Phylogenetic Relationships of Agaric Fungi
Based on Nuclear Large Subunit Ribosomal DNA Sequences

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Abstract.—Phylogenetic relationships of mushrooms and their relatives within the order Agaricales were addressed by using nuclear large subunit ribosomal DNA sequences. Approximately 900 bases of the 5′ end of the nucleus-encoded large subunit RNA gene were sequenced for 154 selected taxa representing most families within the Agaricales. Several phylogenetic methods were used, including weighted and equally weighted parsimony (MP), maximum likelihood (ML), and distance methods (NJ). The starting tree for branch swapping in the ML analyses was the tree with the highest ML score among previously produced MP and NJ trees. A high degree of consensus was observed between phylogenetic estimates obtained through MP and ML. NJ trees differed according to the distance model that was used; however, all NJ trees still supported most of the same terminal groupings as the MP and ML trees did. NJ trees were always significantly suboptimal when evaluated against the best MP and ML trees, by both parsimony and likelihood tests. Our analyses suggest that weighted MP and ML provide the best estimates of Agaricales phylogeny. Similar support was observed between bootstrapping and jackknifing methods for evaluation of tree robustness. Phylogenetic analyses revealed many groups of agaricoid fungi that are supported by moderate to high bootstrap or jackknife values or are consistent with morphology-based classification schemes. Analyses also support separate placement of the boletes and russules, which are basal to the main core group of gilled mushrooms (the Agaricineae of Singer). Examples of monophyletic groups include the families Amanitaceae, Coprinaceae (excluding *Coprinus comatus* and subfamily Panaeolideae), Agaricaceae (excluding the Cystodermateae), and Strophariaceae pro parte (*Stropharia, Pholiota,* and *Hypholoma*); the mycorrhizal species of *Tricholoma* (including *Leucopaxillus,* also mycorrhizal); *Mycena* and *Resinomycena; Termitomyces, Podabrella,* and *Lyophyllum;* and *Pleurotus* with *Hohenbuehelia.* Several groups revealed by these data to be nonmonophyletic include the families Tricholomataceae, Cortinariaceae, and Hygrophoraceae and the genera *Clitocybe, Omphalina,* and *Marasmius.* This study provides a framework for future systematics studies in the Agaricales and suggestions for analyzing large molecular data sets. {Fungal evolution; higher phylogeny; homobasidiomycete; large-scale molecular phylogeny; tree support.}

It is particularly hoped . . . that DNA analysis can be methodically extended to generic taxonomy and that ways will be discovered to add new approaches to the solution of problems. Singer, (1986:viii)

Whence cometh the Agarics? Miller and Watling (1987)

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Gilled mushrooms and their allies (Basidiomycota: Agaricales) are among the most familiar of fungi. Often cryptic, they are actually a prominent component of most terrestrial ecosystems and perform a wide variety of ecological roles as saprophytes, mutualists, and parasites. Taxonomy of mushrooms has traditionally relied on morphological characters that are known to be subject to parallel evolution and phenotypic plasticity; as a result, many modern genera and families are artificial, and mycologists still disagree about taxonomic limits of the Agaricales and the identity of natural groups within the order.

The earliest classification system for agarics and other basidiomycetes by Fries (1821) was most notable for its clarity, logical simplicity, and complete artificiality. In his last general work, Fries (1874) recognized one

family and 20 genera of gilled (agaricoid) fungi. Beginning with Patouillard (1887, 1900), mycologists began to revise Fries's taxonomy and to recognize increasingly larger numbers of segregate genera based on ever-more-restricted sets of characters. A century after Fries, modern taxonomic systems recognize up to 230 genera (Singer, 1986) that have been classified into as many as 80 families and 25 orders (Singer, 1962, 1975, 1986; Moser, 1967; Kreisel, 1969; Kühner, 1980; Jülich, 1981; Locquin, 1984).

The most comprehensive modern taxonomic treatment for the Agaricales is that of Singer (1986; see also Appendix), based on that author's exhaustive first-hand knowledge of mushrooms from virtually all regions of the world. In Singer's (1986) system the Agaricales encompass three suborders. Most modern mycological floristic treatments largely follow Singer's system (Moser, 1967; Pegler, 1977, 1983; Bas et al., 1988). However, at least several alternatives to Singer's classification system have been proposed, most notably by Kühner and Romagnesi (1978), Kühner (1980), Jülich (1981), and Redhead (1986). Until recently, it has been difficult to evaluate the weaknesses and strengths of each classification system in the absence of knowledge about phylogenetic relationships.

A growing number of phylogenetic studies have started to address the evolutionary relationships of mushrooms and their relatives, using evidence from ribosomal RNA gene sequences (Bruns et al., 1992, 1998; Moncalvo et al., 1993, 1995; Chapela et al., 1994; Hopple and Vilgalys, 1999; Hibbett and Donoghue, 1995; Binder et al., 1997; Hibbett et al., 1997; Johnson and Vilgalys, 1998; Lutzoni, 1997). Monophyly of Singer's Agaricales has been rejected by several of these studies (Hibbett and Vilgalys, 1993; Hibbett et al., 1997; Thorn et al., 2000; Hibbett and Thorn, in press), which instead support the recognition of a clade of "euagarics" (gilled mushrooms) corresponding largely to Singer's concept for the Agaricineae but also including a number of nonagaric fungi such as puffballs and coral mushrooms.

In this paper we analyze phylogenetic relationships among the major evolutionary lines of agaric fungi, using sequence data from nuclear-encoded large subunit ribosomal RNA genes (nLSU-rDNA) from 154 diverse taxa. Taxonomic sampling for this study has been focused on the Agaricineae (as circumscribed by Singer, 1986), which also constitutes the core group of euagarics recognized by Hibbett et al. (1997). Phylogenetic analyses were conducted with several methods, including maximum parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ). Our results provide molecular support for many well-defined groups of agarics and also help to resolve phylogenetic relationships for some controversial taxa.

We also explored several general aspects of large-scale phylogenetic analysis involving molecular data by using a variety of search strategies aimed at finding optimal trees. The reconstruction of complex phylogenies is one of the most rapidly changing and controversial areas of systematics, and many uncertainties surround the choice of appropriate algorithms, models, and search strategies for dealing with ever-larger data sets, for which optimal solutions might never be known (Hillis, 1996; Rice et al., 1997; Graybeal, 1998; Kim, 1998; Soltis et al., 1998). Earlier studies that have reported on details and problems associated with large DNA sequence data sets were mainly from plant groups (Chase et al., 1993; Soltis et al., 1997, 1998; Soltis and Soltis, 1997). This study is the first to explore this issue by using an intensive sampling of sequences from a single group of fungi.

MATERIALS AND METHODS

Taxonomic Sampling

Taxa for this study were classified according to Singer's (1986) *Agaricales in Modern Taxonomy.* A large collection of nLSU sequences obtained from previous studies (Chapela et al., 1994; Vilgalys and Sun, 1994; Lutzoni, 1997; Johnson and Vilgalys, 1998; Hopple and Vilgalys, 1999) was combined into a single data matrix. Sequences of additional taxa were sampled to include representatives from each of Singer's (1986) families; only two families (Crepidotaceae and Gomphidiaceae) were not sampled. The choice of taxa for phylogenetic analysis

was eventually made from a database of >300 sequences and was based on three criteria: (1) taxonomic diversity (emphasis on current classifications), (2) sequence quality (absence of regions with missing data, hypervariability, or insertions/deletions {indels}), and (3) relative sequence diversity and nonredundancy (emphasis on sequence divergence and phylogenetic breadth). The species selected for phylogenetic analysis are listed in Appendix, along with taxonomic classification according to Singer (1986) and GenBank accession numbers of the nLSU-rDNA sequences. For rooting purposes, two species of the polypore genus *Ganoderma* were included in the analysis as an outgroup to represent the Polyporaceae, which has been suggested as a possible outgroup to the Agaricales according to the molecular phylogeny of homobasidiomycetes (Hibbett et al., 1997).

Molecular Techniques

The region targeted for phylogenetic analysis of sequence data was the 5′end of the nLSU-rDNA gene, which encompasses divergent domains D1 to D3 as defined in Michot et al. (1984, 1990). The D1–D3 region has been shown to contain most of the phylogenetically informative sites in this portion of the nLSU gene (Hillis and Dixon, 1991; Kuzoff et al., 1998; Hopple and Vilgalys, 1999). Sequences were produced by a variety of different enzymatic sequencing strategies over a period of nearly 10 years, including both manual and automated methods (see Appendix for details of the molecular techniques used to produce previously published sequences). Standard DNA isolation procedures were used with either CTAB (hexadecyltrimethyl-ammonium bromide; Zolan and Pukkila, 1986) or sodium dodecyl sulfate (SDS) buffers (Lee and Taylor, 1990). Polymerase chain reaction (PCR) amplifications were as described by Vilgalys and Hester (1990). PCR and sequencing reactions used the primers 5.8SR (5′-TCGATGAAGAACGCAGCG-3′), LROR (5′-ACCCGCTGAACTTAAGC-3′), LR3R (5′-GTCTTGAAACACGGACC-3′), LR5 (5′-TCCTGAGGGAAACTTCG-3′), LR7 (5′-TACTACCACCAAGATCT-3′), and LR16 (5′-TTCCACCCAAACACTCG-3′); additional information about these primers is given at http://www.botany.duke.edu/fungi/mycolab/primers.htm. Most of the sequences were produced with the use of automated sequencers (model ABI373 or ABI377; Perkin-Elmer) and dye terminator sequencing chemistries (Perkin-Elmer/ABI), according to the manufacturer's instructions. Sequence chromatograms generated by the automated sequencers were compiled by using Sequencher software version 2.0 (Gene Codes Corp.).

Phylogenetic Analyses

Sequences were aligned manually by using the editor window of PAUP* (Swofford, 1998). DNA regions with ambiguous alignment were initially removed from the analyses, and regions with distinctive patterns of length variation were recoded to provide additional information for parsimony analysis through the use of several strategies (Bruns et al., 1992; Hibbett et al., 1995; Moncalvo et al., 1995): single gaps that aligned unambiguously were scored as a "fifth character state", gaps forming larger indels were scored as "missing" except for one position scored as "fifth state" (to score an indel represented by several contiguous gaps as a single evolutionary event), and all ambiguously aligned gaps in nonexcluded regions were treated as "missing". Phylogenetic analyses were conducted by using MP with changes among character states having equal weights ("equally weighted parsimony"; UP) or different weights ("weighted parsimony"; WP), maximum likelihood (ML), and distance methods (NJ), as performed in PAUP* (Swofford, 1998) with a Power Macintosh 8600/300 MHz or a UNIX Sun Sparc 20 Station with dual 150 MHz hypersparc processors.

