

A new species of *Lepiota* from China

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Abstract: In this report we describe the three species in *Lepiota* sect. *Lepiota* occurring in tropical China. *Lepiota attenuata* is a new species and is characterized by a pileus with brownish yellow squamules and radially sulcate striate margin, penguin-shaped spores that are distinctively narrowed toward the apex and inflated submoniliform or catenulate elements in the pileus covering. We compared the type specimens of *L. metulispota* and *L. thrombophora* with tropical Chinese specimens; both taxa occur in the study area. Phylogenetic relationships among the tropical Chinese species and other closely related species in the genus were inferred based on DNA sequences of the nuclear ribosomal genes (ITS, LSU and IGS) and the mitochondrial small ribosomal RNA gene (mtSSU).

Key words: Agaricales, Agaricaceae, lepiotaceae fungi, taxonomy, tropics

INTRODUCTION

Genus *Lepiota* (Pers.:Fr.) S.F. Gray sensu stricto contains more than 400 described species (Kirk et al. 2008), with nearly 70 species reported from China (Teng 1936; Chiu 1948; Li et al. 1993; Bi et al. 1994; Yuan and Sun 1995; Mao 1998; Wang 2004; Wang and Yang 2005a, b; Yang et al. 2005; Liang 2007; Liang and Yang 2007; Liang et al. 2010). The genus has considerable variation in the shape of basidiospores and the structure of the pileus covering. While spore shapes range from broadly ellipsoid to spurred, fusiform or penguin-shaped, the types of pileus

covering range from a hymeniderm, a cutis, a trichoderm to a special type of epithelium in which chains of inflated elements agglutinate to form acute spines (Vellinga 2001a). Based on morphological and molecular data, several studies have demonstrated that *Lepiota* is not monophyletic (Johnson and Vilgalys 1998, Johnson 1999, Vellinga 2003). It forms a monophyletic group with genera *Cystolepiota*, *Melanophyllum* and *Pulverolepiota* (Vellinga 2004). Genus *Pulverolepiota* was regarded as a section of *Cystolepiota* by Vellinga (2001a). Common characters of the above are globose to oblong or elongate, inflated elements in the pileus covering. Genus *Melanophyllum* differs from *Cystolepiota* in the coloration of spores (Vellinga 2001a). *Lepiota* sect. *Echinatae* also have globose to oblong or elongate, inflated elements in the pileus covering. The section has even been taken as a section of *Cystolepiota* by Knudsen (1978) and subsequently removed from genus *Lepiota* (Knudsen 1980) and treated as a separate genus, *Echinoderma* (Locq. ex M. Bon) M. Bon by Bon (1991). Vellinga (2003) however regarded as *Lepiota* sect. *Echinatae*. The section differed from *Cystolepiota* in the structure of the veil and binucleate spores (Knudsen 1980, Vellinga 2001a).

Most taxonomists split genus *Lepiota* into several sections based on morphological differences (Kühner 1936, Pegler 1986, Singer 1986, Candusso and Lanzoni 1990, Bon 1996, Vellinga 2001a). At present nearly all taxonomists have accepted the concept of sect. *Lepiota* Singer (Singer 1986), which is characterized by fusiform or penguin-shaped basidiospores and a pileus covering of trichodermium that is composed of long cylindrical or narrowly clavate elements, often intermixed with short elements.

Vellinga (2003) demonstrated that all species from sect. *Lepiota* and a part of sect. *Ovisporae* formed a clade that had relatively high bootstrap support. Her phylogeny showed that the morphological features were not completely consistent with molecular phylogenetic evidence.

The species diversity of *Lepiota* sect. *Lepiota* has been investigated in temperate regions of Europe (Candusso and Lanzoni 1990, Bon 1996, Vellinga 2001a), however little is known about the section in tropical areas. Pegler (1972, 1986) has studied two species in this section, namely *L. metulispota* (Berk. & Broome) Sacc. and *L. thrombophora* (Berk. & Broome) Sacc. from Sri Lanka. One specimen, HMAS 32519, collected from northeastern China, was

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reported as *L. metulispora* by Teng (1963). However a re-examination of the collection revealed that it is *L. clypeolaria* (Bull.: Fr.) P. Kumm.

Morphological observations of some specimens from tropical China show traits that are similar to *L. metulispora* and *L. thrombophora*. Based on comparative studies of the holotypes of the two species, we determined there were three species in tropical China including an undescribed species. To confirm the phylogenetic uniqueness of these species sequences of their internal transcribed spacer (ITS), the nuclear large subunit ribosomal RNA gene (LSU), intergenic spacer (IGS) regions of the nuclear rRNA genes and part of the mitochondrial small ribosomal RNA subunit (mtSSU) were generated and analyzed jointly with sequences from recent molecular phylogenetic studies of lepiotaceous fungi.

MATERIALS AND METHODS

Specimens and morphological descriptions.—Collections were obtained and macromorphological features were noted in the field. All material, unless otherwise noted, is deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). We received some collections tentatively identified as *L. metulispora* and other *Lepiota* species from Herbarium Mycologicum Academiae Sinicae (HMAS). The generic names of *Lepiota*, *Leucoagaricus* and *Macrolepiota* are abbreviated respectively as *L.*, *La.* and *M.* Terminology for descriptive terms follows Vellinga (2001a). Color designations are from Kornerup and Wanscher (1981). Herbarium abbreviations follow Thiers (2010 continuously updated).

For microscopic observations sections of specimens were cut by hand and mounted in 5% KOH, Congo red, and Melzer's reagent. Basidiospores were mounted in cresyl blue to test for a metachromatic reaction (Singer 1986). Sizes were determined for basidia, basidiospores, cheilocystidia and elements of the pileus covering based on ocular micrometer measurements for at least 20 elements of each character. The abbreviation [n/m/p] indicates that measurements were made on n basidiospores in m samples from p collections. Dimensions of basidiospores are given with notation in the form (a)b–c(d). The range b–c contains a minimum of 90% of the measured values. Extreme values are given in parentheses. We used these abbreviations: Q refers to the length/breadth ratio of basidiospores; **Q** refers to the average Q of all basidiospores \pm sample standard deviation.

