A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota)

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Abstract: Chytridiomycota (chytrids) is the only phylum of true Fungi that reproduces with motile spores (zoospores). Chytrids currently are classified into five orders based on habitat, zoospore characters and life cycles. In this paper we estimate the phylogeny of the chytrids with DNA sequences from the ribosomal RNA operon (18S + 5.8S + 28S subunits). To our surprise the morphologically reduced parasites Olpidium and Rozella comprise two entirely new, and separate, lineages on the fungal tree. Olpidium brassicae groups among the Zygomycota, and Rozella spp. are the earliest branch to diverge in the fungal kingdom. The phylogeny also suggests that Chytridiomycota is not monophyletic and there are four major lineages of chytrids: Rozella spp., Olpidium brassicae, the Blastocladiales and a “core chytrid clade” containing the remaining orders and families and the majority of flagellated fungi. Within the core chytrid group 11 subclades can be identified, each of which correlates well with zoospore ultrastructure or morphology. We provide a synopsis of each clade and its morphological circumscription. The Blastocladiales appears to be the sister taxon of most nonflagellated fungi. Based on molecular phylogenetic and ultrastructural characters this order is elevated to a phylum, the Blastocladiomycota.

Key words: Chytridiomycota, Blastocladiomycota, holocarpic, kinetosome, phylogeny, zoospore ultrastructure

INTRODUCTION

The Chytridiomycota is a phylum of Fungi that reproduces through the production of motile spores (zoospores), typically propelled by a single, posteriorly directed flagellum. These organisms, often referred to as chytrid fungi or chytrids, have a global distribution with approximately 1000 described species. Based on biochemical characteristics, including chitin in cell walls, the \( \alpha \)-aminoadipic acid lysine synthetic pathway and storage carbohydrates as glycogen, Bartnicki-Garcia (1970) classified the Chytridiomycota as true Fungi. Others considered chytrids as a transitional group between protists and Fungi because of their production of motile zoospores (Barr 1990). Phylogenetic studies with ribosomal RNA genes unified these views and conclusively demonstrated that chytrids were true Fungi that occupied a basal position in the fungal tree (e.g. Förster et al 1990).

Chytrids are essentially ubiquitous, occurring in diverse and unique habitats from the tropics to the arctic regions (Powell 1993). They are found in aquatic systems such as streams, ponds, and estuarine and marine ecosystems primarily as parasites of algae and plankton components. Perhaps the majority of chytrid species occur in terrestrial habitats (Barr 2001) such as forest, agricultural and desert soils, and acidic bogs as saprotrophs of refractory substrata including pollen, chitin, keratin and cellulose. In soil chytrids are obligate parasites of a wide variety of vascular plants, including potatoes (Sycnchytrium) and curcurbits (Olpidium). The only known chytrid parasite of vertebrates is Batrachochytrium dendrobatidis (Longcore et al 1999), considered the causative agent of die-offs and population declines of amphibian species (Berger et al 1998). In most habitats a few chytrid species are relatively frequent and abundant (i.e. Chytriomycetes hyalinus in freshwater and Rhizophycis rosea in agricultural and perturbed soils),
Chytridiales is the largest of the orders (more than 75 genera) and the classification of this group has been variously approached using developmental, sporangial and more recently ultrastructural characters. Sparrow (1960) considered the operculum to be a defining characteristic and created two series of families based on whether sporangia were operculate. In contrast Whiffen (1944), Roane and Paterson (1974) and Barr (1978) suggested development could be used to divide the Chytridiales into families. Systems of classification based on morphology and those based on development of the thallus and sporangium both have been shown by molecular phylogenetics to be inaccurate in defining genera and families of Chytridiales (James et al 2000, Letcher et al 2004b).

Molecular phylogenies with 18S rDNA have suggested that both the Chytridiomycota and Chytridiales might not be monophyletic (James et al 2000). Phylogenies based on data from entire mitochondrial genomes (Seif et al 2005) have suggested strongly that two main lineages exist within the Chytridiomycota—the Blastocladiales and the remaining four orders (the “core chytrid clade”). In this paper we present a new dataset that includes an analysis of most chytrid 18S rDNA data in conjunction with sequence data for the entire rRNA operon (18S, 28S and 5.8S). These data provide a comprehensive view of chytrid phylogeny and define four major lineages of flagellated Fungi.

