The human isolate of *Brachiola algerae* (Phylum Microspora): development in SCID mice and description of its fine structure features

B. KOUDELA[1,2]*, G. S. VISVESVARA[3], H. MOURA[3] *and* J. VÁVRA[1,4]

[1] *Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic*
[2] *Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic*
[3] *Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30341-3724, USA*
[4] *Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague, Czech Republic*

(*Received 30 October 2000; revised 23 February 2001; accepted 25 February 2001*)

SUMMARY

Ocular, peroral, intraperitoneal, intramuscular, and subcutaneous inoculation of severe combined immunodeficient (SCID) mice with spores of the human isolate (CDC: V404) of *Brachiola algerae* (syn. *Nosema algerae*) (Phylum Microspora) revealed that the microsporidium develops in viscera of the immunodeficient mouse host, but only after the ocular administration of spores. It is hypothesized that the physico-chemical milieu of the conjunctiva and cornea helped to adapt the originally 'poikilothermic microsporidian' to the conditions within the homoiothermic organism. Ocular application of spores caused no clinical signs of disease at the application site. However, severe infection in the liver was found 60 days after infection, manifested as hepatosplenomegaly and multifocal miliary necroses and granulomas containing parasites. No microsporidia were found in any other tissues. Transmission electron microscopy revealed characteristic tubulovesicular 'secretory materials' on the plasma membrane of all developmental stages of *B. algerae* except sporoblasts and spores. These formations increase the parasite surface and allow more efficient metabolic communication of the parasite with the host cell. It is hypothesized that the presence of these structures is a factor helping the parasite to grow in a variety of hosts and tissues. Ultrastructural characters support the likelihood that *B. algerae* and *B. vesicularum* are conspecific, and that there exists a relationship between species of the genera *Brachiola* and *Anncaliia*.

Key words: microsporidia, *Nosema algerae*, *Brachiola algerae*, SCID, eye infection.

INTRODUCTION

The phylum Microspora Sprague, 1977 represents intracellular protistan parasites of members of most of the animal phyla (Sprague, 1977). They are either closely related to fungi (Keeling & McFadden, 1998; Weiss & Vossbrinck, 1999) or have actually evolved from within the fungi (Keeling, Luker & Palmer, 2000). Most microsporidia are parasites of arthropods and fish (Wittner, 1999). However, some microsporidia are known to be parasites of homoiothermic vertebrates, humans included (Didier, Snowden & Shadduck, 1998). Of special importance are microsporidia that are able to infect humans because of immunodeficiencies caused by viral infections (HIV), innate malfunctioning of the immune system and by immunosuppressive therapy (Wittner, 1999). Out of the 14 microsporidian species of 8 genera listed as parasites of humans (Curry, 1999), some species are evidently specific parasites of homoiothermic vertebrates, including man (*Enterocytozoon bieneusi*, *Encephalitozoon cuniculi*, *Encephalitozoon hellem*, *Encephalitozoon intestinalis*). Other listed species are either incompletely known or occur sporadically and the probability that they represent opportunists acquired by humans from another vertebrate or invertebrate host cannot be ruled out. Of particular interest is the question whether some microsporidia of invertebrates or poikilothermic vertebrates can adapt to life within a warm-blooded vertebrate (humans included), provided that other conditions (e.g. immunity and nutrition needs) allow. For example, *Nosema algerae* (Vávra & Undeen, 1970), a microsporidian originally described as a mosquito parasite, was recently found to infect humans (Visvesvara *et al*. 1999) and was subsequently relocated to the genus *Brachiola* and named *B. algerae* (Lowman, Takvorian & Cali, 2000).

The present paper explores the opportunistic capacity of *B. algerae* in a model of the severe combined immunodeficient (SCID) mouse. Data on the fine structure of *B. algerae* and the host–parasite relationship at the fine structure level, are also reported.

* Corresponding author: Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic. Tel: +420 5 41562262. Fax: +420 5 748841. E-mail: koudelab@vfu.cz

 © 2001 Cambridge University Press
DOI: 10.1017/S0031182001008228 Printed in the United Kingdom
https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

