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Ultrastructure of the Microsporidium, *Duboscqia legeri*, the Type Species of the Genus *Duboscqia* Perez, 1908

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Summary. The type species of the genus *Duboscqia* Perez, 1908 (Opisthokonta, Microspora), *D. legeri* is a pathogen of termites. It was found again in *Zootermopsis angusticollis* in British Columbia and the material is used for emendation of data on ultrastructures of this old genus. The sporogony of this microsporidian ends with 16 oval spores closed in sporophorous vesicles. The isofilar polar filament coiled in 13 coils, the arched anchoring disc and the polaroplast with tightly packed lamellae are typical for the ultrastructures of uninucleate spores. The sporophorous vesicle is persistent. The microsporidian infects cyst-like lobes of the fat body hanging free in the body cavity. Relations to other related genera are discussed.

Key words: *Duboscqia legeri*, microsporidium, ultrastructures.

INTRODUCTION

Duboscquia legeri Perez, 1908 was described from the body cavity of an infected termite, *Reticulitermes lucifugus* (Rossi, 1792) (Perez 1908), and is also known from the fat body of *R. flavipes* (Kollar, 1837) (Kudo 1942). It was designated as the type species of *Duboscqia* Perez, 1908, with typical pansporoblasts containing 16 spores (Perez 1908). Another species, *D. sidae* (Jírovec, 1942), found in the body cavity of *Sida crystallina* (Müller, 1776) (Jírovec 1942) and in fat body cells of *Daphnia pulex* Leydig, 1860 and *D. magna* Straus, 1820 (Weiser 1945), was included in the genus *Duboscqia*. Later, new species of this genus living in different hosts were described. *Duboscqia chironomi* (Voronin, 1975) was described from fat body cells of *Chironomus plumosus* (Linnaeus, 1758) (Voronin 1975). *D. coptotermi* Kalavati et Narasinhamurti, 1976 heavily infects the midgut of an Indian termite *Coptotermes heimi* (Wasmann, 1902) (Kalavati and Narasimhamurti 1976).

D. sidae infecting fat body cells and hemocytes of *Holopedium gibberum* Zaddach, 1855 was used for a detailed study of ultrastructure (Larsson and Yan 1988). Larsson and Yan (1988) differentiated the studied microsporidium "from the type species and all other genera with polysporoblastic sporogony in sporophorous vesicles" and proposed a new genus, *Agglomerata*

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Larsson et Yan, 1988. This genus contains four other *Duboscqia*-like species: *A. sidae* (Jírovec, 1942), *A. cladocera* (Pfeiffer, 1895), *A. volgensae* Larsson et Voronin, 2000, and *A. lacrima* Bronnvall et Larsson, 2001 (Larsson and Yan 1988, Larsson *et al.* 1996). Another new genus, *Tardivesicula* Larsson and Bylén, 1992, with *T. duplicata* Larsson et Bylen, 1992 as type species, was included in the family Duboscqiidae Sprague 1977 (Larsson and Yan 1988). *T. duplicata* infects the hypoderm and fat body of a caddis fly (Trichoptera). Two last members of Duboscqidae are species of genera *Trichoduboscqia* Leger, 1926 and *Mitoplistophora* Codreanu, 1966 (Leger 1926, Codreanu 1966).

The current paper describes the ultrastructure of the type species, *D. legeri*, infecting a termite and compares its ultrastructure with that reported for representatives of genera *Agglomerata* and *Tardivesicula*.

