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Phylogenetic analyses of the *Lyophylleae* (*Agaricales*, *Basidiomycota*) based on nuclear and mitochondrial rDNA sequences

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Current classifications of the *Lyophylleae* and the importance of siderophilous granulation in the basidia for the classification of agaricoid fungi were evaluated using parsimony analyses of sequence data from the nuclear ribosomal large subunit gene (nLSU), the internal transcribed spacer region of the nuclear ribosomal array (ITS), and the mitochondrial ribosomal small subunit gene (mtSSU). These three different data partitions were phylogenetically congruent on the basis of the Mickevich–Farris statistical test, but not from the ILD and the Templeton tests. Bootstrap supports for nodes in phylogenetic trees generated from combined nLSU, ITS, and mtSSU sequence data were generally higher than those in trees generated from individual data sets. This suggests a lack of major conflict in the phylogenetic signal among the different data sets. We conclude that the Mickevich–Farris test is more appropriate for estimating congruence and combinability between different sources of molecular data than the more widely used ILD and Templeton tests, at least when the different data sets have their respective resolution power at different depths in the phylogeny. Results of the combined analyses show that the *Entolomataceae* are a sister group to a clade composed of the *Lyophylleae*, *Termitomyceteae*, and *Tricholomateae p.p.* This implies that presence of siderophilous granulation in the basidia of agaric fungi has probably a single origin, and would have been lost in the *Tricholomateae*. Inclusion of the *Termitomyceteae* within the *Lyophylleae* suggests homology of the macro type granulation. Because the exact placement of *Tricholomateae* pro parte remains uncertain, it remains unclear whether the *Lyophylleae* (including *Termitomyceteae*) are monophyletic or paraphyletic. Within the *Lyophylleae*, genera *Lyophyllum* and *Calocybe* are shown to be artificial, as are *Lyophyllum* sections *Lyophyllum*, *Difformia*, and *Tephrophana*. Four main natural groups of *Lyophylleae* have been identified that should serve as a basis for developing a more natural classification system for these fungi.

INTRODUCTION

Development of a natural classification system for gilled fungi and their allies (*Agaricales*, *Basidiomycota*) is increasingly relying on molecular data. Morphological data have been shown to be of limited value for fungal systematics due to their inherent simplicity, evolutionary convergence, parallelisms, and phenotypic plasticity (Bruns, White & Taylor 1991, Bruns *et al.* 1992, Hibbett & Vilgalys 1993, Vilgalys, Hopple & Hibbett 1994, Hibbett & Donoghue 1995, Hibbett *et al.* 1997, Johnson & Vilgalys 1998, Johnson 1999, Drehmel, Vilgalys & Moncalvo 1999, Hopple & Vilgalys 1999, Pine, Hibbett & Donoghue 1999, Moncalvo *et al.* 2000a, Wagner & Fisher 2001, Hibbett & Thorn 2001, Moncalvo *et al.* 2002). In this study we use nucleotide sequence data from the nuclear ribosomal large subunit gene (nLSU), the internal transcribed spacer region of the nuclear ribosomal array (ITS), and the mitochondrial ribosomal small subunit gene (mtSSU) to infer phylogenetic relationships in the *Lyophylleae* and allied taxa.

The tribe *Lyophylleae* (Kühner 1938) has traditionally been classified in the *Tricholomataceae*, a family that contains an artificial assembly of white or pale spored mushrooms (Moncalvo *et al.* 2000a). The *Lyophylleae* was introduced to accommodate taxa characterized by basidia having siderophilous granulation (Kühner 1938). However, electron microscopy has revealed several different types of siderophilous granules, which are more widely distributed in the *Hymenomycetes* than previously thought (Clémençon 1978). For instance, the fact that the *Termitomyceteae*, previously classified in the *Amanitaceae* (Singer 1962, Pegler 1977), possess

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granulation of the macro-type found also in the *Lyophylleae* (Clémençon 1984) was advanced by Singer (1986) as one reason for transferring the tribe to the *Tricholomataceae* near the *Lyophylleae* (Singer 1986). Several members of the *Entolomataceae* (Singer 1986) possess a granulation of the micro-type, that might also imply a relationship with the *Lyophylleae* (Kühner & Romagnesi 1953). Overall, the presence of siderophilous granulation in fungi other than the *Lyophylleae* suggests that the tribus as previously delimited may not represent a natural group.

Within the *Lyophylleae* itself, taxonomic circumscription has also been controversial (Kühner & Romagnesi 1953, Moser 1978, Singer 1986, Bon 1999a). Singer (1986) recognized four genera, *Lyophyllum* (Karsten 1881), *Asterophora* Ditmar ex Link (Ditmar 1809), *Calocybe* (Donk 1962) and *Hypsizygus* (Singer 1947), and segregated *Lyophyllum* into three sections: sect. *Lyophyllum* for blackening types; sect. *Difformia* for tricholomatoid to clitocyboid types; and sect. *Tephrophana* for collybioid types. Other authors placed greater taxonomic significance on the collybioid habit, and ranked the last section at the genus (*Tephrocybe*; Moser 1978) or tribe (*Tephrocybeae*; Bon 1999a) levels. Taxonomists have also disagreed about the limits between *Calocybe* and *Lyophyllum*. Singer (1986) treated these as two distinct genera, while Kühner & Romagnesi (1953) and Kühner (1980) consider *Calocybe* as a section of *Lyophyllum*. Recently, Bon (1999a) transferred several species of *Calocybe* into *Rugosomyces*, retained *Calocybe constricta* (recognized here as *Tricholomella constricta*), and transferred two blackening *Lyophyllum* species (*L. favrei* and *L. ochraceum*) to *Calocybe* (Bon 1999b). Overall, differences in taxonomic delimitation, which reflect divergences in perception of significant taxonomic characters, emphasize the need for more natural groupings. The difficulties inherent to *Lyophyllum* taxonomy were highlighted by Clémençon & Smith (1983), who were forced to cite key characters from different sections in descriptions of new species from North America, prompting the authors to acknowledge the need for revision of the genus. While section *Difformia* has been revised (Moncalvo, Toriola & Clémençon 1991, Moncalvo, Rehner & Vilgalys 1993, Moncalvo & Clémençon 1994) and reduced to a single complex surrounding *Lyophyllum decastes*, a broader phylogenetic study of the entire tribe *Lyophylleae* is still lacking.

The main questions addressed in this study are: (1) Are siderophilous granules in the basidia homologous among gilled fungi, and can this character be used to define taxonomic groups? (2) Do the *Lyophylleae* form a natural group, and if so, what are its phylogenetic affinities? (3) What are the natural groups within the *Lyophylleae*, and do they correspond to those defined by morphology based systematics?

We also used our three independent molecular data sets to explore the relevancy and limits of several tests (Mickevitch *et al*. 1981, Templeton 1983, Farris *et al*. 1995a, b) commonly used to determine congruence and combinability between different data partitions in an effort to resolve issues still debated in systematics (Myamoto 1985, Kluge 1989, Bull *et al*. 1993, de Queiroz 1993, Huelsenbeck & Bull 1996, Lutzoni 1997, Cunningham 1997a, b, Soltis *et al*. 1998, Slowinsky & Page 1999, McCracken *et al*. 1999, Moncalvo, Drehmel & Vilgalys 2000b).

MATERIALS AND METHODS

Taxon sampling

The taxa considered in this study (Table 1) include 28 species representing all the segregated genera, subgenera and sections of the tribe *Lyophylleae* (Kühner 1953, 1980, Moser 1978, Singer 1986, Bon 1999a) with the exception of the small subgenus *Lyophyllopsis* (syn. *Gerhardtia*). Eighteen of the 68 species listed within *Lyophyllum* by Singer (1986) were sampled. To test for monophyly of the *Lyophylleae*, we also sampled species of tribes *Tricholomateae* and *Termitomyceteae*, which both morphology (Singer 1986) and molecular (Moncalvo *et al*. 2000a) evidence have shown to be closely related to *Lyophylleae*. Members of the *Entolomataceae* were also sampled in order to explore the putative homology between micro and macro-type of siderophilous granulation in the basidia. For global rooting purposes (Farris 1972, Maddison, Donoghue & Maddison 1984), species from presumably more distantly related genera were sampled: *Amanita*, *Gymnopus*, *Agaricus*, *Coprinus*, *Cystolepiota*, *Lepiota*, *Ripartitella*, and *Cystoderma*. Several nLSU and mtSSU sequences for these outgroup taxa were already available from earlier studies (Johnson & Vilgalys 1998, Johnson 1999, Moncalvo *et al*. 2000b).

Molecular techniques

DNA was isolated from lyophilized mycelia grown in liquid culture (in malt or cherry extracts), fresh fruit bodies, or dried herbarium material (Table 1). Standard DNA isolation methods employing CTAB lysis buffer (Zolan & Pukkila 1986) or mini columns filtration (Dneasy Plant Mini Prep, Qiagen) were used. The second procedure was found to be most helpful for DNA isolation from strains producing pigments or polysaccharides in liquid culture. For material with more resistant hyphae, cell walls were first broken in a microwave oven (Goodwin & Lee 1993). PCR amplification followed a modified Vilgalys & Hester (1990) procedure using 2 mM $MgCl_2$, 0.4 μg μl^{-1} of bovine serum albumin (Hillis, Moritz & Mable 1996), and chemistries from Qiagen or Perkin Elmer. Amplified PCR products were purified using either Genclean® II Kit (Bio 101 Inc.) prior to manual sequencing or Ultrafree-MC filters (Millipore) prior to automated sequencing. Manual sequencing was conducted using

Table 1. Organisms used and GenBank accession nos.

