

## Phylogeny and taxonomy of *Macrolepiota* (Agaricaceae)

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**Abstract:** The position and composition of *Macrolepiota* within the Agaricaceae and its phylogenetic relationships with other members of the family were investigated, using both molecular (ITS and LSU rDNA sequences) and morphological characters. The molecular data separate the genus into two clades. The first clade comprises *M. procera*, *M. mastoidea*, *M. clelandii* and allies and is a sister group of *Leucoagaricus* and *Leucocoprinus*. The second, more diverse, clade, with *M. rachodes* and allies, *M. globosa*, *Chlorophyllum molybdites*, *Leucoagaricus hortensis* and *Endoptychum agaricoides*, is a sister group of *Agaricus*. The separation of the two clades is supported by morphological characters, such as the structure of the pileus covering, the stipitipellis and the shape of the germ pore and the spore apex. The two clades are regarded as genera for which the names *Macrolepiota* and *Chlorophyllum* are proposed. *Macrolepiota nymphaeum* does not belong to either clade but is assigned to the genus *Leucoagaricus*, close to *L. leucothites*. *Endoptychum depressum* is transferred to the genus *Agaricus* as *A. inapertus*.

**Key words:** Agaricaceae, *Chlorophyllum*, *Endoptychum*, ITS and LSU rDNA, phylogeny

### INTRODUCTION

*Macrolepiota* Singer belongs to the family Agaricaceae (Fr.) Chev., one of the most diverse families in the

Agaricales. Spore print color in the family varies from white to brown, green and blue; the hymenophoral trama is regular or trabecular; and the structure of the pileus covering (of velar or pileipellicular origin) ranges from epithelioid to trichodermal or cuticular. Despite this morphological diversity, the family was shown to be monophyletic, based on studies of nuclear ribosomal ITS, LSU, and mitochondrial SSU (Johnson and Vilgalys 1998, Johnson 1999, Moncalvo et al 2000, 2002). The molecular data support Singer's morphological concept of the family (1986), although tribus Cystodermatae was excluded and the family Lycoperdaceae was included (Kirk et al 2001, Moncalvo et al 2002). A separate family for the white-spored taxa, Lepiotaceae Over., as advocated by some authors, was ruled out by the molecular data. However, relationships within the family Agaricaceae remained largely unresolved, due to the small sample of this diverse family, conservatively estimated to comprise more than 900 species (Kirk et al 2001).

In the studies of Johnson (1999) and Johnson and Vilgalys (1998), *Macrolepiota* comprising five species, is polyphyletic but taxonomic conclusions were not drawn. Morphologically, *Macrolepiota* has been recognized by these characters: big and fleshy basidiocarps; hymenidermal or trichodermal universal veil that splits up into coarse-to-fine squamules on the pileus; an often complicated, double annulus, and white-to-pink, thick-walled spores with a germ pore that are dextrinoid, metachromatic in Cresyl blue and congophilous. The lamella trama is trabecular (Buller 1924, Heinemann 1989, Cléménçon 1997), a character shared with *Leucoagaricus* Singer, *Leucocoprinus* Pat., and *Chlorophyllum* Mass.

Several genera are very similar to *Macrolepiota*. *Chlorophyllum* differs only in the distinctly green or ochre spores; these colors obscure the staining reactions of the spores. The genus *Chlorolepiota* Sathe & Deshpande (1979) hypothetically occupies an intermediate position between *Chlorophyllum* and *Macrolepiota* because the spore print is primrose yellow and the spores are provided with a germ pore but are not truncate; clamp connections are said to be absent. *Volvolepiota* Singer also closely resembles *Macrolepiota*; a volva is present, the pileus covering is trichodermal, clamp connections are present (in the context of the stipe) and the spores have a germ pore (Heinemann and De Meijer 1996).

The position of *Leucoagaricus hortensis* (Murrill) Pegler is anomalous and has been the subject of recent discussion (Akers and Sundberg 1997, Johnson 1999). This species was placed in the genus *Leucoagaricus* by Pegler (1983) because the spores lack a germ pore; yet clamp connections are present, a character state that does not occur elsewhere in the genus *Leucoagaricus*. The structure of the veil resembles that found in *Macrolepiota rachodes* (Vittad.) Singer.

The secotioid genus *Endoptychum* was considered a member of the Agaricaceae by some authors (e.g., Moser 1983). Singer (1986), on the other hand, regarded it as a Gasteromycete and as such an ancestor of the Agaricales.

Two competing infrageneric classifications of the genus *Macrolepiota* have been proposed: i) a division into two sections based on the presence or absence of clamp connections in which section *Macrolepiota* has clamp connections and section *Macrosporae* (Singer) Bon is lacking clamp connections in the trama (Singer 1986, Candusso and Lanzoni 1990, Balero and Contu 1991, though the last authors treated *Macrolepiota* and *Lepiota* as belonging to one genus); ii) a classification with three sections, *Macrolepiota*, *Macrosporae* and *Laevistipedes* (Pázmány) Bon (Bon 1993), based on diverse macroscopical and microscopical characters, such as the shape of the spore and the germ pore, the structure of the annulus and the covering of the stipe. According to Singer's (1986) classification, *M. rachodes* and *M. procera* belong to the same section, though according to the molecular analyses of Johnson (1999), and our own, these taxa belong to different lineages.

Several toxic species are known within *Macrolepiota* and *Chlorophyllum*, and a natural question is whether these species form one clade, setting them apart from the edible species, sharing characters that facilitate recognition and medical treatment. *Chlorophyllum molybdites* (G. Meyer : Fr.) Mass. causes gastro-intestinal problems and is a particular threat to children, because it frequents lawns and other man-made habitats in tropical, subtropical areas and other places with humid summers. It is very common in urban and suburban areas in the eastern and southern parts of the United States. An extensive list of references to this species and its toxicity can be found in Reid and Eicker (1991). The toxic component of this species is unknown (Lehmann and Khazan 1992). *Endoptychum agaricoides* Czern. caused hemolytic anemia in a dog, which had eaten a mature basidiocarp (DE Desjardin pers obs). *Macrolepiota neomastoides* (Hongo) Hongo and *M. venenata* Bon are reportedly toxic (in the case of the former, by Yokoyama and Yamaji 1981; in the case of the latter, by Bon et al

1979, Mazzolai 1989), whereas many other species, especially *M. rachodes* and *M. procera*, are considered excellent edibles. Some species are commercially grown or sold in cultivation kits. Arora (1986) suspected that *Leucoagaricus hortensis* (as *Lepiota humei*) might be very toxic, but this species is sold on the market in Bolivia (E Boa pers obs; identification by senior author).

