CHAPTER 4

DIVERSITY AND PHYLOGENY OF BOLETES IN NORTHERN THAILAND

Knowledge of boletes diversity is important because of their major roles in natural and managed ecosystems as ectomycorrhizal fungi. This fungal group becomes an important factor for reforestation program worldwide. Furthermore, they are important as food sources for human being and animals (Brundrett *et al*., 1996). In addition fungal diversity as well as boletes can also be used as a bio-indicator of environmental quality. Studies on the diversity and taxonomy (base on morphological characters and molecular analyses) of boletes and other macro-fungi of Thailand are interested and need more investigate.

The present study aims to more complete study of diversity and molecular relation of some stipe-tubulate boletes in family *Boletaceae*, *Strobilomycetaceae*, *Boletinellaceae*, *Gyroporaceae*, and *Suillaceae* in three major suborders of *Boletales* (*Boletineae*, *Sclerodermatineae* and *Suillineae*) collected from reserved rainforests in 8 national parks of upper northern Thailand.

4.1 DIVERSITY OF BOLETES IN UPPER NORTHERN THAILAND

The results of this study have provided overviews of the diversity and molecular phylogeny of ectomycorrhizal boletes in the upper northern Thailand. Approximately 128 collections were recorded from 8 national parks including 94 taxa over a two-year period from 2005 to 2006 during wet and cool dry season (May-December). Species classifications follow Corner (1972) and Singer (1986) with modification by Binder and Hibbett (2004, 2006) and Halling (2008). A summary of the classification and distribution of the identified boletes is present in table 4.1. It is important to note that the systematics of some genera and species of *Boletales* is still under analyses, such as *Boletellus*, *Boletus*, *Gyroporus*, *Heimioporus*, *Tylopilus*, *Suillus* and *Xerocomus*, and that the present classification may change with the incorporation of new data.

The most abundant family was the *Boletaceae* (76 taxa), and the second and third abundances were the *Strobilomycetaceae* (10 taxa) and the *Suillaceae* (4 taxa) (Table 4.1, Figure 4.1). The large number of taxa in *Boletaceae* is similar to those collections of boletes worldwide such as China (Zang *et al*., 2001), Belize and the Dominican Republic (Ortiz-Santana *et al*., 2007), Central America (Singer *et al*, 1983; Halling, 2008), Japan (Komiyama and Yamada, 2000), Malaysia (Corner, 1972) and also in Thailand (Soythong, 1994; Rodtong *et al*., 1998; Tongglam, 1999; Rodtong and Teaumroong, 2000; Sanmee, 2004; Chandrasrikul *et al*., 2008).

The most numerous collections of boletes taxa were found in Doi Suthep Pui national park (70 taxa) particularly forests at Sangasabhasri Lane to Huai Kok Ma village which dominated by the *Fagaceae* (e.g. *Castanopsis*, *Lithocarpus* and *Quercus*). The second and third of high boletes species collections are Doi Inthanon national park (16 taxa)and Doi Phuka national park (10 taxa) (Table 4.1). The collection sites in all those national parks are covered with *Fagaceae* and *Pinaceae*. The similar results have also been found in Doi Suthep Pui national park by Tongglam (1999) and Sanmee (2004) and in Nam Nao and Phu Rua national parks by Chantorn *et al*. (2007), however more number of boletes taxa were recovered in this study.

The most common boletes species appearing in rain forests of northern Thailand were *Phlebopus siamensis* sp.nov. (10.6% of total 94 taxa) and *Boletellus ananus* (6.4%). *Boletus changensis* like, *B. chrysenteron*, *B. chrysops* like, *Heimioporus retisporus*, *Pulveroboletus ravenelii*, *Tylopilus virens* and *Suillus granulatus* were found 2.1-4.3% (Table 4.1, Figure 4.2). The common genera found in this study almost similar to those investigated from forests in Thailand (Ruksawong and Flegel, 2001; Chantorn *et al*., 2007).

Table 4.1 Distribution of boletes taxa collected from 14 sites of 8 national parks in Northern Thailand during 2005-2006 (2 collections from each site).

Name	Distribution of bolete in 14 sites													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Suborder Boletineae														
Boletaceae														
Aureoboletus sp.1								1						
Austroboletus subvirens?								2						
Boletus borneensis								1						
Boletus chrysenteron					1		1							
Boletus coccineinanus									2					
Boletus fragrans								1						
Boletus grieseus									1					
Boletus impolitus								2						
Boletus leptospermi									1					
Boletus magnificans								1						
Boletus queletii								1						
Boletus quercinus	1													
Boletus radicans								1						

Table 4.1 (Continued)

Name	Distribution of bolete in 14 sites													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Boletus rebellus									1					
Boletus regius								1						
Boletus speciosus								1						
Boletus subvirens								1						
Boletus sp.1			2											
Boletus sp. 2			1											
Boletus sp.3			1											
Boletus sp.4			1											
Boletus sp.5						1								
Boletus sp.6						1								
Boletus sp.7						1								
Boletus sp.8						1								
Boletus sp.9								1						
Boletus sp.10								1						
Boletus sp.11								1						
Boletus sp.12								1						
Boletus sp.13								1						
Boletus sp.14									1					
Boletus sp.15									1					
Boletus sp.16									1					
Boletus sp.17									1					
Boletus 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						
Boletus sp.25*	1													
Boletus changensis like		1						1	2					
Boletus chrysops like*	1				1		1							
Boletus edulis like									1					
Boletus satanus like									1					
Boletus subtomentosus like								1						
Chalciporus piperatoides								2						
Heimioporus retisporus								1			1			
Leccinum crocipodium								1						
Leccinum griseum								1						
Leccinum holopus			1											
Leccinum oxydabile								1						
Leccinum scabrum								1						
Leccinum thalassinum								1						
Leccinum sp.1	1													
Leccinum sp.2								1						
Leccinum sp.3									1					
Pulveroboletus ravanelii			1					1						
Pulveroboletus sp.1									1					
Rubinoboletus ballouii								2	2					
Rubinoboletus sp.									1					

