วิวัฒนาการชาติพันธุ์ระดับโมเลกุลของราเอคโตไมคอร์ไรซาในวงศ์ Boletaceae จากภาคเหนือ และภาคตะวันออกเฉียงเหนือของประเทศไทย

นางสาวปวราย์ ปาจิตร์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาพฤกษศาสตร์ ภาควิชาพฤกษศาสตร์ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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MOLECULAR PHYLOGENY OF ECTOMYCORRHIZAL FUNGI IN THE FAMILY BOLETACEAE FROM NORTH AND NORTHEASTERN OF THAILAND

Miss Pawara Pachit

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science Program in Botany Department of Botany Faculty of Science Chulalongkorn University Academic Year 2011 Copyright of Chulalongkorn University

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ปวราย์ ปาจิตร์ : วิวัฒนาการชาติพันธุ์ระดับโมเลกุลของราเอคโตไมคอร์ไรซาในวงศ์ Boletaceae จากภาคเหนือและภาคตะวันออกเฉียงเหนือของประเทศไทย. (MOLECULAR PHYLOGENY OF ECTOMYCORRHIZAL FUNGI IN THE FAMILY BOLETACEAE FROM NORTH AND NORTHEASTERN OF THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.จิตรตรา เพียภูเขียว, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : อาจารย์ ดร.เชิดชัย โพธิ์ศรี, 140 หน้า.

ทำการเก็บตัวอย่างดอกเห็ดของราเอคโตไมคอร์ไรซาในวงศ์ Boletaceae จากแหล่งต่างๆ ใน 5 จังหวัด คือ ชัยภูมิ เชียงใหม่ น่าน พิษณุโลก และอุบลราชธานี ได้ตัวอย่างดอกเห็ดเป็นจำนวน 95 ตัวอย่าง ถูกจัดจำแนก อยู่ใน 8 สกุล Boletus (24 ชนิด) Tylopilus (14 ชนิด) Boletellus (5 ชนิด) Strobilomyces (4 ชนิด) Heimioporus (3 ชนิด) Lecinum (2 ชนิด) Pulveroboletus (1 ชนิด) และ Zangia (1 ชนิด)

ศึกษาความสัมพันธ์เชิงวิวัฒการชาติพันธุ์ของราเอคโตไมคอร์ไรซาในวงศ์ Boletaceae จากตำแหน่ง ของไรโบโซมอลดีเอ็นเอ 2 ตำแหน่ง คือ internal transcribed spacer (ITS) และ large subunit (LSU) ทำการ เปรียบเทียบลำดับนิวคลีโอไทด์ทั้งสองตำแหน่งของตัวอย่างที่ได้กับลำดับนิวคลีโอไทด์จากฐานข้อมูล GenBank จากผลการวิเคราะห์ความสัมพันธ์เชิงวิวัฒนาการชาติพันธุ์จากตำแหน่ง ITS พบว่าสกุล Boletellus Heimioporus Pulveroboletus และ Strobilomyces เป็นกลุ่มแบบวงศ์วานเดี่ยวในขณะที่สกุล Boletus และ Tylopilus ไม่ใช่กลุ่มแบบวงศ์วานเดี่ยว ซึ่งแตกต่างจากผลการวิเคราะห์ความสัมพันธ์เชิงวิวัฒนาการชาติพันธุ์ จากตำแหน่ง LSU ที่แสดงให้เห็นว่าเฉพาะสกุล Heimioporus Pulveroboletus และ Strobilomyces เป็นกลุ่ม แบบวงศ์วานเดี่ยว โดยความแตกต่างนี้อาจมาจากจำนวนลำดับนิวคลีโอไทด์ในแต่ละสกุลที่ใช้วิเคราะห์จาก ้ตำแหน่ง ITS มีน้อยเกินไป แต่อย่างไรก็ตาม ความสัมพันธ์ระหว่างสกุลในวงศ์นี้ยังไม่ชัดเจน นอกจากนี้ ทำการศึกษาวิวัฒนาการชาติพันธุ์โดยใช้ไรโบโซมอลทั้งสองตำแหน่งของสกุล Tylopilus ซึ่งเป็นสกุลที่มีจำนวน สมาชิกมาก จากผลการวิเคราะห์เชิงวิวัฒนาการชาติพันธุ์ของทั้งสองตำแหน่งมีความสอดคล้องกันและแสดงให้ เห็นว่า *Tylopilus* สามารถแบ่งเป็นอย่างน้อย 4 กลุ่ม นอกจากนี้ความสัมพันธ์ระหว่างชนิดของสกุล Tylopilus ้ยังสัมพันธ์กับวงศ์ของพืชอาศัยด้วย จากการศึกษาครั้งนี้ พบว่า มีราสกุล Tylopilus อย่างน้อย 14 ชนิดใน ประเทศไทย นอกจากนี้การศึกษาในครั้งนี้ยังให้ข้อมูลของราในวงศ์ Boletaceae ในเขตร้อนทั้งทางด้านสัณฐาน ้วิทยาและข้อมูลเชิงโมเลกุล แต่อย่างไรก็ตาม ควรมีการศึกษาราในวงศ์นี้เพิ่มเติมโดยเฉพาะอย่างยิ่งในเขตร้อน เพื่อเติมเต็มองค์ความรู้ทางด้านระบบวิทยาของราวงศ์ Boletaceae

ภาควิชา	พฤกษศาสตร์	ลายมือชื่อนิสิต
สาขาวิชา	พฤกษศาสตร์	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก
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PAWARA PACHIT : MOLECULAR PHYLOGENY OF ECTOMYCORRHIZAL FUNGI IN THE FAMILY BOLETACEAE FROM NORTH AND NORTHEASTERN OF THAILAND. ADVISOR : ASST.PROF. JITTRA PIAPUKIEW, Ph.D., CO-ADVISOR : CHERDCHAI PHOSRI, Ph.D., 140 pp.

The ectomycorrhizal basidiocarps in the family Boletaceae were collected from various forests and plantations in five provinces, Chiang Mai, Chaiyaphum, Nan, Phitsanulok and Ubon Ratchathani. Ninety-five basidiocarps were classified in 8 genera, *Boletus* (24 species), *Tylopilus* (14 species), *Boletellus* (5 species), *Strobilomyces* (4 species), *Heimioporus* (3 species), *Lecinum* (2 species), *Pulveroboletus* (1 species) and *Zangia* (1 species).

Phylogenetic relationships among ectomycorrhizal Boletaceae were studied based on both ribosomal DNA regions, internal transcribed spacer (ITS) and large subunit (LSU). ITS and LSU sequences of Thai specimens were compared with some species in Boletaceae available in GenBank database. Phylogenetic analysis based on ITS suggested that *Boletellus, Heimioporus, Pulveroboletus* and *Strobilomyces* were monophyletic groups while *Boletus* and *Tylopilus* were not monophyletic groups. It was dissimilar to phylogenetic tree based on LSU which indicated that only *Heimioporus, Pulveroboletus* and *Strobilomyces* were monophyletic groups. This inconsistence might be from the few ITS sequences in each genus. However, the relationships among genera were still unclear. In addition, the phylogenetic analyses showed that *Tylopilus* could be clearly divided into at least 4 clades. Moreover, the relationships among *Tylopilus* species corresponded to their host plant families. Fourteen *Tylopilus* species existed in Thailand. Moreover, this study provided the important morphological and molecular studies in this family especially in tropical region is significantly needed to fulfill the systematic study of Boletaceae.

Department : Botany	Stu	dent's Signature
Field of Study : Botany	, Ad	visor's Signature
nold of olday i <u>Bolany</u>		
Academic Year : 2011	Co-	advisor's Signature

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LIST OF ABBREVIATIONS

- G Gram
- µl Microliter
- mg Milligram
- ml Milliliter
- M Molar
- s Second
- h Hour
- ITS Internal Transcribed Spacer
- LSU Nuclear Large Subunit
- BS Bootstrap supported

CHAPTER I

INTRODUCTION

Ectomycorrhiza is a mutualistic relationship between higher plants (Angiosperms and Gymnosperms) and soil-borne fungi which are not plant pathogen (Brundrett and Cairney, 2002; Tagu *et al.*, 2002). Two partners reciprocate advantages. The plants allocate photosynthetic carbohydrates to the heterotrophic fungi. The hyphae of the fungi explore the soil and absorb both water and minerals which are particularly a low mobility in the soil such as phosphorous and nitrogen and then water and these elements are transferred to the roots (Tagu *et al.*, 2002; Taylor and Alexander, 2005; Courty *et al.*, 2010). Apart from the ectomycorrhizal fungi are essential to the health and growth of host plants by nutrient uptake; they can protect root systems from pathogenic attacks, and adverse abiotic soil conditions like water-stress and heavy metal contamination (Tagu *et al.*, 2002; Ray *et al.*, 2005).

The major group of ectomycorrhizal fungi is family Boletaceae which represent 18-25% of all (Halling *et al.*, 2008). The diversity of Boletaceae has long been researched worldwide, especially in North America and Europe. Recently, family Boletaceae consists of approximate 39 genera and more than 700 species. The basidiocarps of Boletaceae are diverse forms. The fruiting bodies include conspicuous stipitate-pileate forms that mostly have tubular hymenophores and some genera have lamellate or intermediate hymenophores. In several genera, the fruiting bodies are gasteroid (puffball-like forms). This fungal family distributes across temperate and tropical regions (Halling *et al.*, 2007; Kirk *et al.*, 2008; Desjardin *et al.*, 2009; Orihara *et al.*, 2010; Li *et al.*, 2011).

In Thailand, the diversity of Boletaceae is abundant particularly in Northern and Northeastern (Klinhom and Klinhom, 2007; Thangklam, 2008). Chantorn *et al.* (2007) reported the diversity of Boletaceae in Nam Nao and Phu Rua National Parks during rainy seasons of 2005 and 2006. There were fifty-two specimens which belonged to nine genera. Nine species were new records to Thailand. One hundred and three species of Boletaceae from 11 genera in Northeast part were reported and described by Klinhom and Klinhom (2007). Forty-four species of Boletaceae in Thailand were described and illustrated by Chandrasrikul *et al.* (2008). Thongklam (2008) studied Boletes diversity in eight national parks of upper northern Thailand during year 2005-2006. Eighty-three species from 13 genera were found. Twenty-nine species were new recorded in Thailand. Moreover, new genus, *Spongiforma*, was found in Khao Yai National Park (Desjardin *et al.*, 2009). Although several reports demonstrated high diversity in Boletaceae in Thailand but they have never been well studied owning to lack of experienced experts (Chantorn *et al.*, 2007). Moreover, little knowledge of the systematics of this family based on molecular phylogeny has been obtained.

According to molecular phylogeny of Boletales (Binder and Hibbett, 2006), the family Boletaceae was monophyletic group but the relationship among this family was unclear and the large genera such as *Boletus*, *Xerocomus* and *Tylopilus* were not monophyletic group. As the phylogeny of Boletes (Drehmel *et al.*, 2008), *Leccinum* and *Suillus* were monophyletic group but *Boletus*, *Xerocomus* and *Tylopilus* were not. Aside from the relationship among genera in this family, the phylogeny between the species in each genus was investigated.

Therefore, the main objectives of this study are

To study phylogenetic relationships between the genera and species of ectomycorrhizal fungi in the family Boletaceae in Thailand based on nuclear large subunit rDNA and Internal transcribed spacer.

CHAPTER II

LITERATURE REVIEW

2.1 Overview of Ectomycorrhiza

Ectomycorrhiza is a mutualistic association between higher plants (Angiosperms and Gymnosperms) and soil-borne fungi which are not plant pathogen. This mutualism is beneficial to plants. The fungal mycelium increases the absorptive surface of the root and intensifies the entry of water and nutrients such as phosphorus and nitrogen into the plant, as a consequence, promotes plant growth. In return, the plant allocates photosynthetic carbohydrates such as glucose to the fungus. (Brundrett and Cairney, 2002; Tagu *et al.*, 2002; Taylor and Alexander, 2005; Courty *et al.*, 2010).

Ectomycorrhizas consist of three structural components: (1) a sheath or mantle of fungal tissues which encloses the root tip; (2) a labyrinthine inward growth of hyphae between the epidermal and cortical cells called the Hartig net (Figure 2.1); (3) an outwardly growing system of hyphal elements called an extraradical mycelium which forms essential connections of the Hartig net both with the soil and reproductive structures (Smith and Read, 1997). These relationships are formed predominantly on the fine root tips of the host plants. The fungal differentiation processes in the plant induce architectural changes at the tissues and organ levels of root (e.g. enhanced formation of root tips, root hair suppression) as well as cellular differentiation that includes cell-wall and cytoskeleton reorganization. These roots are usually short and rise to racemose system of branching. Ectomycorrhizal development is also accompanied with the differentiation of specialized interfaces between the hosts and mycobionts, resulting in a highly coordinated metabolic interplay. (Lakhanpal, 2000; Brundrett and Cairney, 2002; Tagu *et al.*, 2002; Taylor and Alexander, 2005).



Figure 2.1 Section of *Populus tremuloides* ectomycorrhizal root, the mantle (M), cortex (C), endodermis (En) and Hartig net (arrows) are visible. (Brundrett and Cairney, 2002)

According to the modification of morphology and physiology of ectomycorrhizal roots, the ectomycorrhizal fungi can provide several non-nutritional benefits to the host plants particularly the seedlings. Ectomycorrhizal plants are often more resistant to diseases, such as those caused by microbial soil-borne pathogens, and are also more resistant to the effects of drought. Moreover, several ectomycorrhizal fungi can ameliorate the toxicity of heavy metal and increase the tolerance of their hosts (Carnery and Chambers, 1999; Tagu *et al.*, 2002; Ray *et al.*, 2005).

Ectomycorrhizal fungi serve many important roles in forest ecosystem. They contribute to a number of key ecosystem functions such as carbon cycling and nutrient cycling (Taylor and Alexander, 2005; Courty *et al.*, 2010). Since ectomycorrhizal fungi play an important role in seedling establishment (Tedersoo *et al.*, 2010), these fungi are used as inocula in reforestation programs. The inoculated seedlings of host plants exhibit better growth than non-inoculated seedlings in the nurseries as well as the survival rate of inoculated seedlings is higher than non-inoculated seedlings after transplanting (Lakhanpal, 2000).

2.2 Diversity of Host Plants

Around 8,000 species or approximately 3% of seed plants form ectomycorrhizas (Taylor and Alexander, 2005). Eventhough number of plant species is minor; these plant families, Pinaceae, Abietaceae, Fagaceae, Betulaceae, Nothofagaceae, Myrtaceae, Dipterocarpaceae and Caesalpiniaceae, are ecologically and economically important forest trees by dominating woodland and forest communities in boreal, Mediterranean, and temperate forests of the Northern Hemisphere and parts of South America, seasonal savanna and rain forest habitats in Africa, India and Indo-Malay as well as temperate rain forest and seasonal woodland communities of Australia (Table 2.1) (Tedersoo *et al.*, 2010).

Host taxon	Europe	North	Temperate	South-	India and	Africa	Northern	Southern	Australia	New
		America	Asia	East	Sri		South	South		Zealand
				Asia	Lanka		America	America		
Acacia									Х	
Arbutoideae	х	х	Х	х			х			
Betulaceae	Х	Х	Х	Х				Х		
Bossiaeeae									Х	
Caesalpinioideae				Х	Х	х	Х		Х	
Casuarinaceae				Х					Х	
Cistaceae	х	х	Х							
Coccoloba							Х			
Dipterocarpaceae				х	Х	Х	Х			
Fagaceae	х	х	Х	Х			Х			
Leptospermoideae				Х					Х	Х
Nothofagaceae								х	х	Х
Pinaceae	Х	Х	Х	х			Х			
Pisoniae							Х		х	
Pomaderreae									Х	Х
Salicaceae	х	х	Х	х			х	х		
Uapacaceae						Х				

 Table 2.1 Distribution of host taxa in biogeographic realms (Tedersoo *et al.*, 2010)

Wide taxonomic distribution of ectomycorrhizal plants and fungi in all continents (except Antarctica) and large continental islands suggests an ancient evolution of the ectyomycorrhiza. Pinaceae is certainly the oldest extant plant family that associates with ectomycorrhizal fungi. The oldest Pinaceae fossils originated 156 million years ago and the oldest ectomycorrhizal root fossils were found with the roots of *Pinus* from the middle Ecocene Princeton chert (Raina *et al.*, 2000; Tedersoo *et al.*, 2010).

2.3 Diversity of Ectomycorrhizal Fungi

For the mycobionts, the number of fungal species in ectomycorrhizal symbiosis is estimated to be approximately 20,000-25,000. Most of ectomycorrhizal fungi belong to Phylum Basidiomycota and other belong to Phylum Ascomycota and Zygomycota (only 1 genus, *Endogone*) (Agerer, 2006; Tedersoo *et al.*, 2010). The list of ectomycorrhizal fungal genera is shown in Table 2.2. Tedersoo *et al.* (2010) have reported that 216 fungal genera were considered ectomycorrhizal fungi and the largest number of ectomycorrhizal fungi was found in the order Pezizales, Agaricales, Boletales, Cantharellales and Helotiales. Molecular phylogenetic and identification studies suggest that ectomycorrhiza has arisen independently and persisted at least 66 times in 3 fungal phyla.