Equally weighted parsimony.—The following heuristic search settings in PAUP* were used: steepest descent option not in effect, branches allowed to collapse (creating polytomies) if maximum branch length equals 0, multistate taxa interpreted as uncertainty using polymorphism coding in the formal IUPAC codes (which PAUP* recognizes in "datatype = DNA"), and all characters of type unordered. Heuristic searches are subject to local optima (tree is-

lands) and may not always find the shortest trees (Maddison, 1991; Stewart, 1993; Swofford et al., 1996); in addition, many searches from our data matrix could not be completed with MAXTREES unlimited and tree-bisection-reconnection (TBR) branch swapping. We therefore used several strategies to find most-parsimonious trees. We conducted parsimony searches with TBR branch swapping, using starting trees obtained by way of (1) simple addition sequences with MULPARS "on" and MAXTREES unlimited; (2) random addition sequences along with different MAXTREES settings, including searches with MULPARS off; or (3) multiple random trees (Maddison et al., 1992) with low MAXTREES (typically 1–100). Several sets of trees obtained with low MAXTREES settings were used as starting trees in recursive searches (Olmstead et al., 1993; Olmstead and Palmer, 1994) in which we alternated swapping algorithms between searches (TBR, subtree pruning-regrafting {SPG}, and nearest neighbor interchanges {NNI}) along with increased MAXTREES (typically 1,000–5,000). If there was improvement in tree scores, the resulting best trees were saved and then used as starting trees for TBR branch swapping with MAXTREES set to higher values (from >10,000 to unlimited). Though many searches could not be completed, more than a year of CPU time was logged during the course of these analyses. Finally, the strict consensus tree of the most-parsimonious trees obtained in UP searches was used as a constraint for a search of 500 replicates of random addition sequences with TBR branch swapping and MAXTREES set to 5, and PAUP was instructed to save only trees that did not match the constraint; the resulting best trees were used as starting trees for TBR branch swapping with unlimited MAXTREES and the same constraint and also in a search where the constraint was removed. This search strategy was developed to sample a large neighborhood of equal-length trees without exhaustive searching (Catalan et al., 1997; Rice et al., 1997).

Support for phylogenetic groups was estimated by bootstrapping (Felsenstein, 1985) and parsimony jackknifing (Farris et al., 1996) with 50% character deletion,

with inclusion of parsimony-uninformative characters during resampling. Bootstrap and jackknife analyses were conducted with 100 replications of random addition sequences, with the use of "fast" procedures (no branch swapping and MULPARS off) and more-optimized procedures with TBR branch swapping and MAXTREES set to 100. Groups compatible with 50% majority-rule consensus were retained in the resulting consensus trees.

Weighted parsimony.—Weighted parsimony is often used to compensate for unequal base frequencies, transition/transversion biases, or rate heterogeneity in sequence data sets (Albert and Mishler, 1992; Allard and Carpenter, 1996; Yoder et al., 1996; Cunningham, 1997). To avoid arbitrary weighting of nucleotide substitution ratios and circularity in estimating these ratios from a given tree, we developed weightings based on nucleotide frequencies and substitution biases through pairwise comparison of sequences in the data matrix, using the option "pairwise base difference" in PAUP*. Base differences were estimated for all possible pairwise combinations of the 154 taxa in the data matrix, and weights for each substitution type were averaged across all comparisons and scored as a stepmatrix, as suggested by Maddison and Maddison (1992:60; when a substitution type was not observed between two taxa, that substitution type was arbitrarily scored as 0.5 in the calculation of the ln value; gaps scored as fifth character states were given higher weights to account for their rarity). The WP analysis used simple addition sequence with TBR branch swapping and MAXTREES unlimited. Additional searches were conducted by using trees found in UP and ML searches as starting trees.

Maximum likelihood.—A variety of increasingly complex models of molecular evolution were evaluated by using likelihood ratio tests (LRT; Goldman, 1993; Huelsenbeck and Rannala, 1997) to identify a simple and robust substitution model for the nLSU-rDNA data set. LRT were performed by using trees obtained from MP and NJ methods.

The large size of our data matrix made it impractical for PAUP* to use starting trees generated from sequence additions in ML

analyses. Therefore, starting trees for branch swapping were selected from among earlier MP and NJ analyses by choosing as starting trees those with the best ML score. ML parameter settings were reoptimized for each starting tree and later held constant during branch swapping to find more optimal trees (invariable sites were included in all ML analyses). Most branch swapping with ML used NNI because it is the least computationally intensive swapping algorithm available in PAUP*; subsequent searches using TBR swapping had to be aborted before completion (on a Power Macintosh Computer with 300 MHz processor, TBR searches on a single tree with 154 taxa were estimated to take as long as 3 or 4 months to complete).

Distance methods.—NJ trees (Saitou and Nei, 1987) were estimated under a variety of distance measures, based on a priori assumptions about rates and modes of nucleotide substitutions (distances described in Swofford et al., 1996, and estimated in PAUP*), including uncorrected distance "p", LogDet/paralinear distance (Lockhart et al., 1994), Jukes–Cantor (JC) distances (Jukes and Cantor, 1969), Hasegawa–Kishino–Yano (HKY85) distance (Hasegawa et al., 1985), Kimura two-parameter (K2) distance (Kimura, 1980), and Tajima–Nei (TN) distance (Tajima and Nei, 1984). Several distance measures incorporating rate heterogeneity were also included, using a gamma distribution (shape parameter arbitrarily assigned as 0.5) based on JC, HKY85, K2, and TN distances, as well as the general-time-reversible model (GTR) with nucleotide frequencies estimated from the data, as many as six categories of nucleotide substitution types with substitution rate parameters estimated by ML, and as many as eight rate categories with rates assumed to follow a gamma distribution with shape parameter 0.5. Ties encountered during tree reconstruction were set to break systematically (taxon-order dependent).

Estimation of topological differences between trees.—Statistical significance of topological differences among trees was estimated by using MP as the optimality criterion, with the Templeton (1983) and winning-sites tests implemented by PAUP*. Significant topological differences under ML criteria were estimated from the Kishino–Hasegawa test (Kishino and Hasegawa, 1989).

To compare tree lengths and topologies under alternative phylogenetic hypotheses, we also searched for trees by using phylogenetic constraints (Swofford, 1991). Constraint searches used UP with simple addition sequence, TBR branch swapping, and MAXTREES set to 10. The resulting trees were evaluated against the best MP trees.

RESULTS

Sequence Alignment and Nucleotide Sequence Variation

An alignment of 154 nLSU-rDNA sequences was 1,190 positions long. Overall, there was excellent agreement in sequence alignment between sequences produced manually and by computer, with all discrepancies in alignment occurring within hypervariable regions. After removal of the extreme 5′ and 3′ positions, which were incomplete for several taxa, 934 positions remained in the analyses. Alignment over a broad taxonomic sampling was not attainable within three hypervariable, indel-rich regions, and these regions were also removed from the analyses: positions 126–138 in variable domain D1, and 475–492 and 635–686 in variable domain D2 (however, subsets of related taxa aligned nicely within these divergent domains, suggesting that these regions have phylogenetic information at lower taxonomic levels). A few remaining single-gap regions were removed before sequence analysis, because of the possibility that they represented sequencing errors, as suggested from their occurrence in only one or a very few sequences. In contrast, several gap regions with short indels could be recoded as phylogenetically informative characters (mostly by scoring single gaps as a "fifth character state"). In all, 140 positions corresponding to regions with problematic alignments were removed, and 35 indel positions were recoded. Of the 826 characters included in the analyses, 422 characters were constant, 93 variable characters were parsimony-uninformative, and 311 characters were parsimony-informative. The final alignment for 154 taxa has been deposited on the internet as a NEXUS file, together with more de-

tailed explanations about the recoding strategies used, at http://www.botany.duke.edu/fungi/mycolab/agarical.htm.

Data Analysis

Equally weighted parsimony.—Results of UP searches using simple addition sequences, TBR branch swapping, and MAX-TREES unlimited yielded 60 equally parsimonious trees of length 3,100 (consistency index {CI} = 0.207, retention index {RI} = 0.555). Searches using random addition sequences with TBR and MAXTREES unlimited were aborted during the sixth replicate (and >300 hr of CPU time), and yielded trees of length 3,102–3,112. In subsequent searches MAXTREES settings were reduced to allow searches to continue to completion within a reasonable amount of time. Two searches using 100 replicates of random-addition sequences with MAXTREES set to 100 and 10 were completed within 71 and 20 hr, respectively; both searches produced trees 3,101 steps long, which were potentially located in three different tree islands (Maddison, 1991).

Another search strategy was attempted with MAXTREES set to 1 (= MULPARS off) and TBR branch swapping. Searches beginning with either 100 or 500 replicates of random addition sequences yielded slightly longer trees than previous UP analyses (lengths 3,104–3,121 and 3,102–3,130, respectively); searches starting from random trees (100 replicates) yielded trees of length 3,103–3,120, which were also very similar in length to trees produced by searches using random addition sequences. In contrast, when no branch swapping was performed, trees produced from random addition sequences were at least 43 steps longer than the shortest trees even though the number of replicates used was high (10,000).

Recursive swapping with unlimited MAXTREES and using starting trees from one of the three islands of score 3,101 yielded 72 trees of length 3,100. These 72 trees were topologically different from the first 60 MP trees (of equal length), according to comparisons of symmetric tree distances (data not shown), and therefore belong to a different tree island (Maddison, 1991). We also eventually found a third tree island consisting of 180 trees of length 3,100 when trees produced by WP searches (see below) were used as starting trees for TBR branch swapping in a UP search. No other sets of trees of length 3,100 or shorter were found, although numerous sets of trees were used as starting trees for recursive swapping. We subsequently refer to the three equally parsimonious tree islands found by using UP as UP60, UP72, and UP180 (referring to the number of equally parsimonious trees present in each island).

Symmetric differences among trees from these islands were calculated in PAUP*. Most differences occurred between trees from island UP60 and those from UP72 or UP180. Little average symmetric differences were found between trees from the islands UP72 and UP180. Within a single tree island, strict consensus trees were relatively well resolved. The strict consensus tree from the 252 trees of the combined islands UP72 and UP180 was also well resolved (Fig. 1a), which reflected lower average symmetric differences between trees from these two islands. In contrast, a strict consensus tree containing trees from all three islands contains many collapsed (especially deep) branches (Fig. 1b), largely because of differences between UP60 and the other two islands. When the strict consensus tree from all three islands was used as a negative constraint in a search with 500 random addition sequences, TBR branch swapping, and MAXTREES set to 5, the shortest trees produced were of length 3,103; when these trees were used as starting trees with TBR branch swapping and MAXTREES unlimited, 2,700 trees of length 3,101 (i.e., one step longer than the best trees in UP60, UP72, and UP180) were produced in searches that either kept, or removed, the constraint. The latter results provide a high degree of confidence (Catalan et al., 1997) that islands UP60, UP72, and UP180 represent sets of shortest trees.

