7 Blastocladiomycota

Timothy Y. James[1], Teresita M. Porter[2], W. Wallace Martin[3]

CONTENTS

[1]Department of Ecology and Evolutionary Biology, University of Michigan, 830 N. University, Ann Arbor, MI 48109, USA; e-mail: tyjames@umich.edu
[2]Biology Department, Duke University, Durham, NC 27708, USA and Department of Biology, McMaster University, Hamilton, ON L8S 4K1, Canada; e-mail: terri@evol.mcmaster.ca
[3]Department of Biology, Randolph-Macon College, Ashland, VA 23005, USA; e-mail: wmartin@rmc.edu

I. Introduction

Blastocladiomycota are **zoosporic fungi** that comprise an early-diverging branch on the tree of true fungi possessing a number of distinguishing morphological and life history characteristics. These aquatic and soil fungi include genera typically considered so-called water molds, occurring as saprotrophs on decaying plants and animals. Other members of the clade are obligate parasites of invertebrates, plants, and algae. Beyond a basic understanding of global biodiversity, Blastocladiomycota present avenues for exciting research: they have served as models for fungal genetics and physiology (Olson 1984; Ribichich et al. 2005), they have potential as biocontrol agents of plant pests and disease vectors (Chapman 1985; Garcia 1983; Singh et al. 2007), and they interact with aquatic food webs and nutrient cycling through parasitism and consumption (Johnson et al. 2006). Convenient terms for the group include ***blastodad*** and ***blastoclad***, and we adopt the latter in reference to any fungus belonging to this phylum.

The blastoclads were historically considered to be closely related to other zoosporic true fungi, the Chytridiomycota (chytrids), because they reproduce with zoospores possessing a **single posteriorly directed flagellum** (Sparrow 1960). Blastocladiomycota, as well as the two zoosporic fungal phyla Chytridiomycota and Neocallimastigomycota, were shown to be members of the fungal kingdom in the earliest ribosomal DNA–based molecular phylogenies (Bowman et al. 1992; Bruns et al. 1992;

Systematics and Evolution, 2nd Edition
The Mycota VII Part A
D.J. McLaughlin and J.W. Spatafora (Eds.)

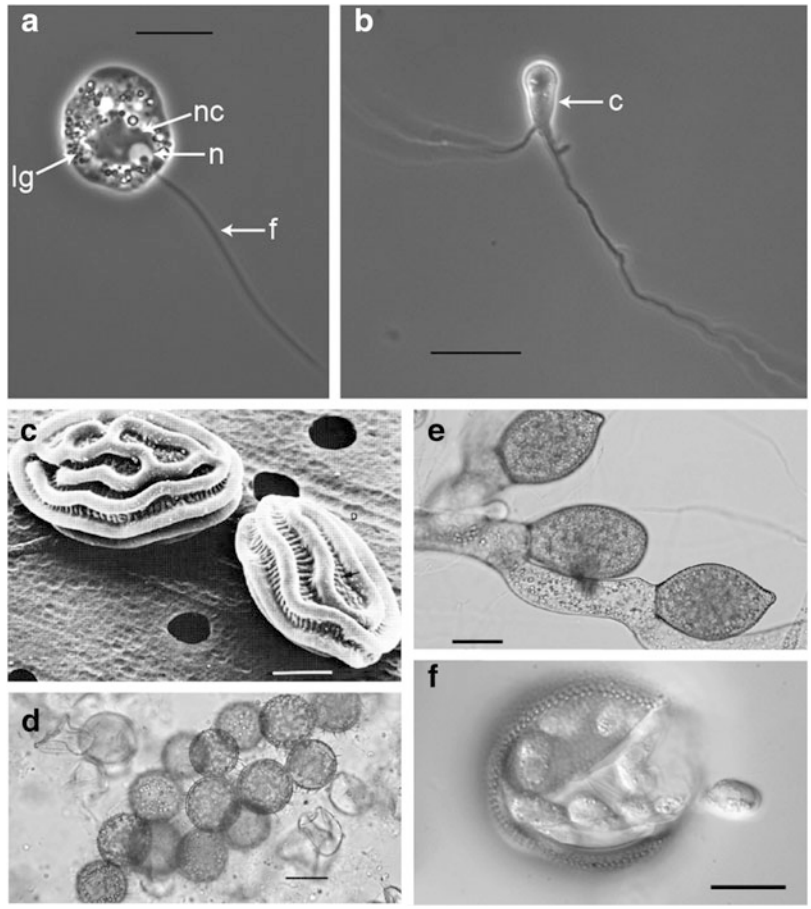

Fig. 7.1 Characteristic stages of blastoclads include zoospores and bipolar germination of encysted spores. (**a**) Uniflagellate zoospore of *Allomyces javanicus*. *f* flagellum, *n* nucleus, *nc* nuclear cap, *lg* lipoid granules. (**b**) Cyst (c) demonstrating bipolar germination with one end enlarging to form a hypha and the other end composed of thin rhizoids involved in substrate attachment and resource extraction. (**c**) Scanning electron micrograph of resting sporangia of *Coelomomyces iliensis* var. *iliensis*, a parasite of the mosquito *Culex antennatus* [photo from Couch and Bland (1985), used with permission from Elsevier]. (**d**) Spiny resting sporangia of *Catenaria spinosa*, a parasite of the midge *Chironomus decorus*. (**e**) Cluster of resting sporangia of *Allomyces moniliformis* demonstrating pitted outer wall. (**f**) Germinating resting sporangium of *A. javanicus* releasing meiospores. Scale bar in A–C = 10 μM; D, F = 30 μM; E = 20 μM

Förster et al. 1990). While it was obvious that posteriorly uniflagellate "phycomycetes" were allied with true fungi, molecular phylogenetic studies immediately detected a large phylogenetic distance between the blastoclads and the chytrids (Bruns et al. 1992; Nagahama et al. 1995). Despite an uncertain phylogenetic placement, the composition of blastoclads has remained largely consistent over time (with the exception of the genus *Physoderma*), and it has been accepted as monophyletic because of the presence of several distinctive characteristics. The most consistent characteristic of the group is reproduction by a zoospore with a prominent nuclear cap that in hyphal species often demonstrates bipolar germination (Fig. 7.1a, b). Another distinguishing feature is the thick-walled, darkly pigmented **resting sporangium** that is often ornamented with pits, ridges, or spines (Fig. 7.1c–f) and undergoes germination by the cracking of an outer wall through which an endosporangium protrudes. The resting sporangium is sometimes referred to as a resting spore; however, because the

sporelike structure germinates to produce zoospores by internal cleavage, the term *sporangium* is more accurate. Also, in our discussion we refer to this structure as a *resting sporangium* rather than a *resistant sporangium* or *meiosporangium*.

Blastoclads are the only fungal group known to demonstrate an alternation of haploid and diploid generations. On the basis of this life cycle, ultrastructural characters, and overall phylogenetic distinctness from chytrids (James et al. 2000; Nagahama et al. 1995; Seif et al. 2005), the blastoclads have been considered a separate phylum, Blastocladiomycota, with a single class and order, Blastocladiomycetes and Blastocladiales, respectively (James et al. 2006b). A distinct life cycle, diverse ecological roles, and phylogenetics all suggest that the group is ancient. This ancient divergence is also corroborated by fossil evidence demonstrating an alternation of generations in the Devonian blastoclad ***Paleoblastocladia milleri*** (Remy et al. 1994) and *Allomyces*-like resting sporangia from the Devonian that are remarkably similar to those of extant species (Taylor et al. 1994).

Here, we provide a basic introduction to the blastoclads, covering their distribution and ecology, phylogenetic relationships, and morphological characteristics of their spores, and we present an overview of their unique life cycles and genetics.

II. Occurrence and Distribution

Blastoclads are globally distributed in numerous aquatic and terrestrial habitats. Within these habitats, blastoclads can be observed on decaying plant or insect material or as pathogens of aquatic organisms. However, other unique terrestrial habitats, such as in the photosynthetic tissues of vascular plants, and as parasites of terrestrial invertebrates, such as tardigrades and nematodes, are important habitats for blastoclads. Some genera, such as *Blastocladiella* and *Allomyces*, seem to be more common and speciose in tropical or subtropical habitats (Emerson 1941; Whisler 1987), a general trend in the diversity of zoosporic fungi and pseudo-fungi (Sparrow 1960). No marine or halophytic species are known.

The geographic distribution of certain species within well-studied genera suggests many widespread species of *Allomyces*, *Coelomomyces*, and *Blastocladia*. *Allomyces arbusculus* is known to occur on most continents, including temperate climates, while *A. moniliformis* and the hybrid species *Allomyces javanicus* are reported in both Old and New World tropical and subtropical habitats (Emerson 1941; Sparrow 1960; Wolf 1941). The species *Blastocladia pringsheimii* is thought to be widespread and ubiquitous and is known from all continents except Antarctica (Nascimento and Pires-Zottarelli 2010; Sparrow 1960). Numerous other species of *Blastocladia* are newly described and only known from India (Dasgupta and John 1988). It is important to note, however, that species concepts among blastoclads are largely untested, and current phylogenetic evidence suggests that species concepts in *Allomyces* are in need of revision (Porter et al. 2011). Other genera, for example, *Coelomomyces* spp., have species with rather distinct resting sporangium ornamentation, suggesting that they should be readily diagnosable taxa; these species also have been found on multiple continents (Couch and Bland 1985).

A. Saprobic Species

Most saprobic species are known from studies in which water or soil is baited with appropriate substrates such as hemp seeds, rosaceous fruits, pollen, or insect body parts. These species are thus likely to perform an active role in the decomposition of cellulosic, chitinous, or keratinic substrates within the ecosystem. Because the majority of saprobic blastoclads are known to produce a resistant sporangium, they are readily recovered from soils that are air-dried and then baited using an appropriate substrate (Whisler 1987). Soils that are periodically inundated represent good collecting locations for *Allomyces* (Sparrow 1960). The blastoclad-resistant sporangium may either provide an advantage in these habitats that undergo cycles

of flooding and drying or merely explain the reason they are so readily recovered. Some *Blastocladia* spp. are **facultatively anaerobic** (Gleason and Gordon 1989) and are recovered from submerged twigs and fruits in stagnant water or by baiting under anaerobic conditions (Whisler 1987).

B. Invertebrate Parasitic Species

Both aquatic and terrestrial invertebrates are hosts for parasitic blastoclads. In cold streams, the larvae of black flies may be host to *Coelomycidium*. Midge eggs and mosquito larvae may be collected along the edges of ponds and streams and are frequently parasitized by *Catenaria* spp. and *Coelomomyces*, respectively (Martin 1987). Infected larvae typically die before metamorphosing into adults; however, fourth-instar female larvae infected by *Coelomomyces* may pupate into adults, become sterilized by the infection, and shed *Coelomomyces* resting sporangia when they attempt to oviposit (Lucarotti 1987). Additional hosts of parasitic blastoclads include nematodes, copepods, cladocerans, and trichopterans, where the parasites grow internally as walled or wall-less thalli, but they may be most readily spotted as gametangia or resting sporangia that may fill the entire body cavity. *Sorochytrium* is only known from the tardigrade *Milnesium* (Dewel et al. 1985) and has been collected only from the type locality in North Carolina. The genus *Myiophagus* is recorded from larvae and pupae from dipterans and scale insects (Karling 1977; Sparrow 1939). The wide distribution of blastoclad pathogens across Crustacea and Arthropoda suggests that many more genera and species remain to be detected. A recent review discussed all of the known genera and species of invertebrate blastoclad parasites (Gleason et al. 2010).

C. Plant-Pathogenic Species

The genera *Physoderma* and *Urophlyctis* are obligate parasites of vascular plants with a worldwide distribution. Symptoms include pustules and leaf streaks of darkly pigmented resting sporangia. The pathogen is observed primarily on the leaves and shoots of aquatic or terrestrial plants but may also appear in the root system (Sparrow 1965). Hosts are diverse and include water ferns, sedges, composites, and the crops *Zea mays* and *Medicago sativa*, but the economic importance of these pathogens seems minimal. Experimental inoculation studies have demonstrated that one unnamed *Physoderma* species from *Agropyron repens*, common quack grass, could infect all tested congeneric hosts but not nine other sympatric species from other families (Sparrow and Griffin 1964). Unlike many Chytridiomycota, only one monotypic genus is known as a parasite of algae, *Paraphysoderma* (Hoffman et al. 2008).

D. Mycoparasites

A single mycoparasitic species is known, *Catenaria allomycis*, which grows endobiotically in *Allomyces* spp. or *Blastocladiella simplex*. Experimental inoculations (Couch 1945) showed the parasite could infect all *Allomyces* spp. (to varying degrees), only one *Blastocladiella* sp., but not *Blastocladia parva*, *Catenaria anguillulae*, or any saprolegnialean hosts.

E. DNA-Based Evidence

Because most of what is known of the distribution and diversity of blastoclads is known from cultured studies using baiting rather than direct observation of samples, the investigation of **environmental DNA** has great potential to enlighten our understanding of the suitable habitats and diversity of blastoclads, as it has done in other fungal lineages in soil and plant roots (Freeman et al. 2009; Schadt et al. 2003; Vandenkoornhuyse et al. 2002). These studies involve obtaining an environmental sample, for example, soil or pond water, which is then subjected to DNA extraction, polymerase chain reaction using primers targeting ribosomal RNA genes, and analysis of DNA sequences to determine the organisms present in the sample. At least three published environmental DNA studies have detected blastoclads. One study

(Slapeta et al. 2005) recovered two sequence types of unknown blastoclads from the sediment of an anoxic pond that clustered with *Allomyces* and *Blastocladiella*. Later, when Porter et al. (2011) produced the first sequences of *Blastocladia*, these sequences were shown to be closely related to the sequences identified by Slapeta et al. (2005). These results are consistent with the role of *Blastocladia* as an obligately fermenting saprobic species. Other studies have revealed blastoclads from the oxycline (region of lower oxygen concentration) of a deep lake in France (Lefèvre et al. 2007) and from the surface water of a tropical lake (Chen et al. 2008). New sequence data from Porter et al. (2011) suggest that these two sequences are related to *Blastocladiella*.

III. Structure of Thallus and Reproductive Characters

Thalli of blastoclads vary greatly in size, extent, and position in relation to the substratum. Simple thalli may be **monocentric** (producing a single reproductive body) or **epibiotic** (with the reproductive body produced outside the substratum). A tube from a young epibiotic thallus penetrates the substratum and branches distally to form an **endobiotic** system of smaller branching tubes (**rhizoids**) that functions in the absorption of nutrients. The complex thalli of some genera are **mycelial** (filamentous with tubular hyphae) and **polycentric** (produce multiple reproductive bodies). Blastoclad thalli generally consist of a larger basal axis attached to the substratum by rhizoids, which may exhibit **determinate** (limited growth) or **indeterminate** (unlimited growth) apical branching and growth. The hyphae may be **septate** (with incomplete septa having central and lateral perforations) or **aseptate** and **coenocytic** (without septa). Polycentric thalli in some parasites take the form of slender rhizoidal elements alternating with spindle-shaped swellings (a **rhizomycelium**), while in other parasites the thalli may be reduced to unwalled coenocytic thalli (**hyphal bodies**) that lack rhizoids. Thalli lacking rhizoids that are completely converted into reproductive structures are **holocarpic**, whereas thalli bearing rhizoids or vegetative portions not contributing to reproductive structures are **eucarpic**.

