

Lepiota* (Agaricales) in northern Thailand-2 *Lepiota* section *Lepiota

Phongseun SYSOUPHANHONG^{a,d*}, Kevin D. HYDE^{a*,b,d},
Ekachai CHUKEATIROTE^a, Ali H. BAHKALI^b & E.C. VELLINGA^c

^a School of Science, Mae Fah Luang University, 57100 Chiang Rai, Thailand,
email: psphongseun@gmail.com

^b Botany and Microbiology Department, College of Science, King Saud University,
P.O. Box: 2455, Riyadh 1145, Saudi Arabia

^c Department of Plant and Microbial Biology, University of California at Berkeley,
Berkeley, CA 94720-3102, U.S.A

^d Mushroom Research Foundation, Ban Pa Deng, Pa Pae, Chaing Mai, Thailand

Abstract – In this report, we investigate the presence of *Lepiota* species in section *Lepiota* in Northern Thailand (Chiang Mai and Chiang Rai). Five *Lepiota* species are characterised by distinct fusiform-amygdaliform spores. Of these, three species, namely *L. euryasperma*, *L. microcarpa*, and *L. pongduadensis*, are proposed as new species; *L. metulispora* is a new record for Thailand. Macro- and microscopic descriptions, illustrations, and a key to the species are provided. In addition, the identity of these *Lepiota* species is also clarified based on molecular data.

Agaricaceae / biodiversity / nrITS sequence / taxonomy

INTRODUCTION

The genus *Lepiota* (Pers.) S.F. Gray is presently divided into six sections, based on morphology. *Lepiota* section *Lepiota* harbours species with fusiform-amygdaliform spores with convex abaxial and convex adaxial sides or with straight abaxial sides, a trichodermal pileus covering made up of narrowly cylindrical with or without short clavate elements at the base of the long elements, and with clamp-connections (Vellinga 2001).

Section *Lepiota* differs from the other sections in the combination of shape of the basidiospores and the pileus covering, but the placement of some species has been controversial. *Lepiota cortinarius* J.E. Lange has narrow spores that are slightly spurred at base, and it has been placed in sect. *Stenosporae* (J.E. Lange) Kühner (Bon 1981; Candusso & Lanzoni 1990), but the structure of the pileus covering, and phylogenetic analyses based on molecular data place it in sect. *Lepiota* (Vellinga 1992, 2003). *Lepiota aspera* (Pers.: Fr.) Quél., *L. perplexa* Knudsen and *L. hemisclera* (Berk. & M.A. Curtis) Sacc. have similar narrow spores, and are placed in sect. *Echinatae* Fay. because of the pileus covering which is made up of chains of rounded to ellipsoid elements unit to form pyramidal spines (Knudsen 1980; Montoya & Bandala 2005; Vellinga 2001, 2003).

* Corresponding author.

Phylogenetic analyses of the ITS and LSU regions of *Lepiota* sect. *Lepiota* revealed that the species with ellipsoid spores and a trichodermal pileus covering are closely related to the other species with a trichodermal pileus covering with fusiform to 'penguin-shaped' spores (Vellinga 2003; Liang *et al.* 2011).

The diversity and species of *Lepiota* and its section *Lepiota* in Asia are not well investigated. A recent article by Liang *et al.* (2011) focuses on the species of section *Lepiota* in tropical China, and describes three species in detail, one of them new. Wang (2004) reported on *L. cortinarius* from China; *L. clypeolaria* (Bull.: Fr.) Kummer, *L. metulispورا* (Berk. & Broome) Sacc., and *L. thrombophora* (Berk. & Broome) Sacc. are known from Sri Lanka, India, Nepal and tropical China (Petch 1950; Majula & Natarajan 1983; Majula 1983; Pegler 1972, 1986; Liang *et al.* 2011). Only a few species have been recorded from Thailand, such as *L. clypeolaria* and *L. cortinarius* (Chandrasrikul *et al.* 2008). However, these two species were identified by macrocharacters only.

We are studying the macrofungi in northern Thailand and around the Mushroom Research Centre (Desjardin *et al.* 2009; Kerekes & Desjardin 2009; Wannathes *et al.* 2009; Zhao *et al.* 2010) and in this paper describe the *Lepiota* species in section *Lepiota* from northern Thailand, based on their morphology and in a few cases also on molecular data.

MATERIALS AND METHODS

This study is based on collecting in Chiang Mai and Chiang Rai Provinces of Northern Thailand from 2007-2010 (Sysouphanhong *et al.* 2010). We recorded location, forest type, soil and substrate for each collection. Samples were also photographed in the field, and they were transferred in plastic boxes or aluminum foil to the laboratory. Macromorphological characters were noted, using standard procedures and terminology (Vellinga & Noordeloos 2001). Colour annotations in the macroscopical descriptions are from Kornerup & Wanscher (1978); samples were dried on hot air dryer, at 40-50°C to preserve tissues. The specimens were deposited in the herbarium of Mae Fah Luang University (MFU). Microscopic characters were measured and illustrated from dry specimens, using a drawing tube attached to an Olympus CX-41 research compound microscope. Characters concerning colour were observed in water or in 2.5-10% of KOH, chemical reactions in Melzer's reagent, Cotton blue and Cresyl blue, and drawing of all characters using Congo red in ammonia. Twenty-five spores were observed and measured in side view per collection. The notation [100,5,4] indicates that measurements were made on 100 spores in five samples in four collections. The following abbreviations are used: L for lamellae, l for lamellulae, avl for average of spore length, avw for average of spore width, Q for quotient of length and width and avQ for average quotient. All species were illustrated and compared with species from Asia, and with European and American species.

Two collections belonging to two new species were investigated for sequences of internal transcribed spacer 1 and 2 (ITS1 & ITS2). DNA was extracted from dried herbarium collections using the instructions of the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China). Primers ITS1-F and ITS4 were used for PCR following the protocol of Gardes & Bruns (1993). PCR amplified products were cleaned and sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.). All new sequences were deposited in GenBank.

Sequences were edited and contigs assembled using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences of taxa belonging to *Lepiota* section *Lepiota* present in GenBank were downloaded, and the data set was augmented with sequences of other Thai *Lepiota* species, and their close relatives from elsewhere; all sections of *Lepiota* are represented (**Table 1** – see online supplementary material). A sequence of *Macrolepiota procera* from China was used as outgroup. The complete data set, 49 sequences in total, was aligned using MAFFT (Kato *et al.* 2002; Kato & Toh 2008) and minimally manually adjusted. Heuristic search was performed using the maximum parsimony (MP) option of the program PAUP* 4.0 b10 (Swofford 2004), using 1000 heuristic searches, employing TBR branch swapping and random sequence addition. Other settings were as follows: gaps were treated as missing data; all characters are of type unordered and equally weighted; multistate taxa interpreted as uncertainty; starting trees were obtained via stepwise addition; one tree was held at each step during stepwise addition; the steepest descent option was not in effect, branches were collapsed (creating polytomies) if minimum branch length was zero, and MulTrees option was in effect. Bootstrap supports were evaluated using 1000 bootstrap replicates with 10 heuristic searches per replicate, random sequence addition and TBR branch swapping.

RESULTS AND DISCUSSION

Phylogenetic study

The Maximum Parsimony analysis of the nrITS data set, comprising 48 species with *M. procera* as outgroup, was composed of 725 characters and resulted in a relatively well resolved phylogeny (Fig. 1). Clade I (with 99% bootstrap support) was made up of *Lepiota* species with fusiform and ellipsoid spores and a trichoderm pileus covering made up of long elements, often with short cells at the base. Clade II (with 100% bootstrap support) comprised species with spurred or ellipsoid spores, and a pileus covering which is either a cutis or a trichoderm, but always lacking short elements at the base of the long ones. The two new taxa in sect. *Lepiota* cluster together, despite their different spore morphology, and form a clade separate from those of temperate regions of Europe and North America (Fig. 1).

Other clades that are recovered harbour species with a hymeniform pileus covering (Clade III) and species with a pileus covering made up of rounded elements in chains (sect. *Echinatae*; Clade IV).