Table 4.1 (Continued)

Name	Distribution of bolete in 14 sites													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Tylopilus humilis							1							
Tylopilus nigerlimus	1							1						
Tylopilus virens			2					1						
Tylopilus sp.1				1										
Tylopilus sp.2						1								
Tylopilus sp.3								1						
Tylopilus sp.4									1					
Tylopilus sp.5										1				
Tylopilus sp.6										1				
Xerocomus rubellus								1						
Boletus/Leccinum ?								1						
Boletus/Suillus ?								1						
Tylopilus/Leccinum ?									1					
Tylopilus/Porphyrellus ?									1					
Strobilomycetaceae?														
Boletellus ananus	1				2		1	1	1					
B. emodensis								1						
Boletellus sp.														
Porphyrellus porphyrosporus								1						
Porphyrellus pseudoscaber									1					
Strobilomyces mollis								1						
S. strobilaceus								1						
S. velutipes									1					
Strobilomyces sp.1			1											
Strobilomyces sp.2										1				
Suborder Sclerodermatineae														
Boletinellaceae														
Phlebopus portentosus						1					1			
P. siamensis sp. nov.					1	2		2			2		2	1
Gyroporaceae														
Gyroporus castaneus								1						
Gyroporus sp.1						1								
Suborder Suillineae														
Suillaceae														
Suillus granulatus									1	1				
Suillus grevillei									1					
Suillus luteus									1					
Suillus placidus								1						
Total	**6**	**1**	**10**	**1**	**5**	**11**	**4**	**48**	**30**	**5**	**4**	**0**	**2**	**1**
Total number of taxa	**6**	**1**	**8**	**1**	**4**	**10**	**4**	**43**	**27**	**5**	**3**	**0**	**1**	**1**

* = probably a new taxa.

1= Doi Inthanon National Park, Mae Ya waterfall
2= Doi Inthanon National Park, Wachiratan waterfall
3= Doi Inthanon National Park, km 25 on highway road 1009
4= Doi Inthanon National Park, Ang Ka
5= Doi Luang National Park, Jumpa Thong Waterfall in Pha Yao
6= Doi Phuka National Park, nature trail
7= Doi Suthep-Pui National Park, Pha Lad
8= Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma village 9= Doi Suthep-Pui National Park, Doi Pui
10= Huay Nam Dang National Park, nature trail
11= Khun Chae National Park, nature trail
12= Pha Daeng (Chiang Dao) National Park, Sri Sang Wan Waterfall
13= Pha Daeng (Chiang Dao) National Park, Longan orchard at Muang Ngai-Ban
14= Sri-Lanna National Park, near Mae Ngad Dam, on Hwy 1323

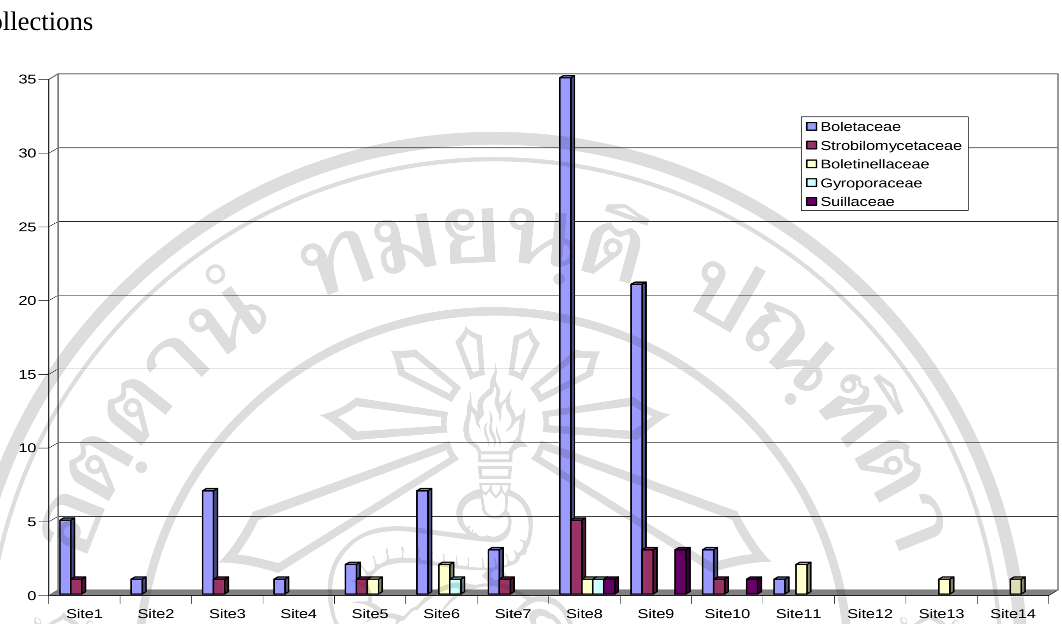


Figure 4.1 Frequency of boletes taxa found in 14 studied sites.