MATERIALS AND METHODS

Our dataset consists of taxa for which most of the rRNA operon was sequenced (18S+28S+5.8S subunits, n = 54) as well as chytrid taxa represented only by 18S data (n = 49) and additional fungal, animal and Mesomycetozoea taxa (n = 21; complete operon with one exception, Diaphanoeca grandis). GenBank accession numbers and strain/voucher data are provided (SUPPLEMENTARY TABLE 1). Data gathered for the full operon were obtained by PCR, primarily with primers SR1R and LR12 (Vilgalys and Hester 1990) using LAq (TaKaRa), with these thermocycling conditions: 94 C for 1 min followed by 35 cycles of denaturing at 94 C for 30 s, annealing at 55 C for 30 s, extension at 72 C for 5 min and a 10 min final extension at 72 C. Amplicons generally were cloned into pCR2.1-TOPO (Invitrogen).

The three gene regions were aligned by eye with GeneDoc v2.6 (http://www.crisi.com/~ketchup/genedoc.shtml) and combined into one supermatrix with MacClade 4.05 (Maddison and Maddison 2002). Regions of ambiguous alignment were excluded from further analysis leaving 4190 included characters, 1388 of which are parsimony informative. We estimated the phylogeny with MrBayes v3.1 (Huelsenbeck and Ronquist 2001) by four independent runs with the GTR+I+Γ model of evolution, sampling trees every 500 generations for 10 × 10⁶ generations. We also assessed
FIG. 1. Light micrographs of representative chytrids. a. *Rozella allomyces* isolate UCB-47-54 (Clade 1) parasitizing hyphae of *Allomyces*. The parasite grows inside the host and causes it to produce hypertrophied, highly septic cells within which the parasite may form thick-walled resting spores (RS) or unwalled zoosporangia (ZS) that use the host’s cell wall as its own. b. *Neocallimastix* sp. (Clade 2A) monocentric thallus with profusely branched rhizoids, inset: multiflagellate zoospores (by Gary Easton). c. *Monoblepharella polyomorpha* (Clade 2B) mature zygote or oospore (OS), empty and mature antheridia (AN) and antherozoids (AT) or male gametes emerging from antheridium; photo by Marilyn M. N. Mollicone. d. *Polychytrium aggregatum* isolate JEL109 (Clade 2C) finely branched rhizomycelium. e. *Catenochytridium* sp. (Clade 2D) monocentric, operculate (OP) zoosporangium with catenulate rhizoids (RH). f. *Chytriumyces angularis* isolate JEL45 (Clade 2F) monocentric, operculate sporangium with thread-like rhizoids that branch several μm from the sporangial base (arrow). g. *Terramyces subangulosum* isolate PL 076 (Clade 2G) monocentric, inoperculate sporangium with a thick rhizoidal axis, and densely branched rhizoids (RH) that taper to ~0.5 μm at tips. h. *Blyttiomycis helicus* (Clade 2H) forming epibiotic,
support for nodes with parsimony bootstrap as implemented in PAUP v4.0b10 (Swofford 2002) and maximum likelihood bootstrap as implemented in RAxML-VI-HPC v2.0 (Stamatakis et al 2005).

RESULTS AND DISCUSSION

The phylogeny (Fig. 2) is the consensus of credible trees derived from the Bayesian analysis of rDNA genes from Chytridiomycota. These analyses confirm the basal position of this group among the Fungi and provide support for at least four major clades of Chytridiomycota. Clade 1 is the earliest diverging lineage in the Fungi and comprises Rozella spp. The largest, Clade 2 or the “core chytrid clade,” comprises Chytridiales, Spizellomycetales pro parte, Monoblepharidales and Neocallimastigales and can be subdivided further into 11 clades (clades 2A–2K). Clade 3 represents the Blastocladiales, grouping sister of the Dikarya+Glomeromycota+Zygomyctae, and Clade 4 is represented solely by Olpidium brassicae, which groups among the Zygomyctae. These results further suggest that Chytridiomycota is polyphyletic or at least paraphyletic. As suggested by a phylogenetic study of Ophiokonts, the amoeba Nuclearia simplex appears to be the closest known sister taxon of the Fungi (Steenkamp et al 2005). Below we discuss the membership of each clade, providing a brief statement on the systematics of each group in light of our phylogenetic findings.

