

CHAPTER 2

LITERATURE REVIEW

2.1. BASIDIOMYCOTA (MACROFUNGI)

Representatives of the fungi *sensu stricto* include four phyla: *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Zygomycota* (McLaughlin *et al.*, 2001; Seifert and Gams, 2001). *Chytridiomycota* and *Zygomycota* are described as lower fungi. They are characterized by vegetative mycelium with no septa, complete septa are only found in reproductive structures. Asexual and sexual reproductions are by sporangia and zygospore formation respectively. *Ascomycota* and *Basidiomycota* are higher fungi and have a more complex mycelium with elaborate, perforate septa. Members of *Ascomycota* produce sexual ascospores in sac-shaped cells (asci) while fungi in *Basidiomycota* produce sexual basidiospores on club-shaped basidia in complex fruit bodies. Anamorphic fungi are anamorphs of *Ascomycota* and *Basidiomycota* and usually produce asexual conidia (Nicklin *et al.*, 1999; Kirk *et al.*, 2001).

The *Basidiomycota* contains about 30,000 described species, which is 37% of the described species of true Fungi (Kirk *et al.*, 2001). They have a huge impact on human affairs and ecosystem functioning. Many *Basidiomycota* obtain nutrition by decaying dead organic matter, including wood and leaf litter. Thus, *Basidiomycota* play a significant role in the carbon cycle. Unfortunately, *Basidiomycota* frequently

attack the wood in buildings and other structures, which has negative economic consequences for humans.

2.1.1 LIFE CYCLE OF MUSHROOM (BASIDIOMYCOTA)

The life cycle of mushroom (Figure 2.1) is beginning at the site of meiosis. The basidium is the cell in which karyogamy (nuclear fusion) and meiosis occur, and on which haploid basidiospores are formed (basidia are not produced by asexual *Basidiomycota*). Mushroom produce basidia on multicellular fruiting bodies. There is a great range of variation in morphology of the basidium, the number of spores formed, and how the spores are borne on the surface of the basidium (Ingold, 1991). Typically, four spores are produced on each basidium, at the tips of minute stalks called sterigmata. Each spore usually contains one or two of the haploid meiotic products. Basidiospores germinate to form hyphae (filaments) or yeast cells that are typically haploid and uninucleate. The hyphae of mushroom are septate. The hypha extends by tip growth (apical growth) and multiplies by branching, creating a fine network, or mycelium.

2.1.2 CLASSIFICATION OF BASIDIOMYCOTA

As generally known that the most diagnostic feature of *Basidiomycota* is the production of **basidia** (sing. basidium), which are the cells on which sexual spores are produced, and from which the group takes its name. A long-lived **dikaryon**, in which each cell in the thallus contains two haploid nuclei resulting from a mating event, is another characteristic feature. Finally, **clamp connections** are hyphal outgrowths that form when cells in dikaryotic hyphae divide. All fungi that produce clamp

connections are members of the *Basidiomycota*, but not all *Basidiomycota* produce clamp connections. The regular formation of clamp connections must have developed early in basidiomycete evolution, because they are found in all the major clades of *Basidiomycota*.

Indeed, *Basidiomycota* are so variable that it is impossible to identify any morphological characteristics that are both unique to the group and constant in the group. The phylum was traditionally divided into the true mushrooms of *Homobasidiomycetes* and the rusts and smuts of *Heterobasidiomycetes*.

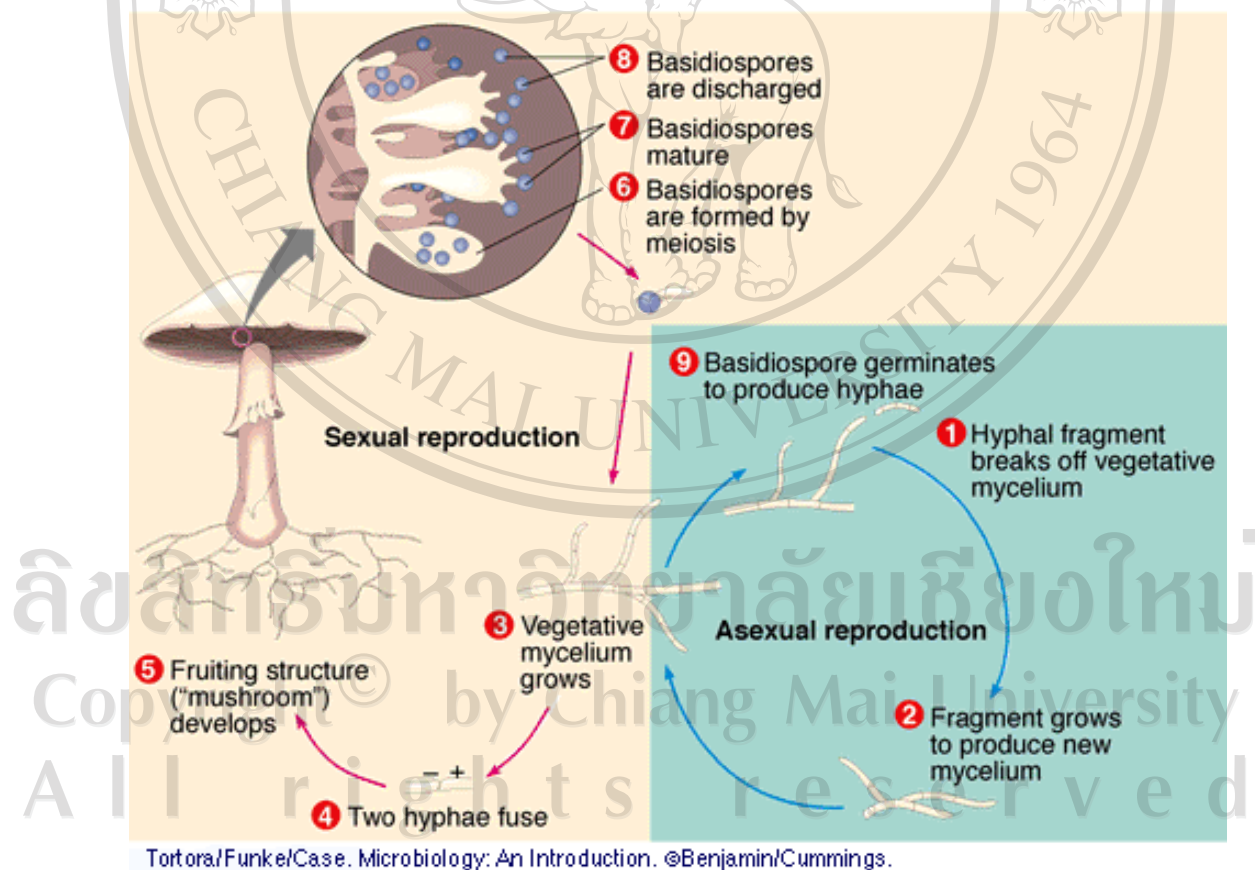


Figure 2.1 Life cycle of mushroom (Tortora *et al.*, 2007).

The Dictionary of the Fungi classified members of mushroom into 16 orders, 112 families, 1037 genera, and 20,391 species. By accepting the distinction made by Talbot (1968) between 'primary' and 'adventitious' septa two subclasses were distinguished including subclass *Tremellomycetidae* and *Agaricomycetidae* (Kirk *et al.*, 2001).

Tremellomycetidae is approximate synonym with *Phragmobasidiomycetidae*, *Heterobasidiomycetes*, in which the metabasidium is divided by primary septa that are usually cruciate or horizontal. This subclass includes 8 orders of *Auriculariales*, *Ceratobasidiales*, *Christianseniales*, *Cystofilobasidiales*, *Dacrymycetales*, *Filobasidiales*, *Tremellales* and *Tulasnellales*. While, *Agaricomycetidae* is approximate synonym with *Hymenomycetidae* or *Homobasidiomycetes* in which the metabasidium is not divided by primary septa but may sometimes become adventitiously septate, and contains 8 orders of *Agaricales*, *Boletales*, *Cantharellales*, *Hymenochaetales*, *Phallales*, *Polyporales*, *Russulales* and *Thelephorales*.

2.2 THE BOLETES

Boletes are known as fleshy pore fungi in order *Boletales*. They are the most fascinating and highly prized of edible macrofungi. They have a cap, a stalk, and a sponge-like layer of tubes on the undersurface of the cap. Except for the genus *Gastroboletus*, the species of which have enclosed and irregularly arranged tubes, boletes have vertically arranged tubes, each of which terminates in a pore. The tube layer is easily detached and typically separates cleanly from the cap flesh (Bessette *et al.*, 1997). Spores are produced on basidia that line the inside of the tubes. The life cycle of boletes is shown in Figure 2.2. Many boletes have a strong color reaction

when the flesh or tubes are damaged, such as blue color (Brundrett *et al.*, 1996). They have been classified in the order *Agaricales*, the family *Boletaceae*, for the reason that the basidiocarps of this order are fleshy to subfleshy (Ainsworth *et al.*, 1973). Hawksworth *et al.* (1995) placed them in *Boletales*, because the poroid basidiocarps differs from that of the poroid ‘aphyllophorales’ by having a soft, fleshy cortex similar to the *Agaricales* and the tubes, are easily separated from the pileus, which usually has a central stipe. Only a few can be collected in open fields or grassy areas (Bessette *et al.*, 1997; Kirk *et al.*, 2001). Boletes are often ectomycorrhizal with trees in the families, *Dipterocarpaceae*, *Fagaceae*, and *Pinaceae*. Some species need a specific host such as *Boletus edulis* with pines, *Suillus caerulescens* with Douglas firs and *Boletus aureus* with oaks (Thiers, 1975). Ectomycorrhizal fungi are classified ‘non-protein’ fungi, with no apparent ability to use protein, and ‘protein’ fungi that are able to use protein as an N source. They were also grouped accordingly into ‘early-stage’ and ‘late-stage’ fungi to describe succession at different stages of forest development, where early and late stages of forest development are associated with predominantly inorganic and organic soil (Sangtuan and Schmidt, 2002). Knowledge of boletes diversity is important because of their roles in natural and managed ecosystems such as supplying plant nutrients and moisture through mycorrhizal roots and antagonism of parasitic organisms. Other roles in ecosystems are recycling and conserving nutrients by soil mycelia, food sources for many animals, improving soil structure and transporting carbon from plant roots to other soil organisms. In Thailand, boletes are found in every region, especially in the upper northern provinces: Mae Hong Son, Chiang Mai, Chiang Rai, Phayao, Lamphun, Lampang, Nan and Phrae. They are sought during the rainy season for food and goods. These

economic ectomycorrhizal mushrooms cannot form fruiting bodies when they are cultivated, only the mycelia are produced due to lack of some nutrients from the plant host.