MATERIALS AND METHODS

Three swarming alate reproductive adults of the Pacific dampwood termite, *Zootermopsis angusticollis* (Hagen, 1858), were collected on September 4th 2001 in suburban Burnaby, BC, Canada. They were brought alive to the laboratory. Microsporidia infecting the fat cells were observed during study of the flagellate fauna in the midgut of one of the three termites. The microsporidia-containing "cysts" (for detailed description, see "results") were excised and fixed in 2.5% glutaraldehyde (Fluka AG, Buchs, CH) in 0.2 M sodium cacodylate buffer (pH 7.2) for 4 h at 4°C. The tissues (three lobes of the fat body fixed to the midgut) were washed in cacodylate buffer, fixed in 2% osmium tetroxide in cacodylate buffer (1 h at 4°C), dehydrated in an ascending series of ethanol solutions, and embedded in Vestopal W (EMC, Haltfield, PA). Sections were stained with uranyl acetate and lead citrate. Semithin sections were stained with Victoria blue (EMC, Haltfield, PA) and used for evaluation with the light microscope. The slide on which we dissected the insect was used as a dry smear and was fixed with methanol and stained with Giemsa (Sigma, St. Louis MO). Part of the material was fixed in 2.5% glutaraldehyde and prepared for TEM (Weiser *et al.* 1998). Ultrathin sections were studied using a Philips EM 100 TEM.

RESULTS

Pathology of the host

As noted, only one of the three specimens of *Z. angusticollis* was infected. Spherical "cysts" were attached to the midgut by a thin bridge of connective tissue. Trachea deriving from the tracheal system of the midgut, were submerged into this tissue. Xenomas were 100–150 μm in diameter and were composed of conglomerates of adipocytes surrounded by the layer of connective tissue. Borders of the adipocytes heavily loaded with mature spores, were not visible, indicating formation of syncytium. Some "cysts" were only partially infected; in this case integrity of individual adypocytes was preserved; infected cells in such "cysts" contained predominantly vegetative stages, intermixed with occasional pansporoblasts with spores. There was no evident decrease in fat droplets and albuminoid granules in those adypocytes compared with not-infected fat body cells. The plasmodia were usually half-moon shaped, rounded at one side and lobate on the other. Some isolated spores were present in connective tissues close to the midgut, but the midgut was not infected. Some oenocytes on the coelomic surface of the gut were filled with developingstages of the microsporidium. The pansporoblasts with mature spores had a persistent wall, and no free single spores were liberated into the hemolymph during dissection.

Morphology of *Duboscqia legeri*. Light microscopy

The vegetative stages observed in the termite in fixed squash preparation and semithin sections were irregular bodies with 2 to 16 nuclei. The early meronts were small oval bodies with divisions of nuclei. The sporont membrane remained as a spherical sac around the multinucleate plasmodium during the formation of sporoblasts. Its nuclei were arranged on the surface, and uninucleate finger-like protrusions matured to free sporoblasts and maturing spores. Elongate sporoblasts were arranged in a dense half-moon-shaped cluster that was smooth on one side (Fig. 2). The groups of 16 spores were very regular, and spores remained firmly aggregated. Pansporoblasts with 16 sporoblasts and spores measured 8 × 10 μm or 10 μm in diameter. Some vesicles contained 32 spores. Some single spores were elongated or oval (4.5–5.0 × 2.0–2.5 μm) in the water mount, with the anterior end slightly constricted. The sporophorous vesicles were persistent and do not released spores in smears. Therefore the measurement of spores in sporophorous vesicles brought reduced data (3.5 – 4 × 2.5 μm) after fixation and Giemsa staining. The vesicles maintained the original space of the host cells but the cell membranes were dissolved, and individual host nuclei hypertrophied into irregular masses with individual sporonts and pansporoblasts penetrating deep into the nucleus.

