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Unikaryon montanum sp. n. (Protista: Microspora), a new pathogen of the spruce bark beetle, *Ips typographus* (Coleoptera: Scolytidae)

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Abstract. *Unikaryon montanum* sp. n. infects the fat body, muscle, Malpighian tubules and ovaries of adult *Ips typographus* L. The development is haplokaryotic, with separate nuclei during the schizogony and sporogony. Sporonts have the cellular envelope with added layer of electron dense material. Two types of spores are formed: small broad-oval primary spores, 1.5 × 1.0 µm, with warty surface of spore wall, uninucleate, with isofilar polar filament in 5/6 coils and elongated-oval environmental spores, 0.8-1 × 2 µm, with warty spore wall attenuated at the anterior end, uninucleate, with spore polar filament in 8 coils. Both types have a dual polaroplast with the anterior part of a layer of confluent fine lamellae ending posteriorly in bulbous processes, and posterior part composed of coil of tubules.

In adult spruce bark beetles, *Ips typographus* L., collected recently from some localities in SW Germany, Austria and Czech Republic, a new microsporidian was present with minute spores infecting different tissues of the beetle. Its description is presented in this paper.

MATERIALS NAD METHODS

Adults where the infection was present were collected from nuptial chambers of trap trees and dissected in the laboratory. The gut was removed together with parts of the adhering fat body, the Malpighian tubules and the ovaries and inspected under the microscope. Where the infection was present, part of the material was used for preparation of dry smears and another part was fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4) at 4°C for 4 h, washed in the same buffer and postfixed in 1% osmic acid in buffer 1 h at 4°C. After dehydration in alcohol-aceton series it was embedded in Vestopal W. Dry smears were fixed with metanol and stained with Giemsa. Semithin sections of resin blocks were stained with toluidin blue.

RESULTS

The spores of the microsporidian were present in the fat body, the tissues of the ovary and in egg follicles, the muscles and in Malpighian tubules of adult beetles. Stages were distributed in tissues in irregular groups (Fig. 1). In water-mounts there was no evidence of any groups closed in sporophorous vesicles. Morphology of stages from all three localities was identical.

Unikaryon montanum sp. n. Figs. 1-11

Description: Vegetative stages were rare in smears and sections, most stages were sporoblasts and spores. Sporoblasts were elongate oval cells 2.5-3 × 1.5-2 µm, with a single nucleus in the posterior part. Mature spores were present in two types (measures of fixed material): short broad oval spores 1.5 ± 0.1 × 1.0 ± 0.1 µm (n = 20) and less stained elongate oval spores 2.0 ± 0.1 × 0.9 ± 0.1 µm. There was evidence of only one type of sporoblast for both types of spores. Both types of spores were free in the cytoplasm of host cells, not in membrane-bound groups.

Electron microscopy shows sporonts as uninucleate stages (Fig. 2) with a layer of electron-dense material added externally to the plasma membrane, with some interruptions. In young spores this covering material (Fig. 2) changes into a thick homogenous layer of the spore wall, partially electron-dense and wrinkled on its surface (Figs. 4-6). The electron-dense material of the sporoblast wall is spread diffusely in the electron-lucent incrustation of the wall and is more distinct on the outer and inner surface. In empty spores (Fig. 3) the electron-dense material is evident in the outer surface layer when the rigid interior wall disappears. Empty spores are wider than non-activated spores. The germ in broad oval spores has a single spherical nucleus (Fig. 4) enclosed in a distinct double unit membrane, located in the centre of the spore. A multiple system of ribosomes fixed to membranes of the rough endoplasmic reticulum encircles the nucleus. The polar filament is fixed in an arched anchoring disc (Fig. 8), inserted in a depression

