5 Microsporidia

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I. Introduction

The phylum Microsporidia Balbiani 1882 (Weiser 1977) is comprised of a diverse group of over 1,400 species (Didier and Weiss 2006). These organisms are obligate intracellular pathogenic protists uniquely characterized by a **specialized invasion organelle, the polar tube**, through which the cytoplasm and nucleus of these organisms pass during the infection of their host cells. Long considered early branching eukaryotes classified with the Archezoa, the microsporidia are now considered fungi based on accumulated data and more sophisticated analyses (Hibbett et al. 2007; James et al. 2006). **Microsporidia infect commercially significant animals, such as honey bees, salmon, silkworms, farm animals, and companion pets, and are of medical importance because they cause emerging opportunistic infections in humans.** A wide range of animals that are less commercially relevant can also be infected with microsporidia and thus pose a risk as environmental reservoirs of infection (Santín and Fayer 2009a). As a consequence of studies to better characterize the tree of life, the microsporidia have undergone a major transition from placement with the earliest diverging eukaryotes to

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now being classified with the deep-branching fungi, closely related to the zygomycetes (Hibbett et al. 2007; James et al. 2006; Keeling 2009; Lee et al. 2008, 2010b). Questions remain, however, about the true relationship between the microsporidia and fungi (Koestler and Ebersberger 2011). Particularly noteworthy is the **evolution of gene compaction and reduction** observed among the microsporidia (Corradi and Keeling 2009; Keeling 2009; Keeling et al. 2010; Lee et al. 2010a; Texier et al. 2010; Vossbrinck et al. 2004). In this regard, microsporidia are highly efficient parasites, to the point that at least one species, *Enterocytozoon bieneusi*, even lacks genes for core carbon metabolism and depends fully on host-cell ATP import (Keeling et al. 2010).

II. Occurrence and Distribution

Microsporidia infect hosts ranging from protists to invertebrates (mainly insects) and vertebrates (mainly fish and mammals, including humans). **The only extracellular stage that survives is the spore, which is relatively resistant to environmental stress**. Because of the wide host range and the environmentally resistant spore wall, it is not surprising that microsporidia exhibit a worldwide distribution and can be found in aqueous (fresh and salt water) and terrestrial environments.

A. Arthropod Hosts

An extensive knowledge base on microsporidia isolated from terrestrial arthropod hosts has been dominated by species from insects, but other host groups include, but are not restricted to, cestodes, trematodes, nematodes, oligochaetes, isopods, myriapods, arachnids, and anaplurans (Sprague 1977). One indicator of the importance of microsporidia isolated from this group is that **nearly half of the approximately 186 described genera shown in what follows are from terrestrial arthropod hosts, with the majority of these from insects** (Becnel and Andreadis 1999). Prior to classification as fungi, the genera of microsporidia were established by the International Code of Zoological Nomenclature, and new genera are listed below in boldface type (Sprague and Becnel 1999). A request to exclude microsporidia from the International Code of Botanical Nomenclature was submitted and accepted and is now part of the revised code (Redhead et al. 2009). As such, the microsporidia are considered fungi, but descriptions of species remain subject to the International Code of Zoological Nomenclature.

The most commonly encountered microsporidia from insects are from Lepidoptera (within the *Nosema/Vairimorpha* clade) and Diptera, including the well-known genera *Amblyospora* and *Parathelohania* (Becnel and Andreadis 1999). It is likely that the large number of genera and species described from Lepidoptera and Diptera is due to the extensive studies searching for biological control agents in these groups of pest insects. Microsporidia in insects and other terrestrial arthropods have been studied extensively where they are important as natural control factors, have potential as manipulated microbial control agents for pest species, and cause chronic infections in beneficial arthropods.

Microsporidia **as important natural pest control agents** have been most extensively studied in insect pests. *Nosema pyrausta* in the European corn borer, *Ostrinia nubilalis*, is one of the most important regulators of larval populations in the USA (Andreadis 1984; Siegel et al. 1986). Populations of an important forest pest, the spruce budworm, *Choristoneura fumiferana*, are suppressed by epizootics caused by *Nosema fumiferanae* (Wilson 1973, 1981). A more recent example of natural control is in the red imported fire ant, *Solenopsis invicta*, where *Kneallhazia* (syn. *Thelohania*) *solenopsae* has become widespread in US populations of this invasive species (Oi et al. 2004).

Perhaps the best example of a species of microsporidia used as a microbial pesticide was a program for the control of rangeland grasshoppers with *Paranosema* (syns. *Nosema* and *Antonospora*) *locustae* (Henry and Oma 1981). *P. locustae* infects over 90 species of grasshoppers in the family Acrididae (Brooks 1988) and was registered by the US Environmental Protection Agency in 1989 as a microbial insecticide. Whole-organism tech-

1. *Abelspora* Azevedo 1987
2. ***Aedispora*** Kilochitskii 1997
3. *Agglomerata* Larsson & Yan 1988
4. *Agmasoma* Hazard & Oldacre 1975
5. *Alfvenia* Larsson 1983
6. *Alloglugea* Paperna & Lainson 1995
7. ***Amazonspora*** Azevedo & Matos 2003
8. *Amblyospora* Hazard & Oldacre 1975
9. *Ameson* Sprague 1977
10. *Amphiacantha* Caullery & Mesnil 1914
11. *Amphiamblys* Caullery & Mesnil 1914
12. ***Andreanna*** Simakova, Vossbrinck & Andreadis 2008
13. ***Anisofilariata*** Tokarev, Voronin, Seliverstova, Dolgikh, Pavlova, Ignatieva & Issi 2010
14. *Anncaliia* Issi, Krylova & Nicolaeva 1993
15. ***Antonospora*** Fries, Paxton, Tengo, Slemenda, da Silva & Pieniazek 1999
16. *Auraspora* Weiser & Purrini 1980
17. *Bacillidium* Janda 1928
18. *Baculea* Loubes & Akbarieh 1978
19. ***Becnelia*** Tonka & Weiser 2000
20. *Berwaldia* Larsson 1981
21. ***Binucleata*** Refardt, Decaestecker, Johnson & Vávra 2008
22. *Binucleospora* Bronnvall & Larsson 1995
23. *Bohuslavia* Larsson 1985
24. *Brachiola* Cali, Takvorian & Weiss 1998
25. ***Bryonosema*** Canning, Refardt, Vossbrinck & Curry 2002
26. *Burenella* Jouvenaz & Hazard 1978
27. *Burkea* Sprague 1977
98. *Mariona* Stempell 1909
99. *Marssoniella* Lemmermann 1900
100. *Merocinta* Pell & Canning 1993
101. *Metchnikovella* Caullery & Mesnil 1897
102. *Microfilum* Faye, Toguebaye & Bouix 1991
103. *Microgemma* Ralphs & Matthews 1986
104. *Microsporidium* Balbiani 1884
105. *Microsporidyopsis* Schereschewsky 1925
106. *Mitoplistophora* Codreanu 1966
107. ***Mockfordia*** Sokolova, Sokolov & Carlton 2010
108. *Mrazekia* Léger & Hesse 1916
109. ***Myospora*** Stentiford, Bateman, Small, Moss, Shields, Reece & Tuck 2010
110. ***Myosporidium*** Baquero, Rubio, Moura, Pieniazek & Jordana 2005
111. *Myxocystis* Mrazek 1897
112. *Nadelspora* Olson, Tiekotter & Reno 1994
113. *Napamichum* Larsson 1990
114. *Nelliemelba* Larsson 1983
115. ***Neoflabelliforma*** Morris & Freeman 2010
116. *Neoperezia* Issi & Voronin 1979
117. *Neonosemoides* Faye, Toguebaye & Bouix 1996
118. *Nolleria* Beard, Butler & Becnel 1990
119. *Norlevinea* Vávra 1984
120. *Nosema* Naegeli 1857
121. *Nosemoides* Vinckier 1975
122. ***Novothelohania*** Andreadis, Simakova, Vossbrinck, Shepard & Yurchenko 2011
28. *Buxtehudea* Larsson 1980
29. *Campanulospora* Issi, Radischcheva & Dolzhenko 1983
30. *Canningia* Weiser, Wegensteiner & Zizka 1995
31. *Caudospora* Weiser 1946
32. *Caulleryetta* Dogiel 1922
33. *Chapmanium* Hazard & Oldacre 1975
34. *Chytridioides* Trégouboff 1913
35. *Chytridiopsis* Schneider 1884
36. *Ciliatosporidium* Foissner & Foissner 1995
37. *Coccospora* Kudo 1925
38. *Cougourdella* Hesse 1935
39. ***Crepidulospora*** Simakova, Pankova & Issi 2004
40. ***Crispospora*** Tokarev, Voronin, Seliverstova, Pavlova & Issi 2010
41. *Cristulospora* Khodzhaeva & Issi 1989
42. *Cryptosporina* Hazard & Oldacre 1975
43. ***Cucumispora*** Ovcharenko, Bacela, Wilkinson, Ironside, Rigaud & Wattier 2009
44. *Culicospora* Weiser 1977
45. *Culicosporella* Weiser 1977
46. *Cylindrospora* Issi & Voronin 1986
47. *Cystosporogenes* Canning, Barker, Nicholas & Page 1985
48. ***Dasyatispora*** Diamant, Goren, Yokeş, Galil, Klopman, Huchon, Szitenberg & Karhan 2010
49. ***Desmozoon*** Freeman & Sommerville 2009
50. *Desportesia* Issi & Voronin 1986
51. ***Dimeispora*** Simakova, Pankova & Issi 2004
52. *Duboscqia* Perez 1908
123. *Nucleospora* Docker, Kent & Devlin 1996
124. *Nudispora* Larsson 1990
125. *Octosporea* Flu 1911
126. ***Octotetrasporea*** Issi, Kadyrova, Pushkar, Khodzhaeva & Krylova 1990
127. *Oligosporidium* Codreanu-Bălcescu, Codreanu & Traciuc 1981
128. *Ordospora* Larsson, Ebert & Vávra 1997
129. *Ormieresia* Vivares, Bouix & Manier 1977
130. *Orthosomella* Canning, Wigley & Barker 1991
131. *Orthothelohania* Codreanu & Bălcescu-Codreanu 1974
132. *Ovavesicula* Andreadis & Hanula 1987
133. ***Ovipleistophora*** Pekkarinen, Lom & Nilsen 2002
134. ***Pankovaia*** Simakova, Tokarev & Issi 2009
135. ***Paraepiseptum*** Hyliš, Oborník, Nebesářová & Vávra 2007
136. ***Paranosema*** Sokolova, Dolgikh, Morzhina, Nassonova, Issi, Terry, Ironside, Smith & Vossbrinck 2003
137. ***Paranucleospora*** Nylund, Nylund, Watanabe, Arnesen & Karlsbakk 2010
138. ***Parapleistophora*** Issi, Kadyrova, Pushkar, Khodzhaeva & Krylova 1990
139. ***Parastempellia*** Issi, Kadyrova, Pushkar, Khodzhaeva & Krylova 1990
140. *Parathelohania* Codreanu 1966
141. ***Paratuzetia*** Poddubnaya, Tokarev & Issi 2006
142. *Pegmatheca* Hazard &