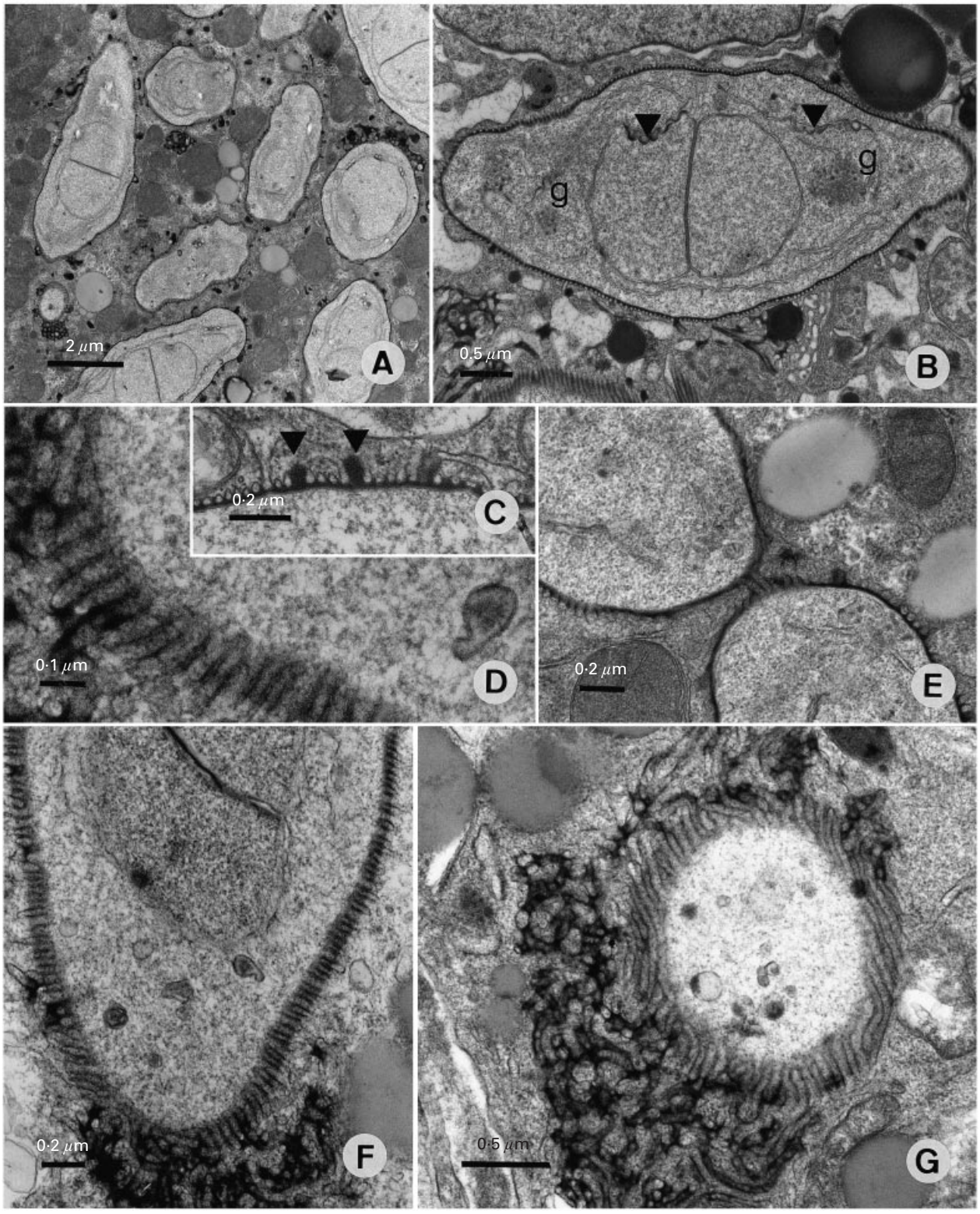


Fig. 1. Fine structure of *Brachiola algerae* meronts in liver cells of SCID mice. (Abbreviations: Do, dense outgrows: TF, tubular fleece, TT, tubulo-vesicular tuft, DS, dense substance). (A) Individual meronts in the cytoplasm of a hepatocyte. (B) Meront showing the regular pattern of cross-sectioned tubules forming a kind of TF on meront surface. Electron-dense material is located in the perinuclear cisterna (arrowheads) and in the ER. Two Golgi areas are located at opposite poles of the cell (g). (C) Detail of the meront surface shows the DS covering the plasma membrane and underlying the elements of TF. The DS protrudes at some points as DO throughout the TF layer into the host cell cytoplasm (arrowheads). (D) Tangential section throughout the tubules of the TF. (E) The tubules of the TF layer connect temporarily 2 daughter meronts after cytokinensis (E, F). Tubules of the TT extend from one pole of the meront into the host cell cytoplasm in longitudinal (E) and grazing section of the meront pole (F). Note the continuity between the TF and TT tubules, the electron density of TT tubules and higher electron density of the ground cytoplasm surrounding the tuft. At some points the TF tubules extend as narrow channels into the host cell cytoplasm.

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

MATERIALS AND METHODS

Parasite

The human isolate of *Brachiola algerae* (syn. *Nosema algerae*) used in this study was grown from corneal scrapings of an HIV-negative, 67-year-old man from Mexico (isolate CDC: V404). The organism was continuously cultivated in monkey kidney cell (E-6) monolayers at 37 °C as previously described (Visvesvara *et al*. 1999). Spores were obtained from the supernatant in culture flasks centrifuged at 2000 ***g*** at 4 °C for 10 min and were suspended in sterile phosphate-buffered saline (PBS, pH 7·2) and counted using a haemocytometer.

Animals and inoculations

SCID mice were housed in groups of 3 animals per cage in flexible isolators (BEM, Znojmo, Czech Republic) with high-efficiency particulate air (HEPA) filters. All cages, food, water, and bedding were sterilized before use. A total of 18, 8 to 11-week-old SCID mice of both sexes were used in this study, with 3 mice in each inoculation group. Inocula (10^7 *B. algerae* spores suspended in a 0·5 ml of PBS) were applied in drops to the eye surface or inoculated perorally by tube. Mice were also injected intraperitoneally, intramuscularly in the gluteal muscle, or subcutaneously in the tail with the same inoculum. Three SCID mice served as controls. All mice were monitored daily for the development of clinical signs. On 60 days post-infection (p.i.), mice from all groups were euthanized and necropsied.

Light microscopy

At necropsy, fresh peritoneal fluid and urine samples of infected SCID mice were examined for the presence of microsporidia by staining with the optical brightener Calcofluor White M2R (Vávra *et al*. 1993). Twenty-eight tissue samples (liver, gall bladder, pancreas, lung, cardiac muscle, spleen, brain, kidney, urinary bladder, diaphragm, stomach, duodenum, middle jejunum, ileum, cecum, colon, rectum, mesenteric lymph node, upper cheek, eyelid, nose, foot pads, tongue, jowl, scrotum or external genitalia of females, gluteal muscles, triceps brachii, inguinal mesenteric lymph nodes) from each mouse were fixed in 10% neutral buffered formalin and processed for light microscopy using standard methods. Paraffin sections were stained with haemotoxylin-eosin, Gram's method, and with the optical brightener Calcofluor White M2R as cited above.

Electron microscopy

For transmission electron microscopy (TEM), tissue samples from liver were fixed in 2·5% glutaraldehyde in cacodylate buffer (0·1 M, pH 7·4) at 4 °C and post-fixed in 1% osmium tetroxide in the same buffer. Tissue samples were then washed 3 times in cacodylate buffer, dehydrated in a graded ethanol series and embedded in Durcupan epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined using a JEOL 1010 transmission electron microscope.