Some *Macrolepiota* species are widespread, and reported from all over the world, others, such as *M. excoriata* (Schaeff. : Fr) Wasser, and *M. phaeodisca* Bellù, are restricted in their area of distribution (Courtecuisse and Duhem 1994, Nauta and Vellinga 1995; resp. Candusso and Lanzoni 1990). Many species occur in man-made habitats, such as gardens, lawns, compost-heaps; others occur in grasslands or open places in woods. There are striking disparities in the number of species in different areas. These differences are genuine and are not artifacts of the extent to which they have been studied or of taxonomic perspective. Western Europe is rich, with 11 to 19 species recorded, depending on the author. In North America, on the other hand, only two species generally are recorded, although the true number might be seven (senior author pers obs). Despite the fact that many species form big, conspicuous basidiocarps, several species are still undescribed. It is important to note that European names often have been applied prematurely to similar-looking species in other parts of the world, complicating clear understanding of this group. For example, the name *M. procera* has been misapplied to a North American entity (designated *M. spec. nov. 5* in this study) and to the Australian *M. clelandii* Grgur. (Grgurinovic 1997).

This study has three themes. First, it focuses on phylogeny of *Macrolepiota* as inferred from ribosomal DNA data and how this phylogeny relates to the classification, based on morphology; second, on the placement of the taxa within the family Agaricaceae; and third, on the relationship of the secotioid genus *Endoptychum* Czern. to the Agaricaceae. In the course of the study several new taxa were discovered; they will be described separately. Many collections from a large area (including representatives from Africa, and Australia) were examined, and ITS and LSU sequences, and morphological characters were used. The choice deliberately was made to use a high number of taxa and samples instead of data from other genes from a small sample (e.g., Greybeal 1998).

#### MATERIAL AND METHODS

*Material*.—Initially 92 collections of taxa belonging to *Macrolepiota* (sensu Singer 1986) or to putative species in this

group were used for molecular screening, along with an additional seven sequences, which were accessed through Genbank. These 99 collections represent at least 24 taxa or monophyletic species complexes, and one sample of each (i.e., 24 in total) was arbitrarily chosen from each set of sequences with up to five differences in base pairs and used in these analyses; in a few cases the ITS sequence was taken from a different specimen than the LSU-sequence. Material of 28 species representing the genera *Agaricus*, *Allopsalliota*, *Endoptychum*, *Lepiota*, *Leucoagaricus*, *Leucocoprinus*, and *Microsalliota* was used for comparison (see TABLE I for collections and species analyzed).

*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 and Bruns 1993) for the ITS region, and primers LR0R, LR3R, LR7, and LR16 for LSU (see <http://www.biology.duke.edu/fungi/mycolab/primers.htm>). Sequencing of both strands was performed with an ABI model 377 sequencer (Applied Biosystems, Foster City, CA, U.S.A.) using a Thermo Sequenase<sup>®</sup> Dye terminator Cycle Sequencing Pre-Mix Kit (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.) or a BigDye<sup>®</sup> Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Co.); the primers ITS1 or ITS5, ITS2, ITS3, and ITS4 were used for the ITS region, and primers LR0R, LR3R, LR7, and LR16 for the LSU-region. Raw data were processed with DNA Sequencing Analysis v.2.1.2 and Sequence Navigator v.1.0.1 (also of Applied Biosystems). The sequences thus obtained were aligned in ClustalW (Thompson et al 1994), with these settings: opening gap penalty 10, end gap penalty 10, extending gap and separation gap penalty both 0.05. Visual alignment was the next step. The alignments have been deposited in TreeBASE under number S782. Sequences have been deposited in Genbank, and the accession numbers are given in TABLE I.

*Morphological data.*—Nine morphological character states were scored for all taxa and added to the databases (TABLE II).

*Phylogenetic analysis.*—Maximum-parsimony analyses were performed with PAUP\* version 4.0b8 (Swofford 2001). 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 datasets (ITS data, LSU sequences, and ITS and LSU combined). Three members of tribus Lepioteae, *L. brunneoincarnata*, *L. cristata* and *L. rufipes*, were chosen as outgroup. Noninformative characters and part of the ITS1 region (190 base pairs) that was not unambiguously alignable were excluded from the analyses. Gaps were treated as missing data. To measure relative support for the resulting clades, 500 bootstrap replications (Felsenstein 1985) were performed with the same parameters as for the parsimony analyses.

To test alternative phylogenetic relationships, the Kishino-Hasegawa maximum-likelihood ratio test (Kishino and Hasegawa 1989) was performed, as implemented in PAUP\* with default settings, under the model developed by Hasegawa et al (1985). The hypotheses tested are enumerated in TABLE III under results.

## RESULTS

*Analyses of ITS dataset.*—The aligned data set includes a total of 906 base pairs (including gaps); 273 parsimony-informative characters were analyzed (a stretch of 190 base pairs in ITS1 was excluded from the analyses). A total of 90 most-parsimonious trees was recovered, with these characteristics:  $l = 1353$ ,  $CI = 0.3836$ ,  $CR = 0.2449$ .

Four lineages were recovered (FIG. 1): (i) Lineage 1, composed of *Agaricus*, *Microsalliota*, and *Endoptychum depressum*. (ii) Lineage 2 included *M. rachodes* and allies, *M. neomastoidea*, *Chlorophyllum molybdites*, *M. globosa*, *Endoptychum agaricoides* and *Leucoagaricus hortensis*. This lineage shows relatively long branches. Lineages 1 and 2 are sister groups of each other. (iii) Lineage 3 to which *Leucoagaricus* and *Leucocoprinus* species belonged. (iv) Lineage 4 comprising *M. procerata*, *M. mastoidea*, *M. excoriata*, and allies; the branches within this lineage in general are short.

All four lineages are present in the same topology on the consensus tree (not shown). However, Lineage 3, the *Leucoagaricus-Leucocoprinus* assemblage, is not bootstrap supported. Lineage 1 has 100% bootstrap support, whereas Lineage 2 has a bootstrap support of 76%, and Lineage 4 has 73% (however, Lineage 4 is 100% bootstrap supported when *Macrolepiota* spec. nov. 1 is excluded (data not shown)).

The topological constraint enforcing *Macrolepiota* sensu Singer (1986), i.e., without *Chlorophyllum molybdites* and *M. globosa*, without *Leucoagaricus hortensis*, and without *Endoptychum agaricoides*, does not yield a less likely tree (TABLE III). But, enforcing *Leucoagaricus hortensis* with Lineage 3 has to be rejected ( $P < 0.05$ ). Significantly less likely than the best tree are the options in which *Endoptychum agaricoides* is excluded from Lineage 2, *Macrolepiota nymphaeum* is included in *Macrolepiota*, or *E. depressum* and *E. agaricoides* form a monophyletic group. A position of *Macrolepiota* (lineages 2 and 4 together) together with Lineage 3 (*Leucocoprinus* and *Leucoagaricus*), is significantly worse than the option of a monophyletic group of lineages 1, 2 and 4, which is the most likely topology recovered.

*Analyses of LSU dataset.*—The aligned dataset shows a total of 934 characters, of which 82 are parsimony informative. One hundred most-parsimonious trees, occurring in four islands, were recovered ( $l = 225$ ).

Only a few distinct lineages were recognized in all four: *Agaricus* and *Endoptychum depressum*; the group of *M. rachodes*; *M. procer*a plus two closely related species; all other species are paraphyletic throughout the phylogram, in different topologies (the tree with the highest  $-\ln$  likelihood value is given in FIG. 2). The consensus tree also is highly unresolved, and only the lineages of *Micropsalliota* and *Allopsalliota*, *Leucoagaricus meleagris* and *L. americanus*, and *L. leucothites* and *M. nymphaeum* respectively have a bootstrap support higher than 70% (consensus tree not shown; bootstrap values given in FIG. 2).

