

## RESEARCH NOTE

## “POLAR VESICLES” OF MICROSPORIDIA ARE MITOCHONDRIAL REMNANTS (“MITOSOMES”)?

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**Abstract.** Conventional transmission electron microscopy was used to localise double-membrane vesicles probably representing mitochondrial remnants (“mitosomes”) in four species of microsporidia. Very few such vesicles were found dispersed throughout cytoplasm with no relationship to other cell organelles. Several double-membrane vesicles per ultrathin section, however, occurred regularly close to the nuclear spindle plaque. These vesicles are identical with the “polar vesicles” typically associated with the microsporidian spindle plaque and known since 1971. The reason for mitosome accumulation near the spindle plaque is unknown. Possibly the spindle plaques are involved in mitosome segregation during cell division.

Microsporidia, highly reduced intracellular parasites, have traditionally been considered to be amitochondriate. In 1997, nuclear genes encoding orthologues of typical mitochondrial heat-shock Hsp70 proteins were detected, suggesting the presence of mitochondria (Germot et al. 1997, Hirt et al. 1997). Also the phylogenetic studies associating microsporidia with fungi (Keeling et al. 2000) and the finding that microsporidia contain genes related to mitochondrial functions (Fast and Keeling 2001, Katinka et al. 2001) implied that microsporidia have cryptic mitochondria or mitochondrial remnants. Williams et al. (2000) immunolocalised by confocal microscopy the mitochondrial Hsp70 protein in meronts of *Trachipleistophora hominis* Hollister, Canning, Weidner, Field, Kench et Marriott, 1996, observing between 7 and 47 (mean  $28 \pm 13$ ) structures dispersed in the cytoplasm of the microsporidian cell. These structures, representing mitochondrial remnants, were shown by transmission electron microscopy to be double-membrane vesicles measuring  $50 \times 90$  nm. This conclusively demonstrated that microsporidia have atypical, reduced mitochondria, similar to mitochondrial remnants (“mitosomes”) of several anaerobic protists (van der Giezen and Tovar 2004).

In order to determine whether mitosomes occur in other microsporidian species, we investigated meronts and sporonts of four species of microsporidia: *Vavraia culicis* Weiser, 1977 produced in *Spodoptera exigua* (Hübner), *Amblyospora* sp. from *Cyclops strenuus* Fischer (formerly reported as *Stempel- lia* sp.; see Vávra 1976), *Vairimorpha* sp. from *Lymantria dispar* L., and *Marssoniella elegans* Lemmermann, 1900 parasitizing *Cyclops vicinus* Uljanin. Ultrathin sections of glutaraldehyde-osmium fixed (Hirsch and Fedorko 1968), epoxy resin-embedded material, were examined by transmission electron microscopy for the presence of double-membrane vesicles representing mitosomes, using a JEOL 1010 electron microscope equipped with a Megaview 3 CD camera, enabling us to work at high resolution.

Double-membrane vesicles were found in all microsporidia evaluated (Figs. 1–8) either as individual vesicles dispersed in the cytoplasm without spatial relationship to other cytoplasmic organelles or groups of vesicles regularly associated with the spindle plaque (a lentil-like electron-dense structure situated in a depression of the nuclear membrane, the mitotic spindle organisation centre in microsporidia) (Vávra 1976, Vávra and Larsson 1999). Vesicles dispersed in the cytoplasm were rare, with a maximum of one vesicle observed per ultrathin section (Figs. 1–3); in many sections no vesicles were observed. Vesicles associated with the spindle plaque occurred regularly, numbering 3–5 per section in close proximity to each spindle plaque (Figs. 4–8).

The double-membrane vesicles in most species investigated were oval, about  $150\text{--}200 \times 100$  nm in size, without internal structures (Figs. 6–8). In *Vavraia culicis*, however, they were more conspicuous, being long, lobed, and  $200\text{--}500 \times 100\text{--}250$  nm in size. The interior of the vesicles was slightly more electron-dense than the surrounding cytoplasm. In some vesicles it contained electron-dense granules and in some vesicles structures resembling membranous folds were seen (Figs. 1–5).