RESULTS

Pathology and infected tissues

No clinical signs of disease were seen in experimental animals, whatever the application or inoculation method. At necropsy (60 days p.i.), only the SCID mice infected via the eye showed ascites in the abdominal cavity and hepatomegaly with numerous greyish spots throughout the liver. Spores of *B. algerae* were sporadically detected in smears prepared from peritoneal macrophages of these eye-infected SCID mice. In the liver, the infection was concentrated at the periphery of the organ. Histologically, lesions consisted of multifocal miliary granulomas and necroses containing parasites or intracellular clusters of microsporidia without associated inflammatory response (Fig. 3F). The granulomas and necroses were usually located in the tissue adjacent to the peritoneum.

Microsporidial spores were identified with Gram's stain as gram-positive rod-shaped bodies, free within foci of granulomas or necroses, or located intracellularly within hepatocytes or macrophages. No spores were found in conjunctiva and cornea of eye-infected SCID mice. Peroral infection as well as intraperitoneal, intramuscular, and subcutaneous inoculations did not cause infection in any tissue examined.

Fine structural features of the human isolate of Brachiola algerae

General characters. All developmental stages observed were spindle-shaped or oval cells with one diplokaryon, dispersed singly within the cytoplasm of the host liver cells (Fig. 1A, B). Parasite cells containing more than 1 diplokaryon were not observed. The cytoplasm of parasite cells contained numerous ribosomes, most of them freely dispersed and some of them attached to the few existing lamellae of endoplasmic reticulum (ER). Some ER lamellae, including the perinuclear cisterna, contained electron-dense material on lamellar membranes and in the cisternal lumen (Fig. 1B). The cytoplasmic membrane surrounding individual cells of all life-cycle stages was covered with characteristically structured wall material. This material is homologous with the 'secretions' appearing during the life-cycle on the outer face of cytoplasmic membrane of other microsporidia (Vávra & Larsson, 1999). The wall material encrusting the plasma-

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

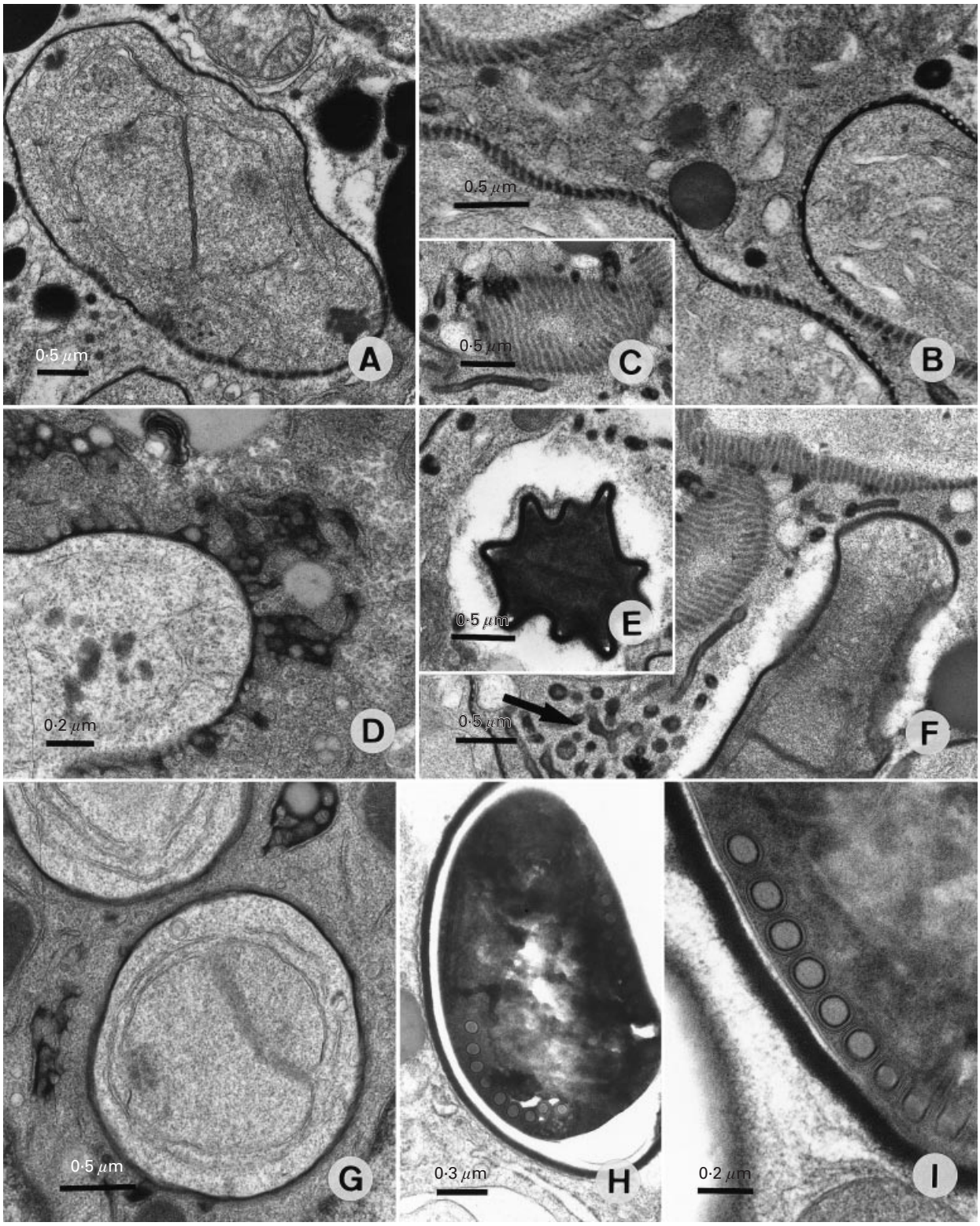


Fig. 2. Fine structure of sporogonial stages of *Brachiola algerae* in liver cells of SCID mice. (Abbreviations: TF, tubular fleece; TT, tubulo-vesicular tuft; WM, wall material.) (A) Sporont showing the thickening of the electron-dense WM, while the elements of TF become less prominent. The cytoplasm of the sporont contains electron-dense material close to the cell poles. (B) Higher magnification of the cell surface of sporonts. (C) Grazing section of the sporont surface. (D) The tubules of TT fuse during sporogony into irregular blebs of electron-dense material with circular electron-lucent inclusions. (E, F) Sporoblasts sectioned transversally (E) and longitudinally (F). A single layer of electron-dense material covers the cell. The material remaining from the TT now forms irregular streaks and granules (arrow). (G) Late sporonts are covered by 2 layers of material of different electron density with no tubules present. (H) Mature spore showing 6 thicker and 3 thinner polar filament coils. (I) The structure of the spore surface and polar filament coils at higher magnification.

