

Genera in the family *Agaricaceae*: evidence from nrITS and nrLSU sequences

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Received 25 August 2003; accepted 25 January 2004.

The circumscription of the family *Agaricaceae*, its genera and their characters are investigated. Analyses of the ITS and LSU sequence data, separately and in combination result in exclusions and reorganization. *Notholepiota areolata*, a secotioid species from New Zealand, *Cystoagaricus* with brown, irregular-knobby spores, and *Leucopholiota* with gelatinized hymenophoral trama and amyloid spores, fall outside the family *Agaricaceae*. In the reorganization, long accepted tribe and genus definitions are amended, viz.: (1) *Macrolepiota* and *Chlorophyllum* separately are monophyletic, but not together; (2) *Leucoagaricus* and *Leucocoprinus* together form one large monophyletic clade, that also includes polyphyletic *Sericeomyces*; (3) *Lepiota*, *Cystolepiota* and *Melanophyllum* together form a monophyletic clade; (4) Secotiation has happened within several clades (e.g. *Agaricus* and *Chlorophyllum*), though not in the *Leucoagaricus/Leucocoprinus* clade, nor in the lineage of *Macrolepiota* proper; gasteroid and most secotioid species in the course of adaptation acquire coloured spores; and (5) the monovelangiocarpic *Chamaemyces fracidus* appears to be basal to the family and provides clues to the morphology of the ancestral *Agaricaceae*. Trama and developmental characters characterize the different clades, whereas spore characters play a minor role.

INTRODUCTION

The family *Agaricaceae* is a widely distributed monophyletic group of saprotrophic fungi that exhibits huge diversity in spore colour, and in structure of the pileus covering. Agaricoid, as well as secotioid and gasteroid taxa are included; see Singer (1986) for agaricoid members, Redhead *et al.* (2001) for *Coprinus s. str.*, and Kirk *et al.* (2001), Krüger *et al.* (2001), and Moncalvo *et al.* (2002) for gasteroid and secotioid taxa.

This paper sets out to examine the circumscription of the family as a whole (which genera do belong to it, and which do not), the relationships among the genera, and the characterization of the genera. The study focuses on the lepiotoid taxa and the emphasis is on the non-secotioid and non-gasteroid, north-temperate members of the family with white spores. Many taxa exist in the family, especially in the tropics, with other character combinations than those covered here. To give just one example, *Heinemannomyces* is a Malaysian genus characterized by a cobalt blue spore print (Watling 1999).

Some specific questions which are considered are: (1) Do *Leucopholiota*, *Cystoagaricus*, *Chamaemyces*, and *Notholepiota* belong to the *Agaricaceae*? (2) Is a

separate family *Lepiotaceae* for the white-spored taxa warranted? (3) Are the tribes, recognized on morphological characters, supported by molecular evidence? (4) Are the genera *Lepiota*, *Cystolepiota*, *Leucoagaricus*, *Leucocoprinus*, *Macrolepiota*, and *Sericeomyces* monophyletic? And (5) What are the morphological characters which are important at the deeper levels of the classification?

The family *Agaricaceae* in the morphological sense, i.e. restricted to taxa with an agaricoid habit, has been divided into four tribes (Singer 1986, Wasser 1993). However, one of the tribes, *Cystodermateae*, was excluded from the family on morphological grounds (Bas 1988), and later the exclusion was confirmed by sequence analyses (Johnson & Vilgalys 1998, Moncalvo *et al.* 2002). The remaining tribes are:

(1) *Agariceae*: characterized by a brown spore print, a regular lamella trama, and the absence of clamp-connections; there is some evidence that the exceptional *Heinemannomyces* with blue spores might belong to this tribe. Singer (1975) stated that the spore wall was not metachromatic in Cresyl Blue. Representatives of *Agaricus* subgen. *Agaricus*, *Allopsalliota*, *Cystoagaricus*, and *Micropsalliota*, as well as the secotioid *A. inapertus* (syn. *Endoptychum depressum*), *Gyrophragmium*

dunalii and *Longula texensis*, were examined for the present study.

(2) *Lepioteae*: comprising species with a regular lamella trama and whitish spores that are not metachromatic in Cresyl Blue (but see p. 374). Some authors (e.g. Bon 1981, 1993), have included *Melanophyllum*, with coloured spores, in this tribe, while other authors have placed that genus close to *Agaricus*, (e.g. Kerrigan 1986, Singer 1986, Wasser 2002). Pegler (1986) considered *Melanophyllum* part of tribe *Cystodermateae*. *Chamaemyces*, *Cystolepiota* (including *Pulverolepiota*), *Lepiota* (including *Echinoderma*), and *Melanophyllum* were sampled for this study.

(3) *Leucocoprineae*: containing taxa with a trabecular lamella trama and a complex spore wall which sometimes has a germ pore, and is metachromatic in Cresyl Blue; the spores are whitish, rarely green. *Chlorophyllum*, *Leucoagaricus*, *Leucocoprinus*, *Macrolepiota*, and *Sericeomyces* were sampled.

Many authors (e.g. Moser 1983), followed Singer's family concepts after they had appeared in an earlier edition of his synopsis of the *Agaricales* (Singer 1975). However, Bon (1993) placed the two tribes *Lepioteae* and *Leucocoprineae* in a separate family, *Lepiotaceae*, which he distinguished from the *Agaricaceae* in the white, rarely green, spore print. Ballero & Contu (1991) adopted the same family concept, while deviating from all other contemporary authors in regarding *Macrolepiota* as a synonym of *Lepiota*. Jülich (1982) raised the three tribes to the rank of family, and this concept was followed by Grgurinovic (1997); Jülich (1982) also treated secotioid genera in families of their own (*Podaxaceae*, *Montagneaceae*, *Secotiaceae*).

In previous molecular studies (Johnson & Vilgalys 1998, Johnson 1999, Moncalvo *et al.* 2002) it was shown that *Leucoagaricus* and *Leucocoprinus* are not jointly monophyletic, with *L. flammeatincta* and *La. rubrotinctus*¹ outside the clade made up of the other *Leucoagaricus* and *Leucocoprinus* species examined in those studies. This clade also includes the taxa which are cultivated by the attine ants, and in which basidiome formation is suppressed (Mueller, Rehner & Schultz 1998).

Likewise, an analysis of LSU data (Moncalvo *et al.* 2002) indicated that the genus *Lepiota* does not form a monophyletic clade. This study assigned five species, morphologically considered *Lepiota* (according to Singer 1986), to four different clades. It also found that *Cystolepiota* and *Melanophyllum* species, together with *L. aspera* (as *L. acutesquamosa*), form a clade separate from the other *Lepiota* species. Two of the taxa used in this study are designated *Lepiota* although they belong

morphologically to the *Leucocoprinus* group or to *Chlorophyllum* (*viz.* *L. flammeatincta*, *L. humei*); however, the appropriate combinations had not yet been made.

MATERIAL AND METHODS

Material

This study is based on 160 specimens representing *ca* 150 taxa. Sequences were derived from fresh and herbarium specimens or were retrieved from GenBank. Most collections originated in Europe or North America; tropical regions and the Southern Hemisphere are strongly under-represented. Type species of genera were included wherever possible, but this was not feasible for *Cystolepiota* and *Micropsalliota*. A single taxon represents the *Lycoperdaceae*, a group of *ca* 190 species, in the ITS and ITS-LSU analyses; nine species are included in the big LSU data set. *Limacella glioderma* was chosen as outgroup; several bolete species served as an alternate outgroup for a separate LSU data set of 54 species to test the position of several species formerly assigned to the family. Sequences of the species cultivated by the attine ants were taken from GenBank. Additional GenBank sequences represent unidentified Central American taxa, for which morphological data are lacking. In a few cases the ITS-sequence was taken from a different specimen than the LSU-sequence (see Table 1 for collections and species analysed).

Most of the sequences obtained from GenBank were in the *Agaricaceae* and a few appeared to be misidentified, based on sequence comparisons with the present author's data. Considerable effort was made to trace and examine the material on which the suspect sequences had been based but this effort was not always successful. However for non-lepiotaceous fungi the identity declared in GenBank was not questioned.

Molecular identification

DNA was extracted from fresh and herbarium material; the internal transcribed spacer (ITS), and part of the large subunit (LSU) of the nuclear ribosomal repeat were amplified by the polymerase chain reaction (PCR) with the fungal specific primers ITS1F and ITS4 (Gardes & Bruns 1993) for the ITS-region, and primers LR0R, LR3R, LR7 and LR16 (www.biology.duke.edu/fungi/mycolab/) for LSU. Sequencing of both strands was performed with an ABI model 377 sequencer or an ABI model 3100 automated sequencer (Applied Biosystems, Foster City, CA) using a Thermo SequenaseTM Dye terminator Cycle Sequencing Pre-Mix Kit (Amersham Pharmacia Biotech, Piscataway, NJ) or a BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems); the primers ITS1 or ITS5, ITS2, ITS3, and ITS4 were used for the ITS-region, and primers LR0R, LR3R,

¹ The following abbreviations are used throughout the paper to indicate genus names: *Ag.*, *Agaricus*; *Ch.*, *Chamaemyces*; *Chl.*, *Chlorophyllum*; *Cl.*, *Cystolepiota*; *L.*, *Lepiota*; *La.*, *Leucoagaricus*; *Lc.*, *Leucocoprinus*; *M.*, *Macrolepiota*; *Me.*, *Melanophyllum*. Some taxa, morphologically belonging to *Leucoagaricus* or *Leucocoprinus*, are still listed under *Lepiota*, as the appropriate combination has not been made yet (see also Table 1).

Table 1. Overview of species and collections and the GenBank accession numbers for the ITS and LSU sequences. Type species of genera in the *Agaricaceae* are indicated with an asterisk (*). Note that many species are still listed under *Lepiota*, though the correct placement would be in *Leucoagaricus/Leucocoprinus*; these species are marked with a dagger (†).

Species	Collection and herbarium	Location and date of collection	GenBank accession nos.	
			ITS	LSU
<i>Agaricus bitorquis</i>	<i>E. C. Vellinga 2462</i> (UC)	USA: Michigan: Washtenaw Co., Ann Arbor, 10 Aug. 2000	AF482829	
<i>Ag. bitorquis</i>	GenBank			AF059217
<i>Ag. californicus</i>	<i>E. C. Vellinga 2319</i> (UC)	USA: California: Alameda Co., Berkeley, U.C. Berkeley campus, 29 Jan. 1999	AF482830	
<i>Ag. californicus</i>	<i>E. C. Vellinga 2337</i> (UC)	USA: California: Alameda Co., Berkeley, U.C. Berkeley campus, 3 Aug. 1999		AF482876
<i>Ag. campestris</i> *	GenBank		U85307	U85273
<i>Ag. diminutivus</i>	<i>E. C. Vellinga 2360</i> (UC)	USA: Washington: Olympic Peninsula, Grays Harbor Co., north side of Quinault Lake, July Creek Campground, 16 Oct. 1999	AF482831	AF482877
<i>Ag. inapertus</i> (syn. <i>Endoptychum depressum</i>)	<i>E. C. Vellinga 2339</i> (UC)	USA: California: Sierra Nevada, Fresno Co., Dinkey Creek Rd, 6 Aug. 1999	AF482834	AF482878
<i>Allopsalliota geesterani</i> *	<i>E. C. Vellinga 2263</i> (L)	The Netherlands: <i>Prov. Noord-Holland:</i> Amsterdam, Amsterdamse Bos, 23 Sept. 1998	AF482857	AF482888
<i>Amanita phalloides</i>	GenBank			AF261435
<i>Austropaxillus infundibiliformis</i>	GenBank			AF098393
<i>Battarrea laciniata</i>	GenBank			AF208534
<i>Boletellus mirabilis</i>	GenBank			AF050652
<i>Boletus edulis</i>	GenBank			AF071457
<i>Bovista</i> sp.	GenBank			AF261483
<i>Calvatia</i> sp.	GenBank			AF261482
<i>Chamaemyces fracidus</i> *	<i>T. W. Kuyper 960</i> (L)	Belgium: <i>Prov. Luxembourg:</i> Ave et Auffe, Le Roptai, Oct. 1977	AY176343	AY176344
<i>Chlorophyllum brunneum</i>	<i>E. C. Vellinga 2317</i> (UC)	USA: California: Alameda Co., Berkeley, U.C.-Berkeley campus, 25 Jan. 1999	AF482856	
<i>Chl. brunneum</i>	<i>E. C. Vellinga 2361</i> (UC)	USA: Oregon: Multnomah Co., Portland, S.E. Locust Ave, 20 Oct. 1999		AF482886
<i>Chl. hortense</i>	<i>D. E. Hemmes 1365</i> (SFSU)	USA: Hawai'i Islands: Hawai'i, Waipio Valley, 10 Dec. 1996	AF482843	
<i>Chl. hortense</i>	GenBank			U85284
<i>Chl. molybdites</i> *	<i>J. States AEF1097</i> (MICH)	USA: Arizona: Maricopa Co., Peoria, 4 Sept. 1994	AF482836	
<i>Chl. molybdites</i>	GenBank			U85274
<i>Chl. rachodes</i>	<i>E. C. Vellinga 2106</i> (L)	The Netherlands: <i>prov. Zuid-Holland:</i> Leiden, Sept. 1997	AF482849	
<i>Chl. rachodes</i>	<i>M. Enderle</i> (L)	Germany: <i>Baden-Württemberg:</i> between Altheim and Gerstetten, 10 Oct. 1998		AY176345
<i>Coprinopsis semitalis</i>	GenBank			AF041508
<i>Coprinus comatus</i> *	<i>E. C. Vellinga 2766</i> (UC)	USA: California: Alameda Co., Berkeley campus, University of California at Berkeley, near Warren Hall, 3 Dec. 2001	AY176346	

