J Pest Sci (2006) 79: 127–135
DOI 10.1007/s10340-006-0124-y

ORIGINAL PAPER

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Larssoniella duplicati n.sp. (Microsporidia, Unikaryonidae), a newly described pathogen infecting the double-spined spruce bark beetle, *Ips duplicatus* (Coleoptera, Scolytidae) in the Czech Republic

Received: 19 April 2005 / Revised: 23 December 2005 / Accepted: 17 January 2006 / Published online: 7 June 2006

Abstract *Larssoniella duplicati n.sp.* infects the midgut muscularis, the Malpighian tubules, and the ovaries of adult *Ips duplicatus* (Sahlb.) in the Czech Republic. The microsporidian attacks up to 50% of the population. Oval spores of two sizes, 3–3.5×1.5–2 and 2–2.5×1.5 μm have the polar filament coiled in 6/7 coils, representing primary and environmental spores, respectively. In early sporogony the young spores produce long electron dense threads and tubules of secretions, which remain fixed around the spore and avoid their free release during dissection of infected hosts. The microsporidian was not found in associated bark beetles such as *Ips typographus* (L.), or *I. amitinus* (Eichh.) and others.

Keywords Microsporidia · *Larssoniella duplicati* · *Ips duplicatus* · Scolytidae

Introduction

Investigations of diseases of bark beetles were focused during the last 15 years on *Ips typographus* (L.), the major pest of spruce stands in Central Europe (Wegensteiner 2004). The specificity of this pest is in its fast massive growth of populations in wind-broken or snow-broken stands and the difficult establishment of natural balance by biological means especially in protected reserves and national parks (Skuhravý 2002). During the last decade another bark beetle, the double-spined spruce bark beetle, *Ips duplicatus* (Sahl.), invaded the spruce forests in the eastern part of the Czech Republic. The pest was first spread mainly in spruce stands in northern boreal Euro-Sibirian taiga and almost unknown in Central Europe (survey see Holuša et al. 2003). Its recent massive outbreak followed the massive distribution of *I. typographus* was initiated by damages of environment due to irregularities in rainfall and periods of higher summer temperatures connected to infestation of forests by honey root fungus, *Armillaria ostoyae* (Romangn.) Herink. (Holuša and Liška 2002). Both bark beetles appear together in the same type of damaged trees: *I. duplicatus* attacks upper parts and major branches of weakened trees and *I. typographus* is present in the lower part (Holuša et al. 2003). The invasion of *I. duplicatus* in the new area seems to be possibly connected with some changes in its populations responding to new climatic conditions in Central Europe (Grodzki 1997).

The pathogens of the double-spined spruce bark beetle (*I. duplicatus*) in its new invaded area include a new microsporidian, which is described in this study.

Materials and methods

Material of adult beetles was collected by digging from nuptial chambers in freshly invaded trees and only additionally, in case of very difficult or impossible collection of beetles under the bark, we studied beetles from Theysohn® type pheromone traps in several localities in the eastern part of the Czech Republic in 2002–2003. We studied only beetles collected in the field.

Due to time-consuming dissection of beetles, fifty beetles were sampled from several trees three times a

Communicated by Jürgen Gross

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year in accordance with the method proposed by Wegensteiner (personal communication). The sampling was built on the idea that both species have two or three generations per year in the studied area, with the main peaks of bark beetle flight in April/May, July and August/September (Holusa et al. 2003).

Under outbreak conditions, trees were scattered about 100 m apart. Due to intensive cutting and logging of infested trees it was sometimes difficult to find the required numbers of beetles, especially in 2003. In the spring, adult mature beetles of the parental generation were collected during their flight and boring of nuptial chambers. In summer, we collected the beetles of the parental generation when they were still in the nuptial chamber but it was also possible to collect offspring beetles of F1 generation; it means the beetles of the next generation from the same trees (in all cases offspring beetles from several colonies). Beetles of F1 generation were studied at the end of mature feeding, in the stage of dark brown beetles, in which we can suppose that the pathogen have already developed (see Wegensteiner and Weiser 1996). At the end of summer, we supposed that we collected mature beetles of F1 generation after mating and egg laying and the beetles of F2 generation after maturation feeding. Beetles were stored in plastic vials in the refrigerator. Beetles were dissected in the same way used with *Ips typographus* (Wegensteiner et al. 1996). Their abdomens were cut off and the midgut was extracted with the last segment from the rest of abdomen in a droplet of water. The extracted gut with adherent Malphigians, ovary and fat body with adherent tissues were inspected under the cover slip in the optical microscope at magnification 100× and 450×. In case of infection, the material was immediately transferred to 2.5% glutaraldehyde in cacodylate buffer (pH = 7.2) and stored for further processing. Smears of the infected gut were prepared as dry smears, fixed in methanol and stained with Giemsa. Part of material was stored in water at 4°C. Measurements of pathogens were performed in water mounts and on Giemsa stained dry smears. For electron microscopy beetles were dissected in 2.5% glutaraldehyde in cacodylate buffer and the material was processed for embedding in resin for TEM. After 12 h at 4°C the material was washed in cacodylate buffer and stored in buffer at 4°C for refixation with 2% osmic acid and transfer to vestopal W. Blocks were cut in semithin sections for histological evaluation and in ultrathin sections for TEM. These were contrasted routinely in uranyl acetate and lead citrate and inspected in Philips EM300 TEM. All *I. duplicatus* were dissected and inspected individually.