Molecular identification.—We extracted genomic DNA from herbarium or silica-dried materials with a CTAB protocol (Xu et al. 1994). For each sample we analyzed four loci, ITS, LSU, IGS and mtSSU. The amplification primers were ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for the ITS region, LR0R and LR7 for the LSU region (Vilgalys and Hester 1990), LR12R and 5SRNA for the IGS region (White et al. 1990) and MS3 and MS4 for mtSSU (Liang et al. 2009). We purified PCR with the Bioteke DNA Purification Kit (Bioteke Corp., Beijing). Sequencing of

both strands was performed with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology & Services Co. Ltd, Shanghai). We submitted these sequences to GenBank (SUPPLEMENTARY TABLE I). Aside from the 56 sequences produced in this study, for broad comparisons we also included 90 sequences from isolates of genera *Lepiota* and *Leucoagaricus* (as outgroup) obtained in Vellinga (2001b, c, 2003), Liang (2007), Liange et al. (2009, 2010).

Sequence alignment and phylogenetic analyses.—DNA sequences were edited and aligned with SeqMan (DNASTar package) and Clustal X (Thompson et al. 1997) and manually checked and modified. Ambiguously aligned positions were excluded from the matrix. Gaps were treated as missing data. All unambiguous characters and character transformations were weighted equally. We used *Leucoagaricus naucinus*, *La. nymphaeum* and *Macrolepiota mastoidea* as outgroup. The dataset was analyzed with Bayesian inference using the parallel version of MrBayes 3.1.2 with default priors (Ronquist and Huelsenbeck 2003, Altekar et al. 2004) and maximum likelihood with the RAXML BlackBox online server (Stamatakis et al. 2008).

Bayesian analysis was implemented on a Linux cluster with Intel Pentium 4 processors under a general-time-reversible (GTR) model (nst = 6) and rate heterogeneity parameters (gamma-distributed substitution rate and a proportion of invariable sites) following the AIC criterion in Modeltest 3.06 (Posada and Crandall 1998). Bayesian analyses were run with six chains 10 000 000 generations and sampled every 500 generations. A burn-in period of 1000 trees was discarded. The remaining trees were pooled together and used to generate a 50% majority rule consensus tree with averaged branch lengths. Nodes that were recovered more than 95% of the time were considered to have a significant posterior probability (PP).

Maximum likelihood tree generation and bootstrap analyses were performed with RAXML 2.2.3 (Stamatakis et al. 2008). One hundred bootstrap analyses were done to affirm consistency of results. A GTR model of substitution and GAMMA + P-Invar rate heterogeneity parameters were employed. A bootstrap proportion greater than 70% was considered significant. The results were exported to Dendroscope for tree viewing (Huson et al. 2007).

TAXONOMY

Lepiota attenuata J. F. Liang & Zhu L. Yang, sp. nov.

FIG. 1

MycoBank MB516037

Pileus sordide albus, squamulis flavo-brunneis, striatus. Stipes subcylindricus, sursum attenuatus, cremeus, squamulis flavo-aurantiis. Basidiosporae 14.5–17.0 \times 4.0–5.0 μ m, ventricosus-fusiformes, apice contractae, hyalinae, dextrinoidae. Cheilocystidia clavata. Pleurocystidia nulla. Squamulae pilei ex trichodermiis subcylindricis terminalibus compositae. Fibulae praesentes.

Holotypus. “J.F. Liang 394 (HKAS 50110), 11 July 2006, Lushilin Park, Mengla County, Yunnan Province, China.”

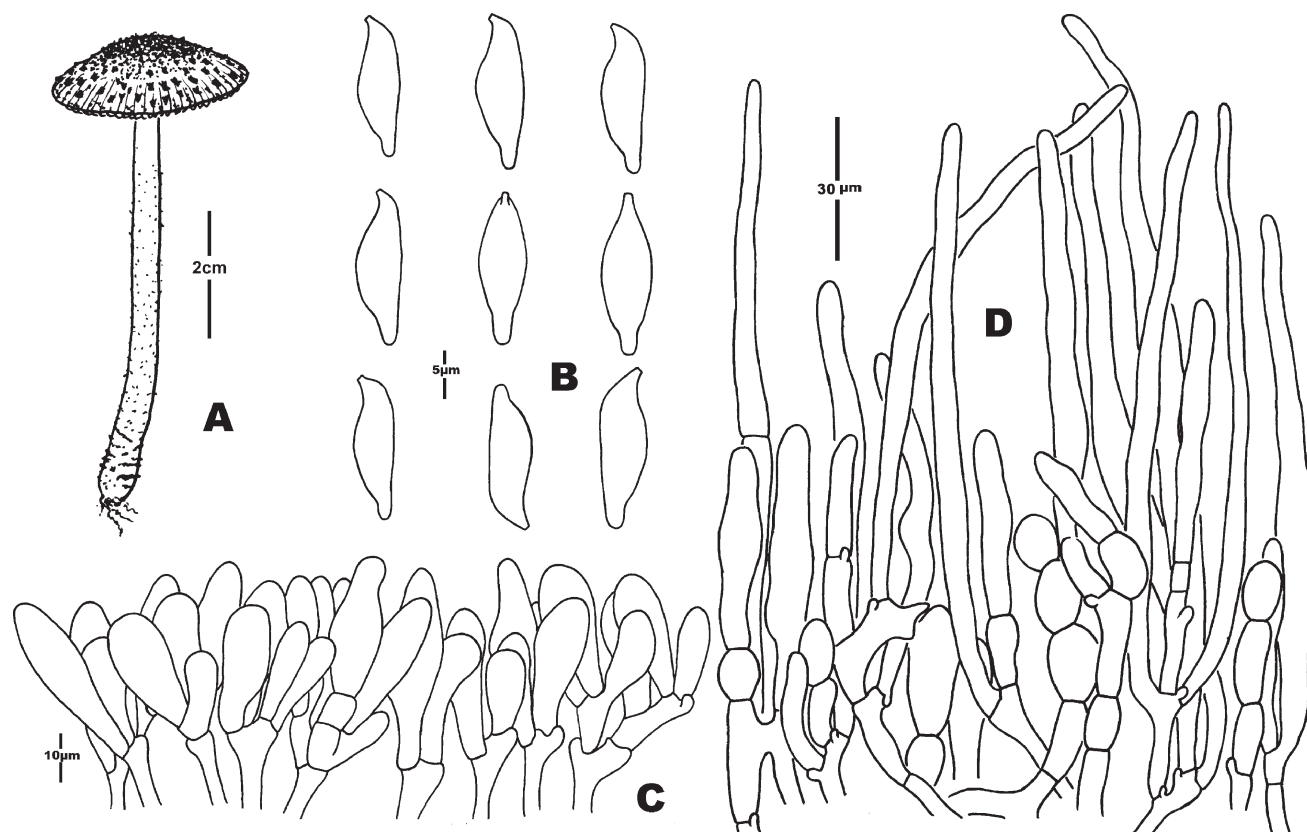


FIG. 1. *Lepiota attenuate*. A. Basidioma. B. Basidiospores. C. Cheilocystidia. D. Pileus covering. All from Holotype.

