DIVERSITY OF *Lepiota* (BASIDIOMYCOTA) IN NORTHERN THAILAND

PHONGEUN SYSOUPHANTHONG

MASTER OF SCIENCE
IN
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THESIS COMMITTEE

Rampai Kodsueb
.....CHAIRPERSON
(Dr. Rampai Kodsueb)

.....ADVISOR
(Assoc. Prof. Dr. Kevin D. Hyde)

.....CO-ADVISOR
(Asst. Prof. Dr. Ekachai Chukeatirote)

.....EXAMINER
(Dr. Itthayakorn Promputtha)

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Phongeun Sysouphanthong

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Author	Phongeun Sysouphanthong
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Advisor	Assoc. Prof. Dr. Kevin D. Hyde
Co-Advisor	Assist. Prof. Dr. Ekachai Chukeatirote

ABSTRACT

The mushroom genus *Lepiota* known as 'lepiotaceous fungi' belongs to the family *Agaricaceae*. Mushrooms in this group are diverse and have a worldwide distribution but most reports are from the USA and Europe while records for Asia are limited. Most *Lepiota* species are poisonous containing amatoxins (clyclopeptides) which are also found in *Amanita*, *Conocybe* and *Galerina*. Some species might be noted as edible in a region but they are known to be fatal or poisonous in other places such as *L. americana, L. friesii, L naucinoides* and *L. clypeolaria*. In terms of the *Lepiota*'s taxonomy, key morphological characters are used including basidiospore, basidiocarp, pluteus, pileus covering a cutis to trichroderm, white and free gills. Recently, a development of molecular phylogenetics based on the DNA and protein sequences have also been widely used in *Lepiota*'s identification and relationship.

A study of diversity of *Lepiota* species in northern Thailand was carried out in Chiang Mai and Chiang Rai Provinces during the period 2007-2010. In total, 72 specimens of *Lepiota* were collected; these represented 33 different species consisting 5 sections (11 species in section *Stenosporae*, 8 species in section *Ovoisporae*, 6 species in section *Lepiota*, 5 species in section *Lilaceae*, and 3 species in section *Echinatae*).

In this study, eleven species of section *Stenosporae* and five of section *Lepiota* are provided with full descriptions. This published document is known as the first record to Thailand. Major results obtained from our work are to reveal 5 new species namely *L. aureofulvella* and *L. papilata* (section *Stenosporae*), *L. eurysperma*, *Lepiota micrpcarpa* and *L. pongduadensis* (section *Lepiota*). Their species identification is confirmed by the ITS sequencing data.

Keywords: Basidiomycotina/Agaricales/*Lepiota*/Diversity/Distribution/Taxonomy/ Thailand

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Mushroom genera with white spores such as *Chamaemyces, Chlorophyllum, Coniolepiota, Cystolepiota, Eriocybe, Lepiota, Leucoagaricus*, *Leucocoprinus*, and *Macrolepiota* are called lepiotaceous fungi and they show varied forms and morphological characters (Vellinga, 2004a, 2004b). Historically mycologists characterised and defined these lepiotaceous taxa mostly from Europe (Bon, 1993; Fries, 1821). Candusso and Lanzoni (1990) studied the lepiotaceous fungi including some herbaria material from Europe and estimated there were 156 species belonging to seven genera. The main characters of this group are thus mostly based on the characters of temperate species with a few considerations from tropical species (Vellinga, 2001). Studies of tropical lepiotaceous taxa are less. Pegler (1972) provided a revision of *Lepiota* species from Sri Lanka, and Manjula (1983) carried out a study in India; these studies also included *Macrolepiota* and *Leucocoprinus*. However, most of mycologists did not accept a narrow definition of lepiotaceous fungi. The presently accepted characters of lepiotaceous fungi are mostly derived from the literature by Vellinga (2001); Vellinga and Huijser (1993; 1990) and main characters are as follows:

1. Basidiomata: The fruiting bodies can be solitary to gregarious, rarely in fairy rings; the basidiomata vary from tiny to large and from pluteoid (free lamellae, context of pileus discontinuous with context of stipe and the stipe is longer than width of pileus) or collybioid (pileus not umbilicate, not conical; free or adnate lamellae, tough context and context of pileus continuous with context of stipe). The persistence of basidiomata is dependent on their size.

2. Pileus: Pileus can be less than 5 mm and up to 40 cm diameter, paraboloid, campanulate or umbonate, planoconvex to planoconcave; the covering of the pileus can be smooth, granulose, with patches, squamules or pyramidal spines, and ranges from white, grey to almost black, pink, purplish, yellow, orange, to olivaceous and a wide variety of browns.

3. Lamellae: Lamellae are always free, moderately crowded to crowded, ventricose to distinctly ventricose, white to sulfur-yellow or pale green, sometimes deep pink.

4. Stipe: Stipe is always hollow, cylindrical with a wider base or bulbous; a membranous, annulus is present in all taxa of *Leucoagaricus, Leucocoprinus, Macrolepiota* and *Chlorophyllum*.

5. Annulus: Annulus is usually present in all genera, the shapes are membranous or cuff-like with or without flaring part; some species of *Lepiota*, *Chamaemyces*, *Coniolepiota*, *Cystolepiota* and *Eriocybe* have an annular zone with fibrils or squamules on the stipe which are same as the structures on the pileus covering.

6. Context: Context of pileus is usually dull; context of stipe is shiny.

Smell and Taste: The smell and taste varies and is very distinctive or less distinctive in different species and may be sweet, fruity, rubber-like, soapy, or fungoid astringent.

7. Spore print: Spore print is white to cream or pale yellow, rarely pale pink; it is dull green in *Chlorophyllum* species.

8. Colour reactions: In *Lepiota* species might develop orange tinges with age; several groups of species in *Leucoagaricus* turn red when touched. Chemical reactions include a red-brown or a green reaction with ammonia or KOH or a colour change with iron salts.

9. Spores: Spores show a range of size and shapes among species or genera such as ellipsoid-ovoid in most genera, spurred or fusiform in some *Lepiota* species, with a germ pore in *Chlorophyllum, Leucocoprinus* and *Macrolepiota*, and some *Leucoagaricus* species; and spores are ellipsoid and finely verrucose in *Melanophyllum*. The spore wall of most species turn red brown in Melzer's reagent, blue in Cotton blue and red in Congo red; in *Leucoagaricus, Leucocoprinus, Macrolepiota* and *Chlorophyllum*, and a few *Lepiota* species the inner spore wall turns pink in cresyl blue.

10. Basidia: Basidia are 4-spored, in a few species 2-spored; mostly narrowly clavate, but in *Leucocoprinus* relatively wide and heteromorphous.

11. Cheilocystidia and pleurocystidia: Cheilocystidia are present in most species, with the exception of some *Cystolepiota* species; pleuracystidia are absent in most species, but present in some *Leucoagaricus* species and in *Chamaemyces*. The cheilocystidia are clavate, utriform, sphaeropedunculate to irregular and with or without apical crystals, without or with apical excrescence, and in some cases a septum is present; generally the cystidia are hyaline.

12. Trama: There are two kinds of hymenophoral trama in lepiotaceous fungi; a regular trama is present in some genera such as *Chamaemyces*, *Cystolepiota*, *Lepiota* and *Melanophyllum*, and a more irregular, often trabecular trama is present in *Leucoagaricus, Leucocoprinus, Macrolepiota* and *Chlorophyllum.*

13. Pileus- and stipe covering: The structure of the pileus covering is very important, it can be a cutis, a hymeniderm, an epithelium, a trichoderm or variations on any of those. The covering can be of velar origin or part of the pileipellis.

14. Veils: A universal and a partial veil are always present.

1.2 Introduction to Genus *Lepiota*

Lepiota (Pers.: Fr.) S.F. Gray is a large and diverse genus of white spored *Agaricales* which commonly occur in the tropics (Dennis, 1952). The genus *Lepiota*

belongs to the family *Agaricaceae* (Kirk, Cannon, Minter & Stalpers, 2008; Singer, 1986) and the classification of the genus is shown in Fig. 1.1.

The first definition of *Lepiota* was given by Fries (1821) and this definition included *Cystolepiota, Leucoagaricus, Leucocoprinus* and *Macrolepiota*. Many mycologists divided *Lepiota* into different sections according to characters concerning the spores and the pileus covering (e.g. Bon, 1993; Candusso & Lanzoni, 1990; Vellinga, 2001). The current divisions and characterizations of the genus are mostly based on European collections and studies. In addition, a few mycologists have studied *Lepiota* in Asia, e.g. Pegler (1972) revised genus *Lepiota* in Sri Lanka and nine sections were present, and Manjula (1983) gave a revised list of *Lepiota* from India and Nepal and five section were found comprising *Stenosporae, Ovisporae, Cristata, Echinatae* and *sericellae*. However, *Lepiota* species in these and many other studies also included species from the other genera in the family, such as *Coniolepiota*, *Cystolepiota*, *Leucoagaricus* and *Leucocoprinus*. The currently accepted definition of *Lepiota* is provided by Vellinga (2001) and the classification of *Lepiota* species is mostly based on morphology and anatomy of spores and cheilocystidia, and the structure of the pileus covering.

Domain: Eukaryota

Kingdom: Fungi

Phylum: Basidiomycota

Subphylum: Agaricomycotina

Class: Agaricomycetes

Subclass: Agaricomycetidae

Order: Agaricales

Family: Agaricaceae

Genus: *Lepiota, Leucoagaricus, Leucocoprinus, Macrolepiota, Chlorophyllum, Cystolepiota,*

From Kirk, P. M, Cannon, P. F, Minter, D. W, Stalpers, J. A. (2008). **Ainsworth & Bisby's Dictionary of the fungi(10th Ed)**.Wallingford, U.K: CAB International

Figure 1.1 Classifications of *Lepiota* and Related Genera of Lepiotaceous Fungi

1.3 General Characteristics of *Lepiota*

Lepiota was based on *Agaricus* sect. *Lepiota* Persoon (Persoon, 1797). The type species of *Lepiota* was not indicated at that time and *L. procera* (Scop. : Fr.) Gray [= *Macrolepiota procera* (Scop. : Fr.) Singer] was considered to be type by Earle in 1909, but *M. procera* is not presently included in *Lepiota*. Another species, *Agaricus colubrinus* Bull. [current name = *L. clypeolaria* (Bull. : Fr.) P. Kumm.] was also suggested to be type species of *Lepiota* (Singer & Smith, 1946), and this is now the accepted type of the genus.

Figure 1.2 Some Species of *Lepiota* in tropical East Asia

Vellinga (2001) provided a definition for the genus based on macro- and microcharacters. Characteristic macrocharacters for *Lepiota* include pluteoid basidiomata with a persistent universal veil and a pileus surface that in most species is squamulose. The lamellae are free and white to cream, the annulus or annular zone presents the remnants of the partial veil and the spore print is white to cream. Typical microcharacters in *Lepiota* are ellispsoid, fusiform or spurred basidiospores in most species, which are smooth or minutely rough in a few species. The walls are hyaline, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue or, rarely, with a pink inner wall in Cresyl blue (Vellinga, 2001), and the spore walls swell slightly or do not swell in ammonia [NH3 (aq)] and acetic acid (CH3COOH (aq)); spores are mostly binucleate, although uninucleate in a few species. Cheilocystidia are present in most species. Pleurocystidia are rarely present. The hymenophoral trama is regular. The pileus covering is a trichoderm, a cutis, a hymeniderm or an epithetlium and clamp-connections are present in most species. Some tropical species of *Lepiota* are given in Fig. 1.2.

1.4 General Classification and Division of Genus *Lepiota*

1.4.1 Key to Sections of Genus *Lepiota*

1. Pileus covering a trichoderm or a cutis.....2
1. Pileus covering not a trichoderm or a cutis.....3
2. Basidiospores spurred, or with straight base.....(3) Sect. *Stenosporae*
2. Basidiospores ellip not spurred, or without straight base.....4
3. Pileus covering a hymeniderm and basidiospores ovoid or spurred…….. ……...(5) Sect. *Lilaceae*
3. Pileus covering not a hymeniderm 5
4. Basidiospores ellipsoid, fusiform-amygdaliform with straight or convex abaxial side and suprahilar depression..... (1) Sect. *Lepiota*
4. Basidiospores ellipsoid to ovoid; annulus present or as annular zone…... ……..... (2) Sect. *Ovisporae*
5. Pileus covering a cutis made up of articulate and cylindrical, repent to ascending elements; clamp connection absent; basidiospores ellipsoid to ovoid……..……..…….....(4) Sect. *Fuscovinaceae*
5. Pileus with acute squamules; pileus covering made up of globose to ellipsoid elements in agglutinated chains; basidiospores ellipsoid, or spurred…….……...……(6) Sect. *Echinatae*

1.4.2 Section *Lepiota*

Members of this section are charac-terized by fusiform-amygdaliform basidiospores with convex abaxial and convex adaxial sides, or with a straight abaxial side, in combination with a pileus covering made up of long cylindrical elements with or without short clavate elements at the base and with clamp-connections (Vellinga, 2001). Candusso and Lanzoni (1990) divided this section into 2 subsections, species with subamygdaliform basidiospores belong to subsection *Latisporinae* Bon with *Lepiota latispora* Kühner ex Wasser as the type species. Species with largely ellipsoid

or fusiform basidiospores were placed in subsection *Lepiota*. The taxonomy of this section does not include *Lepiota cortinarius* J.E. Lange which was placed in section *Stenosporae* because of the long fusiform basidiospores and slightly spurred base. However, section *Lepiota* is not divided into subsections in the present classification and *Lepiota cortinarius* is presently considered to be a member of this section based on morphology and molecular data (Vellinga, 2001; 2003a).

1.4.3 Section *Ovisporae* (J.E. Lange) Kühner

This section includes species with ellipsoid to oblong spores, a trichoderma pileus covering made up of long elements with or without short clavate elements at the base of these long elements and having clamp connections. Two subsections are separated by structures of elements of the pileus covering. Species whose pileus covering is made up of long erect elements with short clavate elements belong to the subsection *Felininae* Bon while species whose pileus covering are made up of long erect elements without short clavate elements belong to subsection *Helveolinae* Bon & Boiffard (Candusso & Lanzoni, 1990; Vellinga, 2001).

1.4.4 Section *Stenosporae* (J.E. Lange) Kühner

The species in this section are distinguished from other groups in having cylindrical spores with a spurred base, the pileus covering is a cutis or a trichoderm made up of long erect and slender elements and with clamp connections (Vellinga, 2001). There are two forms of pileus covering (cutis and trichoderm) in this section but it is not divided into subsections.

1.4.5 Section *Fuscovinaceae* Bon and Candusso

This section comprises species only *Lepiota fuscovinacea* (J.E. Lange) Kühner, a species without clamp connections, with articulate and cylindrical elements in the pileus covering, and ovoid basidiospores (Candusso & Lanzoni, 1990; Vellinga, 2001).

1.4.6 Section *Lilaceae* M. Bon

Species in this section are characterized by a hymeniderm pileus covering which is made up of tightly packed clavate to narrowly clavate elements, and by subglobose to ellipsoid or spurred basidiospores (Vellinga, 2001). Candusso and Lanzoni (1990) put *Lepiota cristata* (species with spurred spores and hymenodermal pileus covering) into Section *Stenosporae*, but Vellinga (2001) put every species with hymenodermal pileus covering into Section *Lilaceae*; this is supported by phylogenetic studies based on molecular characters (Vellinga, 2003a).

1.4.7 Section *Echinatae* Fay

Species in this section are characterized by a pileus covering with acute squamules, made up of globose to ellipsoid elements in agglutinated chains, transient to long and coloured hyphae on the pileus surface; basidiospores are dextrinoid and rarely not reacting with Melzer‘s Regent, ovoid to ellipsoid, ovoid or cylindrical with a spurred base; clamp connections are present or rarely absent (Vellinga, 2001). This section has been recognized at genus level as *Echinoderma* (Locq. ex Bon) Bon.

1.5 Phylogenetic Studies of *Lepiota*

Phylogenies of the genera in family Agaricaceae were presented by Vellinga (2004b) and Vellinga, Sysouphanthong & Hyde (2011). Some of the main findings of these studies are: *Leucoagaricus* and *Leucocoprinus* form together one large monophyletic cade, while *Lepiota*, *Cystolepiota* and *Melanophyllum* also form together one monophyletic cade. The phylogeny of the genus *Lepiota* and its satellite genera was the subject of a study by Vellinga (2003b), using evidence from nrITS and LSU sequences. Four clades could be recognized: Clade (I) is characterized by a trichodermal pileus covering with fusiform, penguin-shaped and broadly fusiform to ellipsoid spores, accommodating species from sect. *Lepiota* and *Ovisporae*. In clade (II) are species with 2 types of pileus covering, trichodermal and a cutis pileus covering and the spores are ellipsoid or spurred spores, comprising species from sect. *Stenosporae* and sect. *Ovisporae*. In clade (III), species with hymenidermal pileus covering with variable spore shape of sect. *Lilaceae*. The fourth clade is not monophyletic based on ITS-data and species in this group are in sect. *Echinatae* Fay., but in a phylogenetic tree based on ITS and LSU data, sect. *Echinatae* is split into 2 subclades.

1.6 Significance, Toxicity, Medical Application and Cultivation of *Lepiota*

Many *Lepiota* species are reported to be poisonous, with only a few species eaten. However, *Leuoagaricus americanus, Leucoagaricus leucothites, Macrolepiota* species and *Chlorophyllum rachodes* are noted as good edible species (e.g. Arora, 1986; Marshall, 2008; Fischer & Bessette, 1992; Christensen, 1972; 1992). Boa (2004) gave a global overview of the use and importance to humans of wild mushrooms; *Macrolepiota procera, M. madirokenlensis, M. mastoidea* and *Chl. rachodes* are economically important edible fungi, and some edible or medicinal species are *L. aspera, L. grassei, L. henningsii,* and *L. magnispora.*

Some species that are reported to be edible are sometimes toxic when eaten in different regions; this includes species such as *Leucoagaricus americanus, L. clypeolaria* and *Chl. rachodes* (Boa, 2004; Christensen, 1972; 1992; Fischer & Bessette, 1992; Marshall, 2008). It is not clear whether these are the same species or if they are wrongly identified or whether species have different properties depending on where they grow. Many species of *Lepiota* are poisonous as they contain amatoxins (cyclopeptides) as in species of *Amanita*, *Pholiotina*, and *Galerina*, these toxins are generally considered the world's most toxic substance in mushrooms (Benjamin, 1995). For *Amanita* species the genes encoding for these major toxins have been discovered and described (Hallen, Luy, Scott-Craig & Walton, 2007), but the genes and the pathways in the other genera are not known yet. The amatoxins from *Lepiota* species have been implicated in human and animals poisonings which ended in hepatic failure and death (e.g. Wieland, 1986; Bettin, Marcon, Scevola, Dona &

Carlassara, 1993; Haines, Lichstein & Glickerman, 1986; Khelil et al., 2010; Bresinsky & Besl, 1985; Enjalbert et al., 2002). Most toxic *Lepiota* species (Table 1.1) belong to sections *Ovisporae* and *Stenosporae* (Gérault & Girre, 1975; 1977). Because of possible confusion with those species consumption of any *Lepiota* species is generally not recommended. There are no reports on cultivation efforts of *Lepiota* species; however, some *Macrolepiota* species are cultivated (Coetzee, Trendler & Eicker, 1980; Manz, 1971; Gblolagade, Ajayi, Oku & Wankasi, 2006; Jones, Tantichareon & Hyde, 2004). Gblolagade, Ajayi, Oku & Wankasi (2006) studied the effect of different nutrient sources on biomass production of *M. procera* in submerged liquid cultures by comparing mycelial dry weight of *M. procera* in different nutrient sources; fruitbodies were not produced in these media. Jones, Tantichareon & Hyde (2004) cultivated *M. gracilenta* by using a growth medium, composed of a sterilized mixture of composted straw, rice bran, gypsum, urea and calcium carbonate; this substrate produced fruitbodies well.

Table 1.1 *Lepiota* Species Reported as Poisonous or Edible

Species	Edible	Poisonous
L. aspera (Pers.) Quél.	+	
L. clypeolaria (Bull.) P. Kumm.	+	
L. brunneoincarnata Chodat & Martin		+
L. brunneolilacea Bon & Boiffard		+
L. castanea Quél.		+
L. clypeolarioides Rea		+
L. elaiophylla Vellinga & Huijser		+
L. felina (Pers. : Fr.) Karsten		+
L. fulvella Rea (*L. boudieri*)		+
L. friesii (Lasch) Quél.	+	
L. grassei R. Heim	+	
L. griseovirens Maire		+
L. helveola Bres.		+
L. henningsii Sacc. & P. Syd.	+	
L. josserandii Bon & Boiffard		+
L. kuehneri Huijsm. ex Hora		+
L. langei Locq.		+
L. ochraceofulva Orton		+
L. pseudohelveola Kühner ex Hora		+
L. subincarnata J.E. Lange		+
L. ventriosospora D.A. Reid	+	

1.7 Distribution of *Lepiota* in South and Southeast Asia

Kirk et al. (2008) estimated that there are 400 *Lepiota* species worldwide, and though it was speculated that in tropics the *Leucoagaricus/Leucocorpinus* clade is more species rich than *Lepiota*, and that *Lepiota* species are more numerous in temperate regions (Vellinga, 2004a), many species have been reported from eastern Asia. Interpretation of these names and reports are hampered by the fact that many early reports only give a few sentences per species, modern type studies have not been carried out for all taxa, and literature is not always easily accessible, European names have been used, and even in very recent literature, *Leucoagar*icus species have been included in the genus *Lepiota* s. str. (e.g. Pegler, 1972; Kumar & Manimohan, 2009; Wang & Yang, 2005). We present an overview of *Lepiota* species reported from eastern Asia in Table 1.2, which is meant as a first attempt to compile the available data; probably some reports and literature has not been included; *Cystolepiota*, *Coniolepiota*, *Macrolepiota* and *Leucoagaricus* taxa are not treated here. We were unable to find any records for Bangladesh, Cambodia, Laos, and Myanmar.

The earliest study from India was started by Berkeley (1850; 1852; 1854) and all *Lepiota* species were placed in *Agaricus* at this time, and for this reason, some reports of *Lepiota* are not verified. The next study was carried out by Massee (1912) and he described four new species from India, *Lepiota mimica*, *L. punicea*, *L. flavophylla* and *L. sericea*. Other substantial studies were from South India and Nepal, with many species being described and illustrated (Natarajan & Manjula, 1983) 14 species and two varieties of *Lepiota* were described and illustrated and all species were new records for India; Manjula (1983) gave a revised list and a key to 30 species Indian species of *Lepiota*; Rawla & Arya (1991) described *Lepiota sulphurea* and *L. nainitala* as new species from north west India; six species of *Lepiota* have been recorded from Nepal (Adhikari, 1990; 1991; Manandhar & Adhikari, 1994). Natarajan, Kumaresan, & Narayanan (2005) provided a checklist of Indian Agarics and Boletes, this included 18 species. Kumar & Manimohan (2009) recently studied 22 *Lepiota* taxa from Kerala state, and they recorded eight new species and one new variety; however, several of these clearly belong to *Leucoagaricus*; because of the absence of clamp connections and the structure of the pileus covering; these have not been included in Table 1.2.

Other substantial studies were in Sri Lanka; Berkeley (1847) who first studied *Lepiota* from Sri Lanka and Berkeley and Broome (1871) studied materials from Gardner and Thwaites. Over thirty years later, Petch (1910; 1917) revised fungi of Sri Lanka and described *L. viridiflava* as new species to Sri Lanka, and Petch & Bisby (1950) gave the checklist of *Lepiota* at that time. The connected study of *Lepiota* was done again by Pegler (1972) who studied the type collections of the species described by Berkeley & Broome (1871) and he recorded 53 species from Sri Lanka, many of them belonging to other genera than *Lepiota*; it also appeared that several of Berkeley & Broome‘s species had to be accommodated in unrelated genera such as *Pluteus* or *Amanita*. After Pegler‘s study it seems that no mycologist worked on *Lepiota* in Sri Lanka.

The study of *Lepiota* in Indonesia is poor. A few studies of the genus were done by Hennings (1900a; 1900b; 1901) and three new species were described to

Indonesia which are *L. aurantiaca, L. verrucosa* and *L. celebica,* and Boedijn (1940) recorded *L. flavophylla* and *L. oenopoda* from the region. Twenty species of *Lepiota* with spurred spores were studied in Papua New Guinea; those species are included sections *Echinatae, Stenosporae* and *Lilaceae* (Horak, 1981).

For the close area of Indonesia, Singapore, *L. semivestita, L. carneorubra, L. ochracea, L. ferruginosa* and *L. albida* were reported (Massee, 1914). Some *Lepiota* species are known from the Philippines by Copeland (1905) and 4 species were reported; Graff (1914) provided 7 species and 2 new species; Mendoza and Leus-Palo (1934; 1938) published *L. americana* as edible species and they revised 12 species of Philippine *Lepiota*.

Very few *Lepiota* species are known from Vietnam (Kiet, 1998; Yang, 2000). Yang (2000) studied the type collections of the species described by Patouillard (1892; 1907; 1917), and only *Lepiota demangei* is clearly a *Lepiota* species, all others are accommodated in *Leucoagaricus*, *Leucocoprinus* or *Micropsalliota*.

Several reports of macrofungi in Thailand have been carried out (Anong Chandrasrikul, 1996; Anong Chandrasrikul et al., 2008; Ruksavong & Flegel, 2001; Soytong, 1994; Høiland & Schumacher, 1982), but the lepiotaceous fungi have rarely been studied and are poorly known. Some mycologists have studied Basidiomycota in general, and have reported a few lepiotaceous species, such as *Lepiota cristata*, *L. clypeolaria*, *L. cortinarius*, and *L. pseudohelveola* (Anong Chandrasrikul, 1996, Chandrasrikul et al., 2008; Ruksawong & Flegel, 2001; Soytong, 1994). Sysouphanthong, Hyde, Chukeatirote, Bahkali, & Vellinga (2011a) and Sysouphanthong, Hyde, Chukeatirote, Bahkali, & Vellinga (2011b) illustrated 15 species of *Lepiota* section *Stenosporae* and *Lepiota* in northern Thailand and 5 species were described as new for science. However, relatively little attention has been given to the distribution and diversity of the lepiotaceous fungi in Thailand. In the area of Northeast Asia, there are many species of *Lepiota* recorded an described as new from Japan (e.g. Hongo, 1956a; 1956b; Hongo, 1958; Hongo, 1959; Hongo, 1965; Hongo, 1973; Imai, 1938; Kasuya & Knudsen, 2003). *Lepiota grangei* and *L. cristata* are listed from Korea (Kim, 1998; Wojewoda, Heinrich & Komorowska, 2004). Yang, Ge & Liang (2005) gave a list of species diversity of Lepiotoid fungi in China and they provided 50 species of *Lepiota*., and in recent years several new species have been described, and type collections restudied (Liang, 2007; Liang & Yang, 2011; Liang et al., 2011). In addition, Liang et al. (2009) studied the population structure of *Lepiota cristata* in China by using three genes. Some other studies are those by Wang & Yang (2005; 2006), and a more general overview by Mao (2000). All species review is presented in Table 1.2.