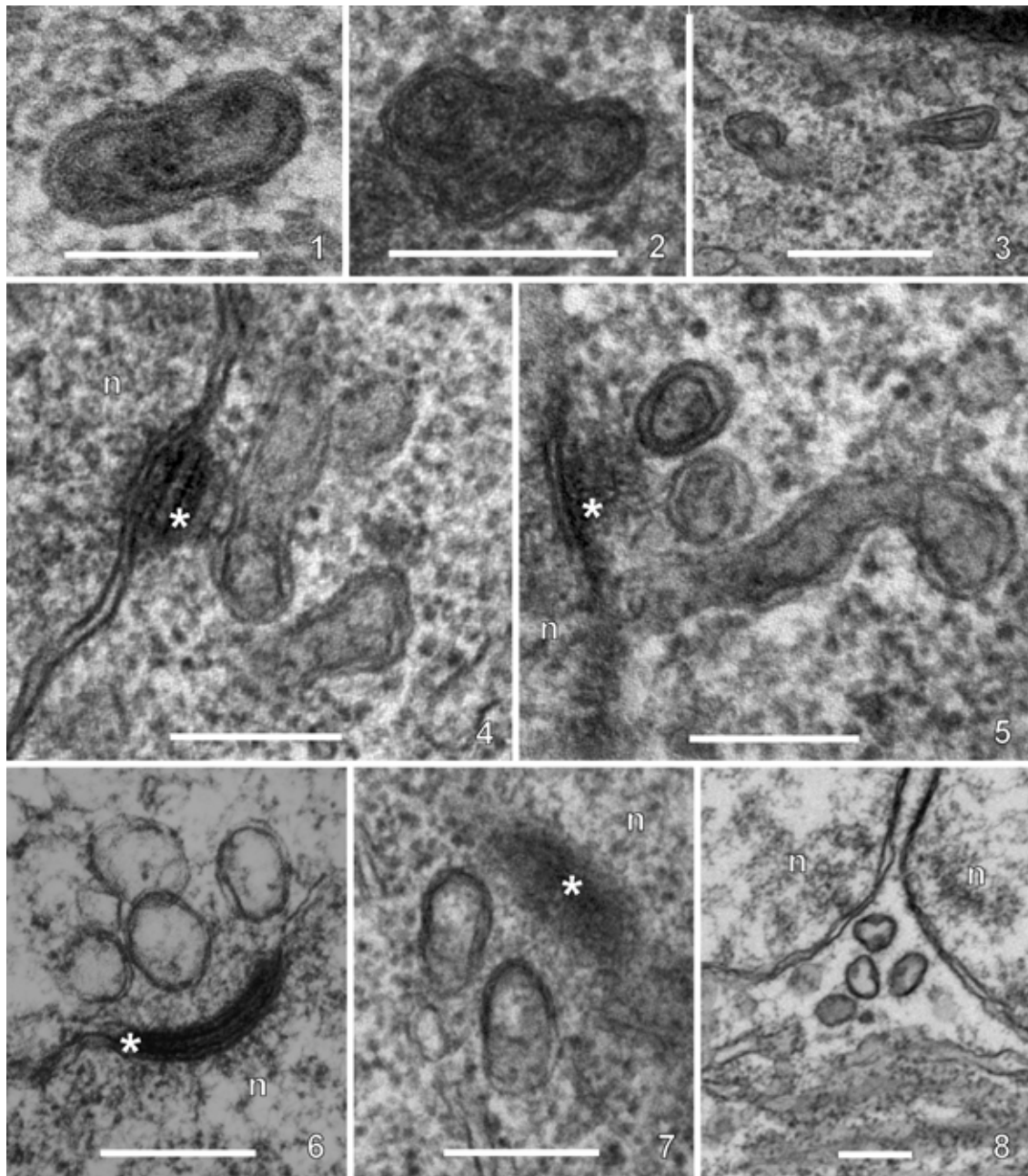
The vesicles scattered freely in cytoplasm correspond to the mitochondrial remnants seen by Williams et al. (2000). The double-membrane vesicles associated with the spindle plaque, although probably also mitochondrial remnants, correspond to “polar vesicles”, a well-known entity from past ultrastructural studies of microsporidia. They were first reported by Youssef and Hammond (1971) and were later recorded by a number of authors (see Vávra 1976, Vávra and Larsson 1999).

Previous observations describe polar vesicles as 3–6 round structures,  $100\text{--}200$  nm in size, enveloped by two membranes and localised in the proximity of the microsporidian spindle plaque. The polar vesicles regularly occur close to the spindle terminus cytoplasmic face, but have no direct contact with it. It is possible that the unusually long polar vesicles observed in some cells of *Vavraia culicis* (Figs. 4, 5) were mitosomes commencing division. They occurred near the spindle plaques, which have a layered structure, possibly indicating that the spindle plaques started the duplication process.

We propose that the polar vesicles of microsporidia are mitochondrial remnants (mitosomes) specifically assembled near the spindle microtubular organizing centre. The reason for this association is not known. The possibility that spindle plaques are involved in mitosome segregation during cell division should be considered. Configuration somewhat similar to polar vesicles in microsporidia occurs in yeasts, which also have spindle plaques. In yeasts, mitochondria line the dividing nucleus (Zickler and Olson 1975).

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**Figs. 1–8.** Microsporidian mitosomes. **Figs. 1–3.** *Vavraia culicis* mitosomes lying freely in the cytoplasm. Note internal granules and membrane folds shown respectively in Figs. 1 and 2. **Figs. 4–8.** Polar vesicles (presumed microsporidian mitosomes) associated with the spindle plaque. **Figs. 4, 5.** *Vavraia culicis*. **Fig. 6.** *Amblyospora* sp. **Fig. 7.** *Marssoniella elegans*. **Fig. 8.** *Vairimorpha* sp. Explanation: n – nucleus; asterisk – spindle plaque. Scale bars = 200 nm. (Fig. 6 reprinted with permission from Vávra 1976.)

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