Etymology. Named because of its spores that narrow toward the apex.

Basidiomata (FIG. 1A). Pileus 3–6 cm diam, plano-convex to applanate, surface covered with small, brownish yellow to yellowish brown (5C7–5D8) squamules on whitish background, yellowish brown (5D8) at the disk; margin with radially sulcate striations. Context whitish, thin. Lamellae free, whitish, moderately crowded, lamellulae present. Stipe 7.5–8.5 × 0.3–0.4 cm, subcylindrical, attenuate, hollow, whitish at the apical part, more or less glabrous, covered whitish to grayish orange (5B3–5B5) squamules at lower part; mycelium cords at base white. Annulus fugacious. Odor none. Flavor not recorded.

Basidiospores (FIG. 1B) [43/2/2] (14.0)14.5–19.0 × 4.0–5.5 µm [$Q = (3.10) 3.22–3.88 (4.00)$, $Q = 3.53 \pm 0.22$], penguin-shaped with straight abaxial side and a suprahilar depression in side view, distinctly narrowed at the apex, without germ pore, fusiform in frontal view, hyaline, smooth, slightly thick-walled, dextrinoid, congophilic, not metachromatic in cresyl blue. Basidia 20–27 × 9–11 µm, clavate, four-spored. Lamella edge sterile. Cheilocystidia (FIG. 1C) 21–43 × 7–13 µm, clavate or fusiform, sometimes narrowed at middle apical part; walls smooth, thin to slightly

thick, hyaline in KOH, congophilic. Pleurocystidia absent. Pileus covering (FIG. 1D) a trichodermial palisade with elongate subcylindrical or attenuate, terminal elements 80–231 × 8–13 µm, with short clavate elements between and sometimes with inflated submoniliform or catenulate elements; pigment brownish yellow, parietal and intracellular in upper part, sometimes in lower part of elements also encrusting with a fine brown pigment. Clamp connections common.

Material examined. China: Yunnan Province, Mengla County, Menglun, Lüshilin Park, 650 m, 11 Jul 2006, *J. F. Liang* 394 (HKAS 50110, Holotype); Taiwan Province: Nantou County, Jiujiufeng, 400–700 m 10 Sep 2002, *J.M. Chen* 3586 (HKAS 42320).

Habitat. Solitary, saprotrophic and terrestrial on calcareous soil, in tropical montane rain forests.

Distribution. Known only from southern China.

Notes. *Lepiota attenuata* is characterized by its pileus with brownish yellow squamules and radially sulcate striate margin, penguin-shaped spores distinctively narrowed toward the apex, and inflated submoniliform or catenulate elements of pileus covering.

Lepiota attenuata is close to *L. metulispora*, but the latter lacks inflated submoniliform or catenulate elements in the pileus covering and the basidiospores

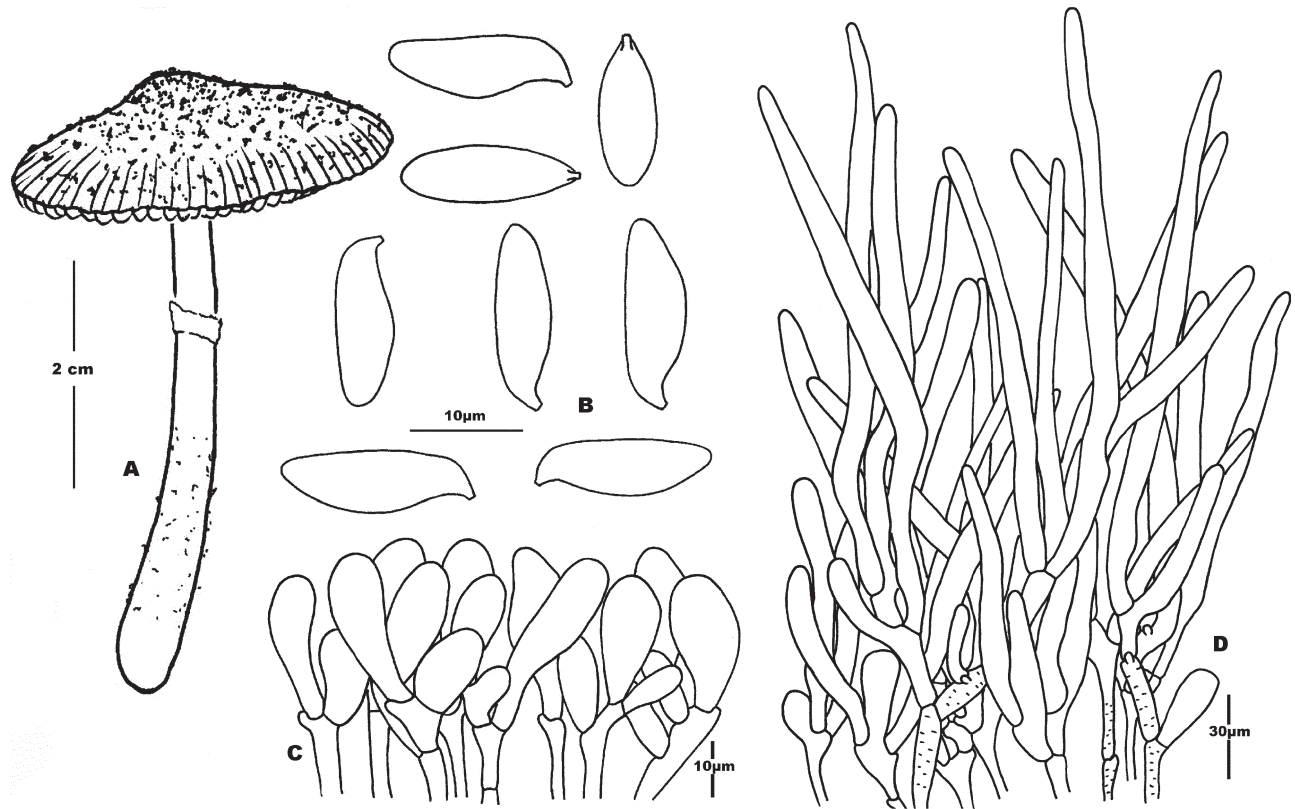


FIG. 2. *Lepiota metulispora*. A. Basidioma. B. Basidiospores. C. Cheilocystidia. D. Pileus covering. All from HMAS 61859.

are not attenuate at their apex. *Lepiota thrombophora* differs from *L. attenuata* in having darker pileus squamules, basidiospores that are never narrowed at apex and the absence of inflated submoniliform or catenulate elements in the pileus covering.