1.8 Objectives

1.8.1 To study diversity and distribution of *Lepiota* species in Northern Thailand.

1.8.2 To determine phylogenetic relationships among species of *Lepiota* in Northern Thailand.

Table 1.2 Distribution of *Lepiota* in South Asia and Southeast Asia

Species	CN	ID	IN	JP	NP	Ko	Ph	SG	SL	TH	VN	PN
L. adusta (Horak) E. Horak												+
L. alba Beeli	+											
L. albida Massee								+				
L. albocitrina Pat. [probably in *Leucoagaricus*, see Yang 2000]											+	
L. alopochroa (Berk. & Br.) Sacc.		+							+	+		+
L. altissima Massee	+	+										
L. anax (Berk.) Sacc.		+										
L. apalochroa (Berk. & Br.) Sacc.		+							+	+		
L. aspera (Pers.) Quél. (*L. acutesquamosa* (Weinm.) P. Kumm.)	+			+	+					+		+
L. atrata E. Horak												+
L. atrosquamulosa Hongo	+			+								
L. attenuata J.F. Liang & Zhu L. Yang	+											
L. aureofulvella Sysouphanthong et al.										+		
L. aurola E. Horak												+
L. azalearum (Murril) Dennis	+											
L. babruka Kumar & Manim.		+										
L. boudieri Bres.	+											
(*L. fulvella* Rea; *L. fulvella* f. *gracilis* J.E. Lange)												
L. brevipes Murrill		+										
L. brevipes var. *distincta* Kumar & Manim.		+										
L. bichroma E. Horak												+
L. brunneoincarnata Chodat & Martín	+											
L. calcarata (Horak) E. Horak												+
L. candida Copeland							+					
L. carneorubra Masse								+				

Note. CN= China, ID= India, IN= Indonesia, JP= Japan, Ko= North and South Korea, Ph= Philippines, SG= Singapore, SL= Sri Lanka, TH= Thailand, VN= Vietnam, PN= Papua New Guinea

Table 1.2 (continued)

Species	CN	ID	IN	JP	NP	Ko	Ph	SG	SL	TH	VN	PN
L. castanea Quél.	+	+		+	+					+		+
L. catenariocystidiata Wang & Yang	+											
L. celebica Henn.			+									
L. ceramogenes (Berk. & Br.) Sacc.	+						+					
L. cf. *hispida* (Lasch.) Fr.		+										
L. chichuensis W.F. Chiu	+											
L. cinnamomea Hongo	+											
L. citrophylla (Berk. & Br.) Sacc.	+	+							+	+		
L. clypeolaria (Bull. : Fr.) P. Kumm.	+	+		+						+		
L. cortinarius J.E. Lange	+									+		
L. crepusculata E. Horak												+
L. cristata (Bolt. : Fr.) P. Kumm.	+	+		+	+	+	+			+		
L. cristata var. *macrospora* Zhu L. Yang	+											
L. cristatanea J.F. Liang & Zhu L. Yang	+											
L. deliciolum (Berk.) Sacc.		+										
L. demangei Pat.											+	
L. disseminata E. Horak												+
L. echinacea J.E. Lange		+										
L. elaiophylla Vellinga & Huijser		+										
L. elata Copel.							+					
L. epicharis (Berk. & Br.) Sacc.	+	+							+			
L. erminea (Fr.) Gillet	+	+			+							
L. erythrogramma (Berk & Br.) Sacc. (*L. alborussea* (Berk. & Broome) Sacc.	+	+							+			

Note. CN= China, ID= India, IN= Indonesia, JP= Japan, Ko= North and South Korea, Ph= Philippines, SG= Singapore, SL= Sri Lanka, TH= Thailand, VN= Vietnam, PN= Papua New Guinea

Table 1.2 (continued)

Species	CN	ID	IN	JP	NP	Ko	Ph	SG	SL	TH	VN	PN
L. erythrosticta (Berk. & Broome) Sacc.		+							+	+		+
L. eurysperma Sysouphanthong et al.										+		
L. exocarpi Cleland												+
L. felina (Pers.) P. Karst.	+				+						+	
L. felinoides var. *macrospora* W. F. Chiu	+											
L. ferruginosa Massee								+				
L. flavophylla Massee			+		+				+			
L. fraterna E. Horak												+
L. friesii (Lasch) Quél.					+							
L. fuscosquamea Peck	+						+					
L. fuscovinacea F.H. Møller & J.E.Lange	+											
L. grangei (Eyre) J.E. Lange						+				+		+
L. cf. *griseorubescens* Dennis	+											
L. griseovirens Maire		+								+		
L. helveola Bres.	+											
L. hispida Lasch							+					
L. hystrix F.H. Møller & J.E.Lange	+											
L. ianthinosquamosa Pegler	+	+										
L. ignivolva Bousset & Joss.	+											
L. implana (Berk.) Sacc. [probably an *Amanita* species]		+										
L. infelix E. Horak										+		+
L. insimulata E. Horak	+											+
L. jacobi Vellinga & Knudsen	+											
L. leontoderes (Berk. & Br.) Sacc.		+							+			
L. lepidophora (Berk. & Br.) Sacc.		+							+			

Note. CN= China, ID= India, IN= Indonesia, JP= Japan, Ko= North and South Korea, Ph= Philippines, SG= Singapore, SL= Sri Lanka, TH= Thailand, VN= Vietnam, PN= Papua New Guinea

Table 1.2 (continued).

Species	CN	ID	IN	JP	NP	Ko	Ph	SG	SL	TH	VN	PN
L. leprica (Berk. & Br.) Sacc.									+			
L. lilacea Bres.	+											
L. longicauda Henn.			+									
L. luteophylla Sundberg		+										
L. luteocastanea E. Horak												+
L. luteocephala Beeli		+										
L. magnispora Murrill (*L. ventriosospora* D. Reid)	+			+								
L. mammosa Henn.			+									
L. manilensis Copeland.							+					
L. metulispora (Berk. & Broome) Sacc.	+	+							+	+		
L. micropholis (Berk. & Br.) Sacc.	+								+			
L. microspila Berk.												+
L. microcarpa Sysouphanthong et al.										+		
L. microspora Massee		+										
L. mimica Massee		+										
L. montosa (Berk.) Sacc. [probably an *Amanita* species]		+										
L. murinocapitata Dennis [probably in *Leucoagaricus*]		+										
L. nainitala Rawla		+										
L. nigricans Pat.											+	
L. nirupama Kumar & Manim.		+										
L. nivalis W.F. Chiu	+											
L. ochracea Massee								+				
L. oenocephala (Berk. & Br.) Sacc.									+			
L. oreadiformis Velen.	+											

Note. CN= China, ID= India, IN= Indonesia, JP= Japan, Ko= North and South Korea, Ph= Philippines, SG= Singapore, SL= Sri Lanka, TH= Thailand, VN= Vietnam, PN= Papua New Guinea

Table 1.2 (continued).

Species	CN	ID	IN	JP	NP	Ko	Ph	SG	SL	TH	VN	PN
L. pardalota (Berk. & Br.) Sacc.									+			
L. papillata Sysouphanthong et al.										+		
L. parvannulata (Lasch) Fr.	+	+										
L. philipinensis Mendoza							+					
L. phlyctaenodes (Berk. & Br.) Sacc.	+	+							+			
L. plumbicolor (Berk. & Br.) Sacc.		+							+			
L. poliochloodes Vellinga & Huijser										+		
L. pongduadensis Sysouphanthong et al.										+		
L. pselliophora (Berk. & Br.) Sacc.									+			
L. pseudoasperula (Knudsen) Knudsen		+										
L. pseudohelveola Kuehner ex Hora (*L. pseudolilacea* Huijsman)										+		
L. pulcherrima P.W. Graff							+					
L. punicea Massee		+										
L. purpurata (G. Stev.) E. Horak												+
L. pyrrhaes (Berk. & Br.) Sacc.		+							+			
L. rhyparophora (Berk. & Br.) Sacc.									+			
L. roseoalba P. Henn.			+									
L. sanguinea Sathe & Deshp.		+										
L. semivestita Massee								+				
L. shixingensis Bi & Li	+											
L. shveta Kumar & Manim,		+										
L. spiculata Pegler	+											
L. squamatula E. Horak												+
L. squamulosa B. Tolgor & Yu Li	+											
L. subamanitiformis Dennis	+	+										

Note. CN= China, ID= India, IN= Indonesia, JP= Japan, Ko= North and South Korea, Ph= Philippines, SG= Singapore, SL= Sri Lanka, TH= Thailand, VN= Vietnam, PN= Papua New Guinea

Table 1.2 (continued).

Species	CN	ID	IN	JP	NP	Ko	Ph	SG	SL	TH	VN	PN
L. subgracillis Kühner		+										
L. subincarnata J.E. Lange (*L. josserandii* Bon & Boiff.)	+	+										
L. subclypeolaria (Berk. & M.A. Curtis) Sacc.	+											
L. subrufa Natarajan & Manjula		+										
L. sulphopenita P.W. Graff							+					
L. sulphurea Rawla		+										
L. thiersii Sundberg		+										
L. thrombophora (Berk. & Br.) Sacc.	+	+							+			
L. verrucosa Henn.			+									
L. viridiflava Petch									+			
L. xanthophylla P.D. Orton		+										
L. zalkavritha Kumar & Manim.		+										

Note. CN= China, ID= India, IN= Indonesia, JP= Japan, Ko= North and South Korea, Ph= Philippines, SG= Singapore, SL= Sri Lanka, TH= Thailand, VN= Vietnam, PN= Papua New Guinea**.**

CHAPTER 2

DIVERSITY OF *Lepiota* SPECIES IN NORTHERN THAILAND

2.1 Introduction

Basidiomycytes known as macrofungi are a very large group which play an important role in evolution, ecosystem and function of human improvement. As a result, numerous studies relevant to this family group have been carried out throughout the world including America, Europe, Africa and Asia (Kirk, Cannon, David, & Stalpers, 2001). Presently, the Basidiomycetes are classified into diverse families. The family Agaricaceae is one of the most diverse and large groups of Basidomycetes comprising of many important edible species. Many of them can be cultivated for commercial purpose; these include *Agaricus* spp., *Macrolepiota procera*, *M. rachodes*, *M. madirokenlensis*, *M. mastoidea*, and *Leucoagaricus naucinus* (Kendrick, 2000; Boa, 2004).

Lepiota, one of the mushroom genera in the family *Agaricaceae* (Singer, 1986; Kirk et al., 2008), is known to consist of several poisonous species. They have been shown to produce amatoxins (cyclopeptides) similar to those synthesized in *Amanita*, *Pholiotina* and *Galerina* species. The amatoxins are generally considered the world's most toxic substance found in mushrooms (Benjamin, 1995).

Formerly, *Macrolepiota* were classified as *Lepiota*. This classification has currently been revised based on their morphology and molecular means. The difference between both genera is also in agreement with the edible species (*Macrolepiota*) and the inedible species (*Lepiota*).

The genus *Lepiota* can be divided into different sections according to characters concerning the spores and the pileus covering. Presently, there are 6 sections: *Echinatae, Lepiota, Lilaceae, Fuscovinacae, Ovisporae* and *Stenosporae* (Bon, 1993; Candusso & Lanzoni, 1990; Vellinga, 2001). There is only a limited study of the *Lepiota* genus in Asia. For example, Pegler (1972) revised the genus *Lepiota* in Sri Lanka and, and Manjula (1983) gave a revised list of *Lepiota* from India. However, *Lepiota* species described in this work also included species from the other genera such as *Coniolepiota*, *Cystolepiota*, *Leucoagaricus* and *Leucocoprinus*. The currently accepted definition of *Lepiota* is provided by Vellinga (2001) (see Section 1.4).

Several reports of macrofungi in Thailand have been carried out (Anong Candrasrikul, 1996; Ruksavong & Flegel, 2001; Soytong, 1994; Jones, Tanticharoen, & Hyde, 2004; Desjardin, Flegel & Boonpratuang, 2004; Anong Candrasrikul et al.,

2008), but the genus *Lepiota* has rarely been studied and are poorly known. Anong Candrasrikul et al. (2011) provide a checklist of macrofung in Thailand and there are 24 species of *Lepiota* present but the species are confused with *Macrolepiota, Leucoagaricus, Leucocoprinus* and *Cystolepiota*. Moreover, Sysouphanthong, Hyde, Chukeatirote, Bahkali, & Vellinga (2011a) and Sysouphanthong, Hyde, Chukeatirote, Bahkali, & Vellinga (2012) studied on *Lepiota* sections *Lepiota* and *Stenosporae* by morphology and molecular data, and sixteen species were new records including five new species to Thailand.

As a consequence, this study has been carried out as part of the survey of the *Lepiota* diversity in Thailand. The data are expected to provide an overview of the diversity of *Lepiota* in northern Thailand. Moreover, it is also interesting to study if there is any difference in *Lepiota* diversity between Thailand and other countries (i.e., Sri Lank, India and Nepal, The Philippines, and Japan). Overall, our data obtained will provide an insight to a better understanding of the distribution and diversity of the lepiotaceous mushrooms in Asia.

2.2 Materials and Methods

2.2.1 Collecting Sites and Mushroom Sampling

In order to investigate the diversity and distribution of *Lepiota* species in northern Thailand, fresh samples were collected from 16 localities in three Districts of Chiang Mai Province and three Districts of Chiang Rai Province in northern Thailand (Table 2.1 and Figure 2.1). Localities details of GPRS reading, plant species, type of soils, substrates, habit, habitats and forest were recorded. The collection was carried out during the rainy season (May to October) of 2007 to 2010.

Table 2.1 Sampling Sites in This Study

Sites	Coordinate/Altitude	Forest types
1. Forest of Mae Sae village, Pa Pae Sub-district, Mae Taeng District, Chiang Mai Province (FM)	N 19˚ 14.59', E 98˚, 39. 45'/ 962 m.	Deciduous mixed rain forest dominated by *Castanopsis armata*, *Castanopsis* sp., *Pinus* sp., *Lithocarpus* sp. etc.
2. Forest of Tung Jaw Village, Pa Pae Sub-district, Mae Taeng District, Chiang Mai Province (FT)	N 19˚ 08.07', E 98˚ 38.90'/ 1300 m.	Deciduous mixed rain forest dominated by *Pinus kesiya, Castanopsis armata* etc.
Forest of Pong Duaed Village, Pa Pae Sub-district, Mae Taeng District, Chiang Mai Province (FP)	N 16˚ 06. 16', E 99˚ 43 07'/ 805 m.	Deciduous mixed rain forest with several kind of tree species and *Bambosa* spp.
Pha Deng village, Pa Pae Sub-district, Mae Taeng District, Chiang Mai Province (PV)	N 19˚ 07.13', E 98˚43. 52'/ 905 m.	Deciduous mixed rain forest dominated by *Castanopsis armata, Lithocarpus* spp. etc.

Table 2.1 (continued)

Sites	Coordinate/Altitude	Forest types
Mork Fah Waterfall, Mae Taeng District, Chiang Mai Province (MF)	N 20˚ 02. 43', E 99˚ 52. 35'/ 596 m.	Mixed forest dominated by *bambusa* spp., *Lithocarpus polystachyus* and other trees
Huai Kok Ma, Suthep Sub-diatrict, Muaeng District, Chiang Mai Province (HK)	N18°48.62' E098°54.60'/ 1145 m.	Rain forest dominated by *Castanopsis* spp., *Lithocarpus polystachyus* and other trees.
Forest at Junction of Highway 1009 and Road to Mae Cheam, Chom Thong District, Chiang Mai Province (JM)	N 19˚ 31.58', E 98˚ 29.64'/ 1700 m.	National Park with deciduous rain forest dominated by *Castanopsis, Lithocarpus echinops* etc.
Forest of Highway 1009 at 25 km marker, Chom Thong District, Chiang Mai Province (HW)	N 18˚ 32.54', E 98˚ 33.51'/ 1076 m.	National Park with deciduous rain forest dominated by *Castanopsis armata and Pinus* sp.
Huai Nam Dang, Mae Taeng District, Chiang Mai Province (HN)	N 18˚ 18.21', E 98˚ 36.01'/ 1550 m.	National Park with deciduous rain forest dominated by *Pinus kesiya*, *Dipterocarpus* spp., Bamboo & scattered *Castanopsis* sp.
Mai Mai Lai Village, Mae Taeng District, Chiang Mai Province (ML)	N 19˚ 06.12', E 98˚ 53.21'/ 500 m.	Deciduous forest dominated by *Dipterocarpus* spp.
Mae Sa Village, Mae Rim District, Chiang Mai Province (MV)	Not recorded	Deciduous forest dominated by *Pinus kesiya*, *Dipterocarpus* spp.
Coffee garden with forest of *Pinus kesiya* at Doi Tung, Mae Fah Luang District, Chiang Rai Province (CG)	N 20˚ 17.37', E 99˚ 49.08'/ 860 m.	Deciduous rain forest dominated by *Pinus kesiya* and coffee
Forest of Doi Nang Norn, Mae Sai District, Chiang Rai Province (DN)	N 20 ° 22.5 ', E 99° 52,1'/ 450 m.	Mixed rain forest
Khun Kon Waterfall, Muang District, Chiang Rai Province (KK)	N 19˚ 52.36', E 99˚ 37.31'/ 710 m.	National Park with deciduous rain forest dominated by *Lithocarpus* spp., Bamboo, etc.
Huai Mae Sack Waterfall, Vieng Xieng Rung District, Chiang Rai Province (HM)	Not recorded	Mixed rain forest with various tree species
Forest of Doi Ngaem of MaeFah Luang University, Muang District, Chiang Rai Province (FD)	N 20˚ 02.45', E 99˚ 53.59'/ 500 m.	Forest dominated by *Ficus* spp.

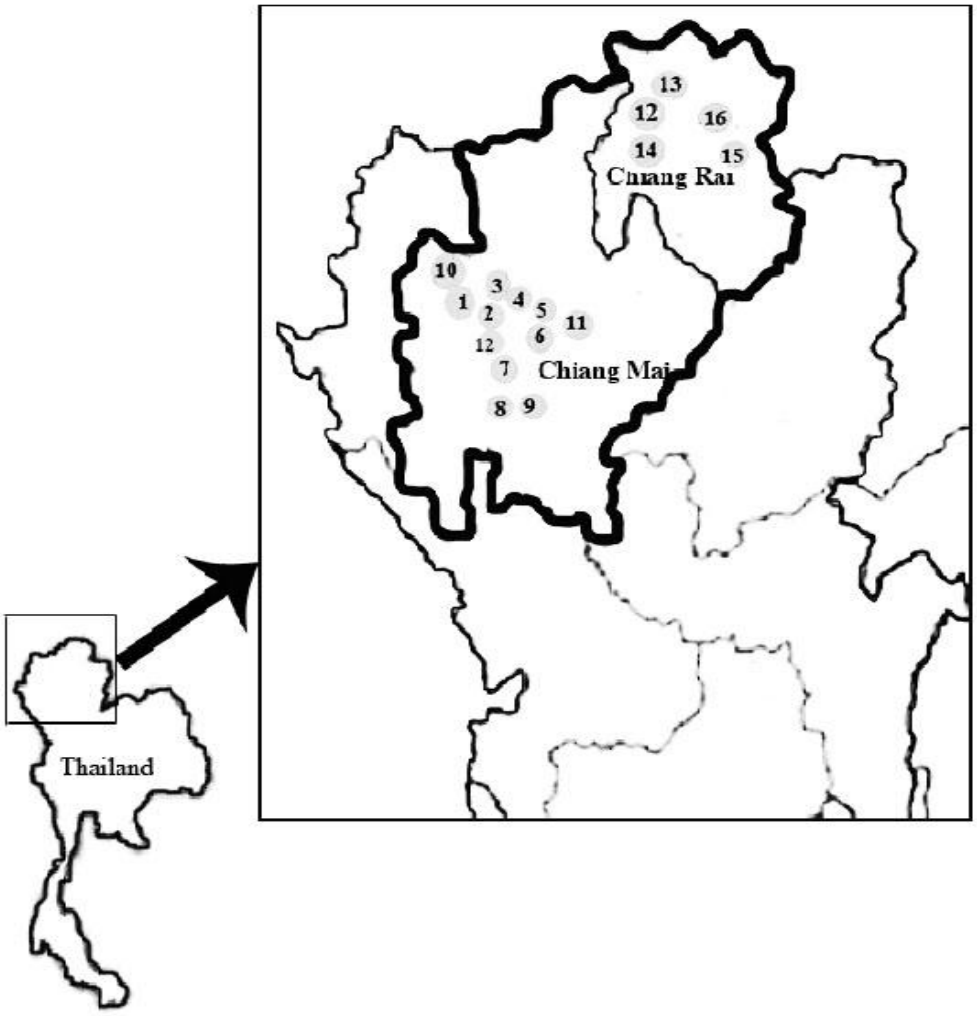

Figure 2.1 Thailand Map, The Studied Locations (Chiang Mai and Chiang Rai)

2.2.2 Morphological Characterization

Freshly collected specimens were brought to Mushroom Research Center's laboratory for morphological study and photographing. Both young and old specimens were studied using macrocharacters for morphology with development and maturation. Specimens were dried (40C for 24h) for long term herbarium storage to avoid deterioration of tissue and degeneration of DNA. Dried specimens were stored in the plastic bags with collection numbers and deposited to MFLU Herbarium**.**

All mushroom specimens were characterized morphologically based on macrocharacters such as pileus, lamellae, stipe, annulus or annular zone, context, spore print and colour reaction (see Fig. 2.3 and Table 2.2). The smell and taste of the mushroom specimens were also evaluated.

Microcharacters were used for identifying mushrooms to genus, section and species level. The microcharacters were illustrated with the aid of a drawing tube

attached to an Olympus CX-41research compound microscope. The important characters were basidiopores, basidia, and cheilocystidia. Pleurocystidia were observed in hymenium trama. Pileus covering was observed on pileu surface and stipe covering were observed on stipe surface. All characters were illustrated and prepared for writing up the description (see Fig. 2.3 and Table 2.2).

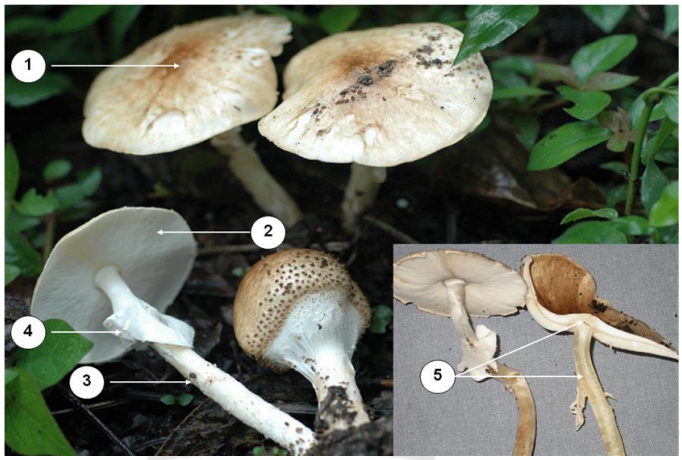

Figure 2.2 Basidiomata and a Section of Samples for Macromorphological Study. (1) Pileus, (2) Lamellae, (3) Stipe, (4) Annulus, (5) Context of Pileus and Stipe

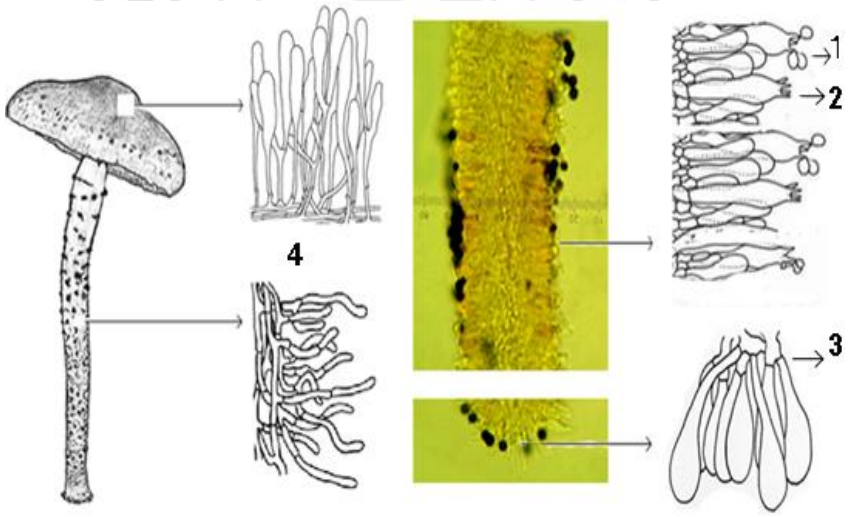

Figure 2.3 Micromorphological Character. (1) Basidiospores, (2) Basidia, (3) Cheilo-cytidia, (4) Pileus Covering and Stipe Covering

Table 2.2 Detailed Descriptions of Mraco and Microcharacters Used in This study

Characters	Description
Pileus (cap)	The important characters recorded for distinguishing taxa are size (mm), shape, form,structure and type of scales with velar remnants, colour and the colour change of the surfaces upon bruising and cutting flesh or colour reaction
Lamellae (gill)	The lamellae features recorded are attachment to stipe or pileus, shape, length and width (mm), number of lamellulae, consistency, edge and colour change of surface bruising or reaction with ammonia. These characters distinguish *Lepiota* and *Leucoagaricus* from other mushrooms.
Stipe (stalk)	Stipe is also a pileus term and important for identifying taxa to species level. The characters noted are size of length and width, shape, surface and pileus covering, and colour of scales and background and colour changes
Annulus (ring) or annular zone	The characters of annulus or annular zone are used to distinguish between *Lepiota* and *Leucoagaricus*. The important features are shape, colour, attachment to a part of stipe, and covering of scale with the velar remnants.
Context	The context of pileus and stipe were observed as thickness (mm), colour and colour change. The context of the stipe is usually hollow.
Smell and taste	Smell and taste were observed in some species with large basidiocarps. These characters are recorded as soft to distinct smell and tasteless to strong taste.
Spore print	Spore print was observed with black paper for 3-6 hours and colour of spores on the paper was recorded.
Colour reaction	Samples were tested for chemical reactions, such as adding ammonia or iron sulfate and colour changes recorded for the different species. (Largent, 1986; Largent & Thiers, 1977; Vellinga, 2003).
Basidiospores	There are several shapes of basidiospore of *Lepiota* amd *Leucoagaricus*. The original colour of basidiospores were observed in sterile water (H_2O) or 2.5-10% of KOH; in Congo red for measurements, drawing and reaction; in Melzer's reagent, Cotton blue and Crezyl blue for reactions. Twenty-five spores were measured in side of length and width in micrometer (µm) for calculating quotient (Q)
Basidia	Basidia size was measured, and shape, number of sterigma and contents and wall were recorded.
Cheilocystidia:	Cheilocystidia were observed as sterile or fertile on the lamella-edge, and their shape, size, pigment, contents and attachment were also recorded
Pileus and Stipe covering	Pileus and stipe coverings were noted on structures or layers of elements and hyphae as shapes, size, pigments, wall thickness and clamp connections
Clamp connections	Clamp connections are mostly present in *Lepiota* species but absent in *Leucoagaricua* species

2.3 Data Analysis

The percentage of occurrence frequency (% OF) for each species was calculated by the formula:

% OF = OSa x 100/ TN where OSa is occurrence of species A and TN is total number of all species

To compare the number of species for each locality, the number of all species was calculated and then final numbers of species were compared. The species (section) diversity was calculated using Shannon's diversity index, *H'* (Shannon and Weaver, 1963):

$$H' = -\sum_{i=1}^{n} P_i \log_e P_i \quad \text{where } P_i = \frac{N_i}{N}$$

- N_i is individual number of *i* species
- *N* is individual number of all species
- P_i is the proportion of *i* species
- *n* is the number of species.

