



รายงานวิจัยฉบับสมบูรณ์

โครงการวิจัยด้านวิวัฒนาการและความหลากหลายทางชีวโมเลกุลของรา
ในสกุล *Lentinus* ในประเทศไทย (Taxonomy, phylogeny
and cultivation of *Lentinus* species in northern
Thailand)

By

Associate Professor Kevin David Hyde
Assistant Professor Ekachai Chukeatirote
et al. 2011

This research was made possible by support of
Mae Fah Luang University 2011

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Associate Prof. Kevin Hyde



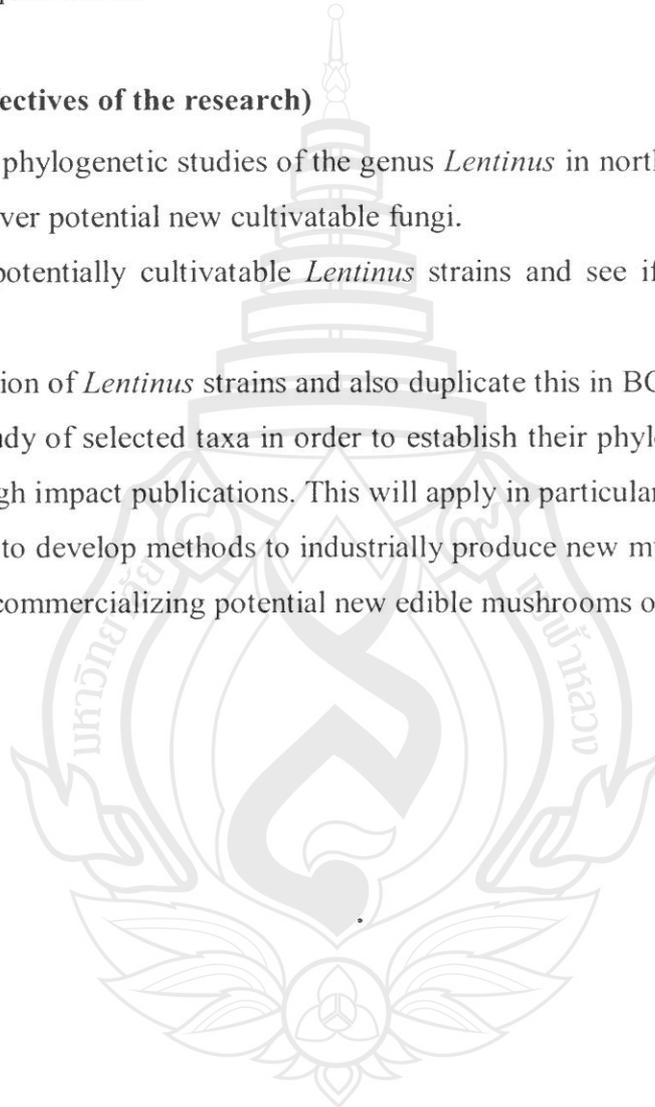
บทสรุปผู้บริหาร (EXECUTIVE SUMMARY)

1. ความสำคัญและที่มาของปัญหาในการการวิจัย (Rationale and review)

The genus *Lentinus* contains three currently cultivated species but also many species that are not presently cultivated and have this potential. We will therefore carry out a taxonomic study of *Lentinus* species in Thailand and establish which species are present and describe any new species. We will isolate strains and try to grow the newly found species and introduce them for industrial production.

2. วัตถุประสงค์ของโครงการวิจัย (Objectives of the research)

1. Carry out taxonomic and phylogenetic studies of the genus *Lentinus* in northern Thailand and in this way will discover potential new cultivatable fungi.
2. Develop ways to grow potentially cultivatable *Lentinus* strains and see if they can be cultivated.
3. Maintain a culture collection of *Lentinus* strains and also duplicate this in BCC.
4. Carry out a molecular study of selected taxa in order to establish their phylogeny, which will result in relatively high impact publications. This will apply in particular to new taxa.
5. Carry out initial research to develop methods to industrially produce new mushroom on a laboratory scale and, for commercializing potential new edible mushrooms or strains.



3. ขอบเขตของโครงการวิจัย (Scope of the research)

The genus *Lentinus* Fr. is characterized with decurrent lamellae, dimitic sporocarp tissues, hyaline, elliptic to cylindrical spores, and in most cases, hyphal pegs. This genus is cosmopolitan with circa 63 described species, which are frequently encountered in temperate regions, and abundant in the tropics. The species are wood-decaying, and easily found on fallen tree trunks and decaying timber. Generally they are xeromorphic with a tough, firm texture when dry and having a long life span.

We will carry out biodiversity, taxonomy and phylogenetic studies on the macrofungi of Thailand with emphasis on edible genus *Lentinus*. We will isolate numerous strains of potentially cultivatable mushrooms in the genus *Lentinus*, will maintain a culture collection of strains of these fungi in Mae Fah Lunag University and also deposit them at BIOTEC (BCC). A major output of this project will be international publications describing and documenting these new species.

As well as documenting species we will plan to use selected potential cultivatable strains and develop laboratory scale methods to grow them. Successful strains can then be further developed at the industrial scale through further research.

4. ระเบียบวิธีวิจัยและผลผลิตจากการวิจัย (Methodology and the research output)

Research Plan from October 2011 to September 2012: (12 months).

During the first year of the project we went to the field on more than 50 occasions and collected more than 110 collections of macrofungi. Of these 110 collections belonged to *Lentinus* and related genera, we were able to identify these to 16 *Lentinus* species based on macro and micro characters, and nr-ITS sequence data: *L. similis* Berk. & Br., *L. sajor-caju* (Fr); *L. velutinus* Fr.; *L. tigrinus* (Bull. : Fr.) *L. polychrous* Fr., *L. tuber-regium* (Fr.) Fr., *L. squarrosulus* (Mont.) Singer, *L. polychrous* Lév., *L. stupeus* Klotzsch, *L. swartzii* Berk., *L. zeyheri* Berk. and three new species of *Lentinus* sensu stricto: *L. roseus*, *L. concentricus* and *L. megacystidiatus* and one new record *L. giganteus* Berk. (Tab. 1, Fig. 1). We have attempted to grow some of these strains, and were successful in growing *Pleurotus giganteus* (syn: *Lentinus giganteus*), *Lentinus connatus* and *Lentinus roseus*. We are developing growing methods further. We published our first article from the project in an SCI journal entitled "Domestication of wild strain of *Pleurotus giganteus*. Thirteen *Lentinus* cultures were deposited in the MFLU culture collection, while seven were deposited in the BIOTEC culture collection. In year two we will collect, isolate and identify further *Lentinus* species

which should result in further new species and new records and SCI publications. We will also continue to carry out growing experiments for the potential edible new *Lentinus* species which are in culture and which will be isolated in the future.

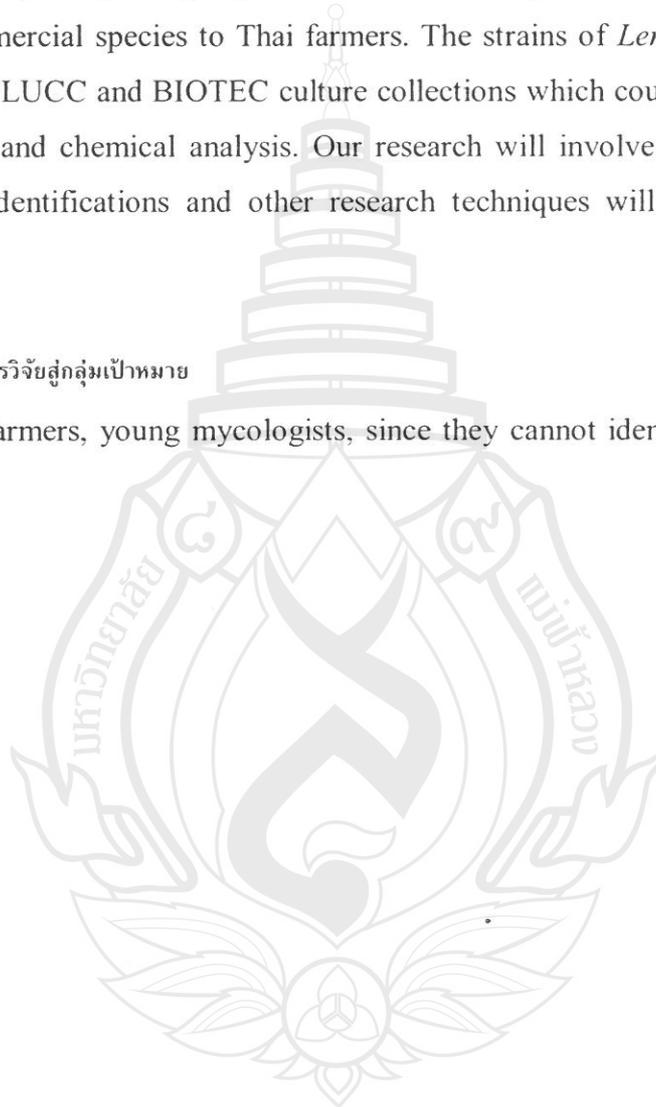


5 ประโยชน์ที่ได้รับ (Benefit)

Almost all the *Lentinus* species are edible except the once have hard texture, and some are medicinal. In Thailand, there have not been done much research on taxonomy, phylogeny and cultivation of *Lentinus*. So it is worth noting to do taxonomy, phylogeny and domestication of *Lentinus* in Thailand to accurately identify the species based on macro- and micro characters and, DNA sequence data, and describe new species and new records and make important publications. We also will carry out growing experiments for the new potential edible species and try to introduce as commercial species to Thai farmers. The strains of *Lentinus* will be isolated and deposited in MFLUCC and BIOTEC culture collections which could be used in future growing experiments and chemical analysis. Our research will involve international collaborations so that our identifications and other research techniques will be accepted globally.

6. แผนการถ่ายทอดเทคโนโลยีหรือผลการวิจัยสู่กลุ่มเป้าหมาย

Systematicians, mushroom farmers, young mycologists, since they cannot identify *Lentinus* confidently



ABSTRACT

There have been few studies on the taxonomy and biodiversity of the genus *Lentinus* in Thailand, which is a genus of edible mushrooms. Collections from 17 sites in northern Thailand yielded 110 specimens of *Lentinus* sensu lato belonging to 16 species: *L. similis* Berk. & Br., *L. sajor-caju* (Fr); *L. velutinus* Fr.; *L. tigrinus* (Bull. : Fr.) *L. polychrous* Fr., *L. tuber-regium* (Fr.) Fr., *L. squarrosulus* (Mont.) Singer, *L. polychrous* Lév., *L. stupeus* Klotzsch, *L. swartzii* Berk., *L. zeyheri* Berk. and three new species of *Lentinus* sensu stricto: *L. roseus*, *L. concentricus* and *L. megacystidiatus* and one new record *L. giganteus* Berk. based on phylogenetic analysis of the nrITS sequences. We were able to identify sixteen *Lentinus* species during the first year of the project based on macro- and micro characters, and nr-ITS sequences. In addition we were able to get successful results for growing *Pleurotus giganteus* (syn: *Lentinus giganteus*), *Lentinus connatus* and *Lentinus roseus*. Thirteen *Lentinus* cultures deposited in the MFLU culture collection while seven in BIOTEC culture collection. We are in the process of collecting, identifying, isolating and doing growing experiments for *Lentinus* which will result more new species and new records, and SCI papers.

Key words: biodiversity, edible, nrITS, phylogenetic analysis

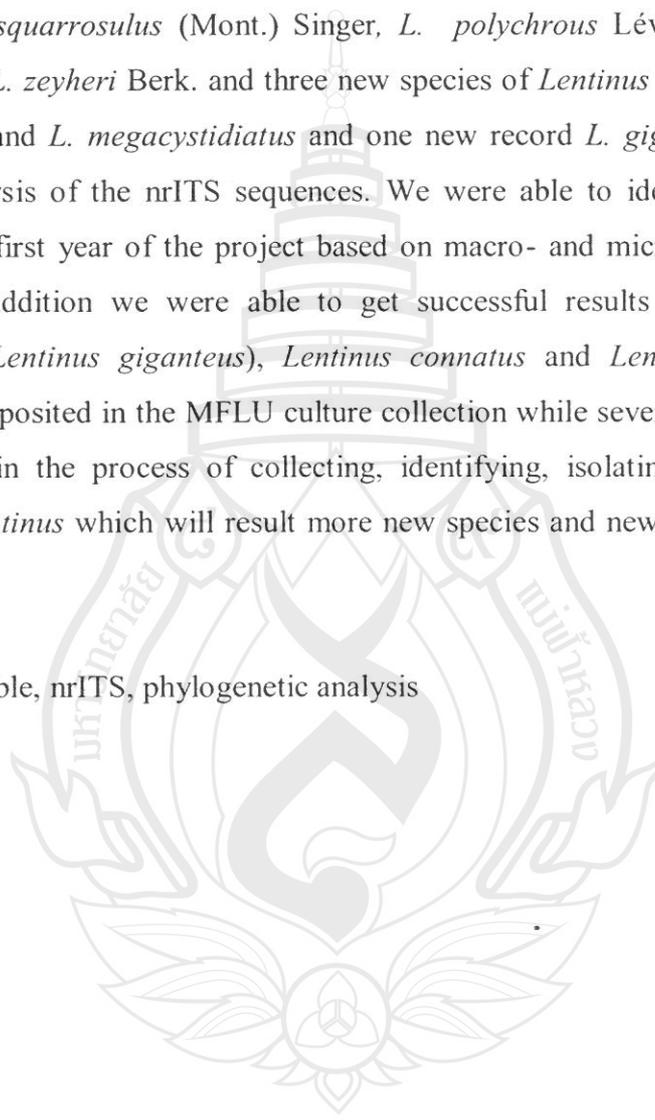


TABLE OF CONTENTS

| | |
|-----------------------------------|------|
| Acknowledgements | I |
| Executive Summary | II |
| Abstract | VI |
| Table of Contents | VII |
| List of Table | VIII |
| List of Figures | VX |
| | |
| Chapter 1: Introduction | 1 |
| Chapter 2: Literature Reviews | 2 |
| Chapter 3: Methodology | 5 |
| Chapter 4: Results and Discussion | 8 |
| Chapter 5: Conclusions | 21 |
| | |
| References | 44 |



LIST OF TABLES

| | |
|--|----|
| Tab. 1. Taxon information and GenBank accession numbers in molecular work. | 24 |
| Tab. 2. List of publications during October 2011 – September 2012 | 25 |



