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Large-Subunit rRNA Sequence of the Chytridiomycete *Blastocladiella emersonii,* and Implications for the Evolution of Zoosporic Fungi

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Abstract. The 5.8S and 28S ribosomal RNA sequences of the chytridiomycete *Blastocladiella emersonii* were determined. These data were combined with 18S rRNA sequences in order to carry out a phylogenetic analysis based on distance matrix, parsimony, and maximum likelihood methods. The new data confirmed that chytridiomycetes are true fungi and not protists, as was already suggested on the basis of biochemical, ultrastructural, and 18S rRNA data. Within the fungal clade, *B. emersonii* formed the first line of divergence. The position of the fungi within the eukaryotic ''crown'' taxa was also reassessed, and the alveolate-stramenopile cluster appeared as their sister group. The stramenopiles also comprise a number of zoosporic fungi, which resemble chytridiomycetes in so many respects, e.g., production of motile spores, thallus morphology, and absorptive nutrition, that they have been classified together with them in the past. This suggests that the possible common ancestor of the fungi, stramenopiles, and alveolates may have been a zoosporic fungus, which would mean that zoosporic fungi are paraphyletic instead of polyphyletic as previously suggested.

Key words: Chytridiomycetes — Stramenopiles — Alveolates — Fungi — Mastigomycetes — rRNA phylogeny — *Blastocladiella emersonii*

Abbreviations: SSU rRNA: small-subunit rRNA, viz. 18S rRNA; LSU rRNA: large-subunit rRNA, viz. 5.8S and 28S rRNA; PCR: polymerase chain reaction; MUCL: Mycothèque de l'Université Catholique de Louvain; ATCC: American Type Culture Collection

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Introduction

Zoosporic fungi are characterized by the production of motile reproductive cells which bear one or two flagella. They used to be classified as fungi because of their typical fungal thallus morphology and mode of nutrition (Ainsworth 1973), and as protists because of the motile nature of their spores (Corliss 1984; Margulis and Schwartz 1988). Chytridiomycetes are zoosporic fungi typified by zoospores which are posteriorly uniflagellated. On the basis of 18S rRNA data it became clear that chytridiomycetes together with zygomycetes, ascomycetes, and basidiomycetes form a monophyletic clade referred to as ''true'' fungi, Eumycota, or kingdom Fungi, whereas other zoosporic fungi—viz., hyphochytriomycetes and oomycetes—cluster with the chromophyte algae (Förster et al. 1990; Bruns et al. 1991; Schlegel 1991; Bowman et al. 1992; Paquin et al. 1995; Van der Auwera et al. 1995). This result is consistent with ultrastructural and biochemical data, such as cell-wall composition, enzyme characteristics, lysine synthesis pathway, shape of mitochondrial cristae, and flagellar apparatus (e.g., Vogel 1965; Léjohn 1974; Taylor 1978; Cavalier-Smith 1981; Barr 1983; Lange and Olson 1983; Bartnicki-Garcia 1987; Tehler 1988).

Although it is clear now that chytridiomycetes are true fungi, the relationships between the various fungal taxa are less well resolved. Based on SSU rRNA data, chytridiomycetes and zygomycetes are situated at the base of the fungal tree, followed by the divergence of basidiomycetes and ascomycetes, which are sister groups (Bruns et al. 1992; Van de Peer et al. 1993; Paquin et al. 1995). However, the relationship between chytridiomy-

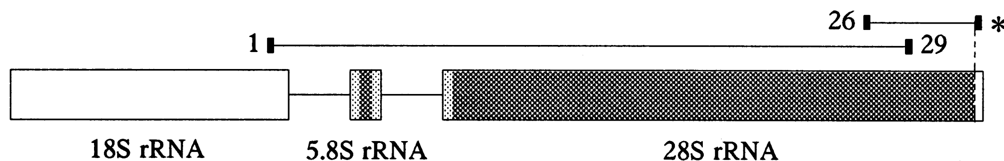

Fig. 1. Schematic representation of an rDNA transcription unit, with an indication of the two PCR-amplified regions together with the position of the primers (see text for details). The *dark shaded regions* were sequenced on both strands, while the *light shaded regions* were only sequenced on one strand due to the lack of primers in the other direction. The *white regions* were not sequenced in this study.