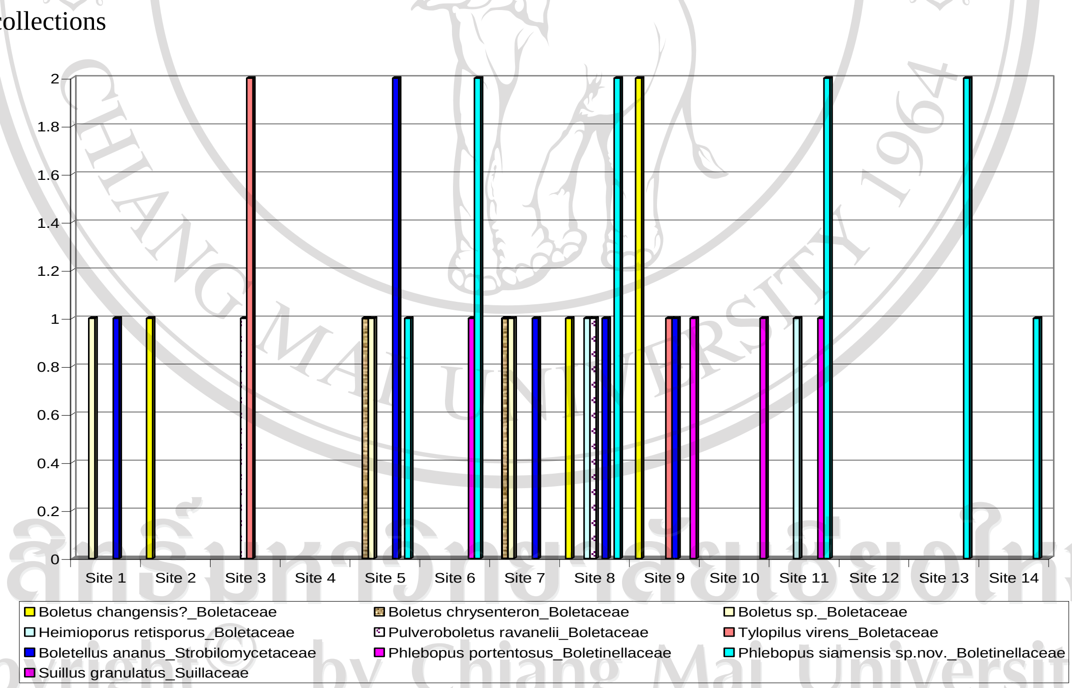


Figure 4.2 Common species of boletes found in 14 studied sites.

The number of overlapping boletes taxa from different national park pair are shown in the Table 4.2. The number of overlapping boletes taxa is high between Chiang Dao national park and Sri-Lanna national park (50%), followed by 25%

between Chiang Dao national park and Khun Chae national park, Khun Chae national park and Sri-Lanna national park,; 20% between Doi Luang national park and Sri-Lanna national park, Chiang Dao national park and Doi Luang national park; 15.4% between Khun Chae national park and Phuka national park; 10% between Doi Luang national park and Doi Inthanon national park; 9.1% between Chiang Dao national park and Doi Inthanon national park, Phuka national park and Sri-Lanna national park.

Table 4.2 Overlapping boletes taxa found in eight national parks.

National park	CD	DL	HD	IT	KC	PK	SL
DL	20						
HD	0	0					
IT	0	10	0				
KC	25	0	0	0			
PK	9.1	7.1	0	0	15.4		
SL	50	20	0	0	25	9.1	
ST	1.4	5.4	1.3	7.0	1.4	1.3	1.4

CD = Pha Daeng (Chiang Dao) National Park
DL = Doi Luang National Park
HD = Huay Nam Dang National Park
IT = Doi Inthanon National Park
KC = Khun Chae National Park
PK = Doi Phuka National Park
SL = Sri-Lanna National Park
ST = Doi Suthep-Pui National Park

The correspondence analysis (Figure 3.2) showed that boletes communities from Doi Luang national park, Inthanon national park and Suthep Pui national park were similar to each other than to those from other national parks. While the boletes communities from Chiang Dao national park, Khun Chae national park and Sri-Lanna national park were also similar to each other with a sister group of Doi Phuka national park. While the community of boletes found in Huai Nam Dang differ from those of all other national parks. The number of boletes found in Dipterocarp and Pine forest in Doi Luang national park, Khun Chae national park and Sri Lanna national park are quite low and this may have been due to the visiting time in each site that far from

Chiang Mai were inappropriate. Sometime of collections the forest soils were too dried or in the beginning of raining period so the fruit bodies of boletes were not growing yet.

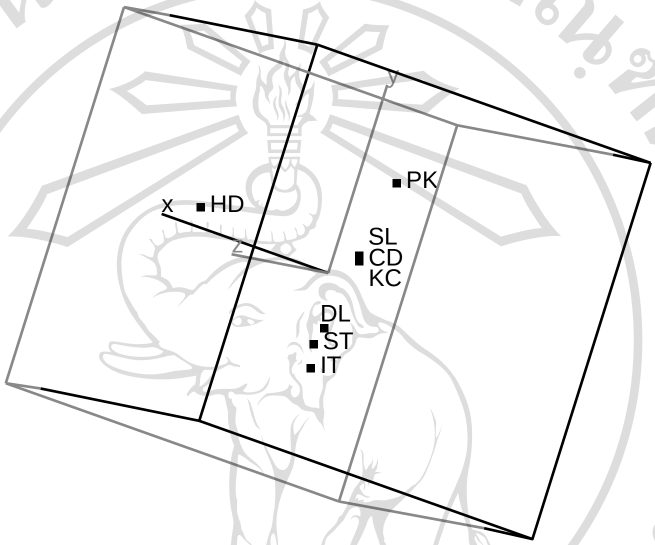


Figure 4.3 Three-dimensional correspondence analysis of boletes species recorded from 8 national parks of northern Thailand. A percentage of total variance explained by the model is 85.80%.

