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Higher-Level Phylogenetic Relationships of Homobasidiomycetes (Mushroom-Forming Fungi) Inferred from Four rDNA Regions

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Homobasidiomycetes include approximately 13,000 described species of mushroom-forming fungi and related taxa. The higher-level classification of this ecologically important group has been unsettled for over 100 years. The goals of the present study were to evaluate a recent phylogenetic classification by Hibbett and Thorn that divided the homobasidiomycetes into eight major unranked clades, and to infer the higher-order relationships among these clades. A dataset of 93 species that represent all eight previously recognized clades was assembled, with 3800 bp of sequence data from nuclear and mitochondrial large and small subunit rDNAs for each taxon. Parsimony and maximum-likelihood analyses support the monophyly of the eight major clades recognized by Hibbett and Thorn. Most groups are strongly supported in bootstrapped parsimony analyses, but the polyporoid clade remains weakly supported. For the first time, the sister-group relationship of the euagarics clade and bolete clade is strongly supported, and the Hygrophoraceae is strongly supported as the sister group of the rest of the euagarics clade. Nevertheless, the backbone of the homobasidiomycete phylogeny, and the internal structure of several clades, remain poorly resolved. © 2002 Elsevier Science

Key Words: **basidiomycota; evolution; combining data; ribosomal DNA.**

INTRODUCTION

The higher-level taxonomy of homobasidiomycetes (mushroom-forming fungi) has been in flux for over 100 years. In the 19th century, higher taxa of homobasidiomycetes (Fries, 1874) were delimited on the basis of the gross morphology of the fruiting bodies (whether gilled, poroid, toothed, coralloid, etc.). Although such groupings were easy to conceptualize and to apply in the field, it was soon recognized, based on anatomical characters, that they do not reflect monophyletic taxa (Fayod, 1889; Patouilliard, 1900). The dominant classifications of homobasidiomycetes of the 20th century were based primarily on anatomical features, and often combined outwardly dissimilar taxa into families, orders, etc. (e.g., Donk, 1964; Oberwinkler, 1977; Jülich, 1981). Since the late 1980s, and especially following the seminal work of White *et al.* (1990), phylogenetic studies of homobasidiomycetes have been based almost entirely on sequences of nuclear and mitochondrial large and small subunit ribosomal RNA genes (nuc-lsu, nuc-ssu, mt-lsu, mt-ssu rDNA), although protein-coding genes have also started to come into play (e.g., Kretzer and Bruns, 1999).

Different groups of molecular systematists working on homobasidiomycetes have tended to focus on different rDNA regions and taxa, and extensive but largely nonoverlapping datasets have accumulated. Examples include the mt-lsu rDNA dataset of boletes and other ectomycorrhizal taxa generated by Bruns and colleagues (Bruns *et al.,* 1998), the nuc-lsu rDNA dataset of agarics generated by Vilgalys and colleagues (Moncalvo *et al.,* 2000), and the nuc-ssu rDNA and mt-ssu rDNA dataset of polypores and other wood-decaying taxa generated by Hibbett and colleagues (Hibbett and Donoghue, 2001). To achieve a comprehensive phylogenetic classification of homobasidiomycetes it is necessary to integrate these datasets and reconcile their conflicts.

Recently, Hibbett and Thorn (2001) proposed a preliminary phylogenetic outline of homobasidiomycetes, which they suggested can be divided into eight mutually exclusive clades: the polyporoid clade, euagarics clade, bolete clade, russuloid clade, thelephoroid clade, hymenochaetoid clade, cantharelloid clade, and gomphoid–phalloid clade. The primary analysis on which Hibbett and Thorn based their classification was that of Hibbett *et al.* (1997), which used nuc-ssu and mt-ssu rDNA sequences of 76 genera of homobasidiomycetes. However, Hibbett and Thorn also incorporated the results of diverse studies using all four rDNA regions, and their classification thus includes over 250 genera. Ambiguities in the classification of Hibbett and Thorn derive largely from weak bootstrap support for many nodes in the analysis of Hibbett *et al.* (1997). For

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example, the polyporoid and hymenochaetoid clades were both supported by less than 50% of the bootstrap trees (Hibbett *et al.* 1997). In addition, nodes along the "backbone" of the phylogeny were weakly supported, so higher-order relationships among the eight major clades of homobasidiomycetes were not resolved with confidence. Another source of ambiguity in the classification of Hibbett and Thorn was conflict among the different phylogenetic studies that they attempted to synthesize. For example, the nuc-lsu rDNA analysis of Moncalvo *et al.* (2000) suggested that the Hygrophoraceae are nested among other agarics (in the euagarics clade *sensu* Hibbett and Thorn), but the mt-lsu rDNA analysis of Bruns *et al.* (1998) suggested that the Hygrophoraceae are not in this group.

The goals of the present study were to test support for the eight major clades of homobasidiomycetes recognized by Hibbett and Thorn and assess their higher-order relationships. A dataset of all four commonly analyzed rDNA coding regions was constructed, with taxa representing all eight clades recognized by Hibbett and Thorn. Analyses of subpartitions of the data were used to determine which regions or combinations of regions provide support for different clades within the homobasidiomycetes.

MATERIALS AND METHODS

A data matrix containing 91 species of homobasidiomycetes and two heterobasidiomycetes ("jelly fungi") was constructed, with sequences of nuclear and mitochondrial large and small subunit rDNA sequences for each taxon (Table 1). Two hundred fifty-eight sequences were published previously and 114 sequences were newly generated for this study (Table 1). In 23 species, the sequences of the different rDNA regions represent more than one isolate (Table 1).

DNA was isolated from fresh, frozen, or freeze-dried mycelium or fruiting bodies using a SDS-NaCl extraction buffer, with phenol-chloroform extractions and ethanol precipitation or GeneClean purification (Bio101, La Jolla, CA). The PCR was used to amplify four rDNA regions: nuc-ssu rDNA (bounded by primers PNS1 and NS8; however, 19 taxa included only 0.6–1.4 kb nuc-ssu rDNA sequences; Table 1), partial nuc-lsu rDNA (bounded by primers LR0R and LR5), partial mt-ssu rDNA (bounded by primers MS1 and MS2), and partial mt-lsu rDNA (bounded by primers ML5 and ML6). Amplified rDNAs were sequenced using standard primers and protocols for automated fluorescent cycle sequencing (White *et al.,* 1990; Bruns and Szaro, 1992; Hibbett and Donoghue, 1995; Hibbett, 1996; for primer sequences, see these publications and http://plantbio.berkeley.edu/~bruns/ and http://www.botany.duke.edu/fungi/mycolab/default.htm).

Sequences were assembled using Sequencher 3.0 (GeneCodes, Ann Arbor, MI) and aligned by hand in the PAUP* 4.0 data editor (Swofford, 1999). Sequences were deposited in GenBank (Accession Nos. AF393043–AF393156, AF393373), and the alignment has been deposited in TreeBASE (Accession No. 5644). Regions of ambiguous alignment were excluded, and gaps were treated as missing data. Phylogenetic analyses were performed using PAUP*. The heterobasidiomycetes *Dacrymyces chrysospermus* and *Auricularia auricula-judae* were used for rooting purposes. All parsimony analyses used unordered characters, with equal weighting of characters and transformations, random taxon addition sequences, and TBR branch swapping. Other settings for specific analyses are described below.

Prior to combining the data, a series of analyses was performed to assess whether there is significant conflict among the data partitions. Briefly, the tests of conflict involved a pair of intragenomic tests of conflict (i.e., nuc-ssu vs nuc-lsu and mt-ssu vs mt-lsu), followed by an intergenomic test of conflict (nuc-ssu + nuc-lsu vs mt-ssu + mt-lsu). The intragenomic test was performed as follows: (1) Bootstrapped parsimony analyses of each individual region were performed, and positively conflicting nodes supported by at least 90% of the bootstrap trees in both data partitions were selected for further evaluation (bootstrap analyses used 100 replicates, with one heuristic search per replicate, keeping up to 100 trees per replicate, and MAXTREES set to 10,000). (2) Unconstrained parsimony analyses of each region were performed (a two-step search protocol was employed: step one used 100 heuristic searches, keeping up to 10 trees per replicate; step two used TBR branch swapping on the shortest trees found in step one, with MAXTREES set to 1000). (3) Constraint trees that forced monophyly of the strongly supported conflicting nodes were used to perform constrained analyses of the data partitions, using the same settings as the unconstrained analyses (only one node was constrained in each analysis). (4) Constrained and unconstrained trees were compared using the Kishino-Hasegawa (1989) maximum-likelihood test (see description of ML analyses for settings, below) and the Templeton (1983) nonparametric parsimony test, both implemented in PAUP*. A significant result with either test was taken as evidence of strongly supported conflict among the data partitions.