Then the Evenness (E) is calculate as formula: Evenness (E) = *H′*/lnS.

Simpson's Diversity Index (1-D) is used to compare with Shannon's diversity index and the formula is below: D= Σ n(n-1)/N(N-1) where n is the total number of organisms of a particular species and N is the total number of organisms of all species.

2.3 Results and Discussions

2.3.1 Diversity of *Lepiota* in Northern Thailand

In total, 73 collections were recorded from 16 sites in two Provinces (Chiang Mai and Chiang Rai) during 2007-2009. This included 33 taxa in five sections of *Lepiota*, 11 species (33.33 %) in section *Stenosporae* (J. Lange) Külner, eight species (24.24 %) in section *Ovoisporae* (J. Lange) Külner, six species (18.2 %) in section *Lepiota*, five species (15.2 %) in section *Lilaceae* M. Bon and three species (9.1 %) in section *Echinatae* Fay (see Table 2.3). According to Shannon's diversity index (H'), the highest diversity is found in the section *Stenosporae* with an H' of 2.20, followed by *Lepiota* with an H' of 1.7. Sections *Ovisporae*, *Lilaceae* and *Echinatae* have lower diversity with *H'* of 1.54, 1.42 and 0.87 respectively. The results obtained from the Simpson's diversity index is also similar to those of the Shannon's diversity index (see Table 2.4).

A summary of section diversity in *Lepiota* collections at the 16 sites is shown in Table 2.2. The highest diversity of *Lepiota* mushrooms was found in Pha Deng Village where five sections were found, followed by Mae Sa Village with 4 sections. Forest of Pong Duaed Village, Mae Sae Village, Coffee garden, Hui Kok Ma and Doi Ngaem with three sections while Thung Jaw Village, Junction to Mae Jaem, to Doi Inthanon, Mae Ma Lai, Khun kon Waterfall and Doi Nang Non had the lowest diversities (see Table 2.3). The species numbers were high in Mae Sa Valley (8), Forest of Pha Deng Village (7), Forest of Doi Ngaem (6), Forest of Pong Duad (5), respectively while there were few species present in other localities. According to Shannon's diversity index (H'), the diversity of species in each collecting site is highest in Mae Sa Valley with an H' of 2.8, followed by Pha Deng Village and Forest of Doi Ngaem with an H' of 1.83 and 1.7 respectively. Forest of Pong Duad Village and Coffee garden are identical in diversity with H' of 1.6. The lower diversities are in Forest of Mae Sae Village, Mok Fah Waterfall, Huai Kok Ma, Huai Mae Sak and Forest of Thung Jaw with H' of 1.4, 1.24, 1.04, 0.7 and 0.5 respectively. The diversity in other sites is too low and unable for calculating. The result of Simpson's diversity index is also identical to Shannon's diversity index (see Table 2.3). The generally highest concurrent species is *L. furfuraceipes* (14.1 %), *L.* sp. 6 (10.9 %), *L. cithrophylla* (7.81%), *L. apera* and *fraterna* (6.25%), and *L. aureofulvella, L. poliochloodes, L. eurysperma, L. microcarpa and L. pongduadensis* (4.69).

In conclusion, this study provided an extensive survey of *Lepiota* species in Thailand during the three year period. It should be noted however that the frequency of sample collections for each sites was not consistent due to unsuitable or inaccessible conditions.

2.3.2 Comparing The Diversity of *Lepiota* in Thailand with Some Countries in Asia

A summary of total species recorded from Thailand and species from counties close to Thailand is given in Table 2.3. There are 34 identified *Lepiota* species known from Thailand including species recorded in this study and the previous studies (Anong Candrasrikul, 1996; Anong Candrasrikul et al., 2008; Ruksavong & Flegel., 2001; Soythong 1994; Sysouphanthong, Hyde, Chukeatirote, Bahkali, & Vellinga, 2011a; 2012; Sysouphanthong, Hyde, Chukeatirote, & Vellinga, 2011b;). According to Sysouphanthong, Hyde, Chukeatirote, & Vellinga (2011b), the number of *Lepiota* species recorded in Thailand is lower than those of Sri Lanka (56), India (55) and China (41). However, known species diversity is presently higher than those of the Philippines (20), Vietnam (11), Nepal (7) and North Korea (2). Diversity of *Lepiota* species in other countries is dependent on number of studies; there are several studies of *Lepiota* from Sri Lanka, India, China, Thailand and the Philippines; there are however, few known species from Vietnam, Japan and North Korea; and there is no record and study of *Lepiota* present in other Asian countries.

In conclusion, the present study represents the first comprehensive assessment of the diversity of *Lepiota* in northern Thailand with the morphology illustration. A total of five species such as *L. aureofulvella* and *L. papillata* (section *Stenosporae*), and *L. eurysperma, L. microcarpa,* and *L. pongduadensis* (section *Lepiota*) were described as new to science (Sysouphanthong, Hyde, Chukeatirote, Bahkali, & Vellinga, 2011a; 2012). Moreover, thirty two species were new records for Thailand

while *L. clypeolaris*, *L. cortinarius*, *L. pseudohelveola* and. *L. rubrotincta* described by Anong Candrasrikul et al. (2008) are not present in this study. Based on the taxonomic key of *Lepiota* from Asia, 14 species discovered from this study are new records for Asia. Besides, there are 7 species remained unidentified due to incomplete data in which further work is expected to perform (see Table 2.3).

2.3.3 Appearance of *Lepiota* Species throughout the Season

Appearance of *Lepiota* species throughout the season is given in Table 2.5. It was found that the highest species number was 20 in July with a rainfall average of 188 mm, followed by 14 species in August (rainfall 244 mm), and 10 species in June (rainfall 161.3 mm). The low values of the species number was 2 and 3 in May (rainfall 214.0) and September (rainfall 152.1), respectively. Some species (i.e., *L. aspera*, *L. furfuraceipes* and *L. lilacea*) were common and found throughout the season. It was showed that most species were found in the middle of raining season (June-August).

Species occurrence is dependent on rain and humidity. The highest number of species of *Lepiota* appeared in July, August, and then June, when high rainfall occurs (Wannathes, Desjardin, Retnowati, Tan & Lumyong, 2004; Wannathes, Desjardin & Lumyong, 2007; 2009; Sysouphanthong, Thongkantha, Zhao, Soytong & Hyde, 2009; Sysouphanthong, Hyde, Chukeatirote, Bahkali & Vellinga, 2011a; 2012; Zhao, Desjardin, Soytong & Hyde, 2006; Zhao, Jeewon, Desjardin, Soytong & Hyde, 2007; Zhao, Desjardin, Soytong, Perry & Hyde, 2010). Sysouphanthong, Thongkantha, Zhao, Soytong & Hyde (2009) studied mushroom diversity in sustainable shade tea forests and found that mushrooms appeared during the rainfall of 132-161 mm between May to July, but number of mushrooms was lower at rainfall higher than 228 mm in August and September, and when rain was lacking in April.

Table 2.3 Distribution of *Lepiota* taxa Collected from 16 Sites in Northern Thailand During 2007-2010

Taxa	FM	FT	FP	PV	MF	HK	JM	HW	HN	ML	MV	CG	KK	DN	HM	FD	T	OF
Section *Stenosporae*																		
L. castanea							2					1					3	4.11
L. aureofulvella					3												3	4.11
L. alopochroa					1												1	1.37
L. cithrophylla	1		2									1			1		5	6.85
L. poliochloodes	1				1						1						3	4.11
L. eryctoticta			1														1	1.37
L. griseovirens												1					1	1.37
L. papillata											1						1	1.37
L. infelix				1													1	1.37
L. sp. 1											1						1	1.37
L. sp. 2											1						1	1.37
Section *Lepiota*																		
L. metulispora				1												1	2	2.74
L.. sp. 3										1							1	1.37
L. sp. 4														1			1	1.37
L. eurysperma			2										1				3	4.11
L. microcarpa						2						1					3	4.11
L. pongduadensis			3														3	4.11
Section *Echinatae*																		
L. aspera				2	1		1										4	5.48
L. echinacea											1						1	1.37
L. babruka																1	1	1.37
Section *Lilaceae*																		
L. cristata						1											1	1.37

Table 2.3 (continued).

Taxa	FM	FT	FP	PV	MF	HK	JM	HW	HN	ML	MV	CG	KK	DN	HM	FD	T	OF
L. cf. *hymenoderma*				1													1	1.37
L. fraterna			3								1						4	5.48
L.a lilacea																3	3	4.11
L. sp. 5											1						1	1.37
Section *Ovisporae*																		
L. furfuraceipes	1	4						2				2				1	10	13.7
L. pseudolilacea		1															1	1.37
L. rhodorhiza				1													1	1.37
L. shixingensis									1								1	1.37
L. sp. 6	1			3		1					1				1		7	9.59
L. sp. 7																1	1	1.37
L. subincarnata				1													1	1.37
L. xanthrohylla																1	1	1.37
Number of mushroom samples	**4**	**5**	**11**	**10**	**6**	**4**	**3**	**2**	**1**	**1**	**8**	**6**	**1**	**1**	**2**	**8**	**73**	
Species richness (S)	**4**	**2**	**5**	**7**	**4**	**3**	**2**	**1**	**1**	**1**	**8**	**5**	**1**	**1**	**2**	**6**		
Shannon's diversity index (H')	**1.4**	**0.5**	**1.6**	**1.83**	**1.24**	**1.04**	**0.64**	**0**	**0**	**0**	**2.8**	**1.6**	**0**	**0**	**0.7**	**1.7**		
Simpson's diversity index (1-D)	**0.75**	**0.32**	**0.78**	**0.82**	**0.67**	**0.63**	**0.44**	**0**	**0**	**0**	**0.88**	**0.78**	**0**	**0**	**0.5**	**0.78**		
Evenness (E)	**0.99**	**0.72**	**0.96**	**0.94**	**0.89**	**0.95**	**0.92**	**0**	**0**	**0**	**1.35**	**0.99**	**0**	**0**	**1.01**	**0.95**		

Notes. FM= Forest of Mae Sae Village, FT= Forest of Thung Jaw Village, FP= Forest of Pong Duad Village, PV= Pha Deng Village, MF= Mork Fah Waterfall, MV= Mae Sa Village, HM= Huai Mae Sak Waterfall, FD= Forest of Doi Ngaem of Mae Fah Luang University, HK= Huai Kok Ma, JM= Junction to Mae Jaem, FH= Forest of Highway 1009 at 25 km marker to Doi Inthanon , HN= Huai Nam Dang, ML= Mae Ma Lai Village, CG= Coffee garden at Doi Tung, DN= Doi Nang Non, OF = Percentage of occurrence frequency (%), S= Species richness, T= Total.

Table 2.4 Diversity of *Lepiota* in Each Section

	Stenosporae	***Lepiota***	***Echinatae***	***Lilacea***	***Ovisporae***
Species richness (S)	11	6	3	5	8
Shannon's diversity index (H')	2.20	1.7	0.87	1.42	1.54
Simpson's diversity index (1-D)	0.90	0.8	0.5	0.72	0.71
Evenness (E)	0.91	0.94	0.80	0.90	0.74

Table 2.5 Distribution of *Lepiota* Throughout the Season (2007-2010)

Taxa	**May**	**June**	**July**	**August**	**September**
Lepiota alopochroa				*	
Lepiota aspera	*		*	*	
Lepiota aureofulvella		*		*	
Lepiota babruka			*		
Lepiota castanea		*	*		
Lepiota cithrophylla			*	*	
Lepiota cristata		*			
Lepiota cf. echinacea			*		
Lepiota eryctoticta				*	
Lepiota eurysperma				*	*
Lepiota fraterna			*		
Lepiota furfuraceipes	*	*	*	*	
Lepiota griseovirens			*		
Lepiota cf hymenoderma				*	
Lepiota infelix			*		
Lepiota lilacea		*		*	*
Lepiota metulispora		*	*		
Lepiota microcarpa		*			
Lepiota papillata				*	
Lepiota poliochloodes			*	*	
Lepiota pongduadensis		*	*	*	
Lepiota pseudolilacea		*			
Lepiota rhodorhiza			*		
Lepiota shixingensis			*		
Lepiota subincarnata			*		
Lepiota xanthrohylla		*			
Lepiota sp. 1			*		

Table 2.5 (continued).

Taxa	May	June	July	August	September
Lepiota sp. 2			*		
Lepiota sp. 3				*	
Lepiota sp. 4			*		
Lepiota sp. 5			*		
Lepiota sp. 6			*		*
Lepiota sp. 7				*	
Total species	**2**	**10**	**20**	**14**	**3**
Rainfall average (2008-2010)	**152.1mm**	**161.3mm**	**188.0mm**	**244.0mm**	**214.0mm**

Note. Source of rainfall (May-September) in northern Thailand from 2008-2010 (http://www.tmd.go.th/climate/-climate.php?FileID=7)

2.5 Conclusion

In this study, 73 *Lepiota* mushrooms were collected representing 33 species from 5 sections. The distribution of these *Lepiota* species were as follows: 11 species of section *Stenosporae*, 8 of *Ovisporae*, 6 of *Lepiota*, 5 of *Lilaceae*, and 3 of *Echinacea*. The higheset diversity of *Lepiota* species was found in the section *Stenosporae* as indicated by the H' index (2.20), followed by *Lepiota* (1.7), *Ovisporae* (1.54), *Lilaceae* (1.42), and *Echinaceae* (0.87). The species number of *Lepiota* was generally high in Mae Sa Valley (8), Forest of Pha Deng Village (7), Forest of Doi Ngaem (6), Forest of Pong Duaed (5). The most commonly found species were *L. furfuraceipes* (14.1 %), *L.* sp. 6 (10.9 %), *L. cithrophylla* (7.81%), *L. apera* and *fraterna* (6.25%). This present study is one of the pioneering work dealing with the *Lepiota* species in Thailand in terms of their diversity and distribution.

CHAPTER 3

Lepiota SECTION *Stenosporae* IN NORTHERN THAILAND

3.1 Introduction

Lepiota had been split on morphological grounds into a number of sections, based on spore shape, and secondly on pileus covering structure. Sect. *Stenosporae* (J.E. Lange) Kühner with *L. pseudofelina* J. Lange as its type is characterized by spores with a distinct outgrowth (spur) at the base, and a trichodermal or cutis-like pileus covering (e.g., Singer, 1986; Bon, 1993). Molecular-phylogenetic analyses have reorganised the morphologically defined sections (Vellinga, 2003b). Species with spurred spores and a hymenidermal pileus covering are closely related to species with ellipsoid spores and a hymeniderm (Vellinga, 2003b), and species such as *L. cortinarius* J.E. Lange with spores that lack a distinct spur and are slender, and have a trichoderm were shown to group with species with fusiform spores (Vellinga, 2003b). In fact phylogenetic analyses of the nrITS and LSU regions show that taxa with ellipsoid spores and a trichoderm without basal short elements form one clade together with the spurred-spored species with a trichoderm or cutis (Vellinga 2003b).

Only a few *Lepiota* species have previously been reported from Thailand. These include *L. clypeolaria* (Bull. : Fr.) P. Kumm., *L. cortinarius* J.E. Lange, *L. cristata* (Bolton : Fr.) P. Kumm., and *L. pseudohelveola* Hora; none of them belongs to *Lepiota* sect. *Stenosporae*: (Soytong, 1994, Anong Chandrasrikul, 1996, Anong Chandrasrikul et al., 2008). However, the following species in *Lepiota* sect. *Stenosporae* are known from other parts of tropical Asia: *L. alopochroa* (Berk. & Broome) Sacc., *L. citrophylla* (Berk. & Broome) Sacc., *L. erythrosticta* (Berk. & Broome) Sacc., *L. leontoderes* (Berk. & Broome) Sacc., and *L. pyrrhaes* (Berk. & Broome) Sacc. from Sri Lanka (Pegler, 1972; 1986; Petch & Bisby, 1950), *L. castanea* Quél., *L. erythrosticta* (Berk. & Broome) Sacc., and *L. griseovirens* Maire from southern India (Kumar & Manimohan, 2009); *L. aurora* E. Horak, *L. castanea*, *L. crepusculata* E. Horak, *L. erythrosticta*, *L. infelix* E. Horak, *L. luteocastanea* E. Horak, and *L. squamatula* E. Horak from Papua New Guinea (Horak, 1981).

The present study focuses on *Lepiota* sect. *Stenosporae* in northern Thailand, in our ongoing efforts to document the diversity of Agaricaceae from this area (Le, Nuytinck, Verbeken, Lumyong & Desjardin, 2007; Le et al., 2007; Sanmee et al., 2008; Kerekes & Desjardin, 2009; Wannathes, Desjardin & Lumyoung, 2007; 2009; Zhao, Desjardin, Soytong, Perry & Hyde, 2010). We used morphological and molecular methods to identify and circumscribe our taxa.

3.2 Materials and Methods

3.2.1 Collecting and examination methods

All mushroom samples used in this study were collected from Chiang Mai and Chiang Rai. The specimens were then characterised morphologically as described in Section 2.2.

3.2.2 Molecular and phylogenetic methods

3.2.2.1 DNA extraction, polymerase chain reaction and sequencing: DNA was extracted from dried mushroom samples collected in Thailand (see Table 3.1) using Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China). The genomic DNA was then used as the template in the amplification of the rRNA gene. For this, the PCR reaction was carried out in 50 µl consisting of 25mM $MgCl_2$, 10 Mg-free buffer, 2.5 µM dNTPs, 1.5 µM primers, 1.5 unit of *Taq* Polymerase and appropriate volume of sterile dd water. The primers used were ITS1-F (CTTGGTCATTTAGAGGAAGTAA) (Gardes & Bruns, 1993), ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTAGATAT-GC) (White, Bruns, Lee & Taylor, 1990). The PCR conditions were as follows: 94°C for 5 min. in denaturation step, followed by 35 cycles of 94°C for 30 sec., 55°C for 30 sec., and 70°C for 45 sec. The products then were sent to Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.) for sequencing. Sequencing was performed using Big Dye chemistry, with the same primers as for PCR, and an ABI PRISM 3100 Genetic Analyzer (both from Applied Biosystems, Foster City, CA, USA). All new sequences were deposited in GenBank (see Table 3.1).

3.2.2.2 Data analysis: Sequences were edited and contigs assembled using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, MI, USA). The sequences of all taxa belonging to *Lepiota* section *Stenosporae* available in GenBank were downloaded, and the complete data set aligned using MAFFT (Katoh, Misawa, Kuma & Miyata, 2002; Katoh & Toh 2008) and minimally manually adjusted. Heuristic search of ITS 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 setting 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. A Maximum Likelihood analysis was performed with the on line program RAxML (Stamatakis, Hoover & Rougemont, 2008). All free model parameters were estimated by RAxML using a general time-reversible (GTR) substitution matrix and a proportion of invariable sites estimate. One hundred rapid ML bootstraps were performed. The ML tree was visualized with the program Figtree v. 1.3.1 (Rambaut, 2009).

Table 3.1 Overview of Species, Collections and Herbarium with GenBank Accession Numbers for the ITS Sequences Used for the Phylogenetic Analyses

Species	Country	Collection & Herbarium	ITS GenBank number
Lepiota andegavensis	France	P.D.H. Roux 2121 (herb. Roux)	AY176461
Lepiota boudieri	The Netherlands	E.C. Vellinga 1180 (L)	AF391025
Lepiota boudieri	Italy	MCVE: 474	FJ998388
Lepiota cf. *boudieri*	U.S.A	E.C. Vellinga 2601 (UC)	AY176479
Lepiota aureofulvella	Thailand	MFLU090183	HQ647293
Lepiota castanea	The Netherlands	N.J. Dam 97020 (herb. Dam)	AY176463
Lepiota castanea	The Netherlands	H.A. Huijser (herb. Huijser)	AF391026
Lepiota alopochroa	Thailand	MFLU090178	HQ647294
Lepiota cingulum	Germany	M. Enderle (L)	AY176359
Lepiota citrophylla	Thailand	MFLU090172	HQ647295
Lepiota cristata	U.S.A	P.B. Matheny 1958 (WTU)	AF391051
Lepiota griseovirens	Italy	MCVE:13747	FJ998403
Lepiota grangei	Belgium	H.A. Huijser (herb. Huijser)	AY176471
Lepiota grangei	Italy	MCVE:4666	FJ998399
Lepiota pilodes	The Netherlands	H.A. Huijser (herb. Huijser)	AY176476
Lepiota pilodes	U.S.A	E.C. Vellinga 3234 (UC)	EF080865
Lepiota cf. *ignipes*	U.S.A	MCVE: 480	FJ998390
Lepiota ignicolor	The Netherlands	H.A. Huijser (herb. Huijser)	AY176472
Lepiota poliochloodes	Thailand	E.C. Vellinga 3877 (MFLU)	HQ647296
Lepiota rhodophylla	U.S.A	E.C. Vellinga 2610 (UC)	AY176480
Lepiota rhodophylla	U.S.A	E.C. Vellinga 3026 (UC)	EF080864
Lepiota sp.	Thailand	E.C. Vellinga 3881 (MFLU)	HQ647297
Lepiota pseudofelina	Italy	MCVE: 3553	FJ998398
Lepiota sp.	U.S.A	E.C. Vellinga 3327 (UC)	EF080867
Lepiota sp.	U.S.A	E.C. Vellinga 2574 (UC)	AY176484
Lepiota sp.	U.S.A	E.C. Vellinga 3014 (UC)	EF080866
Lepiota subalba	The Netherlands	E.C. Vellinga 2242 (L)	AY176489
Lepiota sp.	U.S.A	E.C. Vellinga 2603 (UCB)	AY176481
Lepiota tomentella	The Netherlands	H.A.Huijser (L)	EF080868

3.3 Results and Discussion

Nine named species and two unnamed species of *Lepiota* sect. *Stenosporae* were recognized in this study. A key to the eleven taxa is provided and each taxon is provided with a full description, colored photographs and illustrations. An overview of morphological characters of the Thai species is given in Table 3.1. Two species

have a cutis as pileus covering, viz. *L. aureofulvella* and *L.* sp. 2; most species, except *L. papillata*, have dextrinoid spores.

Sequence data are provided for the ITS region for five species from Thailand and compared with 23 specimens from Europe and U.S.A.; *Lepiota cristata* with spurred spores and a hymeniform pileus covering is used as outgroup (see Table 3.1). The topology of a tree based on Maximum Likelihood analysis is identical to the one presented here (Fig. 3.1) and two clades are present in the tree. The Bootstrap values ≥ 60 % are indicated below the branches. Clade I is composed of species with spurred spores and a trichodermal pileus covering; the Thai species *L. citrophylla* and *L. poliochloodes* are grouped together and sister to *L. grangei, L. griseovirens* and *L. pseudofelina* from Europe with low bootstrap support; *L. alopochroa* is in a group of *L. castanea* and forming a clade with *L.* cf. *ignipes* (from North America) with high bootstrap support. Clade II is composed of species with spurred spores and a cutis-like pileus covering including *L. andegavensis* and *L. boudieri* (Europe), *L.* cf. *boudieri* and *L. rhodophylla* (U.S.A.) and *L. aureofulvella* (Thailand), and *L. aureofulvella* is in the group of *L. boudieri* with low bootstrap support (Fig. 3.1).

Table 3.2 Overview of some Basidiospore Characters in Collections from Thailand

Species	Spore sizes (µm)	Avl × avw	Q	avQ	D	M	Ch	PC
L. alopochroa	7.5–9.3 × 3.8–4.0	8.5 × 3.9	2.0–2.3	2.2	+	-	+	T
L. aureofulvella	5.8–7.5 × 3.0–4.8	7.2 × 3.8	1.6–2.1	1.9	+	-	+	C
L. castanea	9.5–13.0 × 3.8–5.2	11.5 × 4.5	2.4–2.6	2.5	+	-	+	T
L. citrophylla	6.0–8.5 × 3.8–4.3	7.2 × 4.0	1.5–2.0	1.8	+	-	+	T
L. erythrosticta	7.8–11.3 × 3.8–4.8	9.8 × 4.2	2.2–2.8	2.3	+	-	+	T
L. griseovirens	6.2–7.0 × 3.5–4.2	6.7 × 3.8	1.5–1.8	1.7	+	-	+	T
L. infelix	7.5–9.0 × 4.0–4.2	8.3 × 4.0	1.7–2.2	2.0	+	-	+	T
L. poliochloodes	6.5–8.2 × 3.0–3.8	7.2 × 3.5	1.6–2.4	2.0	+	-	+	T
L. papilata	7.2–11.8 × 4.0–4.8	8.2 × 4.1	1.8–2.5	2.0	-	-	+	T
Lepiota sp.1	7.1–8.4 × 2.8–4.2	7.8 × 3.2	1.9–2.8	2.4	+	-	+	T
Lepiota sp.2	6.2–8.0 × 3.2–4.0	7.3 × 3.7	1.6–2.5	1.9	+	-	+	C

Note. D= Dextrinoid, M= Metachromatic, Ch= Cheilocystidia, PC= Pileus Covering, C= Cutis, T= Trichoderm

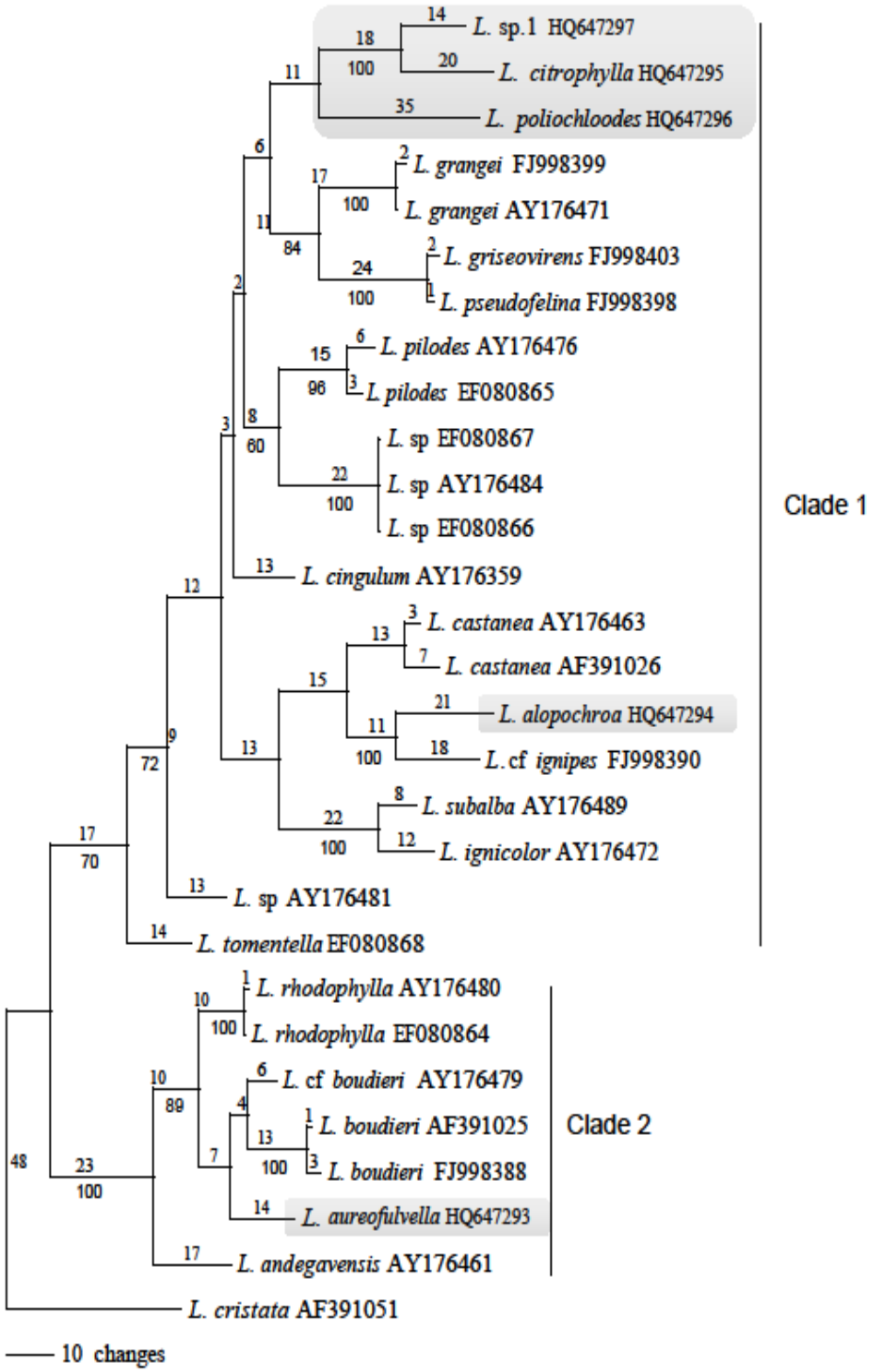

Figure 3.1 Phylogenetic Relationships of *Lepiota* Section *Stenosporae*, Based on a Maximum Parsimony Analysis of nrITS Sequences.

