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Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale

Kerry O'Donnell [1]

Microbial Properties Research Unit, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Agricultural Research Service, 1815 North University Street, Peoria, Illinois, USA 61604-3999

François M. Lutzoni

Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, Illinois, USA 60605-2496

Todd J. Ward

Microbial Properties Research Unit, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Agricultural Research Service, 1815 North University Street, Peoria, Illinois, USA 61604-3999

Gerald L. Benny

Department of Plant Pathology, 1453 Fifield Hall, University of Florida, Gainesville, Florida, USA 32611-0680

Abstract: Mucorales (Zygomycota) are ubiquitous, morphologically simple terrestrial fungi that are united taxonomically by possession of a coenocytic mycelium upon which nonmotile mitotic spores are produced asexually in uni- to multispored sporangia, and zygospores, where known, are produced following fusion of sexually compatible hyphae. Here we report the first comprehensive phylogenetic analysis of essentially all genera of Mucorales (63 species, 54 genera and 13 families) based on partial nucleotide sequence data of nuclear small subunit (18S) ribosomal RNA and nuclear large subunit (28S) ribosomal RNA genes, translation elongation factor-1α gene exons, and a morphological data set consisting of 1826, 389, 1092 and 11 characters, respectively. Individual and combined data sets were analyzed by unequally weighted maximum parsimony (MP) to investigate evolutionary relationships among and within mucoralean families. A *Micromucor–Umbelopsis* clade, traditionally included in the Mortierellaceae, was identified as the basal sister-group to all other Mucorales. A major discovery of this study is that traditional family-level classification schemes for this order appear to be highly artificial as evidenced by polyphyly of four of the seven families containing two or more genera. As presently circumscribed, these four families include 83% of the Mucorales. In addition, the largest and best known genera, *Mucor* and *Absidia*, were resolved as polyphyletic. The results provide a robust phylogenetic framework for additional evolutionary studies of the Mucorales.

Key Words: phylogeny, rDNA, systematics, translation elongation factor

INTRODUCTION

Historically, fungal systematists have been faced with the daunting task of circumscribing monophyletic taxa for morphologically simple microorganisms in the near absence of a fossil record (Berbee and Taylor 1993). However, theoretical and technical advances in molecular systematics and phylogeny reconstruction during the past decade have made it possible to test and confirm the hypotheses that the monophyletic true Fungi is defined by the synapomorphic production of chitinous cell walls and a unique lysine biosynthesis pathway (Bartnicki-Garcia 1987). While much attention has been focused on the origin of the Fungi (Bruns et al 1992, Baldauf and Palmer 1993, Wainright et al 1993, Keeling et al 2000), and evolutionary relationships among and within the so-called higher fungi represented by the Ascomycota (Spatafora and Blackwell 1994, Taylor et al 1994, Berbee 1996, Sugiyama 1998, Liu et al 1999) and Basidiomycota (Bruns et al 1992, Swann and Taylor 1993, Hibbett et al 1997, Moncalvo et al 2000), very little effort has been made to develop a general phylogenetic framework for the Chytridiomycota and Zygomycota, which occupy the basal most branches within the Fungi (Simon et al 1993, Nagahama et al 1995, Jensen et al 1998, James et al 2000, Tanabe et al 2000). Preliminary estimates suggest that the Chytridiomycota or zoosporic fungi diverged from the progenitor of the Zygomycota in the Ordovician (Berbee and Taylor 1993) and that these taxa are very likely nonmonophyletic (Bruns et al 1992, Jensen et al 1998). Perhaps reflective of their putative polyphyly, the Zygomycota comprise 10 morphologically and ecologically diverse orders that include endo- and ectomycorrhizal symbionts of vascular plants (Redecker et al 2000), obligate symbionts of

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[1] Corresponding author: email: kodonnell@sunca.ncaur.usda.gov

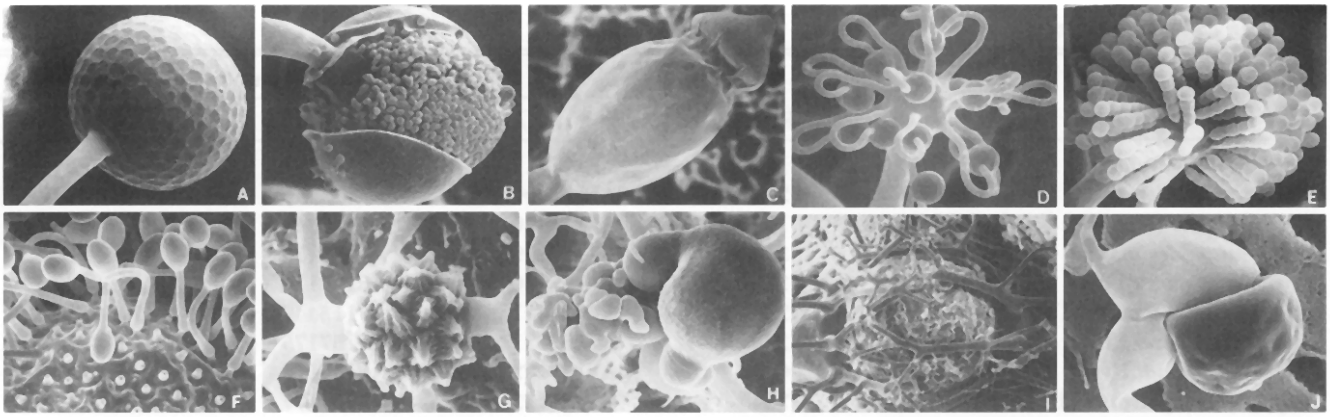

FIG. 1. Morphological diversity of asexual and sexual reproductive structures in the Mucorales and putative convergence of zygospore form in *Mortierella*. A–C. Multispored sporangia. A. *Micromucor ramannianus*. ×950. B. *Gilbertella persicaria*. ×300. C. *Pilobolus umbonatus*, 'the hat-thrower,' unique among Mucorales for its forcible discharge of sporangia. ×65. D-E. Few-spored sporangia. D. *Cokeromyces recurvatus*. ×500. E. *Syncephalastrum racemosum*, uniquely evolved uniseriate sporangia (also called merosporangia). ×625. F. Unispored sporangia of *Benjaminiella poitrasii*. ×1100. G-J. Zygospores formed during the sexual cycle. G. *Mycotypha africana*, ornate zygospore between opposed suspensors. ×375. H. *Choanephora cucurbitarum*, smooth zygospore between apposed suspensors. ×300. I. *Phycomyces blakesleeanus*, antler-like appendages on apposed suspensors. ×60. J. *Mortierella epigama*, suspensors apposed. ×500.

aquatic insect larvae (Lichtwardt 1986), entomopathogens (Jensen et al 1998), obligate mycoparasites (Tanabe et al 2000), and the ubiquitous saprobes of the order Mucorales noted for their intricately beautiful reproductive morphology (FIG. 1A–J). An eleventh order of Zygomycota, the Amoebidiales, has recently been shown to be nested within the protozoa (Benny and O'Donnell 2000, Ustinova et al 2000).

Members of the Mucorales are among the most common microorganisms encountered in soil, organic debris, dung and air, in large part because they are among the fastest growing fungi and the first to colonize and sporulate on diverse terrestrial substrates rich in simple carbohydrates. Distinguished from the Ascomycota and Basidiomycota by their coenocytic mycelia, mucoralean fungi are responsible for a number of opportunistic infections in immunocompromised humans and other animals (Rinaldi 1989), and many economically-important postharvest storage rots of fruits and vegetables (Hesseltine and Ellis 1973). This order also includes beneficial species that are used in the fermentation of the traditional east Asian soybean-based foods sufu (i.e., Chinese cheese) and tempeh, production of diverse organic compounds (Hesseltine 1991), as well as the model system organism, *Phycomyces blakesleeanus* (FIG. 1I), used in the molecular genetic dissection of intracellular sensory transduction processes (Eslava and Alvarez 1996).