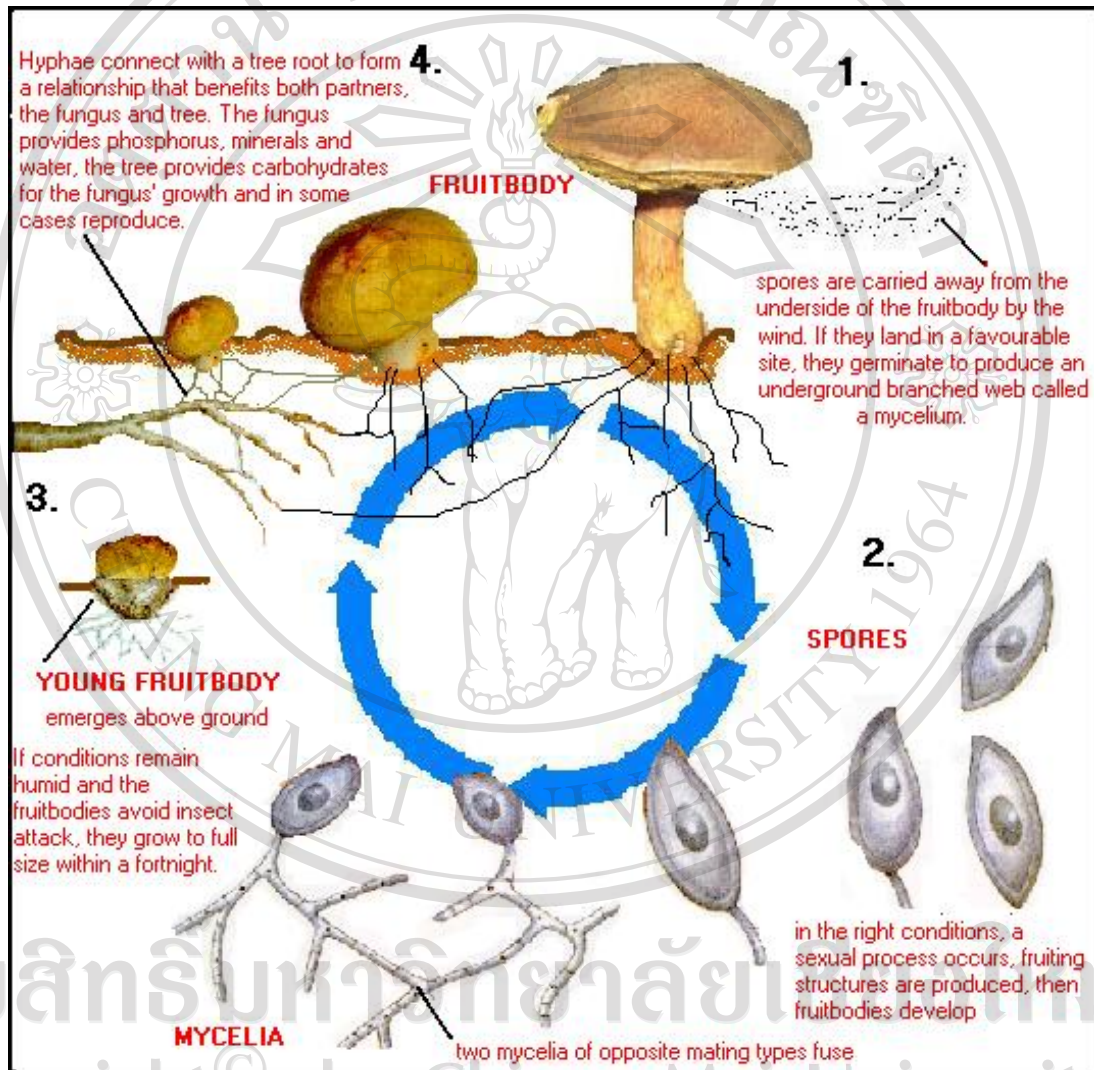


Figure 2.2 Life cycle of boletes (Tortora *et al.*, 2007).

2.3 TAXONOMY OF BOLETES

The boletes are members of the large group of fungi characterized primarily by the formation of spores, or reproductive bodies, on a highly specialized, microscopic structure known as a basidium (plural, basidia). The fruit body of the boletes is similar in appearance to the typical mushroom except that, in the boletes, tubes have replaced the gills, or lamellae, on the under surface of the cap. Because of the presence of these tubes and their pores, or openings, boletes are often called fleshy pore fungi.

2.3.1 TAXONOMY OF BOLETES BASED ON MORPHOLOGICAL CHARACTERS

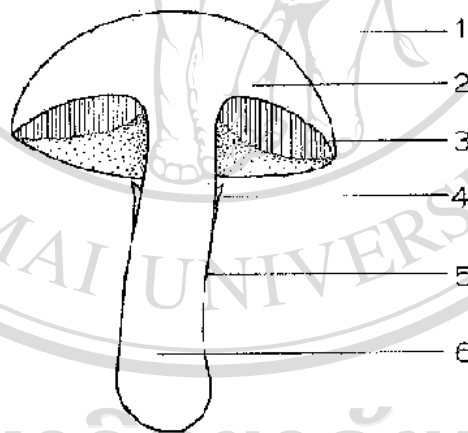
The classification of the *Boletales* was initially based on the morphology of macrocharacters, then combining the morphology of macro- and microscopic characters with anatomical and biochemical criteria.

Singer (1981) classified the *Boletales* in the kingdom *Fungi*, phylum *Basidiomycota*, class *Basidiomycetes*, order *Agaricales* and suborder *Boletineae*, with three families, six subfamilies and 33 genera; this classification was based on their poroid or sublamellate hymenophore. Later in 1986, Singer grouped them into one family, the *Boletaceae*, and six subfamilies within the order *Agaricales*.

The major morphological characters are including basidiocarp (pileus, pileus trama, tubes or hymenophore, annulus, stipe cuticle and stipe trama) (Figure 2.3) and basidiospore. Some species also needed to find clamp connection appearance. Details are shown as following (Thiers, 1975).

The Basidiocarp

The major difference between the boletes and gill fungi is that in the boletes the basidia are located on the inner surface of numerous tubes, which are typically vertically arranged on the lower surface of the pileus (except in *Gastroboletus*). These tubes, or gills in the case of mushrooms, are commonly designated as the hymenophore, or the part of the basidiocarp bearing the hymenium. The hymenium, in turn, is a layer of rather closely packed basidia plus distinctive sterile cells called cystidia. Another difference noted in the field is that, although some mushrooms grow on logs or other woody substrates, only a few boletes are found consistently on such substrates, and most occur in the soil or humus in the vicinity of woody plants.



Figures 2.3 Longitudinal section through bolete basidiocarp 1.Pileus. 2.Pileus Trama. 3 Tubes (Hymenophore). 4. Annulus. 5. Stipe cuticle. 6. Stipe trama (Thiers, 1975).

The Pileus

Bolete pilei are typically large, often reaching 15 cm or more in diam., and are rarely as small as 2-4 cm in diameter. Characteristically, they are more or less convex

or bulbous in outline when young, becoming plane or plano-convex when mature. Colors range from almost black to many different shades of brown, pink, or bright red. When the surface of the pileus is bruised, many boletes show some type of color change.

The nature of the surface of the pileus is of considerable taxonomic significance in the boletes. In most species of *Suillus* and in a few belonging to other genera it is viscid, or "sticky" or "slimy," to the touch. In the nonviscid species the surface may be either moist or dry to the touch. The surface may vary from glabrous, or bare, to fibrillose, or covered with a layer of fibrils that may be closely appressed or loosely and irregularly arranged. In some species the arrangement of the fibrils may give a tomentose, or velvety, appearance. There are several boletes in which the fibrils become agglutinated into scales. Most commonly these scales remain closely attached to the surface, but in some boletes the tips may break free. In a few they become quite large and give the pileus a squarrose or squamulose appearance. Boletes, as they grow older, often show a tendency to become split, or rimose, on the margin and to become checked, or areolate, elsewhere on the surface. The areolations sometimes become deep and strongly pronounced in prolonged periods of dry weather, which results in a frustose condition of the pileus.

The margin or edge of the young pileus in most boletes is entire, that is, more or less smooth and even and with no ornamentations. In the *Suillus*, however, there is frequently a noticeable cottony roll of veil tissue closely attached to the margin of the young pilei. This roll, commonly referred to as a false veil, is typically white or whitish and usually disappears as the pileus matures. Also in the genus *Suillus* and in some other genera, a partial veil is sometimes present which, when breaking free from

the pileus, often leaves fragments, or appendiculations, hanging from the margin. Typical of the members of the genus *Leccinum* is the continued growth of the outer layer or cuticle beyond the edge of the pileus, resulting in the formation of a band of tissue around the margin. This band usually breaks into fragments, or "flaps," as the pileus expands. These fragments seem to serve no function and eventually become either inconspicuous or completely disappear.

The flesh or context of the pileus is usually relatively soft and putrescent, and with a high water content. It varies in thickness from 0.5-2 cm, but in exceptional cases may be as thick as 4-5cm or as thin as 2-4 mm. Most commonly, the context is some shade of yellow or white, with other colors such as pink or tan rarely evident. The context of many boletes changes color when exposed to the air or damaged. The characteristic blue discoloration, or bluing, seen in a number of species may result from the oxidation by an enzyme of a compound known as boletol. Other color changes, such as the reddening, browning or blackening seen in many species, are probably similar oxidative reactions, but the compounds and chemical pathways are largely unknown.

The Hymenophore

The hymenophore, which consists of soft, moist, putrescent tubes, is the most distinctive feature of the boletes. The hymenium forms the inner lining of these tubes.

There is a superficial resemblance between the hymenophore of polypores and boletes.

The basidiocarps of polypores, however, are tough, dry, and woody.

In most boletes the hymenophore separates readily and cleanly from the pileus, and in many the tubes are easily separated from each other; however, in some cases of

the *Boletus* and *Suillus*, the tubes appear to develop in a different manner and can be separated only by tearing the walls apart. Except for *Gastroboletus*, the tubes are more or less vertically arranged in an orderly fashion, and a spore print is readily obtainable. Most often the tubes are either shallowly or deeply depressed at the stipe, but in some boletes, especially species of *Suillus*, they extend down the surface in a decurrent fashion.