Lepiota aspericepes Murrill differs from *L. attenuata* by its subfusiform to narrowly ovoid basidiospores (Akers and Sundberg 2000). *L. cultorum* (Berk. et Curt.) Sacc. and *L. floralis* (Berk. et Rav.) Sacc. share some characters, according to the descriptions of Dennis (1952). However *L. floralis* is distinguished by its smaller basidiospores and undifferentiated terminal elements of pileus covering while *L. cultorum* differs by its smaller and elongated pip-shaped basidiospores and absence of inflated elements in the pileus covering.

Lepiota cortinarius J.E. Lange, *L. magnispora* Murrill and *L. spheniscispora* Vellinga also have penguin-shaped basidiospores and a trichodermis pileus covering (Vellinga 2001a, b; Wang 2004). However the larger basidiomata, basidiospores without a narrowed apex and absence of inflated elements of pileus covering can be used to separate these taxa from *L. attenuata*.

Lepiota metulispora (Berk. & Broome) Sacc., Syll. Fung. 5:38, 1887.

FIG. 2

Basionym: *Agaricus metulisporus* Berk. & Broome, J. Linn. Soc. Bot. 11:512, 1871.

≡ *Lepiota clypeolaria* var. *metulispora* (Berk. & Broome) Babos, Annls hist.-nat. Mus. natn. hung. 53:198, 1961 (as *metulaespora*).

Basidiomata (FIG. 2A). Pileus 2–3 cm diam, convex, more or less umbonate, surface covered with small, appressed, ochraceous buff squamules on a whitish background, brownish yellow at the disk; margin radially sulcate striate half-way to center. Lamellae free, whitish, crowded with lamellulae of two lengths. Stipe 3–4.5 × 0.2–0.3 cm, subcylindrical, hollow, whitish and glabrous at the apical part, lemon yellow to brown at lower part, with white to cream flocculose squamules, inflated at base. Annulus whitish, membranous, evanescent.

Basidiospores (FIG. 2B) [60/3/3] (12.0)13.0–17.0 × (3.5)4.0–5.5 µm [$Q = (2.60)2.70–3.30(3.75)$, $Q = 2.92 \pm 0.18$], penguin-shaped with straight abaxial side and a suprahilar depression in side view, without germ pore, fusiform to subcylindrical in frontal view; hyaline, smooth, slightly thick-walled, strongly dex-

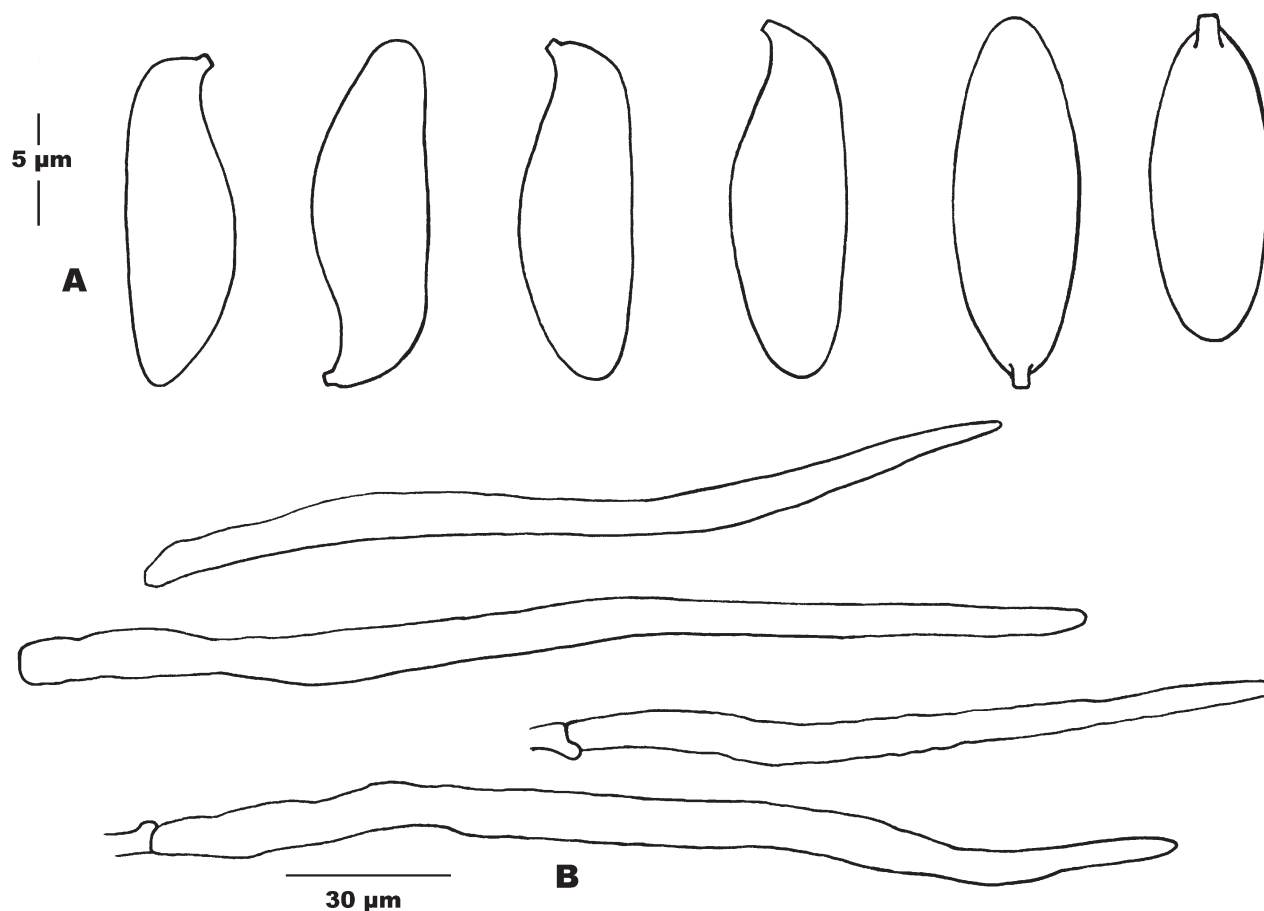


FIG. 3. Holotype of *Lepiota metulispora*. A. Basidiospores. B. Pileus covering.