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

lemma was in direct contact with the cytoplasm of the host cell. In each cell, there were usually 2 areas of small aggregated, electron-dense vesicles situated close to each of the twin nuclei forming the diplokaryon and positioned along the long axis of the cell. These aggregates corresponded to what is known as microsporidian Golgi apparatus (Fig. 1B).

Life-cycle stages. The terms 'meront' and 'sporont' have been used in the present study as an approximation because the special structure of the cell surface sometimes prevented the unambiguous identification of individual developmental stages into either the merogonic or sporogonic part of the life-cycle (Vávra & Undeen, 1970). In this situation, minor changes in the thickness and structure of the wall material covering the outer face of the parasite plasmalemma (Canning & Sinden, 1973) were used as the feature distinguishing different life-cycle stages.

Meronts. Those *B. algerae* cells that had the following features were considered as meronts. Cells that were elongate (approx. 5–8 μm in size) and spindle-shaped (Fig. 1A, B), with the cell membrane covered with a rather uniform coat of wall material consisting of 2 structural elements: a thin (approx. 20 nm) inner layer of dense, structureless substance ('dense substance', DS), covered on the outside by a single layer of tightly packed, parallel tubules forming a sheet or fleece covering the cell surface ('tubular fleece', TF) (Fig. 1B, C). The tubules were revealed in tangential sections as a ladder-like structure of wide, less dense strips (= interior of tubules), alternating with 2 closely adjacent dense lines (= walls of the 2 adjacent tubules) (Fig. 1D). The TF tubules temporarily connected the twin meronts during cytokinesis (Fig. 1E). The TF tubules were mostly of the same diameter (40–50 nm in cross-section), but occasionally larger tubules were interposed. Some tubules also expanded, in the form of irregular, electron-lucent, tubulo-vesicular channels (TVC), from the parasite surface deep into the host cell cytoplasm (Fig. 3C, D, E). The DS of the wall material covering the plasmalemma protruded at some points throughout the TF layer and formed irregular dense outgrowths (DO) invaginated into the host cell cytoplasm. (Fig. 1C). A conspicuous structure seen in some meronts was a meshwork of branching electron-dense tubules and vesicles emerging from a pole of the meront cell and reaching deep as a 'tubulo-vesicular tuft' (TT) into the host cell cytoplasm (Fig. 1E, F). The TF and TT tubules seemed to be continuous in some places, but their exact relationship could not be determined because of the complexity of the tubulo-vesicular meshwork. The dense aspect of the TT tubules was due to an electron-dense material covering the tubules and interspersed among the elements of the TT. Also the optical superposition of the tubules contributed to their apparent density (Figs 1E, F and 3A–C).

Sporogonial stages. Those cells in which the layer of the DS of the wall material increased in thickness were labelled as sporonts. At the same time the TF tubules on the plasmalemma became progressively less prominent and less abundant (Fig. 2A, B). As the TF tubules became more deeply embedded in the electron-dense DS matrix, they were no longer revealed in tangential sections of the cell surface as discrete units and the parasite cell seemed to be covered by alternating fuzzy strips of more or less electron-dense material (Fig. 2B, C). In late sporonts the TF tubules, the TVC, and the dense outgrowths (DO) disappeared entirely and the parasite cells were surrounded by a thick layer of dense material with a structureless less dense layer on top (Fig. 2G). At the sporoblast stage, the top less-dense layer disappeared and the parasite cell was covered by structureless dark material only (Fig. 2E, F). Early sporoblasts were elongate cells with homogeneously dense material on their surface, 1 diplokaryon and a relatively dense cytoplasm (Fig. 2F). Also present were extremely distorted cells of high electron density that prevented the recognition of internal cell structure (Fig. 2E). A clear halo was present around the sporoblasts that were in the process of maturing into spores (Fig. 2E). The halo probably represented the area occupied by the parasite before its volume condensation during sporogony. In the course of sporulation, the tubules of the TT fused into an irregular, dense mass with some lucent inclusions (Fig 2D). Mature, non-extruded spores were extremely rare in our material, most spores were extruded, and looked empty or with some cytoplasmic debris inside (Fig. 3G, H). Spores showed a trilaminar spore envelope, consisting of an approx. 110 nm layer of high electron density, a 160–220 nm layer of electron-lucent material and a plasma membrane (Fig. 3G). The polar filament was of anisofilar type, showing 9 coils, 6 of them slightly thicker than the 3 posterior ones (Fig. 2H, I). Polaroplast structure was not recognized in our material. During sporoblast and spore formation some electron-dense material produced originally at the surface of parasite cells (probably the material of the TT) was now completely detached in the degenerating cytoplasm of the host cell, being represented by long, dense, thick and branching tubules or electron-dense globules (Fig. 2F). In some cells, the empty extruded spores showed signs of degradation. The exospore layer was thinner, the endospore was less transparent, the spore interior looked finely granular. The spore was surrounded by a large vacuole containing finely granular material (Fig. 3I).

