Parasitology Research (2020) 119:915–923
https://doi.org/10.1007/s00436-020-06611-9

GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Ultrastructure, molecular phylogeny, and prevalence rates of *Alternosema bostrichidis* gen. nov. sp. nov. (Microsporidia, Terresporidia), a parasite of *Prostephanus truncatus* and *Dinoderus* spp. (Coleoptera, Bostrichidae)

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Received: 11 September 2019 / Accepted: 15 January 2020 / Published online: 22 January 2020

Abstract
A new species and a new genus of a microsporidium *Alternosema bostrichidis* isolated from an adult *Prostephanus truncatus* in Mexico and from three species of the genus *Dinoderus* in Nigeria are described. The microsporidium is monomorphic, monoxenic, and develops in direct contact with host cell cytoplasm. The infection first appears with thoracic muscles, followed by a generalized invasion of the host. All developmental stages are diplokaryotic. Sporogony is disporoblastic. Mature spores are ovoid. Unfixed spores measure 3.7–4.2 × 2.0–2.6 μm, fixed and stained spores 3.5–5.0 × 2.4–2.8 μm. The polaroplast consists of dense lamellae and rare lamellae. The polar tube is slightly anisofilar, consisting of 11–17 coils, with 9–14 proximal (130 nm in diameter) and 2–3 distal coils (120 nm in diameter) arranged in one layer. Molecular phylogenetic analysis based upon a short portion of small-subunit ribosomal RNA gene (Genbank accession # KP455651) placed the new microsporidium within *Liebermannia-Orthosomella* lineage, which contains multiple undescribed parasites. In particular, *A. bostrichidis* showed maximal sequence similarity of 95% to *Microsporidium* sp. BBRE2 (# FJ755987) from Baikalian *Diplacanthus brevispinus* (Amphipoda: Acanthogammaridae) and *Microsporidium* sp. Comp CD Van 2 (# KC111784) from compost and soil in Canada. Frequent, devastating epizootics of laboratory cultures of *A. bostrichidis* support its potential as a biological control agent of grain borers.

Keywords *Alternosema bostrichidis* · Microsporidia · *Prostephanus truncatus* · Bostrichidae · Ultrastructure · Molecular phylogenetics

Introduction

Beetles of the family Bostrichidae are well known as pests of stored food products, and they are also destructive to wood constructions. The large grain borer, *Prostephanus truncatus* has recently become of great concern in Africa. Earlier this species was only known as a minor pest in North, Central, and South America. After being accidentally introduced into Africa, in short time it became a very serious pest in the East and West African countries, spreading quickly from one region to another (Hodges et al. 1983). Due to economical, technical, and sociological reasons, *P. truncatus* cannot be controlled by intensive chemical methods in African rural areas. Therefore effective biological control programs, initiated by governmental or international agencies, and based on introduced pathogens, predators, and parasites, would be the most attractive and suitable way to control this beetle.

Microsporidia are among the most widely occurring pathogens of insects and are referred to as potential biocontrol agents. Their biodiversity, however, is still underestimated, as becoming increasingly evident from molecular phylogenetic and metagenomics/transcriptomics studies (Ardila-Garcia et al. 2013; Johansson et al. 2013; de Carvalho et al. 2014). Elucidation of phylogenic relationships is of great importance not only for correct species description and identification but also for estimation of microsporidia biohazard, as certain

Section Editor: Yaoyu Feng

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phylogenetic lineages demonstrate ability to switch from insect to human hosts (Franzen et al. 2006; Choudhary et al. 2011; Meissner et al. 2012).

The majority of microsporidia which parasitize beetles have been attributed to the genus *Nosema* Naegeli. However, recent studies revealed polyphyletic nature of this taxon, and it has been split into several new genera (Issi et al. 1993; Silveira and Canning 1995; Canning et al. 2002; Sokolova et al. 2003; Franzen et al. 2005, 2006). Differences in ultrastructural features (first of all, in exospore and extrusion apparatus) of these taxa corresponded well to molecular phylogenetics, showing distant placing of *Pseudonosema (Nosema) cristatellae, Paranosema (Nosema) locustae, Tubulinosema (Nosema) kingi, Vittaforma corneae (Nosema corneum)*, and *Anncaliia (Brachiola, Nosema) meligethi* in relation to *Nosema bombycis* which is the type species of the "true *Nosema*" sensu Vossbrinck and Debrunner-Vossbrinck (2005). It stresses the necessity of careful examination of *Nosema*-like species of microsporidia using modern approaches of ultrastructural and molecular analysis to attain the modern taxonomic criteria.

In the present paper, we describe a new microsporidium, *Alternosema bostrichidis* gen. nov., sp. nov., which is a potential agent for the biological control of *P. truncatus* and *Dinoderus* spp.