Many blastoclads have been shown to share a life history involving the alternation of two developmentally associated phases: a haploid **gametophyte generation** (which produces gametes) and a diploid **sporophyte generation** (which produces asexual spores). Organisms in which the vegetative features of gametophyte and sporophyte are very similar have an **isomorphic alternation of generations**, whereas those in which the vegetative features of one generation differ substantially from those of the next have a **heteromorphic alternation of generations**. Sexual reproduction occurs when **gametes** produced from gametophytic thalli undergo **syngamy** (fusion) to form diploid **planozygotes** that give rise to sporophytic thalli. Sporophytic thalli carry out asexual reproduction through the formation of **thin-walled zoosporangia** (which produce zoospores that renew the sporophyte generation) and thick-walled **resistant sporangia** (which typically undergo **meiosis** and produce **meiospores** that renew the gametophyte generation). In some publications thin-walled zoosporangia and zoospores are referred to as mitosporangia and mitospores, respectively, whereas resistant sporangia are referred to as **resting spores**, **resting sporangia**, or **meiosporangia** and their products variously as **meiospores**, **RS zoospores**, or **RS planospores**. The resistant sporangium has a thick, pigmented outer wall that may be smooth or ornamented with pits, punctae, or a complex series of ridges (Fig. 7.1c). At germination the outer wall of the resistant sporangium cracks open at undetermined points in some species, while in others it opens along a preformed **germination slit** or a **circumcissile lid**. An elastic **inner wall** (also called the **endosporangium**) may swell and protrude through the outer wall at the time of germination. **Discharge papillae** are dome-shaped protrusions of gelatinous material that form temporary plugs in **discharge pores** or slits in the walls of zoosporangia, gametangia, and the inner wall of the resistant sporangium. Upon dissolution of the plug the spores pass through the pore or slit to the outside. Species producing gametangia on a

single thallus whose nuclei are self-compatible are said to be **homothallic**, whereas those producing gametangia on different thalli whose nuclei are self-incompatible but cross-compatible are **heterothallic**. Morphologically distinct male and female **gametangia** or gametes occur in both homothallic and heterothallic species and may be distinguished by differences in size, color, and mating behavior. The alternative forms of physiologically distinguishable (but morphologically indistinguishable) heterothallic species are designated as **plus (+)** and **minus (–) mating types**. Sexual reproduction by fusion of flagellated gametes that are equal in size is referred to as **isogamy**, while fusion of flagellated gametes that are unequal in size is **anisogamy**. With reference to animal systems, the smaller gamete is designated as male while the larger is female.

IV. Phylogeny and Systematics

A. Phylogenetic Placement of Blastocladiomycota

The history of Blastocladiomycota began with the description of the first so-called *chytrid* genus, *Physoderma*, by Wallroth in 1833. In 1878, P. F. Reinsch described *Blastocladia pringheimii* as the single member of a new genus whose unusual features were puzzling to systematists who included the organism variously among the Saprolegniaceae (Fischer 1892) or the Leptomitaceae (Schroeter 1893). Petersen felt that these fungi differed substantially from the Saprolegniales and in 1909 established the order Blastocladiales containing the single family Blastocladiaceae to accommodate the genus *Blastocladia* (Petersen 1909), and a second genus, *Allomyces*, was soon added to the family (Butler 1911). Little additional knowledge of the group occurred until Kniep's researches on *Allomyces* (Kniep 1930), which resulted in the **discovery of a life cycle with sporic meiosis and a new type of sexuality (anisogamy) previously unknown in the fungi**. These findings stimulated further research and quickly led to a more refined concept of the Blastocladiales as a morphologically distinct group with greatest affinities to Scherffel's uniflagellate "Chytridineen" series of fungi (Scherffel 1925). This concept was followed by Sparrow in his monographic treatments in 1943 and 1960. Sparrow regarded the posteriorly uniflagellate Chytridiales, Blastocladiales, and Monoblepharidales as one of four lines of descent among the aquatic fungi and erected the class Chytridiomycetes into which they were transferred (Sparrow 1960).

Since the onset of molecular phylogenetic-based systematics, numerous studies have investigated the phylogenetic placement of the blastoclads in the fungal tree of life. Currently, the placement is disputed and ranges from being sister to the Chytridiomycota to being related to terrestrial zygomycete fungi (Fig. 7.2). The implications for the placement of the group within the fungal kingdom are important for understanding the traits of the most recent common ancestor of all fungi and of evolutionary trends in life cycles. Recently, Porter et al. (2011) produced the first comprehensive molecular phylogenetic study of genera and families of blastoclads. The results of these phylogenetic studies and additional ultrastructural studies led to the establishment of a new phylum, the Blastocladiomycota (James et al. 2006b), with the single class Blastocladiomycetes, into which were placed the genera included in Blastocladiales.

The tool of choice for fungal molecular phylogenetics has been nuclear-encoded ribosomal DNA (rDNA) because this multicopy region contains both conserved and divergent regions capable of providing multiple levels of phylogenetic resolution and is easy to amplify using conserved polymerase chain reaction primers (Bruns et al. 1991). Early phylogenetic studies of zoosporic fungi focused on ultrastructural characters of the zoospore that have been used to delimit the orders, families, and genera; these studies suggested a good correlation among ultrastructural characters and phylogenetic relationships based on rDNA (James et al. 2000; Letcher et al. 2006, 2008; Simmons et al. 2009). Subsequently, the rDNA operon (18S+5.8S+28S) also provided a framework for suggesting taxonomic revisions in the Blastocladiomycota (Porter et al. 2011).

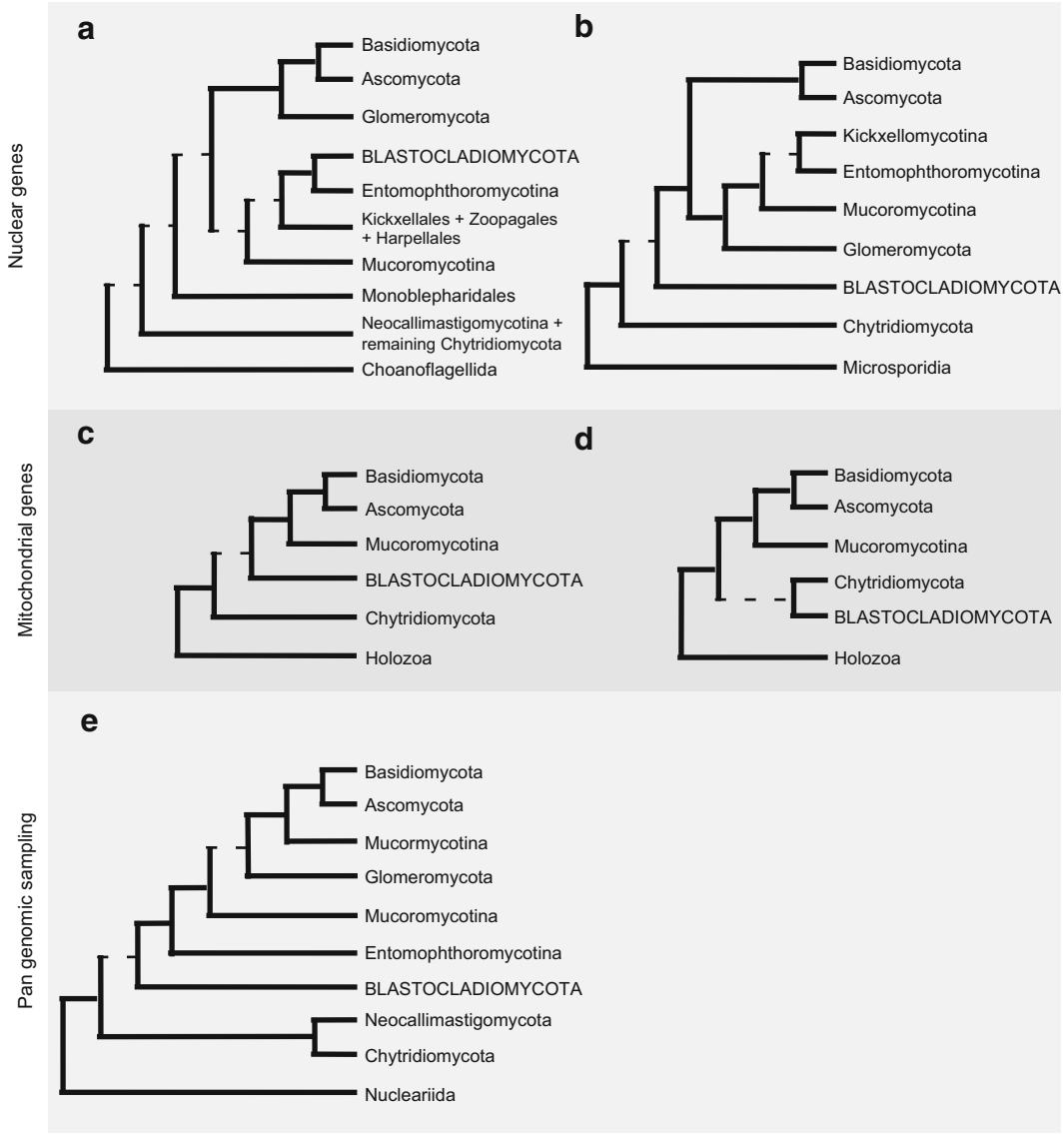

Fig. 7.2 Hypothesized phylogenetic placements of Blastocladiomycota among fungi. (**a**) James et al. (2000) with SSU rDNA analyzed using maximum parsimony. (**b**) Liu et al. (2006) with RPB1+RPB2 concatenated protein sequences analyzed using Bayesian inference and maximum parsimony. (**c**, **d**) Lang et al. (2002) with 11 concatenated mtDNA proteins analyzed using maximum likelihood (**c**) or a distance method (**d**). (**e**) Liu et al. (2009) with 150 concatenated proteins (40,925 amino acids) analyzed using Bayesian inference and maximum likelihood. Dashed lines: branches whose placement in phylogeny was not strongly supported by bootstrap analyses (<70 %)

In many of the following cited studies, species in the Blastocladiales are still classified with the Chytridiomycota; however, in Fig. 7.2 they are classified according to their present placement in Blastocladiomycota. Nuclear genes, such as rDNA and protein-coding genes, have been used with varying levels of success to classify the basal fungi. James et al. (2000) used small subunit (SSU) rDNA (18S rDNA) sequences to produce the first well-sampled phylogeny of zoosporic true fungi and addressed the phylogenetic consistency of zoospore discharge type, thallus

development, and ultrastructural features of the zoospore (Fig. 7.2a). Three members of Blastocladiomycota were included in this study, and they clustered separate from the other Chytridiomycota as sister to Entomophthoromycotina (zygomycetes); however, this placement was not statistically supported. Another study used Bayesian inference of aligned amino acids from the RPB1+RPB2 (the two largest subunits of RNA polymerase II) nuclear protein coding sequences from representatives of the major fungal lineages (Liu et al. 2006). They recovered high Bayesian posterior probability but low maximum parsimony bootstrap support for the clustering of Blastocladiomycota as a sister group to the nonzoosporic fungi, i.e., Dikarya+zygomycetes (Fig. 7.2b).

Altogether, a large number of studies have included taxa from the Blastocladiomycota in phylogenies of the basal fungal lineages using a variety of markers, such as SSU rDNA (Tanabe et al. 2000, 2005), RPB1 (Tanabe et al. 2004), complete mitochondrial sequences (Bullerwell et al. 2003; Lang et al. 2002), and elongation factor subunit 1-alpha (EF1-α) indels (Tanabe et al. 2002). Taking these results into account, Tanabe et al. (2005) proposed a new supraordinal phylogeny with unresolved basal nodes and placement of Blastocladiomycota with Entomophthoromycotina. A combined gene approach used maximum likelihood and Bayesian inference with the rDNA operon (SSU+5.8S +LSU rDNA)+EF1-α+RPBI+RPB2 for a representative collection of fungal sequences (James et al. 2006a). The resulting phylogeny placed the blastoclads sister to nonzoosporic fungi. Altogether, studies using multiple combinations of phylogenetic markers have repeatedly found that the Blastocladiomycota are monophyletic and usually group separately from other lineages of zoosporic fungi.

Until 2006, the blastoclads were treated as an order (Blastocladiales) in Chytridiomycota. The distinctiveness of Blastocladiales from Chytridiomycota was formally addressed by James et al. (2006b), who described Blastocladiales as a separate phylum, Blastocladiomycota. This was based on rDNA phylogenetic analysis and ultrastructural characters. Blastocladiomycota was also recognized as a separate phylum in the most recent classification of the Fungi (Hibbett et al. 2007). Currently, the International Committee on Botanical Nomenclature is considering whether description of a phylum of the same name by Doweld (2001) meets standards for valid publication and has priority.

The Fungal Mitochondrial Genome Sequencing Project has targeted several key representatives of the basal fungal lineages (Paquin et al. 1997). In the most inclusive analysis, Lang et al. (2002) used maximum likelihood and neighbor-joining phylogenetic analyses with 11 mitochondrial genes from representatives from the major fungal lineages. Depending on the method and taxon set used for phylogenetic reconstruction, Blastocladiomycota branches sister to either Dikarya+Mucoromycotina or Chytridiomycota (Fig. 7.2c, d). In each case, the statistical support for placement of Blastocladiomycota is low, while support for the placement of other groups is quite high.

With the increasing publication of fungal genomes, it is now possible to include larger combinations of markers in phylogenetic analyses. Liu et al. (2009) used Bayesian inference and maximum likelihood phylogenetic analyses to target 150 nuclear protein-coding genes comprising 40,925 amino acids from representatives of the major fungal lineages. The placement of the Blastocladiomycota was sister to the nonzoosporic fungi but with low statistical support (Fig. 7.2e). In conclusion, the ancient Blastocladiomycota seem to be monophyletic in each of the numerous phylogenetic studies. However, the precise placement of the lineage relative to other phyla and subphyla of both zoosporic and nonzoosporic fungi is uncertain. In the future, additional genome and large-scale expressed sequence tag (EST) sequencing of many more representatives of the basal fungal lineages will be needed to resolve the question of when the blastoclads diverged among the fungi.

B. Phylogenetic Classification of Blastocladiomycota

Blastoclads comprise 5 families (Blastocladiaceae, Catenariaceae, Coelomomycetaceae, Physodermataceae and Sorochytriaceae), 14

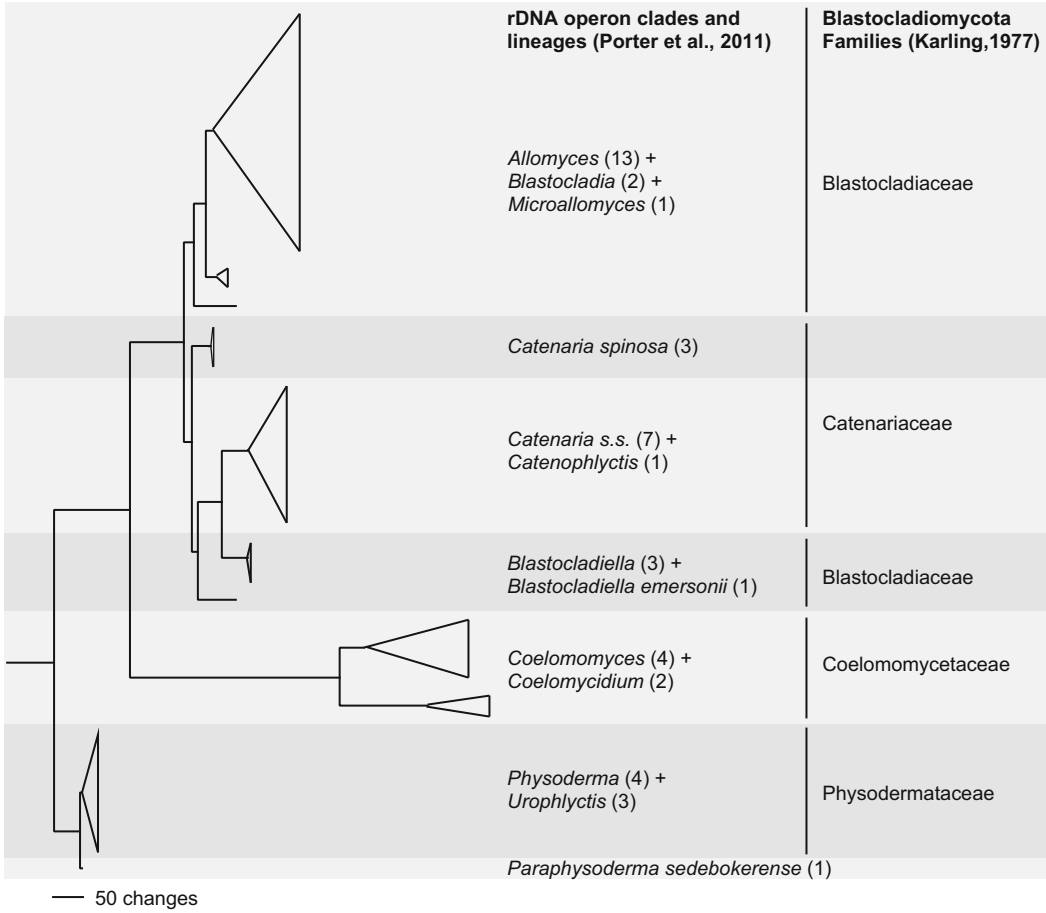

Fig. 7.3 Schematic phylogeny of Blastocladiomycota based on rRNA operon sequences (Porter et al. 2011) with taxonomic classifications from Karling (1977). Numbers following generic names: number of sequences included

genera, and approximately 180 species. It is likely that many additional species, genera, and higher taxa remain to be discovered and described. Figure 7.3 summarizes the results from a Bayesian rDNA (SSU+5.8S+LSU) phylogeny, including 45 gene sequences from the Blastocladiomycota representing 4 of the 5 families (Porter et al. 2011). Here we review the phylogenetic placement of the various families, the evidence of monophyly of genera, and the morphological characters that separate the various genera.