53. *Edhazardia* Becnel, Sprague & Fukuda 1989
54. *Encephalitozoon* Levaditi, Nicolau & Schoen 1923
55. *Endoreticulatus* Brooks, Becnel & Kennedy 1988
56. *Enterocytozoon* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisse & Modigliani 1985
57. ***Enterospora*** Stentiford, Bateman, Longshaw & Feist 2007
58. *Episeptum* Larsson 1986
59. ***Euplotespora*** Fokin, Giuseppe, Erra & Dini 2008
60. *Evlachovaia* Voronin & Issi 1986
61. ***Fibrillanosema*** Galbreath, Smith, Terry, Becnel & Dunn 2004
62. *Flabelliforma* Canning, Killick-Kendrick & Killick-Kendrick 1991
63. *Geusia* Rühl & Korn 1979
64. *Glugea* Thélohan 1891
65. *Glugoides* Larsson, Ebert, Vávra & Voronin 1996
66. *Golbergia* Weiser 1977
67. *Gurleya* Doflein 1898
68. *Gurleyides* Voronin 1986
69. ***Hamiltosporidium*** Haag, Larsson, Refardt & Ebert 2010
70. *Hazardia* Weiser 1977
71. *Helmichia* Larsson 1982
72. ***Hepatospora*** Stentiford, Bateman, Dubuffet, Chambers & Stone 2011
73. *Hessea* Ormières & Sprague 1973
74. *Heterosporis* Schubert 1969
75. *Heterovesicula* Lange, Macvean, Henry & Streett 1995
76. *Hirsutusporos* Batson 1983
77. *Holobispora* Voronin 1986
78. *Hrabyeia* Lom & Dyková 1990
79. *Hyalinocysta* Hazard & Oldacre 1975
80. *Ichthyosporidium* Caullery & Mesnil 1905
81. *Inodosporus* Overstreet & Weidner 1974
82. *Intexta* Larsson, Steiner & Bjørnson 1997
83. ***Intrapredatorus*** Chen, Kuo & Wu 1998
84. *Issia* Weiser 1977
85. *Janacekia* Larsson 1983
86. *Jirovecia* Weiser 1977
87. *Jiroveciana* Larsson 1980
88. *Johenrea* Lange, Becnel, Razafindratiana, Przybyszewski & Razafindrafara 1996
89. ***Kabatana*** Lom, Dyková & Tonguthai, 2000
90. *Kinorhynchospora* Adrianov & Rybakov 1991
91. ***Kneallhazia*** Sokolova & Fuxa 2008
92. ***Krishtalia*** Kilochitskii 1997
93. *Lanatospora* Voronin 1986
94. *Larssonia* Vidtman & Sokolova 1994
95. *Larssoniella* Weiser & David 1997
96. ***Liebermannia*** Sokolova, Lange & Fuxa 2006
97. *Loma* Morrison & Sprague 1981
143. *Perezia* Léger & Duboscq 1909
144. *Pernicivesicula* Bylén & Larsson 1994
145. *Pilosporella* Hazard & Oldacre 1975
146. *Pleistophora* Gurley 1893
147. *Pleistophoridium* Codreanu-Bălcescu & Codreanu 1982
148. *Polydispyrenia* Canning & Hazard 1982
149. ***Potaspora*** Casal, Matos, Teles-Grilo & Azevedo 2008
150. ***Pseudoloma*** Matthews, Brown, Larison, Bishop-Stewart, Rogers & Kent 2001
151. ***Pseudonosema*** Canning, Refardt, Vossbrinck, Okamura & Curry 2002
152. *Pseudopleistophora* Sprague 1977
153. *Pulcispora* Vedmed, Krylova & Issi 1991
154. *Pyrotheca* Hesse 1935
155. *Rectispora* Larsson 1990
156. *Resiomeria* Larsson 1986
157. *Ringueletium* Garcia 1990
158. ***Schroedera*** Morris & Adams 2002
159. *Scipionospora* Bylén & Larsson 1996
160. *Semenovaia* Voronin & Issi 1986
161. ***Senoma*** Simakova, Pankova, Tokarev & Issi 2005
162. *Septata* Cali, Kotler & Orenstein 1993
163. ***Simuliospora*** Khodzhaeva, Krylova & Issi 1990
164. *Spherospora* Garcia 1991
165. *Spiroglugea* Léger & Hesse 1924
166. *Spraguea* Weissenberg 1976
167. *Steinhausia* Sprague, Ormières & Manier 1972
168. *Stempellia* Léger & Hesse 1910
169. *Striatospora* Issi & Voronin 1986
170. *Systenostrema* Hazard & Oldacre 1975
171. *Tardivesicula* Larsson & Bylén 1992
172. *Telomyxa* Léger & Hesse 1910
173. *Tetramicra* Matthews & Mattews 1980
174. *Thelohania* Henneguy 1892
175. *Toxoglugea* Léger & Hesse 1924
176. *Toxospora* Voronin 1993
177. *Trachipleistophora* Hollister, Canning, Weidner, Field, Kench & Marriott 1996
178. *Trichoctosporea* Larsson 1994
179. *Trichoduboscqia* Léger 1926
180. ***Trichonosema*** Canning, Refardt, Vossbrinck, Okamura & Curry 2002
181. *Trichotuzetia* Vávra, Larsson & Baker 1997
182. *Tricornia* Pell & Canning 1992
183. ***Tubulinosema*** Franzen, Fischer, Schroeder, Schölmerich & Schneuwly 2005
184. *Tuzetia* Maurand, Fize, Fenwik & Michel 1971
185. *Unikaryon* Canning, Lai & Lie 1974
186. *Vairimorpha* Pilley 1976
187. *Vavraia* Weiser 1977
188. *Vittaforma* Silveira & Canning 1995
189. *Weiseria* Doby & Saguez 1964
190. *Wittmannia* Czaker 1997

nology was used to produce large numbers of *P. locustae* spores in grasshoppers that were formulated into bait and applied by air (Henry and Oma 1981). *P. locustae* does not cause rapid mortality but has a debilitating effect on the host that can have long-term control implications when introduced.

Microsporidia are perhaps best known because several prominent species are the causative agents of **chronic disease in beneficial insects such as silkworms (*Bombyx mori*) and honey bees (*Apis melifera*).** The first named species of microsporidia was *Nosema bombycis* from *B. mori* and was the subject of landmark studies by Louis Pasteur, who established this pathogen as the etiological agent of "**pébrine**" **or silkworm disease** (Pasteur 1870). Pasteur proved that *N. bombycis* was transmitted from adult to progeny via the egg (transovarial transmission) and by the ingestion of spores, and he developed preventive methods that saved the silkworm industry worldwide. Adult honey bees worldwide are afflicted by nosemosis, which has been caused historically by *Nosema apis*, and more recently the Asian species, *Nosema ceranae*, has been implicated as playing a major role (Chen et al. 2008). Interactions of *Nosema* spp. with other bee pathogens have been implicated in contributing to **colony collapse disorder** and declines in honey bee colonies worldwide (Ratnieks and Carreck 2010). In addition, numerous microsporidia are implicated in reducing the effectiveness of commercially produced biological control agents. A few select examples are *Nosema muscidifuracis*, which reduced the fitness of the muscoid fly parasitoid *Muscidifurax raptor* (Geden et al. 1995), and *Oligosporidium occidentalis* from the predatory mite *Metaseiulus occidentalis*, which has a negative impact on the overall fitness of this predator (Becnel et al. 2002).

B. Aquatic Hosts (Marine and Freshwater)

Microsporidia infect a broad range of aquatic organisms, including crustaceans and amphipods, and freshwater, saltwater, and anadromous fish. The impacts of microsporidian parasites on fish in aquaculture, wild populations, and research have been documented on several occasions (Lom and Dyková 1992; Shaw and Kent 1999), and microsporidian species belonging to some 18 genera have been described in fishes (Lom 2002; Lom and Nilsen 2003). Most of these infections seem to be chronic, with minimal host mortality. Infections by some species, however, can have a profound **economic impact on wild fish and aquaculture hosts** in terms of mortality and commercial quality of fish**.** Several of these microsporidia have been shown to impact fish either by directly killing the host or indirectly by reducing fecundity (Ramsay et al. 2009; Wiklund et al. 1996) or decreasing the commercial quality of farmed fish. For example, Becko disease in yellowtail is caused by the formation of cysts in skeletal muscle by the microsporidium *Kabatana seriolae* Egusa 1982.

With the rapid growth of aquaculture, microsporidian pathogens in fish have increased in importance, and three species of microsporidia are the main causes of **disease in seawater-reared salmon**. *Loma salmonae* results in high mortality of salmonids reared in freshwater hatcheries and in seawater netpens in North America and Europe due to chronic gill infections (Kent and Poppe 1998). *Nucleospora salmonis*, also a microsporidian parasite of the Chinook salmon *Oncorhynchus tshawytscha*, is unique in that it infects the host cell nucleus and results in lymphoblastosis and a leukemia-like condition in fish (Chilmonczyk et al. 1991). Another intranuclear microsporidium, *Paranucleospora theridion*, infects the rainbow trout, *Oncorhynchus mykiss*, and salmonids (e.g., Atlantic salmon, *Salmo salar*), causing up to 80 % mortality in Atlantic salmon farms in Norway (Nylund et al. 2010). Additionally, *P. theridion* can infect the salmon louse *L. salmonis*, providing a potential reservoir for this parasite.

Microsporidia are also common **pathogens of baitfish**. The shiner, *Notemigonus crysoleucas*, and fathead minnow, *Pimephales promelas*, are frequently infected by *Ovipleistophora ovariae*, which generally does not result in acute mortalities but significantly affects the fecundity of spawning fish. Additionally, due to the

increased use of fish in research, infections by microsporidia can have a confounding impact on experimental results using such fish (Kent et al. 2011). Zebrafish, *Danio rerio*, are also affected by microsporidia, with the first report describing infection of the spinal cord in fish purchased from a pet store for use in toxicological studies (de Kinkelin 1980). After further characterization, this microsporidian was assigned to a new genus and species, *Pseudoloma neurophilia* (Matthews et al. 2001). *P. neurophilia* infections are widespread in laboratory facilities (Kent et al. 2011) and are generally characterized as chronic and occasionally associated with spinal deformities and emaciation. *Pleistophora hyphessobryconis*, a muscle-infecting microsporidian, has also been identified in laboratory populations of zebrafish (Sanders et al. 2010). Commonly known as *neon tetra disease* for its type host, *Paracheirodon innesi*, this parasite is a frequent problem in the aquarium trade, often resulting in considerable mortality in a wide range of fishes. *P. hyphessobryconis* has a remarkably broad host range, infecting some 20 species of fishes in 4 orders (Lom and Dyková 1992; Schäperclaus 1991; Steffens 1962). As with *P. neurophilia*, *P. hyphessobryconis* can be harbored by otherwise healthy-appearing fish that may show clinical signs of the infection only after experiencing immunosuppressive events. Clinical presentation of the disease includes massive infections of myocytes resulting in liquefactive necrosis of the muscle tissue that almost invariably leads to the death of the fish. This example highlights the importance of obtaining fish used in research from reputable sources and the potential for introducing a microsporidian with a broad host range to new or accidental hosts.

In contrast to *P. hyphessobryconis*, many other microsporidia of fish are host-specific, at least at the family or genus level. One example, *L. salmonae*, infects all species of Pacific salmon, *Onchorynchus* spp., but does not infect the Atlantic salmon, *S. salar*, based on results from experimental exposure of fish to *L. salmonae*-infected gill tissue (Shaw et al. 2000). Using polymerase chain reaction to monitor infection of intestine, heart, spleen, and gill tissues, experimental exposure to *L. salmonae* showed an aberrant progression in Atlantic salmon, *S. salar*, compared to that seen in the rainbow trout, *O. mykiss* (Sanchez et al. 2001). Parasite DNA was detected in all tissues tested until week 3, at which point, rather than progressing to the gills to complete the life cycle by forming mature spores, the parasite was apparently cleared. This illustrates an abortive life cycle by *L. salmonae* infection in a nonpermissive host whereby the parasite was able to invade certain tissues and proliferate to some extent but was unable to progress to sporogony.

C. Mammalian and Avian Hosts

Members of the genus *Encephalitozoon* are the most common microsporidia infecting mammals and birds. The type species of this genus, *Enc. cuniculi*, was first identified in rabbits with motor paralysis in 1922 (Wright and Craighead 1922) and was also the first microsporidian genome to be sequenced (Katinka et al. 2001). There are now several sequenced microsporidian genomes, and the data can be found at http://microsporidiadb.org/micro/. *Enc. cuniculi* has an extraordinarily wide host range among mammals, such as rodents, lagomorphs, canines, equines, nonhuman primates, and humans. *Enc. hellem* Didier et al. 1991 and *Enc.* (syn. *Septata*) *intestinalis* Cali et al. 1993 were later isolated and identified from AIDS patients (Cali et al. 1993; Didier and Weiss 2006; Didier et al. 1991). *Enc. intestinalis* is still considered more common in humans, whereas *Enc. hellem* is more common in birds with humans believed to be zoonotic hosts. Since the *Encephalitozoon* species are indistinguishable by light microscopy, reports of *Enc. cuniculi* in birds prior to the AIDS pandemic may actually have been due to *Enc. hellem* (Didier et al. 1998; Snowden and Logan 1999; Snowden et al. 2000). *Encephalitozoon* species may infect enteric sites and contribute to diarrhea, but they more typically cause systemic infections to persist over the life of the host unless treated with effective drugs (e.g., albendazole). **Disease occurs predominantly in immune-deficient hosts** (e.g., AIDS patients, organ transplant recipients undergoing

immunosuppressive therapy) and occurs sporadically in immune-competent hosts (Kotler and Orenstein 1998; Weber et al. 2000).

The most prevalent microsporidian in humans, *Ent. bieneusi* Desportes et al. 1985, was first identified in an AIDS patient in Haiti and is primarily associated with persistent and self-limiting diarrhea in immune-deficient and immune-competent humans, respectively (Desportes et al. 1985; Didier and Weiss 2006). The host range of *Ent. bieneusi* seems to be far wider than first believed and now includes wild, farm, and companion pet animals (Santín and Fayer 2009a, b). In addition, *Ent. bieneusi* has been increasingly identified in avian hosts such as chickens, pigeons, falcons, and exotic birds (Graczyk et al. 2008; Haro et al. 2005; Muller et al. 2008; Reetz et al. 2002). Currently the genus *Enterocytozoon* contains only a single species, *Ent. bieneusi*.

It is possible, however, that this organism is a species complex, and as additional information is obtained, it may be split into separate species, as was done with *Cryptosporidium parvum*. It should also be appreciated that the family Enterocytozoonidae contains the genus *Nucleospora*, which has several species, including *N. salmonis*, previously named *Ent. salmonis*.

The pathogenesis of *Ent. bieneusi* infections in immune-competent humans and nonhuman hosts has not been well characterized. For example, it is unknown whether *Ent. bieneusi* persists in otherwise healthy people and reactivates under conditions of immune deficiency. Additional species of microsporidia less frequently identified in mammals and birds include *Vittaforma corneae*, *Trachipleistophora* spp., *Anncaliia algerae*, *Pleistophora ronneaifiei*, *Nosema ocularum*, and *Microsporidium* spp. (Didier and Weiss 2006).