Taxa[a]	Collection[b]	DNA source[c]	GenBank accession nos.[d] nLSU	mtSSU	ITS
Family *Tricholomataceae*					
Tribe *Lyophylleae*					
Genus *Lyophyllum*					
sect. *Lyophyllum*					
L. favrei	IE-BSG-BSI94cp2	Cpph	AF223182	AF357103	AF357035
L. favrei	IE-BSG-HAe234.97	Cpph	AF223183	AF357102	AF357034
L. favrei	IE-BSG-HC96cp4	Cpph	AF223184	AF357104	–
L. semitale	CBS 369.47	MAA	AF223207	AF357124	AF357048
L. semitale	IE-BSG-HC85/13	MAA	AF042581	AF357125	AF357049
L. sykosporum	IE-BSG-HCM3	MAA	AF357073	AF357127	AF357051
L. sykosporum	CBS 319.80/IFO 30978	MAA	AF223208	AF357126	AF357050
L. caerulescens	IE-BSG-HC80/140	MAA	AF223209	AF357128	AF357052
L. ochraceum	IE-BSG-BSI94.cp1	Cpph	AF223185	AF357143	AF357033
'*L. leucophaeatum*'[e] (= *L. gangraenosum*)	CBS 695.87	MAA	AF357074	–	–
L. leucophaeatum (= *L. gangraenosum*)	IE-BSG-HAe251.97	Cpph	AF223202	AF357101	AF357032
sect. *Tephrophana*					
'*T. tylicolor*'[e]	SAR4735	MAA	AF357070	–	–
T. tylicolor	IE-BSG-Sag5-27/11	MAA	AF223194	AF357111	AF357039
T. tylicolor	IE-BSG-Sag5/27lyo9	MAA	AF223193	AF357113	–
T. tylicolor	IE-BSG-BSI92/245	MAA	AF223195	AF357112	AF357040
'*T. tylicolor*'[e]	CBS 165.50	MAA	AF357072	–	–
T. tylicolor	CBS 362.80	MAA	AF223192	AF357114	–
T. anthracophila	IE-BSG-BSI94/88	MAA	AF223211	AF357130	AF357054
'*T. anthracophila*'[e]	CBS 930.72	MAA	AF357064	–	–
'*T. anthracophila*'[e]	CBS 823.87	MAA	AF357076	–	–
'*T. anthracophila*'[e]	CBS 825.87	MAA	AF357075	–	–
T. anthracophila	IE-BSG-HC79/132	MAA	AF223212	AF357132	AF357055
T. anthracophila	CBS 156.44	MAA	AF223213	AF357131	AF357056
'*T. anthracophila*'[e]	CBS 325.80/IFO 30976	MAA	AF357077	–	–
T. ambusta	CBS 450.87	MAA	AF223214	AF357135	AF357058
T. ambusta	CBS 451.87	MAA	AF223215	AF357134	–
T. ambusta	CBS 452.87	MAA	AF223216	AF357133	AF357057
T. gibberosa (≠ *T. ambusta*)	CBS 320.80/IFO 30977	MAA	AF223198	AF357117	AF357042
T. gibberosa (≠ *T. ambusta*)	CBS 321.80/IFO 30331	MAA	AF223196	AF357116	–
T. gibberosa (≠ *T. ambusta*)	CBS 328.50	MAA	AF223197	AF357115	AF357041
T. atrata	CBS 709.87	MAA	AF223210	AF357129	AF357053
'*T. atrata*'[e]	CBS 710.87	MAA	AF357069	–	–
'*T. atrata*'[e]	CBS 712.87	MAA	AF357068	–	–
'*T. mephitica*'[e]	CBS 168.50	CHA	AF357079	–	–
T. palustris	CBS 714.87	CHA	AF223199	AF357118	AF357043
'*T. palustris*'[e]	CBS 715.87	CHA	AF357071	–	–
T. palustris	CBS 717.87	CHA	AF223200	AF357119	AF357044
T. inolens	CBS 330.85	CHA	AF223201	AF357120	AF357045
T. rancida	CBS 204.47	CHA	AF223203	AF357094	AF357025
T. boudieri	IE-BSG-HC78U	CHA	AF223206	AF357121	AF357046
T. boudieri	CBS 379.88	CHA	AF223205	AF357123	–
'*T. boudieri*'[e]	CBS 369.82	CHA	AF357067	–	–
T. boudieri	IE-BSG-BSI96/84	CHA	AF223204	AF357122	AF357047
'*T. boudieri*'[e]	CBS 563.85	CHA	AF357066	–	–
sect. *Difformia*					
L. decastes	IE-BSG-Lc4-2(T5P)	MAA	AF357078	AF357137	AF357060
L. decastes	IE-BSG-JM 87/16 (T1)	MAA	AF042583	AF357136	AF357059
Genus *Asterophora*					
A. parasitica	CBS 683.82	MAA	AF223191	AF357110	AF357038
A. lycoperdoides	CBS 170.86	MAA	AF223190	AF357109	AF357037
Genus *Calocybe*					
C. fallax	IE-BSG-HC80/103	CHA	AF223180	AF357099	AF357030
C. persicolor	IE-BSG-HC80/99	CHA	AF223176	AF357095	AF357026
C. ionides	IE-BSG-HC77/133	CHA	AF223179	AF357098	AF357029
C. obscurissima	IE-BSG-HC79/181	CHA	AF223181	AF357100	AF357031
C. carnea	CBS 552.50	CHA	AF223178	AF357097	AF357028
C. gambosa	IE-BSG-HC78/64	CHA	AF223177	AF357096	AF357027
Genus *Tricholomella*[f]					
T. constricta (= *Calocybe constricta*)[f]	IE-BSG-HC80/148	MAA	AF223187	AF357106	–

[*continues overleaf*

Table 1 (*cont.*)

Taxa[a]	Collection[b]	DNA source[c]	GenBank accession nos.[d]		
			nLSU	mtSSU	ITS
T. constricta (= *Calocybe constricta*)[f]	CBS 660.87	MAA	AF223186	AF357108	–
T. constricta (= *Calocybe constricta*)[f]	CBS 320.85	MAA	AF223189	AF357107	–
T. constricta (= *Calocybe constricta*)[f]	IE-BSG-HC84/75	MAA	AF223188	AF357105	AF357036
Genus *Hypsizygus*					
H. ulmarius	DUKE-JM/HW	DNA	AF042584	AF357140	–
Tribe *Termitomyceteae*					
Genus *Termitomyces*					
T. cylindricus	DUKE-JMleg.HSEUs.n.	–	AF042585	–	–
T. heimii	DUKE-JMleg.MUIDs.n.	Cpph	AF042586	AF357091	AF357022
T. clypeatus	DUKE-JMleg.MUIDs.n.	–	AF261398	–	–
T. sp.	IE-BSG-BSIsp.1	MAA	AF223174	AF357093	AF357024
T. microcarpus (= *Podabrella microcarpa*)[g]	DUKE-PRU3900	Cpph	AF042578	AF357092	AF357023
Tribe *Tricholomateae* (*p.p.*)					
Clitocybe dealbata	IE-BSG-HC95.cp3	Cpph	AF223175	AF357138	AF357061
C. dealbata	DUKE-JMs.n.	–	AF042589	–	–
C. connata (= *Lyophyllum connatum*)[h]	DUKE-JM 90 c	–	AF042590	AF357139	–
C. ramigena	DUKE-SRs.n.	–	AF042648	–	–
Lepista nebularis (= *Clitocybe nebularis*)	CBS 362.65	MAA	AF223217	AF357142	AF357063
L. nuda	DUKE-RV84/1	DNA	AF042624	AF357141	AF357062
Tricholoma pardinum	KMS278	DNA	U76462	AF357080	AF357014
T. portentosum	KMS591	DNA	U76464	AF357081	AF357015
T. subaureum	KMS590	DNA	U76466	AF357082	AF357016
T. atroviolaceum	KMS400	–	U76457	–	–
T. venenatum	KMS393	–	U76463	–	–
T. imbricatum	KMS356	–	U76458	–	–
T. caligatum	KMS452	–	U76467	–	–
Leucopaxillus albissimus	SAR1/2/90	–	AF042592	–	–
Tribe *Collybieae*[i]					
Gymnopus polyphilus[i]	DUKE-RV182.01	–	AF042596	–	–
G. acervatus[i]	CBS 174.48	MAA	AF223172	–	–
G. dryophilus[i]	DUKE-RV83.180	–	AF042595	–	–
G. peronatus[i]	CBS 426.79	MAA	AF223173	AF357090	–
Family *Entolomataceae*					
Rhodocybe truncata	CBS 482/50	CHA	AF223167	AF357086	–
R. truncata	CBS 604/79	CHA	AF223168	AF357085	–
R. hirneola	CBS 576.87	CHA	AF223164	–	–
R. hirneola	CBS 577.87	CHA	AF223163	–	–
R. fallax	CBS 605.79	CHA	AF223165	AF357084	AF357018
R. fallax	CBS 129.63	CHA	AF223166	AF357083	AF357017
Clitopilus prunulus	DUKE-RV88/109	–	AF042645	–	–
Entoloma giganteum (= *E. abortivum*)	CBS 143.34	CHA	AF223169	AF357087	AF357019
E. strictius	DUKE-JM96/10	–	AF042620	–	–
E. sericeum	CBS 153.46	CHA	AF223171	AF357088	AF357020
E. sericeum f. nolaniforme	CBS 237.50	CHA	AF223170	AF357089	AF357021
'*E. mammosum*'[e]	CBS 599.79	CHA	AF357065	–	–
Family *Agaricaceae* (*sensu* Redhead *et al.* 2000)[j]					
Coprinus comatus	DUKE-C116	–	AF041529	AF026655	–
Lepiota aspera (= *L. acutesquamosa*)	DUKE-JJ177	–	U85293	U85360	–
Cystolepiota cystidiosa	MICH18884	–	U85298	U85365	–
Agaricus pocillator	DUKE-J173	–	AF04152	U85340	–
Tribe *Cystodermateae* (excluded from *Agariceae*; Moncalvo *et al.* 2000a)					
Cystoderma granulosum	NY-BPI752511	–	U85299	U85369	–
Ripartitella brasiliensis	NY-EFM744	–	U85300	U85370	–
Family *Amanitaceae*					
Amanita muscaria	DUKE-RV/SR	–	AF097367	AF159064	–
A. ceciliae	DUKE-RV6Jul94	–	AF097372	AF159068	–
A. virosa	DUKE-JM97/42	–	AF159086	AF159084	–
A. peckiana	DUKE-RV94.143	–	AF042608	AF159078	–

[a] We follow Singer's (1986) systematic frame. However, because this paper contains a comparison of different analyses in molecular taxonomy rather than a proposal of a new systematic arrangement, we use the generic names currently in use (authorities of names can be found in FUNINDEX (http://194.131.255.3/cabipages/Names/NAMES.ASP)). To facilitate comparison with other publications, we mention selected older names in parentheses. This does not imply that we accept all those names because it is evident from the results presented in Figs 1–5 that the systematics of the *Lyophylleae* are in need of serious corrections.