The pores of the tubes are worthy of special mention. Most are angular, varying from almost square to somewhat rectangular, and range in size from 0.5-2 mm in diameter. In some boletes the arrangement of the pores is radial, or boletinoid and they radiate from the stipe much like the spokes of a wheel. Large pores compounded with internal partitions are seen in several species of *Suillus*. In a few boletes the pores are so large and elongated that they resemble lamellae and are described as lamellate. The pores are usually concolorous with the tubes; however, in some species they are pink or red in contrast to the yellow color of the body of the tube or, less commonly, they may be a contrasting shade of brown or black. The pores ordinarily show the same color changes as the remainder of the hymenophore.

The Stipe

The boletes are stipitate and no sessile species are known. The stipe is central. An eccentric attachment is found rarely, except in *Gastroboletus*, and species with truly lateral stipes are still unknown. The overall shape of the stipe is sometimes equal or more or less the same size from apex to base. More commonly, it is clavate with a gradual enlargement toward the base. In some boletes, best exemplified by *Boletus satanas*, there is a conspicuous and abruptly bulbous base, which is sometimes as

much as 5 cm in diameter. There are others in which the stipe is ventricose, or larger in the midportion than at the apex or base. Generally, the range in length is from 4-5 cm to a maximum of 14-15 cm, although *Suillus brevipes*, for example, may have a stipe as short as 1 cm and that of *Boletus mirabilis* may sometimes reach 20 cm. The diameter of the apex of the stipe, on an average, varies from 0.5-2 cm, but the more massive species, such as *Boletus regius* and *B. edulis*, may exceed 3-4 cm, whereas *B. piperatus*, *Gyroporus castaneus* and some *Suillus* species may be less than 0.5 cm in diameter.

The color of the bolete stipe ranges from white to yellow to pink to darker colors, such as red, brown, or almost black. Frequently the background color is overlain by some other pigment, resulting in a blending of colors. As in the pileus, some colors show a tendency to fade or, conversely, become more intense with age. Often the fading or darkening is uneven, which results in the formation of bands or blotches of color. Ornamentations on the surface of the stipe are often a contrasting color, and usually darken with age. Usually, the same series of color changes occurring in the pileus upon bruising or exposure takes place in the stipe, except that the changes may be more intense.

The surface of the stipe in a major percentage of boletes is either dry, moist, or, less commonly, viscid. Typically, it is glabrous, tomentose, fibrillose, or fibrillose-scaly. Sometimes it is reticulate, perhaps best demonstrated by *Boletus edulis* or *B. eastwoodiae*, in which there is a noticeable network of raised lines on the surface. A reticulum at the apical portion may be formed by the extension of the tubes down the stipe. In such cases the ridges forming the reticulum are composed of basidia and cystidia. In *Leccinum* the surface is furfuraceous, that is, covered with squamules, or

scales. These are usually white or pallid when young, but change to dark brown or black with age, and are composed largely of caulocystidia, or large sterile cells. In *Suillus* the surface often has glandulae, or small raised dots, which vary considerably in size, are typically colored some shade of brown, and may stain the fingers when handled. These glandulae are composed of clusters, of fascicles, of caulocystidia. Frequently, the basal portion of the bolete stipe is clothed with coarse hairs, which may be distinctively colored. Another important taxonomic feature associated with the surface of the stipe is the annulus, or remnants of the partial veil. It is sometimes evanescent; that is, it disappears as the basidiocarp matures or is represented merely by a fibrillose zone. On the other hand, it may be massive and constitute a conspicuous part of the mature basidiocarp. In some boletes, particularly in the genus *Suillus*, the stipe may be peronate, a condition in which the lower part of the stipe is completely surrounded by velar tissue. In *Pulveroboletus ravenelii* the veil and annulus are dry, copious, brilliant yellow, and floccose in texture. In some species of *Suillus*, the annulus is white or pallid, frequently inconspicuous, and may be noticeably viscid.

The context of the stipe is typically similar in structure and texture to that of the pileus. The sequence of color changes in the stipe apex when exposed is of considerable taxonomic value in the genus *Leccinum*. The stipe is typically solid, but in a few species, such as *Gyroporus castaneus*, it is hollow, at least in the basal portion.

The Basidiospore

The basidiospores are of considerable diagnostic value in the taxonomy and systematics of the boletes and must be checked before positive determinations can be made. At the generic level the color of the spores in mass is of significance, and spore prints are often necessary. The spore print varies in color from a shade of brown in such genera as *Boletus*, *Leccinum*, and *Suillus* to yellow in *Gyroporus* and flesh or dark pink in *Tylopilus*. The individual spore ranges from hyaline or colorless to brown. The shape of the spores is, in a broad sense. Generally, in face view they appear elongate and cylindric to fusoid (spindle-shaped) or ellipsoid (Figure 2.4a). The bulge is located near the apiculate end, that is, the end attached to the basidium. Occasionally, oddly shaped or pleomorphic spores are produced, often noted when the basidium is not four-spored. Since the germination of bolete spores is exceedingly difficult, it is not known whether these atypical spores are viable or not. In *Boletus truncatus* and sometimes in other species the apical end is often abruptly terminated and appears as if it had been cut off or truncated. A germ pore or thin spot in the wall is sometimes apparent in this region.

The spore length averages between 9-15 μm and the width from 4-5 μm . Smaller spores are characteristic of *B. orovillus* and several species of *Suillus*. On the other hand, spores are as long as 17-20 μm in species of *Leccinum* and in some boletes, such as *B. mirabilis*. The spore wall is generally thin, averaging about 1 μm , but some spores, such as in *B. mirabilis*, possess thickened walls.

The bolete spores show little or no change in color when mounted in water, dilute solutions of potassium hydroxide, or similar basic compounds. When mounted in Melzer's reagent, however, they may either remain unchanged or one of several

color changes may occur. Most commonly, the brown color of the walls is merely intensified or becomes a deep rusty brown. In some the spores give a dextrinoid reaction in which they become bright rust red or tawny. The chemical reaction responsible for this color change is not fully understood, especially since rarely do all the spores show the same reaction. Recently, several species have been found in which the spore wall gives a typical blue-black, or amyloid, reaction when mounted in Melzer's reagent. Spores of *Boletus amyloideus* and *Tylopilus amylosporus* show this reaction. Like the dextrinoid reaction, the chemistry of the amyloid reaction is poorly understood, and all spores from a given species usually will not react positively.

The hymenium is the palisade or layer of basidia and associated cells that form the inner lining of the tubes and, in some instances, may extend down the stipe if the surface is reticulate (Figure 2.5). The most important cells in this layer are the basidia, which develop as terminal cells of the hyphae that form the tissue of the tube. Nuclear fusion (karyogamy), reduction division (meiosis), and subsequent spore formation occur in the basidia. The basidia are rather large, club-shaped cells ranging from 20-30 μm in length and from 7-12 μm in width. At the apex four short, somewhat hooked branches develop. These are sterigmata, and eventually each will bear a single basidiospore.

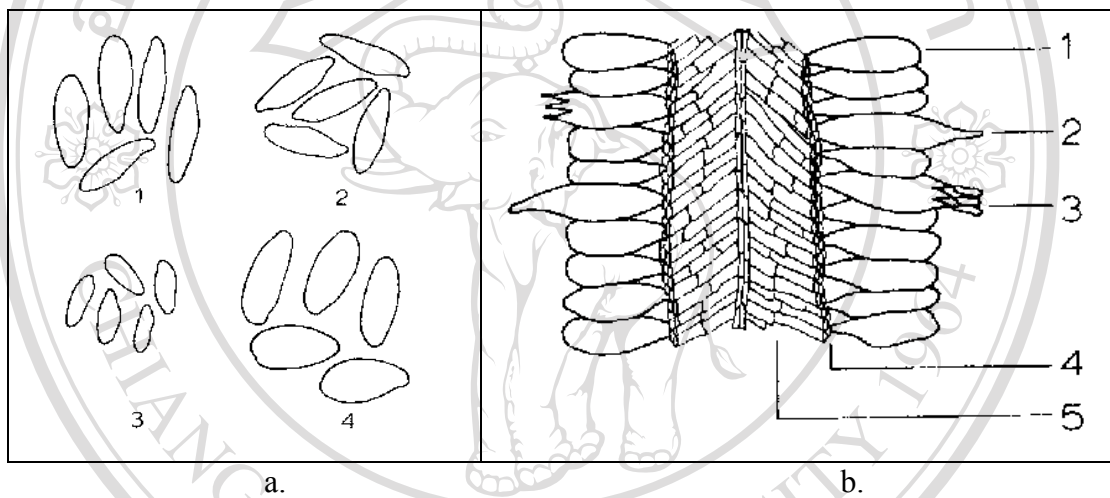
Basidioles or brachybasidioles are also commonly seen in the hymenium. These may be undeveloped basidia or basidioid cells that never produce basidiospores. They appear to have the same origin as the fertile basidia, but their true function is not understood. Perhaps they serve as lateral support for the spore-bearing basidia (Figure 2.4b).

A third type of cell commonly found in the hymenium of most boletes is the cystidium (Figure 2.4b). These cells are sterile and, like the basidia, arise as differentiated hyphal tips. They are often highly distinctive in size and shape, and frequently extend well beyond the basidial layer into the tube cavity. Most often they are clavate to fusoid or ventricose in shape with an elongated to obtuse or mucronate apex. Cystidia reach 50-75 μm in length and 10-15 μm in diameter. These cells are usually thin-walled, but may become noticeably thickened or incrustated with amorphous materials. When mounted in water, the cystidia are typically hyaline or only weakly pigmented; however, in potassium hydroxide or in Melzer's reagent there is often a marked color change to reddish brown or black.