Phyla	Families	Genera
Zygomycota	Endogonaceae	Endogone
Ascomycota	Discinaceae	Gymnohydnotria, Gyromitra
	Elaphomycetaceae	Elaphomyces
	Geoglossaceae	Geoglossum, Spathularia
	Helotiaceae	Hymenoscyphus, Neocudoniella
	Helvellaceae	Balsamia, Barssia, Fischerula, Helvella, Hydnotria,
		Leucangium, Underwoodia, Picoa, Wynnella
	Morchellaceae	Morchella, Verpa
	Pezizaceae	Amylascus, Boudiera, Hydnobolites, Hydnotryopsis,
		Pachyphloeus, Peziza, Plicaria, Ruhlandiella,
		Sphaerozone, Tirmania

Table 2.2 Genera of ectomycorrhizal fungi (Agen	er, 2006)
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Phyla	Families	Genera
Ascomycota	Pyronemataceae	Genea, Geopora, Humaria, Hydnocystis, Nothojafnea,
		Phaeangium, Pulvinula, Sphaerosoma, Sphaerosporella,
		Tricharina, Wilcoxina
	Terfeziaceae	Cazia, Delastria, Loculotuber, Terfezia
	Tuberaceae	Choiromyces, Dingleya, Labyrinthomyces, Paradoxa,
		Reddellomyces, Tuber
Basidiomycota	Albatrellaceae	Albatrellus, Polyporoletus, Scutiger
	Amanitaceae	Amanita
	Atheliaceae	Amphinema, Byssocorticium, Byssoporia, Piloderma,
		Tylospora
	Bankeraceae	Bankera, Boletopsis, Hydnellum, Phellodon, Sarcodon
	Bolbitiaceae	Descomyces, Descolea
	Boletaceae	Afroboletus, Aureoboletus, Austroboletus, Boletellus,
		Boletus, Chalciporus, Chamonixia,Gastroboletus,
		Gastroleccinum, Gastrotylopilus, Leccinum, Paxillogaster,
		Phylloboletellus, Phylloporus, Porphyrellus,
		Pulveroboletus, Royoungia, Strobilomyces, Tubosaeta,
		Tylopilus, Veloporphyrellus, Xerocomus
	Cantharellaceae	Cantharellus, Craterellus
	Chondrogastraceae	Chondrogaster
	Clavulinaceae	Clavulina
	Cortinariaceae	Annamika, Cortinarius, Cuphocybe, Dermocybe,
		Destuntzia, Hebeloma, Inocybe, Mackintoshia,
		Mycoamaranthus, Naucoria, Rozites, Setchelliogaster,
		Stephanopus, Thaxterogaster
	Cribbeaceae	Cribbea, Mycolevis
	Entolomataceae	Clitopilus, Entoloma
	Geastraceae	Geastrum, Radiigera
	Gomphaceae	Clavariadelphus, Gomphus

Table 2.2 (continued) Genera of ectomycorrhizal fungi (Agerer, 2006)

Phyla	Families	Genera		
Basidiomycota	Gomphidiaceae	Brauniellula, Chroogomphus, Cystogomphus,		
		Gomphidius, Gomphogaster		
	Gyroporaceae	Gyroporus, Rubinoboletus		
	Hydnaceae	Hydnum		
	Hydnangiaceae	Hydnangium, Laccaria, Maccangia, Podohydnangium		
	Hygrophoraceae	Camarophyllus, Hygrophorus		
	Hymenochaetaceae	Coltricia		
	Hymenogastraceae	Hymenogaster, Quadrispora		
	Hysterangiaceae	Hysterangium, Trappea		
	Leucogastraceae	Leucogaster, Leucophlebs		
	Marasmiaceae	Rhodocollybia		
	Melanogastraceae	Alpova, Corditubera, Hoehnelogaster, Melanaogaster		
	Mesophelliaceae	Andebbia, Castoreum, Gummiglobus, Mesophellia		
	Octavianiaceae	Octaviania, Sclerogaster		
	Paxillaceae	Austrogaster, Austropaxillus, Gymnopaxillus, Gyrodon,		
		Paxillus		
	Ramariaceae	Austrogautieria, Gautieria, Ramaria		
	Rhizopogonaceae	Rhizopogon		
	Russulaceae	Arcangeliella, Cystangium, Elasmomyces, Gymnomyces,		
		Lactarius, Macowanites, Martellia, Russula, Zelleromyces		
	Sebacinaceae	Sebacina		
	Sclerodermataceae	Astraeus, Calostoma, Pisolithus, Scleroderma		
	Suillaceae	Boletinus, Gastrosuillus, Psiloboletinus, Suillus		
	Thelephoraceae	Amaurodon, Lenzitopsis, Pseudotomentella, Thelephora,		
		Tomentella, Tomentellopsis		
	Tricholomataceae	Catathelasma, Leucopaxillus, Lyophyllum, Tricholoma		
	Truncocolumellaceae	Truncocolumella		

Table 2.2 (continued) Genera of ectomycorrhizal fungi (Agerer, 2006)

2.4 Boletales

Boletales is the one of major group of mushroom-forming fungi that worldwide distribution in various forest ecosystems. Currently, this order is classified in Kingdom Fungi, Phylum Basidiomycota and Class Agaricomycetes (Binder and Hibbett, 2006; Kirk et al., 2008). The basidiocarps of Boletales are in diverse forms (Figure 2.2). The fruiting bodies include conspicuous stipitate-pileate forms that mostly have tubular hymenophores and some genera have lamellate or intermediate hymenophores. In several genera, the fruiting bodies are gasteroid (puffball-like forms) or resupinate. Moreover, species in Boletales pursue various habits. Saprotrophs among this order have developed a unique mode of brown-rot while white-rot saprotrophy is absent in this group. Ectomycorrhiza are established by the greater of Boletales. This group is associated with various plant families such as Betulaceae, Casuarinaceae, Dipterocarpaceae, Ericaceae, Fabaceae, Fagaceae, Mimosaceae, Myrtaceae, Pinaceae and Salicaceae. Few species in Boletales are mycoparasites (Binder and Hibbett, 2006).



Figure 2.2 Morphological diversity of basidiocarps in Boletales, A. stipitate-pileate with pores (tubular hymenophores), B. stipitate-pileate with gills (lamellate hymenophores) and C. gasteroid.

According to molecular systematic studies, Binder and Hibbett (2006) found that the Boletales was strongly supported as monophyletic group and had closely relationship with Agaricales and Atheliales. Six major lineages of Boletales that currently were recognized on subordinal level, Boletineae, Paxillineae, Sclerodermatineae, Suillineae, Tapinellineae, and Coniophorineae received varied support values. Boletineae and Suillineae received the highest support values but other lineages were not consistently resolves as monophyly. The basal group in the Boletales was Tapinellineae which consists of brown-rotting fungi. However, the relationships among genera in Boletinae were poorly resolved and most of the larger genera were not monophyletic.

2.5 Boletaceae: Diversity and Biology

Boletaceae is the one of main genera in Boletales. This family includes obvious stipitate-pileate forms which mainly have tubular hymenophores (Binder and Hibbett, 2006; Halling *et al.*, 2007). But the basidiocarps of some genera may be gasteroid (Yang *et al.*, 2007; Kirk *et al.*, 2008). Moreover, some species in Boletaceae are saprotrophs or parasites (Binder and Hibbett, 2006). But most of all are ectomycorrhizal fungi (Halling *et al.*, 2008)

Family Boletaceae is the major group of ectomycorrhizal fungi and may represent 18-25% of all ectomycorrhizal fungi (Halling *et al.*, 2008). Agerer (2006) reported the genera of ectomycorrhizal fungi and several genera in family Boletaceae were recorded as follows: *Afroboletus, Aureoboletus, Austroboletus, Boletellus, Boletus, Chalciporus, Chamonixia, Fistulinella, Gastroboletus, Gastroleccinum, Leccinum, Paxillogaster, Phylloboletellus, Phylloporus, Pulveroboletus, Royoungia, Strobilomyces, Tubosaeta, Tylopilus, Veloporphyrellus and Xerocomus. In addition, 4 newly genera in Boletaceae, <i>Bothia* (Halling *et al.*, 2007), *Heliogaster* (Orihara *et al.*, 2010), *Spongiforma* (Desjardin *et al.*, 2009) and *Zangia* (Li *et al.*, 2011) have been recognized as ectomycorrhizal fungi. The morphology, distribution and host plant families of some genera in Boletaceae were described by Halling (2011) (Table2.3).

Fungal Genera	Host Plant Families								
	Fagaceae	Dipterocarpaceae	Pinaceae	Myrtaceae	Casuarinaceae	Caesalpinoidae	Betulaceae	Nothofagaceae	Sapotaceae
Afroboletus	Х	Х				Х			
Aureoboletus	х		х						
Austroboletus	х	Х	х	Х	Х				
Boletellus	х	Х	х	х	Х	Х			
Boletochaete		Х				Х			
Boletus	Х	Х	Х	Х	Х	Х	Х		
Bothia	Х								
Chalciporus	Х		Х						
Chamonixia			х						
Fistulinella	х			Х		Х		Х	Х
Heimioporus	х	х		Х	Х				
Leccinellum	Х						Х		
Leccinum	Х	х	х			Х	Х		
Phylloporus	х	х	х	Х	Х				
Pulveroboletus	Х	Х	Х	Х		Х			
Retiboletus	Х								
Rhodactina		Х							
Royoungia				Х	Х				
Spongiforma		Х							
Strobilomyces	Х	Х		Х	Х	Х			
Tuboseta	Х	Х				Х			
Tylopilus	Х	Х	Х	Х	Х	Х	Х	Х	
Veloporphyrellus	Х								
Xanthoconium	Х		х	Х	Х				
Zangia	Х		Х						

Table2.3 Some ectomycorrhizal genera and their host plant families (Halling, 2011)

The diversity of Boletaceae has long been researched worldwide, especially in North America and Europe. Recently, family Boletaceae consists of approximate 39 genera and more than 700 species. This fungal family distribute across temperate and tropical regions (Halling *et al.*, 2007; Kirk *et al.*, 2008; Desjardin *et al.*, 2009; Orihara *et al.*, 2010; Li *et al.*, 2011). In Thailand, the diversity of Boletaceae is abundant particularly in Northern and Northeastern (Klinhom and Klinhom, 2007; Thangklam, 2008) but it has never been well studied owning to lack of experienced experts (Chantorn *et al.*, 2007). Chantorn *et al.* (2007) reported the diversity of Boletaceae in Nam Nao and Phu Rua National Parks during rainy seasons of 2005 and 2006. There were fifty-two specimens which belonged to nine genera as follows: *Boletellus, Boletus, Heimiella* (*Heimioporus*), *Leccinum, Phylloporus, Pulveroboletus, Strobilomyces, Tylopilus* and *Xerocomus*. Nine species were new records to Thailand. One hundred

and three species of Boletaceae from 11 genera in Northeast part were reported and described by Klinhom and Klinhom (2007). Forty-four species of Boletaceae in Thailand were described and illustrated by Chandrasrikul *et al.* (2008). Thongklam (2008) studied Boletes diversity in eight national parks of upper northern Thailand during year 2005-2006. Eighty-three species from 13 genera (*Aureoboletus, Austroboletus, Boletellus, Boletus, Chalciporus, Heimioporus, Leccinum, Porphyrellus, Pulveroboletus, Rubinoboletus, Strobilomyces, Tylopilus, and Xerocomus*) were found. Twenty-nine species were new recorded in Thailand. Moreover, new genus, *Spongiforma*, was found in Khao Yai National Park (Desjardin *et al.*, 2009).

2.6 Systematics of Boletaceae

The systematic of Boletales (include Boletaceae) have been widely studied in recent years (Binder and Hibbett, 2006) based on morphology (Corner, 1972; Moser, 1978; Agerer, 1999) and pigment chemistry (Besl and Bresinsky, 1997). But the relationships of some group of fungi were controversial and unresolved. For example, chemosystematic study of *Boletinus*, *Suillus*, *Gastroboletus*, *Gomphidius*, and Chroogomphus suggested that Suillus is more closely related to the Gomphidiaceae and Rhizopogonaceae than to other boletes. Therefore, a new family, Suillaceae was established and combined with Gomphidiaceae and Rhizopogonaceae into new suborder based on pigment (Besl and Bresinsky, 1997) although their morphology of basidiocarps were different. For the relationship resovling, since 1990, phylogenetic studies of various groups of fungi began to appear (Hibbett, 2007). These phylogenetic studies based on molecular data almost have used ribosomal gene, both of mitochondrial and nuclear origin such as LSU (nuclear large subunit rDNA), SSU (nuclear small subunit rDNA), ITS (Internal transcribed spacer) and atp6 (ATPase subunit 6 gene) (Hibbett, 2007).



Figure 2.3 The organization of ribosomal DNA. (Schlötterer and Tautz, 2004)

rDNA (ribosomal DNA) encode 5S, 5.8S, small subunit (SSU), and large subunit (LSU) rDNAs (Figure 2.3). rDNA has been shown to be remarkably conserved between different organisms and highly repetitive. Thus, they are widely used for the inference of phylogenetic relationships among both closely and distantly related species. Among them, LSU rDNA gene is the largest one carrying a wide range of informative characters for phylogenetic study at higher taxonomic levels (Gupta and Satyanarayana, 2000; Hwang and Kim, 2000). The other well known nuclear region in the field of molecular ecology and fungal systematic is ITS. This region lies between SSU and LSU rDNA and contains two noncoding spacer regions separated by the 5.8S rDNA. In fungi it is typically about 650–900 bp in size, including the 5.8S gene (Horton and Bruns, 2001).

According to molecular phylogeny of Boletales (Binder and Hibbett, 2006), the family Boletaceae was monophyletic group but the relationship among this family was unclear and the large genera such as *Boletus*, *Xerocomus* and *Tylopilus* were not monophyletic group. As the phylogeny of Boletes (Drehmel *et al.*, 2008), *Leccinum* and *Suillus* were monophyletic group but *Boletus*, *Xerocomus* and *Tylopilus* were not. Aside from the relationship among genera in this family, the phylogeny between the species in each genus was investigated.

In genus *Leccinum*, phylogeny of European *Leccinum* species was investigated based on ITS and LSU by maximum parsimony, maximum likelihood and Bayesian approaches. The results suggested that several traditional sections were artificial. Furthermore, the minisatellite in first internal transcribed spacer (ITS1) region was unsuitable for phylogenetic analysis of relations above the species level in this genus (den Bakker *et al.*, 2004a). Moreover, the level of host specificity of some *Leccinum* was assessed by phylogenetic analysis. den Bakker *et al.* (2004b) determined the phylogenetic relationships among *Leccinum* species from Europe and North America based on second internal transcribed spacer (ITS2) and glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) by maximum likelihood and parsimony analyses. The results showed that most of all *Leccinum* species were highly host tree specific, except *L. aurantiacum*.

In genus *Tylopilus*, one cryptic species, *T. bllouii*, was studied by using LSU rDNA and largest subunit of DNA-dependent RNA polymerase II (RPB1) sequence data. The LSU data suggested geographic structuring of the tested accessions. However, RPB1 data indicated that long-distance dispersal events are possible (Halling *et al.*, 2007).

In genus *Strobilomyces*, the phylogeny of some cryptic species, *S. confuses*, *S. seminudus*, *S. strobilaceus* and *S. mirandus* was analysed using ITS2, RPB1 and ATPase subunit 6 (*atp6*). The results indicated that *Strobilomyces* was monophyletic group and this genus related to Fagaceae species over Pinaceae species as host plants. In addition, this study demonstrated that molecular data could help to detect species boundaries. (Sato *et al.*, 2008)

The widely investigated genus is *Boletus* (include *Xerocomus*) especially in Europe. Dentinger *et al.* (2010) studied the phylogeny of *Boletus* section *Boletus* using LSU, *atp6* and RPB1. The phylogenetic study from RPB1 dataset showed that this section was monophyletic group. Molecular phylogeny of *Boletus* section *Boletus* was also studied by Beugelsdijk *et al.* (2008) using ITS and glyceraldehyde 3-phosphate dehydrogenase gene (Gapdh). The phylogenetic tree of European *Boletus* suggested

that *Boletus edulis* was a variable species with a wide morphological, ecological and geographic range, and includes several specific and subspecific taxa, while, three other European species (*B. aereus*, *B. pinophilus* and *B. reticulatus*) were well delimited species based on morphology and molecular data. This result conformed to the studies of Leonardi *et al.* (2005). Other study of species delimitation was *Xerocomus chrysenteron* complex (Peintner *et al.*, 2003). This phylogenetic analyses based on LSU demonstrated that the *X. chrysenteron* complex is a monophyletic group and clearly confirmed species concept.

CHAPTER III

MATERIALS AND METHODS

3.1 Chemicals Used in This Study

- Agarose molecular biology grade (ISC Bio Express)
- Cetyltrimethylammonum bromide (CTAB) (Serva)
- Chloroform (Merck, Germany)
- EmeraldAmp GT PCR Master Mix (Takara)
- Ethanol (Merck, Germany)
- Ethylenediamine tetraacetic acid (EDTA) (Scharlau)
- Gel star (Lonza, USA)
- Isoamyl alcohol (Carbo Erba)
- Iso-propylthio-β-galactoside (IPTG) (Fermentas)
- Silica gel
- Sterile distilled water
- 10X Tris Boric acid Disodium Ethylenediamine Tetracetic Acid (10X TBE buffer)
- 100 bp+1.5 Kb DNA lader (ISC Bio Express)

3.2 Instruments Used in This Study

- Compound microscope (Model CH30, Olympus, Japan)
- Gel-Doc (Model ECX-26.MX, Vilber Lourmat, France)
- Micro refrigerated centrifuge (Model 3700/Kubota, Kubota Corporation, Japan)
- Authorized thermal cycler (Model TP 600 ,TAKARA)
- pH meter (Model 2000, Cyberscan)
- Electrophoresis chamber set (Mupid-ex, Bruker BioSpin, Switzerland)
- Vortex mixer (Model G-560E, Scientific Industries, USA)

3.3 Basidiocarp Sampling

The putative ectomycorrhizal basidiocarps in family Boletaceae were collected during the rainy season, July to August, in 2010 and 2011 from various localities in four Provinces namely Chiang Mai, Phitsanulok, Nan and Chaiyaphum. Some samples were provided by Natural Medicinal Mushroom Museum, Faculty of Science, Mahasarakram University. Young and mature individual basidiocarps were collected as many as possible and placed in the paper bags. The fresh collected basidiocarps were photographed and characterized some morphological characters. Host plant families were also recorded.

All collected basidiocarps were divided into two parts. One part of collected basidiocarps was dried in hot-air oven at 70-80 °C in 24-48 hours or until the basidiocarps were completely dried. Then, the dried basidiocarps were kept in the plastic boxes with silica gel for voucher specimens and deposited in herbarium at Department of Botany, Faculty of Science, Chulalongkorn University. For the remaining part of the basidiocarps, several tissue pieces of stem from each basidiome were cut into small pieces (1×1 cm²) and dried with silica gel in a polyethylene bag for DNA extraction.

3.4 Morphological Study

The collected basidiocarps were conventionally assighed to morphospecies based on morphological characters, both macroscopic and microscopic features. Identification of ectomycorrhizal fungi in Baletaceae was followed the key described by Corner (1972) and Moser (1978).

Most of macroscopic features of the basidiocarps such as color, shape, margin, surface and sizes of the stems and caps were evaluated by eye observation before drying. In addition to a spore print, a small piece of cap was cut from the mature fresh basidiome and placed on the slide in the petridish then the piece of cap was removed in the next day and the color of spore mass the slide was also noted.

