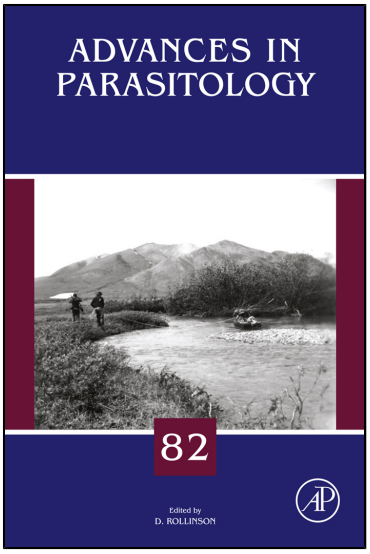

From Vávra, J., Lukeš, J., 2013. Microsporidia and 'The Art of Living Together'.
In: Rollinson, D. (Ed.), Advances in Parasitology, Academic Press, pp. 253–320.
ISBN: 9780124077065

Academic Press

CHAPTER FOUR

Microsporidia and 'The Art of Living Together'

Jiří Vávra*,†,1, Julius Lukeš*,

*Biology Centre, Institute of Parasitology, Czech Academy of Sciences, and Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
†Faculty of Science, Charles University in Prague, Prague, Czech Republic
[1]Corresponding author: E-mail: jiri.vavra@natur.cuni.cz

Contents

Advances in Parasitology, Volume 82
ISSN 0065-308X, http://dx.doi.org/10.1016/B978-0-12-407706-5.00004-6

Advances in Parasitology, First Edition, 2013, 253-319

Abstract

Parasitism, aptly defined as one of the 'living-together' strategies (Trager, 1986), presents a dynamic system in which the parasite and its host are under evolutionary pressure to evolve new and specific adaptations, thus enabling the coexistence of the two closely interacting partners. Microsporidia are very frequently encountered obligatory intracellular protistan parasites that can infect both animals and some protists and are a consummate example of various aspects of the 'living-together' strategy. Microsporidia, relatives of fungi in the superkingdom Opisthokonta, belong to the relatively small group of parasites for which the host cell cytoplasm is the site of both reproduction and maturation. The structural and physiological reduction of their vegetative stage, together with the manipulation of host cell physiology, enables microsporidia to live in the cytosolic environment for most of their life cycle in a way resembling endocytobionts. The ability to form structurally complex spores and the invention and assembly of a unique injection mechanism enable microsporidia to disperse within host tissues and between host organisms, resulting in long-lasting infections. Microsporidia have adapted their genomes to the intracellular way of life, evolved strategies how to obtain nutrients directly from the host and how to manipulate not only the infected cells, but also the hosts themselves. The enormous variability of host organisms and their tissues provide microsporidian parasites a virtually limitless terrain for diversification and ecological expansion. This review attempts to present a general overview of microsporidia, emphasising some less known and/or more recently discovered facets of their biology.

1. DEFINING MICROSPORIDIA

1.1. Basic Characteristics

Microsporidia are protistan (=single cell eukaryotes) parasites of animals and, less frequently, of protists belonging to the 'Sar' kingdom as defined by Adl et al. (2012). Microsporidia have been known to science for about 150 years, and 1300 to 1500 species in 187 genera have been described. These unique organisms are strictly intracellular parasites with relatively uniform life cycle (Cali and Takvorian, 1999). The germinating spore injects the spore contents in the form of a small cell, the 'sporoplasm', into the cytoplasm of a host cell by means of an explosively evaginable 'injection tube' (usually referred to as a polar tube, polar filament or invasion tube) (Delbac and Polonais, 2008; Franzen, 2004, 2005; Weidner, 1972; Xu and Weiss, 2005) (see Section 3.1, p. 275). The sporoplasm grows into cells called meronts, which divide by 'merogony' into daughter meronts. The meronts progressively fill the cytoplasm of the host cell. Then, after an unknown signal, the synthesis of proteins that will constitute the spore wall is activated and the cell wall material consisting of chitin and microsporidia-specific proteins is progressively deposited on the plasma membrane of stages called sporonts

(Bohne et al., 2000; Brosson et al., 2005; Hayman et al., 2001; Li et al., 2009; Peuvel-Fanget et al., 2006; Southern et al., 2007; Wu et al., 2008, 2009; Xu et al., 2006). Sporonts, depending on the species, may continue to divide and produce daughter sporonts but, finally, each sporont cell matures by a process termed sporogony into a complex infective spore equipped with an injection apparatus (see Section 3.1, p. 275). The spore is thus the product of internal differentiation of a single cell (Vávra and Larsson, 1999). It is the only stage that can survive in the environment and is responsible for the dissemination of the parasite. The presence of the injection apparatus in the spore is an autapomorphic character that sharply delineates microsporidia as a monophyletic taxon presently classified as the phylum Microsporidia.

1.2. What Is in a Name?

The taxonomy of the phylum Microsporidia is, by tradition, considered under the Code of Zoological Nomenclature (ICZN). It has been proposed that this tradition continues (Redhead et al., 2009) despite the fact that Microsporidia are now believed to be related to Fungi, the taxonomy of which is formally subjected to the Botanical Code [ICBN]. Practical reasons favour this solution; the Zoological and Botanical Codes are technically incompatible and the nature of microsporidia–fungi relationship is unresolved (see Section 1.3, p. 257 and 1.3.4, p. 262) (Note that in this paper 'Fungi' and 'Microsporidia' are used when meaning the respective taxons, otherwise the vernacular names 'fungi' and 'microsporidia' are used).

Although microsporidia represent a well-defined monophyletic group of organisms, it is paradoxically the phylum name that is problematic. The French embryologist Edouard-Gerard Balbiani was the first to use the name 'microsporidies' (Balbiani, 1882), but did not specify their taxonomic level. This led to a taxonomic confusion and a discussion concerning the proper name for the phylum representing these unicells. Most authors presently use the name-author-date combination for the phylum Microsporidia Balbiani 1882 (the name used in this paper); some authors, however, prefer Microspora Sprague 1977 as the proper name (see discussion in Sprague and Becnel, 1998). For more details on microsporidia early taxonomy and its revisions, see Corradi and Keeling 2009.

Classification of microsporidia is primarily based on structural characters observed under light and electron microscopy (Issi, 1986; Sprague, 1977; Sprague et al., 1992; Weiser, 1977); however, rRNA gene sequences are now currently used as a supporting and sometimes even the principal

tool in defining taxa (see Section 2.2.3, p. 269). Consequently, some new taxons are insufficiently characterised because the procedures for protist taxa description, recently summarised by Lynn and Simpson (2009), were ignored. As far as higher taxonomic categories are concerned, host ecology has been proposed as a means to categorise microsporidian classes (Vossbrinck and Debrunner-Vossbrinck, 2005) (see Section 4.2, p. 289). The most recent summary of microsporidia identification is that of Larsson (1986, 1988, 1999). The latest census of genera dates from 1999 to 2000 (Canning and Vávra, 2000; Larsson, 1999; Sprague and Becnel, 1999). It is inevitably incomplete and subject to change because at least 43 genera have been added since 1999, increasing the total number of existing genera to 187 (as of February 2013).

A consistent problem for classifying microsporidia is that many structural characters either do not bear a phylogenetic signal or the potential as a signal has not been recognised. Thus, the formal classification of microsporidia based on morphology and host range is in many cases incongruent with phylogenetic relationships revealed by molecular methods. On the other hand, the use of these methods facilitated the recognition that some structural characters are phylogenetically informative and prompted the reclassification and renaming of a number of microsporidian species. The correct names of several species frequently used in current investigations of microsporidian biology are presented in Table 4.1. Other examples

Table 4.1 Taxonomically valid names of some microsporidia frequently used in contemporary research that have been recently reclassified

Present valid name	Older synonym(s)
Anncaliia algerae Franzen et al., 2006	*Nosema algerae* Vávra & Undeen, 1970 *Brachiola algerae* Lowman, Takvorian & Cali, 2000
Paranosema locustae Sokolova et al., 2003	*Nosema locustae* Canning, 1953 Antonospora *locustae* Slamovits, Williams & Keeling, 2004
Hamiltosporidium tvaerminnensis Haag et al., 2011*	*Octosporea bayeri* Jírovec, 1936*
Tubulinosema kingi Franzen et al., 2005	*Nosema kingi* Kramer, 1964
Tubulinosema acridophagus Franzen et al., 2005	*Nosema acridophagus* Henry, 1967

*These two microsporidia are not identical; however, all molecular and population biology data on *O. bayeri* reported to the year 2011 actually concern *H. tvaerminnensis*.

of microsporidia reclassified to new genera could be cited here but these transfers were made sufficiently long ago that the new correct names are now in common usage.

1.3. The Fungi that Do Not Look like Fungi, or Sisters of Fungi?

The structure and biology of microsporidia is so unique that their phylogenetic relationship with other organisms is not obvious. Historically, the taxon Microsporidia appeared as an isolated group of organisms (Vávra, 1966). Due to their protistan nature, microsporidia were considered to be parasitic protozoans and at a certain time, they were considered to represent eukaryotic organisms lacking mitochondria, the Archezoa of Cavalier-Smith (1983) (Corradi and Keeling, 2009; Keeling, 2009). Presently, microsporidia are firmly anchored within the superkingdom Opisthokonta (Adl et al., 2005, 2012). The evidence supporting phylogenetic position of microsporidia within the opisthokonts is based exclusively on molecular biology characters because microsporidia lack flagella, a hallmark of opisthokonts (though secondarily lost in a number of their representatives). Like other opisthokonts, microsporidia have a unique 11 amino acid-long insertion in the EF-1α gene and their THS-DHFR genes are separated (Stechmann and Cavalier-Smith, 2003; Steenkamp et al., 2006; Vivares et al., 1996).

While several molecular characters indicate that microsporidia are related to fungi (Section 1.3.4. p. 262), microsporidia do not resemble fungi morphologically, and no extant 'missing link' between these two groups of organisms is known. Meaningful structural similarity between microsporidia and fungi is limited to the deposit of cell wall material on the cell membranes of certain developmental stages and the structure of spindle pole bodies from which spindle microtubules emerge. The latter character resembles the spindle plaque of yeast and ascomycetous fungi (Desportes, 1976; Desportes and Theodorides, 1979; Vávra and Larsson, 1999). Other characters shared by microsporidia and fungi, and claimed to reflect their relationship, include the formation of spores, presence of chitin in spores, cryptomitosis, presence (in some species) of a formation of two adjacent nuclei called diplokaryon, some features of meiosis and the presence of trehalose (Cavalier-Smith, 2001; Thomarat et al., 2004). These characters, however, are not exclusive for fungi and occur in a number of protist groups (Flegel and Pasharawipas, 1995; Hine et al., 2007; Iturriaga et al., 2009; Mulisch, 1993; Raikov, 1982).

Because microsporidia are structurally so dissimilar to other protists, molecular phylogenies seemed to be the useful tool for revealing their evolutionary relationships. However, the first sequences of ribosomal genes (Vossbrinck et al., 1987) demonstrated the limitations of using gene sequencing for the inference of evolutionary relationships of microsporidia. Due to their extremely accelerated evolutionary rates (Katinka et al., 2001; Slamovits et al., 2004a; Thomarat et al., 2004), the trees based on microsporidian genes are prone to the artefact of 'long-branch attraction' (LBA), in which divergent lineages (i.e. those having long evolutionary branches) are invariably drawn to the base of the tree. This prompted Vossbrinck et al. (1987) to suggest that microsporidia are extremely ancient eukaryotes, and Cavalier-Smith (1983) to propose the now obsolete taxon Archezoa.

That the deep position of microsporidia in trees based on amino acid sequences is an artefact was first convincingly shown by Hirt et al. (1999), and it serves as one of the most striking examples of LBA in eukaryotic trees (Brinkmann et al., 2005; Philippe and Adoutte, 1998). Today, we know that microsporidia are relatively modern organisms (Keeling, 2009). Their branches may be up to 8–10 times longer than those of most other fungal organisms (Cavalier-Smith, 2001). The problem associated with the LBA has been to some extent alleviated by the use of protein-coding genes with relatively low evolutionary rates, or by the implementation of computational methods, which restrict the LBA artefact. However, as described below, a degree of uncertainty concerning microsporidian phylogeny persists in nearly all studies, in which protein sequences have been used.

Although microsporidia do not resemble fungi in terms of structure, the molecular evidence supporting their relationship is overwhelming. Sequences of many of their protein-coding genes (e.g. α, β tubulin; hsp-70; EF-1α; valyl, glutamyl and seryl synthases; RPB1; vacuolar ATPase; TATA box binding protein; TF-II; mitochondrial pyruvate dehydrogenase subunits α, β) and the rRNA gene are related to those of fungi (Arisue et al. 2002; Brown and Doolittle, 1999; Edlind et al., 1996; Fast et al., 1999; Germot et al., 1997; Fischer and Palmer, 2005; Hirt et al., 1997, 1999; Katinka et al., 2001; Van de Peer et al., 2000; Williams et al. 2002). Furthermore, microsporidia possess a three-component mRNA-capping system similar to that of fungi (Hausmann et al., 2002; Texier et al., 2005) and, like fungi, the microsporidian SSU rRNA gene lacks a paromomycin-binding site (Katiyar et al., 1995). Recently, the battery of molecular features supporting the microsporidia–fungi relationship was strengthened by a synteny of ribosomal protein genes RPS9 and RPL21 unique to fungi and microsporidia

and by the absence in microsporidia of gene fusion between glutamyl-prolyl tRNA synthetase and the ubiqutin–ribosomal subunit S30 present in fungi, but absent in other opisthokonts (Lee et al., 2010). In addition, the types of microsporidia septins, GTPases involved in organising the sites of cell division, vesicle trafficking, apoptosis and cell movement support fungal affiliation to microsporidia. Interestingly, however, microsporidia septins are closely related to septins of yeast but not of other fungi (Pan et al., 2007) (The reader is further referred to Fast and Keeling (2005), Van de Peer et al. (2000) and Vossbrinck et al. (2004) for a more detailed account of the relationship between microsporidia and fungi).

Although the concept that microsporidia are related to fungi is presently generally accepted, some authors have denied that such a relationship exists. Microsporidial origin was sought near the Animal-Fungi divergence because microsporidia lack a two amino acid fungi-specific indel present in the EF-1α gene (Tanabe et al., 2002, 2005). Ebersberger et al. (2009) nested microsporidia between Mycetozoa and Amoebozoa, a rather improbable placement. Voigt and Kirk (2011) published a tree based on 1262 aligned amino acids comprising actin, β-tubulin and translation elongation factor 1-α from 80 eukaryotic taxa, including numerous fungi. In this tree, the microsporidia branch off close to the root of the tree with no specific relationship to fungi, although the length of the microsporidian branch is indicative of the LBA involvement.

Although the view that microsporidia are related to fungi seems to be generally accepted, it is the exact nature of the relationship that is uncertain. Are microsporidia nested within the fungi, derived from a specific fungal group or do they represent their sister group? Three different approaches, based respectively on the phylogenetic signal of gene sequences, the genome architecture involving gene synteny, and the presence of specific gene products, can be used to address this question.

1.3.1. Gene Sequences and Microsporidia Phylogeny

Although numerous genes mentioned above show that microsporidia are affiliated to fungi, some gene trees seemed to pinpoint the position of microsporidia within fungi with more precision. Tubulin phylogenies indicated the emergence of microsporidia after the chytrid fungi (Keeling et al., 2000) or from within Zygomycetes (Keeling, 2003). An eight-protein-concatenated tree showed that microsporidia are related to the combined ascomycetes/basidiomycetes clade, the Dikarya (Gill and Fast, 2006). Other phylogenies, however, suggest that microsporidia emerged close to

the origin of fungi. Thomarat et al. (2004) could not decide if microsporidia branched off right after the basal chytrid fungi or represent a sister group outside the fungi. Similarly other fungal trees show microsporidia as either a sister group to fungi (Liu et al., 2006) or interpret them as their earliest diverging branch (Wang et al., 2009). McLaughlin et al. (2009) present fungal classification in which microsporidia are a part of a basal fungal lineage including non-monophyletic group of 'chytrids and zygomycetes'. Alternatively, microsporidia were proposed to be derived from a basal endoparasitic chytrid ancestor similar to *Rozella allomycis* (James et al., 2006) (see Section 1.3, p. 259). Recently, the position of microsporidia as branching close to the origin of fungi has been further strengthened by a study exploiting the available 121 sequenced 'fungal genomes' (six of them microsporidian), by large sampling and the use of specific methods overcoming the limitations of other phylogenies, possibly affected by the LBA (Capella-Gutierrez et al., 2012). Ensuing analysis of the phylomes (assemblage of gene phylogenies of an organism) and of 53 concatenated genes of six microsporidian species, showed topology with microsporidia constituting either the earliest diverging branch of fungi or their sister group. The authors believe that these two positions cannot be resolved on a factual basis. Due to 'the existence of a number of synapomorphies between microsporidia and fungi', Capella-Gutierrez et al., 2012, favour the former placement. Authors of another extensive phylogenomic study that compared the *Nematocida parisii* genome to seven other microsporidian and 13 fungal genomes arrived to the same conclusion (Cuomo et al., 2012). Finally, another study claims that together with *R. allomycis* and an algal intracellular parasite *Amoeboaphelidium protococcarum,* microsporidia form a monophyletic sister clade (ARM or Cryptomycota) to Fungi (Letcher et al., 2013; Karpov et al., 2013). It should be pointed here, however, that none of the four above-mentioned publications provides any evidence that microsporidia are fungi as opposed to being related to fungi (see Section 1.3.4, p. 262).

1.3.2. Gene Order Analysis and Microsporidia Phylogeny

Gene order has been used as a method for overcoming the limitations imposed on phylogeny studies by the LBA. Gene order (synteny) in microsporidia seems relatively stable and allows informative comparison with other organisms (Cornman et al., 2009; Corradi et al., 2007; Peyretaillade et al., 2012; Slamovits et al., 2004a). This approach indicated a significant similarity between microsporidia and zygomycete fungi (Dyer, 2008; Lee et al., 2008), as they share a synteny of genes encoding a triose-phosphate

transporter, transcription factor high mobility group (involved in sexuality of zygomycetes) and an RNA helicase. This seemed to confirm previous indications that microsporidia are related to zygomycetes based on the analysis of tubulin genes (Keeling, 2003). However, this synteny-based evidence has been challenged on the basis of the evolutionary history of genes involved. First, it was suggested that the genes in microsporidia/zygomycetes syntenic clusters may not be orthologous but paralogous and hence allow no conclusion about the evolutionary relationship of the two taxa (Ebersberger and Koestler, 2009; Lee et al., 2010). Further, it was shown that the shared synteny of the respective clusters represents at best a shared ancestral character (plesiomorphy), and is not phylogenetically informative. Similarity in gene order between microsporidia and zygomycetes does not exceed that between microsporidia and other fungi and even some animals (Koestler and Ebersberger, 2011). That a reliable phylogeny of microsporidia cannot be inferred from the gene neighbourhood analysis has recently been confirmed by additional authors. Heinz et al. (2012) reported a lack of synteny or orthology among the relevant genes of two microsporidia (*Trachipleistophora hominis* and *Nosema ceranae*) and zygomycetes. Capella-Gutierrez et al. (2012) found that there is a similarly low level of gene neighbourhood conservation between fungal groups and microsporidia. Such observation makes the gene order conservation not sufficiently informative.

1.3.3. Gene Products and Microsporidia Phylogeny

While both the gene sequences and genome architecture failed to provide convincing evidence regarding the evolutionary history of microsporidia, the presence/absence of certain gene products may serve as possible phylogenetic markers and indicate more precisely if microsporidia are early fungi or their sister group. Cell wall components could possibly serve in this context because cell wall chemistry and structure have some importance in fungal taxonomy. Indeed cell wall polysaccharides were used for delimitation of high level taxa and their role in fungal evolution (Bartnicki-Garcia, 1970; Ruiz-Herrera et al., 2002). In addition to some specific proteins, α-chitin is the major component of the microsporidian spore wall (Vávra, 1976; Wu et al., 2008). While there is a single type of chitin synthase gene per species in a typical animal genome, in fungi the chitin synthesis is based on the regulation of distinct chitin synthase (CHS) isoenzymes with one to five classes in filamentous fungi (Ruiz-Herrera et al., 2002). Individual isoenzymes are involved in respective morphogenetic

events of fungal life cycles (Roncero, 2002). Recent analyses of the evolution of structural elements of the fungal cell wall and of genes involved in their synthesis indicate that there is an evolutionary signal in the way how the fungal cell wall is built (Xie and Lipke, 2010). In particular, it is the evolution of CHSs, which accompanied fungal evolution (Ruiz-Herrera and Ortiz-Castellanos, 2010). In addition to chitin deacetylases (Brosson et al., 2005), microsporidia possess a gene for a single type of CHS (class IV CHS-1), which is believed to represent an ancestral form of CHS, that existed prior to the split of fungi from other opisthokonts (Ruiz-Herrera and Ortiz-Castellanos, 2010) and is a member of CHS supergroup 2 to which CHS of animals belong (Latge, 2007; Ruiz-Herrera et al., 2002). So far this ancestral type of CHS has been found in *Encephalitozoon cuniculi*, *Encephalitozoon intestinalis*, *Hamiltosporidium tvaerminnensis*, *N. ceranae* and *Spraguea lophii* (Cornman et al., 2009; Corradi et al., 2009, 2010; Hinkle et al., 1997; Katinka et al., 2001) thus suggesting that microsporidia seem to have branched off close to the origin of fungi. Moreover, microsporidia lack β-1,3, β-1,6 glucans and mannans, common matrix components associated with chitin in cell walls of fungi (Ruiz-Herrera and Ortiz-Castellanos, 2010). Rather in the microsporidian cell wall, chitin is complexed with glycoproteins, as it is in many organisms other than fungi (Xie and Lipke, 2010). Further research is needed to ascertain if the presence of the single ancestral type of CHS-1 class IV truly reflects an early-branching position of microsporidia near the animal-fungi divergence, and is not the result of the loss of other CHS types, which has occurred (although very rarely) in some fungi (Bowen et al., 1992).

1.3.4. Are Microsporidia Sisters of Fungi?

In summary, the origin of microsporidia and the nature of their relationship to fungi remain unresolved. It is almost certain that microsporidia are not derived from within a certain group of existing fungi, e.g. Zygomycetes or the Dikarya, while deep-branching of microsporidia in relation to fungi is now firmly supported. The view that microsporidia are a sister group to fungi (lastly pronounced by Heinz et al., 2012), and indicated also by the nature of their cell wall material and its corresponding genes discussed above, seems to be the best current hypothesis worthy of further investigation, especially in situations where newly found organisms affiliated with fungi ('cryptomycota') (James and Berbee, 2011; Jones et al., 2011) promise to contribute to our understanding of the origin of fungi. According to the view postulating that microsporidia are sisters to fungi, it is suggested

here that in articles dealing with these parasites, the systematic affiliation '(Opisthokonta: Microsporidia)' is used, instead of the usual '(Fungi: Microsporidia)'.

2. THE 'ART OF LIVING INSIDE THE CELL'

2.1. Microsporidia Are Not Only Intracellular, but Truly Intracytoplasmic Parasites

As described below in more detail (see Section 3.1, p. 275), the first step of the microsporidian life cycle is the injection of the germplasm contained in the spore into the cytoplasm of the host cell. This is a highly unusual mechanism of cell infection among eukaryotic pathogens. Somewhat similar infection mechanisms are used by some basal oomycetes for injection of the germplasm into the body cavity of nematodes, tardigrades and rotifers (Glockling and Beakes, 2002; Hakariya et al., 2002; Robb and Barron, 1982) and by rhizarian plasmodiophorids for injection into plant roots (Aist and Williams, 1971). These respective injection mechanisms evidently arose independently during the course of evolution, since microsporidia, oomycetes and rhizarians belong to different kingdoms (Adl et al., 2005). Direct introduction of the sporoplasm into the host cell means that, in contrast to intracellular parasites that live in vacuoles (a kind of "growth chamber") derived from host-cell plasmalemma (e.g. the parasitophorous vacuole of Apicomplexa, phagosome-like vacuoles of some kinetoplastid flagellates or the symbiontophoric vacuoles of many intracellular prokaryotic or eukaryotic symbionts), microsporidia are not only intracelullar but are genuinely intracytoplasmic (cytosolic) parasites.

The sole interface between the injected sporoplasm and the host-cell cytoplasm is the parasite plasma membrane often covered with glycocalyx. This glycocalyx is highly developed in some microsporidia, forming structures deeply intruding into the host cytoplasm, suggesting that the glycocalyx plays a role in the transport of nutrients into the microsporidian parasite (Koudela et al., 2001). Different interfacial relationships of microsporidia with host cells were categorised by Cali and Takvorian (1999), however, the basic fact is that the parasite resides among host-cell organelles, often surrounded by accumulated mitochondria and by lamella of the endoplasmic reticulum. A sole exception to this rule seems to be the intracellular location of *Encephalitozoon* spp. (and possibly also of *Endoreticulatus* spp.) in what resembles a parasitophorous vacuole (i.e. vacuole formed by the host cell). At present its origin is far from resolved (Bohne et al., 2011; Fasshauer

et al., 2005; Rönnebäumer et al., 2008), and is beyond the scope of this review. The intimate relationship between the parasite and the host cell is maintained throughout merogony, during which the parasite does not cause visible harm to the host cell except for some loosening and disintegration of myofibrils in muscle infections. During merogonial growth and division, microsporidia thus resemble an intracytoplasmic symbiont rather than a typical parasite. The integrity of the host-cell cytoplasm in proximity to the parasite cells is altered only when the microsporidium begins to form a cell wall and enters the sporulation phase. The entire life cycle takes place within the host cell into which the sporoplasm has been injected; there is no evidence of vegetative stages actively moving among the host cells (see also discussion below). It is a unique character of microsporidia that they remain hidden within host cells and their vegetative stages remain sheltered from extracellular milieu, thus limiting their recognition by the host immune system.

