DOI: 10.1515/ap-2016-0083

Acta Parasitologica, 2016, 61(3), 621–628; ISSN 1230-2821

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# Occurrence of *Microsporidium* sp. and other pathogens in *Ips amitinus* (Coleoptera: Curculionidae)

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## Abstract

A new microsporidium is reported from the small spruce bark beetle, *Ips amitinus*: *Microsporidium* sp. with uninucleate oval spores measuring 3.5 × 2.5 µm; infecting cells of the midgut epithelium, midgut muscles, the fat body, the Malpighian tubules, and the gonads of adult beetles collected in Austria. Seven other pathogens were found in beetles collected from Austria, the Czech Republic, and Finland. Six of them were already known from *I. amitinus*. *Nosema* cf. *typographi* is recorded for the first time in the overwintering generation of *I. amitinus* from the Czech Republic.

## Keywords

Small spruce bark beetle, Scolytinae, microsporidia, protozoa, fungi, virus

## Introduction

The small spruce bark beetle, *Ips amitinus* Eichh., develop on *Picea abies* (L. (Karst) and *Pinus cembra* (L.). This species attacks stressed trees and produces up to two generations per year. Its primary gallery system consists of three to five maternal galleries, which are inhabited by individual female beetles. *I. amitinus* is more abundant than *Ips typographu*s (L.) on Norway spruce at altitudes above 1000 m, but the two species can occur sympatrically at high and low altitudes (Holuša *et al.* 2012). Because these species may often build their galleries close together, and it seems possible that specimens of each species may cross galleries during maturation feeding of callow adults or after hibernation.

To date, only seven pathogens have been detected in *I. amitinus*: an entomopoxvirus (probably the same virus that is found in *I. typographus*); the protozoan *Gregarina typographi* (Fuchs, 1915) (Sporozoa, Gregarinidae); the microsporidian *Chytridiopsis typographi* (Weiser, 1954,1970) (Microspora, Chytridiopsidae); an unidentified microsporidian that was only vaguely described and that might be a species of *Unikaryon* (Microspora, Unikaryonidae); the ascomycete fungus *Metschnikowia typographi* Weiser, Wegensteiner, Handel, Žižka 2003 (Ascomycota, Saccharomycotina); the amoeba *Malamoeba scolyti* (Purrini, 1980) (Rhizopoda, Amoebidae) (Händel and Wegensteiner 2005); and the neogregarine *Mattesia schwenkei* (Purrini, 1970) (Sporozoa, Lipotrophidae) (Haidler 1998; Händel 2001; Haidler *et al.* 2003; Weiser *et al.* 2003; Lukášová *et al.* 2013). The pathogens of *Ips* spp. were discussed in a recent review (Lukášová and Holuša 2012).

In evaluating the pathogen complex of *I. amitinus*, it is important to consider that this species may have contact with the frequently occurring bark beetles *Ips typographus* (L.) and *Pityogenes chalcographus* (L.), or with other less common bark beetles that breed in the fresh phloem of spruce trees. The pathogens that have been reported from *I. typographus* include the entomopoxvirus ItEPV, the amoeba *M. scolyti*, the eugregarine *G. typographi*, the neogregarines *M. schwenkei* and *Menzbieria chalcographi* (Weiser, 1955) (Sporozoa, Ophryocystidae), and the microsporidians *Nosema typographi* (Weiser, 1955) (Microspora, Nosematidae), *U. montanum*, and *C. typographi*. The following insect pathogenic fungi also attack *I. typographus*: *Beauveria bassiana* (Bals.), *Isaria farinosa* (Zimm), *Isaria fumosorosea* Wize (Ifr), and *Metschnikowia typographi* (Lukášová and Holuša 2012; We-

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gensteiner *et al.* 2015). We suspect that these pathogens of *I. typographus* can also infect *I. amitinus.*

In this paper, we report the results of an investigation of pathogens of *I. amitinus* collected from Austria, the Czech Republic, and Finland. A new microsporidian species infecting *I. amitinus* in Austria is described.

## Materials and Methods

Details on the collection of adults of *I. amitinus* are provided in Table 1. Most of the collection locations were forests dominated by *P. abies*, except the site in Finland was dominated by *Pinus sylvestris* L. (Table I).