The microscopic features especially hymenophores were study from dried basidiocarps. Free hand longitudinal and transverse of approximately 0.1 mm thick were

made from dried basidiocarps with a sharp razor blade. The sections were rehydrated by soaking in 3% KOH and water, respectively before analyzing their morphology. The sections were then stained with 1% Congo Red (Largent *et al.*, 1977). The thininest sections were selected and placed on glass slides and covered with cover slips. Low power (×40) objectives of a standard light microscope were used to observe the sections. Internal basidiome regions including basidia, basidiospores, cystidia and hymenophoral trama were recorded by mounted photography. Basidiospore size was determined by measuring the diameter of 30 spores and calculating their size ranges. Characteristics of hymenophores and basidiospores were critically analysed using scanning electron microscopy (SEM). Samples were air-dried and sputter-coated with gold with a JSM-5410 LV scanning electron microscope.

3.5 DNA Extraction and Sequencing

Genomic DNA was extracted from dried pieces of stem tissue with cetyltrimethylammonium bromide (CTAB) method as described in Zhou *et al.* (1999). Briefly, each sample was grinded to powder in a 2 ml microtube containing 5 beads using a homogenizer (VX-100) for 2 min. The powdered samples were homogenized in washing buffer (Appendix A). After centrifugation at 15,000 rpm for 2min, the pellet was washed 1-2 times by homogenization in the washing buffer and centrifuged at 15,000 rpm for 3 min. The washed pellet was suspended and incubated in CTAB buffer (Appendix A) at 65°C for 1 hour, and added an equal volume of chloroform-isoamyl alcohol mixture (24:1, v/v). After centrifuged at 1,500 rpm for 8 min., the supernatant was removed into a new 1.5 ml microtube and added an equal volume of chloroform-isoamyl alcohol mixture (24:1, v/v) again. DNA was precipitated by adding an equal volume of isopropanol and was incubated in -20°C for 30 min. After centrifugation at 8,000 rpm for 10 min., DNA was washed by 70% ethanol and resuspended in 50 µl of sterilized distilled water. DNA solution was stored at -20°C until use.

Two regions of nuclear ribosomal DNA, internal transcribed spacer (ITS) and large subunit (LSU) rDNA, of each sample were amplified with two pairs of primers

ITS1F (Gardes and Bruns, 1993) and ITS 4 (White *et al.*, 1990) for ITS region or LR0R and LR7 (Vilgalys and Hester, 1990) for LSU region. PCR amplification was performed in a 30 µl of reaction mixture containing 15 µl of EmeraldAmp GT PCR Master Mix , 11.4 µl of sterilized distilled water, 0.3 µl of each 20 µM primer and 3 µl of DNA solution by a TP600 Authorized thermal cycler. Amplification was started with a heat of 98°C for 1 min., followed by 38 cycles of a denaturing step at 98°C for 10 sec, an annealing 51°C for 30 sec in ITS reaction and for 45 °C in LSU reaction, and extension step at 72°C for 1 min and ended with an additional 5 min-extension step at 72 °C. All of PCR products were sent to Macrogen (Soul, Korea) for sequencing.

3.6 Phylogenetic Analysis

All ITS and LSU sequences were compared with the available sequences in GenBank database (http://www.ncbi.nlm.nih.gov/) or the UNITE database (http://www.unite.zbi.ee/) using BLAST version 2.2.18. The sequences were automatically aligned with some sequences obtained from the DNA database. The alignment was carried out using MUSCLE (Edgar, 2004) in Mega 5 program and then manually improved. ITS and LSU sequences of Rhizopogon species were included as outgroup species. The phylogenetic trees between genera in Boletaceae or among species in two large genera, Boletus and Tylopilus were constructed by using Maximum Likelihood of Mega 5 (Tamura et al., 2011). Maximum Likelihood analysis was performed after suitable model. Bootstrap values were calculated by 100 replications.

CHAPTER IV

RESULTS

4.1 Collecting Sites

The collecting sites in this study were in four Provinces: Chaiyaphum, Chiang Mai, Nan and Phitsanulok. The putative host trees were the member of Dipterocarpaceae, *Eucalyptus* (Myrtaceae), Fagaceae and Pinaceae. The details of the study sites in each location were represent in Table 4.1 and Figure 4.1. Moreover, some basidiocarps were brought from the local market in Ubon Ratchathani province.

Provinces	Sites	Forest Types	Location	Altitudes	Ectomycorrhizal Host
				(m-amsl)	Plants
Chaiyaphum	Nong Bua	Dipterocarp	N 16 11.085	360	Dipterocarpaceae
	Daeng District	forest	E101 30.152		
		Eucalyptus	N 16 10.398	280	Eucalyptus
		plantation	E101 41.406		
	Thep Sathit	evergreen forest	N 15 41.832	290	Fagaceae
	District		E101 24.115		
	Nam Nao	Hill evergreen	N16 73.964	830	Fagaceae
		forest	E101 57.378		
Chiang Mai	Mae Jam	Dipterocarp	N 18 31.374	550-570	Dipterocarpaceae
	District	forest	E 98 23.496		
		Dipterocarp and	N 18 31.981	860-870	Dipterocarpaceae
		Pinus forest	E 98 24.939		Pinus
Nan	Wiang Sa	Dipterocarp	N 18 33.661	220	Dipterocarpaceae
	District	forest	E 100 47.883		
Phitsanulok	Phu Hin Rong	Pinus Plantation	N 17 00.184	550	Pinus
	Khla		E100 59.597		
		evergreen forest	N 17 12.056	480	Fagaceae
			E100 63.788		
Ubon	unknown	unknown	unknown	unknown	unknown
Ratchathani					

Table 4.1 The list of collecting sites in each province



Figure 4.1 The collecting sites include coniferous plantation (A), evergreen forest (B), *Eucalyptus* plantation (C) and diptercarp forest (D).

4.2 Diversity of Boletaceae and Morphological Identification

The results of this study have provided overviews of the diversity of ectomycorrhizal fungi in family Boletaceae in some part of Thailand over a two-year period from 2010 and 2011 during rainy season (July-Semtember). Approximately 95 collections were identified in 54 species based on morphology. A summary of the classification and distribution of the identified boletes was presented in table 4.2.

In this study, most basidiocarps were classified in 7 genera of Boletaceae, The most abundant genus was the *Boletus* (24 species), and the second and third abundances were the *Tylopilus* (15 species) and the *Boletellus* (5 species). The number of species in other genera, *Strobilomyces* and *Heimioporus* and *Leccinum* were 4, 3 and 2 species respectively while *Pulveroboletus* species represented only 1 species. The most numerous collections of boletes taxa were found in Chaiyaphum Province particularly forests which dominated by the Fagaceae and Dipterocarpaceae.

Species	Chaiyaphum	Chiang Mai	Nan	Phitsanulok	Ubon
					Ratchathani
Boletellus ananas	2	1		1	2
Boletellus sp.1				1	
Boletellus sp.2		2			
Boletellus sp.3		1			
Boletellus sp.4		2	1	1	
Boletus sp.1	2			1	
Boletus sp.2	2				
Boletus sp.3	1				
Boletus sp.4	3				
Boletus sp.5		1			
Boletus sp.6	1				
Boletus sp.7		1			
Boletus sp.8	1				
Boletus sp.9	1				
Boletus sp.10	1				
<i>Boletus</i> sp. 11	1				
<i>Boletus</i> sp. 12				1	

 Table 4.2 The number of specimens in Boletaceae from 5 provinces.

Species	Chaiyaphum	Chiang Mai	Nan	Phitsanulok	Ubon
					Ratchathani
Boletus sp. 13	3				
Boletus sp. 14				1	
Boletus sp. 15	1				
Boletus sp. 16	1				
Boletus sp. 17				2	1
<i>Boletus</i> sp. 18		1			
Boletus sp. 19	1				
Boletus sp. 20	1				
Boletus sp. 21	1				
Boletus sp. 22	1				
Boletus sp. 23	1				
Boletus sp. 24	1	1			
Heimioporus sp.1					1
Heimioporus sp.2	4	2			
Heimioporus sp.3	1				
Leccinum	1				
extremiorientale					
Leccinum sp.	1				
Pulveroboletus sp.	3	1			
Strobilomyces				1	
mirandus					
Strobilomyces sp.1				1	
Strobilomyces sp.2				2	
Strobilomyces sp.3	1				
Tylopilus eximius	1				
<i>Tylopilus</i> sp. 1	1				
Tylopilus sp. 2	1				
Tylopilus sp. 3				1	
<i>Tylopilus</i> sp. 4	4	1			
Tylopilus sp. 5	1				
<i>Tylopilus</i> sp. 6	1		1		
Tylopilus sp. 7	2	1			
<i>Tylopilus</i> sp. 8	1				
Tylopilus sp.9	1				
<i>Tylopilus</i> sp.10	3				
<i>Tylopilus</i> sp.11	1				

 Table 4.2 (continued)
 The number of specimens in Boletaceae from 5 provinces.
Species	Chaiyaphum	Chiang Mai	Nan	Phitsanulok	Ubon
					Ratchathani
Tylopilus sp.12	1				
Tylopilus sp.13	5				
<i>Tylopilus</i> sp.14				1	

Table 4.2 (continued) The number of specimens in Boletaceae from 5 provinces.

The results of morphological study of eight genera in Boletaceae were shown in Table 4.3. and Figure 4.2

Genera		Pile	us		Stem	Basidi	ospores
	surface	margin	change of color	color of pore	surface	shape	ornamentation
Boletellus	appressed-	appendiculate or	blue	yellow	glabrous or	ellipsoid	longitudinally
	squamulose or not	not			reticulate		ridged
Boletus	glabrous	entire	often blue	greenish yellow or	glabrous or	subfusiform or	smooth
				yellow or cream	subpruinose or	ellipsoid	
					reticulate		
Heimioporus	glabrous or	entire	Often blue	yellow	glabrous or	ellipsoid	reticulate or
	alveolate				reticulate		perforate
Leccinum	glabrous to	appendiculate or	unchanging	tan or yellow	scabrous with brown	subfusiform	smooth
	subtomentose	entire			scales		
Pulveroboletus	pulverulent	appendiculate	blue	yellow	glabrous	ellipsoid	smooth
Strobilomyces	coarsely fibrillose	often	red then dark	white then gray or	glabrous or	globose	reticulate to
	to squamulose	appendiculate	gray	pale purple	squamose		irregularly
							echinate
Tylopilus	glabrous to	entire or slightly	pale brown	white then pinkish	glabrous or slightly	subfusiform or	smooth
	subtomentose	appendiculate		or pale purple	fibrillose	ellipsoid	
Zangia	glabrous	entire	unchanging	white then pinkish	glabrous	subfusiform	smooth
				or pale purple	yellow at base		

Table 4.3 Specific morphological characters of genera in Boletaceae



Figure 4.2 Basidiospores of Boletaceae. *Boletellus* spp. (A-B); *Boletus* sp. (C); *Heimioporus* sp.(D); *Leccinum* sp. (E); *Pulveroboletus* sp. (F); *Tylopilus* sp. (G); *Strobilomyces* sp. (H)

Boletellus ananas (CP12, MJ03, NN4, P13, UB7, and UB10)

Pileus 5-11 cm, convex, pale yellow, appressed-squamulose, fuscous tan, margin irregularly appendiculated with the woolly remains of the veil; tubes 8-20 mm, sinuate, golden yellow; pores 0.5-1 mm, subrounded, golden yellow, cyanescent. Stem 5-9 cm x 8-18mm, equal, longitudinally fibrillose, pale fuscous downwards, and apex reddish.



Figure 4.3 basidiocarps of Boletellus ananas, scale bar = 2 cm

Boletellus sp.1 (PH34)

Pileus 7 cm, convex, crimson, areolate, margin entire; tubes 8-10 mm, yellow; pores 0.8-1 mm, subrounded, yellow. Stem 6.5 cm x 15 mm, equal, longitudinally fibrillose, pale red, base pallid and white.



Figure 4.4 basidiocarps of *Boletellus* sp.1, scale bar = 2 cm

Boletellus sp.2 (MJ12 and MJ15)

Pileus 5-6.5 cm, convex to broadly convex, reddish brown, fine appressedsquamose when young, margin entire or rimose; tubes 5-8 mm, yellow; pores 0.8-1 mm, subrounded, yellow, cyanescent. Stem 4 cm x 6-10 mm, equal, longitudinally fibrillose, minutely pubescent, dark reddish brown, yellow at apex, cyanescent.



Figure 4.5 basidiocarps of *Boletellus* sp.2, scale bar = 2 cm

Boletellus sp.3 (MJ26)

Pileus 4-5.3 cm, convex, pale reddish brown, smooth, entire; tubes 5-8 mm, decurrent, yellow; pores 0.3-1 mm, subrounded to angular, yellow. Stem 8-9 cm x 10-20 mm at apex, 15-25 mm at base, slightly clavate, pale yellow, reddish reticulate.



Figure 4.6 basidiocarps of *Boletellus* sp.3, scale bar = 2 cm

Boletellus sp.4 (CH02,MJ04, P00, and WS02)

Pileus 6-7.5 cm, convex, pale yellow, appressed-squamulose, reddish brown, margin irregularly appendiculated with the woolly remains of the veil; tubes 8-15 mm, yellow; pores 0.5 mm, rouded to subrounded, yellow. Stem 8-10 cm x 15 mm at apex, 20 mm at base, slightly clavate, reticulate, dark red.



Figure 4.7 basidiocarps of *Boletellus* sp.4, scale bar = 2 cm

Boletus sp.1 (CP31, CP47, P12)

Pileus 5-15 cm, convex to broadly convex, orange to brown, smooth, margin entire; tubes 5-8 mm, greenish yellow; pores 0.8-1 mm, subrounded, greenish yellow. Stem 4-11 cm x 13-20 mm, equal, glabrous, orange to brown, yellow at apex, base pallid and white.



Figure 4.8 basidiocarps of *Boletus* sp.1, scale bar = 2 cm

Boletus sp.2 (CP18 and CP25)

Pileus 3.5-6.5 cm, convex to broadly convex, orange brown, smooth, margin entire; tubes 5-8 mm, yellow; pores 0.3-0.5 mm, subrounded, white then yellow. Stem 4-6 cm x 10-13 mm, equal, longitudinally fibrillose, minutely pubescent, pale orange brown, base pallid and white.



Figure 4.9 basidiocarps of *Boletus* sp.2, scale bar = 2 cm

Boletus sp.3 (CP01)

Pileus 5.5 cm, broadly convex, cream, smooth, margin entire; tubes 3-5 mm, yellow; pores 0.3-0.5 mm, subrounded, red, cyanescent. Stem 3.5 cm x 18 mm, equal, dark red, cyanescent.



Figure 4.10 basidiocarps of *Boletus* sp.3, scale bar = 2 cm

Boletus sp.4 (CP15, CP20 and CP51)

Pileus 2-5 cm, convex to broadly convex, yellowish brown or reddish brown to dark brown, smooth, margin entire; tubes 3-5 mm, yellow; pores 0.3 mm, subrounded, white then yellow. Stem 4-6 cm x 8-15 mm, equal to clavate, cream, reticulate, brown ridge.



Figure 4.11 basidiocarps of *Boletus* sp.4, scale bar = 2 cm

Boletus sp.5 (MJ23)

Pileus 3.5-4 cm, convex, pale reddish brown, smooth, margin entire; tubes 5 mm, yellow; pores 0.2 mm, subrounded, cream. Stem 3.5 cm x 21 mm at apex, 18 mm at base, slightly clavate, cream at apex, pale brown at base, reticulate.



Figure 4.12 basidiocarps of *Boletus* sp.5, scale bar = 2 cm

Boletus sp.6 (CP03)

Pileus 5.5 cm, convex, reddish brown, smooth, margin entire; tubes 5 mm, yellow; pores 0.8-1 mm, subrounded, yellow, cyanescent. Stem 2.5 cm x 8 mm, equal, longitudinally fibrillose, dark reddish brown, yellow at apex, cyanescent.

Boletus sp.7 (MJ27)

Pileus 2.8 cm, convex, reddish brown, rivulose, margin entire; tubes 3 mm, cream; pores 0.25 mm, subrounded, cream. Stem 3 cm x 12 mm, flatten, equal, longitudinally fibrillose, cream, base pallid and white.



Figure 4.13 basidiocarps of *Boletus* sp.7, scale bar = 2 cm

Boletus sp.8 (CP40)

Pileus 3-3.5 cm, convex, orange brown, smooth, margin entire; tubes 5 mm, yellow; pores 1-1.2 mm, subrounded, yellow. Stem 2.5-3 cm x 5-8 mm, equal, longitudinally fibrillose, pale brown, yellow at apex, base pallid and white.



Figure 4.14 basidiocarps of *Boletus* sp.8, scale bar = 2 cm

Boletus sp.9 (NN05)

Pileus 7 cm, convex, dark brown, smooth, margin entire; tubes 8 mm, bright yellow; pores 0.3-0.5 mm, subrounded, bright yellow, cyanescent. Stem 9.5 cm x 18 mm, equal, longitudinally fibrillose, minutely pubescent at apex, pale reddish brown, pale yellow at apex, base pallid and white.



Figure 4.15 basidiocarps of *Boletus* sp.9, scale bar = 2 cm

Boletus sp.10 (CP11)

Pileus 2.5 cm, convex, orange, rivulose, margin entire; tubes 5 mm, orange; pores 0.3 mm, subrounded, orange, cyanescent. Stem 2.5 cm x 7 mm, equal, minutely pubescent, dark orange, yellow at apex, cyanescent.



Figure 4.16 basidiocarps of *Boletus* sp.10, scale bar = 2 cm

Boletus sp.11 (CP8)

Pileus 9.5 cm, broadly convex, creamy brown, fine areolate, margin entire; tubes 15-20 mm, yellow; pores 0.8-1 mm, subrounded, pale reddish brown. Stem 7 cm x 25 mm at apex, 40 mm at base, clavate, longitudinally fibrillose, dark reddish brown.



Figure 4.17 basidiocarps of *Boletus* sp.11, scale bar = 2 cm

Boletus sp.12 (P05)

Pileus 6-9 cm, convex, reddish brown to dark brown, smooth, margin entire or rimose; tubes 5 mm, white; pores 0.3-0.5 mm, subrounded, white. Stem 4 cm x 25-30 mm, tapered at base, reticulate at apex, olive-green at apex, dark brown at base.



