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분자 염기 서열분석에 기초한 고약버섯류의 계통학적 연구

Systematic Study of Corticioid Fungi based on Molecular Sequence Analyses

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Systematic Study of Corticioid Fungi based on Molecular Sequence Analyses

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ABSTRACT

Corticioid fungi of simple resupinate fruitbodies belong to the Hymenomycetes of Basidiomycota. Due to their saprobic activity as a decomposer in degradation of cellulose and lignin of forest wood, they play an important role in forest ecology. In spite of this importance, there have been no comprehensive phylogenetic studies on corticioid fungi and no generally accepted taxonomic systems for this group except for fragmentary studies on some specific taxa. To infer the phylogenetic relationships of corticioid fungi, diverse taxa were included and, based on morphological and molecular data, phylogenetic analyses were performed in this study.

The species number of Korean corticioid fungi (mainly from the family Corticiaceae) reported until recently is 83 in 39 genera of 7 families. Through morphological studies, total 40 corticioid fungi that corresponded to 27 genera of 6 families were confirmed as new or unrecorded to Korea and an annotated list of these fungi was presented in an alphabetical order of species. For molecular studies, the nuclear small subunit ribosomal DNA (nuc-ssu rDNA) region was used to infer higher level relationships and the internal transcribed spacer (ITS) region was used to infer lower level relationships of corticioid fungi. Phylogenetic analyses based on nuc-ssu rDNA showed that ten major clades were recognized in Homobasidiomycetes. These results were congruent with those of recent molecular phylogenetic studies. Corticioid forms occurred in eight major clades: (1) russuloid clade, (2) peniophoroid clade, (3)

hymenochaetoid clade, (4) euagarics clade, (5) bolete clade, (6) laeticorticioid clade, (7) polyporoid clade and (8) botryobasidioid clade. Three of them were newly recognized in this study. The peniophoroid clade possessed non-amyloid spores and formed a sister taxon to the russuloid clade. The laeticorticioid clade was characterized by large basidia, basidiospores and dendrohyphidia. The botryobasidioid clade was characterized by urniform basidia with 6 to 8 sterigmata.

Phylogenetic analyses of closely related genera were performed for the ITS region. In the russuloid clade, molecular phylogeny of *Stereum* and *Xylobolus* was mostly discussed, which indicated that hymenium bleeding was an important character in defining *Stereum*. Type of hyphidia showed a trend to reduce and fruitbodies an evolutionary direction from resupinate to fan-shaped or spathulate forms in *Stereum-Xylobolus* phylogeny. In the hymenochaetoid clade, *Hyphodontia* was closely placed to *Schizopora* and *Resinicium* whose relationships were also supported by anatomical characters. In the euagarics clade, phylogenetic analysis suggested a possibility that the ancestor of the euagarics clade might be *P. crispa*. Toothed hymenophores and globose basidiospores supported a relationship between *M. copelandii* and *D. fragilis* that again grouped with *Hypochnicium* species at 100% bootstrap support.

According to hymenium type, the bolete clade was divided into two groups. One group contained *Serpula* and *Pseudomerulius* with merulioid hymenium and the other group *Coniophora* with smooth hymenium. In the laeticorticioid clade, *Vuilleminia*, *Laeticorticium* and *Dendrocorticium* species form resupinate fruitbodies but

Punctularia forms distinctly fan-shaped fruitbodies. The evolutionary direction of fruitbodies from resupinate form to spatulate form was also shown in this clade. The polyporoid clade was divided into six groups: *Trametes* group, *Steccherinum* group, *Irpex* group, *Phlebia* group, *Phanerochaete* group and brown rot group. Corticioid fungi of the polyporoid clade have distinctive microscopic characters: dendrohyphidia in *Trametes* group, simple-septate hyphal systems in *Irpex* group, capitate leptocystida in *Phlebia* group, incrusted lamprocystidia and simple-septate hyphal systems in *Phanerochaete* group and brown rot habit in brown rot group. The sequence analysis, along with morphological and cultural characteristics, suggested that *Irpex hydnoides* of the *Irpex* group is a new species.

Based on type of hyphidia, hymenium form, fruitbody morphology, microscopic structures, and other related characters of phylogenetic significance like mitic system, presence or absence of clamp connections, amyloidity of basidiospores, or rot type, corticioid fungi might have convergently developed their morphologies that are simple in appearance but complex under microscope or nutritional modes to utilize ligno-cellulose complex of wood during the history of evolution.

■ **Key words** : corticiaceae, corticioid fungi, taxonomy, molecular phylogeny, ITS, Small subunit rDNA.

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TABLE OF CONTENS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CBS	Centraalbureau voor Schimmelcultures, Netherlands
CI	Consistency index
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
IFO	Institute for Fermentation, Osaka
IMSNU	Institute of Microbiology, Seoul National University
ITS	internal transcribed spacer
KCTC	Korean Collection for Type Cultures
MP	most parsimony
NJ	neighbor joining
Nuc-SSU	nuclear small subunit
PAUP	phylogenetic analysis using parsimony
PCR	polymerase chain reaction
rDNA	ribosomal DNA
RC	re-scaled consistency index
RI	retention index
SFC	Seoul National University Fungus Collections
SSU	small subunit

CHAPTER 1

General Introduction

1. Categories of Corticioid Fungi

Corticioid fungi are characterized by simple fruitbodies, usually resupinate, crust-like and closely attached to their substrate, but their microscopic features vary more widely than those of other families in Aphyllophorales. As the term "corticioid" refers to a genearal resemblance of typically effuse and thin basidiomes, the corticioid fungi are widely distributed in Hymenomycetes. Eriksson and his colleagues (1973-1988) included many families of the Aphyllophorales as well as the family Corticiaceae in their magnum opus "The Corticiaceae of North Europe." The Corticiaceae sensu lato was very heterogeneous, for which reason it was impossible to place the family in a single phylogenetic scheme. To create some kind of a family tree, reflecting the supposed natural relationships of taxa, Jülich (1981) divided the Basidiomycota into two classes, the Heterobiasidiomycetes with 13 orders and the Homobasidiomycetes with 49 orders. Jülich placed the corticioid fungi into 15 orders and 42 families. Ginns and Lefebvre (1993) included 1,163 species into the corticioiod fungi that distributed among 21 orders and 54 families. Among them, a few genera like *Auricularia, Climacodon, Creolophus, Hericum, Tremella* and cyphelloid genera that did not have corticioid basidiomata were included together because they were taxonomically or ecologically related to the corticioid group.

Therefore, the lignicolous corticioid fungi have been thought to be a taxonomically heterogeneous group. The corticioid fungi are here taken in a very wide sense in order

to cover all the species collected by the methods described here.

2. Ecology and Economic Importance of Corticioid Fungi

When people go out in a forest in order to collect fungi, they can easily find large mushrooms, but it is difficult for them to find a corticioid fungus. This is because the corticioid fungi largely live a hidden existence at the lower sides of dead branches lying on the floor of the forest. Most of these fungi are capable of breaking down cellulose and lignin and have been accepted as a saprophyte because they fruit on dead wood of fallen branches, logs, or barks and so on. However, in many cases, the association of a basidiome with dead wood is the only evidence that they are saprophytic. Many corticioid species decay wood of trees that are already dead, but some are specialized to enter wounds in living trees and make their way to the heartwood. In the wound of the tree, a number of organisms such as bacteria, yeasts and conidial fungi precede the secondary aphyllophoralean wood decayers. However, many trees compartmentalize the rot so that microorganims do not invade other parts.

The species decaying wood cause either a white rot or a brown rot. In general, white rot fungi degrade cellulose and hemicellulose at an approximately same rate and

the lignin is usually degraded at a faster rate. That resulted in bleaching the wood to white or light colors. The brown rot fungi attack almost exclusively the cellulose and give the wood a brown color. The infamous dry rot of buildings is a brown rot caused by several fungi. Two of well-better known dry rot species are *Coniophora puteana* and *Serpula lacrymans* (Ginns and Lefebvre, 1993). The vast majortiy of the corticioid fungi are white rot fungi. Only a few genera and species are brown rot fungi and, of these, almost all fungi attack or live on coniferous wood (Hjortstam *et al.*, 1987). Brown rot genera in the corticioid fungi contain following ones; *Coniophora, Columnocystis, Crustoderma, Dacryobolus* and *Pseudomerulius* (Gilbertson, 1981).

The ecological roles of corticioid species are more diverse than saprophytism. Some are parasitic, causing a variety of diseases from root rot to leaf blights. *Rhizoctonia* root is caused by *Thanatephorus cucumeris* (best known under its anamorph name, *Rhizoctonia solani*) and violet root rot is caused by *Helicobasidium brebissonii* (anamorph name *R. crocorum*). Species in several corticioid genera such as *Athelia, Botryobasidium, Ceratobasidium, Thanatephorus, Uthatobasidium* and *Waitea* form *Rhizoctonia* anamorphs. *Chondrostereum purpureum* causes the notorious silver leaf disease of fruit trees (Ginns and Lefebvre, 1993). Some speices occur on the bedlog of shiitake (*Lentinula edodes*) and reduce fruiting body yield (Maekawa and Arita, 1984). And the most economically important corticioid fungi were *Echinodontium tinctorium, Stereum sanguinolentum* and *Scytinostroma galactinum* (Hepting, 1971). Several genera, particularly *Aleurodiscus, Corticum* and *Dendrothele,*

occupy xeric habitats, typically the bark of living trees, but in most cases do not penetrate to the cambium or cause injury to the tree (Ginns and Lefebvre, 1993).

Several species are excellent examples of how fungi can be utilized by man. *Phlebiopsis gigantea*, which in nature fruits on old conifer logs, has been used on a commercial scale as a biological control agent to prevent *Heterobasdion annosum* causing annosus root rot from entering freshly cut conifer stumps and spreading into live trees (Deacon, 1997). The antibiotic merulidial, produced by *Phlebia tremellosus*, suggests that other useful compounds will be found when this group is better studied (Quack *et al.*, 1978). *Phanerochaete chrysosporium* has been extensively used in studies: 1) on the enzymatic activity in decaying wood, especially in lignin degradation; 2) on the biopulping of wood; and 3) on the bioremediation of contaminated water and soil (Ginns and Lefebvre, 1993)

3. Taxonomic Problems in the Corticioid Fungi

Corticioid fungi (Hymenomycetes, Basidiomycotina) were described by Persoon at Classi secunda Gymocarpi, Ordo quintus Hymenothecii. Fries treated the corticioid fungi as a tribe Resupinatus of the genus *Thelephora* (Jülich, 1981). Patouillard who first broke down the Friesian classification considered the genus as a taxon with smooth or toothed hymenophores (Jülich, 1981). In his classfication system, the

corticioid fungi were distributed through the three Series of two Tribu in Aphyllophorales. The corticioid fungi were later used in uniting holobasidiomyceteous fungi with usually resupinate basidomata and smooth hymenium without tubes, gills or spines. After emphasis had been laid on microscopic structures, the traditional Thelephoraceae became split all over into the Corticiaceae, the Cyphellaceae, the Stereaceae and the Thelephoraceae. Strictly defined Thelephoraceae is mainly characterized by its brownish spores which are ornamented and either globose or irregularly lobbed and by the thelephoric acid which has been found in nearly all the species studied yet (Donk, 1964).

Donk (1964) put previous taxonomic works together and suggested a completely new classification of the Aphyllophorales comprising of 23 modern families instead of 5 groups of the Friesian system. Most taxonomists working in that field have accepted Donk's work. In Donk's system, hymenial configuration was no longer accepted as a familial character, but microscopic characters such as the mitic system, spore morphology, basidium morphology and sterile elements were emphasized in defining new families. Although Donk's work greatly improved the taxonomy of the Aphyllophorales, several families remained artificial and wanted revisions.

The corticiaceae has been called an artificial family, a chaotic mass or an artificial taxon but an assembalge of species with similar habit (Hjortstam *et al.*, 1987). Several attempts have been made to delimit monophyletic groups of corticioid taxa from other alien members and, over the years, many such genera were described by Boidin,

Eriksson, Hjortstam, Parmasto and other mycologists. In these early studies, primary attention was given to identifying differences between the new genera and the old ones. Much less interest has been directed toward understanding how the genera are related with each other.

In another general system for Basidiomycetes, Jülich (1981) placed the corticioid fungi into 15 orders and 42 families. This system was severely criticized for some inconsistencies and statements that were not supported by arguments and for noncritical devaluation of taxonomic categories (Parmasto, 1985; Ryvarden, 1991). Jülich's classification has been used by some mycologists, sometimes with modifications (Ginns and Lefebvre, 1993; Krieglsteiner, 1991). At present, there is no generally acceptable taxonomic system for corticioid fungi and, in most papers dealing with this group, the genera are in alphabetical order and any attempt for hierarchical classification is avoided.

A proper placement of corticioid fungi within a more general classification of hymenomycetes raises a theoretical question. Two different viewpoints have prevailed concerning the origins of corticioid fungi (Parmasto, 1986). According to one view, the corticioid fungi are the most primitive family of Hymenomycetes (Parmasto, 1968, 1986, 1995). Although many other families are also claimed to be primitive, this viewpoint is not in accordance with present-day knowledge of macroevolution that suprageneric taxa may not be developed by other suprageneric taxa but by speciation from species. As a result, there is no such thing as a primitive taxon. According to

another hypothesis, the primitive hymenomycetes had clavarioid and cantharelloid basidiomata with inflating hyphae and thickened hymenium (Corner 1954, 1989; Jülich 1981) or gastroid basidiomata (Kreisel, 1969). The corticioid habit arose through secondary simplification (reduction) numerous times. Based on this view, the corticioid fungi is a polyphyletic group that has been repeatedly derived by reduction (Corner, 1991).