Species found to have significant intragenomic conflicts were deleted and the intergenomic test of conflict was performed (following the same protocol as the intragenomic tests). After testing for conflict, *Gloeophyllum sepiarium* and *Dentocorticium sulphurellum* were pruned from the dataset. Neither exhibited strongly supported conflict among data partitions, but the placement of *G. sepiarium* in prior analyses has been labile (Hibbett and Donoghue, 2001), and it was thought that it could have a destabilizing effect on the topology.

Parsimony analyses were performed using the

TABLE 1

Species Examined (with Each Unique Isolate on a Separate Line), Collection Numbers, and GenBank Accession Numbers

Species	No.	nuc-ssu	nuc-lsu	mt-ssu	mt-lsu
Abortiporus biennis	KEW 210	AF334899	AF287842	AF334868	AF393087[a]
Agaricus bisporus	DSH 96-057	—	—	AF026656	—
A. bisporus	SAR 88/411	—	U11911	—	AD001538
A. bisporus	no ID	L36658	—	—	—
Albatrellus skamanius	DAOM 220694	AF287829	AF393044[a]	AF287817	—
A. skamanius	JL 92-89	—	—	—	AD001542
Albatrellus syringae	CBS 728.85	AF026632	AF393045[1]	AF026674	—
A. syringae	DAOM 216918	—	—	—	AD001543
Amanita muscaria	Moncalvo 96/63	AF026631	—	AF026673	—
A. muscaria	AR s.n.	—	AF042643	—	—
A. muscaria	TDB 1513	—	—	—	AD001549
Amylostereum laevigatum	CBS 623.84	AF334901	AF287843	AF334871	AF393088[a]
Antrodia carbonica	DAOM197828	AF026570	AF287844	U27023	AF393089[a]
Auricularia auricula-judae	FPL11504	—	—	U27022	AF393090[a]
A. auricula-judae	no ID	L22254	—	—	—
A. auricula-judae	GJW-855-10	—	L20278	—	—
Auriporia aurea	FPL7026	AF334903	AF287846	AF334873	AF393091[a]
Auriscalpium vulgare	DAOM128994	AF026581	AF287847	U27024	AF393092[a]
Bankera fuligineo-alba	DAOM184178	AF287831[c]	AF393046[a]	AF287819	AF393093[a]
Bjerkandera adusta	DAOM215869	AF026592	AF287848	U27025	AF393094[a]
Boletus satanas	TDB 1000	M94337	AF071528	M91009	AD001566
Bondarzewia berkleyi	DSH 93-190	AF026575	—	U27026	AF393095[a]
B. berkleyi	73BO	—	AF218563	—	—
Bondarzewia montana	DAOM 415	U59063[c]	—	AF393082[a]	AF393096[a]
B. montana	SAR s.n.	—	AF042646	—	—
Botryobasidium isabellinum	GEL 2109	AF026610	AF393047[c]	AF393083[a]	AF393097[a]
Botryobasidium subcoronatum	GEL 1286	AF026609	AF393048[a]	AF026651	AF393098[a]
Calostoma cinnabarina[b]	MSC 362913	AF093122[c]	AF093123	AF093120	AF093121
Cantharellus tubaeformis	DSH 93-209	AF026636	AF287851	AF026678	AF393099[a]
Ceriporia purpurea	DAOM21318	AF026594	AF287852	U27029	AF393100[a]
Ceriporia viridans	FPL7440	AF334905[c]	AF393049[a]	AF393084[a]	AF393101[a]
Ceriporiopsis subvermispora	FPL 90031-sp.	AF334906	AF287853	AF334874	AF393102[a]
Chroogomphus vinicolor	TDB 1010	M90822[c]	AF071529	M91010	AD001578
Coltricia perennis	DSH 93-198	AF026583	AF287854	U27028	AF393103[a]
Cortinarius iodes	Moncalvo 96/23	AF026633	AF042613	AF026675	AF393104[a]
Cryptoporus volvatus[b]	DAOM211791	AF334907[c]	AF393050[a]	U27031	AF393105[a]
Dacrymyces chrysospermus	FPL11353	—	AF287855	AF026642	AF393106[a]
D. chrysospermus	UC 1475112	L22257	—	—	—
Daedaleopsis confragosa	DSH 93-182	AF334908[c]	AF393051[a]	—	AF393107[a]
D. confragosa	DAOM180496	—	—	AF069637	—
Datronia mollis[b]	DAOM211792	AF334909[c]	AF393052[a]	U27033	AF393108[a]
Dendrocorticium roseocarneum	FPL1800	AF334910	AF393053[a]	AF334875	AF393109[a]
Dentipellis separans	CBS 538.90	AF334911	AF393054[a]	AF334876	AF393110[a]
Dentocorticium sulphurellum	FPL11801	AF026604	AF393055[a]	AF026647	AF393111[a]
Echinodontium tinctorium	DAOM16666	AF026578	AF393056[a]	U27035	AF393112[a]
Entoloma strictius	Moncalvo 96/10	AF287832	AF393057[a]	AF287820	AF393113[a]
Fomes fomentarius[b]	DAOM129034	AF026574	AF287857	U27036	AF393114[a]
Fomitopsis pinicola	DAOM189134	AF026599	AF287858	U27038	AF393115[a]
Ganoderma australe	Moncalvo 0705	AF026629	X78780	AF026672	AF393116[a]
Gautieria otthii	REG 636	AF393043[a]	AF393058[a]	AF393085[a]	AF393117[a]
Gloeocystidiellum leucoxanthum[b]	CBS 454.86	AF026602	AF287860	AF026645	AF393118[a]
Gloeophyllum sepiarium	DAOM137861	AF026608	AF393059[a]	U27041	AF393119[a]
Gloeoporus taxicola	KEW 213	AF334913	AF287861	AF334879	AF393120[a]
Gomphidius glutinosus	TDB 953	M90823	AF071530	M91011	—
G. glutinosus	TDB 957	—	—	—	AD001587
Gomphus floccosus	DSH 94-002	AF026637	AF287862	AF026679	AF393121[a]
Henningsomyces candidus	Thorn-156	AF334916	AF287864	AF334882	AF334749
Hericium ramosum	DSH 93-199	AF026577	AF287865	U27043	AF393122[a]
Heterobasidion annosum	DAOM73191	AF026576	—	U27042	—
H. annosum	T841	—	AF139949	—	—
H. annosum	KV 340	—	—	—	AD001593
Humidicutis marginata	Moncalvo 96/32	AF287833	AF393060[a]	AF287821	AF393123[a]

