Parasitology Research (2018) 117:759–766
https://doi.org/10.1007/s00436-018-5749-2

ORIGINAL PAPER

A new microsporidium *Fibrillaspora daphniae* g. n. sp. n. infecting *Daphnia magna* (Crustacea: Cladocera) in Siberia and its taxonomic placing within a new family Fibrillasporidae and new superfamily Tubulinosematoidea (Opisthosporidia: Microsporidia)

Anastasia V. Simakova[1,2] · Yuri S. Tokarev[2] · Irma V. Issi[2]

Received: 5 October 2017 / Accepted: 3 January 2018 / Published online: 10 January 2018

Abstract
Infection with a new microsporidium, *Fibrillaspora daphniae* g. n. sp. n., was found in a local *Daphnia magna* population in Tomsk region (Western Siberia, Russia) at the prevalence rate of 52%. Histological sections showed parasite cells entirely encompassing the host haemocoel. Methanol-fixed spores were elongate, oval, 4.8 ± 0.3 μm × 2.3 ± 0.2 μm in size. All developmental stages were in direct contact with the host cell cytoplasm, with single nuclei, and division by binary fission. The sporont surface was covered with an additional outer layer composed of fine tubules. The spores possessed a thick endospore, large posterior vacuole filled with electron-dense granules, and a bipartite polaroplast composed of anterior lamellar and posterior globular elements. The polar tube was slightly anisofilar, with 13–19 coils arranged in one row; the two posterior coils were of lesser diameter. The small subunit ribosomal RNA gene sequence was deposited in Genbank under accession # MF278272. Considering the sister relationship between *Fibrillanosema crangonycis* and our new isolate described here as *Fibrillaspora daphniae*, we propose a new family Fibrillasporidae fam. n. to contain these two genera and the descendants of their common ancestor. A new superfamily Tubulinosematoidea superfam. n. is proposed as a monophyletic assemblage of Fibrillasporidae fam. n. and Tubulinosematidae.

Keywords Microsporidia · Ultrastructure · Molecular phylogeny · Systematics

Introduction

Water fleas of the genus *Daphnia* are common inhabitants of permanent and temporary bodies of water. They represent one of the major components in the trophic food chain and often highly abundant in lakes and ponds including those of importance to the fish industry. Microsporidia are among the most prevalent parasitic groups of these microcrustaceans. Over 30 species of microsporidia have been described from Cladocera (Green 1974; Vidtmann and Sokolova 1994; Ebert 1995; Larsson et al. 1996; Lukyantsev and Simakova 2014; Refardt et al. 2002; Ebert 2005; Refardt et al. 2008; Weigl et al. 2012; Vavra et al. 2016). These species belong to diverse phylogenetic lineages from two major clades, Aquasporidia (parasites of predominately aquatic hosts) and Terresporidia (primarily parasites of terrestrial but also of aquatic hosts), referred to as classes (Vossbrinck and Debrunner-Vossbrinck 2005). Moreover, a recently discovered *Mitosporidium daphniae* which is placed at the root of the Microsporidia tree of life also develops in *Daphnia* (Haag et al. 2014). Because of the diversity of microsporidia infecting the water fleas, studying their phylogenetic relationships may give new insights into host-parasite ecology and co-evolutionary process of this group. In particular, a close phylogenetic relationship is observed between two species of microsporidia, *Binucleata daphniae* from *Daphnia magna* and *Senoma globulifera* from *Anopheles messae* (Diptera: Culicidae), with 96% sequence similarity of small subunit (SSU) rRNA gene. This suggests the possibility of a complex life cycle involving hosts of both taxa (mosquito and *Daphnia*) in microsporidia belonging to this phylogenetic

✉ Yuri S. Tokarev
jumacro@yahoo.com

[1] Tomsk State University, pr. Lenina 36, Tomsk 634050, Russia

[2] All-Russian Institute for Plant Protection, sh. Podbelskogo 3, St. Petersburg, Pushkin 196608, Russia

lineage (Refardt et al. 2008). Developing a molecular phylogeny for various microsporidian groups is essential for building a reliable taxonomy. While the classification of the microsporidia has been based upon morphological characters over the last 100 years (Weiser 1977; Issi 1986; Sprague et al. 1992), these characters do not provide reliable or consistent information to advance evolutionary relationships for the microsporidia (Vossbrinck et al. 2014).

In the present study, we describe a novel microsporidium infecting *Daphnia* in Western Siberia and propose new taxa of species, genus, family, and superfamily ranks.

Material and methods

Field sampling and light microscopy

Specimens of *Daphnia magna* were collected on May 25, 2014 from a temporary inland pool in the Kuzovlevo settlement, Tomsk region, Western Siberia, Russia (N 56° 34′ 28″, E0 85° 01′ 14″). The pool was an open swampy meadow, with depth of 1 m or less, bottom covered with fallen grass, leaves, and needles, and surrounded by infrequent willow bushes. Dark field microscopy MBS-10 (LOMO, St. Petersburg) was used for morphological analysis of the water fleas and detection of microsporidia-infected specimens. Morphological identification *D. magna* was performed according to the key of Manuilova (1964) and confirmed by molecular genetic methods (see below). The ultrastructural and corroborating molecular investigations were conducted on the same specimen. A single infected *D. magna* was cut in half on a glass slide; one half was fixed with 70% ethanol for molecular genetic analysis, and the other half with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for electron microscopy. For light microscopy analysis, smears of the dissected tissues were made on glass slides, air-dried, and fixed with absolute methanol for 1–2 min followed by Giemsa staining (Voronin and Issi 1974). Examination was performed in bright field (×1200) using an Axiostar Plus microscope (Carl Zeiss, Germany). Dimensions of the stained spores ($n = 25$) were estimated using AxioVision Rel. 1.0 software.