4. Molecular Phylogenetics

4-1. Ribosomal RNA genes as a tool of systematics

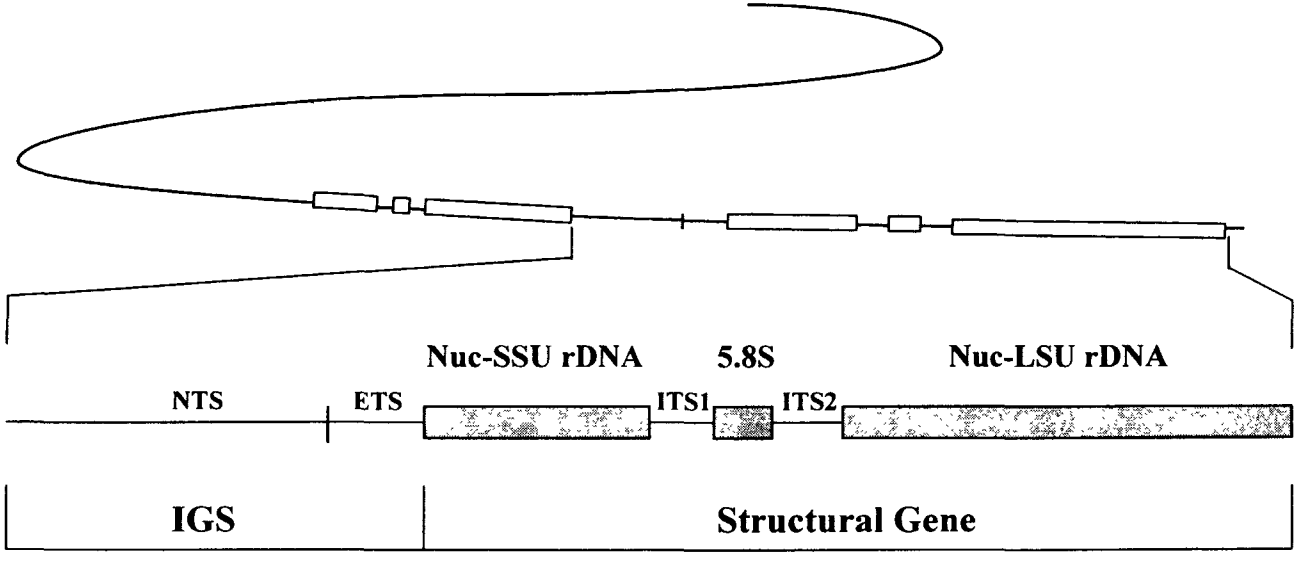
Molecular techniques are becoming more important than ever as means for studying taxonomic and phylogenetic relationships among fungi. Molecular phylogeny is based on the hypothesis that sequences of molecules vary constantly in all lineages and can represent the history of molecules and organisms. Therefore, such a molecular sequence is called a molecular clock. To be a useful molecular clock, following specifications must be satisfied: 1) the molecule should evolve at an approximately same rate through all the lineages examined, 2) the molecule has to have the same function in all taxa, 3) the molecule should evolve at an appropriate rate for the comparison of interest, 4) the size of the molecule has to be large enough to provide an

adequate amount of information and 5) the molecule has to be present only once in a genome or behaves like a single copy region (Mitchell *et al.*, 1995; Woese, 1987).

The ribosomal RNA gene is a very old ancient gene, which all organisms have. Ribosomal RNA gene forms a mosaic pattern of conserved and variable regions that make taxonomic analysis possible at many levels. The rDNA found in the nuclear genome of eukaryotes generally consists of tandem repeated units. The rDNA unit usually consists of external transcribed spacer (ETS), small subunit rDNA, internal transcribed spacer 1 (ITS1), 5.8S rDNA, internal transcribed spacer 2 (ITS2), large subunit rDNA and non-transcribed spacer (NTS) (Fig. 1.1). It is generally considered that the rDNA arrays tend to be homogenized through the mechanism called concerted evolution (Hillis and Dixon, 1991). The mechanism of concerted evolution is not certain, although models such as unequal crossing-over and gene conversion have been proposed to explain the homogenization of the repeated units (Li *et al*, 1985).

Regions most commonly used for phylogenetic analyses are nuclear or mitochodrial small subunit rDNAs and large subunit rDNAs, internal transcribed spacers (ITSs) that occur between coding regions for nuclear small and large-subunit rRNAs, and intergenic spacers (IGS) (Moncalvo *et al.*, 1995; Zambino and Szabo, 1993). The nuclear small-subunit rRNA region is appropriate for analysis at or above the level of orders (Berbee and Taylor, 1992). Nuclear and mitochondrial large-subunit rRNA regions may be useful within and among species and genera of fungi (Bruns *et al.*, 1990). As noncoding portions may be more variable than coding regions (White *et*

Figure 1.1. Structure of regions encoding ribosomal RNA genes. The rRNA gene clusters consist of tandem repeated units. The rDNA unit usually consists of non-transcribed spacer (NTS), external transcribed spacer (ETS), Nuc-SSU rDNA, internal transcribed spacer 1 (ITS1), 5.8S rDNA, internal transcribed spacer 2 (ITS2) and Nuc-LSU rDNA. Transcribed regions are indicated by bars and internal or non-transcribed spacer regions by lines.



Ribosomal RNA gene cluster

al., 1990), they are appropriate for analysis of closely related species of fungi (Zambino and Szabo, 1993) and the IGS can be used for race identification in some fungal species (Moncalvo *et al.*, 1995).

4-2. Molecular phylogenetic studies on corticioid fungi

As there have been no comprehensive studies of the entire corticioid group, there is no generally accepted system of higher ranks for this group. Only fragmentary phylogenetic studies have been accomplished on some specific corticioid taxa. The most taxonomically inclusive phylogenetic study in homobasidiomycetes done so far is that of Hibbett and Donoghue (1995) that was based on mt-ssu rDNA sequences. In their analyses, only 6 corticioid species were included and one of their conclusions was that there was considerable homoplasy in morphological characters. After that, to draw conclusions on the importance of certain morphological characters in the practical delimitation of corticioid genera, phylogenetic studies based on morphological data and partial nuc-lsu rDNA sequence data were performed by Hallenberg and Parmasto (1998). However, their conclustions were almost same of Hibbett and Donoghue (1995). Boidin *et al.* (1998) tried to evaluate the phylogenetic relationships within the order Aphyllophorales using internal trasncribed spacer (ITS1-5.8S-ITS2) sequences. And they classified and revised the Aphyllophorales into twenty orders, creating five new orders; Peniphorales, Phlebiales, Podoscyphales, Coriolales and Vuilleminiales

(Boidin *et al.*, 1998; Boidin and Korf, 1998). Their results showed that the Aphyllophorales represented different independent lineages and that traditional classifications were not supported with some exceptions like the Hericiales with amyloid spores. The corticioid fungi were also scattered among the phylogenetic tree in their study. However, ITS regions are known to evolve rapidly, so they are not thought to be appropriate in study of higher relationships of fungi. *Peniphora*, a well-known genus of corticioid fungi, was studied phylogentically by Hallenberg *et al.* (1996) on the basis of ITS sequences. Lignin-degrading corticioid fungi, *Phlebia* and *Phaenerochaete*, based on RFLP analysis of nuc-ssu rDNA and ITS region were perfomed by Dresler-Nurmi and his colleagues (1999). There are another molecular works accomplished for some corticioid fungi; Aphyllophorales by Kim and Jung (2000), Coniophoraceae by Gardes and Bruns (1996), ectomycorrhizal basidiomycetes by Bruns *et al.* (1998), Corticiaceae by Lee and Jung (1998) and Stereaceae by Yoon *et al.* (2001).

Recently, most taxonomically inclusive phylogenetic study in homobasidiomycetes was achieved by Hibbett and Thorn (2001). Referring to the results of recent molecular studies, they tentatively divided the homobasidiomycetes into eigth major clades. Corticioid forms occurred in each of eight major clades. However, because of uncertain higher level relationships, limited sampling of taxa and choice of inappropriate molecules, their delimination and phylogenetic placements of corticioid fungi were not clear as much as expected.

4-3. Phylogenetic methods

Methods of DNA sequences analyses can be categorized as three groups. These three methods are all implemented in PAUP*4.0b4a (Swofford, 1999). In the following, each method is briefly explained.

4-3-1. Distance methods

Distance methods are algorithmic in their approach and calculate pairwise distances between taxa for seeking phylogenetic trees. In calculating pairwise distances, simple dissimilarities or evolutionarily adjusted distances (Jukes and Cantor, 1969; Kimura, 1980) are calculated. Merits of distance-based methods are that they produce an exact tree and require relatively short time to perform overall analysis. Total analysis time increases only slightly when many new taxa are added. Several tree-building algorithms have been developed in seeking phylogenetic trees from pairwise distances. UPGMA (Sokal and Michener, 1958), Fitch and Margoliash method (Fitch and Margoliash, 1967), neighbor-joining method (Saitou and Nei, 1987) and distance Wagner method (Farris, 1972) are examples of currently favored tree-building algorithms.

4-3-2 Parsimony methods

The governing assumption of parsimony methods-based trees is that a true tree is the one that requires the fewest number of mutational changes to explain the differences observed between the gene sequences (Priest and Austin, 1993). The score for each tree is based on the minimum number of changes in character states that are required to explain the data. Parsimony methods are described as character state methods to distinguish them from those based on distance methods sinch they use raw data, rather than estimates of distance or similarity, and the concept of informative sites. Several different parsimony criteria, for example, Wagner parsimony, Fitch parsimony, Dollo parsimony, Calmin and Sokal parsimony, and generalized parsimony, exist according to the constraint placed on given situation (Forey *et al.*, 1992; Hillis *et al.*, 1996).

4-3-3. Likelihood methods

Likelihood methods are an statistical approach and find out a tree with the highest probability from a given data set and model of sequence evolution (Felsenstein, 1988). Recently, it is reported that the maximum likelihood method is the best of the three (Huelsenbeck, 1995). Likelihood methods have the advantage of both character-based parsimony and model-based distance methods. Another advantage of likelihood methods is that a statistical test called the likelihood ratio can be used to evaluate many

properties of tree (Huelsenbeck, 1995; Kishino and Hasegawa, 1989). However, owing to the enormous calculation time, the method is not routinely used when inferring trees from data sets. Therefore, likelihood searches are usually practical with only relatively small numbers of sequences, even when using heuristic search strategies. Instead, the method is frequently used when statistical comparison of trees with different topologies is required (Kishino and Hasegawa, 1989).

4-3-4. Robustness of internal branches

When developing taxonomic discussions from given phylogenetic trees, it is the statistical support on each branch of the tree rather than the tree topology itself that has any real significance. Many concepts and methods have been developed to evaluate statistical supports on branches in a given tree. The most widely used method is the bootstrap (Felsenstein, 1985). Bootstrap analysis is a resampling tree evaluation method that works with distance, parsimony, likelihood and any other tree deviation method. Originally the value over 95% consensus was suggested as a significant one, but later simulation studies have reported that the branch nodes are significant when received above 70% supports (Hillis and Bull, 1993). Although the significance of bootstrap value varies according to the data set and the method of phylogenetic inference, a bootstrap value of 70-80% is often taken to indicate strong support for a cluster of sequences.

CHAPTER 2

Annotated List of the New Corticioid Fungi to Korea

Introduction

In the last century, taxomony of the corticioid fungi was based on their hymenial configuration. Around the turn of the 20th century, micromorphological characters were introduced to delimit taxa of the corticioid fungi. Since then, with the advance of microscopical studies, many new genera and species have been reported mainly from Europe and North America. A modern taxonomic framework of corticioid fungi was constructed by Eriksson (1958), Donk (1964) and Parmasto (1968). Subsequently, Parmasto (1986) transferred many heterogeneous genera to other families and proposed a more restricted concept of Corticiaceae: basidiomata effused, rarely effused-reflexed; hymenial surface smooth, merulioid, granular, rarely toothed or poroid; hyphal system monomitic; basidia aseptate (holobasidia); basidiospores non-repetitious, non-budding and non-septate. Many mycologists have basically accepted this restricted concept. The number of previously known corticioid fungi based on the Parmasto's concept is more than 1,100 species, which distribute among about 170 genera (Maekawa, 1993).

The corticioid fungi of Korea are still very poorly known and are believed that only a small part of the existing fungal biodiversity has been discovered. Many of Korean corticioid fungi have been reported by Jung (1994-1996b) and, subsequently, by Lim and Jung (1998b-2000). The total number of species of the corticioid fungi (mainly from the family Corticiaceae) reported in Korea is 83, assigned in 39 genera and 7

families. This study is aimed to provide descriptions, illustrations and phylogenetic notes for the new corticioid and poroid fungi in Korea. A few genera that had poroid basidiomata like *Ceriporia, Gloeoporus* and *Schizopora* were treated together because they are taxonomically related to the corticioid fungi based on morphological or molecular characters and it is logical that they need to be included here for a comprehensive study. Those fungi are presented in alphabetical order of species names regardless of their taxonomic ranks.

Materials and Methods

Fresh matrerials were collected from the field and fruitbody morphology, color, hymenial surface and marginal zone were noted after taking photographs on the spot. Collected materials were brought to the laboratory and completely dried over mild heat up to several days. Dried specimens were deposited in the Seoul National University Fungus Collections (SFC).

Speciemens were observed by naked eyes or under a stereomicroscope. In describing color names, the color book of Kornerup and Wanscher (1978) was used. The measurements and drawings were made from slide preparations mounted with KOH for 3% potassium hydroxide (Hawksworth *et al.* 1995) and stained with aqueous phloxine or cotton blue (Largent *et al.*, 1977). An iodine reaction was most commonly

Table 2.1. Staining reagents used in this study.

Reagent	Formula, methods and reaction results
Water	
	Use : For ascertaining the true color of spores and other observing structures which are dissolved or altered by alkalis or acids
KOH	
	Formula: Potassium hydroxide 3-5% in water in 95-97 ml water
	Use : For soaking preparations made from dried specimens, liquefying or soaking hyphae, cystidia, etc., altering shape and color, clearing dark structure, also specifically for macroscopic color reactions e.g. in polypores, corticioids and Ramarias Positive – permanent dark reaction
Phloxine	
	Formula: 1% aqueous solution
	Use : Phloxine is taken up by the diffused cytoplasm into hyphae and used to stain the interior of hyphae. Positive – red color reaction
Sulphovanillin (Sulfovanillin)	
	Formula: 25g vanillin, 2ml conc. sulphuric acid and 2ml water
	Use : For coloring the contents of gloeocystidia. Positive – black reaction
Cotton blue	
	Formula: 0.1% cotton blue in 60% lactic acid
	Use : For staining principally cell contents and certain spore ornaments Cyanophililous – cell wall or spore ornamentation are stained dark purple and distinctly contrasted against backgrounds

Reagent	Formula, methods and reaction results

Melzer's reagent

Formula: 0.5g iodine, 1.5g KI, 22g chloral hydrate and 20 ml water

Use : For determining the amyloidity or dextrinoidity of spores, basidia, cystidia or pieces of tissue

Amyloid – blue to black reaction (positive)

Dextrinoid (Pseudoamyloid) – brownish to reddish brown reaction

Nonamyloid – yellow to hyaline reaction (negative)

noticed using the Melzer's reagent (Gilbertson and Ryvarden, 1986). The staining reagents used in this study and their reaction results are listed in Table 2.1. Microscopic examinations of specimens were observed with an optical microscope at magnification of 100X, 400X and 1000X.

