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[1]*Institute of Forest Entomology, Forest Pathology and Forest Protection, BOKU-University of Natural Resources and Applied Life Sciences, Vienna, Austria;* [2]*Insect Pathology, Institute of Entomology, Academy of Sciences of the Czech Republic, Ceske Budejovice, Czech Republic;* [3]*Electron Microscopy, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

Infection of *Tomicus piniperda* (Col., Scolytidae) with *Canningia tomici* sp. n. (Microsporidia, Unikaryonidae)

By B. KOHLMAYR[1], J. WEISER[2], R. WEGENSTEINER[1], U. HÄNDEL[1] and Z. ZIZKA[3]

Abstract

***Canningia tomici* sp. n. (Microsporidia, Unikaryonidae) infects the midgut epithelium, the gut muscules, Malpighian tubules, connective tissues, adipose tissues and the gonads of the pine shoot beetle, *Tomicus piniperda* (L.) (Coleoptera, Scolytidae). The infection is present in populations of *Tomicus piniperda* in Europe and in the United States. Uninucleate oval single spores occur in two sizes: 2.8 ± 0.4 × 1.4 ± 0.4 µm and 3.8 ± 0.3 × 2.0 ± 0.2 µm. The polar filament of this microsporidium is fixed subapically in a flat anchoring disc. The thick posterior lamellae of the binary polaroplast are asymmetric due to the lateral fixation of the polar filament.**

1 Introduction

Tomicus piniperda (L.) (Coleoptera, Scolytidae), the pine shoot beetle, is a widely distributed pest of *Pinus*, especially of the Scots pine (*Pinus sylvestris*), throughout Europe and more recently in North America, (HAACK, 1997). Scots pine is used for reforestation in vast areas of Europe, where it is often the predominant tree species. Environmental stress leads to infestation, and mature stands often suffer from poor site, insufficient ecological conditions, and damage from this and other bark beetle species. *Tomicus* species (*T. piniperda, T. minor* and *T. destruens*) overwinter as adults, emerge in early spring and are attracted to wind-thrown trees to serve as sites of infestation. Females emerge from the breeding systems after laying one batch of eggs. After subsequent ("regeneration") feeding in one- and two-year-old twigs in the crown of old trees, they initiate a second or "sister brood". Emerging callow adults perform maturation feeding in one-year-old twigs of mature trees. Maturation feeding occurs frequently at forest sites next to timber yards or close to saw mills, wherever infested trees allow completion of the bark beetles' development (LÅNGSTRÖM and HELLQUIST, 1990).

Control of bark beetles is difficult because of their secluded life. Bark beetles are controlled by means of the trap tree method and by removal of all breeding material. Biological control with the use of predators, parasitoids or pathogens may help with this problem, however, pathogens are the least studied aspect of bark beetle biology. A few microsporidian species are recorded from different bark beetle species, including e.g.: *Nosema typographi* (WEISER, 1955), *Chytridiopsis typographi* (WEISER, 1970), *Unikaryon minutum* (KNELL and ALLEN, 1978), *Canningia spinidentis* (WEISER et al., 1995), *Unikaryon montanum* (WEISER et al., 1998) and *Unikaryon polygraphi* (WEISER et al., 2002).

This is the first report of a microsporidium isolated from *T. piniperda*. The only other entomopathogen from *T. piniperda* is the fungus *Beauveria bassiana* (NUORTEVA and SALONEN, 1968; LUTYK and SWIEZYNSKA, 1984).

The aim of this project was to examine pathogen prevalence in *T. piniperda* and *T. minor* from various locations in Europe and through different generations (parental and filial beetles). The results of our study presented in this paper describe the occurrence of a previously unknown microsporidium species which may be of importance in limiting populations of *T. piniperda* in different regions of Europe and in North America.

2 Material and methods

Starting with our investigations in 1999, adult bark beetles (*Tomicus destruens, T. minor* and *T. piniperda*) were collected during April and May from infested pine logs in managed forests from 33 stands in seven European countries (Austria, Czech Republic, Finland, Greece, Hungary, Italy and Poland) and from two sampling localities in the USA (Michigan) (table 1).

Tomicus-infested log sections from Austria, Czech Republic, Greece and Italy were cut from trap logs and stored in breeding chambers at 21.6° to 23.4°C (±1°C) under long day conditions (L:D = 16:8) in the laboratory. Emerging beetles, both dark colored parental and pale colored "callow adult" filial beetles, were removed daily and stored in an incubator together with some pine twig pieces at 15 °C until microscopical examination for Microsporidia (≤ one week). Bark beetles of the parental generation from Finland, Poland and the USA were collected by hand over a period of a few days in the field (in spring 1995, 1997, 1999 and 2000). At one sampling plot at Hyytiälä, Finland, larvae were collected by opening breeding galleries and collecting by hand.

Live beetles, with exception of beetles from the USA (frozen immediately after sampling), were dissected according to the method described in WEGENSTEINER and WEISER (1996). The thorax was cut and the gut was pulled out by the last abdominal segments and placed on a microscope slide in a drop of water. Vegetative stages were transferred to Ringer's solution (7.5 g NaCl + 3.5 g KCl + 2.8 g $CaCl_2$ ($2H_2O$) in 1000 ml with distilled water). The whole gut, Malpighian tubules, gonads, adipose tissues and haemolymphe were inspected using a light microscope. Infected tissues were fixed in 2 % glutaraldehyde in 0.2 M sodium cacodylate buffer (pH = 7.2) at 4 °C for 24 h,

Table 1. Data of locations and forest stands where microsporidia infected *T. piniperda* were found.