<i>C. comatus*</i>	GenBank			AF041529
<i>C. sterquilinus</i>	GenBank		AF345821	AF041530
<i>Crucibulum laeve</i>	GenBank			AF336246
<i>Cyathus striatus</i>	GenBank			AF336247
<i>Cystoagaricus strobilomyces</i>	<i>E. Nagasawa 97-40</i> (TMI)	Japan: <i>Tottori Pref.:</i> Tottori City, Kokoge (Exp. For.), 30 May 1997	AY176347	AY176348
<i>Cystoderma amianthinum</i>	GenBank			AF261473
<i>Cystolepiota cystidiosa</i>	GenBank		U85333	U85298
<i>Chl. pulverulenta</i> (type species of <i>Pulverolepiota</i>)	<i>E. C. Vellinga 1872</i> (L)	UK: <i>England:</i> Surrey, Richmond, Kew, Royal Botanic Garden, 8 Sept. 1992	AF391036	AY176349
<i>Chl. seminuda</i>	<i>H. A. Huijser s.n.</i> (herb. Huijser) 6126	The Netherlands: <i>Prov. Limburg:</i> Savelsbos, 4 Oct. 1989	AY176350	AY176351
<i>Endoptychum agaricoides*</i>	<i>R. Brotzu</i> (herb. Brotzu)	Italy: <i>Sardinia:</i> Nuoro, Oct. 1990	AF482837	AF482885
<i>Entoloma nidorosum</i>	GenBank			AF261296
<i>Lepiota aspera</i>	<i>E. C. Vellinga 2233</i> (L)	The Netherlands: <i>Prov. Limburg:</i> Elsloo-Geulle, Bunderbos, 19 Sept. 1998	AY176354	
<i>L. aspera</i>	GenBank			U85293
<i>L. brunneoincarnata</i>	<i>E. C. Vellinga 2295</i> (L)	The Netherlands: <i>Prov. Zeeland:</i> Schouwen-Duiveland, Verklikkerduinen, 7 Oct. 1998	AY176355	
<i>L. brunneoincarnata</i>	<i>M. Enderle</i> (L)	Germany: <i>Baden-Württemberg:</i> Ulm-Grimmelfingen, 'Wald "Hörnle"', 29 Aug. 1996		AF482896
<i>L. castaneidisca</i>	<i>E. C. Vellinga 2411</i> (UC)	USA: <i>California:</i> San Mateo Co., Skyline Blv, 13 Dec. 1999		AY176356
<i>L. castaneidisca</i>	<i>F. Stevens</i> (coll. <i>E. C. Vellinga 2300</i>) (UC)	USA: <i>California:</i> San Mateo Co., San Mateo County Memorial Park, 4 Dec. 1998	AF391057	
<i>L. cingulum</i>	<i>M. Enderle</i> (L) 6174	Germany: <i>Bavaria:</i> 20 Sept. 1995	AY176359	AY176360
<i>L. clypeolaria*</i>	<i>E. C. Vellinga 1683</i> (L)	Germany: <i>Rheinland-Pfalz:</i> Eifel, Gerolstein, Berlinger Bach, 17 Sept. 1990	AY176361	
<i>L. clypeolaria*</i>	<i>H. A. Huijser s.n.</i> (herb. Huijser) 6129	Belgium: <i>Prov. Luxembourg:</i> Resteigne, Bois de Resteigne, 4 Oct. 1998		AY176362
<i>L. cristata</i>	<i>E. C. Vellinga 1445</i> (L)	Luxembourg: Hollenfels, 28 Sept. 1988	AF391027	
<i>L. cristata</i>	GenBank			U85292
<i>L. cristatoides</i>	<i>H. A. Huijser s.n.</i> (herb. Huijser) 6116	The Netherlands: <i>Prov. Limburg:</i> Cadier en Keer, Riesenberg, 5 Sept. 1996	AY176363	AY176364
<i>L. cystophoroides</i>	<i>E. C. Vellinga 2142</i> (L)	France: <i>Dept l'Hérault:</i> Cédraie d'Escandorgue, 20 Oct. 1997	AF391031	AY176365
<i>L. echinella</i>	<i>H. A. Huijser s.n.</i> (herb. Huijser) 6146	Belgium: <i>Prov. Luxembourg:</i> Bois de Resteigne, 4 Oct. 1998	AY176366	AY176367
<i>L. cfr erminea</i>	<i>N. S. Weber 2947</i> (MICH)	USA: <i>Idaho:</i> Owyhee Co., Cow Creek, 12 June 1972	AY176357	AY176358
<i>L. farinolens</i>	<i>E. C. Vellinga 2146</i> (L)	France: <i>Dépt. L'Hérault:</i> Graissessac, 21 Oct. 1997	AY176368	AY176369
<i>L. felina</i>	GenBank		U85330	U85295
<i>L. flammeatincta†</i>	GenBank		U85331	U85296
<i>L. flammeatincta†</i>	<i>E. C. Vellinga 2746</i> (UC)	USA: <i>California:</i> Marin Co., Tomales Bay State Park, 28 Nov. 2001	AY176440	
<i>L. flammeatincta†</i>	<i>E. C. Vellinga 2533</i> (UC)	USA: <i>California:</i> Mendocino Co., Mendocino, Jackson State Forest, 18 Nov. 2000		AY176441
<i>L. forquignonii</i>	<i>E. C. Vellinga 2284</i> (L)	The Netherlands: <i>Prov. Limburg:</i> Valkenburg, Schaelsberg, 10 Oct. 1998	AY176370	AY176371

Table 1. (Cont.)

Species	Collection and herbarium	Location and date of collection	GenBank accession nos.	
			ITS	LSU
<i>L. fuscovinacea</i>	<i>E. C. Vellinga</i> 2255 (L)	The Netherlands : <i>Prov. Noord-Holland</i> : Amsterdam, Amstelpark, 23 Sept. 1998	AY176372	AY176373
<i>L. haemorrhagica</i> †	<i>K. R. Thiele</i> 2652 (MEL)	Australia : <i>Victoria</i> : 52 km No of Orbost on the Bonang Road, Martins Creek, 320 m asl, 17 Nov. 2000	AY176374	AY176375
<i>L. hymenoderma</i>	<i>E. C. Vellinga</i> 2017 (L)	The Netherlands : <i>Prov. Limburg</i> : Elsloo-Geulle, Bunderbos, 7 Sept. 1996	AF391083	AY176376
<i>L. hystrix</i>	<i>H. A. Huijser</i> 6114 (herb. Huijser)	France : <i>Beaulieu-en-Argonne</i> , Triancourt, 25 Oct. 1998	AY176377	AY176378
<i>L. lilacea</i>	<i>E. C. Vellinga</i> 2451 (UC)	USA : <i>Michigan</i> : Washtenaw Co., Ann Arbor, 6 Aug. 2000	AY176379	AY176380
<i>L. magnispora</i>	<i>E. C. Vellinga</i> 2246 (L)	The Netherlands : <i>Prov. Limburg</i> : Hopel-Kerkrade, mine Laura-Julia, 19 Sept. 1998	AF391021	
<i>L. magnispora</i>	<i>J. States</i> 1830 (AEF1015) (MICH)	USA : <i>Arizona</i> : Cochise Co., Chiricahua Mountains, Onion Pass, 4 Sept. 1993		AY176381
<i>L. nigrescentipes</i>	<i>G. Rioussset</i> 93051001 (herb. Rioussset)	France : <i>Dépt. Bouche du Rhône</i> : Maillane, 5 Oct. 1993	AY176382	AY176383
<i>L. oculata</i> †	<i>E. C. Vellinga</i> 2366 (UC)	USA : <i>Oregon</i> : Lincoln Co., Lincoln City, Devils Lake Park, 22 Oct. 1999	AY176384	AY176385
<i>L. ochraceofulva</i>	<i>E. C. Vellinga</i> 2273 (L)	The Netherlands : <i>Prov. Zuid-Holland</i> : Rijswijk, Ter Werve, 30 Sept. 1998	AY176386	
<i>L. ochraceofulva</i>	<i>E. C. Vellinga</i> 2267 (L)	The Netherlands : <i>Prov. Flevoland</i> : Noordoostpolder, Urkerbos, 23 Sept. 1998		AY176387
<i>L. perplexa</i>	<i>E. C. Vellinga</i> 2228 (L)	The Netherlands : <i>Prov. Limburg</i> : Elsloo-Geulle, Bunderbos, 19 Sept. 1998	AY176388	AY176389
<i>L. psalion</i>	<i>H. A. Huijser</i> 6177 (herb. Huijser)	The Netherlands : <i>Prov. Limburg</i> : Valkenburg, Schaelsberg, 15 Aug. 1999	AY176390	
<i>L. psalion</i>	<i>H. A. Huijser</i> 6153 (herb. Huijser)	The Netherlands : <i>Prov. Limburg</i> : Valkenburg, Schaelsberg, 23 Aug. 1999		AY176391
<i>L. pseudolilacea</i>	<i>E. C. Vellinga</i> 2278 (L)	The Netherlands : <i>Prov. Noord-Holland</i> : Amsterdamse Waterleidingduinen, 4 Oct. 1998	AY176392	
<i>L. pseudolilacea</i>	<i>H. A. Huijser</i> 6147 (herb. Huijser)	The Netherlands : <i>Prov. Limburg</i> : Brunssum, mine Hendrik, 7 Oct. 1998		AY176393
<i>L. roseolivida</i> †	<i>E. C. Vellinga</i> 2475 (UC)	USA : <i>California</i> : San Mateo Co., San Mateo Co. Memorial Park, 31 Oct. 2000	AY176394	
<i>L. roseolivida</i> †	<i>E. C. Vellinga</i> 2486 (UC)	USA : <i>California</i> : Contra Costa Co., Tilden Regional Park, 4 Nov. 2000		AY176395
<i>L. rufipes</i> sensu [auct. Europe.]	<i>H. A. Huijser</i> s.n. (herb. Huijser)	The Netherlands : <i>Prov. Limburg</i> : Bemelen, 9 Oct. 1991		AF482897
<i>L. scaberula</i>	<i>E. C. Vellinga</i> 2595 (UC)	USA : <i>California</i> : San Mateo Co., San Francisco Watershed, near Pulgas Water Temple, 8 Dec. 2000	AF391030	
<i>L. scaberula</i>	<i>E. C. Vellinga</i> 2307 (UC)	USA : <i>California</i> : San Mateo Co., Wunderlich County Park, 26 Dec. 1998		AY176396