LIST OF FIGURES

- Fig 1. Maximum parsimony phylogram showing phylogenetic relationships among three new *Lentinus* species *L. concentricus* (MFLU08 1375), *L. megacystidiatus* (MFLU08 1388) and *L. roseus* (MFLU08 1389) with some selected *Lentinus sensu stricto* and *Polyporus* species based on ITS sequences. Data were analysed with random addition sequence, unweighted parsimony and gaps were treated as missing data. Values above the branches are parsimony bootstrap ($\geq 50\%$). The tree is rooted with *Pleurotus ostreatus* (EU520193). 23
- Fig. 2. *Lentinus roseus* sp. nov. (MFU08 1376) a: Basidiospores; b: Cheilocystidia; c: Basidia d: Sclerocystidia; e: Skeletal hyphae; f: Generative hyphae Scale bars: a, b, c, d = 20 μm ; e, f = 10 μm 26
- Fig. 3. Fruiting bodies of *Lentinus roseus* sp. nov. (MFU08 1376) in the field 27
- Fig. 4. *Lentinus megasclerocystidiatus* sp. nov. (MFU08 1388). a: Spores; b: Basidia; c: Sclerocystidia; d: Cheilocystidia; e: Hairs on the pileus; f: Skeletal hyphae; g: Generative hyphae; Scale bars: a, b, c, d = 20 μm ; e, f, g = 10 μm 28
- Fig. 5. The fruiting bodies of *Lentinus megasclerocystidiatus* sp. nov. (MFU08 1388) in the field 29
- Fig. 6. *Lentinus zonabasioma* sp. nov. (MFU08 1375). a: Spores; b: Cheilocystidia; c: Basidia d: Generative hyphae; e: Skeletal hyphae; Scale bars: a, b, c = 20 μm ; d, e = 10 μm 30
- Fig. 7. Fruiting bodies of *Lentinus zonabasioma* sp. nov. (MFU08 1375) in the field 31
- Fig. 8. *Lentinus giganteus* (MFU08 1382). a: Basidiospores; b: Cheilocystidia; c: Basidia; d: Generative hyphae e: Skeletal hyphae; Scale bars: a, b, c = 20 μm ; d, e = 10 μm 32
- Fig. 9. Fruiting bodies of *Lentinus giganteus* (MFU08 1382) 33
- Fig. 10. *Lentinus connatus* Berk. A: Cheilocystidia; B: Basidiospores; C: Basidia; D: Generative hyphae and Skeletal Hyphae; Scale bars: A, C= 20 μm ; B, D = 10 μm 34
- Fig. 11. Fruiting bodies of *Lentinus connatus* Berk in the field 35
- Fig. 12. *Lentinus similis* Berk. A: Basidiospores; B: Basidia; C: Cheilocystidia; D: Sclerocystidia; E: Generative hyphae and Skeletal Hyphae; F: Hairs on pileus Scale bars: B, C, D = 20 μm ; A, E, F = 10 μm 36
- Fig. 13. Fruiting bodies of *Lentinus similis* Berk in the field 37
- Fig.14. *Lentinus sajor-caju* (Fr.) A: Cheilocystidia; B: Basidiospores; C: Basidia; D: Skeletal Hyphae; E: Generative hyphae Scale bars: A, C = 20 μm ; B, D, E = 10 μm 38

Fig. 15. Fruiting bodies of *Lentinus sajor-caju* (Fr.) in the field

Fig. 16. *Lentinus velutinus* (Fr.) A: Basidia; B: Basidiospores; C: Cheilocystidia; D: Sclerocystidia; E: Generative hyphae & Skeletal Hyphae; F: Hairs on pileus Scale bars: A, C = 20 μ m; B, D, E = 10 μ m

Fig. 17. Fruiting bodies of *Lentinus velutinus* (Fr.)

Fig. 18. *Lentinus tigrinus* (Bull. : Fr.) A: Basidia; B: Basidiospores; C: Cheilocystidia; D: Generative hyphae & Skeletal Hyphae; Scale bars: A, C = 20 μ m; B, D = 10 μ m

Fig. 19. Fruiting bodies of *Lentinus tigrinus* (Bull. : Fr.)



CHAPTER 1

INTRODUCTION

The Goals

We will carry out biodiversity, taxonomy and phylogenetic studies on the macrofungi of Thailand with emphasis on edible genus *Lentinus*. We will isolate numerous strains of potentially cultivatable mushrooms in the genus *Lentinus*, will maintain a culture collection of strains of these fungi in Mae Fah Lunag University and also deposit them at BIOTEC (BCC). A major output of this project will be international publications describing and documenting these new species.

As well as documenting species we will plan to use selected potential cultivatable strains and develop laboratory scale methods to grow them. Successful strains can then be further developed at the industrial scale through further research.

Objectives

We will:

1. Carry out taxonomic and phylogenetic studies of the genus *Lentinus* in northern Thailand and in this way will discover potential new cultivatable fungi.
2. Develop ways to grow potentially cultivatable *Lentinus* strains and see if they can be cultivated.
3. Maintain a culture collection of *Lentinus* strains and also duplicate this in BCC.
4. Carry out a molecular study of selected taxa in order to establish their phylogeny, which will result in relatively high impact publications. This will apply in particular to new taxa.
5. Carry out initial research to develop methods to industrially produce new mushroom on a laboratory scale and 3) for commercializing potential new edible mushrooms or strains.

CHAPTER 2

LITERATURE REVIEWS

The macrofungi of the northern Thailand are presently being studied (Le et al. 2007a, b; Sanmee et al. 2008; Kerekes and Desjardin 2009; Wannathes et al. 2009a, b). The genus *Lentinus* is cosmopolitan with ca. 63 described species (Kirk et al., 2008) and species are able to survive over a wide temperature range, are abundant in the tropics and are frequently found in temperate regions (Pegler 1983). Species of *Lentinus* are normally wood-decaying basidiomycetes and have decurrent lamellae, dimitic tissues in the basidiome, and hyaline, ellipsoid to cylindrical spores, and species in subgenus *Lentinus* have hyphal pegs (Corner 1981; Pegler 1983). The basidiomes are generally xeromorphic with a tough, firm texture when dry and they have a long life span. In Thailand they fruit only for a short period, early in the summer rainy season (Sysouphanthong et al. 2010). Traditionally, *Lentinus* has been placed in the agaric family Tricholomataceae because species possess a lamellate hymenophore and white spore print (e.g. Miller 1973). Recent studies using restriction analysis of nuclear-encoded ribosomal RNA genes (rDNA) show that *Lentinus tigrinus* is more closely related to the Polyporaceae than to the Tricholomataceae whereas other *Lentinus* appear to be intermediate between the Polyporaceae and Tricholomataceae and could not be clearly assigned to either family (Hibbet and Vilgalys, 1991). A close relationship has long been observed between *Lentinus* and certain polypores (Corner 1981; Pegler 1983; Singer 1986). *Lentinus* has been grouped under the family Polyporaceae based on the presence of dimitic and amphimitic hyphal systems (Moser, 1978; Kühner, 1980; Pegler, 1983; Singer, 1986). Moreover, hyphal pegs, fascicles of sterile hyphae coming out from the hymenium surface are some of the common features present in some genera of the Polyporaceae and in *Lentinus* subg. *Lentinus* (Pegler, 1983; Corner, 1981). Recent research (e.g., Redhead & Ginns 1985; Hibbett & Vilgalys 1993; Hibbett & Thorn 1994; Hibbett et al. 1993) has shown that *Lentinus sensu* Pegler is polyphyletic, *Lentinus-Pleurotus-Panus* is a complex, which comprises 4 monophyletic genera: *Panus* Fr., *Pleurotus* Fr., *Lentinus* (Fr.) Quel., and *Neolentinus* Redhead & Ginns (Fleming 1994; Hibbett et al. 1994). These genera have been treated differently by Corner (1981), Kühner (1980), Pegler (1975, 1983) and Singer (1986). Two brown rot genera, *Neolentinus* Redhead & Ginns, and *Heliocybe* Redhead & Ginns were separated from *Lentinus* which otherwise comprises white rot species (Gilbertson, 1980; Redhead & Ginns, 1985). Recent molecular studies suggest that *Lentinus*

subg. *Lentinus sensu* Pegler (1983) is monophyletic, *Panus*, *Pleurotus*, *Neolentinus* and *Heliocybe* are not in the same lineage (Hibbett and Vilgalys 1991, 1993). Here *Lentinus* will be used to mean *Lentinus* subg. *Lentinus sensu* Pegler (1983), which is essentially equivalent to *Lentinus sensu* Corner (1981). Molecular studies support the view that *Lentinus* is derived from the Polyporales (Pegler, 1983) and suggest that *Polyporus arcularius* Batsch : Fr. is a closely related outgroup (Hibbett and Vilgalys, 1991, 1993). Also according to restriction analysis of nuclear-encoded ribosomal RNA genes (rDNA) it has been proved that *Lentinus*, at least in part, is more closely related to the polypores than to certain agarics (Hibbett, 1991). *Lentinus sensu stricto* is separated as it is characterized by binding hyphae and hyphal pegs. *Panus* s. str. is characterized by the skeletal hyphae, the radial trama, and the purplish pigment of the young basidiomes but absence of metuloides and gloeocystidia are an abnormal feature. Species of *Lentinus* are found on fallen tree trunks and decaying timber. Thailand is rich in species of *Lentinus* based on the records by Pegler (1983) *L. badius* (Berk.) Berk., *L. squarrosulus* Mont., *L. polychrous* Lev., *L. cladopus* Lev., *L. sajor-caju* (Fr.) Fr., *L. strigosus* (Schwein.) Fr., *L. velutinus* Fr., *L. similis* Berk. & Br. and *L. connatus* Berk. but no systematic study of the genus has been undertaken so far.

Wild mushrooms are one of the high value of non-timber forest products in the northern Thailand. They provide locals with seasonal food, medicine and an alternative income. The richness of wild mushrooms is also one bio-indicator of ecosystem health. The cultivated, non-mycorrhizal mushrooms, such as *Volvariella* spp., *Lentinula edodes*, *Pleurotus sajor-caju*, *Pleurotus* spp., *Auricularia* spp., and *Agaricus* spp., are available throughout the year in markets of northern Thailand, however, edible wild mushrooms can only be found in the wet season from June to September (Sysouphanthong et al. 2010). In genus *Lentinus* almost all the members are edible except those which have a tough texture. It is important to attempt to introduce members of this genus as commercial species as only *Lentinus sajor-caju* is grown in Thailand in this genus. Other cultivatable fungi in Thailand are *Lentinula edodes* (shitake), *Volvariella volvacea* (straw mushroom), *Pleurotus ostreatus* (oyster mushroom), *Flammulina velutipes* (golden needle), *Auricularia auricular* (jew's ear) and *Agaricus bisporus* (button mushroom). Although there are many studies on cultivated and wild edible mushrooms in the northern hemisphere and their nutritional value (Aletor 1995; Dermirbas 2000; Latiff et al. 1996; Manzi et al. 2001; Manzi et al., 1999), there is little information available about the taxonomy, biodiversity and potential to introduce for cultivation of the genus *Lentinus* in

northern Thailand or the Asean region. Scientific information on wild mushrooms is essential for the introduction of new species to the table.



CHAPTER 3

METHODOLOGY

3.1. Taxonomy study

- 3.1.1 Sample collection:** The collection work will be carried out during the warm wet season. The collection sites will be chosen by altitude and degree of humidity. All types of habitat will be chosen including lowland forests (Alt.<800m), mid-elevation forests (Alt.800-1200m) and highland forests (Alt.1200-2565m). Every site will be located using GPS and the type of vegetation, soil and the climate will recorded. Collection of samples will be conducted over three years (2010-2013).
- 3.1.2 Holotype study:** Examination of holotypes is an important tool for correct identification and will be carried out as needed. Holotypes will be borrowed from herbaria around the world. Every specimen will be carefully studied in detail.
- 3.1.3 Identification:** Wild collections of macrofungi will be described in detail with macro characters being recorded at collection and micro characters being recorded later from dried material. Macromorphological characters include the structure of the ring and the velar remnants, the type of scaliness of the cuticle, the color change of the surfaces upon bruising and the flesh on cutting, and the smell. Notes will be taken on those item mentioned above and also the morphological characters, such as cap, gills, context, stipe and annulus. After taking the collection notes and photographic documentation the specimens will be dried rapidly on a food drier. Micromorphological analysis will document pertinent anatomical structures and will be carried out in the laboratory as time permits.

3.2 Molecular phylogenetics

- 3.2.1 DNA extraction:** DNA extraction will be carried out using CTAB lysis buffer and phenol chloroform as outlined by Jeewon et al. (2003). DNA samples will be checked for purity and integrity by gel electrophoresis.
- 3.2.2 PCR Amplification:** PCR amplification will be amplified in Thermal controlable Cycler DNA amplification using at least 3 different gene. PRC program will be

optimized for each studied gene. Products will be purified using the QIAquick DNA purification Kit (QIAGEN, LTD.)

3.2.3 Sequencing: Both strands of the DNA will be sequenced in an automated sequencer following the manufacturer's protocols.

3.2.4 Alignment: All sequences will be aligned using Clustal X with default settings (Thomson et al., 1997) in preliminary. Then they will be manually adjusted in BioEdit.

3.2.5 Phylogenetic analysis: Different analyzing methods, MP, ML and NJ, will be used. Support values for NJ and MP will be established by bootstrapping with 1000 replicates. The distance matrix for NJ will be calculated under the Kimura two-parameter model. All of them will be implemented in PAUP (Swofford 1998).

3.2.6 Analysis of data: ClustalX will be used for sequence alignment and PAUP*4.0b10 for phylogenetic analysis.

3.3 Isolation of pure cultures

Pure cultures were isolated from the sterile internal fungal tissues. About 5 ml of PDA medium was poured into 50 ml culture tubes followed by tight capping with sterile cotton wool, sterilization, and kept as slants. Fresh juvenile fruiting bodies of *Pleurotus giganteus* (*Lentinus giganteus*), *L. connatus* & *L. roseus* were collected from the Mushroom Research Center, Chiang Mai, Thailand (MFLU10 0154), (MFLU12 0216), (MFLU12 0219) respectively and used for tissue culture. Small pieces of the internal tissue of the broken mushroom was cut and removed with a flamed needle. The needle with the tissue was then transferred into a culture-tube slant and the tissue was laid on the agar surface, and incubated at 30 °C. After 3 to 4 days, the agar surface was covered with a white mycelium as pure culture.

3.4 Cultivation methods

Standard media will be prepared for testing strains of *Lentinus* to establish fruiting body formation. Media will use sawdust and other ingredients as presently use to grow cultivated *Lentinus* species. The correct water content will be checked by pressing the media by hand. The medium will be placed in polypropylene bags with about 800 g of medium being packed into each bag. Bags will be then sealed, autoclaved and inoculated with a small agar piece

from the fully grown cultures as four replicates from one strain and one bag will be inoculated with *Pleurotus ostreatus* (Oyster mushroom) as a control.