None of the five taxa of sect. *Lepiota* in northern Thailand have fusiform spores with a convex abaxial side (such as in *L. clypeolaria*), but four have spores shaped like those of *L. magnispora*, and *L. pachysperma* has much shorter spores, resembling those of *L. ignivolvata* and *L. subgracilis*.

Taxonomy

Key to species of section Lepiota in Thailand

1. Basidiospores relatively broad ($Q = 1.3-2.4$, $avQ < 2.0$), without straight abaxial side and with or without suprahilar depression *L. euryasperma*

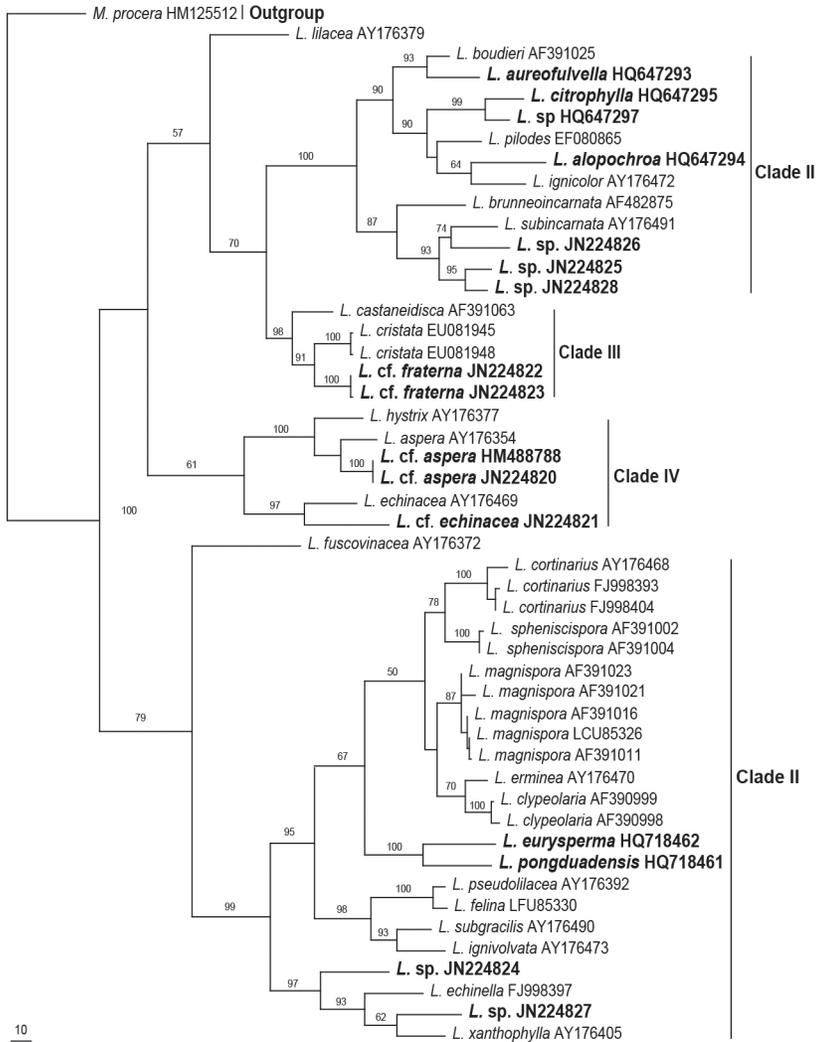


Fig. 1. MP Phylogeny of *Lepiota* in northern Thailand based on ITS sequences. Bootstrap values of 50% and higher are above the branches. *Macrolepiota procerata* from China is the outgroup. Parsimony tree scores for the consistency index (CI) = 0.417, retention index (RI) = 0.764, rescaled consistency index (RC) = 0.311, and homoplasy index (HI) = 0.583, length = 1828. All species with bold marks are specimens from Thailand and the two species (*L. eurosperma* and *L. pongduadensis*) in clade (I) are described as new.

1. Basidiospores relatively long and fusiform ($Q = 2.2-3.6$, $avQ > 2.5$) with straight abaxial side and suprahilar depression. 2
2. Pileus covering made up of narrowly cylindrical elements with rounded apical apex; stipe with a cuff-like annulus *L. sp.3*
2. Pileus covering made up of narrowly cylindrical elements, tapering towards apex; stipe with an annular zone 3



Fig. 2. *Lepiota* section *Lepiota* in the field, a. *Lepiota metulispora* (MFLU090165), b. *Lepiota euryserma* (MFLU090184), c. *Lepiota pongduadensis* (MFLU090184), d. *Lepiota microcarpa* (MFLU100491), e. *Lepiota* sp. (MFLU10 0502).

- 3. Basidiomata tiny (pileus 3.5-6 mm) *L. microcarpa*
- 3. Basidiomata medium to large (pileus >10 mm 4
 - 4. Pileus covered by light brown to brown squamules, striate; pileus covering with abundant short clavate elements *L. metulispora*
 - 4. Pileus covered by brown to dark brown squamules, not striate but surface breaking open in radial streaks around umbo towards margin; short clavate elements in pileus covering rare *L. pongduadensis*

***Lepiota euryserma* Sysouphanthong, K.D. Hyde & Vellinga, sp. nov Fig. 3**

Mycobank MB 519960

Lepiota clypeolariae similis, sed in basidiosporis latis et brevibus, et in spatii interni transcripta sequentia (“ITS”) differt.

Holotype: Thailand: Chiang Mai Province, Mae Taeng District, near Forest of Pong Duad Village, N 16° 06' 16.1”, E 99° 43' 07.9”, alt. 780-805 m, 8 August 2008, P. Sysouphanthong, MFLU 0900035.

Etymology: The name of this species comes from its broad basidiospores.

Table 2. Overview of some characters of basidiospores and pileus covering structure in collections from Thailand

<i>Secies</i>	<i>Spore sizes (µm)</i>	<i>Quotient</i>	<i>Spore shape</i>	<i>Pileus covering</i>
<i>Lepiota eurysperma</i>	7.8-11.5 × 4.5-6.5 (8.9 × 5.2)	1.3-2.4 (1.7)	Broadly amygdaliform to oblong without suprahilar depression and straight abaxial side	With short clavate elements
<i>Lepiota metulispora</i>	13.5-17.0 × 4.0-5.2 (15.0 × 4.7)	2.7-3.4 (3.1)	Long fusiform, with straight abaxial side and suprahilar depression	With short clavate elements
<i>Lepiota microcarpa</i>	10.8-16.5 × 4.0-5.3 (13.5 × 4.7)	2.5-3.6 (2.9)	Long fusiform, with straight abaxial side and suprahilar depression	With short clavate elements
<i>Lepiota pongduadensis</i>	13.0-16.0 × 4.5-5.5 (14.6 × 5.2)	2.2-3.2 (2.8)	Long fusiform, with straight abaxial side and suprahilar depression	Without short clavate elements
<i>Lepiota</i> sp.	11.3-16.0 × 4.0-5.0 (14.6 × 4.8)	2.7-3.2 (2.9)	Long fusiform, with straight abaxial side and suprahilar depression	Without short clavate elements

Pileus 45-60 mm, when young parabolic, expanding via conical to campanulate to plano-convex, with wide umbo, with inflexed margin, when young felted or glabrous, dark brown (8F4-6), soon breaking up into patches and squamules, brown (7E6-7) at umbo and with light brown (7D6) to brown (6E6) fibrillose squamules, very crowded around centre, with light brown (7D6) irregularly concentric squamules around umbo towards margin on white to yellowish-white (4A2) background; margin cortinate and connected with stipe when young, when mature floccose, squamulose, fringed. **Lamellae** free, crowded, ventricose, up to 4 mm wide, white, with floccose edge. **Stipe** 30-70 × 4-5 mm, cylindrical, wider at bulb, 6 mm wide, covered completely with white floccose fibrillose covering, densely so at annular zone at middle to upper part of stipe, below annular zone to base pale yellow brown (4A2) fibrillose and darker downwards to base, with brown (7E4) fibrillose squamules, sometimes with drops on stipe, hollow. Annulus an annular zone, fibrillose or cortinate, white. **Context** in pileus white to yellowish white (4A2), 2-2.3 mm wide, in stipe, white to light brown (6D4), with white fibrils in central cavity. **Smell** very strong, sweet. **Taste** mild, a little bit spicy. **Spore print** white.