Most topological constraints do not yield significantly less likely trees than the unconstrained tree (TABLE III under LSU data). However, enforcing *M. nymphaeum* with *Macrolepiota* sensu Singer (1986), enforcing the two *Endoptychum* species together and enforcing a monophyletic clade containing all *Macrolepiota*, *Chlorophyllum*, and *Agaricus* species are significantly less likely.

*Analyses of the combined ITS and LSU dataset.*—The combined dataset was tested for incongruence with the partition homogeneity test, as implemented in PAUP\* 4.0b8, as advocated by Cunningham (1997). The original ITS-dataset was incongruent with the LSU-dataset ( $P = 0.0200$ ), because of the placement of *Allopsalliota geesterani*. This species is a sister species of *Micropsalliota* spec. in the phylogram, based on the LSU-data set, but appears in the *Leucoagaricus-Leucocoprinus* lineage in the analyses of the ITS-data set. The position of this species based on morphological characters is also rather enigmatic; it shares characters with *Agaricus* and *Micropsalliota* (Nauta 1999) and has some chemical reactions in common with *Leucoagaricus americanus* and its allies. A similar situation was found in the analyses of several molecular datasets illuminating the phylogeny of the tribus Triticeae within the Gramineae (Mason-Gamer and Kellogg 1996), where the position of *Triticum monococcum* caused incongruence. After removal of *A. geesterani* in these datasets, the two datasets were congruent ( $P = 0.4100$ ) and were used for further analysis.

The combined dataset of ITS and LSU sequences yields 287 parsimony-informative characters (out of 1883); 20 most-parsimonious trees could be recovered, with a length of 988 (CI = 0.4615, CR = 0.2969), and all show the same four lineages as derived from the ITS-data set (the strict consensus tree is given in FIG. 3). These phylograms differ from the ones based on ITS data only in the fact that lineages 3 and 4 are now sister groups. *Leucoagaricus hortensis* is a sister taxon of the *Endoptychum agaricoides*-*Chlorophyllum* clade in half of the phylograms, and a sister

group to the *M. rachodes* clade in the other 10 phylograms. The bootstrap support for Lineage 1 is 100%, 75% for Lineage 2, and 95% for Lineage 4; again Lineage 3 gets very low bootstrap support (<50%). All four lineages are present in the consensus tree (FIG. 3) in the same topology as in the individual most parsimonious trees.

The most likely tree recovered is the one in which lineages 1, 2 and 4 form a monophyletic group, followed by the unconstrained tree (lineages 1 and 2 monophyletic and sister groups, Lineage 4 monophyletic and a sister group to Lineage 3) (TABLE III). The topological constraint enforcing lineages 2 and 4 together does yield an acceptable tree ( $P = 0.2$ ), but all the other topological constraints yield much less likely trees (TABLE III). The hypothesis that lineages 2, 3 and 4 form a monophyletic group (i.e., *Macrolepiota* in a wide sense is a sister group to the *Leucoagaricus-Leucocoprinus* assemblage) cannot be rejected, based on these data.

Adding morphological data to the data set or treating the gaps as fifth characters does not change the topology of the trees (data not shown), although bootstrap values might change slightly.

## DISCUSSION

*Concept of Macrolepiota.*—The traditional concept of *Macrolepiota*, as an agaricoid genus comprising white to pink-spored species with a germ pore in the spores, cannot be maintained, based on the results of the analyses of LSU data and combined ITS-LSU data. *Leucoagaricus hortensis* whose spores are not provided with a germ pore, *Chlorophyllum molybdites* and *M. globosa* with greenish spores, and the sectoid *Endoptychum agaricoides* appear to belong to the same lineage as some *Macrolepiota* taxa. *Macrolepiota nymphaeum*, on the other hand, is a sister taxon of *Leucoagaricus leucothites*, according to these analyses.

The phylograms based on the ITS sequences, and ITS-LSU-data (FIGS. 1 and 3), show two distinct clades within *Macrolepiota*, viz. lineages 2 and 4, which do not form a monophyletic group. However, topological constraints forcing the two lineages together (analyses of all three data sets) that result in one monophyletic “big” *Macrolepiota* clade is neither significantly better, nor worse, than the two-clade option. These alternatives cannot be discriminated on the present molecular grounds alone.

*Morphological considerations.*—The number of morphological characters of mushrooms available for a phylogenetic analysis on the generic level is unfortunately very small in general. Nevertheless, several morphological characters do support recognition of

TABLE I. Overview of species and collections and the GenBank accession numbers for the ITS and LSU sequences

Species	Collection & herbarium	Location & date of collection	ITS GenBank accession #	LSU GenBank accession #
<i>Agaricus arvensis</i> Schaeff.	GenBank		AF161015	U11910
<i>Agaricus bisporus</i> (J.E. Lange) Imbach	GenBank		AF161014	U11911
<i>Agaricus bitorquus</i> (Quél.) Sacc.	E.C. Vellinga 2462 (UCB)	U.S.A., Michigan, Washtenaw Co., Ann Arbor, 10-VIII-2000	AF482829	
<i>Agaricus californicus</i> Peck	E.C. Vellinga 2319 (UCB)	U.S.A., California, Alameda Co., Berkeley, U.C. Berkeley campus, 29-I-1999	AF482830	
	E.C. Vellinga 2337 (UCB)	U.S.A., California, Alameda Co., Berkeley, U.C. Berkeley campus, 3-VIII-1999		AF482876
<i>Agaricus campestris</i> L.: Fr.	GenBank		U85307	U85273
<i>Agaricus diminutives</i> Peck	E.C. Vellinga 2360 (UCB)	U.S.A., Washington, Olympic Peninsula, Grays Harbor Co., north side of Quinault Lake, July Creek Campground, 16-X-1999	AF482831	AF482877
<i>Agaricus subrutilescens</i> (Kauffman) Hotson & Stuntz	E.C. Vellinga 2418 (UCB)	U.S.A., California, Sonoma Co., Salt Point State Park, 15-XII-1999	AF482832	
<i>Agaricus</i> spec.	N.L. Bougher H6271 (CSIRO-Wembley)	Australia, West Australia, 86.2 km east of Broome along the Great Northern Hg, 10-II-1993	AF482833	
<i>Allopsalliota geesterani</i> (Bas & Heinem.) Nauta	E.C. Vellinga 2263 (L)	Netherlands, prov. Noord-Holland, Amsterdam, Amsterdamse Bos, 23-IX-1998	AF482857	AF482888
<i>Chlorophyllum molybdites</i> (G. Meyer: Fr.) Massee	J. States AEF1097 (MICH) GenBank	U.S.A., Arizona, Maricopa Co., Peoria, 4-IX-1994	AF482836	U85274
<i>Endophyllum agaricoides</i> Czern.	R. Brotzu (herb. Brotzu)	Italy, Sardinia, Nuoro, X-1990	AF482837	AF482885
<i>Endophyllum depressum</i> Singer & A.H. Sm.	E.C. Vellinga 2339 (UCB)	U.S.A., California, Sierra Nevada, Fresno Co., Dinkey Creek Rd, 6-VIII-1999	AF482834	AF482878
<i>Lepiota brunneoincarnata</i> Chodat & Martin	E.C. Vellinga 2260 (L)	Netherlands, prov. Noord-Holland, Amsterdam, Amsterdamse Bos, 23-IX-1998	AF482875	
	M. Enderle (L)	Germany, Baden-Württemberg, "Hörnle" close to Ulm-Grimmelfingen, 29-VIII-1996		AF482896
<i>Lepiota "carmineobasidiata"</i> Sundberg (1967)	E.C. Vellinga 2596 (UCB)	U.S.A., California, San Mateo Co., San Francisco watershed, 8-XII-2000	AF482860	
<i>Lepiota cristata</i> (Bolt.: Fr.) Kummer	E.C. Vellinga 1445 (L) GenBank	Luxembourg, Hollenfels, 28-IX-1988	AF391027	U85292
<i>Lepiota rufipes</i> Morgan sensu European authors	H.A. Huijser (L)	Netherlands, prov. Limburg, Bemelen, 9-X-1991	AF391066	AF482897