[α-^{33}P]dATP with the fmol ™DNA Sequencing Kit (Promega), and automated sequencing was conducted using fluorescent dye terminator chemistries on sequencer ABI 373 (Perkin-Elmer). Primers used for PCR amplification and sequencing of the ITS and nrLSU-rDNA regions were SR6R, LR7, LR5, LR16, LR0R, LR3R, LR6, LR4, LR21, LR0, 5.8SR, and ITS2[#] (a location map and oligonucleotide sequences of these primers can be found at http://www.biology.duke.edu/fungi/mycolab/primers.htm). Primers used for PCR amplification and sequencing of the mtSSU were MS1 and MS2 (White *et al.* 1990). Autoradiograms were read with BioMax® BandScanner™SQ sequence image analysis software (Kodak, Scientific Imaging Systems) and assembled with SeqApp (David Gilbert, version 1.9a 169). Sequences obtained by automated sequencing were assembled and edited using the software package Sequencher 3.0 (Gene Codes Corp.).

Phylogenetic analyses

Alignment of nucleotide sequences was performed by eye. Gap regions with ambiguous alignment were excluded from the analyses. nLSU, mtSSU and ITS sequence data were analyzed both separately and in combination. Phylogenetic analyses were conducted in PAUP* version 40b2a (Swofford 1998) with a Power Macintosh G3 computer. Searches for optimal trees used equally weighted parsimony (MP) and/or maximum-likelihood (ML). MP analyses were performed using 100 heuristic searches with random addition sequence and tree bisection-reconnection (TBR) branch swapping. Other search settings in PAUP* were as follows: all characters of type unordered, multistate taxa interpreted as uncertainty, one tree held at each step during stepwise addition, steepest descent option not in effect, branches collapsed if minimum branch length were zero, MAXTREES unlimited, and MULPARS option in effect. Branch robustness was evaluated by bootstrap analyses (Felsenstein 1985, Hillis & Bull 1993), and also by decay analyses (Bremer 1988) when searches generated less than 5000 equally parsimonious trees. Bootstrap analyses included 100 replicates, each consisting of 10 heuristic searches using random addition sequences and TBR branch swapping with MAXTREES unlimited. Faster bootstrap procedures were also used, in which only 10 trees per replicate were retained for TBR branch swapping. Likelihood ratio tests (LRT) were conducted to determine for each data set the 'best-fit model' (Cunningham, Zhu & Hillis 1998, Lio & Goldman 1998, Yang, Goldman & Friday 1994) for ML analyses, following the procedure outlined in Moncalvo *et al.* (2000b). ML analyses used heuristic searches with the settings suggested by LRT, 'asis' addition sequences, and TBR branch swapping.

Congruence tests

Congruence and combinability between the nLSU, mtSSU and ITS data sets were estimated using the incongruence length difference (ILD) test (Farris *et al.* 1995a, b), which corresponds to the partition homogeneity test in PAUP*. Sensitivity of the ILD test to the number of trees retained for branch swapping (MAXTREES setting) and to the number of heuristic replicates performed for each character resampling was explored. Data congruence was also estimated from the Mickevich–Farris index (Mickevich *et al.* 1981) as suggested by Kluge (1989). The Templeton (1983) test, under MP criterion, was used to evaluate topological congruence between trees produced from the different data partitions and to compare topologies between trees produced from unconstrained searches and trees obtained in constraining monophyly of particular groups. The Kishino–Hasegawa (1989) test shown by Goldman, Anderson & Rodrigo (2000) to mislead in many cases when used for topological comparisons was not used in this study. Finally, data congruence was also inferred by comparing bootstrap values for clades revealed in the separated and combined analyses, as suggested by several authors (Kluge 1989, Cunningham 1997a, b, Soltis *et al.* 1998, McCracken *et al.* 1999, Moncalvo *et al.* 2000b).

RESULTS

Data sampling

Over 2100 nucleotides were sequenced for most taxa of the *Lyophylleae* and closely related groups, corre-

[b] Collection sources: CBS, Centraalbureau foor Schimmelcultures, Utrecht; DUKE, Duke University, Durham NC; IE-BSG, Institut d'Ecologie-Botanique Systématique et Géobotanique, University of Lausanne; MICH, University of Michigan; NY, New York Botanical Garden; SAR, Agricultural Research Service, USDA.

[c] DNA source: Cpph, carpophores; MAA, mycelia grown on malt-asparagine-agar; CHA, mycelia grown on cherry-agar; DNA, aliquots of DNA stored at DUKE.

[d] Sequenced regions: nLSU, nuclear ribosomal large subunit; mtSSU, mitochondrial ribosomal small subunit; ITS, internal transcribed spacers 1 and 2 and nuclear ribosomal 5.8S.

[e] Misidentified taxa excluded from the final analyses (see text).

[f] Classification following Kalamees (1992); *Calocybe constricta* in Singer (1986).

[g] Included in *Termitomyces* (Moncalvo *et al.* 2000a).

[h] *Clitocybe connata* (excluded from the *Lyophylleae*; Moncalvo *et al.* 2000a).

[i] Classification following Hughes *et al.* (2000); *Collybia* in Singer (1986).

[j] Classification following Redhead *et al.* (2000); in *Coprinaceae* in Singer (1986).

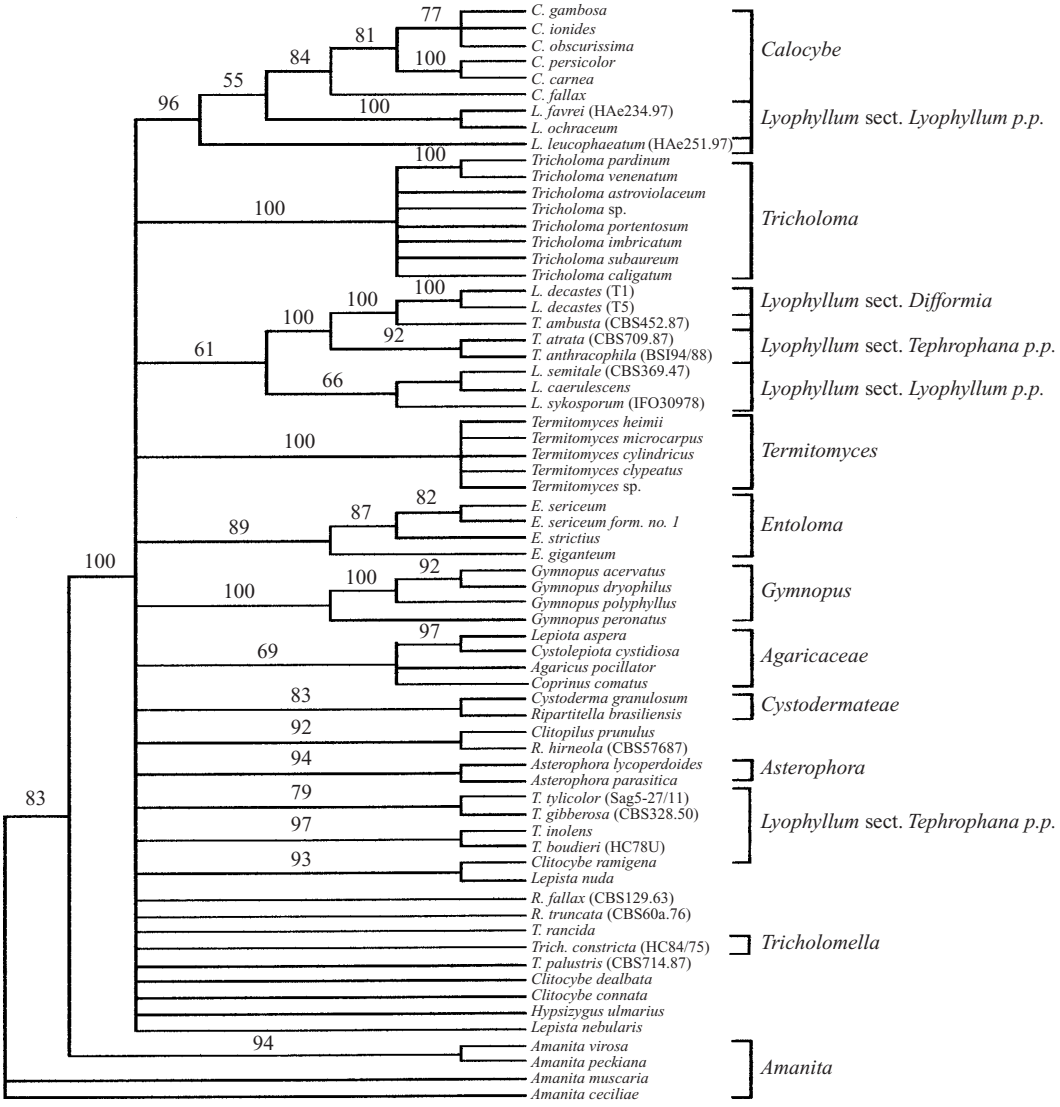

Fig. 1. Strict consensus of the 2596 equally parsimonious trees produced from nLSU sequence data. Abbreviations: *C.*, *Calocybe*; *L.*, *Lyophyllum*; *E.*, *Entoloma*; *R.*, *Rhodocybe*; *T.*, *Tephrocybe*; *Trich.*, *Tricholomella*. Bootstrap values greater than 50 % are shown above their respective branches. They were obtained using a fast bootstrap procedure.

sponding approximately to 1100 bp at the 5′ end of the nLSU, 500 bp of the mtSSU, and 500 bp of the ITS rDNA. GenBank accession numbers of the sequences are given in Table 1. Ambiguously aligned regions that were excluded from the analyses include 5 short regions in the nLSU data set, two short and one longer insert (varying in size from 50 bp in *Amanita* to 260 bp in *Tricholoma*) in the mtSSU alignment, and seventeen gap regions in the ITS alignment. The percent of parsimony informative characters to alignable bases for the nLSU, mtSSU and ITS data sets were 21.0 % (235/1119), 24.0 % (104/433) and 30.7 % (166/541) respectively. nLSU sequences were also obtained from multiple different collections for several *Lyophylleae* species. In several cases, nLSU sequences from different collections of the same putative species did not group together. Analyses of these problematic sequences within a global nLSU database for basidiomycetes indicated that 14 strains from culture collections were misidentified (Table 1). For instance, nLSU sequences of strains CBS 563.85, CBS 369.82 (both labelled *Tephrocybe boudieri*) and CBS 712.87 (labelled *T. atrata*) were found to represent *Armillaria* species (data not shown). In culture, these strains also developed the typical rhizomorph structure that characterizes *Armillaria* species (Cooke & Rayner 1984). Analysis of nLSU data also indicated that SAR4735 (as *T. tylicolor*) is probably a polypore species, CBS 715.87 (as *T. palustris*) a russuloid species, CBS 695.87 (as *L. leucophaeatum*) a *Clitocybe* species, and CBS 599.79 (as *E. mammosum*) a *Hypholoma* species (data not shown). Additionally, cultures CBS 325.80, CBS 823.87 and CBS 825.87, all labelled *T. anthracophila* in CBS, did not cluster with collections identified as *T. anthracophila* by (H. C. L.) on the basis of morphological similarity to the type of that species. Other problematic sequences were from strain CBS 165.50 (as *T. tylicolor*), which clustered with *T. gibberosa* isolates and not with the other *T. tylicolor* strains sampled, and CBS 168.50 (as *T. mephitica*), which nested within *T. boudieri* isolates.

