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Orthosomella lipae sp. n. (Microsporidia) a parasite of the weevil, *Liophloeus lentus* Germar, 1824 (Coleoptera: Curculionidae)

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ABSTRACT

A new microsporidium, *Orthosomella lipae* sp. n., was isolated from the outer ovariole sheath, trophic chambers, oocytes, somatic tissues and eggs of adults of the weevil, *Liophloeus lentus* Germar, 1824 (Coleoptera: Curculionidae) from southern Poland. Morphological and life cycle characteristics revealed using light and electron microscopy, place this new species within the Unikaryonidae. However, the 16S rDNA phylogeny indicates that it is associated with the genus *Orthosomella*.

1. Introduction

Weevils (Coleoptera: Curculionoidea) are common pests of wild and cultivated plants, stored grains and other foodstuffs. Microsporidia are widespread pathogens of insects and are known to regulate populations of several weevil species (Streett et al., 1975; Bruck et al., 2008). Thirteen microsporidian species have been mentioned in weevils so far. Most descriptions are incomplete and include only morphological features observed under the light microscope (Table 1). Seven of these species were described as belonging to the genus *Nosema* (Weiser, 1951; Maddox and Luckmann, 1966; Issi and Lipa, 1968; Drea et al., 1969; McLaughlin, 1969; Streett et al., 1975; Youssef, 1974), two were provisionally placed within the genera *Canningia* (Bruck and Solter, 2005; Bruck et al., 2008) and *Unikaryon* (Świątek and Górkiewicz, 2006) and four were placed within the collective genus *Microsporidium* (Hesse, 1905; Malone, 1985; Ghosh, 1990; Lewis et al., 2003). Among microsporidian parasites of weevils only *Microsporidium itiiti* infecting Argentine stem weevil *Listronotus bonariensis*, have been studied ultrastucturally (Malone, 1985).

The weevil *Liophloeus lentus* Germar, 1824 (Coleoptera: Curculionoidea) occurs in forests, meadows and riparian habitats (Anderson, 1993). It is a pest of *Chaerophyllum hirsutum* L., and other small herbaceous plants, including *Aegopodium podagraria* L., *Petasites albus* (L.) Gaertn and *P. kablikianus* Tausch ex Bercht (Dieckmann, 1980). A new microsporidium was discovered during studies of oogenesis in *L. lentus* from forest meadows in the Gorce Mountains of Poland. On the basis of its gross morphology, this new microsporidian species was initially placed within the genus *Unikaryon* (Świątek and Górkiewicz, 2006). However, phylogenetic analysis places the new microsporidium with *Orthosomella* spp.

2. Materials and methods

2.1. Field studies, sampling, and light microscopy

Adult specimens of *Liophloeus lentus* were collected from riparian meadows of Kamienica stream in the Gorce mountains, southern Poland (49°34.943′N 20°13.291′E), in May 2004. The density of the host, *L. lentus*, was high and insects were collected by hand. The ovarioles and trofaria of 2 of 12 studied specimens of *L. lentus* were infected by microsporidia (Świątek and Górkiewicz, 2006). Additional samples of infected weevils were collected in the same locality in summer 2005–2006. Collected

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Table 1
Summary of species of microsporidia reported in weevils (Curculionidae).

Microsporidium	Host	Spore form and size (μm)	Tissue	Locality	References
Canningia sp.	*Otiorhynchus sulcatus* (F.), adults	No data	Alimentary canal	USA	Bruck and Solter (2005), Bruck et al. (2008)
Nosema gasti	*Anthonomus grandis* Boheman, adults	4.3 × 2.3 (fresh)	Alimentary canal, other organs	USA	McLaughlin (1969), Streett et al. (1975)
N. hyperae	*Hypera postica* (Gyllenhal), adults and larvae	3.1 × 1.7 (fresh)	Malpighian tubules, muscles, epidermal cells, fat body, trachea	USA	Youssef (1974), Sprague (1977)
N. otiorrynchi	*Otiorrhynchus ligustici* (L.), adults	3.8–4 × 1.8–2	Malpighian tubules, fat body, muscles	Europe (Czech Republic)	Weiser (1951), David (1993)
Nosema sp.	*Pissodes strobi*, adults and larvae	3.5–4.2 × 1.8–2.2 or 5.0–6.0 × 1.8–2.5	Muscles, fat body, other tissues excluding reproductive organs	USA	Streett et al. (1975)
Nosema sp.	*Hypera postica* larvae	4.3–5.7 × 1.9–2.9 (fresh)	Muscles, fat body, salivary glands, trachea	USA	Maddox and Luckmann (1966)
Nosema sp.	*H. postica*, adults	6 × 4.5 (fresh)	Reproductive organs, fat body	USA	Drea et al. (1969)
Nosema sp.	*Pissoides picea*	3.6–5 × 2–2.9 (stained)	No data	Russia (Leningrad)	Issi and Lipa (1968)
Unikaryon sp.	*Liophloeus lentus* Germar, adult	3.4–5.2 × 1.2–2.0 (fresh)	Ovarioles, eggs, fat body, muscle cells	Poland	Świątek and Górkiewicz (2006), present paper
Microsporidium itiiti	*Listronotus bonariensis* (Kuschel), larvae and adults	2.5 × 1.4 (fresh)	Midgut (mainly), epidermis, fat body, muscles, ovaries	New Zealand	Malone (1985)
M. longifilum	*Otiorrhynchus fuscipes*, adults	4–5 × 3, 6 × 4 (macrospores)	Fat body	France	Hesse (1905), Sprague (1977)
Microsporidium strobi	*Sitophilus oryzae*, adults	No data	No data	India	Ghosh (1990)
Microsporidium sp.	*Neochetina eichhorniae*, *N. bruchi*, adults	No data	No data	USA	Lewis et al. (2003)