Results

The host

Ips duplicatus attacks upright standing stems of damaged trees (Holuša et al. 2003). Its galleries are often separated from other associated bark beetles such as *I. typographus* and *I. amitinus* but there are limited zones of overlapping of late larval galleries or feeding tunnels where contacts are possible.

In dissected larvae, pupae and callow beetles of *I. duplicatus* all repeated dissections were negative and there was no evident mortality in galleries of these stages.

A total of 1,471 *I. duplicatus* were dissected and inspected for pathogens. Some pathogens known from *I. typographus* were also present in *I. duplicatus* (Table 1). Nematode invasions infections were present in all localities, without any evident connection to other pathogens. *Contortylenchus dispar* (Fuchs) and *Polymorphotylenchus typographi* (Fuchs) were present in *I. typographus* and *Contortylenchus amitini* Rühm in *I. amitinus*. *I. duplicatus* was infected with species of genus *Contortylenchus* and *Polymorphotylenchus*. Due to their active migrations in collected beetles in vials their presence did not characterize the real situation in beetles in their bark galleries.

The new microsporidian was present in the majority of samples of *I. duplicatus* in NE Czech Republic. Totally, 18.3% of beetles were infected (Table 1).

The microsporidian

Light microscopy

The infection is localized in the midgut, the Malpighian tubules and the ovaries of *I. duplicatus*. In the midgut the pathogen infection is localized in the longitudinal and

Table 1 The survey of total infection level of pathogens (frequency in %) and presence in samples (constancy in %) in *Ips typographus* and *I. duplicatus* in the eastern part of the Czech Republic in 2002–2003 (*S* number of samples, *N* number of beetles, *C.t. Chytridiopsis typographi*, *N.t. Nosema typographi*, *L. Larssoniella duplicati*, *G.t. Gregarina typographi*)

	S	*N*	*C.t.*	*N.t.*	*M.ch.*	*L.*	*G.t.*
Ips typographus							
Constancy in localities (in %)	39	1,418	17.5	5.0	5.0	0	35.0
Frequency (in %)			1.1	0.1	0.1	0	2.2
Ips duplicatus							
Constancy in localities (in %)	49	1,471	10.0	0	0	78.0	10.0
Frequency (in %)			0.4	0	0	18.3	0.7

circular muscle fibers of the muscularis in the whole length of the gut. Both genders are infected. The spores and vegetative stages are concentrated in coherent masses in the central part of the muscle. Infected fibers are scattered in groups in all parts of the midgut. The infection evidently spreads from the body cavity; there are no infection centers in the epithelial layer of columnal cells. Mature and maturing spores are mixed with vegetative stages and were present also in gastric caeca and cryptae in their muscle layers. Some infected elements on the surface of the midgut are infected oenocytes. Minor ultracerating centers and spores of infection appear in the Malphigian tubules and spores from destroyed cells of the epithel leave the tubules together with urate secretions and are released in the posterior part of the gut. In the ovary we find ovarioles with minute groups of spores which are matured from germs injected evidently to the chorion and were not forming centers of further development. The source of germs can be migrating infected oenocytes or minute ligaments of connective tissue with local infection holding the midgut position.

The infected muscle fibers hold the spores in position when crushed in watermount and individual spores do not leave the groups as is the case in microsporidia of other genera in bark beetles (*Nosema typographi* (Weiser), *Canningia spinidentis*) Weiser, Wegensteiner, Žižka (Wegensteiner 2004; Weiser et al. 1995).

The spores are broad oval, regular in size and shape. Differences in size are evident in dry smears, where spores with softer wall are broader when dried to the slide. In Giemsa stained materials the spores are stained faintly, with a distinct metachromatic granule in the posterior part in the area of the Golgi system. The sporoblasts are oval, 3–4×3 μm, with one central nucleus. In water mount spores are broad oval refringent bodies, 3–3.5×2–2.5 μm in size, without size variation. Measurements of fixed and stained spores in smears show two major groups of spores: up to 60% are spores 3–3.5×1.5–2 μm, 30% are minor spores, 2–2.5×1.5 μm and the other 10% are doubled teratospores (in the sense by Weiser 1966) 4–5×2–2.5 μm. In some beetles the minor spores were less numerous. The characteristic for this microsporidian is the faint staining of spores and their minimum release from infected tissues.

Electron microscopy

The infected tissues contain spores in rather dense aglomeration, without formation of empty parasitophorous vacuoles. They are in close contact with the host tissues. In electron microscopy they can be divided in two groups, the dark, electron dense environmental spores and the less dense primary spores. This second type contains also empty spores with extruded germ and can be defined as spore providing spread of germs in the same host. The primary spores are mainly in the muscularis and the oenocytes of the midgut (Figs. 1, 2, 3)