<i>Lepiota</i> sp.	GenBank PA409		AF079752	AF079670
<i>Lepiota</i> sp.	<i>R. Douwes</i> 25 (WAG)	Cameroon: <i>South Province:</i> T.C.P. area, Bitsili, 20 Nov. 1997		AY176398
<i>Lepiota</i> sp.	<i>P. B. Matheny</i> 1827 (WTU)	USA: <i>Hawaii,</i> Kawai, Koke'e State Park, Waimea Canyon, 28 Dec. 1999	AY176402	AY176403
<i>Lepiota</i> sp.	GenBank (as <i>L. subincarnata</i>)		U85329	U85294
<i>Lepiota</i> sp.	<i>P. B. Matheny</i> 1486 (WTU)	USA: <i>Missouri:</i> Taney Co., Coy Bald at Hercules Glade, 4 Jun. 1999	AY176400	AY176401
<i>Lepiota</i> sp.	<i>R. Douwes</i> 42 (WAG)	Cameroon: <i>South Province:</i> T.C.P. area, Ebimingang, 24 Nov. 1997		AY176399
<i>Lepiota</i> sp.	<i>E. C. Vellinga</i> 2582 (UC)	USA: <i>California:</i> San Mateo Co., San Francisco Watershed, near Pulgas Water Temple, 8 Dec. 2000	AY176397	
<i>L. spheniscispora</i>	<i>E. C. Vellinga</i> 2298 (UC)	USA: <i>California:</i> San Mateo Co., San Francisco Watershed, near Pulgas Water Temple, 4 Dec. 1998	AF391000	
<i>L. spheniscispora</i>	<i>E. C. Vellinga</i> 2438 (UC)	USA: <i>California:</i> Marin Co., Mt. Tamalpais, 15 Feb. 2000		AY176404
<i>L. cf subclypeolaria</i> Sacc.†	GenBank		AF079735	AF079653
<i>L. xanthophylla</i>	<i>E. C. Vellinga</i> 2240 (L)	The Netherlands: <i>Prov. Limburg:</i> Elsloo-Geulle, Bunderbos, 19 Sept. 1998	AY176405	AY176406
<i>L. cf zamurensis</i> †	GenBank		AF079753	AF079671
<i>Leucoagaricus americanus</i>	<i>E. C. Vellinga</i> 2454 (UC)	USA: <i>Michigan:</i> Washtenaw Co., Ann Arbor, campus University of Michigan, 6 Aug. 2000	AY176407	AF482891
<i>La. barssii</i> *	<i>E. C. Vellinga</i> 2342 (UC)	USA: <i>California:</i> Alameda Co., San Leandro, 21 Sept. 1999	AF295931	
<i>La. barssii</i> *	<i>E. C. Vellinga</i> 2268 (L)	The Netherlands: <i>Prov. Noord-Holland:</i> Texel, Loosmansduinen, 24 Sept. 1998		AF482894
<i>La. cinerascens</i>	<i>P. B. Matheny</i> 1830 (WTU)	USA: <i>Hawaii:</i> Kawai, Koke'e State Park, Halemann-Koke'e Trail, 28 Dec. 1999	AY176408	AY176409
<i>La. cinerascens</i>	<i>P. B. Matheny</i> 1831 (WTU)	USA: <i>Hawaii:</i> Kawai, Koke'e State Park, Halemann-Koke'e Trail, 28 Dec. 1999	AY176410	AY176411
<i>La. croceovelutinus</i>	<i>E. C. Vellinga</i> 2243 (L)	The Netherlands: <i>Prov. Limburg:</i> Elsloo-Geulle, Bunderbos, 19 Sept. 1998	AF482862	AF482889
<i>La. crystallifer</i>	<i>H. A. Huijser s.n.</i> (herb. Huijser)	Germany: <i>Baden-Württemberg:</i> Gottenheim, Wasenweiler Wald, 3 Sept. 1998	AF482863	AY176412
<i>La. georginae</i>	<i>E. C. Vellinga</i> 2238 (L)	The Netherlands: <i>Prov. Limburg:</i> Elsloo-Geulle, Bunderbos, 19 Sept. 1998	AY176413	AY176414
<i>La. ionidicolor</i>	<i>E. C. Vellinga</i> 2280 (L)	The Netherlands: <i>Prov. Limburg:</i> Stokhem, 10 Oct. 1998	AY176415	AY176416
<i>La. leucothites</i>	<i>E. C. Vellinga</i> 2050 (L)	The Netherlands: <i>Prov. Noord-Holland:</i> Amsterdamse Waterleidingduinen, Van Lennepkanaal, 15 Oct. 1996	AF482865	
<i>La. leucothites</i>	GenBank			U85280
<i>La. marriagei</i>	<i>E. C. Vellinga</i> 2005 (L)	The Netherlands: <i>Prov. Limburg:</i> Elsloo-Geulle, Bunderbos, 9 Sept. 1996	AF482866	
<i>La. melanotrichus</i>	<i>E. C. Vellinga</i> 2262 (L)	The Netherlands: <i>Prov. Noord-Holland,</i> Amsterdam, Amsterdamse Bos, 23 Sept. 1998	AY176417	AY176418
<i>La. meleagris</i>	<i>E. C. Vellinga</i> 1990 (L)	The Netherlands: <i>Prov. Noord-Holland:</i> Amsterdam, Zorgvlied, 30 July 1996	AY176419	AF482890

Table 1. (Cont.)

Species	Collection and herbarium	Location and date of collection	GenBank accession nos.	
			ITS	LSU
<i>La. nympharum</i>	<i>C. Bas</i> 9269 (L)	Germany : <i>Rheinland-Pfalz</i> : Eifel, near Gerolstein, 21 Sept. 1990	AF482868	
<i>La. nympharum</i>	<i>R. P. J. de Kok</i> (L)	Germany : <i>Rheinland-Pfalz</i> : Eifel, near Dohm, 24 Sept. 1990		AF482895
<i>La. serenus</i>	<i>A. Bizzi</i> 369/98 (herb. Bizzi)	Italy : <i>Vicenza</i> : Grancona, Val del Gazzo, 2 Oct. 1998	AY176420	
<i>La. serenus</i>	<i>E. C. Vellinga</i> 1930 (L)	Belgium : <i>Prov. Liège</i> : Tilff, Vallon de la Chawresse, 11 Sept. 1995		AY176421
<i>La. sericatellus</i>	<i>E. C. Vellinga</i> 2013 (L)	The Netherlands : <i>Prov. Limburg</i> : Elsloo-Geulle, Bunderbos, 7 Sept. 1996	AY176422	AY176423
<i>La. sericatellus</i>	<i>C. Bas</i> 8842 (L)	The Netherlands : <i>Prov. Overijssel</i> : Zwolle, Zalker Bos, 12 Sept. 1992	AY176424	AY176425
<i>La. sericifer</i>	<i>E. C. Vellinga</i> 2116 (L)	The Netherlands : <i>Prov. Utrecht</i> : Breukelen, estate Nijenrode, 1 Oct. 1997	AY176426	AY176427
<i>Leucoagaricus</i> sp. (<i>rubrotinctus</i> group)	<i>E. C. Vellinga</i> 2457 (UC)	USA : <i>Michigan</i> : Washtenaw Co., Ann Arbor, 7 Aug. 2000	AY176428	AY176429
<i>Leucoagaricus</i> sp. (<i>rubrotinctus</i> group)	<i>E. C. Vellinga</i> 2561 (UC)	USA : <i>California</i> : Contra Costa Co., Tilden Regional Park, 26 Nov. 2000	AY176430	AY176431
<i>Leucoagaricus</i> sp. (<i>rubrotinctus</i> group)	<i>K. R. Thiele</i> 2646 (MEL)	Australia : <i>Victoria</i> : 53 km No by road of Orbost, Martins Creek, 320 m asl, 20 March 2000	AY176432	AY176433
<i>Leucoagaricus</i> sp. (<i>rubrotinctus</i> group)	GenBank (as <i>L. rubrotinctus</i>)		U85316	U85281
<i>Leucoagaricus</i> sp. (<i>rubrotinctus</i> group) a	<i>M. M. Rogers</i> (UC) 6184	USA : <i>Oregon</i> : Lane Co., Eugene, Mount Pisgah Arboretum, 4 Nov. 2000	AY176434	
<i>Leucoagaricus</i> sp. (<i>rubrotinctus</i> group) a	<i>E. C. Vellinga</i> 2499 (UC)	USA : <i>California</i> : Marin Co., Mt. Tamalpais, 9 Nov. 2000		AY176435
<i>Leucoagaricus</i> sp.	<i>K. R. Thiele</i> 2645 (MEL)	Australia : <i>Victoria</i> : 53 km No by road of Orbost, Martins Creek, 320 m asl, 20 March 2000	AY176436	AY176437
<i>Leucoagaricus</i> sp.	GenBank (as <i>L. americanus</i>)		U85317	U85282
<i>Leucoagaricus</i> sp. a	<i>E. C. Vellinga</i> 2607 (UC)	USA : <i>California</i> : San Mateo Co., San Francisco Watershed, 8 Dec. 2000	AY176438	
<i>Leucoagaricus</i> sp. a	<i>E. C. Vellinga</i> 2498 (UC)	USA : <i>California</i> : Marin Co., Mt. Tamalpais, 9 Nov. 2000		AY176439
<i>La. sublittoralis</i>	<i>E. C. Vellinga</i> 2235 (L)	The Netherlands : <i>Prov. Limburg</i> : Elsloo-Geulle, Bunderbos, 19 Sept. 1998	AY176442	AY176443
<i>La. tener</i>	<i>E. C. Vellinga</i> 2261 (L)	The Netherlands : <i>Prov. Noord-Holland</i> : Amsterdam, Amstelpark, 23 Sept. 1998	AY176444	AY176445
<i>Leucocoprinus brebissonii</i>	<i>E. C. Vellinga</i> 1784 (L)	France : <i>Dept Pas-de-Calais</i> : Forêt de Boulogne, 13 Oct. 1991	AF482859	AY176446
<i>Lc. cepaestipes</i> *	GenBank		U85338	U85306
<i>Lc. cretaceus</i>	<i>T. Læssøe & J. Omar</i> TL-6171 (C)	Malaysia : <i>Sabah</i> : Tabin Wildlife Reserve, Mud Volcano trails 3 & 4, 9 Feb. 1999	AY176447	
<i>Lc. cretaceus</i>	<i>J. Engelen</i> s.n. (L)	The Netherlands : <i>Prov. Gelderland</i> : Apeldoorn, 9 Sept. 1997		AY176448
<i>Lc. fragilissimus</i>	GenBank		U85324	U85289
<i>Lc. fragilissimus</i>	GenBank		AF079738	AF079656
<i>Leucocoprinus</i> sp.	GenBank PA147		AF079729	AF079647