Country/location	Data of location					Data of forest stand	
	Altitude [m]	Coordinates East/West	Coordinates North	Gradient [%]	Orientation	Age group*	Tree population
A/Altenburg	310	15°37′00″E	48°38′28″	<10	SO	III	*P. sylvestris*
A/Mattersburg	540	16°21′16″E	47°41′38″	<30	NNO	III	*P. sylvestris*
CZ/Otradovice2	250	14°46′E	50°12′	0	–	III	*P. sylvestris*
CZ/Stara Boleslav	250	14°44′E	50°12′	0	–	IV	*Quercus* sp., *P. sylvestris*
FIN/Hyytiälä	~100	24°17′E	61°50′	<10	S	II	*Picea abies*, *P. sylvestris*
FIN/Joensuu	~100	29°46′E	62°36′	0	–	V	*P. sylvestris*
PL/Sekocin	~100	20°52′59″E	52°06′59″	0	–	–	*P. sylvestris*
USA Mi/Snowcap Ingham C	~200	84°30′ W	42°41′	0	–	–	*P. sylvestris*
USA, Mi/Fenner Arboretum	~200	84°30′ W	42°41′	0	–	–	*P. sylvestris*

* age group II: <40 years; III: <60 years; IV: <80 years; V: <100 years.

Table 2. Infections in *T. piniperda* (%) in different years and localities.

Country/location	Year	$\sum$ dissected total	Microsporidia infection in *T. piniperda*							
			Total	%	N♂♂	%	N♀♀	%	N??	%
A/Altenburg	1995	34	1	2.9	1	6.7	0	0.0	0	0.0
A/Mattersburg	2000	353	32	9.1	16	10.3	15	7.7	1	33.3
CZ/Otradovice2	2000	80	1	1.3	0	0.0	1	1.6	0	0.0
CZ/Stara Boleslav	1999	206	20	9.7	7	7.8	13	11.6	0	0.0
	2000	260	1	0.4	1	1.0	0	0.0	0	0.0
FIN/Hyytiälä	1997	12	1	8.3	0	0.0	1	10.0	0	0.0
	1999	263	24	9.1	1	3.1	22	9.0	1	50.0
	2000*	189	3	1.6	0	0.0	0	0.0	3	0.0
	2000	24	1	4.2	0	0.0	1	5.0	0	0.0
FIN/Joensuu	2000	161	20	12.4	6	10.2	13	13.3	1	25.0
PL/Sekocin	2000	138	3	2.2	0	0.0	3	3.6	0	0.0
USA/Mi/Fenner Aboretum	2000	355	99	28.0	35	31.0	34	23.6	30	30.6
USA/Mi/Snowcap Lansing	2000	121	41	33.9	17	34.7	23	32.9	1	50.0

* larvae.

washed twice and stored in cacodylate buffer. The tissues were postfixed in 2 % osmic acid for 4 h at 4 °C, then dehydrated gradually in a series of ethanol and acetone and finally embedded in Vestopal W. Ultrathin sections were made and stained in uranyl acetate and lead citrate. Semithin sections were used for histology and were stained with toluidine blue and enclosed in Eukitt for light microscopy.

The dissecting droplet was used for preparation of a dry smear, which was subsequently fixed in methanol and stained with Giemsa (ROMEIS, 1968; WEISER, 1977). The size of Giemsa-stained spores was measured under the light microscope with an ocular micrometer (magnification 1000×). These data (length and width of the spores) were used for K Mean Cluster analysis in SPSS 8.0 for Windows. Mean value and standard deviation of length and width were calculated and two defined groups (WEBER, 1986; BROSIUS, 1998) were determined.

3 Results

3.1 Prevalence of Canningia tomici

Our study revealed a new microsporidium in *T. piniperda*. The gut epithelium, the muscularis of the midgut, the Malpighian tubules, the adipose tissues and the gonads were infected with this pathogen. Infections with Microsporidia were recorded in beetles from nine different locations (table 2), reaching the highest prevalence in beetles from Snowcap (33.9 %) and Fenner Arboretum (28.0 %) (both in USA). Only one infected specimen was found in beetles from Altenburg (2.9 %), Otradovice 2 (1.3 %), Stara Boleslav (2000: 0.4 %), and Hyytiälä (1997: 8.3 %; 2000: 4.2 %). In addition, the number of infected male and female beetles was recorded. In two of the localities, microsporidian infections were recorded in multiple years (table 2). 189 *T. piniperda* larvae from Hyytiälä were inspected in the year 2000 (table 2); three of those larvae (1.6 %) were infected with the microsporidium. None of the infected larvae or adults exhibited any externally visible symptoms, such as obvious changes in mobility, growth or color compared with uninfected ones.

3.2 Seasonal occurrence of Canningia tomici

Phenology of *T. piniperda* differed slightly comparing beetles' emergence from log sections from Stara Boleslav, CZ (1999) and from Mattersburg, A (2000) (fig. 1). Phenology of infections in the beetles from these two localities differed also slightly, starting in week 23 in 1999 (one female, from Stara Boleslav) and in week 22 in 2000 (one female and one male, from Mattersburg) (fig. 1); in both cases, these emerging beetles belong