cetes and zygomycetes remains doubtful. In many SSU rRNA trees, the divergence of the chytridiomycete *Blastocladiella emersonii* (order Blastocladiales) and the zygomycete *Mucor racemosus* (order Mucorales) precedes that of the remaining chytridiomycetes and zygomycetes (Bruns et al. 1992; Nishida and Sugiyama 1993; Cavalier-Smith et al. 1994). On the basis of ultrastructure and characteristics of nuclear division, some authors have already suggested the removal of the Blastocladiales from the chytridiomycetes (Cavalier-Smith 1981; Heath 1986). Based mainly on morphological data, Tehler (1988) also treated the chytridiomycetes as a paraphyletic group, and Heath (1986) indicated that the zygomycetes might not be monophyletic either.

When *Blastocladiella emersonii* and *Mucor racemosus* are found as the first lineages in the fungal SSU rRNA tree, they appear either as a cluster or as two successively diverging lineages, in which case the order of divergence cannot be established (Bruns et al. 1992; Nishida and Sugiyama 1993; Cavalier-Smith et al. 1994). Also, bootstrap support for the inferred phylogenies is often low. Since almost the entire LSU rRNA sequence of *M. racemosus* is already known, we determined the LSU rRNA sequence of *B. emersonii* in order to further elucidate the phylogenetic relationships between these organisms and the higher fungi. To our knowledge, this is the first chytridiomycete LSU rRNA sequence reported.

Because *Blastocladiella emersonii* has much in common with protists as well as with fungi, and is situated at the base of the fungal tree, the relationship between the fungi and the other eukaryotic taxa on the basis of SSU and LSU rRNA sequences is also evaluated.

Materials and Methods

Cloning of the LSU rDNA. Biomass of *Blastocladiella emersonii* culture MUCL 35075 (ATCC 22665) was obtained from the MUCL (Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium) culture collection. DNA was isolated as described in Van der Auwera et al. (1994). Since only small amounts of DNA were obtained, the rRNA genes had to be amplified by PCR, and two overlapping fragments were needed. The position of these overlapping fragments together with the location of the primers is shown schematically in Fig. 1. Primers are numbered as in Van der Auwera et al. (1994). The first reaction was performed with primers 1 and 29 in 100 μl of 10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, and 0.1% Triton X-100, containing 0.2 mM of each dNTP, 10 ng template DNA, 0.5 μM of each primer, and 0.5 U SuperTaq (HT Biotechnology Ltd., Cambridge, UK). Thirty cycles were performed, each consisting of 1 min at 94°C, 1 min at 55°C, and 3 min 30 s at 72°C. Cycling was preceded by 2 min at 94°C and followed by 10 min at 72°C. The hot-start PCR technique was used. In the second amplification primer 26 was used in combination with a primer (Van der Auwera et al. 1995) situated approximately 40–20 bases from the 3′-end of the 28S rDNA (indicated with * in Fig. 1). Conditions for this PCR were nearly identical, except that the primer extension step took 2 min and 2.5 U Taq DNA polymerase (Boehringer Mannheim, Mannheim) was used in buffer containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.3. Both PCR products were cloned in either vector pGEM-T (Promega, Madison, Wisconsin) or pBluescriptIISK+ (Stratagene, La Jolla, CA).

Sequencing. The 5.8S and 28S rDNA were sequenced completely, except for 40 or so 3′-terminal nucleotides of the 28S rDNA, which were not determined because they could not be amplified by PCR due to the lack of a suitable primer beyond the 3′-end (Fig. 1). Sequencing was done on both strands, except for ±40 terminal nucleotides on both sides of each gene, as shown in Fig. 1. Sequences were determined on a pool of a minimum of ten clones to minimize sequencing errors introduced by Taq DNA polymerase during PCR. Sequencing was performed with the Sequenase sequencing kit from USB (Cleveland, OH) and the T7 sequencing kit from Pharmacia Biotech (Uppsala, Sweden), all according to the manufacturers' instructions.