trinoid, congophilic, not metachromatic in cresyl blue, with guttules. Basidia $15\text{--}35 \times 7\text{--}12\text{ }\mu\text{m}$, clavate, four-spored, rarely intermixed two-spored. Lamella edge sterile. Cheilocystidia (FIG. 2C) $19\text{--}34 \times 8\text{--}11\text{ }\mu\text{m}$, narrowly clavate to fusiform, sometimes utriform and subcylindrical, walls smooth, thin, hyaline in KOH, congophilic. Pleurocystidia absent. Pileus covering (FIG. 2D) a trichodermial palisade of elongate, apically attenuate terminal elements, $75\text{--}218 \times 7\text{--}13\text{ }\mu\text{m}$, often between with some short elements; pigment pale yellow brown, parietal and intracellular in upper part, sometimes in basal part of elements also finely encrusting. Clamp connections common.

Type study. Basidiospores (FIG. 3A) [$20/1/1$] $13.5\text{--}15.5 \times 4.5\text{--}5.5\text{ }\mu\text{m}$ [$Q = (2.55) 2.73\text{--}3.33(3.50)$, $Q = 2.83 \pm 0.24$], penguin-shaped with straight abaxial side and a suprahilar depression in side view, without germ pore, fusiform in frontal view; hyaline, smooth, slightly thick-walled, strongly dextrinoid, congophilic, not metachromatic in cresyl blue, with guttules. Basidia $20\text{--}29 \times 7\text{--}10\text{ }\mu\text{m}$, clavate, four-spored. Cheilocystidia unknown. Pileus covering (FIG. 3B) a trichodermial palisade of elongate, apically attenuate terminal

elements $95\text{--}230 \times 7\text{--}12\text{ }\mu\text{m}$, between often with some short elements, with pale yellow brown pigment, sometimes in basal part also encrusted with a fine brown pigment. Clamp connections common.

Material examined. Sri Lanka: Kandy District, Peradeniya, Jul 1869, *Thwaites 1180 cum icon.* (Holotype, K139635); China: Hong Kong, campus of Chinese University of Hong Kong, 7 Aug 1990, *X.L. Mao 3617* (HMAS 61859); Hunan Province, Zhangjiajie, Sangzhi County, Badagong Mount, 1300 m, 18 Jul 2003, *H.C. Wang 307* (HKAS 43044).

Distribution. Sri Lanka (Pegler 1986), India (Natarajan and Manjula 1983, Kumar and Manimohan 2009), Tanzania (Pegler 1977) and China.

Notes. *Lepiota metulispora* is characterized by small basidiomata, radially sulcate striate pileus with brownish yellow to ochraceous-buff squamules and penguin-shaped spores. Type study shows that basidiospores of the type (average $14.5 \times 4.8\text{ }\mu\text{m}$) are relatively smaller and broader than those in Pegler (1972) ($15.7 \times 4.3\text{ }\mu\text{m}$), and terminal elements of pileus covering are apically attenuate but not rounded obtuse.

Lepiota thrombophora differs from *L. metulispora* by dark brown squamules and the rarely occurring short

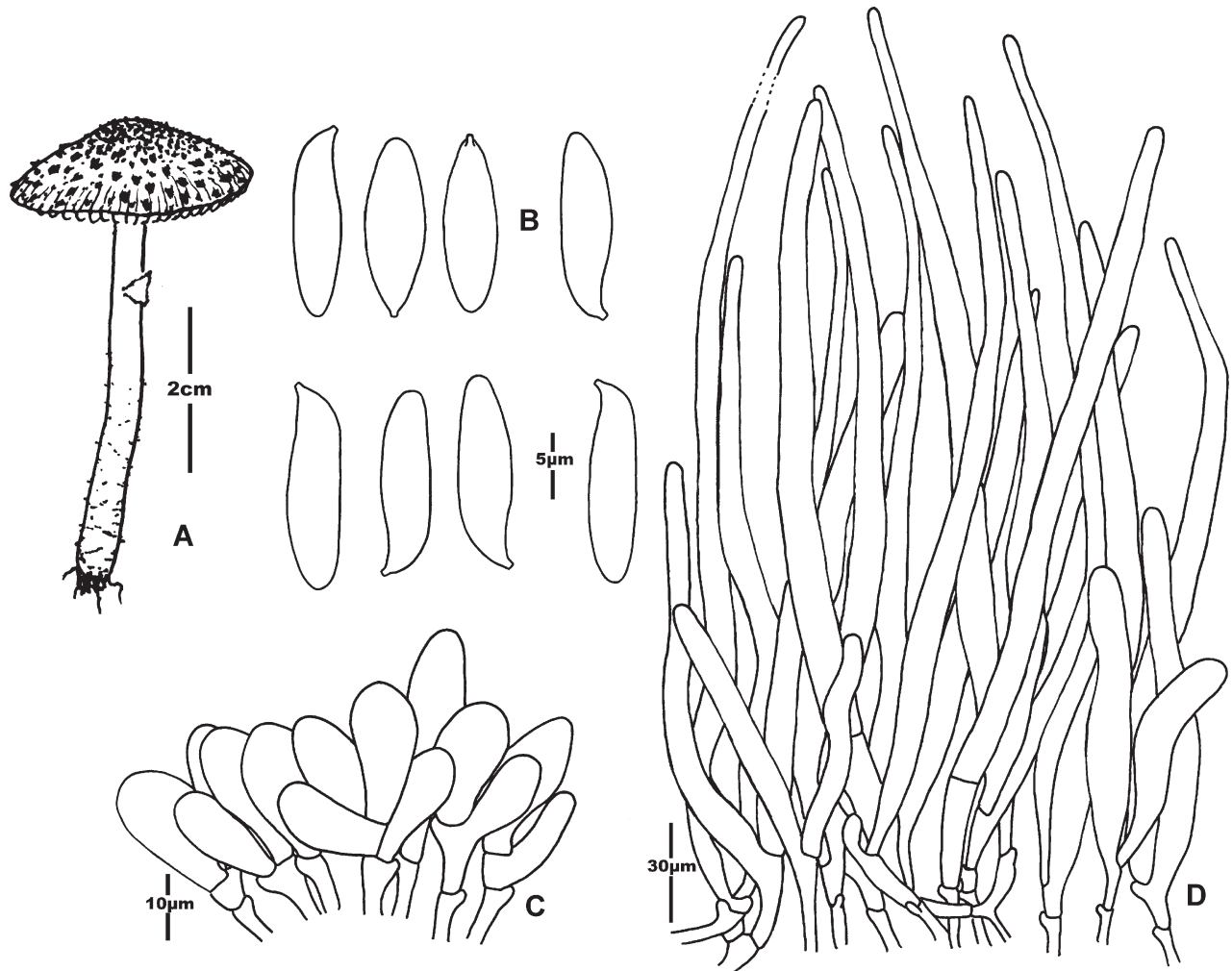


FIG. 4. *Lepiota thrombophora*. A. Basidioma. B. Basidiospores. C. Cheilocystidia. D. Pileus covering. All from HKAS 48451.

clavate elements between elements in the pileus covering.