3.3.1 Key to Species and Identification

1. Pileus covering a cutis made up of cylindrical elements.....……... …….2
1. Pileus covering a trichoderm made up of erect cylindrical to narrowly clavate elements.....3
2. Pileus covering yellowish brown, golden-brown to brown; pileus covering elements cylindrical, septate and not branched...*L. aureofulvella*
2. Pileus covering grayish yellow, grayish brown, or slightly reddish brown; pileus covering elements cylindrical, septate and sometimes branched..*L.* sp. 2
3. Basidiomata pale yellow to light yellow...……..….*L. citrophylla*
3. Basidiomata not yellow…..4
4. Basidiomata pinkish; pileus with reddish white to pinkish squamules... …….....……...*L. erythrosticta*
4. Basidiomata not pinkish; pileus without reddish white to pinkish squamules, but with gray, brown, greenish to dark colors…….....5
5. Pileus covered with gray to grayish brown, dark gray or olive brown squamules ….....6
5. Pileus covered with brown to dark brown or reddish brown squamules.....8
6. Pileus umbonate with high umbo or papilla, covered with brownish gray to olive brown squamules…….....*L. papillata*
6. Pileus umbonate with wide umbo, covered with gray to grayish brown, sometimes olivaceous squamules...……..... ……..7
7. Pileus with gray to grayish brown small plush-like squamules or floccules; smell mild; basidiospores 6.5–8.2 × 3.0–3.8 µm.....*L. poliochloodes*
7. Pileus with small plush-like squamules or floccules, grayish brown to black, sometimes with a hue of green to olive-brown; smell fruity; basidiospores 6.2–7.0 × 3.5–4.2 µm….....….*L. griseovirens*
8. Pileus covered with reddish brown small scales or squamules.....*L.* sp. 1
8. Pileus covered with light brown to dark brown squamules.....….9
9. Pileus convex without or with low umbo……*L. infelix*
9. Pileus umbonate or with distinct umbo ….....…10
10. Pileus covered with dark brown to dark at center (umbo), with brown squamules around center toward margin; smell spicy; basidiospores 9.5–13 × 3.8–5.2 µm.....*L. castanea*
10. Pileus umbonate, covered with brown squamules toward margin; smell fruity; basidiospores 7.5–9.3 × 3.8–4.0 µm…….....……....*L. alopochroa*

Figure 3.2 Basidiomata of *Lepiota* Species Section *Stenosporae* in Nature, a. *L. aureofulvella,* b. *L. citriphylla,* c. *L. castanea,* d. *L. alopochroa,* e. *L. poliochloodes,* f. *L. infelix,* g. *L. papilata,* h. *L. sp. 2,* i. *L. sp.1* and j. *L. erythrostica.*

1. ***Lepiota alopochroa*** (Berk. & Br.) Sacc. Syll. Fung. 5: 63. 1887. (Fig. 3.3) ≡ *Agaricus alopochrous* Berk. & Broome, J. linn. Soc., Bot. 11: 510. 1871. *Lepiotula alopochroa* (Berk. & Broome) E. Horak, N.Z. J. Bot. 18: 185. 1980.

Pileus 14 mm, umbonate, expanding to plano-concave with low umbo, with inflexed margin, brown (7E6–7) at umbo, rough or with crowded squamules, with surface breaking up around umbo, with brown (7E6–7) fibrillose squamules toward margin, on light brown (6D5–6) fibrillose background, with peeling surface at margin and white background, with white fibrillose remnants of partial veil and margin exceeding lamellae. Lamellae free, crowded, ventricose, 1 mm wide, white or pale yellow to orange-white (4A3, 5A2), with white serrulate edge. Stipe 35 × 2.5–4 mm, cylindrical or slightly tapering to apex and wider at base, with grayish orange to brownish orange (5B4–5, 6C5) background, white fibrillose at annular zone, with brown (7E6–7) squamules or fibrillose squamules at middle zone downward base, with white rhizomorphs at base, hollow. Annulus as an annular zone. Context in pileus white, 0.5 mm wide; in stipe concolorous with surface. Smell fruity. Taste unknown. Spore print white.

Basidiospores [25,1,1] 7.5–9.3 × 3.8–4.0 µm, avl × avw = 8.5 × 3.9 µm, Q = 2.0–2.3, avQ = 2.2, in side view oblong to cylindrical, spurred, truncate or with outgrown base, with acute apex, in frontal view fusiform to oblong, thick-walled, hyaline, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 13–17 × 6.0–8.0 µm, clavate, narrowly clavate, rarely subclavate, 4-spored, thick-walled, hyaline. Lamella edge sterile. Cheilocystidia 25–37 × 5–7 µm, narrowly clavate to cylindrical, with rounded apex, rarely utriform, thin-walled, hyaline. Pleurocystidia absent. Pileus covering a trichoderm made up of narrowly clavate to cylindrical elements, 50–113 × 3.5–18 µm, brown and thick-walled, with parietal pale brown pigment; underlayer with hyaline to pale brown hyphae, 2.5–4 µm wide. Clamp connections present in all tissue.

Habitat and distribution: Solitary, saprotrophic and terrestrial on soil, on dead leaves in bamboo forest. Known from one locality in northern Thailand.

Material examined:Thailand, Chiang Mai Prov., Mae Taeng Distr., Mok Fah Waterfall, N 20° 02’ 43.1”, E 99° 52’ 35.0”, 596 m alt., 6.VIII.2008, P. Sysouphanthong, MFLU090178.

Discussion: This species in the *Lepiota castanea* group is characterized by relatively short spores (on average 8.5 × 3.9 µm), and relatively short elements in the pileus covering; the cheilocystidia are narrowly clavate to cylindrical. Despite these short spores and short pileus covering elements it differs from *L. ignicolor* Bres. in ITS sequences (Figure3.1).

The Thai collections of *L. castanea* have longer spores and longer elements in the pileus covering, and the basidiomata are darker in color.

The collection fits the description of *L. alopochroa* (Berk. & Broome) Sacc. reasonably well, based on the descriptions by Pegler (1972; 1986) and Horak (1981), however molecular evidence is not available to support this conclusion.

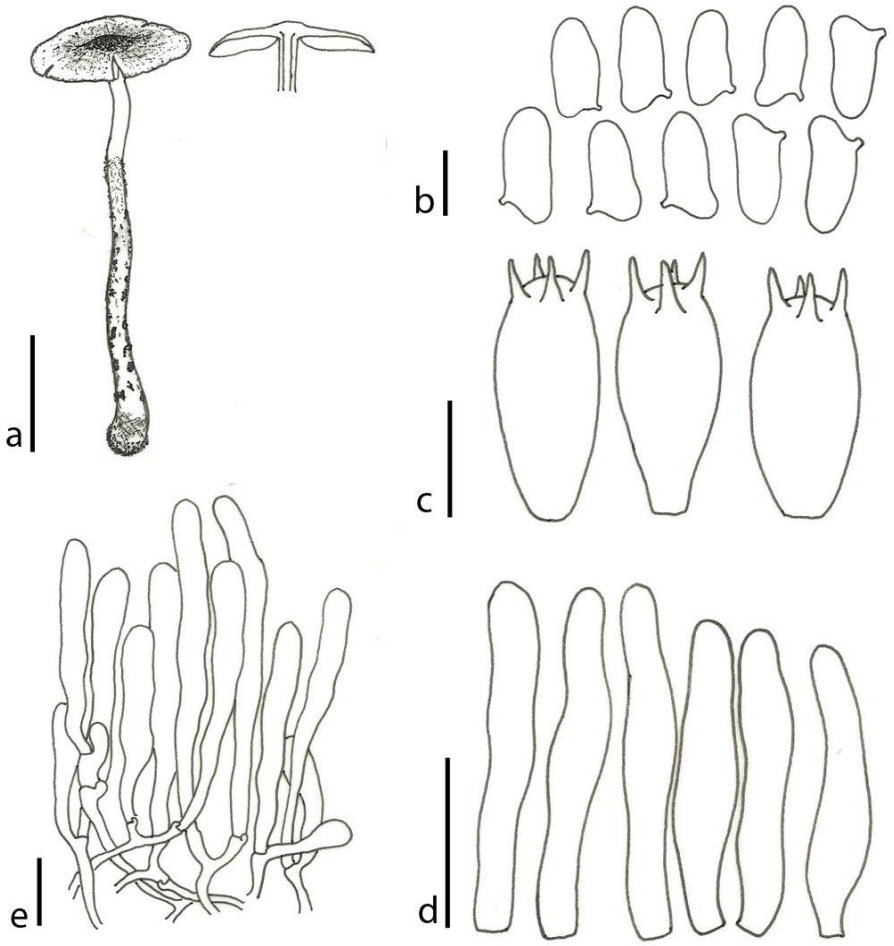

Figure 3.3 *Lepiota alopochroa* (MFLU090178). a. basidiomata, b. basidiospores, c. ba-sidia, d. cheilocystidia, e. pileus covering. Scale bars: a = 10 mm, b–d = 10 µm, e = 20 µm.

2. *Lepiota aureofulvella* Sysou., Hyde., Chuk., & Vellinga. Mycotaxon, 117: 53-85. 2011. (Fig. 3.4)

Pileus 15–40 mm, convex to umbonate, expanding to plane or plano-concave, with straight margin, with crowded brown (6E7–8) fibrils at umbo, with concentrically crowded fibrillose squamules from umbo toward margin, yellowish brown to golden brown (5D5–7), on white background, when mature surface peeling from background; margin fringed and exceeding lamellae. Lamellae free, crowded, ventricose, 5–6 mm wide, white, with white serrulate edge. Stipe 25–45 × 2.5–5 mm, cylindrical to subclavate, with white to light brown (6D4–5) background, white fibrillose in upper zone, at base with slightly brown (6E4–8) fibrillose squamules, with white rhizomorphs at base, hollow. Annulus an annular zone, with white fibrils. Context in pileus white and 4.5–5 mm wide; in stipe concolorous with surface. Smell fruity. Taste unknown. Spore print white.

Basidiospores [60,3,3] 5.8–7.5 × 3.0–4.8 µm, avl × avw = 7.2 × 3.8 µm, Q = 1.6–2.1, Qav = 1.9, cylindrical to oblong, with truncate to spurred base, triangular or with curved abaxial side, in frontal view cylindrical, thick-walled, hyaline, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 16.3–21.5 × 5.5–6.3 µm, clavate, slightly thick-walled, 4-spored. Lamella edge sterile. Cheilocystidia 17.5–27.5 × 5–10 µm, clavate, narrowly clavate, utriform, cylindrical, hyaline, slightly thick-walled. Pleurocystidia absent. Pileus covering a cutis made up of cylindrical elements, sometimes with narrowly clavate terminal elements, 40–130 × 5.5–14 µm, thick-walled, with brown parietal pigment; underlayer with hyaline to pale brown hyphae, 3–4 septate, 5.0–13 µm wide. Clamp connections present in all tissues.

Habitat and distribution: growing in a small to large group; saprotrophic on decayed wood, soil rich in humus, and found in forest with dominant bamboo; in one locality in northern Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Distr., Mok Fah Waterfall National Park, N 20° 02’ 43.1”, E 99° 52’ 35.0”, 596 m. alt., 8. VIII.2008, P. Sysouphanthong, MFLU090183 (holotype); ibidem, 24.VI.2010, P. Sysouphanthong, MFLU100592; ibidem, 9.VI.2010, P. Sysouphanthong, MFLU100621.

Disscussion: *Lepiota aureofulvella* is characterized by the yellowish brown or light brown to golden brown color of the pileus covering, the clavate to utriform cheilocystidia and the cutis-like pileus covering.

The only similar species is *L. alopochroa* which resembles *L. aureofulvella* in color and general stature, but differs because of the trichodermal pileus covering.

Lepiota squamatula E. Horak from Papua New Guinea, which also has a cutis-like pileus covering judging from its description and illustrations, though in the Latin description it is called a trichodermial pileus covering, differs by its orange-brown squamules and fibrils on the pileus, the longer (8.0–9.0 µm) and narrower (2.5–3.5 µm) basidiospores, and the pileus covering which is “composed of cylindric interwoven hyphae forming a cutis or trichoderm” (Horak, 1981).

Some north temperate species with a cutis-like pileus covering and spurred basidiospores are similar to *L. aureofulvella; L. boudieri* Bres. is similar in morphology, but the colors are somewhat lighter and more golden in *L. aureofulvella*, basidiospores are shorter (5.8–7.5 µm) in *L. boudieri* (Vellinga, 2001); the western North American *L. rhodophylla* Vellinga is distinguished from *L. aureofulvella* by a pinkish-brownish pileus covering with a pinkish brown margin, and pink to pale brownish lamellae (Vellinga, 2006), and the French species, *L. andegavensis* Mornand (1982) with very dark pileus colors.

The nrITS based phylogeny represented in (Figure3.1) shows clearly that *L. aureofulvella* differs from all the other species with a cutis-like pileus covering in clade 2, for which sequence data are available.

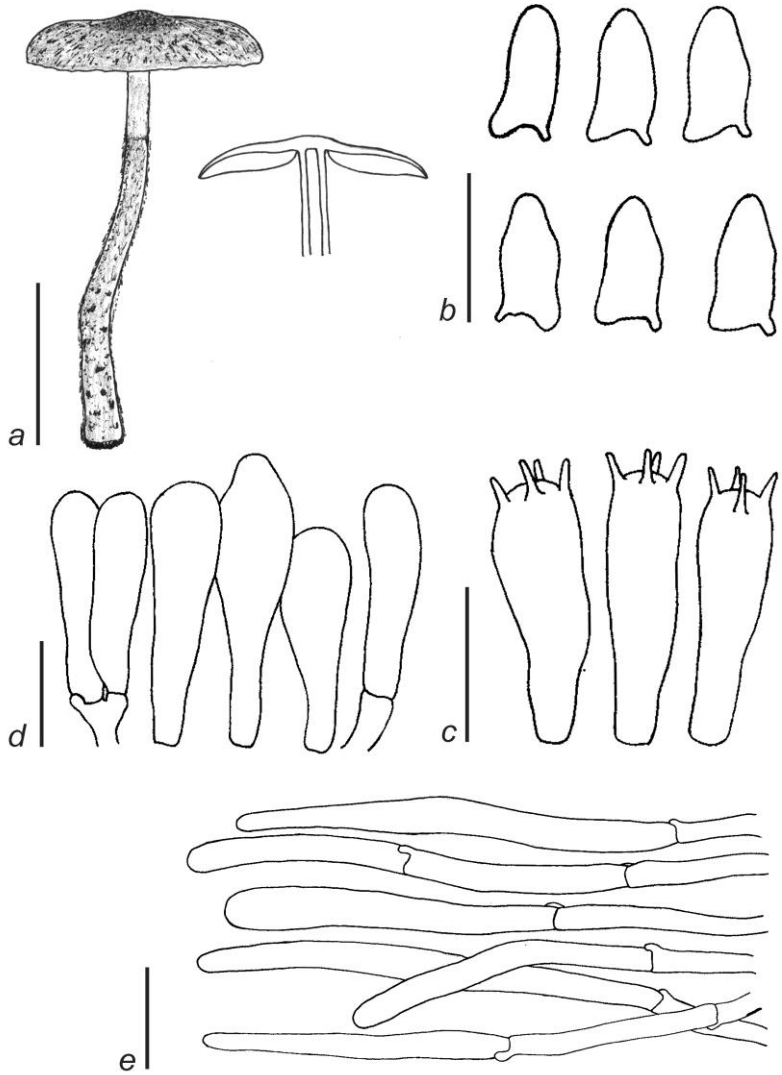

Figure 3.4 *Lepiota aureofulvella* (MFLU090183). a. basidioma and a section, b. basidiospores, c. basidia, d. cheilocystidia, e. elements of pileus covering. Scale bars: a = 10 mm, b–e =10 µm.

3. *Lepiota castanea* Quél., C. r. Ass. Franç. Av. Sci. (Reims, 1880) 9: 661. 1881 (Suppl. Champ. Jura Vosges 10) (Fig. 3.5)

Pileus 15–20 mm, umbonate to campanulate with wide umbo, with inflexed margin, with crowded fibrils at umbo, brown (7E6–8) and changing to dark brown to dark when dry (7F6–8), with rough umbo, with rough surface around umbo to margin, brown (6E5–6) and paler at margin (6D5–7), with surface soon breaking up and becoming squamules or patch-like squames with up curved tips, on white background, at margin white fibrillose and with light brown fibrillose squamules. Lamellae free, crowded, ventricose, 3 mm wide, white, with smooth edge. Stipe 40–58 × 1.8–3 mm, cylindrical or slightly tapering to apex, white from apex to 1/3 of length, white or pale

orange to light orange from annular zone to base with squamule-like, light brown to brown (6D5–7, 6E6) patches and bands, hollow, with white rhizomorphs at base. Annulus an annular zone, with squamules or fibrillose partial veil. Context in pileus white and dull, 2–2.5 mm wide, in stipe white. Smell spicy. Taste unknown. Spore print white.

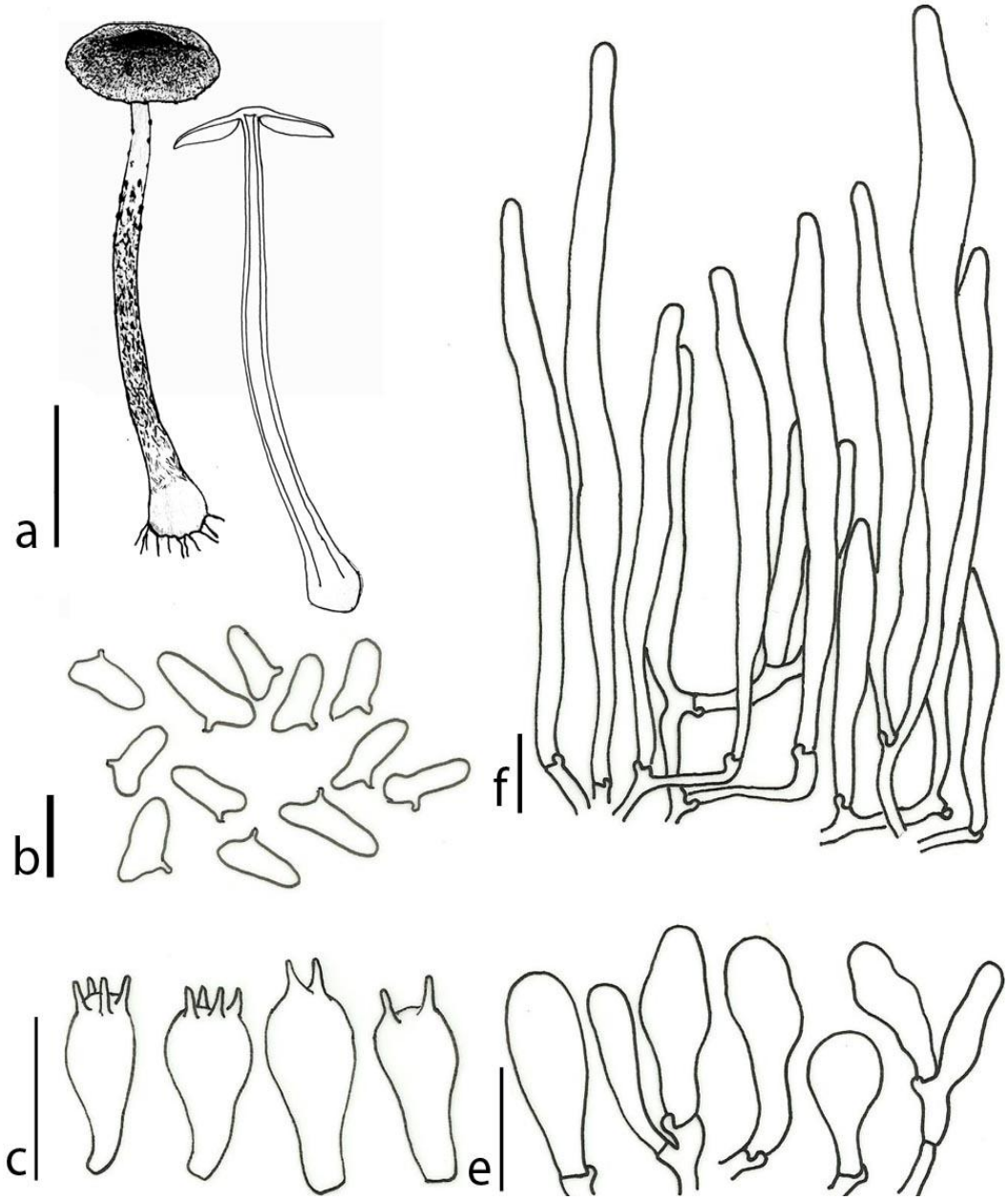

Figure 3.5 *Lepiota castanea* (MFLU090106). a. basidioma and a section, b. basidiospo-res, c. basidia, d. cheilocystidia, e. pileus covering. Scale bars: a = 10 mm, b–e = 10 µm.

Basidiospores [75,2,2] in side view 9.5–13 × 3.8–5.2 µm, avl × avw = 11.5 × 4.5 µm, Q = 2.4–2.6, Qav = 2.5, in side view cylindrical, with truncate to distinctly spurred base, mostly with outgrown broad basal spur and long hilar appendage, some with a straight base, in frontal view oblong to cylindrical, hyaline, slightly thick-walled, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 18–23 × 9.0–10 µm, clavate, 4 – spored, with or without mucilaginous containts. Lamella edge sterile. Cheilocystidia 18–36 × 5.5–12.5 µm, usually clavate to narrowly clavate, with acute or rounded apex, often utriform, thin–walled. Pleurocystidia absent. Pileus covering and stipe covering a trichoderm made up of narrowly clavate to cylindrical elements, wider at middle and narrowing to apex and base, 40–375 × 6.5–35 µm, with slightly thickened walls, pale brown to brown-walled, with parietal brown pigment, with rare short clavate elements; under layer with cylindrical hyphae, hyaline to pale brown-walled, 2.5–5 µm wide. Clamp connections present in all tissues.

Habitat and distribution: Growing solitary or in a small group, saprotrophic and terrestrial on forest floor with high amount of humus, only found in deciduous forest at high elevation (Doi Inthanon National Park, Chiang Mai Province, Thailand). Widely distributed in Europe and North America and temperate parts of Asia (Vellinga, 2001), also recorded from India and Nepal (Manandhar & Adhikari, 1994), and from Papua New Guinea (Horak, 1981). This is a new record for Thailand.

Material examined:Chiang Mai Prov., Chom Thong Distr., Doi Inthanon National Park, Junction of Highway 1009 and Road to Mae Cheam, N 19° 31.58, E 98° 29.64', 1700 m alt., 20.VI.2008, P. Sysouphanthong MFLU090106; ibidem, 15.VII.2008, P. Sysouphanthong, MFLU090154.

Discussion: The complex of *L. castanea* is in need of a combined morphological-molecular approach to resolve the species borders. Vellinga (2001) took a pragmatic view, and considered *L. castanea* a very variable species, variable in terms of spore length and length of pileus covering elements. Migliozzi & Zecchin (1997; 2000) recognized several species in the complex: *L. rufidula* Bres. for the long-spored taxon, commonly and also by us named *L. castanea*; *L. ignipes* Bon is considered a synonym of *L. rufidula*; *L. castanea* s. str. for a short-spored species and *L. ignicolor* Bres. for a taxon with small basidiomata, with spores that tend to be more ovoid. A collection tentatively called *Lepiota ignicolor* is sister to *L. subalba* P.D. Orton in the phylogenetic analyses based on nrITS sequences (Figure 3.1); *L. subalba* has short elements in the pileus covering, but whitish basidiomata. The characters of the Italian collection named *L. ignipes* in GenBank (accession number FJ998390) are not known, but the molecular data indicates that it is different from the other taxa in this complex.

Lepiota cingulum Kelderman resembles *L. castanea*, but its basidiomata have predominantly pinkish brown colors, and the terminal elements of the pileus covering tend to be tapering towards the tips (Kelderman, 1994).

The record of *L. castanea* in Nepal (Manandhar & Adhikari 1994) refers to another species, as the spores are stated to be ellipsoid.

Lepiota castanea is rare in northern Thailand, and was found only at one locality, at 1700 m asl on the highest mountain of Thailand, early in the rainy season. It represents the temperate, Eurasian element of the Thai *Lepiota* mycota.