Alignment and Tree Construction. The LSU rDNA sequence of *Blastocladiella emersonii* was included in an alignment kept in our research group (De Rijk et al. 1994), which is based both on primary and secondary structure of the rRNA. This alignment includes the 5.8S rRNA as well as the 28S rRNA. For the phylogenetic analysis, only 3,015 well-aligned positions were used, comprising 2,899 out of the 3,693 available nucleotides from *B. emersonii.* Most phylogenetic trees were constructed on a combination of the LSU rRNA alignment with 1,806 well-aligned positions of the SSU rRNA alignment (Van de Peer et al. 1994), and this will be referred to as the SSU+LSU rRNA alignment. The combination of both alignments in order to obtain a larger and therefore more reliable data set is justified because characters in both data sets have similar properties and can be analyzed using exactly the same methods. The SSU and LSU rRNA alignments, with an indication of the sites that were left out of the analysis, can be obtained from the authors on request.

Neighbor-joining trees (Saitou and Nei 1987) were constructed using the software package TREECON (Van de Peer and De Wachter 1994). Dissimilarities were converted into evolutionary distances using an equation (Van de Peer et al. 1990) based on the Jukes and Cantor model (Jukes and Cantor 1969) but also taking into account insertions and deletions. Bootstrap analysis (Felsenstein 1985) was performed on 2,000 samples. Maximum parsimony trees were constructed using the software package PAUP 3.1.1 (Swofford 1993). A heuristic search was used, and bootstrap was performed with 100 replicates. Further analysis of the parsimony trees—viz., the calculation of the total number of steps needed for alternative tree topologies—was carried out with the aid of MACCLADE 3.04 (Maddison and Maddison 1992). A maximum likelihood tree (Felsenstein 1981) was constructed with the soft-