CHAPTER 4

RESULTS AND DISCUSSION

Taxonomic study

The *Lentinus* collections have been described in detailed with Macromorphological characters and Micromorphological characters. The detailed descriptions of identified nine species are stated below. The phylogenetic placement of the three new species *L. roseus*, *L. concentricus* and *L. megacystidiatus* found out using nrITS sequence data (Tab. 1, Fig. 1).

01) *Lentinus roseus* Karunaratna, K.D. Hyde & Zhu L. Yang, sp. nov.

Pileus 5—5.5 cm *latus coriaceus profunde cyathiformis centro pallide alboluteolo marginem versus fuscatum ipsum pallide roseum siccus estriatus ezonatus. Lamellae profunde decurrentes lamellulis 5 seriebus. Stipes* 1.5—2 cm *longus 0.5—0.7 cm latus centralis fusiformis. Basidiosporae* 5—7(--9) μm *longae 3—4(--6) μm latae ellipsoideae ad elongatae. Basidia* 18—24 μm *longa 5—7 μm lata anguste clavata sterigmatibus 4. Cheilocystidia* 19—37 μm *longa 5.5—8 μm lata clavata hyalina parietate tenui. Sclerocystidia* 36—45(--60) μm *longa 9—15 μm lata clavata apice late rotundato pariete crasso hyalino.*

Type collection: THAILAND, Chiang Mai Province, Mae Taeng District, Hot Spring National Park, Pong Duad Pa Pae 40 kms off Chiangmai. 35 kms from the Highway Route No 1095: Mae Malai-Pai and turn right for 6.5 kms, humid montane rainforest with *Quercus*, *Castanopsis*, *Lithocarpus echinops*, 05 July 2008, Samantha C. Karunaratna (MFU08 1376)

Etymology: *roseus*, in reference to the distinguishable colour of the mature fruiting body.

Description: Basidiomes relatively small. Pileus 5.0-5.5 cm in diameter, leathery, deeply cyathiform, when seen from above rounded flabelliform; margin eroded; surface pale yellow cream (4A3) at centre, darker towards margin and there reddish grey (11B2), not changing on bruising, virgate, dry, neither striate nor zonate. Lamellae deeply decurrent, with 5 tiers of lamellulae, 0.5 mm wide, reddish grey (11B2) close to pileus margin, pale yellow cream (4A3), towards stipe in old and young specimens, with entire edge. Stipe 1.5-2.0 × 0.5-0.7

Cm, central, 1.5-2 × 5 mm at apex, expanding towards base 1.5-2 × 7 mm attached to a discoid base, solid, fusiform, yellowish white (4A2), punctuate, leathery, solid, with white cottony context. Generative hyphae (Fig. 3f) 4-5 µm diameter, inflated with a slightly thickened wall, more or less radially parallel but frequently branching and with large clamp connections. Skeletal hyphae (Fig. 3e) 5-6 µm in diameter, hyaline, very thick walled with only a very narrow lumen, typically unbranched. Basidiospores (Fig. 3a) 5-7 (-9) × 3-4 (-6) µm [n=20, L^m=5.93 µm, W^m=3.72 µm, Q=1.36-1.85, Q=1.59], ellipsoid to elongate, hyaline, thin walled, with few contents. Basidia (Fig. 3c) 18-24 × 5-7 µm, elongate clavate, bearing 4 sterigmata. Cheilocystidia (Fig. 3b) 19-37 × 5.5-8 µm, clavate, hyaline, thin walled. Sclerocystidia (Fig. 3d) 36-45 (-60) × 9-15 µm, clavate with a broadly rounded apex, thick walled, hyaline. Clamp connections are prominent in skeletal hyphae.

Habitat: clustered on rotten wood, in forest with *Quercus*, *Castanopsis* and *Lithocarpus echinops*

Specimens examined: THAILAND, Chiang Mai Province, Mae Taeng District, Hot Spring National Park, Pong Duad Pa Pae 40 kms off Chiangmai. 35 kms from the Highway Route No 1095: Mae Malai-Pai and turn right for 6.5 kms, humid montane rainforest with *Quercus*, *Castanopsis*, *Lithocarpus echinops*, 05 July 2008, Sam 017 (MFU08 1376) (holotype), THAILAND, Chiang Rai Province, Tambon Mae Korn and Tambon Huay Chompoo, Muang District, Khun Korn Waterfall, N19°51-54' E 99°35.39', elevation 1208 m, moist upper mixed deciduous forest (Royal forest Department, 1962), 22 July 2009, Sam T-09 (MFLU10 0145).

Distribution: Only known from northern Thailand.

Notes: This new species is distinguished by its relatively small basidiomes, leathery, deeply cyathiform and pink pileus, very large, clavate sclerocystidia with a broad rounded apex, ellipsoid to elongate basidiospores with a hyaline thin wall, a dimitic hyphal system with very thick walled unbranched skeletal hyphae. In many aspects it bears superficial resemblance to *Pl. sajor-caju*. The latter however, has larger basidiomes with a whitish cream to mottled grey pileus, an annulate stipe and relatively smaller basidiospores. This new species forms clusters of basidiomes on dead and decaying wood. Initially the young fruiting bodies are whitish pink, and they become pink with maturity.

02) *Lentinus megasclerocystidiatus* Karunarathna, K.D. Hyde & Zhu L. Yang, sp. nov.

Pileus 0.6—2 cm *latus tenuis convexus coriaceus umbilicatus ad infundibuliformis*. *Lamellae arcuatae profunde decurrentes confertae*. *Stipes centralis rarissime lateralis cylindricus*. *Basidiosporae* 6—8 μm *longae* 3—4 μm *latae ellipsoideae ad cylindricae*. *Basidia* 18—24 μm *longa* 5—7 μm *lata clavata sterigmatibus* 4. *Cheilocystidia* 19—35 μm *longa* 4—6.5 μm *lata inflata clavata*. *Sclerocystidia dispersa* 37—52 μm *longa* 10—16 μm *lata pariete incrassato (ad 3 μm) hyalino vel pallide brunneo*.

Type collection: THAILAND, Chiang Mai Province, Mae Taeng District, Ban Pha Deng village, Pathummikaram Temple, forest trail, N 19°06.288' E 98°44.473', elevation 1050 m., rain forest dominated by *Castanopsis armata* and *Dipterocarpus* sp. 15 July 2008, Samantha C. Karunarathna (MFU08 1388)

Etymology: *megasclerocystidiatus*, in reference to very large sclerocystidia.

Description: Basidiomes very small to quite large. Pileus 0.6-2.0 cm in diameter, thin, convex, coriaceous, umbilicate to infundibuliform; margin inflexed, entire, thin at first reflexed, involute at maturity; surface dry, uniformly velutinous to slightly long-hispid or sub-squamulose furfuraceous, zonate, densely ciliate; hairs dark brown (6F7); cap surface grayish orange (5B4). Lamellae arcuate, deeply decurrent, up to 0.25mm wide, crowded with 11 tiers of lamellulae, grayish orange (5B4), with entire edge. Stipe 0.2-1.4 cm \times 2-4 mm, central, excentric, rarely lateral, cylindrical, slender, solid, yellowish brown (5E8), uniformly and persistently velutinous, with dark brown (6F7) hairs; context 2-3 mm, white in color, consisting of a dimitic hyphal system with generative and skeletal hyphae. Generative hyphae 5-6 μm diameter, hyaline, very thin walled, frequently branched, with prominent clamp connexions. Skeletal hyphae 9-10 μm diameter, hyaline with a thickened wall, unbranched or with an occasional short, lateral branch, either terminal or intercalary in origin. Basidiospores (Fig. 4a) 6.0-8.0 \times 3.0-4.0 μm [n=40, $L^m=6.37 \mu\text{m}$, $W^m=3.60 \mu\text{m}$, $Q=1.50-2.16$, $Q=1.76$], ellipsoid to cylindric, hyaline, thin walled, with few contents. Basidia (Fig. 4b) 18-24 \times 5-7 μm , clavate, bearing 4 sterigmata. Cheilocystidia (Fig. 4d) 19-35 \times 4-6.5 μm , narrowly clavate, to utriform, hyaline, thin-walled with some contents. Sclerocystidia (Fig. 4c)

scattered, $37\text{--}52 \times 10\text{--}16 \mu\text{m}$, with a thickened, hyaline or pale brown wall up to $3 \mu\text{m}$ thick. Clamp connections are prominent in generative hyphae.

Habitat: On dead wood, in clusters, in rain forest dominated by *Castanopsis armata*, *Dipterocarpus* sp., *Ficus* sp. and *Tabebuia chrysantha*.

Specimens examined: THAILAND, Chiang Mai Province, Mae Taeng District, Ban Pha Deng village, Pathummikaram Temple, forest trail, N $19^{\circ}06.288'$ E $98^{\circ}44.473'$, elevation 1050 m., rain forest dominated by *Castanopsis armata* and *Dipterocarpus* sp. 15 July 2008, Sam 029 (MFU08 1388), (holotype) THAILAND, Chiang Rai Province., Thasud, Muang Dist., Mae Fah Luang University park, N $18^{\circ}05'59.1''$, E $102^{\circ}40'02.9''$, elevation 488 m., dominated by *Dipterocarpus* sp., *Ficus* sp., and *Tabebuia chrysantha*. 18 June 2009, BJP 021 (MFLU10 0151).

Distribution: Only known from northern Thailand.

Notes: This new species is characterized by its small basidiomes, and very big, scattered, clavate sclerocystidia $37\text{--}52 \times 10\text{--}16 \mu\text{m}$ with a broadly rounded apex. In many aspects *Lentinus megasclerocystidiatus* bears superficial resemblance to *L. strigosus* Fr. which has a whitish to pallid ochraceous brownish pileus 1.5–7(-10) cm in diameter, and ovoid to ellipsoid basidiospores $4.5\text{--}6.0 \times 2.5\text{--}3.7 \mu\text{m}$. In contrast the basidiospores of *L. megasclerocystidiatus* are ellipsoid to cylindric $6.0\text{--}8.0 \times 3.0\text{--}4.0 \mu\text{m}$. The basidiome of *L. megasclerocystidiatus* is also much smaller, laterally attached, zonate, dry, hard, uniformly velutinous to slightly long hispid or sub-squamulose furfuraceous. This new species forms clusters of basidiomes on dead and decaying wood.

03) *Lentinus zonabasioma* Karunarathna, K.D. Hyde & Zhu L. Yang, sp. nov.

Pileus 6.5–7 cm diam. *tenuis centro profunde umbilicatus velutinus mollis zonatus. Lamellae profunde decurrentes lamellulis 5 seriebus confertis. Stipes* 4–4.5 mm longus 5–6 mm latus cylindricus. *Basidiosporae* 5–7 μm longae 2.5–4 μm latae ellipsoideae ad cylindricae. *Basidia* 19–25 μm longa 5–6.5 μm lata sterigmatibus 4. *Cheilocystidia* 20–30 μm longa

5–7 μm lata anguste clavata aliquot appendicibus apicalibus (10–12 μm longis) hyalina parietate tenui. Pagina pilei pilis 5–5.5 μm diam.

Type Collection: THAILAND, Chiang Mai Province, Mae Taeng District, Ban Pha Deng, Mushroom Research Centre, N 19°17.123' E 98°44.009', elevation 900 m, rainforest dominated by *Castanopsis armata*, *Erythrina* sp, and *Dipterocarpus* sp., 15 July 2008, Samantha C. Karunarathna (MFU08 1375)

Etymology: *zonabasidioma*, in reference to the prominent zonation on the pileus surface.

Description: Basidiomes medium to large. Pileus 6.5-7.0 cm in diameter, thin, with deeply umbilicate centre, when seen from above circular, yellowish brown-clay (5D5) at margin, yellowish brown (5E8) towards centre, surface from centre to margin velvety, soft, zonate, with brownish grey (6F8) hairs at margin; hairs grayish orange (5B4) towards centre. Lamellae deeply decurrent, 0.5 mm broad, with 5 tiers of lamellulae, pompeian yellow (5C6) when young and old, with entire edge. Stipe 4-4.5 \times 5-6 mm, cylindric, solid, leathery, dry, yellowish brown-clay (5D5) when young, raw umber (5F8) when old, surface velvety/velutinous, short/soft hairs; brownish grey color (6F8); with white context up to 2 mm thick at the disk, consisting of a dimitic hyphal systems with skeletal hyphae. Generative hyphae 3-4 μm diameter, not inflated, hyaline, thin-walled, frequently branched, with clamp connections. Skeletal hyphae 3-4.5 μm diameter, sinuous, cylindric, hyaline, with a thickened wall and narrow lumen, unbranched. Diameter of hairs on the surface of pileus 5-5.5 μm . Basidiospores 5.0-7.0 \times 2.5-4.0 μm [n=20, L^m=6.78 μm , W^m=3.75 μm , Q=1.5-2.4, Q=1.8] ellipsoid to cylindric, hyaline, thin-walled, with few contents. Basidia 19-25 \times 5-6.5 μm , clavate, bearing 4 sterigmata. Lamella edge sterile with crowded cheilocystidia. Cheilocystidia 20-30 \times 5-7 μm , narrowly clavate, some with apical excrescence (10-12 μm long) hyaline, thin-walled.

Habitat: On dead wood, in cluster, in rain forest dominated by *Castanopsis armata*.

Specimen examined: THAILAND, Chiang Mai Province, Mae Taeng District, Ban Pha Deng village, N 19°17.123' E 98°44.009', elevation 900 m., rainforest dominated by *Castanopsis armata*, 15 July 2008, Sam 016 (MFU08 1375) (holotype)

Distribution: Only known from northern Thailand.

Notes: This new species is characterized by its yellowish brown-clay, velvety, zonate, leathery, solid, dry and hard small basidiomes. The prominent zonation of the pileus is the main characteristic feature. In many aspects it bears superficial resemblance to *L. fasciatus* Berk. which has an off-whitish to pale ochraceous pileus with cylindrical basidiospores but, in *Lentinus zonabasioma* the basidiospores are slightly smaller and mostly ellipsoid $5-7 \times 2.5-4 \mu\text{m}$ and sclerocystidia are absent. This new species forms clusters of basidiomes on dead and decaying wood.

04) *Lentinus giganteus* Berk., J. Bot., London 6: 493 bis (1847).