Basidiospores [75,3,3] 7.8-11.5 × 4.5-6.5 µm, avl × avw = 8.9 × 5.2 µm, Q = 1.3-2.4, avQ = 1.7, in side-view amygdaliform, without or without suprahilar depression, in frontal view more or less fusiform, hyaline, thick-walled, dextrinoid, congophilous, cyanophilous, not metachromatic. **Basidia** 24-40 × 9.5-11.5 µm, clavate, 4-spored, sometimes 2-spored, rarely 1-spored, hyaline, thick walled, with clamp-connections. **Lamella edge** sterile. **Cheilocystidia** 14-35 × 7.0-17 µm, clavate or sphaeropedunculate, (sub)utriform, hyaline, thick-walled, rarely with apical excrescence. **Pleurocystidia** absent. **Pileus covering** a trichoderm made up of narrowly cylindrical elements, 60-255 × 5.0-11 µm, sometimes septate, pale brown and thick-walled; pigment pale brown and parietal; with short cylindrical to narrowly clavate elements, 35-45 × 5.0-10.5 µm, with hyaline hyphae up to 2.5-4 µm wide in under layer. **Stipe covering** in squamules a trichoderm similar to pileus covering. **Clamp-connections** present in all tissues.

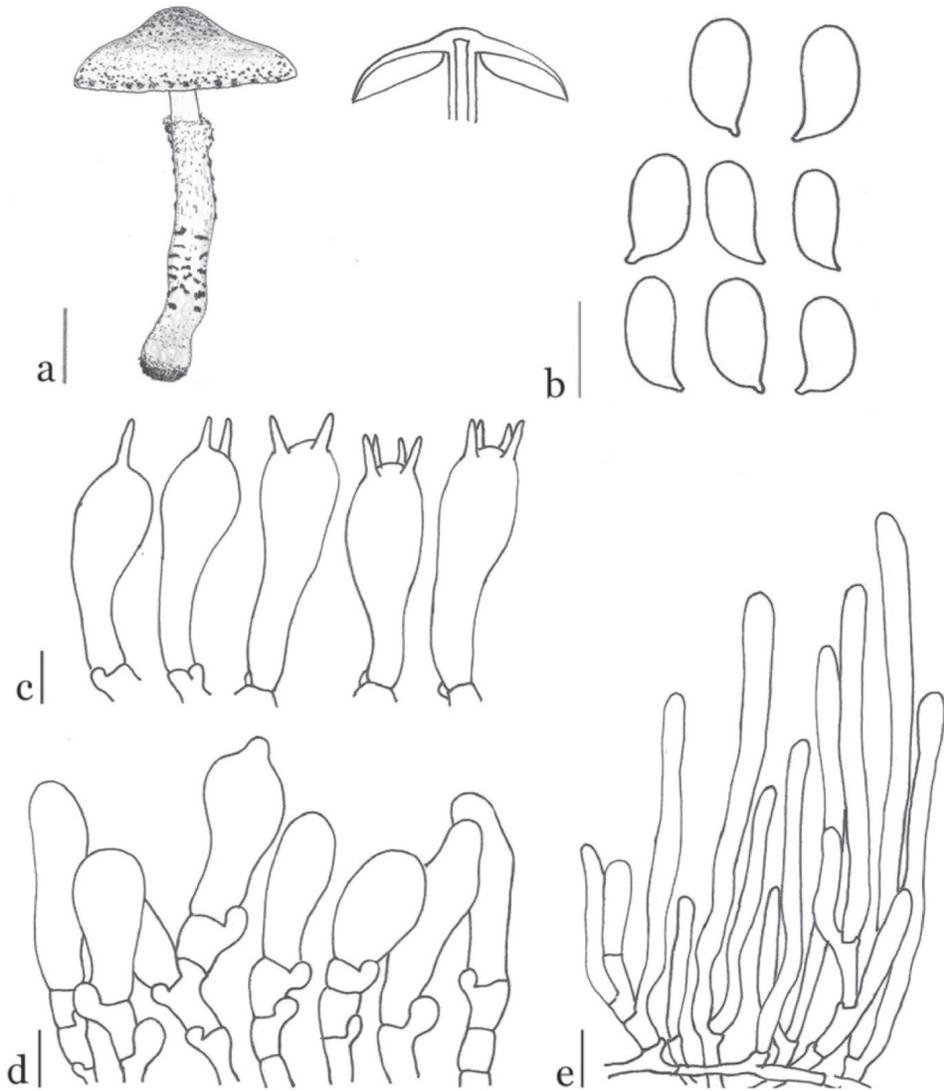


Fig. 3. Morphological characters of *Lepiota eurysperma* (MFLU090035), a. basidiomata and a section, b. basidiospores, c. basidia, d. cheilocystidia, e. pileus covering. Scale bars a = 10 mm, b-f = 10 μ m.

Habitat and distribution: Growing in a small or large group, on humus-rich soil with decaying leaves in deciduous wood, in mixed rain forest with several kinds of tree species and bamboo; only known from Mae Taeng District, Chiang Mai Province, Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Dist., near Forest of Pong Duad Vill., N 16° 06' 16.1", E 99° 43' 07.9", alt., 780-805 m, 10 August 2007, P. Sysouphanthong, MFLU 0900035 (holotype); *ibidem*, 4 August 2007, P. Sysouphanthong, MFLU 090003; *ibidem*, 17 September 2007,

P. Sysouphanhong, MFLU 0900069; *ibidem*, 8 August 2008, P. Sysouphanhong, MFLU 0900198.

Discussion: The colour of the basidiomata in *Lepiota eurysperma* is variable, ranging from yellowish-white to distinctly brown squamulose on the pileus. It is characterized by the cortinate pileus margin and zone on stipe, the relatively short and broad spores, the cylindrical to narrowly clavate cystidia which often have an apical excrescence and the trichodermal pileus and stipe coverings with short very narrowly clavate elements at the base of the long cells. It differs from all other Thai species by the short spores.

Lepiota pongduadensis differs from *Lepiota eurysperma* in the dark brown to brown pileus squamules, and the longer basidiospores. However, despite their different spore morphology they group together in one clade, separate from all other species in section *Lepiota* (Fig. 1).

Lepiota ignivolvata Bousset & Joss., a species from Europe, comes morphologically close to *Lepiota eurysperma* but differs in the more robust basidiomata with an orange stipe. *Lepiota clypeolaria* (Bull.: Fr.) P. Kumm. is macroscopically similar, but its basidiospores and pileus covering elements were described as much longer than in *L. eurysperma* (Vellinga 2001; Candusso & Lanzoni 1990). ITS data show that *L. eurysperma* is not identical to *Lepiota ignivolvata*, nor *Lepiota clypeolaria* (material from Germany and USA), or *L. subgracilis* (collection from the Netherlands).

Lepiota subgracilis Kühner is distinguished from *Lepiota eurysperma* by greyish pinkish-brown pyramidal squamules on the pileus, and a band-forming annulus, but is very similar in micromorphology (Kühner 1936; Vellinga 2001).

The north-temperate *L. magnispora* Murrill (syn. *L. ventriosospora* D.A. Reid), and the western north American *L. spheniscispora* Vellinga resemble *L. eurysperma* macroscopically, but in general are more woolly on the stipe and with thicker squamules on the pileus, and are microscopically very different because of the long 'penguin-shaped' spores with a straight abaxial side, and the long pileus covering elements.

Lepiota metulispora (Berk. & Broome) Sacc., Syll. Fung. 5: 38. 1887. **Fig. 4**

≡ *Agaricus metulisporus* Berk. & Broome in J. Linn. Soc. Bot. 11: 512. 1871.