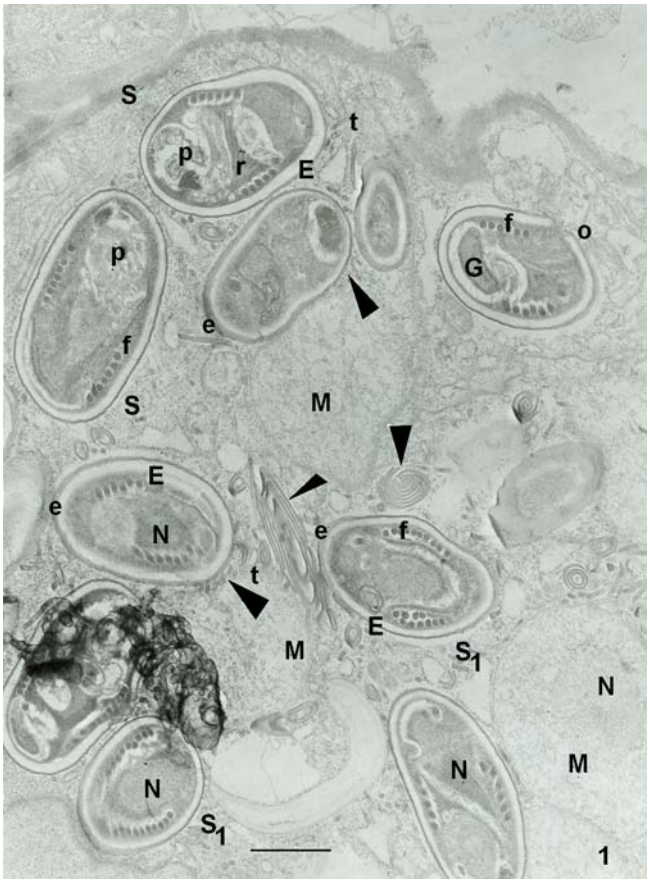

Fig. 1 *Larssoniella duplicati*, primary-type spores in the midgut of *Ips duplicatus*. Meronts (*M*) with nucleus (*N*) and thin outer membrane. Spores (*S*) with single nucleus (*N*), Golgi (*G*), regular electron lucid endospore (*E*) and thin exospore (*e*). Polaroplast (*p*) and coiled polar filament (*f*). Secretion tubules (*t*) arising at the posterior end of the spores (*arrowheads*). Spore S_1 with long polar filament and broken spore wall (*o*). *Scale bar* 1 μm

and spores of the dark dense type are present in oenocytes (Fig. 4), the Malpighian tubules and in some parts of the muscularis of the gut (Figs. 7, 8). Limited numbers of primary spores are present in all infected tissues.

Vegetative stages are uninucleate meronts (Figs. 1, 2, M) with thin outer membrane, 2.5–3 μm in diameter, in direct contact with the host cytoplasm. After division, at the end of schizogony their covering membrane is thickened (Fig. 2, M_1) and further transformation into sporoblasts and spores is going so fast that there is no evidence of any stage of early formation of spores and polar filament in a large EM material. The stage of wrinkled sporont, which is typical for other microsporidia is totally absent in our material.

During maturation in primary spores (Figs. 1, 2 S), a smooth, regular electron lucent endospore (E) is formed. In persistent environmental spores, the electron-lucent endospore is not formed (Figs. 5a, 6). With the maturation of both types of spores, secretion tubules are formed (Figs. 1, 4, 6, 7, t). They are 20–25 nm in diameter with tubule-wall 8–10 nm thick and of different length, up to 2–3 μm. They arise mainly on the posterior

Fig. 2 Infected cells of the midgut with primary spores and several meronts (*M*). Meront M_1 with thickened outer membrane in transformation to sporont. Wall of empty spore (*es*). *Scale bar* 5 μm

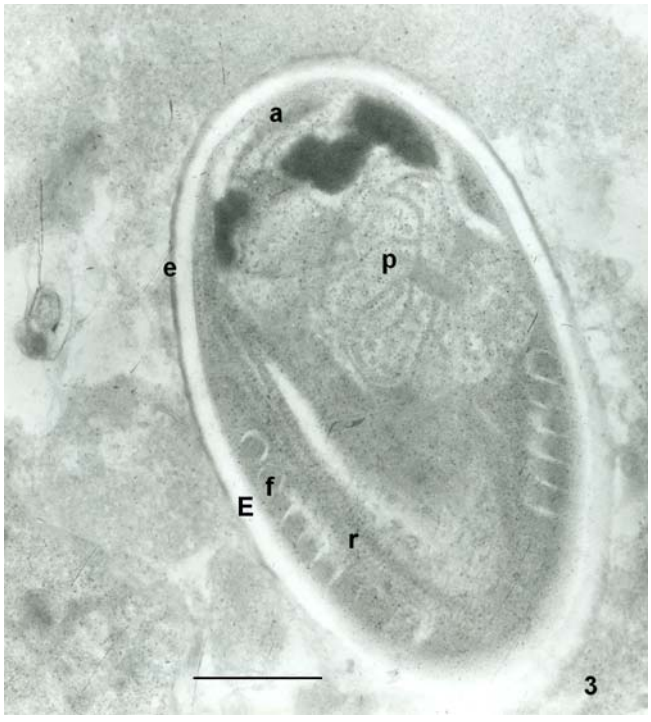

Fig. 3 Primary spore with smooth exospore (*e*) and regular layer of endospore (*E*). In part of anchoring disc (*a*) and tubular polaroplast (*p*). Polar filament in 6 coils (*f*). Ribosomal "basket" (*r*). *Scale bar* 500 nm

end of maturing spores (Fig. 6). There is some evidence of their early formation as dense granulations on surface of the exospore (Fig. 5a). Some tubules are coiled in spiral wicks. They are not resorbed or dissolved after maturation of the spores and they possibly hold the spores together.