The Blastocladiaceae Petersen 1909 currently comprises five genera: *Allomyces* Butler 1911, *Blastocladia*, *Microallomyces* Emerson and Robertson 1974, *Blastocladiella* Matthews 1937, and *Blastocladiopsis* Sparrow 1950 (Karling 1977). Genera can be separated on the basis of thallus organization. *Allomyces* is the only genus that displays truly indeterminate growth as a mycelium with septa, and *Microallomyces* is similar to *Allomyces* but smaller in stature and lacking pseudosepta. Members of *Blastocladia* typically have a single trunklike basal cell, with septations only at reproductive organs. The poorly known *Blastocladiopsis* is distinguished from *Blastocladia* on the basis of an unpitted, loose resting sporangium. In Fig. 7.3, strains from *Allomyces*, *Blastocladia*, and *Microallomyces* form a clade, three strains of *Blastocladiella* and *Blastocladiella emersonii* Cantino and Hyatt 1953 cluster separately, and the genus *Blastocladiopsis* has yet to be placed in a molecular phylogeny. Though *Allomyces* forms a clade, Porter et al. (2011) showed that these taxa do not necessarily group according

to current subgenera defined by life cycle characteristics (Emerson 1938, 1941). Instead, Porter et al. recovered two major *Allomyces* clades with multiple strains of the type species *A. arbusculus* found in each. The only subgenus that formed a clade was *Cystogenes*, whereas the **subgenera *Euallomyces* and *Brachyallomyces* were recovered as paraphyletic.**

Catenariaceae can be distinguished from Blastocladiaceae by the presence of a catenulate (chainlike) rhizomycelium with swellings or sporangia separated by narrow isthmuses. The Catenariaceae Couch 1945 currently comprises three genera: *Catenaria* Sorokin 1889, *Catenophlyctis* Karling 1965, and *Catenomyces* A.M. c 1944 (Karling 1965). *Catenophlyctis* is distinguished from *Catenaria* by having a more chytridlike monocentric growth form, though some isolates of the former are highly polycentric. In Fig. 7.3 *Catenaria* and *Catenophlyctis* form a clade that includes the type species of *Catenaria*, *Catenaria anguillulae* Sorokin 1876. The sole isolate of *Catenophlyctis* groups among *Catenaria*, suggesting the distinction of the genera is likely artificial. *Catenomyces*, currently classified in the Catenariaceae, however, clusters with Chytridiomycota (James et al. 2006b). Two additional strains of *Catenaria* isolated from midge larvae, *Catenaria spinosa* and *Catenaria uncinata*, form a separate clade, suggesting that ***Catenaria* is polyphyletic** (Martin 1975, 1978; Porter et al. 2011).

Coelomomycetaceae Couch ex Couch 1962 currently comprises two invertebrate pathogenic genera, *Coelomomyces* Keilin 1921 and *Coelomycidium* Debais 1919. Both genera grow inside their hosts in the form of naked protoplasts. *Coelomomyces* is distinguished from *Coelomycidium* on the basis of hosts, mosquitoes, and ostracods or copepods in the former and blackflies in the latter. In Fig. 7.3 ***Coelomomyces* and *Coelomycidium* are reciprocally monophyletic sister clades.**

Physodermataceae Sparrow 1952 comprises two plant pathogenic genera, *Physoderma* Wallr. 1833 and *Urophlyctis* J. Schrot. 1886. Both genera form an epibiotic, monocentric, sporangial stage and an endobiotic, polycentric phase. These two genera were synonymized (Karling 1950) and are generally still considered synonymous to this day (Karling 1977; Kirk et al. 2008). *Physoderma* includes some 80 or more species that are obligate parasites of plants whose effects on stems, leaves, and inflorescences may vary from simple discoloration to significant hypertrophy. Those species that were known to induce gall formation in the host were segregated into *Urophlyctis* (Sparrow 1962). The synonymy of *Physoderma* and *Urophlyctis* has been debated (Karling 1977; Sparrow 1962), but *Urophlyctis* differs from *Physoderma* in several microscopic characters as well as in inducing gall formation in its host. Lange and Olson (1980) studied the ultrastructure of motile cells of *Physoderma* and transferred the family Physodermataceae to Blastocladiales from Chytridiales. *Physoderma* and *Urophlyctis* are closely related (Fig. 7.3), and Porter et al. (2011) showed that *Urophlyctis* is nested within *Physoderma*, which together form a monophyletic clade. Additional sampling of taxa and markers/loci will be required to determine whether *Urophlyctis* and all gall-inducing species are monophyletic. A newly described genus, *Paraphysoderma*, is only known as a parasite of the Chlorophycean alga *Haematococcus*. It clusters sister to *Physoderma*+*Urophlyctis* (Hoffman et al. 2008; James et al. 2011). *Paraphysoderma* is further distinguished by producing nonflagellated aplanospores rather than zoospores.

Sorochytriaceae (Dewel et al. 1985) currently contains a single species, *Sorochytrium milnesiophthora*, which grows endobiotically within the tardigrade host and typically forms a polycentric rhizomycelium. The species has yet to be placed in a molecular phylogeny; however, a study of the ultrastructure of the zoospores of *S. milnesiophthora* clearly places the family with Blastocladiales (Dewel and Dewel 1990).

The time-consuming nature and specialist knowledge required to collect and identify new isolates means that many described members of Blastocladiomycota, particularly pathogenic species, have yet to be sequenced and placed in a molecular phylogeny, echoing a common pattern in science (Hibbett et al. 2007). Several additional organisms have been described in recent years whose affinities are clearly with the blastoclads but whose life cycles or development are incompletely known or understood. These include *Polycaryum laeve*, an endoparasite of *Daphnia* previously considered a haplosporidian. Phylogenetic evidence was used to

affiliate *Polycaryum* with blastoclads, but only a partial sequence is available, precluding more precise placement (Johnson et al. 2006). An effort to collect and place type species in a molecular phylogeny would help to catalog the phylogenetic diversity in Blastocladiomycota and provide a framework for further ecological studies. Mention should also be made of *Callimastix cyclopis, a* parasite of the copepod *Cyclops* whose zoospore structure most resembles that of *Coelomomyces* (Vavra and Joyon 1966).

V. Life Cycles

A. Historical Perspective

By the early twentieth century the concept of an alternation of haploid and diploid generations in the life histories of lower plants and major algal groups was firmly established. However, the discovery by Kniep of an alternation of sporophyte and gametophyte generations in a new zoosporic fungus (*A. javanicus*) was unexpected and aroused great interest in the mycological community (Kniep 1930). Kniep established ploidy in the new fungus by reported **nuclear volume ratios of 1:2 between gametophyte and sporophyte**, and Emerson and Wilson (1949) and Wilson (1952) provided the cytological proof of "sporic" meiosis in the resistant sporangium. These studies, along with the later electron microscopic observations, confirmed that meiosis in *A. macrogynus* begins during early resistant sporangium formation with the appearance of a synaptonemal complex, is halted as **the resistant sporangium enters the resting phase in the late prophase (diplotene) of meiosis I, and is completed during germination** (Olson 1974).

Emerson's 1941 monograph was a seminal work detailing the results of a 6-year study involving the comparative development of 51 *Allomyces* isolates from around the world (Emerson 1941). In this work Emerson recognized three life cycle types that formed the basis of his classification of the genus into three subgenera: ***Euallomyces*** (to include so-called long-cycled species in which there is an isomorphic alternation of generations), ***Brachyallomyces*** (to include so-called short-cycled isolates in which there is no indication of sexuality), and ***Cystogenes*** (to include isolates in which the gametophyte thallus is reduced to a single-celled cyst). Since that time, new members of the Blastocladiomycota have been interpreted and described in relation to the life cycles of *Allomyces*, and this practice is reflected in much of the present classification. To understand the diversity of life histories now known for the blastoclads, it is necessary to review in some detail earlier research on the sexuality and life cycles of *Allomyces*.

B. Life Cycles of *Allomyces*

Euallomyces The subgenus *Euallomyces* includes long-cycled *Allomyces* species such as *A. javanicus* and *A. arbuscula* in which the vegetative thalli of both gametophyte and sporophyte generations are the same in appearance (an isomorphic alternation of generations). The life history of *A. arbuscula* (Emerson 1941; Hatch 1935) is shown in Fig. 7.4. A simplified diagram of the *Euallomyces* life cycle is presented in Fig. 7.6c. In these species the homothallic gametophyte generation bears gametangia that are typically paired and sexually dimorphic with smaller orange male and larger colorless female gametangia. Smaller, more active orange gametes released from male gametangia undergo anisogamous fusion with larger colorless gametes from female gametangia to produce biflagellate planozygotes that give rise to the diploid sporophyte generation. Sporophytes produce a mixture of thin-walled zoosporangia and thick-walled, brown, pitted, resistant sporangia. Zoospores from zoosporangia give rise asexually to additional sporophytic thalli, and resistant sporangia undergo meiosis and release haploid meiospores that produce the gametophyte generation. Deviations from these so-called normal patterns have been observed frequently, including the parthenogenetic development of both gametophyte and sporophyte thalli from single (nonfusing) female gametes and the development of sporophytic thalli from meiospores (Emerson 1941).

Cystogenes Emerson (1938, 1941) and Wilson (1952) described a very different type of life cycle in *A. moniliformis* and *A. neo-moniliformis* (=*A. cystogenus*) (Emerson 1941), a heteromorphic

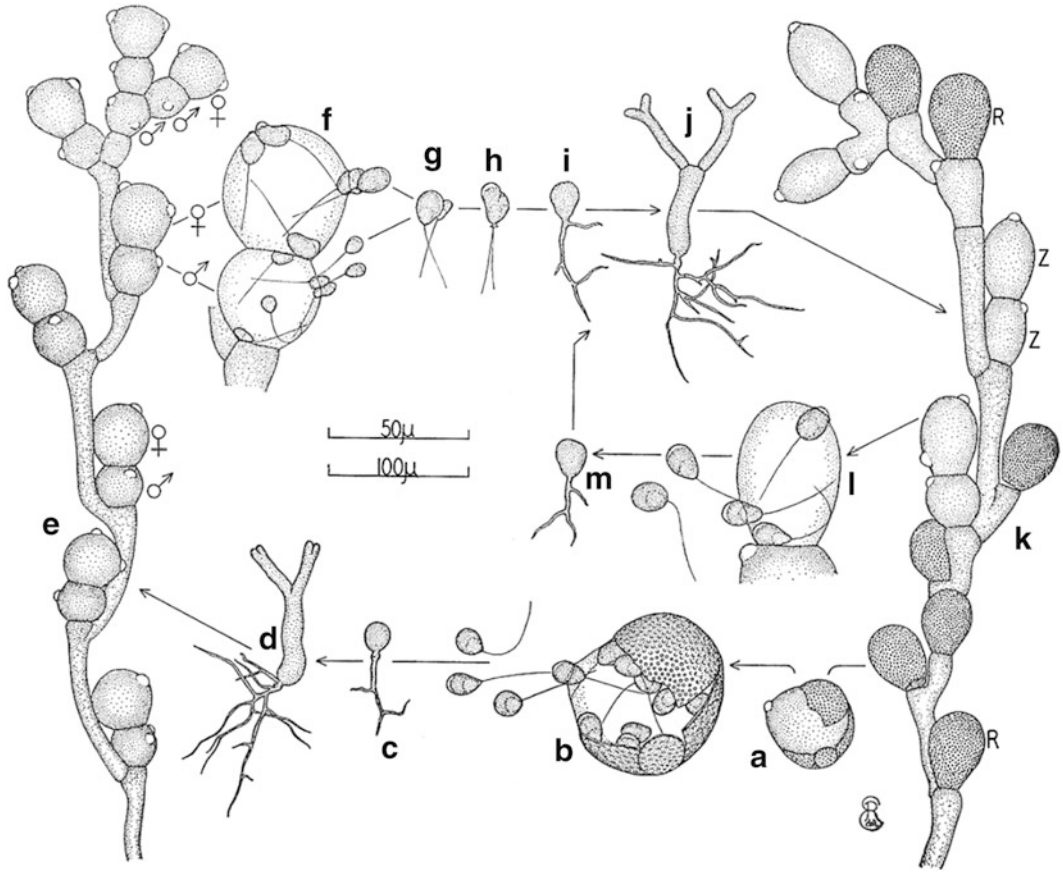

Fig. 7.4 (**a**–**m**) Life cycle of *Allomyces arbuscula*. (**a**) Germinating resistant sporangium with ruptured outer wall and swollen inner wall with discharge papilla. (**b**) Release of uniflagellate meiospores. (**c**–**e**) Stages in growth of young thallus (**c**, **d**) into mature gametophytic thallus bearing papillate male (♂) and female (♀) gametangia (**e**). (**f**) Release of male and female gametes from paired gametangia. (**g**) Syngamy of anisogametes. (**h**) Biflagellate planozygote. (**i**) Germination of encysted planozygote. (**j**, **k**) Young thallus (**j**) develops into mature sporophytic thallus (**k**) bearing resistant sporangia (R) and thin-walled zoosporangia (Z). (**l**) Release of zoospores from thin-walled zoosporangia. (**m**) Germination of encysted zoospore to form young thallus (**j**). Drawing from Emerson (1941), used with permission from Lloyd Library and Museum; American Society of Pharmacognosy

alternation of generations in which the gametophyte thallus is reduced to a single-celled cyst (Figs. 7.5 and 7.6d). The sporophytic thalli of *Cystogenes* species are like those of *Euallomyces* in structure and size, and meiosis takes place in the resistant sporangium (Emerson and Wilson 1949; Olson 1980; Wilson 1952). However, in *Cystogenes* isolates meiosis is followed by a pairing of haploid nuclei prior to cleavage into spores. Meiospores exit the resistant sporangium as binucleate cells that move sluggishly as biflagellate motile cells or as nonflagellated amoeboid cells before rapid encystment. The two cyst nuclei undergo a single mitotic division to produce four haploid cells (isogametes) that exit upon deliquescence of the single papilla. The fate of the quartet of cells emerging from the cyst was unknown to Emerson; however, the cells were shown to function as uniflagellate or aflagellate amoeboid isogametes that fuse to form zygotes (McCrainie 1942; Teter 1944).