TABLE I. Continued

Species	Collection & herbarium	Location & date of collection	ITS GenBank accession #	LSU GenBank accession #
<i>Leptota viridincta</i> (Berk. & Broome) Sacc.	R.P.J. de Kok F 61 (L)	Indonesia, East Kalimantan, Wanariset, 1-III-1993	AF482873	
<i>Leucoagaricus americanus</i> (Peck) Vellinga	S.J.W. Verduin (L) E.C. Vellinga 2454 (UCB)	U.S.A., North Carolina, Durham, Duke University campus, Duke Medical Center, 29-VII-1998 U.S.A., Michigan, Washtenaw Co., Ann Arbor campus, University of Michigan, 6-VIII-2000	AF295928	AF482891
<i>Leucoagaricus barssii</i> (Zeller) Vellinga	E.C. Vellinga 2342 (UCB) E.C. Vellinga 2268 (L)	U.S.A., California, Alameda Co., San Leandro, 21-IX-1999 Netherlands, prov. Noord-Holland, Texel, Loosmansduinen, 24-IX-1998	AF295931	AF482894
<i>Leucoagaricus croceovelutinus</i> (Bon & Boiffard) Bon & Boiffard	E.C. Vellinga 2243 (L)	Netherlands, prov. Limburg, Elsloo-Geulle, Bunderbos, 19-IX-1998	AF482862	AF482889
<i>Leucoagaricus crystallifer</i> Vellinga	H.A. Huijser (L)	Germany, Baden-Württemberg, Gottenheim, Wasenweiler Wald, 3-IX-1998	AF482863	
<i>Leucoagaricus hortensis</i> (Murrill) Pegler	D.E. Hemmes 1365 (SFSU) GenBank	U.S.A., Hawai'i Islands, Hawai'i, Waipio Valley, 10-XII-1996	AF482843	U85284
<i>Leucoagaricus leucothites</i> (Vittad.) Wasser	E.C. Vellinga 2050 (L) GenBank	Netherlands, prov. Noord-Holland, Amsterdamse Waterleidingduinen, Van Lennepkanaal, 15-X-1996	AF482865	
<i>Leucoagaricus marriagei</i> D.A. Reid	E.C. Vellinga 2005 (L)	Netherlands, prov. Limburg, Elsloo-Geulle, Bunderbos, 9-IX-1996	AF482866	U85280
<i>Leucoagaricus meleagris</i> (Sow.) Singer	E.C. Vellinga 2095 (L) E.C. Vellinga 1990 (L)	Netherlands, prov. Zuid-Holland, Capelle aan den IJssel, 18&19-VIII-1997 Netherlands, prov. Noord-Holland, Amsterdam, Zorgvlied, 30-VII-1996	AF482867	AF482890
<i>Macrolepiota nympharum</i> (Kalchbr.) Wasser	C. Bas 9269 (L) R.P.J. de Kok 3 (L) E.C. Vellinga 2291 (L)	Germany, Rheinland-Pfalz, Eifel, near Gerolstein, 21-IX-1990 Germany, Rheinland-Pfalz, Eifel, near Dohm, 24-IX-1990 Netherlands, prov. Zeeland, Schouwen-Duiveland, near Haamstede, 6-XI-1998	AF482868	AF482895
<i>Leucoagaricus purpureobilacianus</i> Huijsman	E.C. Vellinga 1930 (L)	Belgium, prov. Liège, Tilff, Vallon de la Chavresse, 11-IX-1995	AF482871	AF482893
<i>Leucoagaricus serenus</i> (Fr.) Bon & Boiffard	E.C. Vellinga 2116 (L)	Netherlands, prov. Utrecht, Breukelen, Nijenrode, 6-X-1997	AF482872	
<i>Leucoagaricus sericifer</i> (Locq.) Vellinga	H.A. Huijser (L)	Netherlands, prov. Limburg, Neercanne, Cannerbos, IX/X-1987	AF482874	