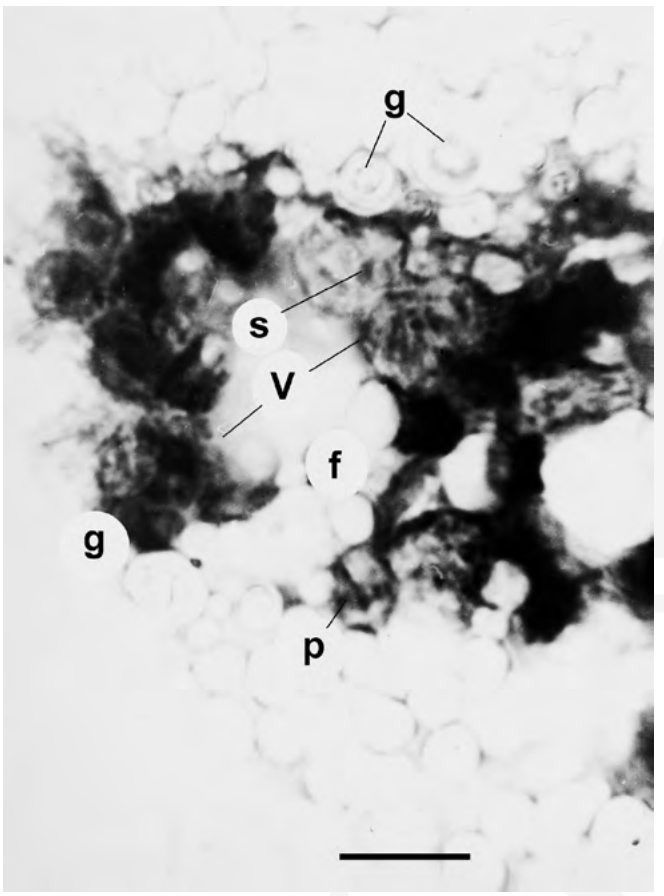

Fig. 1. Sporogony of *Duboscqia legeri* with plasmodia – *p*, maturing spores – *S* and young vesicles – *V*, fat droplets – *f* and protein granules – *g*. Giemsa. Scale bar: 10 μm.

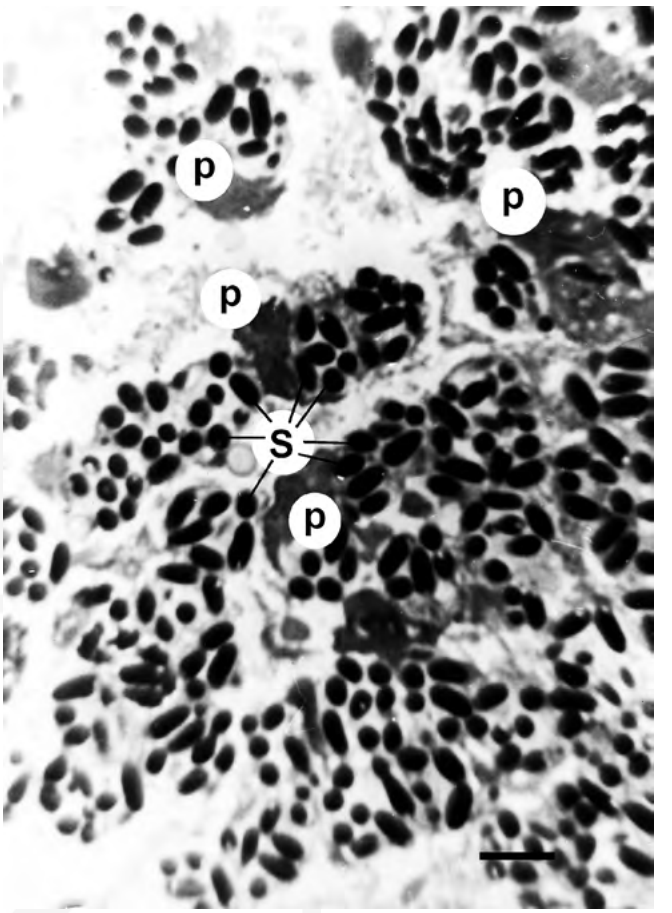

Fig. 2. Mature spores of *Duboscqia legeri* – *S* in sporophorous vesicles. Sporogonial plasmodia – *p* with sporoblasts adhering to one side of the vesicle. Semithin s. Scale bar: 10 μm.