Figure 4.18 basidiocarps of *Boletus* sp.12, scale bar = 2 cm

Boletus sp.13 (CP17, CP21and CP49)

Pileus 5-7.5 cm, convex to broadly convex, cream to pale gray, smooth, margin entire; tubes 5-8 mm, yellow; pores 0.3-0.5 mm, subrounded, white then yellow. Stem 3-6 cm x 15-18 mm, equal, longitudinally fibrillose, concolorous with pileus.



Figure 4.19 basidiocarps of *Boletus* sp.13, scale bar = 2 cm

Boletus sp.14 (PH37)

Pileus 2-3 cm, broadly convex, reddish brown, smooth, margin incurve; tubes 2-3 mm, yellow; pores 0.3 mm, subrounded, yellow. Stem 3 cm x 5 mm, equal, longitudinally fibrillose, dark brown, yellow at apex.



Figure 4.20 basidiocarps of *Boletus* sp.14, scale bar = 2 cm

Boletus sp.15 (NN12)

Pileus 13 cm, broadly convex, orange brown, smooth, margin entire; tubes 20 mm, yellow; pores 0.8-1 mm, subrounded, yellow. Stem 9 cm x 20-23 mm, equal, longitudinally fibrillose, reticulate at apex, pale orange.



Figure 4.21 basidiocarps of *Boletus* sp.15, scale bar = 2 cm

Boletus sp.16 (NN16)

Pileus 10 cm, broadly convex, pale brown, smooth, margin entire; tubes 5-8 mm, yellow; pores 0.5 mm, subrounded, yellow, cyanescent. Stem 13 cm x 15-18 mm, equal, longitudinally fibrillose, cream, pale brown at base, cyanescent.



Figure 4.22 basidiocarps of *Boletus* sp.16, scale bar = 2 cm

Boletus sp.17 (P15, PH41 and UB04)

Pileus 5-6cm, convex to broadly convex, reddish brown to dark brown, smooth, margin entire; tubes 5 mm, pale brown; pores 0.5 mm, subrounded, pale brown. Stem 6-8 cm x 10 mm, equal, longitudinally fibrillose, white to pale brown.



Figure 4.23 basidiocarps of *Boletus* sp.17, scale bar = 2 cm

Boletus sp.18 (MJ16)

Pileus 12-14 cm, broadly convex, dark brown, slightly uneven, margin entire or rimose; tubes 8 mm, yellow; pores 1-1.2 mm, subrounded, yellow, cyanescent. Stem 7 cm x 20 mm at apex, 23 mm at base, slightly clavate, longitudinally fibrillose, minutely pubescent, dark brown, yellow at apex, pale cyanescent.



Figure 4.24 basidiocarps of *Boletus* sp.18, scale bar = 2 cm

Boletus sp.19 (CP34)

Pileus 5.5 cm, broadly convex, purplish brown, smooth, margin entire; tubes 5 mm, yellow; pores 0.8-1.2 mm, subrounded, yellow. Stem 5 cm x 5 mm, equal, longitudinally fibrillose, cream, pale brown at apex.



Figure 4.25 basidiocarps of *Boletus* sp.19, scale bar = 2 cm

Boletus sp.20 (NN02)

Pileus 8.5 cm, broadly convex, orange brown, smooth, margin recurved; tubes 3-5 mm, yellow; pores 0.8-1 mm, subrounded, cream to yellow. Stem 6.5 cm x 10 mm, equal, longitudinally fibrillose, pale reddish brown, yellow at base.



Figure 4.26 basidiocarps of *Boletus* sp.20, scale bar = 2 cm

Boletus sp.21 (CP53)

Pileus 9 cm, broadly convex, dark brown, fine areolate, margin entire; tubes 8 mm, yellow; pores 0.5-0.8 mm, subrounded, yellow. Stem 7 cm x 40 mm, clavate, reticulate, cream to pale brown, pale brown at apex.



Figure 4.27 basidiocarps of *Boletus* sp.21, scale bar = 2 cm

Boletus sp.22 (CP44)

Pileus 7.5 cm, convex to broadly convex, dark brown, smooth, margin entire; tubes 5-8 mm, purplish brown; pores 0.5 mm, subrounded, purplish brown. Stem 6 cm x 10-15 mm, equal to slightly clavate, minutely pubescent, pale brown to dark brown.



Figure 4.28 basidiocarps of *Boletus* sp.22, scale bar = 2 cm

Boletus sp.23 (CP19)

Pileus 3-3.5 cm, convex, yellowish brown, rivulose, margin entire; tubes 5 mm, yellow; pores 0.3-0.5 mm, subrounded, yellow. Stem 3.5-4 cm x 8 mm, equal, longitudinally fibrillose, pale brown.



Figure 4.29 basidiocarps of *Boletus* sp.23, scale bar = 2 cm

Boletus sp.24 (SN01 and NN11)

Pileus 4-5 cm, convex, brown, dark brown in the center, fine areolate, margin entire; tubes 5 mm, yellow; pores 0.8-1 mm, subrounded, yellow, cyanescent. Stem 5 cm x 8 mm, equal, longitudinally fibrillose, dark brown, yellow at apex.



Figure 4.30 basidiocarps of *Boletus* sp.24, scale bar = 2 cm

Heimioporus sp.1 (UB01)

Pileus 4-7 cm, convex, crimson, smooth, entire; tubes 5-8 mm, yellow; pores 0.5-1 mm, subrounded, yellow. Stem 8-12 cm x 8-10 mm at apex, 15-18 mm at base, slightly clavate, crimson, fuscous at base, reticulate.



Figure 4.31 basidiocarps of *Heimioporus* sp.1, scale bar = 2 cm

Heimioporus sp.2 (CP13, CP13.2, CP48, CP52, MJ06 and MJ25)

Pileus 6-11.5 cm, convex, crimson to yellowish pink, entire; tubes 10-20 mm, yellow; pores 0.5-1 mm, subrounded, yellow. Stem 8-16 cm x 15-20 mm at apex, 18-27 mm at base, equal, longitudinally fibrillose, crimson to pale pink, yellow at apex.



Figure 4.32 basidiocarps of *Heimioporus* sp.2, scale bar = 2 cm

Heimioporus sp.3 (NN03)

Pileus 7 cm, convex, crimson, smooth, entire; tubes 8 mm, yellow; pores 0.5-1 mm, subrounded, yellow. Stem 10.5 cm x 18 mm at apex, 22 mm at base, equal, reticulate, crimson, yellowish red at apex.



Figure 4.33 basidiocarps of *Heimioporus* sp.3, scale bar = 2 cm

Leccinum extremiorientale (NN18)

Pileus 10 cm, convex, orange brown, areolate, margin appendiculated with the remains of the veil; tubes 15-20 mm, yellow; pores 0.8-1 mm, subrounded, yellow. Stem 6 cm x 25 mm, equal, orange brown, longitudinally fibrillose, finely scabrous, brown scale.



Figure 4.34 basidiocarps of *Leccinum extremiorientale*, scale bar = 2 cm

Leccinum sp. (NN13)

Pileus 4-4.5 cm, convex, dark reddish brown, margin entire; tubes 5 mm, purplish brown; pores 0.5-1 mm, subrounded, purplish brown. Stem 5.5-6 cm x 10-12 mm, equal, cream, scabrous, dark brown scale.



Figure 4.35 basidiocarps of *Leccinum* sp., scale bar = 2 cm

Pulveroboletus sp. (039, CP16, MJ33 and NN21)

Pileus 5-7 cm, convex, pulverulent, brilliant sulphur yellow, margin appendiculate with fragments of the yellow and friable veil; tubes 5 mm, yellowish to rufescent; pores 0.5-1 mm, subrounded, yellow to rufescent, pale cyanescent. Stem 5-6 cm x 10-12 mm, equal, pulverulent, brilliant sulphur yellow, apical annulus.



Figure 4.36 basidiocarps of *Pulveroboletus* sp., scale bar = 2 cm

Strobilomyces mirandus (PH32)

Pileus 6-8 cm, convex to broadly convex, golden tawny, appressed-squamulose, fuscous tan, margin irregularly appendiculated with the woolly remains of the veil; tubes white, blackening at maturity; pores 0.5 mm, subrounded, white then blackening. Stem 6-9 cm x 10 mm, equal, golden orange, covered with irregular elongate shallow reticulations more or less thinly floccoso-squamulose. All parts of the basidiocarp reddening slightly, then blackening on bruising.



Figure 4.37 basidiocarps of Strobilomyces mirandus, scale bar = 2 cm

Strobilomyces sp.1 (P04)

Pileus 17 cm, broadly convex, cracked, brown, black in the center, appressedsquamulose, fuscous, margin irregularly appendiculated with the woolly remains of the veil; tubes cream then blackening; pores 0.8-1 mm, subrounded, cream then blackening. Stem 2 cm x 40 mm, equal, reticulate, black at apex, white at base. All parts of the basidiocarp reddening slightly, then blackening on bruising.



Figure 4.38 basidiocarps of Strobilomyces sp.1, scale bar = 2 cm

Strobilomyces sp.2 (P01 and P14)

Pileus 5-7 cm, convex, dark brown, finely appressed-squamulose, black; tubes 10 mm, cream then blackening; pores 1-2 mm, subrounded to angular, cream then blackening. Stem 5.5-8 cm x 5-15 mm, equal, dark gray, covered with irregular elongate shallow reticulations more or less thinly floccoso-squamulose. All parts of the basidiocarp reddening slightly, then blackening on bruising.



Figure 4.39 basidiocarps of Strobilomyces sp.2, scale bar = 2 cm

Strobilomyces sp.3 (CP43)

Pileus 11 cm, plane, pale gray, finely appressed-squamulose, black; tubes cream then blackening; pores 1-2 mm, subrounded to angular, cream then blackening. Stem 6.5 cm x 15 mm, equal, dark gray to black, reticulate. All parts of the basidiocarp reddening slightly, then blackening on bruising.



Figure 4.40 basidiocarps of *Strobilomyces* sp.3, scale bar = 2 cm

4.3 Identification of the Basidiocarps Based on ITS and LSU Regions

Ninety-one of 95 collected basidiocarps were successfully amplified ITS (ITS1, 5.8S and ITS2) regions. The size of ITS fragments varied in length from 500 to 1000 nucleotides (Figure 4.41). Only 61 ITS sequences were obtained. LSU amplification of 95 samples was successful and 84 LSU amplified products were successfully sequenced. The lengths of LSU fragments were approximately 1500 nucleotides (Figure 4.42). Generally, the ITS amplification resulted in less amount DNA product than LSU. Totally, 52 sequences of both ITS and LSU regions were obtained.



Figure 4.41 PCR product of ITS sequences in gel electrophoresis. M was marker.



Figure 4.42 PCR product of LSU sequences in gel electrophoresis. M was marker.

The similarity comparisons of ITS and LSU sequences in this study with available sequences in GenBank database are given in Table 4.4. The BLAST results of sequence affinity showed that all sequences of both regions were identical for the members of Boletaceae with 80%-99% similarity for ITS sequences and 85%-99% similarity for LSU sequences. The closest species matches included genera *Aureoboletus, Boletellus, Boletus, Leccinum, Phylloporus, Pulveroboletus, Strobilomyces, Tylopilus, Xanthoconium, Xerocomus, and Zangia.*

The ITS sequences in this study were also compared with the sequences of known species in the taxonomic reliable database, UNITE, as shown in Table 4.5. The result represented that most sequences shared high percentages of similarity (90%-100%) with various members of Boletaceae such as *Aureoboletus Boletus*, *Buchwaldoboletus*, *Leccinum*, *Porphyrellus*, *Strobilomyces* and *Xerocomus*. However the low overlap value of all sequences reflected that the closest species matches did not closely related with the sequences in this study.

Species	code		ITS re	gion				LSU region				
		base pair	closest species match	accession	overlap	%similarity	base pair	Closest species match	accession	overlap	%similarity	
				no.					no.			
Boletellus ananas	UB07	<u>618</u>	Unculture ectomycorrhiza	DQ146391	617/618	99%	795	Zangia olivacea	HQ326946	744/804	93%	
Boletellus ananas	UB10	-	-	-	-	-	795	Zangia olivacea	HQ326946	744/804	93%	
Boletellus ananas	CP12	-	-	-	-	-	789	Boletellus ananas	AY612799	689/733	94%	
Boletellus ananas	NN04	-	-	-	-	-	790	Boletellus ananas	AY612799	692/735	94%	
Boletellus ananas	MJ03	-	-	-	-	-	789	Boletellus ananas	AY612799	690/733	94%	
Boletellus ananas	P13	-	-	-	-	-	790	Boletellus ananas	AY612799	692/735	94%	
<i>Boletellus</i> sp.1	PH34	834	Boletellus obscurococcineus	AB509989	488/508	97%	786	Boletellus projectellus	NG027638	733/791	93%	
Boletellus sp.2	MJ15	913	Boletus sp.	FJ480441	787/916	86%	782	Boletellus projectellus	NG027638	746/788	95%	
Boletellus sp.2	MJ12	-	-	-	-	-	782	Boletellus projectellus	NG027638	745/788	95%	
Boletellus sp.3	MJ26	<u>582</u>	Unculture ectomycorrhiza	AM113453	373/437	85%	791	Tylopilus ballouii	EU430732	748/794	94%	
Boletellus sp.4	WS02	<u>846</u>	Unculture Boletaceae	GQ268578	797/863	92%	-	-	-	-	-	
Boletellus sp.4	P00	445	Uncultured Boletaceae	GQ268578	432/458	94%	789	Boletellus projectellus	NG027638	733/791	93%	
Boletellus sp.4	MJ04	<u>635</u>	Uncultured Boletaceae	GQ268578	601/642	94%	789	Boletellus projectellus	NG027638	733/791	93%	
Boletellus sp.4	CH02	-	-	-	-	-	789	Boletellus projectellus	NG027638	733/791	93%	
Boletus sp.1	P12	804	Boletus queletii	JF907785	388/461	84%	788	Boletellus projectellus	NG027638	736/790	93%	
Boletus sp.1	CP31	-	-	-	-	-	788	Boletellus projectellus	NG027638	736/790	93%	
Boletus sp.1	CP47	<u>328</u>	Uncultured ectomycorrhizal	AM412264	261/296	90%	790	Aureoboletus thibetanus	AY700189	744/801	93%	
			fungus									
Boletus sp.2	CP18	<u>502</u>	Boletus bicolor	GQ166877	371/414	90%	788	Boletellus projectellus	NG027638	753/789	95%	
Boletus sp.2	CP25	-	-	-	-	-	788	Boletellus projectellus	NG027638	753/789	95%	
Boletus sp.3	CP01	770	Boletus bicolor	GQ166877	656/779	84%	787	Tylopilus ballouii	EU430737	749/792	95%	
Boletus sp.4	CP15	<u>659</u>	Boletus bicolor	GQ166877	373/442	84%	799	Boletellus projectellus	NG027638	726/809	90%	
Boletus sp.4	CP51	<u>516</u>	Boletus bicolor	GQ166877	373/442	84%	799	Boletellus projectellus	NG027638	727/809	90%	

Table 4.4 Sequence affinity of basidiocarps in this study based on Genbank Database

Species	code		ITS re		LSU region						
		base pair	closest species match	accession	overlap	%similarity	base pair	Closest species match	accession	score (bit)	%similarity
				no.					no.		
Boletus sp.4	CP20	-	-	-	-	-	799	Boletellus projectellus	NG027638	726/809	90%
Boletus sp.5	MJ23	-	-	-	-	-	798	Boletellus projectellus	NG027638	727/809	90%
Boletus sp.6	CP03	783	Boletus brunneissimus	DQ407249	630/673	94%	782	Xerocomus pruinatus	AF514827	725/745	97%
Boletus sp.7	MJ27	860	Boletus edulis subsp.	EU231978	281/322	87%	793	Tylopilus felleus	HQ326934	740/802	92%
			Aurantioruber								
Boletus sp.8	CP40	720	Boletus erythropus	HM347643	486/581	84%	783	Tylopilus felleus	HQ326934	746/788	95%
Boletus sp.9	NN05	699	Uncultured Boletus clone	HM146797	521/604	86%	783	Tylopilus felleus	HQ326934	754/790	95%
Boletus sp.10	CP11	941	Boletus fragrans	JF907800	404/466	87%	787	Tylopilus ballouii	EU430732	709/801	89%
Boletus sp.11	CP08	573	Boletus pallidus	JN020986	202/215	94%	787	Tylopilus felleus	HQ326934	745/795	94%
Boletus sp.12	P05	629	Boletus pinophilus	DQ679803	357/421	85%	787	Tylopilus felleus	HQ326934	735/798	92%
Boletus sp.13	CP17	728	Boletus pinophilus	DQ131626	447/535	84%	786	Zangia erythrocephala	HQ326943	750/795	94%
Boletus sp.13	CP21	731	Boletus pinophilus	DQ131626	448/536	84%	-	-	-	-	-
Boletus sp.13	CP49	717	Boletus sp.	JN020990	604/663	91%	-	-	-	-	-
Boletus sp.14	PH37	<u>370</u>	Boletus rubellus	EU819460	330/360	91%	<u>503</u>	Tylopilus ballouii	EU430737	489/504	97%
Boletus sp.15	NN12	848	Boletus sp.	EU569236	579/694	83%	789	Boletellus projectellus	NG027638	753/791	95%
Boletus sp.16	NN16	826	Boletus sp.	AB509789	462/474	97%	783	Boletellus projectellus	NG027638	743/785	95%
Boletus sp.17	P15	621	Boletus sp.	FJ480436	563/657	86%	783	Boletellus projectellus	NG027638	748/785	95%
Boletus sp.17	PH41	617	Boletus sp.	FJ480436	561/655	86%	<u>502</u>	Boletellus shichianus	NG027636	490/502	98%
Boletus sp.17	UB04	637	Boletus sp.	FJ480436	560/650	87%	783	Xanthoconium affine	AY612838	705/720	98%
Boletus sp.18	MJ16	<u>537</u>	Boletus sp.	EU569236	299/334	90%	793	Boletellus projectellus	NG027638	749/790	94%
Boletus sp.19	CP34	813	Uncultured ectomycorrhizal	AB218099	592/685	87%	793	Xerocomus illudens	AY612840	686/705	97%
			fungus								
Boletus sp. 20	NN02	741	Uncultured fungus	FM999554	441/485	85%	787	Boletellus projectellus	NG027638	735/791	93%

Table 4.4 (continued) Sequence affinity of basidiocarps in this study based on Genbank Database