Clade 1. Rozella.—Two isolates of Rozella are supported strongly as the earliest diverging lineage in the fungi. Rozella is a holocarpic parasite, primarily of chytrids and Oomycetes, that grows inside its host as a wallless trophic form (Held 1981) until it produces thick-walled resting spores or zoosporangia that fill the host cell (Fig. 1a). The two strains in the rDNA phylogeny are parasites on other chytrids (Allomyces and Rhizoclosmatium). The ultrastructure of R. allomyces zoospores presents a unique combination of characters including a striated rhizoplast connecting the kinetosome (the basal body of the flagellum) to a large, spheroidal mitochondrion, which abuts a helmet-shaped nucleus (Held 1975). Phylogenetic analyses using the two largest subunits of RNA polymerase II suggest that microsporidia might belong to this lineage (T.Y. James and R. Vilgalys, unpublished data).

Clade 2A. Neocallimastigales.—It is supported as the most basal group of the core chytrid clade (Clade 2) by Bayesian posterior probability. Members of this clade are characterized by obligate anaerobic growth and are found in the rumen and hindgut of many larger mammalian herbivores. They are morphologically similar to other chytrid orders and may be monocentric or polycentric. The rumen chytrids possess an organelle called a hydrogenosome that generates ATP and appears to be a degenerate mitochondrion lacking a genome (van der Giezen 2002). Rumen chytrid zoospores may be multiflagellate or uniflagellate (Fig. 1b). The zoospore of rumen chytrids lacks the nonflagellated centrioles (nfc) and lipid globules that are found in most other members of Clade 2 but possesses a microtubular root emanating from a kinetosome associated spur (Heath et al 1983). Six described genera are circumscribed on the basis of number of flagella and sporangial characters (Ho and Barr 1995, Ozkose et al 2001).

Clade 2B. Monoblepharidales.—It is monophyletic and sister of the clade of Chytridiales+Spizellomycetales. Monoblepharidales is a small order consisting only of six genera. The earliest diverging lineage is the species Hyaloraphidium curvatum, once believed to be a colorless, unicellular green alga (Ustinova et al 2000). The planktonic H. curvatum has a lunate thallus superficially similar to that of some Harpochytrium species, but zoospores have not been observed in H. curvatum. The genera Oedogoniomyces, Hyaloraphidium and Harpochytrium all grow vegetatively as simple, unbranched thalli lacking rhizoids, whereas the remaining three genera, Gonapodya, Monoblepharis and Monoblepharella, are polycentric species with a well-developed coenocytic mycelium. In polycentric genera oogamous reproduction occurs, in which a motile male gamete (antherozoid) fuses with a nonmotile female gamete (egg, Fig. 1c). Hyphae of Monoblepharidales have a foamy or reticulate appearance. Zoospores of Monoblepharidales have a fenestrated cisterna called a rumposome (Fuller...
FIG. 2. Phylogeny of chytrid fungi based on rDNA operon. Shown is a majority-rule consensus phylogram computed from the set of 36,000 credible trees. A ‘++’ above a node indicates branch was supported by ≥ 95% Bayesian posterior probability (BPP); ‘+++’ indicates branch supported by ≥ 95% BPP and maximum likelihood bootstrap (MLBS) ≥ 70%. Branches supported by BPP, MLBS and parsimony bootstrap ≥ 70% are indicated by thickened lines. Branches with double diagonal hash marks have been shortened four times to aid viewing. The schematic illustrations diagram the ultrastructural details of...
Ultrastructures are indicated for all clades except 2H (Cladochytrium and Nowakowskiella and Allochytridium). Polychytrium aggregatum is found in acidic lakes and bogs; Lacostromyces hiemalis occurs in lakes or commonly wet areas and Karlingiomyces can be isolated from aquatic or terrestrial habitats. Both the monocentric and polycentric species of this clade grow on chitin baits. Zoospores are spherical when in motion and usually larger than 4 μm diam. The unique ultrastructural feature of the clade is the composition of the kinetosome and its associated structures (Longcore 1993 unpublished). The nfcs are as long as the kinetosome and the connection of the nfc to the kinetosome is distinctive. Densely staining material extends about 0.1 μm into the zoospore from kinetosome triplets 1 and 9; the primary microtubule root arises between these extensions. At the same level within the kinetosome is a scalloped ring (Longcore 1993).