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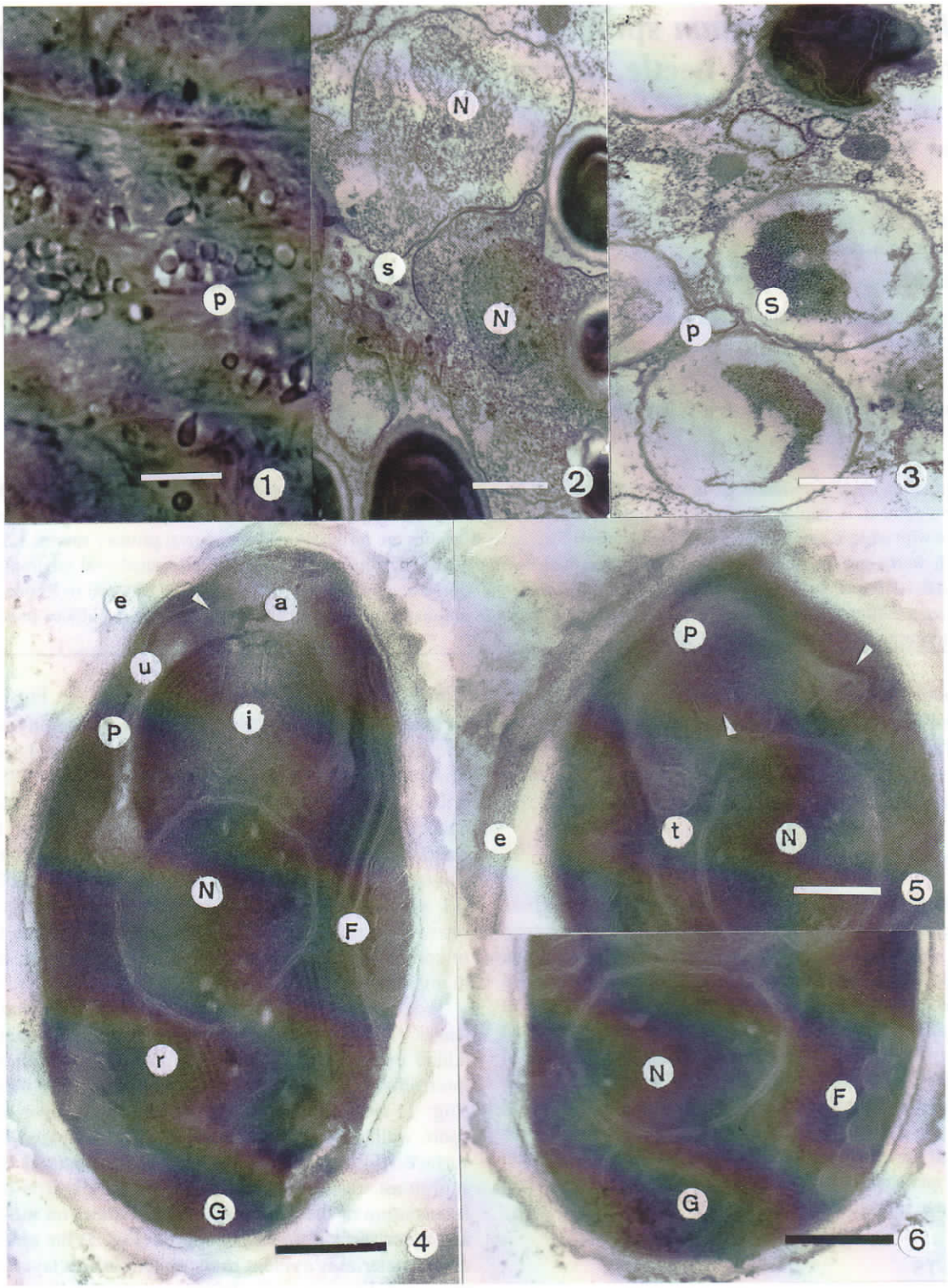

Fig. 1. Primary (p) and environmental (E) spores and vegetative stages of *Unikaryon montanum* in muscle fibres of adult *Ips typographus*. **Fig. 2.** Early sporonts with nucleus (N) and thickened membranes (s). **Fig. 3.** Empty primary spores (p) with remains of polaroplast material (S) after filament extrusion. Attenuated spore wall. **Fig. 4.** Primary spore. N - nucleus, e - spore wall, F - polar filament, G - Golgi system, P - outer polaroplast, i - inner polaroplast, r - ribosomal system, a - anchoring disc with umbrellar border (u). **Fig. 5.** Section of the anterior end of a primary spore with structures of the outer polaroplast (P) divided posteriorly into tubular coils (t). N - nucleus, e - spore wall. **Fig. 6.** Posterior end of primary spore with cross sections of the isofilar polar filament (F) and details of tubules of the Golgi systém (G), N - nucleus. Scale bars 5 µm (Fig. 1), 0.5 µm (Figs. 2,3) and 0.25 µm (Figs. 4-6).