Transmission electron microscopy

Merogonial stages were not detected under the electron microscope. The sporogonial stages were enclosed in a solid sporophorous vesicle, which contained a lobate sporont, growing into a multinucleate plasmodium. The surface of the plasmodium was wrinkled and lobed (Fig. 3), and it later cleaves into 16 elongate sporoblasts. The sporophorous vesicles adhered to the

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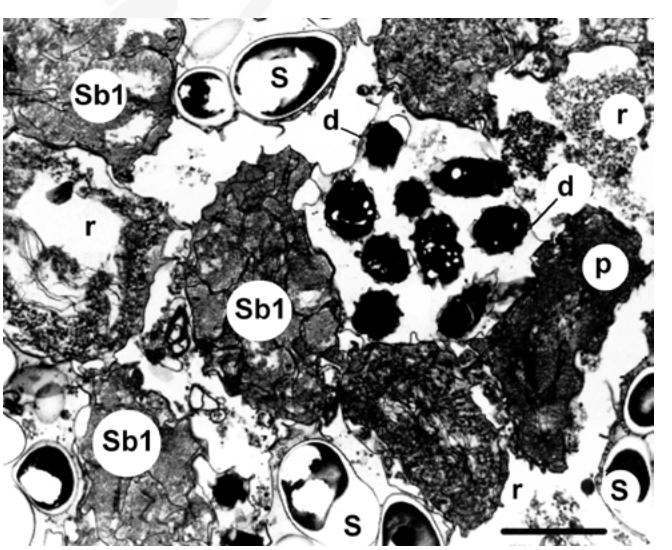

Fig. 3. Portion of infected cell containing sporophorous vesicles at different development of *Duboscqia legeri*: (i) sprorophorous vesicle with sporogonial plasmodia – *p*; (ii) sporophorous vesicles with tightly compressed sporoblasts – *Sb1*; (iii) sporophorous vesicles with mature spores – *S*; exospores of which display numerous outwards projections – *d*. The space between the sporoblasts was filled with very fine granule – *r*. TEM. Scale bar: 5 μm.

mass of sporoblasts. The space between the sporoblasts was filled with very fine granules (Fig. 3). The sporoblasts were uninucleate, elongate, smooth, and oval stages bent in a complicated way (Fig. 3), and they form (eventually under influence of fixation) a dense plait of bodies (Fig. 3). The next stage in maturation was the corrugated dark sporoblast (Fig. 3), which occurred after formation of the hard exospore and before endospore secretion. Mature spores were of a single type are oval (1.5–1.7 × 3.0–3.5 μm) with equal blunt ends in ultrathin sections. Their exospore (20 nm) consisted of three layers, with a homogenous dense layer in the center. The electron-lucent endospore (Fig. 4) was of equal thickness (80 nm) and attenuated at the apical end with an adhering anchoring disc. The polar filament was coiled in 13 turns, isofilar, 80 nm in diameter, with a broader bulbus adhering to the anchoring disc (Fig. 5). The single nucleus was in the center of the spore. The Golgi system was connected to the spherical vacuole of the posterosome (Fig. 4). The polaroplast filled the anterior third of the spore. It was composed of fine lamellae in the anterior part and an indistinct posterior granular zone (Fig. 5). The membrane of the sporophorous vesicle was a uniform membrane 20 nm thick, which persists after maturation of the spores (Fig. 3). Neither individual spores nor pansporoblasts had any gelatinous capsule (mucocalix) when examined in India-ink suspension.

DISCUSSION

Host

Z. angusticollis is the most economically important dampwood termite in its range from British Columbia to Lower California. It can attack any wooden struc-