Species	code		ITS r	egion			LSU region				
		base pair	closest species match	accession	overlap	%similarity	base pair	Closest species match	accession	score (bit)	%similarity
				no.					no.		
Boletus sp.21	CP53	-	-	-	-	-	794	Boletus edulis	DQ071747	751/802	94%
Boletus sp.22	CP44	-	-	-	-	-	789	Zangia olivacea	HQ326946	734/798	92%
Boletus sp.23	CP19	-	-	-	-	-	507	Tylopilus ballouii	EU430732	471/510	92%
Boletus sp.24	SN01	-	-	-	-	-	783	Phylloporus bogoriensis	JQ003680	758/797	95%
Boletus sp.24	NN11	-	-	-	-	-	785	Phylloporus bogoriensis	JQ003680	758/799	95%
Heimioporus sp.1	UB01	<u>495</u>	Unculture Boletus	HM105532	372/434	86%	784	Tylopilus felleus	HQ326934	748/790	95%
Heimioporus sp.2	MJ06	849	Xerocomus sp. Nan MN4	AB453026	736/746	99%	-	-	-	-	-
Heimioporus sp.2	MJ25	720	Xerocomus sp. Nan MN4	AB453026	712/720	99%	784	Boletellus shichianus	NG027636	755/794	95%
Heimioporus sp.2	CP13	848	Xerocomus sp. Nan MN4	AB453026	735/744	99%	784	Boletellus shichianus	NG027636	755/794	95%
Heimioporus sp.2	CP13.2	846	Xerocomus sp. Nan MN4	AB453027	737/742	99%	-	-	-	-	-
Heimioporus sp.2	CP48	600	Xerocomus sp Nan MN4	AB453026	553/602	92%	784	Boletellus shichianus	NG027636	755/794	95%
Heimioporus sp.2	CP52	-	-	-	-	-	785	Tylopilus felleus	HQ326934	755/793	95%
Heimioporus sp.3	NN03	823	Xerocomus sp. Nan MN4	AB453026	617/740	83%	784	Tylopilus felleus	HQ326934	750/790	95%
Leccinum	NN18	633	Leccinum extremiorientale	DQ407262	615/633	97%	790	Tylopilus felleus	HQ326934	735/799	94%
extremiorientale											
Leccinum sp.	NN13	-	-	-	-	-	790	Leccinum holopus	HQ326928	729/798	91%
Pulveroboletus sp.	MJ33	-	-	-	-	-	791	Tylopilus ballouii	EU430732	748/794	94%
Pulveroboletus sp.	CP16	480	Boletus sp.	EU569234	412/436	94%	791	Tylopilus ballouii	EU430732	748/794	94%
Pulveroboletus sp.	NN21	731	Boletus sp.	EU569234	525/578	91%	791	Tylopilus ballouii	EU430732	748/794	94%
Pulveroboletus sp.	039	736	Boletus sp.	EU569234	516/568	91%	791	Tylopilus ballouii	EU430732	748/794	94%
Strobilomyces	PH32	595	Strobilomyces mirandus	AB275218	338/341	99%	790	Strobilomyces floccopus	AY645053	758/798	95%
mirandus											
Strobilomyces sp.1	P04	785	Strobilomyces seminudus	DQ407255	726/750	97%	783	Tylopilus ballouii	EU430732	722/799	90%
Strobilomyces sp.2	P01	752	Strobilomyces sp.	JF273544	728/735	99%	775	Tylopilus ballouii	EU430737	710/795	89%

Table 4.4 (continued) Sequence affinity of basidiocarps in this study based on Genbank Database

Species	code		Γ	TS region			LSU region					
		base pair	closest species match	accession	overlap	%similarity	base pair	Closest species match	accession	score (bit)	%similarity	
				no.					no.			
Strobilomyces sp.2	P14	747	Strobilomyces sp.	JF273544	720/735	98%	776	Tylopilus ballouii	EU430737	711/796	89%	
Strobilomyces sp.3	CP43	-	-	-	-	-	786	Strobilomyces floccopus	AY645053	707/804	88%	
Tylopilus eximius	CP35	904	uncultured Boletaceae	GQ268585	735/919	80%	763	Boletellus projectellus	NG027638	749/795	94%	
<i>Tylopilus</i> sp.1	NN22	-	-	-	-	-	787	Tylopilus ballouii	EU430737	749/792	95%	
Tylopilus sp.2	CP41	705	Tylopilus felleus		591/717	83%	783	Tylopilus felleus	HQ326934	750/787	95%	
<i>Tylopilus</i> sp.3	PH40	<u>584</u>	Tylopilus formosus	HM060320	512/615	83%	783	Tylopilus felleus	HQ326934	750/787	95%	
Tylopilus sp.4	CP09	<u>481</u>	Tylopilus formosus	HM060320	378/477	85%	-	-	-	-	-	
<i>Tylopilus</i> sp.4	CP10	<u>576</u>	Tylopilus formosus	HM060320	474/573	83%	794	Tylopilus violatinctus	HQ326935	751/808	93%	
<i>Tylopilus</i> sp.4	MJ01	-	-	-	-	-	792	Tylopilus violatinctus	HQ326935	754/806	94%	
<i>Tylopilus</i> sp.4	K04	-	-	-	-	-	792	Tylopilus violatinctus	HQ326935	753/806	93%	
<i>Tylopilus</i> sp.4	CP24	-	-	-	-	-	792	Tylopilus violatinctus	HQ326935	754/806	94%	
Tylopilus sp.5	NN10	-	-	-	-	-	785	Tylopillus felleus	AY586723	749/788	95%	
<i>Tylopilus</i> sp.6	CPB	-	-	-	-	-	783	Tylopilus felleus	HQ326934	746/788	95%	
<i>Tylopilus</i> sp.6	WS01	630	Unculture Boletaceae	GQ268587	391/430	91%	-	-	-	-	-	
Tylopilus sp.7	NN01	682	<i>Xerocomus</i> sp.	JF723274	499/611	82%	788	Tylopilus felleus	HQ326934	758/791	96%	
<i>Tylopilus</i> sp.7	PK01	<u>664</u>	Boletus rhodopurpureus	HM347667	361/415	87%	788	Tylopilus felleus	HQ326934	758/791	96%	
<i>Tylopilus</i> sp.7	CM03	-	-	-	-	-	788	Tylopilus felleus	HQ326934	757/791	96%	
Tylopilus sp.8	NN07	-	-	-	-	-	797	Tylopillus neofelleus	HQ326936	749/810	92%	
<i>Tylopilus</i> sp.9	CP39	-	-	-	-	-	789	Tylopilus neofelleus	HQ326936	736/804	92%	
<i>Tylopilus</i> sp.10	CP23	-	-	-	-	-	795	Tylopilus violatinctus	HQ326935	745/810	92%	
<i>Tylopilus</i> sp.10	CP45	-	-	-	-	-	795	Tylopilus violatinctus	HQ326935	747/810	92%	
<i>Tylopilus</i> sp.10	CP46	<u>597</u>	Tylopilus formosus	HM060320	513/619	83%	-	-	-	-	-	
<i>Tylopilus</i> sp.11	CP14	<u>490</u>	Retiboletus nigerrimus	AB509860	278/315	88%	-	-	-	-	-	
<i>Tylopilus</i> sp.12	NN06	-	-	-	-	-	788	Tylopilus rhoadsiae	AY612836	676/718	94%	

Table 4.4 (continued) Sequence affinity of basidiocarps in this study based on Genbank Database

Species	code		ľ	TS region		LSU region					
		base pair	base pair closest species match		overlap	%similarity	base pair	Closest species match	accession	score (bit)	%similarity
				no.					no.		
<i>Tylopilus</i> sp.13	CP05	516	Tylopilus ballouii	AB509625	298/304	98%	792	Tylopilus ballouii	EU430740	778/792	98%
<i>Tylopilus</i> sp.13	CP06	403	Tylopilus ballouii	AB509626	298/304	98%	-	-	-	-	-
<i>Tylopilus</i> sp.13	CP37	537	Tylopilus ballouii	AB509735	312/321	98%	794	Tylopilus ballouii	EU430740	780/79	99%
<i>Tylopilus</i> sp.13	035	539	Tylopilus ballouii	AB509735	318/321	99%	-	-	-	-	-
<i>Tylopilus</i> sp.13	NN17	-	-	-	-	-	793	Tylopilus ballouii	EU430740	781/793	98%
<i>Tylopilus</i> sp.14	P02	-	-	-	-	-	785	Zangia citrina	HQ326941	779/785	99%

Table 4.4 (continued) Sequence affinity of basidiocarps in this study based on Genbank Database

Species	code	ITS region				
		base pair	closest species match	accession no.	overlap	%similarity
Boletellus ananas	UB07	<u>618</u>	Boletus edulis	UDB011153	196/205	95%
Boletellus sp.1	PH34	834	Xerocomus cisalpinus	UDB011447	186/190	97%
Boletellus sp.2	MJ15	913	Buchwaldoboletus	UDB000647	249/263	94%
			hemichrysus			
Boletellus sp.3	MJ26	<u>582</u>	Leccinum pseudoscabrum	UDB011422	183/191	95%
Boletellus sp.4	WS02	846	Xerocomus ferrugineus	UDB011448	171/173	98%
Boletellus sp.4	P00	445	Xerocomus ferrugineus	UDB011448	171/173	98%
Boletellus sp.4	MJ04	<u>635</u>	Xerocomus ferrugineus	UDB011448	171/173	98%
Boletus sp.1	P12	804	Boletus luridus	UDB002401	174/179	97%
Boletus sp.1	CP47	<u>328</u>	Boletus queletii	UDB000760	176/184	95%
Boletus sp.2	CP18	<u>502</u>	Boletus calopus	UDB000079	301/333	90%
Boletus sp.3	CP01	770	Boletus rubrosanguineus	UDB000410	309/323	95%
Boletus sp.4	CP15	659	Xerocomus cisalpinus	UDB011447	209/219	95%
Boletus sp.4	CP51	<u>516</u>	Xerocomus cisalpinus	UDB011447	209/219	95%
Boletus sp.6	CP03	783	Boletus radicans	UDB003224	550/607	90%
Boletus sp.7	MJ27	860	Boletus pinophilus	UDB011150	192/201	95%
Boletus sp.8	CP40	720	Boletus erythropus	UDB001523	296/325	91%
Boletus sp.9	NN05	699	Boletus erythropus	UDB001523	387/432	89%
Boletus sp.10	CP11	941	Boletus erythropus	UDB001523	289/318	90%
Boletus sp.11	CP08	573	Xerocomus ferrugineus	UDB011448	168/171	98%
Boletus sp.12	P05	629	Melanogaster variegatus	UDB001487	171/175	97%
Boletus sp.13	CP17	728	Boletus edulis	UDB011153	179/179	100%
Boletus sp.13	CP21	731	Boletus edulis	UDB011153	179/179	100%
Boletus sp.13	CP49	717	Boletus edulis	UDB011153	179/179	100%
Boletus sp.14	PH37	370	Boletus erythropus	UDB001523	178/180	98%
Boletus sp.15	NN12	848	Boletus legaliae	UDB001115	303/332	91%
Boletus sp.16	NN16	826	Aureoboletus gentilis	UDB000687	186/191	97%
Boletus sp.17	P15	621	Aureoboletus gentilis	UDB000687	178/179	99%
Boletus sp.17	PH41	617	Aureoboletus gentilis	UDB000687	178/179	99%
Boletus sp.17	UB04	637	Aureoboletus gentilis	UDB000687	234/250	93%
Boletus sp.18	MJ16	<u>537</u>	Xerocomus silwoodensis	UDB002290	176/179	98%
Boletus sp.19	CP34	813	Xerocomus ferrugineus	UDB011448	180/180	100%
Boletus sp. 20	NN02	741	Boletaceae	UDB011023	199/202	98%
Heimioporus sp.1	UB01	<u>495</u>	Xerocomus cisalpinus	UDB011447	248/265	93%
Heimioporus sp.2	MJ06	849	Xerocomus cisalpinus	UDB011447	242/260	93%
Heimioporus sp.2	MJ25	720	Xerocomus cisalpinus	UDB011447	242/260	93%
Heimioporus sp.2	CP13	848	Xerocomus cisalpinus	UDB011447	242/260	93%
Heimioporus sp.2	CP13.2	846	Xerocomus cisalpinus	UDB011447	242/260	93%
Heimioporus sp.2	CP48	<u>600</u>	Xerocomus cisalpinus	UDB011447	242/260	93%
Heimioporus sp.3	NN03	823	Boletus satanas	UDB000419	247/265	93%
Leccinum	NN18	633	Boletus luridus	UDB002401	303/325	93%
extremiorientale						

Table 4.5 Sequence affinity of basidiocarps in this study based on UNITE Database

The underline (_) in column base pair indicates incomplete full length in sequencing.

Table 4.5 (continued) Sequence affinity of basidiocarps in this study based on UNITE

Database

Species	code	ITS region							
		base pair	closest species match	accession no.	overlap	%similarity			
Pulveroboletus sp.	CP16	<u>480</u>	Boletus luridus	UDB002401	250/265	94%			
Pulveroboletus sp.	NN21	731	Boletus queletii	UDB000760	306/332	92%			
Pulveroboletus sp.	039	736	Boletus queletii	UDB000760	292/318	91%			
Strobilomyces	PH32	595	Strobilomyces strobilaceus	UDB000662	172/172	100%			
mirandus									
Strobilomyces sp.1	P04	785	Boletus erythropus	UDB001523	236/258	91%			
Strobilomyces sp.2	P01	752	Porphyrellus porphyrosporus	UDB001485	153/157	97%			
Strobilomyces sp.2	P14	747	Porphyrellus porphyrosporus	UDB001485	150/157	95%			
Tylopilus eximius	CP35	904	Xerocomus badius	UDB011681	211/219	96%			
Tylopilus sp.2	CP41	705	Xerocomus cisalpinus	UDB011447	243/258	94%			
Tylopilus sp.3	PH40	<u>584</u>	Serpula lacrymans	UDB003334	169/174	97%			
Tylopilus sp.4	CP09	<u>481</u>	Serpula lacrymans	UDB003334	168/173	97%			
<i>Tylopilus</i> sp.4	CP10	<u>576</u>	Serpula lacrymans	UDB003334	168/173	97%			
<i>Tylopilus</i> sp.6	WS01	630	Hygrophoropsis aurantiaca	UDB011685	159/164	96%			
Tylopilus sp.7	NN01	682	Boletus luridus	UDB002401	179/181	98%			
<i>Tylopilus</i> sp.7	PK01	<u>664</u>	Boletus luridus	UDB002401	179/181	98%			
<i>Tylopilus</i> sp.10	CP46	<u>597</u>	Leucopaxillus giganteus	UDB011853	170/174	97%			
<i>Tylopilus</i> sp.11	CP14	<u>490</u>	Pisolithus arhizus	UDB001206	158/161	98%			
<i>Tylopilus</i> sp.13	CP05	516	Boletus luridus	UDB002401	171/174	98%			
<i>Tylopilus</i> sp.13	CP06	<u>403</u>	Boletus luridus	UDB002401	171/174	98%			
<i>Tylopilus</i> sp.13	CP37	537	Xerocomus ferrugineus	UDB011448	171/178	98%			
<i>Tylopilus</i> sp.13	035	539	Xerocomus chrysonemus	UDB002257	175/181	96%			

The underline (_) in column base pair indicates incomplete full length in sequencing.

4.4 Phylogeny of Boletaceae

To study phylogenetic relationship among the family Boletaceae, ITS and LSU sequences of some *Boletellus, Boletus, Heimioporus, Leccinum, Pulveroboletus, Strobilomyces* and *Tylopilus* species were analysed with available sequences from GenBank Database. Twenty-nine sequences of twenty-two local species were used for construction of phylogenetic tree based on ITS region, while, Forty-four sequences of twenty-eight local species were use in analyses based on LSU region.

According to phylogenetic tree based on ITS regions (Figure 4.43), five genera, *Boletellus, Heimioporus, Leccinum, Pulveroboletus* and *Strobilomyces* were monophyletic groups while *Boletus* and *Tylopilus* were not. Both *Boletus* and *Tylopilus* were also separated in three clades.

Within genus *Boletellus*, the bootstrap value was 87%. *Boletellus* sp.2 (MJ15) corresponded to *B.* mirabilis while *Boletellus* sp.1 (PH34) closely related to *B. obscurococcineus* with highly supported bootstrap value (95%). However, most relationships among this genus were unclear.

Genus *Pulveroboletus* in this study composed of four species with 72% bootstrap value. Within this clade, Thai *Pulveroboletus* sp. (NN21) showed the close relationship to *Pulveroboletus ravenelii*. The sister group of *Pulveroboletus, Leccinum* clade comprised only two species, *L. extremiorientale* (including NN18) and *L. rugosiceps* with 67 % bootstrap supported.

Heimioporus clade had strong supported (96% BS). Thai samples were divided into 2 subclades. First subclade contained *Heimioporus* sp.2 (MJ25 CP13 and MJ06) and *Heimioporus* sp. from Genbank with highest supported 99% bootstrap value. Within other subclades, *Heimioporus* sp.3 closely related to *H. japonica* with 91% bootstrap supported.

Strobilomyces clade was represented with 78% bootstrap value. Three Thai species were placed in this clade. *Strobilomyces* sp.2 (P14 and P01) showed highly corresponded to *S. seminudus* and *Strobilomyces* sp. with 99% bootstrap. *S. mirandus* (PH32) from Thailand closely related to the sequence of same species from GenBank and this species was sister group of *Strobilomyces* sp.1(P04). This Thai species closely related to other *S. seminudus* with highest supported 99% bootstrap value.

Boletus clade I was separated into 2 subclades and each subclade contained one species from Thailand. *Boletus* sp.19 (CP34) demonstrated closed relationship with ectomycorrhizal fungus (AB218099) with highly supported bootstrap value (96%). The other Thai species, *Boletus* sp.17 (UB04 P15 and PH41) was placed with uncultured Basidomycota clone (GU328591) and bootstrap value was 99%.

The relationship among *Boletus* clade II was unclear. *Boletus* sp.6 (CP03) closely related to *B. subvelutipes* (81% bootstrap value) and *Boletus* sp.3 (CP01) closely related to *B. bicolor* (84% bootstrap value). While *Boletus* sp.9 (NN05) and *Boletus* sp.11 (NN12) show corresponded to *B. erythropus* and *Boletus* sp. respectively with weak supported.

