# 6 Chytridiomycota, Monoblepharidomycota, and Neocallimastigomycota

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## I. Introduction

Phylogenetic analyses of molecular sequences (James et al. 2000, 2006a, b) have generated monumental growth in our understanding of evolutionary relationships among zoosporic fungi since Barr's (2001) review of the morphology, life history, and occurrence of Chytridiomycota over a decade ago. New understandings of evolutionary relationships among zoosporic fungi have sharpened our focus on the value of

zoospore ultrastructural characters in systematic analyses (Letcher et al. 2008a, c; Simmons 2011) and have given us insights into the convergence of thallus features (Letcher et al. 2005; Mozley-Standridge et al. 2009) once used as primary taxonomic characters (Sparrow 1960). Molecular techniques allow us to detect uncultured and unseen chytrids in environmental samples and to demonstrate that zoosporic fungi are essentially ubiquitous and abundant in a wide range of habitats, including temperate soils and aquatic environments (Chen et al. 2008; Lefèvre et al. 2008, 2012; Lepère et al. 2008; Miki et al. 2011; Monchy et al. 2011; Sime-Ngando et al. 2011) as well as especially stressful environments such as anoxic deep-sea cold seeps and hydrothermal vent ecosystems (LeCalvez et al. 2009; Nagahama et al. 2011; Stoeck and Epstein 2003), exposed soils at high elevations (Freeman et al. 2009; Schmidt et al. 2012), and soils at Arctic latitudes (Stoeck et al. 2007). A renaissance of interest in zoosporic fungi is occurring because, as basal members in the evolution of fungi, they hold the key to reconstructing ancestral forms and forces that may have driven the evolutionary radiation of fungi (Amaral Zettler et al. 2001; Stajich et al. 2009; Steenkamp et al. 2006). Moreover, their roles as parasites of phytoplankton (Bruning et al. 1992; Holfeld 2000) and amphibians (Longcore et al. 1999, 2007; Piotrowski et al. 2004; Voyles 2011) cause concern among conservationists (Bai et al. 2010; Rosenblum et al. 2008; Schloegel et al. 2012; Weldon et al. 2004); their recognition as key players in food webs alerts ecologists of their potential impact on aquatic and terrestrial sustainability (Gleason et al. 2008; Kagami et al.

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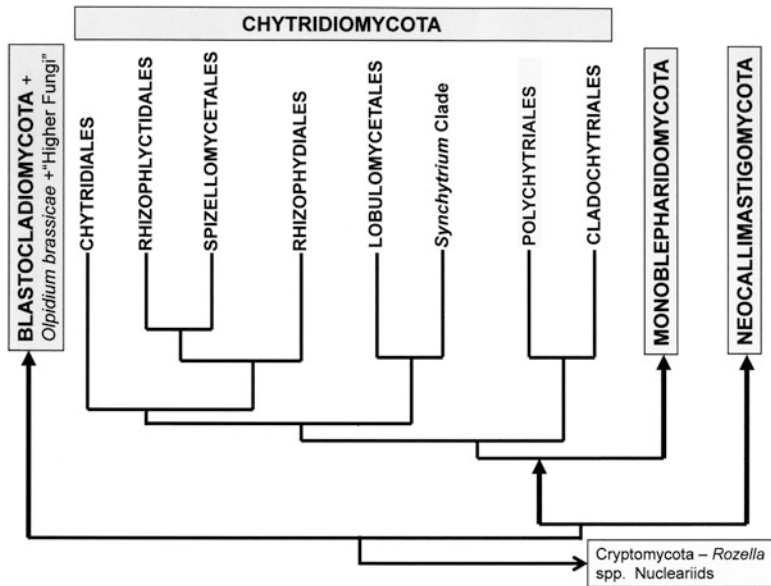
**Fig. 6.1** Phylogenetic hypothesis for zoosporic fungi based on James et al. (2006b). Four phyla have been formally circumscribed: Blastocladiomycota, Chytridiomycota, Monoblepharidomycota, and Neocallimas-tigomycota. The phylum Cryptomycota, which is considered either a fungal or protistian phylum, includes the zoosporic genus *Rozella* spp., earlier classified in the Chytridiomycota

2007, 2011, 2012; Miki et al. 2011; Sime-Ngando et al. 2011).

This chapter updates Barr's (2001) review of zoosporic fungi, with an emphasis on how molecular and ultrastructural phylogenetic analyses have revolutionized the taxonomy of zoosporic fungi. As we rapidly learn more about the biology, diversity, and global distribution of zoosporic fungi, we are revising their systematics, which, as a consequence, is in a state of flux (Powell and Letcher 2012). The phylum Chytridiomycota as circumscribed by Barr (2001) has now been separated into three additional validly published phyla: Blastocladiomycota (James et al. 2006b), Monoblepharidomycota (Doweld 2001), and Neocallimastigomycota (Hibbett et al. 2007), each circumscribing a monophyletic lineage (Fig. 6.1, Table 6.1). Moreover, *Rozella* species, once classified in the Chytridiomycota, are now amalgamated with filose pseudopodiate (pseudociliate) organisms in the **phylum Cryptomycota** (Jones et al. 2011; Karpov et al. 2013). **Blasto-cladiomycota** is commonly placed as the sister group of zygomycetous fungi (Fig. 6.1) and

diverges from other groups of zoosporic fungi [James et al. 2006b; see James et al. (2014)]. However, in different analyses, other placements may be found (Ebersberger et al. 2012; Sekimoto et al. 2011). We summarize progress in the systematics of **Chytridiomycota** (chytrids), **Monoblepharidomycota** (monoblephs), and **Neocallimastigomycota** (neocallimastigos).

## II. Occurrence and Dispersal

Zoosporic fungi are common members of aquatic and soil microbial communities and can be isolated from or detected on leaf litter and tree-canopy detritus (Bandoni and Barr 1976; Bills et al. 2004; Letcher and Powell 2001, 2002b; Longcore 2005; Nikolcheva and Barlocher 2004; Powell 1993; Shearer et al. 2004). **Chytrids** are microscopic, and their thalli may be observed from environmental samples of algae, other hosts, or organic substrates. **Monoblephs** typically occur on totally submerged waterlogged twigs, decorticated

**Table 6.1** Classification of Zoosporic Fungi from 1990 to 2013

| Barr (1990) | Barr (2001)[a] | This volume |
|---|---|---|
| **Chytridiomycota** | **Chytridiomycota** | **Chytridiomycota** |
| Chytridiomycetes | Chytridiomycetes | Chytridiomycetes |
| Chytridiales | Chytridiales | Chytridiales |
| | | Rhizophydiales |
| | | Lobulomycetales |
| | | Cladochytriales |
| | | Polychytriales |
| Spizellomycetales | Spizellomycetales | Spizellomycetales |
| | | Rhizophlyctidales |
| Monoblepharidales | Monoblepharidales | **Monoblepharidomycota** |
| | | Monoblepharidomycetes |
| | | Monoblepharidales |
| | | Harpochytriales |
| | | Hyaloraphidiomycetes |
| | | Hyaloraphidiales |
| | Neocallimastigales | **Neocallimastigomycota** |
| | | Neocallimastigomycetes |
| | | Neocallimastigales |
| Blastocladiales | Blastocladiales | **Blastocladiomycota** |
| | | Blastocladiomycetes |
| | | Blastocladiales |

[a]*Olpidium* and *Rozella*, previously classified in the Spizellomycetales and Olpidiaceae, place outside of the Chytridiomycota in molecular phylogenetic studies. Gene sequences of the type species of these two genera have not been obtained; consequently, the genera and family are *incertae sedis*. *Rozella allomycis* has been classified in a nomenclaturally validly described phylum, Cryptomycota (Jones et al. 2011)

twigs, fruits, or insect material in shallow freshwater habitats, and as filamentous growth, often as tufts with a slimy texture. **Neocallimastigos** are adapted for growth in the rumen and digestive tracts of animals, including sheep, goats, cows, horses, deer, elephants, and buffalo. They may be even more widespread among herbivores than previously recognized; they have recently been found associated with the digestive system of the green iguana, a herbivorous reptile (Liggenstoffer et al. 2010). Molecular techniques have detected them outside of host animals in anoxic landfills rich in cellulosic materials (Lockhart et al. 2006), and resistant spores can survive outside of their hosts in dried feces (Milne et al. 1989; Wubah et al. 1991).

The notion that zoosporic fungi are strictly aquatic fungi has been dispelled because there are essentially terrestrial groups such as Spizellomycetales and Rhizophlyctidales (Letcher et al. 2008a; Wakefield et al. 2010). Their **adaptations** for dispersal and survival are more complex than generally recognized. Clearly, chytrids require water or humidity to trigger zoospore release from sporangia and for zoospores to disperse. Although the zoospore is covered with a cell coat of varying prominence (Dorward and Powell 1983; Powell 1994; Shields and Fuller 1996), the zoospore is unwalled and becomes desiccated if left out of water for any extended period of time before it encysts. Zoospores of many chytrids, especially those of the Spizellomycetales, are capable of a squirming amoeboid-type motion that can advance the zoospore in a thin water film, but some moisture is still required for zoospore motility.

How far zoospores (Fig. 6.2A, E) can swim under their own power is not known, but distant dispersal by individual zoospores seems to be limited to a few centimeters (Hampson and Coombes 1989). Zoospores can remain motile after release from sporangia for a few seconds, hours, or even (rarely) days; but typically zoospore motility is ultimately limited in time because of their dependence on endogenous
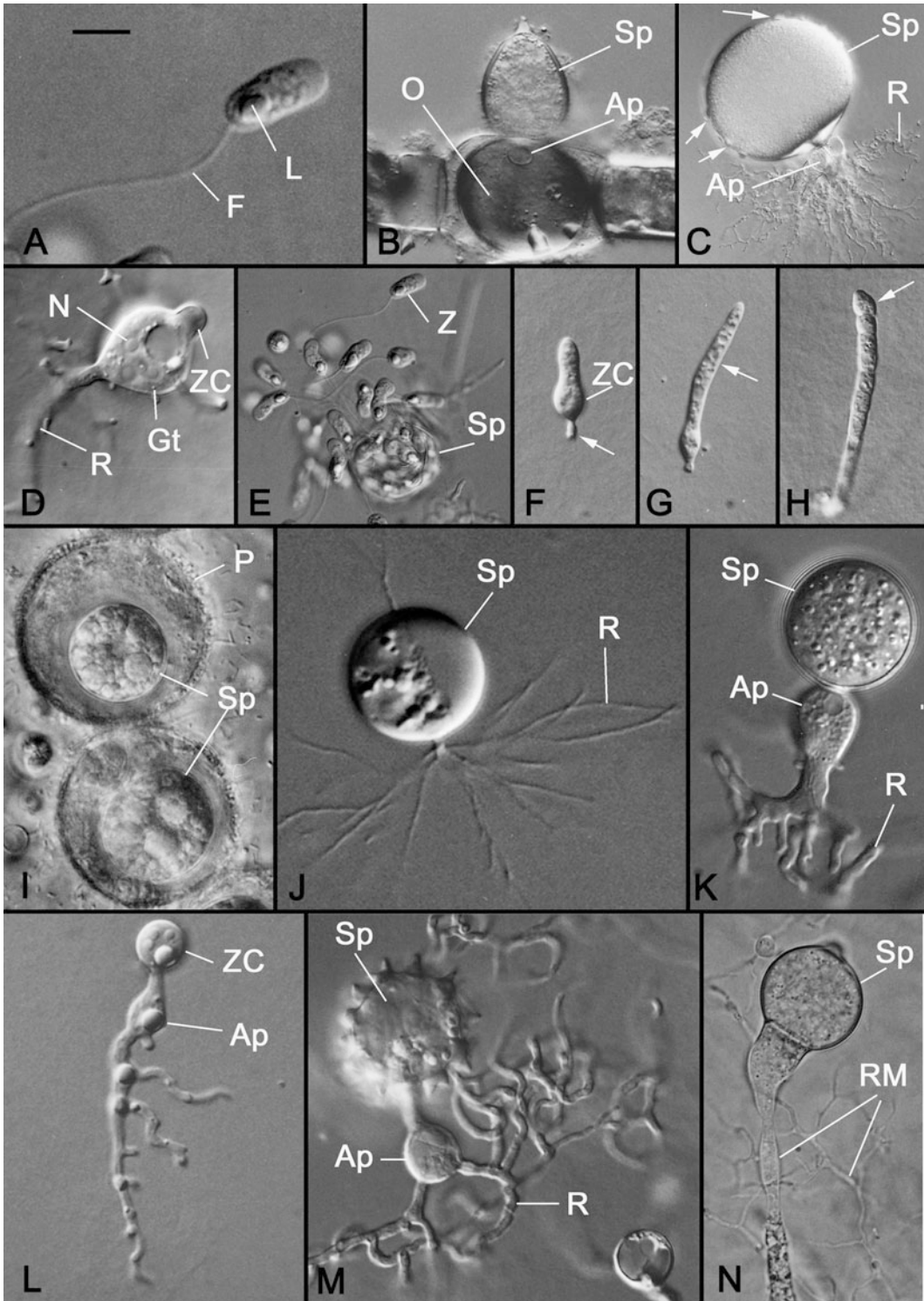
**Fig. 6.2** Light microscopic morphological features of Chytridiomycota and Monoblepharidomycota. **A.** Oblong zoospore with prominent lipid globule (L) and posterior flagellum (F). **B.** *Chytridium olla* thallus growing on oospore in oogonium (O) of the green alga *Oedogonium*. The sporangium (Sp) bears an apical

reserves for energy (Powell 1976b). Their **pattern of swimming** is not one of a direct trajectory to a substrate but rather one of abrupt changes in direction, colliding into other chytrid zoospores and substrates before attachment, encystment, and germination. Chytrid and neocallimastigo zoospores are **attracted** to specific nutrient sources, and chytrid zoospores move toward blue wavelengths of light (Kazama 1972; Moss et al. 2008; Muehlstein et al. 1987, 1988; Orpin and Bountiff 1978; Strasburger 1878). In addition to dispersal, it seems that a primary role of the zoospore is the location of a suitable substrate or host on which to grow.

The fact that chytrids with identical ribosomal gene sequences can be isolated from soils in Australia and North America (Letcher et al. 2004) argues that they are not solely dependent upon zoospores for dispersal. Chytrid **resting spores** are thick-walled structures filled with glycogen, lipid, and protein reserves, and they may arise vegetatively or after sexual reproduction. Because they are commonly spherical, with or without wall ornamentation, and smaller than sporangia, they might easily be disseminated in soil, water, or air with a wider distribution than zoospores can provide. The ability of the two-celled resting spore of *Septosperma* to disarticulate from its substrate also argues for the importance of chytrid resting spores in wide dissemination (Powell and Blackwell 1991). Neocallimastigos seem to spread from mother to offspring through saliva

during grooming and licking activities, and survival of resistant spores in dry dung may also enhance dispersal (Milne et al. 1989; Wubah et al. 1991).

**Dung** is widely recognized as an excellent substrate for a range of higher fungi (Webster 1970). As would be expected, neocallimastigos are found in dung; however, the occurrence of herbivore dung-inhabiting chytrids has only recently been discerned and is limited to Spizellomycetales and Lobulomycetales (Simmons et al. 2012; Wakefield et al. 2010). The presence of viable chytrids in freshly voided horse feces, as discovered in baiting experiments, indicates that chytrids can survive the digestive system of herbivores (Wakefield et al. 2010). Studies suggesting that birds and earthworms are **vectors** for chytrids are especially relevant in considerations of chytrids as dung fungi. Thornton (1970) demonstrated that earthworms could transport viable chytrids in two ways: among debris clinging to their mucilaginous surface or in casts they discharge after consuming soil. Supporting earthworms as dispersal agents for chytrids, Hampson and Coombes (1989) demonstrated that *Synchytrium endobioticum* dispersed greater distances when earthworms were present than when only zoospores were present. Birds have also been implicated in long-distance dispersal of chytrids, indicating that chytrids can survive the digestive system of birds and remain viable in bird dung (Thornton 1971). Whether birds acquire chytrids from

**Fig. 6.2** (continued) apiculus. The chytrid penetrates the host with a haustorium-like apophysis (Ap). C. Thallus of *Gaertneriomyces semiglobifer* with multiple discharge pores (*arrows*) around sporangium (Sp) and finely branched rhizoidal system (R) bearing an apophysis (Ap). D. Germination of *Geranomyces variabilis* with exogenous development. Nuclei (N) have migrated from the zoospore cyst (ZC) into the germ tube (Gt), which expands and forms a sporangium with rhizoids (R). E. Release of zoospores (Z) from sporangium (Sp). F. Germination of zoospore cysts (ZC) of *Harpochytrium* sp. in a uniaxial thallus with basal holdfast (*arrow*). G. *Harpochytrium* sp. thallus with highly vacuolated, foamy appearing cytoplasm (*arrow*). H. *Harpochytrium* sp. cleaving zoospores beginning at apex (*arrow*) of thallus. I. *Olpidium* sp. monocentric holocarpic thalli endobiotic in pollen (P); the thallus is totally converted into a sporangium (Sp). J. *Alphamyces* *chaetifer* eucarpic thallus with spherical sporangium (Sp) and finely branched rhizoids (R) arising from a single axis. K. *Spizellomyces punctatus* eucarpic thallus with spherical sporangium (Sp) and coarsely branched rhizoids arising from a single apophysis (Ap); notice that the rhizoid tips are rounded and blunt. L. Germination of *Phlyctochytrium aureliae* with endogenous development. The nucleus remains in the zoospore cyst (ZC) and the germ tube branches into a rhizoidal system with an apophysis (Ap). M. *Phlyctochytrium aureliae* eucarpic thallus bearing sporangial (Sp) ornamentation and tubular rhizoids (R). A spherical apophysis (Ap) is far from the sporangium. N. Rhizomycelium (RM) of *Polychytrium aggregatum* is tubular with finely branched rhizoids and spherical sporangia (Sp). *Scale bar* shown in A = 3 μm in A; 6 μm in L; 10 μm in E–H; 15 μm in D, J, K, M, N; 20 μm in B, C, I

eating earthworms or plant debris harboring chytrids is not known. Aquatic birds carry thalli of the amphibian parasite *Batrachochytrium dendrobatidis* on the keratinous webbing of their feet and may also provide long-range dispersal of chytrids (Garmyn et al. 2012).

**Plant pathogenic** chytrids can be distributed by transport of contaminated plants and soil. *S. endobioticum*, the causal agent of potato wart disease, is readily introduced through infected seed potatoes and soils containing resting spores. There is no evidence that zoospores are effective at broad-scale dispersal of this disease (Hampson and Coombes 1989). *B. dendrobatidis* can be **transmitted between animals** (Rachowicz and Vredenburg 2004) and is believed to have been spread globally through movement of animals for food, medicine, research, and the global pet trade (Bai et al. 2010; Schloegel et al. 2012; Weldon et al. 2004).