Material and methods

The adult beetles *P. truncatus, Dinoderus bifoveolatus, Dinoderus minutus,* and *Dinoderus porcellus* were collected during 1985 to 1989 from granaries or under field conditions in Europe, Africa, and North America (Table 1). Collected insects were examined using light microscopy. Permanent squash preparations were air-dried and fixed with methanol for 2–5 min or (b) Bouin-Duboscq-Brasil (BDB) solution for 1–2 h and consequently stained with Giemsa, methylene blue or Heidenhain's iron hematoxylin, respectively. For paraffin sectioning, the cuticle was dissected and removed, and the internal tissues were fixed with BDB overnight. Then the tissue samples were washed with distilled water and dehydrated in ascending ethanol series, cleared with butanol and embedded in Paraplast (Lancer St. Louis, MO, USA). Sections were cut at 10 μm and stained with hematoxylin in combination with a modification of the polychromatic staining (Vetterling and Thompson 1972). Measurements were made with an eyepiece micrometer at ×1000 magnification.

The description of ultrastructural features of this microsporidium is based upon micrographs kindly provided by Prof. R.I. Ronny Larsson. For transmission electron microscopy, beetles with the cuticle removed were cut into halves, fixed, embedded, sectioned, stained routinely (Reynolds 1963) and viewed using EM at accelerating voltage of 80 mV. As the first set of fixations did not preserve all spores in a good shape a second attempt was made several years later. The spores from dead beetles stored at 4 °C for 11 years were fixed in 4% formaldehyde solution at 60 °C for 2 h, washed in cacodylate buffer and further handled identically to the samples of fresh tissue.

For the molecular phylogenetic studies, a subsample of 10 heavily infected adult *P. truncatus* beetles from Mexico were crushed with a plastic pestle in an Eppendorf tube with 100 μl of lysis buffer containing 2% CTAB, 1.4 M NaCl, 100 mM EDTA, and 100 mM Tris-Cl (pH 8.0). Then 500 μl of the lysis buffer, as above with addition of 0.2% β-mercaptoethanol and 10 μl proteinase K (20 mg mL^{-1}), were added, and the sample was incubated at 65 °C for 3 h. DNA was extracted routinely with phenol-chloroform followed by isopropanol precipitation and ethanol washing (Sambrook et al. 1989) and resuspended in 50 μl UHQ water. PCR was run using a Bio-Rad iCycler in 20 μl volume containing 5 μl DNA template; PCR buffer; dNTPs, 0.25 mM; Taq-polymerase (Sileks, Russia), 1 U; forward and reverse primers (Evrogen, Russia), 1 pMol each. The PCR program included (i) a first cycle of denaturation at 92 °C for 3 min, (ii) 30 cycles of denaturation at 92 °C for 30 s, annealing at 54 °C for 30 s and elongation at 72 °C for 30–60 s (depending upon the expected size of the product), and (iii) a last cycle of extension at 72 °C for 10 min. To amplify the small subunit (SSU) ribosomal RNA gene of microsporidia, the following combinations of the universal microsporidia primers were tested: 18f:530r, 18f:1492r, 1061f:1492r, and 1061f:ls580r (Weiss and Vossbrinck 1999). However, only the 18f:530r primers were successful in obtaining a positive PCR signal. The amplified band of *ca* 450 bp was gel purified and cloned into pAL-TA vector (Evrogen, Russia). The resulting plasmid, purified with phenol-chloroform, was sequenced in both directions using M13F and M13R primers. After obtaining the first rRNA gene sequence, a subsample of 10 adult *D. bifoveolatus* beetles from Nigeria was used to repeat the whole experimental cycle from DNA extraction to sequence analysis with the identical results.

Newly obtained sequence, submitted to Genbank under accession # KP455651, was compared to those available in NCBI using the built-in BLAST utility (www.ncbi.nlm.nih.gov/Blast.cgi). The alignment of the new sequence with those showing significant homology (Table 1) was done automatically using CLUSTAL W algorithm and edited by eye in BioEdit v7.0.8.0 (Hall 1999). Regions containing gaps and ambiguous sites were removed, leaving an alignment of 326 bp length. Phylogenetic reconstructions were carried out with Bayesian Inference (BI) using MrBayes v3.1.2 under GTR + I + G evolutionary model (Ronquist and Huelsenbeck 2003). MrBayes was run for 100,000 generations, and every 100th generation was sampled. The first 25% of samples was

Table 1 Laboratory populations of *Prostephanus truncatus* and *Dinoderus* spp. examined for microsporidia infection