Clade 2C. Chytridiales.—Polychytrium clade. This clade (formerly Novakowskiiella) (James et al 2000) is a well supported lineage with a zoospore ultrastructure similar to Novakowskiiella elegans and Cladochytrium replicatum (Lucarotti 1981). It includes eight genera: Allochytridium, Catenochytridium, Chytriomyces (? pro parte, Cladochytrium, Endochytridium, Nephrochytrium, Novakowskiiella and Septochytrium. Species in this group may be monocentric or polycentric and often are characterized by the presence of swellings in the rhizoids or rhizomycelium (Fig. 1e). They occur primarily on cellulose-rich substrates in aquatic and soil habitats. Members of the Cladochytrium clade have a zoospore that is typical for Chytridiales and most similar to that of the Chytriomyces clade (2K). One distinctive feature of the zoospore is that the microtubular root arising from the kinetosome is a cord-like bundle interconnected by fine fibrillar bridges (Lucarotti 1981, Barr 1986). Two major subclades were recovered in our phylogenetic analysis. Nephrochytrium and Cladochytrium form a clade that is sister of a clade containing the remaining genera.

Clade 2D. Chytridiales.—Cladochytrium. This clade (formerly Nowakowskiiella) (James et al 2000) is a well supported lineage with a zoospore ultrastructure similar to Novakowskiiella elegans and Cladochytrium replicatum (Lucarotti 1981). It includes eight genera: Allochytridium, Catenochytridium, Chytriomyces (? pro parte, Cladochytrium, Endochytridium, Nephrochytrium, Novakowskiiella and Septochytrium. Species in this group may be monocentric or polycentric and often are characterized by the presence of swellings in the rhizoids or rhizomycelium (Fig. 1e). They occur primarily on cellulose-rich substrates in aquatic and soil habitats. Members of the Cladochytrium clade have a zoospore that is typical for Chytridiales and most similar to that of the Chytriomyces clade (2K). One distinctive feature of the zoospore is that the microtubular root arising from the kinetosome is a cord-like bundle interconnected by fine fibrillar bridges (Lucarotti 1981, Barr 1986). Two major subclades were recovered in our phylogenetic analysis. Nephrochytrium and Cladochytrium form a clade that is sister of a clade containing the remaining genera.

Clade 2E. Chytridiales.—Synchytrium. This genus contains more than 200 species that are obligate parasites of green plants, particularly angiosperms. The four species of Synchytrium are monophyletic in the rDNA phylogeny (Fig. 2). The distinguishing feature of Synchytrium is colonial asexual reproduction by a sorus, a cluster of sporangia. The sorus of Synchytrium is derived by the internal cleavage of the common membrane of a single thallus into multiple sporangia. Synchytrium is included among Chytridiales, and the zoospore ultrastructure of two of the species generally conforms to the chytridial type (Lange and Olson 1978, Montecillo et al 1980). The S. endobioticum (Lange and Olson 1978) zoospore contains an electron opaque plug at the base of the flagellum but lacks a fenestrated cisterna; in contrast the S. macrosporum zoospore has a fenestrated cisterna but lacks the electron opaque plug (Montecillo et al 1980). Both species lack aggregated ribosomes observed in most other Chytridiales. Synchytrium spp. host ranges vary from broad (Karling 1964, S. macrosporum can be inoculated onto 165 different plant families) to narrow (S. decipiens is restricted to the genus Amphicarpaea).

Clade 2F. Chytridiales.—C. angularis. Chytriomyces angularis is a monocentric, epibiotic, operculate fungus distinguished by a gibbous zoosporangium with thread-like rhizoids (Fig. 1f). Secondary rhizoids usually form several micrometers from the sporangial base and branch perpendicularly from the initial rhizoidal axis (Fig. 1f). In the literature fungi with this description were considered possibly to be an alternate form of C. poculatus. These fungi are recovered on pollen and snakeskin baits from soils or boggy areas and seem to be more abundant in acidic environments. This clade also includes Chytriomyces polypsiniae, a parasite of the marine brown alga Pyliaella littoralis. Although C. angularis was classified a member of Chytriomyces based on its morphology and development, its zoospore (Longcore 1992) lacks features found in the Chytridiaceae (Letcher et al 2005); instead it is characterized by a unique connection of the kinetosome and nfcs (Longcore 1992).