## III. Culture and Maintenance

Several informative references detail methods and techniques for isolation, culture, and growth of chytrids and monoblephs (Bills et al. 2004; Fuller and Jaworski 1987; Shearer et al. 2004). Most **chytrids** are extracted from a habitat by way of enrichment of environmental samples with heat-killed algae, chitin, cellulose, keratin, or pollen substrates and incubation at ambient temperatures for 2–3 days (Couch 1939; Barr and Désaulniers 1987). **Monoblephs** are frequently isolated from the surfaces of algae, fruits, and twigs in water (Emerson 1958, 1964; Emerson and Whisler 1968; Perrott 1955, 1958). **Neocallimastigos**, as obligate anaerobic fungi, must be grown under anaerobic conditions (Orpin 1975; Rezaeian et al. 2004). Most isolation studies extract neocallimastigos directly using a cannula collection system that aseptically penetrates into the rumen or other regions of the alimentary canal of herbivores (Orpin 1975).

A large number of zoosporic fungi are in culture, and the majority of these are in university-managed collections. Many zoosporic fungi survive on agar slants stored at 4 °C for up to 6 months. Advances have been made in **cryo-storage** of chytrid cultures. Cultures grown in broth on cotton tips, transferred to a glycerin solution, and stored at −80 °C or in liquid nitrogen (Barr and Babcock 1994; Gleason et al. 2007) have been recovered after 15 years (C. E. Babcock, personal communication). Freezing techniques also facilitate the storage of plant-pathogenic chytrids. *Synchytrium solstitiale* stored in 0.5 M sucrose at −2 °C remained viable in host tissue for 3 months (Widmer 2006), and it may also remain viable as air-dried tissue for over 2 years (Bruckart et al. 2011).

## IV. Phylogenetic Concepts of Zoosporic Fungi

The broadest ranging molecular phylogenetic analysis of zoosporic fungi was conducted by James et al. (2006b), and Fig. 6.1, which is based on that study, depicts our current phylogenetic hypothesis. Although sharing a common ancestor, the lineage including Neocallimastigomycota, Monoblepharidomycota, and Chytridiomycota diverges from the lineage that gave rise to the Blastocladiomycota and higher fungi (Fig. 6.1). Thus, it was unexpected that the Blastocladiomycota and the plant parasite *Olpidium brassicae* placed in a clade with non-zoosporic fungi. **Cellular characteristics** support the relationship of Blastocladiomycota with filamentous fungi, including sharing the loss of Golgi apparatus cisternal stacking (Powell and Letcher 2012). The phylogenetic placement of *Olpidium* spp. (James et al. 2006b; Sekimoto et al. 2011) based on molecular analyses is still perplexing when the mode of zoospore formation and zoospore structure are considered (Barr and Hartmann 1977; Lange and Olson 1979). *Rozella* spp., once classified in the Chytridiomycota (Barr 1980; Held 1975, 1981), are placed within the sister clade of all other fungi (James and Berbee 2011; James et al. 2006a, b).

Whereas the traditional classification of zoosporic fungi relied on **morphological features** of the thallus (Karling 1977; Sparrow 1960; Whiffen 1944), analyses of **zoospore ultrastructural features** and **molecular sequences** have revealed that many classically

used morphological character states appear in multiple lineages and are convergent. For example, Sparrow (1960) used differences in zoospore discharge openings as a primary taxonomic characteristic for two series of chytrids (Operculatae and Inoperculatae), but we now know that operculate and inoperculate thalli may occur within a single evolutionary lineage. Only members of the Rhizophlyctidales and Spizellomycetales discharge zoospores exclusively through inoperculate openings (Powell 1976a). Five orders of Chytridiomycota (Chytridiales, Rhizophydiales, Cladochytriales, Lobulomycetales, and Polychytriales) include some members with operculate discharge and others with inoperculate discharge. Whether or not the underlying developmental mechanism for the production of an operculum in all orders is the same or different is not known, but differences have been described (Beakes et al. 1992; Powell et al. 2011; Taylor and Fuller 1981). As a second example, polycentric versus monocentric thallus complexity (Whiffen 1944) was used to distinguish families within Sparrow's (1960) two series of chytrids. However, recent phylogenetic analyses of the Chytridiales, Rhizophlyctidales, Cladochytriales, and Polychytriales have revealed members with polycentric and monocentric thalli within the same order. A third example is the so-called *Entophlyctis*-type of development, in which the germ tube rather than the zoospore cyst gives rise to the sporangium (Fig. 6.2D) (Blackwell et al. 2006). This exogenous type of development, along with endogenous development, is found in several lineages of Chytridiomycetes. Thus, it is clear that organisms within diverse evolutionary lineages, but with simple thalli growing in similar habitats and exposed to similar selective pressures, adapt with similar morphological phenotypes, resulting in a convergence of thallus features.

Contemporary taxa of chytrids are now delineated based on **molecular monophyly**. With this approach we look at a snapshot in time of the evolution of a species, with gene sequences serving as the primary taxonomic character. Because genes, zoospore ultrastructural characters, and thallus features evolve at different rates, we use a constellation of zoospore ultrastructural characters and thallus features to define taxa within monophyletic clades (Fig. 6.1). Zoosporic fungi are an ancient group of eukaryotes, and plesiomorphic character states shared with a common ancestor (=descent-based similarity; Hörandle and Stuessy 2010) may appear within diverging lineages only to be modified repeatedly or lost in multiple lineages. We have made great advances in delineating monophyletic orders, especially in circumscribing the limits of a monophyletic Chytridiales (Vélez et al. 2011) in the Chytridiomycota. Table 6.1 summarizes progress in the classification of zoosporic fungi with greater insights into their phylogenetic relationships.

## V. Identification of Zoospore Ultrastructural Characters and Character States

Because of the stability of ultrastructural character states, ultrastructure is instrumental in understanding relationships among zoosporic fungi. Zoosporic fungi are notoriously apt at **thallus phenotypic diversity** (Powell and Koch 1977), which may adapt them well to changing environments but makes thallus-based identification challenging. With the added insight of molecular phylogenetic analyses, we have been able to identify and describe zoospore ultrastructural features useful for characterizing and delineating taxa. Koch (1961) first emphasized the "surprising diversity" of zoospore types in chytrids when he illustrated six major types, and from that beginning we now recognize a tremendous diversity in the architectural forms of chytrid zoospores. The two main regions of the zoospore that afford the richest supply of characters are the flagellar apparatus (Barr 1980, 2001; Barr and Désaulniers 1988) and the microbody–lipid globule complex (MLC) (Powell 1976b, 1978; Powell and Roychoudhury 1992).

The **flagellar apparatus** and auxiliary structures provide a range of characters and character states. Morphologies of **kinetosome-associated structures** (KASs) (Figs. 6.3F–H

and 6.4F, G, I, J) are applicable in the determination of families (Letcher et al. 2008c; Vélez et al. 2011) and genera (Letcher and Powell 2005a; Longcore and Simmons 2012; Simmons 2011). In analyses of the precise configuration of the flagellar apparatus, the position of the KASs and microtubule roots has been described for several organisms and seems to be useful in generic delimitations (Barr and Désaulniers 1988; Roychoudhury and Powell 1992). The presence (Fig. 6.4D, E) or absence (Fig. 6.4C) of an electron-opaque core in the transition zone through which the axoneme doublets pass is a signature for several orders.

The **microbody–lipid globule complex** (MLC) is an assemblage of organelles consisting of lipid globules, a cisterna, mitochondria, and microbodies and is involved in the conversion of stored lipid into energy for zoospores (Powell 1976b, 1978). Because calcium may be sequestered in the MLC cisterna, which is positioned adjacent to the plasma membrane (Dorward and Powell 1982) and near the flagellar apparatus, it has been proposed to regulate flagellar beat and zoospore directionality (Powell 1983). How closely and the manner in which the organelles are linked in the MLC appear to be conserved indicators of phylogenetic relationships. Other features, such as the extensiveness or lobed nature of the microbody, are taxonomically informative (Barr and Désaulniers 1987; Letcher et al. 2008c). The MLC cisterna also provides character states for systematic comparisons. The MLC cisterna may be a simple cisterna (Fig. 6.3A) with no fenestrations, or it may contain a disk of honeycomb-patterned fenestrae (Fig. 6.3C) (Dorward and Powell 1982), termed the rumposome when first reported in the posterior portion of the zoospores of monoblephs (Fuller 1966; Fuller and Reichle 1968). Electron microscopic studies have eloquently demonstrated that the fenestrated disk of the cisterna is continuous with a nonfenestrated cisterna (Barr and Désaulniers 1987; McNitt 1974; Montecillo et al. 1980). The degree of fenestration may range from inconspicuous and minimal (Fig. 6.3B) to conspicuous and extensive (Fig. 6.3C), or it may be even more complex and multitiered (Barr and Désaulniers 1987;

Fuller and Reichle 1968; Letcher et al. 2008c; Reichle 1972; Simmons et al. 2012).

## VI. Characterization of Phyla

Molecular phylogenetic analyses have validated the application of zoospore ultrastructural characters in systematic considerations (James et al. 2006b; Letcher et al. 2008a, c; Longcore and Simmons 2012; Simmons 2011). We have repeatedly found that **molecular-based phylogenetic hypotheses predict zoospore ultrastructural types**. Using a constellation of character states, we can assign an organism to an order based on zoospore ultrastructural characters (Fig. 6.5). As we characterize a genetically more diverse sampling within orders, we are also uncovering more variation in zoospore architecture and can define more character states for each character (Letcher et al. 2008c, 2012a, b; Longcore and Simmons 2012; Picard et al. 2009; Simmons 2011; Simmons et al. 2012). Hence, **zoospore ultrastructural character states** can also be used to define families within orders and genera within families (Letcher et al. 2006, 2008a, c, 2012b; Longcore and Simmons 2012; Simmons 2011). There are zoospore types found in described species for which phylogenetic placement and classification into an order have not been resolved, and these species remain classified as *incertae sedis* (Beakes et al. 1988, 1993; Karpov et al. 2010; Nyvall et al. 1999; Powell 1981a, b).

Zoospores of each order are distinguished by a **suite of characters**, rather than a single defining feature. The constellation of ultrastructural states allows one to identify the order based on zoospore ultrastructural characters (Fig. 6.5). What complicates using zoospore ultrastructural characters alone to define orders is that, because of evolutionary descent, ancestral character states may be lost or transformed within multiple lineages with shared ancestry. For example, a MLC cisterna with fenestrae seems to be a character state shared with the last common ancestor of monoblephs and chytrids because it is present in both lineages. However, within diverging lineages,
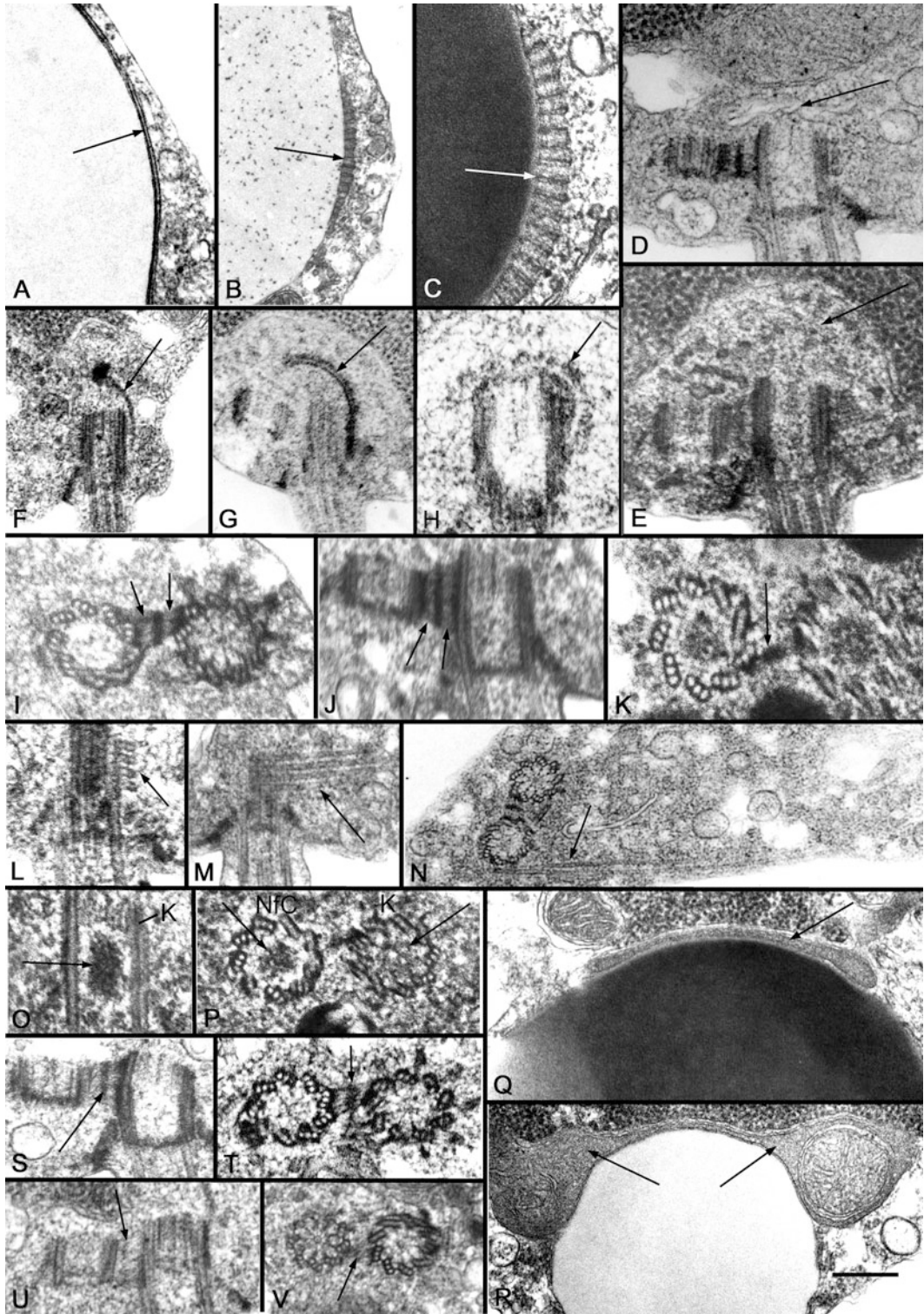
**Fig. 6.3** Ultrastructural characters and character states in Rhizophydiales. *Arrows* indicate illustrated feature. A–C. Microbody–lipid globule complex cisterna. A. Simple. B. Inconspicuously fenestrated. C. Conspicuously fenestrated. D, E. Vesiculated region adjacent to kinetosome. D. Absent. E. Present. F–H. Kinetosome-

the fenestrae may be reduced or lost (Letcher et al. 2008c), and the cisterna may be absent (Longcore et al. 1999). When a fenestrated MLC cisterna is present, microtubule roots are typically also present (Barr and Désaulniers 1988; Dorward and Powell 1982). Conversely, when the MLC cisterna lacks fenestrae (=simple cisterna) or is absent, an organized microtubule root is typically absent (e.g., Picard et al. 2009; Powell et al. 2011). Thus, in using the concept of a characteristic zoospore type for each order, it is recognized that genes and morphology do not evolve at the same rate and molecular-based phylogenies allow tracking patterns of character state evolution.

## A. Chytridiomycota

The Chytridiomycota is circumscribed as a monophyletic phylum containing a single class, Chytridiomycetes, with seven orders and two additional lineages. Doweld (2001) recognized the subclass Spizellomyceti-dae [=Spizomycetidae in Cavalier-Smith (1998)], but we do not use this subclass at this time (Table 6.1) because it would render subclass Chytridiomycetidae (Doweld 2001) polyphyletic (Fig. 6.1). The thalli of chytrids may grow endobiotically (Fig. 6.2I) or epibiotically (Fig. 6.2B) on a substrate or host, and the thallus may consist solely of a sporangium (holocarpic, monocentric) (Fig. 6.2I), a sporangium with rhizoids (eucarpic, monocentric) (Fig. 6.2C, J, K, M), or multiple sporangia (eucarpic, polycentric) growing along a filamentous, branching rhizoidal system (rhizomycelium) (Fig. 6.2N).