2.2. Structural and Genomic Reduction

2.2.1. Extensive Structural Reduction

The injected microsporidian sporoplasm, which is the beginning of the life cycle in the host cell, consists of the contents of the infective spore, which, although being forcibly passed through a long (up to several hundreds μm) and very narrow (0.1–0.2 μm) injection tube, has maintained its integrity. The sporoplasm, a few micrometres in diameter, is surrounded by a plasma membrane of a peculiar origin – it was originally folded within the mature spore as an accordion-like membranous stack known as the polaroplast (see Section 3.1.1, p. 275). During germination, the polaroplast membranes are drawn into the injection tube together with the spore cytoplasm and nucleus, and are in part ejected from the end of the injection tube as a germplasm-containing vesicle (see Section 3.1.3, p. 281). The origin of the sporoplasm cell membrane is thus quite unusual, as it would involve a reversal of membrane polarity (the former cytoplasmic side of the membrane is now exposed to the environment). It was reported that the cytoplasmic proteins tubulin and dynactin appear at the outer surface of the sporoplasm plasma membrane and, consequently, its outer leaf lacks cholesterol and lectin-binding molecules (Weidner, 2000, 2001; Weidner and Findley, 1999), yet these data need to be verified.

The freshly injected sporoplasm can be considered as a kind of 'minimal eukaryotic cell'. Its cytoplasm is dense with ribosomes and contains an inconspicuous nucleus (or two adhering nuclei forming a diplokaryon

in some species – see Section 2.4, p. 272), non-specific vesicles, and some membrane whorls and fragments that are not yet organised into cellular compartments. There is no visible Golgi apparatus and no structures reminiscent of cristae bearing mitochondria or other organelles (Takvorian et al., 2005; Weidner, 1972). Cell membranes and organelles appear progressively in later stages but structural reduction remains the general feature of vegetative cells. Microsporidia have lost peroxisomes, their mitochondria are reduced to mitosomes (see below) and their membrane trafficking machinery is highly reduced (Dacks and Field, 2007; Mironov et al., 2006). The Golgi apparatus of microsporidia is unstacked (Vávra, 1965; Mowbrey and Dacks, 2009), reduced to a clump of varicose tubules embedded in an electron opaque material (Beznoussenko et al., 2007; Dolgikh et al., 2010; Vávra and Larsson, 1999). Although it is rather inconspicuous in vegetative stages, the Golgi apparatus plays a central role in elaborating components of the extrusion apparatus during sporogenesis (Vávra, 1976) (see Section 3.1.1, p. 275).

The highly reduced mitochondria, called mitosomes, appear as small, double-membrane vesicles distributed close to the terminus of the microtubular division spindle and also scattered in the cytoplasm of vegetative stages (Vávra, 2005; Willwswiams et al., 2002, 2008a). Mitosomes are minimalistic organelles with fewer than 20 mitosomal proteins identified, as compared to yeast mitochondria with well over 1000 proteins (Paldi et al., 2010). Mitosomes lack a genome, oxidative phosphorylation and Krebs cycle proteins and their protein import machinery is much reduced (Waller et al., 2009). The Fe–S cluster assembly machinery seems to be the only presently known function of microsporidian mitosomes (Goldberg et al., 2008; Heinz et al., 2012; Williams, 2009; Williams et al., 2002). In contrast, however, the gene for alternative oxidase (AOX) was found in genomes of several other microsporidia (Heinz et al., 2012; Williams et al., 2010), and the respective protein and glycerol-phosphate dehydrogenase were localised to the mitosomes, indicating that these organelles are involved in reoxidising the reducing equivalents produced by glycolysis (Dolgikh et al., 2011). Structures corresponding to lysosomes and peroxisomes, which are components of a typical eukaryotic cell, are missing in the vegetative stages of microsporidia (Vávra and Larsson, 1999); however, the posterior vacuole, which plays an important role in spore germination, may be a primitive or extremely specialised peroxisomal organelle (Findley et al., 2005; Weidner and Findley, 2002) (see Section 3.1.3, p. 281).

2.2.2. Reduced Genomes

Interestingly, the general structural simplification of microsporidian cell is reflected in reduction and compaction at the molecular level, which is most obvious in the organisation of their genomes (Corradi and Selman, 2013). Microsporidian genomes are characterised by gene loss (mostly of metabolic genes, the products of which can be obtained from the host – see below), reduction of gene length and by genome compaction, with these events varying in different lineages (Corradi and Slamovits, 2011; Heinz et al., 2012; Keeling et al., 2005; Keeling and Slamovits, 2004, 2005; Texier et al., 2005; Vivares and Metenier, 2000). Initial data on microsporidian genomics have been obtained from the analysis of the small, 2.9 Mbp genome of *E. cuniculi* (Katinka et al., 2001; Vivares et al., 2002). The recently sequenced genomes of other *Encephalitozoon* spp. are even smaller (*E.intestinalis,* 2.3 Mbp – Corradi et al., 2010; *Encephalitozoon hellem* and *Encephalitozoon romaleae,* 2.5 Mbp – Pombert et al., 2012; Selman et al., 2011). Additionally, several surveys based on low coverage assemblies of microsporidia with larger genomes are now available (Gill et al., 2008; Hinkle et al., 1997; Mittleider et al., 2002; Slamovits et al., 2004a; Williams et al., 2008b) as well as the following draft quality genomes: (*Enterocytozoon bieneusi* <6.0 Mbp [Akiyoshi et al., 2009]; *N. ceranae* <7.86 Mbp [Cornman et al., 2009]; *Hamiltosporidium tvaerminnensis* <24.2 Mbp [Corradi et al., 2010]; *N. parisii* <4.1 Mbp [Cuomo et al., 2012]). These have been complemented recently by an in-depth coverage of 8.5 Mbp from the *T. hominis* genome which is about 11.6 Mbp in size (Heinz et al., 2012) and by high-quality annotation of a large (23 Mbp) genome of *Anncaliia algerae* (Peyretaillade et al., 2012). If the smallest genomes of *Encephalitozoon* spp. are disregarded, the mean genome size calculated from the available data exceeds 12 Mbp. Thus, the typical microsporidia genome is much larger (up to tenfold) than the extremely small genomes of *Encephalitozoon* spp., which represent rather an exception. The size differences of microsporidia genomes are, however, not reflected in the number of their genes. The smallest (2.9–2.3 Mbp) genomes of *E. cuniculi* and *E. intestinalis* contain 2094 and 1907 genes, respectively, while the so far largest (23 Mbp) genome of *A. algerae* contains 2075 genes (this number has been established by annotation using transcriptional signals – see Peyretaillade et al., 2012). Currently the highest number of genes in sequenced microsporidia is represented by the 3266 ORFs in the 11.6 Mbp genome of *T. hominis* (Heinz et al., 2012). This number is still quite low when compared to the

genome of *Saccharomyces cerevisiae,* which contains ~6000 genes (Corradi and Slamovits, 2011).

The size of microsporidia genes is generally smaller than of their eukaryotic counterparts (see below), but again there is no rule that small microsporidia genomes have smaller genes than the larger ones; for example, the *A. algerae* genes are generally shorter than those found in *E. cuniculi* and *E. intestinalis* (Peyretaillade et al., 2012). Hence, the tenfold variation in genome sizes cannot be attributed to the size and number of genes, but to highly variable gene density, ranging from 119 bp in *E. cuniculi* to 1.18 Kbp in *T. hominis* (Heinz et al., 2012). The gene density depends on the length variation of intergenic regions, the number of sequence repeats and transposable elements and the size of telomeric regions (Cuomo et al., 2012; Peyretaillade et al., 2012). The difference between large genomes and the streamlined ones can to some extent also be attributed to the presence of overlapping genes in the latter genomes (Corradi and Slamovits, 2010; Heinz et al., 2012).

The genome compaction is evidently a product of the parasitic life style. Many widespread and conserved eukaryotic genes, whose functions can be provided by the host cell, such as the genes for biosynthesis of nucleotides, genes of the tricarboxylic acid cycle, and electron-transport respiratory chain, are missing (Katinka et al., 2001). In the extreme case, the 1833 and 1750 protein-coding genes identified in the respective *E. intestinalis* and *E. bieneusi* genomes could represent the lower limits of a functional eukaryotic genome (Corradi et al., 2010; Peyretaillade et al., 2012).

Moreover, microsporidian protein-coding genes are about 20% shorter than their yeast orthologues. Intergenic regions are truncated and gene transcription is modified as demonstrated by overlapping mRNAs between contiguous genes in some microsporidia (Corradi et al., 2008, 2009, 2010; Williams et al. 2005). In comparison with meronts, this 'multi-gene transcription', in which mRNA transcripts of neigbouring genes partially and in different degree overlap, occurs at a higher rate during the spore stage of the life cycle, suggesting that it might play a role in accelerating and streamlining proteosynthesis right after host infection (Corradi and Slamovits, 2011; Gill et al., 2010; Grisdale and Fast, 2011) (see Section 2.4, p. 272).

Regardless of their size, even evolutionary distant microsporidian genomes retain a high degree of gene order (synteny) (Slamovits et al., 2004a). Thus, despite the rapid evolution of the coding sequences (Thomarat et al., 2004), the genome architecture seems to be rather stable and conserved in some microsporidia up to the point that homologous, yet divergent genes can

be identified due to their location in the genome (Polonais et al., 2005). Gene compaction and overlapping transcription may contribute to this maintenance of gene order (Corradi et al., 2007). From the evolutionary point of view, it is probable that the gene loss responsible for the relative paucity of microsporidian genomes may have been an ancestral feature that emerged as a consequence of intracellular parasitism (Heinz et al., 2012; Williams et al., 2008b). However, due to evolutionary pressures concurrent with intracellular parasitism, the evolution of microsporidian genomes has not been marked only by losses and reductions, as some new genes and protein families have been gained, which enabled microsporidia to become successful intracellular parasites (Heinz et al., 2012). Microsporidia perfected the strategy of acquiring metabolites and nutrients from host cells, amplified the number of transport proteins in their genome and acquired some essential genes (ATP transporters, one class of nucleoside transporters and some genes with their products involved in metabolism) by HGT from prokaryotes or in rare cases from animal hosts (Cuomo et al., 2012; Lee et al., 2009; Pombert et al., 2012; Tsaousis et al. 2008) (see Section 2.3, p. 270). All this resulted in a massive loss of dispensable genes and the retention of an assemblage of the 'core of further irreducible sets of genes preserved throughout microsporidian evolution' (Corradi and Slamovits, 2011). Establishing this 'core' will require a broad sampling of genomes across microsporidia diversity.

Additional specific differences in individual genomes may be due to their adaptation to different hosts and to different genomic environments (Texier et al., 2010). One such example is the genome of *E. bieneusi,* the most frequently recovered mammalian microsporidium. In contrast to other species, which contain a full complement of genes for carbon metabolic pathways (glycolysis, trehalose metabolism and pentose-phosphate pathway), it appears that *E. bieneusi* has lost nearly all these genes and has no known mechanism to produce energy on its own (Keeling et al., 2010); however, since the *E.bieneusi* genome remains currently incomplete, this view has to be considered preliminary. The loss of genes for energy production makes *Enterocytozoon* extremely dependent on the host cell and possibly explains why this species has so far resisted in vitro cultivation (Corradi and Slamovits, 2011). In addition to the ability to generate energy from sugars, *E. bieneusi* has also lost introns and the spliceosome (Keeling et al., 2010). Surprisingly, despite all these losses, the *E. bieneusi* genome seemed to contain a relatively large set of novel protein-coding genes as compared to other microsporidia (Akiyoshi et al., 2009). Following comparative analysis, however, pointed to the absence of typical microsporidian promoters for a total of 387 these genes (Peyretaillade et al., 2012). While microsporidia

were proved to acquire genes from their hosts as well as from other organisms (see Section 2.3.1), conclusions about large-scale acquisitions seem to be premature. Thus, it is most probable that the existing *E. bieneusi* assembly has been contaminated with bacterial sequences (Heinz et al., 2012). Actually, as shown above, the genome of *E. bieneusi* with its 1750 genes (after re-annotation by Peyretaillade et al., 2012) is one of the smallest of Microsporidia genomes (if not the smallest one). One can speculate that the extreme streamlining of the *E. bieneusi* genome may allow the parasite to accelerate its life cycle, a necessity if enterocytes with a life span of mere 3–5 days are parasitised (see Section 2.4, p. 272).

Numerous whole-genome sequencing initiatives that are currently under way are bringing more data of individual microsporidian species. Indeed, it is expected that as more microsporidia genomes are analysed (Corradi and Slamovits, 2011; Texier et al., 2010), more genomic differences reflecting individual life histories of respective microsporidia will be discovered. The loss of splicing machinery that happened independently in several microsporidian lineages is one of such results (Cuomo et al., 2012).

2.2.3. Reduced Ribosomes

Considering the highly reduced nature of microsporidian genomes, it is not surprising that the respective ribosomal RNA genes are highly truncated, with the sedimentation coefficient of microsporidian ribosomes resembling that of the prokaryotic 70S ribosomes (Curgy et al., 1980; Ishihara and Hayashi, 1968). Microsporidian large subunit (LSU) rRNA is reduced to the universal core (De Rijk et al., 1998), ITS-2 is missing altogether and the 5.8S rRNA is covalently linked either to LSU or, less frequently, to the small subunit (SSU) rRNA (Peyretaillade et al., 1998; Vossbrinck and Woese, 1986). In the first case, the rRNA unit has the typical eukaryotic organisation (SSU-ITS-LSU), while for *Nosema* spp. and a few other microsporidia an alternative and very unusual reverse organisation (LSU-ITS-LSU) is characteristic (Huang et al., 2004; Refardt and Mouton, 2007). Microsporidia are the only eukaryotes known to lack an individual 5.8S rRNA molecule; however, the relevance of this fact is unknown (Torres-Machorro et al., 2010). It is of interest that not all ribosomes inside one cell are necessarily the same. The rRNA genes are multicopy and distributed on different chromosomes (Katinka et al., 2001), and are either identical (*E. cuniculi*), or exist in multiple variants, mostly differing in the ITS region (Tay et al., 2005). In *Nosema bombi*, a bumblebee parasite, two such rRNA variants were found to co-exist in almost equal proportions in the same cell (spore), and it was hypothesised that they are

restricted to separate nuclei of the diplokaryon (O'Mahony et al., 2007). Significance of such variability is not known and its occurrence implies that the concerted evolution of microsporidian rRNA genes is uniquely relaxed. The co-occurrence of rRNA variants has, however, two important implications. First, the occurrence and distribution of variants suggests that recombination among them is taking place (Ironside, 2013), implying that a sexual haplo–diploid cycle producing new haplotypes occurs in species such as *N. ceranae* (Sagastume et al., 2011). Second, care has to be taken when multicopy rRNA genes are used for population genetics and phylogenetic analyses (O'Mahony et al., 2007) or in barcoding (Ironside, 2013).

2.3. Full Dependence on the Host Cell

The structural and genomic reduction mentioned above is only possible because in terms of the metabolic and energy needs, microsporidia fully rely on the host cell (Williams, 2009). Besides the partial or total loss of energy-producing pathways mentioned above, the absolute dependence on the host-cell metabolism is documented by the so far unprecedented total loss of all components of the Tor pathway, a nutrient-sensing signalling cascade that is otherwise universally present in eukaryotes. Microsporidia probably relaxed their control of nutrient sensing and lost the Tor genes while modifying their cellular machinery to adjust to intracellular growth in a nutrient-rich environment (Shertz et al., 2010).

Once inside the host cell, microsporidia can proliferate very quickly because the sporoplasm and the early meronts are loaded with ribosomes that are needed for rapid proteosynthesis. Indeed, about 14% of proteins encoded by the *E. cuniculi* genome are involved in proteosynthesis (Vivares et al., 2002). The cell cycle of microsporidia is possibly accelerated by the loss in microsporidia of the tumour suppressor gene Retinoblastoma, and in this respect, microsporidia resemble some cancer cells (Cuomo et al., 2012). Some species are able to complete their entire life cycle and form mature spores within 3–4 days (Leitch and Ceballos, 2008; Wasson and Barry, 2003) and, in accordance, time course analysis of selected transcripts of *N. parisii* indicated a doubling time of 3.3 h, comparable to the doubling time of yeasts in a rich culture medium (Cuomo et al., 2012). Considerable amounts of nutrients and energy are needed for such rapid intracellular growth. Although microsporidia have lost in various degrees the genes required for key energy-generating reactions, and their ATP production is only possible by substrate level phosphorylation (Katinka et al. 2001; Weidner et al., 1999b;), microsporidia have developed a unique capacity to

appropriate ATP from the host cell, and became 'energy parasites' (Vivares et al., 2002). For this purpose, they use a series of recently discovered ATP transporters, which were acquired from intracellular bacteria such as *Rickettsia* or *Chlamydia* (Tsaousis et al., 2008).

An additional cunning mechanism that might help microsporidia to cope with energy and nutrient demands has recently been proposed by Cuomo et al. (2012). These authors found that microsporidian hexokinases gained a microsporidia-specific secretion signal sequence, possibly allowing these enzymes to be exported into host cells and reprogram them towards biosynthesis. Microsporidia thus could stimulate the energy balance of infected tissues. Indeed, in the fat body of crickets infected by *Paranosema grylli*, the ATP content and the ATP/ADP concentrations are increased about four times, while at the same time, the infection causes depletion of glycogen in the host (Dolgikh et al., 2002). Sugar metabolism is upregulated in midgut cells of bees infected by *N. ceranae* (Dussaubat et al., 2012). The metabolic needs of microsporidia are evidently the basis of the tendency for these parasites to infect diverse metabolically active tissues including muscles, fat bodies, ovaries, and salivary and silk glands. The capacity to 'steal' ATP mentioned above likely explains the frequently observed accumulation of host-cell mitochondria around the vegetative stages of the parasite (Scanlon et al., 2004), as well as the propensity of a few species of microsporidia to live in host-cell nuclei, a rich source of ATP and of nucleoside triphosphates (Metenier and Vivares, 2001; see Section 2.4, p. 272). To facilitate nucleoside uptake, microsporidia acquired nucleoside transporters, one new class of them probably hijacked from bacteria (Cuomo et al., 2012). The high demand for energy also explains why these protists survive in a kind of dormancy within cells that are temporarily metabolically inactive, such as the germ-line cells, before initiation of embryonic development (Weiser, 1961). Regulation of gene expression is clearly needed in order for such an adaptation to the metabolic state of the host cell to occur. RNA interference, recently shown to be functional in *N. ceranae* (Paldi et al., 2010) and likely present also in *T. hominis* (Heinz et al., 2012), yet absent from *E. cuniculi* (Katinka et al., 2001), might be one example of a gene expression-regulating mechanisms.

2.3.1. Gene Transfer from the Host

Although microsporidia live in an extremely intimate relationship with the host-cell cytoplasm or, as mentioned above in host-cell nucleus, the transfer of genetic material between the animal host and the parasite is evidently

a rare event. Only two cases of a horizontal gene transfer (HGT) from a host have been fully documented. In one case, two sister microsporidia, *E. romaleae* and *E. hellem* from insects and vertebrates, respectively, contain a gene for purine nucleotide phosphorylase (PNP), evidently acquired from an unknown insect host in the *E. hellem* – *E. romaleae* ancestor (other known members of the genus *Encephalitozoon* lack the PNP gene – Selman et al., 2011). However, further investigations are needed to establish the extent of the HGT from animal hosts. It will certainly be worth analysing the evolutionary history of genes involved in folate and purine metabolism specific for *E. hellem* and *E. romaleae*, which were evidently acquired by their ancestor in several HGT events, involving different organismal donors, some of them probably eukaryotic (Pombert et al., 2012). In a highly unusual HGT event, a transposable element in the genome of *T. hominis* was evidently obtained from an ant or a close relative (Heinz et al., 2012).

2.4. Influence on the Host Cell

Microsporidia have mastered both the entry into host cell and the exploitation of its metabolism, and have developed a number of physiological adaptations enabling them to subvert host-cellular machinery to their benefit (see Section 2.3, p. 270). Another peculiar feature is the capacity of microsporidia to synchronise their life cycle with that of the host cell, and even modify its cytoskeleton and organelle arrangement (Scanlon et al., 2000, 2004; Weidner et al., 1999b). Specifically, the normal cell cycle of infected host cells is blocked at multiple points, their division is often arrested and their susceptibility to apoptosis is reduced (Scanlon et al., 1999, 2000) because the parasite inhibits activation of the p53 apoptotic pathway (del Aguila et al., 2006). Delayed apoptosis allows the parasite to increase its numbers and complete its development to the mature spore stage.

Very little is known about the interaction between microsporidia and the host-cell cytoskeleton, which is presumed to play a role in the enlargement of infected cells and in the intracellular positioning of the parasite (see below). Because the microsporidian tubulin and dynactin appear at the surface of the freshly discharged sporoplasm, it was hypothetised that the latter protein may be responsible for the positioning of the parasite within the host cell (Weidner, 2000). Some species occupy a specific location in the host cell, e.g. *E. bieneusi* is usually abutted to the enterocyte nucleus and is often situated inside the concavity of the invaginated nuclear membrane (Desportes-Livage et al., 1996; Chalifoux et al., 1998). Members of the genera *Nucleospora* and *Enterospora* are nucleotropic (Lom and Dyková,

2002; Stentiford and Bateman, 2007; Stentiford et al., 2007), but how they reach their destination remains unknown. The interaction with the host cytoskeleton was described for *N. parisii*, which destroys the spectrin–yosin terminal web of infected gut enterocytes of its host, the model nematode *Caenorhabditis elegans* (Troemel et al., 2008).

The enlargement of the host cells post-invasion reflects the ability of microsporidia to subvert the host-cell cytoskeleton and volume control in order to secure a sufficiently spatial and protected niche. Hypertrophy of infected host cells is characteristic of microsporidian infections in general (Lom and Dyková, 2005) and is accompanied by several secondary effects; individual infected cells may dedifferentiate into a syncytium, undergo nuclear hypertrophy and fragmentation, increase RNA synthesis, and sometimes produce an increased number of cell nuclei (Canning and Hazard, 1982; Leitch et al., 2005a; Martins and Perondini, 1977). Another notable effect is the enlargement and increase in the degree of polyteny of giant chromosomes in *Diptera*, signalling the generalised hyperfunction of the cell genome under the influence of microsporidian infection (Pavan et al., 1969) and the inactivation of cell-type-specific Balbiani rings and puffs signalling a change in the cell-type-specific genetic program (Staiber, 1994). Infected lymphocytes of annelids form multiple microvilli on their surface, which probably serve to increase the absorptive capacity of the cell (Janiszewska et al., 1978).

The most spectacular host-parasite interaction as far as the increase of cell volume is concerned is represented by giant, usually macroscopic, spore-filled formations termed xenoma, characteristic for numerous microsporidian species infecting fish. Xenoma are not limited to fish; giant cells induced by microsporidia occur in number of other hosts including oligochaetes, crustaceans and insects (Lom and Dyková, 2005). Xenomas in fish may reach several millimetres in size but remain a single cell with one, albeit significantly modified, nucleus, cytoplasm containing developmental stages of the parasite and, finally, mature spores. The host cell and the parasite are morphologically and physiologically integrated in the xenoma and it is supposed that this alliance shelters the parasite from the host's immune system. Indeed, the inflammatory reaction in host tissues occurs when the xenoma reaches maturity, looses its integrity and the spores are released (Kent and Speare, 2005). The xenomas in fish supposedly originate in many cases in infected leukocytes and they occur in many forms, differing mostly in the nature and thickness of the wall surrounding the xenoma cell (Lom and Dyková, 2005). So far, it is not known how the cell volume of infected cells

is controlled, how the parasite regulates the metabolism of its host cell, and how it recognises the critical mass of its life stages inside the cell. As the host cell has a limited parasite-carrying capacity, signals must exist to induce the parasite to complete merogony and initiate sporogony in order to form mature spores before cell death. No data on such signalling exist. Regrettably, the macroscopic xenomas have so far been ignored by molecular biologists despite their relative amenability for molecular analyses, the lack of their availability in culture being the probable reason.