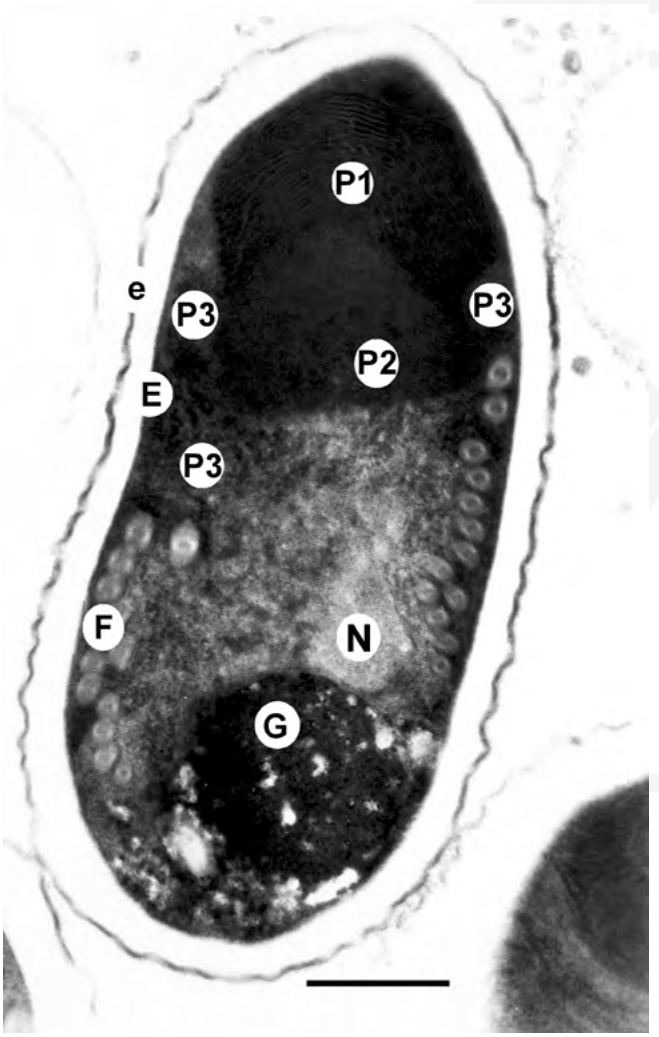

Fig. 4. Mature spore of *Duboscqia legeri.* Exospore – *e*, endospore – *E*. Polaroplast with P_1 lamellar, P_2 granular and P_3 tubular part, nucleus – *N*, polar filament – *F* and Golgi system – *G*. TEM. Scale bar: 500 nm.

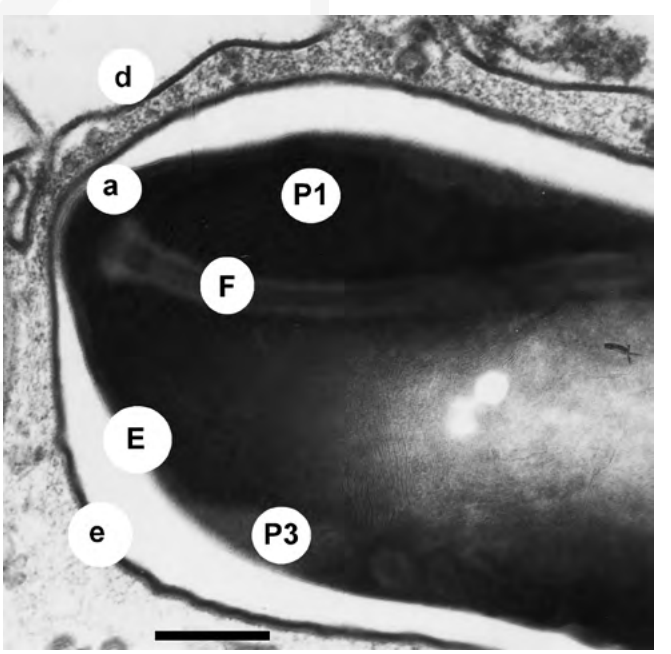

Fig. 5. Anterior end of the mature spore of *Duboscqia legeri* with exospore – *e* and endospore – *E*. Anchoring disc – *a* of the polar filament – *F*. Polaroplast with lamellar – P_1 and tubular – P_3 part. Wall of the sporophorous vesicle – *d*. TEM. Scale bar: 200 nm.