## 1. Rhizophydiales

*Rhizophydium* is among the larger and more complex genera of Chytridiomycetes and was traditionally classified in the Chytridiales (Letcher and Powell 2012; Sparrow 1960). *Rhizophydium* characteristically produces a monocentric thallus bearing a single tubular rhizoidal axis and a sporangium varying in shape from spherical, to oval, to pyriform, to irregularly lobed (Letcher and Powell 2012). Zoospores (Fig. 6.5A) are typically spherical and are released from one to several inoperculate discharge pores or tubes and, more rarely, from operculate openings. It was unexpected when *Rhizophydium* placed outside the Chytridiales clade in the James et al. (2000) molecular phylogenetic study. Thus, to explore the diversity in this genus, Letcher et al. (2004, 2006, 2008b, c, 2012b) conducted a broad-based global inventory of chytrids and revealed great molecular divergence and distinctive zoospore ultrastructural architectures. As the first step in the taxonomic revision of the polyphyletic Chytridiales (James et al. 2006a, b), Letcher et al. (2006) delineated the Rhizophydiales as a new order in the Chytridiomycota and designated a culture of *Rhizophydium globosum* as the epitype species of the genus. Rhizophydiales includes a large number of commonly collected and isolated chytrids as well as rare species (Letcher and Powell 2005a; Letcher et al. 2008b, c, 2012b; Longcore 2004; Longcore et al. 2011; Powell et al. 2011). Thus, what had once been a single genus with over 200 species (Letcher and Powell 2012) is now an order with 10 families, 18 genera, and lineages of unknown alliances. This clade also includes a wider range of thallus forms than previously realized. Thus far, all are monocentric except for *B. dendrobatidis*, which may be colonial. Several have multiple rhizoidal axes arising from the sporangium (Longcore et al. 1999, 2011). Although *Rhizophydium* species had been considered inoperculate, two operculate genera

**Fig. 6.3** (continued) associated structure. F. Solid spur. G. Laminated spur. H. Shield. I–K. Fibrillar bridge between kinetosome and nonflagellated centriole. I. Fibrillar bridge perpendicular to two structures, transverse section. J. Fibrillar bridge perpendicular to two structures, longitudinal section. K. Fibrillar bridge diagonal between two structures, transverse section. L–N. Microtubular root. L. Oblique longitudinal section. M. Medial longitudinal section. N. Transverse section. O, P. Granular cylinder in kinetosome or nonflagellated centriole. O. Medial longitudinal section of kinetosome (K). P. Transverse section of nonflagellated centriole (NfC) and kinetosome (K). Q, R. Microbody. Q. Simple. R. Lobed. S–V. Zone of convergence of fibrils in fibrillar bridge between kinetosome and nonflagellated centriole. S. Wide (~0.075 μm), longitudinal section. T. Wide, transverse section U. Narrow (0.010–0.025 μm), longitudinal section. V. Narrow, transverse section. *Scale bar* shown in R = 0.15 μm in K; 0.16 μm in H, I; 0.20 μm in A–E, J, L, M, O–V; 0.25 μm in N; 0.33 μm in F, G
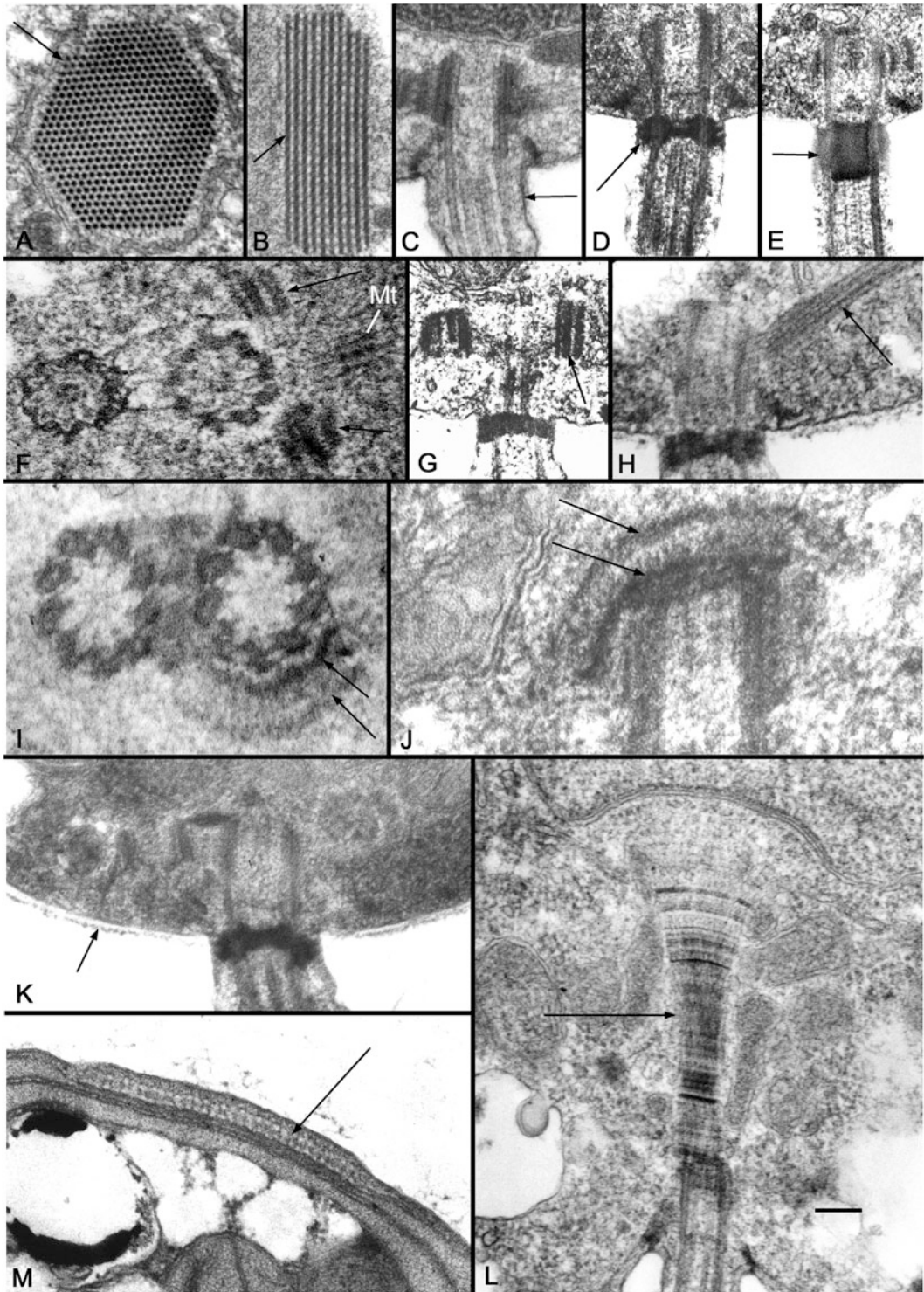
**Fig. 6.4** Ultrastructural characters and character states in Chytridiales (A, B, D–K), Rhizophydiales (C), Rhizophlyctidales (L), and Monoblepharidomycota (M). *Arrows* indicate illustrated feature. A, B. Paracrystalline inclusion. A. Transverse section. B. Longitudinal section. C–E. Flagellar plug in base of flagellum. C. Absent.

have now been described for this order (Letcher et al. 2008c; Powell et al. 2011).

Members of the Rhizophydiales are environmentally diverse and commonly grow as **saprotrophs** on pollen and keratin but are also found on cellulose and chitin substrates. Rhizophydiales are also parasites of a wide range of organisms, especially planktonic microinvertebrates and algae (Canter and Lund 1951). A few are found in marine environments, and *Rhizophydium littoreum* has been reported as a **parasite** on crab eggs (Shields 1990) and algae (Kazama 1972). *Rhizophydium graminis* is a root parasite of higher plants, such as wheat, grasses, and a few dicots (Barr 1973). Although not generally considered degraders of animal tissue, Kiziewicz (2004) reported *Rhizophydium keratinophilum* growing on muscles of vendace fish in lakes. The only known **chytrid parasite of vertebrates** is *B. dendrobatidis*, the highly destructive pathogen of amphibians (Bai et al. 2010; Longcore et al. 1999, 2007; Piotrowski et al. 2004; Rosenblum et al. 2008; Schloegel et al. 2012; Voyles 2011). Evidence suggests that pathogenesis was acquired by **lateral gene transfers** from bacteria and oomycete pathogens rather than by evolving within the Rhizophydiales lineage (Sun et al. 2011).

**Molecular-based ecological inventories** of chytrids in lakes commonly detect novel clades and known species within the Rhizophydiales, indicating they may be a major component of fungal aquatic communities (Lefèvre et al. 2008, 2012; Monchy et al. 2011). It is possible that some of the novel phylotypes are chytrid parasites of plankton for which genes have not yet been sequenced and, hence, are not retrieved from public databases in BLAST searches (Lepère et al. 2008; Sønstebø and Rohrlack 2011).

The revision of the Rhizophydiales is an example of the value of zoospore ultrastructural characters and character states. Broad sampling

has now demonstrated over 18 unique zoospore configurations in the order (e.g., Fig. 6.5 in Letcher et al. 2008c, 2012b; Powell et al. 2011), whereas in earlier studies *Rhizophydium* species were characterized as having a Group III-type zoospore (Barr and Hadland-Hartmann 1978). The key following the list of 14 characters below demonstrates how suites of zoospore ultrastructural character states distinguish families. Within the Rhizophydiales several lineages and subclades with distinctive zoospore types have now been described taxonomically and await greater sampling (Letcher et al. 2008b; Longcore et al. 1999, 2011; Powell et al. 2011; Powell and Roychoudhury 1992); these are not included in the key.

Characters and Character States of Zoospores in Rhizophydiales

1. Location of nucleus: 0, outside ribosomal aggregation; 1, embedded in ribosomal aggregation.
2. Endoplasmic reticulum ramifying through ribosomal aggregation: 0, absent; 1, present.
3. Kinetosome-associated structure: 0, absent; 1, solid spur; 2, laminated spur; 3, shield (Fig. 6.3E–H).
4. Microtubular root: 0, absent; 1, present (Fig. 6.3L–N)
5. Fibrillar bridge between kinetosome and nonflagellated centriole: 0, perpendicular to the two structures; 1, diagonal between the two structures (Fig. 6.3I, K).
6. Perpendicular zone of convergence in fibrillar bridge between kinetosome and nonflagellated centriole: 0, absent; 1, narrow (0.01–0.025 μm); 2, wide (approximately 0.075 μm; greater than 0.025 μm) (Fig. 6.3S–V).
7. Granular cylinder in core of kinetosome or nonflagellated centriole: 0, absent; 1, present (Fig. 6.3O, P).
8. Vesiculated region adjacent to kinetosome: 0, absent; 1, present (Fig. 6.3D, E).
9. Microbody–lipid globule complex cisterna: 0, absent; 1, simple (no fenestrations); 2, inconspicuously fenestrated; 3, conspicuously fenestrated (Fig. 6.3A–C).
10. Number of lipid globules: 0, predominantly one; 1, multiple.

**Fig. 6.4** (continued) D. Present, Chytriomycetaceae. E. Present, Chytridiaceae. F, G. Kinetosome-associated structure a pair of stacked plates on either side of microtubular root. F. Transverse section, with microtubular root (Mt). G. Longitudinal section. H. Microtubular root, longitudinal section. I, J. Kinetosome-associated structure, a caplike body over kinetosome. I. Transverse section. J. Longitudinal section. K. Cell coat. L. Fibrillar rhizoplast between kinetosome and nucleus, longitudinal section. M. Rumposome (fenestrated cisterna) backed by microbody, longitudinal section. *Scale bar* shown in L = 0.08 μm in I, J; 0.10 μm in F; 0.12 μm in K, M; 0.13 μm in H; 0.15 μm in B–E; 0.18 μm in A, G; 0.20 μm in L

Key to Families in the Rhizophydiales using Ultrastructural Characters

A. Kinetosome associated, crescent-shaped structure a spur ………………….……….…B

    B. Spur solid (Fig. 6.3F) ……………….…………………..………Terramycetaceae

    BB. Spur layered (Fig. 6.3G) …..……………….…………….…..…Rhizophydiaceae

AA. Kinetosome-associated-crescent-shaped spur absent (Fig. 6.3D)…….….……….…C

    C. Fibrillar bridge between kinetosome and nonflagellated centriole

    diagonal and electron-opaque core in kinetosome (Fig. 6.3K) …………………D

        D. Electron-opaque core in kinetosome and in nonflagellated

        centriole (Fig. 6.3K) ………..………………... Kappamycetaceae

        DD. Electron-opaque core in kinetosome and no electron-opaque core

        in nonflagellated centriole (Fig. 6.3O,P) ………...……...Alphamycetaceae

    CC. Fibrillar bridge between kinetosome and nonflagellated centriole

    perpendicular and no electron-opaque core in kinetosome or nonflagellated

    centriole (Fig. 6.3I, J) …………….……………………...…………….….…E

        E. Nucleus embedded within ribosomal aggregation ……………….…F

            F. Perpendicular zone of convergence in fibrillar bridge between

            kinetosome and nonflagellated centriole wide, greater than

            $0.025 \ \mu m$ (Fig. 6.3S, T) …………………….Globomycetaceae

            FF. Perpendicular zone of convergence in fibrillar bridge between

            kinetosome and nonflagellated centriole narrow, less than 0.025

            $\mu m$ (Fig. 6.3U, V) ………………………Gorgonomycetaceae

        EE. Nucleus at surface of ribosomal aggregation ……………….…...…G

            G. Close association of a portion of the microbody with the

            kinetosome ………………………….…. Angulomycetaceae

            GG. No close association of a portion of the microbody with the

            kinetosome ………………………………………………….…H

                H. Perpendicular zone of convergence in fibrillar bridge

                between kinetosome and nonflagellated centriole wide,

                greater than $0.025 \ \mu m$ …..………...….Aquamycetaceae

                HH. Perpendicular zone of convergence in fibrillar bridge

                between kinetosome and nonflagellated centriole

                narrow, less than $0.025 \ \mu m$ ……………..……….….…I

                    I. Microbody not branching extensively away from

                    lipid globule (Fig. 6.3Q) …….......Pateramycetaceae

                    II. Microbody branching extensively away

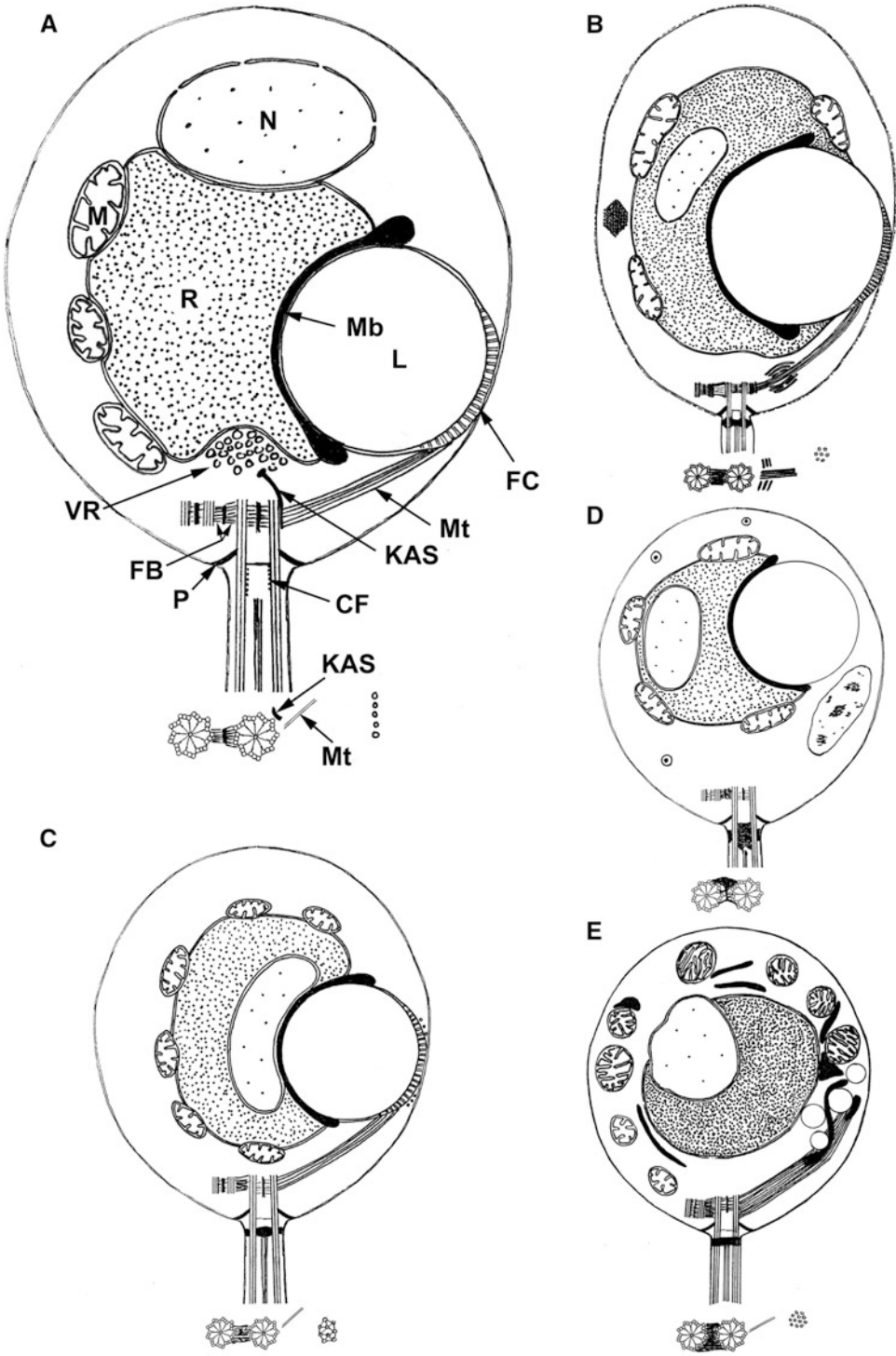                    from lipid globule (Fig. 6.3R) ....Protrudomycetaceae
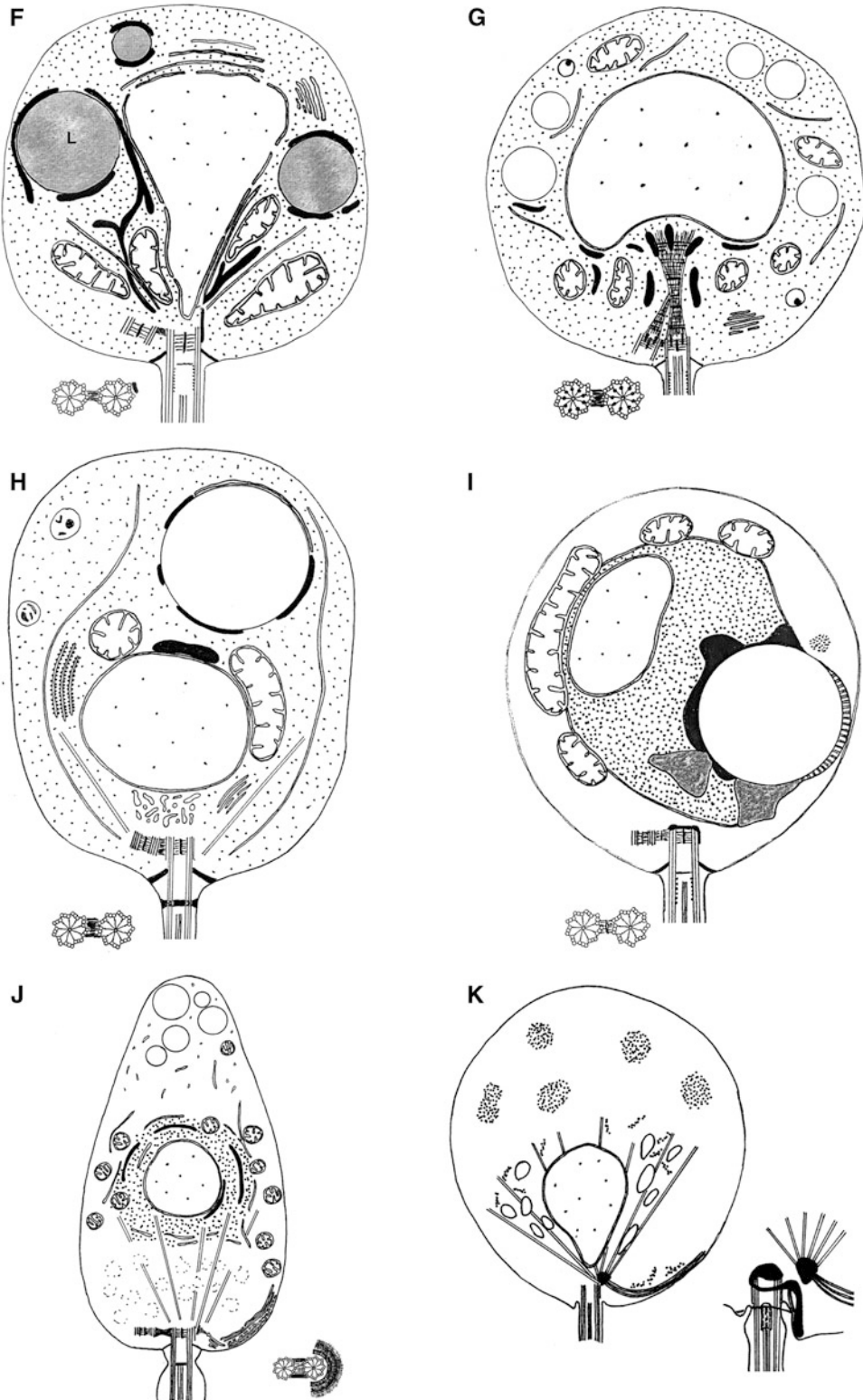
**Fig. 6.5** (continued)

**Fig. 6.5** Schematics of longitudinal sections through zoospores representative of 11 lineages of zoosporic fungi (A–K), with transverse sections through kineto- some, nonflagellated centriole, and microtubular root when present (A–J), and longitudinal section through kinetosome (K). A. Rhizophydiales. B. Chytridiales. C.