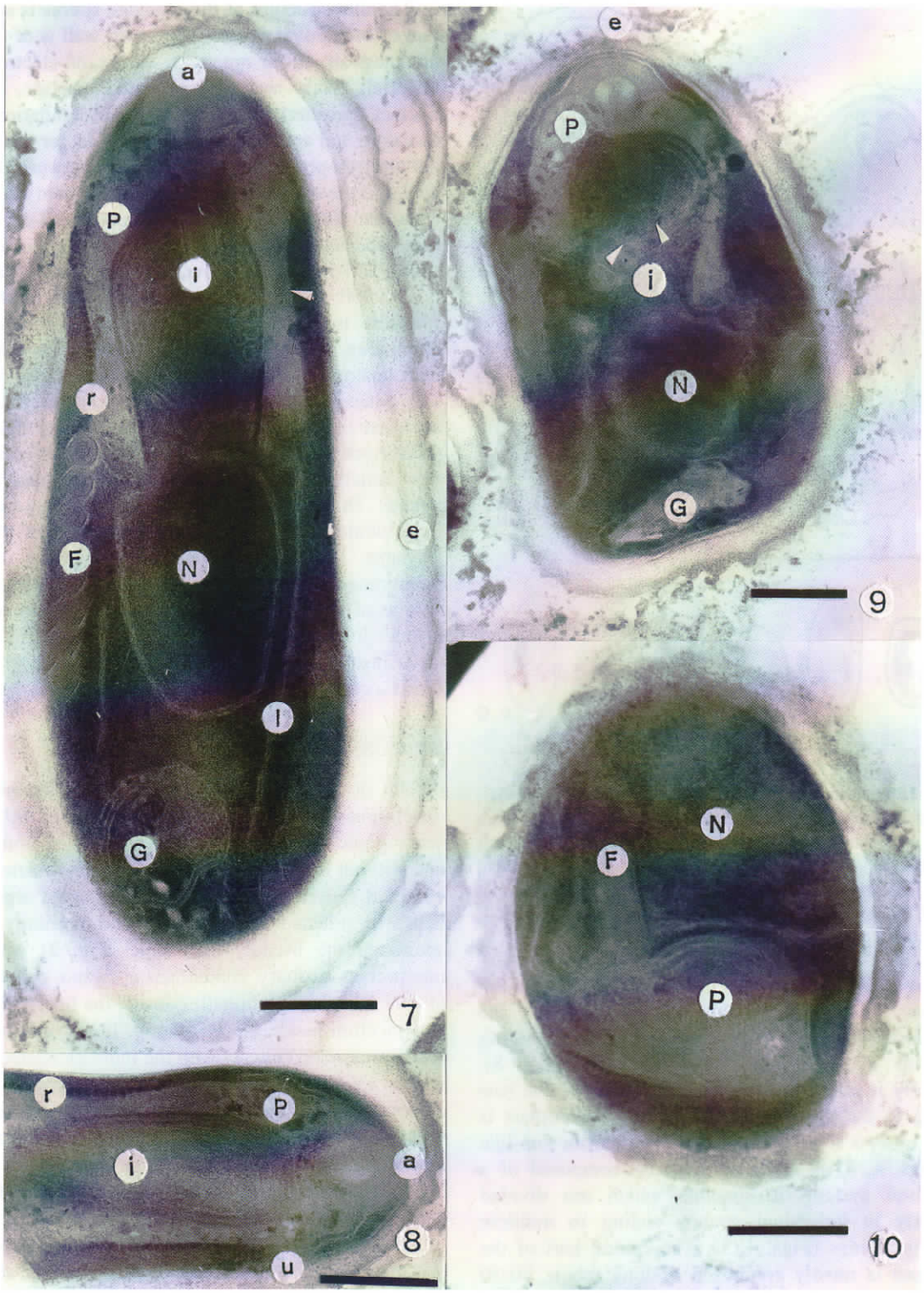

Fig. 7. Elongate environmental spore of *Unikaryon montanum*. N - nucleus, e - vrinkled spore wall, F - isofilar polar filament, G - Golgi apparatus, r - ribosomal basket with longitudinal lamella (I), P - outer polaroplast, i - inner polaroplast of closely packed tubuli, a - anchoring disc. **Fig. 8.** Apical end of environmental spore with ribosomes (r), outer (P) and inner (i) polaroplast. Anchoring disc (a) with umbrellar border (u). **Fig. 9.** Primary spore with details of apical fixation of polar filament. P - outer polaroplast with cracks in lamellar part and tubular ending, i - inner polaroplast with details of cross sections of tubules (arrowheads) filled with granular secretion, N - nucleus, G - Golgi system. **Fig. 10**. Primary spore with forming system of polaroplast (P) adhering to nucleus (N) and connected with polar filament (F). Scale bars = 0.25 μm.

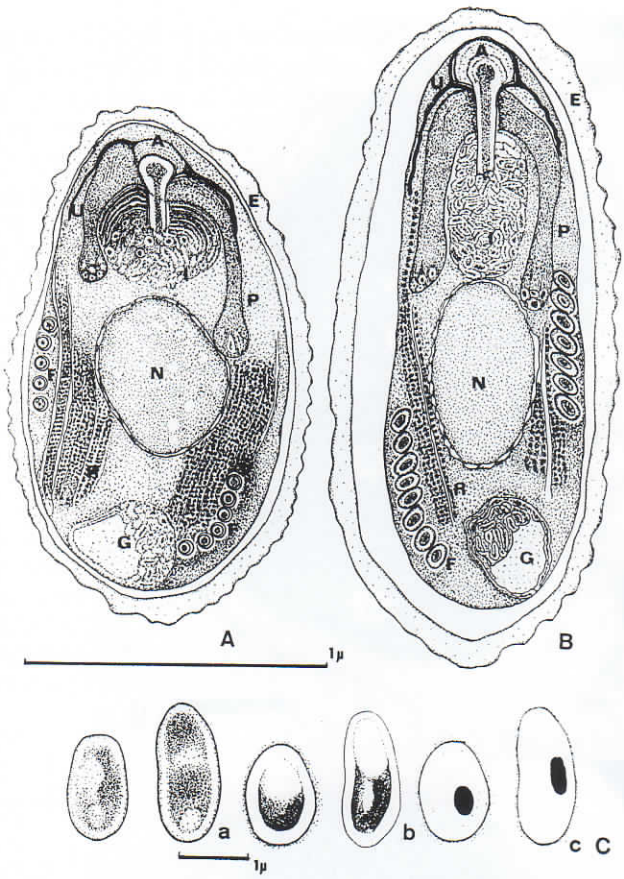

Fig. 11. Ultrastructure of primary (A) and environmental (B) spores of *Unikaryon montanum*. A - anchoring disc, E - spore wall, F - polar filament, G - Golgi system, I - inner polaroplast, N - nucleus, P - outer polaroplast, R - ribosomal basket, U - umbrellar border. Primary and environmental spores (C) in: a - watermount, b - Giemsa smear, c - after HCL nuclear staining.

of the spore wall. The anchoring disc extends a flat umbrellar border over the anterior part of the polaroplast. The polar filament is fixed in the anchoring disc with a pestle-like thickened head (Fig. 8). Posteriorly it is coiled in 5/6 isofilar turns in one row adhering to the spore wall. The binary polaroplast is formed by two separate systems. The anterior cup-like part (Figs. 4, 8) is in its apical mass composed of a compressed system of lamellae which are divided posteriorly in individual strands ending in multiple tubular groupings (Figs. 4, 5). The central part of the polaroplast is mostly composed of long tubuli, 20-30 nm in diameter and up to 350 nm long. They are fixed to the external membrane of the manubrioid part of the polar filament and are turned posteriorly (Fig. 4). This complex system is formed in young spores from a common coil. of tubules and lamellae close to the nucleus, adhering to the anterior end of the filament (Fig. 10). In the posterior pole of the spore the Golgi system is rather indistinct.