III. Morphology of the Microsporidian Spore

A. General Description and Common Features

Microsporidian spores are generally small and vary from 1 to 20 μm in length (Fig. 5.1). Spores of most species of microsporidia are oval in shape but may also exhibit pyriform, spherical, or rod shapes. The spore wall provides resistance to environmental influences and allows for the increase in hydrostatic pressure that causes spore discharge (see below; Frixione et al. 1997). The spore wall is surrounded by a glycoproteinaceous electron-dense exospore and electron-lucent endospore composed primarily of chitin (Vavrá and Larsson 1999). Ultrastructural studies of the genus *Encephalitozoon* using transmission electron microscopy, freeze fracture, and deep etching demonstrated that **the exospore is very complex and consists of three layers: an outer spiny layer, an intermediate electron-lucent lamina endospore, and an inner fibrous layer** (Bigliardi et al. 1996). The endospore is observed as a space crossed by bridges connecting the exospore to the plasma membrane. It has been suggested that **chitin, a major component of the endospore**, comprises the fibrils forming the bridges across the endospore and is part of the fibrillar system of the exospore (Bigliardi et al. 1996; Erickson and Blanquet 1969; Prigneau et al. 2000; Vavrá 1976). It is possible to distinguish subcompartments within the spore wall using polyclonal antisera against partially purified microsporidial proteins. A glycine- and serine-rich 51-kDa protein named SWP1 is localized to the exospore in *Enc. cuniculi* (Bohne et al. 2000) and *Enc. intestinalis* (Hayman et al. 2001). The corresponding gene, *swp1*, has been identified in *Enc. cuniculi*, *Enc. hellem*, and *Enc. intestinalis* (Bohne et al. 2000; Hayman et al. 2001). SWP1 is absent in meronts (proliferating stages) and first seen in early sporonts (stages that differentiate into spores) at a time when organisms translocate from the periphery to the center of the parasitophorous vacuole (PV) (Bohne et al. 2000). A 150-kDa glycoprotein in the spore wall named SWP2 was identified in *Enc. intestinalis* (Hayman et al. 2001). In addition, a putative glycophosphatidylinositol (GPI)-anchored chitin deacetylase has been localized to the plasmalemma endospore interface. Using proteomic techniques, a new spore wall protein, SWP3/EnP2, corresponding to ECU01_1270, was identified and localized to the endospore (Peuvel-

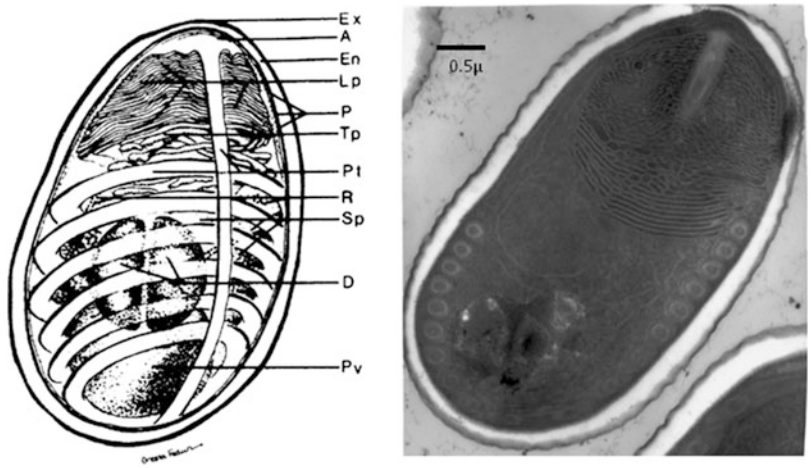

Fig. 5.1 Diagram of internal structure of a microsporidian spore (*left*) and a transmission electron micrograph of *Vavraia culicis floridensis* from *Aedes albopictus* (*right*). The spore coat has an outer electron-dense region called the exospore (Ex) and an inner thicker electron-lucent region known as the endospore (En). A unit membrane (P) separates the spore coat from the spore contents. The extrusion apparatus—anchoring disk (A), polar tubule (Pt), lamellar polaroplast (Lp), and tubular polaroplast (Tp)—dominates the spore contents and is diagnostic for microsporidian identification. The posterior vacuole (Pv) is a membrane-bound vesicle that sometimes contains a membrane whirl, a glomerular structure, flocculent material, or some combination of these structures. The spore cytoplasm is dense and contains ribosomes (R) in a tightly coiled helical array. The nucleation may consist of a single nucleus or pair of abutted nuclei, a diplokaryon (D). The size of the spore depends on the particular species and can vary from less than 1 μm to more than 10 μm. The number of polar tubule coils also varies from a few to 30 or more, again depending on the species observed. Reprinted with permission from Cali and Owen (1988)

Fanget et al. 2006; Xu et al. 2006). By immunoelectron microscopy this protein was found on the cell surface during sporogony and in the endospore in mature spores. SWP3 has several potential O-glycosylation sites and is likely a mannosylated protein like the major polar tube protein (PTP1). EnP1, corresponding to ECU01_0820, has also been localized to the endospore and demonstrated to be involved in adhesion of the spore to host cells (Peuvel-Fanget et al. 2006; Southern et al. 2007).

Under light microscopy, viable spores are refractile, and after histochemical staining (e.g., chromotrope, Gram), a **posterior vacuole** may be observed. The **unique structure that characterizes all microsporidia is the polar tube or filament** that coils within the spore and is part of the germination and infection apparatus (see below). The arrangement and number of coils of the polar filament within the spore vary among the microsporidia species. Long considered to be amitochondriate, the microsporidia have been found to possess **reduced mitochondria called mitosomes**, as well as **atypical Golgi** that lack the classical stacked dictyosome structure but instead are comprised of vesicular tubules that connect with the endoplasmic reticulum, plasma membrane, and developing polar tube. An **anchoring disk** with a membranous lamellar polaroplast is located at the anterior end of the spore and functions to anchor and fuel the extruding polar filament during germination (Keeling 2009; Vavrá and Larsson 1999). Ribosomes found along the endoplasmic reticulum are smaller than those of most eukaryotes (70S rather than 80S), being more similar to those of bacteria, and **the microsporidian nucleus may exist in a monokaryon or diplokaryon arrangement**.

B. Species (Spores) Infecting Arthropod Hosts

Studies on microsporidia in insects have been instrumental in establishing many aspects of microsporidian biology, but perhaps none more important than basic information about

spores and spore types. For more than 100 years following the first microsporidian species *N. bombycis* from silk worms was named, the one spore/one species concept was almost universally accepted. Over 100 years later, Hazard and Weiser (1968) discovered that **some microsporidian species infecting mosquitoes were observed to exhibit spore dimorphism, where two spore types with distinctive morphology and function were formed over the course of the life cycle.** They reported that a binucleate spore formed in the adult female was responsible for transmitting pathogens to progeny. These studies revealed that in infected male larval progeny, uninucleate spores (meiospores) were produced, while spore development was delayed until pupation and adult emergence in infected female progeny. Binucleate spores of the original type were produced in these infected females to repeat the cycle. This proved that the two morphologically distinctive spores found in larvae and adult hosts (formerly believed to belong to two genera) may represent a single species. The means by which these microsporidia were transmitted horizontally remained a mystery until the discovery that meiospores formed in larvae were infectious to a copepod intermediate host (Sweeney et al. 1985). When ingested by mosquito larvae, the spores from the copepod intermediate host initiate a sequence of development that ends with binucleate spores in the adult female mosquito. Multiple spore types within the same species have also been documented for *K. solenopsae* infecting the fire ant *Solenopsis invicta* where four different spore types have been reported (Sokolova and Fuxa 2008), suggesting that this trait may be common in many genera.

C. Species (Spores) Infecting Aquatic Hosts

Spores of microsporidia infecting fish are generally spherical or ovoid to pyriform in shape and contain a sporoplasm that is either monokaryotic or diplokaryotic. **Most species form spores of relatively uniform size and shape, but highly variable spore sizes (macrospores and microspores) occur together in tissues of hosts infected with members of the genera *Heterosporis*, *Pleistophora*, and *Ichthyosporidium*.** The genus *Pleistophora* comprises numerous species that generally infect skeletal muscle of fish. Several of these species have been found to produce two and sometimes three spore types of different sizes (Canning et al. 1986). The type species of this genus, *Pleistophora typicalis*, produces elongate macrospores averaging 7.5×3.0 μm and, more commonly, microspores that are ovoid and average 4.4×2.3 μm. Spores of *Pleistophora* spp. generally have a large posterior vacuole, occupying over half the total spore volume.

Other microsporidia of fish produce polymorphic spores that vary by host and even tissue within the same host in which they develop. For example, the intranuclear microsporidian *P. theridion* develops spherical monokaryotic spores of 2.2–2.5 μm in the salmon louse, *Lepeophtheirus salmonis*. In the fish host, *P. theridion* produces spherical, diplokaryotic spores of 0.9–1.2 μm in diameter in reticuloendothelial cells and ovoid spores of $2.4–2.7 \times 2.0–2.1$ μm in gill and skin epithelial cells (Nylund et al. 2010). *Nucleospora salmonis*, another intranuclear microsporidian, develops similarly small, ovoid spores (1×2 μm), but other spore forms have not been seen for this organism (Chilmonczyk et al. 1991). Spores of *Glugea anomala* are elongate and oval, and spore size varies minimally in the same host species. However, there are some variations in sizes of spores taken from different hosts such as *Gasterosteus aculeatus* ($3–6 \times 1.9–1.7$ μm) or *Pungitius pungitius* ($3.5–5.1 \times 1.9–2.6$ μm), and here also the posterior vacuole is relatively large, taking up approximately half of the spore volume.

D. Species (Spores) Infecting Mammalian and Avian Hosts

Encephalitozoon spores measure approximately $1–2 \times 2–4$ μm and exhibit a typical microsporidian spore configuration of a glycoproteinaceous electron-dense exospore, electron-lucent endospore composed of chitin, and a plasma membrane containing the cyto-

plasmic organelles. The polar filament typically coils five to seven times in single row arrangement, and the nucleus is monokaryotic. Mature spores usually contain a prominent posterior vacuole that often is visible by light microscopy of histochemically stained organisms. Additional organelles include the membranous anterior anchoring disk, lamellar polaroplast with Golgi-like vesicles, endoplasmic reticulum, and ribosomes.

Spores of *Ent. bieneusi* are among the smallest of the microsporidia measuring 1 × 1.5 μm and the chitinous endospore in *Ent. bieneusi* is somewhat thinner than found in *Encephalitozoon* spores. The polar filament coils five to seven times and commonly is observed to align in two rows. A prominent posterior vacuole may be observed, and the nucleus is monokaryotic.

IV. Microsporidian Invasion Apparatus

The invasion apparatus of the microsporidia consists of a polar tube, also referred to as the polar filament prior to discharge (Lom and Vávra 1963; Takvorian and Cali 1986; Weidner 1972, 1976, 1982), that consists of two domains: an anterior straight region surrounded by a lamellar polaroplast that is attached to the inside of the anterior end of the spore by an anchoring disk and a posterior coiled region that forms from 4 to approximately 30 coils around the sporoplasm in the spore, depending on the species (Wittner and Weiss 1999). In cross section, the polar filament inside the spore is composed of electron-dense and electron-lucent concentric layers that can range from as few as 3 to as many as 20 different layers (Cali et al. 2002; Chioralia et al. 1998; Lom 1972; Sinden and Canning 1974; Vavrá 1976; Weidner 1972, 1976). **During germination the polar filament (tube) is discharged from the anterior of the spore and forms a hollow tube that remains attached to the spore and facilitates passage of its sporoplasm and nucleus (or diplokaryon) into its host cell (Frixione et al. 1992; Lom and Vávra 1963; Ohshima 1937; Walters 1958; Weidner 1972).** Electron microscopy has demonstrated elongated sporoplasm in sections of extruded polar tube and the piercing of host cell membranes by the polar tube (Lom 1972; Weidner 1976). This process serves as a unique mechanism of infection, resulting in sporoplasm transfer directly into the host cell cytoplasm (Frixione et al. 1992; Lom and Vávra 1963; Ohshima 1937; Weidner 1972). In *A. algerae*, polar tube discharge is associated with the appearance of membrane infoldings surrounding the polar tube (Cali et al. 2002). These ultrastructural observations suggest that the polar tube is actually extracytoplasmic in the spore and explains how the sporoplasm can remain intact during the explosive germination reaction.