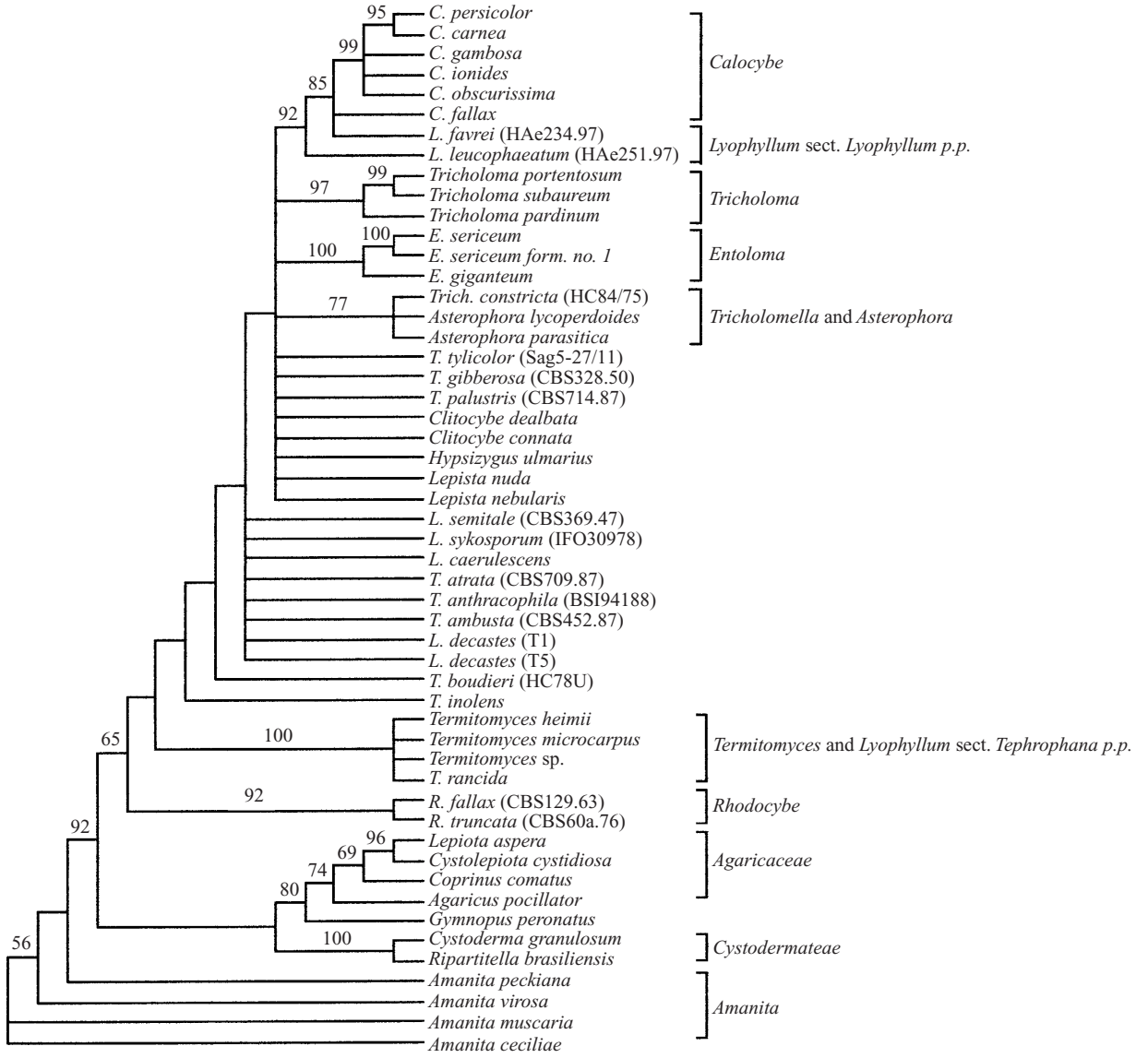

Fig. 2. Strict consensus of the 5592 equally parsimonious trees produced from mtSSU sequence data. See Fig. 1 for abbreviations and bootstrap values above branches.

All problematic taxa were excluded from further analyses (see Table 1).

Analysis of the nLSU data set

MP analyses of 67 nLSU sequences produced 2596 equally parsimonious trees of length (L) = 1187 (CI = 0.415; rescaled consistency index, RC = 0.266). The strict consensus tree is shown in Fig. 1 with associated bootstrap supports (bs) for branches. In this tree, many deeper branches collapse and the *Lyophylleae* do not form a distinct clade. However, within the *Lyophylleae* and putative allies there is strong support for monophyly of *Asterophora* (bs = 94%), *Tephrocybe gibberosa* and *T. tylicolor* (bs = 79%), *T. inolens* and *T. boudieri* (bs = 97%), *Termitomyces* (bs = 100%), *Tricholoma* (bs = 100%), and *Entoloma* (bs = 89%). Within *Lyophyllum*, results indicate that neither sect. *Lyophyllum* nor sect. *Tephrophana sensu* Singer (1986; Table 1) are monophyletic: several members of sect. *Lyophyllum* nest with taxa from both sections *Tephophrana p.p.* and *Difformia* (bs = 61%) while others cluster with *Calocybe* species (bs = 96%). *Calocybe* is monophyletic (bs = 84%). The tree in Fig. 1 supports monophyly of the outgroup taxa *Cystodermateae* (bs = 83%), *Gymnopus* (bs = 100%), *Agaricaceae* (bs = 69%), and *Amanita* (bs = 100%). LTR tests indicate that the best fit model for the nLSU data matrix corresponds to the Tamura–Nei model (Tamura & Nei 1993) with an estimate proportion of invariable site of 0.275, a gamma distribution with shape parameter of 0.385 for the variable sites, and seven rate categories. The topology of the ML tree produced with these settings ($-\ln = 7978.609$) is congruent with the topology of the MP trees (Templeton test: $P = 0.245$).

Analysis of the mtSSU data set

Fig. 2 shows the strict consensus of 5592 equally parsimonious trees (L = 410, CI = 0.436, RC = 0.372)

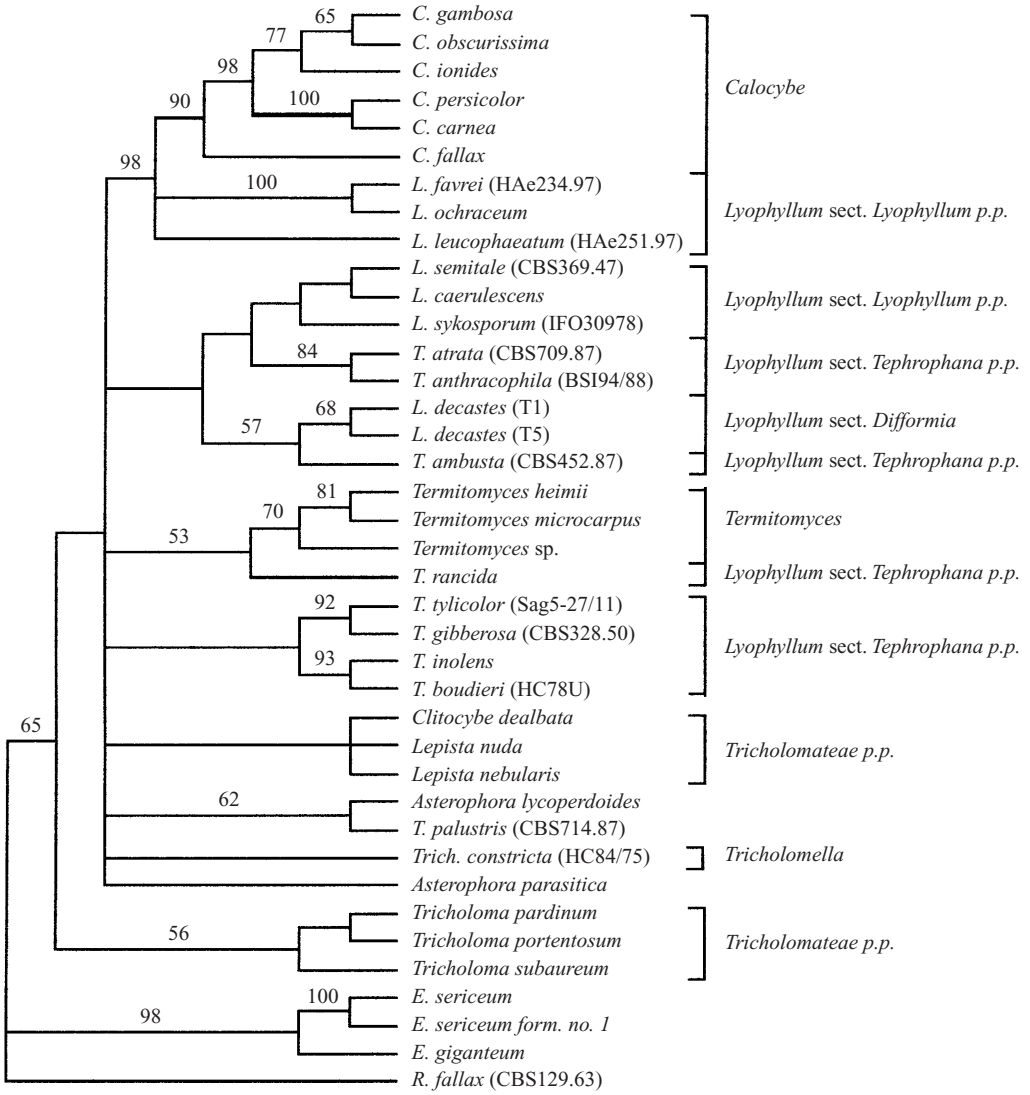

Fig. 3. Strict consensus of the six equally parsimonious trees produced from ITS sequence data. See Fig. 1 for abbreviations and bootstrap values above branches.

