SYSTEMATICS OF AGARICUS, CYATHUS AND MICROPSALLIOTA (AGARICOMYCETES, BASIDIOMYCOTA) IN NORTHERN THAILAND

RUI-LIN ZHAO

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ชื่อวิทยานิพนธ์	การจัดระเบียบของเห็ดในสกุล *Agaricus*, *Cyathus* และ *Micropsalliota* (Agaricomycetes, Basidiomycota) ในภาคเหนือของประเทศไทย
ชื่อนักศึกษา	Miss Rui-Lin Zhao
รหัสประจำตัว	47067401
ปริญญา	ปรัชญาดุษฎีบัณฑิต
สาขาวิชา	เทคโนโลยีชีวภาพทางโรคพืช
พ.ศ.	2551
อาจารย์ที่ปรึกษาวิทยานิพนธ์	รศ.ดร. เกษม สร้อยทอง
อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม	Prof.Dr. Kevin D. Hyde
	Prof.Dr. Dennis E. Desjardin

บทคัดย่อ

วิทยานิพนธ์นี้ทำการศึกษาเกี่ยวกับการจัดระเบียบของเห็ดในสกุล *Agaricus*, *Cyathus* และ *Micropsalliota* ซึ่งเก็บตัวอย่างเห็ดในภาคเหนือของประเทศไทยระหว่างปี พ.ศ. 2547-2550 ทั้งหมดจำนวน 323 ตัวอย่าง จาก 19 แห่ง และได้ยืมตัวอย่างเห็ดจาก herbarium ต่างๆทั่วโลก จำนวน 115 ตัวอย่าง มาศึกษาเปรียบเทียบ จากการศึกษาพบว่ามีการจัดลำดับใหม่ของ ITS และ LSU rDNA ในเห็ดที่ทำการศึกษา จึงจัดส่งข้อมูลไปไว้ที่ GenBank

เห็ดในสกุล *Agaricus* เป็นเห็ดที่ดำรงชีวิตโดยอาศัยซากพืชที่เน่าเปื่อยเป็นแหล่งอาหาร และบางชนิดสามารถนำมาเพาะเลี้ยงและรับประทานได้ ในการศึกษาครั้งนี้ เห็ดจำนวน 200 ตัวอย่าง ได้มีการตรวจสอบลักษณะทางสัณฐานวิทยา และการวิเคราะห์ลำดับเบส จากการศึกษาพบเห็ดจำนวน 27 ชนิด พบว่ามี 15 ชนิดที่มีรายการค้นพบแล้ว ส่วนอีก 12 ชนิดคาดว่าเป็นเห็ดชนิดใหม่หรือได้ถูกค้นพบแล้ว ในการวิจัยจึงได้จัดทำความสัมพันธ์ทางวิวัฒนาการขึ้นมาใหม่ของเห็ดในสกุล *Agaricus* โดยใช้พื้นฐานการจัดลำดับเบสของตัวอย่างที่เก็บได้ในประเทศไทย รวมทั้งสกุลย่อย *Lanagaricus* และการจัดลำดับเบสจาก GenBank

เห็ดในสกุล *Cyathus* จำนวน 115 ตัวอย่าง ได้ยืมมาศึกษาเปรียบเทียบจาก herbarium นานาชาติ และตัวอย่างสด 16 ตัวอย่างที่ได้จากการเก็บในภาคเหนือของประเทศไทย ซึ่งในวิทยานิพนธ์นี้ได้บรรยายลักษณะของเห็ดในสกุลนี้จำนวน 46 ตัวอย่าง และมี 1 ชนิดที่รายงานว่าเป็นชนิดใหม่ (new species) และอีก 4 ชนิดที่ถูกเสนอให้มีความคล้ายคลึงกับเห็ดชนิดอื่น และอีก 1 ชนิดที่ได้เสนอให้มีการจัดลำดับ taxon ใหม่ จากการศึกษาการวิเคราะห์วิวัฒนาการของสิ่งมีชีวิตโดยอาศัยระบบการจัด

จำแนก infrageneric ของ Brodie พบว่า 7 กลุ่มของ infrageneric ไม่ได้สนับสนุนข้อมูลระดับโมเลกุล โดยการจัดทำข้อมูล ITS และ LSU เพื่อสนับสนุนการยอมรับให้จัดเป็น 3 infrageneric groups รวมถึงการนำลักษณะสัณฐานวิทยาต่างๆ ขนาดของสปอร์เห็ด สีของดอกเห็ด และลักษณะกายวิภาค เพื่อช่วยในการจำแนกเห็ดในกลุ่มเหล่านี้

"Small Agaricus" เป็นคำอธิบายลักษณะของเห็ดในสกุล *Micropsalliota* เนื่องจากมีลักษณะที่คล้ายคลึงกับเห็ดในสกุล *Agaricus* ถึงแม้ว่าดอกเห็ดส่วนใหญ่จะมีขนาดเล็ก จากการเก็บตัวอย่างเห็ด *Micropsalliota* ในประเทศไทย จำนวน 104 ตัวอย่าง จัดจำแนกได้ 24 ชนิด เป็นเห็ดชนิดใหม่ 8 ชนิด (new spicies) และ 2 new varieties จึงได้มีการตั้งชื่อและเขียนรายละเอียดของเห็ดไว้ ส่วนอีก 4 ชนิดยังไม่ได้ตั้งชื่อ แต่มีการเขียนรายละเอียดไว้แล้วซึ่งคาดว่าจะเป็นชนิดใหม่ หรือได้ถูกค้นพบแล้ว การศึกษานี้จึงถือว่าเป็นการนำข้อเสนอเป็นครั้งแรกในการนำ phylogenic analyses มาใช้ในการจัดจำแนกระดับ genus ของเห็ดกลุ่มนี้ นอกจากนี้ยังพบว่าเห็ด 4 ชนิด (รวมทั้ง 2 ชนิดใหม่) จากเห็ดในสกุล Heinemannomyces และ Hymenagaricus มีความสัมพันธ์กับเห็ดในสกุล *Agaricus* และ *Micropsalliota* ทางสัณฐานวิทยาและวิวัฒนาการของสิ่งมีชีวิต

คำสำคัญ : Agaricaceae, Nidulariaceae, วิวัฒนาการของสิ่งมีชีวิต อนุกรมวิธาน

Thesis	Systematics of *Agaricus*, *Cyathus* and *Micropsalliota* (Agaricomycetes, Basidiomycota) in Northern Thailand
Student	Miss Rui-Lin Zhao
Student ID.	47067401
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Program	Biotechnology in Plant Pathology
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Thesis Advisor	Assoc. Prof. Dr. Kasem Soytong
Thesis Co-advisor	Prof. Dr. Kevin D. Hyde
	Prof. Dr. Dennis E. Desjardin

ABSTRACT

This thesis reports on a systematic study of *Agaricus*, *Cyathus* and *Micropsalliota* species collected in northern Thailand from 2005-2007. Three hundred and twenty three specimens were collected from19 sites and 115 specimens borrowed from worldwide herbaria. Two hundred and twenty two new ITS and LSU rDNA sequences were generated and will be submitted to GenBank.

Agaricus species are saprobes and some species are important cultivated edible species. In this study 200 specimens were examined morphologically and subjected to sequence analyses. Full descriptions are given for 27 species of which 15 are recorded species including two new to science. The other 12 species are suspected to be new or known species but are not provided with descriptions in this thesis because of paucity of material. A phylogenetic reconstruction of *Agaricus* is produced based on sequences from Thailand collections including the subgenus *Lanagaricus*, plus sequences from GenBank .

Cyathus specimens (115) were borrowed from international herbaria and 16 fresh specimens were collected from northern Thailand. In this thesis 46 species are fully described, one is reported as new, four taxa are proposed as synonyms of other species and one taxon rank change is proposed. Phylogenetic analyses have been conducted. The current infrageneric classification system of Brodie that recognizes seven infrageneric groups is not supported by molecular data. Instead, the ITS and LSU datasets support recognition of three infrageneric groups.

Morphological characters useful in distinguishing these groups include basidiospore size, fruit body coloration, and peridium anatomy.

The *Micropsalliota* epithet translates to "small *Agaricus*" because they possess characters similar to *Agaricus* although the fruitbodies are relatively small. One hundred and four specimens of *Micropsalliota* were collected in Thailand. Twenty two taxa including 8 new species and 2 new varieties are named with full descriptions, figures and colored plates, and 4 species are unnamed and suspected as new or known species and are documented with full descriptions. The first phylogenetic analyses of this genus are presented. In addition, four species (including 2 new species) from the genera *Heinemannomyces* and *Hymenagaricus* that are allied with *Agaricus* and *Micropsalliota* in morphology and phylogeny are fully described.

Key words: Agaricaceae, Nidulariaceae, phylogeny, taxonomy.

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Rui-Lin Zhao

Contents

Contents (continued)

Contents (continued)

Contents (continued)

Contents (continued)

Contents (continued)

Contents (continued)

Contents (continued)

List of Tables

List of Figures

List of Figures (continued)

List of Figures (continued)

List of Figures (continued)

List of color plates

Chapter 1

INTRODUCTION

1.1 General introduction

1.1.1 Introduction to systematics

Wherever we look in nature, we find a uniqueness of life, and this uniqueness comprises biodiversity. To determine such uniqueness is the task of taxonomy. Presently, more than 1 million animal species and 500,000 plant and microorganism species have been described; the estimates of undescribed species range from 3 to 10 million or even higher. The amount of diversity is unmistakably immense (Mayr & Ashlock 1991).

The application of the term taxonomy and systematics often overlap, but they have a subtle difference in meaning: "taxonomy is the theory and practice of classifying organisms" (Mayr & Ashlock 1991), whereas "Systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them" (Simpson 1961). More simply, systematics is the science of diversity of organisms and deals with populations, species and higher taxa. It not only provides urgently needed information concerning these levels, but also and more importantly, cultivates a way of thinking, a way of approaching biology as a whole (Mayr 1968, 1982).

The major tasks of systematics is to determine by means of comparison what the unique properties of each species and higher taxa are; to determine what properties certain taxa have in common and establish the biological causes of the differences or shared characters; and to determine the variation within taxa (Mayr & Ashlock 1991). The requirements of systematic study involve species recognition and arrangement of the different taxa into a classification that will show their natural relationships. The three terms most used in systematic literature are: systematics, taxonomy and nomenclature (Kaul 1997).

Systematics is one of the major subdivisions of biology, and is as broad-based as genetics or molecular biology. It includes not only identification, but also the comparative study of all aspects of organisms as well as their evolutionary history. Based on the advance in DNA sequencing techniques and analytic methods in the last decade, the knowledge of molecular phylogenetics and systematics of fungi have progressed rapidly.

1.1.2 General introduction to Basidiomycota

Basidiomycota are characterized by a multi-layered cell walls, barrel-shaped structures or pulley wheel occlusions at the septa of hyphae (dolipore septa), an extended dikaryophase, clamp connections that often develop on septa, and the formation of meiosporangia (basidia) that produce meiospores (basidiospores) at the tips of sterigmata (Kendrick 2000). Almost 30,000 species had been described (Kirk *et al.* 2001).

Historically Basidiomycota was treated as a subphylum Basidiomycotina and comprised 3 classes: Teliomycetes (rust and smut fungi), Hymenomycetes (mostly gilled mushrooms) and Gasteromycetes (puffballs, bird's nest fungi, earth stars, stinkhorns) in a 5 subphyla classification system (Ainsworth *et al.* 1973). In the latest edition of the "Dictionary of Fungi", Basidiomycota comprise 3 classes: 1) Basidiomycetes (including members of Hymenomycetes and Gasteromycetes), 2) Urediniomycetes and Ustilaginomycetes, and 3) "hymenomycetes" and "gasteromycetes" were taken as informal and not monophyletic categories (Kirk *et al.* 2001).

With the exception of Urediniomycetes and Ustilaginomycetes that contain important plant pathogens, the Basidiomycetes mostly are saprobes and symbionts, and play ecologically important roles, such as oxygen, carbon and nitrogen cycling. Humans were first attracted to mushrooms since ancient times because of their edible or poisonous traits. Mushrooms are an important group in the biosphere and their significance in diversity and conservation issues have been recognized extensively (Kaul 2001).

1.2 Advance of phylogenetic systematics of Basidiomycota and Agaricomycotina

Swann and Taylor (1993) carried out pioneering studies on the phylogeny of Basidiomycota by sequencing and analyzing the 18S rRNA gene of 19 species. Swann & Taylor (1995) increased the number of species to 54 using the same gene. Both publications revealed 3 major groups: Hymenomycetes, Ustilaginomycetes and Urediniomycetes in Basidiomycota. Subsequent researchers (e.g., James *et al.* 2006, Blackwell *et al.* 2006) have shown Swann and Taylor's classification to be remarkably stable, but the term of Hymenomycetes is no longer accepted, because it is a hononym of "hymenomycetes" (Fries 1874), which had been shown to be polyphyletic and should be used only informally.

The "Deep Hypha" project which continued as the "Assembling the Fungal Tree of Life (AFTOL)" project aims to establish the phylogeny of the entire fungal kingdom. Several publications, milestones in the classification of fungi have been published as a result (James *et al.* 2006, Blackwell *et al.* 2006, Deep Hypha issue of Mycologia Dec. 2006 "2007"). All publications consistently revealed similar major clades in the monophyletic fungal kingdom. These discoveries are summarized by Hibbett *et al.* (2007) as "the higher-level classification of fungi", which includes a comprehensive phylogenetic classification of the kingdom Fungi. The classification accepts 1 Kingdom, 1 subkingdom, 7 Phyla, 10 subphyla, 35 classes, 12 subclasses and 129 orders. Ascomycota and Basidiomycota constitute the subkingdom Dikarya, which comprise taxa with dikaryotic hyphae. The formerly named Basidiomycetes, Urediniomycetes and Ustilaginomycetes (Kirk *et al.* 2001) are accepted as Agaricomycotina, Pucciniomycotina and Ustilaginomycotina, respectively.

The classification of major groups within the Agaricomycotina remains unsettled. It has been divided into Heterobasidiomycetes (jelly fungi) and Homobasidiomycetes (mushroom-forming fungi) based on the structure of the septal pore apparatus and the spindle pole body in GenBank 2006 and Mycota VIIb (Hibbett & Thorn 2001), while they are called Tremellomycetidae and Agaricomycetidae under the class Basidiomycetes in the last edition of Dictionary of Fungi (Kirk *et al.* 2001). In the Homobasidiomycetes, a preliminary phylogenetic outline with 8 major clades was revealed using nuc-ssu and mt-ssu rDNA sequences (Hibbett *et al.* 1997). These major clades coincided with Polyporoid, Euagarics, Bolete, Russuloid, Thelephoroid Hymenochaetoid, Cantharelloid and Gomphoid-phalloid clades. This classification was not congruent with Swann and Taylor's (1995) findings. Recently Homobasidiomycetes was shown to be polyphyletic (Binder *et al.* 2005; Moncalvo *et al.* 2006).

Presently, the most accepted classification of Agaricomycotina comprisies 5 independent clades that are supported with Bayesian statistics (Hibbett 2007 '2006'), and they are recognized as the 5 classes Agaricomycetes, Tremellomycetes, Dacrymycetes, Wallemiomycetes and Entorrhizomycetes (James *et al.* 2006; Hibbett *et al.* 2007, and Matheny *et al.* 2007; 2007 '2006').

Lack of, or having septa within basidia, the type of basidia division and the basidiospore discharge mechanism were previously considered as important higher level characters , but this is not reflected by phylogenetic reconstructions. Characters associated with the septal pore apparatus, nuclear division and the spindle pole body are in agreement with current molecular

phylogenetic analyses (Celio 2007 "2006") based on the Structural and Biochemical Database for AFTOL.

1.3 Advances in phylogenetic systematics of Agaricomycetes and Agaricales

The class Agaricomycetes is divided into two subclasses; Agaricomycetidae and Phallomycetidae (Binder 2005; Matheny *et al.* 2007). Agaricomycetidae comprises 3 orders: Agaricales, Atheliales and Boletales; while Phallomycetidae comprises 13 orders (Matheny 2007 '2006' and 2007). In many orders, especially those representing larger groups, such as Agaricales, there is still not enough resolution or taxon sampling to develop comprehensive family-level classifications (Hibbett *et al.* 2007).

Agaricales comprises the so-called mushrooms and toadstools, and is the largest clade of mushroom-forming fungi. More than 9000 species in more than 300 genera, and 26 families had been described (Kirk *et al.* 2001). Mostly they are terrestrial, lignicolous and saprobic, and many are mycorrhizal.

An early classification of mushrooms was developed by Fries (1874). In his classification 12 genera of gilled mushrooms (agarics) were recognized based on macroscopic features of basidiocarps and colour of spore prints (white, pink, brown, purple-brown and black). His system was widely used as it had the advantage that many genera could be identified on field characters. The system was relatively unchallenged until Fayod studied the anatomy and microscopic features of basidiocarps and consequently recognized 108 genera in 1889.

The most influential systematic treatment of the Agaricales is *The Agaricales in Modern Taxonomy* by Singer (1986). Singer utilized Fayod's anatomic characters and Fries's macroscopic characters in reorganizing families and genera. The term "Agaricales" in his scheme refers to the order containing the type genus *Agaricus* and the type family Agaricaceae. In his system there were 3 majors groups in the order Agaricales s. l.: Agaricales s. str., Boletales, and Russulales. Those 3 groups were accepted as the euagaric clades, bolete clade and russuloid clade based on molecular data (Hibbett & Thorn 2001). Totally 18 families and 230 genera were distinguished in his system (Singer 1986).

Recent molecular phylogenetic research has revealed that Singer's Agaricales roughly parallels the euagarics clade (Hibbett *et al.* 1997; Moncalvo *et al.* 2000, 2002). However phylogenetic data have shown that an overemphasis on spore print color, fruit body formation and

some anatomical and cytological traits has led to the establishment of many artificial groups, while some cyphelloid, aphyllophoralean and gasteroid fungi should be included. For example, Hibbett *et al.* (1997) constructed a comprehensive phylogenetic dataset using nuclear and mitochondrial ribosomal DNA sequences of representatives of Agaricales, Aphyllophorales and gasteromycete families (i.e. gilled, nongilled and puffballs). The data showed that fruitbody form and hymenophore type did not reflect phylogenetic relationships well. Later research has also confirmed this (Hibbett 2004). Moncalvo *et al.* (2000) investigated phylogenetic relationships within the order Agaricales with analysis of nuclear large subunit ribosomal DNA sequences. One of their discoveries was to reveal polyphyletic groups, such as in the families Tricholomataceae, Cortinariaceae and Hygrophoraceae and the polyphyletic genera *Clitocybe*, *Omphalina*, and *Marasmius*. Another phylogenetic study using a broader sampling in the euagaric clade (Moncalvo *et al.* 2002) analyzed nuclear large subunit ribosomal DNA sequences to reveal 117 monophyletics clades and at the same time showed that some traditional taxonomic groupings were artificial. Some non-gilled resupinate, cyphelloid and gasteroid taxa were found to be members of the Agaricales (Binder and Bresinsky 2002; Bodensteiner *et al.* 2004; Larsson *et al.* 2004; Binder *et al.* 2005, Moncalvo *et al.* 2002, Hallen *et al.* 2003, Matheny and Bougher 2006, Peintner *et al.* 2001). These discoveries indicate that ecological, biochemical, or tropic habits rather than morphological similarities should also be used as diagnosis characters to understand the natural groupings in this order (Moncalvo *et al.* 2002).

The most recent phylogenetic treatment within Agaricales was conducted by Matheny *et al.* (2007 "2006"). In this study the dataset consisted of 146 genera and 238 species utilizing sequences from 6 gene regions: rpb1, rpb-intron2, rpb2, 18S, 25S and 5.8S rRNA. Bayesian analysis revealed 6 clades labeled the Agaricoid, Tricholomatoid, Marasmioid, Pluteoid, Hygrophoroid and Plicaturopsidoid clades.

The Agaricoid clade (in Euagarics clade) comprised 14 families and tribes of primarily dark-spored agarics and gasteromycetes which clustered together with significant Bayesian support. The same group was also resolved in the Maximum Parsimony tree but with poor bootstrap support (Matheny *et al.* 2007 "2006"). Nidularicaceae and Cystodermateae formed a subclade in the Agaricoid clade, thus the main group comprised 12 families. It is the Agaricoid clade that contains the taxa of focus in this thesis research.

1.4 Recognition of species and advances in strategies for establishing phylogenetic hypotheses

1.4.1 Speciation and recognition of species

Speciation is a process in which genetically cohesive groups diverge into two or more genetically distinct groups of individuals. This progress is due to change and accumulation in genetic differences through the natural process of evolution, including mutation, selection, and genetic drift. When the differences become sufficient, they are recognized as different species (Petersen & Hughes 1999). Polyploidy or chromosomal rearrangements is the main way to form genetic differences and different species may arise from different populations in overlapping geographical ranges (Burnett 2003). Speciation is a process that occurs with time, although there is uncertainty concerning the exact point in this process when a population becomes a species (Petersen & Hughes 1999).

The species concept based on Darwinian evolution and speciation is known as the Evolutionary Species Concept (ESC), which defines a species as "...a single lineage of ancestor-descendent populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate" (Wiley 1978). However, this concept is difficult to operate in practice. Mayden (1997) proposed the Morphological Species Concept (MSC), Biological Species Concept (BSC), and Phylogenetic Species Concept (PSC) as secondary to the ESC, which can be used to diagnose or recognize species.

A species based on the Morphological Species Concept assesses overall similarities of morphological characters. MSC is in the most commonly used system for the identification of organisms. The recognition of species by MSC however, often comprise more than one species (Taylor 2000). In many cases, fruitbody phenotypic differences are subtle and there is disagreement as to whether two groups are different species or just local adaptations within a species. Evidence from fungi shows that genetic isolation precedes reproductive isolation and that morphological differentiation comes last. The rate of nucleotide substitution in fungi, a key factor in phylogenetics, has been shown to be similar to that in bacteria and macroscopic eukaryotes (Kasuga *et al.* 2002).

The Biological Species Concept comprises "...groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups." (Mayr 1940). The greatest limitation of BSC is that it is impossible to apply this concept to all fungi as many lack meiospores or often cannot be cultivated to carry out mating tests. Furthermore, there is evidence (Hibbett *et al.* 1995; Hibbett and Donoghue 1996) that the criterion of reproduction lumps

together groups of fungi that are genetically isolated in nature (e.g. in *Lentinula edodes*). It has been argued that the BSC overemphasizes potential gene flow between populations, whereas the PSC can recognize as distinct species those groups or populations that are genetically isolated from other groups and therefore, represent distinct evolutionary units (Harrison 1998).

The Phylogenetic Species Concept is "...the smallest diagnosable cluster of individual organisms within which there is a pattern of ancestry and descent." (Mayr 1940). PSC requires recognition of monophyletic groups of organisms that share at least one uniquely derived character, and this character is descended from a common ancestor (Taylor *et al.* 2000; Moncalvo 2005). In fungi, applying the PSC in research concerning asexual organisms and in the detection of relationships between anamorphic and teleomorphic stages of a single species has an obvious advantage (Moncalvo 2005).

Different attributes in a species have been regarded as being important, thus some species have been defined differently. The most pragmatic way to proceed is therefore to continue to work with a morphospecies as the basic taxonomic unit, but to include additional supplementary material as supplied by other studies as a guide to both phylogenetic trends and the interpretation of evolutionary potentialities. In operational terms this could most easily be achieved by the use of the MSC, and supplementary reference to the entities defined as BSC or PSC (Burnett 2003). There are many examples where new taxa are recognized based on morphological and molecular phylogenetic data (Blanco-Dios 2006; Bruns 1998; Buyck 2006; Callac 2005; Dai 2006; Desjardin *et al.* 2004b; Miller and Huhndorf 2004; Munnis 2006, Wang 2002).

1.4.2 Advances in strategies for establishing phylogenetic hypotheses

Statements such as "Phylogenetic methods come to age", "The use of molecular phylogenies to examine evolutionary questions has become commonplace with automation of DNA sequencing and availability of efficient computer programs to perform phylogenetic analysis", have evocated the use of sequencing in mushroom taxonomy (Huelsenbeck & Rannala 1997). The goal of phylogenetics is to construct phylogenetic topologies which form assumptions concerning the natural evolutionary relationships of clades. There is an evident need to obtain a robust and accurate phylogeny from the data, and this can be achieved by extending the number of nucleotides sequenced and by choosing the appropriate genes. The use of more nucleotides in phylogenetic analysis has become commonplace (Berbee *et al.* 2000). It also been recognized that single-gene phylogenies may not truly represent organismal phylogenies, but the concordant

phylogenetic genealogies from multiply gene sequences can solve this problem well (Doyle 1992; Maddison 1997; Taylor *et al.* 2000). Furthermore the use of different kinds of genes has been recommended, as the rDNA genes alone could not provide sufficient information to resolve a fungal phylogeny with a satisfactory level of confidence (Bruns *et al.* 1992; Sugiyama 1998; Tehler *et al.* 2000; Berbee *et al.* 2000; Binder and Hibbett 2002; Moncalvo *et al.* 2000, 2002). Multiple genes sequence data from both nuclear and mitochondrial rDNAs has been used in recent papers (Hibbett *et al.* 1997; Hibbett and Binder 2002; Binder and Hibbett 2002; Binder *et al.* 2005, Hughey *et al.* 2000, Wang 2004). The use of data from protein-coding genes for a broad inference of fungal systematics has been hindered due to the difficulty in designing robust primers for PCR amplification across a broad range of diverse taxa, and the need to clone heterozygous loci in dikaryotic organisms (Moncalvo 2005). However progress has been made, as proposed in AFTOL (http://ocid.nacse.org/research/aftol/). The protein-coding genes frequently used in Basidiomycota are as follows: RPB1, the gene that encodes the largest subunit of RNA polymerase II (Kropp & Matheny 2004; Matheny *et al.* 2002, Matheny 2005); RPB2, the gene that encodes the second largest subunit of RNA polymerase II (Liu *et al.* 1999; Liu and Hall 2004; Matheny 2005; Reeb *et al.* 2004; Wang *et al.* 2004; Zhang & Blackwell 2002); tef1, codes for translation elongation factor 1-□ (Baldauf and Palmer 1993; O'Donnell *et al.* 2001; Rehner and Buckley 2005, Matheny 2007), and ATP6, a mitochondrial gene (Kretzer and Bruns 1999, Robinson *et al.* 2001).

1.5 Research background and objectives

1.5.1 The need for mycological knowledge from tropical areas

The magnitude of fungal diversity (including chromistan fungi, lichen-forming fungi, slime moulds and yeasts) has been estimated at 1.5-3 million species, and only 2.5-5% of that figure have been described (Hawksworth 1991). Presently, 75-120,000 species are actually known to science (Kirk *et al.* 2001). Although the figure of 1.5 million was generally accepted this figure has been questioned as being too high or too low (Hawksworth 2001). This is because the estimation of global species numbers relied heavily on data from temperate UK and Europe, and much more basic data is needed from the tropics (Hyde 2001). An increased inventory of tropical

mycological taxa is a vital component of knowledge development (Hawksworth 2001, Subramanian 1982).

Increase in knowledge of the geographic range of a fungal species might offer more phylogenetic information than before. Global geographic distributions of some fungal species defined by morphology have been reported. However, when these species are defined by phylogeny, they have been shown to comprise several to many endemic species (e.g. *Schizophyllum commune* in James *et al.* 1999; James and Vilgalys 2001; *Lentinula* in Hibbett 2001). Thus taxa reported from locations distant from their original distribution and those taxa reported as having a worldwide distribution based only on morphology must be viewed with caution (Taylor 2006). Such discoveries also give mycologists a challenge to provide a more comprehensive recognition of morphospecies in tropical areas and give rise to the need for knowledge about their reproductive or genetic isolation.

1.5.2 Lack of taxonomic knowledge of *Cyathus*, *Agaricus* and *Micropsalliota* from Thailand

The Salween and Mekong rivers pass into northern Thailand, and the dividing ranges between these rivers include all of the highest peaks in Thailand (eg. Doi Intanon at 2565 m). The climate of North Thailand is strongly seasonal (Gardner *et al.* 2000). The seasonal climate of North Thailand coupled with the complex topography has resulted in rich biodiversity, including of fungal diversity.

The genus *Agaricus* L.: Fr. (= *Psalliota* Fr.; family Agaricaceae, order Agaricales) is characterized by fruiting bodies with free lamellae, an annulate stipe and production of a dark brown spore print. *Agaricus* species are widely distributed saprobes and some species are cultivated for food (e.g., *A. bisporus*). The most commonly referenced monographs of *Agaricus* species in tropical areas are those of Heinemann 1978, 1986a). *Micropsalliota* v. Höhnel emend Pegler (family Agaricaceae, order Agaricales) are known as the "small *Agaricus*" because they possess similar field characters to *Agaricus,* but have tiny fruiting bodies. The latter genus is only distributed in tropical areas (Heinemann 1978, 1989, 1991; Pegler 1966, 1969). Two hundred *Agaricus* and 40 *Micropsalliota* species are presently known (Kirk *et al.* 2001). Most reports concerning the systematics of *Agaricus* and *Micropsalliota* however, are from Europe and America. There is no report of *Micropsalliota* species from Thailand and the only previously

reported *Agaricus* species in Thailand are *A. campestris*, *A. bisporus*, *A. bitorquis*, *A trisulphuratus* and *A rufolanosus* (Høiland and Schumacher 1982; Ratchabundithayasatharn 1996; Ruksawong and Flegal 2001; Soytong 1994).

The genus *Cyathus* Haller along with the genera *Crucibulum* Tul., *Mycocalia* Palmer, *Nidula* V.S. White and *Nidularia* Fr. are known as the bird's nest fungi because of their small vase-shaped or nest-like fruiting bodies containing lentil-shaped or egg-like peridioles. *Cyathus* comprises 44 species (Kirk *et al.* 2001) and is the most speciose genus in the family Nidulariaceae (Nidulariales). *Cyathus* is distinguished from the other three genera in the Nidulariaceae based on grey to black peridioles with funicular cords and peridia composed of three layers of tissues (Brodie 1975). In Brodie's monographs (Brodie 1975, 1984) eight species were known from southeast Asia. Four *Cyathus* species have been reported previously from Thailand (Brodie 1975, Desjardin *et al.* 2004a, Dissing 1963; Ellingsen 1982, Soytong 1994).

1.5.3 The need for phylogenetic information from *Agaricus*, *Cyathus* and *Micropsalliota*

The genera *Agaricus* and *Micropsalliota* belong to the Agaricaceae, a family established by Chevall (1828). Traditionally this family consisted of 51 genera characterized by tricholomatoid, collybioid or pluteoid fruitbodies, and pigmented but never rusty-brown or cinnamon-brown spores (Kirk *et al.* 2001, Singer 1986). Early phylogenetic studies revealed that Agaricaceae along with some members of gilled Agaricales, Aphyllophorales and Gasteromycetes (bird's nest fungi, puffballs, false truffles and secotioid fungi) composed a large clade called the Euagarics Clade (Hibbett & Thorn 2001, Hibbett 1997).

Cyathus along with other four similar genera (*Crucibulum*, *Mycocalia*, *Nidula* and *Nidularia*) are placed in the family Nidulariaceae. Historically Nidulariaceae belonged to Nidulariales in the order "Gasteromycetes" (Ainsworth 1973). Phylogenetic data based on rDNA sequences showed the Nidulariaceae to be related to the gilled mushrooms, specifically to the Agaricaceae, even though the bird's nest fruiting body form was unique and unlike gilled fungi (Larsson *et al.* 2004; Moncolvo *et al.* 2002; Matheny *et al.* 2007 "2006"; Hibbett *et al.* 1997; and Hibbett and Thorn 2001).

As mentioned before, Matheny *et al.* (2007 "2006") had hypothesized a phylogenetic framework that contained six major clades in the order Agaricales. In the Agaricoid clade

Agaricaceae, Nidulariaceae and Cystodermateae unite as a subclade that is sister to the other 11 families with 99% posterior probability support (Fig. 1.1). In their, research the bird's nest fungi represented by *Crucibulum laeve* and *Cyathus striatus* were sister to the Agaricaceae and nested in the Agaricoid clade in the order of Agaricales.

In this study, we present phylogenetic hypotheses of the genera *Cyathus*, *Agaricus* and *Micropsalliota*, and include information from allied genera from tropical areas.

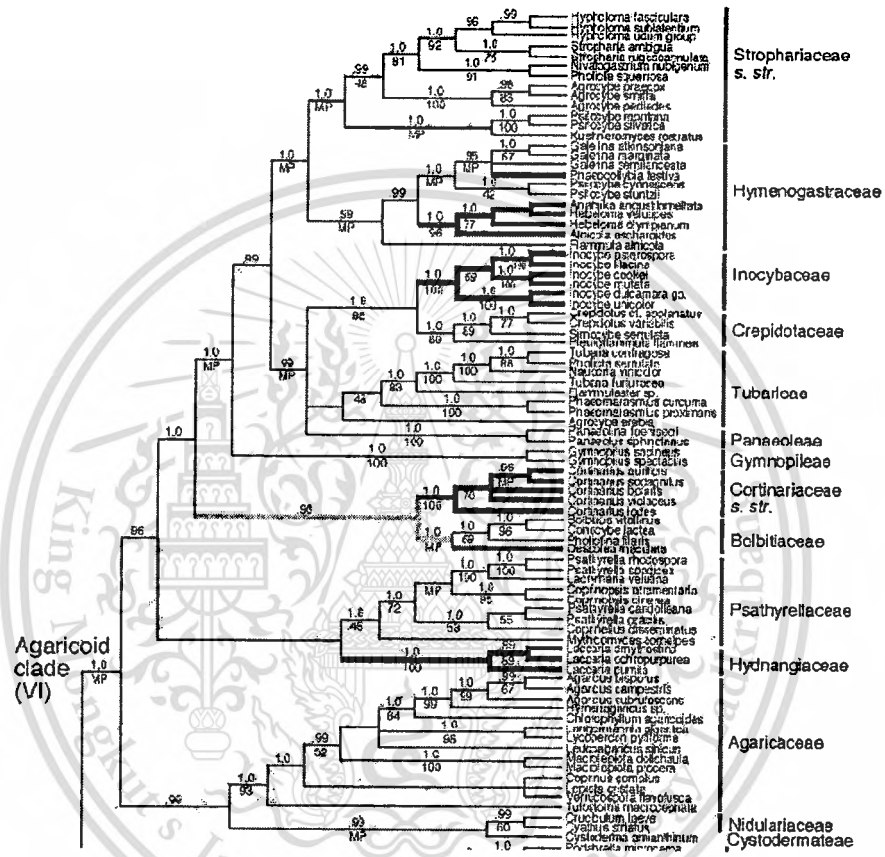

Fig. 1.1: Part of Bayesian cladogram of the Agaricales produced from combined rpb1, rpb1-intron2, 18S, 25S and 5.8S nucleotide sequences showing the relationships within the Agaricoid clade. (Matheny *et al.* 2007 "2006")

1.5.4 Research objectives

This research provides data on the genera *Agaricus* and *Micropsalliota* in northern Thailand, and *Cyathus* from Thailand based on the comparison of other *Cyathus* species from the world. Both morphological and molecular data (DNA sequences) will contribute to a better understanding of species delimitations and their phylogenetic relationships. Furthermore the

morphological and phylogenetic information on these three genera not only contributes to increasing knowledge, but also contributes to phylogenetic knowledge of the subclade (mainly consisting of Agaricaceae and Nidulariaceae) in the Agaricoid clade (Matheny 2007 "2006") along with allied genera. The objectives of this study are:

1) To produce a worldwide monograph of *Cyathus* by investigating the genus *Cyathus* through morphological study of world specimens and northern Thailand specimens.
2) To establish the phylogeny of *Cyathus* based on analyses of ITS and LSU rDNA sequences from type and authentic specimens, and indicate which morphological characters are important in species recognition.
3) To investigate the genus *Agaricus* in northern Thailand, and produce a monograph of *Agaricus* species in northern Thailand.
4) To sample all 3 subgenera of *Agaricus* to produce a comprehensive phylogenetic reconstruction in this genus.
5) To investigate the genus *Micropsalliota* in northern Thailand, and produce a monograph of *Micropsalliota* in northern Thailand.
6) To develop a phylogenetic reconstruction of the genus *Micropsalliota*.
7) To build a phylogenetic hypothesis for *Cyathus, Agaricus* and *Micropsalliota* plus allied genera to obtain a better understanding of the subclade (Agaricaceae + Nidulariceceae + Cystodermateae) in the Agaricoid clade.

Chapter 2

LITERATURE REVIEW

2.1 The genus *Agaricus*

The genus *Agaricus* L.: Fr. (= *Psalliota* Fr.) is a famous edible mushroom group, and it is the type genus of the family Agaricaceae Cohn ss. Singer, which is the type family of the order Agaricales Clements. This genus can be recognized in the wild by dark-colored spores (brown to black), free gill attachment and the presence of a partial veil during fruitbody development that manifests itself as an annulus or ring around the stem. They are saprotrophic fungi and gregarious in woods, forests, gardens, on roadside, fields, pasture-land, grass-land, rubbish dumps, manure heaps, alluvial soils, and occur from sea level up to the vegetation limit in mountainous areas. They are common in tropical areas and almost cosmopolitan in distribution. In the temperate zone of the Northern Hemisphere, only one other mushroom genus -- *Melanophyllum* -- may show similar features, however the species are rare and have other unusual characteristics such as red gills, greenish spores, and distinctive microscopic features. In the tropics, the genus *Micropsalliota* has macromorphological characters similar to *Agaricus* (except for their smaller size), but its elongate-capitate cheilocystidia and often incrusted hyphae of the pileipellis are characters distinguishing *Micropsalliota* from *Agaricus*.

2.1.1 *Agaricus* species have high edibility and medicinal values

Agaricus comprises more than 200 species (Kirk 2001). A few species are poisonous, and some edible species cause digestive upsets in certain people. Many other species are choice edibles and as far as we know no species have caused fatalities. In *Agaricus* the most important edible species is probably the button mushroom, *A. bisporus* (J.E. Lange) Pilát, which is the most widely cultivated species of edible mushroom comprising about 32% of world production in 1997 (Chang 1999). The other commercial species are *A. subrufescens* Peck (the almond mushroom), which was cultivated in the northeastern USA from about 1890 to 1910 and the reason it faded from commercial production is beacuse a small number of people cannot digest it easily (Kerrigan 2005). Another commercial species is *A. bitorquis* (Quélet) Saccardo (= *A. edulis* & *A. rodmani*), which is frequently found in hard-packed soils, and prefers warmer conditions. It was first

regions with hotter climates, including India (Kerrigan 1986; Cappelli 1984; Möller 1950; Heinemann 1978). *Agaricus subperonatus* (J.E. Lange) Singer was cultivated experimentally at Hazel Dell Mushrooms around 1982, and was cultivated semi-commercially in several laboratories. It is regarded as a high-quality mushroom (Noble *et al.* 1995). *Agaricus brasiliensis* (= *A. blasei* Murill) was named as the sun mushroom, god's mushroom, Brazil's mushroom, royal sun *Agaricus* or Himematsutate, and originated from the village of Piedade in the state of São Paulo, in Brazil. It has been gaining worldwide attention because it contains the highest level of □-(1-6) D glucan of any mushroom known in the world, a polysaccharide, known to enhance the body's immune system (Ikekawa 2005). Recently, Kerrigan (2005) provided sufficient molecular evidence that *A. brasiliensis* is a synonynm of the older epithet *A. subrufescens*. Furthermore numerous *Agaricus* species have been known as edible wild mushrooms, such as *A. campestris* L.: Fr. (the meadow mushroom), *A. augustus* Fr. (the prince) and *A. arvensis* Schaef. (the horse mushroom).

However most of the cultivated species require low temperatures and are difficult to grow in tropical areas. The discovery of local *Agaricus* species with potential commercial value would be helpful in local economy.

2.1.2 Morphological research in *Agaricus*

Although the genus *Agaricus* is familiar as important cultivated mushrooms, the circumscription and delimitation of species in the genus is complex (Cappelli 1984).

Linnaeus accepted all gilled mushroom in the genus *Agaricus* and were assigned to the family of Agaricaceae in 1753. In "Systema Mycologicum" by Fries in 1821, Linnaeus' large *Agaricus* was separated to two new genera *Cantharellus* and *Schizophyllum* and the remaining species were subdivided into tribes. *Psalliota* first appeared with reference to the tribes with annulate fruitbodies and purple spores, and included *Agaricus* as we now know it, plus *Stropharia* species. In Fries' (1838) next work "Epicrisis", he subdivided the tribe *Psalliota* into two sections: *Lepiotideae* with completely free gills (the present *Agaricus*) and *Pholiotidei* with more or less adnate gills (the present *Stropharia*). Later in "Monographia", Fries (1857) recognized the two previous sections as the separated subgenera, but did not recognize them at generic rank. Kummer (1871) elevated *Psalliota* to generic level, but still in the broad sense including *Stropharia*. It was in 1872 that Quélet, in "Champignons du Lura et des Vosges", raised all Friesian subgenera to

generic rank and *Psalliota* made its appearance as a distinct genus, replacing *Agaricus* in Quélet's sense. In 1879 Karsten decided to reinstate the epithet *Agaricus* as a substitute for *Psalloita.* for the current delimitation of the genus *Agaricus* was derived from the Friesian subgenus *Psalliota* (Cappelli 1984).

Two-hundred *Agaricus* species have been reported (Kirk, 2001), while there are 6,000 records with the name of "Agaricus" (http://www.indexfungorum.org). However most reports about *Agaricus* come from America, Australia, Chili, Europe, India and New Zealand (Albertó 1994, 1998; Bohus 1975, 1990, and 1995; Callae 1993; Crgurinovic 1997; Esteve-Raventós 1998; Flower *et al.* 1997; Freeman 1979a,b; Geml *et al.* 2007; Hotson 1938; Huijsman 1960; Kerrican 1985, 1989; Lacheva and Stoichev 2004; Lanconelli 2002; Nauta 2000; Mitchell and Walter 1999; Murrill 1912,1918 and 1941; Natarajan 2005; Naauta 1999; Ortox 1960; Parra 2003; Pegler 1983, 1990; Peterson *et al.* 2000; Saini 1997; Smith 1944; Valenzuela *et al.* 1997).

The taxonomic system for *Agaricus* is also complex. There are several systems proposed by different mycologists based on their subjective judgments concerning the significance of morphological characters in the recognition of species and sections in this genus (Cappelli 1984; Heinemann 1978; Kerrigan 1986; Konrad et Maublanc 1952; KÜhner et Romagnesi 1953; Möller 1950; Pilat 1951; Moser 1967-1983; Singer 1986; Wasser 1980). In all systems three main characters are used to distinguish between taxa within *Agaricus*: 1) the Schäffer's cross-reaction (chemical test with aniline and concentrated HNO_3); 2) the alkali test (application of strong alkali, either NaOH or KOH); and 3) basidiome color change when bruised or broken (Heinemann 1987).

The most commonly referenced monographs of *Agaricus* species in tropical areas are those of Heinemann (Heinemann 1952-1993, especially in 1978 and 1986). Knowledge of *Agaricus* species is still very limited in Southeast Asia regions. For example in Thailand only *A. campestris*, *A. bisporus*, *A. bitorquis*, *A. trisulphuratus* and *A rufolanosus* have been reported (Høiland and Schumacher 1982; Ruksawong 2001).

2.1.3 The molecular phylogenetic research in *Agaricus*

"Phylon" means stem and "genesis" means origin. In molecular phylogeny the relationships among organisms or genes are studied by comparing homologues of DNA or protein sequences. Dissimilarities among the sequences indicate genetic divergence as a result of molecular evolution during the course of time. Molecular phylogenetic methods are based on assumptions

about the processes of molecular change in DNA or protein sequences. The evolution of a sequence into several lineages can be simulated and the resulting dataset can be subjected to phylogenetic analyses. Under these conditions, the true phylogenetic tree is known and can be used to test the accuracy, consistency and robustness of phylogenetic methods and evolutionary models (Nei 1979). Therefore phylogenetic analyses of DNA sequences have allowed us to begin testing morphological assumptions and resolving the morphological problems.

There are a few published papers that include *Agaricus* molecular phylogenies. Mitchell and Bresinsky (1999) conducted the first phylogenetic research on this genus. The ITS region plus a portion of the 28S gene was used to study the phylogenetic relationships of 16 *Agaricus* species selected from a range of sections contained in the subgenus *Agaricus*. In *Agaricus,* 2 sections are well supported by molecular phylogenetic data: 1) section Duploannulatae comprises the group of species allied with *A. bisporus* and *A. bitorquis*, and DNA sequence date from ITS segments of the nuclear ribosomal DNA region were used from samples of European and North America isolates (Challen *et al.* . 2003); and 2) section Xanthodermatei contains many yellow-staining, phenol-odored taxa (Kerrigan *et al.* 2006 '2005'). With the development of phylogenetic analyses some species/varieties have been reported as new to science based on morphological and molecular data (Callac et Guinberteau 2005, Callac *et al.* 2003, Kerrigan *et al.* 1999, Xu *et al.* 1998, and Callac 1993). All published reports concerning *Agaricus* phylogeny are based on material collected from European and the Americas. Data from Southeast Asia are lacking.

2.2 The genus *Cyathus*

The genus *Cyathus,* along with the genera *Crucibulum, Mycocalia, Nidula* and *Nidularia,* are known as the bird's nest fungi because of their small vase-shaped or nest-like fruiting bodies containing lentil-shaped or egg-like peridioles. Because some *Cyathus* species such as *C. helenae, C. butteri* and *C. intermedium* have been shown to produce antibiotic compounds (Albutt 1971, Aver 1976a-c, Fu *et al.* 2003, Yang 2002), the genus attracted the attention of many mycologists.

All five genera of bird's nest fungi belong to the Nidulariaceae Dumort, and are historically placed in the order "Nidulariales" (Ainsworth *et al.* . 1973, Miller and Miller 1988). However, molecular phylogenetic research has shown that Nidulariaceae is related to the Agaricales (gilled mushrooms; Moncalvo *et al.* 2002, Minder and Hibbett 2002, Hibbett *et al.* 1997, Hibbett and Thorn 2001, Matheny *et al.* 2007 "2006").

Cyathus and *Crucibulum* are distinguished from the other "bird's nest" genera in the Nidulariaceae based on the character that the peridioles are attached to the inner peridial surface by a thread-like funiculus. *Cyathus* differs from *Crucibulum* in that the peridium consists of more than one layer, and the peridioles are gray to black (Brodie 1975, Pegler 1995).

Cyathus is the most speciose genus in the family Nidulariaceae and comprises 44 species (Kirk *et al.* 2001). There are 171 epithets in *Cyathus* at Index Fungorum (http://speciesfungorum.org/Names/Names.asp). The first monograph on *Cyathus* was published by Lloyd (1906), and later Brodie did remarkable research on this group (Brodie 1952-1984). Of special significance are his his two monographs (Brodie 1975, 1984) on Bird's Nest Fungi wherein he included four genera (*Crucibulum* Tul. & C. Tul., *Cyathus* Haller, *Nidula* V.S. White and *Nidularia* Fr.) and 49 species of *Cyathus*. Recognition of *Cyathus* species was based on morphological characters, such as the shape of the fruiting body, peridial coverings and plications, anatomy of peridioles, and the size and shape of basidiospores (Brodie 1975). Although Brodie's monographs have been followed by most mycologists, some *Cyathus* species (such as *C. ambiguus*, *C. olivaceo-brunneus*, *C. cheliensis*, and *C. confusus*) in his monographs are questionable because he did not examine all type specimens. In addition, since the last monograph of Brodie was published in 1984, 22 additional species of *Cyathus* and five varieties have been published (Chen *et al.* 2003; Liu and Li 1989; Ren and Zhou 1992; Zang 1980; Yang *et al.* 2002; Zhou *et al.* 2004; Zhou and Ren 2004; Shinners *et al.* 1998; Liu and Cao 1986; Gómez and Pérez-Silva1988), many of which await critical comparisons with extant type specimens.

The taxonomic system for *Cyathus* was first subdivided into two infrageneric groups (Tulasne 1844), and later was subdivided into five infrageneric groups (Lloyd 1906). H.J. Brodie subdivided *Cyathus* into 7 groups (Brodie 1975, 1984), and his system was adopted by most mycologists for a long period. All taxonomic systems were based on morphological and anatomic of characters, and distinct plications on the peridium of the fruiting body were considered to be a major diagnostic character for separating groups and species (Brodie 1975). Species with plications were placed into the "striatus" and "poeppigii" groups, while species lacking plications were placed in the "olla", "pallidus", "triplex", "gracilis" and "stercoreus" groups (Brodie 1975, 1984). In recent molecular research on this genus, RAPD analysis provided stable hypothesis about the taxonomic system (Zhao *et al.* 2004). Phylogenetic research using rDNA sequences however, did not support this system, and a new infrageneric system with

recognition of 3 groups (ollum, pallidum and striatum groups) has been proposed based on molecular and morphological data (Zhao *et al.* 2006 and Chapter 3).

In this research as many type and authentic specimens as possible, especially the holotypes of questionable and recently described species, were borrowed from herbaria along with samples collected from Thailand, and were examined using Brodie's protocol and current molecular techniques.

2.3 The genus *Micropsalliota*

The genus *Micropsalliota* Höhn. (Agaricaceae/Agaricales) was established in 1914. The name "*Micropsalliota*" indicates this genus is similar to *Agaricus* (from term of "psalliota") and produces small carpophores. *Micropsalliota pseudovolvulata* Höhn is the type species and was described from Java by Höhnel (1914). Subsequently Donk (1962) included *Agaricus plumarium* Berk. & Br. from Java, and *A. arginea* Berk. & Br. and *A. microcosmus* Berk. & Br. from Ceylon in the *Micropsalliota*, but new combinations were not formally proposed.

Singer (1947), however, based on his observation that the spores of the type specimen of *M. pseudovolvulata* are pseudoamyloid (= dextrinoid), reduced *Micropsalliota* to a synonym of *Lepiota* (Pers.:Fr.) S.F. Gray which has mostly white spore but also has a wide range of spore colors.

Pegler and Rayner (1969) emended the genus *Micropsalliota* and accepted it with the distinguishing characters of elongate-capitate cheilocystidia, the absence of clamp-connections and dextrinoid spores. They thought the position of the genus *Micropsalliota* was intermediate between the tribe *Agaricus* Pat. and the tribe *Lepiota* Fayod. *Micropsalliota* was considered close to *Agaricus* based on the presence of an annulus on the stipe, dark pigmented lamellae and hyphae lacking clamp-connections, but differed from *Agaricus* in having much smaller fruitbodies, distinctive capitate cheilocystidia and dextrinoid spores (Pegler and Rayner 1969). A key with 4 *Micropsalliota* species (*M. arginea* = *A. microcosmus, M. brunneosperma, M. plumaria* and *M. pseudovolvulata*) was presented. Pegler and Rayner's emendation (1969) of *Micropsalliota* based on anatomical and chemical characters was accepted by Singer (Singer 1986).

Heinemann was another mycologist who did considerable work on this genus *Micropsalliota* (Heinemann 1976, 1978'1977', 1980, 1983, 1988, 1989 and 1990; Heinemann and Flower 1983;

Heinemann and Leelavathy 1991). In 1976 he investigated the diagnostic characters of *Micropsallitoa* and compared those characters with *Agaricus* and *Lepiota* species. He mounted spores from *Micropsalliota*, *Agaricus* and *Lepiota* species in water, ammonia, Melzer's reagent and chloral hydrate to detect the spore color (chemical characters). He found that the spores of both *Micropsalliota* and *Agaricus* species were dextrinoid. Heinemann thought the basis for separation from *Agaricus* was anatomical (cheilocystidia shape, thickness of spore wall, encrusting pigments on pileipellis hyphae), and by no means the dextrinoid reaction of the spores (Heinemann 1976).

The genus *Micropsalliota* consists of 40 species in Dictionary of Fungi (Kirk *et al.* 2001), and 56 epithets are recorded in Index Fungorum (http://www.indexfungirum.org). Forty-four taxa of this genus were described by Heinemann from India (Heinemann 1983, Heinemann and Flower 1983; Heinemann and Leelavathy 1991), Malaysia and Indonesia (Heinemann 1980), Africa (Heinemann 1988) and the American tropics, such as Argentina, Brazil and Mexico (Heinemann 1989). Another 12 *Micropsalliota* taxa were described from Java (Höhnel 1914), Ceylon (Singer 1977), Sri Lanka (Pegler 1986), India (Natarajan and Manjula 1982), Mexico (Guzmán-Dávalos 1992 and 1994) and East Africa (Pegler 1977). To date there are no published data on *Micropsalliota* species from Thailand. Molecular phylogenetics data on this genus are also lacking.

Chapter 3

RESEARCH AND METHODOLOGY

3.1 Morphological research

3.1.1 Sample collection

GPS coordinate of each collection site was recorded. Each collection once collected was wrapped by foil or kept in box separately in order to avoid the mixture and crush. In each collection the young and mature carpophores are collected if them appearance; and the all part of fruiting body are collected including the base of stipe, fell annulus. For the *Agaricus* species the odor and color change on bruising were detected when they were collected.

Samples were collected during the raining season in those 19 sites of 3 provinces in Northern Thailand. The collected sites are as following:

1) Chiang Mai Province, Chiang Dao Cave.

2) Chiang Mai Province, Mae Taeng District, Mok Fa Waterfall.

3) Chiang Mai Province, Mae Taeng District, Ban Pha Deng village, Mushroom Research Centre, N 19°17.12', E 98°44. 01', elevation 900 meter.

4) Chiang Mai Province, Mae Taeng District, New Waterfall.

5) Chiang Mai Province, Chom Thong, Ob Luang National Park, elevation 1812 meter.

6) Chiang Mai Province, Mae Taeng District, Ban Pha Deng village, Pathummikaram Temple, forest trail, N 19°06.29', E 98°44.47', elevation 1050 meter.

7) Chiang Mai Province, Mae Teng Dist., Tung Joaw village, forest trail, N19°08.07' E98°38.90', elevation 1300 meter.

8) Chiang Rai Province, Ban Chom Bom, Um Poo, Vieng Chai, N19°53.25', E100°07.35", elevation 408 meter.

9) Chiang Rai Province, Mae Sai, Doi Tung.

10) Chiang Rai Province, Mae Souy District, Pamae Lao National Park.

11) Mae Hong Son Province, on Highway 1095 near 198km marker, opposite with Suan Im Chay 100 m, elevation 288 meter.

12) Chiang Mai Province, Doi Inthanon National Park, Highway 1009 at 25 km marker, N18°32.54' E98°33.51', elevation 1076 meter.

13) Chiang Mai Province, Doi Inthanon National Park, junction of Highway 1009 and road to Mae Chem, N19°31.58' E 98°29.64', elevation 1700 meter.

14) Chiang Mai Province, Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma village, N18°48.62' E98°54.60', elevation 1145 meter.

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

16) Chiang Mai Province, Mae Teng District, Huai Nam Dang National Park, nature trail, N19°18.29' E98°35.88', elevation 1530 meter.

17) Chiang Mai Province, Mae Mai Lai, Huan Rai Temple, N19°06.170' E98°53.363', elevation 515 m.

18) Chiang Mai Province, Mae Teng District, Highway 1095 at 22 km marker, N19°07.57' E98°45.65', elevation 750 meter.

19) Chiang Mai Province, Mae Taeng, Ban Mae Sae village, on Highway 1095 near 50 km marker, N19°14. 60' E98°39.46', elevation 962 meter.

On the other hand the specimens of those genera included holotypes or isotypes were borrowed from BPI, BR, DAOM, HMAS, MICH and SWFC (Holmgren and Holmgren 1998).

3.1.2 Morphological character examination

The macrocharacters, chemical test and photograph of fresh sample were carried out as soon as possible after came back from the field trip and follow the instruction described by Largent (1986) . The colour terms follow Kornerup and Wanscher (1978). If could not finish all samples in short time the specimens were stored in the 4° C freezer waiting for examination later. Then the examined specimens were put into the drier at least overnight to dehydration, sealed in the plastic bag, and kept in the herbarium. Duplicates of the specimen were deposited in the BIOTEC Bangkok Herbarium (BBH), Bangkok, Thailand, the H.D. Thiers Herbarium (SFSU) at San Francisco State University, San Francisco, California, USA, and the herbarium of the Mushroom Research Centre (MRC), Chiang Mai, Thailand.

The microcharacters were examined from the dry specimen (Largent 1986). If the slide of a basidiomate is made, the following regions or areas were determined: the surface of pileus which is the outermost layer of hyphae of the pileus to detect the pileipillis features; the surface of stipe to detect the stipitipellis features; the hymenophoral trame; the hymenium, a lay of consisting of spores, basidia and cystidia; and the annulus. Before make a slide, take a small piece of issue from each region as the above, then use pure ethanol to refresh them. After made the cross slide,

the materials was mounted into 3% KOH or distilled water to examine, while in the determination of pigment type of pileipillis the materials was mounted in water then 3% KOH in order.

Measurements of anatomical features (Spores, basidia, cheilocystidia, and pluerocystidia) were presented based on at least 20 measurments of each item and include x, the mean of length by width ± SD, Q, the quotient of length and spore width, and Q_m, the mean of Q-values ± SD.

3.2 Molecular phylogenetic research

3.2.1 Fungal samples

The samples included into the molecular research see Table 3.1.

3.2.2 DNA extraction, PCR and sequencing

Generally following the described by White *et al.* (1990), some modification had been made. Two different methods of DNA extraction were used. The first one used fungal mycelia harvested from cultures that were grown on potato dextrose agar (PDA) for 2 weeks, following the procedures: scrape off fresh mycelia (50mg) from the surface of the culture plate and transferred into 1.5 ml centrifuge tubes with pre-heated (60 C) 2X Cetyl Trimethyl Ammonoum Bromide (CTAB) extraction buffer and 0.2g sterilized quartz sand; grind materials using a glass pestle; then add 300 µl of pre-heated (60 C) 2X CTAB extraction buffer; incubate in 60 C water bath for 30 min. with occasionally gentle swirling; add 600 µl of phenol:choloform (1:1) and mix well by inversion to form an emulsion; centrifuge at 13,000 rpm for 15-30 mins. Then remove the aqueous phase into a fresh 1.5 ml tube; reextract DNA by adding phenol:Chloroform (1:1) and mix by inversion; centrifuge at 13,000 rpm for 15-30 mins, then transfer the upper phase to a clean tube; add 1 ml cold absolute ethanol and invert gently and keep in –20 C freezer overnight; centrifuge at 11,000 rpm for 15-30 mins, then 4 C to precipitate DNA; wash DNA pellet by 70% ethanol twice; after dry add 100 µl TE buffer (with Rnase 10 µl/ml) to resuspend DNA. The second extraction method involved the use of a commercial DNA extraction kit (E. Z. N. A. Forensic Kit, D3591-01, Omega Bio-Tek) for dried fungal specimens.

Most samples were sequenced in the University of Hong Kong. The PCR reactions were performed in a 50 µl volume [0.3 mM primers (LROR: 5'-ACCCGCTGAACTTAAGC-3' and LR5: 5'-TCCTGAGGGAAACTTCG-3';or ITS4: 5'-TCCTCCGCTTATTGATATGC-3' and

ITS5: 5'-GGAAGTAAAAGTCGTAACAAGG-3'), 10-20 ng DNA template, 1× buffer, 0.2 mM dNTPs, 1.5 units Taq and sterile water]. The thermal cycles consisted of 94 C for 3 min, 30-35 cycles of 94 C for 1 min, 52 C for 50 sec and 72 C for 1 min, with a final extension step of 72 C for 10 min. PCR products were checked by electrophoresis gels (1% agarose) stained with ethidium bromide in 1 × Tris-boric acid EDTA buffer. They were purified using minicolumns according to the manufacturer's protocol (GFX PCR DNA and Gel Band Purification Kit, 27-9602-01, Amersham Biosciences). Primers ITS4, ITS5, LROR and LR5 were used to sequence both strands of the DNA molecule in an automated sequencer at the Genome Research Centre, the University of Hong Kong.

Some more samples or the samples failed in sequencing from the University of Hong Kong were conducted in San Francisco State University. The PCR reactions were performed in a 13 µl volume [0.3 mM primers (LROR and LR5; or ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA--3' and ITS4B: 5'-CAGGAGACTTGTACACGGTCCAG -3'), 10-20 ng DNA template, 1× buffer, 0.2 mM dNTPs, 1.5 units Taq and sterile water]. The thermal cycles consisted of 95 C for 5 min, 30-35 cycles of 94 C for 30 sec, 52 C for 30 sec and 72 C for 90 sec, with a final extension step of 72 C for 5 min. PCR products were checked by electrophoresis gels (1% agarose) stained with ethidium bromide in 1 × Tris-boric acid EDTA buffer. The successful PCR amplified products were cleaned up: transfer 5 µl of each PCR product from PCR tube to strip tube, add 0.5-1 µl of the ExoSAP-it Mix into and mix well, incubate 37 C 30 min to perform the digestion and 80 C 10 min to inactive the enzymes. Once the ExoSAP-it reaction is finished, set up a cycle-sequencing reaction (1-2 µl cleaned template, 1 µl 5 µm primer, 5 × buffer, 0.5 µl BigDye and 7.3 µl sterile water) as following: 94 C for 1 min 30 sec, 30-35 cycles of 94 C for 20 sec, 55 C for 30 sec and 60 C for 4 sec. After the thermal cycle finished, add 5 µl of 125 mM EDTA to each tube, then add 50 µl of 0.11 M Sodium acetate in Ethanol, cortex well, spin 45 min at 4000 rpm, dump the liquid, then spin 1 min at 700 rpm, add 50 µl of 0.11 M Sodium acetate in Ethanol again and vortex, spin 5 min at 4000 rpm, dump the liquid, then spin 1 min at 700 rpm again, dry in vacuum concentrator for 5 mins, resuspend in 15 µl Hi-Di, then denature 2 mins at 95 C. Then the ready samples were set in ABI PRISM 3100 Genetic Analyzer to sequence. Primers ITS1F, ITS4B, LROR and LR5 were used to sequence both strands of the DNA molecule in the lab.

3.2.3 Sequence alignment and phylogenetic analysis.

3.2.3 Sequence alignment and phylogenetic analysis.

All sequences were separated as different databases for analysis based on the previous research result (Matheny 2006 and 2007) and the nature that ITS gene is much more variable than LSU genes. In order to having the proper alignment and use as many as possible sequences, they are *Cyathus* ITS, LSU and ITS + LSU datasets; *Agaricus* + *Micropsalliota* LSU datasets; *Agaricus* ITS, LSU and ITS + LSU datasets; and *Micropsalliota* ITS, LSU and ITS + LSU datasets.

The sequences in each dataset initially aligned using Clustal X with default settings (Thomson *et al.* 1997). Then they were manually adjusted in BioEdit and gaps were introduced to improve alignments. The alignments will be submitted to TreeBase.

Phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2004). Heuristic searches of the ITS, LSU and combined (ITS + LSU) datasets were performed under 4 optimality criteria: weighed parsimony (WP) for small dataset or maximum parsimony (MP) for big dataset, maximum likelihood (ML), neighbor joining (NJ) and MrBayes (Huelsenbeck and Ronquist 2001, Huelsenbeck *et al.* 2001). Unordered characters, random taxon addition sequences, gaps treated as missing data, and the tree bisection-reconnection (TBR) branch swapping were used in the analyses. For weighted maximum parsimony, maxtrees was limited to 5000 trees with 1000 replications. The weighted parameters were produced using Stmatrix (François Lutzoni & Stefan Zoller, Duke University) as described in Miadlikowska *et al.* (2002). The best nucleotide substitution models for maximum likelihood were chosen by using MrModeltest2.2 (Nylander 2004). For neighbor joining, all characters were weighted equally. Bootstrap values (BS) were obtained from 1000 replicates. Unconstrained trees (WP/MP, ML and NJ trees) were compared in PAUP* using Kishino-Hasegawa and Shimodaira-Hasegawa tests (Kishino and Hasegawa 1989). Bayesian posterior probability (PP) was calculated using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). One million generations were run for four Markov chains and sampled every 100th generation resulting in 10,000 trees. The first 2000 trees were discarded as part of the burn-in phase, and the remaining 8000 trees were used to calculate posterior probabilities in a 50% majority rule consensus tree. Trees were viewed in TreeView and exported to graphics programs (Page 1996).

Table 3.1: Taxa information and GenBank accession numbers in molecular work

Taxa	Resource & Herbarium accession number	Origin	DNA extract Material	GenBank accession numbers ITS	LSU
Agaricus abruptibulbu	GenBank		-	AY484673	AF059228
A. albolutescen	GenBank		-	AY484675	-
A. angusticystidiata sp nov	2043		DS	R	R
	2085		DS	R	R
A. arvensis	GenBank		-	AY484690	-
A. augustus	GenBank		-	AY484672	AF291286
A. bambusae	2036		DS	R	-
A. bernardii	GenBank		-	AF432880	-
A. bernardiifor	GenBank		-	AJ884632	-
A. bisporus	GenBank		-	DQ404388	-
A. bitorquis	GenBank		-	AF432898	AY176342
A. boisseletii	GenBank		-	DQ182531	-
A. bresadolianu	GenBank		-	DQ185572	-
A. aff. *brunneolus*	3007		DS	-	R
A. californicus	GenBank		-	AY484679	AF482876
A. campestris	GenBank		-	AF432877	AF059221
A. comtulus	GenBank		-	AJ887992	-
A. cupreobrunne	GenBank		-	DQ182532	-
A. cupressicola	GenBank		-	AF432903	-
A. devoniensis	GenBank		-	AF432896	-
A. diminutives	GenBank		-	AF482831	AF482877
A. dulcidulus	3101		DS	R	R
A. duplocingulatus	3031		DS	R	-
	3041		DS	R	R
	3064		DS	R	R

Table 3.1 (continued)

A. endoxanthus	GenBank	-	DQ182511	-
	3094	DS	R	R
	3095	DS	R	R
A. essettei	GenBank	-	-	AY207136
A. excellens	GenBank	-	AY484682	-
A. fiardii	2134	DS	R	R
A. fissuratus	GenBank	-	AY484683	-
A. freirei	GenBank	-	DQ182512	-
A. fuscofibrill	GenBank	-	AY484684	-
A. fuscovelatus	GenBank	-	AY484677	-
A. gennadii	GenBank	-	AJ884633	-
A. hondensis	GenBank	-	DQ182515	-
A. impudicus	3020	DS	R	-
A. iodosmus	GenBank	-	DQ182516	-
A. johnstonii	3005	DS	R	R
	4017	DS	R	R
	4036	DS	-	R
A. laskibarii	GenBank	-	AY943975	-
A. lilaceps	GenBank	-	AY484676	-
A. macrocarpus	GenBank	-	AY484686	-
	GenBank	-	AY484687	-
A. maeseaensis	2048	DS	-	R
A. martineziens	GenBank	-	-	AY668957
A. maskae	GenBank	-	AJ884642	-
A. menieri	GenBank	-	DQ182520	-
A. moelleri	GenBank	-	AY899264	-
A. nivescens	GenBank	-	AY484670	-
A. ochrascens	2053	DS	R	R
	2127	DS	R	R
	3028	DS	R	-

Table 3.1 (continued)

A. parvitigrinu	GenBank	-	AY899266	-
A. pattersonae	GenBank	-	AY943974	-
A. pequinii	GenBank	-	AJ884639	-
A. phaeolepidot	GenBank	-	DQ185551	-
A. placomyces	GenBank	-	DQ182525	-
A. porphyrizon	2044	DS	R	R
	3056	DS	R	R
A. pseudopraten	GenBank	-	DQ182526	-
A. rollanii	GenBank	-	AJ884631	-
A. romagnesii	GenBank	-	AJ884640	-
A. rotalis	GenBank	-	DQ182528	-
A. semotus	GenBank	-	-	AF207136
A. silicola	GenBank	-	-	AF059223
A. spissicaulis	GenBank	-	-	AF059220
A. subfloccosus	GenBank	-	AF432887	-
A. subperonatus	GenBank	-	AF432889	-
A. subrufescens	GenBank	-	AY818650	-
	GenBank	-	AY818651	-
A. subrutilesce	GenBank	-	AY943973	-
A. sylvaticus	GenBank	-	-	AY207137
A. tollocanensis	GenBank	-	AY703912	-
A. trisulphuratus	2123	DS	R	R
	2128	DS	-	R
	3014	DS	R	R
	3072	DS	R	-
	4034	DS	-	R
A. vaporarius	GenBank	-	AF432900	-
	GenBank	-	AF432902	-
A. xanthoderma	GenBank	-	-	AF059222
A. xanthodermul	GenBank	-	AY899273	-

Table 3.1 (continued)

A. xanthodermus	GenBank		-	DQ182534	-
A. xantholepis	2110		DS	R	R
	3039		DS	R	R
	3088		DS	R	R
A. sp 1	2124		DS	R	R
	2125		DS	R	-
A. sp 2	3102		DS	R	R
A. sp 3	3080		DS	R	R
A. sp 4	3077		DS	R	-
A. sp 5	3093		DS	R	R
A. sp 6	2109		DS	R	R
	2136		DS	R	-
A. sp 7	3012		DS	R	R
	2137		DS	-	R
A. sp 8	3044		DS	R	R
A. sp 9	3032		DS	-	R
	3086		DS	R	R
A. sp 10	3034		DS	R	-
	3099		DS	R	R
A. sp 11	2132		DS	R	R
A. sp 12	3091		DS	R	R
Chlorophyllum molybdites	GenBank		-	AY081243	U85303
Crucibulum laeve	SWFC 21261	China	DS	DQ463357	–
	GenBank		-		AF336246
Cystoderma amianthinum	GenBank		-	AY207195	DQ192177
*Cyathus africanus**	DAOM 200370	Tanzania	DS	DQ463347	DQ463330
	SWFC 20782	China	CB	DQ463340	–
C. africanus var. *latisporus**	SWFC 21187	China	DS	DQ463342	DQ463328
C. annulatus *	DAOM 200366	Canada	DS	DQ463351	DQ463332

Table 3.1 (continued)

C. berkeleyanus	SWFC 20789	China	DS	DQ463355	–
C. colensoi [B]	DAOM 200423	India	DS	DQ463344	–
C. crassimurus *	DAOM 200372	Hawaii	DS	DQ463350	–
C. gansuensis *	SWFC 20880	China	DS	DQ463348	DQ463335
C. griseocarpus *	DAOM 200396	India	DS	–	DQ463324
C. guandishanensis *	HMAS 81896	China	DS	–	DQ463329
C. helenae *	DAOM 200384	Canada	DS	–	DQ463334
C. hookeri	SWFC 20799	China	CB	DQ463346	–
C. jiayuguanensis *	SWFC 20802	China	DS	DQ463341	DQ463325
C. olla f. *olla* [B]	BPI 727227	Canada	DS	DQ463345	DQ463327
C. olla f. *anglicus**	BPI 727225	USA	DS	–	DQ463326
C. olla f. *brodiensis*	SWFC 21137	China	DS	DQ463343	–
C. olla f. *lanatus**	DAOM 200704	USA	DS	–	DQ463337
C. pallidus	SWFC 21160	China	DS	DQ463356	DQ463336
C. poeppigii	SWFC 21357	China	DS	–	DQ463339
C. renweii *	SWFC 21406	China	CB	DQ463352	DQ463333
C. setosus *	DAOM 200815	Jamaica	DS	DQ463349	DQ463331
C. stercoreus	SWFC 21386	China	CB	DQ463354	DQ463338
C. triplex	SWFC 21077	China	CB	DQ463353	–
Heinemanomyces splendiissima	3043			R	-
	3062			-	R
Heinemanomyces sp. sp nov	3103	Thailand	DS	R	R
Hymenagaricus epipastus	3045			R	R
Hymenagaricus sp.	2047			-	R
Macrolepiota procera			-	-	AM946456
Micropsalliota albosericea	3049	Thailand	DS	R	R
		Thailand	DS	R	R
		Thailand	DS	R	R

Table 3.1 (continued)

M. allantoidea sp. nov.	2038	Thailand	DS	R	R
M. arginea	3090	Thailand	DS	-	R
M. arginophaea	3110	Thailand	DS	R	R
	2089	Thailand	DS	R	R
	2091	Thailand	DS	R	R
	2088	Thailand	DS	R	R
	2027	Thailand	DS	R	R
	3097	Thailand	DS	-	R
	3106	Thailand	DS	-	R
		Thailand	DS	R	R
M. bifida sp. nov.	2057	Thailand	DS	R	R
	2103	Thailand	DS	R	R
	3076	Thailand	DS	R	R
		Thailand	DS	R	R
	3067	Thailand	DS	R	R
M. brunneosperma var *cortinata*	2129	Thailand	DS	R	R
M. furfuracea sp nov	2119	Thailand	DS	R	R
	3006	Thailand	DS	R	R
M. globocystis	3004	Thailand	DS	R	R
	3030			-	R
	2060			-	R
	2126	Thailand	DS	R	-
M. gracilis	2041	Thailand	DS	R	R
M. lateritia var. *vinaceipes* var. nov.	2073	Thailand	DS	R	R
M. megarubescens sp nov	2008	Thailand	DS	R	R
	2009	Thailand	DS	R	R
	2086	Thailand	DS	R	R
M. megaspore sp. nov.	2051	Thailand	DS	R	R

Chapter 4

RESULTS AND DISCUSSION Part A: the systematics of *Agaricus* in northern Thailand

4.1 Generic description and type species

Agaricus L. ex Fr. Syst. Mycol. 1:5 1821, emend Karst Bidr. Finl. Nat. Folk. 32: xxv 1879.

Syn.: *Agaricus* tribus *Psalliota* Fr., Syst. Mycol. 1: 280;

Psalliota (Fr.) Kumm., Führ. Pilzk.: 23 1871;

Agaricus # *Pratella* Pers., Syn. Meth. Fung.: XVI 1801;

Pratella (Pers.) S.F. Gray, Nat. Arr. Br. Pl. 1: 626 1821.

Type species: *Agaricus campestris* L. ex Fr.

Generic desceiption: basidiomata middle to large, occasionally small; pileus conic, convex, plano-convex, mostly umbonate, covered by fibrillose squamoses, some glabrous, yellow, orange, grayish-brown, brown, reddish-brown, dark brown, yellow or red staining in most cases on touching or cutting. Context flesh, white, thick, with color staining on cutting. Lamellae free, crowded to less crowded, white, pink, light brown, brown, dark brown in age, lamellulae with several lengths, normal to ventricose. Stipe cylindrical, subbulbous to bulbous, surface smooth, tomentose to squamulose, white or with other color tone. Annulus present, mostly membranous, some double or cortinate, white, persistent mostly. Smell typically almond or phenol.

Spores smooth, brown to slightly reddish-brown, pseudoamyloid, ellipsoid, thick walled, variable in size, with a distinct or indistinct spore germ. Basidia hyaline, smooth, 2- spored or 4-spored. Cheilocystidia present or absent, if present mostly inflated as broad cylindrical, clavate, pyriform, smooth, hyaline, thin walled. Pleurocystidia rarely present. Pileipellis cutis and consisting of smooth hyphae. Hymenophoral trame regular to irregular in age. Hyphae without clamp connections. (Single 1986, Cappelli 1984).

Habit, habitat and distribution: solitary, scattered and gregarious in the soil, dung, tan, humus, some on rotted wood in grassland and forests.

4.2 Taxonomical and informative features

4.2.1 Pileus

Size is variable and generally middle to large in *Agaricus*, and it is one of important diagnostic characters in the recognition of sections and subsections (e.g. subsection Minores only has small fruiting bodies). The shape of the cap is variable, generally hemispherical, conic when young, then expending to convex, plano-convex, applanate with age. The umbo and subumbo at the disc is common, but never depressed or with uplifted margin. The surface of pileus mostly fibrillose, fibrillose-scales to shaggy with variable color (mainly white, brown, reddish-brown, yellow and orange), which is an important character at the species level. and very the main colors are pure white, white with brown, reddish-brown and purple tones at the disc. Color staining on touching and cutting is important character.

4.2.2 Lamellae

The lamellae of all species are free, with close or crowded spacing, and range from narrow to ventricose. The color of lamellae is variable with age (white, pink, light brown, brown and dark brown), and often has pink or red tone stage with the mature of the gills.

4.2.3 Stipe

Shape is variable, e.g. cylindrical, clavate, bulbous, subbulbous. The surface of the stipe is glabrous, tomentose or fibrillose, squamulose without pigments or colored below the annulus. Color staining on touching and cutting is often happened, which is important characters in section and species level.

4.2.4 Partial Veil

A partial veil is formed by all species. It is membranous in the most case and relatively persistent, and a double-annulus appearing in some species and sections. It can be narrow to very large, smooth to floccose surface. Those are important characters to recognize the sections and species.

4.2.5 Context

The context of the pileus and stipe is usually white, but some species stain yellow or red when cut.

4.2.6 Chemical reaction

The surface of pileus must be tested with 3% KOH. In a number of species a positive yellow or red reaction occurs and these are taxonomically important features.

4.2.7 Smell

Smell is a taxonomically significant character, which can be almond, phonel, iodine.

4.2.8 Basidiospores

The spore color in *Agaricus* is brown to reddish-brown, dextrinoid in Melzer's reagent, and they are smooth and without a germ pore. The spores are generally broadly ellipsoid or ellipsoid. Basidiospore size is taxonomically important. Care must be taken to measure at least 20 spores per specimen to accurately assess spore size.

4.2.9 Cheilocystidia

Cheilocystidia are present or absent in this genus species. The shape is variable among different species, and range from clavate to broad clavate, pyriform is most frequent. However the narrow flexuous cylindrical shape appears in the new species from this research. Capitate or subcapitate with the capitulum is never detected in this genus. The size is variable but taxonomically significant.

4.2.10 Pleurocystidia

Pleurocystidia are usually absent.

4.2.11 Pileipellis

The pileipellis of all species is a cutis composed of more or less parallel hyphae. Significant micromorphological characters include: i) width of hyphae; ii) shape of hyphae; and iii) type of pigments. In this genus pileipellis hyphae is never encrusted.

4.3 Molecular phylogeny

4.3.1 The relationship of *Agaricus* with *Micropsalliota* and allied genera

The relationship of *Agaricus, Micropsalliota* and allied genera was demonstrated using nLSU sequences. The dataset consisted of 99 sequences representing 36 *Agaricus* species, 19 *Micropsalliota* taxa (17 species and 2 varieties), 1 *Hymenagaricus* species and 2 *Heinemanomyces* species. *Macrolepiota procera* and *Chlorophyllum molybdites* were chosen as outgroups based on data presented by Moncalvo et al. (2002) and Matheny et al. (2007). There were 750 characters of which 582 characters were constant; 70 characters were parsimony-uninformative; and 99 characters were parsimony-informative.

In the maximum parsimony analysis (MP), equal parameters were used and gaps were treated as missing data. Maximum likelihood analysis resulted in the best tree with a likelihood score of

3487.24832 after rearrangements tried 398,935 times using the GTR+I+G model selected from MrModeltest 2.2. The Kishino-Hasegawa and Shimodaira-Hasegawa tests among topologies obtained from ML and MP indicated that the ML tree was best. ML and Bayesian trees were nearly identical except in the positions of a few members of some subclades. The ML tree is shown in Fig. 4.1.

The phylogenetic topologies indicate that the genera *Micropsalliota* and *Agaricus* are monophyletic with high to moderate posterior probability support (100% and 74% respectively). *Micropsalliota* is sister to *Hymenagaricus*, and together are sister to *Agaricus*, but this relationship is not well supported (61% PP). The *Heinemanomyces* clade consists of 2 species and forms an unresolved position basal to the other in-group genera. (see Fig. 4.1).

4.3.2 Molecular phylogeny within *Agaricus*

4.3.2.1 The ITS dataset

The phylogeny of *Agricus* is produced from 97 ITS sequences, which present 80 *Agaricus* taxa, 2 *Heinemanomyces* species and 2 outgroup taxa (*Chlorophyllum molybdites* and *Micropsalliota arginophaea*). The ITS dataset includes of 759 characters of which 60 ambiguous characters were excluded, 369 characters are constant, 72 variable characters are uninformative and 257 variable characters are parsimony-informative. The unweighted maximum parsimony analyses was preformed, and the bootstrap support was produced using 1000 replications. The maximum likelihood tree was produced with a score of 8017.52183 after 192755 rearrangements (showed in Fig. 4.2).

The subgenus *Lanagaricus* (consists of *A. trisulphuratus* and *Agaricus* sp 11) is basal in the *Agaricus* dendrogram. In topology, this subgenus is well supported with 99% BS support and sister with the subgenus *Agaricus*. The *Agaricus* species (e.g. *A. duplocingulatus*, *A. johstonii*, *A. dulcidulus* and *A. angusticystidiata*) from Thailand are distant with the species from Europe and America, and mostly compress their own subclades. There are only a few species which nest with the European and American species, such as *A. endoxanthus* and *A. impudicus*.

4.3.2.2 The LSU dataset

In this dataset totally have 748 characters of which 627 characters are constant, 58 variable characters are parsimony-uninformative and 63 characters are parsimony-informative. Those sequences represent 54 sequences including 38 *Agaricus* species, 2 *Heinemanomyces* species and

the outgroup *Chlorohyllum molybdites.* The Bayesian tree was produced and showed in Fig. 4.3, which is almost identical with the ML tree.

The genus *Agaricus* is monophyletic with full support, and *Heinemanomyces* clade is sister to it. In the *Agaricus* clade, subclade of subgenus *Lanagaricus* (comprising *A. trisulphuratus* and *Agaricus* sp 11), subclade of those species which have narrow flexuous cylindrical cheilocystidia (*A. angusticystidiata*, *A. maeseaensis*, and *Agaricus* sp 9), subclade of *Agaricus* sp 7, subclade of *A. duplocingulatus* + *A. johnstinii*, subclade of *A. xantholepis* and subclade of *A. brunneolus* and *Agaricus* sp 10 are full supported.

4.3.2.3 The ITS+LSU dataset

This dataset comprised of 33 sequences which represent 30 *Agaricus* species, 2 *Heinemanomyces* species and the outgroup *Chlorophyllum molybdites.* In this dataset totally have 1479 characters of which 24 ambiguous characters are excluded, and 1100 characters are constant, 114 variable characters are parsimony-uninformative, and 241 characters are parsimony-informative. The phylogenetic analysis were produced with Bayesian and ML, and the topologies almost identical. (Fig. 4.4)

The genus *Agaricus* is monophyletic in full support, and the subclades which well supported in the LSU and ITS datasets also are well supported in this combined dataset.

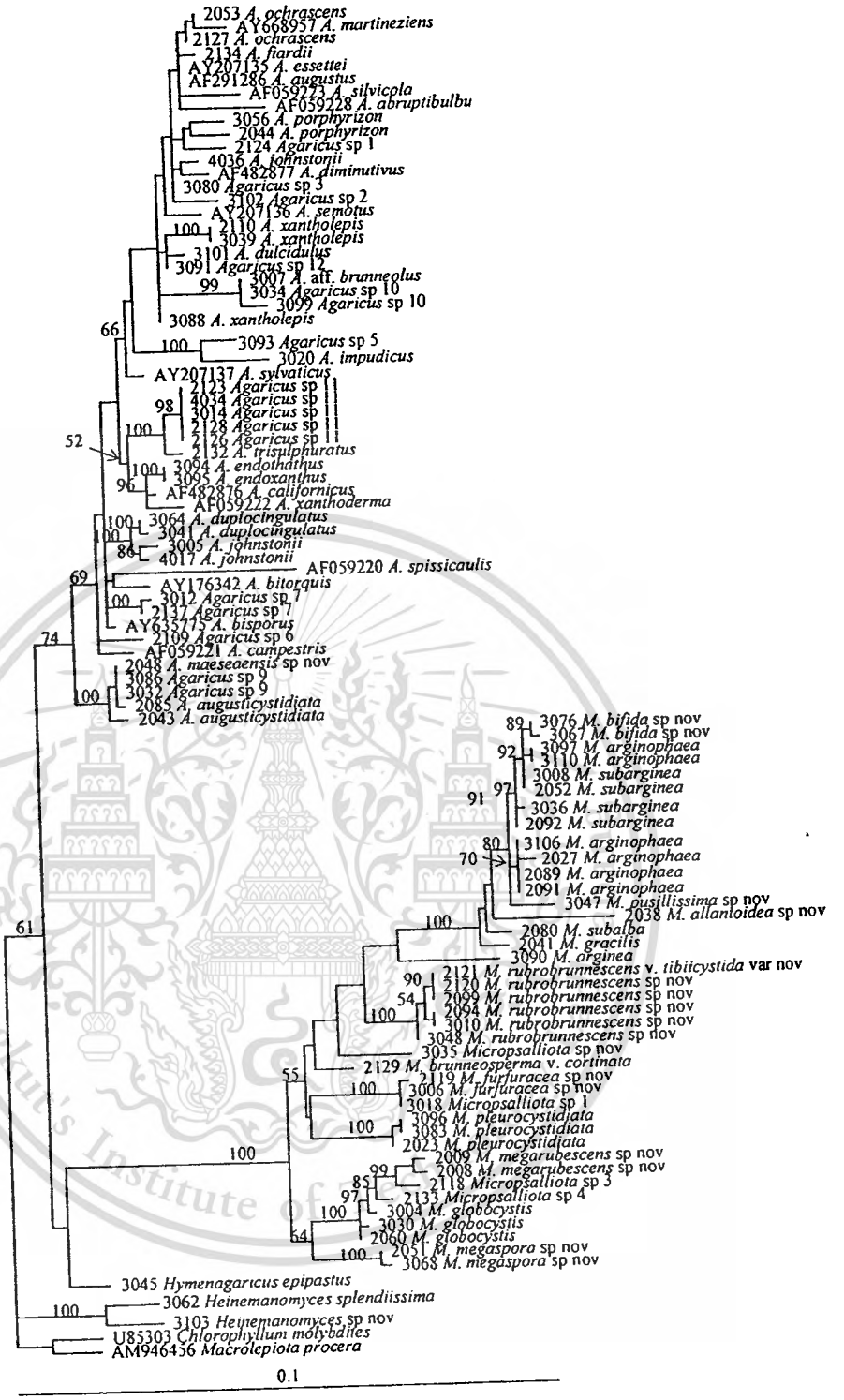

Fig 4.1 Phylogeny of *Agaricus*, *Micropsalliota* and allied species generated from Maximum Likelihood analyses based on LSU sequences. *Chlorophyllum molybdites* and *Macrolepiota procera* are outgroups. Bayesian posterior probability (PP) values (from Bayesian analyses) > 50% are given at the internodes.

Name in "**Bold**" are samples from Thailand, and other are from Europe or America.

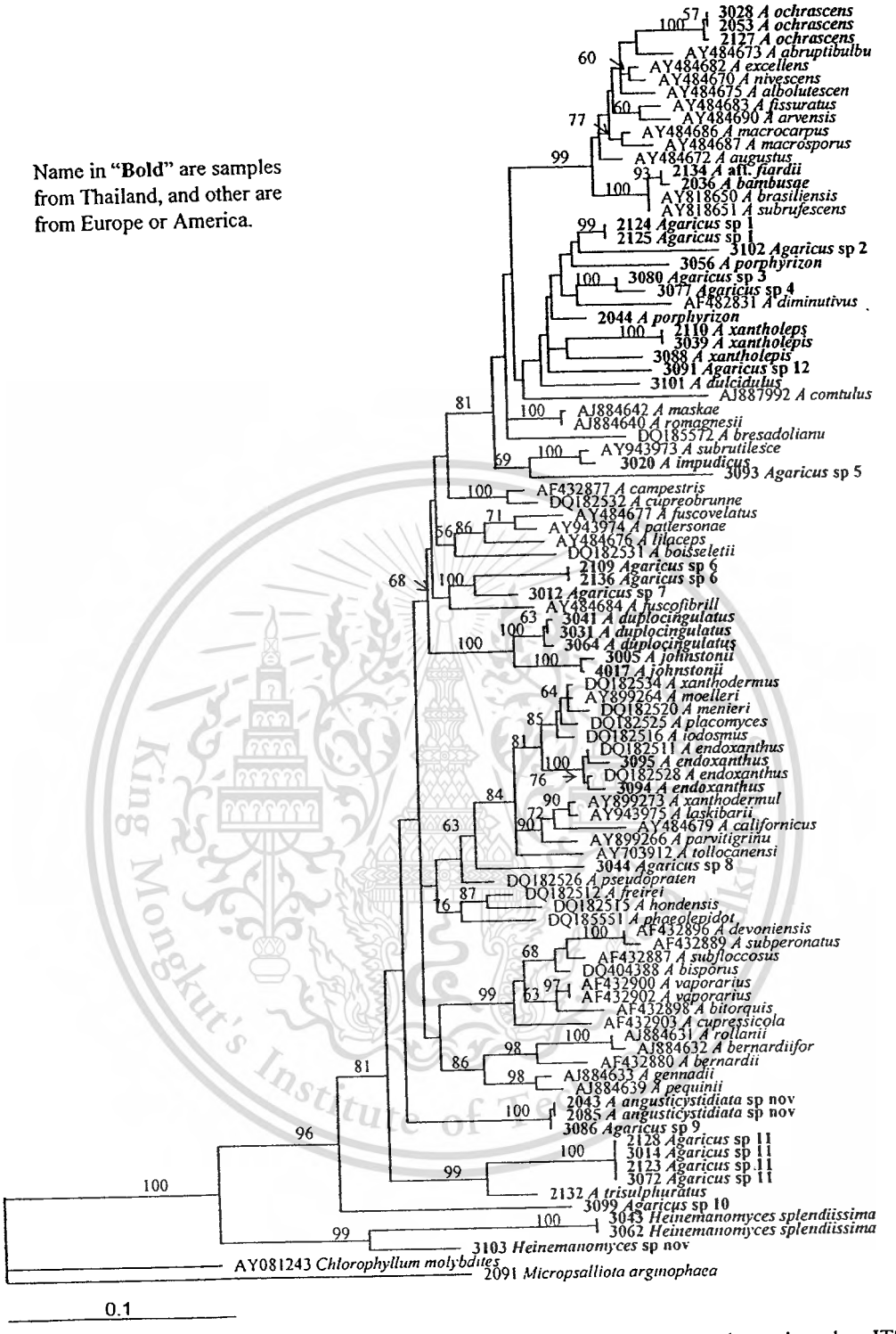

Fig. 4.2 The phylogeny of Agaricus generated from Maximum Likelihood analyses based on ITS sequences, rooted by Chlorophyllum molybditea and Micropsalliota arginophae. Parsimony bootstrap support (BS) value from unequally weighted parsimony analyses > 50% are given at the internodes.

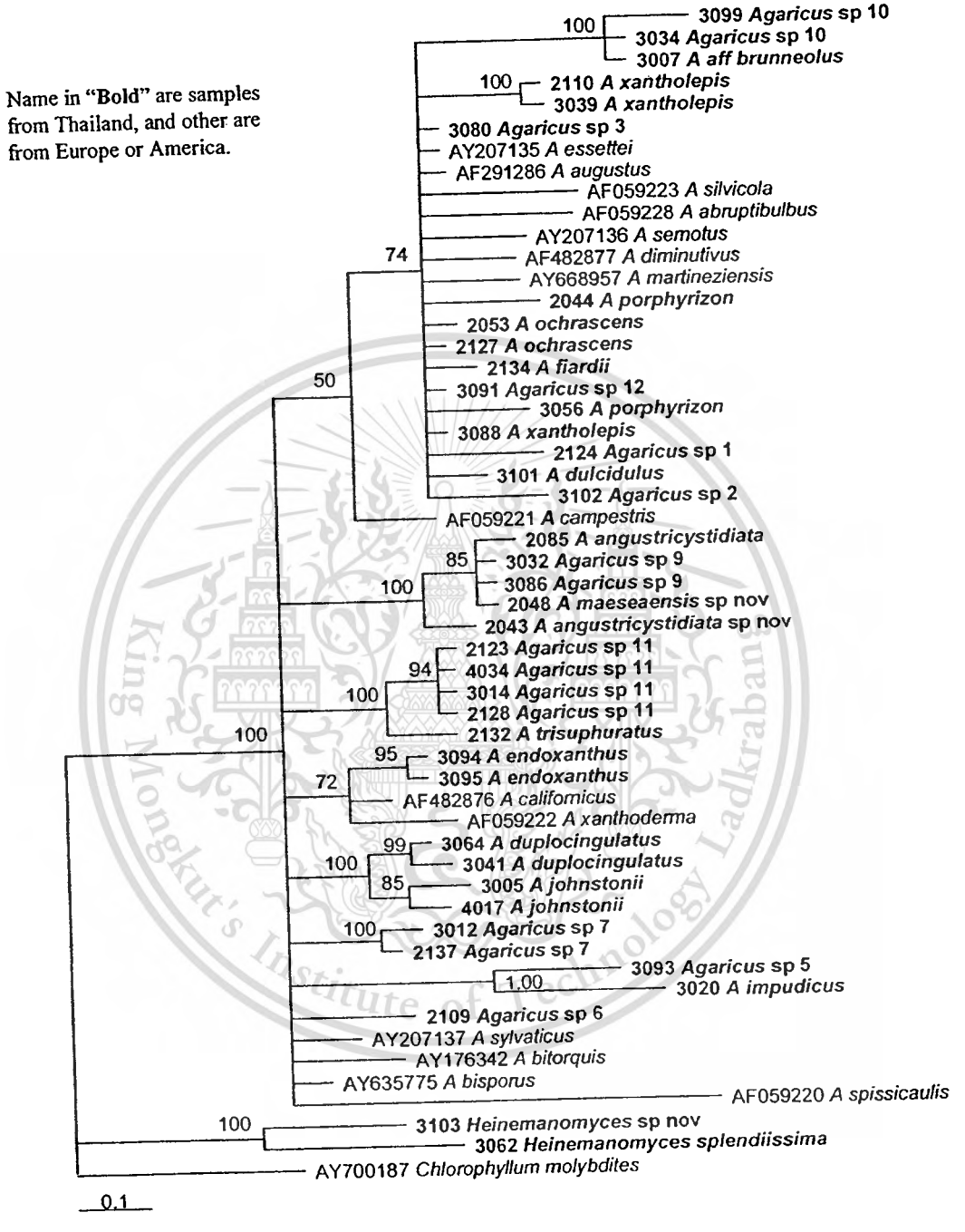

Fig. 4.3 The phylogeny of Agaricus generated from Bayesian analyses based on LSU sequences, rooted by Chlorophyllum molybditea. Bayesian posterior probability (PP) values (from Bayesian analyses) > 50% are given at the internodes.

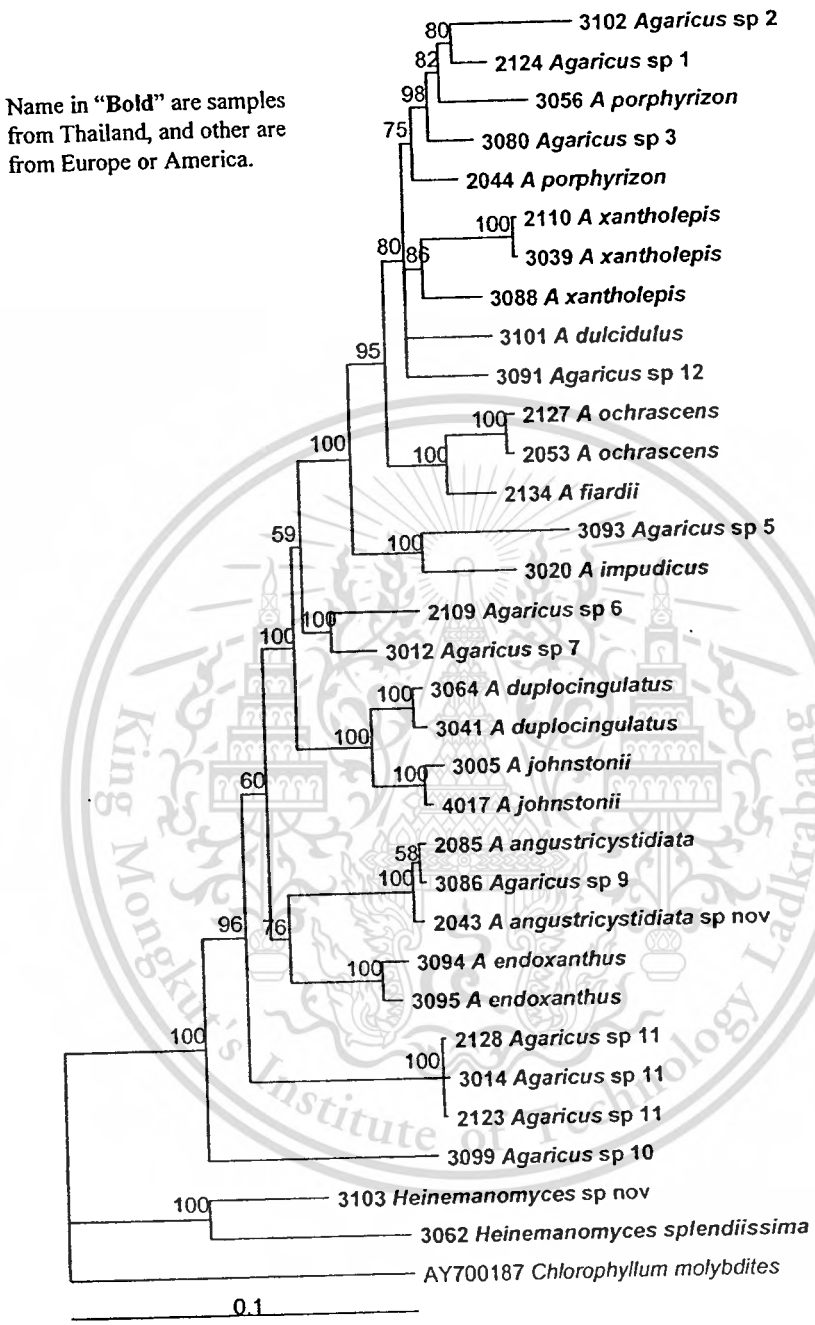

Fig. 4.4 The phylogeny of Agaricus generated from Bayesian analyses based on ITS + LSU sequences, rooted by Chlorophyllum molybditea. Bayesian posterior probability (PP) values (from Bayesian analyses) > 50% are given at the internodes.

4.4 species descriptions

1. ***Agaricus angusticystidiata*** R.L. Zhao, Desjardin, K. Soytong & K.D. Hyde sp. nov. Fig. A.1; Plate E.12.b.

Pileus 40-80 mm diam., plano-convex, applanate, broadly umbonate; surface concentric squamulose and small skull-cup at disc, appressed, slightly fissured, light brown (6D8), brown, grayish brown (5D5), dark brown (6D6) against the gray background. *Context* 4-5 mm thick at disc, fragile, white to gray in age. *Lamellae* free, crowded, lamellulae with 3-4 lengths, 3-4 mm broad, normal to slightly ventricose, brown (7E5) to dark brown (7F7-8), edge color similar to the gill itself. *Stipe* 55-100 × 5-8 (base 8-15) mm, cylindrical bulbous, with rhizomorphs in most cases, hollow, surface glabrous to silky, white to dark brown. *Annulus* perdent or percurrent; single; upper side membraneous, white; lower side surface powdery, light yellow grain-like dots in circulate; superior, persistent, edge entire, up to 5 mm broad. Smell iodoform. No color change in touching; light brown, light dull red, grayish brown (7D4) in cutting.