tures in contact with soil or other sources of moisture. In its natural habitat, the termite attacks and breaks down fallen trees and dead roots in the coastal forests. Its winged sexual stages swarm from July through October (Ebeling 1975). The female prepares a nuptial gallery where she lays about 12 eggs. A second group of eggs is laid the following spring. Each female can form colonies with up to 4000 individuals. Their activity is evident from pellets formed with wood dust and liquid feces. They clean their body by mutual grooming, and members of the colony that are injured, diseased, or dead are eaten by other members; most pathogens, including microsporidia, are transmitted in this way. The microsporidia do not cause immediate mortality but reduce the activity and survival of infected insects (Mallis 1954). In the current study, only three termites were collected because the appearance of the pathogen was unexpected. A larger number of specimens are necessary for evaluation of the infection level.

Pathology

Most pathogens, including microsporidia, are transmitted in mentioned way (Weiser 1976). The transmission undergoes two periods: (i) pathogen invades after ingestion a part of the midgut and (ii) they are consequently distributed to their target tissues. Type material of the microsporidian in *R. lucifugus* in France is not available. The cyst-like segments of the fat body of *Z. angusticollis*, which are the seat of the infection, are identical to those described in *R. flavipes* (Kudo 1942). They are connected with a fine trachea to the tracheal system on the surface of the gut. The cysts are enclosed in a connective tissue sheet covering a group of cells of the fat body. In some cysts, the host cells formed a syncytium. Thus the cyst-like segments of the fat body observed in midgut of *R. flavipes* by Kudo (1942), and of *Z. angusticollis* by us, fell well under the definition of syncytial xenomas with host cells confluen into one common syncytium (Weiser 1976). In *Z. angusticollis* the hypertrophic host nuclei were not evident and the lobe contains granular remains of the host tissue (Fig. 3). These cyst-like groups of cells originating in the fat body of insects are also known from larval beetles infected with some types of neogregarines, for example *Pseudomonocystis hopliae* Weiser, Wille, 1960 in white grubs (Weiser and Wille 1960, Weiser 1976). The spread of microsporidium infection in insect hosts after opening of the spores and the injection of germs in the midgut epithel causes a temporary infection of the midgut epithel. The further colonization of the host is usually mediated by hemocytes (David and Weiser 1994) which are infected during their migration on the surface of the midgut. This may be the way of infection in the termite. The identical type of infection in the cases of Perez (1908) and Kudo (1942) and in our case strongly supports the identity of *Duboscqia* in all three infections even when reactions in the infected tissue (nuclear hypertrophy of infected cells) can be reduced. With the rather low reproduction rate of *Zootermopsis* species the microsporidium plays a role in limiting its population density. Mainly solitary cysts were found in *R. flavipes* but even cases with 10 and 11 cysts were observed (Kudo 1942). In our material, the number of cysts varied from about 4 to 5 per cluster.

Ultrastructural characteristics of *Duboscqia*

In addition to the short characteristic of the genus *Duboscqia* Perez, 1908 by Sprague (1977) as microsporidium with "sporogony resulting in 16 sporoblasts that develop into 16 spores" we have to include many further details. The studies of Kudo (1942) brought many morphological details which have limited connection with ultrastructures distinguished in TEM. The sporophorous vesicle with 16 spores is persistent, unornamented, the space between the young spores is filled with a thin suspension of granular secretions which disappear during spore maturation. The uninucleate broad oval spores have a thin smooth exospore with a distinct dense central layer and soft outer layers. The isofilar polar filament is coiled in 13 coils, usually in one irregular row. It is fixed in an arched anchoring disc with narrow border. The spherical coil of Golgi tubules is connected with a reduced posterior vacuole. The structure of the polaroplast corresponds with other microsporidia. Supporting characteristic is the localization of the infection in "cyst"-like lobes of the fat body hanging on a stalk connected with the midgut tracheal system.