insects were dissected. Fresh preparations of infected tissues and Giemsa stained smears were studied. Smears were air dried, fixed in methanol and stained with Giemsa-stain solution. Live and methanol-fixed Giemsa-stained spores were observed and measured under Olympus BX50F4 microscope equipped with Analysis Pro 2.11 software. In total, 96 live spores were measured.

2.2. Transmission electron microscopy

For transmission electron microscopy (TEM), samples of infected tissues were fixed in 2.5% glutaraldehyde in 0.1 M. phosphate buffer (pH 7.4) for 3–7 days. After washing in phosphate buffer, the material was postfixed for 1 h in 1% OsO_4 in the same buffer, dehydrated in a graded series of ethanol and acetone, and

Table 2
Hosts, GenBank Accession numbers and sequence similarities to the new sequence from *L. lentus* for the SSU rDNA sequences of 28 microsporidian species used in the phylogenetic analyses.

Species	Accession	Host	Similarity to new sequence (%)
Orthosomella operophterae	AJ302316	*Operophtera brumata* (Insecta, Lepidoptera)	97
	AJ302317		96
Orthosomella sp.	GU299512	*Conistra vaccinii* (Insecta, Lepidoptera)	97
Microsporidium sp.	AJ871393	*Planorbis vortex* (Mollusca, Gastropoda)	87
Liebermannia dichroplusae	EF016249	*Dichroplus elongates* (Insecta Orthoptera)	82
Liebermannia sp.	EU709818	*Covasacris pallidinota* (Insecta Orthoptera)	82
Liebermannia patagonica	DQ239917	*Tristira magellanica* (Insecta Orthoptera)	78
Desmozoon lepeophtherii	AJ431366	*Lepeophtheirus salmonis* (Crustacea, Copepoda)	77
Paranucleospora theridion	FJ594990	*Lepeophtheirus salmonis* (Crustacea, Copepoda)	77
Enterocytozoon bieneusi	AF024657	*Homo sapiens* (Primates)	77
Nucleospora salmonis	AF185991	*Salmo salar* (Pisces, Salmoniformes)	79
Endoreticulatus bombycis	AY009115	*Bombyx mori* (Insecta, Lepidoptera)	78
Endoreticulatus schubergi	L39109	*Lymantria dispar* (Insecta, Lepidoptera)	78
Pleistophora sp.	U10342	None specified	78
Glugoides intestinalis	AF394525	Daphnia magna (Branchiopoda, Cladocera)	76
Vittaforma corneae	U11046	Homo sapiens (Primates)	76
Ordospora colligata	AF394529	*Daphnia magna* (Branchiopoda, Cladocera)	72
Encephalitozoon lacertae	AF067144	*Mabuya perrotetii* (Reptilia, Squamata)	73
Encephalitozoon hellem	AF177920	*Agapornis roseicollis* (Aves, Psittaciformes)	70
Encephalitozoon intestinalis	L19567	None specified	71
Vairimorpha necatrix	DQ996241	*Pseudaletia unipuncta* (Insecta, Lepidoptera)	67
Nosema apis	U97150	*Apis mellifera* (Insecta, Hymenoptera)	68
Nosema bombi	AY008373	*Bombus terrestris* (Insecta, Hymenoptera)	68
Oligosporidium occidentalis	AF495379	*Metaseiulus occidentalis* (Arachnida, Acarina)	68
Vairimorpha cheracis	AF327408	*Cherax destructor* (Crustacea, Decapoda)	68
Nosema bombycis	L39111	*Bombyx mori* (Insecta, Lepidoptera))	69
Nosema granulosis	AJ011833	*Gammarus duebeni* (Crustacea, Amphipoda)	69
Bryonosema plumatellae	AF484690	*Plumatella nitens* (Phylactolaemata, Plumatellida)	70

embedded in Epon 812 (Fullam Inc., Latham, NY, USA). Semithin sections (0.7 μm thick) were stained with methylene blue and examined with Olympus BX60 microscope. Ultrathin sections were cut on a Leica ultracut UCT ultramicrotome. After contrasting with uranyl acetate (10 min) and lead citrate (15 min), the sections were examined in Hitachi H500 and JEOL 1010 electron microscopes at 75–80 kV.

2.3. DNA sequencing

Weevils were dissected individually under a stereoscopic dissection microscope to remove all parts of the exoskeleton and obtain as much soft tissue as possible. DNA was extracted using a Qiagen DNeasy tissue kit. Each sample was subjected to PCR to amplify a portion of the 16S rRNA gene, using primers V1 and 1492 (Baker et al., 1994). PCR was performed using Invitrogen native Taq polymerase with an initial denaturing step at 95 °C for 5 min followed by 35 cycles of 95 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 5 min. All reactions were performed in a Primus Thermo Cycler. PCR products were sequenced directly using capillary array sequencing on an ABI 3100 DNA analyzer.