In some cases, the key factor that determines if a microsporidium is able to infect a certain tissue is the rate of its development related to the host-cell cycle and cellular turnover. This is best demonstrated in species infecting enterocytes of digestive systems. The turnover time for enterocytes is in the range of 3–5 days (and there is no data suggesting that the infected enterocyte lives longer), into which the microsporidium has to complete several multiplication cycles along with the formation of spores. As mentioned in Section 2.3 (p. 270) some microsporidia develop in their host cells with astonishing speed. *E. intestinalis* and *E. hellem* are able to form spores within 3 days (Leitch and Ceballos, 2008), a fact confirmed by real-time quantitative PCR showing that *E. intestinalis* DNA is detectable in colonic enterocytes in tissue culture at 4 h post-inoculation, replication is completed by 36 h and spores are released from host cells at 72 h post-inoculation (Wasson and Barry, 2003). Spore-specific mRNAs of *N. parisii* are detectable already at 40 h post-infection (Cuomo et al., 2012), while in *E. cuniculi* mRNAs involved in spore chitin synthesis are induced 20–48 h post-infection (Rönnebäumer et al., 2006). The same high rate of development must exist in *E. bieneusi,* in which the precocious formation of the extrusion apparatus begins already during the plasmodial stage (Desportes-Livage et al., 1991). Within the short life span of a gut enterocyte, this microsporidium will produce about 60 spores from a single sporoplasm (Vávra and Larsson, 1999).

As mentioned in the Section 2.3 (p. 270), physiological factors in microsporidia are set up to promote rapid growth of the parasite. It is however possible that also other, less obvious factors allow microsporidia to develop quickly in order to exploit the limited viability of host cells. One might speculate that specific features such as the size of ribosomes (Curgy et al., 1980; Ishihara and Hayashi, 1968), truncated genes (Katinka et al., 2001) and peculiarities in genomic expression (Gill et al., 2010; Grisdale and Fast, 2011) may contribute to the rapid rate at which microsporidia develop. There are two structural characters that provide evidence to support the potential for rapid cell cycling. One, previously mentioned, is the high

abundance of ribosomes (isolated ribosomes in meronts, polyribosome aggregates in spores), and the other is the frequent occurrence (either permanent or transitory) of two adhering haploid nuclei that form the so-called diplokaryon (Canning, 1988; Vávra and Larsson, 1999). Although the physiological reason for the presence of two nuclei in a cell remains to be established (and in the much-reduced microsporidian cell is somewhat surprising), one may speculate that having two nuclei facilitates the nucleus/cytoplasm transport and makes the mRNA 'ratcheting' (Stewart, 2007) out of the nucleus more efficient. The presence of two nuclei in a cell also increases its ploidy and confers advantage to the organism as the ploidy-levels regulate gene expression (Galitski et al., 1999; Hieter and Griffiths, 1999) and influence rates of evolution (Otto, 2007). It should be mentioned here that the presence of two nuclei in cells of some microsporidia raises the question of how the genetic complementarity in both nuclei is maintained and its putative association with sexual processes. This is a contentious subject that would require a detailed treatment that is beyond of the scope of this review.

3. THE 'ART OF DISSEMINATION'

3.1. Spore Is a Gun Cell Powering the Missile Projection

Because of their obligate intracellular life stages and lack of motility, microsporidia became adapted to solving the problem of how to disperse among hosts and how to disseminate within their host. To that end they evolved a form of dispersal, which might be appropriately called the 'missile projection'. The germinating spore functions as a 'gun cell', which by means of an evaginable 'injection tube' (conventionally called the polar tube) nearly instantly propels the sporoplasm to a distance from several to hundreds of μm from the germinating spore. This tube, first described by the French protistologist Thélohan (1892), has a tremendous penetration power. It pierces host tissues with ease and injects the microsporidian sporoplasm directly into the host cells as documented by early TEM observations (Ishihara, 1968). This missile form of infection is used for new host invasions as well as for dissemination within the host tissues. For this dual purpose, a single microsporidian species produces either spores that look similar but differ in germination readiness, or in some cases spores that differ both physiologically and morphologically (see Section 3.1.2, p. 280).

3.1.1. Microsporidian Spore: a Masterpiece of Compaction

The microsporidian spore is a highly compact product of a single cell differentiation. Spore cytoplasm, one or two nuclei and a three-part

injection apparatus, which consists of the injection tube, the polaroplast (an accordion-like membrane storage organelle) and a posterior vacuole (a pressure-building organelle), fill a body of only several microns in length and diameter; the smallest spores are similar in size to a bacterium (1.2 × 0.9 μm in *E. bieneusi*, 4 × 2.5 μm in a typical *Nosema*). All parts of the injection apparatus and the spore case are secretory products processed through the classical endoplasmic reticulum-Golgi synthetic pathway and are fully formed in the mature spore (Delbac et al., 2001). Spore structure and function has been the subject of several exhaustive reviews (Delbac and Polonais, 2008; Franzen, 2004, 2005; Keohane and Weiss, 1999; Vávra, 1976; Vávra and Larsson, 1999; Xu and Weiss, 2005).

The spore is essentially a miniature pressure vessel with a thick and complex cell wall that consists of two layers: the internal endospore layer, which is composed of a complex of proteins and α-chitin, and the external proteinaceous exospore (Vávra, 1976). The shapes of spores (e.g. oval, round, pyriform, rod-like) are ideal for withstanding the high internal pressure (>7.0 Mpa) that is generated inside the spore during germination (Lom and Vávra, 1963) The endospore is the pressure-resistant layer of the spore envelope, while the exospore is believed to mediate contact with environmental stimuli. This is most evident in species from aquatic hosts, which often bear spines, tubules, fibrils or mucous layers on their exospores (Vávra and Larsson, 1999) (see Section 4.5, p. 294). The spore wall proteins have no homologues in protein databases outside of microsporidia. Some of them bear a heparin-binding motif, indicating a possible interaction of spores with glycosoaminoglycans, that occurs on the surface of host cells and which may influence spore infectivity (Bohne et al., 2000; Brosson et al., 2005; Hayman et al., 2001; Li et al., 2009; Peuvel-Fanget et al., 2006; Southern et al., 2007; Wu et al., 2008, 2009; Xu et al. 2006). Proteins associated with the spore wall are typically found in either the exo- or endospore layer of the spore envelope but curiously, in one instance, the protein SWP5 of *Nosema bombycis* was found not only in the exospore, but also inside the spore in association with the coils of the injection tube. This protein is thought to interact with injection tube proteins PTP 2 and 3 and can be detected immunologically in the extruded injection tubes (Li et al., 2012). Considerable inter- and intraspecific variability of the spore wall proteins exists, and it has been suggested that these proteins might be used in diagnostics and in molecular epidemiology studies (Xiao et al., 2001).

The injection tube, which is ejected from the spore during germination and serves to make the missile projection possible, is the most characteristic structure within the spore. It is also the only component of the injection apparatus that is universally present in microsporidian spores, while other structures such as the polaroplast and the posterior vacuole are occasionally lacking (or exist in an unusual, as yet unrecognised form?), which is the case of some putatively primitive microsporidia of the order Metchnikovellida (Desportes and Theodorides, 1979; Larsson, 2000; Larsson and Koie, 2006). The tube has been referred to as 'polar filament', 'polar tube' and 'invasion tube'; however, the term 'injection tube' expresses best its function and is our preferred use for the structure. It has to be stressed here that the injection tube, while inside the spore, does not resemble a hollow tube, as it looks rather like a solid structure (hence 'polar filament' in older descriptions) (see below). However, its tubular nature is revealed during spore germination.

The tube is usually coiled into one or multiple coils to fit within the spore volume and is quite long in some species. The bacterium-sized spore of *E. bieneusi* contains an injection tube that, according to only rough calculations, should be between 10 and 20 μm in length when extruded (no data have been published concerning its actual length). The spore of the well-known honey bee-infecting *Nosema apis* is approximately 5 μm in length and extrudes an injection tube that exceeds 300 μm in length (Olsen et al., 1986). However, about 20% of microsporidian genera have spores that contain straight, uncoiled, rod-like tubes, which in the unextruded state just fit within the length of the spore (Canning and Vávra, 2000). The tube is anchored to the inner apex of the spore by a mushroom-like structure called the polar cap, consisting of filament mushroom-like terminal (anchoring disc), enveloped by a membraneous bag-like structure called the polar sac. The polar cap area (sometimes called polar sac – anchoring disc complex) is the major site for mannose-rich glycoproteins (Taupin et al., 2006; Vávra, 1972; Vávra and Larsson, 1999) and is present in all microsporidia. It represents an autapomorphic character that can be elucidated by staining and can be used in light microscopic diagnostics to differentiate microsporidian spores from other spore-forming organisms (Vávra and Maddox, 1976). The polar cap anchors the injection tube to the spore apex during eversion (Lom, 1972) and it is speculated that the abundance of hydrophilic sugars associated with the polar cap facilitates exit of the tube from the spore (Taupin et al., 2006). The polar cap region is also rich in the spore wall protein EnP1, which might assist in adherence of spores to host cells (Southern et al., 2007).

From the spore apex, the injection tube descends in a straight path throughout the apical volume of the spore and is eventually coiled in the posterior region. The PTP proteins, best characterised in *E. cuniculi, E. intestinalis* and *E. hellem* (Delbac et al., 2001), constitute a novel protein family (Delbac and Polonais, 2008). Three proteins labelled PTP1, PTP2 and PTP3 represent the major and, so far, the only defined components of the injection tube (Delbac et al., 1998; Keohane and Weiss, 1998; Peuvel et al., 2002; Xu and Weiss, 2005) and their structural interaction in injection tube assembly remains to be elucidated (Bouzahzah et al., 2010). The major component (70%) of the injection tube is PTP1, a proline-cysteine-rich O-mannosylated protein. O-mannosylation may protect the injection tube from degradation in the gastrointestinal tract of the hosts, and may mediate its interaction with mannose receptors of the host-cell membrane, and thus facilitate the adherence of the tube to the host cells (Bouzahzah and Weiss, 2010; Xu et al., 2004).

PTP1 is a highly immunogenic; it induces a strong immune response in vaccinated animals and was sufficient for inducing protective immunity against *E. cuniculi* (Moretto et al., 2010). Moreover, this protein is claimed to be responsible for the relatively frequent occurrence of antibodies to microsporidia in healthy humans (Furuya et al., 2008; Peek et al., 2005). The high cysteine content of PTP1 and PTP2, which is also rich in lysine, suggests that disulphide bridges play an essential role in the injection tube assembly and are responsible for its high tensile strength. Finally, the PTP3 protein lacks cysteine residues and its role in tube construction is not clear. The PTP proteins are conserved and the respective genes of their homologues have been identified in a number of microsporidian species (Akiyoshi et al., 2009; Cornman et al., 2009; Corradi et al. 2009). Their general presence is likely due to the unique character of the microsporidian injection mechanism. The PTP proteins 1 and 2 are suitable for phylogenetic inference within Microsporidia. For example, PTPs from *N. ceranae* share only ~20% identity with orthologues from the distantly related *E. cuniculi* (Cornman et al., 2009), whereas they display 67% and 85% identity with PTPs from more closely related *Paranosema locustae* and *P. grylli,* respectively (Polonais et al., 2005). Interestingly, the high variability of the PTP locus corresponds with the existence of three epidemiological genotypes of *E. cuniculi*, namely those of "rabbit", 'mouse' and 'dog', which are known to differ in other molecular biology markers as well (Didier et al., 1995; Peuvel et al., 2000; Xiao et al., 2001). Extensive PTP polymorphism was recorded in *N. ceranae* (Hatjina et al., 2011) and among different isolates of *E. hellem* (Haro et al., 2003; Peuvel et al., 2000). This polymorphism may be the cause of differential

infectivity of various isolates of *E. hellem* (Haro et al., 2006). As a practical outcome, the PTP polymorphism may be used as a suitable marker for tracing sources of infection of some microsporidia (Peuvel et al., 2000). It is of interest that despite a careful search for proteins with structural organisation reminiscent of PTP1, Heinz et al. (2012) failed to identify its homologue in the *T. hominis* genome. Further research is needed to prove that the extensive polymorphism of PTP proteins is not responsible for this 'absence'.

Even though the fine structure of the injection tube is well known and its main proteins have been characterised, many aspects of its structure and function remain to be elucidated. The fine structure of the tube, when coiled in the resting spore, typically consists of 3–20 concentric layers (Vávra and Larsson, 1999; Xu and Weiss, 2005) of materials of different electron density. One of the layers is glycoprotein-rich. As already stated above, the tube-like nature of the injection tube is not evident from ultrastructural studies of non-germinated spores (Cali et al., 2002; Lom, 1972; Vávra, 1976; Vávra and Larsson, 1999), yet the structure functions during germination as an actual tube (see Section 3.1.3.1, p. 281). The fine structure of the tube markedly differs among individual species and the above-mentioned variability of PTPs is probably a basis for these differences. Moreover, the injection tube structure may also vary among different spore morphs of the same species. *Vairimorpha disparis* forms three types of spores, each with a specific length and polar tube structure (5, 12 and 30 filament coils in respective spore types) (Vávra et al., 2006) (see Section 3.1.2, p. 280). The structure of the individual tube also varies along the length of the unextruded tube, being uniformly thick along the entire length (isofilar tubes), or being thicker at its proximal part (anisofilar tubes). If the proximal part is very thick, it is called manubrium (Lom, 1972; Vávra and Larsson, 1999). There is no explanation for these differences in the injection tube structure.

It is worth mentioning that the extruded tube is usually longer than estimated from TEM photomicrographs of spore coils in the dormant spore. It remains to be determined whether the tube is fully preformed in the dormant spore (this is most probable) and is extruded as such, or if it is actually partly assembled during eversion from the spore. Lom and Corliss (1967) calculated the length of the tube from ultrathin sections of a fish microsporidium to be in the range of 100–120 μm, while, after extrusion, the tube was estimated to be 300–500 μm long. Such an expansion of length suggested that the tube may actually grow during eversion by the addition, and polymerisation on its growing tip, of material deposited in the interior of the unextruded tube (Keohane and Weiss, 1999;

Weidner, 1982). However, elongation of the tube might be simply due to its extensive elasticity, witnessed as it momentarily thickens during sporoplasm passage (up to several μm – Olsen et al., 1986), and its recoiling by 5–10% of length when germination pressure ceases. The elasticity might reside in the tube substructure since extruded tubes have a complex substructure (Cali et al., 2002), and negatively stained extruded tubes (after tryptic digestions) appear to possess a fibrillar ultrastructure reminiscent of the meshwork structure of a firefighter's hose (Vávra, 1976).

The evolutionary origin of the injection tube is not known. Due to the 'fungal connection' of microsporidia, attempts were made to interpret the injection tube as homologous to fungal structures involved in host invasion, such as the polar bodies of the 'holdfast' appressoria of trichomycete harpellaelan fungi (Cavalier-Smith, 1998), spore appressorium and penetration tube-like peg of the "chytridiomycete" *R. allomycis* (James et al., 2006) (similar penetration tube-like formation exists in *Amoeboaphelidium*, see Karpov et al., 2013; Letcher et al., 2013), and the sporangiophore of zygomycetous fungi (Lee et al. 2008). These fungal structures, however, are basically modified hyphae formed by the cell wall expansion and are likely to have a completely different chemical composition than that of the injection tube. Hence, they cannot be easily compared with the injection tube preformed inside the spore by cytoplasmic differentiation that does not involve the cell wall of the spore (Williams, 2009). Kugrens et al. (1994) and Vivares and Metenier (2001) remarked that the microsporidian injection apparatus resembles extrusomes, which are membrane-bound ejectable organelles common in some protists, especially ciliates and dinoflagellates, and capable of discharging their contents under certain conditions to the outside of the cell (Hausmann, 1978). The fundamentally unique feature of the microsporidian injection tube resides in the fact that it serves for emptying the entire spore contents and not only the specific extrusome material as occurs in protist extrusomes. It can be concluded that the origin of microsporidian injection apparatus remains to be unknown and appears to be a specific evolutionary apomorphy of these parasites.

3.1.2. Single Species but Different Spores

The extreme adaptation of microsporidia to their host physiology and life cycle is reflected in the facts that even within a single-host species, spores may be formed that are different both physiologically and structurally. When two or more different spore types are produced, the given species is termed to be dimorphic or polymorphic respectively. Distinct

spore types are formed either in different hosts through which the organism completes its life cycle, or as an outcome of different life cycle paths occurring in the same host. For example, *V. disparis,* a parasite of the gypsy moth (*Lymantria dispar* L.), forms three spore types: one for quick dissemination within the host, and two other types that serve for interhost transmission (Vávra et al., 2006). Some species parasitising mosquitoes form different spores either in the same or in different hosts (Becnel et al., 2005; Lukeš and Vávra, 1990). The life cycle of *Amblyospora* spp., involves three hosts: a copepod, a male mosquito larva and a female mosquito adult. A different spore type is formed in each host (Andreadis, 2007). The individual types differ to the extent that they would be assigned to different genera in conventional classification. *Kneallhazia solenopsae* forms four distinct spore types in various life cycle stages and tissues of its host, the fire ant *Solenopsis invicta* (Sokolova and Fuxa, 2008). An interesting example is that of the human microsporidian opportunist *Trachipleistophora anthropophthera*, which forms two structurally very distinct spore types that are probably involved in intra- and interhost dissemination, respectively. Both types are formed in the same brain tissue and sometimes within a single cell (Vávra et al., 1998). It is perhaps of evolutionary importance that the capacity to form several distinct spore types occurs in the putatively primitive microsporidia, class Metchnikovellidea (Weiser, 1977), hyperparasites of gregarines and some insects (Desportes and Theodorides, 1979; Larsson, 2000; Larsson and Koie, 2006; Purrini and Weiser, 1985). However, even in monomorphic species, not all spores produced are identical. As discussed below, spore variability expressed as a differential response to germination stimuli probably plays an important role in microsporidia evolution.

3.1.3. Spore Germination and Its Evolutionary Significance

3.1.3.1. The Germination Event

Although spore germination has been well documented (Frixione et al., 1992; Lom and Vávra, 1963), several aspects of the process are only partially understood. What initiates spore germination under natural conditions is unknown, although several experimental stimuli such as shifts in pH and ionic concentration have been identified (Keohane and Weiss, 1999). Germination can be triggered in some species by simply wetting dried spores (Olsen et al., 1986). It is generally accepted that germination is an osmotic event and it was proposed that the first step of the signalling cascade that triggers germination is calcium/calmodulin binding at the spore surface (Weidner and Byrd, 1982; Williams, 2009). It was postulated that the

next step in spore germination is the influx of water into the spore, possibly due to the activity of ionophore molecules (Dall, 1983) or aquaporins in the spore membrane (Frixione et al., 1997). Indeed, aquaporin-like genes were identified in genomes of several microsporidia (Akiyoshi et al., 2009; Corradi et al., 2009; Ghosh et al., 2006).

Germination begins by swelling of the polaroplast area during which the polaroplast membranous stacks dissociate. The pressure inside the spore rises to a point where the thinner apex of the spore case ruptures and the injection tube is expelled explosively while turning inside out during the process ('finger of a glove' model). While the tube everts, the posterior vacuole in the spore expands and forces the cytoplasm and the nucleus of the spore through the tube. The small cell appearing at the tip of the fully everted tube is enveloped by plasma membrane that is derived from the folded membrane system of the polaroplast and which was forced into the tube along with the cytoplasm and the nucleus (Weidner et al., 1984). After germinating, the spore case remains essentially empty but is lined inside by the former plasma membrane. During germination, the tip of the evaginating injection tube advances through the environment and penetrates any obstacle in its way, including other microsporidian spores (Weidner, 1972), and, injects the sporoplasm like a hypodermic needle, into the cytoplasm of an encountered host cell (Ishihara, 1968). The penetration power of the injection tube is enormous, e.g. spores of *Pleistophora hyphessobryconis* ingested by the ciliate *Paramecium* germinate in its food vacuoles, shoot the tubes throughout the ciliate cytoplasm and cell pellicle and deliver the sporoplasms to the ciliate surroundings (J. Vávra and J. Lom, unpublished observations). The penetration power resides in the fact that it is only the tip of the evaginating tube that advances through the environment while the entire length of the tube behind the tip is immobile (Franzen, 2004, 2005; Lom and Vávra, 1963; Weidner, 1972). The process of germination is so rapid that it takes approximately 2 s for the tip of the tube to advance through environment at a 'rocket speed' estimated to be more than 100 μm per second (Frixione et al., 1992).

Spore germination requires energy; however, the source of this energy remains unknown. Rapid breakdown of trehalose into glucose and the concommitant increase of osmotic pressure within the spore had been proposed as a source of the energy required for germination (Undeen, 1990). Both trehalose and the enzyme trehalase occur in microsporidia (Dolgikh and Semenov 2003; Metenier and Vivares, 2001), and trehalose is known to accumulate at the final stage of spore maturation (Undeen and Solter, 1996).

However, it is unlikely that trehalose powers the germination process because Undeen and Van der Meer (1999) reported that the level of trehalose decreases during in vitro spore germination in microsporidia from aquatic hosts but not in those from terrestrial hosts. It is quite counterintuitive that different microsporidia would use several osmolytes for germination instead of a single and universal source. It is also questionable to distinguish aquatic and terrestrial microsporidia because there are species that have different spore morphs in both types of hosts. Thus, it seems more reasonable to suppose that, in microsporidian spores, trehalose serves as an energy source for spore survival and as an anti-desiccation agent (Metenier and Vivares, 2001). The depletion of trehalose that occurs in spores exposed to stress factors and to long-term storage corroborates this proposed function (Undeen and Solter, 1996).

A different source of energy for spore germination was suggested to be located in the posterior vacuole. Findley et al. (2005) suggested that the posterior vacuole is a giant peroxisome in which hydrogen peroxide produced by the decomposition of long-chain fatty acids is converted by catalase into water and oxygen. This causes the posterior vacuole to swell and eject the spore contents from the spore. Although catalase was histochemically detected in spores of *Spraguea lophii* (Weidner and Findley, 2002, 2003), the general presence of catalase in microsporidia remains questionable; the gene for catalase seems to be missing from the *E. cuniculi* genome but, in contrast, *P. locustae* possesses a catalase gene of prokaryotic origin (Fast et al., 2003). The uncertainty concerning the presence of catalase in microsporidia requires further investigation as one could reasonably expect that all microsporidia should utilise the same germination mechanism. In summary, the source of energy for spore germination has yet to be determined.

Microsporidia spread via spore germination either within cells of an individual host (intrahost dissemination) or by invading another host usually when spores are ingested and germinate within digestive tract (interhost dissemination). Some microsporidian species form special spore types for intrahost dissemination but it seems that, in most microsporidian species, spores are essentially similar but differ in their readiness to germinate. Spores that could be called 'precocious germinators' germinate as soon as they are formed inside the host cells or released from bursting cells. For example, many *E. cuniculi* spores, freshly liberated from bursting infected cells into a cultivation medium, immediately germinate while other spores remain unchanged (Vávra et al., 1972). When this occurs in infected tissue, the sporoplasms are introduced into surrounding host tissues and

thus parasite disseminates within the host. Systematic data addressing this scenario in vivo are not available because inside-the-host germination is usually not recorded; empty spores are quickly degraded by the host and/or spore remains are reported as artefacts. The stimuli that promote intracellular germination are currently unknown; however, ionic changes that accompany cell death are suspect as a candidate. In general, the formation of precociously germinating spores is a very little known phenomenon, despite that it is probably of common occurrence.

As mentioned previously, some microsporidia that infect *Diptera*, *Hymenoptera* and *Lepidoptera* form special generation(s) of precociously germinating spores, which are structurally distinct and which are produced at an early stage of infection. Such 'early' or 'primary' spores have thin spore walls, large posterior vacuoles and relatively short injection tubes. They germinate spontaneously, immediately after formation, and inject their sporoplasms into distant target tissues of the host where another generation(s) of 'secondary' or 'environmental' spores is formed. A microsporidium that infects the gypsy moth *L. dispar* represents an example of this strategy. Ingested 'secondary' spores are acquired from the environment by larvae and germinate in their midgut tissues. The first generation of spores is produced very quickly (30 h post-infection) in muscle cells surrounding the gut. These early spores germinate spontaneously upon maturation and their sporoplasms are injected into fat body, salivary and silk glands, and other tissues where environmental spores destined for interhost transmission are produced. These secondarily formed spores have a smaller posterior vacuole, thicker spore walls and a long injection tube (Solter and Maddox, 1998b; Vávra et al., 2006). It seems that the production of primary spores is controlled genetically rather than by the host, because they are produced also in microsporidia that are grown in tissue cultures (Iwano and Ishihara, 1991; Iwano and Kurtti, 1995).