11. Number of mitochondria in longitudinal section: 0, one; 1, multiple.
12. Close association of a lobe of a mitochondrion with kinetosome: 0, absent; 1, present.
13. Close association of a lobe of a microbody with kinetosome: 0, absent; 1, present.
14. Microbody morphology: 0, simple 1, lobed and branched (Fig. 6.3Q, R).

## 2. Chytridiales

One of the greatest impacts of the James et al. (2006b) molecular phylogenetic analyses of Chytridiomycota was the revelation that the Chytridiales as described (Barr 1980) was polyphyletic. The type species for the Chytridiomycota and Chytridiales is *Chytridium olla* (Fig. 6.2B), a chytrid Braun (1851, 1855) described as growing parasitically on the oospore of *Oedogonium* (Fig. 6.2B). Thus, finding and culturing *C. olla* was vital for defining the phylum Chytridiomycota and establishing the limits of the order Chytridiales. Vélez et al. (2011) were able to grow *C. olla* in culture with its host (Fig. 6.2B), facilitating characterization of zoospore ultrastructure and analyses of ribosomal genes. Chytridiales has now been circumscribed as a monophyletic order that includes the type species (Vélez et al. 2011). Of the four families Barr (1980) included in Chytridiales, only Chytridiaceae remains. Endochytriaceae and Cladochytriaceae have been transferred to a newly erected Cladochytriales (Mozley-Standridge et al. 2009). *Synchytrium* species form a distinct clade (James et al. 2006b), and the family Synchytriaceae will likely reside with this clade outside of the Chytridiales (*Synchytrium taraxaci*, the type species, however, has not been characterized molecularly).

Chytridiales is morphologically diverse (Letcher et al. 2005) and contains two monophyletic families, each defined based on zoospore ultrastructure and gene sequence analyses. Members of the Chytridiaceae have a Group II-type zoospore (Fig. 6.5B) (Barr 1980; Barr and Hartmann 1976) and include *C. olla* (Fig. 6.2B), *C. lagenaria*, *Polyphlyctis unispina*, *Phlyctochytrium planicorne*, and *Phlyctochytrium aureliae* (Fig. 6.2L, M) (Letcher and Powell 2005b; Letcher et al. 2012a; Vélez et al. 2011). All members produce thalli that are monocentric, eucarpic, and epibiotic, and zoospore discharge occurs through either operculate or inoperculate openings. Members of the Chytriomycetaceae have a Group I-type zoospore (Barr 1980; Barr and Hartmann 1976) and include species in the monocentric, eucarpic, epibiotic/interbiotic genera *Asterophlyctis*, *Chytriomyces*, *Obelidium*, *Phlyctorhiza*, *Podochytrium*, *Rhizidium*, *Rhizoclosmatium*, and *Siphonaria*; the monocentric, eucarpic, endobiotic *Entophlyctis luteolus*; and the polycentric *Physocladia obscura*. Molecular phylogenetics reveal that *Chytriomyces*, *Entophlyctis*, and *Rhizidium* are polyphyletic as circumscribed (Letcher et al. 2005; Picard et al. 2009; Vélez et al. 2011). *Chytridium*, *Chytriomyces* (Letcher and Powell 2002a), and *Phlyctochytrium* are genera with relatively large numbers of species (Longcore 1996; Sparrow 1960). The appearance of operculate genera among inoperculate genera and the intermediate expression of this characteristic in this order (Letcher et al. 2012a) demonstrate that the nature of discharge is not a reliable character for distinguishing orders (Sparrow 1960; Whiffen 1944).

Members of Chytridiales are more common in aquatic habitats than in soil. Many are obligate parasites of algae, including the type species, *C. olla* (Vélez et al. 2011). *P. planicorne* is a commonly reported facultative parasite of algae (Letcher and Powell 2005b). *Rhizoclosmatium globosum* and *Chytriomyces hyalinus* are

**Fig. 6.5** (continued) Cladochytriales. D. Lobulomycetales. E. Polychytriales. F. Spizellomycetales. G. Rhizophlyctidales. H. *Synchytrium* clade. I. *Blyttiomyces helicus*. J. Monoblepharidomycota. K. Neocallimastigomycota. Abbreviations in A: CF, concentric fiber; FB, fibrillar bridge; FC, fenestrated cisterna; KAS, kinetosome-associated structure; L, lipid; M, mitochondrion; Mb, microbody; Mt, microtubular root; N, nucleus; P, flagellar prop; R, ribosomal aggregation; VR, vesicle region. Illustrations based on the following studies: A. Letcher et al. (2006); B. Letcher et al. (2005); C. Lucarotti (1981); D. Simmons et al. (2009); E. Letcher (unpublished), Longcore and Simmons (2012); F. Barr (1984a); G. Letcher et al. (2008a); H. Lange and Olson (1978); I. Letcher (unpublished); J. Fuller and Reichle (1968); K. Gold et al. (1988)

among the most commonly reported and isolated chitinophilic chytrids from aquatic habitats. Isolated from soil, *Rhizidium phycophilum* grows in culture only in the company of a coccoid green alga, suggesting a symbiotic partnership (Picard et al. 2009).

Four features distinguish the zoospore of Chytridiales (Fig. 6.5B) from that of other orders: (1) the cordlike microtubule root is composed of approximately six to eight microtubules that are bundled together like a fist full of soda straws and extends laterally (Fig. 6.4F, H); (2) the kinetosome to nonflagellate centriole bridge is layered, with more electron-dense material at the anterior edge (Fig. 6.4K); (3) a paracrystalline structure composed of linear stacks of rods is present in the peripheral cytoplasm (Fig. 6.4A, B); and (4) a prominent cell coat (Dorward and Powell 1983) surrounds the zoospore body, but not the flagellar membrane (Fig. 6.4K). In their zoospores ribosomes aggregate at the center of the zoospore body, and organelles of the MLC are tightly packaged (Fig. 6.5B). When a microtubule root is present, it extends between the kinetosome and MLC cisterna, which is typically fenestrated. An axonemal basal plug is present in the transition region of the axoneme with axonemal microtubules passing through it, and the kinetosome and nonflagellated centriole are usually parallel (Fig. 6.5B) (Barr 1980; Barr and Désaulniers 1987, 1988; Barr and Hartmann 1976; Dorward and Powell 1982, 1983; Letcher and Powell 2005b; Letcher et al. 2005, 2012a; Longcore 1992b, 1995; Picard et al. 2009; Vélez et al. 2011).

KASs and the morphology of the electron-opaque plug in the transition region of the flagellum (FP) distinguish the Group I- and Group II-type zoospores (Barr 1980). In Chytridiaceae (Group II-type zoospore) the KASs are layered caplike structures that typically cover the anterior end and side of the kinetosome (Fig. 6.4I, J), and the FP is as long as it is wide (Fig. 6.4E). In Chytriomycetaceae (Group I-type zoospore) the KASs are stacked plates (Fig. 6.4F, G) between which the microtubule root extends from the kinetosome (Fig. 6.4F) to the MLC cisterna (Fig. 6.5B), and the FP is biconcave, shaped like a dog bone (Fig. 6.4D).

Investigations of genetically more diverse taxa within the two families of Chytridiales are revealing additional variations in each type of zoospore, with either modification or loss of a character. For example, *Phlyctochytrium aureliae* (Chytridiaceae) zoospores are patterned on the Group II-type zoospore, but in place of the caplike KAS there is an amorphous anvil-shaped KAS; and the fenestrations in the MLC cisternae are reduced in diameter (Letcher et al. 2012a). *R. phycophilum* (Chytriomycetaceae) zoospores are patterned on the Group I-type zoospore but have lost the stacked-plate KAS, microtubule root, and fenestrations in the MLC cisternae (Picard et al. 2009).

### 3. Cladochytriales

Cladochytriales was erected as a segregate from Chytridiales based on molecular monophyly and distinct zoospore ultrastructural characters (Mozley-Standridge et al. 2009). Molecular phylogenetic analyses (James et al. 2006b; Mozley-Standridge et al. 2009; Steiger et al. 2011) revealed that the order includes species of eight described genera, which are assigned to four families or are considered *incertae sedis*: *Catenochytridium (incertae sedis)*, *Cladochytrium* (Cladochytriaceae), *Cylindrochytridium (incertae sedis)*, *Nowakowskiella* (Nowakowskiellaceae), *Septochytrium* (Septochytriaceae), *Endochytrium* (Endochytriaceae), *Nephrochytrium (incertae sedis)*, and *Allochytridium (incertae sedis)*. However, in these analyses, *Allochytridium*, *Endochytrium*, and *Nephrochytrium* were polyphyletic (Mozley-Standridge et al. 2009). The order includes members with monocentric and polycentric thalli, epibiotic or endobiotic habits, apophysate and nonapophysate rhizoids, and operculate and inoperculate sporangia. The thallus structure may be variable as in *Septochytrium*, which is capable of producing either monocentric or polycentric thalli. The presence of **catenulate rhizoidal swellings** and intercalary swellings (=spindle organs, turbinate swellings) along the rhizomycelium appear to be a morphological feature characteristic of members of this order.

Members of this order are most commonly found on decaying plants and algae from aquatic habitats, suggesting they have a role in the initial degradation of cellulose-containing materials. With robust and extensively branched rhizoids or rhizomycelia, they are readily isolated from cellulosic baits and cultured on dilute soluble starch agar (Mozley-Standridge et al. 2009).

The distinguishing characteristic of the zoospore (Fig. 6.5C) is the structure of the lateral root, which consists of bundles of up to 25 microtubules with spaces between microtubules cross-linked with lateral fibrillar links (Barr and Désaulniers 1987, 1988; Lucarotti 1981; Mozley-Standridge et al. 2009). The basic zoospore design for this order is similar to that in the Chytridiales: a lateral root joins the fenestrated cisterna and kinetosome; an electron-opaque flagellar plug occupies the transition zone of the flagellar axoneme; ribosomes are aggregated in the core of the body of the zoospore; organelles of the MLC are tightly packaged; and the nonflagellated centriole is parallel to the kinetosome and joined by a dense fibrillar bridge. Variations in the states of some of these characters will be useful in distinguishing genera. For example, the MLC cisterna may have a thickened cisternal area containing the fenestrae (Barr 1986; Lucarotti 1981) or a narrow cisterna with a small fenestrated area (Barr et al. 1987), or it may contain two or three tiers of fenestrae in the cisterna (Barr and Désaulniers 1987). Structures associated with the kinetosome seem to distinguish genera and will be useful as the ultrastructure of more zoospores of this order is characterized. For example, in zoospores of *Allochytridium luteum* the microtubule root originates from a u-shaped structure connected to kinetosomal triplet 1, and in zoospores of *Catenochytridium hemicysti* rods are parallel and linked to kinetosomal triplets 9 and 2 with a bridge partially encircling the kinetosome and joining the two rods (Barr and Désaulniers 1988).

## 4. Lobulomycetales

In the James et al. (2006b) molecular analysis of Chytridiomycota, two species of *Chytriomyces*,

*C. angularis* (Longcore 1992a) and *C. poculatus* (Willoughby and Townley 1961), placed outside of the clade that included the type of the genus, *Chytriomyces hyalinus* (Letcher and Powell 2002a). Comparative studies of *C. angularis* (Longcore 1992a) substantiated that the zoospore ultrastructure differed from that of chytridialian zoospores, and thallus features (fine, sparsely branched rhizoidal system and absence of a rhizoidal subsporangial swelling) were not characteristic of the type for *Chytriomyces*.

Additional collections and molecular environmental sequencing illuminated the diversity within this clade, leading to the establishment of a new order, Lobulomycetales, which includes four genera (*Alogomyces*, *Clydaea*, *Lobulomyces*, *Maunachytrium*) and six species (Simmons et al. 2009, 2012). Based on nuclear small subunit (SSU) ribosomal DNA sequence analysis (Müller et al. 1999), the marine algal parasite *Chytridium polysiphoniae* has been assigned to this order (Simmons et al. 2009). All members of the order are monocentric and include operculate or inoperculate organisms. They have been collected or their phylotypes detected from springs, *Sphagnum* in acidic lakes, ice-fed lakes, alpine barren soil, crop soils, acidic forest soils, tree-canopy detritus, and horse manure (Simmons et al. 2009, 2012). Environmental molecular sequencing studies often identify members of this order in lakes (Monchy et al. 2011) and deep-sea habitats (LeCalvez et al. 2009). Although the Lobulomycetales is a small group at this time, the extreme range in habitats in which its members are found suggests that this order is more diverse than presently described and is a common member of soil and aquatic microbial communities.

The most distinguishing zoospore ultrastructural characters (Fig. 6.5D) in this order are the anterior extensions on the electron-opaque plug in the transition region of the axoneme and dense amorphous material bridging the flagellum and nonflagellated centriole (Fig. 6.5D) (Longcore 1992a; Simmons et al. 2009). When Simmons et al. (2009) originally established the order, they reported the absence of MLC cisternae. However, a MLC cisterna

with small fenestrae, morphologically quite distinct from the large honeycomb-patterned fenestrae in Chytridiales, was recently observed in zoospores of *Alogomyces* (Simmons et al. 2012). Zoospores contain a ribosomal aggregation around the nucleus and one to several lipid globules in the MLC. No organized microtubule root has been observed in any of the zoospores.

## 5. Polychytriales

The Polychytriales (Longcore and Simmons 2012) was erected based on the *Polychytrium* clade (James et al. 2006b). Its members are rhizophlyctoid chytrids (Dogma 1973) in which rhizoids emanate from multiple sites on the sporangium. All members grow on chitin, and all except *Karlingiomyces asterocystis* are able to grow on cellulose and keratin. The order consists of five genera (*Arkaya*, *Karlingiomyces*, *Lacustromyces*, *Neokarlingia*, *Polychytrium*), two of which are newly described and include new combinations with existing species: *Arkaya* with *Rhizophlyctis serpentina* and *Neokarlingia* with *Rhizophlyctis (Karlingia) chitinophila*. Three of the genera are monocentric, and two genera, *Polychytrium* and *Lacustromyces*, are polycentric with broadly tubular rhizomyceliums (Fig. 6.2N) **lacking the turbinate swellings** characteristic of polycentric members of the Cladochytriales. The only operculate genus is *Karlingiomyces* (Blackwell et al. 2004); the other genera release zoospores through inoperculate openings. Polychytriales is the sister group of Cladochytriales (James et al. 2006b), a clade that also includes monocentric and polycentric thalli but is characterized by growth on cellulose rather than chitin.

Each genus has a distinct suite of zoospore ultrastructural characters. The zoospore ultrastructure (Fig. 6.5E) is remarkably varied in this order (Longcore 1993; Longcore and Simmons 2012) and harkens diversity that is likely to be discovered. The zoospores are spherical and relatively large, typically greater than 4 μm in diameter. The zoospores are distinctive because the nonflagellated centriole is longer than that in other orders, with its length equal to or

exceeding its diameter and with copious densely packaged fibrillar material joining the kinetosome the full length of the nonflagellated centriole. Microtubule roots range from three to none, and an electron-opaque plug in the transition region of the flagellum occurs in three of the five genera. *Lacustromyces* has the most extensive microtubule root system with three roots, one of which is massive and embedded in dense material (Longcore 1993). The MLC is varied and includes multiple lipid globules surrounded by or embedded in an extensive microbody in *Polychytrium aggregatum* and *Lacustromyces hiemalis*. The MLC cisterna is fenestrated only in zoospores of *Arkaya*, and the MLC cisterna is reported to be absent in the other genera.

## 6. Spizellomycetales

The earliest chytrids described were aquatic parasites of algae and were discovered by botanists observing algae (Braun 1851, 1855). Barr (1980) and Longcore et al. (1995) recognized that zoospores of more recently described soil-inhabiting species of two historic genera, *Phlyctochytrium* and *Entophlyctis*, were different from zoospores of the type species of these genera, which were algal parasites. Consequently, new genera were erected for soil-inhabiting species of *Phlyctochytrium* and *Entophlyctis* and were classified in a newly established order, Spizellomycetales (Barr 1980, 1984b; Longcore et al. 1995). Spizellomycetales was the first order separated from Chytridiales based on fundamental differences in zoospore ultrastructure (Barr 1980). Members of Spizellomycetales are distinct from other chytrids because they lack the translation elongation factor 1-alpha gene (EF-1 alpha) and instead possess the paralog, **elongation factor-like gene** (EFL) (James et al. 2006a; Keeling and Inagaki 2004; Simmons 2011; Simmons and Longcore 2012). Whether or not the paralog EFL is due to lateral gene transfer or to gene duplication and loss (Keeling and Inagaki 2004), its presence in all Spizellomycetales examined so far suggests a single evolutionary event corresponding to a major radiation of a chytrid lineage in soil.

Barr (1980) provisionally placed *Rhizoph-lyctis*, *Rozella*, *Olpidium*, and *Caulochytrium* in Spizellomycetales because ribosomes were dispersed in their zoospores and the nucleus was bridged to the kinetosome by either a striated rhizoplast or mitochondrion (Barr and Hadland 1977; Held 1975, 1981; Powell 1981b). Barr (2001) later questioned the relatedness of these taxa, emphasizing marked differences in nuclear features. *Rhizophlyctis*, *Rozella*, *Olpidium*, and *Caulochytrium* are now excluded from Spizellomycetales because phylogenetic placement in molecular analyses confirms Barr's (2001) doubts (James and Berbee 2011; James et al. 2006b; Karpov et al. 2010).