Both bootstrapping and jackknifing provided good support for many terminal clades as well as several internal nodes (Figs. 2 and 3a). As expected for statistically related procedures, both bootstrap and jackknife values are strongly correlated (Fig. 3a; Pearson R^2 = 0.98). Both bootstrap and jackknife trees showed nearly identical

(a)

(b)

A
"B"
C
D
E
F
G
H
I
"J"
K
L
M
N
O
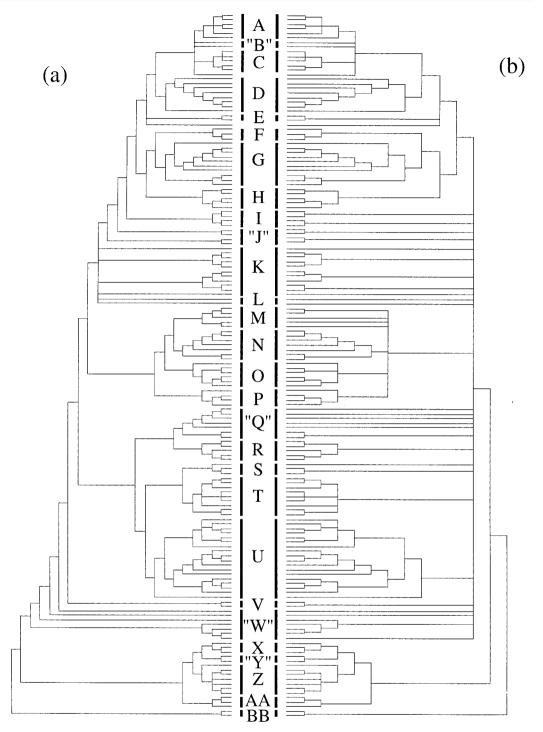P
"Q"
R
S
T
U
V
"W"
X
"Y"
Z
AA
BB

FIGURE 1. Tree topologies for (a) the strict consensus of 252 trees for two tree islands UP72 and UP180 and (b) the strict consensus of 312 trees for all three tree islands UP72, UP180, and UP60. Identities of groups A to BB are given in Figures 2 and 5. Groups that are paraphyletic in Figure 2 or 5 are labeled in quotation marks.

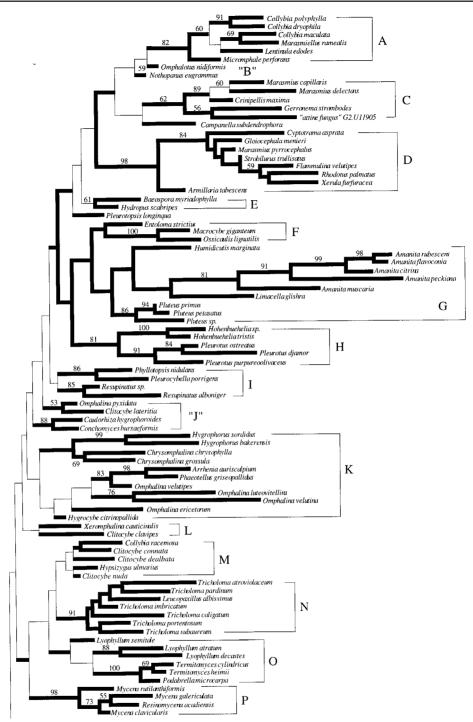FIGURE 2.   Agaricales phylogeny based on MP analysis of large nuclear subunit rDNA sequences. The figure shows one most-parsimonious tree found in both equally weighted parsimony (UP) and weighted parsimony (WP) searches. Bold lines indicate branches present in the strict consensus of all most-parsimonious UP and WP trees. UP searches yielded 312 equally parsimonious trees of length 3,100 (CI = 0.207, RI = 0.555) located in three
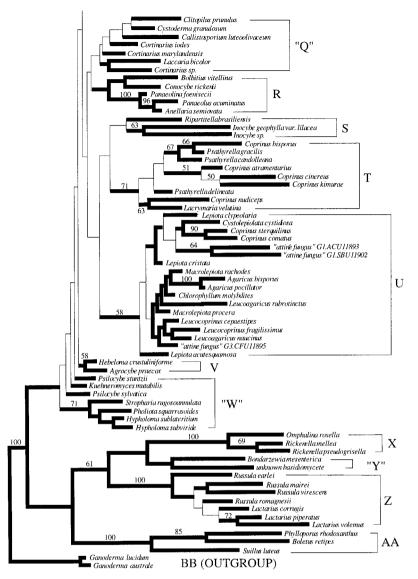
FIGURE 2. (continued) different tree islands. WP searches yielded four equally parsimonious trees of score 12,655.9 (CI = 0.220, RI = 0.563). Branch lengths were estimated by using ACCTRAN with character-state changes having equal weight. Bootstrap values ≥50% are shown above each branch (bootstrap values based on 100 replicates with MAXTREE set to 100 in each replicate, random addition sequence, TBR branch swapping, and character-state changes having equal weight).

topologies at nodes supported at >50% confidence level, though below this value some branches that are present in one tree may be absent in the other (data not shown). Several computationally faster alternative procedures for calculating bootstrap and jackknife values were also implemented in PAUP*, including "fast" bootstrapping and "fastjac" jackknifing, which yielded very similar topologies to those of the more opti-

mized (and more computationally intensive) search strategies, albeit with slightly lower support values for most nodes (Fig. 3b; results are shown only for fast bootstrap vs. normal bootstrap).

The Templeton and winning-sites tests were used to evaluate trees obtained with different search strategies. All searches that used TBR branch swapping were able to find trees within 30 steps of the most-parsi-
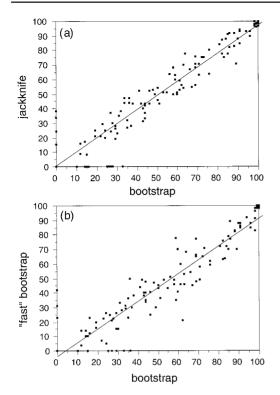
FIGURE 3. Comparison between bootstrap and jack-knife measures of support (based on 100 replicates). (a) Pairwise comparison between bootstrap and jackknife with 50% character deletion, TBR branch swapping, random addition sequence, and MAXTREE set to 100 in each replicate (Pearson R^2 = 0.94). (b) Pairwise comparison between "fast" bootstrap (no branch swapping) versus bootstrap with TBR branch swapping, random addition sequence, and MAXTREE set to 100 in each replicate (Pearson R^2 = 0.93).

monious trees, and nearly all of these trees (>99.9 %) were not markedly different from the most-parsimonious trees. Heuristic searches performed without swapping, however, always yielded trees that were at least 43 steps longer and always significantly worse than the best tree (P < 0.02 in the Templeton test). The efficiency of each search strategy can also be gauged by examining the computer time required (using either UNIX or PowerMac computer). With MAXTREES set at 10 or higher, both computers were able to find trees within 2 steps of the most-parsimonious tree after TBR swapping—but only after considerable time (at least 19 hr of CPU time). In contrast, searches storing a single tree (MAXTREES = 1 or MULPARS off) at each step of TBR swapping ran very quickly on a Power

Macintosh computer (as little as 7 hr for 100 replicates) and quickly converged toward a set of near most-parsimonious trees that were not statistically different from the three islands of most-parsimonious trees.

Weighted parsimony.—A stepmatrix based on dinucleotide frequencies (Fig. 4a) was used to perform WP analyses. Nine equally parsimonious trees of length 12,670.4 were produced from a search using simple addition sequences. When these trees were evaluated under the UP criterion, the length of all nine trees was 3,106 steps (six steps longer than the best UP trees). Both Templeton and winning-sites tests found no significant differences between UP and WP trees under either UP (P = 0.565–0.834, Templeton test, and P = 0.460–1.000, winning-sites

(a)

	[A]	[C]	[G]	[T]	[–]
[A]	1.4	5.7	3.8	5.1	6.7
[C]	5.7	1.7	5.9	3.5	6.9
[G]	3.8	5.9	1.3	5.2	6.9
[T]	5.1	3.5	5.2	1.5	6.2
[–]	6.7	6.9	6.9	6.2	2.7

(b)

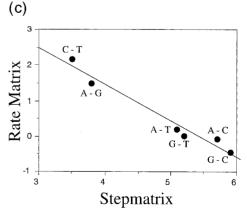|	[A]	[C]	[G]	[T]
[A]	-6.765	0.919	4.613	1.229
[C]	0.919	-10.342	0.651	8.772
[G]	4.613	0.651	-6.264	1.000
[T]	1.229	8.772	1.000	-11.000

(c)

FIGURE 4. Nucleotide substitution matrices used for WP and ML analyses. (a) Stepmatrix based on dinucleotide frequencies used for WP analysis (see text for treatment of gaps: {–}). (b) Substitution rate matrix estimated from ML with a GTR-6 model of evolution. (c) Matrix correlation plot for (a) and (b) above (Pearson R^2 = 0.98).

test) or WP models (P = 0.273– 1.000, Templeton test, and P = 0.526–1.000, winning-sites test).

Under the WP model, 17 of the UP60 and UP72 trees were shorter than the best WP tree obtained with simple addition sequences (12,670.4 steps in length), and the four equally shortest trees from this set (12,669.3 steps) were used as starting trees for TBR branch swapping in a new WP search. That search found four slightly shorter trees (12,655.9 steps), which scored 3,100 under the UP criterion (same as the best UP trees). These trees eventually yielded the tree island UP180 (see above).

One of the four best WP trees (length 12,655.9 steps) revealed by these analyses is shown in Figure 2. This tree is also one of the 312 best UP trees (length 3,100, located in island UP180). Because it is supported by both UP and WP analyses, this tree represents our best hypothesis of Agaricales phylogeny based on parsimony (MP tree).

Distance methods.—Eleven NJ trees were estimated from distance matrices calculated by using various evolutionary models. Though many topological differences were observed between these trees, many terminal groups were generally similar and correspond well with clades also found in parsimony analyses (data not shown). When scored under the UP criterion, however, all NJ trees (length = 3,166–3,179 steps) were significantly worse than the most-parsimonious trees under both the Templeton ($P \leq$ 0.001) and winning-sites tests ($P \leq$ 0.028).

Maximum likelihood.—Table 1 outlines the procedure followed in choosing the most adequate ML model for this data set. For every evolutionary model investigated, the token UP tree always had higher likelihood scores than the NJ–JC tree (Table 1). LRT based on either tree suggest the use of a similar model of evolution with five categories for base-substitution types and seven rate categories for the variable sites distributed according to a gamma-parameter (Table 1). In evaluating model complexity, the greatest improvements in ML scores in the LRT were obtained by increasing the number of substitution types from 1 to 2 to account for transition/transversion bias (6.8% improvement), and in taking into account among-site rate variation (17% improvement: 10% improvement after distin-

guishing variable vs. invariable sites, and an additional 7% improvement when variable sites were allowed to vary according to a gamma distribution). ML estimates of substitution rates for a model with different rate categories for each substitution type (GTR-6 model; Yang, 1994) are shown in Figure 4b. The smallest difference in substitution rates was between A-C and G-T substitutions, and combining these two kinds of substitution into a single rate category did not significantly affect ML scores (Table 1); therefore, a GTR-5 model of evolution was chosen for subsequent ML analyses.

ML estimates of substitution rates for each substitution type (Fig. 4b) were compared with the weights assigned to the same substitution types for WP (determined from dinucleotide frequencies) (Fig. 4a). Both methods gave estimates that were inversely correlated (Pearson R^2 = 0.98), with the lowest ML estimate of substitution rate (G-C) having the most weight in WP analyses (Fig. 4c).