Brachyallomyces Isolates in which the motile spores from the resistant sporangia regularly gave rise to asexual (sporophytic) rather than sexual (gametophytic) thalli were placed in a new species, *A. anomalus*, and included in the subgenus

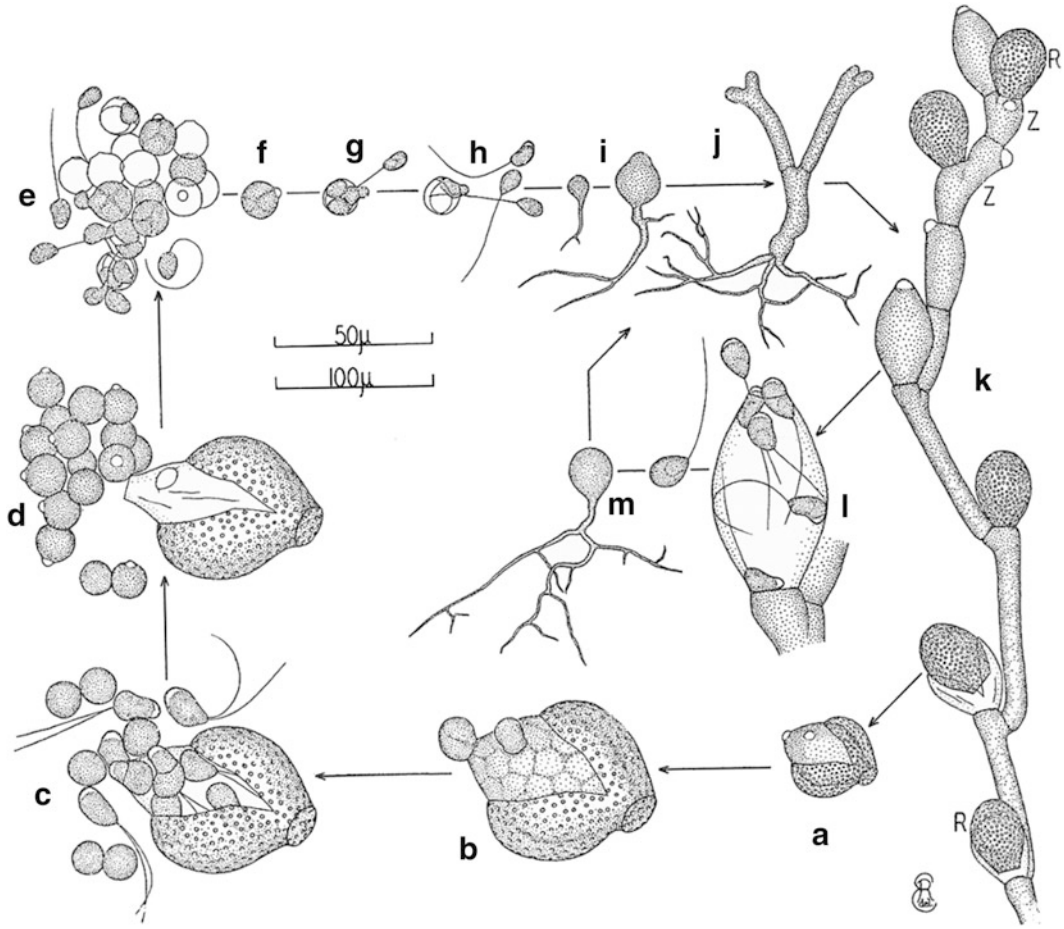

Fig. 7.5 (**a**–**m**) Life cycle of *Allomyces neo-moniliformis*. (**a**) Germinating resistant sporangium with ruptured outer wall and swollen inner wall with two discharge papillae. (**b**) Beginning of meiospore release. (**c**) Biflagellate meiospores exiting resistant sporangium; some have encysted. (**d**) Cysts, each with single papilla, clustered at mouth of empty resistant sporangium. (**e**–**h**) Stages in emergence of uniflagellate gametes from cysts; syngamy not shown. (**i**) Germination of encysted zygotes. (**j, k**) Young thallus (**j**) develops into mature sporophytic thallus (**k**) bearing resistant sporangia (R) and thin-walled zoosporangia (Z). (**l**) Release of zoospores from thin-walled zoosporangia. (**m**) Germination of encysted zoospore to form young thallus (**j**). Drawing from Emerson (1941), used with permission from Lloyd Library and Museum; American Society of Pharmacognosy

Brachyallomyces (Emerson 1941). Emerson found that by varying the substrate he could induce some putative *Brachyallomyces* isolates to form gametophytes, and he was careful to ascribe only those isolates that remained consistently asexual to the subgenus. It was later revealed that asexuality was maintained by mitosis in the resistant sporangia of some *A. anomalus* isolates (Fig. 7.6e), while in other isolates meiosis was presumably followed by endomitosis (nuclear replication without division) in germinating meiospores to reestablish the diploid condition (Fig. 7.6f) (Wilson and Flanagan 1968). The sporophytic thalli of *A. anomalus* isolates are entirely like those of *Euallomyces* species but lack a sexual stage.

C. Life Cycles of Other Blastocladiomycota

In the 70 years since the publication of Emerson's monograph on *Allomyces*, many new blastoclads have been discovered, and the diversity

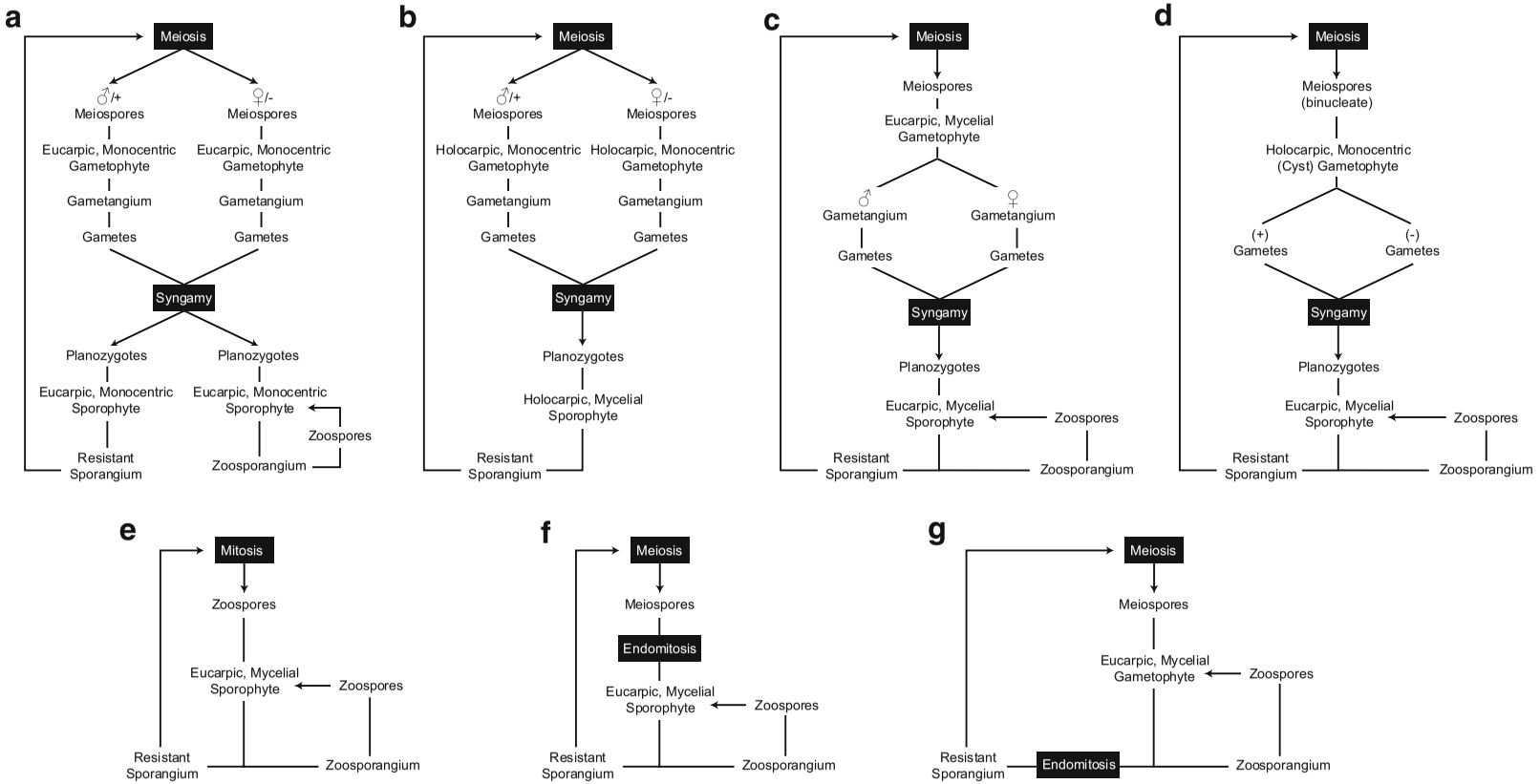

Fig. 7.6 Diagrams illustrating life cycles and patterns of sexuality in selected members of Blastocladiomycota. RS, resistant sporangia, TWZ, thin-walled zoosporangia. (**a**) Isomorphic alternation of generations, gametophytes heterothallic with isogamy, separate sporophytes bear RS or TWZ; *Blastocladiella variabilis*. (**b**) Isomorphic alternation of generations, gametophytes heterothallic with isogamy, sporophyte bearing RS only; *Coelomomyces punctatus*. (**c**) Isomorphic alternation of generations, gametophyte homothallic with anisogamy, sporophyte with RS and TWZ; *Allomyces arbusculus*. (**d**) Heteromorphic alternation of generations, gametophyte a monocentric cyst with isogamy, sporophyte produces RS and TWZ; *Allomyces neo-moniliformis*. (**e**) Asexual zoospores give rise directly by mitosis to sporophytes bearing RS and TWZ; some *Allomyces anomalus*. (**f**) Meiospores resulting from meiosis in RS undergo endomitosis at germination to form sporophytes; sporophyte produces RS and TWZ; some *A. anomalus*. (**g**) Meiospores resulting from meiosis in RS give rise to gametophytes bearing TWZ and young RS; endomitosis or selfing in early RS development restores diploid condition; *Catenaria anguillulae*

of life cycle types has increased. The *Euallomyces* life cycle of *Allomyces* (Fig. 7.6c) has an isomorphic alternation of generations with homothallic mating of morphologically distinct male and female gametes. **Anisogamy has been reported only in *Euallomyces* species.** Homothallism of the type displayed by *Allomyces* has not been reported in other isomorphic genera, but such a pattern may have existed in *Paleoblastocladia milleri* (Remy et al. 1994), a fossil blastoclad from the 400 million-year-old Rhynie chert. In addition to sporophytic thalli bearing zoosporangia and resistant sporangia there are similar (gametophytic?) thalli bearing paired cells that resemble rather remarkably the gametangia of *A. arbuscula*.

The *Cystogenes* life cycle of *Allomyces* (Fig. 7.6d) is a heteromorphic alternation of generations with homothallic mating of isogametes. This pattern has been reported in species of *Blastocladiella* (subgenus *Cystocladiella*) and in the mycoparasite *Catenaria allomycis*. However, the life cycle of these fungi differs slightly from that of *Cystogenes* in producing uninucleate and uniflagellate meiospores that undergo two mitotic divisions prior to gamete formation.

An asexual or *Brachyallomyces* life cycle seems to be widespread in many blastoclad genera, particularly those of the Blastocladiaceae. The types represented by mitosis in the resistant sporangium (Fig. 7.6e) and by meiosis followed by diploidization by endomitosis in germinating meiospores (Fig. 7.6f) have been reported only for *A. anomalus* isolates. A third asexual pattern (Fig. 7.6g) has been reported in *C. anguillulae* (Olson and Reichle 1978a), and a modification of this pattern is likely present in *B. emersonii* (Olson and Reichle 1978b). In both organisms synaptonemal complexes and meiotic divisions are present during resistant sporangium formation and germination; however, both meiospores and zoospores are reported to be haploid. Induction of diploidization and resistant sporangium formation in *C. anguillulae* occurred when the haploid zoosporangial thalli were transferred from a nutrient medium lacking starch to one containing it. A similar transformation of zoosporangia into resistant sporangia occurred in *B. emersonii* when bicarbonate and other salts were added to media (Cantino 1956) and in *B. brittanica* in response to darkness (Horenstein and Cantino 1962).

The life cycle of the *Coelomomyces* species that have been thoroughly studied is that of an isomorphic alternation of generations involving heterothallic mating of isogametes (Fig. 7.6b). While this heteroecious life cycle is classified as isomorphic, the gametophytic thalli in crustacean hosts are smaller and with fewer branches than sporophytic thalli in dipteran hosts. Some species of *Coelomomyces* have strikingly dimorphic gametangia, with the male mating type bright orange and the female amber or colorless. In other species the + and – gametangia have similar pigmentation or are colorless. The thin-walled zoosporangia produced in some *Coelomomyces* species do not seem to be homologous to the thin-walled zoosporangia of other blastoclads as they are structurally similar to resistant sporangia and, like them, produce meiospores rather than zoospores.

Physoderma species reported to be heterothallic have a life cycle similar to that of *Coelomomyces*, with notable exceptions. In *Physoderma* there is a heteromorphic alternation of generations owing to significant differences in the size and structure of the monocentric and epibiotic gametophyte thalli and the polycentric and endobiotic sporophyte thalli. In some species of *Physoderma* the epibiotic thalli are reported to mature into gametangia that produce isogametes or zoosporangia whose zoospores form additional epibiotic thalli. As in *Coelomomyces*, gametangia may be distinguished as orange or crimson males and colorless females in reportedly heterothallic species (Karling 1977).

Blastocladiella is similar to *Allomyces* but is monocentric and thus may produce only sporangia or gametangia at any given phase of the life cycle. Karling (1977) noted the similarity between the life cycles of *Blastocladiella* and *Allomyces* and erected subgenera of *Blastocladiella* corresponding to those of *Allomyces*: *Eucladiella* (corresponding to Emerson's *Euallomyces*), in which there is an isomorphic alternation of generations, *Cystocladiella* (corresponding to *Cystogenes*), in which the gametophyte generation is a single-celled cyst, and *Blastocladiella* (corresponding to *Brachyallomyces*), for short-cycled or asexual forms. The sporophyte generation of *Blastocladiella* generally consists of separate thalli bearing zoosporangia or resistant sporangia, although in some

species zoosporangial thalli are lacking. **In species of *Eucladiella* the gametophyte generation consists of separate but equal-sized male and female thalli.** While the life cycles of *Cystocladiella* species such as *B. cystogena* are similar to those of *Cystogenes*, they differ in that they lack thin-walled zoosporangia (they produce thalli bearing resistant sporangia only) and produce cells from resistant sporangia that are uniflagellate and uninucleate rather than biflagellate and binucleate. The subgenus *Blastocladiella* includes short-cycled species such as *B. simplex* (Matthews 1937), *B. britannica* (Willoughby 1959), and *B. emersonii* (Cantino and Hyatt 1953), in which no sexual reproduction has been reported. Although much has been learned about the physiology of *B. emersonii*, questions remain about its sexuality and life cycle. Even though flagellated swarmers from orange and colorless (OC) cells were never observed to undergo karyogamy, they were reported to undergo plasmogamy and cytoplasmic exchange (Cantino and Horenstein 1954). Investigators have generally agreed that *B. emersonii* has a *Brachyallomyces* (or subgenus *Blastocladiella*) type of life cycle. Olson and Reichle (1978) found synaptonemal complexes and meiotic nuclear divisions in germinating *B. emersonii*-resistant sporangia, and Horgen et al. (1985) studied the fluorescence of mithramycin-stained nuclei and determined that meiospores contained half the DNA of zoospores of ordinary colorless cells (thin-walled zoosporangia). **The life cycle of *B. emersonii* is most similar to the *Brachyallomyces* pattern** in which meiosis is present (Fig. 7.6f).

The life cycle of *Blastocladiella variabilis* (Fig. 7.6a) is worthy of note because it perhaps has the greatest potential for development as a model genetic system among the flagellated fungi. Like *Coelomomyces* and *Physoderma*, its life cycle is an isomorphic alternation of generations with heterothallic mating. However, in *Blastocladiella* the thalli of both gametophyte and sporophyte generations are monocentric, epibiotic, and, thus, separate. Four distinct reproductive structures are produced on separate thalli in *B. variabilis*: two distinct heterothallic gametangia (one with reddish male gametangia and the other with colorless female gametangia) and two distinct sporophytic thalli (one bearing zoosporangia and the other bearing resistant sporangia) (Harder and Sörgel 1938).

VI. Zoospore Ultrastructure

A. Historical Perspective

In 1896 Thaxter examined the zoospores of *B. pringsheimii* and noted the posterior cilium and the broad and distinct mass of granular protoplasm in front of the large and subtriangular nucleus (Fig. 7.1a). Couch and Whiffen (1942) provided excellent illustrations of the zoospores and meiospores of *Blastocladiella cystogena* and rather prophetically stated, "This cap is undoubtedly of phylogenetic importance," and noted that such a structure had been reported previously in *Coelomycidium simulii* (Debaisieux 1920). Subsequently, the discovery of a new zoosporic fungus with posteriorly uniflagellate cells with a nuclear cap of the *Blastocladia* type became regarded as clear evidence of a relationship to the Blastocladiales.

Zoospores of the Chytridiales and Blastocladiales were among the earliest biological specimens to be examined with the electron microscope (Manton et al. 1952; Koch 1956). As new zoosporic fungi were discovered and the extent of variation in their vegetative and reproductive structures became known, the validity of morphology as revealed by light microscopy for taxonomic distinctions was questioned (Powell and Koch 1977a, b). Beginning in the 1960s detailed ultrastructural studies of the motile cells of various chytrid and blastoclad genera were conducted in search of additional characters of taxonomic and phylogenetic value. These studies revealed the identities of organelles previously observed with the light microscope and demonstrated new cytoplasmic similarities and differences among blastoclad genera. The nuclear cap was observed to be a membrane-bound cluster of ribosomes at the tip of a linear **axial assembly** found in all members of Blastocladiales. The side body (or Seitenkörper) of earlier studies was seen to be a mitochondrion and a component of a side body complex also found in all members of Blastocladiales.