Lepiota thrombophora (Berk. & Broome) Sacc., Syll. Fung. 5:53. 1887. FIG. 4

Basionym. *Agaricus thrombophorus* Berk. & Broome, J. linn. Soc. 11:500. 1871.

Basidiomata (FIG. 4A). Pileus 1–3 cm diam, plano-concave to plano-convex, surface covered with small, uplifted, red-brown to fuscous brown (6D6–6D4) concentrically arranged squamules on a whitish background, dark brown at the disk; margin radially sulcate striate. Context whitish, thin, about 2–4 mm thick. Lamellae free, whitish, ventricose, up to 3.5 mm wide, moderately crowded with lamellulae of two lengths. Stipe 3–4 × 0.3–0.4 cm, subcylindrical, attenuate, hollow, whitish and glabrous at the apical part, covered with light brown to dark brown minute squamules on a whitish background at lower parts.

Annulus whitish, membranous, superior, 0.8–1.0 cm from apex.

Basidiospores (FIG. 4B) [81/4/4] 11.0–15.5(17.0) × (3.0)3.5–4.5 μm [$Q = (3.11)3.25–4.00(4.25)$, $Q = 3.53 \pm 0.24$], penguin-shaped with straight abaxial side and a suprahilar depression in side view, without germ pore, fusiform to cylindrical in frontal view, hyaline, smooth, slightly thick-walled, dextrinoid, congophilic, not metachromatic in cresyl blue, with 0–3 guttules. Basidia 16–33 × 6–11 μm, clavate, four-spored. Lamella edge sterile. Cheilocystidia (FIG. 4C) 18–35 × 9–15.5 μm, clavate, sometimes narrowed to subfusiform, rarely broadly clavate, with smooth, thin to slightly thick walls, hyaline in KOH, congophilous. Pleurocystidia absent. Pileus covering (FIG. 4D) a trichodermial palisade of elongate, apically attenuate, terminal elements 83–330 × 8–17 μm, sometimes with one septum; short elements at base rare; pigment brownish yellow, parietal and intracellular in upper

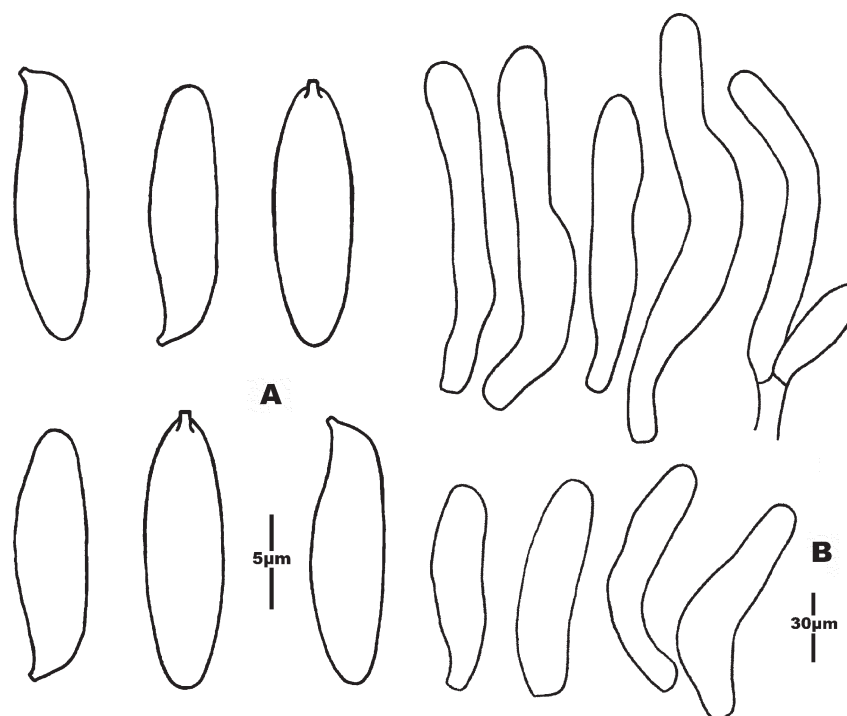


FIG. 5. Holotype of *Lepiota thrombophora*. A. Basidiospores. B. Pileus covering.

part, sometimes in lower part also encrusting as a fine brown pigment. Clamp connections common.

Type study. Basidiospores (FIG. 5A) [$20/1/1$] (13.0) 13.5 – 16.0 (17.0) \times 4.0 – 4.5 μm [$Q = (3.11)3.25$ – 3.86 , $Q = 3.63 \pm 0.22$], penguin-shaped with straight abaxial side and a suprahilar depression in side view, without germ pore, fusiform in frontal view; hyaline, smooth, slightly thick-walled, dextrinoid, congophilic, not metachromatic in cresyl blue. Basidia 21 – 24×7 – 11 μm , clavate, four-spored. Cheilocystidia unknown. Pileus covering (FIG. 5B) a trichodermial palisade of clavate inflated terminal elements 60 – 125×9 – 16 μm , with obtusely rounded apex; pigment yellow brown. Clamp connections common.

Material examined. Sri Lanka: Kandy District, Peradeniya, on the ground, Jan 1869, *Thwaites 903 cum icon.* (Holotype, K139634); China: Hainan Province, Ledong County, Jianfengling, Gouguyulin, 650 m, 14 Jul 2005, *J.F. Liang 111* (HKAS 48451); Mingfengguo, 950 m, 13 Jul 2005, *J.F. Liang 97* (HKAS 41003); Yunnan Province, Mengla County, Xishuangbanna Tropical Botanical Garden, 650 m, 10 Jul 2006, *J.F. Liang 370* (HKAS 50086).