TABLE I. Continued

Species	Collection & herbarium	Location & date of collection	ITS GenBank accession #	LSU GenBank accession #
<i>Leucoagaricus</i> spec.	E.C. Vellinga 2777 (UCB)	U.S.A., California, Alameda Co., Berkeley, U.C. Berkeley campus, 6-XII-2001	AF482858	
<i>Leucocoprinus brebissonii</i> (Godey) Locq.	E.C. Vellinga 1784 (L)	France, dept Pas-de-Calais, Forêt de Boulogne, 13-X-1991	AF482859	
<i>Leucocoprinus cretaceus</i> (Bull.: Fr.) Locq.	T. Læssøe 6171 (C)	Malaysia, Sabah, Tabin Wildlife Reserve, Mud Volcano trails 3 & 4, 9-II-1999	AF482861	
<i>Leucocoprinus heinemannii</i> Migl.	J. Engelen (L) E.C. Vellinga 2101 (L)	Netherlands, prov. Gelderland, Apeldoorn, 9-IX-1997 Netherlands, prov. Zuid-Holland, Leiden, Hortus Botanicus, 29-VIII-1997	AF482864	AF482892
<i>Leucocoprinus stramineus</i> (Bagl.) Narducci & Caroti	E.C. Vellinga 2080 (L)	Netherlands, prov. Zuid-Holland, Leiden, Hortus Botanicus, 16-VI-1997	AF482870	
<i>Macrolepiota clelandii</i> Grguri-novic	K.R. Thiele 2650 (MEL)	Australia, Victoria, 52 km north of Orhost on the Bonang Road, Martins Creek, 17-V-2000	AF482838	AF482882
<i>Macrolepiota colombiana</i> Franco-Molano	GenBank		U85311	U85276
<i>Macrolepiota dolichaula</i> (Berk.) Pegler & Rayner	E.M. Canning 6603 (CANB)	Australia, New South Wales, between Blakney Creek and Bendvale, just off road inside entrance to 'Kunama Cottage', 4-IV-1989	AF482839	
<i>Macrolepiota excoriata</i> (Schaeff.: Fr) Wasser	R.P.J. de Kok 901 (CANB)	Australia, ACT, Canberra, Black Mountain, CSIRO site, 14-IV-2000		AF482883
<i>Macrolepiota fuliginosa</i> (Barla) Bon	R. Chrispijn (L) E.C. Vellinga 2275 (L)	Netherlands, prov. Groningen, Dollarddijk, 11-IX-1997 Netherlands, prov. Limburg, Elsloo, Bunderbos, 29-IX-1998	AF482840 AF482841	
<i>Macrolepiota globosa</i> Mossebo	H. Neda N421 (TFM)	Nigeria, Edo State, Benin City, University of Benin campus, 24-V-2000	AF482842	
<i>Macrolepiota mastoidea</i> (Fr.: Fr.) Singer	E.C. Vellinga 1685 (L) GenBank	Germany, Rheinland-Pfalz, Eifel, west of Wiesbaum, 18-IX-1990	AF482844	
<i>Macrolepiota neomastoidea</i> (Hongo) Hongo	E. Nagasawa (TMI 14182)	Japan, Shimane Pref., Naka-gun, Yasaka-son, Nishikawauchi, 23-IX-1990	AF482845	U85279
<i>Macrolepiota olivieri</i> (Barla) Wasser	E.C. Vellinga 2230 (L)	Netherlands, prov. Limburg, Elsloo-Geulle, Bunderbos, 19-IX-1998	AF482846	AF482887
<i>Macrolepiota phaeodisca</i> Bellù	P. Roux 994 (L)	France, Corsica, Porticcio, 6-XI-1990	AF482847	
<i>Macrolepiota procera</i> (Scop.: Fr.) Singer	E.C. Vellinga 2293 (L)	Netherlands, prov. Zeeland, Schouwen-Duiveland, Haamstede, 7-XI-1998	AF482848	
<i>Macrolepiota rachodes</i> (Vittad.) Singer	R.P.J. de Kok s.n. (L) E.C. Vellinga 2106 (L)	Netherlands, prov. Groningen, Ter Apel, Ter Haar, 18-X-1990 Netherlands, prov. Zuid-Holland, Leiden, 24-IX-1997		AF482880

TABLE I. Continued

Species	Collection & herbarium	Location & date of collection	ITS GenBank accession #	LSU GenBank accession #
<i>Macrolepiota</i> spec. nov. 1	GenBank Z.L. Yang 2286 (HKAS)	China, Sichuan Prov., Xiangcheng, Co., in the vicinity of Shagong, 11-VIII-1998	AF482850	U85277
<i>Macrolepiota</i> spec. nov. 2	O. Oku (herb, Oku)	Japan, Ibaraki Pref., Tsukuba, X-2000	AF482851	AF482884
<i>Macrolepiota</i> spec. nov. 3	Z.L. Yang 2172 (HKAS)	China, Yunnan Prov., Jinghong, Damenglon, 14-VIII-1995	AF482853	
<i>Macrolepiota</i> spec. nov. 4	B. Bayliss, E4505 (CSIRO-Wembley)	Australia, Queensland, Mareeba, Davies Creek Road, 3-II-1992	AF482854 (ITS1)	
<i>Macrolepiota</i> spec. nov. 5	R.E. Tulloss (UCB)	U.S.A., Connecticut, Tolland Co., Gay City State Park, 23-IX-2000	AF482852	
	R.E. Tulloss RET 9-25-99-H (UCB)	U.S.A., Connecticut, Middlesex Co, Meshomasic State Forest, Cobalt, 25-IX-1999		AF482881
<i>Macrolepiota</i> spec. nov. A	H. Lepp 1142 (CANB)	Australia, Australian Capital Territory, Striling, 8 km SW of Capital Hill, Canberra, 19-I-1995	AF482855	
<i>Macrolepiota</i> spec. nov. B	E.C. Vellinga 2317 (UCB)	U.S.A., California, Alameda Co, Berkeley, UC-Berkeley campus, 25-I-1999	AF482856	
	E.C. Vellinga 2361 (UCB)	U.S.A., Oregon, Multnomah Co., Portland, S.E. Locust Ave, 20-X-1999		AF482886
<i>Microspalliota</i> spec.	T. Læssøe 6025 (C)	Malaysia, Sabah, Danum Valley, Field Centre, Main East Trail, 30-I-1999	AF482835	AF482879

TABLE II. Overview of morphological characters

Clamp-connections	absent	present	
Spore colour	white to pink	green	brown
Germ pore	absent	present	
Cap over germ pore	absent	present	missing
Basidia	2-spored	4-spored	
Hymenophoral trama	regular	trabecular	
Spore apex	truncate	rounded	
Habit	agaricoid	secotioid	
Causing gastrointestinal problems	no	yes	

lineages 2 and 4 at genus level. (i) The veil structure is hymenidermal in Lineage 2 (with clavate to lageniform terminal elements) versus trichodermal (all elements cylindrical and elongate) in Lineage 4; (ii) a stipe covering is absent in Lineage 2, although present in all taxa of Lineage 4, giving the stipes of the species a striking, banded appearance; (iii) the spores of the taxa of Lineage 2 lack either a germ pore, or have a truncate to rounded apex, without a hyaline cap covering the germ pore; the pore is caused by a depression of the episporium (Meléndez-Howell 1967); the spores of the Lineage 4 taxa always have a rounded apex, with a germ pore, covered by a hyaline cap; the germ pore in this case is caused by an interruption of the episporium, and it is filled by a refringent plug (Meléndez-Howell 1967).

The data suggest that representatives of Lineage 2 are more thermophilic or thermotolerant than those of Lineage 4. Lineage 2 includes species with a wide distribution in the tropics, or with a preference for compost heaps.

All agaricoid taxa of lineages 2 and 4 share the general *Macrolepiota* features: The basidiocarps are relatively big and fleshy (though the two undescribed taxa basal to Lineage 4 form relatively small basidiocarps), with a more or less complex annulus on the stipe, made up of both universal and partial veil remnants. The spores of all taxa are ellipsoid to amygdaloid-ellipsoid, and relatively large (8.0–22  $\mu\text{m}$  long).

Presence or absence of clamp connections previously was considered a good character to distinguish sections within the genus *Macrolepiota* (Singer 1986, Pázmány 1985). In our observations, clamp connections were found, at least at the base of the basidia, in almost all investigated taxa of Lineage 4 (they are absent to rare in collections belonging to the complex of *M. mastoidea* (Vellinga 2001)). Clamp connections are absent or rare in some taxa of Lineage 2, e.g., *Chlorophyllum molybdites* (Sundberg 1971), and absent in others (*M. venenata*, *E. agaricoides*, and

*M. spec. nov.* A (senior author pers obs). In short, clamp connections are present and absent in both lineages, as indicated in FIG. 3, and clearly this character cannot be used to distinguish the two lineages.

*Taxonomic implications.*—Morphological characters, particularly features of the covering layers, and the spore apex, provide the decisive factor to rule out the “big” *Macrolepiota* clade alternative and to regard the two lineages as separate genera.