Primary spores (Figs. 1, 2, 3, 4) are regular broad oval bodies with a smooth endospore (E) 80–100 nm thick and a thin electron dense exospore (e), 20 nm thick. The single nucleus (N) is located in a basket of ribosomes (r) and the polar filament (f) is coiled in 6/7 coils on the spore wall in the central part of the spore. The anchoring disc fills the apical end of the spore (a). The polaroplast (p) is tubular, with a posterior lamellar part. A large Golgi system is located in the posterior vacuole (G). The variability in size of spores is not evident in the TEM pictures, but polar filaments of double length are present in spores of regular size (Fig. 1, S_1).

Persistent spores (Figs. 4, S_2, 5, 6, 7) are characterized by their electron dense staining and lack of differentiation of spore ultrastructures. After the formation of the electron lucent endospore the whole spore is soft and bent in different ways, the spore wall is on surface corrugated and the internal structures are identical with the structures in primary spores. They are in the same size and shape ranges.

Discussion

The host and infection rates

Ips duplicatus is known as a pest of *Picea abies, P. obovata* Led. and *P. jezoensis (= ajanensis)* (S. et Z.) Carr. throughout their distribution in the taiga of the European part of Siberia, the Yakutia and Primorie (Maritime Far East). It is less common on pine (*Pinus silvestris* (L.), *P. sibirica* (Du Tour) and *P. koraiensis* (Siebold et Zucc.). In Central Europe its occurrence on spruce is disjunctive to its main distribution area and possibly the populations in southern Poland and border mountains of the Czech Republic are specific (Grodzki 1997). Its distribution and abundance in alpine forests did not attract attention of investigators. The bark beetle does not form groups of infested trees as

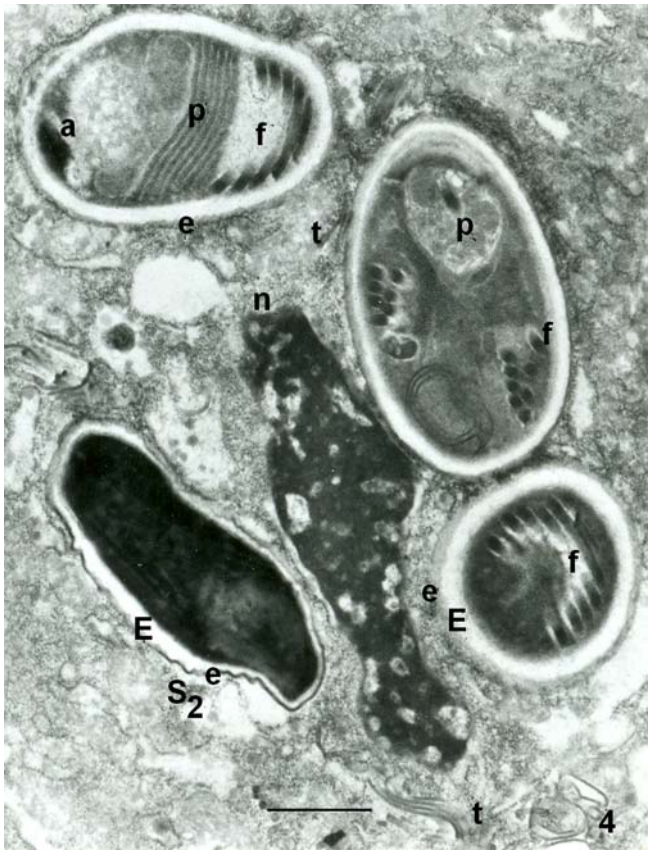

Fig. 4 Three primary spores and one environmental spore (S_2) located close to the nucleus (*n*) of the oenocyte. Polar filaments (*f*) in *single and double row*. Secretion tubules (*t*) fill the plasm of the host cell, without parasitophorous vacuoles. Exospore (*e*) and endospore (*E*) of the environmental spore are *wavy and bent* (*a* anchoring disc, *p* polaroplast). *Scale bar* 1 μm