<i>Leucocoprinus</i> sp.	GenBank PA148		AF079730	AF079648
<i>Leucocoprinus</i> sp.	GenBank PA152		AF079731	AF079649
<i>Leucocoprinus</i> sp.	GenBank PA165		AF079733	AF079651
<i>Leucocoprinus</i> sp.	GenBank PA170		AF079734	AF079652
<i>Leucocoprinus</i> sp.	GenBank PA205		AF079736	AF079654
<i>Leucocoprinus</i> sp.	GenBank PA234		AF079737	AF079655
<i>Leucocoprinus</i> sp.	GenBank PA272		AF079740	AF079658
<i>Leucocoprinus</i> sp.	GenBank PA280		AF079741	AF079659
<i>Leucocoprinus</i> sp.	GenBank PA294		AF079743	AF079661
<i>Leucocoprinus</i> sp.	GenBank PA300		AF079744	AF079662
<i>Leucocoprinus</i> sp.	GenBank PA302		AF079745	AF079663
<i>Leucocoprinus</i> sp.	GenBank PA309		AF079746	AF079664
<i>Leucocoprinus</i> sp.	GenBank PA341		AF079747	AF079665
<i>Leucocoprinus</i> sp.	GenBank PA 351		AF079748	AF079666
<i>Leucocoprinus</i> sp.	GenBank PA356		AF079749	AF079667
<i>Leucocoprinus</i> sp.	GenBank PA363		AF079750	AF079668
<i>Leucocoprinus</i> sp.	GenBank PA375		AF079751	AF079669
<i>Leucopholiota decorosa</i>	<i>H. Kotiranta & T. Læssøe TL4781 (C)</i>	Russia: Kamchatka: 12 Aug. 1997	AY176449	AY176450
<i>Limacella glioderma</i>	<i>E. C. Vellinga 2241 (L)</i>	The Netherlands: Prov. Limburg: Elsloo-Geulle, Bunderbos, 19 Sept. 1998	AY176451	AY176452
<i>L. glioderma</i>	<i>E. C. Vellinga 2456 (UC)</i>	USA: Michigan: Washtenaw Co., Ann Arbor, 7 Aug. 2000	AY176453	AY176454
<i>Lycoperdon marginatum</i>	GenBank			AF261485
<i>L. perlatum*</i>	GenBank		AJ237627	AJ406583
<i>L. pyriforme</i>	GenBank		AJ237620	
<i>Lycoperdon</i> sp.	GenBank			AF287873
<i>Macrolepiota clelandii</i>	<i>K. R. Thiele 2650 (MEL)</i>	Australia: Victoria: 52 km North of Orbost on the Bonang Road, Martins Creek, 17 May 2000	AF482838	AF482882
<i>M. dolichaula</i>	<i>E. M. Canning 6603 (CANB)</i>	Australia: New South Wales: between Blakney Creek and Bevendale, just off road inside entrance to 'Kunama Cottage', 4 April 1989	AF482839	
<i>M. dolichaula</i>	<i>R. P. J. de Kok 901 (CANB)</i>	Australia: ACT: Canberra, Black Mountain, CSIRO site, 14 April 2000		AF482883
<i>M. mastoidea</i>	<i>E. C. Vellinga 1685 (L)</i>	Germany: Rheinland-Pfalz: Eifel, west of Wiesbaum, 18 Sept. 1990	AF482844	
<i>M. mastoidea</i>	GenBank			U85279
<i>M. procera*</i>	<i>E. C. Vellinga 2293 (L)</i>	The Netherlands: Prov. Zeeland: Schouwen-Duiveland, Haamstede, 7 Nov. 1998	AF482848	
<i>M. procera*</i>	<i>R. P. J. de Kok s.n. (L)</i>	The Netherlands: Prov. Groningen: Ter Apel, Ter Haar, 18 Oct. 1990		AF482880
<i>Melanophyllum haematospermum*</i>	<i>E. C. Vellinga 2249 (L)</i>	The Netherlands: Prov. Limburg: Hopel-Kerkrade, mine waste heap Laura-Julia, 19 Sept. 1998	AF391038	AY176455
<i>Me. haematospermum*</i>	<i>E. C. Vellinga 2517 (UC)</i>	USA: California: Mendocino Co., Navarro River Redwoods State Park, 19 Nov. 2000		AY176456
<i>Micropsalliota</i> sp.	<i>T. Læssøe & J. Omar TL-6025 (C)</i>	Malaysia: Sabah: Danum Valley, Field Centre, Main East Trail, 30 Jan. 1999	AF482835	AF482879
<i>Montagnea arenarius*</i>	GenBank			AF041538
<i>M. candollei</i>	GenBank			AF261581
<i>M. radiosus</i>	GenBank			AF261480

Table 1. (Cont.)

Species	Collection and herbarium	Location and date of collection	GenBank accession nos.	
			ITS	LSU
<i>Notholepiota areolata</i>	<i>C. Shirley CSAK004</i> (herb. Shirley)	New Zealand : North Island		AY253721
<i>Phaeolepiota aurea</i>	GenBank			AF261474
<i>Podaxis pistillaris</i>	GenBank		U85336	AF041539
<i>Psathyrella delineata</i>	GenBank			AF041532
<i>Pseudobaeospora pyrifer</i>	<i>L. Krieglsteiner</i> (isotype, REG)	Germany : Bavaria: Kitzinger, Klosterforst, 10 Sept. 1995	AF391034	AY176457
<i>Suillus luteus</i>	GenBank			AF042622
<i>Termiticola</i> sp.	<i>T. Læssøe TL-6106</i> (C)	Malaysia : Sabah: Danum Valley, Borneo Jungle Lodge, 2 Feb. 1999	AY176352	AY176353
<i>Thaxterogaster violaceus</i>	GenBank			AF261552
<i>Tricholoma focale</i>	GenBank			U76460
<i>Tulostoma brumale</i>	GenBank			AF336272
<i>T. simulans</i>	GenBank			AF26148
Attine symbiont	GenBank S60		AF079701	AF079619
Attine symbiont	GenBank G26		AF079721	AF079639
Attine symbiont	GenBank DA373		AF079754	AF079672
Attine symbiont	GenBank G9		AF079693	AF079611
Attine symbiont	GenBank S16		AF079715	AF079633
Attine symbiont	GenBank S36		AF079679	AF079597
Attine symbiont	GenBank G28		AF079724	AF079642
Attine symbiont	GenBank G11		AF079716	AF079634
Attine symbiont	GenBank S28		AF079708	AF079626
Attine symbiont	GenBank OC29		AF079727	AF079645
Attine symbiont	GenBank G15		AF079677	AF079595

LR7, and LR16 for the LSU-region. The raw data were processed with the use of DNA Sequencing Analysis v.2.1.2 and Sequence Navigator v.1.0.1 (Applied Biosystems). All sequences were deposited in GenBank with the accession numbers given in Table 1.

Alignment

Special attention was paid to the alignments; partial order alignment (POA) (Lee, Grasso & Sharlow 2002) was used with minimal visual adjustment. By contrast, alignments made with CLUSTAL X 1.81 (Thompson *et al.* 1997) needed extremely heavy editing and were deemed unsatisfactory (before correction, identical sequences sometimes ended up in different clades.) A large part of the ITS1-region is very variable, and was excluded from the phylogenetic analysis.

Phylogenetic analysis

Maximum parsimony analyses were performed using PAUP* version 4.0b10 (Swofford 2002). One hundred heuristic searches were conducted with random sequence addition and tree bisection-reconnection (TBR) branch-swapping algorithms, collapsing zero-length branches and saving all minimal length trees (MulTrees) on different data sets (ITS data, combined ITS and LSU; LSU data sets). *Limacella glioderma* was chosen as outgroup, except for a separate analysis of a LSU data set of 54 taxa, in which several boletoid species (*viz.* *Boletus edulis*, *Boletellus mirabilis*, *Suillus luteus*, and *Austropaxillus infundibuliformis*) constituted the outgroup. Non-informative characters and a large part of the ITS1-region that was not unambiguously alignable were excluded from the analyses. Gaps were treated as missing data. To measure relative support for the resulting clades, 3000 bootstrap replications (Felsenstein 1985) were performed under the 'fast stepwise addition' option. The combined data set was tested for incongruence with the partition homogeneity test, as implemented in PAUP* 4.0b10, as advocated by Cunningham (1997). To test alternative phylogenetic relationships, the Kishino-Hasegawa maximum likelihood ratio test (Kishino & Hasegawa 1989) (under default settings) was performed with several constraints, as implemented in PAUP*. Pairwise genetic distances were calculated with the standard setting in PAUP* 4.0b10, and divided into intrageneric and intergeneric groups, according to two classification schemes. Similar distance methods have been used to evaluate existing genus definitions in mammals (Castresana 2001) and *Eusascomycetes* (Lumbsch 2002).

RESULTS

General

The ITS sequence data set, including the 5.8S gene, comprises 128 sequences, and is 929 characters long with 377 parsimony informative characters, after

alignment. The ITS data yield 190 most parsimonious trees in two islands, with $l=4818$, $CI=0.1696$, and $RC=0.0848$. The tree with the smallest $-\ln L$ is depicted in Fig. 1, and the strict consensus tree in Fig. 2. The same main clades as in the ITS-LSU trees (Fig. 5) are recovered, but the topology is slightly different and some taxa end up in different clades than in the combined data set (e.g. *Allopsalliota geesterani*). The ITS sequence of *Notholepiota areolata* was not included, as it was not alignable with the other sequences.

The LSU data set is made up of 149 taxa and 639 characters of which 188 are parsimony informative. The LSU data do give quite different results than the ITS data set, with many fewer resolved clades, and only a few small species groups that are bootstrap supported. The particulars of the trees are $l=1343$, $CI=0.2107$, $RC=0.1181$ (the maximum number was set to 5000; Fig. 3). Striking disagreements with the results of the analyses based on the ITS sequences are (i) the placement of some *Lepiota* species outside the main *Lepiota* clade, and (ii) *Leucoagaricus* not monophyletic. Neighbour joining methods yield slightly different topologies, depending on the distance parameters. Adding or omitting taxa significantly changes the topologies, and the composition of the clades (see also Fig. 4).

The smaller LSU data set, comprising 54 species with an outgroup of four bolete and bolete-related taxa, yielded the maximum number of trees, based on 238 parsimony informative characters ($l=1042$, $CI=0.3484$, $RC=0.1853$). One tree is shown in Fig. 4, and provides evidence that *Notholepiota areolata*, *Cystoagaricus strobilomyces*, *Pseudobaeospora pyrifer*, and *Leucopholiota decorosa* do not belong to the *Agaricaceae*. The position of the *Lycoperdaceae* is different here than in the phylograms based on a data set with more members of the *Agaricaceae*.

The ITS and LSU data sets are congruent ($P=0.2$), and were combined in one data base. The POA-aligned ITS-LSU data set comprises 1533 characters, of which 548 are parsimony informative. The ITS-LSU data set yields 2299 most parsimonious trees in four islands ($l=6088$, $CI=0.1759$, $RC=0.0874$). Tree 194 has the smallest $-\ln L$ and is depicted in Fig. 5. The principal clades of this tree are also recovered in the strict consensus tree based on all 2299 trees. The differences between these trees are not in number of clades but in the configuration of the taxa within them.

Bootstrap values in general are very low, and not many clades are bootstrap-supported. The family *Agaricaceae* has a bootstrap support of 95%, excluding *Cystoagaricus* and *Leucopholiota*. Four clades do get reasonably high bootstrap support, *viz.* *Agaricus* plus satellite genera (clade 1), *Chlorophyllum* (clade 2), *Macrolepiota s. str.* (clade 4), and some parts of clade 3, namely the *Leucoagaricus sericifer* group, and part of the *Leucoagaricus rubrotinctus/Sericomyces* group. But all major clades are present in the strict consensus tree (see below).



Fig. 1. Best (–ln L) of 190 equally most parsimonious trees, based on ITS data. The circled numbers on the right refer to the clade numbers, as used throughout the text.