Electron microscopy

For transmission electron microscopy (TEM), infected *D. magna* were fixed for 24 h in a 2.5% glutharaldehyde solution, washed twice with 0.2 M cacodylate buffer (pH 7.4) for 15 min at RT, and postfixed with 1% cacodylate-buffered osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 1 h. Samples were dehydrated in an ascending ethanol series and absolute acetone and embedded in epon-araldite resin (Undeen and Vavra 1997; Larsson, 2005). Ultrathin sections 60–100 nm thick were cut using Ultratome III (LKB, Sweden), stained with 2% uranylacetate in 50% ethanol and lead citrate for 10–20 min (Reynolds 1963), and examined using an JEM-100 CX II electron microscope (JEOL, Japan) at an accelerating voltage of 80 kV. Dimensions of the cell structures were obtained with 30 measurements.

Molecular phylogenetics

The samples were homogenized with a plastic pestle in 100 μl lysis buffer (2% CTAB, 1.4 M NaCl, 100 mM EDTA, 100 mM Tris-Cl, pH 8.0). After homogenizing, 500 μl lysis buffer as above with addition of 0.2% β-mercaptoethanol and 10 μl proteinase K (20 mg mL^{-1}) was added, and the samples were incubated at 65 °C for 3 h. DNA was extracted routinely with phenol-chloroform (Sambrook et al. 1989) and resuspended in 50 μl UHQ water. To amplify the SSU rRNA gene of microsporidia, several combinations of universal microsporidian primers 18f, 530r, 530f, 1047r, 1061f, and 1492r (Weiss and Vossbrinck 1999) were used. Due to low DNA quantities in the sample, neither 18f:1492r nor 18f:1047r primer pairs produced positive results, but shorter fragments were amplified using 18f:530r, 530f:1047r, and 1061f:1492r pairs.

PCR was run using a "Tertsik" DNA amplifier (DNA Technology) in 10 μl volume containing 5 μl DNA template; PCR buffer; dNTPs, 0.25 mM; Taq-polymerase (Sileks, Russia), 0.5 U; forward and reverse primers (Evrogen, Russia), 1 pMol each. A first cycle of denaturation was carried out at 95 °C for 3 min, and a last cycle of extension was carried out at 72 °C for 10 min. Samples were amplified for 30 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and elongation at 72 °C for 30 s. The PCR products were gel purified and sequenced in both directions.

Newly obtained SSU rRNA gene sequences were compared to those available in NCBI using the built-in BLAST utility (www.ncbi.nlm.nih.gov/Blast.cgi). Among these, only one fragment showed significant similarity to Microsporidia (see "Results and discussion" section) and was submitted to Genbank under accession number MF278272. The sequence was aligned with closely related sequences from Genbank (Table 1) 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 403-bp length. Phylogenetic reconstruction was performed using maximum likelihood algorithm with GTR+I+G model settings in MEGA 7 (Kumar et al. 2015). Pairwise evolutionary distances were calculated using Kimura-2 model (Kimura 1980) in MEGA 7.

Table 1 Estimates of evolutionary divergence between 14 sequences of Microsporidia indicating the number of base substitutions per site using the Kimura-2 model (Kimura 1980) on the basis of the 403-bp-long dataset in MEGA 7 (Kumar et al. 2015)

#	Microsporidia species (Genbank accession no.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Anncaliia meligethi* (AY894423)	ID	–	–	–	–	–	–	–	–	–	–	–	–	–
2	*Anncaliia algerae* (AF069063)	0.002	ID	–	–	–	–	–	–	–	–	–	–	–	–
3	*Anncaliia azovica* (KY288064)	0.000	0.002	ID	–	–	–	–	–	–	–	–	–	–	–
4	*Kneallhazia solenopsae* (AY312502)	0.136	0.139	0.136	ID	–	–	–	–	–	–	–	–	–	–
5	*Kneallhazia carolinensis* (GU173849)	0.130	0.133	0.130	0.079	ID	–	–	–	–	–	–	–	–	–
6	*Tubulinosema acridophagus* (AF024658)	0.208	0.212	0.208	0.208	0.215	ID	–	–	–	–	–	–	–	–
7	*Tubulinosema ratisbonensis* (AY695845)	0.205	0.208	0.205	0.201	0.215	0.005	ID	–	–	–	–	–	–	–
8	*Tubulinosema kingi* (DQ019419)	0.215	0.218	0.215	0.214	0.222	0.005	0.010	ID	–	–	–	–	–	–
9	*Tubulinosema hippodamiae* (JQ082890)	0.215	0.218	0.215	0.215	0.222	0.005	0.010	0.010	ID	–	–	–	–	–
10	*Tubulinosema loxostegi* (JQ906779)	0.208	0.212	0.208	0.208	0.215	0.000	0.005	0.005	0.005	ID	–	–	–	–
11	*Tubulinosema pampeana* (KM883008)	0.208	0.212	0.208	0.208	0.215	0.000	0.005	0.005	0.005	0.000	ID	–	–	–
12	*Fibrillanosema crangonycis* (AY364089)	0.242	0.242	0.242	0.211	0.222	0.198	0.204	0.204	0.205	0.198	0.198	ID	–	–
13	*Microsporidium* sp. Mic6 (JQ012757)	0.268	0.268	0.268	0.239	0.254	0.257	0.250	0.264	0.265	0.257	0.257	0.128	ID	–
14	*Fibrillaspora daphniae* sp. n. (MF278272)	0.267	0.267	0.267	0.235	0.247	0.246	0.250	0.253	0.254	0.246	0.246	0.116	0.023	ID
15	*Janacekia debaisieuxi* (AJ252950)	0.359	0.359	0.359	0.376	0.382	0.406	0.397	0.414	0.415	0.406	0.406	0.335	0.378	0.389