As Spizellomycetales is currently circumscribed (Simmons 2011; Wakefield et al. 2010), all are eucarpic, monocentric, and inoperculate. A great amount of genetic variation and diversity within Spizellomycetales is apparent, even for isolates collected within the same geographic location (Simmons 2011; Simmons and Longcore 2012; Wakefield et al. 2010). There are two monophyletic families, each corresponding to a specific mode of thallus development. Thalli in the Spizellomycetaceae grow epibiotically on substrates and exhibit endogenous development (the nucleus remains in the zoospore cyst, which develops into the sporangium, Fig. 6.2K) (Wakefield et al. 2010). Rhizoids often have a subsporangial swelling (apophysis), and the tips tend to be rounded or blunt (Fig. 6.2K). Spizellomycetaceae contains 4 genera (*Spizellomyces*, *Kochiomyces*, *Gaertneriomyces*, *Triparticalcar*) with 12 validly published species, but *Spizellomyces* and *Gaertneriomyces* are polyphyletic (Wakefield et al. 2010). Thalli in the Powellomycetaceae (Simmons 2011; Simmons and Longcore 2012) grow endobiotically and display exogenous development (the nucleus migrates from the zoospore cyst into the germ tube, and the germ tube grows into the sporangium with rhizoids) (Fig. 6.2D). Generally, the zoospore cyst persists attached to the sporangium and may function as the discharge tube (Powell and Koch 1977; Simmons 2011; Simmons and Longcore 2012). Powellomycetaceae contains

four genera (*Fimicolochytrium*, *Geranomyces*, *Powellomyces*, *Thoreauomyces*) with eight species.

Spizellomycetalean chytrid are essentially ubiquitous in soils (Barr 1980; Wakefield et al. 2010). They are common saprobes of pollen and are found even in harsh and arid environments and in dung. Studies are beginning to explore the dynamics of Spizellomycetales in soil microbial communities and in nutrient dynamics and sustainability (Midgley et al. 2006). From studies focused on molecular detection of fungi from exposed soils at high elevations, spizellomycetalean chytrid phylotypes are prominent components of the fungal community (Freeman et al. 2009; Schmidt et al. 2012). As parasites of nematodes and oospores of downy mildews, they may have a beneficial impact on plants. On the other hand, as parasites of arbuscular mycorrhizae, they may be detrimental to plants [reviewed in Powell and Letcher (2012) and Wakefield et al. (2010)].

Zoospores of the Spizellomycetales can be recognized with a light microscope because they may become polymorphic even while swimming, shifting between elongate, round, or amoeboid. They sometimes swim with their flagellar insertion anterior, trailing the flagellum alongside the zoospore body (Fuller and Jaworski 1987). The constellation of their zoospore ultrastructural characters (Fig. 6.5F) is also distinctive because their ribosomes are dispersed, a portion of the nucleus is positioned adjacent to the kinetosome, an electron-opaque plug is absent from the flagellar transition zone, the nonflagellated centriole is at an acute to right angle with the kinetosome, organelles of the MLC are loosely packaged and the MLC cisterna is never fenestrated, and microtubule roots originate from kinetosome-associated structures and extend anteriorly but are not associated with the MLC. A constellation of zoospore ultrastructural character states distinguishes each genus in Spizellomycetales (Barr 1980, 1981, 1984a, b; Barr and Allan 1981; Longcore et al. 1995; Simmons 2011; Simmons and Longcore 2012). Where multiple types of kinetosome-associated structures were used within a single

genus (*Spizellomyces* and *Gaertneriomyces*), molecular phylogenetics has demonstrated that the genus was polyphyletic (Barr 1980; Simmons 2011; Simmons and Longcore 2012; Wakefield et al. 2010). The variation in zoospore ultrastructure in the Spizellomycetales demonstrates well the intrinsic value of analyzing zoospore ultrastructure when describing new chytrid species.

## 7. Rhizophlyctidales

Rhizophlyctidales (Letcher et al. 2008a) was established as an order delineated from the Spizellomycetales (Barr 1980). In earlier electron microscopic analyses of zoospores of isolates putatively identified as *Rhizophlyctis rosea*, Barr and Désaulniers (1986) discovered four distinct zoospore subtypes. These observations presaged the great diversity Letcher et al. (2008a) later found in their analyses of morphology, zoospore ultrastructure, and nuclear large subunit (LSU) and internal transcribed spacer region (ITS) rRNA gene sequences of 49 isolates in the *R. rosea* complex from globally distributed soil samples, a study that included isolates previously studied ultrastructurally (Barr and Désaulniers 1986; Barr and Hartmann 1977). In molecular phylogenetic studies, Rhizophlyctidales places as the sister group of Spizellomycetales (Fig. 6.1) (James et al. 2006b), but the thalli, with multiple rhizoids emanating from the sporangial surface, and the distinctive zoospore ultrastructure distinguish its members from those in the Spizellomycetales (Letcher et al. 2008a). In addition to members with monocentric thalli, *Catenomyces persicinus* is a polycentric taxon in the order (James et al. 2006b). Rhizophlyctidales also differs from Spizellomycetales genetically, as evidenced by the possession of the translation elongation factor 1-alpha gene instead of the paralog elongation factor-like gene characteristic of Spizellomycetales (James et al. 2006a; Keeling and Inagaki 2004; Simmons 2011).

Rhizophlyctidales (Letcher et al. 2008a) includes four monophyletic families, each distinguishable morphologically and corresponding to one of the four zoospore subtypes (Barr and Désaulniers 1986). Each of the four families contains a single described genus (Rhizophlyctidaceae: *Rhizophlyctis*, Sonoraphlyctidaceae: *Sonoraphlyctis*, Arizonaphlyctidaceae: *Arizonaphlyctis*, and Borealophlyctidaceae: *Borealophlyctis*), but many isolates remain uncharacterized taxonomically. *R. rosea* is the type of *Rhizophlyctis* (Blackwell and Powell 1999), but the genus is not monophyletic because several of its species are known to reside in other orders (Letcher et al. 2006; Longcore and Simmons 2012). *R. rosea* is by far the most commonly collected and studied species within the order [reviewed in Letcher et al. (2008a)], and species of the other three genera are more rarely found (Letcher et al. 2008a). *R. rosea* can be considered a morphospecies because among 42 isolates in Rhizophlyctidaceae sequences were highly divergent, LSU > 91% and ITS > 60% (Letcher et al. 2008a). Despite the divergence of the morphospecies, phylotypes can be either cosmopolitan, with similar phylotypes found on different continents, or divergent, with dissimilar phylotypes found in the same location (Letcher et al. 2008a).

Members of Rhizophlyctidales are primarily terrestrial saprobes of cellulosic substrates. *R. rosea* is ubiquitous in agricultural and horticultural soils and vegetative debris matter and may survive periods of extended soil drying as desiccated sporangia (Gleason et al. 2004; Willoughby 1998; 2001) or in a resistive, resting stage (Johanson 1944). Thus, it was unexpected that molecular sequence analyses of environmental samples detected phylotypes of this clade in lakes (Monchy et al. 2011). It is possible that dispersion of propagules of these fungi may occur from soil perturbation via agricultural runoff or airborne desiccated sporangia or resting spores.

Although morphologically diverse, the four zoospore types in Rhizophlyctidales have in common a unique suite of ultrastructural features [see Fig. 6 in Letcher et al. (2008a)]. Their zoospores (Fig. 6.5G) lack a transition zone plug, organelles of the MLC are loosely arranged, the nonflagellated centriole is positioned at an acute angle to the kinetosome, and

neither microtubule roots nor microtubules have been observed. In addition, the zoospore of *R. rosea* is characterized by dispersed ribosomes, multiple lipid globules, numerous mitochondria closely associated with the nucleus, a centrally located nucleus, and a fibrillar rhizoplast (Fig. 6.4L) extending from the kinetosome and nonflagellated centriole to the posterior end of the nucleus with closely clustered microbodies (Barr and Hartmann 1977; Letcher et al. 2008a).

## 8. *Synchytrium* Lineage

In sorting taxa formerly classified in the Chytridiales into monophyletic orders, several groups await official circumscriptions. One of these is the *Synchytrium* clade positioned as the sister group of the Lobulomycetales (James et al. 2006b). The family Synchytriaceae is excluded from Chytridiales because of a lack of monophyly with this order. The genus *Synchytrium* is composed of more than 250 described species of obligate plant and algal parasites (Karling 1964). At some stage of development the thallus is colonial, producing sporangia in sori, and sexual reproduction occurs with the fusion of motile gametes (Karling 1964). *S. endobioticum*, the causal agent of **potato wart disease**, has the potential to destroy crops, make soils unusable for potato crops for years, and result in the quarantine of a district's potatoes (Powell 1993). Although essentially eliminated from the USA (Putnam and Hampson 1989), *S. endobioticum* remains a threatening **pathogen of potatoes** in many regions of the world despite strict global quarantine regulations. *S. endobioticum* is on the US list of select agents and toxins that pose threats of economic damage to major agricultural crops if reintroduced (Rossman et al. 2006). On the other hand, species are being explored as potential **biocontrol agents** of invasive plants. Reports of *Synchytrium minutum* on kudzu (*Pueraria lobata*) vines in Korea demonstrate that this species is widespread in Asia and has generated interest as a biocontrol agent for kudzu in regions where it is invasive with no

natural controls (Yun et al. 2011). Another species, *Synchytrium solstitiale*, is being evaluated as a biological control agent for the yellow starthistle, an invasive plant in the western USA (Bruckart et al. 2011).

The *Synchytrium* clade includes *S. decipiens*, *S. endobioticum*, *S. macrosporum*, *Synchytrium* sp. (James et al. 2006b), and *S. minutum* (Yun et al. 2011). Partial SSU sequences of these five isolates of *Synchytrium* are 85–95 % similar, while two strains of *S. endobioticum* (P-58 and AS-1) are only 90 % similar. *S. endobioticum* contains at least 20 pathotypes, and a cooperative global effort has attempted to unify the coding of pathotypes (Baayen et al. 2006; Stachewicz and De Boer 2002). Comparisons of sequence similarities indicate significant molecular divergence among *Synchytrium* taxa and potential issues with taxon identification based on morphology.

The zoospore ultrastructure is known only for *S. endobioticum* (Fig. 6.5H) (Lange and Olson 1978) and *S. macrosporum* (Montecillo et al. 1980), and the zoospores of these two species are remarkably different. Zoospores of these taxa have a suite of ultrastructural features that includes a single lipid globule, dispersed ribosomes, microbodies associated with both the lipid globule and the nucleus, an angled orientation of the nonflagellated centriole relative to the kinetosome, and microtubules that radiate from the kinetosome into the cytoplasm. The zoospore of *S. endobioticum* has an electron-opaque plug at the base of the flagellum and a simple MLC cisterna, while the zoospore of *S. macrosporum* has no plug in the flagellum base and has a fenestrated MLC cisterna.

## 9. *Blyttiomyces helicus* Lineage

*Blyttiomyces helicus* is the single taxon in a lineage sister of the group composed of Spizellomycetales and Rhizophlyctidales; and the inclusive grouping of *B. helicus*, Spizellomycetales, and Rhizophlyctidales is a sister group of Rhizophydiales (James et al. 2006b). *B. helicus* is morphologically stunning among chytrids, having a golden-brown sporangium ornamented with spiral bands (Sparrow and Barr 1955).

A saprobe of pollen, it has not been isolated into pure culture, and its SSU sequence was obtained from an enriched unifungal culture on pollen. From a different isolate on pollen, ultrastructural studies of the zoospore were conducted by sectioning multiple sporangia containing cleaved zoospores

The zoospore ultrastructure (Fig. 6.5I) of *B. helicus* is quite different from that of either Spizellomycetales or Rhizophlyctidales and more closely resembles that of Rhizophydiales. Ultrastructural features that are common to *B. helicus* and Rhizophydiales include aggregated ribosomes, a single lipid globule, a fenestrated MLC cisterna, shieldlike KASs, and the absence of an electron-opaque plug at the base of the flagellum. The zoospore of *B. helicus* is morphologically distinct, however, in having one or more prominent, often anvil-shaped, cisternae with granular matrices adjacent to the lipid globule, which seem to be continuous with the fenestrated MLC cisterna (Fig. 6.5I).

*Blyttiomyces spinulosus* is the type species of the genus and has not been isolated or characterized molecularly or ultrastructurally (Blackwell et al. 2011). Thus, whether *B. helicus* is molecularly and ultrastructurally representative of the genus is uncertain at this time.

## B. Monoblepharidomycota

Among extensive nomenclatural revisions, Doweld (2001) elevated the order Monoblepharidales and formally described it as a phylum with a new class, order, and family (Table 6.1). Monoblepharidomycota is monophyletic and typically placed as the sister group of the Chytridiomycota (Bullerwell et al. 2003; James et al. 2006b) (Fig. 6.1), although the position of monoblephs in phylogenetic trees is not stable (Bullerwell and Lang 2005; Einax and Voigt 2003; Sekimoto et al. 2011). However, the phylum seems to be monophyletic in studies that include a broad range of zoosporic fungi (James et al. 2006b; Sekimoto et al. 2011). Determining whether proposed subphylum groupings are monophyletic awaits molecular analyses of a broader range of taxa.

Monoblepharidomycota is a distinctive group among zoosporic fungi because of their **oogamous sexual reproduction**, and this feature supports Doweld's (2001) recognition of monoblephs as a phylum. Monoblephs are

saprotrophs, and no parasites are known (Emerson 1958, 1964; Emerson and Whisler 1968; Perrott 1955, 1958). Monoblephs and chytrids are also similar in a number of features, including **plasmodesmata** in septa (Powell 1974; Powell and Gillette 1987), **mitosis** with the nuclear envelope opened only at the poles at metaphase (Dolan and Fuller 1985; Powell 1975, 1980; Roychoudhury and Powell 1991; Whisler and Travland 1973), and initiation of **zoospore cleavage** before elongation of the flagellar axoneme (McNitt 1974). Monobleph thalli produce terminal sporangia and are filamentous with a basal holdfast or rhizoidal system, and vacuolated cytoplasm gives the thallus a foamy appearance (Fig. 6.2G). The filament may be short and mostly occupied by the sporangium as in *Harpochytrium* (Fig. 6.2F–H) or hypha-like as in *Monoblepharella*. The thallus of *Oedogoniomyces* attaches to a variety of substrates, including snail shells, seeds, and algae without penetration (Emerson and Whisler 1968). Molecular phylogenetic analysis unexpectedly revealed that the colorless green alga *Hyaloraphidium curvatum* placed as the sister group of all other taxa in the monoblephs (Forget et al. 2002; Ustinova et al. 2000). Its thallus is similar to *Harpochytrium*, but its sporangium releases **autospores** (Ustinova et al. 2000). Thus, it seems that loss of zoospore motility has occurred in monoblephs and chytrids (e.g., *Amoebochytrium*, *Sporophlyctis*).

As monobleph zoospores swim, they are elongate and tapered toward the anterior end. The zoospore ultrastructure (Fig. 6.5J) of all five genera that produce zoospores (*Gonapodya*, *Harpochytrium*, *Oedogoniomyces*, *Monoblepharis*, *Monoblepharella*) has been studied (Fuller 1966; Fuller and Reichle 1968; Gauriloff et al. 1980a, b; Mollicone and Longcore 1994, 1999; Reichle 1972; Travland and Whisler 1971). The most distinguishing features of the zoospore are their spherical mitochondria and the position of the MLC cisterna, which lies adjacent to the plasma membrane but backs the microbody, instead of lipid globules (Fig. 6.4M) (Dorward and Powell 1980) as in chytrids (Powell 1978). The MLC is fenestrated and was initially named the rumposome, but the complexity of the cisterna varies from a cisterna

with shallow pores (Fuller and Reichle 1968) to deep pores (Reichle 1972). In zoospores of *Harpochytrium* the fenestrated cisterna connects to the striated rootlet (Travland and Whisler 1971). An electron-opaque plug is in the transition region of the flagellum but is lost in *Gonapodya polymorpha* (Mollicone and Longcore 1999), a lineage distinct from *Gonapodya prolifera* and characterized by a greater number of bases in the LSU C1_3 helix (Chambers 2003) and by the unique presence of a paraxonemal structure (Mollicone and Longcore 1999). Consistent with these observations, in initial molecular analyses, *G. polymorpha* and *G. prolifera* are not monophyletic (Chambers 2003). Zoospores of all genera are similar in that the endoplasmic reticulum both binds and penetrates the ribosomal aggregation surrounding the nucleus and a microtubule root radiates anteriorly from a striated disk that partially encircles the kinetosome. Lipids vary in their locations from predominantly posterior (Travland and Whisler 1971) to anterior (Fuller and Reichle 1968), but the reticulate microbody consistently extends between lipids and mitochondria and the area of the flagellar apparatus (Dorward and Powell 1980; Gauriloff et al. 1980a, b; Mollicone and Longcore 1999). Thus, despite the scattered nature of organelles of the MLC, they are interconnected, which is important for their functions (Powell 1976b, 1978).

## C. Neocallimastigomycota

Neocallimastigomycota is comprised of obligate anaerobic zoosporic fungi and specialized commensals growing in the digestive system of herbivores, and their zoospores may bear from 1 to 20 posterior flagella. Flagella of polyflagellate neocallimastigos often adhere together, which might be adaptive to swimming through viscous digestive fluids (Gold et al. 1988). In most molecular phylogenetic analyses, the Neocallimastigomycota is placed as the sister group of the Monoblepharidomycota + Chytridiomycota (James et al. 2006b).

Searching for the true taxonomic affinities of these organisms, Orpin (1975, 1977) detected chitin in the cell walls of *Neocallimastix*, astutely ascribing their kinship to fungi.

About a decade later, Heath et al. (1983) recognized that their posteriorly uniflagellate zoospores and microscopic thalli allied them to chytrid fungi. After another decade, Li et al. (1993) established the order Neocallimastigales within the Chytridiomycota. However, neocallimastigos are distinctive from all other groups of zoosporic fungi in the absence of flagellar props in zoospores (Gold et al. 1988). They also differ from monoblephs and chytrids in many developmental characteristics: the nuclear envelope is totally closed at metaphase [reviewed in Li et al. (1993)], plasmodesmata (Powell 1974) have not been observed in septa (Gold et al. 1988; Heath et al. 1983), during zoospore cleavage axonemes extend into flagellar vesicles prior to cleavage of zoospore bodies (Gold et al. 1988; Heath et al. 1983), the whole flagellum including the kinetosome is shed (Gold et al. 1988; Orpin 1975) instead of retracted during zoospore encystment (Koch 1968), no centrioles are associated with vegetative cell nuclei (Heath et al. 1986), and zoospores contain no nonflagellated centrioles (Heath et al. 1983; Li et al. 1991). Because of these differences and their position as a monophyletic group, neocallimastigos have recently been formally established as a phylum (Hibbett et al. 2007).