Macrochemical reaction: KOH reaction nagetive.

Spores 5-6.5 × 3-4 (-4.5) µm [$\bar{x}$ = 5.6 ± 0.5 × 3.8 ± 0.4, Q = 1.1-2.2, Q_m = 1.52 ± 0.7, n = 20], cymbiform, occasionally endosporioum, no germ pore, brown. *Basidia* 10-15 × 5.5-7 µm, clavate, hyaline, smooth, 4-spored. *Pleurocystidia* absent. *Cheilocystidia* 20-30 (-45) × 5-8 µm, occasionally one septa, clavate, some with elongated top, rarely subcapitate, hyaline, smooth. *Pileipellis* cutis consists of 3-5 µm diam. hyphae, hyaline, smooth, non-constricted at septa. *Annulus* hyphae same to pileipellis.

Habit: gregarious in soil.

Etymology: refers to the narrow clavate cheilocystidia.

Distribution: THAILAND (type distribution).

Materials examination: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2005, collected by Jennifer Kerekes, ZRL2043 (Holotype: SFSU); same location, 3 July 2004, collected by Thitiya Boonpratuang, ZRL2085 (SFSU).

Notes: one of distinct features is its long clavate cheilocystidia, which is similar to the genus *Allopsalliota* (Nauta 1998, 2001), but the macrochemical reaction is not match the determination of *Allopsalliota*, furthermore the ITS analysis indicate this new species is far away from *Allopsalliota* and nests in the *Agaricus* clade. This species similar to *A. phaeolepidetus*, but this forms larger basidiomata with pileus 55-120 mm diam., stipe 60-130 × 7-14 mm, cheilocystidia in

chain with terminal cells 8-20 µm, ellipsoid spores. *Agaricus iodolens* is another similar species from Cogon, but this has a non-bulbous stipe base, boarder cheilocystidia (9-12 µm), different spore shape and boarder pileipellis hayphae 7-17 (-25) µm.

2. *Agaricus bambusae* Beeli, Bull. Soc. Roy. Bot. Belg., LXI: 93, 1928 Fig. A.2; Plate E.13.a.

Pileus 60 mm diam., plano-convex with flatten disc, margin appendiculated white denticulate remnant of annulus; surface dry, covered by fibrillose squamulose, appressed and dense at the contre, slight hirsute and sparsely to the margin; reddish-brown (8E4) against light color background. *Lamellae* free, crowded, lamellulae 3-4 series, 4 mm broad, normal, white, pink and dull red (10C3) to brown with age. *Stipe* 90 × 6-7 (base 15) mm, cylindrically subbulbous with rhizomorphs, smooth above annulua and fine tomentose and scattered small fibrillose nodules below annulus, hollow, white. *Annulus* membraneous, flaky on the lower side, perdent, superior, single, relaxed, up to 11 mm broad, white. *Contex* 4 mm thick at disc fleshy, white. Small weak, taste a little astringent. Staining light yellow on stipe on touching.

Macrichemical reaction: KOH-reaction unknown.

Spores 4-5 × 3-3.5 µm [$\bar{x}$ = 4.7 ± 0.4 × 3.1 ± 0.2, Q = 1.1-1.7, Q_m = 1.51 ± 0.41, n = 20], ellipsoid, some ovate, apiculus distinct. *Basidia* 11-15 × 5-6.5 µm, clavate, 4-spored. *Cheilocystidia* consists of 7-13 × 7-11 µm elements, sphere or subsphere, 2-3 (-4) elements in chain (catenulate), smooth, hyaline. *Pileipellis* a cutis composed of 5-7.5 µm diam. hyphae, repent in net-like, brow or light brown, smooth, constricted at the sepeta in most cases. *Annulus* consists of 5-6.2 µm diam. hyphae, cylindrical, smooth, hyaline.

Habit: solitary in soil near the bamboo.

Distribution: Argentina, Congo and Thailand.

Material examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Mok Fa Waterfall, 25 June 2005, collected by Ruilin Zhao, ZRL2036 (SFSU).

Notes: *Agaricus bambusae* (Heinemann 1956c and 1990a) belongs in section Arvenses; subsection Augusti by the characters of yellow staining, pleasant almond smell, large fruiting bodies and annulus. It differs from *A. augustus* Fries which has larger spores (7.7-9.4 × 5.1-6 µm) (Kerrigan 1986). Among the species with 4-7 × 3-4 µm spores, catenulate cheilocystidia and fibrillose or squamules pileus, *A. bambusae* is distinguished by copper pileus from *A. niger* Heinem. & Gooss. (brownish-black pileus), and *A. heterocystis* Heinem. & Gooss. (light brown pileus) (Heinemann 1956c and 1978).

3. ***Agaricus*** **aff. *brunneolus*** (Lange) Pilát, Acta Mus. Nat. Pragae 7B(1): 10, 1951

Fig. A.3; Plate E.18.c.

=*Psalliota brunneola* Lange, Studies Agar. Denm. 12: 90 1938.

Pileus 40-60 mm diam., broadly parabolic, conic, expending to convex, plano-convex with broadly umbo or slightly depressed top, surface dry, silky, scattered with appressed dark blond (5D4), pitch-black squame against the white background and the squamose easy to be rub off. *Context* firm, white and slightly ocherous at disc. *Lamellae* free, crowded, lamellulae of more than 6 series, 3 mm broad, narrow, white, pink and brown. *Stipe* 100-140 × 5-7 (base 9-20) mm, cylindrically bulbous with rhizomorphs, tomentum, white or with slightly pink tinge near the top, fibrous, hollow. *Annulus* up to 20 mm broad, membraneous and low side with flakes, perdent, single, superior, fugacious, edge entire, relaxed. Smell almonds. Staining slightly yellow on stipe on cutting.

Macrochemical reaction: KOH reaction negative.

Spores 5-6×3 -3.5 µm [$\bar{x}$ = 5.4 ± 0.3 × 3.1 ± 0.2, Q = 1.5-1.9, Q_m = 1.74 ± 0.24, n = 20], without germ pore, brown. Basidia 15-20 × 5-7 µm, clavate, 4-spored. *Cheilocystidia* (11-) 14-20 × (7-) 10-15 µm diam., pyriform, turbinate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis consisted of hyphea of 5-10 µm diam., with light yellow pigments in dots, smooth, mostly without constriction on septa; mixing with oleiferous hyphae of 5-10 µm diam., which light olive or hyaline, constricted at septa, long cylindrical. *Annulus* consisted of hyphae of 4-9 µm diam., cylindrical, smooth, hyaline, no constriction at the septa, while the flakes consisted of disarticulatingly cylindrical hyphae, hyaline. *Stipitipellis* hyphae similar to pileipellis hyphae but without pigments.

Habit: solitary or gregarious in rich soil.

Distribution: Europe, Malaysia, Thailand.

Examined materials: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 13 June 2005, collected by Jacques Fournier, ZRL2003 (SFSU); same location, 29 May 2006, collected by Ruilin Zhao, ZRL3007 (SFSU).

4. ***Agaricus caribaeus*** Pegler, Kew Bull. Additional Series IX: 436. Fig. A.4; Plate E.13.b.

Pileus 20-40 mm diam., convex, soon expending plano-convex, applanate, applanate with uplifted margin, some broad umbonate; surface dry, covered with fibrils, lacking squame, disc

greenish-brown (4E4, 5F4), dark brown (7E6), near black, disrupted into minute, innate squamules towards margin against cream or white background. *Context* thin, 1.5-2 mm thick, white; stipe, white, grayish-brown, brown with age. *Lamellae* free, crowded, lamellulae 3-4 series, 3-5 mm broad, grayish-orange (5B3), reddish brown (8E4), brown, dark brown (8F4) with age. *Stipe* 45-70 × 2-5 (base 4-8) mm, long subclavate, long cylindrical, rhzimorphes, smooth or slightly tomentose, hollow, white. *Annulus* percurrent, white, superior, thick. Smell almonds or not distinct. No staining on stipe on touching.

Macrochemical reaction: unknown.

Spores 6-8 × 3-4 µm [$\bar{x}$ = 6.7 ± 0.5 × 3.6 ± 0.4, Q = 1.5-2.3, Q_m = 1.88 ± 0.58, n = 20], elongate, oblong, with narrow apex end, endosporum, without germ pore, brown. *Basida* 10-15 × 6-8 µm, clavate, 4-spored. *Cheilocystidia* 8-28 × 7-16 µm, pyriform, subglobular, smooth, hyaline. *Pileipellis* a cutis consisted of hyphae of 3-7.5 µm diam., cylindrical, branched, hyaline or with dark brown vacuolated pigments. *Annulus* consisted of hyphae of 2.5-5 µm diam., hyaline, branched, constricted at some septa.

Habit: solitary or scattered in small group in rich soil.

Distribution:Tartane (type distribution);Thailand.

Material examination: THAILAND, Chiang Mai Prov., Chom Thong, Ob Luang National Park, elev. 1812 m., 23 June 2004, collected by Thanh Huyen Le, ZRL2025 (SFSU); Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 24 June 2004, collected by Dennis E Desjardin, ZRL2031 (SFSU); same location and collection date, collected by Jennifer Kerekes; ZRL2032 (SFSU).

Notes: *Agaricus caribaeus* is distinguished by long slender stipe, white pileus bearing near greenish-black to near black, minute squamules at the disc, which are unique in *Agaricus*. Pegler thought this species should come to section Brunneopicti Heinem. by its munite squamules (Pelger 1983).

5. *Agaricus dulcidulus* S. Schulz. In Kalchbr., Ic. Sel. Hymenomyc. Hung.: 29 1874.

Fig. A.5; Plate E.13.c.

= *Psalliota purpurella* F. Møller in Friesia 4: 193. 1952.

= *Agaricus purpurellus* (F. Møller) F. Møller in Friesia 4: 204. 1952

Pileus 40-50 mm diam., convex, plano-convex, applanate, margin cuticle exceeding and appear white edge; surface dry, covered with fibrillose, appressed, thick and felt-like at disc then

broken to margin, reddish-brown (8D4, 8E4, 9E4, 9E5) or "vinaceous brown" against the white background. *Context* 3-4 mm thick at disc, firm, white or light brown at disc. *Lamellae* free, crowded, lamellulae 6-8 series, 3 -4 mm broad, broad or slightly ventricose, light brown, dull brown, brownish-grey (9C2, 9E2), brown with age. *Stipe* 40-60 × 5-8 (base 11-15) mm, cylindrically bulbous, smooth upper of annulus and fine tomentose below annulus, narrow hollow (1.5 mm diam.), white. *Annulus* membraneous, perdent, single, supervior, persistent, stretched, white, 2-6 mm broad. Smell pleasant almonds. Yellow staining at both pileus and stipe on touching and cutting.

Macrochemical reaction: KOH-reaction yellow (positive).

Spores 5-7×4-4.2 µm [$\bar{x}$ = 6.1 ± 0.5 × 4 ± 0, Q = 1.3-1.8, Q_m = 1.53 ± 0.27, n = 20], ellipsoid, without germ pores. *Basidia* 13-22 × 6-9 µm, clavate, 4-spored. *Cheilocystidia* (16-) 20-35 (- 40) × 9-15 µm, clavate, pyriform, mostly with short handle at base, hyaline or orange. *Pileipellis* a cutis composed of hyphae of 5-12.5 µm diam., brown pigment distributed irregular, smooth, slightly constricted at the septa. *Annulus* consisted of hyphae of 3-7.5 µm diam., cylindrical, hyaline, smooth, branched. *Stipitipellis* consisted of same hyphae of annulus.

Habit: solitary in soil side of road.

Distribution: worldwide.

Materials examined: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 2 July 2004, collected by Amy Honan, ZRL2071 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 23 June 2006, collected by Ruilin Zhao, ZRL3071 (SFSU); same location, 14 July 2006, collected by RuiLin Zhao, ZRL3085 (SFSU); same location, 20 July 2006,collected by Ruilin Zhao, ZRL3084 (SFSU); same location, 13 September 2006, collected by Ruilin Zhao, ZRL3101 (SFSU); same location, 9 October 2006, collected by Ruilin Zhao, ZRL3108 (SFSU).

Notes: *Agaricus dulcidulus* (Nauta 2000) has a common synonym *A. purpurellus* (Pegler 1983). Yellow staining, almond smell and small fruiting bodies show it belong in section Arvenses, subsection Minores (Heinemann 1978). The most similar species is *A. johnstonii* in the similar sized and shaped spores and cheilocystidia, however *A. johnstonii* has copper brown pileus (Heinemann 1962a).

6. ***Agaricus duplocingulatus*** Heinem., Bull. Jard. Bot. Nat. Belg. 50: 32 1980.

Fig. A.6; Plate E.14.a-b.

Pilleus 30-80 mm diam., campanulate, convex, plano-convex, disc applanate or broadly umbonate, margin incurved when young then straight; surface dry, covered by scales in circularity and sparsing to margin, light brown, brown (6E6, 7D5, 7D6), appressed, backgroud cream or dirty cream. *Context* firm, white, cream, ocherous to reddish-gray at the disc. *Lamellae* free, crowded, lamellulae more than 4 series, 4-7 mm broad, normal to slightly ventricose, white, light brown (7D4), orange-grey (5B2), brownish-orange (6C3, 7C4), brown (7E6). *Stipe* mm, mm diam., 60-120 × 4-7 (base 10) mm, cylindrical, long clavate, some bulbous with rhizomorphs, white or cream, smooth or slightly tomentose, narrowly hollow. *Annulus* double, upper one membraneous, perdent, stretched, white, low side floccose, up to 8 mm broad; lower one bracelet-like, thick, movable, white except brown tinge at low side. Smell strong almond. Staining basically is not sensitive, and varied in different collections.

Macrochemical reaction: KOH-reaction negative.

Spores 5-6×3.5-4.5 μm [$\bar{x}$ = 5.5 ± 0.5 × 4 ± 0.5, Q = 1.3-1.6, Q_m = 1.45 ± 0.15, n = 20], broadly ellipsoid, without germ pore, brown. *Basidia* (13-) 17-21 × 6-9 μm, clavate, 4-spored. *Cheilocystidia* 14- 30 (-34) × 9-20 μm, turbinate, pyriform, subspherial, hyaline, smooth. *Pileipellis* a cutis consisted of hyphae of 5-8 μm diam., cylindrical, smooth, reddish brown pigments distributed in plots. *Membransous Annulus* consisted of hyphae of 12.5-12 μm diam., cylindrical, hyaline, smooth; *blacelet-like annulus* consisted of hyphae of 5-7.5 μm diam., smooth, hyaline or reddish brown (same with pileipellus hyphae). *Stipitipellis* consisted of the hyphae is same with the hyphae in blacelet-like annulus.

Habit: solitary in the forest.

Distribution: Singapore (type distribution), Thailand.

Examined materials: THAILAND, Chiang Mai Prov., Doi Inthanon National Park, Hwy 1009 at 25 km marker, N18°32.54' E98°33.51', elev. 1076 m., 5 June 2004, collected by Tim Baroni, ZRL3023 and ZRL3024 (SFSU); Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 13 June 2004, collected by Todd Osmundson, ZRL3064 and ZRL3065 (SFSU); same location, 7 June 2006, collected by Dennis E Desjardin, ZRL3031 and ZRL3038 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 23 May 2004, collected by Ruilin Zhao, ZRL3003 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Highway 1095 at 22 km

marker, N19°07.57' E98°45.65', elev.750 m., 11 June 2004, collected by Thanh Huyen Le, ZRL3051 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8" E 98°44'47.3", elev. 1050 m., 8 June 2004, collected by Thanh Huyen Le, ZRL3041 (SFSU).

Notes: *Agaricus duplocingulatus* is distinguished by double annulus which are quite different in morphology each other (membraneous and blacelet-like) (Heinemann 1980), and also different the double ring in section Bitorques (Cappelli 1984). Even Heinemann grouped it in the section Xanthodermatei, he also mentioned it was ambiguous that it should belong in section Xanthodermatei or section Arvenses (Heinemann 1980). Catenulate cheilocystidia did not be detected in the Thai materals, even the shape and size of elements are matched the original description from Heinemann. However catenulate cheilocystidia are easily separated in age.

Agaricus hypophaeus Heinem. (Heinemann 1980) is the most similar species to *A. duplocingulatus*, but the former has darker pileus and clavate cheilocystidia.

7. ***Agaricus endoxanthus*** Berk.& Br., Journ. Linn. Soc., Bot. 11: 548 1871.

Fig. A.7; Plate E.17.c.

Pileus 60-70 mm diam., convex, plano-convex, disc broad umbo with flattened top or slightly depressed, margin cuticle exceeding as white edge; surface dry, completely covered by brownish-grey (8E2), grey (8E5) fibrils and radiatively broken so appearing the white context, pink tone in wet; at disc fibrils darker and aggregated into small fibrillose nodes, scattered. *Context* firm, white. *Lamellae* free, crowded, lamellulae of more than 10 series, 5 mm broad, normal or ventricose, dull red (8C3), brown to dark brown (8F4) with age, edge color is lighter than gill itself. *Stipe* 4-12 mm in diam, 50-70 mm high, cylindrical but slight taping to the apex, rhizomorphes; surface both above and below ring smooth, brownish grey (8E2), hollow. *Annulus* up to 11 mm broad, low side slightly floccose, perdent, single, superior or near apex of stipe, persistent, entire, stretched, white and some with brown grains. Smell indistinct, not almond. Base of stipe strong yellow staining on cutting, no color change on touching.

Macrochemical reaction: KOH-reaction yellow; no color change with ammonia.

Spores 5-6×3-4 µm [$\bar{x}$ = 5.4 ± 0.3 × 3.5 ± 0.4, Q = 1.3-2, Q_m = 1.55 ± 0.45, n = 20], ellipsoid, narrowing one end in most cases. *Basidia* 12-17 × 5-7 µm, clavate, 4-spored. *Cheilocystidia* (9-) 11-17 × 8-12 µm, rarely with age, mostly pyriform, some turbinate, subsphearial, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of elements which are variable in size and

shape, 25-44 × 17-25 µm or 17-25 × 5-7.5 µm, locus root like or subspherical in chain, distinctly constricted at the septa, dark brown vacuolated pigments against the hyaline. *Annulus* consisted of hyphae of 5-7.5 µm diam., hyaline, smooth, curved. *Stipipellis* a cutis composed of hyphae same with pileipellis' except sligtly smaller size.

Habit: solitary or gregarious in soil.

Distribution: Singapore, Sri Lanka, Thailand.

Materials examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 9 August 2006, collected by Ruilin Zhao, ZRL3094 (SFSU); same location, 13 August 2004, collected by Ruilin Zhao, ZRL3095 (SFSU).

Notes: Yellow staining and no almond smell show Thai materials belong to section Xanthodermatei (Heinemann 1978). *Agaricus endoxanthus* is distinguished by dark pileus, appessed fibrillose squames, large membraneous-floccose annulus, small spores and cheilocystidia, vacuolar pigments on the pileipellis hyphae (Pegler 1977;1986; Heinemann 1980). It differs from *A. pseudoniger* Heinem. & Gooss. which has brownish-black pileus and larger cheilocysitidia (18-30 × 9.5-18.5 µm) (Heinemann 1980; 1956c). It differs from another dark pilsus species, *A. hypophaeus* Heinem. which has double annulus (Heinemann 1980).

8. ***Agaricus fiardii*** Pegler, Ag. Lesser Antilles: 447 1983. Fig. A.8; Plate E.15.a.

Pileus 78 mm diam., plano-convex, and margin up-lifted with flatten disc; edge appendiculate the remnant of part viel, denticulate, white; surface dry, entirely covered with fibrils, fibrillose-squamulose, purple-brown (6E5), appressed. *Context* 6 mm thick at disc, generally white, except the disc and stipe with light brown tinge, firm. *Lamellae* free, crowded, lamellulae 4 series, 5 mm broad, normal, brown, dark brown. *Stipe* 80 × 6 -8 (base 14) mm, long clavate, subbulbous with rhizomorphes, smooth above annulua and fine tomemtose below annulus, with light brown tinge, hollow. *Annulus* membranceus, lower side with flaks, perdent, single, superior, persistent, entire, up to 20 mm broad, white. Smell slightly almonds. No color change on touching and cutting. Exsiccant sample appear strong yellow in context.

Macrochemical reaction: KOH-reaction negative.

Spores 5-6.5 × 4-4.5 µm [$\bar{x}$ = 5.9 ± 0.4 × 4.1 ± 0.2, Q = 1.3-1.6, Q_m = 1.44 ± 0.16, n = 20], ellipsoid with undistinct hyaline appendage, without germ pore, brown. *Basidia* 12-18 × 6-8 µm, clavate, 4-spored. *Cheilocystidia* consisted of 8-18 × 7-12 µm elements, catenulate, pyriform in base element and other ellipsoid, subglobe, globe, hyaline, smooth. *Pileipellis* a cutis consisted of

hyphae of 10-15 µm diam., hyaline or yellowish brown, cylindrical, slightly constricted at the septa. *Annulus* consisted of hyphae of 4-7.5 µm diam., hyaline, smooth, curved. *Stipitipellis* consisted of elements of 4-8 µm diam. in chain, ellipsoid, subglobe, cylindrical, hyaline, smooth,.

Habit: solitary in rich soil of flower pot.

Distribution: Congo, Martinique (type distribution), Thailand.

Material examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 28 August 2004, collected by Kevin D Hyde, ZRL2134 (SFSU).

Notes: This material having large annulus, catenulate cheilocystidia and yellow staining indicate it belong in section Arventsus, subsection Augusti. The features are quite matched the definition of *A. fiardii* (Pegler 1983), except the exsiccant specimen of Thai appear yellow, but not brown (Heinemann 1990a).

9. *Agaricus impudicus* (Rea) Pilát, Klíč urč. Hub hřib. Bedl.: 403 1952. Fig. A.9; Plate E.15.b-c.

= *Psalliota impudica* Rea, Appendix II to British Basidiomycetes-Trans. Brit. Mycol. Soc., 17, p50, 1932 (Basionymum).

= *Psalliota brunneola* J. Lange, Flora Agaricina Danica, p. VII, 1940.

= *Psalliota variegate* Möller, Friesia, IV p31, 1950.

= *Agaricus variegatus* (Möller) Pilat, Acta Mussei Nationalis Pragae, VII B, 1, p8, 1951 (Nom. inval.)

non *Agaricus variegates* Persoon: Fries 1821.

= *Agaricus brunneolus* (J. Lange) Pilat, Acta Musei Nationalis Pragae, VII B, 1, p10, 1951.

= *Psalliota impudica* Rea sensu Möller, Friesia, IV p196, 1952.

= *Agaricus impudicus* (Rea) M. Lange, Bot. Tidsskrift, 71, p95, 1976.

= *Agaricus brunneolus* (J. Lange) Pilat sensu M. Lange, Bot. Tidsskrift, 71 p95, 1976.

= *Agaricus reai* Bon, Documents mycologiques, XI, 44 p28, 1981 (Nom. inval.)

Pileus 85-125 mm diam., convex, plano-convex with broad umbo, top truncate, margin deflexed; surface dry, entirely covered with fibrils, thick and dark at the centre, then broken towards margin into more minutely squamulose-fibrillose, not concentrically arranged all around (which is one character of this species), appressed, brown (6E6, 6E8), with red tinge in wet. *Context* 6-7 mm thick at disc, generally white, but with light brown or pinkish grey tinge in the disc, and apex of stipe. *Lamellae* free, crowded, lamellulae 2-3 series, 6 mm broad, normal, white,

light brown (7D4), brown, dark brown. *Stipe* 120-150 × 8-10 (base 15-16) mm, clavate, cylindrically subbulbous, smooth above the annulus, large wives squamulose brown or white against the white background. *Annulus* membraneous, 10-25 mm broad, large, perdent, single, superior, entire, relaxed, lower side floccose. No color change on touching; base of stipe slightly yellow on cutting.

Macrochemical reaction: KOH-reaction negative.

Spores 4.2-6 × 3-3.5 µm [$\bar{x}$ = 4.9 ± 0.4 × 3.1 ± 0.2, Q = 1.3-2, Q_m = 1.58 ± 0.42, n = 20], ellipsoid, no germ pore, brown. *Basidia* 13-20 × 5-7 µm, clavate, 4-spored. *Cheilocystidia* 15-28 × 10-19 µm, pyriform, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis consisted of hyphae of 3-7.5 µm diam., brownish-grey, brown, smooth, constricted at the septa in most cases. *Annulus* consisted of hyphae of 3-10 µm diam., hyaline, cylindrical, some inflated into clavate, smooth, constricted at the septa. *Stipitipellis* consisted of hyphae of 5-12.5 diam., hyaline, smooth, elongate cylindrical, curved and branched.

Habit: Solitary in rich soil of forest.

Distribution: Europe, northern Africa, South America, Asian (Cappelli 1984; Nauta 2000).

Materials examination: THAILAND, Chiang Mai Prov., Doi Inthanon National Park, junction of Highway 1009 and road to Mae Chem, N19°31.58' E 98°29.64', elev. 1700 m., 27 June 2005, collected by Dennis E Desjardin, ZRL2054 and ZRL2055 (SFSU); same location, 5 June 2006, collected by Todd Osmundson, ZRL3020 (SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2005, collected by Ruilin Zhao, ZRL2042 (SFSU).

10. ***Agaricus johnstonii*** Murr, Mycologia 10: 75, 1918. Fig. A.10; Plate E.16.a.

Pileus 50-55 mm diam., convex, plano-convex, disc flatten, surface dry, covered by fibrils, light brown or "ochraceous buff", thick and broken to margin, appressed, background cream, light grey. *Context* 4 mm thick at disc, firm, pale pink brown. *Lamellae* free, crowded, lamellulae 6-8 series, 5 mm broad, ventricose, light brown, dull brown, brown with age. *Stipe* 40-50 × 6-8 (base 11) mm, clavate-subbulbous with one rhizomorph, smooth to silky above annulus, lightly tomentose or smooth below annulus, white. *Annulus* perdent, single, superior, fagacious, relaxed, low side slightly floccose, white, up to 11 mm broad. Smell strong almonds. No color change on touching and cutting.

Macrochemical reaction: KOH-reaction negative.

Spores 5-6×4-4.2 µm [$\bar{x}$ = 5.6 ± 0.3 × 4.1 ± 0.1, Q = 1.2-1.5, Q_m = 1.39 ± 0.19, n = 20], broad ellipsoid, without germ pore, brown. *Basidia* 16-17 × 7-8 µm, clavate, 4-spored. *Cheilocystidia* 11-26 × 9-19 µm, inflated clavate, pyriform, smooth, hyaline. *Pleurocystidia* absent. *Pilleipellis* a cutis composed of hyphae of 6-8 µm diam., cylindrical, without constricted at the septa, smooth, brown pigments scattered in cell. *Annulus* consisted of hyphae of 5-7.5 µm diam., cylindrical, hyaline, smooth, without constriction at the septa. *Stipitipellis* consisted of same hyphae of annulus.

Habit: scattered in small group or solitary in forest.

Distribution: Martinique, Thailand.

Material examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 26 May 2006, collected by Ruilin Zhao, ZRL3005 (SFSU).

Notes: Even the only collection did not give the color staining information, but the molecular analyses show this material belong in section Arvenses. Furthermore small fruiting body, almond smell indicate it should belong in subsection Minores (Heinemann 1978). Compared overall character, this material match A. johnstonii (Pegler 1983; Heinemann 1962a).

11. ***Agaricus maesaecnsis*** R. L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov. Fig. A.11; Plate E.16.b.

Pileus 19 mm diam., hemispherical, convex, surface covered erected or recurved squamulose, hirsute, reddish brown (9E4) on the gray white background, no color staining. *Context* thin, 1 mm thick, white to gray at pileus, brown to dark brown on stipe. Lamellae free, crowded, lamellulae with 2 lengths, breadth 2 mm broad, narrow, brown, dark brown. *Stipe* 1.5 mm and 2.5 mm at base diam., 22 mm long, cylindrical-subbulbous, with rhizomorphes, above ring smooth to silky, white, and below ring slightly squamulose, white to yellowish white. *Annulus* thick, and surface floccose, pernent, persistent, superior, rigid, white to light yellow. Smell odorless.

Macrochemical reaction: KOH reaction unknown.

Spores 5-6 × 3-4 µm [$\bar{x}$ = 5.7 ± 0.4 × 3.6 ± 0.4, Q = 1.3-1.8, Q_m = 1.57 ± 0.27, n = 20], ellipoid to subcymbiform, some amygdaliform, without germ pore. *Basidia* 11-14 × 3-4 µm, broad clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 25-46 × 3-5 µm, occasional with one septa, irregular narrow clavate, often with long neck. *Pleurocystidia* absent. *Pileipellis* cutis,

hyphae 3-5 (-7.5) µm diam., light yellow, smooth. *Annulus* hyphae 3-5 µm diam., light yellow, smooth, discardulate.

Habit: Solitary in soil.

Etymology: refers to the original location of this species.

Type distribution: Thailand.

Materials examination: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2004, collected by Ruilin Zhao, ZRL2048 (Holotype: SFSU).

Notes: This species is distinct by tiny fruiting bodies, brown hirsute scaly pileus and narrow clavate cheilocystidia with long curved neck. And the ITS and LSU sequences indicate it is the member of *Agaricus*.

12. ***Agaricus ochrascens*** Heinem. & Gooss., Bull. Jard. Bot Etat. Brux. 26: 37 Fig. A.12; Plate E.16.c.

Pileus 110-180 mm diam., hemispherical, convex, plano-convex, disc applanate; surface covered with small, grayish-brown (6D3), brownish-orange (5C5), light brown (6D5) grain-like squamules (not fibrillose) against cream color background; squamules closed congregated at disc, and sparse to margin, punctiform, cricoid, appressed; lubricous and with pink color under the moist condition; margin appendiculate remnants of partial veil. *Context* 8 mm thick, firm, white with a little pink tinge. *Lamellis* free, crowded, 6 - 10 mm broad, normal to ventricose, white, pink, then light brown with age. *Stipe* 130-170 × 10-20 (base 20-40) mm, cylindrical-bulbous, rhizomorphs, smooth upper ring, filbrilous and scattered flakes below ring, narrow hollow, white. *Annulus* 20 mm broad, membraneous, lower side floccose, perdent, white. Smell pleasant almonds. No color staining on touching; slightly yellow staining on cutting.

Macrochemical reaction: KOH-reaction strong yellow.

Spores 5-6.2 ×3.5-4.2 µm [$\bar{x}$ = 5.6 ± 0.4 × 4 ± 0.2, Q = 1.3-1.6, Q_m = 1.42 ± 0.18, n = 20], ellipsoid, without germ pore, reddish brown. *Basidia* 13-17 × 6-7 (-8) µm, clavate, 4-spored. *Cheilocystidia* consisted of element of 13-23 × 8-15 µm, ellipsoid, subspherical or clavate, in chain or not, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis consisted of hyphae of 5-7.5 µm diam., curved, cylindrical, hyaline or light brown. *Annulus* consisted of hyphae of 5-10 µm diam., cylindrical, hyaline, smooth, separated at the septa easily. *Stipitipellis* consisted of

hyphae of 32-40 × 15-25 µm at apex, lotus root like or ellipsoid elements in chain, saccate, hyaline, smooth.

Habit: solitary, scattered or gregarious in rich soil in forest.

Distribution: Congo (type distribution), Thailand.

Material examination: THAILAND, Chiang Mai Prov., Doi Inthanon National Park, junction of Highway 1009 and road to Mae Chem, N19°31.58' E 98°29.64', elev. 1700 m, 27 June 2005, collected by Dennis E. Desjardin, ZRL2053 (SFSU); same location, 5 June 2006, collected by Todd Osmundson, ZRL3021 (SFSU); same location, 5 June 2006, collected by Chan Hong Twu, ZRL3022 (SFSU); same location, 5 June 2006, collected by Ruilin Zhao, ZRL3028 (SFSU).

Notes: Yellow staining, almond smell, catenulate cheilocystidia show the materials belong in section Arvenses, subsection Augusti. The grain-like ocherous squamules on pileus and no constriction at the septa of pileipellis hyphae made *A. ochrascens* distinguished in this section. *Agaricus agrocyboides* Heinem. & Gooss. is the most similar, however which has larger cheilocystidia (30-40 × 11-22 µm)and brown spores (Heinemamm 1956c).

13. ***Agaricus porphyrizon*** P.D. Orton, Trans. Br. Mycol. Soc. 43: 174. 1960. Fig. A.13; Plate E.17.a-b.

= *A. purpurascens* (Cooke) Pilát in Acta Mus. nat. Prag. 7B (1): 10. 1951, non A. purpurascens A. & S. 1805:Fr.

= *A. arvensis* var. *purpurascens* Cooke, Ill. Brit. Fungi 4: pl. 584. 1885.

= *Psalliota purpurascens* (Cooke) F. Møller in Friesia 4: 187. 1952.

Pileus 50-75 mm diam., at first bell-shaped, expanding to obtuse conic, convex, plano-convex, disc applanate never umbonate or depressed, margin appendiculate part veil remnants, white; surface covered with fibrillose, thick, appressed, purple or reddish brown (7E5, 6E6) squame on pale background; squame thick at the disc and becoming fibrillose towards the margin. *Context* white, firm. *Lamellae* free, crowded, lamellulae with 3-4 series, slightly ventricose, 5-6 mm broad, normal or slightly broader, white, pink, reddish-grey, finally brown. *Stipe* 50-70 × 8-12 (base 20-21) mm, distinctly clavate- bulbous with white and short rhizomorphs; smooth above ring, white; fibrillose-squamulose below ring occasionally with light brown tinges, stuffed or narrowly fistulose. *Annulus* perdent, single, superior, persistent, edge torn, stretched, white, mostly around 5 mm (up to 15 mm) broad. Smell strong almonds. Staining yellow to orange on touching and cutting at both pileus and stipe.

Macrochemical reaction: KOH-reaction strong yellow.

Spores 5-6×3.5-4.5 µm [$\bar{x}$ = 5.9 ± 0.3 × 3.9 ± 0.2, Q = 1.3-1.7, Q_m = 1.49 ± 0.21, n = 20], ellipsoid, without germ pore, reddish brown. *Basidia* (16-) 18-24 (-27) × (6-) 7-8 µm, clavate, 4-spored. *Cheilocystidia* 15-36 × (9-) 12-22 µm, pyriform, turbinate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 7-12 µm diam., cylindrical or slightly inflated (up to 30 µm diam.), constricted at the septa, with light brown or brown intracellular pigments. *Annulus* consisted of hyphae of 5-12.5 µm diam., cylindrical, curved, branched, hyaline, smooth. *Stipitipellis* consisted of the same hyphae of annulus.

Habit: solitary in rich soil on the bank of road.

Distribution: Europe, south America, Thailand.

Examined materials: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2005, collected by Ruilin Zhao, ZRL2044 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Highway 1095 at 22 km marker, N19°07.57' E98°45.65', elev.750 m., 11 June 2006, collected by Dennis E Desjardin, ZRL3055 (SFSU); THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 12 June 2006, collected by Ruilin Zhao, ZRL3056 (SFSU).

Notes: *Agricus porphyrizon* belong in section Arvenses; subsection Minores based on the characters of yellow staining, single and relatively small annulus and undeveloped universal veil (Cappelli, 1984; Heinemann 1990; Nauta 2001). *Agaricus dulcidulus* is the most similar spicies because both having vinaceous-brown, purple-brown fibrillose squames pileus, and it mainly differs from *A. porphyrizon* in having smaller fruiting bodies (mostly less than 50 mm diam. pileus) (Heinemamm 1978).

14. ***Agaricus trisulphuratus*** Berk., Ann. Mag. Nat. Hist. V, 15: 386, 1885.

Fig. A.14; Plate E.18.b.

= *A. nothus* Berk. in l.c. 1885.

= *A. mephistopheles* Cooke in Grevillea 19: 7. 1890.

= *Stropharia mephistopheles* (Cooke) Sacc., Syll. Fung. 9P: 139, 1891.

= *S. stuhlmanni* P. Henn. in Engl., Bot. Jahrb. 17: 33, 1893.

= *Psalliota burkillii* Massee in Bull. Misc. Inf. Kew 1907: 123, 1907.

= *A. burkillii* (Massee) Sacc. & Trott. in Sacc., Syll. Fung. 23: 302, 1925.

= *Cystoagaricus trisulphuratus* (Berk.) Single in Mycologia 39: 87, 1947.

Pileus 55 mm diam., broadly conic, convex; surface dry, flake-squamulose to sharp warty squamulose, erected at the disc then recurved to margin, deep orange, and fading by raining. *Context* 2 mm broad, white to cream. *Lamellae* free, more crowded, lamellulae with 3 series, 4-5 mm broad, narrow to normal, white, grayish pink, dull red (8B3). *Stipe* 22 × 5-7 mm, cylindrical, curved, with rhizomorphes, smooth and white above the annulus; thick flake squamulose and deep orange below annulus, hollow. *Annulus* perdent, superior, and mix with the stipe squame, deep orange. No staining on touching and cutting. Smell mushroomy.

Macrochemical reaction: KOH reaction negative.

Spores 5-6 × 3-4 µm [$\bar{x}$ =5.4 ± 0.4 × 3.6 ± 0.4, Q = 1.3-2, Q_m = 1.53 ± 0.47, n = 20], ellipsoid, smooth, reddish-brown. *Basidia* 12-16 × 6-7 µm, clavate, smooth, hyaline. *Cheilocystidia* 21-30 × 7-13 µm, clavate, broadly clavate, mostly subcapitate with apex of 4-7 µm diam., smooth or slightly fine spiny, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 2.5-5 µm diam., equal, branched, even light yellow, smooth or fine spiny, without constriction at the septa. *Annulus* and *stipitipellis* hyphae same to those of pileipellis.

Habit: solitary in soil of bank of road.

Material examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44. 009', elev. 900 m, 21 August 2005, collected by Ruilin Zhao, ZRL2132 (SFSU).

Note: *Agaricus trisulphuratus* is type species of subgenus Lanagaricus; section Lanagaricus. The definitions of *A. crocopeplus* Berk. & Br. is very close to *A. trisulphuratus*. *Agaricus trisulphuratus* is red, like fire color in fruiting bodies, the shape of spore is with an applanate adaxial surface (a little ship-shape); and *A. crocopeplus* is orange color, spores without obvious supra-apical depression, more round in outline (normal ellipsoid) (Heinemann 1956c; 1980). But those are difficult to apply in them identification. The Thai material has subcapitate cheilocystida in some cases is the uncommon place with Heinemann's description.

15. *Agaricus xantholepis* (F. Møller) F. Møller, Friesia 4: 204 1952, Fig. A.15; Plate E.18.a.

= *Psalliota xantholepis* F. Møller, Friesia 4: 191 1952.

Pileus 30-60 mm diam., hemispherical, then expending convex, plano-convex, disc truncated or slightly depressed; surface dry, covered with fibrillose squamulose, orange, greynish-orange (5B4) at the disc, pale to margin as lemon yellow against white background, appressed, radical,

glabrescent. *Context* firm, generally white, ocherous at disc. *Lamellae* free, crowded, lamellulae of 4-6 series, 4 -5 mm broad, normal or slightly ventricose, white, pink, light orangish-grey (8B2), brown, dark brown with age. *Stipe* 45-120 × 6-12 (base 10-25) mm, cylindrical, elongate clavate, bulbous and rhizomorphes; surface smooth, silky, white with pink tints above the annulus, while heavy squamulose, white with light yellow tints towards base below annulus, hollow, fibrous. *Annulus* 12-15 mm broad, cortinate when young then membranaceus, radiacal stiate, perdent, single, superior, persistent, edge torn, stretched, white or brownish orange with age. Smell almonds. No color staining on touching and cutting.

Macrochemical reaction: KOH-reaction yellow.

Spores 4.5-6 × 3-4 μm [$\bar{x}$ = 5.3 ± 0.4 × 3.5 ± 0.3, Q = 1.3-1.8, Q_m = 1.52 ± 0.28, n = 20], ellipsoid, without germ pore, brown. *Basidia* 14-22 × 6-8 μm, clavate, 4-spored. *Cheilocystidia* 16-30 × 9-18 μm, pyriform, hyaline or scattered orange vacuolar pigments. *Pleurocystidia* absent. *Pileipillis* a cutis consisted of hyphae of 5-8 μm diam., light yellow, smooth, cylindrical, no constriction or slightly constricted at the septa. *Annulus* consisted of hyphae of 4-10 μm diam., hyaline, smooth, branched. *Stipitipellis* consisted of the same hyphae of annulus.

Habit: Caespitose, gregarious in soil of forest.

Distribution: Europe, south America, Thailand.

Materials examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 8 June 2006, collected by Tim Baroni, ZRL3039 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw Village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 3 August 2004, collected by Ruilin Zhao, ZRL2110 (SFSU); Chiang Rai Prov., Mae Souy Dist., Pamae Lao Nat. Park, 2 August 2004, collected by Ruilin Zhao, ZRL3088 (SFSU).

Notes: KOH-reaction yellow, small fruiting bodies and almond smell indicate Thai materials belong in section Arvenses, subsection Minores (Heinemann 1978). *Agaricus xantholepis* is distinguished by lemon yellow pileus (Nauta 2000), and it differs from another yellow pileus species *A. tornocephalus* Berk. & Br. which has floccose-furfuraceous stipe and large annulus (Pelger 1986). *Agaricus lutosus* (F. Møller) F. Møller also has yellow pileus, but its tapering base of stipe and vaguely purplish tone at the disc make it different from Thai materials (Heinemann 1978, 1990a).

16 *Agaricus* sp 1

Pileus 55-110 mm diam., convex to plano-convex, flat or slightly depressed at the disc; surface dry, fibrillose scales, dense at the disc, then scattered to margin, sometimes scales rubbed by rain, light brown (7D4) to brown (7D7) against white background, with pink tone in wet. *Context* 5-7 mm broad, firm, white. *Lamellae* free, crowded, lamellulae with 3-5 series, 6-7 mm broad, normal, white, pink, pale red (7A3), light brown (6D4), brown, dark brown with age. *Stipe* 50-110 × 8-11 (base 10-17) mm, cylindrical-bulbous, smooth about annulus, squamulus below annulus, white, small hollow. *Annulus* membraneous, perdant, single, superior, upper side smooth and lower side fibrillose, fugacious, white, up to 10 mm broad. Smell almond. Staining light yellow to yellow on stipe (pileus not distinct) on touching and cutting.

Macrochemical reaction: KOH reaction yellow.

Spores (5.5-) 6-7.5 × 3.5-4 µm [$\bar{x}$ = 6.4 ± 0.6 × 3.9 ± 0.2, Q = 1.4-1.9, Q_m = 1.63 ± 0.27, n = 20], ellipsoid, smooth, brown. *Basidia* 15-22 × 7-9 µm, clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 20-25 × 10-13 µm, no abounded, and lacked in some samples, broad clavate to pyriform, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 5-10 µm diam., long cylindrical, light brown, smooth, slightly contricted at the septa. *Annulus* hyphae of 5-8 µm diam., apex inflated into 10-13 µm diam., cylindrical to long clavate, hyaline, smooth.

Habit: scattered or gregarious in the opened areas of forest.

Materials examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44. 009', elev. 900 m., 12 August 2005, collected by Kevin D Hyde, ZRL2124 (SFSU); same location, 13 August 2005, collected by Kevin D Hyde, ZRL2125 (SFSU).

Notes: This species is distinguished by middle to large size, light brown pileus and bulbous stipe. Yellow staining on touching and cutting and yellow KOH reaction, along with the almond smell suggest the materials should belong in subsection Arvenses.

17 *Agaricus* sp 2

Pileus 20-25 mm diam. (around 10 mm in young age), conic, hemispherical, broad conic, convex, then quickly expending to aplanate, top flat; surface floccose-scales brown against white background in young, then scales more appressed, fibrillose-scales against cream to light gray background in age, dense at the disc and scattered to margin, partial veil appendulated at the edge. *Context* 1 mm broad at the disc, white to light gray. *Lamellae* free, crowded, lamellulae with 3 series, 3 mm broad, normal to slightly ventricose, white, grayish white, brown, edge color lighter than gill itself. *Stipe* 30-40 × 2-3 mm, long cylindrical, curved, smooth to tomentose above

annulus, slightly squamulose when young, then fibrillose in age, white, hollow. *Annulus* membraneous, fugacious, perdent, single, superior, white, 2-3 mm broad. Staining yellow on touching and cutting, especial on stipe. Smell slightly almond.

Macrochemical reaction: KOH reaction yellow on pileus surface and context.

Spores 4.2-5.2 × 3.2-4 µm [$\bar{x}$ = 3.9 ± 0.2 × 3.6 ± 0.2, Q = 1.2-1.6, Q_m = 1.38 ± 0.22, n = 20], ellipsoid, smooth, reddish brown. *Basidia* 12-15 × 6-8 µm, stout clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 12-30 (-40) × 7-13 µm, clavate, broad clavate to pyriform, hyaline or with orange pigments, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 4-12 µm diam., smooth, slightly constricted at the septa, dark gray pigments distribution unevenly. *Annulus* hyphae of 3-5 µm diam., hyphae-like, hyaline to light yellow, arranged in regular parallel, unbranched.

Habit: gregarious in sandy soil of forest.

Material examination: THAILAND, Chiang Mai Prov., Chom Thong, Ob Luang National Park, elev. 1812 m., 15 September 2006, collected by Ruilin Zhao, ZRL3102 (SFSU).

Notes: This material has yellow staining, almond smell and small basidiome. That indicates it should belong in the subsection Minores of section Arvenses.

18 *Agaricus* sp 3

Pileus 27 mm diam., broadly conic, applanate; surface radical fibrillose scales, dense at the disc, reddish-brown against white background, cuticle exceeding in edge, split at the margin. *Context* 1 mm at the disc, white and ocherous at the centre of pileus. Lamellae free, crowded, lamellulae with 2-4 series, 2 mm browd, normal, white, pink, light brown to brown. *Stipe* 45 × 3 (base 6) mm, cylindrical, smooth to tomemtose both above and below annulus, white, hollow. *Annulus* fibrillose-membraneous, up to 6 mm broad, fugacious, torn, perdent, superior, stretched, white. Staining yellow on touching and cutting. Smell almond.

Macrochemical reaction: KOH reaction bright yellow.

Spores 4-5 × 3-3.5 µm [$\bar{x}$ = 4.7 ± 0.4 × 3.1 ± 0.2, Q = 1.3-1.7, Q_m = 1.52 ± 0.22, n = 20], ellipsoid, smooth, brown. *Basidia* 16-22 × 6-7 µm, hyaline, smooth, 4-spored. *Cheilocystidia* 15-22 × 10-14 µm, broad clavate, pyriform, hyaline, smooth, some samples lacked. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 7-13 µm diam., cylindrical, slightly constricted at the septa, brown to dark gray pigments distributed in dots and uneven, smooth. *Annulus* hyphae of 3-6 µm diam., cylindrical, hyaline, smooth, slightly constricted at the septa or not.

Habit: solitary in rich soil of forest.

Material examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44. 009', elev. 900 m., 10 July 2006, collected by Thanh Huyen Le, ZRL3080 (SFSU).

Notes: This species is similar to *Micropsalliota globocystidia* (Heinemann 1980) because they shared the reddish-brown pileus and small fruiting body (*M. globocystidia* has middle size fruiting bodies also) in the field. However *Agaricus* sp3 has pink gill, and *M. globocystidia* lacks pink color in the procedure of gill/spores mature. The endosporium spores, larger spores (6-7 × 3.5-4.2 µm), and incrusted pileipellus hyphae of *M. globocystidia* make difference from *Agaricus* sp3.

19 *Agaricus* sp 4

Pileus 30-96 mm diam., applanate, and applanate with uplifted margin, infundibuliform, appendiculate the remnants of veil at edge, some splitting, disc flat or slightly broad umbonate; surface dry, generally white, cream color, except disc covered with fibrils aggrageted into small fibrillose nodal, then sparse towards margin, no squame, disc yellowish brown (5E5) to dark grey, turn pink or reddish brown in wet. *Context* 6 mm broad at disc, firm, white and grayish brown. *Lamellae* far free, crowded, 3-6 series, breadth 5-7 mm, normal, pink, reddish brown, dull bown, brown. *Stipe* 120 × 8-10 (base 30) mm, clavate, cylindrical abruptly bulbous, smooth except base velvet, white, light brown, slightly yellow towards base, hollow. *Annulus* tough, thick, perdent or subperonate, superior, persistent, entire, rigid, up to 10 mm broad and 6 mm thick, pure white. Smell slightly almonds or mushroomy, not strong. No color staining on touching, slight yellow staining at the disc on cutting.

Macrochemical reaction: KOH-reaction bright yellow.

Spores 4.5-5.5× 3-4 µm [$\overline{x}$ = 5.1 ± 0.2 × 3.2 ± 0.3, Q = 1.3-1.7, Q_m = 1.61 ± 0.31, n = 20], ellipsoid, without germ pore, smooth, brown. *Basidia* 13-16 × 5-6 µm, mostly cylindrical, some clavate, hyaline, smooth, 4-spored. *Cheilocystidia* absent or basidia-like. *Pleurocystidia* absent. *Pileipillis* a cutis consisted of hyphae of 5-7 µm diam., long cylindrical, occasionally constricted at septa, brown vacuolated pigments. *Annulus* hyphae of 5-10 µm diam., inflated towards terminal, up to 20 µm diam., hyaline, cylindrical, smooth, branched, occasionally constricted at septa.

Habit: solitary in rich soil of forest.

Material examination: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma village, N18°48.62' E98°54.60', elev. 1145 m., 5 July 2006, collected by Ruilin Zhao, ZRL3077 (SFSU).

20 *Agaricus* sp 5

Pileus 40-45 mm diam., convex with truncate top, cuticle exceeding; surface dry, fibrillose scales dense at the disc, areola to margin, background cream to light grayish white, edge color lighter than gill itself. *Context* 2 mm broad at the disc, white and ocherous at the centre of pileus and stipe out layer. *Lamellae* free, crowded, lamellulae with 5 series, 3 mm broad, narrow to normal, and apex deflexed, white, light brown, brown. *Stipe* 75×5 mm, cylindrical, curved, with small rhizonorphes, smooth above the annulus, fibrillose to fibrillose scales below the annulus, white. *Annulus* membranous and lower side floccose, perdent, single, superior, fugacious, torn, stretched, white, up to 7 mm broad. Staining yellow at stipe on touching. Smell almond.

Macrochemical reaction: KOH reaction and Ammonia reaction negative.

Spores 5-6 × 3-4 µm [$\bar{x}$ = 5.6 ± 0.4 × 3.5 ± 0.3, Q = 1.4-1.9, Q_m = 1.63 ± 0.27, n = 20], ellipsoid, smooth, reddish-browm. *Basidia* 12-14× 5-7 µm, clavate, smooth, hyaline, 4-spored. *Cheilocystidia* 10-18× 6-8 µm, broad clavate, ellipsoid, some pyriform, hyaline, smooth. Pleurocystidia absent. *Pileipellis* a cutis of hyphae of 5-8 µm diam., long cylindrical, dark gray pigment distributed in plots unevenly, smooth. *Annulus* hyphae of elements of 5-10 µm diam, ellipsoid, cylindrical, hyaline, smooth. *Stipitipellis* hyphae similar to those of annulus.

Habit: scattered in rich soil of forest.

Material examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Hot Spring Nat. Park., 8 August 2006, collected by Ohnmar Myo Aung, ZRL3093 (SFSU).

21 *Agaricus* sp 6

Pileus 40-70 mm in diam., convex with inrolled margin, or concave with flared margin in age, cuticle exceeding, covered by floccose scale, snow white. *Context* 5-6 mm broad, firm, white. *Lamellae* free, crowded, lamellulae with 2-3 series, 4.5-6 mm broad, normal to slightly ventricose, dull red or reddish-brown, edge color distinct lighter than gill itself. *Stipe* 40-70 × 11-12 µm, cylindrical, taping to base, smooth above the annulus, floccose below the ring, but easy rubbed so appear smooth in the old fruiting bodies, white, small hollow. *Annulus* fugacious, and often

appendiculate the pileus margin but not stipe, middle or superior, torn. No staining on touching, rubescence on cutting.

Macrochemical reaction: negative.

Spores 5-7 × 4-5 µm [$\bar{x}$ = 5.9 ± 0.4 × 4.6 ± 0.4, Q = 1.1-1.5, Q_m = 1.28 ± 0.22, n = 20], ovate with apiculus, broad ellipsoid, brown, reddish-brown, smooth. *Basidia* 17-20 × 7-8 µm, clavate, smooth, hyaline, 4-spored. *Cheilocystidia* 16-21× 9-14 µm, broad clavate, pyriform, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 7.5-10 µm diam., elongate cylindrical arranged in parallel, smooth, hyaline. *Annulus* consisted by slender haphae of 2.5-5 µm diam., loose interwoven, hyaline, smooth, branched.

Habit: scattered in dung soil.

Material examination: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44. 009', elev. 900 m, 2 Aug. 2005, collected by Ruilin Zhao, ZR2109 (SFSU); same location, 19 September 2005, collected by Ruilin Zhao, ZRL2136 (SFSU).

Note: This species is similar to A. campetris, but differs from it which possessing larger spores (6.7-8× 4.6-5.3 µm) and cheilocystidia absent (Kerrigan 1986).

22 ***Agaricus* sp 7**

Pileus 60-80 mm diam., hemispherical, convex, plano-convex, some slightly depressed at the disc; surface floccose, and broken to scales to margin, light brown (6C3, 6B2). *Context* 7-9 mm, firm, white. *Lamellae* free, crowded, lamellulae with 7 series, 4 mm broad, narrow to normal, white, dull red (8C3), brown, edge color lighter than gill itself. *Stipe* 50-90 × 8-12 (base 14-16) mm, cylindrical to subbulbous, long clavate, smooth to silky, white. *Annulus* thick, rigid, perdent, white, entire, up to 12 mm broad. No staining on touching, strong scarlet staining on context on cutting. Smell mushroomy.

Macrochemical reaction: KOH reaction negative.

Spores 5.5-7 × 4.5-5.2 µm [$\bar{x}$ =6.3 ± 0.5 × 5 ± 0.2, Q = 1.1-1.4, Q_m = 1.27 ± 0.17, n = 20], ovoid to broad ellipsoid, brown, smooth. *Basidia* 21-26 × 7-9 µm, clavate, hyaline, smooth. *Cheilocystidia* and *Pleurocystidia* absent. *Pileipellis* a cutis of hyphae of 7 –15 µm diam., cylindrical, light brown, smooth. *Annulus* hyphae of 7 –15 µm diam., cylindrical, hyaline, smooth.

Habit: solitary or scattered in small group in dung soil.

Material examination: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 3 June 2006, collected by Ruilin Zhao, ZRL3012 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44. 009', elev. 900 m, 3 October 2005, collected by Ruilin Zhao, ZRL2137 (SFSU); same location, 5 August 2005, collected by Ruilin Zhao, ZRL2122 (SFSU).

23 *Agaricus* sp 8

Pileus 30 mm when young, then expending 50 – 55 mm in diam., convex with distinct broad umbo, margin deflexed, splitting with age, but never uplifted, surface dry, covered with fibrils, lacking squamulose, generally white or cream but grayish brown (5F5), black, some with green tongs at umbo, then disrupted towards the margin so appearing minute pulverulences. *Context* 3 mm thick at disc, white. *Lamellae* free, crowded, lamellulae with 4 series, 5 – 6 mm broad, ventricose, white, light orange grey (6B2), grayish brown (8D3), dull brown, dark brown. *Stipe* 40-70 × 4-5 (base 5 – 10) mm, cylindrical, subclavate, subbulbous, surface smooth, silky, white, hollow. *Annulus* 2 mm broad, bracelet-like, ephemeral, white. Smell pleasant, close to almonds. Staining reddish-brown on touching, no color change on cutting.

Macrochemical reaction: KOH-reaction bright yellow.

Spores 5.5-7 × 3-4 µm [$\bar{x}$ =6± 0.4 × 3.5 ± 0.3, Q = 1.4-2, Q_m = 1.75 ± 0.35, n = 20], elongate ellipsoid, without germ pore, smooth, brown. *Basidia* 12-19 × 6-8 µm, hyaline, smooth, 4-spored. *Cheilocystidia* 9-24 × 8-13 µm, scattered at edge, pyriform, ellipsoid, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis consisted of hyphae of 5-10 µm diam., brown vacuolated pigments on hyaline cell, cylindrical, some constricted at septa. *Annulus* hyphae consisted of 4-7 µm diam. hyphae, smooth, hyaline, branched.

Habit: solitary or scattered in small group in opening areas of forest.

Materials examination: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 10 June 2006, collected by Todd Osmundson, ZRL3044 (SFSU); Chiang Rai Prov., Mae Sai, Doi Tung, 3 August 2006, collected by Ruilin Zhao, ZRL3092 (SFSU).

24 *Agaricus* sp 9

Pileus 10-20 mm diam., broadly conic, convex, with broad subumbo, some truncate at the disc; surface floccose, broken in to floccose-squamulose to margin, orange brown at the disc then

fade to margin, yellowish-brown, orange, light brown against the white background. *Context* 1 mm thick, white, firm. *Lamellae* free, crowded, lamellulae with 4 series, 1 mm broad, normal, white, grayish-pink, brownish-orange (7C4), brown. *Stipe* 30-35× 2-3 mm, cylindrical, curved, smooth above the annulus, white; floccose squamulose below the annulus, especially near the base, orange brown against white background, hollow. *Annulus* rigid membraneous, upper side smooth, white; lower side fibrillose to floccose, with orange tone, peronate, single, superior, persistent, edge entire, up to 2 mm broad. No staining on touching and cutting.

Macrochemical reaction: KOH reaction yellow; Ammonia reaction fugacious gray on pileus surface and negative on context.

Spores 4.5-6 × 3-4 µm [$\bar{x}$ =5.2± 0.4 × 3.3 ± 0.3, Q = 1.4-1.8, Q_m = 1.6 ± 0.2, n = 20], ellipsoid, occasionally with slightly subaymbiform, smooth, reddish-brown. *Basidia* 15-17 × 6-7 µm, clavate, hyaline, smooth, 4-spored. *Cheilocystidia* (15-) 20-42 × 3-5 µm, elongate clavate, long stick-like, flexed, mostly with one septa, hyaline, smooth. *Pleurocystidia* absent. *Pileipellus* a cutis composed of hyphae of 5-7 µm diam., long cylindrical, without constriction at the septa, smooth, hyaline to light brown. *Annulus* hyphae of 3-5 µm diam., smooth, hyaline, no constriction at the septa.

Habit: solitary or scattered in small group in rich soil of forest.

Material examination: THAILAND, Chiang Mai Prov., Chiang Dao Cave, 22 July 2006, collected by Ruilin Zhao, ZRL3086 (SFSU); Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma village, N18°48.62' E98°54.60', elev. 1145 m., 7 June, 2006, collected by Maria Alice Neves, ZRL3032 (SFSU).

Notes: this species is distinguished by small orange brown, floccose-squamulose pileus, and stick-liked cheilocystidia. The molecular analysis showed this species is close to *A. angusticystidiata* and *A. maeseaensis* which both have flexed stick-like cheilocystidia. All them comprised a subclade which is sister to the clode of subgenus *Agaricus* in the *Agaricus* clade.

25 *Agaricus* sp 10

Pileus 12 – 36 mm in diam., hemispherical, expending broad conic, convex with broad umbo and deflexed margin in most cases, some without umbo, cuticle exceeding; surface dry, glabrous to fibrillose, lacking squame, generally white or cream, disc light orange, brownish orange (6C5), fading to margin. *Context* 2 mm thick at disc, firm, white with grayish brown tinge. *Lamellae* free, less crowded or slightly distant, lamellulae with 3 series, 4 – 5 mm broad, broad and

ventricose, grayish brown (7D2, 7D3), brownish grey, dark brown. *Stipe* 18-80 × 2-4 (base 4-5) mm, cylindrical, subclavate, rhizomorphes, smooth above the annulus, tomentose below the annulus and heavier towards the base, white to gray tinge, hollow or staffed. *Annulus* membranaceus, perdent, single, superior, fugacious, torn, stretched, with radiate stiate, white, up to 4 mm broad. Smell carbolic acid and ink. No staining on touching and cutting.

Macrochemical reaction: KOH-reaction brightly yellow.

Spores 5.5-6.5 × 3.2-4 µm [$\bar{x}$ =6± 0.2 × 3.8 ± 0.3, Q = 1.4-2, Q_m = 1.61 ± 0.39, n = 20], cymbiform to ellipsoid, some endosporium, without germ pore, smooth, reddish-brown. *Badia* 13-18 (-22) × 4-7 µm, hyaline, smooth, 4-spored. *Cheilocystidia* 12-32 × 7-16 µm, pyriform, broad clavate, smooth, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis consisted of hyphae of 4 – 9 µm diam., light yellow or hyaline, smooth, branched, no constricted at the septa. *Annulus* consisted of same hyphae of pileipellis.

Habit: solitary or scattered in small group in red soil.

Materials examination: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma village, N18°48.62' E98°54.60', elev. 1145 m., 2 July 2005, collected by Thanh Huyen Le, ZRL2081 (SFSU); same location, 7 June 2006, collected by Thanh Huyen Le, ZRL3034 (SFSU); same location, 13 June 2006, collected by Todd Osmundson, ZRL3063 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 30 June 2006, collected by Ruilin Zhao, ZRL3074 (SFSU); same location, 5 September 2006, collected by Ruilin Zhao, ZRL3098 (SFSU) and ZRL3099 (SFSU).

Notes: This species has the characters of *Micropsalliota* and *Agaricus* together: having cymbiform and endosporium spores (like *Micropsalliota*) and non-capitate cheilocystidia and smooth pileipellis hyphae (like *Agaricus*). But the molecular analysis indicated it should belong in *Agaricus.*

26 *Agaricus* sp 11

Pileus mostly 30-60 mm diam., hemispherical, conical, broadly conical, convex, plano-convex with subunbonate in age; surface dry, covered with acute squame, floccose, erect at center and recurved to margin part; golden yellow (5B8), orange, reddish-orange (7A8), deep orange (5A8), and fading with age and raining. *Context* firm, white. *Lamellae* free, crowded to more crowded, narrow to normal, white, orange-white (6A2), reddish-brown (8E3), grayish-pink,

grayish-brown (8D3, 6D3), brown (7E8), dark brown with age, lighter-colored at edge. *Stipe* 20-90 × 3-7 mm, cylindrical, mostly with rhizomorphs, smooth, white above ring, floccose, flaky, shaggy, same color the pileus below ring, hollow. *Annulus* perdent, same color with the flake of stipe's surface, in most cases difficult to be recognized from those flakes. Smell odourless or mushroomy. No color staining on handing and cutting.

Macrochemical reaction: KOH-reaction yellow at pileus.

Spores 3.5-4.5×3-4μm [$\bar{x}$ = 5.1 ± 0.3 × 3.3 ± 0.3, Q = 1.3-1.8, Q_m = 1.57 ± 0.27, n = 20], ovoid, ellipsoid, with an applanate adaxial surface, reddish brown. *Basidia* 12-20 (-23) × 5.5-7 (-8) μm, clavate, 4-spored. *Cheilocystidia* 18-36 × 6-13 (-15) μm, clavate, cylindrical with taping base, occasionally with 1 sepeta, surface spiny in some cases, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 3-5 μm diam., long cylindrical, curved, brunched, fine spiny. *Annulus* and *stipitipellis* consisted by the same hyphae of pileipellis.

Habit: mostly solitary, some scattered in small group in soil.

Material examination: THAILAND, Chiang Mai, collection data collector unknown, ZRL1003 (SFSU); Chiang Mai Prov., Chiang Dao Cave, 22 July 2004, collected by Ruilin Zhao, ZRL3089 (SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2005, collected by Jennifer Kerekes, ZRL2045 (SFSU); same location, 3 July 2005, collected by Ruilin Zhao, ZRL2087 (SFSU); same location, 18 August 2004, collected by Edward Grand, ZRL2128 (SFSU); same location, 3 June 2006, collected by Ruilin Zhao, ZRL3014 (SFSU); same location, 18 June 2006, collected by Ruilin Zhao, ZRL3070 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 11 August 2004, collected by Kevin D Hyde, ZRL2123 (SFSU); same location, 22 May 2006, collected by Ruilin Zhao, ZRL3002 (SFSU); same location, 29 May 2006, collected by Ruilin Zhao, ZRL3009 (SFSU); same location, 24 June 2006, collected by Ruilin Zhao, ZRL3072 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw Village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 3 August 2004, collected by Edward Grand, ZRL2111 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 12 June 2004, collected by Ruilin Zhao, ZRL3054 (SFSU).

Notes: this species is similar to *A. trisulphuratus* and *A. crocopeplus* in deep orange, flake squamulose fruiting bodies. However those 2 known species has larger spores (5.3-6.2 × 3.6-4.3 μm and 4.9-5.7 × 3.4-3.9 μm respectively) (Heinemann 1956c). The molecular analysis also

support *Agaricus* sp11 is different from *A. trisulphuratus*. Furthermore, even those 4 samples of *Agaricus* sp 11 are slightly morphological varieties in cheilocystidia shape (from clavate to cylindrical) and pileus shape (umbonate or flat top in pileus), their molecular phylogeny are identical.

27 ***Agaricus*** **sp 12**

Pileus 15-20 mm diam., comvex, plano-convex, slightly subumbo; surface radical fibrillose, reddish-brown at the disc, and scattered fine fibrillose nodes, white background, margin slightly splitting, cuticle exceeding. *Context* 1 mm thick, white. *Lamellae* free, crowded, lamellulae 2 series, 2 mm broad, normal, white, light pink, reddish-brown. *Stipe* 30-35 × 2 mm, cylindrical, straight, smooth above annulus, tomemtose below the ring, white, hollow. *Annulus* membraneous, perdent, single, superior, fugacious, white, up to 2 mm broad. Staining light yellow on the base of stipe on cutting, and no staining on touching. Smell almond.

Macrochemical reaction: KOH reaction yellow on the pileus surface and context.

Spores 4.5-5.5 × 3-4 µm [$\bar{x}$ = 5 ± 0.2 × 3.3 ± 0.3, Q = 1.1-1.7, Q_m = 1.53 ± 0.42, n = 20], ellipsoid, smooth, brown. *Basidia* 11-17 × 7-8 µm, clavate, smooth, hyaline, 4-spored. *Cheilocystidia* and *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 5-8 µm diam., long cylindrical, some constricted at the septa, hyaline or gray. *Annulus* hyphae similar to those of pileipellis except of only having hyaline hyphae.

Habit: solitary in soil of forest.

Material examination: THAILAND, Chiang Rai Prov., Mae Souy Dist., Pamae Lao Nat. Park., 2 August 2006, collected by Ruilin Zhao, ZRL3091 (SFSU).

Notes: *Agaricus* sp 12 is similar to *Agaricus* sp 3, but differs in species 3 having fibrillose scales and having cheilocystidia.

Chapter 5

RESULTS AND DISCUSSION Part B: a worldwide monograph of *Cyathus* and its molecular phylogeny

5.1 Generic description and type species

Cyathus Haller, Strip. Helvet., 3: 127, 1768; ex Pers. Syn. Meth. Fung.: 236, 1801.

- *Cyathia* P. Brown, Civ. and Nat. Hist. Jamaica: 78, 1756.

Type species: *Cyathus striatus* (Huds.: Pers.) Willd.

Generic description: fruiting bodies distinctly cup-shaped or goblet-shaped; peridium consisted of 3 layers, tough; outside of peridium coved with hairs, tomentose, hirsute or shaggy, and inner surface smooth or grooved; the month closed until maturity by a membrane (the epiphram) which disappear with age; Peridiole (gleba) lens-shaped and attached with peridium with thread-like cord (funiculus), mostly brown or dark brown color, some covered with tunica; basidia scattered irregularly throughout a large central area of peridiole, and not forming a homogeneous hymenium, basidia become empty and gelatinize with the forming of spores. Basidiospore hyaline, smooth and mostly with thick wall, while the shape and size are varied.

Peridioles release passive by water-splash action, and basidiospore spread by the discomposed of cortex of perodioles. The members of bird's nest fungi possess the funnel-shape or vase-shape, which is splash-cups. Peridioles contain inside, which ejected to a distance of several feet by the force of falling raindrops which land in the open mouth of the cups. However the information about the long-distance dispersal is limited.

Habit, habitat and distribution: all *Cyathus* species are saprophytic and usually can be found in moist, partly shaded location, such as edge of forest, on trails; also some growing in such places as pastures and grain field where are moist; a few species grow under moderate desert conditions. The more likely to find *Cyathus* are decaying twigs, wood, dung or rich soil.

This genus is widely distributed, through temperate, subtropical and tropical areas.

5.2 Taxonomical and informative features

5.2.1 Fruiting body

Shape. The fruiting bodies are all basically infundibuliform, and there slightly varied among the fruiting bodies within one collection. But main shape of fruiting bodies in every collection or species always fall into 3 kinds of forms generally: i) slender inverted cone, which the height is much longer than those of width of mouth, and base constricted as a long stipe; ii) broad inverted cone, which the height and width at mouth almost are equal, and base constricted as a very short stipe or directly touch the substrate; and iii) porcelain crucible shape, which often is with curved sides (Fig. 5.1). Furthermore the mouth character also is important in species identification. Mostly mouth is straight, but some species can be flared (e.g. *C. olla*), or incurved (e.g. *C. limbatus*) (Fig. 5.2).

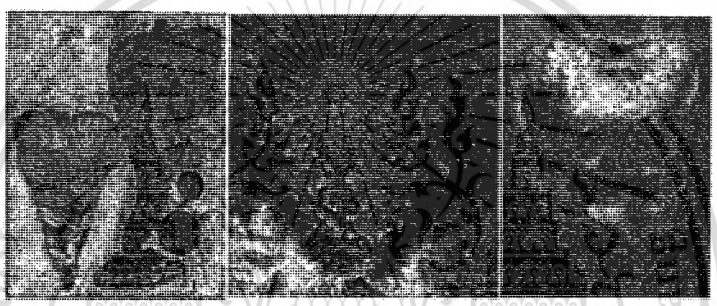

Fig. 5.1 Main shapes of fruiting bodies in *Cyathus*. 1. slender inverted cone; 2. broad inverted cone; 3. porcelain crucible shape.

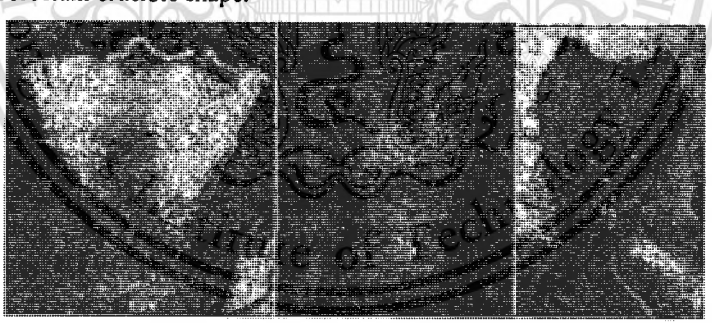

Fig. 5.2 The mouth of fruiting bodies and hairs types in *Cyathus*. 1. straight mouth with hirsute hairs. 2. flared mouth with tomentum hairs. 3. incurved mouth with shaggy hairs.

Size. The dimensions of the fruiting bodies in terms of widths at the top (mouth) and total height are given in description.

Color. The color of inner and outer side of peridium need be documented. Generally the colors are basically identical among different fruiting bodies in same collection, but may change

Peridium. Peridium is a term applies to the outer bounding wall of fruiting body. The outer surface of peridium wall is covering the hairs. The term of tomentum suggests the hairs are fine woven, velvet like; hirsute suggests the coarse hairs aggregate into small tufts; and shaggy or wooly suggests the long and coarse hairs aggregate into long clusters (Fig.5. 2), but the hairs on some specimens might be rubbed off or fell down by age. The wall of the peridium in *Cyathus* may appear, either externally or internally (or both), to be smooth (inner surface), striate, sulcate or plicate. The microscopy of peridium wall have not used in the species identification, but it is an important character in distinction of different genera in Nudilareaceae.

Setae. Hyphae at the lip of fruiting bodies are aggrageted into very conspicuous bristles called setae, which is an diagnosis character in some species e.g. *C. striatus*, *C. setosus.* If those hyphae aggragated into tufts which is not so distinct as setae, called fimbriate, and most *Cyathus* species possess it.

Emplacement. The emplacement refers the solid mass of hyphae at the base of the fruiting body, and it is conspicuous in some species.

5.2.2 Peridiole

Peridioles are refered to the numerous lenticular bodies within the peridium, and each peridioles is a hymenial layer consisting of basidia, basidiospores, and intermingled with paraphyses. When the peridiole is mature, the central part of it contains mass of basidiospores, most of which have separated from their basidia and lie free from each other.

Macrocharacters. The shape, size and color are documented in description.

Tunica. The outermost layer of the peridiole called tunica. Under the microscope tunica consists of grey, light brown or brown hyphae which interwoven looser than those of cortex, and some species (e.g. *C. stercoreus*) lack tunica. The thickness of tunica is varied.

Cortex. The layer or layers beneath tunica or directly exposed as outmost layer, which is hard dark-colored coat of compacted hyphae. Two main types of cortex had been recognized: i) single cortex, only one layered cortex; and ii) double cortex, the cortex consists of two distinct dark layers of compacted hyphae, which separated by a band of light colored and loosely interwoven hyphae.

5.2.3 Basidiospores

All *Cyathus* species possess smooth and hyaline spores, so only size and shape of basidiospores have the value of species identification. The phylogenetic research based on rDNA sequences had pointed the size of spore has remarkable significance (Zhao et al 2006).

5.3 Molecular phylogeny

5.3.1 Phylogeny based on ITS sequence data

The ITS dataset consisted of 17 samples representing 16 *Cyathus* taxa, *Crucibulum laeve, Nidula niveotomentosa,* and *Cystoderma amianthinum* as an outgroup. This dataset consisted of 776 characters of which 125 characters were ambiguous and were excluded in the analysis, and 168 characters were parsimony-informative.

For the weighted parsimony analysis, Stmatrix was used to assign appropriate parameters and gaps were treated as missing data. This yielded 1 equally parsimonious tree with a length of 468 steps (CI=0.776, HI=0.224, RI=0.746). For maximum likelihood analysis the model selected by MrModeltest 2.2 was GTR+G, and the resulting tree has a likelihood score of 2969.71694. Table 2 shows the results of the Kishino-Hasegawa and Shimodaira-Hasegawa tests among topologies obtained from ML, NJ and WP. The ML tree (Fig. 5.3) was the best tree and therefore chosen to represent ITS phylogenies.

Trees generated from MrBayes yielded similar topologies as those from ML and WP and were not significantly different. All gene trees were characterized by 3 major clades (herein designated Clades A, B and C; Fig. 5.3), although there were slight differences in the topological arrangement within the clades. Clade A includes *C. africanus* (type specimen and a second specimen), *C. africanus* var. *latisporus, C. colensoi, C. hookeri, C. jiayuguanensis, C. olla* and *C. olla* f. *brodiensis* with 100% PP and 98% BS supports. Clade B is composed of *C. annulatus, C. crassimurus, C. renweii, C. setosus, C. stercoreus* and *C. triplex* but had PP and BS supports of lower than 50%. Clade C comprises *C. berkeleyanus, C. gansuensis* and *C. pallidus* with 100% PP and 83% BS supports.

In the ML ITS tree (Fig. 5.3), *C. hookeri* nested with *C. olla* and *C. olla* f. *brodienensis* with 95% PP and 67% BS supports, and of 776 aligned nucleotide sites, *C. hookeri, C. olla* and *C. olla* f. *brodienensis* differed by only 5 base pairs.

In the ML ITS tree (Fig. 5.3), *C. hookeri* nested with *C. olla* and *C. olla* f. *brodienensis* with 95% PP and 67% BS supports, and of 776 aligned nucleotide sites, *C. hookeri*, *C. olla* and *C. olla* f. *brodienensis* differed by only 5 base pairs.

4.3.2 Phylogeny based on LSU sequence data

In the LSU dataset, 19 sequences were included, consisting of 16 *Cyathus* taxa, *Crucibulum laeve*, *Nidula niveotomentosa* and the trees were rooted with *Cystoderma amianthinum*. This dataset consisted of 797 characters of which 10 characters were excluded, and 69 were parsimony-informative.

Weighted parsimony analysis treated gaps as missing data and yielded a single tree with length of 193 steps (CI=0.736, HI=0.264, RI=0.669). Maximum likelihood analysis with likelihood settings with the best-fit model (GTR+G) resulted in a tree with a score of 2020.58832. Results of the Kishino-Hasegawa and Shimodaira-Hasegawa tests indicated that the ML analysis yielded the best tree (Fig. 5.4), although not topologically different from the WP results.

Maximum likelihood analysis of the LSU dataset shows that *Cyathus* species group into 3 Clades (A, B and C) that are essentially the same as those obtained from ITS dataset, albeit with different statistical support. Clade A with 69% BS support and less than 50% PP support comprises *C. griseocarpus*, *C. guandishanensis* and *C. olla* f. *anglicus*, together with four species from Clade A in the ITS tree, viz., *Cyathus africanus* (type), *C. africanus* var. *latisporus*, *C. jiayuguanensis* and *C. olla*. Clade B with 99% PP and 61% BS support consists of *Cyathus helenae* and *C. poeppigii* together with three species from Clade B of the ITS tree, viz., *C. annulatus*, *C. renweii* and *C. stercoreus*. Clade C with 93% PP and 73% BS support comprises *C. olla* f. *lanatus* and two species from Clade C of the ITS tree, viz. *C. gansuensis* and *C. pallidus*. One major topological difference between the ITS and LSU trees concerns the position of *C. setosus*. In all ITS analyses, *C. setosus* was nested in Clade B. However, in all LSU analyses, *C. setosus* was a sister taxon of clades A, B and C in an unresolved polytomy.

5.3.3 Phylogeny of the combined datasets

The *Cyathus* taxa that successfully provided both ITS and LSU sequences were used to construct the combined dataset. In this data matrix there were 10 *Cyathus* taxa, *Crucibulum laeve*,

Nidula niveotomentosa and outgroup *Cystoderma amianthinum* with a total of 1567 characters out of which 216 were parsimony-informative and 138 characters were excluded.

WP analysis resulted in a single tree with a length of 594 steps (CI=0.798, HI=0.202, RI=0.709). Maximum likelihood settings were from the best-fit model (GTR+I+G) and ML analysis yielded a tree with the score of 4781.34621. The Kishino-Hasegawa and Shimodaira-Hasegawa tests indicate that WP, ML and NJ trees were not significantly different, and ML tree (Fig. 5.5) is the best. The Bayesian tree also resulted in 3 major clades with strong PP support and had the same internal topologies as the WP, NJ and ML trees, although the relationships amongst the clades were not strongly supported. Species in Clade A grouped with 100% PP and 100% BS support; species in Clade B grouped with 100% PP and 69% BS support; while those in Clade C grouped with 100% PP and 99% BS support. *Cyathus setosus* was outside all three major clades. In all three analyses (ITS, LSU, combined ITS-LSU) species of *Cyathus* formed a monophyletic clade with 100% PP and 100% BS support, although the relationship with other bird's nest fungi was unresolved.

5.3.4 Phylogeny of *Cyathus olla* and its forms

In this research, the holotypes of *C. olla* f. *lanatus* and *C. olla* f. *anglicus* (= *C. anglicus*), authentic material (Brodie-identified) of *C. olla* f. *olla*, and material of *C. olla* f. *brodiensis* identified by R. Zhao were used in the molecular study. Our phylogenetic analyses showed that *C. olla* f. *olla*, *C. olla* f. *anglicus* and *C. olla* f. *brodiensis* are closely related and all cluster in Clade A (Figs. 5.3-5). However, the position of *C. olla* f. *lanatus* in the LSU tree (Fig. 5.4) was in Clade C with 93% PP and 73 BS supports.

5.3.5 Phylogeny of *Cyathus jiayuguanensis, C. africanus and C. africanus* var. *latisporus.*

The results of ITS, LSU and the combined dataset (totaling 1567 bases) analyses using sequences from holotype specimens showed that *C. jiayuguanensis* and *C. africanus* var. *latisporus* have identical sequences. *Cyathus africanus* (SWFC 20782) nested with *C. jiayuguanensis* and *C. africanus var. latisporus* in the ITS tree (Fig. 5.3), but was distant from the holotype of *C. africanus*.

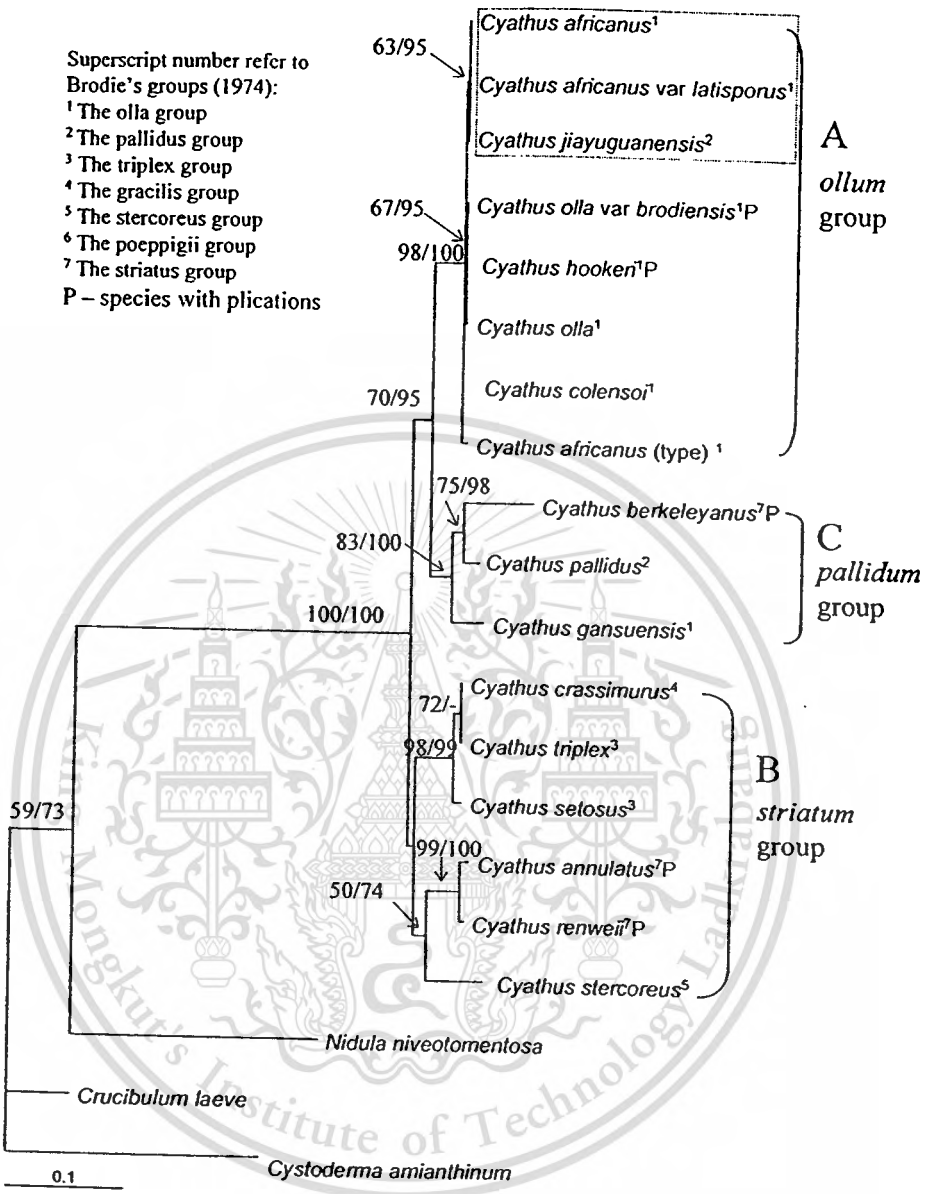

Fig. 5.3 Phylogeny of *Cyathus* inferred from ITS rDNA sequences. The maximum likelihood tree was rooted with the outgroup species *Cystoderma amianthinum*. Bootstrap support (BS) from 1000 replicates in a heuristic search and Bayesian posterior probabilities (PP) values of more than 50% are shown above nodes (BS/PP) and "-" refers to values less than 50%. Branch length is proportional to number of substitutions. *Cyathus jiayuguanensis* = *Cyathus africanus* var. *latisporus* is shown within the dotted box.

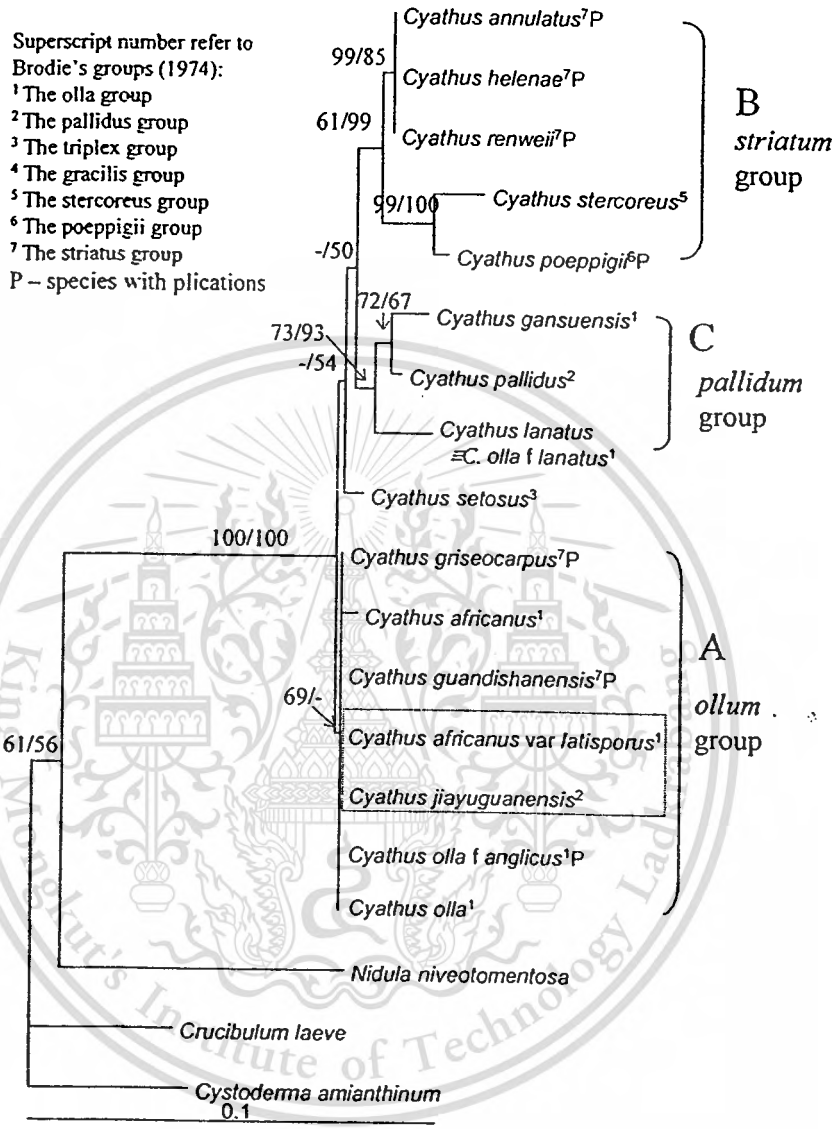

Fig. 5.4 Phylogeny of Cyathus inferred from LSU rDNA sequences. The maximum likelihood tree was rooted with Cystoderma amianthinum. Bootstrap support (BS) from 1000 replicates in a heuristic search and Bayesian posterior probabilities (PP) values of more than 50% are shown above nodes (BS/PP) and "-" refers to values less than 50%. Branch length is proportional to number of substitutions. Cyathus jiayuguanensis = Cyathus africanus var. latisporus is shown within the dotted box.

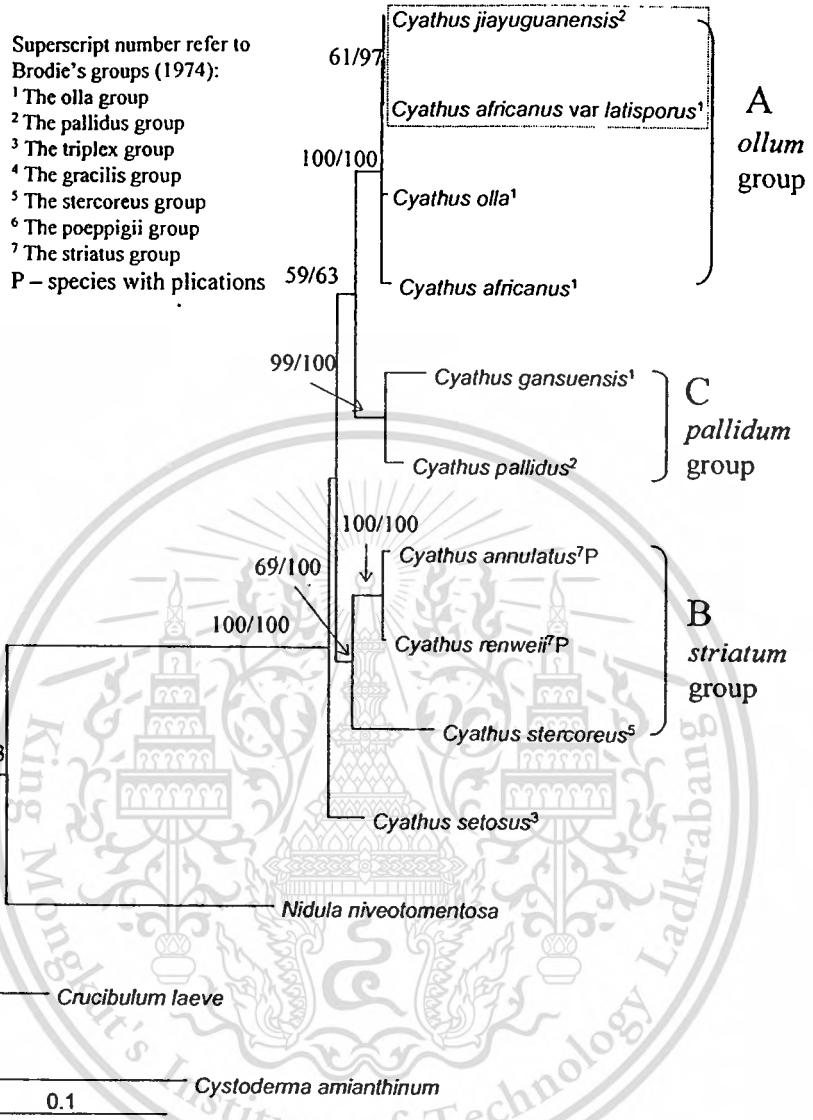

Fig. 5.5 Phylogeny of Cyathus inferred from combined ITS-LSU rDNA sequences. The maximum likelihood tree was rooted with the outgroup species Cystodrma amianthinum. Bootstrap support (BS) from 1000 replicates in a heuristic search and Bayesian posterior probabilities (PP) values of more than 50% are showed at the nodes (BS/PP) and "-" refers to values less than 50%. Branch length is proportional to number of substitutions. Cyathus jiayuguanensis = Cyathus africanus var. latisporus is shown within the dotted box.

5.4 Keys to *Cyathus* species

5.4.1 Key to new groups of *Cyathus*

Based on analyses of the morphology of 115 *Cyathus* specimens and on phylogenetic analyses of ITS, LSU and combined ITS-LSU datasets of a subset of these specimens, *Cyathus* were segregated into 3 species groups. To avoid confusion with the species group names of Brodie (1975), the new groups are named the "ollum" group, "striatum" group and "pallidum" group (Zhao et al. 2007). Those groups were mainly defined by basidiospores size and covering characters of the outside of peridium, then supplement by the color and base shape of fruiting body. Herein those *Cyathus* species were arranged based on the new 3-sproups.

The species without molecular data are fit into this 3-groups only based on the morphological definitions of those 3 groups. *Cyathus crispus, C. microporus* grouped in ollum and pallidus groups respectively are "phylogenetic affinity uncertain", because of the ambiguous morphological characters in grouping.

1 Basidiospores 15 µm or longer (at least the mean equal or longer than 15 µm); fruiting body brown, reddish-brown or dark brown in most case, less light-colored peridium..... *striatum* group

1 Basidiospores less than 15 µm long (at least the mean equal or longer than 15 µm); fruiting body light yellow, orange, gold, gray or yellowish-brown in most cases, rare dark colored.....2

2 Covering of the outside of peridium of fine, short and soft hairs, sometime aggregated into tufts, hirsute; the base of fruiting body usually abruptly constricted into a distinct stipe; ..... *ollum* group

2 Covering of the outside of peridium with a thick tomentum, felt-like and usually shaggy or woolly; the fruiting body not abruptly constricted, with a broad attachment in most cases..... *pallidum* group

5.4.2 Key to species of striatum group

1 Inner surface of peridium smooth or distant shallow sulcate.....2

1 Inner surface of peridium disctinct crowded placation.....9

2 Basidiospores huge, 27.5-35 × 23.8-38.8 µm.....*C. stercoceus*

2 Basidiospores smaller than above, never more than 30 µm long.....3

3 Lip of peridium distinct stiff setae..... *C. setusus*

3 Lip of peridium smooth or fimbriate.....4

4 Peridioles single cortex with tunica.....5

4 Peridioles double cortex with or without tunica.....6

5 Fruiting bodies small 4-5 × 4-5 mm, cortex thick 70-80 µm, tunica 25 µm.....*C. minmus*

5 Fruiting bodies larger 5-6 × 4-7 µm, cortex thin 20 µm, tunica thick 40-50 µm...*C. crassimurus*

6 External surface of peridium near black and internal surface white; basidiospores 21-28 × 14-18 µm.....*C. nigroalbus*

6 External and internal surfaces of peridium not like above; basidiospores 14-20 × 10-14 µm.....7

7 Fruiting bodies with long stipe (one third of height of fruiting body).....*C. gracilis*

7 Fruiting bodies without long stipe.....8

8 Peridioles double cortexes with tunica.....*C. elmeri*

8 Peridioles double cortexes without tinica.....*C. triplex*

9 Peridioles double cortexes with or without tunica.....10

9 Peridioles single cortex with tunica.....12

10 Tunica present; basidiospores 10-17.5 × 7.5-10.6 µm (mean = 15.4 × 8.6 µm)....*C. luxiensis*

10 Tunica absent; basidiospores larger than above.....11

11 Basidiospores 31-39 × 19-25 µm.....*C. peoppigii*

11 Basidiospores 18-22 × 12-15 µm.....*C. limbatus*

12 Basidospores globe, subglobe (Q_m< 1.4) 13

12 Basidiospores ellipsoid to long ellipsoid (Q_m > 1.4).....15

13 Both surfaces of peridium pale color, Q_m = 1.12 ± 0.19.....*C. subglobisporus*

13 Peridium dark color, Q_m = 1.2-1.4.....14

14 Basidiospores 18.8-25 × 15-20 µm.....*C. yunnanensis*

14 Basidiospores 12.5-21 × 11-15 µm.....*C. olivaceobrunneus*

15 Fruiting bodies distinctly flared at the mouth.....16

15 Fruiting bodies straight at the mouth 17

16 Plication distant on the inner surface of peridium; lip smooth.....*C. durus*

16 Plication crowded on the inner surface of peridium; lip dark brown fimbriate.....*C. helenae*

17 Basidiospores long, more than 20 µm.....18

17 Basidiospores 14-20 µm long.....19

18 Basidiospores long ellipsoid to cylindrical, 21.2-26.7 × 7-10 µm; Q_m=2.75.....*C. hirtulus*

18 Basidiospores ellipsoid, Q_m=2 .....*C. renweii*

19 Peridioles plump and hard; tunica thick 30-40 µm.....*C. montagnei*

19 Peridioles thin and not hard; tunica thin 10-25 µm.....20

20 Fruiting bodies brown, bleach brown, light brown; lip fimbriate.....*C. annulatus*

20 Fruiting bodies dark brown, chocolate brown; lip setae.....*C. striatus*

5.4.3 Key to species of ollum group

1 Peridium distinct and crowded plicate.....2

1 Peridium smooth or faintly distant plicate.....8

2 Peridioles double cortexes without tunica.....*C. crispus*

2 Peridioles single cortex with tunica.....3

3 External surface of peridium near black.....4

3 External surface of peridium pale brown, brown to reddish brown.....5

4 Fruiting bodies 7-9 × 4-6 µm; crowded plications on the inner surface of peridium.....*C. lijiangensis*

4 Fruiting bodies huge 11-14 × 11-14 µm; distant grooved on the inner surface of peridium .....*C. olla* f. *anglicus*

5 Basidiospores 7-8 × 4-6 µm; peridium ivory, brownish yellow with long hirsute hairs.....*C. griseocarpus*

5 Basidiospores 8-14 × 5.5-10 µm.....6

6 Basidiospores ellipsoid; peridioles with thick tunica (60 µm).....*C. olla* f. *brodiensis*

6 Basidiospores ovoid, mostly with an distinct apiculus.....7

7 Basidiospores 10.6-13.8 × 8.8-10 µm; tunica near black.....*C. pullus*

7 Basidiospores 8.8-12.5 × 5.6-8.2 µm; tunica light brown.....*C. tianshanensis*

8 Basidiospores with distinct apitulus in most cases.....9

8 Basidiospores without apitulus in most cases.....11

9 Basidiospores more close to globe, Q = 1.1-1.5, Q_m=1.3; inner surface of peridium smooth.....*C. jiayuguanensis*

9 Basidiospores longer, Q = (1.1) 1.3-2; inner surface of peridium faintly plicate.....10

10 Inner surface of peridium light color; lip minutely fimbriate; Q_m= 1.6.....*C. aficanus*

10 Inner surface of peridium dark gray; lip distinct fimbriate, dark brown;

Q_m=1.38.....*C. guandishanensis*

11 Fruiting bodies curved in side view; mouth distinct flared outwards.....*C. olla*

11 Fruiting bodies straight in side view; mouth straight.....12

12 Basidiospores 12.5-16.2 × 8 –12.8 µm.....*C. intermedia*

12 Basidiospores less than 10 µm long.....13

13 Peridium surface faintly plicate.....*C. hookeri*

13 Peridium surfaces completely smooth.....14

14 Fruiting bodies light color, outside covered fine hairs, tomemtose.....*C. colensoi*

14 Fruiting bodies light color also, outside covering hirsute.....*C. julietae*

5.4.4 Key to species of pallidum group

1 Peridium distinctly plicate.....2

1 Peridium smooth or faintly plicate on the inner surface.....4

2 Basidiospores globe to subglobe Q_m = 1.1; peridium pale color.....*C. bulleri*

2 Basidiospores ellipsoid Q_m = 1.5-1.7; peridium brown, dark brown.....3

3 Fruiting bodies 7-12 × 4-6 mm; mouth straight.....*C.* aff. *berkeleyanus*

3 Fruiting bodies 14-23 × 8-10 mm; mouth flared outwards.....*C. cornucopioides*

4 Inner surface of peridium faintly plicate; basidiospores less than 10 µm long.....5

4 Inner surface of peridium completely smooth; basidiospores more than 10 µm long.....6

5 Peridium dark brown, chocolate brown; fruiting bodies height and width almost

equal.....*C. microporus*

5 Peridium pale brown, yellowish brown; fruiting bodies height much longer than

width.....*C. confuses*

6 Fruiting bodies funnel-shaped; basidiospores 10.5-13.5 × 8.5-10.5 µm.....*C. gansuensis*

6 Fruiting bodies crucible-shaped; basidiospores width 5 –8.8 µm.....7

7 Peridioles tunic and cortex totally 80-100 µm broad .....*C. lanatus*

7 Peridioles tunica and cortex totally 30-35 µm broad.....*C. pallidus*

5.5 Description of species

1 ***Cyathus africanus*** H.J. Brodie, Can. J. Bot. 45:1653, 1967. Fig. B.1

Fruiting bodies 4-7 × 5-9 mm, abconic, funnel-shaped with age, base abrubtly constricted into a distinct short stipe; exterior of peridium yellowish-brown and brown, the old one dark brown, covering of peridium with fine hairs, and longer towards the base, some agerated into small nodules; inside of peridium usually gray, some dark gray or yellow-gray, no plications or faint plications on the inner of wall; lip minutely brown fimbriate, no setae; attach substrate with emplacement, up to 7 mm diam. *Peridioles* 1.5-2.5 mm diam., thin, circular or subcircular shape, silvery; one cortex 20-40 µm with tunica 20-30 µm. *Basidiospores* 7.5-12.5 × (5.6-) 6-8 µm [$\bar{x}$ = 10.5 ± 1.4 × 6.6 ± 0.8, Q = 1.3-2, Q_m = 1.3 ± 0.4, n = 20], ovoid or broadly ellipsoid, some ellipsoid, with distinct apiculus, hyaline, smooth.

