

## The pantropical genus *Macrocybe* gen. nov.

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**Abstract:** Analysis of morphological and ecological characters plus ribosomal DNA sequences support the removal of seven related tropical species from the genus *Tricholoma*. This group of apparently nonmycorrhizal species that have abundant clamp connections on all hyphae but lack siderophilous granulation in the basidia cannot be accommodated in other genera, such as *Calocybe* or *Megatracholoma*. We therefore propose *Macrocybe* as a new genus in the Tricholomataceae for these taxa.

**Key Words:** Agaricales, *Calocybe*, ribosomal DNA, *Tricholoma*, Tricholomataceae, *Tricholomataceae*

### INTRODUCTION

Singer (1986) included only one genus, *Tricholoma* (Fr.) Staude, when he proposed the subtribe *Tricholomatinae*. This is characterized by basidiomata that form obligate ectomycorrhizal associations with forest trees, and hyphae that lack clamp connections. Exceptions to this definition are found in subgenus *Contextocutis* Singer, Section *Leucorigida* Singer, as proposed by Singer (1945). Singer (1986) included *T. titans* (as *T. cystidiosum*), *T. giganteum* and *T. prae-*

*grande* in section *Leucorigida*. Both the type species of this section, *T. mongolicum* S. Imai, and the second cited species, *T. altaicum* Singer, are common species in northern China and Mongolia, where they form fairy rings in grass. They are remarkably similar to the North temperate species, *Calocybe gambosa* (Fr.) Donk and its allies, and must be regarded as belonging to *Calocybe* Kühner ex Donk.

The remaining species form large, robust basidiomata that are tricholomatoid in habit, but their precise position within the genus nevertheless remains problematical. In many ways, these species closely resemble those of *Calocybe*, in tribe Lyophylleae Kühner ex Bas. However, siderophilous granules are absent from the basidia of fresh and dried specimens (C. Ovrebø, pers. comm.; Pegler, 1983, respectively), and the presence of these granules characterizes tribe *Lyophylleae*. Siderophilous granules may be weakly stained in the basidiospores. However, the presence of clamp connections throughout the basidiomata, cyanophilic spores, and the nonectomycorrhizal nature, provide a unique combination of characters. Kost (1984) proposed the genus *Megatracholoma* to accommodate the European species, *Tricholoma colossium* (Fr.) Quél. This species forms large basidiomata but differs from both *Tricholoma* (Fr.) Staude and the tropical species in its hemiangiocarpic development resulting in an annulate stipe, together with differences in the subhymenial layer and basidia, and the presence of true cheilocystidia and pleurocystidia. Furthermore, *M. colossium* lacks clamp connections.

The striking appearance of these large, pale, nonectomycorrhizal, tropical and subtropical agarics, which are distinct from *Calocybe*, *Megatracholoma* and *Tricholoma* sect. *Leucorigida* sensu stricto, require recognition at the generic level. We present a molecular analysis by Nakasone and Lodge of the 5'-end of the ribosomal large subunit DNA sequences of *T. titans*, four ectomycorrhizal species of *Tricholoma* (*T. cf. flavovirens*, *T. imbricatum*, *T. cf. intermedium*, and *T. vaccinum*), *Clitocybe clavipes* and *Ca. gambosa*. The morphological and ecological data, considered together with molecular analyses by us and others are consistent with our hypothesis that *T. titans* falls outside the *Tricholoma* sensu stricto clade, and placement of *T. titans* in the genus *Calocybe* of Tribe *Lyophylleae* is

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also not strongly supported. A new genus, *Macrocybe*, is therefore proposed.

Some of the tropical species belonging to the proposed new genus have had a complex and confusing history. Petch (1912: 268) supplied a revised account of *T. crassum* (Berk.) Sacc., based on material collected in the Kandy District of Central Province, Sri Lanka. At that time, he placed another species that was also originally described from Sri Lanka, *T. pachymeres* (Berk. & Broome) Sacc., in synonymy. This caused confusion between the two species that has persisted until now. *Tricholoma pachymeres* has been recorded on a number of occasions (Baker and Dale, 1951: 94, pl. 1g; Pegler, 1983: 111, pl. 3D), and was regarded as a pantropical species. Cifuentes and Guzman (1981) described a similar species from Mexico as *T. cystidiosum* Cifuentes & Guzmán, which presumably differed in the presence of hymenial cystidia. However, the type material of the paleotropical species, *T. pachymeres*, is in poor condition so it is not possible to confirm the presence of pleurocystidia and hence whether or not it is conspecific with *T. titans*. Courtecuisse renamed the neotropical species as *T. cifuentesii* in 1985 to avoid confusion with an earlier species, *T. cystidiosum* A. H. Sm. Singer (1990) synonymized *T. cystidiosum* Cifuentes & Guzmán with *T. titans* H. E. Bigelow & Kimbr., a species described one year earlier from Florida (Bigelow and Kimbrough, 1980), again emphasizing the presence of hymenial cystidia.

Recent Puerto Rican collections by one of us (DJL) match the characteristics of *T. titans* well. The characters of *T. titans* are distinct from those of *T. giganteum* Massee from West Bengal, India, and *T. praegrande* from Brazil. The latter two species lack the squarrose appearance of the stipe associated with the numerous small, recurved squamules as found in *T. titans*, *T. crassum* and *T. pachymeres*, and they also lack pseudocystidia. A reexamination of all the neotropical collections deposited at Kew under the name *T. pachymeres* has demonstrated the presence of pseudocystidia and other characteristics consistent with *T. titans*. An examination of all the collections from Costa Rica deposited at the Field Museum of Natural History under the name '*T. praegrande*' also demonstrated the presence of cystidia and scaly stipes consistent with *T. titans*. In addition to describing a new genus to accommodate these tropical and subtropical species, we provide the correct placement of various collections under the material examined section for each species.

#### MATERIALS AND METHODS

Color names in quotation marks are those in Ridgway (1912) or Ridgway colors as reproduced by Smithe

(1975). Spore dimensions, means, standard deviations and Q's are given for 25 spores taken from the type or an authentic collection, except for two collections that were sequenced and are described below.

The following taxa were included in the molecular study: *T. titans* (Lodge PR-1637, see specimens examined for data; GenBank U8637-U8638), *T. cf. flavovirens* (Pers. : Fr.) Lundell (USA, WISCONSIN: Sauk County, County Highway PF, in *Picea* plantation, Oct. 1994, *T. J. Volk TJV 94-134*, CFMR, GenBank U86672), *T. vaccinum* (Pers. : Fr.) Kummer (USA, WISCONSIN: Oct. 1994, *T. J. Volk TJV 94-133*, CFMR, GenBank U86443-U86444), *Ca. gambosa* (Fr. : Fr.) Singer ex Donk (ENGLAND, SURREY Esher, West End Common, copse under young *Ulmus* and *Sambucus*, B. M. Spooner, K(M)22752; GenBank U86440-U86441), *Cl. clavipes* (Pers. : Fr.) Kummer (USA, WISCONSIN: Sauk County, Oct. 1994, *T. J. Volk TJV 94-122*, CFMR, GenBank U86439-86440). The following sequences obtained from GenBank were also included in the analysis: *T. cf. intermedium* Pk. (USA, CALIFORNIA: Sierra County, Tahoe National Forest, Rd. 54 off Highway 49, near Yuba Pass, buried groups under old-growth *Abies magnifica*, 15 Oct. 1995, N. Wilson, ledger K.M. Shanks KMS 593, SFSU; GenBank U76465), *T. imbricatum* (USA, CALIFORNIA: Marin County, Pt. Reyes, 9 Jan. 1993, K.M. Shanks KMS 356, SFSU; U76458), *Pleurotus pulmonarius* (Fr. : Fr.) Quél. (U04153) and *P. cornucopiae* (Paulet) Rolland (U04135). The sequences for *T. intermedium* and *T. imbricatum* were provided by K. M. Shanks before submission to GenBank.