3.1.3.2. Evolutionary Aspects of Spore Germination

We can only speculate about the potential evolutionary aspects of spores that are formed in the same host but endowed with a different sensitivity to germination stimuli. Spores with a capacity for delayed germination may pass through (even numerous) non-specific hosts before they eventually release their sporoplasms in a host that provides the most favourable conditions. Kramer (1973) noted that most spores of *Octosporea muscae-domesticae,* which parasitise the gut epithelium of the house fly, germinate during the first passage through the alimentary tract; some spores, however, germinate

only after they have undergone two to four passages. The combination of delayed germination and a lack of specific germination stimuli (meaning that germination would occur only in the most favourable and specific host) may thus be a powerful factor inherent to microsporidian evolution. Human infections caused by opportunistic microsporidia of probable insect or fish origin (see Section 4.3, p. 291, for the list and references) and the present expansion of the Asian honeybee, *Apis cerana,* parasite *N. ceranae* among populations of the European honey bee *Apis mellifera* (Fries, 2010) provide a cogent testimony that potential incidents of host-switching and of host range expansions continuously occur. The peculiar germination mechanism that microsporidia possess also allows them to successfully infect parasitic organisms that do not possess a functional digestive tube. Members of the supposedly primitive order Metchnikovellida are examples of such a strategy, as they live as hyperparasites in gregarines that inhabit the alimentary tract of polychaetes, sipunculids and echiurids (Canning and Vávra, 2000; Larsson, 2000; Larsson and Koie, 2006). One microsporidian lives as a hyperparasite in the protist *Marteilia*, parasite of oysters (Comps et al., 1979). Several microsporidian species are known to be hyperparasites of parasitic platyhelminths, tapeworms and trematodes (Canning, 1975; Sene et al., 1997). In each of these hosts, the sporoplasm must be injected into the host from outside, through the host's cell membrane complex (gregarines, *Marteilia*) or tegument (platyhelminths). The stimulus for spores to germinate must be provided in the digestive tract of the host that harbours the gregarine, tapeworm or trematode. This peculiar germination–injection mechanism explains why the larvae (rediae) of the fluke *Fasciola hepatica* become infected when the snail *Lymnea*, hosting the trematode, is fed spores of the mosquito microsporidium *A. algerae* (Costa and Bradley, 1980).

3.1.3.3. Spore Germination: the Unknowns

Many questions need to be answered in order to satisfactorily understand the spore germination event and its impact on the distribution of microsporidia and their host and tissue specificity. Even the basic question of how the injection tube penetrates the cell has been debated. Although direct mechanical piercing represents the generally accepted explanation, Agaud et al. (1997) and Rönnebäumer et al. (2008) suggested that the extrusion of the injection tube of *E. cuniculi* into an invagination of the host-cell membrane might be integral in the infection process, meaning that it is different from that of other microsporidia. This is an improbable contention, especially if one realises that the penetration power of the tube is such,

that a cell membrane does not present any obstacle to the germ injection (see Section 3.1.3.1., p. 281). Together with the question of how the parasitophorous vacuole in which *Encephalitozoon* develops is formed, the issue needs further research at best and is beyond the scope of the present review. Additional questions include the following: Is the spore somehow oriented before discharge and do the spores germinate preferentially when adjacent to the host tissues? *In vitro* experiments demonstrated that the adherence of spores to glycosoaminoglycans of target cell is related to the success of the infection (Hayman et al., 2005; Leitch et al., 2005b; Southern et al., 2007). However, no contact with host tissue was found to be required in germination of *N. apis* and *N. ceranae* spores (Gisder et al., 2011). There is also some evidence that intestinal mucus may influence spore orientation (Leitch and Ceballos, 2008). Spores of *E. intestinalis* were shown to express an endospore-targeted protein (EnP1) on their coat with most of this protein detected in the area of the anchoring disc, which is the apex of the injection tube. Potentially, EnP1 mediates spore attachment to the host-cell glycosoaminoglycans (Hayman et al., 2005).

3.1.3.4. Within-Host Dissemination: More Unknowns

Very little data is extant on the within-host dissemination of microsporidia. As mentioned previously, within-host spore germination is involved in dissemination (at least in some species); however, it seems that there should be another mechanism enabling the parasite to spread among host cells. A remarkable characteristic common in many microsporidian infections is the massive invasion of host tissues. This is most obvious in small invertebrate hosts where the host often becomes a living 'bag' of spores. Yet, how the infection progresses rapidly from cell to cell is not understood and, for some species, internally germinating spores have never been observed. A typical case is that of *Amblyospora* spp. which progressively infects the oocytes of copepods. The entire host ovary appears to become one large mass of spores, all of them at the same stage of maturation, yet no germinated spores are present (J. Vávra, unpublished observation). This phenomenon certainly requires further research.

The tissue tropism and specificity of microsporidia also remain largely obscure. *Enterospora canceri* and *Enterospora* sp. are confined to the nuclei of epithelial cells lining the hepatopancreas of the European edible crab, *Cancer pagurus* (Stentiford et al., 2007). These hepatopacreatocytes are not in direct contact with the food consumed by the host, so it is quite enigmatic as to how the parasite finds way to its specific location. Similarly, *Nucleospora*

salmonis, Nucleospora secunda and *Enterospora* sp. specifically infect host cell nuclei (Chilmonczyk et al., 1991; Lom and Dyková, 2002; Stentiford and Bateman, 2007). Do these species occur in the nucleus because the injection tube strikes the nucleus either by chance or due to an unknown targeting mechanism? Or were they injected first into the cytoplasm and then translocated into the nucleus? The notion that microsporidia can be translocated secondarily into a specific cell compartment seems unlikely, but cannot be excluded. Most intriguing is the case of several species of the genus *Spraguea*, which infect neural tissues of the angler fish *Lophius*. The parasite evidently travels from cutaneous mucous gland to medulla oblongata, and then to various nerves, with spores being finally excreted in urine (Freeman et al., 2011). How the parasite finds the way to its locations is not known.

4. THE 'ART OF LIVING IN THE HOST'

4.1. Well-Adapted Pathogens

Microsporidia qualify as parasites, sine qua non as they 'infect host cells, exploit what is in there to replicate within them, produce spores and then transmit themselves to other cells within the same host or to a new host' (Agnew et al., 2003). However, during the life cycle, each individual microsporidium passes through a less extreme form of symbiotic relationship, behaving first as a seemingly harmless, intracellular commensal. Infected host cells are ultimately destroyed during the period when the parasite forms spores. To what extent the host is harmed during this last phase of the microsporidian life cycle depends on many factors, several of which involve the host itself. This makes the host–parasite relationships strongly context-dependent and subject to variation between deleterious (including host mortality), more or less neutral, or even positive in some cases (Ryan and Kohler, 2010). Consequently, the variety of host–microsporidian interactions, categorised for the first time for mosquito microsporidia by Kellen et al. (1965), is enormous. It depends on the respective parasite and host species, the degree of their co-adaptation, tissue tropism, genotype or sex of the host, environmental conditions, means of transmission and other factors (Agnew et al., 2003; Becnel et al., 2005; Ebert, 1995, 2008; Issi, 2002; Little and Ebert, 1999; Ryan and Kohler, 2010; Smith, 2009).

In retrospect, microsporidia have mastered the means to maximise reproduction and transmission by producing large numbers of infectious spores and/or by efficient transmission mechanisms that maintain the pathogens in host populations. This is demonstrated by some infections in insects. Many

insect microsporidia cause infections that initially do not appear to injure the host in obvious ways, even when massive multiplication of the parasite in host organs takes place. For example, some species that infect lepidopteran larvae progressively destroy the fat bodies of their hosts as the cells become filled with spores. Infected larvae still feed and behave in a more or less normal way; however, they usually die during molt or pupation, when energy reserves of the host's fat body tissues needed for molting and metamorphosis are unavailable (Agnew et al., 2003; Goertz and Hoch, 2008a; Siegel et al., 1986; Weiser, 1961). Delaying the death of the host until the late stage molt or pupation allows the parasite to maximise the production of spores; this reservoir of spores released by the cadavers provides the inoculum to infect new hosts (Goertz and Hoch, 2008a,b). In some Lepidopteran species that produce silk, microsporidian spores may be transmitted along the silk secreted by larvae from infected silk glands (Jeffords et al., 1987).

The maximum production of spores and their dissemination into the environment is also the strategy employed when the microsporidian pathogen targets a tissue that has high regenerative capacity (gut or excretory epithelia), thus allowing the continuous production of spores. In such case, even a massive infection leaves the host apparently unaffected, at least temporarily. This occurs in midgut infections of gypsy moth larvae by *Endoreticulatus schubergi* (Hoch et al., 2009) or in the nematode *Caenorhabditis* infected with *N. parisii* ('the nematode-killer from Paris'). The nematode host can carry a large parasite burden in its gut epithelial cells and still feed and behave relatively normally, at least for some time. Ultimately, however, its life span is shortened (Troemel, 2011).

In mammals (and probably true for many vertebrates), microsporidian infections have a chronic character, provided that the host is immunocompetent and the infection remains localised (Ghosh and Weiss, 2012). On the contrary, immunocompromised hosts may develop a lethal disease (Texier et al., 2010). The apparent benign nature of chronic infections is, however, relative. This is best demonstrated in rabbit encephalitozoonosis, a very frequent microsporidiosis caused by *E. cuniculi* in farm-bred rabbits worldwide. The spores of the pathogen are excreted in the urine and the infection is easily transmitted from cage to cage by contamination in intensive breeding situations (Vávra et al., 1986, 1987). Rabbit encephalitozoonosis usually follows a subclinical course (no preventive or curative measures are applied by breeders) and infected animals appear healthy (Künzel and Joachim, 2010). However, a detailed examination of a group of well-fed, clinically healthy broiler rabbits, with relatively high titres of antibodies against *E. cuniculi,*

showed 11% reduction in the weight of the meat carcass at slaughter compared with serologically negative animals (Vávra et al., 1980).

Extending the life span of the host is beneficial for many pathogens and microsporidia are a prime example of such a strategy as they usually do not excessively or prematurely harm the host. However, physiological weakening of the host's immune defences may lead to explosive colonisation of the host by the parasite with ensuing deleterious consequences. In such cases, microsporidia behave as opportunists that are not only able to excessively colonise their usual host, but also potentially infect atypical hosts. Microsporidioses are thus included in the Emerging Diseases category (Didier, 2005). Their opportunism is best shown in HIV/AIDS-positive or severely immunosuppressed humans, in whom a cryptic infection can be exacerbated or an infection by a species of unknown source can occur (Anane and Attouchi, 2010; Didier et al., 2004; Didier and Weiss, 2006, 2011). The immune status dependency of microsporidian infections is reflected in the recently improved immune status of HIV-infected humans; due to the availability of more efficient anti-HIV drug cocktails, a notable decrease has occurred in the number of publications addressing microsporidia/HIV co-infections (Heyworth, 2012). The opportunistic character of microsporidia is also revealed in infections of the eye, an immunoprivileged site. Human and animal keratoconjuctivitis and uveitis caused by microsporidia are not a rarity. Exposure to dust and muddy water, minor injuries, hot springs exposure and warm and wet climates are risk factors for ocular microsporidiosis (Alkatan et al., 2012; Fan et al., 2012; Juarez et al., 2005; Kunzel and Joachim, 2010; Quek et al., 2011; Sharma et al., 2011). Eye infections are in principle, dangerous as they can progress to systemic diseases (Koudela et al., 2001).

4.2. Host-Specific Parasites

Most microsporidia are host-specific parasites which evolved and co-speciated with their original hosts. Their specificity is typically restricted to a single host species or to a group of affiliated species (Baker et al., 1998; Shafer et al., 2009; Smith, 2009). It is the concensus among traditional taxonomists that the host is an important indicator of microsporidian species. Two good examples of microsporidian co-speciation with their hosts is the phylogeny of *Loma* spp., which mirrors the evolutionary course of gaddid fish in the Pacific Basin (Brown et al., 2010) and the phylogeny of mosquito microsporidia, which reflects the evolutionary relationships of respective genera with their hosts (Andreadis et al., 2012).

Since microsporidia tend to group by hosts (Brown et al., 2010), and host phylogeny and ecology are mostly compatible, an attempt was made to define formal microsporidia taxons according to their host habitat. Hence, classes 'Aquasporidia', 'Marinosporidia' and 'Terresporidia' were proposed for species parasitising hosts in the respective freshwater, marine and terrestrial habitats (Vossbrinck and Debrunner-Vossbrinck, 2005). These classes have been occasionally used in actual classification (Stentiford et al., 2010), despite criticism raised after their creation by those who respect formal taxonomy rules (Larsson, 2005).

Host ecology also seems to play a role in the maintenance of host specificity and microsporidian diversity. Some microsporidia that are able to infect multiple host species under experimental conditions (when the general compatibility between the pathogen and the host, termed 'physiological specificity' is determined) do not infect the same host in nature because ecological barriers between atypical hosts and the parasite exist. These barriers determine the 'ecological specificity' of the pathogen (Solter and Maddox, 1998a). Vertically transmitted species parasitising forest Lepidoptera represent parasites for which the physiological and the ecological specificity are often not congruent (Solter, 2006; Solter et al., 2005). Frequently, host and tissue specificity are related, e.g. *N. apis* is confined to the midgut enterocytes of the honeybee (although it is also able to infect gypsy moth cells in a tissue culture – Gisder et al., 2011), and *S. lophii* parasitises the neural ganglia of the angler fish *Lophius* sp. (Freeman et al., 2004, 2011). Other microsporidia, however, parasitise broader groups of hosts, and are less tissue-specific; mammalian microsporidia are a good example. For the widespread *E. cuniculi, E. hellem* and *E. intestinalis*, low host and practically no tissue specificity are characteristic, whereas *E. bieneusi* is mostly but not totally confined to the enterocytes and occasionally infects epithelial cells of the respiratory and urinary tracts of its multiple mamalian hosts (Botterel et al., 2002; Sak et al., 2011b,c).

The physiological basis of host and tissue specificity in individual microsporidian species is not known, but one possible reason is genome reduction leading to the close dependency of the parasite on host metabolism (Peyretaillade et al., 2011). Interestingly, investigation of several human-infective species, including those that parasitise mammals or are simply opportunists, showed that these microsporidia contain a specific inter-B gene family, located in the subterminal chromosomal regions. This inter-B multigene locus is absent in a number of species not implicated in human infections (Dia et al., 2007). Several members of the inter-B gene family

possess predictable transmembrane domains and N-terminal signal peptides, suggesting that respective proteins are targeted to parasite surface (Williams, 2009), and hence likely have an impact on host–parasite relationships.

4.3. Ubiquitous Parasites

The ability to inject their germplasm (sporoplasm) into the intracellular milieu of a host opened for microsporidia an ecological niche represented by a vast variety of hosts and their respective tissues, and allowed them to rapidly radiate. Microsporidia are recognised as one of the most frequently observed parasites of both invertebrates and vertebrates, including humans. While they have been found to occur in representatives of about half of metazoan phyla, it is without doubt that many, if not most, species still await discovery, given the low number of experts studying these parasites and the plethora of prospective animal hosts. True microsporidian diversity might approach and or surpass the number of animal species (Keeling and Slamovits, 2004); parasitism of a single-host species by several microsporidian species is not uncommon (Krebes et al., 2010).

Most microsporidian diversity is found in invertebrates, with crustaceans and insects being the most frequent hosts. In the paper of Vossbrinck and Debrunner-Vossbrinck (2005) proposing microsporidian classes based on host ecology, 69% out of the 125 species listed have crustacea and/or insects as their hosts. Although this number has only a limited value due to low sampling, it reflects the relative abundance of microsporidia in respective hosts.

Insect microsporidia are memorable as the agent of the collapse of European silk industry in the mid-nineteenth century, caused by *N. bombycis,* the first microsporidian described (Nägeli, 1857). This species is unquestionably the most important parasite of the silkworm as even today it is a threat to the silkworm industry (Bhat et al., 2009; Kawarabata, 2003). *Nosema bombi* infections of bumblebees and *N. apis* and *N. ceranae* infections of honey bees exemplify widespread pathogens of principal pollinators (Cameron et al., 2011; Chen et al., 2009; Otti and Schmid-Hempel, 2007). *Nosema ceranae*, originally the parasite of the Asian *Apis cerana*, has progressively invaded on the world-wide scale the colonies of the European honey bee, *A. mellifera* (Fries, 2010). This parasite has a significant pathogenic effect on its new host. It disturbs gut tissue regeneration and homeostasis and influences a number of physiological functions of the bee gut (Dussaubat et al. 2012). It is of considerable economic importance that even the sublethal levels of pesticide residues, now commonly occurring in agriculture,

significantly increase the sensitivity of honey bees to *Nosema* infections and contribute to bee colonies mortality (Pettis et al., 2012; Wu et al., 2012). More than 150 microsporidian species have been described from different mosquito genera worldwide, and it is believed that most if not all mosquito species serve as hosts for at least one microsporidian parasite (Andreadis, 2007; Andreadis et al., 2012). Some microsporidia act as regulators and/or agents of biological control for noxious insects of economic or health importance (Bargielowski and Koella, 2009; Hajek and Delalibera, 2010; Lomer et al., 2001). *Nosema pyrausta*, which infects the European corn borer, *Ostrinia nubilalis*, is a classical example of a parasite that effectively helps to regulate an important agricultural insect pest (Lewis et al., 2009), while *P. locustae* (better known to public by its original name *Nosema locustae*) is used as a biological pesticide for grasshopper control (Henry and Oma, 1981; Lomer et al., 2001).

The second most numerous group of microsporidia are found in crustacean hosts. Economically significant representatives are *Enterocytozoon hepatopenaei* and *Myospora metanephrops* from shrimps and lobsters, respectively (Tourtip et al., 2009; Stentiford et al., 2010).

Fish, the most common vertebrate hosts of microsporidia, with more than 160 described species assigned to more than 17 genera (Canning and Lom, 1986; Lom, 2002; Lom and Nilsen, 2003), are known to cause important losses to fisheries, both in natural conditions and in aquaculture (Abdel-Ghaffar et al., 2011; Dyková, 2006;). *Loma salmonae* has a significant negative impact on commercial cultures of salmonids (Shaw et al., 2000), while *Pseudoloma neurophilia* and *P. hyphessobryconis* occasionally plague the research colonies of the laboratory model zebrafish *Danio rerio* (Matthews et al., 2001; Sanders et al., 2010). Several microsporidian species are known to parasitise mammals (Canning and Lom, 1986). Of about 14 microsporidian species found infecting humans, only four (*E. cuniculi*, *E. hellem, E. intestinalis* and *E. bieneusi*) are specific to mammals, while others (e.g. *Vittaforma corneae*, *T. hominis, T. anthropophthera, A. algerae, Tubulinosema* spp., *Endoreticulatus*-like microsporidium, and several organisms assembled under the collective genus *Microsporidium*) are opportunists that have been acquired by humans from insects or unknown hosts (Anane and Attouchi, 2010; Cali and Takvorian, 2004; Canning and Lom, 1986; Coyle et al., 2004; Curry et al., 2005; Didier, 2005; Didier et al., 2004; Didier and Weiss, 2006; Cali et al., 2010; Choudhary et al., 2011; Fan et al., 2012; Field et al., 2012; Juarez et al., 2005; Mathis et al., 2005; Meissner et al., 2012; Suankratay et al., 2012; Vávra et al., 1998), or as in the case of *Pleistophora ronneafiei,* probably from

fish (Cali and Takvorian, 2003). The ability of some mammalian microsporidia to infect insect hosts (and thus possibly be of insect origin) has been experimentally proved (Vávra et al., 2011; Weidner et al., 1999a).

4.4. Cryptic Existence Masks Wide Occurrence

Because of their cryptic existence in their hosts, only a relatively small fraction of putatively extant microsporidia has been discovered to date. Many infections pass unnoticed, either permanently or at least temporarily, when the infections are mild and spore loads are low. This cryptic existence of microsporidia is shown most clearly by the use of molecular techniques that allow sensitive diagnosis of low-level infections (Accoceberry and D'Almeida-Fourquet, 2012; Franzen and Müller, 1999; El-Matbouli and Soliman, 2006; Hamiduzzaman et al., 2010; Refardt and Ebert, 2006; Valenčáková et al., 2012; Weiss and Vossbrinck, 1999). Indeed, molecular techniques have shown microsporidia to be unexpectedly common parasites. The human intestinal microsporidium *E. bieneusi* was originally described as a rare, HIV-associated pathogen (Desportes et al., 1985). Presently, more than 90 genotypes of this organism, distinguished on the basis of the hypervariable ITS region of the SSU rRNA gene, have been recognised from a number of mammalian hosts, most of which exhibit no overt clinical symptoms (Santin and Fayer, 2009a,b; 2011; Thellier and Breton, 2008). *E. bieneusi* actually may be one of the most common human parasites with a prevalence attaining 58% in some populations (Nkinin et al., 2007). Although most infections are inapparent, the association of this parasite with diarrhoeal disease is well known (Akinbo et al., 2012; Ojuromi et al., 2012), and recently the capacity of *E. bieneusi* to cause small food-borne epidemics in immunocompetent subjects has been documented (Decraene et al., 2012). The number of its genotypes recovered from humans continues to rise (Sak et al., 2011b,c; Zhang et al., 2011). The widespread prevalence of *E. bieneusi* is evidently due to its low host specificity, since no clear-cut association exists between its genotypes and specific hosts. Many genotypes, the human-ones included, occur in several hosts: out of 12 genotypes identified in humans in China, nearly one half occur also in animals, strongly indicating the possibility of zoonotic transmission (Zhang et al., 2011). Overall, the tendency of genotypes to segregate according to the host species is weak and is revealed only through the use of quantitative phylogenetic tests (Widmer and Akiyoshi, 2010).

Another example of a cryptic, yet extensive occurrence is the presence of the *E. bieneusi, E. cuniculi, E. intestinalis* and *E. hellem* DNA in birds.

Eleven percent of feral pigeons in Amsterdam excrete spores of these microsporidia in fecal droppings (Bart et al., 2008) and nearly 40% of droppings of 300 healthy pet birds were PCR-positive for the above species (Kašicková et al., 2009). The actual prevalence of these cryptic infections may be even higher as shown by PCR-based daily examination of individual droppings of a group of budgerigars (*Mellopsittacus undulatus*) (Sak et al., 2010). All birds excreted DNA of several microsporidian species during the whole investigation period (one month), but respective parasite species DNAs were excreted at irregular intervals in individual birds. The birds seemed healthy and the necropsy search for microsporidia by histopathology was negative, although various internal organs were PCR-positive (Sak et al., 2010). Another example of the widespread occurrence of microsporidia is that of wild mice. Sak et al. (2011c) examined by PCR the intestinal contents of nearly 300 specimens of the wild mouse, *Mus musculus* trapped in Central Europe, and found the respective DNA of *E. cuniculi, E. hellem* and *E. bieneusi* in 33% of the sampled individuals. Six percent of infected animals contained more than one microsporidian species (Sak et al. 2011c).

The cited examples of broad microsporidian occurrence suggest that humans are exposed to microsporidia to a degree that is not fully appreciated. This is documented in a recent survey for microsporidian DNA in a healthy human population in the Czech Republic, a country with good hygienic standards. Among 400 healthy people, 42% excreted microsporidian DNA in their feces, with *Encephalitozoon* spp. being the most frequently identified, followed by *E. bieneusi* (Sak et al., 2011a). In another longitudinal study, a group of healthy persons with some occupational risk of contact with animals was followed for three months on a weekly basis using PCR and immunological methods. The study demonstrated convincingly that microsporidian infections are widespread and long lasting: out of the 15 monitored individuals, 13 excreted spores in their feces and urine (13 and 7 of *Encephalitozoon* spp. and *E. bieneusi*, respectively), yet no clinical symptoms were observed during the examination period (Sak et al., 2011b).

4.5. Strong Adaptation to Host Life Cycles and Biology

The mechanism for spore germination provides two ways for microsporidia to infect the host. Most frequently, the host is infected horizontally by spores that are acquired from the environment. Alternatively, infection occurs vertically by transovarial transmission when sporoplasms are injected into germinal cells of the gonads inside the parental organism. In some cases, mature spores are found in eggs (Phelps and Goodwin, 2008). Remarkably, however,

the sporoplasms injected into the germinal cells usually postpone their development to the beginning of embryogenesis (Weiser, 1961). Although horizontal transmission is most common, vertical transmission is apparently underreported, and in many cases, microsporidia are transmitted both horizontally and vertically (Dunn and Smith, 2001; Solter, 2006). The difference in virulence of ensuing infections is attributed to these respective transmission ways. Species that rely principally on vertical transmission tend to produce more cryptic infections and best represent the intimate relationship between parasite and host. Several microsporidian species that infect gammarids (*Crustacea: Amphipoda*) are primarily transmitted vertically, and cause feminisation of host males by preventing the development of their androgenic gland, thereby abolishing production of the hormone that is essential for male development (Haine et al., 2004; Rodgers-Gray et al., 2004; Terry et al., 2004). Feminisation of the host benefits the parasite because it is transovarially transmitted via the gonadal tissues of females (Bandi et al., 2001). In developing embryos, microsporidia ensure their distribution in the host by being segregated to specific daughter embryonal cells (Weedall et al., 2006). The infection of daughter embryonal cells is secured by positioning of the parasites along the axis of the nuclear spindle (Terry et al., 1999). Feminisation not only favours the parasite by augmenting the number of female individuals bearing infection, but unexpectedly seems to be advantageous for the host because infected females of *Gammarus roeseli* breed earlier in the reproductive season. This is the first case in which a positive effect of a microsporidian infection on the host has been recorded (Haine et al., 2004, 2007). Surprisingly, however, this positive effect is dependent on infection intensity: female gammarids have higher fecundity and more reproductive bouts only at intermediate infection intensity. Neither light nor high intesnsity infections have the same effect (Ryan and Kohler, 2010).