Insect species, sampling date, location	Origin of population	Number of insects	
		Examined	Infected (%)
Dinoderus bifoveolatus, 26.02.1985, Institute of Stored Products Protection BBA, Berlin, Germany	Unknown	26	16 (62%)
Dinoderus minutus, 6.10.1986, Department of Biological Sciences Rivers State University, Port Harcourt, Nigeria	Corn from storehouse	11	5 (45%)
Dinoderus porcellus, 6–7.10.1986, Nigerian Stored Products Research Institute, Port Harcourt Substation, Nigeria	Yam chips from market	20	2 (10%)
Prostephanus truncatus, 5.07.1989, Instituto de Biologia UNAM, Mexico City, Mexico	CIMMYT	60	60 (100%)
P. truncatus, 7.07.1989, International Center of Improvement of Maize and Wheat (CIMMYT), El Batan, Mexico	Collected in field and from granary in 1987–1989	120	80 (67%)
P. truncatus, 10.11.1989, Center for Research and Advanced Studies, Irapuato, Mexico	From granary	41	34 (83%)

discarded as burn-in, parameter values were summarized, and a consensus tree was constructed. Standard deviation of split frequencies, which estimates the precision of the clade probabilities, reached 0.009 after 100,000 generations.

Results

Host pathology and infection prevalence

Microsporidian infections were detected in *P. truncatus* with provenance from Mexico and in three species of *Dinoderus* from Nigeria (Table 1). No externally visible signs of infection could be noticed in infected adults of *P. truncatus* and *Dinoderus* spp. In dissected specimens, however, the infection was obvious, visible as white strands in the thoracic muscles. The microscopic examination revealed infection of multiple tissues (generalized invasion), being prominent in the fat body, thoracic muscles, gut epithelium, Malpighian tubules, testes, seminal ducts, and ovaries. The infected tissues were hypertrophied and filled with the parasite (Fig. 1A, B). The prevalence rates usually exceeded 50% and sometimes reached 100% (Table 1).

Laboratory rearing *of D. bifoveolatus* started at the Laboratory of Insect Pathology in Poznan from insects received from Prof. R. Wohlgemuth (Berlin) collapsed due to 100% infection. Rearings of *P. truncatus*, kept at the Instituto de Biologia UNAM, collapsed in 1989 (Dr. Julietta Ramos, personal communication). Dead specimens were 100% infected with this microsporidium.

Light microscopy

The semithin sections of fat body and Malpighian tubules showed presence of multiple intensively stained spores interlaced with faintly stained round zones of larger size, corresponding to the presporogonic developmental stages of the parasite (Fig. 1A, B). Mature spores were ovoid with one or both poles blunt (Fig. 1C–E). A small proportion (*ca* 1–2%) of abnormally large spores were also observed. Unfixed spores harvested from *P. truncatus* measured 3.7–4.2 × 2.0–2.6 μm. Fixed and stained spores measured 3.5–5.0 × 2.4–2.8 μm, mean size was 3.82 × 2.52 μm. The size range and mean measurements of the parasite spores were similar in the four hosts (Table 2).

Electron microscopy

All developmental stages of parasite were in direct contact with the host cell cytoplasm. Diplokaryotic meronts and sporonts and rarely sporoblasts were present on the ultrathin sections (Fig. 2). Early meront was a rounded cell with a large diplokaryon measuring up to 2.1 μm in diameter. Its electron-dense cytoplasm contained numerous free ribosomes and Golgi complex structures and was devoid of visible endoplasmic reticulum (ER) (Fig. 2A). Late meronts were irregularly rounded cells, their cytoplasm being considerably less electron dense (Fig. 2B–D). Diplokarya of late meronts measured up to 3.4 μm in diameter. The process of nuclear division was indicated by the presence of centriolar plaques, up to 280 nm wide, on the external side of the nuclear membrane (Fig. 2C). The meronts with two diplokarya were also observed (Fig. 2D).

The onset of sporogony was associated with formation of a discontinuous electron-dense layer, about 30 nm thick, on the surface of meront/sporont transitional stage (Fig. 2E). In sporont, this layer, which is the primordium of the exospore, became continuous. During the sporogony, distinct strands of rough ER were formed, and in mature sporont, they surrounded the nucleus. The shape of the sporont cell became

