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Budding: A new stage in the development of *Chytridiopsis typographi* (Zygomycetes: Microsporidia)

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ABSTRACT

Chytridiopsis typographi Weiser, 1954, the microsporidian pathogen of the spruce bark beetle, *Ips typographus* L. (Coleoptera: Scolytidae), has an early developmental period with plurinucleate mother cells, each of which produces a single bud. The globular bud is connected with the mother cell by a collar and the cellular constituents are pushed to the distant end of the bud. Both the mother cell and the bud continue to develop; the bud then separates from the mother cell and grows to produce a cell of the same type. Both cells then continue sporogonial development and produce sporophorous vesicles with 16–32 spores. The process of a single mother cell producing a single bud that grows to an identical stage is new in the development of *C. typographi* and has no analogy in other Microsporidia.

1. Introduction

The production of the microsporidium *Chytridiopsis typographi* for biological control of *Ips typographus* (Tonka et al., 2009) provided an opportunity to study the early phases in the development of the pathogen. Beetles infected with *C. typhographi* were examined to obtain ultrastructural information in addition to details previously provided by Purrini and Weiser (1984, 1985). Specifically, we were interested in the sporogonial stage ending with two types of sporophorous vesicles (thin-walled and thick-walled) and two types of spores. Vesicles of the thin-walled type dehisce in the gut of the host and release thin-walled spores that extrude their sporoplasms under the effect of the gut juices. These spores serve primarily to spread the infection in the gut of the same host. The rest of the thin-walled spores exit the gut with the faeces and soon lose their activity in the external environment. The thick-walled vesicles are cyst-like structures with resistant walls containing 16–32 spherical thick-walled spores. These vesicles do not release spores but exit the host in the faeces of the beetle, serving to infect new hosts. In our experiments the suspension of stages used for the laboratory infection was freshly prepared from dissected infected beetles and contained both types of sporophorous vesicles. The composition of the spore suspension and immediate transfer to susceptible hosts evidently resulted in increased development of a specific type of early-stage sporogony. These stages were present to a degree exceeding their normal occurrence in field-collected infections. The cells, which are evidently products of sporoplasms from thin-walled spores, form specific mother cells that produce a single bud. The bud grows to a size and structure identical to that of the mother cell. This process is described in the present study.

2. Materials and methods

The microsporidian *C. typographi* is frequently recovered in collections of bark beetles, *Ips typographi* from the Šumava National Park, near to the southwest border of the Czech Republic. Beetles collected from the bark galleries of spruce trees were dissected and the midgut was excised and inspected in a drop of insect saline under a cover slip (Wegensteiner et al., 1996). For production of the pathogen, infected gut tissues were crushed in saline and used to inoculate a group of young adult *Ips typographus* reared in the laboratory. Young (brown) beetles were kept in plastic vials 8 mm in diameter, 100 mm long, with a chip of bark exposed at the end of the tube. The tubes, containing a maximum of 10 beetles per tube, were held at 25 ± 1 °C in a 16:8 light/dark regime. A drop of saline with suspended stages of *C. typographi* from crushed infected gut tissues was placed on the chip of bark fed to the beetles. New clean bark chips were provided every 2 days and dead beetles were removed. The vials were held for 50 days with periodic replacement of the bark. Mortality usually began after day 12 with maximum mortality occurring on day 25 and a second peak on day 35. Mortality ceased after day 45. Dead

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beetles were dissected in saline and positive midguts were stored in water at 4 °C until use.

For the present study, beetles were inoculated as above and active beetles were removed from the rearing tube on day 8 after inoculation. They were decapitated in a droplet of saline and the gut tissues were excised and inspected for infection. The infected tissues were transferred into 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer of pH 7.4, cut into segments, transferred into fresh glutaraldehyde solution, and held overnight at 4 °C. After several washes in cacodylate buffer, the material was post-fixed in 2% aqueous OsO_4 at 4 °C for 2 h. Fixed tissues were embedded in Polybed 812 epoxy resin, stained with uranyl acetate and lead citrate and examined and photographed using a JEOL-JEM 1010 electron microscope at 80 kV equipped with Megaview 3 CD camera.

3. Results

3.1. Pathology

Multiple thin-walled developmental stages were observed in the anterior midgut epithelial cells of the host. The stages were scattered throughout the midgut tissues from the epithelial brush border cells to the basal membrane (Figs. 1–3, bb, bm) and were not concentrated in any layer. Late sporogonial stages, sporophorous vesicles of thin-walled and thick-walled type and mature spores were rare. In Fig. 1, more than 46 early developmental stages in the development to budding mother cells (Bm) and buds (b) are present, and only two thick-walled sporophorous vesicles (SpT) are observed. All stages of the mother cell have a distinct type of cytoplasm that contrasts with the higher density midgut tissue cells of the bark beetle. The initial stages (x) are small spherical stages, 1–2 μm in diameter, with a single nucleus and appear in groups. These stages grow to oval mother cells, each approximately 7 × 5 μm in size, with four nuclei. The thin cytoplasm of these cells contains a system of endoplasmic reticulum and multiple vacuoles enclosed within a thin plasma membrane.