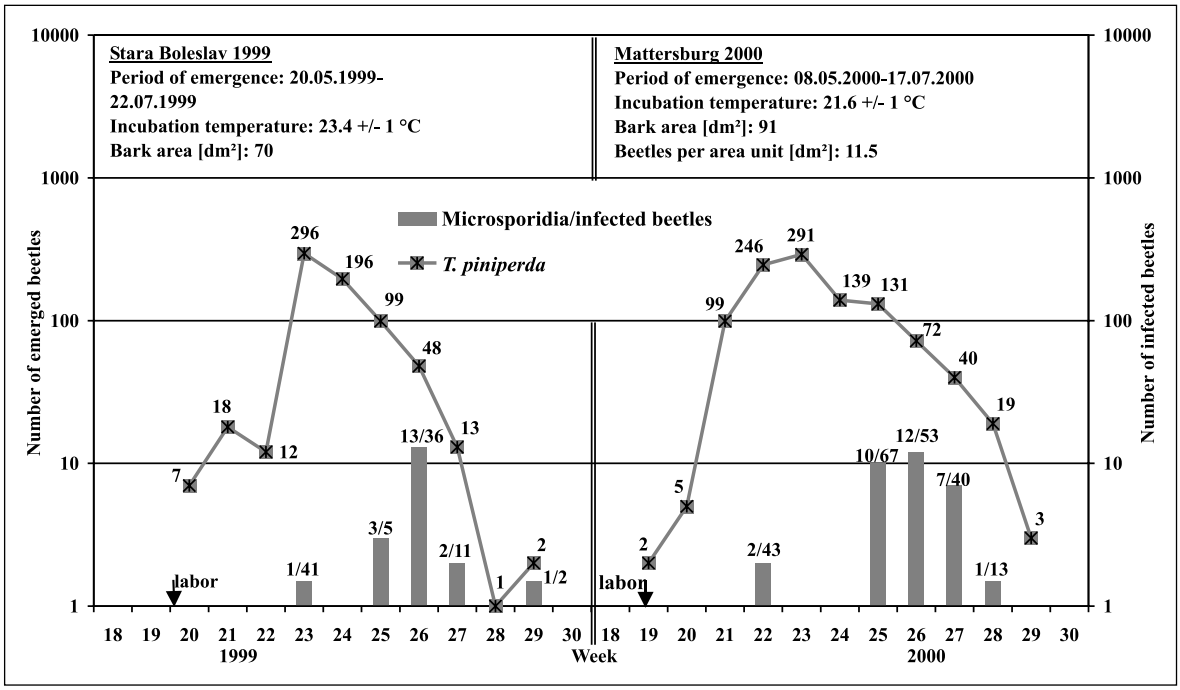


Fig. 1. Phenology of *T. piniperda* at the locations Stara Boleslav (1999) and Mattersburg (2000); the squares combined with a line show the numbers of emerging beetles (left log-scale) during the whole observation period and the bars show the numbers of microsporidia infected *T. piniperda* (right log-scale).

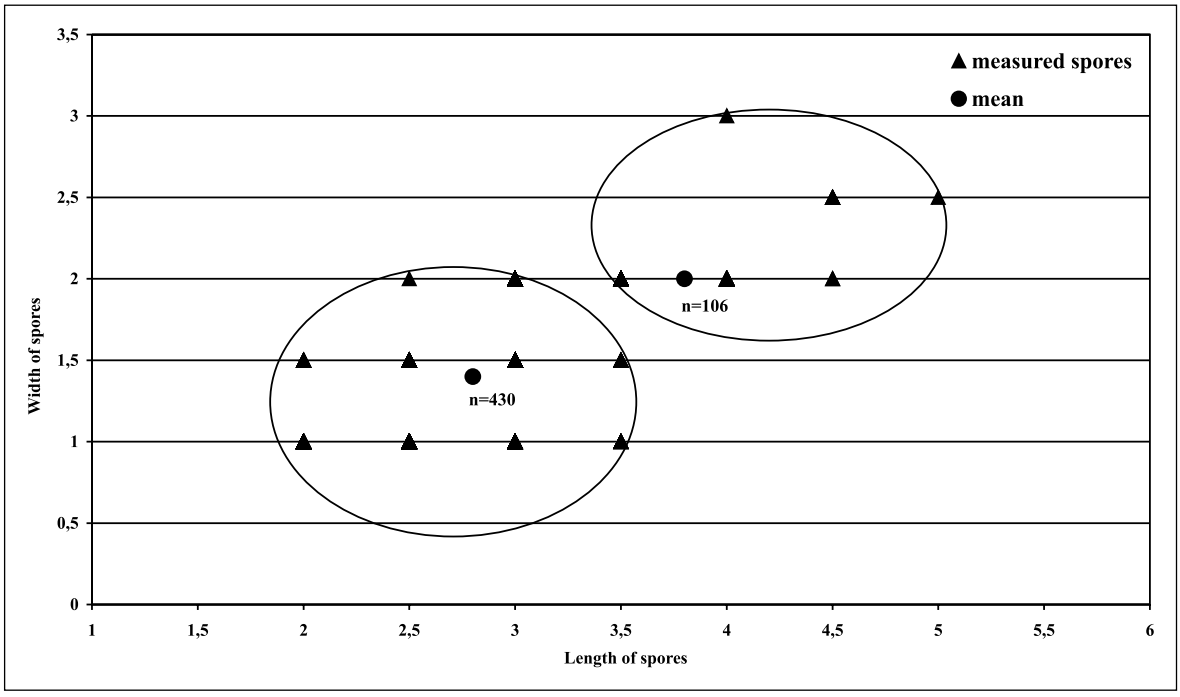
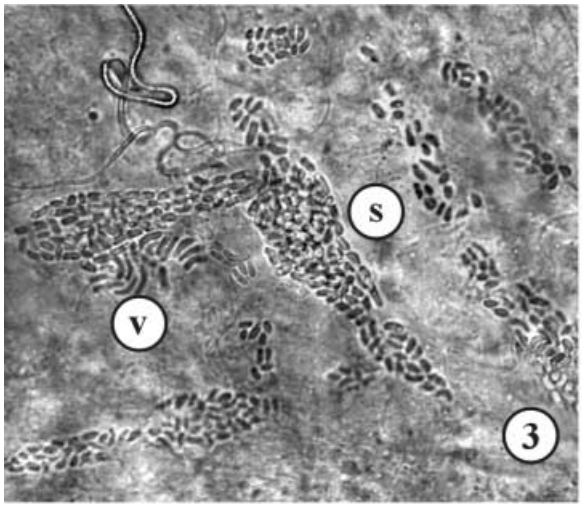