TABLE 1—*Continued*

Species	No.	nuc-ssu	nuc-lsu	mt-ssu	mt-lsu
Hydnum repandum	DSH 97-320	AF026641	—	AF026683	AF393124[a]
H. repandum	"Sichuan"	—	AJ279576	—	—
Hygrophorus sordidus	RV 94/178	AF287834	AF042562	AF287822	—
H. sordidus	TDB 727	—	—	—	AD001597
Hyphodontia alutaria	GEL 2071	AF026615	AF393061[a]	AF026660	AF393125[a]
Laccaria amethystina	DSH s.n.	AF287837	AF393062[a]	AF287824	AF393126[a]
Laccaria pumila	DSH s.n.	AF287838	AF287869	AF287825	AF393127[a]
Laetiporus sulphureus[b]	DSH 93-194	AF026597	AF287870	U27049	AF393128[a]
Laxitextum bicolor	CBS 284.73	AF026605	AF287871	AF026648	AF393129[a]
Lenzites betulina	DAOM180504	AF334919[c]	AF393063[a]	U27045	AF393130[a]
Lycoperdon sp.	DSH 96-054	AF026619	AF287873	AF026663	AF393131[a]
Nia vibrissa	REG M200	AF334754	AF334750	AF334753	AF334748
Oligoporus rennyi	KEW 57	AF334922	AF287876	AF334885	AF393132[a]
Panus rudis	DSH 92-139	AF026569	AF287878	AF026644	AF393133[a]
Paragyrodon sphaerosporus	TDB 420	M90826[c]	AF071531	M91014	AD001613
Peniophora nuda	FPL4756	AF026586	AF287880	U27063	AF393134[a]
Phellinus igniarius	FPL5599	AF026614	AF287884	U27061	AF393135[a]
Phlebia radiata[b]	FPL6140	AF026606	AF287885	AF026649	AF393136[a]
Phylloporus rhodoxanthus	TDB 540	M90825[c]	—	M91013	AD001618
P. rhodoxanthus	SAR 89/475	—	U11925	—	—
Pleurotus ostreatus[b]	VB 477	—	—	—	AF393137[a]
P. ostreatus[b]	D261	—	U04140	—	—
P. ostreatus[b]	DSH 93-214	—	—	U27064	—
P. ostreatus[b]	no ID	U23544	—	—	—
Pleurotus tuberregium[b]	DSH 92-155	U59091	AF393064[a]	U27071	AF393138[a]
Pluteus sp.	Moncaivo 96/28	AF026634	AF393065[a]	AF026676	AF393139[a]
Polyporoletus sublividus	DAOM221078	AF287840	AF393066[a]	AF287827	—
P. sublividus	DAOM 194363	—	—	—	AD001621
Polyporus arcularius	VT959	AF334928[c]	AF393067[a]	U27055	AF393140[a]
Polyporus melanopus	DAOM212269	AF334929[c]	AF393068[a]	U27062	AF393141[a]
Polyporus squamosus	FPL6846	AF026573	AF393069[a]	U27068	AF393142[a]
Polyporus tuberaster	DAOM7997B	AF334930[c]	AF393070[a]	U27070	AF393143[a]
Polyporus varius	DSH 93-195	AF334931[c]	AF393071[a]	AF393086[a]	AF393373[a]
Postia leucomallela	KEW 29	AF334932	AF393072[a]	AF334889	AF393144[a]
Pulcherricium caeruleum	FPL7658	U59083	AF393073[a]	U27057	AF393145[a]
Pycnoporus cinnabarinus	DAOM72065	AF334934[c]	AF393074[a]	U27059	AF393146[a]
Ramaria stricta	TENN HDT-5474	AF026638	AF287887	AF026680	AF393147[a]
Rhizopogon subcaerulescens	F-2882	M90827[c]	AF071534	M91015	AD001629
Russula compacta	Duke s.n.	AF026582	AF287888	U27074	AF393148[a]
Schizophyllum commune	DSH 96-026	—	—	AF069638	AF334747
S. commune	REG Sco1	—	AF334751	—	—
S. commune	no ID	X54865	—	—	—
Scleroderma citrinum	DSH 96-011	AF026621	—	AF026664	AF393149[a]
S. citrinum	REG Sc1	—	AF336266	—	—
Scytinostroma alutum	CBS 762.81	AF026607	AF393075[a]	AF026650	AF393150[a]
Sistotrema eximum	Thorn-429	AF334935	AF393076[a]	AF334891	AF393151[a]
Sphaerobolus stellatus	DSH 96-015	AF026618	AF393077[a]	AF026618	AF393152[a]
Stereum hirsutum	FPL8805	AF026588	AF393078[a]	U27076	AF393153[a]
Suillus cavipes	TDB 646	M90828[c]	AF071535	M91016	—
S. cavipes	TDB 645	—	—	—	AD001641
Suillus sinuspaulianus	DAOM 66995	M90829[c]	AF071536	X16137	—
S. sinuspaulianus	DAOM 66996	—	—	—	AD001643
Tapinella atrotomentosa	TDB 782	M90824[c]	—	M91012	AD001614
T. atrotomentosa	310	—	AF042014	—	—
Tapinella panuoides	DSH 96-043	AF026628	—	AF026671	—
T. panuoides	318	—	AF098394	—	AD001645
Thelephora sp.	DSH 96-010	AF026627	AF287890	AF026670	—
T. sp.	TDB 1504	—	—	—	AD001646
Typhula phacorhiza	DSH 96-059	AF026630	AF393079[a]	AF026687	AF393154[a]
Tyromyces chioneus	KEW 141	AF334938	AF393080[a]	AF334896	AF393155[a]
Wolfiporia cocos	FPL4198	AF334940	AF393081[a]	AF334898	AF393156[a]

[a] New sequences generated in this study.
[b] Species found to have significant conflict in intragenomic tests of congruence.
[c] 18S incomplete (571–1439 bp).

pruned "core" dataset as well as a "complete" dataset with all taxa included (using 1000 heuristic searches with MAXTREES set to autoincrease; bootstrap analyses used the same settings described previously). In addition, maximum-likelihood analyses of the core and complete datasets were performed using the HKY 85 model of sequence evolution, with empirical base frequencies, transition:transversion bias set to 2, and site-to-site rate heterogeneity modeled on a "discrete gamma" distribution, with four rate classes and α = 0.5. Maximum-likelihood searches used TBR branch swapping with one of the most parsimonious trees as the starting tree.

To evaluate the resolving power of each data partition, a series of parsimony and bootstrapped parsimony analyses of the core taxa was performed using all four single-region data partitions, all six two-region data partitions, and all four three-region data partitions (using the same PAUP* settings as in the tests of conflict). Results from each analysis were compared in terms of the number of most parsimonious trees recovered, the number of nodes resolved in the strict consensus of all most parsimonious trees, the number of nodes supported at 90–100% bootstrap support, and bootstrap support for 11 focal clades, which include the eight major clades of homobasidiomycetes recognized by Hibbett and Thorn (2001; Table 2) and several other groups that are discussed below.

RESULTS

PCR product sizes conformed with expectations based on results in other taxa (nuc-ssu = ca. 1.8 kb, nuc-lsu = ca. 0.95 kb, mt-ssu = ca. 0.6 kb, mt-lsu = ca. 0.5 kb). Group 1 introns have been reported from the rDNAs of some homobasidiomycetes (Hibbett, 1996). However, no introns were observed in the taxa sequenced for this study, except for one insertion of 45 bp in the nuc-lsu rDNA of *Bankera fuligineo-alba*. In the core dataset, the four-region data partition had an aligned length of 3800 bp, with 1664 variable positions, and 1111 parsimony-informative positions. Three hundred thirty-seven parsimony-informative positions are in the nuc-ssu rDNA data partition, 390 are in the nuc-lsu rDNA, 247 are in the mt-ssu rDNA, and 140 are in the mt-lsu rDNA (Table 2). One hundred thirty-six bp of the aligned sequences were deemed to be ambiguously aligned, including a 5-bp region of nuc-ssu rDNA, 60 bp of nuc-lsu rDNA, 50 bp of mt-lsu rDNA, and 21 bp of mt-lsu rDNA.

No strongly supported conflicts were observed between the nuc-ssu and nuc-lsu data partitions. However, strongly supported conflicts were detected between the mt-ssu and mt-lsu data partitions, involving nine species (*Gloeocystidiellum leucoxanthum, Laetiporus sulphureus, Phlebia radiata, Pleurotus ostreatus, P. tuberregium, Fomes fomentarius, Cryptoporus volvatus, Calostoma cinnabarina,* and *Datronia mollis*), which were pruned from the dataset. No significant conflicts were detected between the combined nuclear and mitochondrial datasets. As noted previously, *Gloeophyllum sepiarium* and *Dentocorticium sulphurellum* were also pruned to yield the core dataset, which includes 82 species, whereas the complete dataset includes 93 species.

Parsimony analysis of the core dataset yielded one tree (8436 steps, CI=0.315, RI = 0.492; Fig. 1, Table 2). The eight major clades of homobasidiomycetes recognized by Hibbett and Thorn (2001) were all resolved as monophyletic, with bootstrap support ranging from 58% (polyporoid clade) to 100% (bolete clade, gomphoid–phalloid clade; Fig. 1; Table 2). In general, the higher-order relationships among the eight major clades were not resolved with confidence (Fig. 1). However, one major group that includes the bolete clade and euagarics clade was strongly supported (bootstrap = 94%). We call this group the "euagarics/bolete clade." Monophyly of the homobasidiomycetes relative to the two heterobasidiomycete outgroup taxa was supported at 79%. *Dendrocorticium roseocarneum* was placed as the sister group of the rest of the homobasidiomycetes, but this placement received weak support (Fig. 1).

Support for the internal topologies of the major clades varied considerably. Among the more densely sampled groups, the bolete clade (11 species) was the most robust, with only two internal nodes supported at less than 70% bootstrap frequency (Fig. 1). In contrast, 31 internal nodes in the polyporoid clade (25 species), euagarics clade (14 species), and russuloid clade (15 species) were supported at less than 70% bootstrap frequency (Fig. 1). Nevertheless, several strongly supported groups were resolved within these clades, including a clade that contains all representatives of the euagarics clade except the Hygrophoraceae, which we call the "core euagarics clade" (bootstrap = 97%). Other noteworthy groups include two clades in the polyporoid clade that correspond to "Group 1" and "Group 5" described by Hibbett and Donoghue (1995). The least densely sampled groups include the thelephoroid clade (two species), hymenochaetoid clade (three species), cantharelloid clade (five species), and gomphoid–phalloid clade (four species). Within these groups, no internal node received less than 77% bootstrap support (Fig. 1).