Results and discussion

Host pathology and parasite morphology

The infected water fleas displayed microsporidiosis symptoms typical for many aquatic arthropods with transparent bodies, appearing opaque white or light orange in color due to spore masses occupying the inner tissues. Histological sections revealed parasite-infected cells occupying the entire host haemocoel (Fig. 1a). We recorded a 52% prevalence rate of infection in field-collected *D. magna* ($n = 2380$). Elongated oval spores with a densely stained cytoplasm were observed in Giemsa-stained smears (Fig. 1b). A small proportion of spores appeared void. Methanol-fixed spores measured 4.8 ± 0.3 μm × 2.3 ± 0.2 μm.

Late meronts, meront/sporont transitional stages, sporoblasts, and spores were observed in ulthrathin section (Figs. 2, 3, and 4). Meronts and sporonts appeared to divide by binary fission. All developmental stages were in direct contact with the host cell cytoplasm and contained endoplasmic reticulum (ER) with numerous ribosomes attached to ER or freely distributed within the cytoplasm (Fig. 3b, c, for example). Late meronts contained one or two unpaired nuclei. Cells with two nuclei were 3.4–3.6 × 1.5–1.9 μm in size, while daughter cells resulting from their division and containing one nucleus were 2.7–4.1 μm in diameter. Nuclei of meronts measured up to 0.9–1.6 μm in diameter, and were typically surrounded with several rows of ER (Fig. 2a, b). Immediately following binary division of meronts into uninucleate cells, their cytoplasmic membranes transformed into a multilayer cell wall of complex structural characteristics associated with sporogonial stages. An electron-dense coat with an additional thin membranous layer was deposited on separated regions of the cell surface (Fig. 2c) and further encompassed the entire cell. The outer membrane consequently transformed into a system of fine tubules laid in parallel (Fig. 3c). The tubules were attached to the electron-dense coat. The tubule diameter measured approximately 20 nm. Sporonts were 3.2–4.1 × 2.0–2.7 μm in size (Fig. 2d). The outer tubular layer was usually tightly attached to the cell surface or was partially detached forming "bubbles" in dividing cells (Fig. 3a). Each sporont divided into two uninucleate sporoblasts 1.9–2.2 × 1.1–1.5 μm in size (Fig. 3a, b). Sporoblast nuclei were elongated oval, up to 1.5 μm in diameter, occupying a significant portion of the parasite cell volume. Formation of the spore structures began with appearance of primordial endospore and polar tube coils (Fig. 3d). These stages were infrequently observed suggesting that the sporoblast to spore transformation is a relatively fast process. Groups of host cells mitochondria were observed in the vicinity of parasite prespore stages and mature spores (Fig. 3e, f).

Spore ultrastructure of the examined microsporidium combined both "primitive" and "advanced" characters. Spores were uninucleate. Nuclei were of moderate electron density, either rounded or of irregular angular shape, up to 0.8–1.0 μm in diameter. They were located in the spore center and were surrounded with two rows of granular ER and numerous ribosomes. The structure of polaroplast was unique. Its anterior

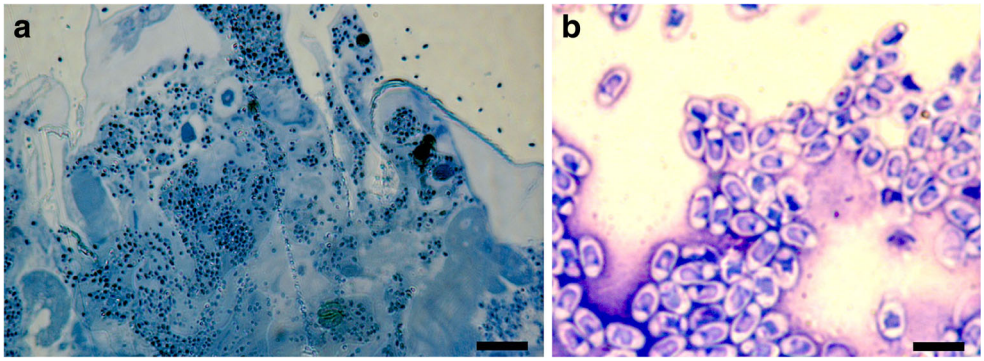

Fig. 1 Light microscopy of *Fibrillaspora daphniae* g. n. sp. n. from *Daphnia magna.* **a** Localization of parasite spores in a dorsoventral section of *D. magna*. **b** Mature spores of *Fibrillaspora daphniae* g. n. sp. n. on a methanol fixed and Giemsa-stained smear. Scale bar = 50 μm (**a**), 5 μm (**b**)

and posterior portions consisted of tightly packed lamellae and globules of low electron density, respectively. The diameter of the globules was within the range of 50–80 nm. At the posterior pole of the spore, there was large posterior vacuole limited by a two-layer membrane and filled with granular material (Fig. 4a, b). The polar tube was slightly anisofilar, 110 nm in diameter, and formed 13–19 coils arranged in a single layer. The two posterior coils were lesser in diameter and possessed fewer inner membraneous layers and core of higher electron density as compared to the anterior coils (Fig. 4c). The spore wall was composed of a cytoplasmic membrane, electron-transclucent endospore (100 nm), electron-dense granulated exospore (20 nm), and a surface layer composed of thin tubular structures 20 nm in diameter (Fig. 4d). The tubular layer was only partially attached to the exospore (Fig. 4a, b).