Morphological Characters of the Corticioid Fungi used in This Study

From a taxonomical point of view, the corticioid fungi are not certainly a well-defined group. They have, however, certain characters of gross morphology in common. The fruitbody is not greatly differentiated and is appressed and attached to the substratum. The margin is rarely free or even recurved. But their microscopic features are various depending on genera. Specialized literatures of Breitenbach and Kränzlin (1986) and Hjortstam *et al.* (1987) are available for the most striking macro- and microscopic structures. Authors' names are often abbreviated following the lists of Ginns and Lefebvre (1993) and Breitenbach and Kränzlin (1986) (Table 2.2). Microscopic structures like basidia, basidiospores and cystidia used in this study are represented in Figs. 2.1-2.2.

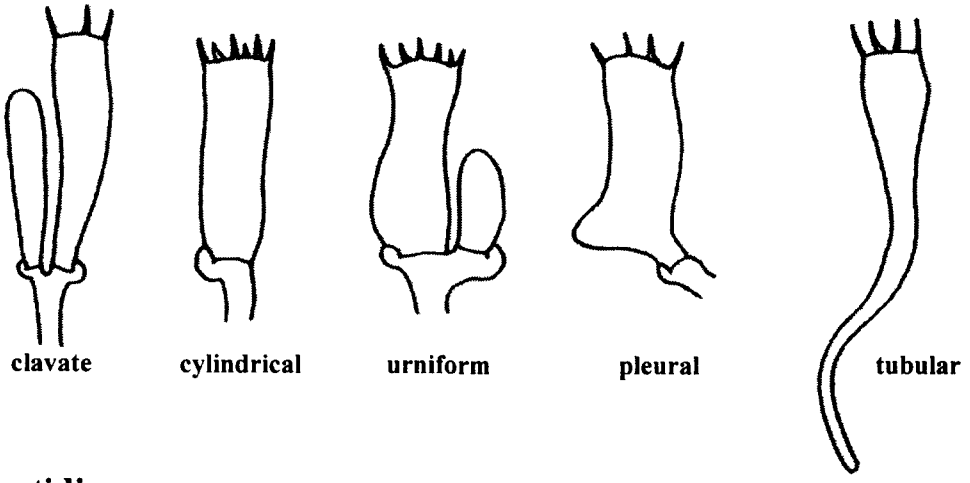
Table 2.2. Abbreviations for authors of fungal names.

Abb[a]	Name	Abb	Name
Alb.	J.B.A. Albertini	Imaz.	Imazeki
Berk.	M. J. Berkeley	Jül.	W. Jülich
Boid.	J. Boidin	Karst.	P.A. Karsten
Bourd./B.	H. Bourdot	Larsen	M.J. Larsen
Bres.	G. Bresadola	Lund.	S. Lundell
Cke.	M.C. Cooke	Mass.	G.E. Massee
Curt.	M.A. Curtis	Oberw.	F. Oberwinkler
Ell.	J.B. Ellis	Parm.	E. Parmasto
Erikss.	J. Eriksson	Pat.	N.T. Patouillard
Fr.	E.M. Fries	Pers.	C.H. Persoon
Galz./G.	A. Galzin	Pouz.	Z. Pouzar
Gilbn.	R.L. Gilbertson	Ryv.	L. Ryvarden
Hjortst.	K. Hjortstam	Schw.	L.D. Schweinitz
Hol.-Jech.	V. Holubova-Jechova		

[a] abbreviations

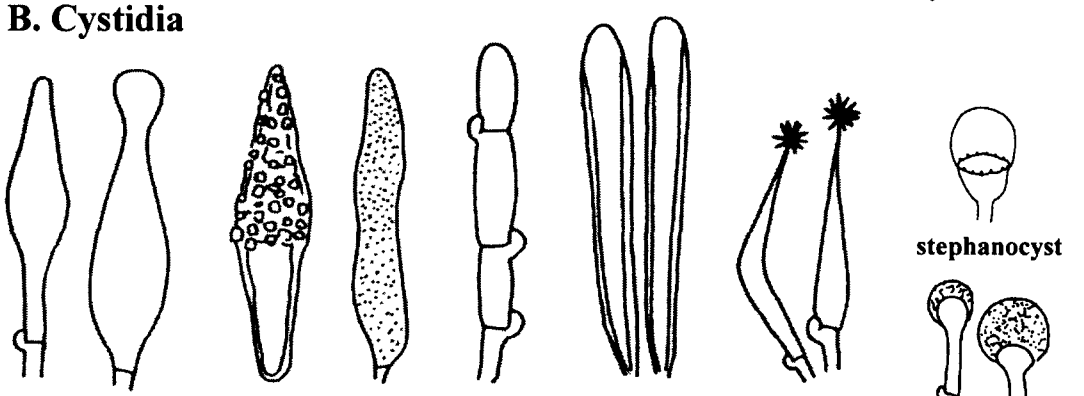
Figure 2.1. Microscopic structures of basidia, cystidia and sterile hymenial elements observed in corticioid fungi.

A. Basidia



clavate cylindrical urniform pleural tubular

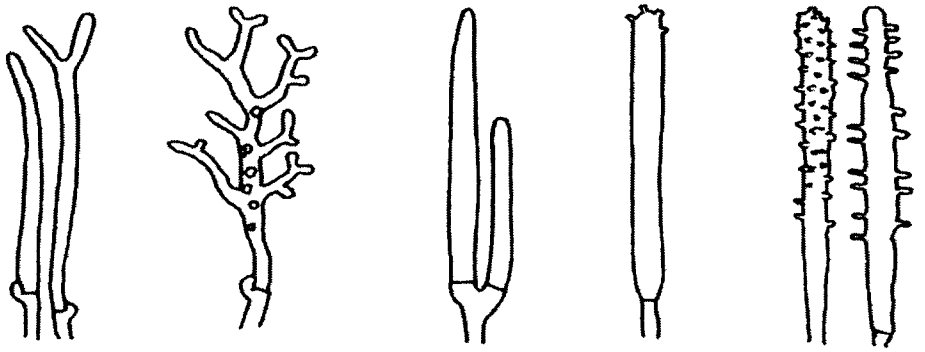
B. Cystidia



fusiform- capitate- lamprocystidia septocystidia asterocystidia stephanocyst

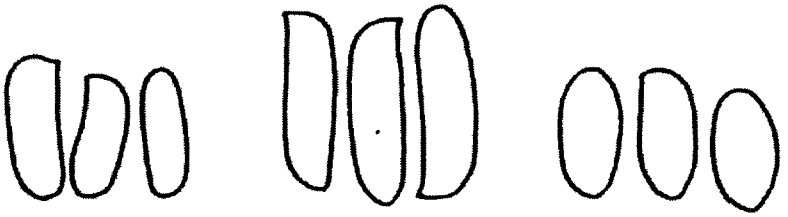
leptocystidia gloeocystidia pseudocystidia halocystidia

C. Sterile hymenial elements



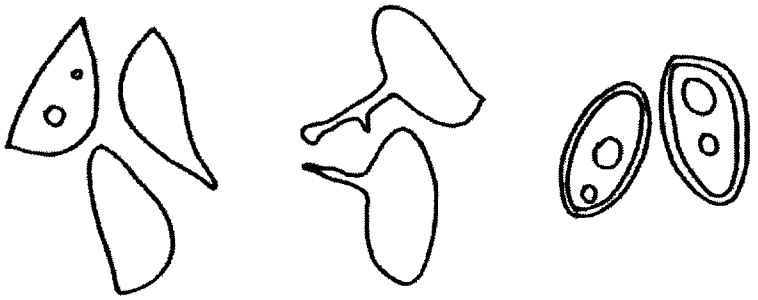
Paraphysoid Dendrohyphidia simple hyphidia pseudoacantho-hyphidia acantho-hyphidia

Figure 2.2. Various microscopic types of basidiospores.

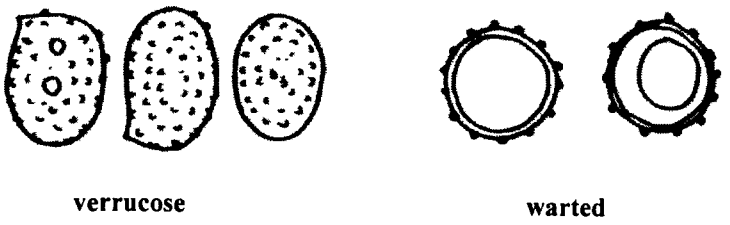


allantoid cylindrical ellipsoid

ovoid subglobose globose



navicular repetetive thick-walled



verrucose warted

Annotated List of Corticioid Fungi to Korea

1. *Australohydnum dregeanum* (Berk.) Hjortst. & Ryv.

Basidiomes resupinate on the underside of the substrate, semipileate to peleate on vertical and laterally extending substrates, imbricate, up to 6 cm wide, up to 2.5 cm long; upper surface hairy, hispid from tomentum, violate when fresh and brown when old, zonate; hymenophore merulioid, daedaloid to reticulate-porose, white to cream-colored.

Hyphal system dimitic; generative hyphae hyaline in KOH, simple-septate, thin-walled, frequently branched, 3.4-5.14 μm thick; skeletal hyphae predominant, sinuous to straight, frequent branched, thick-walled, 5-8 μm thick; cystidia conspicuous, abundant, thick-walled, heavily incrusted like lamprocystidia, 39-102 × 6.2-9.2 μm; basidia clavate, with 4 sterigmata, 25-31 × 5.1-6.9 μm, simple-septate at the base; basidiospores elliptical, smooth, hyaline, 5-6 × 3.1-3.7 μm, non-amlyoid.

Specimens examined: Dead wood of *Quercus*, Chungpyoung, Kyonggido (SFC 980804-04); dead wood of *Quercus*, Kangwha Island (SFC 981115-09).

Remarks: Macroscopically, this species resembles *Punctularia strigoso-zonatus*, but the latter species has smooth hymenophore. Conspicuously incrusted cystidia and simple-septate hyphae are the diagnostic characters of *A. dregeanum*. The phylogenetic position of this species is the *Phanerochaete* group in the polyporoid clade.

2. *Botryobasdium medium* Erikss.

Basidiomes fully resupinate, closely appressed to the substrate; margin thin, filamentous; hymenophore smooth, farinose, light-ochre; consisteny soft.

Hyphal system monomitic, hyphae thick-walled, 4-9.7 μm across, occasionally with multiple clamps, light brownish, occasionally incrusted; cystidia not seen; basidia not observed; basidiospores navicular, biapiculate, smooth, hyaline, 8.5-9.14 × 4.5-5.1 μm, non-amyloid, cyanophilic.

Specimens examined: On the fallen branch of a deciduous tree, Ullung Island (SFC 900807-05).

Remarks: This species is characterized by ochraceous hymenophore and occasional septa with multiple clamps. Its phylogenetic placement is thought to be in the botryobasidioid clade.

3. *Ceriporia purpureum* (Fr.) Donk

Basidiomes resupinate, tightly attached to the substrate, pale to pinkish when fresh, purple to dark brownish purple with age; hymenophore poroid, 4-5 per mm, soft in KOH; margin white, finely tomentose, narrowly sterile.

Hyphal system monomitic without clamps, 2.2-4.0 μm thick, some lightly incrusted; cystidia absent; basidia clavate, with 4 sterigmata, 17-21 × 5.7-6.3 μm; basidiospores allantoid, hyaline, 5.7-6.5 × 1.8-2.3 μm, non-amyloid.

Specimens examined: On the fallen branch of a dead hardwood, Mungyong,

Kyongbuk (SFC 990505-02); On the fallen branch of a dead hardwood, Chungpyoung, Kyonggi (SFC 961227-19); On the fallen branch of a dead hardwood, Kumak, Kangwon (SFC 951007-13, SFC 951007-14).

Remarks: This species is macroscopically recognized by the purple color and characterized by the waxlike texture becoming soft in KOH. Phylogenetic analysis revealed that *Ceriporia* is closely related to the *Irpex* group.

4. *Coniophora* sp.

Basidiomes fully resupinate, tightly attached to the substrate; hymenophore smooth, ochre to yellowish brown; margin lighter, finely filamentous; consistency membranous.

Hyphal system monomitic, thin-walled, hyaline to yellowish, 2.5-5.7 μm thick without clamps, rarely up to 14 μm thick with scattered multiple clamps; cystidia not seen; basidia sinuous, clavate, 4 sterigmata, 42.8-45.7 × 9.7-10.3 μm, simple-septate at the base; basidiospores elliptical, smooth, light brown, thick-wall, frequently with oil drops, 10.2-14.2 × 5.7-8.5 μm, non-amyloid, dextrinoid.

Specimens examined: On the underside of barkless *Pinus densiflora* lying on the ground, Kangchon, Kangwondo (SFC 20000928-23); on the underside of barkless *Pinus koraiensis* lying on the ground, Kangwha Island (SFC 991008-30).

Remarks: The species described here has practically same macro- and microscopic features as those of *Coniophora arida*. But basidia were smaller than those of *C. arida* and the genetic distance of ITS region between two species was very long up to

15.52% (Chapter 3, V). This species belongs to the bolete clade.

5. *Cytidiophorus castaneus* (Lloyd) Imaz.

Basidiomes fully resupinate, tightly attached to the substrate; hymenophore merulioid, irregularly labyrinthine-reticulate, orange to gold-brown; marginial zone narrow, sterile, smooth; consistency fibrous when fresh, strongly tight, tough when old.

Hyphal system dimitic; generative hyphae simple-septate, thin-walled, 2.3-4.0 μm thick; skeletal hyphae predominant, straight, thich-walled, up to 6 μm thick; lamprocystidia conspicuous, abundant, thick-walled, upper half heavily incrusted, 30-46 (12-20) × 9-11 μm (incrusted part); basidia clavate, 4 sterigmata 18-20 × 3.4-5.1 μm, simple-septate at the base; basidiospores allantoid, smooth, 6.3-7.5 × 2.5-3 μm.

Specimens examined: On a dead branch of conifer wood, mainly *Pinus densiflora*, Kyongbuk (SFC 980119-02); on dead branches of conifer wood, mainly *Pinus densiflora*, Munkyong, Kyongbuk (SFC 990326-07, SFC 991003-01, SFC 991120-02); on a fallen trunk of conifer wood, mainly *P. densiflora*, Kangwha Island (SFC 981128-15).

Remarks: This species is similar to a resupinate form of *Leucogyrophana* which lacks incrusted lamprocystidia and has hyphae with clamps. *Cytidiophorus castaneus* is also reminiscent of *Phanerochaete* by simple-setate hyphae and lamprocystida. In the phylogenetic analysis, this species is grouped into the *Phanerochaete* group of the polyporoid clade.

6. *Cytidia salicina* (Fr.) Burt

Basidiomes resupinate, becoming cupuliform, looking like a discomycete, coriaceous to gelatinous, hard and brittle when dry; hymenophore orange red to violaceous red when fresh; margin concolorous with the hymenial surface.