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

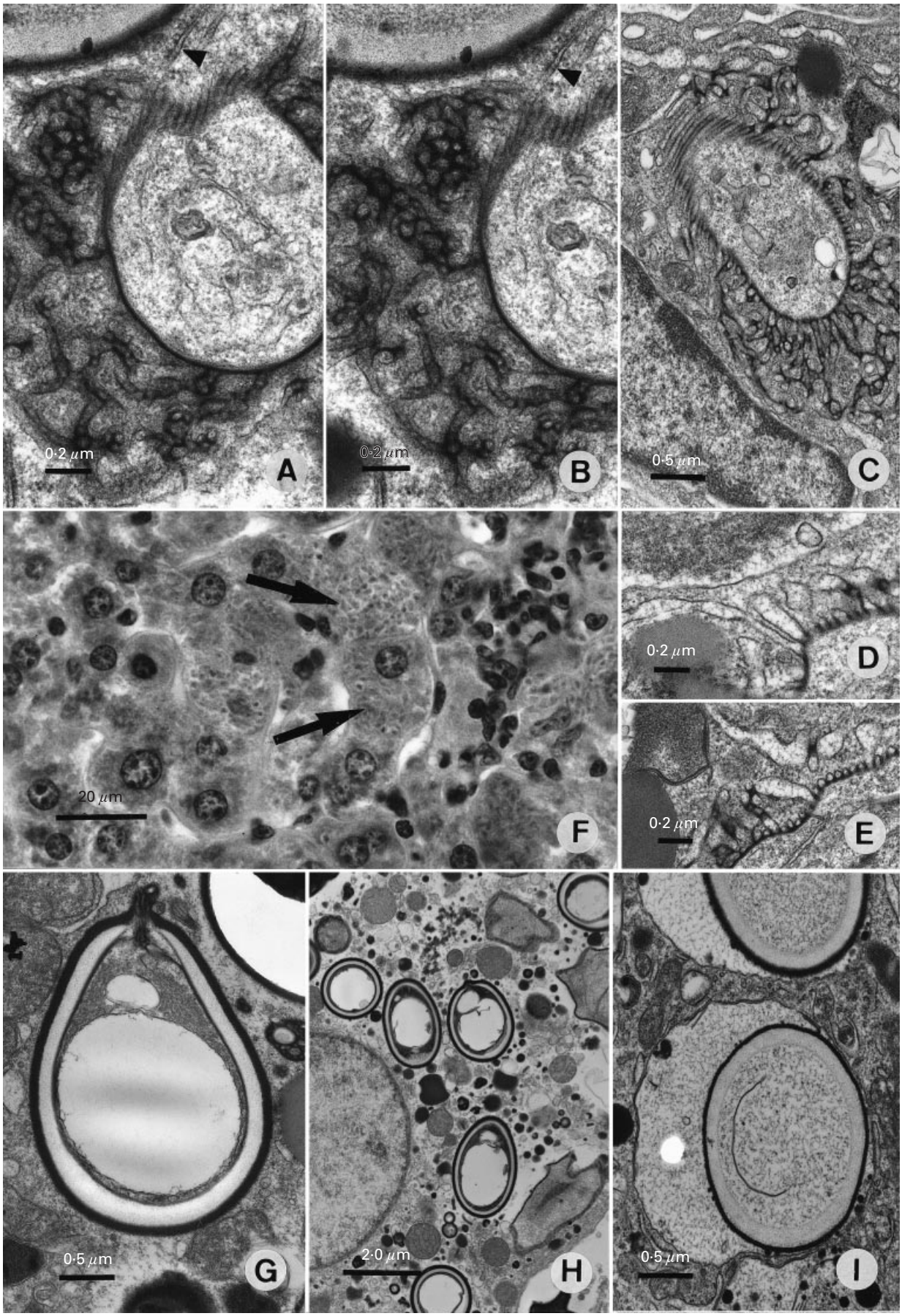


Fig. 3. Host–parasite cellular relationships of *Brachiola algerae* in the liver of SCID mice. (Abbreviations: TF, tubular fleece; TT, tubulo-vesicular tuft; TVC, tubulo-vesicular channels). (A, B) Stereocouple image of the TT

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

DISCUSSION

Epidemiology

It is of interest that only the application of spores to the eyeball surface led to a successive infection of the internal organ of SCID mice. A similar phenomenon was noticed during experimental infections of SCID mice with the microsporidian *Vittaforma corneae* (Koudela *et al*. 1999). We believe that the relatively low temperature of the eye and conjunctival surface (around 35 °C) favoured the microsporidian that was originally from a poikilothermic invertebrate host, and thus helped its adaptation to the conditions within the mammalian body. The temperature itself, however, is not the only favourable factor, because the microsporidian isolate used in our experiments has been continuously cultivated in cell culture at 37 °C and thus had adapted to that temperature (Visvesvara *et al*. 1999). It is also possible that the tears stimulated the spores in the inoculum to extrude the sporoplasms and to start a milk, local (transitory?) infection (not detected in our experiments), that was later carried by macrophages into the internal organs. Whatever the mechanism, our results suggest that the eye is an important portal of entry of microsporidia into vertebrate hosts. Ocular infections by microsporidia in humans are relatively common and in several of them the pathogen has been identified as not belonging to the usual spectrum of human-infecting species (Friedberg & Ritterband, 1999; Font *et al*. 2000). Such infections are evidently accidental but, as our results show, their potential to disseminate from the eye into visceral organs cannot be ignored.

Tissue specificity and growth

We have no explanation for the fact that the only infected organ after a long post-infection period (60 days) was the liver. We noted that practically all the spores of *B. algerae* present in the liver tissue were 'empty' i.e. extruded, and in the process of degradation by the host cells. Also practically, all developmental stages observed contained a single diplokaryon only and actively dividing cells were rarely seen. Thus, the overall aspect of the infection was a slow, chronic process. Both this and the limited organ distribution may reflect the fact that the conditions within a homoiothermic vertebrate are not fully favourable for *B. algerae*.