Neocallimastigos produce monocentric and polycentric thalli with extensive rhizoids or a bulbous haustorium-like structure (Gold et al. 1988; Ho and Barr 1995), and they discharge zoospores bearing one to several posterior flagella. Zoospores may be spherical, oval, or pyriform and are capable of amoeboid movement during which their form is irregular (Orpin 1975). Even within the same isolate, zoospore diameters vary, but those with single flagella are typically smaller than zoospores with multiple flagella. Evidence of sexual reproduction has never been observed among these organisms. Ho and Barr (1995) produced the most current monograph of the neocallimastigos, and only *Cyllamyces* has been added since then (Ozkose et al. 2001). The neocallimastigos constitute a relatively small group with 3 monocentric genera (*Caecomyces*, *Neocallimastix*, *Piromyces*), 3 polycentric genera (*Anaeromyces*, *Cyllamyces*, *Orpinomyces*), and 21 species (Eck-

art et al. 2010; Ho and Barr 1995; Ozkose et al. 2001). Most molecular phylogenetic studies of Neocallimastigomycota have utilized SSU and ITS1 ribosomal genes, where described genera have been supported but with genera showing varying degrees of divergence among species, as predicted with light and electron microscopic observations (Brookman et al. 2000; Ho and Barr 1995; Li et al. 1993). Molecular environmental studies have revealed that the diversity of this group is much greater than has been described, with more taxa to be characterized (Fliegerová et al. 2010; Liggenstoffer et al. 2010; Nicholson et al. 2010).

The neocallimastigos have attracted interest because of their **biotechnology** potentials in industrial applications (Chu et al. 2011), conversion of plant materials into **biofuels** [reviewed in Elshahed (2010)], and increased food efficiency when low-grade fibrous plant material is used as feed for herbivores (Ho and Barr 1995; Nagpal et al. 2011). As early colonizers of plant material in the rumen, neocallimastigos extensive rhizoidal system physically penetrates refractory, cellulose-containing fibrous plant materials and chemically degrades cellulose and other wall compounds with a whole battery of wall-degrading enzymes, allowing an increased surface area for additional degradation by cellulolytic bacteria and protozoa (Ho and Barr 1995; Montford and Orpin 1994; Orpin and Letcher 1979; Tachezy 2008). Because herbivorous mammals lack the enzymes to break down fibrous lignocellulosic-containing feed, neocallimastigos are vital to the **feed efficiency** of substrates that would otherwise be undigestible by host animals. Genome-sequencing projects, such as those for *Piromyces* and *Orpinomyces*, will greatly facilitate our understanding of genes that are potentially useful in biofuel production and the breakdown of cellulose (Griffith et al. 2010; Nagpal et al. 2011).

Zoospores of neocallimastigos (Fig. 6.5K) were once thought to be flagellated protozoa, but careful developmental studies link two life history stages, the zoospore stage and the monocentric or polycentric thallus stage found attached to fibrous feed (Orpin 1975, 1977; Orpin and Bountiff 1978). Like all flagellated

opisthokonts, neocallimastigos have posteriorly directed flagella, and the possession of a transitional helix (=concentric fiber) (Barr 2001; Heath et al. 1983; Li et al. 1991) is a symplesiomorphic character shared with chytrids, monoblephs, and Blastocladiomycota. The ultrastructure of the zoospore differs in the cellular architecture and the range of flagella numbers from those of all other zoosporic fungi. Zoospores of neocallimastigos often have a protrusion opposite the flagellum, and the flagellum is inserted into a concave invagination at the posterior end of the zoospore (Gold et al. 1988). Unique to neocallimastigos zoospore, megatubules form a posterior dome. Instead of flagellar props characteristic of other flagellated fungi, they have a novel kinetosome/flagellar-associated complex with a circumflagellar ring lying just under the plasma membrane where the flagellum emerges from the zoospore body (Gold et al. 1988). A cup-shaped scoop covers the anterior end of the kinetosome, and several struts link the scoop to the circumflagellar ring (Gold et al. 1988). From a globular spur of electron-dense material near the anterior side of the kinetosome several microtubule roots arise, one root extending as a lateral sheet along the plasma membrane and another root flaring anteriorly toward the nucleus (Gold et al. 1988; Heath et al. 1983; Li et al. 1991). Hydrogenosomes cluster in the posterior end of the zoospore and along the side of the beaked extension of the nucleus (Fig. 6.5K). Ribosomes reportedly occur toward the anterior end of the cell as clusters and helices (Gold et al. 1988).

## VII. Evolution

*Rozella* spp., once classified in Chytridiomycota, is placed as the sister group of all other fungi (Fig. 6.1). *Rozella* is an unwalled obligate endoparasite of other fungi and oomycetes (Held 1975, 1981). In some molecular studies (James and Berbee 2011; Karpov et al. 2013) *Rozella* spp. are affiliated with aphelids and the unwalled endoparasites of animals, Microsporidia. However, the phylogenetic placement

of Microsporidia is still controversial (Corradi and Keeling 2009). The clade that includes *Rozella* spp. also includes a genetically diverse array of phylotypes detected from environmental samples of freshwater, marine sediments, and peat bogs and was informally named the Rozellida (Lara et al. 2010; Lepère et al. 2008; Nagahama et al. 2011) and formally circumscribed as the Cryptomycota (Jones et al. 2011). Cryptomycota is a highly derived and diverse group of organisms, and whether they are considered fungi is a matter of where the basal limits are drawn. Brown et al. (2009) proposed the Nucletmycea as a supergroup for the large clade that includes the filose-pseudo-podia-forming nucleariids, the cellular slime mold *Fonticula alba*, the **Cryptomycota**, and fungi.

**Neocallimastigos** seem to share a common posteriorly uniflagellate aerobic ancestor with chytrids but diverged from chytrids in a lineage that adapted them to an anaerobic habitat (Fig. 6.1). Rather than respiration with mitochondria, they utilize hydrogenosomes that produce ATP by substrate-level phosphorylation (van der Giezen et al. 2003). **Hydrogenosomes** seem to be secondarily derived from mitochondria. Although these organelles have lost their mitochondrial genome (Bullerwell and Lang 2005), they retain two surrounding membranes and share protein-importing mechanisms (van der Giezen 2009; van der Giezen et al. 2003). From the close interaction between rumen bacteria and neocallimastigos in the gut of herbivores and the facility bacteria have in transferring genes, it seems that in the divergence of neocallimastigos from other zoosporic fungi they obtained numerous genes for enzymes important in the degradation of plant material by horizontal gene transfer from bacteria. This seems to be the case for glycosyl hydrolases (Garcia-Vallvé et al. 2000) and, perhaps, cellulase because of the presence of cellulosomes, multienzyme complexes that in neocallimastigos degrade crystal cellulose directly into glucose (Ljungdahl 2008).

Phylogenetic hypotheses based on the analyses of molecular sequences suggest that the ancestors of zoosporic fungi were unwalled nucleariid amoebae (Amaral Zettler et al. 2001; Brown et al. 2009; Bullerwell and Lang 2005; Liu et al. 2009; Steenkamp et al. 2006). Like fungi, nucleariid amoebae have flattened discoid mitochondrial cristae. With the production of a cyst wall and polarized growth, the filamentous (hyphalike) thallus form may have evolved leading to two basal and diverging zoosporic fungal groups, Blastocladiomycota and Monoblepharidomycota. Among the basal filamentous zoosporic fungi, a Spitzenkörper-like assemblage, which is characteristic of higher fungal hyphae, has only been found in *Allomyces* (Blastocladiomycota) (Vargas et al. 1993). The simpler eucarpic, monocentric thallus found among chytrids (Fig. 6.2C, J, K) may have been derived from ancestors with filamentous thalli consisting of short filaments bearing a basal holdfast and terminal sporangium, as in the monoblephs (Fig. 6.2 F–H). Thalli composed of anucleate rhizoids and a terminal sporangium occur in multiple lineages of chytrids. Much of the developmental variation among chytrid thalli depends upon the behavior of the nucleus following zoospore encystment and germination (Blackwell et al. 2006; Powell and Koch 1977). Whether the nucleus remains in the zoospore cyst (as in *Rhizophydium*), migrates out of the cyst into its germ tube (as in *Powellomyces*), moves with the protoplast into a host cell (as in *Synchytrium*), or continues to migrate along a rhizomycelium (as in *Nowakowskiella*) determines thallus morphology. Even within a clonal chytrid isolate, the pattern of nuclear migration can vary with differing environmental conditions (Powell and Koch 1977). The fact that monocentric and polycentric thallus forms occur within the same monophyletic order (such as Chytridiales, Cladochytriales, and Polychytriales) suggests that there is not great phylogenetic significance in the differences in thallus complexity within an order. Whether a chytrid is monocentric or polycentric may be a matter of how **nuclear migration genes** are regulated, and understanding the evolution and regulation of genes associated with nuclear positioning (Morris

2000) is key to understanding the evolutionary basis of chytrid development.

**Substrate utilization** may have been a selective factor in the evolution of saprobic chytrids. Rhizophlyctidales and Cladochytriales primarily use cellulose as a substrate, and Polychytriales and Chytridiales have lineages that use chitin exclusively. **Habitat** also seems to have exerted selective pressure in zoosporic evolution. Terminal groups (Fig. 6.1), such as Rhizophlyctidales and Spizellomycetales, are more often found in soil and dung than in bodies of water. No verification of sexual reproduction has been discovered in either of these orders, which means their adaptation to soil is primarily clonal.

Evidence suggests that basal fungi evolved in **freshwater aquatic systems** because no marine Blastocladiomycota or Monoblepharidomycota have been discovered, and most aquatic chytrids are found in freshwater. However, marine representatives are scattered among the orders of Chytridiomycota, having been described in genera now classified in Chytridiales, Rhizophydiales, Lobulomycetales, and Cladochytriales. Freshwater forms may have become secondarily adapted to halophytic soils, brackish waters, and marine habitats. There is a gradation of organisms that grow in estuarine areas (with great fluctuation in salinity) to authentic marine fungi growing on algal hosts or crab eggs (Amon 1976; Booth 1971; Johnson and Sparrow 1961; Karling 1977; Müller et al. 1999; Nyvall et al. 1999; Shields 1990; Sparrow 1960; reviewed in Gleason et al. 2011). The presence of chytrid phylotypes detected in deep-sea hydrothermal vents and cold seep sediments highlights the underexplored diversity of marine chytrids (LeCalvez et al. 2009; Nagahama et al. 2011).

With molecular-based phylogenetics we can begin to trace the pattern of inheritance of **zoospore ultrastructural features**, with character states being transformed or lost. In the phylogentic hypothesis with monoblephs as the sister group of chytrids (Fig. 6.1), the shared common ancestor would be aquatic with a filamentous/hypha-like thallus. Zoospores would

have contained a fenestrated MLC cisterna, ribosomal aggregation, and electron-opaque plug in the flagellar transition region. Thus, in **terminal clades** such as Spizellomycetales, we find that in their divergence each of these characters has been lost and the organisms are well adapted to terrestrial habitats primarily as saprobes (Wakefield et al. 2010).

## VIII. Conclusions

Exploration of new habitats and refinements in recognition of diversity among zoosporic fungi has revealed the untapped diversity of these organisms. Emerging molecular techniques for rapid sequencing of genes and total genomes will transform our understanding of zoosporic fungi in the next 10 years, as have applications of gene sequences and ultrastructural characters in the past decade. The genomes of only a few chytrids (*B. dendrobatidis* isolates JEL 423 and JAM 81; *Homolaphlyctis polyrhiza* JEL 142; *Spizellomyces punctatus* isolate BR 117), monoblephs (*Gonapodya* sp. isolate JEL 183), and neocallimastigos (*Piromyces* sp., *Orpinomyces* sp. isolate OUS 1) have been sequenced (Joneson et al. 2011; Stajich 2011). But even with the scant knowledge we have, access to sequenced genomes of zoosporic fungi is impacting our views of the evolution of genes in fungi. Rosenblum et al. (2008) have exploited the sequenced genome of *B. dendrobatidis* to use whole-genome arrays and track differential gene expression in zoospores and sporangia. Idnurm et al. (2010) used comparative genomics to identify putative light-receptive genes from the sequenced genome of *S. punctatus*. Zoosporic fungi have been considered intractable to genetic transformation because no procedures have been successfully developed with Chytridiomycetes, thwarting our ability to compare gene functions. However, a recently developed transformational system for Blastocladiomycota might prove useful for chytrids as well (Vieira and Camilo 2011). Recognizing the importance of zoosporic fungi in soil fertility (Midgley et al. 2006), in food webs (Kagami

et al. 2007, 2011, 2012; Sime-Ngando et al. 2011), as pollution indicators (Dileo et al. 2010), and in biotechnology invites more extensive exploration of the diversity and ecological roles of these fascinating fungi.

# References

Amaral Zettler LA, Nerad TA, O'Kelly CJ, Sogin ML (2001) The nucleariid amoebae: more protists at the animal-fungal boundary. J Eukaryot Microbiol 48:293–297

Amon JP (1976) An estuarine species of *Phlyctochytrium* (Chytridiales) having a transient requirement for sodium. Mycologia 68:470–480

Baayen RP, Cochius G, Hendriks H, Meffert JP, Bakker J, Bekker M, van den Boogert PHJF, Stachewicz H, van Leeuwen GCM (2006) History of potato wart disease in Europe—a proposal for harmonization in defining pathotypes. Eur J Plant Pathol 116:21–31

Bai C, Garner TWJ, Li Y (2010) First evidence of *Batrachochytrium dendrobatidis* in China: discovery of chytridiomycosis in introduced American bullfrogs and native amphibians in the Yunnan Province, China. EcoHealth 7:127–134

Bandoni RJ, Barr DJS (1976) On some zoosporic fungi from washed terrestrial litter. Trans Mycol Soc Japan 17:220–225

Barr DJS (1973) *Rhizophydium graminis* (Chytridiales): morphology, host range, and temperature effect. Can Plant Dis Surv 53:191–193

Barr DJS (1980) An outline for the reclassification of the Chytridiales, and for a new order, the Spizellomycetales. Can J Bot 58:2380–2394

Barr DJS (1981) Ultrastructure of the *Gaertneriomyces* zoospore (Spizellomycetales, Chytridiomycetes). Can J Bot 59:83–90

Barr DJS (1984a) Cytological variation in zoospores of *Spizellomyces* (Chytridiomycetes). Can J Bot 62:1202–1208

Barr DJS (1984b) The classification of *Spizellomyces*, *Gaertneriomyces*, *Triparticalcar*, and *Kochiomyces*. Can J Bot 62:1171–1201

Barr DJS (1986) *Allochytridium expandens* rediscovered: morphology, physiology and zoospore ultrastructure. Mycologia 78:439–448

Barr DJS (1990) Phylum Chytridiomycota. In: Margulis L, Corliss JO, Melkonian M, Chapman DJ (eds) Handbook of Protoctista. Jones and Bartlett, Boston, MA, pp 454–466

Barr DJS (2001) Chytridiomycota. In: Esser K, Lemke PA (eds) The mycota, systematics and evolution, vol VIIA. Springer, New York, pp 93–112

Barr DJS, Allan PME (1981) Ultrastructure of *Kochiomyces* and *Triparticalcar* zoospores (Spizellomycetales, Chytridiomycetes). Can J Bot 59:649–661

Barr DJS, Babcock CE (1994) Culture collection information: cryopreservation of unicellular zoosporic fungi. US Fed Cult Collections Newslett 24:6

Barr DJS, Désaulniers NL (1986) Four zoospore subtypes in the *Rhizophlyctis-Karlingia* complex (Chytridiomycetes). Can J Bot 64:561–572

Barr DJS, Désaulniers NL (1987) *Allochytridium luteum* n. sp.: morphology, physiology and zoospore ultrastructure. Mycologia 79:193–199

Barr DJS, Désaulniers NL (1988) Precise configuration of the chytrid zoospore. Can J Bot 66:869–876

Barr DJS, Hadland-Hartmann VE (1978) Zoospore ultrastructure in the genus *Rhizophydium* (Chytridiales). Can J Bot 56:2380–2404

Barr DJS, Hartmann VE (1976) Zoospore ultrastructure of three *Chytridium* species and *Rhizoclosmatium globosum*. Can J Bot 54:2000–2013

Barr DJS, Hartmann VE (1977) Zoospore ultrastructure of *Olpidium brassicae* and *Rhizophlyctis rosea*. Can J Bot 55:1221–1235

Barr DJS, Désaulniers NL, Knox JS (1987) *Catenochytridium hemicysti* n. sp.: morphology, physiology and zoospore ultrastructure. Mycologia 79:587–594

Beakes GW, Canter HM, Jaworski GHM (1988) Zoospore ultrastructure of *Zygorhizidium affluens* and *Z. planktonicum*, two chytrids parasitizing the diatom *Asterionella formosa*. Can J Bot 66:1054–1067

Beakes GW, Canter HM, Jaworski GHM (1992) Ultrastructural study of operculation (discharge apparatus) and zoospore discharge in zoosporangia of *Zygorhizidium affluens* and *Z. planktonicum*, chytrid parasites of *Asterionella formosa*. Mycol Res 96:1060–1067

Beakes GW, Canter HM, Jaworski GHM (1993) Sporangium differentiation and zoospore fine-structure of the chytrid *Rhizophydium planktonicum*, a fungal parasite of *Asterionella formosa*. Mycol Res 97:1059–1074

Bills GF, Christensen M, Powell MJ, Thorn G (2004) Saprobic soil fungi. In: Mueller GM, Bills GF, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier, Oxford, pp 271–302

Blackwell WH, Powell MJ (1999) The nomenclatural propriety of *Rhizophlyctis rosea*. Mycotaxon 70:213–217

Blackwell WH, Letcher PM, Powell MJ (2004) Synopsis and systematic reconsideration of *Karlingiomyces* (Chytridiomycota). Mycotaxon 89:259–276

Blackwell WH, Letcher PM, Powell MJ (2006) Thallus development and the systematics of Chytridiomycota: an additional developmental pattern represented by *Podochytrium*. Mycotaxon 97:91–109

Blackwell WH, Letcher PM, Powell MJ, Vélez CG (2011) The occurrence of *Blyttiomyces spinulosus* in Alabama and Argentina, and comments on the genus *Blyttiomyces* (Chytridiomycota). Phytologia 93:304–315

Booth T (1971) Ecotypic responses of chytrid and chytridiaceous species to various salinity and temperature combinations. Can J Bot 49:1757–1767

Braun A (1851) Betrachtungen über die Erscheinung der Verjüngung in der Natur, insbesondere in der Lebens- und Bildungsgeschichte der Pflanze, Leipzig

Braun A (1855) Über *Chytridium*, eine Gattung einzelner Schmarotzergewächse auf Algen und Infusorien. Monatsber Berlin Akad 1855:378–384

Brookman JL, Mennim G, Trinci APJ, Theodorou MK, Tuckwell DS (2000) Identification and characterization of anerobic gut fungi using molecular methologies based on ribosomal ITS1 and 18S rRNA. Microbiology 146:393–403

Brown MW, Spiegel FW, Silberman JD (2009) Phylogeny of the "forgotten" cellular slime mold, *Fonticula alba*, reveals a key evolutionary branch within Opisthokonta. Mol Biol Evol 26:2699–2709