Fig. 2. Mean length and width of spores, allowing the distinction of two groups of spores. The number of measured spores in the respective size group is denoted by n.

most probably to the parental generation, whereas all other infected beetles (in both cases) belong to the filial generation (fig. 1). The highest infection rates were found in offspring beetles. At both locations, the total number of infected beetles increased in the second part of the emerging period, and the highest number of infected beetles was found in week 26. Although host density differed in the two locations (5.9; 11.5 beetles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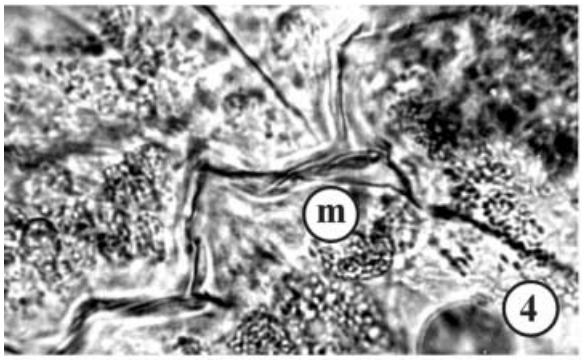
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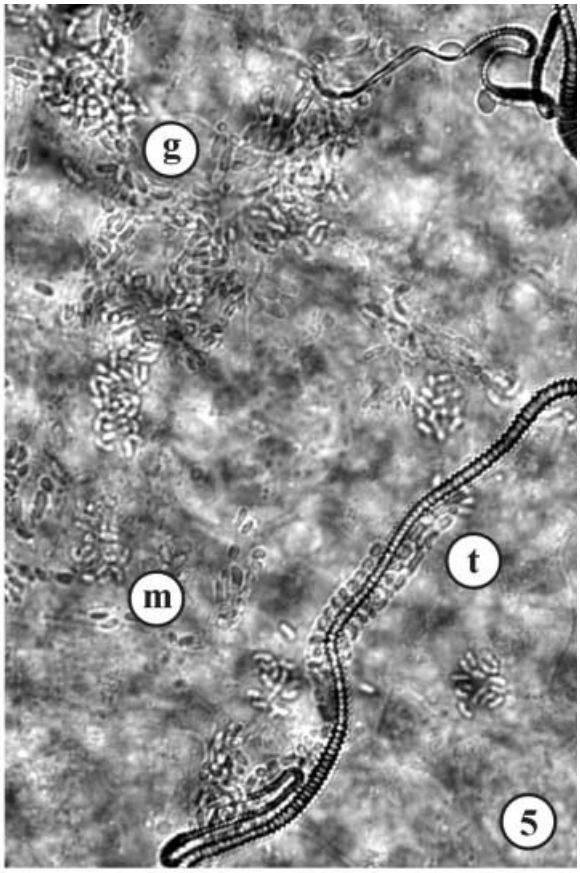
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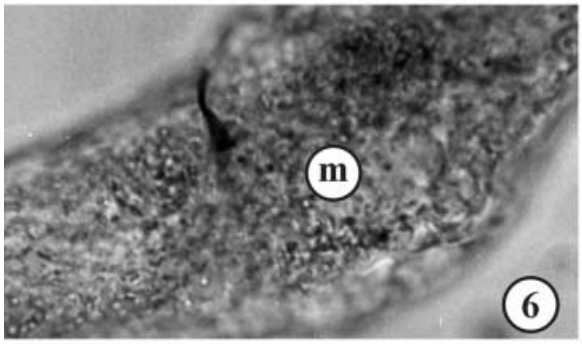
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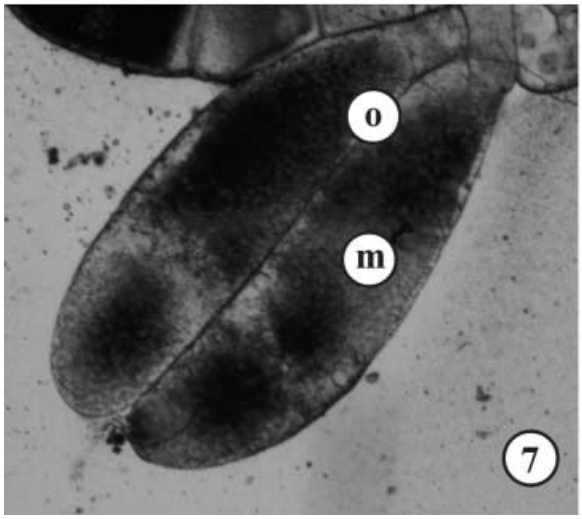
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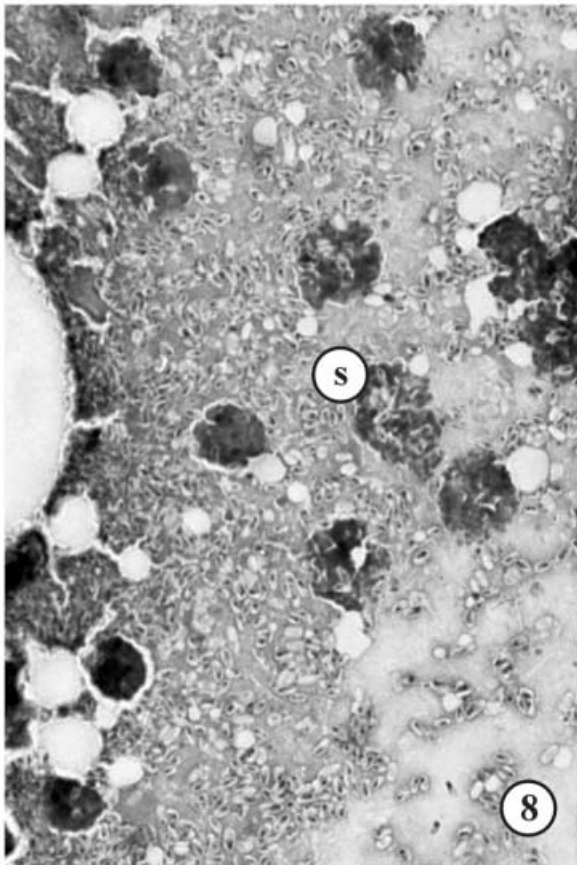
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per dm^2), we did not find a correlation between host density and infection prevalence.