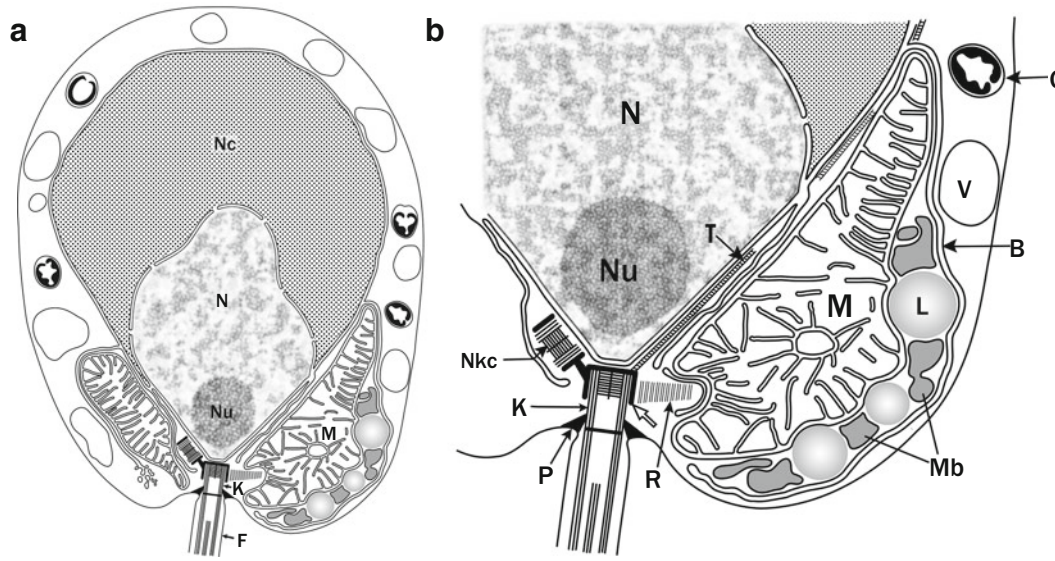

Fig. 7.7 (**a**, **b**) *Blastocladiella emersonii*. Interpretive drawings of zoospore showing ultrastructural details based primarily on several studies (Cantino and Truesdell 1970; Lessie and Lovett 1968; Reichle and Fuller 1967; Shaw and Cantino 1969). (**a**) Median longitudinal section through zoospore. (**b**) Detail of kinetosomal region and side body complex. *N* nucleus; *Nu* nucleolus; *Nc* nuclear cap; *F* flagellum; *K* functional kinetosome, *Nkc* nonkinetosomal centriole, *P* prop, *R* rhizoplast, *M* mitochondrion, *Mb* microbody, *L* lipid globule, *B* backing membrane, *G* gamma body, *T* cytoplasmic microtubule, *V* vacuole, *open arrow*, amorphous perikinetosomal material

B. Generalized Structure of Motile Cells of Blastocladiomycota

Surveys of ultrastructural characters in motile cells of the Chytridiomycetes have found four basic patterns of side body complexes or **microbody–lipid globule complexes (MLCs)** corresponding to the various orders of posteriorly uniflagellate fungi (Lange and Olson 1979; Powell 1978). The MLC of the Blastocladiales (type 4) was distinguished as an ordered arrangement of one or more mitochondria, microbodies, and lipid globules located along one side of an axially arranged nucleus and nuclear cap and enclosed by a backing membrane. Molecular phylogenetic studies provided most of the evidence by which Blastocladiales was raised to phylum status (James et al. 2006b); however, the formal description of phylum Blastocladiomycota was based largely on ultrastructural details. In the following account ultrastructural characters defining the phylum are discussed along with characters believed to be of systematic and phylogenetic importance in distinguishing major clades, subclades, and component genera.

1. Axial Assembly

The remarkable homogeneity of the nucleus, nuclear cap, and associated structures is revealed in drawings and photographs of motile cells of representatives of major clades of Blastocladiomycota, including a zoospore of *B. emersonii* (Fig. 7.7a, b), a zoospore of *Allomyces macrogynus* (Fig. 7.8a, b), a meiospore of *Coelomomyces punctatus* (Fig. 7.9), and a meiospore of *Physoderma maydis* (Fig. 7.10). The flagellated cells of all known members of Blastocladiomycota are characterized by a linear arrangement of organelles (axial assembly) consisting of a posterior flagellum with contained axoneme, functional **kinetosome**, nucleus with contained nucleolus, and anterior nuclear cap. The flagellum has the nine-plus-two arrangement typical of microtubules and narrows distally to a short whiplash portion. At the point where the flagellum joins the spore

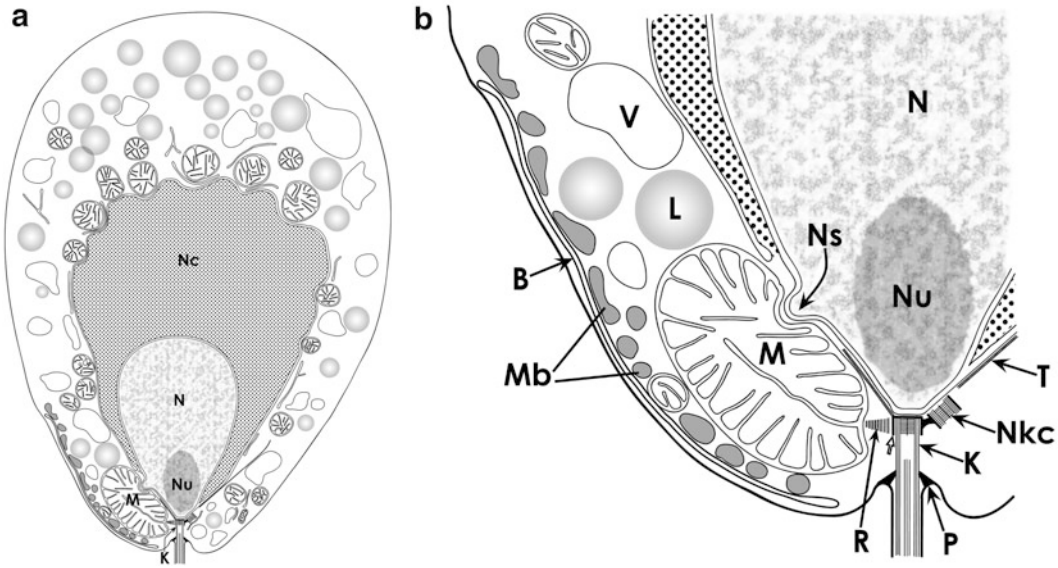

Fig. 7.8 (**a**, **b**) *Allomyces macrogynus*. Interpretive drawings of the zoospore showing ultrastructural details based primarily on two studies (Fuller and Olson 1971; Hill 1969). (**a**) Median longitudinal section through zoospore. (**b**) Detail of kinetosomal region and side body complex. *N* nucleus, *Nu* nucleolus, *Nc* nuclear cap, *Ns* nuclear spur, *K* functional kinetosome, *Nkc* nonkinetosomal centriole, *P* prop, *R* rhizoplast, *M* mitochondrion, *Mb* microbodies, *L* lipid globule, *B* backing membrane, *T* cytoplasmic microtubule, *V* vacuole, *open arrow* amorphous perikinetosomal material

body, nine electron-dense props extend inward to the axoneme or kinetosome. In all genera except *Coelomomyces* a **nonkinetosomal centriole** is present alongside the nucleus and is attached to the functional kinetosome by an electron-opaque bridge. A cap of electron-dense amorphous material surrounds the top of the functional kinetosome and extends down along the sides of the cartwheel region. In all genera cytoplasmic microtubules arise from the amorphous material at the extreme proximal end of the functional kinetosome and pass up and around the periphery of the nucleus and nuclear cap and into the cytoplasm (Figs. 7.7, 7.8, 7.9, and 7.10). It has been suggested that this area is a so-called organizing center for kinetosome-related microtubule formation (Dewel and Dewel 1990; Fuller and Calhoun 1968). In cross sections distal to the kinetosome the cytoplasmic microtubules typically occur in nine groups of three (triplets). The conical shape of the nucleus and nuclear cap is thought to be maintained by this basketlike arrangement of cytoplasmic microtubules. The ring or semicircle of amorphous material immediately below the organizing center is part of a flagellar apparatus that connects with the mitochondrion. The nuclear cap is composed of a dense cluster of dormant 80S ribosomes surrounded by double membranes, the outermost of which is continuous with the nuclear membrane (Jaworski and Stumhofer 1984).

2. Microbody–Lipid Globule Complex

Powell (1978) separated the type 4 MLC of Blastocladiomycota into two subtypes. Subtype 4A contains a single large mitochondrion that extends primarily along one side of the nucleus and nuclear cap and includes motile cells of *B. emersonii* (Fig. 7.7a, b), *C. punctatus* (Fig. 7.9), and *P. maydis* (Fig. 7.10). In *B. emersonii* and *C. punctatus* a cluster of lipid globules is located external to the long side of the mitochondrion and internal to a microbody, which is distended or completely penetrated by the rounded lipid globules. MLC subtype 4B occurs in motile cells of *A. macrogynus* (Fig. 7.8a, b) and *Allomyces*

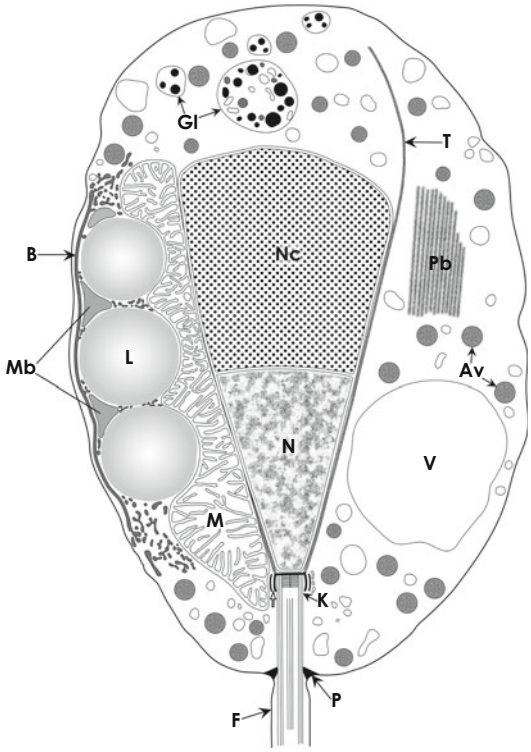

Fig. 7.9 *Coelomomyces punctatus*. Interpretive drawing of median longitudinal section through meiospore showing ultrastructural details based primarily on Martin (1971). *Av* adhesion vesicle, *B* backing membrane (contrasted with microbody to show tubular network), *Gl* gammalike body, *K* functional kinetosome, *L* lipid globule, *M* mitochondrion, *Mb* microbody, *N* nucleus, *Nc* nuclear cap, *P* prop, *F* flagellum, *Pb* paracrystalline body, *T* cytoplasmic microtubule, *V* vacuole, *open arrow* perikinetosomal striations

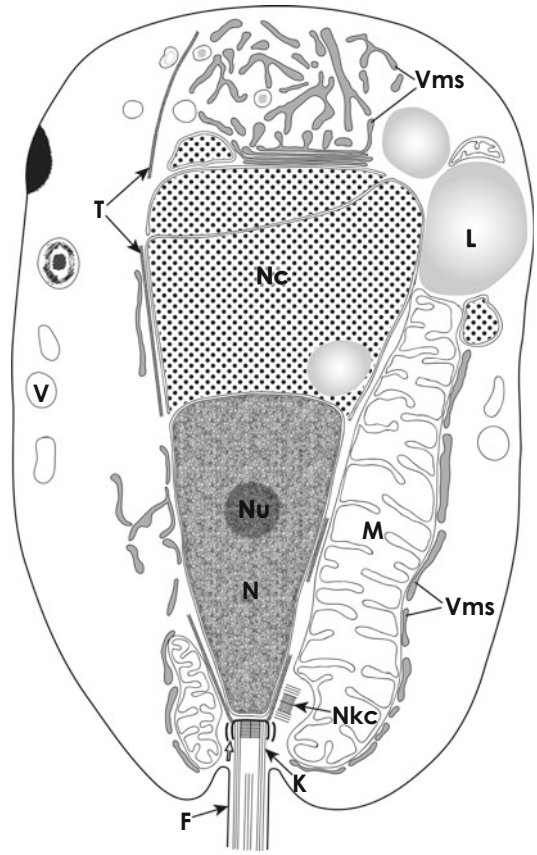

Fig. 7.10 *Physoderma maydis*. Interpretive drawing of median longitudinal section through meiospore showing ultrastructural details based primarily on Olson and Lange (1978) and Lange and Olson (1980). *F* flagellum, *K* functional kinetosome, *L* lipid globule, *M* mitochondrion, *N* nucleus, *Nc* nuclear cap, *Nkc* nonkinetosomal centriole, *Nu* nucleolus, *T* cytoplasmic microtubule, *V* vacuole, *Vms* vesicular–microbody system, *open arrow* perikinetosomal striations

neo-moniliformis (Fuller and Olson 1971; Olson 1980) and is unlike other blastocladian genera in having components that are highly divided and less closely associated. Numerous rounded mitochondria are present in zoospores of *A. macrogynus*, and many are partially embedded in the nuclear cap. A larger cup-shaped mitochondrion partially surrounds the functional kinetosome and is the main component of a reduced and poorly organized MLC that contains several microbodies, a small number of lipid globules, and additional mitochondria. Motile cells of *Blastocladia ramosa* contain multiple mitochondria but lack an organized MLC (Lingle and Barstow 1983). The outermost component of the MLC in subtypes 4A and 4B is a backing membrane that is usually continuous at several points with the outer membrane of the nuclear cap. The motile cells of *P. maydis* have a modified subtype 4A MLC in which the number of lipid globules may be reduced to one, and a **vesicular-microbody system** is present in place of a discrete backing membrane and microbody.

3. Flagellar Apparatus

In all members of the phylum there is a close association between the functional kinetosome and a completely or partially encircling mitochondrion. The **flagellar apparatus consists of perikinetosomal structures (striations, rhizoplasts, and fibrils)** that appear to form connections between the functional kinetosome and mitochondrion. In motile cells of *Sorochytrium milnesiopthora* (Dewel and Dewel 1990), *P. maydis* (Lange and Olson 1980), and *C. punctatus* (Martin 1971) the amorphous band of material surrounding the cartwheel portion of functional kinetosome may be partially resolved in cross sections as a complete or partial circle of discrete electron-dense projections. In longitudinal sections the projections often appear as discrete striations, each a component of an inner electron-dense band and one or more outer diffuse bands (Figs. 7.7 and 7.8, open arrows). Thin fibrils are often observed connecting the inner and outer perikinetosomal striations and fanning out to connect with the surface of a mitochondrion or a membrane cisterna. Olson and Lange (1978) referred to the striations as bridges, and presumably they are homologous with the transition fibers of *Coelomomyces dodgei* (Lucarotti and Federici 1984). The motile cells of *A. macrogynus* (Fig. 7.8), *A. neo-moniliformis* (Fuller and Olson 1971), *B. emersonii* (Fig. 7.7), *Blastocladiella brittanica* (Cantino and Truesdell 1971), and *C. anguillulae* (Olson et al. 1978) contain a rhizoplast (also called a striated or banded rootlet). The **rhizoplast** is a bar- or ribbon-shaped structure composed of a lateral series of equally spaced striae that is located laterally and in close proximity to the cartwheel portion of the functional kinetosome. Double membranes in the form of sheets or cisternae believed to originate from the outer nuclear or nuclear cap membrane are important components of the flagellar apparatus in many, if not all, blastoclad genera (Figs. 7.7b, 7.8b, 7.9). Powell (1983) has suggested that such cisternae may function in signal reception and transport between the cell surface and the flagellar apparatus.