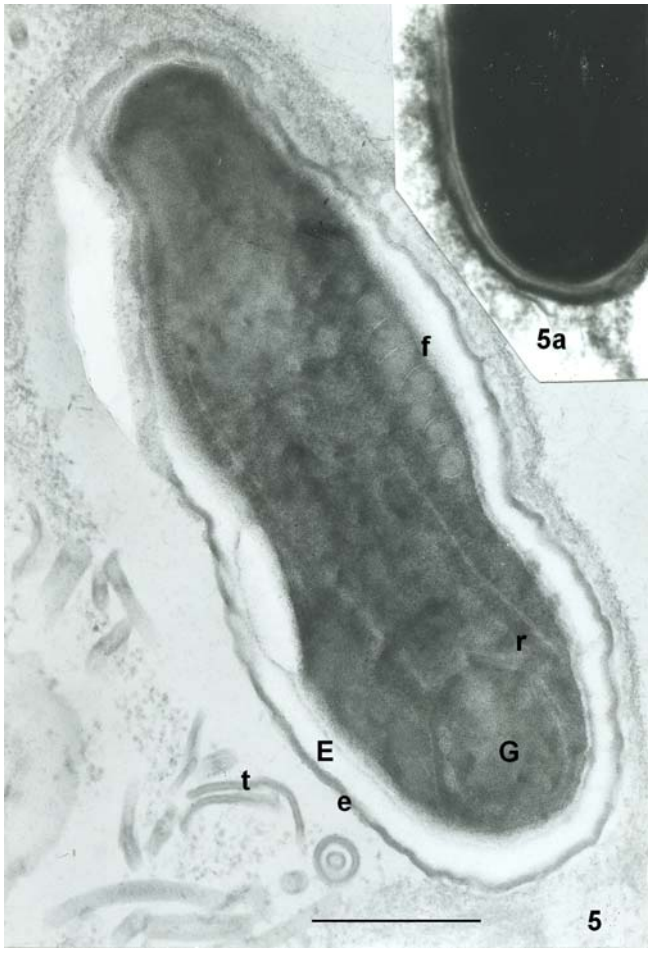

Fig. 5 Environmental spores with typical electron dense disturbed interior. Visible location of the polar filament (*f*) in eight coils adhering to the wall, wavy and flexible endospore (*E*), exospore (*e*) adhering to its surface. Part of anchoring disc (*a*) is adhering to the anterior pole and the Golgi (*G*) is in the posterior pole of the spore. Nucleus is not differentiated, the longitudinal line is limiting the "ribosomal basket" (*arrowhead*). Secretion tubules (*t*) are coiled around the spore end. *Scale bar* 500 nm. End of young environmental spore with distinct plasmalemma and absent lucent endospore (**a**). Exospore (*e*) with granular secretion product appearing in regular release points

I. typographus does. It occurs in single crowns of spruce trees and is out of attention. The formation of local populations with a common identical level of infection in the area is suppressed and the arrays of pathogens of beetles in individual tree-centers are autonomous. In our study area the trees infested by bark beetles are removed the same year and the development of next generations of bark beetles in the same tree is impossible. Therefore it is surprising that there is a parasitization with this one microsporidian presented in the majority of studied populations of *I. duplicatus* and was found in 18.3% of studied beetles (Table 1). Very often more than 10% beetles were infected and the infection reaches up to more than 50% in some samples (Table 2). The callow beetles were negative in the same localities.

In *I. typographus* in the same sampling areas, there is no infestation by microsporidia except sporadic *Chytridiopsis typographi* (Weiser) and *Gregarina typographi* (Fuchs) infection (Table 1). On the other hand this confirms that the close contact of both bark beetles in infected trees does not produce cross infections with the microsporidian although their zones of galleries are sometimes overlaping and evidently the described microsporidian in *I. duplicatus* does not infect *I. typographus*. On the other hand, the rather high level of infection in all populations of *I. duplicatus* could support the possibility that this basic infection is mainly a result of the transovarial infection. The spores of the microsporidian appear in the feces of infected adults after the infection centers appear in the Malpighian tubules. Per os infections can be expected only in the case of callow beetles entry during maturation feeding into mature galleries or nuptial chambers.

The pathogenicity of the microsporidian does not cause evident decreases of populations in the next generations. Due to fixation of masses of spores in infected tissues in solid packing, the infected midgut musculature is not destroyed totally and the covering layer of muscle fibers produces most probably some activity and provides compressions necessary for motion of food pellets.

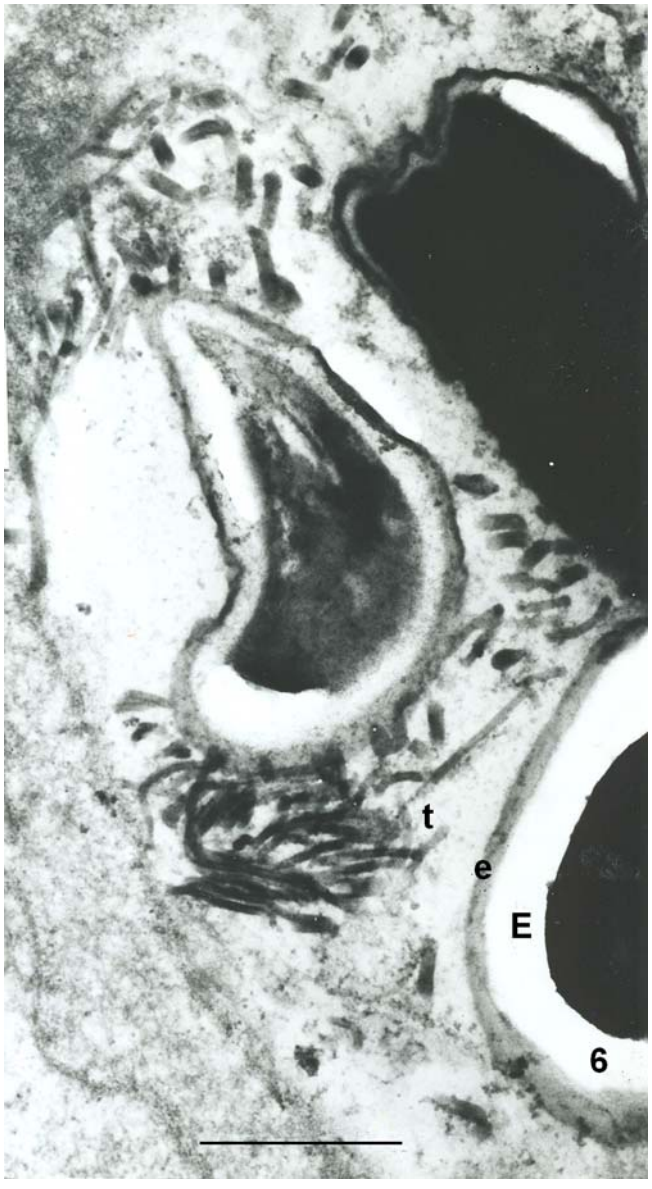

Fig. 6 Three environmental spores, one without lucent endospore, other two with well formed lucent endospore (*E*), foamy thick exospore (*e*) and fresh masses of secretion threads arising from the exospore (*t*). *Scale bar* 500 nm