3.3 Light microscopic observations on Canningia tomici

The midgut was infected in its epithelial part to a minor extent, and the infected cylindrical cells were not hypertrophic and prominent. In the part with flat epithelial cells, the spores are arranged in rows in peristaltic nodules (figs. 3, 5) of the gut wall. In other regions of the midgut, a rather massive development in columnar cells (fig. 4) is not affected by any peristaltic muscles. In the area of gastric caeca, the microsporidia form sack-like agglomerations in individual cells (fig. 3). These individual cells are more likely part of the regenerative nodules and connective sheath of the gut. The development of the microsporidium seems to be continued in the smooth part in the connective tissue cells and on the surface of the basement membrane (figs. 3, 5). Spores were found in the strands of the circular and longitudinal midgut muscles (fig. 5). In some parts, the spread of the infection seems to be mediated by infected cells of the tracheal matrix (fig. 5). The strands of the longitudinal and circular muscular system are infected in their central part, and the microsporidian spores are arranged in long rows of vegetative stages and spores beneath the sarcolemma replacing the sarcoplasma (fig. 5). The sarcolemma does not burst, and the stages of the microsporidium do not spread from these tubular arrangements. In the Malpighian tubules (fig. 6), the infection is localized in the upper, secretory part, with stages distributed in spots in the epithelial cells, without massive local development.

The microsporidium seems to be introduced from connective sheaths of the gonads into testicles in male beetles, and there is some development in the somatic mesodermal cells covering the developing spermatocytes (fig. 7). In the ovaries, the entrance of infection is presumed to occur via connective tissue cells of the peritoneal coat. Dense groups of sporoblasts are visible in irregular spots in mature eggs (fig. 7). The development in young follicles is a diffuse dispersion.

No massive destruction could be found in any organ. The regions with gastric caeca are damaged only to a limited extent. The secretion of the glandular upper part of the Malpighian tubules is not totally suppressed. The infection appears in all organs, even in the adipose tissues, only in spots.

3.4 Morphology of Canningia tomici

We observed mainly spores and only few sporonts using the light microscope. Spores in fresh smears were oval bodies, varying in length and width. Fixed and stained spores can be divided into two groups (fig. 2). The first group, measuring 2.8 ± 0.4 by 1.4 ± 0.4 µm (range: 2 to 3.5 µm in length and 1.0 to 2.0 µm in width), was more frequent and formed approximately 80 % of measured spores (n = 536). The second group, measuring 3.8 ± 0.3 by 2.0 ± 0.2 µm (range: 3.5 to 5.0 µm in length and 2.0 to 3.0 µm in width), formed approximately 20 % of spores. Spores of both sizes are present in all infected tissues.

On Giemsa-stained smears (fig. 8), the sporoplasm formed a c-shaped structure and was positioned subapically in the anterior end of the spore. Spores were single, not enclosed in a pansporoblastic membrane. We found few vegetative stages: stages of schizogony are uninucleate, round, varying in diameter from 4 to 5 µm; sporoblasts are elongate oval uninucleate stages with a size of 3 × 5 µm.

3.5 Ultrastructure of Canningia tomici

In ultrathin sections, spores were elongate oval and slightly curbed bodies. The polar filament was fixed subapically with a distinctly arched anchoring disc (figs. 9, 10, 14). The spore wall consists of the exospore (fig. 12) (100 nm thick) which was seen as an electron-dense layer tightly adhering to the electron-lucent layer of the endospore (fig. 12). The polar filament was coiled in the posterior part in 5 to 6 or 4 to 5 turns (figs. 9, 11). It consists of 6 distinct concentric layers, with an electron-dense 3rd layer which is prominent in cross section. The polaroplast (figs. 11, 13) has two lamellar parts. The first part, composed of thin dense lamellae, is symmetrical and adheres to the anchoring disc (figs. 13, 14), forming a collar under the spore wall. The second, posterior part of the polaroplast is composed of 6 to 10 flat vesicular lamellae with an electron-dense core and electron-lucent cover layer. Due to the subapical fixation of the filament, it is asymmetric, the lateral part protruding close to the single nucleus (figs. 11, 12, 13). The posterior vacuole and the Golgi system is not prominent, but in its posterior region, spores appeared "dented" during fixation (fig. 12). The two different size classes of spores were not different in ultrastructure, except that the filament of the larger spores is coiled in 5 to 6 coils (fig. 11), whereas the smaller spores have only 4 to 5 coils. The interior of the spore is filled with ribosomes arranged between longitudinal endoplasmic lamellae (Figs. 11, 13, 14). Spores with some fine granular content are common. Their electron-dense surface layer appears foamy.

Fig. 3. Part of the midgut of *Tomicus piniperda* with circular and longitudinal muscles filled with mature spores (s) and with groups of sporoblasts (sp) in the basal membrane.