Habitat. Solitary, on tropical forest floor.

Distribution. Sri Lanka (Pegler 1986), India (Natarajan and Manjula 1983), China.

Notes. *Lepiota thrombophora* is characterized by its pileus with red-brown to fuscous brown squamules and a radially sulcate striate margin, penguin-shaped spores, and pileus covering a trichodermial palisade

of elongate, apically attenuate elements, rarely between short clavate elements in mature basidiomata.

Type study shows that basidiospores of the type (average 14.5×4.0 μm) are relatively bigger than those in the description of Pegler (1972) (13.5×4.0 μm).

Observations on the collections cited above revealed that *L. thrombophora* shows considerable variation in the pileus and its structures. In young collections (FIG. 6A–C) the pileus is not sulcate striate and the terminal elements of the pileus covering are distinctly shorter and thicker than in mature collections (FIG. 3D). While small clavate cells between elements of pileus covering are common in young collections, they are rare in mature ones. Basidiospores of a young collection [11.0 – 14.0 (17.0) \times 3.5 – 4.0 μm] [$Q = (3.14)3.25$ – 4.00 , $Q = 3.55 \pm 0.28$] also are smaller than those of mature collections. Although there are differences between young and mature collections, we identified them as the same species based on same habitat and phylogenetic analyses in which the similarity of the ITS sequences of young and mature specimens was almost 99%. The holotype of *L. thrombophora* (FIG. 5A, B) is similar to young collections from China in macromorphological characters, elements of pileus covering and shape of spores, and similar to mature Chinese collections in size and shape of spores. In view of the above we identified the Chinese collections as *L. thrombophora*.

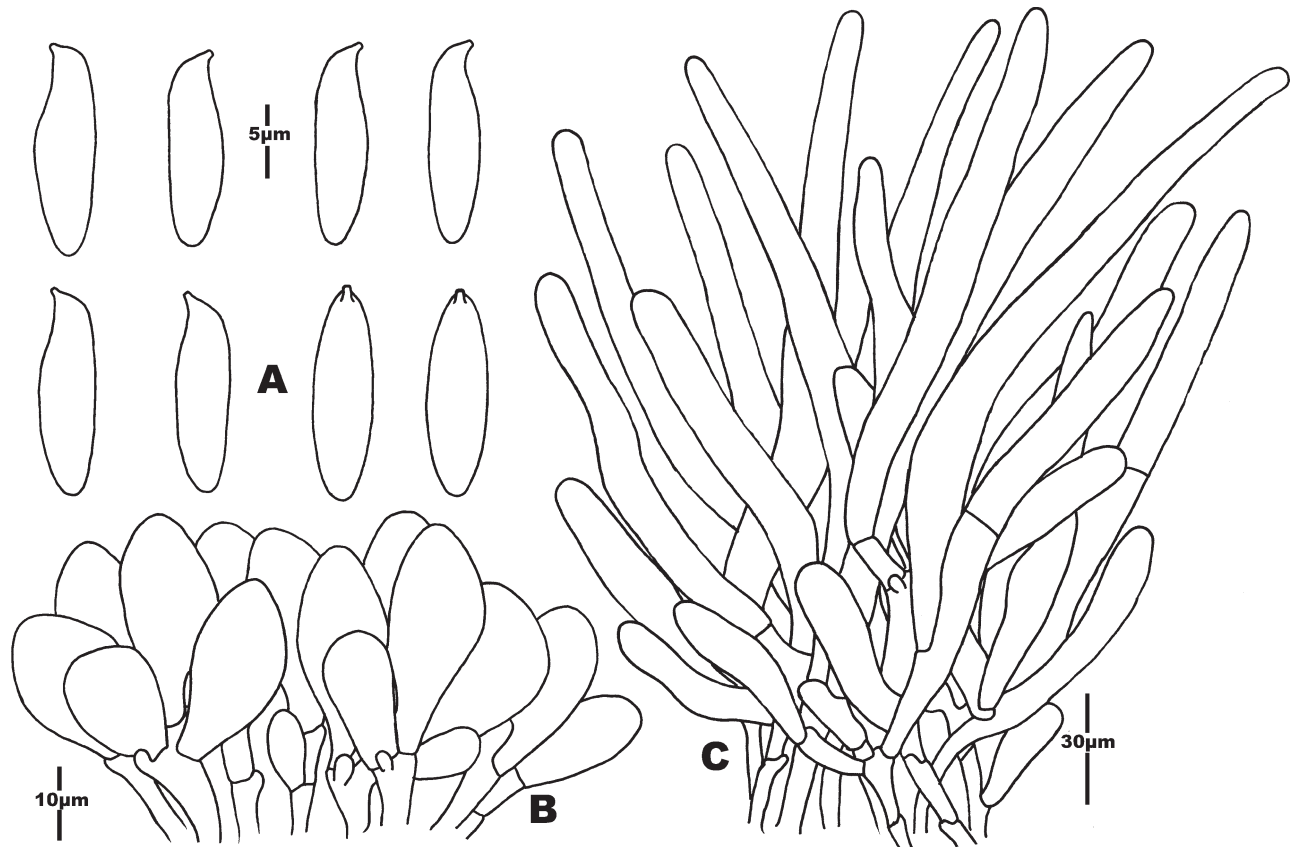


FIG. 6. *Lepiota thrombophora*. A. Basidiospores. B. Cheilocystidia. C. Pileus covering. All from HKAS 41003.

PHYLOGENETIC RESULTS

The combined dataset of ITS, LSU, IGS and mtSSU consisted of 58 taxa and 3150 characters. A wide representation of *Lepiota* sequences was retrieved from GenBank. Bayesian and RAxML phylogenetic analyses (FIG. 7) showed that *L. metulisporea*, *L. thrombophora* and the new species, *L. attenuata*, fell into a single group (clade I). Clade I was supported with 1.0 posterior probability and included most species that shared with trichodermial pileus covering and fusiform-amygdaliform (e.g. *L. clypeolaria* and *L. erminea*) or penguin-shaped spores (e.g. *L. magnisporea* and *L. cortinarius*). Clade II, with 100% ML bootstrap value and 1.0 posterior probability, contained two species (*L. subgracilis* and *L. ignivolvata*) with trichodermial pileus covering and broadly fusiform to amygdaliform spores that were placed in section *Lepiota* (Vellinga 2001a). Clade III contained two species (*L. felina* and *L. pseudolilacea*) that shared a trichodermial pileus covering and ellipsoid to oblong spores and received 100% ML bootstrap value and 1.0 posterior probability. The above three clades formed a monophyletic group with 0.97 posterior probability. Singer (1986) and Vellinga (2001a, 2003) said that *Lepiota* sect. *Lepiota* included the species

with fusiform to penguin-shaped spores and a trichoderm as pileus covering. According to the definition, the species in clades I and II belonged to sect. *Lepiota* but the species in clade III belonged to sect. *Ovisporae*.