The culture of *Cl. clavipes* and the specimens of *Cl. clavipes*, *T. vaccinum* and *T. cf. flavovirens* are deposited at CFMR. The specimen of *Ca. gambosa* is at K, and the specimens of *T. intermedium* and *T. vaccinum* are deposited at SFSU. The specimens were typical for their species, except for *T. cf. flavovirens* and *T. cf. intermedium*, which are briefly described below.

*Extraction of total DNA.*—Total DNA was obtained from basidiospore drops, dried basidiomata, or lyophilized cultures. For *T. vaccinum* and *M. titans*, a basidiospore suspension in 50 ml of TE (10 mM Tris, 1 mM EDTA, pH 8) was placed in an 1.8-ml microcentrifuge tube with a small amount of glass beads (Sigma G-4649), vortexed at high speed for 2 min, and stored at -20 C.

Dried lamellae or small sections (1 X 1 cm) of the pileus of *T. cf. flavovirens* and *Ca. gambosa* were ground to a fine powder with a small amount of sand with a mortar and pestle, and DNA was extracted following Cenis (1992). In the case of *Cl. clavipes*, a culture was grown on 50 mL of sterile 2% malt extract supplemented with 1% glucose at 25 C for 1 wk. The culture was harvested by filtration onto Miracloth (Chicopee Mills, Inc., La Jolla, California), ly-

ophilized, and stored at -20 C in plastic bags. Total DNA was extracted following Cenis (1992).

*Amplification, sequencing and analysis of DNA segments.*—Procedures followed are described in Nakasone (1996) except that to amplify the 5'-end of the nuclear large ribosomal RNA gene, primers LR0R (5'-ACCCGCTGAACTTAAGC-3', position 26-42 with respect to *Saccharomyces cerevisiae*) and LR 7 (5'-TAC-TACCACCAAGATCT-3', position 1448-14322 with respect to *S. cerevisiae*; Vilgalys and Hester, 1990) were used. Sequencing primers used were: LR0R, LR2R (5'-AAGAACTTTGAAAAGAG-3'), LR3R (5'-GTCTTGAAACACGGACC-3'), LR17R (5'-TAACCT-ATTCTCAAACCTT-3'), and LR22 (5'-CCTCACGGT-ACTTGTCGCT-3'). The primers were prepared by Operon Technologies, Inc. (Alameda, California) based on sequences obtained from Rytas Vilgalys (Duke University). Methods used for sequencing are found in Nakasone (1996).

Sequences were manually aligned, and the alignment that produced the fewest number of informative characters was selected in ambiguous cases. Analysis procedures outlined in Nakasone (1996) were followed except that insertion/deletion data were not analyzed as a separate character set. Baseline maximum parsimony trees with unordered nucleotide data (Fitch, 1971) were obtained using PAUP, version 3.1.1 (Swofford, 1993). Tree searches were conducted using branch swapping options of branch and bound and tree bisection and reconnection with accelerated transformation optimization. The consistency index (Klug and Farris, 1969) and retention index (Farris, 1989) are reported for base-line trees (uninformative characters were excluded). Branch lengths include autapomorphic characters. Different alignments were analyzed to accommodate ambiguous areas and insertion/deletions, as well as the effects of applying greater weights to transversions than to transitions. Support for monophyletic clades were estimated with bootstrap (Felsenstein, 1985) using 1000 replicates of heuristic searches as well as decay analysis (Bremer, 1988).

#### TAXONOMY

##### **Macrocybe** Pegler & Lodge, gen. nov.

Basidioma illo generis *Tricholoma* (Fr.) Staude habitu generale est. Pileus albidus, cremeus vel griseo-ochraceus; hyphae omnes fibulatae; spora subglobosae vel breve ellipsoideae, hyalinae, cyanophilae, inamyloideae, tenuitunicatae, laeves; basidia granulis siderophilis destituta; cutis valde intermixtae. Nonec-

tomycorrizoidea. Species typicae: *Tricholoma titans* H. E. Bigelow & Kimbr.

Basidiomata large, fleshy, often forming connate or caespitose clusters. Pileus convex, umbonate to depressed; surface white, cream to grayish ochraceous, smooth, dry; margin incurved when young. Lamellae sinuate, pale, crowded, with numerous lamellulae. Stipe cylindrical to obclavate, often with distinct swollen base, solid; surface smooth and fibrillose or squarrose; arising from a cottony mycelium or a pseudosclerotium. Context white, unchanging on exposure; generative hyphae thin-walled, inflated; clamp connections always present. Spore deposit white to cream. Basidiospores less than 10 µm long, subglobose to broadly ellipsoid, hyaline, cyanophilic, inamyloid, thin-walled, smooth, often containing one or more guttules; hilum nodulose (ultrastructurally). Basidia clavate, tetrasporic, lacking siderophilous granulation. Cheilocystidia absent, pseudocystidia absent or present and then gloeocystidioid. Hymenophoral trama regular; subhymenial layer narrow. Pileipellis a well developed, repent cutis, of narrow, slightly inflated, thin-walled hyphae, with clamp connections; sometimes with gloeo-hyphae. Gymnocarpic. Terrestrial, in grassland or associated with decayed wood, nonectomycorrhizal.

TYPE. *Tricholoma titans* H. E. Bigelow & Kimbr. 1980. USA: FLORIDA: Lake Co., on sandy soil beneath oaks, 14 Aug. 1978, J. A. Hadox (FLAS F51990).