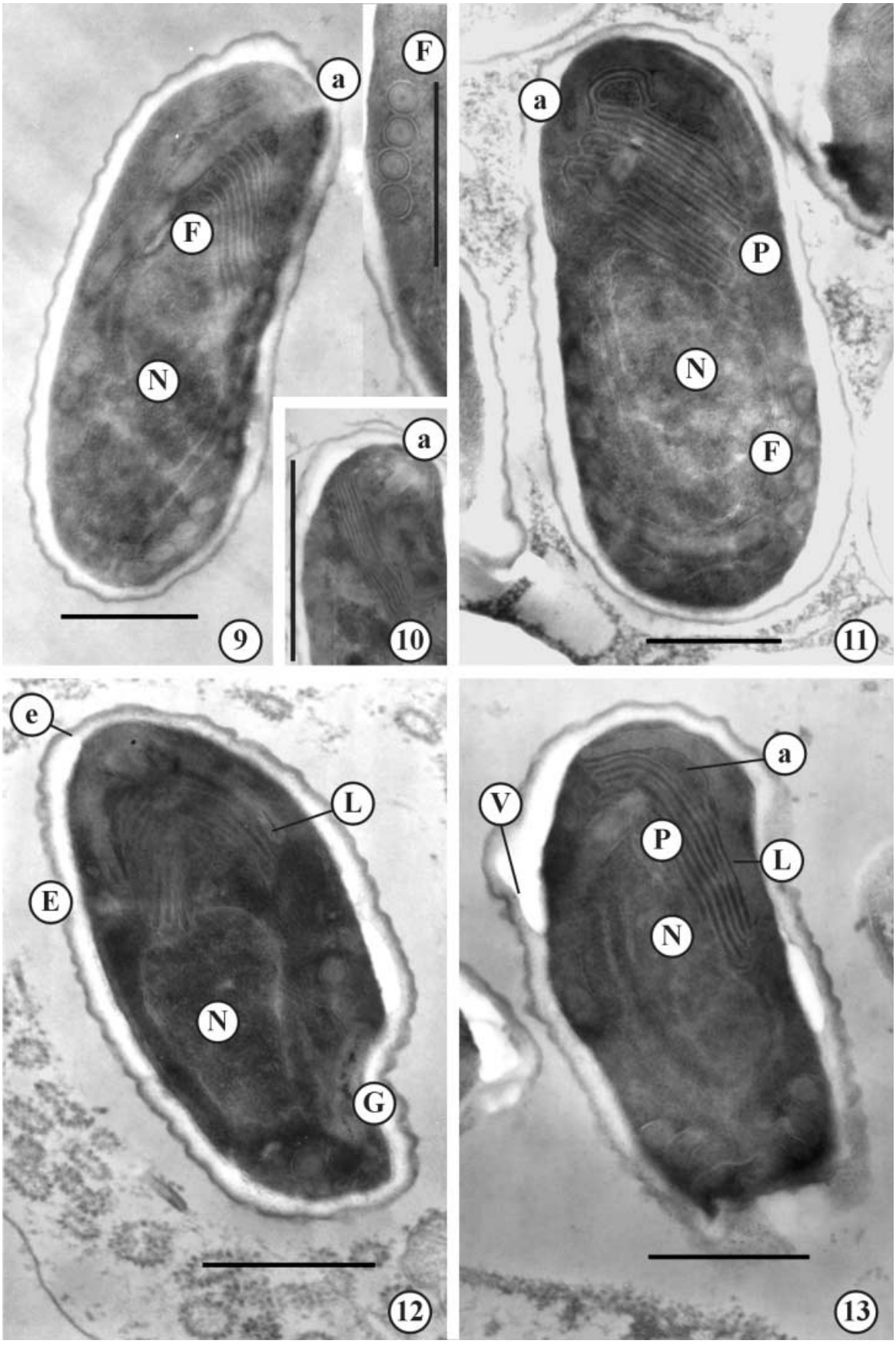
Fig. 4. Massive development (m) of the microsporidium in columnar cells of the midgut.

Fig. 5. Microsporidian infection in the basement region of the midgut with centers in the basement membrane (g) in infected basal ends of columnar cells and the labyrinth and typical infections of tracheal matrix cells (t) which are transferring the microsporidian infection in the connective tissue of the midgut. Vegetative stages (m) are present in the regenerative cells under the basement membrane and in the circular and longitudinal muscles of the midgut.

Fig. 6. Malpighian tubule, its secretory upper part with spores spread in the disorganized cells.

Fig. 7. Two eggs in the ovary (o) of *T. piniperda*. Vegetative stages of *Canningia* are visible in the dark spots (m).

Fig. 8. Massive development of the microsporidium and spores (s) in the haemolymphe and adipose tissue in Giemsa-stained smear.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4 Discussion

4.1 General

This microsporidium evidently belongs to the genus *Canningia*. The genus *Canningia* was used for a rather similar microsporidium (*C. spinidentis*) infecting the fir bark beetle, *Pityokteines spinidens* (WEISER et al., 1995). In our material, the posterior part of the polaroplast is asymmetric, due to the subapical fixation of the polar filament, and the spore is oval with equally round ends. The anchoring disc is broader than in *C. spinidentis* and arched, on its border is a short umbrella part. The structure of the spore wall is identical, without separation of the electron-dense exospore from the layer of the electron-lucent endospore. *C. spinidentis* (WEISER et al., 1995) and *U. minutum* (KNELL and ALLEN, 1978) differ from the *Canningia* in *T. piniperda* in spore size and shape. Spores of *C. spinidentis* measure 1.9–2 × 0.8–1.0 µm, those of *U. minutum* 2–2.5 × 0.8–1.0 µm compared to 2.8 × 1.4 µm and 3.8 × 2.0 µm for the mirosporidium in *T. piniperda*. The distribution in the host tissues is very similar.

These uninucleate microsporidia are probably a different species, as a consequence of permanent separation of different bark beetle species, monophagous on different tree species. *U. minutum* infects the southern pine beetle, *Dendroctonus frontalis*, which infests different types of pine in parts of the USA. *U. minutum* was also recorded from Central America. ATKINSON and WILKINSON (1979) documented high infection rates of 79 % with *U. minutum* in Florida, whereas populations from Georgia had only an infection rate of 2 %. Other samplings showed a variable distribution of the infection rate from 2.3 to 70.0 %. *U. minutum* and an undescribed microsporidium were isolated from southern pine beetles collected in Mississippi and Alabama by SIKOROWSKI et al. (1979).