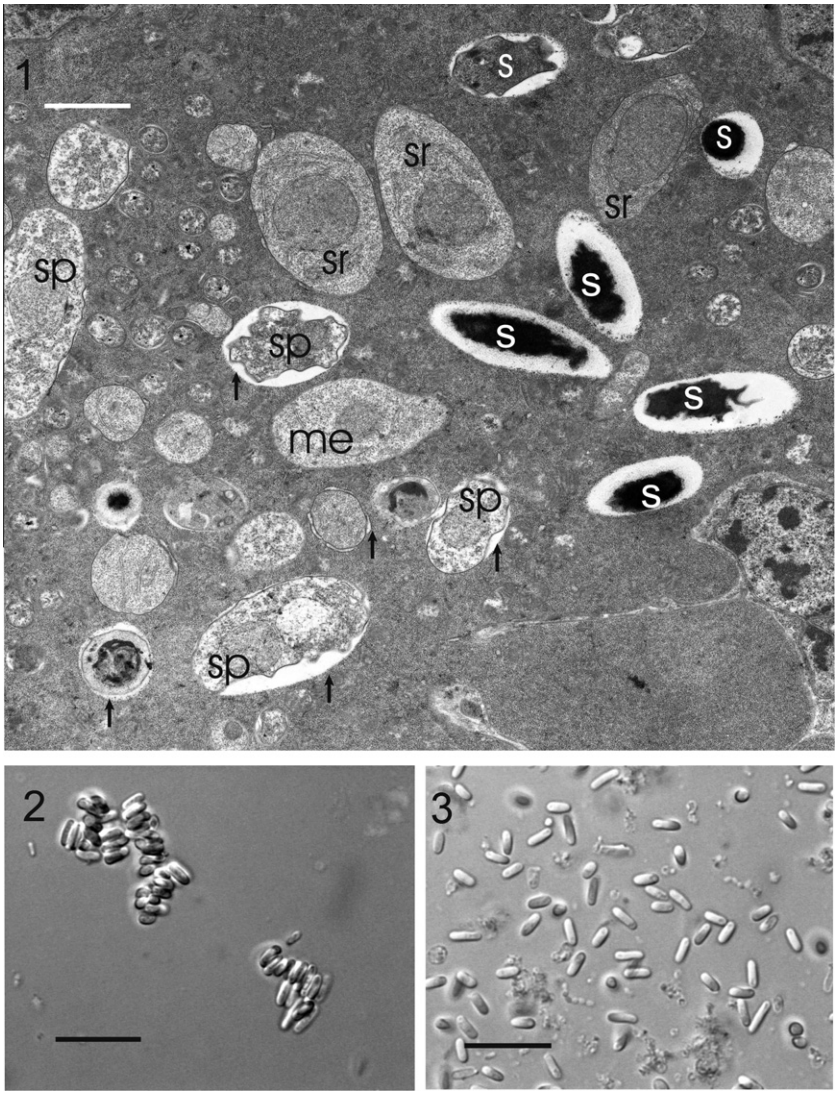
2.4. Sequence analysis

Sequence similarity was estimated by NCBI search tool Nucleotide Blast. Twenty-nine microsporidian sequences (Table 2) were aligned with CLUSTALL W implemented in BioEdit Version 7.0.9. Bayesian interference phylogenetic analyses was performed with MRBAYES v 3.1.2 (Huelsenbeck and Ronquist, 2001) using the General Time Reversible model with gamma variance and a proportion of invariant sites (GTR + I + G), as selected by MrModeltest 2.2. The analysis ran on two chains for 200,000 generations with one tree retained every 10 generations and a burn in period of 50,000 generations. A consensus tree was constructed from 570 trees remaining after the burn in period. A Kimura 2-Parameter model implemented in MEGA 5 Software (Tamura et al., 2011) was used to construct a DNA distance matrix for the sequences used in the analysis. *Bryonosema plumatella* was used as an outgroup.

3. Results

3.1. Pathogenicity

All inspected specimens lacked evidence of gross pathology. Microsporidia were observed within the cytoplasm of host cells