The genera with large, pale, tricholomatoid basidiomata in the Tricholomataceae may be separated as follows:

#### KEY TO SIMILAR GENERA OF TRICHALOMATOID AGARICS

1. Mature basidia containing siderophilous granulation; clamp connections present on all hyphae; mainly grassland species, saprotrophic, never obligately ectomycorrhizal; temperate and tropical . . . . . Tribus *Lyophylleae*, *Calocybe* Kühner ex Donk
1. Mature basidia lacking siderophilous granulation . . . . . Tribus *Tricholomataceae*, 2
  2. Clamp connections present on all hyphae; gloeocystidioid pseudocystidia present or absent; development gymnocarpic; tropical and subtropical; saprotrophic, not ectomycorrhizal. . . . . *Macrocybe* gen. nov.
  2. Clamp connections absent on all hyphae; obligately ectomycorrhizal with forest trees. . . . . 3
3. Development hemiangiocarpic, leaving an annulus on the stipe; well differentiated cheilocystidia and pleurocystidia present . . . . . *Megatracholoma* Kost
3. Development gymnocarpic; pleurocystidia absent; inconspicuous pseudocystidia or poorly differentiated cheilocystidia rarely present . . . *Tricholoma* (Fr.) Staude

KEY TO SPECIES OF *MACROCYBE*

1. Stipe smooth to fibrillose-striate, but never forming recurved squamules ..... 3
1. Stipe with recurved squamules ..... 2
  2. Abundant fusoid pseudocystidia with refractive contents present; tropical and subtropical America (-1) . . . *M. titans*
  2. Presence of pseudocystidia uncertain; Sri Lanka, India (-1) ..... *M. pachymeres*
3. Largest expanded pilei exceeding 20 cm diam (-1) ..... 5
3. Pileus less than 20 cm diam (-1) ..... 4
  4. Pileus 6-12(-20) cm diam, white to ivory, sometimes spotted ochre or red, with narrow lamellae up to 3 mm broad, arising in small tufts from a pseudosclerotium; odor of coumarin or 'bitter almonds'; in grass, West Africa (-3) ..... *M. lobayensis*
  4. Pileus darker, or lamellae broader than 3 mm, or basidiomata not arising from a pseudosclerotium (-3) ..... 5
5. Basidiomata in large tufts arising from a pseudosclerotium, often more than 50 in a cluster; pileus pure white, 7-40 (-60) cm diam; lamellae 5-20 mm broad; odor strongly cyanic; associated with *Saccharum*; Mauritius, Japan (-3,4) ..... *M. spectabilis*
5. Basidiomata solitary or few, without a pseudosclerotium; pileus cream, ochraceous, pale brown or gray; odor not cyanic; not associated with *Saccharum* (-3,4) ..... 6
  6. Stipe cylindrical, ca. 6 cm diam; pileus initially white, soon pale gray; lamellae straw yellow (-5) ..... *M. gigantea*
  6. Stipe base strongly swollen, 6-13 cm diam; lamellae white to pale cream ..... 7
7. Pileus and stipe cream to dark cream; tropical America (-6) ..... *M. praegrandidis*
7. Pileus pale cream to yellowish brown or grayish brown in center; stipe off-white with brown fibrillose streaks; paleo-tropics (-6) ..... *M. crassa*

**Macrocybe crassa** (Berk.) Pegler & Lodge, comb. nov.

≡ *Agaricus crassus* Berk. in Hooker, Lond. Journ. Bot. 6: 483 bis. 1847.

≡ *Tricholoma crassum* (Berk.) Sacc., Syll. Fung. 5: 109. 1887.

Basidiomata solitary or caespitose. Pileus 14-24 (-40) cm diam, convex to obtusely umbonate or almost appanate, finally becoming slightly depressed; surface pale cream, yellowish brown to grayish brown, darker at center, smooth, drying minutely cracked and sometimes splitting radially; margin involute, weakly crenate. Lamellae adnexed to sinuate, white to pale cream, 4-10 mm broad, crowded, with lamellulae of two lengths. Stipe 15-25 × 1.4-5 cm, cylindrical, swollen at base; solid then fistulose; surface off-white, with brown fibrillose streaks. Context up to 3.5 cm thick at disc, white, firm; consisting of thin-walled hyphae, 2-6 μm diam, inflated to 20 μm diam, with clamp connections; taste slightly bitter; odor 'ammoniacal'. Spore deposit pale cream. Spores 5.0-6.5 × 3.7-4.5 (5.60 ± 0.15 × 4.20 ± 0.32) μm, Q = 1.36, ovoid, hyaline, inamyloid, thin-walled, smooth. Basidia 25-30 × 7-8 μm, clavate, bearing four sterigmata. Hymenial cystidia none. Hymenophoral trama regular, with hyphae 3-5 μm diam. Subhymenial layer 7-12 μm thick, hyphae narrow, interwoven. Pileipellis a repent cutis, of thin-walled, uninflated hyphae, 5-7 μm diam.

HOLOTYPE. SRI LANKA, CENTRAL PROV.: Kandy Distr., Peradeniya, on the ground, June 1844, Gardner 53 (K(M) 36629).

*Additional specimens examined.* KUALA LUMPUR: May 1995, Muid (K(M) 30560). INDIA, KERALA STATE: Calicut, 22 Jun. 1980, Zachariah (K(M)37713). MALAYSIA: Selangor, Jelun Kebun, 20 Sept. 1986, Muid & Samani 407 (K(M)37709); Bangi, 1 Aug. 1986, Graham 3 (K(M)37711). THAILAND: Bangkok, Bangkok, cultivated on horse manure and sawdust, 8 Aug. 1969, Nutalaya (K(M)37710); March 1871, Nutalaya (K(M)37709); Sanpakhoy, sold in market, 27 Jun. 1989, Graham (K(M)37712); Chiangma, sold in market, 10 July 1991, Graham (K(M)17439).

*Observations.* This species and *M. gigantea* represent the two largest, tricholomatoid agarics of south-east Asia. The two species can be distinguished by the hymenophore color, the lamellae of *M. gigantea* being straw yellow rather than white, and the stipe that is cylindrical in *M. gigantea* rather than being expanded at the base. Collections from Malaysia are said to be edible, with a slightly bitter taste that is removed by cooking. Large basidiomata can weigh up to 1.25 kg.

**Macrocybe gigantea** (Masse) Pegler & Lodge comb. nov.

≡ *Tricholoma giganteum* Masse in Bull. Misc. Inf. Kew 1912: 254. 1912.

Pileus 30-35 cm diam, conicoconvex then expanding; surface initially white, soon gray with a glaucous tint, paler towards the margin, glabrous and silky smooth but cracking on drying; margin slightly incurved, scurfy, often cracking. Lamellae emarginate, sinuate, straw yellow, ventricose, densely crowded, with lamellulae of four lengths. Stipe 15-18 × 6 cm,

cylindrical, often elongate, solid finally fistulose; surface concolorous with pileus, fibrillose-striate. Context up to 3 cm thick at disc, white, firm; consisting of thin-walled hyphae, 2-8  $\mu\text{m}$  diam, inflated to 25  $\mu\text{m}$  diam, with clamp connections; odor recalling 'brewer's grains'. Spore deposit white. Spores 5.7-7.5  $\times$  4.0-5.3 (6.70  $\pm$  0.90  $\times$  4.60  $\pm$  0.38)  $\mu\text{m}$ ,  $Q = 1.46$ , ovoid to short ellipsoid, hyaline, inamyloid, thin-walled. Basidia 25-37  $\times$  5-8  $\mu\text{m}$ , narrowly clavate to subcylindrical, bearing four sterigmata; with basal clamp connection. Lamellar edge fertile; cystidia absent. Hymenophoral trama regular, of parallel, thin-walled hyphae, 2-5  $\mu\text{m}$  diam, with clamp connections. Subhymenial layer narrow, 5-9  $\mu\text{m}$  wide, interwoven. Pileipellis a compact, repent cutis of interwoven hyphae, 2-8  $\mu\text{m}$  diam.