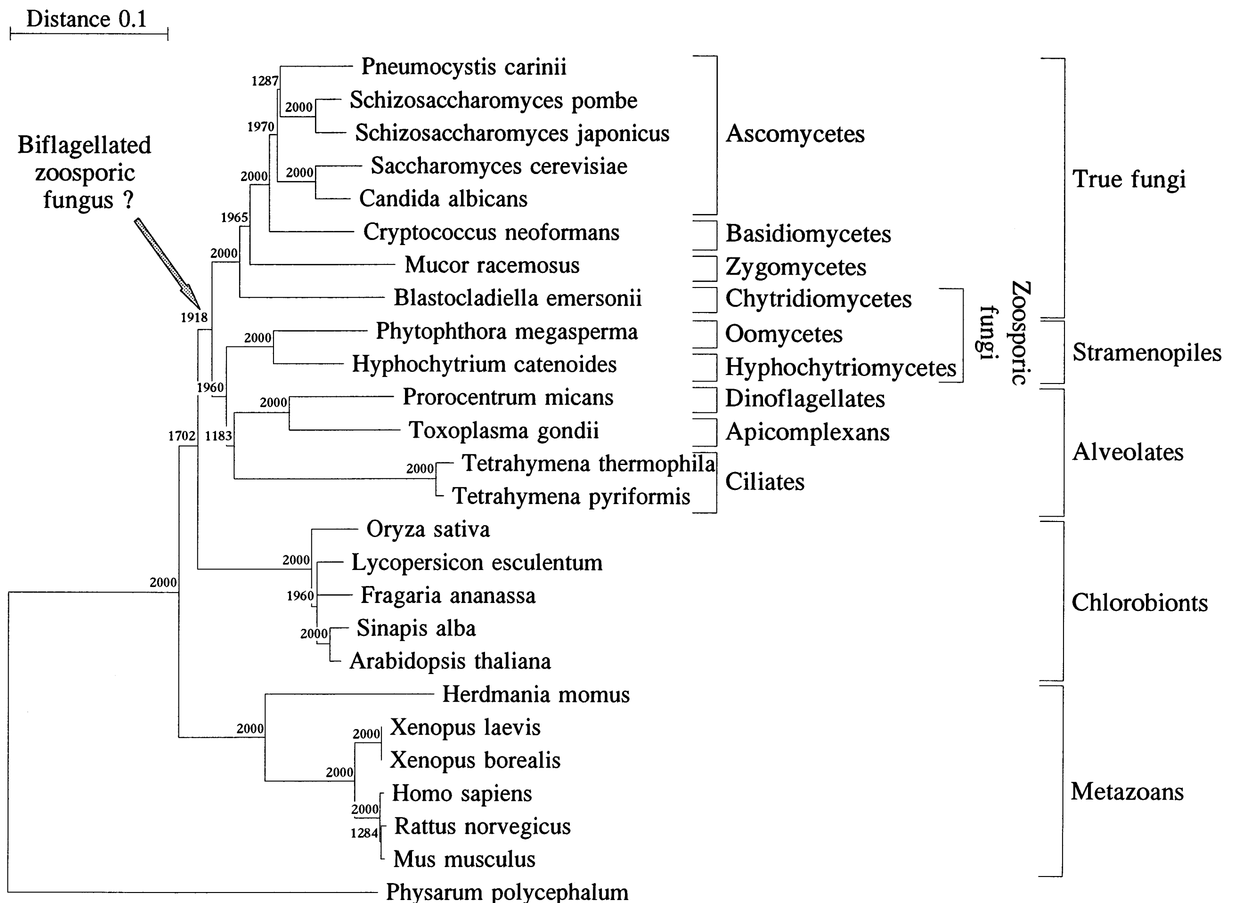

Fig. 2. Neighbor-joining tree constructed on the basis of a combination of SSU and LSU rRNA sequences. Only species of the crown taxa and for which both sequences are known are included in the tree. The evolutionary distance between two sequences is obtained by summing the lengths of the connecting branches along the horizontal axis, using the *scale* at the top. Bootstrap analysis was performed on 2,000 samples, and *values are shown at the internodes.*

ware package fastDNAml (Olsen et al. 1994). The time needed to construct a phylogenetic tree from the SSU+LSU rRNA alignment of 26 species is in the order of seconds for a neighbor-joining tree, hours for a maximum parsimony tree, and days for a maximum likelihood tree. As a consequence, no bootstrap analysis could be carried out for the maximum likelihood tree, and it was impossible to examine the influence of alternative outgroups on tree topology. Therefore the latter tree is not shown but only described in the Results section.

Results

Primary Structure of the LSU rRNA

The entire 28S and 5.8S rRNA sequences of *Blastocladiella emersonii* were determined, except for approximately 40 nucleotides at the 3′-end of the 28S rRNA, as shown in Fig. 1. The 5.8S rRNA has a length of 155 nucleotides; the determined part of the 28S rRNA is 3,538 nucleotides long. The location of the termini was estimated for both molecules by alignment with the other LSU rRNA sequences in the database of De Rijk et al. (1994). Both sequences were deposited at the EMBL Nucleotide Sequence Database and were assigned accession numbers X90410 (5.8S rDNA) and X90411 (28S rDNA).

Phylogenetic Analysis

Figure 2 shows a phylogenetic tree constructed by the neighbor-joining method on the basis of the SSU + LSU rRNA alignment. The tree includes organisms of the eukaryotic ‘‘crown’’ taxa (Knoll 1992), of which both the complete or nearly complete SSU and LSU rRNA sequences are known. The crown taxa comprise all organisms that have originated as a result of a radiation in the eukaryotic tree that gave rise to fungi, metazoans, chlorobionts, rhodophytes, and a number of protist phyla such as stramenopiles and alveolates. As can be seen in this tree, *Blastocladiella emersonii* forms a monophyletic clade with the other true fungi, a group that is very well supported by a bootstrap value of 2,000/2,000. Also, the clustering of *Mucor racemosus* with the higher fungi—viz., ascomycetes and basidiomycetes—is supported by a bootstrap value of 1,965/2,000 (98%), indicating that *B. emersonii* is not a sister group of *M. racemosus,* but