Likelihood scores were calculated for trees from all of the previous UP, WP, and NJ searches by using likelihood parameters optimized for either a UP or NJ model tree. The tree with the highest likelihood score was also one of the nine best trees obtained from WP with simple addition sequence (ML-WP tree). The likelihood parameters were reoptimized to fit the ML-WP tree under the GTR-5 model of evolution, resulting in the following parameters: assumed nucleotide frequencies A = 0.270, C = 0.194, G = 0.298, and T = 0.238; substitution-rate matrix with G-C substitutions = 0.677, A-C and G-T = 1, A-T = 1.275, A-G = 4.792, and C-T = 9.110; proportion of sites assumed to be invariable = 0.455; rates for variable sites assumed to follow a gamma distribution with shape parameter = 0.749, and number of rate categories = 7. With these settings, the ML-WP tree was used as a starting tree for NNI branch swapping. This search was completed within 2 days of computation and resulted in a single tree of score 15,689.295, which was only 0.15% better than the input tree. This tree was used as a starting tree for TBR branch swapping. After 1,009 hr of CPU time on a UNIX Sun Sparc 20 Station (150 MHz), 132,748 rearrangements were performed, and the ML tree still had the same score. This best ML

TABLE 1. Alternative evolutionary models used to estimate likelihoods scores (−ln L) and likelihood ratio tests for one of the most-parsimonious tree of the unweighted parsimony analysis with simple addition sequence (tree UP1) and for a neighbor-joining tree based on Jukes–Cantor distances (tree NJ–JC). Data for the best-fit model are given in bold.

Evolution model[a]	No. of substitution types	Assumed base frequencies	Among-sites rate variation			Tree UP1		Tree NJ-JC	
			Invariable sites	Rates of variable sites[b]	No. of rate categories	−ln L	P[c]	−ln L	P[c]
JC	1	Equal				19,730.522		19,986.075	
F81	1	Estimated[d]				19,797.629	–	20,059.049	–
K2P	2	Equal				18,541.052	<0.001	18,760.684	<0.001
HKY85	2	Estimated[d]				18,649.802	–	18,875.148	–
GTR-3	3	Equal				18,425.134	<0.001	18,633.799	<0.001
GTR-4	4	Equal				18,416.157	<0.001	18,625.174	<0.001
GTR-5	5	Equal				18,409.873	<0.001	18,616.892	<0.001
GTR-6	6	Equal				18,409.584	n.s.	18,615.695	n.s.
GTR-5	5	Equal	Estimated[e]	Equal		16,715.065	<0.001	16,866.660	<0.001
GTR-5	5	Equal	0	Estimated[e]	4	15,865.837	<0.001	15,961.686	<0.001
GTR-5	5	Equal	Estimated[e]	Estimated[e]	4	15,772.353	<0.01	15,865.462	<0.05
GTR-5	5	Estimated[d]	Set[f]	Set[f]	4	15,766.148	<0.01	15,857.715	<0.01
GTR-5	5	Estimated[d]	Set[f]	Set[f]	5	15,756.607	<0.001	15,847.704	<0.001
GTR-5	5	Estimated[d]	Set[f]	Set[f]	6	15,751.878	<0.01	15,843.074	<0.01
GTR-5	**5**	**Estimated[d]**	**Set[f]**	**Set[f]**	**7**	**15,749.336**	**<0.05**	**15,840.546**	**<0.05**
GTR-5	5	Estimated[d]	Set[f]	Set[f]	8	15,747.874	n.s.	15,838.987	n.s.

[a] Listed in order of increasing complexity. GTR-3 = two categories for transitions and one for transversions; GTR-4 = two categories for transitions and two for transversions: (A-T), (A-C, G-T, G-C); GTR-5 = two categories for transitions and three for transversions: (A-T), (G-C), (A-C, G-T); GTR-6 = two categories for transitions and four for transversions.
[b] Variable sites assumed to follow a gamma distribution with a gamma shape parameter.
[c] Compared with the best value previously calculated, likelihood was either significantly improved, not significantly improved (n.s.), or worse (−).
[d] Estimated empirically from the data.
[e] Likelihood estimate from the data.
[f] Set as estimated above.

tree is shown in Figure 5. When scored under the UP criterion, this ML tree is 3,128 steps in length, which is not significantly different from the UP trees (3,100 steps) in both the Templeton ($P = 0.167$) and winning-sites ($P = 0.262$) tests. Reciprocally, trees found in the most-parsimonious islands UP60, UP72, UP180 and in the WP analysis were also not significantly different from the ML tree under the Kishino–Hasegawa test (scores 15,717.375–15,755.618, $P = 0.358–0.065$). In contrast, NJ trees were always significantly worse than the ML tree ($P < 0.010$, Kishino–Hasegawa test).

Comparison between Topologies of MP, ML, and Distance Trees

Results of the Templeton, winning-sites, and Kishino–Hasegawa tests (see above) indicated that trees produced by UP, WP, and ML analyses were all highly congruent, whereas unoptimized distance trees were all significantly worse than the best UP, WP, and ML trees. We therefore consider the optimal MP and ML trees (Figs. 2 and 5) to represent our best current estimates of Agaricales phylogeny. To facilitate discussion, the groups in Figures 1, 2, and 5 have been labeled A to BB. We use quotation marks to refer to groups that were paraphyletic in Figure 2 or 5 (e.g., group "B").

DISCUSSION

Phylogenetic Relationships in the Agaricales

The phylogeny of the Agaricales is still a controversial field. Singer (1986:124)

Progress toward a phylogenetic system of classification for the agaricoid fungi has been slower than for other organisms and is still hampered by many factors, most notably our still-too-poor knowledge of undiscovered taxa, especially in the tropics. This study represents a first comprehensive attempt to analyze phylogenetic relationships within the Agaricales, including exemplars from 16 of the 18 families recognized by Singer (1986). Our results demonstrate that nLSU-rDNA sequences provide suitable resolution for identifying major lineages of agaric fungi, with good support for many terminal clades and many internal branches (Fig. 2). The evolutionary lineages

identified from this study (see Figs. 2 and 5) may also be considered as a starting point for defining phylogenetic taxa. In most instances, phylogenetic groupings recognized by our analysis already correspond in whole or part to existing taxonomic groups (e.g., Amanitaceae and Russulaceae). Of course, results of this study also suggest new relationships among certain groups, or otherwise suggest that some traditional taxonomic views are in need of revision.

Our two best estimates of phylogenetic relationships (Figs. 2 and 5) are largely consistent with Singer's system (1986). Both parsimony and likelihood trees support Singer's division of the Agaricales into three major lineages, corresponding to the suborders Agaricineae, Boletineae, and Russulineae. Relationships among these three groups are not completely resolved, however, because MP trees (Fig. 2) suggest sister-group relationships between the Boletineae and the Russulineae lineages (group X-AA), whereas the ML analysis places the Boletineae lineage basal to the Agaricineae (Fig. 5). The ML tree (Fig. 5) agrees with a recently published MP tree of homobasidiomycetes that is based on 18S nuclear and 12S mitochondrial rDNA sequences (Hibbett et al., 1997) and supports a sister-group relationship between boletoid and agaricoid lineages. That study was based on analysis that would be expected to resolve phylogenetic groupings at higher levels (i.e., at deeper levels of branching) than the faster-evolving nLSU-rDNA gene. Combined analysis using data from a more slowly evolving gene (18S RNA) along with our nLSU-rDNA should offer better resolution of the basal relationships in this agaric-bolete-russuloid triangle.

The family Russulaceae (group Z), containing *Russula* and *Lactarius*, forms the core group of suborder Russulineae (Singer, 1986) and represents a distinct clade with 100% bootstrap support (Figs. 2 and 5). Phylogenetic analysis also supports grouping of the Russulaceae into a larger clade that includes a polypore (*Bondarzewia*), a group of small omphalinoid agarics (*Rickenella* spp., placed by Singer in the Agaricineae), and an unidentified basidiomycete (group X-Z). This "russuloid" clade (Hibbett and Thorn, in press) is supported by both MP (61% bootstrap support) and ML
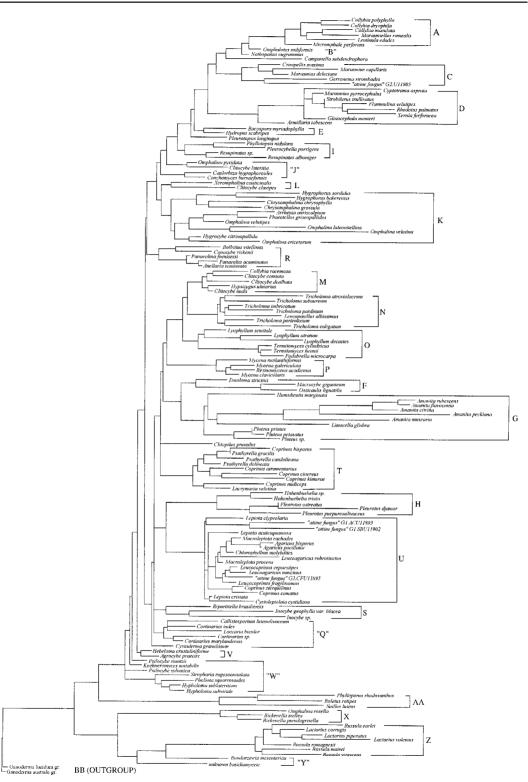
FIGURE 5. Agaricales phylogeny estimated by using ML. Tree score = 15,689.295. Groups labeled A to BB are the same as in Figures 1 and 2.

analyses (MP and ML trees differ in the branching order within the Russulaceae–*Bondarzewia*–*Rickenella* clade). *Bondarzewia* has been proposed to be closely related to the Russulaceae on the basis of basidiospore morphology and the presence of laticiferous hyphae (Redhead and Norvell, 1993; Singer, 1986). The grouping of several *Rickenella* species (group X) with other russuloid fungi is surprising from a morphological standpoint, because *Rickenella* does not possess any distinctive characters (amyloid ornamented spores, heteromerous trama, spherocysts, laticifers) that might show evidence of recent coancestry. Because of their simple agaric morphology, *Rickenella* spp. instead have traditionally been classified in the Agaricineae (family Tricholomataceae), "doubtfully distinct from *Mycena*" according to Kühner (1980:730), and as a synonym of *Gerronema* in Singer (1986).

The boletes and their relatives (group AA) include mostly poroid (*Boletus* and *Suillus*) as well as gilled (*Phylloporus*) mushrooms, which Singer placed in the Boletineae (Figs. 2 and 5). Although our sampling of boletoid fungi for this study was limited, the Boletineae (represented by the three genera named) are supported as a monophyletic group with 100% bootstrap support in all analyses. Monophyly of the Boletaceae and their close agaricoid relatives has been demonstrated in several previous molecular phylogenetic studies (Bruns and Szaro, 1992; Bruns et al., 1992, 1998; Hibbett et al., 1997). Our results do not support a close evolutionary relationship between *Omphalotus* (an agaric, group "B") with other boletes as once suggested (Bresinsky and Besl, 1979; see also discussion of *Omphalotus* below).

The majority of taxa sampled for this study belong to the Agaricineae sensu Singer (1986), which includes most species of agaric fungi. Except for the *Rickenella* clade (group X), monophyly of the Agaricineae (groups A–W) is supported by all analyses (including bootstrap and jackknife consensus trees). The node for monophyly of the Agaricineae, however, is not supported beyond 30% in either bootstrap or jackknife analyses (data not shown). Even though the Agaricineae are the largest and best known group of mushroom taxa, we are not aware of any obvious morphologi-

cal synapomorphies for this group, because most of the traditional characters used to define these fungi (lamellae, fleshy fruit bodies, etc.) are known to have evolved multiple times in other groups of fungi (Hibbett et al., 1997). Agreement supporting the monophyly of the Agaricineae between this study and earlier ones suggests that additional characters may be found that unambiguously define this lineage.