Molecular phylogenetics and taxonomic placing of the parasite

Among three obtained DNA fragments, two (primer pairs 18f:530r and 1061f:1492r) were identical to the host ribosomal RNA gene (*Daphnia magna*). This is likely due to non-specific amplification of host DNA, which can occur when attempting to amplify Microsporidia from different arthropod hosts (Tokarev et al. 2010). Only the fragment (495 bp long) obtained with the primer pair 530f:1047r showed attribution to Microsporidia. The closest Genbank entry with high sequence similarity (about 97%) belonged to an undescribed parasite referred to as *Microsporidium* sp. isolate Mic6 (Genbank accession # JQ012757) from *Daphnia longispina* complex. Among the valid species, the closest relative was

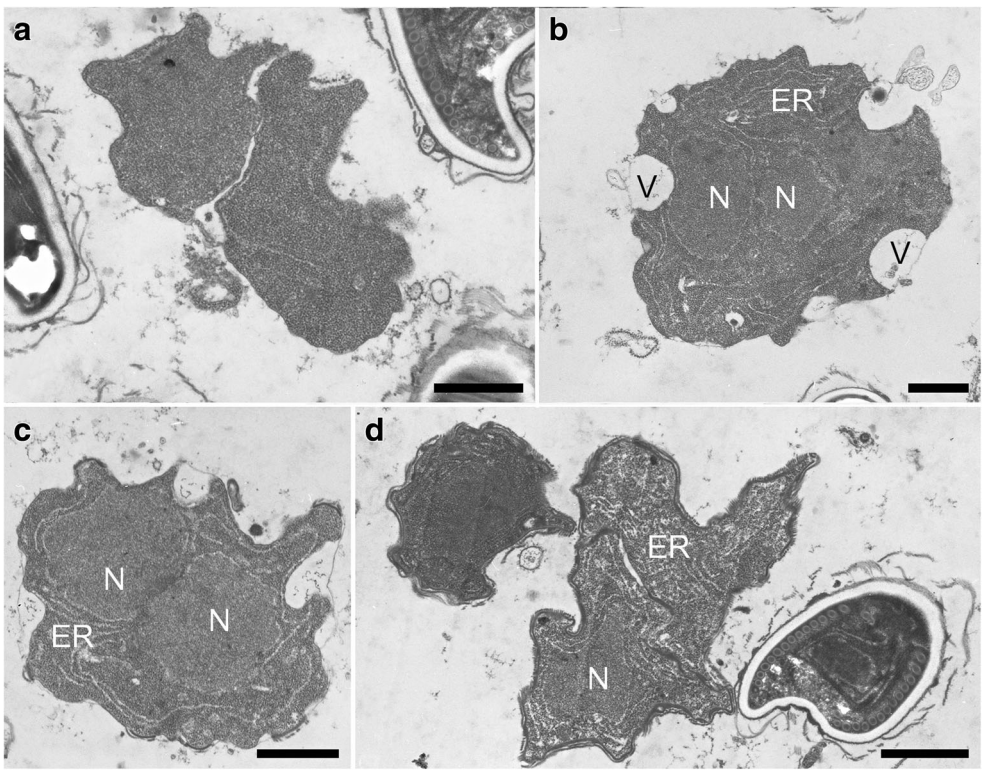

Fig. 2 Transmission electron microscopy of early developmental stages of *Fibrillaspora daphniae* g. n. sp. n. **a** Late meronts with a single nucleus. **b** Late meront with two unpaired nuclei. **c** Early sporont. **d** Sporonts. N nucleus, V vacuole, ER endoplasmic reticulum. Scale bar = 1 μm