Polar tubes range from 50 to 100 μm in length and 0.1 to 0.15 μm in diameter (Frixione et al. 1992). A germinated spore is shown in Fig. 5.2. The polar tube discharges from the anterior pole of the spore in an **explosive reaction occurring in less than 2 s** (Frixione et al. 1992; Lom and Vávra 1963; Ohshima 1937; Weidner 1972). Spore discharge occurs through phases of (1) activation, (2) increase in intrasporal osmotic pressure, (3) eversion of the polar tube, and (4) passage of sporoplasm through the polar tube. The exact mechanism of this process is not well understood. **Conditions that lead to spore germination vary widely among species, presumably reflecting the adaptation of each microsporidian to its host and external environment (Undeen and Epsky 1990; Wittner and Weiss 1999).** Since microsporidia are found in a wide range of terrestrial and aquatic hosts, different species may require unique activation conditions for spore discharge. These specific conditions are also probably important to prevent accidental discharge in the environment (Undeen and Avery 1988; Undeen and Epsky 1990). It has been theorized that, regardless of the mode of activation, microsporidia exhibit the same response to stimuli by increasing the intrasporal osmotic pressure (Lom and Vávra 1963; Ohshima 1937; Undeen and Frixione 1990, 1991). The increase in osmotic pressure results in an influx of water into the spore

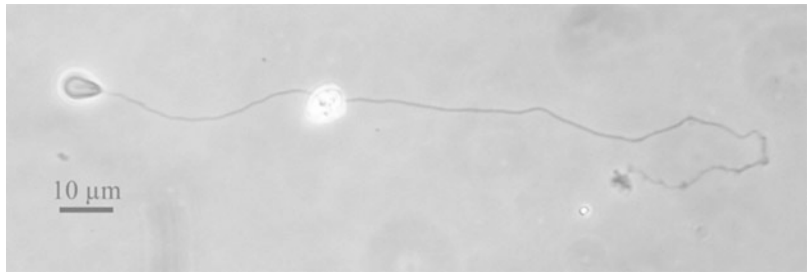

Fig. 5.2 Germinated spore of *Edhazardia aedis* from mosquito *Aedes aegypti*. The emptied spore (*left*) is shown attached to an extruded polar tube. The sporoplasm, or cytoplasmic contents of the spore, is propelled through the everting polar tube and is shown on the *right side* of the image. Under appropriate conditions, the sporoplasm is introduced into a new host cell to initiate infection. Note that the extruded polar filament is approximately 20 times the length of the spore

accompanied by swelling of the polaroplast and posterior vacuole prior to spore discharge (Frixione et al. 1992; Lom and Vávra 1963; Weidner and Byrd 1982). This pressure forces the eversion of the polar tube and expulsion of sporoplasm (Undeen 1990). In hyperosmotic solutions, polar tube discharge is inhibited or slowed down, and sporoplasm passage does not occur, thus providing indirect support for the osmotic pressure theory (Frixione et al. 1992; Lom and Vávra 1963; Ohshima 1937; Weidner 1976; Undeen and Frixione 1990).

The polar tube has flexibility, varies in diameter from 0.1 to 0.25 µm during discharge, can increase to 0.4 µm in diameter during sporoplasm passage, and shortens in length by 5–10 % after sporoplasm passage (Frixione et al. 1992; Lom and Vávra 1963; Ohshima 1937; Weidner 1972). The hollow discharged tubes appear to be two to three times as long as the dense, coiled tube inside the spore, and it has been suggested that the internal contents of the tube are incorporated at its growing tip during discharge (Frixione et al. 1992; Weidner 1972, 1976, 1982). The evagination of the polar filament has been likened to reversing a finger of a glove (Lom and Vávra 1963; Ohshima 1937; Weidner 1972, 1982; Weidner and Byrd 1982; Weidner et al. 1995). The polar tube is essentially a delivery mechanism for transversing the intestinal lumen to deliver the spore contents into intimate association with the host cell. It is not clear whether the polar tube pierces the host cell or invagination and internalization are driven by an interaction of the sporoplasm at the tip of the polar tube with host cell membranes. Although it is accepted that the sporoplasm flows through the discharged polar tube and into the host cell, the mechanisms of activation and tube formation during discharge remain unclear.

Studies have demonstrated that the polar tube has unusual solubility properties and resists dissociation in 1–3 % sodium dodecyl sulfate (SDS), 1 % Triton X-100, 1–10 % H_2O_2, 5-8 N H_2SO_4, 1-2 N HCl, chloroform, 1 % guanidine HCl, 0.1 M proteinase K, 8–10 M urea, 50 mM $NaCO_3$, and 50 mM $MgCl_2$ (Weidner 1972, 1976; Weidner and Byrd 1982). The polar tube, however, dissociates in various concentrations of 2-mercaptoethanol (2-ME) and dithiothreitol (DTT) (Keohane et al. 1994; Weidner 1972, 1976). This has allowed proteomic investigations of the composition of the polar tube (Ghosh et al. 2011). A procedure was developed for the isolation and purification of the major polar tube protein (PTP1) from the spores of microsporidia (Keohane et al. 1994; Keohane and Weiss 1998). Soluble polar tube preparation of *Glugea americanus*, *Enc. hellem*, *Enc. cuniculi*, *Enc. intestinalis*, and *A. algerae* were prepared by sequentially extracting glass-bead-disrupted spores with 1 % SDS and 9 M urea, followed by solubilization of the residual polar tubes in 2 % DTT (Keohane et al. 1994, 1999b; Weiss 2001). PTP1 in the DTT-solubilized material was then purified to homogeneity using reverse phase high-performance liquid chromatography (HPLC) (Keohane and Weiss 1998; Keohane et al. 1996a). By SDS-PAGE and silver staining, this purified fraction migrated at 43 kDa for *G. americanus*, 45 kDa for *Enc. cuniculi* and *Enc. intestinalis*, and 55 kDa for *Enc. hellem* (Keohane et al. 1999a, b). Monoclonal or polyclonal antibodies raised against the purified PTP1 demonstrated reactivity with polar tubes by immunofluorescence (IF) and immunogold electron microscopy (EM) and demonstrated cross reactivity among the species by

immunoblotting and immunogold EM (Keohane and Weiss 1998; Keohane et al. 1994, 1996a, b, c).

All of the **major polar tube proteins (PTP1)** purified to date demonstrate similarities in mass, hydrophobicity, high proline content, and immunologic epitopes. The major polar tube protein, PTP1, from both *Enc. cuniculi* and *Enc. hellem* was identified in 1998 (Delbac et al. 1998a; Keohane et al. 1998). It is somewhat surprising that the translated proteins have only limited identity in amino acid sequences (Weiss 2001). Further comparisons, however, **strikingly reveal that these proteins are proline-rich and have a similar percentage of cysteine (Weiss 2001). PTP1 proteins have central amino acid repeat regions that are predominantly hydrophilic.** However, the repeats differ in composition and number. It is possible that this region is not important for the assembly of the polar tube and may function as an immunologic mask. In the process of evolution a similar duplication of internal sequences has been noted in malaria and other protozoan genes, and this mechanism may be operative in the microsporidia PTP gene (Rich and Ayala 2000). Analysis of PTP1 from other isolates of *Enc. hellem* supports this view, as the number of repeats is variable (Weiss 2001). Post-translational o-linked mannosylation occurs on PTP1, and this modification is probably involved in the ability of PTP1 to interact with the surface of host cells (Xu et al. 2004).

While PTP1 is the major component of the polar tube, other polar-tube-associated proteins (PTPs) are clearly present in the DTT-solubilized polar tube fraction.

For example, several putative PTPs of 23, 27, and 34 kDa have been identified in *G. americanus* using monoclonal antibodies produced to the DTT-solubilized polar tube (Keohane et al. 1994). Using two-dimensional SDS-PAGE one can also demonstrate other PTPs in DTT-solubilized *Enc. hellem* polar tube (Weiss 2001). In addition, several polyclonal and monoclonal antibodies have localized to the polar tube by IFA and immunogold EM and recognized proteins of 34, 75, and 170 kDa in *G. atherinae* and 35, 52/55, and 150 kDa in *Enc. cuniculi* and 60 and 120 kDa in *Enc. intestinalis* (Beckers et al. 1996; Delbac et al. 1998b). This resulted in the identification of PTP2, a 35-kDa protein, in *Enc. cuniculi* (Delbac et al. 2001). *Enc. cuniculi* PTP2 exists as a single copy per haploid genome and is located on the same chromosome as the *Ecptp1* gene, i.e., chromosome VI (Delbac et al. 2001), and has been found as a PTP1–PTP2 gene cluster in several other microsporidia (Delbac et al. 2001). By immunoscreening of a cDNA library of *Enc. cuniculi*, another polar tube protein, PTP3, was found (Peuvel et al. 2002). This protein, predicted to be synthesized as a 1,256-amino-acid precursor (136 kDa) with a cleavable signal peptide, is encoded by a single transcription unit (3,990 bp) located on chromosome XI of *Enc. cuniculi* (Peuvel et al. 2002). PTP3 is solubilized in the presence of SDS alone (Peuvel et al. 2002). Considering that PTP3 is extractable from *Enc. cuniculi* spores in the absence of thiol-reducing agent, lacks cysteine, but is rich in charged residues, it has been suggested that PTP3 interacts with PTP1 or PTP2 via ionic bonds and may play a role in the control of the conformational state of PTP1–PTP2 polymers (Peuvel et al. 2002). For example, when the polar tube exists as a coiled structure inside a spore, interactions with PTP3 may permit the maintenance of PTP1–PTP2 polymers in a condensed form (Peuvel et al. 2002).

It was found that DSP, a chemical cross linker that creates disulfide linkages between proteins, could mediate the purification of a large multimolecular complex from polar tubes that contained PTP1, PTP2, and PTP3 (Peuvel et al. 2002). Studies using yeast two hybrid vectors have confirmed the interaction of PTP1, PTP2, and PTP3 and determined that both the N-terminal and C-terminal regions of PTP1 are involved in these interactions, but that the central repeat region of PTP1 is not involved in these protein–protein interactions (Bouzahzah et al. 2010). It is likely that the regular multilayered organization of the microsporidian polar tube is dependent on specific interactions between its protein components.

V. Life Cycle

Microsporidia generally undergo three phases of development (Cali and Takvorian 1999). The **infective phase** occurs following the release of

spores into the environment or tissues where, under suitable conditions, spores germinate and inject their spore contents through the everting polar filament to infect the host cell. Organisms then continue through a **proliferative phase** within the host cells (often referred to as *merogony*), which is followed by **sporogony, during which organisms commit to maturation and spore formation**. The modes of transmission, intracellular sites of development, number of proliferation cycles, and maturation vary widely among the species of microsporidia. Selected examples of the more common species infecting arthropod, aquatic, mammalian, and avian hosts are described in what follows.

A. Species Infecting Arthropod Hosts

The complete life cycles of many microsporidia in insects are well documented and have shown great diversity from the very simple to the complex, with some involving an intermediate host (Becnel et al. 2005). Microsporidia with **simple life cycles** are generally characterized as having a single sporulation sequence (sometimes with a second sporulation sequence) that occurs in a single host or host group. *Vavraia culicis* in mosquitoes is an example of a species that has only uninucleate stages throughout the life cycle and produces only one spore type (Vavrá and Becnel 2007). *Nosema apis* in honey bees has only binucleate (diplokaryotic) stages throughout the life cycle but is a bit more complex with the production of a primary (early) binucleate spore in the midgut epithelium that serves to spread the infection (autoinfection) to other midgut cells (de Graaf et al. 1994). These infections lead to the production of a second thick-walled (environmental) spore that can be released into the environment to infect a new host (Fries 1993).

Some species are characterized by complex life cycles involving multiple spore types responsible for horizontal and vertical transmission. They often affect two generations of the definitive host and some involve an obligate intermediate host. These microsporidia (often termed polymorphic or heterosporous) are generally very host-specific with complex developmental sequences that can be characterized by specialized stages and high levels of tissue specificity, as well. *Amblyospora californica* (Kellen and Lipa 1960) parasitizes the mosquito *Culex tarsalis* and is representative of a species with a complex life cycle that involves an intermediate host (Becnel 1992). Binucleate spores are formed in oenocytes of adult female *C. tarsalis* following a blood meal. These oenocytes invade the ovaries, where germinations occur, to infect the developing eggs. Developmental sequences in progeny are sex-dependent where females carry benign infection throughout larval development, which leads to the formation of binucleate spores in adults capable of initiating another round of transovarial transmission. Male progeny from infected adults undergo a distinctively different development where the pathogen invades fat bodies with rapid vegetative reproduction that terminates with meiosis and the production of meiospores. These male larvae die, releasing massive numbers of spores into the larval habitat. Meiospores are not infectious to mosquito larvae, but they are horizontally transmitted when ingested by females of the copepod intermediate host *Macrocyclops albidus*. Uninucleate stages replicate in ovaries of the female copepods, which terminates with the production of uninucleate spores and the death of the host. These spores are infectious when ingested by *C. tarsalis* mosquito larvae where the uninucleate stages invade larval oenocytes and remain dormant until pupation and adult emergence. In female adult mosquitoes, binucleate spores that are responsible for transovarial transmission are produced to complete the life cycle. **To date, it has been determined that the involvement of an intermediate host in the life cycles of microsporidia in insects is restricted to mosquitoes and copepod intermediate hosts** and has been documented in a number of species and genera such as *Amblyospora dyxenoides* (Sweeney et al. 1985), *Amblyospora connecticus* (Andreadis 1988), *Parathelohania anophelis* (Avery and Undeen 1990), *Culicospora magna* (Becnel et al. 1987), *Hyalinocysta chapmani* (Andreadis 2002), and *Edhazardia aedis* (Becnel et al. 1989).