CD = Pha Daeng (Chiang Dao) National Park
DL = Doi Luang National Park
HD = Huay Nam Dang National Park
IT = Doi Inthanon National Park
KC = Khun Chae National Park
PK = Doi Phuka National Park
SL = Sri-Lanna National Park
ST = Doi Suthep-Pui National Park

Thirty two new record taxa were found after checklist of Soytong (1994), Klingesorn *et al*. (1998a, b), Rodtong *et al*. (1998), Thaithatgoon *et al*. (1998), Watling (1998), Tongglam (1999), Ruksawong and Flegel (2001), Jones and Hyde (2004), Chantorn *et al*. (2007) and Chandrasrikul *et al*. (2008). There were including *Boletus borneensis*, *B. chrysenteron*, *B. coccineinanus*, *B. fragrans*, *B. grieseus*, *B. impolitus*, *B. leptospermi*, *B. magnificans*, *B. queletii*, *B. quercinus*, *B. radicans*, *B. rebellus*, *B. regius*, *B. speciosus*, *B. subvirens*, *Chalciporus piperatoides*, *Gyroporus* *castaneus*, *Leccinum crocipodium*, *L. griseum*, *L. oxydabile*, *L. scabrum*, *L. thalassinum*, *Porphyrellus porphyrosporus*, *P. pseudoscaber*, *Strobilomyces mollis*, *S. strobilaceus*, *Suillus grevillei*, *S. placidus*, *Tylopilus humilis*, *T. nigerlimus* and *Xerocomus rubellus*.

Table 4.3 shows the list reported of all identified Thai species eaten of boletes by local people also in China, Japan, Malaysia and Europe with local name of almost species in Thai. When compared with Europe and some other Asian countries as China and Japan, where the known edible species are numerous, the number of the edible species in Thailand is less. This may be due to the diversity and ethnology studies of forest fungi in parts of northern and also other parts are less than those in other country mention as above (Corner, 1972; Zang, 1999; Maow, 2000; Halling, 2008). In addition the extra same local names of Hed Tub Tao as shown in Table 4.3 may also be confusing to understanding in the same species of boletes.

Table 4.3 Thai boletes species observed to be eaten by local people and knowledge from literature data. (L=eaten by locally; K=eaten by knowledge; *eaten by local people or the mushroom hunter nearby collection sites of this surveyed)

No.	Species	Local name	Comment
1.	*Aureoboletus thibetanus*	Hed Tub Tao Thi Bet	L
2.	*Austroboletus subflavidus*	Hed Kra Bong Pet Khao	K
3.	*Boletellus ananus*	Hed Phug Bua Hang	K
4.	*Boletellus chrysenteroides*	Hed Tub Tao Lai Kra	K
5.	*Boletellus emodensis*	Hed Tub Tao Klet Dang Klump	K
6.	*Boletellus russellii*	Hed Kra Bong Pet Laeung	K
7.	*Boletinus cavipes*	Hed Pod Nok Kamin	K
8.	*Boletus appendiculatus*	Hed Tub Tao Sri Naey	K
9.	*Boletus aureissimus*	Hed Tub Tao Sri Thong	K
10.	*Boletus auripes*	Hed Tub Tao Thong Dang	K
11.	*Boletus bicolor*	Hed Tub Tao Song Sri	K
12.	*Boletus chrysenteron*	Hed Tub Tao Kra Dang	K
13.	*Boletus chromapes*	Hed Tub Tao Leaung	K
14.	*Boletus colossus*	Hed Pheung	L
15.	*Boletus dimocarpicola*	Hed Har Lum Yai	L

Table 4.3 (Continued).

No.	Species	Local name	Comment
16.	*Boletus edulis*	Hed Tub Tao Ra Cha	L
17.	*Boletus firmus*	Hed Pheung Ru Dang	K
18.	*Boletus fraternus*	Hed Tao Dang	K
19.	*Boletus griseipurpureus*	Hed Sa Med	L
20.	*Boletus griseus*	Hed Tub Tao Tao Dum	K
21.	*Boletus nobilis*	Hed Tub Tao Phu Luang	K
22.	*Boletus pallidus*	Hed Tub Tao Khao	K
23.	*Boletus reticulatus*	Hed Tub Tao Nong Ta Kai	K
24.	*Chalciporus piperatus*	Hed Cherry	K
25.	*Heimiella japonica*	Hed Pod Ma Ta Kai Dang	K
26.	*Heimiella mandarina*	Hed Pod Ma Ru Dang	K
27.	*Heimiella retispora*	Hed Pod Ma	L*
28.	*Leccinum holopus*	Hed Tub Tao Khao-Khaew	K
29.	*Leccinum intusrubens*	Hed Ta Bai Hua Nam Tal	K
30.	*Phaeogyroporus braunii* (Current name: *Phlebopus braunii*)	Hed Har	L*
31.	*Phaeogyroporus portentosus* (Current name: *Phlebopus portentosus*)	Hed Har	L*
32.	*Pulveroboletus ravenelii*	Hed Tang Kum Ma Thun	K
33.	*Strobilomyces floccopus*	Hed Ta Thao	K
34.	*Strobilomyces seminudus*	Hed Pod Dum	K
35.	*Suillus bovinus*	Hed Tub Tao Som-Chompu	K
36.	*Suillus granulatus*	Hed Tub Tao Nam Nom	K
37.	*Suillus intermedius*	Hed Tub Tao Naeu Preao	K
38.	*Suillus luteus*	Hed Tub Kai	L
39.	*Suillus pictus*	Hed Tub Tao Klet Pla	K
40.	*Tylopilus ballouii*	Hed Tub Tao Sri Som	K
41.	*Tylopilus chromapes*	Hed Tub Tao Teen Laeung	L
42.	*Tylopilus eximius*	Hed Tub Tao Nam Tal Muang	K
43.	*Tylopilus felleus*	Hed Sa Med	L
44.	*Tylopilus nigerrimus*	-	K
45.	*Tylopilus nigropurpureus*	-	K
46.	*Tylopilus otsuensis*	Hed Tub Kai Tong	K
47.	*Tylopilus vinosobrunneus*	Hed Tub Toa Sri Mung Kut	K
48.	*Tylopilus virens*	-	K

4.2 CULTURING OF SOME BOLETES FROM UPPER NORTHERN THAILAND

Approximately 20 taxa of boletes collected from several national parks and Longan orchards nearby Chiang Dao national park in this study could be cultured for vegetative mycelium by using a small piece of tissue from flesh basidiocarp (Figure 4.4). The mycelium of the edible species such as *Phlebopus portentosus* and *P.*

siamensis sp. nov. grew well on a half strength concentrate medium of PDA that commonly used for commercial cultivation of saprotrophic mushrooms. However, fruiting body have no found in Longan (*Dimocarpus longan*) seedling bags after inoculated with the inoculation of sorghum seed covered with species of *Phlebopus* mycelia for two years (Figure 4.3g).