The microsporidian

The specific symptom, which characterizes the difference of this microsporidian in *I. duplicatus* from others in bark beetles, is the compact formation of spore groupings, without spread of spores all over the dissected material. The only factor, which may explain this compactness, is the formation of secretion tubules. These are tubular structures, usually rather long, sometimes coiled and are adjacent to the apical and the posterior part of forming spores. They are around primary spores as well as around persistent spores and there is no evidence of any fixing point in the exospore of the spore wall where they are produced except a granulation, as evident in Fig. 5a.

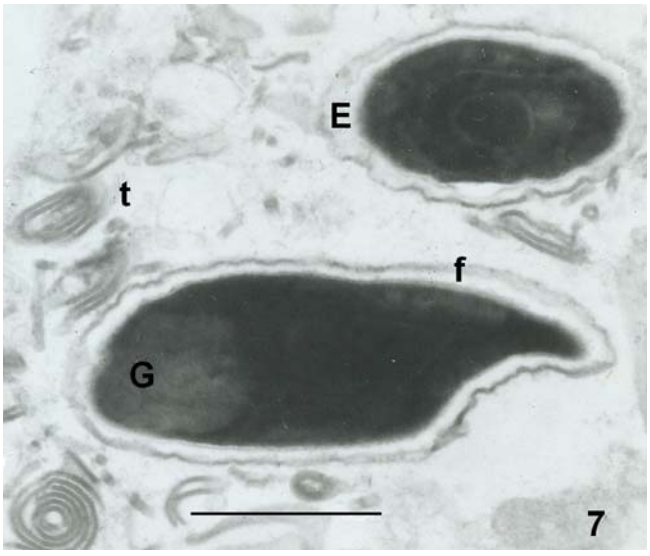

Fig. 7 Two environmental spores with dense interior, and the single nucleus in the center of the minor cross section. Five coils of filament (*f*) on the spore wall (*E* endospore), the spherical Golgi system (*G*) is in the posterior end of the spore. Surface of the spores is wrinkled and bent. The mass of spores is fixed together with secretion tubules (*t*). *Scale bar* 1 μm

Measurements of spores conducted on dry smears have shown that there are usually present two types of spores, 3–3.5×1.5–2 and 2–2.5×1.5 μm. In fresh material in water mount the two size types are not evident. On ultrathin sections it is difficult to distinguish any different size or shape. In the fixed material the early spores are well differentiated and with firm smooth walls. The environmental spores are all bent, corrugated or with waved spore wall and the differentiation of the interior is difficult. All are with electron dense material. In both spore types the polar filament is coiled in 5/6 to 6/7 turns, but there are exceptions with double length filament and 10 turns. The early spores are not a

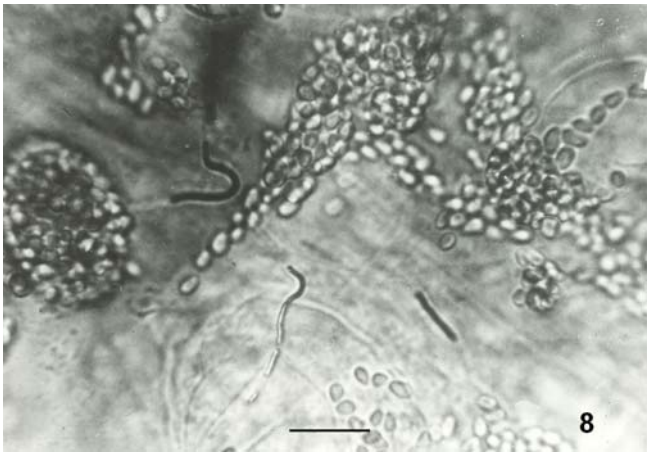

Fig. 8 Surface of the midgut with spores of *Larssoniella duplicati* in the muscle strands of the muscularis and end cells of tracheolae. *Scale bar* 10 μm

Table 2 Infection levels of pathogens (in%) in the locality of Václavovice (300–310 m a.s.l., 49°44′N; 18°21′E) in five consequent generation of *Ips typographus* and *I. duplicatus* (*S* number of samples, *N* number of beetles, *C.t. Chytridiopsis typographi*, *N.t. Nosema typographi*, *L. Larssoniella duplicati*, *G.t. Gregarina typographi*, *P* parental generation, *F1–F4* filial generations)

Generation	Date of collection	*Ips typographus*							*Ips duplicatus*				
		S	*N*	*C.t.*	*N.t.*	*M.ch.*	*L.*	*G.t.*	S	N	*C.t.*	*L.*	*G.t.*
P	25.5.2002	2	71	0	0	0	0	1					
P	1.8.2002	1	50	0	0	0	0	0	1	30	0	57	0
F1	1.8.2002								1	50	0	14	0
F1	23.9.2002	1	50	0	0	0	0	0	2	37	0	24	0
F2	23.9.2002	2	60	0	0	0	0	0	2	90	0	24	1
F2	16.5.2003	2	67	0	0	0	0	0	1	46	0	26	0
F2	27.6.2003								1	20	0	0	0
F3	27.6.2003	1	30	0	0	0	0	0	3	40	0	15	8
F3	26.8.2003	2	45	0	0	0	0	1	2	47	0	34	0
F4	26.8.2003	1	20	0	0	0	0	1	1	10	0	10	0

developmental stage of persistent spores, when stored in tissues. Their content is emptied and autolyzed.