Hyphal system monomitic; hyphae thin-walled, mostly straight, rarely curved 1.7-3.5 μm thick; cystidia absent; dendrohyphidia numerous, richly branched, forming a dense layer on the hymenial surface, up to 1.5-3 μm thick; basidia narrowly clavate, very long, with 4 curved sterigmata, 80-90 × 8.5-11 μm, with a basal clamp; basidiospores allantoid, smooth, 13.7-19.5 × 4-5.5 μm, non-amyloid, some forming secondary spores.

Specimens examined: On attached twigs and branches of *Salix* species, Kwangduk Mt., Kyonggido (SFC 971231-06).

Remarks: In dry condition, this fungus is easily confused with *Exidia* species, but the distinguishing features of *C. salicina* are reddish color, discoid, coriaceous to gelatinous basidiomata, large basidia, large spores and dentrohyphida. Its phylogenetic position is in the laticorticioid clade.

7. *Dacryobolus karstenii* (Bres.) Oberw. & Parm.

Basidiomes fully resupinate, becoming confluent, membranous to coriaceous, up to 3 mm thick; hymenophore smooth, white to dark cream; margin abrupt.

Hyphal system dimitic; generative hyphae thin-walled, hyaline, 1.7-2.8 μm thick with clamps, some hyphae thick-walled and sparsely septated; leptocystidia very long, 50-85 × 8.5-10 μm, protruding up to 50 μm, apically thin-walled, gradually thickening toward the base; basidia narrowly clavate, with 4 sterigmata, 35-45 × 2.3-3.2 μm, with a basal clmap; basidiospores allantoid, smooth, 5.5-6.8 × 1.4-1.7 μm, non-amyloid.

Specimens examined: On the underside of barkless *Pinus densiflora* lying on the ground, Myongji Mt., Kyonggi (SFC 971006-13); on the underside of barkless *Pinus densiflora* lying on the ground, Kangwha Island (SFC 970710-26); on the underside of barkless *Pinus densiflora* lying on the ground, Munkyong, Kyongbuk (SFC 990326-03, SFC 990422-22); on attached branches of barkless *Pinus densiflora*, Chunkwan Mt., Chunnam (SFC 990729-16); on the underside of barkless *Pinus densiflora*, Bangtae Mt., Kangwon (SFC 990914-11); on a dead *Prunus sargentii*, Kwanak Mt., Seoul (SFC 980929-17).

Remarks: *Dacryobolus karstenii* is characterized by having long and thick-walled leptocystidia, smooth resupinae basidiocarps on dead or fallen branches of coniferous trees. This species is also associated with a brown cubical rot (Ginns and Lefebvre, 1993). Its phylogenetic position is nested in the polyporoid clade and showed that this species was closely related to brown rot fungi such as *Fomitopsis*, *Antrodia* and *Daedalea*.

8. *Dendrocorticium violaceum* Larsen & Gilbn.

Basidiomes resupinate, effused-relexed, fibrous, thick; hymenophore smooth, violaceous, fading in time; margin distinct, without rhizomorphs.

Hyphal system monimitic; generative hyphae thin-walled, hyaline, 2.8-3.4 μm thick with clamps; cystidia none; dendrohyphidia numerous, richly encrusted; basidia tubular, with 4 sterigmata, 40-45 × 6.8-8.6 μm, with a basal clmap; basidiospores ellipsoid, smooth, 7.7-8.6 × 4.9-5.5 μm, non-amyloid.

Specimens examined: On dead fallen trunks and branches of *Betula* and *Quercus*, Myongji Mt., Kyonggi (SFC 970823-19).

Remarks: This species is macroscopically similar to *Phanerochaete crassa* but is distinguished by microscopic characters. Its phylogenetic placement is in the laeticorticioid clade.

9. *Dendrocorticium* sp. No. 1

Basidiomes fully resupinate, tightly attached to the substrate, thick; hymenophore smooth, pale pinkish, fading in time; margin rhizomorphic.

Hyphal system monimitic; generative hyphae thin-walled, hyaline, 2.8-3.6 μm thick with clamps; cystidia none; dendrohyphidia numerous, richly encrusted; basidia tubular, with 4 sterigmata, broad in the middle of base (probasidium), 82-95 × 8-9 μm, with a basal clamp; basidiospores ellipsoid, smooth, 12-14 × 7.8-9.1 μm, non-amyloid.

Specimens examined: On a dead fallen trunk of *Zelkova serrata*, Myongji Mt., Kyonggi (SFC 990326-29); on a dead fallen trunk of *Acer*, Kwangduk Mt., Kyonggi

(SFC 971231-7, SFC 960127-10).

Remarks: This species is characterized by large probasidia, spores and pinkish resupinate fruitbodies with marginal rhizomorphs. These characters were distinctive and not congruent with others of *Dendrocorticium* or *Laeticorticum*. However, molecular data revealed that this species was closely related to *D. violaceium* and their ITS sequence similarity was almost the same. Therefore, this species is thought to be a new species or cryptic species of *D. violaceium*.

10. *Dendrocorticium* sp. No. 2

Basidiomes fully resupinate, tightly attached to the substrate; hymenophore smooth, violaceous; margin distinct.

Hyphal system monimitic; generative hyphae thin-walled, hyaline, 2-4 μm thick with clamps; cystidia none; dendrohyphidia numerous, slightly encrusted; basidia tubular, with 4 sterigmata, 57-95 × 8-13 μm, with a basal clmap; basidiospores subglobose to globose, smooth, thin-walled, 15.4-18 × 12-14 μm, non-amyloid.

Specimens examined: On dead fallen trunks of *Larix leptolepis*, Kangwha Island (SFC 990527-09).

Remarks: This species is characterized by large basidia and spores. Phylogenetic position is in the laeticorticioid clade. Its distinctive morphological characters and molecular data showed that it might be another new species of *Dendrocorticium*.

11. *Dentipellis fragillis* (Pers. : Fr.) Donk

Basidiomes fully resupinate, tightly attached to the substate; hymenophore thin, membranous, densely crowded with fine spines up to 1 cm long, white to cream, soft, fragile when dry.

Hyphal system monimitic; generative hyphae thin-walled, hyaline, up to 4 μm thick with clamps; gloeocystidia cylindrical, 28-43 × 6.3-7.4 μm; dendrohyphidia occasionally present; basidia clavate, with 4 sterigmata, 31-37 × 6-8 μm, with a basal clamp; basidiospores subglobose to globose, smooth, with one large oil drop, 5.1-6.4 × 5.0-6.2 μm, amyloid.

Specimens examined: On a dead hardwood, Daemo Mt., Seoul (SFC 980929-17, SFC 970830-09).

Remarks: Resupinate fungi with spinose hymenophores are found in diverse genera like *Mycoacia, Hyphodontia* and *Steccherinum* and so on. Spore amyloidity is reminiscent of the russuloid clade. In the study of Boidin *et al.* (1998), this species was grouped in the order Hericales, which is included in the russuloid clade. However, this specimens (SFC 970830-09) was grouped in the euagarics clade and closely related to *Mycoacia copelandii*.

12. *Gloeocystidiellum porosum* (Berk. & Curt.) Donk

Basidiomes fully resupinate, tightly attached to the substrate, thin, membranous; hymenophore smooth, white to cream, waxy, soft.

Hyphal system monomitic; generative hyphae thin-walled, hyaline, 2.8-4 μm thick with clamps; gloeocystidia cylindrical to fusiform, abruptly narrowing in the middle of apex, 64-71(up to 150) × 8.5-12 μm; basidia clavate, with 4 sterigmata, 26-28 × 3.4-4 μm, with a basal clamp; basidiospores elliptical, finely verrucose, hyaline, 4.5-5.1 × 3.0-3.7 μm, amyloid.

Specimens examined: On the underside of branches of deciduous wood lying on the ground, Bukhan Mt., Seoul (SFC 971011-06); on the underside of branches of deciduous wood lying on the ground, Ullung Island (SFC 900807-16, SFC 900807-23).

Remarks: Gloeocystidia and finely verrucose spores are characteristic of this species. Those features were stronly supported by molecular data and this species is included in the russuloid clade.

13. *Haplotricum conspersum* (Pers.) Hol.-Jech.

Basidiomes fully resupinate, loosely appressed, downy, arachnoid, thin, filamentous; hymenophore smooth, cottony, ochre-yellow to brown, soft.

Hyphal system monomitic; generative hyphae simple-septate, up to 12 μm thick; cystidia absent; conidiophores with transverse septa, leaving many bud scars; basidia and basidiospores not observed; conidia globose, 12.8-15 μm diam.

Specimens examined: On the underside of *Pinus densiflora* lying on the ground, Kangwha Island (SFC 990123-15).

Remarks: This species is the anamorph of *Botryobasidium conspersum* Erikss. and is

phylogenetically nested in the botryobasidioid clade.

14. *Hyphoderma odontiaeforme* Boidin & Berthet

Basidiomes fully resupinate, adnate; hymenophore white to cream or yellowish, odontoid; margin concolorous.

Hyphal system monomitic, genertive hyphae thick-walled, nodose-septate with frequent simple septa between clamps, 2.3-6 μm daim; cystidia of two types, leptocystidia fusoid to subcylindrical, tapering to the apex, with a basal clamp, 63-94 × 8-10 μm, stephanocysts rarely present, with a basal clmap, 14.2-16.6 × 9.14-11.4 μm; basidia clavate, with 4 sterigmata, 32-35 × 6.8-9.7 μm, with a basal clamp; basidiospores suballantoid to cylindrical, smooth, thin-walled, 8-9 × 3.5-4.6 μm, non-amyloid.

Specimens examined: On dead deciduous wood of *Quercus*, Bukhan Mt., Seoul (SFC 971008-03); on dead deciduous wood of *Quercus* and *Castanea crenata*, Kangwha Island (SFC 980129-01, SFC 980201-17).

Remarks: This species is easily confused with *Hyphodontia* speices due to its odontoid surface but characterized by large gloeocystidia and stephanocysts. Its phylogenetic placement is not clear but occurred near the *Hyphoderma* and *Hypochnicium* within the polyporoid clade.

15. *Hyphodonita pallidula* (Bres.) Erikss.

Basidiomes fully resupinate, adnate; hymenophore pale yellowish to buff, finely pilose, warty; margin concolorous.

Hyphal system monomitic; generative hyphae thick-walled, with clamps, 2.2-2.5 μm thick; septocystidia numerous, with several septations with clamps, up to 65 × 3.4-3.8 μm; basidia subclavate, with 4 sterigmata, 12-16 × 3.2-3.4 μm, with a basal clamp; basidiospores ellipsoid, smooth, thin-walled, 3.6-4 × 1.8-2.1 μm, non-amyloid.

Specimens examined: On dead coniferous wood of barkless *Pinus densiflora*, Kangwha Island (SFC 981128-06).

Remarks: This species is characterized by small spores and septocystidia. Its phylogenetic placement is on the basal branch of *Hyphodontia* and *Schizopora* within the hymenochaetoid clade.

16. *Hyphodontia sambuci* (Pers.) Erikss.

Basidiomes fully resupinate, tightly attached to the substrate; hymenophore cream to yellow, smooth, somewhat fissured when dry; margin thinning out to distinctly bounded.

Hyphal system monomitic; generative hyphae thin-walled, with with clamps, 1.7-4 μm thick; leptocystidia capitate, with a basal clamp, 37-40 × 4-5.7 μm, projecting up to 19 μm; basidia suburniform, with 4 sterigmata, 23-29 × 4-5.2 μm, with a basal clamp; basidiospores ellipsoid to ovoid, with one oil drop, smooth, thin-walled, 5-6.3 × 3.4-3.8 μm, non-amyloid.

Specimens examined: On dead deciduous wood, Chiak Mt., Kangwon (SFC 910715-59).

Remarks: The capitate leptocystidia are a good identification character. The species has been treated as a member of *Hyphoderma* Wallr. by Eriksson and Ryvarden (1975) and Ginns and Lefebvre (1993). According to Langer and Oberwinkler (1993), the septal ultrastructure of *H. sambuci* is a dolipore type with imperforeate parenthesomes same as the one of *Hyphodontia*.

17. *Hypochnicium vellereum* (Ell. & Cragin) Parm.

Basidiomes fully resupinate, adnate; hymenophore whitish with a rose tint to rose red, smooth, somewhat pulverulent.

Hyphal system monomitic; generative hyphae thin-walled, with clamps, 2.8-4 μm thick; cystidia absent; basidia tubular, tapering towards the base, with 4 sterigmata, 42-65 × 4-7.42 μm, with a basal clamp; basidiospores globose, thick-walled, irregularly uneven or finely rough, 6.8-7.4 × 5.7-6.2 μm, cyanophilic, non-amyloid.

Specimens examined: On dead deciduous wood, Sobaek Mt., Kyongbuk (SFC 900626-63).

Remarks: This species is easily recognized by lacking cystidia, having globose, finely rough spores, and reddish fruitbodies. According to Eriksson and Ryvarden (1976), chlamydospores also characterize this species. Phylogenetic position of *Hypochnicium* occurred near the *Hyphoderma* within the polyporoid clade.

18. *Irpex hydnoides* Y. W. Lim & H. S. Jung, sp. nov.

Basidiocarpus annus, resupinatus vel effuso-reflexus; superficies pilei albido, tomentose-hirsute; hymenio cremeo-lutea vel ochraceo, hydnoid, 4 mm longis; systema hypharum dimiticum; hyphae generativae hyalinae, tenui-tunicatae, 2.5-5.14 μm latae; hyphae skeleticae hyalinae, crassetunicatae; basdia hyalina, clavata, 4-sterigmatibus, 28-32 × 6-7 μm; basidiospores ellipsoideae, levis, hyalinae, non-amyloideae.

Holotype: Korea, Kangwondo, Chiak Mt. On *Quercus* sp. 15, Dec. 1997, Y. W. Lim, SFC-971215-19.

Etymology: The species is named after its hymenophoral type with long teeth.

Basidiomes annual, usually resupinate or effused-reflexed; pilei usually laterally fused, up to 5 mm long, upper surface white-colored, densely tomentose to hirsute; hymenophore cream to deep yellow (3A3 - 3A5), hydnoid, tooth up to 4 mm long.

Hyphal system dimitic; contextual generative hyphae thin-walled with frequent branching, simple-septate, 2.5-5.14 μm in diam; skeletal hyphae hyaline, thick-walled with rare simple septa, 4-6 μm in diam; lamprocystida conspicuous, abundant, thick-walled, heavily incrusted at apex, 25-45 × 6.7-11 μm (incrusted part), originating in the subhymenium from tramal skeletal hyphae; basidia clavate, with 4 sterigmata, 28-32 × 6-7 μm, simple-septate at the base; basidiospores subglobose to ellipsoid, hyaline, smooth, 5.7-6.5 × 3.7-3.8 μm, non-amyloid.

Specimens examined: On dead wood of *Quercus*, Chiak Mt., Kangwon (SFC 971215-19); on dead wood of *Quercus*, Odae Mt., Kangwon (SFC 971011-12).