4. Cytoplasmic Inclusions

A variety of inclusions have been reported in the cytoplasm of various blastoclad motile cells, including concentric granules, **vacuoles, adhesion vesicles, phosphate granules, gammalike bodies, gamma bodies or granules, and paracrystalline bodies.** Inclusions that appear homologous or analogous to gamma bodies or gammalike bodies have been found in motile cells of all blastocladian genera. Gamma bodies of *B. emersonii* are formed during zoosporogenesis by the coalescence of small granule-containing cisternae to form larger cisternae with many distinct granules (gammalike bodies) and a final aggregation stage that results in a distinctive cup-shaped inclusion (Barstow and Lovett 1975; Cantino and Truesdell 1971; Lessie and Lovett 1968; Lovett 1975; Mills and Cantino 1979). Mobilization or breakdown of gamma bodies typically occurs shortly after the beginning of zoospore encystment and results in the vesiculation of the contents and the translocation of vesicles to the cell surface. **Gamma bodies were once thought to function in the transport of chitin synthetase for cyst wall formation** (Barstow and Pommerville 1980; Mills and Cantino 1981); **however, later studies failed to support this hypothesis** (Dalley and Sonneborn 1982; Hohn et al. 1984). Olson and Lange (1983) interpreted the gamma bodies in motile spores of *Allomyces* as vesicle-generating structures that performed multiple functions upon mobilization or breakdown, including (1) the formation of water-expulsion vacuoles to maintain osmotic balance during zoospore motility, (2) production of vesicles that fuse to form axonemal and plasma membranes during sporogenesis, and (3) formation of vesicles that appear to be involved in cyst wall creation. The widespread occurrence of gamma bodies in the Blastocladiomycota prompted Dewel and Dewel (1990) to suggest that the gamma body should be considered a synapomorphy of the phylum. Adhesion vesicles are present in motile cells of *Coelomomyces* and are distinguished from gamma particles by their fine granular background and indistinct fibrous core (Fig. 7.9).

The contents of adhesion vesicles are released to form an adhesive plate that attaches meiospores to the intersegmental membranes of copepods and zygotes to the cuticle of mosquito larvae (Federici and Lucarotti 1986; Travland 1979).

A most unusual feature of both meiospores and gametes of *Coelomomyces* is the presence of one or more rod-shaped paracrystalline bodies that lie alongside the axial components opposite the MLC (Fig. 7.9) A similar structure has been reported in motile cells of *Callimastix cyclopis* (Manier and Loubes 1978; Vavra and Joyon 1966) and various members of the *Chytriomyces* clade of Chytridiomycota (Barr and Hartmann 1976; Picard et al. 2009; Taylor and Fuller 1981). The function of the paracrystalline body is unknown, but it has been speculated that it plays a role in infection (Madelin and Beckett 1972).

VII. Genetics and Physiology

A. Hybridization

The phenomenon of hybridization is not extensively documented in fungi, and verified instances of interspecific hybridization are rather rare (Brasier 2001; Schardl and Craven 2003). The classic work of Emerson and colleagues on hybridization between *A. arbusculus* and *A. macrogynus* provides convincing evidence for the existence of interspecific hybrids in the wild, but very little work has been conducted since the landmark paper by Emerson and Wilson (1954). Emerson and Wilson's work utilized clear differences between *A. arbusculus* and *A. macrogynus* in the arrangement of the pairs of male and female gametangia at hyphal tips. **Male gametangia (distinctively orange from gamma-carotene) are terminal or epigynous in *A. macrogynus*, while male gametangia are hypogynous in *A. arbusculus*.** Using controlled crosses between the two species, Emerson and Wilson found that F1 sporophytes were readily obtained, but the viability of meiospores produced by the sporophytes was greatly reduced. Among the viable meiospores, the F1 gametophyte generation displayed a range of gametangial arrangements, and putative hybrids typically showed a mixture of epigynous and hypogynous arrangements. This intermediate arrangement is also observed in **natural isolates of *A. javanicus*, which Emerson and Wilson hypothesized were hybrids of *A. arbusculus* x *A. macrogynus*.**

Emerson and Wilson further used cytology to verify the hybrid nature of the intermediate F1 gametophytes. Comparison of natural isolates suggested that *A. arbusculus* isolates were a polyploid series with a base chromosome number of 8, with the most common haploid (gametophyte) chromosome number of 16, implying that most *A. arbusculus* sporophytes are tetraploid. It was suggested that *A. macrogynus* had a base chromosome number of 14, but the common chromosomal types used by Emerson and Wilson possessed 28 chromosomes. Artificially produced *A. javanicus* would thus be expected to have 44 chromosomes before meiosis, and in crosses in which haploid *A. arbusculus* × *A macrogynus* fused, 44 chromosomes were observed, but only 1–5 bivalents were seen, indicating a lack of extensive homology between the chromosomes of the two species. This lack of pairing explains both the wide range of chromosome numbers in the artificial hybrid F1 gametophytes (improper segregation) and their low viability and agrees with the highly variable numbers of chromosomes seen in *A. javanicus* wild isolates. However, it was unclear why *A. javanicus* wild isolates had a variable but much lower (13–21) chromosome number than the artificial hybrids (20–44) (Emerson and Wilson 1954). It is also unclear whether the polyploid series within *A. arbusculus* and *A. macrogynus* are frequently generated by doubling or rarely generated and actually represent different species. Evidence that prolonged growth of *A. macrogynus* at 35 °C leads to a reduction in chromosome number that can be restored by growth at 23 °C (Borkhardt and Olson 1979; Olson and Borkhardt 1978) suggests that autopolyploidy by endomitosis could occur readily, but **the absence of many bivalents in F1 hybrid meiosis suggests, possibly, a more ancient origin.** Olson and Borkhardt additionally showed that when tetraploid resting sporangia are germinated and meiosis is blocked to induce the generation of sporophytic colonies with increased ploidy, they are usually unstable (Olson and Borkhardt 1978).

The highly selfing nature of *Allomyces* allows for the rapid stabilization and fertility of hybrids in the F2 and F3 generations as each haploid chromosome of viable F1 hybrids would find an identical homologous chromosome to pair with following the fusion of genetically identical selfed male and female gametes. Thus, selfing may have facilitated the recovery of natural hybrids in *Allomyces*. Phylogenetic analyses have now begun to shed light on the relationships between these hybridizing species of section *Euallomyces*. Neither *A. arbusculus* nor *A. macrogynus* seems to be monophyletic (Porter et al. 2011). Thus, the simple designations used to designate the species based on gametangial arrangements seem to be artificial, and the extensive morphological variation and polyploid series must be reevaluated by a combined study of chromosomes, phylogenies, and crosses.

Experimental hybridization has also been conducted in *Coelomomyces*, between *C. dodgei* and *C. punctatus* (Federici 1979, 1982). Utilizing the orange pigment of male gametangia and a common gametophyte host, the copepod *Cyclops vernalis*, Federici fused isogamous gametes of opposite mating type between the two species and then demonstrated that the hybrids could infect a common mosquito host (either *Anopheles freeborni* or *Anopheles quadrimaculatus*), proliferate as a sporophyte, and produce meiosporangia. The resting sporangia produced by the hybrid sporophytes displayed a wide range of characteristics but were mostly similar to one or the other parental species. The resting sporangia dehisced and released meiospores that encysted on the copepod host; however, **no gametophytes were ever produced**. These results demonstrate that the germination and growth of the haploid gametophyte is the most disrupted phase among hybrids of both *Coelomomyces* and *Allomyces*, as predicted by genetics. These studies also produce a working model for testing biological species; however, most of the crossing manipulations are extremely laborious.

B. Mitosis

Mitosis in the Blastocladiomycota has been well characterized using a combination of light and electron microscopy, and several innovations have been developed to study the process in the group. The process of nuclear division was often described as a part of a larger description of the development of hyphal, zoosporangial, or gametangial development from germinating zoospores at a time when *Allomyces* and *Blastocladiella* were still considered model organisms in genetics. Kniep (1930) observed that, although hyphal nuclei of *Allomyces* are large compared with other fungi, they were difficult subjects for the study of mitosis. Another early light-microscopy study described nuclear behavior in detail for *A. arbusculus* (Hatch 1935). Hatch described the development of germinating spores into coenocytic multinucleate hyphae that in turn develop into either a gametophyte bearing gametangia or into a sporophyte bearing zoosporangia. The nuclear count at the start of septation, when the first septum forms on a hypha, delimiting the apical female gametangium, and the second septum that forms further behind on the hypha, delimiting the male gametangium, showed roughly equal numbers of nuclei in each gametangium. At the end of gametangial differentiation a twofold increase in the number of nuclei in the male gametangium was observed as a result of repeated nuclear divisions. It was also observed that mitotic divisions were not synchronous, and drawings of actively dividing nuclei with a spindle as well as anaphase and telophase chromosome configurations were provided. Though nuclei in the female gametangium were about twice the size of nuclei in the male gametangium, both gametes contain only six chromosomes. Hatch (1935) suggested that the size difference between male and female nuclei may be related to maintaining a particular nuclear-plasma ratio, though how this was related to an increased number of mitotic divisions in the male gametangium could not be

explained. Another light-microscopy study by Wilson and Flanagan (1968) followed mitosis in resistant sporangia and hyphae in *Brachyallomyces* strains and noted that somatic nuclei were smaller than those in resistant sporangia.

Either closed mitosis or partially open mitosis has been shown in fungi. So far, **only closed mitosis, or intranuclear division, has been described in blastoclads**. In closed mitosis the nuclear membrane remains intact or largely intact and the spindle forms inside the nucleus (DeSouza and Osmani 2007; Heath 1980). A persistent nuclear membrane during somatic mitosis has been demonstrated in *Allomyces* spp. (Olson 1984), *C. anguillulae* (Ichida and Fuller 1968), and *Coelomomyces indicus* (Madelin and Beckett 1972). Lessie and Lovett (1968) reported intranuclear mitosis in *B. emersonii* comprised of a typical microtubular spindle apparatus and paired but unequal extranuclear centrioles at each pole.

C. Taxis

Taxis refers to the ability of motile cells or organisms to move across a gradient in a directed manner. Such behavior is clearly advantageous for zoospores and gametes of blastoclads as they disperse to find a new food source or mate. Both phototaxis and chemotaxis have been well documented in blastoclads. Positive phototaxis toward light was demonstrated in both *Allomyces* spp. (Olson 1984; Robertson 1972) and *Coelomomyces* (Martin 1970). Positive phototaxis may provide a mechanism by which gametes or zoospores may emerge from sediments. The attraction of zoospores of *Allomyces* to cellulose and chitin irrespective of light has been shown (Mitchell and Deacon 1986), as has positive chemotaxis toward several amino acids (Machlis 1969; Stumm et al. 1976).

Chemotaxis during mating should facilitate motile gametes seeking a compatible partner. The diffusible hormone **sirenin** is produced by female gametes of *Allomyces* (Machlis 1958a, b) and **has activity at very low concentrations** (10^{-10} to 10^{-5} M) (Carlile and Machlis 1965). Sirenin was the first fungal hormone to be chemically characterized and shown to be a **bicyclic sequiterpenediol** (Machlis 1968). Pommerville has also provided evidence that male gametes produce a hormone, though the swimming ability of female gametes is much reduced compared to those of males (Pommerville 1977, 1978).

D. Substrate Utilization and Respiration

Completely defined media have been constructed for studying nutrition in *Allomyces* (Ingraham and Emerson 1954). Growth on glucose, maltose, and starch as a sole carbon source has been shown for *Allomyces* (Ingraham and Emerson 1954), *C. anguillulae* (Nolan 1970), and *B. pringsheimii* (Crasemann 1957; Emerson and Cantino 1948; Gleason and Gordon 1989). Nitrogen utilization varies among taxa, with ***Allomyces* capable of using inorganic nitrogen and *Blastocladiella* and *Catenaria* using only organic sources** (Barner and Cantino 1952). An absolute requirement for an organic source of sulfur in the medium has also been demonstrated for the saprotrophic genera (Cantino and Turian 1959; Nolan 1969). Nutritional studies have facilitated the isolation of auxotrophic mutants; however, many mutants reported in the literature have apparently been unstable or displayed non-Mendelian inheritance due to ploidy (Olson 1984).

The obligately biotrophic parasites have been nearly impossible to isolate into pure culture. Numerous methods employing a "shotgun" approach have been tried for the growth of *Coelomomyces*, which would have clear benefits for biocontrol (Bland 1985; Nolan 1985). Several media, such as BHM (comprised of brain-heart infusion, mosquito larval extract, fetal bovine serum, and corn stunt spiroplasma media, to name a few ingredients!), have supported the growth of *Coelomomyces*, including the production of inviable sporangia (Bland 1985; Castillo and Roberts 1980). Key to the successful deployment of *Coelomomyces* inoculum as a biocontrol agent will be the in vitro culture of the gametophyte stage from copepods, needed to produce infective

zygotes. Alternatively, the development of species such as *Coelomomyces iliensis* var. *iliensis* whose sporophyte stage makes asexual diploid zoospores that can reinfect the mosquito host should be pursued. Among the plant parasitic genera, the algal parasite *Paraphysoderma* is the only one that can be grown in vitro (Hoffman et al. 2008). This observation suggests inroads to cultivating *Physoderma* may be found by careful study of the nutritional requirements of the former.

The genus *Blastocladia* has been shown to be obligately fermentative and facultatively anaerobic using with at least one species, *B. ramosa,* conforming to its observed niche of stagnant waters (Held et al. 1969). *Blastocladia* cultures responded positively to the addition of CO_2 to 20 %, suggesting they may be able to convert CO_2 into organic acids (Tabak and Cooke 1968). Interestingly, electron micrographs of *B. ramosa* showed double-membrane structures like mitochondria lacking any cristae in germlings (Held et al. 1969); however, with improved fixation techniques the single mitochondrion of the zoospores of ***B. ramosa*** **did indeed have cristae but not as many as the obligately aerobic genera** (Lingle and Barstow 1983). Microaerophily, or improved development under low oxygen conditions, has been suggested for other members of Blastocladiaceae: *Allomyces reticulatus* and *Microallomyces dendroideus* (Emerson and Robertson 1974).

E. Genomics

Our knowledge of fungal genomes is biased toward mainly Ascomycota species, particularly model organisms and pathogenic species. The only Blastocladiomycota genome in progress is for ***A. macrogynus*** **ATCC38327**, sequenced by the Broad Institute's Origins of Multicellularity project (Ruiz-Trillo et al. 2007). Data on the expressed portion of Blastocladiomycota genomes are available from EST projects such as through the Taxonomically Broad EST Database (TBestDB) for *A. macrogynus* (submitted by B.F. Lang, University of Montreal) and the National Center for Bioinformatics Information (NCBI) for *B. emersonii* (Ribichich et al. 2005). Our knowledge of fungal mitochondrial genomes is similarly biased, but mitochondrial genomes of the basal fungal lineages are better represented. Two Blastocladiomycota mitochondrial genomes have been completed for *A. macrogynus* and *B. emersonii* (Paquin and Lang 1996; Tambor et al. 2008).

Mitochondrial chromosomes (mtDNA) usually encode proteins involved in the electron transport chain, adenosine triphosphate (ATP) synthesis, structural proteins, and proteins of unknown function that may be found as open reading frames in introns (Griffiths 1996). Though mitochondrial function is basically the same in all organisms, fungal mitochondrial genomes may show great differences in size and gene organization due to the presence of introns and size variation in intergenic spacer regions (Lang et al. 2007). The mitochondrial genomes examined so far in Blastocladiomycota have been shown by electron microscopy, restriction enzyme analysis, and sequencing to be circular, although more recently it has been shown that linear forms may also be present in vivo for many fungi (Bendich 1993, 1996, 2010; Burger et al. 2003).

Allomyces macrogynus The first mtDNA physical maps for the aquatic fungi were for *A. macrogynus* (Borkhardt and Delius 1983; Borkhardt et al. 1988). The mitochondrial genome sequence for *A. macrogynus* confirmed its circular nature, total size of 57,473 bp, and slightly enriched A+T base content of 60.5 % (Paquin and Lang 1996). All mitochondrial genes seemed to be transcribed from the same DNA strand, similar to many other fungi (Paquin and Lang 1996).

It has been hypothesized that the universal mitochondrial code is an ancestral trait in fungal mitochondria (Paquin et al. 1997). Similar to plants and protists, *A. macrogynus* only uses the UGG codon for tryptophan (Paquin and Lang 1996). Other fungi that share this trait include the blastoclad *B. emersonii*, the zygomycete *Rhizopus stolonifer*, the chytrids *Spizellomyces*, *Monoblepharella*, and *Harpochytrium*, and the basal Ascomycete fission yeast *Schizosaccharomyces pombe* (Massey and Garey 2007; Paquin et al. 1997; Tambor et al. 2008). In most other

fungi and animals, the UGA stop codon has been reassigned to code for tryptophan. Additionally, *A. macrogynus* uses the UAG and UAA stop codons equally to signal termination (*A. macrogynus* does not use UGA), whereas most other fungi uniquely or preferentially use UAA (Paquin and Lang 1996). The only other organism known to use both stop codons equally is *Paramecium* (Pritchard et al. 1990).