For Austrian and Finnish locations, log sections of infested spruce trap trees were removed from the forest and incubated in the insectary at the Institute of Forest Entomology, Vienna, at 23°C (± 2.5°C) under long-day conditions (L:D = 16:8). Emerging beetles were collected daily and stored in an incubator at 15°C (± 1°C) without light until they were inspected (within 1 week of emergence).

In locations in the Czech Republic, maternal beetles were collected in the field from the individual galleries in trap trees. The individual beetles were placed in 2-ml Eppendorf tubes and stored at –4°C.

Each beetle was then dissected by removing the whole gut together with parts of the muscles, the fat body, Malpighian tubules, and ovaries or testes (Wegensteiner *et al*. 1996). After the dissected tissue was inspected with a light microscope (at 40x to 400x), infected organs were transferred to 2% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4) and stored at 4°C for 4 h before they were washed in buffer and postfixed in 1% osmic acid (1 h at 4°C). After dehydration in an alcohol and acetone series, the guts were embedded in Vestopal W. Ultra-thin sections were examined by transmission electron microscopy (TEM) (Philips EM 300). Remains of dissected material on slides were used for preparation of dry smears, which were fixed with methanol and stained with Giemsa dye. Spores were measured at 1000x with a light microscope equipped with an ocular micrometer and were also measured on electron micrographs.

## Results

### Occurrence of pathogens in *I. amitinus*

The detection of pathogens in *I. amitinus* is summarized in Table 2. *It*EPV, *M. scolyti*, *Mattesia* sp., and *N.* cf. *typographi* were found in only a small number of *I. amitinus* and at a few locations. *G. typographi* was found in 4 of the 6 years at the Tamsweg location, from all locations in Oberleibnig, and from one location in the Czech Republic (collected in spring). *C. typographi* was found at most locations and in most years. The reported microsporidium, *Microsporidium* sp., was found only at the Tamsweg location and in 4 of the 6 years. *M. typographi* was found at two locations. The entomopoxvirus was found in the cells of the midgut epithelium, *M. scolyti* in the cells of the midgut epithelium and in the Malpighian tubes, *Mattesia* sp. in the adipose tissue, *G. typographi* in the midgut lumen (some trophozoits were attached to midgut cells), *C. typographi* in the cells of the midgut epithelium, and *M. typographi* in the cells of the anterior part of the midgut epithelium. *Nosema* cf. *typographi* and the new microsporidium were found in different organs of *I. amitinus*.

**Table I**. Locations in Austria, Finland, and Czech Republic where *Ips amitinus* was collected

| Location | Nation | Latitude | Longitude | Elevation (m) | Year of collection |
|---|---|---|---|---|---|
| Tamsweg | Austria | 47.11551°N | 13.84662°E | 1600 | 1994-1999 |
| Kobernaußer Wald 1 | | 48.08782°N | 13.31436°E | 660 | 1998-1999 |
| Kobernaußer Wald 2 | | 48.06274°N | 13.29686°E | 600 | 1998 |
| Kobernaußer Wald 3 | | 48.07024°N | 13.24943°E | 585 | 1999 |
| Kobernaußer Wald 4 | | 48.05825°N | 13.23522°E | 630 | 1998-1999 |
| Oberleibnig 1 | | 46.93084°N | 12.63569°E | 1775 | 2001 |
| Oberleibnig 2 | | 46.92935°N | 12.64193°E | 1875 | 2001 |
| Oberleibnig 3 | | 46.93098°N | 12.64152°E | 1925 | 2001 |
| Město Albrechtice | Czech Republic | 50.17435°N | 17.56245°E | 450 | 2008 |
| Šenov | | 49.79938°N | 18.36996°E | 270 | 2010 |
| Podvihov | | 49.86061°N | 17.98041°E | 450 | 2010 |
| Kozlov | | 49.60319°N | 17.52324°E | 625 | 2011 |
| Hlubočec (spring)* | | 49.84681°N | 17.95076°E | 475 | 2012 |
| Hlubočec(summer)** | | 49.84681°N | 17.95076°E | 475 | 2012 |
| Hyytiälä | Finland | 61.84673°N | 24.29828°E | 170 | 1997 |