Lineage 4 comprises the type species of *Macrolepiota*, *M. procera*, and keeps the name *Macrolepiota*. *Macrolepiota* in this restricted sense comprises section *Macrolepiota* and section *Macrosporae* with subsections *Excoriatae* Bon and *Microsquamatae* (Pázmány) Bellù & Lanzoni of the infrageneric classification advocated by Bon (1993).

Lineage 2 is more diverse, with relatively long-branch lengths in the phylograms based on the sequence data, and the species morphologically are more diverse than those in Lineage 4. Lineage 2 is made up of *Macrolepiota* section *Laevistipedes* (Pázmány) Bon, *M. globosa*, the genus *Chlorophyllum*, *Leucoagaricus hortensis*, and *Endoptychum agaricoides*. *Macrolepiota neomastoidea* is basal to this lineage. Moreno et al (1995) concluded on morphological grounds that *Chlorophyllum* and *Macrolepiota* should be merged, the only differences between the two genera being the different spore color. The green pigments in *Chlorophyllum* spores obscure the staining reactions that nevertheless are similar to those of spores of *Macrolepiota* species. Moreno et al (1995) used the name *Macrolepiota* for the combined group, though the genus name *Chlorophyllum* (Masse 1898) predates *Macrolepiota* (Singer 1948) by 50 years.

*Endoptychum agaricoides* is the type species of the genus *Endoptychum* (Czernajew 1845), which means that Lineage 2 should be called *Endoptychum*, because it is the oldest generic name available. However, it would be infelicitous to call Lineage 2 *Endoptychum*, because this name is associated in usage and etymology with the secotioid habit of only one taxon in Lineage 2. Furthermore, the name is not well known and has been used for species now shown to belong to several genera (discussed further below). Vellinga and De Kok (2002) officially proposed to conserve *Chlorophyllum* against *Endoptychum*.

Akers and Sundberg (1997) placed the species *Leucoagaricus hortensis* in the genus *Leucoagaricus* on account of the absence of the germ pore, despite the presence of clamp connections. In all analyses, that position is significantly worse than including *L. hortensis* within Lineage 2, the *M. rachodes*-*Chlorophyllum*-*Endoptychum* clade.

*Macrolepiota nympharum* is shown to belong to nei-

TABLE III. Results from Kishino-Hasegawa tests for the three data sets.  $P$  is the probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two-tailed test)

Constraints	ITS data			LSU data			ITS+LSU data					
	Tree length	No. trees	$-\ln L$	$P$	Tree length	No. trees	$-\ln L$	$P$	Tree length	No. trees	$-\ln L$	$P$
Unconstrained	1353	90	6167.58989	0.5992	225	100	1049.61794	(best)	988	20	4401.49163	0.5343
<i>Macrolepiota</i> including <i>Chlorophyllum</i> species, <i>Endophyllum agaricoides</i> , and <i>Leucoagaricus hortensis</i> , but excluding <i>L. nymphaeum</i> , is monophyletic	1359	84	6171.74242	0.1287	229	306	1064.59536	0.1187	994	12	4413.93499	0.1863
<i>Macrolepiota</i> sensu Singer (1986) is monophyletic, i.e., excluding <i>Chlorophyllum</i> , <i>Endophyllum agaricoides</i> , and <i>Leucoagaricus hortensis</i> (but also excluding <i>L. nymphaeum</i> )	1369	120	6194.14041	0.0681	231	204	1068.66069	0.0823	1001	9	4439.71153	0.0012*
<i>Macrolepiota</i> including <i>Chlorophyllum</i> , but excluding <i>E. agaricoides</i> and <i>L. hortensis</i> is monophyletic	1373	120	6199.17163	0.0363*	231	165	1069.43603	0.0544	1012	28	4443.77166	0.0002*
<i>Macrolepiota</i> including <i>E. agaricoides</i> and <i>Chlorophyllum</i> , excluding <i>Leucoagaricus hortensis</i> is monophyletic	1387	58	6200.62042	0.0304*	229	66	1064.14571	0.1132	1004	20	4441.08814	0.0102*
<i>Leucoagaricus nymphaeum</i> is not a sister taxon of <i>L. leucothites</i> but belongs to <i>Macrolepiota</i> (lineages 2 and 4)	1357	1170	6230.88738	0.0042*	239	48	1106.47892	<0.0001*	1060	2	4607.29662	<0.0001*
<i>Macrolepiota</i> (lineages 2 and 4) form one clade with <i>Agaricus</i>	1357	120	6159.82350	(best)	245	408	1088.52286	0.0034*	991	6	4398.41866	(best)
<i>Macrolepiota</i> (lineages 2 and 4) is a sister group to the <i>Leucoagaricus-Leucocoprinus</i> assemblage	1383	669	6234.02119	0.0030*	230	237	1060.50652	0.2053	994	3	4410.9952	0.2396
<i>Endophyllum</i> forms a monophyletic group, comprising <i>E. depressum</i> and <i>E. agaricoides</i>	1427	180	6345.85121	<0.0001*	237	116	1075.09145	0.0242*	1068	6	4582.82402	<0.0001*
<i>Leucoagaricus hortensis</i> belongs in the <i>Leucoagaricus-Leucocoprinus</i> assemblage	1394	62	6263.64613	<0.0001*	228	117	1055.32346	0.4337	1006	10	4444.83291	0.0060*

\*  $P < 0.05$ .