The polypores and their relatives have been suggested at different times to be closely related with agarics. Singer (1986) even included several genera of gilled agarics together with the genus *Polyporus* in the family Polyporaceae on the basis of similarities in dimitic hyphal structure. Although the outgroup chosen for this study (*Ganoderma*) appears to be a good exemplar from the Polyporaceae, based on molecular evidence (Hibbett and Vilgalys, 1993; Hibbett and Donoghue, 1995), our results support the view that several taxa placed by Singer in the Polyporaceae belong instead to the Agaricineae, including *Lentinula* (group A), *Nothopanus* (group "B"), *Phyllotopsis* (group I), and *Pleurotus* (group H).

Phylogeny within the Agaricineae

Within the Agaricineae, many terminal and higher-level phylogenetic groupings are supported by the nLSU-rDNA data. The phylogenetic hypothesis based on MP in Figure 2 divides the Agaricineae into two major groups that correspond with spore print color: Groups A to P include only pale-spored agarics, which are derived from within a paraphyletic group (R to "W") that includes mostly dark-spored species. This pattern is similar to the earliest Friesian classifications (Fries, 1821, 1874), which emphasized spore print color as an important character at higher taxonomic levels. A different set of relationships, however, is suggested by ML analysis (Fig. 5), which places a group of dark-spored agarics (clade R) within the pale-spored agarics, and the *Pleurotus* clade (pale-spored; clade H) within the dark-spored. Statistical tests (Templeton and winning-sites test under MP criterion and Kishino–Hasegawa test under ML model) of MP versus ML trees did not show any difference between these alternative hy-

potheses. We agree with Singer's (1986:3) observation that "Fries' discovery of spore print colors as a taxonomic character of first grade importance was certainly a fortunate and valuable contribution to the systematics of the Agaricales, however it should be used with reason".

Tricholomataceae (groups A–F, H–P, X).— The Tricholomataceae is the largest family of the Agaricales. In Figures 2 and 5, members of the Tricholomataceae are not monophyletic: They composed the core of groups A through F, H through P, and X (*Rickenella*), with *Laccaria* and *Callistosporium* being in group "Q". Group "Q" forms a heterogeneous, poorly supported group that also includes members of the Cortinariaceae, Entolomataceae, and Agaricaceae (it is monophyletic in the MP tree in Fig. 2, but does not include *Clitopilus prunulus;* it is paraphyletic in the ML analysis in Fig. 5). Trees constraining monophyly of the Tricholomataceae (including *Phyllotopsis, Lentinula*, and *Omphalotus* but not *Hohenbuehelia*, which strongly groups with *Pleurotus* in clade H) are 3,135 steps in length and significantly different from MP trees (Templeton test, *P* = 0.038–0.047). If only the *Rickenella* clade (group X) is excluded, constraint trees (3,123 steps) are no longer significantly different from MP trees (Templeton test, *P* = 0.184–0.209). In summary, though our analysis cannot unequivocally support or reject monophyly of the Tricholomataceae, phylogenetic analysis strongly suggest placement of certain taxa in other families. Also, although attempts have been made to redivide the Tricholomataceae into smaller families (Kühner, 1980; Pegler, 1983; Redhead, 1986), none of the clades in Figure 2 or 5 fully corresponds with any of these. However, further study of cases where rDNA phylogeny and supporting characters from anatomy are in concordance are likely to provide additional justification for redividing the Tricholomataceae based on monophyletic lineages. Several examples are discussed below.

All MP trees (Figs. 1 and 2) and the ML tree (Fig. 5) show exemplar sequences from the Tricholomataceae divided into two smaller clades (groups A–E with inclusion of *Pleurotopsis longiqua*, and M–P), with similar sets of relationships among groups within each clade. The first clade (A–E) includes exemplars from Singer's tribes Collybieae, Marasmieae, Biannularieae, Pseudohiatuleae, and Rhodoteae, whereas the second clade (M–P) mostly contains members of the Lyophylleae, Termitomyceteae, Leucopaxilleae, Tricholomatinae, and Clitocybinae (see Appendix). In Figures 2 and 5, exemplar sequences for tribe Myceneae fall into two monophyletic groups, one (*Baeospora* with *Hydropus,* clade E) being sister to the A–D clade, the other (*Mycena* and *Resinomycena,* clade P) sister to the M–O clade. This overall topology does not fully correspond with Singer's (1986) proposed tribes and subtribes (see Appendix) or any other proposed classifications aimed at dividing the Tricholomataceae. However, Kühner's (1980) families Marasmiaceae (with the inclusion of the Rhodotaceae) and Tricholomataceae roughly correspond to groups A–D and M–O, respectively. Not all Tricholomataceae are unambiguously resolved, particularly groups F (*Macrocybe* and *Ossicaulis*), I (*Resupinatus, Pleurocybella,* and *Phyllotopsis*), "J" (*Clitocybe lateritia, Caulorhiza,* and *Conchomyces*), and L (*Xeromphalina* and *Clitocybe clavipes*), for which we are unaware of any significant morphological synapomorphies. In the absence of stronger evidence, we consider these last groupings based on nLSU-rDNA phylogeny to be provisional.

Phylogenetic analyses also show that several genera placed in the Tricholomataceae by Singer (1986) are not monophyletic. For example, exemplar taxa representing different sections of *Collybia* are placed into two phylogenetically separate groups (sections *Levipedes* and *Striipedes* within clade A, and section *Collybia* within clade M). The genera *Omphalina, Marasmius, Tricholoma,* and *Clitocybe* are also polyphyletic according to our analyses. *Marasmius* species possessing broom cells form a monophyletic group (in clade C), whereas those lacking broom cells (*M. pyrrocephalus,* in section *Chordales*) belong to clade D, close to *Gloiocephala.* Similarly, the mycorrhizal species of genus *Tricholoma* form a monophyletic group with *Leucopaxillus* (also a mycorrhizal genus—clade N, 91% support), whereas *Tricholoma giganteum* (a nonmycorrhizal species) is sister to *Ossicaulis lignatilis*—group F, 100% support. Our re-

sults support a recent phylogenetic analysis of partial nLSU-rDNA sequence data by Pegler et al. (1998), who transferred *T. giganteum* to a new genus, *Macrocybe.*

The taxonomic position of the termite-associated agaric mushroom *Termitomyces* (Heim, 1941, 1977) has seen a certain amount of controversy. The original description of *Termitomyces* (Heim, 1942) indicated morphological similarities with both *Amanita* and *Lepiota. Termitomyces* species are characterized by a bilateral hymenophore trama, as in *Amanita* and *Pluteus;* pink spores, as in *Pluteus* and *Entoloma;* a spore hilum of the open-type, as in *Pluteus* (Pegler and Young, 1971); and cyanophilic spores and siderophilous granulation in the mature basidia, as in *Lyophyllum* (Clémen on, 1978, 1985). Consequently, the genus has been tentatively classified in the Amanitaceae (Singer, 1962; Pegler, 1977), Pluteaceae (Pegler and Young, 1971), and Tricholomataceae (Singer, 1975). In our study both MP and ML analyses suggest that *Termitomyces* is best classified close to *Lyophyllum* (in clade O) in the Tricholomataceae.

The genus *Omphalotus* includes several species of pleurotoid, white-spored, bioluminescent, toxin-containing mushrooms, including the well-known Jack-o-lantern mushroom (*O. olearius*). Before 1970, mycologists often placed *Omphalotus* in the Tricholomataceae, often as a synonym of other taxa, including *Clitocybe, Panus, Armillaria,* or *Pleurotus* (see Corner, 1981; Singer, 1986). Similarities in pigment chemistry not found in other agarics led Bresinsky (1974) and Bresinsky and Besl (1979) to suggest a link between *Omphalotus* and the boletes, a proposal accepted by later taxonomists, who followed in placing *Omphalotus* in the Paxillaceae within the Boletineae (Singer, 1986; Bas et al., 1988). Our analyses place *Omphalotus* with another bioluminescent fungus, *Nothopanus eugrammus,* in the paraphyletic group "B", which is deeply nested within the A–E clade (Figs. 2 and 5). Bioluminescence is not well documented in fungi but has often been reported for other agaric fungi, including *Armillaria, Flammulina, Panellus,* and *Mycena.* Another recent analysis of nLSU-rDNA phylogeny supports conclusions similar to ours, suggesting that *Omphalotus* is better placed

with white-spored agarics than with the boletes (Binder et al., 1997).

Amanitaceae and Pluteaceae (group G).— The Amanitaceae (*Amanita* and *Limacella*) are monophyletic in both MP and ML trees (Figs. 2 and 5). Monophyly of the genus *Amanita* is strongly supported (81% bootstrap support). Mycologists have disagreed about placement for the genus *Limacella,* which was included in the Pluteaceae by Locquin (1984) and later transferred to the Amanitaceae by Singer (1986). Both MP and ML analyses support placement of *Limacella* as a sister-group to other Amanitaceae. The three members of the genus *Pluteus* were monophyletic (86% bootstrap support) and nested with the Amanitaceae (in clade G) in the MP and ML trees, although with lower support. The Amanitaceae and Pluteaceae differ in their spore anatomy (Kühner, 1984; Singer, 1986; Clémen on, 1997) but share similarly structured lamellar trama, free and crowded lamellae, and inamyloid hyphae in the context tissue (Bas, 1969; Clémen on, 1997). A close relationship between these two families was suspected by earlier taxonomic treatments, with *Pluteus* classified in the Amanitaceae by Maire (1933) and Pegler (1977), and by Singer's earlier taxonomic system for the Agaricales (Singer, 1962).

The placement of *Humidicutis marginata* as a sister-group of the Amanitaceae in both the MP and ML trees is surprising, given that most taxonomic treatments place it with other members of the Hygrophoraceae. Given that none of the exemplar taxa from the Hygrophoraceae were clearly placed by our analyses, this odd placement of *H. marginata* may be the result of poor resolution for nLSU-rDNA data or even possible misidentification. Additional taxon and data sampling should help address this issue.

Entolomataceae. —The two members of Entolomataceae sampled (*Entoloma strictius* and *Clitopilus prunulus*) were placed into groups F and "Q" by the MP analysis (Fig. 2) but do not cluster strongly with any taxon. In the ML tree (Fig. 5), *Entoloma strictius* is also in group F but *Clitopilus prunulus* stands alone between groups G and T. The Entolomataceae differ from other agarics in having spores that are angular and have a characteristically complex wall structure as

seen in electron microscopy (Clémen on, 1997); the monophyly of the family has never been challenged. We attribute to a sampling artifact the nonmonophyly of the two Entolomataceae included here; the Entolomataceae appear to be monophyletic from ongoing analyses from a larger data set that includes many more taxa from this family (Baroni, Moncalvo, and Vilgalys, data not shown).