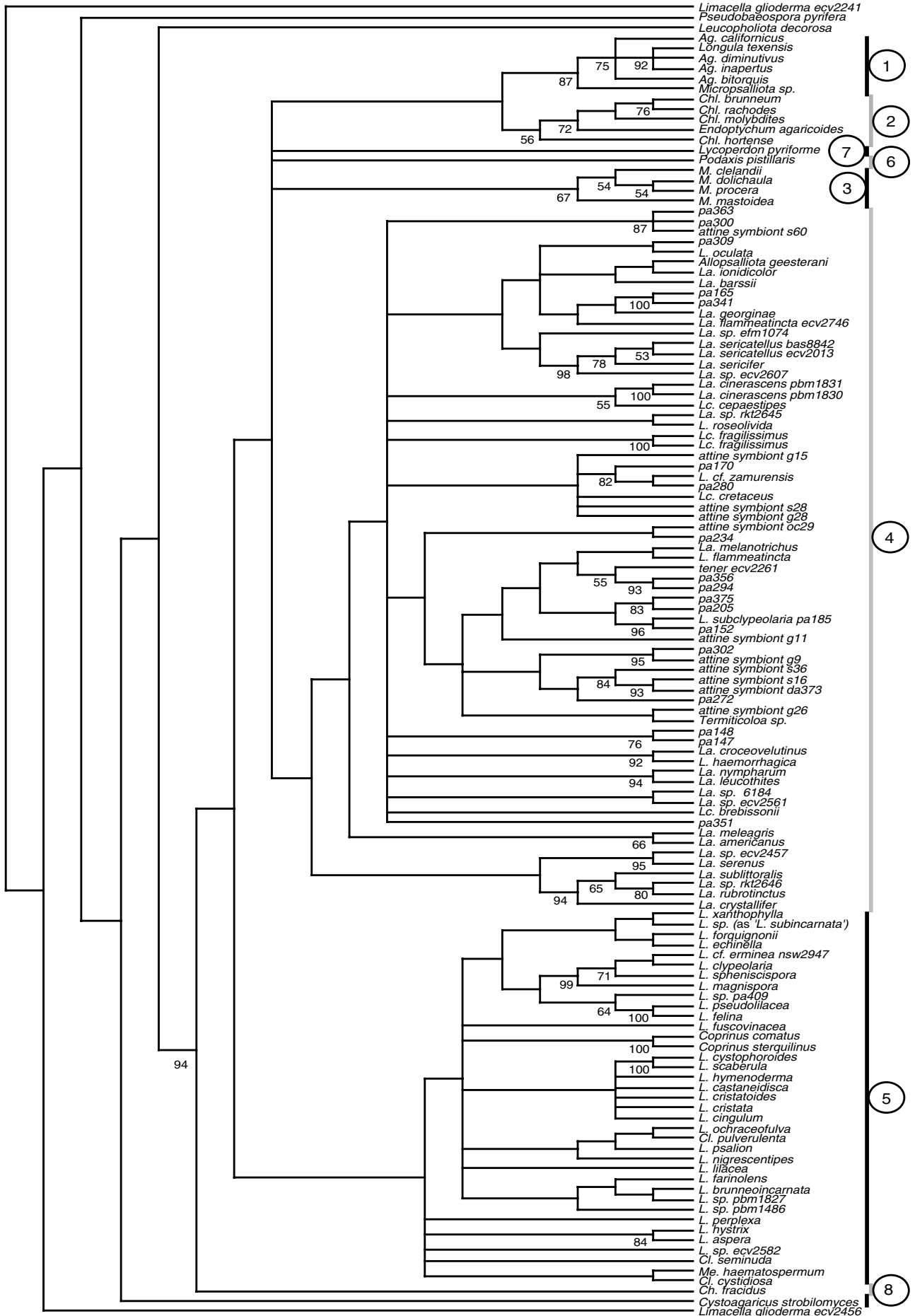


Fig. 2. Strict consensus trees of 190 most parsimonious trees, based on ITS data. Bootstrap values $\geq 50\%$ are indicated below branches.

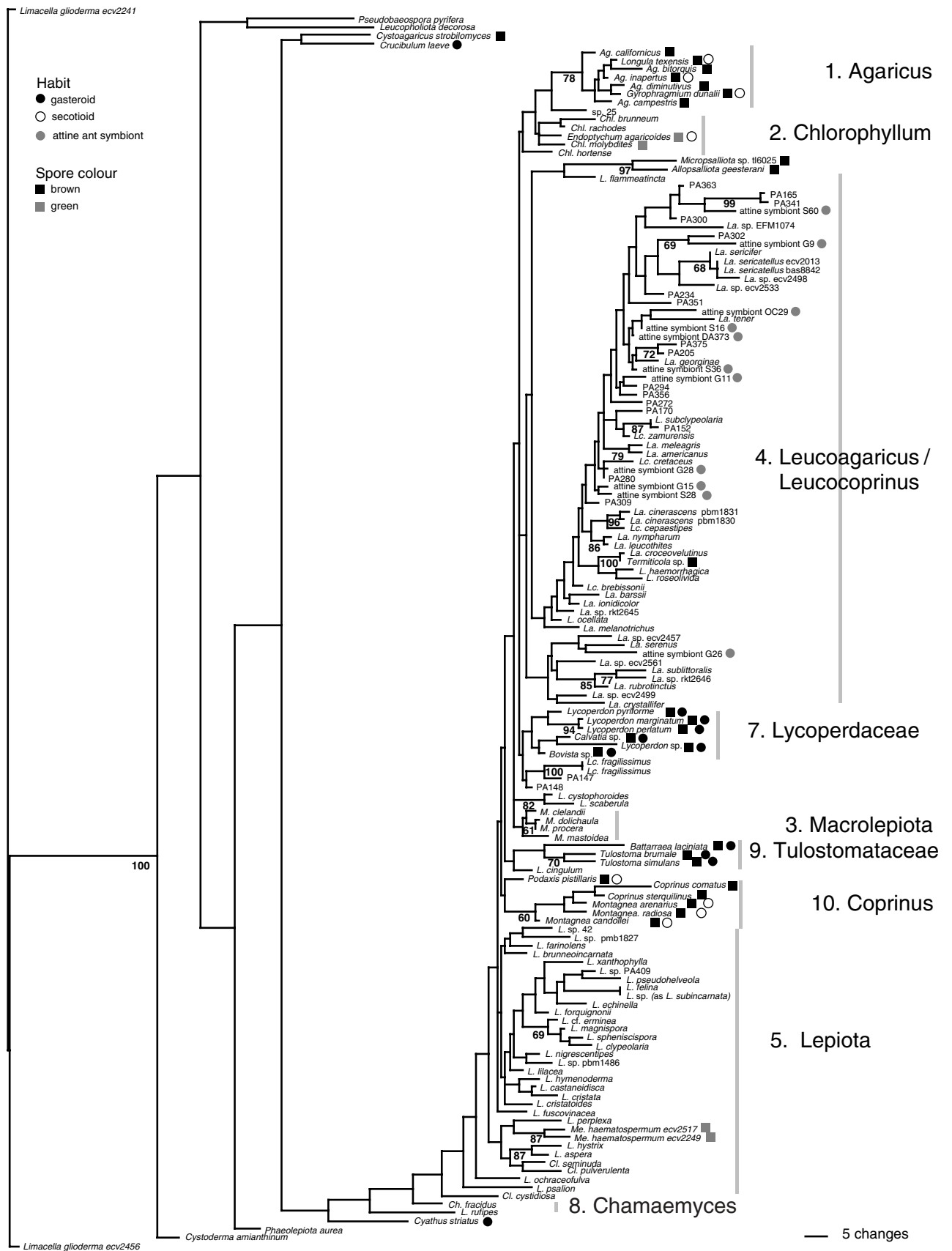


Fig. 3. One of 5000 equally parsimonious trees, based on LSU data. Life form and spore colour are indicated. Bootstrap values $\geq 60\%$ are given in bold below the branches.

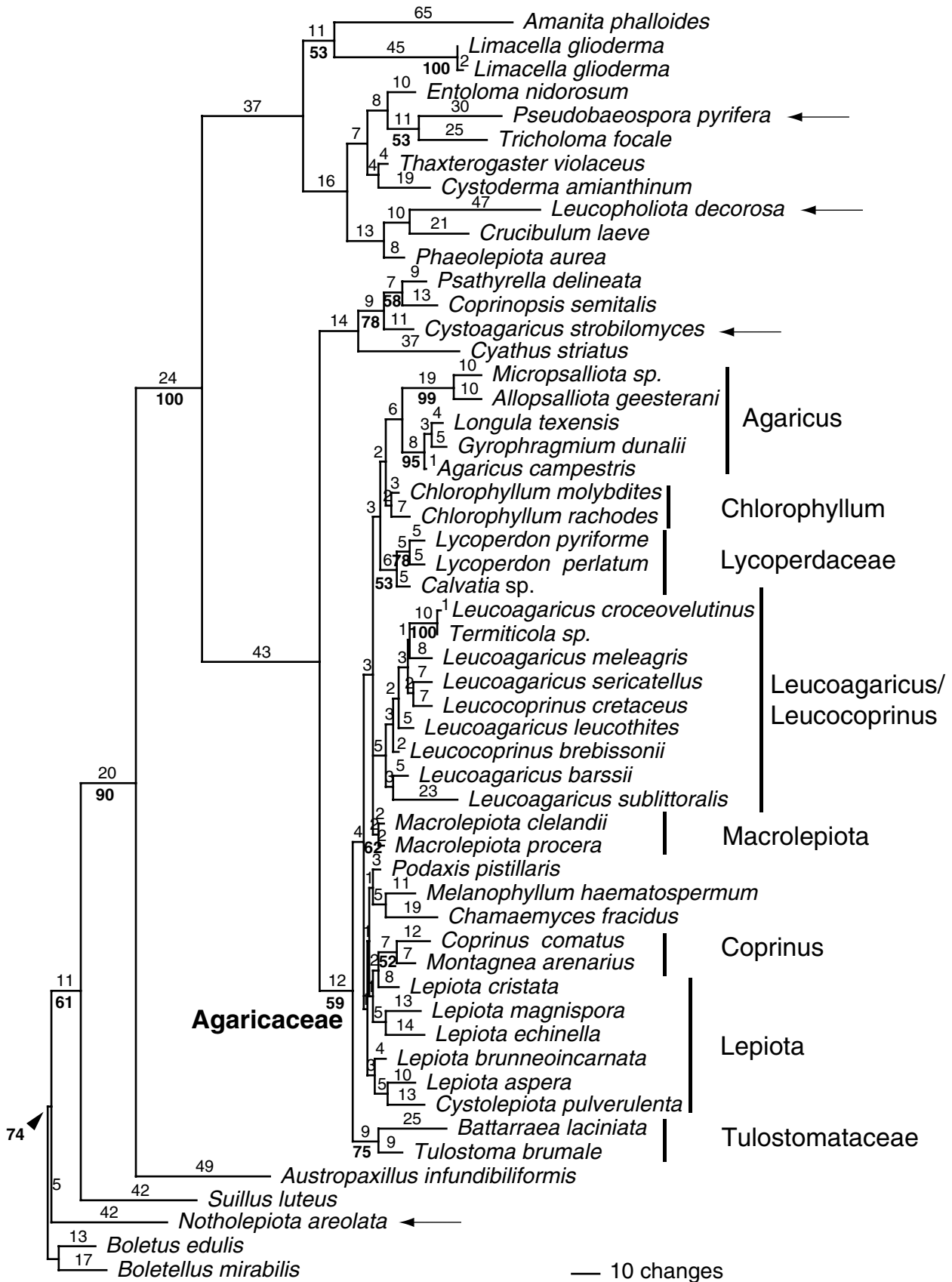


Fig. 4. One of 5000 most parsimonious trees, based on LSU data of 54 species. The arrows indicate species formerly assigned to the Agaricaceae. Bootstrap values $\geq 50\%$ are given below the branches.