Maximum-likelihood analysis of the core dataset was aborted after 56,200 rearrangements. The tree (score = 45772.67828) supports the monophyly of all eight major clades of homobasidiomycetes recognized by Hibbett and Thorn (2001; Fig. 3). It also supports the sister-group relationship of the euagarics clade and bolete clade, and places *Dendrocorticium roseocarneum* as the sister group of all other homobasidiomycetes, as in the parsimony tree. However, the

TABLE 2

Comparison of Performance of Data Partitions

	1-region				2-region						3-region				4-region	
	Core dataset															Complete dataset
nuc-ssu	√				√	√	√				√	√	√		√	√
nuc-lsu		√			√				√	√	√	√		√	√	√
mt-ssu			√			√		√	√		√		√	√	√	√
mt-lsu				√			√	√		√		√	√	√	√	√
Aligned length	1860	1076	486	382	2936	2346	2242	868	1562	1458	3422	3318	2728	1944	3800	3800
No. variable sites[a]	602	554	310	202	1156	912	804	512	864	756	1466	1358	1114	1066	1668	1732
No. parsimony-informative sites[a]	337	390	247	140	727	584	477	387	637	530	974	867	724	777	1114	1153
No. steps	2056	3303	1821	877	5473	3994	3074	2842	5263	4348	7419	6551	6376	6330	8436	9390
CI	0.406	0.292	0.297	0.383	0.328	0.344	0.376	0.303	0.286	0.293	0.315	0.323	0.328	0.288	0.315	0.298
No. MP trees	40	10	10	80	11	90	40	30	3	23	20	22	20	12	1	2
No. nodes resolved in strict consensus	58	48	71	30	62	55	69	36	76	57	70	60	69	67	79	88
No. nodes with bootstrap ≥90%	9	13	17	7	18	24	16	17	21	15	26	17	24	23	28	34
	Bootstrap support for focal clades															
polyporoid clade	—	—	—	—	—	—	—	—	—	—	—	—	—	—	58	—
euagarics clade	—	—	—	—	72	89	79	—	—	—	72	74	92	—	73	85
core euagarics	—	—	—	—	—	86	56	66	64	—	82	70	96	91	97	78
bolete clade	87	63	—	—	98	98	95	—	65	76	100	100	83	73	100	100
euagarics/bolete clade	—	—	—	—	80	52	—	—	67	—	93	74	—	58	94	90
Russuloid clade	55	—	—	—	—	73	90	—	—	—	73	71	87	—	86	93
Thelephoroid clade	80	54	—	—	92	—	—	—	—	—	90	74	—	—	76	72
Hymenochaetoid clade	—	—	—	81	—	—	62	93	69	75	88	82	93	90	98	95
Gomphoid–phalloid clade	100	—	67	61	100	100	100	86	—	72	100	100	100	93	100	100
Cantharelloid clade	—	—	76	—	—	91	—	—	79	—	86	—	58	73	93	92
Homobasidiomycetes	—	—	99	—	—	79	—	—	91	—	93	—	—	78	79	61

[a] After excluding ambiguously aligned regions.

maximum-likelihood and parsimony trees disagree regarding other aspects of the higher-order topology (Fig. 2). The internal topologies of the bolete clade, gomphoid–phalloid clade, cantharelloid clade, and hymenochaetoid clade were the same in the maximum-likelihood and parsimony trees, but there were a number of differences in the euagarics clade, russuloid clade, and polyporoid clade (Figs. 1 and 3).

Parsimony analysis of the complete dataset yielded two trees (9390 steps, CI = 0.298, RI = 0.490; Fig. 4), which differ only in a minor rearrangement in the terminal clade that includes *Ceriporia* spp. and *Gloeopoprus taxicola*. The trees support the monophyly of all eight major clades of homobasidiomycetes recognized by Hibbett and Thorn (2001). For seven of the focal clades, bootstrap support was within 10 bootstrap percentage points of that in the core analysis (Table 2). However, support for the polyporoid clade, core euagarics clade, and homobasidiomycetes clade was at least 10 bootstrap percentage points lower in the complete analysis than in the core analysis, while support for the euagarics clade was 12 bootstrap percentage points higher in the complete analysis than in the core analysis (Table 2). Two species could not be placed in any of the eight major clades recognized by Hibbett and Thorn: *Gloeophyllum sepiarium,* which was excluded from the core dataset, and *Dendrocorticium roseocarneum,* which was again weakly supported as the sister group of the rest of the homobasidiomycetes (Fig. 4). Higher-order relationships were not resolved with confidence, except for the euagarics/bolete clade, which was strongly supported (bootstrap = 90%). Levels of resolution and support within the major clades of homobasidiomycetes were comparable to those in the parsimony analysis of the core dataset (Figs. 1 and 4).

Maximum-likelihood analysis of the complete dataset was aborted after 130,500 rearrangements. The tree (score = 50492.66349) supports the monophyly of

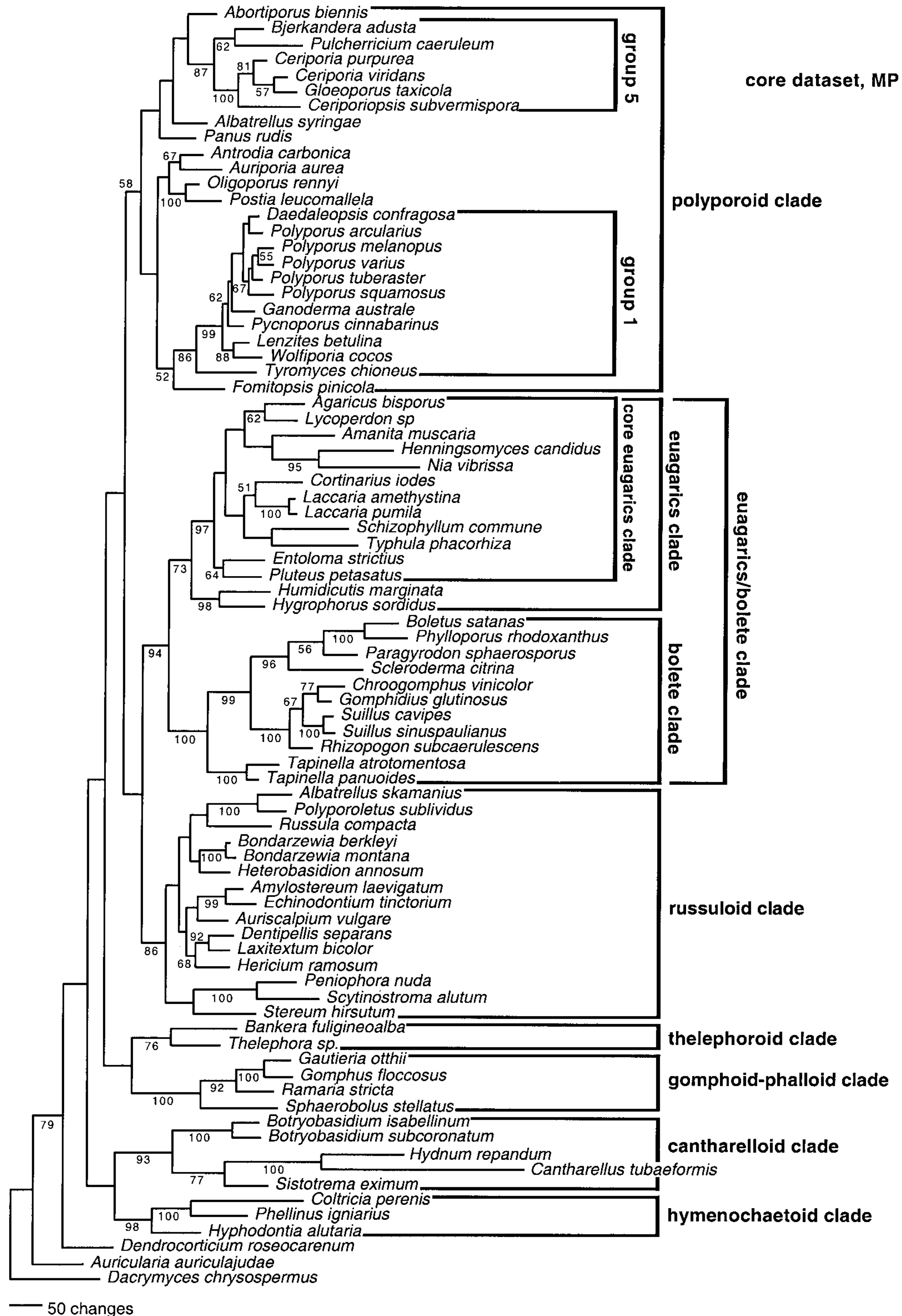

FIG. 1. Maximum-parsimony tree from analysis of the core dataset. Tree 1/1.

the eight major clades recognized by Hibbett and Thorn (Fig. 5). The higher-order relationships among the eight major clades of homobasidiomycetes are the same as in the maximum-likelihood tree obtained from the core dataset, but differ from those in the parsimony trees obtained from the core and complete datasets (Figs. 2 and 5).