B. Species Infecting Aquatic Hosts

Several hundred described species of microsporidia infect fish, but the **life cycles of only a few have been described**. Many of the fish microsporidia that have been investigated to date can **be transmitted directly by ingestion of free spores or spores from infected tissues** (Baxa-Antonio et al. 1992; Kent and Bishop-Stewart 2003; Kent and Speare 2005; McVicar 1975; Sanders et al. 2010; Weissenberg 1968). **Autoinfection** has been suggested or demonstrated for some *Loma* species, in which spores within tissues establish new infections (Matos et al. 2003; Rodriguez-Tovar et al. 2003; Shaw et al. 1998). The potential for **maternal transmission**, either transovum or transovarial, has been reported for *L. salmonae* (Sanchez et al. 2001) and *P. neurophilia* (Kent and Bishop-Stewart 2003). Phelps and Goodwin (2008) provided the most conclusive evidence for vertical transmission, showing the presence of the *Ovipleistophora ovariae* DNA by polymerase chain reaction from within the eggs of infected golden shiners, and similar results were obtained with *P. neurophilia* (Sanders and Kent 2011).

The sequential development from early infection to the site of sporulation is poorly understood for almost all microsporidia of fish. The development of *L. salmonae* has been elucidated and serves as an example for the development of microsporidia in fish (Kent and Speare 2005). The initial site of infection by *L. salmonae* is the mucosal epithelium of the stomach and intestine. Within 4 h of exposure, spores are seen in close association with the stomach epithelium and parasite DNA is present in the cytoplasm of epithelial cells and lamina propria of the small intestine by 12 h post exposure (Sprague and Hussey 1980). Two days post exposure, infected cells with dividing stages of *L. salmonae* can be visualized in the endocardium of the heart by in situ hybridization. After 2–3 weeks, uninucleate or binucleate merogonial stages can be seen developing within the endothelial cells or pillar cells of the blood vessels in the gills, the primary site of sporulation. Meronts are located at the periphery of the host cytoplasm, and the parasite (meront) cell membrane is in close proximity with the surrounding host cell membrane.

The sporogonic stages of *L. salmonae* occur in hypertrophic host cells of gills to generate a xenoparasitic complex or xenoma. **Xenomas are host cells with a radically altered structure in which the microsporidia have integrated into the host cell cytoplasm to undergo massive proliferation while isolated from the body's defense mechanisms (Lom and Dyková 2005).** Other xenoma-forming species, such as members of the genus *Glugea*, can produce very large xenomas (up to 3 mm) in many organs, especially in the subepithelium of the intestine, resulting in grossly visible tumorlike structures that are derived from a single hypertrophic host cell. Another type of xenoma develops with infection by *Ichthyosporidium giganteum*, which forms a large syncytium from the coalescence of several host cells, resulting in a large, lobular cyst (Rodriguez-Tovar et al. 2003). **In contrast to xenoma-forming species, skeletal-muscle-infecting microsporidia of the genus *Pleistophora* develop within host cells, replacing the sarcoplasm and destroying infected cells without inducing the hypertrophy characteristic of xenomas.**

Early during the first 2–3 weeks of xenoma formation, meronts of *L. salmonae* occupy the periphery of the host cell, and by 5 weeks this area begins to be occupied by mature spores (Lom and Dyková 2005). Eventually, spores can be seen throughout the xenoma that can reach a diameter of up to 0.4 mm. With other genera, sporogony occurs asynchronously throughout the xenoma (Morrison and Sprague 1983). A parasite-derived sporophorous vesicle forms prior to sporogonial division, and sporogony proceeds by binary fission, resulting in two uninucleate sporoblasts per vacuole (Chilmonczyk et al. 1991). The formation of this vacuole is absent in the genus *Spraguea*, which instead develops in direct contact with the host cytoplasm. The intranuclear microsporidia *N. salmonis* (Rodriguez-Tovar et al. 2003) and *P. theridion* (Nylund et al. 2010) develop in direct contact with the host cell nucleoplasm. Eventually, sporoblasts develop into mature spores. **The intact xenoma is surrounded by numerous inflammatory cells but**

seems to elicit little, if any, response by those cells (Rodriguez-Tovar et al. 2003). Eventual rupture of the xenoma results in the release of mature spores, elicitation of a severe proliferative inflammatory reaction, and uptake of spores by infiltrating phagocytes.

C. Species Infecting Mammalian and Avian Hosts

The most common modes of transmission of *Encephalitozoon* species in mammals, and presumably birds, are by **ingestion and inhalation of spores shed from urine, feces, or other fluids.** Transmission may occur through **direct contact (e.g., trauma), and vector-borne, sexual, and horizontal routes** have also been reported to occur in mammals. The life cycle of *Encephalitozoon* is relatively simple in comparison to that of other microsporidia. After germination and introduction of the spore cytoplasmic contents into the host cell, *Encephalitozoon* species undergo multiple cycles of binary division within a PV, the membrane of which seems to be host-cell-derived (Rönnebäumer et al. 2008). The proliferative stages or meronts tend to be larger than the mature spore and appear to adhere to the inner PV membrane. In some cases, karyokinesis occurs slightly faster than cytokinesis such that ribbons of dividing multinucleated meronts can be observed. Sporogony or spore maturation occurs as the parasite plasma membrane thickens and differentiates to form the exospore and endospore layers. These stages separate from the PV membrane and may continue to undergo a limited number of cell divisions. During this phase, the polar filament develops and the organisms become smaller and more electron-dense. Eventually, the PV becomes full of organisms leading to host cell and PV rupture and release of organisms. Among the sites of infection in mammals and birds are kidney, small intestine, and liver, so spores are commonly shed with urine and feces.

Ent. bieneusi infections typically occur in cells lining the small intestine in which organisms replicate in direct contact with the host cell cytoplasm (Cali and Owen 1990). Merogony is characterized by nuclear division without cytokinesis to generate a multinucleated plasmodium. During sporogony, electron-dense disks are observed to stack and eventually fuse to form the polar filaments in association with each nucleus. The individual nuclei become more defined, and the plasmalemma of the plasmodium begins to thicken and invaginate to surround the individual nucleus and polar filament units. Maturation continues with the thickening and differentiation of the spore wall, release of spores into the intestinal lumen, and shedding with feces.

VI. Systematics and Evolution

Microsporidia possess prokaryote-sized 70S ribosomes and lack typical mitochondria and Golgi. Early molecular biology studies also demonstrated fusion of the 5.8S and large subunit rRNAs similar to that in prokaryotes. These observations and the initial phylogenetic analyses of microsporidian small subunit rRNA genes supported divergence of the microsporidia prior to the symbiotic origin of mitochondria and placed microsporidia at the earliest and deepest branch of the eukaryotic tree (Vossbrinck et al. 1987). Evidence began to mount, however, that microsporidia are more highly evolved. Nuclear-encoded genes that target mitochondrial proteins (e.g., mHSP70, alpha and beta subunits of pyruvate dehyrdrogenase E1) were discovered and antibodies to mHSP70 identified membrane-bound organelles called mitosomes that function as mitochondrial remnants for iron–sulfur cluster assembly (Fast and Keeling 2001; Germot et al. 1997; Hirt et al. 1997; Williams and Keeling 2005; Williams et al. 2002). Genome-sequence studies of several microsporidia species (e.g., *Enc. cuniculi*, *Enc. intestinalis*, *Ent. bieneusi*, *P. locustae*, *A. algerae*) and improved phylogenetic analyses on additional genes have shed further light in demonstrating a close relationship between the microsporidia and the fungi (Akiyoshi et al. 2009; Burri et al. 2006; Cornman et al. 2009; Corradi and Slamovits 2011; Corradi et al. 2007; Katinka et al. 2001; Keeling et al.

2010; Williams et al. 2008). Microsporidia are highly efficient parasites and have undergone significant gene reduction and compaction (Keeling 2009; Keeling et al. 2010). In addition, microsporidia seem to have **evolved relatively quickly and exhibit a high degree of gene sequence divergence**, so phylogenetic analyses to address their evolution are problematic. However, a strong conservation of gene order, or synteny, among several gene clusters, including the *sex* locus, was reported among distantly related microsporidia and the zygomycetes (zygomycete mating type, *MAT*) (Dyer 2008; Lee et al. 2008, 2010b; Corradi and Keeling 2009; Corradi and Slamovits 2011). These findings were used to support a deep-branching fungal origin of the microsporidia from a zygomycete ancestor and suggest that microsporidia may have a genetically controlled sexual cycle. Concerns have been raised, however, about whether this shared syntenic relationship truly supports a microsporidial–fungal relationship because the gene cluster of the microsporidia that resemble the zygomycete sex-related loci traces back to an ancient gene cluster in the common ancestor of plants, animals, and fungi (Koestler and Ebersberger 2011).

VII. Classification

Newer molecular biology analyses and approaches being applied to better understanding evolution of the microsporidia also have impacted their taxonomy and classification. The microsporidia are now fairly well accepted for classification with Kingdom Fungi (Corradi and Keeling 2009; Hibbett et al. 2007; James et al. 2006), but some analyses question this association (Koestler and Ebersberger 2011), suggesting that further studies are required. Classification of fungi is based on the International Code of Botanical Nomenclature, but microsporidia had been described using the International Code of Zoological Nomenclature. To avoid nullification, a formal request to accept the current nomenclature of the microsporidia was presented at the last taxonomy meeting at the International Botanical Congress and was approved (Redhead et al. 2009). As a result, **microsporidia are considered fungi but remain subject to the rules of the International Code for Zoological Nomenclature.** A broad-based consensus classification (Hibbett et al. 2007) did not subdivide the microsporidia within the fungi due to a lack of well-sampled multilocus analyses at that time. More recently, gene order (i.e., synteny) between several unrelated microsporidia and the zygomycetes was highly conserved (Corradi and Keeling 2009; Dyer 2008; Lee et al. 2008), but again, others suggest that synteny was not more similar between microsporidia and the zygomycetes than with any other fungal taxon (Koestler and Ebersberger 2011). The phylum name Zygomycota is considered invalid because the interrelationships among the major clades are still unresolved, and it was named without a Latin description, so further classification of the basal fungi to relate microsporidia to the zygomycetes or another fungal group is still in progress (Hibbett et al. 2007). Based on the complexity of microsporidian evolution, it is also possible that the microsporidia might represent a sister group to the fungi. As newer analytical tools incorporate additional genomic and proteomic information, a better picture will emerge regarding the classification of the microsporidia.

Primary classification of organisms into the phylum Microsporidia was based on the presence of the polar tube. Further classification was based on morphological and ultrastructural features, as well as host and habitat (Larsson 1986; Sprague et al. 1992). More specific characteristics used to classify the microsporidia include host cell, spore size, nucleus configuration (i.e., monokaryon, diplokaryon), number and configuration of the polar filament coils, type of nuclear and cellular division (e.g., binary division, plasmotomy), interface with the host cell (e.g., replication within a PV, direct contact with host cell cytoplasm), and whether a sporophorous vesicle is formed. Microsporidia initially fell into two groups based on the presence or absence of a sporoblast vesicle (Pansporoblastina and Apansporoblastina, respectively) and then were divided into groups based on

nuclear configuration as single (Haplophasea) or double (Dihaplophasea) nuclear arrangement, the latter being grouped on the basis of diplokaryon formation through meiosis or nuclear dissociation.

With the advancement of molecular biology technology, classifications within the phylum incorporated phylogenetic analyses (Vossbrinck and Debrunner-Vossbrinck 2005), and genera accepted to date are found in Sect. II.A. A comparative molecular phylogenetic analysis using ssrDNA sequences of 125 species in relation to host and habitat led to a proposal for grouping microsporidia into five clades among three new classes: the Aquasporidia (clades I, II, and V), the Marinosporidia (clade III), and the Terresporidia (clade IV). This new classification, however, is considered to be under development due to the relatively small representation for analyzing only 125 of over 1,200 species of microsporidia, the as yet undescribed microsporidia that are likely to be found, and a need to account for features related to morphology, life cycle, and host–parasite relationship (Larsson 2005; Vossbrinck and Debrunner-Vossbrinck 2005).

VIII. Maintenance and Culture

A. Species Infecting Arthropod Hosts

Brooks (1988) presents an excellent review of spore storage and maintenance of microsporidia infecting arthropods, but the optimal storage conditions must be determined experimentally for each isolate. **There are no standard guidelines on the best practices to preserve spore viability.** There is general agreement, however, that many microsporidian spores from terrestrial hosts will tolerate freezing or desiccation, whereas spores from aquatic hosts do not but in some cases can be maintained long term under other conditions. Spores to be stored are most commonly handled as **intact infected cadavers or as purified suspensions.**

Many species of microsporidia can be maintained for extended periods (months to years) as highly purified spores held in deionized water at 5 °C (±3). Antibiotics and fungicides are routinely added to the suspensions to retard microbial growth, which can reduce spore viability. Highly purified spores of *A. algerae* cannot survive freezing but have maintained viability after being held at 5 °C (±3) for more than 10 years. Many terrestrial species of microsporidia can be frozen (−30 to −20 °C) as cadavers, or purified spores can be placed into liquid nitrogen for long-term storage. The addition of 50 % glycerol to the pure spore suspensions as a cryoprotectant is often beneficial. Some spores can also be stored in the dried host cadaver for extended periods, such as *Nosema whitei*, a pathogen of flour beetles. Spores that can be dried can often be successfully lyophilized. If information on storage parameters for a species is not available, it is suggested that highly purified spores be held in deionized water at 5 °C (±3).