Taxonomy

The genus *Unikaryon* was used for description of microsporidia with uninucleate thick-walled spores and with development presenting only uninucleate and binucleate immediately dividing schizonts. Sporonts are spherical, with thickened covering membrane and they form oval spores (2–3×1–2 μm) rather thick-walled, with waved surface, with two types of spores: primary spores opening inside the host extruding their filament, and environmental type with electron dense interior, serving for spread with feces in the environment the outside distribution and new infections. Polar filament is coiled in 5/6 to 10 coils in one layer adhering to the spore wall. The 16 or more species included in this genus are described from Trematodes (4 species), Copepods (1 species), ticks (1 species), chrysomelid beetles (4 species) and bark beetles (5 species) (see below). A closely related species is *Larssoniella* infecting Lepidoptera and *Oligosporidium* infecting a spider. They also have environmental spores with dark interior but they produce secretion tubules or granules.

Codreanu et al. (1981) proposed the genus *Oligosporidium* for a microsporidian infecting a spider *X. ysticus cambridgei* (Blackwall). *O. arachnicolum* Codreanu-Balcescu, Codreanu et Traciuc was proposed first as member of the genus *Unikaryon* (Codreanu et al. 1981). Compared with species presented in our review it has many analogies. Development, type of schizogonial stages and sporogony are of the same type as our actual microsporidian. The spores are of the same type and size range, with polar filament in 7/8 coils. Codreanu et al. (1981) present in their description only one spore type which is identical with our environmetntal type of spores. But in their paper (Codreanu et al. 1981) in Fig. 2 we find the same complex of spores of the primary and environmental type as we find in our Fig. 4. Instead of production of tubular secretions as in *Larssoniella, Oligosporidium* produces a mass of fine globular secretion granules, which fill a parasitophorous vacuole formed in the cytoplasm of the oocytes of the spider. All developmental stages are present in this mass. With these characteristics it is evident that *Oligosporium* is in morphology very closely related to *Larssoniella* and it belongs to the Unikaryonidae.

Survey of microsporidia of the family Unikaryonidae is given below. The species described from beetles are very similar in morphology. The species infecting bark beetles are close in general morphology and type of attack, but in nature they are rather host specific and do not appear as common mutual infections in hosts although in case of dense infestation of tree, adult beetles may cross galleries. Other microsporidia in bark beetles are less specific (*Chytridiopsis typographi, Nosema typographi*) (Wegensteiner 2004) and cross infections of associated bark beetles are rather common. But in some bark beetle populations these microsporidia are quite rare. On the contrary, the microsporidian in *I. duplicatus* is common in the majority of inspected populations and infection level reaches very often more than 10% of infected beetles. This fact is difficult to explain because in general pathology in *I. duplicatus* does not differ from the less common *Unikaryon minutum* Knell et allen in *Dendroctonus frontalis* Zimm. (Knell and Allen 1978), *Unikaryon montanum* Weiser, Wegensteiner et Žižka in *I. typographus* (Weiser et al. 1998), *U. polygraphi* Weiser, Haendel, Wegensteiner et Žižka in *Polygraphus poligraphus* Linné (Weiser et al. 2002) or *U. amitini* Haendel in *I. amitinus* (Haendel 2001).

The main difference in the *Unikaryon*—like microsporidian in *I. duplicatus* is the formation of secretion tubules around primary and environmental spores. The same system of secretion was recorded only in *Larssoniella resinellae* Weiser et David (Weiser and David 1997). This microsporidian was ranged into Unikaryonidae and its development is identical with that of bark beetle species including the formation of a tuft of secretion tubules. *Larssoniella* infects a Lepidopteran, *Retinia (Petrova) resinella* (L.) and is present in its silk gland, the Malpighian tubules, the fat body, and the ovary. The infection is distributed in organs, which are not typical

for *Unikaryon* in bark beetles. The secretion tubules are formed mainly on the posterior end of the sporont and maturing spore and they are dissolved before maturation of the spores. This possibly explains the free distribution of spores in water mounts of organs with *Larssoniella resinellae*. In *I. duplicatus* the secretions remain persistent and hold together the spores produced in infected tissues. In *Larssoniella* the environmental spores remain electron dense as it is in the microsporidian of *I. duplicatus*. Therefore we believe that the microsporidian in the midgut muscularis, Malpighian tubules and gonads of *I. duplicatus* in morphological diagnosis belongs to the genus *Larssoniella* and we suggest *Larssoniella duplicati n.sp.* as a new member of the genus *Larssoniella*, Weiser et David, Unikaryonidae Sprague.