Hygrophoraceae (groups G, K).—Members of the Hygrophoraceae are nested among groups G (*Humidicutis*) and K (*Hygrophorus* and *Hygrocybe*) but do not cluster strongly with any taxa (Fig. 2). A MP analysis constraining the Hygrophoraceae to be monophyletic produced a tree of length 3,108, which is not significantly different from the shortest unconstrained trees of length 3,100 (Templeton test, *P* = 0.893–0.943). Since the description of *Hygrophorus* by Fries (1835, 1838) and the creation of the family Hygrophoraceae by Roze (1876), the monophyly of this assemblage of white-spored, waxy agarics with long and narrow basidia has never been questioned. Trees in Figs. 2 and 5 (group K) suggest that the Hygrophoraceae may be related to *Omphalina* and allied genera; these taxa share bright colored pigments and decurrent lamellae that are sometimes thick and distant.

Pleurotaceae (group H).—Although several pleurotoid fungi were included in this study (*Pleurotus, Pleurotopsis, Conchomyces, Resupinatus, Hohenbeuhelia, Phyllotopsis*), only *Pleurotus* and *Hohenbeuhelia* species are supported as monophyletic groups, with 81% bootstrap support. Both *Pleurotus* and *Hohenbuehelia* share the ability to trap and digest nematodes (Thorn, 1986; Thorn and Tsuneda, 1993), which could be a synapomorphy for recognizing the family Pleurotaceae (Thorn et al., 2000).

Cortinariaceae (groups "Q", S, V).—Members of the Cortinariaceae (*Cortinarius* in group "Q", *Inocybe* in group S, and *Hebeloma* in group V) were polyphyletic in both MP and ML analyses. However, nLSU data provide only weak support for most relationships. Constraining the Cortinariaceae to be monophyletic resulted in trees 3,117 steps in length, not significantly different from the shortest unconstrained trees of length 3,100 (Templeton test, *P* = 0.187–0.234). However, if relationships of *Corti-*

narius and *Inocybe* to other agarics remain largely unresolved, members of *Hebeloma* cluster with *Agrocybe* in both ML and MP trees (clade V) with moderate (58%) bootstrap support in MP analyses.

Bolbitiaceae (groups R, V).—The small family Bolbitiaceae includes *Bolbitius, Conocybe,* and *Agrocybe*. In both MP (Fig. 2) and ML (Fig. 5) analyses, *Bolbitius* and *Conocybe* are monophyletic and form the sister group (group R) of the Panaeoloideae (Coprinaceae), whereas *Agrocybe* groups with *Hebeloma* (Cortinariaceae) in group V close to a paraphyletic *Strophariaceae* (group "W").

Coprinaceae (group T).—The Coprinaceae (group T), with the exclusion of subfamily Panaeoloideae (group R) and two species (*C. comatus* and *C. sterquilinus,* both placed in the Agaricaceae and discussed below), are monophyletic in both ML and MP (71% bootstrap support) trees. This result is also in agreement with earlier studies by Hopple and Vilgalys (1994; 1999). Subfamily Panaeoloideae (*Panaeolina, Panaeolus,* and *Anelleria*) form a monophyletic group with 100% bootstrap support and clustered with *Bolbitius* and *Conocybe* (Bolbitiaceae pro parte) in both ML and MP trees but with low bootstrap support in the MP analysis. Constraining the monophyly of the Panaeoloideae with the Coprinaceae (excluding *Coprinus comatus* and *C. sterquilinus*) resulted in a tree of length 3,112 that is not significantly different from the shortest unconstrained trees of length 3,100 (*P* = 0.593–0.683, Templeton test). Therefore, although our results suggest closer relationships between the Panaeoloideae and *Bolbitius* rather than between the Panaeoloideae and *Coprinus,* our results do not fully resolve the controversy about whether the Panaeoloideae should be classified in the Coprinaceae (Singer, 1986; Bas et al., 1988; Kemp, 1995) or in the Bolbitiaceae (Kühner and Romagnesi, 1978).

Agaricaceae (group U).—The light- and dark-spored family Agaricaceae (excluding tribes Cystodermateae and including *Coprinus comatus* and *C. sterquilinus*) form a monophyletic group (group U) in both ML and MP (58% bootstrap support) trees. Phylogenetic evidence linking *C. comatus* and *C. sterquilinus* with the Agaricaceae has been presented previously (Johnson and Vilgalys, 1998; Hopple and Vilgalys, 1999).

In our analysis, two groups of ant symbionts (G1 and G3 from Chapela et al., 1994) are also included in the Agaricaceae. The position of both *Cystoderma* (in group "Q") and *Ripartitella* (in group S) remains unresolved. Constraining the monophyly of *Cystoderma* and *Ripartitella* with the Agaricaceae resulted in a tree of length 3,118, which is not significantly different from the shortest MP trees (Templeton test, P = 0.148–0.173).

Strophariaceae (group "W").—Members of the Strophariaceae form a paraphyletic group (group "W") at the base of the Agaricineae in both MP and ML analyses (Figs. 2 and 5). Monophyly of the genera *Stropharia, Pholiota,* and *Hypholoma* is fairly well supported by bootstrapping (71% of bootstrap confidence level); thus, neither subfamilies Stropharioideae nor Pholiotideae sensu Singer (1986) (see Appendix) appear to be monophyletic.

One application for the nLSU database is for identification and taxonomic placement of unknown sequences originating from fungi, including mycorrhizas, plant tissues, and other environmental samples. During the course of this study we identified one instance of misidentification involving a culture originally identified as *Asterophora parasita,* a parasitic mushroom that often occurs on decaying fruit bodies of Russulaceae, which was later determined to be an unidentified environmental isolate. Phylogenetic analysis places "unknown basidiomycete" together with *Bondarzewia* (group "Y") (Figs. 2 and 5), not with other Tricholomataceae (in the Agaricineae) as suggested by morphological evidence (Kühner, 1980; Singer, 1986). Because evidence from other molecular studies (Bruns et al., 1998; Cullings et al., 1996) also supports placement of *Asterophora* in the Tricholomataceae, we consider this sequence to represent an environmental contaminant. Subsequent analyses of independently derived nLSU sequences from bona fide material of *Asterophora* have confirmed this conclusion (V. Hofstetter and H. Clémen on, pers. comm.).

Phylogenetic Inference of Large Data Sets

With the recent birth of automated sequencing, analysis of large molecular data sets has come within the reach of many laboratories. At the same time, systematists are collectively realizing the importance of both adequate taxon and data sampling for accurate phylogenetic inference (Hillis, 1996; Lecointre et al., 1993; Graybeal, 1998; Poe, 1998). In this study we performed extensive analyses on a moderately large molecular data set (154 taxa and 826 characters) to determine empirically which heuristic methods might also be useful for future phylogenetic studies involving larger numbers of taxa and sequence data. Below we discuss several aspects of complex phylogenetic data sets that relate to our data.

In this study, taxa were selected from observation of branch lengths in preliminary analyses, to avoid pitfalls resulting from unequal branch lengths. Unequal branch lengths may have two origins: taxon sampling and among-taxa variation in molecular rates of evolution.

Taxon sampling.—Taxon sampling can have a strong effect on phylogenetic inference (Lecointre et al., 1993; Poe, 1998). We observed this frequently during preliminary analyses in which the bolete (group AA) and *Amanita* (group G) clades were always placed together within the Agaricineae. A sister-group relationship between boletes and *Amanita* conflicted with nearly every other classification system proposed to date and made little sense from a morphological standpoint. Examination of branch lengths showed that both the boletes and *Amanita* spp. occurred on long branches (Figs. 2 and 5), which suggested that "long branch attraction" might cause spurious grouping of these taxa (Felsenstein, 1978). One solution proposed for the long branch attraction problem was sampling of additional taxa, which was expected to break up long branches and result in more stable phylogenetic estimates (Swofford et al., 1996; Graybeal, 1998). We therefore sampled *Limacella* (which had been suggested to be the sister taxon of *Amanita* in Singer, 1986) and *Suillus* (representing another bolete lineage) for inclusion in this study. Phylogenetic analysis of the resulting data matrix (Figs. 2 and 5) separated the bolete and *Amanita* clades, in agreement with morphologically based classifications. Although this was a particularly noteworthy example of how taxon

sampling can affect tree topologies, other examples appear to be present in our data. Our observations agree with those of Hillis (1996), who has demonstrated that increased taxon sampling density appears to also increase phylogenetic accuracy.

Evaluating support: bootstrapping or jackknifing?—One commonly encountered problem with large data sets concerns the applicability or accuracy of standard descriptors used to assess branch robustness. The simplest measure of support used for parsimony trees, the decay or support index (Bremer, 1994), is not practical for large data sets because of the large number of trees that cannot be sampled. Other measures of "goodness of fit", such as CI and homoplasy, are also sensitive to sample size (Sanderson and Donoghue, 1989). Even bootstrapping has come under increased scrutiny because of questions regarding what constitutes a "significant" value (Hillis and Bull, 1993; Sanderson, 1989) and also because bootstrap replication often requires longer amounts of computing time as the number of taxa increases. As an alternative to bootstrapping, Farris et al. (1996) proposed the use of jackknife resampling to infer branch support.

In this study little difference was observed between support values obtained by bootstrap or jackknife analyses (Fig. 3a). Also, bootstrapping with fast search strategies (no branch swapping) gave values that were slightly lower (and thus more conservative) than more-optimized methods (Fig. 3b), which is probably a consequence of both quick convergence of parsimony searches toward the shortest trees and inclusion of suboptimal trees in the bootstrap consensus. Overall topologies of bootstrap and jackknife trees were similar to that of the strict consensus tree of combined islands UP60, UP72, and UP180 (Fig. 1b). Therefore, we regard bootstrapping and jackknifing (including "fast" methods) as two similar tools for assessment of branch robustness, both of which are useful (although not fully satisfactory) in analyses of large molecular data sets.

Choice of evolutionary models and search strategies.—Controversies abound concerning the choice of appropriate algorithms, models, or search strategies in reconstructing complex phylogenies. The applicability

of parsimony to large data sets has been questioned because of the large number of trees that must be examined in searching for shortest trees (other problems of parsimony in reconstructing phylogenies have been discussed by Felsenstein, 1978, and Stewart, 1993). ML searches appear to be even more limited by computational constraints than parsimony. Though distance methods such as NJ have the advantage of rapidity for phylogenetic reconstruction, NJ performed poorly when compared against other methods that used MP or ML as optimality criteria.

To reduce the time necessary for heuristic searches to find islands of shortest trees and also to explore the universe of parsimonious solutions, we conducted UP searches from many different starting trees, using various settings for MAXTREES and branch swapping. Two major observations regarding analysis of large data sets are also evident from this study: (1) Multiple islands of most-parsimonious trees (Maddison, 1991) were found; the trees from two of them (UP72 and UP180) were very similar to each other, whereas trees from the third island (UP60) were quite different from those from UP72 and UP180. Discovery of additional islands would also be expected to reduce the overall resolution of basal relationships in strict consensus trees (see Fig. 1). (2) Several search strategies are necessary when analyzing large data sets. In particular, we found it useful to vary several parameters for heuristic searches, including different settings for MAXTREES ($\leq$100 versus unlimited), as well as different swapping algorithms (TBR and NNI) while performing successive searches, saving the best trees at each. In a recursive fashion, initial searches use less-greedy algorithms first, which quickly converge toward local optima; these are followed by more computationally extensive algorithms, which are more likely to find optimal trees. In this study, all quick searches with 100 replicates of random addition sequences (as well as a search starting from random trees) that used TBR branch swapping and MAXTREES in the range 1–100 yielded at least one tree within 0.13% of the length of MP trees. In addition, all trees were <1% longer than the length of the MP trees; though not optimal, 99.9% of these trees were statisti-

cally similar to the MP trees, according to the Templeton and winning-sites tests, and scores generally quickly improved when these trees were recursively submitted to NNI swapping with higher MAXTREES and then again to TBR swapping (data not shown). We do not know whether the quick convergence toward MP trees as observed in our analyses resulted from our taxon-intensive sampling strategy or is a general property of large data sets, or both; but if this is a general property of large data sets, then increasing the number of taxa would be expected to increase accuracy of phylogenies without necessarily increasing computation time, as has already been suggested by Hillis (1996, 1998).