≡ *Lepiota clypeolaria* var. *metulispora* (Berk. & Broome) Babos, in Anns hist.-nat. Mus. Natn. hung. 53: 198. 1961 (basonym not mentioned; as var. *metulaespora*). Sri Lanka. Holotypus Thwaites 1180 (K).

Pileus 20-60 mm, first subglobose to conical, expanding to parabolic, convex, umbonate, plano-convex, with inflexed margin, when young glabrous, light brown to brown (6D7-8) at center, paler toward margin, orange-white (5A2) to white at margin, when mature glabrous at umbo, light brown to brown (6D7-8), orange-white (5A2) around umbo, with orange-white (5A2) concentric squamules around umbo and toward margin, on white fibrillose background; at margin peeling from surface, with orange-white (5A2) squamules, sulcate, white woolly or cobwebby, with partial veil remnants. **Lamellae** free, broadly ventricose, 4 mm wide, white, slightly crowded, with serrulate edge. **Stipe** 30-50 × 2.8-5 mm, cylindrical, wider at base, 5.5-6 mm wide; completely fibrillose, crowded at annular zone down toward base, white, white to orange-white (5A2), on pale yellow to

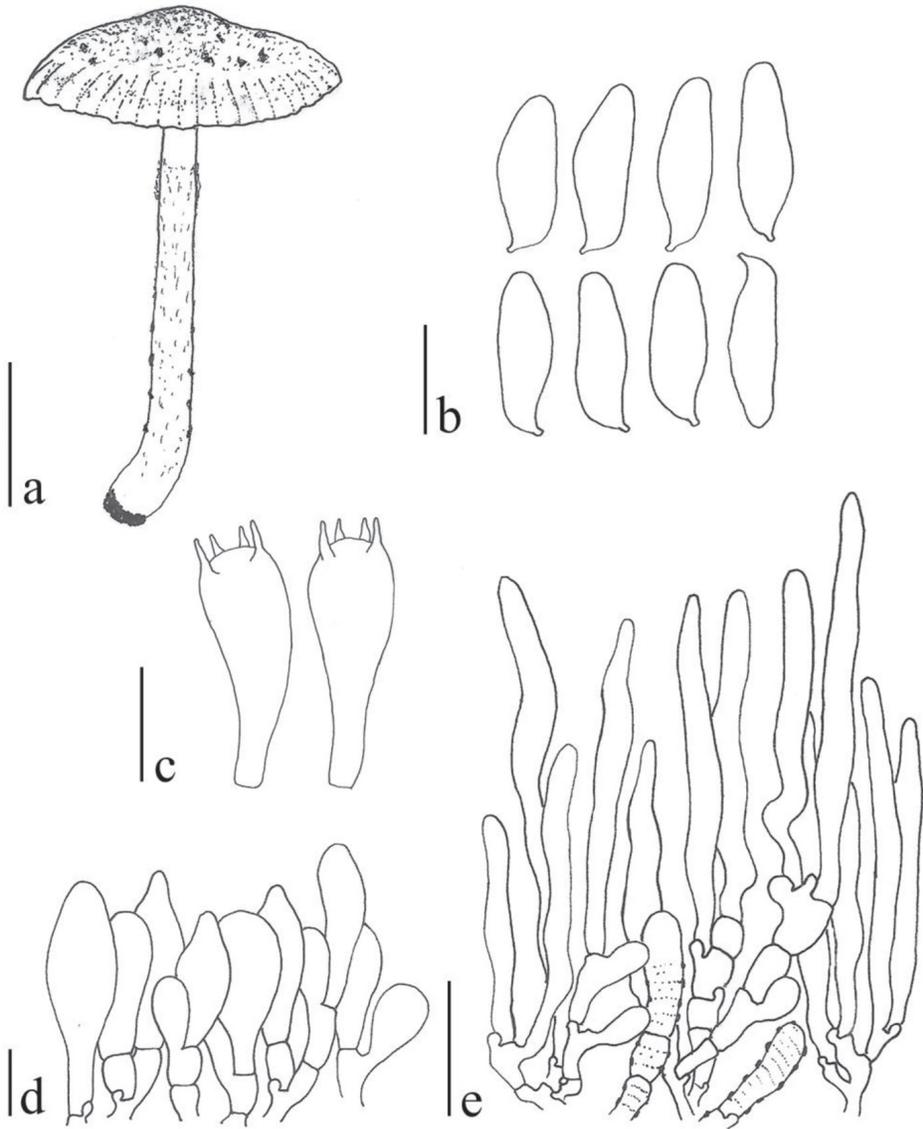


Fig. 4. Morphological characters of *Lepiota metulispora* (MFLU090165), a. basidiomata and a section, b. basidiospores, c. basidia, d. pileus covering, e. cheilocystidia. Scale bar. a = 10 mm, b, c and e = 10 μ m, d = 20 μ m.

orange-white (4A3, 5A2) background. **Annulus** an annular zone, cortinate, or fibrillose, white, sometimes with remnants, orange-white (5A2), granulose at base. **Context** in pileus white, 2.5-3 mm wide; hollow, concolorous with surface. **Smell** fruity. **Taste** unknown. **Spore print** white.

Basidiospores [50,2,2] 13.5-17.0 \times 4.0-5.2 μ m, avl \times avw = 15.0 \times 4.7 μ m, Q = 2.7-3.4, Qav = 3.1, in side-view cylindrical, with more or less straight abaxial side, inflexed hilar appendage, and suprahilar depression, in frontal view fusiform

to cylindrical, thick-walled, dextrinoid, congophilous, cyanophilous, not meta-chromatic. **Basidia** 18-30 × 8.5-10 µm, clavate, slightly thick-walled, 4-spored. **Lamella edge** sterile. **Cheilocystidia** 18-37 × 7.0-17.5 µm, utriform, clavate to narrowly clavate, rarely broadly clavate, slightly thick-walled. **Pileus covering** a trichoderm made up of cylindrical elements with rounded apex or slightly tapering to apex, 50-180 × 6.0-11.5 µm, hyaline to pale brown, thick-walled, mostly smooth-walled, sometimes rough-walled, with parietal pale brown pigment, with short clavate elements under these cylindrical elements, with parietal pale brown pigment; with hyaline hyphae under the layer of upright elements, 3-12 µm wide, thick-walled, smooth or rough-walled. **Stipe covering** of annular zone similar to pileus covering. **Clamp connections** present in all tissues.

Habitat and distribution: Growing in a small group or solitary, on dead leaves, saprotrophic and terrestrial on humus soil; in deciduous rain forest with dominant *Lithocarpus* spp. and grassland of national Park. Distributed in Sri Lanka (Pegler 1972), India (Natarajan and Manjula 1983, Kumar and Manimohan 2009), China (Liang *et al.* 2011), and this is a first report to Thailand.

Material examined: Thailand, Chiang Mai Province, Mae Taeng District, Forest of Pha Deng Village, N 19° 07' 13.7", E 98° 43' 52.9", 905 m, 25 July 2008, P. Sysouphanhong, MFLU 090165; Chiang Rai Province, Mueng District, Forest of Doi Ngaem near Mae Fah Luang University, N 18° 05' 59.1", E 102° 40' 02.9", 488 m, 18 June 2009, P. Sysouphanhong, MFLU 100495.

Discussion: This is the first record of *Lepiota metulispora* for Thailand. The species is rare and was only found twice, in a forest, and in a grassland, in the middle of the rainy season. Both collections have predominantly white basidiomata with an orange to orange-brown centre of the pileus, a woolly or cobwebby pileus margin, and a white fibrillose stipe.

Lepiota metulispora, originally described from Sri Lanka, is characterized by a pileus with a brown surface, ochraceous buff squamules, striate or sulcate surface toward the margin, an appendiculate margin, an equal stipe with evanescent annulus, elongate basidiospores with a suprahilar depression (11-19 × 3.7-5 µm), and a trichodermal pileus covering made up of elongate elements (25-170 × 5.5-14 µm) (Pegler 1972). Pegler (1972) and Liang *et al.* (2011) studied the type material and unfortunately cheilocystidia were not recovered. The Thai material fits this description well, and also the description by Liang *et al.* (2011) based on modern material from tropical China (Hong Kong and Hunan Province).