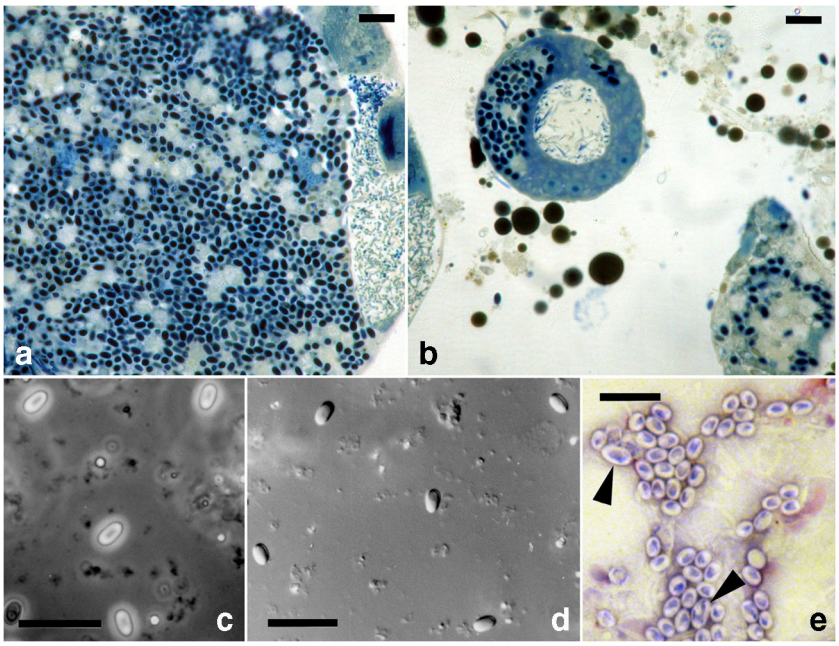

Fig. 1 Light microscopy of *Alternosema bostrichidis* gen. nov., sp. nov. from *Prostephanus truncatus*. A, B – semithin sections of testis (A) and seminal duct (B) filled with prospore stages and spores, stained with methylene blue. C, D – fresh spores under phase contrast (C) and Nomarski contrast (D). E – mature spores on a Giemsa-stained smear. Scale bar = 10 μm

irregular with protrusions resembling amoeboid pseudopodia (Fig. 2F).

The young spore possessed all structures typical of the microsporidia spores, including thick endospore and complex exospore (see below), anchoring disc, developed bipartite polaroplast, and polar tube with several proximal coils arranged in 1–2 layers on lateral sides of the spore and one distal coil of lesser diameter laying freely in the cytoplasm, closer to the nucleus surrounded with 2–3 layers of ER (Fig. 3A).

The glutaraldehyde fixation used on fresh spores was not always successful for mature spores with very thick endospore, and longitudinally sectioned, perfectly fixed spores were not obtained. However, the main details of the ultrastructural cytology of the spore could be visualized. The second attempt using formaldehyde was more successful and no visible signs of destruction were observed. The spore wall was 250–280 nm thick, with a thinning over the anchoring disc (Fig. 3B, C). There were three layers of the spore wall: (a) internal unit membrane, up to 9 nm thick, (b) electron-translucent endospore, 190–220 nm thick (and up to 30 nm over the anchoring disc), and (c) the two-layered exospore, 49–55 nm thick (Fig. 3 C–F). In the mature spore, two subdivisions of the exospore were visible: a basal, 26–32 nm wide, prominently electron-dense layer with indistinct border to the endospore and the two-layered membrane associated with tubular structures located close to the spore surface (Fig. 3 D–F).

Table 2 Spore measurements of *Alternosema bostrichidis* gen. nov. sp. nov. (fixed and stained) from four different hosts (number of spores measured $n = 50$)

Host	Length × width, μm	
	Range	Mean
Dinoderus bifoveolatus	3.00–4.80 × 2.16–2.84	3.78 × 2.52
Dinoderus minutus	3.30–4.85 × 2.20–2.80	3.78 × 2.48
Dinoderus porcellus	3.60–4.72 × 2.40–2.52	3.94 × 2.52
Prostephanus truncatus	3.48–5.04 × 2.40–2.76	3.82 × 2.52

The polar tube is slightly anisofilar with 11–12 coils, 9–10 proximal (130 nm in diameter), and 2–3 distal coils (120 nm in diameter) arranged in one layer close to the spore wall. The ultrastructure of the 2–3 most posterior coils often differed slightly from the rest of the coils (Fig. 3E, F). The angle of tilt of the anterior coil to the long axis of the spore was about 55^0. The transversely sectioned coils exhibited layers of variable thickness and electron density (Fig. 3C, F).

The polaroplast occupied a lateral side of the anterior part of the spore (Fig. 3B, D). Its anterior and posterior parts consisted of thin lamellae and thick lamellae interlaced with layers of electron-dense material, respectively (Fig. 3D). The polaroplast is enclosed within the membrane of the distal portion of the polar sac (Fig. 3A, D). The anchoring disc was almost semicircular, up to 360 nm wide, composed of layers of varying electron density (Fig.3A). The diplokaryotic nuclear apparatus occupied the center of the spore (Fig. 3B). Maximum length of the nuclear contact zone reached 1.5 μm. The cytoplasm was fairly electron-dense and granular. Strands of polyribosomes surrounded the diplokaryon (Fig. 3B).

On the outer spore surface, an additional layer of electron-dense material was seen forming irregular protrusions into the host cell cytoplasm in the form of small clumps (Fig. 3D, E).