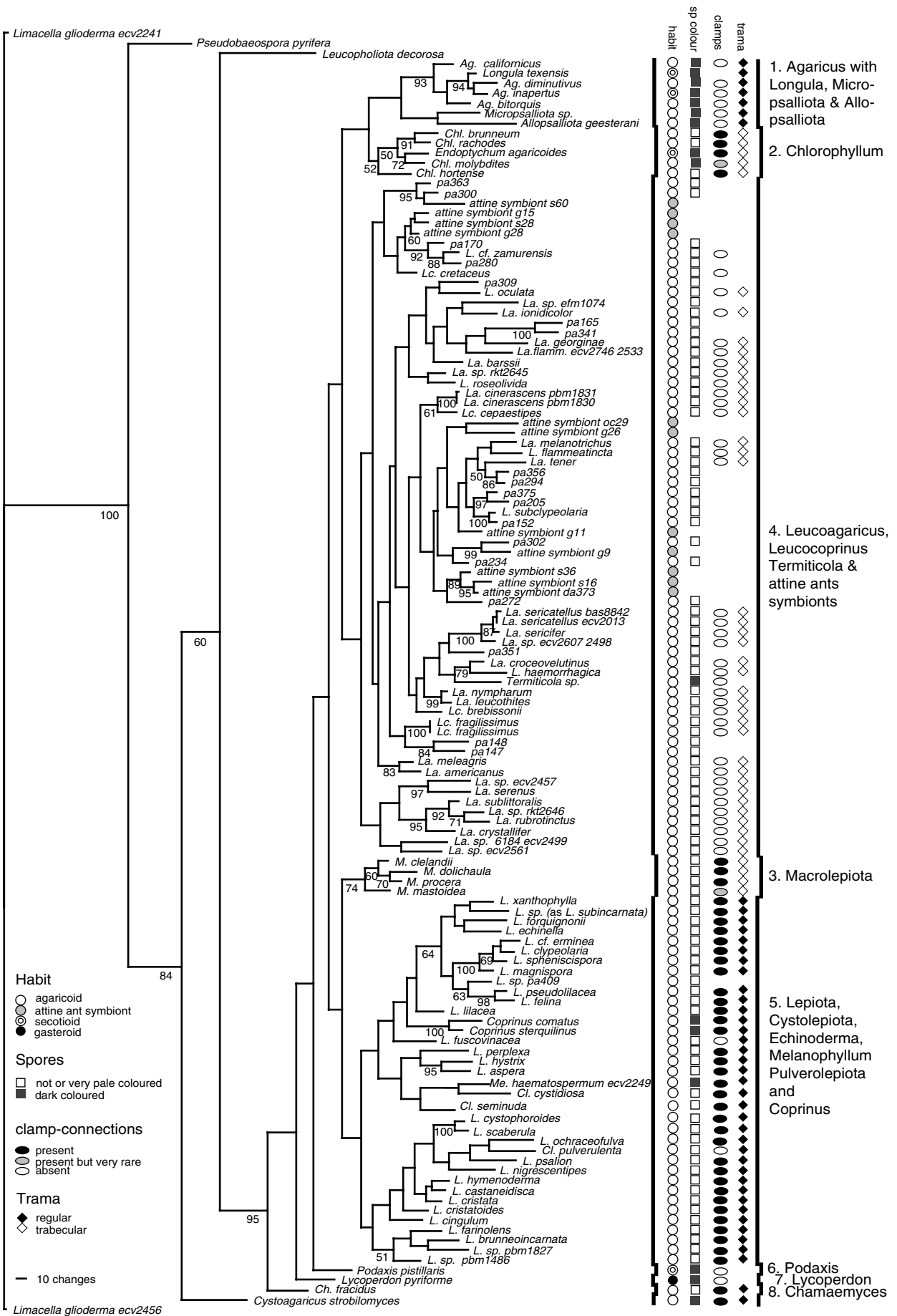


Fig. 5. Best (–ln L) of 2299 equally most parsimonious trees, based on ITS-LSU data. Bootstrap values $\geq 50\%$ are indicated below branches. Several morphological characters are indicated.

Table 2. Results from two-tailed Kishino–Hasegawa tests for the ITS-LSU data set, using RELL bootstrap; the number of bootstrap replicates = 1000. The tests were performed on a data set without the GenBank sequences of unidentified species and ant-cultivars.

Constraint	–ln L	Diff –ln L	P
Unconstrained	22733.18587	2.40336	0.364
<i>Chamaemyces fracidus</i> within <i>Lepiota</i>	22843.85187	113.06936	0.004*
<i>Coprinus comatus</i> and allies within <i>Lepiota</i>	22802.50293	71.72042	0.103
<i>Chamaemyces fracidus</i> and <i>Coprinus comatus</i> -group within <i>Lepiota</i>	22786.99627	56.21376	0.153
<i>Lepiota</i> (including sect. <i>Echinatae</i> , <i>Cystolepiota</i> , <i>Melanophyllum haematospermum</i> and <i>Pulverolepiota</i>) monophyletic	22792.99150	62.20899	0.197
<i>Cystolepiota</i> , <i>Melanophyllum haematospermum</i> , <i>Lepiota</i> sect. <i>Echinatae</i> and <i>Pulverolepiota</i> together monophyletic	22746.19879	15.41628	0.532
<i>Chlorophyllum</i> monophyletic	22738.30896	7.52645	0.806
<i>Leucoagaricus</i> (including <i>Sericeomyces</i>) monophyletic	22856.66240	125.87990	0.017*
<i>Leucoagaricus</i> (including <i>Sericeomyces</i>), but excluding <i>La. meleagris</i> and <i>La. americanus</i> , monophyletic	22855.48275	124.70024	0.012*
<i>Sericeomyces</i> monophyletic	22873.55549	142.77299	0.000*
<i>Leucocoprinus</i> monophyletic	22783.01293	52.23043	0.115
<i>Leucoagaricus</i> and <i>Leucocoprinus</i> together monophyletic	22731.92431	1.14180	0.979
<i>Leucoagaricus</i> and <i>Leucocoprinus</i> , excluding <i>La. meleagris</i> and <i>La. americanus</i> , monophyletic	22742.72927	11.94676	0.669
<i>Macrolepiota</i> and <i>Chlorophyllum</i> together monophyletic	22924.98389	194.20138	0.000*
<i>Macrolepiota</i> monophyletic (Fig. 6)	22730.78251	(best)	

P is the probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two-tailed test).

* P < 0.05.

Composition of the family

The ITS sequence of *Notholepiota areolata* was not alignable with the *Agaricaceae* sequences. When the LSU sequence was blasted against the available sequences in GenBank, it fitted best with *Boletus edulis*, and its position with the boletes is shown in Fig. 4.

The ITS and LSU sequences of *Leucopholiota decorosa* (syn. *Lepiota lignicola*) were alignable with those of the *Agaricaceae*, but the species does not belong within the family on molecular and morphological grounds. Its LSU sequence indicates a closer relationship with *Phaeolepiota aurea*, also outside the *Agaricaceae*.

The type species of *Cystoagaricus*, *C. strobilomyces*, was examined. Its LSU sequence is close to that of *Psathyrella delineata*, *Coprinopsis trispora*, and *C. semitalis* (Fig. 4). In the present ITS-LSU analyses its position is not with *Agaricus* and *Micropsalliota*, as proposed by Singer (1947).

Pseudobaespora pyrifer does not belong to the *Agaricaceae* either, though its sequences were alignable with those of the family. This species falls outside the 95% bootstrap supported group, and the best matches for its LSU sequences are with *Thaxterogaster*, and other *Cortinari* species, and with *Entoloma* and *Nolanea* species; in Fig. 4 it groups with *Tricholoma focale*.

Lineages within the *Agaricaceae proper*

Within the monophyletic *Agaricaceae* the following clades can be recognized, mainly on the basis of the ITS-LSU data set (Fig. 5):

(1) *Agaricus*, including *Longula texensis* and *Gyrophragmium dunalii*, with *Micropsalliota* and *Allopsalliota*.

(2) *Chlorophyllum molybdites*, *Endoptychum agarioides* and *Macrolepiota rachodes* sect. *Laevistipedes*.

(3) *Macrolepiota proper*, excluding sect. *Laevistipedes*.

(4) A species rich lineage comprising taxa of *Leucoagaricus* and *Leucocoprinus*, including the taxa cultivated by the attine ants of Central and South America. A dark-spored species, growing near termite mounds, tentatively assigned to the genus *Termiticola* also belongs to this clade.

(5) *Lepiota* including *Coprinus comatus* and *C. sterquilinus*, *Cystolepiota*, *Melanophyllum*, and *Pulverolepiota*.

(6) Three small clades, each represented by one species: (6) *Podaxis pistillaris*; (7) *Lycoperdon* as representative of the *Lycoperdaceae* (*Calvatia* and *Bovista* belong here as well, based on LSU data); and (8) *Chamaemyces fracidus*.

(9) The LSU data yield a second gasteroid clade, clade 9, with genera of the *Tulostomataceae*, viz. *Tulostoma* and *Battarraea*.

A set of 14 different phylogenetic hypotheses has been tested for the combined data set (Table 2). These hypotheses were tested on a data set without the GenBank sequences of unidentified *Leucoagaricus/Leucocoprinus* species and the attine ant cultivars. Monophyly of *Leucoagaricus* (including *Sericeomyces* species, but excluding *Leucocoprinus* species), of *Sericeomyces* alone, of *Macrolepiota* and *Chlorophyllum* together, and a position of *Ch. fracidus* within *Lepiota proper* have to be rejected. Also, monophyly of *Leucoagaricus* without *L. americanus* and *L. meleagris* has to be rejected. However, monophyly of *Leucocoprinus*, of *Macrolepiota*, of *Chlorophyllum* and of *Lepiota* including sect. *Echinatae* (genus *Echinoderma*), *Cystolepiota*, *Pulverolepiota*, and *Melanophyllum*, are acceptable.

Table 3. Results from two-tailed Kishino–Hasegawa tests for the LSU data set (same taxa as in the combined ITS–LSU data set), using RELL bootstrap; the number of bootstrap replicates = 1000. *P* is the probability of getting a more extreme *T*-value under the null hypothesis of no difference between the two trees (two-tailed test).

Constraint	–ln L	Diff –ln L	<i>P</i>
Unconstrained	6809.11618	20.36774	0.510
<i>Lepiota</i> (including sect. <i>Echinatae</i> , <i>Cystolepiota</i> , <i>Melanophyllum haematospermum</i> and <i>Pulverolepiota</i>) monophyletic	6835.42919	46.68075	0.105
<i>Macrolepiota</i> monophyletic	6808.58255	19.83411	0.572
<i>Leucoagaricus</i> , <i>Leucocoprinus</i> and <i>Sericeomyces</i> combined monophyletic	6853.74469	64.99625	0.069
<i>Chlorophyllum</i> monophyletic	6788.74844	(best)	

Several different phylogenetic hypotheses have been tested for the full LSU data set (Table 3), and none of the proposed monophylies of *Lepiota* (including *Cystolepiota* and *Melanophyllum*), of *Macrolepiota s. str.*, of *Chlorophyllum* (both in the sense of Vellinga, de Kok & Bruns 2003), and of *Leucoagaricus* and *Leucocoprinus* together, can be rejected.

DISCUSSION

Characteristics of the data sets

The low resolution of the LSU data and the changing topologies in the resulting phylograms are probably caused by the combination of a high number of taxa and a low number of parsimony informative characters. Use of a group of outgroup taxa did not resolve this problem completely (*cfr* Figs 3–4).

LSU and ITS have relatively low resolution power at the deeper parts of the phylogeny, leaving the branching order and the grouping of the lineages unresolved (*cfr* Hofstetter *et al.* 2002 for the *Lyophylleae*).

The position of several *Lepiota* species in the phylogram based on the LSU data set is aberrant from that in the phylograms based on either ITS or ITS and LSU. Especially striking is the position of *L. rufipes*, at the base of the family (Fig. 3); in ITS based phylograms it groups with other *Lepiota* species with a hymeniderm (Vellinga 2003). *Allopsalliota geesterani* changes from a position in the *Leucoagaricus* clade to a sister group of *Micropsalliota*, dependent on data set.

The family definition

The results of the combined ITS–LSU data set show a 95% bootstrap supported core group of *Agaricaceae*. Several taxa previously assigned to the *Agaricaceae* do not belong to this family.

The exclusion of *Notholepiota areolata*, and its place in suborder *Boletineae* of the *Boletales*, do come as a surprise. Horak (1971) characterized his new secotioid genus ‘by the form of the fruiting bodies, the thick-walled, dextrinoid spores (without a germ pore), and the hymeniform cuticle.’ Few morphological characters are available within the secotioid taxa and the breakdown of this description illustrates the danger

of relying upon them. However, there were already indications of a problem. All *Agaricaceae* have a saprotrophic lifestyle, but that *Notholepiota areolata* invariably occurs with *Leptospermum* (*Myrtaceae*) suggests that it is ectomycorrhizal. A second species of *Notholepiota*, *N. sardoa*, was described from Sardinia (Italy) by Padovan & Contu (2001); on account of the morphology it has recently been placed in the genus *Lepiota* (Vila & Castellón 2004), though molecular evidence is still wanting.

The structure of the hymenophoral trama (a regular trama with a gelatinized median stratum; data not shown), and the amyloid thin-walled spores set *Leucopholiota decorosa* apart from the *Agaricaceae*. It was included in *Lepiota* sect. *Amyloideae* as *L. lignicola* by Knudsen (1980) and Singer (1986). Harmaja (2002) erected the separate genus *Amylolepiota* for *L. lignicola*, and assumed a close relationship to *Lepiota*. He also disputed the synonymy of *L. lignicola* with *L. decorosa*. Miller, Volk & Bessette (1996) placed *Leucopholiota decorosa* in the family *Tricholomataceae*. The LSU sequence of the collection from Kamchatka (Russia) studied here is close to that of *Phaeolepiota aurea*, a species whose position in the LSU-based overview of the *Agaricales* is not well-established (Moncalvo *et al.* 2002).