Phylogenetic relationships of the 82 species in the

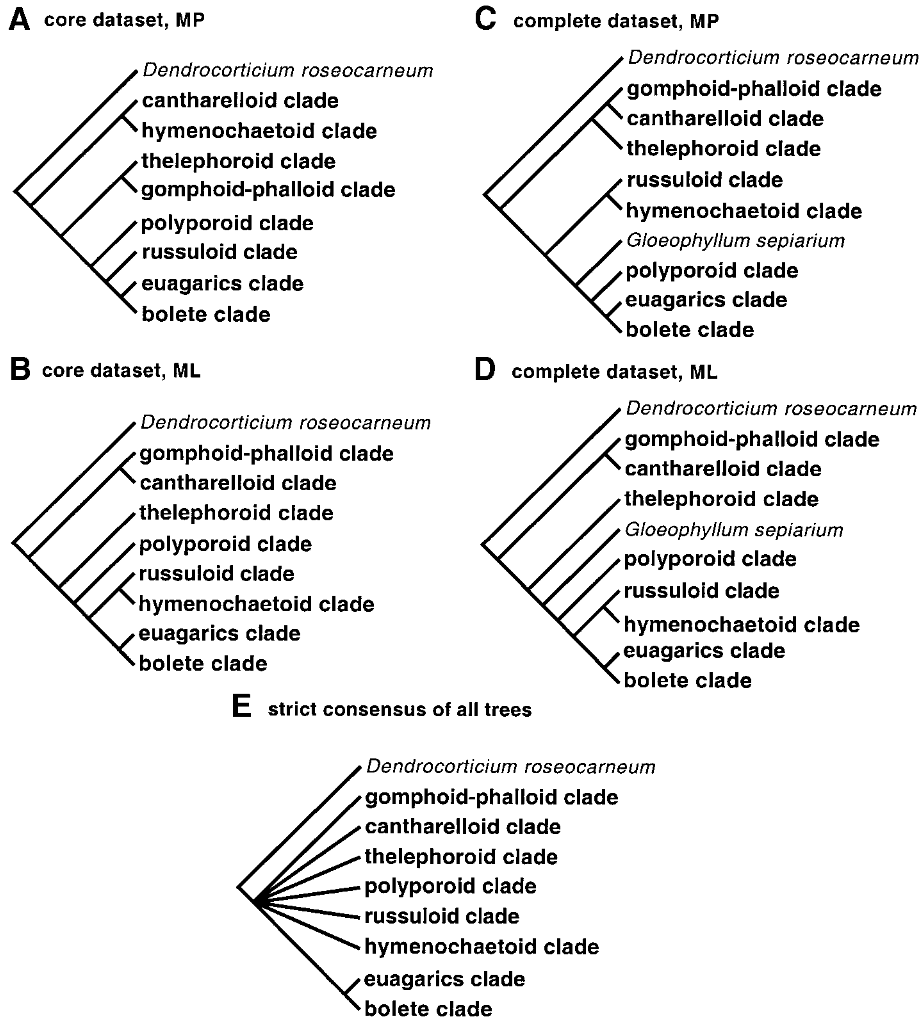

FIG. 2. Higher-level phylogenetic relationships of homobasidiomycetes inferred from parsimony and maximum-likelihood analyses of the core and complete datasets. (A) Parsimony tree from core dataset. (B) Maximum-likelihood tree from core dataset. (C) Strict consensus of parsimony trees from complete dataset. (D) Maximum-likelihood tree from complete dataset. (E) Strict consensus of all trees from analyses of the core and complete datasets

core dataset were estimated using the four-region dataset and all 14 possible subpartitions of the data (Table 2). Based on the number of nodes supported at or above 90% bootstrap frequency, the most robust single-region data partition was the mt-ssu rDNA (17 strongly supported nodes); the most robust two-region data partition was the mt-ssu/nuc-ssu data partition (24 strongly supported nodes); and the most robust three-region data partition was the mt-ssu/nuc-ssu/nuc-lsu data partition (26 strongly supported nodes; Table 2). The four-region dataset was the most robust overall; it supported 28 nodes at or above 90%, and it is the only data partition that supported all 11 of the focal clades at or above 50% bootstrap frequency (Table 2).

DISCUSSION

This study had two main purposes, to evaluate the monophyly of the eight major clades of homobasidiomycetes recognized by Hibbett and Thorn (2001) and to estimate their higher-order relationships. Monophyly of each of the eight clades, as represented in the present sample of taxa, was strongly supported (bootstrap $\geq$ 90%), except for the polyporoid clade, which remains weakly supported (bootstrap = 58%; Fig. 1; Table 2). Surprisingly, the strongest support for some clades was obtained not with the four-region dataset, but with various subpartitions of the data (as discussed below; Table 2).

Evaluation of Intragenomic Conflicts

We cannot say for certain why the placements of nine species (*Gloeocystidiellum leucoxanthum, Laetiporus sulphureus, Phlebia radiata, Pleurotus ostreatus, P. tuberregium, Fomes fomentarius, Cryptoporus volvatus, Calostoma cinnabarina,* and *Datronia mollis*) showed significant conflicts in the mt-lsu rDNA vs mt-ssu rDNA partitions. It is possible that the mt-lsu

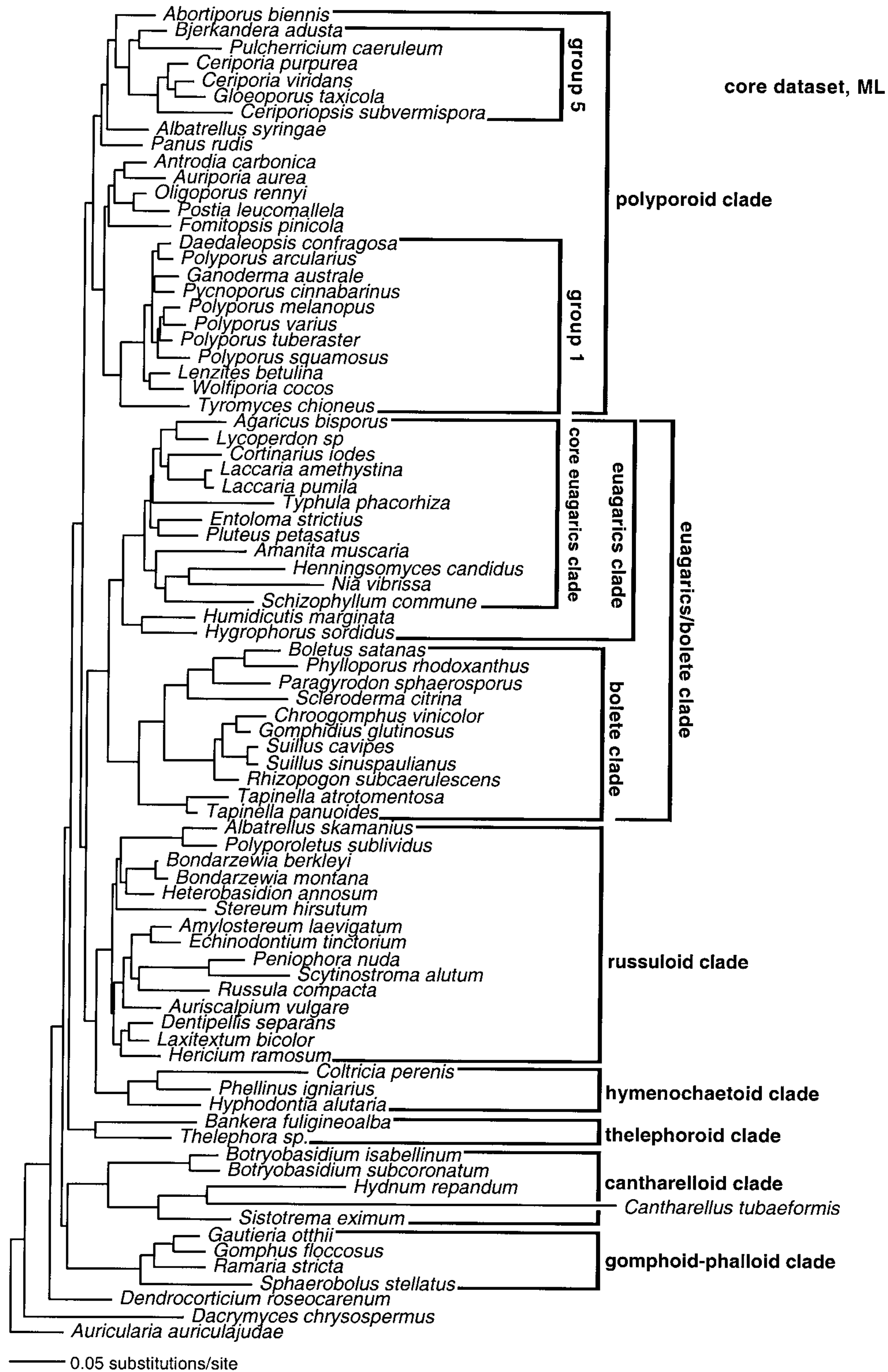

FIG. 3. Maximum-likelihood tree derived from analysis of the core dataset.

rDNA and mt-ssu rDNA have different phylogenies, but this is unlikely because the two regions are closely linked in the mitochondrial genome. The statistical validity of the Kishino-Hasegawa (1989) and Templeton (1983) tests that we used to detect conflict has recently been questioned (Goldman *et al.,* 2000). Therefore, another possibility is that the intragenomic conflicts we detected are statistical artifacts.