produced from 52 mtSSU sequences. In contrast to nLSU data, mtSSU nucleotide sequences provide relatively good phylogenetic resolution at deeper nodes, and indicate monophyly of a larger clade composed of *Lyophylleae*, *Termitomyceteae*, *Tricholomateae* and *Entolomataceae* (bs = 65%) relative to the outgroup taxa Agaricaceae (monophyletic; bs = 74%), *Cystodermateae* (monophyletic; bs = 100%), and *Amanita* (monophyletic; bs = 92%). However, the mtSSU phylogeny does not support monophyly of *Lyophylleae*: several *Tricholomateae* (e.g. *Tricholoma* and *Clitocybe* spp.) and *Entoloma* species are scattered among this tribe, and *Tephrocybe rancida* strongly clusters with *Termitomyceteae* (bs = 100%). Results also indicate non-monophyly of genus *Lyophyllum* and sections *Lyophyllum* and *Tephrophana*. *Calocybe* is monophyletic (bs = 85%) only if *Lyophyllum favrei* is included in the clade. In turn, *L. favrei* clusters with *Asterophora* with moderate bootstrap support (bs = 55%). Finally, there is a good support for monophyly of *Tricholoma* (bs = 97%), but support for a monophyletic Entolomataceae is lacking, although both *Entoloma* (bs = 100%) and *Rhodocybe* (bs = 92%) appear to be monophyletic. The best-fit ML model for the mtSSU data set was determined to be the General Time Reversible (GTR) model (Yang 1994), with three substitution classes (one for transitions, and two for transversions, respectively AT and AC, GT, GC), an estimated proportion of invariable sites of 0.314, a gamma distribution with shape parameter of 0.511 for the variable sites, and six rate categories. The ML analysis produced one tree of score $-\ln = 2683.420$. Based on the Templeton ($P = 0.543$) test, ML and MP trees produced from mtSSU data are not significantly different from each other.

Analysis of the ITS data set

Alignment of ITS sequences was not attainable among more distantly related taxa selected as outgroups in the

Table 2. Results of the Templeton test (1983) for nLSU[a] and mtSSU[a].

	LSU trees	SSU trees	LSU/SSU combined trees
LSU matrix			
Tree length	997	1119–1154	1008–1011
Templeton	[b]$P = 0.768$–1.0	$P < 0.05$	$P = 0.12$–0.19
SSU matrix			
Tree length	443–452	400	418–421
Templeton	$P < 0.05$	$P = 1.0$	$P < 0.05$

[a] Abbreviations: LSU, nuclear ribosomal large subunit; SSU, mitochondrial ribosomal small subunit.

[b] *P* values inferior to 0.05 indicate topological incongruence (Templeton 1983).

nLSU and mtSSU analyses (data not shown). Therefore, the ITS data set was restricted to members of *Lyophylleae*, *Termitomyceteae*, *Tricholomateae* and Entolomataceae (39 taxa included). *Rhodocybe* was chosen as the outgroup to root the phylogeny on the basis of the results shown in Fig. 2. The strict consensus tree of the 6 equally parsimonious trees (L = 783, CI = 0.506, RC = 0.299) produced from MP analyses (Fig. 3) exhibits resolution and support for most clades previously identified in the nLSU (Fig. 1) and/or mtSSU (Fig. 2) phylogenies. For instance, the placement of *Lyophyllum ochraceum* with *L. favrei* (bs = 100 %) and of *L. leucophaeatum* close to *Calocybe* (bs = 98 %) agrees with both nLSU and mtSSU data. Also, the placement of *Tephrocybe rancida* with *Termitomyces* (bs = 53 %), and the presence of a larger clade composed of sections *Lyophyllum p.p.*, *Tephrophana p.p.*, and *Differmia* (Fig. 3) are respectively in agreement with mtSSU (Fig. 2) and nLSU (Fig. 1) data. ITS phylogeny also suggests (but without significant statistical support) new relationships, including: (1) the possible monophyly of *Tephrocybe palustris* with *Asterophora* (bs = 62 %; Fig. 3); (2) a more basal position of *Tricholoma*; and (3) monophyly of *Lyophylleae*, *Termitomyceteae*, *Clitocybe* and *Lepista* species. LRT tests indicate that the best-fit model for ML analysis of ITS data is the TN model with an estimated proportion of invariable sites to be 0.497, a gamma distribution for variable sites of 0.418, and six rate categories. The ML analysis produces one tree of score $-\ln = 4561.588$. Topologies of the ML and MP trees are found to be congruent (Templeton test: $P = 0.242$)

Congruence and combinability of the data sets

nLSU and mtSSU sequences of 51 isolates were merged into a single matrix to conduct the Templeton, Mickevich–Farris and ILD tests for data congruence and combinability. The Templeton test (Table 2) indicated that nLSU trees and mtSSU trees are topologically incongruent. Topologies of nLSU trees are congruent with trees obtained from the combined data set, but mtSSU trees are not (Table 2). The ILD test also indicated incongruence between the nLSU and mtSSU data sets ($P = 0.01$). In contrast, the Mickevich–Farris test produced an index of 4.80 %, which indicates that data are congruent.

Table 3. Comparative bootstrap support (%) for monophyly of selected groups obtained from separated and combined analyses of nLSU[a] and mtSSU[a] sequence data for 51 taxa.

Groups (labelled as in Figs 1–5)	nLSU	mtSSU	nLSU + mtSSU
Amanita	100	96	100
Amanita/*Agaricaceae*	75	?[b]	52*
Agaricaceae	62	94	99
Cystodermateae	86	100	100
Entolomataceae/*Tricholomateae*/*Lyophylleae*/*Termitomyces*	55	?	55
Entoloma	98	99	100
Calocybe/sect. *Lyophyllum p.p.*	95	87	99
Calocybe	68	67	87
sect. *Differmia*/sect. *Lyophyllum p.p.*/ sect. *Tephrophana p.p.*	65	?	83
sect. *Differmia*/sect. *Tephrophana p.p.*	100	?	100
sect. *Differmia*	98	?	100
Termitomyces/*L. rancida*	?	99	96*
Termitomyces	100	?	100
Tricholoma	100	93	100
Asterophora/*Calocybe constricta*	?	63	52*
Asterophora	100	?	100

[a] Abbreviations: LSU, nuclear ribosomal large subunit; SSU, mitochondrial ribosomal small subunit.

[b] Bootstrap support < 50 %.

* Groups for which bootstrap support was higher in either the nLSU or mtSSU analysis than in the combined analysis.

Table 3 shows comparison of bootstrap supports (bs > 50 %) for 16 clades present in the combined analysis of nLSU and mtSSU data. Seven of these clades were also supported in the separated analyses of both nLSU and mtSSU data with bs > 50 %, but in all cases bs was not higher than in the combined analysis. Nine clades were supported with bs > 50 % from one data set only. Bootstrap support increased for six and decreased for three clades when data were combined. In the three cases where bootstrap support decreased in the combined analysis, they decreased from 75 to 52 % (*Amanita*/*Agaricaceae*; nLSU data), 99 to 96 % (*Termitomyces*/*Lyophyllum rancida*; mtSSU data), and 63 to 52 % (*Asterophora*/*Calocybe constricta*; mtSSU data) (Table 3). Using the Templeton test, topologies of the trees produced separately from nLSU, mtSSU and ITS data generally differ significantly from one another (Table 4). However, results show topological compatibility between the nLSU trees and trees obtained with all the combined matrices that include nLSU data. Topologies of the mtSSU trees significantly differ from all the topologies obtained when using another data set, and ITS trees show topological congruence only with mtSSU + ITS trees. Topologies of the trees obtained from combined data sets are more often compatible with topologies obtained from individual data sets. For instance, the nLSU + mtSSU trees are congruent with all other trees except those obtained from ITS, ITS +

Table 4. Topological comparisons of nLSU, mtSSU, ITS/5.8S and combined trees (Templeton 1983).

	nLSU[b] trees	mtSSU[b] trees	ITS[b] trees	nLSU+mtSSU trees	nLSU+ITS trees	mtSSU+ITS trees	nLSU+mtSSU+ ITS trees
nLSU matrix	L = 575 $P = 1.00$	L = 705–726 $P < 0.05$[a]	L = 620–627 $P < 0.05$[a]	L = 583 $P = 0.19$	L = 580–581 $P = 0.11$–0.17	L = 599–636 $P < 0.05$[a]	L = 580–583 $P = 0.03$–0.22
mtSSU matrix	L = 226–228 $P < 0.05$[a]	L = 205 $P = 1.00$	L = 233–236 $P < 0.05$[a]	L = 213 $P = 0.07$	L = 228–229 $P < 0.05$[a]	L = 214–216 $P = 0.02$–0.06	L = 217–220 $P = 0.01$–0.14
ITS matrix	L = 788–792 $P < 0.05$[a]	L = 850–860 $P < 0.05$[a]	L = 767 $P = 1.00$	L = 791 $P < 0.05$[a]	L = 776–777 $P = 0.11$–0.15	L = 776–778 $P = 0.02$–0.28	L = 780–783 $P < 0.05$[a]
nLSU+mtSSU matrix	L = 801–803 $P = 0.33$–0.54	L = 910–931 $P < 0.05$[a]	L = 853–863 $P < 0.05$[a]	L = 796 $P = 1.00$	L = 808–810 $P = 0.08$–0.15	L = 816–841 $P < 0.05$[a]	L = 798–801 $P = 0.18$–0.37
nLSU+ITS matrix	L = 1363–1367 $P = 0.08$–0.22	L = 1556–1586 $P < 0.05$[a]	L = 1385–1394 $P < 0.05$[a]	L = 1374 $P < 0.05$[a]	L = 1357 $P = 1.00$	L = 1384–1404 $P < 0.05$[a]	L = 1361–1364 $P = 0.13$–0.27
mtSSU+ITS matrix	L = 1015–1019 $P < 0.05$[a]	L = 1057–1065 $P < 0.05$[a]	L = 1000–1003 $P = 0.14$–0.23	L = 1004 $P < 0.05$[a]	L = 1005 $P = 0.07$	L = 992 $P = 1.00$	L = 998–1001 $P = 0.13$–0.27
nLSU+mtSSU +ITS matrix	L = 1590–1594 $P = 0.07$–0.18	L = 1764–1791 $P < 0.05$[a]	L = 1620–1630 $P < 0.05$[a]	L = 1587 $P = 0.42$	L = 1585–1586 $P = 0.46$–0.54	L = 1594–1620 $P < 0.05$[a]	L = 1581 $P = 1.00$
Number of equally parsimonious trees	4	5655	6	1	2	88	6

[a] P values inferior to 0.05 indicate topological incongruence (Templeton 1983).