Pileus (Fig. 2a-b) 100-110 mm in diameter, strongly convex to applanate becoming slightly depressed in the centre, dark brown (7F5), towards the margin light brown camel (6D4), grayish orange (5B4) at the marginal area, at centre fibrillose-scaly, surface initially uniformly dark, fuscous brown, fuliginous or black, then fading with age to pale ochraceous or yellowish brown (E8), with a darker centre although sometimes remaining dark, dry, disrupted into small, indefinite, radial, innate squamulus, overlain by scanty, pale grey or blackish, verrucose-floccose, concentrically arranged remnants of the veil; margin strongly involute then straight, thin, slightly sulcate-striate. Lamellae moderately crowded with lamellulae of five lengths, decurrent, slightly interveined and anastomosing over the stipe apex, 2-3 mm broad, white to cream colour (3A2); edge entire, pale ochraceous or yellowish brown (5E8). Stipe $6.5-7 \times 0.7-0.8$ cm at the apex, $6.5-7 \times 1.0-1.1$ cm at the base, fusiform, with radicating base, up to 10-12 cm long, solid, with surface concolorous with the pileus, paler at the apex, finely tomentose with indefinite zones of paler velar remnants in the early stages; veil thin, floccose, pale to dark brown (6F6), soon reduced to floccose remnants but never forming an annulus on the stipe. Context 2-3 mm thick at the disk, submembranous at the pileal margin, white in pileus and stipe, fleshy-spongy, consisting of a dimitic hyphal system with skeletal hyphae. Generative hyphae (Fig. 1e) $4-6 \mu\text{m}$ in diameter, inflated with a thick or slightly thickened wall, more or less radially parallel but frequently branching and with large clamp connections. Skeletal hyphae (Fig. 1d) $6-8 \mu\text{m}$ in diameter, hyaline of intercalary or terminal origin, becoming very thick-walled with a narrow lumen, tending to taper apically, occasionally with a limited lateral branch. Spores (Fig. 1a) $7-9 \times 6-7 \mu\text{m}$ [$n =$

20, $L^m = 8.3 \mu\text{m}$, $W^m = 6.36 \mu\text{m}$, $Q = 1.18-1.46$, $Q = 1.33$] broadly ellipsoid to ellipsoid, white in mass, smooth, with one large oil drop or multiguttulate, inamyloid, thin-walled. The large spores are not cylindrical but rather broadly ellipsoid, although the largest spores become oblong ellipsoid. Basidia (Fig. 1c) $25-40 \times 8-10 \mu\text{m}$, elongate, clavate, bearing 4 sterigmata. Lamella edge sterile with a broad layer of cheilocystidia (Fig. 1b) $15-30 \times 6-10 \mu\text{m}$, more or less lecythiform with a ventricose base and a small capitellum ($3-4 \mu\text{m}$) subtended by a narrow neck, hyaline, thin-walled.

Habitat: on buried rotten wood, solitary, rain forest dominated by *Castanopsis armata*, *Dipterocarpus* sp. and deciduous forest dominated by *Xylia xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) Niels. (Leguminosae, Mimosoideae), *Pterocarpus macrocarpus* Kurz (Leguminosae, Papilionoideae), *Afzelia xylocarpa* (Kurz) Craib and *Sindora siamensis* Teysm. ex Miq. var. *siamensis* (both Leguminosae, Caesalpinioideae).

Specimens examined: THAILAND, Chiang Mai Province, Mae Taeng District, Ban Pha Deng Village, Pathummikaram Temple, forest trail, N $19^{\circ}06.288'$ E $98^{\circ}44.473'$, elevation 1050 m., rain forest dominated by *Castanopsis armata*, *Dipterocarpus* sp., *Pinus kesiya*, 8 July 2008, Sam 023 (MFU08 1382); *ibid.*, 27 June 2008, Sam 012 (MFU08 1371); *ibid.*, 12 July 2009, BJP 024 (MFLU 100136), THAILAND, Chiang Rai Province, Mae Sai District, Highway No.110 to Mae Sai, Doi Tung, N $20^{\circ}20'45''$ E $99^{\circ}50'04''$, elevation 950 m, deciduous forest dominated by *Xylia xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) Niels. (Leguminosae, Mimosoideae), *Pterocarpus macrocarpus* Kurz (Leguminosae, Papilionoideae), *Afzelia xylocarpa* (Kurz) Craib and *Sindora siamensis* Teysm. ex Miq. var. *siamensis* (both Leguminosae, Caesalpinioideae), 10 June 2009, BJP 045 (MFLU10 0137), *ibid.*, 08 August 2009, Sam 35 (MFLU10 0138); *ibid.*, 10 June 2009, Sam 27 (MFLU10 0140); *ibid.* 15 July 2009, Sam 31 (MFLU10 0141); *ibid.*, 08 August 2009, Sam 34 (MFLU10 0142).

Distribution: Sri Lanka, Malay Peninsula, Sabah, Australia, Vietnam, China (Pegler 1983).

New to Thailand.

Notes: *Lentinus giganteus* possesses many structures which are not typical of the genus *Lentinus*, and its taxonomic position remains uncertain. Corner (1981:54) proposed it as the type and only species of a new subgenus, *Gigantopanus* Corner, of the genus *Panus*. Pegler (1983) moved this subgenus into *Lentinus* as a section: *Lentinus* sect. *Gigantopanus* (Corner) Pegler. The large basidiospores are not cylindrical, but rather broadly ellipsoid, although the largest spores become oblong ellipsoid. The oil-guttule found within the spore is typical for *Lentinus*. The generative hyphae are less wide than the skeletal hyphae. The lamella-edge is well defined with a broad, sterile layer of differentiated cheilocystidia, similar to those found in some *Pleurotus* species. The lamellae are broad and well spaced and the development is metavelangiocarpic. In many respects this species might be more appropriately placed in *Pleurotus*, rather than in *Lentinus*. However the skeletal hyphae of the dimitic hyphal system become dominant to produce a tough basidiome and the radiating construction of the hymenophoral trama contrasts with the descending trama found in *Pleurotus*. However, phylogenetic analyses of the ITS data suggest that *L. giganteus* is more related to *Pleurotus* than to *Lentinus*. More data from other genes are necessary to decide this issue.

This species was originally described from Sri Lanka (Berkeley 1847). When fully grown, the basidiome is typically infundibuliform measuring up to 35 cm in diameter and 28 cm in height.

Petch (1910) listed *L. stenophyllus* Berk. as a synonym of *L. giganteus* but this was a reflection of an error originating from Berkeley & Broome (1873) who determined the Sri Lankan collection, Thwaites 688, as *L. stenophyllus* and placed it in the same type cover amongst the collections at Kew. Examination of both these collections and the accompanying water-color illustration showed that it is quite distinct from *L. stenophyllus* (*L. connatus* Berk.) and to have the thick, radicant stipe, and subdistant broad lamellae which are typical of *L. giganteus* (Pegler 1983). *L. velatus* Berk. & Broome was based on small, immature specimens which possess the velar remnants of *L. giganteus*, and are quite distinct from *L. connatus* as was suggested by Petch (1916: 146).

Yang (2000) studied the type of *Lentinus mollipes* Pat., and concluded that the latter species is conspecific with *L. giganteus*, which is common in tropical regions of China (Yang & Zang 2003).

05) *Lentinus connatus* Berk. In Hooker. Journ. Bot. 1: 145 (1842), non *Panus connatus* Berk. (1852).

Distribution in Thailand:

THAILAND, Chiang Mai Province., Mae Taeng District., Ban Pha Deng village,
Pathummikaram Temple.

Mae Sae Village and Hot Spring National Park, Mae Taeng District, Chiang Mai

A. Macromorphology

-Cap: 2-15 (-20) Cm diam., appanate to deeply cyathiform, white to pale ochraceous,
white in context.

-Lamellae: deeply decurrent.

-Stipe: cylindrical, 2-15 Cm x 3-15 mm, excentric or lateral, surface white soon becoming
greyish brown or darker.

- Taste and Smell: smell mushroomy.

- Edibility: edible when it is young

b. Cheilocystidia: 15-45 x 6-9 μ m, clavate, narrowly clavate to cylindrical

c. Basidiospores: 6-8 x 3-4 μ m, Oblong to Cylindrical, brown in mass

d. Basidia: 15-45 x 6-9 μ m, clavate, 4-spored

Lentinus connatus Berk. A: Cheilocystidia; B: Basidiospores; C: Basidia; D: Generative
hyphae and Skeletal Hyphae; Scale bars: A, C= 20 μ m; B, D = 10 μ m

06) *Lentinus similis* Berk. & Br. In Journ. Linn. Soc., Bot. 14:43 (1873).

Distribution in Thailand:

THAILAND, Chiang Mai Province., Mae Taeng District., Ban Pha Deng village,
Pathummikaram Temple.

Inthanon National Park and Mork Fah Waterfall National Park, Thailand

A. Macromorphology

-Cap: 3-15 Cm diam., deeply infundibuliform expanding to cyathiform, cinnamon brown to dark chestnut brown, white in context.

-Lamellae: decurrent.

-Stipe: cylindrical, 2-15 Cm x 2-15 mm, excentric or sublateral, surface concolorous with pileus.

- Taste and Smell: smell mushroomy.

- Edibility: edible when it is young

b. Cheilocystidia: 18-25 x 3.5-5 μm , crowded, nodulose clavate, irregular, hyaline, thin walled.

c. Basidiospores: 5-6.5 x 2.5-3.2 μm , elongate ellipsoid to Oblong cylindric, hyaline, thin walled

d. Basidia: 18-28 x 4-5 μm , clavate cylindric, 4-spored

e. Sclerocystidia: very abundant, often appearing crowded, 20-40 x 4-9 μm

07) *Lentinus sajor-caju* (Fr) Fr., Epicrisis: 393 (1838).

Distribution in Thailand:

Inthanon National Park and Mork Fah Waterfall National Park, Thailand

A. Macromorphology

-Cap: 3-9 (-20) Cm diam., convex with a deeply umbilicate centre then cyathiform to infundiliform, or excentric and flabelliform, surface very variable in colour, cream colour, pale ochraceous.

-Lamellae: deeply decurrent.

-Stipe: central, excentric or lateral, short, 0.8-3 x 0.5-1.5 Cm, surface concolorous with pileus.

- Taste and Smell: smell mushroomy.

- Edibility: Excellent

b. Cheilocystidia: 20-25 x 4-6 μm , clavate, often sinuous or nodulose, hyaline, thin walled

c. Basidiospores: 5-9 x 1.5-2.5 μm , narrowly cylindrical, often curved, hyaline, thin walled

d. Basidia: 15-20 x 3.5-4.5 μm , very narrow, clavate cylindrical, 4-spored

08) *Lentinus velutinus* Fr. in *Linnaea* 5: 510 (1830), non *Agaricus (Omphalia) velutinus* Fr., loc. Cit.: 508.

Distribution in Thailand:

Pha Deng Village, Mae Taeng District, Thailand

THAILAND, Chiang Mai Province., Mae Taeng District., Ban Pha Deng village,
Pathummikaram Temple.

A. Macromorphology

-Cap: 2-8 Cm diam., deeply umbilicate to broadly infundibuliform or cyathiform; surface uniformly pale greyish cinnamon to rufous or tawny brown, uniformly velutinate to short hispid.

-Lamellae: arcuate, short decurrent.

-Stipe: 2-25 Cm x 2-10 mm, slender, elongate, cylindric, surface concolorous with pileus or more often darker.

- Taste and Smell: no special smell.

- Edibility: edible when it is young

b. Sclerocystidia: 20-64 x 3-12 μm , present on lamellae edge, initially clavate and thin walled, soon developing a thickened wall.

c. Basidiospores: 5-7 (-8) x 3-3.8 μm , Oblong -cylindric, hyaline, thin walled

d. Basidia: 18-22 x 4-5 μm , narrowly cylindric, 4-spored

09) *Lentinus tigrinus* (Bull. : Fr.) Fr., Syst. Orb. Veg. : 78(1825).

Distribution in Thailand:

Hot Spring National Park, Mae Taeng District, Thailand

THAILAND, Chiang Mai Province., Mae Taeng District., Ban Pha Deng village,
Pathummikaram Temple.

A. Macromorphology

-Cap: 1-10 Cm diam., fleshy coriaceous, pliant, strongly convex then concave-umbilicate to deeply infundibuliform, surface at first greyish brown to blackish brown owing to crowded.

-Lamellae: decurrent.

-Stipe: 1.5-5 (-10) Cm x 2-10 mm, central or excentric, slender, cylindric, tapering below, solid, surface soon yellowish.

- Taste and Smell: smell mushroomy.

- Edibility: Excellent

b. Cheilocystidia: 20-28 x 3-6 μ m, sinuous clavate, often constricted or nodulose, hyaline, thin walled.

c. Basidiospores: 6-9.5 x 2-3.5 μ m, narrowly-cylindric, hyaline, thin walled

d. Basidia: 24-30 x 4-6 μ m, elongate clavate, 4-spored

Molecular phylogenetic analysis results

We investigated the position of *L. concentricus*, *L. megacystidiatus* and *L. roseus* based on phylogenetic analyses of the nrITS sequences (Fig. 1). The gen bank numbers of the sequences used for the phylogenetic analysis are in the table 1.