Lepiota attenuata comes close to *L. metulispora* from Thailand in the light colour and striate pileus, size of basidiospores and cheilocystidia but its elements on pileus covering are longer (231 × 8-13 µm), and the spores are attenuated at the apex (Liang *et al.* 2011).

Lepiota thrombophora described from Sri Lanka is also quite close to *L. metulispora*, but differs in the dark brown squamules; Pegler (1972) studied the type and noted that the pileus covering elements are inflated and rather short (25-100 × 5-14 µm). Liang *et al.* (2011) also studied the type, and modern material from China and postulated that the type specimen is rather young, and that in older material the pileus covering elements are longer (83-330 × 7-18 µm).

Lepiota oreadiformis Velenovsky from Europe is close to *L. metulispora* but its basidiospores are shorter and do not have a straight abaxial side (Candusso & Lanzoni 1990; Vellinga 2001).

Lepiota clypeolaria differs from *L. metulispora* in the (pale) brown scaly pileus, breaking up into a central patch ('calotte') and surrounding scales, and in the shape of the spores (not penguin-shaped in *L. clypeolaria*).

The completely white or pale yellow European grassland species *L. erminea* and *L. alba* have longer elements in the pileus covering (200-300 μm long), and slightly wider spores (5.5-6.0 μm), that again have a convex abaxial side (Vellinga 2001).

Lepiota microcarpa Sysouphanthong, K.D. Hyde & Vellinga **sp. nov.**

Fig. 5

Mycobank MB 519962

Pileus 3.5-6 mm, convexus vel umbonatus, applanatus, squamis brunneis vel ochraceo-brunneis, Stipes 8.5-20 \times 0.8-1.2 mm, cylindricus, albus, fibrillosus, squamis ad basim. Sporae 10.8-16.5 \times 4.0-5.3 μm , fusiformes vel cylindricae.

Holotype: Thailand, Chiang Rai Prov., Mae Fah Luang District, Forest of Doi Tung, 10 June 2009, P. Sysouphanthong (MFLU 100491).

Etymology: named because of its tiny basidiomata.

Pileus 3.5-6 mm, convex, with small umbo, or umbonate to applanate, with straight to slightly inflexed margin, at center brown to red-brown (7E5-8, 8E5-6), glabrous to felted, densely fibrillose, or rarely with pyramid-liked squamules, around umbo with irregularly concentric squamules toward margin, squamules distant or absent at margin, squamules brown (7E5-8) on white fibrillose background; margin white fibrillose to cortinate, sulcate or fringed, exceeding lamellae. **Lamellae** free, sub-distant to slightly crowded, broadly ventricose, 2.0-3 mm wide, white with white eroded edge. **Stipe** 8.5-20 \times 0.8-1.2 mm, cylindrical, covered with white fibrils, crowded at annular zone downward base, on light brown (6D5-6) background, with or without brown (7E5-8) squamules at base zone. **Annulus** an annular zone, cortinate or fibrillose, white. **Context** in pileus thin, white to light brown, less than 1 mm wide; in stipe concolorous with surface, hollow. **Taste** and **smell** not observed. **Spore print** white.

Basidiospores [75,3,3] 10.8-16.5 \times 4.0-5.3 μm , $\text{avl} \times \text{avw} = 13.5 \times 4.7 \mu\text{m}$, $Q = 2.5-3.6$, $\text{av}Q = 2.9$, in side-view with more or less straight abaxial side, strongly inflexed above hilar appendage ('penguin-shaped'), in frontal view fusiform to cylindrical, hyaline and thick-walled, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl Blue. **Basidia** 18-23 \times 8.2-10.5 μm , narrowly clavate, 4-spored. **Lamella edge** sterile. **Cheilocystidia** 15.5-30 \times 6.5-12.5 μm , mostly fusiform or utriform, clavate to narrowly clavate, hyaline and thin-walled. **Pleurocystidia** absent. **Pileus covering** a trichoderm made up of narrowly cylindrical elements, narrow and tapering to apex, curved at base of elements, 62-240 \times 10-12.5 μm , brown and thin-walled, with brown parietal and intracellular pigments, with short clavate elements, 8.5-37.5 \times 4.5-12.5 μm , brown and slightly thick-walled; under layer with hyaline to pale brown hyphae, up to 4.5 μm wide. **Stipe covering** of squamules at base a trichoderm similar to pileus covering. **Clamp connections** present in all tissues.

Habitat and distribution: growing solitary or on a small group, mostly on humus-rich soil; under shade of deciduous forest dominated by *Dipterocarpus* spp. or in forest of *Pinus kesiya*. Found in rainy season (June to October) of mountainous areas of Northern Thailand.

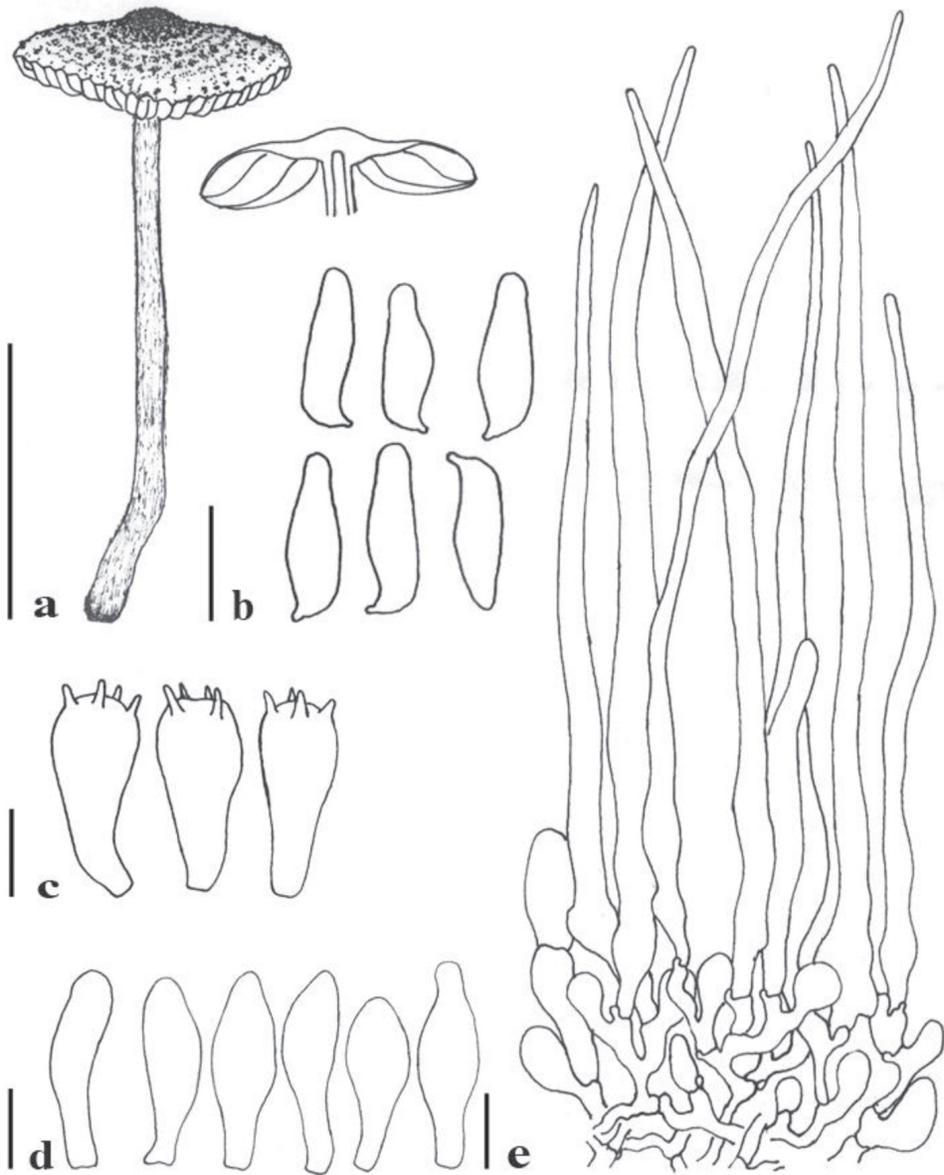


Fig. 5. Morphological characters of *Lepiota microcarpa* (MFLU100491), a. basidioma and a section, b. basidiospores, c. basidia, d. cheilocystidia, e. pileus and stipe covering. Scale bars. a = 1 mm, b-d = 10 µm, e = 20 µm.