Remarks: This new species is easily recognized by its effused-reflexed fruitbodies with yellowish long hyhnoid hymenium. But to the naked eyes, it is quite similar to *Irpex lacteus* and is confused with this species in the field. It is differentiated from *I. lacteus*, the type species of *Irpex*, due to the hymenium with long teeth and pale or deep yellowish color, large basidia and basidiospores, rather slow growth rate in MEA media and the dissimilarity of sequences to those of *I. lacteus*. Its phylogenetic position is in the polyporoid clade.

19. *Irpex* sp.

Basidiomata annual, usually resupinate or effused-reflexed; hymenophore cream to ochre, hydnoid, tooth up to 2 mm long; margin loose and often somewhat rolled upward.

Hyphal system monomitic; generative hyphae thin-walled with frequent branching, simple-septate, 2.2-3.4 μm in diam; lamprocystida conspicuous, abundant, thick-walled, heavily incrusted at apex, 24-27 × 5.7-6.2 μm; basidia clavate, with 4 sterigmata, 25-31 × 5-6 μm, simple-septate at the base; basidiospores ellipsoid, hyaline, smooth, 5.2-6.3 × 2.8-4.5 μm.

Specimens examined: On a fallen branch of *Pinus densiflora*, Munkyong, Kyongbuk (SFC 990326-16).

Remarks: To the naked eyes, this species is quite similar to *Irpex lacteus* and is confused with *I. lacteus* in the field. Coniferous host, small lamprocystidia and

monomitic hyphal system were distinct characters of this species. Its phylogenetic position is in the polyporoid clade.

20. *Jacksonomyces furfurellus* (Bres.) S.H. Wu & Z.C. Chen

Basidiomes fully resupinate, adnate; hymenophore white to pale yellowish, smooth to finely pilose under 10 × lens; margin white, thinning out.

Hyphal system monomitic; generative hyphae thin-walled, with clamps, 1.7-2.8 μm thick; leptocystidia capitate, with a basal clamp, 20-28 × 2.8-3.5 μm, projecting up to 15 μm; basidia subclavate, with 4 sterigmata, 14-18 × 4-5.7 μm, with a basal clamp; basidiospores allantoid, smooth, thin-walled, 4.5-5.2 × 1-1.2 μm, non-amyloid.

Specimens examined: On barkless decaying wood of *Pinus densiflora*, Munkyong, Kyongbuk (SFC 90326-33, SFC 990505-03, SFC 990521-05, SFC 991003-12).

Remarks: Jülich (1979) removed *Phlebia phlebioides* from the genus *Phlebia* because of its stalked, clavate, small and partly pleural basidia, and established a monotypic genus *Jacksonomyces* Jülich for the species (Maekawa, 1993). Wu and Chen (1992) transferred six species of *Phlebia* to this genus on the basis of their morphological and cultural characters. Because this species was morphologically similar to *Hyphodontia*, however, it was often misidentifed as a species of *Hyphodontia*. Capitate leptocystidia and small allantoid spores characterize this species.

21. *Laurilia sulcata* (Burt) Pouz.

Basidiomes perennial, resupinate, leathery, orbiculate at first, confluent with time, thick; upperside brown to blackish, often with concentric furrows and ridges; hymenophore smooth to somewhat tuberculate, white to light yelowish.

Hyphal system dimitic; generative hyphae with clamps, hyaline, 2.8-4 μm diam; skeletal hyphae thick-walled, 4-6 μm thick; lamprocystidia numerous, thick-walled, apically round, encrusted, 37-45 (22) × 9.7-11.5 μm (incrusted part); basidia clavate to tubular, with 4 sterigmata, 42-52 × 6.8-7.5 μm, with a basal clamp; basidiospores subglobose, echinulate, with somewhat thickened walls, 6.8-7.4 × 5.1-5.7 μm, amyloid.

Specimens examined: On living wood of *Torreya nucifera*, Naezang Mt., Chunbuk (SFC 950218-15, SFC 991030-01).

Remarks: This species is readily distinguishable by the echinulate spores and the unique living host of *Torreya nucifera*. Microscopic features such as echinulate and amyloid spores were congruent with the result of molecular phylogeny that grouped *L. sulcata* into the russuloid clade.

22. *Megalocystidium lactescens* (Berk.) Jül.

Basidiomes fully resupinate, tightly attached to the substrate, thick; hymenophore smooth to somewhat tuberculate, white to cream; margin distinct, slightly cracked when dry.

Hyphal system monomitic; generative hyphae hyaline, without clamps, 2.3-3.5 μm

thick; gloeocystidia numerous, sinuous, cylidrical, 130-214 × 7-9 μm; dendrohyphidia thin, 0.7-1.7 μm thick; basidia tubular, with 4 sterigmata, without a basal clamp, 68-75 × 6.8-8 μm; basidiospores ellipsoid to subglobose, smooth, thin-walled, 7.4-9.2 × 5-6.3 μm, amyloid.

Specimens examined: On dead wood of a deciduous tree, Naezang Mt., Chunbuk (SFC 910821-01).

Remarks: Distinguishing characteristics of this species are simple-septate hyphae and dendrohyphidia in the hymenium and thick cracked fruitbodies.

23. *Meruliopsis corium* (Fr.) Ginns

Basidiomes resupinate, effused-reflexed to somewhat semipileate, growing in patches, 0.5-1 mm thick, membranaceous; pilei projecting outward to the side, forming narrow and continuous edges; upperside whitish to creamish, fibrillose-tomentose, inconspicuously zoned; hymenophore whitich to ocherish, initially smooth, then reticulate-poroid to meruloid, verrucose; margin finely byssoid, texture leathery membranaceous, rather tough.

Hyphal system monomitic; generative hyphae simple-septate, rarely clamped, thin- to thick-walled, 2.5-4.5 μm thick, commonly branched and intertwined; cystidia absent; basidia slenderly clavate, with 4 sterigmata, 25-30 × 4-6 μm, without a basal clamp; basidiospores narrowly ellipsoid, smooth, 5-6 × 2-3 μm, non-amyloid.

Specimens examined: On a fallen twig of *Prunus sargentii*, Gunwi, Kyongbuk (SFC

970918-1, SFC 990123-3).

Remarks: This species is also called *Byssomerulius corium* depending on authors (Eriksson and Ryvarden, 1973). Its basidiocarp is rather various from resupinate to semipileate forms in shape but is very simple and has no particular characters under the microscope. Its relationship with other merulioid fungi is uncertain but the species is placed in the *Irpex* group within the polyporoid clade.

24. *Phanerochaete calotricha* (Karst.) Eriksss. & Ryv.

Basidiomes resupinate, effused, confluent, membranaceous, thin, less than 0.3 mm thick; hymenophore white, then turning yellowish or pale ochraceous, smooth, somewhat cracking on drying; margin variable but often partly fibrillose and with whitish yellow rhizomorphs.

Hyphal system monomitic; subhymenial hyphae simple-septate, thin-walled, 2-3 μm thick, richly and irregularly branched; subicular hyphae infrequently nodose-septate, somewhat thick-walled, 4-6 μm thick, sometimes up to 10 μm thick, sparsely branched, somewhat horizontally arranged; leptocystidia abundant, more or less subulate, 40-74 × 4-6 μm, commonly projecting up to 35 μm; basidia narrowly clavate, with 4 sterigmata, 25-35 × 4-6 μm, without a basal clamp, non-amyloid; basidiospores narrowly ellipsoid, straight or adaxially somewhat convex, smooth, generally with two oil drops, 4.5-5.5 × 2-2.5 μm, non-amyloid.

Specimens examined: On the underside of rotten wood of *Pinus rigida*, Gunwi,

Kyongbuk (SFC 991008-20); on the underside of rotten wood of *Pinus densiflora*, Kangwha-Island (SFC 970918-2).

Remarks: This fungus develops whitish rhizomorphs from the fibrillose margin and, microscopically, has numerous subulate cystidia. It is often treated as a pale form of *P. sanguinea* (Burdsall, 1985) but, as the color used to be a steady character in *Phanerochaete*, it would be better to treat this species as a separate taxon of its own. This species is placed in the *Phanerochaete* group within the polyporoid clade.

25. *Phanerochaete chrysorhiza* (Torrey) Budington & Gilbn.

Basidiomes resupinate, effused, thin, becoming membranaceous, easily separable; hymenophore reddish orange, hydnaceous, with spines up to 2.5 mm long, subulate to cylindirical with a tapering apex; margin orange white, fibrillose to rhizomorphic with well-developed cordons up to 1 mm thick.

Hyphal system monomitic; subhymenial hyphae simple-septate, thin-walled, 3-5 μm thick, frequently branched; subicular hyphae mostly simple-septate, occasionally septate with clamps, thick-walled, 4-7 μm thick, frequently branched usually at right angles, commonly encrusted with hyaline crystals; leptocystidia common, ventricose, 45-60 × 4-6 μm, commonly projecting up to 42 μm; basidia clavate, with 4 sterigmata, 15-20 × 4-6 μm, without a basal clamp; basidiospores ellipsoid to narrowly ellipsoid, smooth, 4-5 × 2-2.5 μm, non-amyloid.

Specimens examined: On dead wood of broadleaved trees, Daemo Mt., Seoul (SFC

970830-21); on dead wood of broadleaved trees, Munkyong, Kyongbuk (SFC 990911-60).

Remarks: Due to the conspicuous orange fruitbody with subulate spines and rhizomorphic cordons, this fungus is easily identified by the naked eyes. Microsopically, it has encrusted hyphae, leptocystidia, and spores typical of the genus *Phanerochaete*. However, this species is placed in the *Phlebia* group within the polyporoid clade.

26. *Phanerochaete sanguinea* (Fr.) Pouz.

Basidiomes fully resupinae; henophore white or cream-colored, turning reddish with time, membranous, adnate.

Hyphal system monomitic; generative hyphae simple-septate, 5-7 μm thick, hyaline, regularly branching at nearly right angles, mostly simple-septate, sometimes with clamps; lamprocystidia easy to find, thin-walled to somewhat thick-walled, cylindrical or tapering, naked or with varying degree of incrustation, 40-49 × 5.1-8 μm; basidia clavate, thin-walled, 23-25 × 4-6 μm; basidiospores narrowly ellipsoid, hyaline, 5.5-6.2 × 2.5-3.0 μm, non-amyloid, acyanophilous.

Specimens examined: On dead deciduous trees, Naezang Mt., Chunbuk (SFC 910821-19); on dead deciduous trees, Kangchon, Kangwon (SFC 990617-05).

Remarks: This species is generally easily recognized due to its red color. Young specimens are wholly white and then resemble very much *Pha. sordida*, but old

specimens are wholly red. Burdsall had treated this species as a synonym of *Pha. calotricha*. However, they may be separated by the size, shape and number of the cystidia, and the basidiospore. This species is placed in the *Phanerochaete* group within the polyporoid clade.

27. *Phanerochaete velutina* (Fr.) Karst.

Basidiomata resupinate, adnate; hymenophore white to reddish or even orange red, mainly smooth, often with some rhizomorphs.

Hyphal system monomitic; generative hyphae mostly simple-septate, 7-10 μm thick, hyaline; lamprocystidia very numerous, easily visible, forming a velutinous surface of a whitish color in old specimens from rich incrustations, 42-48 × 14-18 μm; basidia clavate, thin-walled, 32-38 × 6-7 μm; basidiospores broadly ellipsoid, hyaline, 6.2-7 × 3.5-4.3 μm, non-amyloid, acyanophilous.

Specimens examined: In mostly humid deciduous forests, Munkyong, Kyongbuk (SFC 990422-05); in mostly humid deciduous forests, Myongji Mt., Kyonggi (SFC 970816-20).

Remarks: The typical specimens of this species are easily recognized but the variation is normally very great. The subiculum, consisting of broad, more or less encrusted hyphae in an open texture, is considerably thick and visible in the crack of the hymenium. There is usually no color reaction to KOH except for some of orange-colored specimens, in which there is a weak red color. This species is placed in the

Phanerochaete group within the polyporoid clade.

28. *Phanerochaete xerophila* Burdsall

Basidiomes fully resupinate; hymenophore ochre-colored, membranous, adherent, cracking extensively.

Hyphal system monomitic; generative hyphae mostly simple-septate, 3.5-4.5 μm thick, mostly curved, hyaline, regularly branching at nearly right angles; cystidia absent; basidia clavate, 42-52× 5.7-6.8 μm, thin-walled; basidiospores broadly ellipsoid, hyaline, 7.4-7.9× 3.5-3.9 μm, non-amyloid, acyanophilous.

Specimens examined: On a dead deciduous tree, Kangwha Island (SFC 981128-3-2); on a dead deciduous tree, Munkyong, Kyongbuk (SFC 990326-37).

Remarks: This species has been frequently reported as *Phlbebia chrysocrea* for its morphological similarity. It differs from *Phl. chrysocrea* in its hyphal system without clamp connection. This species is placed in the *Phanerochaete* group within the polyporoid clade.

29. *Pseudomerulius aureus* (Fr.) Jül.

Basidiomes resupinate, effused or partly reflexed, about 1 mm thick, adnate, becoming loosely attached and separable on drying, orbicular, often confluent; hymenophore yellow, orange, or yellow brown, becoming dark on touching or aging, irregularly folded into a net of composed pores, pores 1-2 mm wide; margin distinct,

white or yellow, narrow.

Hyphal system monomitic; generative hyphae with clamps, sometimes with ampullate clamps, thin-walled, somewhat thick-walled in the subiculum, 2-3 μm thick in the subhymenium, 3-5 μm thick in the subiculum, richly branched; cystidia not seen; basidia clavate, with 4 sterigmata, 15-20 × 3.5-4.5 μm, without a basal clamp; basidiospores cylindrical, straight or slightly curved, smooth, 2.9-3.9 × 1.1-1.5 μm, cyanophilic, non-amyloid.

Specimens examined: On fallen twigs or dead branches of *Pinus densiflora*, Munkyong, Kyongbuk (SFC 990326-04, SFC 990505-26, SFC 990623-17); on a fallen twig or a dead branch of *P. koraiensis*, Myongji Mt., Kyonggi (SFC 990626-16).

Remarks: In the field, this fungus is easily recognized because of its bright gold hymenophore and irregularly composed pores. This species has been reported to grow exculsively on conifers (Eriksson *et al.*, 1981). Phylogenetic position of this species was found in the bolete clade.

30. *Schizopora flavipora* (Cke.) Ryv.

Basidiomes annual, resupinate, leathery to tough fibrous; hymenophore poroid, white to cream, in an appressed form, angular to daedaleoid or irpicoid, 5-6 per mm, with thin dissepiments; margin usually sterile, whitish.