HOLOTYPE. INDIA, WEST BENGAL: Calcutta, Shamnagar, Oct. 1911, *Burkill 44*, (K(M)36628).

*Additional specimens examined.* INDIA, WEST BENGAL: Calcutta, Shamnagar, 25 Oct. 1977, *Samajpati 22* (K(M)37720); 29 May 1978, *Samajpati 10*, (K(M)377819); 9 June 1978, *Samajpati 12* (K(M)37715); 27 June 1978, *Samajpati 47* (K(M)37718); 13 July 1978, *Samajpati 57* (K(M)37721); 22 July 1978, *Samajpati 64* (K(M)37724); Burdwar, 20 May 1968, *Mehur 100* (K(M)37714); Shillong, Manipur, Dec. 1979, *Verma 9* (K(M)37716); Tripura, Agartale, 4 June 1989, *Bhattacharya 61* (K(M)13491); NEPAL: Kathmandu, 1972, *Singh 1060* (K(M)37722); Jabalpur, Nov. 1979, *Jamaluddin 8* (K(M)37723). PAKISTAN: Changa Manga, 31 Oct. 1981, *Ahmad 27931* (K(M)7725).

*Observations.* Although Massee (1912) mentioned figures with the material in his original account, these have not been found at Kew or NY. The spores and basidia of this species were illustrated by Manjula (1983: 97, figs. 148-9). Exsiccate material can be separated from that of *M. crassa* in having a darkened pileus and lamellae. The 'Nioo-shimeji' fungus from Japan, *Tricholoma giganteum sensu* Nagasawa and Hongo (1981), may represent an additional species within *Macrocybe* that is close to but distinct from *M. gigantea*. The collections were originally described as lacking hymenial cystidia, but Cléménçon (pers. comm.) observed that material collected in Kyushi, Japan had 'numerous cystidia'. In addition to the cystidia, this fungus differs from *M. gigantea* in the densely caespitose habit and the absence of any glaucous tint on the pileus (Cléménçon, pers. comm.). Although *M. spectabilis* also occurs in Japan, it is always associated with *Saccharum*, and it lacks hymenial cystidia. The smooth stipe of the 'Nioo-shimeji' fungus precludes classifying it as either *M. titans* or *M. pachymeres*. Recognition of a new species awaits detailed examination of the 'Nioo-shimeji' fungus.

**Macrocybe lobayensis** (R. Heim) Pegler & Lodge, comb. nov.

$\equiv$  *Tricholoma lobayense* Heim in Rev. Mycol. 34: 346. 1970; Cah. La Maboké 7: 77 (1969, nom. non rite publ.).

Pileus 6-12(-20) cm diam, fleshy, convex to applanate; surface pure white to ivory white, at times spotted ochre to reddish ochre, glabrous, smooth finally cracking; margin lobate, nonstriate, inrolled. Lamellae adnexo-adnate to sinuate, cream-color with pale pink tints, narrow, up to 3 mm broad, moderately crowded, with lamellulae of five to six lengths; edge entire. Stipe 5-15(-30)  $\times$  1.5-3(-6) cm, cylindrical or obclavate with a swollen base, solid finally fistulose; surface ochraceous, often discolored pale gray, fibrilloso-rugose, glabrous; arising from a buried pseudosclerotium. Context up to 2 cm thick, pure white, unchanging, firm-fibrillose; consisting of very thin-walled hyphae, 2-5  $\mu\text{m}$  diam, inflated to 12  $\mu\text{m}$  diam, with clamp connections; odor of coumarin or 'bitter almonds'; taste farinaceous. Spores 5.5-6.7  $\times$  3.5-4.3 (5.8  $\pm$  0.2  $\times$  3.8  $\pm$  0.2)  $\mu\text{m}$ ,  $Q = 1.53$ , ovoid to broadly ellipsoid, hyaline, thin-walled, containing a single, large, refractive guttule. Basidia 27-32  $\times$  4-6  $\mu\text{m}$ , narrowly clavate, bearing four sterigmata, with a basal clamp connection. Lamellar edge fertile; cystidia none. Hymenophoral trama regular, hyaline, with thin-walled hyphae, 2-8  $\mu\text{m}$  diam. Subhymenial layer 8-10  $\mu\text{m}$  wide, interwoven. Pileipellis a thick cutis of closely interwoven hyphae, 1.5-4  $\mu\text{m}$  diam, thin-walled, much branched, with oleaginous contents, and clamp connections.

*Specimens examined.* GHANA: Cape Coast University campus, on ground in grassland, 10 June 1973, *Rose 7308* (K(M)37017). IVORY COAST: Abidjan, Forêt de la Besso, 31 Mar. 1975, *Aké Assi 342* (K(M)37015); Parc National de Tai, 9 Mar. 1976, *Aké Assi 463* (K(M)37016). Nigeria, Ibadan, Ife Biol. Gard., 1968, *Zoberi 327* (K(M)37018); Cross River State, Oban Forest, 6 June 1991, *Nicholson 850* (K(M)23858).

*Observations.* This species was originally described from the Central African Republic and recent collections received at Kew have confirmed further records from the Ivory Coast, Ghana and Nigeria. Similar to *M. spectabilis*, the stipes originate from an underground pseudosclerotium but the number of basidiomata is generally far fewer, and the host association is not specific.

**Macrocybe pachymeres** (Berk. & Broome) Pegler & Lodge, comb. nov.

$\equiv$  *Agaricus pachymeres* Berk. & Broome in Journ. Linn. Soc., Bot. 11: 515. 1871.

= *Tricholoma pachymeres* (Berk. & Broome) Sacc., Syll. Fung. 5: 120. 1887.

Basidiomata caespitose. Pileus 4-10 cm diam, hemispherical, convex to obtusely umbonate, finally slightly depressed; surface pale ochraceous to grayish brown, paler towards the margin, smooth, finally tessellately cracking when dry; margin smooth, involute. Lamellae arcuate, narrowly sinuate, almost white to straw yellow, 5-8 mm broad, crowded, with lamellulae of two lengths. Stipe 5-11 × 1.5-4 cm, cylindrical to obclavate, with swollen base, solid; surface pale gray, covered with scattered, small, recurved squamules. Context up to 2.5 cm thick, white, unchanging, consisting of thin-walled, inflated hyphae, 4-12 µm diam, with clamp connections. Spores 5.5-6.5 × 4.0-4.7 (5.96 ± 0.33 × 4.30 ± 0.28) µm, Q = 1.39, subglobose to broadly ovoid, hyaline, inamyloid, thin-walled, smooth, often containing a large, refractive guttule. Basidia 20-27 × 4-7 µm, clavate, bearing four sterigmata. Lamella-edge probably fertile; cystidia not observed. Hymenophoral trama regular, broad, hyaline, consisting of parallel, thin-walled hyphae, 3-12 µm diam. Subhymenial layer narrow, 7-12 µm wide, interwoven. Pileipellis a thick cutis of interwoven hyphae, 3-6 µm diam.