Habit: on rotted wood.

Distribution: Tanzania.

Material examined: TANZANIA, Mt Killimangoro, elev. 2700 m., March 1966, collected by Drake Hocking, 200370 (**Holotype:** DAOM).

Notess: This species is distinguished by light fruiting bodies, fluffy peridium and spores with distinct apiculus. There are some specimens collected from the western of China [20782, 21163 and 21259 (SWFC)], which were named as *C. africanus*. However they probably should be *C. jiayuguanensis*, which is another species with apiculate spores, but much gray peridium (Yang et al 2002). Futhermore in molecular analysis, the sample 20782 (SWFC) named as *C. africanus* is nested in the subclade of *C. jiayuguanensis,* and far from the holotype of *C. africanus* 200370 (DAOM) (Fig. 5.3).

2 ***Cyathus annulatus*** H.J. Brodie, Can. J. Bot. 48:749, 1970 Fig. B.2

Fruiting bodies 8-10 × 6-9 mm, broadly obconic, flaring at upper 1/3-1/4, base constrict and formed distinct short stipes; outside of peridium brown, inside gray, brownish gray, covering long shaggy hairs, apex bleach brown or light brown; inner of peridium with distinct plications; Lip fimbriate, no setae, dark brown; base attaching soil directly. *Peridioles* 1.5-2 mm diam., circular or subtrianglar, thin, brownish gray, one cortex with tunica, thickness of 15-25 µm in both.

Basidiospores 17-20 × 9-11 µm [$\bar{x}$ = 18.8 ± 1.2 × 10.4 ± 0.7, Q = 1.6-2, Q_m = 1.81 ± 0.21, n = 20], regular long ellipsoid, thick wall, hyaline, smooth.

Habit: in fine argillaceous soil among grasses.

Distribution: Canada.

Material examined: CANADA, Alberta, Cypress Hills, above Elkwater lake, 8 August 1968, collected by H. J. Brodie, 200366 (**Holotype**: DAOM).

Notess: Examining the holotype of *C. annulatus* showed the size of spores was 17-20 × 9-11 µm. That is different from the description of Brodie (1978): spores 15.5-17 × 15-19 µm. *Cyathus annulatus* is similar to *C. striatus* and *C. helenae* because they share the distinct plicated peridium and simlar shape of spores. However it differs from *C. helenae* which has broadly ellipsoid spore (Q = 1.1-1.6, rarely 2). *Cyathus striatus* has the same spores with *C. annulatus* in size and shape. But the fruiting bodies of *C. striatus* has distinct setae at the mouth, and much darker peridia color.

3 ***Cyathus*** aff. ***berkeleyanus*** (Tul. & C. Tul.) Lloyd, Nidulariaceae:19, 1906. Fig. B.3

Cyathus microsporus var. *berkeleyanus* Tul. & C. Tul., Ann. Sci. Nat., Bot., sér. III, 1:74, 1844

Cyathia berkeleyana (Tul. & C. Tul.) V.S. White, Bull. Torrey Bot. Club 29: 258, 1902

Fruiting bodies 7-12 (-15) × 4-6 mm, cylindrical to obconic without abruptly constrict at the base in side view, no distinct stipe, attached to the substrate by a mycelial pad; exterior dark brown or brownish orange covered with lone hairs, and agrageted into long wooly curls, strongly sulcate plications, from apex extending down, half peridium long; inner of peridium grayish brown, distinct plications; lip smooth, without fimbria or setae. *Peridioles* 1.5-2 mm diam., flattened, mostly subtriangular, brownish gray, one cortex 20-25 µm thick and covered by tunica of 15-20 µm thick. *Basidiospores* 7.8-12.5 × 5-6.2 µm [$\bar{x}$ = 9.4 ± 1.6 × 5.5 ± 0.5, Q = 1.4-2, Q_m = 1.7 ± 0.3, n = 20], mostly ellipsoid, ovoid, rarely subglobose, hyaline, smooth.

Habit: on the delayed wood, gregarious.

Distribution: widespread in tropic areas, such as Bolivia, Brazil, Cuba, Florida, Hawaiian Islands, Mexico, South China, West India.

Material examined: CHINA, Sichuan Prov., Chengdu, November 1999, collected by Tongxin Zhou, 20789 (SWFC).

Notes: The morphology of this material generally matched the descriptions of Brodie (1975), except those two points: 1) the fruiting bodies of this material is cylindrical shape, which did not mention before for this species; 2) the thick wooly hairs covered the peridium. *Cyathus berkeleyanus* is similar to *C. pallidus* based on they share the thick wooly hairs, similar sized and shaped spores. However *C. pallidus* has smooth peridium and much lighter color in fruiting bodies.

4 *Cyathus bulleri* H.J. Brodie, Bull. Torrey Bot. Club 94:68, 1967. Fig. B.4.

Fruiting bodies 5-8 × 5-8 mm, broad obconic, upper part flared outwards, base constrict into short stipe; external surface light brown yellow, linen yellow, covered by long but soft hairs, some interweaved, appear wooly or shaggy; internal surface grayish-white, cream color, with distingct but not deep striate in wide distance; lip smooth or fine fimbriate, same color with peridial hairs. *Peridioles* 1.2-1.8 mm diam., circular, thin, gray, single cortex of 30-40 µm thick with dark tunica of 20 µm thick. *Basidiospores* 6-9 × 5-8 µm [$\bar{x}$ = 7.3 ± 0.9 × 6.8 ± 1.1, Q = 1-1.2, Q_m = 1.08 ± 0.12, n = 20], globose, subglobose, thick wall, hyaline, smooth.

Habit: On dead wood.

Distribution: West India, Hawaiian Island and Mexico.

Material examination: INDIA, West India, Guadeloupe, 8 Feb 1966, collected by H.J. Brodie, 727126 (BPI: **Isotype**).

Notes: In the Brodie's description, the epiphragm was " snow-white, beset with fawn-coloured vertical tufts of hairs", but in the present specimen, those characters have not been showed. Very light-colored fruiting bodies covered by shaggy hairs make *C. bulleri* looks like *C. pallidus*. However, the flared mouth, plications on the inner surface of peridium and globose spores of *C. bulleri* are distinct characters to separated from *C. pallidus*, which is never flared mouth, absolutely smooth surface and ovoid spores.

5 *Cyathus colensoi* Berk. Fl. Nov.-Zel. vol. 2:192, 1855. Fig. B.5

Fruiting bodies 4-7 (-10) × 4-7 mm, campanulate, some obconic, with slightly wide stipe; exterior of peridium light brown, brownish orange or brown, covered appressed fine tomenta, without plications; inner peridium gray, light gray, brownish gray, smooth; lip smooth, no fimbria and setae. *Peridioles* 1-2 mm diam., circular, dark gray, one cortex of 30 µm thick, covered by

tunica, thick, near 50 µm. *Basiodiospores* 7-9 × 4.5-9 µm [$\bar{x}$ = 8 ± 0.7 × 6.2 ± 1.2, Q = 1-1.6, Q_m = 1.33 ± 0.33, n = 20], subglobose, some broadly ellipsoid,, thick wall, hyaline, smooth.

Habit: on rotting twig.

Distribution: Australia, China, India, New Zealand.

Material examined: AUSTRALIA, 1880, collected by Muelkei, 200422 (DAOM); AUSTRALIA, Kalgoarlie, 11 November 1953, collected by R. Melvelle, 198718 (DAOM); CHINA, Xingjiang Prov., 1 Octorber1999, collected by L. Z. Zhao, 20860 (SWFC); INDIA, Siligusi, 9 October 1980, collected by B. M. Sharme, 200423 (DAOM).

Notes: *Cyathus olla*, *C. africanus* and *C. colensoi* Berk. are covered by the fine-textured on the outer peridium. The sizes of basidiospores are same. According to the description (Brodie, 1975), *C. africanus* is differed from *C. olla* and *C. colensoi* by its broadly ovoid with a diatinct apiculus basidiospore. The spores of *C. olla* are broadly ellipsoid and *C. conlensoi* are subglobose. The fruit bodies of *C. olla* tend to be markedly expended or flared outwards near the mouth, the peridioles are conspicuously large (up to 3mm wide) (Brodie, 1975), while *C. colensoi* was campaculate in most case and did not expand near the mouth of fruit bodies and with small peridioles.

6 *Cyathus confusus* F.L. Tai & Hung, Sci. Rep. Natl. Tsing Hua Univ., ser. B 3(2): 36, 1948 Fig. B.6.

Fruiting bodies 9-12 × 6-10 mm, obconic, long obconic, base abcute constricted into short stipe, outline from side view straight, or slightly curvered near the base; exterior light brown, yellowish brown and brown with age, covered with shaggy hairs, but rubbing off easily and appear smooth surface; interior of peridium light brown, smooth or faintly plications; lip fimbriate, no setae. *Peridioles* 1.8-3 mm diam., variable in shape, such as broadly ellipsoid, semicircle, brown, light brown, one cortex 30 µm thick, with thin tunica of 10-15 µm thick. *Basidiospores* 6.2-11.2 × 5-6.2 µm [$\bar{x}$ = 8.7 ± 1.4 × 5.7 ± 0.5, Q = 1.2-1.8, Q_m = 1.6 ± 0.4, n = 20], ellipsoid, elongated ovoid with sharp apex, thick wall, hyaline, smooth.

Habit: on rotting wood.

Distribution: China.

Material examined: CHINA, Yunnan Prov., Kunming, 1 December 1942, collected by W. F. Chui, 03490 (HMAS: **Holotype**).

Notes: There is a munite unidentical bwtween my examination and In the original description (Tai and Hung 1948) *C. confuses* up to 17 mm tall, which is the only unidnetical to my examination of this holotype. Tai and Hung thought *C. confuses* was similar to *C. hookeri*, which has smaller fruiting bodies (up to 14 mm tall), and wider spores (8-11 × 6-8 µm) (Brode 1975). However the peridium of *C. confusus* covered by shaggy hairs, which is another diagnostic character to separate from *C. hookeri* (which covered by tomentose hairs on the peridium).

Brodie according to the illustration and description of *C. confusus* suggested it propably was a tall forma of *C. intermedius* (Brodie, 1975). However *C. intermedius* has larger spores ($\bar{x}$ =16 × 10 µm) (Lloyd 1906). *Cyathus confusus* might be related to *C. novae-zealandian*, *C. berkeleyanus* and *C. pullus* in morphology because they share the similar shaped and sized spores. Strong plications of *C. berkeleyanus* and *C. pullus* is a useful character for separating them from *C. confuses*, while the preidium of *C. novae-zealandian* and *C. pullus* cover with appressed tomenta, and that is quite different the shaggy hairs of *C. confusus*.

7 ***Cyathus cornucopioides*** T.X. Zhou & W. Ren, Acta. Mycol. Sin. 11(1):23, 1992 Fig. B.7.

Fruiting bodies 14-23 × 8-10 mm, long campanulate, upper 1/3 remarkable flared; exterior dark brown, covered with shaggy, wooly hairs, in some cased faint plictions after the haris rubbed off; inner surface of peridium grayish brown, clearly plicate; lip smooth, no fimbria or setae. *Peridioles* 2.5 mm diam., mostly circular, brown, one cortex of 50 µm thick, with thin tunica of 10 µm thick. *Basidiospores* 6.2-10.6 × 5-6.5 µm [$\bar{x}$ = 8.5 ± 1.3 × 5.6 ± 0.6, Q = 1.2-1.8, Q_m = 1.51 ± 0.31, n = 20], ovoid or broadly ellipsoid, occasionally with one narrow end, hyaline, smooth.

Habit: gregarious in soil.

Distribution: China.

Material examination: CHINA, Yunnan Prov., Luxi county, 7 July 1983, collected by L.Z. Zhao, 20414 (**Holotype**: SWFC).

Notes: *Cyathus cornucopioides* is similar to *C. berkeleyanus*, because they both have dark brown, distinct plications and shaggy peridia, same shaped and sized basidiospores. However *Cyathus cornucopioides* has at least 14 mm high, and for the fresh fruiting bodies can up to 29 mm (Ren and Zhou 1992), and *C. berkeleyanus* is 4-14 mm tall. Furthermore, the fruiting body of *C. cornucopioides* has distinct flaring mouth and *C. berkeleyanus* is straight mouth.

8 ***Cyathus crassimurus*** H.J. Brodie, Can. J. Bot. 49:1609, 1971. Fig. B.8.

Fruiting bodies 5-6 × 4-7 mm, width of mouth equal or longer than the height of fruiting bodies, broad obconic, funnel-shaped, arose from a mass of 3-5 mm diam. comsisted of mycelia and soil; exterior brownish gray, buff, pale buff or light brown, covered with stiff tufts or nodules arised from felt-liked fibrils, 0.3 mm thick; interior brownish gray, gray, smooth; lip fimbriate, dark brown. *Peridioles* 1-1.2 mm diam., circular or fan-shaped, brown, grayish brown, one cortex of 20 µm thick, with thick tunica of 40 µm thick, dark brown. *Basidiospores* 14-20 × 11-13 µm [$\overline{x}$ = 17.4 ± 1.7 × 11.8 ± 0.6, Q = 1.2-1.6, Q_m = 1.48 ± 0.28, n = 20], ellipsoid, thick wall (up to 4 µm), hyaline, smooth.

Habit: on rotting wood and sawdust.

Distribution: Island of Hawaii.

Material examination: THE USA, Island of Hawaii, near Captain cook, 23 June 1968, collected by H.J. Brodie, 200372 (**Holotype**: DAOM); same location, collection date and collector, 198720 (DAOM).

Notes: The name of this species was named from the thick walls of fruiting bodies and spores (Brodie, 1971b). The species similar to *C. crassimurus* are *C. graclis*, *C. intermedius* and *C. elmeri*, because they share the similar sized and shaped spores, spores having thick wall and smooth peridia. However, the fruiting body of *C. gracilis* is slender with long stipe; the spores of *C. intermedius* are smaller ($\overline{x}$ =16 × 10 µm) (Lloyd 1906); and *C. elmeri* has dark colored and short tomentose peridium.

9 ***Cyathus crispus*** H.J. Brodie, Can. J. Bot. 52:1661, 1974. Fig. B.9.

Fruiting bodies 5.5-7 × 6-6.5 mm, obconic, mouth incurved in dry, base abruptly constricted into short stipe; exterior peridium light brown, yellowish brown, covered with curls aggretated by reddish brown tomenta, with clearly striates and distant; interior peridium gray, distant plicate; mouth smooth, neither fimbriate nor setae. *Peridioles* 1.2-1.5 mm diam., mostly circular, some subtrianglar, dark gray or brownish gray, double cortex of totally 80 µm thick, without tunica. *Basidiospores* 12-17 × 7-10 µm [$\overline{x}$ = 14.4 ± 1.5 × 8.4 ± 0.8, Q = 1.5-2.3, Q_m = 1.73 ± 0.57, n = 20], long ellipsoid, thick wall, hyaline, smooth.

Habit: on rotten wood.

Distribution: Ghana.

Material examination: GHANA, Bunsu, 17 June 1949, collected by S.T. Hughes, 200373 (**Holotype**: DAOM).

Notes: *Cyathus limbatus* is most similar to *C. crispus* in morphology, because both they have the same sized and shaped fruiting bodies, same colored pperidium, plicted, and similar shaped spores (Brodie 1975a). However *C. limbatus* have a remarkable dark brown fimbriate at the lip and larger spores (16-22 × 10-12 µm).

10 ***Cyathus durus*** (V.S. White) Sacc. & D. Sacc., Syll. fung. XVII:215, 1905. Fig. B.10.

Fruiting bodies 7-11 × 4.5-11 mm, long obconic, upper half or third flaring outwards heavily, base constrict into long stipe; peridium near 1 mm in thickness, external surface brown, dark brown, covered densely hairs and aggregated into tufts, hirsute; internal surface lighter color than outside, brownish cream, plication appear on the upper third part, regular, wide distance each other; mouth smooth. *Peridioles* 1-2 mm diam., shape variable, circular, fan-shaped, angular, brownish gray, single cortex of 18-20 µm thick, with tunica of 10-20 µm thick. *Basidiospores* 14-20 × 11-14 µm [$\bar{x}$ = 18 ± 1.7 × 12.6 ±1, Q = 1.2-1.7, Q_m = 1.43 ± 0.27, n = 20], ellipsoid, broad ellipsoid, thick wall, hyaline, smooth.

Habit: in soil and rotten wood piece.

Distribution: The USA.

Material examination: THE USA, Colorado, Denver, 1900, collected by E. Bethel, 727135 (**Isotype**: BPI) and 727134 (**Isotype**: BPI).

Notes: *Cyathus helenae* is species mostly similar to *C. durus* in morphology, because they have the wide plications on the inner surface of peridium, same shaped and sized spores and flaring mouth. However *C. durus* plication are distant, and smooth mouth which differ from *C. helenae* (close plications and dark brown fimbriate at mouth).

11 ***Cyathus elmeri*** Bres., Hedwigia 51:324, 1912. Fig. B.11.

Fruiting bodies 7-9 × 8-9 mm, obconic, funnel-shaped or campanulate; exterior peridium brown, covered with aggressed tomenta, without plications; interior peridium brownish gray, smooth to faintly ridges near the mouth; lip fimbriate, dark brown. *Peridioles* 1.5-2 mm diam., circular, brown, double cortex of 44-60 µm thick, with thin and dark brown tunica of 10 µm thick. *Basidiospores* 17-21 × 10-13 µm [$\bar{x}$ = 19 ± 1.2 × 11.6 ±1, Q = 1.4-2, Q_m = 1.67 ± 0.33, n = 20], long ellipsoid, round at both ends, thick wall (up to 8 µm), hyaline, smooth.

Habit: on dead stems of palms.

Distribution: Philippine.

Materials examination: PHILIPPINE, Palo Island, June 1906, collected by A. D. E. Elmer, 198717 (**Holotype**: DAOM) and 198717a (DAOM).

Notes: See the notes of *C. crassimurus*.

12 ***Cyathus gracilis*** H.J. Brodie, Can. J. Bot. 51:1393, 1973. Fig. B.12.

Fruiting bodies 7-10 × 5-8 mm, funnel-shaped, obconic, constricted into long stipe (mostly one third of fruiting body tall), slender; exterior peridium brown, rust, reddish brown, covered by hairs, aggregated into tufts, hirsute, without striate; interior peridium concolorous or lighter than those of exterior, smooth; lip fimbriate, dark brown; emplacement spreading, up to 5 mm diam. *Peridioles* 1.2-2 mm diam., circular, dark brown, double cortex of 70-80 µm thick, without tunica. *Basidiospores* 14-20 × 12-13 µm [$\bar{x}$ = 17.4 ± 1.9 × 12.3 ± 0.5, Q = 1.2-1.7, Q_m = 1.41 ± 0.29, n = 20], long ellipsoid, ellipsoid, thick wall, hyaline, smooth.

Habit: gregarious on the rotten wood.

Distribution: Philippine.

Materials examination: PHILIPPINE, Luzon, Lamao River, December 1903, collected by R. S. Williams, 200380 (**Holotype**: DAOM).

Notes: This species is easily recognized by its long stipe, double cortex of peridioles and ellipsoid spores.

13 ***Cyathus griseocarpus*** H.J. Brodie & B.M. Sharma, Bot. Notiser 133:343. Fig. B.13.

Fruiting bodies 7-12 × 6-8 mm, obconic, occasionally campanulate with slightly curved sides, base abruptly constrict into short stipe; exterior peridium ivory, brownish orange, grayish orange, brownish yellow, covered with long downward-pointed, shaggy hairs; interior peridium yellowish white, brownish yellow, brownish orange, plications distinct, occasionally smooth; lip fimbriate, same color with peridial hairs; emplacement very thin and spreading, up to 4 mm diam. *Peridioles* 2-3 × 1.8-2 mm, ovoid, broadly ellipsoid, gray, single cortex of 25- 35 µm thick, with lighter brown tunica of 12-20 µm thick. *Basidiospores* 7-8 × 4-6 µm [$\bar{x}$ = 7.3 ± 0.5 × 5.3 ± 0.5, Q = 1.2-2, Q_m = 1.41 ± 0.59, n = 20], ellipsoid, ovoid some with one narrow end, hyaline, smooth.

Habit: on twigs and soil in mixed forest.

Distribution: India, southern China, Thailand.

Material examination: INDIA, Manipur State, Ukhrul, 29 August 1978, collected by H.J. Brodie, 200396 (**Holotype**: DAOM); CHINA, Yunnan Prov., 13 August 1991, collected by Y.L. Liu, 20630 (SWFC); same location, July 2000, collected by L.Z. Zhao, 21359 (SWFC) and 21533 (SWFC); THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m, 26 June 2005, collected by Amy Honan, ZRL c002 (SFSU); Chiang Mai Prov., Mae Taeng Dist., New Waterfall, 30 June 2005, collected by Dennis E Desjardin, ZRL c003 (SFSU); same location and date, collected by Jennifer Kerekes, ZRL c004 (SFSU); same location and date, collected by Ruilin Zhao, ZRL c005 (SFSU); same location, 2 July 2005, collected by Ruilin Zhao, ZRL c007 (SFSU); Chiang Mai Prov., 2007, collected by Dirk Stubbe, ZRL c015 (SFSU); Mae Hong Son Prov., on Highway 1095 near 198km marker, opposite with Suan Im Chay 100 m, elev. 288m, 23 July 2005, collected by Ruilin Zhao, ZRL c008 and ZRL c010 (SFSU).

Notes: *Cyathus griseocarpus* is similar to *C. pallidus,* because they share light-colored and shaggy peridium, same sized and shaped spores (Brodie 1984). However *C. pallidus* is absolutely without plications. *Cyathus bulleri* is another species which has pale-colored and shaggyl hairs peridium. But it differs from *C. griseosrpus* in its strong plications on both sides of peridium and spherical or subglobose spores.

14 ***Cyathus guandishanensis*** B. Liu & Y.M. Li, Acta. Mycol. Sin. 8(2):101, 1989. Fig. B.14.

Fruiting bodies 8-11 × 7-9 mm, obconic, funnel-shaped, base constricted into short stipe; exterior peridium brown, yellowish brown, covered by fine tomenta, and aggregated into small tufts; inner surface of peridium grayish brown, with shallow plications; mouth fimbriate, dark brown, short; emplacement spreaded, up to 5 mm diam. *Peridioles* 1.8-2.5 mm diam., circular, or subcircular, grayish brown, one cortex of 20-25 µm thick with light brown tunica of 25 µm. *Basidiospores* 10-13.8 × 7.5-10 µm [$\bar{x}$ = 11.7 ± 1.3 × 8.6 ± 0.9, Q = 1.1-1.8, Q_m = 1.38 ± 0.42, n = 20], mostly ovoid with an apiculus, some broadly ellipsoid, thin wall, hyaline, smooth.

Habit: on dead twigs or soil.

Distribution: China.

Materials examination: CHINA, Shanxi Prov., Guandishan, 11 August 1987, collected by M.C. Chang, 81896 (**Holotype**: HMAS).

Notes: this species is very similar to *C. africanus*, especially they share the similar sized and shaped spores. However *C. guandishanensis* has distinct plications, and that differs from *C. africanus* which mostly smooth peridium.

15 ***Cyathus helenae*** H.J. Brodie, Can. J. Bot. 44:1235, 1966. Fig: B.15.

Fruiting bodies 5-9 × 4-6 mm, obconic, and upper one third part flared, and base constricted into stout stipe; exterior peridium pale brown to brown, covered with hairs and aggregated into tufts, hirsute; inner surface gray, light brown gray, with plications, some faint to smooth; lip fimbriate, dark brown; emplacement large, mix with siol, up to 7 mm diam. *Peridioles* 1.5-2.2 mm diam., ovoid, aome angular, brown gray, single cortex of 10-25 µm thick, with thin tunic of 15-20 µm thick. *Basidiospores* 14-20 × 10-14 µm [$\bar{x}$ = 16.6 ± 1.7 × 11.4 ± 1.2, Q = 1.1-2, Q_m = 1.47 ± 0.53, n = 20], mostly ellipsoid, some ovoid, thick wall, hyaline, smooth.

Habit: on dead stems and roots on mountain scree.

Distribution: Canada, the USA.

Material examination: CANADA, Alberta, 20 August 1965, collected by H.J. Brodie, 200384 **(Holotype:** DAOM); CANADA, Alberta, 8 June 1947, collected by H.J. Brodie, 200386 (DAOM); THE USA, Idaho, Elmore county, Alexandor Flats, 16 Novenber 1965, collected by E. Trueblood, 200395 (DAOM); location unknown, 6 September 1981, collected by H.J. Brodie, 200387 (DAOM).

Notes: *Cyathis helenae* has special ecological character, such as Brodie mentioned "*C. helenae* invades alpine regions, the far north and desert areas in northwestern American" (Brodie, 1975). In the morphology, this species is similar to *C. striatus*. However it differs from *C. striatus* which has darker peridium, deep striata and much longer spores (Q>2).

16 ***Cyathus hirtulus*** B. Liu & Y.M. Li, Acta Mycol. Sin. 8(2):108, 1989. Fig. B.16.

Fruiting bodies 10-13 × 8-10 mm, long obconic, cup-shaped, base abruptly constrict into short stipe; outer surface of peridium dark brown, chockelet color, covered long, shaggy, down-pointed hairs; inner surface of peridium brown, grayish brown, distinct and very crowded plications; mouth setae, dark brown; emplacement large and spreaded, up to 8 mm diam. *Peridioles* 3 mm diam., variable in shape, mostly fan-shaped and subtrianglor, brown, grayish brown, single cortex of 15-20 µm thick, with tunica of 15-30 µm thick. *Basidiospores* 21.2-26.2

× 7-10 µm [$\overline{x}$ = 23.3 ± 1.7 × 8.5 ± 0.8, Q = 2.1-3.1, Q_m = 2.75 ± 0.65, n = 20], cylindrical with round ends, long ellipsoid, thick wall, hyaline, smooth.

Habit: gregarious on the dead twigs.

Distribution: China.

Materials examination: CHINA, Jilin Prov., 11 August 1986, collected by Y.M. Li and K. Tao, 59607 (**Holotype:** HMAS).

Notes: The most similar species is *C. striatus*, because they share similar fruiting shape, same color and distinct plicate peridium and setae lip. But *C. striatus* has smaller and fater spores (18-20 × 8-10 µm, Q = 2).

17 ***Cyathus hookeri*** Berk., Hooker's J. Bot. Kew Gard. Misc. 6:204, 1854. Fig. B.17.

Fruiting bodies 8-11 × 8-10 mm, obconic, outline straight from side view; outer surface of peridium light brown, brownish orange, covered with tomenta and aggregated into nodules, some shaggy; inner surface of peridium grayish orange, faint plicate; lip fine fimbriate, same color with hairs; emplacement mix with soil. *Peridioles* 1.8-2 mm diam., lenticular, gray, brownish gray, one cortex of 15-25 µm, with thin tunica of 12-15 µm thick. *Basidiospores* 6-10 × 4-6 µm [$\overline{x}$ = 7.8 ± 1.5 × 5.1 ± 0.7, Q = 1.2-1.8, Q_m = 1.53 ± 0.33, n = 20], ovoid, some ellipsoid, hyaline, smooth.

Habit: on the dead twig.

Distribution: China, India and New Zealand.

Materials examination: INDIA, Meghalaya, 26 June 1978, collected by B. M. Sharma, 200435 (DAOM).

Notes: This species is similar to *C. africanus* and *C. microsporus*. *Cyathus microsporus* has smaller fruiting bodies (5-7 × 6 mm) and samler spores (5-6 × 4 µm) than those of *C. hookeri*, and *C. africanus* has distinct apiculus spores, which does not appear in *C. hookeri*.

18 ***Cyathus intermedius*** (Mont.) Tul. & C. Tul., Ann. Sci. Nat.; Bot., sér. III, 1:72, 1844. Fig. B.18.

Nidularia intermedia Mont., in Sagra, Hist. Phys., Pol & Nat. Cuba 2: 321, "1838-1842" (1842)

Cyathus intermedia (Mont.) White, Bull. Torrey Bot. Club 29: 258, 1902 (superfl.)

Fruiting bodies 6-7 (-9) × 4.5-7 mm, broad funnel-shaped, with short and slander stipe; exterior surface of peridium light brown, pale fawn and brown with age, covered low mounds composed by hairs, hirsute; interior surface of peridium light brown, brownish gray, smooth, occasionally faint plications; lip fine fimbriate, dark brown; emplacement 2-3 mm diam. *Peridioles* 1.2-1.5 (-2) mm diam., near circular, broadly ellipsoid, single cortex of 15-25 µm thick, with thin tunica of 10-15 µm thick. *Basidiospores* 12.5-16.2 × 8-12.8 µm [$\bar{x}$ = 14.1 ± 1.2 × 9.8 ± 1.3, Q = 1.1-1.7, Q_m = 1.46 ± 0.36, n = 20], ellipsoid or ovoid, thick wall, hyaline, smooth.

Habit: on the rotten wood.

Distribution: Florida, Mexico, Venezuela, Colombia, Philippine and China.

Materials examination: GUYANA, Geosge town, December 1908, collected by A. W. Baitlett, 727151(BPI); JAMAICA, Kingston, Hope Bot. Gdns., collect date and collector unknown, 727153 (BPI); THE USA, Florida, Gaineeville, 11 April 1939, collector unknown, 198716 (DAOM); location and collect data unknown, collected by C. Wright, 727154 (BPI).

Notes: *Cyathus crassimurus* is the most similar species to *C. intermedius*, and almost share all morphological characters, except *C. crassimurus* has thicker peridium and slightly larger spores.

19 ***Cyathus jiayuguanensis*** J. Yu, T.X. Zhou & L.Z. Zhao, Mycosystema 21:314, 2002. Fig. B.19.

= *Cyathus africanus* var. *latisporus* Y.H. Chen & J. Yu, Mycosystema 22:345-348, 2003.

Fruiting bodies 6-8 × (4-) 5-6 mm, cup-like, obconic, with slender stipe, exterior of peridium light brown, grayish-orange and covered with tufts or nodules aggregated by a fine tomentum; inside of peridium brownish-gray, or gray, smooth. *Peridioles* 1.5-2 × 1.5-1.8 mm, subcircular, circular or broadly ellipsoid; grayish-brown, or lighter, cortex a single layer, 20-25 µm wide, with tunica of 10-20 µm wide. *Basidiospores* 8.8-12.5 × 6.9-8.2 µm [$\bar{x}$ = 10.1 ± 1.1 × 7.8 ± 0.5, Q = 1.1-1.5, Q_m = 1.3 ± 0.2, n = 20], ovoid or ovoid with apiculus, hyaline, smooth.

Habit: on dead twig.

Distribution: China.

Specimen examined: CHINA, Gansu Prov., Jiayuguan, 12 Oct. 1999, collected by Zhou, T.X. & Zhao, L.Z. 20802 (**Holotype**: SWFC) and 20846 (SWFC); CHINA, Neimenggu, 13 September 2000, collected by T.X. Zhou, 21187 (**Holotype** of *C. africanus* var. *latisporus:* SWFC).

Notess: Because the fruiting bodies of *C. jiayuguanensis* lacked plications and are covered with shaggy hairs, Yang (2002) treated it as the member of the "pallidus" group. The fruiting

bodies of *C. africanus* var. *latisporus* lack plications and are covered with tufts of fine hairs, which are shorter than *C. jiayuguanensis'*. So it was treaded as a member of the "olla" group (Chen 2003). Apparently Yang (2002) and Chen (2003) followed Brodie's taxonomic system when introducing *C. jiayuguanensis* as a new species and *C. aficanus* var. *latisporus* as new variety.

However, morphological examinations of the holotypes of *C. jiayuguanensis* and *C. africanus* var. *latisporus* (HMSFC 20802 and 21187) showed that they share many morphological characters, such as a similar color and size of fruiting bodies, lack of plications, thin peridial wall, single cortex with thin tunica, and importantly the shape and size of spores. The only difference is that *C. jiayuguanensis* has longer hairs on the outside of the peridium than those of *C. africanus* var. *latisporus*, and this characters also is one of main differences between the "olla" group and "pallidus" group (Brodie 1975a). In fact, however, differentiation between woolly curls or nodules is subjective and affected by the age of the fruiting body.

We consider that the *C. africanus* var. *latisporus* should be synonymized with *C. jiayugusnensis* based not only on morphological characters but also our molecular data. *Cyathus jayuguanensis* has identical ITS sequences to those of *C. africanus* var. *latisporus* suggesting that *C. africanus* var. *latisporus* and *C. jiayuguanensis* are synonymous.

Cyathus africanus is morphologically the most similar species to *C. jiayuguanensis* especially as both spores have an apitulus. Morphological examination of the holotype of *C. africanus* (DAOM 200370) revealed spores of *C. africanus* to be 8.5-12 × 6.5-8.5 µm in most cases, while in *C. jiayuguanensis* (holotype SWFC 20802) and *C. africanus* var. *latisporus* (holotype MHSEC 21187) spores were broader being up to 10 µm wide.

20 *Cyathus julietae* H.J. Brodie, Svensk Bot. Tidskr. 61:94, 1967. Fig. B.20.

Fruiting bodies 5.5-7 × 7-8 mm, broad obconic, outline straight, base constrict and form short and slander stipe; exterior surface of peridium bark blond, light brown, yellow, covered with hairs and aggregated into tufts, hirsute; inner surface of peridium yellow, brownish gray, smooth, glossy; lip fine fimbriate, same color with hair, and covered by epiphragm when young, snown white, membraneous; emplacement 3-4 mm diam. *Peridioles* 1.5-2 mm diam., broad ellipsoid, subglobose, brown, single cortex of 15-20 µm thick, with tunica of 15-20 µm thick. *Basidiospores* 7.5-10 × 5-6.2 µm [$\bar{x}$ = 8.7 ± 0.8 × 5.8 ± 0.6, Q = 1.2-1.8, Q_m = 1.5 ± 0.3, n = 20], broad ellipsoid, subglobose, thick wall, hyaline, smooth.

Habit: on rotten wood.

Distribution: China, Jamaica.

Material examination: JAMAICA, Hardwar Gap, collect data unknown, collected by H.J. Brodie, 727156 (**Holotype**: BPI) and 200436 (**Isotype**: DAOM).

Notes: This species is charactered by glossy inner surface of peridium, yellow or light brown colored fruiting body, and middle sized spores. *Cyathus pallidus* is similer to this species, which both have light colored fruiting bodies and smooth peridium. However *C. julietae* has funnel-shaped fruiting body with straight outline from side view, while those of *C. pallidus* is crucible-shaped with curved outline. Furthermore the spores of *C. juliatae* are regular broadly ellipsoid, but spores of *C. pallidus* are ovoid with one narrow end. *Cyathus jiayuguanensis* is another similar species with *C. julietae* because they has smooth peridium. But *C. jiayuguanensis* is gray color on the peridium and has ovoid with an apiculus spores.

21 ***Cyathus lanatus*** (H.J. Brodie) R.L. Zhao, Mycologia 99:394, 2007. Fig. B.21.

≡ *Cyathus olla* f. *lanatus* H.J. Brodie, Bot. Notiser 131: 31-34, 1978.

Fruiting bodies 5-7 mm × 5-6 mm, crucible-shaped, without a distinct stipe, broadly attached to a firm thickened base, some flared at the mouth; Peridia light gray, light buff or grayish-yellow, lacking plications on the peridium, peridium covered with upward-pointing tufts or radiating tufts arranged by thick and fine hairs; inside smooth, gray and shiny; lip distinctly fimbriate. *Peridioles* diameter very variable, 1.2-3.5 mm, gray to light buff, lenticular or irregular in outline, some plump, shiny, tunica thick, cortex a single layer, up to 80-100 µm, funiculus stout and short. *Basidiospores* 9-13 × 7-8 µm [$\bar{x}$ = 10.8 ± 1.4 × 7.5 ± 0.5, Q = 1.3-1.7, Q_m = 1.44 ± 0.26, n = 20], mostly ovate, occasionally provided with apiculus, thick-walled, hyaline, smooth.

Habit: gregarious on dead deserts plants on soil.

Specimens examined: USA, Idaho, Owyhee county, Reynold's Creek, 4 November 1976, collected by E. Trueblood, 200703 (**Holotype:** DAOM).

Notess: We found some minor differences from the description of Brodie (1978a) when examining the holotype: i) In Brodie's description the tunica was described as up to 60 µm thick with a contex of 60-80 µm thick (in total 120-140 µm), whereas we found the tunica plus contex to be 80-100 µm wide. ii) The spores were smaller than in Brodie (1978a) description, 9-13 µm long and 7-8 µm wide vs Brodie's 12-15 µm long and 7.5-9 µm wide.

Brodie (1978a) stated that she doubted that the type of *C. olla* f. *lanatus*, USA, Idaho) should be identified as *C. olla* as the fruiting bodies are "very small", "often short, thick-walled and bleached". At the first glance, they might easily be mistakenly identified as "*Crucibulum laeve*", because what was " as thick and tough as is the tunica of *Crucibulum*". Brodie therefore dealt with this sample as a form of *C. olla* mainly based on the fruiting body shape (some fruit bodies were flared at the mouth) and the size of spores, lack of plications on the peridium and *C. olla* "exists in many variations", although "no test of possible fertility between *C. olla* f. *lanatus* and *C. olla* has been carried out because all attempts to germinate spores of *C. olla* f. *lanatus* have been unsuccessful."

In this study analysis based on the LSU dataset showed *C. lanatus* to nest with *C. pallidus* and *C. gansuensis* which have thick and fine hairs and make up the group pallidum (Clade C). *Cyathus olla* and its forms nest with other species and comprise the group ollum (Clade A). *Cythus lanatus* differs from *C. olla* f. *olla* in having a thick and fine hairs and crucible-shaped fruiting bodies, from *C. olla* f. *brodiensis* lacks plications and is distinguished from *C. olla* var. *anglicus* in having smaller fruiting bodies and being lighter.

The differences between *C. lanatus* and other species of the pallidum group are mainly plication characters, thickness of fine hairs and the spore shape (See key to the pallidum group). *Cyathus colensoi* has similar sized and shaped spores as those of *C. lanatus*, but the covering of peridium of the *C. colensoi* is thin, with a short appressed and fine hairs. *Cyathus africanus* is another species with the similar spores to those of *C. lanatus*, but can be easily distinguished as the fruiting body of *C. africanus* has a distinct stipe and is gray and *C. lanatus* attaches the substrate broadly without a distinct stipe and is yellowish-brown. *Cyathus julietae* has yellowish-brown fruiting bodies, lacking plications and the same shape spores as *C. lanatus*. The fruiting body of *C. julietae* is however, obconic and not crucible-shaped, and also has smaller spores than those of *C. lanatus*.

22 *Cyathus lijiangensis* T.X. Zhou & R.L. Zhao, Fungal Diversity 17:243, 2004. Fig. B.22

Fruiting bodies 7-9 × 4-6 mm, cup-shaped, campaniform with curved outline, base constrict and form short stipe; exterior of peridium dark brown or near black, covered with grayish white tufts, short but hirsute, faint striate or smooth; interior surface of peridium gray, dark gray and fading to white toeards the base, distinct plications; lip fimbriate, gray. *Peridioles* 1.8-2.2 mm diam., variable in shape, ovoid or subcircular, grayish brown or black, single cortex of 20-25 µm,

with tunica of 15-20 µm. *Basidiospores* 10-13 × 8-10 µm [$\bar{x}$ = 11.4 ± 0.9 × 9.1 ± 1, Q = 1.1-1.5, Q_m = 1.26 ± 0.24, n = 20], subglobose, thick or thin wall, hyaline, smooth.

Habit: on dead wood.

Distribution: China

Material examination: CHINA, Yunnan Prov., Yulong Snow Mountain, 5 November 2000, collected by Y. Z. Pan, 21081 (**Holotype**: SWFC).

Notes: My measure of spores is different from original description which is (14-) 15.5-18.5 (-21) × (11-) 13-15 (-16) µm (Zhou et al 2004). *Cyathus lijiangensis* is distinguished by its near black peridium, which is rare in *Cyathus* species. *Cyathus nigro-albus* is the only another species which has near black peridium in known *Cyathus* species, and its spores is 16-22 × 12 µm (Brodie 1975a). The differences between them are inner surface of *C. nigro-albus* is white and faint plicate, while those of *C. lijiangensis* is dark color and distinctly crowded plications.

23 ***Cyathus limbatus*** Tul. & C. Tul., Ann. Sci. Nat., Bot., sér. III, 1:78, 1844. Fig. B.23.

= *Cyathus cheliensis* F.L. Tai & Hung, Sci. Rep. Natl. Tsing Hua Univ., ser. B 3(2): 39, 1948

= *Nidularia striata* var. *pusilla* Berk., Ann. Nat. Hist. III:397, 1839.

Fruiting bodies 6-10 × 5-7 mm, obconic, mostly incurved at the mouth with age; exterior of peridium brown, reddish brown, dark brown, but afte rubbed the hairs, white, cream, hairs hirsute, plications distinct; inner surface of peridium mostly gray, some darker, brownish gray, dark gray, distinct crowded plications; lip fimbriate, dark brown; emplacement 3-6 mm diam. *Peridioles* 1.5-2.5 mm diam., mostly circular or subcircular, sometimes broadly ellipsoid, double cortex of 80-100 µm thick, without tunica. *Basidiospores* 18-22 × 12-15 µm [$\bar{x}$ = 20.5 ± 1.9 × 13.3 ± 1.5, Q = 1.3-1.8, Q_m = 1.56 ± 0.26, n = 20], ellipsoid, broadly ellipsoid, both ends round, thick wall, hyaline, smooth.

Habit: gregarious on dead wood.

Distribution: widely distributed in warm areas. Africa, British Guiana, China, Hawaiian Island, India, Pacific Island, South America, Thailand.

Materials examination: CHINA, 1940, collected by H.S. Yao, 02755 (**Holotype** of *C. cheliensis*: HMAS); CHINA, Yunnan Prov., Menglun, collector unknown, 11 September 1994, 20009 (SWFC); CONGO, Katanga, 24 Novenber 1960, collector unknown, 200492 (DAOM); JAMAICA, 3 September 1955, collected by D. A. Powell, 727165 (BPI); JAMAICA, Kingston, Hope Bot Gdns, 14 January 1966, collected by H.J. Brodie, 727167 (BPI); KENYA, Naisafi,

Novenber 1953, collected by R. M. Nattiaes, 200496 (DAOM);location and collector unknown, 200494 (DAOM); THAILAND, Chiang Mai Prov., Mae Taeng Dist., Hot Spring Nat. Park., 31 August 2005, collected by Kevin D Hyde, ZRL c011 (SFSU); UGANDA, collect data unknown, collected by R. A. Dummer, 727166 (BPI); USA, Neola, Seoisa haone, 29 August 1956, collected by H.J. Brodie, 200493 (DAOM).

Notes: this species has a variable size of spores based on the descriptions from different person. "Spores 15 × 10 µm in the type but 16-22 × 10-12 µm in other collections" is from Brodie's description (1975). My measure of five specimens identified by Brodie showed the size of spore was 17-23 µm long, 11-14 (-16) µm wide. However distinct plications on both surfaces of peridium, incurvered mouth, thick and double cortex, ellipsoid spores are stable diagnostic characters of *C. limbatus*. *Cyathus poeppigii* is the most closed to *C. limbatus* in field. But it has huge spores (30-42 × 20-28 µm).

24 ***Cyathus luxiensis*** T.X. Zhou, J.Yu & Y.Hui Chen, Mycosystema 22:345, 2003. Fig. B.24.

Fruiting bodies 6-9 (-12) × 6-9 mm, funnel-shaped, some incurved at mouth, stipe slender and short; exterior of peridium brown, reddish brown, yellowish brown, covered with tufts, hirsute, some plicated; inner surface of peridium grayish white, brownish gray, shinny, distinct crowded and long plications; lip fimbriate, dark brown. *Peridioles* 1.8-2.2 mm diam., subcircular, circular, thin, dark gray, brown, double cortex (inner lay dense, black and thin; the outer lay thick, gray, staffed by interwaved reddish haphae), up to 100 µm thick in total, with vary thin and fragile tunica of 10 µm thick. *Basidiospores* 10-17.5 × 7.5-10.6 µm [$\overline{x}$ = 15.4 ± 2.3 × 8.6 ± 1.1, Q = 1.3-2.3, Q_m = 1.81 ± 0.5, n = 20], ellipsoid, occasionally broad ellipsoid or ovoid, hyaline, smooth.

Habit: on the rotten wood.

Distribution: China

Material examination: CHINA, Yunnan Prov., collect data unknown, collected by L.Z. Zhao, 21355 (**Isotype**: HSFC) and 21362 (SWFC).

Notes: This species is similar to *C. limbatus* in field. However the double cortex of peridoles in *C. limbatus* is composed of 2 black and dense layers, and there are some interwoved reddish brown hyphae between those two layers. This kind of double cortex is common in the genus *Cyathus*. However the double cortex of *C. luxiensis* is different: inner lay is black and dense,

while the outer lay is mixture of reddish brown haphae and gray metter. Futhurmore, *C. limbatus* has larger spores than those of *C. luxiensis*.

25 *Cyathus microsporus* Tul. & C. Tul., Ann. Sci. Nat., Bot., ser. III, 1:73, 1844. Fig. B.25.

Fruiting bodies 5-8 × 5-7 (-9) mm, obconic, with slender and short stipe; exterior of peridium brown, dark brown or chocolate brown with age, without plication in most case or occasionally faint plicate, hairs shaggy, hirsute or appressed; interior of peridium lighter color than outside, brownish gray, gray, mostly soomth, some faint plication; lip fimbriate, dark brown; emplacement inconspicuous. *Peridioles* 1.5-2.2 mm diam., lenticular, circular, dark brown, black, single cortex of 40 µm thick, with thin tunica of 8-10 µm thick. *Basidiospores* 6-10 × 5-6 µm [$\overline{x}$ = 8.2 ± 1.1 × 5.8 ± 0.4, Q = 1.2-2, Q_m = 1.42 ± 0.58, n = 20], broad ellipsoid, ovoid, some with one narrow end, thick wall, hyaline, smooth.

Habit: on dead wood.

Distribution: Costa Rica, Cuba, Florida, Hawaii, Jamaica, San Domingo (type origin).

Material examination: CUBA, collect data and collector unknown, 200594 (DOAM); location unknown, 16 March 1954, collected by Marie L. Farr, 200593 (DAOM); location unknown, 30 July 1972 by K.P. Dwmont, R.F. Cain, G.J. Samuels and B. Manara, 200595 (DAOM); USA, Costa Rica, 23 October 1953, collected by C. B. Necien, 200592 (DAOM).

Notes: *Cyathus hookeri* is the most similar species to *C. microsporus*, and there were aguement about wheather *C. hookeri* should be a synonym of *C. microsporus* or not. If *C. hookeri* was accepted as a separated species, it differs from *C. microsporus* in its larger fruing bodies (up to 14 × 10 mm) and spores (8-11 × 6-8 µm) (Brodie 1975a). However the examination of the above determined specimens by Brodie showed the spores' size were overlapped with those of *C. hookeri*. Just like the comments of Brodie, "This species is not readily distinguished from other small dark brown species of *Cyathus*".

26 *Cyathus minimus* Pat., J. Bot. (Morot), 11:345, 1897. Fig. B.26.

Fruiting bodies 4-5 × 4-5 mm, obconic, with short stipe, some mouth flaring; exterior of peridium lighter brown, hairs tomentose to slightly hirsute, no plication; interior peridium yellowish cream or yellowish brown, smooth; lip smooth or fine fimbriate, dark brown; emplacement not distinct. *Peridioles* 1-1.2 (-1.8) mm diam., mostly circular, brown, single cortex of 70-80 µm thick, very thick and dark grey, with tunica of 25 µm thick. *Basidiospores* 18-23 ×

9-14 µm [$\overline{x}$ = 20.1 ± 1.2 × 12.1 ± 1.4, Q = 1.4-2, Q_m = 1.68 ± 0.32, n = 20], ellipsoid with round ends, occasionally long ellipsoid, thick wall, hyaline, smooth.

Habit: on rotten wood.

Distribution: Japan, Tonkin (northern Vietnam).

Material examination: VIETNAM, Tonkin, collector and collect data unknown, 703447 (**Holotype**: BPI); JAPAN, Novenber 1906, collector unknown, 703448 (BPI).

Notes: Fruiting bodies small, light yellow peridium, hairs tomemose to hirsute, single cortex with tunica and ellpsoid spore are the main characters to recognize this species. *Cyathus pallidus* and *C. julietae* are similar this species by the light-colored and near smooth peridium. But both them have has smaller spores (*C. pallidus* 7.5-15 × 4-8.5 µm; *C. julietae* 5-9 × 5-7 µm) than those of *C. minimus*.

27 ***Cyathus montagnei*** Tul. & C. Tul., Ann. Sci. Nat., Bot., ser. 3, 1:70, 1844. Fig. B.27.

Fruiting bodies 6-12 × 4-7 mm, obconic, some mouth flaring, base constricted into short stipe; exterior surface of peridium brown, reddish brown or chocolate brown, covering with hairs long hirsute, some faint plication; interior surface lighter color than outside, brownish gray, dark gray, shinny, clear striate; lip fimbriate or short setae, dark brown; emplacement distinct, 3-7 (-9) mm diam. *Peridioles* 1.5-2.5 mm diam., circular, subcircular, plump and hard, dark brown, black, single cortex of 30-40 µm thick, with thick tunica of 20-50 µm. *Basidiospores* 14.4-18.8 × 8.8-11.2 µm [$\overline{x}$ = 17.2 ± 1.3 × 9.5 ± 0.9, Q = 1.4-2.1, Q_m = 1.81 ± 0.41, n = 20], broad ellipsoid, ellipsoid, thick wall, hyaline, smooth.

Habit: gregarious on the dead wood.

Distribution: Brazil, west Idia, central America, Venezuela, Congo, Philippines, Thailand, Colombia, Guadeloupe.

Material examination: BRASIL, Rio Grande do Sul, Sao Leopoldo, 1932, collected by J. Rick, 727176 (BPI); COLOMBIA, near Purerto Lopez, 31 July 1944, collected by Ruby Little, 727179 (BPI); same location, 24 July 1944, collected by Ruby Little, 727180 (BPI); location unknown, 15 Febraury 1966, collected by H.J. Brodie, 727175 (BPI) and 727177 (BPI); location unknown, 2 May 1981, collected by M. Guariglia and R. Liesner, 200609 (DAOM); location unknown, 29 June 1975, collected by K.P. Qumont, 200610 (DAOM); USA, Costa Rica, Punto Arenas, collect data unknown, collected by C. B. Heiser, 727174 (BPI); VENEZUELA, 30 June

1972, collected by Decmont, 727178 (BPI); VENEZULA, edo Tachuia, 29 July 1971, collected by K.P. Qumont, 200605 (DAOM), 200607 (DAOM) and 200608 (DAOM).

Notes: Bump and hard peridioles is the unique character of *C. montagnei. Cyathus striatus*, *C. limbatus* are the similar species with *C. montagnei* in morphology, because all them have distinct plications on the peridium, dark brown fruiting body, hirsute or shaggy hairs, and similar sized and shaped spores. The spores of *Cyathus striatus* are long ellpsoid, while those of *C. limbatus* and *C. montagnei* are ellipsoid or broad ellpsoid. In addition, the peridioles of *C. limbatus* is double cortex with reddish brown hyphae between 2 layers, no tunica, while those of *C. montagnei* are single cortex with distinct tunica.

28 ***Cyathus nigroalbus*** Lloyd, Nidulariaceae: 18, 1906. Fig. B.28.

Fruiting bodies 5-8 × 3.5-5 mm, narrow obconic, long bell-shaped, mouth straight or flared, base constricated into slender stipe; external surface of peridium dark brown, chocolate color, covering haires, long tufts, hirsute to shaggy, not down-pointed; interior peridium white, cream, or light brownish-gray, mostly smooth and shinning, occasionally faint plications; epiphragn white, smooth, membraneous in the young fruiting bodies; lip slightly fimbriate; emplacement not developed. *Peridioles* 1.5-1.8 mm diam., circular, broad ellipsoid or ovoid, gray, brownish gray, double cortex of 35-40 µm thick, without tunica. *Basidiospores* 21-28 × 14-18 µm [$\overline{x}$ = 24.3 ± 2 × 16.3 ± 1.4, Q = 1.3-1.8, Q_m = 1.5 ± 0.3, n = 20], broad ellipsoid, rarely ovoid, thick wall, hhaline, smooth.

Habit: gregarious on the dead wood.

Distribution: Fiji, Samoa.

Material examination:SAMOA, collect data and collector unknown, 703968 (**Holotype**: BPI).

Notes: *Cyathus nigroalbus* is distinguished by near black exterior peridium against the white internal surface of peridium, covering hairs shaggy.

29 ***Cyathus olivaceobrunneus*** F.L. Tai & Hung, Sci. Rep. Natl. Tsing Hua Univ., ser. B 3(2):39, 1948, Fig. B.29.

Fruiting bodies 7-9 × 6-7 mm, obconic, base constricted into short stipe; exterior of peridium brown, grayish brown, plicate, slitted with plications, covered with hairs, felt, and aggregated into reddish- brown and small tufts, rubbed off easily; internal surface grayish brown, distinct plications of upper half of peridium long; lip fimbriate, dark brown; emplacement up to 3 mm

diam. *Peridioles* 1.5-2 mm diam., subcircular, dark brown, wrinkled, single cortex of 30-15 µm thick, with light brown tunica, 35-50 µm thick. *Basidiospores* 12.5-21.2 × 11.2-15 µm [$\overline{x}$ = 17.9 ± 2.3 × 13.2 ± 1.1, Q = 1.1-1.7, Q_m = 1.36 ± 0.36, n = 20], mostly ovoid, some with one narrow end, or broad ellipsoid, wall not very thick, hyaline, smooth.

Habit: on the dead twig, grown with lichen.

Distribution: China.

Material examination: CHINA, Yunnan Prov., Dali, 28 August 1939, collected by H.S.Yao, 01518 (**Holotype**: HMAS).

Notes: *Cyathus poeppigii*, *C. limbatus*, *C. striatus* and *C. montagnei* are similar species with *C. olivaceobrunneus* in morphology, because all of their fruiting bodies are brown color, clearly striate on the peridium and similar character of hairs. Brodie thought *C. olivaceobrunneus* should be synonym of *C. poeppigii* based on the description of this species. But the holotype examination of *C. olivaceobrunneus* showed it has undoubted single cortex with tunica, which differs from double cortex of *C. poeppigii*. Furthermore *C. olivaeobrunneus* has smaller spore than those of *C. poeppigii* (30-42 × 20-28 µm). Similarly, although *C. limbatus* has the same range of spore size with *C. olivaceobrunneus*, its double cortex is mainly difference from *C. olivaeobrunneus*. The peridioles of *C. striatus* and *C. montagnei* are single cortex with tunica. The typical ellipsoid spores of *C. striatus* differs from ovoid spores of *C. olivaeobrunneus*. *Cyathus montagnei* have the similar shape and size of spores with *C. olivaeobrunneus*, but its fruiting bodies are much darker than those of *C. olivaeobrunneus*, bump and hard peridioles and hirsute hair are differences from *C. olivaeobrunneus*.

30 *Cyathus olla* (Batsch: Pers.) Pers., Syn. Meth. Fung.:237, 1801. Fig. B.30.

≡ Peziza olla Batsch, Elench. Fung.: 127, 1783

= *Cyathus vernicosus* DC., Fl. Fr. 2: 270, 1805

= *Cyathus dasypus* Nees, Horae Phys. Berol.:41, 1820

= *Cyathia lentifera* (L.) V.S. White, Bull. Torrey Bot. Club 29: 264, 1902

= *Crucibulum albosaccum* Lloyd, Mycol. Notess 7: 1118, 1922

Fruiting bodies (6-) 7-13 (-16) × (5-) 7-11 mm, cup-shaped, mouth flared outwards in mostly collections, base constricted into short stipe; exterior of peridium gray, fawn, yellowish brown, covered by short and soft hairs, tomentose, sometime aggregated into fine tufts; inner surface of peridium silver gray, gray or grayish brown, smooth, transversely ridges occasionally; mouth

smooth and outline wavy; epiphragm fawn or light yellow; emplacement 3-5 mm diam. *Peridioles* relatively bigger than other species, 2-3 mm diam., shape variable, ellipsoid, trianglar, irregular, grayish brown, single cortex of 20-50 µm thick, with tunica of 20-35 µm thick. *Basidiospores* 10-14.4 × 7.5-9.4 µm [$\overline{x}$ = 12.1 ± 1.4 × 8 ± 0.7, Q = 1.3-1.9, Q_m = 1.52 ± 0.38, n = 20], ovoid, broad ellipsoid, occasionally with angulus, thick wall, hyaline, smooth.

Habit: gregarious on dead wood or soil.

Distribution: common species in Europe (type location, Germany), America, Australia, China, Iran, South Africa. There are no records from tropic areas.

Material examination: CANADA, Ottawa, 23 September 1981, collected by H.J. Brodie, 727227 (BPI); CHINA, Ningxia Prov. Liupanshang, collect data unknown, collected by L.Z. Zhao, 21269 (SWFC); location unknown, 24 October 1950, collected by Mrshd Oswald, 727228 (BPI); location unknown, 8 September 1941, collected by A.H.R. Boller, 727224 (BPI); UK, Kew, 8 July 1958, collector unknown, 727226 (BPI); USA, Aherwood Park, Octorber 1968, collected by H.J. Brodie, 727223 (BPI); USA, Califonia, 8 Febraury 1978, collected by V. G. Cooke and L. Bodine, 727229 (BPI); Winnepeg, Octorber 1947, collected by H.J. Brodie, 727222 (BPI).

Notes: Mouth of fruiting bodies is flaring, gray color, covering tomentose and big peridioles make this species to be recognized easily. Ovoid spores without angulus is key character to separate it from *C. africanus*.

31 ***Cyathus olla* f. *anglicus*** (Lloyd) H.J. Brodie, Mycologia 44:417, 1952. Fig. B.31.

≡ *Cyathus anglicus* Lloyd, Nidulariaceae: 25 (1906)

=***Cyathia dura*** V.S. White, Bull. Torrey Bot. Club 29: 261, 1902

Fruiting bodies 11-14 × 11-14 mm, fennel-shaped, mouth reflex with dentate or lacerated outline, base constricted into short stipe; external surface of peridium dark gray, covered short and soft hairs, never tufts or shaggy, grooved near the mouth; internal surface of peridium brown, grayish brown, shinny, grooved; lip smooth, emplacement not remarkable. *Peridioles* large, 2-3.5 mm diam., broad ellipsoid, subtrianglar, brown, dark brown, single cortex of 30-40 µm thick with dark colored tunica of 25-35 µm thick. *Basidiospores* 8.2-12.5 × 7.5-8.8 µm [$\overline{x}$ = 11.1 ± 1.5 × 8.2 ± 0.6, Q = 1.1-1.7, Q_m = 1.35 ± 0.35, n = 20], ovoid, broad ellipsoid, hyaline, smooth.

Habit: in soil of pasture.

Distribution: Argentina, England, USA.

Material examination: USA, Oregon, 5 November 1950, collected by L. D. Carson, 727225 (**Holotype**: BPI).

Notes: the fruiting bodies of *C. olla* f. *anglicus* could be 18 mm tall in the previous description (Brodie 1975a). However there is a tiny unidentical that the examination of holotype showed the fruiting bodies up to 14 mm tall.

Lloyd's monograph of the Nidulariaceae, *C. olla* f. *anglicus* was elevated to species level (as *C. anglicus*), because of its large fruiting body and markedly sulcate mouth. Later, Brodie (1952) established it as a form of *C. olla* because single-spore mycelia of typical *C. olla* were found to be sexually compatible with those of form *anglicus*.

32 ***Cyathus olla* f. *brodiensis*** T.C. Shinners & J.P. Tewari, Mycologia 90:986, 1998. Fig.B.32.

Fruiting bodies 8-11 × 7-10 mm, broad obconic, some mouth reflex, base constricted into stipe; external surface of peridium brown, covered by thick tomentum, light brown; internal surface grayish brown, light brown-silvery, with distinct plications; mouth smooth. *Peridioles* 2-2.8 mm diam., subcircular, broad ellipsoid, gray, grayish brown, single cortex of 30 µm thick, with thick and dark tunica of 60 µm. *Basidiospores* 10-11.2 × 6.2-8.8 µm [$\bar{x}$ = 10.3 ± 0.4 × 7.7 ± 0.8, Q = 1.1-1.5 (-1.7), Q_m = 1.36 ± 0.34, n = 20], mostly broad ellipsoid, occasionally ovoid, hyaline, smooth.

Habit: on dead twig.

Distribution: Canada, China.

Material examination: CHINA, Neimonggu, 24 September 1999, collected by L.Z. Zhao, 21137 (SWFC).

Notes: *Cyathus olla* f. *brodiensis* was described by Shinners & Tewari (1998), which differs from other forms in having distinct plications on the inside of the peridium and a unique RAPD fingerprint. The examination of this specimen from China showed its the plicated inner surface of peridium along with other morphological characters quite match the definition of *Cyathus olla* f. *brodiensis*.

33 ***Cyathus pallidus*** Berk. & M.A. Curt., J. Linn. Soc., Bot. 10:346, 1868. Fig. B.33.

≡ *Cyathia pallida* (Berk. & M.A. Curt.) V.S. White, Bull. Torrey Bot. Club 29: 263, 1902.

≡ *Cyathus sphaerosporus* Lloyd, Nidulariaceae: 23, 1906.

Fruiting bodies 5-7.5 × 5-7.5 mm, campanulate, crucible-shaped, outline curved, base constricated into short and broad attachment, stipe not distinct; external surface of peridium pale buff, brownish orange, covered by felt like tonmentum, shaggy, no plication; internal surface grayish orange, shinny, smooth and completely smooth; lip smooth; emplacement not developed. *Peridioles* broad 1.8-2.5 mm diam., ellipsoid, subcircular, grayish white, single cortex of 20 µm with thin tunica of 10-15 µm thick. *Basidiospores* 8.2-16.2 × 5-8.8 µm [$\bar{x}$ = 11.1 ± 2.3 × 6.5 ± 1.1, Q = 1.1-2.2, Q_m = 1.74 ± 0.64, n = 20], shape variable, ellipsoid, ovoid, some taping in one end, hyaline, smooth.

Habit: on dead twig in moist ground.

Distribution: America tropic area, China, Cuba (type location), Mexico, south Hawaiian island, West Idias.

Material examination: CHINA, Guizhou Prov., August 2000, collected by S.K. Bai, 21160 (SWFC); CHINA, Yunnan Prov., Kunming, 19 August 1986, collector unknown, 20440 (SWFC); CHINA, Yunnan Prov., Tongbiguan, 20 June 1981, collected by Y.F. Wang, 20772 (SWFC).

Notes: Crucible-shape, light color, thick tomentum, plications not at all and small but shape variable spores make *C. pallidus* easy to be recognized. *Cyathus griseocarpus* is most similar species to *C. pallidus* in morphology. But *C. griseocarpus* has distinct plicated peridium.

34 ***Cyathus poeppigii*** Tul. & C. Tul., Ann. Sci. Nat., Bot., ser. III, 1:77, 1844. Fig. B.34.

=*Cyathus sulcatus* Kalchbr., Grevillea 10: 107, 1882.

= *Cyathus megasporus* W. Ren & T.X. Zhou, Acta Micol. Sin. 11(1): 23-27, 1992.

Fruiting bodies 6-8 × 4-5 mm, obconic or narrow obconic, base constricted into stipe, thin; external surface of peridium dark brown, reddish brown, hairs shaggy or hirsute, ridges distinct; internal surface dark brown, dark gray, deeply plications, some splitting along the plications; lip fimbriate, dark brown. *Peridioles* 1.8-2 mm diam., circular, subsircular, dark brown, double cortex of 50-60 µm thick with interwoved reddish brown hyphae between those two layers, without tunica. *Basidiospores* large, 31.2-39 × 18.8-25 µm [$\bar{x}$ = 35.1 ± 3.2 × 22.5 ± 2.7, Q = 1.2-1.9, Q_m = 1.58 ± 0.38, n = 20], broad ellipsoid, ovoid, thick or thin wall, hyaline, smooth.

Habit: on the dead wood and old fibrous mats.

Distribution: common species in the tropic area, such as Africa, Cuba (type location), Hawaiian Islands, south America, south China, West Indias.

Material examination: CHINA, Hunan Prov., Jiuyishan, 4 Janaurary 2001 by L.Z. Zhao, 21400 (SWFC); CHINA, Yunnan Prov., Longchuan, 5 July 2000, collected by L. Z. Zhao 21357 (SWFC); CHINA, Yunnan Prov., Kunming, 23 Novenber 1987, collected by X. Xing, 20448 (**Holotype** of *C. megasporus:* SWFC).

Notes: *Cyathus poeppigii* is variable in spores size, for example the spores length is 20-42 µm (Teng 1964); 15.3-44 µm (Bottomley 1948); 30-42 µm (Brodie 1975a). *Cyathus limbatus* is the most similar species to *C. poeppigii* in field. However *C. limbatus* has much smaller spores (18-22 × 12-15 µm).

35 ***Cyathus pullus*** F.L. Tai & Hung, Sci. Rep. Natl. Tsing Hua Univ., ser. B 3(2):38, 1948. Fig. B.35.

Fruiting bodies 8-14 × 6-11 mm, obconic, stipe short but clear; external surface of peridium brown, yellowish brown, covered by fine, short and soft hairs (tomentose), some aggregated into small tufts, plicated near the mouth; internal surface light brown, grayish brown, with distinct long plications on the upper half peridium; mouth smooth; emplacement not developed. *Peridioles* 1.5-2 mm diam., circular, thin, brown, grayish brown, single cortex of 20-40 µm, with dark color tunica of 30-40 µm thick. *Basidiospores* 10.6-13.8 × 8.8-10 µm [$\overline{x}$ = 11.8 ± 1 × 9.1 ± 0.5, Q = 1.1-1.5, Q_m = 1.3 ± 0.2, n = 20], ovoid, mostly with an apiculus, thick wall, hyaline, smooth.

Habit: on dead wood, twig or soil.

Distribution: China.

Material examination: CHINA, Kunming, 28 August 1937, collector unknown, 01510 (**Holotype**: HMAS); CHINA, Yunnan Prov., Xiangyun, collect data unknown, collected by G.Z. Xu, 20727 (SWFC); CHINA, Yunnnan Prov., Kunming, collect data unknown, collected by R. X. Sheng, 20027 (SWFC).

Notes: This species is common in the south China, especially in the Yunnan Provine (also type location). In the original description of this species (Tai & Hung 1948), this species was thought to be similar with *C. hookeri* and *C. novae-zeelandiae* because they share the same size of spores. However *C. hookeri* has smooth or faint plicated peridium, which differs from *C. pullidus* which has distinct plicatons. *Cyathus novae-zeelandiae* has pilacted peridium, but its spores have "elliptical, somewhtat pointed at both ends", and white tunica in peridiole, which differs from *C.*

pullus. Most spores of *C. pullidus* have an apiculus, and that is similar to spores of *C. africanus*, but the latter does not appear any plication on the peridium.

36 ***Cyathus pygmaeus*** Lloyd, Nidulariaceae: 26, 1906. Fig. B.36.

= *Cyathus gansuensis* B. Yang, J. Yu & T.X. Zhou, Mycosystema 21(3): 313-315, 2002.

Fruiting bodies small, 3.5-5 × 4-5 mm, shape variable, obconic with slender and short stipe or crucible-shaped without distinct stipe; external peridium light brown, covered appressed and thick tomenta, aggregated into tufts, bleach color; internal surface dark gray, smooth, not plications; lip smooth; emplacement not developed, spreading mycelia. *Peridioles* 0.8-1 mm diam., circular, thin, dark gray, single cortex of 16-24 µm thick, with tunica of 16-20 µm thick. *Basidiospores* 12-16 × 6.5-10 µm [$\bar{x}$ = 13.9 ± 0.9 × 9.3 ± 0.9, Q = 1.3-2, Q_m = 1.5 ± 0.5, n = 20], broad ellipsoid, ovoid, hyaline, smooth.

Habit: on dead twigs of arid areas.

Distribution: Chile, China, USA.

Material examination: USA, Washington, June1909, collected by J.S. Cotton, 703514 (**Holotype**: BPI); USA, California, Los Angeles, collect data unknown, collected by Stewart S. Towne, 703515 (BPI); CHILE, Santiago, collect data unknown, collected by M. R Espinosa, 703513 (BPI); CHINA, Gansu Prov., 15 September 1999, collected by L.Z. Zhao, 20880 (**Holotype** of *C. gansuensis*: SWFC).

Notes: This species is one of the smallest *Cyathus*. Brodie thought there were 3 characters of *C. pygmaeus* should be attended in the recognition of this species: i) the flaring rim of fruiting bodies; ii) the very dark interior of the cup; iii) the white, unusually durable epiphragm (Brodie, 1975). My morphological examination of holotype and 2 specimens which identified by Lloyd showed that there are not fruiting bodies with flared mouth in the holotype, but the specimen from California is flaring at mouth. So the flared mouth is questioned as an important character of recognition of this species. Epiphragm is fragile for most *Cyathus* collection, so it is difficult to be used in the identification of herbaria specimen. But the dark internal surface of peridium is a good and durable character in the identification of this species. In addition, the habit is an useful information for recognition of this species. The species was found on the dead twig of shrubby plants of arid areas.

37 ***Cyathus renweii*** T.X. Zhou & L.Z. Zhao, Fungal Diversity 17:245, 2004. Fig.B.37.

Fruiting bodies 8-10 × 5-6 mm, obconic or cup-shaped, base constricted in stipe, Outer surface of peridium brownish, clothed with yellowish to yellowish-pink hairs and narrow tufts, weakly plicate only near the mouth, inner greyish, strongly plicate (1-2 per mm), lip fimbriate, not setose; emplacement small (about 2 mm diam.) or not obvious. *Peridioles* 2 mm diam., depressed, round, greyish, cortex single and with tunica of 20-25 µm thick, brown. *Basidiospores* (18-) 21-31 (-34.5) × 10.5-13.5(-16) µm [$\overline{x}$ = 26.6 × 13.2, Q_m = 2.02, n = n = 83/3], ellipsoid to elongate-ellipsoid, rarely ovoid, exospore thin or slightly thicker, hyaline, smooth.

Habitat: on remnant stakes in the woods.

Materials examination: CHINA, Hunan Prov., Tianpingshan Hill, Badagongshan National Nature Reserve, Octorber 2001, collected by Lizhong Zhao, 21406 (**Holotype**: SWFC) and 21381 (Paratype: SWFC).

Notess: The new species belongs to the *striatum*-group. The large, ellipsoid to elongate-ellipsoid spores (usually 21-31 × 10.5-13.5 µm) are the main distinguishing characteristics. In contrast, the spores of *C. striatus* are typically ellipsoid and usually 13-24 × 8-12 µm, obviously smaller than those of the new species. The peridium is also wider than in *C. renweii*, which is usually 8-15 mm high and 5-9(-12) mm wide at mouth, the inside and outside are dark-brown, the plicates are long, and the lip setose are dark brown. In the same group, two other species with large spores resemble the new species, but the spores of *Cyathus yunnanensis* B. Liu & Y.M. Li are broadly ellipsoid to subglobose, 14.5-22.5(-26) × 10.5-18(-20) µm; *Cyathus hirtulus* B. Liu & Y.M. Li has ellipsoid, cylindrical spores, some of which are slightly curved in the form of allantospores, 18-25.5(-29) × 7.5-9(-11) µm, and its basidiocarp and peridioles are larger than those of *C. renweii*, and have setae around the lip.

38 *Cyathus setosus* H.J. Brodie, Can. J. Bot. 45:1, 1967. Fig. B.38.

Fruiting bodies 6-9 × 6-10 mm, broad obconic, constricted at the base into short and slender stipe; external peridium dark reddish brown, chocolate brown, hirsute,; internal surface brownish gray, silvery, faint plications; lip setae, dark brown, stiff; emplacement distinct, 1.5-3 mm diam. *Peridioles* 1.5-2 mm diam., lenticular, circular or angular, dark brown or black, double cortex of 50 µm thick, without tunica. *Basidiospores* 14-20 × 8-11 µm [$\overline{x}$ = 16.3 ± 2.3 × 9.9 ± 0.9, Q = 1.3-2, Q_m = 1.66± 0.34, n = 20], ellipsoid, both ends round, with very thick wall (almost 10 µm thick), hyaline, smooth.

Habit: On the dead wood under *Xanthosoma* plants.

Distribution: Bolivia, Guadeloupe, Jamaica, Mexico, St Lucia and Trinidad.

Material examination: JAMAICA, 9 Janurary 1966, collected by H.J. Brodie, 200815 (**Holotype**: DOAM).

Notes: Stiff setae at the rim is the most distinguished morphological characters for recognition *C. setosus*. *Cyathus striatus* is another species with setae and dark colored peridium. However deep and clear plications on the peridium and single cortex of *C. striatus* are main differences from *C. setosus*.

39 ***Cyathus stercoceus*** (Schwein.) De Toni, in Sacc., Syll. Fung. 7:40, 1888. Fig: B.39.

≡ *Nidularia stercorea* Schwein., Trans. Am Phil. Soc. 4: 253, "1834" 1832.

Fruiting bodies 5-15 × 4-8 mm, obconic with straight outline or campanulate with curved outline, base constrict into distinct, short or long stipe; external surface of peridium light color, such as brown, yellowish brown, or darker, such as dark brown, near black, covered hairs aggregated into tufts, hirsute to shaggy, never tomentose, hairs rubbed off and appear smooth in some cares; internal surface gray, dark gray, smooth; lip smooth. *Peridioles* 2-2.5 × 1.5-1.8 mm, ovoid, broad ellipsoid, angular, subcircular, double cortex with introwavon reddish brown hyphae between two layers, 40-60 µm, without tunica. *Basidiospores* huge, 27.5-35 × 23.8-38.8 µm [$\bar{x}$ = 32 ± 3.1 × 25.8 ± 1.9, Q = 1-1.4, Q_m = 1.24± 0.24, n = 20], globose, subglobose, thick wall, hyaline, smooth.

Habit: Chiefly coprophilous, on manure.

Distribution: world-wide distribution.

Material examination: CHINA, Neimenggu Prov., Hailaer, September 1999, collected by L.Z. Zhao, 21140 (SWFC); CHINA, Lilin Prov., Changchun, September 1998, collector unknown, 20714 (SWFC).

Notes: This species is great variable in size and color of fruiting bodies, and spore size. However for this very common species can be recognized by shaggy or hirsute non-plication cups, black and shinny peridioles and huge subglobose spores.

40 ***Cyathus striatus*** (Huds.: Pers.) Willd., Fl. Berol. Prod.:339. 1787. Fig. B.40.

≡ *Peziza* striata Huds., Fl. Angl., Edn. 2, 2: 634, 1778

= Cyathus *striatus* var. *schweinitzii* Tul. & C. Tul., Ann. Sci. Nat., Bot., ser. III, 1: 68, 1844

= *Cyathus griseus* Pers.

= *Cyathia hirsuta* (Schaeff.) V.S. White, Bull. Torrey Bot. Club 29: 259, 1902

= *Cyathus hirsutus* (Schaeff.) Quél. Enchir. Fung 232, 1886

Fruiting bodies (6-) 9-14 × (4-) 4-9 mm, narrow obconic, upper part flaring outwards or straight, never incurred, with distinct stipe; exterior of peridium mostly dark brown, reddish brown, chocolate brown, brown, occationally brownish yellow, covered by long and shaggy hairs, mostly down-pointed, some plicated; internal surface sliver white, grayish brown or brown, dark brown, distinct plications; lip setae, stiff, dark brown; emplacement even, spreading, diameter variable; epiphragm fragile, white, membraneous in young fruiting bodies. *Peridioles* (1.2-) 1.5-2.5 mm diam., subcircular, broad ellipsoid, grayish brown, dark brown, single cortex of 15-30 µm thick, with tunica of 10-20 µm thick. *Basidiospores* 15-21.2 × 8.8-12.5 µm [$\overline{x}$ = 18.5 ± 2.3 × 10.5 ± 1.2, Q = 1.6-2, Q_m = 1.77± 0.23, n = 20], ellipsoid, thick wall, hyaline, smooth.

Habit: on the dead wood or twigs.

Distribution: common species in the temperate areas, such as America, China, Europe, India, Japan, Mexico and Thailand.

Material examination: CANADA, collect data unknown, collected by H.J. Brodie, 727640 (BPI); CANADA, Ottawa, 18 Octorber 1987, collected by H.J. Brodie, 727643 (BPI); CHINA, Guizhou Prov., Anshun, collect data unknown, collected by M.H. Liu, 21588 (SWFC); CHINA, Hunan Prov., Daweishang, 2000, collected by L.Z. Zhao, 21431 (SWFC); GREEK, Ithaca, South Hill, August 1902, collected by J.M. Van Hook,727639 (BPI); INDIA, Bloomiugton, June 1951, collected by H.J. Brodie, 727642 (BPI); THAILAND, Chiang Mai Prov., Mae Taeng Dist., New Waterfall, 30 July 2005, collected by Ruilin Zhao, ZRL c006 (SFSU); USA, Ohawa, 13 August 1987, collected by H.J. Brodie, 727641 (BPI).

Notes: Lloyd (1906) had separated this species into two forms: one is *C. striatus* f. *striatus* from Europe, which was dark and pronounced tunica; another is *C. striatus* f. *schweinitzii* Tul. from America, which pale and thin tunica. I did not study those holotypes, but Brodie had seen both forms and believed no such difference as Lloyd's description (Brodie, 1975). This species, however, is variable in the color and mouth shape of fruiting bodies. For example, not only the color of outside of peridium is from lighter color to dark color, but also the inside surface from near white (BPI 727641, 727642, 727643) to dark brown (BPI 727640). In addition, mostly fruiting bodies have flaring mouth, but also some specimens appear narrow obconic with stright outline, cylindrical, such as specimen (SWFC) 21431. But anyway, this species is easy to be recognized by its shaggy or wooly hairs, clearly striates and ellipsoid spores.

41 ***Cyathus subglobisporus*** R.L. Zhao, Desjardin & K.D. Hyde, sp. nov. (in prep.) Fig.B.41

Fruiting bodies 7-10 × 5-8 mm with the quotient of height by width 1-1.4, clavate when young, then opening, extending and becoming obconic to infundibuliform with relatively straight sides in side view; external peridium covered by hairs aggregated into shaggy or hirsute clusters, ivory, light yellow or buff when young, sometimes with a hint of pale orange, then darkening to pale brown with age and the hairs remain pallid. Inner surface of peridium grey to brownish-grey, darkening with age, distinctly plicate when young, becoming striate to smooth in age. Epiphragm membranous, white, covered by buff to pale orangish white hairs similar to those on the external surface of the peridium, cracking irregularly during maturation and disappearing or leaving a minutely fimbriate lip along the top edge of the peridium. Base of the fruiting bodies narrower than the rest of the fruiting body but lacking a distinct stipe. *Peridioles* 1.5-2 mm diam., lenticular, grayish brown to light brown. Peridiole covering composed of two layers; a black inner corte× layer 15 - 25 µm thick, and a yellowish-brown or dark brown outer tunica layer 25 - 50 µm thick. *Basidiospores* 13–18 × 12-16 µm, [$\overline{x}$ = 15.8 ± 2.8 × 14.1 ± 2.1, Q = 1-1.31, Q_m = 1.12 ± 0.19, n = 50], subglobose or rarely broadly ellipsoid, hyaline, smooth, thick walled (1.5-2 (-3) µm).

Habit: gregarious on rotten bamboo stem.

Habitat: in moist bamboo forest.

Materials examination: THAILAND, Chiang Mai Province, Chiang Dao, 22 July 2006, collected by Rui-Lin Zhao, Kevin D Hyde, Hong- Li Hu, Jia-Ning Liu, Wulandari Nilam and Cheewangkoon Ratchadawan, ZRL c013 (**Holotype:** SFSU) and 18348 (**Isotype:** BBH).

Etymology: refers to the subglobose spores.

Notess: At a first glance, the proposed new species of *Cyathus* looks like *Cyathus griseocarpus* (Brodie 1984) a species commonly encountered in northern Thailand. Both species share features of light yellow or light brown fruiting bodies, external peridium covered by light yellow hairs that aggregate into conic mounds, inner surface of peridium striate to plicate, and grey-toned peridioles. The subglobose basidiospores and light brown peridioles of *C. subglobisporus* however, can differentiate it from *C. griseocarpus*, because the latter species possesses much smaller, more ellipsoid basidiospores ($\overline{x}$ = 7.3 ± 0.7 X5.3 ± 1.3 µm, Q_m = 1.4 ± 0.6, from holotype) and pale grey peridioles. The LSU tree (Fig. 2) clearly indicates that *C. griseocarpus* belongs to the Ollum group and is distantly related to *C. subglobisporus*.

Species of *Cyathus* whose fruiting bodies are light yellow or light brown and have a distinctly plicate inner peridium include *C. annulatus*, *C. berkeleyanus*, *C. bulleri*, *C. cornucopioides*, *C. crispus*, *C. durus*, *C. guandishanensis*, *C. helenae*, *C. pallidus*, *C. setosus*, *C. tianshanensis* and *C. yunnanensis*. Only *C. bulleri*, *C. guandishanensis* and *C. yunnanensis* however, possess subglobose or globose spores. After comparison of their spore size, *C. subglobisporus* is distinct in possessing larger spores ($\bar{x}$ = 15.8 ± 2.8 × 14.1 ± 2.1, Q_m = 1.12 ± 0.19) than those of *C. bulleri* ($\bar{x}$ = 7.3 ± 1.7 × 6.8 ± 1.8 µm, Q_m = 1.08 ± 0.15, from isotype) and *C. guandishanensis* ($\bar{x}$ = 11.6 ± 2.15 × 8.6 ± 1.4 µm, Q_m = 1.37 ± 0.46, from holotype), and smaller spores than those of *C. yunnanensis* ($\bar{x}$ = 22.38 ± 3.6 × 18 ± 3, Q_m = 1.25 ± 0.42, from holotype). Of the latter three species, sequence data was obtained successfully only from *C. guandishanensis*. The LSU tree (Fig. 2) clearly indicates that *C. guandishanensis* belongs to the Ollum group and is distantly related to *C. subglobisporus*.

The spores of *Cyathus olivaceobrunneus* (etymologized from the olive-brown peridium) are similar to those of *C. subglobisporus* in shape and size, and the former species has been suspected to be a synonym of *C. poeppigii* (Brodie 1975a). Examination of the type specimen of *C. olivaceobrunneus* showed the color of its fruiting bodies to be much darker and the spores longer ($\bar{x}$ = 17.88 ± 8.75 × 13.2 ± 2, Q_m = 1.36 ± 0.34, from holotype) than those of *C. subglobisporus*. We were unable to generate quality sequence data from *C. olivaceobrunneus* for comparison with *C. subglobisporus*.

42 *Cyathus tianshanensis* B. Liu & J.Z. Cao, Acta. Mycol. Sin. Suppl. 1:316, 1986. Fig: B.42.

Cyathus tianshanensis var. *tomentosus* B. Liu, J.Z. Cao et Y.M. Li, Acta. Mycol. Sin. 8: 101-112, 1989.

Fruiting bodies 7-10 × 6-8 mm, campanulate, some rim incurred, base contract into short stipe; exterior of peridium grayish brown, yellowish brown, sand color, covered by appressed tomentose, some aggregated into small mounds, after the hairs rubbed off, plications clear; internal surface brown to dark brown, with deep plications; lip smooth; emplacement up to 5 mm diam. *Peridioles* 1.5-2 mm diam., subcircular, some subangular, dark brown, single cortex of 50 µm thick, with lighter colored tunica of 25 µm thick. *Basidiospores* 8.8–12.5 × 5.6-8.2 µm, [$\bar{x}$ = 10.8 ± 1.7 × 6.7 ± 0.9, Q = 1.3-2.2, Q_m = 1.64 ± 0.56, n = 20], ovoid, mostly with an apiculus, hyaline, smooth.

Habit: in soil.

Distribution: the northwestern China.

Material examination: CHINA, Neimenggu, collect data unknown, collected by Y.Z. Shang, 21157 (SWFC).

Notes: Comparing this species with all species with deep and clear plications, its light colored, campanulate fruiting bodies and appressed tomentose are distinct morphological characters to recognize it. *Cyathus griseocarpus* has the same sized and shaped spores with *C. tianshanensis*, but its faint plication and never campanulate fruiting bodies are different from this species.

43 ***Cyathus triplex*** Lloyd, Nidulariaceae: 23, 1906. Fig. B.43.

Fruiting bodies 5-6.5 × 5-6.5 mm, height almost equal to the width of mouth, broad obconic, outline straight, with slender and short stipe; external peridium brown, dark brown and some bleach, covered by low mounds or tufts compared by hairs, coarse, and top of mound bleach color; internal surface cream to brownish gray, smooth or very faint plications; lip fimbriate, dark brown, not setose; emplacement well developed, consisted by mycelia and soil, firm and mummer-liked, up to 6 mm diam.. *Peridioles* 1.5-2 mm diam., mostly circular, gray or brown, dark brown, double cortex of 60-75 µm thick, without tunica. *Basidiospores* 16-20 × 11-14 µm, [$\overline{x}$ = 18.9 ± 1.2 × 12.4 ± 0.8, Q = 1.2-1.8, Q_m = 1.53 ± 0.33, n = 20], broad ellipsoid, hyaline, smooth.

Habit: On rotten twigs and soil, gregarious.

Distribution: China, Hawaii Island, Mauritius Island, Philippine, Thailand, USA, Venezuela, West India.

Material examination: PHILIPPINE, Mauaniu, 1919, collected by P. Nelovn, 200857 (DAOM); PHILIPPINE, May 1928, collector unknown, 703992 (**Holotype**: BPI); THAILAND, 10 November 1961, collector unknown, 198709 (DAOM).

Notes: Peridiole has double cortex with tunica in the original description of *C. triplex*, however the examination of holotype did not find the tunica. The specimen DAOM 200857 which was identified by Brodie, "tunica" appeared, but only limited in the area near the funicalor cord. So it is suspicious weather is true tunica or spreading hyphae of funicular cord.

Cyathus intermedius, *C. limbatus* and *C. setosus* are similar species with *C. triplex* in morphology, because the height of all their fruiting bodies are almost equal to the width of mouth, and they have the similar colored peridium, the similar shaped and sized spores. *Cyathus intermedius* can be separated from *C. triplex* by its single cortex with tunica; *C. limbatus* has deep and clear plications; and *C. setosus* is characterized by stiff setae at the lip.

44 *Cyathus yunnanensis* B. Liu & Y.M. Li, Acta. Mycol. Sin. 8:292, 1989, Fig. B.44.

Fruiting bodies 5-7 × 5-7 mm, broad obconic, base constrict into short stipe, external peridium brown, dark brown, covered by appressed hairs, tomentose, some consisted into small tufts, light yellow or brown with age, plications distinct; internal surface dark brown or dark gray, deep and distinct plicatios; lip fimbriate, dark brown; emplacement not distinct. *Peridioles* 1.5-1.7 mm diam., circular, brown, dark brown, single cortex of 20-45 µm thick, with light brown tunica of 20-30 µm thick. *Basidiospores* 18.8–25 × 15-20 µm, [$\overline{x}$ = 22.4 ± 2.2 × 18 ± 1.7, Q = 1-1.7, Q_m = 1.25 ± 0.45, n = 20], broadly ellipsoid or subglobose, thick wall, hyaline, smooth.

Habit: gregarious on the rotten wood.

Distribution: China,Yunnan Province.

Material examination: CHINA, Yunnan Prov., 13 September 1935, collected by Q. W. Wang, 17373 (**Holotype**: HMAS).

Notes: *Cyathus yunnanensis* is characterized by clear plications on the peridium and big subglobose spores. *Cyathus gayanus* Tul. has the same size of subglobose spores with those of *C. yunnanensis*. But *C. gayanus* has narrow obconic fruiting bodies, tall and slender, and peridioles double cortex.

5.6 Suspicious species

1 *Cyathus affinis* Pat., Bull. Soc. Mycol. France 11: xxx, 1895. Fig.B.45.

Fruiting bodies 6-9 × 5-6 mm, narrow obconic, long campanulate, base constrict into short stipe; external surface brown, yellowish brown, covered by appressed tomentose; internal surface light brownish gray, shinny, smooth; lip smooth, emplacement developed, firm, up to 5 mm diam. *Peridioles* 1.5-2 mm diam., circular, ellipsoid, dark brownish gray, dark gray, double cortex of 80-120 µm thick, without tunica. *Basidiospores* 24-30 × 16-21 µm [$\overline{x}$ = 26.3 ± 2.4 × 18.6 ± 1.8, Q = 1.2-1.7, Q_m = 1.42 ± 0.28, n = 20], broadly ellipsoid, ovoid, thick wall, hyaline, smooth.

Habit: in soil.

Distribution: unknown.

Material examination: 703390 (**Holotype**: BPI) location and collector unknown.

Notes: This species has light-colored fruiting bodies without plications and large ovoid spores. Those characters are quite similar to *C. stercoceus*, so this species is suspicious as a synonym of *C. stercoceus* (Brodie 1975a).

2 ***Cyathus rufipes*** Ellis & Everh., Bull. Torrey Bot. Club 24:125, 1897. Fig. B.46.

Fruiting bodies 9 × 5.5 mm, long and narrow obconic, outline straight, base constricted in stipe; external peridium light brown, yellow, bleach, hairs hirsute, no plication; internal surface gray, smooth, shinny; lip smooth; emplacement well developed and firmed, darker color than peridium, brown, up to 6 mm diam. *Peridioles* 1.5-2 mm diam., subcircular, dark brown or black, bump, clearly double cortex of 70-80 µm thick, with interwaved reddish brown hyphae between those 2 layers, without tunica. *Basidiospores* 22-34 × 16-26 µm [$\overline{x}$ = 29.2 ± 3.59 × 23.7 ± 2.2, Q = 1-1.7, Q_m = 1.24± 0.46, n = 20], subglobose, broad ovoid, some globose, wall middle thickness, hhaline, smooth.

Habit: gregarious on the rotten wood.

Distribution: Florida and Kansasn of the USA.

Material examination: USA, Florida, collect data unknown, collected by C.E. Please, 703517 (BPI); USA, Kansas, 17 July 1893, collected by E. Bartholomew, 703516 (**Holotype:** BPI).

Notes: This species is distinguished by the large spores along with the light color fruiting bodies, smooth at both surface of peridium and attaching substrate with well developed emplacement. Those characters are quite similar to *C. stercoceus*.

Chapter 6

RESULTS AND DISCUSSION Part C: the systematics of *Micropsalliota* in northern Thailand

6.1 Generic description and type species

Micropsalliota v. Höhnel in Akad. Wiss. Wien Math.-naturw. Klasse 123:31 (1914) emend. Pegler in Kew Bull. 23:340 (1969).

Type species: *M. pseudovolvulata* Höhnel

Generic desceiption: carpophores small; pileus mostly convex to plano-convex, seldom umbonate; surface dull, dry, silky to fibrillose with white, brown, reddish-brown or red groundcolor; context thin-fleshy to membranous, sometimes bruising yellow or reddish-brown when cut or injured. Lamellae free, close to crowded, with multiple series of lamellulae, narrow to broad, thin, white to light brown when young, becoming yellowish brown, umber or dark brown in age. Stipe central, cylindrical, slender, equal, hollow, surface smooth to fibrillose, white, sometimes staining yellow or reddish-brown when bruised. Partial veil membranous or rarely cortinoid, leaving a more or less persistent superior or median annulus, rarely exannulate. Odor not distinctive.

Basidiospores ellipsoid to irregular ellipsoid, amygdaliform or cymbiform, smooth, thick-walled, some with a thicker wall at the distal end, without a germ pore, brown to dark brown in deposit, dextrinoid. Basidia clavate, hyaline, 4-spored. Lamellae trama irregular, hyphae hyaline, unclamped. Lamellae edge sterile; cheilocystidia numerous, variable in shape but generally capitate. Pleurocystidia absent or rare. Pileipellis a cutis on disc and margin, composed of hyaline or pigmented hyphae, with membranous, vacuolar or incrusting pigments.

Habit, habitat and distribution: mostly gregarious, some scattered or solitary, in soil in forests or along road cuts. Common in tropical habitats; reported from Africa, Argentina, Brazil, Ceylon, India, Indonesia, Java, Malysia, Mexico and Sri Lanka.

6.2 Taxonomical and informative features

6.2.1 Pileus

Size is variable and generally less than 20 mm diam., although a few species form more robust basidiomes (e.g., *M. pleurocystidiata*). In some species the size of the pileus is a key diagnostic character, such as in *M. pusillissima* that possesses a pileus less than 3 mm diam. The shape of the cap is variable, ranging in side view from parabolic to campanulate, obtusely conical, convex or plano-convex, sometimes broadly or acutely umbonate, but never depressed or with uplifted margin. The color of pileus is an important character at the species level, and the main colors are pure white, white with brown, reddish-brown and purple tones at the disc. Some species (e.g. *M. rubrobrunnescens*) change color due to bruising of the pileus surface and appear evenly darkly pigmented in age. The margin of pileus is normally straight, entire, occasionally appendiculate with partial veil remnants, and in some species is striate surface at the margin. The surface of pileus is dry, glabrous to silky or more commonly fibrillose or with small fibrillose squamules.

6.2.2 Lamellae

The lamellae of all species are free, with close or crowded spacing, and range from narrow (1 mm) to moderately broad. The color of lamellae is variable: white when young, then darker with age as the basidiospores mature, becoming light brown, yellowish-brown or dark brown.

6.2.3 Stipe

Most stipes are slender, cylindrical, and attached at the centre of the pileus. The surface of the stipe is glabrous, tomentose or fibrillose below the annulus, typically white overall, or sometimes staining yellow or reddish-brown where bruised.

6.2.4 Partial Veil

A partial veil is formed by all species. It is membranous and relatively persistent in most species, although it may be cortinoid (cobweb-like; e.g., *M. brunneosperma* var. *cortinata*) and ephemeral. Typically the partial veil forms a superior or median annulus that is fragile and disappears in age. Rarely remnants of a non-persistent partial veil remain as small fibrils on the pileus margin.

6.2.5 Context

The context of the pileus and stipe is usually white, but some species stain yellow or red when cut.

6.2.6 Chemical reaction

The surface of pileus must be tested with 3% KOH. In a number of species a positive yellow or red reaction occurs and these are taxonomically important features.

6.2.7 Basidiospores

For all species the spore color is brown, dextrinoid in Melzer's reagent, and they are smooth and without a germ pore. The spores are generally broadly ellipsoid or ellipsoid, but in some species they are cybiform (with one side straight and the another side curved) or amygdaliform (Fig. 5.1). Basidiospore shape is an important feature useful in distinguishing species. The wall of the spore in some species is thickened at the apex, which is called the endosporium (Fig. 5.1). Basidiospore size is taxonomically important. Although most spores are relatively small, the species may be grouped into small-spored (<5 µm long) or long-spored (>5 µm long). Care must be taken to measure at least 20 spores per specimen to accurately assess spore size.

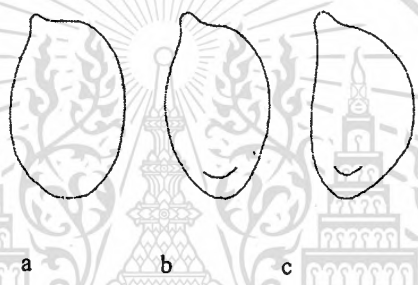

Fig. 6.1. The main spore shapes in *Micropsalliota*. a. ellipsoid; b. amgydaliform; c. cymbiform

6.2.8 Cheilocystidia

Cheilocystidia are present in almost all species in the genus. The shape is variable even within species, and and range from clavate to tibiiform or ventriose, and are usually capitate or subcapitate with the capitulum often elevated on a long neck. The size is variable but taxonomically significant.

6.2.9 Pleurocystidia

Pleurocystidia are usually absent. They are present and rare in a few species (*M. pleurocystidiata*, *M. xanthorubescens*), where they are similar in shape to the cheilocystidia.

6.2.10 Pileipellis

The pileipellis of all species is a cutis composed of more or less parallel hyphae, sometimes with uplifted clusters of terminal cells (pileus squamules). Significant micromorphological characters include: i) width of hyphae; ii) shape of hyphae; and iii) type of pigments. There are

three kinds of pigments. Determination of pigment type requires emersion in water and basic solutions (3% KOH). One is termed a *membranous pigment* in which the pigment is present in the hyphal walls in water but is soluble in basic solution. A second type is termed a *vacuolar pigment* in which the pigment is located in discrete cellular vacuoles, and hence appears unevenly distributed in water mounts. Vacuolar pigments will disperse into the whole cell when treated with basic solutions, and then appear evenly distributed in the cell. The third type of pigment is termed *instructed pigment* which appears as small pigmented granules attached to the outside surface of the cell wall in water, and these incrusting pigments are soluble in basic solution.