The 56 genera of Mucorales have been variously sorted into 13 families based primarily on differences in asexual and sexual reproductive morphology. However, because homologies are difficult to assess for some of the phenotypically simple and microscopic characters used to circumscribe families, several conflicting family-level classification schemes have been proposed for the Mucorales (Hesseltine and Ellis 1973, Benjamin 1979, O'Donnell 1979, von Arx 1982, Hawksworth et al 1995). Here we report the first comprehensive molecular phylogenetic analysis of all 13 families, including 54 genera and 63 species of Mucorales. This analysis is based on partial nucleotide sequences from three genes and a morphological data set, and is used to assess the monophyly of mucoralean families and several of the largest genera.

MATERIALS AND METHODS

Biological materials.—Exemplars were selected from 54 of the 56 genera of Mucorales, representing all 13 families, to develop a comprehensive taxonomic data set. In addition, two to four exemplars were chosen from seven genera to make a preliminary assessment of their monophyly. The 63 strains used in this study listed in TABLE I are stored by lyophilization or in liquid nitrogen vapor at −175 C in the Agricultural Research Service Culture Collection (National Center for Agricultural Utilization Research, Peoria, Illinois). All strains were cultured in yeast-malt broth (O'Donnell et al 1997).

Molecular biology.—Methods for extracting genomic DNA from lyophilized mycelium, PCR amplification, and automated DNA sequencing have been described (O'Donnell and Cigelnik 1997). Published oligonucleotide primers were used to generate sequences of the nuclear small subunit (SSU) rDNA and domains D1 and D2 of the large subunit (LSU) rDNA (White et al 1990, O'Donnell et al

TABLE I. Species analyzed in this study

Species[a]	NRRL #	GenBank accession No.		
		18S rDNA	28S rDNA	EF-1α
Absidia blakesleeana	1304	AF157117	AF157171	AF157225
Absidia corymbifera	2982	AF113407	AF113445	AF157227
Absidia repens [T]	1336	AF113410	AF113448	AF157228
Actinomucor elegans [T]	3104	AF157119	AF157173	AF157229
Amylomyces rouxii [T]	3139	AF157120	AF157174	AF157230
Apophysomyces elegans [T]	22325	AF113411	AF113449	AF157231
Backusella circina [T]	2446	AF157121	AF157175	AF157232
Backusella ctenidia	6238	AF157122	AF157176	AF157233
Benjaminiella poitrasii [T]	2845	AF157123	AF157177	AF127234
Blakeslea trispora [T]	2456	AF157124	AF157178	AF157235
Chaetocladium brefeldii	1349	AF157125	AF157179	AF157236
Chaetocladium jonesii [T]	2343	AF157126	AF157180	AF157237
Chlamydoabsidia padenii [T]	2977	AF113415	AF113453	AF157238
Choanephora cucurbitarum	2744	AF157127	AF157181	AF157239
Circinella umbellata [T]	1351	AF157128	AF157182	AF157240
Cokeromyces recurvatus [T]	2243	AF113416	AF113434	AF157242
Cunninghamella echinulata [T]	1382	AF157130	AF157184	AF157244
Dichotomocladium elegans [T]	6236	AF157131	AF157185	AF157245
Dicranophora fulva [T]	22204	AF157132	AF157186	AF157246
Dissophora decumbens [T]	22416	AF157133	AF157187	AF157247
Echinosporangium transversale [T]	3116	AF113424	AF113462	AF157248
Ellisomyces anomalus [T]	2749	AF157134	AF157188	AF157249
Fennellomyces linderi [T]	2342	AF157135	AF157189	AF157250
Gilbertella persicaria [T]	2357	AF157136	AF157190	AF157251
Gongronella butleri [T]	1340	AF157137	AF157191	AF157252
Halteromyces radiatus [T]	6197	AF157138	AF157192	AF157253
Helicostylum elegans [T]	2568	AF157139	AF157193	AF157254
Hesseltinella vesiculosa [T]	3301	AF157140	AF157194	AF157255
Hyphomucor assamensis [T]	22324	AF157141	AF157195	AF157256
Kirkomyces cordense [T]	22618	AF157142	AF157196	AF157257
Micromucor ramannianus [T]	5844	X89435	AF113463	AF157258
Mortierella verticillata	6337	AF157145	AF157199	AF157262
Mucor circinelloides	22899	AF157129	AF157183	AF157241
Mucor mucedo [T]	3635	X89434	AF113470	AF157267
Mucor racemosus	3640	AF113430	AF113471	AF157268
Mucor recurvus	3247	AF157146	AF157200	AF157270
Mycotypha africana	2978	AF157147	AF157201	AF157271
Mycotypha microspora [T]	1572	AF157148	AF157202	AF157272
Parasitella parasitica [T]	2501	AF157149	AF157203	AF157273
Phascolomyces articulosus [T]	2880	AF157150	AF157204	AF157274
Phycomyces blakesleeanus	1465	AF157151	AF157205	AF157275
Pilaira anomala [T]	2526	AF157152	AF157206	AF157276
Pilobolus umbonatus	6349	AF157153	AF157207	AF157277
Pirella circinans [T]	2402	AF157154	AF157208	AF157278
Poitrasia circinans [T]	2546	AF157155	AF157209	AF157279
Protomycocladus faisalabadensis [T]	22826	AF157156	AF157210	AF157280
Radiomyces spectabilis [T]	2753	AF157157	AF157211	AF157281
Rhizomucor pusillus [T]	2543	AF113433	AF113474	AF157283
Rhizopus oligosporus	2710	AF157158	AF157212	AF157288
Rhizopus stolonifer [T]	1477	AF113441	AF113482	AF157290
Saksenaea vasiformis [T]	2443	AF113442	AF113483	AF157291
Spinellus fusiger [T]	22323	AF157159	AF157213	AF157292
Sporodiniella umbellata [T]	20824	AF157160	AF157214	AF157293
Syncephalastrum monosporum	22812	AF157161	AF157215	AF157294
Syncephalastrum racemosum [T]	2496	X89437	AF113484	AF157295

TABLE I. Continued

Species[a]	NRRL #	GenBank accession No. 18S rDNA	28S rDNA	EF-1α
Syzygites megalocarpus [T]	6288	AF157162	AF157216	AF157296
Thamnidium elegans [T]	2467	AF157163	AF157217	AF157297
Thamnostylum piriforme [T]	6240	AF157164	AF157218	AF157298
Thermomucor indicae-seudaticae [T]	6429	AF157165	AF157219	AF157299
Umbelopsis isabellina	1757	AF157166	AF157220	AF157300
Utharomyces epallocaulus [T]	3168	AF157168	AF157222	AF157302
Zychaea mexicana [T]	6237	AF157169	AF157223	AF157303
Zygorhynchus heterogamus [T]	1489	AF157170	AF157224	AF15730

[a] [T] = strain authentic for the type species of the genus. Strains of five genera (i.e., *Choanephora, Mortierella, Phycomyces, Pilobolus* and *Umbelopsis*) are not the type species, but they are nevertheless authentic for each genus.

1998a). Sequences of the translation elongation factor 1α (EF-1α) gene were obtained using primers from conserved exons (FIG. 2). The sequences reported in this paper have been deposited in GenBank as AF157117-AF157304 (see TABLE I). Several of the DNA sequences used in the present study were deposited in GenBank under accession numbers reported in a previous study (Gehrig et al 1996). The data matrix has been deposited in TreeBASE as accessions S528 and M774. Also included in the matrix are 11 morphological characters (see Appendix 1) that have been used widely as family-level characters within the Mucorales.

Phylogenetic analysis.—TSE, a DOS text editor software package (SemWare, Marietta, Georgia) and Sequencher versions 3.0 and 4.0.5 (Gene Codes Corp., Ann Arbor, Michigan) were used to prepare the aligned DNA sequences. Alignments of the nuclear SSU 18S and LSU 28S rDNA were inspected for the presence of ambiguously aligned regions caused by the insertion of gaps. These regions were unequivocally coded to form a new set of characters replacing these regions in the phylogenetic analyses. Each of these characters, resulting from the coding of ambiguous regions, were subjected to a specific step matrix taking into account the optimal number of steps to transform one ambiguous sequence into another. Coding and the elaboration of symmetric step matrices for each of these coded regions was generated using the program INAASE 0.2c1 (Lutzoni et al 2000).