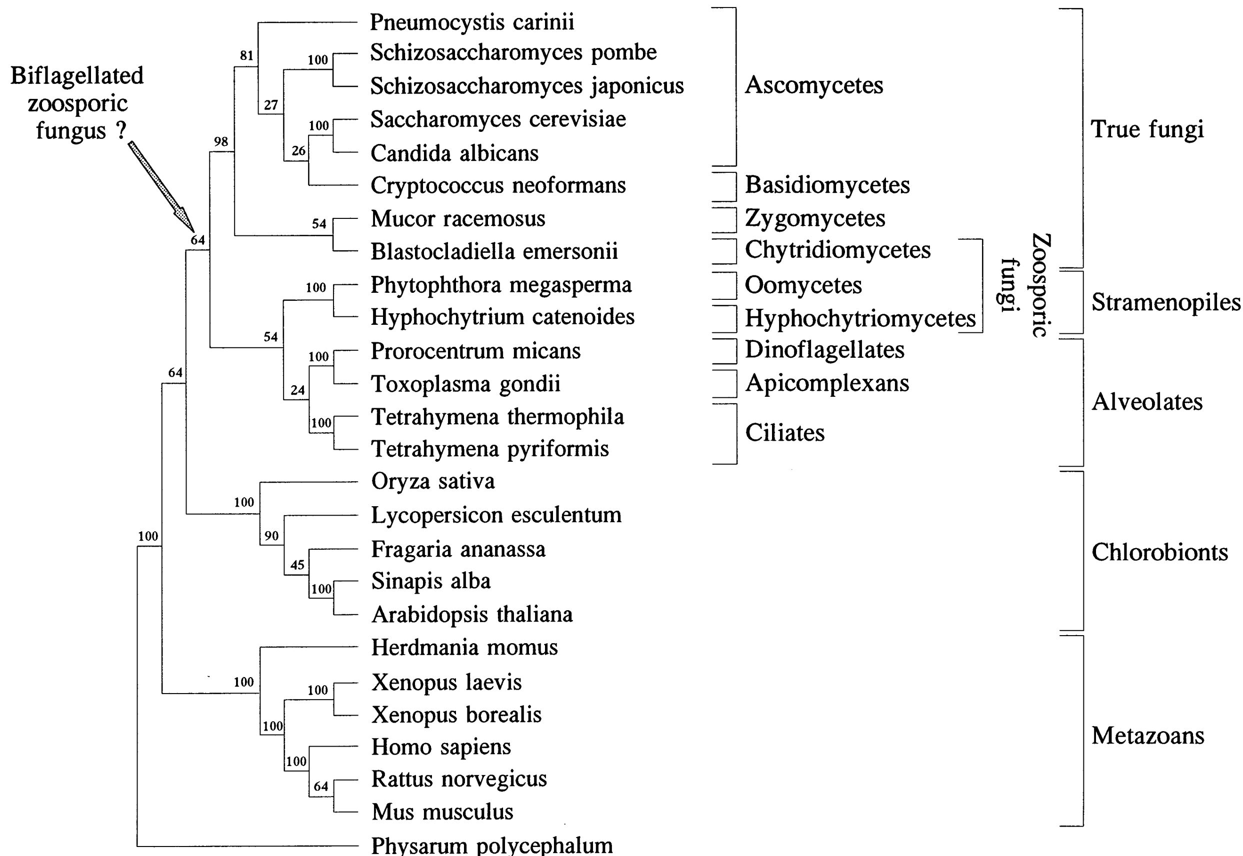

Fig. 3. Maximum parsimony tree of the eukaryotic crown taxa, constructed on the basis of 1,851 informative positions of a combination of SSU and LSU rRNA data. A heuristic search was used, and the tree has a total length of 6,871 steps. A bootstrap tree from 100 replicates was also constructed (not shown), and the *support for each cluster is indicated at the internodes.* Branch lengths are meaningless.

forms the first true fungal branch. A neighbor-joining tree including the same species as in Fig. 2 but based on the LSU rRNA alignment only (not shown) gave the same results.

Figure 3 shows a maximum parsimony tree containing the same species as the neighbor-joining tree in Fig. 2, and is based only on those positions of the SSU + LSU rRNA alignment that are informative for parsimony analysis (Nei 1987). These sites amount to 1,851 of a total of 4,821. The tree has a total length of 6,871 steps. The bootstrap score of each cluster, obtained from a 100 replicate bootstrap analysis, is given at the internodes. As in the neighbor-joining tree, *Blastocladiella emersonii* clusters with the true fungi, a relationship supported by a bootstrap value of 98/100. As opposed to the tree in Fig. 2, it clusters together with *Mucor racemosus,* but a bootstrap value of 54/100 cannot support this relationship. As for the monophyly of the ascomycetes with the basidiomycetes, this is poorly supported by a bootstrap value of 81/100, and the position of the basidiomycete *Cryptococcus neoformans,* though not bootstrap supported at all, renders the ascomycetes paraphyletic. Moreover, only two additional steps, or a total of 6,873, are needed to obtain exactly the same tree topology as encountered in Fig. 2. In conclusion, maximum parsimony gives a confused fungal phylogeny, insufficiently supported to contradict the fungal relationships as deduced from neighbor-joining. In a maximum parsimony tree based on the informative positions of the LSU rRNA alignment only (not shown), *Blastocladiella emersonii* diverged first in the fungal cluster, but also in this case there was no bootstrap support.