Material examined: Thailand, Chiang Rai Prov, Mae Fah Luang Dist., Forest of Doi Tung, 10 June 2009, P. Sysouphanhong (MFLU100491) (Holotype). Chiang Mai Prov., Mueng Dist., Forest of Doi Suthep, N 18°48.62', E 98° 54.60' 1455 m. alt, 6 June 2010, P. Sysouphanhong (MFLU100521), ibidem, 12 June 2010, P. Sysouphanhong (MFLU 100526).

Discussion: Material of this species is easily recognized by the tiny basidiomata, brown to red-brown squamules on pileus, a white fibrillose stipe with light brown background, with or without brown squamules at base, ‘penguin-shaped’ basidiospores, and a trichodermal pileus covering made up of narrow cylindrical elements.

Lepiota microcarpa has consistently very small basidiomata and the basidiospore morphology is similar to that in the *L. magnispora* group. There are no species with such small basidiomata known from Europe or North America. In the field, it can be confused with a yet undescribed *Lepiota* species (MFLU 090126, MFLU 090120, and MFLU 090130) in section *Ovisporae*, with ellipsoid basidiospores and a trichodermal pileus covering.

Lepiota thrombophora from Sri Lanka comes close because of the dark brown squamules on pileus and the basidiospore shapes and sizes, but differs from this species by larger basidiomata and clavate inflated terminal elements in the pileus covering of young basidiomata (Pegler 1972; Liang *et al.* 2011).

It also shares some characters with *L. attenuata* from southern China and Taiwan in micromorphology such as shape of basidiospores, cheilocystidia and elements in pileus covering, but *L. attenuata* shows larger basidiomata (30-60 mm in pileus) and has paler squamules color on pileus (brownish yellow to yellowish brown) (Liang *et al.* 2011).

Lepiota pongduadensis Sysouphanthong, K.D. Hyde & Vellinga **sp. nov.** **Fig. 6**

Mycobank MB 519961

Pileus 19-35 mm, umbonatus, vel campanulatus, applanatus, atrobrunneus, tomentosus vel scaber, striatus. Lamellae albae. Stipes 32-55 × 2-3.2 mm, cylindricus vel subclavaus, pallide brunneis vel atrobrunneis squamis. Sporae 13.0-16.0 × 4.5-5.5 μm, fusiformes.

Holotype: Thailand, Chiang Mai Prov, Mae Taeng Dist, Forest of Pong Duad Vill., N 16° 06' 16.1", E 99° 43' 07.9", 780-805 m alt., 22 June 2009, P. Sysouphanthong, MFLU100497

Etymology: The name is derived from the first collecting site of this species close to Pong Duad Village.

Pileus 19-35 mm, umbonate, expanding to capitate, applanate with low umbo and straight margin, when young matted or glabrous, dark brown (6F7-8, 7F8) at umbo, brown (6E7-8, 7E7-8) around umbo to margin, this covering breaks open when mature, at umbo tomentose, felted to rough, dark brown to dark (7F4-5; 8F4), in radial streaks from umbo towards margin, with white surface in splits and yellowish-white (4A2) fibrillose background when mature, with brown (6E7-8, 7E7-8) squamules between splits or in radial streaks; marginal zone split or striate, fringed or fibrillose, exceeding lamellae. **Lamellae** free, moderately crowded, ventricose, 2.5-4.5 mm wide, cream-white, with white floccose edge. **Stipe** 32-55 × 2-3.2 mm, cylindrical, sometimes wider at middle and slightly tapering to apex and base, with subbulbous base, up to 4 mm wide, white from middle to apex, light brown (6D6-7) at middle zone and darker brown (7E6-7) down toward base, completely white fibrillose and floccose at middle zone to annular zone, squamulose from middle to base and with white to brown crowded squamules at base, hollow. **Annulus** an annular zone, white floccose or cortinate and brown squamulose. **Context** white to light brown (6D6-7) in pileus, in stipe

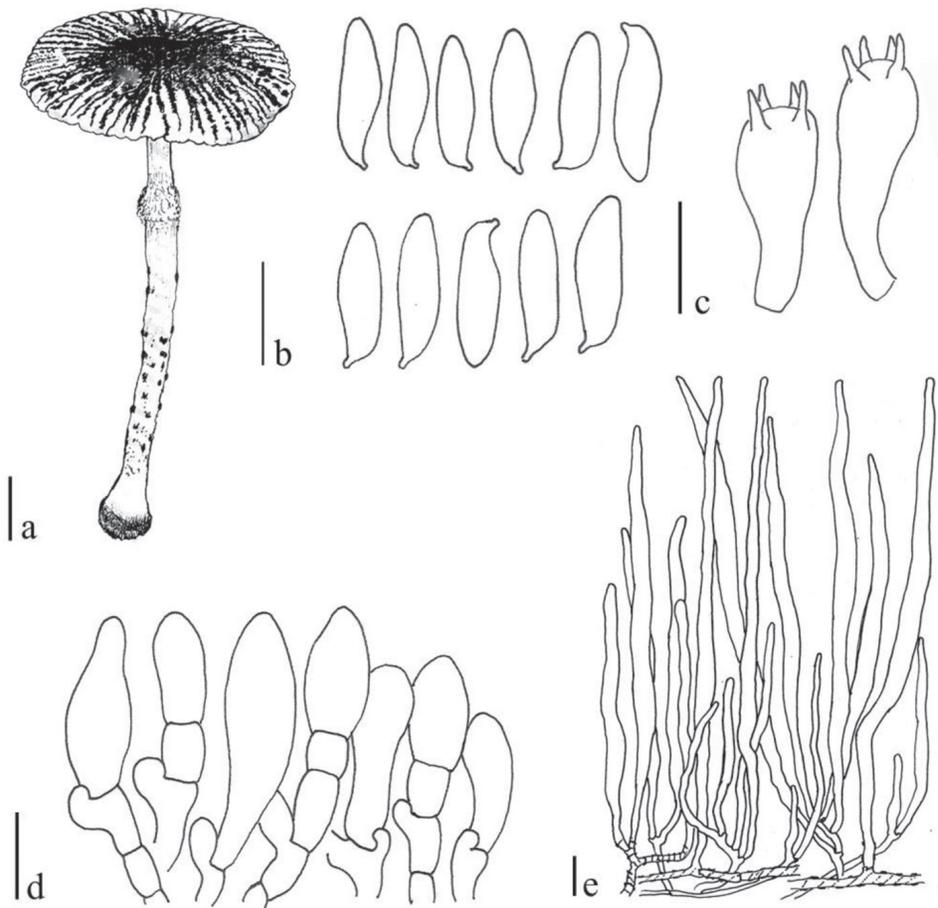


Fig. 6. Morphological characters of *Lepiota pongduadensis*. b, c, d (MFLU100497); a, e (MFLU090184); a. basidioma and a section, b. basidiospores, c. basidia, d. cheilocystidia, e. pileus and stipe covering. Scale bars. a = 10 mm, b-e = 10 μ m.

white at apex to middle, becoming brown (6E7-8) downward. **Smell** and **taste** not observed. Spore print white.