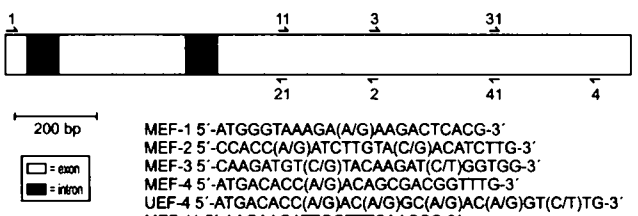

FIG. 2. Map of EF-1α gene in *Absidia glauca* showing exons and introns. The position of PCR and sequencing primers are indicated by numbered half-arrows (→). Conserved mucoralean sequences in the EF-1α gene of *A. glauca* and *Mucor circinelloides* f. *lusitanicus* (GenBank accessions nos. X54730 and J02605/M16352) were used to design oligonucleotides.

Unambiguous portions of the alignments were subjected to symmetric step matrices taking into consideration the frequency of each class of possible changes as described in Fernandez et al (1999). Step matrices were generated for the 18S and 28S rDNA, and first, second and third position of the EF-1α gene exons. Changes among morphological character states were equally weighted in all phylogenetic analyses. Maximum parsimony analyses were performed on individual and combined data sets for the 63 taxon data matrix using PAUP* 4.0b4a for the Macintosh (Swofford 1998).

Conflicts among partitions were first detected by inspecting bootstrap scores above 70% (Mason-Gamer et al 1996). If two bootstrap analyses, derived from two different partitions (A and B), provided support ≥ 70% for two different phylogenetic relationships, this was interpreted as a potential incongruence between the two partitions. The significance of this partition heterogeneity was first tested using the incongruence length difference test (Farris et al 1994) as implemented in PAUP*. The incongruence was also tested by building a constrained topology representing only internodes with ≥ 70% bootstrap support using partition A. A phylogenetic search was performed on partition B using this constraint derived from partition A. If the length of this most-parsimonious constrained tree was found to be shorter than at least 10 of 1000 sub-optimal trees derived from the unconstrained bootstrap analysis on partition B, based on 1000 bootstrap replicates where only one tree was kept for each resampled data set, two partitions were interpreted as being not significantly incongruent and were therefore combined. For this test, the lengths of the trees generated by the bootstrap analysis were calculated from the original data set. For incongruence between two partitions to be interpreted as significant, the two reciprocal analyses would have to reject the null hypothesis at $P < 0.01$.

The combined analysis was performed using the heuristic search option of PAUP* with 1000 random addition sequences and tree bisection-reconnection branch swapping. Sequences of the Mortierellaceae (i.e., *Mortierella, Disso-*

phora and *Echinosporangium*) were selected for rooting the tree by the outgroup method based on published phylogenetic analyses of more inclusive data sets (Tanabe et al 2000, Gehrig et al 1996) which suggest that this family and the Mucorales may not form a monophyletic group. Clade stability was assessed by 750 parsimony bootstrap replications implemented in PAUP*, using 10 random addition sequences per replicate. The historical pattern of evolution for each of 11 characters in the morphological data set was examined using MacClade version 3.08a (Maddison and Maddison 1999). In addition, the ancestral character states for asexually produced sporangia and sexual reproductive mode were reconstructed using both MacClade and Discrete version 1.01b (Pagel 1994, 1997, 1999), which employ maximum parsimony and maximum likelihood methods, respectively.

The reconstruction of ancestral character states using maximum parsimony was implemented using a 1:1 gain to loss cost ratio. For the reconstruction of ancestral character states using Discrete, branch lengths (i.e., rates of nucleotide substitutions per site) of the most-parsimonious tree derived from the combined analysis were estimated using maximum likelihood. The maximum likelihood ratio test and the most-parsimonious tree derived from the combined analysis were used to select an evolutionary model and estimate all parameters needed to calculate these branch lengths (Huelsenbeck and Crandall 1997). A four-parameter evolutionary model reflecting the step matrix implemented on the third position of the EF-1α gene, estimated nucleotide frequencies, and site-to-site rate heterogeneity following a gamma distribution with three rate categories, were the optimal settings to generate branch lengths for the Discrete analysis.

In order to determine if instances of nonmonophyletic families and genera, indicated by the phylogenetic analysis, could be the result of sampling error, phylogenetic searches were performed as described above to find the length of the most-parsimonious trees under one-internode constraints of monophyly for each instance of nonmonophyly in the unconstrained MP trees. If the length of the most-parsimonious constrained tree was found to be equal to or less than 1% of trees derived from 1000 unconstrained bootstrap replicates as described above for detecting heterogeneity among partitions, the difference in tree length between the unconstrained MP trees and the constraint trees was interpreted as being not significantly different, and the monophyly of this taxon could not be rejected.

RESULTS

We constructed comprehensive molecular and morphological data sets for 63 species representing 54 of the 56 genera and all 13 families of Mucorales sensu Hawksworth et al (1995). The aligned partial nuclear SSU 18S rDNA, nuclear LSU 28S rDNA, EF-1α gene exons, and morphological data sets consisted of 1826, 389, 1092 and 11 characters, respectively. Although several EF-1α gene amplicons needed to be cloned to obtain readable sequence data, paralogs were never detected. A comparison of bipartitions with bootstrap scores ≥ 70% for trees generated from different data sets did not reveal any potential heterogeneity among data sets except between the two data sets with the most resolving power, i.e., between the SSU 18S rDNA and EF-1α gene. To determine if the incongruence between these two data sets was significant, we first implemented the incongruence length difference test (Farris et al 1994). These two partitions were found to be incongruent at $P = 0.01$. Because it has been shown that when the incongruence length difference test receives a P value greater than or equal to 0.01, combining the data improves or does not reduce phylogenetic accuracy (Cunningham 1997), a second homogeneity partition test was implemented using constrained topologies as described in the Materials and Methods. When imposing a constraint derived from the EF-Iα gene for a phylogenetic search on the 18S rDNA, the most-parsimonious constrained tree was found to be significantly longer than expected due to sampling error. When the reciprocal test was performed using a constrained topology derived from the 18S rDNA for a phylogenetic search on the EF-1α gene, 21 trees from the bootstrap analyses were found to be longer than the constrained tree, meaning that the conflict could result from sampling error (Lutzoni 1997, Lutzoni and Barker 1999). Based on these results the individual data sets were combined and analyzed phylogenetically. The maximum parsimony analysis of the combined data set conducted with PAUP* (Swofford 1998) yielded a single most-parsimonious tree (MPT, FIG. 3) 6423.72 steps in length (consistency index excluding uninformative characters, CI = 0.3304; retention index = 0.5673; rescaled consistency index = 0.2081). The MPT provides a completely resolved and well supported estimate of mucoralean phylogeny. As expected, compared with analyses of the individual partitions (data not shown), there was an increase in bootstrap support and run times were faster when the combined data set was used.