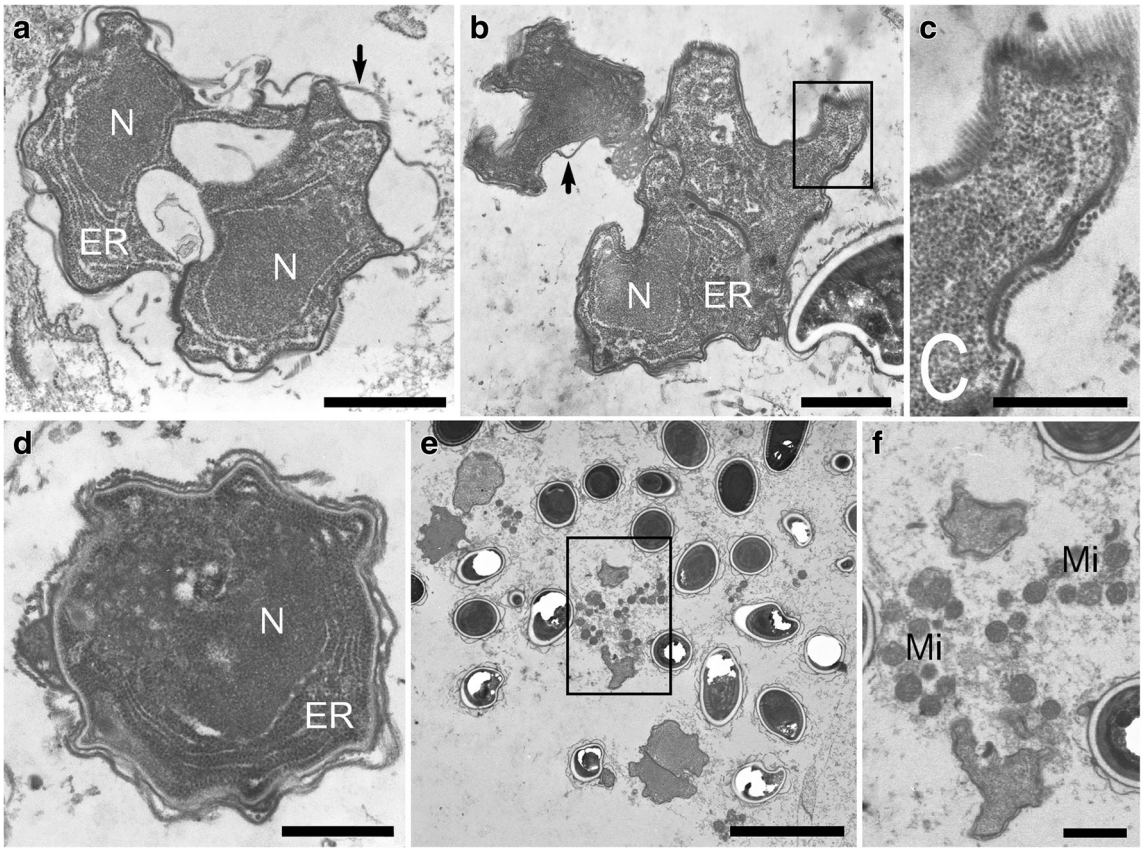

Fig. 3 Transmission electron microscopy of sporogonial stages of *Fibrillaspora daphniae* g. n. sp. n. **a** Sporont division into two sporoblasts. **b** A sporoblast. **c** An enlarged detail of Fig. 3b showing tubular layer (arrows) on the outer membrane of the sporoblast. **d** A young spore with primordial endospore (arrows) and polar tube coils (double arrows). **e** Developmental stages within the host cell cytoplasm. **f** An enlarged detail of **e** showing groups of host cell mitochondria (arrows). Mi mitochondria of the host cell; other abbreviations as in Fig. 2. Arrows indicate "bubbles" (detached outer layer) on the surface on dividing cells. Scale bar = 1 μm (**a**, **b**, **f**), 0.5 μm (**c**, **d**), 5 μm (**e**)

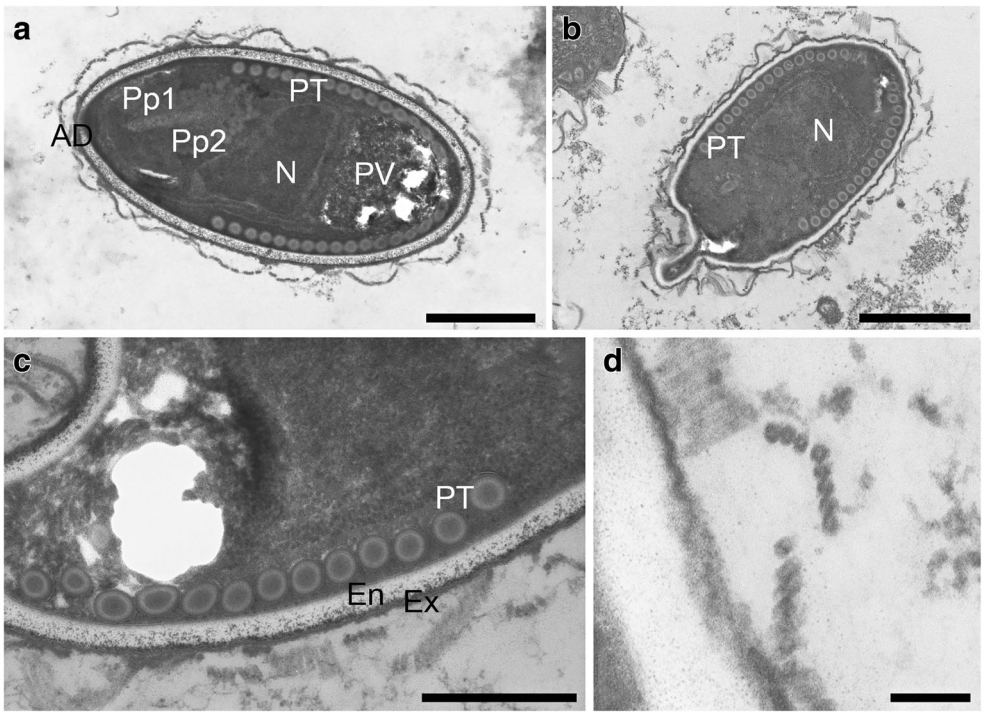

Fig. 4 Transmission electron microscopy of mature spores of *Fibrillaspora daphniae* g. n. sp. n. **a**, **b** Mature spores. **c** Spore wall, polar tube coils, and posterior vacuole of a mature spore. **d** Spore wall with a tubular layer on its outer surface. AD anchoring disk, En endospore, Ex exospore, Pp1 anterior lamellar part of polaroplast, Pp2 posterior globular part of polaroplast, PT polar tube coils, PV posterior vacuole; other abbreviations as in Fig. 2. Scale bar = 1 μm (**a**, **b**), 0.5 μm (**c**), 0.1 μm (**d**)

Fibrillanosema crangonycis (#AY364089) from *Crangonyx pseudogracilis* (Crustacea: Amphipoda) (Slothouber Galbreath et al. 2004), showing 88% sequence similarity.

In the phylogenetic reconstruction, *Fibrillanosema crangonycis* was in basal position to the lineage comprised by the new microsporidium and *Microsporidium* sp. Mic6 while the whole branch containing these taxa was in sister position to the branch including *Tubulinosema*, *Anncaliia*, and *Kneallhazia* (Fig. 5). Evolutionary divergence of the novel microsporidium as compared to the undescribed isolate Mic6, *Fibrillanosema crangonycis* and the *Anncaliia/Kneallhazia/Tubulinosema* lineage reached 0.023, 0.116, and 0.235–0.267 changes per site, respectively (Table 1).