Our observation is the first one to demonstrate the capability of *B. algerae* to infect visceral organs of mammalian hosts. Previous experiments, in which *Nosema algerae* spores were subcutanously injected into the feet and tails of mice, showed that the parasite is able to grow for a limited period of time in tissues close to the infection site (Undeen & Alger, 1976; Trammer *et al*. 1997). These results seemed to indicate that temperature was the factor preventing the infection of tissues deep within the body, allowing the microsporidian to grow only at sites with lower than 37 °C temperature. This explanation, however, probably is not quite sufficient. The same strain of *B. algerae* used by Trammer *et al*. (1997) was able to grow at 37 °C in tissue culture (Trammer *et al*. 1999). Lowman *et al*. (2000) showed that another strain of *B. algerae* (the species type strain) was also able to grow at 37 °C in tissue culture, but that the generation time was considerably extended at that temperature. It is possible that *B. algerae* has the general ability to grow at 37 °C (disregarding strain origin). However, this ability is near the temperature limit of the organism and is more easily accomplished in tissue culture than in the living organism, where the inoculation dosage is lower than in tissue culture and where immune reactions of the host intervene.

Ultrastructure

The human isolate of *B. algerae* grown in SCID mice is structurally similar to the type material of *N. algerae* described from the tissues of *Anopheles stephensi* mosquitoes (Vávra & Undeen, 1970), but displays more extensive tubulo-vesicular outgrowths of the cell surface. The original description of the species, as well as subsequent electron microscope studies, all described the vesiculotubular structures, forming the external layer of parasite developmental stages (Vávra & Undeen, 1970; Canning & Sinden, 1973), including the sporoplasms (Avery & Anthony, 1983). Isolated tubules and tubular whorls of parasite origin located in the host cell cytoplasm were also previously observed (Vávra & Undeen, 1970; Moura *et al*. 1999; Visvesvara *et al*. 1999). The present material, however, thanks to the compact character of the host tissue (liver cells), showed better the complexity of the 'secretory material' covering the

extending from the meront surface into the host cell cytoplasm. Note the branching pattern of the tubules, their density and fusion into globular formations. The host cell cytoplasm interwoven in the TT is more electron dense. The TF tubules extend into the host cell cytoplasm (arrowheads). (C) Total view of cell surface extensions of the parasite into the host cell. (D, E) High-power view of TVC into the host cell. (F) Histological section of liver tissue shows numerous developmental stages in hepatocytes (arrows). (G) Mature spore during extrusion. (H) Empty spore ghosts are found in infected liver cells. (I) Spore ghosts in the process of degradation by host cells.

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

parasite cells and its dynamics during the life-cycle. It is evident from our observations, that the tubular coat on the parasite surface increases enormously the surface area of the microsporidian cell. The TF and TT tubules, which branch off of the parasite surface, penetrate deeply into the host cell cytoplasm, sometimes for a distance of several microns.

This function and chemistry of the electron-dense 'secretory' material occurring at the plasmalemma surface is unknown. However, it seems that this material could have a dual function. The homogeneous DS probably represents the material of the exospore layer of the future spore wall and corresponds in this respect to the electron-dense secretions occurring in other microsporidia on the plasmalemma of sporonts and sporulation stages (Vávra & Larsson, 1999). The tubular material (TF, TT and TVC) on the parasite cell surface probably mediates the 'metabolic contact' of the parasite with host cell cytoplasm and possibly takes part in the transport of nutrients into the parasite.

Later in the life-cycle, when the parasite approaches the final stages of development, the need for contact with the host cell evidently diminishes. The tubules on the parasite cells progressively disappear and the tubular material stretching into host cell cytoplasm 'degenerates' into dense clumps interspersed with the host cell cytoplasm. The early (already in merogony) occurrence of wall material on cells *B. algerae* places this microsporidian among those rare species in which the cell wall is precociously laid down (Vávra & Larsson, 1999). Three other opportunistic human microsporidia belong to the same category: *Trachipleistophora hominis* Hollister, Canning, Weidner, Field, Kench et Mariott 1996, *T. anthropophthera* Vávra, Yachnis, Shadduck et Orenstein 1998 and *Brachiola vesicularum* Cali, Takvorian, Lewin, Rendel, Sian, Wittner, Tanowitz, Keohane et Weiss 1998. While *B. vesicularum* might be conspecific with *B. algerae* (see below), the original host for both of the *Trachipleistophora* species is not known. Transmission experiments suggest, however, that *T. hominis* might be originally an insect (dipteran) parasite (Weidner *et al.* 1999). The question arises whether a low degree of host specificity is not in some way associated with the presence of cell wall material enabling the parasite to satisfy its metabolic needs from a wide range of cell types. It has to be mentioned that *B. algerae* has a very low host specificity that is, it is able to infect (besides anopheline mosquitoes, original hosts) (Vávra & Undeen, 1970; Canning & Sinden, 1973) a wide range of insects (Undeen & Maddox, 1973), tissue culture cells (Undeen, 1975; Streett, Ralph & Hink 1980; Moura *et al.* 1999; Trammer *et al.* 1999; Lowman *et al.* 2000), mice (Undeen & Alger, 1976; Trammer *et al.* 1997) and humans (Visvesvara *et al.* 1999).

Brachiola algerae, *its phylogenetic position and relationship to* Brachiola vesicularum

Nosema algerae Vávra et Undeen, 1970, was recently renamed *Brachiola algerae* n. comb. by Lowman *et al.* (2000). This act was justified by (1) previous molecular phylogeny data, indicating that *N. algerae* does not cluswter with typical representative of the genus *Nosema* (Baker *et al.* 1994; Müller *et al.* 2000) and (2) similarity of structures decorating the cytoplasmic membrane to those present on cells of *B. vesicularum*, the type species of the genus *Brachiola*.