Based on results of more inclusive phylogenetic analyses that indicate the Mortierellaceae may be a sister-group to the Endogonales (Gehrig et al 1996), rather than nested within the Mucorales where it has been classified traditionally (Benjamin 1979), sequences of the three mortierellaceous taxa (i.e., *Mortierella, Dissophora* and *Echinosporangium*) tested as outgroups proved to be excellent for rooting the mucoralean tree (FIG. 3). Furthermore, the root of the outgroup in all analyses always joined the tree with a strongly supported *Micromucor–Umbelopsis* clade forming the most basal branch within the Mucorales (bootstrap = 100%, clade 1). Of the remaining core Mucorales (bootstrap = 100%), phylogenetic results

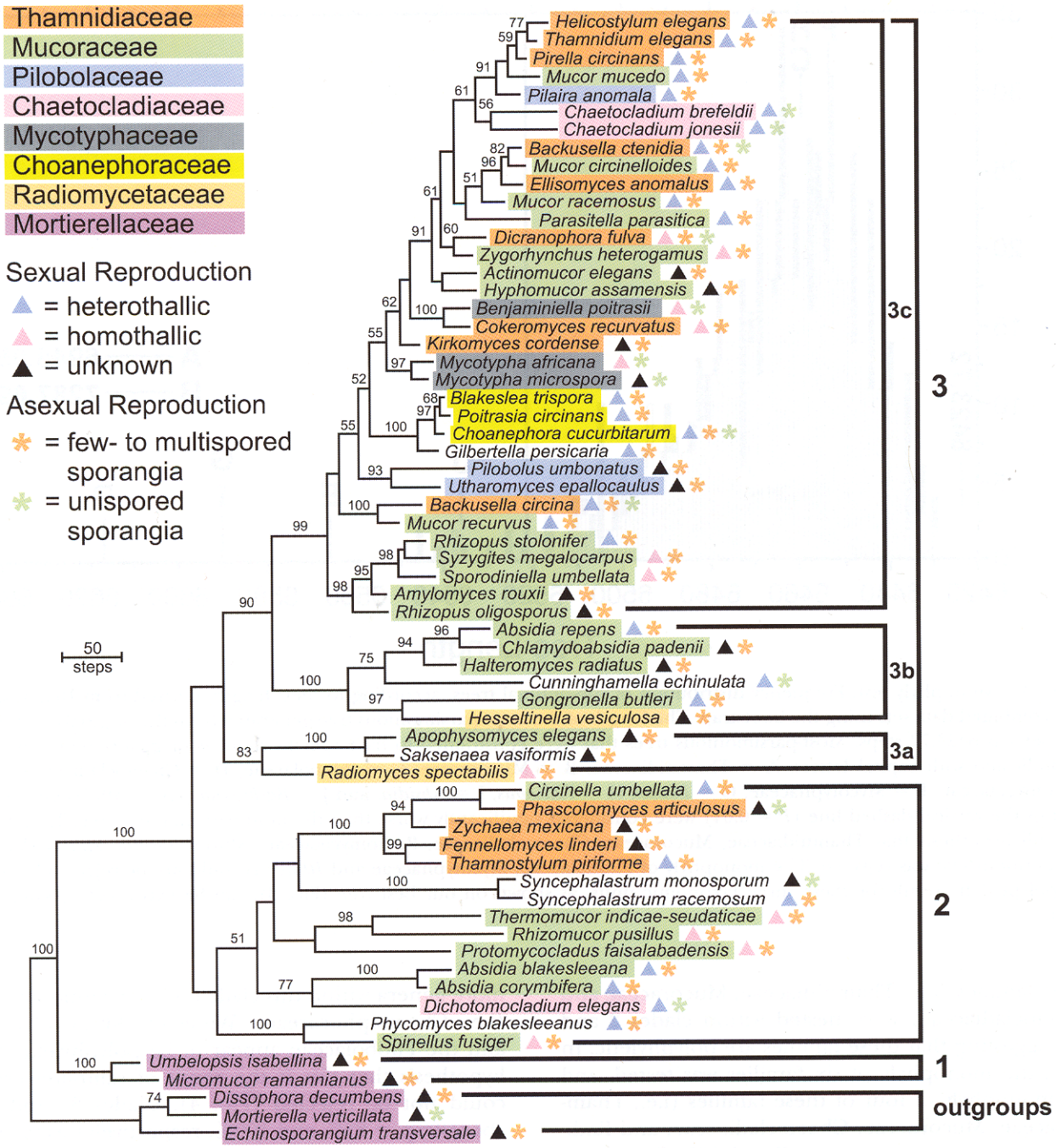

FIG. 3. Single most-parsimonious unequally weighted phylogram of 6423.72 steps depicting phylogenetic relationships for 63 species representing 54 (virtually all) genera of Mucorales (Zygomycota) based on the combined data set of 784 parsimony-informative characters. Sequences of three mortierellaceous taxa were used to root the tree by the outgroup method. Taxa in the eight families that contain two or more genera are color-coded. The six taxa that are not color-coded are classified in monogeneric families. Symbols by taxon names indicate sexual reproductive mode or asexually produced sporangial types according to the legend. Bootstrap values ≥50% are indicated on nodes.

support a sister-group relationship between the early diverging clades 2 and 3. Neither of these clades received bootstrap support greater than 50% because of the unstable placement of clade 3a that was oscillating between clades 2 and 3.

Color-coding of taxa in the eight families that contain two or more genera suggests that many clades of core Mucorales identified here cut across family boundaries established in previous classifications (FIG. 3). For example, representatives of the same