Clade 2G. Chytridiales.—Rhizophydium. This clade is composed primarily of members of the morphogenus Rhizophydium, which is characterized by a monocentric, epibiotic, inoperculate sporangium, an endobiotic rhizoidal axis that branches (Fig. 1g) and an epibiotic resting spore. Molecular studies with 28S
rDNA indicate that the genus *Rhizophydatum* is more variable than previously understood and actually represents multiple genera (Letcher and Powell 2005b). The genus *Kappamyces* was circumscribed on the bases of molecular monophyly and zoospore ultrastructure (Letcher and Powell 2005b). Species showing none of the usual *Rhizophydatum* generic characters also are in this clade (Fig. 2), including endobiotic species (*Batrachochytrium dendrobatidis* and *Entophlyctis heliiformis*), species with multiple rhizoidal axes (JEL142), species with swollen basal rhizoids (e.g. *Rhizophydatum brooksianum*) and oculpture species (e.g. JEL223).

Although thallus morphology among most members of the *Rhizophydatum* clade is relatively conserved, zoospore ultrastructure is divergent. A key feature of the zoospore is the absence of an electron opaque plug in the base of the flagellum, which distinguishes it from most members of the Chytridiales (Letcher et al 2006). Other zoospore ultrastructural features that may be present include a kinetosome-associated structure (KAS) as a spur or shield (Letcher et al 2004b). a microtubular root in which four to five microtubules are stacked and a vesiculated, cup-shaped, invaginated region of the endoplasmic reticulum that surrounds the ribosomal aggregation, adjacent to the proximal end of the kinetosome. Character states of zoospore ultrastructural features are correlated with well supported clades of isolates within the *Rhizophydatum* clade. The diversity of the *Rhizophydatum* clade, as revealed by 18S, 28S, and ITS1–5.8S–ITS2 rDNA molecular analyses and zoospore ultrastructural analyses (Letcher et al 2004b), support naming the clade a new order Rhizophydatumales, which contains many families and genera (Letcher et al 2006).

**Clade 2I. Spizellomyces clade.** This is a distinctive species that has a sporangium traversed by spiral thickenings. It grows saprotrophically on pollen (Fig. 1h) but has not been isolated into pure culture (J.E. Longcore unpublished). The rDNA sequencing for the species was from an enriched unifungal culture on pollen. The placement of *B. helicus* in our analysis is uncertain, but additional data suggest a grouping with *Catamonymes persicinus* (see Clade 2J. Spizellomycesales.—Rhizophydatum clade, below, T.Y. James unpublished).

**Clade 2L. Spizellomycesales.—Spizellomyces clade.** This is a well supported lineage that appears to have diverged from among the lineages of Chytridiales. The clade contains the majority of the genera in the Spizellomycesales: *Gaertneriomyces, Kochiomyces, Powellomyces, Spizellomyces* and *Triparticalcar*. Most members of this clade are saprophytic in soil. All these genera have counterparts in the Chytridiales with similar morphology and development (e.g. *Spizellomyces* has *Phytochelytrium* morphology and development (Fig. 1l) but each possesses the distinctive Spizellomycesalean zoospore). At the ultrastructural level the nucleus is spatially or structurally associated with the kinetosome, the nfc lies at an angle to the kinetosome, organelles of the MLC are loosely associated and ribosomes are not aggregated around the nucleus (Barr 1980). Under a light microscope spizellomycesalean zoospores are recognizable because they can be amoeboid while swimming, are irregularly shaped and the flagellar insertion may move to a lateral position. The rDNA phylogeny (Fig. 2) suggests two groupings within this clade, one in which the nucleus possesses a heel that extends to the kinetosome (*Powellomyces* and *Spizellomyces*) and another in which this character is absent (*Gaertneriomyces* and *Triparticalcar*).
microscopy. Species identified as being in the genus *Chytriomycetes* occur in several separate, well supported clades indicating that *Chytriomycetes* as currently defined is polyphyletic (Fig. 2).

Although thallus morphology is divergent zoospore ultrastructure is conserved among the members of this clade. Most isolates from the *Chytriomycetes* clade possess a Group I-type zoospore (Barr 1980) and form a clade that has been delineated as the Chytridiaceae (Letcher et al. 2005). Features of that zoospore include a fenestrated cisterna, a lateral microtubular root composed of a bundle of approximately seven microtubules in a cord-like arrangement, and a pair of three stacked, flat, electron opaque plates adjacent to the kinetosome, a paracrystalline inclusion in the peripheral cytoplasm and an electron opaque plug in the base of the flagellum. *Phlyctochytrium planicorne* (Letcher and Powell 2005a) and *Polyphlyctis unispina* (Letcher et al. 2005) have a Group II-type zoospore (Barr 1980), distinguishable from the zoospore of the Chytridiaceae primarily by the structure of the electron opaque plates adjacent to the kinetosome. Isolates with a Group II-type zoospore probably represent a separate family in the Chytridiales.