Basidiospores [75,3,3] 13.0-16.0 \times 4.5-5.5 μ m, al \times aw = 14.6 \times 5.2 μ m, Q = 2.2-3.2, Qav = 2.8, in side-view fusiform to amygdaliform, with or without confluent hilar appendage and with or without superhilar depression, in frontal view fusiform to subcylindrical, hyaline, thick-walled, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl Blue. Basidia 18-30 \times 8-10 μ m, clavate, hyaline, slightly thick-walled, 4-spored. **Lamella edge** sterile, with crowded cheilocystidia. **Cheilocystidia** 20-30 \times 8-13 μ m, variable in shape, utriform, clavate or irregularly clavate, slightly thick-walled and colourless. **Pleurocystidia** absent. **Pileus covering** a trichoderm made up of narrowly cylindrical elements, mostly wider at middle and narrow at base and apex, 50-425 \times 5-11.5 μ m, slightly thick-walled and brown, with parietal and intracellular brown pigment, without short clavate elements, with cylindrical hyphae in under layer, smooth and rough-

walled, 2.5-7.5 μm wide. **Stipe covering** of squamules a trichoderm with same structure as pileus covering. **Clamp-connections** present in all tissues.

Habitat and distribution: Growing solitary to a large group; saprotrophic and terrestrial on humus-rich soil, sandy soil with decaying leaves and wood; in deciduous mixed rain forest dominated by *Castanopsis armata*, *Lithocarpus* spp; only known from Northern Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Dist., Forest of Pong Duad Vill., N 16° 06' 16.1", E 99° 43' 07.9", 780-805 m, 8 August 2008, P. Sysouphanthong, MFLU090184; ibidem, 22 June 2009, P. Sysouphanthong, MFLU10 0497; Chiang Rai Prov., Mae Lao Distr., Forest of Khun Kon Waterfall, 22 July 2009, P. Sysouphanthong, MFLU100500.

Discussion: *Lepiota pongduadensis* is found at Forest of Pong Duad Village, Chiang Mai Province and Forest of Khun Kon Waterfall of Chiang Rai Province during rainy season (June to July). Materials were examined in good condition in every stage. The species is recognized by a pileus with distinctly radial streaks of brown squamules and a dark brown umbo, and a white fibrillose or floccose stipe with squamules.

Lepiota microcarpa has some microscopically characters in common with *L. pongduadensis*, but its basidiomata are tiny (pileus 3.5-6 mm wide), and there are clavate cells at the base of the long pileus covering elements.

Lepiota sp. in this study is also similar to *Lepiota pongduadensis*, especially in shape and size of the basidiospores and cheilocystidia, but macroscopically it is quite different (Fig. 4), as it lacks the radial aspect of the pileus squamules, and has a distinct cuff-like annulus.

Lepiota cortinarius J.E. Lange has sturdier basidiomata with a distinctly cortinate pileus margin, and has small, relatively narrow spores (7.5-9.5 \times 3.0-4.0 μm) that are truncate or spurred at base; it has been recorded from Yunnan Province in southwest China (Wang 2004), and is widespread in Europe (Candusso & Lanzoni 1990; Vellinga 2001).

Lepiota clypeolaria has lighter coloured squamules on the pileus, and the spores are amygdaliform with convex sides, measuring 11.0-18.5 \times 4.0-6.5 μm (Vellinga 2001). These latter two taxa also differ in their ITS sequences from *L. pongduadensis* (Fig. 1).

Lepiota sp.

Fig. 7

Pileus 20 mm, paraboloid to convex, with small umbo, with straight margin, at center felted to glabrous and dark brown (7F6-8), around center with concentrically arranged brown squamules and with or without uplifted squamules at margin, on to light brown (6D4-6) fibrillose background around center toward margin; at margin squamulose and brown (7E6-8), white fibrillose and fringed at edge. **Lamellae** free, remote from stipe, ventricose, 2.8 mm wide, white, with slightly white floccose edge. **Stipe** 55 \times 2.8-4 mm, cylindrical to tapering to apex, white from apex to annular zone and white fibrillose or floccose, at center pale yellow (4A3) and darker down towards base, with pale yellow (4A3) to light brown 6D4-6) squamules. **Annulus** like a cuff around stipe, white and set as a band of squamules or patches, concolorous with those on pileus, with white background, margin at upper-side white and slightly fringed. **Context** white in pileus, 1.8 mm wide; hollow and white in stipe. **Smell** pleasant, mild. **Taste** unknown. **Spore print** white.

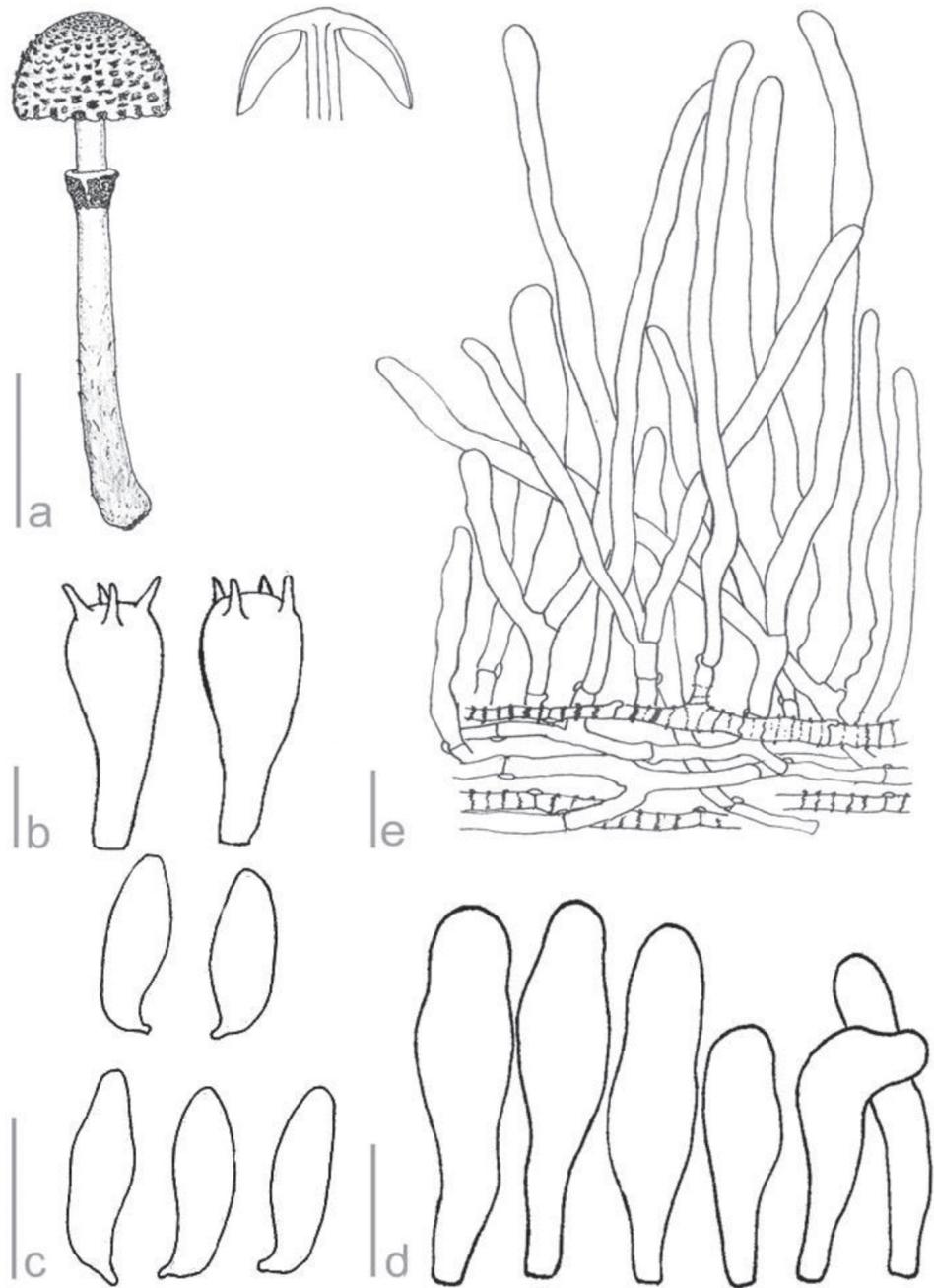


Fig. 7. Morphological characters of *Lepiota* sp. (MFLU10 0502), a. basidioma and section, b. basidia, c. basidiospores, d. cheilocystidia, e. pileus covering. Scale bars. a = 20 mm, b-e = 10 μ m.