List of Unikaryonidae considered in this study
Unikaryon, Unikaryonidae

1. *Unikaryon* species in Trematodes (Trematoda)

Unikaryon piriformis Canning, Lai et Lie in *Echinoparyphium duni* (Lie et Umakhevy) and *Echinostoma audyi* (Kanev); piriform spores 3.8×2.7 μm; body tissues (Canning and Vávra 2000).
Unikaryon legeri (Dolfus, 1912) Canning et Nicholas in *Meigymonophallus minutus;* oval spores 3×1.6 μm, polar filament in 6/7 coils; body tissues (Canning and Nicholas 1974).
Unikaryon allocreadii Canning et Madhavi in *Allocreadium fasciatusi* Kakaji; cylindrical spores 2.3×0.9 μm; body tissues (Canning and Madhavi 1977).
Unikaryon slaptonleyi Canning, Barker, Hammond et Nicholas in *Echinoparyphium recurvatum* (Linstow); spores piriform, 5×2.8 μm, with polar filament in 17/21 coils (Canning et al. 1983).

2. *Unikaryon* in a Copepode (Copepoda)

Unikaryon mytilicolae Durfort, Vallmitjana et Vivares in *Mytilicola intestinalis* (Steuer); spores oval, 2×0.8 μm, polar filament in 6 coils; body tissues (Durford et al. 1980).

3. *Unikaryon* in ticks (Acarina)

Unikaryon ixodis (Weiser) in *Ixodes ricinus* (L.), *Dermacentor reticulates* (Sulzer, 1776); spores oval, 2.6×1.5 μm, polar filament four coils, 1.6–2×1.4 μm and polar filament in six coils; gut and ovary (Weiser et al. 1999).

4. *Unikaryon* in beetles (Coleoptera)

Chrysomelidae

Unikaryon bouixi Toguebaye et Marchand in *Euryope rubra* Latreille, 1807; spore oval, 1.6–2.5×1.4–1.6 μm, polar filament in 3/4 coils; midgut, Malpighian tubules (Toguebaye and Marchand 1983).
Unikaryon mattei Toguebaye et Marchand in *Nisotra sp.*; spores oval, 3.7×1.9 μm, polar filament in 5 and 12 coils; midgut, Malpighian tubules, muscle, fat body (Toguebaye and Marchand 1984).
Unikaryon nisotrae Toguebaye in *Nisotra sjoestedti* Jacoby; oval spores, 2.3×1.6 μm; midgut and fat body (Canning and Vávra 2000).

Meloidae

Unikaryon euzeti Toguebaye et Marchand in *Mylabris vestita* Reiche; oval spores 2–2.3×1.3–1.5 μm, polar filament in 7/9 coils; midgut, Malpighian tubules (Toguebaye and Marchand 1988).

Scolytidae

Unikaryon minutum in *Dendroctonus frontalis*; spores cylindrical, 2.3×0.9 μm, polar filament in 6 coils; midgut, Malpighian tubules, muscle, fat body (Knell and Allen 1978).
Unikaryon montanum Weiser, Wegensteiner et Žižka in *I. typographus;* spores oval, 2×0.8–1 μm, polar filament in 8 coils and 1.5×1 μm; midgut, Malpighian tubules, ovary (Weiser et al. 1998).
Unikaryon polygraphi Weiser, Haendel, Wegensteiner et Žižka in *Polygraphus poligraphus*; oval spores 2–2.5×1 μm, polar filament in 5 coils; midgut, Malpighian tubules, ovary (Weiser et al. 2002).
Unikaryon amitini Haendel in *I. amitinus*; spores oval, 1.5–2×1 μm, polar filament in 5/6 coils; midgut, Malpighian tubules, fat body, ovary (Haendel 2001).

5. *Larssoniella*, Unikaryonidae in Lepidoptera

Larssoniella resinellae in *Retinia resinella*; spores cylindrical, 4.5–5×1.7–2 μm, polar filament coiled in 10/11 coils; salivary glands, Malpighian tubules, fat body, ovary. Spores with tufts of secretion tubules (Weiser and David 1997).
Larssoniella duplicati n.sp. (this paper) in *I. duplicatus*; spores oval, 3.5×2 μm, polar filament 6/7 coils and 2×1.5 μm with polar filament 5/6 coils; midgut, Malpighian tubules, ovary.

6. *Oligosporidium* Unikaryonidae in Acarina

Oligosporidium arachnicolum Codreanu-Balcescu, Codreanu et Traciuc in *Xysticus cambridgei* (Blackwall); spores oblong, 3.6×2 μm, polar filament in 7/8 coils, fine granular secretion; ovary, oocytes, and pedicular cells (Codreanu-Balcescu et al. 1981).

7. *Canningia*, Unikaryonidae in Coleoptera

Canningia spinidentis in *Pityokteines spinidens* (Reitter); spores cylindrical, 1.9–2×0.8–1 μm, polar filament coiled

in 5/6 coils; fat body, Malpighian tubules, muscles, and connective tissue. Spores with polar filament fixed and extruded subapically (Weiser et al. 1995).

Canningia tomici Kohlmayr, Weiser, Wegensteiner, Haendel et Žižka in *Tomicus piniperda* (Linné, 1758); spores 2.5–3×1.5–2 and 3.5–4×2 μm, polar filament in 4/5 and 5/6 coils; infected are muscles, midgut, Malpighian tubules, fat body, and eggs. Spores with polar filament fixed and extruded subapically (Kohlmayer et al. 2003).

Acknowledgements The authors wish to thank Jitka Koláčková, Laboratory of Electron microscopy, Institute of Microbiology, Academy of Sciences, Praha, for perfect technical assistance. This study was supported by the Institutional Research Concept AVOZ 50200510 and by grant No. 526/02/D149 from the Grant Agency of the Czech Republic.

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