In addition to the influence on the host sexual orientation mentioned above, there are multiple additional impacts of microsporidia on hosts, and consequently only a few examples can be given here. Microsporidia were reported to influence the host transcriptome (Dussaubat et al., 2012) and proteome (Biron et al., 2005), alter the courtship and shoaling of infected fish (Pelabon et al., 2005; Ward et al., 2005) and change the swimming behaviour of cladoceran crustacea (Makrushin, 2010; Vávra and Pražáková, 1983). In insects, infection by microsporidia may extend the developmental period of the host and lead to host-larval gigantism (Blaser and Schmid-Hempel, 2005; Down et al., 2008; Henn and Solter, 2000). The physiological reasons for such extension are multiple and depend on the specific host, the parasite,

the timing of infection and its dose (Henn and Solter, 2000). Analysis of these factors is beyond the scope of this chapter but it is worth mentioning that some infections in insects, leading to extended larval development and host gigantism, were originally explained by the parasite's capacity to physiologically manipulate production of the juvenile hormone in the host. This may lead to the delay in moulting (Fisher and Sanborn, 1962, 1964). This issue has been debated extensively and the metabolic stress caused by the infection was proposed to be the factor that mimics the effect of the juvenile hormone (Henn and Solter, 2000; Issi and Tokarev, 2002; Seleznev, 2003; Karlhofer et al., 2012). Other reports, however, claim that infected insect larvae have indeed elevated levels of the juvenile hormone, causing extension of their pre-moulting time. This may allow the parasite to produce more spores and increase its reproductive fitness (Down et al., 2008).

Many interesting adaptations of microsporidia to their hosts and to their life cycles have been described for species inhabiting the freshwater environment. The aquatic habitat significantly influences the distribution of infective spores in the environment. Spores of numerous aquatic species are equipped with mucous layers, sporal hairs, tubules or tails formed as outgrowth of the exospore layer (Canning and Vávra, 2000; Vávra et al., 2005). The likely function of these apendages is to serve as aids in buoyancy, thus helping to retain the spores in the water column and influencing their accessibility and uptake by the host. There are no experimental data in support of this function, except for an anecdotal case when microsporidian spores floating in pond water were originally described as a planktonic alga (Lemmermann, 1900). However, their true identity was revealed only much later (Vávra et al., 2005). In the aquatic environment, spores can pass from host to host more easily, thus facilitating the complex life cycles involving several host species. Such life cycles have been described (with several modifications) for *Amblyospora* spp. that complete their life cycle by cycling between mosquito larvae, copepods and mosquito female adults. Spore morphs, structurally so distinct that they would be ranked into different genera, are formed in each host and male killing allows transmission of infection from mosquito larvae to copepods (Andreadis, 2005, 2007; Lukeš and Vávra, 1990; Sweeney et al., 1985). Similar life cycles may exist in many other aquatic microsporidia but have yet to be discovered. For example, in some aquatic microcrustacea, the spores fed to the original host do not cause infection. This provides circumstantial evidence that host exchange is evidently needed to perpetuate the life cycle (Mangin et al., 1995; Refardt et al., 2002, 2008; Vávra, 1964; Vávra and Larsson, 1994; Vávra et al., 2005; Wolinska et al., 2009). The examination

of life cycles in this context would appear to be a major issue of future microsporidian research.

5. ENVOI – THE 'MASTER PARASITES'

Microsporidia, a distinct form of life identified more than 150 years ago, are an unprecedented resource for the investigating the mechanisms involved in host-parasite interactions. These organisms developed morphological characteristics and life-cycle strategies that are totally different from fungi (probably their sister taxon) and to some extent, are also unique in the world of protists. Evolution of the injection apparatus and its projectile movement enabled microsporidia to colonise the intracellular niche of animal cells, to become intracytoplasmic parasites totally dependent on the host cell, and resembling, at a certain phase of the life cycle, intracytoplasmic symbionts. Upon entering into the new environment of the intracytoplasmic space, microsporidia gained an unprecedented ecological opportunity, which enabled them to quickly radiate. Their intracellular existence also allowed them to reduce certain elements of their cell structures and genomes, in some cases to the minimum required for eukaryotic existence. The availability of a wide variety of animal and protistan hosts and host tissues provided microsporidians with a vast opportunity to adapt to individual hosts and tissues and also to evolve rapidly and readily disseminate from host to host. We suggest that the accelerated evolution of these ubiquitous parasites was facilitated by their ability to produce infective spores that vary in their readiness to germinate. Microsporidia are considered to be very specific parasites, intimately adapted to their host species, specific tissues and even specific cell compartments. Nevertheless, they are constantly probing the environment by stochastically germinating spores, thus providing the potential to infect new hosts and new tissue sites. We speculate that the ability to produce spores of different germination readiness has been the key factor allowing microsporidia to radiate and to become one of the most common parasites of animals.

Microsporidia also provide a rich ground for further research. Having undergone dramatic reductive evolutionary events, microsporidia represent a fascinating model to study molecular, genome and cellular evolution. Vast numbers of microsporidia species remain to be discovered. Despite their abundance in nature, their relationship to the fungi has not yet been fully resolved, life cycles of many species remain to be clarified and interactions with their hosts need to be more precisely analysed using modern methods.

6. CONCLUSION

Microsporidia are unique organisms that can serve as models for a more thorough understanding of the, living-together, biological phenomenon.

ACKNOWLEDGEMENTS

This review is dedicated to the memory of two outstanding Czech Microsporidia researchers: Prof. Otto Jírovec (1907–1972), founder of the Czech parasitology school, and Dr Jaroslav Weiser (1920–2012), 'the father of modern insect pathology in Europe'. The following colleagues provided invaluable assistance to improve this paper: Dr Leellen Solter (Illinois Natural History Survey, University of Illinois, Urbana, USA), Dr Michael L. McManus (USDA Forest Service [retired] Hamden, USA), Dr David J. Morris (University of Strathclyde, UK), Prof. John. Lucocq (University of St Andrews, UK), Prof. T. Martin Embley and Dr Robert Hirt (Newcastle University, UK), Dr Toni Gabaldon (Centre for Genomic Regulation, Barcelona, Spain), Prof. Ronny Larsson (University of Lund, Sweden) and Prof. Andreas Linde (Hochschule für Nachhaltige Entwicklung, Eberswalde, Germany). Their critical comments and suggestions have been deeply appreciated. J.L. is a Fellow of the Canadian Institute for Advanced Research and was supported by the Praemium Academiae Award.

REFERENCES

Abdel-Ghaffar, F., Bashtar, A.R., Mehlhorn, H., Al-Rasheid, K., Morsy, K., 2011. Microsporidian parasites: a danger facing marine fishes of the Red Sea. Parasitol. Res. 108, 219–225.

Accoceberry, I., D'Almeida-Fourquet, M., 2012. Diagnosis of intestinal microsporidia. Revue Francophone des Laboratoires 440, 27–34.

Adl, S.M., Simpson, A.G., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., James, T.Y., Karpov, S., Kugrens, P., Krug, J., Lane, C.E., Lewis, L.A., Lodge, J., Lynn, D.H., Mann, D.G., MCCourt, R.M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S.E., Nerad, T.A., Shearer, C.A., Smirnov, A.V., Spiegel, F.W., Taylor, M.F.J.R., 2005. The new higher level classification of Eukaryotes with emphasis on the taxonomy of protists. J. Eukaryot. Microbiol. 52, 399–451.

Adl, S.M., Simpson, A.G., Lane, C.E., Lukeš, J., Bass, D., Bowser, S.E., Brown, M., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., leGall, L., Lynn, D.H., McManus, H., Mitchell, E.A.D., Mozley-Standridge, S.E., Wegener Parfrey, L., Pawlowski, J., Rueckert, S., Shadwick, L., Schoch, C., Smirnov, A., Spiegel, F.W., 2012. The revised classification of eukaryotes. J. Eukaryot. Microbiol. 59, 429–493.

Agaud, A.M., Achbarou, A., Desportes-Livage, I., 1997. Cell invasion by the microsporidium *Encephalitozoon intestinalis*. J. Eukaryot. Microbiol. 44, 81S.

Agnew, P., Becnel, J.J., Ebert, D., Michalakis, Y., 2003. Symbiosis of microsporidia and insects. In: Bourtzis, K., Miller, T. (Eds.), Insect Symbiosis, CRC Press LLC, Florida, USA, pp. 145–163.

Aist, J.R., Williams, P.H., 1971. The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. Can. J. Bot. 49, 2023–2034.

Akinbo, F.O., Okaka, C.E., Omoregie, R., Dearen, T., Leon, E.T., Xiao, L., 2012. Molecular epidemiologic characterization of *Enterocytozoon bieneusi* in HIV-infected persons in Benin City, Nigeria. Am. J. Trop. Med. Hyg. 86, 441–445.

Akiyoshi, D.E., Morrison, H.G., Lei, S., Feng, X., Zhang, Q., Corradi, N., Mayanja, H., Tumwine, J.K., Keeling, P.J., Weiss, L.M., Tzipori, S., 2009. Genomic survey of the non-cultivatable opportunistic human pathogen, Enterocytozoon bieneusi. PLoS Pathog. 5, e1000261.

Alkatan, H.M., Al-Zaaidi, S., Athmanathan, S., 2012. Microsporidial keratitis: literature review and report of 2 cases in a tertiary eye care center. Saudi J. Ophthalmol. 26, 199–203.

Anane, S., Attouchi, H., 2010. Microsporidiosis: epidemiology, clinical data and therapy. Gastroenterologie Clinique et Biologique 34, 450–464.

Andreadis, T.G., 2005. Evolutionary strategies and adaptations for survival between mosquito-parasitic microsporidia and their intermediate copepod hosts: a comparative examination of *Amblyospora connecticut* and *Hyalinocysta chapmani* (Microsporidia: Amblyosporidae). Folia Parasitol. 52, 23–35.

Andreadis, T.G., 2007. Microsporidian parasites of mosquitoes. J. Am. Mosq. Control Assoc. 23, 3–29.

Andreadis, T.G., Simakova, A.V., Vossbrinck, C.R., Shepard, J.J., Yurchenko, Y.A., 2012. Ultrastructural characterization and comparative phylogenetic analysis of new microsporidia from Siberian mosquitoes: evidence for coevolution and host switching. J. Invertebr. Pathol. 109, 59–75.

Arisue, N., Sanchez, L.B., Weiss, L.M., Muller, M., Hashimoto, T., 2002. Mitochondrial-type hsp70 genes of the amitochondriate protists, *Giardia intestinalis, Entamoeba histolytica* and two microsporidians. Parasitol. Int. 51, 9–16.

Baker, M.D., Vossbrinck, C.R., Becnel, J.J., Andreadis, T.G., 1998. Phylogeny of *Amblyospora* (Microsporida: Amblyosporidae) and related genera based on small subunit ribosomal DNA data: a possible example of host parasite cospeciation. J. Invertebr. Pathol. 71, 199–206.

Balbiani, G., 1882. Sur les microsporidies ou psorospermies des articules. C. R. Acad. Sci. Paris 95, 1168–1171.

Bandi, C., Dunn, A.M., Hurst, G.D.D., Rigaud, T., 2001. Inherited microorganisms, sex-specific virulence and reproductive parasitism. Trends Parasitol. 17, 88–94.

Bargielowski, I., Koella, J.C., 2009. A possible mechanism for the suppression of *Plasmodium berghei* development in the mosquito *Anopheles gambiae* by the microsporidian *Vavraia culicis*. PLoS One 4 e4676.

Bart, A., Wentink-Bonnema, E.M., Heddema, E.R., Buijs, J., Van Gool, T., 2008. Frequent occurrence of human-associated microsporidia in fecal droppings of urban pigeons in Amsterdam, the Netherlands. Appl. Environ. Microbiol. 74, 7056–7058.

Bartnicki-Garcia, S., 1970. Cell wall composition and other biochemical markers in fungal phylogeny. In: Harborne, J.B. (Ed.), Phytochemical Phylogeny, Academic Press, London, pp. 81–103.

Becnel, J.J., White, S.E., Shapiro, A.M., 2005. Review of microsporidia-mosquito relationships: from the simple to the complex. Folia Parasitol. 52, 41–50.

Beznoussenko, G.V., Dolgikh, V.V., Seliverstova, E.V., Semenov, P.B., Tokarev, Y.S., Trucco, A., Micaroni, M., Di Giandomenico, D., Auinger, P., Senderskly, I.V., Skarlato, S.O., Snigirevskaya, E.S., Komissarchik, Y.Yu., Pavelka, M., De Matteis, M.A., Luini, A., Sokolova, Y.Ya., Mironov, A.A., 2007. Analogs of the Golgi complex in microsporidia: structure and avesicular mechanisms of function. J. Cell Sci. 120, 1288–1298.

Bhat, S.A., Bashir, I., Kamili, A.F., 2009. Microsporidiosis of silkworm, *Bombyx mori* L. (Lepidoptera-Bombycidae): a review. Afr. J. Agric. Res. 4, 1519–1523.

Biron, D.G., Agnew, P., Marché, L., Renault, L., Sidobre, C., Michalakis, Y., 2005. Proteome of *Aedes aegypti* larvae in response to infection by the intracellular parasite *Vavraia culicis*. Int. J. Parasitol. 35, 1385–1397.

Blaser, M., Schmid-Hempel, P., 2005. Determinants of virulence for the parasite *Nosema whitei* in its host *Tribolium castaneum*. J. Invertebr. Pathol. 89, 251–257.

Bohne, W., Ferguson, D.J.P., Kohler, K., Gross, U., 2000. Developmental expression of a tandemly repeated, glycine- and serine-rich spore wall protein in the microsporidian pathogen *Encephalitozoon cuniculi*. Infect. Immun. 68, 2268–2275.

Bohne, W., Böttcher, K., Groß, U., 2011. The parasitophorous vacuole of *Encephalitozoon cuniculi*: biogenesis and characteristics of the host cell-pathogen interface. Int. J. Med. Microbiol. 301, 395–399.

Botterel, F., Minozzi, C., Vittecoq, D., Bouree, P., 2002. Pulmonary localization of *Enterocytozoon bieneusi* in an AIDS patient: case report and review. J. Clin. Microbiol. 40, 4800–4801.

Bouzahzah, B., Weiss, L.M., 2010. Glycosylation of the major polar tube protein of *Encephalitozoon cuniculi*. Parasitol. Res. 107, 761–764.

Bouzahzah, B., Nagajyothi, F., Ghosh, K., Takvorian, P.M., Cali, A., Tanowitz, H.B., Weiss, L.M., 2010. Interactions of *Encephalitozoon cuniculi* polar tube proteins. Infect. Immun. 78, 2745–2753.

Bowen, A.R., Chen-Wu, J.L., Momany, M., Young, R., Szaniszlo, P.J., Robbins, P.W., 1992. Classification of fungal chitin synthases. Proc. Natl. Acad. Sci. U.S.A. 89, 519–523.

Brinkmann, H., Van Der Giezen, M., Zhou, Y., De Rancourt, G.P., Philippe, H., 2005. An empirical assessment of long-branch attraction artefacts in deep eukaryotic phylogenomics. Syst. Biol. 54, 743–757.

Brosson, D., Kuhn, L., Prensier, G., Vivares, C.P., Texier, C., 2005. The putative chitin deacetylase of *Encephalitozoon cuniculi*: a surface protein implicated in microsporidian spore-wall formation. FEMS Microbiol. Lett. 247, 81–90.

Brown, J.R., Doolittle, W.F., 1999. Gene descent, duplication and horizontal transfer in the evolution of glutamyl-tRNA synthetases. J. Mol. Biol. 49, 485–495.

Brown, A.M.V., Kent, M.L., Adamson, M.L., 2010. Description of five new *Loma* (microsporidia) species in pacific fishes with redesignation of the type species *Loma morhua* Morrison & Sprague, 1981, based on morphological and molecular species-boundaries tests. J. Eukaryot. Microbiol. 57, 529–553.

Cali, A., Takvorian, P.M., 1999. Developmental morphology and life cycles of the microsporidia. In: Wittner, M., Weiss, L.M. (Eds.), The Microsporidia and Microsporidiosis, ASM Press, Washington, D.C., pp. 85–128.

Cali, A., Takvorian, P.M., 2003. Ultrastructure and development of *Pleistophora ronneafiei* n. sp., a microsporidium (Protista) in the skeletal muscle of an immuno-compromised individual. J. Eukaryot. Microbiol. 50, 77–85.

Cali, A., Takvorian, P.M., 2004. The microsporidia: pathology in man and occurrence in nature. Southeast Asian J. Trop. Med. Public Health 35, 58–64.

Cali, A., Takvorian, P.M., Weiss, L.M., 2002. *Brachiola algerae* spore membrane systems, their activity during extrusion, and a new structural entity, the multilayered interlaced network, associated with the polar tube and the sporoplasm. J. Eukaryot. Microbiol. 49, 164–174.

Cali, A., Neafie, R., Weiss, L.M., Ghosh, K., Vergara, R.B., Gupta, R., Takvorian, P.M., 2010. Human vocal cord infection with the microsporidium *Anncaliia algerae*. J. Eukaryot. Microbiol. 57, 562–567.

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., Griswold, T.L., 2011. Patterns of widespread decline in North American bumble bees. Proc. Natl. Acad. Sci. U.S.A. 108, 662–667.

Canning, E.U., 1975. The Microsporidian parasites of Platyhelminthes: their morphology, development, transmission and pathogenicity. CIH Miscellaneous Publication No.2, Commonwealth Agricultural Bureau, Great Britain, p. 32.

Canning, E.U., 1988. Nuclear division and chromosome cycle in microsporidia. Biosystems 21, 333–340.

Canning, E.U., Hazard, E.I., 1982. Genus *Pleistophora* Gurley, 1893: an assemblage of at least three genera. J. Protozool. 29, 39–49.
Canning, E.U., Lom, J., 1986. The Microsporidia of Vertebrates. Academic Press, London.
Canning, E.U., Vávra, J., 2000. Phylum microsporidia balbiani, 1882. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.), second ed. The Illustrated Guide to the Protozoa, vol. 1. Society of Protozoologists, Lawrence, Kansas, pp. 39–126.
Capella-Gutierrez, S., Marcet-Houben, M., Gabaldon, T., 2012. Phylogenomics supports microsporidia as the earliest diverging clade of sequenced fungi. BMC Biol. 10, 47.
Cavalier-Smith, T., 1983. A 6-kingdom classification and a unified phylogeny. In: Schenk, H.E.A., Schwemmler, W. (Eds.), Endocytobiology II: Intracellular Space as Oligogenetic Ecosystem, Walter de Gruyter, Berlin, pp. 1027–1034.
Cavalier-Smith, T., 1998. A revised six-kingdom system of life. Biol. Rev. 73, 203–266.
Cavalier-Smith, T., 2001. What are fungi? In: McLaughlin, D.J., McLaughlin, E.G., Lemke, P.A. (Eds.), The Mycota, Part A. Systematics and Evolution, vol. VII. Springer Vrlg, Berlin, pp. 3–37.
Chalifoux, L.V., MacKey, J., Carville, A., Shvetz, D., Lin, K.C., Lackner, A., Mansfield, K.G., 1998. Ultrastructural morphology of *Enterocytozoon bieneusi* in biliary epithelium of *Rhesus* macaques (*Macaca mulatta*). Vet. Pathol. 35, 292–296.
Chen, Y., Evans, J.D., Zhou, L., Boncristiani, H., Kimura, K., Xiao, T., Litkowski, A.M., Pettis, J.S., 2009. Asymmetrical coexistence of *Nosema ceranae* and *Nosema apis* in honey bees. J. Invertebr. Pathol. 101, 204–209.
Chilmonczyk, S., Cox, W.T., Hedrick, R.P., 1991. *Enterocytozoon salmonis* n. sp.: an intranuclear microsporidium from salmonid fish. J. Protozool. 38, 264–269.
Choudhary, M.M., Metcalfe, M.G., Arrambide, K., Bern, C., Visvesvara, G.S., Pieniazek, N.J., Bandea, R.D., Deleon-Carnes, M., Adem, P., Choudhary, M.M., Zaki, S.R., Saeed, M.U., 2011. *Tubulinosema* sp. microsporidian myositis in immunosuppressed patient. Emerg. Infect. Dis. 17, 1727–1730.
Comps, M., Pichot, Y., Deltreil, J.-P., 1979. Mise en evidence d' une microsporidie parasite de *Marteilia refringens* agent de la maladie de la glande digestive de *Ostrea edulis*. L. Rev. Trav. Inst. Peches Marit. 43, 409–412.
Cornman, R.S., Chen, Y.P., Schatz, M.C., Street, C., Zhao, Y., et al., 2009. Genomic analyses of the microsporidian *Nosema ceranae*, an emergent pathogen of honey bees. PLoS Pathog. 5, e1000466.
Corradi, N., Keeling, P.J., 2009. Microsporidia: a journey through radical taxonomical revisions. Fungal Biol. Rev. 23, 1–8.
Corradi, N., Selman, M., 2013. Latest progress in microsporidian genome research. J. Eukaryot. Microbiol. doi: 10.1111/jeu. 12030
Corradi, N., Slamovits, C.H., 2011. The intriguing nature of microsporidian genomes. Brief. Funct. Genomics 10, 115–124.
Corradi, N., Akiyoshi, D.E., Morrison, H.G., Feng, X., Weiss, L.M., Tzipori, S., Keeling, P.J., 2007. Patterns of genome evolution among the microsporidian parasites *Encephalitozoon cuniculi, Antonospora locustae* and *Enterocytozoon bieneusi*. PLoS One 2, e1277.
Corradi, N., Gangaeva, A., Keeling, P.J., 2008. Comparative profiling of overlapping transcription in the compacted genomes of microsporidia *Antonospora locustae* and *Encephalitozoon cuniculi*. Genomics 91, 388–393.
Corradi, N., Haag, K.L., Pombert, J.-F., Ebert, D., Keeling, P.J., 2009. Draft genome sequence of *Daphnia* pathogen *Octosporea bayeri*: insights into the gene content of a large microsporidian genome and a model for host-parasite interactions. Genome Biol. 10, R106.
Corradi, N., Pombert, J.-F., Farinelli, L., Didier, E.S., Keeling, P.J., 2010. The complete sequence of the smallest known nuclear genome from the microsporidian *Encephalitozoon intestinalis*. Nat. Commun. 1, 77.
Costa, C.A.F., Bradley, R.E., 1980. Hyperparasitism of intrasnail stages of *Fasciola hepatica* by a mosquito microsporidian parasite. J. Invertebr. Pathol. 35, 175–181.

Coyle, C.M., Weiss, L.M., Rhodes III, L.V., Cali, A., Takvorian, P.M., Brown, D.F., Visvesvara, G.S., Xiao, L., Naktin, J., Young, E., Gareca, M., Colasante, G., Wittner, M., 2004. Fatal myositis due to the microsporidian *Brachiola algerae*, a mosquito pathogen. N. Engl. J. Med. 351, 42–47.

Cuomo, C.A., Desjardins, C.A., Bakowski, M.A., Goldberg, J., Ma, A.T., Becnel, J.J., Didier, E.S., Fan, L., Heiman, D.I., Levin, J.Z., Young, S., Zeng, Q., Troemel, E.R., 2012. Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. Genome Res. 22, 2478–2488 gr. 142802.112.

Curgy, J.J., Vávra, J., Vivares, C., 1980. Presence of ribosomal RNAs with prokaryotic properties in Microsporidia, eukaryotic organisms. Biol. Cell. 38, 49–52.

Curry, A., Beeching, N.J., Gilbert, J.D., Scott, G., Rowland, P.L., Currie, B.J., 2005. *Trachipleistophora hominis* infection in the myocardium and skeletal muscle of a patient with AIDS. J. Infect. 51, e139–e144.

Dacks, J.B., Field, M.C., 2007. Evolution of the eukaryotic membrane-trafficking system: origins, tempo and mode. J. Cell Sci. 120, 2977–2985.

Dall, D.J., 1983. A theory for the mechanism of polar filament extrusion in the microspore. J. Theor. Biol. 105, 647–659.

Decraene, V., Lebbad, M., Botero-Kleiven, S., Gustavsson, A.-M., Löfdahl, M., 2012. First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. Epidemiol. Infect. 140, 519–527.

De Rijk, P., Gatehouse, H.S., De Wachter, R., 1998. The secondary structure of *Nosema apis* large subunit ribosomal RNA. Biochim. Biophys. Acta – Gene Struct. Expr. 1442, 326–328.

del Aguila, C., Izquierdo, F., Granja, A.G., Hurtado, C., Fenoy, S., Fresno, M., Revilla, Y., 2006. *Encephalitozoon* microsporidia modulates p.53-mediated apoptosis in infected cells. Int. J. Parasitol. 36, 869–876.

Delbac, F., Polonais, V., 2008. The microsporidian polar tube and its role in invasion. Sub-cellular Biochem. 47, 208–220.

Delbac, F., Peyret, P., Metenier, G., David, D., Danchin, A., Vivares, C.P., 1998. On proteins of the microsporidian invasive apparatus: complete sequence of a polar tube protein of *Encephalitozoon cuniculi*. Mol. Microbiol. 29, 825–834.

Delbac, F., Peuvel, I., Metenier, G., Peyretaillade, E., Vivares, C.P., 2001. Microsporidian invasion apparatus: Identification of a novel polar tube protein and evidence for clustering of ptp1 and ptp2 genes in three *Encephalitozoon* species. Infect. Immun. 69, 1016–1024.