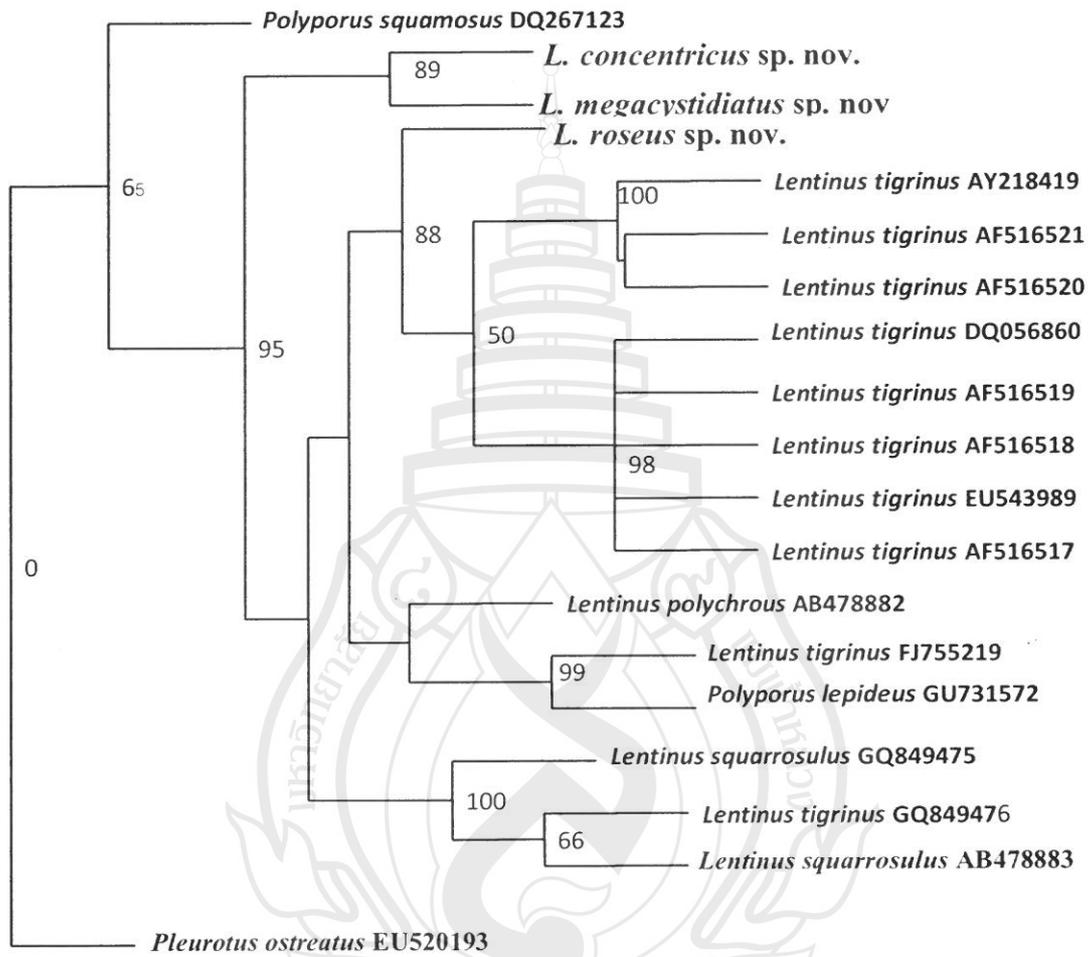
CHAPTER 5

CONCLUSION

We collected 110 *Lentinus* specimens from 17 sites in northern Thailand, belonging to 16 species of *Lentinus sensu lato*: *L. similis* Berk. & Br., *L. sajor-caju* (Fr); *L. velutinus* Fr.; *L. tigrinus* (Bull. : Fr.) *L. polychrous* Fr., *L. tuber-regium* (Fr.) Fr., *L. squarrosulus* (Mont.) Singer, *L. polychrous* Lév., *L. stupeus* Klotzsch, *L. swartzii* Berk., *L. zeyheri* Berk. and three new species of *Lentinus sensu stricto*: *L. roseus*, *L. concentricus* and *L. megacystidiatus* and one new record *L. giganteus* Berk. based on phylogenetic analyses of the nrITS sequences. We have fully described and illustrated 9 species with line drawings. We investigated the position of *L. concentricus*, *L. megacystidiatus* and *L. giganteus* based on phylogenetic analyses of the nrITS sequences (Fig. 1, Tab. 1). The results from these analyses are preliminary as the nrITS region is very variable and it is not the best region to use for phylogenetic inference (Bruns 2001). A multi-gene phylogeny of *Lentinus* is needed based on a sampling of a wide range of species. The morphological data, however, supports the introduction of the new species. *Lentinus concentricus*, *L. megacystidiatus* and *L. roseus* are positioned, with high bootstrap support. This group is in need of further phylogenetic research, and it has to be seen whether the genus *Lentinus* should be maintained as a separate genus. The main focus of the first year of this project has been on biodiversity and taxonomy, however, it is worth noting that basidiomes of most *Lentinus* species are potentially edible and cultivatable. In most countries, there is a well-established consumer acceptance for cultivated mushrooms (e.g. *Agaricus bisporus*, *Auricularia* spp., *Pleurotus* spp., *Lentinula edodes* and *Volvariella volvacea*) (Sanmee et al. 2003). *Lentinus tuber-regium* and *L. squarrosulus* are popular throughout central Africa as a source of food (Watling 1993), while Chin (1981) stated that *Pleurotus sajor-caju* and *Lentinus strigosus* are edible. *Pleurotus sajor-caju* is also widely sold at the local markets in Thailand. Mushrooms have been shown to have great potential for anti-oxidant activity (Yang et al. 2002; Vattem et al. 2004; Mau et al. 2005). Burkill (1966) stated that *Lentinus subnudus* is an edible species. *Pleurotus giganteus* (syn: *Lentinus giganteus*) locally referred to as “Uru Paha” in Sri Lanka, is one of the largest of edible mushrooms, and has been treated as a special food since ancient times as mentioned in Buddhist literature (Udugama et al. 1991; Berk 1947). Furthermore *Lentinus tuber-regium* is a very valuable medicine against diarrhea (Burkill 1966). In this study we

collected many new strains of edible cultivatable fungi (e.g. *L. connatus*, *L. giganteus*, *L. polychrous*, *L. similis*, *L. tigrinus*, *L. velutinus* and *Pl. sajor-caju*), and one new species which might also provide edible cultivatable strains (*Lentinus roseus* sp. nov.). In addition we were successful in growing *Pleurotus giganteus* (syn: *Lentinus giganteus*), *Lentinus connatus* and *Lentinus roseus* and came up with a SCI publication entitled “Domestication of wild strain of *Pleurotus giganteus* and in addition four International papers were published during October 2011 to September 2012 and three were in SCI journals (Table 2). Our study has shown that it is possible to domesticate local strains of *P. giganteus* (synonym: *L. giganteus*) that can grow at temperature consistent with Thailand farm productions. This mushroom was successfully domesticated in the 1980’s and strains of this mushroom are now extensively grown in China. We isolated numerous strains of *Lentinus* and deposited in MFUCC and BIOTEC, which will be used to carry out future growing experiments.





Maximum parsimony phylogenetic tree showing phylogenetic relationships among three new *Lentinus* species *L. concentricus* (MFLU08 1387), *L. megacystidiatus* (MFLU08 1388) and *L. roseus* (MFLU08 1389) with some selected *Lentinus sensu stricto* and *Polyporus* species ITS sequences. Data were analysed with random addition sequence, unweighted parsimony and gaps were treated as missing data. Bootstrap values above the branches are parsimony bootstrap ($\geq 50\%$). The tree is rooted with *Pleurotus ostreatus* (EU520193).

| Taxa | Resource & Herbarium accession number | GenBank accession numbers (ITS) |
|------------------------------------|--|--|
| <i>Pleurotus ostreatus</i> | GenBank | EU520193 (Unpublished data) |
| <i>Lentinus squarrosulus</i> | GenBank | GQ849475 (Unpublished data) |
| <i>Lentinus squarrosulus</i> | GenBank | AB478883 (Sotome et al. 2009) |
| <i>Lentinus tigrinus</i> | GenBank | GQ849476 (Unpublished data) |
| <i>Lentinus tigrinus</i> | GenBank | FJ755219 (Unpublished data) |
| <i>Lentinus tigrinus</i> | GenBank | EU543989 (Unpublished data) |
| <i>Lentinus tigrinus</i> | GenBank | DQ056860 (Unpublished data) |
| <i>Lentinus tigrinus</i> | GenBank | AF516521 (Krueger et al. 2002) |
| <i>Lentinus tigrinus</i> | GenBank | AF516520 (Krueger et al. 2002) |
| <i>Lentinus tigrinus</i> | GenBank | AF516519 (Krueger et al. 2002) |
| <i>Lentinus tigrinus</i> | GenBank | AF516518 (Krueger et al. 2002) |
| <i>Lentinus tigrinus</i> | GenBank | AF516517 (Krueger et al. 2002) |
| <i>Lentinus tigrinus</i> | GenBank | AY218419 (Unpublished data) |
| <i>Lentinus polychrous</i> | GenBank | AB478882 (Sotome et al. 2009) |
| <i>L. concentricus</i> sp. nov. | MFLU08 1375 | (new sequence) |
| <i>L. megacystidiatus</i> sp. nov. | MFLU08 1388 | (new sequence) |
| <i>L. roseus</i> sp. nov. | MFLU08 1389 | (new sequence) |
| <i>Polyporus lepideus</i> | GenBank | GU731572 (Unpublished data) |
| <i>Polyporus squamosus</i> | GenBank | DQ267123 (Unpublished data) |

Tab. 1. Taxon information and GenBank accession numbers in molecular work.

| Year | SCI publication |
|------|---|
| 2012 | Klomklung N., Karunarathna S. C., Chukeatirote E., Hyde K. D. (2012) Domestication of wild strain of <i>Pleurotus giganteus</i> – <i>Sydowia</i> 64 (1): 39-53 |
| | Mortimer PE, Karunarathna SC, Xue-qing Y, Yang X, He J, Sysouphanthong P & Hyde KD (2012) Prized edible Asian mushrooms; value, ecology, conservation, sustainability and effects of global warming. <i>Fungal Diversity</i> DOI: 10.1007/s13225-012-0196-3 |
| | Wisitrassameewong K., Karunarathna S.C., Thongklang N., Zhao R., Callac P., Moukha S., Férandon C., Chukeatirote E., Hyde K.D. (2012) <i>Agaricus subrufescens</i> : A review. <i>Saudi Journal of Biological Sciences</i> 19:131-146. |
| | De Silva DD, Rapior S, Hyde KD, Bahkali AH (2012). Medicinal mushrooms in prevention and control of diabetes mellitus. <i>Fungal Diversity</i> 56: 1-29 |
| | Karunarathna SC, Udayanga D, Maharachchikumbura SN, Pilkington M, Manamgoda DS, Wijayawardene DNN, Ariyawansa HA, Bandara AR, Chukeatirote E, McKenzie EHC, Hyde KD 2012 – Current status of knowledge of Sri Lankan mycota. <i>Current Research in Environmental & Applied Mycology</i> 2(1), 18-29, Doi 10.5943/cream/2/1/2 |

Tab. 2 List of publications during October 2011 – September 2012



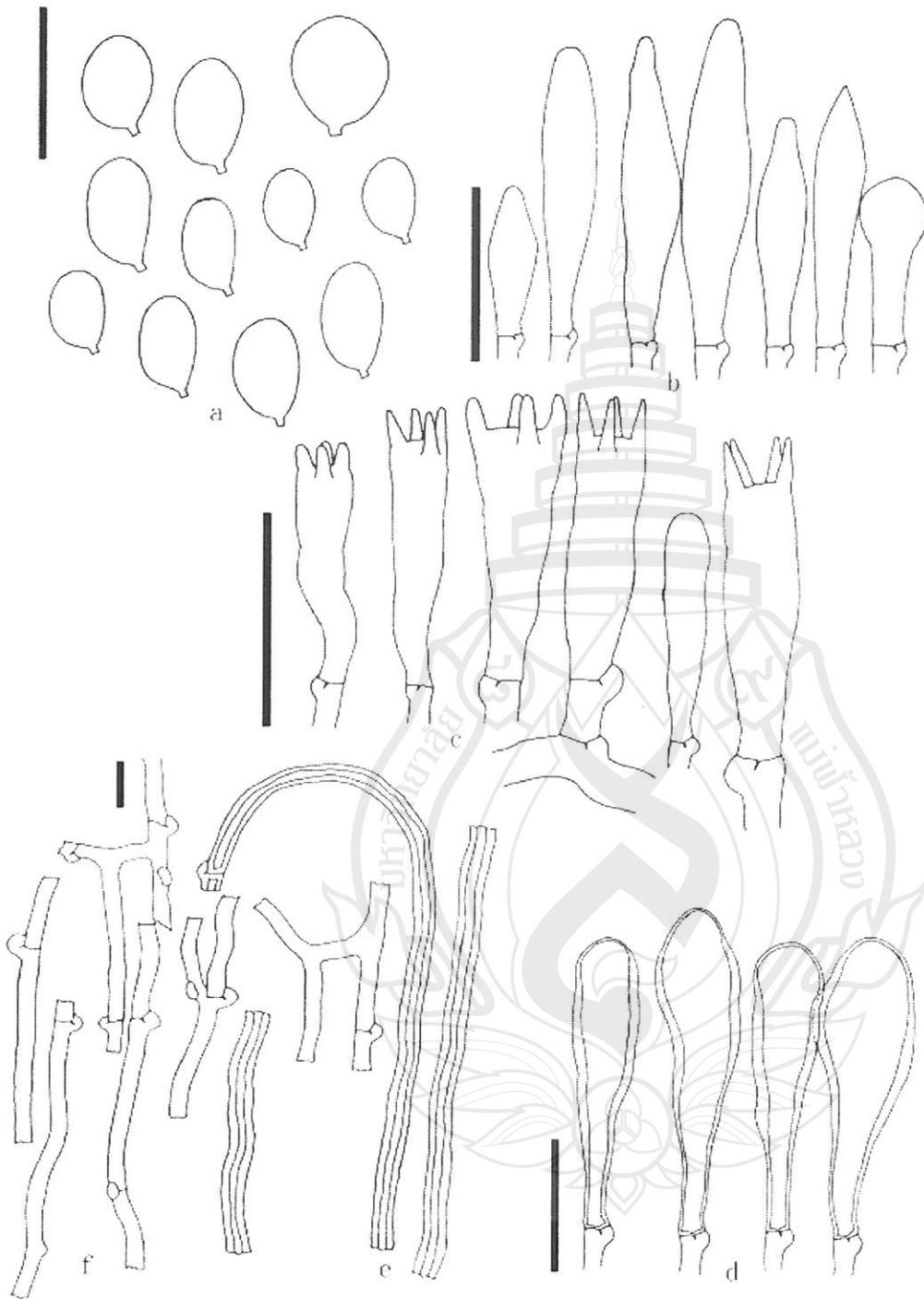


Fig. 2. *Lentinus roseus* sp. nov. (MFU08 1376) a: Basidiospores; b: Cheilocystidia; c: Basidia
d: Sclerocystidia; e: Skeletal hyphae; f: Generative hyphae Scale bars: a, b, c, d = 20 μ m; e, f = 10 μ m