The fruit bodies of *P. portentosus* have been recovered with high distribution in natural or organic Longan orchard (Sanmee, 2004; Lumyong *et al*., 2007), Mango orchards, *Syzygium* species (Whar or Har) and *Fagaceae* forests scatted with *Pinus* (Sanmee, 2004). Furthermore, there were several reports in successful of cultivation of a popular edible boletes as Hed Tabtao in Thailand with various kinds of fruit tree. (See more detail in Sanmee, 2004). Cultivation of some edible species especially ectomycorrhizal boletes in large scale may need fully understanding in nutritional requirements and some other factors on morphogenesis, such as host species, light, temperature, and CO_2.

4.3 MOLECULAR STUDY OF SOME BOLETES FOUND IN UPPER NORTHERN THAILAND

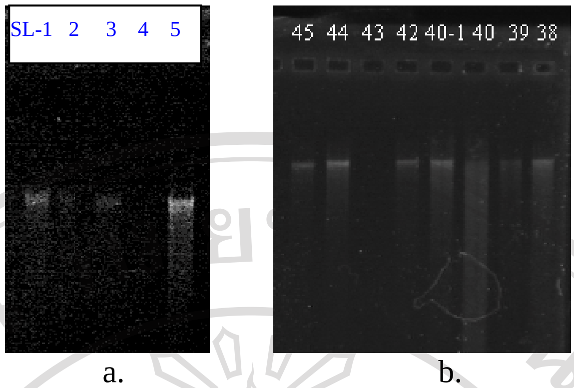
Phylogenetic relationships of some boletes collected from natural rain forests in upper northern of Thailand include the genus belong to suborders, *Sclerodermatineae* and *Suillineae*. This study combines the effort from previous studies (especially the taxa investigated by Binder and Hibbett, 2006) and provides 64 new sequences for 41 taxa of boletes from northern Thai national parks to resolve or confirm the morphological base identification of some confusing species based on 28S rDNA and ITS sequence analyses. The high level of boletes classification (suborder, family) was discussed as below, while in genera and species level will be discussed alternate with description and illustration in chapter 5-7 for some interest taxa. Table 4.4 lists all boletes that were included for sequencing analyses with the result of sequences obtained in this study.

4.3.1 DNA EXTRACTION, PCR AMPLIFICATION AND PCR PRODUCT PURIFICATION

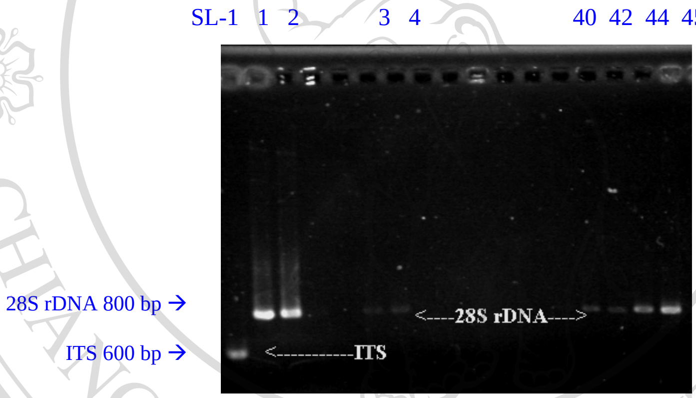
Genomic DNA of approximately 44 selected boletes collections can be extracted by using DNA extraction methods (see in chapter 3) and DNA extraction Kits (NucleoSpin® Plants II, Macherey Nagel, Catalog no. 740770.50) (Figure 4.5). However the DNA extracted from the DNA extraction Kits may increase cost but it's more comfortable or easier. This method may be appropriate for the small project that includes samples less than 50.

Figure 4.6 shown an example result of the PCR product of 28S rDNA and ITS region that were obtained after amplified following manual's protocol by using the primer pair of LROR/LR5 and ITS4/ITS5 in the present study. The purified PCR products were also got after following manufacturer's protocol of NucleoSpin® Extract II PCR clean-up Kit (Macherey Nagel, Catalog no. 740609.50) (Figure 4.7). The amplified 28S rDNA and ITSs fragments were directly sequenced by Macrogen Company in Korea using sequencing reactions with PCR primers mentioned above. The result of that was shown in Table 4.4.

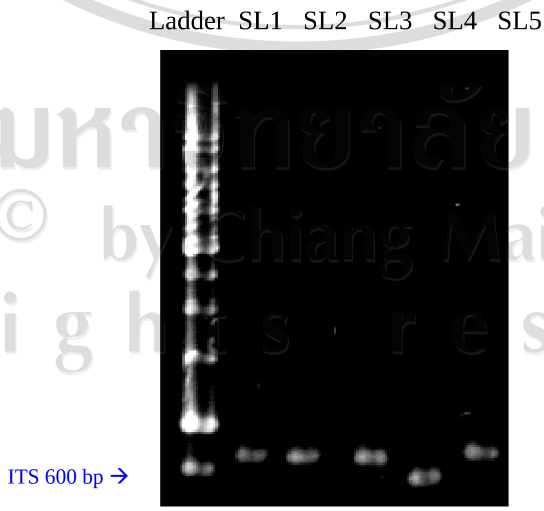
Figures 4.4 Cultivation of some boletes. a-b. Basidiocarp. c-f. Mycelia on ½ concentrate PDA plate. g. Mycelia on ½ concentrate PDA slant. Mycelial inoculation systems of Longan with some edible boletes in seedling bag.