All the aforementioned species are parasites of arthropods. In particular, *Fibrillanosema crangonycis* isolates 1971 and Mic6 were found in Crustacea (Amphipoda or Cladocera) while the members of *Anncaliia/Kneallhazia/Tubulinosema*—in terrestrial insects (locusts, flies, beetles, moths, bumble bees, ants) with the exception of *A. algerae* from an amphibiont mosquito host and another species of *Anncaliia* (not shown) isolated from a brackish-water gammarid host (Tokarev et al. 2014). It should also be noted that certain members of *Anncaliia* and *Tubulinosema* may also infect and cause disease in humans (Franzen et al. 2006; Choudhary et al. 2011; Meissner et al. 2012) thus extending the host range of this group of microsporidia to vertebrates.

One of the key unique ultrastructural features of the isolate 1971 is the posterior portion of the polaroplast, which is globular (Fig. 4a), as opposed to the majority of previously described genera. The only other microsporidium with a globular polaroplast is *Globulispora mitoportans* (Vavra et al. 2016) which belongs to clade 4 in the crown of the Microsporidia tree of life (Vossbrinck et al. 2014), also known as class Terresporidia (Vossbrinck and Debrunner-Vossbrinck 2005) while the parasites presented in the phylogenetic reconstruction (Fig. 5) are branched within clade 3 which is close to the base of Microsporidia tree of life (Vossbrinck et al. 2014). The appearance of globules comprising the polaroplast in the new species and *G. mitoportans* is strikingly different, which is consistent with their distant phylogenetic placement. As for *Fibrillanosema*, which is the closest relative of isolate 1971 among described species with ultrastructural data available, the resolution of published micrographs do not allow concluding about possible globular structure of posterior part of the polaroplast.

These findings prompt us to establish a new species and a new genus for this microsporidium, *Fibrillaspora daphniae* g. n. sp. n., described below. Its closest relative, undescribed isolate Mic6, also a parasite of *Daphnia*, can be provisionally placed into this genus as *Fibrillaspora* sp. due to high level of SSU rRNA gene sequence similarity (97%) to the newly established type species of *Fibrillaspora* g. n. As the *Anncaliia/Tubulinosema* cluster members are allocated to the family Tubulinosematidae (Franzen et al. 2005) and *Fibrillanosema/Fibrillaspora* lineage is in sister position to this group, we find it appropriate to establish a new family Fibrillasporidae fam. n. High branch support of the whole *Fibrillaspora/Tubulinosema* lineage in sister position to

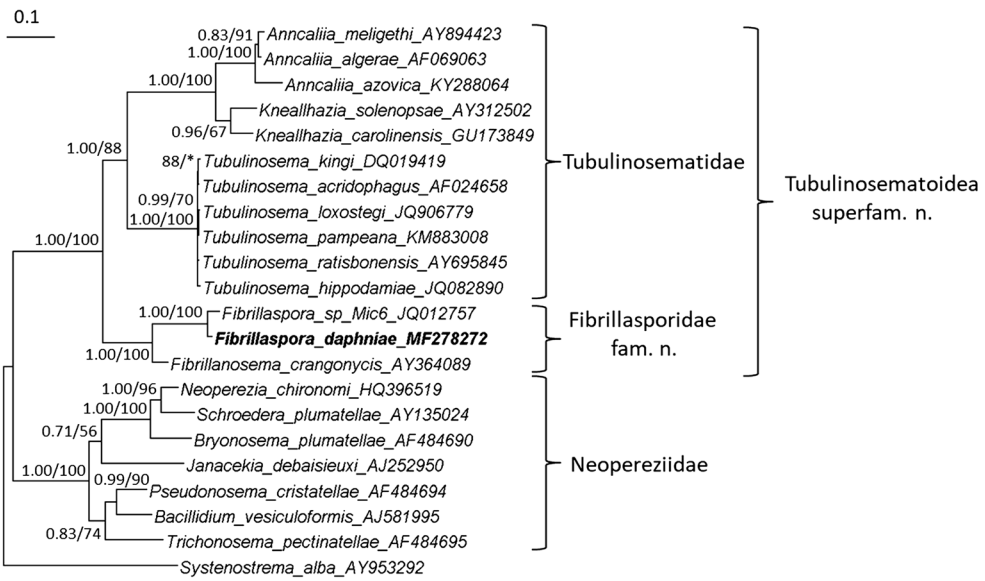

Fig. 5 Phylogenetic reconstruction showing position of the new microsporidium (in bold) and related species. The phylogram is obtained using 1493-bp-long alignment (including gaps as missing data for shorter sequences) of small subunit ribosomal RNA gene portion (respective Genbank accession numbers are shown in taxa annotation) with maximum likelihood (ML) in MEGA 7 (Kumar et al. 2015) and Bayesian inference (BI) in MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). Numbers at the nodes indicate posterior probabilities/bootstrap support for BI/ML, respectively. Asterisk indicates node for which the bootstrap support (ML) was below 50 resulting in a collapsed branch. Scalebar = 0.1 expected changes per site

Neopereziidae (Fig. 5) and apparent structural similarity of members of Fibrillasporidae fam. n. and Tubulinosematidae make it logical to establish a new taxon of superfamily rank to unify these two families: Tubulinosematoidea superfam. n. In terms of structural characteristics, Tubulinosematidae and Fibrillasporidae fam. n. are quite similar; main difference is the time when ornamentation of the cell surface appears. In former, it appears in meronts and disappears from mature spores, while in the latter, it is observed from sporonts to mature spores.

Fibrillaspora g. n.

Development in direct contact with host cell cytoplasm. Division by binary fission. Sporont surface with additional outer layer composed of fine tubules. Spore with thick endospore, large posterior vacuole filled with electron-dense granules, and bipartite polaroplast composed of anterior lamellar and posterior globular parts. Polar tube slightly anisofilar, coils arranged in one row, two posterior coils of lesser diameter and higher electron density.