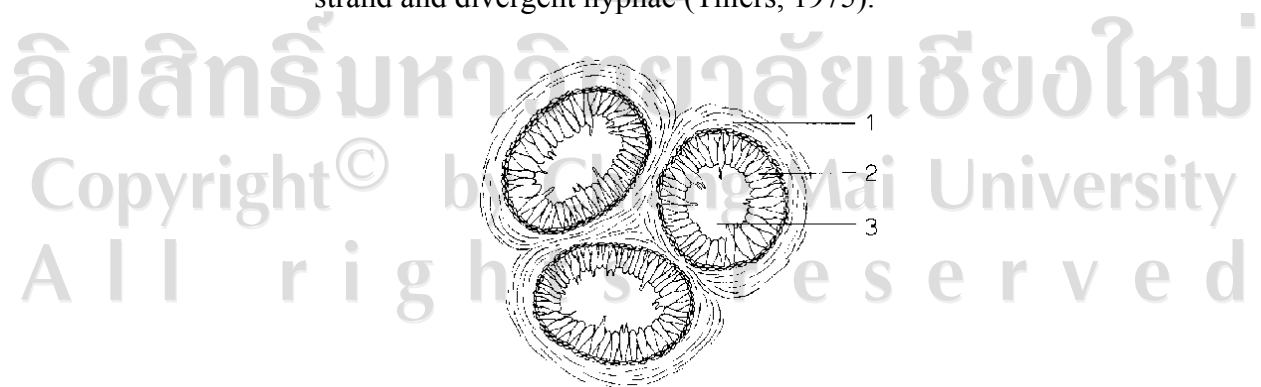
The Trama

The body of the pileus, hymenophore, and stipe is composed of filamentous, thin-walled hyphae that are undifferentiated, except for the surface, or cuticular, hyphae. These internal hyphae are referred to collectively as the trama (Figure 2.4b). In the tubes the hyphae are characteristically arranged in a compact central strand with noticeable filaments diverging from it, except in those few boletes in which the hyphae are arranged parallel to one another. This divergent, or bilateral, trama is often apparent only in young basidiocarps and at maturity may appear tangled or interwoven. The tube trama is generally hyaline when mounted in potassium hydroxide, but may change to some shade of brown, especially in the central strand. The tramal hyphae are often relatively large and range from 4-6 μm in width. In several species the walls of the hyphae seem to gelatinize or dissolve when mounted in water or weak base solution (KOH).

The pileus trama is generally similar in all bolete species and of little taxonomic or phylogenetic value. Its typical appearance is one of tangled hyphae, which are more or less loosely interwoven and usually homogeneous. Laticiferous hyphae are sometimes irregularly interspersed, but are not seen as frequently as in the tube trama. The stipe trama in all boletes is composed of masses of more or less parallel hyphae that may be loosely or tightly packed, except in *Gyroporus* where the hyphae are transversely arranged.



Figures 2.4 a. **Bolete basidiospores** 1. *Boletus*; 2. *Leccinum*; 3. *Suillus*; 4. *Tylopilus*, b. **Longitudinal section through the bolete hymenophore** 1. Basidium; 2. Hymenial cystidium; 3. Basidium with sterigmata and Basidiospores; 4. Subhymenium; Bilateral trama showing central strand and divergent hyphae (Thiers, 1975).



Figures 2.5 **Cross-section of the bolete hymenophore** 1. Tube (hymenophoral) trama. 2. Hymenium. 3. Pore (Thiers, 1975).

The Cuticle

One of the most important and useful anatomical features from a taxonomic point of view in the boletes is the microscopic structure of the cuticular or external layer of the pileus, and to a lesser extent of the stipe. The cuticle is usually easily distinguished from the pileus trama.

The simplest and perhaps most primitive type of cuticle is the cutis in which there is a layer of appressed, filamentous hyphae over the surface of the pileus. The cells of these hyphae are more or less similar in shape and size and seldom break free from one another. Such a cuticle is only rarely seen in the boletes.

The cuticle of the stipe shows less variation than the pileus cuticle. Most commonly, the surface is differentiated as a cutis, but trichodermial or ixotrichodermial types also occur (Figure 2.6). In *Suillus* the surface of the stipe of many species is noticeably dotted with differently colored, often irregularly shaped, resinous globules. Because of their appearance, these globules are designated as glandulae; however, structurally they are composed of masses of caulocystidia similar to the cystidia in the hymenium and give similar color changes when mounted in potassium hydroxide.

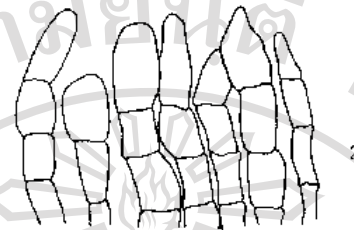


Figure 2.6 Types of bolete cuticle 1. Trichodermium composed of interwoven hyphae and hyphal tips; 2. Trichodermium composed of erect hyphal tips; 3. Ixotrichodermium in which the walls of the hyphae are gelatinizing (Thiers, 1975).

Clamp connections are short, inconspicuous branches located at the cross walls or septa of the hyphae and form a by-pass around the septations. They are present in some of the boletes and are most readily found in the cuticular hyphae, at the base of the basidium or, less frequently, in the basal tomentum of the stipe. The significance of clamp connections is not fully understood since they are often not present at every septation. Their significance has been further confused by the recent observation that the vegetative hyphae of at least some species of *Suillus* when grown in pure culture develop clamps, but in the basidiocarps of the same species all hyphae are devoid of such structures.

2.3.2 TAXONOMY OF BOLETES BASED ON MOLECULAR PHYLOGENY

The application of polymerase chain reaction (PCR) in mycology was firstly described by White *et al.* (1990) and concerned the amplification and direct sequencing of ribosomal RNA gene (rDNA) to establish the taxonomic and phylogenetic relationship of fungi. There are three major steps in a PCR including denaturation, annealing and extension, which are repeated for 30 or 40 cycles (Figure 2.7). This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time. During PCR processes, both strands are copied, there is an exponential increase of the number of copies of the gene (Figure 2.8) and the PCR product could be verified by using gel electrophoresis (Figure 2.9).

With the advent of PCR and its associated methodologies e.g. random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and DNA sequencing have frequently been used for the construction of phylogenetic relationships of fungi at different classification levels. RAPD is a simple and rapid method for detecting genetic diversity as well as it is a powerful tool used to differentiate morphologically similar microorganisms (Welsh and McClelland, 1990). AFLP is a modified technique of simple PCR. There is amplification of fragments from restriction enzyme digestion of genomic DNA. AFLP is similar to RAPD in that it analyses the whole genome but is different in that it uses stringent PCR conditions and produces more reproducible results. AFLP has been applied to the detection of inter- and intraspecific genetic variation in fungi and has more advantages than restriction fragment length polymorphism analysis (RFLP) (Majer *et al.*, 1996). The maximum degree of polymorphism can be detected by sequencing appropriate region of DNA and identifying which of the four possible

nucleotides (ATGC) occurs at each position (Talbot, 2001). The simply in sequencing of DNA and computerization of analytic methods together with the rapid increasing amount of sequences in database (GenBank, EMBL and DDBJ), therefore sequenced data are usually used to infer phylogenies of fungi.

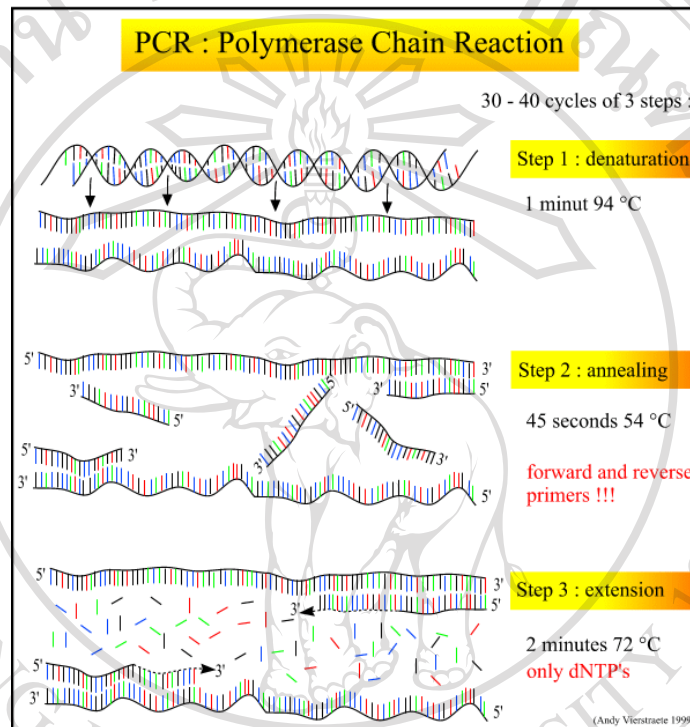


Figure 2.7 The different steps in PCR (Anonymous, 2008).

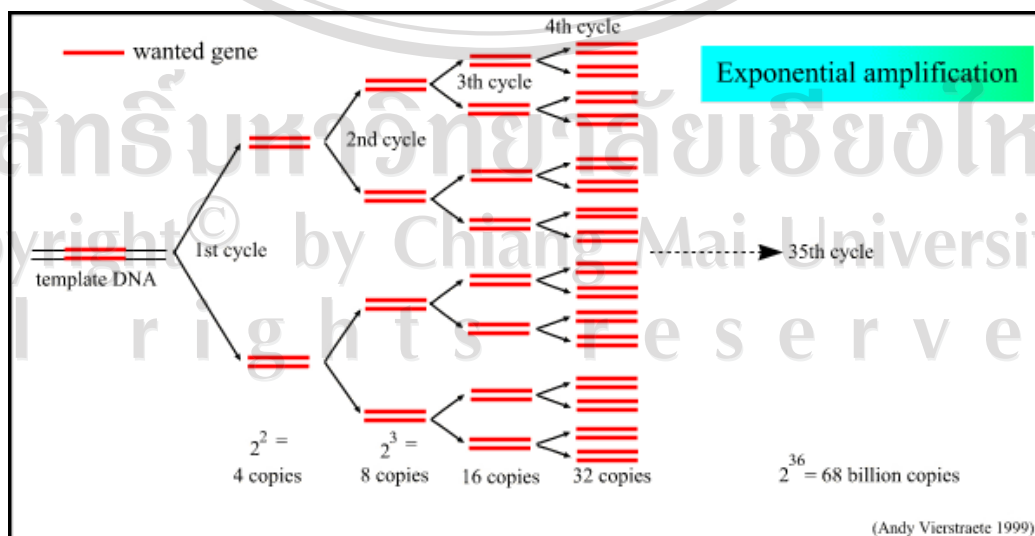


Figure 2.8 The exponential amplification of the gene in PCR (Anonymous, 2008).