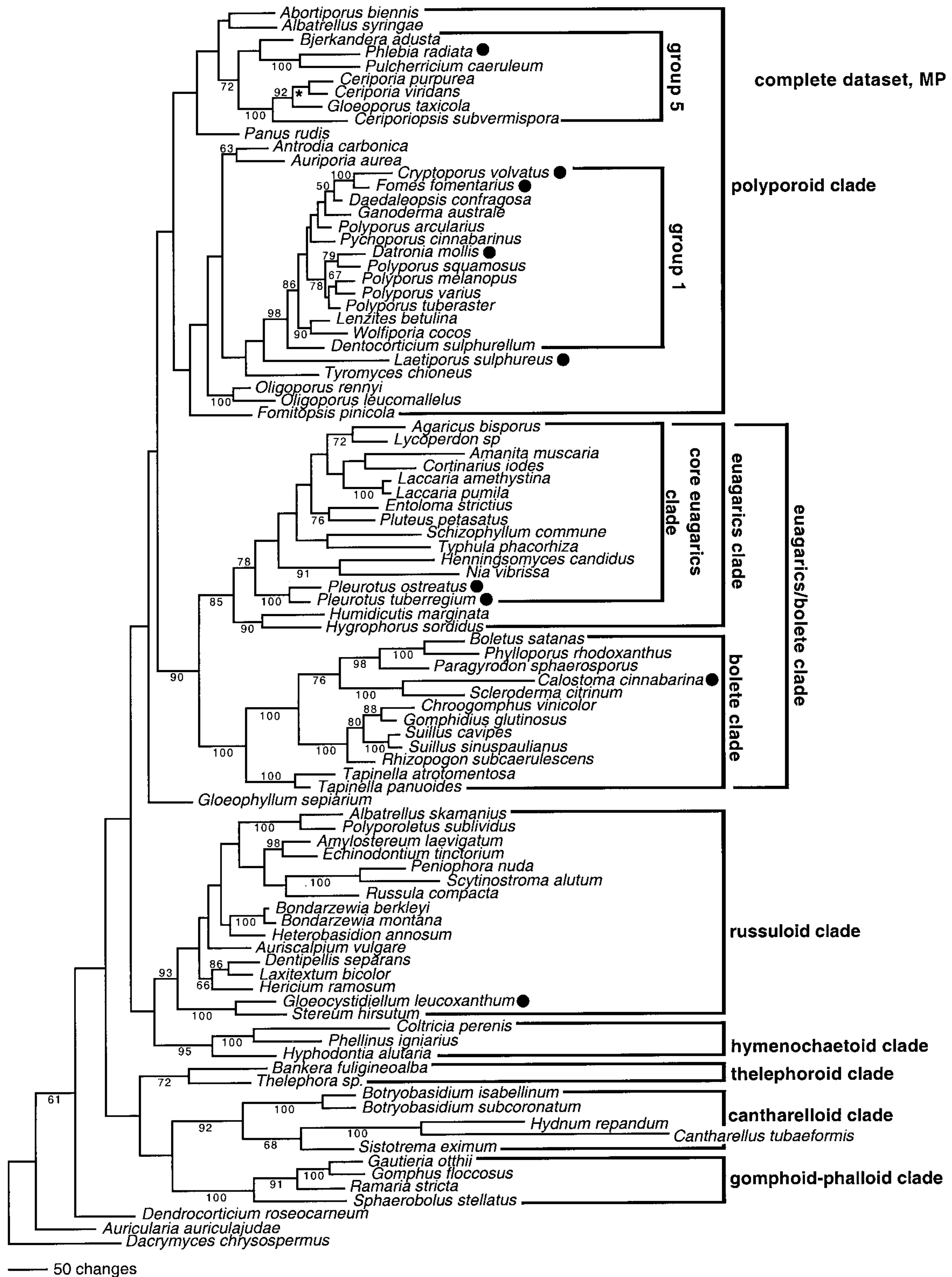

FIG. 4. Maximum-parsimony tree from analysis of complete dataset. Tree 1/2. Asterisk indicates node that collapses in strict consensus of both equally parsimonious trees. Dots next to taxon names indicate species that are involved in apparent conflicts between the mt-ssu rDNA and mt-lsu rDNA data partitions.

The resolution of the major clades of homobasidiomycetes was not sensitive to the inclusion of the nine species that were involved in the conflicts, and in most cases support for the major clades was within 10 bootstrap percentage points in both the core and complete analyses. Moreover, the placements of most of the spe-

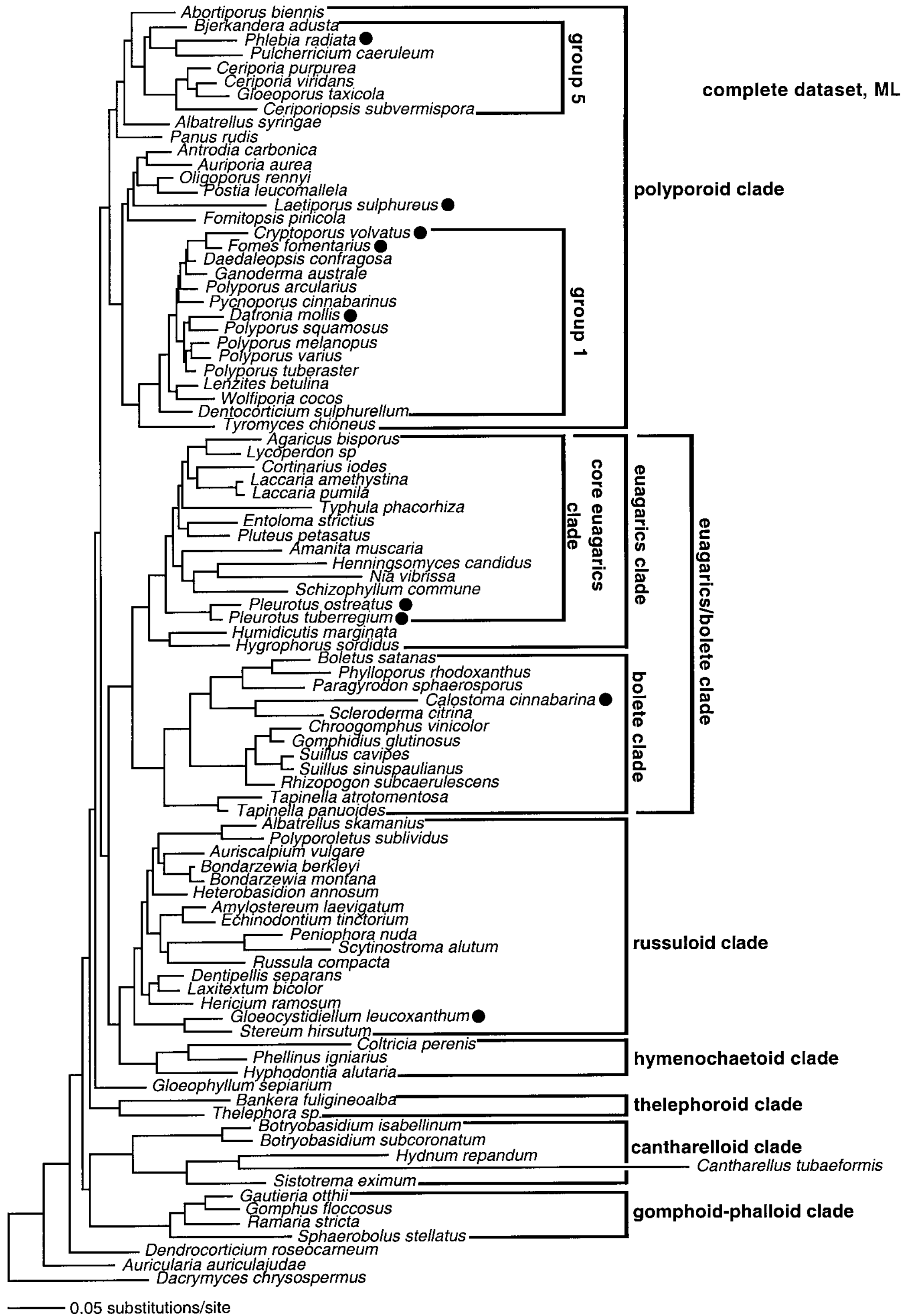

FIG. 5. Maximum-likelihood tree derived from analysis of the complete dataset. Dots indicate taxa that are involved in apparent conflicts between the mt-ssu rDNA and mt-lsu rDNA data partitions.