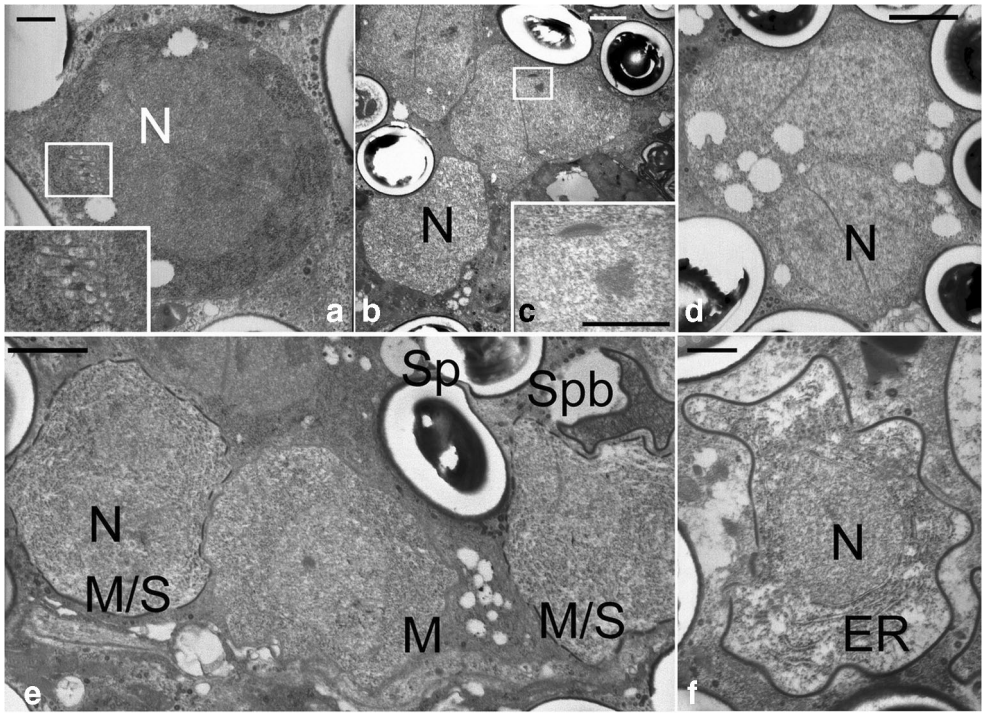

Fig. 2 Transmission electron microscopy of prespore developmental stages of *Alternosema bostrichidis* gen. nov. sp. nov. A – diplokaryotic meront of first merogony with electron-dense cytoplasm and Golgi complex structures. B – meronts of second merogony with less dense cytoplasm and nuclei with centriolar placues. C – an enlarged detail of Fig. 2B showing a centriolar placue on the nuclear membrane and a nucleolus. D – dividing late meront with two diplokarya. E – meront/sporont transitional stage. F – late sporont. ER – endoplasmatic reticulum, M – meront, M/S – meront/sporont transitional stage, N – nucleus, Sp – spore, Spb – sporoblast. Scale bar = 1 μm (A, B, D-F), 0.5 μm (C). Based upon images kindly provided by Prof. R.I. Ronny Larsson