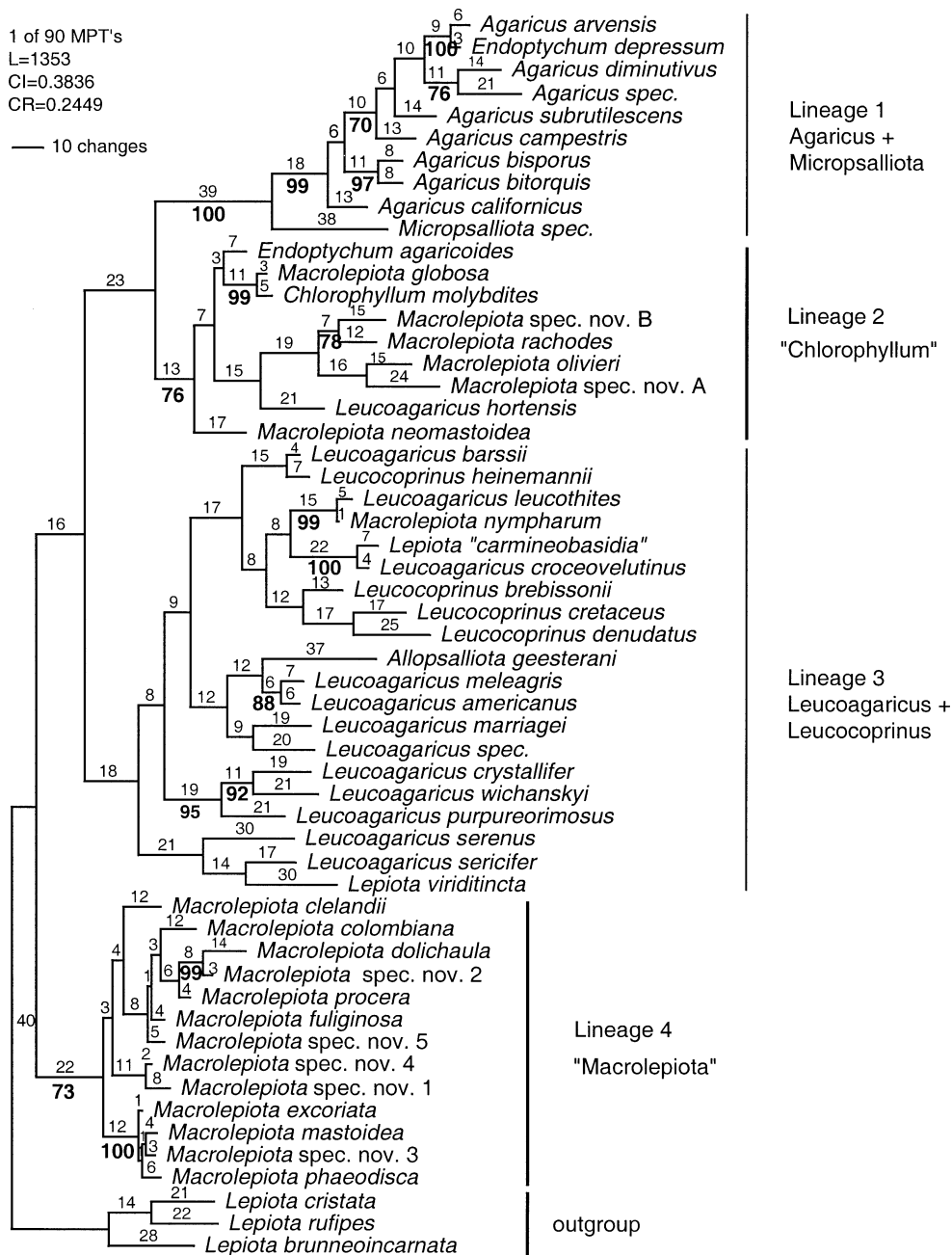


FIG. 1. Maximum-parsimony analysis of ITS data, one of 90 most parsimonious trees. Bootstrap values over 70% are indicated in bold below the branches.

ther of the two *Macrolepiota* lineages, but to be a sister taxon of *Leucoagaricus leucothites*. Migliozi and Bizzi (1994) suggested on morphological grounds that *M. nymphaeum* (as *M. puellaris*) was an intermediate between *Macrolepiota* and *Leucoagaricus*. The structure of the pileus covering is much looser than in *Macrolepiota* species; the shape of the cheilocystidia and, in particular, the absence of clamp connections support placement in *Leucoagaricus* (see also Vellinga 2001).

*The position of Endoptychum.*—It is not a new observation that secotioid fungi do not form a monophyletic group but are either mutants of normally agaricoid taxa (Hibbett et al 1994) or recently derived from diverse agaricoid and boletoid ancestors. *Endoptychum depressum* and *E. agaricoides* both recently are derived from representatives of Agaricales, it appears. In such cases, taxonomic conclusions have been drawn in different ways. Kretzer and Bruns (1997) combined the genera *Gastrovillus* Thiers and

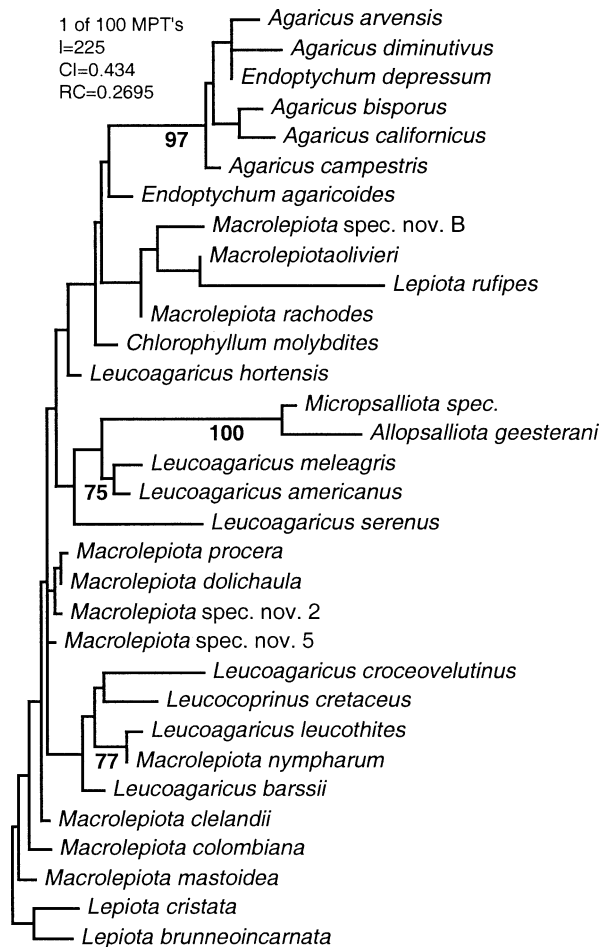


FIG. 2. Best ( $-\ln$  Likelihood) of 100 equally most-parsimonious trees, based on LSU data. Bootstrap values over 70% are indicated in bold below the branches.

*Suillus* Gray, but Redhead et al (2001) kept *Montagnea* Fr. and *Coprinus* Pers. s. str. as separate genera within the Agaricaceae. *Endoptychum depressum* in Lineage 1 is morphologically an *Agaricus* species in which the lamellae are not exposed and the basidia do not actively discharge spores. It is closely related to *Agaricus arvensis*. The name *Agaricus depressus* Léveillé, used for a small marasmioid species from Java, Indonesia, predates a new combination by at least 150 years, so the new name *Agaricus inapertus* Vellinga is proposed; basionym: *Endoptychum depressum* Singer & A.H. Sm. in *Brittonia* 10: 216. 1958.

*Endoptychum agaricoides* is a sister taxon of *Macrolepiota globosa* and *Chlorophyllum molybdites*. Conard (1915) had presumed a close relationship with *Agaricus campestris*, because of similarities in development, although this opinion was disputed strongly by Lohweg (1924), who interpreted the agaricoid fungi as derived from secotioid forms. A relationship with

*Chlorophyllum* already was suggested by Singer and Smith (1958), on account of similar spore colors. The spores of *E. agaricoides*, the secotioid member of Lineage 2, are greenish to yellowish brown under the microscope, not gray-green like the spores of *Chl. molybdites*. A germ pore is lacking or vaguely visible. Young, uncolored spores have all the characteristic staining reactions: red in Congo Red, blue in Cotton Blue, red-brown in Melzer's reagent, and with a pink inner wall in Cresyl Blue. Clamp connections are absent at the base of the basidia and on hyphal septa, and the hymenophoral trama hyphae are inflated, character states shared with *Chl. molybdites*.

Singer and Smith (1958) listed two more *Endoptychum* species: *E. melanosporum* (Berk.) Singer & A.H. Sm. with black spores, and *E. arizonicum* (Shear & Griffiths) Singer & A.H. Sm. with white, globose spores, and clamp connections in the trama (senior author pers obs). These taxa await sequencing for correct taxonomic placement.