cies involved in the intragenomic conflicts were strongly supported by bootstrapping in the complete analysis, and were consistent with expectations based on morphology. For example, *Phlebia radiata* was strongly supported as the sister group of *Pulcherricium caeruleum* in the complete analysis (bootstrap = 100%;

Fig. 4). *Phlebia* and *Pulcherricium* have a number of attributes in common, including a bipolar mating system, which is a relatively uncommon, apomorphic condition in homobasidiomycetes (Hibbett and Donoghue, 2001; Gilbertson and Ryvarden, 1986; Nakasone, 1990; Stalpers, 1978). Similarly, *Fomes fomentarius, Cryptoporus volvatus,* and *Datronia mollis* are all placed in a strongly supported (bootstrap = 98%) clade that is united by the possession of binding hyphae or branched skeletal hyphae, which confer toughness to the fruiting bodies (Gilbertson and Ryvarden, 1986; Hibbett and Donoghue, 1995; Figs. 4 and 5). The placements of *Gloeocystidiellum leucoxantha, Calostoma cinnabarina,* and *Pleurotus ostreatus* and *P. tuberregium* are also strongly supported in the complete analysis (Fig. 4). The only species involved in intragenomic conflicts whose placement was not strongly supported in the complete analysis is the polypore, *Laetiporus sulphureus* (Fig. 4). In the parsimony analysis of the complete dataset, *L. sulphureus* was weakly supported as the sister group of the "group 1" species, which is surprising because all members of group 1 (except *Wolfiporia cocos*) can decay lignin, while *L. sulphureus* lacks this ability. However, in the ML analysis of the complete dataset *L. sulphureus* is nested among other taxa that lack the ability to decay lignin (*Antrodia carbonica, Auriporia aurea, Oligoporus rennyi*; Fig. 5), which is more likely the correct placement.

In summary, the overall topology of the homobasidiomycetes is not sensitive to the inclusion or exclusion of the nine species involved in the apparent conflicts between mt-ssu rDNA and mt-lsu rDNA. While we cannot rule out the possibility that these regions have different underlying phylogenies, this does not appear to be a significant source of error.

Higher-Level Relationships of Homobasidiomycetes

Two major groups that have not previously been resolved with confidence were supported in this analysis: the core euagarics clade (all members of the euagarics clade except the Hygrophoraceae; bootstrap = 97–78%) and the euagarics/bolete clade (bootstrap = 94–90%; Figs. 1–5, Table 2). The inclusion of the Hygrophoraceae in the euagarics clade is consistent with the nuc-lsu rDNA-based results of Moncalvo *et al.* (2000; *contra* Bruns *et al.,* 1998). However, whereas the analysis of Moncalvo *et al.* (2000) suggested that the Hygrophoraceae is polyphyletic and nested within the euagarics clade, the present results suggest that it is the sister group of the rest of the euagarics clade. This finding should empower future studies of the euagarics clade by providing an outgroup for analyses of the core euagarics clade. It also has implications for the hypothesized morphology and nutritional mode of the ancestors of the euagarics clade and euagarics/bolete clade, as discussed below.

The sister-group relationship of the euagarics clade and bolete clade has been resolved previously, but never with strong bootstrap support (Hibbett *et al.,* 1997; Moncalvo *et al.,* 2000). The euagarics clade has been estimated to include about 8400 described species, and the bolete clade has been estimated to contain about 840 described species (Hibbett and Thorn, 2001). Thus, the euagarics/bolete clade contains about 70% of the roughly 13,000 described species of homobasidiomycetes (Hawksworth *et al.,* 1995), including the vast majority of stipitate–pileate mushrooms.

Although the sampling of the euagarics clade in the present study is limited, the position of the Hygrophoraceae as the sister group of the core euagarics clade suggests that the ancestral morphology of the euagarics clade was agaricoid. The ancestral morphology of the bolete clade is more ambiguous, however. In the present analysis, the bolete clade contains three strongly supported clades: a clade containing *Tapinella atrotomentosa* and *T. panuoides,* and two clades that represent the "suilloid group" and "boletoid group" described by Bruns *et al.* (1998). The *Tapinella* clade is strongly supported as the sister group of the rest of the bolete clade (Figs. 1–5). *Tapinella atrotomentosa* and *T. panuoides* both have lamellate hymenophores, which would seem to suggest that the ancestral morphology of both the bolete clade and euagarics/bolete clade was agaricoid. However, more inclusive studies in the bolete clade have demonstrated that the clade that includes *Tapinella* also includes the corticioid *Coniophora, Serpula,* and *Leucogyrophana,* which develop smooth or merulioid hymenophores (Bruns *et al.,* 1998; Bresinsky *et al.,* 1999; Binder and Bresinsky, unpublished). Moreover, analyses by Hibbett *et al.* (2000) suggest that the corticioid *Amphinema byssoides* could be the sister group of the entire bolete clade, although this result was not strongly supported. Thus, the present sample of taxa suggests that the ancestor of the euagarics/bolete clade was a gilled mushroom, but consideration of unsampled taxa suggests that it might also have been a resupinate fungus.

The finding that the euagarics clade and bolete clade are sister taxa also has implications for the evolution of nutritional modes in homobasidiomycetes. Recently, Hibbett *et al.* (2000) presented a tree that showed the euagarics clade and bolete clades as sister taxa, and suggested that the nutritional mode of their most recent common ancestor was ectomycorrhizal. If so, the saprotrophic members of the euagarics clade and bolete clade (which comprise over half of all known saprotrophic homobasidiomycetes) are ultimately derived from ectomycorrhizal forms (Hibbett *et al.,* 2000). The present analysis supports the view that the euagarics clade and bolete clade are sister taxa, but does not resolve the nutritional mode of their most recent common ancestor. One source of ambiguity is the limited sampling and lack of robustness in the core euagarics clade (Figs. 1 and 3–5). In addition, the plesi-

omorphic nutritional mode of the Hygrophoraceae is poorly understood and potentially of critical importance to resolving the plesiomorphic condition of the euagarics clade as a whole (*Hygrophorus* is regarded as ectomycorrhizal, but *Hygrocybe* is probably saprotrophic). The unsampled *Amphinema byssoides* could again be pivotal. In the present trees, the saprotrophic *Tapinella* clade is the sister group of the rest of the bolete clade, which might suggest that the plesiomorphic nutritional mode of the bolete clade was saprotrophic. However, this could change with the inclusion of *Amphinema,* which is an ectomycorrhizal associate of *Picea* (Danielson and Pruden, 1989).

Except for the sister-group relationship of the euagarics clade and bolete clade, the higher-order relationships among the major clades of homobasidiomycetes were not resolved (Fig. 2). The internal nodes along the backbone of the homobasidiomycete phylogeny appear to be quite short relative to more terminal branches, suggesting that the divergences among the major clades of homobasidiomycetes may have occurred rapidly. If so, it may be very difficult to resolve relationships among these groups with molecular sequence data from any source. Resolving the backbone of the homobasidiomycete phylogeny may require characters that do not evolve in a clocklike fashion, but rather evolve episodically, perhaps including major molecular rearrangements, gene duplications, or anatomical features. So far, there are few morphological characters that have shown promise for resolving higher-order relationships in homobasidiomycetes. Examples include septal pore ultrastructure and cytological aspects of basidiosporogenesis (Hibbett and Thorn, 2001). Biochemical characters may also provide clues to higher-order relationships in homobasidiomycetes. For example, the related compounds, thelephoric acid and atromentin, occur in the thelephoroid clade and in some members of the bolete clade (e.g., *Boletus separans*) and in the russuloid clade (e.g., *Albatrellus* spp.), suggesting that these groups might be closely related (Gill and Steglich, 1987).

Dendrocorticium roseocarneum and *Gloeophyllum sepiarium* are the only species that could not be placed in any of the major clades of homobasidiomycetes. Both species have been problematical in previous studies using various rDNA regions (Hibbett and Donoghue, 1995; Hibbett *et al.,* 1997; Thorn *et al.,* 2000; Hibbett and Donoghue, 2001). *Gloeophyllum sepiarium* has been shown to be closely related to *Neolentinus* and *Heliocybe,* with which it shares a brown rot mode of wood decay, a bipolar mating system, and binucleate basidiospores (Hibbett *et al.,* 1995; Hibbett and Donoghue, 2001; Thorn *et al.,* 2000). However, the placement of the *Gloeophyllum/Neolentinus/Heliocybe* clade has not been resolved with confidence. Analyses of rDNA internal transcribed spacer (ITS) sequences by Boidin *et al.* (1998) and nuc-lsu rDNA sequences by E. Langer (personal communication) suggest that *Dendrocorticium* is closely related to *Vuilleminia* and *Punctularia*. Based on the results of Boidin *et al.* (1998), Hibbett and Thorn (2001) tentatively concluded that these taxa are in the polyporoid clade. This is inconsistent with the present results, which weakly support *Dendrocorticium* as the sister group of the rest of the homobasidiomycetes. To infer the plesiomorphic form of the homobasidiomycetes it is vital to resolve their basal relationships. Therefore, we will continue to investigate the position of *Dendrocorticium* and its putative relatives, *Vuilleminia* and *Punctularia*.