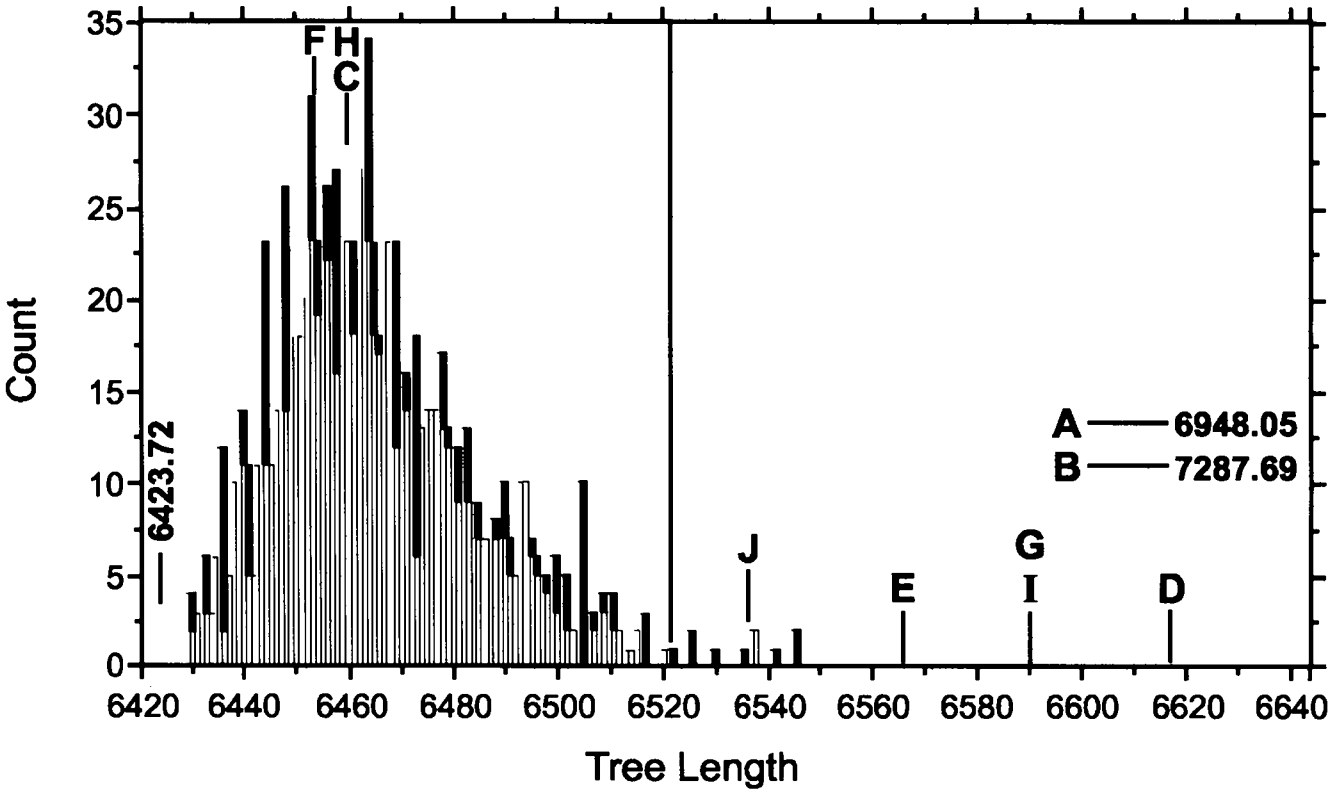

FIG. 4. Monophyly test. Frequency distribution of sub-optimal trees, according to their length, derived from 1000 resampled combined data sets using the bootstrap. The length of the most-parsimonious tree generated from the original combined data set was 6423.72 steps. Most-parsimonious trees that were constrained to have specific families or genera monophyletic are indicated with capital letters: A = Thamnidiaceae, B = Mucoraceae, C = Pilobolaceae, D = Chaetocladiaceae, E = Radiomycetaceae, F = Mycotyphaceae, G = *Mucor*, H = *Rhizopus*, I = *Absidia*, and J = *Backusella*. Trees with length to the right of the vertical dashed line ($P = 0.01$) were considered significantly worse than the most-parsimonious tree. Therefore, the null hypothesis that Thamnidiaceae, Mucoraceae, Chaetocladiaceae, Radiomycetaceae, *Mucor*, *Absidia*, and *Backusella* are monophyletic was rejected. The monophyly of Pilobolaceae, Mycotyphaceae and *Rhizopus* could not be rejected because the polyphyletic result for these three taxonomic units, as shown on our best tree (FIG. 3), can be explained by sampling error.

three families (i.e., Thamnidiaceae, Mucoraceae and Chaetocladiaceae) were nested within clades 2 and 3c, suggesting that these families are polyphyletic. In total, the monophyly of six families was tested, and the monophyly of four of these families (i.e., Thamnidiaceae, Mucoraceae, Chaetocladiaceae, and Radiomycetaceae) was rejected at $P \ll 0.001$ (FIG. 4). As currently circumscribed these four families include 83% of the Mucorales, excluding the Mortierellaceae, and 15% of the entire Zygomycota diversity. The null hypothesis that the genera *Mucor*, *Absidia* and *Backusella* are monophyletic was rejected at $P \leq 0.005$. However, monophyly of *Rhizopus* could not be rejected with the data included in this study.

Phylogenetic analyses identified clade 3 as the most evolutionarily and taxonomically diverse lineage, containing approximately three-fourths of the core Mucorales and representatives of 10 of the 13 families. However, only one of the seven families with two or more genera within this clade is clearly monophyletic (i.e., Choanephoraceae). While the Mycotyphaceae and the Pilobolaceae appear to be polyphyletic, the hypothesis that this is the result of sampling error could not be rejected (FIG. 4). Clades 3a and 3b (bootstrap = 83% and 100%, respectively) each contain representatives of three families, including Mucoraceae and Radiomycetaceae, while seven different families were nested within clade 3c (bootstrap = 99%). Overall, many of the strongly supported lineages conflict with traditional family-level classification of the Mucorales.

This phylogenetic study provides a framework for examining the historical pattern of evolution for morphological characters and reproductive modes. With the exception of trophocyst formation, which is synapomorphic for the Pilobolaceae excluding *Pilaira*, the morphological characters exhibited homoplastic patterns of evolution on the most-parsi-

Erratum

For the article "Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale" by Kerry O'Donnell, Francois M. Lutzoni, Todd J. Ward and Gerald L. Benny, which appeared in Mycologia 93(2), 2001, Fig. 4 was printed incorrectly. The correct figure and its legend appear below.

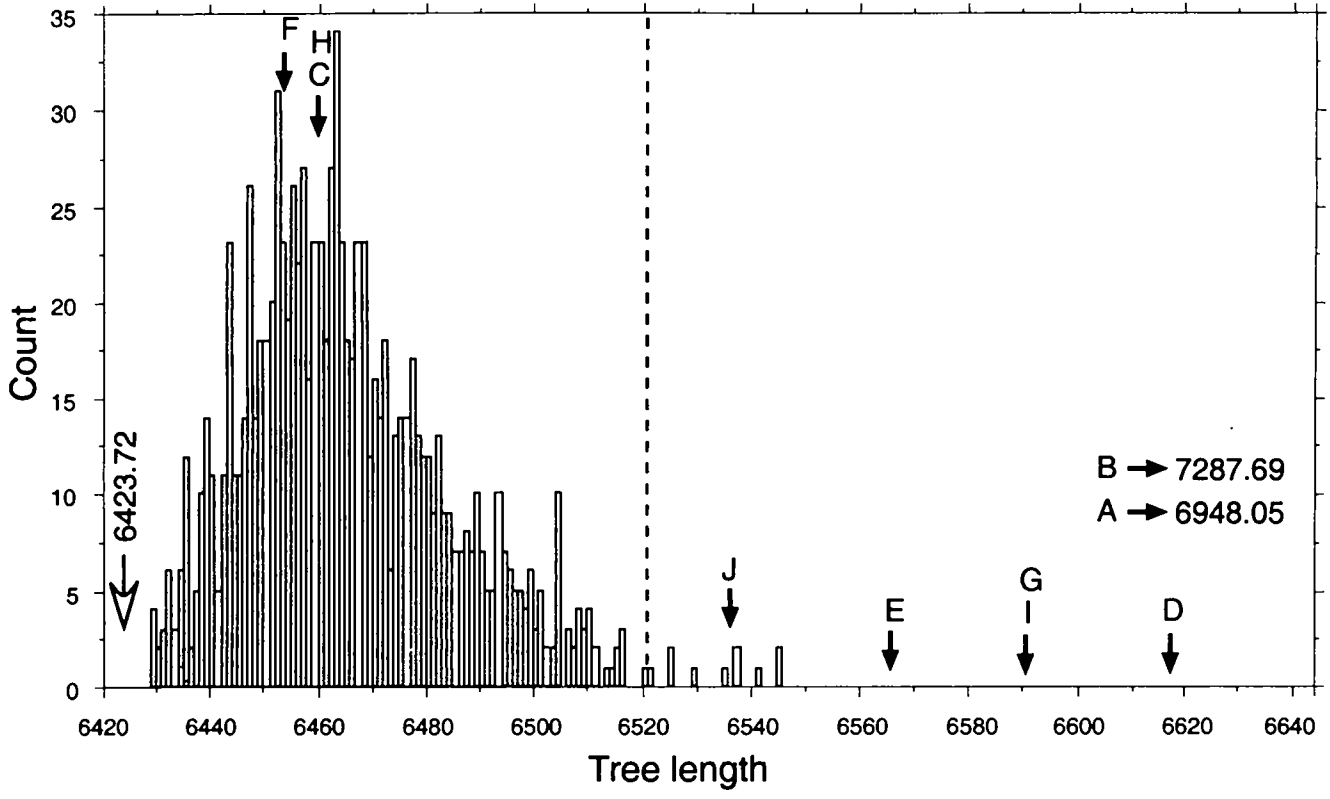

Fig. 4. Monophyly test. Frequency distribution of sub-optimal trees, according to their length, derived from 1000 resampled combined data sets using the bootstrap. The length of the most-parsimonious tree generated from the original combined data set was 6423.72 steps. Most-parsimonious trees that were constrained to have specific families or genera monophyletic are indicated with capital letters: A = Thamnidiaceae, B = Mucoraceae, C = Pilobolaceae, D = Chaetocladiaceae, E = Radiomycetaceae, F = Mycotyphaceae, G = *Mucor*, H = *Rhizopus*, I = *Absidia*, and J = *Backusella*. Trees with length to the right of the vertical dashed line ($P = 0.01$) were considered significantly worse than the most-parsimonious tree. Therefore, the null hypothesis that Thamnidiaceae, Mucoraceae, Chaetocladiaceae, Radiomycetaceae, *Mucor*, *Absidia*, and *Backusella* are monophyletic was rejected. The monophyly of Pilobolaceae, Mycotyphaceae and *Rhizopus* could not be rejected because the polyphyletic result for these three taxonomic units, as shown on our best tree (Fig. 3), can be explained by sampling error.

monious tree. For instance, asexual reproductive sporangia, which has been widely used as a character in family-level classifications within the Mucorales (Benjamin 1979, von Arx 1982, O'Donnell 1979, Hawksworth et al 1995), had a consistency index of 0.08 on the most-parsimonious tree (FIG. 3) Using either MacClade or Discrete, few- to multispored sporangia was identified as the plesiomorphic condition for this character, indicating that unispored sporangia were independently derived several times during mucoralean evolution. A homoplastic pattern of evolution (consistency index = 0.14) was also indicated for sexual reproductive mode (FIG. 3), which has not been used in family-level classifications within the Mucorales, but is an important part of the biology of these organisms. Maximum parsimony analysis conducted with MacClade indicated that heterothallism was the plesiomorphic character state for mucorlaean fungi. However, 97% of the probability from a maximum likelihood analysis conducted with Discrete was associated with homothallism as the plesiomorphic condition.