Desportes, I., 1976. Ultrastructure de *Stempellia mutabilis* Leger et Hesse, microsporidie parasite de l'éphemere *Ephemera vulgata*. L. Protistologica 12, 121–150.

Desportes, I., Theodorides, J., 1979. Etude ultrastructurale d' *Amphiamblys laubieri* n. sp., (Microsporidie, Metchnikovellidae) parasite d'une gregarine (*Lecudina* sp.) d'un echiurien abyssal. Protistologica 15, 435–457.

Desportes, I., Le Charpentier, Y., Galian, A., Bernard, F., Cochand-Priollet, B., Lavergne, A., Ravisse, P., Modigliani, R., 1985. Occurrence of a new microsporidan: *Enterocytozoon bieneusi* n.g., n. sp., in the enterocytes of a human patient with AIDS. J. Protozool. 32, 250–254.

Desportes-Livage, I., Hilmarsdottir, I., Romana, C., Tanguy, S., Datry, A., Gentilini, M., 1991. Characteristics of the microsporidian *Enterocytozoon bieneusi*: a consequence of its development within short-living enterocytes. J. Protozool. 38, 111S–113S.

Desportes-Livage, I., Chilmonczyk, S., Hedrick, R., Ombrouck, C., Monge, D., Maiga, I., Gentilini, M., 1996. Comparative development of two microsporidian species: *Enterocytozoon bieneusi* and *Enterocytozoon salmonis*, reported in AIDS patients and salmonid fish, respectively. J. Eukaryot. Microbiol. 43, 49–60.

Dia, N., Lavie, L., Méténier, G., Toguebaye, B.S., Vivares, C.P., Cornillot, E., 2007. InterB multigenic family, a gene repertoire associated with subterminal chromosome regions of *Encephalitozoon cuniculi* and conserved in several human-infecting microsporidian species. Curr. Genet. 51, 171–186.

Didier, E.S., 2005. Microsporidiosis: an emerging and opportunistic infection in humans and animals. Acta Trop. 94, 61–76.
Didier, E.S., Weiss, L.M., 2006. Microsporidiosis: current status. Curr. Opin. Infect. Dis. 19, 485–492.
Didier, E.S., Weiss, L.M., 2011. Microsporidiosis: not just in AIDS patients. Curr. Opin. Infect. Dis. 24, 490–495.
Didier, E.S., Vossbrinck, C.R., Baker, M.D., Rogers, L.B., Bertucci, D.C., Shadduck, J.A., 1995. Identification and characterization of three *Encephalitozoon cuniculi* strains. Parasitology 111, 411–421.
Didier, E.S., Stovall, M.E., Green, L.C., Brindley, P.J., Sestak, K., Didier, P.J., 2004. Epidemiology of microsporidiosis: sources and modes of transmission. Vet. Parasitol. 126, 145–166.
Dolgikh, V.V., Semenov, P.B., 2003. Trehalose catabolism in microsporidia *Nosema grylli*. Parazitologiya 37, 333–342.
Dolgikh, V.V., Semenov, P.S., Grigoriev, M.V., 2002. Peculiarities of metabolism of the microsporidia *Nosema grylli* during the intracellular development. Parazitologiya 36, 493–501.
Dolgikh, V.V., Senderski, I.V., Pavlova, O.A., Beznoussenko, G.V., 2010. Expression of vesicular transport genes in avesicular cells of microsporidia *Paranosema* (*Antonospora*) *locustae*. Cell Tissue Biol. 4, 136–142.
Dolgikh, V.V., Senderskiy, I.V., Pavlova, O.A., Naumov, A.M., Beznoussenko, G.V., 2011. Immunolocalization of an alternative respiratory chain in *Antonospora* (*Paranosema*) *locustae* spores: Mitosomes retain their role in microsporidial energy metabolism. Eukaryot. Cell 10, 588–593.
Down, R.E., Bell, H.A., Bryning, G., Kirkbride-Smith, A.E., Edwards, J.P., Weaver, R.J., 2008. Infection by the microsporidium *Vairimorpha necatrix* (Microspora: Microsporidia) elevates juvenile hormone titres in larvae of the tomato moth, *Lacanobia oleracea* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 97, 223–229.
Dunn, A., Smith, J.E., 2001. Microsporidian life cycles and diversity: the relationship between virulence and transmission. Microbe. Infect. 3, 381–388.
Dussaubat, C., Brunet, J.-L., Higes, M., Colbourne, J.K., Lopez, J., Choi, J.-H., Martin-Hernandez, R., Botias, C., Cousin, M., McDonell, C., Bonnet, M., Belzunces, L.P., Moritz, L.P.A., Le Conte, Y., Alaux, C., 2012. Gut pathology and responses to the microsporidium *Nosema ceranae* in the honey bee *Apis mellifera*. PLoS One 7, e37017.
Dyer, P.S., 2008. Evolutionary biology: Microsporidia sex – a missing link to fungi. Curr. Biol. 18, R1012–R1014.
Dyková, I., 2006. Phylum Microspora. In: Woo, P.T.K. (Ed.), Fish Diseases and Disorders, second ed. Protozoan and Metazoan Infections, vol. 1. CABI Publishing, Wallingford, Oxfordshire, U.K. pp. 203–227.
Ebersberger, I., Koestler, T., 2009. Personal communication based on the presentation "Is sex determination in zygomycetes evolutionary ancient?". (EMBO Conference "Comparative Genomics of Eukaryotic Organisms", San Felieu de Guixoes, October 2009).
Ebersberger, I., De Matos Simoes, R., Kupczok, A., Gube, M., Kothe, E., Voigt, K., Von Haeseler, A., 2012. A consistent Phylogenetic backbone for the fungi. Mol. Biol. Evol. 29, 1319–1334.
Ebert, D., 1995. The ecological interactions between a microsporidian parasite and its host *Daphnia magna*. J. Anim. Ecol. 64, 361–369.
Ebert, D., 2008. Host-parasite coevolution: Insights from the *Daphnia*-parasite model system. Curr. Opin. Microbiol. 11, 290–301.
Edlind, T.D., Li, J., Visvesvara, G.S., Vodkin, M.H., McLaughlin, G.L., Katiyar, S.K., 1996. Phylogenetic analysis of beta-tubulin sequences from amitochondrial protozoa. Mol. Phylogenet. Evol. 5, 357–367.
El-Matbouli, M., Soliman, H., 2006. Molecular diagnostic methods for detection of *Thelohania contejeani* (Microsporidia), the causative agent of porcelain disease in crayfish. Dis. Aquat. Org. 69, 205–211.

Fan, N.W., Wu, C.C., Chen, T.L., Yu, W.K., Chen, C.P., Lee, S.M., Lin, P.Y., 2012. Microsporidial keratitis in patients with hot springs exposure. J. Clin. Microbiol. 50, 414–418.

Fasshauer, V., Gross, U., Bohne, W., 2005. The parasitophorous vacuole membrane of *Encephalitozoon cuniculi* lacks host cell membrane proteins immediately after invasion. Eukaryot. Cell. 4, 221–224.

Fast, N.M., Keeling, P.J., 2005. The fungal roots of microsporidian parasites. In: Vega, F.E., Blackwell, M. (Eds.), Insect-fungal Associations, Oxford Press, New York, pp. 97–118.

Fast, N.M., Logsdon, J.M., Doolittle, W.F., 1999. Phylogenetic analysis of the TATA box binding protein (TBP) gene from *Nosema locustae*: evidence for a microsporidia-fungi relationship and splicosomal intron loss. Mol. Biol. Evol. 6, 1415–1419.

Fast, N., Law, J.S., Williams, B.A.P., Keeling, P.J., 2003. Bacterial catalase in the microsporidian *Nosema locustae*: implications for microsporidian metabolism and genome evolution. Eukaryot. Cell 2, 1069–1075.

Field, A.S., Paik, J.Y., Stark, D., Qiu, M.R., Morey, A., Plit, M.L., Canning, E.U., Glanville, A.R., 2012. Myositis due to the microsporidian *Anncaliia* (*Brachiola*) *algerae* in a lung transplant recipient. Transplant Infect. Dis. 14, 169–176.

Findley, A.M., Weidner, E.H., Carman, K.R., Xu, Z., Godbar, J.S., 2005. Role of the posterior vacuole in *Spraguea lophii* (Microsporidia) spore hatching. Folia Parasitol. 52, 111–117.

Fisher Jr., F.M., Sanborn, R.C., 1962. Production of insect juvenile hormone by the microsporidian parasite *Nosema*. Nature 194, 1193.

Fisher Jr., F.M., Sanborn, R.C., 1964. *Nosema* as a source of juvenile hormone in parasitized insects. Biol. Bull. 126, 235–252.

Fischer, W.M., Palmer, J.D., 2005. Evidence from small-subunit ribosomal RNA sequences for a fungal origin of Microsporidia. Mol. Phylogenet. Evol. 36, 606–622.

Flegel, T.W., Pasharawipas, T., 1995. A proposal for typical eukaryotic meiosis in microsporidians. Can. J. Microbiol. 41, 1–11.

Franzen, C., 2004. Microsporidia: how can they invade other cells? Trends Parasitol. 20, 275–279.

Franzen, C., 2005. How do microsporidia invade cells? Folia Parasitol. 52, 36–40.

Franzen, C., Müller, A., 1999. Molecular techniques for detection, species differentiation, and phylogenetic analysis of microsporidia. Clin. Microbiol. Rev. 12, 243–285.

Franzen, C., Fischer, S., Schroeder, J., Scholmerich, 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, 141–152.

Franzen, C., Nassonova, E.S., Scholmerich, J., Issi, I.V., 2006. Transfer of the members of the genus *Brachiola* (Microsporidia) to the genus *Anncaliia* based on ultrastructural and molecular data. J. Eukaryot. Microbiol. 53, 26–35.

Freeman, M.A., Yokoyama, H., Ogawa, K.A., 2004. Microsporidian parasite of the genus *Spraguea* in the nervous tissues of the Japanese anglerfish *Lophius litulon*. Folia Parasitol. 51, 167–176.

Freeman, M.A., Yokoyama, H., Osada, A., Yoshida, T., Yamanobe, A., Ogawa, K., 2011. *Spraguea* (Microsporida: Spraguidae) infections in the nervous system of the Japanese anglerfish, *Lophius litulon* (Jordan), with comments on transmission routes and host pathology. J. Fish Dis. 34, 445–452.

Fries, I., 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). J. Invertebr. Pathol. 103 (Suppl. 1), S73–S79.

Frixione, E., Ruiz, L., Santillan, M., De Vargas, L.V., Tejero, J.M., Undeen, A.H., 1992. Dynamics of polar filament discharge and sporoplasm expulsion by microsporidian spores. Cell. Motil. Cytoskelet. 22, 38–50.

Frixione, E., Ruiz, L., Cerbon, J., Undeen, A.H., 1997. Germination of *Nosema algerae* (Microspora) spores: conditional inhibition by D_2O, ethanol and Hg^{2+} suggests dependence of water influx upon membrane hydration and specific transmembrane pathways. J. Eukaryot. Microbiol. 44, 109–116.

Furuya, K., Omura, M., Kudo, S., Sugiura, W., Azuma, H., 2008. Recognition profiles of microsporidian *Encephalitozoon cuniculi* polar tube protein 1 with human immunoglobulin M antibodies. Parasite Immunol. 30, 13–21.

Galitski, T., Saldanha, A.J., Styles, C.A., Lander, E.S., Fink, G.R., 1999. Ploidy regulation of gene expression. Science 285, 251–254.

Germot, A., Philippe, H., LeGuyader, H., 1997. Evidence for loss of mitochondria in microsporidia from a mitochondrial-type HSP 70 in *Nosema locustae*. Mol. Biochem. Parasitol. 87, 159–168.

Ghosh, K., Weiss, L.M., 2012. T cell response and persistence of the microsporidia. FEMS Microbiol. Rev. 36, 748–760.

Ghosh, K., Cappiello, C.D., McBride, S.M., Occi, J.L., Cali, A., Takvorian, P.M., McDonald, T.V., Weiss, L.M., 2006. Functional characterization of a putative aquaporin from *Encephalitozoon cuniculi*, a microsporidia pathogenic to humans. Int. J. Parasitol. 36, 57–62.

Gill, E.E., Fast, N.M., 2006. Assessing the microsporidia-fungi relationship: combined phylogenetic analysis of eight genes. Gene 375, 103–109.

Gill, E.E., Becnel, J.J., Fast, N.M., 2008. ESTs from the microsporidian *Edhazardia aedis*. BMC Genomics 9, 296.

Gill, E.E., Lee, R.C.H., Corradi, N., Grisdale, C.J., Limpright, V.O., Keeling, P.J., Fast, N.M., 2010. Splicing and transcription differ between spore and intracellular life stages in the parasitic microsporidia. Mol. Biol. Evol. 27, 1579–1584.

Gisder, S., Möckel, N., Linde, A., Genersch, E., 2011. A cell culture model for *Nosema ceranae* and *Nosema apis* allows new insights into the life cycle of these important honey bee-pathogenic microsporidia. Environ. Microbiol. 13, 404–413.

Glockling, S.L., Beakes, G.W., 2002. Ultrastructural morphogenesis of dimorphic arcuate infection (gun) cells of *Haptoglossa erumpens* an obligate parasite of *Bunonema* nematodes. Fungal Genet. Biol. 37, 250–262.

Goertz, D., Hoch, G., 2008a. Horizontal transmission pathways of terrestrial microsporidia: a quantitative comparison of three pathogens infecting different organs in *Lymantria dispar* L. (Lepidoptera: Lymantriidae) larvae. Biol. Control 44, 196–206.

Goertz, D., Hoch, G., 2008b. Vertical transmission and overwintering of microsporidia in the gypsy moth, *Lymantria dispar*. J. Invertebr. Pathol. 99, 43–48.

Goldberg, A.V., Molik, S., Tsaousis, A.D., Neumann, K., Kuhnke, G., Delbac, F., Vivares, C.P., Hirt, R.P., Lill, R., Embley, T.M., 2008. Localization and functionality of microsporidian iron-sulphur cluster assembly proteins. Nature 452, 624–628.

Grisdale, C.J., Fast, N.M., 2011. Patterns of 5' untranslated region length distribution in *Encephalitozoon cuniculi*: Implication for gene regulation and potential links between transcription and splicing. J. Eukaryot. Microbiol. 58, 68–74.

Haag, K.L., Larsson, J.I.R., Refardt, D., Ebert, D., 2011. Cytological and molecular description of *Hamiltosporidium tvaerminnensis* gen. et sp. nov., a microsporidian parasite of *Daphnia magna*, and establishment of *Hamiltosporidium magnivora* comb. nov. Parasitology 139, 447–462.

Haine, E.R., Brondani, E., Hume, K.D., Perrot-Minnot, M.-J., Gaillard, M., Rigaud, T., 2004. Coexistence of three microsporidia parasites in populations of the freshwater amphipod *Gammarus roeseli*: evidence for vertical transmission and positive effect on reproduction. Int. J. Parasitol. 34, 1137–1146.

Haine, E.R., Motreuil, S., Rigaud, T., 2007. Infection by a vertically-transmitted microsporidian parasite is associated with a female-biased sex ratio and survival advantage in the amphipod *Gammarus roeseli*. Parasitology 134, 1363–1367.

Hajek, A.E., Delalibera Jr., I., 2010. Fungal pathogens as classical biological control agents against arthropods. BioControl 55, 147–158.

Hakariya, M., Masuyama, N., Saikawa, M., 2002. Shooting of sporidium by "gun" cells in *Haptoglossa heterospora* and *H. zoospora* and secondary zoospore formation in *H. zoospora*. Mycoscience 43, 119–125.

Hamiduzzaman, M.M., Guzman-Novoa, E., Goodwin, P.H., 2010. A multiplex PCR assay to diagnose and quantify *Nosema* infections in honey bees (*Apis mellifera*). J. Invertebr. Pathol. 105, 151–155.

Haro, M., Del Aguila, C., Fenoy, S., Henriques-Gil, N., 2003. Intraspecies genotype variability of the microsporidian parasite *Encephalitozoon hellem*. J. Clin. Microbiol. 41, 4166–4171.

Haro, M., Del Aguila, C., Fenoy, S., Henriques-Gil, N., 2006. Variability in infection efficiency *in vitro* of different strains of the microsporidian *Encephalitozoon hellem*. J. Eukaryot. Microbiol. 53, 46–48.

Hatjina, F., Tsoktouridis, G., Bouga, M., Charistos, L., Evangelou, V., Avtzis, D., Meeus, I., Brunain, M., Smagghe, G., de Graaf, D.C., 2011. Polar tube protein gene diversity among *Nosema ceranae* strains derived from a Greek honey bee health study. J. Invertebr. Pathol. 108, 131–134.

Hausmann, K., 1978. Extrusive organelles in protists. Int. Rev. Cytol. 52 197–176.

Hausmann, S., Vivares, C.P., Shuman, S., 2002. Characterization of the mRNA capping apparatus of the microsporidian parasite *Encephalitozoon cuniculi*. J. Biol. Chem. 277, 96–103.

Hayman, J.R., Hayes, S.F., Amon, J., Nash, T.E., 2001. Developmental expression of two spore wall proteins during maturation of the microsporidian *Encephalitozoon intestinalis*. Infect. Immun. 69, 7057–7066.

Hayman, J.R., Southern, T.R., Nash, T.E., 2005. Role of sulfated glycans in adherence of the microsporidian *Encephalitozoon intestinalis* to host cells in vitro. Infect. Immun. 73, 841–848.

Heinz, E., Williams, T.A., Nakjang, S., Noël, C.J., Swan, D.C., Goldberg, A.V., Harris, S.R., Weinmaier, T., Markert, S., Becher, D., Bernhardt, J., Dagan, T., Hacker, C., Lucocq, J.M., Schweder, T., Rattei, T., Hall, N., Hirt, R.P., Embley, T.M., 2012. The genome of the obligate intracelllar parasite *Trachipleistophora hominis*: New insights into microsporidian genome dynamics and reductive evolution. PLoS Pathog. 8, e1002979.

Henn, M.W., Solter, L.F., 2000. Food utilization values of gypsy moth *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae infected with the microsporidium *Vairimorpha sp*. (Microsporidia: Burenellidae). J. Invertebr. Pathol. 76, 263–269.

Henry, J.E., Oma, E.A., 1981. Pest control by *Nosema locustae*, a pathogen of grasshoppers and crickets. J. Invertebr. Pathol. 23, 371–377.

Heyworth, M.F., 2012. Changing prevalence of human microsporidiosis. Trans. R. Soc. Trop. Med. Hyg. 106, 202–204.

Hieter, P., Griffiths, T., 1999. Polyploidy-more is more or less. Science 285, 210–211.

Hine, P.M., Engelsma, M.Y., Wakefield, St.J., 2007. Ultrastructure of sporulation in *Haplosporidium armoricanum*. Dis. Aquat. Org. 77, 225–233.

Hinkle, G., Morrison, H.G., Sogin, M.L., 1997. Genes coding for reverse transcriptase, DNA-directed RNA polymerase, and chitin synthase from the microsporidian *Spraguea lophii*. Biol. Bull. 193, 250–251.

Hirt, R.P., Healy, B., Vossbrinck, C.R., Canning, E.U., Embley, T.M., 1997. A mitochondrial HSP70 orthologue in *Vairimorpha necatrix*: molecular evidence that microsporidia once contained mitochondria. Curr. Biol. 7, 995–998.

Hirt, R.P., Logsdon Jr., J.M., Healy, B., Dorey, M.W., Doolittle, W.F., Embley, T.M., 1999. Microsporidia are related to fungi: evidence from the largest subunit of RNA polymerase II and other proteins. Proc. Natl. Acad. Sci. U.S.A. 96, 580–585.

Hoch, G., Pilarska, D.K., Dobart, N., 2009. Effect of midgut infection with the microsporidium *Endoreticulatus schubergi* on carbohydrate and lipid levels in *Lymantria dispar* larvae. J. Pest Sci. 82, 351–356.

Huang, W.F., Tsai, S.J., Lo, C.F., Soichi, Y., Wang, C.H., 2004. The novel organization and complete sequence of the ribosomal RNA gene of *Nosema bombycis*. Fungal Genet. Biol. 41, 473–481.

Ironside, J.E., 2013. Diversity and recombination of dispersed ribosomal DNA and protein coding genes in microsporidia. PLoS ONE 8, e55878.

Ishihara, R., 1968. Some observations on the fine structure of sporoplasms discharged from spores of a microsporidian, *Nosema bombycis*. J. Invertebr. Pathol. 12, 245–258.

Ishihara, R., Hayashi, Y., 1968. Some properties of ribosomes from the sporoplasm of *Nosema bombycis*. J. Invertebr. Pathol. 11, 377–385.

Issi, I.V., 1986. Microsporidia as a phylum of parasitic protozoa. Protozoology 10, 6–136.

Issi, I.V., 2002. Parasitic systems of Microsporidia: descriptions and terminology questions. Parazitologiya 36, 478–492.

Issi, I.V., Tokarev, Yu, S., 2002. Impact of the microsporidia on hormonal balance in insect hosts. Parazitologiya 36, 418–421.

Iturriaga, G., Suarez, R., Nova-Franco, B., 2009. Trehalose metabolism: from osmoprotection to signaling. Int. J. Mol. Sci. 10, 3793–3810.

Iwano, H., Ishihara, R., 1991. Dimorphism of spores of *Nosema* spp. in cultured cell. J. Invertebr. Pathol. 57, 211–219.

Iwano, H., Kurtti, T.J., 1995. Identification and isolation of dimorphic spores from *Nosema furnacalis* (Microspora: Nosematidae). J. Invertebr. Pathol. 65, 230–236.

James, T.Y., Berbee, M.L., 2011. No jacket required – new fungal lineage defies dress code. Bioessayes 34, 94–102.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.-H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schussler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lucking, R., Budel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W., Vilgalys, R., 2006. Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443, 818–822.

Janiszewska, J., Kassner, J., Modrdzejewska, M., 1978. Fine structure of a phagocyte surface of *Limnodrillus hoffmeisteri* Claparede, 1862 (Oligochaeta) infected with *Mrazekia limnodrilli* Jirovec, 1936 (Microsporida). Biol. Poloniae 27, 135–143.

Jeffords, M.R., Maddox, J.V., O'Hayer, K.W., 1987. Microsporidian spores in gypsy moth larval silk: a possible route of horizontal transmission. J. Invertebr. Pathol. 49, 332–333.

Jones, M.D.M., Forn, I., Gadelha, C.., Egan, M.J., Bass, D., Massana, R., Richards, T.A., 2011. Discovery of novel intermediate forms redefines the fungal tree of life. Nature 474, 200–203.

Juarez, S.I., Putaporntip, C., Jongwutiwes, S., Ichinose, A., Yanagi, T., Kanbara, H., 2005. *In vitro* cultivation and electron microscopy characterization of *Trachipleistophora anthropophthera* isolated from the cornea of an AIDS patient. J. Eukaryot. Microbiol. 52, 179–190.

Karlhofer, J., Schafellner, C., Hoch, G., 2012. Reduced activity of juvenile hormone esterase in microsporidia-infected *Lymantria dispar* larvae. J. Invertebr. Pathol. 110, 126–128.

Karpov, S.A., Mikhailov, K.V., Mirzaeva, G.S., Mirabdullaev, I.M., Mamkaeva, K.A., Titova, N.N., Aleoshin, V.V., 2013. Obligately phagotrophic aphelids turned out to branch with the earliest-diverging fungi. Protist 164, 195–205.

Kašicková, D., Sak, B., Kváč, M., Ditrich, O., 2009. Sources of potentially infectious human microsporidia: molecular characterisation of microsporidia isolates from exotic birds in the Czech Republic, prevalence study and importance of birds in epidemiology of the human microsporidial infections. Vet. Parasitol. 165, 125–130.

Katinka, M.D., Duprat, S., Cornillott, E., Metenier, G., Thomarat, F., Prensier, G., Barbe, V., Peyretaillade, E., Brottier, P., Wincker, P., Delbac, F., El Alaoui, H., Peyret, P., Saurin, W., Gouy, M., Weissenbach, J., Vivares, C.P., 2001. Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. Nature 414, 450–453.

Katiyar, S.K., Visvesvara, G.S., Edlind, T.D., 1995. Comparisons of ribosomal RNA sequences from amitochondrial protozoa: implications for processing, mRNA binding and paromomycin susceptibility. Gene 152, 27–33.

Kawarabata, T., 2003. Biology of microsporidians infecting the silkworm, *Bombyx mori*, in Japan. J. Biotechnol. Sericol. 72, 1–32.

Keeling, P.J., 2003. Congruent evidence from alpha-tubulin and beta-tubulin gene phylogenies for a zygomycete origin of microsporidia. Fungal Genet. Biol. 38, 298–309.

Keeling, P.J., 2009. Five questions about Microsporidia. PLoS Pathog. 5, e1000489.

Keeling, P.J., Slamovits, C.H., 2004. Simplicity and complexity of microsporidian genomes. Eukaryot. Cell 3, 1363–1369.

Keeling, P.J., Slamovits, C.H., 2005. Causes and effects of nuclear genome reduction. Curr. Opin. Genet. Dev. 15, 601–608.