4.2 Two spore sizes

Several species of *Nosema* and *Vairimorpha* have been reported to develop two morphologically and functionally distinct spore types. For example IWANO and ISHIHARA (1991) described "few-" and "many-coiled" spores in *Nosema furnacalis*, SAGERS et al. (1996) denominated their dimorphic spores in *N. furnacalis* and *Nosema pyrausta* "early" and "late" spores and SOLTER and MADDOX (1998) proposed the name in *Vairimorpha* spp. "primary" and "environmental" spores. It is believed that one spore type is involved in infecting the insect *per os* (many coiled-, late-, environmental spores) with the other spore type infecting tissues progressively within the host (few coiled-, early-, primary spores). The two spore sizes found in *T. piniperda* are not only morphologically distinct, but may also represent two functionally different spore types of this microsporidium. A similar observation of different spore sizes was also described for *Unikaryon montanum* (WEISER et al., 1998) and *Unikaryon polygraphi* (WEISER et al., 2002).

In our Giemsa-stained smears, empty spores or destroyed spores with a crumby structure were common. These might be empty spore sheets or maybe primary spores which did not survive the procedure of fixation and staining as a consequence of low resilience.

4.3 Vertical transmission

A reliable diagnosis of an infection in larvae or in callow adults requires highly infected tissues; this situation is only available when the infection takes place in the first days after hatching out of the egg. However, larval feeding takes place under sterile conditions in the phloem, and young larvae do not contact their mother galleries. Therefore, we expected vertical transmission from the parental to the filial generation. We found most of the developed eggs were infected with this microsporidium in centers of developing spores in the chorion and in the yolk, thus vertical transmission is transovarial. The follicular cells, nurse cells and oocytes were less infected. In the studied cases, there were no spores in the calyx, oviducts and accessory glands. The microsporidian infection of the gonads as shown in fig. 11 may cause a decrease in nutrient supply, but it is evident that the infection does not affect their function and most probably does not kill the embryo. Infected eggs are expected to allow embryonic development until egg hatch of larvae, possibly with some increased mortality. Larval development and pupation of infected hosts seems to be normal, and we suspect that the filial beetles shown in fig. 1 were vertically infected, deriving from one female (parental) beetle. High infection rates, up to 12 % in Europe and up to 33.9 % in USA, in this monogamous beetle species favors the pathway of vertical transmission as the most important. Assuming that all infected filial beetles in our study belong to the offspring from one infected female beetle, leads to the calculation that the proportion of infected adult female

Fig. 9. Longitudinal section of a spore of *Canningia tomici*. The anchoring disc (a) of the polar filament is fixed subapically, the manubrial part of the filament (F) crosses the broad lamellar part of the polaroplast and the filament is coiled in 3–4 turns in the posterior part. The single elongate nucleus (N) is in the central region. Bar = 500 nm.

Fig. 10. The fixation of the polar filament in the anchoring disc (a) is with a thickened electron- lucent terminal layer; the electron-dense layer is extended and covers the thickening. Cross sections of the filament (F) show a second electron-lucent layer enclosing the electron-dense layer and the interior structures of the filament. Bar = 500 nm.

Fig. 11. Spore with well differentiated layers of the polaroplast (P) asymmetric to the crossing polar filament. The anchoring disc (a) is cut tangentially and forms a loop. On the side of the nucleus (N) is the polar filament, coiled in five turns (F). Bar = 500 nm.

Fig. 12. Mature spore of *Canningia* with anchoring point turned to the observer, polaroplast with the series of broad lamellae close to the nucleus (N) and another series of fine lamellae (L) connected with the anchoring system. The Golgi system (G) is compressed in the area of the posterior vacuole. The electron-dense layer of the exospore (e) is adhering to the endospore (E) without visible partition. Bar = 500 nm.

Fig. 13. Two types of lamellae, the broad lamellae (P_2) and dense lamellae (P_1) in the polaroplast of the spore. A mass connected with the anchoring disc between both systems (a). The plasticity of the spore wall is evident in vacuolated parts (V). Bar = 500 nm.

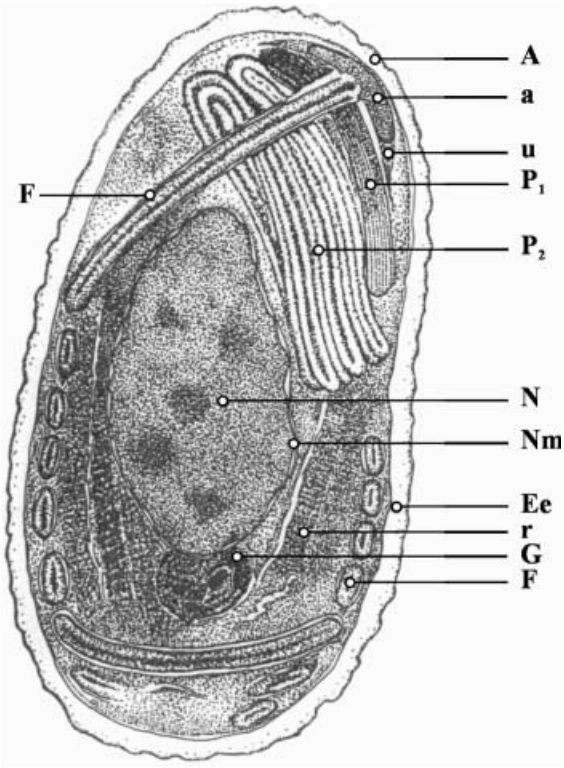

Fig. 14. Canningia tomici, ultrastructure of the spore typical for the family Unikaryonidae and for the genus *Canningia*. A – Anterior end with subapical fixation of the polar filament and laterally attenuated spore wall; the presented spore is an immature stage, with long immature filament; a – anchoring disc with reduced umbrella border (u); P_1 – anterior dense lamellar part of the polaroplast; P_2 – posterior broad lamellar part of the polaroplast, asymmetric formation; N – single nucleus, oval, in central part; Nm – nuclear membrane; Ee – spore wall without distinct delimitation of the exospore; r – columns of ribosomes with long endoplasmic lamellae; G – Golgi system of membranes and tubules; F – polar filament in cross and longitudinal section, isofilar, short, in 5 to 6 coils.