DISCUSSION

Molecular and morphological data were used to investigate evolutionary relationships among and within all 13 morphologically-defined families of the Mucorales (Benjamin 1979, O'Donnell 1979, von Arx 1982, Hawksworth et al 1995). Dense taxon sampling was possible because exemplars of 54 of the 56 currently recognized genera easily grow and sporulate in pure culture. As such, the phylogeny reconstruction provides the first detailed information of the evolutionary history of the Mucorales. One of the most important finding to emerge from this study is that the most-parsimonious tree (MPT) shows little similarity to current mucoralean family-level classification (FIG. 3).

Of the few traditional taxonomic groupings supported by the phylogeny (FIG. 3), Choanephoraceae–Gilbertellaceae are unique among the Mucorales in that they possess distinctive morphological synapomorphies, including a persistent sporangial wall that splits in half at maturity (FIG. 1B) and sporangiospores with polar setae. A monotypic Gilbertellaceae with opposed zygospore suspensors was only recently segregated from the other Choanephoraceae (Benny 1991) that produce zygospores between apposed suspensors (FIG. 1H). However, results of the phylogeny indicate that the transformation from opposed to apposed zygospore suspensors is homoplasious, having occurred independently at least three times.

The reconstruction of ancestral character states using maximum parsimony (Swofford 1998) indicated that homothallic species were derived from heterothallic ancestors, which is consistent with previous reports from other groups of fungi (Yun et al 1999, O'Donnell 2000) and algae (Coleman 1999). However, maximum likelihood analysis (Pagel 1997) strongly supported homothallism as the plesiomorphic condition for mucoralean fungi, which suggests that the mode of sexual reproduction in mucoralean fungi may have a different genetic architecture than that reported for other groups. This result is incongruent with population genetics models which demonstrate that evolution from homothallism to heterothallism is unlikely (Nauta and Hoekstra 1992). However, the mode of sexual reproduction is unknown for 22 of the taxa in this study, including all three members of the Mortierellaceae used to root the tree. Given this fact, and the incongruence between reconstructions based on parsimony and likelihood methods, the plesiomorphic character state for sexual reproductive mode in the mucoralean fungi remains to be resolved.

Our results add to a growing number of studies that have demonstrated the utility of EF-1α nucleotide or protein sequences for molecular phylogenetics (Cho et al 1995, Baldauf and Doolittle 1997, O'Donnell et al 1998b). Of the loci we sequenced, EF-1α was the most informative with 441 parsimony-informative sites. Although paralogous EF-1α genes have been reported for *Mucor circinelloides* f. *lusitanicus* (as *M. racemosus,* et al 1986) and honeybees (Danforth and Ji 1998), duplicated EF-1α genes were not encountered in the present study. Moreover, phylogenetic analyses that included the three paralogous EF-1α genes of *M. circinelloides* demonstrated that they have a monophyletic origin (data not shown).

Results of the present study have important implications for developing a classification that reflects the evolutionary history of the Mucorales. Homoplastic evolution of morphologically simple asexual reproductive characters has contributed significantly to the difficulty in circumscribing monophyletic mucoralean families. Clearly, highly polyphyletic families such as the Mucoraceae and Thamnidiaceae, which collectively account for close to two-thirds of the ingroup, were circumscribed by characters with homoplasious distributions on the MPT such as exclusive production of mostly globose-to-pyriforme multispored sporangia (Mucoraceae) or few-spored to unispored sporangia with or without multispored sporangia (Thamnidiaceae) (Benjamin 1979). The extraordinary convergence of asexual reproductive morphologies implies that these characters have experienced strong selective pressure associated with limited spore dispersal mechanisms (Ingold and Zoberi 1963).

Mucor with its putative plesiomorphic multispored sporangia and opposed zygospore suspensors has been generally regarded as the most ancestral member of the Mucorales (Hesseltine and Ellis 1973); however, results of the phylogeny clearly indicate that the type species, *M. mucedo,* is one of the most derived mucoralean taxa as evidenced by its terminal position within clade 3c. In contrast to *Mucor,* polyphyly of *Absidia* can be eliminated easily by recognizing the two *Absidia* species nested within clade 2 as *Mycocladus* (Hesseltine and Ellis 1964). Because life-threatening infections in humans caused by thermophilic mucoralean species such as *Absidia* (*Mycocladus*) *corymbifera* are increasing in importance due to the dramatic rise in the number of immunosuppressed and immunocompromised patients (Rinaldi 1989), greater knowledge of the systematics and evolutionary relationships of these medically important fungi may have important public health implications for more accurately predicting differences in their resistance to antifungal drugs.

While the trend in recent years has been to circumscribe monogeneric families within the Mucorales (e.g., Saksenaceae, Cunninghamellaceae, Phycomycetaceae and Gilbertellaceae), the phylogeny clearly shows that these taxa add little to the classification. Paramount among the difficulties of trying to translate the phylogeny into Linnaean categories is that a number of strongly supported clades cannot be defined by morphological apomorphies, and those that can would result in a cumbersome classification due to the proliferation of family names (Hibbett and Donoghue 1998). For these reasons, we have chosen to use the phylogeny to represent the classification in tree format (Vilgalys and Hibbett 1993) by numbering the three major clades of Mucorales for the purpose of the present discussion and by giving informal names only to those strongly supported clades discussed in the present study (e.g., *Micromucor–Umbelopsis* clade, core Mucorales = clades 2 + 3, choanephoraceous clade, *Absidia* clade = 3b). Higher taxa recently proposed for these fungi, including the subclass Mucoromycetidae for the Mucorales and Mortierellales (Cavalier-Smith 1998), are premature given the uncertain phylogenetic relationship of these taxa. Furthermore, the proposed monogeneric order Cunninghamellales and its subclass Meromycetidae that includes the unispored Cunninghamellaceae (Cavalier-Smith 1998) is strongly rejected because the type species, *Cunninghamella echinulata,* is nested within the *Absidia* clade (= 3b, bootstrap = 100%).

In summary, phylogenetic analysis of three genes and a morphological data set constructed for essentially all genera of Mucorales has provided the first robust phylogenetic framework for testing traditional morphology-based classification schemes and for elucidating patterns of genotypic and phenotypic diversification within this important group of microfungi. The most important result to emerge from the present phylogenetic study is that most of the monophyletic groupings identified are not reflected in family-level classifications proposed in the past. In part because morphological apomorphies are not currently available for a number of the strongly supported clades, we anticipate that the aligned DNA sequence data base, like similar electronically portable data bases being developed for morphologically simple fungi (Bruns et al 1998), will find utility beyond its systematic value as a tool for studying these ecologically diverse fungi and for the identification of unknown isolates of importance to agriculture, industry and medicine.

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LITERATURE CITED

Baldauf SL, Doolittle WF. 1997. Origin and evolution of the slime molds (Mycetozoa). Proc Natl Acad Sci USA 94: 12007–12012.

———, Palmer JD. 1993. Animals and fungi are each other's closest relatives: congruent evidence from multiple proteins. Proc Natl Acad Sci USA 90:11558–11562.

Bartnicki-Garcia S. 1987. The cell wall: a crucial structure in fungal evolution. In: Rayner ADM, Brasier CM, Moore D, eds. Evolutionary biology of the fungi. Cambridge: Cambridge University Press. p 389–403.