HOLOTYPE. SRI LANKA, CENTRAL PROV.: Am-begamowa, on the ground, Nov. 1868, *Thwaites* 797 cum icon. (K(M)36555).

*Additional specimen examined.* INDIA: Madhya Pradesh, Jabalpur, Nov. 1979, *Jamaluddin* 9 (K(M)377).

*Observations.* Berkeley and Broome (1871) commented that this species differs from *M. crassa* 'in the even not sinuato-plicate margin, the scaly stem and other characters'. The macroappearance of the basidiomata, except for the even pileus margin, closely resembles that of *M. titans* in both pigmentation and the squarrose stipe surface. Although no pleurocystidia were found in the type, it is in poor condition (consisting of slices of the basidiomata which are badly contaminated with molds), and thus their presence cannot be confirmed microscopically. A fungus from Malaya, however, that was described and illustrated by Corner (1994, pl. 7) as '*T. crassum*' resembles *T. pachymeres* and was stated to have pleurocystidia. Because the distributions of *M. pachymeres* and *M. titans* are so widely separated geographically, we prefer to leave them as separate taxa until new material of *T. pachymeres* from the type locality in Sri Lanka can be examined.

**Macrocybe praegrans** (Berk.) Pegler & Lodge  
comb. nov.

= *Agaricus praegrans* Berk. in Hooker, Lond. Journ. Bot. 2: 629. 1843.

= *Tricholoma praegrans* (Berk.) Sacc., Syll. Fung. 5: 141. 1887.

Pileus 20-50 cm diam, convex, finally depressed at center; surface cream colored, smooth, with an irregular, wavy margin. Lamellae adnexed to sinuate, concolorous with pileus, at times discoloring brown at edge, moderately broad. Stipe 17-30 × 2.5-4 cm, with a swollen base (6-13 cm), solid; surface cream-colored, smooth, with a basal cottony mycelium. Context 1-2 cm thick, pure white, firm, consisting of thin-walled hyphae, 2.5-6.5 µm diam, inflated to 23 µm diam, with clamp connections; taste slightly bitter. Spore deposit white. Spores 5.5-7.5 × 3.5-5.3 (6.45 ± 0.48 × 4.58 ± 0.32) µm, Q = 1.4, subglobose to broadly ovoid, hyaline, inamyloid, thin-walled, containing numerous guttules. Basidia 27-35 × 6-9 µm, clavate, bearing four sterigmata; with a basal clamp connection. Lamella-edge fertile; cystidia absent. Hymenophoral trama regular, with thin-walled, hyaline, inflated hyphae, 3-11 µm diam. Subhymenial layer 7-12 µm thin, narrow, interwoven. Pileipellis a thick cutis, 150-200 µm thick, of closely interwoven hyphae, 3-7 µm diam, thin-walled, much branched, with oleaginous contents, and clamp connections.

HOLOTYPE. BRAZIL, MINAS GERAES: amongst grass, Oct. 1840, *Gardner*; Herbs. Hooker & Berk. (K(M)36458).

*Additional specimens examined.* BRAZIL, SAO PAULO STATE: Mun. Cotia, 25 Feb. 1968, *Murano* (SP102098. F); Parque del Estado do Sao Paulo, March 1971, *S. Skvortsov* (F 1016811); Parque do Estado, Jan. 1971, *Skvortsov* (K(M)36483); Jardim do Cicade Universitaria, 25 Nov. 1982, *Bicudo* (SP177880).

*Observations.* This is one of the largest and most robust agaric species, growing singly or in tufts of two or more basidiomata, often amongst grass. A study of the type collection of this species, preserved in Herb. Kew, was published by Pegler (1988).

**Macrocybe spectabilis** (Peerally & Sutra) Pegler,  
comb. nov.

= *Tricholoma spectabilis* Peerally & Sutra in Rev. Agric. Suer. Ile Maurice 53: 117. 1973.

Pileus 7-40(-60) cm diam, convex, broadly umbonate to appanate then expanding, finally depressed; surface pure white to 'Buff', glabrous, smooth, silky, dry, not viscid; margin undulate, entire, involute. Lamellae sinuate, white, 5-20 mm broad, crowded, with lamellulae of two lengths. Stipe 5-30 (-40) × 4-7 cm (at base), cylindrical to obclavate, solid becoming finally fistulose; surface pure white,

smooth, with longitudinal fibrils; arising from a white, cottony mycelium. Context white, 1-2.5 cm thick, abruptly thinner at the margin, firm; odor strong, cyanic. Spore deposit pure white. Spores 6.2-7.2 × 4.5-5.5 (6.88 ± 0.20 × 4.82 ± 0.46) μm; Q = 1.43, subglobose to broadly ovoid, hyaline, inamyloid, thin-walled, smooth, containing a single, large refractive guttule. Basidia 24-26 × 6-8 μm, clavate, bearing four sterigmata. Cystidia none. Pileipellis of narrow, interwoven hyphae with clamp connections.

*Specimen examined.* MAURITIUS: Riche-en-eau, under *Saccharum*, 1972, *Peerally* ISOTYPE (K(M) 36557). JAPAN, YOMITAN-SON: Nago-city, Okinawa-pref., on soil under *Saccharum*, June 1979, ex-Herb. *Tottori* (K(M) 36645; K(M) 36646).

*Observations.* Although the type is deposited at Herb. Kew, it is composed of a set of photographs, together with a heavy spore deposit. The full details of the microstructure are therefore not available for study. *Macrocybe spectabilis* appears to be always associated with sugar-cane, growing at the base of the stems in enormous tufts, frequently with 50 or more basidiomata, and often with confluent stipes. It is closely allied to *M. lobayensis*, from West Africa, but regarded as distinct by virtue of the much larger overall dimensions and massive habit. A more complete, illustrated description can be found in *Peerally and Sutra* (1972: 142-145, Figs. 4-10).

**Macrocybe titans** (H.E. Bigelow & Kimbr.) Pegler, Lodge & Nakasone comb. nov.

= *Tricholoma titans* H.E. Bigelow & Kimbr. in *Mycotaxon* 11: 426. 1980.

= *T. cystidiosum* Cifuentes & Guzmán in *Bol. Soc. Mex. Micol.* 16: 38. 1981, as *T. 'cystidiosa'*, non *T. cystidiosum* A. H. Sm. 1941.

= *T. cifuentesii* Courtec. in *Docum. Mycol.* 16 (61): 49. 1985.