*21May2012; **24July2012

**Table II.** Prevalence of pathogen species detected in *I. amitinus* collected from locations in Austria (A), Finland (SF), and the Czech Republic (CZ). Prevalence was calculated as the percentage of positive specimens relative to the total number of specimens examined from a specific location in a specific year. N = number of examined beetles; *It*EPV = *Ips typographus*-Entomopoxvirus; M.s. = *Malamoeba scolyti*; Mat. = *Mattesia* sp.; G.t. = *Gregarina* cf. *typographi*; C.t. = *Chytridiopsis* cf. *typographi*; N.t. = *Nosema* cf. *typographi*; M.n. = *Microsporidium* n.sp.; Met. = *Metschnikowia typographi*

| Location | Year | Nation | N | *It*EPV | M.s. | Mat. | G.t. | C.t. | N.t. | M.n. | Met. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tamsweg | 1994 | A | 30 | – | – | – | – | 3.3 | – | – | – |
| | 1995 | | 276 | – | – | – | 6.2 | 8.0 | – | 2.5 | – |
| | 1996* | | 448 | – | – | – | 0.9 | 15.8 | – | 7.8 | – |
| | 1997 | | 4 | – | – | – | – | – | – | 50.0 | – |
| | 1998 | | 133 | – | – | – | 0.8 | 8.3 | – | – | – |
| | 1999 | | 307 | 0.3 | – | – | 10.7 | 15.6 | – | 0.7 | – |
| Kobernaußer Wald 1 | 1998 | | 44 | – | – | – | – | 15.9 | – | – | – |
| | 1999 | | 8 | – | – | – | – | – | – | – | – |
| Kobernaußer Wald 2 | 1998 | | 91 | – | – | – | – | 5.5 | – | – | – |
| Kobernaußer Wald 3 | 1999 | | 292 | – | – | – | – | 4.5 | – | – | 5.1 |
| Kobernaußer Wald 4 | 1998 | | 14 | – | – | – | – | – | – | – | – |
| | 1999 | | 1 | – | – | – | – | – | – | – | – |
| Oberleibnig 1 | 2001** | | 188 | – | 0.5 | – | 18.6 | 11.2 | – | – | – |
| Oberleibnig 2 | 2001** | | 231 | – | – | 0.9 | 26.0 | 13.9 | – | – | – |
| Oberleibnig 3 | 2001** | | 129 | – | – | – | 3.1 | – | – | – | – |
| Město Albrechtice | 2008 | CZ | 50 | – | – | – | – | 10.0 | – | – | – |
| Šenov | 2010 | | 7 | – | – | – | – | – | – | – | – |
| Podvihov | 2010 | | 23 | – | – | – | – | 4.4 | – | – | – |
| Kozlov | 2011 | | 20 | – | – | – | – | – | – | – | – |
| Hlubočec (spring) | 2012 | | 196 | – | – | – | 0.5 | 0.5 | 0.5 | – | – |
| Hlubočec (summer) | 2012 | | 284 | – | – | – | – | 1.4 | – | – | – |
| Hyytiälä | 1997 | SF | 30 | 3.3 | – | – | – | 6.7 | – | – | 13.3 |

*Data were mentioned by Haidler *et al.* (2003). **Data were mentioned by Händel and Wegensteiner (2005)

The number of inspected beetles was extremely low, and so only a small number of beetles could be dissected at the following locations in the following years: Tamsweg in 1997, Kobernaußer Wald 1 in 1999, Kobernaußer Wald 4 in 1998 and 1999, and Šenov in 2010. *G. typographi* was the most prevalent species in the locations where it occurred (its prevalence at these locations ranged from 0.5 to 26.0%) but it was not detected in the Kobernaußer Wald and Finland locations and was found in only one location in the Czech Republic. *C. typographi* was the most frequently detected species (it was found in 11 of the 22 locations), and it was sometimes prevalent, with prevalence ranging from 0.5 – 15.9%. *M. typographi* was found at only two locations (Kobernaußer Wald 3 and Hyytiälä) but with relatively high prevalence (5.1 and 13.3%). All of the other pathogens occurred at only a few locations and usually with low prevalance. The prevalance of the new microsporidian species *Microsporidium* sp. ranged from 0.7 to 7.8% when