[b] List of abbreviations: nLSU, nuclear ribosomal large subunit; mtSSU, mitochondrial ribosomal small subunit; ITS, internal transcribed spacers 1 and 2 and 5.8S coding region.

Table 5. Results of the Partition homogeneity test (ILD) (Farris 1995a, b).

	10 replicates Maxtrees = 10	10 replicates Maxtrees = 100	100 replicates Maxtrees = 10
LSU+SSU[a]	$P = 0.01$[b]	$P = 0.01$	$P = 0.04$
LSU+ITS[a]	$P = 0.13$	$P = 0.10$	$P = 0.09$
SSU+ITS	$P = 0.04$	$P = 0.05$	$P = 0.06$

[a] Abbreviations: LSU, nuclear ribosomal large subunit; SSU, mitochondrial ribosomal small subunit; ITS, internal transcribed spacers 1 and 2 and 5.8S coding region.

[b] P values inferior to 0.05 indicate that null hypothesis of congruence is rejected.

nLSU, and ITS+mtSSU matrices. The nLSU+ITS trees are compatible with all the other trees except those produced from mtSSU data alone. The mtSSU +ITS trees are congruent with all trees except those produced with the inclusion of nLSU data. Finally, the trees generated by combining all the data are congruent with all trees produced from other data sets except with trees produced from ITS data alone.

ILD tests indicate incongruence between nLSU and mtSSU data sets ($P = 0.01$–0.04), and congruence between the nLSU and ITS data sets ($P = 0.09$–0.13) regardless of the search strategy employed (Table 5). When testing for congruence between SSU and ITS data, however, results of the test were influenced by the search strategies employed to find the most parsimonious trees ($P = 0.04$–0.06; Table 5).

The Mickevich–Farris test produced indices of 4.16% between nLSU and mtSSU data, 2.51% between nLSU and ITS data, 4.38% between mtSSU and ITS data, and 4.82% among the three data sets combined, indicating no significant incongruence among the three different data partitions.

Combined analyses

We followed the 'total evidence' principle of Kluge (1989) and combined the nLSU, mtSSU and ITS data sets for further analyses, even though some statistical tests indicated incongruence between the different data partitions (Tables 2, 4–5). LRT tests indicated that the best-fit model for each data set is very different (see above), making the choice of ML model for the combined data set impractical (Moncalvo *et al*. 2000b). Therefore, combined analyses were only conducted using maximum-parsimony.

Combination of nLSU and mtSSU data

The strict consensus tree of 26 equally parsimonious trees (L = 1368, CI = 0.474, RC = 0.304) produced by combining nLSU and mtSSU nucleotide sequence data from 51 taxa is depicted in Fig. 4. Clades supported from this analysis are largely similar to those revealed in the separate analyses (Figs 1–2), and support monophyly of the outgroup taxa *Amanita* (bs = 52%; decay index = 2 [= d2]), *Cystodermateae* (bs = 100; d3+) and *Agaricaceae* (bs = 99; d3+). As in the mtSSU analysis (Fig. 2), these are reciprocally monophyletic (bs = 55%) to a larger group that includes Entolomataceae, *Tricholomateae*, *Termitomyceteae*, and *Lyophylleae*. In contrast to the mtSSU analysis (Fig. 2), all Entolomataceae taxa are basal (bs = 57%) to *Tricholomateae*, *Termitomyceteae*, and *Lyophylleae* (Fig. 4). Phylogenetic relationships among the latter three tribes are only partially resolved, but seven major

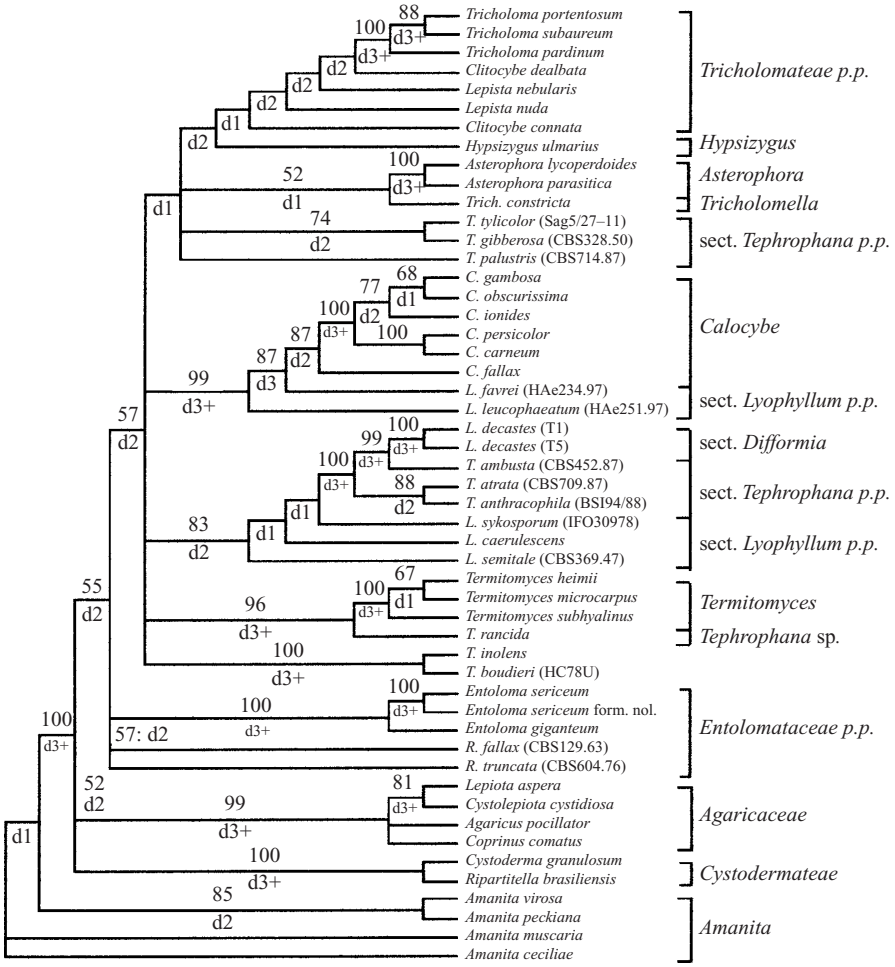

Fig. 4. Strict consensus of the 26 most parsimonious trees produced in combining nLSU and mtSSU sequence data. See Fig. 1 for abbreviations and bootstrap values above branches. Decay indices are indicated below their respective branches.

clades are well supported: (1) *Tricholoma* (bs = 100 %, d3+); (2) *Asterophora* (monophyletic, bs = 100 %, d3+) and *Tricholomella constricta* (bs = 52 %; d1); (3) *T. tylicolor* and *T. gibberosa* (bs = 74 %, d2); (4) *Calocybe* and two species of section *Lyophyllum* (bs = 99 %, d3+); (5) members of *Lyophyllum* from sections *Lyophyllum*, *Tephrophana*, and *Difformia* (bs = 83 %; d2); (6) *Termitomyceteae* (monophyletic, bs = 100 %; d3+) and *Tephrocybe rancida* (bs = 96 %; d3+); (7) *Tephrocybe inolens* and *T. boudieri* (bs = 100 %, d3+). Unconstrained trees do not differ significantly from trees produced either by constraining monophyly of Entolomataceae with *Amanita* (Templeton: $P = 0.454$–0.473) or by constraining monophyly of the *Lyophylleae–Termitomyceteae–Tricholomateae* clade with either Agaricaceae (Templeton: $P = 0.375$) or *Cystodermateae* (Templeton: $P = 0.107$–0.116).

Combined analyses of nLSU, mtSSU and ITS data

The nLSU + mtSSU + ITS combined data set included complete sequences for 40 taxa, except for *L. ochraceum* (mtSSU data missing) and *Hypsizygus ulmarius* (ITS data missing). MP analyses yielded 3 equally parsimonious trees (L = 1611, CI = 0.523, RC = 0.341). The strict consensus tree (Fig. 5) is fully resolved except for the clade including *Clitocybe* and *Lepista* (6B in Fig. 5). When the phylogeny is rooted with *Entolomataceae* (as suggested in Figs 2–4), both *Termitomyceteae* (clade 12B) and *Tricholomateae* (clade 4B) appear to have been derived from within *Lyophylleae* (clades 1B, 2B, 3B and 4A; Fig. 5). The derived position of *Termitomyceteae* within *Lyophylleae* is strongly supported by its placement as sister group to *T. rancida* (bs = 100 %, d3+). In contrast, the derived position of *Tricholomateae* within the *Lyophylleae* is weakly to moderately supported at all deeper nodes (bs < 50 %; d1–d3), and a search constraining monophyly of the clade *Lyophylleae–Termitomyceteae* with *Tricholomateae* produced trees that do not differ significantly from the unconstrained trees (Templeton test: $P = 0.493$–0.551). In contrast, searches constraining monophyly of *Lyophylleae* ss. Singer (i.e. excluding *Termitomyceteae*) produced trees significantly different from the unconstrained trees (Templeton test: $P = 0.011$–0.016). Within *Lyophylleae*, analyses of the combined nLSU