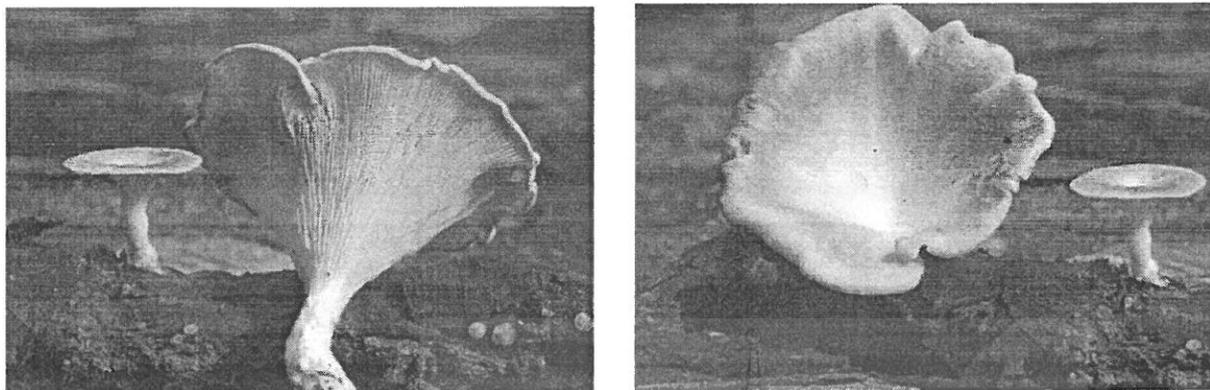


Fig. 3. Fruiting bodies of *Lentinus roseus* sp. nov. (MFU08 1376) in the field



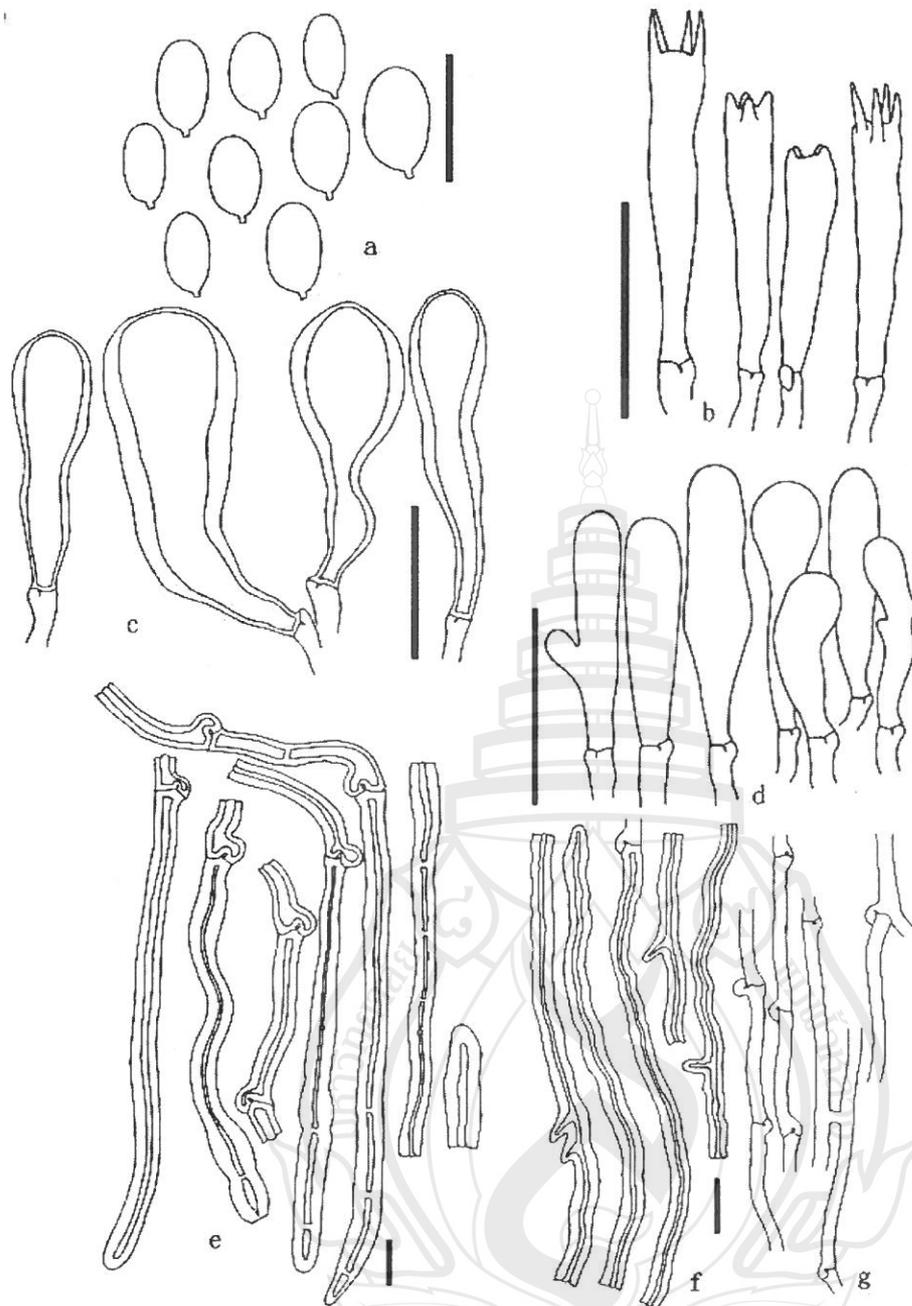


Fig. 4. *Lentinus megasclerocystidiatus* sp. nov. (MFU08 1388). a: Spores; b: Basidia; c: Sclerocystidia; d: Cheilocystidia; e: Hairs on the pileus; f: Skeletal hyphae; g: Generative hyphae; Scale bars: a, b, c, d = 20 μ m; e, f, g = 10 μ m

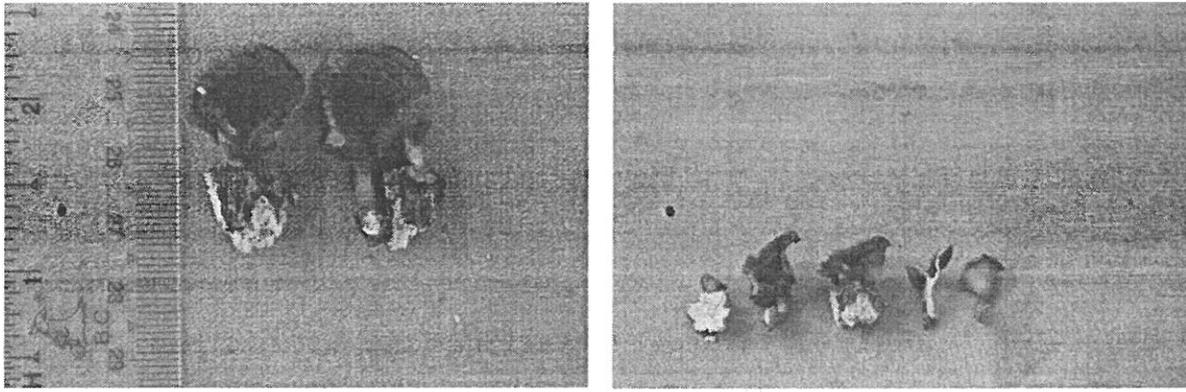


Fig. 5. The fruiting bodies of *Lentinus megasclerocystidiatus* sp. nov. (MFU08 1388) in the field



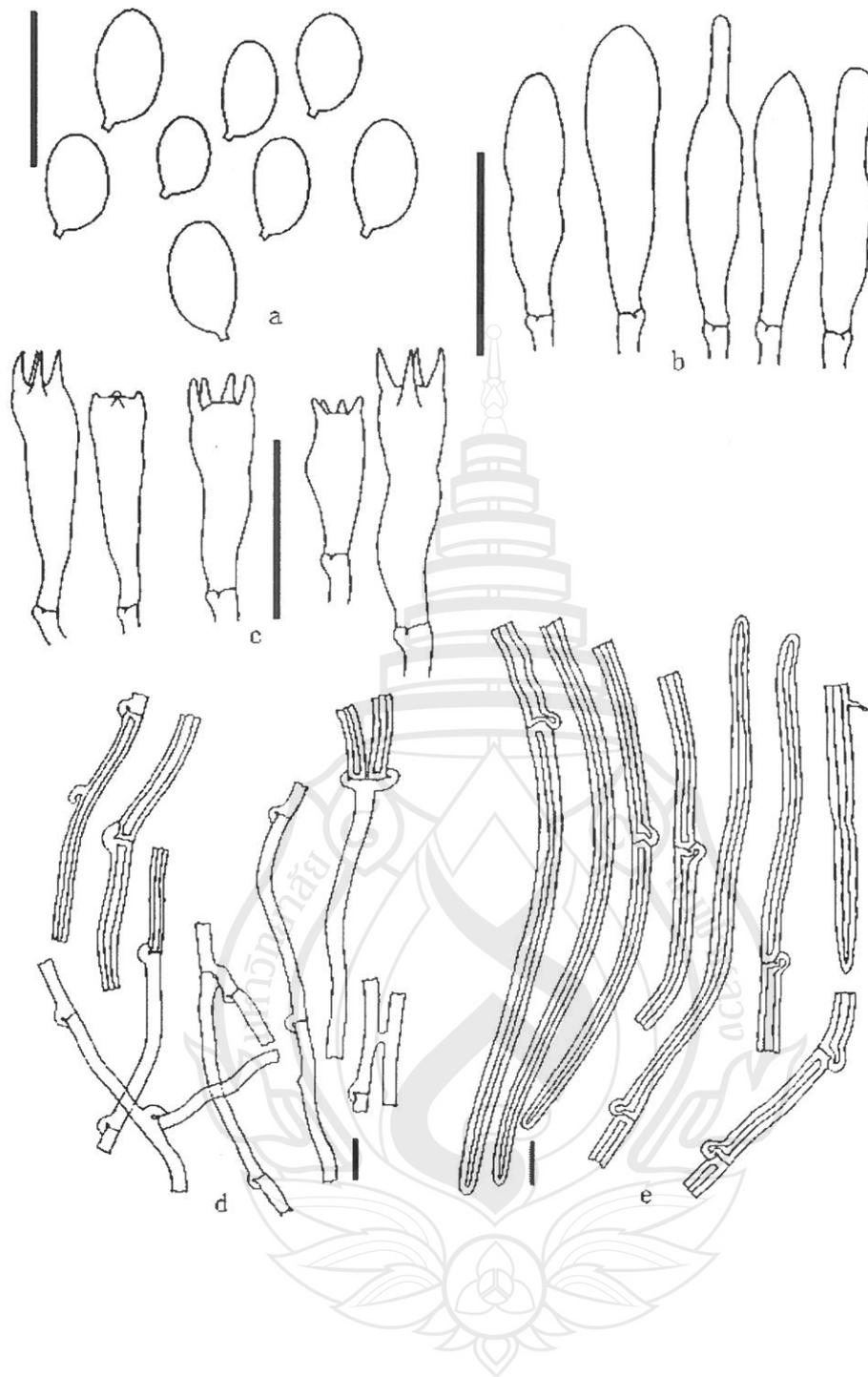


Fig. 6. *Lentinus zonabasioma* sp. nov. (MFU08 1375). a: Spores; b: Cheilocystidia; c: Basidia d: Generative hyphae; e: Skeletal hyphae; Scale bars: a, b, c = 20 μ m; d, e = 10 μ m

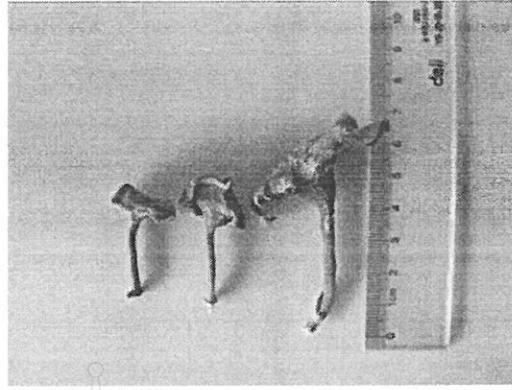
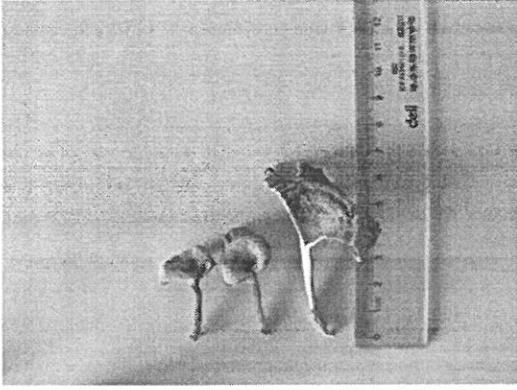


Fig. 7. Fruiting bodies of *Lentinus zonabasioma* sp. nov. (MFU08 1375) in the field



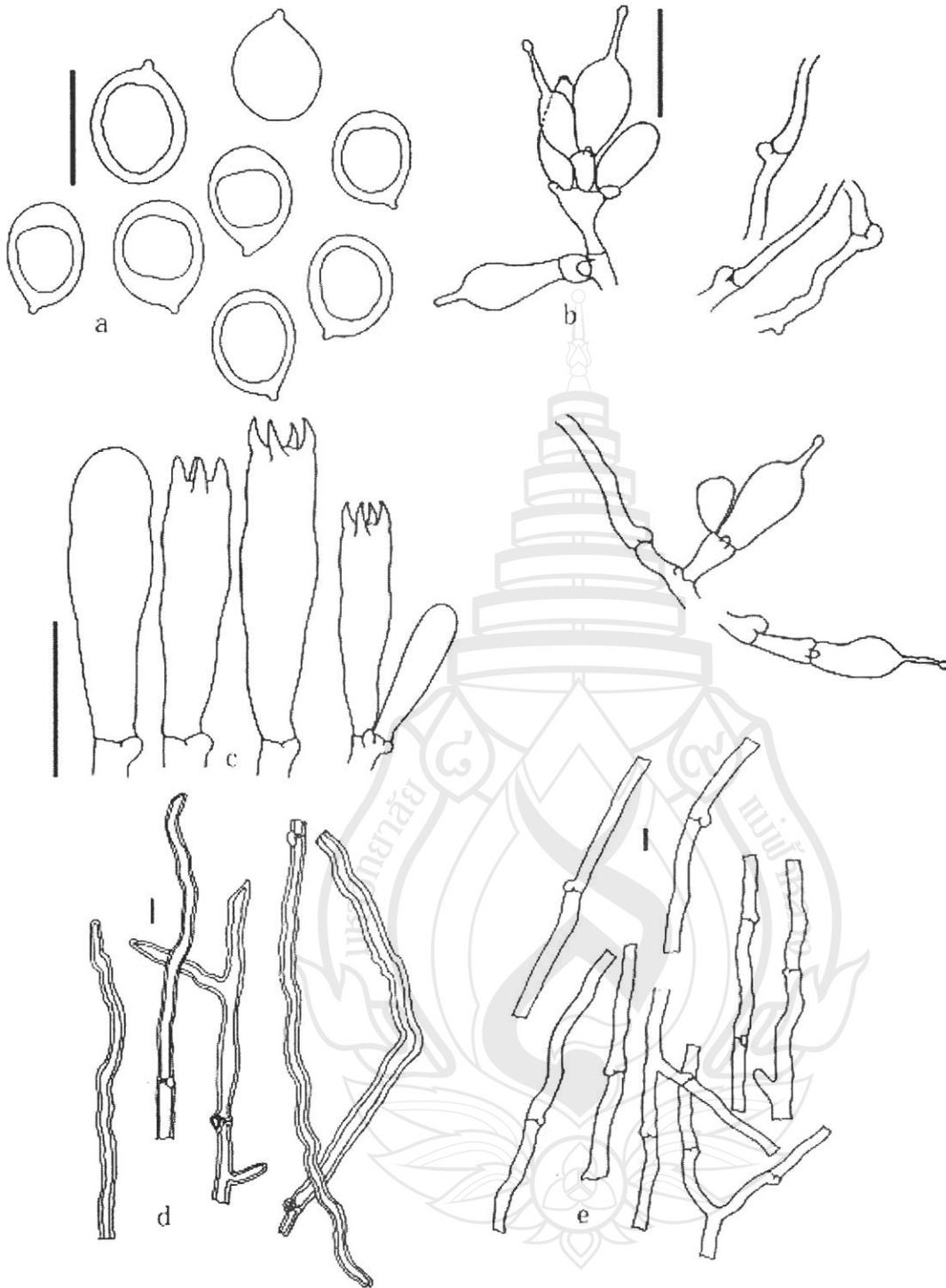


Fig. 8. *Lentinus giganteus* (MFU08 1382). a: Basidiospores; b: Cheilocystidia; c: Basidia; d: Generative hyphae
 e: Skeletal hyphae; Scale bars: a, b, c = 20 μ m; d, e = 10 μ m