Etymology: Prefix "fibrilla-" indicates relatedness to *Fibrillanosema.*

Type species: *Fibrillaspora daphniae* sp. n.

Fibrillaspora daphniae sp. n.

Life cycle and cell structure as in genus description. All developmental stages with single nuclei. Fixed spores elongated oval, $4.8 \pm 0.3 \times 2.3 \pm 0.2$ μm in size. Polar tube with 13–19 coils.

Etymology: The species name alludes to the host genus.

Type host: *Daphnia magna* (Crustacea: Cladocera: Daphniidae).

Type material: Glass slides with stained smears and histological sections (# 1971), epon-araldyte embeddings, and electron micrographs (## 46149-46150, 49208-49210, 49331-49352) are deposited at the Chair of Invertebrate Zoology, Tomsk State University (pr. Lenina 36, Tomsk, 634050, Russia); frozen genomic DNA sample (# SIMAK1971) is stored at Laboratory of Microbiological Control, All-Russian Institute for Plant Protection (sh. Podbelskogo 3, St. Petersburg, Pushkin, 196608, Russia).

Genbank accession number: MF278272 (small subunit ribosomal RNA gene, partial sequence).

Fibrillasporidae fam. n.

The family is defined as a monophyletic group including Fibrillaspora (type genus) and Fibrillanosema in sister position to members of the family Tubulinosematidae. All stages in direct contact with the host cell cytoplasm. Sporogonial division presumably by binary fission. Surface of sporoblasts and exospore of immature spores bears additional ornamentation: coated bundles of fibrils or tight rows of thin tubular structures. Polaroplast bipartite with lamellar anterior and globular posterior parts. Polar tube slightly anisofilar.

Tubulinosematoidea superfam. n.

The superfamily defined as a monophyletic group including families Fibrillasporidae and Tubulinosematidae. Development in direct contact with host cell cytoplasm. The outer layer of developmental stages composed of fine tubules or fibrils (meronts to immature spores in Tubulinosematidae and sporonts to mature spores in Fibrillasporidae). The polaroplast bipartite. The polar tube slightly anisofilar.

Taxonomic synopsis for Tubulinosematoidea superfam. n.
Family 1. Tubulinosematidae
Genus 1. *Tubulinosema* (type)
Genus 2. *Anncaliia*
Genus 3. *Kneallhazia*
Family 2. Fibrillasporidae fam. n.
Genus 1. *Fibrillaspora* g. n. (type)
Genus 2. *Fibrillanosema*

Acknowledgements Authors are indebted to A.A. Miller (Institute of Strength Physics and Materials Science of Siberian Branch Russian Academy of Sciences, Tomsk, Russia) for assistance with transmission electron microscopy and to Theodore G. Andreadis (The Connecticut Agricultural Experiment Station, CT, USA) for language correction of the manuscript. The research is performed within the frames of Governmental Contract from Ministry of Education and Science of Russian Federation # 6.7525.2017/8.9 and a grant from Russian Foundation of Basic Research # 17-04-00871.

References

Choudhary MM, Metcalfe MG, Arrambide K, Bern C, Visvesvara GS, Pieniazek NJ, Bandea RD, Deleon-Carnes M, Adem P, Choudhary MM, Zaki SR, Saeed MU (2011) *Tubulinosema* sp. microsporidian myositis in immunosuppressed patient. Emerg Infect Dis 17(9): 1727–1730. https://doi.org/10.3201/eid1709.101926

Ebert D (1995) The ecological interactions between a microsporidian parasite and its host *Daphnia magna*. J Animal Ecol 64(3):361–369. https://doi.org/10.2307/5897

Ebert D (2005) Ecology, epidemiology, and evolution of parasitism in *Daphnia*. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. **Available from:** http://www.ncbi. nlm.nih.gov/books/NBK2036

Franzen C, Fischer S, Schroeder J, Schölmerich J, Schneuwly S (2005) Morphological and molecular investigations of *Tubulinosema ratisbonensis* gen. nov., sp. nov. (Microsporidia: Tubulinosematidae fam. nov.), a parasite infecting a laboratory colony of *Drosophila melanogaster* (Diptera: Drosophilidae). J Eukaryot Microbiol 52(2):141–152. https://doi.org/10.1111/j.1550-7408.2005.04-3324.x

Franzen C, Futerman PH, Schroeder J, Salzberger B, Kraaijeveld AR (2006) An ultrastructural and molecular study of *Tubulinosema*

kingi Kramer (Microsporidia: Tubulinosematidae) from *Drosophila melanogaster* (Diptera: Drosophilidae) and its parasitoid *Asobara tabida* (Hymenoptera: Braconidae). J Invertebr Pathol 91(3):158–167. https://doi.org/10.1016/j.jip.2005.11.007

Green J (1974) Parasites and epibionts of Cladocera. Trans Zool Soc London 32:417–515

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp 41:95–98

Haag KL, James TY, Pombert JF, Larsson R, Schaer TM, Refardt D, Ebert D (2014) Evolution of a morphological novelty occurred before genome compaction in a lineage of extreme parasites. Proc Natl Acad Sci U S A 111(43):15480–15485. https://doi.org/10.1073/pnas.1410442111

Issi IV (1986) Microsporidia as a phylum of parasitic protozoa. In: Beyer TV, Issi IV (eds) Protozoology 10. Leningrad. Nauka, pp 1–136

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evolut 16(2):111–120. https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Tamura K (2015) MEGA7: molecular evolutionary genetics analysis version 7.0. Mol Biol Evol 33(7):1870–1874