The relationship among *Tylopilus* was non-monophyletic group. *Tylopilus* clade I consisted of two species, *T. eximius* (CP35) from Thailand and *T. felleus* with 72% bootstrap support. Within *Tylopilus* clade II, *Tylopilus* sp.4 (CP04) showed closely relationship to uncultured Boletaceae type (GQ268587) and *Tylopilus* sp.10 (CP46) was placed with *T. formosus*. The other *Tylopilus* clade was the clade of *T. ballouii* that contain *Tylopilus* sp.13 (CP05 and CP37) from Thailand. Bootstrap value of this clade was 95%.



Figure 4.43 Phylogenetic tree based on ITS sequences of Boletaceae, closely related species and outgroups (*Rhizopogon* spp.) using Maximum Likelihood, The numbers at the nodes are boostrap values based 100 replications. The bold letters denote Thai samples in this study. The scale bar indicates 0.5 of the genetic distance.



Figure 4.43 (continued) Phylogenetic tree based on ITS sequences of Boletaceae, closely related species and outgroups (*Rhizopogon* spp.) using Maximum Likelihood,The numbers at the nodes are boostrap values based 100 replications. The bold letters denote Thai samples in this study. The scale bar indicates 0.5 of the genetic distance.

The relationship among Boletaceae based on LSU region was represented in Figure 4.44 The phylogenetic tree clearly divided into 16 clades. Only three genera, *Heimioporus, Pulveroboletus* and *Strobilomyces* were monophyletic groups.

The strong supported clade was *Heimioporus* with 94% bootstrap value. The relationship in this clade was clearly resolved. *Heimioporus* sp.1 (UB01) closely related to *H. retispora* (96% BS) and was sister group of *Heimioporus* sp.3 (NN03). While 2 sequences of *Heimioporus* sp.2 (CP52 and MJ27) were grouped together (96% bootstrap value).

Pulveroboletus clade contained only one Thai species (039 CP16 MJ33) which closely related to *P. ravenelii* (98% bootstrap value). While *Strobilomyces* clade comprised 4 species from Thailand with high supported (80% BS) and was separated into 3 subclades. *S. mirandus* (PH32), *S. floccopus* and *Strobilomyces sp.* were place together in first subclade with 78% bootstrap value. Within second subclade, *Strobilomyces* sp.3 (P01) related to *Strobilomyces* sp.1 (P04) with weak supported (54% bootstrap value). The last subclade comprised *Strobilomyces* sp.2 (P01 and P04) and *Strobilomyces* sp. with 77% bootstrap support.

Leccinum was non-monophyly. *Leccinum* sp. (NN13) from Thai sample was placed in *Leccinum* clade I together with *L. holopus* and *L. rugosiceps* (98% bootstrap value) while *L. extremiorientale* was separated.

Boletellus could be divided into three clades. *Boletellus* sp.2 (MJ12 and MJ15) related to *B. chrysenteroides* closely within *Boletellus* clade I with 95% bootstrap value. While *B. ananas* (including MJ03 P13 and UB7) and *Boletellus* sp.4 (CP32 MJ04 and P00) were clearly separately with strongly supported (95% and 99% respectively)

Boletus could be seperated into 5 clades. Within *Boletus* clade I, two subclades were grouped together with strongest supported (99%). First subclade comprised *B. edulis* and *B. rex-veris* (99% bootstrap value). While, *Boletus* sp. 21 (CP53) closely related to *B. quercophilus* and were placed together in second subclade (99% BS). Within *Boletus* clade II, the relationship was still unclear. This clade contained two Thai samples, *Boletus* sp.3 (CP01) and *Boletus* sp.6 (CP03). *Boletus* clade III comprised only
two species. *Boletus* sp.1 (CP47 and P12) demonstrated closed relationship with *B. viridiflavus* with highly supported (97% BS). *Boletus* sp. 24 (SN1) and *Boletus* sp.19 (CP34) were placed together with other two *Boletus* species in *Boletus* clade IV with 87% bootstrap support. Within Boletus clade V, *Boletus* sp.4 (CP15 CP20 and CP51) and *Boletus* sp.5 (MJ23) were grouped together with strongest supported (99% bootstrap value).

The relationship among *Tylopilus*, this genus could be separated into 4 clades. *Tylopilus* clade I was highly supported clade (92% BS) and could be divided into 2 subclades. Within first subclade with 98% bootstrap value, *Tylopilus* sp.3 (PH40) and *Tylopilus* sp.4 (CP10 and MJ01) were grouped with *T. violatinctus* (85% bootstrap value) while *Tylopilus* sp. 10 (CP23 and CP45) closely related to *T. formosus* with highly supported (96% bootstrap value). *Tylopilus* sp.8 (NN07) was placed with *T. intermedius*, *T. rubrobrunneus* and *T. neofelleus* in other subclade with weakly supported. *Tylopilus* clade II contained only one Thai species (*Tylopilus* sp.14) and the relationship in this clade was unclear. Within *Tylopilus* clade III, *Tylopilus* sp.13 (CP05 CP37 and NN17) was closely corresponded with *T. ballouii* (99% bootstrap value) and this species could be separated into 2 subclades. *Tylopilus* clade IV comprised only one species, *T. eximius* (including CP35) with 94 % bootstrap value.



Figure 4.44 Phylogenetic tree based on LSU sequences of Boletaceae, closely related species and outgroups (*Rhizopogon* spp.) using Maximum Likelihood, The numbers at the nodes are boostrap values based 100 replications. The bold letters denote Thai samples in this study. The scale bar indicates 0.05 of the genetic distance.



Figure 4.44 (continued) Phylogenetic tree based on LSU sequences of Boletaceae, closely related species and outgroups (*Rhizopogon* spp.) using Maximum Likelihood, The numbers at the nodes are boostrap values based 100 replications. The bold letters denote Thai samples in this study. The scale bar indicates 0.05 of the genetic distance.

4.5 Phylogeny of Tylopilus

To study phylogenetic relationship among one of large genus, *Tylopilus*, ITS and LSU sequences of some *Tylopilus* and *Zangia* species were analysed with available sequences from GenBank Database. Fourteen sequences of nine local species were used for construction of phylogenetic tree based on ITS region, while, twenty sequences of twelve local species including one *Zangia* species were use in analyses based on LSU region. The detail of sequences from GenBank Database from GenBank Database including host plant families were demonstrated in Table 4.6 and 4.7.

Table 4.6 ITS sequences of Tylopilus and Retiboletus from Genbank Database

species	Accession no.	host plant families	country
Tylopilus ballouii	AB509625	unknown	Japan
Tylopilus ballouii	AB509735	unknown	Japan
Tylopilus felleus	HM190016	Pinaceae and Fagaceae	Germany
Tylopilus felleus	HM190015	Pinaceae and Fagaceae	Germany
Tylopilus rubrobrunneus	GQ166869	Fagaceae	USA
Tylopilus formosus	HM060320	Casuarinaceae and Myrtaceae	New Zealand
Uncultured Boletaceae	GQ268587	Dipterocarpaceae	Malaysia
Retiboletus nigerrimus	AB509860	unknown	Japan

species	Accession no.	host plant families	country
Tylopilus violatinctus	HQ326935	Pinaceae and Fagaceae	China
Tylopilus formosus	HM060319	Casuarinaceae and Myrtaceae	New Zealand
Tylopilus intermedius	HQ161875	Fagaceae	USA
Tylopilus rubrobrunneus	HQ161876	Fagaceae	USA
Tylopilus neofelleus	HQ326936	Fagaceae	China
Tylopilus rhoadsiae	AY612836	Pinaceae and Fagaceae	USA
Tylopilus eximius	AF139684	Pinaceae and Fagaceae	USA
Tylopilus virens	DQ534621	Fagaceae	USA
Zangia citrina	HQ326940	Pinaceae and Fagaceae	China
Zangia citrina	HQ326941	Pinaceae and Fagaceae	China
Zangia olivaceobrunnea	HQ326947	Pinaceae and Fagaceae	China
Zangia olivaceobrunnea	HQ326948	Pinaceae and Fagaceae	China
Tylopilus ballouii	EU430731	Fagaceae	Costa Rica
Tylopilus ballouii	EU430732	Fagaceae	Costa Rica
Tylopilus ballouii	EU430734	Fagaceae	USA
Tylopilus ballouii	EU430737	Fagaceae	USA
Tylopilus ballouii	EU430736	Pinaceae	Belize
Tylopilus ballouii	EU430735	Fagaceae	Belize
Tylopilus ballouii	EU430733	unknown	Mexico
Tylopilus ballouii	EU430741	Casuarinaceae and Myrtaceae	Australia
Tylopilus ballouii	EU430738	Casuarinaceae and Myrtaceae	Australia
Tylopilus ballouii	EU430740	Dipterocarpaceae	Thailand

Table 4.7 LSU sequences of Tylopilus and Zangia from Genbank Database

A phylogenetic dendrogram based on ITS sequences of *Tylopilus* samples collected from various localities in Thailand and those of registered ones in Genbank database was represented in Figure 4.45 All sequences in the dendrogram were divided into four major clades (clade I, clade II, clade III and clade IV). Sequences of all Thai basidiocarps were distributed in all clades.

Clade I was divided into two subclade, A and B, with highly supported value, 97%. ITS sequences of Thai samples, CP05 and CP06 were grouped together with sequence of *T. ballouii* (AB509625) in subclade A (80% BS). Other Thai sample, CP37 and 035 closely related to *T. ballouii* (AB509735) with 83% bootstrap value in subclade B.

Clade II (64% bootstrap value) was separated into two subclades (C and D). Thai samples, CP4, PK01 and NN01 *Tylopilus* sp.7, were placed in subclade D and associated with Fagaceae while *T. felleus* in subclade C were reported that could also associate with Pinaceae.

Clade III was separated into 3 subclades (E, F and G). The samples in this clade had different host plant families. Within subclade F, CP46 and *T. formosus* associated with *Eucalyptus*. Dipterocarpaceae was the host plant of members in subclade E (CP09 and CP10) and subclade G (WS01 and Boletaceae sequence). *T. rubrobrunneus* and Thai sample, PH40 associated with Fagaceae.

Members in clade IV associated with different host plants. *T. eximius* was found in association with Fagaceae while in subclade H, CP11 which closely related to *Retiboletus nigerrimus* was found in a *Eucalyptus* plantation.



Figure 4.45 Phylogenetic tree based on ITS sequences of *Tylopilus*, closely related species and outgroups (*Rhizopogon* spp.) using Maximum Likihood,The numbers at the nodes are boostrap values based 100 replications. The bold letters denote Thai samples in this study. The scale bar indicates 0.2 of the genetic distance.

The relationship among *Tylopilus* based on LSU region was represented in Figure 4.46 The phylogenetic tree clearly divided into 5 clades. The sequences of Thai basidocarps in two highly supported clades (clade III and IV) were grouped together with the sequences of *T. ballouii* (96% and 100% BS respectively). Our samples (CP05, NN07 and CP37) were placed within subclade M including one Thai sample from GenBank Database (EU430740). In addition, three subclades were resolved in clade IV. It was appear that each Tylopilus species was confined to host plant families.

Within strongly supported clade, clade I could be divided into 5 subclades. Subclade A (100% BS) comprised of Thai samples, CP24, K04, CP10 and MJ01, and associated with plant family Dipterocarpaceae. Within subclade B, PH40 was related to *T. violatinctus* with 52% bootstrap supported and associated with Fagaceae and Pinaceae. These two subclades were separated with 99% bootstrap value. Subclade C comprised *Eucalyptus* associated species, *T. formosus*, CP23 and CP45 while subclade D and E consisted of five species that associated with Fagaceae, *T. intermedius*, *T.rubrobrunneus*, *T. neofelleus*, and Thai samples (NN07 and CP09).

All species in clade II shared the same host plant family, Fagaceae and Pinaceae despite of low bootstrap supported. The two *Tylopilus* samples from Thailand were placed into subclade F and G. NN06 and *T. rhoadsiae* were represented close relationship. CP35 from this study was closely related with *T. eximius*. While the relationship between NN22 and other species was unresolved. Furthermore, the relationship in this clade might affirm the monophyly of new closely related genus, *Zangia*, with high supported bootstrap value (98%) including Thai sample, P02 in subclade H.

The last clade wasseparated from other groups and composed of only Thai samples, CM03, PL01 and NN01 which associated with Fagaceae.



Figure 4.46 Phylogenetic tree based on LSU sequences of *Tylopilus*, closely related species and outgroups (*Rhizopogon* spp.) using Maximum Likelihood, The numbers at the nodes are boostrap values based 100 replications. The bold letters denote Thai samples in this study. The scale bar indicates 0.05 of the genetic distance.

According to phylogenetic study in *Tylopilus* based on ITS and LSU sequences, it reveals that at least fourteen *Tylopilus* species exist in Thailand as the following:

Tylopilus ballouii (Peck) Singer (CP05, CP06, CP37 and 035)

Pileus 5-7 cm, convex to broadly convex, bright ochraceous orange to reddish orange, often fulvous in the centre; tubes 3-8mm, adnate, cream white ; pores 1-2mm, subrounded, radially elongate near the stem, cream white. Stem 3-7cm x 15-20mm at the apex, 5-8mm at the base, equal, strongly attenuate downwards, orange to paler concolorous, base pallid and slightly villous with the white mycelium.

Basidia clavate, 4-spored. Basidiospores 3.91 (6.09 \pm 0.77) 8.32 μ m x 2.77 (3.98 \pm 0.60) 5.78 μ m, broadly ellipsoid, smooth. Pleurocystidia ventricose-rostrate, hyaline in KOH.



Figure 4.47 basidiocarp characteristics of Tylopilus ballouii

Tylopilus sp.1 (NN22)

Pileus 4.5cm, convex, smooth, pale gray, dark gray in the center; tubes 7mm pale purple; pores purplish red when young. Stem 5cm x 15mm, equal, slightly longitudinal-straite, apex purphish cream, base dark gray.



Figure 4.48 basidiocarp characteristics of Tylopilus sp.1

Tylopilus sp.2 (CP41)

Pileus 5.5 cm, convex, smooth, dark brown; tubes 3 mm, rufescent ; pores 0.5 mm, subrounded, rufescent, pale near the stem. Stem 4cm x 8mm at the apex, 15mm at the base, clavate, smooth, reddish brown, base dark brown.



Figure 4.49 basidiocarp characteristics of Tylopilus sp.2

Tylopilus sp.3 (PH40)

Pileus 2 cm, convex, smooth, dark purple; pores 0.2mm subrounded, pale purplish pink. Stem 5.5cm x 8mm at the apex, 10mm at the base, clavate, tapered at base, apex dark purple, base pallid.

Pleurocystidia ventricose-rostrate, hyaline in KOH.



Figure 4.50 basidiocarp characteristics of Tylopilus sp.3

Tylopilus sp.4 (CP09, CP10, CP24, K04 and MJ01)

Pileus 4.5-7.5 cm, convex to broadly convex, smooth, bright pinkish purple to purple, subviscid when moist; tubes 5-8mm, purplish cream; pores rounded to subrounded, pale purple. Stem 5-8cm x 12-17mm at the apex, 12-20mm at the base, equal or slightly clavate, pinkish purple, base pallid and slightly villous with the white mycelium.

Basidia clavate, 4-spored. Basidiospores subfusiform, smooth. Pleurocystidia ventricose-rostrate, hyaline in KOH.



Figure 4.51 basidiocarp characteristics of Tylopilus sp.4

Tylopilus sp.5 (NN10)

Pileus 10 cm, convex to broadly convex, smooth, dark purple with dark spots;
pores 0.3-0.4mm rounded, pale purple. Stem 6cm x 20mm, equal, dark purplish brown.
Basidia clavate, 4-spored. Basidiospores 6.76 (8.09 ± 0.80) 6.76 μm x 3.70 (4.61 ± 0.53) 5.44 μm, elipsoid, smooth. Pleurocystidia ventricose-rostrate, dark in KOH.



Figure 4.52 basidiocarp characteristics of Tylopilus sp.5

Tylopilus sp.6 (WS01 and CPB)

Pileus 8 cm, convex, smooth, pale purple; tubes 10mm, rufescent; pores 1-2mm, rounded to subrounded, rufescent. Stem 7.5cm x 20mm, equal, punctuate, pinkish purple, base white and slightly villous with the white mycelium.

Basidia clavate, 4-spored. Basidiospores 10.65 (11.88 \pm 0.70) 13.73 μ m x 3.59 (4.33 \pm 0.39) 5.24 μ m, subfusiform, smooth. Pleurocystidia ventricose-rostrate, hyaline in KOH.



Figure 4.53 basidiocarp characteristics of Tylopilus sp.6

Tylopilus sp.7 (CM03, PK01 and NN01)

Pileus 5-7.5cm, convex, smooth, dark brown; tubes 5mm, pale purple; pores rounded to subrounded, white then pale purple. Stem 5-6cm x 10mm, equal, slightly fibrillose, dark brown, apex pallid.

Basidia clavate, 4-spored. Basidiospores 9.10 (10.28 \pm 0.64) 11.26 μ m x 3.20 (3.74 \pm 0.26) 4.37 μ m, subfusiform, smooth. Pleurocystidia ventricose-rostrate, hyaline in KOH.



Figure 4.54 basidiocarp characteristics of Tylopilus sp.7

Tylopilus sp.8 (NN07)

Pileus 5-7cm, convex, smooth, dark purple; tubes 4mm, pale purple; pores rounded to subrounded, cream then pale purple. Stem 4-5cm x 10-15mm, equal, dark purple.



Figure 4.55 basidiocarp characteristics of Tylopilus sp.8

Tylopilus sp.9 (CP39)

Pileus 6.5-13.5 cm, convex to broadly convex, smooth, brown to yellow brown; tubes 10mm, purplish cream; pores rounded to subrounded, cream then pale purple. Stem 4-6cm x 12-15mm at the apex, 15-20mm at the base, equal or slightly clavate, dark yellow brown, base pallid and slightly villous with the white mycelium.

Basidia clavate, 4-spored. Basidiospores 5.68 (6.75 \pm 0.49) 8.12 µm x 3.91 (4.85 \pm 0.44) 6.20 µm, ellipsoid, smooth. Pleurocystidia ventricose, dark in KOH.



Figure 4.56 basidiocarp characteristics of Tylopilus sp.9

Tylopilus sp.10 (CP23, CP45)

Pileus 6-8.5cm, broadly convex, smooth, brown to purplish brown; tubes 10mm, pale purplish brown; pores rounded to subrounded, pale purplish brown. Stem 3-3.5cm x 15-20mm, equal, brown to purplish brown, base white and slightly villous with the white mycelium.

Basidia clavate, 4-spored. Basidiospores 5.59 (9.53 \pm 1.01) 11.33 μ m x 3.39 (4.36 \pm 0.46) 5.37 μ m, subfusiform, smooth. Pleurocystidia ventricose, dark in KOH.