The transfer of *N. algerae* into the genus *Brachiola* opens the question of possible identity of *B. vesicularum* and *B. algerae*. Originally, it was argued that both these species are different because *N. algerae* had a different tissue specificity since it was not able to grow at mammalian body temperature and its spores had different arrangement of the polar filament and because a molecular probe for *N. algerae* failed to amplify *B. vesicularum* DNA (Cali *et al.* 1998). However, further research showed that several of these distinguishing characters are not valid (see Cheney, Lafranchi-Tristem and Canning (2000) for discussion of this topic). Trammer *et al.* (1999) indicated the *N. algerae* and *B. vesicularum* might actually be conspecific, a view further discussed and corroborated by Cheney *et al.* (2000). Presently, only 2 characters differentiate *N. algerae* and *B. vesicularum*: the presence in *B. vesicularum* of 'protoplasmic extensions' (long, contorted, tubulovesicular extensions of a portion of the cell) and the failure of primers specific for *N. algerae* to amplify the *B. vesicularum* DNA. It is impossible to estimate rightfully the significance of the last-mentioned negative character in which fixed, paraffin embedded biopsy material was used for DNA extraction and the PCR reaction by Cali *et al.* (1998). Thus, presently the only significant difference between *B. vesicularum* and *B. algerae* is the presence of 'protoplasmic extensions' in the former species. In our opinion, these 'protoplasmic extensions' are a weak character and probably do not justify the separate species status of *B. vesicularum* and *B. algerae*. The reasons are 2 fold: the 'protoplasmic extensions' seem to be of rare occurrence in *B. vesicularum* (see photomicrographs by Cali *et al.* 1998). *B. algerae* (the species type strain) forms long cells of contorted outline, similar in some respects to 'protoplasmic extensions', when grown at 37 °C (see Figs 15 and 16 in Lowman *et al.* 2000).

Another question that should be discussed is the relationship of the genus *Brachiola* to other microsporidia having precocious secretory wall material on plasma membrane and the life-cycle of the *Nosema* type. Such is the genus *Anncaliia* Issi, Krylova et Nicolaeva 1993, established for microsporidia, the life-cycle stages of which have a supplementary external layer on the cell envelope

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

consisting of tubules and secretory granules. External tubulovesicular structures, very similar to that of *B. algerae*, were described on meronts of *A. varivestis* (syn. *Nosema varivestis*) (Brooks, Hazard & Becnel, 1985). Cali *et al.* (1998) dismissed the consideration that their microsporidium, presently known as *Brachiola vesicularum*, belongs to the genus *Anncaliia*, arguing that thermophily is the distinguishing character. This argument is no longer tenable because of the experience with thermotolerance of *B. algerae*. The possibility that *Brachiola* and *Anncaliia* are cogeneric have to be decided by future studies involving molecular biology methods.

REFERENCES

AVERY, S. W. & ANTHONY, D. W. (1983). Ultrastructural study of early development of *Nosema algerae* in *Anopheles albimanus*. *Journal of Invertebrate Pathology* **42**, 87–95.

BAKER, M. D., VOSSBRINCK, C. R., MADDOX, J. V. & UNDEEN, A. H. (1994). Phylogenetic relationships among *Vairimorpha* and *Nosema* species (Microspora) based on ribosomal RNA sequence data. *Journal of Invertebrate Pathology* **64**, 100–106.

BROOKS, W. M., HAZARD, E. I. & BECNEL, J. J. (1985). Two new species of *Nosema* (Microsporida: Nosematidae) from the Mexican Bean Beetle *Epilachna varivestis* (Coleoptera: Coccinellidae). *Journal of Protozoology* **32**, 525–535.

CALI, A., TAKVORIAN, P. M., LEWIN, S., RENDEL, M., SIAN, C. S., WITTNER, M., TANOWITZ, H. B., KEOHANE, E. & WEISS, L. M. (1998). *Brachiola vesicularum*, n.g., n.sp., a new microsporidium associated with AIDS and myositis. *Journal of Eukaryotic Microbiology* **45**, 240–251.

CANNING, E. U. & SINDEN, R. E. (1973). Ultrastructural observations on the development of *Nosema algerae* Vávra and Undeen (Microporida, Nosematidae) in the mosquito *Anopheles stephensi* Liston. *Protistologica* **9**, 405–415.

CHENEY, S. A., LAFRANCHI-TRISTEM, N. J. & CANNING, E. U. (2000). Phylogenetic relationships of *Pleistophora*-like microsporidia based on small subunit ribosomal DNA sequences and implications for the source of *Trachipleistophora hominis* infections. *Journal of Eukaryotic Microbiology* **45**, 280–287.

CURRY, A. (1999). Human microsporidial infections and possible animal sources. *Current Opinions in Infectious Diseases* **12**, 473–480.

DIDIER, E. S., SNOWDEN, K. F. & SHADDUCK, J. A. (1998). Biology of microsporidian species infecting mammals. *Advances in Parasitology* **40**, 283–320.

FONT, R. L., SAMAHA, A. N., KEENER, M. J., CHEVEZ-BARIOZ, P. & GOOSEY, J. D. (2000). Corneal microsporidiosis – Report of case, including electron microscopy observations. *Ophthalmology* **107**, 1769–1775.

FRIEDBERG, D. A. & RITTERBAND, D. C. (1999). Ocular microsporidiosis. In *The Microsporidia and Microsporidiosis* (ed. Wittner, M. & Weiss, L. M.), pp. 293–314. ASM Press, Washington DC.

HOLLISTER, W. S., CANNING, E. U., WEIDNER, E., FIELD, A. S., KENCH, J. & MARIOTT, D. J. (1996). Development and ultrastructure of *Trachipleistophora hominis* n.g., n.sp. after *in vitro* isolation from an AIDS patient and inoculation into athymic mice. *Parasitology* **112**, 143–154.

ISSI, I. V., KRYLOVA, S. V. & NICOLAEVA, V. M. (1993). The ultrastructure of the microsporidium *Nosema meligethi* and establishment of the new genus. *Anncaliia Parazitologyia* **27**, 127–133.