In vitro culture of microsporidia in insects has a long history and began with the successful infection of a *B. mori* cell line with *N. bombycis* Trager 1937, but few additional species have been established in cell culture. Until the mid to late 1980s, only about eight species of microsporidia from insects had been cultured in insect cell systems that included *A. algerae*, *N. apis*, *N. bombycis*, *N. disstriae*, *N. heliothidis*, *N. mesnili*, *Vairimorpha necatrix*, and *Vavraia culicis* (Brooks 1988; Jaronski 1984). More recently, a few additional species have been cultured, including *Cystosporogenes operophterae*, *N. furnacalis*, and a *Vairimorpha* sp. (Becnel and Andreadis 1999). The species with the broadest host range and ability to grow in both invertebrate and vertebrate cell lines is *A. algerae*. It has been grown in many insect cell lines (Brooks 1988) and in pig kidney cells (Undeen 1975), rabbit kidney cells (Lowman et al. 2000), several warm-water fish cell lines (Monaghan et al. 2011), and human muscle fibroblasts (Trammer et al. 1999). *A. algerae* has also been grown at elevated temperatures (37 °C), which is unique for insect microsporidia (Lowman et al. 2000).

B. Species Infecting Aquatic Hosts

Few microsporidia that infect aquatic organisms have been successfully propagated in long-term cell culture, and these generally depend upon maintaining groups of infected hosts or obtaining infected hosts from the wild. **The presence of microsporidia in non-mammalian model organisms, such as the zebrafish and the nematode *Caenorhabditis elegans*, provides researchers the opportunity to study these parasites in well-described systems with numerous genetic tools (Troemel**

2011) to elucidate host responses to microsporidia infections. The in vitro **propagation of microsporidia infecting fish has proven difficult**, and the use of fish cell cultures in the long-term maintenance of fish microsporidia was recently reviewed (Monaghan et al. 2009).

For example, the intranuclear microsporidium *N. salmonis* has been successfully maintained in a long-term primary culture of salmonid mononuclear leukocytes grown in supplemented Iscove's modified Dulbecco's medium by adding small numbers of infected leukocytes to uninfected leukocytes (Wongtavatchai et al. 1994). Infected cultures can be preserved long term by freezing in liquid nitrogen with cryoprotectant.

A **continuous cell line, EP-1**, derived from the Japanese eel, *Anguilla japonica*, is persistently infected with *Heterosporis anguillarum*. Whereas this cell line was passaged over 223 times in vitro for maintaining intracellular merogonic stages of the parasite, no spore stages were observed to develop, yet eels inoculated with cells from this culture system became infected and exhibited intramuscular cysts consistent with *H. anguillarum* infection (Kou et al. 1995). To date, this **remains the only cell line developed to be persistently infected with a microsporidian parasite of fish.**

Four fish cell lines—channel catfish ovary, zebrafish caudal fin fibroblast, carp epithelioma, and fathead minnow—have been shown to support limited growth of the microsporidian parasite of zebrafish, *P. neurophilia*. Whereas sporogony occurs in all cell lines, development to the spore stages is limited, and the parasites could not be passaged into new cultures (Watral et al. 2006). Similarly, spores of *Glugea* spp. were internalized by Chinook salmon embryo cells and, while meronts were detected, development ceased by 48 h and no sporogony was observed (Lores et al. 2003). The same parasite did develop in a mosquito cell line (ECACC 90100401), producing spores within 72 h post inoculation, illustrating the potential for insect cell lines in the propagation of fish microsporidia in vitro.

C. Species Infecting Mammalian and Avian Hosts

Enc. cuniculi was the first mammalian microsporidian to be isolated from a rabbit and grown in long-term tissue culture (Shadduck 1969). Since then, *Enc. hellem*, *Enc. intestinalis*, *A. algerae*, *V. corneae*, and *Trachipleistophora hominis* isolates from humans have been grown in culture, but unfortunately long-term culture of *Ent. bieneusi* still has not been accomplished (Braunfuchsová et al. 1999; Didier et al. 1991, 1996; Juarez et al. 2005; Lafranchi-Tristem et al. 2001; Monaghan et al. 2009; Trammer et al. 1999; Visvesvara 2002).

Cultures are typically initiated via coculture of source specimen (tissue biopsy or fluids such as urine, feces, or sputum) and host cells such as Vero, RK-13, MDCK, and other epithelial cells. Examples of tissue culture media that support the growth of the host cells and facilitate propagation of the microsporidia include RPMI 1604 or D-MEM supplemented with 2 mM L-glutamine, 5–10 % fetal bovine serum, and antibiotics (e.g., penicillin, streptomycin, and amphotericin B). The medium is typically changed twice a week and the supernatants can be collected in sterile bottles for short-term storage at 4 °C.

Encephalitozoon-infected cells appear to contain vacuoles filled with organisms. *V. corneae* replicates in the cytoplasm of the host cell, and infected cells may appear larger and multinucleated when filled with organisms. Individual microsporidia suspended in the supernatants after release from ruptured host cells can be observed approximately 2–4 weeks after initiation of coculture, but sometimes longer periods of time are required if the initial inoculum dose of organisms is low. In the case of *V. corneae*, large aggregates of parasite-laden host cells are often also observed in the culture supernatants and can be separated by vortexing or washing the collected culture supernatants. Host cells tend to replicate and replace the ruptured infected cells, but if overgrowth of microsporidia occurs, fresh host cells can be added to the culture flasks.

To enrich microsporidia from host cell debris, the sedimented culture supernatants can be washed sequentially in distilled water, tris-buffered saline (TBS)\ containing 0.3 % Tween 20 (TBS-TW), and TBS (400× *g* for 15 min). The pellets can be further enriched by centrifugation through 50 % Percoll (i.e., mixing equal volumes of spores in TBS and 100 % Percoll) at 400 × *g* for 30–45 min. Extraneous host cell debris remains in the top layers, and the spores centrifuge to the pellet (Didier et al. 1996). Microsporidia to be used for extracting DNA or RNA require additional washing with mild ionic detergent (e.g., 0.5–1 % sodium dodecyl sulfate) to remove host cell DNA that can adhere to the spore surface (Corradi et al. 2010).

Cryopreservation of mammalian microsporidia can be accomplished most efficiently by "scraping" or trypsinizing infected host cells, centrifuging at 400 × *g* (15 min at 4 °C), and resuspending in fetal bovine serum (FBS) containing 10 % dimethyl sulfoxide (DMSO). The vials are then frozen slowly (1 °C per minute) using commercially available cryopreservation containers, followed by final storage of vials in liquid nitrogen. To reestablish culture from cryopreserved spores, flasks of host cells at approximately 50 % confluence should be prepared. The frozen vial of microsporidia should be thawed quickly and added directly to the host cells; a few hours later, after the microsporidia have had an opportunity to infect the host cells, the culture medium should be changed to remove the DMSO. Alternatively, the inoculum of cryopreserved spores can be washed (i.e., centrifuged) and the pellet resuspended in a small volume of medium to remove the DMSO prior to inoculation of the culture flasks. If host cells other than those used to generate the cyropreserved spores are used, the spore inoculum will need to be washed with mild detergent (e.g., 0.5 % SDS) to prevent growth of cryopreserved host cells in the new culture.

IX. Conclusions

The microsporidia comprise a fascinating group of organisms that infect their hosts through an unusual spore germination process of polar filament extrusion and direct inoculation of the sporoplasm into the cell. They are an extremely successful group of organisms that are widespread in both vertebrate and invertebrate hosts and are highly efficient parasites, as noted by their gene compaction and reduction. Over the past 10 years, molecular studies have reshaped our understanding of phylogeny and led to the classification of microsporidia as fungi, although knowledge of the exact relationship is still in flux (Hibbett et al. 2007; Koestler and Ebersberger 2011). Whereas previously the microsporidia had been recognized pathogens of agriculturally and commercially relevant insects, fish, companion pets, domestic animals, and food-producing animals, it was only recently, during the AIDS pandemic, that these organisms came to be seen as common causes of opportunistic and emerging infections in humans. The tremendous increase in the recognition of new species of microsporidia in such a wide host range and the application of newer molecular tools will now need to be applied to improving diagnostics, developing intervention and chemotherapeutic strategies, and learning more about the basic biology and phylogeny of the microsporidia.

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References

Akiyoshi DE, Morrison HG, Lei S, Feng X, Zhang Q, Corradi N, Mayanja H, Tumwine JK, Keeling PJ, Weiss LM, Tzipori S (2009) Genomic survey of the non-cultivatable opportunistic human pathogen, *Enterocytozoon bieneusi*. PLoS Pathog 5:e1000261

Andreadis TG (1984) Epizootiology of *Nosema pyrausta* in field populations of the European corn borer. Environ Entomol 13:882–887

Andreadis TG (1988) Comparative susceptibility of the copepod *Acanthocyclops vernalis* to a microsporidian parasite, *Amblyospora connecticus*, from the mosquito *Aedes cantator*. J Invertebr Pathol 52:73–77

Andreadis TG (2002) Epizootiology of *Hyalinocysta chapmani* (Microsporidia: Thelohaniidae) infections in field populations of *Culiseta melanura* (Diptera: Culicidae) and *Orthocyclops modestus* (Copepoda: Cyclopidae): a three-year investigation. J Invertebr Pathol 81:114–121

Avery SW, Undeen AH (1990) Horizontal transmission of *Parathelohania anophelis* to the copepod, *Microcyclops varicans*, and the mosquito, *Anopheles quadrimaculatus*. J Invertebr Pathol 56:98–105

Baxa-Antonio D, Groff JM, Hedrick RP (1992) Experimental horizontal transmission of *Enterocytozoon salmonis* to chinook salmon, *Oncorhynchus tshawytscha*. J Protozool 39:699–702

Beckers PJ, Derks GJ, van Gool T, Rietveld FJ, Sauerwein RW (1996) *Encephalocytozoon intestinalis*-specific monoclonal antibodies for laboratory diagnosis of microsporidiosis. J Clin Microbiol 34:282–285

Becnel JJ (1992) Horizontal transmission and subsequent development of *Amblyospora californica* (Microsporida: Amblyosporiae) in the intermediate and definitive hosts. Dis Aquat Org 13:17–28

Becnel JJ, Andreadis TG (1999) Microsporidia in insects. In: Wittner M, Weiss LM (eds) The microsporidia and microsporidiosis. American Society for Microbiology Press, Washington, DC, pp 447–501

Becnel JJ, Hazard EI, Fukuda T, Sprague V (1987) Life cycle of *Culicospora magna* (Kudo, 1920) (Microsporida: Culicosporidae) in *Culex restuans* Theobald with special reference to sexuality. J Eukaryot Microbiol 34:313–322

Becnel JJ, Sprague V, Fukuda T, Hazard EI (1989) Development of *Edhazardia aedis* (Kudo, 1930) n. g., n. comb. (Microsporida: Amblyosporidae) in the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). J Protozool 36:119–130

Becnel JJ, Jeyaprakash A, Hoy MA, Shapiro A (2002) Morphological and molecular characterization of a new microsporidian species from the predatory mite *Metaseiulus occidentalis* (Nesbitt) (Acari, Phytoseiidae). J Invertebr Pathol 79:163–172

Becnel JJ, White SE, Shapiro AM (2005) Review of microsporidia-mosquito relationships: from the simple to the complex. Folia Parasitol (Praha) 52:41–50

Bigliardi E, Selmi MG, Lupetti P, Corona S, Gatti S, Scaglia M, Sacchi L (1996) Microsporidian spore wall: ultrastructural findings on *Encephalitozoon hellem* exospore. J Eukaryot Microbiol 43:181–186

Bohne W, Ferguson DJ, 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

Bouzahzah B, Nagajyothi F, Ghosh K, Takvorian PM, Cali A, Tanowitz HB, Weiss LM (2010) Interactions of *Encephalitozoon cuniculi* polar tube proteins. Infect Immun 78:2745–2753

Braunfuchsová P, Kopecký J, Ditrich O, Koudela B (1999) Cytokine response to infection with the microsporidian, *Encephalitozoon cuniculi*. Folia Parasitol (Praha) 46:91–95

Brooks WM (1988) Entomogenous Protozoa. In: Ignoffo CM (ed) Handbook of natural pesticides, Vol. V, microbial insecticides Part A, entomogenous protozoa and fungi. CRC, Boca Raton, FL, pp 1–149

Burri L, Williams BA, Bursac D, Lithgow T, Keeling PJ (2006) Microsporidian mitosomes retain elements of the general mitochondrial targeting system. Proc Natl Acad Sci USA 103:15916–15920

Cali A, Owen RL (1988) Microsporidiosis. In: Balows WJ, Hausler WJ, Ohashi M, Turano A (eds) Laboratory diagnosis of infectious diseases: principles and practice. Springer, New York, pp 929–946

Cali A, Owen RL (1990) Intracellular development of *Enterocytozoon*, a unique microsporidian found in the intestine of AIDS patients. J Protozool 37:145–155