Figure 4.57 basidiocarp characteristics of Tylopilus sp.10

Tylopilus sp.11(CP14)

Pileus 5-11cm, convex to broadly convex, smooth, appenduculate, brown; tubes pale purplish brown; pores rounded to subrounded, pale purplish brown. Stem 2.5-3cm x 15-20mm, equal, brown, apex pale, base white and slightly villous with the white mycelium.

Basidia clavate, 4-spored. Basidiospores 5.74 (9.05 \pm 1.24) 11.50 μ m x 3.25 (3.90 \pm 0.36) 4.56 μ m, subfusiform, smooth. Pleurocystidia ventricose, hyaline in KOH.



Figure 4.58 basidiocarp characteristics of *Tylopilus* sp.11

Tylopilus sp.12 (NN06)

Pileus 7cm, convex to broadly convex, slightly alveolate, pale yellow; tubes pale cream; pores rounded to subrounded, cream. Stem 5cm x 18mm, equal, reticulate, cream.



Figure 4.59 basidiocarp characteristric of Tylopilus sp.12

Tylopilus eximius (Peck) Singer (CP35)

Pileus 8cm, convex, smooth, purplish brown, subviscid when moist; tubes pale purple; pores rounded to subrounded, purple. Stem 3-3.5cm x 25mm at apex, 35 at base, clavate, pale purple minutely punctate with purplish brown dots.

Basidia clavate, 4-spored. Basidiospores subfusiform, smooth. Pleurocystidia ventricose, hyaline in KOH.



Figure 4.60 basidiocarp characteristics of Tylopilus eximius

Zangia sp. (P02)

Pileus 5-7cm, convex, smooth, orange brown to brown; tubes pale purple; pores rounded to subrounded, purple. Stem 3-4cm x 15mm,equal, pale purple, base yellow and slightly villous with the yellow mycelium.

Basidia clavate, 4-spored. Basidiospores 10.15 (12.01 \pm 0.81) 13.70 μ m x 3.70 (4.50 \pm 0.41) 5.56 μ m, subfusiform, smooth. Pleurocystidia ventricose, hyaline in KOH. Pileipellis ixohyphoepithelium.



Figure 4.61 basidiocarp characteristics of Zangia sp.

CHAPTER V

DISCUSSION

5.1 Diversity of Boletaceae and Morphological Identification

Boletaceae is the one of large genera in Boletales and consists of approximate 39 genera and more than 700 species. This fungal family distribute across temperate and tropical regions. (Binder and Hibbett, 2006; Halling *et al.*, 2007; Kirk *et al.*, 2008). In Thailand, the diversity of Boletaceae is abundant particularly in Northern and Northeastern (Klinhom and Klinhom, 2007; Thangklam, 2008)

In this study, the most abundant genus was *Boletus* which consisted of 24 species or 44.44 % of all Boletaceae. The most abundant of *Boletus* was similar to Thangklam (2008). Forty-five *Boletus* species were recorded (54.87% of all Boletaceae) in Northern Thailand. *Boletus* is the largest genus in Boletaceae and comprises of approximately 300 species around the world (Kirk *et al.*, 2008). The other abundant genus was *Tylopilus*. Fourteen *Tylopilus* species was found in this study. Thangklam (2008) reported that the number of *Tylopilus* species in the North was nine as well as *Leccinum* species. Two large genera consist of around 75 species and widespread (Kirk *et al.*, 2008). In contrast, only two species of *Leccinum* were found in this study because the collecting time might not be the time of basidiocarp formation.

Within the difference of eight genera in this study (*Boletellus, Boletus, Heimioporus, Leccinum, Pulveroboletus, Strobilomyces, Tylopilus* and *Zangia*), the morphology of basidiospores was the main character to recognize these genera. In addition, the color of pores and the change of color were still important characters. Several characters such as surface and color of pileus and stem including the margin of pileus were used to species identification but various species were variation in color.

5.2 Identification of the Basidiocarps Based on ITS and LSU Regions

When the success of sequencing in both rDNA regions was compared, more LSU sequences were successful in sequencing. Even though, two regions are close in position but LSU is more conserve while ITS also has higher variation. That may cause failure in direct sequencing and the sequences would not be clear in the position that has variation. In several previous studies (Binder and Hibbett, 2006; Sato *et al.*, 2008; Dentinger *et al.*, 2010), cloning technique were used to resolve this problem.

According to more accuracy in species identification based on ITS region, it indicated that ITS had become the primary genetic marker for molecular identification and other species-level pursuits in many groups of fungi (Nilsson *et al.*, 2011). In contrast to ITS, LSU region was not discriminative at the species-level but this region could be used to assign specimens to a higher taxonomic level when a good ITS match was absent (Abarenkov *et al.*, 2010).

In several specimens, the overlap value based on ITS was quit low. This result reflected the lack of molecular database in the family Boletaceae especially tropical Boletes. Moreover, the results of ITS demonstrated misidentified fungal species in Genbank database for example *Heimioporus* sp.2. Nilsson *et al.* (2006) reported that several fungal ITS sequences in the International Nucleotide Sequence Database comprising GenBank database did not have full species name and 10% had incorrect names. The other database which contain the taxonomic reliable sequences was UNITE database (Abarenkov *et al.*, 2010; Nilsson *et al.*, 2011). In 2011, UNITE holds 2968 reference sequences from 1120 fungal species in 155 genera, primarily of ectomycorrhizal fungi from North Europe and the primary genetic marker targeted is ITS region (Nilsson *et al.*, 2011). Because of different groups of specimens from this study, the overlap value of closest species match was very low.

5.3 Phylogeny of Boletaceae

In this study, the relationship among Boletaceae was investigated based on two rDNA region, ITS and LSU. According to phylogenetic tree based on both regions, *Heimioporus, Pulveroboletus* and *Strobilomyces* were monophyletic groups

Within the relationship among *Heimioporus* species based on ITS region, *Heimioporus* sp. 2 was grouped together with *Heimioporus* sp. (AB453025 and AB453026) with strongest bootstrap value. This result suggested that two species might be the same species and was similar to the BLAST result (Table 4.4). While *Heimioporus* sp.3 was placed with *H. japonica* in other subclade and both species shared the same character of stem, reticulate. The phylogenetic relationship of this genus based on LSU was similar to the ITS result. *Heimioporus* were separated into 2 subclades and conform to the character of stem. First subclade consisted of *Heimioporus* sp.1, *Heimioporus* sp.3 and *H. retispora* which their stems were reticulate. *Heimioporus* sp.2 which had fibrillose stem was placed into other subclade.

In genus *Pulveroboletus*, only one species was found in this study. Both the phylogentic tress based on ITS and LSU demonstrated that Thai species closely related to *P. ravenelii*. These two species were similar except the color of mature pileus. *P. ravenelii* had raddish to raddish brown pileus (Corner, 1972) while the pileus of Thai species was yellow.

According to the relationship among *Strobilomyces*, this genus could be divided into 3 subclades based on both ITS and LSU. Based on ITS, *Strobilomyces* sp.2 was grouped together with *S. seminudus* and *Strobilomyces* sp. (JF273544) in first subclade with highest supported (99% BS). This result indicated that these samples might be the same species. In second subclade demonstrated closely relationship between *Strobilomyces* sp.1 and *S. seminudus*. The present of *S. seminudus* in 2 subclades reflected that *S. seminudus* sequences were misidentified or this species might be complex species. In the last subclade, Thai *S. mirandus* corresponded to the same species from Genbank. This result could confirm correct identification. Based on LSU, even though *Strobilomyces* could be separated into 3 subclades, the bootstrap supported in each subclade was not high as ITS.

The phylogenetic relationship based on ITS suggested that *Boletellus* and *Leccinum* were monophyly but their relationship based on LSU was contrast. This different result was from different number of sequences in each genus.

Boletellus could be separated into 2 subclades based on ITS. Boletellus sp.2 was placed in first subclade but the relationship in this subclade was unclear because of weak support. While *Boletellus* sp.1 closely related to *B. obscurococcineus* in other subclade. Dissimilar to ITS, the phylogenetic relationship based on LSU demonstrated that *Boletellus* was non-monophyletic group and divided into 3 clades. *Boletellus* clade I contained *Boletellus* sp. 2 and *B. chrysenteroides*. The pileus of two species was not squamose like other *Boletellus* species. *Boletellus* clade II comprised only one species, *B. ananas*. This clade could be divided into 3 subclades and suggested that this species might be cryptic species. Contrast with this clade, *Boletellus* sp.4 was grouped together with highest supported (99% BS) in *Boletellus* clade III.

In the genus *Leccinum*, Thai *L. extremiorientale* closely related to the same species based on ITS. This result could confirm correct identification. But this species was separated to other *Leccinum* based on LSU.

However, the number sequences especially *Heimioporus* and *Leccinum* was fewer to investigate the phylogenetic relationship. So ITS and LSU sequences from more taxa should be add in the analysis to confirm the monophyly of genus.

Although two phylogenetic trees were inconsistent in some genera but in large genera both *Tylopilus* and *Boletus* showed the same result. Two genera were non-monophyletic groups and could be divided at least 3 clades. This result was similar to the previous studies (Binder and Hibbett, 2006; Drehmel *et al.*, 2008) and suggested that two genera should be revised.

5.4 Phylogeny of Tylopilus

Tylopilus is one of large genus in the family Boletaceae which composes of 75 species (Kirk *et al.*, 2008). This genus distributes across the subtropics and tropics, especially in the America and Australia continents or East Asia including Southeast Asia. (Halling *et al.*, 2007)

According to this study, fourteen species of *Tylopilus* were classified based on both morphological and molecular data. The phylogenetic analysis of *Tylopilus* based on ITS and LSU regions demonstrated that *Tylopilus* was clearly separated into at least 4 clades. These two phylogenetic trees shared most similarity particularly clade III based on ITS and clade I based on LSU which composed of almost same species. It indicated that these two regions from nuclear rDNA conformed to each other when were used for molecular phylogenetic studies.

When the dataset of two phylogenetic tree were compared, the phylogenetic tree based on LSU which larger dataset was more completed and represented two clades of cryptic species, *Tylopilus ballouii*. This result was similar to the study of Halling *et al.* (2008) which indicated that long-distance dispersal events were possible and the populations have been isolated for long periods.

Moreover, the dataset of two rDNA regions were from mostly temperate species while the molecular data of tropical species was scant. This result reflected that more molecular information especially including tropical species can resolve and provide complete and accurate phylogenetic relationships among *Tylopilus*.

According to phylogenetic tree of *Tylopilus* based on LSU region, the result demonstrated the existence of *Zangia* in Thailand. This genus was reported by Li *et al.* (2011). The basidiocarp of *Zangia* species are similar to genus *Tylopilus* such as pinkish to pink hymenophore, pink to pinkish brown spore deposit and chrome yellow to golden yellow stipe base. The consistent and unique character within *Zangia* species are the present of ixohyphoepithelium pileipellis. Currently, *Zangia* species are only known from southern parts of China and associate with Fagaceae and Pinaceae (Li *et al.*, 2011). In the study, one *Zangia* species were found in coniferous plantation, Phitsanulok

provinces. This interesting result suggested the existence of *Zangia* species in Thailand, so the investigation of this genus in Thailand is needed.

Within morphological identification, the lack of reliable key and database in Thailand including misidentification are concerned. The monograph of *Boletus* in Malaysia which described by Corner (1972) could not use for species identification in this study. It emphasized the revision of this genus in Thailand is needed.

This study could provide some of important database in tropical *Tylopilus* based on both morphological and molecular data. However, more molecular database including morphology of *Tylopilus* particularly from tropical region is really needed to fulfill the systematic studies of this genus.

CHAPTER VI

CONCLUSIONS

According to ninety-five ectomycorrhizal basidiocarp samples from 5 provinces (Chaiyaphum, Chiang Mai, Nan and Phitsanulok), fifty-four species were classified in eight genera (*Boletus, Boletellus, Heimioporus, Lecinum, Pulveroboletus, Strobilomyces, Tylopilus* and *Zangia*) based on morphology and molecular data. The most abundant genera was the *Boletus* (24 species), and the second and third abundances were the *Tylopilus* (14 species) and the *Boletellus* (5 species). The number of species in other genera, *Strobilomyces* and *Heimioporus* and *Leccinum* were 4, 3 and 2 species, respectively while *Pulveroboletus* and *Zangia* represented only 1 species.

Phylogenetic relationships among ectomycorrhizal Boletaceae were studied based on both ribosomal DNA regions, ITS and LSU. The sequences of Thai specimens were compared with some species in Boletaceae available in GenBank database. Phylogenetic analysis based on ITS suggested that *Boletellus, Heimioporus, Pulveroboletus* and *Strobilomyces* were monophyletic groups while *Boletus* and *Tylopilus* were not monophyletic groups. The phylogenetic tree based on LSU which indicated that only *Heimioporus, Pulveroboletus* and *Strobilomyce, Pulveroboletus* and *Strobilomyces*, were monophyletic groups.

In addition, the phylogeny of the large genus, *Tylopilus* was investigated based on both ITS and LSU. Two similar phylogenetic analyses showed that *Tylopilus* could be clearly divided at least 4 clades. Moreover, the relationships among *Tylopilus* species corresponded to their host plant families. Fourteen *Tylopilus* species and one *Zangia* species, the closely genus, existed in Thailand.

This study indicated an importance to revise the genera in family Boletaceae especially *Boletus* and *Tylopilus*. The insufficient data based on both morphological and molecular studies of Topical Boletaceae is an important issue in systematic studies of Boletaceae.

Considerations for future studies:

Some issues remain to be addressed in the future studies. At first, according to high variation in ITS region, the cloning technique should be chosen for resolve the failure in sequencing. Moreover, phylogenetic relationship among several species or genera was still unclear, thus more taxa should be added in the analysis. In addition, different molecular information such as mitochondrial DNA or other nuclear DNA region should be combined to the analysis as well as more taxa.

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APPENDICES

APPENDIX A

CHEMICAL REAGENTS

1. Tris-Cl pH 8

Tris base	121	g
Distilled water	800	ml

Dissolve Tris base thoroughly and adjust pH with HCl to pH 8. After that the distilled water was added to reach 1000 ml. Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes. Keep at room temperature.

2. 0.5 M EDTA (Ethylenediamine tetraacetic acid)

EDTA	186.10	g
Distilled water	800	ml

Dissolve EDTA thoroughly and adjust pH with NaOH to pH 8. After that the distilled water was added to reach 1000 ml. Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes. Keep at 4°C.

3. Washing buffer

PVP (Polyvinylpyrrolidone)	2	g
Ascorbic acid	1.76	g
1 M Tris-HCI (pH 8.0)	20	ml
2-mercaptoethanol	4	ml

Mix PVP, Ascorbic acid, Tris-HCl and 2-mercaptoethanol. After that the distilled water was added to reach 2000 ml and mix thoroughly. Keep at 4°C.

4. 2X CTAB lysis buffer

СТАВ	4	g
1 M Tris-HCI (pH 8.0)	20	ml
0.5 M EDTA (pH 8.0)	8	ml
Sodium chloride (NaCl)	16.36	g
2-mercaptoethanol	1	ml

Mix CTAB, 0.5 M EDTA, Tris-HCI, NaCI and 2-mercaptoethanol. After that the distilled water was added to reach 2000 ml and mix thoroughly. Keep at room temperature.

5. Choloroform/isoamyl alcohol (24:1 V/v)

Choloroform	192	ml
Isoamyl alcohol	8	ml

6. Tris-EDTA buffer (TE buffer)

1 M Tris-Cl (pH 7.4, 7.5 or 8)	10	ml
0.5 M EDTA (pH 8.0)	2	ml

Mix 1 M Tris-Cl and 0.5 M EDTA. After that the distilled water was added to reach 1000 ml. Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes. Keep at room temperature.

7. 10X Tris-boric acid EDTA (10X TBE)

Tris (hydroxymethyl) amino methane	54	g
EDTA	4.65	g
Boric acid	27.50	g

Mix Tris amino methane, EDTA and Boric acid. After that the distilled water was added to reach 500 ml and mix thoroughly. Keep at room temperature.