Figures 4.5 Comparison of DNA products extracted by using a. DNA extraction following methodology provided in Chapter 3. b. DNA extraction Kits (NucleoSpin® Plants II, Macherey Nagel, Catalog no. 740770.50).



Figures 4.6 PCR amplification using LROR/LR5 and ITS4/ITS5 for amplified 28S rDNA and ITS region of some boletes.



Figures 4.7 PCR product of some boletes species after purified by using NucleoSpin® Extract II PCR clean-up Kit (Macherey Nagel, Catalog no. 740609.50).

Table 4.4 28S rDNA and ITS sequences of selected boletes taxa investigated in this study.

No.	Taxa name	Sequence length (bp)		National Park
	(Morphological identification)	28S rDNA	ITS	
SL001	*Tylopilus* sp.2	926		Phuka
SL002	*Boletus* sp.5	927	789	Phuka
SL003	*Gyroporus* sp.1	920	643	Phuka
SL004	*Boletus* sp.6	928	879	Phuka
SL005	*Boletus* sp.8		653	Phuka
SL006	*Phlebopus siamensis* sp.nov.	982		Phuka
SL007	*Phlebopus siamensis* sp.nov.	986	698	Phuka
SL008	*Phlebopus siamensis* sp.nov.		771	Phuka
SL009	*Phlebopus portentosus*		Noise sq.	Phuka
SL010	*Phlebopus siamensis* sp.nov.	819	Noise sq.	Phuka
SL013	*Phlebopus siamensis* sp.nov.	916	Noise sq.	Chiang Dao
SL014	*Phlebopus siamensis* sp.nov.	976	Noise sq.	Chiang Dao, Longan orchard
SL015	*Phlebopus siamensis* sp.nov.	978	Noise sq.	Chiang Dao, Longan orchard
SL018	*Boletus* sp.10	967		Suthep Pui
SL019	*Boletus borneensis*		Noise sq.	Suthep Pui
SL020	*Phlebopus portentosus*		539	Khun Chae
SL021	*Phlebopus siamensis* sp.nov.		771	Khun Chae
SL023	*Boletus* sp.16		773	Suthep Pui
SL025	*Rubinoboletus* sp.	972	412	Suthep Pui
SL026	*Boletus edulis?*	930	635	Suthep Pui
SL027	*Boletus sp.17*	952	Noise sq.	Suthep Pui
SL028	*Tylopilus/Leccinum*	930	949	Suthep Pui
SL029	*Boletus* sp.18	945	806	Suthep Pui
SL030	*Boletus* sp.19	880	821	Suthep Pui
SL031	*Boletus* sp.20	820	Noise sq.	Suthep Pui
SL032	*Rubinoboletus ballouii*	933	528	Suthep Pui
SL034	*Boletus changensis*?	962	736	Suthep Pui
SL035	*Boletus* sp.nov.	964	644	Suthep Pui
SL036	*Leccinum* sp.2	929	742	Suthep Pui
SL037	*Rubinoboletus ballouii*	941	528	Suthep Pui
SL038	*Tylopilus* sp.4	Noise sq.	Noise sq.	Suthep Pui
SL039	*Rubinoboletus ballouii*	901	532	Suthep Pui
SL040	*Boletus changensis* like	880	Noise sq.	Suthep Pui
SL040-1	*Boletus changensis* like	939	743	Suthep Pui
SL041	*Tylopilus/Porphyrellus*	977	418	Suthep Pui
SL042	*Rubinoboletus ballouii*	936	518	Suthep Pui
SL043	*Boletus leptospermi*	960	762	Suthep Pui
SL044	*Boletus coccineinanus*	874	Noise sq.	Suthep Pui
SL045	*Boletus satanas* like	956	782	Suthep Pui
SL046	*Boletus rubellus/ Xerocomus rubellus*	925	Noise sq.	Suthep Pui
SL052	*Suillus granulatus*	937 (+ NNN)	Noise sq.	Huai Nam Dang
SL053	*Tylopilus virens*	916	926	Suthep Pui
SL072	*Boletus chrysops* like*	910	812	Doi Luang
SL073	*Heimioporus retisporus*	958	453	Khun Chae

4.3.2 HIGHER LEVEL RELATIONSHIPS OF BOLETALES

Recent phylogenies studies of boletes based on the increased taxon sampling and multiple gene loci (Binder and Hibbett, 2002; Larsson *et al*., 2004; Binder *et al*., 2006; Binder and Hibbett, 2006; Matheny *et al*., 2006) consistently resolve a large clade of macro-fungi in subclass *Agaricomycetidae* that contains *Agaricales*, *Atheliales* and *Boletales*. As in previous studies, the *Atheliales* was little resolved as sister group of *Boletales* based on multigene analyses (nuclear small subunit, nuclear large subunit, 5.8S, atp6, and mitochondrial large subunit), established by Binder and Hibbett (2006) which is similar to this study. This relationship is supported strongly by posterior probabilities (PP), how ever bootstrap support (BS) is weak (<50%) (Figure 4.8). Inferences of the 28S rDNA data in this study show that extended taxon sampling the overall resolution of major groups in Boletales but still is not answering all questions about sister relationships.