In both Figs. 2 and 3, *Physarum polycephalum* was used as outgroup to root the tree. It should be noted, however, that *Dictyostelium discoideum* and *Entamoeba histolytica* diverge closer to the ingroup, but place the root of the tree elsewhere. Figure 4 shows the unrooted topology of the crown taxa in the neighbor-joining and maximum parsimony trees (Figs. 2 and 3). When the tree is rooted by means of a eukaryote diverging prior to the crown taxa, the place of the root depends on the outgroup used. Most outgroup species draw the root of the tree on either the branch leading to the Metazoa (C in Fig. 4), or the one separating the Chlorobionta and Metazoa from the rest (B in Fig. 4). However, whenever *D. discoideum* or *E. histolytica* is included in the tree, the root is placed on the branch leading to the two ciliate species (A in Fig. 4). Thereby, the alveolate monophyly (Gajadhar et al. 1991; Wolters 1991; Patterson and Sogin 1992) is destroyed, and since dinoflagellates are the most probable

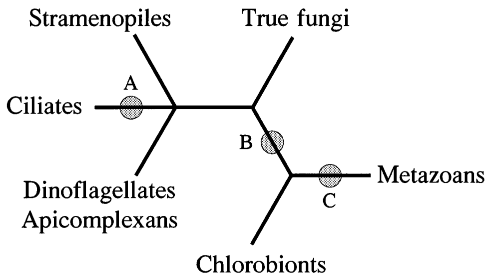

Fig. 4. Unrooted tree topology of the crown taxa, consistent with all neighbor-joining and maximum parsimony tree topologies encountered, regardless of the outgroup, and using the ingroup species of Figs. 2 and 3. Branch lengths have no meaning. The different rooting positions are indicated by *dots*: (*A*) root placement if *Dictyostelium discoideum* and/or *Entamoeba histolytica* is added to the tree or substituted for *Physarum polycephalum* as outgroup; (*B*) and *(C)* position of the root in other cases (see text for details), e.g., position C in Figs. 2 and 3. The topology was also found in a maximum likelihood tree with the same species as in Figs. 2 and 3, although the outgroup *P. polycephalum* rooted the tree in position A.

ancestors of the ciliates (Lee and Kugrens 1992), this would be rather contradictory. Moreover, when *D. discoideum* or *E. histolytica* is included in the tree, it was observed that the crown taxa are not rooted consistently, in that the root changed from position A to B, C, and other locations (Fig. 4), depending on the species included in the ingroup.

As can be seen in Figs. 2 and 3, the true fungi form the sister group of the alveolates and stramenopiles on the basis of SSU + LSU rRNA data, a relationship that is very well supported by a bootstrap value of 1,918/2,000 (96%) in the neighbor-joining tree, but only 64/100 in the maximum parsimony tree. The low bootstrap values for the relationships between the fungi, metazoans, chlorobionts, stramenopiles, and alveolates in the maximum parsimony tree indicate that these relationships cannot be clearly resolved using this method on our data set, although the topology is in agreement with that of the neighbor-joining tree. The same relationships between the crown taxa are also encountered in trees based on the LSU rRNA alignment only (not shown).

The relationships between the crown taxa deduced from a maximum likelihood analysis (results not shown) including the same species as the neighbor-joining and maximum parsimony trees are also as in Fig. 4. Using *Physarum polycephalum* as an outgroup, the root was placed in location A. The influence of other outgroups on the place of the root could not be investigated due to computational time limitations, as explained in the Methods section. The fungal phylogeny was the same as that deduced from the neighbor-joining tree (Fig. 2), except that *B. emersonii* and *M. racemosus* clustered together as they do in the maximum parsimony tree (Fig. 3). However, since no bootstrap analysis could be carried out, it is hard to assess the reliability of the maximum likelihood tree.

Discussion

The clustering of the chytridiomycete *Blastocladiella emersonii* with the true fungi is in agreement with SSU rRNA trees (Bowman et al. 1992; Bruns et al. 1992; Van de Peer et al. 1993), although bootstrap values in these trees are usually lower. It is thus confirmed that chytridiomycetes are true fungi, and not protists.