Larsson JIR, Ebert D, Vávra J, Voronin VN (1996) Redescription of *Pleistophora intestinalis* Chatton, 1907, a microsporidian parasite of *Daphnia magna* and *Daphnia pulex*, with establishment of the new genus *Glugoides* (Microspora, Glugeidae). Europ J Protistol 32(2):251–261. https://doi.org/10.1016/S0932-4739(96)80024-1

Larsson RJI (2005) Fixation of microsporidian spores for electron microscopy. J Invertebr Pathol 90(1):47–50. https://doi.org/10.1016/j.jip.2005.06.016

Lukyantsev VV, Simakova AV (2014) Infestation of lower crustaceans (Copepoda, Cladocera) with microsporidians (Microsporidia) in Western Siberia. Parazitologiya 48(5):358–372

Manuilova EF (1964) Cladocerans of fauna of the USSR. M.; L: Nauka. 318 p

Meissner EG, Bennett JE, Qvarnstrom Y, da Silva A, Chu EY, Tsokos M, Gea-Banacloche J (2012) Disseminated microsporidiosis in an immunosuppressed patient. Emerg Infect Dis 18(7):1155–1158. https://doi.org/10.3201/eid1807.120047

Refardt D, Canning EU, Marthis A, Cheney SA, Lafranchi-Tristem NJ, Ebert D (2002) Small subunit ribosomal DNA phylogeny of microsporidia that infect *Daphnia* (Crustacea: Cladocera). Parasitology 124(Pt 4):381–389

Refardt D, Decaestecker B, Jonson PTJ, Vavra J (2008) Morphology, molecular phylogeny, and ecology of *Binucleata daphniae* n. g., n. sp. (Fungi: Microsporidia), a parasite of *Daphnia magna* Straus, 1820 (Crustacea: Branchiopoda). Eukaryot Microbiol 55(5):393–408. https://doi.org/10.1111/j.1550-7408.2008.00341.x

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17(1):208–212. https://doi.org/10.1083/jcb.17.1.208

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12):1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sambrook J, Fritsch E, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Slothouber Galbreath JGM, Smith JE, Terry RS, Becnel JJ, Dunn AM (2004) Invasion success of *Fibrillanosema crangonycis* n.sp., n,g.: a novel vertically transmitted microsporidian parasite from the invasive amphipod host *Crangonyx pseudogracilis*. Internat. J Parasitol 34:235–244

Sprague V, Becnel JJ, Hazard EI (1992) Taxonomy of phylum Microspora. Crit Rev Microbiol 18(5-6):285–395. https://doi.org/10.3109/10408419209113519

Tokarev Y, Sitnikova N, Pistone D, Lizhi Luo, Huang Shaozhe, Ignatieva A, Senderskiy I, Toderas I, Frolov A (2010) Microsporidia PCR detection artifacts due to non-specific binding of the universal microsporidia primers to the rDNA of arthropod hosts. Buletinul ASM Stiintele Vietii 2010. 310, 72–77

Tokarev Y, Voronin V, Rusakovich E, Issi I (2014) Detection of microsporidia in Gammarids in the Delta of the Kuban River (Azov Sea, Russia). 47th annual meeting of the Society of Invertebrate Pathology. Julius Kuhn Institute, p 86

Undeen AH, Vavra J (1997) Research methods for entomopathogenic Protozoa. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, pp 117–151. https://doi.org/10.1016/B978-012432555-5/50010-5

Vavra J, Larsson JIR (1994) *Berwaldia schaefernai* (Jirovec, 1937) comb. n. (Protozoa, Microsporidia), fine structure, life cycle and relationship to *Bervaldia singularis* Larsson, 1981. Europ J Protistol 30(1):45–54. https://doi.org/10.1016/S0932-4739(11)80197-5

Vavra J, Hylis M, Fiala J, Nebesarova J (2016) *Globulispora mitoportans* n.g., n.sp. (Opisthosporidia: Microsporidia) a microsporidian parasite of daphnids with spore organization and prominent mitosome-like vesicles. J Invertebr Pathol 135:43–52. https://doi.org/10.1016/j.jip.2016.02.003

Vidtmann SS, Sokolova YY (1994) The description of the new genus *Larssonia* gen. n. based on the ultrastructural analysis of *Microsporidium (Pleistophora) obtusa* from *Daphnia pulex* (Cladocera). Parazitologyia 28:202–213

Voronin VN, Issi IV (1974) Methods of working with microsporidians. Parazitologiya 8(3):272–273

Vossbrinck CR, Debrunner-Vossbrinck BA (2005) Molecular phylogeny of the microsporidia: ecological, ultrastructural and taxonomic considerations. Folia Parasitol 52(1):131–142. https://doi.org/10.14411/fp.2005.017

Vossbrinck CR, Debrunner-Vossbrinck BA, Weiss LM (2014) Molecular phylogeny of the Microsporidia. In: Weiss LM, Becnel JJ (eds.). Microsporidia: Pathogens of Opportunity. Wiley-Blackwell 203–220

Weigl S, Korner H, Petrusek A, Seda J, Wolinska J (2012) Natural distribution and co-infection patterns of microsporidia parasites in the *Daphnia longispina* complex. Parasitology 139(07):870–880. https://doi.org/10.1017/S0031182012000303

Weiser J (1977) Contribution of the classification of Microsporidia. Vestn Cs spolec zool 41(4):308–320

Weiss LH, Vossbrinck CR (1999) Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the microsporidia. In: The Microsporidia and Microsporidiosis (Eds. M. Wittner, L. M. Weiss). ASM Press, Washington, 129–171, DOI: https://doi.org/10.1128/9781555818227.ch4