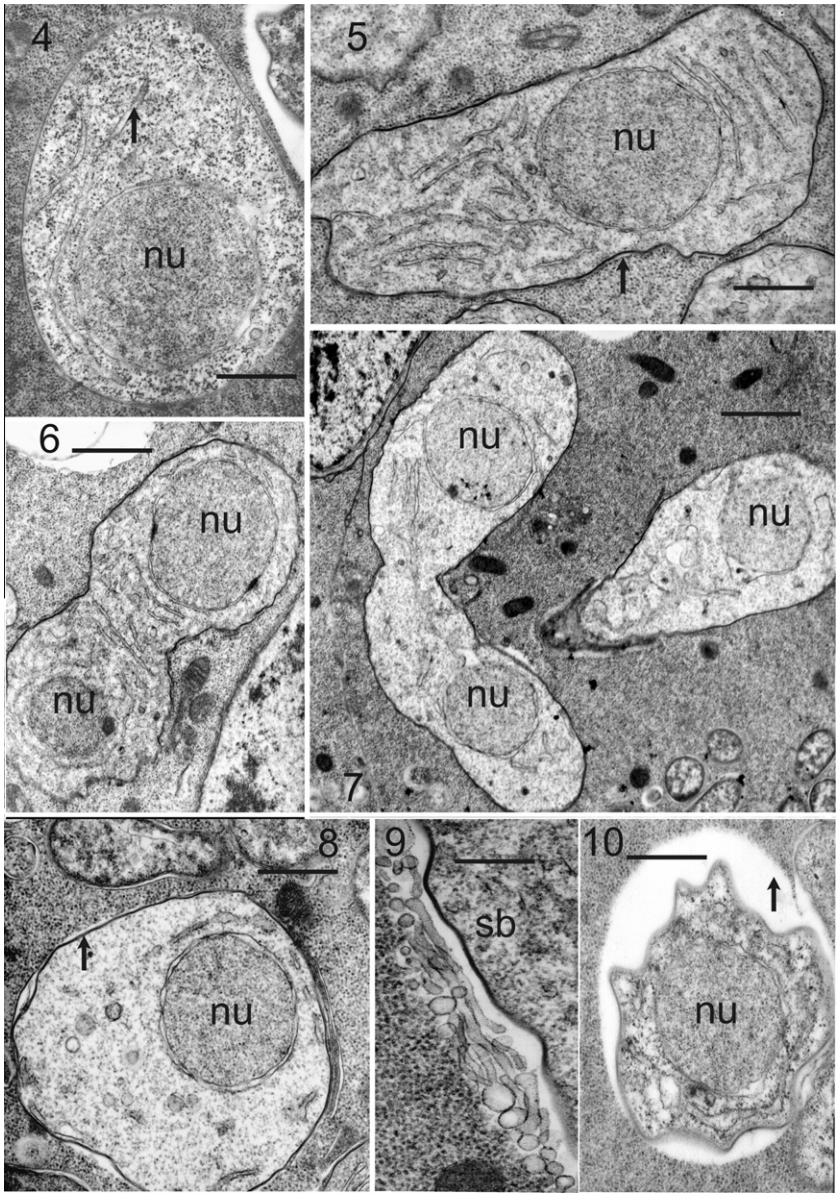


Figs. 1–3. (1) Section through tropharium cell of *L. lentus* infected with microsporidia. Meronts (me), sporonts (sr), sporoblasts (sp) and spores (s) are located in direct contact with host cell cytoplasm or inside parasitophorous vacuoles (arrows). Scale bar: 1.0 μm. (2) Light microscopy of fresh spores. Nomarski contrast. Scale bar: 5.0 μm.

(Fig. 1). Sporogonial stages and spores occurred within germline cells in trophic chambers (tropharia) (Figs. 1, 11, 16), in the cells of the outer ovariole sheath (Fig. 13), and within developing oocytes (Fig. 15). They were located in direct contact with host cell cytoplasm (Figs. 1, 4–7, 12) or inside parasitophorous vacuoles (Figs. 1 and 16). Isolated spores were found within eggs lying in lateral oviducts (for detailed descriptions of the ovary structure and pathogenicity see Świątek, 1999, 2002; Świątek and Górkiewicz, 2006). A lot of developmental stages of the microsporidium were surrounded by host cell mitochondria (Fig. 16). No detectable pathological differences in ovariole morphology between infected and non-infected specimens of *L. lentus* were observed. Spores inside host eggs and somatic tissues (Figs. 12–14) rested within electron transparent areas which contacted host cytoplasm directly without visible interfacial envelopes. Interestingly, some sporogonial stages and spores developing in tropharia, were surrounded by electron dense envelopes, whereas others resided within electron lucent zone without visible bordering lines. Sporogonial stages and spores developing in tropharia were either enclosed in a host-derived coat (Figs. 1, 15, 16) or surrounded by an electron lucent zone without visible bordering (Fig. 1).

3.2. Light and electron microscopy

Live spores were single, rod-shaped with equally rounded ends and measured 4.2 ± 0.4 (3.4–5.2) × 1.6 ± 0.2 (1.2–2.0) μm in size



Figs. 4–10. Electron micrographs of early developmental stages of the new microsporidium from tropharium (Figs. 4–9) and somatic cells (Fig. 10) of *Liophloeus lentus*. (4) Ultrastructure of a uninucleate meront with numerous free ribosomes, few profiles of endoplasmic reticulum (arrow), and a single nucleus (nu). Meront is surrounded by a simple plasmalemma. (5) Electron-dense material covers the outer surface of the early uninicleate sporont. Additional thin-walled envelope stacked with ribosomes, delineate the sporont surface (arrow). (6) Sporont with two separate nuclei (nu) undergoing cytokinesis. The spindle plaques are visible. (7) Cytokinesis of binucleate sausage-like late sporonts. (8) Cross section through the late sporont. The space between the sporont wall and additional envelope, i.e. episporal space (arrow), is expanded. (9) The structural changes in the adjacent host cytoplasm coinciding in time with expansion of episporal space are displayed. Cross section through the late sporont, located in tropharium. (10) Cross section through a young sporoblast with a single nucleus, located in the cytoplasm of the somatic cell. Scale bars: 500 nm (4); 600 nm (5); 1 μm (6, 7), 100 nm (9); 1.1 μm (8, 10).