Bruckart WL III, Eskandari FM, Widmer TL (2011) *Synchytrium solstitiale*: reclassification based on the function and role of resting spores. Mycologia 103:775–778

Bruning K, Lingeman R, Ringelberg J (1992) Estimating the impact of fungal parasites on phytoplankton populations. Limnol Oceanogr 37:252–260

Bullerwell CE, Lang BF (2005) Fungal evolution: the case of the vanishing mitochondrion. Curr Opin Microbiol 8:362–369

Bullerwell CE, Forget L, Lang BF (2003) Evolution of monobleparidalean fungi based on complete mitochondrial genome sequences. Nucleic Acids Res 31:1614–1623

Canter HM, Lund JWG (1951) Studies on plankton parasites. III. Examples of the interaction between parasitism and other factors determining the growth of diatoms. Ann Bot (Oxford) 15:359–371

Cavalier-Smith T (1998) A revised six-kingdom system of life. Biol Rev 73:203–266

Chambers JG (2003) Ribosomal DNA, secondary structure, and phylogenetic relationships among the Chytridiomycota. Ph.D. dissertation, University of Alabama, Tuscaloosa, AL

Chen M, Chen F, Yu Y, Ji J, Kong F (2008) Genetic diversity of eukaryotic microorganisms in Lake Taihu, a large shallow subtropical lake in China. Microb Ecol 56:572–583

Chu C-Y, Tseng C-W, Yueh P-Y, Duan C-H, Liu J-R (2011) Molecular cloning and characterization of a β-glucanase from *Piromyces rhizinflatus*. J Biosci Bioeng 111:541–546

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

Couch JN (1939) Technic for collection, isolation and culture of chytrids. J Elisha Mitchell Sci Soc 55:208–214

Dileo K, Donat K, Min-Venditti A, Dighton J (2010) A correlation between chytrid abundance and ecological integrity in New Jersey pine barrens waters. Fungal Ecol 3:295–301

Dogma IJ (1973) Developmental and taxonomic studies on rhizophlyctoid fungi, Chytridiales I. Dehiscence mechanisms and generic concepts. Nova Hedwigia 24:393–411

Dolan TE, Fuller MS (1985) The ultrastructure of nuclear division in *Monoblepharella* sp. Mycologia 77:791–809

Dorward DW, Powell MJ (1980) Microbodies in *Monoblepharella* sp. Mycologia 72:549–557

Dorward DW, Powell MJ (1982) Cross-linking bridges associated with the microbody-lipid globule complex in *Chytriomyces aureus* and *Chytriomyces hyalinus*. Protoplasma 112:181–188

Dorward DW, Powell MJ (1983) Cytochemical detection of polysaccharides and the ultrastructure of the cell coat of zoospores of *Chytriomyces aureus* and *Chytriomyces hyalinus*. Mycologia 75:209–220

Doweld A (2001) Prosyllabus tracheophytorum: Tentamen systematis plantarum vascularium (Tracheophyta). Geos, Moscow

Ebersberger I, Simoes R, Kupczok A, Gube M, Kothe E, Voigt K, von Haeseler A (2012) A consistent phylogenetic backbone for the fungi. Mol Biol Evol 29:1319–1334

Eckart M, Fliegerová K, Hoffmann K, Voigt K (2010) Molecular identification of anaerobic rumen fungi. In: Gherbawy Y, Voigt K (eds) Molecular identification of fungi. Springer, Berlin, Heidelberg, New York, pp 297–313

Einax E, Voigt K (2003) Oligonucleotide primers for the universal amplification of b-tubulin genes facilitate phylogenetic analyses in the regnum Fungi. Org Divers Evol 3:185–194

Elshahed MS (2010) Microbiological aspects of biofuel production: current status and future directions. J Adv Res 1:103–111

Emerson R (1958) Mycological organization. Mycologia 5:589–621

Emerson R (1964) Performing fungi. Am Biol Teach 26:90–100

Emerson R, Whisler HC (1968) Cultural studies of *Oedogoniomyces* and *Harpochytrium*, and a proposal to place them in a new order of aquatic phycomycetes. Arch Mikrobiol 61:195–211

Fliegerová K, Mrázek F, Hoffmann K, Zábranská J, Voigt K (2010) Diversity of anaerobic fungi within cow manure determined by ITS1 analysis. Folia Microbiol 55:319–325

Forget L, Ustinova J, Wang Z, Huss VAR, Lang BF (2002) *Hyaloraphidium curvatum*: a linear mitochondrial genome, tRNA editing, and an evolutionary link to lower fungi. Mol Biol Evol 19:310–319

Freeman KR, Martin AP, Karki D, Lynch RC, Mitter MS, Meyer AF, Longcore JE, Simmons DR, Schmidt SK (2009) Evidence that chytrids dominate fungal communities in high-elevation soils. Proc Natl Acad Sci USA 106:18315–18320

Fuller MS (1966) Structure of the uniflagellate zoospores of aquatic phycomycetes. In: Madelin MF (ed) The fungus spore, vol 18, Proceedings symposium of the Colston Research Society. Butterworth, London, pp 67–84

Fuller MS, Jaworski A (1987) Zoosporic fungi in teaching and research. Southeastern Publishing, Athens, GA

Fuller MS, Reichle RE (1968) The fine structure of *Monoblepharella* sp. zoospores. Can J Bot 46:279–283

Garcia-Vallvé S, Romeu A, Palau J (2000) Horizontal gene transfer of glycosyl hydrolases of the rumen fungi. Mol Biol Evol 17:352–361

Garmyn A, Van Rooij P, Pasmans F, Hellebuyck T, Van Den Broeck W, Haesebrouck F, Martel A (2012) Waterfowl: potential environmental reservoirs of the chytrid fungus *Batrachochytrium dendrobatidis*. PLoS One 7(4):e35038. doi:10.1371/journal.pone.0035038

Gauriloff LP, Delay RJ, Fuller MS (1980a) Comparative ultrastructure and biochemistry of chytridiomycetous fungi and the future of the Harpochytriales. Can J Bot 58:2098–2109

Gauriloff LP, Delay RJ, Fuller MS (1980b) The fine structure of zoospores of *Harpochytrium hedinii*. Can J Bot 58:2090–2097

Gleason FH, Letcher PM, McGee PA (2004) Some Chytridiomycota in soil recover from drying and high temperatures. Mycol Res 108:583–589

Gleason FH, Mozley-Standridge SE, Porter D, Boyle DG, Hyatt AD (2007) Preservation of Chytridiomycota in culture collections. Mycol Res 111:129–136

Gleason FH, Kagami M, Lefèvre E, Sime-Ngando T (2008) The ecology of chytrids in aquatic ecosystems: roles in food web dynamics. Fungal Biol Rev 22:17–25

Gleason FH, Küpper FC, Amon JP, Picard K, Gachon CMM, Marano AB, Sime-Ngando T, Lilje O (2011) Zoosporic true fungi in marine ecosystems: a review. Mar Freshw Res 62:383–393

Gold JJ, Heath IB, Bauchop T (1988) Ultrastructural description of a new chytrid genus of caecum anaerobe, *Caecomyces equi* gen. nov., sp. nov., assigned to the Neocallimasticaceae. BioSystems 21:403–415

Griffith GW, Baker S, Fliegerova K, Liggenstoffer A, van der Giezen M, Voigt K, Beakes G (2010) Anaerobic fungi: Neocallimastogomycota. IMA Fungus 1:181–185

Hampson MC, Coombes JW (1989) Pathogenesis of *Synchytrium endobioticum*. VII. Earthworms as vectors of wart disease of potato. Plant Soil 116:147–150

Heath IB, Bauchop T, Skipp RA (1983) Assignment of the rumen anerobe *Neocallimastix frontalis* to the Spizellomycetales (Chytridiomycetes) on the basis of its polyflagellate zoospore ultrastructure. Can J Bot 61:295–307

Heath IB, Kaminskyj SG, Bauchop T (1986) Basal body loss during fungal zoospore encystment: evidence against cenriole autonomy. J Cell Sci 83:135–140

Held AA (1975) The zoospore of *Rozella allomycis*: ultrastructure. Can J Bot 53:2212–2232

Held AA (1981) *Rozella* and *Rozellopsis*: naked endoparasitic fungi which dress-up as their hosts. Bot Rev 47:451–515

Hibbett DS, Binder M, Bischoff J, Blackwell M, Cannon P, Eriksson O, Huhndorf S, James T, Kirk P, Lucking R, Lumbsch T, Lutzoni F, Matheny P, McLaughlin D, Powell M, Redhead S, Schoch C, Spatafora J, Stalpers J, Vilgalys R et al (2007) A higher level phylogenetic classification of the fungi. Mycol Res 111:509–547

Ho YW, Barr DJS (1995) Classification of anaerobic gut fungi from herbivores with emphasis on rumen fungi from Malaysia. Mycologia 87:655–677

Holfeld H (2000) Relative abundance, rate of increase, and fungal infections of freshwater phytoplankton. J Plankton Res 22:987–995

Hörandle E, Stuessy TF (2010) Paraphyletic groups as natural units of biological classification. Taxon 59:1641–1653

Idnurm A, Verma S, Corrochano LM (2010) A glimpse into the basis of vision in the kingdom Mycota. Fungal Genet Biol 47:881–892

James TY, Berbee ML (2011) No jacket required—new fungal lineage defies dress code. Bioessays 34:94–102. doi:10.1002/bies.201100110

James TY, Porter D, Leander CA, Vilgalys R, Longcore JE (2000) Molecular phylogenetics of the Chytriomycota supports the utility of ultrastructural data in chytrid systematics. Can J Bot 78:336–350

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 G, Johnson D, O'Rourke M, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler 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, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006a) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822

James TY, Letcher PM, Longcore JE, Mozley-Standridge SE, Porter D, Powell MJ, Griffith GW, Vilgalys R (2006b) A molecular phylogeny of the flagellated Fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia 98:860–871

James TY, Porter TM, Martin WW (2014) Blastocladiomycota. In: McLaughlin DJ, Spatafora JW (eds) Systematics and evolution. Springer, Heidelberg

Johanson AE (1944) An endo-operculate chytridiaceous fungus: *Karlingia rosea* gen. nov. Am J Bot 31:397–404

Johnson TW, Sparrow FW (1961) Fungi in oceans and estuaries. J. Cramer, Weinheim, Germany

Jones MDM, Richards TA, Hawksworth DJ, Bass D (2011) Validation of the phylum name Cryptomycota phyl. nov. with notes on its recognition. IMA Fungus 2:173–175

Joneson S, Stajich JE, Shiu S-H, Rosenblum EB (2011) Genomic transition to pathogenicity in chytrid fungi. PLoS Pathog 7(11):e1002338. doi:10.1371/journal.ppat.1002338

Kagami M, deBruin A, Ibelings BW, van Donk E (2007) Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. Hydrobiologia 578:113–129

Kagami M, Helmsing NR, van Donk E (2011) Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms. Hydrobiologia 569:49–54

Kagami M, Amano Y, Ishii N (2012) Community structure of planktonic fungi and the impact of parasitic chytrids on phytoplankton in Lake Inba, Japan. Microb Ecol 63:358–368

Karling JS (1964) *Synchytrium*. Academic, New York

Karling JS (1977) Chytridiomycetarum Iconographia. Lubrecht and Cramer, Monticello, NY

Karpov SA, Letcher PM, Mamkaeva MA, Mamkaeva KA (2010) Phylogenetic position of the genus *Mesochytrium* (Chytridiomycota) based on zoospore ultrastructure and sequences from the 18S and 28S rRNA gene. Nova Hedwigia 90:81–94

Karpov SA, Mikhailov KV, Mirzaeva GS, Mirabdullaev IM, Mamkaeva KA, Titova NN, Aleoshin VV (2013) Obligately phagotrophic aphelids turned out to branch with the earliest-diverging fungi. Protist 164(2):195–205, http://dx.doi.org/10.1016/j.protis.2012.08.001

Kazama F (1972) Ultrastructure and phototaxis of the zoospores of *Phlyctochytrium* sp., an estuarine chytrid. J Gen Microbiol 71:555–566

Keeling PJ, Inagaki Y (2004) A class of eukaryotic GTPase with a punctate distribution suggesting multiple functional replacements of translation elongation factor 1alpha. Proc Natl Acad Sci USA 101:15380–15385

Kiziewicz B (2004) Aquatic fungi growing on the muscle of vendace (*Coregonus albula* L.), alpine bullhead (*Cottus poecilopus* H.) and lake trout (*Salmo trutta lacustris* L.) from Lake Hańcza (NE Poland). Zool Poloniae 49:85–95

Koch WJ (1961) Studies of the motile cells of chytrids. III. Major types. Am J Bot 48:786–788

Koch WJ (1968) Studies of the motile cells of chytrids. V. Flagellar retraction in posteriorly uniflagellate fungi. Am J Bot 55:841–856

Lange L, Olson LW (1978) The zoospore of *Synchytrium endobioticum*. Can J Bot 56:1229–1239

Lange L, Olson LW (1979) The uniflagellate Phycomycete zoospore. Dansk Bot Arkiv 33:1–95

Lara E, Moreira D, López-Garcia P (2010) The environmental clade LKM11 and *Rozella* form the deepest branching clade of fungi. Protist 161:116–121

LeCalvez T, Burgaud G, Mahé S, Barbier G, Vandenkoornhuyse P (2009) Fungal diversity in deep-sea hydrothermal ecosystems. Appl Environ Microbiol 75:6415–6421

Lefèvre E, Roussel B, Amblard C, Sime-Ngando T (2008) The molecular diversity of freshwater picoeukaryotes reveals high occurrence of putative parasitoids in the plankton. PLoS One 3(6):e2324. doi:10.1371/journal.pone.0002324

Lefèvre E, Letcher PM, Powell MJ (2012) Temporal variation of the small eukaryotic community in two freshwater lakes: emphasis on the zoosporic fungal community. Aquat Microb Ecol 67:91–105

Lepère C, Domaizon I, Debroas D (2008) Unexpected importance of potential parasites in the composition of the freshwater small-eukaryote community. Appl Environ Microbiol 74:2940–2949

Letcher PM, Powell MJ (2001) Distribution of zoosporic fungi in forest soils of the Blue Ridge and Appalachian Mountains of Virginia. Mycologia 93:1029–1041

Letcher PM, Powell MJ (2002a) A taxonomic summary of *Chytriomyces* (Chytridiomycota). Mycotaxon 84:447–487

Letcher PM, Powell MJ (2002b) Frequency and distribution patterns of zoosporic fungi from moss-covered and exposed soils. Mycologia 94:761–771

Letcher PM, Powell MJ (2005a) *Kappamyces*, a new genus in the Chytridiales (Chytridiomycota). Nova Hedwigia 80:115–133

Letcher PM, Powell MJ (2005b) Phylogenetic position of *Phlyctochytrium planicorne* (Chytridiales, Chytridiomycota) based on zoospore ultrastructure and partial nuclear LSU rRNA gene sequence analysis. Nova Hedwigia 80:134–146

Letcher PM, Powell MJ (2012) A taxonomic summary and revision of *Rhizophydium* (Rhizophydiales, Chytridiomycota). University Printing, Tuscaloosa, AL

Letcher PM, Powell MJ, Chambers JG, Holznagel WE (2004) Phylogenetic relationships among *Rhizophydium* isolates from North America and Australia. Mycologia 96:1339–1351

Letcher PM, Powell MJ, Chambers JG, Longcore JE, Churchill PF, Harris PM (2005) Ultrastructural

and molecular delineation of the Chytridiaceae (Chytridiales). Mycologia 83:1561–1573

Letcher PM, Powell MJ, Churchill PF, Chambers JG (2006) Ultrastructural and molecular phylogenetic delineation of a new order, the Rhizophydiales (Chytridiomycota). Mycol Res 110:898–915

Letcher PM, Powell MJ, Barr DJS, Churchill PF, Wakefield WS, Picard KT (2008a) Rhizophlyctidales—a new order in Chytridiomycota. Mycol Res 112:1031–1048

Letcher PM, Powell MJ, Viusent MC (2008b) Rediscovery of an unusual chytridiaceous fungus new to the order Rhizophydiales. Mycologia 100: 325–334

Letcher PM, Vélez CG, Barrantes ME, Powell MJ, Churchill PF, Wakefield WS (2008c) Ultrastructural and molecular analyses of Rhizophydiales (Chytridiomycota) isolates from North America and Argentina. Mycol Res 112:759–782

Letcher PM, Powell MJ, Picard KT (2012a) Zoospore ultrastructure and phylogenetic position of *Phlyctochytrium aureliae* Ajello is revealed (Chytridiaceae, Chytridiales, Chytridiomycota. Mycologia 104:410–418

Letcher PM, Vélez CG, Schultz S, Powell MJ (2012b) New taxa are delineated in Alphamycetaceae (Rhizophydiales, Chytridiomycota). Nova Hedwigia 94:9–29

Li J, Heath IB, Cheng K-J (1991) The development and zoospore ultrastructure of a polycentric chytridiomycete gut fungus, *Orphinomyces joyonii comb. nov*. Can J Bot 69:580–589

Li J, Heath IB, Packer L (1993) The phylogenetic relationships of the anaerobic chytridiomycetous gut fungi (Neocallimasticaceae) and the Chytridiomycota. II. Cladistic analysis of structural data and description of Neocallimasticales *ord. nov*. Can J Bot 71:393–407

Liggenstoffer AS, Youssef NH, Couger MB, Elshahed MS (2010) Phylogenetic diversity and community structure of anaerobic gut fungi (phylum Neocallimastigomycota) in ruminant and non-ruminant herbivores. ISME J 4:1225–1235

Liu Y, Steenkamp ET, Brinkmann H, Forget L, Philippe H, Lang BF (2009) Phylogenomic analyses predict sister group relationship of nucleariids and Fungi and paraphyly of zygomycetes with significant support. BMC Evol Biol 9:272. doi:10.1186/1471-2148-9-272

Ljungdahl LG (2008) The cellulose/hemicellulase system of the anaerobic fungus *Orpinomyces* PC-2 and aspects of its applied use. Ann N Y Acad Sci 1125:308–321

Lockhart RJ, Van Dyke MK, Beadle IR, Humphreys P, McCarthy AJ (2006) Molecular biological detection of anaerobic gut fungi (Neocallimastigales) from landfill sites. Appl Environ Microbiol 72:5659–5661

Longcore JE (1992a) Morphology and zoospore ultrastructure of *Chytriomyces angularis* sp. nov. (Chytridiales). Mycologia 84:442–451

Longcore JE (1992b) Morphology and zoospore ultrastructure of *Podochytrium dentatus* sp. nov. (Chytridiales). Mycologia 84:183–192