4. ***Lepiota citrophylla*** (Berk. & Br.) Sacc., Syll. Fung. 5: 57. 1887. (Fig. 3.6)

≡ *Agaricus citrophyllus* Berk. & Broome, J. Linn. Soc., Bot. 11: 509. 1871. Excluded: *Lepiota citrophylla* sensu Rea, British Basidiomycotae: 72. 1922; sensu Akers & Sundberg, Mycotaxon 69: 433–434. 1998; sensu Liang et al., Journal of Fungal Research 8: 63–65. 2010.

Pileus 15–35 mm, subglobose to conical, expanding to campanulate, with moderately wide umbo, with inflexed margin, at umbo covered with fine squamules to floccules, brown (6D7) at umbo and with light brown to yellowish brown (5D6–8) squamules toward margin on pale yellow to light yellow (4A3–4) background; margin with distant squamules, sometimes with partial veil. Lamellae free, crowded, ventricose, 2.5–3.8 mm wide, pale yellow to light yellow (3A4–4A4), with concolorous serrulate edge. Stipe 60–65 × 2–3 mm, cylindrical, slightly wider at annular zone; surface covered with squamules from annular zone to middle and with small light brown to brown (5D6–6D7) squamules at middle to base, on light yellow (4D4) background; hollow. Annulus an annular zone or made up of squamules. Context in pileus 1.8–2 mm wide and pale yellow (3A3), in stipe light yellow (4D4). Smell as burnt rubber. Taste unknown. Spore print white.

Basidiospores [100, 5, 5] in side view 6.0–8.5 × 3.8–4.3 µm, avl × avw = 7.2 × 4.0 µm, Q = 1.5–2.0, avQ = 1.8, in side view with straight or outgrown spur at base, ellipsoidal to cylindrical with rounded or more acute apex, in frontal view oval, cylindrical, hyaline, thick-walled, strongly dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl Blue. Basidia 19–37 × 6.5–8.5 µm, clavate to slightly narrowly clavate, 4-spored, hyaline, with thin wall. Lamella edge sterile. Cheilocystidia 15–60 × 5.0–10 µm, narrowly clavate, sometimes clavate, utriform, rarely narrowly fusiform, or wider at center. Pleurocystidia absent. Pileus covering a trichoderm made up of erect narrowly clavate elements, often narrowed into pedicel, 50–160 × 8.5–17 µm, with thin brown wall, with parietal and intracellular brown pigment, with an underlayer of cylindrical hyphae, 2–6.5 µm wide, with thin hyaline to slightly pale brown walls, with intracellular pigment. Stipe covering with same structure as pileus covering. Clamp connections present in all tissues.

Habitat and distribution: Solitary or in small groups, terrestrial in soil with decaying leaves and wood, found in deciduous forest and pine forests of northern Thailand. Widely distributed in the tropics: Sri Lanka (Pegler, 1972), Trinidad (Dennis 1952), Kenya and East Africa (Pegler, 1997), and India (Natarajan & Manjula 1983). This is the first record from Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Distr., Hot Spring National Park, N 16° 06’ 16.1”, E 99° 43’ 07,9”, 780–805 m alt., 30.VII.2008, P. Sysouphanthong, MFLU090172; ibidem, 8.VIII.2010, MFLU090185; Chiang Mai Prov., Mae Taeng Distr., Mae Sae Village, N 19° 14.599’, E 98°, 962 m alt., 14.VI.2008, P. Sysouphanthong, MFLU090101; Chiang Rai Prov., Mae Fa Luang Distr., Doi Tung National Park, 10.VI.2009, P. Sysouphanthong, MFLU100405; Chiang Rai Prov., Wiang Chiang Rung Distr., Hui Mae Sack Waterfall National Park, 26.VIII.2009, P. Sysouphanthong, MFLU100424.

Disscussion: This species is found during the whole rainy season and is widespread in northern Thailand. It is quite variable in size of basidiomata and color of pileus covering. *Lepiota citrophylla* is the only species of sect. *Stenosporae* in

northern Thailand which is characterized by predominant yellow colors in the basidiomata. The Thai material is very similar to collections from Sri Lanka (Pegler, 1972; 1986), but Pegler (1972, 1986) did not recover cheilocystidia in the material from Sri Lanka, and according to his measurements the pileus covering elements are shorter (30–80 × 12–17 µm). There can be two reasons for this: the material he studied is young, or the longer elements were degraded, broken, or otherwise not easily found and measured.

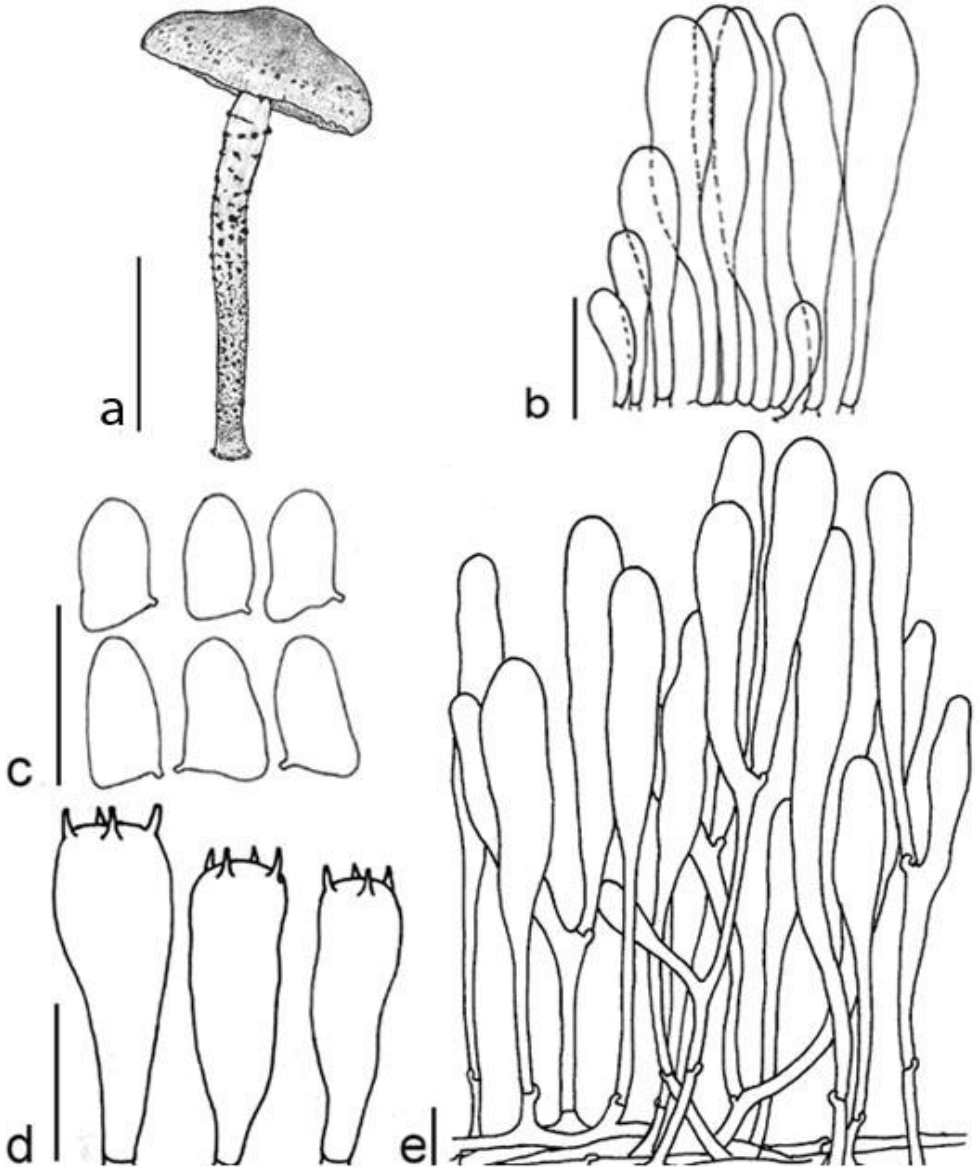

Figure 3.6 *Lepiota citrophylla* (MFLU090101). a. basidiomata, b. cheilocystidia, c. basi-diospores, d. basidia, e. pileus and stipe covering. Scale bars a = 10 mm, b–e = 10 µm

The name *L. citrophylla* was applied to a European species (e.g. Rea 1922; Kühner 1934), and an American species (Akers & Sundberg 1998), but those have non-spurred spores, and the European species was described as *L. xanthophylla* P.D. Orton.

Lepiota luteocastanea E. Horak is very close to *L. citrophylla* as both species have yellow basidiomata, but *L. luteocastanea* is distinguished by the distinct purple tinges in the pileus and fusoid-cylindric elements in squamules of pileus covering (Horak, 1981). *Lepiota subcitrophylla* Hongo described from Japan by Hongo (1956A) comes also close to *L. citrophylla*, but *L. subcitrophylla* turns blue when bruised or damaged. *Verrucospora vulgaris* Pegler is similar to *L. citrophylla* in the brown squamules and yellow tinges in the basidiomata, and the persistent annulus, but it does not have free lamellae, and the basidiospores are angular (Pegler, 1997).

Lepiota citrophylla is only known from tropical parts of Asia, and is first reported from Thailand here. A recent report on the occurrence of this species in China refers to a different species belonging to *Lepiota* sect. *Lepiota*, as the spores are not spurred (Liang et al., 2011).

5. *Lepiota erythrosticta* (Berk. & Br.) Sacc., Syll. Fung. 5: 62. 1887.(Fig. 3.7)
≡ *Agaricus erythrostictus* Berk. & Br., J. Linn. Soc., Bot. 11: 508. 1871.

Pileus 14 mm, parabolic, with straight margin, with crowded reddish or pinkish (8A2–3) squamules at center and toward margin, sometimes with fibrillose squamules, on white to pale red (8A3) background; margin sulcate, with reddish white (8A2–3) fibrillose squamules and partial veil remnants. Lamellae free, slightly crowded, broadly ventricose, white to pale orange (6A3), with white floccose edge. Stipe 32 × 2–2.5 mm, cylindrical, with, 3–3.5 mm wide basal bulb, white (6A2) at apex, covered with light brown to reddish or pinkish (6D4, 8A2) fibrillose squamules on orange fibrillose background from annular zone downward, hollow. Annulus an annular zone, with fibrils and fibrillose squamules similar to those on pileus. Context in pileus white, turning orange white to pale orange (5A2–3), 1 mm wide; in stipe orange white (5A2) in apical zone, grayish red to reddish brown (8C5–8D5) from midway downward. Taste unknown. Smell unknown. Spore print white.

Basidiospores [25,1,1] 7.8–11.3 × 3.8–4.8 µm, on average 9.8 × 4.2 µm, Q = 2.2–2.8, avQ = 2.3, in side-view cylindrical with truncate to spurred base, narrowly triangular, in frontal view cylindrical, thick-walled, hyaline, not dextrinoid, congophilous, cyanophilous, metachromatic in Cresyl blue. Basidia 16–28 × 5–8 µm, clavate, 4-spored, rarely 2- spored. Lamella edge sterile. Cheilocystidia 19–32 × 8–13 µm, irregularly clavate or sphaeropedunculate, utriform, often narrowly fusiform, thick-walled, hyaline. Pleurocystidia absent. Pileus covering a trichoderm made up of long cylindrical elements, 70–170 × 4.5–12.0 µm, hyaline to pale pink-walled, with parietal and intracellular pink pigment, with underlayer made up of hyaline hyphae, 4–10 µm wide. Stipe covering a trichoderm similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution: Growing solitary, on humus-rich soil with decayed leaves and wood, in forest with dominant bamboo; found in one locality in northern Thailand. Distributed in Sri Lanka (Pegler, 1972), Brazil (Wartchow, Putzke & Cavalcanti, 2008), and Trinidad (Dennis, 1952). This is a new record for Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Distr., Hot Spring National Park, N 16° 06' 16.1", E 99° 43' 07.9", 780–805 m alt., 4.VIII.2007, P. Sysouphanthong, MFLU090034.

Discussion: *Lepiota erythrosticta* is recognized by the reddish or pinkish squamules on pileus and stipe, and the pinkish colors in the lamellae. It differs from all other species in sect. *Stenosporae* by the non-dextrinoid spore wall and the metachromatic reaction of the spore wall in Cresyl blue.

The one and only Thai collection consisted of young basidiomata. The sizes of the elements of the pileus and stipe coverings are recorded as longer for material from Brazil (Wartchow et al., 2008). Dennis (1952) recorded this species from Trinidad and he noted basidiospores smaller than those in material from Thailand. Pegler (1972) studied the type material from Sri Lanka but did not report the reaction of the basidiospores in Cresyl blue, he also noted slightly smaller basidiospores. It is well possible that the reports from South America represent a different taxon.

This species is rare in Thailand and it was only found one time in the middle of rainy season (August, 2007) at Hot Spring National Park and it was not found in 2008, 2009, and 2010. It is, like *L. citrophylla*, a species with a tropical distribution pattern.

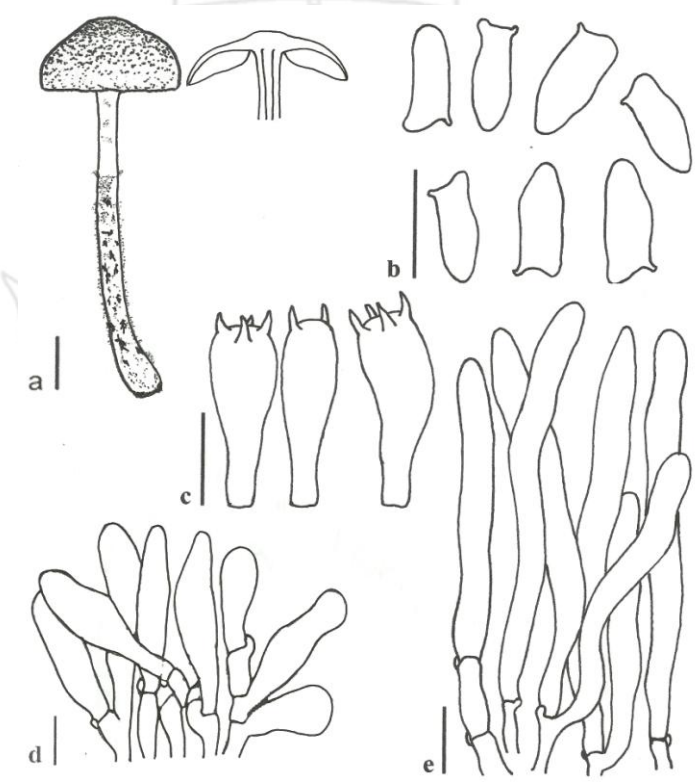

Figure 3.7 *Lepiota erythrosticta* (MFLU090034). a. basidioma, b. basidiospores, c. Basidia, d. cheilocystidia, e. pileus covering. Scale bars: a = 1 mm, b–d = 10µm, e = 20 µm.

6. ***Lepiota griseovirens*** Maire, Bul. trim. Soc. myc. Fr. 44: 37. 1928.(Fig. 3.8)

Excluded: *Lepiota griseovirens* sensu D. Reid, Fungi rar. Ic. col. 6: 14–16. 1972; sensu Bon, Fl. mycol. Eur. 3, Lépiotes: 55. 1993 (=*Lepiota poliochloodes*). Misapplied name: *Lepiota pseudofelina* sensu Bon, Fl. mycol. Eur. 3, Lépiotes: 55. 1993; sensu Candusso & Lanzoni, Fungi Europaei 4 *Lepiota*: 222–225. 1990.

Pileus 8–13 mm, conico-campanulate, expanding to umbonate, plano-convex with broad umbo, with straight margin, at centre covered with tomentose tufts, grayish brown (7F3, 7E3, 7D3) to black, sometimes with a hue of green to olive-brown (4F4–5), toward margin cracked into small squamules, paler than at center, on orange-white to pale orange (5A2–3) background; margin slightly fringed, exceeding lamellae in mature specimen. Lamellae free, slightly crowded, ventricose, and rounded near stipe, 2 mm wide, orange-white to pale orange (5A2), with white eroded to flocculose edge. Stipe 16–20 × 1.5–2.0 mm, with 2.0–2.5 mm wide base, cylindrical, slightly wider at base, white to orange-white (5A2) innately fibrillose above annular zone and below annular zone downward toward base, with scattered grayish brown (7F3, 7E3, 7D3) to black squamules as on pileus, with brownish-orange (6C4–5) background, with white mycelium cords, hollow. Context in pileus white, 1.5–2 mm wide, in stipe concolorous with surface. Smell fruity. Taste unknown. Spore print white.

Basidiospores [50,2,1] in side view 6.2–7.0 × 3.5–4.2 µm, avl × avw 6.7 × 3.8 µm, Q = 1.5–1.8, avQ = 1.7, oblong, with truncate base, with hilar appendage, thick-walled, strongly dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 17–20 × 5.5–7 µm, clavate, 4 – spored, often with 2 sterigmata. Lamella edge sterile. Cheilocystidia 22–30 × 6–8 µm, clavate, narrowly clavate, narrowly utriform. Pleurocystidia absent. Pileus covering a trichoderm made up of cylindrical to narrowly fusiform elements, without short clavate elements at base, 40–140 × 7.0–13 µm, thin–walled, with brown parietal pigment; basal hyphae cylindrical and hyaline, 7.5–12.5 µm wide. Stipe covering (squamules) a trichoderm as pileus covering. Clamp connections present in all tissues.

Habitat and distribution: Growing in a small group with few basidiomata, terrestrial, and saprotrophic on ground of rain forest with dominant pine. This is the first record for Thailand. Also known from Europe (e.g., Vellinga, 2001; Bon, 1993).

Material examined: Thailand, Chiang Rai Prov., Mae Fa Luang Distr., Doi Tung National Park, 17.VII.2010, P. Sysouphanthong, MFLU100554.

Discussion: *Lepiota griseovirens* is rare in Thailand and was only found once, in Doi Tung National Park.

The Thai collection is characterized by grayish brown to black squamules on pileus, sometimes with a hue of green to olive-brown. Its spores are in the lower part of the range recorded for European collections (Vellinga, 2001), but all other characters (including shape of the cheilocystidia) fit well with the European concept of the species. Vellinga and Huijser (1993) discussed the naming of this species, which was mainly known as *L. pseudofelina*. The two nrITS collections in Fig. 3.1 representing this taxon had been named differently, as *L. pseudofelina* and *L. griseovirens* respectively.

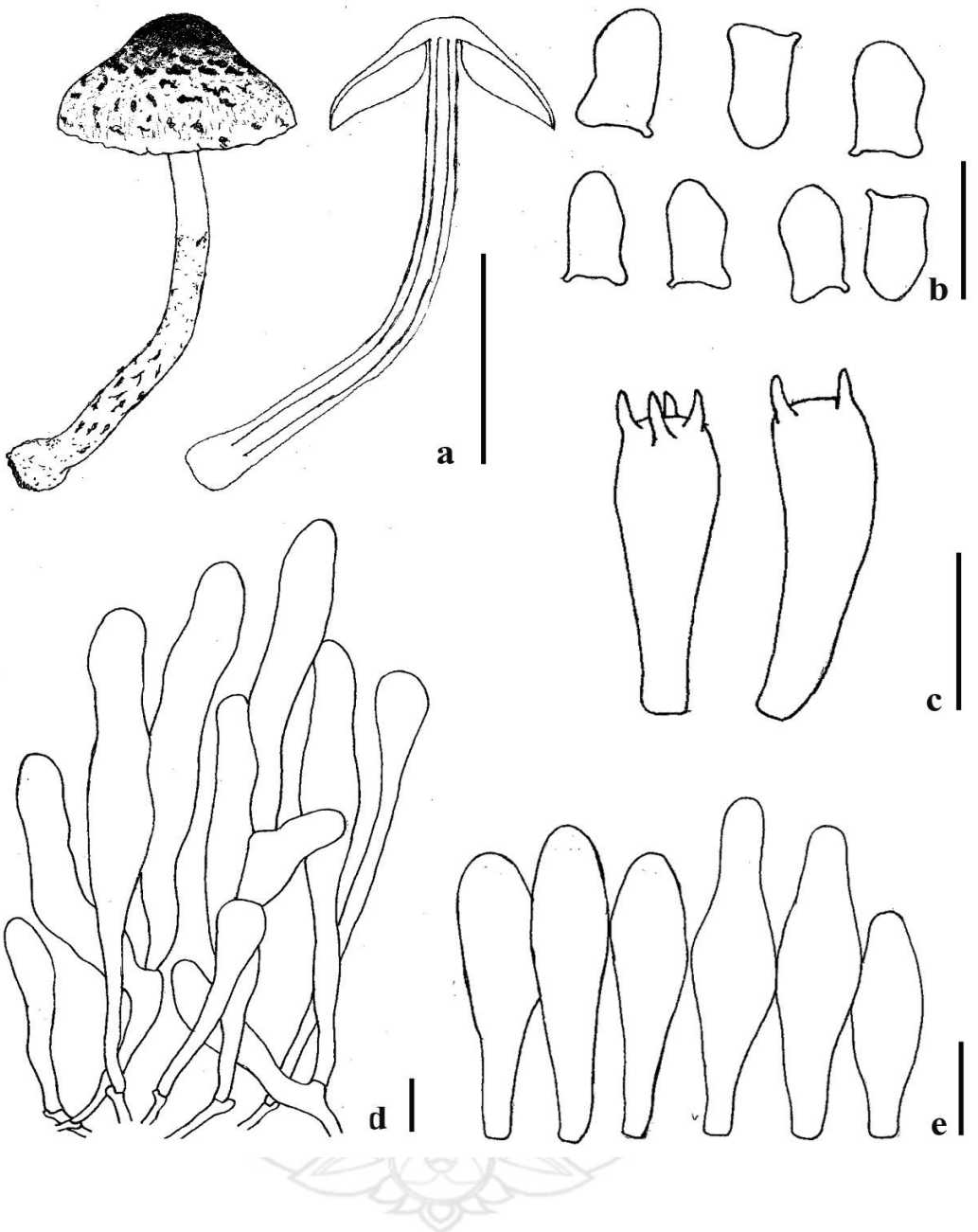

Figure 3.8 *Lepiota griseovirens* (MFLU100554). a. basidiomata and a section, b. basi-diospores, c. basidia, d. pileus covering, e. cheilocystidia. Scale bars: a = 10 mm, b–e = 10 µm.

7. *Lepiota infelix* E. Horak, Sydowia 33: 133. 1981 ('1980'). (Fig. 3.9)

Pileus 19–23 mm, when young convex or with low umbo, expanding to plano-concave with low umbo, with straight or slightly inflexed margin, when young dark brown (7F5–6), soon breaking open into squamules, crowded and dark brown (7F5–6) at centre and umbo, with fibrillose to fibrillose light brown to brown (6D7–8) squamules around centre toward margin, on white fibrillose background; margin split,

fringed, exceeding lamellae when mature. Lamellae free, slightly crowded, ventricose, 3 mm wide, white, fragile, with white eroded edge. Stipe 20–25 × 1.2–1.5 mm, cylindrical, slightly wider at base; background white at apical zone, darker down towards base, light brown (6D7) at base, when mature turning grayish orange to brownish orange (6B4, 6C4–6), with brown (6E5–7) squamules from centre down towards base, hollow. Annulus as annular zone, with squamules. Context in pileus white to pale yellow (3A2), 1 mm wide; in stipe concolorous with surface. Smell mild. Taste unknown. Spore print white.

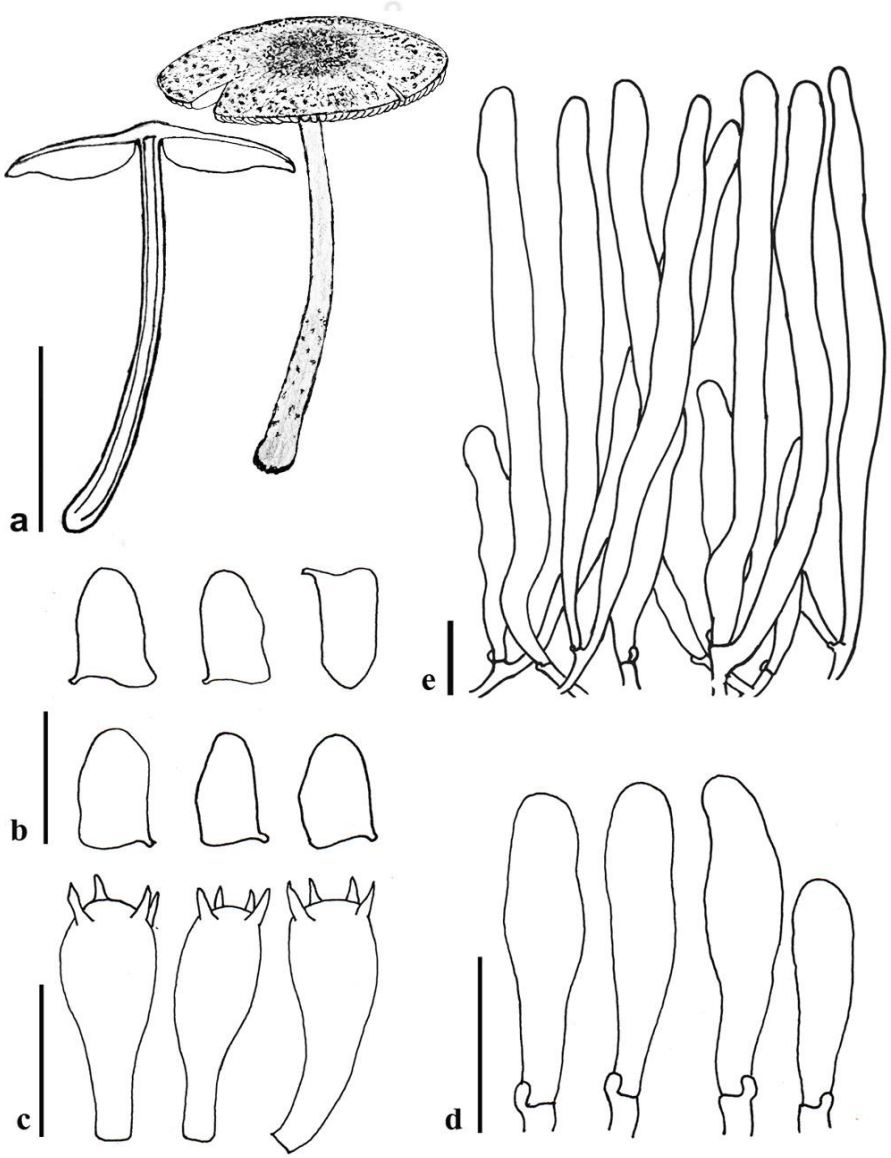

Figure 3.9 *Lepiota infelix* (MFLU090014). a. basidioma, b. basidiospores, c. basidia, d. pileus covering, e. chelocystidia. Scale bars: a = 20 mm., b–e = 10 µm.