**Table III.** Infections by *Microsporidium* n. sp. in males and females of *I. amitinus* collected from Tamsweg in different years. N ♂♂ and N ♀♀ refer to the number of inspected males and females, respectively. % inf. ♂♂ and % inf. ♀♀ refer to the percentage of males and females infected by the pathogen. S = total number of inspected and infected males and females and the mean infection rate (Ø%)

| Year | N ♂♂ | N inf. ♂♂ | % inf. ♂♂ | N ♀♀ | N inf. ♀♀ | % inf. ♀♀ |
|---|---|---|---|---|---|---|
| 1994 | 9 | – | – | 21 | – | – |
| 1995 | 119 | 3 | 2.5 | 157 | 4 | 2.6 |
| 1996 | 207 | 25 | 12.1 | 231 | 11 | 4.8 |
| 1997 | 2 | 1 | 50.0 | 2 | 1 | 50.0 |
| 1998 | 62 | – | – | 71 | – | – |
| 1999 | 110 | – | – | 197 | 2 | 1.0 |
| ∑/Ø% | 509 | 29 | 5.7 | 679 | 18 | 2.7 |

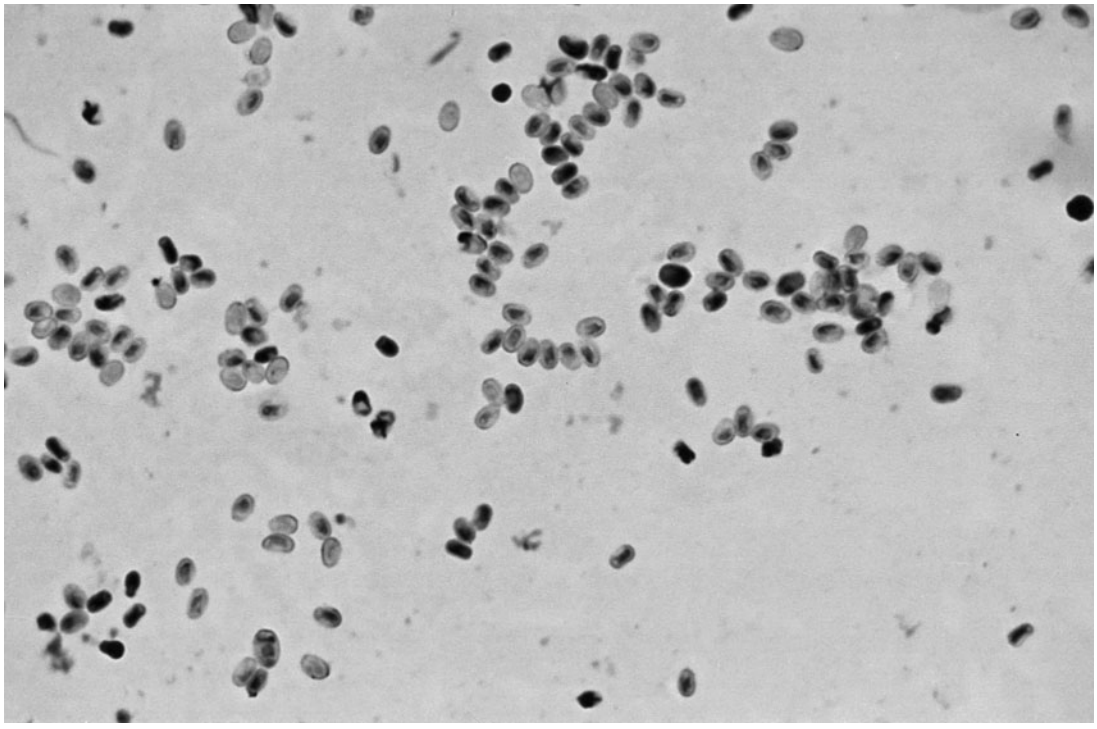

**Fig. 1.** Giemsa-stained spores of *Microsporidium* sp. in the haemolymph of *I. amitinus*

a substanial number of beetles were dissected but had a prevalance of 50.0% in 1997 when only four beetles were collected and dissected at Tamsweg.

Prevalence differed among years, but there was no clear pattern of increase or decrease from one year to the next. *G. typographi* prevalence decreased significantly from 1995 to 1996 ($p<0.001$) and increased significantly from 1998 to 1999 ($p<0.001$). *C. typographi* prevalence tended to increase from 1994 to 1995 ($p>0.05$) and increased significantly from 1995 to 1996 ($p<0.01$) and from 1998 to 1999 ($p<0.05$). *Microsporidium* sp. prevalence increased significantly from 1995 to 1996 ($p<0.01$).