beetles to infected filial beetles is an increase from 1 single infected female parental beetle to 15–20 infected offspring beetles. Furthermore MADDOX et al. (1998) assumed that infected larvae may develop more slowly than healthy ones. This thesis might also fit in our case where the number of infected beetles increased in the last weeks of developing time, although the pine logs were infested in the same week. But it is also possible, that propagation of infection starts in the midgut epithelium and transfer to other tissues (e.g. gonads) and formation of spores needs two to three weeks.

4.4 Horizontal transmission

In contrast to the development of other bark beetle species, especially polygamous ones on pine, spruce or fir, *Tomicus* spp. do not remain in their galleries for a long period. Therefore, there are no or very limited opportunities to contact other bark beetles or their faeces, with exception of the breeding partners in late winter and early spring, thus allowing an additional infection by horizontal transmission of pathogens. Spores might be released within the galleries together with faeces of infected beetles. Infection of the midgut epithelium and of the Malpighian tubules will result in spore dissemination probably via faeces excreted in the gallery; other bark beetle species are suspected of feeding on spore contaminated faeces (WEISER et al., 1995; WEGENSTEINER and WEISER, 1996). Both parental beetles (females and males) become infected and may distribute the microsporidiosis by sister breeding or by initiating a second brood after a second hibernation.

4.5 Course of disease

The microsporidiosis is distributed especially in the midgut epithelium of the central part whereas regions with gastric caeca are infected only during a later phase of the infection. The infection in the midgut may be caused by direct activity of the primary invasion of the microsporidia into the columnar cells. After entry from the midgut lumen, meronts penetrate the midgut cells through to the basement membrane, spores develop in the basal membrane of the midgut epithelium, then infected adjacent tissues such as the muscle cells of the circular and longitudinal muscles of the midgut; early spores or sporoplasms may also enter blood cells adhering to the surface of the midgut in the body cavity. The microsporidia fill the muscle fibrils, destroying the sarcoplasm but keeping the sarcolemma intact (fig. 5). In other microsporidian infections (*Nosema stegomyiae:* FOX and WEISER, 1958; *Vavraia culicis:* WEISER and COLUZZI 1964), the sarcolemma is broken and the muscle becomes a mass of spores. In our case, the infection of organs does not cause massive tissue destruction, as evident from the presented figures, although there may be a decrease of peristaltic movement due to deactivation of the circular and longitudinal muscular sheath of the central part of the gut.

4.6 Prevalence of C. tomici

The two ways of transmission (vertical and horizontal) may cause a rather high prevalence of *C. tomici* in *T. piniperda* populations in European and also in North American locations, compared to microsporidian infections in other bark beetle species. The origin of the beetles introduced into North America as well as the mode of their introduction (single or multiple) is not clear at the moment, but apparently originates from separate introductions (HAACK, 1997). Therefore, it is not possible to make an assumption on the originality of microsporidian spores at the moment. The use of molecular techniques seems to be the only possibility to find an answer to this question.

Furthermore, the occurrence of microsporidia in a population observed over several years might reflect the epidemic status of disease in beetle populations, which may affect the population dynamic of bark beetles, especially during the innocuous phase, but with long-term regulation capacity.

The newly found spores in *T. piniperda* are evidently a new microsporidian species. We propose for it the name *Canningia tomici* sp. n.

Notes on the new species: *Canningia tomici* sp. n.

Host and infected tissue: *Tomicus piniperda* (L.) (Coleoptera, Scolytidae), midgut columnar cells and circular and longitudinal muscles, matrix of tracheal end cells, Malpighian tubules, connective tissue, adipose tissues and gonads.

Locality: Colonies of the host in Scots pine, *Pinus sylvestris* in forests in Europe (Hyytiälä, Joensuu – Finland; Sekocin – Poland; Stara Boleslav, Otradovice – Czech Republic; Altenburg, Mattersburg - Austria) and Northeastern USA (Snowcap and Fenner Arboretum, Lansing, Michigan).

Transmission: transovariole transmission; release in faeces and contamination of food in galleries.

Development: all developmental stages uninucleate, sporoblasts and spores single, in contact with cytoplasm of host cells. Spores oval, in two size classes: $2.8 \pm 0.4 \times 1.4 \pm 0.4$ µm and $3.8 \pm 0.3 \times 2.0 \pm 0.2$ µm. Subapically fixed anchoring disc of the polar filament. Filament coiled in 4 to 5 and 5 to 6 coils, respectively. Binary polaroplast with anterior dense thin lamellae and posterior rather thick asymmetric lamellae. Single nucleus, ribosomes fill the central part and are distributed between two longitudinal endoplasmatic lamellae. Empty spores common.

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Correspondence: Prof. Dr. RUDOLF WEGENSTEINER, Inst. of Forest Entomology, Forest pathology and Forest Protection, BOKU-University of Natural Resources and Applied Life Sciences, Vienna, Hasenauer Str 38, 1190 Vienna, Austria. Fax: +43 1 368 63 52 97, E-mail: rudolf.wegensteiner@boku.ac.at