Our results from neighbor-joining analysis strongly indicate that *B. emersonii* diverges first in the fungal clade, before *M. racemosus,* which clarifies the uncertain phylogeny obtained on the basis of SSU rRNA data. And although maximum parsimony and maximum likelihood cannot support this phylogeny, it is in agreement with trees based on cytochrome oxidase protein sequences (Paquin et al. 1995), in which the divergence of the chytridiomycete *Allomyces macrogynus* (order Blastocladiales) precedes that of the zygomycete *Rhizopus stolonifer* (order Mucorales). Therefore, it is probable that a chytridiomycete-like fungus was the ancestor of all true fungi, of which the zygomycetes and higher fungi have lost their spore flagellum. This scenario was also suggested by Tehler (1988), who performed a cladistic analysis of the true fungi, and by Cavalier-Smith (1987).

Because *B. emersonii* has much in common with protists as well as with fungi, it may be useful to reevaluate the phylogenetic position of the fungi relative to other crown taxa on the basis of SSU + LSU rRNA sequences. The clustering of the true fungi with the alveolates and stramenopiles, as found in Figs. 2 and 3, is consistent with previously found LSU and SSU + LSU rRNA-based phylogenies (Baroin et al. 1988; Lenaers et al. 1989; De Rijk et al. 1995; Van der Auwera et al. 1995). The taxon stramenopiles (Patterson 1989) is practically a synonym for Heterokonta as defined by Cavalier-Smith (1989). It is a monophyletic group constituted of heterokont algae (mainly xanthophytes, chrysophytes, phaeophytes, bacillariophytes, and eustigmatophytes), heterokont fungi (hyphochytriomycetes and oomycetes), and a number of other heterotrophic organisms such as thraustochytrids, which used to be classified with fungi, labyrinthulids, and bicosoecids. *Hyphochytrium catenoides* and *Phytophthora megasperma* are the only stramenopiles included in the trees because no other stramenopile LSU rRNA sequences are known yet. ''Alveolates'' is the term used to designate dinoflagellates, ciliates, and apicomplexans, which also form a monophyletic group (Gajadhar et al. 1991; Wolters 1991; Patterson and Sogin 1992). The alveolate-stramenopile cluster is found in SSU rRNA trees as well (e.g., Schlegel 1991; Wolters 1991; Van de Peer et al. 1993), although bootstrap support is not as high as the 1,960/2,000 (98%) in Fig. 2.

Special attention should be drawn to the fact that the stramenopile-alveolate cluster as well as the true fungal cluster contain zoosporic fungi or mastigomycetes. This group consists of chytridiomycetes, hyphochytriomycetes, and oomycetes (Ainsworth 1973; Corliss 1984), and

all these taxa use absorptive nutrition, have similar thallus morphology, and produce motile reproductive cells called zoospores. Moreover, these zoospores have some structural features in common (Taylor 1978; Barr 1983; Lange and Olson 1983). Some authors have included other taxa in the mastigomycetes as well, but since our analysis does not involve these they will be ignored here. The three mastigomycete taxa are each typified by their zoospore morphology: Chytridiomycete zoospores have one posterior naked flagellum, zoospores of hyphochytriomycetes have an anterior flagellum that bears flagellar hairs, and oomycete spores have both. Despite their resemblance, it was shown mainly on the basis of biochemical, ultrastructural, and SSU rRNA data that the mastigomycetes are polyphyletic and therefore an artificial group (Vogel 1965; Léjohn 1974; Taylor 1978; Cavalier-Smith 1981; Barr 1983; Lange and Olson 1983; Bartnicki-Garcia 1987; Tehler 1988; Förster et al. 1990; Paquin et al. 1995; Van der Auwera et al. 1995): Chytridiomycetes are true fungi, while hyphochytriomycetes and oomycetes are related to the heterokont algae. The similarities between the mastigomycete taxa were thus explained as a case of convergent evolution (e.g., Barr 1983; Bartnicki-Garcia 1987). However, according to the trees in Figs. 2 and 3, true fungi, stramenopiles, and alveolates form a monophyletic clade, indicating that they all originate from the same ancestor. This means that, if the topology of these trees is correct, the shared characteristics uniting the mastigomycetes do not necessarily have to be explained by convergent evolution.