### *Microsporidium* sp. in *Ips amitinus*

The new microsporidian was detected only in black-brown *I. amitinus* adults collected at the Tamsweg locations in 1995, 1996, 1997, and 1999. *Microsporidium*-infected beetles were found through the entire period of beetle emergence; the new microsporidian was found in parental beetles and also in mature offspring beetles. As noted earlier, the prevalence of *Microsporidium* was relatively low except in one case when only four beetles were dissected (Table II). Infection rates were variable in male and female beetles among the collection years (Table III). About the same percentage of male and female beetles were infected in 1995 ($p>0.05$), but significantly more males than females were infected in 1996 ($p<0.01$). The same numbers of infected males and females were found in 1997 ($p> 0.05$), but infection was detected in only two female bee-

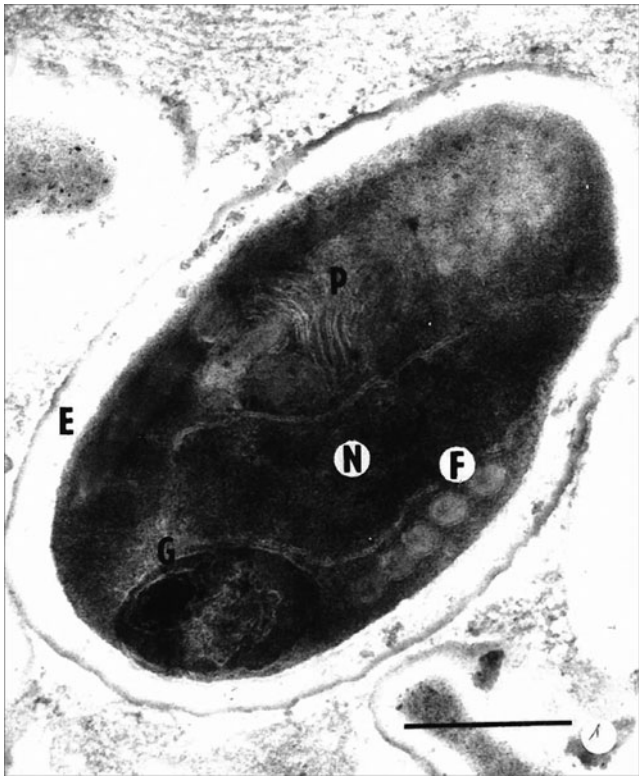


**Fig. 2.** Mature environmental spore of *Microsporidium* sp. with a single elongate nucleus (N), a polar filament (F) coiled in four to five turns, and a Golgi system (G) in the posterior part. The polaroplast (P) is binary, and the spore wall (E) is composed of electron-lucent material with a thin electron-dense surface layer. Bar: 1 μm

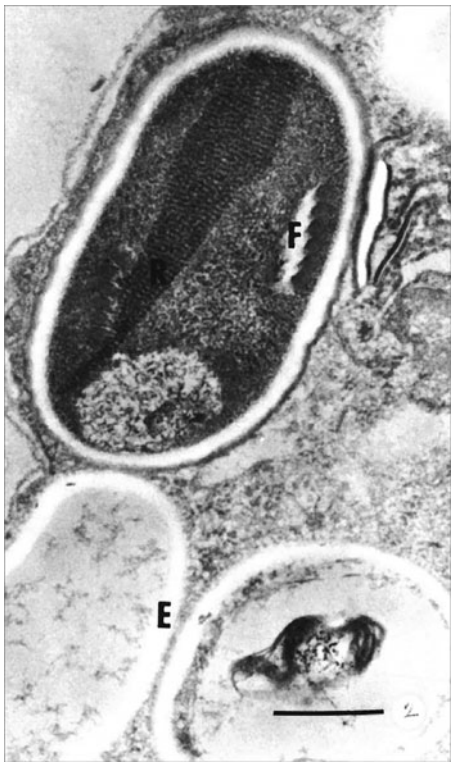


**Fig. 3.** A primary spore of *Microsporidium* sp. with large masses of ribosomes (R) and a polar filament (F) with six turns. The micrograph also shows two empty spores (E) with internal granulated remains and with smooth walls lacking an outer electron-dense layer. Bar: 1 μm


**Fig. 4**. Three primary spores of *Microsporidium* sp. with different configurations and different numbers of turns of the polar filament, and transverse and longitudinal sections of polar filament coils (F). (E – spore wall) Bar: 1 μm

tles in 1999. Overall, significantly more male beetles were infected than female beetles ($p < 0.01$) (Table III).