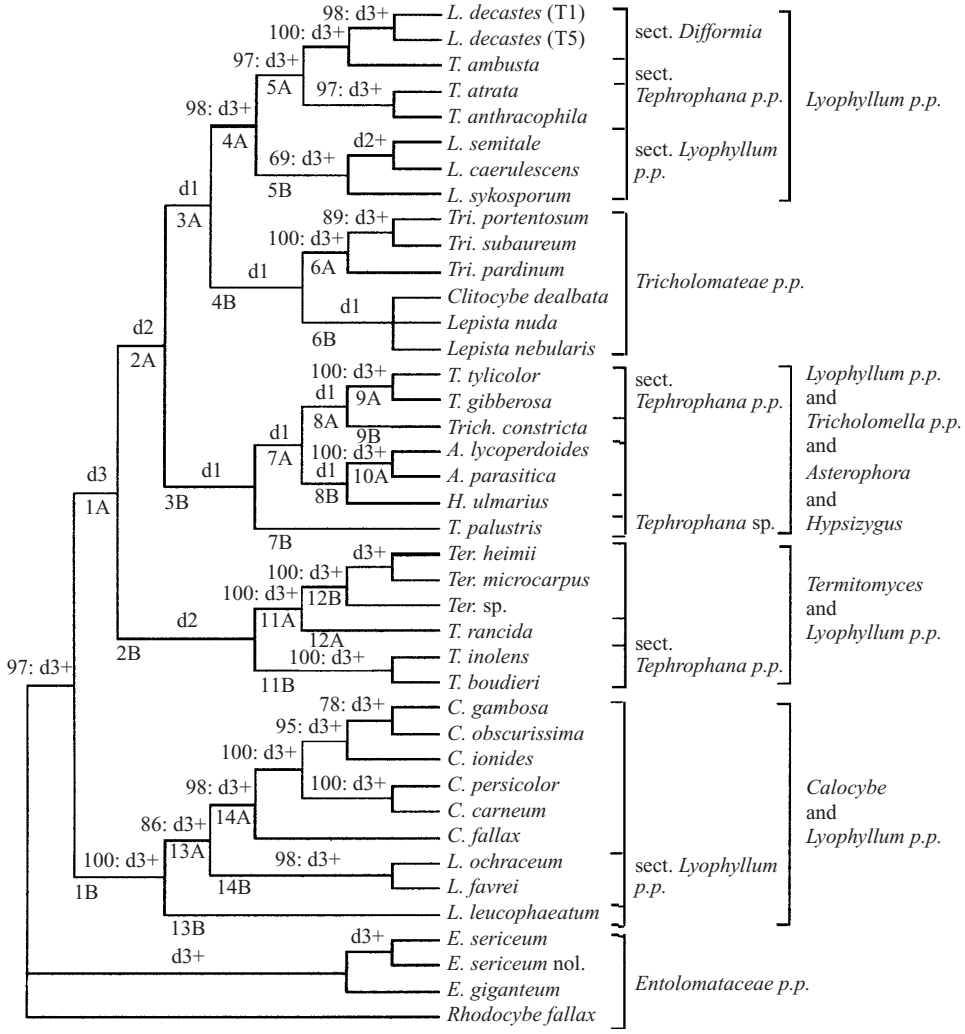

Fig. 5. Strict consensus of the 3 most parsimonious trees produced in combining nLSU, mtSSU and ITS-rDNA sequence data. Abbreviations: *C.*, *Calocybe*; *L.*, *Lyophyllum*; *E.*, *Entoloma*; *R.*, *Rhodocybe*; *T.*, *Tephrocybe*; *Trich.*, *Tricholomella*; *Tri.*, *Tricholoma*; *A.*, *Asterophora*; *Ter.*, *Termitomyces*. Numbers and letters above branches show clades discussed in the text. Bootstrap values greater than 50 % and decay indices are indicated above their respective branches.

+mtSSU+ITS data (Fig. 5) produce clades that are largely similar to those revealed in earlier analyses (Figs 1–4). A strongly supported clade (1B: bs = 100 %, d3+; Fig. 5) encloses members of *Lyophyllum* section *Lyophyllum* (clades 13B and 14B) and *Calocybe* (clade 14A). Within this clade, *L. leucophaeatum* (13B) is basal to a clade (13A: bs = 81 %, d3+) in which *L. favrei* and *L. ochraceum* (14B: bs = 100 %; d3+) are sister to *Calocybe* (14A: bs = 99 %, d3+). A weakly supported clade (2B: bs < 50 %, d2) includes two species of section *Tephrophana* (11B: bs = 100 %; d3+), *T. inolens* and *T. boudieri*, as a sister group to *T. rancida* and the *Termitomyceteae* (clade 11A: bs = 100 %; d3+). *Termitomyceteae* is monophyletic (clade 12A: bs = 100 %, d3+). Another weakly supported clade (3B) includes part of section *Tephrophana* (7B, 9A), *Hypsizygus ulmarius* (10B), *Asterophora* (10A), and *Tricholomella constricta* (9B). *Asterophora* and *Hypsizygus* are monophyletic (clade 8B) and a sister group to *T. constricta*, *T. tylicolor* and *T. palustris* (clade 8A). In this lineage monophyly of *Asterophora* (clade 10A), and of *T. gibberosa* and *T. tylicolor* (9A), are both strongly supported (bs = 100 %; d3+ in both cases). Finally, a strongly supported clade (4A: bs = 98 %; d3+) encloses part of section *Lyophyllum* (5B: bs = 69 %; d3) as sister group of another strongly supported group (5B: bs = 98 %; d3+) that includes species of both sections *Tephrophana* and *Difformia*.

DISCUSSION

Data congruence and combinability

There is still a considerable debate in phylogenetic systematics concerning congruence and combinability of data from different sources. Whereas 'total evidence' is strongly advocated by some authors (Kluge 1989, Myamoto 1985, Soltis *et al.* 1998), others suggest combining different data sets only when data from the different partitions are found to be statistically hom-

ogenous (Bull *et al.* 1993, Lutzoni 1997), or when separate analyses of the different data partition show no obvious phylogenetic conflict among each other (Myamoto & Fitch 1995, de Queiroz 1993). Consequently, as suggested in several recent studies (McCracken *et al.* 1999, Slowinski *et al.* 1999, Moncalvo *et al.* 2000b), we first analyzed data from the different partitions separately.

In the separate analyses of the nLSU, mtSSU or ITS data, statistically well-supported phylogenetic groupings are either present in all trees or unresolved in others (Figs 1–3). Therefore, separate analyses of these three gene regions show no major conflict and indicate no obvious incongruence between the different data partitions (Doyle 1992). These separate analyses also show that nLSU data support several terminal clades, but provide little resolution for more basal relationships, as shown by the collapse of many deeper branches (Fig. 1). In contrast, mtSSU data support only a limited number of terminal clades but resolve several more basal relationships (Fig. 2). It would seem, therefore, that the phylogenetic signals of the nLSU and mtSSU data sets are complementary. Consequently, the differing topologies of the trees produced through separate analyses need not indicate that the data sets are incongruent (Soltis *et al.* 1998).

Results of the Templeton test indicate that tree topologies produced by nLSU or mtSSU data significantly differ (Tables 3–4), suggesting that these data sets should not be combined in a phylogenetic analysis. We observed that trees produced from the combined nLSU + mtSSU data matrix are compatible with nLSU trees but incompatible with mtSSU trees, suggesting that the combined topology may be dominated by the data set with the higher resolution power, here the nLSU data set (Table 2). This result suggests that it is appropriate to use the Templeton test only to estimate congruence among data sets with high resolution. The same observation can be made when the Templeton test is applied to the three data subsets (Table 4). On one hand, the mtSSU data set, which (in contrast to nLSU and ITS data) contains primarily characters resolving basal relationships, is incompatible with topologies of all the combined trees. On the other hand, nLSU + ITS trees are congruent with all trees excepted the mtSSU trees. Therefore, the Templeton test for topological congruence seems to be more adapted to indicate the influence of each data set on the analyses than to establish their putative congruence.

If the Templeton test largely indicates incongruence among the nLSU, ITS and mtSSU data sets (Table 4), the ILD test indicates congruence at least between the nLSU and ITS, and the mtSSU and ITS, data sets (Table 5). However, Table 5 also shows that results of the ILD test can be influenced by the type of search conducted to find the shortest trees when characters are resampled. In our study, both the nLSU and mtSSU data matrices have low overall resolution, and the two matrices provide higher phylogenetic signal at different phylogenetic depths. Therefore, resampling informative characters between such matrices may easily lead to a situation in which the resulting matrices show very little resolution for all ranks. Also, when the number of taxa is high (as in this study), the fact that only suboptimal heuristic searches can be conducted may result in a failure to find the most parsimonious trees. This can lead to an overestimation of incongruence between the data sets. A higher number of character resampling replicates allows searches to start from a higher number of different reference trees, thus increases the likelihood of finding topologies equal or more parsimonious than those obtained with the original matrices (Table 5). These results show the necessity of performing several rounds of random addition sequence to find the most parsimonious trees when the data sets have low resolution power, and to a use an optimized branch swapping algorithm (e.g. TBR). Therefore, the ILD test and the Templeton test both appear to be very sensitive to the resolution power and resolution levels of the different data partitions analyzed.

The Mickevich–Farris index was the only explicit test to assess congruence between all the different data sets. Absence of evident conflict in the phylogenetic signal of the data sets investigated here could also be gauged empirically from the overall increase of phylogenetic resolution and bootstrap supports in the combined *vs.* separated analyses (Table 3; compare also Figs 1–3 with Figs 4–5) (Kluge 1989, Cunningham 1997a, b; Soltis *et al.* 1998, McCracken *et al.* 1999, Moncalvo *et al.* 2000b). In this study, in all but three cases combining nLSU and mtSSU data result in increased statistical supports (Table 3), indicating that data support the same clades, i.e. are fully congruent. Cases for which bs decreased in the combined analyses are as follows (Table 3): (1) mtSSU data strongly supports relationships between *Termitomyces* spp. and *Tephrocybe rancida* (bs = 99% *vs* 96% in the combined analysis; Table 3), where nLSU data do not resolve relationships of either taxon (branches collapsed, Fig. 1); (2) similarly, mtSSU data support relationships between *Asterophora* spp. and *Tricholomella constricta* (bs = 63% *vs* 52% in the combined analysis; Table 3), whereas nLSU data do not resolve relationships of either taxon (branches collapsed, Fig. 1); (3) nLSU data support relationships between *Amanita* and Agaricaceae (bs = 75% *vs* 52% in the combined analysis; Table 3), whereas mtSSU data weakly support the alternative placement of the latter with *Cystodermateae* (bs < 50%). Overall, it appears that decrease in bootstrap supports in the combined *vs.* separated analyses result from differences in the resolution rank of the two data sets rather than contradictory phylogenetic signal, i.e. there appears to be no major incongruence between the two data sets. Therefore, the Mickevich–Farris index (Kluge 1989) seems to be more appropriate for estimating data congruence and combinability than the more widely used ILD (Farris 1995a, b) and Templeton (1983) tests, at least for data

sets with low resolution power and that have higher phylogenetic signal at different phylogenetic levels.