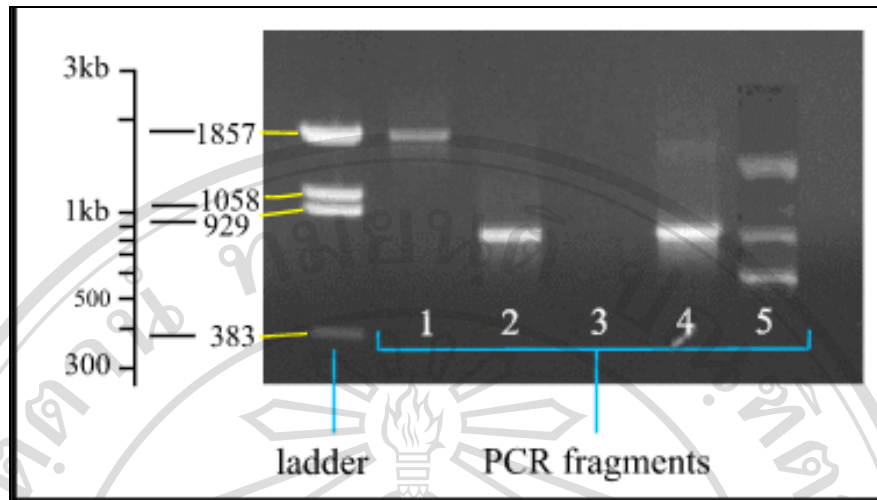


Figure 2.9 Verification of the PCR product on gel. The ladder is a mixture of fragments with known size to compare with the PCR fragments. Notice that the distance between the different fragments of the ladder is logarithmic. Lane 1: PCR fragment is approximately 1850 bases long. Lane 2 and 4: the fragments are approximately 800 bases long. Lane 3: no product is formed, so the PCR failed. Lane 5: multiple bands are formed because one of the primers fits on different places (Anonymous, 2008).

Many boletes are easily identified using macroscopic features only, although, a few of them are very difficult to identify even with the aid of chemical tests and the microscope (Bessette *et al.*, 1997). Nowadays, they are trying to use phylogenetic information gained from sequence analyses, especially of the nuclear rRNA gene (rDNA) (Figure 2.10), to arrange the phylogeny of fungi. The nuclear rRNA gene is transcribed as single molecule with eukaryotic RNA polymerase I. After transcription, the transcripts are processed into 3 molecules (28S, 18S and 5.8S RNA molecules) and then they are use for ribosome construction. The nuclear rRNA genes of fungi are present in the genome as tandem connected operon and as high copy number. The copy number of eukaryotic rDNA is from 400-2000 copy in single genome. They are in nuclei and mitochondria, comprising highly conserved and variable base arrangement. These highly conserved regions include the large subunit (LSU: 28S),

the small subunit (SSU: 18S) and 5.8S that are usually used for estimating the phylogeny of fungi to Orders and the Kingdom levels. The spacer region between subunits is internal transcribed spacer (ITS), and the spacer between gene clusters is intergenic spacer (IGS), which have variable base arrangements, and usually be used for studying the fungal phylogeny at Genera and the Sub-species levels (Duncan *et al.*, 1998). Evidence from molecular studies in the phylum *Basidiomycota* has confirmed the decision to integrate the gasteromycetous and hymenomycetous fungi into a single system (Kirk *et al.*, 2001). Molecular tools using ITS rDNA, small and large subunit rDNA, *atp6*, β -tubulin and histone gene sequences are now being established to prove the relations within various levels (e.g. class, order, family, genus, species) of fungi (Baura *et al.*, 1992; Kreuzinger *et al.*, 1996; Kretzer *et al.*, 1996; Kretzer and Bruns, 1999; Hibbett *et al.*, 1997; Binder and Bresinsky, 2002a, b; den Bakker *et al.*, 2004).

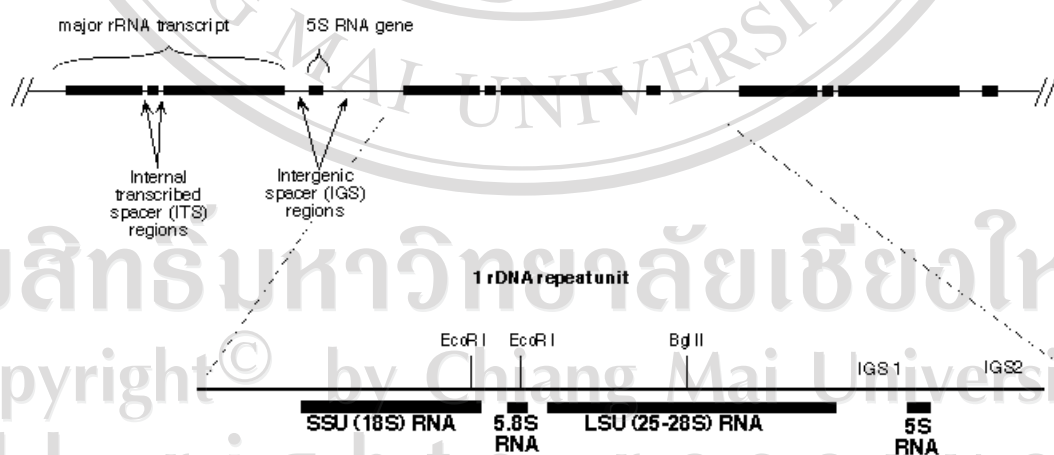


Figure 2.10 Ribosomal RNA gene (rDNA) diagrams (White *et al.*, 1990).

Moncalvo *et al.* (2002) provided a first broad systematic treatment of the euagarics as they have emerged in phylogenetic systematics. The sample consisted of

877 homobasidiomycete taxa and included approximately one tenth (ca. 700 species) of the known number of species of gilled mushrooms that were traditionally classified in the Order *Agaricales*. About 1000 nucleotide sequences at the 5' end of 28S rDNA were produced for each taxon. Phylogenetic analyses of nucleotide sequence data employed unequally weighted parsimony and bootstrap methods. Clades revealed by the analyses support the recognition of eight major groups of homobasidiomycetes that cut across traditional lines of classification, in agreement with other recent phylogenetic studies. The recognition of monophyletic euagarics results in the exclusion from the clades of several groups of gilled fungi that have been traditionally classified in the *Agaricales* and necessitates the inclusion of several clavarioid, poroid, secotioid, gasteroid, and reduced forms that were traditionally classified as in the other basidiomycete orders. Newly discovered phylogenetic affinities include for instance relationships of the true puffballs (*Lycoperdales*) with *Agaricaceae*, of *Panellus* and the poroid fungi *Dictyopanus* and *Favolaschia* with *Mycena*, and of the reduced fungus *Caripia* with *Gymnopus*. Several clades are best supported by ecological, biochemical, or trophic habits rather than by morphological similarities.

According to the previous studies, the phylogenetic relationships among boletes species have not been established in Thailand. Moreover, fewer studies of diversity, physiology, and ecological relationships of boletes been published here than in other countries. Thus a study in the diversity of boletes and phylogenetic relationships is an interesting topic to guide the understanding and sustainable utilization.

2.3.3 THE CURRENT TAXONOMY OF BOLETES

Recent revisions have resulted from the integration of molecular characters and phylogenetic analyses with morphological data. Currently, the *Boletales* has been divided into six suborders (*Boletineae*, *Paxillineae*, *Sclerodermatineae*, *Suillineae*, *Coniophorineae*, and *Tapinellineae*), approximately 19 families and 70 genera, grouping taxa with different fruit body morphology including poroid, gilled, resupinate, hypogeous and epigeous gasteroid forms (Kretzer *et al.*, 1996; Hibbett *et al.*, 1997; Bruns *et al.*, 1998; Kretzer and Bruns, 1999; Grubisha *et al.*, 2001; Binder and Bresinsky, 2002a,b; Binder and Hibbet, 2002; 2004, 2006; Binder *et al.*, 2006).

2.4 DIVERSITY OF BOLETES WORLDWIDE

2.4.1 DIVERSITY OF BOLETES IN AMERICA, EUROPE, AND AFRICA

The diversity of boletes has long been investigated worldwide. There are several studies that document distribution and diversity of boletes from North America to northern South America and the Caribbean region. The region of North America (excluding Mexico) has the highest number of described boletes with approximately 300 species associated with several host plants including 60 species of oak and 35 of pine. Most of the studies have been performed in the eastern part from eastern Canada, New York to Florida and California (Singer, 1947; Smith and Thiers, 1964, 1971; Snell and Dick, 1970; Grund and Harrison, 1976; Phillips, 1991; Singer and Williams, 1992; Both, 1993; Bessette *et al.*, 2000). Thiers (1975) classified several species of boletes from Florida including *Boletus* (31), *Gastroboletus* (5), *Gyroporus* (1), *Leccinum* (13), *Pulveroboletus* (1), *Suillus* (28), and *Tylopilus* (6). McKnight and McKnight (1987) found 35 species of *Austroboletus*, *Boletellus*,

Boletinellus, *Boletus*, *Fuscoboletinus*, *Gyroporus*, *Strobilomyces*, *Suillus*, *Tylopilus*, and *Leccinum*. Approximately 87 species of boletes associated with approximately 12 species of oaks have been studied in Costa Rica of Central America (Singer *et al.*, 1983, 1990a, b, 1991, 1992; Singer and Gómez, 1984; Gómez, 1996; Halling, 1999; Halling and Mueller, 1999; 2003, 2005; Halling *et al.*, 1999). In Belize approximately 13 bolete species were noted in association with *Pinus caribaea* and *Quercus* spp. (Singer *et al.*, 1983, 1991; 1992; García *et al.*, 1986; Kropp, 2001). Seven species associated with *P. caribaea* and *Quercus* spp. have been mentioned by Singer *et al.* (1983), Gómez (1996) and Flores and Simonini (2000) in Guatemala. In Nicaragua, only six species associated with *P. caribaea* were reported (Singer *et al.*, 1983, 1990a, 1991, 1992; Gómez, 1996). Twelve species associated with *P. caribaea* were reported from Honduras (Singer *et al.*, 1983, 1992; Gómez, 1996; Flores and Simonini, 2000). Recently, a survey of *Boletales* was conducted in Belize on the Yucatan Peninsula in northern Central America and the Dominican Republic on the island of Hispaniola in the Caribbean by Ortiz-Santana *et al.* (2007).

In South America, approximately 23 species of boletes associated mainly with *Quercus humboldtii* Bonpl., were described from Colombia (Halling, 1989, 1992; Singer *et al.*, 1990b; Halling *et al.*, 1999; Franco and Uribe, 2000). In the Caribbean region, approximately 18 species of boletes associated with *Pinus* spp., *Coccoloba* spp. and *Quercus* spp. were reported from Cuba (Portales *et al.*, 1999).