8. 1.5% Agarose gel (w/w)

Agarose	1.5	g
1X TBE	100	ml
Gel star	1	μl

APPENDIX B

SEQUENCES OF THE SPECIMENS

1. ITS region

Boletellus ananas (UB7)

Boletellus sp.1 (PH34)

Boletellus sp.2 (MJ15)

Boletellus sp.3 (MJ26)

Boletellus sp.4 (WS02)

Boletellus sp.4 (P00)
Boletellus sp.4 (MJ04)

Boletus sp.1 (P12)

Boletus sp.1 (CP47)

Boletus sp.2 (CP18)

CTAGTAGCTGAAATGGCTTAGGCCCAAATTCGAACTCCAATCGCAAGCGATCTATATCAAAAAGCAAGGGCTTCCCTT GGCATCCAAGACACTCCAGCCACGACGATCATTATCACGTCGAAAGGCCGTGCATGTCAAAGACTAGCCAACCTTT GCTAATGCTTTTCAGAAGAGCTGACAGCCTTTGGCTGCCAGCAACCCCCCAAAAACTCCAAGCCATTTGTTCTCGAAA ATGATCGAGACATGGGTTGAGAATTCACTGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAAGGTG CGTTCAAAGATTCGATGATTCACTGGAAATCTGCAATTCACATTACTTATCGCAATTCGCTGCGTTCTTCATCGATGCG AGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATGATTCATATTTGTAACACACATTCTATGGACATACGATAGGGTGT GATATGAGAGAAAACATAGATCCTCTTTCGAAGACCT

Boletus sp.3 (CP01)

Boletus sp.4 (CP15)

Boletus sp.4 (CP51)

Boletus sp.6 (CP03)

ACACCCCTAGGCTAGCCCTTCAGGTGTCGCTTAGCTACTAGTCGGCCGTGAGGCTGACGAACGTTGGGCGAGCCA CGCTTGGCAGGGCTTGTCTGTTCCGAAACTTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAA

Boletus sp.7 (MJ27)

Boletus sp.8 (CP40)

Boletus sp.9 (NN05)

Boletus sp.10 (CP11)

Boletus sp.11 (CP08)

Boletus sp.12 (P05)

Boletus sp.13 (CP17)

ACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAATTGCGATAAGTAATGTGAATTGCAGATTTTCAGTGAA TCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATCGAATTCTCAA CCATGTCCTCTCTCTTTGGAGAAGGCAACATGGCTTGGACTTGGGGGGTTGCTGGCCGCCTCGTCAGCTCTCCTGAAA TGCATTAGCGGTCGCCAGCAAGTCTCGACATGCACGGCCTTTTGACGTGATAACGATCGTCATGGGCTGTGGAGTG GTCAAGGACAAGCATGAATGGGCCTGATTCGCTTCTAATCCCCACCACTTCTTGGACAGCACTTTAGTTACTAGTCGA GCCCCTCGACGAACACAAGGCGAGCGGTCCTTTGAAGTCTTTGAACGCTTGACCTCAAATCAGGTAGGACTACCCG CTGAACTTAA

Boletus sp.13 (CP21)

Boletus sp.13 (CP49)

Boletus sp.14 (PH37)

AATTGCGATAAGTAATGTGAATTGCAGATTTTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTCGGTATTC CGAGGAGCATGCCTGTTTGAGTGTCATTCGAATTCTCAACCATGTCTCATGACGAGGCTT

Boletus sp.15 (NN12)

Boletus sp.16 (NN16)

Boletus sp.17 (P15)

ACGTGATAATGATCGTCGTGGCTGGAGTGTCAGATCGCCCATGTTGCTTCTAATCAAAAGAGATGGGGGCAGTAGCC TTGTCCTTCTTGACAACTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAA

Boletus sp.17 (PH41)

Boletus sp.17 (UB04)

Boletus sp.18 (MJ16)

CGCATCGATGACGAACGCAGCGAATTGCGATAAGTAATGTGAATTGCAGATTTCCAGTGAATCATCGAATCTTTGAAC GCACCTTGCGCTCCTTGGTATTCCGAGGGGCATGCCTATTTGAGTGTCATTGAATTCTCAACCATGGATCAGAGTTCA TTCTCTGGAACATGGCTTGGAGTTGGGGGGTTGCTGGCAGCCAAGTGGGGCAGTCGGCTCTCCTGAAATGCATTAGT GATGGTTCACTCGTCTGTGGACATGCACGGCCTTCTGACATGATAATGATTGTCATGGGCTGGAAGTGTTCAAGGGC ATGCAATTGGACCATGGCTTCTAACCTGGTTGTCCGCTCTACTTCTCTATCAAAGTCTAGCCTTAGCTATGGAC CTGCAAGGTCTGGCAAACACAAGGCAGGACAAAGGCTTT

Boletus sp.19 (CP34)

Boletus sp.20 (NN02)

Heimioporus sp.1 (UB01)

Heimioporus sp.2 (MJ06)

Heimioporus sp.2 (MJ25)

Heimioporus sp.2 (CP13)

Heimioporus sp.2 (CP13.2)

Heimioporus sp.2 (CP48)

Heimioporus sp.3 (NN03)

Leccinum extremiorientale (NN18)

Pulveroboletus sp. (CP16)

GTGTGTGTGGACGTCTTTTGGCGTGCACGGCCTTCGACGTGATAATGATCGTCGTGGCTGGAGCGCCTGGAATATAA TCTCCCTCTCCCTCATGCTTCTAGTCTGTATT

Pulveroboletus sp. (NN21)

Pulveroboletus sp. (039)

Strobilomyces mirandus (PH32)

Strobilomyces sp.1 (P04)

Strobilomyces sp.2 (P01)

Strobilomyces sp.2 (P14)

Tylopilus eximius (CP35)

Tylopilus sp.2 (CP41)

Tylopilus sp.3 (PH40)

Tylopilus sp.4 (CP09)

GCATCGATGAAGAACGCAGCGAATCGCGATATGTAATGTGAATTGCAGATATTCAGTGAATCATCGAATCTTTGAACG CACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTAATTTCTCAACCATGGTGGTTTTCTCGAC CGCCATGGCTTGGACTTTGAGGGTTGCCGGCCTTGAGACGTTTCGCACTGGTGAACGTCGTCGTCGTCGGCGCTCTCTT GAAATGCATTAGTGGATCGACCTTGCAATCCAGGACAGTATGGTCCGAGACATGCATTCTGTCTCGTCGACGTGATG ATGATCGTCGTCGGGGCCTGTGTGTGTGTGATCCG

Tylopilus sp.4 (CP10)

Tylopilus sp.6 (WS01)

Tylopilus sp.7 (NN01)

Tylopilus sp.7 (PK01)

Tylopilus sp.10 (CP46)

Tylopilus sp.11 (CP14)

Tylopilus sp.13 (CP05)

Tylopilus sp.13 (CP06)

GCATCGATGAAGAACGCAGCGAATTGCGATAAGTAATGTGAATTGCAGATTTCAGTGAATCATCGAATCTTTGAACG CACCTTGCGCTCCTTGGTATTCCGAGGAGCATGTCTGTTTGAGTGTCGTCGAGTTCTCAACCAAGCCCTTGGGTTTGG CTTGGATCTGGAAGCTGCTGGCGGAGGTCGGCTCTTCTGAAATGCATTAGCGATCTACTACGGCCTTCCGGCGTGAT AACGATCGTCGACGACGGCTTTGATCGCTTCCAACGGAACCGATATATAATCTTGACCTCAAATCAGACAGGACTAC CCGCTGAACTTAA

Tylopilus sp.13 (CP37)

Tylopilus sp.13 (035)

2. LSU region

Boletellus ananas (UB7)

Boletellus ananas (UB10)

Boletellus ananas (CP12)

CTCAGGATAGCAGAAACTCGCGCGTGTCAGATTTATGTGGTAAAGCGAATGATTAGAGGCATTGGGGTTGAAACAAC CTCGACCTATTCTCAAACTTTAAATATGTAAGAACGGGCCGTCGCTCGATTGGACCGCCCGGCGATTGAGAGTTTCTA GTGGGCCATTTTTGGTAAGCAGAACTGGCG

Boletellus ananas (NN04)

Boletellus ananas (MJ03)

Boletellus ananas (P13)

AAAGATGGTGAACTATGCCTGAATAGGGCGAAGCCAGAGGAAACTCTGGTGGAGGCTCGTAGCGATTCTGACGTGC AAATCGATCGTCGAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTGCCGAAGTTTCC CTCAGGATAGCAGAAACTCGCATGTATCAGATTTATGTGGTAAAGCGAATGATTAGAGGCATTGGGGTTGAAACAAC CTCGACCTATTCTCAAACTTTAAATATGTAAGAACGGGCCGTCGCTCGATTGGACCGTCCGGCGATTGAGAGTTTCTA GTGGGCCATTTTTGGTAAGCAGAACTGGCG

Boletellus sp.1 (PH34)

Boletellus sp.2 (MJ12)

Boletellus sp.2 (MJ15)

GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGACGTCAGTCGCGTTGGC CAGGGATCAACCTTGCTTCTCGCTGGGTGCACTTCCTGGCTGACGGGTCAGCATCGGTTTCGATCGGGATAGAATG GCCAAGGGAACGTGGCACTCTTCGGAGTGTGTTATAGCCCATGGTCGTATGTGTCGATGGGGACCGAGGAACTCGG CACGACTCCGGTCTGTGTCTAGGATGCTGGCATAATGGCGTTAAGCGACCCGTCTTGAAACACGGACCAAGGAGTC

Boletellus sp.3 (MJ26)

Boletellus sp.4 (P00)

GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGATGTCAGTCTTGTTAGCT AGGGGTCAGCCTTGCTTTCGTTAGCTTGGCGTACTTCCTAGTTCGACAGGGTCAGCATCAGTTTCGATCGCGGTACAA AAGCGAAGGGAATGTGGCACTCTTTCTTGGAGTGTGTTATAGACTTTCGTTGTATGCAGTGGTCGAGACTGAGGTACT CGGCACGACTCAAGTCTGTGTCTAGGATGCTGGCGAAATGGCCTTAAGCGACCCGTCTTGAAACACGGACCCAAGGA GTCTAACATGCTTGCGAGTGTTTGGGTGGAAAACCCAAGTGCGAAATGAAAGTGAACGTCGAGATCTCTGTCGTGGA GAGCATCGACGCCCGGACCCGAGTCTTTGACAAAGGATCTGCGGTAGAGCATGCACGTTAGGACCCCGAAAGATG TGAACTATGCCTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTCGTAGCGATTCTGACGTGCAAATCGAT CGTCGAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTGCCGAAGTTTCCCTCAGGAT AGCAGAAACTTGTATATCAGATTTATGTGGTAAAGCGAATGATTAGAGGCCTTGGGGTTGAAACAACCTTAACCTATTC TCAAACTTTAAATATGTAAGAACGAGCCGTCTCTCAGTTGGACCGCTCGGCGATTGTGAGTTTCTAGTGGGCCATTTTT GGTAAGCAGAACTGGCG

Boletellus sp.4 (MJ04)

GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGATGTCAGTCTTGTTAGCT AGGGGTCAGCCTTGCTTTCGTTAGCTTGGCGTACTTCCTAGTTCGACAGGGTCAGCATCAGTTTCGATCGCGGTACAA AAGCGAAGGGAATGTGGCACTCTTTCTTGGAGTGTGTTATAGACTTTCGTTGTATGCAGTGGTCGAGACTGAGGTACT CGGCACGACTCAAGTCTGTGTCTAGGATGCTGGCGAAATGGCCTTAAGCGACCCGTCTTGAAACACGGACCCAAGGA GTCTAACATGCTTGCGAGTGTTTGGGTGGAAAACCCAAGTGCGAAATGAAAGTGAACGTCGAGATCTCTGTCGTGGA GAGCATCGACGCCCGGACCCGAGTCTTTGACAAAGGATCTGCGGTAGAGCATGCACGTTAGGACCCCGAAAGATG TGAACTATGCCTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTCGTAGCGATTCTGACGTGCAAATCGAT CGTCGAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTGCCGAAGTTTCCCTCAGGAT AGCAGAAACTTGTATATCAGATTTATGTGGTAAAGCGAATGATTAGAGGCCTTGGGGTTGAAACAACCTTAACCTATTC TCAAACTTTAAATATGTAAGAACGAGCCGTCTCTCAGTTGGACCGCTCGGCGATTGTGAGTTTCTAGTGGGCCATTTTT GGTAAGCAGAACTGGCG

Boletellus sp.4 (CH02)

GAAAAGAACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGATGTCAGTCTTGTTAGCT AGGGGTCAGCCTTGCTTTCGTTAGCTTGGCGTACTTCCTAGTTCGACAGGGTCAGCATCAGTTTCGATCGCGGTACAA AAGCGAAGGGAATGTGGCACTCTTTCTTGGAGTGGTGTGTATAGACTTTCGTTGTATGCAGTGGTCGAGACTGAGGTACT CGGCACGACTCAAGTCTGTGTCTAGGATGCTGGCGAAATGGCCTTAAGCGACCCGTCTTGAAACACGGACCCAAGGA GTCTAACATGCTTGCGAGTGTTTGGGTGGAAAACCCAAGTGCGAAATGAAAGTGAACGTCGAGATCTCTGTCGTGGA GAGCATCGACGCCCGGACCCGAGTCTTTGACAAAGGATCTGCGGTAGAGCATGCACGTTAGGACCCCGAAAGATG TGAACTATGCCTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTCGTAGCGATTCTGACGTGCAAATCGAT CGTCGAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTGCCGAAGTTTCCCTCAGGAT AGCAGAAACTTGTATATCAGATTTATGTGGTAAAGCGAATGATTAGAGGCCTTGGGGTTGAAACAACCTTAACCTATTC TCAAACTTTAAATATGTAAGAACGAGCCGTCTCTCAGTTGGACCGCTCGGCGATTGTGAGTTTCTAGTGGGCCATTTTT GGTAAGCAGAACTGGCG

Boletus sp.1 (P12)

Boletus sp.1(CP31)

Boletus sp.1 (CP47)

Boletus sp.2 (CP18)

Boletus sp.2 (CP25)

Boletus sp.3 (CP01)

Boletus sp.4 (CP15)

Boletus sp.4 (CP20)

Boletus sp.4 (CP51)

Boletus sp.5 (MJ23)

Boletus sp.6 (CP03)

Boletus sp.7 (MJ27)

Boletus sp.8 (CP40)

Boletus sp.9 (NN05)

Boletus sp.10 (CP11)

Boletus sp.11 (CP08)

Boletus sp.12 (P05)

Boletus sp.13 (CP17)

Boletus sp.14 (PH37)

GTCTTGAAACACGGACCAAGGAGTCTTACATGCATGCGAGTGTTCGGGTGGTAAACCCGTGCGCGAAACGAAAGTG AAAGTCGAGACCTCTGTCATGGAGGGCACCGACGCCGGACCTGAGTCTTNGACGACGGATCTGCGGTAGAGCCAT GCATGTTGGGACCGAAAGATGGTGAACTATGCCTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTCGTAG CGATTCTGACGTGCAAATCGATCGTCGAATTGGGTATAGGGGCGAAAGACTAATCGAACCGTCTAGTAGCTGGTTC CTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCGTGTATGTCAGATTTATGTGGTAAAGCGAATGATTAGAGGCCTTG GGGTTGAAACAACCTTAACCTATTCTCAAACTTTAAATATGTAAGAACGAGCCGTCGCTCGATTGGACCGCTCGGCG ATTGAGAGTTTCTAGTGGGCCATTTTTGGTAAGCAGAACTGGCG

Boletus sp.15 (NN12)

Boletus sp.16 (NN16)

Boletus sp.17 (P15)

Boletus sp.17 (PH41)

GTCTTGAAACACGGACCAAGGAGTCTAACATGTCTGCGAGTGTTTGGGTGGCAAACCCGAGCGCACAACGAAAGTG AAAGTCGAGACCTCTGTCATGGAGGGCATCGACGCCGGACCTGAGTCTTTGACGACGGATCTGCGGTAGAGCAT GCATGTTGGGACCCGAAAGATGGTGAACTATGCCTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTCGTA GCGATTCTGACGTGCAAATCGATCGTCGAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTT CCTGCCGAAGTTTCCCTCAGGATAGCAGAAACTCGTGTATCAGATTTATGTGGTAAAGCGAATGATTAGAGGCCTTG GGGTTGAAACAACCTTAACCTATTCTCAAACTTTAAATATGTAAGAACGAGCCGTCACTTTGTTGGACCGCTCGGCGA TTGAGAGTTTCTAGTGGGCCATTTTTGGTAAGCAGAACTGGCG

Boletus sp.17 (UB04)

Boletus sp.18 (MJ16)

Boletus sp.19 (CP34)

Boletus sp.20 (NN02)

Boletus sp.21 (CP53)

Boletus sp.22 (CP44)

Boletus sp.23 (CP19)

Boletus sp.24 (SN01)

Boletus sp.24 (NN11)

Heimioporus sp.1 (UB01)

Heimioporus sp.2 (MJ25)

Heimioporus sp.2 (CP13)

Heimioporus sp.2 (CP48)

Heimioporus sp.3 (NN03)

Leccinum extremiorientale (NN18)

Leccinum sp. (NN13)

Pulveroboletus sp. (MJ33)

Pulveroboletus sp. (CP16)

Pulveroboletus sp. (NN21)

Pulveroboletus sp. (039)

Strobilomyces mirandus (PH32)

Strobilomyces sp.1 (P04)

Strobilomyces sp.2 (P01)
Strobilomyces sp.2 (P14)

Strobilomyces sp.3 (CP43)

Tylopilus eximius (CP35)

Tylopilus sp.1 (NN22)

Tylopilus sp.2 (CP41)

Tylopilus sp.3 (PH40)

Tylopilus sp.4 (CP10)

Tylopilus sp.4 (MJ01)

Tylopilus sp.4 (K04)

Tylopilus sp.4 (CP24)

Tylopilus sp.5 (NN10)

Tylopilus sp.6 (CPB)

Tylopilus sp.7 (NN01)

Tylopilus sp.7 (PK01)

Tylopilus sp.7 (CM03)

Tylopilus sp.8 (NN07)

GAAAAGAACTTTGAAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTCGATGTCAGTCGCGTCGGT CAGGGATCAACCTTGCTTTTTTCGCTGGGCGTATTTCCTGGTCGACGGGTCAGCATCAGTTTCGGTTCGCCGTACAAA GGCGAGAGTGAATGTGCCACGCTTCGGCGTGCGTTATAGCCTCTCGTCGGATGCGGCGGTCGGGACTGAGGAACT CGGCGGTGCATCCCTCGTGGGTGTATCGCTCAGGATGCTGGCATAATGGCCTTGAGCGACCCGTCTTGAAACACGG ACCAAGGAGTCTAACATGCATGCGAGGTGTTTGGGTGCAAAACTCAAGCGCGCGAATGAAAGTGAACGTCGAGACCTT CGTCGTGGAGGGCATCGACGCCGGGACCGGACTCTTTCGACGATGGATCCGCGGGTAGAGCATGCACGTTGGGACC CGAAAGATGGTGAACTATGCCTGAATAGGGTGAAGCCAGAGGGAAACTCTGGTGGAAGCTCGTAGCGATTCTGACGT GCAAATCGATCGTCGAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTGCCGAAGTTT CCCTCAGGATAGCAGAAACTCGTCATGCTCAGATTTATGTGGTAAAGCGAATGATTAGAGGCCTTGGGGGCGAAACG ACCTTAACCTATTCTCAAACTTTAAATATGTAAGAACGGGCCGTCTCTCTTTTGGACCGCCCGGCGATTGGGAGTTTCT AGTGGGCCATTTTTGGTAAGCAGAACTGGCG

Tylopilus sp.9 (CP39)

Tylopilus sp.10 (CP23)

Tylopilus sp.10 (CP45)

Tylopilus sp.12 (NN06)

Tylopilus sp.13 (CP05)

Tylopilus sp.13 (CP37)

Tylopilus sp.13 (NN17)

Zangia sp. (P02)

BIOGRAPHY

Miss Pawara Pachit was born on September 18, 1986 in Phitsanulok province, Thailand. She graduated with Bachelor Degree of Science in Botany (2008), Department of Botany, Faculty of Science, Chulalongkorn University. After gradution B. Sc., she continued her Master Degree in Botany Department of Botany, Faculty of Science, Chulalongkorn University. Throughout her M. Sc. Study, she had received the financial support from the Development and Promotion of Talented Science and Technology Scholarship, CU.Graduate School Thesis Grant and The Thai government budget 2011, under the Research Program on Conservation and Utilization of Biodiversity and the Center of Excellence in Biodiversity, Faculty of Science, Chulalongkorn University.

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