Basidiomata caespitose. Pileus 8-50(-100) cm diam, strongly convex to applanate, finally expanded to depressed with undulating margin; surface pale ochraceous, 'Buff', 'Warm Buff', 'Buff-Yellow' to 'Cinnamon-Buff' at center, paling to pale gray at margin, finally white at maturity, smooth, cracking when dry; margin inrolled until late maturity. Lamellae strongly sinuate, white, slightly gray to very pale brown, up to 2 cm broad, densely crowded, with lamellulae of four lengths; edge entire. Stipe 6-15 (-38) × 1.5-4(-12.7) cm, cylindrical to obclavate, up to 12 cm diam at base; solid; surface off-white to pale gray, dry, soon disrupting into numerous, small, reflexed squamules, often caespitose. Context up to 4 cm thick at disk, white, firm-fleshy, consisting of very

thin-walled hyphae, 4-6 μm diam, inflated to 17 μm diam, with small, inconspicuous clamp connections; odor strong, pleasant to disagreeable, fragrant, mushroomlike. Spore deposit cream color. Spores 5.5-7.0 × 4.0-5.0 (6.12 ± 0.90 × 4.32 ± 0.29) μm, Q = 1.42, subglobose to ovoid, hyaline, inamyloid, cyanophilous, thin-walled, usually containing a single, large, refractive guttule. Basidia 25-38 × 6.5-10 μm, narrowly clavate, bearing four sterigmata; with a basal clamp connection. Lamellar edge fertile. Pseudocystidia scattered, 35-50 × 7-10 μm, fusoid with narrow, tapering apex, lanceolate or filiform, thin-walled, originating below the basidia and attached to conducting elements, gloeocystidioid, containing amorphous, refractive contents; may or may not project far beyond the basidia. Hymenophoral trama regular, hyaline, consisting of thin-walled hyphae, 3-5 μm diam, inflated to 15 μm diam, with small, inconspicuous clamp connections. Subhymenial layer narrow, 5-9 μm wide, interwoven. Pileipellis a repent cutis, 150-180 μm thick, not gelatinized, consisting of firmly interwoven, narrow hyphae, 2-5 μm diam, thin-walled, often filled with refractive contents, and with conspicuous clamp connections.

*HOLOTYPE.* *Tricholoma titans* H. E. Bigelow & Kimbr. 1980. USA: FLORIDA: Lake Co., on sandy soil beneath oaks, 14 Aug. 1978, *J. A. Hadox* (FLAS F51990).

*Specimens examined.* COSTA RICA, GUANACASTE PROV.: Estación Santa Rosa, Guanacaste Nat. Park, 16 June 1995, on grassy ground between trees, *G. Mueller*, annot. by D.J. Lodge & T. O'Dell, *Lodge CR-483* (F); Parque Nacional Santa Rosa, 15 December 1981; Santa Rosa Nat. Park, Sector ACG, growing from active leaf cutter ant nest in moist tropical forest, 23 Sept. 1996, as *T. praegrande*, *Cano-595* (F 1117212); same loc., 23 Nov. 1996, *G.M. Mueller GMM-5932* (F 1117436). ECUADOR?: 21 July 1975, as *T. praegrande*, *K.P. Dumont, S.E. Carpenter & P. Buritica EC-1029* (F 1022388). FRENCH WEST INDIES, MARTINIQUE: Morne Accur, Oct. 1974, as *T. pachymeres*, *Fiard 7* (K(M)36482); 4 Nov. 1975 *Fiard 7c* (K(M)36481). TRINIDAD: St. Augustine, at foot of *Melicocca bijuga*, 18 July 1947, as *T. pachymeres*, *Baker 1481* (K(M)36485). USA, FLORIDA: Sarasota Co., in grass close to residential wall, 30 July 1986, *R.S. Williams 301* (K(M)54379) (authentic collection, det. H.E. Bigelow). USA, PUERTO RICO: Luquillo Mountains, Mun. Rio Grande, Urbanizacion Colinas de l Yunque, nr Rte 191, on ground around dead *Lagerstromea*, 17 Nov. 1992, as *T. pachymeres*, *D.J. Lodge PR-906* (CFMR); same loc., 13 May 1993, *D.J. Lodge & L. Fish PR-1130* (CFMR); same loc., 22 June 1993, *M. Boyd PR-1212.1* (CFMR); same loc., 15 July 1993, *D.J. Lodge PR-1212.2* (CFMR); same loc., 15 Feb. 1994, *D.J. Lodge PR-1637* (CFMR & K(M)36486); Mun. Naguabo, Rt. 191 near Rio Cubuy, Lat. 65°47'50", Long. 18°15'26", solitary fruit body deeply rooted in ground inside a garage above large root of a *Spondis dulcis* Parkinson tree

(Jobo, Anacardiaceae), 16 Sept., *C. Laboy PR-4688* (CMFR & K(M)). VENEZUELA, MIRANDA; Parque Nacional Guatopo, 22 Jan. 1958, *Dennis & Aristegineta 1095* (K(M)36484).

**Observations.** This species was originally described as *T. titans* from Florida, U.S.A. (Bigelow and Kimbrough, 1980), and was described again as *T. cystidiosum* from Mexico (Cifuentes and Guzmán, 1981). Later, Courtecuisse (1985) renamed *T. cystidiosum* as *T. cifuentisii* because the former was a homonym, and Singer (1990) synonymized it with *T. titans*. *Macrocybe titans* is mainly distinguished macroscopically by the distinctly squamose stipe surface, and microscopically by the numerous, refractive pseudocystidia. This is the fungus previously reported under the name of *T. pachymeres* (Berk. & Broome) Sacc., by Baker and Dale (1951: 94, pl. 1g) and Pegler (1983: 111, pl. 3D). The habit of the Costa Rican collections of growing from active *Atta* ant gardens is distinctive. Except for the presence of cheilocystidia on some of the Costa Rican collections, they were indistinguishable from other collections of *M. titans*.

**Tricholoma.**—The two collections of *Tricholoma* that were selected and sequenced to represent Section *Tricholoma*, the type section of the genus, do not exactly match the type descriptions for *T. flavovirens* (the type species) and *T. intermedium*. Although these collections are good representatives of Section *Tricholoma*, they may represent undescribed varieties or sibling species. While the description of new *Tricholoma* taxa is beyond the scope of this paper, the descriptions of these two collections are provided below to prevent future confusion regarding their rDNA sequences, which are available from GenBank.

*Tricholoma cf. flavovirens.* Pileus 25-35 mm diam, broadly convex with rolled margin, 'Tawny Olive' with a slight 'Robin Rufus' tint in the center of the young one, somewhat viscid; lamellae 'Buff-Yellow', adnexed, seceding; stipe 23-43 × 8-11 mm, 'Buff-Yellow' at apex, white with a 'Tawny Olive' tint below, cylindrical to slightly expanded at apex, fibrillose. Spores hyaline, (5.6-)6.4-8 × 3.2-4.3 μm, guttulate; basidia 2- and 4-spored, 22-25 × 3-5 μm, clavate; some hyphae of the lamellar context with contents and encrusting material stained strongly pink in KOH, with some of these attached to similarly staining pseudocystidia that penetrate the subhymenium and hymenium, only rarely reaching the level of the tips of the basidia; lamellar edge mostly sterile, with a mixture of hyaline, clavate to contorted cheilocystidia of the type found in *T. intermedium*, and pseudocystidia stained pink in KOH; pileipellis with a thin ixocutis with slender interwoven hyphae; larger diam hyphae below ixocutis, including a high concentra-

tion of hyphae near the ixocutis with contents and coarse crystalline to banded encrustations that stained pink in KOH.