In Mexico, approximately 212 taxa of boletes have been documented, mainly from the southeastern state of Veracruz, associated with 52 host plants, primarily pines and oaks (Singer *et al.*, 1983, 1990a, 1991, 1992; García, *et al.* 1986, 1987; Gómez, 1996; García, 1999; García and Garza, 2001). Many *Leccinum* species were

investigated in Australia and England by Bougher and Bougher (1991) and Watling (1999), respectively. Boletes in England were investigated by Laessle and Conte (1996) and they found 39 species of 7 genera.

As most boletes are obligate ectomycorrhizal symbionts, their biogeographical distribution depends on the distribution of their host plants. In North and parts of Central America, boletes are associated mainly with members of the *Betulaceae* (birch and alder), *Fagaceae* (oaks and beech), *Pinaceae* (fir, hemlock, larch, pine, and spruce) and *Salicaceae* (willows). In the Neotropics, members of the *Fabaceae* (caesalpinoid legumes), *Nyctaginaceae* (e.g., *Neea* and *Pisonia* spp.) and *Polygonaceae* (especially *Coccoloba* spp.) are the predominant ectomycorrhizal hosts (Alexander and Hogberg, 1986; Newman and Reddell, 1987; Moyerson, 1993; Lodge, 1996; Henkel *et al.*, 2002).

2.4.2 DIVERSITY OF BOLETES IN ASIA

The distribution of the major components of the bolete mycobiota of South East Asia was discussed by Watling (2001). He reported that final number of species of boletes in South East Asia is likely to be well in excess of the number compiled in Corner's 1972 monograph.

Zang (1992) presented *Sinoboletus* as a new genus of *Boletaceae* from China. Recently, many new species of boletes have also been described such as *Boletus sinoaurantiacus*, *B. hainanensis*, *Pulveroboletus reticulopileus*, *Sinoboletus maekawae* and *Xerocomus sinensis* (Zang *et al.*, 2001). *Boletus gansunensis* was described from Gansu Province of China by Wang *et al.* (2003).

Boletes has still never been well studied in Laos even this country is a landlocked country in Indochina, lies just between tropical and subtropical zone. And intricate geographic feature is forming an affluent biota. Then this district is precious area as the nature in meaning which is still true and undeveloped virgin forest has been preserved. But the natural matter is not widely known so natural research is not being carried out for various problems. Especially fungi as mushroom, these are known just a little. There is a few websites presents pictures and name lists of some boletes (*Boletellus*, *Phylloporus*, *Gyrodon*, *Suillus*, *Xerocomus*, *Pulveroboletus*, *Boletus*, *Strobilomyces*, *Leccinum* and *Tylopilus*) that found in Laos e.g. http://www.geocities.com/laofungi_2000/ and <http://giechgroup.hp.infoseek.co.jp/kinoko/eng.html>.

2.4.3 DIVERSITY OF BOLETES IN THAILAND

The diversity of macrofungi in Thailand is extremely high, and might be the highest diversity in Asia because of the natural geography of the country (Thaithatgoon *et al.*, 1998). In the North and Northeast Thailand, the diversity of mushrooms at Doi Suthep National Forest, Doi Inthanon National Forest and Khao Yai National Park have preciously been investigated (Watling, 1998).

Soytong (1994) found 5 species of *Boletus aestivalis*, *B. edulis*, *Suillus americanus*, *S. pictus* and *Strobilomyces floccopus*. Chandrasrikul (1996) reported *Heimiella retispora*, *B. edulis* and *Porphyrellus fusisporus* from northern areas. *Boletellus emodensis*, *Boletus coccineinanus*, *B. ornatipes*, *Gyroporus castaneus*, *G. heterosporus*, *Heimiella retispora*, *Pulveroboletus ravenelii*, *Rubinoboletus ballouii*, *Strobilomyces velutipes* and *S. mollis* are apparently found at Doi Inthanon and Doi

Suthep-Pui National Parks. Some new recorded species, e.g. *Boletus coccineinanus* and *Rubinoboletus ballouii*, have been found at Khao Yai and Doi Suthep (Watling, 1998).

Rodtong *et al.* (1998) and Rodtong and Teaumroong (2000) have intensively studied the diversity of mushrooms at Nong-ravieng Plant Genetics Forest and Tup Lan National Park in the Northeastern Thailand. At Nong-ravieng Plant Genetics Forest composed of dry dipterocarp and mixed deciduous forests, diverse genera of macrofungi occur. *Boletus* species have been found in the dry dipterocarp forest of Tup Lan National Park.

In Eastern Thailand, the diversity of some boletes and other macrofungi has been reported in two tropical areas, Khao Kitcha Koot National Park and Khao Soi Dao wildlife sanctuary (Klingsorn *et al.*, 1998a, b).

Tongglam (1999) reported the diversity of boletes in Doi Suthep-Pui National Park, during June 1997-July 1998; the ranges of temperature and relative humidity were 20-27°C and 68-100%, respectively. One hundred and ten specimens were collected and identified to 12 genera and 36 species, whilst 2 and 35 specimens could not be identified to genera or species. Thirty-one species, belonging to 9 genera in the family *Boletaceae*: *Boletus*, *Gyrodon*, *Gyroporus*, *Leccinum*, *Rubinoboletus*, *Pulveroboletus*, *Suillus*, *Tylopilus* and *Xerocomus* were recorded. Moreover, 5 species belonging to 3 genera in the family *Strobilomycetaceae*: *Boletellus*, *Porphyrellus* and *Strobilomyces* were also noted. Huaikokma, evergreen forest at altitudes of 1000-1500 m amsl, pH of soil 4-6 has had the most diverse of boletes. Boletes were regularly found during June-December, but the high frequency was demonstrated during June-July. Ruksawong and Flegel (2001) reported 9 species: *Boletellus emodensis*, *Boletus*

cf. edulis, *B. crassipediis*, *Gyrodon meruloides*, *Pulveroboletus ravenelii*, *Strobilomyces velutipes*, *Suillus luteus*, *Tylopilus fumosipes* and *T. nigropurpureus*, from the forests of Thailand. Jones and Hyde (2004) described some new species of fungi of Thailand including 8 species of boletes. In total, there are 63 recorded species of boletes in Thailand. Chantorn *et al.* (2007) reported the diversity of boletes in Nam Nao and Phu Rua National Parks, Thailand during the wet seasons of 2005 and 2006. There were fifty-two specimens, 40 from Nam Nao and 12 from Phu Rua, which belonged to nine genera as follows: *Boletellus*, *Boletus*, *Heimiella*, *Leccinum*, *Phylloporus*, *Pulveroboletus*, *Strobilomyces*, *Tylopilus* and *Xerocomus*. Nine species were new records to Thailand including *Boletus laetissimus* Hongo, *B. obscureumbrinus* Hongo, *B. subvelutipes* Peck., *Heimiella japonica* Hongo, *Leccinum extremiorientale* (L. Vass) Sing., *Phylloporus cf. rhodoxanthus* (Schw.) Bres., *Strobilomyces confusus* Sing., *Tylopilus eximius* (Peck) Sing. and *Xerocomus subtomentosus* (L.:Fr.) Quel. Recently, 67 species of *Boletales* in Thailand were described and illustrated by Chandrasrikul *et al.* (2008).

Table 2.1 Some boletes species reported from some Asian countries and Thailand (Corner, 1972; Hongo and Izawa, 1998; Komiyama and Yamada, 2000; Zang *et al.*, 2001; Takahashi, 2003; Wang *et al.*, 2003; Chantorn *et al.*, 2007; Srihanan *et al.*, 2007; Chandrasrikul *et al.*, 2008)

Name	China	Japan	Laos	Malaysia	Thailand
<i>Aureoboletus thibetanus</i>		*			*
<i>Austroboletus dictyotus</i>				*	
<i>A. fuisporus</i>		*			
<i>A. gracilis</i>		*			
<i>A. malaccensis</i>				*	
<i>A. mucosus</i>				*	
<i>A. rarus</i>				*	
<i>A. rubiicolor</i>				*	
<i>A. subflavidus</i>					*
<i>A. subvirens</i>					
<i>A. thibetanus</i>	*	*			
<i>Boletellus ananas</i>	*			*	*
<i>B. betula</i>	*				
<i>B. chrysenteroides</i>		*			*
<i>B. dissiliens</i>				*	
<i>B. elatus</i>		*			*
<i>B. emodensis</i>	*	*	+/-	*	*
<i>B. fallax</i>				*	
<i>Boletellus longicollis</i>	*	*		*	
<i>B. mirabilis</i>	*	*			
<i>B. obscurecoccineus</i>		*		*	
<i>B. ridiculus</i>				*	
<i>B. russellii</i>		*			*
<i>B. schichianus</i>		*			
<i>Boletinus asiaticus</i>	*	*			
<i>B. cavipes</i>	*	*			*
<i>B. kunmingensis</i>	*				
<i>B. paluster</i>	*	*			
<i>B. pinetorum</i>	*				
<i>B. spectabilis</i>	*	*			
<i>Boletus aereus</i>	*	*			
<i>B. aokii</i>		*			
<i>B. appendiculatus</i>					*
<i>B. aureomycelinus</i>				*	
<i>B. aureissimus</i>					*

Table 2.1 (Continued).

Name	China	Japan	Laos	Malaysia	Thailand
<i>B. auripes</i>	*	*			*
<i>B. bicolor</i>	*				*
<i>B. brunneissimus</i>	*				
<i>B. calopus</i>	*	*			
<i>B. craspedius</i>	*			*	
<i>B. chrysenteron</i>					*
<i>B. edulis</i>	*	*			*
<i>B. erythropus</i>	*				
<i>B. farinolens</i>				*	
<i>B. firmus</i>					*
<i>B. flammans</i>	*				
<i>B. formosus</i>				*	
<i>B. fraternus</i>	*	*			*
<i>B. fuscopunctus</i>		*			
<i>B. gansunensis</i>	*				
<i>B. graveolens</i>				*	
<i>B. griseipurpureus</i>					*
<i>B. griseus</i>	*	*			*
<i>B. hainanensis</i>	*				
<i>B. havilandii</i>				*	
<i>B. hiratsukae</i>		*			
<i>B. impolitus</i>	*				
<i>B. jocosus</i>				*	
<i>B. kumaeus</i>				*	
<i>B. laetissimus</i>		*			*
<i>B. luridus</i>	*	*			
<i>B. magnificus</i>	*				
<i>B. manicus</i>				*	
<i>B. monsfraseri</i>				*	
<i>B. nobilis</i>					*
<i>B. obscureumbrinus</i>	*	*			*
<i>B. odaiensis</i>	*	*			
<i>B. ornatipes</i>	*	*			
<i>B. pallidus</i>					*
<i>B. patouillardii</i>				*	
<i>B. peltatus</i>				*	
<i>B. persimilis</i>				*	
<i>B. phaeocephalus</i>				*	
<i>B. pinopilus</i>	*				
<i>B. poeticus</i>				*	

Table 2.1 (Continued).