(Figs. 2 and 3). On Giemsa-stained smears, the measurements of the spores were reduced to 3.1 ± 0.2 (2.5–4.7) × 1.6 ± 0.2 (1.2–1.9) 2–2.5 μm.

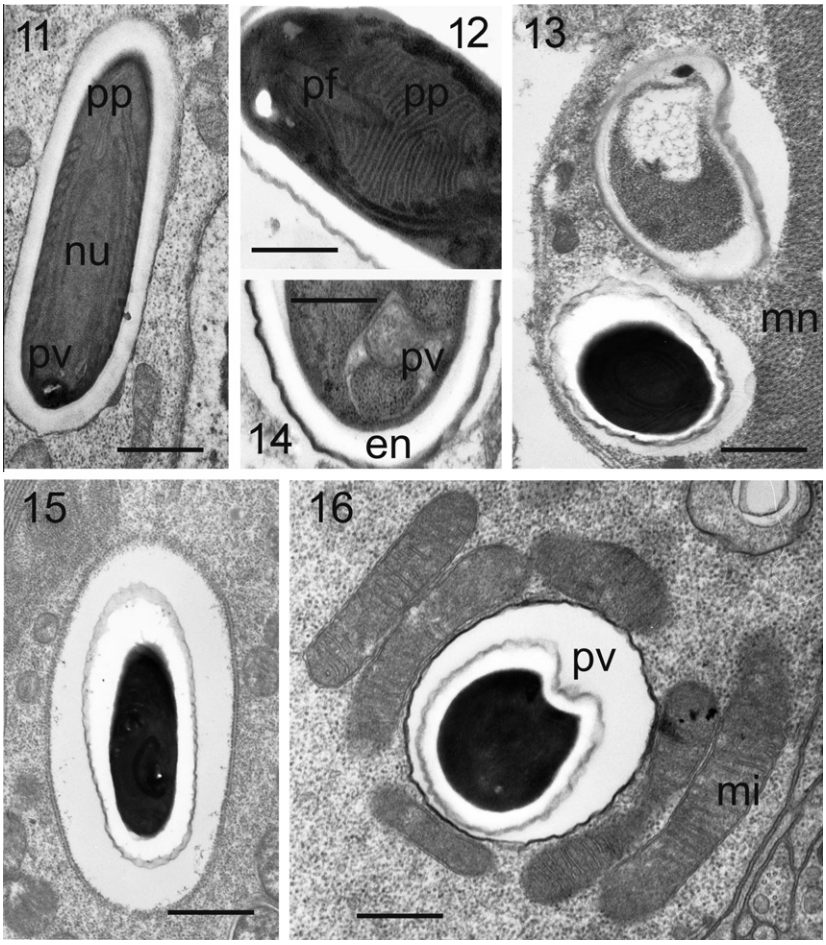
Meronts on ultrathin sections always displayed one nucleus and were roundish or oval in shape (Fig. 4). A nucleus measured 1.7–1.8 um in diameter and regularly occupied more than a half of the cell. The cytoplasm of meronts contained ribosomes and few cisternae of rough cytoplasmic reticulum.

Deposition of electron-dense material at the parasite cell surface indicated stages which were entering sporogony (Figs. 5–9). In sporonts rough endoplasmic reticulum and vacuolar structures increased in number indicating higher metabolic activity (Figs. 5 and 9). Electron-dense walls of sporonts developing in tropharia and oocytes (Fig. 5) were delineated with additional thin membrane stacked with ribosomes (presumably flattened ER cisternae). In late sporonts the space between the sporont wall and additional envelope (episporal space) expanded (Figs. 8 and 9). In somatic cells, however, such additional envelope surrounding sporonts was not observed. Binucleate sporonts were often seen on sections through tropharia (Figs. 6 and 7), as well as in oocytes and somatic cells (not shown). Binucleate sporonts underwent binary fission to produce uninuclear cells, which further developed into sporoblasts. Sporoblasts and spores were surrounded by electron-lucid area (Fig. 1). No membranes or layers separated this area from host cytoplasm, were visible (Fig. 10).

Mature spores were uninucleate (Fig. 11), thickwalled (Fig. 11–16) and filled by ribosomes arranged in a crystalline pattern like polyribosomes. The electron-lucid endospore was 150–180 nm thick; two-layered exospore – 40–50 nm (Fig. 12 and 14). The polar filament (pf) crossed the polaroplast (Fig. 12) and was regularly coiled in 12–14 turns tightly arranged in one row (Fig. 11). The isofilar polar filament was 60–70 nm in diameter. In a mature spore, an electron dense lamella embraced the posterior part of the polaroplast (Fig. 12). The posterior vacuole was about 200 nm in diameter and was filled with electron dense granules (Figs. 11 and 14).