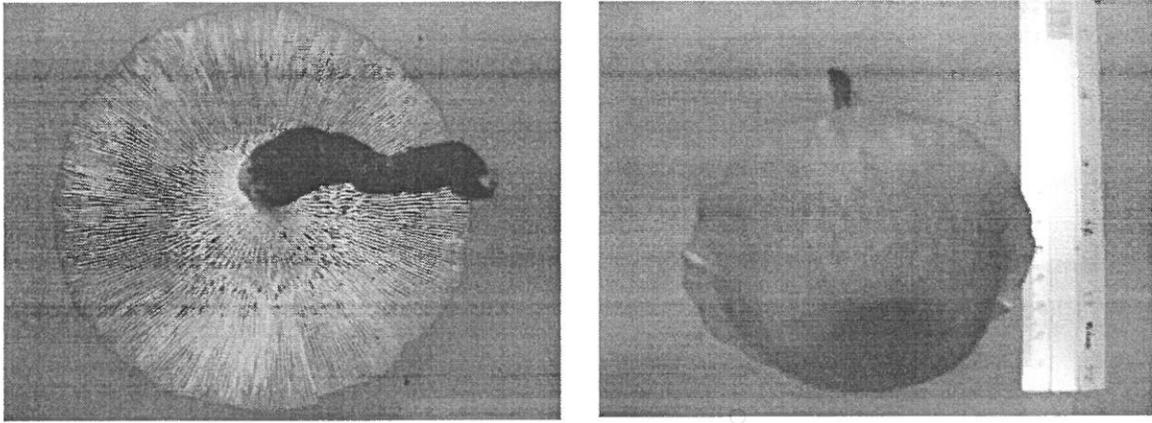


Fig. 9. Fruiting bodies of *Lentinus giganteus* (MFU08 1382)



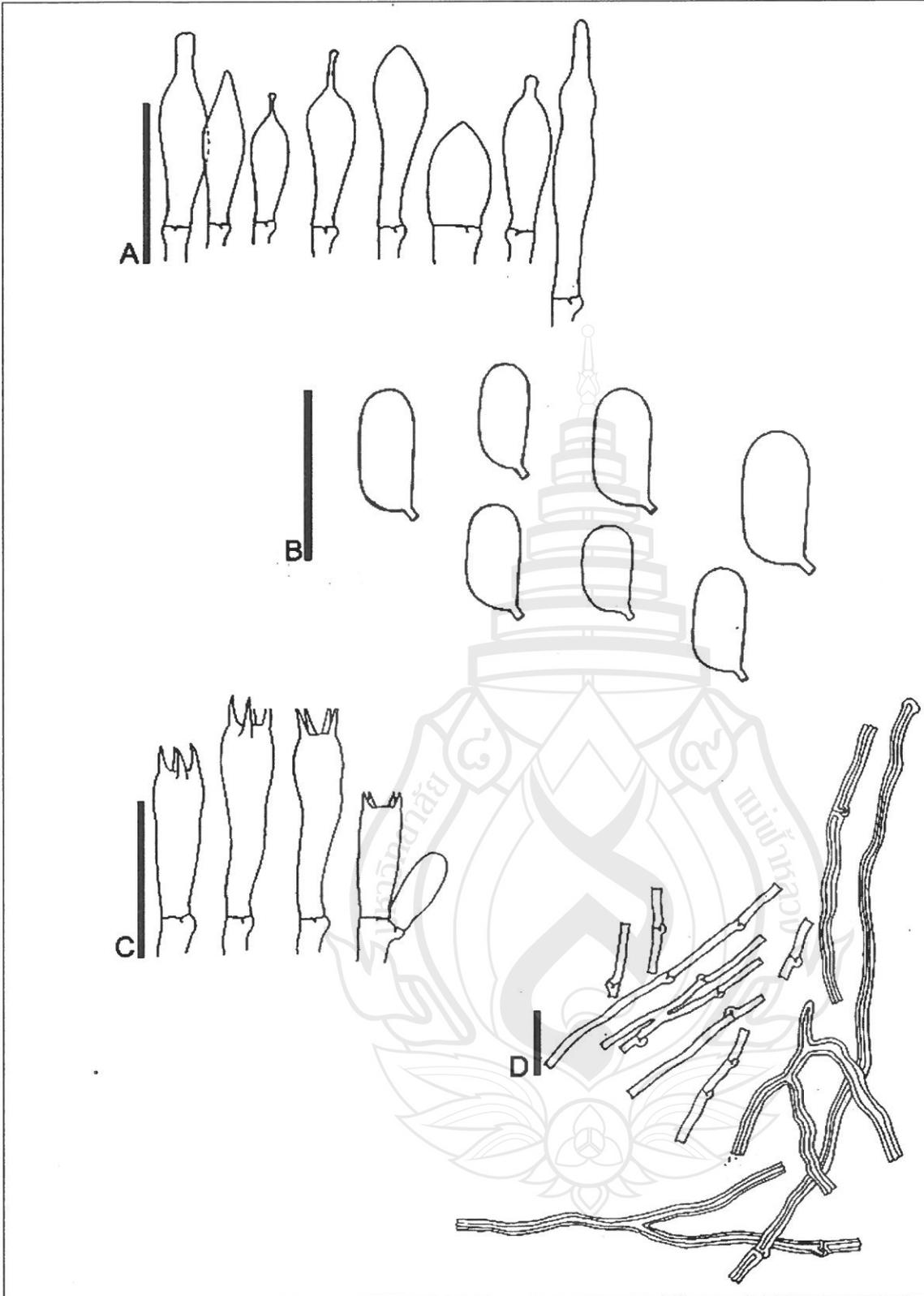


Fig. 10. *Lentinus connatus* Berk. A: Cheilocystidia; B: Basidiospores; C: Basidia; D: Generative hyphae and Skeletal Hyphae; Scale bars: A, C= 20 μ m; B, D = 10 μ m

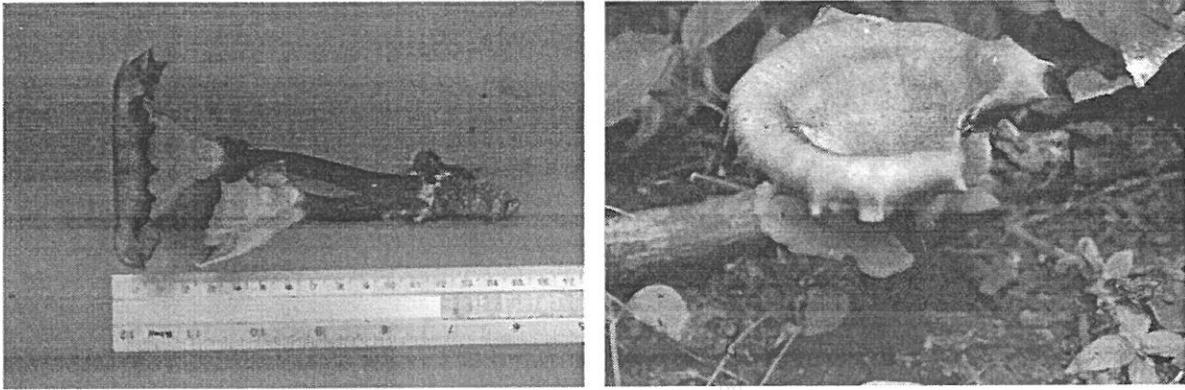


Fig. 11. Fruiting bodies of *Lentinus connatus* Berk in the field



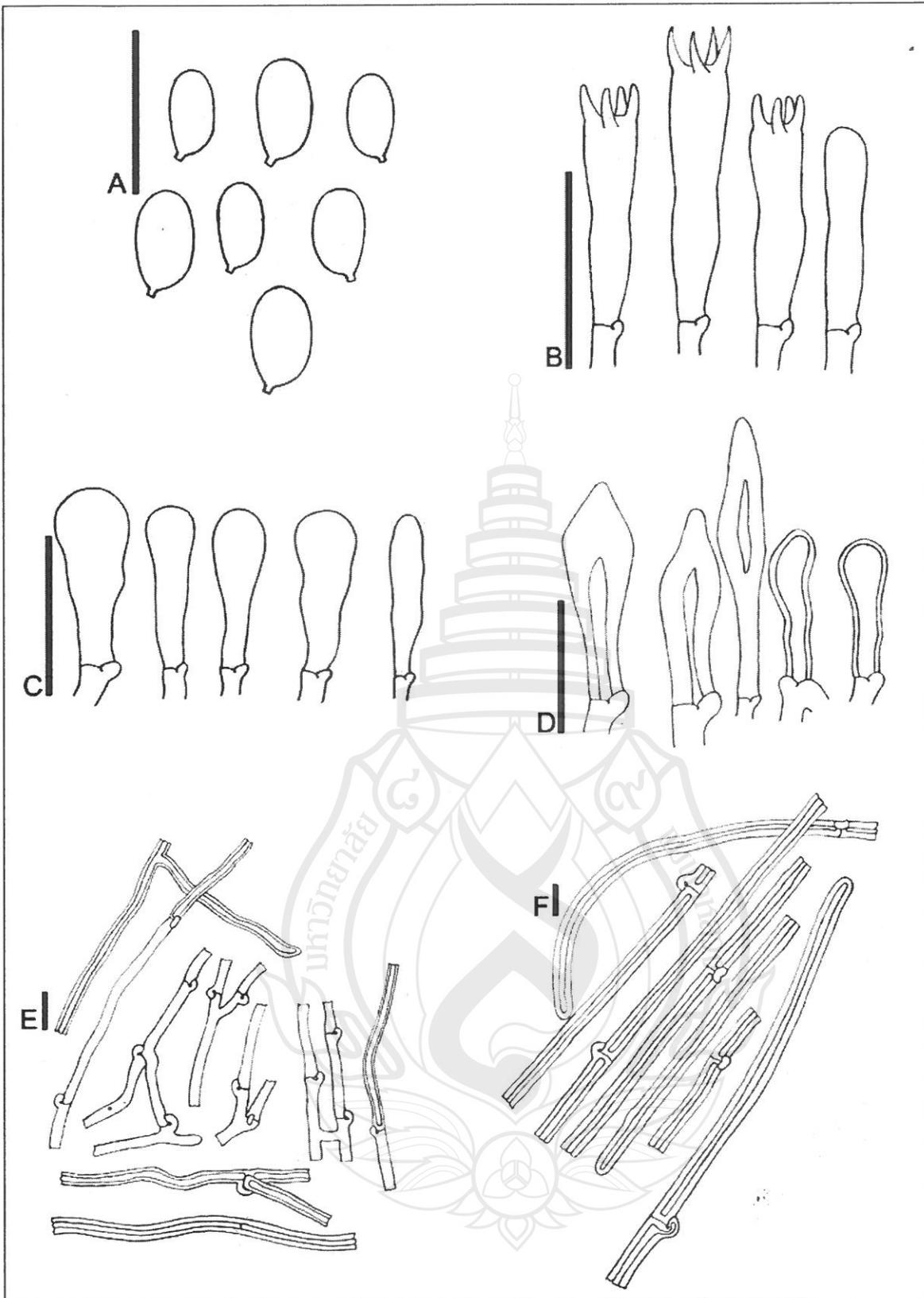


Fig. 12. *Lentinus similis* Berk. A: Basidiospores; B: Basidia; C: Cheilocystidia; D: Sclerocystidia E: Generative hyphae and Skeletal Hyphae; F: Hairs on pileus Scale bars: B, C, D = 20 µm; A, E, F = 10 µm