Hyphal system monomitic; generative hyphae with clamps, hyaline, thin- to thick-walled, 3.2-4 μm in diam, somewhat capitate ending in a globose, swollen apex, up to

10 μm wide; cystidia absent; basidia clavate, with 4 sterigmata, 12-15 × 3-5 μm, with a basal clamp; basidiospores ellipsoid, hyaline, smooth, 3.5-4.5 × 2.5-3.5 μm, non-amlyoid.

Specimens examined: On dead standing wood of *Quercus*, Bukhan Mt., Seoul (SFC 970926-04); on dead standing wood of *Pinus densiflora*, Ilyong, Kyonggi (SFC 980125-7).

Remarks: The small spores, basidia and the appressed form of pores are distinctive features of this species. In phylogenetic analysis, this species is nested in the hymenochaetoid clade.

31. *Sistotrema diademiferum* (Bourd. & Galz.) Donk

Basidiomes fully resupinate, adnate, thin; hymenophore smooth, cream-colored; margin arachnoid.

Hyphal system monomitic; generative hyphae with clamps, 5-5.7 μm thick; cystidia absent; basidia urniform, with 6 sterigmata, 19-21 × 5.7-6.3 μm; basidiospores ellipsoid, smooth, 5.1-5.7 × 2.9-3.4 μm. non-amyloid, acyanophilous.

Specimens examined: On a fallen branch of *Quercus*, Munkyong, Kyongbuk (SFC 990521-13).

Remarks: Urniform basidia with 6 sterigmata characterize this species. This species was placed in the botryobasidioid clade.

32. *Steccherinum fimbriatum* (Pers.:Fr.) Erikss.

Basidiomata fully resupinate, loosely adnate; hymenophore odontioid, fairly soft but tough, usually pale violaceous or grey-reddish in the living state, dark ochraceous, sometimes grayish, or even yellowish-grey in the herbarium, aculei conical, penicillate; margin more or less filamentous to rhizomorphic.

Hyphal system dimitic; generative with clamps, 2-3 μm thick; skeletal hyphae 2.8-4 μm thick; lamprocystidia numerous, strongly incrusted, 27-57 × 8-11 μm; basidia clavate, 18-20 × 4-4.5 μm; basidiospores ellipsoid, smooth, hyaline, 3.5-4.0 × 2.2-2.5 μm, non-amyloid, acyanophilous.

Specimens examined: On the bark of a dead deciduous tree, Ullung Island (SFC 901002-12).

Remarks: Due to the pale violaceous color, rhizomorphic margin and resupinate basidiocarp, this species is easily identified. Phylogenetic position of this speices is in the *Steccherinum* group within the polyporoid clade.

33. *Steccherinum murashkinskyi* (Burt) Maas.

Basidiomes sessile; upper face marked by concentrical shallow grooves and hispid zones, glabrescent at margin, ochraceous yellow-brown or flushed with warmer shade of brown; hymenophore hydnaceous, spines 4-6mm long.

Hyphal system dimitic; generative hyphae 1.7-2.5 μm thick with clamps; skeletal 2.8-4.5 μm thick, hyaline; lamprocystidia up to 5 μm wide, mostly of tramal origin;

basidia clavate, thin-walled, 11-13× 3.4-4.6 μm; basidiospores ellipsoid, smooth, hyaline, 2.8-3.2× 1.8-2.0 μm, non-amyloid, acyanophilous.

Specimens examined: On a dead *Betula*, Bangtae Mt., Kangwon (SFC 990914-17); On a dead *Quercus*, Chungwon Mt., Kyonggi (SFC 960806-06).

Remarks: This species is commonly recognized by its sessile, thick and hard basidiocarps.

34. *Steccherinum robustius* (Erikss. & Lund.) Erikss.

Basidiomes resupinate, widely effuse-reflexed, slightly loosening and detachable in pieces at the margin; hymenophore odontioid to hydnoid, reddish-orange in the living state, fading into grayish color in the herbarium; aculei elongate-conical; consistency firm; margin rarely fimbriate.

Hyphal system dimitic; generative hyphae with clamps, 2.3-4.5 μm thick; skeletal hyphae 4-5.5 μm thick; lamprocystidia numerous, strongly encrusted, 17-30 × 8-10 μm; basidia clavate, 24-28 × 5.1-5.4 μm; basidiospores ellipsoid, smooth, hyaline, 5.1-5.7 × 3.1-3.4 μm, non-amyloid, acyanophilous.

Specimens examined: On dead deciduous fallen branch, Munkyong, Kyongbuk (SFC 990320-13, SFC 990521-08).

Remarks: According to large fructifications, a bright reddish orange color in fresh specimens, scattered irregular aculei, large spore and basidia easily characterize this species. Phylogenetic placement of this speices was in the *Steccherinum* group within

the polyporoid clade.

35. *Stereum complicatum* (Fr.) Fr.

Basidiomes annual, effuso-reflexed to sessile, sometimes umbonate, confluent, sometimes imbricate on vertical substrata, coriaceous-papery; pileus narrowly attached, becoming laterally extended, radially plicate to complicate, thin and lacerate at the margin, usually < 2 cm; upper surface matted-tomentose with a thin, transient, tomentum with strigose-hirsute patches at the point of attachment, zonate, inner part gray or grayish brown, outer part ochraceous white, buff at the margin; hymenophore even, light yellow to buff, 0.5-0.8 mm thick in section; cutis brown; context cream to ochraceous, thickening.

Hyphal system monomitic; generative hyphae simple-septate, thin- to thick-walled, 2-4 μm thick; conducting hyphae thick-walled, hyaline, 4-8 μm thick; pseudocystidia cylindrical to subcylindrical, thick-walled at the base, apically thin-walled, 4-7 μm thick; hyphidia mostly cylindrical, sometimes acuminate, thin-walled, 24-27 × 2-3 μm; basidia narrowly clavate to subcylindrical, with 4 sterigmata, 27-30 × 5.14-6.28 μm; basidiospores ellipsoid to cylindrical, hyaline, smooth, thin-walled, 6.8-7.4 × 2.9-3.4 μm, amyloid.

Specimens examined: Dead corticate limbs and trunks of *Quercus*, Kwangwha Island (SFC 980201-4).

Remarks: This species is common on hardwoods at lower elevations. *Stereum*

complicatum is microscopically very similar to *S. hirsutum*, so they are distinguished from each other on the gross morphology of the tomentum, hymenium, or fruitbody thickness. It is known that such a separation is often impossible because intermediate fruibodies are found. For this reason, Welden (1971) suggested that *S. complicatum* should be maintained as a member of the *Stereum hirsutum* complex. Its phylogenetic placement is in the russuloid clade.

36. *Stereum peculiare* Boidin, Parmasto & Dhingra

Basidiomes annual, occasionally perennial, resupinate, effuso-reflexed, coriaceous, somewhat brittle when dry; pileus laterally extended, up to < 4 mm protruding, upper surface white matted-tomentose at the point of attachment, outer part appressed-hirsute, zonate with thin, alternating bands of reddish brown to grayish white color; hymenophore uneven, tuberculate, with scattered irregular fine teeth, often finely to deeply cracked when dry; margin smooth, brown to pale brown.

Hyphal system monomitic; generative hyphae simple-septate, 3-5 μm thick; acanthohyphidia mostly cylindrical, thin-walled, with numerous cylindrical prongs along the sides, 4-6 μm in diam; basidia rarely seen, narrowly clavate to subcylindrical, with 4 sterigmata, 62-67 × 6.28-7.4 μm; basidiospores ellipsoid to cylindrical, hyaline, smooth, thin-walled, 10-12 × 3.4-4 μm, amyloid.

Specimens examined: On dead corticate stumps of *Quercus*, Chiak Mt., Kangwon (SFC 971215-13); on dead trunks of *Quercus*, Chiri Mt. (SFC 991219-18); on dead

limbs of *Quercus*, Munkyong, Kyongbuk (SFC 990326-25); on large dead limbs of *Quercus*, Soyo Mt., Kyunggi (SFC 980218-13).

Remarks: It is rather easy to recognize this species in the *Quercus* forest because of its scattered irregular fine teeth, brown color of resupinate hymenial surface and finely to deeply cracked fruitbody when dry. Acanthohyphidia are also an important character for the identification of this species. Phylogenetic placement of *S. peculiare* was not clear although it was grouped in the russuloid clade.

37. *Stereum sanguinolentum* (Alb. & Schw.: Fr.) Fr.

Basidiomata annual, resupinate, effuso-reflexed or umbonate-sessile, and subcupulate, confluent, imbricate on vertical substrata, coriaceous; pileus dimidiate, broadly to narrowly attached, becoming laterally extended, up to <1 cm in radius, upper surface matted-tomentose with strigose-hirsute zone, finally nearly glabrous, zonate, tomentum often worn in 1 or 2 thin concentric bands, furrowed to sulcate, grayish orange, brownish orange, blackish brown when dried; hymenophore even, rarely tuberculate, slightly rugose, cracking to reveal paler context, light brown, reddish brown, grayish brown or gray with violaceous tinges, bleeding or bruising red when fresh, white at the margin, 0.2-0.6 μm thick in section; cutis brown; context usually simple, occasionally forming two strata.

Hyphal system monomitic; generative hyphae slightly thick- to thick-walled, with brown oily contents, usually conspicuous in vertical section, 4-8 μm thick;

pseudocystidia cylindrical to subcylindrical, often flexuous, slightly thick-walled to thick-walled at the base, apically thin-walled, with brown oily contents, derived from conducting hyphae or directly from undifferentiated hyphae, 5-10 μm in diam; pseudoacanthohyphidia cylindrical or acuminate, thin-walled, 23-34 × 2-4 μm; basidia subcylindrical to subcylindical, with 4 sterigmata, 28-37 × 4-6 μm; basidiospores ellipsoid to cylindrical, hyaline, smooth, thin-walled, 5-7.5 × 2-3.5 μm, amyloid.

Specimens examined: On a dead conifer, Munkyong, Kyongbuk (SFC 990326-11); on a corticate limb of *Pinus*, Wooi Island, Chunnam (SFC 980816-9).

Remarks: This species is the only species of *Stereum* that normally inhabits coniferous substrata. This character, along with its tendency to bruise red when fresh, and the presence of pseudoacanthohyphidia and colored conducting hyphae make specimens of this species easy to identify. Phylogenetic placement of *S. peculiare* is in the russuloid clade.

38. *Stereum striatum* (Fr.) Fr.

Basidiomes annual, effuso-reflexed to sessile, confluent, coriaceous, somewhat brittle when dry; pileus dimidiate to flabelliform, up to 3.5 × 4.5 cm, occasionally larger, upper surface matted-tomentose with wolly strigose-hirsute patches at the point of attachment, shiny, sericeous, lineate-striate, strongly zonate with thin, alternating bands of light orange, grayish orange, brownish orange to brown color; hymenophore even, sometimes radially wrinkled, light yellow to yellow, brownish orange,

occasionally with central ridges, pale orange, grayish orange, brownish orange to light brown, 150-400 μm thick in section; cutis brown; context subhyaline, pale yellow to yellow brown; hymenium slightly thickening, not stratose.

Hyphal system monomitic; generative hyphae simple-septate, hyaline, 2-4 μm thick; conducting hyphae thick-walled, 4-8 μm thick; pseudocystidia cylindrical to subcylindrical, thick-walled at the base, thin-walled apically, 4-11 μm thick; hyphidia acute, thin-walled, 3-4 μm diam; basidia narrowly clavate to subcylindrical, 4 sterigmata, 25-35 × 4-4.5 μm; basidiospores ellipsoid to cylindrical, hyaline, smooth, thin-walled, 5-7 × 2.8-3.5 μm, amyloid.

Speciemens examined: On dead limbs and trunks of *Carpinus caroliniana* and rarely on other hardwoods, Baekdam Temple, Kangwon (SFC 971030-11).

Remarks: The papery umbonate pilei with a glabrous and shiny upper surface, lack of a cutis and usual habitation on *Carpinus caroliniana* make this species easy to identify. *Stereum sriatum* is related to *S. ochraceo-flavum*. The microscopic similarity between *S. striatum* and *S. ochraceo-flavum* led Welden (1971) to suggest that these two taxa are merely forms of a same species. However, they are considered separate here, based on non-overlapping difference in their upper surface texture and ecological specialization exhibited by *S. striatum*. This species is placed in the russuloid clade.

39. *Stereum subtomentosum* Pouz.

Basidiomes annual, sometimes also perennial, coriaceous; pileus dimidiate,

flabelliform to semicircular with usually narrowly attached pilei, applanate to undulate, resembling *S. ostrea* when confluent, individual basidiomata recognizable, usually < 5 cm protruding, upper surface matted-tomentose, occasionally hirsute, tomentum covering the entire surface, or with a few narrow, glabrous zones, grayish to gray buff or ocher yellowish, later greenish from algae, margin lighter to ochraceous; hymenophore even, tuberculate or undulate, slightly wrinkled radially, with concentric ridges, light beige to ochraceous, margin light yellow, bruising yellow when fresh, 0.3-0.8 mm thick in section; cutis reddish brown; context cream to ochraceous.

Hyphal system monomitic; generative hyphae thick-walled, rounded or with a small point at the tip, 3-7 μm thick; pseudocystidia cylindrical to subcylindrical, 1.5-3 μm thick at the base, thin-walled apically, 5-7 μm thick; hyphidia mostly acuminate, sometimes cylindrical, thin-walled, 16-25 × 2-3 μm; basidia narrowly clavate to subcylindrical, with 4 sterigmata, 30-35 × 3-4 μm; basidiospores ellipsoid to cylindrical, hyaline, smooth, thin-walled, 5-6.5 × 2-3 μm, amyloid.

Specimens examined: On dead wood of *Alnus*, Chiri Mt. (SFC 991219-02); on dead wood of *Salix*, Daedun Mt., Chunbuk (SFC 960127-11); on dead wood of *Alnus*, Gunamu Mt., Kyunggi (SFC 960420-26); on dead wood of broad-leaved trees, Munkyong, Kyongbuk (SFC 990326-19); on dead wood of *Fagus*, Sinsunbong, Chungbuk (SFC 960316-18).

Remarks: *Stereum subtomentosum* is easy to recognize in the field because of its similar appearance of *S. ostrea* which has a tropical or subtropical distribution and is

separated by its pseudoacanthohyphidia (Eriksson *et al.*, 1984). This species is placed in the russuloid clade.