Longcore JE (1993) Morphology and zoospore ultrastructure of *Lacustromyces hiemalis* gen. et sp. nov. (Chytridiales). Can J Bot 71:414–425

Longcore JE (1995) Morphology and zoospore ultrastructure of *Entophlyctis luteolus* sp. nov. (Chytridiales): implications for chytrid taxonomy. Mycologia 87:25–33

Longcore JE (1996) Chytridiomycete taxonomy since 1960. Mycotaxon 60:149–174, http://www.umaine.edu/chytrids/Chytrid%20Bibliography/Bibliography-Index.html

Longcore JE (2004) *Rhizophydium brooksianum* sp. nov., a multipored chytrid from soil. Mycologia 96:162–171

Longcore JE (2005) Zoosporic fungi from Australian and New Zealand tree-canopy detritus. Aust J Bot 53:259–272

Longcore JE, Simmons DR (2012) The Polychytriales ord. nov. contains chitinophilic members of the rhizophlyctoid alliance. Mycologia 104:276–294

Longcore JE, Barr DJS, Désaulniers N (1995) *Powellomyces*, a new genus in the Spizellomycetales. Can J Bot 73:1385–1390

Longcore JE, Pessier AP, Nichols D (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227

Longcore JR, Longcore JE, Pessier AP, Halteman WA (2007) Chytridiomycosis widespread in anurans of northeastern United States. J Wildl Manage 71:435–444

Longcore JE, Letcher PM, James TY (2011) *Homolaphlyctis polyrhiza* gen et sp. nov., a species in the Rhizophydiales (Chytridiomycetes) with multiple rhizoidal axes. Mycotaxon 118:433–440

Lucarotti CJ (1981) Zoospore ultrastructure of *Nowakowskiella elegans* and *Cladochytrium replicatum*. Can J Bot 59:137–148

McNitt R (1974) Zoosporogenesis in *Phlyctochytrium irregulare*. Cytobiologie 9:290–306

Midgley DJ, Letcher PM, McGee PA (2006) Access to organic and insoluble sources of phosphorous varies among soil Chytridiomycota. Arch Microbiol 186:211–217

Miki T, Takimoto G, Kagami M (2011) Role of parasitic fungi in aquatic food webs: a theoretical approach. Freshw Biol 56:173–1183

Milne A, Theodorou MK, Jordan MGC, King-Spooner C, Trinci APJ (1989) Survival of anaerobic fungi in feces, in saliva, and in pure culture. Exp Mycol 13:27–37

Mollicone MRN, Longcore JE (1994) Zoospore ultrastructure of *Monoblepharis polymorpha*. Mycologia 86:615–625

Mollicone MRN, Longcore JE (1999) Zoospore ultra-structure of *Gonapodya polymorpha*. Mycologia 91:727–734

Monchy S, Sanciu G, Jobard M, Rasconi S, Gerphagnon M, Chabé M, Cian A, Meloni D, Niquil N, Christaki U, Viscogliosi E, Sime-Ngando T (2011) Exploring and quantifying fungal diversity in freshwater lake ecosystems using rDNA cloning/sequencing and SSU tag pyrosequencing. Environ Microbiol 13:1433–1453

Montecillo CM, Bracker CE, Powell MJ (1980) Ultra-structure of *Synchytrium macrosporum* zoospores. Can J Bot 58:1885–1897

Montford DO, Orpin CG (1994) Anaerobic fungi: biology, ecology, and function. Dekker, New York

Morris NR (2000) Nuclear migration: from fungi to the mammalian brain. J Cell Biol 148:1097–1101

Moss AS, Reddy NS, Dortaj IM, San Francisco MJ (2008) Chemotaxis of the amphibian pathogen *Batrachochytrium dendrobatidis* and its response to a variety of attractants. Mycologia 100:1–5

Mozley-Standridge SE, Letcher PM, Longcore JE, Porter D, Simmons DR (2009) Cladochytriales—a new order in Chytridiomycota. Mycol Res 113:498–507

Muehlstein LK, Amon JP, Leffler DL (1987) Phototaxis in the marine fungus *Rhizophydium littoreum*. Appl Environ Microbiol 53:1819–1821

Muehlstein LK, Amon JP, Leffler DL (1988) Chemotaxis in the marine fungus *Rhizophydium littoreum*. Appl Environ Microbiol 54:1668–1672

Müller DG, Küpper FC, Küpper H (1999) Infection experiments reveal broad host ranges of *Eurychasma dicksonii* (Oomycota) and *Chytridium polysiphoniae* (Chytridiomycota), two eukaryotic parasites in marine brown algae (Phaeophyceae). Phycol Res 47:217–223

Nagahama T, Takahashi E, Nagano Y, Abdel-Wahab MA, Miyazaki M (2011) Molecular evidence that deep-branching fungi are major components in deep-sea methane cold-seep sediments. Environ Microbiol 13:2359–2370

Nagpal R, Puniya AK, Sehgal JP, Singh K (2011) In vitro fibrolytic potential of anaerobic rumen fungi from ruminants and non-ruminant herbivores. Mycoscience 52:31–38

Nicholson MJ, McSweeney CS, Mackie RI, Brookman JL (2010) Diversity of anaerobic gut fungal populations analysed using ribosomal ITS1 sequences in faeces of wild and domesticated herbivores. Anaerobe 16:66–73

Nikolcheva LG, Barlocher F (2004) Taxon specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. Mycol Prog 3:41–49

Nyvall P, Pedersén M, Longcore JE (1999) *Thalassochytrium gracilariopsidis* (Chytridiomycota), gen. et sp. nov., endosymbiotic in *Gracilariopsis* sp. (Rhodophyceae). J Phycol 35:176–185

Orpin CG (1975) Studies on the rumen flagellate *Neocallimastix frontalis*. J Gen Microbiol 91:249–262

Orpin CG (1977) Invasion of plant tissue in the rumen by the flagellate *Neocallimastix frontalis*. J Gen Microbiol 98:423–430

Orpin CB, Bountiff L (1978) Zoospore chemotaxis in the rumen phycomycete *Neocallimastix frontalis*. J Gen Microbiol 104:113–122

Orpin CG, Letcher AJ (1979) Utilization of cellulose, starch, xylan and other hemicelluloses for growth by the rumen phycomycete *Neocallimastix frontalis*. Curr Microbiol 3:121–124

Ozkose E, Thomas BJ, Davies DR, Griffith GW, Theodorou MK (2001) *Cyllamyces aberensis* gen. nov. sp. nov., a new anaerobic gut fungus with branched sporangiophores isolated from cattle. Can J Bot 79:666–673

Perrott E (1955) The genus *Monoblepharis*. Trans Br Mycol Soc 38:247–282

Perrott E (1958) Isolation and pure culture of *Monoblepharis*. Nature 182:1322–1324

Picard KT, Letcher PM, Powell MJ (2009) *Rhizidium phycophilum*, a new species in the Chytridiales. Mycologia 101:696–706

Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15

Powell MJ (1974) Fine structure of plasmodesmata in a chytrid. Mycologia 66:606–614

Powell MJ (1975) Ultrastructural changes in nuclear membranes and organelle associations during mitosis of the aquatic fungus *Entophlyctis* sp. Can J Bot 53:627–646

Powell MJ (1976a) Development of the discharge apparatus in the fungus *Entophlyctis*. Arch Microbiol 111:59–71

Powell MJ (1976b) Ultrastructure and isolation of glyoxysomes (microbodies) in zoospores of the fungus *Entophlyctis* sp. Protoplasma 89:1–27

Powell MJ (1978) Phylogenetic implications of the microbody-lipid globule complex. BioSystems 10:167–180

Powell MJ (1980) Mitosis in the aquatic fungus *Rhizophydium sphaerotheca* (Chytridiales). Am J Bot 67:839–853

Powell MJ (1981a) Ultrastructure of *Polyphagus euglenae* zoospores. Can J Bot 59:2049–2061

Powell MJ (1981b) Zoospore structure of the mycoparasitic chytrid *Caulochytrium protostelioides* Olive. Am J Bot 68:1074–1089

Powell MJ (1983) Localization of antimonate-mediated precipitates of cations in zoospores of *Chytriomyces hyalinus*. Exp Mycol 7:266–277

Powell MJ (1993) Looking at mycology with a Janus face. A glimpse at Chytridiomycetes active in the environment. Mycologia 85:1–20

Powell MJ (1994) Production and modifications of extracellular structures during development of Chytridiomycetes. Protoplasma 181:123–141

Powell MJ, Blackwell WH (1991) A proposed dispersal mechanism for *Septosperma rhizophydii*. Mycologia 83:673–680

Powell MJ, Gillette L (1987) Septal structure of the chytrid *Rhizophlyctis harderi*. Mycologia 79:635–639

Powell MJ, Koch WJ (1977) Morphological variations in a new species of *Entophlyctis*. I. The species concept. Can J Bot 55:1668–1685

Powell MJ, Letcher PM (2012) From zoospores to molecules: the evolution and systematics of Chytridiomycota. In: Misra JK, Tewari JP, Deshmukh SK (eds) Systematics and evolution of fungi. CRC, Boca Raton, FL, pp 29–54

Powell MJ, Roychoudhury S (1992) Ultrastructural organization of *Rhizophlyctis harderi* zoospores and redefinition of the type I microbody-lipid globule complex. Can J Bot 70:750–761

Powell MJ, Letcher PM, Longcore JE (2011) *Operculomyces* is a new genus in the order Rhizophydiales. Mycologia 103:854–862

Putnam ML, Hampson MC (1989) Rediscovery of *Synchytrium endobioticum* in Maryland. Am Potato J 66:495–501

Rachowicz LJ, Vredenburg VT (2004) Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. Dis Aquat Org 61:75–83

Reichle RE (1972) Fine structure of *Oedogoniomyces* zoospores, with comparative observations on *Monoblepharella* zoospores. Can J Bot 50:819–824

Rezaeian M, Beakes G, Parker DS (2004) Methods for the isolation, culture and assessment of the status of anaerobic rumen chytrids in both *in vitro* and *in vivo* systems. Mycol Res 34:1215–1226

Rosenblum EB, Stajich JE, Maddox N, Eisen MB (2008) Global gene-expression profiles for life stages of the deadly amphibian pathogen *Batrachochytrium dendrobatidis*. Proc Natl Acad Sci USA 105:17034–17039

Rossman AY, Britton K, Luster D, Palm M, Royer M, Sherald J (2006) Evaluating the threat posed by fungi on the APHIS list of regulated plant pests. Plant Health Progress. doi:10.1094/PHP-2006-5050-01-PS. Available via Plant management network. http://www.plantmanagementnetwork.org/pub/php/perspective/2006/fungi/. Cited 5 May 2006

Roychoudhury S, Powell MJ (1991) Ultrastructure of mitosis in the algal parasitic fungus *Polyphagus euglenae*. Can J Bot 69:2201–2214

Roychoudhury S, Powell MJ (1992) Precise flagellar configuration of the *Rhizophlyctis harderii* zoospore. Can J Bot 70:762–771

Schloegel LM, Toledo LF, Longcore JE, Greenspan SE, Vieira CA, Lee M, Zhao S, Wangen C, Ferreira CM, Hipolito M, Davies AJ, Cuomo CA, Daszak P, James TY (2012) Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. Mol Ecol 21:5162–5177. doi:10.1111/j.1365-294X.2012.05710.x

Schmidt SK, Naff CS, Lynch RC (2012) Fungal communities at the edge: ecological lessons from high alpine fungi. Fungal Ecol 5:443–452

Sekimoto S, Rochon D, Long JE, Dee JM, Berbee ML (2011) A multigene phylogeny of *Olpidium* and its implications for early fungal evolution. BMC Evol Biol 11:331. doi:10.1186/1471-2148-11-331

Shearer CA, Langsam DM, Longcore JE (2004) Fungi in freshwater habitats. In: Mueller GM, Bills GF, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier, Oxford, pp 513–531

Shields JD (1990) *Rhizophydium littoreum* on the eggs of *Cancer anthonyi*: parasite or saprobe? Biol Bull 179:201–206

Shields JP, Fuller MS (1996) Ultrastructure of chytridiomycete and oomycete zoospores using spray-freeze fixation. Protoplasma 191:84–95

Sime-Ngando T, Lefèvre E, Gleason FH (2011) Hidden diversity among aquatic heterotrophic flagellates: ecological potential of zoosporic fungi. Hydrobiologia 659:5–22

Simmons DR (2011) Phylogeny of Powellomycetaceae fam. nov. and description of *Geranomyces variabilis* gen. et comb. nov. Mycologia 103:1411–1420

Simmons DR, Longcore JE (2012) *Thoreauomyces* gen. nov., *Fimicolochytrium* gen. nov. and additional species in *Geranomyces*. Mycologia 104:1229–1243. doi:10.3852/12-015

Simmons DR, James TY, Meyer AF, Longcore JE (2009) Lobulomycetales, a new order in the Chytridiomycota. Mycol Res 113:450–460

Simmons DR, Letcher PM, Powell MJ, Longcore JE (2012) *Alogomyces tanneri* gen. et sp. nov., a chytrid in Lobulomycetales from horse manure. Mycologia 104:157–163

Sønstebø JH, Rohrlack T (2011) Possible implications of chytrid parasitism for population subdivision in freshwater cyanobacteria of the genus *Planktothrix*. Appl Environ Microbiol 77:1344–1351

Sparrow FK (1960) Aquatic phycomycetes, 2nd edn. University of Michigan Press, Ann Arbor

Sparrow FK, Barr ME (1955) Additions to the phycomycete flora of the Douglas Lake region I. New taxa and records. Mycology 47:546–556

Stachewicz H, De Boer SH (2002) Pathotype determination of potato wart from Prince Edward Island, Canada. Nachrichtebl Deut Pflanzenschutzd 54:269

Stajich JE (2011) Currently available fungal genome sequences. Available at http://fungalgenomes.org/wiki/Fungal_Genome_Links. Cited 4 July 2011

Stajich JE, Berbee ML, Blackwell M, Hibbett DS, James TY, Spatafora JW, Taylor JW (2009) The Fungi. Curr Biol 19:840–845

Steenkamp ET, Wright J, Baldauf SL (2006) The protistan origins of animals and fungi. Mol Biol Evol 23:93–106

Steiger RA, Simmons DR, Longcore JE (2011) *Cylindrochytridium johnstonii* is a member of the Cladochytriales. Mycotaxon 118:293–302

Stoeck T, Epstein S (2003) Novel eukaryotic lineages inferred from small-subunit rRNA analyses of oxygen-depleted marine environments. Appl Environ Microbiol 69:2657–2663

Stoeck T, Kasper J, Bunge J, Leslin C, Ilyin V, Epstein S (2007) Protistan diversity in the Arctic: a case of paleoclimate shaping modern biodiversity? PLoS One 2(8):e728. doi:10.1371/journal.pone.0000728

Strasburger E (1878) Wirkung des Lichtes und der Wärme auf Schwärmsporen. Jenaische Zeitschr f Naturwiss 2:551–625

Sun G, Yang Z, Kosch T, Summers K, Huang J (2011) Evidence for acquisition of virulence effectors in pathogenic chytrids. BMC Evol Biol 11:195. doi:10.1186/1471-2148/11/195

Tachezy J (2008) Hydrogenosomes and mitosomes: mitochondria of anaerobic eukaryotes. Springer, Berlin, Heidelberg, New York

Taylor JW, Fuller MS (1981) The Golgi apparatus, zoosporogenesis, and development of the zoospore discharge apparatus of *Chytridium confervae*. Exp Mycol 5:35–59

Thornton ML (1970) Transport of soil-dwelling aquatic phycomycetes by earthworms. Trans Br Mycol Soc 55:391–397

Thornton ML (1971) Potential for long-range dispersal of aquatic phycomycetes by internal transport in birds. Trans Br Mycol Soc 57:49–59

Travland LB, Whisler HC (1971) Ultrastructure of *Harpochytrium hedinii*. Mycologia 63:767–789

Ustinova I, Krienitz L, Huss VAR (2000) *Hyaloraphidium curvatum* is not a green alga, but a lower fungus; *Amoebidium parasiticum* is not a fungus, but a member of the DRIPs. Protist 151:253–262

van der Giezen M (2009) Hydrogenosomes and mitosomes: conservation and evolution of functions. J Eukaryot Microbiol 56:221–231

van der Giezen M, Birdsey GM, Horner DS, Lococq J, Dyal PL, Benchimol M, Danpure CJ, Embley TM (2003) Fungal hydrogenosomes contain mitochondria heat-shock proteins. Mol Biol Evol 20:1051–1061

Vargas M, Aronson JM, Roberson RW (1993) The cytoplasmic organization of hyphal tip cells in the fungus *Allomyces macrogynus*. Protoplasma 176:43–52

Vélez CG, Letcher PM, Schultz S, Powell MJ, Churchill PF (2011) Molecular phylogenetic and zoospore ultrastructural analyses of *Chytridum olla* establish the limits of a monophyletic Chytridiales. Mycologia 103:118–130

Vieira ALG, Camilo CM (2011) *Agrobacterium tumefasciens*-mediated transformation of the aquatic fungus *Blastocladiella emersonii*. Fungal Genet Biol 48:806–811

Voyles J (2011) Phenotypic profiling of *Batrachochytrium dendrobatidis*, a lethal fungal pathogen of amphibians. Fungal Ecol 4:196–200

Wakefield WS, Powell MJ, Letcher PM, Barr DJS, Churchill PF, Longcore JE, Chen S-F (2010) A molecular phylogenetic evaluation of the Spizellomycetales. Mycologia 102:596–604

Webster J (1970) Coprophilous fungi. Trans Br Mycol Soc 54:161–180

Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R (2004) Origin of the amphibian chytrid fungus. Emerg Infect Dis 10:2100–2105

Whiffen J (1944) A discussion of taxonomic criteria in the Chytridiales. Farlowia 1:583–597

Whisler HC, Travland LB (1973) Mitosis in *Harpochytrium*. Arch Protistenk 115:69–74

Widmer TL (2006) Cryopreservation of *Synchytrium solstitiale* in plants. Plant Dis 90:429–432

Willoughby LG (1998) A quantitative ecological study on the monocentric soil chytrid, *Rhizophlyctis rosea*, in Provence. Mycol Res 102:1338–1342

Willoughby LG (2001) The activity of *Rhizophlyctis rosea* in soil: some deductions from laboratory observations. Mycologist 15:113–117

Willoughby LG, Townley PJ (1961) Two new saprophytic chytrids from the Lake District. Trans Br Mycol Soc 44:177–184

Wubah DA, Fuller MS, Akin DE (1991) Resistant body formation in *Neocallimastix* sp., an anaerobic fungus from the rumen of a cow. Mycologia 83:40–47

Yun HY, Kim YH, James TY (2011) First report of false rust caused by *Synchytrium minutum* on Kudzu in Korea. Dis Notes 95:358