6.3 Molecular phylogeny

6.3.1 The relationship of *Micropsalliota*, *Agaricus* and allied genera

(See Chapter 4, 4.3.1.)

6.3.2 Molecular phylogeny within *Micropsalliota*

6.3.2.1 The ITS dataset

Forty-six sequences representing 22 *Micropsalliota* species, 2 *Hymenagaricus* species and *Allopsalliota geester* are included in this dataset with *Agaricus campestris* as the outgroup for rooting purposes. Of the 742 characters in the dataset, 73 characters were ambiguous and were excluded from the analyses, 417 characters were constant, 88 variable characters were parsimony-uninformative, and 164 characters were parsimony-informative.

Stmatrix was used to assign appropriate parameters and gaps were treated as missing data before the starting of weighted parsimony analysis. A consensus parsimony tree was produced with a length of 869 steps (CI=0.665, HI=0.552, RI=0.335). Maximum likelihood analysis used the GTR+I+G model selected by MrModeltest 2.2 and after rearrangements tried 29,715 times resulted in a best ML tree with a likelihood score of 3428.83191. The Kishino-Hasegawa and Shimodaira-Hasegawa tests among topologies obtained from ML and MP indicated that the ML tree was best. ML and Bayesian trees are almost identical except for the positions of *M. allantoidea* and *M. brunneosperma* var *cortinata*. The Bayesian tree of the ITS dataset is shown in Fig. 6.2.

6.3.2.2 The LSU dataset

This nLSU dataset was derived from 42 samples representing 19 *Micropsalliota* species, one *Hymenagaricus* species, and *Agaricus campestris* was used as the outgroup for rooting purposes. The dataset includes 751 characters (none were excluded): 653 characters were constant, 32 variable characters were parsimony-uninformative, and 66 characters were parsimony-informative.

For the weighted parsimony analysis, Stmatrix was used to assign appropriate parameters and gaps were treated as missing data. This yielded a consensus parsimony tree with a length of 224 steps (CI=0.482, HI=0.368, RI=0.518). Maximum likelihood analysis used the GTR+I+G model selected by MrModeltest 2.2 and after rearrangements tried 26,719 times resulted a best ML tree with a likelihood score of 2092.90946. The Kishino-Hasegawa and Shimodaira-Hasegawa tests among topologies obtained from ML and MP indicated that the ML tree was best. The ML and Bayesian trees were similar except for the positions of *M. furfuracea* + *Micropsalliota* sp1, *M. pleurocystidiata* and *M. gracilis*. The Bayesian tree of the LSU dataset is shown in Fig. 6.3.

6.3.2.3 The combined ITS+LSU dataset

The combined ITS + LSU dataset included sequences from 17 *Micropsalliota* taxa, 1 *Hymenagaricus* species, and *Agaricus campestris* was used as the outgroup for rooting purposes. The data matrix consisted of a total of 1493 characters of which 227 were parsimony-informative and 73 characters were excluded.

The MP analyses resulted in a consensus tree with a length of 736 steps (CI=0.659, HI=0.519, RI=0.341). Maximum likelihood analysis used the GTR+I+G model selected by MrModeltest 2.2 and yielded a best ML tree with a likelihood score of 5028.19803. The Kishino-Hasegawa and Shimodaira-Hasegawa tests indicate that MP and ML trees were not significantly different. The ML and Bayesian trees showed identical topologies. The Bayesian tree of the ITS + LSU dataset is shown in Fig. 6.4.

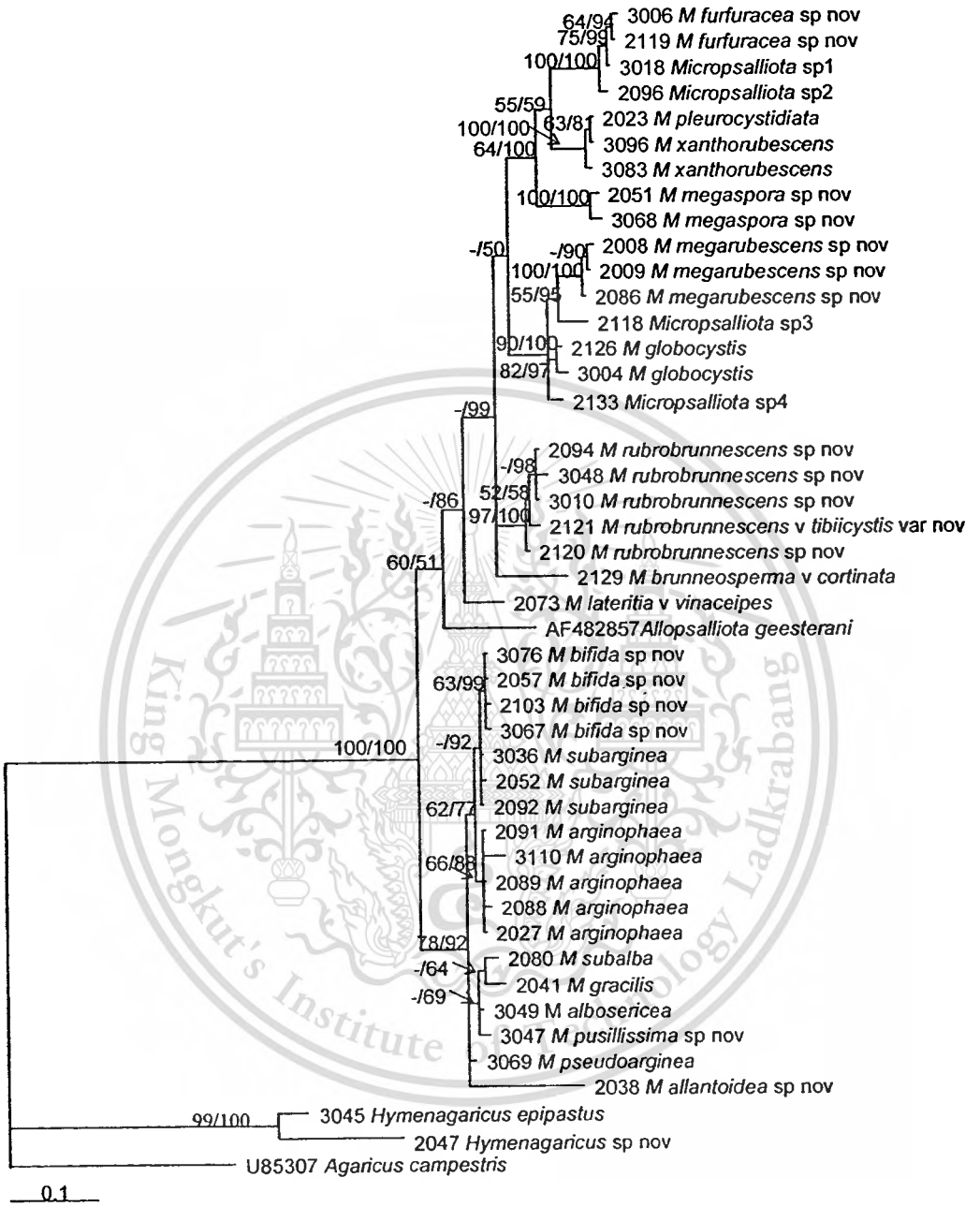

Fig. 6.2. Phylogeny of *Micropsalliota* generated from Bayesian analyses based on ITS sequences, rooted by *Agaricus campestris*. Parsimony bootstrap support (BS) value from unequally weighted parsimony analyses and Bayesian posterior probability (PP) values > 50% are given at the internodes (BS/PP).

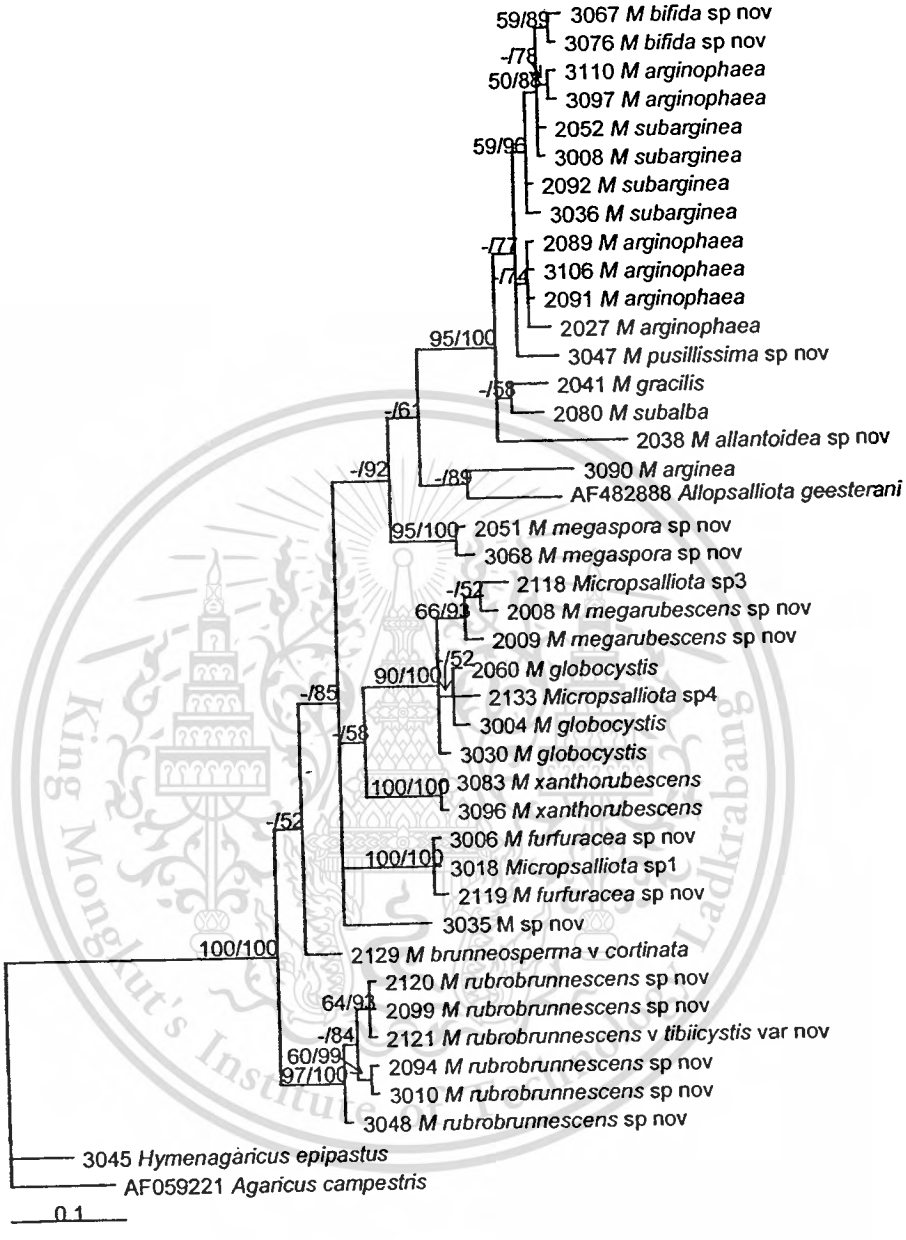

Fig. 6.3. Phylogeny of Micropsalliota generated from Bayesian analyses based on LSU sequences, rooted by Agaricus campestris. Parsimony bootstrap support (BS) value from unequally weighted parsimony analyses and Bayesian posterior probability (PP) values > 50% are given at the internodes (BS/PP).

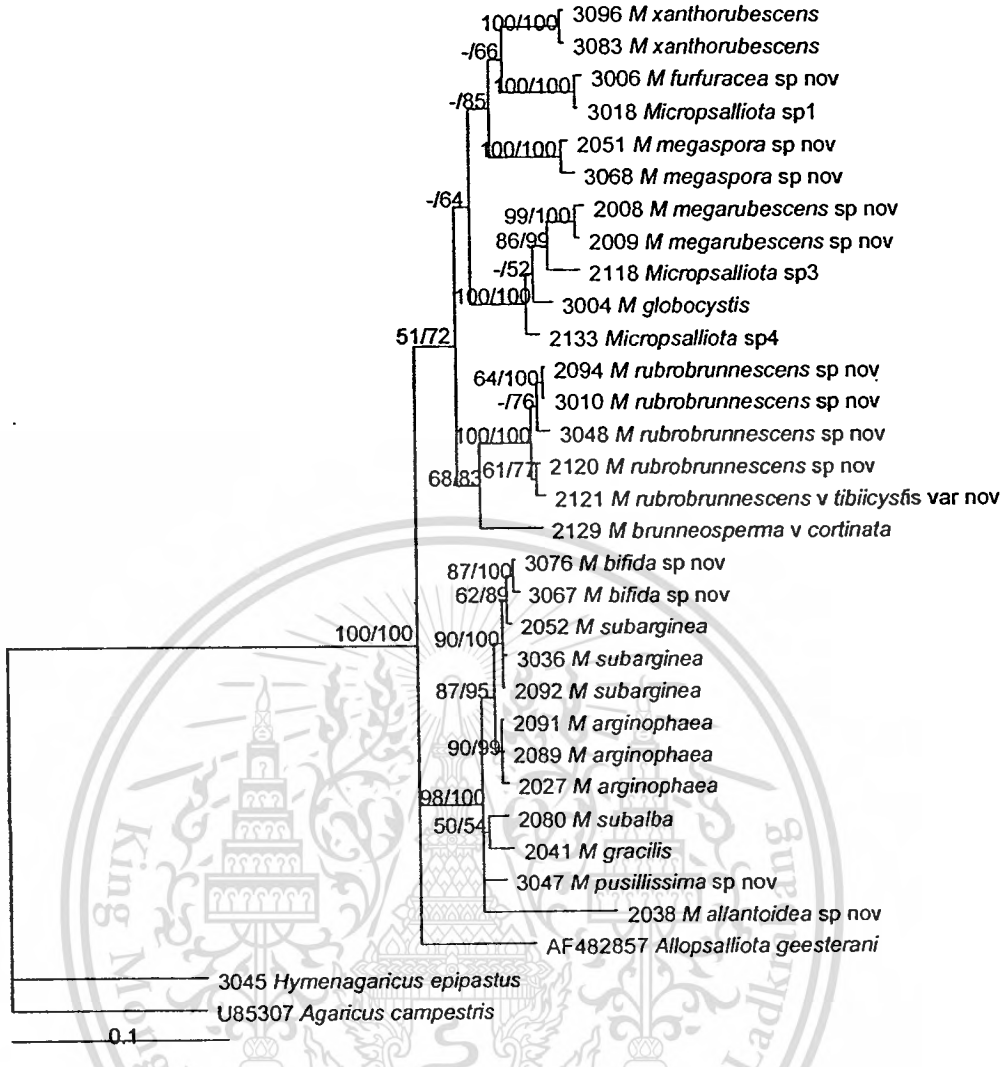

Fig. 6.4. Phylogeny of Micropsalliota generated from Bayesian analyses based on ITS+LSU sequences, rooted by Agaricus campestris. Parsimony bootstrap support (BS) value from unequally weighted parsimony analyses and Bayesian posterior probability (PP) values > 50% are given at the internodes (BS/PP).

6.4 Key to *Micropsalliota* species in Thailand

The only taxonomic system in this genus is from Heinemann (1983). In his system *Micropsalliota* species were separated into 4 groups based on pileus color and ratio of pileus diameter and length of stipe (IG value). The IG value however, is difficult to use for species identification and is not consistent within species. For example, in some collections some fruiting

bodies would be termed "trapu" (stout; Heinemann 1983) which has a low IG value, while other fruiting bodies would be termed "grêle" (slender) because of their high IG value. Therefore in my study, Heinemann's system was not adopted.

1. Carpophores tiny: pileus diameter less than 3 mm (maximum 5 mm); snow white ..... *M. pusillissima* sp. nov.

1. Carpophores small or middle: pileus diameter more than 3 mm; color variable, e.g. white, brown, red, violet 2

2. Carpophores medium-sized: pileus diam. 20-100 mm, stipe 40-150 mm long..... 3

2. Carpophores small-sized: pileus diam. 5-20 mm, stipe less than 40 mm long..... 11

3. Pleurocystidia present; pileus or stipe yellow staining when bruised or cut..... 4

3. Pleurocystidia absent; pileus or stipe reddish-brown or yellow then brown staining when bruised or cut..... 5

4. Stipe less than 70 mm long; flesh staining yellow then red; basidiospore mean = 6 × 4 µm..... *M. xanthorubescens*

4. Stipe mostly longer than 70 mm; flesh staining yellow and remaining so; basidiospore mean = 5.6 × 3.2 µm..... *M. pleurocystidiata*

5. Pileus cream colored to light gray, glabrous to slightly fibrillose..... 6

5. Pileus light brown to brown or reddish brown, squamulose..... 7

6. Pileus or stipe staining reddish-brown when bruised or cut; cheilocystidia clavate, subcapitate to capitate..... *M. megarubescens* sp. nov.

6. Pileus or stipe staining yellow then brown when bruised or cut; cheilocystidia tibiiform..... sp 2

7. Pileus squamules light brown to brown..... 8

7. Pileus squamules reddish-brown 9

8. Oleiferous hyphae abundant in the lamellae, pileus and stipe trama; pileipellis hyphae not incrusted..... sp 1

8. Oleiferous hyphae rare or absent; pileipellis hyphae distinctly incrusted..... *M. furfuracea* sp. nov.

9. Squamules scattered only on the pileus disc overlaying a white background; pileipellis hyphae not incrusted..... sp 4

9. Squamules covering the entire pileus (at least when young) and dense on the disc; pileipellis hyphae distinctly incrusted.....10

10. Basidiospores 6-7 × 3.5-4.2 µm; KOH reaction reddish brown on pileus surface.....*M. globocystis*

10. Basidiospores 4.5-6 × 3-4 µm; KOH reaction yellow on pileus surface.....sp 3

11. Spores cymbiform or subcymbiform.....12

11. Spores amygdaliform or ellipsoid.....14

12. Pileus brown; cheilocystidia capitate with a long flexuous neck.....*M. malabarensis*

12. Pileus white; cheilocystidia without a long neck.....13

13. Basidiospores 4.5-6 × 3–4 µm; cheilocystidia capitate.....*M. albosericea*

13. Basidiospores 4-5 × 2.5-3.2 µm; cheilocystidia clavate, non-capitate.....*M. pseudoarginea*

14. Basidiosporespores in the range 6-8 × 3.8-4.5 µm..... 15

14. Basidiospores smaller, in the range 4-7 × 2.5-4 µm.....17

15. Pileus white to cream; staining reddish-brown 16

15. Pileus brown; not staining.....*M. megaspora* sp. nov.

16. Cheilocystidia ventricose to ventricose-capitate .....

.....*M. rubrobrunnescens* v. *rubrobrunnescens* sp. nov.

16. Cheilocystidia tibiiform.....*M. rubrobrunnescens* v. *tibiicystis* var. nov.

17. Pileus white to cream.....18

17. Pileus more deeply pigmented, at least on the disc (light brown, brown, grayish red, reddish brown).....21

18. Basidiospores 5-7 × 3-4 µm.....19

18. Basidiospores 4-5 × 2.5-3 µm.....20

19. Pileus staining reddish-brown; pileipellis hyphae with membranous pigments.....*M.* aff. *alba*

19. Pileus not staining; pileipellis hyphae with vacuolar pigments.....*M. subalba*

20. Lamellae crowded and narrow; cheilocystidia bifid with 2 heads, capitate to subcapitate.....*M. bifida* sp. nov.

20. Lamellae less crowded and broadly ventricose; cheilocystidia ventricose-capitate with a long flexuous neck.....*M. subarginea*

21. Pileus grayish-red, dull red or with reddish-brown tones on the disc.....22

21. Pileus light brown to brown.....25

22. Pileus reddish-brown on the disc, white elsewhere, staining yellow then reddish-brown; basidiospores 4-5 × 2.5-3 µm, ...*M.* aff. *arginea*

22. Pileus grayish-red overall, not staining; basidiospores 5-6.5 × 3-4 µm 23

23. Cheilocystidia capitate; pileipellis hyphae with vacuolar pigments.....24

23. Cheilocystidia non-capitate; pileipellis hyphae with membranous pigments .....*M. suthepensis* sp. nov.

24. Carpophores slender (pileus 6-22 mm diam., stipe 18-42 mm long); stipe white.....*M. gracilis*

24. Carpophores stout (pileus 10-18 mm diam., stipe 15-18 mm long); stipe violet red.....*M. lateritia* var *vinaceipes* var. nov.

25. Basidiospores 4-4.5 × 2.5-3 µm.....*M. arginophaea*

25. Basidiospores in the range 5.2-6.5 × 3-4 µm 26

26. Annulus membranous; pileipellis hyphae distinctly constricted at the septa, sausage-like, with membranous pigments .....*M. allantoidea* sp. nov.

26. Annulus cortinate; pileipellis hyphae slightly constricted at the septa, with vacuolar pigments .....*M. brunneosperma* var. *cortinata*

6.5 species descriptions

1. ***Micropsalliota* aff. *alba*** Heinem. & Little Flower, Bull. Jard. Bot. Nat. Belg. 53: 75. 1983. **Fig.** C.1; Plate E.1.a.

Pileus 4-8 mm diam., 4-6 mm tall, cuspidate to conical, with a distinct papilla at disc, edge appendiculate with partial veil remnants; surface dry, silky from radial fibrils, striate, white to cream colored. *Context* 0.5 mm thick at disc. *Lamellae* free, crowded, with 2 series of lamellulae, 1 mm broad, ventricose, light brown (6D5). *Stipe* 20-25 × 0.6 mm, cylindrical and slender, fibrous, hollow, smooth, white. *Annulus* membranous, superior, persistent, single, edge entire, 0.2 mm broad, white. *Odor* not distinctive. Pileus and stipe staining brown or reddish-brown on bruising.

Macrochemical reaction: KOH-reaction reddish-brown.

Spores 5.5–7 × 3.2–4 µm [$\bar{x}$ = 6.05 ± 0.95 × 3.5 ± 0.5 µm, Q = 1.57-2, Q_m = 1.75 ± 0.25, n = 20], ellipsoid, without germ pore, brown. *Basidia* 12-16 × 6-7 µm, clavate, 4-spored.

Spores 5.5–7 × 3.2–4 µm [$\bar{x}$ = 6.05 ± 0.95 × 3.5 ± 0.5 µm, Q = 1.57-2, Q_m = 1.75 ± 0.25, n = 20], ellipsoid, without germ pore, brown. *Basidia* 12-16 × 6-7 µm, clavate, 4-spored. *Cheilocystidia* 17-60 × 5-8 (-11) µm [x = 34.4 ± 25.6 × 7 ± 4 µm], irregularly lecythiform; apical capitulum 3.5-6.5 µm diam., arising from a long, sinuous neck, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 5-15 µm diam., smooth or occasionally incrusted, constricted at septa, with membranous pigments. *Annulus* composed of hyphae 3-6 µm diam., cylindrical, terminal cell slightly swollen at apex, hyaline, smooth, branched.

Habit: gregarious in soil in shaded and humid area.

Known distribution: India, Kerala, Calicut University campus (type distribution); Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., New Waterfall trail, 30 June 2005, collected by Ruilin Zhao, ZRL2067 (SFSU).

Notes: Thai specimen is close to *M. pudica* Heinem. & Leelav. (Heinemann and Leelavathy 1991) which is another tiny white species with reddish staining and similar cheilocystidia and basidiospores size, but *M. pudica* lacks an annulus and the basidiospores are conspicuously truncated. We are tentatively identifying the Thai specimen as closest to *M. alba* Heinem. & Little Flower (Heinemann and Flower 1983) because they both share the following features: tiny and fragile basidiomes with white and silky pileus, basidiospores in the range of 5.5-7 × 3-4 µm that are mostly ellipsoid or rarely amygdaliform, capitate cheilocystidia with a long sinuous neck, and rather broad pileipellis hyphae with membranous pigments. The Thai material differs from *M. alba* only in forming a cuspidate pileus and having a reddish-brown staining reaction.

2. ***Micropsalliota albosericea*** Heinem. & Leelavathy, Mycol. Res. 95: 341. 1991. Fig. C.2; Plate E.1.b.

Pileus 3-7 mm diam., conical, expending to convex or plano-convex, cuticle exceeding the sometimes denticular margin; surface dry, covered with fibrils, some aggregated into fine fibrillose strands, pure white. *Context* membranous, white. *Lamellae* free, distinct, with 2 series of lamellulae, 1-1.5 mm broad, broadly ventricose, white, becoming orange-gray to brownish white then brown. *Stipe* 10-20 (-30) × 0.2-0.5 mm, slender, fibrous, hollow, smooth or slightly tomemtose, white. *Annulus* pendent or percurrent, single, superior, fugacious, white. *Odor* not distinctive. Not staining on bruising and cutting. Dried specimens brown or dark brown (not purple).

Macrochemical reaction: KOH-reaction reddish-brown.

Spores 4.5-6 × 3-4 µm [$\bar{x}$ = 5.3 ± 0.4 × 3.2 ± 0.3 µm, Q = 1.4-2, Q_m = 1.72 ± 0.13, n = 20], mostly cymbiform, apiculus projecting, occasionally ellipsoid, without germ pore, brown. *Basidia* 5-6 × 12-14 µm, clavate, 4-spored. *Cheilocystidia* 18-30 × 6-11 µm, clavate to ventricose-capitate or subcapitate, capitulum 5-9 µm diam. *Pileipellis* a cutis composed of hyphae 5-8 µm diam., hyaline, smooth, cylindrical. *Annulus* composed of hyphae 3-7 µm diam., terminal cell slightly swollen, branched, hyaline, smooth.

Habit: gregarious in soil.

Known distribution: India, Kerala, Calicut University campus (type distribution); Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Teng Dist., Highway 1095 at 22 km marker, N19°07.57' E98°45.65', elev.750 m., 11 June 2006, collected by Ruilin Zhao, ZRL3052 (SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 18 August 2005, collected by Ruilin Zhao, ZRL2131 (SFSU); same location, June 2006,collected by Ruilin Zhao, ZRL3049 (SFSU).

Notes: This species is generally white except for an occasional brown tone on the disc. In the field, the distant and ventricose lamellae can distinguish this species from other white tiny *Micropsalliota* species. The clavate to ventricose-capitate or subcapitate cheilocystidia and cymbiform spores are also distinctive characters of this species. *Micropsalliota plumaria* (Berk. & Broome) Höhn. (Pegler 1986) is a similar species to *M. albosericea*, but the former species has a squamulose-floccose pileus that develops rusty brown tones on the disc, has ellipsoid to amygdaliform spores (not cymbiform) and dried herbarium specimens are uniformly purple-brown.

3. ***Micropsalliota allantoidea*** R.L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov.

Fig. C.3; Plate E.2. a-b.

Pileus 5-10 mm diam., hemispheric, expanding to plano-convex; surface densely scaly overall, grayish-brown (7E3). *Lamellae* free, crowded, with 3 series of lamellulae, 1-1.5 mm broad, brown (6F4). *Context* thickened at the disc and thin at the margin, white. *Stipe* 10-20 × 0.5 mm, cylindrical, straight, smooth, white when young, with a light brown tone with age. *Annulus* membranous, persistent, pendent, white. Odor not distinctive. No color staining when bruised or cut.

Macrochemical reaction: KOH-reaction unknown.

Spores 5.2-6.5 × 3-3.8 µm [$\bar{x}$ = 5.8 ± 0.3 × 3.4 ± 0.2 µm, Q = 1.6-2, Q_m = 1.73 ± 0.27, n = 20], ellipsoid, some amygdaliform, wall thickening at apex, no germ pore, brown. *Basidia* 13-19 × 6-7 µm, clavate, 4-spored. *Cheilocystidia* 24-38 × 4-11 µm, ventricose-capitate with an elongate neck, apical capitulum 5-11 µm diam., hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 20-44 × 8-17 µm, cells sausage-like, catenulate, smooth, brown, pigments membranous or sometimes granular-incrusted. *Annulus* composed of hyphae 2-5 µm diam., hyaline, smooth, branched.

Habit: solitary or scattered in soil.

Etymology: "allantoidea" refers to the sausage-shaped pileipellis cells.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Mok Fa Waterfall, 25 June 2005, collected by Jennifer Kerekes. ZRL2038 (Holotype: SFSU).

Notes: The new species is distinguished by its small fruiting bodies with scaly grayish-brown pileus, lack of staining when bruised or cut, and the distinctive sausage-shaped cells with membraneous pigments in the pileipellis. *Micropsalliota allantoidea* differs from *M. bogoriensis* Heinem. (Heinemann 1983) which has a reddish-brown pileus, smaller spores (4-5 × 2.8-3.2 µm) and pileipellis hyphae 40-140 × 7-8 µm without constrictions and with conspicuous pigment incrustationa. *Micropsalliota pholiotinoides* Heinem. (Heinemann 1983) differs in having a purplish-brown pileus 20-30 mm diameter, a stipe 50-90 × 3-4 mm, slightly smaller spores 4.5-5.7 × 2.7-3.3 µm, and pilleipellis hyphae 5-11 µm diameter with vacuolar pigments.

4. ***Micropsalliota* aff. *arginea*** (Berk. & Broome) Pegler & R.W. Rayner, Kew Bull. 23: 367. 1969. Fig. C.4; Plate E.2. c.

Pileus 5-12 mm diam., campanulate or convex, expanding to broadly convex with age; surface silky-fibrillose, white overall when young, sometimes becoming cream to pale gray when old, staining yellow then red to reddish-brown. *Context* white, thickened at the disc and thin at the margin. *Lamellae* free, crowded to close, with 2-3 series of lamellulae, 1 mm broad, straight to slightly ventricose, white becoming yellowish-brown (5D5) to brown, edges paler. *Stipe* 17-25 × 1 mm, cylindrical, smooth or slightly fibrillose, white. *Annulus* present, fugacious, single, superior, edge torn, white. Odor not distinctive. Pileus flesh and stipe staining yellow then reddish-brown.

Macrochemical reaction: KOH reaction reddish-brown then dark brown.

Spores 4-5 × 2.5-3 µm [$\bar{x}$ = 4.7 ± 0.4 × 3 ± 0.1 µm, Q = 1.3-2, Q_m = 1.58 ± 0.42, n = 20], ellipsoid, ellipso-amygdaliform, with apical thickening, no germ pore, brown. *Basidia* 13-16.5 × 4-6.5 µm, clavate, 4-spored, sterigma very long (up to 3.5 µm). *Cheilocystidia* 25-35 × 3-6 µm, tibiiform, base and middle cylindrical or slightly swollen, with a narrower neck (2.5-3.5 µm) and apical capitulum 6-9 µm diam., hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of repent hyphae 5-18 µm diam., hyaline or bearing reddish-brown granular incrustations, constricted at the septa on some hyphae. *Annulus* composed of branched, light brown hyphae with apical cell tibiiform (capitulum 3-9 µm diam.). *Stipitipellis* with tibiiform caulocystidia like the cheilocystidia, some bearing reddish-brown granular incrustations.

Habit: gregarious in soil along side of road.

Known distribution: Sri Lanka (type distribution), Thailand.

Material examined: THAILAND, Chiang Mai Prov., Chiang Dao Cave, 22 July 2006, collected by Ruilin Zhao, ZRL3090 (SFSU).

Notes: The species most similar to the single Thai specimen is *M. arginea* (Pegler 1986). *Micropsalliota arginea*, described from Sri Lanka, is only subtly different in having cheilocystidia with a broader ventricose base (5-9 µm diam.) and a longer neck below the capitulum, and the context has not been reported as staining yellow to red. In the Thai specimen, the cheilocystidia base is cylindrical to subcylindrical, 3-6 µm diam, and the context stains distinctly yellow then red. Until further specimens are collected from Thailand, our determination as *M. arginea* is tentative. Amongst other white, small-spored species, the Thai material is closest to *M. plumaria*, *M. subalba* Heinem. & Little Flower, and *M. pseudoarginea* Heinem. The Thai material differs from *M. plumaria* which has subcapitate, broadly ventricose cheilocystidia lacking an elongated neck (Pegler 1986). *Micropsalliota subalba* differs in having larger spores 5.6-6.5 × 3.5-4.1 µm and broader ventricose-capitate cheilocystidia 7-13 µm diam, but does show the same staining reaction (Heinemann and Flower 1983). *Micropsalliota pseudoarginea* differs in having clavate and non-capitate cheilocystidia 7-9 µm diameter (Heinemann 1982).

5. ***Micropsalliota arginophaea*** Heinem., Bull. Jard. Bot. Nat. Belg. 50:51. 1980. Fig. C.5; Plate E.3.a.

Pileus 6-20 mm diam., hemispheric to convex or campanulate, expanding to plano-convex, often subumbonate to umbonate; surface fibrillose-squamulose, densely so on the disc, sometimes glabrescent, brown. *Context* 0.5-0.8 mm thick, white. *Lamellae* free, crowded, with 3 series of

lamellulae, 1-1.5 mm broad, straight to slightly ventricose, light brown to brownish-gray (7C2) or brown (7E4), edges paler. *Stipe* size variable, 7-40 × 0.5-2 mm, cylindrical, slender or stout, smooth above annulus, fibrillose to floccose below the annulus, white. *Annulus* persistent, pendent, single, rigid, superior, membranous, 0.5-2 mm broad, white. Odor not distinctive. No color staining when bruised or cut.

Macrochemicial reaction: KOH reaction reddish-brown.

Spores 4-5.5 × 2.5-3 µm [$\bar{x}$ = 4.8 ± 0.5 × 2.8 ± 0.3 µm Q = 1.3-2, Q_m = 1.74 ± 0.44, n = 20], ellipsoid to amygdaliform, no germ pore, brown. *Basidia* 9-12 × 4-5 µm, clavate, 4-spored. *Cheilocystidia* 17-30 (-42) × 4-8 µm, apex 3.5-6 µm diam., shape variable, subcylindrical to clavate, mostly with long elongated neck, some subcapitate with swollen base, some with a spear-like apex, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 3-8 (-12) µm diam., cylindrical, light brown or brown, sometimes slightly constricted at septa, with membranous and incrusting pigments. *Annulus* hyphae 5-8 µm diam., hyaline, smooth.

Habit: 2-4 fruiting bodies cespitose, clusters gregarious in red soil.

Known distribution: Malaysia (type distribution); Thailand.

Material examined: THAILAND, Chiang Mai Prov., Chom Thong, Ob Luang National Park, elev. 1812 m, 23 June 2005, collected by Thanh Huyen Le, ZRL2027 (SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m, 3 July 2005, collected by Thitiya Boonpratuang, ZRL2088 (SFSU) and ZRL2091 (SFSU); same location, 3 July 2005, collected by Amy Honan, ZRL2089 (SFSU); same location, 18 August 2005, collected by Ruilin Zhao ZRL2130 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m, 2 September 2006, collected by Thanh Huyen Le, ZRL3097 (SFSU); same location, 20 September 2006, collected by Ruilin Zhao, ZRL3106 (SFSU); same location, 10 October 2006, collected by Ruilin Zhao, ZRL3110 (SFSU).

Notes: *Micropsalliota arginophaea* Heinem. (Heinemann 1980) is distinguished by a brown fibrillose-squamulose pileus, small spores, no staining reaction when bruised or cut, but a strong reddish-brown reaction with KOH. Although the fruitbody features and cheilocystidia shape are quite variable in this species, the ITS data indicate that this variability can be accepted as infraspecific variability. The LSU data, however suggest that specimens ZRL 3097 and ZRL 3110 fall outside of the clade containing other specimens of *M. arginophaea*, but without statistical support. *Micropsalliota arginophaea* is similar in pileus coloration and ornamentation

and spore size to *M. brunneola* Heinem. and *M. brunneosperma* (Singer) Pegler. *Micropsalliota brunneola* differs, however, in forming a larger, more strongly squamose pileus (20-30 mm diam. with erect or recurved squamules up to 1 mm high), and broader pileipellis hyphae (8-20 µm diam.). Unfortunately, there are no published data on cheilocystidia morphology for *M. brunneola* (Heinemann 1980). *Micropsalliota brunneosperma* differs in having broader spores (3.2-4.5 µm diam.), and consistently capitate cheilocystidia with a long and narrow neck (Pegler 1977).

6. ***Micropsalliota bifida*** R.L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov. Fig. C.6; Plate E.3.b.

Pileus 9-15 (- 25) mm diam., obtusely conical to convex, becoming plano-convex with slightly incurved margin in age; surface dry, glabrous to finely powdery-fibrillose, pure white overall. *Context* membranous, white. *Lamellae* free, very crowded, with 4 series of lamellulae, narrow (0.5-0.8 mm), orange-gray to brownish-yellow, becoming grayish-brown (8E3) to light brown (6B4), brown (7E4) or dark brown (7F5) in age, edges paler. *Stipe* 15-30 (-40) × 1-2 mm, cylindrical, smooth or silky, white. *Annulus* persistent, pendent or percurrent, membranous, superior, edge entire or torn. Odor not distinctive. No color staining when bruised or cut. Dried specimens brown.

Macrochemicial reaction: KOH reaction strong reddish-brown; ammonia reaction blue then green.

Spores 3.8-5 × (2-) 2.5-3.2 µm [$\bar{x}$ = 4.5 ± 0.2 × 2.7 ± 0.1 µm, Q = 1.3-2.1, Q_m = 1.67 ± 0.34, n = 80], ellipsoid to amygdaliform, some with apical thickening, no germ pore, brown. *Basidia* 8-12 × 4-5 µm, short-clavate, 4-spored. *Cheilocystidia* 14-27 (-30) ×5-9 µm broadly clavate to subcapitate, bifid with two irregular toe-like lobes, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 3-5 µm diam., hyaline, smooth.

Etymology: "bifida", meaning forked, referring to the two-lobed cheilocystidia.

Habit: gregarious on red soil along road sides.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m, 13 June 2006, collected by Maria Alice Neves, ZRL3067 (Holotype: SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962

m, 17 July 2005, collected by Ruilin Zhao, ZRL2103 (SFSU); Chiang Mai Prov., Chom Thong, Ob Luang National Park, elev. 1812 m. 23 June 2005, collected by Thanh Huyen Le, ZRL2026 (SFSU); Chiang Mai Prov., Doi Inthanon National Park, Highway 1009 at 25 km marker, N18°32.54' E98°33.51', elev. 1076 m, 27 June 2005, collected by Amy Honan, ZRL2057 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m. 8 June 2006, collected by Ruilin Zhao, ZRL3042 (SFSU);Chiang Mai Prov., Mae Taeng Dist., New Waterfall, 30 June 2005, collected by Thanh Huyen Le, ZRL2070 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw Village, forest trail, N19°08.07' E98°38.90', elev. 1300 m, 30 June 2006, collected by Ruilin Zhao, ZRL3076 (SFSU).

Notes: This new species is distinguished by a white glabrous pileus, a red KOH-reaction and blue-green ammonia reaction, small spores and bifid cheilocystidia. *Micropsalliota bifida* is similar to a number of small white species, but differs as itemized below. *Micropsalliota subarginea* Heinem. has long, cylindrical, flexuous, subcapitate cheilocystidia 30-60 × 4-8 µm (Heinemann 1980). *Micropsalliota arginea* has cheilocystidia with a broadly ventricose base and a distinct apical capitulum that arises from a long elongate neck (Pegler 1986). *Micropsalliota subalba* has larger spores 5.5-6.5 × 3.5-4 µm, and ventricose-capitate cheilocystidia 32-40 × 7-13 µm (Heinemann and Flower 1983). *Micropsalliota lateritia* Heinem. has slightly longer spores 4.5-5.8 × 2.6-3.2 µm, clavate to ventricose cheilocystidia 22-40 × 9-18 µm, and pilei that stain reddish brown with age (Heinemann 1980). *Micropsalliota pseudoarginea* has clavate, basidia-like cheilocystidia, and a yellow KOH reaction (Heinemann 1982). *Micropsalliota plumaria* has smaller fruiting bodies (pileus 4-10 mm diam., stipe 10-25 × 0.5-1 mm), pilei with rusty brown disc, broadly clavate, subcapitate cheilocystidia, and fruit bodies that dry purple brown (Pegler 1986). *Micropsalliota alba* has smaller pilei (4-6 mm diam.), larger spores 5.8-6.6 × 3.3-3.6 µm, and broadly capitate cheilocystidia (Heinemann and Flower 1983). Finally, *M. albosericea* Heinem. & Leelav. has smaller fruiting bodies (pileus 5 mm diam., stipe 8-12 × 0.5 mm), cymbiform spores and ventricose-capitate cheilocystida (Heinemann and Leelavathy 1991).

7. ***Micropsalliota brunneosperma*** (Singer) Pegler var. ***cortinata*** Heinem., Bull. Jard. Bot. Nat. Belg. 50: 52. 1980. Fig. C.7; Plate E.4.a.

Pileus 10-20 mm diam., obtusely conical, expanding to convex or plano-convex; disc brown, squamulose; paler towards the white, fibrillose, margin; partial veil remnants remaining as fibrillose patches along pileus margin. *Context* 0.5 mm thick, white. *Lamellae* free, close to

crowded, with 2 series of lamellulae, 3 mm broad, ventricose, orange-gray (6B2) to brownish-orange (6C4), edges concolorous. *Stipe* 11-20 × 1-1.5 mm, cylindrical, fibrillose, white. *Annulus* cortinate, usually absent on stipe surface, remaining as fibrils on margin of pileus. Odor iodoform. No color staining when bruised or cut.

Macrochemicial reaction: KOH reaction negative.

Spores 5.2-6.5 × 3-4 µm [$\bar{x}$ = 6 ± 0.3 × 3.6 ± 0.3 µm, Q = 1.5-2, Q_m = 1.72 ± 0.28, n = 20], ellipsoid, no germ pore, brown. *Basidia* 12-16 × 6-7 µm, broadly clavate, 4-spored. *Cheilocystidia* 26-46 × 5-10 µm, clavate to ventricose with a long flexuous neck and subcapitate apex 3-5.5 µm diam, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis of hyphae 7-15 µm diam., constricted at the septa, light brown, with membranous pigments. *Annulus* hyphae cylindrical, 5-10 µm wide, hyaline, smooth, branched, not constriction at the septa. *Stipitipellis* hyphae same as annulus hyphae.

Habit: gregarious in red soil.

Known distribution: Singapore (type distribution); Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m, 18 August 2005, collected by Ruilin Zhao, ZRL2129 (SFSU).

Notes: This variety is distinguished by pilei with brown floccose scales on the disc over a white background and white fibrillose margin, and by cortinate partial veil that leaves remnants only on the pileus margin. It differs from *M. arginea* and *M. subarginea* which have silky pileus that are white with a pale disc (gray to yellow), smaller spores (4.5-5 × 2.5-3.5 µm), and a well-developed, persistent, membranous annulus (Pegler 1986). This variety is also similar to *M. subalba* which has the same spore range, absence of an annulus, an appendiculate margin (from partial veil remnants) and capitate cheilocystidia (Heinemann and Flower 1983). The Thai material however has a squamulose-fibrillose pileus, ventricose cheilocystidia with a long narrow neck and subcapitate apex, and a negative KOH reaction. In *M. subalba*, the pileus is smooth to silky, cheilocystidia are ventricose-capitate with a capitulum7-10 µm diam., and a brown KOH reaction (Heinemann and Flower 1983).

8. ***Micropsalliota furfuracea*** R.L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov.

Fig. C.8; Plate E.4. b-d.

Pileus 20-50 mm diam., obtusely conic to convex, becoming plano-convex, mostly with a distinct broad umbo; margin decurved and striate; disc densely covered with large flake-like, appressed scales, margin furfuraceous, ornamentation light brown (6D4) over cream-coloured background. *Context* 1-2 mm thick, white. *Lamellae* free, close, with 3-6 series of lamellulae, 3-4 mm broad, yellowish-brown to brownish-orange (5C4) or light brown (6D4). *Stipe* (35-) 60-130 × 2-5 mm, cylindrical, curved, hollow, tough and flexuous, smooth to silky, white to cream. *Annulus* pendent, superior, single, persistent, edge entire, striate, up to 7 mm broad, white. Odor fungal or of seaweed. Pileus, lamellae, annulus and stipe strongly staining red when bruised.

Macrochemical reaction: KOH reaction reddish-brown then dark brown on surface of pileus, dark green on context.

Spores 6-7 × 3.5-4 µm [$\bar{x}$ = 6.6 ± 0.3 × 3.9 ± 0.2 µm, Q = 1.5-2, Q_m = 1.69 ± 0.32, n = 20], ellipsoid, with apical thickening, no germ pore, brown. *Basidia* 15-21 × 6-9 µm, broadly cylindrical to subclavate, 4-spored. *Cheilocystidia* 20-50 (-63) × 7-12 µm, apex 4-12 µm diam., shape exceedingly variable, ranging from clavate to ventricose-capitate, lageniform or strangulate-tibiiform, some having a long narrow neck, some with narrow base and large capitulum, occasionally broadly clavate-subcapitate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 5-13 µm diam., slightly constricted at septa, distinctly incrusted, walls reddish-brown. *Annulus* hyphae 2.5-5 µm diam., cylindrical, hyaline, smooth, branched.

Habit: 2-3 fruiting bodies cespitose, clusters gregarious in soil in forest.

Etymology: "furfuraceus" – covered with bran-like scales, referring to the pileus surface ornamentation.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 26 May 2006, collected by Ruilin Zhao, ZRL3006 (Holotype: SFSU); Chiang Mai Prov., Doi Inthanon National Park, Highway 1009 at 25 km marker, N18°32.54' E98°33.51', elev. 1076 m., 4 August 2005, collected by Tran Thi My Hanh, ZRL2119 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 24 May 2007, collected by Phongeus Sysouphanthong, ZRL4028 (SFSU).

Notes: *Micropsalliota furfuracea* looks like an *Agaricus* species in macromorphology, but its capitate cheilocystidia, incrusted pileipellis hyphae and spore endosporium (thickened apex)

indicate that it belongs in the genus *Micropsalliota* (Heinemann 1976). The ITS and LSU data confirm this taxonomic placement (Figs. 6.2-6.4). The relatively large fruiting bodies, brown flake-like scales on the pileus, vivid red to reddish-brown staining and large spores distinguish it from other species. Similar species include: *M. brunneola* which has erect or recurved squamules on the pileus, much smaller fruiting bodies (pilei 20-30 mm diam.) and spores 4-5 × 2.5-3 µm (Heinemann 1980); *M. lateritia* which has smaller fruiting bodies (pilei 8-15 mm diam.) and spores 5-6.5 × 3 µm, and stains yellow. The most phenetically similar species is *M. brunneosperma,* reported from Argentina and Uganda, which shares with *M. furfuracea* red staining, similar sized and shaped cheilocystidia, and similar spore size (Pegler 1977). However, in *M. brunneosperma* the fruiting bodies are smaller (pilei 5-25 mm diam.), the pileus surface is fibrillose, and the pileipellis hyphae are composed of chains with short cylindrical cells.

Another specimen, ZRL 3018, has been retained temporarily as distinct from *M. furfuracea* and denoted *M. sp.* 1, because it lacks incrusted pileipellis hyphae and has copious oleiferous hyphae in all tissues. In other features it is nearly indistinguishable from *M. furfuracea* and the ITS and LSU data support its close relationship (Figs. 6.2-6.4).

9. ***Micropsalliota globocystis*** Heinem., Bull. Jard. Bot. Nat. Belg. 50: 57. 1980.

Fig. C.9; Plate E.5.a-b.

Pileus with a wide range of size, 17-60 (-80) mm diam., conical to broadly conical, convex or plano-convex and umbonate; disc with dense, erect squamules, elsewhere hirsute to fibrillose-scaly, purple to purplish brown, grayish-brown (8E3) or reddish-brown (8E4). *Context* firm, up to 3 mm thick, white. *Lamellae* free, crowded, with 2-4 series of lamellulae, 2-4 mm broad, white at first, becoming orange-white to grayish-white, orange-gray (6B2) finally brown, edges paler. *Stipe* 40-120 × 3-6 (-12) mm, cylindrical, hollow, smooth to tomentose, white or with reddish-brown tone. *Annulus* pendent or percurrent, single, superior, edge entire, persistent, membranous, rigid, up to 5 mm broad, white. Odor of seaweed. Staining yellow then reddish-brown when bruised.

Macrochemical reaction: KOH reaction strongly reddish-brown on surface of pileus, dark green in context.

Spores 6-7 (-8) × 3.5-4.2 µm [$\bar{x}$ = 6.3 ± 0.4 × 4 ± 0.1, Q = 1.4-1.8, Q_m = 1.59 ± 0.21, n = 20], ellipsoid, with apical thickening, no germ pore, brown. *Basidia* 13-21 × 6-8 µm, broadly clavate, hyaline, 4-spored. *Cheilocystidia* 30-60 × 9-14 µm, apex 9-15 µm diam., broadly clavate to

clavate-capitate, some subcapitate, rarely ventricose, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis a* cutis composed of hyphae 10-25 µm diam., constricted at the septa, with reddish-brown, distinctly incrusting and membranous pigments. *Annulus* hyphae hyaline, smooth, terminal cells 65-80 × 15-20 µm, clavate. *Stipitipellis* hyphae similar to pileipellis, except in some samples with capitate caulocystidia (apex 15-20 µm diam.).

Habit: cespitose and gregarious or occasionally solitary, in soil.

Known distribution: Singapore (type distribution); Thailand.

Material examined: THAILAND, Chiang Mai Prov., Doi Inthanon National Park, Highway 1009 at 25 km marker, N18°32.54' E98°33.51', elev. 1076 m., 5 June 2006, collected by Ruilin Zhao, ZRL3027 (SFSU); Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 2 July 2005, collected by Jennifer Kerekes, ZRL2072 (SFSU); Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 7 June 2006, collected by Maria Alice Neves, ZRL3029 (SFSU) and ZRL3030 (SFSU); same location, 7 June 2006, collected by Dennis Desjardin, ZRL3033 (SFSU); Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m, 13 June 2005, collected by Todd Osmundson, ZRL3066 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 13 August 2005, collected by Kevin D Hyde, ZRL2126 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 14 July 2006, collected by Ruilin Zhao, ZRL3082 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 16 June 2005, collected by Thanh Huyen Le, ZRL2021 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 26 May 2006, collected by Ruilin Zhao, ZRL3004 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 8 June 2006 by, collected Tim Baroni, ZRL3040 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 12 June 2006, collected by Maria Alice Neves, ZRL3060 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Highway 1095 at 22 km marker, N19°07.57' E98°45.65', elev.750 m., 4 June 2006, collected by Ruilin Zhao, ZRL3019 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Huai Nam Dang National Park, nature trail, N19°18.29' E98°35.88', elev. 1530 m., 29 June 2005, collector

unknown, ZRL2060 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw Village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 16 July 2005, collected by Ruilin Zhao, ZRL2100 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw Village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 3 August 2005, collected by Ruilin Zhao, ZRL2113 (SFSU);

Notes: *Micropsalliota globocystis* Heinem. is charactered by purplish brown pilei covered with erect hirsute scales, tissue that stains yellow then reddish-brown, large spores, very large and apically broad cheilocystidia, and membranous-incrusting pigments (Heinemann 1980). It is a rather robust species for a *Micropsalliota*, often resembling an *Agaricus* species. Similar species include: *M. vinaceoumbrinus* (A.H. Sm.) Heinem. which has broadly ellipsoid spores 6.2-7.3 × 4.8-5.4 µm, and much narrower cheilocystidia 38-52 × 4-5 µm (Heinemann 1977); *M. gracilis* Heinem. and *M. roseipes* Heinem. which have vacuolar pigments, and much smaller fruiting bodies(Heinemann 1980); *M. brunneosperma* which has smaller spores (4.8-6.5 × 3.2-4.5 µm), narrower cheilocystidia (16-45 × 2.5-6 µm) and smaller fruiting bodies (Pegler 1977). In the same publication where he described *M. globocystis*, Heinemann (1980) described *M. repanda* Heinem. and indicated that it differed only in forming stouter basidiomes; e.g., in *M. globocystis* pilei measured 25-35 mm diam. and stipes 50-90 × 2-3 mm, whereas in *M. repanda* pilei were 15-45 mm diam. and stipes 25-40 × 2.5-7 mm. The Thai specimens encompassed both of these size ranges and some fruitbodies were even larger. We can find no taxonomically distinguishing differences between these two species and consider them to represent the same taxon; we use the epithet *M. globocystis* to represent the Thai material.

10. ***Micropsalliota gracilis*** Heinem., Bull. Jard. Bot. Nat. Belg. 50: 60 1980.

Fig. C.10; PlateE. 5.c.

Pileus 6-22 mm diam., obtusely conical to convex or plano-convex, sometimes applanate or umbonate, striate or non-striate; surface squamulose to furfuraceous, grayish-red to brownish red (9C5, 10C-D5-6) overall when young, disc remaining so in age, margin fading to dull red (11B-C4) or paler.. *Context* 0.5 mm thick, white. *Lamellae* free, crowded or close, with 3 sries of lamellulae, 1.5-3 mm broad, slightly ventricose, light brown to brownish-gray, brown (7E3-4) with age, edges paler. *Stipe* 18-42 × 0.5-1.5 mm, cylindrical, slender, hollow, smooth to tomentose, white. *Annulus* pendent, single, superior or median, persistent, edge entire, membranous, white. Odor not distinctive. Not staining when bruised or cut.

Macrochemical reaction: KOH reaction greenish gray.

Spores 5.5-6.5 × 3-4 µm [$\bar{x}$ = 5.9 ± 0.3 × 3.3 ± 0.3 µm, Q = 1.6-2, Q_m = 1.81 ± 0.21, n = 20], amygdaliform to ellipsoid, with apical thickening, no germ pore, brown. *Basidia* 11-15 × 5-7 µm, broadly clavate, hyaline, 4-spored. *Cheilocystidia* 27-43 × 5-9 µm, versiform, ventricose to irregularly tibiiform, capitate or subcapitate with long narrow neck, capitulum 3-8 µm diam., occasionally mucronate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 7-20 µm diam., constricted at septa, reddish-brown, smooth, with vacuolar pigments. *Annulus* hyphae 2-5 µm diam., hyaline, smooth.

Habit: gregarious in soil.

Known distribution: Singapore (type distribution); Thailand.

Material examined: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 2 July 2005, collected by Amy Honan, ZRL2079 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Mok Fa Waterfall., 25 June 2005, collected by Amy Honan, ZRL2041 (SFSU); same location, 7 July 2006, collected by Ruilin Zhao, ZRL3079 (SFSU); same location, 29 June 2004, collected by Dennis E Desjardin, DED7727 (SFSU).

Notes: *Micropsalliota gracilis* is distinguished by its grayish red to red, furfuraceous pileus, slender fruiting bodies, vacuolar pigments and small spores. This species is similar to several other gracile species with red pilei. *Micropsalliota cardinalis* Heinem. from Argentina lacks an annulus, has clavate-subcapitate cheilocystidia, and yellow then reddish-brown staining on cutting (Heinemann 1989). *Micropsalliota laeta* Heinem. has larger spores (6.3-7.1 × 3.6-4 µm), a pale pileus (white to pale rose), and membranous pigments (Heinemann 1980). *Micropsalliota cymbispora* Heinem. & Little Flower has a fugacious-cottony annulus, narrower pileipellis hyphae (4-10 µm diam.) and smaller cymbiform spores 4.4-5.8 × 3-3.6 µm (Heinemann and Flower 1983). *Micropsalliota avellanea* Heinem. & Little Flower has cymbiform spores 5-5.8 × 2.8-3.6 µm, lacks of membranous annulus, and has a distinctly reddish-brown pileus (Heinemann and Flower 1983). *Micropsalliota cephalocystis* (Heinem.) Heinem. has large fruiting bodies darker brownish red pilei up to 25 mm diam., and incrusted pileipellis hyphae (Heinemann 1977).

11 ***Micropsalliota lateritia*** var. ***vinaceipes*** R. L. Zhao, Desjardin, K. Soytong & K. D. Hyde var. nov.

Fig. C.11; Plate E.6.a.

Pileus 10-18 mm diam., campanulate to plano-convex, finally applanate with a broad umbo; surface floccose-squamulose, squamules slightly erect or recurved, violet brown (11E5) overall. *Context* white, 1 mm thick at the disc and thinning at the margin, membranous. *Lamellae* free, close, with 2 series of lamellulae, 2.5 mm broad, ventricose, white to grayish-brown. *Stipe* 15-18 × 1-1.5 mm, cylindrical, curved, hollow in age, smooth above annulus, squamulose beneath annulus, violet red above, violet brown at the base. *Annulus* only present in young fruitbodies, membranous, pendent, single, superior, fugacious, edge torn, white. Odor fungal. No color staining when bruised or cut.

Macrochemical reaction: KOH reaction reddish-brown.

Spores 5-6 × 3-3.5 (- 4) µm [$\bar{x}$ = 5.7 ± 0.4 × 3.4 ± 0.3 µm, Q = 1.4-2, Q_m = 1.7 ± 0.3, n = 20], ellipsoid to amygdaliform, with apical thickening, no germ pore, brown. *Basidia* 11-16 × 5-7 µm, broad cylindrical, hyaline, 4-spored. *Cheilocystidia* 16-45 × 5-12µm, broadly clavate or seldom subcapitate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 6-18 µm diam., slightly constricted at the septa, brown, smooth, with vacuolar pigments. *Annulus* hyphae 5-7.5 µm diam., cylindrical, hyaline, smooth. *Stipitipellis* hyphae same as the pileipellis.

Habit: solitary or scattered in forest soils.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 2 July 2005, collected by Amy Honan, ZRL2073 (Holotype: SFSU).

Notes: The Thai material matches the description of *M. lateritia* Heinem. quite nicely, differing only in forming a vinaceous rather than a white stipe. Because of this obvious distinction and its distribution in northern Thailand (the type variety is known only from Singapore), we have chosen to describe it as a new variety. *Micropsalliota lateritia* var. *vinaceipes* is similar to *M. roseipes* Heinem. but the latter differs in having larger spores 6.2-7.4 × 4-4.8 µm (mean 6.7 × 4.4 µm), pileipellis hyphae up to 21 µm diam, and cheilocystidia that are more consistently capitate (Heinemann 1980).

12. ***Micropsalliota malabarensis*** Heinem. & Little Flower, Bull. Jard. Bot. Nat. Belg. 53: 80. 1983. Fig. C.12; Plate E.7.d-e.

Pileus 13 mm diam., convex subumbonate; surface silky to fibrillose-scaly, scales erect or recurved, light grayish-brown (8E3). *Context* white to cream, thin. *Lamellae* free, crowded, with

2 series of lamellulae, 2 mm broad, dark brown. *Stipe* 20 × 1-1.5 mm, cylindrical, silky, white to cream. *Annulus* fugacious, remnants brown. Odor not distinctive. Staining reactions unknown.

Macrochemical reaction: KOH reaction unknown.

Spores 5-6 × 3-4 µm [$\bar{x}$ = 5.7 ± 0.4 × 3.8 ± 0.3 µm, Q = 1.3-1.7, Q_m = 1.51 ± 0.21, n = 20], cymbiform, with apical thickening, no germ pore, brown. *Basidia* 11-15 × 5-7 µm, broadly cylindrical, hyaline, 4-spored. *Cheilocystidia* 30-58 × 5-8 µm, irregularly cylindrical to subclavate, capitate with a long, narrow and flexuous neck, capitulum 5-7 µm diam., smooth, hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 5-20 µm diam., constricted at septa, brown, with incrusting and membranous pigments. *Annulus* hyphae 2-3 µm diam., hyaline, smooth, branched. *Stipitipellis* hyphae 3-8 µm diam., cylindrical, hyaline, smooth.

Habit: solitary in rich soil.

Known distribution: India, Kerala (type distribution); Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Mok Fa Waterfall, 25 June 2005, collected by Dennis E Desjardin, ZRL2040 (SFSU).

Notes: *Micropsalliota malabarensis* is distinguished by cymbiform spores, a light grayish brown, fibrillose-scaly pileus, and incrusting and membranous pigments in the pileipellis hyphae. Other species possessing cymbiform spores include: *M. avellanea* Heinem. & Little Flower, which differs in having an avellaneous pileus, vacuolar pigments, and cheilocystidia with smaller capitula (4.5-5.5 µm diam.) (Heinemann and Flower 1983); *M. cymbispora* which differs in forming a rose brown pileus with appressed squamules, and cheilocystidia with smaller capitula (3.7-4.5 µm diam.) (Heinemann and Flower 1983).

13 ***Micropsalliota megarubescens*** R.L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov. Fig. C.13; Plate E.6.b.

Pileus 25-80 mm diam., obtusely conical to convex, becoming plano-convex and mostly umbonate with slightly flared margin in age; surface dry, densely fibrillose on the disc, white to cream becoming grayish brown with age. *Context* 3 mm thick, white. *Lamellae* subfree, crowded, with 3 series of lamellulae, 4-8 mm broad, white to light brown or orange-gray (5B2), edges paler. *Stipe* 70-120 × 5-15 mm, cylindrical, sometimes with basal rhizomorph, hollow, smooth to silky or with fine fibrils, white. *Annulus* pendent, membranous, edge entire, stretched, white, 5 –6 mm broad. Odor strongly of seaweed, unpleasant. Pileus staining yellow then reddish-brown, lamellae, stipe and annulus strongly staining reddish-brown when bruised or cut.

Macrochemical reaction: KOH reaction reddish-brown.

Spores 6-8 × 3.5-4.5 µm [$\bar{x}$ = 6.8 ± 0.5 × 4 ± 0.2 µm, Q = 1.5-2, Q_m = 1.71 ± 0.29, n = 20], amygdaliform, with apical thickening, no germ pore, brown. *Basidia* 17-25 × 6-8 µm, clavate, hyaline, 4-spored. *Cheilocystidia* 20-40 × 7-13 µm, typically clustered, clavate to broadly clavate, usually subcapitate, occasionally capitate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 50-120 × 8-15 µm, hyaline, slightly incrusted. *Annulus* hyphae 5-12 µm diam., terminal cells inflated to clavate, hyaline.

Habit: cespitose, gregarious in soil.

Etymology: "mega" – large; "rubescens" – becoming red, referring to the relatively large fruiting bodies that quickly stain red when bruised or cut.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 3 July 2005, collected by Tran Thi My Hanh, ZRL2086 (Holotype: SFSU); same location, 3 June 2005, collected by Edward Grand, ZRL2016 and ZRL2017 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Tung Joaw Village, forest trail, N19°08.07' E98°38.90', elev. 1300 m, 15 June 2005, collected by Ruilin Zhao, ZRL2008 and ZRL2009 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8" E 98°44'47.3", elev. 1050 m., 16 June 2005, collected by Thanh Huyen Le, ZRL2024 (SFSU).

Notes: Macromorphologically, this new species resembles an *Agaricus*, with robust fruitbodies and large pendent annulus. Subcapitate cheilocystidia, incrusted pileipellis hyphae and spores with an apical thickening (endosporium), however, indicate that this taxon belongs in *Micropsalliota* (Heinemann 1976). The ITS and LSU data support placement in *Micropsalliota.* *Micropsalliota subalba* is the most phenetically similar species because it has the same pileus features, a strong red staining reaction and similar sized and shaped cheilocystidia. *Micropsalliota subalba* differs, however, in forming smaller fruiting bodies (pilei 8-23 mm diam., stipes 30 × 2 mm), smaller spores (5.5-6.5 × 3.5-4 µm), and lacks an obvious annulus (Heinemann and Little Flower 1983).

14 ***Micropsalliota megaspora*** R.L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov. Fig. C.14; Plate E.7.a-c.

Pileus 5-12 mm diam., conical to convex or plano convex, applanate with a broad umbo in age; surface fibrillose or floccose to squamulose overall, squamules erect or recurved, brown (7E6) to dark brown (8E4). *Context* 0.5 mm thick, white to light grayish-brown. *Lamellae* free, crowded, with 3 series of lamellulae, 1 mm broad, light brown to brown, edges concolorous. *Stipe* 20-30 × 1-1.5 mm, cylindrical, slender, hollow in age, glabrous above annulus, finely fibrillose to squamulose below annulus, light brown to light gray. *Annulus* fugacious, pendent, single, superior, stretched, white with light brown edge, up to 0.5 mm broad. Odor not distinctive. Context stains pinkish red when bruised or cut.

Macrochemical reaction: KOH reaction at first negative, after 1 minute turning green on pileus; ammonia reaction dark green.

Spores 6-8 × 3.8-4.5 µm [$\bar{x}$ = 6.9 ± 0.5 × 4.1 ± 0.2 µm, Q = 1.4-1.9, Q_m = 1.7 ± 0.3, n = 20], amygdaliform to ellipsoid, with apical thickening, no germ pore, brown. *Basidia* 11-15 × 6-7.5 µm, broadly clavate, hyaline, 4-spored. *Cheilocystidia* (12-) 20-38 × 6-12 µm, ventricose with a long obtuse neck to pyriform, apex merely obtuse or seldom subcapitate, 3-6 µm diam., hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 10-22 µm diam., some cells swollen and constricted at the septa, brown, smooth to incrusted. *Annulus* hyphae 5-8 µm diam., cylindrical, hyaline, smooth to slightly incrusted. *Stipitipellis* hyphae 7.5-15 µm diam., cylindrical, hyaline, smooth to slightly incrusted.

Habit: solitary or scattered in red soil along road banks.

Etymology: "mega" – large; "spora" – spores, refers to the large basidiospores.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 18 June 2006, collected by Ruilin Zhao, ZRL3068 (Holotype: SFSU); same location, 26 June 2005, collected by Ruilin Zhao, ZRL2051 (SFSU).

Notes: *Micropsalliota megaspora* is distinguished by a brown, squamulose pileus, gracile and finely squamulose stipe, a context that stains pink when bruised and has no KOH reaction but a dark green ammonia reaction, relatively large basidiospores, and ventricose-rostrate to pyriform cheilocystidia. *Micropsalliota brunneosperma* differs in forming smaller spores (4.8-6.5 × 3-4.5 µm), distinctly capitate cheilocystidia, and a pileus with more reddish to purplish tones (Pegler

1986). *Micropsalliota globocystis* differs in forming larger fruiting bodies (pilei 25-35 mm diam., stipe 50-90 × 2-3 mm), and large, clavate-subcapitate cheilocystidia 45-65 × 10-20 µm (Heinemann 1980). *Micropsalliota brunneola* differs in forming more robust basidiomes (pilei 20-30 mm diam., stipe 25 × 3.5 mm), smaller spores (4-5 × 3-3.5 µm) and brown cheilocystidia or unknown shape and size (Heinemann 1980). *Micropsalliota repanda* has also differs in forming larger fruiting bodies (pilei 15-45 mm diam., stipe 25-40 × 2-5 mm) with purplish red squamules, and larger capitate cheilocystidia 30-40 (- 70) µm long with capitulum 8-11 µm diam. (Heinemann 1980).

15. ***Micropsalliota pleurocystidiata*** Heinem. & Little Flower, Bull. Jard. Bot. Nat. Belg. 53: 81. 1983. Fig. C.15; Plate E.8.a-b.

Pileus 50-100 (-130) mm diam., conical to broadly conical, soon convex with a broad umbo, expanding to plano-convex, applanate or umbonate; surface entirely covered by appressed fibrillose squamules, brown (7E5) to reddish-brown (8E5-6). *Context* 4-5 mm thick on disc, white,. *Lamellae* free, crowded, with about 6 series of lamellulae, 3-8 mm broad, straight to slightly ventricose, white, then light brown (6D4) to brown. *Stipe* (50-) 70-150 × 5-8 mm, cylindrical to subclavate, base up to 15 mm diam., with basal rhizomorphs, hollow, smooth above annulus, squamulose below annulus, white or with light brown tones. *Annulus* pendent, single, superior, persistent, edge entire, stretched, white, membranous, up to 12 mm broad. Odor fungal. Staining yellow when bruised or cut.

Macrochemical reaction: KOH reaction reddish-brown; ammonia reaction dark blue.

Spores 5-6.5 × 3-4 µm [$\bar{x}$ = 5.6 ± 0.5 × 3.2 ± 0.3 µm, Q = 1.4-2.2, Q_m = 1.77 ± 0.43, n = 20], amygdaliform, with apical thickening, no germ pore, brown. *Basidia* 11-17 × 5-6 µm broadly clavate, hyaline, 4-spored. *Cheilocystidia* and *pleurocystidia* common, similar, 25-55 × 10-15 (-19) µm, subcylindrical to broadly clavate or broadly ventricose, short-pedicellate, often subcapitate, hyaline, smooth. *Pileipellis* a cutis composed of hyphae 7-12 µm diam., slightly constricted at septa, reddish-brown, incrusted. *Annulus* hyphae 3-5 µm diam., hyaline, smooth, branched, terminal cells swollen to clavate, 12 -24 × 8-13 µm. *Stipitipellis* hyphae 3-5 µm diam., hyaline to light brown, smooth; caulocystidia 20-24 × 3-4 µm, broadly clavate, capitate or subcapitate.

Habit: solitary or scattered in grassland or forest.

Known distribution: India, Kerala (type distribution), Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 14 June 2005, collected by Ruilin Zhao, ZRL2006 (SFSU); same location, 11 July 2006, collected by Ruilin Zhao, ZRL3081 (SFSU); same location, 8 September 2006, collected by Ruilin Zhao, ZRL3100 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 16 June 2005, collected by Thanh Huyen Le, ZRL2023 (SFSU).

Notes: Fruitbodies of *M. pleurocystidiata* resemble an *Agaricus* species, but the incrusted pileipellis hyphae, subcapitate cheilocystidia and spores with an apical thickening (endosporium) indicating that the species belongs in the genus *Micropsalliota* (Heinemann 1976). Furthermore, the ITS data support this generic placement. *Micropsalliota pleurocystidiata* is characterized by a brown to reddish brown, fibrillose-squamulose pileus on relatively large fruitbodies, a yellow staining reaction when bruised or cut, and distinctive pleurocystidia. The Thai specimens match the protologue very well except that some fruitbodies are larger than reported previously. *Micropsalliota xanthorubescens* is the species most similar to *M. pleurocystidia*, both species sharing robust fruitbodies, pileus features, and pleurocystidia. The former species differs, however, in having slightly larger spores (6.1-6.9 × 3.5-4 µm) and in staining yellow then turning red (Heinemann and Little Flower 1983).

16 ***Micropsalliota pseudoarginea*** Heinem., Bull. Jard. Bot. Nat. Belg. 52: 412. 1982. Fig. C.16; Plate E.8.c.

Pileus 5-12 mm diam., conical when young, soon expanding to plano-convex and umbonate; margin striate, cuticle exceeding; surface dry, smooth to silky or velutinous, pure white. *Context* 0.4 mm thick at the disc, white. *Lamellae* free, distant, with 3 series of lamellulae, 1.5 mm broad, ventricose, white, becoming pink to orange-gray (6B2) then brown. *Stipe* 15-20 × 0.5-1 mm, cylindrical, slender and curved, hollow, smooth above annulus, fibrillose below annulus, white. *Annulus* fugacious, leaving scale-like remnants, white. Odor not distinctive. No color staining when bruised or cut.

Macrochemical reaction: KOH-reaction red.

Spores 4-5 × 2.5-3.2 µm [$\bar{x}$ = 4.5 ± 0.3 × 3 ± 0.1 µm, Q = 1.3-1.8, Q_m = 1.6 ± 0.3, n = 20], ellipsoid to sub-cymbiform, with apical thickening, no germ pore, brown. *Basidia* 11-14 × 5-6 µm, 4-spored, hyaline. *Cheilocystidia* 15-24 × 6.5-12 µm, cylindrical to broadly clavate or ventricose-clavate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of

hyphae 7-13 µm diam., constricted at septa, hyaline to light brown, smooth or occasionally slightly incrustation, with membranous pigments. *Annulus* composed of hyphae 4-6 µm diam., cylindrical, hyaline, smooth, branched. *Stipitipellis* composed of hyphae similar to those of the annulus.

Habit: gregarious on red soil.

Known distribution: Papua New Guinea, Morobe district (type distribution), Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 18 June 2006, collected by Ruilin Zhao, ZRL3069 (SFSU).

Notes: *Micropsalliota pseudoarginea* is distinguished by a small, pure white, velutinous pileus, distant gills and broadly clavate cheilocystidia. Similar species include: *M. arginea* that differs primarily in forming ventricose-capitate cheilocystidia (Pegler 1986); *M. subarginea* differs in forming capitate cheilocystidia with a long flexuous neck and subamygdaliform spores (Heinemann 1980); *M. lateritia* differs in forming a fibrillose-scaly, rose toned pileus, and pileipellis hyphae with vacuolar pigments (Heinemann 1980).

17 ***Micropsalliota pusillissima*** R. L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov. Fig. C.17; Plate E.9.a-b.

Pilues 1-3 mm diam., conical to campanulate, striatulate or non-striate; surface dry, smooth to finely fibrillose, white overall or sometimes with light brown tones on the disc. *Context* white. *Lamellae* free, crowded to close, with 1-2 series of lamellulae, 0.2-0.5 mm broad, orange-gray to brown, edges paler. *Stipe* 5-12 × 0.2 mm, cylindrical, slender, tomentose, white. *Annulus* persistent, membranous, pendent or percurrent, single, superior, edge entire, white. Odor not distinctive. Pileus unchanged when bruised or cut, stipe staining slightly reddish-brown when bruised or cut.

Macrochemical reaction: KOH reaction strongly reddish-brown.

Spores 4-5.5 × 2.5-3.5 µm [$\bar{x}$ = 4.7 ± 0.4 × 3 ± 0.1 µm, Q = 1.3-1.7, Q_m = 1.6 ± 0.3, n = 20], ellipsoid to sub-cymbiform, with apical thickening, no germ pore, brown. *Basidia* 9-13 × 5-6 µm, broadly clavate, hyaline, 4-spored. *Cheilocystidia* 20-30 × 6-11 µm, broadly ventricose-capitate, base narrow and pedicellate, capitulkum 7-11 µm diam., hyaline, smooth or apically granular. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 5-15 µm diam., cylindrical, hyaline, smooth. *Annulus* hyphae 3-7 µm diam., hyaline, smooth, branched.

Habit: gregarious in red soil on bank along the road.

Etymology: "pusillissimus" – very small, referring to the tiny fruiting bodies.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 10 June 2006, collected by Thanh Huyen Le, ZRL3047 (Holotype: SFSU); same location, 3 July 2005, collected by Thitiya Boonpratuang, ZRL2082 (SFSU).

Notes: Micropsalliota pusillissima is distinguished by very tiny fruitbodies with white, smooth to finely fibrillose pilei less than 3 mm diam., ventricose-capitate cheilocystidia and small spores. The only other known *Micropsalliota* species of similar size is the type species of the genus, *M. pseudovolvulata* Höhn., described from Java and not reported since the protologue in 1914. Based on the few available details provided in the literature, *M. pseudovolvulata* differs in forming a chestnut brown, flocculose pileus about 3 mm diam., very small basidia (7 x 3 µm), and distinct, honey-colored cystidia (*fide* Saccardo 1925). Pegler and Rayner (1969) gave spore measurements as 4-5.2 × 2.5-3.4 µm, the same to those of the Thai specimen. *Micropsalliota pusillissima* differs from *M. albosericea* which has larger fruiting bodies (pilei 3-7 mm diam.) that do not stain reddish-brown and cymbiform spores (Heinemann and Leelavathy 1991).

18 ***Micropsalliota rubrobrunnescens*** R.L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov.

18a ***Micropsalliota rubrobrunnescens*** var. ***rubrobrunnescens*** Fig. C.18; Plate E.9.c.

Pileus 8-20 mm diam., hemispherical to conical, expanding to convex or plano-convex in age, often broadly subumbonate, striatulate or non-striate; surface dry, glabrous or silky, white with reddish brown stains. *Context* white, 0.5 mm thick at the disc. *Lamellae* free, crowded, with 2-3 series of lamellulae, 1-1.5 mm broad, straight to slightly ventricose, white to cream, orange-gray, light brown then brown, edges paler. *Stipe* 12-40 × 1-2 mm, cylindrical, hollow in age, silky to finely fibrillose, white with reddish brown stains. *Annulus* persistent, membranous, pendent, single, superior, striate, torn, white, 1-1.5 mm broad. Odor fungal or of seaweed. Pileus, stipe and flesh staining reddish-brown when bruised or cut, sometimes staining yellow then quickly red.

Macrochemical reaction: KOH reaction reddish-brown then dark brown.

Spores (5.5-) 6-7.5 × 3.5-4.5 µm [$\bar{x}$ = 6.6 ± 0.5 × 4.1 ± 0.2 µm, Q = 1.4-1.8, Q_m = 1.62 ± 0.22, n = 20], ellipsoid to slightly amygdaliform, with apical thickening, no germ pore, brown. *Basidia* 13-19 × 6-8 µm, clavate, hyaline, 4-spored. *Cheilocystidia* (20-) 24-48 × 6-10 (-15) µm,

irregularly cylindrical to broadly clavate or broadly lageniform, capitate or non-capitate, apex (3.5-) 5-10 µm diam., hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 4-12 (-15) µm diam. (mean diam. 6-10 µm), smooth, constricted slightly at septa, with reddish-brown vacuolar pigments. *Annulus* hyphae 3-7 µm diam., smooth, hyaline, terminal cells broadly clavate, 20-50 × 7-15 µm. Stipitipellis hyphae similar to the pileipellis hyphae.

Habit: cespitose, gregarious in soil.

Etymology: "rubro" – red, "brunnescens" – becoming brown, referring to the reddish-brown staining of fruiting bodies when bruised or cut.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Doi Inthanon National Park, Highway 1009 at 25 km marker, N18°32.54' E98°33.51', elev. 1076 m., 4 August 2005, collected by Ruilin Zhao, ZRL2120 (Holotype: SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 3 July 2005, collected by Thitiya Boonpratuang, ZRL2094 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 29 May 2006, collected by Ruilin Zhao, ZRL3010 (SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 17 July 2005, collected by Ruilin Zhao, ZRL2105 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 3 June 2006, collected by Dennis E Desjardin, ZRL3015 (SFSU); same location, 10 June 2006, collected by Ruilin Zhao, ZRL3048 (SFSU);Chiang Mai Prov., Mae Teng Dist., Tung Joaw Village, forest trail, N19°08.07' E98°38.90', elev. 1300 m., 16 July 2005, collected by Ruilin Zhao, ZRL2099 (SFSU).

Notes: The new Thai species, *M. rubrobrunnescens* is characterized by moderately sized fruitbodies with white and silky pilei that stain reddish-brown, gracile white stipes with persistent annulus, clavate-capitate cheilocystidia, vacuolar pigments on pileipellis hyphae, and rather large spores with mean 6.6 × 4.1 µm. The most phenetically similar species is *M. laeta* Heinem., but the latter differs in forming pilei that become rose coloured at maturity, and in forming pileipellis hyphae 12-24 µm diam. with membranous pigments (Heinemann 1980). In comparison, the pilei of *M. rubrobrunnescens* are white with reddish brown stains in age, and the pileipellis hyphae have a mean diam. of 6-10 µm and form vacuolar pigments.

18b ***Micropsalliota rubrobrunnescens*** var. ***tibiicystis*** R. L. Zhao, Desjardin, K. Soytong & K. D. Hyde var. nov. Fig. C.19; Plate E.10.a.

We recognize the following specimen as a distinct variety of *M. rubrobrunnescens*, differing from the type variety in forming distinctly tibiiform cheilocystidia and in forming more campanulate and distinctly umbonate pilei.

Etymology: "tibiicystis", referring to the tibia-shaped cystidia.

Habit: gregarious on soil.

Known distribution: THAILAND.

Material examined: THAILAND, Chiang Mai Prov., Doi Inthanon National Park, Highway 1009 at 25 km marker, N18°32.54' E98°33.51', elev. 1076 m., 4 August 2005, collected by Ruilin Zhao, ZRL2121 (Holotype: SFSU).

Notes. *Micropsalliota rubrobrunnescens* var. *tibiicystis* was collected on the same day at the same site as *M. rubrobrunnescens* var. *rubrobrunnescens*, and the ITS and LSU data show them to be sister taxa but with non-identical sequences. Accordingly we retain them conspecific but as distinct varieties.

19 ***Micropsalliota subalba*** Heinem. & Little Flower, Bull. Jard. Bot. Nat. Belg. 53: 83. 1983. Fig. C.20; Plate E.10.b.

Pileus 12-18 mm diam., obtusely conical, expanding to applanate with small umbo; surface silky with fine radiating fibrils, white to light gray overall. *Context* 0.5 mm thick at the disc, white. *Lamellae* free, close, with 3 series of lamellulae, 2.5 mm broad, ventricose, white to grayish brown, edge concolorous. *Stipe* 28-30 × 1-1.5 mm, cylindrical, slender, hollow, surface above annulus glabrous, below annulus fibrillose, white; context white to light brown. *Annulus* pendent or percurrent, single, superior, persistent, entire, rigid, white. Odor not distinctive. Not staining when bruised or cut.

Macrochemical reaction: KOH reaction brown.

Spores 5.2-7 × 3-4 µm [$\bar{x}$ = 6 ± 0.4 × 3.5 ± 0.4 µm, Q = 1.4-2.2, Q_m = 1.72 ± 0.5, n = 20], ellipsoid, some with apical thickening, no germ pore, brown. *Basidia* 11-17 × 5-8 µm, clavate, hyaline, 4-spored. *Cheilocystidia* (22-) 30-58 × 6-15 µm, clavate to ventricose, capitate, capitulum 7-12 µm diam., hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 6.5-16 (- 20) µm diam., hyaline, smooth, constricted slightly at septa, with light brown vacuolar pigments. *Annulus* hyphae 5-7 µm diam., cylindrical, smooth.

Habit: gregarious on rich soil.

Known distribution: India, Kerala (type distribution), Thailand.

Material examined: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 2 June 2005, collected by Ruilin Zhao, ZRL2080 (SFSU).

Notes: The Thai specimen matches quite well with the protologue of *M. subalba* Heinem. & Little Flower, described from India, although in Thailand the species forms a persistent annulus (Heinemann and Flower 1983). *Micropsalliota subalba* is sister to *M. gracilis* in the ITS, LSU and ITS + LSU phylogenetic trees. *Micropsalliota gracilis* differs primarily in forming a grayish red to red, furfuraceous pileus; the shapes and sizes of fruitbodies, spores and cheilocystidia are indistinguishable, and both species do not stain when bruised.

20 ***Micropsalliota subarginea*** Heinem., Bull. Jard. Bot. Nat. Belg. 50: 48. 1980. Fig. C.21; Plate E.11.a.

Pileus 5-15 mm diam., conical, expanding to broadly conical or convex-umbonate; surface dry, fibrillose to finely squamulose , white overall, discoloring light brown to reddish brown in age. *Context* white, 0.8-1 mm thick at the disc. *Lamellae* free, crowded, with 4 series of lamellulae, 0.5-1 mm broad, brownish orange (6C3) to light brown then brown, edges paler. *Stipe* 10 –25 × 0.6-1.5 mm, cylindrical, hollow, fibrillose, white. *Annulus* membranous, fugacious, pendent or percurrent, single, superior, torn, white. Odor of seaweed or not distinctive. Not staining or staining yellow then reddish-brown in time.

Macrochemical reaction: KOH reaction reddish-brown.

Spores 4-5 × 2.5-3 µm [$\bar{x}$ = 4.7 ± 0.3 × 3 ± 0.2 µm, Q = 1.3-2, Q_m = 1.6 ± 0.4, n = 20], amygdaliform to ellipsoid, some with apical thickening, no germ pore, brown. *Basidia* 9-12 × 5-6 µm, clavate, hyaline, 4-spored. *Cheilocystidia* 32-50 × 4-8 µm, with a ventricose base, long flexuous neck and capitate apex, capitulum 2.5-4 µm diam., hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 7.5-12.5 µm diam., cylindrical, constricted slightly at septa, hyaline, smooth. *Annulus* hyphae 3-5 µm diam., smooth, hyaline, branched. *Stipitipellis* hyphae similar to annulus hyphae.

Habit: gregarious on red soil.

Known distribution: Singapore (type distribution); THAILAND.

Material examined: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m., 7 June 2006, collected by Tim Baroni, ZRL3036 (SFSU); Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 12 June 2006, collected by Ruilin Zhao, ZRL3058 (SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2005, collected by Ruilin Zhao, ZRL2052 (SFSU); same location, 3 July 2005, collected by Thitiya Boonpratuang, ZRL2092 (SFSU).

Notes: Micropsalliota subarginea is distinguished by small, white, finely fibrillose pilei that may discolor light brown or reddish brown in age, crowded and narrow lamellae with distinctive cheilocystidia that are ventricose with a long, capitate, flexuous neck, and small spores. The most similar species is *M. bifida*, but the latter differs in forming shorter cheilocystidia (14-30 ×5-9 µm) that are bifid and lack the long, flexulous, capitate neck of M. subarginea. Interestingly, *M. bifida* and *M. subarginea* are sister taxa with 87% BS and 99% PP support in the ITS + LSU phylogenetic tree (Fig. 6.5).

21 ***Micropsalliota suthepensis*** R. L. Zhao, Desjardin, K. Soytong & K. D. Hyde sp. nov. Fig. C.22; Plate E.12.a.

Pileus 10-20 mm diam., campanulate to convex, becoming plano-convex and umbonate in age; surface dry, finely squamulose to tomentose-granular, squamules erect or appressed, brownish violet (11D6), to grayish red (10D5) or violet. *Context* white, 0.5-1 mm thick at the disc. *Lamellae* free, crowded to close, with 2 series of lamellulae, 2-3.5 mm broad, ventricose, white to brownish orange (6C5) then grayish brown (7D3). *Stipe* 22-35 ×1.5 –2 mm, cylindrical, straight or curved, hollow, some with basal rhizomorphs, smooth to tomentose, white or brownish violet; context grayish brown (7D3). *Annulus* membranous, fugacious (mostly only remnants attached), superior, white or pink. Odor fungal. Not staining when bruised or cut.

Macrochemical reaction: KOH reaction negative on surface of pileus, greenish brown then brown on pileus context.

Spores (4.5-) 5-6 × 3-3.5 µm [$\bar{x}$ = 5.4 ± 0.5 × 3.4 ± 0.2 µm, Q = 1.4-2, Q_m = 1.6 ± 0.4, n = 20], amygdaliform or subcymbiform, with apical thickening, no germ pore, brown. *Basidia* 10-15 × 4-8 µm, clavate, hyaline, 4-spored. *Cheilocystidia* 25-38 × 4-7 µm, clavate, non-capitate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 8-13 µm diam.,

some cells inflated and up to 20 µm diam., constricted at septa, with brown membranous pigments.

Habit: solitary or gregarious in soil.

Known distribution: Thailand.

Material examined: THAILAND, Chiang Mai Prov., Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma Village, N18°48.62' E98°54.60', elev. 1145 m, 24 June 2005, collected by Dennis E Desjardin, ZRL2029 (SFSU); same location, 7 June 2006, collected by Maria Alice Neves, ZRL3035 (Holotype: SFSU).

Notes: *Micropsalliota suthepensis* is characterized by a moderately-sized, tomentose-granular, brownish violet to reddish brown pileus, small spores, non-capitate cheilocystidia, and pileipellis hyphae 8-20 µm diam. with brown membranous pigments. This species is most phenetically similar to *M. gracilis*, which differs in forming pilei lacking violet tones, and has distinctly capitate cheilocystidia with long narrow necks and vacuolar pigments in the pileipellis hyphae. *Micropsalliota suthepensis* also shows some features similar to the poorly known *M. brunneola* Heinem., but that species has a brown pileus and no data are available on cheilocystidia shape and size.

22 ***Micropsalliota xanthorubescens*** Heinem. Bull. Jard. Bot. Nat. Belg. 50: 63. 1980. Fig. C.23; Plate E.11.b.

Pileus 40-70 (-90) mm diam., hemispherical to convex, becoming plano-convex, occasionally subumbonate or with margin slightly upturned and disc applanate; surface dry, fibrillose to squamulose, usually covered with light brown (6-7D4-6) squamules on a white background; squamules appressed to erect, dense on the disc and scattered on the margin. *Context* white, 4-6 mm thick at the disc. *Lamellae* free, crowded, with 4-6 series of lamellulae, 5-7 mm broad, straight to ventricose, white to pale yellowish white, grayish yellow (4B3), yellowish grey (4B2), brownish orange (7C3), brownish grey (6B2), then brown to dark brown. *Stipe* 35-70 (-90) × 5-10 mm, cylindrical, hollow, with basal rhizomorphs, smooth or finely tomentose overall, occasionally squamulose near the base, white. *Annulus* membranous, pendent, single, superior, persistent, entire, striate, white, up to 8 mm broad. Odor fungal or of seaweeds. Staining yellow when bruised or cut, sometimes changing to reddish brown.

Macrochemical reaction: KOH reaction reddish-brown.

Spores 5.5-6.5 × 3.8-4.2 µm [$\bar{x}$ = 6.1 ± 0.3 × 4 ± 0.1 µm, Q = 1.4-1.6, Q_m = 1.54 ± 0.12, n = 20], amygdaliform, with apical thickening, no germ pore, brown. *Basidia* 13-19 × 6-8 µm, clavate, hyaline, 4-spored. *Cheilocystidia* and *pleurocystidia* common, similar, 25-60 × 10-20 (-25) µm, clavate to ventricose, non-capitate or rarely subcapitate, short-pedicellate, hyaline, smooth. *Pileipellis* a cutis composed of hyphae 5-11 µm diam., cylindrical, slightly constricted at septa, with reddish-brown or light brown incrusting and membranous pigments. *Annulus* composed of hyphae 4-11 µm diam., cylindrical, terminal cells slightly inflated, smooth, hyaline. *Stipitipellis* hyphae same as those of the annulus.

Habit: solitary in grassland.

Known distribution: Singapore (type distribution), Thailand.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng Village, N 19°17.123' E 98°44. 009', elev. 900 m., 22 May 2006, collected by Ruilin Zhao,ZRL3001 (SFSU); same location, 1 June 2006, collected by Ruilin Zhao, ZRL3011 (SFSU); same location, 14 July 2006, collected by Nilam Wulandari, ZRL3083 (SFSU); same location, 13 August 2006, collected by Ruilin Zhao, ZRL3096 (SFSU); Chiang Mai Prov., Mae Taeng, Ban Mae Sae Village, on Highway 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 3 June 2006, collected by Ruili Zhao, ZRL3013 (SFSU).

Notes: Heinemann (1980) placed this large *Agaricus*-like species in *Micropsalliota* because of the presence of large cheilocystidia and pleurocystidia, spores with an endosporium (apical thickening), and incrusting pigments on the pileipellis hyphae. The Thai specimens match nicely with the protologue based on a single specimen from Singapore, although the Thai fruitbodies rarely stain reddish brown. The ITS and LSU data derived from Thai specimens herein identified as *M. xanthorubescens* confirm placement of this species in *Micropsalliota* and not in *Agaricus*. Pleurocystidia are rare in *Micropsalliota*, found only in two species with robust fruiting bodies, viz., *M. pleurocystidiata* and *M. xanthorubescens*. *Micropsalliota pleurocystidiata* differs in forming more gracile fruiting bodies [stipes generally 70-150 × 5-8 mm] that stain yellow but not changing to red, and has slightly smaller spores with mean 5.6 × 3.2 µm (Heinemann and Flower 1983).

23 ***Micropsalliota* sp. 1** [allied with *M. furfuracea*]

Pileus 45-60 mm diam., convex to plano-convex, disc applanate or subumbonate; surface dry, disc with aggregareted fibrillose scales, margin striate, glabrous to fibrillose, ornamentation light

brown over grayish white background. *Context* 0.5 mm thick at disc, membranous, white, brittle. *Lamellae* subfree, close, with 4 series of lamellulae, 8 mm broad, ventricose, grayish-orange, edges concolorous. *Stipe* 40-50 × 7 mm, cylindrical, hollow, tomentose, white or with light brown tone. *Annulus* membranous, pendent, single, superior, fugacious, up to 4 mm broad, white. Staining reddish-brown when bruised or cut. Odor of seaweed.

Macrochemical reaction: KOH reaction reddish-brown.

Spores 6.5-8 × 3.5-4.5 µm [$\bar{x}$ = 7.1 ± 0.4 × 4 ± 0.2 µm, Q = 1.56-2, Q_m = 1.77 ± 0.23, n = 20], ellipsoid to amgydaliform, without endosporium, reddish-brown. *Basidia* 16-20 × 5-9 µm, clavate, 4-spored, hyaline. *Cheilocystidia* 27-50 × 4.5-6 (-8) µm, apex 5-10 µm diam., broadly clavate to tibiiform, rarely one septate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 7-12 µm diam., long-cylindrical, without constriction at the septa, hyaline to light yellowish brown, smooth (non-incrusted). *Annulus* composed of hyphae 5-7.5 µm diam., hyaline to light yellow, smooth. *Stipitipellis* hyphae similar to those of annulus. Oleiferous hyphae abundant in lamellar trama, pileus, annulus and stipe.

Habit: caespitose in soil.

Material examined: THAILAND, Chiang Mai Prov., Mae Teng Dist., Highway 1095 at 22 km marker, N19°07.57' E98°45.65', elev.750 m., 4 June 2006, collected by Tim Baroni, ZRL3018 (SFSU).

Notes: This specimen is distinguished by large spores, copious amounts of oleiferous hyphae, non-incrusted pileipellis hyphae, and reddish brown bruising and KOH reactions. The single specimen is nearly indistinguishable from two specimens of *M. furfuracea* (ZRL2119, ZRL 3006), and the ITS and LSU data suggest that ZRL3018 belongs to the same clade and may represent the same taxon. We will retain it as separate, however, until the significance of oleiferous hyphae and the lack of pigment incrustations is evaluated further.

24 *Micropsalliota* sp 2

Pileus 40-45 mm diam., broad conical becoming plano-convex, umbonate; surface glabous to finely silky, white overall. *Context* 3 mm thick at the disc, white. *Lamellae* free, close, with 3 series of lamelullae, 4.5 mm broad, light brown to grayish brown (5D3) then brown (6D-E4), edges paler. *Stipe* 70-75 × 4-5 mm, cylindrical, straight, hollow, fibrillose to squamulose overall, white. *Annulus* membranous, pendent or percurrent, single, superior, persistent, edge entire, up to

3 mm broad. Odor not distinctive. Staining yellow (3B4) when bruised or cut then turning reddish brown.

Macrochemical reaction: KOH reaction reddish-brown.

Spores 6.5-8 × 4-5 µm [$\bar{x}$ = 7.3 ± 0.6 × 4.2 ± 0.4 µm, Q = 1.6-2, Q_m = 1.7 ± 0.3, n = 20], ellipsoid to amgydaliform, without endosporium, reddish-brown. *Basidia* 15-18 × 7-8 µm, clavate, hyaline, 4-spored. *Cheilocystidia* 22-50 × 5-8 µm, cylindrical to subclavate or commonly broadly tibiiform, capitate, capitulum 7-15 µm diam., hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis of hyphae 5-8 µm diam., cylindrical, smooth or with light brown to brown weakly incrusting and membranous pigments. *Annulus* hyphae 2.5-3.5 µm diam., some inflated 5-12.5µm diam., some slightly constricted at the septa, smooth, hyaline or light brown. *Stipitipellis* hyphae 5-7.5 µm diam., cylindrical, hyaline, smooth or finely incrusted. Oleiferous hyphae present in the stipe and pileus trama.

Habit: scattered in rich soil under bamboo.

Material examined: THAILAND, Chiang Mai Prov., Chom Thong, Ob Luang National Park, elev. 1812 m., 4 July 2005, collected by Ruilin Zhao, ZRL2096 (SFSU).

Notes: The Thai specimen described above is characterized by a white glabrous to silky pileus, yellow then reddish brown staining when bruised or cut, rather large spores with mean 7.3 ×4.2 µm, tibiiform cheilocystidia with very large capitulum, and incrusting and membranous pigments. It is similar to *M. lutescens* Heinem., described from Singapore, but the latter differs in forming smaller fruitbodies (pilei 6-18 mm diam, stipes 40-70 × 1.5-2 mm), and cheilocystidia that are subclavate to lanceolate, subcapitate with capitulum only 4-7 µm diam. (Heinemann 1980). The ITS data suggest that specimen ZRL2096 is allied with *M. furfuracea* (Fig. 6.2). Until further specimens are collected from northern Thailand, we will not formally described the Thai material as new nor tie it to a formal epithet.

25 *Micropsalliota* sp 3

Pileus 15-45 mm diam., obtusely conical becoming plano-convex with a broad umbo in age; surface dry, fibrillose to squamulose, squamules erect, dense on the disc, reddish-brown (8E5). *Context* white, 1 mm thick at the disc. *Lamellae* free, crowded, with 2-3 series of lamellulae, 1.5 mm broad, white, to grayish-orange then light brown (6D4), edges concolorous. *Stipe* 35-100 × 2-5 mm, cylindrical, straight or curved, hollow, tomentose above annulus, fibrillose to woolly below annulus, white. *Annulus* membranous, single, pendent, persistent in most cases, superior,

stretched, white, sometimes with purple edge,1.5-2 mm broad. Staining red to reddish-brown when bruised or cut. Odor fungal.

Macrochemical reaction: KOH reaction yellow on pileus surface, dark green on context.

Spores 4.5-6 × 3-4 µm [$\bar{x}$ = 5.3 ± 0.5 × 3.1 ± 0.3 µm, Q = 1.38-2, Q_m = 1.75 ± 0.3, n = 20], ellipsoid or occasionally amygdaliform, without endosporium, brown. *Basidia* 14-16 × 5-6 µm, clavate, hyaline, 4-spored. *Cheilocystidia* 20-40 (-50) × 7-9 (-12) µm, irregularly cylindrical to clavate, subcapitate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae (5-) 7.5-12.5 µm diam., cylindrical, with light brown to reddish-brown, incrusting and membranous pigments. *Annulus* of hyphae 5-10 µm diam., cylindrical, smooth, hyaline to light yellow. *Stipitipellis* hyphae similar to those of the annulus.

Habit: gregarious in soil in the forest.

Material examined: THAILAND, Chiang Mai Prov., Doi Inthanon National Park, Highway 1009 at 25 km marker, N18°32.54' E98°33.51', elev. 1076 m., 4 August 2005, collected by Thanh Huyen Le, ZRL2118 (SFSU).

Notes: The single Thai specimen described above is characterized by moderately-sized fruitbodies with reddish brown, fibrillose-squamulose pilei, tissues that stain reddish brown when bruised or cut, small spores 4.5-6 × 3-4 µm, subcapitate cheilocystidia, and incrusting pigments. *Micropsalliota globocystis* is macromorphologically similar, but differs in having larger spores (6-7 × 3.5-4.2 µm), and the tissues stain yellow then reddish-brown when bruised or cut. The ITS and LSU data indicate that ZRL2118 is related to *M. megarubescens,* but the latter new species differs in forming larger spores (6-8 × 3.5-4.5 µm), a white to gray pileus, and tissues that stain yellow then reddish-brown staining when bruised or cut.