This study also demonstrates that application of ML approaches to moderately large data sets is feasible with personal computers and small Unix workstations. Using a restricted set of preselected starting trees (based on their likelihood scores), we were able to swap ML trees to completion by using the NNI algorithm. Subsequent TBR swapping for >1,000 hours did not alter the topology of the tree yielded from NNI swapping, suggesting that the ML tree (Fig. 5) is a fairly good estimate of phylogeny under the ML model of evolution. In simulation studies with limited numbers of taxa, ML performs extremely well in reconstructing phylogenies (Huelsenbeck and Hillis, 1993); however, little is known about the behavior of ML with complex, large data sets in comparison with parsimony models. In this study, we observed good congruence between the ML tree and trees produced from either UP or WP.

WP analysis yielded trees with slightly higher ML scores than UP did ($-\ln \geq 15{,}712.679$ versus $\geq 15{,}717.376$), suggesting that WP may perform better than UP when appropriate weighting schemes are applied: This would agree with simulation studies, which have shown that WP can recover the correct phylogeny with fewer characters than UP requires (Hillis et al., 1994). If MP and ML have the same foundation, as suggested by Goldman (1990), then the use of WP with appropriate weighting schemes might also be expected to estimate trees with higher likelihood scores. The high correlation observed between nucleo-tide weighting matrices used in WP versus ML (Fig. 4) also suggests that the stepmatrix values based on dinucleotide frequencies provide a reasonably accurate estimate of nucleotide substitution biases in the data.

In conclusion, our experience with these data suggests several approaches in analyzing large data sets. We recommend the design of a careful taxon sampling that maximizes homogeneity of branch length across phylogenetic trees. For MP, our study suggests the following: (1) WP using a stepmatrix for nucleotide substitution types with parameters derived from the data set or estimated by way of ML; and (2) extensive exploration of the universe of all possible binary trees with TBR branch swapping and multiple replicates of random addition sequences (at least 100) with low MAXTREES (e.g., 1 to 100). Evaluation of topological differences between trees obtained after fastidious searches (e.g., with MAXTREES unlimited) versus those obtained in quick searches (e.g., MULPARS off) provides information about the extent (length range) of the universe of solutions (which are likely to be found in different tree islands) and about topological differences within this universe. For ML searches, we also recommend using as starting trees those MP (or NJ) trees that exhibit higher ML scores, followed by extensive NNI branch swapping and exploration with TBR swapping. Standard descriptors for branch robustness (bootstrapping and jackknifing) may not be fully satisfactory when applied to large data sets; however, even "fast" procedures of these descriptors can quickly reveal strongly supported clades for developing useful phylogenetic hypotheses.

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APPENDIX

Organisms included in the study are listed according to Singer's (1986) taxonomic system.

Taxa	Collection no.[a]	DNA source[b]	GenBank no.
Suborder Agaricineae (11 families)			
Polyporaceae (2 tribes)			
Tribe Lentineae (5 genera)			
Pleurotus ostreatus (Jacqu.: Fr.) Kummer	RV 83/233t = D261	C	U04140[c]
Pleurotus djamor (Fr.) Boedijn	RV 95/920	C	AF042575
Pleurotus purpureoolivaceus (Stev.) Segedin et al.	RHP 3588.8 = D2342	C	AF042576
Nothopanus eugrammus (Mont.) Singer	JL PR1308	C	AF042577
Phyllotopsis nidulans (Pers.: Fr.) Singer	RV 96/1	B	AF042578
Lentinula edodes (Berk.) Pegler[d]	ATCC 42962	C	AF042579
Hygrophoraceae (3 tribes)			
Tribe Hygrophoreae (1 genus)			
Hygrophorus sordidus Peck	RV 94/178	B	AF042562
Hygrophorus bakerensis Smith & Hesler	SAR s.n.	B	AF042623
Tribe Hygrocybeae (5 genera)			
Humidicutis marginata (Peck.) Singer	JM 96/33	B	AF042580
Hygrocybe citrinopallida (Smith & Hesler) Kobay	LUTZ 930731–1	B	U66435[e]
Tricholomataceae (12 tribes)			
Tribe Lyophylleae (4 genera)			
Lyophyllum decastes (Fr.: Fr.) Singer	JM 87/16	C	AF042583
Lyophyllum atratum (Fr.) Singer	HC 79/133	C	AF042582
Lyophyllum semitale (Fr.) Kühner	HC 85/13	C	AF042581
Hypsizygus ulmarius (Bull.: Fr.) Redhead	JM-HW	C	AF042584
Tribe Termitomyceteae (2 genera)			
Termitomyces cylindricus S.E. He	JM leg. R.S.Hseu s.n.	B	AF042585
Termitomyces heimii Natarajan	JM leg. S.Muid s.n.	B	AF042586
Podabrella microcarpa (Berk. & Br.) Singer	PRU 3900	B	AF042587
Tribe Tricholomateae (4 subtribes)			
Subtribe Laccariinae (1 genus)			
Laccaria bicolor (Maire) Orton	JM 96/19	B	AF042588
Subtribe Clitocybinae (3 genera)			
Clitocybe nuda (Bull.: Fr.) Cooke	RV84/1	B	AF042624
Clitocybe connata (Schum.: Fr.) Gillet	JM 90c	B	AF042590
Clitocybe dealbata (Sow.: Fr.) Kummer	JM s.n.	B	AF042589
Clitocybe clavipes (Pers.: Fr.) Kummer	JM 96/22	B	AF042564
Clitocybe lateritia Favre	LUTZ 930803–1	B	U66431[e]
Ossicaulis lignatilis (Pers.: Fr.) Redhead & Ginns[f]	DAOM 191173	C	AF042625
Subtribe Tricholomatinae (1 genus)			
Tricholoma atroviolaceum A.H. Smith	KMS 400	B	U76457[g]
Tricholoma subaureum Ovrebo	KMS 590	B	U76466[g]
Tricholoma imbricatum (Fr.: Fr.) Kummer	KMS 356	B	U76458[g]
Tricholoma pardinum Quélet	KMS 278	B	U76462[g]
Tricholoma portentosum (Fr.) Quélet	KMS 591	B	U76464[g]
Tricholoma caligatum (Viv.) Ricken	KMS 452	B	U76467[g]
Macrocybe gigantea (Berk.) Pegler & Lodge[h]	IFO 31860	C	AF042591
Subtribe Omphalinae (13 genera)			
Arrhenia auriscalpium (Fr.) Fr.	LUTZ 930731–3	B	U66428[e]
Omphalina luteovitellina (Pilat & Nannf.) M. Lange	LUTZ 930816–8	B	U66447[e]
Omphalina rosella (M. Lange) Moser	REDHEAD 7501	B	U66452[e]
Omphalina velutina (Quel.) Quélet	LUTZ 930812–1	B	U66454[e]
Omphalina velutipes Orton	LAM L77–166h11Xh4	C	U66455[e]
Omphalina ericetorum (Fr.) M. Lange	LUTZ 930817–2	B	U66445[e]
Omphalina pyxidata (Pers.: Fr.) Quélet	LAM L66–118h14	C	U66450[e]
Gerronema strombodes (Berk. & Mont.) Singer	KUYPER 2984	B	U66433[e]
Rickenella mellea (Sing. & Clém.) Lamoure[i]	LAM 74–20h 1/9.91	C	U66438[e]
Rickenella pseudogrisella (A.H. Smith) Gulden[i]	LUTZ 930728–3	B	U66437[e]
Chrysomphalina chrysophylla (Fr.) Clémençon[i]	MICH A.H.Smith 76299	B	U66430[e]
Chrysomphalina grossula (Pers.) Novell et al.[j]	MICH A.H.Smith 82899	B	U66457[e]
Phaeotellus griseopallidus (Desm.) Kühner & Lamoure[k]	LUTZ & LAM 910828–4	B	U66436[e]
Callistosporium luteoolivaceum (Berk.& Curt.) Singer	RV 10–1	C	AF042627

APPENDIX (CONTINUED)

Taxa	Collection no.[a]	DNA source[b]	GenBank no.
Tribe Leucopaxilleae (8 genera)			
Leucopaxillus albissimus (Peck) Singer	SAR 1–2-90	B	AF042592
Tribe Biannularieae (2 genera)			
Armillaria tabescens (Scop.: Fr.) Emel	D 290	C	AF042593
Tribe Collybieae (15 genera)			
Pleurocybella porrigens (Pers.: Fr.) Singer	OKM 19644	B	AF042594
Collybia dryophila (Bull.: Fr.) Kummer	RV 83/180	C	AF042595
Collybia polyphylla (Peck) Sing.	RV 182.01	C	AF042596
Collybia maculata (Fr.) Kumm.	RV 94/175	B	AF042597
Collybia racemosa (Pers.: Fr.) Quél.	DED 5575	C	AF042598
Marasmiellus ramealis (Bull.: Fr.) Singer	DED 3973	C	AF042626
Micromphale perforans (Hofm.: Fr.) Singer	RV 83/67	C	AF042628
Campanella subdendrophora Redhead	DAOM 175393	C	AF042629
Tribe Resupinateae (6 genera)			
Hohenbuehelia tristis Stevenson	RV 95/214	C	AF042601
Hohenbuehelia sp.	RV 95/573	C	AF042602
Resupinatus alboniger (Pat.) Singer	RV/JM s.n.	B	AF042600
Resupinatus sp.	VT 1520 = D 596	C	AF042599
Conchomyces bursaeformis (Berk.) Horak[l]	RV 95/302	C	AF042603
Tribe Panelleae (3 genera)			
Pleurotopsis longinqua (Berk.) Horak[m]	RV 95/473	B	AF042604
Tribe Marasmieae (3 subtribes, 18 genera)			
Marasmius delectans Morgan	DED 4518	C	U11922[n]
Marasmius capillaris Morgan	DED 4345	C	AF042631
Marasmius pyrrocephalus Berkeley	DED 4503	C	AF042605
Strobilurus trullisatus (Murr.) Lennox	DAOM 188775	C	AF042633
Gloiocephala menieri (Boud.) Singer	DAOM 170087	C	AF042632
Xerula furfuracea (Peck) Redhead et al. [o]	JM 96/42	B	AF042566
Crinipellis maxima A.H. Smith & Walter	DAOM 196019	C	AF042630
Tribe Myceneae (16 genera)			
Baeospora myriaodophylla (Peck) Singer	DAOM 188774	C	AF042634
Hydropus scabripes (Murr.) Simger	DAOM 192847	C	AF042635
Caulorhiza hygrophoroides (Peck) Halling[p]	DAOM 172075	C	AF042640
Mycena galericulata (Scop.: Fr.) S.F. Gray	RV 87/14.01	C	AF042636
Mycena clavicularis (Fr.) Saccardo	RV 87/6	C	AF042637
Mycena rutilanthiformis (Murr.) Murrill	JM96/26	B	AF042606
Resinomycena acadiensis Redhead & Singer	DAOM 169949	C	AF042638
Xeromphalina cauticinalis (With.: Fr.) Kühner & Maire	RV 86/11	B	AF042639
Tribe Pseudohiatuleae (4 genera)			
Cyptotrama asprata (Berk.) Redhead & Ginns	DAOM 157066	C	AF042642
Flammulina velutipes (Curt.: Fr.) Singer	SAR s.n.	B	AF042641
Tribe Rhodoteae (1 genus)			
Rhodotus palmatus (Bull.: Fr.) Maire	VT 356	C	AF042565
Amanitaceae (2 genera)			
Amanita muscaria (L.: Fr.) Hooker	SAR s.n.	B	AF042643
Amanita citrina (Schaeff.) S.F. Gray	J 188	B	AF041547[q]
Amanita rubescens (Pers.: Fr.) S.F. Gray	RV "5Aug96"	B	AF042607
Amanita flavoconia Atkinson	RV "5Aug96"	B	AF042609
Amanita peckiana Kauffmann	RV 94/143	B	AF042608
Limacella glishra (Morg.) Murrill	VT-GB505	B	U85301[r]
Pluteaceae (3 genera)			
Pluteus primus Bonnard	JM leg. JB 94–24	B	AF042610
Pluteus petasatus (Fr.) Gillet	JM leg. JB 91–21	B	AF042611
Pluteus sp.	JM 96/28	B	AF042612
Agaricaceae (4 tribes)			
Tribe Leucocoprineae (7 genera)			
Leucocoprinus cepaestipes (Sow.: Fr.) Patouillard	EFM 548	C	U85286[r]
Leucocoprinus fragilissimus (Berk. & Rav.) Patouillard	JJ 84	B	U85289[r]
Leucoagaricus rubrotinctus (Peck) Singer	JJ 100	B	U85281[r]
Leucoagaricus naucinus (Fr.) Singer	OKM 15134	B	U85280[r]