40. *Vuilleminia comedens* (Nees: Fr.) Maire

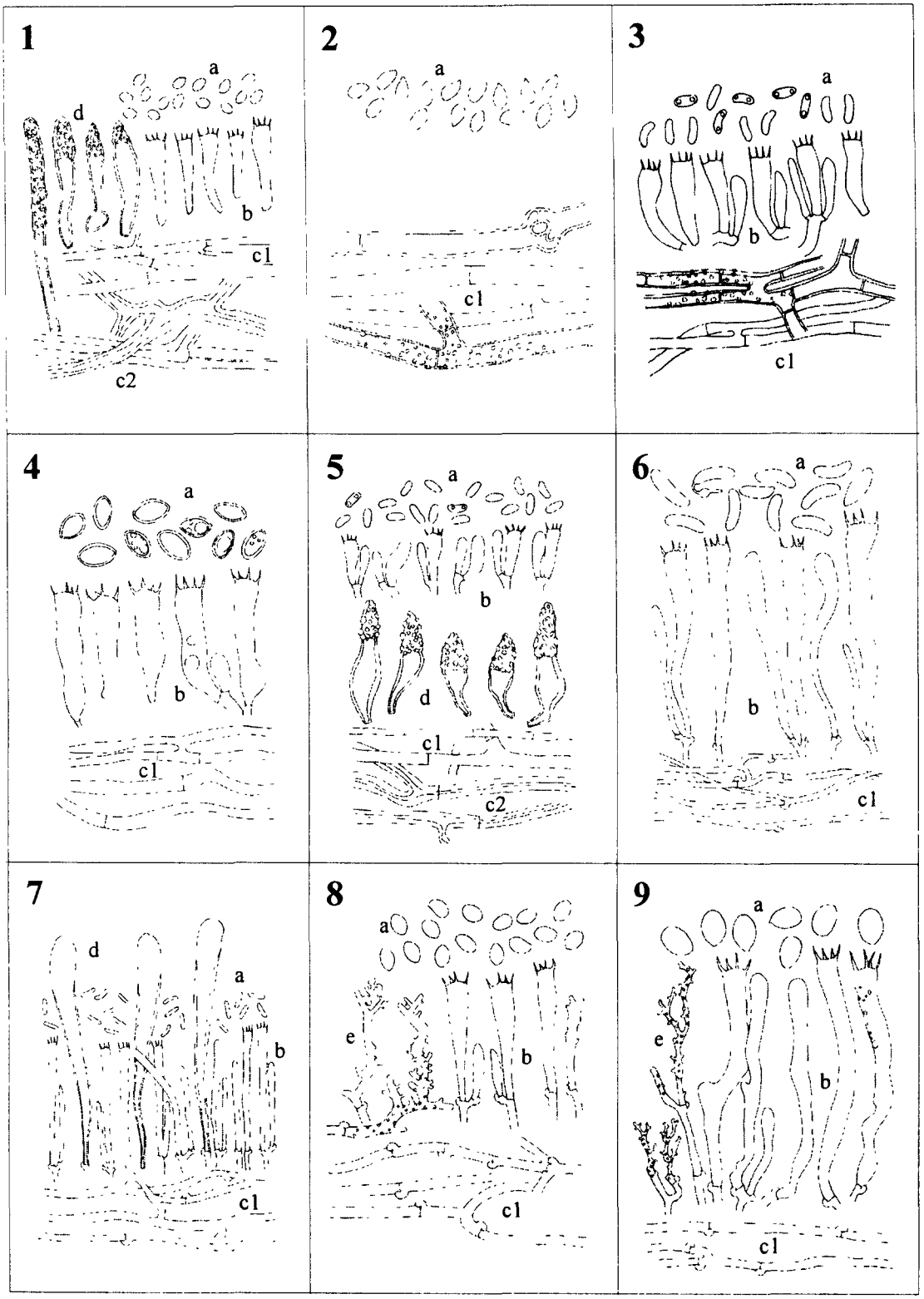
Basidiomes fully resupinate, thin; hymenophore white or cream-colored, gelatinous when fresh.

Hyphal system monomitic; generative hyphae with clamps, 2-3 μm thick; cystidia absent; dendrohyphidia usually numerous, somewhat incrusted; basidia long, tubular, 63-90 × 11-14 μm, with 4 or 6 sterigmata; basidiospores large, allantoid, smooth, hyaline, 17-20 × 7.4-7.9 μm, non-amyloid, acyanophilous.

Specimens examined: On fallen branches of *Quercus*, *Betula schmidtii* and some other deciduous trees, Munkyong, Kyongbuk (SFC 990320-24, SFC 990326-21, SFC 990505-01, SFC 991003-05).

Remarks: This species is characterized by its large basidiospores, large basidia with 4 or 6 sterigmata and gelatinous texture when fresh. Its phylogenetic placement is in the laeticorticioid clade.

Figure 2.3. Microscopic features of corticioid fungi new to Korea. a: basidiospores, a1: condia, b: basidia, c1: generative hyphae, c2: skeletal hyphae, d: cystidia, e: dendrohyphidia, f: pharaphysoid hyphae, g: hyphidia. The figure numbers correspond to those in the annotated list.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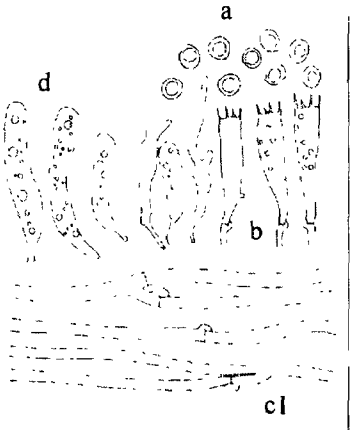

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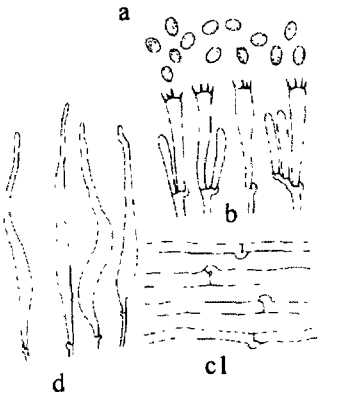

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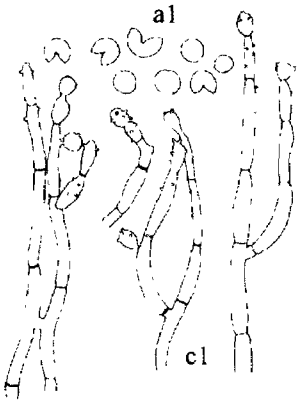

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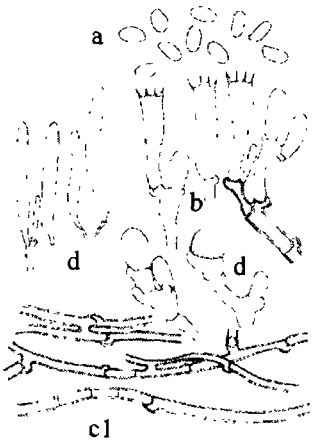

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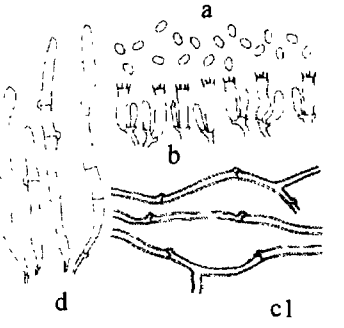

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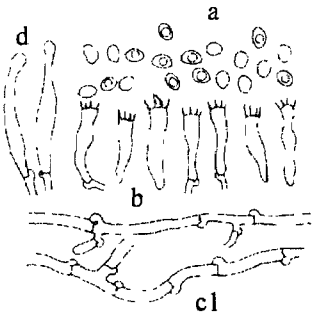

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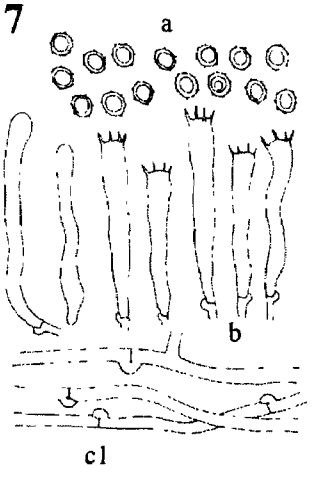

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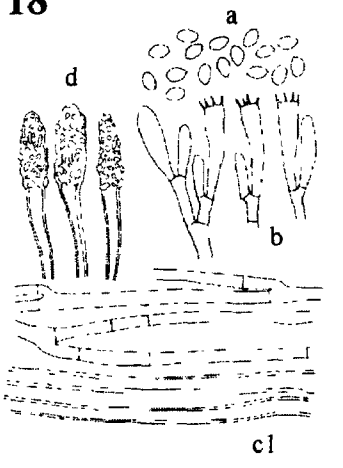

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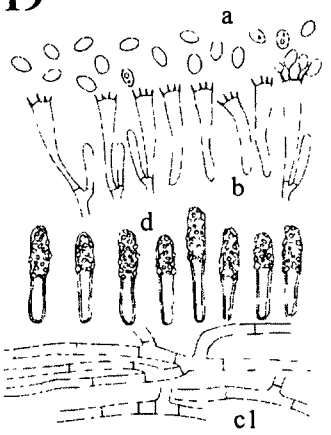

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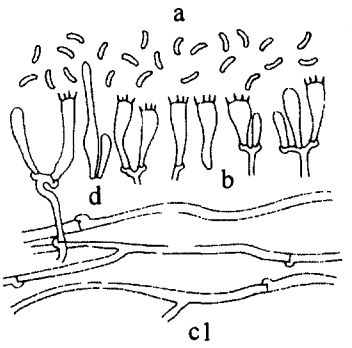

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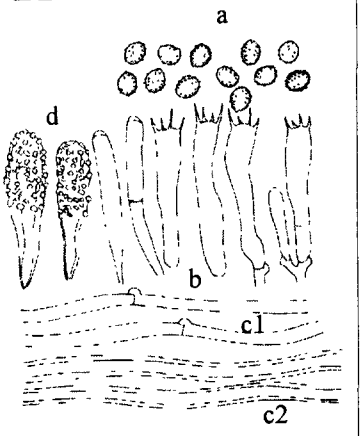

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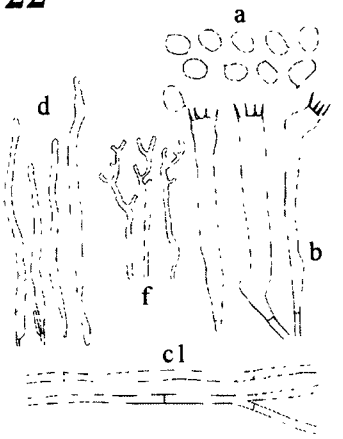

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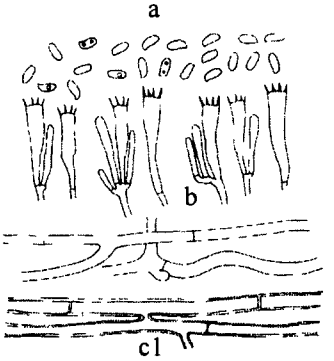

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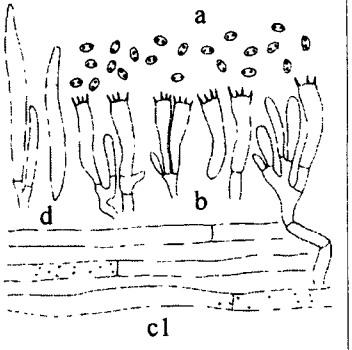

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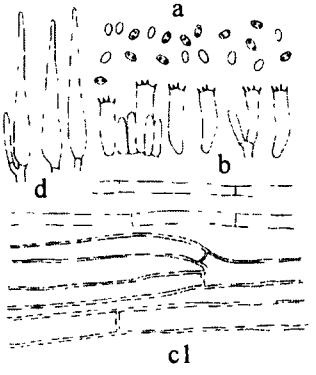

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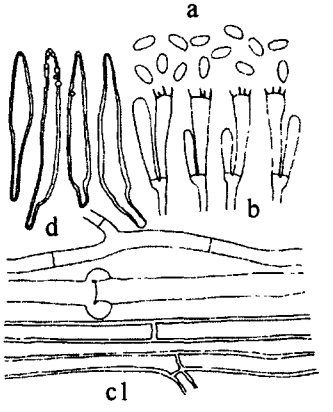

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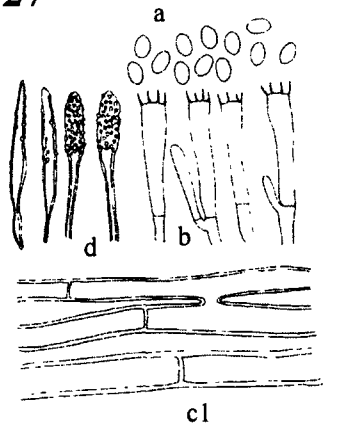

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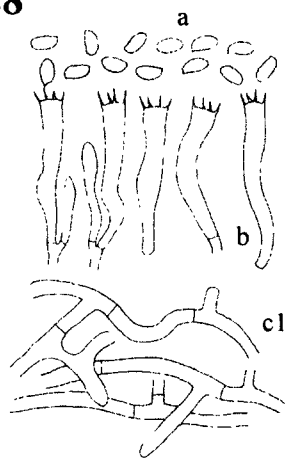

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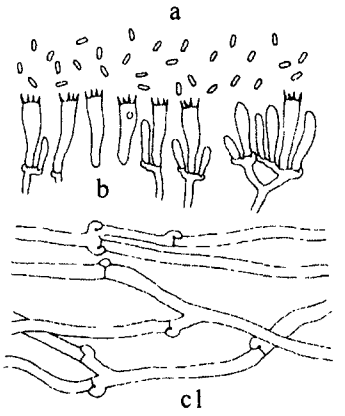

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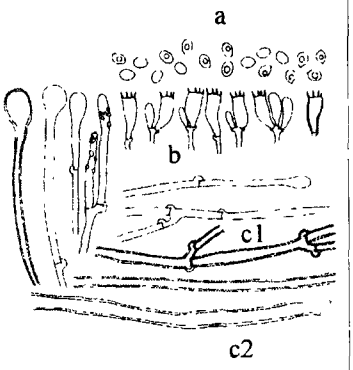

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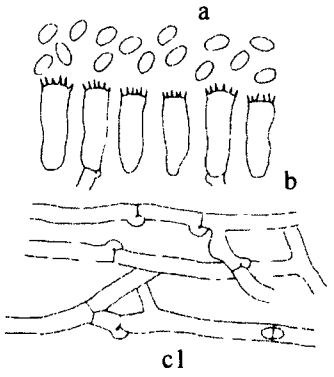

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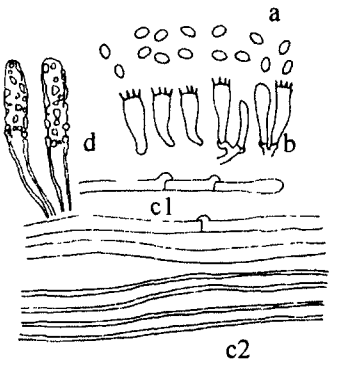