According to our interpretation, the common ancestor of the fungi, alveolates, and stramenopiles would most likely be a biflagellated organism, or at least one that has a biflagellated life stage. This follows from the following considerations. First, the ancestor of the true fungi probably was a chytridiomycete-like organism (Bartnicki-Garcia 1987; Cavalier-Smith 1987; this paper), and the presence of two kinetosomes in the chytridiomycete zoospore, one of which is nonfunctional, may be an indication of a biflagellated ancestry (Olson and Fuller 1968; Lange and Olson 1983). Second, the majority of the stramenopiles are biflagellated, and those that are not have a vestigial kinetosome as a relict of biflagellated ancestry, so the stramenopile ancestor must have been biflagellated, too (Leipe et al. 1994). Third, dinoflagellates are also biflagellated organisms. And since ciliates, which diverge first in the alveolate cluster (Gajadhar et al. 1991; Schlegel 1991; Wolters 1991; this paper), have most probably originated from a dinoflagellate ancestor (Lee and Kugrens 1992), all alveolates originate from biflagellated organisms.

The phylogeny in Figs. 2 and 3 suggests that this biflagellated fungi-alveolate-stramenopile ancestor may have been a zoosporic fungus. Apart from the fact that the fungal ancestor probably was chytridiomycete-like (see previous section), one could imagine the alveolate-stramenopile ancestor to be a zoosporic fungus, too. Indeed, apart from oomycetes and hyphochytriomycetes, stramenopiles also comprise the early diverging thraustochytrids (Cavalier-Smith et al. 1994). These could be derived from zoosporic fungi since they resemble them so well that they have long been classified as such. Moreover, according to Leipe et al. (1994), dinoflagellates probably acquired their plastids independently from the heterokont algae, although both possess chlorophyll a + c, suggesting that the ancestor of alveolates and stramenopiles was nonphotosynthetic. If the proposed fungi-alveolate-stramenopile ancestor was indeed a biflagellated zoosporic fungus, the mastigomycetes would be paraphyletic rather than polyphyletic.

Certainly the hypothesis of a zoosporic fungus being the ancestor of the true fungi, stramenopiles, and alveolates needs further evaluation. First of all, it still remains unclear whether the stramenopile-alveolate ancestor could have been heterotrophic. According to Cavalier-Smith et al. (1994), this is far from evident and the phylogenetic position of the stramenopiles relative to other algal groups such as haptophytes and dinoflagellates, which also contain chlorophyll a + c, needs further investigation. However, even if the stramenopile-alveolate ancestor was photosynthetic, the common ancestor with the fungi could still have been a zoosporic fungus. Second, the shape of the mitochondrial cristae is often used as an indicator of phylogenetic relationships. And while alveolates and stramenopiles have tubular cristae, the true fungi have flattened ones (Cavalier-Smith 1981). However, it is possible that tubular cristae are a shared derived character of the stramenopiles and alveolates (Taylor 1978), which would not contradict their sister relationship with the true fungi. Third, other LSU rRNA sequences will have to be determined in the future to evaluate the correctness of the phylogeny as encountered in Figs. 2 and 3. In SSU rRNA trees, for instance, not the alveolate-stramenopile cluster but the Metazoa appears as the closest neighbor of the true fungi (Wainright et al. 1993; Kumar and Rzhetsky 1996). Although the rooting of the SSU + LSU rRNA trees poses a problem, as mentioned in the Results section, the Fungi are never found as the sister group of the Metazoa, as shown in Fig. 4. The fungal-metazoan relationship is proposed in several studies, but the exact phylogeny among the various crown taxa is still a matter of debate, and results seem to depend on the molecules and methods that are used (Hasegawa et al. 1985; Gouy and Li 1989; Baldauf and Palmer 1993; Wainright et al. 1993; Nikoh et al. 1994; Sidow and Thomas 1994; Gupta 1995; Kumar and Rzhetsky 1996). Also, because many of these analyses do not include crown protists, it cannot be ruled out that the latter are more closely related to either Chlorobionta, or Metazoa, or Fungi, than these three are to each other.

In conclusion, the SSU + LSU rRNA data confirm that *Blastocladiella emersonii* is a true fungus and indi-

cate that it forms the first branch within the fungal cluster. Furthermore, the SSU + LSU rRNA phylogeny that is presently obtained indicates a common ancestry for the fungi, alveolates, and stramenopiles. The fact that the alveolate-stramenopile cluster as well as the true fungal cluster contains zoosporic fungi which are very much alike suggests that the postulated common ancestor was a biflagellated zoosporic fungus. If this hypothesis is correct, it would make the zoosporic fungi a paraphyletic rather than a polyphyletic group.

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