Benjamin, R. K. 1979. Zygomycetes and their spores. In: Kendrick B, ed. The whole fungus. The sexual-asexual synthesis. Vol. 2. Ottawa: Museums of Canada, and the Kananaskis Foundation. p 573–616.

Benny GL. 1982. Zygomycetes. In: Parker SP, ed. *Synopsis and classification of living organisms,* Vol. 1. New York: McGraw Hill Book Company, p 184–195.

———. 1991. Gilbertellaceae, a new family of the Mucorales (Zygomycetes). Mycologia 83:150–157.

———, O'Donnell K. 2000. *Amoebidium parasiticum* is a protozoan, not a Trichomycete. Mycologia 92:1133–1137.

Berbee ML. 1996. Loculoascomycete origins and evolution

of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol Biol Evol 13:462–470.

———, Taylor JW. 1993. Dating the evolutionary radiations of the true fungi. Can J Bot 71:1114–1127.

Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Mol Ecol 7:257–272.

———, 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.

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

Cho S, Mitchell A, Regier JC, Mitter C, Poole RW, Friedlander TP, Zhao S. 1995. A highly conserved nuclear gene for low-level phylogenetics: elongation factor-1α recovers morphological-based tree for heliothine moths. Mol Biol Evol 12:650–656.

Coleman AW. 1999. Phylogenetic analysis of "Volvocacae" for comparative genetic studies. Proc Natl Acad Sci USA 96:13892–13897.

Cunningham CW. 1997. Can three incongruence tests predict when data should be combined? Mol Biol Evol 14: 733–740.

Danforth BN, Ji S. 1998. Elongation factor-1α occurs as two copies in bees: implications for phylogenetic analysis of EF-1α sequences in insects. Mol Biol Evol 15:225–235.

Eslava AP, Alvarez MI. 1996. Genetics of *Phycomyces*. In: Bos CJ, ed. Fungal genetics: principles and practice. New York: Marcel Dekker, Inc. p 385–406.

Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. Cladistics 10:315–319.

Fernández AF, Lutzoni FM, Huhndorf SM. 1999. Teleomorph-anamorph connections: the new pyrenomycetous genus *Carpoligna* and its *Pleurothecium* anamorph. Mycologia 91:251–262.

Gehrig H, Schüßler A, Kluge M. 1996. *Geosiphon pyriforme*, a fungus forming endocytobiosis with *Nostoc* (Cyanobacteria), is an ancestral member of the Glomales: evidence by SSU rRNA analysis. J Mol Evol 43:71–81.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. 1995. Ainsworth & Bisby's dictionary of the fungi. 8th ed. Wallingford, UK: CAB International. 616 p.

Hesseltine CW. 1991. Zygomycetes in food fermentations. Mycologist 5:162–169.

———, Ellis JJ. 1964. The genus *Absidia*: *Gongronella* and cylindrical-spored species of *Absidia*. Mycologia 56: 568–601.

———, ———. 1973. Mucorales. In: Ainsworth GC, Sparrow FK, Sussman AF, eds. The fungi. Vol. IVB. New York: Academic Press. p 187–217.

Hibbett DS, Donoghue MJ. 1998. Integrating phylogenetic analysis and classification in fungi. Mycologia 90:347–356.

———, Pine EM, Langer E, Langer G, Donoghue MJ. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc Natl Acad Sci USA 94:12002–12006.

Huelsenbeck JP, Crandall KA. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annu Rev Ecol Syst 28:437–466.

Ingold CT, Zoberi MH. 1963. The asexual apparatus of Mucorales in relation to spore liberation. Trans Br Mycol Soc 46:115–134.

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.

Jeffries P, Young TWK. 1994. *Interfungal parasitic relationships*. Wallingford, United Kingdom: CAB International. 296 p.

Jensen AB, Gargas A, Eilenberg J, Rosendahl S. 1998. Relationships of the insect-pathogenic order Entomophthorales (Zygomycota, Fungi) based on phylogenetic analyses of nuclear small subunit ribosomal DNA sequences (SSU rDNA). Fungal Genet Biol 24:325–334.

Keeling PJ, Luker MA, Palmer JD. 2000. Evidence from beta-tubulin phylogeny that microsporidia evolved from within the fungi. Mol Biol Evol 17:23–31.

Kirk PM. 1984. A monograph of the Choanephoraceae. Mycol Papers 152:1–61.

———, Benny GL. 1980. The genus *Utharomyces* Boedijn (Pilobolaceae: Zygomycetes). Trans Br Mycol Soc 75: 123–131.

Lichtwardt RW. 1986. The Trichomycetes, fungal associates of arthropods. New York: Springer-Verlag. 343 p.

Linz JE, Katayama C, Sypherd PS. 1986. Three genes for the elongation factor EF-1 alpha in *Mucor racemosus*. Mol Cell Biol 6:593–600.

Liu YJ, Whelen S, Hall BD. 1999. Phylogenetic relationships among Ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol 16:1799–1808.

Lutzoni FM. 1997. Phylogeny of lichen- and non-lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. Syst Biol 46:373–406.

———, Barker FK. 1999. Sampling confidence envelopes of phylogenetic trees for combinability testing: a reply to Rodrigo. Syst Biol 48:596–603.

———, Wagner P, Reeb V. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst Biol 49:628–651.

Maddison WP, Maddison DR. 1999. MacClade Version 3.08. Sunderland, Massachusetts: Sinauer Associates.

Mason-Gamer RJ, Kellogg EA. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst Biol 45:524–545.

Moncalvo J-M, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst Biol 49:278–305.

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

Nauta MJ, Hoekstra RF. 1992. Evolution of reproductive systems in filamentous ascomycetes. I. Evolution of mating types. Heredity 68:405–410.

O'Donnell K. 1979. Zygomycetes in Culture. Athens, GA: Department of Botany, University of Georgia. 257 p.

———. 2000. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. Mycologia 92: 919–938.

———, Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol Phylogenet Evol 7:103–116.

———, ———, Benny GL. 1998a. Phylogenetic relationships among the Harpellales and Kickxellales. Mycologia 90:624–639.

———, ———, Weber NS, Trappe JS. 1997. Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia 89:48–65.

———, Kistler HC, Cigelnik E, Ploetz RC. 1998b. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci USA 95:2044–2049.

Pagel M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proc Roy Soc London B 255:37–45.

———. 1997. Inferring evolutionary processes from phylogenies. Zool Scr 26:331–348.

———. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst Biol 48:612–622.

Redecker D, Morton JB, Bruns TD. 2000. Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). Mol Phylogenet Evol 14:276–284.

Rinaldi MG. 1989. Zygomycosis. Infect Dis Clin N Am 3:19–41.

Simon L, Bousquet J, Lévesque RC, Lalonde M. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67–69.

Spatafora JW, Blackwell M. 1994. The polyphyletic origins of ophiostomatoid fungi. Mycol Res 98:1–9.

Sugiyama J. 1998. Relatedness, phylogeny, and evolution of the fungi. Mycoscience 39:487–511.

Swann EC, Taylor JW. 1993. Higher taxa of Basidiomycetes: an 18S rRNA gene perspective. Mycologia 85:923–936.

Swofford DL. 1998. PAUP*4.0. Phylogenetic Analysis Using Parsimony. Sunderland, Massachusetts: Sinauer Associates.

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 15:253–262.

Taylor JW, Swann EC, Berbee ML. 1994. Molecular evolution of ascomycete fungi: phylogeny and conflict. In: Hawksworth DL, ed. Ascomycete systematics: problems and perspectives in the nineties. New York: Plenum Press. p 201–212.

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

Vilgalys R, Hibbett DS. 1993. Phylogenetic classification of fungi and our linnaean heritage. In: Reynolds DR, Taylor JW, eds. The fungal holomorph: mitotic and pleomorphic speciation in fungal systematics. Wallingford, UK: CAB International. p 255–260.

von Arx JA. 1982. On Mucoraceae s. str. and other families of the Mucorales. Sydowdia 35:10–26.