Spores of *Microsporidium* were found in the cells of the midgut epithelium, in the longitudinal and circular strands of the midgut muscularis, in the cells of the Malpighian tubules (especially in the secretory part), and in those parts of the fat body adhering to the infected midgut. *Microsporidium* development was also evident in the gonads and in oenocytes adhering to the internal surface of the midgut-basement membrane.

Smears contained very few schizogonial stages of *Microsporidium*, and these were usually broad, oval, uninucleate meronts (3 × 4 μm); only a few of the elongated binucleate stages that occur after nuclear division and subsequent separation were observed. Spores (4 × 2–3 μm) were abundant in smears (Fig. 1). In TEM micrographs, the rare meronts appeared as uninucleate oval bodies with a thin plasmalemma and only a few membranes of the endoplasmic reticulum. Stages with more than two round separate nuclei were not evident. Sporogony was represented by single, elongated uninucleate sporonts, usually 5 × 2.5 μm. Initially, the wall of each sporont was only partially thickened; later, all of the wall was thickened and had an electron-dense deposit on its surface. Mature spores were broad, oval, and 3.5 ± 0.5 × 2.5 ± 0.5 μm as measured with an ocular micrometre and a light microscope; they occurred singly or in irregular groups in host cells (Fig. 2). In TEM preparations, uninucleate spores (Fig. 2) measured 2.8–3.2 × 1.3–2 μm after fixation. The wall is formed by a thick electron-lucent endospore and a thin electron-dense exospore visible as a surface impregnation without a distinct separation from the endospore (Figs 2–5). In some smaller oval spores, the polar filament in the posterior part is coiled in five to six turns (Fig. 4); the filament has seven to eight turns in other spores (Fig. 4 and 5). The anterior end of the filament has fixed to a flat anchoring disc that adheres to the centre of the apical end of the spore. A thin umbrella-like border with a lamellar anterior part and a more tubular posterior part covers the dual polaroplast (Fig. 5). Masses of ribosomes are formed in longitudinal polysomal columns around the nucleus (Figures 3 and 5). An oval and distinct Golgi system is evident in the posterior part of the spore (Fig. 2) and contains tubules filled with electron-dense material.

The oval, persistent spores occur along with many shorter and broader (Fig. 4), oval spores that contain lysed debris. The wall of these latter spores is smooth and stretched (Fig. 3); some of these walls have membranous remains in the central part and dissolved exosporal deposits on the surface. They evidently are less resistant to the fixation process and may represent spores that have already extruded their polar filament within the host tissues.

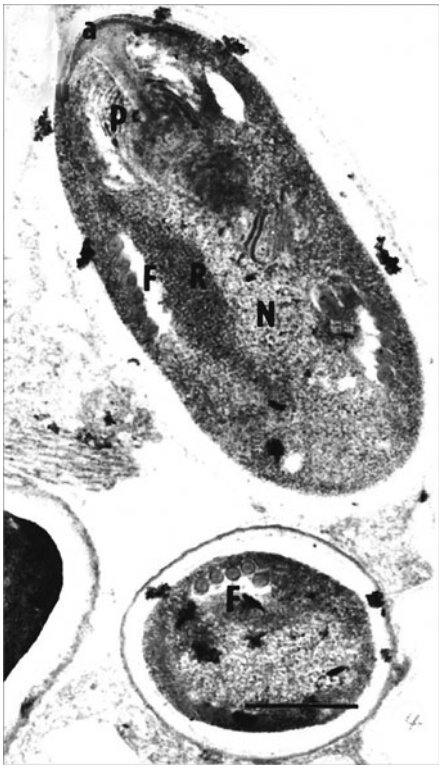


**Fig. 5.** Longitudinal section of a mature environmental *Microsporidium* sp. spore (the top spore) revealing the structure of the apical end. The flat anchoring disc (a) has expanded into an umbrella-like (u), and the hinge area is not differentiated. The basal part of the polar filament is enclosed in a binary polaroplast (P). The polar filament (F) is coiled laterally in six to seven turns, and the ribosome basket (R) and an indistinct nucleus (N) are evident in the central part of the spore. Another mature environmental spore is located at the bottom of figure and is seen in cross section; this spore, has a polar filament with only five turns. Bar: 1 μm