KEELING, P. J. & MCFADDEN, G. I. (1998). Origins of microsporidia. *Trends in Microbiology* **6**, 19–23.

KEELING, P. J., LUKER, M. A. & PALMER, J. D. (2000). Evidence from beta-tubulin phylogeny that microsporidia evolved from within the fungi. *Molecular Biology and Evolution* **17**, 23–31.

KOUDELA, B., ŠLAPÁKOVÁ, D., DIDIER, P. J. & DIDIER, E. S. (1999). Experimental infection of SCID mice as a model of *Vittaforma corneae* microsporidiosis. *Abstracts of the 52nd Annual Meetings, Society of Protozoologists, North Carolina University, Raleigh, May 26–29, 1999, Abstract W67.*

LOWMAN, P. M., TAKVORIAN, P. M. & CALI, A. (2000). The effect of elevated temperatures and various time-temperature combinations on the development of *Brachiola* (*Nosema*) *algerae* n. comb. in mammalian cell culture. *Journal of Eukaryotic Microbiology* **45**, 240–251.

MOURA, H., DA SILVA, A., MOURA, I., SCHWARTZ, D. A., LEITCH, G., WALLACE, S., PIENIAZEK, N. J., WIRTZ, R. A. & VISVESVARA, G. S. (1999). Characterization of *Nosema algerae* isolates after continuous cultivation in mammalian cells at 37 °C. *Journal of Eukaryotic Microbiology* **46**, 14S–16S.

MÜLLER, A., TRAMMER, T., CHIORALIA, G., SEITZ, H. M., DIEHL, V. & FRANZEN, C. (2000). Ribosomal RNA of *Nosema algerae* and phylogenetic relationship to other microsporidia. *Parasitology Research* **86**, 18–23.

SPRAGUE, V. (1977). Systematics of the Microsporidia. In *Comparative Pathobiology* (ed. Bulla, L. A. & Cheng, T. C.), Vol. 2., pp. 1–510. Plenum Press, New York.

STREETT, D. A., RALPH, D. & HINK, W. F. (1980). Replication of *Nosema algerae* in three insect cell lines. *Journal of Protozoology* **27**, 113–117.

TRAMMER, T., CHIORALIA, G., MAIER, W. A. & SEITZ, H. M. (1999). *In vitro* replication of *Nosema algerae* (Microsporidia), a parasite of Anopheline mosquitoes, in human cells above 36 °C. *Journal of Eukaryotic Microbiology* **46**, 464–468.

TRAMMER, T., DOMBROWSKI, F., DOEHRING, M., MAIER, W. A. & SEITZ, H. M. (1997). Opportunistic properties of *Nosema algerae* (Microspora), a mosquito parasite, in immunocompromised mice. *Journal of Eukaryotic Microbiology* **44**, 258–262.

UNDEEN, A. H. (1975). Growth of *Nosema algerae* in pig kidney cell cultures. *Journal of Protozoology* **22**, 107–110.

UNDEEN, A. H. & ALGER, N. E. (1976). *Nosema algerae*: infection of the white mouse by a mosquito parasite. *Experimental Parasitology* **40**, 86–88.

UNDEEN, A. H. & MADDOX, J. V. (1973). The infection of nonmosquito hosts by injection with spores of the microsporidan *Nosema algerae*. *Journal of Invertebrate Pathology* **22**, 258–265.

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press

VÁVRA, J., DAHBIOVÁ, R., HOLLISTER, W. S. & CANNING, E. U. (1993). Staining of microsporidian spores by optical brighteners with remarks on the use of brighteners for the diagnosis of AIDS associated human microsporidioses. *Folia Parasitologica* **40**, 267–272.

VÁVRA, J. & LARSSON, J. I. R. (1999). Structure of microsporidia. In *The Microsporidia and Microsporidiosis* (ed. Witter, M. & Weiss, L. M.), pp. 7–84. ASM Press, Washington DC.

VÁVRA, J. & UNDEEN, A. H. (1970). *Nosema algerae* n. sp. (Cnidospora, Microsporida) a pathogen in a laboratory colony of *Anopheles stephensi* Liston (Diptera, Culicidae). *Journal of Protozoology* **17**, 240–249.

VÁVRA, J., YACHNIS, A. T., SHADDUCK, J. A. & ORENSTEIN, J. M. (1998). Microsporidia of the genus *Trachipleistophora* – causative agents of human microsporidiosis: description of *Trachipleistophora anthropophthera* n. sp. (Protozoa: Microsporidia) *Journal of Eukaryotic Microbiology* **45**, 273–283.

VISVESVARA, G. S., BELLOSO, M., MOURA, H., DA SILVA, A., MOURA, I., LEITCH, G., SCHWARTZ, D. A., CHEVEZ-BARRIOS, P., WALLACE, S., PIENIAZEK, N. J. & GOOSEY, J. (1999). Isolation of *Nosema algerae* from the cornea of an immunocompetent patient. *Journal of Eukaryotic Microbiology* **46**, 10S.

WEIDNER, E., CANNING, E. U., RUTLEDGE, R. & MEEL, L. (1999). Mosquito (Diptera: Culicidae) host compatibility and vector competency for the human myositic parasite *Trachipleistophora hominis* (Phylum Microspora). *Journal of Medical Entomology* **36**, 522–525.

WEISS, L. M. & VOSSBRINCK, C. R. (1999). Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the Microsporidia. In *The Microsporidia and Microsporidiosis* (ed. Wittner, M. & Weiss, L. M.), pp. 129–171. ASM Press, Washington DC.

WITTNER, M. (1999). Historic perspectives on the microsporidia: expanding horizons. In *The Microsporidia and Microsporidiosis* (ed. Wittner, M. & Weiss, L. M.), pp. 1–6. ASM Press, Washington DC.

https://doi.org/10.1017/S0031182001008228 Published online by Cambridge University Press