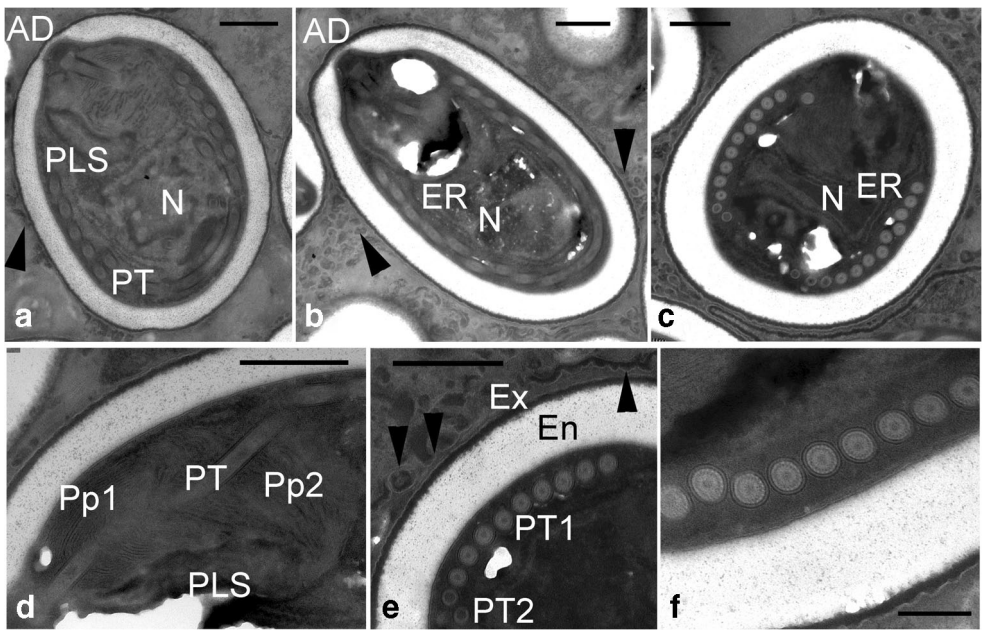

Fig. 3 Transmission electron microscopy of spores of *Alternosema bostrichidis* gen. nov. sp. nov. A – young spore. B, C – mature spores, showing general view (B) and arrangement of polar tube coils (C). D – the polaroplast. E, F – the polar tube coils and the spore wall structure. AD – anchoring disc, En – endospore, ER – endoplasmatic reticulum, Ex – exospore, N – nucleus, Pp1 and Pp2 – anterior and posterior parts polaroplast, respectively; PT – polar tube, PT1 and PT2 – anterior and posterior coils of polar tube, respectively. Arrowheads – additional layer of irregular protrusions associated with the spore surface. Scale bar = 0.5 μm (A–E), 0.2 μm (F). Based upon images kindly provided by Prof. R.I. Ronny Larsson

Molecular phylogeny

Though only a short portion of SSU rRNA gene could be amplified using DNA from a sample of long-term stored dry beetles, the resulted sequences from two independent experimental cycles were identical to each other, thus excluding the possibility of an artifact due to contamination. Analysis of the 396 bp long sequence (Genbank accession # KP455651) showed placing of the new microsporidium within Clade IV, Class Terresporidia sensu Vossbrinck and Debrunner-Vossbrinck (2005), with maximal similarity of 95% to the two environmental sequences of *Microsporidium* sp. BBRE2 (# FJ755987) from Baikalian *Diplacanthus brevispinus* (Amphipoda: Acanthogammaridae) and *Microsporidium* sp. Comp CD Van 2 (# KC111784) from compost and soil in Canada. The closest relatives among described species of microsporidia included *Orthosomella lipae* (#JF960137) from *Liophloeus lentus* (Coleoptera: Curculionidae), *Orthosomella operophterae* (# AJ302317) from *Operophtera brumata* (Lepidoptera: Noctuidae), and *Liebermannia dichroplusiae* (# EF016249) from *Dichroplus elongatus* (Orthoptera: Acrididae), showing maximal sequence similarity to the parasite from *P. truncatus* of 83.3%, 82.2% and 74.3%, respectively. A phylogenetic reconstruction using representatives of the five major clades of Microsporidia "Tree of Life" showed placement of *Liebermannia-Orthosomella* lineage within the Clade IV (Fig. 4A), which is consistent with the previous studies (Vossbrinck and Debrunner-Vossbrinck 2005; Vossbrinck et al. 2014). In a more detailed phylogenetic tree (Fig. 4B) where *L. dichroplusiae* was used as an outgroup, the Genbank entries of closer relatedness were grouped into two sister groups. One of these groups included *Microsporidium* sp. Comp C Van1 from compost in Canada at the basal position and two further subgroups in sister position to each other, each including one species of *Orthosomella. O. operophtherae* was grouped with *Microsporidium* sp. BLAT8 from *Brandtia latissima latior* (Amphipoda: Acanthogammaridae), while *Orthosomella lipae* was closely related to and tightly grouped with three undescribed microsporidia from *Dorogostaiskia parasitica* (Amphipoda: Acanthogammaridae) in lake Baikal, Russia, *Arion subfuscus* (Pulmonata: Arionidae) and invasive *Crangonyx pseudogracilis* (Amphipoda: Crangonyctidae) in UK, respectively. The other group was also composed of undescribed microsporidia from Baikalian amphipods and Canadian compost and soil, forming a polytomic base, which included a branch containing the novel microsporidium from *P. truncatus* and its closest relatives, mentioned above (Fig. 4B).

Discussion

DNA is degraded in archive samples, constraining the capacity of molecular phylogenetic studies. However, short fragments of SSU rRNA of Microsporidia, especially in spore samples, are still available for amplification (Hylis et al. 2005; Malysh et al. 2019). These data are of great importance for matching the novel data and those accumulated previously (Kleespies et al. 2003; Tokarev et al. 2015).

Numerous species of microsporidia described from beetles were primarily allocated to the genus *Nosema.* However, only few isolates of beetle microsporidia examined with molecular phylogenetic tools showed affinity to the "true *Nosema*" clade, which predominately incorporates species parasitizing Lepidoptera, including the type species *Nosema bombycis* from *Bombyx mori*. Other species of *Nosema*-like microsporidia from beetles showed ribosomal RNA gene divergence, placing them either in sister position to the "true *Nosema*" clade in the *Vairimorpha* lineage (*Nosema oulemae*) or distantly within the genera *Anncaliia* (*Nosema meligethi*) and *Paranosema* (*Nosema whitei, Nosema oryzaephili*). Detailed ultrastructural studies were in accordance with these molecular data, showing some distinctive features of these former *Nosema* species (Sokolova et al. 2005; Franzen et al. 2006). Basing upon light microscopy only, the microsporidium examined herein possesses features corresponding the diagnosis of the genus *Nosema* as well. However, fine structure of the cell membrane of its meront/sporont transitional stage and, most notably, spore ultrastructure are remarkably different from those of *Nosema* species.