What Is the Optimal rDNA Data Partition for Phylogenetic Analyses of Homobasidiomycetes?

The four-region dataset provided the most robust support of the homobasidiomycete phylogeny overall, but for many studies it is not feasible to sequence 3.8 kb per individual. Therefore, the following discussion focuses on the phylogenetic utility of subpartitions of the four-region dataset.

Datasets containing single rDNA regions have been used in several broad analyses of homobasidiomycetes, including those of Hibbett and Donoghue (1995), which used mt-ssu rDNA; Bruns *et al.* (1998), which used mt-lsu rDNA; Gargas *et al.* (1996), which used nuc-ssu rDNA; and Thorn *et al.* (2000) and Moncalvo *et al.* (2000), which used nuc-lsu rDNA. The present results (Table 2) suggest that the addition of even a single rDNA region to these datasets could significantly improve their ability to resolve major clades of homobasidiomycetes. Moreover, these results suggest which combinations of regions might provide the greatest improvements in support for the least sequencing effort. For example, if one had a dataset of nuc-lsu rDNA sequences, the addition of mt-ssu rDNA, which is only 0.5 kb, would probably add more support than the addition of nuc-ssu rDNA, which is 1.8 kb (Table 2).

The levels of support conferred by particular data partitions differ from clade to clade. For example, the strongest two-region dataset overall is the mt-ssu/nuc-ssu rDNA data partition, but the hymenochaetoid clade receives much stronger support from the mt-ssu/mt-lsu data partition (93% vs <50%), and the thelephoroid clade receives much stronger support from the nuc-ssu/nuc-lsu rDNA data partition (92% vs <50%). In addition, three of the focal clades—the euagarics clade, thelephoroid clade, and homobasidiomycete clade—receive significantly weaker support from the four-region dataset than they do from certain subpartitions of the data ("significance" being arbitrarily equated with a difference of at least 10 bootstrap percentage points). In these cases, the distribution of bootstrap support among the three-region and four-region partitions suggests which regions could be destabilizing each clade. The euagarics clade receives <50 to 76% bootstrap support from the four-region dataset

and all three-region datasets that include the nuc-lsu rDNA, but receives 92% bootstrap support from the three-region data partition that excludes the nuc-lsu rDNA, which suggests that the nuc-lsu rDNA weakens support for the euagarics clade (Table 2). By the same reasoning, the thelephoroid clade and homobasidiomycete clade appear to be destabilized by the mt-lsu rDNA (Table 2). We do not know why the inclusion of nuc-lsu rDNA and mt-lsu rDNA sequences reduces support for certain clades; it could be due to actual conflicts among gene phylogenies (although these were not detected), excessive homoplasy, or possibly sequencing or alignment error.

CONCLUSIONS

The analyses presented here strongly support the monophyly of the eight major clades of homobasidiomycetes recognized by Hibbett and Thorn (2001), except the polyporoid clade, which is still weakly supported. These analyses also provided strong support for the core euagarics clade (with its sister group, the Hygrophoraceae) and the euagarics/bolete clade, which have not previously been resolved with confidence. Thus, analyses of the four-region dataset improve understanding of the higher-level relationships of homobasidiomycetes. Nevertheless, even with 3.8 kb of sequence per taxon, the backbone of the homobasidiomycete phylogeny and the internal topologies of several major clades remain poorly understood. Resolving these relationships may be among the most challenging problems in homobasidiomycete systematics.

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REFERENCES

Boidin J., Mugnier, J., and Canales, R. (1998). Taxonomie moleculaire des Aphyllophorales. *Mycotaxon* **66:** 445–491.

Bresinsky A., Jarosch, M., Fischer, M., Schönberger, I., and Wittmann-Bresinsky, B. (1999). Phylogenetic relationships within *Paxillus* s.l. (Basidiomycetes, Boletales): Separation of a southern hemisphere genus. *Plant Biol.* **1:** 327–333.

Bruns, T. D., and Szaro, T. M. (1992). Rate and mode differences between nuclear and mitochondrrial small-subunit rRNA genes in mushrooms. *Mol. Biol. Evol.* **9:** 836–855.

Bruns, T. D., Szaro, T. M., Gardes, M., Cullings, K. W., Pan, J. J., Taylor, D. L., Horton, T. R., Kretzer, A., Garbelotto, M., and Li, Y. (1998). A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. *Mol. Ecol.* **7:** 257–272.

Danielson, R. M., and Pruden, M. (1989). The ectomycorrhizal status of urban spruce. *Mycologia* **81:** 335–341.

Donk, M. A. (1964). A conspectus of the families of the Aphyllophorales. *Persoonia* **3:** 199–324.

Fayod, V. (1889). Prodrome d'une histoire naturelle des agaricinées. *Ann. Sci. Bot., Sér. 7,* **9:** 181–411.

Fries, E. M. (1874). "Hymenomycetes Europaei. Upsaliae," E. Berling, Uppsala.

Gargas, A., DePriest, P. T., Grube, M., and Tehler, A. (1996). Multiple origins of lichen symbioses in fungi suggested by ssu rDNA phylogeny. *Science* **268:** 1492–1495.

Gilbertson, R. L., and Ryvarden, L. (1986). "North American Polypores, vol. 1," Fungiflora, Oslo, Norway.

Gill, M., and Steglich, W. (1987). Pigments of fungi (macromycetes). *Progr. Chem. Org. Nat. Prod.* **51:** 1–317.

Goldman, N., Anderson, J. P., and Rodrigo, A. G. (2000). Likelihood-based tests of topologies in phylogenetics. *Syst. Biol.* **49:** 652–670.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C., and Pegler, D. N. (1995). "Dictionary of Fungi, 8th ed.," CAB International, Wallingford, UK.

Hibbett, D. S. (1996). Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. *Mol. Biol. Evol.* **13:** 903–917.

Hibbett, D. S., and Donoghue, M. J. (1995). Progress toward a phylogenetic classification of the Polyporaceae through parsimony analysis of mitochondrial ribosomal DNA sequences *Can. J. Bot.* **73:** 853–861.

Hibbett, D. S., and Donoghue, M. J. (2001). Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. *Syst. Biol.* **49:** 215–242.

Hibbett, D. S., and Thorn, R. G. (2001). Basidiomycota: Homobasidiomycetes. *In* "The Mycota VII Part B, Systematics and Evolution" (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, Eds.), pp 121–168. Springer-Verlag, Berlin.

Hibbett, D. S., Gilbert, L.-B., and Donoghue, M. J. (2000). Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature (London)* **407:** 506–508.

Hibbett, D. S., Pine, E. M., Langer, E., Langer, G., and Donoghue, M. J. (1997). Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proc. Natl. Acad. Sci. USA* **94:** 12002–12006.

Jülich, W. 1981. "Higher Taxa of Basidiomycetes," J. Cramer, Vaduz, Liechtenstein.

Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* **29:** 170–179.

Kretzer, A. M., and Bruns, T. D. (1999). Use of *atp*6 in fungal phylogenetics: An example from the Boletales. *Mol. Phyl. Evol.* **13:** 483–492.

Moncalvo, J. M., Lutzoni, F. M., Rehner, S. A., Johnson, J., and Vilgalys, R. (2000). Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Syst. Biol.* **49:** 278–305.

Nakasone, K. (1990). Cultural studies and identification of wood-inhabiting Corticiaceae and selected Hymenomycetes from North America. *Mycol. Mem.* **15:** 1–412.

Oberwinkler, F. (1977). Das neue System der Basidiomyceten. *In* "Beiträge zur Biologie der Niederen Pflanzen" (H. Frey, H. Hurka, and F. Oberwinkler, Eds.), pp 59–105, G. Fischer, Stuttgart, Germany.

Patouilliard, N. (1900). "Essai Taxonomique sûr les Familles et les Genres des Hyménomycètes," Lucien Declume, Lons-le-Saunier.

Stalpers, J. (1978). Identification of wood-inhabiting Aphyllophorales in pure culture. *Stud. Mycol.* **16:** 1–248.

Swofford, D. L. (1999). PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), Version 4.0, Sinauer Associates, Sunderland, MA.

Templeton, A. R. (1983). Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37:** 221–244.

Thorn, G. R., Moncalvo, J. M., Reddy, C. A., and Vilgalys, R. (2000). Phylogenetic analyses and the distribution of nematophagy support a monophyletic Pleurotaceae within the polyphyletic pleurotoid-lentinoid fungi. *Mycologia* **92:** 241–252.

White T. J., Bruns, T. D., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* "PCR Protocols, A Guide to Methods and Applications" (M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, Eds.), pp 315–322. Academic Press, San Diego, CA.