Cali A, Takvorian P (1999) Developmental morphology and life cycles of the microsporidia. In: Wittner M, Weiss LM (eds) The microsporidia and microsporidiosis. American Society for Microbiology, Washington, DC, pp 85–128

Cali A, Kotler DP, Orenstein JM (1993) *Septata intestinalis* N. G., N. Sp., an intestinal microsporidian associated with chronic diarrhea and dissemination in AIDS patients. J Eukaryot Microbiol 40:101–112

Cali A, Weiss LM, Takvorian PM (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

Canning EU, Lom J, Dyková I (1986) The microsporidia of vertebrates. Academic, London

Chen Y, Evans JD, Smith IB, Pettis JS (2008) *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. J Invertebr Pathol 97:186–188

Chilmonczyk S, Cox WT, Hedrick RP (1991) *Enterocytozoon salmonis* n. sp.: an intranuclear microsporidium from salmonid fish. J Protozool 38:264–269

Chioralia G, Trammer T, Maier WA, Seitz HM (1998) Morphologic changes in Nosema algerae (Microspora) during extrusion. Parasitol Res 84:123–131

Cornman RS, Chen YP, Schatz MC, Street C, Zhao Y, Desany B, Egholm M, Hutchison S, Pettis JS, Lipkin WI, Evans JD (2009) Genomic analyses of the microsporidian *Nosema ceranae*, an emergent pathogen of honey bees. PLoS Pathog 5:e1000466

Corradi N, Keeling PJ (2009) Microsporidia: a journey through radical taxonomical revisions. Fungal Biol Rev 23:1–8

Corradi N, Slamovits CH (2011) The intriguing nature of microsporidian genomes. Brief Funct Genomics 10:115–124

Corradi N, Akiyoshi DE, Morrison HG, Feng X, Weiss LM, Tzipori S, Keeling PJ (2007) Patterns of genome evolution among the microsporidian parasites *Encephalitozoon cuniculi*, *Antonospora*

locustae and *Enterocytozoon bieneusi*. PLoS One 2: e1277
Corradi N, Pombert JF, Farinelli L, Didier ES, Keeling PJ (2010) The complete sequence of the smallest known nuclear genome from the microsporidian *Encephalitozoon intestinalis*. Nat Commun 1:77. doi:10.1038/ncomms1082
de Graaf DC, Raes H, Jacobs FJ (1994) Spore dimorphism in *Nosema apis* (Microsporida, Nosematidae) developmental cycle. J Invertebr Pathol 63:92–94
de Kinkelin P (1980) Occurrence of a microsporidian infection in zebra danio *Brachydanio rerio* (Hamilton-Buchanan). J Fish Dis 3:71–73
Delbac F, Duffieux F, David D, Méténier G, Vivarès CP (1998a) Immunocytochemical identification of spore proteins in two microsporidia, with emphasis on extrusion apparatus. J Eukaryot Microbiol 45:224–231
Delbac F, Peyret P, Méténier G, David D, Danchin A, Vivarès CP (1998b) 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, Méténier G, Peyretaillade E, Vivarès CP (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, 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
Didier ES, Weiss LM (2006) Microsporidiosis: current status. Curr Opin Infect Dis 19:485–492. doi:10.1097/01.qco.0000244055.46382.23
Didier ES, Didier PJ, Friedberg DN, Stenson SM, Orenstein JM, Yee RW, Tio FO, Davis RM, Vossbrinck C, Millichamp N et al (1991) Isolation and characterization of a new human microsporidian, *Encephalitozoon hellem* (n. sp.), from three AIDS patients with keratoconjunctivitis. J Infect Dis 163:617–621
Didier ES, Rogers LB, Orenstein JM, Baker MD, Vossbrinck CR, Van Gool T, Hartskeerl R, Soave R, Beaudet LM (1996) Characterization of *Encephalitozoon (Septata) intestinalis* isolates cultured from nasal mucosa and bronchoalveolar lavage fluids of two AIDS patients. J Eukaryot Microbiol 43:34–43
Didier ES, Snowden KF, Shadduck JA (1998) Biology of microsporidian species infecting mammals. Adv Parasitol 40:283–320
Dyer PS (2008) Evolutionary biology: microsporidia sex–a missing link to fungi. Curr Biol 18:R1012–R1014
Erickson BW Jr, Blanquet RS (1969) The occurrence of chitin in the spore wall of *Glugea weissenbergi*. J Invertebr Pathol 14:358–364
Fast NM, Keeling PJ (2001) Alpha and beta subunits of pyruvate dehydrogenase E1 from the microsporidian *Nosema locustae*: mitochondrion-derived carbon metabolism in microsporidia. Mol Biochem Parasitol 117:201–209
Fries I (1993) *Nosema apis*—a parasite in the honey bee colony. Bee World 74:5–19
Frixione E, Ruiz L, Santillán M, de Vargas LV, Tejero JM, Undeen AH (1992) Dynamics of polar filament discharge and sporoplasm expulsion by microsporidian spores. Cell Motil Cytoskeleton 22:38–50
Frixione E, Ruiz L, Cerbon J, Undeen AH (1997) Germination of *Nosema algerae* (Microspora) spores: conditional inhibition by D2O, ethanol and Hg2+ suggests dependence of water influx upon membrane hydration and specific transmembrane pathways. J Eukaryot Microbiol 44:109–116
Geden CJ, Long SJ, Rutz DA, Becnel JJ (1995) Nosema disease of the parasitoid *Muscidifurax raptor* (Hymenoptera: Pteromalidae): prevalence, patterns of transmission, management, and impact. Biol Control 5:607–614
Germot A, Philippe H, Le Guyader H (1997) Evidence for loss of mitochondria in Microsporidia from a mitochondrial-type HSP70 in *Nosema locustae*. Mol Biochem Parasitol 87:159–168
Ghosh K, Nieves E, Keeling P, Pombert JF, Henrich PP, Cali A, Weiss LM (2011) Branching network of proteinaceous filaments within the parasitophorous vacuole of *Encephalitozoon cuniculi* and *Encephalitozoon hellem*. Infect Immun 79:1374–1385
Graczyk TK, Majewska AC, Schwab KJ (2008) The role of birds in dissemination of human waterborne enteropathogens. Trends Parasitol 24:55–59
Haro M, Izquierdo F, Henriques-Gil N, Andrés I, Alonso F, Fenoy S, del Aguila C (2005) First detection and genotyping of human-associated microsporidia in pigeons from urban parks. Appl Environ Microbiol 71:3153–3157
Hayman JR, Hayes SF, Amon J, Nash TE (2001) Developmental expression of two spore wall proteins during maturation of the microsporidian *Encephalitozoon intestinalis*. Infect Immun 69:7057–7066
Hazard EI, Weiser J (1968) Spores of *Thelohania* in adult female anopheles: development and transovarial transmission, and redescriptions of *T. legeri* Hesse and *T. obesa* Kudo. J Protozool 15:817–823
Henry JE, Oma EA (1981) Pest control by *Nosema locustae*, a pathogen of grasshoppers and crickets. In: Burges HD (ed) Microbial control of pests and plant diseases. Academic, London, pp 573–586
Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lucking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman

CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547

Hirt RP, Healy B, Vossbrinck CR, Canning EU, Embley TM (1997) A mitochondrial Hsp70 orthologue in *Vairimorpha necatrix*: molecular evidence that microsporidia once contained mitochondria. Curr Biol 7:995–998

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822

Jaronski ST (1984) Microsporidia in cell culture. Adv Cell Culture 18:183–229

Juarez SI, 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

Katinka MD, Duprat S, Cornillot E, Méténier 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, Vivarès CP (2001) Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. Nature 414:450–453

Keeling P (2009) Five questions about microsporidia. PLoS Pathog 5:e1000489

Keeling PJ, Corradi N, Morrison HG, Haag KL, Ebert D, Weiss LM, Akiyoshi DE, 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 WR, Lipa JJ (1960) *Thelohania californica* n.sp., a microsporidian parasite of *Culex tarsalis* Coquillet. J Invertebr Pathol 2:1–12

Kent ML, Bishop-Stewart JK (2003) Transmission and tissue distribution of *Pseudoloma neurophilia* (Microsporidia) of zebrafish, *Danio rerio* (Hamilton). J Fish Dis 26:423–426

Kent ML, Poppe TT (1998) Diseases of seawater netpen-reared salmonid fishes. Pacific Biological Station, Department of Fisheries and Oceans, Nanimo, BC

Kent ML, Speare DJ (2005) Review of the sequential development of *Loma salmonae* (Microsporidia) based on experimental infections of rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (O. tshawytscha). Folia Parasitol (Praha) 52:63–68

Kent ML, Buchner C, Watral VG, Sander JL, LaDu J, Peterson TS, Tanguay RL (2011) Development and maintenance of a specific pathogen-free (SPF) zebrafish research facility for *Pseudoloma neurophilia*. Dis Aquat Org 95:73–79

Keohane EM, Weiss LM (1998) Characterization and function of the microsporidian polar tube: a review. Folia Parasitol (Praha) 45:117–127

Keohane E, Takvorian PM, Cali A, Tanowitz HB, Wittner M, Weiss LM (1994) The identification and characterization of a polar tube reactive monoclonal antibody. J Eukaryot Microbiol 41:48S

Keohane EM, Orr GA, Takvorian PM, Cali A, Tanowitz HB, Wittner M, Weiss LM (1996a) Purification and characterization of a microsporidian polar tube protein. Mol Biochem Parasitol 79:255–2559

Keohane EM, Orr GA, Takvorian PM, Cali A, Tanowitz HB, Wittner M, Weiss LM (1996b) Purification and characterization of human microsporidian polar tube proteins. J Eukaryot Microbiol 43:100S

Keohane EM, Takvorian PM, Cali A, Tanowitz HB, Wittner M, Weiss LM (1996c) Identification of a microsporidian polar tube protein reactive monoclonal antibody. J Eukaryot Microbiol 43:26–31

Keohane EM, Orr GA, Zhang HS, Takvorian PM, Cali A, Tanowitz HB, Wittner M, Weiss LM (1998) The molecular characterization of the major polar tube protein from *Encephalitozoon hellem*, a microsporidian parasite of humans. Mol Biochem Parasitol 94:227–2236

Keohane EM, Orr GA, Takvorian PM, Cali A, Tanowitz HB, Wittner M, Weiss LM (1999a) Analysis of the major microsporidian polar tube proteins. J Eukaryot Microbiol 46:29S–30S

Keohane EM, Orr GA, Takvorian PM, Cali A, Tanowitz HB, Wittner M, Weiss LM (1999b) Polar tube proteins of microsporidia of the family encephalitozoonidae. J Eukaryot Microbiol 46:1–5

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

Kotler DP, Orenstein JM (1998) Clinical syndromes associated with microsporidiosis. Adv Parasitol 40:321–349

Kou G-H, Wang C-H, Hung H-W, Jang Y-S, Chou C-M, Lo C-F (1995) A cell line (EP-1 cell line) derived from "Beko disease" affected Japanese eel elver

(*Anguilla japonica*) persistently infected with *Pleistophora anguillarum*. Aquaculture 132:161–173

Lafranchi-Tristem NJ, Curry A, Cheney SA, Canning EU (2001) Growth of Trachipleistophora hominis (Microsporidia: Pleistophoridae) in C2, C12 mouse myoblast cells and response to treatment with albendazole. Folia Parasitol (Praha) 48:192–200

Larsson R (1986) Ultrastructure, function, and classification of microsporidia. Prog Protistol 1:325–390

Larsson JI (2005) Molecular versus morphological approach to microsporidian classification. Folia Parasitol (Praha) 52:143–144

Lee SC, Corradi N, Byrnes EJ 3rd, Torres-Martinez S, Dietrich FS, Keeling PJ, Heitman J (2008) Microsporidia evolved from ancestral sexual fungi. Curr Biol 18:1675–1679

Lee RC, Gill EE, Roy SW, Fast NM (2010a) Constrained intron structures in a microsporidian. Mol Biol Evol 27:1979–1982

Lee SC, Corradi N, Doan S, Dietrich FS, Keeling PJ, Heitman J (2010b) Evolution of the sex-related locus and genomic features shared in microsporidia and fungi. PLoS One 5:e10539

Lom J (1972) On the structure of the extruded microsporidian polar filament. Parasitol Res 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, Dyková I (1992) Protozoan parasites of fishes. Elsevier, Amsterdam

Lom J, Dyková I (2005) Microsporidian xenomas in fish seen in wider perspective. Folia Parasitol (Praha) 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

Lores B, Rosales MJ, Mascaro C, Osuna A (2003) In vitro culture of Glugea sp. Vet Parasitol 112:185–196

Lowman PM, Takvorian PM, Cali A (2000) The effects of elevated temperatures and various time-temperature combinations on the development of *Brachiola (Nosema) algerae* N. Comb in mammalian cell culture. J Eukaryot Microbiol 47:221–234

Matos E, Corral L, Azevedo C (2003) Ultrastructural details of the xenoma of *Loma myrophis* (phylum Microsporidia) and extrusion of the polar tube during autoinfection. Dis Aquat Org 54:203–207

Matthews JL, Brown AM, Larison K, Bishop-Stewart JK, Rogers P, Kent ML (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