4.3.3 THE MAJOR CLADE OF BOLETALES

The 28S rDNA phylogeny in this study support the *Boletales* as a monophyletic order and this result is consistent with previous studies (Kretzer and Bruns, 1999; Jarosch, 2001; Bruns *et al*., 1998; Grubisha *et al*., 2001; Binder and Bresinsky, 2002a; Binder and Hibbett, 2006).

Coniophorineae, *Hygrophoropsidaceae* and *Tapinellineae* form the basal clade of other 4 major groups of boletes (*Boletineae*, *Paxillineae*, *Sclerodermatineae*, and *Suillineae*). This result is consistent with the study of Jorosch and Besl (2002) and Binder and Hibbett (2006).

The *Boletineae* is the most species-rich group of stipitate pileate fungi with tubular hymenophores in the *Boletales* and also includes a few species with lamellate hymenophores and gasteroid forms. That has also been representing with the most abundant distribution in forests in this study as mention above. There were sequences of many taxa of *Boletineae* member expected base on morphological characters were group within the polyphyletic clades of *Boletineae* (Figure 4.8). For example the genus level placement of CMU-SL025, CMU-SL032, CMU-SL039, CMU-SL037 and CMU-SL042 which are little different in color of pileus were confident identified to the *Rubinoboletus* (=*Tylopilus*) because they were claded with in the species complex of *Tylopilus ballouii* with high bootstrap support and posterior probabilities support (Figure 4.9-4.13). While, the result of some selected taxa belong to this suborder will be shown and discussed in Chapter 5. And some other left taxa will be further analyses.

The phylogenetic relationship of the taxa belong to *Sclerodermatineae* sequences will be present in Chapter 6. Unfortunate the sequences of taxa belong to *Suillineae* were not obtained in this study.

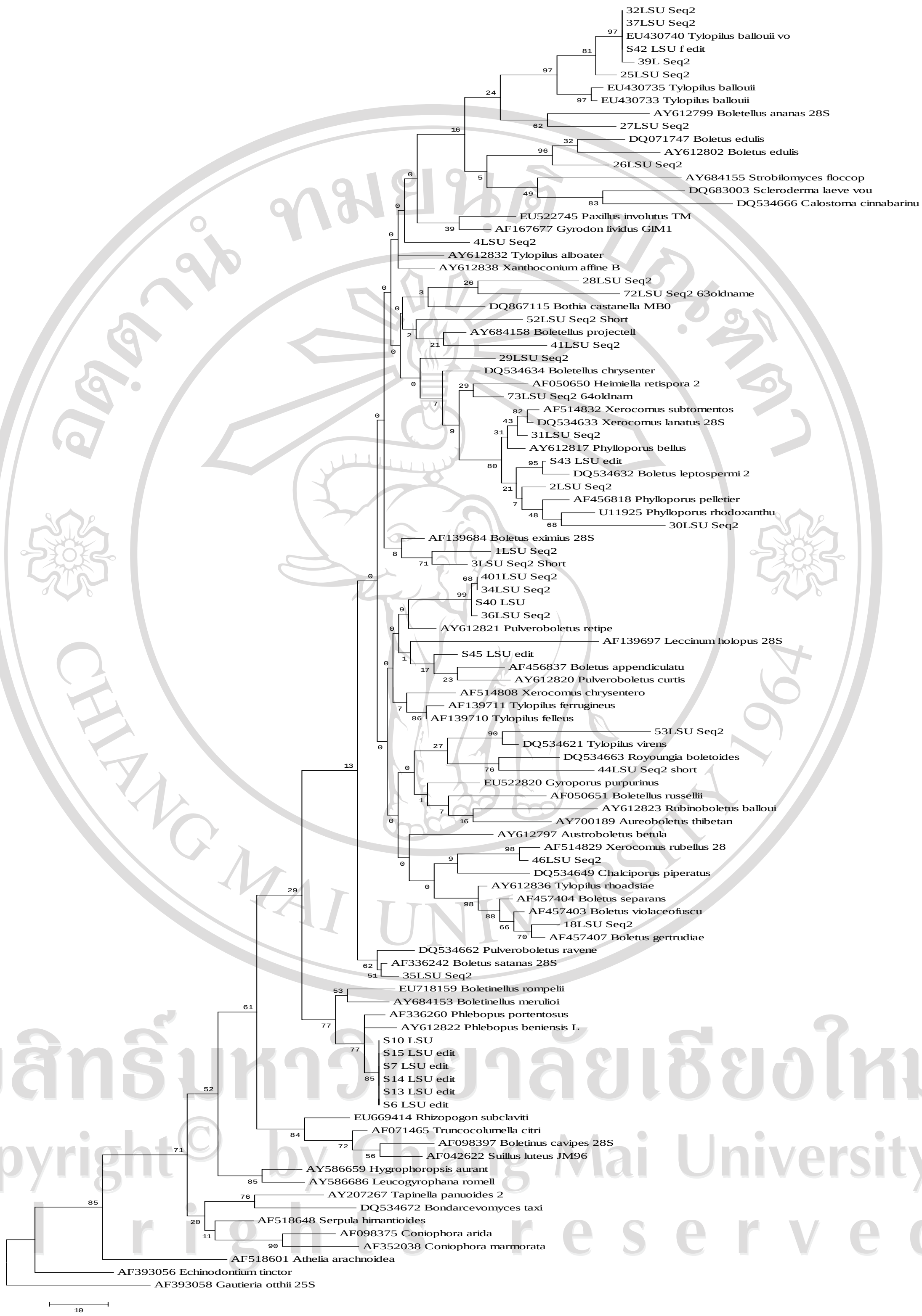


Fig. 4.8 Phylogenetic relationship of 36 boletes in Northern Thailand specimens inferred from the 28S rDNA sequences dataset. Bootstrap percentages > 50% are indicated above the branches.