Wainright PO, Hinkle G, Sogin ML, Stickel SK. 1993. Monophyletic origins of the Metazoa: an evolutionary link with Fungi. Science 260:340–342.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. New York: Academic Press. p 315–322.

Yun, S-H, Berbee ML, Yoder OC, Turgeon BG. 1999. Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proc Natl Acad Sci USA 96: 5592–5597.

Zycha H, Siepmann R, Linnemann G. 1969. *Mucorales eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe.* Lehre, Germany: J. Cramer. 355 p.

APPENDIX

Explanatory notes of our interpretation of the morphological characters are presented below. These characters are discussed in Zycha et al (1969), Benjamin, (1979), O'Donnell (1979), Kirk and Benny (1980), Benny (1982, 1991), Kirk (1984), and Jeffries and Young (1994), or in the references provided by Hawksworth et al (1995). Note that the combination of Characters one (1^1) and two (2^0) results in the complete list of taxa forming few- to multispored sporangia as presented in FIG. 3.

1. *Multispored sporangia with a conspicuous columella.* Not formed (1^0) in the outgroup taxa *Dissophora decumbens, Echinosporangium transversale,* and *Mortierella verticillata* (Mortierellales) or in the ingroup species, *Benjaminiella poitrasii, Chaetocladium brefeldii, C. jonesii, Cokeromyces recurvatus, Cunninghamella echinulata, Dichotomocladium elegans, Ellisomyces anomalus, Hesseltinella vesiculosa, Micromucor ramannianus, Mycotypha africana, M. microspora, Phascolomyces articulosus, Radiomyces spectabilis, Umbelopsis isabellina,* and *Zychaea mexicana* (Mucorales), sporangia are aborted (1^2) in *Amylomyces rouxii,* merosporangia (1^3) are produced in both species of *Syncephalastrum.* The remainder of the taxa in the data set produce multispored sporangia (1^1).
2. *Few-spored sporangia with a persistent wall and usually with a reduced columella.* Produced by the following taxa: *Backusella circina, B. ctenidia, Blakeslea trispora, Cokeromyces recurvatus, Ellisomyces anomalus, Fennellomyces linderi, Helicostylum elegans, Hesseltinella vesiculosa, Kirkomyces cordense, Micromucor ramannianus, Pi-*

rella circinans, Radiomyces spectabilis, Thamnidium elegans, Thamnostylum piriforme, Umbelopsis isabellina and *Zychaea mexicana* (ingroup) and *Dissophora decumbens, Echinosporangium transversale,* and *Mortierella verticillata* (outgroup). The remaining taxa in the ingroup do not produce (2^1) few-spored sporangia with a persistent wall.

3. *Unispored sporangia.* The following taxa produce unispored sporangia (3^0): *Backusella circina, B. ctenidia, Benjaminiella poitrasii, Chaetocladium brefeldii, C. jonesii, Choanephora cucurbitarum, Cunninghamella echinulata, Dichotomocladium elegans, Dicranophora fulva, Mortierella verticilliata, Mycotypha africana, M. microspora, Phascolomyces articulosus* and *Syncephalastrum monosporum.* The remaining species in the data set do not produce unispored sporangia (3^1).
4. *Appendaged sporangiospores.* Sporangiospores bear several long, slender appendages (4^1) in *Blakeslea trispora, Choanephora cucurbitarum, Gilbertella persicaria, and Poitrasia circinans.* The remaining species in the data set do not form appendaged sporangiospores (4^0).
5. *Zygospore suspensors.* Zygospores are unknown in the following taxa and, therefore, the nature of the suspensors cannot be ascertained: *Actinomucor elegans, Amylomyces rouxii, Apophysomyces elegans, Chlamydoabsidia padenii, Halteromyces radiatus, Hesseltinella vesiculosa, Hyphomucor assamensis, Fennellomyces linderi, Kirkomyces cordense, Micromucor ramannianus, Mycotypha microspora, Phascolomyces articulosus, Pilobolus umbonatus, Rhizopus oligosporus, Saksenaea vasiformis, Syncephalastrum monosporum, Umbelopsis isabellina, Utharomyces epallocaulus,* and *Zychaea mexicana* of the ingroup and *Dissophora decumbens, Echinosporangium transversale,* and *Mortierella verticillata* of the outgroup. Zygospore suspensors are apposed (5^1) in *Blakeslea trispora, Choanephora cucurbitarum, Phycomyces blakesleeanus, Pilaira anomala,* and *Poitrasia circinans.* The remaining taxa in the data set produce opposed suspensors (5^0).
6. *Growth temperature.* Some taxa are either 1) thermophile; (6^1): *Rhizomucor pusillus* and *Thermomucor indicae-seudaticae,* 2) psychrophilic (6^2): *Chaetocladium jonesii, Dicranophora fulva, Helicostylum elegans,* and *Spinellus fusiger,* or 3) mesophilic (6^0): the remaining taxa.
7. *Trophocysts.* Trophocysts are formed (7^1) by *Pilobolus umbonatus* and *Utharomyces epallocaulus.* These structures are not formed (7^0) by the remaining species in the data set.
8. *Gall-forming parasites.* Three species of Mucorales (*Chaetocladium brefeldii, C. jonesii,* and *Parasitella parasitica*) are gall-forming, facultative parasites (8^1) in nature but can be grown in axenic culture on ordinary laboratory culture media. The remaining taxa in the data set are saprobes (8^0).
9. *Rhizoids.* Rhizoids are formed (9^1) by the following species: *Absidia* spp., *Actinomucor elegans, Amylomyces rouxii, Apophysomyces elegans, Chaetocladium brefeldii, C. jonesii, Chlamydoabsidia padenii, Gongronella butleri, Halteromyces radiatus, Hyphomucor assamensis, Radiomyces spectabilis, Rhizomucor pusillus, Rhizopus* spp., *Saksenaea vasiformis, Syncephalastrum* spp., *Thamnostylum piriforme, Thermomucor indicae-seudaticae,* and *Zychaea mexicana,* and in *Hesseltinella vesiculosa* rhizoids were reported in the literature but not observed in culture ($9^{0,1}$). The other taxa do not form (9^0) rhizoids.
10. *Suspensor appendages.* Zygospore suspensor appendages are formed (10^1) by *Absidia repens, Parasitella parasitica, Phycomyces blakesleeanus,* and *Radiomyces spectabilis.* The remaining taxa that form zygospores do not produce appendages on the suspensors (10^0). Zygospores are unknown for several species (see "5" above).
11. *Nature of sporangial wall.* The sporangial wall of Mucorales exhibits several variations. The wall is deliquescent (11^0) in *Absidia spp., Apophysomyces elegans, Backusella* spp., *Chamydoabsidia padenii, Dicranophora fulva, Fennellomyces linderi, Gongronella butlerii, Helicostylum elegans, Kirkomyces cordense, Micromucor ramannianus, Mucor circinelloides, M. mucedo, M. recurvus, Parasitella parasitica, Pirella circinans, Protomycocladus faisalabadensis, Phycomyces blakesleeanus, Sporodiniella umbellata, Syzygites megalocarpus, Thamnidium elegans,* and *Thamnostylum piriforme.* The sporangial wall is persistent (11^1) in *Circinella umbellata, Mucor racemosus, Pilaira anomala, Pilobolus umbonatus, Rhizomucor pusillus, Saksenaea vasiformis, Spinellus fusiger, Thermomucor indicae-seudaticae,* and *Utharomyces epallocaulus.* A persistent sporangial wall with one or more longitudinal sutures (11^2) is produced in *Blakeslea trispora, Choanephora cucurbitarum, Gilbertella persicaria,* and *Poitrasia circinans.* The sporangial wall is evanescent (11^3) in both species of *Rhizopus,* and persistent when young but then late deliquescent ($11^{0,1}$) in *Actinomucor elegans, Halteromyces radiatus, Hyphomucor assamensis,* and *Zygorhynchus heterogamous.* The remaining taxa in the data set do not produce a multispored sporangium with a conspicuous columella (11^4).