Basidiospores [25,2,1] 7.5–9.0 × 4.0–4.2 µm, avl × avw = 8.3 × 4.0 µm, Q = 1.7–2.2, avQ = 2.0, oblong to cylindrical, with rounded apex, with distinct spur at base, with or without lateral hilar appendage, in frontal view oblong, hyaline, slightly thick – walled, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 15–18 × 6.0–8.0 µm, clavate, 4-spored. Lamella edge sterile. Cheilocystidia 16–25 × 5–7 µm, clavate to narrowly clavate, often utriform, colorless. Pleurocystidia absent. Pileus covering a trichoderm made up of cylindrical to narrowly clavate elements, 60–150 × 6–15 µm, slightly thick-walled, with pale brown parietal pigment, with under layer made up of hyaline to pale brown-walled, 3–6 µm wide, hyphae. Stipe covering a trichoderm similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution: growing in a small group, saprotrophic and terrestrial on nutrient-rich soil, in deciduous rain forest with dominant Lithocarpus spp. The species is known from one locality in Thaialnd. Found in Papua New Guinea (Horak, 1981), and reported from India (Natarajan & Manjula, 1983). This is a new record for Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Distr., Pha Deng Village, N 19° 07’ 13.7”, E 98° 43’ 52,9”, 905 m alt., 11.VII.2007, P. Sysouphanthong, MFLU090014.

Discussion: *Lepiota infelix* was originally described from Papua New Guinea by Horak (1981) and is characterized by reddish brown squamules on pileus and stipe. The species is closely related to *L. ignipes* by color of pileus covering, but the two taxa are separated by the shape of the basidiospores that are slender in *L. infelix*.

Lepiota infelix is also similar to *L. alopochroa,* described from Sri Lanka, and recorded from Papua New Guinea (Horak, 1981), and now from northern Thailand, but differs in color of squamules of pileus covering

8. ***Lepiota papillata*** Sysou., Hyde., Chuk., & Vellinga. Mycotaxon. 117:53-85. 2011. (Fig. 3.10)

Pileus 9–15 mm, conical at first, expanding to campanulate, umbonate with umbo or high papilla, with inflexed margin, applanate to slightly plano-concave with uplifted margin; background brown (5F4), white to yellowish brown (5E5) on margin, covered with crowded brownish gray to olive brown (4F2–3) squamules at umbo, with brown (6E6) fibrillose squamules around umbo toward margin; margin split, slightly appendiculate, exceeding lamellae. Lamellae free, slightly crowded, ventricose,1.5–2 mm wide, when young white, yellowish white to pale yellow (4A2–3) when mature, turning grayish orange (5B5) when dried, with serrate concolorous edge. Stipe 25–30 × 1.5–2 mm, cylindrical or slightly tapering upward, with orange-white to grayish red (5A2–7B4) background at apex, grayish orange to brownish orange (5B4–6C5) at center to base, turning brown (6D7) when bruised, covered with brownish gray to olive-brown (4F2–3) squamules from annular zone to base, with orange-white to brownish orange (5A2–6C5) fibrils between squamules; hollow. Annulus with annular zone, with squamules as stipe covering. Context in pileus white, less than 1 mm wide; in stipe grayish orange (5–6B6). Smell grass-like. Taste mild. Spore print white.

Basidospores [50,2,1] 7.2–11.8 × 4.0–4.8 µm, avl × avw= 8.2 × 4.1 µm, Q = 1.8–2.5, avQ= 2.0, with straight or outgrown spur, cylindrical to oblong, with rounded or more acute apex, in frontal view oblong to cylindrical fusiform, with or without hilar appendage, hyaline, slightly thick-walled, not strongly dextrinoid in Melzer's reagent, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 12–20 × 5.5–7.5 µm, clavate, 4-spored, rarely 2–spored. Lamella edge sterile. Cheilocystidia 30–52 × 6–29 µm, variable in shape, clavate to narrowly clavate, slightly cylindrical, sometimes pyriform, spathulate, rarely narrowly clavate with long pedicel, hyaline, thin – walled. Pleurocystidia absent. Pileus covering a trichoderm made up of erect narrowly clavate elements 30–130 × 5.5–23 µm, with rounded apex, often with abrupt apical excrescence, pale brown-walled, with parietal and intracellular brown pigment, with cylindrical hyphae in under layer, thin-walled, 2.5–5.0 µm wide. Stipe covering in squamules a trichoderm similar to pileus covering. Clamp connections present in all tissues.

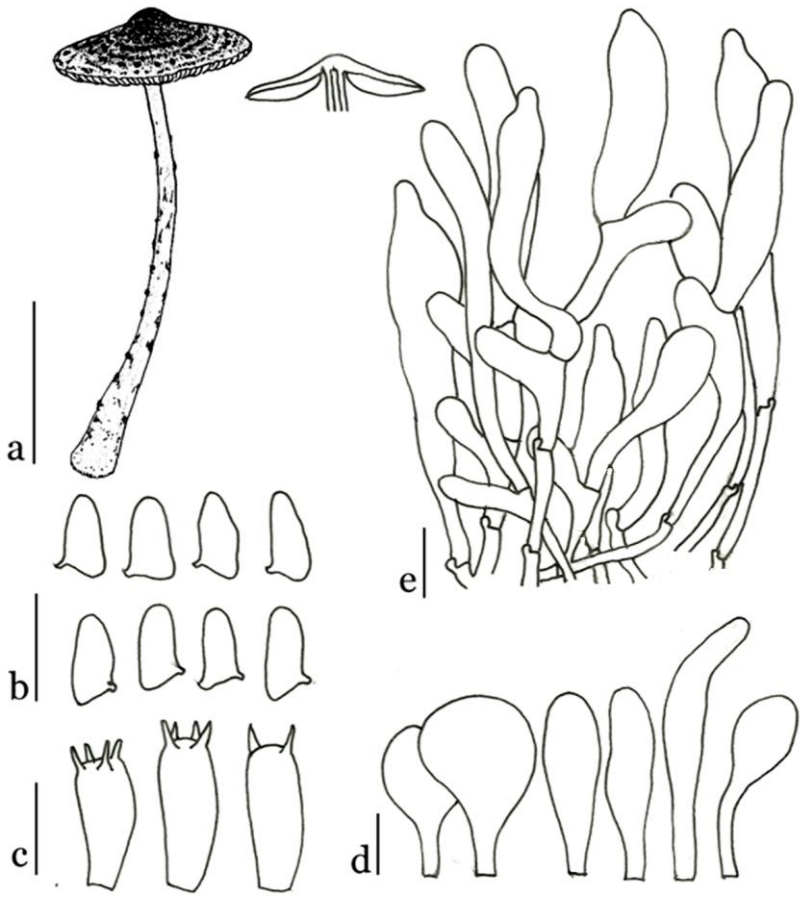

Figure 3.10 *Lepiota papillata* (MFLU090041). a. basidioma, b. basidiospores, c. basidia, d. cheilocystidia, e. pileus covering. Scale bars: a = 10 mm, b–e = 10 µm

Habitat and distribution: Growing in small group, saprotrophic and terrestrial, on humus-rich, nutrient-rich soil and dead leaves, found during rainy season in rain forest with dominant bamboo. Only known from one locality in northern Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Distr., Hot Spring National Park, N 16° 06' 16.1", E 99° 43' 07,9", 780–805 m alt., 9.VIII.2007, P. Sysouphanthong, MFLU090041 (holotype).

Discussion: This species is similar to *L. griseovirens* but it differs especially in the shape of the cheilocystidia: pyriform, spathuliform to narrowly clavate in *Lepiota papillata*, and in the pileus covering elements often having an abrupt apical excrescence.

Lepiota grangei (Eyre) Kühner comes close in morphology, but differs in the dark gray-green, grayish blue, green-brown, or blue-brown to brown pileus, the cylindrical to utriform cheilocystidia, and the intracellular pigment in the long pileus covering elements.

This species comes very close to *L. tomentella* J.E. Lange in the color of pileus covering and size of basidospores, but the elements of pileus covering are longer and without abrupt apical excrescence in *L. tomentella* (Vellinga & Huijser, 1993).

Lepiota pilodes Vellinga & Huijser differs from *L. papillata* by dull orange-brown to dark brown and ochraceous brown colors at centre of pileus, and the elements of pileus- and stipe covering are longer 60–280 (–330) × (6.5) 8.0–21 µm (Vellinga & Huijser, 1993; Vellinga, 2001).

9. *Lepiota poliochloodes* Vellinga & Huijser, Persoon. 15: 229. 93.(Fig.3.11)

Misapplied name: *Lepiota griseovirens* sensu Bon, Fl. mycol. Eur. 3, Lépiotes: 55. 1993; sensu D. Reid, Fung. Rar. Ic. Col. 6: 14–16. 1972; *Lepiota griseovirens* var. *griseovirens* sensu M. Bon, Docum. Myc. 11 (43): 38. 1981.

Pileus 12–25 mm, campanulate to umbonate, applanate to plano-concave with wide umbo, with straight margin, at centre with crowded squamules to small plush-like squamules or floccules, grayish brown, grayish green brown to dark brown (Mu. 2.5 Y 4–5/2, 6E3–6, 6F5–6), with squamules or fibrils around umbo toward margin and distant at marginal zone, on brownish orange, slightly orange or light brown fibrillose background (5C4, 6D4); margin peeling from background when mature, exceeding lamellae. Lamellae, L = around 40, l = 0–3, crowded to moderately crowded, just free, ventricose, 1.7–3 mm wide, white to yellowish white, distinctly pinkish cream (4A2, Mu. 10 YR 8/6), with eroded to floccose, distinctly cystidiose edge. Stipe 25–36 × 1–2.5 mm, cylindrical and slightly wider at middle, with white or yellowish white to orange-white or pale pink (4A2, 5A2) fibrillose background, with dark brown to dark gray-green (6F5–6) squamules from annular zone to base, with white to yellowish white (4A2) fibrils between squamules, hollow, with white rhizomorphs at base. Annulus an annular zone, with gray-green to dark brown squamules. Context in pileus white to pale cream, 1.5–2 mm wide, in stipe white, yellowish white (4A2) to pale orange. Smell mild. Taste unknown. Spore print white.

Basidiospores [70,3,3] 6.2–8.2 × 3.0–3.8 µm, avl × avw = 7.2 × 3.5 µm, Q = 1.6–2.4, Qav = 2.0, in side-view cylindrical or narrowly triangular with or without lateral spur, but spur not protruding abaxially, and with rather abrupt base, in frontal

view cylindrical, with rounded apex or tapering toward apex, with 1 or 2 guttules, congophilous, dextrinoid, cyanophilous, not metachromatic in Cresyl Blue. Basidia 15–19 × 6 – 8 µm, 4-spored, rarely 2-spored, relatively short and plump. Lamella edge sterile. Cheilocystidia 18–40 × 3–9 µm, mostly cylindrical, to very narrowly clavate, some narrowly lageniform or slightly fusiform, strangulate, digitate. Pleurocystidia absent. Pileus and stipe covering a trichoderm or slightly irregular trichoderm made up of erect cylindrical, narrowly clavate to narrowly lageniform elements, (20 –)30–130 × 6.5–15 µm, with pale brown walls, and with intracellular brown pigment, thin-walled, with under layer made up of cylindrical, hyaline, 2.5–9.0 µm wide hyphae. Clamp connections present in all tissues.

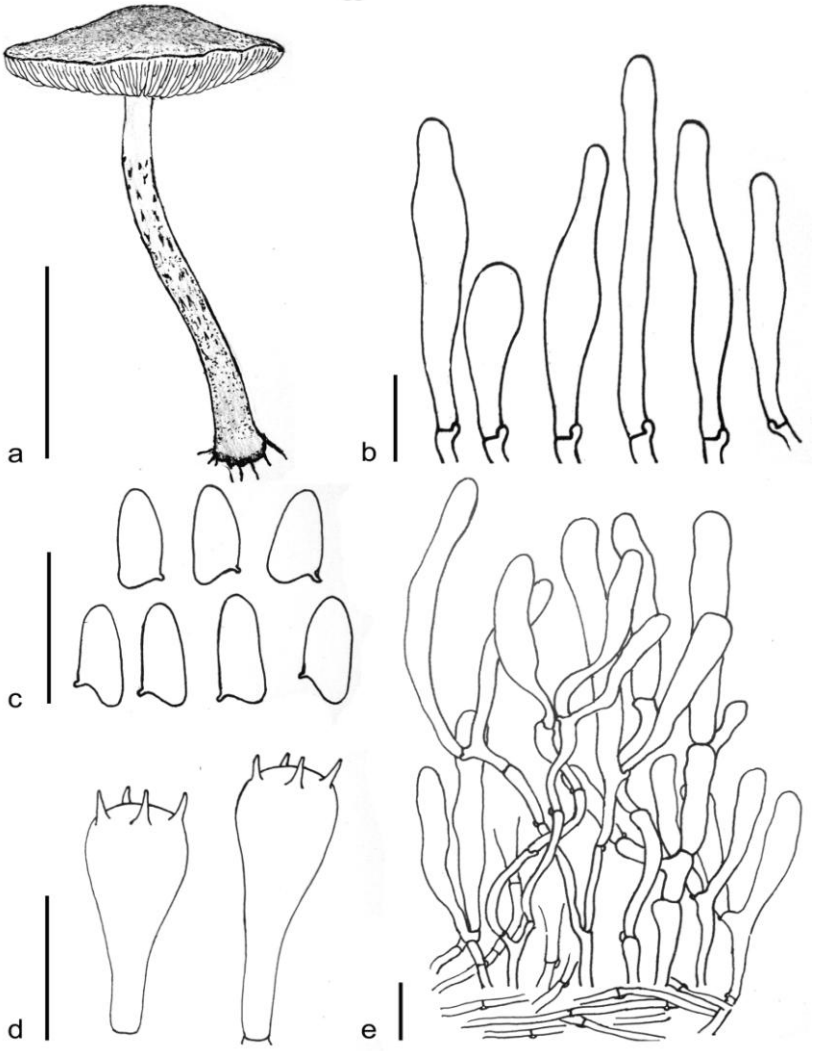

Figure 3.11 *Lepiota poliochloodes* (MFLU090182). a. basidioma, b. cheilocystidia, c. basidiospores, d. basidia. e. pileus covering. Scale bars: a = 20 mm, b–d = 10 µm, e = 20 µm.

Habitat and distribution: Growing solitary, saprotrophic and terrestrial in deciduous forest and bamboo forest. Reported from the Netherlands, Denmark, France, and Great Britain (Vellinga, 2001); this is the first record for Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Distr. Mae Sae village, N 19° 07' 13.7", E 98° 43' 52.9", 905 m alt., 8.VIII.2008, P. Sysouphanthong, MFLU090182; Chiang Mai Prov., Mae Taeng Distr., Mok Fah Waterfall National Park, N 20° 02' 43.1", E 99°52' 35.0", 596 m alt., 5.VII.2008, P. Sysouphanthong, MFLU090118; Chiang Mai Prov., Mae Rim Distr., Mae Sa Valley, 12.VII.2008, J-K. Liu (collection ecv3877; MFLU081272).

Disscussion: This species is rare in Northern Thailand, and only known from three localities. Both macro- and micromorphology of *L. poliochloodes* of material from Thailand agree with the description of *L. poliochloodes* from Europe (Vellinga & Huijser, 1993). Unfortunately, there is no nrITS sequence available from Europe to compare the Thai material with.

The relatively small basidiospores and the short elements in the pileus covering, combined with grayish brown to green pileus covering on a slightly orange background characterize this species.

Lepiota griseovirens Maire is similar in size of basidiospores and cheilocystidia, but differs from *L. poliochloodes* in the much darker colors of the basidiomata and the longer elements in the pileus covering.

10. *Lepiota* sp. 1 (Fig. 3.12)

Pileus 15 mm, plano–convex with umbo and slightly inflexed margin, at umbo not completely closed, with tufted scales; around umbo scales are more spreading out on yellowish background; scales small and reddish brown (Mu. 2.5 YR 3/4–6). Lamellae, L = around 35, l = 1–3, free, not very crowded, slightly ventricose, yellowish (Mu. 10 YR–2.5 Y 8/4) with lighter, slightly cystidiose edge. Stipe 24 × 2 mm, cylindrical, above annular zone innately lengthwise fibrillose, pale brown, below annular zone with small patches, sometimes spiny, of same material and color as on pileus, hollow, with white rhizomorphs. Smell a bit unpleasant, when old astringent.

Basidiospores [20,1,1] in side view 7.1–8.4 × 2.8–4.2 µm, avl × avw = 7.8 × 3.2 µm, Q = 1.9–2.8, avQ = 2.4, with distinct basal spur, in some spores spur also abaxially bulging, with round to slightly amygdaliform apex, in frontal view obovoid to subcylindrical, thick-walled, smooth, dextrinoid, and congophilous. Basidia 15–22 × 6.5–8.0 µm, 4-spored, some 2-spored, with basal clamp connection. Lamella edge sterile. Cheilocystidia 22–28 × 7.0–10.5 µm, utriform, fusi-utriform, and narrowly clavate, with basal clamp-connection. Pleurocystidia absent. Pileus covering trichodermal, made up of erect elements, 52–170 × 7.5–12.5 µm, cylindrical with rounded apex, almost always narrowed into pedicel, some very narrowly lageniform, with intracellular brown pigment. Stipe covering similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution: Solitary, saprotrophic on humus rich soil in orchard with bamboo; only collected once close to Chiang Mai.

Material examined:T hailand, Chiang Mai Prov., Mae Rim Distr., Mae Sa Valley, 12.VII.2008, leg. J-K. Liu (collection E.C. Vellinga 3881; MFLU08-1259).

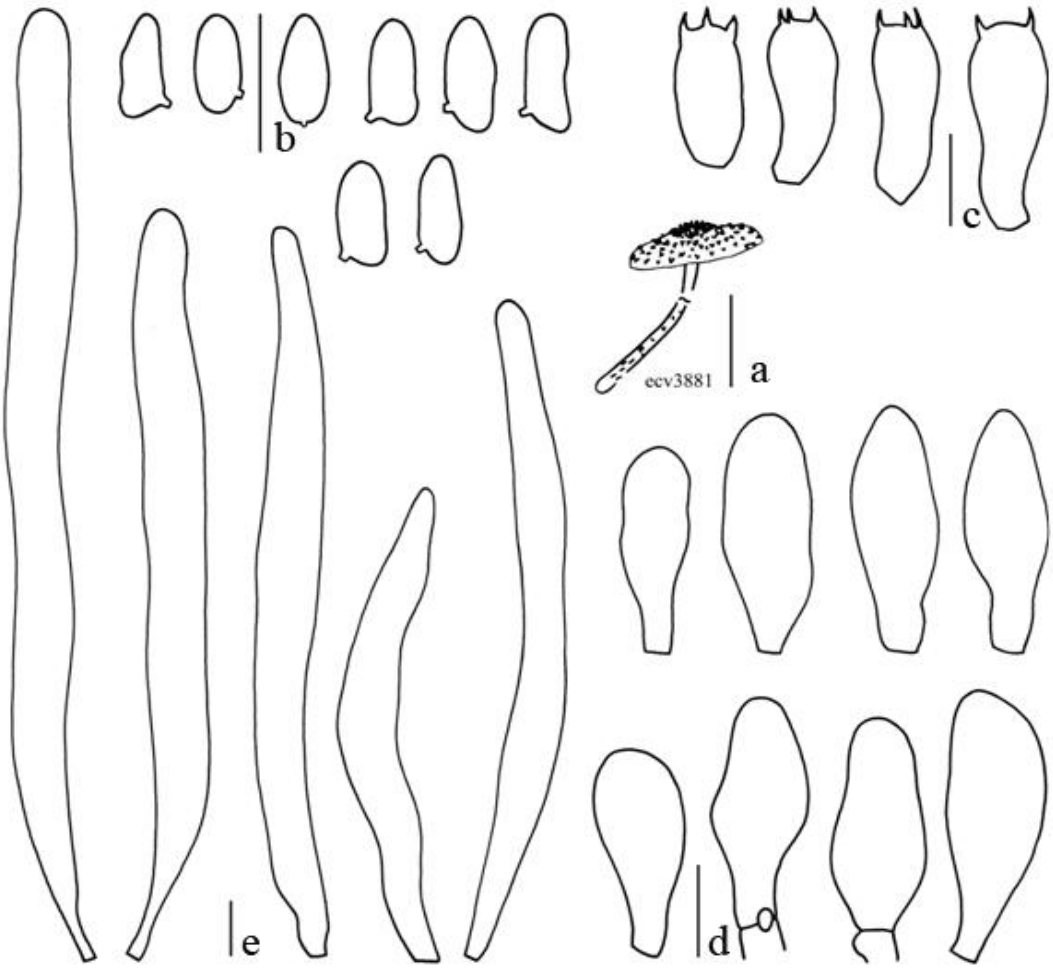

Figure 3.12 *Lepiota* sp. 1 (MFLU081259). a. basidioma, b. cheilocystidia, c.basidiospores, d. basidia, e. pileus covering elements. Scale bars: a = 1 cm, b–d = 10 µm.

Discussion: Macroscopically, this species resembles *L. echinella* Quél. & G.E. Bernard, because of the tufted reddish brown scales on the pileus, but it differs in the spurred spores and the shorter elements in the pileus covering.
We refrain from describing this taxon as a new species, as the one and only collection consisted of one specimen only.

11. *Lepiota* sp. 2 (Fig. 3.13)

Pileus 30 mm, campanulate or umbonate with broad umbo, with straight margin, covered with crowded grayish brown to slightly reddish brown (8F6–8) squamules at center and around umbo toward margin and there more radially fibrillose, on grayish yellow (4B3), white to yellowish white (4A2), radially fibrillose background; margin exceeding lamellae. Lamellae free, crowded, broadly ventricose,

2.5–3 mm wide, white to yellowish white (3A2), with slightly wavy edge. Stipe 40 × 3–4 mm, cylindrical, slightly wider at center and tapering to apex and curved at base; surface with white to pale orange (5A3) fibrils, with squamules from middle downwards, concolorous with squamules on pileus, with yellowish brown (5E5) fibrillose squamules at basal zone. Context in pileus thick at umbo, 4 mm wide, white to yellowish white (3A2); in stipe pale orange (6A3), hollow. Smell Termitomyces-like. Taste sweet. Spore print white.

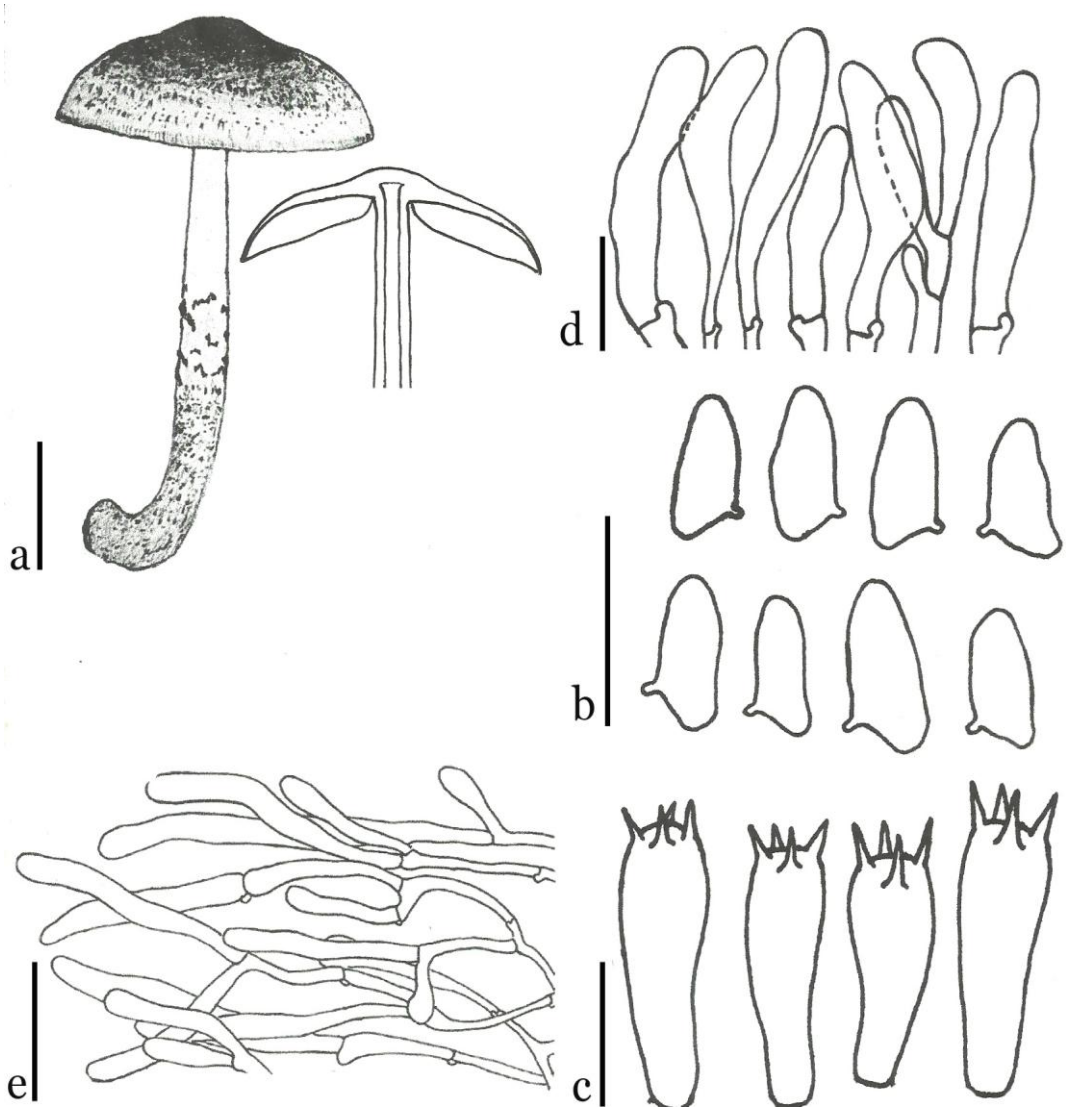

Figure 3.13 *Lepiota* sp. 2 (MFLU090006). a. basidioma and context, b. Cheilocystidia, c. basidiospores, d. basidia, e. pileus covering. Scale bars: a = 10 mm, b–d = 10 µm, e = 20 µm.

Basidiospores [25,1,1] 6.2–8.0 × 3.2–4.0 µm, avl × avw = 7.3 × 3.7 µm, Q = 1.6–2.5, Qav = 1.9, in side-view with truncate to spurred base, oblong to cylindrical triangular or with curved abaxial side, sometimes with rounded or acute apex, in frontal view oval or cylindrical, strongly dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 16–21 × 7.0–8.0 µm, narrowly clavate, 4–spored, hyaline, thin-walled. Lamella edge sterile, with crowded cheilocystidia. Cheilocystidia 12–45 × 5.2–10 µm, cylindrical, sometimes narrowly lageniform, narrowly clavate, with rounded apex, subcapitate, sometimes slightly swollen at center, utriform, colorless, thin-walled. Pleurocystidia absent. Pileus covering a cutis made up of irregular narrowly clavate hyphae, 25–128 × 10–18 µm, usually narrowed into pedicel, with rounded or subcapitate apex, slightly thick-walled, with or without septum, with basal clamp connections, with yellowish brown parietal pigment; under layer made up of cylindrical 3.0–7.0 µm wide hyphae, thin-walled, with yellowish brown walls in upper part, with hyaline walls in lower part. Stipe covering a cutis similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution: Growing solitary, saprotrophic and terrestrial on humus-rich soil with dead leaves and wood, in deciduous forest with Lithocarpus spp. Only found in one locality in northern Thailand.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Distr., Pha Deng Village, N 19° 07’ 13.7”, E 98° 43’ 52,9”, 905 m alt., 19.VII.2007, P. Sysouphanthong, MFLU090006.