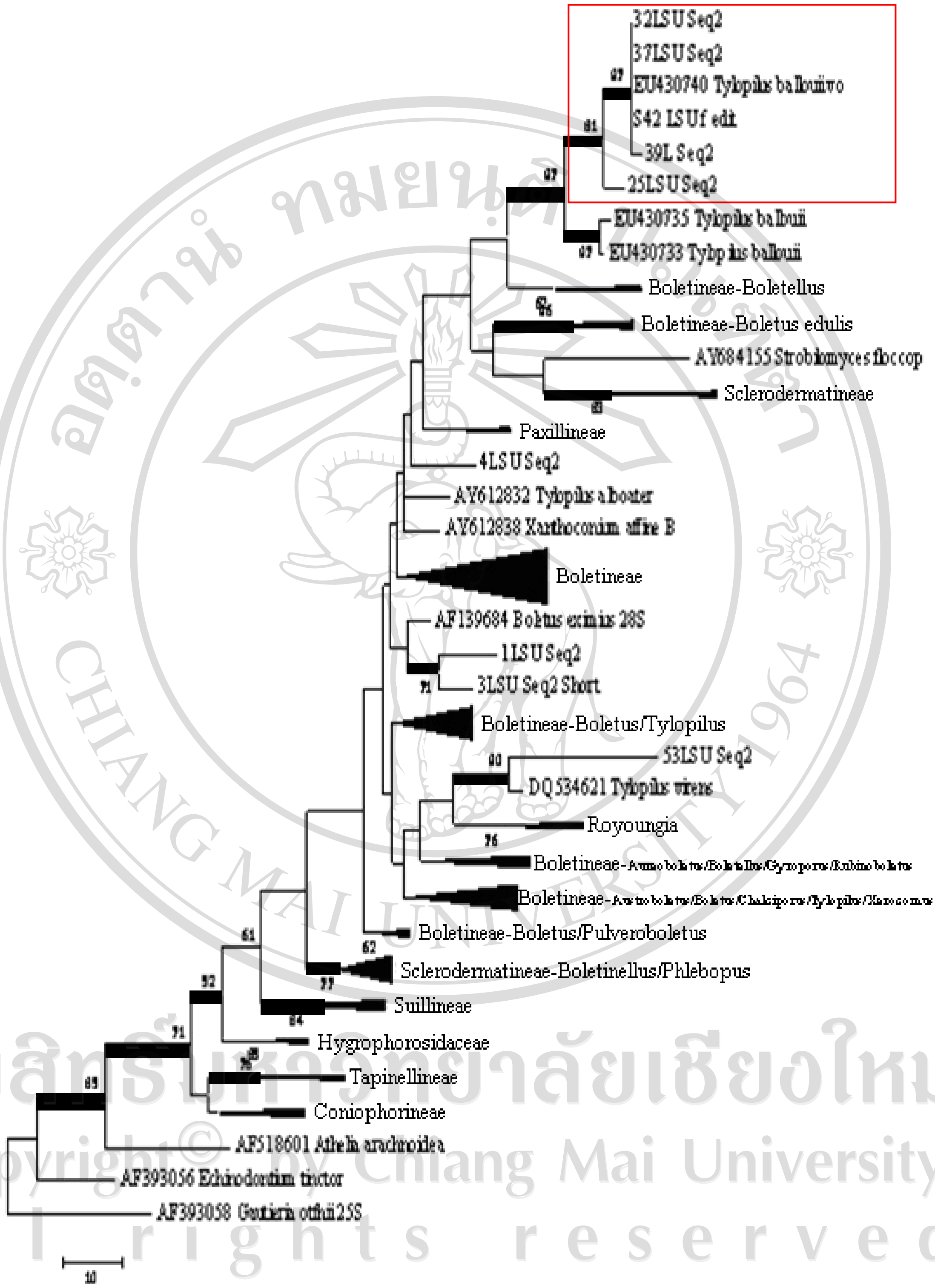


Figure 4.9 Phylogenetic relationship of 5 different taxa of *Rubinoboletus ballouii* (=*Tylopillus ballouii*) specimens collected from Doi Suthep Pui national park inferred from the 28S rDNA sequences dataset. Bootstrap percentages > 50% are indicated above the branches. Posterior probabilities supports are indicated by bold nodes.

Figure 4.10 ***Rubinoboletus ballouii*** CMU-SL032**. a-d.** Basidiocarps; **e-f.** Basidia and basidiospore; Scale bar: d = 20 mm, e-f = 10 µm

Figure 4.11. ***Rubinoboletus ballouii*** CMU-SL037**. a-c.** Basidiocarps; **b.** Basidia and basidiospore. Scale bar: c = 20 mm, d-e = 10 µm

Figure 4.12. ***Rubinoboletus ballouii*** CMU-SL039**. a-e.** Basidiocarps; **f.** Basidia and basidiospores Scale bar = 20 µm.

Figure 4.13 ***Rubinoboletus ballouii*** CMU-SL042**. a.** Basidiospores; **b.** Basidia; **c.** Basidioles; **d.** Pleurocystidia; **e.** Cheilocystidia; **f.** End-cells of pileipellis; **g.** Caulocystidia. Scale bar = 20 µm.

According to the families placement of the boletes member are under studying by many mycology expertise taxonomists worldwide such as Binder M., Hallin R., Hibbett D.S., Larsson E., Langer E., Larger G, Wu Q.X, etc base on both morphological and molecular data. The families placement represents in this study may be changed those of some confusing member within *Strobilomycetaceae*. However, several major genera appearing in the present study which are boletes group distinctly with stipe-tubulate could be identified promptly to genus level by using key to genera that provide by Halling (2008) (see in Chapter 3) combined with the major morphological characters that used to delimited all these genera have also been discussed in a field guide to the boletes by Thiers (1975) which now is available on website of California Mushroom.