The ultrastructure of the elongate spores is similar to that of broad oval spores. The spore wall is of the same thickness of 40-50 nm, with admix of electron-dense material, with warty, corrugated surface, without distinct episporal membrane and differentiation of the exospore and endospore. A distincly larger space without electron-positive material remains between the spore wall and the sporoplasm. The polar filament is coiled in 8 isofilar turns adhering to the spore wall in a single row. The anchoring disc fills the anterior pole of the spore, 200-500 nm in diameter and its umbrellar border is 700-800 nm broad (Fig. 9). The base of the polar filament is pestle-like thickened and composed of alternating layers of different density corresponding with analogous zones on cross section of the filament. The anterior (manubrial) part of the polar filament is enclosed in a binary polaroplast, similar to that of the shorter spores. The outer part is composed of a felt of fine lamellae ending posteriorly in separate bulbous strands. The inner part (Figs. 8, 9) is formed of a coil of long tubules adhering to the straight part of the polar filament. The single nucleus is enclosed in a double membrane and is surrounded by ribosomes adhering to a system of membranes of the endoplasmic reticulum. A distinct longitudinal lamella of the smooth ER divides this ribosomal mass on both sides. The Golgi system forms a coil in the posterior pole of the spore.

DISCUSSION

The described microsporidian has a haplokaryotic development as defined by Sprague et al. (1992), with schizogonic as well as sporogonic stages uninucleate and without formation of a sporophorous vesicle (Vávra 1976). It causes no hypertrophy of infected organs and cells. In infected tissues we found currently empty spores of the broadly oval type (Fig. 2) which we interpret as primary spores *sensu* Iwano and Ishihara (1991). They are germinating inside the host and spread the infection inside the host body. In our material they are intermingled (Fig. 1) with the more elongated spores which we interpret as environmental spores of the authors. The elongated spores provide material surviving outside the host and infecting new hosts after ingestion. There is no evidence that short spores mature into elongate spores and there was no evidence of empty elongate spores on any section of infected tissues.

Among ultrastructural characters of mature spores of this microsporidian most important is the fine structure of the polaroplast. Five types of polaroplasts proposed by Larsson (1986) do not include this type. The binary construction of the polaroplast defined in Vávra (1976) and used in Larsson (1986) as lamellar structures of different density is further developed in our microsporidian and we propose a definition of a sixth type of this tabulation, with distinct outer layer of confluent fine lamellae ending posteriorly in irregular bulbous buds

and the inner part distincly separated and composed of long tubules adhering to the manubrial part of the polar filament. This arrangement is characteristic for the members of the genus *Unikaryon* infecting beetles.

The two spore types characterized as early and environmental differ in shape and size and in the number of coils of the polar filament. As mentioned, the empty spores distributed in infected tissues were all of the shorter broad type. The spore wall of this group of microsporidia does not distinguish an electron-dense exospore and electron-lucent endospore and the single wall contains electron-dense material admixed in the whole lucent mass of the wall.

The taxonomic position of this microsporidian with uninucleate spores and haplokaryotic development corresponds with the genus *Unikaryon* Canning, Lai et Lie, 1974. In a discussion of this genus (Weiser et al. 1995) it was mentioned that the description of the type species *U. pyriformis* Canning, Lai et Lie, 1974 was not based on a satisfactory definition of ultrastructures and further species which were subsequently included in this genus were from very different hosts and not necessarily congeneric. In the drawing of *U. legeri* Azevedo et Canning, 1987, the structure of the polaroplast was not closer specified in species infecting Trematoda. In a recent description of species from Coleoptera by Toguebaye and Marchand (1983, 1984, 1988) structures of the polaroplast are not well distinguished, but the structure of the spore wall in *U. bouixi* (Toguebaye and Marchand 1983) is very similar to our species. This supports our opinion that structures of the spore as described in our material (Fig. 11) are typical at least for Coleoptera infecting species of the genus *Unikaryon*. Therefore, we propose, to include the described microsporidian into the genus *Unikaryon*. It differs from other species described in this group (list see in Weiser et al. 1995) and we consider it a new species, *Unikaryon montanum* sp. n.

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