Structural RNAs included the large and small mitochondrial-encoded rRNA genes (*rnl* and *rns*) and a set of 25 transfer RNAs (tRNAs). Protein-coding genes include three ATPase subunits (*atp6*, *apt8*, and *atp9*), apocytochrome b (*cob*), three subunits of the cytochrome oxidase complex (*cox1*, *cox2*, and *cox3*), and seven subunits of the NADH dehydrogenase complex (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*). Generally, the observed mitochondrial gene content is typical of that found in other fungi (Paquin and Lang 1996). The presence of introns in mitochondrial genes was observed in the first mtDNA genome sequences of fungi, including *A. macrogynus*, and has important consequences for the development of mtDNA genes for DNA barcoding efforts. For example, hybridization studies of the *A. macrogynus cox1* gene suggest the presence of intronic sequences (Borkhardt et al. 1988). The *cox1* gene is now known to contain 12 introns and has a total size of more than 11 kbp (Paquin and Lang 1996).

Blastocladiella emersonii The physical map of the *B. emersonii* mtDNA genome showed this to be the smallest mitochondrial genome of the zoosporic fungi sequenced so far, with a circular structure and a size of 36,503 bp and an enriched A+T base content of 64.9 % (Borkhardt and Olson 1986; Tambor et al. 2008). All mtDNA genes use the universal translation code and are found in the same orientation (Tambor et al. 2008). However, *B. emersonii* mtDNA differs from that of *A. macrogynus* in several ways. First, *B. emersonii* has a smaller mtDNA genome relative to *A. macrogynus*, caused by differences in the number and size of introns, intergenic spacer regions, and double-hairpin DNA elements (DHEs) (Paquin and Lang 1996; Tambor et al. 2008). Specifically, the mitochondrial genome of *B. emersonii* contains only 2 introns (both in the *cox1* gene), compared with 28 introns across the mitochondrial genome of *A. macrogynus*. The intergenic spacer regions comprise 47.9 % of the mitochondrial genome in *B. emersonii*, compared with 22 % in *A. macrogynus*.

VIII. Conclusions and Future Directions

What the blastoclads lack in species number they make up for in phylogenetic and ecological diversity. They are the only group of fungi known to possess an alternation of haploid and diploid generations. This trait, unlike anisogamy, which evolved in the ancestor of subgenus *Euallomyces*, seems to be ancestral to blastoclads. Does a life cycle alternating between haploid and diploid generations suggest that blastoclads might be the first diverging branch in fungi and that they inherited this trait from the most recent common ancestor of all fungi? The answer is, unfortunately, unclear because the phylogenetic placement of the group has yet to be definitively resolved, and the life cycles of the putative outgroups of fungi are not completely known (Brown et al. 2009; Jones et al. 2011). On the other hand, closed mitosis, Golgi equivalents rather than a Golgi apparatus, and true mycelial growth are characteristics of blastoclads that are more similar to the more derived nonzoosporic fungi.

Variations in life cycles are common throughout the group, and they have been used to define subgenera and species in *Allomyces* and *Blastocladiella*. Yet all indications are that life cycle variants can occur within species and that species may hybridize readily and differ greatly in ploidy. Thus, it is no surprise that phylogenetic analysis of *Allomyces* reveals serious problems with traditional species concepts (Porter et al. 2011). All five of the species of *Allomyces* in the phylogeny represented by more than one strain were shown to be nonmonophyletic. In the phylogeny, roughly 12 terminal clades that are suggestive of species were observed, whereas only 9 names are currently valid. Thus, in the minimal sampling employed, additional species must be

described. A major unanswered question is how many good blastoclad species have been proposed and how many are in need of revision. Modern mycology emphasizes a holistic view of fungal species that includes morphological, physiological, phylogenetic, and compatibility data (Cai et al. 2011; Taylor et al. 2000).

While species concepts in blastoclads must be redefined, a concerted effort to enumerate what are likely to be many undescribed species should also be undertaken. The mere 180 species named is obviously a gross underestimate of the true diversity, and future taxonomists of the group will be required to document and describe new species as they will inevitably be encountered. Discovery of these species will be facilitated by DNA-based evidence emerging from sampling of environments likely to be rich in blastoclads, such as suboxic sediments, periodically inundated soils, and invertebrate hosts. How we apply species concepts and delimit taxa in early-diverging fungi is a question that has largely been avoided, and there are very little data to address the question. Several lines of evidence suggest major revisions are warranted. Firstly, hybridization and horizontal gene transfer are poorly documented phenomena in fungi, but recent studies suggest that they may be as common in fungi as in other eukaryotic groups (Brasier 2001; Schardl and Craven 2003). Perhaps the best example of hybridization in fungi comes from experimental and natural hybrids in the species *A. javanicus*, the presumed hybrid of *A. macrogynus* and *A. arbusculus*. However, experiments to characterize *A. javanicus* genetically have never been done, and the role of hybridization in speciation has not been addressed in the blastoclads.

The coevolution of host and pathogen has likely driven diversification in blastoclads. In the future, studies of host specificity must be integrated into studies of taxonomy and systematics so that the simple assumption of one pathogen species per host species does not lead to erroneous classifications. Because most of the *Physoderma* species were named under the assumption that each host species had distinct parasites, and there is evidence to suggest that the species of *Physoderma* are less than host-species-specific in inoculation studies (Sparrow and Griffin 1964), it may be that the species diversity in this group is much lower than the list of taxonomically accepted names. Similar difficulties may arise in other parasitic genera, for example, *Coelomomyces* and *Catenaria*; however, the phylogenetic diversity of *Physoderma* (as measured by branch lengths in a phylogeny) is much shallower than observed in the *Coelomomyces* clade.

References

Barner HD, Cantino EC (1952) Nutritional relationships in a new species of *Blastocladiella*. Am J Bot 39:746–751

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

Barstow WE, Lovett JS (1975) Formation of gamma-particles during zoosporogenesis in *Blastocladiella emersonii*. Mycologia 67:518–529

Barstow WE, Pommerville J (1980) The ultrastructure of cell wall formation and of gamma-particles during encystment of *Allomyces macrogynus* zoospores. Arch Microbiol 128:179–189

Bendich AJ (1993) Reaching for the ring: the study of mitochondrial genome structure. Curr Genet 24:279–290

Bendich AJ (1996) Structural analysis of mitochondrial DNA molecules from Fungi and Plants using moving pictures and pulsed-field gel elctrophoresis. J Mol Biol 255:564–588

Bendich AJ (2010) The end of the circle for yeast mitochondrial DNA. Mol Cell 39:831–832

Bland CE (1985) Culture. In: Couch JN, Bland CE (eds) The genus *Coelomomyces*. Academic, Orlando, FL, pp 349–359

Borkhardt B, Delius H (1983) Physical map of the mitochondrial DNA from the phycomycete *Allomyces macrogynus* including the position of the ribosomal RNA genes and of an intervening sequence in the large rRNA gene. Curr Genet 7:327–333

Borkhardt B, Olson LW (1979) Meiotic prophase in diploid and tetraploid strains of *Allomyces macrogynus*. Protoplasma 100:323–343

Borkhardt B, Olson LW (1986) The mitochondrial genome of the aquatic phycomycete *Blastocladiella emersonii*. Curr Genet 11:139–143

Borkhardt B, Brown TA, Thim P, Olson LW (1988) The mitochondrial genome of the aquatic phycomycete *Allomyces macrogynus*. Physical mapping and mitochondrial DNA instability. Curr Genet 13:41–47

Bowman BH, Taylor JW, Brownlee AG, Lee J, Lu SD, White TJ (1992) Molecular evolution of the fungi-

relationship of the Basidiomycetes, Ascomycetes, and Chytridiomycetes. Mol Biol Evol 9:285–296
Brasier CM (2001) Rapid evolution of introduced plant pathogens via interspecific hybridization. Bioscience 51:123–133
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
Bruns TD, White TJ, Taylor JW (1991) Fungal molecular systematics. Annu Rev Ecol Syst 22:525–564
Bruns TD, Vilgalys R, Barns SM, Gonzalez D, Hibbett DS, Lane DJ, Simon L, Stickel S, Szaro TM, Weisburg WG, Sogin ML (1992) Evolutionary relationships within the Fungi: analyses of nuclear small subunit rRNA sequences. Mol Phylogenet Evol 1:231–241
Bullerwell CE, Forget L, Lang BF (2003) Evolution of monoblepharidalean fungi based on complete mitochondrial genome sequences. Nucleic Acids Res 31:1614–1623
Burger G, Forget L, Zhu Y, Gray MW, Lang BF (2003) Unique mitochondrial genome architecture in unicellular relatives of animals. Proc Natl Acad Sci USA 100:892–897
Butler EJ (1911) On *Allomyces*, a new aquatic fungus. Ann Bot 25:1023–1035
Cai L, Giraud T, Zhang N, Begerow D, Cai GH, Shivas RG (2011) The evolution of species concepts and species recognition criteria in plant pathogenic fungi. Fungal Divers 50:121–133
Cantino EC (1956) The relation between cellular metabolism and morphogenesis in *Blastocladiella*. Mycologia 48:225–240
Cantino EC, Horenstein EA (1954) Cytoplasmic exchange without gametic copulation in the water mold *Blastocladiella emersonii*. Am Nat 88:143–154
Cantino EC, Hyatt MT (1953) Phenotypic "sex" determination in the life history of a new species of *Blastocladiella,* B emersonii. Antonie Van Leeuwenhoek 19:25–70
Cantino EC, Truesdell LC (1970) Organization and fine structure of side body and its lipid sac in zoospore of *Blastocladiella emersonii*. Mycologia 62:548–567
Cantino EC, Truesdell LC (1971) Cytoplasmic gamma-like particles and other ultrastructural aspects of zoospores of *Blastocladiella britannica*. Trans Br Mycol Soc 56:169–179
Cantino EC, Turian GF (1959) Physiology and development of lower fungi (Phycomycetes). Annu Rev Microbiol 13:97–124
Carlile MJ, Machlis L (1965) Response of male gametes of *Allomyces* to sexual hormone sirenin. Am J Bot 52:478–483
Castillo JM, Roberts DW (1980) *In vitro* studies of *Coelomomyces punctatus* from *Anopheles quadrimaculatus* and *Cyclops vernalis*. J Invertebr Pathol 35:144–157
Chapman HC (1985) Ecology and use of *Coelomomyces* species in biological control: a review. In: Couch JN, Bland CE (eds) The genus *Coelomomyces*. Academic, Orlando, FL, pp 361–368
Chen MJ, Chen FZ, Yu Y, Ji J, Kong FX (2008) Genetic diversity of eukaryotic microorganisms in Lake Taihu, a large shallow subtropical lake in China. Microb Ecol 56:572–583
Couch JN (1945) Observations on the genus *Catenaria*. Mycologia 37:163–191
Couch JN, Bland CE (1985) The genus *Coelomomyces*. Academic, New York
Couch JN, Whiffen AJ (1942) Observations on the genus *Blastocladiella*. Am J Bot 29:582–591
Crasemann JM (1957) Comparative nutrition of two species of *Blastocladia*. Am J Bot 44:218–224
Dalley NE, Sonneborn DR (1982) Evidence that *Blastocladiella emersonii* zoospore chitin synthetase is located at the plasma membrane. Biochim Biophys Acta 686:65–76
Dasgupta SN, John R (1988) A contribution to our knowledge of the genus *Blastocladia*. Indian Phytopathol 41:521–547
Debaisieux P (1920) *Coelomycidium simulii* nov. gen., nov. sp. et remarques sur l'*Amoebidium* des larves de *Simulium*. Cellule 30:249–271
DeSouza CPC, Osmani SA (2007) Mitosis, not just open or closed. Eukaryot Cell 6:1521–1527
Dewel RA, Dewel WC (1990) The fine structure of the zoospore of *Sorochytrium milnesiophthora*. Can J Bot 68:1968–1977
Dewel RA, Joines JD, Bond JJ (1985) A new chytridiomycete parasitizing the tardigrade *Milnesium tardigradum*. Can J Bot 63:1525–1534
Doweld AB (2001) Prosyllabus tracheophytorum. Tentamen systematis plantarum vascularium (Tracheophyta). Geos, Moscow
Emerson R (1938) A new life cycle involving cyst-formation in *Allomyces*. Mycologia 30:120–132
Emerson R (1941) An experimental study of the life cycles and taxonomy of *Allomyces*. Lloydia 4:77–144
Emerson R, Cantino EC (1948) The isolation, growth, and metabolism of *Blastocladia* in pure culture. Am J Bot 35:157–171
Emerson R, Robertson JA (1974) Two new members of Blastocladiaceae. I. Taxonomy, with an evaluation of genera and interrelationships in family. Am J Bot 61:303–317
Emerson R, Wilson CM (1949) The significance of meiosis in *Allomyces*. Science 110:86–88
Emerson R, Wilson CM (1954) Interspecific hybrids and the cytogenetics and cytotaxonomy of Euallomyces. Mycologia 46:393–434
Federici BA (1979) Experimental hybridization of *Coelomomyces dodgei* and *Coelomomyces punctatus*. Proc Natl Acad Sci USA 76:4425–4428

Federici BA (1982) Inviability of interspecific hybrids in the *Coelomomyces dodgei* complex. Mycologia 74:555–562

Federici BA, Lucarotti CJ (1986) Structure and behavior of the meiospore of *Coelomomyces dodgei* during encystment on the copepod host, *Acanthocyclops vernalis*. J Invertebr Pathol 48:259–268

Fischer A (1892) Phycomycetes. Die Pilze Deutschlands, Oesterreichs und der Schweiz. Kryptogamen-Fl 1:1–490

Förster H, Coffey MD, Elwood H, Sogin ML (1990) Sequence analysis of the small subunit ribosomal RNAs of three zoosporic fungi and implications for fungal evolution. Mycologia 82:306–312

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, Calhoun SA (1968) Microtubule-kinetosome relationships in motile cells of Blastocladiales. Z Zellforsch Mikrosk Anat 87:526–533

Fuller MS, Olson LW (1971) Zoospore of *Allomyces*. J Gen Microbiol 66:171–183

Garcia R (1983) Mosquito management - ecological approaches. Environ Manage 7:73–78

Gleason FH, Gordon GLR (1989) Anaerobic growth and fermentation in *Blastocladia*. Mycologia 81:811–815

Gleason FH, Marano AV, Johnson P, Martin WW (2010) Blastocladian parasites of invertebrates. Fungal Biol Rev 24:56–67

Griffiths AJF (1996) Mitochondrial inheritance in filamentous fungi. J Genet 75:403–414

Harder R, Sörgel G (1938) Über einen neuen planoisogamen Phycomyceten mit Generationswechel und seine phylogenetische Bedeutung. Nachrichten Gesell Wiss Göttingen Fachgruppe VI (Biol) 3:119–127

Hatch WR (1935) Gametogenesis in *Allomyces arbuscula*. Ann Bot 49:623–649

Heath IB (1980) Variant mitoses in lower eukaryotes: indicators of the evolution of mitosis? Int Rev Cytol 64:1–80

Held AA, Emerson R, Fuller MS, Gleason FH (1969) *Blastocladia* and *Aqualinderella* – fermentative water molds with high carbon dioxide optima. Science 165:706–709

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lucking R, Lumbsch HT, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547

Hill EP (1969) Fine structure of zoospores and cysts of *Allomyces macrogynus*. J Gen Microbiol 56:125–130

Hoffman Y, Aflalo C, Zarka A, Gutman J, James TY, Boussiba S (2008) Isolation and characterization of a novel chytrid species (phylum Blastocladiomycota), parasitic on the green alga *Haematococcus*. Mycol Res 112:70–81

Hohn TM, Lovett JS, Bracker CE (1984) Characterization of the major proteins in gamma particles, cytoplasmic organelles in *Blastocladiella emersonii* zoospores. J Bacteriol 158:253–263

Horenstein EA, Cantino EC (1962) Dark-induced morphogenesis in synchronized cultures of *Blastocladiella britannica*. J Bacteriol 84:37–45

Horgen PA, Meyer RJ, Franklin AL, Anderson JB, Filion WG (1985) Motile spores from resistant sporangia of *Blastocladiella emersonii* possess one-half the DNA of spores from ordinary colorless sporangia. Exp Mycol 9:70–73

Ichida AA, Fuller MS (1968) Ultrastructure of mitosis in the aquatic fungus *Catenaria anguillulae*. Mycologia 60:141–155

Ingraham JL, Emerson R (1954) Studies of the nutrition and metabolism of the aquatic phycomycete, *Allomyces*. Am J Bot 41:146–152