Name	China	Japan	Laos	Malaysia	Thailand
<i>B. pseudocalopus</i>		*			
<i>B. pulverulentus</i>		*			
<i>B. purpureus</i>	*				
<i>B. queletii</i>	*				
<i>B. reayi</i>	*			*	
<i>B. regius</i>	*				
<i>B. reticulatus</i>	*	*			*
<i>B. retipes</i>	*				
<i>B. rhodopurpureus</i>	*				
<i>B. rubellus</i>	*			*	
<i>B. rubrifibrillosus</i>	*			*	
<i>B. rufo-aureus</i>	*			*	*
<i>B. satanas</i>	*				
<i>B. sepiola</i>				*	
<i>B. sensibilis</i>		*			
<i>B. sinapicolor</i>		*		*	
<i>B. sino-aurantiacus</i>	*				
<i>B. speciosus</i>	*	*			
<i>B. subreticulatus</i>				*	
<i>B. subvelutipes</i>	*	*			*
<i>B. tenax</i>					*
<i>B. thibetanus</i>				*	
<i>B. tomentipes</i>	*				
<i>B. umbilicatus</i>	*			*	
<i>B. umbriniporus</i>	*	*			*
<i>B. variipes</i>	*				
<i>B. venenatus</i>		*			
<i>B. violaceofuscus</i>	*	*			
<i>B. xylophilus</i>		*		*	
<i>Chalciporus piperatus</i>		*			*
<i>Gyrodon lividus</i>	*	*			
<i>G. merulioides</i>		*			
<i>G. rompelii</i>					*
<i>Gyroporus atroviolaceus</i>	*			*	
<i>G. castaneus</i>	*	*		*	
<i>G. cyanescens</i>	*	*			
<i>G. longicystidiatus</i>		*			
<i>G. malesicus</i>				*	
<i>G. purpurinus</i>	*				
<i>Heimiella anguiformis</i>				*	
<i>H. japonica</i>		*			*

Table 2.1 (Continued).

Name	China	Japan	Laos	Malaysia	Thailand
<i>H. kinabaluensis</i>				*	
<i>H. mandarina</i>				*	*
<i>H. retispora</i>				*	*
<i>H. subretispora</i>				*	
<i>Ixocomus granulatus</i>				*	
<i>Leccinum atrostripiatum</i>	*				
<i>L. aurantiacum</i>	*				
<i>L. borneensis</i>				*	
<i>L. chromapes</i>	*				
<i>L. crocipodium</i>	*				
<i>L. extremiorientale</i>	*	*	*		*
<i>L. griseum</i>	*	*			
<i>L. holopus</i>	*	*			*
<i>L. hortonii</i>		*			
<i>L. intusrubens</i>		*		*	*
<i>L. oxydabile</i>	*				
<i>L. quercinum</i>	*				
<i>L. rufum</i>	*				
<i>L. rugosiceps</i>	*				
<i>L. scabrum</i>	*	*			
<i>L. subradicatum</i>	*				
<i>L. variicolor</i>	*				
<i>L. versipelle</i>	*	*			
<i>Phlebopus braunii</i>					*
<i>P. portentosus</i>				*	*
<i>Phylloporus rhodoxanthus</i>	*				*
<i>P. bellus</i>	*				*
<i>Porphyrellus orientalis</i>					*
<i>P. pseudoscaber</i>	*				
<i>Pulveroboletus amarellus</i>	*				
<i>P. auriflammeus</i>	*	*			
<i>P. frians</i>	*			*	
<i>P. ravenelii</i>	*	*	*	*	*
<i>P. reticulopileus</i>	*				
<i>P. viridis</i>		*			
<i>P. retipes</i>	*				
<i>Sinoboletus maekawae</i>	*				
<i>Strobilomyces annulatus</i>				*	
<i>S. confusus</i>	*	*			*
<i>S. floccopus</i>					*
<i>S. foveatus</i>				*	

Table 2.1 (Continued).

Name	China	Japan	Laos	Malaysia	Thailand
<i>S. mirandus</i>				*	
<i>S. mollis</i>				*	
<i>S. nigricans</i>				*	
<i>S. polypyramis</i>	*			*	
<i>S. retisporus</i>	*			*	
<i>S. seminudus</i>	*	*			*
<i>S. strobilaceus</i>	*	*			
<i>S. velutipes</i>				*	
<i>Suillus acidus</i>	*				
<i>S. albidipes</i>	*				
<i>S. americanus</i>	*	*			
<i>S. bovinus</i>	*	*			*
<i>S. brevipes</i>	*				
<i>S. collinitus</i>	*				
<i>S. flavidus</i>	*				
<i>S. glandulosipes</i>	*				
<i>S. granulatus</i>	*	*	*		*
<i>S. grevillei</i>	*	*			
<i>S. intermedius</i>	*				*
<i>S. lactifluus</i>	*				
<i>S. laricinus</i>	*	*			
<i>S. luteus</i>	*	*			*
<i>S. pictus</i>	*	*			*
<i>S. placidus</i>	*	*			
<i>S. plorans</i>	*				
<i>S. sibiricus</i>	*				
<i>S. spectabilis</i>	*	*			
<i>S. spraguei</i>	*				
<i>S. subaureus</i>	*	*			
<i>S. subluteus</i>	*	*			
<i>S. tomentosus</i>	*	*			
<i>S. variegatus</i>	*	*			
<i>Tylopilus albirubens</i>				*	
<i>T. albo-ater</i>	*			*	
<i>T. areolatus</i>	*				
<i>T. argentatae</i>				*	
<i>T. ascendens</i>				*	
<i>T. atripurpureus</i>				*	
<i>T. aurantitubus</i>				*	
<i>T. ballouii</i>	*	*		*	*
<i>T. brunneinanus</i>				*	

Table 2.1 (Continued).

Name	China	Japan	Laos	Malaysia	Thailand
<i>T. brunneirubens</i>				*	
<i>T. castaneiceps</i>		*			
<i>T. cervicolor</i>				*	
<i>T. cervinococcineus</i>				*	
<i>T. chromapes</i>	*	*			*
<i>T. coccineinanus</i>				*	
<i>T. cremeus</i>				*	
<i>T. cutifRACTUS</i>				*	
<i>T. eximius</i>	*	*			*
<i>T. felleus</i>	*				*
<i>T. ferrugineus</i>	*	*			
<i>T. fumosipes</i>	*	*			
<i>T. funerarius</i>				*	
<i>T. griseipurpureus</i>				*	
<i>T. holophaeus</i>				*	
<i>T. javanicus</i>				*	
<i>T. levitinetus</i>				*	
<i>T. longipes</i>				*	
<i>T. maculatus</i>				*	
<i>T. manus</i>				*	
<i>T. neofelleus</i>		*			
<i>T. nigerrimus</i>	*	*		*	*
<i>T. nigropurpureus</i>		*		*	*
<i>T. olivaceiluteus</i>				*	
<i>T. olivaceirubens</i>				*	
<i>T. otsuensis</i>		*			*
<i>T. parvus</i>				*	
<i>T. piculinus</i>				*	
<i>T. plumbeoviolaceus</i>	*				
<i>T. porphyrosporus</i>		*			
<i>T. primulinus</i>				*	
<i>T. psittacinus</i>				*	
<i>T. purus</i>				*	
<i>T. quisquiliarum</i>				*	
<i>T. rhodoleucus</i>				*	
<i>T. rigens</i>		*			
<i>T. roseolus</i>	*				
<i>T. spinifer</i>				*	
<i>T. subtostus</i>				*	
<i>T. tristiculus</i>				*	
<i>T. valens</i>		*		*	

Table 2.1 (Continued).

Name	China	Japan	Laos	Malaysia	Thailand
<i>T. velatus</i>	*				
<i>T. vinaceipallidus</i>				*	
<i>T. vinosobrunneus</i>					*
<i>T. virens</i>	*			*	*
<i>T. viridis</i>				*	
<i>Xanthoconium affine</i>	*	*			
<i>X. chrysenferon</i>		*			
<i>Xerocomus aculifer</i>				*	
<i>X. aculifer</i>				*	
<i>X. alutaceus</i>		*			
<i>X. asperipes</i>				*	
<i>X. astraicola</i>		*			
<i>X. badius</i>	*				
<i>X. blanditus</i>				*	
<i>X. calocystides</i>				*	
<i>X. calvus</i>				*	
<i>X. catervatus</i>				*	
<i>X. changensis</i>				*	
<i>X. chlamydosporus</i>				*	
<i>X. chrysenferon</i>	*				
<i>X. chrysops</i>				*	
<i>X. cuticulatus</i>				*	
<i>X. cyaneirufescens</i>				*	
<i>X. destitutus</i>				*	
<i>X. disperses</i>				*	
<i>X. ferruginosporus</i>				*	
<i>X. gyrodontoides</i>				*	
<i>X. hastulifer</i>				*	
<i>X. honestus</i>				*	
<i>X. illudens</i>	*				
<i>X. incertus</i>				*	
<i>X. intentus</i>				*	
<i>X. junghuhnii</i>				*	
<i>X. latisporus</i>				*	
<i>X. lubricus</i>				*	
<i>X. lucescens</i>				*	
<i>X. microcarpoides</i>				*	
<i>X. microcarpus</i>				*	
<i>X. microsporus</i>				*	
<i>X. mirans</i>				*	
<i>X. mugatorius</i>				*	

Table 2.1 (Continued).