Cystoagaricus is another genus that is expelled from the *Agaricaceae*, though Singer (1986) and Kirk *et al.* (2001) positioned it there. Singer (1947) had rejected the possibility of a close relationship of *C. strobilomyces* with *Psathyrella* as the ‘combination of a pseudo-parenchymatic general veil and septate cheilocystidia of a vesiculate type was never found in that genus’. However, microscopic examination of the Japanese specimen included in the present study revealed character combinations that do not fit into the *Agaricaceae*. The hymenophoral trama is made up of relatively short, inflated, wide elements; pleurocystidia are abundant and utriform; cheilocystidia are not septate, but lageniform-fusiform, and slightly incrustated; the pileipellis is hymenidermal with squamules made up of long cylindrical hyphae, and clamp-connections are abundant. The shape of the spores is most extraordinary, and led Murrill (1945) to describe this species in *Nolanea*. *Cystoagaricus strobilomyces* is here assigned to the family *Psathyrellaceae*.

Table 4. Overview of tribes within the family *Agaricaceae* and the genera assigned to those tribes according to Singer (1986).

Tribes (Singer 1986)	Genus (Singer 1986)	Clade or family assignment in the present study
<i>Agariceae</i>	<i>Agaricus</i>	1. <i>Agaricus</i>
	<i>Cystoagaricus</i>	<i>Psathyrellaceae</i> (<i>Coprinaceae</i>)
	<i>Melanophyllum</i>	5. <i>Lepiota</i>
	<i>Micropsalliota</i>	1. <i>Agaricus</i>
	<i>Termiticola</i> (as <i>Agaricus</i> subgen. <i>Conioagaricus</i>)	4. <i>Leucoagaricus/Leucocoprinus</i>
<i>Leucocoprineae</i>	<i>Chlorophyllum</i>	2. <i>Chlorophyllum</i>
	<i>Leucoagaricus</i>	4. <i>Leucoagaricus/Leucocoprinus</i>
	<i>Leucocoprinus</i> (incl. <i>Rugosopora</i>)	4. <i>Leucoagaricus/Leucocoprinus</i>
	<i>Macrolepiota</i>	2. <i>Chlorophyllum</i> and 3. <i>Macrolepiota</i>
	<i>Sericeomyces</i>	4. <i>Leucoagaricus/Leucocoprinus</i>
<i>Lepioteae</i>	<i>Volvolepiota</i>	3. <i>Macrolepiota</i>
	<i>Chamaemyces</i>	8. <i>Chamaemyces</i>
	<i>Cystolepiota</i>	5. <i>Lepiota</i>
<i>Lepioteae</i>	<i>Lepiota</i>	5. <i>Lepiota</i> (excluding section <i>Amyloideae</i> = <i>Leucopholiota</i> in the <i>Tricholomataceae</i> ; excluding section <i>Anomalae</i> p.p. and section <i>Amylosporae</i> , both in 4. <i>Leucoagaricus/Leucocoprinus</i>)
	<i>Cystodermateae</i>	<i>Tricholomataceae</i>

The genera *Clarkeinda*, *Crucispora*, *Hiatulopsis*, *Janauaria* and *Smithiomyces* have not been treated in the present work.

The genera *Heinemannomyces* and *Allopsalliota* have been described after 1986; *Allopsalliota* is treated here and belongs to clade 1 (*Agaricus*).

Singer (1986) and Wasser (2002) included *Pseudobaeospora* in the *Agaricaceae*, because of its superficial resemblance to some of that family's genera, but other authors (e.g. Kühner 1980, Bas 1995, Kirk *et al.* 2001) placed it in the *Tricholomataceae*. Its position is still unsure as the LSU sequence is close to those of two quite different groups of genera, *Thaxterogaster*, and entolomatoid taxa (see also Fig. 4). Very recently the European species were studied in depth, and the number of taxa jumped dramatically from two to 16 (Bas 2002, 2003). *Pseudobaeospora* is a genus with a wide array of pileipellis types (Bas 2002, 2003), and the only species included in the present study is characterized by a rather aberrant pileipellis.

In summary, the agaricoid members of the family can now be defined as having free lamellae, a regular or trabecular, non-gelatinized trama structure with long elements, and white or coloured (brown, green, blue, pale pink, pale yellow) regularly shaped spores.

Comparison with existing classifications: tribes and families

The three widely accepted tribes, *Agariceae*, *Lepioteae*, and *Leucocoprineae*, as proposed by Singer (1986), are not supported by the molecular evidence presented here (see Table 4 for an overview of Singer's tribes and the genera included in each). The *Agaricus* clade, Singer's tribes *Agariceae*, is monophyletic, but is a sister group of the *Chlorophyllum* clade; tribe *Leucocoprineae* is polyphyletic, with *Macrolepiota sensu* Singer (1986) divided into two clades; tribes *Lepioteae* harbours *Lepiota*, *Cystolepiota*, *Melanophyllum*, and *Coprinus comatus* and allies, as well as *Macrolepiota*

proper, according to the combined data set. In separate analyses *Macrolepiota* is actually closer to the *Chlorophyllum* clade (ITS data) or to the *Leucoagaricus/Leucocoprinus* clade (LSU data) (Vellinga *et al.* 2003). *Chamaemyces fracidus*, formerly assigned to tribe *Lepioteae*, is in a clade of its own, basal to the family as a whole, and a position within *Lepiota* has to be rejected (see Table 2). All analyses agree that *Melanophyllum*, with its coloured spores, must be assigned to the same clade as *Cystolepiota*, and that it cannot be closely related to *Agaricus* as suggested by Singer (1986) and Wasser (2002), nor a part of the *Cystodermateae* (Pegler 1986).

Focusing only on taxa with an agaricoid habit, one might suppose that a division into two clades is still possible, though the composition of the two groups differs according to the data sets. Kühner (1980) advocated a division of the *Agaricaceae* into two groups, *viz.* *Psalliotaeae* with *Agaricus*, and *Lepioteae*, comprising *Cystolepiota*, *Melanophyllum*, and *Lepiota*, which in his sense harboured *Chamaemyces*, *Lepiotula*, *Leucocoprinus* and *Macrolepiota* as well. But a clear-cut division into two units is spoiled by the need to accommodate *Podaxis*, the *Lycoperdaceae* and the *Tulostomataceae* within the *Agaricaceae*. More data for the *Lycoperdaceae* and the *Tulostomataceae*, and from examining additional genes will be needed before the deep branching and the number of clades can be resolved.

Comparison with existing classifications: genera

At the genus level, there seems to be more congruence between the morphological classification and the molecular clades.



Fig. 6. Best (–ln L) phylogram, with the constraint of a monophyletic *Macrolepiota* (Table 2), based on the ITS-LSU data set. Note the position of *Leucoagaricus americanus* and *La. meleagris*, and that of *Chlorophyllum* and *Macrolepiota*.

The genus *Agaricus* was only represented by a few taxa in the present study, but it is evident from these data and the data presented by Moncalvo *et al.* (2002), that *Gyrophragmium dunalii* and *Longula texensis* clearly belong to *Agaricus*. The necessary combinations in the genus *Agaricus* will be made in a separate publication.

The genus *Chlorophyllum* has to be amended, as already proposed by Vellinga *et al.* (2003), to include *Macrolepiota* sect. *Laevistipedes* and *La. hortensis*, and consequently, *Macrolepiota* is restricted to sections *Macrolepiota* and *Macrospora*. Representatives of *Volvolepiota* were shown to belong to *Macrolepiota* as well (Vellinga *et al.* 2003, Vellinga & Yang 2003).

Monophyly of *Leucoagaricus* separately has to be rejected, a conclusion also reached by Johnson (1999), though a monophyletic *Leucocoprinus* is acceptable (Table 2). In combination, the two genera do form a monophyletic group (with or without the species *La. americanus* and *La. meleagris*), though without bootstrap support. *Leucocoprinus* had been restricted to taxa with a plicate pileus and with heteromorphic basidia separated from each other by pavement cells (pseudoparaphyses) (Singer 1986, Vellinga 2001). Spore characters and pileipellis structure vary considerably within *Leucocoprinus* and *Leucoagaricus*. *Leucoagaricus* was used to accommodate taxa which did not fit into any of the other, well-defined genera, and as a result is extremely heterogeneous. Some authors (Pegler 1983, 1986, Grgurinovic 1997) treated *Leucoagaricus* in a very restricted sense, placing species, here assigned to the *Leucoagaricus/Leucocoprinus* clade, under *Lepiota* in sects. *Ovisporae*, *Sericellae* and *Cristatae*. Some taxa, in particular those with a reddening reaction after bruising (*La. badhamii*, *La. americanus*, etc.), were transferred back and forth between *Leucoagaricus* and *Leucocoprinus* (*cfr* Moser 1983 and Reid 1990 with Bon 1993). It should also be noted that many species assigned here to clade 4 are still listed as *Lepiota* species in Table 1, as the appropriate new combinations have not been made; these species are marked accordingly. *Rugosospora* with strongly ornamented spores and clamp connections might belong to clade 4 as well (data not shown). One brown-spored taxon, tentatively called *Termiticola* sp. also belongs to this lineage; Singer (1986) suggested that *Termiticola* and *Hymenoagaricus* (his *Agaricus* subgen. *Conioagaricus*) are synonymous and a subgenus of *Agaricus*. The *Leucoagaricus/Leucocoprinus* clade also comprises ant-cultivated taxa, as shown already by Mueller *et al.* (1998) and Mueller (2002).

The genus *Sericeomyces* was split off from *Leucoagaricus* to accommodate white taxa with a silky pileus covering (Heinemann 1978). However, this division is not supported by the present data. *Sericeomyces* is polyphyletic, and a monophyletic *Sericeomyces* has to be rejected (Table 2).

For the time being, one genus for all *Leucoagaricus*, *Leucocoprinus* and *Sericeomyces* taxa seems the best

solution. The numbers of *Leucoagaricus* and *Leucocoprinus* species increase compared to *Lepiota* species along a north-south gradient (Vellinga 2004), and a thorough morphological and molecular analysis of (sub)tropical taxa is necessary before the *Leucoagaricus/Leucocoprinus* clade can be split up into smaller groups.

In contrast to the phylogenies proposed by Johnson (1999) and Moncalvo *et al.* (2002), *Lepiota*, together with *Cystolepiota*, *Melanophyllum*, *Pulverolepiota* and members of *Lepiota* sect. *Echinatae*, forms a monophyletic group. The latter four taxa do form a better supported monophyletic group on their own, and one separate genus for them seems in order. A detailed analysis of clade 5, and the relationships with *Chamaemyces* and *Coprinus* has been published elsewhere (Vellinga 2003).

The position of *Coprinus* and *Montagnea* is enigmatic. According to the LSU data (Fig. 3) they form a separate clade, but according to the combined data set (in which only *C. comatus* and *C. sterquilinus* are included), a place within the *Lepiota* clade can not be rejected (Table 2). The morphology (deliquescence, spore characters, hymenium structure) is completely different from that of *Lepiota* species (see also Redhead *et al.* 2001).

Secotioid taxa, with the exception of *Podaxis*, have relatively recently evolved and are scattered throughout the family, but are absent from the *Lepiota*, *Macrolepiota*, and the *Leucoagaricus/Leucocoprinus* clades. Recognition of separate genera for *Longula*, *Gyrophragmium* and *Endoptychum* taxa results in paraphyletic genera. Gasteroid taxa form separate clades, but are equally present throughout the family (see Krüger *et al.* 2001 and Krüger & Kreisel 2003 for more information on these genera). All the secotioid and gasteroid taxa within the family have coloured brown or olivaceous spores, with the exception of *Notholepiota sardoa*, which is said to have white to light cream spores (Vila & Castellón 2004).