**Observations.** Both *T. flavovirens* and *T. intermedium* have hyphae with contents and encrustations that turn pinkish brown with time in the herbarium (K.S. Shanks, pers. comm.) or in KOH (C. Ovrebo, pers. comm.) and these are sometimes found penetrating the hymenium (C. Ovrebo, pers. comm.). However, typical *T. flavovirens* does not have cheilocystidia. Although many collections of *T. intermedium* have cheilocystidia of the type observed in this collection, that species has white rather than yellow lamellae. *Tricholoma aestuans* (Fr. : Fr.) Gillet is a similar species bearing cheilocystidia, but it lacks an ixocutis and has much greener pigmentation than *T. flavovirens* and the collection that was sequenced.

*Tricholoma cf. intermedium.* Pileus 47-70 mm diam, broadly convex, greenish yellow with some brown tones, glabrous, apparently viscid earlier as indicated by soil adhering to entire surface; lamellae pure white, deeply adnexed to almost free, close; stipe up to 100-110 × 11-23 mm, white, tapering downward, dry, fibrillose with occasional small tufts. Flesh solid; odor mild, pleasant; flavor mild. Spores hyaline, (7.2-)8.3-8.8 × 4.8-5.7 μm, guttulate; basidia mostly 2-spored, some 4-spored; lamellar edge mostly sterile, with a mixture of hyaline, clavate cheilocystidia with or without a fingerlike apical appendage, these also found on the sides of the lamellae with the appendages extending beyond the tips of the basidia; arrangement of lamellar context hyphae regular, these 3.2-10.4 μm diam, lacking clamp connections; pileipellis with a thin ixocutis; hyphae of hypodermium with slight yellow-brown encrustations in KOH.

**Observations.** The spores of this collection are larger than those of the type of *T. intermedium* (i.e., 5.3-7.2 × 3.3-4.3 μm, K. M. Shanks, MA thesis, Dept. Biology, San Francisco State Univ. 1994). The macroscopic and microscopic characters of KMS 593 match a large-spored variant of *T. intermedium* that is the predominant type in Sierra County, California (K. M. Shanks, pers. comm and op. cit.).

**Molecular analysis.**—The LR0R-LR7 region of the nuclear ribosomal DNA region is about 1400 bp long in all the taxa whose selected genomes were amplified and sequenced. Although nearly complete sequences for *M. titans* and *T. cf. flavovirens* were obtained, only about 830-850 bp of sequence downstream from the LR0R primer were obtained for *T. vaccinium*, *Clitocybe clavipes* and *Calocybe gambosa*. The sequences were easily aligned manually and had a length of 1419 bp that included gaps of 1-15 bp.



TABLE I. Mean percent distances between taxa as calculated from ribosomal DNA sequences using PAUP

Taxa	1	2	3	4	5	6	7	8	9
1 <i>Macrocybe titans</i>	—	9.9	8.4	8.4	5.8	5.5	5.8	9.0	9.4
2 <i>Clitocybe clavipes</i>		—	8.2	6.6	6.9	6.4	7.1	8.9	9.6
3 <i>Calocybe gambosa</i>			—	7.2	6.8	6.4	6.9	10.0	11.4
4 <i>Tricholoma vaccinum</i>				—	0.6	2.8	3.2	9.1	9.6
5 <i>T. imbricatum</i>					—	2.2	2.2	7.3	8.4
6 <i>T. cf. flavovirens</i>						—	0.6	7.8	8.9
7 <i>T. cf. intermedium</i>							—	8.0	8.9
8 <i>Pleurotus pulmonarius</i>								—	3.2
9 <i>P. cornucopiae</i>									—

Out of the 1419 nucleotide positions, there were 97 with potentially informative sites. Insertion/deletions events were not considered in the subsequent analyses. *Pleurotus* was chosen as the outgroup genus for the molecular analysis because it is taxonomically distinct from the ingroup taxa but had sequences that were easily aligned with the ingroup taxa (the multiple sequence adjustment is available from KKN).

TABLE I shows the mean distances among the nine taxa as calculated from PAUP. The mean sequence divergence was lowest among the mycorrhizal *Tricholoma* species, ranging from 0.6% for species pairs within a section, to 2.2-3.2% between sections *Tricholoma* and *Genuina*. *Macrocybe titans*, *Ca. gambosa*

and *Cl. clavipes* displayed high sequence divergence values among themselves (8.2-9.9%) but somewhat lower values when compared to the mycorrhizal *Tricholoma* species (6.4-8.4%).

In the parsimony analysis, gaps were treated as missing data, and two baseline trees of 370 steps were recovered. One of the two most parsimonious trees is shown in FIG. 1. The consistency index, excluding uninformative characters is 0.586 with a retention index of 0.699. In this tree, the four ectomycorrhizal *Tricholoma* species (sections *Tricholoma* and *Genuina*) form a clade that is strongly supported by decay and bootstrap values. A second clade paired *M. titans* and *Ca. gambosa* together; however, this branch is

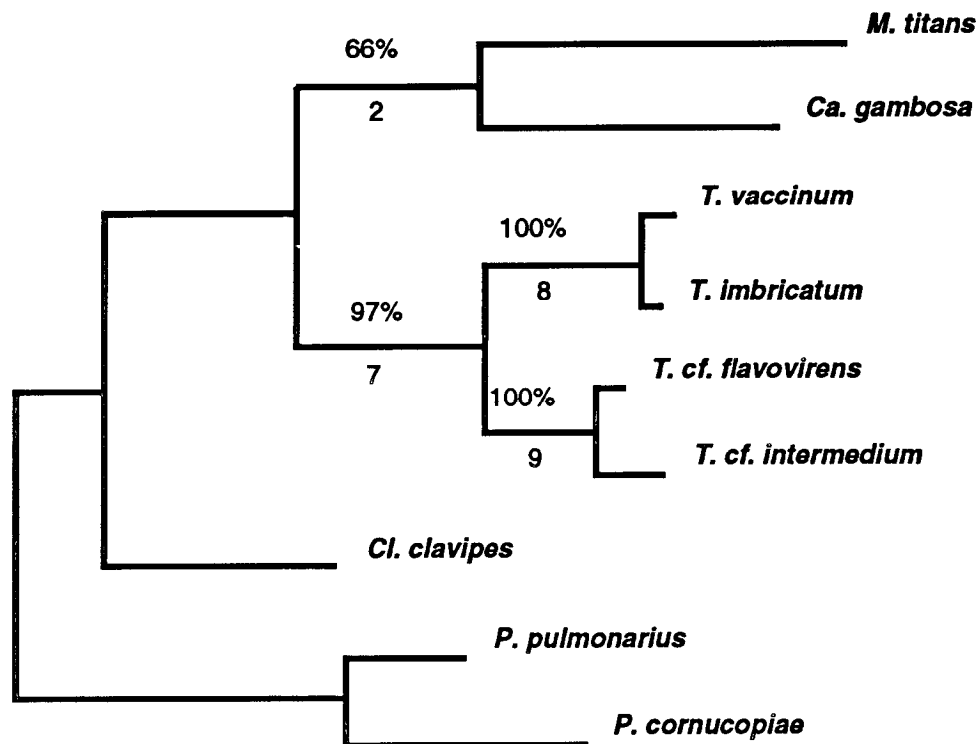


FIG. 1. Phylogram of one of the two most parsimonious trees of the LROR-LR17 of the nuclear large ribosomal RNA gene. This tree is identical to those obtained by various weighting schemes. Bootstrap confidence levels (%) are shown above and decay indices below the branches.

weakly supported by decay and bootstrap values. The position of *Cl. clavipes* is equivocal and may be positioned either at the base of the ingroup taxa (as shown in FIG. 1) or paired with the *Tricholoma* clade. Weighting of transversions more than transitions (1.1:1, 2:1, 3:1) resulted in one tree, which is identical to the tree in FIG. 1. Large areas of missing data were omitted to construct a partial data set consisting of 750 bp. When this partial data set was analyzed, there was no change in the tree topologies and only minor numerical changes in the mean sequence divergence values, consistency index, retention index, and decay and bootstrap values from those reported above.