**Clade 3. Blastocladiomycota.**—Traditionally considered among phylum Chytridiomycota, the Blastocladiales diverges from the core chytrid clade and is sister of a clade including Zygomycota, the chytrid genus *Olpidium*, Glomeromycota and Dikarya (Fig. 2). They are saprotrophs as well as parasites of fungi, algae, plants and invertebrates, and may be facultatively anaerobic. Two major subclades are resolved in molecular phylogenetic analyses (Fig. 2), one composed of the plant parasite *Physoderma* and the other containing the remaining blastocladilean genera. The morphology of thalli of Blastocladiales parallels forms found among the core chytrid clade, ranging from monocentric to polycentric and mycelial. However *Coelomomyces* produces a tubular unwalled thallus in its host, reminiscent of hyphal bodies in the Zygomycete group Entomophthorales. The zoospore is functionally similar to those found among core chytrids with a single posteriorly directed flagellum, stored lipid and glycogen reserves, a characteristic assemblage of lipids, microbodies, membrane cisterna called the side-body complex (=microbody-lipid globule complex) and a membrane-bounded ribosomal cap covering the anterior surface of a cone-shaped nucleus.

Major evolutionary changes have accompanied the divergence of the Blastocladiales from the core chytrids. For example the Blastocladiales have a life cycle with sporic meiosis whereas most core chytrids have zygotic meiosis, where known. The Blastocladiales exhibits several ultrastructural features more characteristic of higher filamentous fungi than of the core chytrids, including closed nuclear poles during mitosis (Fig. 3a) rather than open (fenestrate) poles (Fig. 3b) and Golgi equivalents (Bracker 1967, Fig. 3c) rather than stacked Golgi cisternae (Fig. 3d). A Golgi apparatus with stacked cisternae in *Ponellomyces variabilis* (Spizellomyces clade) near rough endoplasmic reticulum. d. A Golgi apparatus with five stacked cisternae in *Ponellomyces variabilis* (Spizellomyces clade). Notice smooth endoplasmic reticulum and small transport vesicles at the proximal (upper) side of the stacks and larger secretory vesicles at the distal (lower) side of the stack. Methods for sample preparation and microscopy are as previously published (Powell 1975). Bars: a, b = 0.25 μm; c, d = 0.125 μm.

**Blastocladiomycota** T. James, phylum nov.


Archemycota Cavalier-Smith, Biol. Rev. 73:246, 1998 pro parte

Zoosporum cum flagellum unum, corpus-latus complexe, nucleus pileus ex ribosomata membrane confinium, nucleus cum conus forma terminari propinquus kinetosoma, microtubuli ex propinqui-
Allomyces and sexual reproduction through fusion of planogametes, transition zone. Asexual reproduction with zoospores, zoospore flagellum lacks electron-opaque plug in proximal end of the kinetosome around the nucleus, oosome, microtubules radiate anteriorly from the cone-shaped nucleus that terminates near the kinetosome, nuclear cap of membrane-bounded ribosomes, nonsexus cum zoosporae, generare sexus ad conjoin- nucleum, flagellum obturamentum nullum. Generare tate kinetosomatis per zoosporum radiant circum nucleus, flagellum obturamentum nullum. Generare nonsexus cum zoosporae, generare sexus ad conjun- gare planogametum, orbis vita cum meiosis sporae.

Type: Blastocladia Reinsch 1878

Zoospore with a single flagellum, side-body complex, nuclear cap of membrane-bounded ribosomes, cone-shaped nucleus that terminates near the kinetosome, microtubules radiate anteriorly from the proximal end of the kinetosome around the nucleus, zoospore flagellum lacks electron-opaque plug in transition zone. Asexual reproduction with zoospores, sexual reproduction through fusion of planogametes, life cycle with sporic meiosis.

Type: Blastocladia Reinsch 1878

Blastocladiomycetes cl. nov. T. James. Description as for Blastocladiomycota.