Phylogeny of the Lyophylleae *and evolution of siderophilous granulation*

Our study indicates monophyly of the tribes *Lyophylleae*, *Termitomyceteae* and *Tricholomateae* (Figs 4–5), and a possible sister group relationships of this clade with the *Entolomataceae*. In a broader phylogenetic study of the *Agaricales* (Moncalvo *et al*. 2000a) monophyly of these three tribes was already indicated but was statistically weakly supported, and a possible close relationship between these tribes and the Entolomataceae was not evident. All analyses indicate that the tribe *Lyophylleae sensu* Singer (1986) is polyphyletic (Figs 1–5). Its monophyly can also be clearly rejected from our data: constraining monophyly of the *Lyophylleae* did produce trees significantly longer than unconstrained trees (see results). Also, our analyses strongly support monophyly of *T. rancida* with *Termitomyceteae* (Figs 2, 4–5), and the nesting of *Termitomyceteae* within *Lyophylleae* (Fig. 5). Results of the combined analyses (Figs 4–5) suggest that *Lyophylleae–Termitomyceteae* is paraphyletic with respect to *Tricholomateae*; however, trees constraining monophyly of the former group are not significantly different from the unconstrained trees (see results). Consequently, evolutionary relationships between *Lyophylleae s. lat*. (including *Termitomyceteae*) and *Tricholomateae* remain unclear. Our results indicate that *Entolomataceae* is a sister group to the clade *Lyophylleae–Termitomyceteae–Tricholomateae* (Fig. 4). However, unambiguous molecular support for this relationship is still lacking because constraining the monophyly of the Entolomataceae with either *Amanita*, *Agaricaceae* or *Cystodermateae* did not produce trees significantly longer than the unconstrained trees (see results). Nevertheless, a sister group relationship of Entolomataceae with the *Lyophylleae–Termitomyceteae–Tricholomateae* clade can still be considered the best phylogenetic hypothesis from both a molecular and morphological standpoint. Natural relationships between *Tricholoma*, *Termitomyces*, *Lyophylleae*, and/or *Entolomataceae* were inferred by Kühner & Romagnesi (1953) and Clémençon (1978, 1997), based on a number of morphological similarities including the microstructure of the basidiospore cell wall and the presence of siderophilous granules in the basidia of *Lyophylleae*, *Termitomyceteae*, and some *Entolomataceae* taxa.

If the phylogenetic relationships depicted in Figs 4–5 are correct, then siderophilous granulation in the basidia of agaricoid mushrooms is likely to have a single origin. If so, this character appears to have been lost at least once over the course of evolution, in the ancestor of the *Tricholomateae*. It is not possible here to determine in detail the evolution of this character within *Entolomataceae* because our taxonomic sampling in this family was very limited. The strong support for monophyly of *Termitomyceteae* with *T. rancida* (bs = 96%) also implies that siderophilous granulation of the macro-type is homologous between *Lyophylleae* and *Termitomyceteae*. Overall, molecular data support the traditional view that the presence of siderophilous granulation in the basidia of agaricoid mushroom is a good indicator of close phylogenetic relationships, although inferring a phylogeny from a morphological trait must be done with caution.

Limits of the genera Calocybe *and* Lyophyllum

Based on molecular evidence, both genera *Calocybe* and *Lyophyllum sensu* Kühner & Romagnesi (1953), Kühner (1980), Moser (1978), and Singer (1986) are artificial (Figs 1–5). Phylogenetic analyses show that *Calocybe* (clade 14A, Fig. 5) is monophyletic, and that *Tricholoma constricta* (syn. *Calocybe constricta*) (in clade 9B, Fig. 5) should be excluded from this genus (this result is supported in all our analyses; Figs 1–5). This species does not produce phenoxazones in culture (Moncalvo 1991), in contrast to all the other *Calocybe* species tested for the presence or absence of that character (Clémençon 1987, Moncalvo *et al*. 1991). Consequently, both molecular and cultural data support Kalamees (1992), who transferred *Calocybe constricta* to the genus *Tricholomella*. Molecular data also support Bon's (1999b) transfer of both *Lyophyllum favrei* and *L. ochraceum* to *Calocybe*, a possibility already indicated by Kühner & Romagnesi (1953): these two taxa (clade 14B, to Fig. 5) are sister group to the *Calocybe* clade and paraphyletic with *L. leucophaeatum*, the type of *Lyophyllum*. Morphologically, the transfer of both *Lyophyllum favrei* and *L. ochraceum* to *Calocybe* (Bon 1999a) is supported by several shared distinct characters including bright cap colours, yellow lamellae, and a vacuolar pigmentation (Clémençon 1982, 1986). Fruit bodies of both *L. favrei* and *L. ochraceum* turn red before blackening when bruised (Clémençon & Smith 1983), a chemical reaction different from the typical blackening of the other species in section *Lyophyllum*. This character, and the absence of production of phenoxazones in culture, separate these two species from *Calocybe* species. Overall, *L. favrei* and *L. ochraceum* appear to occupy an intermediate position between *L. leucophaeatum* and *Calocybe*.

Molecular evidence also indicates that key characters used to segregate *Lyophyllum* into different sections have been misdiagnosed, as already pointed out by Clémençon & Smith (1983). Sect. *Lyophyllum* is polyphyletic: *L. leucophaeatum*, *L. favrei*, and *L. ochraceum* are related to *Calocybe* (see above), while *L. semitale*, *L. caerulescens*, and *L. sykosporum* (clade 5B, Fig. 5) appear related to species that have been placed in the other two sections of the genus, *Difformia* and *Tephrophana* (all in clade 4A, Fig. 5). This indicates that the blackening of the fruit bodies upon bruising has been a character overemphasized in *Lyophyllum*

taxonomy. Another character overemphasized in morphology-based classifications is fruit body habit: here, sect. *Tephrophana*, created for collybioid species, is shown to be polyphyletic. Some *Tephrophana* species (clade 5A, Fig. 5) cluster with members of the *L. decastes* complex (characterized by tricholomatoid to clitocyboid fruit bodies), while others nests with termitomycetoid (clade 11A, Fig. 5) or pleurotoid (e.g. *Hypsizygus ulmarius*, clade 3B, Fig. 5) species.

Ecology

Molecular analyses suggest that ecology has been underutilized in *Lyophylleae* classification. For instance, clade 3B (Fig. 5) groups all the parasitic species that were included in this study: *Hypsizygus ulmarius* is a tree pathogen (Singer 1986), *Asterophora* is a fungal parasite (Singer 1986), and *Tephrocybe palustris* is necrotrophic on *Sphagnum* (Redhead 1981). This clade also includes *Lyophyllum* species reported growing on mosses (*T. tylicolor* and *T. gibberosa*; Bon 1999a, Neville & Poumarat 1997), that are also thought to be ammonia-fungi (Sagara 1975), and *Tricholomella constricta* which is often found in fertilized fields (Bon 1999a). This implies that members of clade 3B need nitrates supplied either externally (8A) or through parasitism (7B, 8B).

Our results also suggest monophyly of *Tephrocybe rancida* with *Termitomyces* (11A). The association of *Termitomyces* species with termites has been well documented (Heim 1977). There is no evidence so far that *Tephrocybe rancida* could also be associated with insects, but interestingly Métrod (1959) reported that *T. mycenoides*, a species morphologically close to *T. rancida*, was found in groups on piled fir tree-needles which could suggest an association with ants. Based on the latter observation and phylogenetic evidence, a possible insect association of *Tephrocybe* spp. warrants further scrutiny.

Taxonomic implications

Phylogenetic analyses of molecular data divide the *Lyophylleae* into four major clades (Fig. 5) that are not congruent with any of the morphologically based classifications available to date (Kühner & Romagnesi 1953, Moser 1978, Singer 1986, Bon 1999a). These four clades are as follows:

Clade 1B (bs = 100 %, d3+; Fig. 5) includes *Lyophyllum leucophaeatum* (13B; type species of *Lyophyllum*), *L. ochraceum*, *L. favrei* (14B), and *Calocybe* (14A). The limit between these *Lyophyllum* and *Calocybe* species still remain unclear, as discussed above. Several species not included in this study may also belong to this clade. *L. hypoxanthum* (Josserand & Riousset 1974), a non-blackening species, and *L. buxeum* and *L. musashiense* both blackening, all have bright colours similar to *L. favrei*, *L. ochraceum* and *Calocybe* species.

Clade 2B (bs < 50 %, d2; Fig. 5) includes *Termitomyceteae* (clade 12B) and part of sect. *Tephrophana* (*T. boudieri* and *T. inolens* in clade 11B, and *T. rancida* in clade 12A). All these species have free lamellae, distinguishing them from the other *Lyophylleae* species examined here. *T. boudieri* and *T. inolens* form a sister group to the *T. rancida–Termitomyceteae* subclade. One morphological character uniting the latter subclade is the presence of a radicating stipe; also possibly shared is an association with ants and termites, respectively. However, *Termitomyces* spp. and *T. rancida* have different pigments (e.g. they differ in spore print colour).

Clade 3B (bs < 50 %, d1; Fig. 5) includes all parasitic species of *Lyophylleae* that were examined in this study (see above), i.e. *T. palustris* (clade 7B), *Asterophora* (clade 10B), and *Hypsizygus* (clade 10A). Three non-parasitic species also belong to this clade; they form a monophyletic clade (8A) sister to *Asterophora* and *Hypsizygus* (clade 8B). Because non-parasitic species in clade 3B have been shown to be ammonia-fungi or grow on fertilized fields (see above), it appears that all members of this clade live on nitrate derived from an external source. Therefore, this ecological character is a synapomorphy for clade 3B.

Clade 4A (bs = 100 %, d3+; Fig. 5) combines species from three sections of *Lyophyllum sensu* Singer (1986). Two subgroups can be recognized within this clade based on both molecular phylogeny and morphology. The first subgroup (clade 5B) corresponds to '*Nigrescentia*' *sensu* Kühner & Romagnesi (1953), with the exclusion of *L. leucophaeatum*. The second subgroup (clade 5A) comprises members of section *Difformia* (restricted to the *L. decastes* complex; Moncalvo *et al.* 1991, 1993), plus carbonicolous species of section *Tephrophana* (*T. ambusta*, *T. anthracophila* and *T. atrata*; Moser 1978, 1983, Singer 1986, Neville & Poumarat 1997). *T. gibberosa*, morphologically similar and sometimes classified with carbonicolous *Tephrophana* (Moser 1978, 1983, Singer 1986, Neville & Poumarat 1997) is phylogenetically distinct from these 'fireplace' species (Figs 1, 3–5).

In conclusion, this study suggests that both taxonomy and nomenclature of the traditional *Lyophylleae* needs to be revised to reflect a more natural classification system. A publication introducing new genera and taxonomic arrangements is currently in preparation.

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