McVicar AH (1975) Infection of plaice *Pleuronectes platessa* L. with *Glugea (Nosema) stephani* (Hagenmüller 1899) (Protozoa: Microsporidia) in a fish farm and under experimental conditions. J Fish Biol 7:611–619

Monaghan SR, Kent ML, Watral VG, Kaufman RJ, Lee LE, Bols NC (2009) Animal cell cultures in microsporidial research: their general roles and their specific use for fish microsporidia. In Vitro Cell Dev Biol Anim 45:135–147

Monaghan SR, Rumney RL, Vo NT, Bols NC, Lee LE (2011) In vitro growth of microsporidia *Anncaliia algerae* in cell lines from warm water fish. In Vitro Cell Dev Biol Anim 47:104–113

Morrison CM, Sprague V (1983) *Loma salmonae* (Putz, Hoffman and Dunbar, 1965) in the rainbow trout, *Salmo gairdneri* Richardson, and *L. fontinalis* sp. nov. (Microsporida) in the brook trout, *Salvelinus fontinalis* (Mitchill). J Fish Dis 6:345–353

Muller MG, Kinne J, Schuster RK, Walochnik J (2008) Outbreak of microsporidiosis caused by *Enterocytozoon bieneusi* in falcons. Vet Parasitol 152:67–78

Nylund S, Nylund A, Watanabe K, Arnesen CE, Karlsbakk E (2010) *Paranucleospora theridion* n. gen., n. sp. (Microsporidia, *Enterocytozoonidae*) with a life cycle in the salmon louse (*Lepeophtheirus salmonis*, Copepoda) and Atlantic salmon (*Salmo salar*). J Eukaryot Microbiol 57:95–114

Ohshima K (1937) On the function of the polar filament of *Nosema bombycis*. Parasitology 29:220–224

Oi DH, Valles SM, Pereira RM (2004) Prevalence of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection in monogyne and polygyne red imported fire ants (Hymenoptera: Formicidae). Environ Entomol 33:340–345

Pasteur L (1870) Etudes sur la Maladie des vers a Soie. Gauthier-Villars, Paris

Peuvel I, Peyret P, Méténier G, Vivarès CP, 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, Vivarès 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

Phelps NBD, Goodwin AE (2008) Vertical transmission of *Ovipleistophora ovariae* (Microspora) within the eggs of the golden shiner. J Aquat Anim Health 20:45–53

Prigneau O, Achbarou A, Bouladoux N, Mazier D, Desportes-Livage I (2000) Identification of proteins in *Encephalitozoon intestinalis*, a microsporidian pathogen of immunocompromised humans:

an immunoblotting and immunocytochemical study. J Eukaryot Microbiol 47:48–56

Ramsay JM, Watral V, Schreck CB, Kent ML (2009) *Pseudoloma neurophilia* infections in zebrafish *Danio rerio*: effects of stress on survival, growth, and reproduction. Dis Aquat Org 88:69–84

Ratnieks FL, Carreck NL (2010) Ecology. Clarity on honey bee collapse? Science 327:152–153

Redhead SA, Kirk P, Keeling PJ, Weiss LM (2009) Proposals to exclude the phylum Microsporidia from the code. Taxon 58:10–11

Reetz J, Rinder H, Thomschke A, Manke H, Schwebs M, Bruderek A (2002) First detection of the microsporidium *Enterocytozoon bieneusi* in non-mammalian hosts (chickens). Int J Parasitol 32:785–787

Rich SM, Ayala FJ (2000) Population structure and recent evolution of *Plasmodium falciparum*. Proc Natl Acad Sci USA 97:6994–7001

Rodriguez-Tovar LE, Wadowska DW, Wright GM, Groman DB, Speare DJ, Whelan DS (2003) Ultrastructural evidence of autoinfection in the gills of Atlantic cod *Gadus morhua* infected with *Loma* sp. (phylum Microsporidia). Dis Aquat Org 57:227–230

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

Sanchez JG, Speare DJ, Markham RJ, Wright GM, Kibenge FS (2001) Localization of the initial developmental stages of *Loma salmonae* in rainbow trout (*Oncorhynchus mykiss*). Vet Pathol 38:540–546

Sanders JL, Kent ML (2011) Development of a sensitive assay for the detection of *Pseudoloma neurophilia* and laboratory populations of the zebrafish, *Danio rerio*. Dis Aquat Org 96:145–156

Sanders JL, Lawrence C, Nichols DK, Brubaker JF, Peterson TS, Murray KN, Kent ML (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

Schäperclaus W (1991) Plistophora disease of neon and other aquarium fish (Pleistophorosis). In: Schäperclaus W, Kulow H, Schreckenbach K (eds) Fish Diseases (vol 2, 5th ed.), Amerind, New Delh, 1,398 pp

Shadduck JA (1969) *Nosema cuiculi*: in vitro isolation. Science 166:516–517

Shaw R, Kent ML (1999) Fish microsporidia. In: Wittner M, Weiss LM (eds) The microsporidia and microsporidiosis. American Society for Microbiology Press, Washington, DC, pp 418–444

Shaw RW, Kent ML, Adamson ML (1998) Modes of transmission of *Loma salmonae* (Microsporidia). Dis Aquat Org 33:151–156

Shaw RW, Kent ML, Brown AM, Whipps CM, Adamson ML (2000) Experimental and natural host specificity of *Loma salmonae* (Microsporidia). Dis Aquat Org 40:131–136

Siegel JP, Maddox JV, Ruesink WG (1986) Lethal and sublethal effects of *Nosema pyrausta* on the European corn borer (*Ostrinia nubilalis*) in central Illinois. J Invertebr Pathol 48:167–173

Sinden RE, Canning EU (1974) The ultrastructure of the spore of *Nosema algerae* (Protozoa, Microsporida), in relation to the hatching mechanism of microsporidian spores. J Gen Microbiol 85:350–357

Snowden K, Logan K (1999) Molecular identification of *Encephalitozoon hellem* in an ostrich. Avian Dis 43:779–782

Snowden KF, Logan K, Phalen DN (2000) Isolation and characterization of an avian isolate of *Encephalitozoon hellem*. Parasitology 121(Pt 1):9–14

Sokolova YY, Fuxa JR (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

Southern TR, Jolly CE, Lester ME, Hayman JR (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 LA Jr, Cheng TC (eds) Comparative pathobiology. Plenum, New York

Sprague V, Becnel JJ (1999) Appendix: checklist of available generic names for microsporidia with type species and type hosts. In: Wittner M, Weiss LM (eds) The microsporidia and microsporidiosis. American Society for Microbiology Press, Washington, DC, pp 531–539

Sprague V, Hussey KL (1980) Observations on *Ichthyosporidium giganteum* (Microsporida) with particular reference to the host-parasite relations during merogony. J Protozool 27:169–175

Sprague V, Becnel JJ, Hazard EI (1992) Taxonomy of phylum microspora. Crit Rev Microbiol 18:285–395

Steffens W (1962) The current status of distribution of *Plistophora hyphessobryconis* Schaperclaus 1941 (Sporozoa, Microsporidia). Z Parasitenkd 21:535–541

Sweeney AW, Hazard EI, Graham MF (1985) Intermediate host for an *Amblyospora* sp. (microspora) infecting the mosquito, *Culex annulirostris*. J Invertebr Pathol 46:98–102

Takvorian PM, Cali A (1986) The ultrastructure of spores (Protozoa: Microsporida) from *Lophius americanus*, the angler fish. J Protozool 33:570–575

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

Trammer T, Chioralia G, Maier WA, Seitz HM (1999) In vitro replication of *Nosema algerae* (Microsporidia), a parasite of anopheline mosquitoes, in human cells above 36 degrees C. J Eukaryot Microbiol 46:464–468

Troemel ER (2011) New models of microsporidiosis: infections in zebrafish, *C. elegans*, and honey bees. PLoS Pathog 7:e1001243

Undeen AH (1975) Growth of *Nosema algerae* in pig kidney cell cultures. J Protozool 22:107–110

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

Undeen AH, Avery SW (1988) Ammonium chloride inhibition of the germination of spores of *Nosema algerae* (Microspora: Nosematidae). J Invertebr Pathol 52:326–334

Undeen AH, Epsky ND (1990) In vitro and in vivo germination of *Nosema locustae* (Microspora: Nosematidae) spores. J Invertebr Pathol 56:371–379

Undeen AH, Frixione E (1990) The role of osmotic pressure in the germination of *Nosema algerae* spores. J Protozool 37:561–567

Undeen AH, Frixione E (1991) Structural alteration of the plasma membrane in spores of the microsporidium Nosema algerae on germination. J Protozool 38:511–518

Vavrá J (1976) Structure of the microsporidia. In: Bulla LA Jr, Cheng TC (eds) Comparative pathology. Plenum, New York, pp 1–85

Vavrá J, Becnel JJ (2007) *Vavraia culicis* (Weiser, 1947) Weiser, 1977 revisited: cytological characterisation of a *Vavraia culicis*-like microsporidium isolated from mosquitoes in Florida and the establishment of *Vavraia culicis floridensis* subsp. n. Folia Parasitol (Praha) 54:259–271

Vavrá J, Larsson JIR (1999) Structure of the microsporidia. In: Wittner M, Weiss LM (eds) The microsporidia and microsporidiosis. American Society for Microbiology Press, Washington, DC, pp 7–84

Visvesvara GS (2002) In vitro cultivation of microsporidia of clinical importance. Clin Microbiol Rev 15:401–413

Vossbrinck CR, Debrunner-Vossbrinck BA (2005) Molecular phylogeny of the Microsporidia: ecological, ultrastructural and taxonomic considerations. Folia Parasitol (Praha) 52:131–142, discussion p130

Vossbrinck CR, Maddox JV, Friedman S, Debrunner-Vossbrinck BA, Woese CR (1987) Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. Nature 326:411–414

Vossbrinck CR, Andreadis TG, Weiss LM (2004) Phylogenetics: taxonomy and the microsporidia as derived fungi. In: Lindsay DS, Weiss LM (eds) Opportunistic infections: toxoplasma, sarcocystis and microsporidia. Springer, New York, pp 189–213

Walters VA (1958) Structure, hatching and size variation of the spores in a species of *Nosema* (Microsporidia) found in *Hyalophora cecropia* (Lepidoptera). Parasitology 48:113–120

Watral VG, Kauffmann RB, Kent ML (2006) In vitro culture of *Pseudoloma neurophilia*, a common microsporidian of zebrafish (*Danio rerio*). In: IX International workshops on opportunistic protists and the international society of protistologists 57th annual meeting, Lisbon, Portugal

Weber R, Deplazes P, Schwartz D (2000) Diagnosis and clinical aspects of human microsporidiosis. Contrib Microbiol 6:166–192

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

Weidner E (1976) The microsporidian spore invasion tube. The ultrastructure, isolation, and characterization of the protein comprising the tube. J Cell Biol 71:23–34

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

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, Manale SB, Halonen SK, Lynn JW (1995) Protein-membrane interaction is essential to normal assembly of the microsporidian spore invasion tube. Biol Bull 188:128–135

Weiser J (1977) Contribution to the classification of Microsporidia. Vestnik Ceskoslovenske Spolecnosti Zoologicke 41:308–320

Weiss LM (2001) Microsporidia: emerging pathogenic protists. Acta Trop 78:89–102

Weissenberg R (1968) Intracellular development of the Microsporidan *Glugea anomala* Moniez in hypertrophying migratory cells of the fish *Gasterosteus aculeatus* L., an example of the formation of "xenoma" tumors. J Eukaryot Microbiol 15:44–57

Wiklund T, Lounasheimo L, Lom J, Bylund G (1996) Gonadal impairment in roach *Rutilus rutilus* from Finnish coastal areas of the northern Baltic Sea. Dis Aquat Org 26:163–171

Williams BA, Keeling PJ (2005) Microsporidian mitochondrial proteins: expression in *Antonospora locustae* spores and identification of genes coding for two further proteins. J Eukaryot Microbiol 52:271–276

Williams BA, Hirt RP, Lucocq JM, Embley TM (2002) A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. Nature 418:865–869

Williams BA, Lee RC, Becnel JJ, Weiss LM, Fast NM, Keeling PJ (2008) Genome sequence surveys of *Brachiola algerae* and *Edhazardia aedis* reveal microsporidia with low gene densities. BMC Genomics 9:200

Wilson GG (1973) Incidence of microsporidia in a field population of spruce budworm. Can For Serv Bi-Mon Res Notes 29:35

Wilson GG (1981) *Nosema fumiferanae*, a natural pathogen of forest pests: potential for pest management. In: Burges HD (ed) Microbial control of pests and plant diseases. Academic, London, pp 595–602

Wittner M, Weiss LM (1999) The microsporidia and microsporidiosis. American Society for Microbiology Press, Washington, DC

Wongtavatchai J, Conrad PA, Hedrick RP (1994) In vitro cultivation of the microsporidian: *Enterocytozoon salmonis* using a newly developed medium for salmonid lymphocytes. J Tissue Cult Methods 16:125

Wright JH, Craighead EM (1922) Infectious motor paralysis in young rabbits. J Exp Med 36:135–140

Xu Y, Takvorian PM, Cali A, Orr G, Weiss LM (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 LM (2006) Identification of a new spore wall protein from *Encephalitozoon cuniculi*. Infect Immun 74:239–247