26 *Micropsalliota* sp 4

Pileus 15-35 mm diam., obtusely conical becoming plano-convex with a broad umbo; surface dry, fibrillose-squamulose; squamules dense on the disc, rare to scattered on the margin, light brown to pale reddish-brown over a white background. *Context* white, 2 mm thick at the disc. *Lamellae* free, crowded, with 3 series of lamellulae, 3 mm broad, white to yellowish-gray (4B3). *Stipe* 40-70 × 3-4 mm, cylindrical, smooth to tomentose above annulus, squamulose below annulus, white. *Annulus* membranous, pendent or percurrent, single, persistent, superior, edge entire. Staining yellow then brown to reddish-brown when bruised or cut. Odor fungal.

Macrochemical reaction: KOH reaction yellow then dark green on surface of pileus; no color change on context.

Spores 6.5-8 × 3.5-4.5 μm [$\bar{x}$ = 7.1 ± 0.5 × 4 ± 0.3 μm, Q = 1.6-2, Q_m = 1.79 ± 0.26, n = 20], ellipsoid, without endosporium, brown. *Basidia* 15-20 × 7-8 μm, clavate, hyaline, 4-spored. *Cheilocystidia* 25-35 × 7-13 μm, clavate, subcapitate to capitate, capitulum 8-13 μm diam., hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae 7.5-22 μm diam., cylindrical, some constricted at the septa, smooth, with brown membranous pigments. *Annulus* hyphae 5-12.5 μm diam., cylindrical, hyaline, smooth.

Habit: cestipitose in soil in exposed areas of forest.

Material examined: THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44. 009', elev. 900 m., 24 August 2005, collected by Ruilin Zhao, ZRL2133 (SFSU).

Notes: The single Thai specimen is characterized by moderately-sized fruitbodies with light brown to reddish brown, fibrillose-squamulose pilei and tissues that stain yellow then reddish brown when handled, large spores 6.5-8 × 3.5-4.5 μm, clavate-capitate cheilocystidia and membranous pigments. This material is similar to *M. globocystis* in morphology, but the latter species differs in forming pilei that are dark purplish brown, has larger cheilocystidia 30-60 × 9-14 μm, and has distinctly incrusted pileipellis hyphae. The ITS and LSU data (Figs. 6.2-6.4) indicate that ZRL2133 is allied with *M. globocystis* but without statistical support. Until further specimens of this taxon are collected, we will not formally describe it as new.

Chapter 7

RESULTS AND DISCUSSION Part D: allied genera and species of *Micropsalliota* and *Agaricus*

7.1 The genus *Heinemannomyces*

The genus *Heinemannomyces* was established by Roy Watling (1998), and the only described species (*H. splendidissima*) was from Malaysia and northern Thailand. This genus is very unusual by grayish blue spores under the microscope, woolly floccose pileus and stipe.

1. *Heinemannomyces splendidissima* Watling, Belg. Journ. Bot. 131: 133 1998. Fig. D.1; Plate E.19.a.

Pileus 30-40 mm diam., convex, expending to plano-convex, applanate, cuticle exceeding, some dentate; surface dry, completely covered with thick, loose, woolly fibrils, lacking squamulose, reddish-brown (7E2, 8E5, 9E5). *Context* firm, white, surface layer of pileus leather-like, easy separated from context. *Lamellae* free, more crowded, lamellulae 4-6 series, 2-5 mm broad, normal to broad, white to grey (19E1, 20E1) when young, then dark blue (19E5), blackish blue (20F4). *Stipe* 30-60 × 4-6 mm high, equal cylindrical, surface tomentose, white above the annulus, below annulus woolly floccose, reddish brown, heavier and darker towards base, hollow. *Annulus* woolly floccose, with grey tinge. Smell odourless or slightly mushroomy. No color staining on touching, reddish-brown staining on cutting.

Macrochemical reaction: KOH-reaction negative.

Spores 5.5-6.5 × (3.5-) 4-4.5 µm [$\bar{x}$ = 6 ± 0.5 × 4.2 ± 0.3, Q = 1.33-1.63, Q_m = 1.5 ± 0.2, n = 20], ellipsoid, ovoid, some dark brown, mostly violet, violaceous black, without germ pore. *Basidia* 14-18 × 6-8 µm, clavate, 4-spored. *Cheilocystidia* 16-30 (- 40) × 7-11 µm, clavate, or inflate clavate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of (2.5-) 5 - 7.5 µm diam., reddish-brown, incrusted, no constricted at the septa, branched. *Stipitipellis* consisted of the same hyphae of pileipellis.

Habit: solitary in clay soil side of trails.

Materials examination: SFSU ZRL3043 and SFSU ZRL3062 THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, N 19°17.123' E 98°44. 009', elev. 900 m., 10 June 2006

and 13 June 2006, collected by Ruilin Zhao and Dennis E Desjardin; SFSU ZRL3057 THAILAND, Chiang Mai Prov., Mae Taeng Dist., Ban Pha Deng village, Pathummikaram Temple, forest trail, N 19°06'28.8'' E 98°44'47.3", elev. 1050 m., 12 June 2006, collected by Ruilin Zhao.

Notes: Those materials were collected at the same areas as the original description and all features match the definition of this species well.

2. ***Heinemannomyces*** sp. nov. Fig. D.2; Plate E.19.b.

Pileus 18-28 mm diam. (baby 7 mm diam.), hemispherical, bell-like, expending to convex, plano-convex with umbonate, some remnants of veil attached at margin. Surface with skull-cap, then broken to margin in fissure, grainy near margin, brown, grayish brown. *Context* firm, white, 2 mm thick at disc. *Lamellae* free, crowded, lamellulae with 2-3 lengths, 3 mm broad, normal, white, pink, reddish-brown with age, edge color lighter than gill itself. *Stipe* 45-60 × 4-5 mm, cylindrical and taping to base slightly, smooth above ring and scattered fibrillose nodes, brown below the ring, staining red, hollow. *Annulus* firstly close gills, then broken into sheath-like, then membranous, perdent, singer, superior, entire, stretched, white, up to 4 mm board. Staining dull red on touching and cutting. Smell odourless.

Macrochemical reaction: KOH reaction negative on the surface of pileus, dark green on the context; ammonia reaction negative at both.

Spores (5.5-) 6-7 × 4-4.5 µm [$\overline{x}$ = 6.2 ± 0.4 × 4.1 ± 0.2, Q = 1.4-1.8, Q_m = 1.7 ± 0.3, n = 20], ellipsoid, brown, without germ pore, brown, reddish-brown. *Basidia* board clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 20-44 × 5-9 µm, clavate, cylindrical, 0-3 septa, occasional binary branched, and nucleus clear in most cells. *Pleurocystidia* absent. *Pileipellis* a cutis composed of 2 kinds of hyphae: one is 10-15 µm diam., ellipoid or subspherial, brown, smooth, constricted at septa heavily, discarticulous; another 5-7.5 µm diam., cylindrical, brown, smooth, slightly constricted or not. *Annulus* composed of hyphae 3-5 µm diam., hyaline, smooth, branched, curved, hyphae-like. *Stipitipellis* above the ring same with annulus hyphae, below the ring similar to the pileipellis hyphae (having 2 kinds hyphae).

Habit: ceastipose, gregarious in the rich lime soil.

Materials examination: SFSU ZRL3103 THAILAND, Chiang Mai Province, Chom Thong, Ob Luang national Park, elev. 1812m., 15 September 2006, collected by Ruilin Zhao.

7.2 The genus *Hymenagaricus*

The genus *Hymenagaricus* was established through the transfer of *Agaricus* subgen. *Conioagaricus* Heinem. sect. *Hymenopilei*, along with 3 *Hymenagaricus* species (*H. alphitochrous, H. hymenopileus* and *H. nigrovinosus*) (Heinamenn 1981). This genus is character as "pileus with hymeniform layer on the cuticle at least partially or with hymeniform cells, spores large, brown, without endosporial thickening" (Heinemann 1981. There are 10 species described (Kirk et al 2001) and 33 records at Index Fungorum (http://speciesfungorum.org/Names/Names.asp). The Hymenagaricus species had been mainly reported from India and South Africa (Heinemann 1984 and 1985; Reid and Eicher 1995, 1998 and 1999).

1. ***Hynemagaricus epigastus*** (Berk. & Broome) Heinem. & Little Flower Fig. D.3; Plate E.20.a.

Pileus 2-5.5 mm diam., conic, broad conic, convex, margin cuticle exceeding, surface pulverulent, grayish yellow (4B2), putty, and darkening to disc. *Context* 0.2 mm thick, membranous, yellowish-white. *Lamellae* free, crowded, 1 mm board, ventricose, reddish-brown to dark brown (8E4), edge color lighter than gill itself. *Stipe* 5-15 × 0.2 mm, cylindrical, slender, fimbrite, surface smooth to tomentose, yellow. *Annulus* did not detected. No color staining on touching and cutting. Smell odorless.

Macrochemical reaction: KOH reaction negative.

Spores 4-5 × 2.5-3 µm [$\bar{x}$ = 4.4 ± 0.3 × 2.7 ± 0.2, Q = 1.5-1.8, Q_m = 1.65 ± 0.15, n = 20], ellipsoid, without germ pore. *Basidia* 9-12 × 4.5-5 µm, cylindrical, hyaline, smooth, 4-spored. *Cheilocystidia* (13-) 17-32 × 6-12 µm, mostly broad cylindrical, ellipsoid, some ob-pyriform, some subcapitate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* consists of spherical cells in chain, which is 8-20 µm diam., smooth, then compile to mass, hyaline to yellow. *Annulus* consists of hyphae of 5-8 µm diam., terminal cells inflated 13-22 × 7-8 µm, hyaline, smooth. *Stipitipellis* hyphae similar to annulus.

Habit: gregarious in soil with moss on the bank of trail.

Materials examination: SFSU ZRL3016 and SFSU ZRL3045 THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 3 June 2006 and 10 June 2006, collected by Dennis E Desjardin and Thanh Huyen Le.

2. ***Hynemagarucus*** sp. nov. Fig. D.4; Plate E.20.b.

Pileus 2-5 mm diam., hemispherical, broad conical, plano-concex, surface pulverulent, dark violet, dark purple. *Context* thin, membranous, white. *Lamellae* free, crowded, lamellulae with 2 lengths, breadth 0.5 mm broad, light brown to dark brown (9F5). *Stipe* 0.2 mm diam., 10-15 mm long, long cylindrical, slender, straight or curved, white, smooth to powdery. *Annulus* membranous, fugacious, white. Smell unknown.

Macrochemical reaction: unknown.

Spores 3.8-4 × 2.5-3 µm [$\bar{x}$ = 4 ± 0 × 2.7 ± 0.2, Q = 1.3-1.6, Q_m = 1.46 ± 0.16, n = 20], ellipsoid, without germ pore. *Basidia* 7-9 × 4-4.5 µm, broad cylindrical, smooth, 4-spored. *Cheilocystidia* 15-18 × 4-7 µm, cylindrical with narrow basem clavate, ellipsoid, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* consists of spherical to subspherical cells in chain, which 12-30 µm diam., grayish brown, smooth. *Annulus* hyphae 3-5 µm diam., long cylindrical, branched; some cells inflated into clavate, ellipsoid to subspherical, 7 - 15 µm diam., both hyaline, smooth. *Stipitipellis* similar to annulus's.

Habit: gregarious in soil on the bank of trail.

Materials examination: SFSU ZRL2047 THAILAND, Chiang Mai Prov., Mae Taeng, Ban Mae Sae village, on Hwy 1095 near 50 km marker, N19°14. 599' E98°39.456', elev. 962 m., 26 June 2004, collected by Amy Honan.

Chapter 8

CONCLUSIONS AND SUGGESTIONS

8.1 The genus *Agaricus*

8.1.1 *Agaricus* species in northern Thailand

Morphological examination of more than 200 specimens from 19 sites in the northern Thailand yielded 26 species which are fully described. Fourteen species are named and include 2 species new to science (*A. angusticystidiata* and *A. maeseaensis*). Twelve species are not presently named and these may be new or are previously known species. However because of the paucity of material further collections are needed before we can confidently name these taxon. Of the named species, *A. trisulphuratus* is the only species which has previously been reported from Thailand; *Agaricus* aff. *brunneolus*, *A. caribaeus*, *A. dulcidulus*, *A. duplocingulatus*, *A. endoxanthus*, *A.* aff. *impudicus*, *A. johnstonii*, *A. ochrascens*, *A. porphyrizon*, and *A. xantholepis* are new records for Thailand.

8.1.2 Molecular phylogeny of *Agaricus*

Specimens representing the different *Agaricus* taxa were subjected to rDNA sequencing and analyzed using additional sequences from GenBank. The genus *Agaricus* is monophyletic in the ITS and LSU analyses with strong BS and PP support. *Micropsalliota*, *Hemanagaricus* and *Heimenomycetes* are sister to *Agaricus* clade.

For the first time 5 ITS and 5 LSU sequences show *Agaricus trisulphuratus* and *Agaricus* sp. 11 to cluster in the subgenus *Lanagaricus* . This subgenus is stronglysupported (BS and PP values), and nests in the *Agaricus* clade.

Another distinct subclade comprises the 2 new species (*A. angusticystidiata* and *A. maeseaensis*) and *Agaricus* sp. 9, which is full supported in ITS, LSU and ITS+LSU analysis and sister to the members of subgenus *Agaricus*. These species are relatively average to small, and are distinguished by elongate and narrowly clavate, blanch-like cheilocystidia. The cheilocystidia is similar in shape to that of species of *Allopsalliota*, but the LSU data showed them to be distantly related. Therefore these 3 species are members of *Agaricus*, but do not belong in subgenus *Agaricus*.

The species from Thailand are generally distant genealogically from those of the European and American specimens, except for *Agaricus subrufescens* and *A. subrutilescens* from Thailand, which clustered with the material from America (see Fig. 4.2-4, Bold names are from Thailand samples).

8.2 The genus *Cyathus*

8.2.1 The *Cyathus* species

A worldwide monograph of *Cyathus* species was carried out based on morphological examination of 115 loaned specimens. Forty-six taxa are fully described and illustrated. A key to these 46 species is presented using the new 3-group taxonomic system for this genus. Four species are synonymised and the rank of one species is changed based on holotype examination. *Cyathus cheliensis*, *C. gansuensis*, *C. jiayuguanensis* and *C. megasporus* are herein accepted as synonyms of *C. limbatus*, *C. pygmaeus*, *C. africanus* var. *latisporus* and *C. poeppigii*, respectively; *C. olla* f. *lanatus* was named as a specie, *C. lanatus* (Zhao et al 2006, 2007). Sixteen collections of *Cyathus* from northern Thailand resulted in 3 species, with one new to science (*C. subglobosporus*).

8.2.1 Molecular phylogenies of *Cyathus*

Historically, *Cyathus* has been delimited using 3 separate classification systems (Tulasne 1844, Lloyd, 1906, Brodie 1975, 1984). These systems relied heavily on the importance of a plicate peridium, and at which rank this character was used to separate taxa. To date, Brodie's system is most widely accepted although its phylogenetic significance has been untested. In this study we make an important step towards forming a more natural classification of *Cyathus* by combining morphological data with phylogenies generated from rDNA sequence datasets. Taxa incorporated in this study included representatives of all seven groups in Brodie's classification system and most data were obtained from either type specimens or authentic Brodie-determined materials (Table 3.1). Our data indicate that the 7-group morphologically-based system as proposed by Brodie (1975, 1984) is not concordant with the molecular phylogenies proposed here (Figs. 4.3-5).

The presence or absence of plications was the primary character for partitioning groups in traditional taxonomic constructs. The presence or absence of a tunica on the peridioles, types of hairs, and fruiting body shape were of secondary importance (Brodie 1975, 1984). However, results here indicate that plications on the peridium do not appear to be a phylogenetically informative character. Species possessing this character are distributed in all three major clades in our phylogenies (Figs. 4.3-5).

In contrast, the size of basidiospores is a significant morphological character for distinguishing the major clades. In Clade B, there are 8 *Cyathus* species (*C. annulatus*, *C. crassimurus*, *C. helenae*, *C. poeppigii*, *C. renweii*, *C. setosus*, *C. stercoceus* and *C. triplex*). These species are distributed amongst five groups in Brodie's system: the "triplex", "gracilis", "stercoreus", "poeppigii" and "striatus" groups. All members of Clade B have basidiospores with a length greater than 15 µm. In comparison, members of Clade A (*C. africanus*, *C. africanus* f. *latisporus*, *C. conlensoi*, *C. griseocarpus*, *C. guandishanensis*, *C. hookeri*, *C. jiayuguanensis*, *C. olla*, *C. olla* f. *anglicus*, and *C. olla* f. *brodiensis*) and Clade C (*C. berkeleyanus*, *C. gansuensis*, *C. olla* f. *lanatus* and *C. pallidus*) form basidiospores shorter than 15 µm. In previous morphological studies (Tulasne 1844, Lloyd 1906, Brodie 1975, 1984) spore size has been used only in delimitations at the species rank, and no publications reported segregating *Cyathus* species into two major groups based on spores longer or shorter than 15 µm. It should be noted that Clades A and C cannot be distinguished from each other solely on spore measurements.

Colour of the fruiting body has been considered useful in the partitioning of species groups (Brodie 1975, 1984). Our morphological studies indicate that species in Clade B have peridia that are brown, reddish-brown or dark brown on the outside, while species in Clades A and C have peridia that are much lighter, typically yellow, gold or gray. As with basidiospore size, distinguishing amongst members in Clades A and C based solely on peridium coloration is problematical.

Our morphological studies revealed that the best characters for distinguishing members of Clade A from Clade C are the thickness of the tomentum covering the peridium, and the outline of fruiting bodies. Species in Clade C have a thick, felt-like tomentum, usually aggregating into shaggy or woolly hairs covering the peridium. Their fruiting bodies are crucible-shaped without a distinct stipe. In comparison, species in Clade A have a thin tomentum of fine hairs covering the peridium, and the fruiting bodies are funnel-shaped with a constricted base or a distinct stipe.

The problematical taxon *Cyathus setosus* has spores that are 17-24 µm long, has a dark-colored peridium, and nests within Clade B in the ITS analysis (Fig. 1). In the analyses of LSU and combined datasets, however, this species was isolated and sister to Clades A, B and C. The most conspicuous diagnostic feature for *C. setosus* is the very long setae at the mouth of the fruiting body. Whether or not *C. setosus* represents a distinct group within *Cyathus* will require broader sampling and further testing.

In our analyses *Cyathus* resulted as a monophyletic lineage with 100% PP and 100% BS support in ITS, LSU and combined ITS-LSU trees. In addition, in all three analyses, *Nidula niveotomentosa* was sister to *Cyathus* but with low statistical support.

8.3 The genus *Micropsalliota*

8.3.1 *Micropsalliota* species in Northern Thailand

There are 104 *Micropsalliota* specimens collected from northern Thailand. Based on the morphological examination and molecular analysis, twenty six species are fully described (22 species were named with figures and color plates; 4 species suspected as new or known species without figures), including 8 new species and 2 new varieties.

8.3.2 Molecular phylogenies of *Micropsalliota*

All analyses of the ITS, LSU and ITS+LSU datasets (Figs. 6.2, 6.3, 6.4) indicate that *Micropsalliota* is monophyletic with 100% bootstrap (BS) and Bayesian posterior probability (PP) support. Heinemann's definition of *Micropsalliota* stated that the encrusted pileipellis hyphae is an important diagnostic character in the genus; this is well supported by phylogenetic analyses. Elongated capitate cheilocystidia was also considered to be another important diagnostic character (Pegler and Rayner 1969, Heinemann 1976; and Single 1986) and this is supported in this study. The phylogenetic data confirms the definition of *Micropsalliota* (Heinemann 1976), which distinguishes this genus mainly in having elongated capitate cheilocystidia, encrusting pileipellis hyphae and the endosporium of the spore; the pseudoamyloid reaction of the spore is not important. The generic name with the suffix "micro" might leave one to imagine this genus comprises species with small fruiting bodies. However, even though most species are small, this

genus also possesses some species with average or even large fruiting bodies, such as *M. globocystis* and *M. pleurocystidiata*.

Heinemann's taxonomic system within *Micropsalliota*, which based on the IG value [IG = (length of stipe)^2 / (diameter of pileus × diameter of stipe)] and the color of the pileus, does not appear to be supported in the phylogenetic analyses. For example, the clade best supported in all analyses comprises of *M. albosericea*, *M. allantoidea*, *M. arginophaea*, *M. bifida*, *M. gracilis*, *M. pseudoarginea*, *M. pusillissima*, *M. subalba* and *M. subarginea*, and all those species are minute with variable color and spore size.

8.3.3 The genus *Allopsalliota*

Agaricus geesterani was described from Netherlands, and it is characterized well-developed universal veil, long capitate cheilocystidia and *Agaricus bitorquis*-like basidiocarps (Bas & Heinemann 1986), which has the combined morphological characters of both *Agaricus* and *Micropsalliota*. A new section Magici was established for this species in *Agaricus* (Bas & Heinemann 1986). Later, based on the chemical tests of context and lamellae on this species, the re-evaluation of all morphological characters, and comparison those with *Agaricus* and *Micropsalliota*, the genus *Allopsalliota* Nauta & Bas was established in 1998 (Nauta 1998), which is based on the single species *Allopsalliota geesterani* (Bas & Heinemann) Nauta & Bas (≡ *Agaricus geesterani* Bas & Heinemann).

However the molecular data based on ITS and LSU rDNA sequences shows *Allopsalliota geesterani* is nested in *Micropsalliota* clade undoubtedly. Furthermore *Allopsalliota geesterani* has all diagnostic morphological characters of *Micropsalliota*: capitate cheilocystidia, endosporium basidiospores and encrusted pileipellis hyphae (Bas & Heinemann 1986; Heinemann 1976; Nauta 1998 and 2001). So the genus *Allopsalliota* is proposed as a synonym of the genus *Micropsalliota*.

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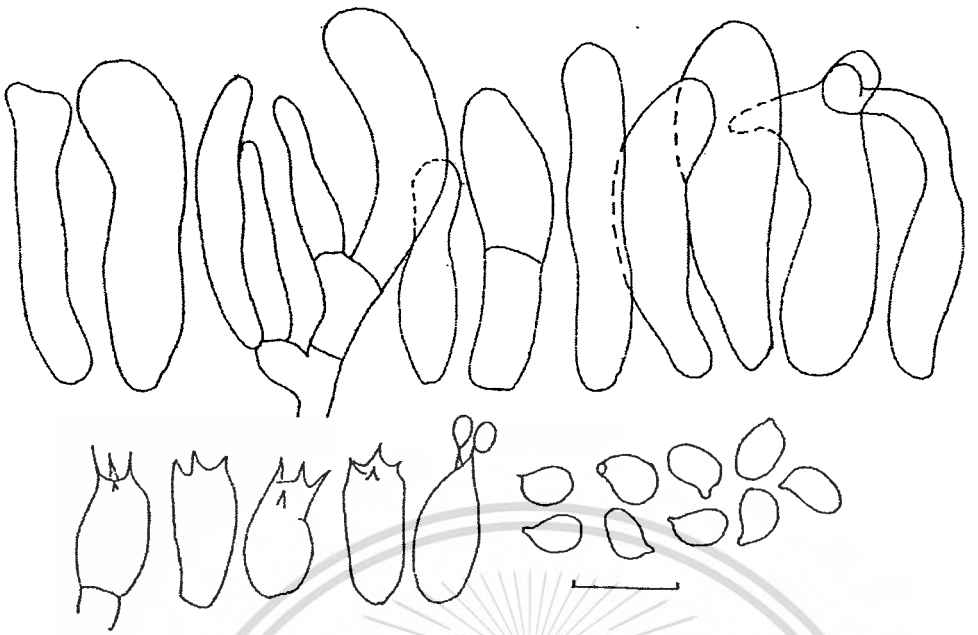

Fig. 4.1 . *Agaricus angusticystidiata* sp. nov., ZRL2043 (Holotype: SFSU). Cheilocystidia, basidia and spores; bar = 10 μm.

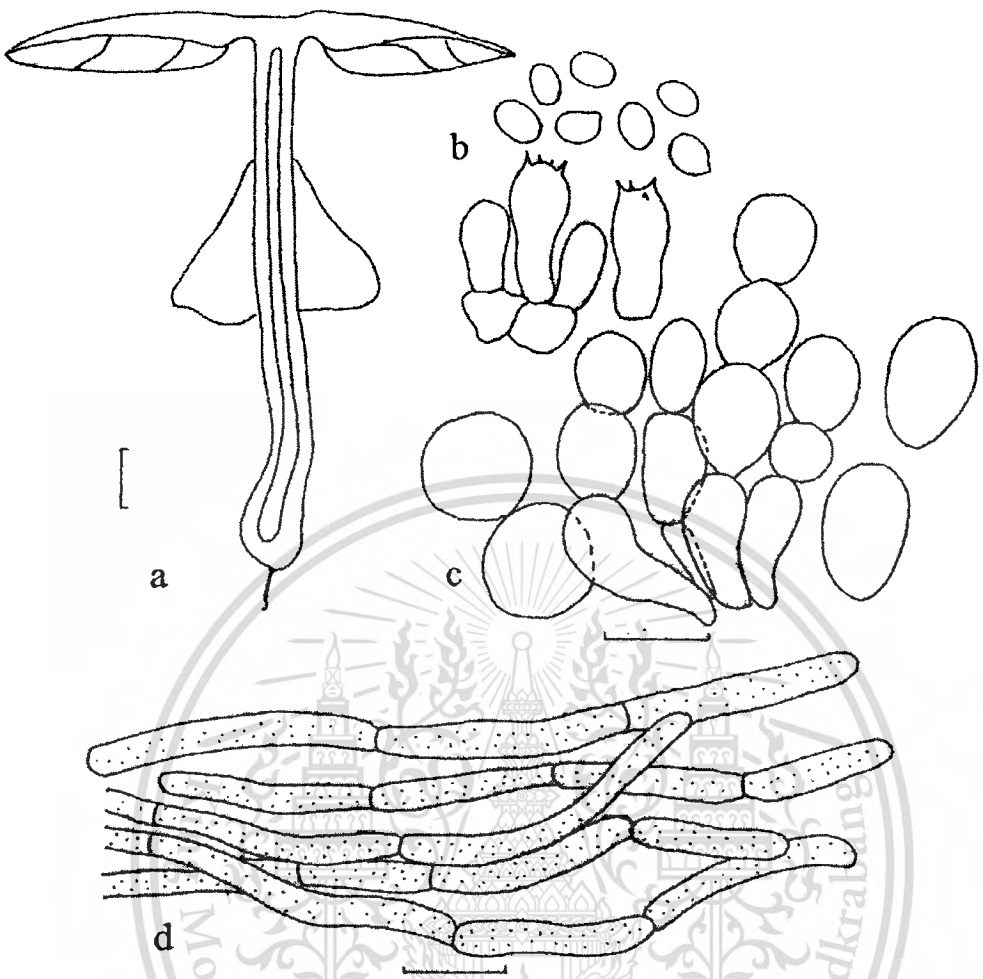

Fig. 4.2:. *Agaricus bambusae,* ZRL2036 (SFSU). a. Fruiting body; b. basidia and spores; c. cheilocystidia; d. pileipellis hyphae; bar a = 8 mm; b and c = 10 µm; d = 25 µm.

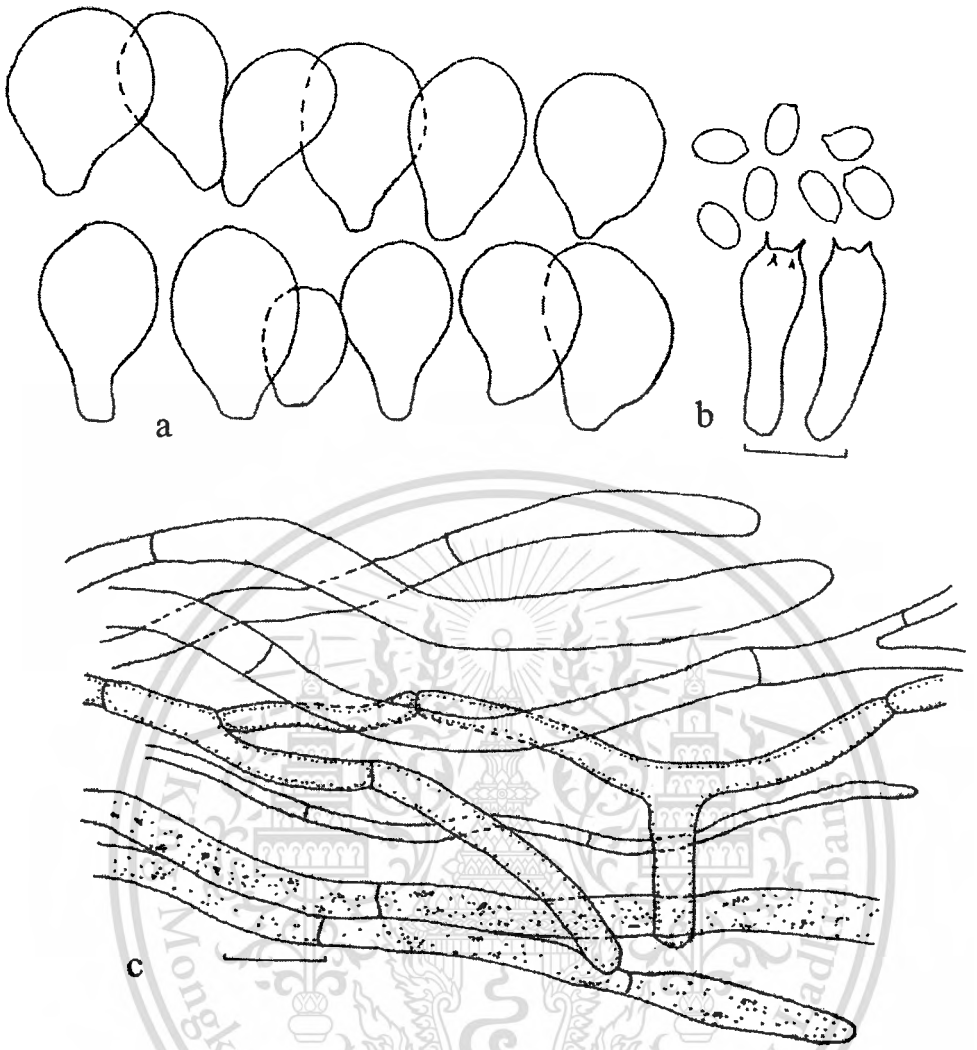

Fig. 4.3. *Agaricus* aff. *brunneolus* ZRL3007 (SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; bar a and b = 10 µm; c = 25 µm.

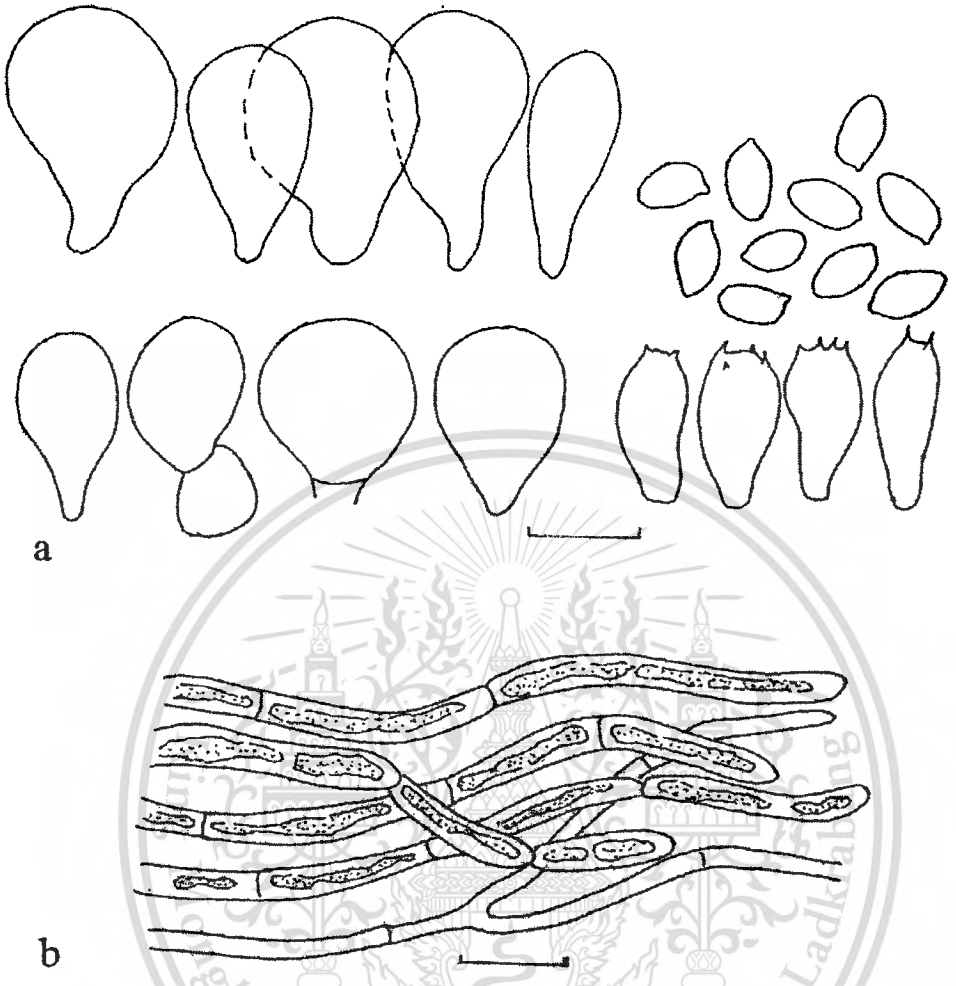

Fig. 4.4. *Agaricus caribaeus* ZRL2032 (SFSU). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar a = 10 µm; b = 25 µm.

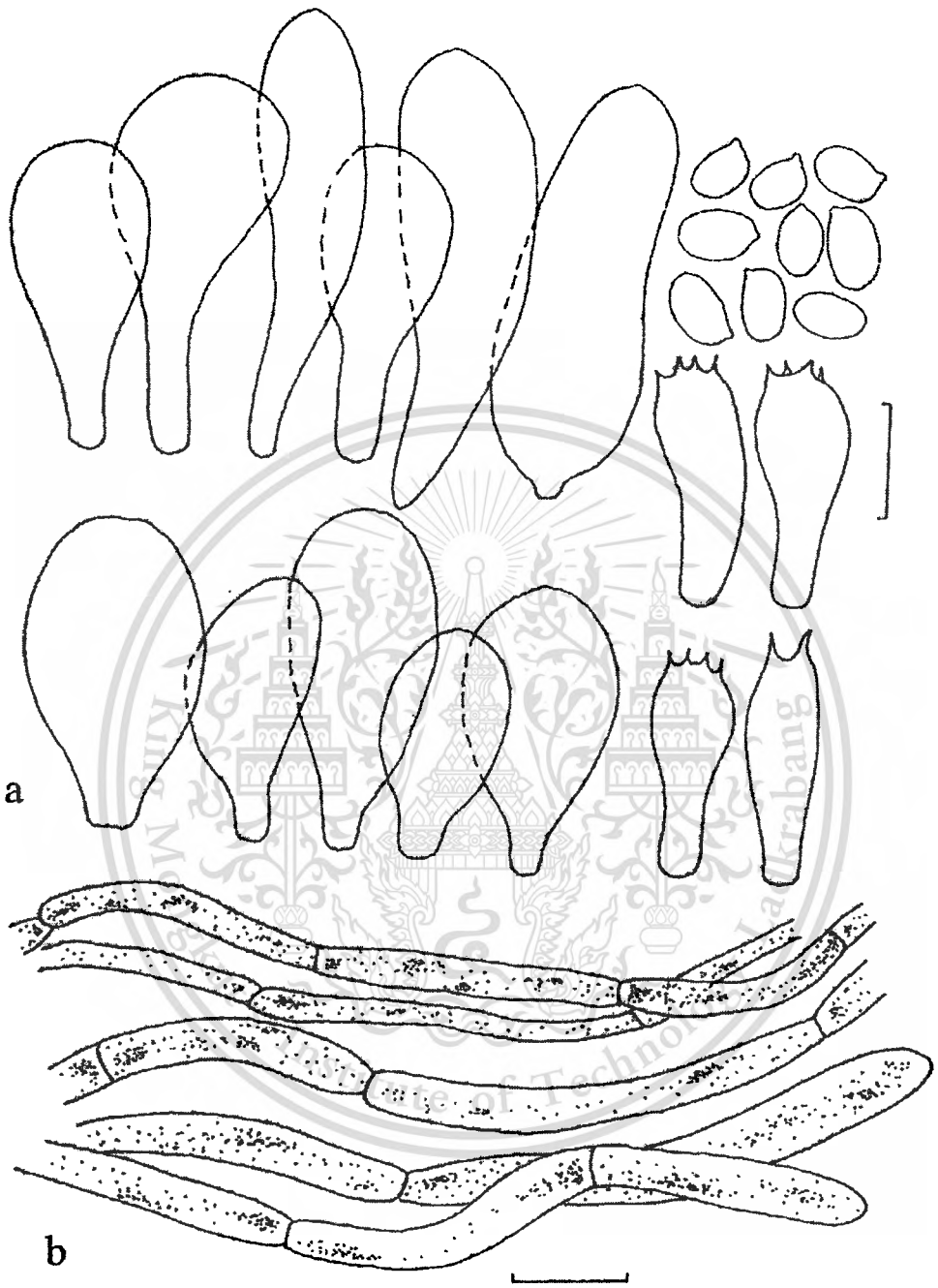

Fig. A.5. *Agaricus dulcidulus* ZRL3071 (SFSU). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar a = 10 µm; b = 25 µm.

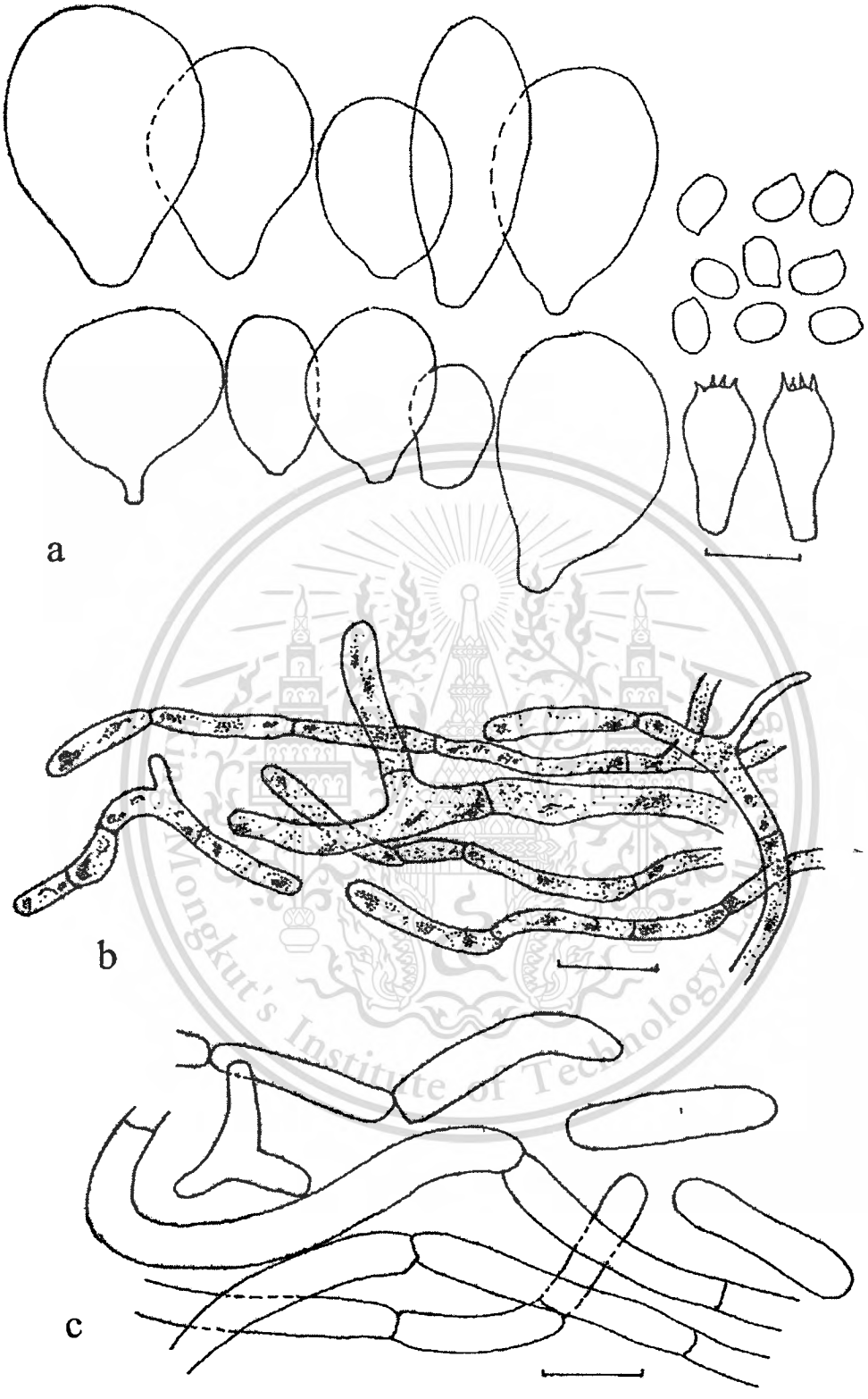

Fig. A.6. *Agaricus duplocingulatus* ZRL3051 (SFSU). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar a = 10 µm; b and c = 25 µm.

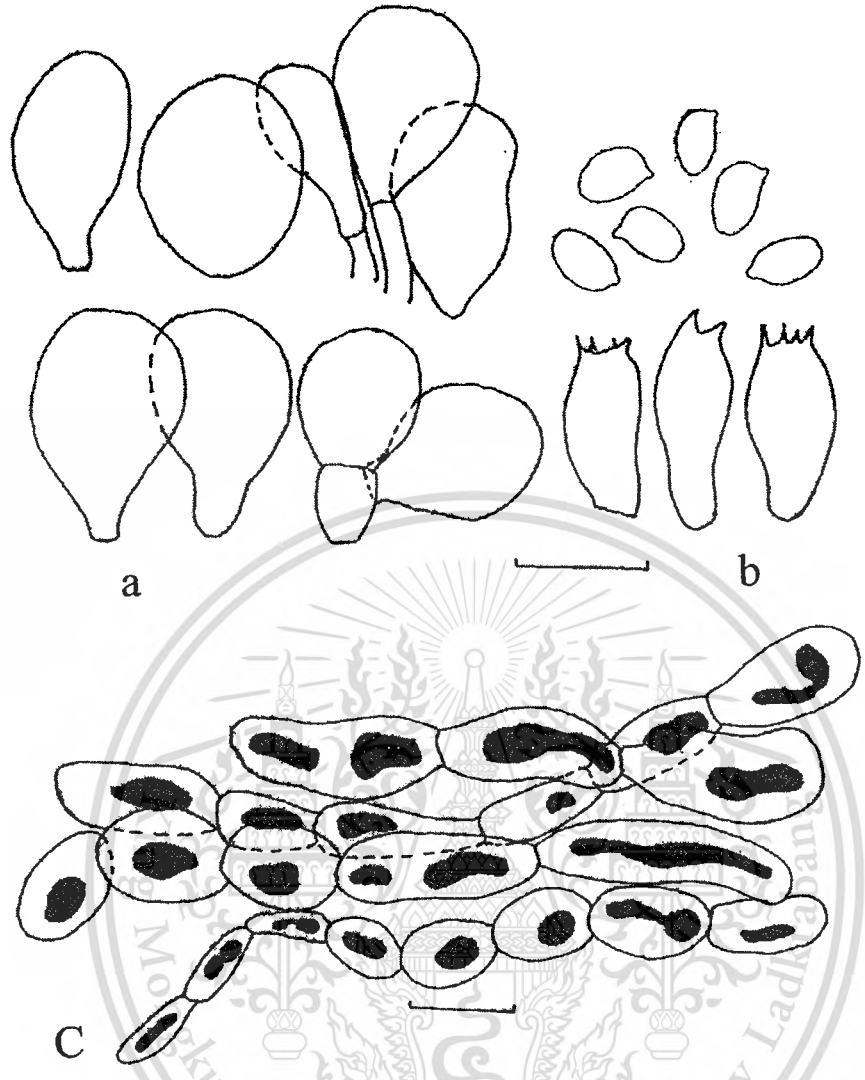

Fig. A.7. *Agaricus endothanthus* ZRL3095 (SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; bar a and b = 10 µm; c = 25 µm.

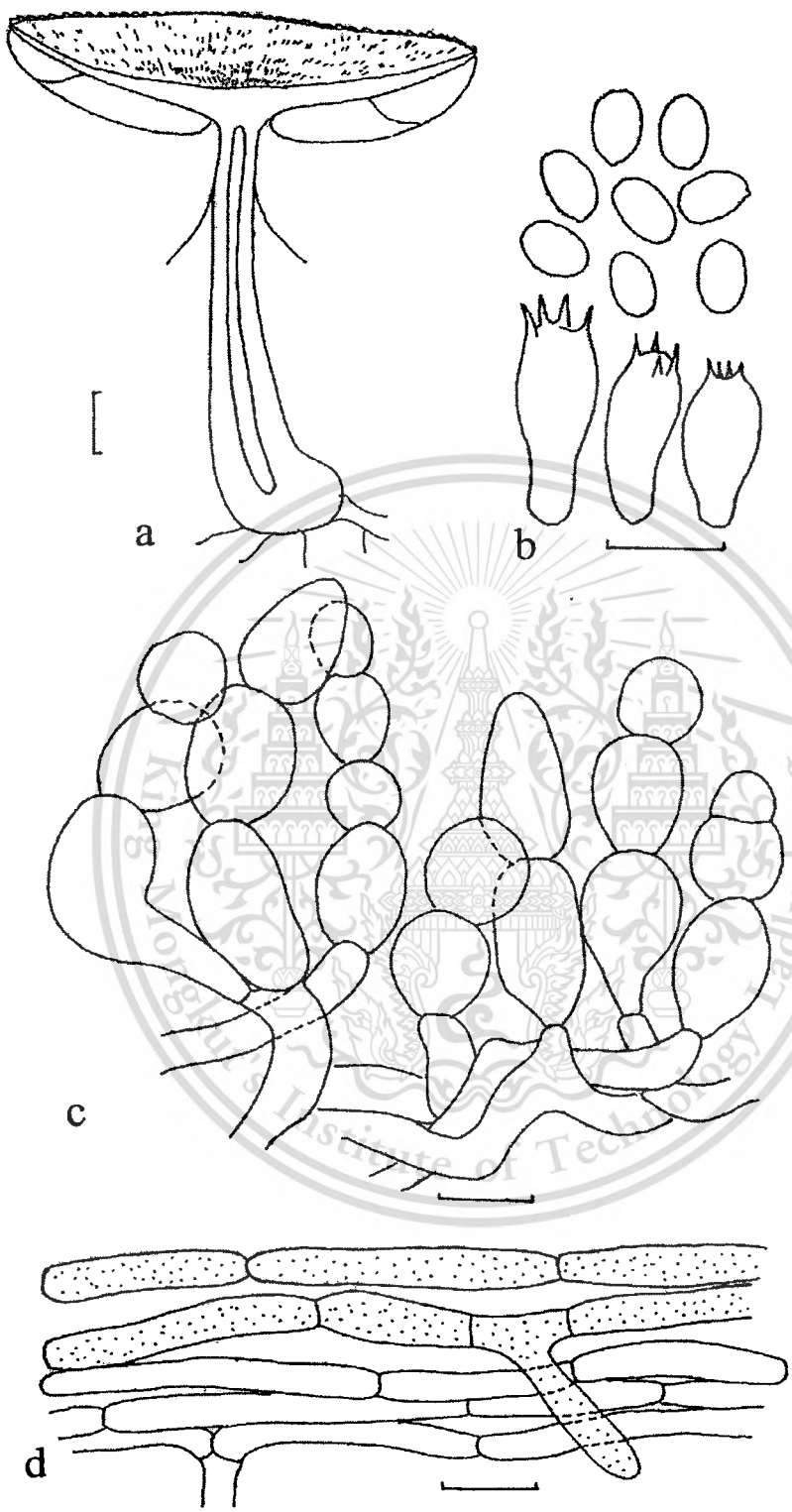

Fig. A.8. *Agaricus fiardii* ZRL2134 (SFSU). a. Fruiting body; b. basidia and spores; c. cheilocystidia; d. pileipellis hyphae; bar a = 8 mm; b and c = 10 μm; d = 25 μm.

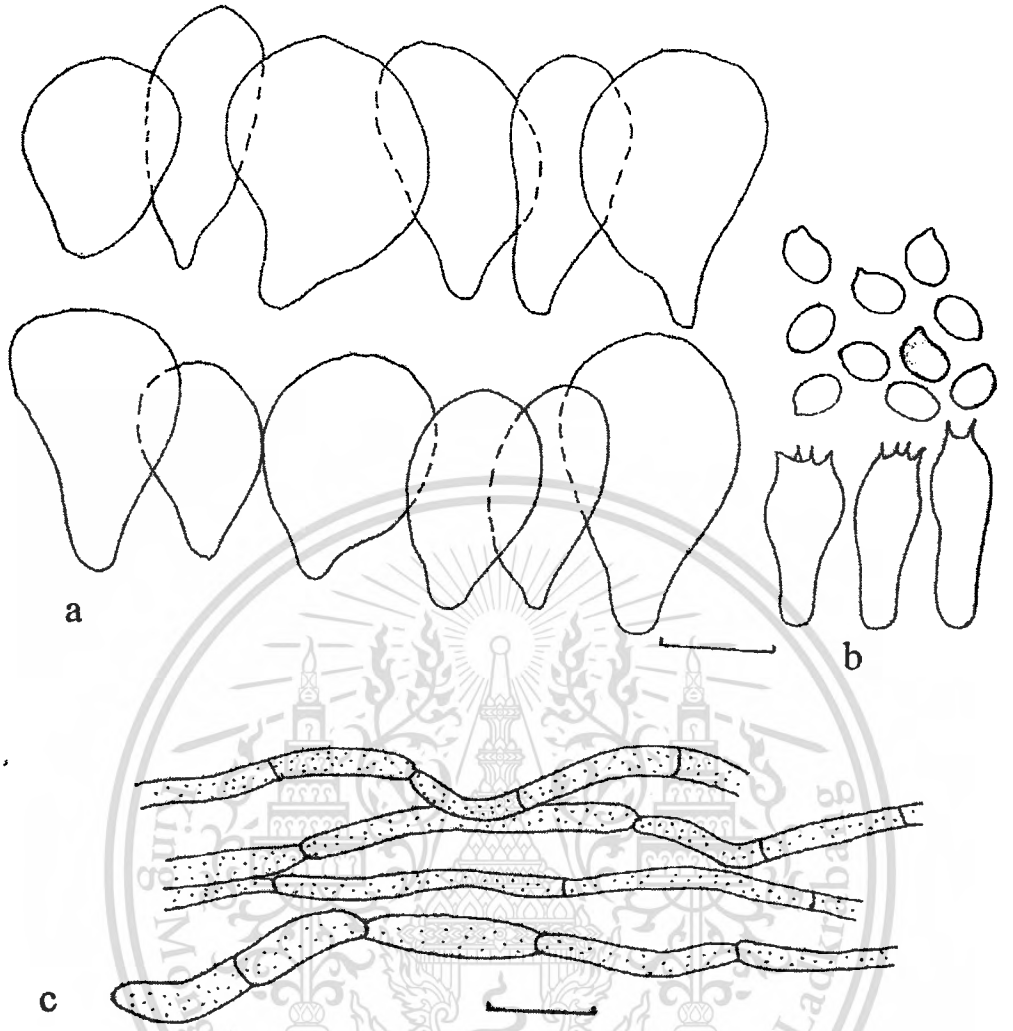

Fig. A.9. *Agaricus impudicus* ZRL3020 (SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; bar a and b = 10 µm; c = 25 µm.

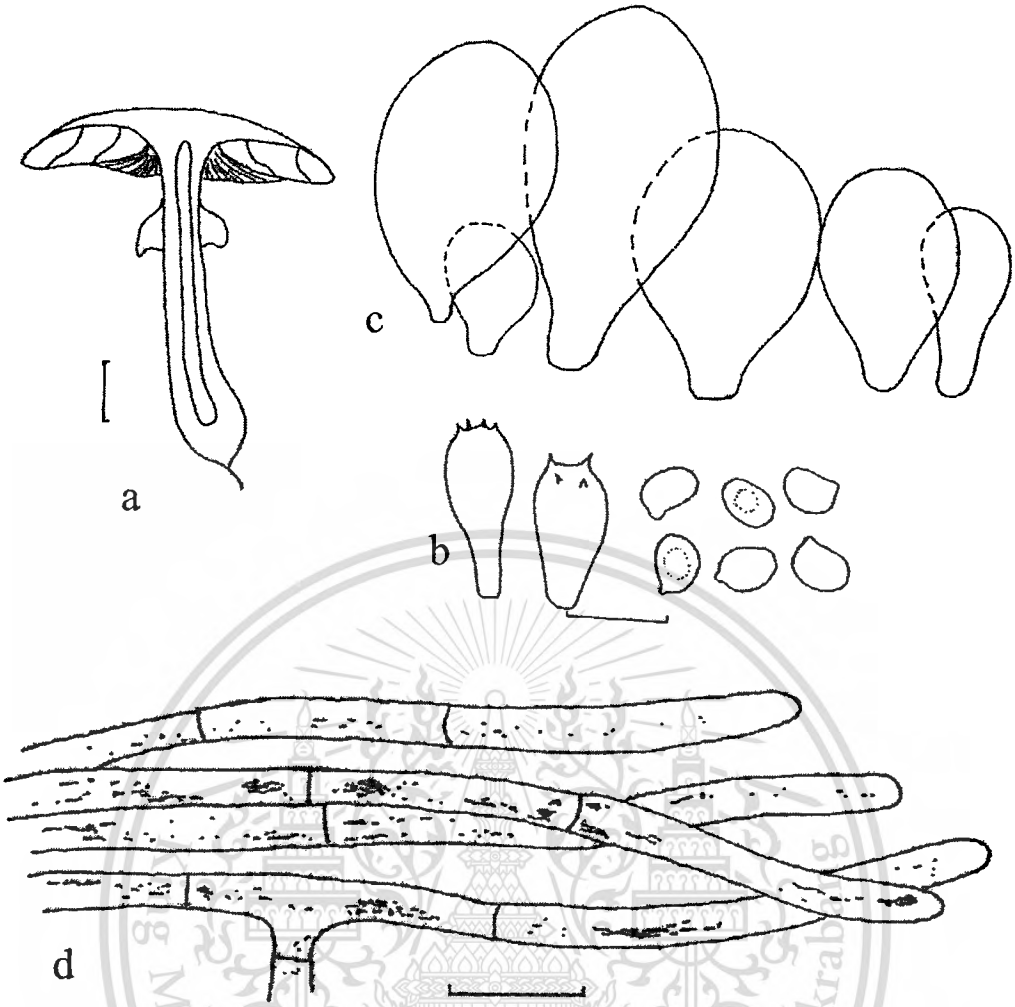

Fig. A.10. *Agaricus johnstonii* ZRL3005 (SFSU). a. Fruiting body; b. basidia and spores; c. cheilocystidia; d. pileipellis hyphae; bar a = 8 mm; b and c = 10 µm; d = 25 µm.

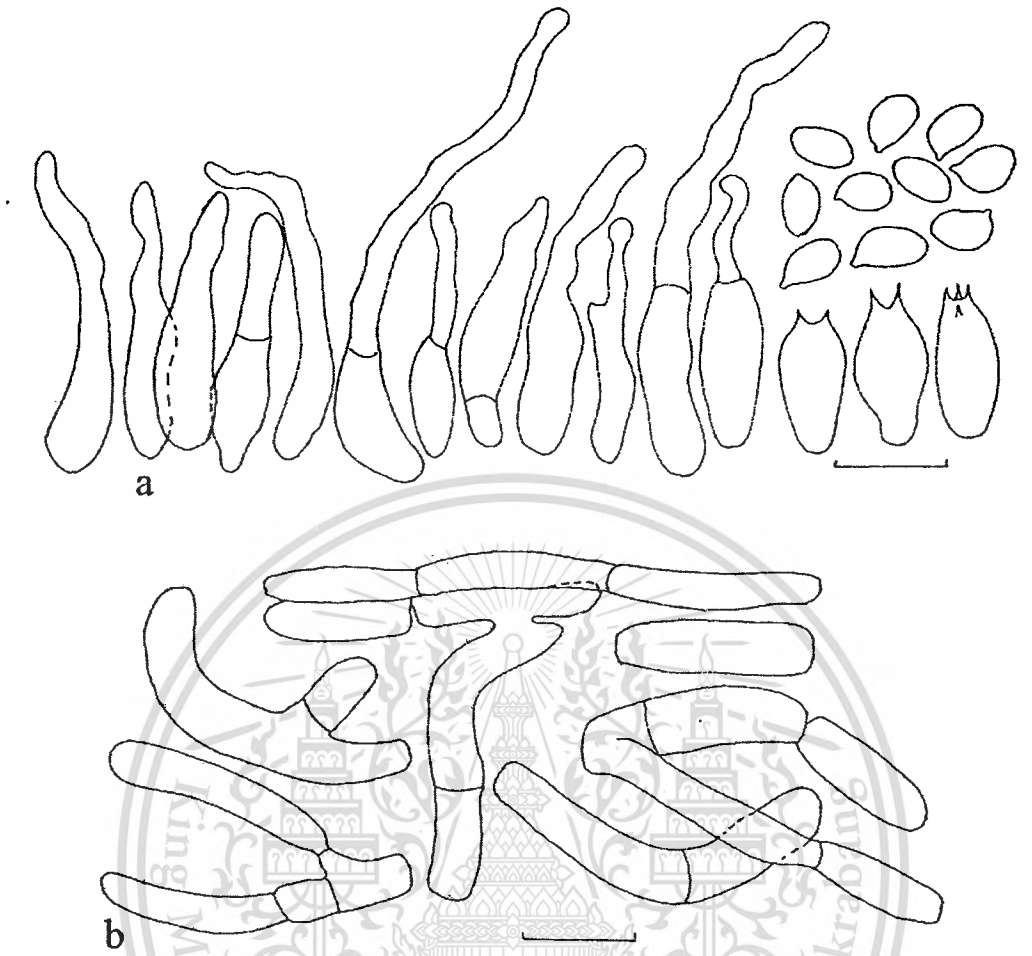

Fig. A.11. *Agaricus maesaeensis* sp nov. ZRL2048 (Holotype: SFSU). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar = 10 µm.

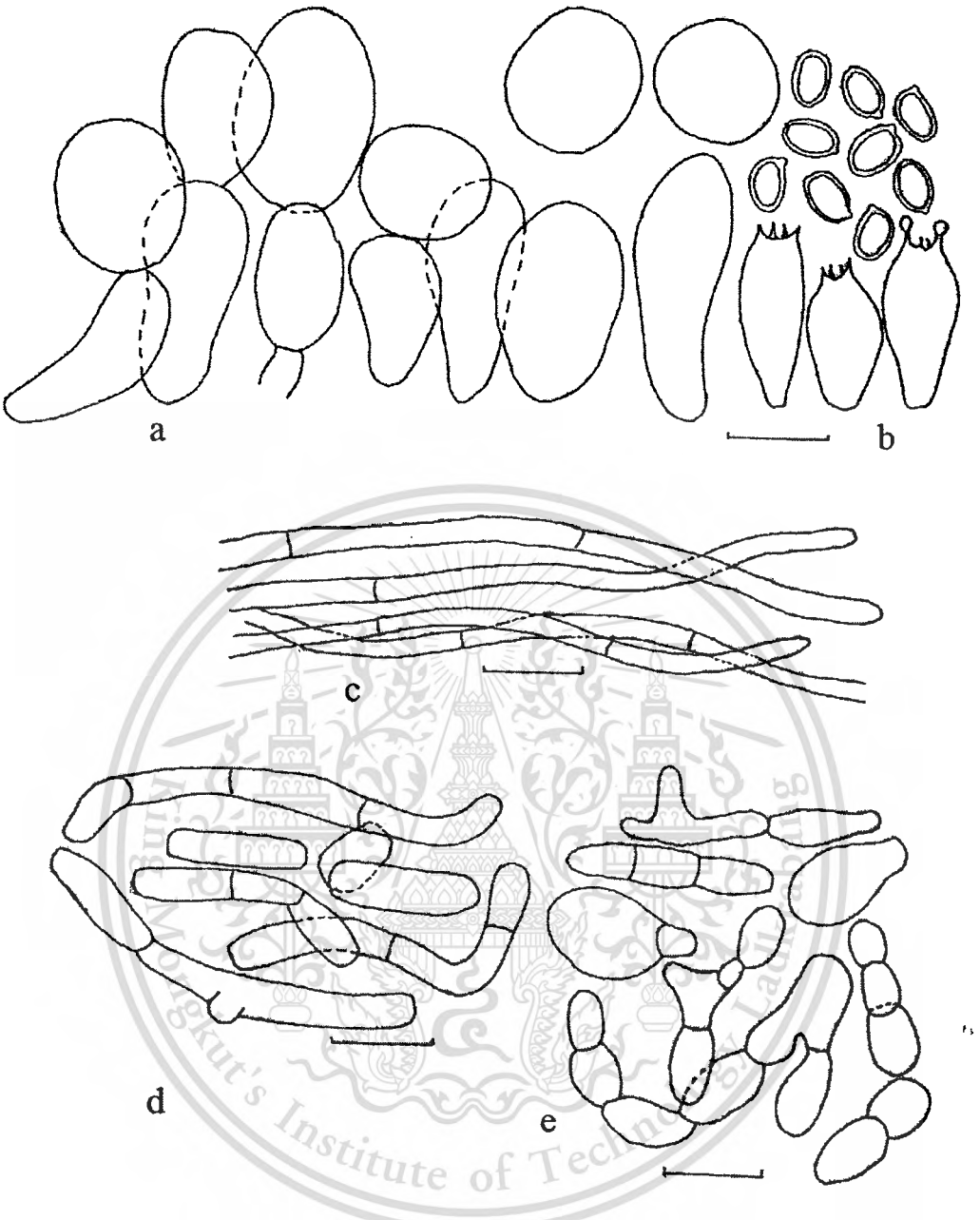

Fig. A.12. *Agaricus ochrascens* ZRL3028 (SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; d. annulus hyphae; e. caulocystidia; bar a and b = 10 µm; c-e = 25 µm.

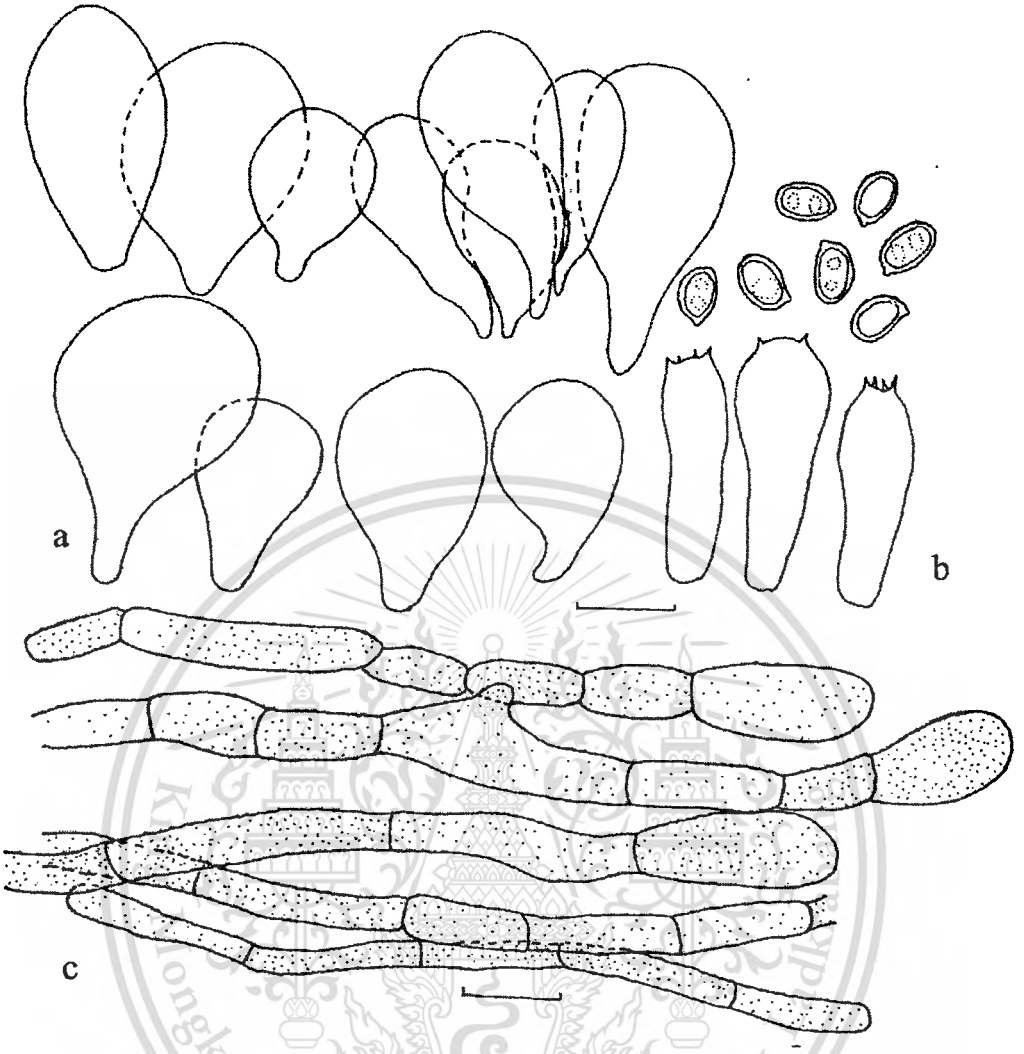

Fig. A.13. *Agaricus porphyrizon* ZRL2044 (SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; bar a and b = 10 µm; c = 25 µm.

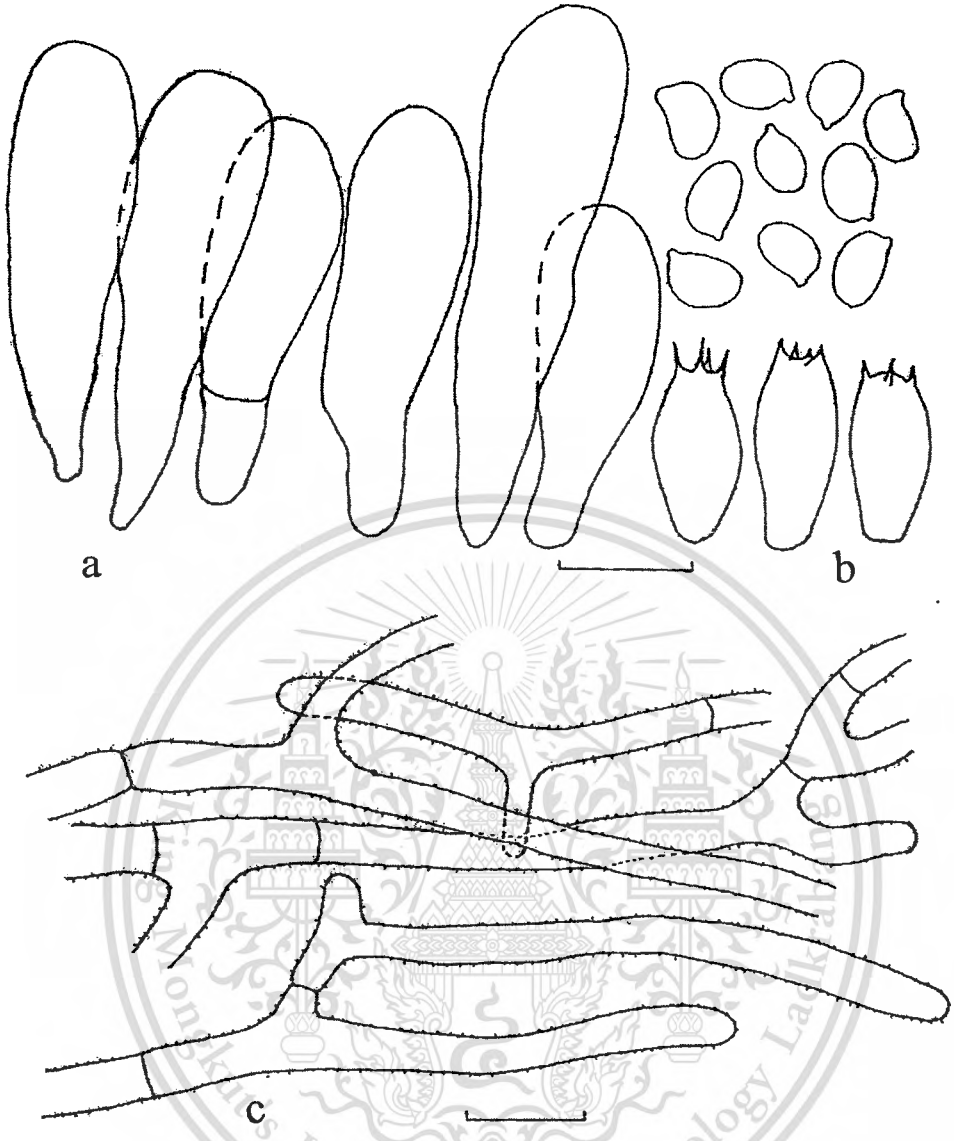

Fig. A.14. *Agaricus trisulphuratus* ZRL2111 (SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; bar = 10 µm.

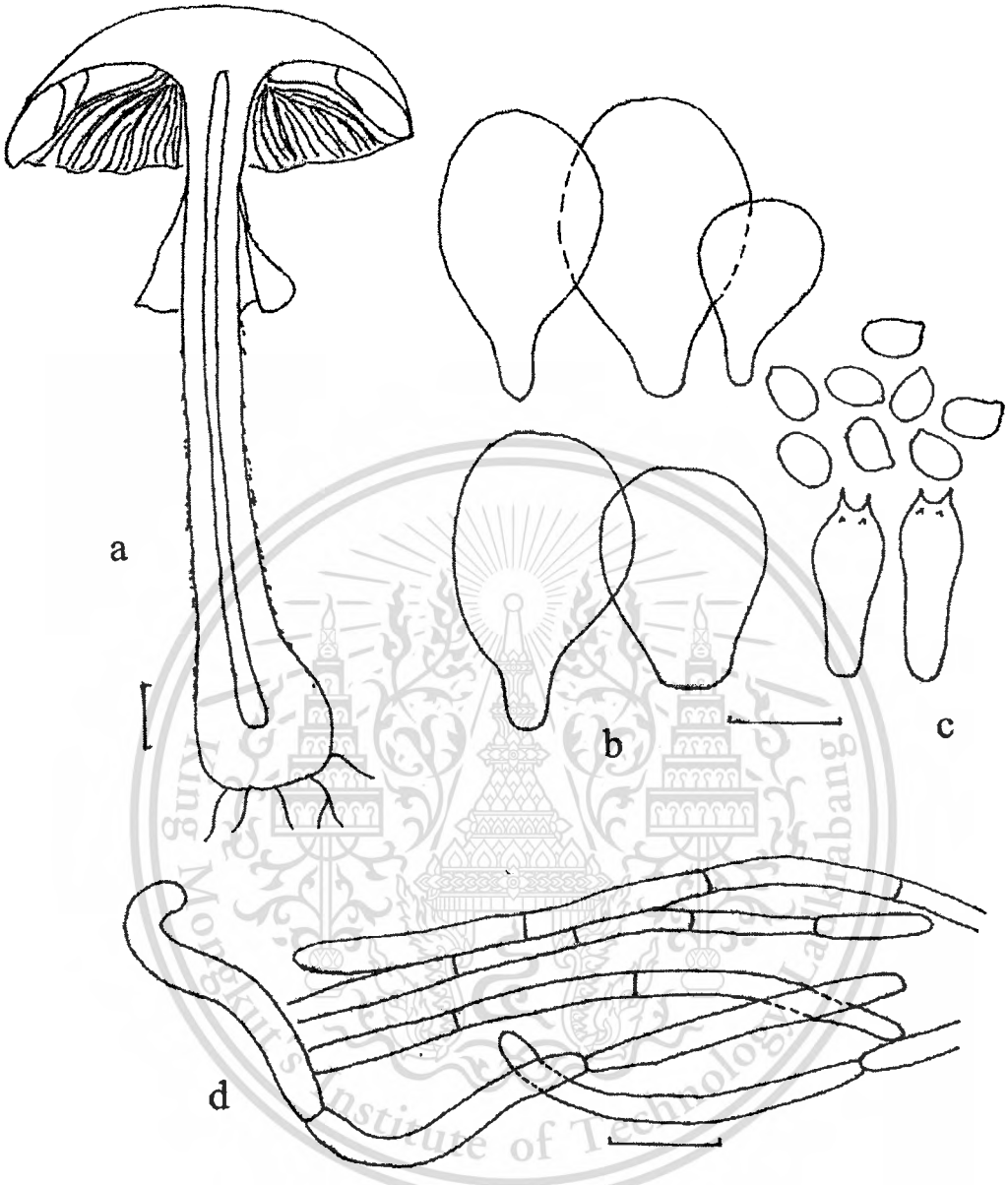

Fig. A.15. *Agaricus xantholepis* ZRL3088 (SFSU). a. Fruiting body; b. cheilocystidia; c. basidia and spores; d. pileipellis hyphae; bar a = 8 mm; b and c = 10 µm; d = 25 µm.

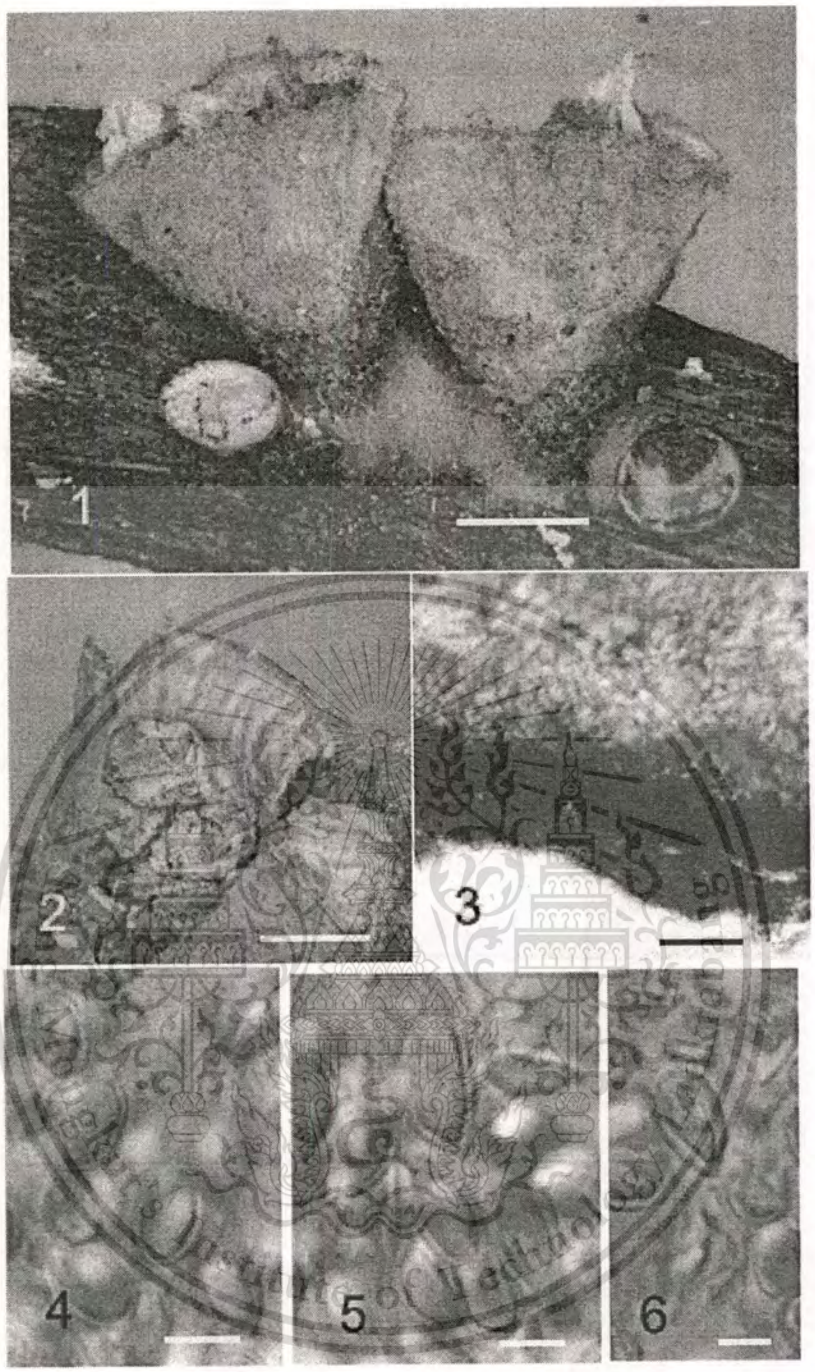

Fig. B.1. *Cyathus africanus* 200370 (Holotype DAOM). 1- 2. Fruiting bodies. 3. Section of peridiole, one cortax with tunica. 4 -5.Basidiospores, ovoid with apiculus.Scale bars: 1,2 = 3 mm, 3 = 25 µm, 4,5,6 = 4 µm.

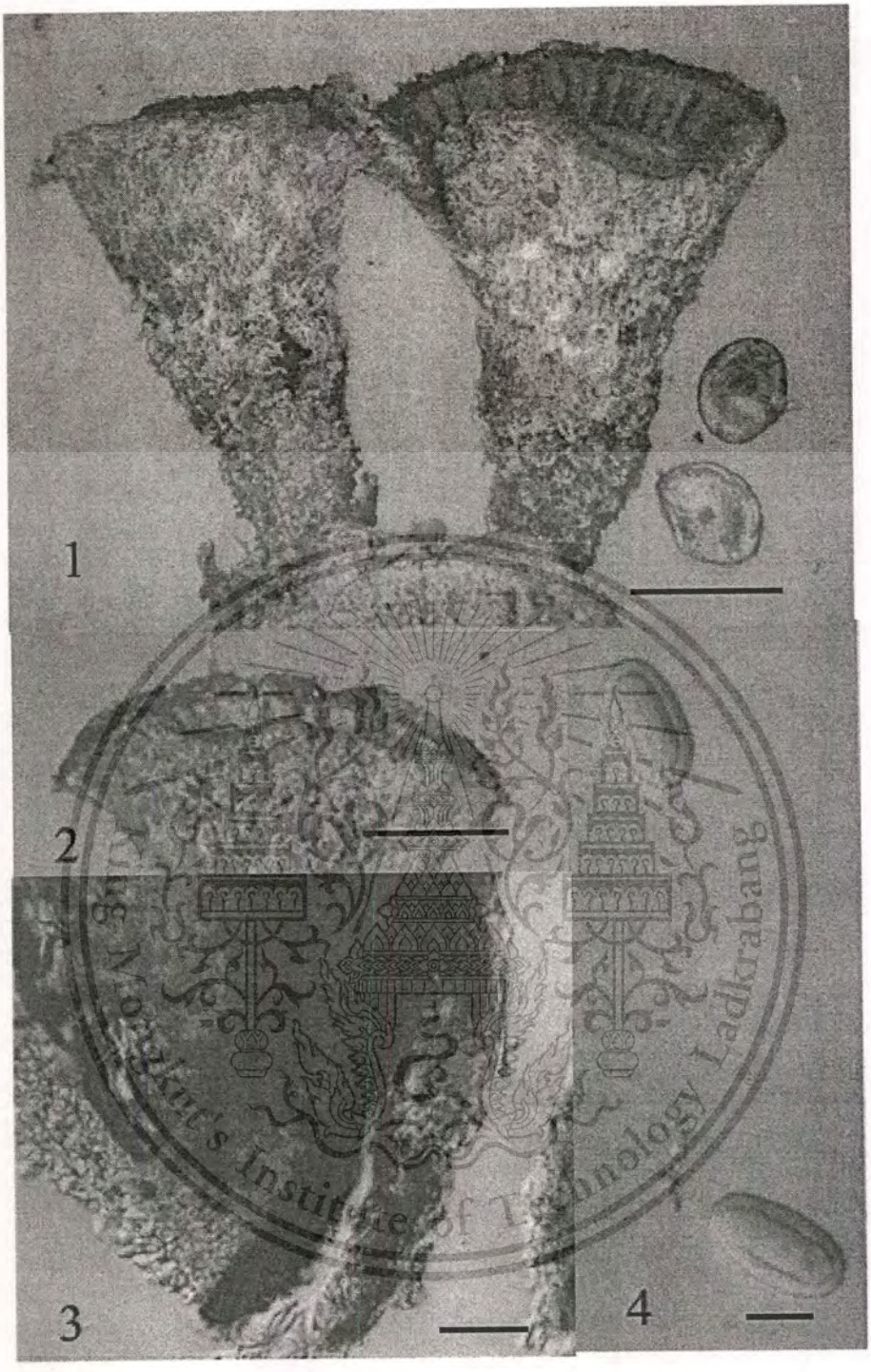

Fig. B.2. *Cyathus annulatus* 200366 (Holotpye DAOM)

1. Fruiting bodies. 2. Brown fimbria at lip. 3. Section of peridiole, one cortex with tunica. 4. Basidiospores Scale bars: 1,2 = 5 mm; 3 = 20 µm; 4 = 10 µm.

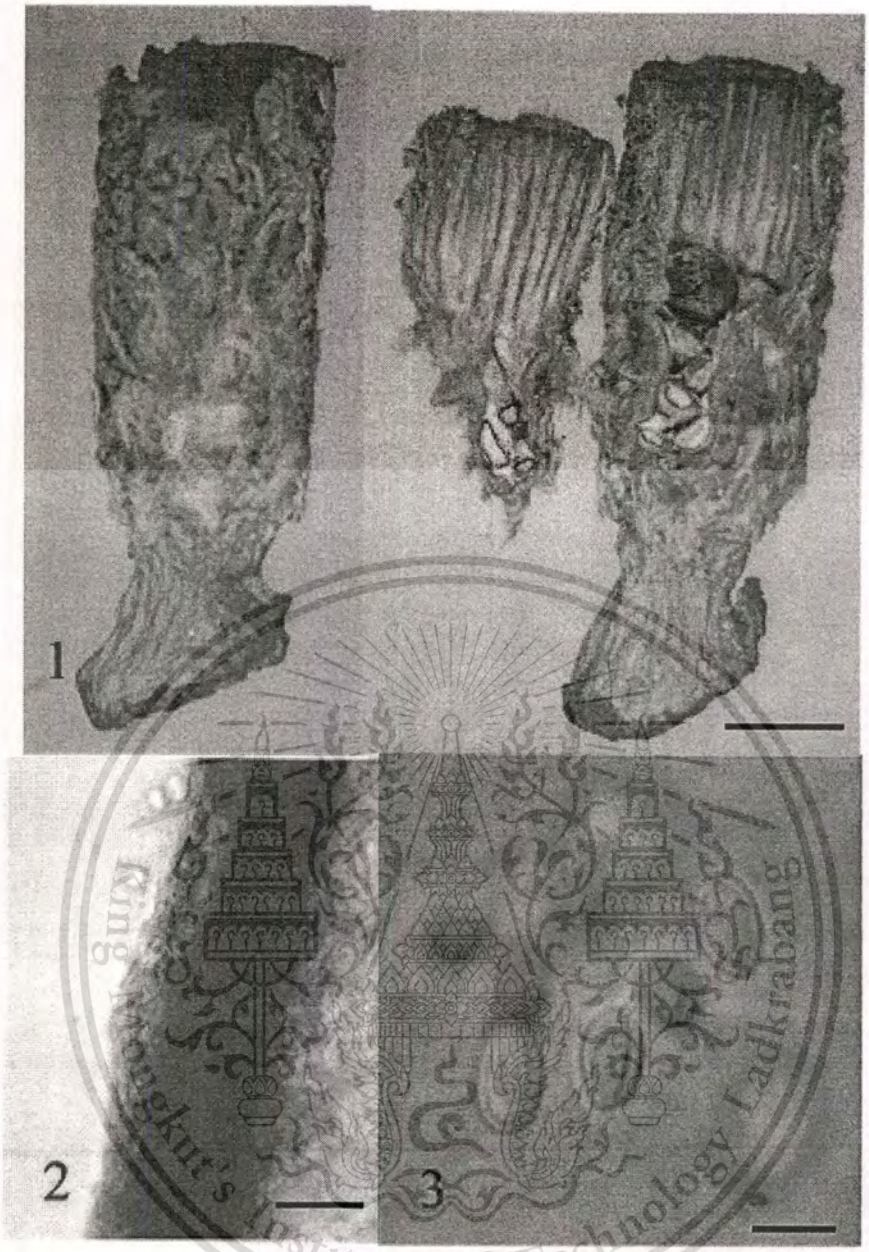

Fig. B.3. *Cyathus* aff. *berkeleyanus* 20789 (SWFC)

1. Fruiting bodies. 2. Section of peridiole, one contex with tunica. 3. Basidiosopres.

Scale bars: 1 = 2 mm, 2 = 20 µm, 3 = 3 µm

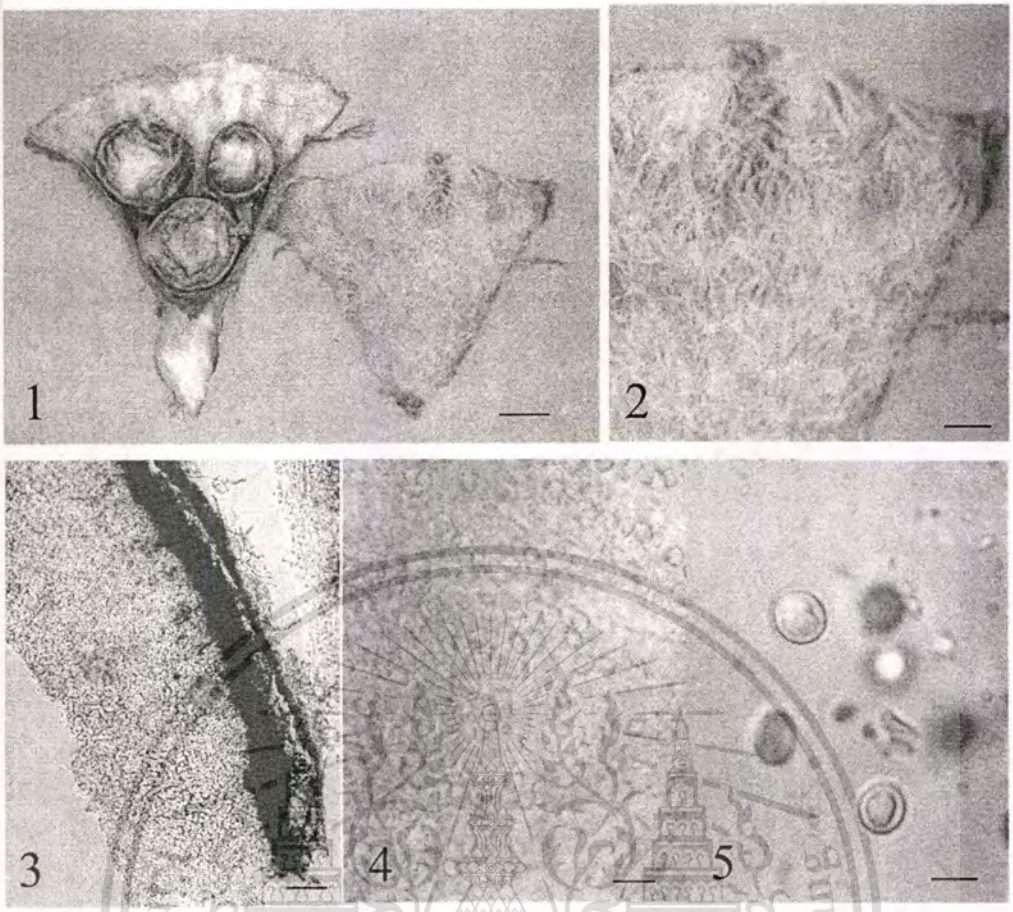

Fig. B.4. *Cyathus bulleri* 727126 (Isotype BPI).

1. Fruiting bodies. 2. Long and soft hairs. 3. Section of peridiole, single cortex with tunica. 4-5. Basidiospores. Scale bars: 1 = 1.2 mm; 2 = 0.5 mm; 3 = 30 μm; 4 = 20 μm; 5 = 8 μm.

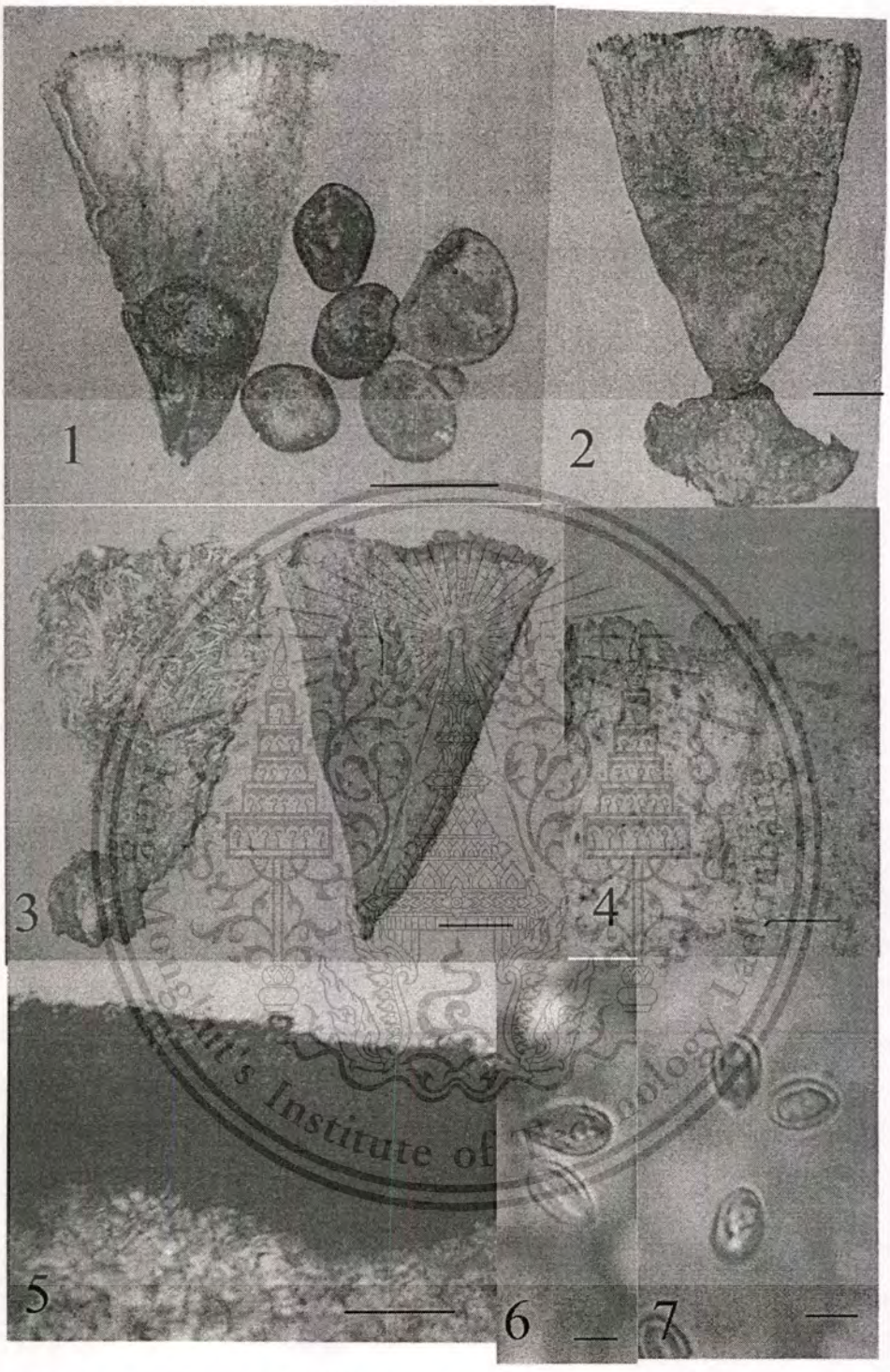

Fig. B.5. *Cyathus confusus* 03490 (Holotype HMAS)

1-3. fruiting bodies (3. Shaggy hairs and smooth surfaces).4. Fimbriate at lip. 5. Section of peridiole, one cortex with tunica. 6-7. Basidiospores.

Scale bars: 1 = 5 mm; 2 , 3 = 3 mm; 5 = 30 μm; 6 = 4 μm; 6 = 8 μm.

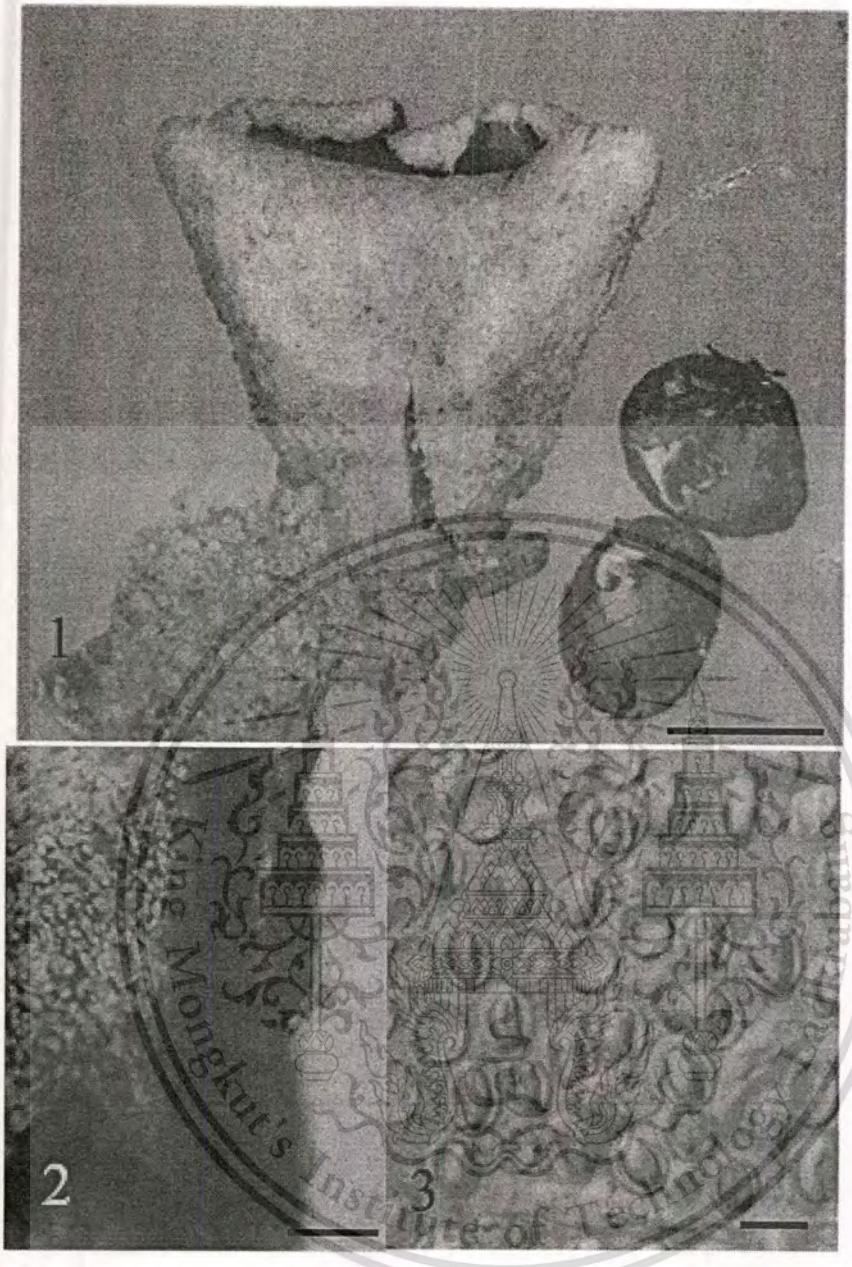

Fig. B.6. *Cyathus conlensoi* 200422 (DAOM)

1. Fruiting bodies. 2. Section of peridiole, one cortex with tunica, 3. Basidiospores.

Scale bars: 1 = 3 mm, 2 = 30 µm, 3 = 6 µm.

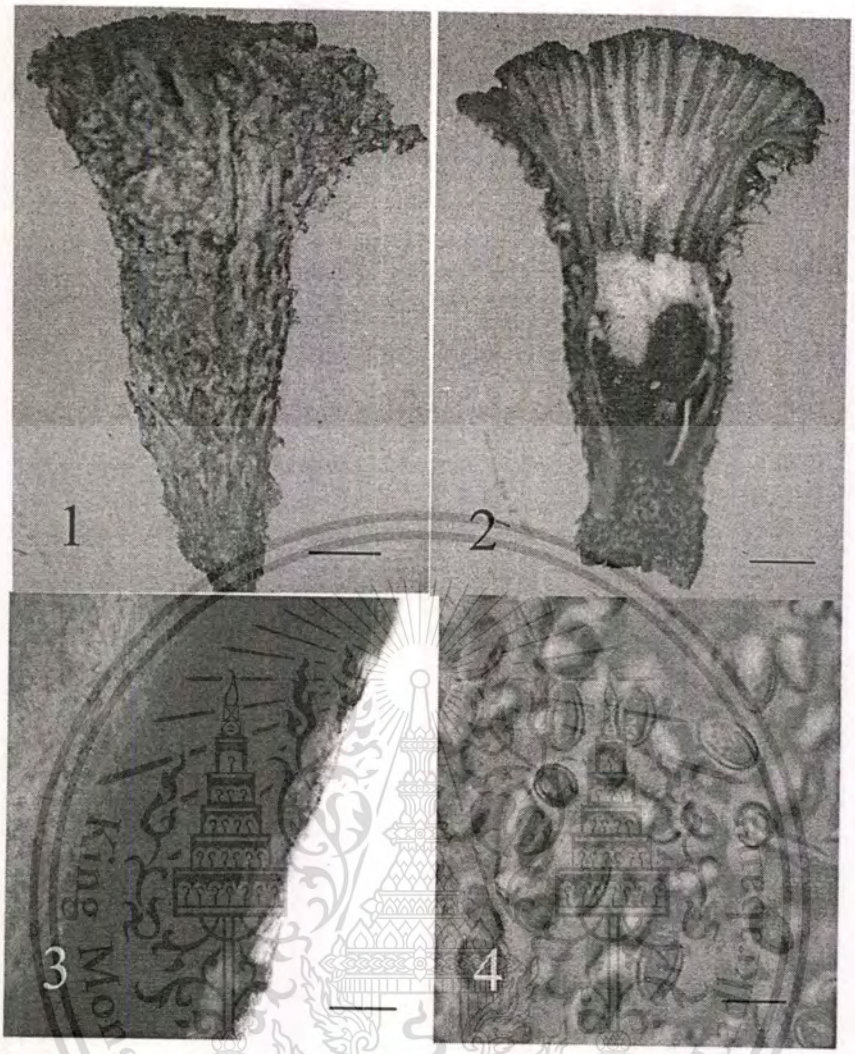

Fig. B.7. *Cyathus cornucopioides* 20414 (Holotype SWFC)

1-2. Fruiting bodies. 3. Section of peridioles, one cortex with tunica. 4. Basidiospores.