## Discussion

The newly reported microsporidian pathogen, *Microsporidium* sp., was found during dissections of *I. amitinus* beetles and occurred in relatively few individuals. Infective spores are probably released with faeces in a form of horizontal transmission. Transovarial transmission also seems possible based the observation of minute centres of microsporidian development in oviposited eggs. Transovarial transmission is typical for other microsporidians; for these pathogens, full development does not become evident until the host matures to the adult stage (ref. in Wegensteiner 2004). Because *Microsporidium* dose not generate massive centres of infection in host insects, infection by these pathogens does not cause a reduction in host functioning and or an increase in host mortality. *Microsporidium* probably does not cause serious harm to *I. amitinus*. In this way, it is probably similar to protozoan pathogens and especially microsporidian pathogens of insects (Tanada and Kaya 1993, Vega and Kaya 2012) (Becnel and Andreadis 1999, Solter *et al*. 2012).

In most cases, prevalence was lower for *Microsporidium* than for *C. typographi*. The higher infection rates of male than female beetles by *Microsporidium* might be important with regard to gender ratio and breeding, because one male beetle mates with three to seven female beetles (Postner 1974); this would enhance the horizontal transfer of the pathogen from male beetles to female beetles. Infections in parental beetles as well as in offspring beetles and the occurrence of spores in the gut (and presumably in the faeces) and in the gonads suggest that *Microsporidium* may be transmitted both horizontally and vertically.

Among the pathogens of bark beetles, some appear to be species-specific in their hosts, and these would include the microsporidians *N. typographi* and *U. montanum*, which develop in the gonads, adipose tissue, and in the midgut epithelium of *I. typographus*. Others appear to be less specific but are restricted to a few species; these would include the *I. typographus*-Entomopoxvirus and the protozoan *M. chalcographi*. In contrast, *M. scolyti*, *G. typographi*, and *C. typographi*, are presumed to be rather nonspecific pathogens that infect several bark beetle species (Wegensteiner 2004; Lukášová and Holuša 2012; Lukášová *et al.* 2013).

The ratio in prevalence of the different pathogen species in *I. amitinus* was similar to that in *I. typographus* in that prevalence was higher for *C. typographi* and *G. typographi* than for other pathogens in most years (Wegensteiner *et al.* 1996).

The morphology and ultrastructure of the uninucleate spores of new species *Microsporidium* indicate that this microsporidium could belong to the family Unikaryonidae and to the genus *Unikaryon* (Table IV), as described by Weiser *et al.* (1995). The development of *Microsporidium* sp. is haplokaryotic as defined by Sprague and Becnel (1992), with uninucleate stages in schizogony and sporogony and without the formation of a sporophorous vesicle. *Microsporidium* sp. develops two types of spores whose morphologies appear to be very similar when fresh material is examined but different when material is fixed and examined with TEM. These spores are probably the two types (primary and environmental spores) mentioned in Weiser *et al.* (1998); primary spores (Figures 3–5), which further the colonization of infected organs and also infect other tissues in the host, and environmental spores (Figures 2, 5), which infect other hosts after spores are released.

The pathology and ultrastructure of *Microsporidium* sp. in *I. amitinus* are somewhat similar to the pathology and ultrastructure *U. montanum* in *I. typographus*, and these microsporidian species differ mainly in the size of spores (Table IV) (Weiser *et al.* 1998). In comparison with species of the genera *Canningia*, *Larssoniella* and *Orthosomella* there are differences in spore shape and size (Table IV). Although both species of bark beetles are often in close contact, there is no evidence that *Microsporidium* sp. infects *I. typographus* or that *U. montanum* infects *I. amitinus*, i.e., spores that measure 3.5 × 2.5 μm have not been reported from *I. typographus*, and spores that measure 2 × 1 μm have not been reported from