The other known genera of microsporidia, characterized by *Nosema*-like spores, tubular structures on the surface of developmental stages, and a slightly anisofilar polar tube, are *Tubulinosema* (Franzen et al. 2005) and *Anncaliia* (Issi et al. 1993; Franzen et al. 2006), both belonging to the family Tubulinosematidae in the Clade V, Class Aquasporidia of the molecular phylogenetic system of Vossbrinck and Debrunner-Vossbrinck (2005). The microsporidium described here, however, is distant from *Nosema, Tubulinosema* and *Anncaliia* as evidenced from molecular phylogenetic study (Fig. 4A). The new genus and species, *Alternosema bostrichidis,* is thus established. Its close relatedness to an undescribed microsporidium from the Baikalian amphipod *Diplacanthus brevispinus* cannot be fruitfully discussed as any descriptive morphological and ultrastructural data are absent for the latter parasite. At least, distant relatedness to genera-like *Tubulinosema* and *Anncaliia* indirectly indicates lower risk of vertebrate infections for this insect parasite.

Although natural populations and laboratory cultures of *P. truncatus* have been surveyed intensively for pathogens, so far only two pathogenic microorganisms have been discovered: a neogregarine of the genus *Mattesia* (Lipa, Wohlgemuth 1986) and the microsporidium *A. bostrichidis* (this paper). Epizootics causing total devastation of pest cultures due to infection with *A. bostrichidis* are frequent, likely resulting from often contacts between insect hosts dwelling in

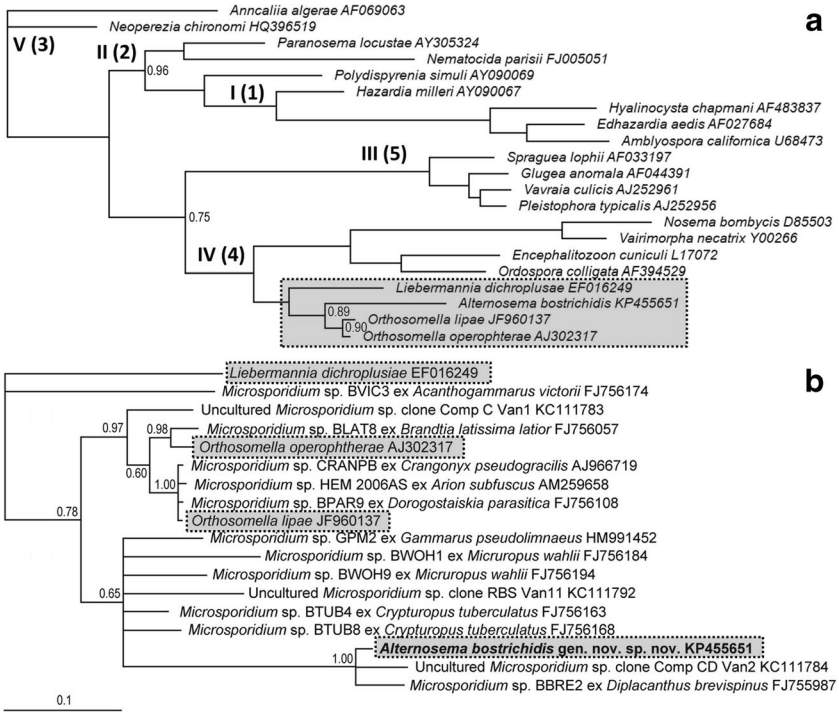

Fig. 4 Molecular phylogenetics of *Alternosema bostrichidis* gen. nov. sp. nov. (in bold) and related taxa, as obtained by Bayesian inference from an alignment of respective ribosomal RNA gene sequences. Taxa are annotatedwith Genbank accession numbers. A – position of *Liebermannia-Orthosomella* lineage (gray rectangle with dotted contour) within the Microsporidia "Tree of Life". Clades are indicated as Arabic (Vossbrinck et al. 2014) and Roman numerals (Vossbrinck and Debrunner-Vossbrinck 2005). Branch support (Bayesian posterior probability) are indicated for clades with values below 1.0. B – Detailed topology of *Liebermannia-Orthosomella* lineage containing the described species (gray rectangles with dotted contour) and undefined isolates of microsporidia (*Microsporidium* sp.), further annotated with isolate names. Scale bar = 0.1 expected nucleotide changes per site

a limited space. It is also probable that *A. bostrichidis* was responsible for the collapse in 1988 of the rearings of *P. truncatus*, kept at the CIMMYT Station in Poza Rica (Dr. J.A. Mihn, personal communication). When the host density is not so high as to provide horizontal dissemination (between the organisms of one generation), we can expect vertical route of transmission (from maternal to filial generations) as the parasite infects ovaries.