3.3. DNA sequence analysis

The amplified 1105 bp fragment of SSUrDNA was sequenced and deposited in GenBank (Accession No. JF960137)). NCBI BLAST search revealed close similarity of the novel sequence to *Orthosomella* spp. (97–98% similarity, 100% coverage). Phylogenetic analyses by Bayesian inference placed the new microsporidium as a sister taxon to the *Orthosomella* clade, however with only moderate node support (Fig. 17). The DNA distance matrix (Table 3) indicated 0.01–0.02 genetic distances among the novel species and the members of the genus *Orthosomella*.



Figs. 11–16. Ultrastructure of the spores developing in tropharia (Figs. 11, 12, and 16), outer ovariole sheath cell (Fig. 13 and 14) and oocytes (Fig. 15). (11) Longitudinal section through the mature spore. Single nucleus (nu), posterior vacuole (pv) and polaroplast (pp) are visible. (12) Section through the posterior part of the spore. Polar filament (pf), posterior part of polaroplast (pp) and spore wall ultrastructure are visible. Anterior part of the polaroplast is also noticeable; lamellae are denser, thus harder to distinguish. (13) Spores located free in cytoplasm of the outer ovariole sheath cells. Numerous mionems (mn) are seen near the parasite spores. (14) Details of the posterior part of the mature spore; posterior vacuole contains electron-dense granular body (posterosome). (15) A spore with a notably thick endospore inside a parasitophorous vacuole. (16) A spore inside a parasitophorous vacuole (pv) with mitochondria (mi) attached to its wall. Scale bars: 200 nm (12, 14); 500 nm (11); 1 μm (13, 15, 16).

4. Discussion

As far as we know, it is the first recorded case of alternative development of the microsporidium either with or without a parasitophorous vacuole, within the same host organism and even in the same tissue. Sporogonial stages and spores in oocytes were surrounded by membranes studded with ribosomes (Fig. 15). Parasite stages in tropharia were also surrounded by membranes of parasitophorous-like vesicles, though spores lay free in the cytoplasm. In contrast, parasitophorous-like vesicles were never observed if sporogony occurred inside somatic cells or eggs (Figs. 10 and 13). This phenomenon may be explained by the existence of two alternative sporogonic sequences, one in direct contact with host cytoplasm and the other in a parasitophorous vesicle. We presume though that the vacuoles may form due to specific defense reaction of certain host cell types against the infection. Encrustation of the PV membrane with ribosomes suggests host origin of this interfacial envelope (References).

The microsporidium studied in *L. lentus* is monokaryotic and disporoblastic. It is differentiated from microsporidia of the genera *Encephalitozoon*, *Endoreticulatus* and *Septata* by the absence of a persistent vacuolar membrane between developmental stages and the host cell cytoplasm. Ultrastructurally the novel microsporidium resembled genera *Unikaryon*, *Oligosporidium*, *Orthosomella*, *Canningia*, and *Larssoniella*. All these genera have unpaired nuclei in all stages and were originally described as developing in direct contact with the host cell cytoplasm (Canning et al., 1983). However, the presence of sporophorous vesicles is mentioned for the