APPENDIX (CONTINUED)

Taxa	Collection no.[a]	DNA source[b]	GenBank no.
Macrolepiota procera (Scop.: Fr.) Singer	JJ 168	B	U85304[r]
Macrolepiota rachodes (Vitt.) Singer	OKM 19588	B	U85277[r]
Chlorophyllum molybdites (Meyer: Fr.) Massee	JJ 162	B	U85274[r]
Tribe Agariceae (5 genera)			
Agaricus bisporus (Lange) Singer	SAR 88/411	B	U11911[n]
Agaricus pocillator Murrill	J 173	B	AF041542[q]
Tribe Lepioteae (6 genera)			
Cystolepiota cystidiosa (A.H. Smith) Bon	MICH 18884	B	U85298[r]
Lepiota acutesquamosa (Weinm.) Singer	JJ 177	B	U85293[r]
Lepiota cristata (Bolt.: Fr.) Kummer	DUKE HN 1582	B	U85292[r]
Lepiota clypeolaria (Bull.: Fr.) Kummer	OKM 22029	B	U85291[r]
Tribe Cystodermateae (7 genera)			
Cystoderma granulosum (Batsch) Fayod	BPI 752511	B	U85299[r]
Ripartitella brasiliensis (Speg.) Singer	EFM 744	C	U85300[r]
Coprinaceae (4 subfamilies)			
Subfamily Coprinoideae (1 genus)			
Coprinus atramentarius (Bull.: Fr.) Fries	C 114 = VT 1131	C	AF041484[q]
Coprinus cinereus (Schaeff.: Fr.) Fries	C 13 (from R.KEMP)	C	AF041494[q]
Coprinus kimurae Hongo & Aoki	C 78 = KEMP 1553	C	AF041500[q]
Coprinus nudiceps P.D. Orton	C 159 = KEMP 737/1	C	AF041517[q]
Coprinus bisporus Lange	C 148 = KEMP 1659/1	C	AF041523[q]
Coprinus comatus (Müll.: Fr.) S.F. Gray	C 116 = D 252	C	AF041529[q]
Coprinus sterquilinus (Fr.) Fries	C 123 = TF916 (VPI)	C	AF041530[q]
Subfamily Psathyrelloideae (2 genera)			
Psathyrella candolleana (Fr.) Maire	J 181	C	AF041531[q]
Psathyrella delineata (Peck) A.H. Smith	J 156	B	AF041532[q]
Psathyrella gracilis (Fr.) Quélet	J 130	C	AF041533[q]
Lacrymaria velutina (Pers.: Fr.) Singer[s]	J 100	C	AF041534[q]
Subfamily Panaeoloideae (4 genera)			
Panaeolina foensecii (Pers.: Fr.) Maire	SAR 87/378	B	U11924[n]
Panaeolus acuminatus (Schaeff.: Secr.) Quélet	J 129	C	AF041535[q]
Anellaria semiovata (Sow.: Fr.) Pearson & Dennis	SAR s.n.	B	AF041536[q]
Bolbitiaceae (6 genera)			
Bolbitius vitellinus (Pers.) Fries	SAR 84/100	B	U11913[n]
Conocybe rickenii (Schaeff.) Kühner	J 183	B	AF041546[q]
Agrocybe praecox (Pers.: Fr.) Fayod	SAR 84/159	B	AF042644
Strophariaceae (2 subfamilies)			
Subfamily Stropharioideae (4 genera)			
Stropharia rugosoannulata Farlow ex. Murrill	D 258	C	AF041544[q]
Psilocybe stuntzii Guzman & Ott	VT 1263 = D 216	C	AF042567
Psilocybe silvatica (Peck) Singer & Smith	RV 5–7-1989	C	AF042618
Hypholoma sublateritium (Fr.) Quélet [t]	JM 96/20	B	AF042569
Hypholoma subviride (Berk. & Curt.) Dennis [t]	JJ 69	B	AF042570
Subfamily Pholiotoideae (5 genera)			
Pholiota squarrosoides Peck	JJ 7	B	AF042568
Kuehneromyces mutabilis (Schaeff.: Fr.) Singer & Smith	DSM 1684	C	AF042619
Cortinariaceae (3 tribes)			
Tribe Inocybeae (1 genus)			
Inocybe sp.	RV 7/4	B	AF042617
Inocybe geophylla (Sow.: Fr.) Kummer	JM96/25	B	AF042616
Tribe Hebelomateae (3 genera)			
Hebeloma crustiliniforme (Bull.: Fr.) Quélet	SAR 87/408	B	U11918[n]
Tribe Cortinarieae (10 genera)			
Cortinarius iodes Berkeley & Curtis	JM 96/23	B	AF042613
Cortinarius sp.	JM 96/40	B	AF042614
Cortinarius marylandensis Ammirati & Smith	JM 96/24	B	AF042615
Entolomataceae (3 genera)			
Clitopilus prunulus (Scop.: Fr.) Kummer	RV 88/109	C	AF042645
Entoloma strictius (Peck) Sacc.	JM 96/10	B	AF042620

APPENDIX (CONTINUED)

Taxa	Collection no.[a]	DNA source[b]	GenBank no.
Suborder Boletineae (3 families)			
Paxillaceae (7 genera)			
Omphalotus nidiformis Berkeley	VT 1946.8 = OKM 23886	C	AF042621
Boletaceae (24 genera)			
Suillus luteus (L.: Fr.) S.F. Gray	JM 96/41	B	AF042622
Phylloporus rhodoxanthus (Schwein.) Bresadola	SAR 89/457	B	U11925[n]
Boletus retipes Berkeley & Curtis[u]	SAR 91/1	B	U11914[n]
Suborder Russulineae (2 families)			
Bondarzewiaceae (1 genus)			
Bondarzewia mesenterica (Schaeff.) Kreisel	SARs.n.	B	AF042646
Russulaceae (2 genera)			
Russula earlei Peck	RV 1sp92	B	AF042571
Russula mairei Singer	RV 89/62	B	U11926[n]
Russula virescens (Schaeff.: Zanted.) Fries	JH s.n.	B	AF041548[q]
Russula romagnesii Singer	JJ 60	B	AF042572
Lactarius corrugis Peck	RV 88/61	B	U11919[n]
Lactarius piperatus (L.: Fr.) S.F. Gray	RV "6 jul. 1994"	B	AF042573
Lactarius volemus (Fr.) Fries	RV 94/150	B	AF042574
Unclassified:			
Attine fungus G1.U11893			U11893[n]
Attine fungus G1.U11902			U11902[n]
Attine fungus G2.U11905			U11905[n]
Attine fungus G3.U11895			U11895[n]
Unknown basidiomycete[v]	RV 94/140	C	AF042563
Outgroups:			
Ganoderma lucidum gr.	JM RSH-RZ	C	X78776[w]
Ganoderma australe gr.	JM RSH-0705	C	X78780[w]

[a] RV, D, JM, SAR, LUTZ, JJ, JH, J, C, KMS = authors' collections or Duke University Herbarium and Culture Collection; VT, VPI, OKM = Orson K. Miller, Virginia Tech.; DED = Dennis Desjardin, San Francisco; RHP = Ron Peterson, University of Tennessee; DAOM, REDHEAD = Scott Redhead, Ottawa, Canada; DSM, HC = Heinz Clémen on, University of Lausanne, Switzerland; JL = Jean Lodge, Puerto Rico; KUYPER = Thomas Kuyper, The Netherlands; LAM = Denise Lamoure, France; MICH = Herbarium of the University of Michigan; EFM = New York Botanical Garden; PRU = Herbarium of the University of Pretoria, South Africa; BPI = U.S. National Fungus Collection; ATCC = American Type Culture Collection; IFO = Institute for Fermentation, Osaka, Japan.

[b] DNA was isolated from culture (C) or basidiome (B) tissues.

[c] Vilgalys and Sun (1994).

[d] *Lentinus* in Singer (1986).

[e] Lutzoni (1997).

[f] *Clitocybe* in Singer (1986).

[g] Shanks and Vilgalys, unpublished.

[h] *Tricholoma* in Singer (1986).

[i] *Gerronema* in Singer (1986).

[j] *Omphalina wynniae* (Berk. & Br.) Ito in Lutzoni, 1997.

[k] *Leptoglossum* in Singer (1986).

[l] *Hohenbuehelia* in Singer (1986).

[m] *Panellus* in Singer (1986).

[n] Chapela et al. (1994).

[o] *Oudemansiella* in Singer (1986).

[p] *Hydropus* in Singer (1986).

[q] Hopple and Vilgalys, 1999.

[r] Johnson and Vilgalys (1998).

[s] *Psathyrella* in Singer (1986).

[t] *Naematoloma* in Singer (1986).

[u] *Pulveroboletus* in Singer (1986).

[v] Unidentified culture isolated from *Asterophora parasitica*.

[w] Moncalvo et al. (1995).