Reinsch described Blastocladia in 1878, and Peter- sen (1910) erected the family Blastocladiaceae to accommodate this single genus, classifying it in the class Oomycetes. Kanouse (1927) first used the order name, Blastocladiales (which she credited to Petersen), and included in it the single family with Allomyces and Gonapodya (now removed). Fitzpatrick (1930) placed the Blastocladiaceae in class Phycomycetes, a classification Sparrow (1960) followed. Alex- opoulos (1966) separated fungi into eight classes, one of which was class Chytridomyces, including Blas- tocladias. This placement for the Blastocladiaceae was retained as Chytridomyces was elevated to the phylum Chytridomycota (not validly published, no Latin diagnosis; p 15 in von Arx 1967, Margulis et al 1989). Chytridomycota will be validated when the phylogeny is more clearly resolved.

Although Tehler (1988) used Blastocladiomycota as a division (phylum) name, the taxon was not validly published because he did not provide or refer to a Latin description or diagnosis or explain the basis of or rationale for this name (Article 36.1, Greuter et al 2000). Consequently it is nomen nudum and furthermore the group that Tehler (1988) circumscribed with this name is not monophyletic. Thus we have used Blastocladiomycota as a phylum for a monophyletic clade (Clade 3), validated the phylum name with a Latin description and have designated a type. A potentially competing phylum name, Allomyces (Cavalier-Smith 1981), is invalid because Cavalier-Smith did not include a Latin diagnosis. We may select the phylum name Blastocladiomycota however because the principle of priority is not mandatory above the rank of family (Article 11.1, 11.9). Cavalier-Smith (1998) eventually validated class Allomycetes with Latin; however this validation is moot to our selection because a name does not have priority outside its own rank (Article 11.2). As we have described the Blastocladiomycota, it is a natural group separate from the more heterogeneous phylum Archymycota (Cavalier-Smith 1998).

Clade 4. Olpidium brassicace.—Olpidium brassicace unexpectedly grouped among the polyphyletic Zygo- mycota and with the nonzoosporic fungi. O. brassicace currently assigned to the Spizellomycetales (Barr 1980), although this placement was considered tenuous (Barr 2001). Few ecological and morphological features unite O. brassicace and Zygomycota: O. brassicace is a root pathogen of cucurbits, whereas most members of the Zygomycota II lineage (Fig. 2) are associated with animals. The zoospore of O. brassicace contains a unique combination of features, cone-shaped striated rhizoplast, gamma-like particles and rough endoplasmic reticulum (Lange and Olson 1976). These ultrastructural features as well as holocarpic, endobiotic sporangia had suggested a relationship between Olpidium and Rozella (Held 1975), but Olpidium differs from Rozella in that sporangia of the former develop a cell wall inside the host cytoplasm while sporangia of Rozella do not and instead use the host’s cell wall (Held 1981). Further data will be needed to determine the exact phyloge- netic position of O. brassicace and whether it shares a common ancestor with the majority of terrestrial fungi. We predict that Caulochytrium protostelioides with a similar zoosporic ultrastructure and aerially produced sporangia (Powell 1981) also will be a member of this currently monotypic clade.

Toward a phylogenetic classification.—The paraphyly of the Chytridiomycota requires that its current circumscription be redefined to produce a phyloge- netic classification system. Placing all flagellate true Fungi into one phylum does not reflect shared ancestry of these fungi because the flagellum was a character possessed by the common ancestor of all Fungi (i.e. it is a pleisomorphous character. Further- more the absence of a flagellated state (as in Hyaloraphidium curvatum) should not prevent the inclusion of nonzoosporic fungi among natural groups of Chytridiomycetes. The phylogeny (Fig. 2) suggests the existence of at least four major lineages of chytrids. The distinction of Blastocladiomycota from other chytrids is clear on the basis of life cycle (Sparrow 1960), ultrastructural characters (Fig. 3) and multiple molecular phylogenetic studies using rDNA (James et al 2000, this study), RPB1 (Tanabe et al 2004), RPB2 (T.Y. James unpublished) and whole mitochondrial genome sequencing (Seif et al 2005). The exact placement of the holocarpic parasites Rozella and Olpidium, formerly included in the
Spizellomycetales, is an open question because of limited sampling of taxa, a paucity of molecular data and phylogenetic uncertainty (in the case of O. brassicae).