Basidiospores [25,1,1] $11.3-16 \times 4.0-5.0 \mu\text{m}$, on average $14.6 \times 4.8 \mu\text{m}$, $Q = 2.7-3.2$, $Q_{\text{av}} = 2.9$, in side-view amygdaliform, oblong to cylindrical, rarely with suprahilar depression, in frontal view fusiform to subcylindrical, hyaline, thick-walled, dextrinoid, congophilous, cyanophilous, not metachromatic. **Basidia** $24-30 \times 9-10 \mu\text{m}$, clavate, 4-spored, hyaline, slightly thick-walled. **Lamella edge** sterile. **Cheilocystidia** $17-30 \times 6-10 \mu\text{m}$, clavate to narrowly clavate, (sub)utriform, rarely cylindrical, thin-walled, hyaline. **Pleurocystidia** absent. **Pileus covering** a trichoderm made up of narrowly cylindrical or elongate elements, $50-250 \times 7-12.5 \mu\text{m}$, slightly thick-walled, brown-walled, without short clavate element between and under cylindrical elements; underlayer with cylindrical hyphae, $4.5-6.5 \mu\text{m}$ wide, hyaline to pale brown-walled, with rough and smooth-walls. **Stipe covering** at squamules of stipe a trichoderm as pileus covering. **Clamp-connections** present in all tissues.

Habitat and distribution: growing solitary, on rich humus soil mixed with dead leaves and wood; in mixed rain forest with several species of deciduous trees (tree species are not recorded); in Thailand known from Doi Nang Non, Mae Sai District of Chiang Rai Province.

Material examined: Thailand, Chiang Rai Prov., Mae Sai District, Forest of Doi Nang, 8 August 2009, P. Sysouphantong, MFLU100502.

Discussion: This species was found only once, in Doi Nang Norn National Park. The young (and only) basidioma collected is characterized by the pileus with a small umbo, the discrete brown squamules on the pileus, the cuff-like annulus with squamules, and the pileus covering that lacks clavate cells at the base of the narrowly cylindrical elements.

Lepiota subgracilis also has a cuff-like annulus, but differs in the relatively broad spores; some other *Lepiota* species, *L. felina*, *L. pseudohelveola* Hora, and *L. lilacea* also have a cuff-like annulus, but again differ in spore shape and in the case of *L. lilacea* also in structure of the pileus covering.

Acknowledgements. We wish to acknowledge Mae Fah Luang University (MFLU) and the Mushroom Research Center (MRC) in Mae Taeng District, Chiang Mai, Thailand for providing funding, and facilities for collecting and study. International Fungal Research and Development Centre, The Research Institute of Insect Resources (RIRI) is thanked for providing materials and laboratory facilities to do molecular work. The Global Research Network for Fungal Biology and King Saud University are also thanked for support. Funding of ECV and PS in 2007-2009 by NSF grant DEB 0618293 is gratefully acknowledged. We would like to express special thank to Dr. Jun Feng Liang for his help and information to identify *Lepiota* species.

REFERENCES

- BON M., 1981 — Clé monographique des « Lépiotes » d'Europe. *Documents Mycologiques* 11(43): 1-77.
- CANDUSSO M. & LANZONI G., 1990 — *Lepiota* s.l. Fungi europaei 4. Giovanna Biella, Saronno. pp. 743.
- CHANDRASRIKUL A., SUWANARIT P., SANGWANRIT U., MORINAGA T., NISHIZAWA Y. & MURAKAMI Y., 2008 — Diversity of mushrooms and macrofungi in Thailand. Kasetsart University [in Thai].
- DESJARDIN D.E., BINDER M., ROEKRING S. & FLEGEL T., 2009 — *Spongiforma*, a new genus of gasteroid boletes from Thailand. *Fungal Diversity* 37: 1-8.

- GARDES M. & BRUNS T.D., 1993 — ITS primers with enhanced specificity for basidiomycetes — application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118. doi: 10.1111/j.1365-294X.1993.tb00005.x
- KNUDSEN H., 1980 — A revision of *Lepiota* sect. *Echinatae* and *Amyloideae* (Agaricaceae) in Europe. *Botanisk Tidsskrift* 75: 121-155.
- KATOH K., MISAWA K., KUMA K. & MIYATA T., 2002 — MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066. doi: 10.1093/nar/gkf436
- KATOH K. & TOH H., 2008 — Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286-298. doi:10.1093/bib/bbn013.
- KEREKES J.F. & DESJARDIN D.E., 2009 — A monograph of the genera *Crinipellis* and *Moniliophthora* from Southeast Asia including a molecular phylogeny of the nrITS region. *Fungal Diversity* 37: 101-152.
- LIANG J.F., YANG Z.L. & XU D.P., 2011 — A new species of *Lepiota* from China. *Mycologia* 103(4): 820-830. doi: 10.3852/10-216.
- MONTOYA L. & BANDALA V.M., 2005 — A new species and a new record of *Lepiota* occurring in the Gulf of Mexico area. *Mycotaxon* 94: 115-125.
- MANJULA B., 1983 — A revised list of the agaricoid and boletoid basidiomycetes from India and Nepal. *Proceedings of Indian Academy of Sciences (Plant Science)* 92: 81-213.
- NATARAJAN K. & MANJULA B., 1983 — South Indian Agaricales XII—*Lepiota*. *Bibliotheca Mycologica* 91: 563–581.
- PEGLER D.N., 1972 — A revision of the genus *Lepiota* from Ceylon. *Kew Bulletin* 27: 155-202.
- PEGLER D.N., 1986 — Agaric flora of Sri Lanka. *Kew Bulletin. Addition Series* XII: 1-519.
- PETCH T. & BISBY G.R., 1950 — The fungi of Ceylon. *Peradeniya Manual* 6: 1-111.
- REID D.A., 1958 — New or interesting records of British hymenomycetes. II. *Transactions of the British Mycological Society* 41 (4): 419-445.
- SWOFFORD D.L., 2004 — PAUP*: Phylogenetic Analysis Using Parsimony, Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- SYSOUPHANTHONG P., THONGKANTHA S., ZHAO R., SOYTONG K. & HYDE K.D., 2010 — Mushroom diversity in sustainable shade tea forest and the effect of fire damage. *Biodiversity and Conservation* 19: 1401-1415. doi: 10.1007/s10531-009-9769-1
- VELLINGA E.C., 1992 — Notulae ad Floram Agaricinam Neerlandicam.18. Some notes on *Cystolepiota* and *Lepiota*. *Persoonia* 14: 407-415.
- VELLINGA E.C., 2000 — Notes on *Lepiota* and *Leucoagaricus*. Type studies on *Lepiota magnispora*, *Lepiota barssii*, and *Agaricus americanus*. *Mycotaxon* 76: 429-438.
- VELLINGA E.C., 2001 — *Lepiota*. In M.E. Noordeloos, Th.W. Kuyper, E.C. Vellinga (eds). *Flora agaricina neerlandica* 5: 109-151.
- VELLINGA E.C., 2003 — Phylogeny of *Lepiota* (Agaricaceae) — Evidence from nrITS and nrLSU sequences. *Mycological Progress* 2: 305-322.
- WANG H.C., 2004 — *Lepiota cortinarius*, a species new to China. *Mycosystema* 23: 439-440.
- WANNATHES N., DESJARDIN D.E., HYDE K.D., PERRY B.A. & LUMYONG S., 2009 — A monograph of *Marasmius* (Basidiomycota) from Northern Thailand based on morphological and molecular (ITS sequences) data. *Fungal Diversity* 37: 209-306.
- ZHAO R.L., DESJARDIN D.E., SOYTONG K., PERRY B.A. & HYDE K.D., 2010 — A monograph of *Micropsalliota* in Northern Thailand based on morphological and molecular data. *Fungal Diversity* 45: 33-79.