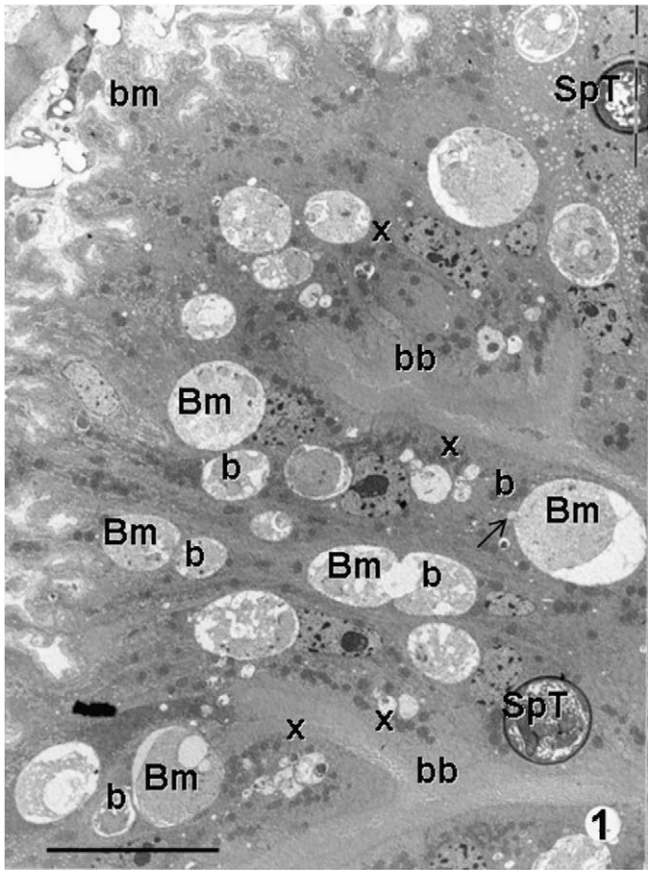

Fig. 1. A section of the midgut of *Ips typographus* with many stages of *Chytridiopsis typographi* maturing to the budding stage. Mother cells (Bm) in the process of budding. Buds (b) in different stages of maturation. First stages (x) in groups. Two thick-walled sporonts (SpT). Basal membrane of the gut (bm), gut epithelia with brush border (bb). Bar = 10 μm.

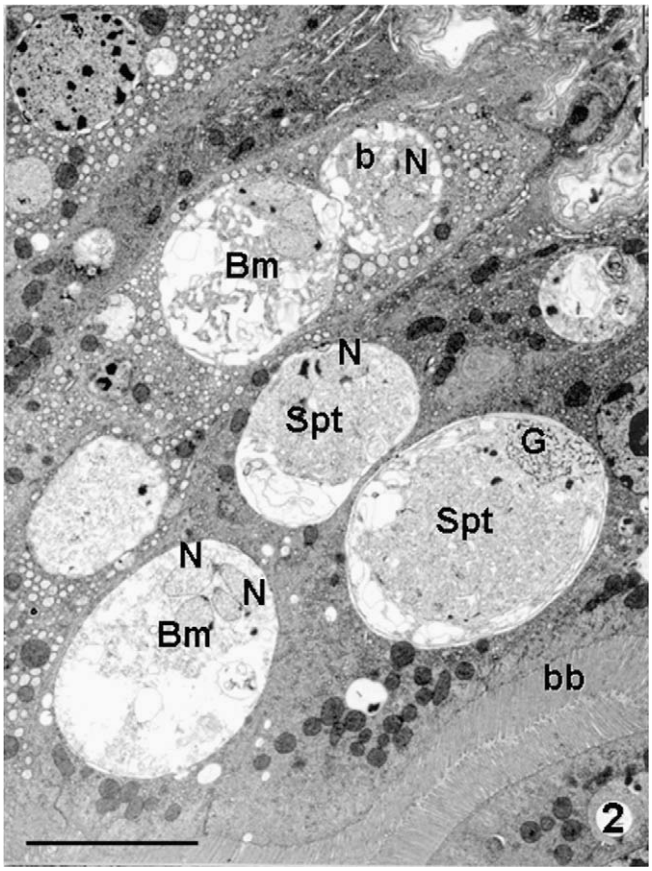

Fig. 2. Mother cell (Bm) ready for budding. Nuclei (N) grouped at one end and vacuolar system prepared for budding (b) at the opposite end. Budding mother cell with multiple Golgi membranes (G) and a bud matured to a thin-walled sporont (Spt). Brush border of midgut epithelium (bb). Bar = 5 μm.

There was no evidence of immune response by the host cells. The thin-walled mother cells were observed near (distance of 2–4 μm) the brush border of the midgut epithelial cells (Fig. 1, bb) or on the opposite side near the basal membrane of the gut (Figs. 1 and 3, bm). Thick-walled vesicles were scarce (Figs. 1 and 3, SpT), without specific orientation to the brush border and the gut lumen. Thin-walled sporonts (Fig. 2, Spt) and developing thin-walled spores were rare in this material and mature thin-walled sporophorous vesicles were not yet formed.

4. Development of bud mother cells

Vacuoles within the maturing mother cell move close to the simple plasma membrane enclosing the stage. This membrane does not thicken during maturation. Mature bud mother cells are broad oval stages 6–8 × 5–6 μm in size (Figs. 1–3, Bm). They usually have four nuclei (N) grouped at one pole of the cell. The nuclei have several flat electron-dense nucleolar plaques fixed on their membrane (Fig. 4, sp) that persist during sporogonial develop-