Discussion: This taxon is characterized by grayish brown to reddish brown squamules on pileus and stipe, truncate to spurred basidiospores, cylindrical to narrowly clavate cheilocystidia, and the cutis-like pileus covering.

Lepiota griseovirens Maire can be confused with this species in the field because of the color of the pileus squamules, but differs in the structure of the pileus covering.

The single collection consisted of one basidioma only, and we were not able to get a nrITS sequence either, so we do not describe this as a new species until we can make further collections

3.4. Conclusions

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. eurysperma*, *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 thespecies are provided. In addition, the identity of these *Lepiota* species is also clarified based on molecular data.

CHAPTER 4

Lepiota SECTION *Lepiota* IN NORTHERN THAILAND

4.1 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, 1993; 2003a). *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 united to form pyramidal spines (Knudsen 1980; Montoya & Bandala 2005; Vellinga 2001; 2003a).

Phylogenetic analyses of the ITS and LSU regions of *Lepiota* sect. *Lepiota* revealed that 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' basidiospores (Vellinga, 200b; Liang, Yang & Xu, 2011).

The diversity and species of *Lepiota* and its section *Lepiota* in Asia are not well investigated yet. 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 *L. cortinarius* from China; *L. clypeolaria* (Bull.: Fr.) Kummer, *L. metulispora* (Berk. & Broome) Sacc., and *L. thrombophora* (Berk. & Broome) Sacc. are known from Sri Lanka, India, Nepal and tropical China (Petch & Bisby, 1950; Natarajana & Manjula, 1983; Manjula, 1983; Pegler, 1972; 1986; Liang et al., 2011). Only a few species have been recorded from Thailand, such as *L. clypeolaria* and *L. cortinarius* (Anong Chandrasrikul et al., 2008). However, these two species were identified by macrocharacters only.

Here we describe the *Lepiota* species in section *Lepiota* from northern Thailand, based on their morphology and in a few cases also on molecular data.

4.2 Materials and Methods

4.2.1 Collecting and Examination Methods

All mushroom samples studied in this chapter were collected from Chiang Mai and Chiang Rai, and the specimens were characterised morphologically as described in section 2.2.

4.2.2 Molecular and Phylogenetic Methods

4.2.2.1 DNA extraction, PCR, sequencing and data analysis: In general, overall procedures were carried out as previously described in Chapter 3 (see Section 3.2). The DNA sequences obtained were then analysised using the procedures as described previously in Section 3.2.2. It should be noted that, for this analysis, the sequences of *Lepiota* section *Lepiota* were used (see Table 4.1). For outgroup, the ITS sequence of *Macrolepiota procera* was used.

Table 4.1 Overview of Species, Collections and Herbarium with GenBank Accession Numbers for the ITS Sequences Used for the Phylogenetic Analyses

Species	Country	Collection & Herbarium	ITS GenBank number
*L.*cf. *aspera*	Thailand	MFLU090061	HM488788
L. cf. *aspera*	Thailand	MFLU090181	JN224820
L. cf. *aspera*	Netherlands	E.C. Vellinga 2233 (L)	AY176354
L. aureofulvella	Thailand	MFLU090183	HQ647293
L. alopochroa	Thailand	MFLU090178 (MFLU)	HQ647294
L. boudieri	Netherlands	E.C. Vellinga 1180 (L)	AF391025
L. brunneoincarnata	Netherlands	E.C. Vellinga 2260 (L)	AF482875
L. castaneidisca	USA	E.C. Vellinga 2411 (UC)	AF391063
L. citrophylla	Thailand	MFLU090172	HQ647295
L. clypeolaria	USA	M.M. Rogers (UC)	AF390999
L. clypeolaria	Germany	E. Schaetzle (L)	AF390998
L. cortinarius	Italy	MCVE:16811	FJ998404
L. cortinarius	Italy	MCVE:865	FJ998393
L. cortinarius	USA	R.E. Tulloss (UC)	AY176468
L. cristata	China	HKAS49449	EU081945
L. cristata	China	HKAS45053	EU081948
L cf. *echinacea.*	Thailand	MFLU090142	JN224821
L. echinacea	Netherlands	H.A. Huijser (herb. Huijser)	AY176469
L. echinella	Italy	MCVE:2320	FJ998397
L. erminea	Netherlands	E.C. Vellinga 2290 (L)	AY176470
L. eurysperma	Thailand	MFLU090035 (MFLU)	HQ718462

Table 4.1 (continued).

Species	Country	Collection & Herbarium	ITS GenBank number
L. felina	USA	VPI-OKM20596	LFU85330
L. cf *fraterna*	Thailand	MFLU090173	JN224823
L. cf *fraterna*	Thailand	MFLU090048	JN224822
L. fuscovinacea	Netherlands	E.C. Vellinga 2255 (L)	AY176372
L. hystrix	France	H.A. Huijser s.n (herb.Huijser)	AY176377
L. ignicolor	The Netherlands	H.A. Huijser (herb. Huijser)	AY176472
L. ignivolvata	France	E.C. Vellinga 2127 (L)	AY176473
L. lilacea	USA	E.C. Vellinga 2451 (UC)	AY176379
L. magnispora	South Korea	VPI-OKM22029	U85326
L. magnispora	USA	C. Ardrey 41 (HSC)	AF391011
L. magnispora	Netherlands	E.C. Vellinga 2246 (L)	AF391021
L. magnispora	Canada	E.C. Vellinga 1574 (L)	AF391016
L. magnispora	Netherlands	H.A. Huijser (herb. Huijser)	AF391023
L. pilodes	USA	E.C. Vellinga 3234 (UC)	EF080865
L. spheniscispora	USA	E.C. Vellinga 2559 (UC)	AF391004
L. spheniscispora	USA	E.C. Vellinga 2429 (UC)	AF391002
L. pongduadensis	Thailand	MFLU090184 (MFLU)	HQ718461
L. pseudolilacea	Netherlands	E.C. Vellinga 2278 (L)	AY176392
L. subgracilis	Netherlands	E.C. Vellinga 1783 (L)	AY176490
L. subincarnata	Netherlands	E.C. Vellinga 2234 (L)	AY176491
L. xanthophylla	Netherlands	E.C. Vellinga 2240 (L)	AY176405
L. sp.	California,USA	E.C. Vellinga 2582 (UC)	AY176397
L. sp.	Thailand	E.C. Vellinga 3881 (MFLU)	HQ647297
L. sp.	Thailand	MFLU090152	JN224826
L. sp.	Thailand	MFLU090143	JN224825
L. sp.	Thailand	MFLU090192	JN224828
L. sp.	Thailand	MFLU090120	JN224824
L. sp.	Thailand	MFLU090167	JN224827
Macrolepiota procera	China	HKAS31471	HM125512

4.3 Results and Discussion

The Maximum Parsimony analysis of the nrITS data set, comprising of 48 species of the ingroup, and *M. procera* as outgroup, composed of 725 characters, resulted in a relatively well resolved phylogeny (Figure 4.1), with Clade I (with high Bootstrap support) made up of the species with fusiform and ellipsoid spores and a trichodermal pileus covering made up of long elements, often with short cells at the base, and Clade II (with 100% Bootstrap support) with species with spurred spores 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.

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* (see Fig. 4.1).

Figure 4.1 *Lepiota* Section *Lepiota* in Nature, a. *L. metulispora* (MFLU09-0165), b. *L. eurysperma* (MFLU090184), c. *L. pongduadensis* (MFLU090-184), d. *L. microcar**p**a* (MFLU100491), e. *L.* sp. (MFLU10 0502)

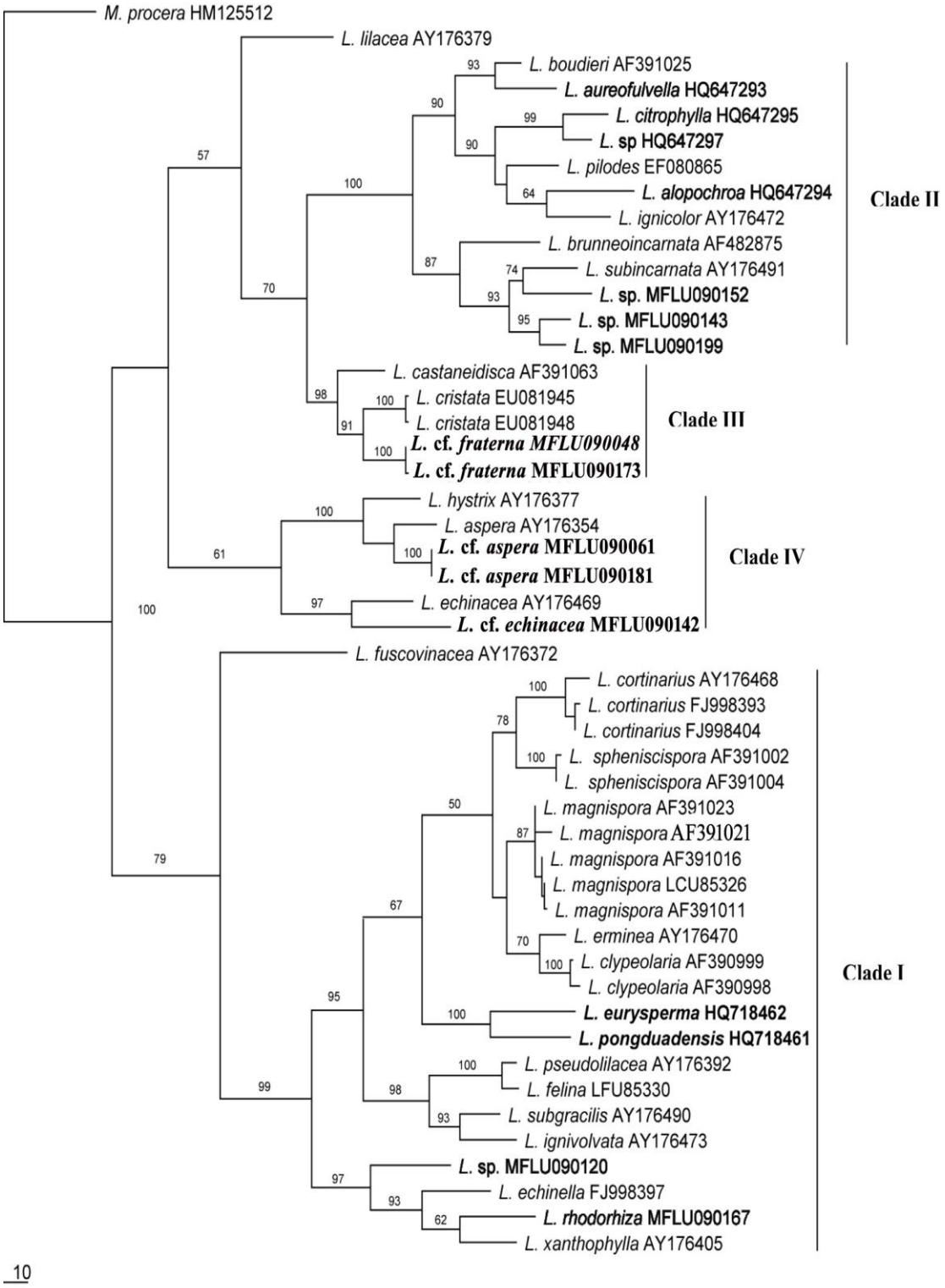

Figure 4. 2 MP Phylogeny of *Lepiota* in Northern Thailand Based on ITS Sequences

Table 4.2 Overview of Some Basidiospore Characters, Size, Shape, and Pileus Covering Structure in Collections from Thailand

Secies	Spore sizes (µm)	Quotient	Spore shape	Pileus covering
L. eurysperma	7.8-11.5 × 4.5-6.5 (8.9 x 5.2)	1.3- 2.4 (1.7)	broadly amygdaliform to oblong without suprahilar depression and straight abaxial side	With short clavate elements
L. metulispoa	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
L. microcarpa	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
L. pongduadensis	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
L. 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

4.3.1 Key to species of *Lepiota* in Northern Thailand

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

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

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*

1. *Lepiota eurysperma* Sysou., Hyde & Vellinga, Cryp. Mycol. 33(1):25. 2012. (Fig. 4.3)

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 concentrical 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 x 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 x 4.5-6.5 µm, avl x avw = 8.9 x 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 x 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 x 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 x 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 x 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.

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 Hot Spring National Park, Mae Taeng District, Chiang Mai Province.

Material examined: Thailand, Chiang Mai Prov., Mae Taeng Dist., Hot Spring National Park, N 16° 06’ 16.1”, E 99° 43’ 07,9”, alt., 780-805 m, 10. VIII. 2007, P. Sysouphanthong, MFLU0900035; ibidem, 4. VIII. 2007, P. Sysouphanthong, MFLU090003; ibidem, 17. IX. 2007, P. Sysouphanthong, MFLU0900069; ibidem, 8. VIII. 2008, P. Sysouphanthong, MFLU0900198.

Discussion: *Lepiota eurysperma* is variable in colour of the basidiomata, 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 sect. *Lepiota* (Fig. 4.1).

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).

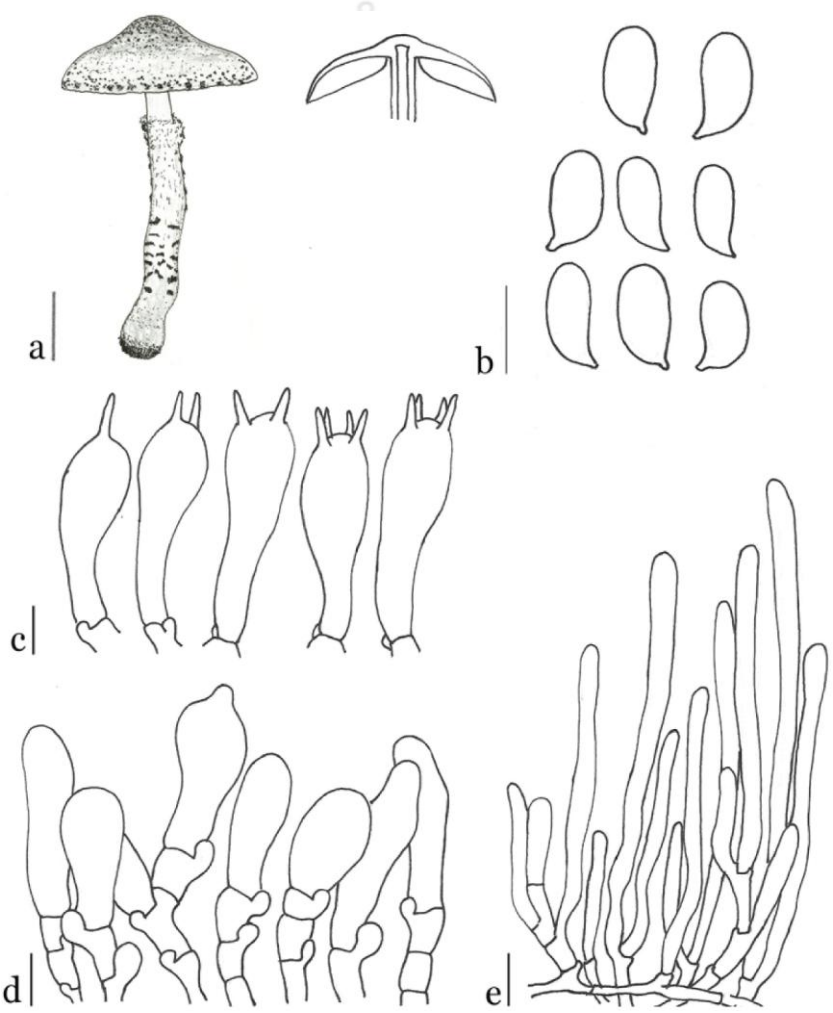

Figure 4.3 *Lepiota eurysperma* (MFLU090035), a. basidio-mata and a section, b. basidiospores, c. basidia, d. cheilocystidia, e. pileus co-vering. Scale bars a =10mm, b-f = 10 µm.

Lepiota subgracilis Kühner is distinguished from *Lepiota eurysperma* by greyish pinkish-brown pyramidal squamules on 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.

2. *Lepiota metulispora* (Berk. & Br.) Sacc., Syll. Fung. 5: 38. 1887. (Fig. 4.4)
≡ *Agaricus metulisporus* Berk. & Broome in J. linn. Soc., Bot. 11: 512. 1871.
≡ *Lepiota clypeolaria* var. *metulispora* (Berk. & Broome) Babos in Annls hist.-nat. Mus. Natn. hung. 53: 198. 1961 (basionym 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) concentrical 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 orange-white (4A3, 5A2) background. Annular 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 × 4.0-5.2 µm, avl × avw=15.0 × 4.7 µm, 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 metachromatic. 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 & Manjula, 1983; Kumar & Manimohan, 2009), China (Liang, Yang & Xu, 2011a), and this is a first report to Thailand.

Material examined: Thailand, Chiang Mai Prov, Mae Taeng Dist., Pha Deng Village, N 19° 07' 13.7", E 98° 43' 52.9", 905 m, 25 VII 2008, P. Sysouphanthong, MFLU090165; Chiang Rai Prov., Mueng Dist., Doi Ngaem of Mae Fah Luang National Park, N 18° 05' 59.1", E 102° 40' 02.9", 488 m, 18. VI. 2009, P. Sysouphanthong, MFLU100495.

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 pilesu with a brown surface, ochraceous buff squamules, striate or sulcate surface toward margin, an appendiculate margin, an equal stipe with evanescent annulus, elongate basidiospores with a suprahilar depression (11-19 x 3.7-5 µm), and trichodermal pileus covering made up of elongate elements (25-170 x 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 Prov.).

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 x 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 x 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 x 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).

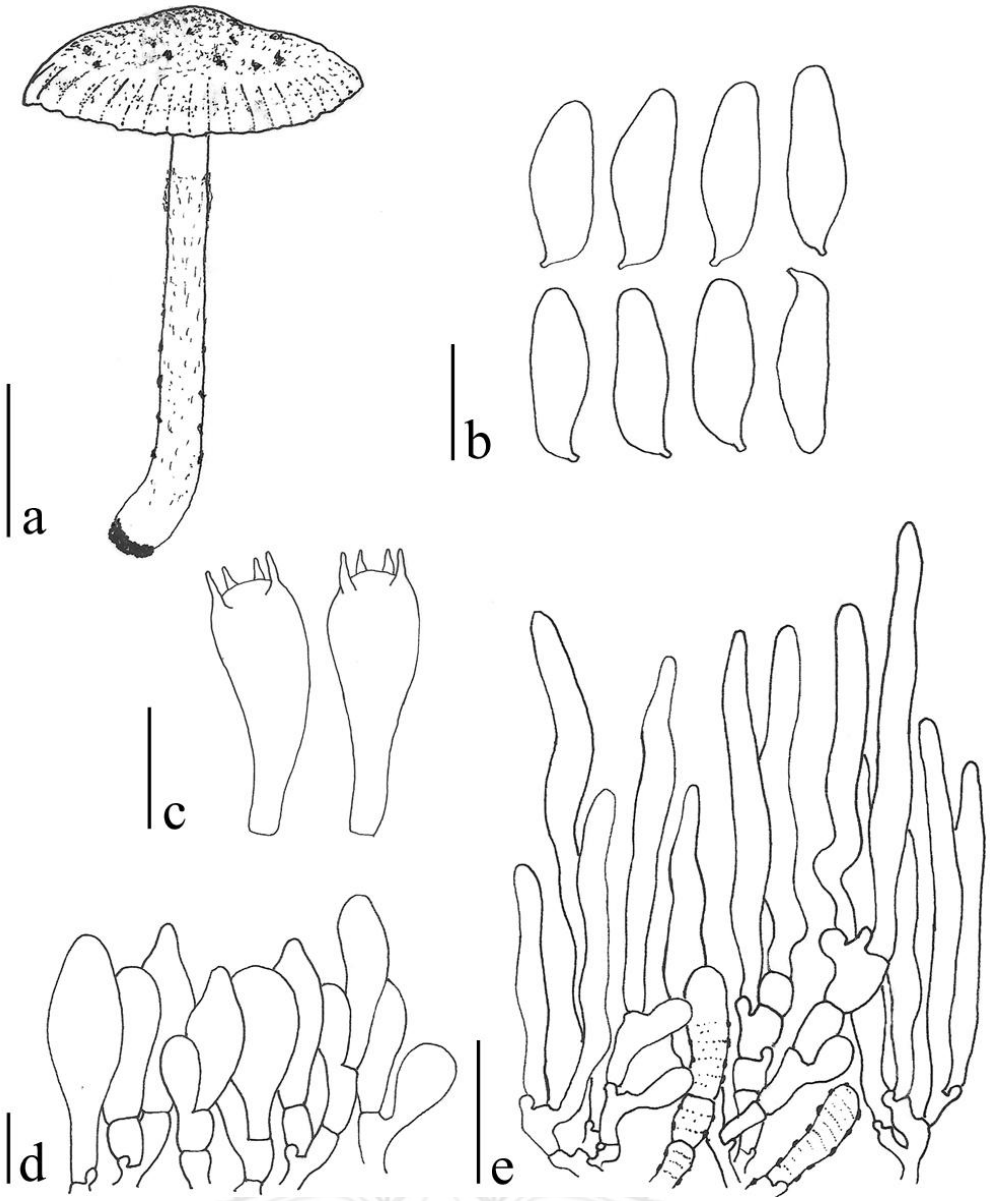

Figure 4.4 *Lepiota metulispora* (MFLU090165), a. basidio-mata and a section, b. basidiospores, c. basidia, d. pileus covering, e. cheilocy-stidia. Scale bar. a = 10 mm, b, c and e = 10 µm, d = 20 µm.

3. *Lepiota microcarpa* Sysou., Hyde, Chuk., & Vellinga, Cryp. Mycol. 33(1): 25. 2012. (Fig. 4.5)

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 concentrical 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 × 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. Lamellaea an annular zone, cortinate or fibrillose, white. Context in pileus thin, white to light brown, less than 1 mm wide; in stipe concoulourous with surface, hollow. Taste and smell not observed. Spore print white.

Basidiospores [75,3,3] 10.8-16.5 × 4.0-5.3 µm, avl × avw= 13.5 × 4.7 µm, Q= 2.5-3.6, avQ= 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 × 8.2-10.5 µm, narrowly clavate, 4-spored. Lamella edge sterile. Cheilocystidia 15.5-30 × 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 × 10-12.5 µm, brown and thin-walled, with brown parietal and intracellular pigments, with short clavate elements, 8.5-37.5 × 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 distributions: 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.

Material examined: Thailand, Chiang Rai Prov, Mae Fah Luang Dist., Doi Tung National Park, 10. VI. 2009, P. Sysouphanthong (MFLU100491). Chiang Mai Prov., Mueng Dist., Doi Suthep Pui National Park, N 18°48.62’, E 98° 54.60’ 1455 m. alt, 6. VI. 2010, P. Sysouphanthong (MFLU10 0521), ibidem, 12. VI. 2010, P. Sysouphanthong (MFLU10 0526).

Discussion: All material of this species is 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; in the field, it can be confused with a yet undescribed *Lepiota* species (MFLU090126, MFLU090120, and MFLU090130) in sect. 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).

There are no species with such small basidiomata known from Europe or North America. It also shares some characters with *L. attenuata* from southern China and Taiwan in micromorphology such as shape of basidiospores, cheilocystidia and elements in pilus covering but differs in all those size, and *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).

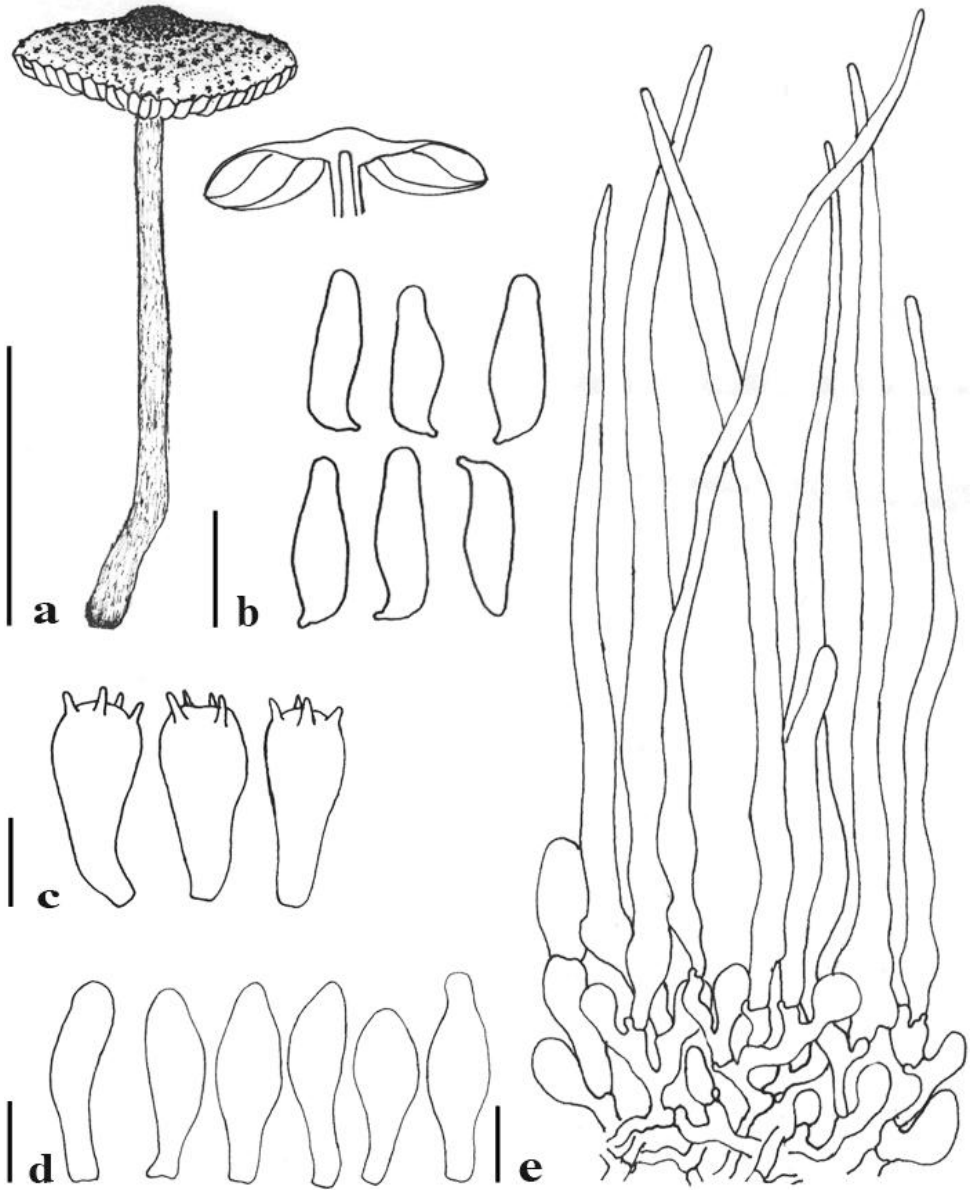

Figure 4.5 *Lepiota microcarpa* (MFLU100491), a. basidio-mata and a section, b. basidiospores, c. basidia, d. cheilocystidia, e. pileus and stipe covering. Scale bars. a= 1 mm, b-d= 10 µm, e= 20 µm

4. *Lepiota pongduadensis* Sysou., Hyde & Vellinga, Cryp. Mycol. 33(1): 25. 2012. (Fig. 4.6)

Pileus 19-35 mm, umbonate, expanding to capanlate, 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 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 × 4.5-5.5 µm, al × aw= 14.6 × 5.2 µm, 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 × 8-10 µm, clavate, hyaline, slightly thick-walled, 4-spored. Lamella edge sterile, with crowded cheilocystidia. Cheilocystidia 20-30 × 8-13 µm, 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 × 5-11.5 µm, 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., Hot Spring (Pong Duad) National Park, N 16° 06’ 16.1”, E 99° 43’ 07,9”, 780-805 m, 8.VIII.2008, P. Sysouphanthong, MFLU090184; ibidem, 22.VI.2009, P. Sysouphanthong, MFLU10 0497; Chiang Rai Prov., Mae Lao Distr., Khun Kon Waterfall National Park, 22.VII.2009, P. Sysouphanthong, MFLU100500.