Scale bars: 1-2 = 2 mm, 3 = 25 µm, 4 = 10 µm.

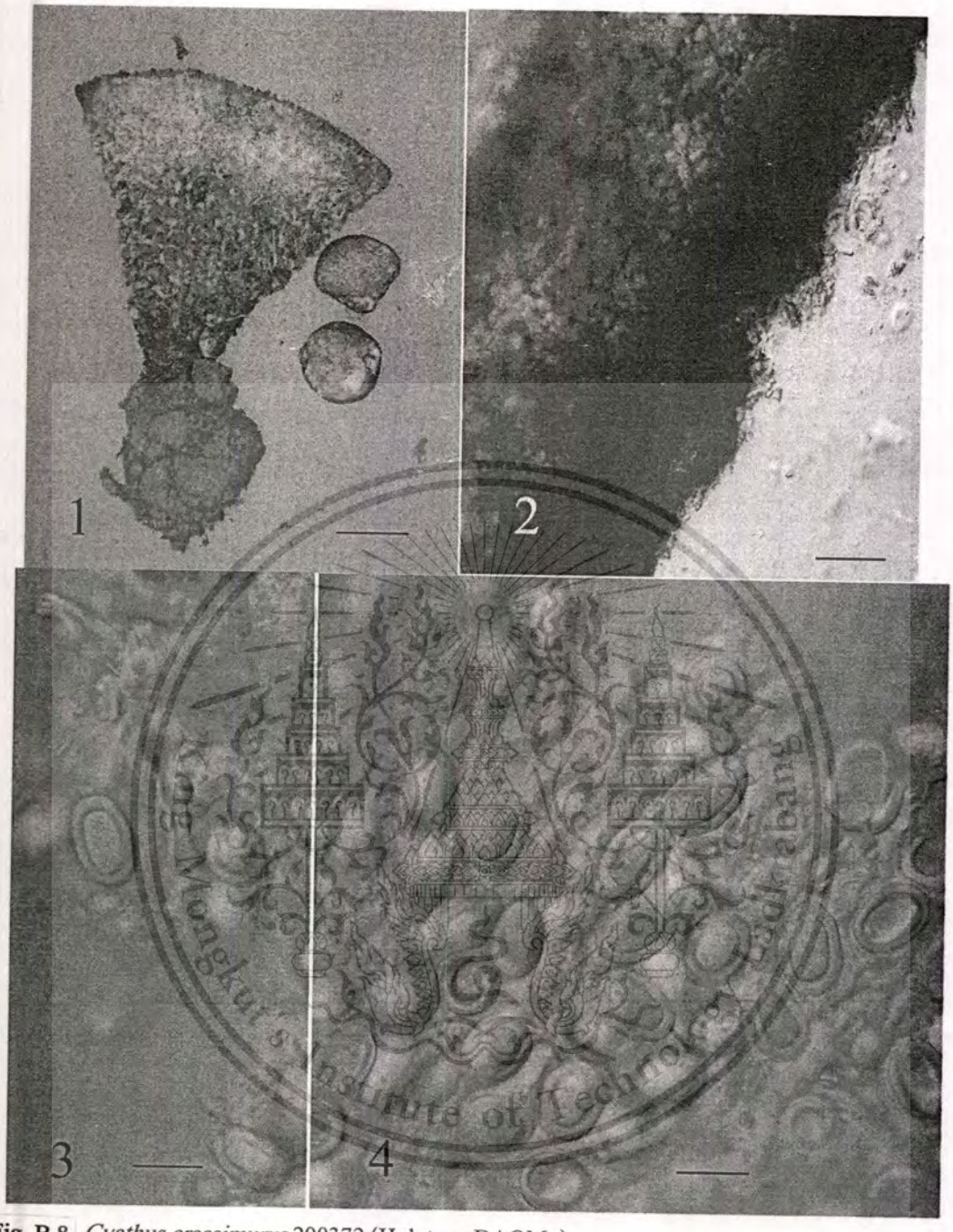

Fig. B.8. *Cyathus crassimurus* 200372 (Holotype DAOM)

1. Fruiting body. 2. Section of peridiole, one cortex with dark brown tunica. 3-4. Basidiospores. Scale bars: 1 = 1 mm, 2 = 30 μm, 3-4 = 15 μm.

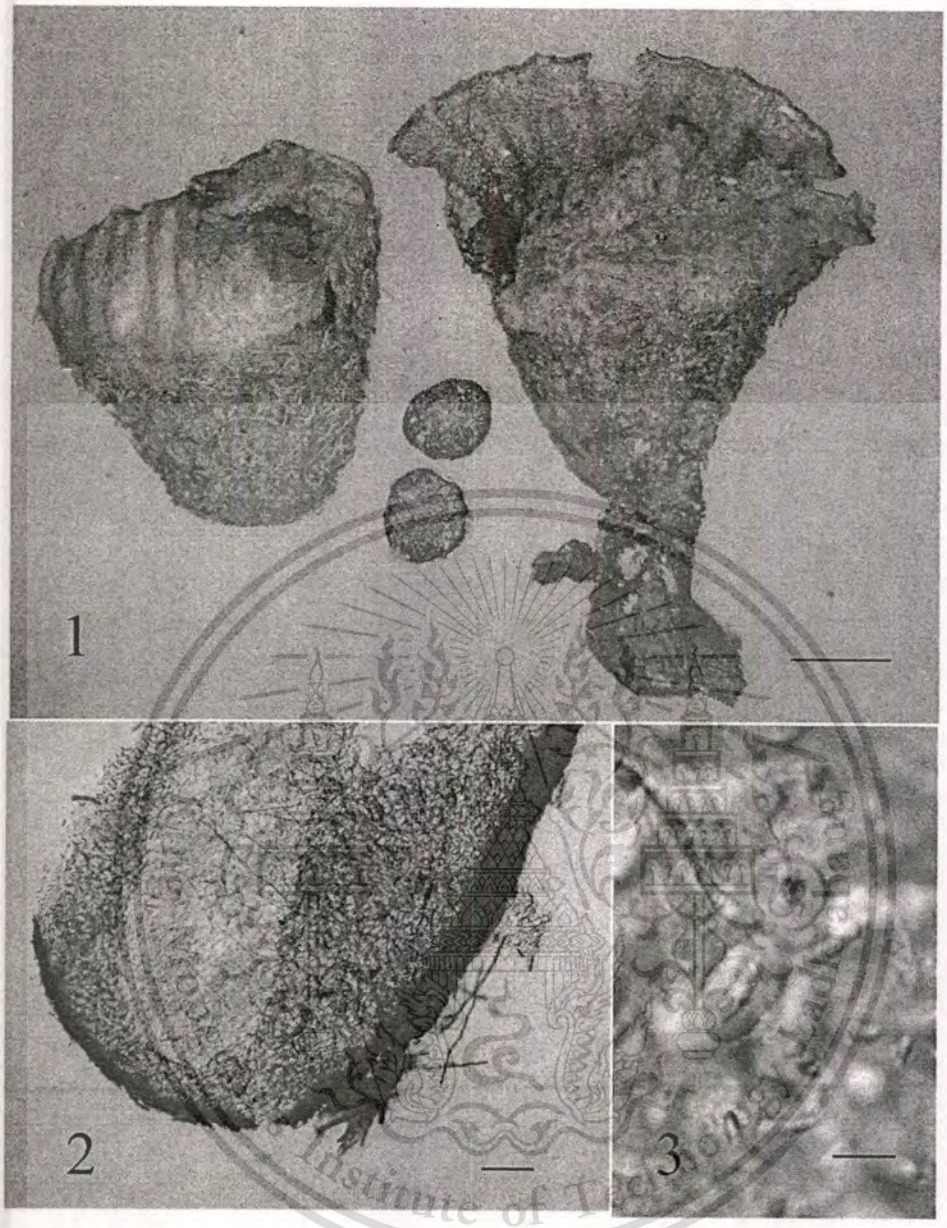

Fig. B.9. *Cyathus crispus* 200373 (Holotype DAOM)

1. Fruiting bodies. 2. Section of peridiole, double cortex, without tunica.

3. Basidiospores. Scale bars: 1 = 1.5 mm, 2 = 80 μm, 3 = 8 μm.

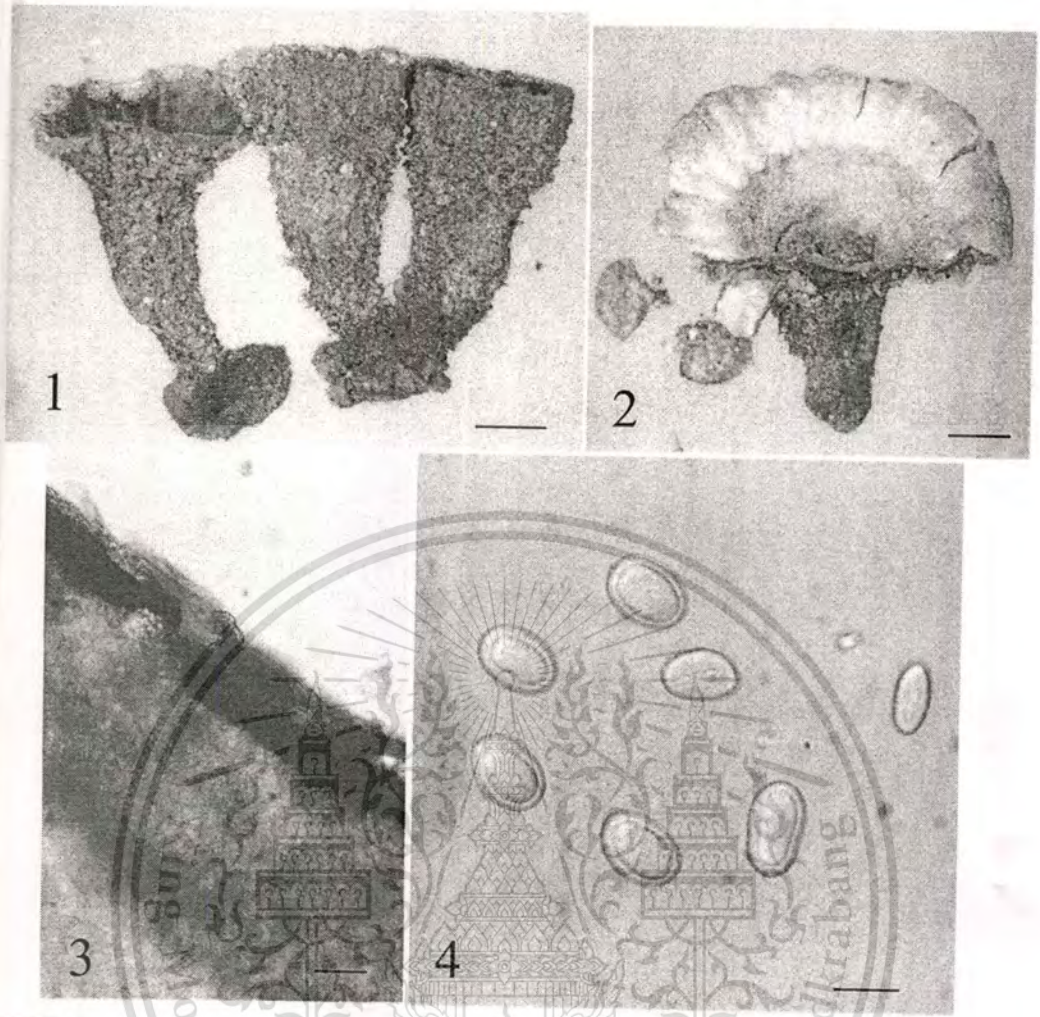

Fig. B.10. *Cyathus durus* 727135 (Isotype BPI).

1. Fruiting bodies. 2 Fruiting body (from 727134 Isotype BPI). 3. Section of peridiole, single cortex with tunica.4. Basidiospores. Scale bars: 1 = 2 mm; 2 = 1.8 mm; 3 = 25 µm; 4 = 15 µm.

Fig. B.11. *Cyathus elmeri* 198717 (Holotype DAOM)

1. Fruiting bodies. 2. Section of peridiole, one cortex with tunica. 3. Basidiospores.

Scale bars: 1 = 2.5 mm, 2 = 40 μm, 3 = 20 μm.

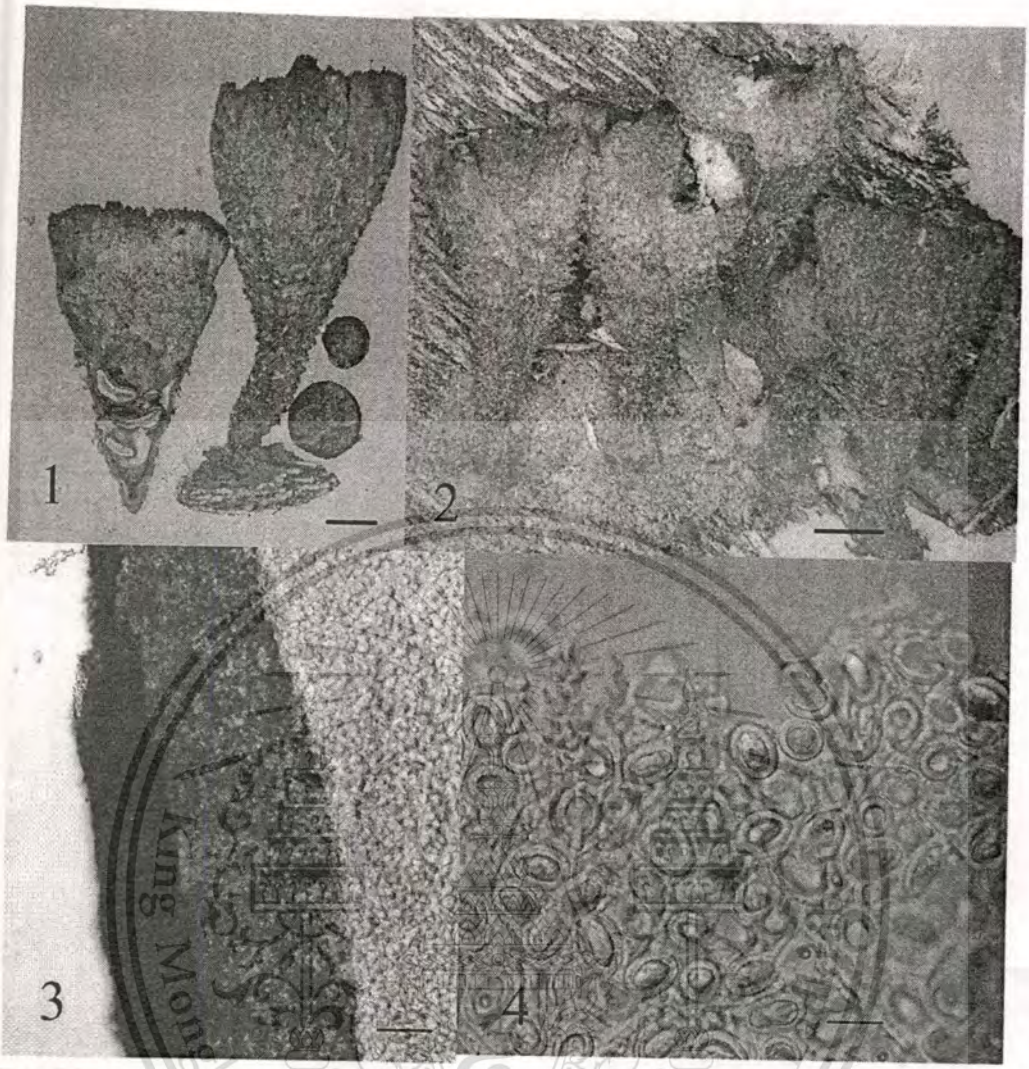

Fig. B.12. *Cyathus gracilis* 200380 (Holotype DAOM)

1-2. Fruiting bodies. 2. Section of peridiole, double cortex. 3. Basidiospores.

Scale bars: 1,2 = 1 mm; 2 = 60 µm; 3 = 20 µm

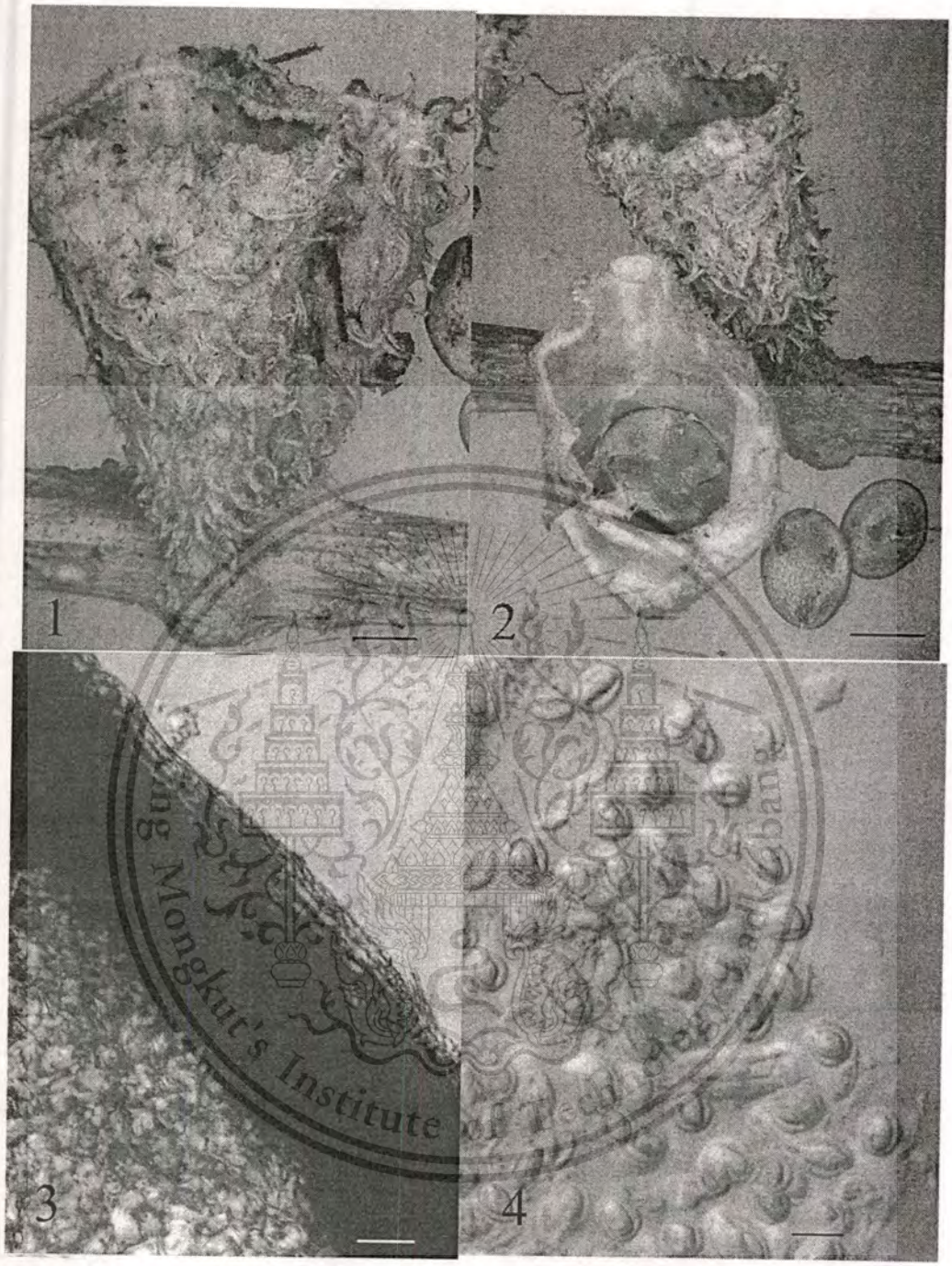

Fig. B.13. *Cyathus griseocarpus* 200396 (Holotype DAOM)

1. Fruiting bodies. 2. Show inner surface smooth or plicate. 3. Section of peridiole, single cortex with tunica.4. Basidiospores. Scale bars: 1 = 1 mm; 2 = 2 mm; 3 = 25 µm; 4 = 8 µm.

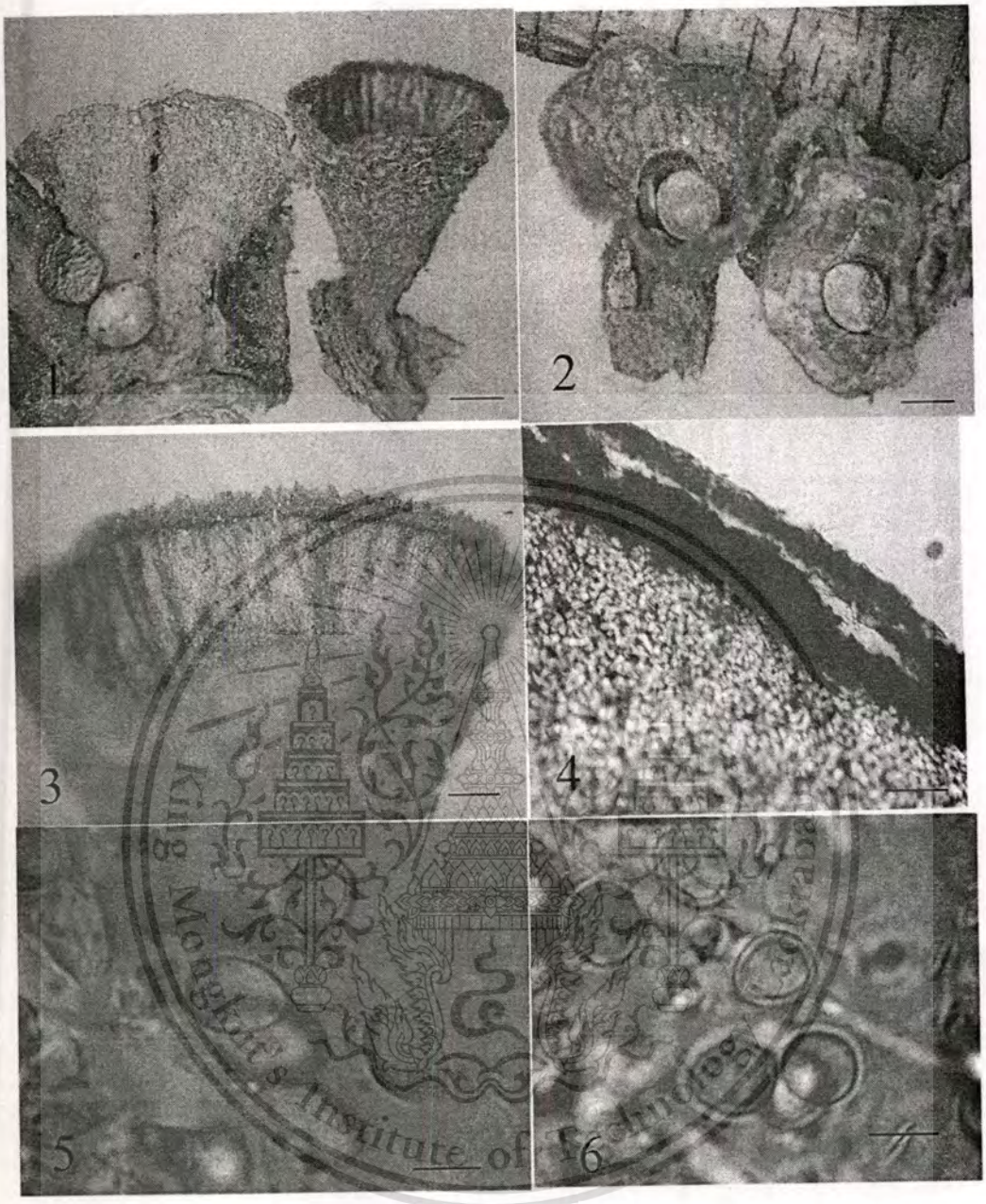

Fig. B.14. *Cyathus guandishanensis* 81896 (Holotype HMAS)

1. Fruiting bodies. 2. Show shallow plications on inner peridium. 3. Fimbriate.

4. Section of peridiole, single cortex with tunica. 5-6. Basidiospores. Scale bars:

1,2 = 2 mm; 3 = 0.5 mm; 4 = 20 µm; 5 = 10 µm; 6 = 8 µm.

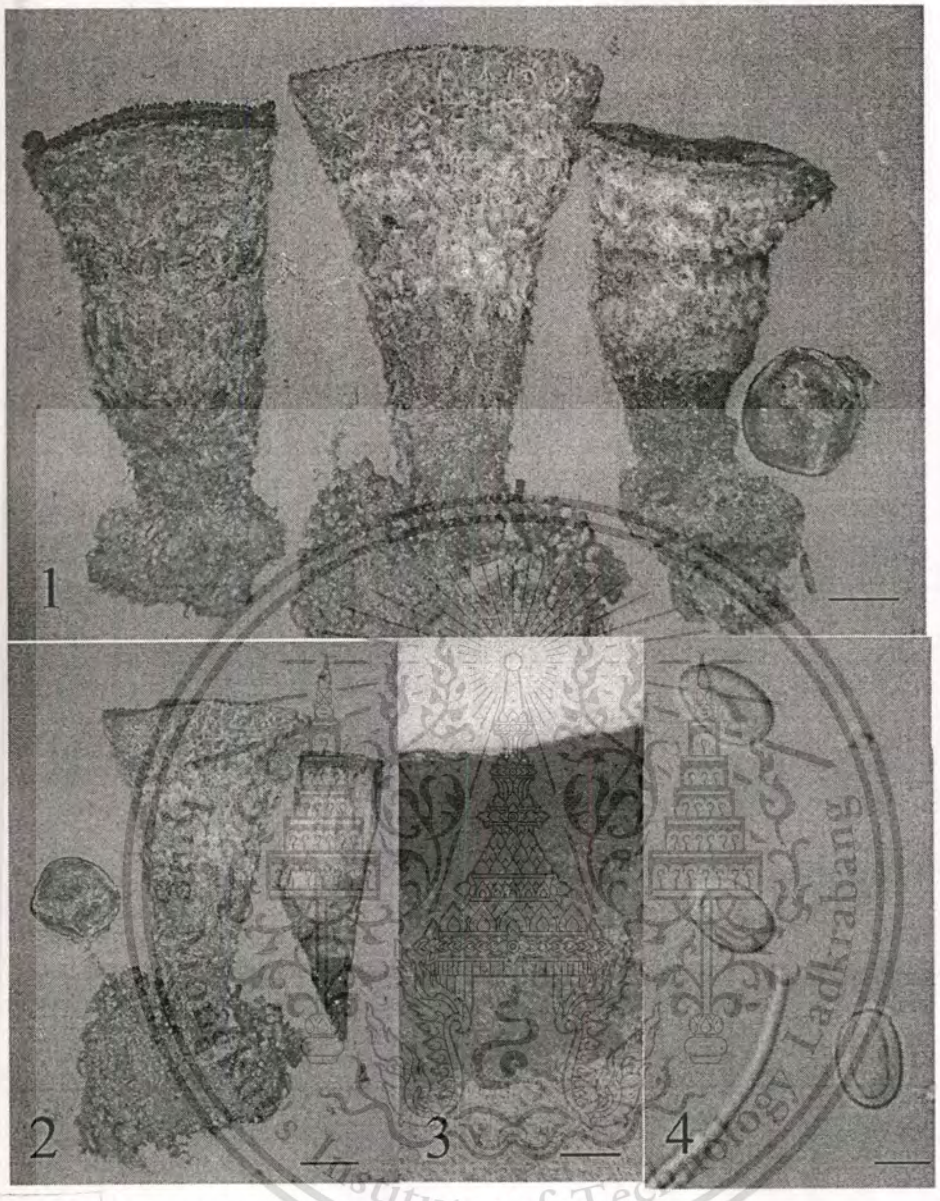

Fig. B.15. *Cyathus helenae* 200384 (Holotype DAOM)

1. Fruiting bodies. 2. Fruiting body and faint plications. 3. Section of peridiole, single cortex with tunica. 4. Basidiospores. Scale bars: 1= 1 mm; 2 = 1.5 mm; 3 = 20 µm; 4 = 10 µm.

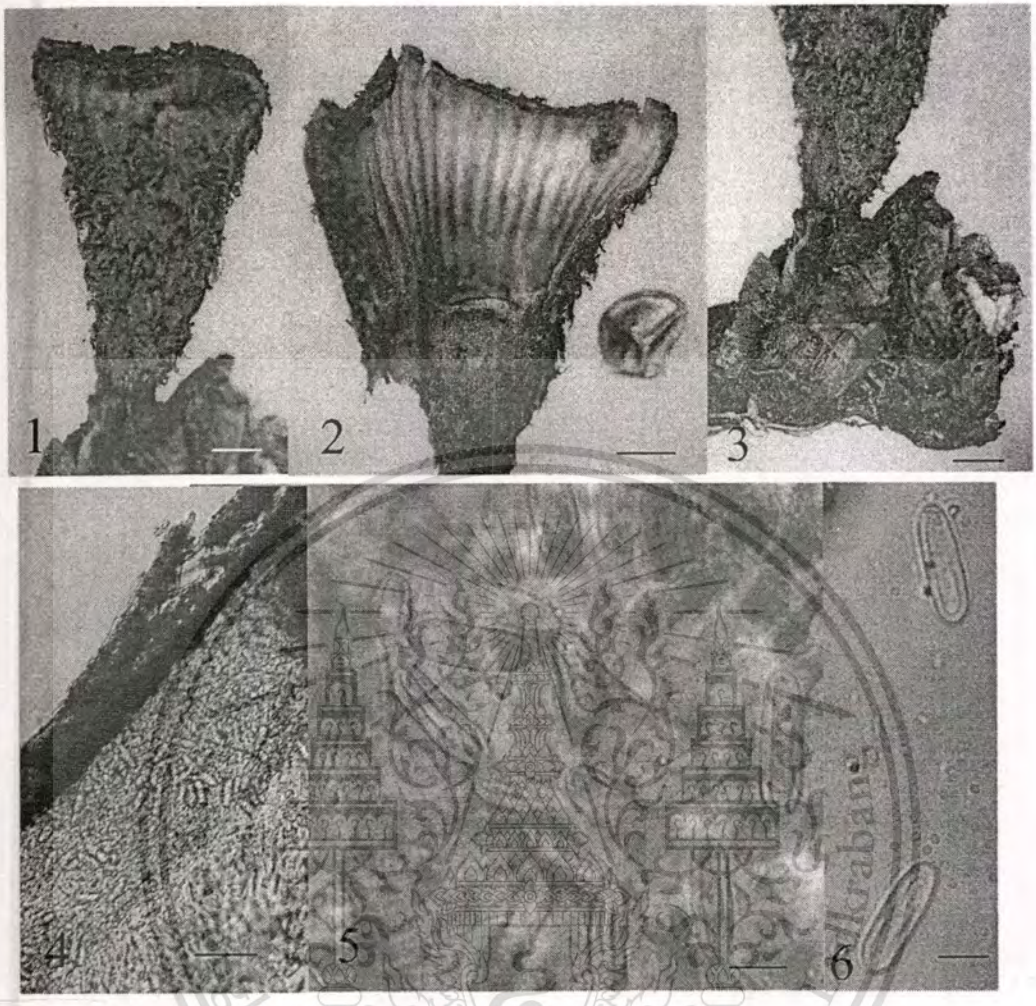

Fig. B.16. *Cyathus hirtulus* 59607 (Holotype HMAS)

1. Fruiting body. 2. Inner surface of peridium. 3. Attachment with substract.

4. Section of peridiole, single cortex with tunica. 5-6. Basidiospores. Scale bar:

1 = 0.6 mm; 2 = 1.5 mm; 3 = 1 mm; 4 = 20 µm; 5-6 = 10 µm.

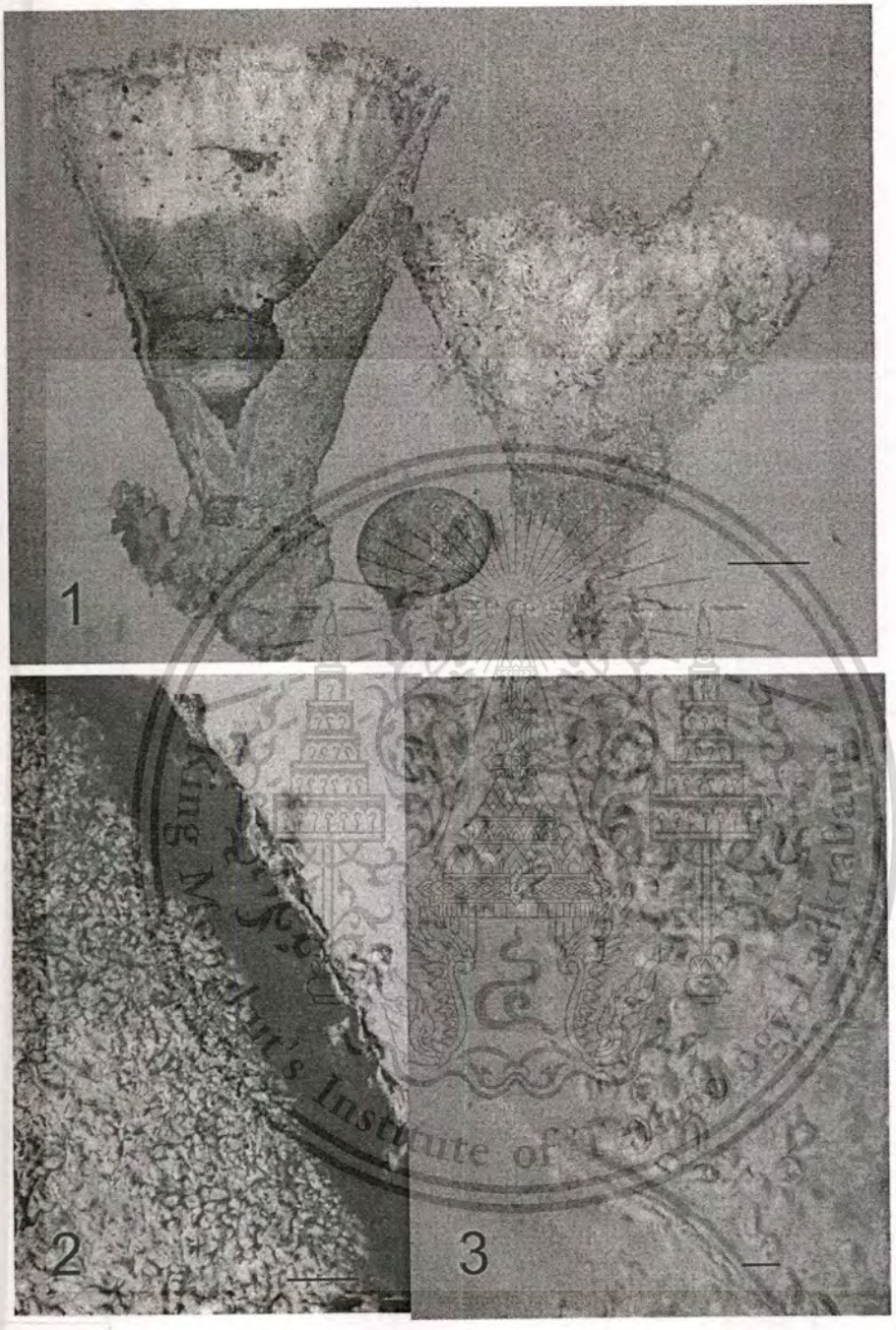

Fig. B.17. *Cyathus hookeri* 200435 (DAOM)

1. Fruiting bodies. 2. Section of peridioles, one cortex with tunica. 3 Basidiospores.

Scale bars: 1 = 1 mm; 2 = 20 µm; 3 = 6 µm.

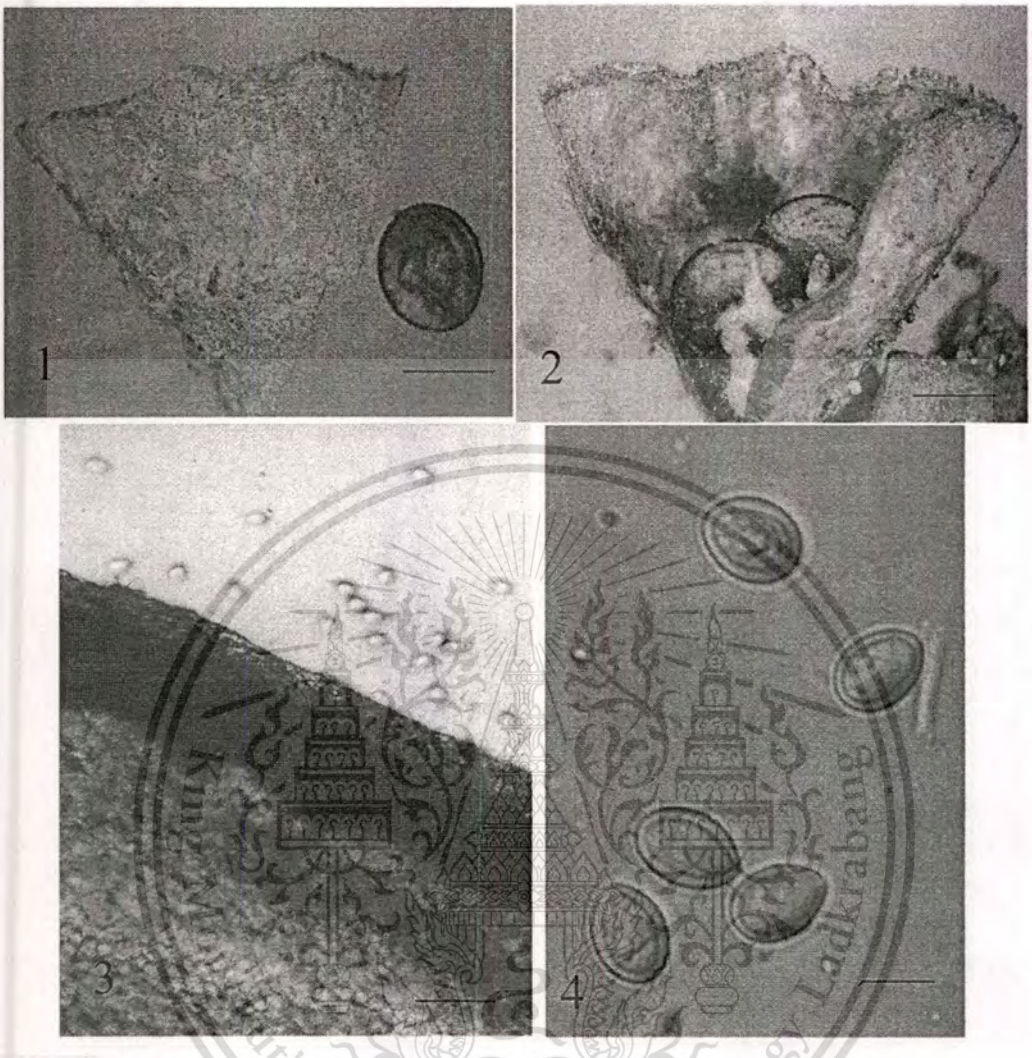

Fig. B.18. *Cyathus intermedius* 727153 (BPI)

1. Outside of fruiting body. 2. Inner of fruiting body.3. Section of peridiole, one cortex with tunica.4. Basidiospores. Scale bars: 1, 2 = 2 mm; 3 = 20 μm; 3 = 12 μm.

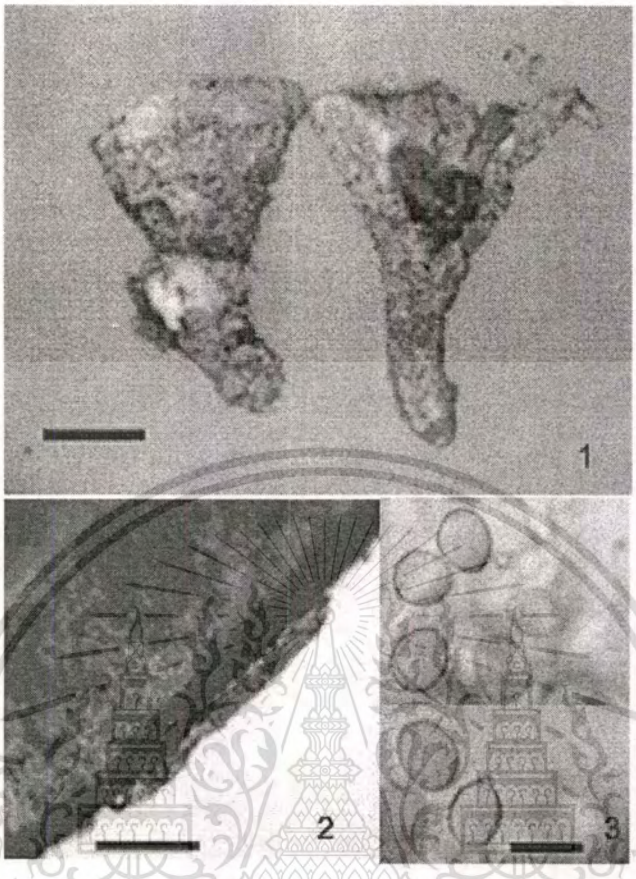

Fig. B.19. *Cyathus jiayuguanensis* 20802 (Holotype SWFC)

1 Fruiting Bodies. 2 Section of periduliole, one cortex with tunica.

3 Basidiospores. Bars: 1= 3 mm; 2 = 100 μm; 3 = 10 μm

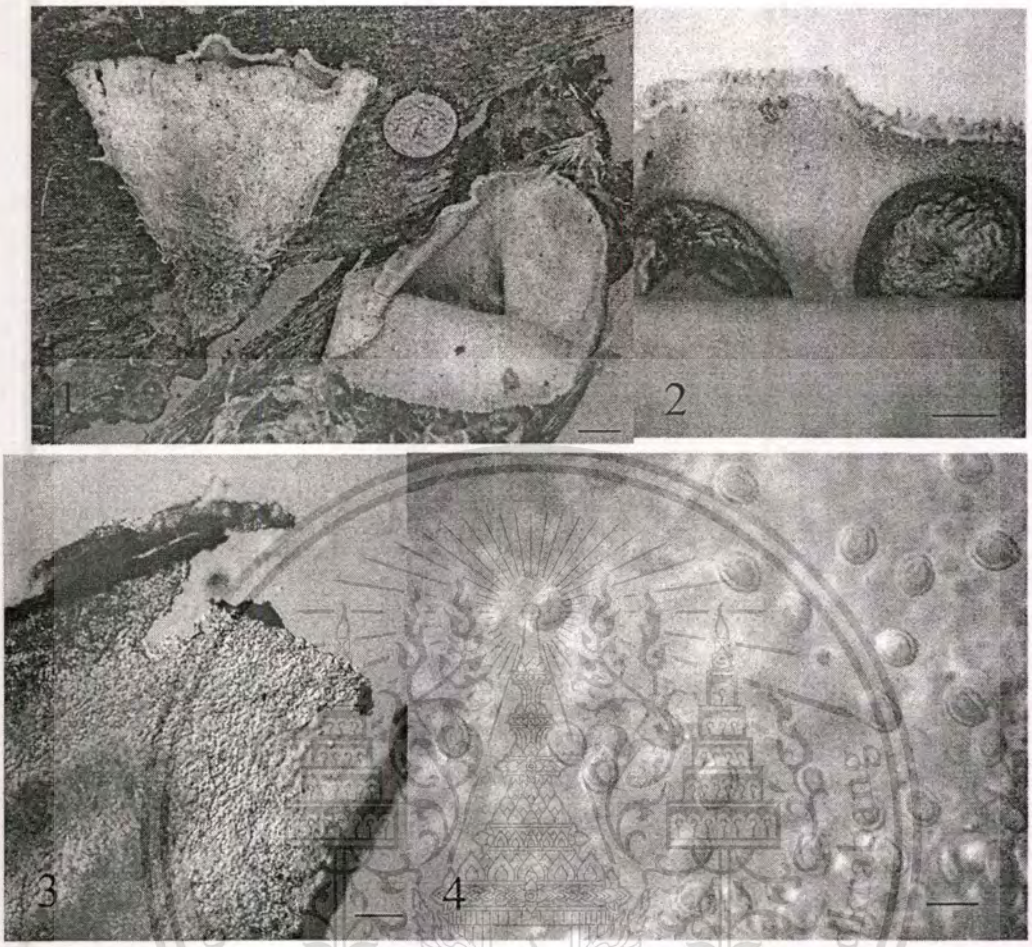

Fig. B.20. *Cyathus julietae* 727156 (BPI Holotype)

1. Fruiting bodies. 2. Lip of fruiting body. 3. Section of peridiole, one cortex with tunica. 4. Basidiospores. Scale bars: 1 = 1 mm; 2 = 2 mm; 3 = 50 µm; 4 = 1 µm.

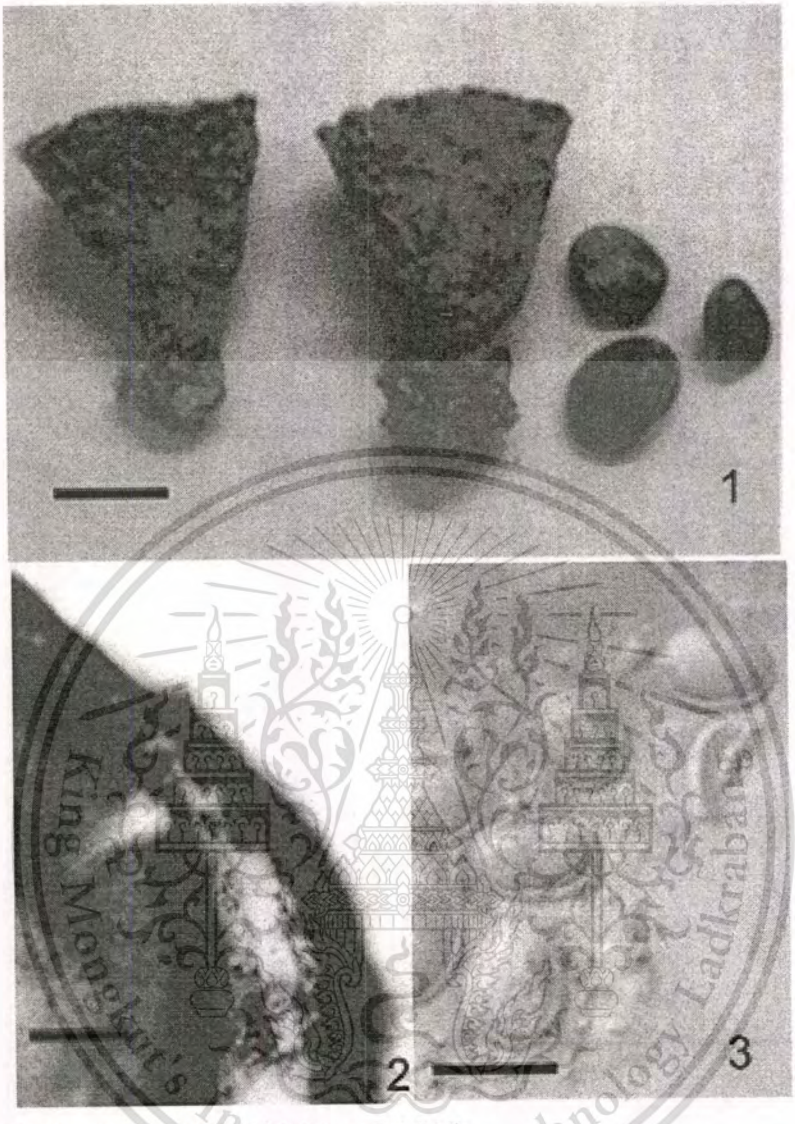

Fig. B.21. *Cyathus lanatus* 200703 (Holotype DAOM)

1 Fruiting Bodies. 2 Section of periduliole, one cortex with tunica. 3 Basidiospores.

Scale bars: 1 = 3 mm; 2 = 100 µm; 3 = 10 µm

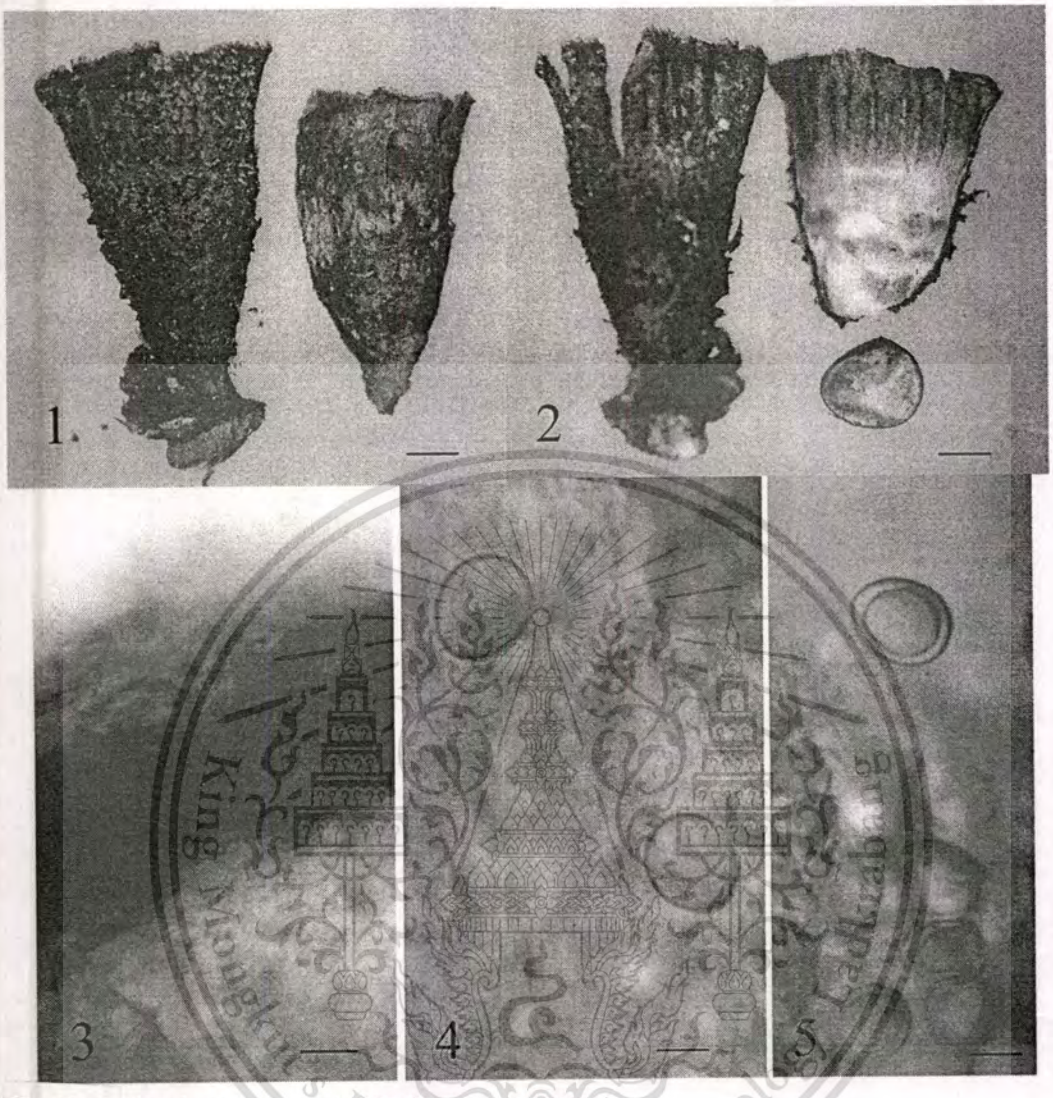

Fig. B.22. *Cyathus lijianensis* 21081 (Holotype SWFC).

1,2. Fruiting bodies. 2. Section of peridiole, one cortex with tunica. 3, 4. Basidiospores, thin and thick wall. Scale bars: 1, 2 = 2 mm; 3 = 15 μm; 4,5 = 10 μm.

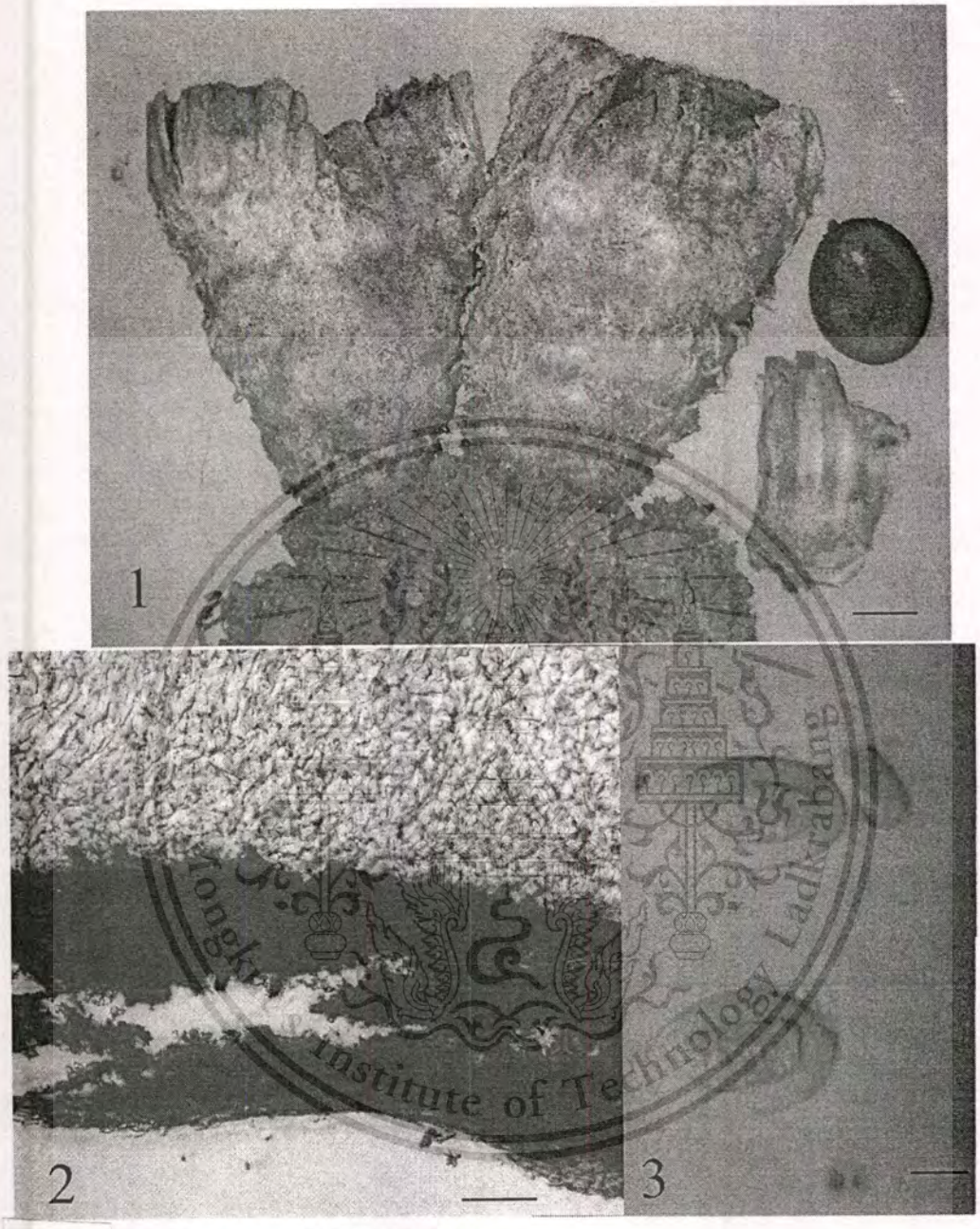

Fig. B.23. *Cyathus limbatus* 200496 (DAOM)

1. Fruiting bodies. 2. Section of peridiole, double cortex.3. Basidiospores.

Scale bars: 1 = 2 mm; 2 = 25 μm; 3 = 5 μm.

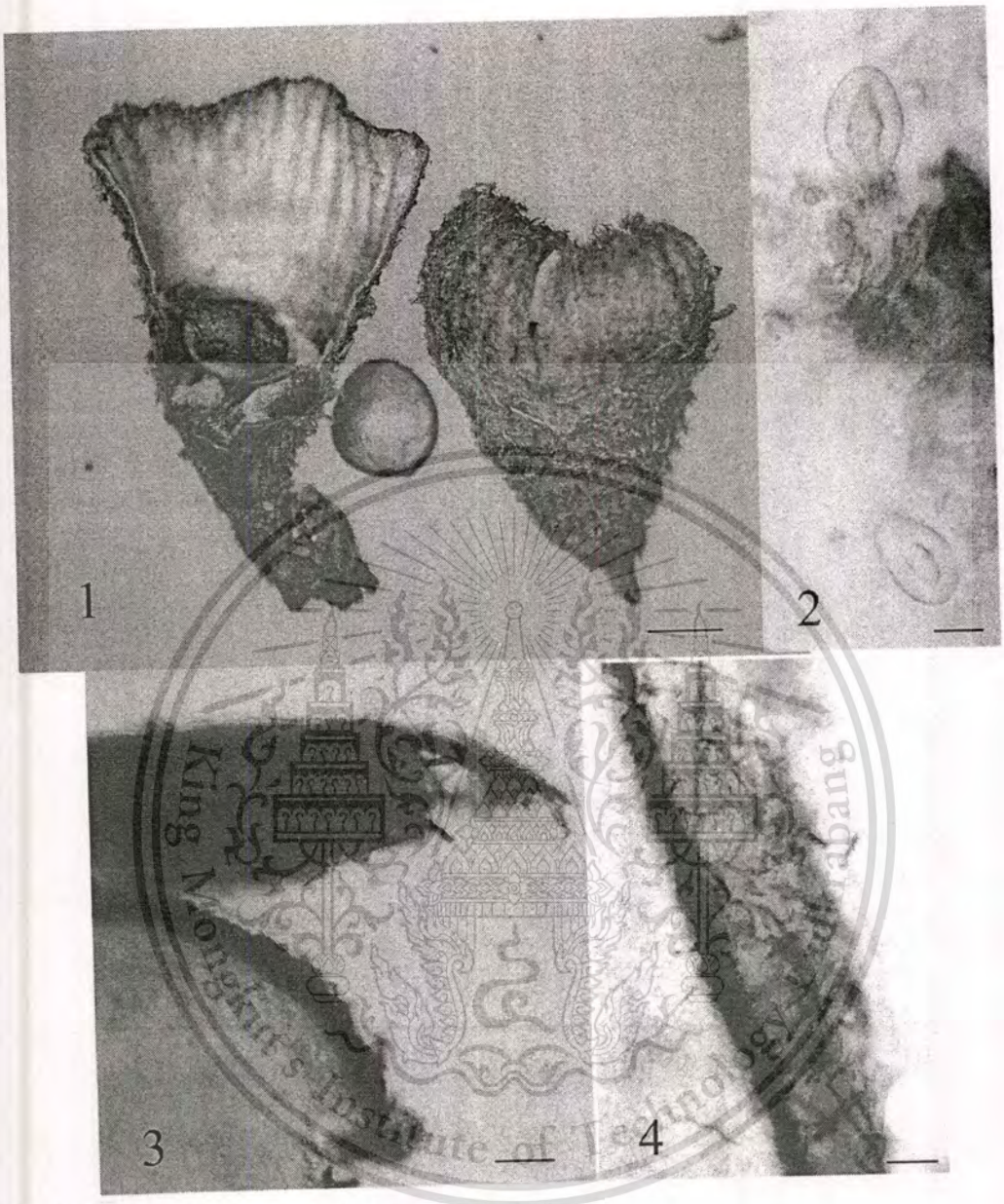

Fig. B.24 *Cyathus luxiensis* 21355 (Isotype SWFC)

1. Fruiting bodies. 2. Basidiospores. 3. Section of peridiole, double cortex with fragile tunica. 4. Structure of cortex. Scale bars: 1 = 2 mm; 2 = 8 μm; 3,4 = 35 μm.

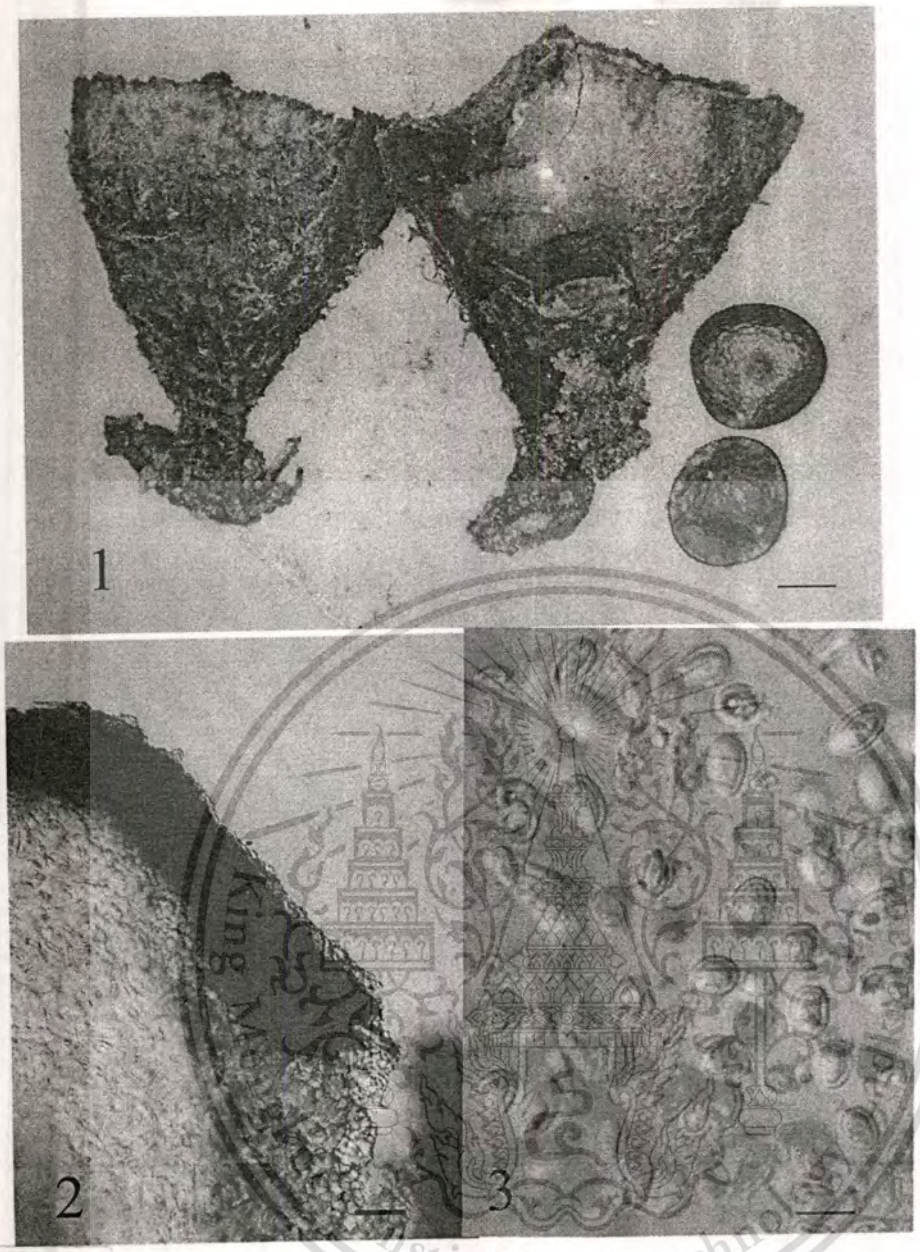

Fig. B.25. *Cyathus microsporus* 200592 (DAOM)

1. Fruiting bodies. 2. Section of peridiole, one thick cortex with fragile tunica.

3. Basidiospores. Scale bars: 1 = 1 mm; 2 = 15 µm; 3 = 6 µm.

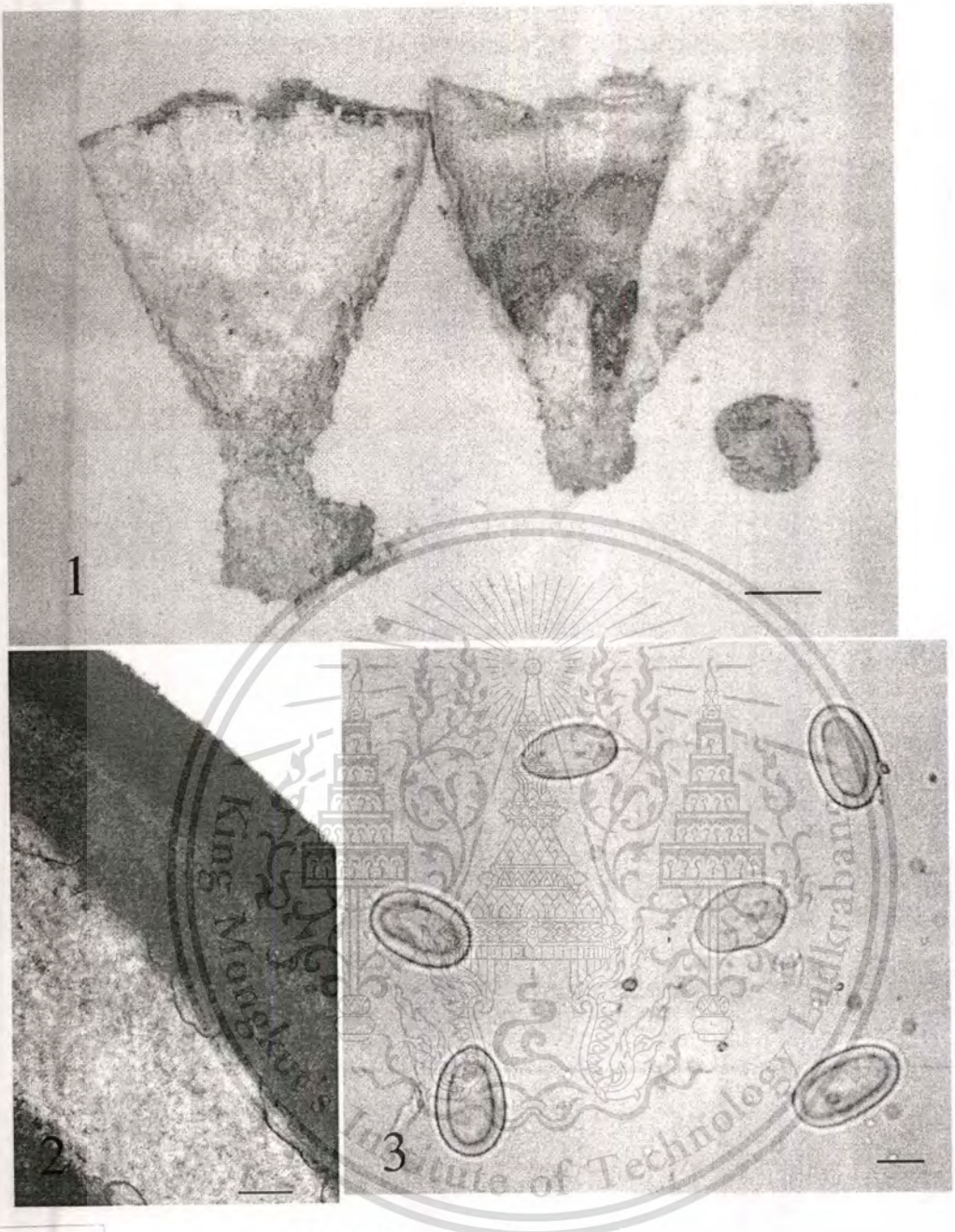

Fig. B.26. *Cyathus minimus* 703447 (Holotype BPI)

1. Fruiting bodies. 2. Section of peridiole, double cortex. 3. Basidiospores. Scale bars: 1 = 1 mm; 2 = 40 µm; 3 = 10 µm.

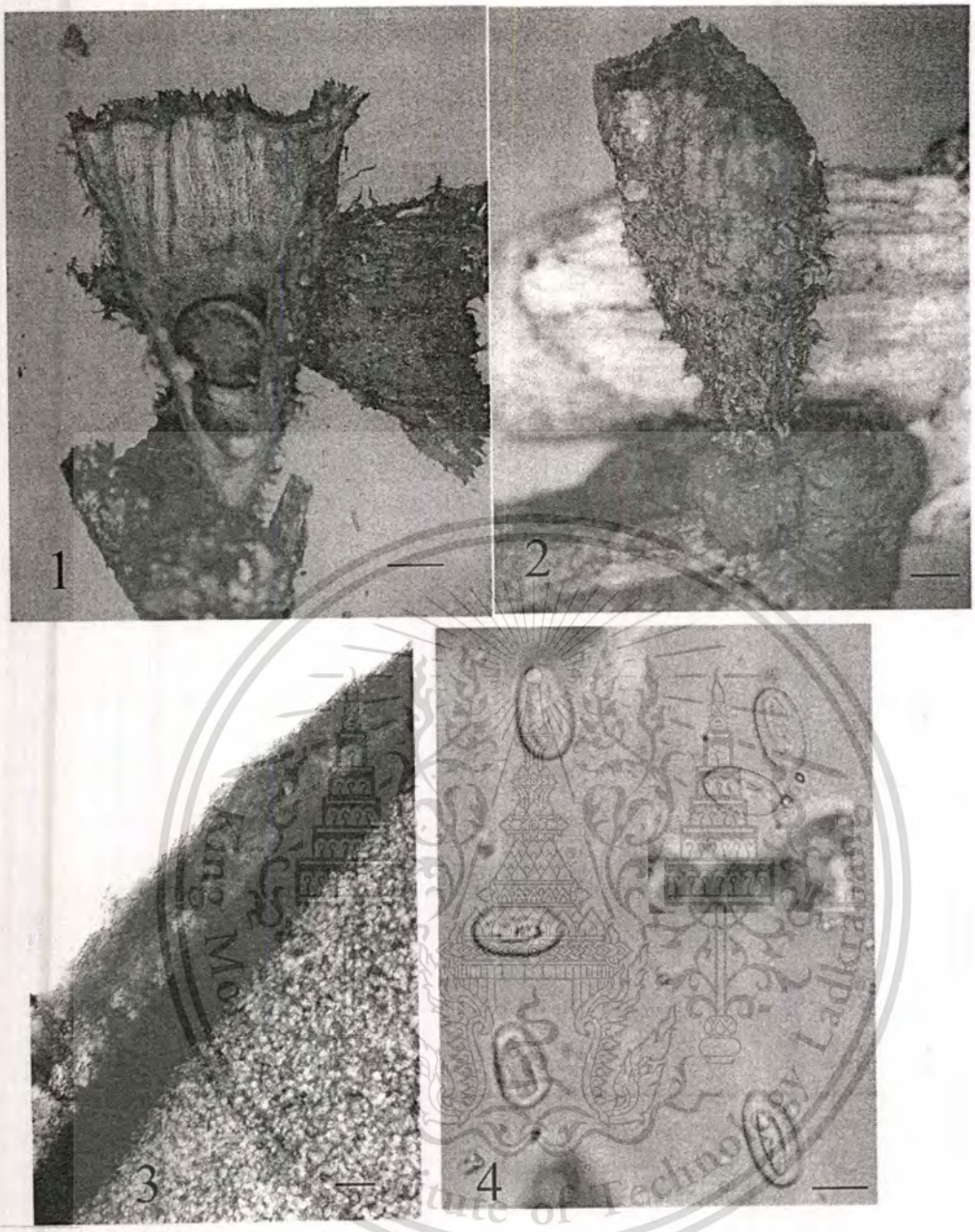

Fig. B.27. *Cyathus montagnei* 727179 (BPI)

1-2. Fruiting bodies. 3. Section of peridiole, single cortex with tunica. 4. Basidiospores.

Scale bars: 1-2 = 1.2 mm; 3 = 30 μm; 4 = 20 μm.

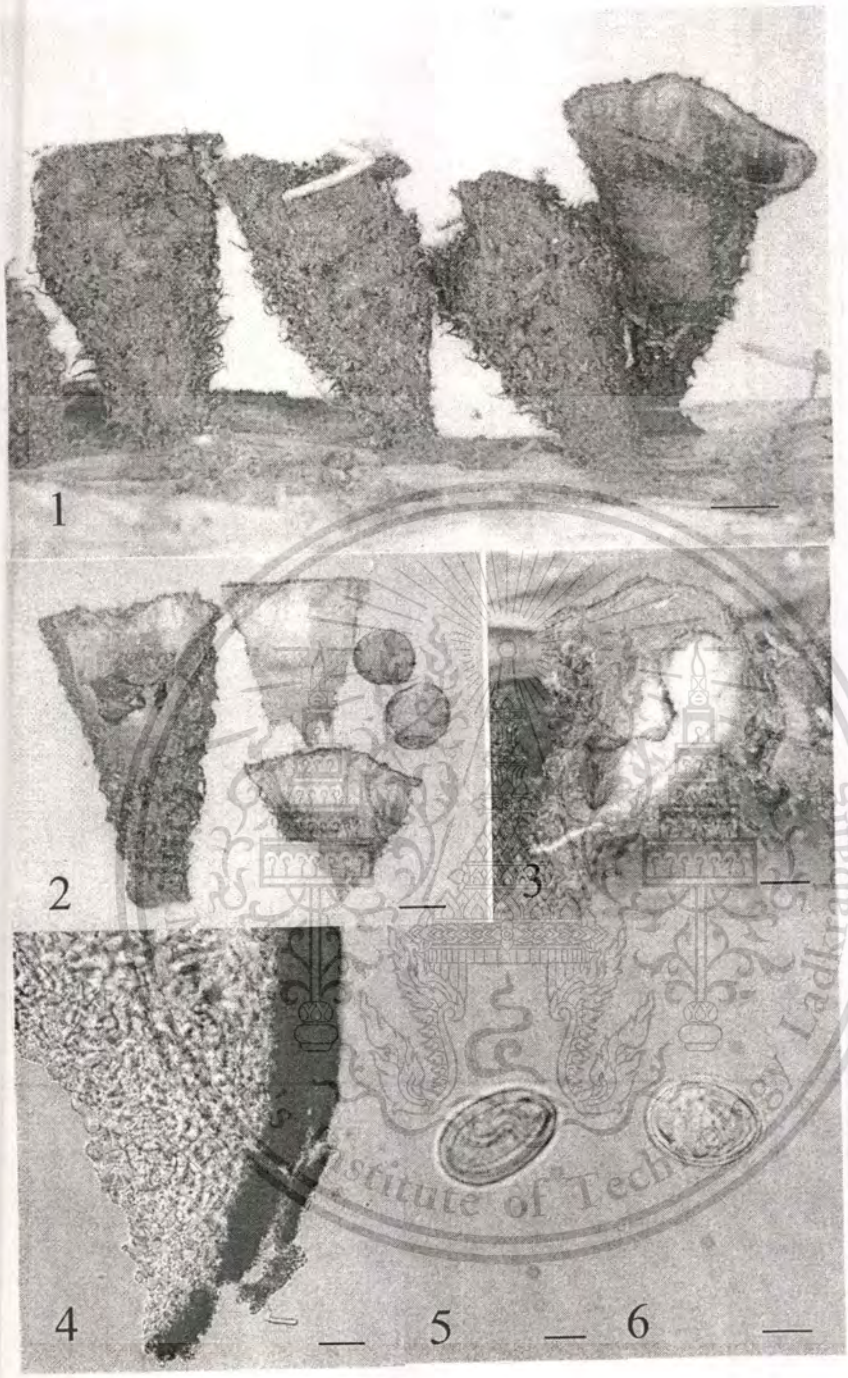

Fig. B.28. *Cyathus nigroalbus* 703968 (Holotype BPI)

1. Fruiting bodies. 2. Inner surface of peridium. 3. Top viem of fruiting bodies.

4. Section of peridiole. 5-6. Basidiospores. Scale bars: 1 = 1.3 mm; 2 = 1 mm;

3 = 0.8 mm; 4 = 20 μm; 5, 6 = 8 μm.

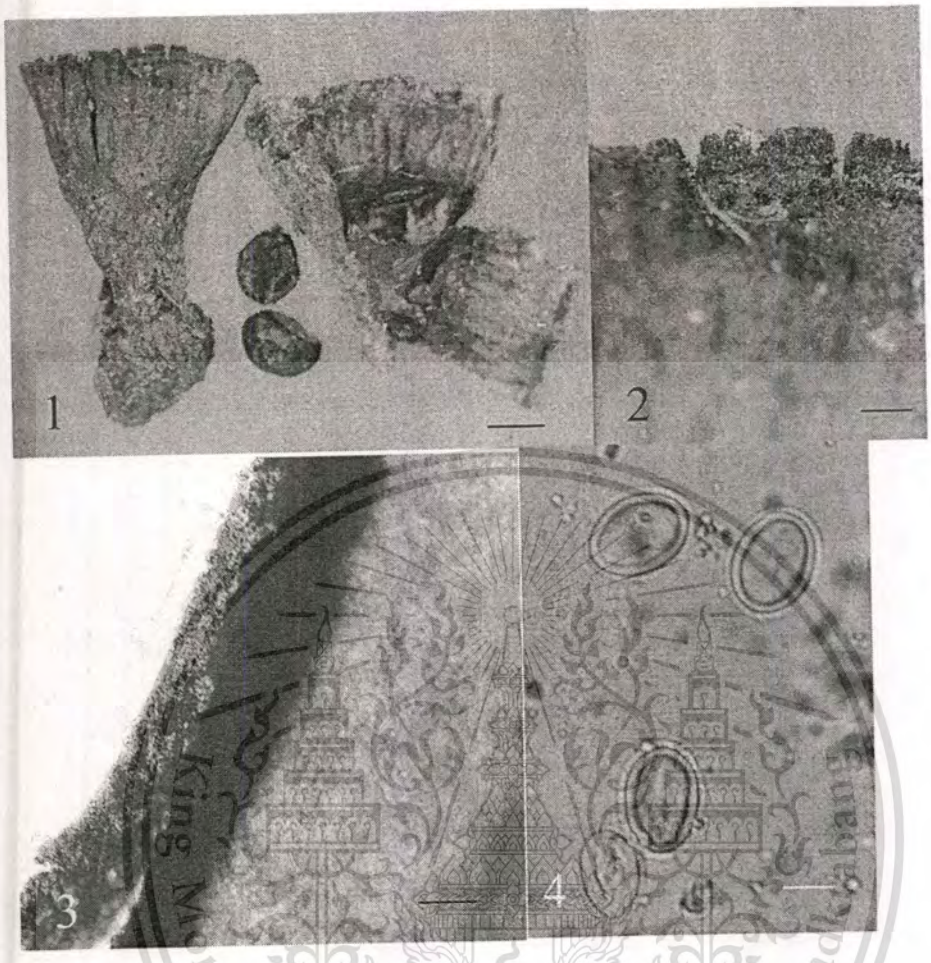

Fig. B.29. *Cyathus olivaceobrunneus* 01518 (Holotype HMAS).

1. Fruiting bodies. 2. Lip fimbriate. 3. Section of peridiole, single cortex with tunica.

4. Basidiospores. Scale bars: 1 = 1.3 mm; 2 = 0.5 mm; 3 = 30 μm; 4 = 10 μm.

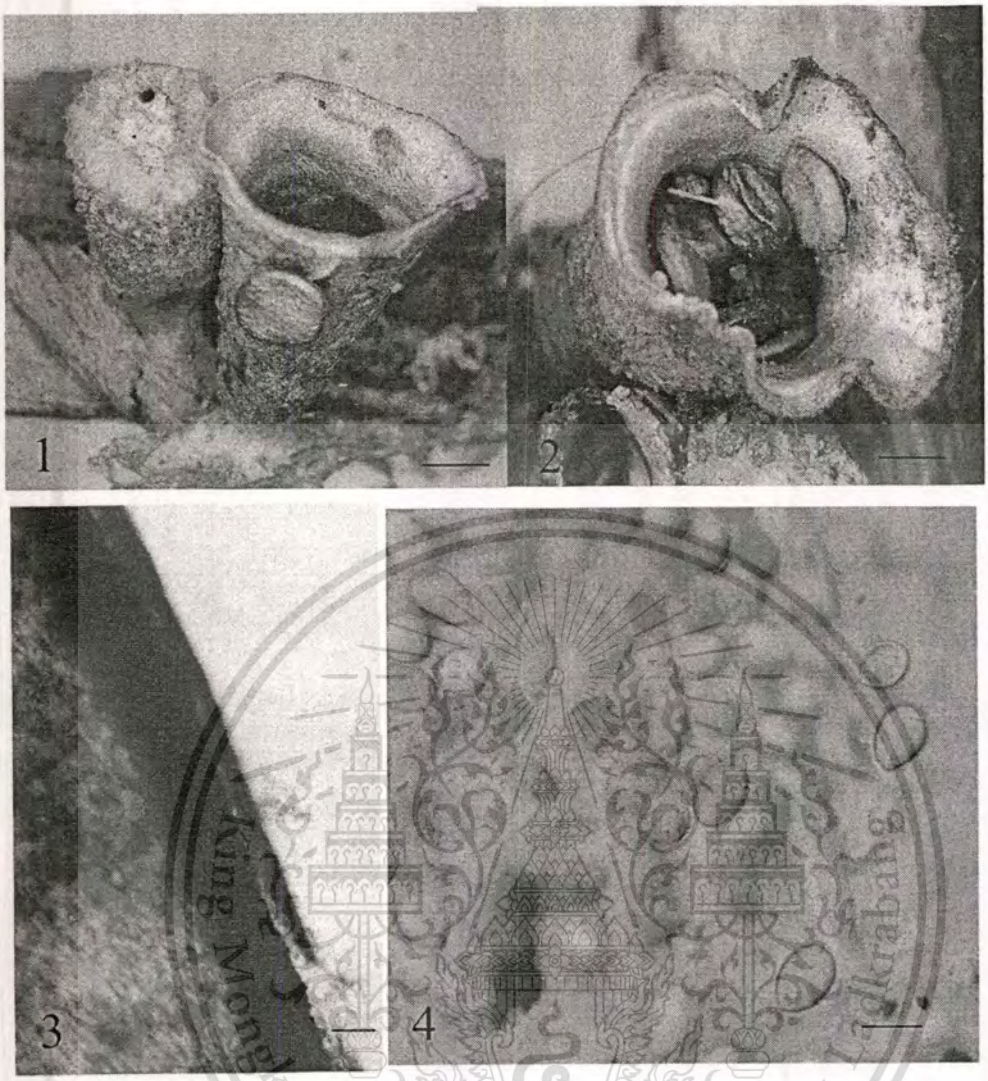

Fig. B.30. *Cyathus olla* 727227 (BPI).

1-2. Fruiting bodies. 3. Section of peridiole, one cortex with tunica.

4. Basidiospores. Scale bars: 1,2 = 1.8 mm; 3 = 40 µm; 4 = 9 µm.

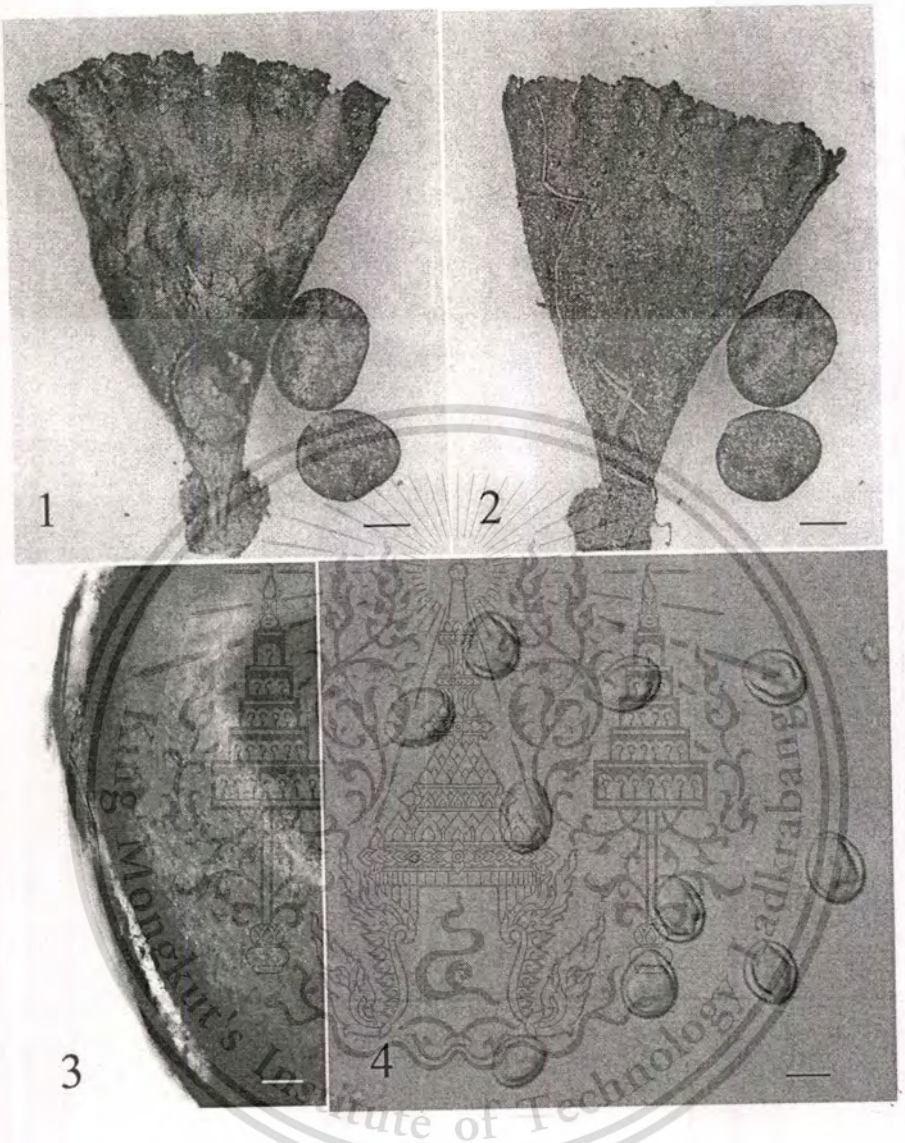

Fig. B.31. *Cyathus olla* f. *anglicus* 727225 (Holotype BPI).

1-2. Fruiting bodies. 2. Section of peridiole, single cortex with tunica. 4. Basidiospores.

Scale bars: 1,2 = 1 mm; 3 = 30 μm; 4 = 8 μm.

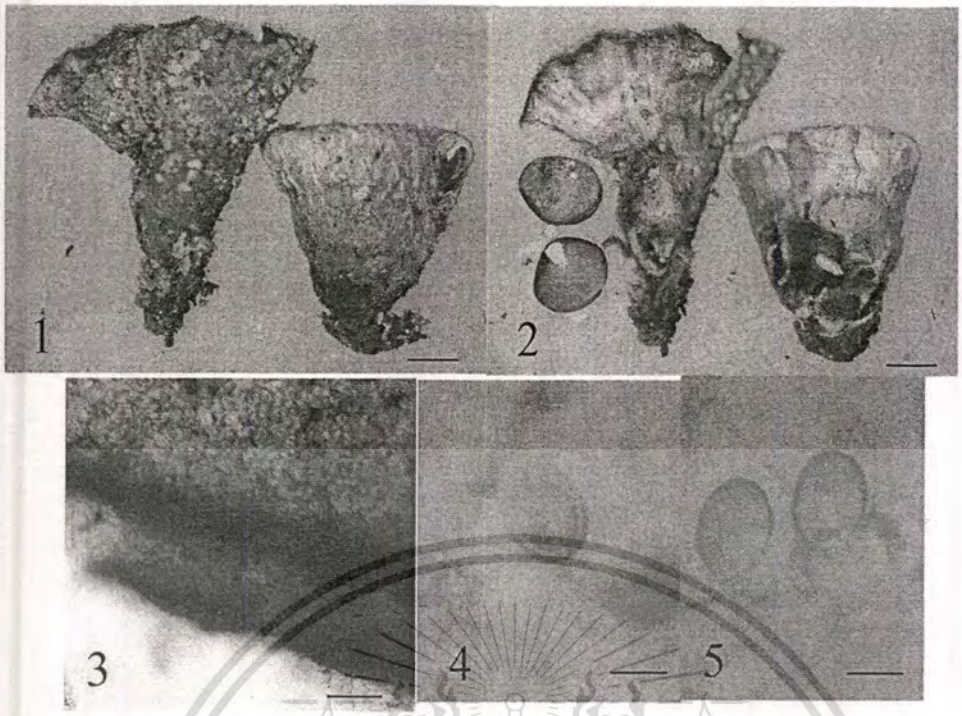

Fig. B.32. *Cyathus olla* f. *brodiensis* 21137 (SWFC)

1-2. fruiting bodies. 3. Section of peridiole, one cortex withtunica. 4-5. Basidiospores.

Scale bars: 1,2 = 1.8 mm; 3 = 30 μm; 4,5 = 6 μm.

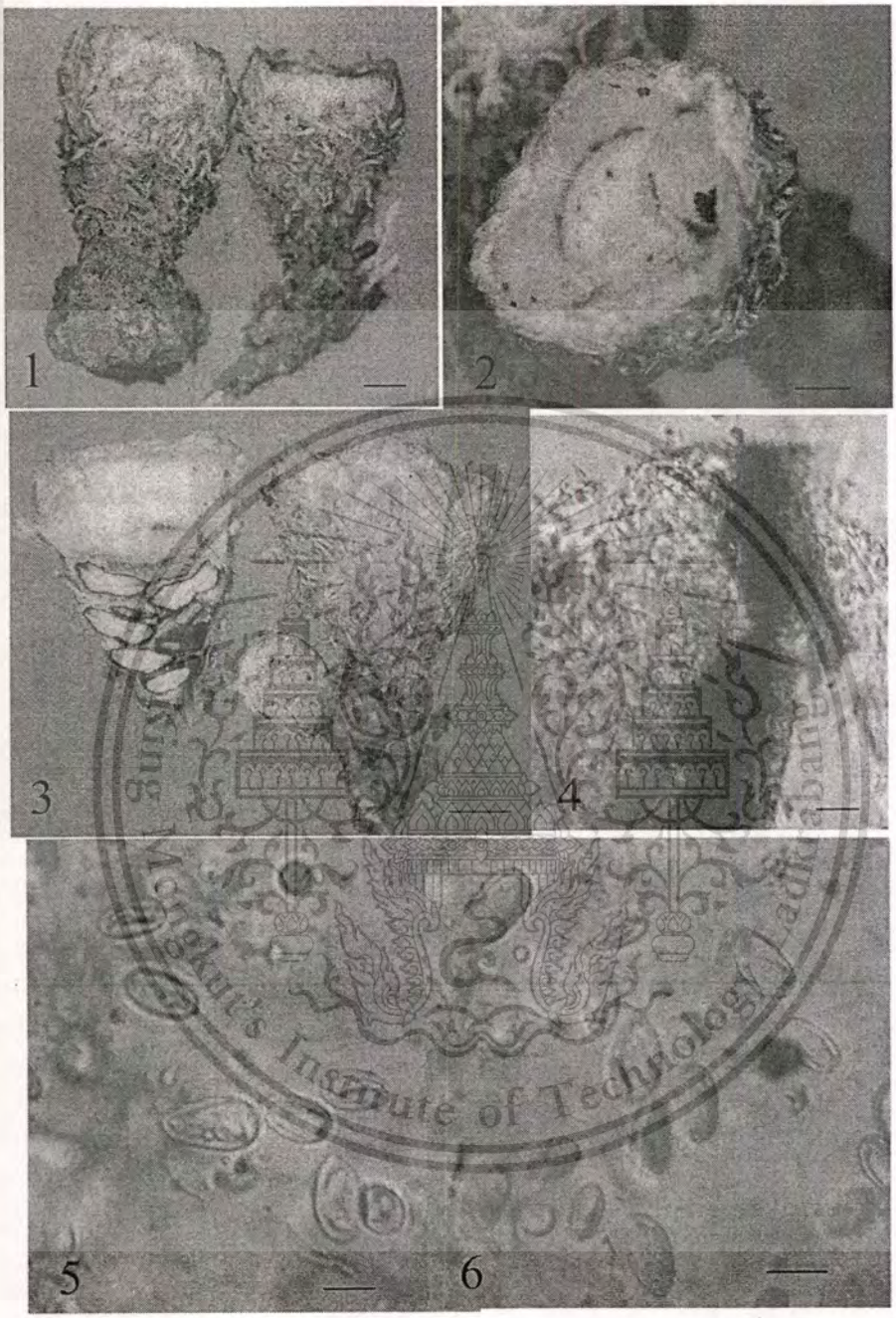

Fig. B.33. *Cyathus pallidus* 21160 (SWFC).

1-3. Fruiting bodies. 4. Section of peridiole, single cortex with tunica. 5-6. Basidiospores. Scale bars: 1 = 1.3 mm; 2 = 1 mm; 3 = 1.1 mm; 4 = 20 µm; 5,6 = 6 µm.

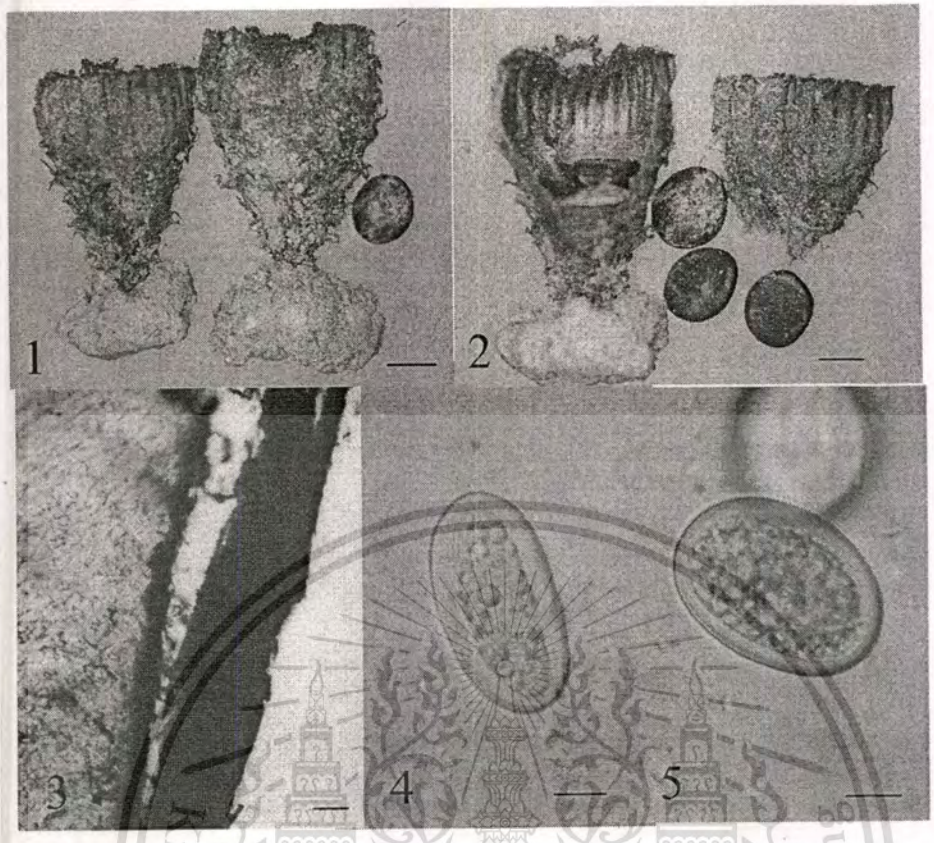

Fig. B.34. *Cyathus poeppigii* 21357 (SWFC).

1-2. Fruiting bodies. 2. Section of peridiole, double cortex. 3-4. Basidiospores.

Scale bars: 1,2 = 2 mm; 3 = 15μm; 4,5 = 7 μm.

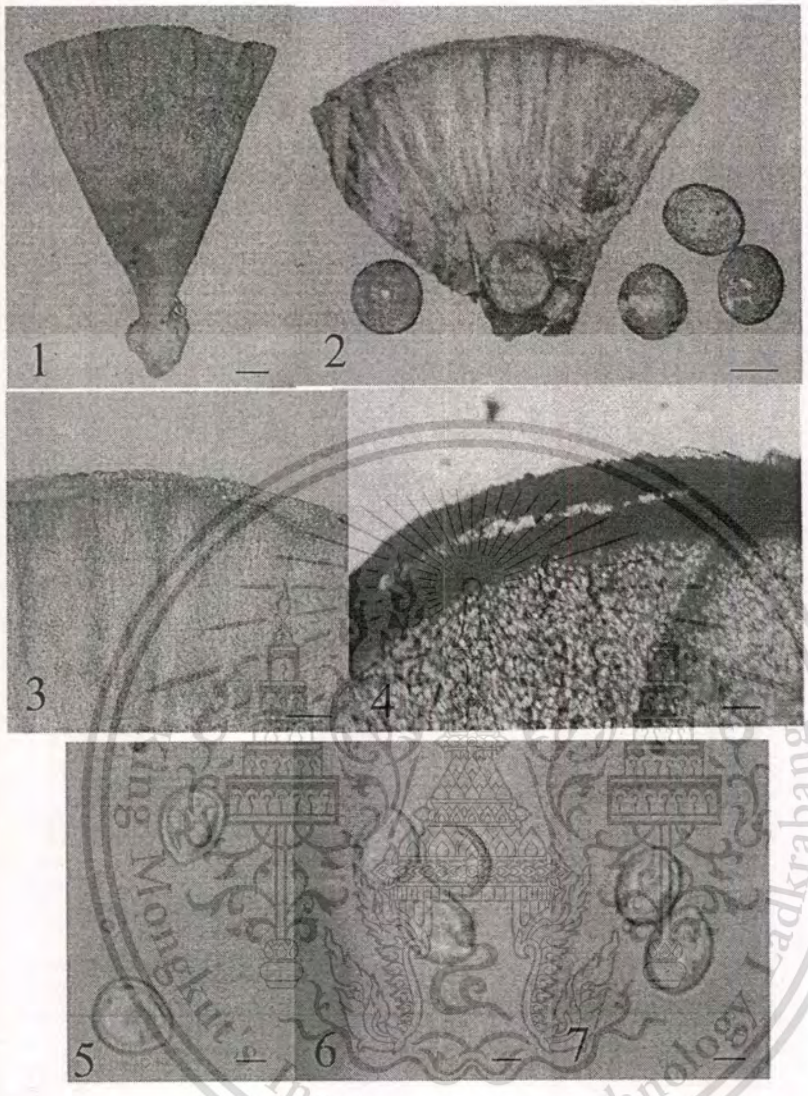

Fig. B.35. *Cyathus pullus* 01510 (Holotype HMAS).

1-2, Fruiting bodies. 3. Smooth lip. 4. Section of peridiole,single cortex with tunica.

5-7. Basidiospores. Scale bars:1 = 1.6 mm; 2 = 2 mm; 3 = 0.8 mm; 4 = 20 µm; 5-7 = 5 µm.