The effects of new genus hypotheses are shown in Figs 7–8, in which the pairwise genetic distances within and among lepiotaceous genera are given as histograms, based on the ITS-LSU data set. The old classification harbours several paraphyletic or polyphyletic genera (e.g. *Leucoagaricus*, *Leucocoprinus*, *Sericeomyces*, *Lepiota*, and *Agaricus*), and a number of small genera (e.g. *Longula*, *Gyrophragmium*, *Endoptychum*) which are nested within bigger genera, rendering those paraphyletic. Fig. 8 is based on wide genus concepts for *Lepiota* (including *Cystolepiota*, *Melanophyllum*, and *Pulverolepiota*), *Leucoagaricus/Leucocoprinus* including *Sericeomyces*, and *Agaricus* (including *Longula*, *Endoptychum depressum* and *Gyrophragmium*), a slimmed concept of *Macrolepiota*, and an expanded view of *Chlorophyllum*. Under the old scheme, almost complete overlap of the intergeneric and intrageneric distance data is evident, and the big distances between the intergeneric pairs point to the existence of

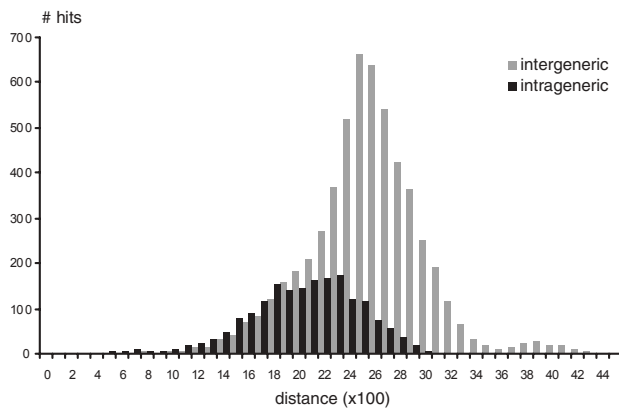


Fig. 7. Histogram of intrageneric and intergeneric pairwise distances based on the ITS-LSU data set, under a classification scheme based on Bon (1993) (European taxa) and Singer (1986) (world-wide coverage).

paraphyly. These artifacts are eliminated in the new classification. The intergeneric distances are still quite big, as now some huge genera are proposed.

Morphological characters

Singer (1986) used spore wall characters (colour, and staining reactions, in particular the reactions in an iodine solution and in Cresyl Blue) to distinguish the three tribes. The data presented here demonstrate that spore colour has no significance at clade level; species with coloured spores belong to a wide variety of clades. Of the agaricoid taxa, the *Agaricus* clade is the only exception, as all species have a pigmented spore wall. Earlier work by Vellinga & Huijser (1999) had already shown that metachromatic reactions of the spore wall do occur within *Lepiota* proper and in *Chamaemyces*, though Singer (1986) confined that reaction to tribus *Leucocoprineae*. Furthermore, this reaction can be seen in young *Agaricus* spores, despite reports to the contrary. The presence of a germ pore, on the other hand, does seem to be restricted to a few clades, but is not universally present within these clades. For instance, *Chl. hortense*, without a germ pore, is grouped with *Chlorophyllum* species with a conspicuous germ pore. The structure of the germ pore in *Macrolepiota s. str.* is fundamentally different from that in *Chl. molybdites* and *Chl. rachodes* (Meléndez-Howell 1967).

The structure of the hymenophoral trama does differ from clade to clade. However, this character has not been described consistently for all taxa. Roughly speaking, it appears that a regular trama occurs in clades 1 (*Agaricus*), 5 (*Lepiota*) and 8 (*Chamaemyces*), and a trabecular trama in clades 2 (*Chlorophyllum*), 3 (*Leucoagaricus/Leucocoprinus*), and 4 (*Macrolepiota*) (Buller 1924, Heinemann 1989, Cléménçon 1997). The truly secotioid and gasteroid taxa do not possess a distinct hymenophoral trama. Neither a gelatinized trama nor a regular trama made up of

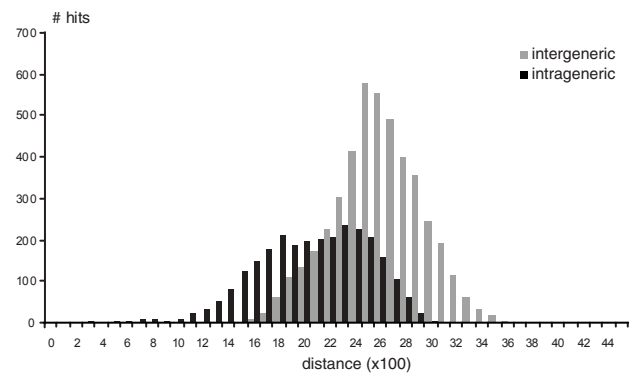


Fig. 8. Histogram of intrageneric and intergeneric pairwise distances based on the ITS-LSU data set, under a proposed classification in which *Leucoagaricus*, *Leucocoprinus* and *Sericeomyces* are taken together, *Macrolepiota* is split up and partly merged with *Chlorophyllum* and *Endoptychum*, *Agaricus* includes *Gyrophragmium*, *Longula* and *E. depressum*, and *Lepiota* harbours *Melanophyllum*, *Cystolepiota*, *Echinoderma* and *Pulverolepiota*.

relatively short inflated elements are found within the *Agaricaceae*.

A universal characteristic of the family is the presence of veils (Reijnders 1975); *Chamaemyces* is monovelangiocarpic, but all other agaricoid taxa are bivelangiocarpic.

The aspect of the pileus is a consequence of the microscopic structure of the covering layers and varies greatly within and between the clades. In many taxa it is not known whether the layers originate as a veil or as a pileipellis. The considerable differences between clades 2 (*Chlorophyllum*) and 3 (*Macrolepiota*) in the structure of their pileus and stipe coverings and in the structure of the germ pore, are reason enough to distinguish them at genus level (Vellinga *et al.* 2003). Within the *Leucoagaricus/Leucocoprinus* clade the pileus covering varies from a cutis to an epithelium or different types of trichoderm; the *Lepiota* clade is equally diverse. In both these lineages, subclades are often characterized by a single type of pileus covering.

The combination of pavement cells (pseudoparaphyses) and basidia of different length is found in the species traditionally assigned to *Leucocoprinus* and in *Coprinus comatus* and *C. sterquilinus*. According to the present analyses this character has evolved several times within the family, and cannot be used at higher taxonomic levels. Outside the *Agaricaceae* it is also widespread, occurring in *Bolbitius*, and in the *Psathyrellaceae* (formerly *Coprinaceae*).

Clamp-connections have been widely used to distinguish taxa at different levels. They are always absent in clades 1 (*Agaricus*), 4 (*Leucoagaricus/Leucocoprinus*), and 7 (*Lycoperdaceae*). Not all taxa in the other clades possess clamp-connections, but presence is the norm.

Agerer (2002) demonstrated that the rhizomorphs of agaricoid *Agaricaceae* and *Lycoperdaceae* are similar

in structure, and this supports a close relationship between those two taxa.

CONCLUSION

Despite the low resolution of the basal branches of the phylogenies based on ITS and LSU sequences, whether separately or in combination, it is clear that existing classifications based on morphological characters, will have to be changed. It is premature, however, to do so in detail, before more tropical and more gasteroid taxa have been studied and other genes are examined. Effort should also go into understanding the development and structure of the covering layers of the basidiocarps which has proved to be important in making decisions on the genus level.

If the basal position of *Ch. fracidus* is supported, this will shed light on the original characters of the family. It is worth noting that in this taxon the pileus and stipe contexts are confluent; the spores are small, binucleate, not dextrinoid, and metachromatic in Cresyl Blue; pleurocystidia are present (and conspicuous); the pileus covering is a slightly gelatinized hymeniderm, and clamp-connections are present and conspicuous. *Chamaemyces* also differs from all other taxa in the *Agaricaceae* by the monovelangiocarpy and the stipitocarpic development.

If the basal positions of the gasteroid taxa *Tulostoma* and *Battarraea*, inferred from the small LSU data set, are confirmed in other gene genealogies, a totally different scenario for the evolution of the family *Agaricaceae* will be needed.

In the phylogeny presented by Moncalvo *et al.* (2002), the family *Agaricaceae* is situated between the pale spored *Cystoderma* and *Ripartitella*, and the birds nest fungi *Crucibulum* and *Cyathus*. Beyond those groups, brown-spored taxa appear as more distant relatives on both sides. A transition from non-pigmented to coloured spores has occurred several times within the family, but the reverse has never happened; this makes a brown spored ancestor seem unlikely. Coloured spores have been viewed as an ecological adaptation to drought, high light intensity or cold. (*cf.* Butler & Day 1998).

To sum it up, short characterizations of the nine clades retrieved in the present study are given. All clades are characterized by a combination of characters. The *Coprinus-Montagnea* clade is added as a tenth group, though it does not form a separate clade in the analysis of the combined ITS-LSU data set.

(1) *Agaricus* clade: spores brown, trama regular, pileus covering a cutis, clamp-connections absent, habit agaricoid, rarely secotioid. Several genera belong to this clade, and *Agaricus*, *Micropsalliota* and *Allopsalliota* are accepted here.

(2) *Chlorophyllum* clade: spores white, green or green-brown, trama trabecular, pileus covering hymenidermal, clamp-connections present or absent, habit agaricoid, in one species secotioid.

(3) *Macrolepiota* clade: spores white with a germ pore, trama trabecular, pileus covering trichodermal, clamp-connections present, habit agaricoid. The genus *Volvolepiota* is included in *Macrolepiota*.

(4) *Leucoagaricus/Leucocoprinus* clade: spores white with or without germ pore, rarely brown, trama trabecular, pileus covering extremely variable, clamp-connections absent, habit agaricoid. This clade can probably be split up into several genera, but extensive taxon sampling in the tropics has to occur first. *Seriaceomyces* and the attine ant cultivated species belong to this clade.

(5) *Lepiota* clade: spores white, rarely green, never with a germ pore, trama regular, pileus covering hymenidermal, epithelial, cutis-like, or trichodermal, clamp-connections present in most species, habit agaricoid, very rarely secotioid. This clade comprises two genera *Lepiota* on the one hand, and a combined *Cystolepiota*, *Echinoderma*, *Melanophyllum*, and *Pulverolepiota*.

(6) *Podaxis* clade: spores very dark brown, with a germ pore, habit secotioid, resembling *Coprinus comatus*.

(7) *Lycoperdaceae* clade: spores brown, ornamented, clamp-connections absent in *Lycoperdon* and allies, habit gasteroid. *Bovista*, *Lycoperdon*, *Morganella* and *Calvatia* belong to this clade.

(8) *Chamaemyces* clade: spores white without germ pore, trama regular, pileus covering an ixohymeniderm, clamp-connections present and conspicuous, habit agaricoid, and context not confluent between stipe and pileus as in clades 2–5.

(9) *Tulostomataceae* clade: spores brown, ornamented, clamp-connections present in *Tulostoma* and *Battarraea*, habit gasteroid. Due to the lack of ITS data this clade was only recovered from the LSU data set.

(10) *Coprinus comatus* clade: spores black with a germ pore, trama regular, pileus covering a cutis, clamp-connections and pleurocystidia absent, stipe with central strand, habit agaricoid (*Coprinus*) or secotioid (*Montagnea*).

ACKNOWLEDGEMENTS

Without fungal collections this study could not have been done. The herbaria of CANB, MICH, and WAG sent material on loan; Kees Bas, Alberto Bizzi, Renato Brotzu, Roel Douwes, Manfred Enderle, Jan Engelen, Henk A. Huijser, Rogier P. J. de Kok, Thomas Læssøe, P. Brandon Matheny, Eiji Nagasawa, Ghisèle Rioussel, Maggie Rogers, Clive Shirley and Kevin R. Thiele provided me with collections. The Alexander H. Smith and Helen V. Smith Award for 2000 of the Mycological Society of America enabled the author to visit the herbarium of the University of Michigan at Ann Arbor. The 'Rijksherbariumfonds Dr E. Kits van Waveren' provided monetary support for the laboratory work in 1999. Support from the Bruns Laboratory (Department of Plant and Microbial Biology, University of California at Berkeley) was crucial. Comments on the manuscript by Pieter Baas, Thomas D. Bruns, David S. Hibbett, Jacqui Johnson, Thomas W. Kuyper, John Lennie, Machiel E. Noordeloos, and an anonymous reviewer are gratefully acknowledged.

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Corresponding Editor: D. S. Hibbett