Keeling, P.J., Luker, M.A., Palmer, J.D., 2000. Evidence from beta-tubulin that microsporidia evolved from fungi. Mol. Biol. Evol. 17, 1–9.

Keeling, P.J., Fast, N.M., Law, J.S., Williams, B.A.P., Slamovits, C.H., 2005. Comparative genomics of microsporidia. Folia Parasitol. 52, 8–14.

Keeling, P.J., Corradi, N., Morrison, H.G., Haag, K.L., Ebert, D., Weiss, L.M., Akiyoshi, D.E., Tzipori, S., 2010. The reduced genome of the parasitic microsporidian *Enterocytozoon bieneusi* lacks genes for core carbon metabolism. Genome Biol. Evol. 2, 304–309.

Kellen, W.R., Chapman, H.C., Clark, T.B., Lindegren, J.E., 1965. Host–parasite relationships of some *Thelohania* from mosquitoes (Nosematidae: Microsporidia). J. Invertebr. Pathol. 7, 161–166.

Kent, M.L., Speare, D.J., 2005. Review of the sequential development of *Loma salmonae* (Microsporidia) based on experimental infections of rainbow trout (*Onchorhynchus mykiss*) and Chinook salmon (*O. tshawytscha*). Folia Parasitol. 52, 63–68.

Keohane, E.M., Weiss, L.M., 1998. Characterization and function of the microsporidian polar tube: a review. Folia Parasitol. 45, 117–127.

Keohane, E.M., Weiss, L.M., 1999. The structure, function and composition of microsporidian polar tube. In: Wittner, M., Weiss, L.M. (Eds.), The Microsporidia and Microsporidiosis, ASM Press, Washington, D.C., pp. 196–224.

Koestler, T., Ebersberger, I., 2011. Zygomycetes, microsporidia, and the evolutionary ancestry of sex determination. Genome Biol. Evol. 3, 186–194.

Koudela, B., Visvesvara, G.S., Moura, H., Vávra, J., 2001. The human isolate of *Brachiola algerae* (Phylum Microspora): development in SCID mice and description of its fine structure features. Parasitology 123, 153–162.

Kramer, J.P., 1973. Differential germination among spores of the microsporidian *Octosporea muscaedomesticae*. Z. Parasitenkd. 41, 61–64.

Krebes, L., Blank, M., Frankowski, J., Bastrop, R., 2010. Molecular characterisation of the Microsporidia of the amphipod *Gammarus duebeni* across its natural range revealed hidden diversity, wide-ranging prevalence and potential for co-evolution. Infection, Genet. Evol. 10, 1027–1038.

Kugrens, P., Lee, R.E., Corliss, J.O., 1994. Ultrastructure, biogenesis, and functions of extrusive organelles in selected non-ciliate protists. Protoplasma 181, 164–190.
Künzel, F., Joachim, A., 2010. Encephalitozoonosis in rabbits. Parasitol. Res. 106, 299–309.
Larsson, R., 1986. Ultrastructure, function and classification of microsporidia. In: Patterson, D.J., Corliss, J.O. (Eds.), Progress in Protistology, vol. 1. Biopress, Bristol, pp. 325–390.
Larsson, J.I.R., 1988. Identification of microsporidian genera (Protozoa, Microspora) – a guide with comments on the taxonomy. Arch. Protistenkd 136, 1–37.
Larsson, J.I.R., 1999. Identification of microsporidia. Acta Protozool. 38, 161–197.
Larsson, J.I.R., 2000. The hyperparasitic microsporidiun *Amphiacantha longa* Caullery et Mesnil, 1914 (Microspora: Metchnikovellidae) – description of the cytology, redescription of the species, emended diagnosis of the genus *Amphiacantha* and establishment of the new family Amphicanthidae. Folia Parasitol. 47, 241–256.
Larsson, J.I.R., 2005. Molecular versus morphological approach to microsporidian classification. Folia Parasitol. 52 (1–2), 143–144.
Larsson, J.I.R., Koie, M., 2006. The ultrastructure and reproduction of *Amphiamblys capitellides* (Microspora, Metchnikovellidae), a parasite of the gregarine *Ancora sagittata* (Apicomplexa, Lecudinidae), with redescription of the species and comments on the taxonomy. Europ. J. Protistol. 42, 233–248.
Latge, J.-P., 2007. The cell wall: a carbohydrate armour for the fungal cell. Mol. Microbiol. 66, 279–290.
Lee, S.C., Weiss, L.M., Heitman, J., 2009. Generation of genetic diversity in microsporidia via sexual reproduction and horizontal gene transfer. Commun. Integr. Biol. 2, 414–417.
Lee, S.C., Corradi, N., Byrnes III, E.J., Torres-Martinez, S., Dietrich, F.S., Keeling, P.J., Heitman, J., 2008. Microsporidia evolved from ancestral sexual fungi. Curr. Biol. 18, 1675–1679.
Lee, S.C., Corradi, N., Doan, S., Dietrich, F.S., Keeling, P.J., Heitman, J., 2010. Evolution of the sex-related locus and genomic features shared in microsporidia and fungi. PLoS One 5, e10539.
Leitch, G.J., Ceballos, C., 2008. Effects of host temperature and gastric and duodenal environments on microsporidia spore germination and infectivity of intestinal epithelial cells. Parasitol. Res. 104, 35–42.
Leitch, G.J., Shaw, A.P., Colden-Stanfield, M., Scanlon, M., Visvesvara, G.S., 2005a. Multinucleate host cells induced by *Vittaforma corneae* (Microsporidia). Folia Parasitol. 52, 103–110.
Leitch, G.J., Ward, T.L., Shaw, A.P., Newman, G., 2005b. Apical spore phagocytosis is not a significant route of infection of differentiated enterocytes by *Encephalitozoon intestinalis*. Infect. Immun. 73, 7697–7704.
Lemmermann, E., 1900. Beitrege zur Kenntnis der Planktonalgen. IX. *Lagerheimia marssonii* nov. spec., *Centratractus belonophora* (Schmidle) nov. gen. et spec. *Synedra limnetica* nov. spec., *Marssoniella elegans* nov. gen. et spec. Ber. Dtsch. Bot. Ges. 18, 272–275.
Letcher, P.M., Lopez, S., Schmieder, R., Lee, P.A., Behnke, C., Powell, M. J., McBride, R.C., 2013. Characterization of *Amoeboaphelidium protococcarum*, an algal parasite new to the Cryptomycota isolated from an outdoor algal pond used for the production of biofuel. PLoS One 8, e56232.
Lewis, L.C., Bruck, D.J., Prasifka, J.R., Raun, E.S., 2009. *Nosema pyrausta*: Its biology, history, and potential role in a landscape of transgenic insecticidal crops. Biol. Control 48, 223–231.
Li, Y., Wu, Z., Pan, G., He, W., Zhang, R., Hu, J., Zhou, Z., 2009. Identification of a novel spore wall protein (SWP26) from microsporidia *Nosema bombycis*. Int. J. Parasitol. 39, 391–398.
Li, Z., Pan, G., Li, T., Huang, W., Chen, J., Geng, L., Yang, D., Wang, L., Zhou, Z., 2012. SWP5, a spore wall protein, interacts with polar tube proteins in the parasitic microsporidian *Nosema bombycis*. Eukaryot. Cell 11, 229–237.
Little, T.J., Ebert, D., 1999. Associations between parasitism and host genotype in natural populations of *Daphnia* (Crustacea: Cladocera). J. Anim. Ecol. 68, 134–149.

Liu, Y.J., Hodson, M.C., Hall, B.D., 2006. Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of kingdom fungi inferred from RNA polymerase II subunit genes. BMC Evol. Biol. 6, 74.
Lom, J., 1972. On the structure of the extruded microsporidian polar filament. Z. Parasitenkd 38, 200–213.
Lom, J., 2002. A catalogue of described genera and species of microsporidians parasitic in fish. Syst. Parasitol. 53, 81–99.
Lom, J., Corliss, J.O., 1967. Ultrastructural observations on the development of the microsporidian protozoon *Plistophora hyphessobryconis* Schaeperclaus. J. Protozool. 14, 141–152.
Lom, J., Dyková, I., 2002. Ultrastructure of *Nucleospora secunda* n. sp. (Microsporidia), parasite of enterocytes of *Nothobranchius rubripinnis*. Eur. J. Protistol. 38, 19–27.
Lom, J., Dyková, I., 2005. Microsporidian xenomas in fish seen in wider perspective. Folia Parasitol. 52, 69–81.
Lom, J., Nilsen, F., 2003. Fish microsporidia: fine structural diversity and phylogeny. Int. J. Parasitol. 33, 107–127.
Lom, J., Vávra, J., 1963. The mode of sporoplasm extrusion in microsporidian spores. Acta Protozool. 1, 81–89.
Lomer, C.J., Bateman, R.P., Johnson, D.L., Langewald, J., Thomas, M., 2001. Biological control of locusts and grasshoppers. Annu. Rev. Entomol. 46, 667–702.
Lukeš, J., Vávra, J., 1990. Life cycle of *Amblyospora weiseri* n. sp. (Microsporidia) in *Aedes cantans* (Diptera, Culicidae). Eur. J. Protistol. 25, 200–208.
Lynn, D.H., Simpson, A.G.B., 2009. From the Editors: describing new taxa of unicellular protists. J. Eukaryot. Microbiol. 56, 403–405.
Makrushin, A.V., 2010. Changes in the behaviour of *Moina macrocopa* (Crustacea: Cladocera) under the influence of *Gurleya sp.* (Microsporidia: Gurleyidae). Parazitologiya 44, 475–477.
Mangin, K.L., Lipsitch, M., Ebert, D., 1995. Virulence and transmission of two microsporidia in *Daphnia magna*. Parasitology 111, 133–142.
Martins, R.R., Perondini, A.L.P., 1977. Effects of microsporidia on the striated parietal muscle of *Rhynchosciara angelae* (Diptera: Sciaridae). J. Invertebr. Pathol. 30, 422–428.
Mathis, A., Weber, R., Deplazes, P., 2005. Zoonotic potential of the microsporidia. Clin. Microbiol. Rev. 18, 423–445.
Matthews, J.L., Brown, A.M.V., Larison, K., Bishop-Stewart, J.K., Rogers, P., Kent, M.L., 2001. *Pseudoloma neurophilia* n. g., n. sp., a new microsporidium from the central nervous system of the zebrafish (*Danio rerio*). J. Eukaryot. Microbiol. 48, 227–233.
McLaughlin, D.J., Hibbett, D.S., Lutzoni, F., Spatafora, J.W., Vilgalys, R., 2009. The search for the fungal tree of life. Trends Microbiol. 17, 488–497.
Meissner, E.G., Bennett, J.E., Qvarnstrom, Y., Dasilva, A., Chu, E.Y., Tsokos, M., Gea-Banacloche, J., 2012. Disseminated microsporidiosis in an immunosuppressed patient. Emerg. Infect. Dis. 18, 1155–1158.
Metenier, G., Vivares, C.P., 2001. Molecular characteristics and physiology of microsporidia. Microbe. Infect. 3, 407–415.
Mironov, A.A., Banin, V.V., Sesorova, I.S., Dolgikh, V.V., Luini, A., Beznoussenko, G.V., 2006. Evolution of the endoplasmic reticulum and the Golgi complex. In: Jekely, G. (Ed.), Origins and Evolution of Eukaryotic Endomembranes and Cytoskeleton, vol. 1. Landes Bioscience, Austin, TX, pp. 61–72.
Mittleider, D., Green, L.C., Mann, V.H., Michael, S.F., Didier, E.S., Brindley, P.J., 2002. Sequence survey of the genome of the opportunistic microsporidian pathogen, *Vittaforma corneae*. J. Eukaryot. Microbiol. 49, 393–401.
Moretto, M.M., Lawlor, E.M., Xu, Y., Khan, I.A., Weiss, L.M., 2010. Purified PTP1 protein induces antigen-specific protective immunity against *Encephalitozoon cuniculi*. Microbe. Infect. 12, 574–579.
Mowbrey, K., Dacks, J.B., 2009. Evolution and diversity of the Golgi body. FEBS Lett. 583, 3738–3745.

Mulisch, M., 1993. Chitin in protistan organisms. Distribution, synthesis and deposition. Eur. J. Protistol. 29, 1–18.
Nägeli, C., 1857. Über die neue Krankheit der Seidenraupe und verwandte Organismen. Bot. Zeitung 15, 60–761.
Nkinin, S.W., Asonganyi, T., Didier, E.S., Kaneshiro, E.S., 2007. Microsporidian infection is prevalent in healthy people in Cameroon. J. Clin. Microbiol. 45, 2841–2846.
Ojuromi, O.T., Izquierdo, F., Fenoy, S., Fagbenro-Beyioku, A., Oyibo, W., Akanmu, A., Odunukwe, N., Henriques-Gil, N., Del Aguila, C., 2012. Identification and characterization of microsporidia from fecal samples of HIV-positive patients from Lagos, Nigeria. PLoS One 7, e35239.
Olsen, P.E., Rice, W.A., Liu, T.P., 1986. *In vitro* germination of *Nosema apis* spores under conditions favorable for the generation and maintenance of sporoplasms. J. Invertebr. Pathol. 47, 65–73.
O'Mahony, E.M., Tay, W.T., Paxton, R.J., 2007. Multiple rRNA variants in a single spore of the microsporidian *Nosema bombi*. J. Eukaryot. Microbiol. 54, 103–109.
Otti, O., Schmid-Hempel, P., 2007. *Nosema bombi*: a pollinator parasite with detrimental fitness effects. J. Invertebr. Pathol. 96, 118–124.
Otto, S.P., 2007. The evolutionary consequences of polyploidy. Cell 131, 452–462.
Paldi, N., Glick, E., Oliva, M., Zilberberg, Y., Aubin, L., Pettis, J., Chen, Y., Evans, J.D., 2010. Effective gene silencing in a microsporidian parasite associated with honeybee (*Apis mellifera*) colony declines. Appl. Environ. Microbiol. 76, 5960–5964.
Pan, F., Malmberg, R.L., Momany, M., 2007. Analysis of septins across kingdoms reveals orthology and new motifs. BMC Evol. Biol. 7, 103.
Pavan, C., Perondini, A.L.P., Picard, T., 1969. Changes in chromosomes and in development of cells of *Sciara ocellaris* induced by microsporidian infections. Chromosoma 28, 328–345.
Peek, R., Delbac, F., Speijer, D., Polonais, V., Greve, S., Wentink-Bonnema, E., Ringrose, J., Van Gool, T., 2005. Carbohydrate moieties of microsporidian polar tube proteins are targeted by immunoglobulin G in immunocompetent individuals. Infect. Immun. 73, 7906–7913.
Pelabon, C., Borg, A.A., Bjelvenmark, J., Barber, I., Forsgren, E., Amundsen, T., 2005. Do microsporidian parasites affect courtship in two-spotted gobies? Marine Biol. 148, 189–196.
Pettis, J.S., Vanengelsdorp, D., Johnson, J., Dively, G., 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. Naturwissenschaften 99, 153–158.
Peuvel, I., Delbac, F., Metenier, G., Peyret, P., Vivares, C.P., 2000. Polymorphism of the gene encoding a major polar tube protein PTP1 in two microsporidia of the genus *Encephalitozoon*. Parasitology 121, 581–587.
Peuvel, I., Peyret, P., Metenier, G., Vivares, C.P., Delbac, F., 2002. The microsporidian polar tube: evidence for a third polar tube protein (PTP3) in *Encephalitozoon cuniculi*. Mol. Biochem. Parasitol. 122, 69–80.
Peuvel-Fanget, I., Polonais, V., Brosson, D., Texier, C., Kuhn, L., Peyret, P., Vivares, C., Delbac, F., 2006. EnP1 and EnP2, two proteins associated with the *Encephalitozoon cuniculi* endospore, the chitin-rich inner layer of the microsporidian spore wall. Int. J. Parasitol. 36, 309–318.
Peyretaillade, E., Biderre, C., Peyret, P., Duffieux, F., Metenier, G., Gouy, M., Michot, B., Vivares, C.P., 1998. Microsporidian *Encephalitozoon cuniculi*, a unicellular eukaryote with an unusual chromosomal dispersion of ribosomal genes and a LSU rRNA reduced to the universal core. Nucl. Acids Res. 26, 3513–3520.
Peyretaillade, E., El Alaoui, H., Diogon, M., Polonais, V., Parisot, N., Biron, D.G., Peyret, P., Delbac, F., 2011. Extreme reduction and compaction of microsporidian genomes. Res. Microbiol. 162, 598–606.

Peyretaillade, E., Parisot, N., Polonais, V., Terrat, S., Denonfoux, J., Dugat-Bony, E., Wawrzyniak, I., Biderre-Petit, C., Mahul, A., Rimour, S., Gonçalves, O., Bornes, S., Delbac, F., Chebance, B., Duprat, S., Samson, G., Katinka, M., Weissenbach, J., Wincker, P., Peyret1, P., 2012. Annotation of microsporidian genomes using transcriptional signals. Nat. Commun. 3, 1137. http: //dx. doi. org/10.1038, ncomms 2156.

Phelps, N.B.D., Goodwin, A.E., 2008. Vertical transmission of *Ovipleistophora ovariae* (Microspora) within the eggs of the goldenshiner. J. Aquat. Anim. Health 20, 45–53.

Philippe, H., Adoutte, A., 1998. The molecular phylogeny of eukaryota: solid facts and uncertainties. In: Coombs, G., Vickerman, K., Sleigh, M., Warren, A. (Eds.), Evolutionary Relationships among Protozoa, Chapman & Hall, London, pp. 25–56.

Polonais, V., Prensier, G., Metenier, G., Vivares, C.P., Delbac, F., 2005. Microsporidian polar tube proteins: highly divergent but closely linked genes encode PTP1 and PTP2 in members of the evolutionarily distant *Antonospora* and *Encephalitozoon* groups. Fungal Genet. Biol. 42, 791–803.

Pombert, J.-F., Selman, M., Burki, F., Bardell, F.T., Farinelli, L., Solter, L.F., Whitman, D.W., Weiss, L.M., Corradi, N., Keeling, P.J., 2012. Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. Proc. Natl. Acad. Sci. U.S.A. 109, 12638–12643.

Purrini, K., Weiser, J., 1985. Ultrastructural study of the microsporidian *Chytridiopsis typographi* (Chytridiopsida: Microspora) infecting the bark beetle, *Ips typographus* (Scolytidae: Coleoptera), with new data on spore dimorphism. J. Invertebr. Pathol. 45, 66–74.

Quek, D.T.-L., Pan, J.C.-H., Krishnan, P.U., Zhao, P.S., Teoh, S.C.B., 2011. Microsporidial keratoconjunctivitis in the tropics: a case series. Open Ophthalmol. J. 5, 42–47.

Raikov, I., 1982. The Protozoan Nucleus, Morphology and Evolution. Cell Biology Monographs. vol. 9. Springer Verlag, Vienna.

Redhead, S.A., Kirk, P.M., Keeling, P.J., Weiss, L.M., 2009. Proposals to exclude the phylum microsporidia from the code. Taxon 58, 669.

Refardt, D., Ebert, D., 2006. Quantitative PCR to detect, discriminate and quantify intracellular parasites in their host: an example from three microsporidians in *Daphnia*. Parasitology 133, 11–18.

Refardt, D., Mouton, L., 2007. Reverse arrangement of rRNA subunits in the microsporidium *Glugoides intestinalis*. J. Eukaryot. Microbiol. 54, 83–85.

Refardt, D., Canning, E.U., Mathis, A., Cheney, S.A., Lafranchi-Tristem, N.J., Ebert, D., 2002. Small subunit ribosomal DNA phylogeny of microsporidia that infect *Daphnia* (Crustacea: Cladocera). Parasitology 124, 381–389.

Refardt, D., Decaestecker, E., Johnson, P.T.J., Vávra, 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). J. Eukaryot. Microbiol. 55, 393–408.

Robb, J.E., Barron, G., 1982. Nature's ballistic missile. Science 218, 1221–1222.

Rodgers-Gray, T.P., Smith, J.E., Ashcroft, A.E., Isaac, R.E., Dunn, A.M., 2004. Mechanisms of parasite-induced sex reversal in *Gammarus duebeni*. Int. J. Parasitol. 34, 747–753.

Roncero, C., 2002. The genetic complexity of chitin synthesis in fungi. Curr. Genet. 41, 367–378.

Rönnebäumer, K., Gross, U., Bohne, W., 2008. The nascent parasitophorous vacuole membrane of *Encephalitozoon cuniculi* is formed by host cell lipids and contains pores which allow nutrient uptake. Eukaryot. Cell. 7, 1001–1008.

Rönnebäumer, K., Wagener, J., Gross, U., Bohne, W., 2006. Identification of a novel developmentally regulated genes in *Encephalitozoon cuniculi*: an endochitinase, a chitin-synthase and two subtilisin-like proteases are induced during meront-to-sporont differentiation. J. Eukaryot. Microbiol. 53, S74–S76.

Ruiz-Herrera, J., Ortiz-Castellanos, L., 2010. Analysis of the phylogenetic relationships and evolution of the cell walls from yeasts and fungi. FEMS Yeast Res. 10, 225–243.

Ruiz-Herrera, J., Manuel Gonzalez-Prieto, J., Ruiz-Medrano, R., 2002. Evolution and phylogenetic relationships of chitin synthases from yeasts and fungi. FEMS Yeast Res. 1, 247–256.

Ryan, J.A., Kohler, S.L., 2010. Virulence is context-dependent in a vertically transmitted aquatic host-microparasite system. Int. J. Parasitol. 40, 1665–1673.

Sagastume, S., del Aguila, C., Martin-Hernandez, R., Higes, M., Henriques-Gil, N., 2011. Polymorphism and recombination for the rDNA in the putatively asexual microsporidian *Nosema ceranae*, a pathogen of honeybees. Environ. Microbiol. 13, 84–95.

Sak, B., Kašičková, D., Kváč, M., Květoňová, D., Ditrich, O., 2010. Microsporidia in exotic birds: Intermittent spore excretion of *Encephalitozoon* spp. in naturally infected budgerigars (*Melopsittacus undulatus*). Vet. Parasitol. 168, 196–200.

Sak, B., Brady, D., Pelikánová, M., Květoňová, D., Rost, M., Kostka, M., Tolarová, V., Hůzová, Z., Kváč, M., 2011a. Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. J. Clin. Microbiol. 49, 1064–1070.

Sak, B., Kváč, M., Kučerová, Z., Květoňová, D., Saková, K., 2011b. Latent microsporidial infection in immunocompetent individuals – a longitudinal study. PLoS Negl. Trop. Dis. 5, e1162.

Sak, B., Kváč, M., Květoňová, D., Albrecht, T., Pialek, J., 2011c. The first report on natural *Enterocytozoon bieneusi* and *Encephalitozoon spp*. infections in wild East-European house mice (*Mus musculus musculus*) and West-European house mice (*M. m. domesticus*) in a hybrid zone across the Czech Republic–Germany border. Vet. Parasitol. 178, 246–250.

Sanders, J.L., Lawrence, C., Nichols, D.K., Brubaker, J.F., Peterson, T.S., Murray, K.N., Kent, M.L., 2010. *Pleistophora hyphessobryconis* (Microsporidia) infecting zebrafish *Danio rerio* in research facilities. Dis. Aquat. Org. 91, 47–56.

Santín, M., Fayer, R., 2009a. *Enterocytozoon bieneusi* genotype nomenclature based on the internal transcribed spacer sequence: a consensus. J. Eukaryot. Microbiol. 56, 34–38.

Santín, M., Fayer, R., 2009b. A longitudinal study of *Enterocytozoon bieneusi* in dairy cattle. Parasitol. Res. 105, 141–144.

Santín, M., Fayer, R., 2011. Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. Res. Vet. Sci. 90, 363–371.

Scanlon, M., Leitch, G.J., Shaw, A.P., Moura, H., Visvesvara, G.S., 1999. Susceptibility to apoptosis is reduced in the microsporidia-infected host cell. J. Eukaryot. Microbiol. 46, 34–35.

Scanlon, M., Shaw, A.P., Zhou, C.J., Visvesvara, G.S., Leitch, G.J., 2000. Infection by microsporidia disrupts the host cell cycle. J. Eukaryot. Microbiol. 47, 525–531.

Scanlon, M., Leitch, G.J., Visvesvara, G.S., Shaw, A.P., 2004. Relationship between the host cell mitochondria and the parasitophorous vacuole in cells infected with *Encephalitozoon* microsporidia. J. Eukaryot. Microbiol. 51, 81–87.

Seleznev, K.V., 2003. The role of microsporidia in the disturbance of the hormonal balance in the infected insects. Parazitologiya 37, 428–435.

Selman, M., Pombert, J.-F., Solter, L., Farinelli, L., Weiss, L.M., Keeling, P., Corradi, N., 2011. Acquisition of an animal gene by microsporidian intracellular parasites. Curr. Biol. 21, 576–577.

Sene, A., Ba, C.T., Marchand, B., Toguebaye, B.S., 1997. Ultrastructure of *Unikaryon nomimoscolexi* n. sp. (Microsporida, Unikaryonidae), a parasite of *Nomimoscolex* sp. (Cestoda, Proteocephalidea) from the gut of *Clarotes laticeps* (Pisces, Teleostei, Bagridae). Dis. Aquat. Org. 29, 35–40.