## DISCUSSION

*Macrocybe* species have a pantropical distribution, and produce exceptionally large, fleshy basidiomata that often grow in large caespitose clusters which may exceed 30 kg fresh weight. The large size and flavorful fruiting bodies of some *Macrocybe* species make them a valuable source of food, though it should be noted that they often contain toxic cyanide compounds that must be driven off by cooking. Species of *Macrocybe* were formerly placed within *Tricholoma* (Tribe *Tricholomatae*) but segregated in Section *Leucorigida* morphologically on the basis of the presence of clamp connections, and ecologically on the absence of ectomycorrhizal associations. Although detailed collection information are unavailable for *M. gigantea* and *M. pachymeres*, *Macrocybe*, species for which this information is known are clearly not mycorrhizal. *Macrocybe praegrans* and *M. lobayensis* are reported in grasslands while *M. spectabilis* is associated with sugar cane, all communities with arbuscular rather than ectomycorrhizal associations. While the native collections of *M. crassa* do not provide enough detail to determine nutritional mode, a collection from Thailand was cultivated on horse manure and sawdust which indicates that it is also saprotrophic. The *M. titans* collections that have been annotated thoroughly in regard to habitat have been reported from grass in Florida, active leaf cutter ant gardens in Costa Rica, and associated with live or dead trees that are not ectomycorrhizal (e.g., *Lagerstromea* and a relative of mango in the Anacardiaceae) in Puerto Rico. A comparison of molecular sequences would be useful for determining if the Costa Rican specimens of *M. titans* growing from leaf-cutting ant gardens are different from the others.

For molecular comparison, we selected four mycorrhizal *Tricholoma* species to represent two sections in the subgenus *Tricholoma*: *T. cf. flavovirens* and *T. cf. intermedium* in section *Tricholoma* Quél., and *T.*

*vaccinum* and *T. imbricatum* in section *Genuina* (Fr.) Sacc. *Tricholoma flavovirens* is the type species of the genus, subgenus and section *Tricholoma*, while *T. vaccinum* is the type species of section *Genuina*. Our molecular analysis showed that these four mycorrhizal representatives of *Tricholoma sensu stricto* formed a strong clade that never included *M. titans*.

*Calocybe gambosa* in the Tribe *Lyophylleae* was selected for comparison because it shares the saprophytic nutritional mode, macroscopic features and abundant clamp connections with the group of tropical species of interest to us. Pegler (1983) previously suggested that *Ca. gambosa* might have an affinity with *M. titans* (as *T. pachymeres*). Although *M. titans* and *Ca. gambosa* are macroscopically similar, our parsimony analysis of the DNA sequences from the 5'-end of the nuclear large ribosomal RNA gene for these two species reveals weak support (66% confidence value) for this association. Although we cannot reject the hypothesis that both *Ca. gambosa* and *M. titans* belong in the same genus or that both belong in the genus *Tricholoma* because our molecular analysis includes so few taxa, this arrangement can be rejected based on other data.

*Calocybe* belongs in the Tribe *Lyophylleae*, characterized by siderophilous granulation in the basidia whereas *Tricholoma* and *Macrocybe* lack siderophilous granulation and are therefore placed in the Tribe *Tricholomatae*. Therefore, *Macrocybe* species cannot be placed in either *Calocybe* or another genus in the Tribe *Lyophylleae* without disregarding the character of siderophilous granulation and combining the Tribes *Lyophylleae* and *Tricholomatae*, a result that few systematists would agree with. Combining *Calocybe* and *Macrocybe* in the genus *Tricholoma* would present the same difficulties. Furthermore, a previous analysis of ribosomal RNA sequences by Moncalvo et al. (1993) indicated that *T. giganteum* ss E. Nagasawa and T. Hongo (1981), a good member of the genus *Macrocybe*, was quite distant from true *Lyophyllum* species, which is concordant with our hypothesis that *Macrocybe* does not belong in the *Lyophylleae*. Additional unpublished molecular analyses by M. Moncalvo and R. Vilgalys (pers. comm.) reaffirm the distant relationship of *Macrocybe* with members of Tribe *Lyophylleae* as well as species of *Tricholoma sensu stricto*. The limited overlap in sequences between our study and Moncalvo et al. (1993) unfortunately precluded us from incorporating their sequences in our analysis. Although *Macrocybe* species were formerly segregated in a Section of *Tricholoma*, raising this Section to the generic level was precluded because the type species of Section *Leucorigida*, belongs to *Calocybe*.

We selected *Cl. clavipes* for molecular comparison to represent a possibly basal, saprophytic genus in the Tribe *Tricholomatae*, and found it was paired 3.8% of

the time with *M. titans* and *Ca. gambosa* in the bootstrap analysis. Branching points near the base of the phylogenetic tree could not be resolved with the sequences we analyzed. Nevertheless, molecular data of ours and others taken together with morphological and ecological evidence supports the erection of the new genus in the Tricholomataceae, *Macrocybe*, to accommodate large, saprotrophic, tricholomatoid, tropical species that have abundant clamp connections, but lack siderophilous granulation in their basidia.

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Dr. Thomas Laessøe provided the original impetus for removing the tropical, nonmycorrhizal species from the genus *Tricholoma*. We are very grateful to Kris Shanks for sharing DNA sequences she obtained for *Tricholoma* cf. *intermedium* and *T. imbricatum*, and providing advice and copies of parts of her Masters thesis and a manuscript that was in press. Dr. G. M. Mueller provided new material and habitat information for *M. cf. titans*. We also thank Dr. Clark Ovrebo for examining fresh material of *M. titans* for siderophilous granulation in the basidia, and providing information on staining reactions and cystidia of *Tricholoma*. Dr. Rytas Vilgalys was very helpful in sharing methodology, sequences and advice. Rita Rentmeester at the USDA-Forest Service, Forest Products Laboratory helped to generate, align and analyze the sequences. We thank Prof. H. Clemençon and Dr. R. Halling for their invaluable insights on *T. giganteum* ss E. Nagasawa & T. Hongo and *T. titans*, respectively. Collecting in Puerto Rico was supported by the Forest Products Laboratory and the International Institute of Tropical Forestry, USDA-Forest Service, and by the US National Science Foundation, Biotic Surveys and Inventories program, grant DEB-95-25902 to the Research Foundation of the State University of New York at Cortland.

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