Molecular studies have revealed that many currently defined genera in Chytridiales are polyphyletic (James et al 2000, Letcher and Powell 2005b, Letcher et al 2005). Our results show that neither developmental characters, such as polycentric growth, or sporangial characters, such as presence of an operculum, will be useful in defining families or even genera. These phylogenies demonstrate a repeated evolution of polycentric growth in nearly every well sampled clade, suggesting the evolution of this form of indeterminant growth does not require a complex genetic change. Some of the largest genera (e.g. Rhizophydium, Entophlyctis and Chytriomyces) are based on few distinctive characters and are dramatically in need of revision. Other genera are highly distinctive (e.g. Loborhiza, Obelidium) but are monotypic. Current efforts are focused on combining morphological, ultrastructural and molecular data to use a holistic approach for redefining genera of Chytridiales (e.g. Letcher and Powell 2005b).

Application of zoospore ultrastructural characters.—A resolved phylogeny of the chytrid fungi now permits the mapping of ultrastructural character states onto the gene tree to further our understanding of character evolution. For example the electron-dense plug in the base of the flagellum has been considered phylogenetically informative (Barr 2001). This structural character state is present in all members of the Chytriomyces (2K, Letcher et al 2005), Cladochytrium (2D) and Polychytrium (2C) clades examined thus far. It is absent in the Rozella (1), Neocallimastigales (2A), Blastocladiomycota (3) and Olpidium (4) clades (Barr 2001). When the presence or absence of this character is mapped onto the rDNA phylogeny, it appears that this character was present in the ancestor of the Chytridiales and Monoblepharidales (clades 2B–K) but has been lost repeatedly in independent lineages (Fig. 2). For example in the Synchytrium clade (2E) this structure may be either present (S. endobioticum, Lange and Olsen 1978) or absent (S. macrosorum, Montecillo et al 1980); in the Monoblepharidales (2B) it is either present (i.e. Monoblepharis polymorpha, Mollicone and Longcore 1994) or lost (Gonapodya polymorpha, Mollicone and Longcore 1999); it has been lost in the Spizellomycetales (2I and 2J, Barr 1980) and Rhizophydium clades (2G; Letcher et al 2004b, 2006). Thus an apparently identical character state, the absence of the flagellar plug, is a convergent character state if applied to organisms from different lineages but it may be a valuable character for taxonomic purposes within a lineage. The heuristic exercise of mapping morphological characters on gene trees suggests that some character states have evolved repeatedly in distinct lineages, thus creating an opportunity for previous assumptions regarding character homology to be reassessed in a phylogenetic context.

Summary of current phylogenetic knowledge and remaining questions.—We have identified four major lineages of chytrid fungi, the largest of which can be subdivided into 11 additional clades. By increasing the sampling of both rRNA characters and species we have greatly expanded our knowledge of phylogenetic diversity and relationships among the groups of chytrids. Our results confirm previous phylogenetic studies and ultrastructural information that suggest the Blastocladias is distinct from the remaining chytrids and possibly more closely related to the nonzoosporic fungi than the core chytrids. Thus we have elevated the order Blastocladiales to the level of phylum (Blastocladiomycota). Based on the present research, as well as the results of the Assembling the Fungal Tree of Life project (http://acet.nace.org/research/aftol/) and complete sequencing of fungal mitochondrial genomes (Seif et al 2005), the phylogeny of the core chytrids is beginning to be resolved with the Neocallimastigales as the earliest diverging lineage and the Monoblepharidales as sister taxon of a clade containing Chytridiales+Spizellomycetales. Phylogenetic investigations in the past three years suggest that much of the diversity in the chytrids is found in species that are not readily cultured (e.g. Olpidium, Rozella, Synchytrium). Remaining questions in the chytrid phylogeny are the exact placement of Olpidium and whether microsporidia are a part of the basal grade of chytrids. Further sampling of DNA sequences from species that are obligate biotrophic parasites with unique zoospore ultrastructure (e.g. Caulochytrium [Powell 1981], Thalassochytrium [Nyvall et al 1999] and Zygorhizidium [Beakes et al 1988]) might yet uncover additional clades. Furthermore, given that a number of chytrid species are facultative or obligate anaerobes, sampling of these environments also might uncover additional phylogenetic diversity.

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LITERATURE CITED


———, McGee PA, Powell MJ. 2004a. Diversity of chytrids
from soils of four vegetation types in New South Wales, Australia. Can J Bot 82:1490–1500.


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