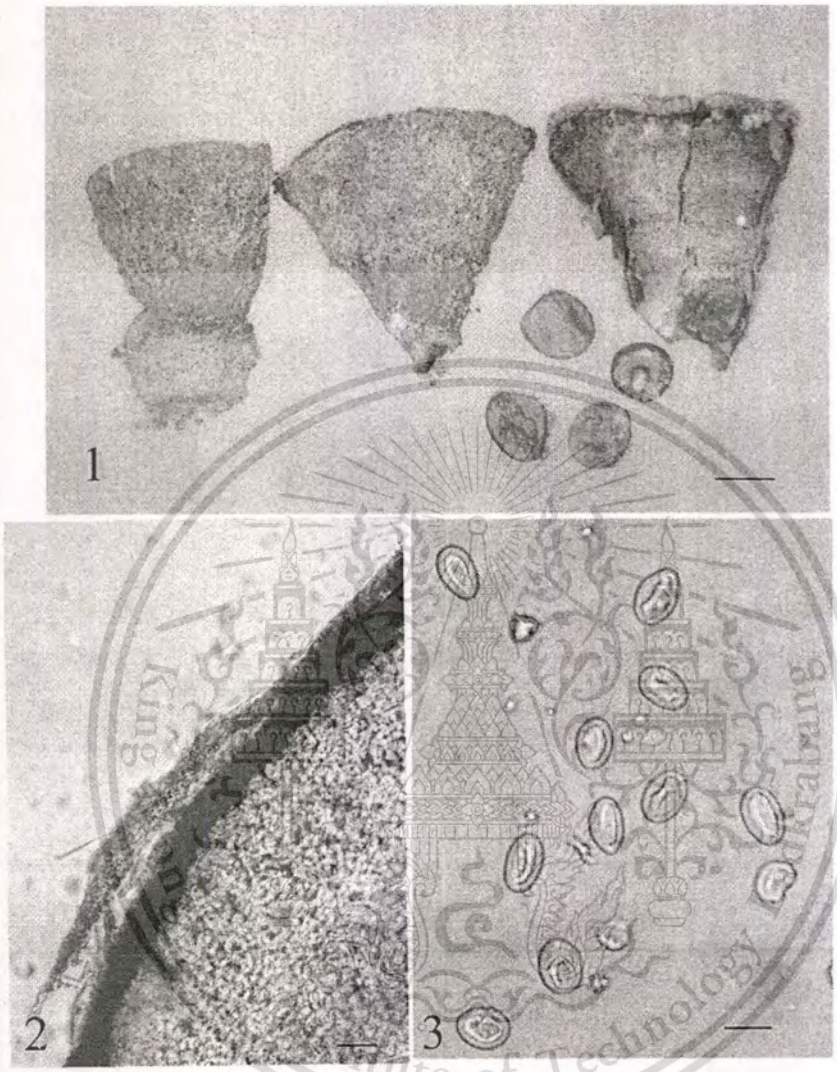

Fig. B.36. *Cyathus pygmaeus* 703514 (Holotype BPI).

1. Fruiting bodies. 2. Section of peridiole, one cortex with tunica. 3. Basidiospores.

Scale bars: 1 = 1.4 mm; 2 = 20 µm; 3 = 12 µm.

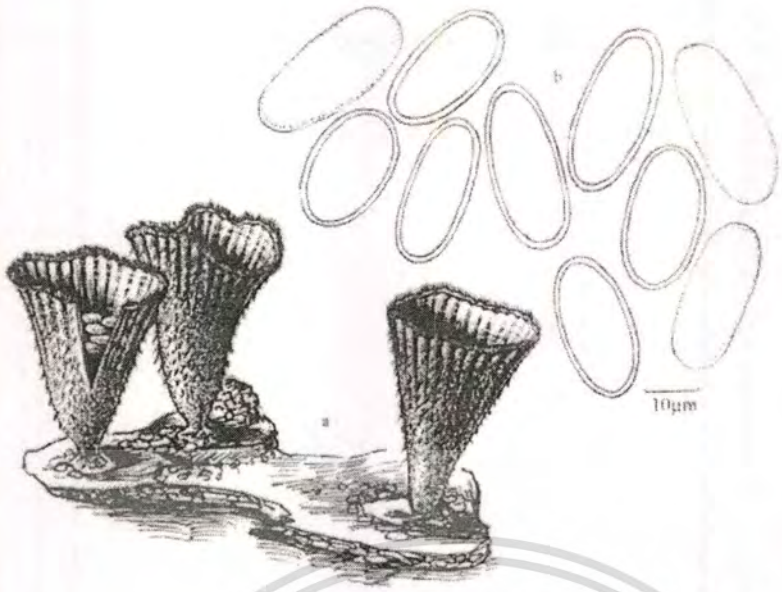

Fig. B.37. *Cyathus renweii.* a. basidiocarps. b. basidiospores (Drawing by Li Nan)

Fig. B.38. *Cyathus setosus* 200815 (DAOM).

1. Fruiting bodies. 2. Section of peridiole, double cortex.3. Basidiospores.

Scale bars: 1 = 1.2 mm; 2 = 50 μm ; 3 = 10 μm .

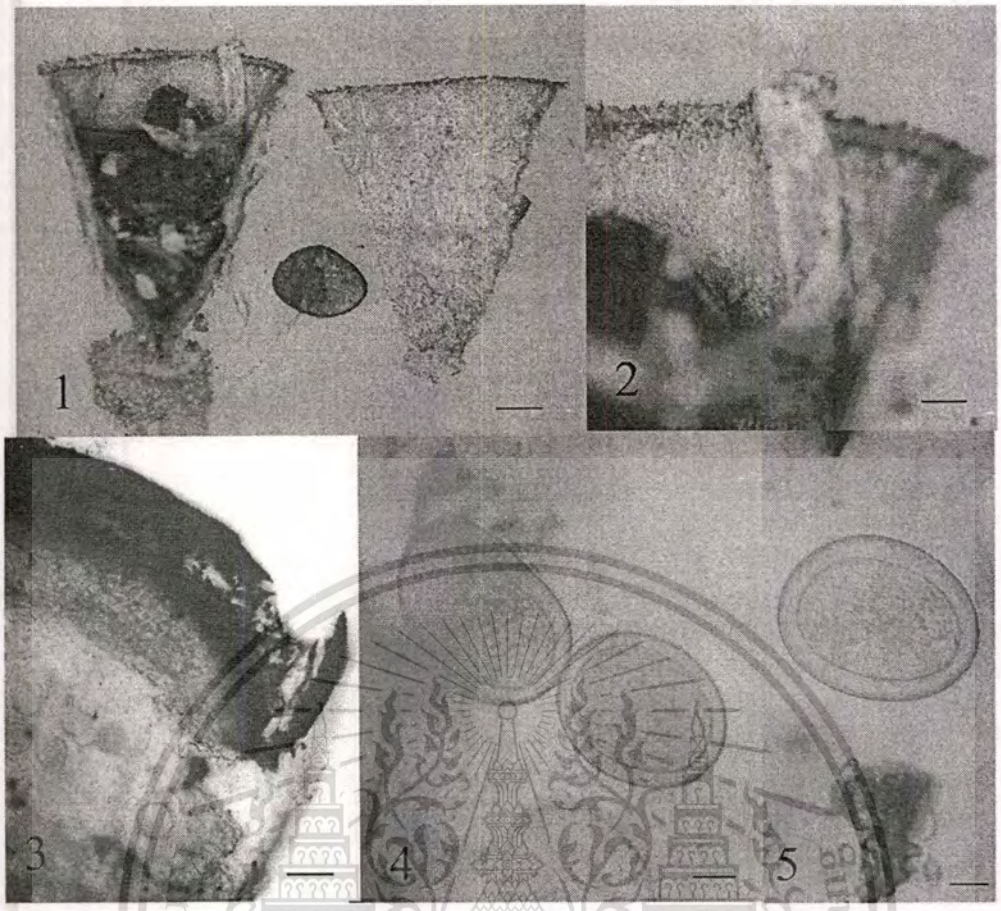

Fig. B.39. *Cyathus stercoceus* 21140 (SWFC)

1. Fruiting bodies. 2 Lip of fruiting body. 3. Section of peridiole, double cortex. 4-5. Basidiospores. Scale bars: 1 = 1.4 mm; 2 = 0.5 mm; 3 = 50 µm; 4,5 = 5 µm.

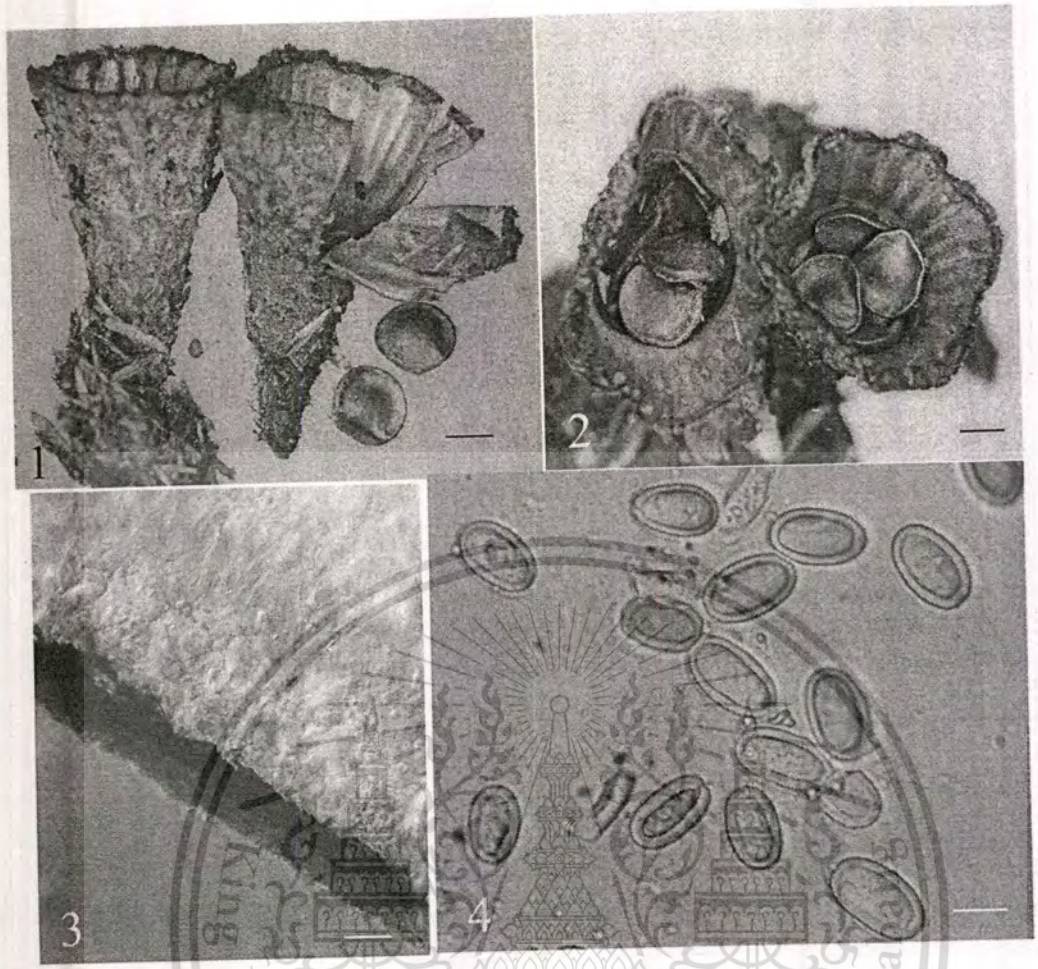

Fig. B.40. *Cyathus striatus* 727641 (BPI)

1. Fruiting bodies. 2. Top view of fruiting bodies. 3. Section of peridiole, single cortex with tunica. 4. Basidiospores. Scale bars: 1 = 1.5 mm; 2 = 1 mm; 3 = 15 µm; 4 = 8 µm.

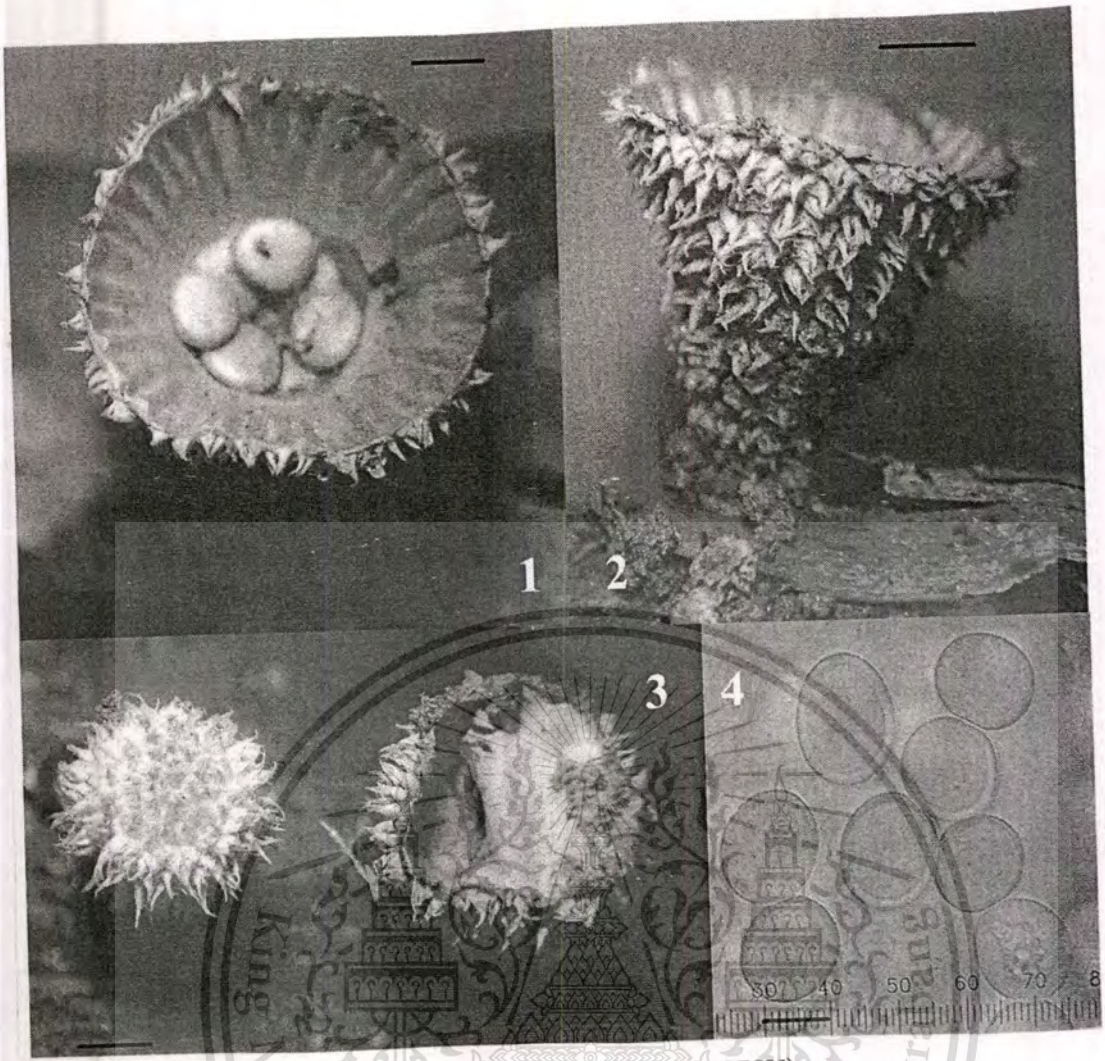

Fig. B.41. *Cyathus subglobosporum* sp. nov. zrl c013 (Holotype SFSU)

1. Fruiting body in top view showing peridioles and plications on the inner surface of peridium. 2. Fruiting body in side view showing shaggy hairs. 3. Opening changes with the development of fruiting bodies. 4. Basidiospores. Scale bars: 1 = 1.5 mm; 2 = 1.8 mm; 3 = 2 mm and 4 = 11 µm.

Fig. B.42. *Cyathus tianshanensis* 21157 (SWFC)

1-2. Fruiting bodies. 3. Section of peridiole, single cortex with tunica. 4-5. Basidiospores. Scale bars: 1 = 1.4 mm; 2 = 1.3 mm; 3 = 80 µm; 4,5 = 8 µm;

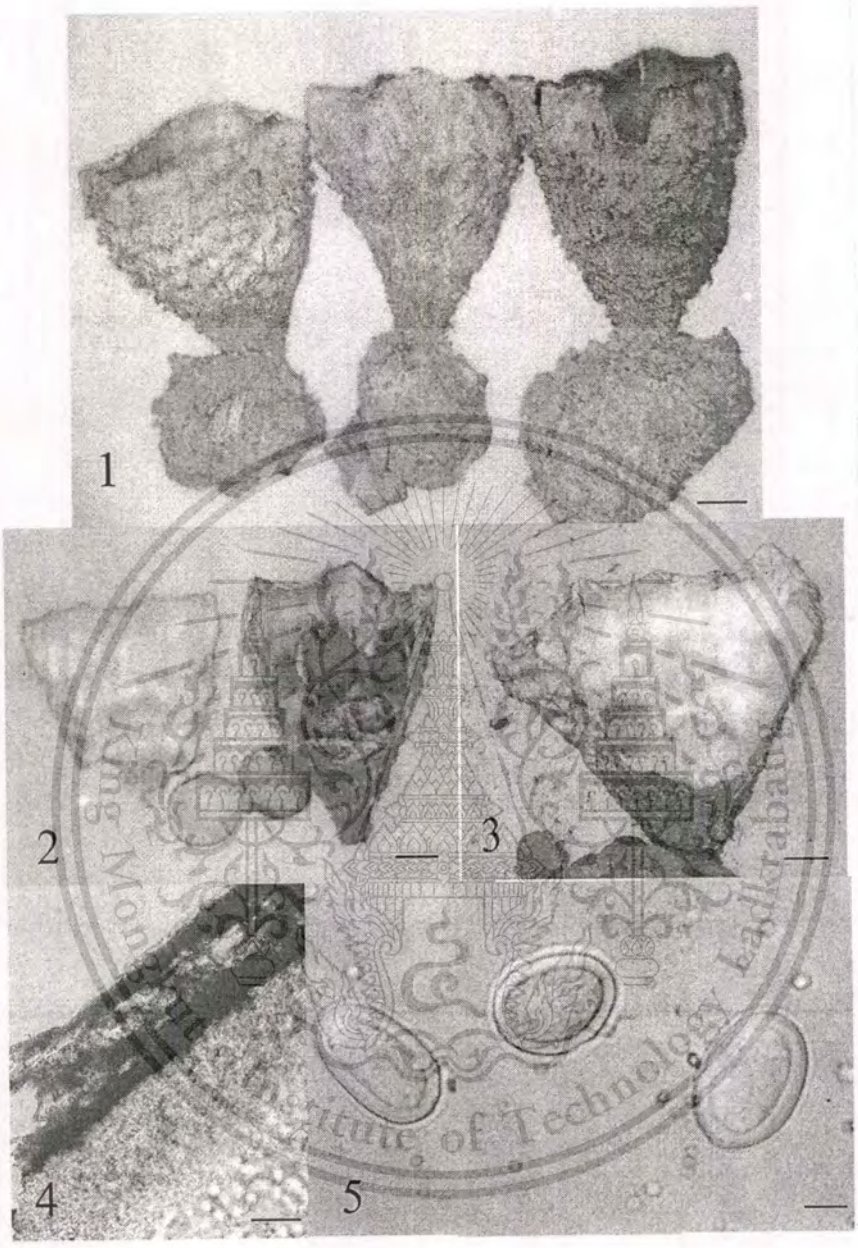

Fig. B.43. *Cyathus triplex* 703992 (Holotype BPI)

1. Fruiting bodies. 2. Inner surface of peridium. 3. Section of peridiole, double cortex.

5. Basidiospores. Scale bars: 1,2 = 1 mm; 3 = 0.8 mm; 4 = 40 µm; 5 = 5 µm.

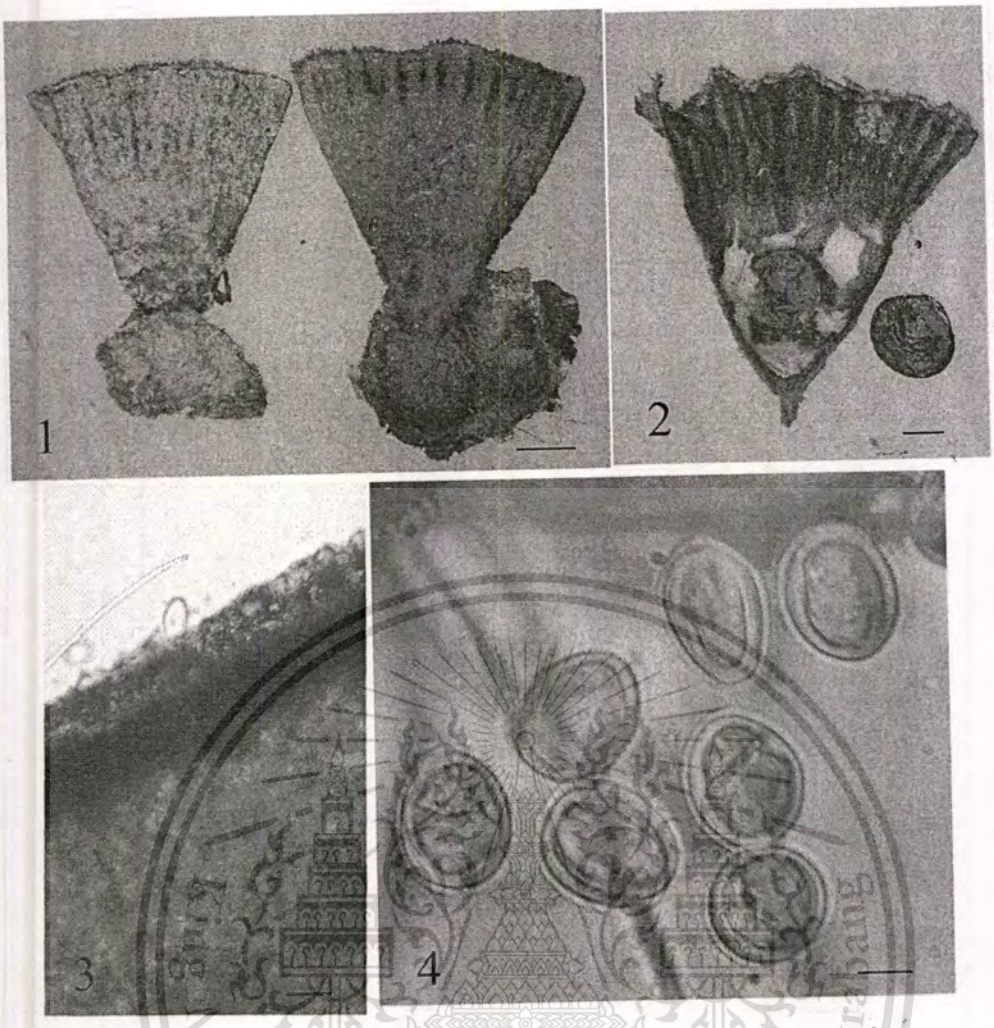

Fig. B.44. *Cyathus yunnanensis* 17373 (Holotype HMAS).

1. Fruiting bodies. 2. Vertical section of bruiting bodies.3. Section of peridiole, single cortex with tunica. 4. Basidiospores. Scale bars: 1 = 1.2 mm; 2 = 0.8 mm; 3 = 25 μm; 4 = 9 μm.

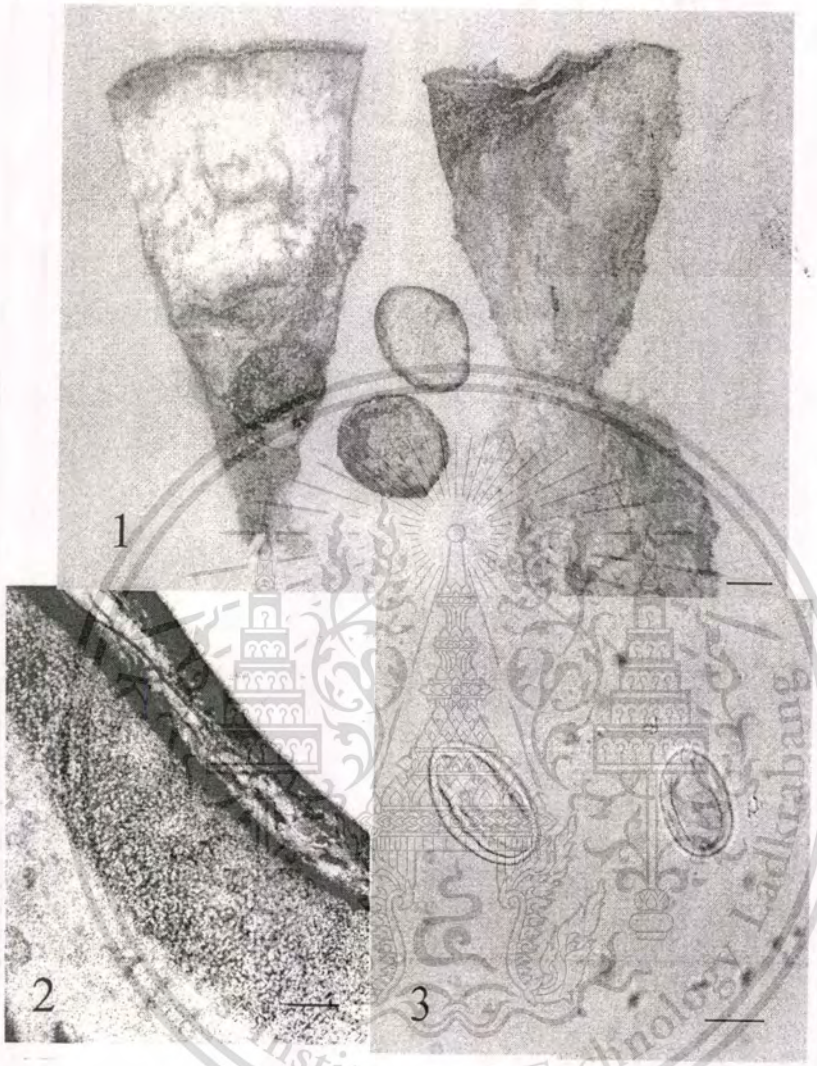

Fig. B.45. *Cyathus affinis* 703390 (Holotype BPI).

1. Fruiting bodies. 2 Section of peridioles, double cortex. 3. Basidiospores.

Scale bar: 1 = 1.3 mm; 2 = 80 µm; 3 = 12 µm.

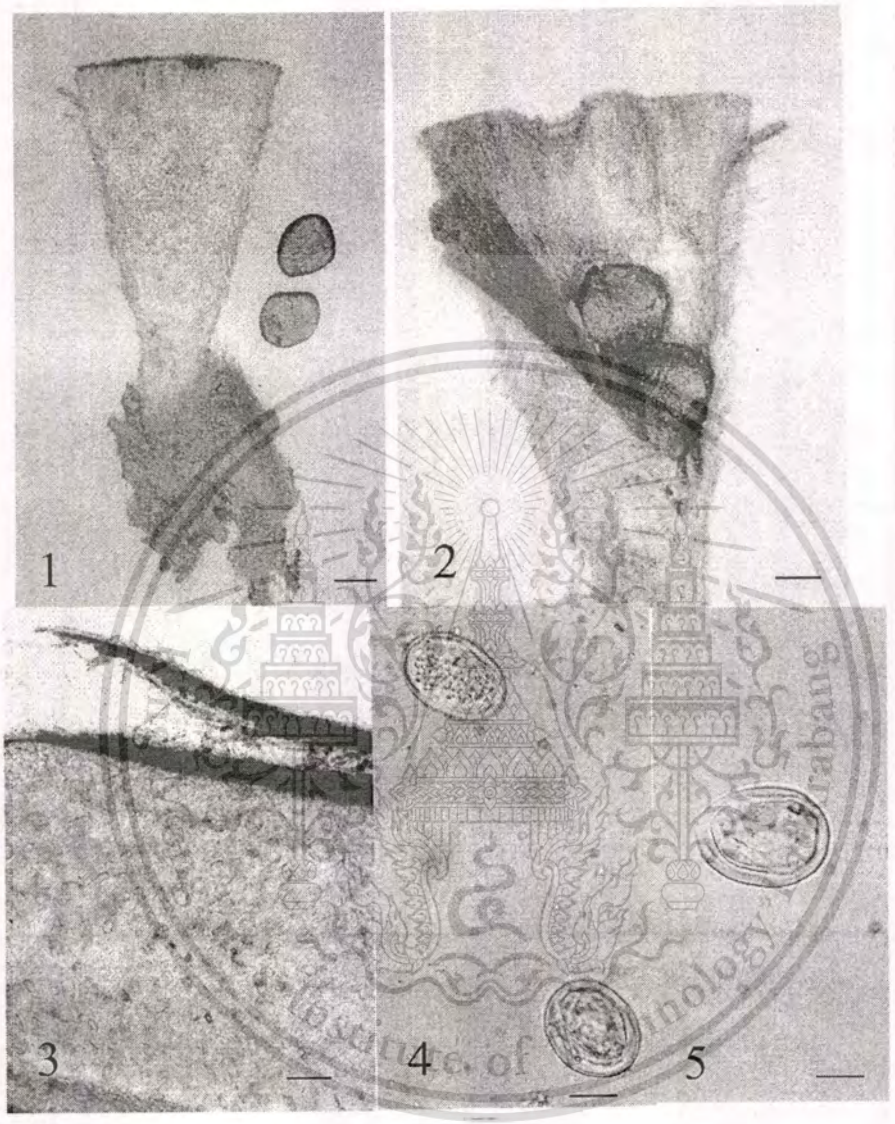

Fig. B.46. *Cyathus rufipes* 703516 (Holotype BPI).

1-2. Fruiting bodies. 3. Section of peridiole, double cortex. 4-5. Basidiospores.

Scale bars: 1 = 1 mm; 2 = 0.8 mm; 3 = 40 µm, 4 = 12 µm; 5 = 10 µm.

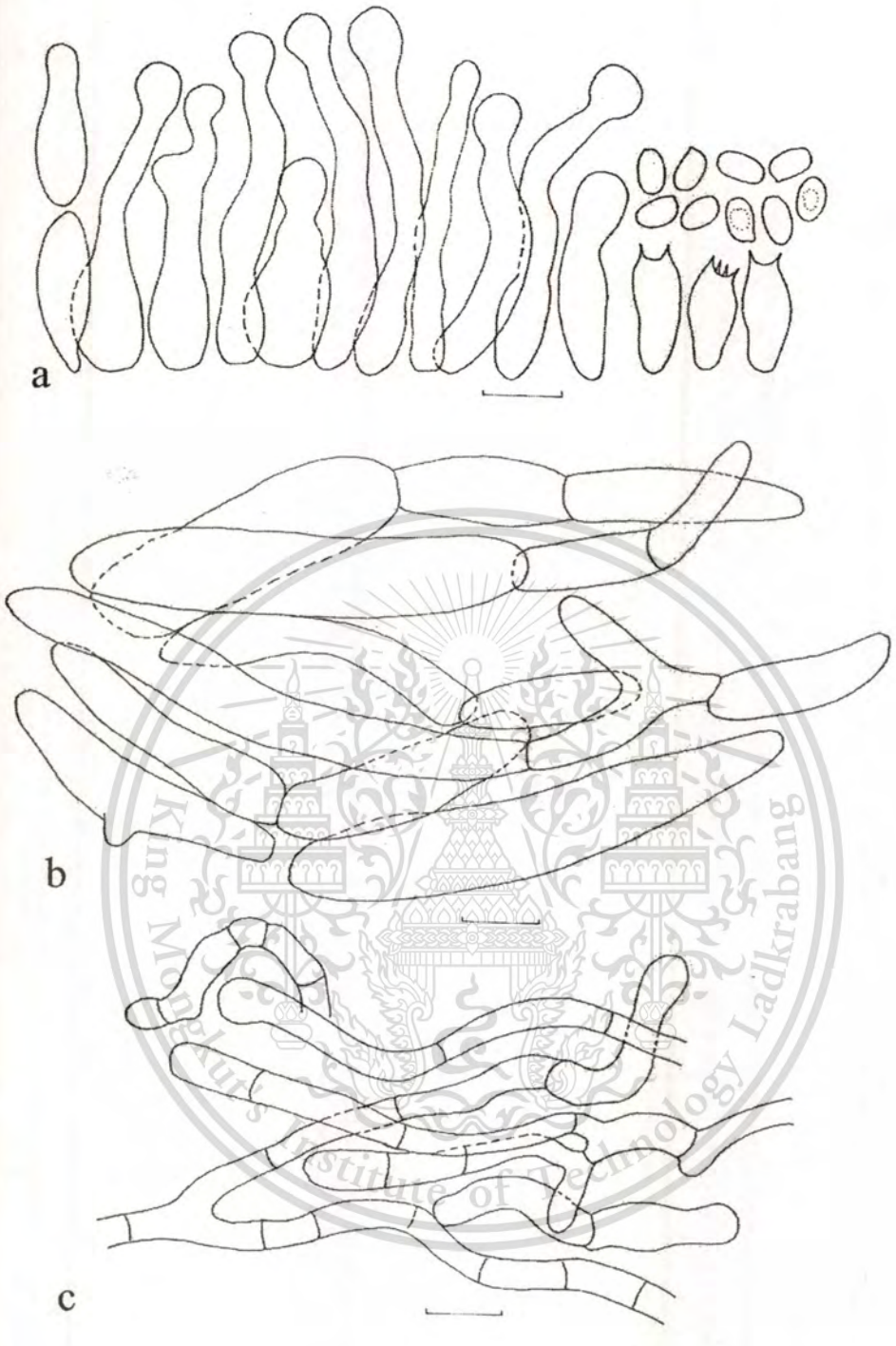

Fig. C.1. *Micropsalliota* aff. *alba* (SFSU ZRL2067). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar = 10 µm.

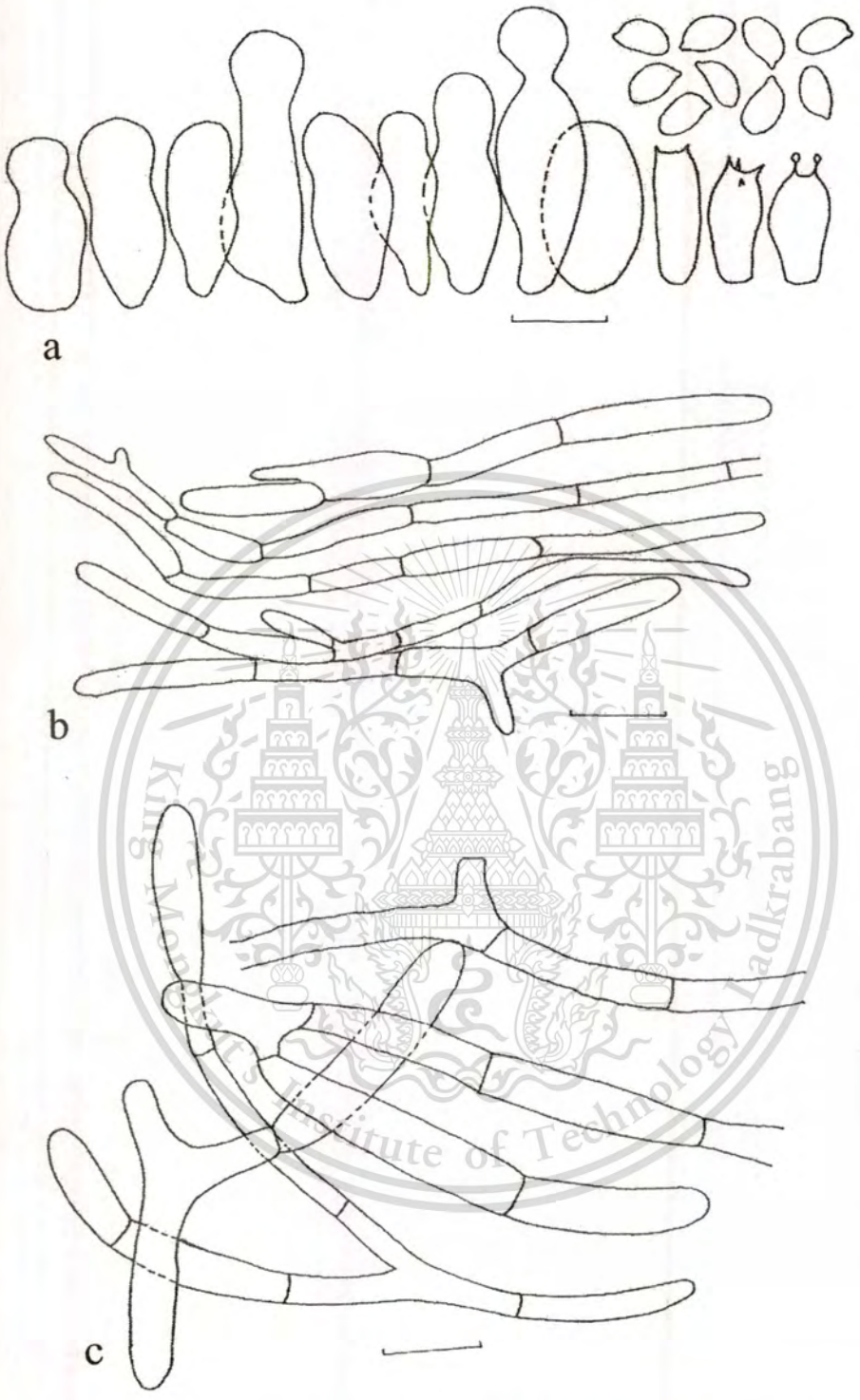

Fig. C.2. *Micropsalliota albosericea* (SFSU ZRL3049). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar a and c = 10 µm, b = 25 µm.

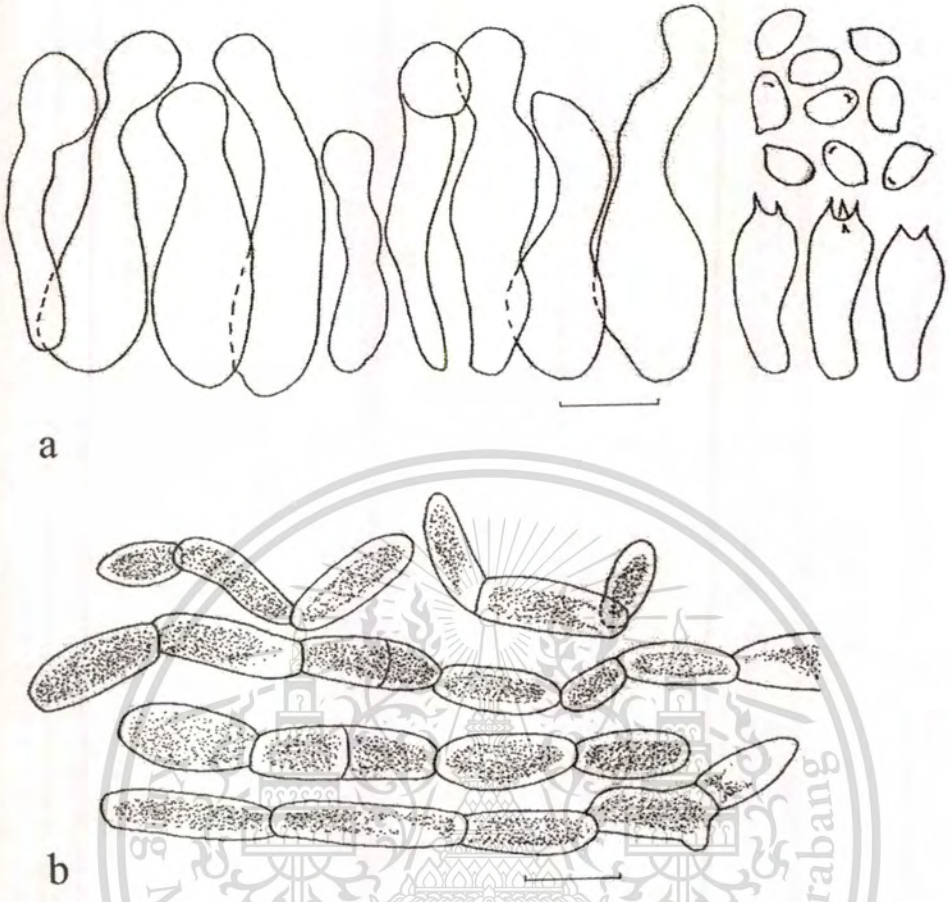

Fig. C.3. *Micropsalliota allantoidea* sp. nov. (Holotype SFSU ZRL2038). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar a = 10 µm; b = 25 µm.

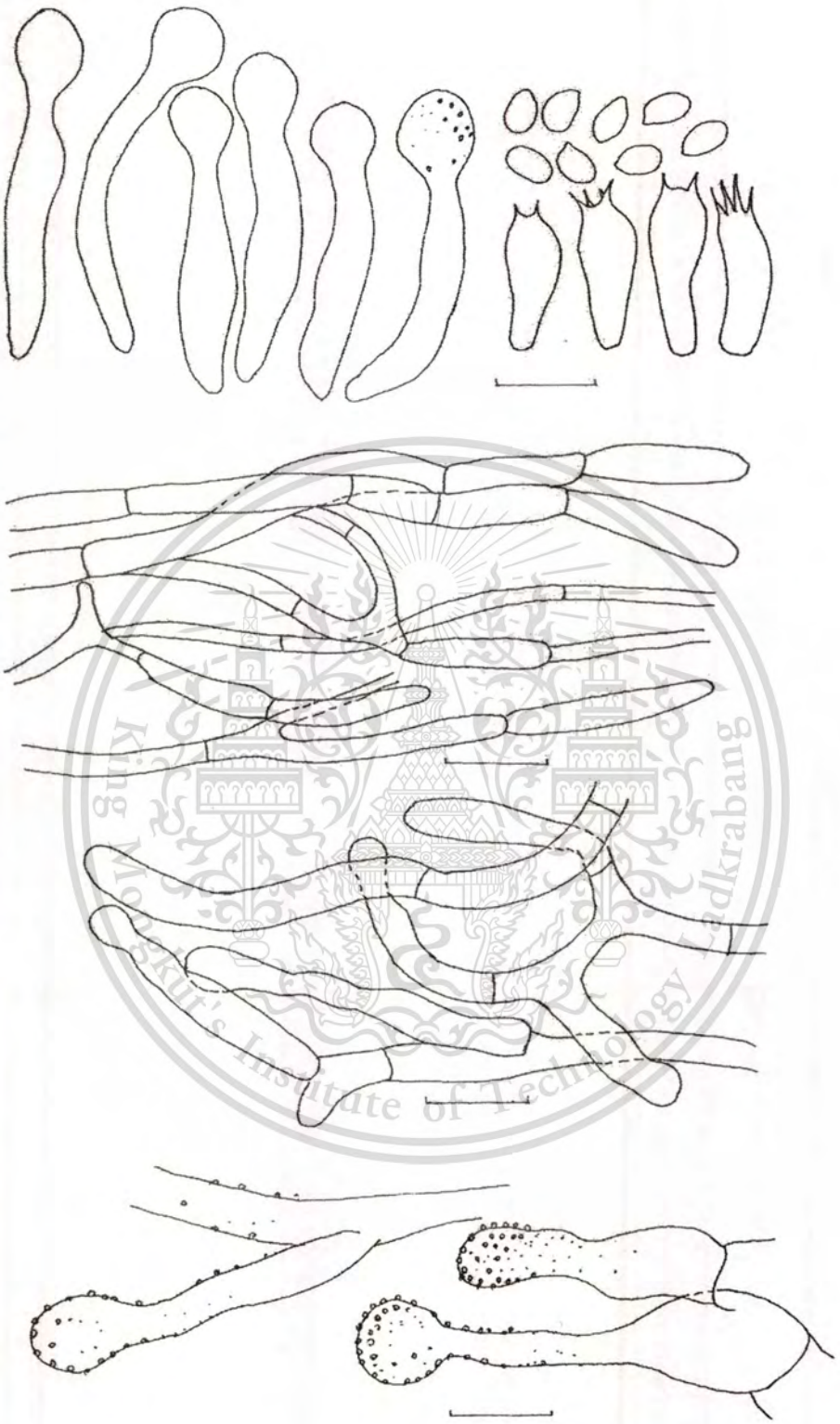

Fig. C.4. *Micropsalliota* aff. *arginea* (SFSU ZRL3090). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; d. caulocystidia; bar in a, c and d = 10 µm; b = 25 µm.

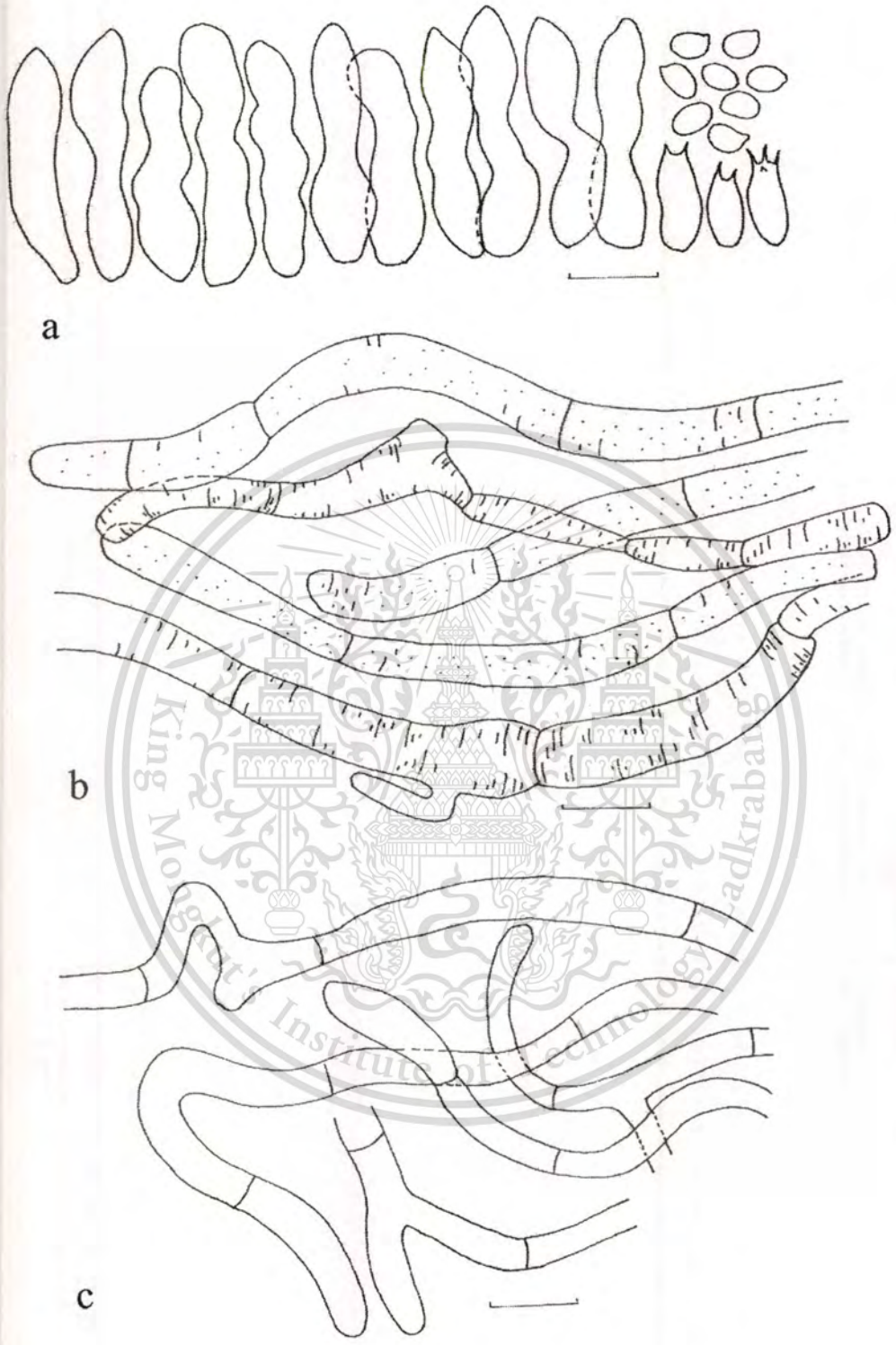

Fig. C.5. *Micropsalliota arginophaea* (SFSU ZRL2089). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar = 10 µm.

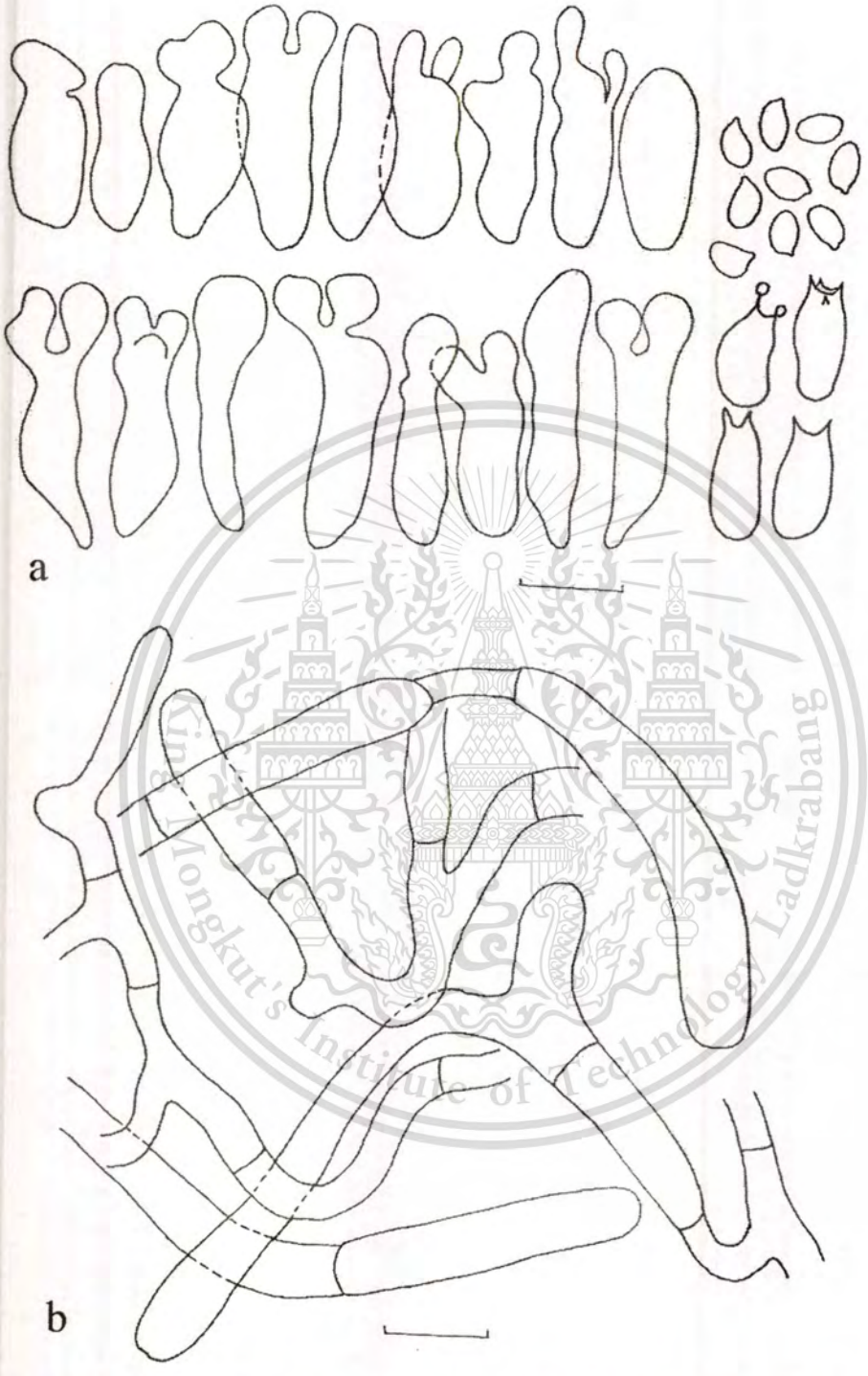

Fig. C.6. *Micropsalliota bifida* sp. nov (SFSU ZRL2103). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar = 10 µm.

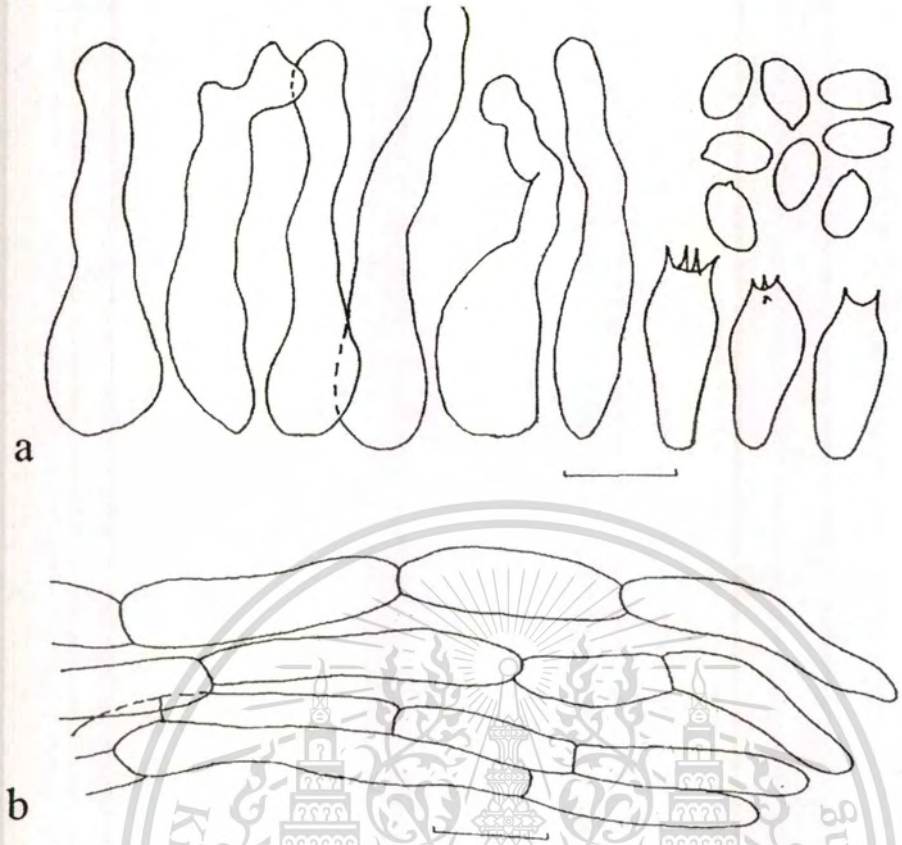

Fig. C.7. *Micropsalliota brunneosperma* var. *cortinata* (SFSU ZRL2129). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar a = 10 µm; b = 25 µm.

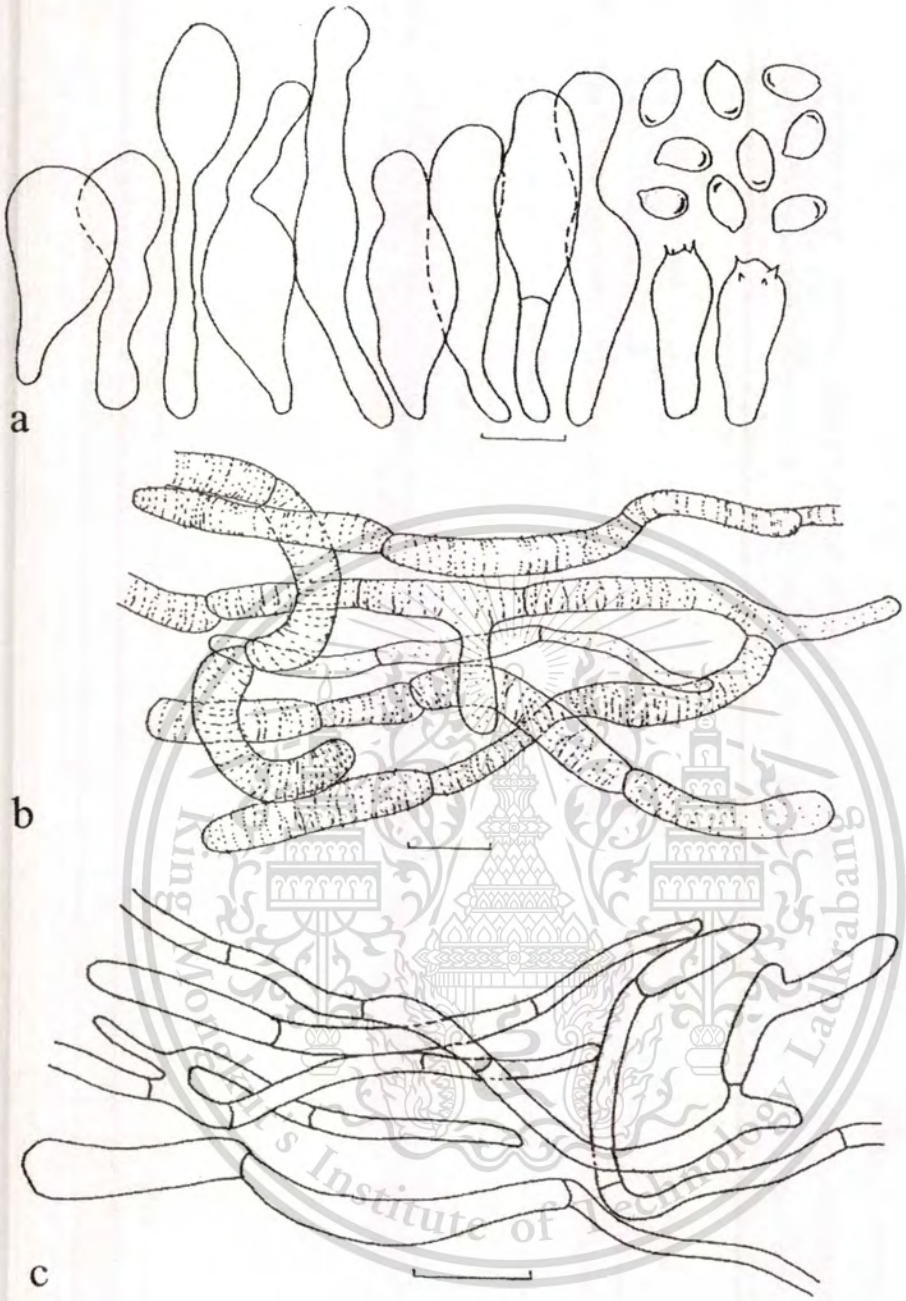

Fig. C.8. *Micropsalliota furfuracea* sp. nov. (Holotype SFSU ZRL3006). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar a and c = 10 μm; b = 25 μm.

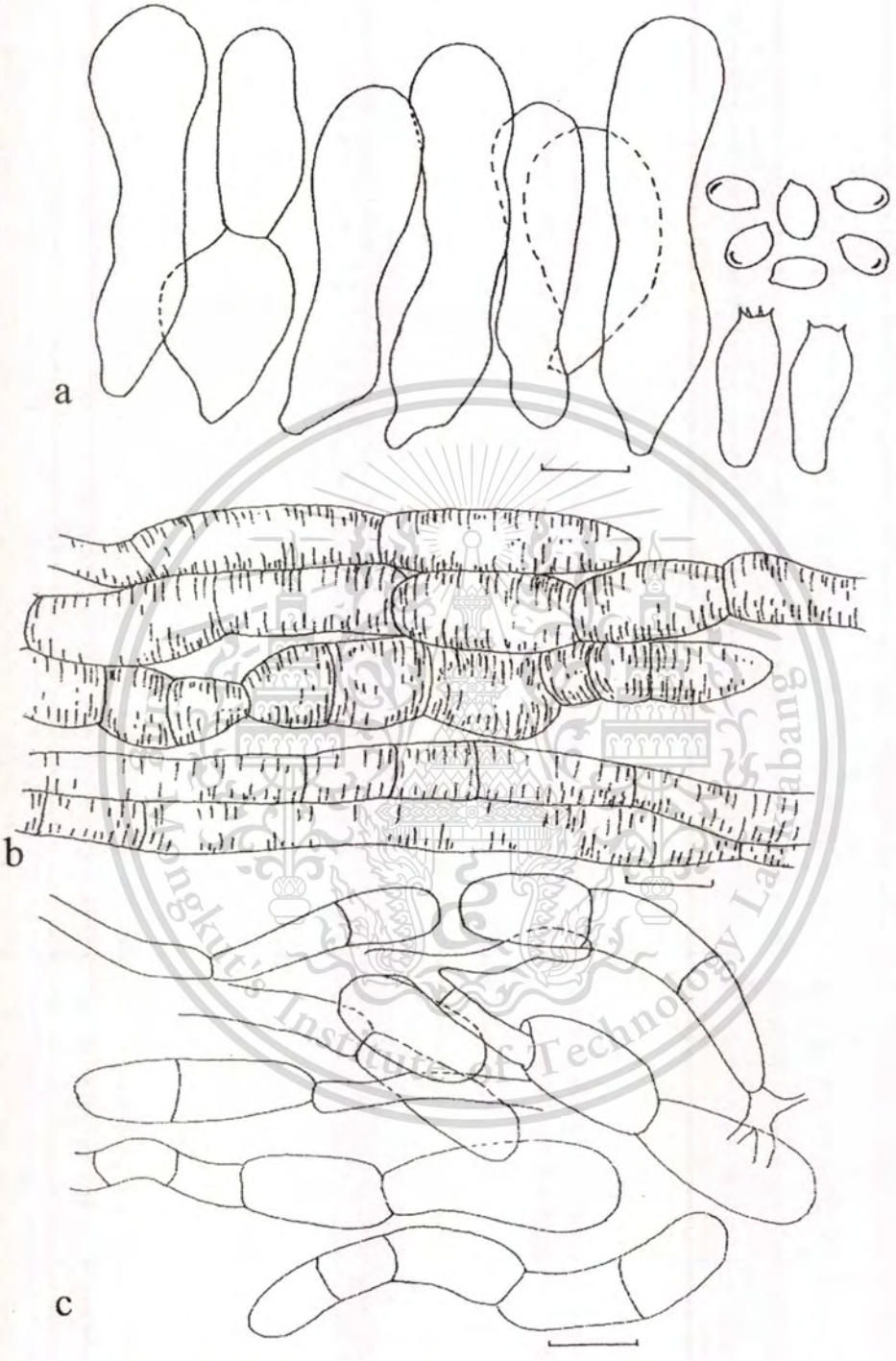

Fig. C.9. *Micropsalliota globocystidia* (SFSU ZRL3004). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar a = 10 µm; b and c = 25 µm.

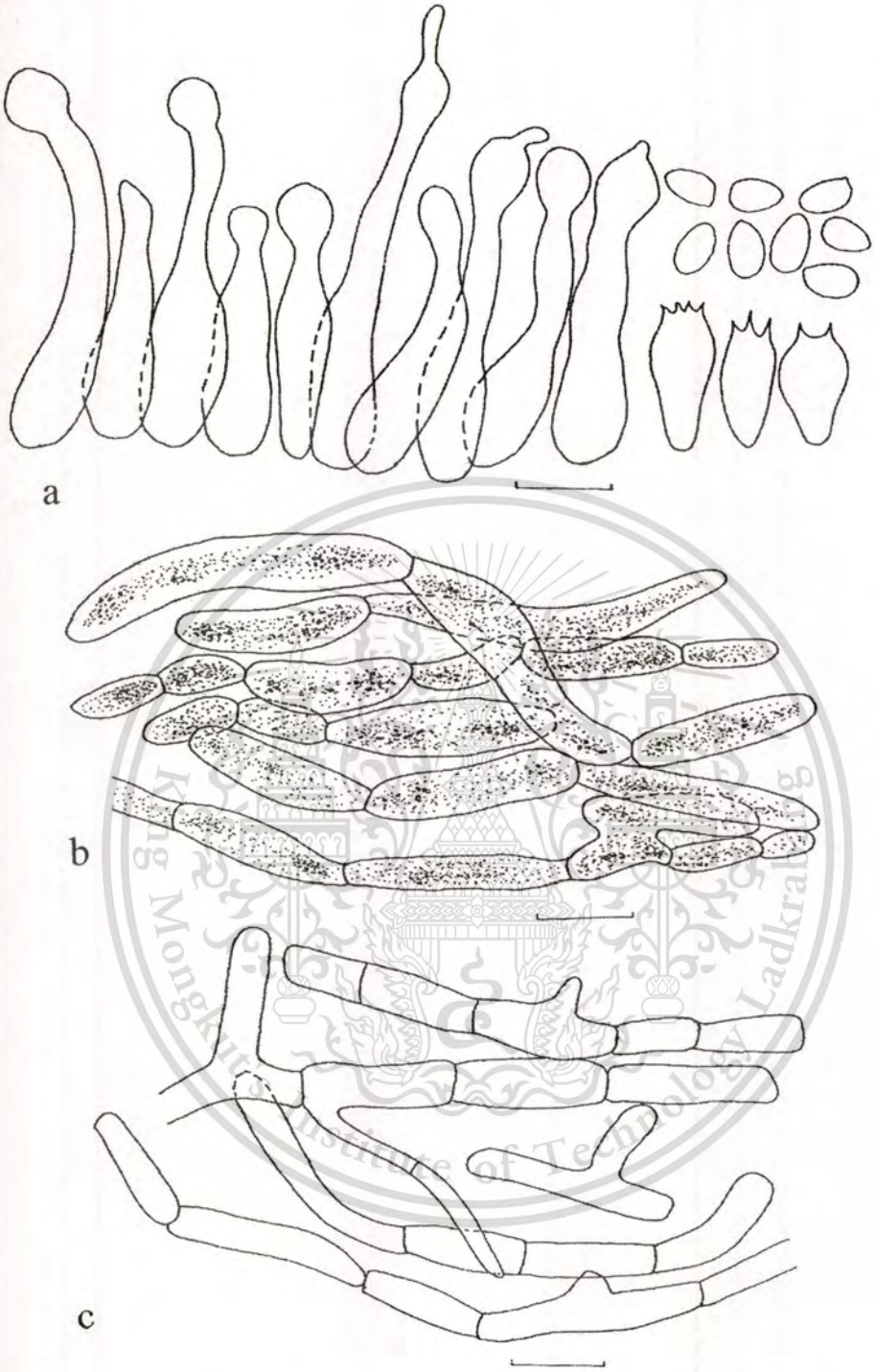

Fig. C.10. *Micropsalliota gracilis* (SFSU ZRL2041). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar a and c = 10 μm; b = 25 μm.

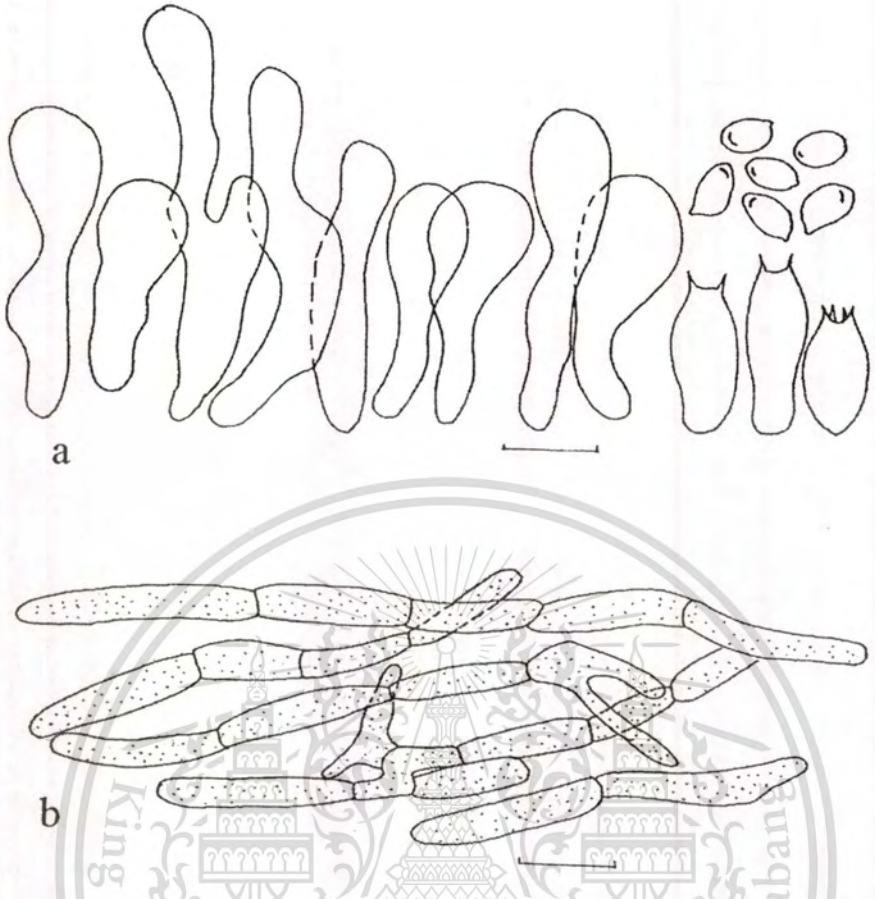

Fig. C.11. *Micropsalliota lateritia* var. *vinaceipes* var. nov. (Holotype SFSU ZRL2073).

a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar a = 10 µm; b = 25 µm.

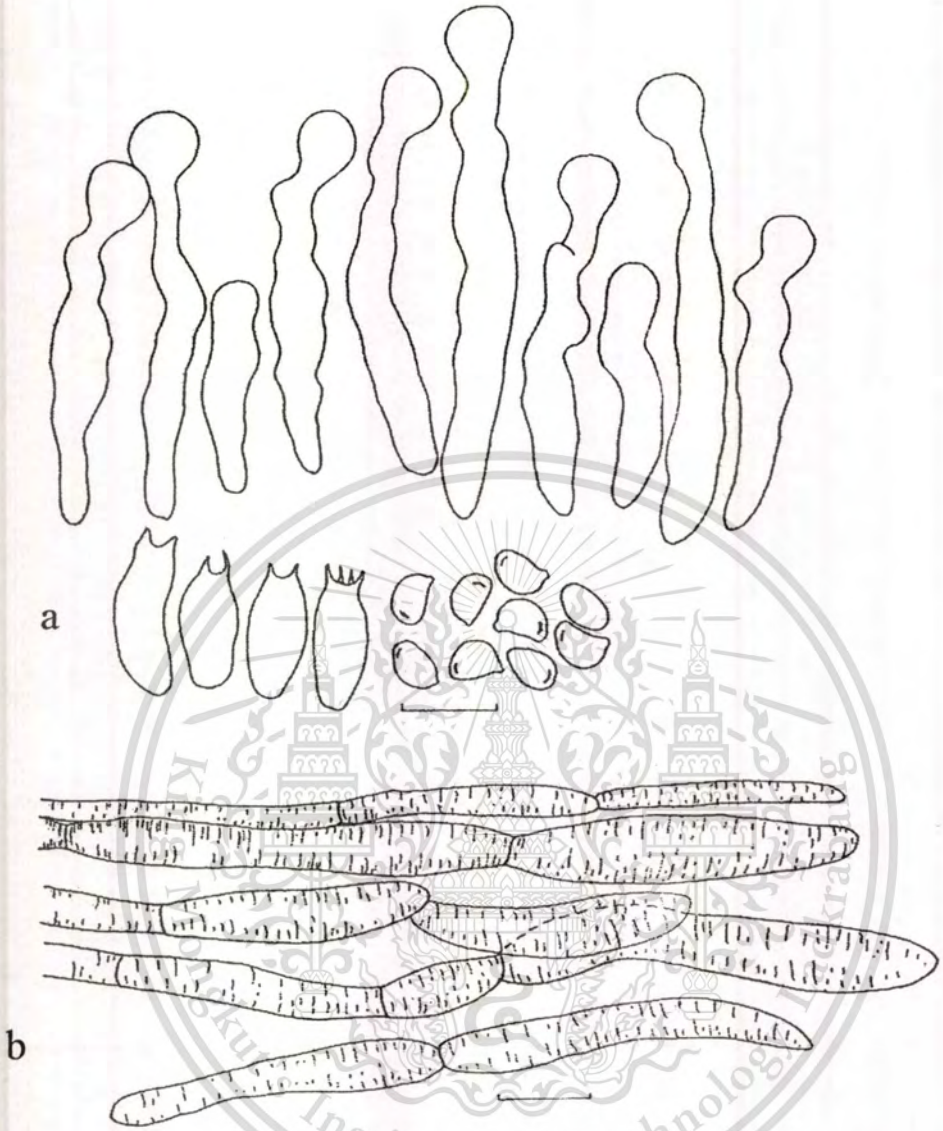

Fig. C.12. *Micropsalliota malabarensis* (SFSU ZRL2040). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar a = 10 µm; b = 25 µm.

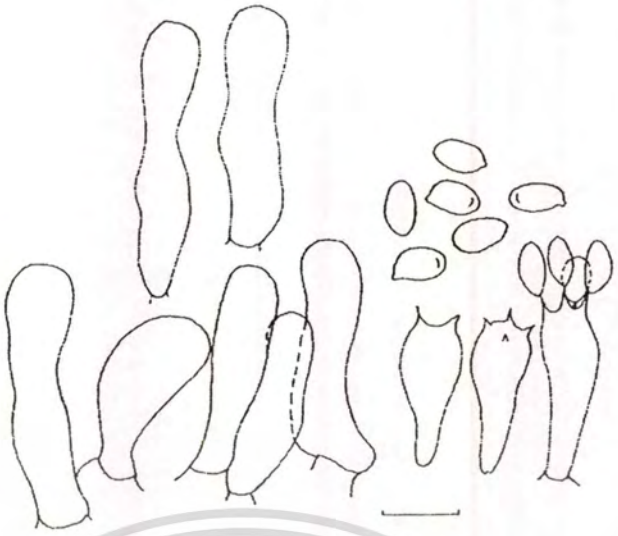

Fig. C.13. *Micropsalliota megarubescens* sp. nov. (Holotype SFSU ZRL2086).

Cheilocystidia, basidia and spores; bar = 10 μm.

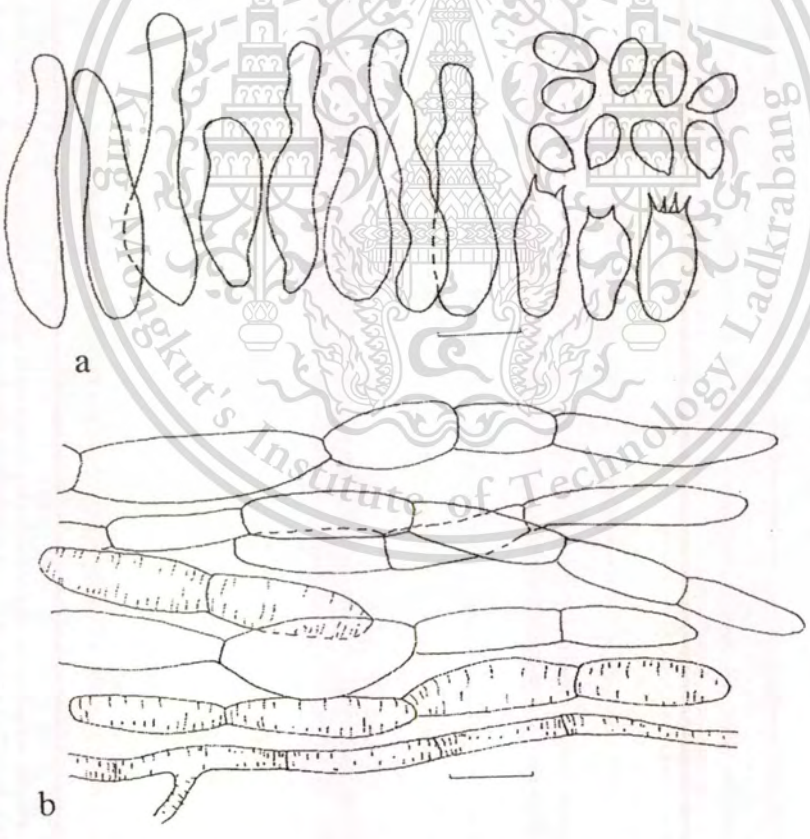

Fig. C.14. *Micropsalliota megaspora* sp. nov. (Holotype SFSU ZRL3068).

a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar a = 10 μm; b = 25 μm.

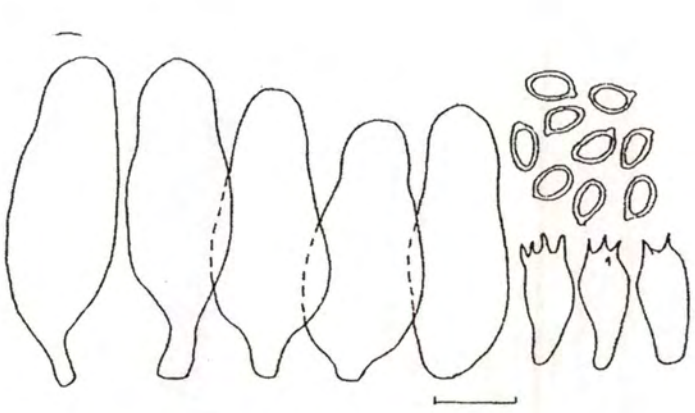

Fig. C.15. *Micropsalliota pleurocystidiata* (SFSU ZRL2023). Cheilocystidia/pluerocystidia, basidia and spores; bar = 10 μm.

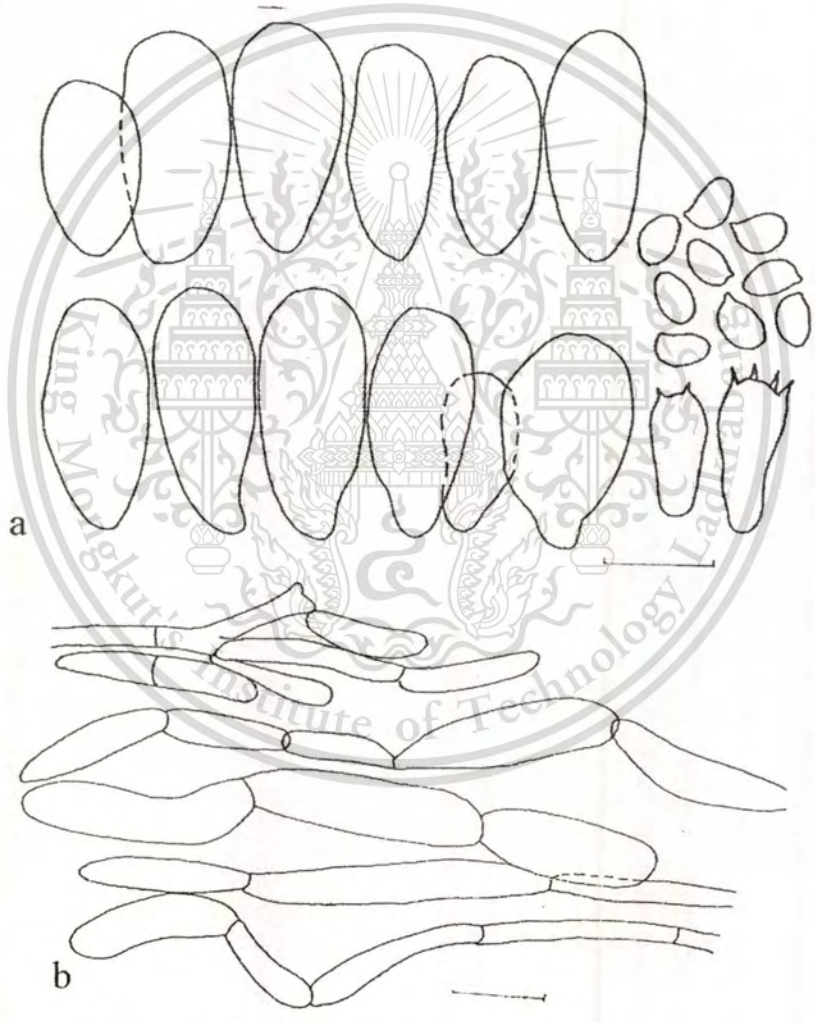

Fig. C.16. *Micropsalliota pseudoarginea* (SFSU ZRL3069). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar a = 10 μm; b = 25 μm.

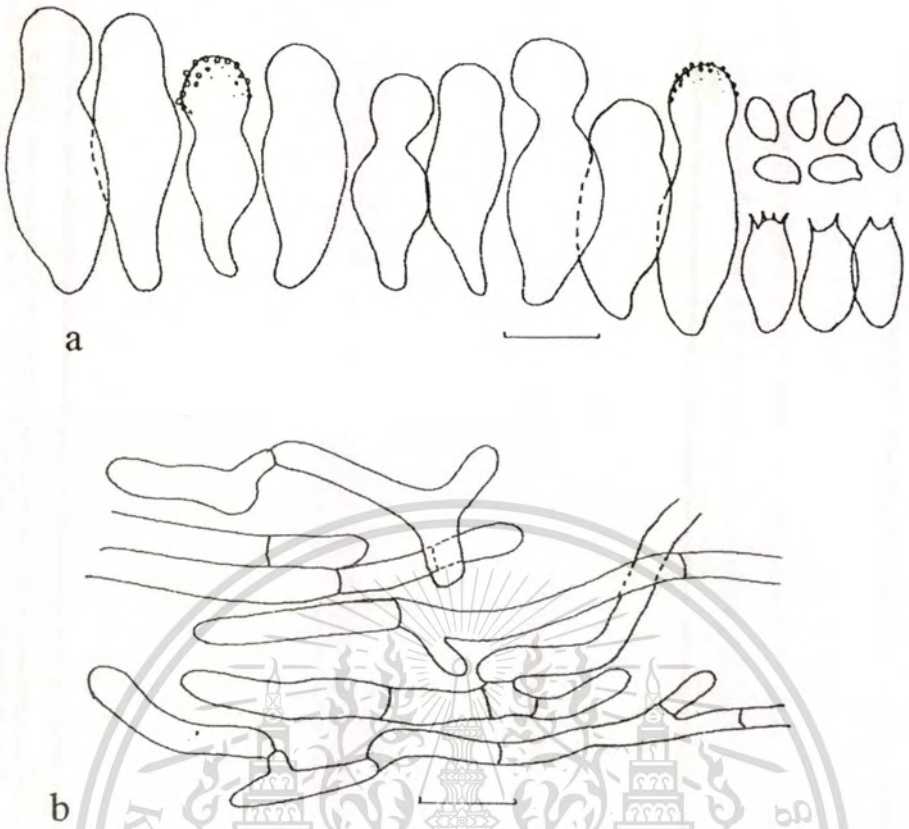

Fig. C.17. *Micropsalliota pusillissima* sp. nov. (Holotype SFSU ZRL3047). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar = 10 µm.

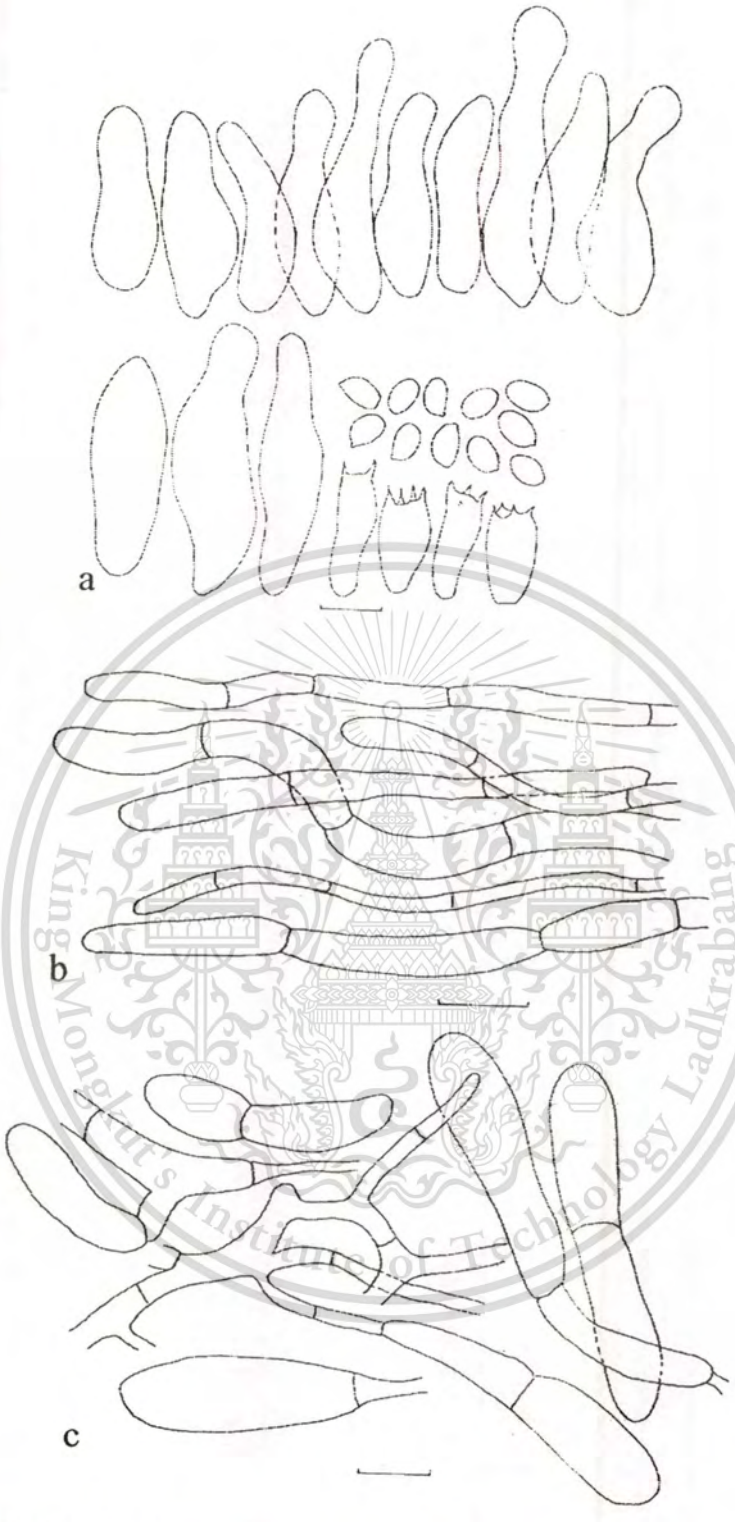

Fig. C.18. *Micropsalliota rubrobrunnescens* sp. nov. (Holotype SFSU ZRL2120).

a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar a and c = 10 µm; b = 25 µm.

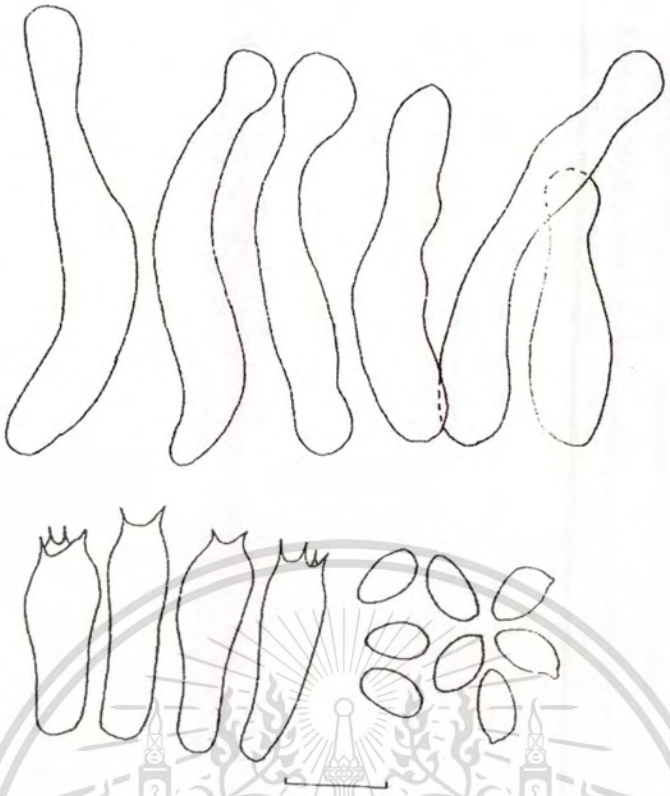

Fig. C.19. *Micropsalliota rubrobrunnescens* var. *tibiicystis* var. nov. (Holotype SFSU ZRL2121) Cheilocystidia, basidia and spores; bar = 10 μm.

Fig. C.20. *Micropsalliota subalba* (SFSU ZRL2080). Cheilocystidia, basidia and spores; bar = 10 μm.

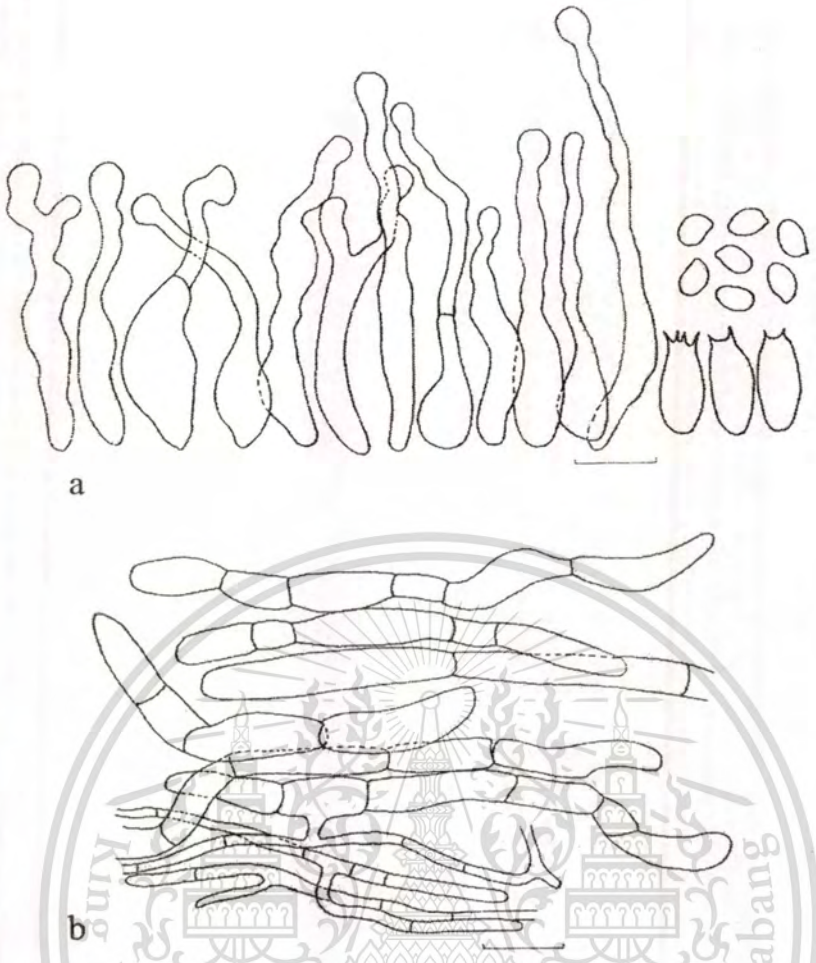

Fig. C.21. *Micropsalliota subarginea* (SFSU ZRL3058). a. Cheilocystidia, basidia and spores; b. pileipellis hyphae; bar a = 10 µm; b = 25 µm.

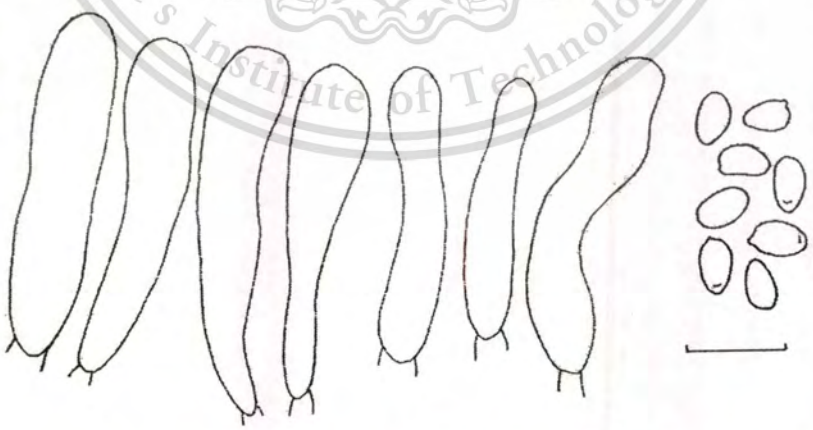

Fig. C.22. *Micropsalliota* suthepensis (Holotype SFSU ZRL2029). Cheilocystidia, basidia and spores; bar = 10 µm.

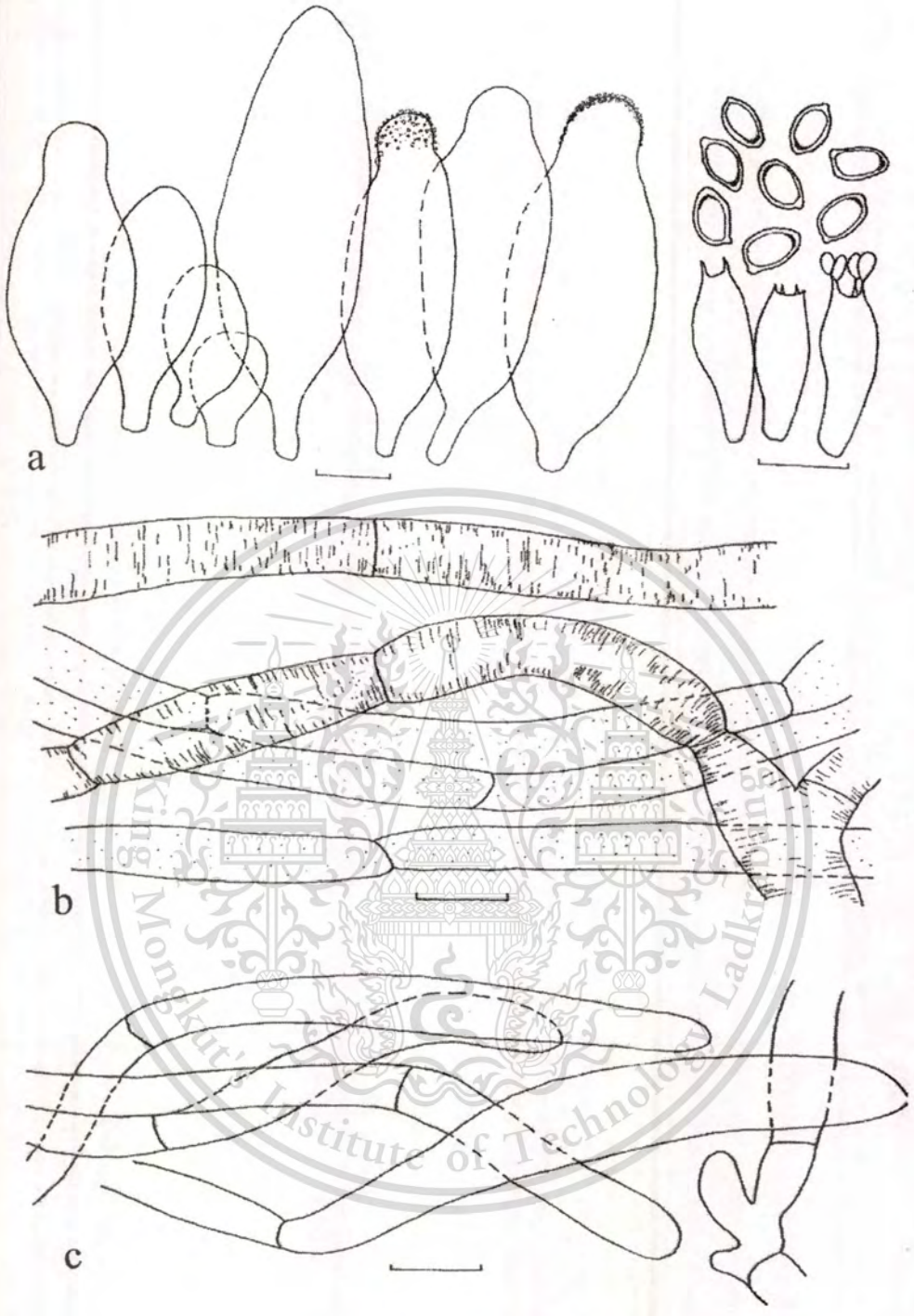

Fig. C.23. *Micropsalliota. xanthorubescens* (SFSU ZRL3096). a. Cheilocystidia/pleurocystidia, basidia and spores; b. pileipellis hyphae; c. annulus hyphae; bar = 10 µm.

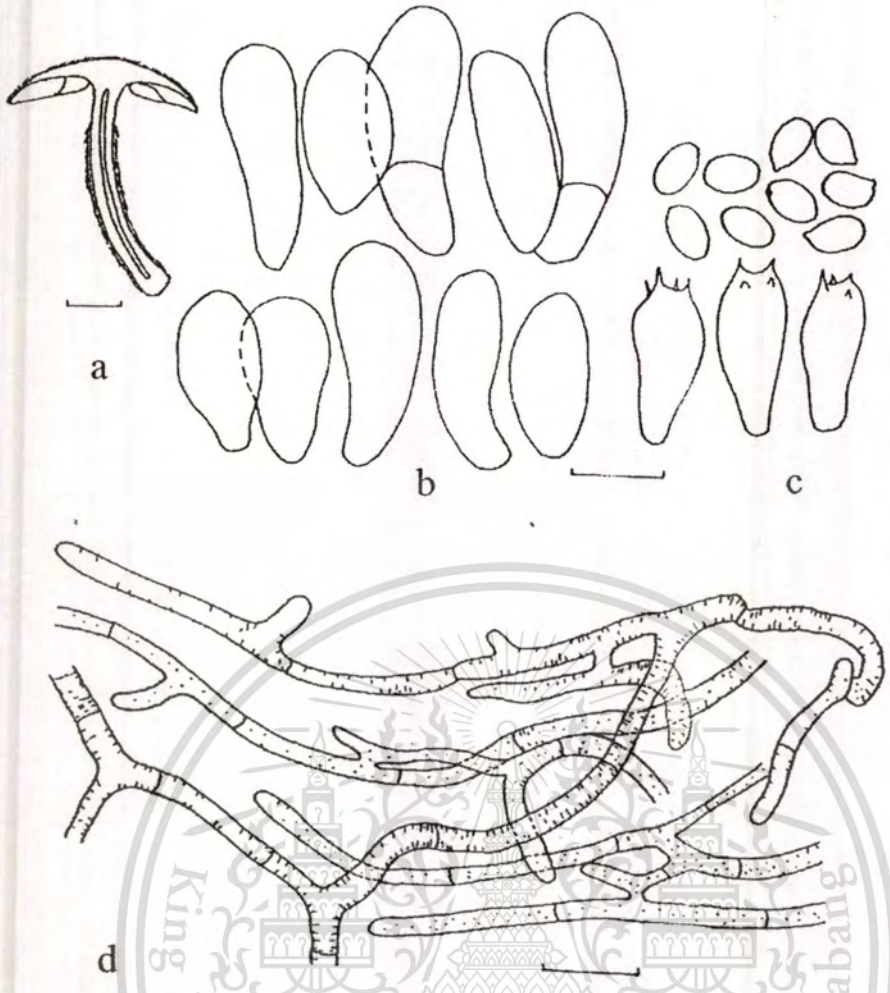

Fig. D.1. *Heinemannomyces splendidissima* ZRL3043 (SFSU). a. Fruiting body; b. cheilocystidia; c. basidia and spores; d. pileipellis hyphae; bar a = 8 mm; b-d = 10 µm.