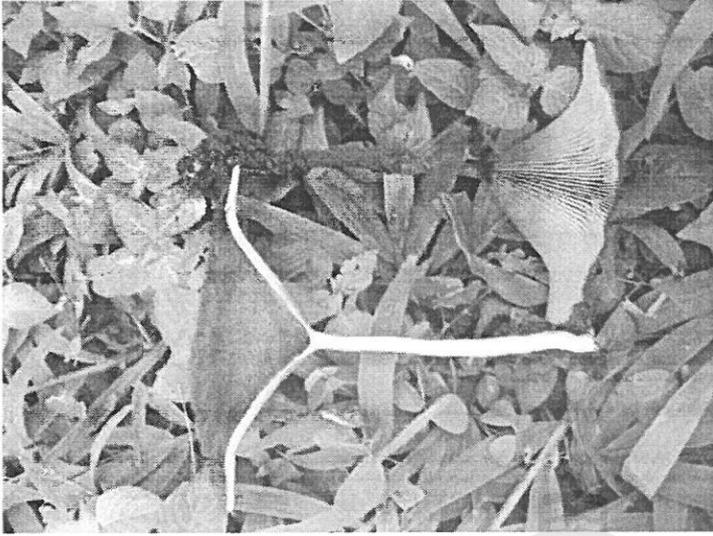


Fig. 13. Fruiting bodies of *Lentinus similis* Berk in the field



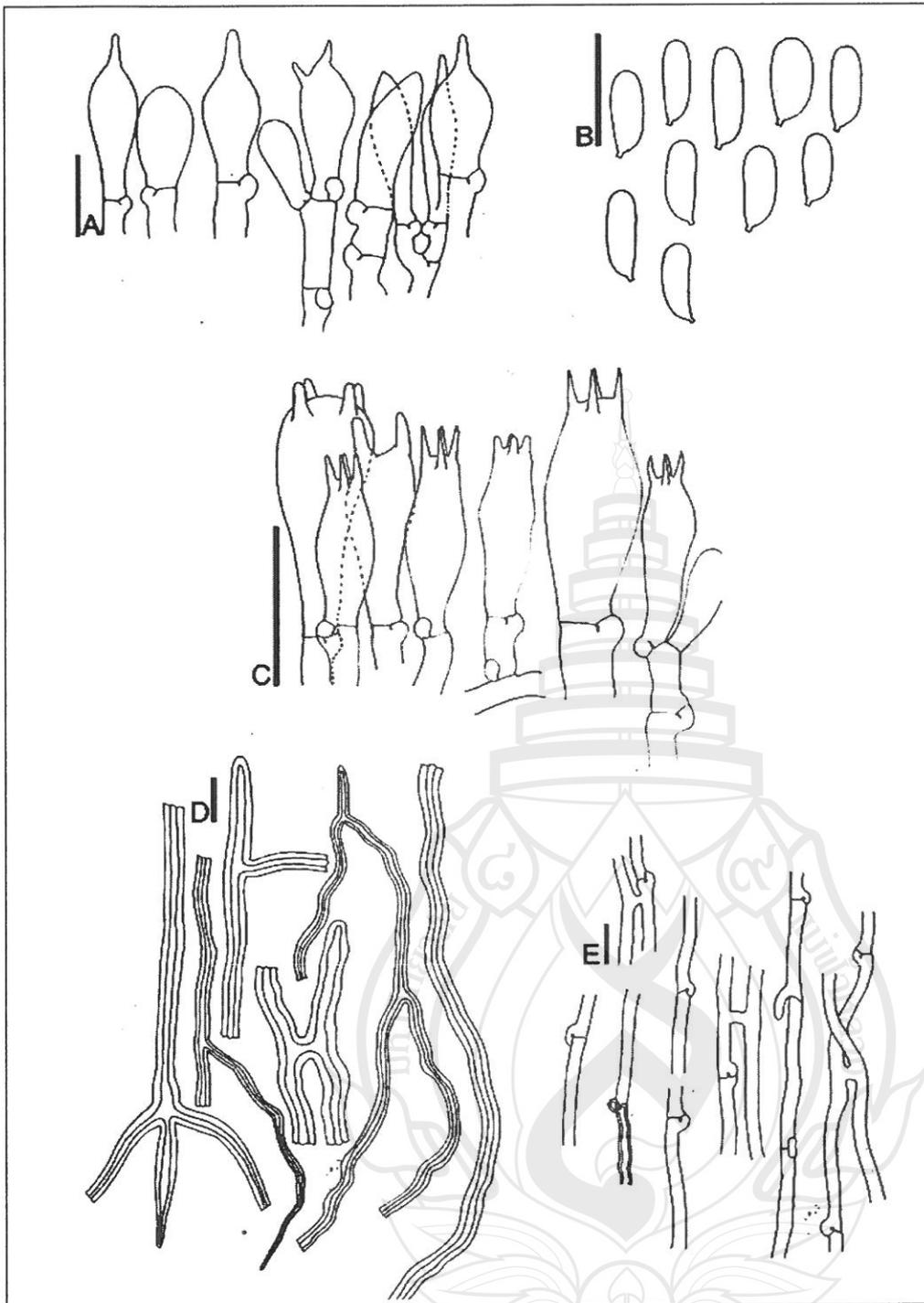


Fig.14. *Lentinus sajor-caju* (Fr.) A: Cheilocystidia; B: Basidiospores; C: Basidia; D: Skeletal Hyphae; E: Generative hyphae Scale bars: A, C = 20 µm; B, D, E = 10 µm

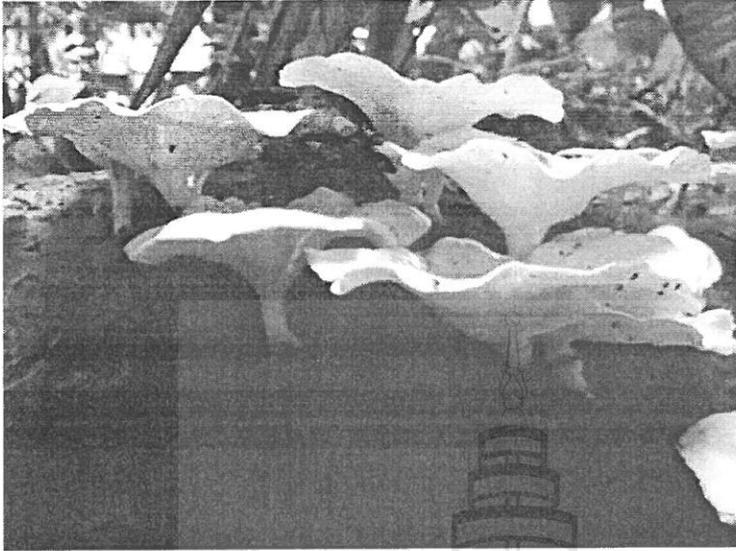


Fig. 15. Fruiting bodies of *Lentinus sajor-caju* (Fr.) in the field



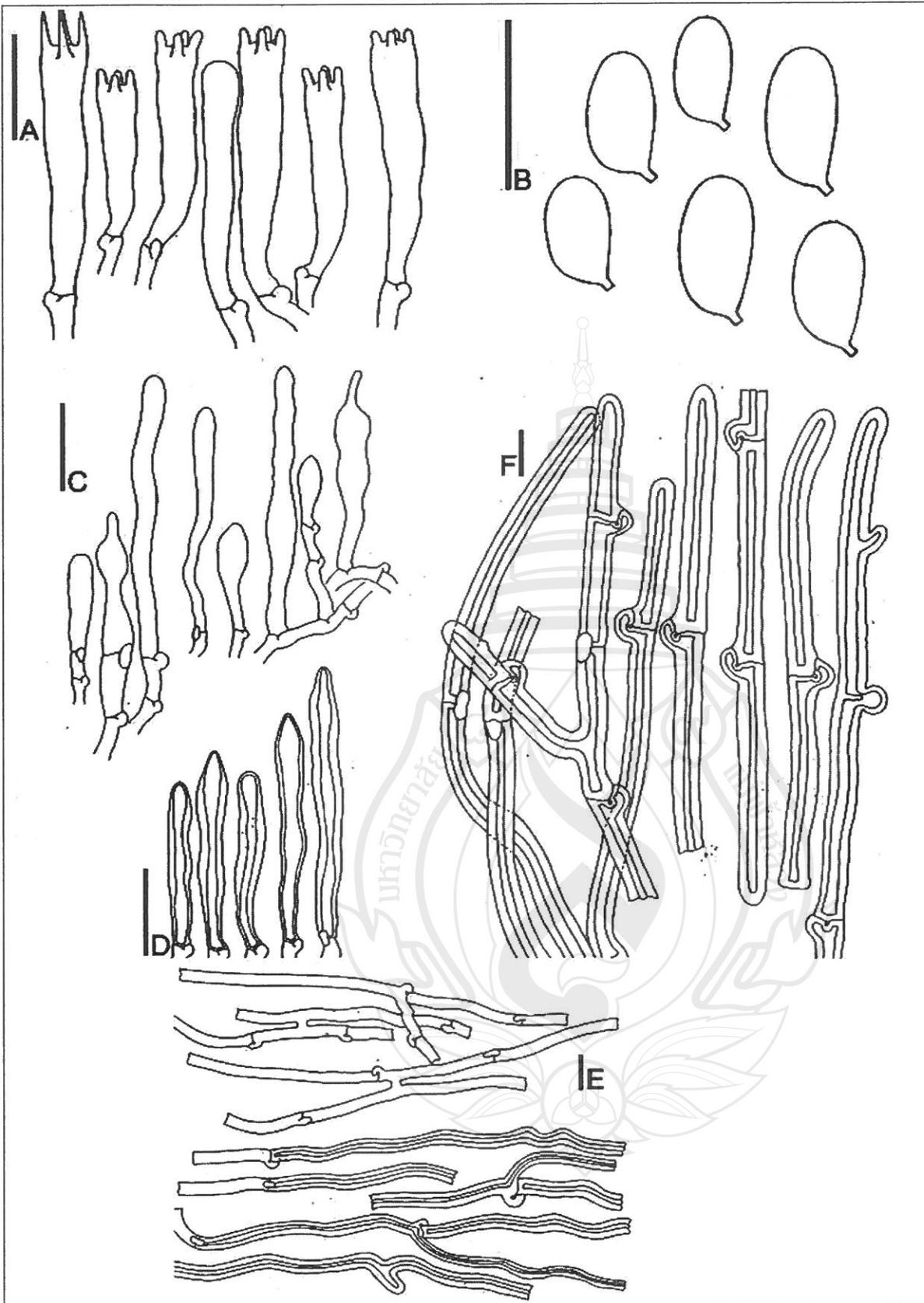


Fig. 16. *Lentinus velutinus* (Fr.) A: Basidia; B: Basidiospores; C: Cheilocystidia; D: Sclerocystidia; E: Generative hyphae & Skeletal Hyphae; F: Hairs on pileus Scale bars: A, C = 20 μm ; B, D, E = 10 μm

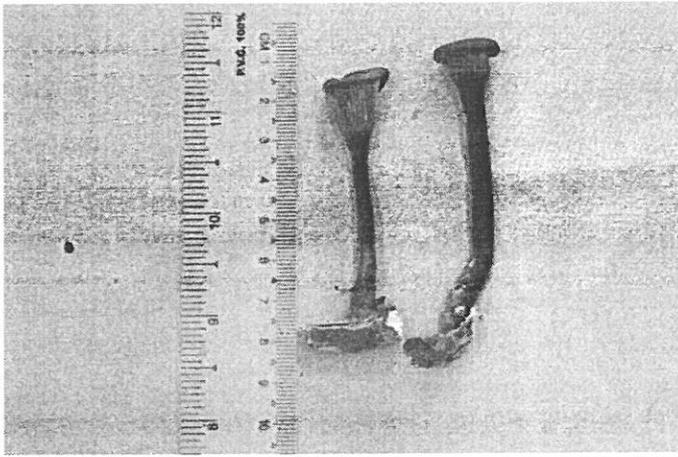


Fig. 17. Fruiting bodies of *Lentinus velutinus* (Fr.)



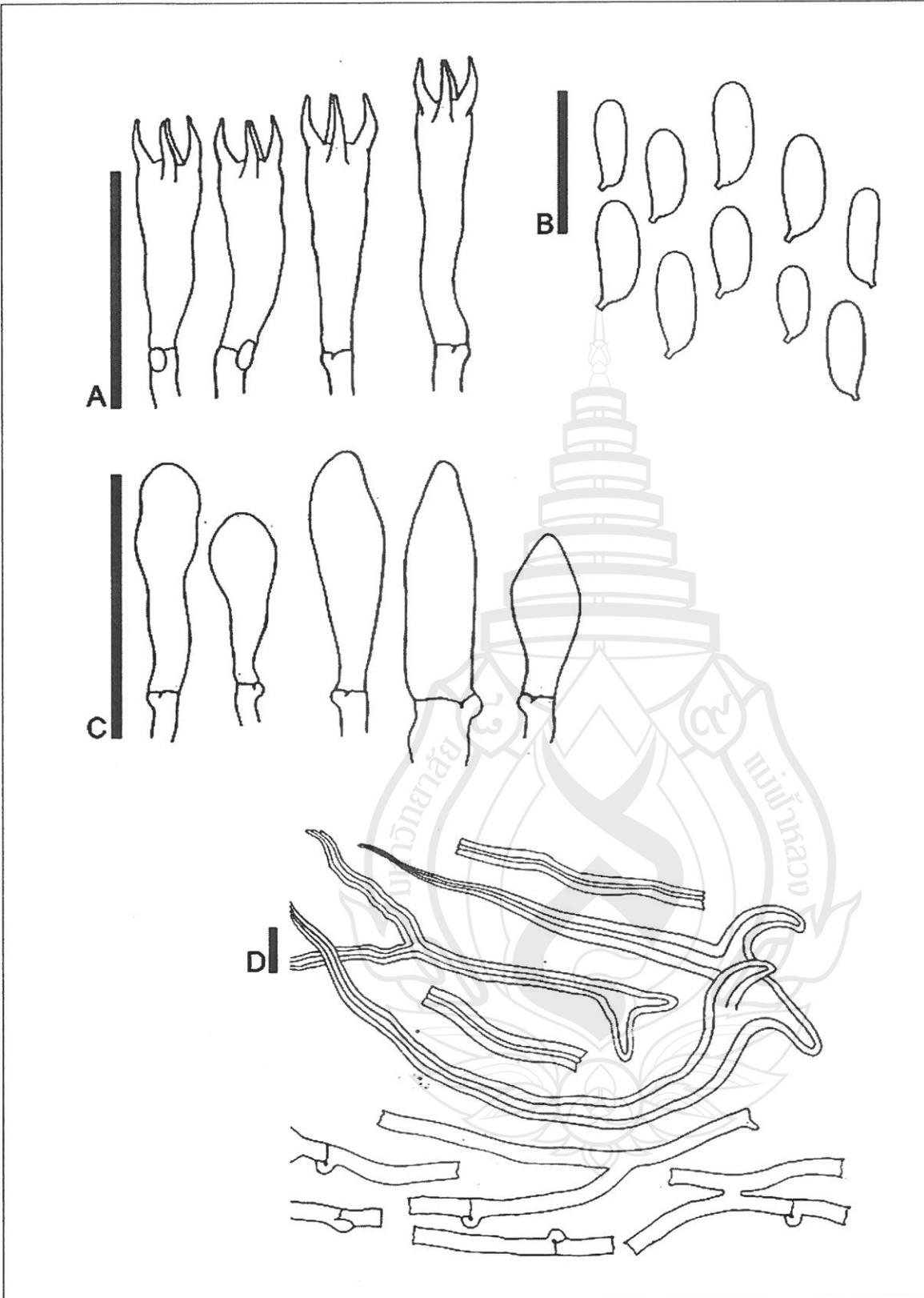


Fig. 18. *Lentinus tigrinus* (Bull. : Fr.) A: Basidia; B: Basidiospores; C: Cheilocystidia; D: Generative hyphae & Skeletal Hyphae; Scale bars: A, C = 20 μ m; B, D = 10 μ m

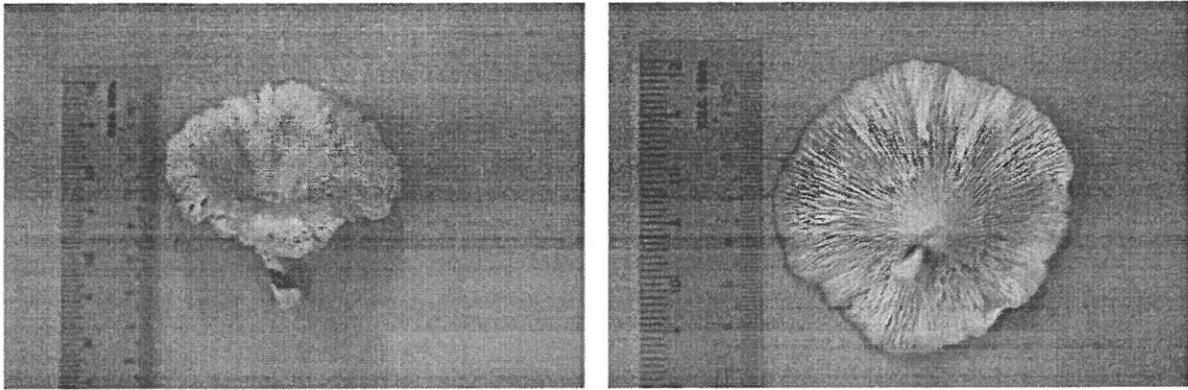


Fig. 19. Fruiting bodies of *Lentinus tigrinus* (Bull. : Fr.)



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