Discussion: *Lepiota pongduadensis* is found at Hot Spring National Park of Chiang Mai Province and Khun Kon Waterfal National Park 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.

*Lepiot*a 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 (Figure 4.4), as it lacks the radial aspect of the pileus squamules, and has a distinct cuff-like annulus.

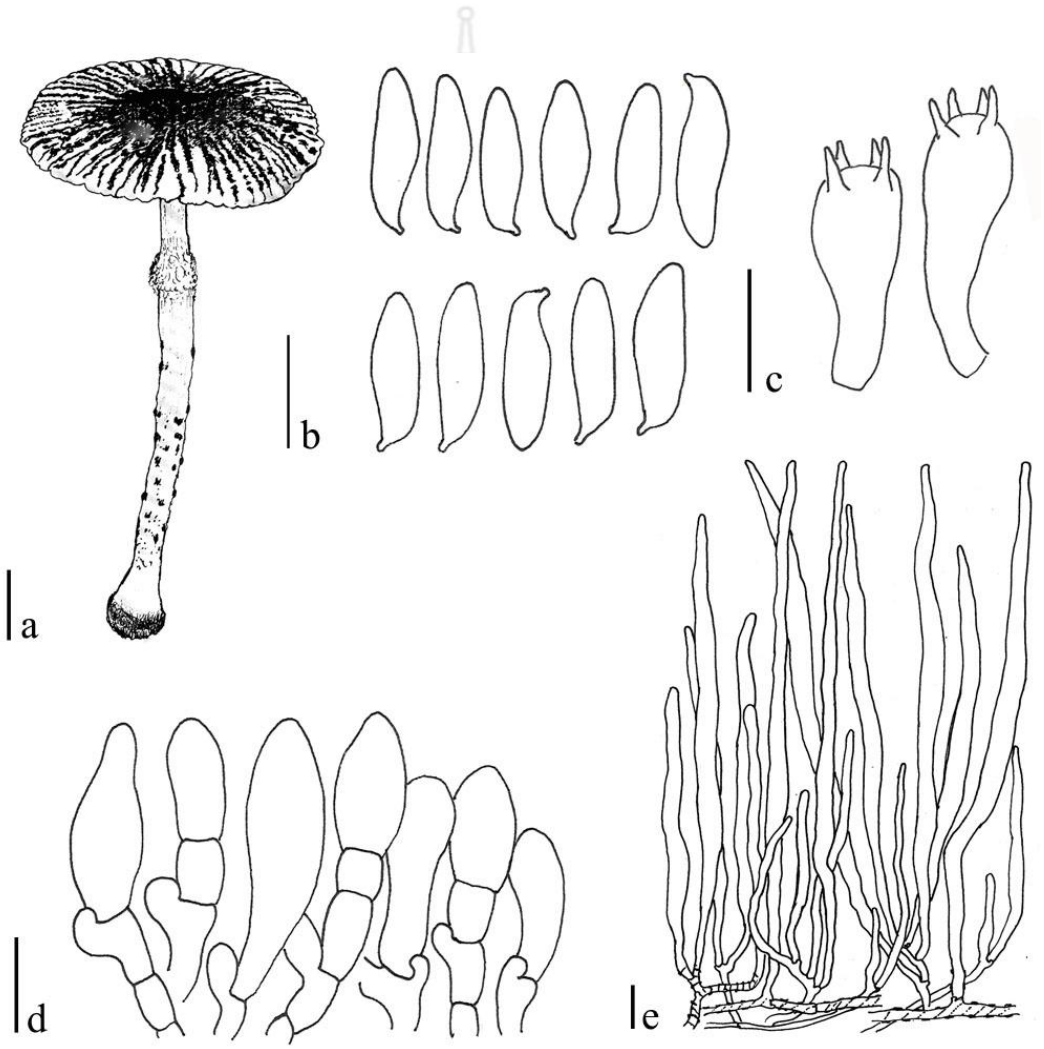

Figure 4.6 Morphological *Lepiota pongduadensis*. b, c, d (MFLU100497); a, e (MF-LU090184); a. basidioma and a section, b. basidiospores, c. basidia, d. cheilocystidia, e. pileus and stipe covering. Scale bars. a=10 mm, b-e= 10 µm.

Lepiota cortinarius J.E. Lange has sturdier basidiomata with a distinctly cortinate pileus margin, and has small, relatively narrow spores (7.5-9.5 x 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 x 4.0-6.5 µm (Vellinga, 2001). These latter two taxa also differ in their ITS sequences from *L. pongduadensis* (Figure 4.2).

5. *Lepiota* sp. (Fig. 4.7)

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 × 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, concolourous 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.

Basidiospores [25,1,1] 11.3-16 × 4-5 µm, on average 14.6 × 4.8 µm, Q = 2.7-3.2, Qav = 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 metachomatic. Basidia 24-30 × 9-10 µm, clavate, 4-spored, hyaline, slightly thick-walled. Lamella edge sterile. Cheilocystidia 17-30 × 6-10 µm, 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 × 7-12.5 µm, slightly thick-walled, brown-walled, without short clavate element between and under cylindrical elements; underlayer with cylindrical hyphae, 4.5-6.5 µm 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 Ching Rai Province.

Material examined: Thailand, Chiang Rai Prov., Mae Sai District, Doi Nang Norn National Park, 8.VIII.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.

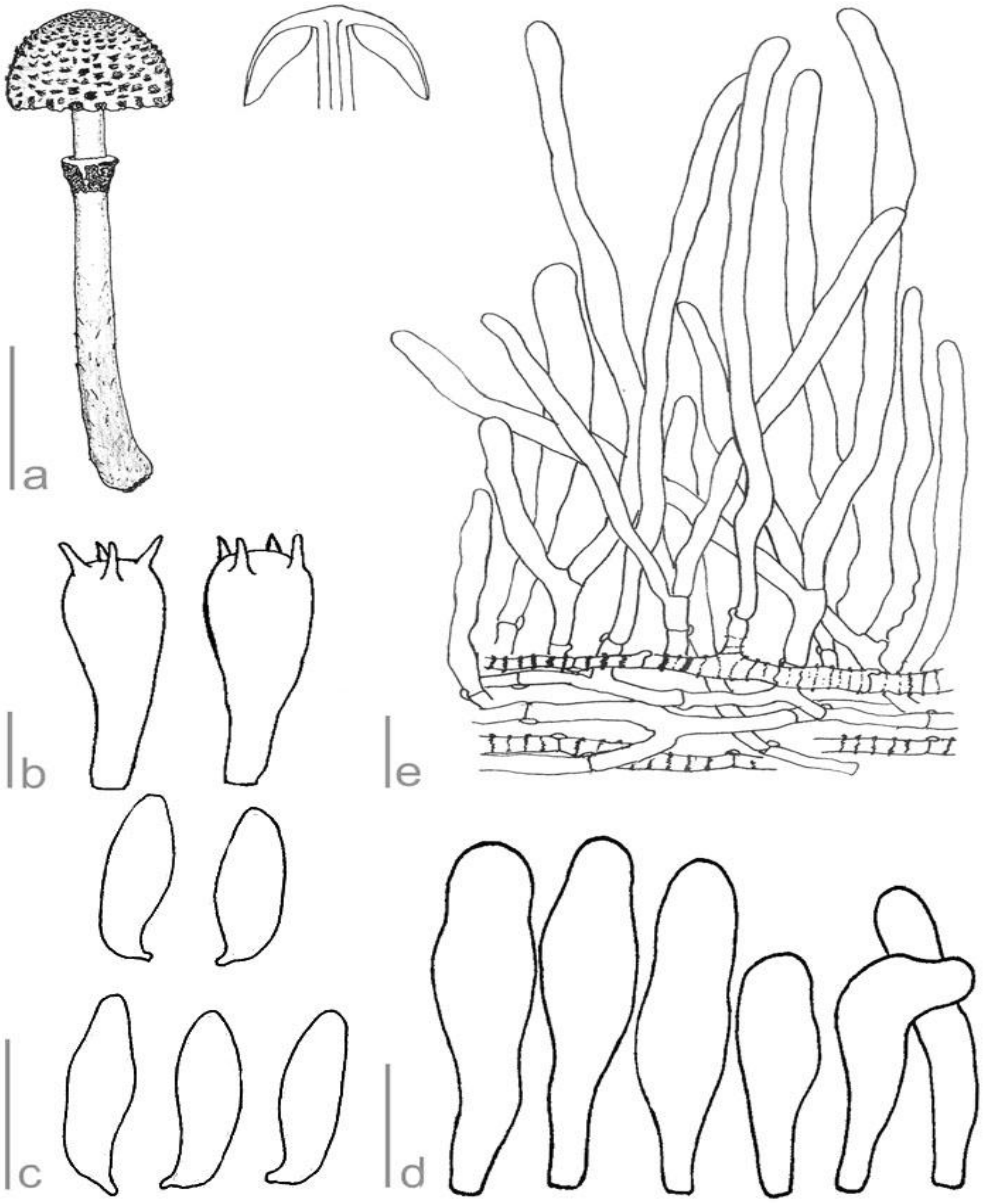

Figure 4.7 *Lepiota* sp. (MFLU10 0502), a. basidioma and section, b. basidia, c. basidiospores, d. cheilocystidia, e. pileus covering.
Scale bars. a = 20 mm, b-e = 10 µm.

4.4 Conclusion

Five *Lepiota* species of section *Lepiota* are present in northern Thailand. Members of this section are characterised by distinct fusiform-amygdaliform basidiospores and trichodermal pileus covering. Three species, *L. eurysperma*, *L. microcarpa* and *L. pongduadensis* are proposed as new species whereas *L. metulispora* is a new record for Thailand. One species remained unidentification and further study on ITS sequence is required. We also provided macro- and microscopic descriptions, illustrations, and a key to these species. The Maximum Parsimony analysis of the ITS data set shows that the section *Lepiota* is related to species with the section *Ovisporae*, in which basidiospores are ovoid and pileus covering is a trichoderm with short clavate elements.

CHAPTER 5

OVERALL CONCLUSIONS

Northern Thailand is a tropical region consisted of evergreen and deciduous forests with abundant native timber, fruit and medicine yielding tree species. The rainy season in Thailand begins in May and ends in October, every year. This indicates high moisture content that make it an important source of sustenance for diverse living organisms (Garner et al., 2000). Many species of saprotrophic fungi are growing well in tropical forests due to high nutrient, high moisture content and suitable substrates that support the growth of fungi. One of the interesting groups of mushrooms are *Lepiota* species and these fungi grow best in rich humus soil, mixed with fallen, decomposing deciduous leaves and woods on forest floor (Vellinga, 2004b). However, information about the diversity of *Lepiota* in Northern Thailand has been scarce and, therefore, urgently needed. This study provides baseline data on the diversity and distribution of *Lepiota* in Thailand.

Sixteen sites in the localities of Chiang Rai and Chiang Mai in Northern Thailand were studied for the diversity and distribution of *Lepiota* spp. The distribution and diversity of these fungi in this study were compared and analysed based on the type of forest present or vegetation type, location and elevation of collecting sites. The taxonomy, and molecular phylogenetic analysis was carried out based on ITS and 5.8S rDNA sequences.

5.1 Diversity and Distribution of *Lepiota* of Thailand

Lepiota spp. were firstly identified to section and species using literature from Europe and USA and some from Asia. Almost all species were mostly identified to species level based on morphological characteristics and some molecular data. However, this study was focused merely on the taxonomy of Sections *Stenosporae* and *Lepiota*. These two sections were investigated based on their clearl morphological characters, diversity and high number of observed species. The data on the distribution and diversity of species included in other sections is also presented in this study. Thirty-three species of *Lepiota* were collected from sixteen (16) sites in Northern Thailand. Based on their morphological characteristics, five (5) sections of this genus have been found in northern Thailand. The highest number of species recorded was in sections *Stenosporae, Ovisporae, Lepiota, Lilaceae Echinatae,* respectively. However, the *Lepiota* section *Fuscovinaceae* was not found in this study. Section *Stenosporae* showed the highest diversity in northern Thailand. The common species of this section were *L. cithrophylla* and *L.* cf. *poliochlodes.*

Although the number of species in section *Ovisporae* was less than that of *Stenosporae*, the highest percentage of occurrence frequency is recorded in this section. The common species found in this section are *L. furfuraceipes* and *L.* sp. 6. section *Lepiota* and *Lilaceae* showed similar diversity and occurrence. The common species recorded in these sections were *L. fraterna, L. metulispora, L. microcarpa* and *L. pongduadensis.* The lowest number of species has been observed in section *Echinatae*. In this study, *L. aspera* was recorded as the most diverse and commonly found species in Northern Thailand.

5.2 Emphasis, Morphology, Molecular and on the Sections *Stenosporae* and *Lepiota*

Actually, species of this genus are not important for edibility, economy and application. Mostly, *Lepiota* species are reported to be poisonous that they contain amatoxins (cyclopeptides), with only a few species eaten but they are not generally recommended for consumption.

The sections *Stenosporae* and *Lepiota* are clearly recognized by morphology and molecular data of ITS gene while other sections are required for more ITS sequences. In the section *Stenosporae*, molecular-data was supported to morphological and taxonomy study to divide into two clades (a clade of species with a cutis pileus covering and another clade species with trichodermal pileus covering). For the section *Lepiota*, it was noticed that species with trichodermal pileus covering and short spores are related to species with trichodermal pileus covering and ovoid spores in section *Ovisporae* by morphology and molecular-data. It has been shown in this study that, the two new species in this section *Lepiota eurysperma* and *L. pongdudensis* are distantly different in morphological characters but their ITS sequences data are identical.

Nevertheless, this study provides baseline information on the diversity and distribution of *Lepiota* spp. in northern Thailand. It is interesting to note that although the study is limited to a few localities in northern Thailand, five of the species found in sections *Stenosporae* and *Lepiota* are new species while eleven are new records. Thus, it is necessary to increase the number of sites in different regions in Thailand to acquire more valuable information that can contribute to the diversity of *Lepiota* spp in Thailand. The related literatures and sequences from GenBank are lacking, thus, the primary information and some sequences from the study were deposited to GenBank can be most useful for future studies.

5.3 New Records and New Species to Thailand

Thirty three species present in this study are new records to Thailand included five new species. These are *L. aureofulvella* and *L. papilata* that belongs to section

Stenosporae and *L. eurysperma*, *Lepiota micrpcarpa* and *L. pongduadensis* that belong to section *Lepiota*. Fifteen species were described, illustrated and published.

5.4 Future Work

The overview of diversity and distribution of *Lepiota* species in northern Thailand is presented in this study. The taxonomy and molecular study of sections *Stenosporae* and *Lepiota* have been published. Further studies are presently being carried out for three other sections, viz. *Ovisporae*, *Lilaceae* and *Echinatae*. These sections were identified based on their morphological characteristics thus, molecular analysis is necessary for verification purposes. It is also very important for these groups to be explored for the presence of bioactive metabolites that may have medical, agricultural, biocontrol and economic significance.

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APPENDICES

APPENDIX A

CHEMICAL REAGENTS

Chemical Reagents using for this study

1) Potassium Hydroxide (KOH): 3-5% aqueous solution (Lagent *et al*., 1977)

- 3 (-5) g of potassium hydroxide
- 97 (-95) ml water.
- Place the material to be studied in a drop of potassium hydroxide on a glass slide; add Congo Red if desired.

Use: 3-5% KOH is the reagent used to revive the hyphae of dried basidioms.

2) Congo Red: 1% aqueous solution (Lagent *et al*., 1977).

- 1 g of Congo Red
- 99 ml water; filter the excess dye.

Use: Usually used in combination with water for fresh material or potassium hydroxide for dried material. Congo Red will stain the wall of hyphae to red and this is used to observe walls hyphae and spores for drawing.

3) Melzer's Reagent (abbreviated as Melzer's) (Lagent *et al*., 1977).

- Iodind 1.5 g,
- Potassium-Iodide 5 g, and
- Chloral hydrate 100g to
- Water 100 ml.

Use: For colour reactions of material. If a blue to black reaction (positive), the material is called "amyloid"; a brownish to reddish-brown reaction in which case the material is called "dextrinoid'; a yellow to hyaline reaction (negative) in which case the material is called "inamyloid".

4) Cresyl Blue:

- 1 gram Cresul Blue
- 100 ml water.

Use: this is used to observe colour reaction of hyphae or spore wall of fungi. This is used to identify *Lepiota* and *Leucoagaricus*, eg. If spore-walled turned violet in Cresyl Blue, the species is belonged to *Leucoagaricus*; if not, the species is belonged to *Lepiota*.

APPENDIX B

ABSTRACT PRESENTED AT CONFERENCE AND PUBLICATIONS

Abstract presented in The Fourth Annual Meeting of Thai Mycological Association (TMA and Mycology Conference Thailand

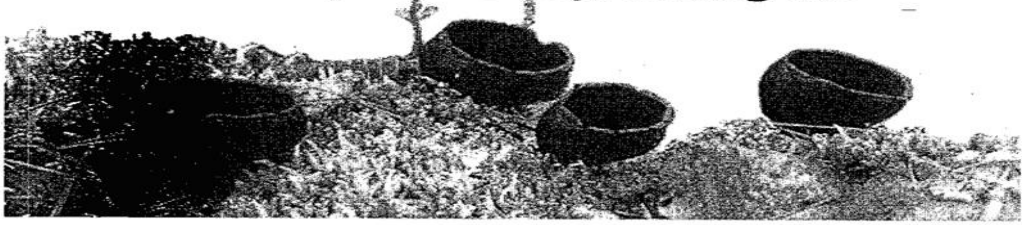
THE FOURTH ANNUAL MEETING OF THAI MYCOLOGICAL ASSOCIATION (TMA) AND MYCOLOGY CONFERENCE THAILAND

October 24, 2009

THEME: BUILDING UP MYCOLOGY IN THAILAND

Organized by

Thai Mycolgical Association (TMA)
Faculty of Agricultural Production
Maejo University, Chiang Mai



Contents

The 4th Annual Meeting of Thai Mycological Association and
Mycology Conference in Thailand. 24 October 2009

Lepiota (Agaricales) in northern Thailand: Species with spurred spores and a trichodermal pileus covering

Phongeun Sysouphanthong[1*], Zhao Ruilin[2], Ekachai Chukeatirote[1], Else C. Vellinga[4] and Kevin D. Hyde[1]

[1]School of Science, Mae Fah Luang University, 333 Moo 1, Muang District, Chiang Rai, 51700, Thailand
[2]Key Laboratory of Forest Disaster Warning and Control in Yunnan Province, Faculty of Biology Conservation, Southwest Forest University, Bailongshi, Kunming, Yunan Province 650224, P.R. China
[3]Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA 94720-3102, U.S.A

Abstract: Samples of *Lepiota* were collected in Chiang Mai and Chiang Rai Provinces during the rainy seasons of 2007 and 2008. Colleting sites were situated at an altitude of 596-1700 m, and comprise different vegetations types. Detailed notes, concerning location, vegetation type, soil and substrate were taken in the field. Most specimens were also photographed in the field. Macromorphological characters were noted in the laboratory, using standard procedures and terms. Microscopical characters were illustrated using a drawing tube attached to an Olympus CX-41research compound microscope. Characters were observed in Congo red in ammonia, water, or in 2.5-10% of KOH. Chemical reactions in Melzer's reagent, Cotton blue and Cresyl blue were noted. At least 25 spores per collection were measured in side view. The notation indicates that measurements were made on 115 spores in seven samples in six collections. The following abbreviations are used: L for lamellae, l for lamellulae, avl for average length, avw for average width, Q for quotient of length and width and avQ for average quotient. Some species was studied on molecular and base on ITS gene. The following 6 species of *Lepiota* with spurred spores and a trichodermal pileus covering are described and studied: *Lepiota castanea, L. cf. poliochloodes, L. citrophylla, Lepiota* sp.1, *Lepiota* sp.2 and *Lepiota* sp.3.

Key words: agaricales, *Lepiota*, spurred spores, Thailand

* Corresponding author: Phongeun Syouohanthong; e-mail: phongeunsysou@gmail.com

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Lepiota (Agaricales) in northern Thailand – 1. *L.* section *Stenosporae*

P. Sysouphanthong[1,3*], K.D. Hyde[1,3,4], E. Chukeatirote[1], A.H. Bahkali[4] & E.C. Vellinga[2]

[1]*School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2]*Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA 94720-3102 U.S.A.*
[3]*International Fungal Research and Development Centre, Research Institute of Insect Resources, Chinese Academy of Forests, Bailongsi, Kunming, Yunnan Province 650224, P.R. China*
[4]*Botany and Microbiology Department, College of Science, King Saud University, P.O. Box: 2455, Riyadh 1145, Saudi Arabia*
[*]Correspondence to: *phongeunsysou@gmail.com*

Abstract —Eleven species of *Lepiota* section *Stenosporae*, reported as new for Thailand, are fully described and illustrated. Five are compared with European and North American collections based on nrITS sequence data. Two species possess a cutis-like pileipellis (*L. aureofulvella*, *L.* sp. 2) and nine are characterized by a trichodermium (*L. alopochroa*, *L. castanea*, *L. citrophylla*, *L. erythrosticta*, *L. griseovirens*, *L. infelix*, *L. papillata*, *L. poliochloodes*, *L.* sp. 1). New to science are *Lepiota aureofulvella* (close to *L. boudieri*) and *L. papillata*, which is characterized by small basidiomata covered with gray-brown to olive brown squamules, an orange-white to brownish-orange context, spurred basidiospores with straight or outgrown base, clavate to cylindrical cheilocystidia, and a trichodermal pileus covering. A key to *Lepiota* sect. *Stenosporae* in northern Thailand is provided.

Key words — *Agaricaceae*, biodiversity, taxonomy, saprotroph

Introduction

Members of the genus *Lepiota* (Pers.) Gray (*Agaricaceae*) are in general saprotrophic forest floor dwellers (Singer 1986, Vellinga 2004b). They occur worldwide in tropical and temperate regions, with only a few species reported from desert and arctic-alpine areas (Vellinga 2004b). Sequence analyses of nrITS and LSU data in the *Agaricaceae* have shown that *Lepiota* forms a monophyletic clade together with *Cystolepiota*, *Echinoderma*, *Melanophyllum*, and *Pulverolepiota* (Vellinga 2003ab, 2004a).

Current Research in Environmental & Applied Mycology Doi 10.5943/cream/1/2/3

A review of genus *Lepiota* and its distribution in east Asia

Sysouphanthong P[1,2*], Hyde KD[1,3], Chukeatirote E[1] and Vellinga EC[4]

[1]Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, 57100 Chiang Rai, Thailand
[2]Mushroom Research Foundation, 333 Moo3, Phadeng Village, T. Pa Pae, A. Mae Taeng, Chiang Mai, 50150, Thailand
[3]Botany and Microbiology Department, College of Science, King Saud University, P.O. Box: 2455, Riyadh 1145, Saudi Arabia
[4]861 Keeler Avenue, Berkeley CA 94708, USA

Sysouphanthong P, Hyde KD, Chukeatirote E, Vellinga EC 2011 – A review of genus *Lepiota* and its distribution in Asia. Current Research in Environmental & Applied Mycology 1(2), 161-176, Doi10.5943/cream/1/2/3

Lepiota is a large genus comprising saprobic species growing under trees on the forest floor or in grasslands and occurs as solitary or gregarious fruiting bodies; there is a high diversity of species in tropical and temperate regions. This study provides a review of the general characteristics and differences of *Lepiota* from related genera, presents the infrageneric classification, discusses phylogenetic studies, and its significance. Several sections of *Lepiota* are diverse and distributed in Asia, and a part of this review provides a preliminary list of *Lepiota* species in countries of east Asia.

Key words – Asia – Agaricales – distribution – diversity – Lepiotaceous fungi.

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*Corresponding Author: e-mail – phongeunsysou@gmail.com

Introduction to lepiotaceous fungi

Mushroom genera with white spores such as *Chamaemyces, Chlorophyllum, Coniolepiota, Cystolepiota, Eriocybe, Lepiota, Leucoagaricus*, *Leucocoprinus,* and *Macrolepiota* are called lepiotaceous fungi and they show varied forms and morphological characters (Vellinga 2004 a,b). Historically mycologists characterised and defined these lepiotaceous taxa mostly from Europe (Bon 1993, Fries 1821). Candusso & Lanzoni (1990) studied the lepiotaceous fungi including some herbaria material from Europe and estimated there were 156 species belonging to seven genera. The main characters of this group are thus mostly based on the characters of temperate species with a few considerations from tropical species (Vellinga 2001). Studies of tropical lepiotaceous taxa are less. Pegler (1972) provided a revision of *Lepiota* species from Sri Lanka and Manjula (1983) carried out a study in India; these studies also included *Macrolepiota* and *Leucocoprinus*. However, most of mycologists did not accept a narrow definition lepiotaceous fungi. The presently accepted characters of lepiotaceous fungi are mostly derived from the literature by Vellinga (2001), Vellinga & Huijser (1993, 1998), Vellinga & Noordeloos (2001) and main characters are as follows.

Basidiomata: The fruiting bodies can be solitary to gregarious, rarely in fairy rings; the basidiomata vary from tiny to large and from pluteoid (free lamellae, context of pileus discontinuous with context of stipe and the stipe is longer than width of pileus) or collybioid (pileus not umbilicate, not conical; free or adnate lamellae, tough context and context of pileus continuous with context of

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

Phongeun 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: psphongeun@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. eurysperma*, *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.

CURRICULUM VITAE

CURRICULUM VITAE

NAME	Mr. Phongeun Sysouphanthong
DATE OF BIRTH	31 March 1981
ADDRESS	147 Xaisavang Village, Xaithany District, Vientian Capital, Laos P D R.
EDUCATION BACKGROUND	Bachelor of Agriculture, Faculty of Agriculture, National University of Laos

PUBLICATION

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