DISCUSSION

Based on Bayesian and RAxML phylogenetic analyses (FIG. 7), we found that species in *Lepiota* sect. *Lepiota* are divided into clades I and II. The monophyletic grouping of clade I, clade II and clade III (species in sect. *Ovisporae*) indicated that sect. *Lepiota* is not monophyletic. This result is consistent with Vellinga (2003) with regard to this group of taxa. Vellinga (2003) found that all species from sect. *Lepiota* and a part of sect. *Ovisporae* formed a monophyletic group with high bootstrap support. The species of sect. *Ovisporae* reported in Vellinga (2003) included *L. felina*, *L. pseudolilacea*, *L. xanthophylla*, *L. echinella*, *L. forquignonii* etc. However in our phylogeny *L. echinella* and *L. forquignonii*, although related to clade III, did not group with these species. Furthermore *L. xanthophylla* clustered with other species in *Lepiota*. The difference between this study and

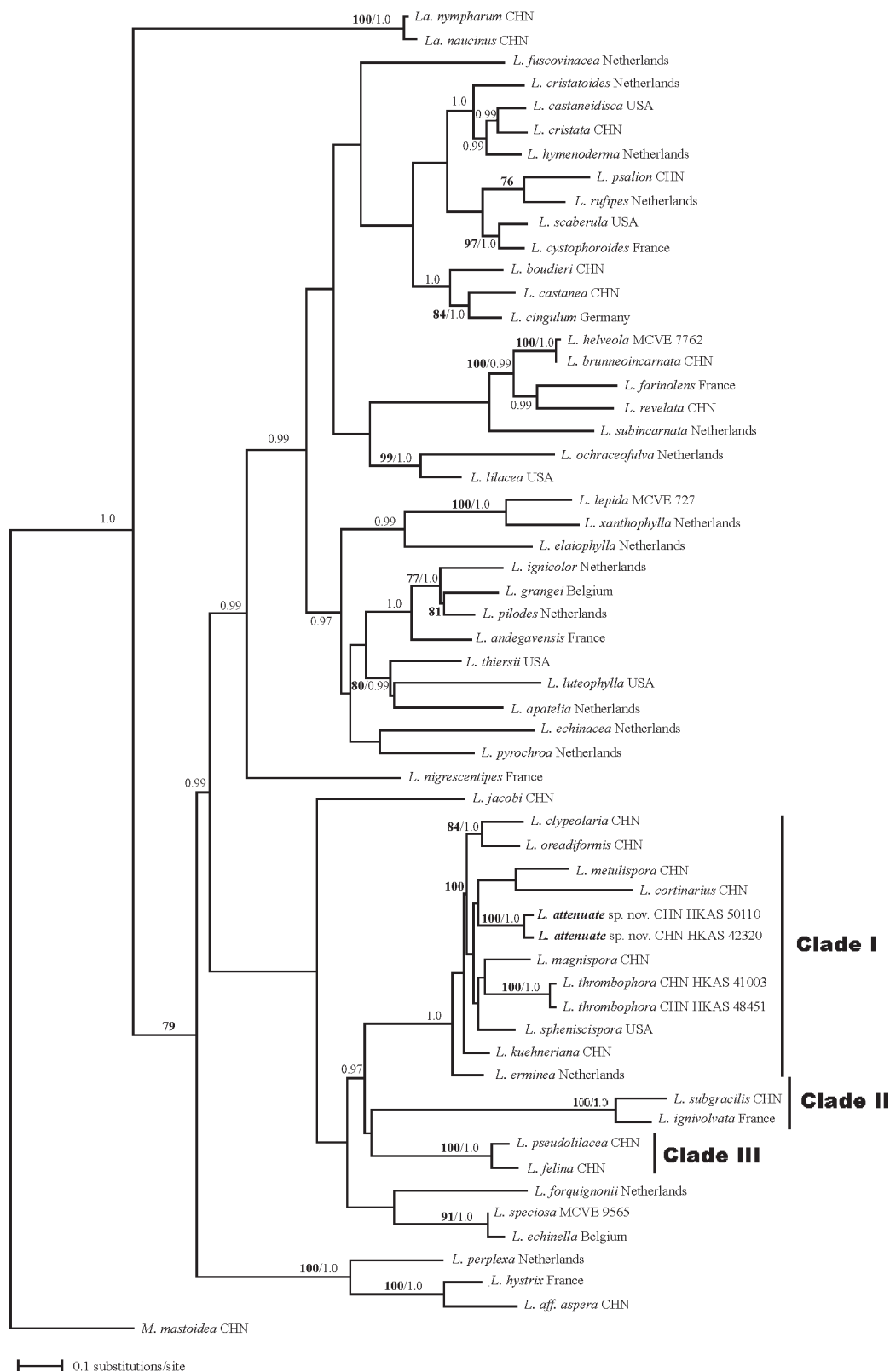


FIG. 7. One of 100 RAxML likelihood trees ($-\ln L$ 29276.323916) based on the combined ITS-LSU-IGS-mtSSU dataset. Support values in boldface are RAxML likelihood bootstrap ($\geq 70\%$). Values in lightface are Bayesian posterior probabilities (≥ 0.95).

previous studies is likely a result of different gene sequences analyzed.

Several tropical species in sect. *Lepiota* did not form a monophyletic clade but were dispersed throughout clade I. These differ from other species in the section in having more fragile basidocarps, a sulcate striate pileus and smaller basidiospores.

Lepiota attenuata grouped into sect. *Lepiota* with high support values (1.0 posterior probability). This indicated that the new species should be placed in the section although the section is not monophyletic. It obviously is different from the known species in the section based on ITS, IGS, mtSSU sequences. The obvious divergence between the new species and the previously described species confirms that it is significantly divergent from these known species, meriting species recognition. *Lepiota attenuata* is allied with *L. cortinarius* and *L. metulisporea*. Long branch lengths among the species suggest that other potentially unknown species might exist and remain to be identified.

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