Name	China	Japan	Laos	Malaysia	Thailand
<i>X. nigromaculatus</i>		*			
<i>X. obscurebrunneus</i>		*			
<i>X. parvulus</i>	*				
<i>X. phoeniculus</i>				*	*
<i>X. phylloporoides</i>				*	*
<i>X. polychrous</i>				*	*
<i>X. prebadius</i>				*	*
<i>X. pseudochrysenteron</i>				*	*
<i>X. pulverulentus</i>	*				
<i>X. puniceus</i>	*				
<i>X. ranunculus</i>				*	*
<i>X. raphanolens</i>				*	*
<i>X. rectus</i>				*	*
<i>X. rubriporus</i>				*	*
<i>X. rufoflavipes</i>				*	*
<i>X. rugosellus</i>	*				
<i>X. sartor</i>				*	*
<i>X. satisfactus</i>				*	*
<i>X. semitarius</i>				*	*
<i>X. sinensis</i>	*				
<i>X. solitarius</i>				*	*
<i>X. spadiceus</i>	*				
<i>X. spiculipes</i>				*	
<i>X. subpaludosus</i>	*				
<i>X. subtomentosus</i>	*	*			*
<i>X. sylvestris</i>				*	
<i>X. tentabundus</i>				*	
<i>X. variisporus</i>				*	
<i>X. versicolor</i>	*				

* = found, +/- = may be found

2.5 SIGNIFICANCE OF BOLETES

Boletes are valuable to people as food resources, medicinal uses, aesthetic values and bio-indicator of environmental quality (Brundrett *et al.*, 1996). A few species of boletes are poisonous whilst some edible boletes such as *B. edulis* in Europe are becoming commercially valuable.

2.5.1 BIOACTIVE COMPOUND AND MEDICINE FROM BOLETES

Antitumor and cytotoxic activity of tylopilan, a fungal polysaccharide from *Tylopilus felleus* fruit bodies has been reported by Grzybek *et al.* (1990). Chumkhunthod (2004) has been reported that the *Strobilomyces mollis* extracts showed powerful anti-human epidermoid carcinoma cells as well as those from other mushroom group (*Amanita* sect. *Vaginatae*, *Lentinus* sp. and *Schizophyllum commune*). Austrogracilin A and B, two 2-naphthoic acid derivatives were isolated from the air-dried fruit bodies of *A. gracilis* (Bartsch *et al.*, 2004).

2.5.2 EDIBLE SPECIES BOLETES

The fruit bodies of higher fungi particularly in group of *Basidiomycota* have been known as a source of food for thousand of years. Many countries they were gathered in their natural environment, and only in the 17th century was their culture in artificial conditions started in France (Mattila *et al.*, 2001).

Several mushrooms as well as boletes species used as food by different way of human. They are used as meat substitute, sold fresh in local markets, or dried for preservation and use during the dry season when meat becomes very scarce. Such mushrooms are usually collected in the wild especially during the rainy season. In Nigeria, it is a practice mainly engaged in by women and children. They were identifying the edible mushrooms from poisonous ones. Mushroom have been made many collections, and so far the following species followed by their local names have been identified included *Pleurotus tuberregium*, *Lentinus squarrosulus*, *Volvariella* sp., *Agaricus* sp., *Auricularia* sp., *Termitomyces* sp., *Schizophyllum commune*, and *Corprinus* sp.

Various species of boletes such as *Boletus edulis* (King Bolete), *B. mirabilis* (Velvet Top), *Suillus lakei* (Lake's Bolete) and *S. luteus* (Slippery Jack) have been reported as the best economic bets of wild edible boletes in British Columbia (Wills and Lipsey, 1999). Fruiting body of *B. edulis* has a distinct aroma reminiscent of fermented dough. It has higher water content than other edible mushrooms. In a good fruiting year, approximately 100,000 kg. of fresh boletes were harvested mainly from Haida Gwaii and the Prince George area. In a bad year there may be virtually no harvest at all. These mushrooms are weather dependent that it is not possible to rank order their source areas. Again boletes grow in many places where they are not harvested. Pickers on average are paid Can. \$2.50/lb for boletes (there are four grades), and exporters receive US \$8-12.00/lb landed and fresh, around US\$75.00/kg dried and landed, and around US\$5.00-6.00/kg frozen. Approximately 90% of all harvested boletes are exported dried or frozen and only around 10% of the harvest is exported fresh. Boletes are one of the first wild food mushrooms to be attacked by pests, (they can change grades in three hours), and there is currently a world shortage of king boletes. In addition *B. smithii* (Smith's Bolete), *B. zelleri* (Zeller's Bolete), *S. brevipes*, *S. caerulescens* (Blue-Staining Slippery Jack), *S. cavipes* (Hollow Stemmed Larch Bolete), *S. granulatus* (Dotted Stalked Slippery Jack), *S. subolivaceus* (Slippery Jill) and *S. tomentosus* (Woolly Capped Bolete) are reported as the edible mushroom by Berch and Cocksedge (2003) (Figure 2.11).

There are many kinds of wild edible boletes in the north of Thailand. Sanmee (2004) reported various species of wild edible mushroom in upper northern Thailand. She also analyzed for nutritive value of Hed Har or *Phlebopus* (= *Phaeogyroporus*) *portentosus*. While *Boletus dimocarpicola*, *Heimioporus retisporus*, *Suillus luteus*,

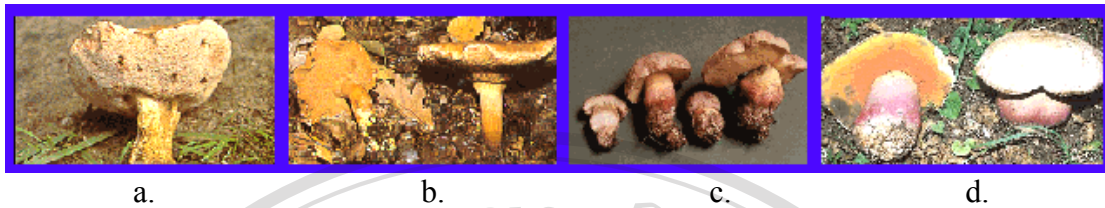
and *Tylopilus felleus* are wild edible boletes species that are also generally sale in local market or roadside market (especially those are nearby high mountain or National Park forests) in northern Thailand during raining season. While some those species may slightly be poisonous when fresh but they are still can be eaten after heating or cooking.

2.5.3 TOXIC AND UNPLEASANT TASTE SPECIES BOLETES

Several boletes are known to cause digestive system irritation. Devil's B. (*Boletus satanas*) causes unpleasant symptoms even when taken in small amounts. Similar poisoning is caused by the Bitter Bolete (*Tylopilus felleus*), Peppery Bolete (*Chalciporus piperatus*) and *B. calopus* all of which have an unpleasant peppery or bitter taste (Figure 2.12).



Figures 2.11 Some edible species boletes a. *Boletus edulis* (King Bolete). b. *B. mirabilis* (Velvet Top). c. *Suillus lakei* (Lake's Bolete). d. *S. luteus* (Slippery Jack). e. A pile of dried porcini.



Figures 2.12. Some toxic and unpleasant taste species boletes. a. Bitter Bolete (*Tylopilus felleus*). b-c. Peppery Bolete. b. *Chalciporus piperatus*. c. *B. calopus*. d. Devil's B. (*B. satanas*).

2.5.4 MYCORRHIZAL ASSOCIATIONS

In worldwide the boletes are exclusively forest-inhabiting fungi. The restriction to the forests is due to the necessity of the formation of mycorrhizal associations in order to survive. This type of association is an intimate relationship between the vegetative mycelium of the fungus and the young roots of the associated tree. The mycelium forms a rather dense layer or mantle on the external surface of the root. This relationship seems to be mutually beneficial. The mushroom profits by obtaining nutrients and perhaps water from the host tree. The benefits afforded to the tree by the fungus are not so readily apparent, but indications are that the fungus accumulates considerable quantities of certain minerals from the soil that are subsequently available to the tree and increase the absorptive surface of the roots.

Obviously, if such associations are essential for the survival or development of the tree, these fungi assume considerable significance in reforestation practices.

Since the fungus hyphae are microscopic except in mass, the difficulty of tracing the connection between the basidiocarp and the roots of the tree is readily apparent. It is understandable, therefore, that most mycorrhizal associations are only presumed and cannot easily be confirmed except by cultural practices. Such

assumptions are based upon repeated observations that a specific boletes always occurs in close proximity to the same kind of tree. In the boletes such associations may be formed with hardwood trees or conifers. Some species appear capable of forming mycorrhizal associations with a rather wide range of trees; others are seemingly highly restricted.

2.6 THE NATIONAL PARK IN NORTHERN THAILAND

In the upper northern Thailand there are many high peaks; Doi Chiang Dao at 2225 m amsl, Doi Inthanon at 2565 m amsl, Doi Suthep-Pui at 1685 m amsl and some major mountains such as Doi Luang, and Doi Phu Kha. Those are 3 different forest types varying by altitudes as lowland forests (<800 m amsl), mid-elevation forests (800-1200 m amsl), and highland forests (1200-2565 m amsl), which are consist of moist areas, intermediate moisture areas, and dry areas in each type of forests.

The seasonal climate of this area coupled with the complex topography result in natural vegetation patterns consisting of an intricate mosaic of both evergreen and deciduous forest patches. Tree species in each forest types in northern Thailand can be similar or different depending on 3 main factors; moisture, altitude and disturbance.

The lowland forests consist of gallery evergreen forests, deciduous/bamboo forests, and dry dipterocarp forests in moist areas, intermediate moisture areas, and dry areas, respectively. Common emergent trees in moist areas of the lowland forests include

Leguminosae, *Meliaceae*, *Dipterocarpaceae*, *Sterculiaceae*, *Datiscaeeae*, and *Moraceae*. The mid-elevation forests contain a proportion of both lowland and highland species. In highland forests, the families *Lauraceae*, *Magnoliaceae* and *Theaceae* are often common in moist sites. Trees of the family *Fagaceae* often form

at least 50% of the main canopy layer in the intermediate sites. In the dry areas with extremely sandy soils, the whole landscape can become dominated by pine-dry evergreen forest (Gardner *et al.* 2000).

Unfortunately, forest cover has been significantly decreased from the last 40 years, from 53.3 % in 1961 to 25.3% in 1998 (Anonymous, 2001). The several main causes of deforestation are logging, communist threat, clearance of land for agriculture and cattle-raising, slash-and-burn agriculture, forest fires, plantations, various tourism development projects (Elliott, 2001; Fehr, 1993). Forest destruction in Thailand lead to the problem of a series of floods, landslides, greenhouse effects, effects on wildlife and biodiversity, affected scenery and its impact on tourism (von Geusau *et al.*, 1992; Fehr, 1993). According to the rich of wildlife and biodiversity in all those large areas (at least 10 km²) of all mountains in northern Thailand the Thai government established them to be the national parks. The main objectives of that are the conservation and reforestation for sustainable forests. The high biodiversity that found in the national park as mentions as above the present study focus on the diversity of boletes from the forests in some national parks of northern part of our country particularly those in Chiang Mai.