Taxonomy

The two infections of *D. legeri* in *R. lucifugus* in France and *R. flavipes* in the U.S.A. were accepted by Kudo (1942) and Sprague (1977) as conspecific and we believe that the infection in *Z. angusticollis* in British Columbia is the same microsporidium. Another microsporidium infecting termites is *D. coptotermi* in *C. heimi* in India (Kalavati et Narasinhamurti 1976). This microsporidium infects the epithelian cells of the midgut of the workers and infected cells and their nuclei showed significant hypertrophy. The infected cells were easily ruptured with slightest pressure. The type of infection

differs from that in *Reticulitermes* species in localization of the infected tissue (Kalavati and Narasinhamurti 1976). The hypertrophy of infected cells and hypertrophy of their nuclei is analogous to the effect of *D. legeri* in *Reticulitermes* species. The sporogonial plasmodia were elongated, measured 23 × 9 µm. Plasmodia with distinct nuclei split in 16 uninucleate sporoblasts and matured in 16 broad oval spores 5.6– 6.6 × 2.5–3.5 µm in spherical vesicles. The extruded uniform polar filament measured 45 to 55 µm (Kalavati and Narasinhamurti 1976). The spores differ in size from *D. legeri* and the polar filament is half the length (Kudo 1942). This may correspond to 5–7 coils of the polar filament. The filament in authors (Kalavati and Narasinhamurti 1976) drawing if complete seems to be isofilar. This type of infection is related to the expected early period in the infection with *D. legeri* where during the passage from the port of entry in the gut to the fat body we must expect at least one cycle of sporogony in the epithel of the midgut.

Two other species, *D. aediphaga* Kettle et Piper, 1988 (Ketlle and Piper 1988) and *D. dengihilli* Sweeney, Doggett et Piper, 1993 (Sweeney *et al.* 1993), were described as a members of the genus *Duboscqia*. Both these species have to belong to family Amblyosporidae, because of existence two types of spores typical for the genus *Amblyospora* Hazard and Oldacre1975 (Sprague *et al.* 1992).

These *Duboscqia* species can be easily differentiated (by ecology, systematic position of hosts, and several ultrastructural features) from aquatic microsporidia, formerly or tentatively placed within the genus *Duboscquia* (or the family *Duboscqiidae*). The main common morphological symptom with Duboscqiidae is the persistent sporophorous vesicle with 16 spores (Larsson and Yan 1988). Based on actual knowledge of the ultrastructures of *D. legeri* (representing the family and genus), we find in *Agglomerata* important differences: (i) the spores are pyriform, (ii) the structure of the exospore is different, (iii) the polar filament is anisofilar and rather short, coiled in 5 to 6 turns, (iv) the interspace in the sporophorous vesicle with mature spores is filled with prominent secretions (Larsson and Yan 1988, Larrson *et al.* 1996, Larsson and Voronin 2000, Bronnwall and Larsson 2001). These differences distinguish the genus *Agglomerata* with *A. cladocera*, *A. sidae*, *A. volgensae* and *A. lacrima* perfectly from genus *Duboscquia*. Genus *Trichoduboscqia*, represented by *T. epeori*, is close to *Agglomerata* because of short polar filament coiled in 5 to 6 turns (Batson 1982). Genus *Tardivesicula*, with *T. duplicata*, often with 32 spores, proposed for inclusion into Duboscqiidae differs in that spores with an isofilar polar filament coiled in 9–10 turns, are tubular (Larsson and Bylen 1992).

In addition we may include into this group also the genus *Mitoplistophora*, with *M. angularis* infecting the fat body of *Ephemera danica* Müller, 1764 (Codreanu 1966). The sporophorous vesicles with 8, 16, eventually 32 spores are fusiform to triangular, each angle prolonged in a short filament (Codreanu 1966) analogous to *Trichoduboscqia.* Spores are pyriform, 4 × 2 µm, and the polar filament is isofilar and coiled in 6 turns. This suggestion has to be support by comparative molecular analyses that will only reveal real relationships among species.

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