This microsporidium is therefore a potential agent for the biological control of *P. truncatus* and *Dinoderus* spp. Further studies are necessary to elaborate mass propagation and application strategies of *A. bostrichidis.*

Diagnosis of Alternosema *gen. nov.*

Type locality: Mexico.

Life cycle: The microsporidium is monoxenous, monomorphic, develops in direct contact with the host cell cytoplasm. Binary fission throughout the life cycle. Two merogonial cycles. The meronts of the first merogony and all the consequent developmental stages are diplokaryotic. The surface of the meront/sporont transitional stage is covered with a thick layer of electron-dense granules – future exospore. Spores are ovoid, with a thick endospore and a two-layered exospore. The latter is composed of granular material and thin electron-translucent structures.

Type host: *Prostephanus truncatus* (Coleoptera, Bostrichidae).

Etymology: Based on *Nosema* with the prefix *Alter-*, implying "other".

Type species: *Alternosema bostrichidis* sp. nov.

Diagnosis of Alternosema bostrichidis *sp. nov.*

Life cycle: The species diagnosis corresponds to that of the genus. Spores are ovoid. Unfixed spores measure 3.7–4.2 × 2.0–2.6 μm, fixed and stained spores 3.5–5.0 × 2.4–2.8 μm (average 3.8 × 2.5 μm). The spore wall is 250–280 nm thick, with a 49–55 nm thick exospore, exhibiting two distinct layers. The anisofilar polar filament arranged in 11–17 (usually 13) 113–122 nm wide coils in one row, The row of coils is approximately half the length of the spore. The angle of tilt is approximately 55^0. The polaroplast is bipartite, the anterior and posterior parts composed of dense lamellae and rare lamellae, respectively. Diplokaryon is in the center of the spore.

Tissue localization: Infection is predominant in the musculature, and frequently seen in gonads of both genders, midgut epithelium, Malpighian tubules vessels, and in the fat body. Infected tissues are hypertrophic without production of syncytia.

Type host: the lesser grain borer *Prostephanus truncatus* (Horn, 1878) (Coleoptera, Bostrichidae).

Additional hosts: *Dinoderus bifoveolatus* Wollaston, 1858, *Dinoderus porcellus* Lesne, 1923, and *Dinoderus minutus* Fabricius, 1775 (Coleoptera, Bostrichidae).

Type locality: Mexico.

Type slides: the syntype slides are deposited at the Collection of Microsporidia, which is a part of the State Collection of Entomopathogenic and Phytopathogenic Microorganisms and their Metabolites affiliated to the All-Russian Institute of Plant Protection RAAS (Podbelsky sh. 3, 196,608 St. Petersburg, Pushkin, Russian Federation) under deposition numbers 931,115-A-2 (Giemsa-stained smears), 931,115-A-4 (hematoxylin-stained smears), and 931,115-A-5 and 931,115-A-6 (methylene blue-stained semithin sections).

Genbank Accession number: KP455651 for partial small subunit rRNA gene sequence.

Etymology: The species name alludes to the previous taxon designation, *Nosema bostrichidis* (Zepeda-Rodriguez and Ibarra 1991), which is considered as *nomen nudum*, conserved for nomenclatural stability.

Acknowledgments Special thanks are expressed to the following persons for help during this study: Dr. Z. D'browski (Ibadan), Prof. R. Kumar and Dr. T. Allotey (Port Harcourt), Prof. Dr. R. Wohlgemuth (Berlin), Dr. Julietta Ramos (Mexico), Dr. J.A. Mihn (El Batan), and Dr. J. Ibarra (Irapuato). The careful technical assistance provided by Mrs. Lina Gefors, Mrs. Birgitta Klefbohm, and Mrs. Inger Norling, all at the Department of Zoology, University of Lund is gratefully acknowledged. The authors are thankful to Prof. J.I. Ronny Larsson for provided electron micrographs and for revision of an early draft of the manuscript.

Author contribution statement JJL processed material and prepared initial draft. YST conducted molecular study and prepared final draft. IVI provided the taxonomic descriptions. All authors read and approved the manuscript.

Funding information The research is supported by Russian Foundation of Basic Research,grant # 17–04-00871.

Compliance with ethical standards

Ethical approval The research is not involving human participants and animals.

Conflict of interests The authors declare that they have no conflict of interests.

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