**Table IV**. Characteristics of the selected uninucleate microsporidian species from beetles

| Species | Spore shape and size (µm) | Infected organ | Polar filament | Host | References |
|---|---|---|---|---|---|
| *Canningia minutum* (Knell et Allen, 1978) | Tubular 2.2 × 0.9 µm | muscle tissue, Malphighian tubules, fat body | 6–7 coils | *Dendroctonus frontalis* Zimmermann, 1868 | Knell et Allen, 1978 |
| *Canningia spinidentis* Weiser, Wegensteiner et Žižka, 1995 | Long oval to tubular 1.9 × 1.0 µm | fat body, muscle tissue, Malpighian tubules, gonads | 5–6 coils | *Pityokteines spinidens* (Reitter, 1894) | Weiser *et al* 1995 |
| *Canningia tomici* Kohlmayr, Weiser, Wegensteiner, Händel et Žižka, 2003 | Oval, two sizes: 2.8 × 1.4 µm and 3.8 × 2.0 µm | midgut epithelium, gut muscules, Malpighian tubules, adipose tissues, gonads | 4–6 coils | *Tomicus piniperda (*Linnaeus, 1758) | Kohlmayr *et al*. 2003 |
| *Larssoniella duplicati* Weiser Holuša et Žižka, 2006 | Oval, two sizes: 3.3 × 1.8 and 2.3 × 1.5 µm | midgut muscularis, Malpighian tubules, ovaries | 6–7 coils | *Ips duplicatus* (C. R. Sahlberg, 1836) | Weiser *et al*.2006 |
| *Orthosomella lipae* Ovcharenko, Świątek, Ironside et Skalski, 2013 | Rod-shaped with equally rounded ends 4.2 × 1.6 µm | outer ovariole sheath, trophic chambers, oocytes, somatic tissues, eggs | 12–14 coils | *Liophloeus lentus* Germar, 1824 | Ovcharenko *et al*. 2013 |
| *Unikaryon bouixi* Toguebaye et Marchand, 1983 | Oval 2.1 × 1.6 µm | gut, Malpighian tubules | 3–4 coils | *Euryope rubra* (Latreille, 1807) | Toguebaye and Marchand, 1983 |
| *Unikaryon matteii* Toguebaye et Marchand, 1984 | Oval 3.7 × 2.0 µm | gut, Malpighian tubules, muscle tissue | 5–12 coils | *Nisotra sp.* | Toguebaye and Marchand, 1984 |
| *Unikaryon montanum* Weiser, Wegensteiner et Žižka, 1998 | Oval 2.1 × 1.3 µm | fat body, muscle tissue, Malpighian tubules, ovaries | 5–8 coils | *Ips typographus* (Linnaeus, 1758) | Weiser *et al*. 1998 |
| *Unikaryon nisotrae* Toguebaye et Marchand, 1986 | Oval 2.3 × 1.7 µm | gut, adipose tissue | 5–7 coils | *Nisotra sjoestedti* (Jacoby, 1903) | Toguebaye and Marchand, 1986 |
| *Unikaryon phyllotretae* Yaman, Radek, Weiser et Toguebaye 2010 | Spherical to oval 3.8× 1.9 µm | Malpighian tubules | 6–7 coils | *Phyllotreta undulata* (Kutschera, 1860) | Yaman *et al*. 2010 |
| *Unikaryon polygraphi* Weiser, Händel, Wegensteiner et Žižka, 2002 | Oval 2.8 × 1.3 µm | midgut, longitudinal and circular muscles, secretory part of Malpighian tubules | 5–7 coils | *Polygraphus poligraphus* (Linnaeus, 1758) | Weiser *et al*. 2002 |
| *Microsporidium* sp. | Oval 3.5 × 2.5 µm | midgut epithelium, midgut muscles, fat body, Malpighian tubules, gonads | 4–7 coils | *Ips amitinus* (Eichhoff, 1871) | this study |

*I. amitinus*. For *Microsporidium* sp., the relatively short polar filament that is coiled with five to six or with seven to eight turns and the apical fixation of the polar filament are typical for the genus *Unikaryon.* In both species the spore wall is formed by a thick electron-lucent endospore and a thin electron-dense exospore.

**Acknowledgements.** We thank the Austrian Federal Forest Company (ÖBf), the Tyrolean federal state forest protection officer Dipl. Ing. Christian Schwaninger (Tyrol, Austria), and Prof. Dr. Kari Heliövaara (University of Helsinki) for providing bark beetle-infested log sections. This research was supported by project B05/16 from the Internal Grant Agency of Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, and by project from Ministry of Education, Youth and Sport of the Czech Republic (LO 1509) and EU (Operational Program Prague – Competitiveness project, CZ.2.16/3.1.00/24023). The authors thank Dr. Bruce Jaffee (USA) for linguistic and editorial improvements.

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**Received**: December 30**,** 2015
**Revised**: April 15, 2016
**Accepted for publication**: April 29, 2016