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

James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox C, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, 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 D, Lutzoni F, McLaughlin D, Spatafora J, Vilgalys R (2006a) Reconstructing the early evolution of the 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, Hoffman Y, Zarka A, Boussiba S (2011) *Paraphysoderma sedebokerense*, gen. et sp. nov., an aplanosporic relative of *Physoderma* (*Blastocladiomycota*). MycoTaxon 118:177–180

Jaworski AJ, Stumhofer P (1984) Dormant ribosomes in *Blastocladiella emersonii* zoospores are arrested at elongation. Exp Mycol 8:13–24

Johnson PTJ, Longcore JE, Stanton DE, Carnegie RB, Shields JD, Preu ER (2006) Chytrid infections of *Daphnia pulicaria*: development, ecology, pathology and phylogeny of *Polycaryum laeve*. Freshw Biol 51:634–648

Jones MDM, Forn I, Gadelha C, Egan MJ, Bass D, Massana R, Richards TA (2011) Discovery of novel intermediate forms redefines the fungal tree of life. Nature 474:200–234

Karling JS (1950) The genus *Physoderma*. Lloydia 13:29–71

Karling JS (1965) *Catenophlyctis*, a new genus of Catenariaceae. Am J Bot 52:133–138

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

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the Fungi, 10th edn. CABI Publishing, The Netherlands

Kniep H (1930) Über den Generationswechsel von *Allomyces*. Zeitschrift für Botanik 22:433–441

Koch WJ (1956) Studies of the motile cells of chytrids. I. Electron microscope observations of the flagellum, blepharoplast and rhizoplast. Am J Bot 43:811–819

Lang BF, O'Kelly C, Nerad T, Gray MW, Burger G (2002) The closest unicellular relatives of animals. Curr Biol 12:1773–1778

Lang BF, Laforest M-J, Burger G (2007) Mitochondrial introns: a critical view. Trends Genet 23:119–125

Lange L, Olson LW (1979) Uniflagellate phycomycete zoospore. Dansk Botanisk Arkiv 33:7–95

Lange L, Olson LW (1980) Transfer of the Physodermataceae from the Chytridiales to the Blastocladiales. Trans Br Mycol Soc 74:449–457

Lefèvre E, Bardot C, Noel C, Carrias JF, Viscogliosi E, Amblard C, Sime-Ngando T (2007) Unveiling fungal zooflagellates as members of freshwater picoeukaryotes: evidence from a molecular diversity study in a deep meromictic lake. Environ Microbiol 9:61–71

Lessie PE, Lovett JS (1968) Ultrastructural changes during sporangium formation and zoospore differentiation in *Blastocladiella emersonii*. Am J Bot 55:220–236

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

Lingle WL, Barstow WE (1983) Ultrastructure of the zoospore of *Blastocladia ramosa* (Blastocladiales). Can J Bot 61:3502–3513

Liu YJ, Hodson MC, Hall BD (2006) Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of Kingdom Fungi inferred from RNA polymerase II subunit genes. BMC Evol Biol 6:13

Liu Y, Steenkamp ET, Brinkmann H, Forget L, Philippe H, Lang BF (2009) Phylogenomic analyses predict sistergroup relationship of nucleariids and Fungi and paraphyly of zygomycetes with significant support. BMC Evol Biol 9:11

Lovett JS (1975) Growth and differentiation of water mold *Blastocladiella emersonii* – cytodifferentiation and role of ribonucleic acid and protein synthesis. Bacteriol Rev 39:345–404

Lucarotti CJ (1987) *Coelomomyces stegomyiae* infection in adult *Aedes aegypti*. Mycologia 79:362–369

Lucarotti CJ, Federici BA (1984) Ultrastructure of the gametes of *Coelomomyces dodgei* Couch (Blastocladiales, Chytridiomycetes). Protoplasma 121:77–86

Machlis L (1958a) A study of sirenin, the chemotactic sexual hormone from the watermold *Allomyces*. Physiol Plant 11:845–854

Machlis L (1958b) Evidence for a sexual hormone in *Allomyces*. Physiol Plant 11:181–192

Machlis L (1968) Response of male gametes of *Allomyces* to sexual hormone sirenin. Plant Physiol 43:1319–1320

Machlis L (1969) Zoospore chemotaxis in the water mold *Allomyces*. Physiol Plant 22:126–139

Madelin MF, Beckett A (1972) Production of planonts by thin-walled sporangia of fungus *Coelomomyces indicus* – parasite of mosquitos. J Gen Microbiol 72:185–200

Manier JF, Loubes C (1978) *Callimastix cyclopis* Weissenberg, 1912 (Phycomycetes, Blastocladiales) a parasite of *Microcyclops claus*, 1893 (Copepoda, Cyclopoida) from Tchad - ultrastructural features. Protistologica 14:493–501

Manton I, Clarke B, Greenwood AD, Flint EA (1952) Further observations on the structure of plant cilia by combination of visual and electron microscopy. J Exp Bot 3:204–215

Martin WW (1970) A morphological and cytological study of *Coelomomyces punctatus*. M.Sc., University of North Carolina, Chapel Hill

Martin WW (1971) The ultrastructure of *Coelomomyces punctatus* zoospores. J Elisha Mitchell Sci Soc 87:209–221

Martin WW (1975) A new species of *Catenaria* parasitic in midge eggs. Mycologia 67:264–272

Martin WW (1978) Two additional species of *Catenaria* (Chytridiomycetes, Blastocladiales) parasitic in midge eggs. Mycologia 70:461–467

Martin WW (1987) Zoosporic parasites of aquatic insects: collection, identification, and culture. In: Fuller MS, Jaworski A (eds) Zoosporic fungi in teaching and research. Southeastern, Athens, GA, pp 137–142

Massey SE, Garey JR (2007) A comparative genomics analysis of codon reassignments reveals a link with mitochondrial proteome size and a mechanism of genetic code change via suppressor tRNAs. J Mol Evol 64:399–410

Matthews VD (1937) A new genus of the Blastocladiaceae. J Elisha Mitchell Sci Soc 53:191–195

McCrainie J (1942) Sexuality in *Allomyces cystogenus*. Mycologia 34:209–213

Mills GL, Cantino EC (1979) Trimodal formation of microbodies and associated biochemical and cytochemical changes during development in *Blastocladiella emersonii*. Exp Mycol 3:53–69

Mills GL, Cantino EC (1981) Chitosome-like vesicles from gamma-particles of *Blastocladiella emersonii* synthesize chitin. Arch Microbiol 130:72–77

Mitchell RT, Deacon JW (1986) Selective accumulation of zoospores of chytridiomycetes and oomycetes on cellulose and chitin. Trans Br Mycol Soc 86:219–223

Nagahama T, Sato H, Shimazu M, Sugiyama J (1995) Phylogenetic divergence of the entomophthoralean fungi: evidence from nuclear 18S ribosomal RNA gene sequences. Mycologia 87:203–209

Nascimento CDA, Pires-Zottarelli CLA (2010) Blastocladiales e Spizellomycetales do Parque Estadual da Serra da Cantareira, São Paulo, Brasil. Revista Brasileira de Botânica 33:693–704

Nolan RA (1969) Nutritional requirements for species of *Allomyces*. Mycologia 61:641–644

Nolan RA (1970) Sulfur source and vitamin requirements of aquatic phycomycete, *Catenaria anguillulae*. Mycologia 62:568–577

Nolan RA (1985) Physiology and biochemistry. In: Couch JN, Bland CE (eds) The genus *Coelomomyces*. Academic, Orlando, FL, pp 321–348

Olson LW (1974) Meiosis in the aquatic Phycomycete *Allomyces macrogynus*. Comptes rendus des travaux du laboratoire Carlsberg 40:113–124

Olson LW (1980) *Allomyces neo-moniliformis* gametogenesis. The Cystogenes life cycle. Protoplasma 105:87–106

Olson LW (1984) *Allomyces* – a different fungus. Opera Bot 73:5–96

Olson LW, Borkhardt B (1978) Polyploidy and its control in *Allomyces macrogynus*. Trans Br Mycol Soc 71:65–76

Olson LW, Lange L (1978) Meiospore of *Physoderma maydis* – causal agent of physoderma disease of maize. Protoplasma 97:275–290

Olson LW, Lange L (1983) The gamma body- a vesicle generating structure. Nord J Bot 3:673–680

Olson LW, Reichle R (1978a) Meiosis and diploidization in the aquatic Phycomycete *Catenaria anguillulae*. Trans Br Mycol Soc 70:423–437

Olson LW, Reichle R (1978b) Synaptonemal complex formation and meiosis in the resting sporangium of *Blastocladiella emersonii*. Protoplasma 97:261–273

Olson LW, Lange L, Reichle R (1978) Zoospore and meiospore of aquatic phycomycete *Catenaria anguillulae*. Protoplasma 94:53–71

Paquin B, Lang BF (1996) The mitochondrial DNA of *Allomyces macrogynus*: the complete genomic sequence from an ancestral fungus. J Mol Biol 255:688–701

Paquin B, Laforest MJ, Forget L, Roewer I, Wang Z, Longcore J, Lang BF (1997) The fungal mitochondrial genome project: evolution of fungal mitochondrial genomes and their gene expression. Curr Genet 31:380–395

Petersen HE (1909) Studier over Ferskvands-Phycomyceter. Bidrag til Kundskaben om de submerse Phykomyceters Biologi og Systematik, samt om deres Udbredelse i Danmark. Bot Tidskrift 29:345–440

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

Pommerville J (1977) Chemotaxis of *Allomyces* gametes. Exp Cell Res 109:43–51

Pommerville J (1978) Analysis of gamete and zygote motility in *Allomyces*. Exp Cell Res 113:161–172

Porter TM, Martin WM, James TY, Longcore JE, Gleason F, Adler PH, Letcher PM, Vilgalys R (2011) Molecular phylogeny of the Blastocladiomycota (Fungi) based on nuclear ribosomal data. Fungal Biol 115:381–392

Powell MJ (1978) Phylogenetic implications of microbody-lipid globule complex in zoosporic fungi. BioSyst 10:167–180

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

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

Powell MJ, Koch WJ (1977b) Morphological variations in a new species of *Entophlyctis*. II. Influence of growth-conditions on morphology. Can J Bot 55:1686–1695

Pritchard AE, Seilhammer JJ, Mahalingam R, Sable CL, Venuti SE, Cummings DJ (1990) Nucleotide sequence of the mitochondrial genome of *Paramecium*. Nucleic Acids Res 18:173–180

Reichle RE, Fuller MS (1967) Fine structure of *Blastocladiella emersonii* zoospores. Am J Bot 54:81–92

Remy W, Taylor TN, Hass H (1994) Early Devonian fungi – a Blastocladalean fungus with sexual reproduction. Am J Bot 81:690–702

Ribichich KF, Salem-Izacc SM, Georg RC, Vencio RZN, Navarro LD, Gomes SL (2005) Gene discovery and expression profile analysis through sequencing of expressed sequence tags from different develop-

mental stages of the chytridiomycete *Blastocladiella emersonii*. Eukaryot Cell 4:455–464
Robertson JA (1972) Phototaxis in a new *Allomyces*. Arch f Mikrobiol 85:259–266
Ruiz-Trillo I, Burger G, Holland PWH, King N, Lang BF, Roger AJ, Gray MW (2007) The origins of multicellularity: a multi-taxon genome initiative. Trends Genet 23:113–118
Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301:1359–1361
Schardl CL, Craven KD (2003) Interspecific hybridization in plant-associated fungi and oomycetes: a review. Mol Ecol 12:2861–2873
Scherffel A (1925) Endophytische Phycomyceten-Parasiten der Bacillariaceen und einege neue Monadinen. Ein Beitrag zur Phylogenie der Oomyceten (Schröter). Archiv Protistenk 52:1–141
Schroeter J (1893) Phycomycetes. Natürlichen Pflanzenfam 1:63–141
Seif E, Leigh J, Liu Y, Roewer I, Forget L, Lang BF (2005) Comparative mitochondrial genomics in zygomycetes: bacteria-like RNase P RNAs, mobile elements and a close source of the group I intron invasion in angiosperms. Nucleic Acids Res 33:734–744
Shaw DS, Cantino EC (1969) An albino mutant of *Blastocladiella emersonii* - comparative studies of zoospore behaviour and fine structure. J Gen Microbiol 59:369–382
Simmons DR, James TY, Meyer AF, Longcore JE (2009) Lobulomycetales, a new order in the Chytridiomycota. Mycol Res 113:450–460
Singh KP, Jaiswal RK, Kumar N (2007) *Catenaria anguillulae* Sorokin: a natural biocontrol agent of *Meloidogyne graminicola* causing root knot disease of rice (Oryza sativa L.). World J Microbiol Biotechnol 23:291–294
Slapeta J, Moreira D, Lopez-Garcia P (2005) The extent of protist diversity: insights from molecular ecology of freshwater eukaryotes. Proc R Soc B Biol Sci 272:2073–2081
Sparrow FK (1939) The entomogenous chytrid *Myrophagus* Thaxter. Mycologia 304:113–116
Sparrow FK (1960) Aquatic phycomycetes. University of Michigan Press, Ann Arbor, MI
Sparrow FK (1962) *Urophlyctis* and *Physoderma*. Trans Mycol Soc Jpn 3:16–18
Sparrow FK (1965) Concerning *Physoderma graminis*. Mycologia 57:624–627
Sparrow FK, Griffin JE (1964) Observation on chytridiaceous parasites of phanerograms. XV. Host range and species concepts studies in Physoderma. Arch f Mikrobiol 40:275–282
Stumm C, Hermans JMH, Croes AF, Bucks JH (1976) Chemotaxis and transport of amino acids in *Allomyces arbuscula*. Ant v Leeuwenhoek 42:203–209
Tabak HH, Cooke WB (1968) Effects of gaseous environments on growth and metabolism of fungi. Bot Rev 34:126–252
Tambor JHM, Ribichich KF, Gomes SL (2008) The mitochondrial view of *Blastocladiella emersonii*. Gene 424:33–39
Tanabe Y, O'Donnell K, Saikawa M, Sugiyama J (2000) Molecular phylogeny of parasitic Zygomycota (Dimargaritales, Zoopagales) based on nuclear small subunit ribosomal DNA sequences. Mol Phylogenet Evol 16:253–262
Tanabe Y, Watanabe MM, Sugiyama J (2002) Are Microsporidia really related to Fungi?: a reappraisal based on additional gene sequences from basal fungi. Mycol Res 106:1380–1391
Tanabe Y, Saikawa M, Watanabe MM, Sugiyama J (2004) Molecular phylogeny of Zygomycota based on EF-1 and RPB1 sequences: limitations and utility of alternative markers to rDNA. Mol Phylogenet Evol 30:438–449
Tanabe Y, Watanabe MM, Sugiyama J (2005) Evolutionary relationships among basal fungi (Chytridiomycota and Zygomycota): insights from molecular phylogenetics. J Gen Appl Microbiol 51:267–276
Taylor JW, Fuller MS (1981) The Golgi apparatus, zoosporogenesis, and development of the zoospore discharge apparatus of *Chytridium confervae*. Exp Mycol 5:35–59
Taylor TN, Remy W, Hass H (1994) *Allomyces* in the Devonian. Nature 367:601–601
Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 31:21–32
Teter HE (1944) Isogamous sexuality in a new strain of *Allomyces*. Mycologia 36:194–210
Travland LB (1979) Structures of the motile cells of *Coelomomyces psorophorae* and function of the zygote in encystment on a host. Can J Bot 57:1021–1035
Vandenkoornhuyse P, Baldauf SL, Leyval C, Straczek J, Young JPW (2002) Evolution - Extensive fungal diversity in plant roots. Science 295:2051–2051
Vavra J, Joyon L (1966) Etude sur la morphologie le cycle evolutif et la position systematique de *Callimastix cyclopis* Weissenberg. Protistologica 2:5–15
Whisler HC (1987) On the isolation and culture of water molds: the Blastocladiales and Monoblepharidales. In: Fuller MS, Jaworski A (eds) Zoosporic fungi in teaching and research. Southeastern, Athens, GA, pp 121–124
Willoughby LG (1959) A new species of *Blastocladiella* from Great Britain. Trans Br Mycol Soc 42:287–291
Wilson CM (1952) Meiosis in *Allomyces*. Bull Torr Bot Club 79:139–160
Wilson CM, Flanagan PW (1968) The life cycle and cytology of *Brachyallomyces*. Can J Bot 46:1361–1367
Wolf FT (1941) A contribution to the life history and geographic distribution of the genus *Allomyces*. Mycologia 33:158–173