Shafer, A.B.A., Williams, G.R., Shutler, D., Rogers, R.L., Stewart, D.T., 2009. Cophylogeny of *Nosema* (Microsporidia: Nosematidae) and bees (Hymenoptera: Apidae) suggests both cospeciation and a host-switch. J. Parasitol. 95, 198–203.

Sharma, S., Das, S., Joseph, J., Vemuganti, G.K., Murthy, S., 2011. Microsporidial keratitis: need for increased awareness. Surv. Ophthalmol. 56, 1–22.

Shaw, R.W., Kent, M.L., Brown, A.M.V., Whipps, C.M., Adamson, M.L., 2000. Experimental and natural host specificity of *Loma salmonae* (Microsporidia). Dis. Aquat. Org. 40, 131–136.

Shertz, C.A., Bastidas, R.J., Li, W., Heitman, J., Cardenas, M.E., 2010. Conservation, duplication, and loss of the Tor signaling pathway in the fungal kingdom. BMC Genomics 11, 510.

Siegel, J.P., Maddox, J.V., Ruesink, W.G., 1986. Lethal and sublethal effects of *Nosema pyrausta* on the European corn borer, *Ostrinia nubilalis* in Central Illinois USA. J. Invertebr. Pathol. 48, 167–173.

Slamovits, C.H., Fast, N.M., Law, J.S., Keeling, P.J., 2004a. Genome compaction and stability in microsporidian intracellular parasites. Curr. Biol. 14, 891–896.

Slamovits, C.H., Williams, B.A.P., Keeling, P.J., 2004b. Transfer of *Nosema locustae* (Microsporidia) to *Antonospora locustae* n. comb. based on molecular and ultrastructural data. J. Eukaryot. Microbiol. 51, 207–213.

Smith, J.E., 2009. The ecology and evolution of microsporidian parasites. Parasitology 136, 1901–1914.

Sokolova, Y.Y., Fuxa, J.R., 2008. Biology and life-cycle of the microsporidium *Kneallhazia solenopsae* Knell Allan Hazard 1977 gen.n., comb. n., from the fire ant *Solenopsis invicta*. Parasitology 135, 903–929.

Sokolova, Y.Y., Dolgikh, V.V., Morzhina, E.V., Nassonova, E.S., Issi, I.V., Terry, R.S., Ironside, J.E., Smith, J.E., Vossbrinck, C.R., 2003. Establishment of the new genus *Paranosema* based on the ultrastructure and molecular phylogeny of the type species *Paranosema grylli* Gen. Nov., Comb. Nov. (Sokolova, Selezniov, Dolgikh, Issi 1994), from the cricket *Gryllus bimaculatus* Deg. J. Invertebr. Pathol. 84, 159–172.

Solter, L.F., 2006. Transmission as a predictor of ecological host specificity with a focus on vertical transmission of microsporidia. J. Invertebr. Pathol. 92, 132–140.

Solter, L.F., Maddox, J.V., 1998a. Physiological host specificity of microsporidia as an indicator of ecological host specificity. J. Invertebr. Pathol. 71, 207–216.

Solter, L.F., Maddox, J.V., 1998b. Timing of an early sporulation sequence of Microsporidia in the genus *Vairimorpha* (Microsporidia: Burenellidae). J. Invertebr. Pathol. 72, 323–329.

Solter, L.F., Maddox, J.V., Vossbrinck, C.R., 2005. Physiological host specificity: a model using the European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae) and microsporidia of row crop and other stalk-boring hosts. J. Invertebr. Pathol. 90, 127–130.

Southern, T.R., Jolly, C.E., Lester, M.E., Hayman, J.R., 2007. EnP1, a microsporidian spore wall protein that enables spores to adhere to and infect host cells *in vitro*. Eukaryot. Cell 6, 1354–1362.

Sprague, V., 1977. Systematics of the microsporidia. In: Bulla Jr., L.A., Cheng, T.C. (Eds.), Comparative Pathobiology, vol. 2. Plenum Press, New York, pp. 1–510.

Sprague, V., Becnel, J.J., 1998. Note on the name-author-date combination for the taxon Microsporidies Balbiani, 1882, when ranked as a phylum. J. Invertebr. Pathol. 71, 91–94.

Sprague, V., Becnel, J.J., 1999. Appendix: checklist of available generic names for microsporidia with type species and type hosts. In: Wittner, M., Weiss, L.M. (Eds.), Microsporidia and Microsporidiosis, ASM Press, Washington, D.C., pp. 517–530.

Sprague, V., Becnel, J.J., Hazard, E.I., 1992. Taxonomy of phylum Microspora. Crit. Rev. Microbiol. 18, 285–395.

Staiber, W., 1994. Effects of *Nosema algerae* infections on the gene activity of the salivary gland chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae). Parasitol. Res. 80, 108–111.

Stechmann, A., Cavalier-Smith, T., 2003. Rooting the eukaryote tree by using a derived gene fusion. Science 297, 89–91.

Steenkamp, E.T., Wright, J., Baldauf, S.L., 2006. The protistan origins of animals and fungi. Mol. Biol. Evol. 23, 93–106.

Stentiford, G.D., Bateman, K.S., 2007. *Enterospora sp.*, an intranuclear microsporidian infection of hermit crab *Eupagurus bernhardus*. Dis. Aquat. Org. 75, 73–78.

Stentiford, G.D., Bateman, K.S., Longshaw, M., Feist, S.W., 2007. *Enterospora canceri* n. gen., n. sp., intranuclear within the hepatopancreatocytes of the European edible crab *Cancer pagurus*. Dis. Aquat. Org. 75, 61–72.

Stentiford, G.D., Bateman, K.S., Small, H.J., Moss, J., Shields, J.D., Reece, K.S., Tuck, I., 2010. *Myospora metanephrops* (n. g., n. sp.) from marine lobsters and a proposal for erection of a new order and family (Crustaceacida; Myosporidae) in the Class Marinosporidia (Phylum Microsporidia). Int. J. Parasitol. 40, 1433–1446.

Stewart, M., 2007. Ratcheting mRNA out of the nucleus. Mol. Cell 25, 327–330.

Suankratay, C., Thiansukhon, E., Nilaratanakul, V., Putaporntip, C., Jongwutiwes, S., 2012. Disseminated infection caused by novel species of Microsporidium, Thailand. Emerg. Infect. Dis. 18, 302–304.

Sweeney, A.W., Hazard, E.I., Graham, M.F., 1985. Intermediate host for an *Amblyospora sp.* infecting the mosquito *Culex annulirostris*. J. Invertebr. Pathol. 46, 98–102.

Takvorian, P.M., Weiss, L.M., Cali, A., 2005. The early events of *Brachiola algerae* (Microsporidia) infection: spore germination, sporoplasm structure, and development within host cells. Folia Parasitol. 52, 118–129.

Tanabe, Y., Watanabe, M.M., Sugiyama, J., 2002. Are microsporidia really related to fungi? A reappraisal based on additional gene sequences from basal fungi. Mycol. Res. 106, 1380–1391.

Tanabe, Y., Watanabe, M.M., Sugiyama, J., 2005. Evolutionary relationships among basal fungi (Chytridiomycota and Zygomycota): Insights from molecular phylogenetics. J. Gen. Appl. Microbiol. 51, 267–276.

Taupin, V., Metenier, G., Delbac, F., Vivares, C.P., Prensier, G., 2006. Expression of two cell wall proteins during the intracellular development of *Encephalitozoon cuniculi*: an immunocytochemical and in situ hybridization study with ultrathin frozen sections. Parasitology 132, 815–825.

Tay, W.T., O'Mahony, E.M., Paxton, R.J., 2005. Complete rRNA gene sequence reveals that the microsporidium *Nosema bombi* infects diverse bumblebee (*Bombus* spp.) hosts and contains multiple polymorphic sites. J. Eukaryot. Microbiol. 52, 505–513.

Terry, R.S., Dunn, A.M., Smith, J.E., 1999. Segregation of a microsporidian parasite during host cell mitosis. Parasitology 118, 43–48.

Terry, R.S., Smith, J.E., Sharpe, R.G., Rigaud, T., Littlewood, D.T.J., Ironside, J.E., Rollinson, D., Bouchon, D., MacNeil, C., Dick, J.T.A., Dunn, A.M., 2004. Widespread vertical transmission and associated host sex-ratio distortion within the eukaryotic phylum microspora. Proc. Roy. Soc. B: Biol. Sci. 271, 1783–1789.

Texier, C., Brosson, D., El Alaoui, H., Metenier, G., Vivares, C.P., 2005. Post-genomics of microsporidia, with emphasis on a model of minimal eukaryotic proteome: a review. Folia Parasitol. 52, 15–22.

Texier, C., Vidau, C., Vigues, B., El Alaoui, H., Delbac, F., 2010. Microsporidia: a model for minimal parasite-host interactions. Curr. Opin. Microbiol. 13, 443–449.

Thellier, M., Breton, J., 2008. *Enterocytozoon bieneusi* in human and animals, focus on laboratory identification and molecular epidemiology. Parasite 15, 349–358.

Thélohan, P., 1892. Observations sur les Myxosporidies et essai de classification de ce organismes. Bull. Soc. Philom. 4, 165–178.

Thomarat, F., Vivares, C.P., Gouy, M., 2004. Phylogenetic analysis of the complete genome sequence of *Encephalitozoon cuniculi* supports the fungal origin of microsporidia and reveals a high frequency of fast-evolving genes. J. Mol. Evol. 59, 780–791.

Torres-Machorro, A.L., Hernandez, R., Cevallos, A.M., Lopez-Villasenor, I., 2010. Ribosomal RNA genes in eukaryotic microorganisms: witnesses of phylogeny? FEMS Microbiol. Rev. 34, 59–86.

Tourtip, S., Wongtripop, S., Stentiford, G.D., Bateman, K.S., Sriurairatana, S., Chavadej, J., Sritunyalucksana, K., Withyachumnarnkul, B., 2009. *Enterocytozoon hepatopenaei* sp. nov. (Microsporida: Enterocytozoonidae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): fine structure and phylogenetic relationships. J. Invertebr. Pathol. 102, 21–29.

Trager, W., 1986. Living Together. The Biology of Animal Parasitism. Plenum Press, New York.

Troemel, E.R., 2011. New models of microsporidiosis: infections in zebrafish, *C. elegans* and honey bee. PLoS Pathog. 7, e1001243.

Troemel, E.R., Felix, M.-A., Whiteman, N.K., Barriere, A., Ausubel, F.M., 2008. Microsporidia are natural intracellular parasites of the nematode *Caenorhabditis elegans*. PLoS Biol. 6, 2736–2752.

Tsaousis, A.D., Kunji, E.R.S., Goldberg, A.V., Lucocq, J.M., Hirt, R.P., Embley, T.M., 2008. A novel route for ATP acquisition by the remnant mitochondria of *Encephalitozoon cuniculi*. Nature 453, 553–556.

Undeen, A.H., 1990. A proposed mechanism for the germination of microsporidian (Protozoa: Microspora) spores. J. Theor. Biol. 142, 223–235.

Undeen, A.H., Solter, L.F., 1996. Sugar content of and density of living and dead microsporidian (Protozoa: Microspora) spores. J. Invertebr. Pathol. 67, 80–91.

Undeen, A.H., Van der Meer, R.K., 1999. Microsporidian intrasporal sugars and their role in germination. J. Invertebr. Pathol. 73, 294–302.

Valenčáková, A., Balent, P., Ravaszová, P., Horák, A., Oborník, M., Halánová, M., Malčeková, B., Novotný, F., Goldov, M., 2012. Molecular identification and genotyping of microsporidia in selected hosts. Parasitol. Res. 110, 689–693.

Van de Peer, Y., Ben Ali, A., Meyer, A., 2000. Microsporidia: accumulating molecular evidence that a group of amitochondriate and suspectedly primitive eukaryotes are just curious fungi. Gene 246, 1–8.

Vávra, J., 1964. A failure to produce an artificial infection in cladoceran microsporidia. J. Protozool. 11, S35–S36.

Vávra, J., 1965. Etude au microscope electronique de la morphologie et du developpement de quelques microsporidies. C. R. Acad. Sci. Paris 261, 3467–3470.

Vávra, J., 1966. Some recent advances in the study of microsporidian spores. In: Corradetti, A. (Ed.), Proceedings of the First International Congress of Parasitology, Pergamon Press, Oxford, pp. 443–444.

Vávra, J., 1972. Detection of polysaccharides in microsporidian spores by means of the periodic acid-thiosemicarbazide-silver proteinate test. J. Microscopie 14, 357–360.

Vávra, J., 1976. Structure of microsporidia. In: Bulla Jr., L.A., Cheng, T.C. (Eds.), Comparative Pathobiology, vol. 1. Plenum Press, New York, pp. 1–86.

Vávra, J., 2005. "Polar vesicles" of microsporidia are mitochondrial remnants ("mitosomes")? Folia Parasitol. 52, 193–195.

Vávra, J., Larsson, J.I.R., 1994. *Berwaldia schaefernai* (Jirovec, 1937) comb.n. (Protozoa, Microsporida), fine structure, life cycle, and relationship to *Berwaldia singularis* Larsson, 1981. Eur. J. Protistol. 30, 45–54.

Vávra, J., Larsson, J.I.R., 1999. Structure of the microsporidia. In: Wittner, M., Weiss, L.M. (Eds.), The Microsporidia and Microsporidiosis, ASM Press, Washington, D.C., pp. 7–84.

Vávra, J., Maddox, J.V., 1976. Methods in microsporidiology. In: Bulla Jr., L.A., Cheng, T.C. (Eds.), Comparative Pathobiology, vol. 1. Plenum Press, New York, pp. 281–319.

Vávra, J., Pražáková, M., 1983. The influence of a microsporidian infection on the behaviour of the infected host. J. Protozool. 30, 32A.

Vávra, J., Bedrník, P., Činátl, J., 1972. Isolation and *in vitro* cultivation of the mammalian microsporidian *Encephalitozoon cuniculi*. Folia Parasitol. 19, 349–354.
Vávra, J., Chalupský, J., Oktábec, J., Bedrník, P., 1980. *Encephalitozoon cuniculi* (EC) in a rabbit farm: transmission and influence on body weight. J. Protozool. 27, 74A–75A.
Vávra, J., Chalupský, J., Bedrník, P., Horvath, G., Varga, I., 1986. Encephalitozoonosis in rabbit colonies. Symp. Biol. Hung. 33, 369–378.
Vávra, J., Chalupský, J., Bedrník, P., Horvath, G., Varga, I., 1987. A review on the *Encephalitozoon cuniculi* infection in rabbits; its incidence in Hungarian rabbit colonies (in Hungarian with English summary). Hung. Vet. J. 42, 499–502.
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). J. Eukaryot. Microbiol. 45, 273–283.
Vávra, J., Hyliš, M., Oborník, M., Vossbrinck, C.R., 2005. Microsporidia in aquatic microcrustacea: the copepod microsporidium *Marssoniella elegans* Lemmermann, 1900 revisited. Folia Parasitol. 52, 163–172.
Vávra, J., Hyliš, M., Vossbrinck, C.R., Pilarska, D.K., Linde, A., Weiser, J., McManus, M.L., Hoch, G., Solter, L.F., 2006. *Vairimorpha disparis* n. comb. (Microsporidia: Burenellidae): a redescription and taxonomic revision of *Thelohania disparis* Timofejeva 1956, a microsporidian parasite of the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). J. Eukaryot. Microbiol. 53, 292–304.
Vávra, J., Kamler, M., Modrý, D., Koudela, B., 2011. Opportunistic nature of the mammalian microsporidia: experimental transmission of *Trachipleistophora extenrec* (Fungi: Microsporidia) between mammalian and insect hosts. Parasitol. Res. 108, 1565–1573.
Vivares, C.P., Metenier, G., 2000. Towards the minimal eukaryotic parasitic genome. Curr. Opin. Microbiol. 3, 463–467.
Vivares, C.P., Metenier, G., 2001. The microsporidian *Encephalitozoon*. BioEssays 23, 194–202.
Vivares, C., Biderre, C., Duffieux, F., Peyretaillade, E., Peyret, P., Metenier, G., Pages, M., 1996. Chromosomal localization of five genes in *Encephalitozoon cuniculi* (microsporidia). J. Eukaryot. Microbiol. 43, 97.
Vivares, C.P., Gouy, M., Thomarat, F., Metenier, G., 2002. Functional and evolutionanary analysis of a eukaryotic parasitic genome. Curr. Opin. Microbiol. 5, 499–505.
Voigt, K., Kirk, P.M., 2011. Recent developments in the taxonomic affiliation and phylogenetic positioning of fungi: Impact in applied microbiology and environmental biotechnology. Appl. Microbiol. Biotechnol. 90, 41–57.
Vossbrinck, C.R., Debrunner-Vossbrinck, B.A., 2005. Molecular phylogeny of the microsporidia: ecological, ultrastructural and taxonomic considerations. Folia Parasitol. 52, 131–142.
Vossbrinck, C.R., Woese, C.R., 1986. Eukaryotic ribosomes that lack a 5.8S RNA. Nature 320, 287–288.
Vossbrinck, C.R., Maddox, J.V., Friedman, S., Debrunner-Vossbrick, B.A., Woese, C.R., 1987. Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. Nature 326, 411–414.
Vossbrinck, C.R., Andreadis, T.G., Weiss, L.M., 2004. Phylogenetics: taxonomy and the microsporidia as derived fungi. In: Lindsay, D.S., Weiss, L.M. (Eds.), Opportunistic Infections: *Toxoplasma, Sarcocystis* and Microsporidia, vol. 9. Kluwer Academic Publishers, N.Y., Boston, Dordrecht, London, Moscow, pp. 189–213.
Waller, R.F., Jabbour, C., Chan, N.C., Celik, N., Likic, V.A., Mulhern, T.D., Lithgow, T., 2009. Evidence of a reduced and modified mitochondrial protein import apparatus in microsporidian mitosomes. Eukaryot. Cell 8, 19–26.
Wang, H., Xu, Z., Gao, L., Hao, B., 2009. A fungal phylogeny based on 82 complete genomes using the composition vector method. BMC Evol. Biol. 9, 195.

Ward, A.J.W., Duff, A.J., Krause, J., Barber, I., 2005. Shoaling behaviour of sticklebacks infected with the microsporidian parasite, *Glugea anomala*. Environ. Biol. Fishes 72, 155–160.

Wasson, K., Barry, P.A., 2003. Molecular characterization of *Encephalitozoon intestinalis* (Microspora) replication kinetics in a murine intestinal cell line. J. Eukaryot. Microbiol. 50, 169–174.

Weedall, R.T., Robinson, M., Smith, J.E., Dunn, A.M., 2006. Targeting of host cell lineages by vertically transmitted, feminising microsporidia. Int. J. Parasitol. 36, 749–756.

Weidner, E., 1972. Ultrastructural study of microsporidian invasion into cells. Z. Parasitenkd 40, 227–242.

Weidner, E., 1982. The microsporidian spore invasion tube. III. Tube extrusion and assembly. J. Cell Biol. 93, 976–979.

Weidner, E., 2000. Cytoplasmic proteins on the surface of discharged microsporidian sporoplasms. Biol. Bull. 199, 208–209.

Weidner, E., 2001. Microsporidian spore/sporoplasm dynactin in *Spraguea*. Biol. Bull. 201, 245–246.

Weidner, E., Byrd, W., 1982. The microsporidian spore invasion tube. II. Role of calcium in the activation of invasion tube discharge. J. Cell Biol. 93, 970–975.

Weidner, E., Findley, A., 1999. Extracellular survival of an intracellular parasite (*Spraguea lophii*, Microsporea). Biol. Bull. 197, 270–271.

Weidner, E., Findley, A., 2002. Peroxisomal catalase in extrusion apparatus posterior vacuole of microsporidian spores. Biol. Bull. 203, 212.

Weidner, E., Findley, A., 2003. Catalase in microsporidian spores before and during discharge. Biol. Bull. 205, 236–237.

Weidner, E., Byrd, W., Scarborough, A., Pleshinger, J., Sibley, D., 1984. Microsporidian spore discharge and the transfer of polaroplast organelle membrane into plasma membrane. J. Protozool. 31, 195–198.

Weidner, E., Canning, E.U., Rutledge, C.R., Meek, C.L., 1999a. Mosquito (Diptera: Culicidae) host compatibility and vector competency for the human myositic parasite *Trachipleistophora hominis* (Phylum Microspora). J. Med. Entomol. 36, 522–525.

Weidner, J., Findley, A.M., Dolgikh, V., Sokolova, J., 1999b. Microsporidian biochemistry and physiology. In: Wittner, M., Weiss, L.M. (Eds.), The Microsporidia and Microsporidiosis, ASM Press, Washington, D.C., pp. 172–195.

Weiser, J., 1961. Die Mikrosporidien als Parasiten der Insekten. Monogr. z. Ang. Ent. (Beihefte Ztschr. Angw. Entomol.). vol. 17. P. Parey Verlag, Hamburg und Berlin pp. 149.

Weiser, J., 1977. Contribution to the classification of microsporidia. Věst. Česk. Spol. Zool. 41, 308–320.

Weiss, L.M., Vossbrinck, C.R., 1999. Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the Microsporidia. In: Wittner, M., Weiss, L.M. (Eds.), The Microsporidia and Microsporidiosis, ASM Press, Washington, D.C., pp. 129–171.

Widmer, G., Akiyoshi, D.E., 2010. Host-specific segregation of ribosomal nucleotide sequence diversity in the microsporidian *Enterocytozoon bieneusi*. Inf. Genet. Evol. 10, 122–128.

Williams, B.A.P., 2009. Unique physiology of host-parasite interactions in microsporidia infections. Cell. Microbiol. 11, 1551–1560.

Williams, B.A.P., Hirt, R.P., Lucocq, J.M., Embley, T.M., 2002. A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. Nature 418, 865–869.

Williams, B.A.P., Slamovits, C.H., Patron, N.J., Fast, N.M., Keeling, P.J., 2005. A high frequency of overlapping gene expression in compacted eukaryotic genomes. Proc. Natl. Acad. Sci. U.S.A. 102, 10936–10941.

Williams, B.A.P., Cali, A., Takvorian, P.M., Keeling, P.J., 2008a. Distinct localization patterns of two putative mitochondrial proteins in the microsporidian *Encephalitozoon cuniculi*. J. Eukaryot. Microbiol. 55, 131–133.

Williams, B.A.P., Lee, R.C.H., Becnel, J.J., Weiss, L.M., Fast, N.M., Keeling, P.J., 2008b. Genome sequence surveys of *Brachiola algerae* and *Edhazardia aedis* reveal microsporidia with low gene densities. BMC Genomics 9, 200.

Williams, B.A.P., Elliot, C., Burri, L., Kido, Y., Kita, K., Moore, A.L., Keeling, P.J., 2010. A broad distribution of the alternative oxidase in microsporidian parasites. PLoS Pathog. 6, e1000761.

Wolinska, J., Giessler, S., Koerner, H., 2009. Molecular identification and hidden diversity of novel *Daphnia* parasites from European lakes. Appl. Environ. Microbiol. 75, 7051–7059.

Wu, J.Y., Smart, M.D., Anelli, C.M., Sheppard, W.S., 2012. Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to *Nosema* (Microsporidia) infection. J. Invertebr. Pathol. 109, 326–329.

Wu, Z., Li, Y., Pan, G., Tan, X., Hu, J., Zhou, Z., Xiang, Z., 2008. Proteomic analysis of spore wall proteins and identification of two spore wall proteins from *Nosema bombycis* (Microsporidia). Proteomics 8, 2447–2461.

Wu, Z., Li, Y., Pan, G., Zhou, Z., Xiang, Z., 2009. SWP25, a novel protein associated with the *Nosema bombycis* endospore. J. Eukaryot. Microbiol. 56, 113–118.

Xiao, L., Li, L., Visvesvara, G.S., Moura, H., Didier, E.S., Lal, A.A., 2001. Genotyping *Encephalitozoon cuniculi* by multilocus analyses of genes with repetitive sequences. J. Clin. Microbiol. 39, 2248–2253.

Xie, X., Lipke, P.N., 2010. On the evolution of fungal and yeast cell walls. Yeast 27, 479–488.

Xu, Y., Weiss, L.M., 2005. The microsporidian polar tube: a highly specialised invasion organelle. Int. J. Parasitol. 35, 941–953.

Xu, Y., Takvorian, P.M., Cali, A., Orr, G., Weiss, L.M., 2004. Glycosylation of the major polar tube protein of *Encephalitozoon hellem*, a microsporidian parasite that infects humans. Infect. Immun. 72, 6341–6350.

Xu, Y., Takvorian, P., Cali, A., Wang, F., Zhang, H., Orr, G., Weiss, L.M., 2006. Identification of a new spore wall protein from *Encephalitozoon cuniculi*. Infect. Immun. 74, 239–247.

Zhang, X., Wang, Z., Su, Y., Liang, X., Sun, X., Peng, S., Lu, H., Jiang, N., Yin, J., Xiang, M., Chen, Q., 2011. Identification and genotyping of *Enterocytozoon bieneusi* in China. J. Clin. Microbiol. 49, 2006–2008.