Taxa other than the ones studied here putatively belonging to the *Chlorophyllum* lineage are *M. abrutilbulba* (Heim) Heinem., *M. brunnea* (Farlow & Burt) S. Wasser, *M. venenata*, *M. subrhacodes* Murrill, and probably *Chlorolepiota mahabaleshwarensis* Sathe & Deshpande.

*Classification above genus level.*—Current molecular data also show that recognition of two families, Lepiotaceae for the non-brown-spored taxa and Agaricaceae for brown-spored taxa, cannot be justified, just as shown in earlier papers (e.g., Johnson 1999, Moncalvo et al 2000). In fact, Lineage 2 appears to be more closely related to the brown-spored genus *Agaricus* than to the white-spored genera *Leucoagaricus* and *Leucocoprinus*, despite discordant morphological data, such as the structure of the trama (regular in *Agaricus* versus trabecular in *Chlorophyllum*, *Macrolepiota*, *Leucoagaricus* and *Leucocoprinus*) and the structure of the pileus coverings.

Classification of the family Agaricaceae into three tribus has to be reconsidered. Division into two tribus, Lepiotae and Agariceae, seems to be better supported by the current molecular data, but analysis of data in the family as a whole is better suited to resolving the intrafamilial classification. *Coprinus comatus* (O.F. Müll. : Fr.) Pers. and *C. sterquilinus* (Fr. : Fr.) Fr., and their secotioid allies *Montagnea arenarius* (DC.) Zeller, and *Podaxis* Desv. should be included in these analyses (Hopple 1994, Johnson 1999) and so should gasteroid taxa in the Lycoperdaceae (Hibbett et al 1997, Krüger et al 2001, Moncalvo et al 2002).

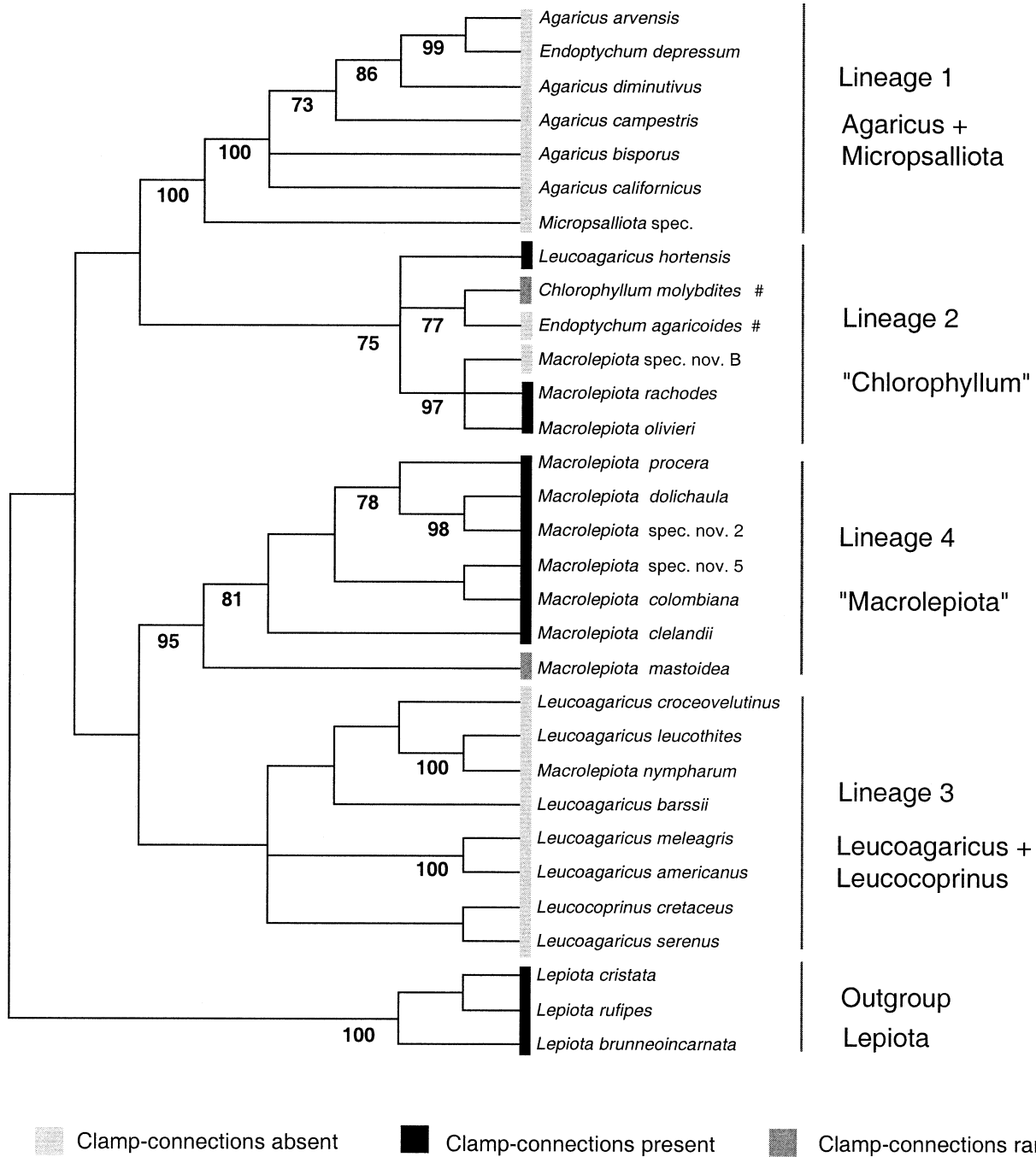


FIG. 3. Strict-consensus tree, based on maximum-parsimony analysis of the combined data set. Presence or absence of clamp connections is indicated. Bootstrap values over 70% are given below the branches. The # sign indicates that the species causes gastro-intestinal problems.

Molecular and morphological characters in Lineage 3, the *Leucoagaricus-Leucocoprinus* assemblage, are highly diverse, and our datasets do not warrant any conclusions on this lineage because a relatively small number of representatives have been chosen for a comparison with *Macrolepiota* and *Chlorophyllum*.

*In conclusion.*—The three questions raised in the introduction now can be answered. The genus *Macrolepiota* has to be emended and now is restricted to species with trichodermal pileus covering, a stipe covering made up of hymeni-trichodermal patches, and spores with a rounded apex with a covered germ pore. *Macrolepiota procera*, *M. dolichaula*, *M. masto-*

*idea* and allies belong here. A second genus comprises *Endoptychum agaricoides*, *Chlorophyllum molybdites*, *M. globosa*, *Leucoagaricus hortensis* and the group of *M. rachodes*. The agaricoid members are characterized by a hymenidermal pileus covering, a smooth stipe, and often truncate spores with an uncovered germ pore, or the germ pore might be absent. We suggest that the generic name *Chlorophyllum* is appropriate for this lineage, pending the outcome of a proposal submitted to Taxon. Toxic species group together in the latter genus, Although they do not constitute a separate clade. It also is shown that secotioid taxa, formerly in the genus *Endoptychum*, belong to different genera: *E. depressum* in the genus *Agaricus*, and *E. agaricoides* in *Chlorophyllum* (proposal pending).

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