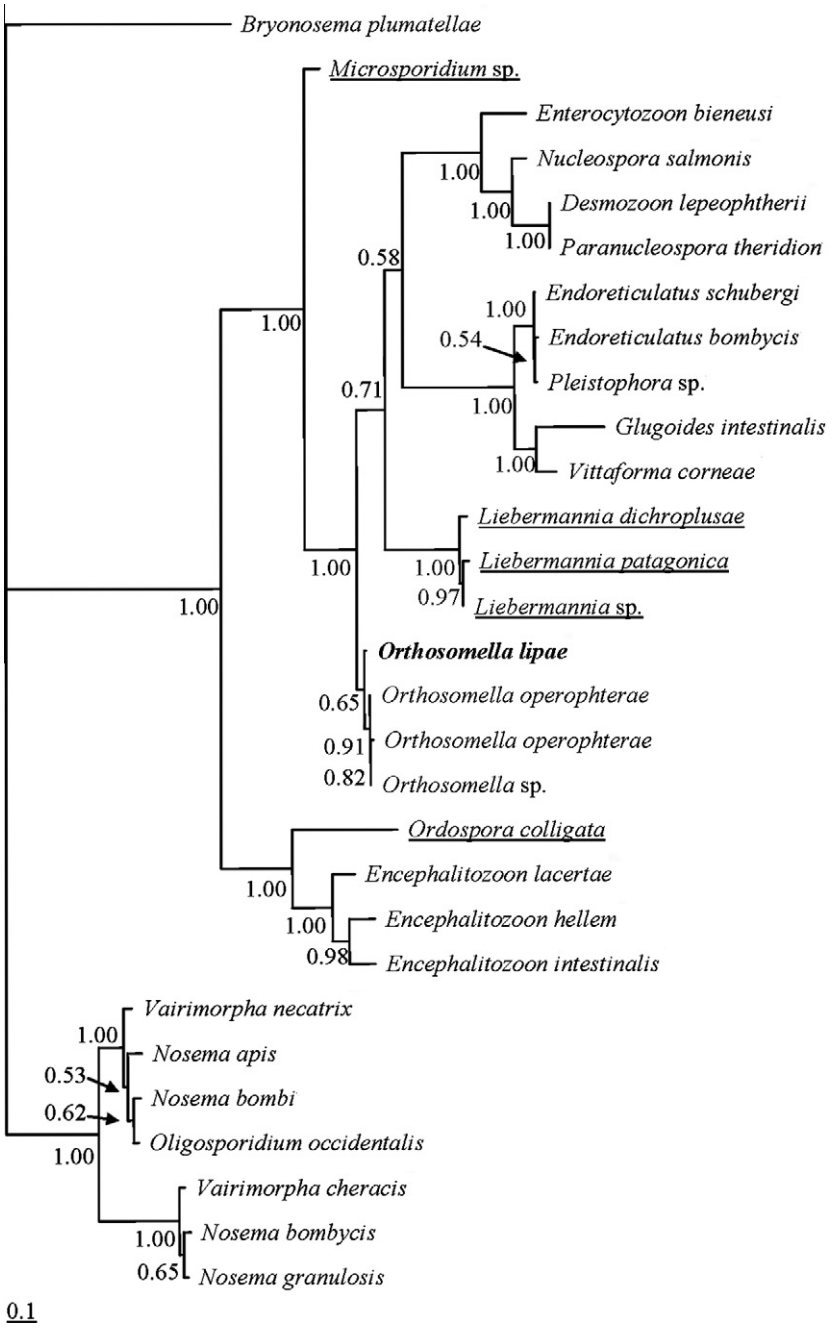


Fig. 17. Bayesian consensus tree inferred from SSUrRNA sequences of 29 microsporidia. *Bryonosema plumatella* is used as an out group. Nodal support is given as Bayesian posterior probability. The new species from *L. lentus*, is indicated in bold.

Table 3
DNA distance matrix for *Orthosomella lipae* and other microsporidian genera. Genetic distances were calculated using the Kimura 2-parameter model, instantiated in MEGA.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. *Orthosomella lipae*																			
2. *Orthosomella operophterae*	0.01																		
3. *Orthosomella operophterae*	0.02	0.01																	
4. *Orthosomella* sp.	0.01	0.00	0.01																
5. *Liebermannia dichroplusae*	0.18	0.17	0.18	0.17															
6. *Liebermannia* sp.	0.18	0.17	0.18	0.17	0.02														
7. *Liebermannia patagonica*	0.19	0.19	0.19	0.18	0.03	0.01													
8. *Endoreticulatus bombycis*	0.23	0.22	0.23	0.22	0.28	0.29	0.30												
9. *Endoreticulatus schubergi*	0.23	0.22	0.23	0.22	0.28	0.29	0.30	0.00											
10. *Pleistophora* sp.	0.23	0.22	0.22	0.22	0.28	0.28	0.30	0.00	0.00										
11. *Encephalitozoon lacertae*	0.33	0.32	0.33	0.33	0.41	0.40	0.42	0.39	0.39	0.39									
12. *Encephalitozoon hellem*	0.39	0.38	0.37	0.38	0.45	0.44	0.46	0.43	0.42	0.43	0.10								
13. *Encephalitozoon intestinalis*	0.35	0.34	0.34	0.35	0.41	0.42	0.44	0.40	0.40	0.39	0.11	0.09							
14. *Vairimorpha necatrix*	0.52	0.52	0.51	0.51	0.59	0.57	0.59	0.51	0.51	0.51	0.44	0.39	0.38						
15. *Nosema apis*	0.51	0.51	0.50	0.50	0.59	0.58	0.60	0.51	0.51	0.51	0.44	0.40	0.39	0.03					
16. *Nosema bombi*	0.49	0.48	0.47	0.47	0.56	0.55	0.57	0.50	0.50	0.50	0.44	0.38	0.38	0.03	0.04				
17. *Oligosporidium occidentalis*	0.51	0.50	0.50	0.50	0.60	0.59	0.61	0.51	0.51	0.51	0.43	0.38	0.38	0.02	0.03	0.02			
18. *Vairimorpha cheracis*	0.61	0.61	0.60	0.60	0.68	0.66	0.68	0.61	0.61	0.60	0.51	0.45	0.45	0.14	0.15	0.14	0.14		
19. *Nosema bombycis*	0.61	0.60	0.59	0.59	0.68	0.66	0.67	0.60	0.60	0.60	0.51	0.45	0.44	0.14	0.15	0.14	0.14	0.02	
20. *Nosema granulosis*	0.60	0.59	0.58	0.58	0.68	0.66	0.68	0.62	0.62	0.61	0.51	0.45	0.46	0.14	0.15	0.14	0.14	0.02	0.02

type species *Unikaryon legeri* (Azevedo and Canning, 1987). Mentioned genera have disporoblastic sporogony, with the exception of *Orthosomella*, in which sporogony is polysporoblastic (Canning, 1960; Canning et al., 1991; Andreadis et al., 1996).

Following traditional taxonomy, the developmental and morphological features of the microsporidium from *L. lentus*, support its placement either into the genus *Unikaryon* or into *Oligosporidium*. No molecular data is currently available for any *Unikaryon* species. However, this genus has an extremely wide host range from trematodes (type host) to chrysomelid beetles, which suggests that it is polyphyletic. SSU-rDNA-based phylogenies indicate that the family Unikaryonidae, to which *Unikaryon* belongs, is definitely polyphyletic, containing the distantly related genera *Microgemma*, *Orthosomella* or *Encephalitozoon* (Vossbrinck and Debrunner-Vossbrinck, 2005).