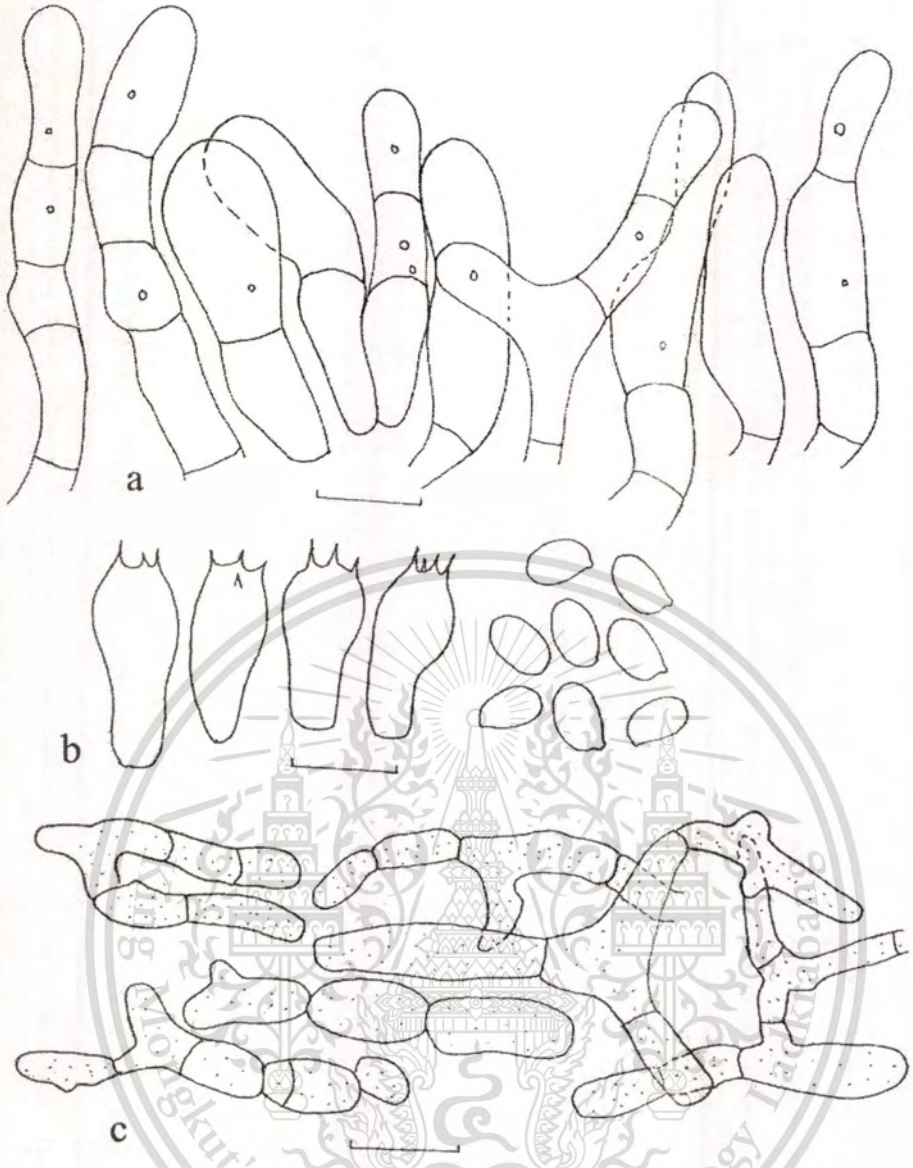

Fig. D.2. *Heinemannomyces* **sp. nov. ZRL3103 (SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; bar a and b = 10 μm; c = 25 μm.**

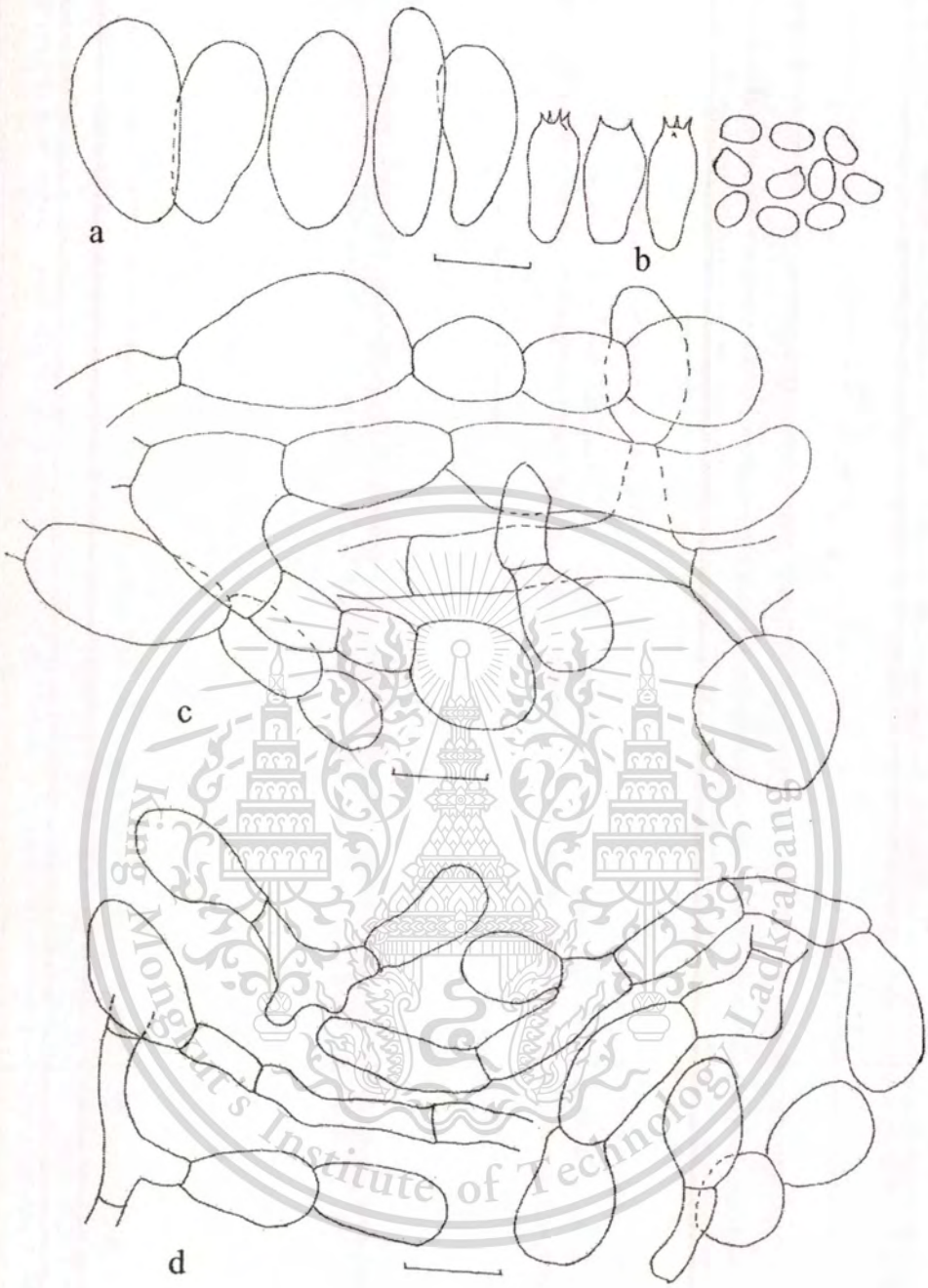

Fig. D.3. *Hynemagaricus epigastus* ZRL3045 (SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; d. annulaus hyphae; bar = 10 μm.

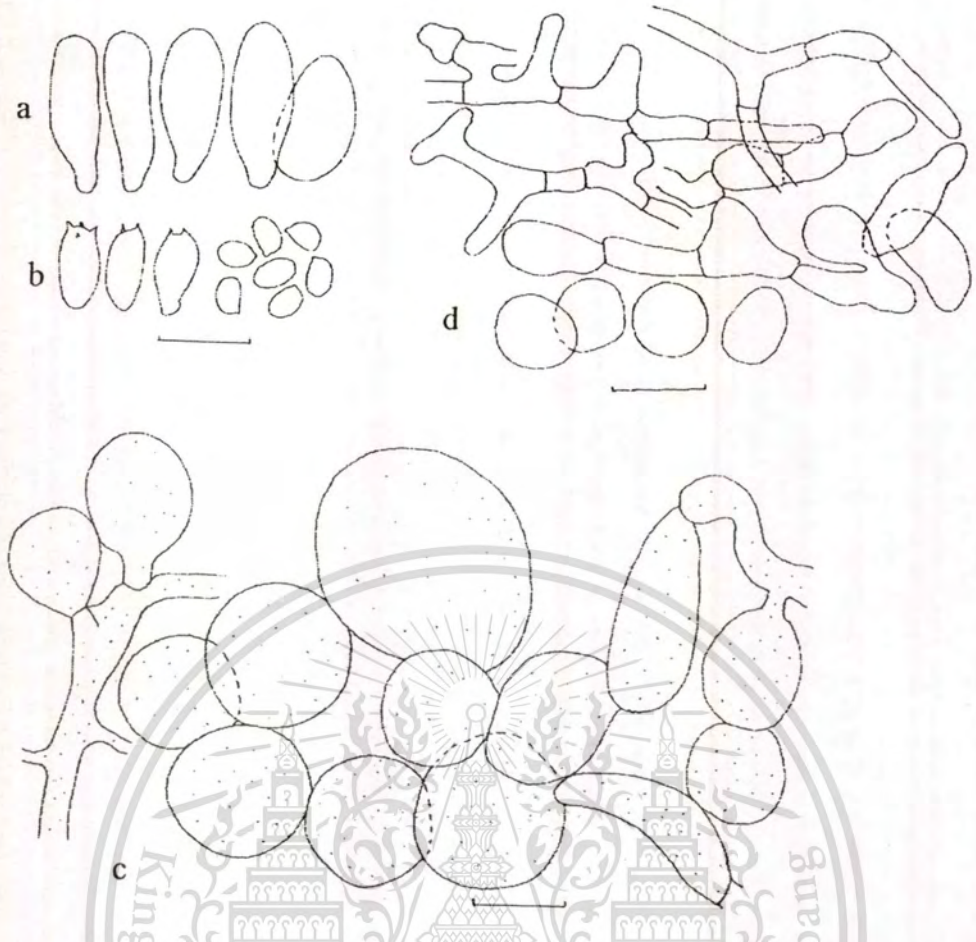

Fig. D.4. *Hynemagarucus* sp. nov. ZRL2047 (Holotype: SFSU). a. Cheilocystidia; b. basidia and spores; c. pileipellis hyphae; d. annulaus hyphae; bar = 10 µm.

Plate E.1. a. ***Micropsalliota* aff. *alba*** ZRL 2067 (SFSU); b. ***Micropsalliota albosericea*** ZRL3049 (SFSU).

Plate E.2. a-b. ***Micropsalliota allantoidea*** sp. nov. ZRL2038 (Holotype SFSU);

c. ***Micropsalliota*** **aff.** ***arginea*** ZRL3090 (SFSU).

Plate E.3. a. ***Micropsalliota arginophaea*** ZRL2089 (SFSU); b. ***Micropsalliota bifida*** sp. nov. ZRL3067 (Holotype SFSU).

Plate E.4. a. ***Micropsalliota brunneosperma*** var. ***cortinata*** ZRL2129 (SFSU); b-d. ***Micropsalliota furfuracea*** sp. nov. ZRL3006 (Holotype SFSU).

Plate E.5. a-b. ***Micropsalliota. globocystidia*** ZRL3004 (SFSU); c. ***Micropsalliota gracilis*** ZRL2041 (SFSU).

Plate E.6. a. ***Micropsalliota lateritia*** var. ***vinaceipes*** var. nov. ZRL 2073 (Holotype SFSU); b. ***Micropsalliota megarubescens*** sp. nov. ZRL 2086 (Holotype SFSU).

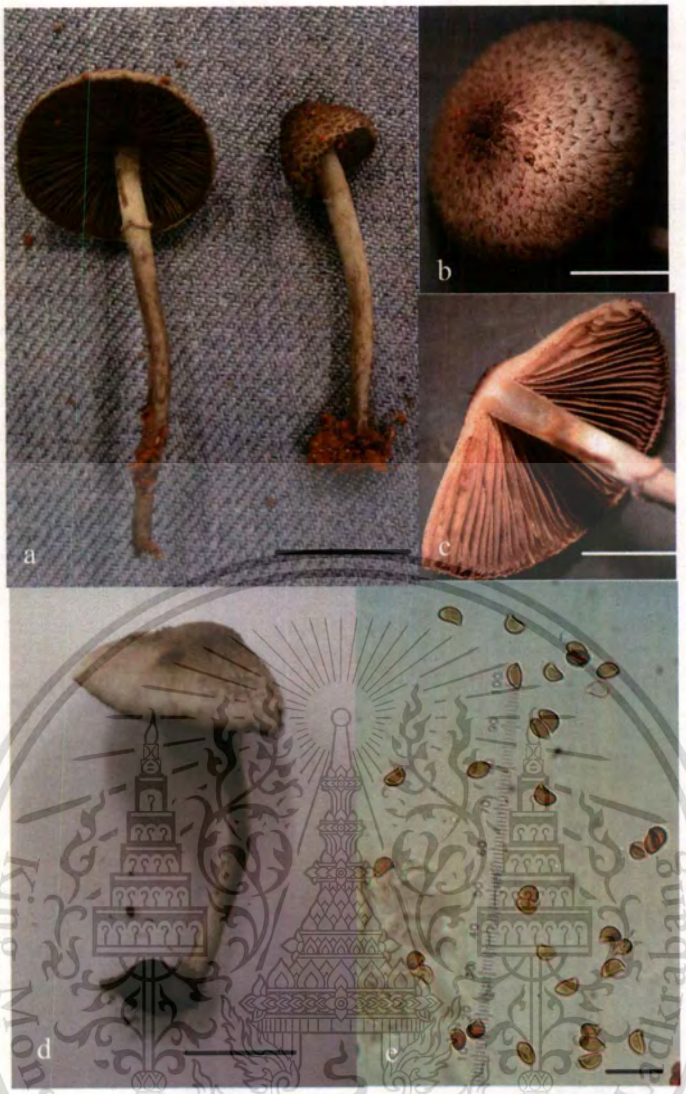

Plate E.7. a-c. ***Micropsalliota megaspore*** sp. nov. ZRL3068 (Holotype SFSU); d-e. ***Micropsalliota malabarensis*** ZRL2040 (SFSU).

Plate E.8. a-b. ***Micropsalliota pleurocystidiata*** (a. ZRL2023 SFSU; b. ZRL3081 SFSU); c. ***Micropsalliota pseudoarginea*** ZRL3069 (SFSU).

Plate E.9. a-b. ***Micropsalliota pusillissima*** sp. nov. ZRL3047 (Holotype SFSU);
c. ***Micropsalliota rubrobrunnescens*** var. ***rubrobrunnescens*** ZRL2120 (Holotype SFSU); bar a & c = 1 mm; b = 1 mm.

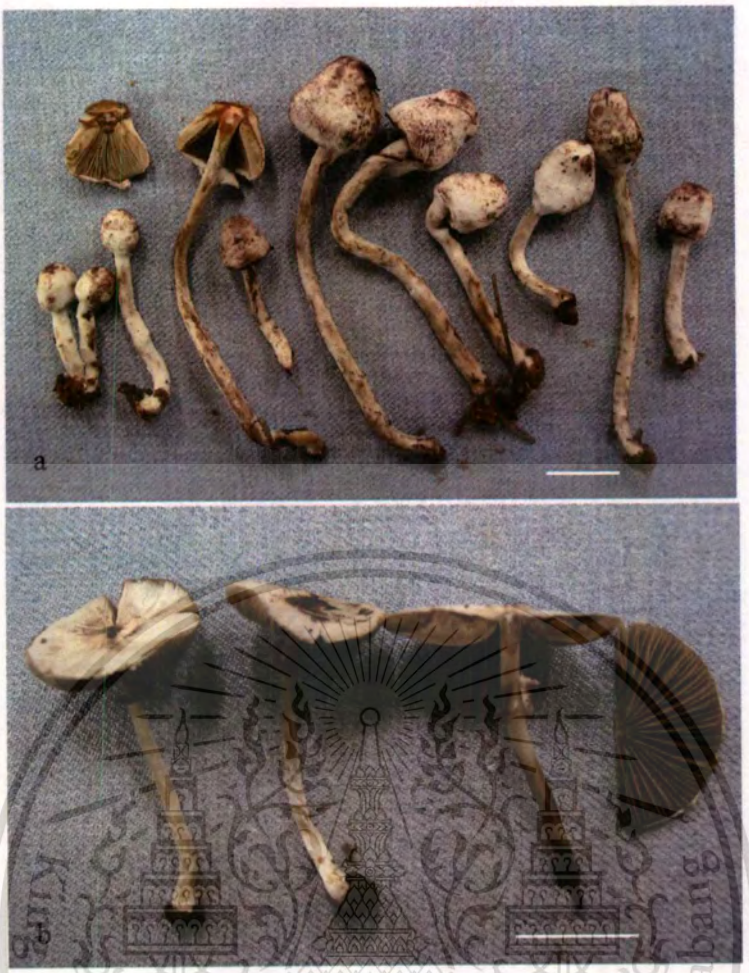

Plate E.10. a. ***Micropsalliota rubrobrunnescens*** var. ***tibiicystis*** var. nov. SFSU ZRL2121 (Holotype); b. ***Micropsalliota subalba*** ZRL2080 (SFSU); bar = 10 mm.

Plate E.11. a. ***Micropsalliota subarginea*** ZRL3058 (SFSU); b. ***Micropsalliota xanthorubescens*** ZRL3058 (SFSU); bar = 10 mm.

Plate E.12. a. ***Micropsalliota suthepensis*** sp. nov. ZRL2029 (Holotype SFSU); b. ***Agaricus angusticystidiata*** sp. nov. ZRL2043 (Holotype SFSU); bar = 10 mm.

Plate E.13. a. ***Agaricus bambusae*** ZRL2038 (SFSU); b. ***Agaricus caribaeus*** ZRL2032 (SFSU); c. ***Agaricus dulcidulus*** ZRL3071 (SFSU); bar = 10 mm.

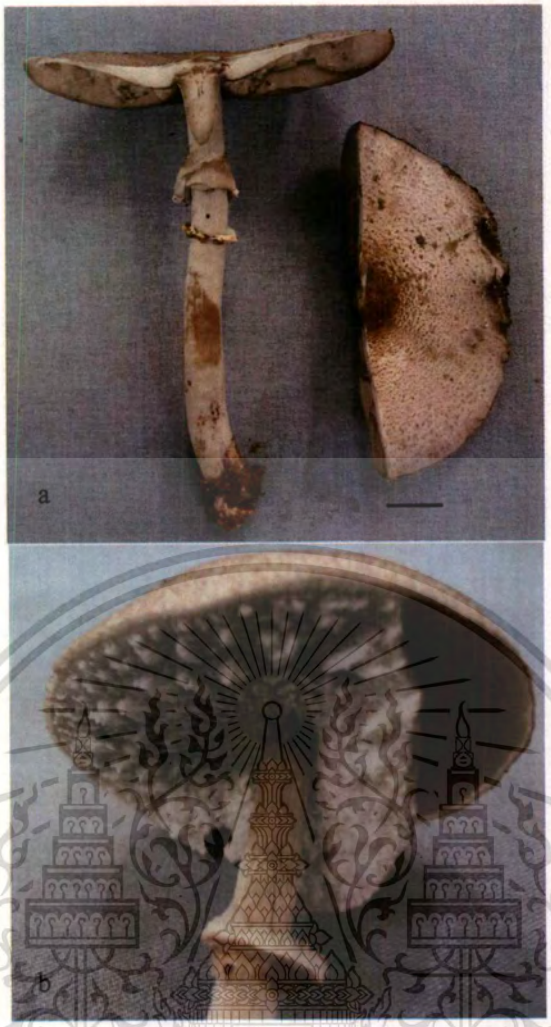

Plate E.14. a-b. ***Agaricus duplocingulatus*** (a. ZRL3051 SFSU; b.ZRL3003 SFSU); bar = 10 mm.

Plate E.15. a. ***Agaricus fiardii*** ZRL2134 (SFSU); b-c. ***Agaricus impudicus*** (b. ZRL2054 SFSU; c. ZRL3020 SFSU); bar = 10 mm.

Plate E.16. a. ***Agaricus johnstonii*** ZRL3005 (SFSU); b. ***Agaricus maesaeensis*** sp. nov. ZRL2048 (Holotype SFSU); c. ***Agaricus ochrascens*** ZRL2053 (SFSU); bar = 10 mm.

Plate E.17. a-b. ***Agaricus porphyrizon*** ZRL2044 (SFSU); c. ***Agaricus endoxanthus*** ZRL3094 (SFSU); bar = 10mm.

Plate E.18. a. ***Agaricus* aff. *xantholepis*** ZRL3088 (SFSU); b. ***Agaricus trisulphuratus*** ZRL2111 (SFSU); c. ***Agaricus*** aff. ***brunneolus*** ZRL3007 (SFSU); bar = 10 mm.

Plate E.19. a. ***Heinemannomyces splendidissima*** ZRL3043 (SFSU); b. ***Heinemannomyces*** sp. nov. ZRL3103 (SFSU); bar = 10 mm.

Plate E.20. a. ***Hynemagaricus epigastus*** ZRL3045 (SFSU); b. ***Hynemagarucus*** sp. nov. ZRL2047 (SFSU); bar = 10 mm.

Published Papers

1) **Rui-Lin Zhao**, Dennis E. Desjardin, Kasem Soytong & Kevin D. Hyde. 2006 (2007). Proposed Synonyms in *Cyathus*. Mycotaxon 97: 327-335. **(SCI)**
2) **Rui-Lin Zhao**, Rajesh Jeewon, Dennis E. Desjardin, Kasem Soytong & Kevin D. Hyde. 2007. Ribosomal DNA phylogenies of *Cyathus*: Is the current infrageneric classification appropriate? Mycologia 99(3):385-395. **(SCI)**

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Ribosomal DNA phylogenies of *Cyathus*: Is the current infrageneric classification appropriate?

Rui-Lin Zhao[1,2]

Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand

Rajesh Jeewon

Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, The University of Hong Kong, SAR, PR China

Dennis E. Desjardin[2]

Department of Biology, San Francisco State University, San Francisco, California

Kasem Soytong

Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand

Kevin D. Hyde

Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, The University of Hong Kong, SAR, PR China

Abstract: Phylogenetic relationships within the genus *Cyathus* (bird's nest fungi) were investigated with neighbor joining, maximum likelihood, weighted maximum parsimony and MrBayes analyses of ITS and LSU ribosomal DNA sequences datasets. Twenty-two taxa of *Cyathus* were used in the analyses based primarily on type and authentic specimens. The current infrageneric classification system of Brodie recognizes seven infrageneric groups based on morphological characters, including peridium plications and variations in peridium hair anatomy, peridiole structure and fruit-body color. These groups are not supported by molecular data. Instead the ITS and LSU datasets support recognition of three infrageneric groups herein named the *ollum*, *pallidum* and *striatum* groups. Morphological characters useful in distinguishing these groups include basidiospore size, fruit-body coloration and peridium anatomy. *Cyathus africanus* var. *latisporus* is considered a synonym of *Cyathus jiayuguanensis*, and a new combination *Cyathus lanatus* (Brodie) R.L. Zhao is proposed based on morphological and molecular data.

Key words: bird's nest fungi, gasteromycetes, Nidulariaceae, phylogenetics, taxonomy

INTRODUCTION

The genus *Cyathus* was established by Haller in 1768 and later subdivided into two infrageneric groups (viz. the "eucyathus" group with plications on the inner surface of the peridium of fruiting bodies and the "olla" group without such plications [Tulasne 1844]). Lloyd (1906) monographed *Cyathus* and accepted five infrageneric groups (viz. two sections in the eucyathus group and three sections in the olla group). No formal names were provided for Lloyd's sections. Later H.J. Brodie published more than 50 papers and two monographs on *Cyathus*, describing 62 species and subdividing *Cyathus* into seven groups (for references refer to Brodie 1975, 1984, and for a key to species accepted by Brodie refer to Brodie 1977).

In Brodie's taxonomic system distinct plications on the peridium of the fruiting body were considered to be a major diagnostic character for separating groups and species (Brodie 1975), following Tulasne and Lloyd before him. Species with plications were placed into the "striatus" and "poeppigii" groups, while species lacking plications were placed in the "olla", "pallidus", "triplex", "gracilis" and "stercoreus" groups (Brodie 1975, 1984). There are, however, several discrepancies with this system. One example occurs in the "olla" group, typified by *C. olla* (Batch) Pers., wherein species reputedly lack plications. *Cyathus olla* f. *anglicus* (Lloyd) H.J. Brodie possesses a distinctly sulcate mouth (Lloyd 1906, Brodie 1952). In addition *C. olla* f. *brodiensis* Shinners & J.P. Tewari, described based on Random Amplified Polymorphic DNA and morphological data, has a remarkably striate inner peridium (Shinners and Tewari 1998). Finally in Brodie's (1975) notes on an isotype specimen of *C. hookeri* Berk., another member of the olla group, fruiting bodies were faintly plicate internally. In another example *C. griseocarpus* H.J. Brodie & B.M. Sharma was placed by Brodie (1984) in group "striatus" because of distinct striations reported on the inner peridium. Examination of the holotype specimen of *C. griseocarpus* however revealed that some fruiting bodies are smooth and lack

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[1] Additional addresses: Mushroom Research Foundation, 128 Moo3, Bahn Pha Deng, T. Pa Pae, A. Mae Taeng, Chiang Mai 50150, Thailand; and Faculty of Conservation Biology, Southwest Forestry College, Kunming, Yunnan Province 650224, PR China.

[2] Corresponding authors. E-mail: ded@sfsu.edu and zhao_ruilin@yahoo.com.cn

plications. The importance of plications as a taxonomic character in the systematics of *Cyathus* therefore is questionable and this issue is addressed herein.

The two major groups based on plications were separated further into groups based on the type of peridial hairs, the structure of peridioles and the color of fruiting bodies (Brodie 1975, 1977, 1984). In general the hairs on the outside of the peridium of *Cyathus* fruiting bodies can be of three types: (i) a tomentum characterized by fine, short and soft hairs; (ii) hairs that are long and aggregated into small tufts or mounds; and (iii) hairs that are long and aggregated resulting in a shaggy or woolly appearance. Characteristics of the peridial hairs are affected by the environment and by the age of fruiting bodies, and its taxonomic significance therefore is questionable.

Brodie's taxonomic organization has been followed by most mycologists. *Cyathus* comprises 44 species (Kirk et al 2001) and is the most speciose genus in the family Nidulariaceae (Nidulariales). *Cyathus* is distinguished from the other three genera in the Nidulariaceae (*Crucibulum* Tul., *Nidula* V.S. White and *Nidularia* Fr.) based on gray to black peridioles with funicular cords and peridia composed of three layers of tissues.

The application of DNA sequence data in phylogenetic analyses of basidiomycetous fungi have let mycologists test taxonomic constructs based on morphology (Binder and Hibbett 2002, Hibbett and Binder 2002, Moncalvo et al 2002, Zhang et al 2004). Previous phylogenetic studies based on LSU datasets and including gasteromycetous fungi have included only a single species of *Cyathus* (e.g. *C. striatus* in Hibbett et al 1997, Hibbett and Thorn 2001, *C. stercoreus* in Moncalvo et al 2002). In those studies *Cyathus* nested within the euagarics clade with numerous lamellate genera and with members of the Lycoperdales. The most recent treatment of the agaricoid clade of basidiomycetes (Matheny et al 2006) included only *Crucibulum laeve* and *C. striatus* as representatives of the Nidulariaceae. In this research the Nidulariaceae was sister of the Cystodermataceae (represented by *Cystoderma amianthinum*) and together these two families formed a well resolved clade with the Agaricaceae *sensu stricto*. In a previous study (Zhao et al 2004) RAPD analyses performed on 43 *Cyathus* isolates representing 18 *Cyathus* taxa resulted in the establishment of two new species (Zhao et al 2004). However this study failed to provide a clear picture of species relationships within *Cyathus*. In addition all isolates used in this study originated from China, thus the results lack worldwide applicability. To date no molecular phylogenetic studies investigating evolutionary relationships among *Cyathus* species have been published. Consequently the current morphological classification proposed by previous researchers has never been tested.

The objectives of this study are to reexamine the genus *Cyathus* with morphology and sequences analyses to determine the phylogenetic importance of various characters used in *Cyathus* taxonomy. We also test whether the classification scheme proposed by Brodie based on plications and hairs on fruiting bodies is phylogenetically supported. Our sampling strategy was to include as many type specimens and authentic material studied by Brodie as was available.

MATERIALS AND METHODS

Fungal samples.—One hundred fifteen specimens included 30 holotypes or isotypes were borrowed from BPI, DAOM, HMAS, MICH and SWFC (Holmgren and Holmgren 1998). Each specimen was examined morphologically following Brodie's protocols (Brodie 1975). Twenty-two taxa (23 strains) of *Cyathus* and two other bird's nest fungi, *Crucibulum laeve* (Huds.) Kambly and *Nidula niveotomentosa* (Henn.) Lloyd, were sequenced (TABLE I). Sequences of *Cystoderma amianthinum* (Scop.) Fayod were retrieved from GenBank and used as outgroup for rooting propose (TABLE I) (cf. Matheny et al 2006). *Cyathus* samples included 13 holotypes or paratypes, two samples identified by J.H. Brodie, five samples identified by R.L. Zhao and three cultures borrowed from the Mycological Herbarium of Southwest Forestry College, China (SWFC).

DNA extraction, PCR and sequencing.—Two methods of DNA extraction were used. The first one used fungal mycelia harvested from cultures that were grown on potato-dextrose agar (PDA) for 2 wk, following the procedures as written by Jeewon et al (2002, 2004) and Cai et al (2005). The second extraction method involved the use of a commercial DNA extraction kit (E.Z.N.A. Forensic Kit, D3591-01, Omega Bio-Tek) for dried fungal specimens.

PCR reactions were performed in a 50 µL volume (0.3 mM primers [LROR: 5'-ACCCGCTGAACTTAAGC-3' and LR5: 5'-TCCTGAGGGAAACTTCG-3';or ITS4: 5'-TCCTCCGCTTATTGATATGC-3' and ITS5: 5'-GGAAGTAAAAGTCGTAACAAGG-3'], 10–20 ng DNA template, 1× buffer, 0.2 mM dNTPs, 1.5 units Taq and sterile water). The thermal cycles consisted of 94 C for 3 min, 30–35 cycles of 94 C for 1 min, 52 C for 50 sec and 72 C for 1 min, with a final extension step of 72 C for 10 min.

PCR products were checked by electrophoresis gels (1% agarose) stained with ethidium bromide in 1× Tris-boric acid EDTA buffer. They were purified with minicolumns according to the manufacturer's protocol (GFX PCR DNA and Gel Band Purification Kit, 27-9602-01, Amersham Biosciences). Primers ITS4, ITS5, LROR and LR5 were used to sequence both strands of the DNA molecule in an automated sequencer at the Genome Research Centre, University of Hong Kong.

Sequence alignment and phylogenetic analysis.—All sequences initially were aligned with Clustal × with default

TABLE I. Taxa information and GenBank accession numbers

Taxa	Herbarium accession number	Origin	DNA extract material	GenBank accession numbers ITS	LSU
Crucibulum laeve	SWFC 21261	China	DS	DQ463357	—
*Cyathus africanus**	DAOM 200370	Tanzania	DS	DQ463347	DQ463330
	SWFC 20782	China	CB	DQ463340	—
C. africanus var. *latisporus**	SWFC 21187	China	DS	DQ463342	DQ463328
*C. annulatus**	DAOM 200366	Canada	DS	DQ463351	DQ463332
C. berkeleyanus	SWFC 20789	China	DS	DQ463355	—
C. colensoi[B]	DAOM 200423	India	DS	DQ463344	—
*C. crassimurus**	DAOM 200372	Hawaii	DS	DQ463350	—
*C. gansuensis**	SWFC 20880	China	DS	DQ463348	DQ463335
*C. griseocarpus**	DAOM 200396	India	DS	—	DQ463324
*C. guandishanensis**	HMAS 81896	China	DS	—	DQ463329
*C. helenae**	DAOM 200384	Canada	DS	—	DQ463334
C. hookeri	SWFC 20799	China	CB	DQ463346	—
*C. jiayuguanensis**	SWFC 20802	China	DS	DQ463341	DQ463325
C. olla f. *olla*[B]	BPI 727227	Canada	DS	DQ463345	DQ463327
C. olla f. *anglicus**	BPI 727225	USA	DS	—	DQ463326
C. olla f. *brodiensis*	SWFC 21137	China	DS	DQ463343	—
C. olla f. *lanatus**	DAOM 200704	USA	DS	—	DQ463337
C. pallidus	SWFC 21160	China	DS	DQ463356	DQ463336
C. poeppigii	SWFC 21357	China	DS	—	DQ463339
*C. renweii**	SWFC 21406	China	CB	DQ463352	DQ463333
*C. setosus**	DAOM 200815	Jamaica	DS	DQ463349	DQ463331
C. stercoreus	SWFC 21386	China	CB	DQ463354	DQ463338
C. triplex	SWFC 21077	China	CB	DQ463353	—
Nidula niveotomentosa	SWFC 3000	China	DS	DQ463358	DQ463323

* refers to the type specimen; [B] indicates that H.J. Brodie determined the specimen; DS indicates that DNA was extracted from a dried specimen; CB indicates that DNA was extracted from a culture.

Sequences retrieved from GenBank: *Crucibulum laeve*, AF336246 (LSU); *Cystoderma amianthinum*, AY207195 (ITS) and DQ192177 (LSU).

settings (Thomson et al 1997). Then they were manually adjusted in BioEdit and gaps were introduced to improve alignments. The alignments were submitted to TreeBase (Submission ID: SN2794).

Phylogenetic analyses were performed with PAUP* 4.0b10 (Swofford 2004). Heuristic searches of the ITS, LSU and combined (ITS + LSU) datasets were performed under four optimality criteria: weighed parsimony (WP), maximum likelihood (ML), neighbor joining (NJ) and MrBayes (Huelsenbeck and Ronquist 2001, Huelsenbeck et al 2001). Unordered characters, random taxon addition sequences, gaps treated as missing data and the tree bisection-reconnection (TBR) branch swapping were used in the analyses. For weighted maximum parsimony MAXTREES was limited to 5000 trees with 1000 replications. The weighted parameters were produced with Stmatrix (François Lutzoni and Stefan Zoller, Duke University) as described in Miadlikowska et al (2002). The best nucleotide substitution models for maximum likelihood were chosen with MrModeltest2.2 (Nylander 2004). All characters were weighted equally for neighbor joining. Bootstrap values (BS) were obtained from 1000 replicates. Unconstrained trees (WP, ML and NJ trees) were compared in PAUP* with Kishino-Hasegawa and Shimodaira-Hasegawa tests (Kishino and Hasegawa 1989). Bayesian posterior probability (PP) was calculated with MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). One million generations were run for four Markov chains and sampled every 100th generation, resulting in 10 000 trees. The first 2000 trees were discarded as part of the burn-in phase, and the remaining 8000 trees were used to calculate posterior probabilities in a 50% majority rule consensus tree. Trees were viewed in TreeView and exported to graphics programs (Page 1996).

RESULTS

Phylogeny based on ITS sequence data.—The ITS dataset consisted of 17 samples representing 16 *Cyathus* taxa, *Crucibulum laeve, Nidula niveotomentosa*, and *Cystoderma amianthinum* as outgroup. This dataset consisted of 776 characters of which 125 characters were ambiguous and were excluded in the analysis and 168 characters were parsimony informative.

Stmatrix was used to assign appropriate parameters and gaps were treated as missing data for weighted parsimony analysis. This yielded one equally parsimo-

TABLE II. Summary of the Kishino-Hasegawa and Shimodaira-Hasegawa tests on the topologies obtained from ML, NJ and WP based on ITS, LSU and ITS-LSU combined datasets

Dataset	Tree	-ln L	Diff -ln L	KH-test *P*	SH-test *P*
ITS	WP	3765.34199	2.62696	0.000*	0.507
	NJ	3770.49979	7.78477	0.000*	0.133
	ML	3762.71503	(best)		
LSU	WP	2189.92422	34.74895	0.000*	0.049*
	NJ	2160.18367	5.00840	0.000*	0.519
	ML	2155.17527	(best)		
ITS-LSU	WP	5668.13037	3.87531	0.000*	0.141
	NJ	5668.13037	3.87531	0.000*	0.141
	ML	5664.25505	(best)		

* $P < 0.05$.

nious tree with a length of 468 steps (CI = 0.776, HI = 0.224, RI = 0.746). For maximum likelihood analysis the model selected by MrModeltest 2.2 was GTR + G, and the resulting tree has a likelihood score of 2969.71694. Results of the Kishino-Hasegawa and Shimodaira-Hasegawa tests among topologies obtained from ML, NJ and WP re provided (TABLE II). The ML tree (FIG. 1) was the best tree and therefore chosen to represent ITS phylogenies.

Trees generated from MrBayes yielded similar topologies as those from ML and WP and were not significantly different. All gene trees were characterized by three major clades (herein designated clades A, B and C; FIG. 1), although there were slight differences in the topological arrangement within the clades. Clade A includes *C. africanus* (type specimen and a second specimen), *C. africanus* var. *latisporus, C. colensoi, C. hookeri, C. jiayuguanensis, C. olla* and *C. olla* f. *brodiensis* with 100% PP and 98% BS supports. Clade B is composed of *C. annulatus, C. crassimurus, C. renweii, C. setosus, C. stercoreus* and *C. triplex* but had PP and BS supports of less than 50%. Clade C comprises *C. berkeleyanus, C. gansuensis* and *C. pallidus* with 100% PP and 83% BS supports.

In the ML ITS tree (FIG. 1) *C. hookeri* nested with *C. olla* and *C. olla* f. *brodienensis* with 95% PP and 67% BS supports, and of 776 aligned nucleotide sites *C. hookeri, C. olla* and *C. olla* f. *brodienensis* differed by only five base pairs.

Phylogeny based on LSU sequence data.—In the LSU dataset 19 sequences were included, consisting of 16 *Cyathus* taxa, *Crucibulum laeve, Nidula niveotomentosa*, and the trees were rooted with *Cystoderma amianthinum*. This dataset consisted of 797 characters of which 10 characters were excluded and 69 were parsimony informative.

Weighted parsimony analysis treated gaps as missing data and yielded a single tree with length of 193 steps (CI = 0.736, HI = 0.264, RI = 0.669). Maximum likelihood analysis with likelihood settings with the best-fit model (GTR + G) resulted in a tree with a score of 2020.58832. Results of the Kishino-Hasegawa and Shimodaira-Hasegawa tests (TABLE II) indicated that the ML analysis yielded the best tree (FIG. 2), although not topologically different from the WP results.

Maximum likelihood analysis of the LSU dataset shows that *Cyathus* species group into three clades (A, B and C) that are essentially the same as those obtained from ITS dataset, albeit with different statistical support. Clade A with 69% BS support and less than 50% PP support comprises *C. griseocarpus, C. guandishanensis* and *C. olla* f. *anglicus*, together with four species from Clade A in the ITS tree (viz. *Cyathus africanus* [type], *C. africanus* var. *latisporus, C. jiayuguanensis* and *C. olla*). Clade B with 99% PP and 61% BS support consists of *Cyathus helenae* and *C. poeppigii* together with three species from Clade B of the ITS tree (viz. *C. annulatus, C. renweii* and *C. stercoreus*). Clade C with 93% PP and 73% BS support comprises *C. olla* f. *lanatus* and two species from Clade C of the ITS tree (viz. *C. gansuensis* and *C. pallidus*). One major topological difference between the ITS and LSU trees concerns the position of *C. setosus*. In all ITS analyses *C. setosus* was nested in Clade B. However in all LSU analyses *C. setosus* was a sister taxon of clades A, B and C in an unresolved polytomy.

Phylogeny of the combined datasets.—The *Cyathus* taxa that successfully provided both ITS and LSU sequences were used to construct the combined dataset. In this data matrix there were 10 *Cyathus* taxa, *Crucibulum laeve, Nidula niveotomentosa* and outgroup *Cystoderma amianthinum* with a total of 1567 characters out of which 216 were parsimony informative and 138 characters were excluded.

WP analysis resulted in a single tree with a length of 594 steps (CI = 0.798, HI = 0.202, RI = 0.709).

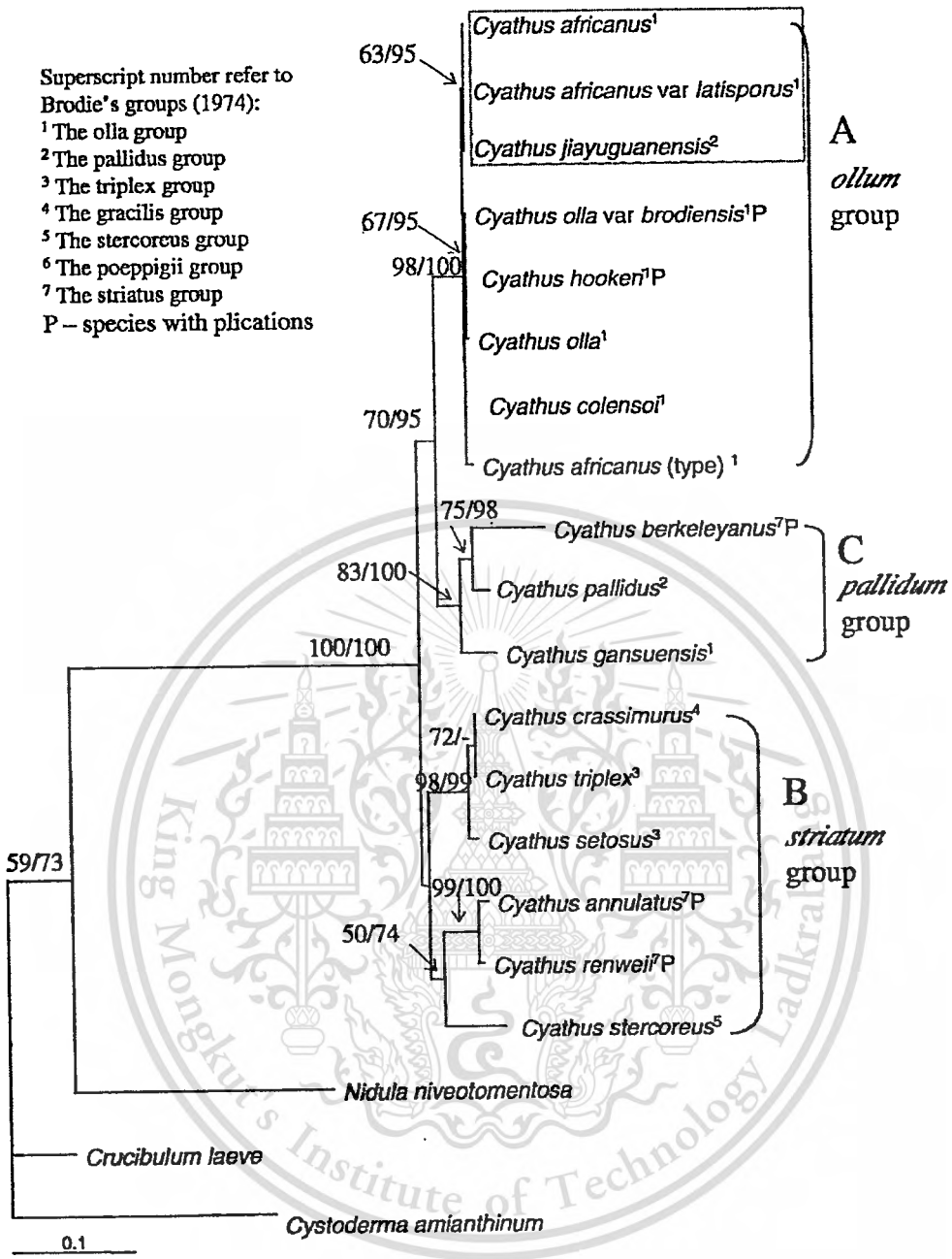

FIG. 1. Phylogeny of *Cyathus* inferred from ITS rDNA sequences. The maximum likelihood tree was rooted with outgroup species *Cystoderma amianthinum*. Bootstrap support (BS) from 1000 replicates in a heuristic search and Bayesian posterior probabilities (PP) values of more than 50% are shown above nodes (BS/PP) and "-" refers to values less than 50%. Branch length is proportional to number of substitutions. *Cyathus jiayuguanensis* = *Cyathus africanus* var. *latisporus* is shown in the dotted box.

Maximum likelihood settings were from the best-fit model (GTR + I + G) and ML analysis yielded a tree with the score of 4781.34621. The Kishino-Hasegawa and Shimodaira-Hasegawa tests indicate that WP, ML and NJ trees were not significantly different and ML tree (FIG. 3) is the best (TABLE II). The MrBayes tree also resulted in three major clades with strong PP support and had the same internal topologies as the WP, NJ and ML trees, although the relationships among the clades were not strongly supported. Species in Clade A grouped with 100% PP and 100% BS support, species in Clade B grouped with 100% PP and 69% BS support, while those in Clade C grouped with 100% PP and 99% BS support. *Cyathus*

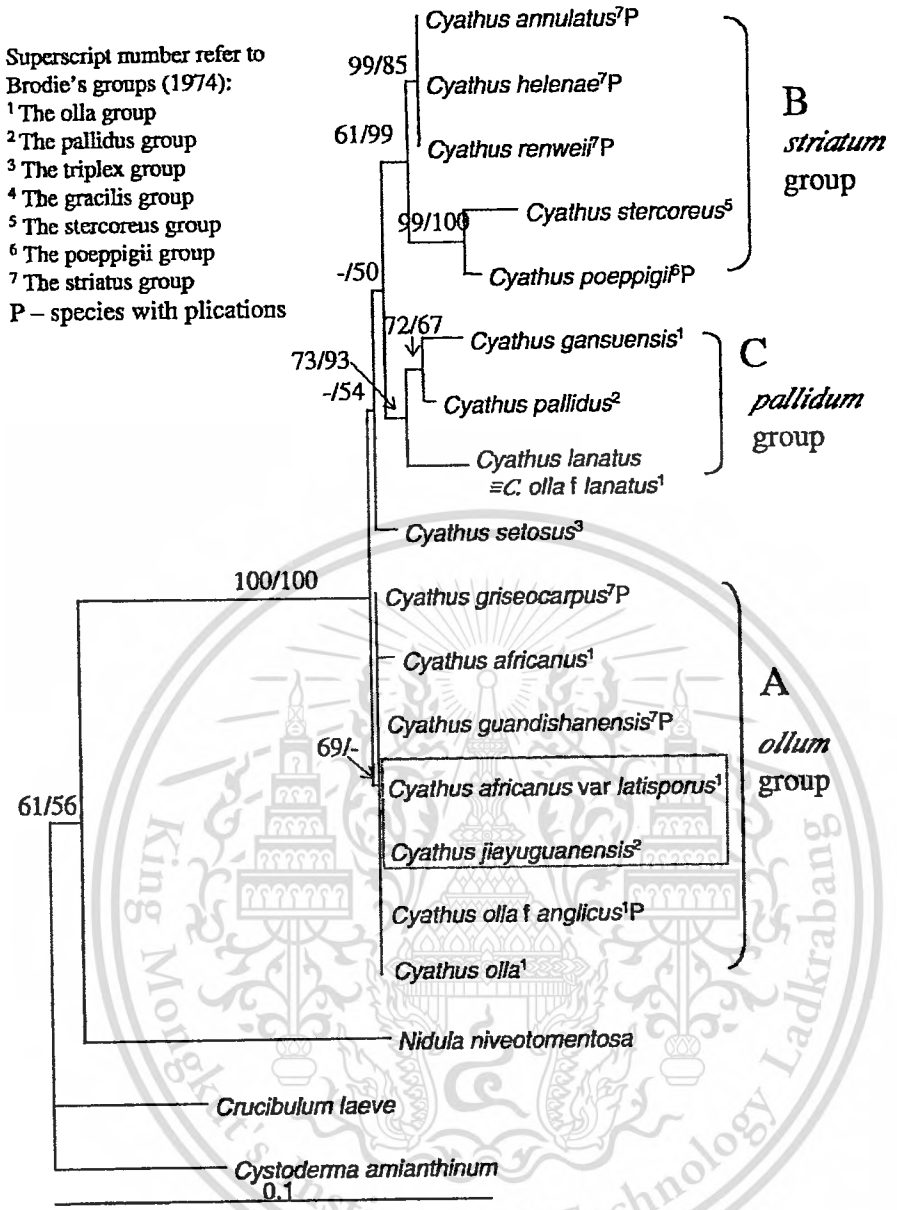

FIG. 2. Phylogeny of *Cyathus* inferred from LSU rDNA sequences. The maximum likelihood tree was rooted with *Cystoderma amianthinum*. Bootstrap support (BS) from 1000 replicates in a heuristic search and Bayesian posterior probabilities (PP) values of more than 50% are shown above nodes (BS/PP) and "-" refers to values less than 50%. Branch length is proportional to number of substitutions. *Cyathus jiayuguanensis* = *Cyathus africanus* var. *latisporus* is shown in the dotted box.

setosus was outside all three major clades. In all three analyses (ITS, LSU, combined ITS-LSU) species of *Cyathus* formed a monophyletic clade with 100% PP and 100% BS support, although the relationship with other bird's nest fungi was unresolved.

Phylogeny of Cyathus olla and its forms.—In this research the holotypes of *C. olla* f. *lanatus* and *C. olla* f. *anglicus* (=*C. anglicus*), authentic material (Brodie identified) of *C. olla* f. *olla* and material of *C. olla* f. *brodiensis* identified by R. Zhao were used in the molecular study. Our phylogenetic analyses showed that *C. olla* f. *olla*, *C. olla* f. *anglicus* and *C. olla* f. *brodiensis* are related closely and all cluster in Clade A (FIGS. 1–3). However the position of *C. olla* f. *lanatus* in the LSU tree (FIG. 2) was in Clade C with 93% PP and 73 BS supports.

Phylogeny of Cyathus jiayuguanensis, C. africanus *and* C. africanus *var.* latisporus. The results of ITS, LSU and the combined dataset (totaling 1567 bases) analyses using sequences from holotype specimens

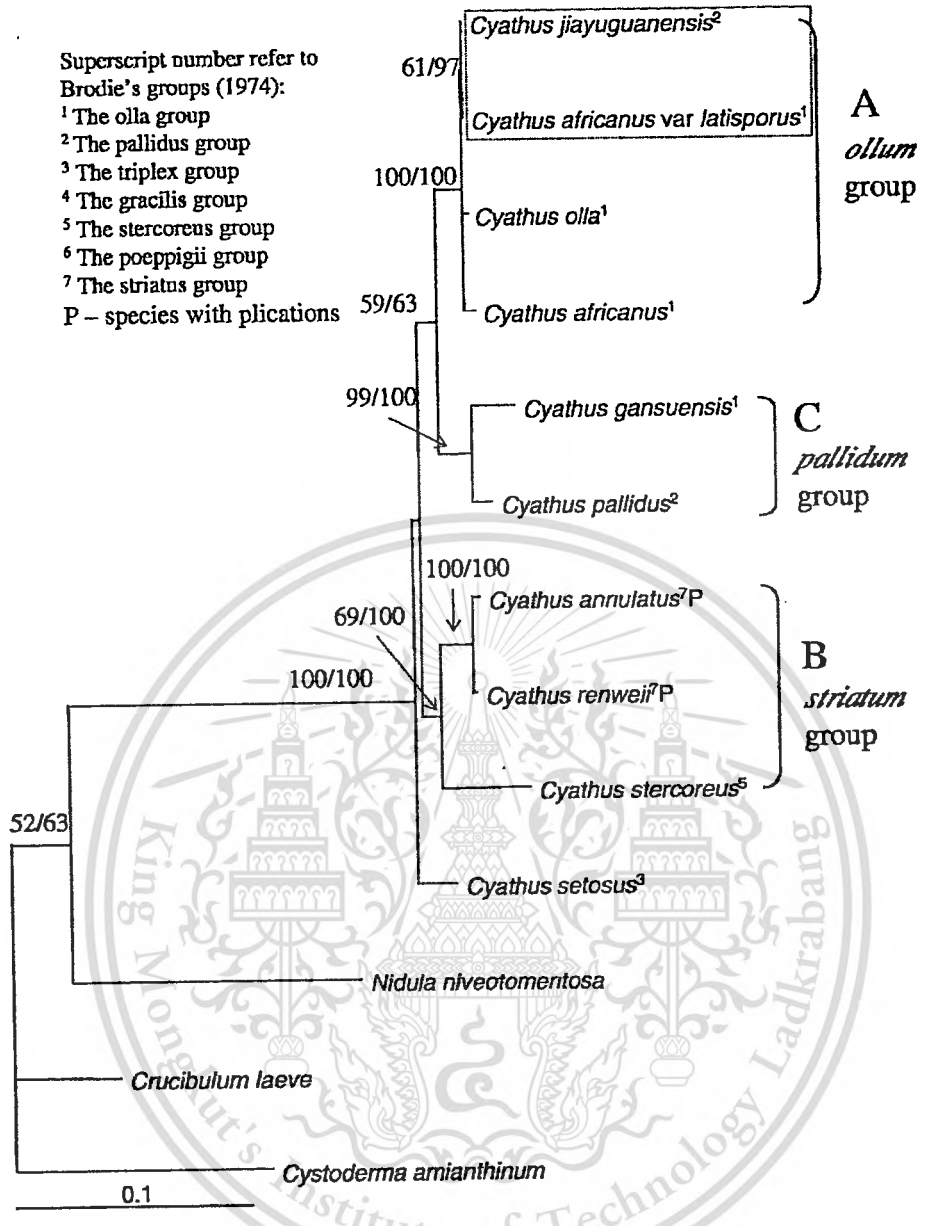

FIG. 3. Phylogeny of *Cyathus* inferred from combined ITS-LSU rDNA sequences. The maximum likelihood tree was rooted with outgroup species *Cystoderma amianthinum*. Bootstrap support (BS) from 1000 replicates in a heuristic search and Bayesian posterior probabilities (PP) values of more than 50% are showed at the nodes (BS/PP) and "-" refers to values less than 50%. Branch length is proportional to number of substitutions. *Cyathus jiayuguanensis* = *Cyathus africanus* var. *latisporus* is shown in the dotted box.

showed that *C. jiayuguanensis* and *C. africanus* var. *latisporus* have identical sequences. *Cyathus africanus* (SWFC 20782) nested with *C. jiayuguanensis* and *C. africanus* var. *latisporus* in the ITS tree (FIG. 1) but was distant from the holotype of *C. africanus*.

DISCUSSION

Systematics of Cyathus.—*Cyathus* has been delimited historically with three separate classification systems (Tulasne 1844, Lloyd 1906, Brodie 1975, 1984). These systems relied heavily on the importance of a plicate peridium and at which rank this character was used to separate taxa. To date Brodie's system is the most widely accepted one, although its phylogenetic significance has been untested. In this study we make an important step toward forming a more natural classification of *Cyathus* by combining morphological data with phylogenies generated from rDNA sequence datasets. Taxa incorporated in this study

included representatives of all seven groups in Brodie's classification system and most data were obtained from either type specimens or authentic Brodie-determined materials (TABLE 1). Phylogenetic trees generated from the ITS, LSU and combined datasets are essentially similar. Our data indicate that the seven-group morphologically based system as proposed by Brodie (1975, 1984) is not concordant with the molecular phylogenies proposed here (FIGS. 1–3).

The presence or absence of plications was the primary character for partitioning groups in traditional taxonomic constructs. The presence or absence of a tunica on the peridioles, types of hairs and fruiting body shape were of secondary importance (Brodie 1975, 1984). However results here indicate that plications on the peridium do not appear to be a phylogenetically informative character. Species possessing this character are distributed in all three major clades in our phylogenies (FIGS. 1–3).

In contrast the size of basidiospores is a significant morphological character for distinguishing the major clades. In Clade B, there are eight *Cyathus* species (*C. annulatus, C. crassimurus, C. helenae, C. poeppigii, C. renweii, C. setosus, C. stercoreus* and *C. triplex*). These species are distributed among five groups in Brodie's system: "triplex", "gracilis", "stercoreus", "poeppigii" and "striatus". All members of Clade B have basidiospores with a length greater than 15 µm. In comparison members of Clade A (*C. africanus, C. africanus* f. *latisporus, C. conlensoi, C. griseocarpus, C. guandishanensis, C. hookeri, C. jiayuguanensis, C. olla, C. olla* f. *anglicus* and *C. olla* f. *brodiensis*), and Clade C (*C. berkeleyanus, C. gansuensis, C. olla* f. *lanatus* and *C. pallidus*) form basidiospores shorter than 15 µm. In previous morphological studies (Tulasne 1844, Lloyd 1906, Brodie 1975, 1984) spore size has been used only in delimitations at the species rank, and no publications reported segregating *Cyathus* species into two major groups based on spores longer or shorter than 15 µm. It should be noted that clades A and C cannot be distinguished from each other solely on spore measurements.

Fruiting body color has been considered useful in the partitioning of species groups (Brodie 1975, 1984). Our morphological studies indicate that species in Clade B have peridia that are brown, reddish-brown or dark brown on the outside while species in clades A and C have peridia that are much lighter, typically yellow, gold or gray. As with basidiospore size, distinguishing among members in clades A and C based solely on peridium coloration is problematical.

Our morphological studies revealed that the best characters for distinguishing members of Clade A from Clade C are the thickness of the tomentum covering the peridium and the outline of fruiting bodies. Species in Clade C have a thick, felt-like tomentum, usually aggregating into shaggy or woolly hairs covering the peridium. Their fruiting bodies are crucible-shaped without a distinct stipe. In comparison species in Clade A have a thin tomentum of fine hairs covering the peridium and the fruiting bodies are funnel-shaped with a constricted base or a distinct stipe.

The problematical taxon *Cyathus setosus* has spores that are 17–24 µm long, has a dark-colored peridium and nests within Clade B in the ITS analysis (FIG. 1). In the analyses of LSU and combined datasets, however, this species was isolated and sister of clades A, B and C. The most conspicuous diagnostic feature for *C. setosus* is the long setae at the mouth of the fruiting body. Whether *C. setosus* represents a distinct group within *Cyathus* will require broader sampling and further testing.

In our analyses *Cyathus* resulted as a monophyletic lineage with 100% PP and 100% BS support in ITS, LSU and combined ITS-LSU trees. In addition, in all three analyses, *Nidula niveotomentosa* was sister of *Cyathus* but with low statistical support. To resolve the relationships among the genera within the Nidulariaceae additional members of the other genera will need to be included; however that was not the focus of this research.

Phylogeny of Cyathus olla *and its forms.*—*Cyathus olla* is the type species of the "olla" group in Brodie's system. In addition to the diagnostic characters of Clade A mentioned above *C. olla* fruiting bodies also have flared mouths, large peridioles, peridia lacking plications and broadly ellipsoid basidiospores. A number of infraspecific taxa have been accepted by various authors, including *C. olla* f. *olla, C. olla* f. *lanatus, C. olla* f. *brodiensis* and *C. olla* f. *anglicus* (Brodie 1952, 1975, 1978, Shinners and Tewari 1998). In Lloyd's (1906) monograph of the Nidulariaceae, *C. anglicus* was described as distinct because of its large fruiting body and markedly sulcate mouth. Later Brodie (1952) accepted it as a form of *C. olla* because single-spore mycelia of typical *C. olla* were found to be sexually compatible with those of *C. anglicus. Cyathus olla* f. *brodiensis*, published by Shinners and Tewari (1998), differs from other forms in having distinct plications on the inside of the peridium and a unique RAPD fingerprint. *Cyathus olla* f. *lanatus* (Brodie 1978) is characterized by having a thick tomentum that aggregates into shaggy or woolly hairs on the outside of the fruiting body. Results from our LSU analyses suggest that *C. olla* f. *lanatus* should be recognized as an independent

species. *Cyathus olla* f. *lanatus* is phylogenetically closer to *C. pallidus* and *C. gansuensis* (Clade C) than to *C. olla* and related species (Clade A). This form therefore is redescribed and a new combination is proposed below.

Phylogeny of Cyathus hookeri *and* C. olla.—The specimens SWFC 20799 of *C. hookeri* and SWFC 21137 of *C. olla* f. *brodiensis* were identified based on the descriptions of Brodie (1975) and Shinners and Tewari (1998), while the specimen BPI 727227 of *C. olla* was identified by Brodie. The ITS sequence analyses reveal a close relationship among *C. hookeri*, *C. olla* and *C. olla* f. *brodiensis*. These taxa form a subclade within Clade A with 95% PP and 67% BS supports. All three taxa are strikingly similar morphologically and are characterized by light-colored peridia, faint plications, and they have the same basidiospore shape and size. These data suggest that *C. hookeri* might represent a synonym of *C. olla*. Before any taxonomic amendment is proposed, further morphological and molecular studies incorporating topotypical isolates of these taxa should be conducted.

Phylogeny of C. jiayuguanensis, C. africanus *and* C. africanus *var.* latisporus.—A similar result was obtained for the species *C. jiayuguanensis* (type), *C. africanus* var. *latisporus* (type) and *C. africanus* (SWFC 20782). The ITS sequences of all three specimens are identical, and in addition they share similar morphologies. The data suggest that they should be treated as one species. However the correct epithet is not *C. africanus*. The phylogenetic placement of the holotype specimen of *C. africanus* (DAOM 200370) is distant from specimen SWFC 20782 that was identified as *C. africanus*. We consider the latter specimen as misidentified. We accept *C. jiayuguanensis* as the correct name for this species and accept *C. africanus* var. *latisporus* as a synonym below.

TAXONOMY

Key to new groups of Cyathus.—Based on analyses of the morphology of 115 *Cyathus* specimens and on phylogenetic analyses of ITS, LSU and combined ITS-LSU datasets of a subset of these specimens, we segregate *Cyathus* into three species groups. To avoid confusion with the species group names of Brodie (1975) the new groups are named herein the "ollum" group (Clade A), "striatum" group (Clade B) and "pallidum" group (Clade C). We tentatively place *Cyathus setosus* in the striatum group based on the ITS analysis, basidiospores >15 µm long, and the dark-colored peridium.

1. Basidiospores 15 µm or longer; fruiting body brown, reddish-brown or dark brown . . . . . *striatum* group
1′. Basidiospores less than 15 µm long; fruiting body light yellow, orange, gold, gray or yellowish-brown 2
2. The base of fruiting body usually abruptly constricted into a distinct stipe; covering of the outside of peridium of fine, short and soft hairs, rarely shaggy . . . . . *ollum* group
2′. Fruiting body not abruptly constricted, with a broad attachment; covering of the outside of peridium with a thick tomentum, felt-like and usually shaggy or woolly . . . . . *pallidum* group

Synonymy and new combination.

Cyathus jiayuguanensis J. Yu, T.X. Zhou et L.Z. Zhao, Mycosystema 21:313. 2002.

= *Cyathus africanus* var. *latisporus* Y.H. Chen et J. Yu, Mycosystema 22:345. 2003.

Fruiting bodies 6–8 mm tall × (4–) 5–6 mm wide at the mouth, cup-like, obconical, with slender stipe; mouth smooth. *Peridia* thin, exterior light brown to grayish-orange and covered with a fine tomentum aggregated into tufts or nodules; inside of peridium brownish-gray or gray, smooth. *Peridioles* 1.5–2 × 1.5–1.8 mm, subcircular, circular or broadly ellipsoid, grayish-brown or lighter; cortex a single layer 20–25 µm thick; tunica 10–20 µm thick. *Basidiospores* 8.5–12.5 × 6.5–8.2 µm, ovoid.

Specimens examined: PR CHINA, GANSU PROVINCE: Jiayuguan, on soil, 12 Oct 1999, *Zhou, T.X. & Zhao, L.Z.* (SWFC 20802; holotype of *C. jiayuguanensis*); PR CHINA, NEIMENGGU PROVINCE: Helingeleqi, on soil, 13 Sep 2000, *Zhou, T.X.* (SWFC 21187; holotype of *C. africanus* var. *latisporus*).

Notes: Because the fruiting bodies of *C. jiayuguanensis* lack plications and are covered with shaggy hairs Yang et al (2002) treated it as the member of the "pallidus" group. The fruiting bodies of *C. africanus* var. *latisporus* lack plications and are covered with tufts of fine hairs that are shorter than in *C. jiayuguanensis*, which resulted in its being treated as a member of the "olla" group (Chen et al 2003). Morphological examinations of the holotype specimens of *C. jiayuguanensis* and *C. africanus* var. *latisporus* showed that they share many morphological characters, such as similar color and size of fruiting bodies, the lack of plications, thin peridial walls, a single cortex with thin tunica and importantly similar basidiospore shape and size. The only distinction between the species is that *C. jiayuguanensis* has longer hairs on the outside of the peridium than

those of *C. africanus* var. *latisporus*. This feature is one of main differences between the "olla" and "pallidus" groups of Brodie (1975). Based on overall morphological similarity and identical ITS sequences we accept *C. africanus* var. *latisporus* as a synonym of *C. jiayuguanensis*.

Cyathus lanatus (H.J. Brodie) R.L. Zhao, comb. et stat. nov.

≡ *Cyathus olla* f. *lanatus* H.J. Brodie, Bot. Notiser 131:31–34, 1978.

Fruiting bodies 5–7 mm high × 5–6 mm wide at mouth, crucible-shaped, without a distinct stipe, broadly attached to a firm thickened base, some flared at the mouth, lip distinctly fimbriate. *Peridia* light gray, light buff or grayish-yellow, externally covered with upward-pointing tufts or radiating tufts formed from thick and fine hairs; interior smooth, lacking plications, gray and shiny. *Peridioles* 1.2–3.5 mm diam, lenticular or irregular in outline, some plump, gray to light buff, shiny. Funiculus stout and short. Tunica thick, cortex a single layer, together 80–100 µm thick. *Basidiospores* 9–13 × 7–8 µm, mostly ovoid, occasionally provided with an apiculus, thick-walled.

Specimens examined: USA, IDAHO: Owyhee County, Reynold's Creek, on dead twigs of *Artemisia* sp., 4 Nov 1976, *T. Trueblood* (DAOM 200703, HOLOTYPE); USA. IDAHO: Owyhee County, West Rabbit Creek, on dead twigs of *Sarcobatus vermiculatus*, 2 Nov 1969, *T. Trueblood* (DAOM 200704, PARATYPE).

Notes: Examination of the holotype specimen revealed some minor differences from those published in the protologue (ibid.). In Brodie's description the tunica was described as up to 60 µm thick with a cortex of 60–80 µm thick (in total 120–140 µm), whereas we found the tunica plus cortex to be 80–100 µm thick. The spores were smaller in our analysis, 9–13 × 7–8 µm vs. 12–15 µm × 7.5–9 µm in the protologue.

Brodie (1978) was reluctant to include this new taxon as a form of *C. olla* because the fruiting bodies were "very small", "often short, thick-walled and bleached." He indicated that at first glance they might be mistaken easily for *Crucibulum laeve*. Brodie included the taxon as a form of *C. olla* based mainly on fruiting-body shape (some fruit bodies were flared at the mouth), on basidiospore size, the lack of plications on the peridium and because *C. olla* "exists in many variations." He stated further that "no test of possible fertility between *C. olla* f. *lanatus* and *C. olla* has been carried out because all attempts to germinate spores of *C. olla* f. *lanatus* have been unsuccessful."

In our analysis based on the LSU dataset *C. lanatus* nested with *C. pallidus* and *C. gansuensis*, species with thick and fine peridial hairs and members of the pallidum group (Clade C). *Cyathus olla* and its forms nest with other species and comprise the group ollum (Clade A). The differences between *C. lanatus* and other species of the pallidum group are mainly plication characters, thickness of fine hairs and basidiospore shape.

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Proposed synonyms in *Cyathus*

RUI-LIN ZHAO[1,2,3], DENNIS E. DESJARDIN[4*], KASEM SOYTONG[1] & KEVIN D. HYDE[5]

ded@sfsu.edu

[1] *Department of Plant Pest Management, Faculty of Agricultural Technology King Mongkut's Institute of Technology Ladkrabang (KMITL) Ladkrabang, Bangkok 10520, Thailand*

[2]*Mushroom Research Foundation 128 Moo3, Bahn Pha Deng, T. Pa Pae, A. Mae Taeng Chiang Mai 50150, Thailand*

[3]*Faculty of Conservation Biology, Southwest Forestry College Kunming, Yunnan Province 650224, PR China*

[4]*Department of Biology, San Francisco State University San Francisco, California, USA*

[5]*Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity The University of Hong Kong, SAR PR China*

Abstract—Based on the morphological analyses of 48 taxa of *Cyathus*, including 30 type specimens, three *Cyathus* species were found to represent synonyms of existing species. *Cyathus cheliensis*, *C. gansuensis* and *C. megasporus* are herein accepted as synonyms of *C. limbatus*, *C. pygmaeus* and *C. poeppigii*, respectively. The latter three species are redescribed and illustrated.

Key words—bird's nest fungi, *Nidulariaceae*, taxonomy

Introduction

The genus *Cyathus* (*Nidulariaceae, Nidulariales*) was established by Haller in 1768. The first monograph on *Cyathus* was published by Lloyd (1906), and later Brodie (1975, 1984) published two monographs on Bird's Nest Fungi wherein he included four genera (*Crucibulum* Tul. & C. Tul., *Cyathus* Haller, *Nidula* V.S. White and *Nidularia* Fr.) and forty nine species of *Cyathus*. Although Brodie's monographs have been followed by most mycologists, some *Cyathus* species in his monographs are questionable, because he did not examine all type specimens. In addition, since the last monograph of Brodie was published in 1984, twenty two additional species of *Cyathus* and five varieties have been published, many of which await critical comparisons with extant type specimens.

328

A systematic study of the genus *Cyathus* was conducted using morphological and molecular approaches (molecular phylogenies will be published elsewhere). The morphological analyses revealed that three recently described *Cyathus* species represent synonyms of older epithets. Although attempts were made to sequence all six taxa referred to in this paper, we were unsuccessful in obtaining useful sequences for *C. megasporus*, *C. limbatus* and *C. pygmaeus*. Our taxonomic conclusions are therefore based on data obtained from examinations of holotype specimens and authentic specimens identified by Brodie.

Materials and Methods

One hundred and fifteen specimens representing forth eight species of *Cyathus*, included thirty type specimens, were borrowed from five herbaria (BPI, DAOM, HMAS, MICH and SWFC; Holmgren & Holmgren 1998) and examined critically following Brodie's (1975) protocols. Pertinent data on peridia and peridiole features, and basidiospore size and shape were documented. A minimum of twenty basidiospores were measured per specimen.

Taxonomy

Cyathus limbatus Tul. & C. Tul., Ann. Sci. Nat., Bot. III, 1: 78, 1844. Figure 1
= *Cyathus cheliensis* F.L. Tai & Hung, Sci. Rep. Nat. Tsing Hua Univ. B, 3: 161, 1948.

Fruiting bodies obconical, mostly incurved at the mouth, constricting abruptly at the base, 6-10 mm high, 5-7 mm wide at mouth; exterior surface of peridium brown, reddish-brown or dark brown, some appearing lighter in color after the hairs are rubbed off; hairs congregated into tufts, appearing hirsute; plications distinct; interior surface of peridium mostly grey, sometimes brownish-grey to dark grey, distinctly striate; lip fimbriate, dark brown; base usually attached to the substrate by a conspicuous mass of mycelia. *Peridioles* mostly circular or subcircular in face view, sometimes broadly ellipsoid, 1.5-2.5 mm diam., with double cortexes 80-100 µm thick, without a tunica. *Basidiospores* hyaline, smooth, ellipsoid to broadly ellipsoid, both ends rounded, thick-walled, 17-23 x 11-14 (-16) µm.

Habit: gregarious on dead wood.

Known distribution: widely distributed in warm countries. Africa, British Guiana (type origin), China, Hawaiian Islands, India, Pacific Island, South America, Thailand, West Indies.

Material examined: One specimen of *C. cheliensis*: CHINA, Yunnan Province, Jinhong, 1939, H.S. Yao, HMAS 02755 (Holotype). Eight specimens of *C. limbatus*: CHINA, Yunnan Province, 11 Sept. 1994, T. X. Zhou, SWFC 20009; Congo, Katanga, 24 Nov. 1960, Schmity Lavecgue, DAOM 200492 (identified by Brodie); place unknown, 1899,

collector unknown, DAOM 200494 (identified by Brodie); JAMAICA, Wedcombe, 3 Sept. 1955, D.A. Powell, BPI 727165 (identified by Brodie); JAMAICA, Kingston, 14 Jan. 1966, H. J. Brodie, BPI 727167 (identified by Brodie); KENYA, Naisafi, Nov. 1953, R. M. Nattiaes, DAOM 200496 (identified by Brodie); UGANDA, time unknown, R. A. Dummer, BPI 727166 (identified by Brodie); USA, Kansas, Neola, 29 Aug. 1956, collector unknown, DAOM 200493 (identified by Brodie).

Notes: *Cyathus limbatus* is characterized by the following combination of features: darkly pigmented fruiting bodies with distinct plications on both peridial surfaces; mouths that are mostly incurved; thick and double cortexes on peridioles; and ellipsoid spores that measure "15 × 10 μm in the type, but 16-22 × 10-12 μm in other collections" (Brodie 1975). Morphological analyses of seven specimens identified by Brodie and of the holotype specimen of *C. cheliensis* indicated that the two taxa are anatomically indistinguishable (See Table 1). We therefore consider *C. cheliensis* to represent a synonym of *C. limbatus*.

Table 1. The comparison of mainly morphological characters between *Cyathus limbatus* and its synonym *Cyathus cheliensis*

Characters	*C. limbatus*	*C. cheliensis*
Shape of fruiting bodies	Obconic, base abruptly constrict, some mouth incurved	Obconic, broadly obconic, base abruptly constrict
Size of fruiting bodies	6-10 × 5-7 mm	7-9 × 7-8 mm
Color of exterior surface of peridium	Brown, reddish brown, dark brown	Russet, reddish brown, brown
Hairs on peridium	Gathered into tufts, hirsute	Gathered into tufts
Plications on peridium	Distinct at exterior and inner surfaces	Distinct at exterior and inner surfaces
Lip of fruiting bodies	fimbriate	fimbriate
Size of peridioles	1.5-2.5 mm in diam.	1.5-1.8 mm in diam.
Structure of peridioles	Double cortexes	Double cortexes
Shape of basidiospores	Ellipsoid, broadly ellipsoid, both ends round	Ellipsoid, broadly ellipsoid, both ends rounded
Size of basidiospores	17-23 × 11-14 (-16) μm	15-20 × 7.8-12.5 μm
Materials source	7 specimens identified by Brodie	Holotype

Tai (1979) omitted structural characters of the peridioles in his description of *C. cheliensis*, which is one of the crucial characters for the determination of *Cyathus* species. *Cyathus olivaceobrunneus* was the only species that was compared with *C. cheliensis* by Tai (1979), which differed from *C. cheliensis* in having lighter colored fruiting bodies, a smooth lip and smaller spores. Affinities of *Cyathus limbatus* to *C. cheliensis* were not addressed by Tai (1979).

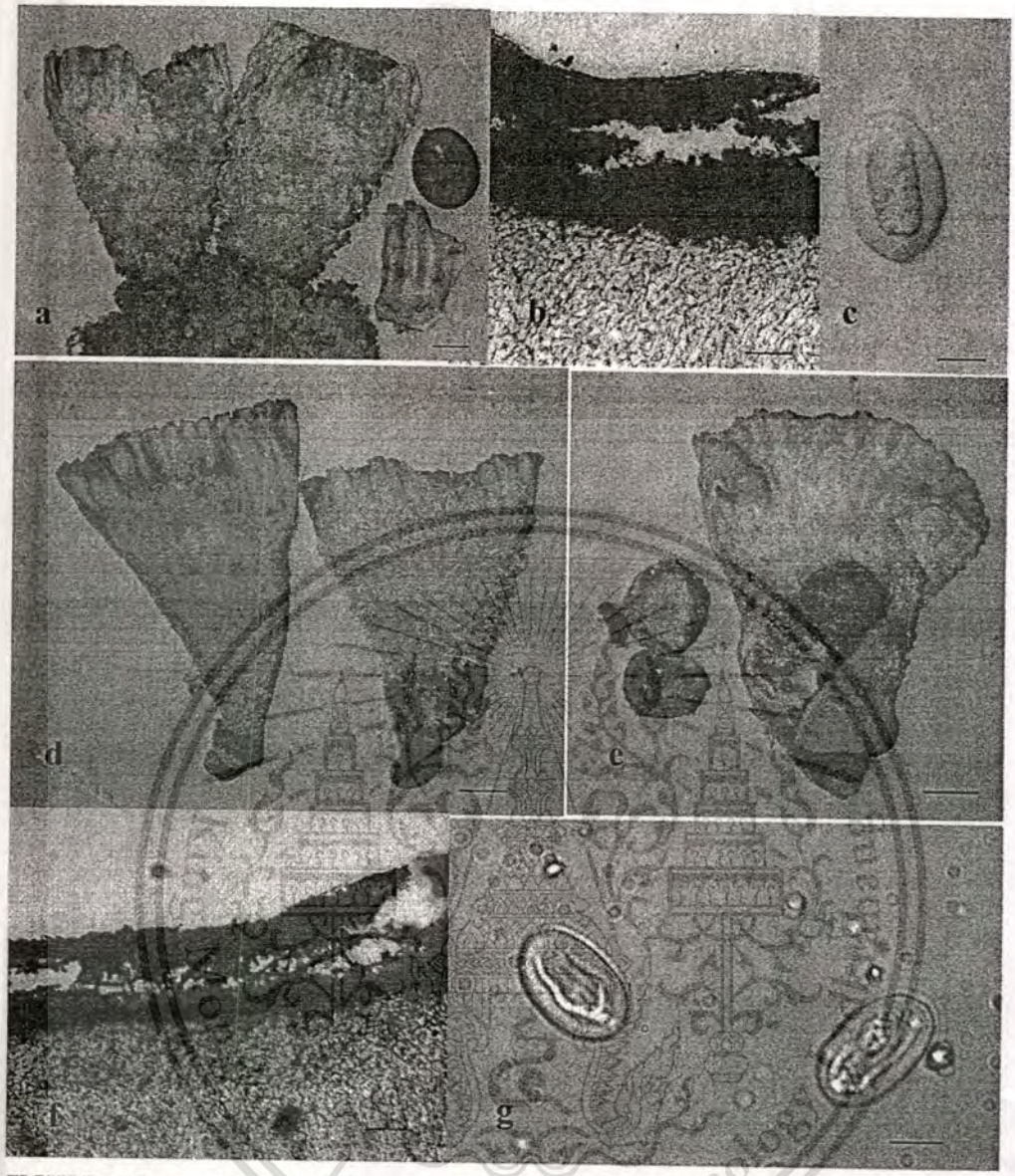

FIGURE 1. Comparison between a-c [*Cyathus limbatus* (DAOM 200496, identified by Brodie)] and proposed synonym d-g [*Cyathus cheliensis* (HMAS 02755 Holotype)]. *a, d, e*—Fruiting bodies, bars = 2 mm; *b & f*—Section of peridioles, double cortex, bars = 25 µm; *c & g*—Basidiospores, bars = 5 µm.

Cyathus pygmaeus Lloyd, Mycological Writings 2, The *Nidulariaceae:* 26, 1906.

Figure 2

= ***Cyathus gansuensis*** B. Yang, J. Yu & T.X. Zhou, Mycosystema 21:313, 2002.

Fruiting bodies variable in shape, obconical with a slender, short stipe or crucible-shaped without a distinct stipe, 3.5-5 mm high, 4-5 mm wide at the straight mouth; exterior surface of peridium light brown, lacking placations,

with hairs soft, short, aggregated into tufts, apex of tufts bleached, felt-like; interior surface of peridium dark gray, smooth, lacking plications; lip smooth; attached to the substrate without a conspicuous mass of mycelia. *Peridioles* circular to broadly ellipsoid, dark grey, 0.8-1.2 mm diam., with a single cortex 16-24 µm thick, and a tunica 16-20 µm thick. *Basidiospores* hyaline, smooth, broadly ellipsoid to ovoid, 11-15(-16) x 8-10 µm.

Habit: gregarious on dead twigs.

Known distribution: Chile, China, USA, typically found in arid habitats.

Material examined: Three specimens of *C. pygmaeus*: CHILE, Santiago, collection time unknown, M. R. Espinosa, BPI 703513 (identified by Lloyd); USA, California, collection time unknown, Stewart S. Towne, BPI 703515 (identified by Lloyd); USA, Washington, June 1909, J. S. Cotton, BPI 703514 (Holotype). One specimen of *C. gansuensis*: CHINA, Gansu Province, Xinglongshan Mountain, 1999, L.Z. Zhao, SWFC 20880 (Holotype).

Table 2. The comparison of mainly morphological characters between *Cyathus pygmaeus* and its synonym *Cyathus gansuensis*

Characters	*C. pygmaeus*	*C. gansuensis*
Shape of fruiting bodies	Obconic with slender, short stipe or crucible-shaped without distinct stipe	Obconic, abruptly constricted at the base
Size of fruiting bodies	3.5-5 × 4-5 mm	2-7 × 4-8 mm
Color of exterior surface of peridium	Light brown	Brown, grayish brown
Color of interior surface of peridium	Dark brown	Dark brown
Hairs on peridium	Tufts aggregated by thick tomenta	Tufts aggregated by tomenta
Plications on peridium	No plications	No plications
Lip of fruiting bodies	Smooth	Smooth
Size of peridioles	0.8-1 mm in diameter	1-1.5 mm in diameter
Structure of peridioles	Single cortex with tunica	Single cortex with tunica
Shape of basidiospores	Broad ellipsoid, ovoid	Ovoid, broadly ellipsoid
Size of basidiospores	12-15 (-16) × 8-10 µm	10-13 × 7.5-10 µm
Materials source	Holotype and 2 specimens identified by Lloyd	Holotype

Notes: *Cyathus pygmaeus* is one of the smallest known *Cyathus* species in terms of fruiting body size. Brodie (1975) stated that three other characters should be used to distinguish *C. pygmaeus* from similar taxa: i) flared rim of fruiting bodies; ii) very dark interior of the cup; and iii) the white, durable epiphragm. Examination of the holotype of *C. pygmaeus* showed that fruiting bodies did not have flared mouths, although a second specimen, BPI 703515 identified by

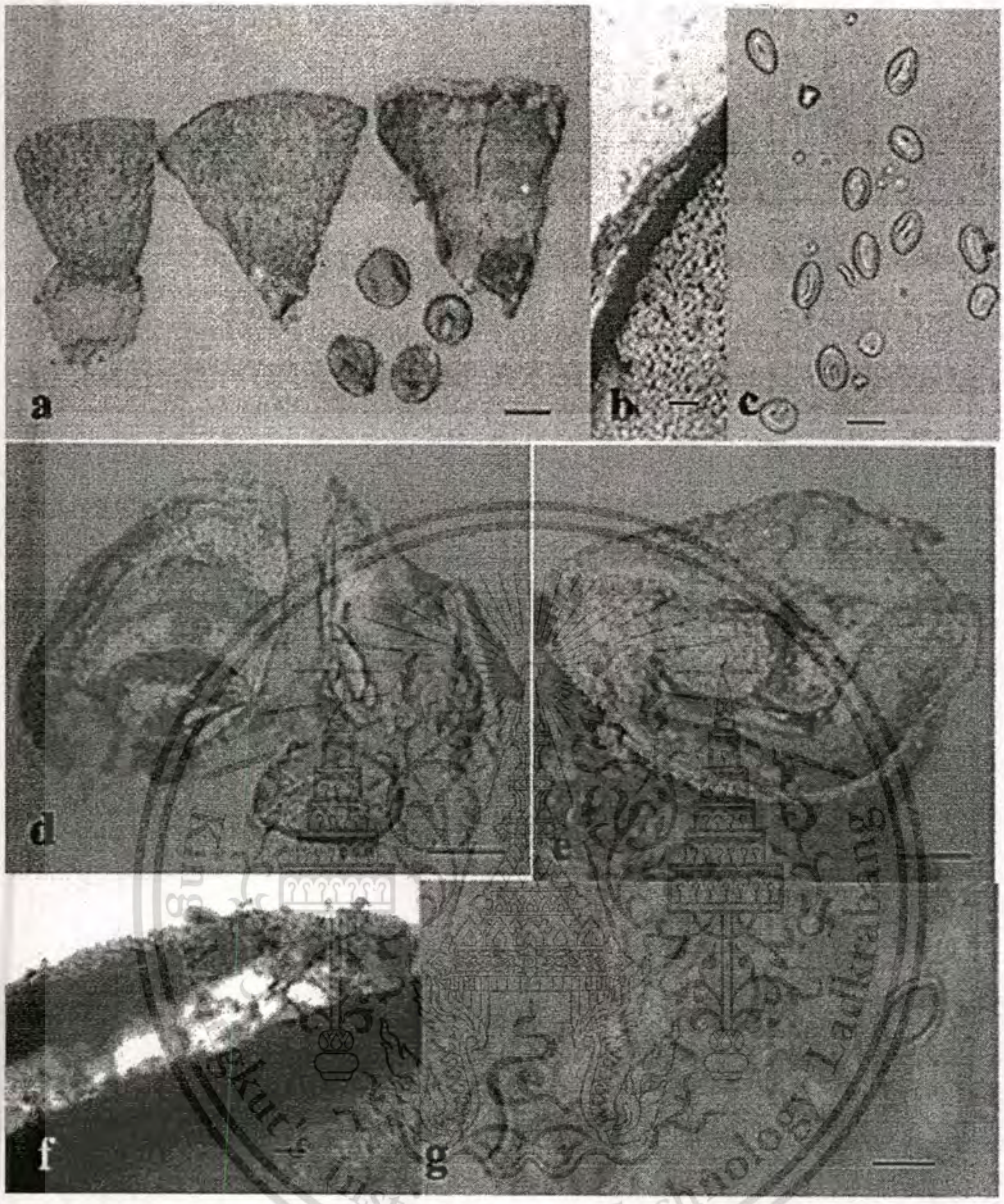

FIGURE 2. Comparison between a-c [*Cyathus pygmaeus* (BPI 703514 Holotype)] and proposed synonym d-g [*Cyathus gansuensis* (SWFC 20880 Holotype)]. *a*—Fruiting bodies, bar = 1.4 mm; *b*—Section of peridiole, one cortex with tunica, bar = 20 µm; *c*—Basidiospores, bar = 9 µm; *d* & *e*—Fruiting bodies, bars = 1 mm; *f*—Section of peridiole, one cortex with tunica, bar = 10 µm; *g*—Basidiospores, bar = 10 µm.

Lloyd, showed a flared mouth on its single fruiting body. Presence of a flared mouth is therefore a variable character in this species. The tiny fruiting bodies, in combination with a dark internal surface of the peridium, however, are reliable characters for species diagnosis. Moreover, the habitat of *C. pygmaeus* in arid areas is also a good diagnostic feature (Brodie 1975).

When *C. gansuensis* was introduced by Yang et al. (2002), it was stated to differ from *C. pygmaeus* in having larger fruiting bodies (4-6 (-9) mm high, (3.5) 5-8 (-9.5) mm wide), larger peridioles (1.5-2 mm long, 0.8-1.5 mm wide) and being darker on the inner surface of peridium. Based on our observations, the fruiting bodies and peridioles of *C. gansuensis* are of similar size to those of *C. pygmaeus*, and inner peridial surfaces are also dark brown. Furthermore the morphology of the peridioles and spores are indistinguishable from those of *C. pygmaeus* (Table 2). *Cyathus gansuensis* was collected from an arid area on Xinglongshan Mountain, Gansu Province. We therefore consider *C. gansuensis* to be synonymous with *C. pygmaeus*.

Cyathus poeppigii Tul. & C. Tul., Ann. Sci. Nat., Bot. III, 1: 77, 1844. Figure 3
= *Cyathus megasporus* W. Ren & T.X. Zhou, Acta Mycol. Sin. 11: 25, 1992.

Fruiting bodies obconical or narrowly obconical, some with mouths incurved, constricting abruptly at the base and forming a slender stipe, 6-8 mm high, 4-5 mm wide; exterior surface of peridium dark brown to reddish-brownwith hairs long, appearing shaggy or hirsute; ridges distinct; internal surface of peridium dark brown to dark grey, deeply plicate, some splitting along the fluted lip; lip fimbriate, dark brown. *Peridioles* circular to subcircular, dark brown, 1.5-2 mm

Table 3. The comparison of mainly morphological characters between *Cyathus poeppigii* and its synonym *Cyathus megasporus*

Characters	*C. poeppigii*	*C. megasporus*
Shape of fruiting bodies	Cup-shaped with slender stipe	Long obconic with slender stipe
Size of fruiting bodies	5-8 × 4-5 mm	5-11 × 3.5-6.5 mm
Color of exterior surface of peridium	Brown, dark brown	Dark brown
Hairs on peridium	Aggregated into long tufts, some shaggy	Shaggy
Plications on peridium	Distinct on exterior and inner surfaces of peridium	Distinct on exterior and inner surfaces of peridium
Lip of fruiting bodies	Smooth or fimbriate	Fimbriate
Size of peridioles	1.5-2 mm in diam.	1.5-2 mm in diam.
Structure of peridioles	Double cortexes	Double cortexes
Shape of basidiospores	Ellipsoid, ovoid	Ellipsoid
Size of basidiospores	(20-)30-45(-50)× (15-)18-30 μm Brodie: 30-42 × 20-28 μm	25-30 × 15-16 μm Ren & Zhou: (24-)31-55(-68) × (15-)18-36.5(-47) μm
Materials source	2 specimens identified by us	Holotype

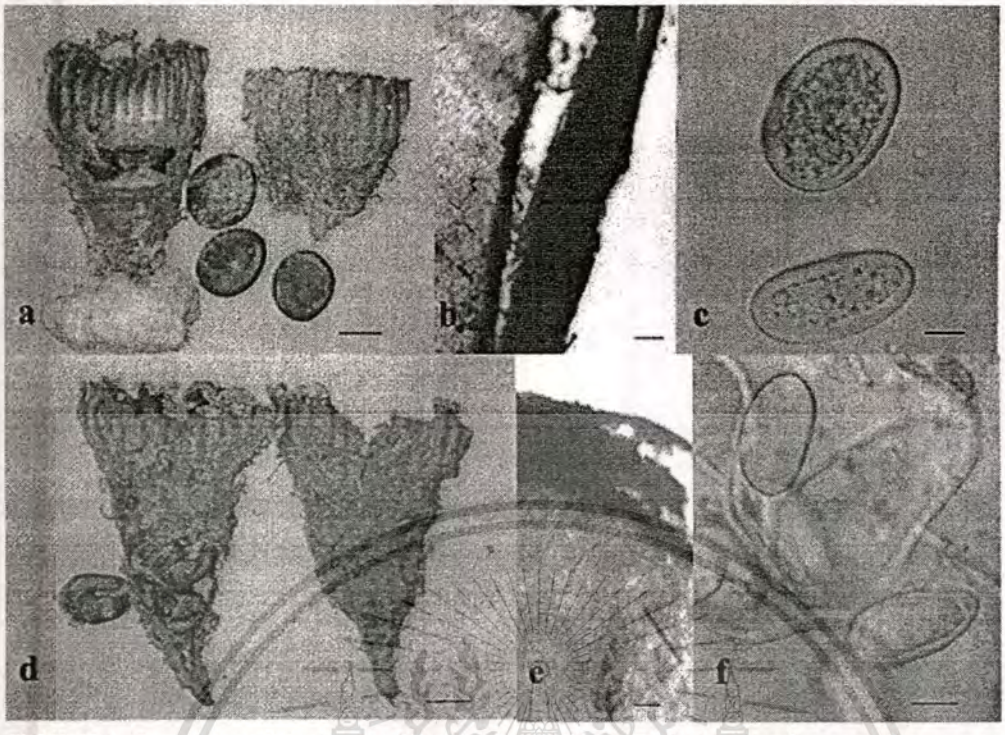

FIGURE 3. Comparison between a-c [*Cyathus poeppigii* (SWFC 21357)] and proposed synonym d-f [*Cyathus megasporus* (SWFC 20448 Holotype)].

a—Fruiting bodies, bar = 2 mm; *b*—Section of peridiole, double cortex, bar = 15 μm; *c*—Basidiospores, bar = 20 μm; *d*—Fruitng bodies, bar = 1.5 mm; *e*—Section of peridiole, double cortex without tunica, bar = 20 μm; *f*—Basidiospores, bar = 16 μm.

diam.; double cortex 50-75 μm thick, composed of interwoven reddish-brown hyphae, without a tunica. *Basidiospores* hyaline, smooth, broadly ellipsoid to ovoid, rounded at both ends, thick or thin walled, (20-)30-45(-50) x (15-)18-30 μm.

Known distribution: Common in tropical areas of Africa, China, Hawaiian Islands, South America and the West Indies (type location: Cuba).

Material examined: Two specimens of *C. poeppigii*: CHINA, Hunan province, Jiuyishan Mountain, 4 Jan. 2001, L.Z. Zhao, SWFC 21400; CHINA, Yunnan Province, Longchuan, 5 July 2000, L.Z. Zhao, SWFC 21357. One specimen of *C. megasporus*: CHINA, Yunnan Province, Kunming, 23 Nov. 1987, X. Xing, SWFC 20448 (Holotype).

Notes: Cyathus poeppigii is characterized by deep plications on the inner peridium, dark brown or reddish brown color, double cortexes in the peridioles and very large basidiospores. The principal variation observed among the collections examined is in basidiospore size. Brodie (1975) stated: "spores of *C. poeppigii* are always large and variable", and "when other characteristics are remarkably constant, the author does not feel that spore size ought to be unduly emphasized as a diagnostic feature of *C. poeppigii*".

Cyathus megasporus was described as a new species by Ren & Zhou (1992) based primarily on its large spore size, reported as (24-) 31-55 (-68) x (15-) 18-36.5 (-47) µm. This is larger than those of *C. poeppigii* whose spores are 30-42 µm x 20-28 µm (Brodie 1975). Re-examination of the holotype of *C. megasporus* revealed that the basidiospores measured 25-30 x 15-16 µm, and other morphological characters were indistinguishable from those of *C poeppigii* (Table 3). We therefore consider *C. megasporus* to be a synonym of *C. poeppigii*.

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Author Biography

Name: Ruilin Zhao

Date of Birth: 4 February 1976

Nationality: Yunnan Province, PR China

Address: Faculty of Conservation Biology, Southwest Forestry University, Kunming, Yunnan Province 650224, PR China.

Education Background:

Sept.1994-Jul.1998 Bachelor of Science in Forestry (Forest Conservation)

Southwest Forestry University, Kunning, Yunnan, PR China

Sept.2000-Jul.2003 Master of Science in Forestry (Forest pathology)

Southwest Forestry University, Kunming, Yunnan, PR China

Oct.2004 up to now Ph. D. student on Technology in Plant Pathology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand

Specialization: Mycology and its molecular phylogeny.

Working experiences and academic activities:

1. Worked at Southwest Forestry University, Kunming, Yunnan Province, PR China as lecturer in Mycology since 2003.
2. 2001, participated the project entitled "the diversity research of higher microfungi in Gaoligong Mountain National Natural Reserve", which was financially supported by Natural Science Finance of Yunnan Province, China
3. 2003-2004, participated the project entitled "Applied Basic Study on Biocontrol the *Cronartium ribicola* Using Biodiversity", which was financially supported by Natural

Science Finance of China.

4. 2004 December to 2005 June, Got **training on molecular phylogenetics of fungi** at the University of Hong Kong.

5. 2004 9-21 August, participated the **Fungal Taxonomy workshop** held at Mushroom Research Foundation, Chiang Mai, Thailand.

6. 2004, 25-26 August, participated the **1st KMITL international Conference on Integration of Science & Technology for Sustainable Development** with an oral presentation entitled *Application of RAPD Analysis in Species Classification of Cyathus,* held at KMITL, Bangkok, Thailand.

7. 2004, 14-19 November participated **The IV Asia-Pacific Mycological Congress & The IX International Marine and Freshwater Mycology Symposium** with an abstract entitled *Four new species of Cladosporium hyperparasitic on other fungi from China,* held in Chiang Mai, Thailand.

8. 2005 14-19 March participated **the GBIF & EASIANET workshop: Proposed Collection/Names/Images digitization** held at The University of Hong Kong, Hong Kong.

9. 2006, 18 Jan -18 May, **Molecular phylogenetics research** at the University of Hong Kong.

10. 2006, 10-19 July, taught *Methods of molecular phylogenetic in mushroom research* in **IV Mycological Taxonomy Workshop** held at Mushroom Research Foundation, Chiang Mai, Thailand.

11. 2006, 21-26 August, Attended **the 8th International Mycological Conference,** with

poster presentations entitled *Ribosomal DNA phylogenies of Cyathus: Is the current infrageneric classification appropriate?* and oral presentation *Mycology in China----expected more international cooperation,* in Cairns, Queensland, Australia

12. 2006, 28-29 October, attended the **Annual Meeting of Thai Mycological Association and Mycological Conference** as co-chair with oral presentation entitled *Ribosomal DNA phylogenies of Cyathus: Is the current infrageneric classification appropriate?* hold in Bangkok, Thailand.
13. 2007 Feb.- April Conducted **Molecular phylogenetics research** of genera *Agaricus* and *Micropsalliota* at The University of Hong Kong.
14. 2007,27-28 April, attended 2nd **KMITL international Conference on Integration of Science & Technology for Sustainable Development** as co-chair with an oral presentation entitled *Cyathus subglobisporus sp. nov. from the northern Thailand based on morphological and molecular data*, held at KMITL, Bangkok, Thailand.
15. 2007, June 23, attended Attended the 2nd **Annual Meeting of Thai Mycological Association and Mycological Conference** hold in Chiang Mai University, Thailand during, as co-chair with oral presentation entitled *The Agaricus and Micropsalliota species from Thailand.*
16. 2007 30 August -31 Dec **Visiting research scholar** engaged in molecular phylogenetic and morphological research of *Agaricus* and *Micropsalliota*, and Mushroom Taxonomy Training at San Francisco State University, San Francisco, California, the USA.
17. 2008 2 – 9 January **Visiting research scholar** about mushroom taxonomy at National Botanic Garden of Belgium (BR), Brussels, Belgium.

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1) **Rui-Lin Zhao**, Guang-Cai Zhao. 2003. Six Hidden Pathogans on Tropical Crops in Yunnan. Journal of Yunnan Tropical Crops Science and Technology. 26(3):5-9.

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