A single 16S rDNA sequence is available for the species *Oligosporidium occidentalis* (AF495379). However, this sequence is only 68% similar to the sequence obtained from the microsporidium from *L. lentus* (Table 2). Furthermore, previous molecular phylogenetic analysis, confirmed by our molecular analysis (Fig. 17), placed *O. occidentalis* within the *Nosema*/*Vairimorpha* clade (Becnel et al., 2002). This clade contains species with very different morphological and life history characters in comparison to *O. occidentalis*. The conflict between molecular phylogeny and traditional taxonomy led Becnel et al. (2002) to question the placement of *Oligosporidium* within the Unikaryonidae.

The level of sequence divergence between the new species and the *Orthosomella* species does not exceed the norm for congeneric microsporidian species. From the DNA sequences listed in Table 2, similar iterspecific distances were calculated for the genera *Orthosomella* (0.00–0.01), *Endoreticulatus* (0.00) and *Liebermania* (0.01–0.03) while substantially greater DNA distances occur within the genera *Encephalitozoon* (0.09–0.11) and *Nosema/Vairimorpha* (0.02–0.15). The molecular data therefore provide no support for the exclusion of the new microsporidium from the genus *Orthosomella*.

All currently described members of the genus *Orthosomella* are parasites of Lepidoptera (Canning, 1960; Canning et al., 1991; Andreadis et al., 1996). However, an isolate from the gastropod *Arion subfuscus* (AM259659) was also identified as *Orthosomella* sp. by molecular analysis, suggesting potential host plasticity of *the Orthosomella* spp. In fact, typical lepidopteran hosts of *Orthosomella* spp., weevils *Liophloeus lentus*, the hosts of the novel species, and slugs *Arion subfuscus*, the hosts of putative *Orthosomella* sp. AM259659 share similar habitat, facilitating the host switch in the course of microsporidian evolution.

Orthosomella spp. have unpaired nuclei during schizogony and sporogony, develop in direct contact with the host cell cytoplasm, and produce multinucleate, sausage-shaped sporogonial plasmodia with linearly arranged nuclei giving rise to moniliform chains of unikaryotic sporoblasts and free spores (Andreadis et al., 1996). In contrast, the weevil microsporidium produces sporonts with paired nuclei and monomorphic spores through disporoblastic sporogony. Such variations prove once again that the nuclear phase, and type of sporogony (polysporoblastic vs. disporoblastic) can vary in closely related species as it has been previously demonstrated for *Liebermannia* spp. (Sokolova et al., 2009).

5. Taxonomic summary

Amended diagnosis of the genus *Orthosomella* Canning, 1960 (Canning, 1960; Canning et al., 1991; Andreadis et al., 1996).

All stages of schizogony and sporogony with unpaired nuclei.

Development free within the hyaloplasm of the host cell. *Sporogony may occur in direct contact with the host cell cytoplasm or within host cell-derived vacuoles*. Sporogonial plasmodia *binucleate* or multinucleate, sausage-shaped, with linearly arranged nuclei. Plasmodia give rise to moniliform chains of unikaryotic sporoblasts and free spores.

Orthosomella lipae n. sp.

Diagnosis. Monokaryotic meronts develop in direct contact with the host cell cytoplasm without interfacial envelopes. Only one sporulation sequence is known. Monokaryotic sporonts undergo nuclear division to produce sausage-like sporogonial stages with two nuclei. Two uninucleate spores are produced as the result of cytokinesis of this stage. Depending upon the host cell type, sporogony may occur in direct contact with the host cell cytoplasm or within host cell-derived vacuoles. Spores are rod shaped, thick walled with equally rounded ends. Spores measure, 4.2 ± 0.4 $(3.4–5.2) \times 1.6 \pm 0.2$ $(1.2–2.0)$ μm (live). Spore wall is composed of 150–180 nm wide endospore and double layered exospore 40–50 nm thick. Lamellar polaroplast occupies the anterior third of the spore. The isofilar polar filament is arranged in a single row of 12–14 rings.

Site of host infection and pathology. Reproductive system of adult females: ovarioles, oocytes, tropharia, somatic tissues, eggs. No detectable pathological differences in the ovariole morphology be-

tween infected and non-infected specimens of *L. lentus* were observed.

Transmission. Transmission unknown, probably via ova.

Type host. *Liophloeus lentus* Germar 1824 (Coleoptera, Curculionidae)

Type locality. Riparian meadow in the Gorce mountains in southern Poland (49°34.943′N 20°13.291′E).

Type material. A syntype slides and TEM blocks have been stored in the collection of senior author, Witold Stefański Institute of Parasitology PAN, Warszawa, Twarda 51/55, Poland 00–818. Syntype material, embedded in TEM blocks are also in collection of Piotr Świątek, Silesian University Katowice, Poland.

Gene sequences. The SSU r DNA sequence of the novel microsporidium has been deposited in the GenBank database under Accession No JF960137.

Etymology. The new species is named in respect of the contribution of Prof. Jerzy Jeremi Lipa to the study of microsporidian parasites in Poland.

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