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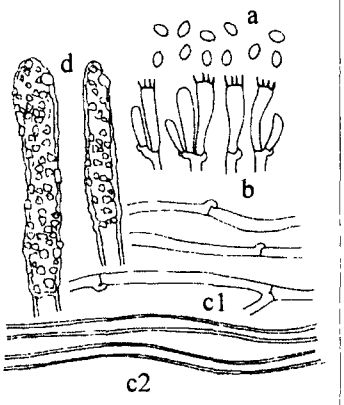

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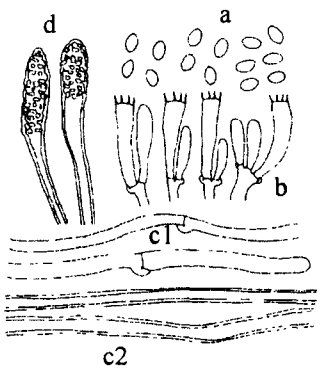

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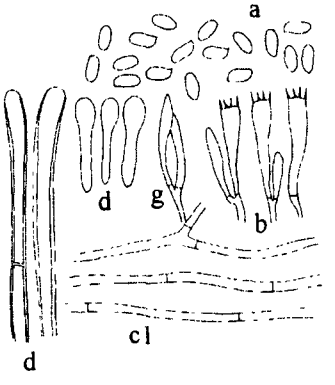

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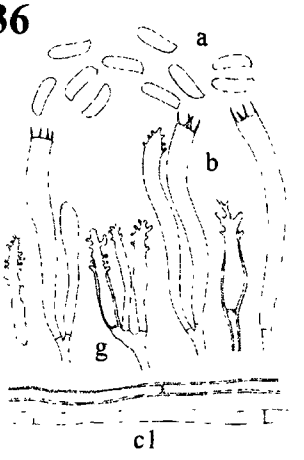

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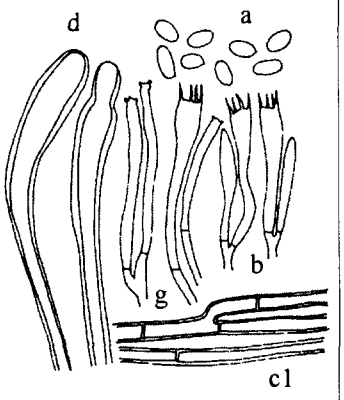

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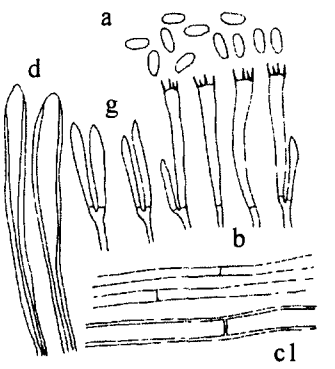

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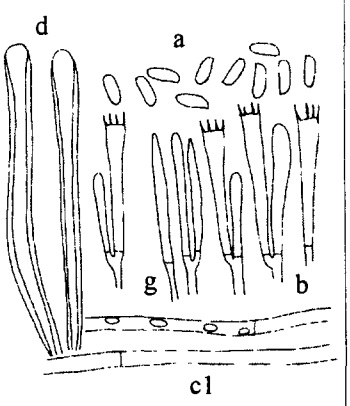

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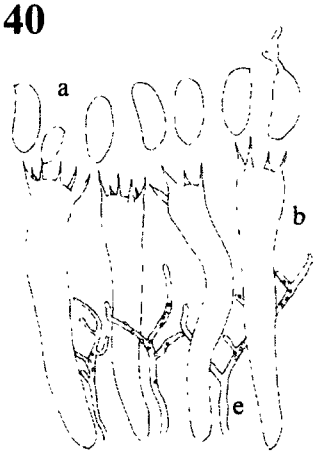

Figure 2.4. Photographs showing various basidiocarps of corticioid fungi new to Korea (Photograph 1 by courtesy of H.S. Jung, 23 by courtesy of S.G. Hong, the others from the author).

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CHAPTER 3

Molecular Phylogenenetic Studies on Corticioid Fungi

Introduction

Most of corticioid fungi play an important role in forests. The major importance of the corticioid fungi is their saprobic activities as decomposers, particularly in the degradation of cellulose and lignin. *Phanerochaete chrysosporium* is an efficient white rotter that has been intensively studied. Although most species are saprobic in soil, litter, bark, dead wood, or nonconducting xylem (heartwood) of living trees, some species may be truly parasitic or pathogenic to shade and forest trees, nonwoody crops and nonvascular plants. A few wood inhabitant fungi, including some members of the corticioid fungi, have been reported to form ectomycorrhizae with forest trees that are similar to those formed by agarics (Alexopoulos *et al*, 1996). A surprisingly large number of the basidiomata of species of the Aphyllophorales have been used medicinally. The antibiotic merulidial has been isolated from the corticioid fungus, *Phlebia tremellosus*, which suggests that other useful compounds will be found when this group is more analytically studied (Quack *et al.*, 1978).

The corticioid fungi are characterized by simple fruitbodies, usually resupinate, crust-like and closely attached to their substrate. Therefore, all members of the Corticiaceae and many other members of Hymenomycetes were included in this group. In Donk's system, eight families among 23 families of Aphyllophorales contained the corticioid fungi (Donk, 1964). While their macroscopic features were very simple, their microscopic ones varied more widely than those of other families in

Aphyllophorales. These results implied that the corticioid fungi were heterogenous and had complex phylogenetic relationships.

Many cultural and morphological studies for the family Corticiaceae have been investigated (Boidin, 1958; Eriksson *et al.*, 1978, 1981, 1984; Eriksson and Ryvarden, 1973, 1975, 1976; Ginns and Lefebvre, 1993; Hjortstam *et al.*, 1987, 1988; Jung, 1987; Nakasone, 1990; Nobles, 1965; Stalpers, 1978,). However, these have never been focused on phylogenetic relationships explicitly. Hence, there is no generally accepted system of higher ranks for this group. But only fragmentary phylogenetic studies about some corticioid groups have been accomplished because they have simple fruitbodies and are limited in sampling all corticioid taxa.

The most taxomoically inclusive phylogenetic study in homobasidiomycetes was accomplished by Hibbett and Donoghue (1995) that was based on mt-ssu rDNA sequences. In their analyses, only 6 corticioid species were included and one of their conclusions was that there was considerable homoplasy in morphological characters. After that, to draw conclusions on the importance of certain morphological characters in the practical delimitation of genera, a phylogenetic study based on morphological data and partial nuc-lsu rDNA sequence data were performed by Hallenberg and Parmasto (1998), but their conclustions were almost same as those of Hibbett and Donoghue (1995). Boidin *et al.* (1998) tried to evaluate the phylogenetic relationships within the order Aphyllophorales using internal trasncribed spacer (ITS1-5.8S-ITS2) sequences. Their results showed that the Aphyllophorales represented different

independent lineages and that traditional classifications were not supported. The corticioid fungi were also scattered among the phylogenetic tree in their study. However, ITS regions are known to evolve rapidly, so they are not thought to be appropriate in study of higher relationships of fungi. Another several molecular works were accomplished for some corticioid fungi: Aphyllophorales by Kim and Jung (2000), Coniophoraceae by Gardes and Bruns (1996), ectomycorrhizal basidiomycetes by Bruns *et al.* (1998), Corticiaceae by Lee and Jung (1998), *Peniphora* by Hallenberg *et al.*, (1996), Stereaceae by Yoon *et al.* (2001) and lignin degrading corticioid fungi, *Phlebia* and *Phaenerochaete* by Dresler-Nurmi and his colleagues (1999).

Refering to the results of recently molecular studies, most taxonomically inclusive phylogenetic study in homobasidiomycetes was achieved by Hibbett and Thorn (2001). According to their results, corticioid forms occurred in each of the eight major clades. However, because of uncertain relationships of fungi at higher levels, limited sampling of corticioid taxa and choice of inappropriate molecules, the deliminations and phylogenetic placements of corticioid fungi are not still clear.

Materials and Methods

A schematic representation of the molecular approach to infer phylogenetic relationships of the corticioid fungi is shown in Figure 3.1.

Strains

The species whose sequences were analyzed and examined in this study were listed in Table 3.1. Fungal specimens from Seoul National University Fungus Collection (SFC) and cultured mycelia from fresh samples and various collection centers (CBS, DSMZ, IFO, IMSNU and KCTC) were used. Cultures were grown at 24℃ on MEA in a BOD incubator. Extracellular oxidase reactions were tested according to Stalpers (1978). Many sequences used in this study were retrieved from the database of GenBank or were kindly donated by Mugnier (Rhône-Poulenc, France).

DNA extraction

Total DNAs were extracted from mycelia grown on agar plates covered with cellophane discs or specimens completely dried over mild heat according to the protocol of Lecellier and Silar (1994) with some modifications (Lim and Jung, 1998a; Lee *et al.*, 2000). Agar plate-grown mycelia or dried specimen pieces were taken into

Figure 3.1. Schematic diagram of molecular approaches used in this study. SFC: Seoul National University Fungus Collection.

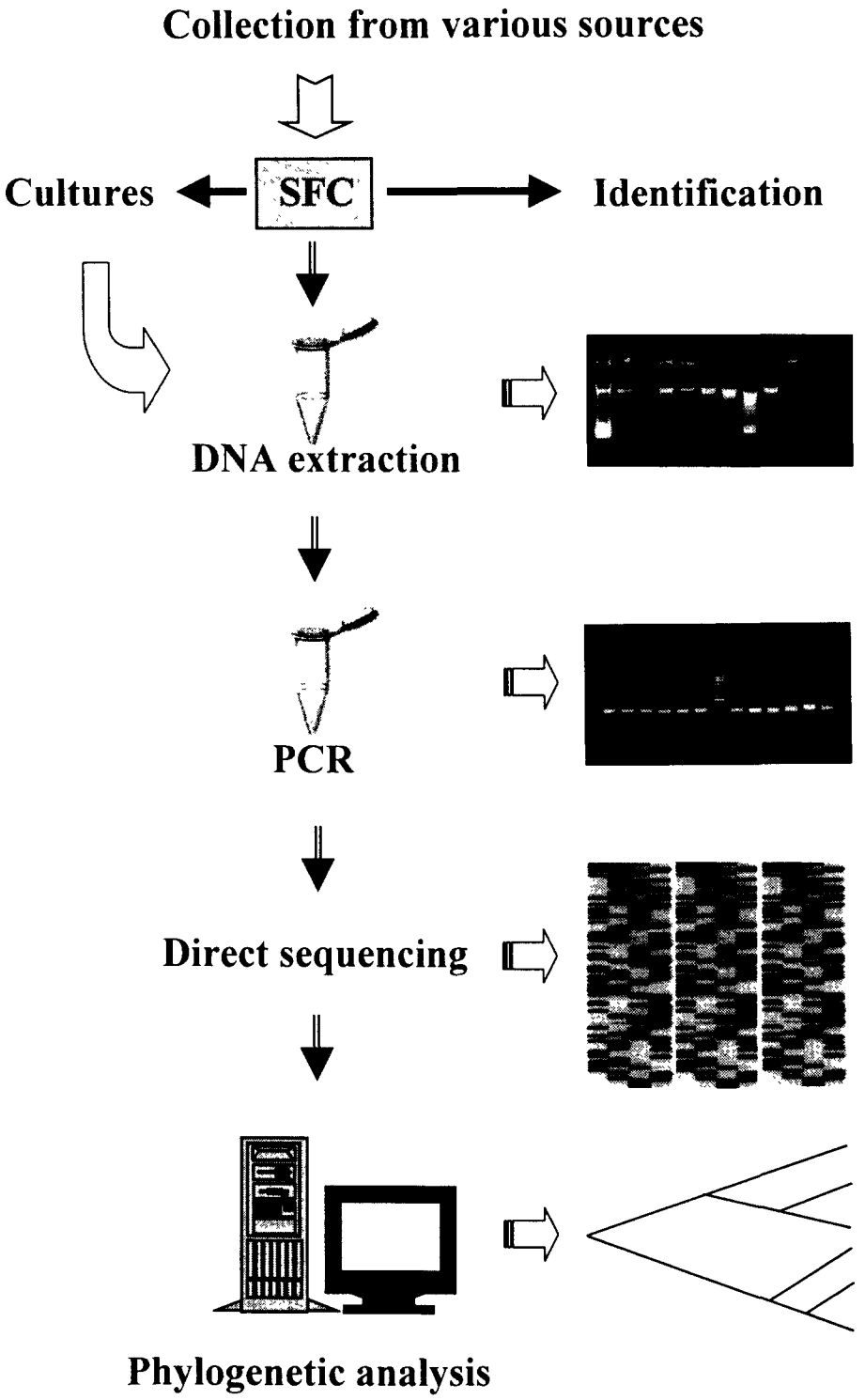

Collection from various sources
Cultures
SFC
Identification
DNA extraction
PCR
Direct sequencing
Phylogenetic analysis

Table 3.1. Strains examined in this study, their source, host, locality and sequence.

Species name	Source	Host	Locality	Sequence
Australohydnum dregeanum	SFC[a] 980804-04	*Quercus* sp.	Chungpyong, Korea	ITS
	SFC 981115-09	*Quercus* sp.	Kangwha Island, Korea	ITS
Chondrosterum purpureum	SFC 971001-13	hardwood	Bukhan Mt., Korea	Nuc-SSU, ITS
Columnocystis ambigua	CBS[b] 136.63	*Picea jezoensis*	Siberia Dzheltulak, Russia	Nuc-SSU
Coniophora arida	SFC 990911-57	*Pinus densiflora*	Munkyong, Korea	Nuc-SSU, ITS
C puteana	IFO[c] 6275			ITS
Coniophora sp.	SFC 991008-30	*Pinus densiflora*	Kangwha Island, Korea	ITS
Cylindrobasidium evolvens	SFC 990121-8	deciduous wood	Kangchon, Korea	Nuc-SSU
	SFC 990320-2	*Zelkova serrata*	Munkyong, Korea	ITS
Cytidia salicina	SFC 9971231-6	*Salix* sp.	Kwangduk Mt., Korea	ITS
Cystidiophorus castaneus	SFC 980119-2	*Pinus densiflora*	Dosan, Korea	Nuc-SSU, ITS
	SFC 990326-7	*Pinus densiflora*	Munkyong, Korea	ITS
Dacryobolus karstenii	SFC 971006-13	*Pinus densiflora*	Myongji Mt., Korea	Nuc-SSU, ITS
Dentipellis fragillis	SFC 970830-9	hardwood	Daemo Mt., Korea	ITS
Dendrocorticium violaceum	SFC 97082309	*Alnus* sp.	Myongji Mt., Korea	ITS
Dendrocorticium sp.(1)	SFC 971231-7	*Zelkova serrata*	Kwangduk Mt., Korea	ITS
	SFC 990326-19	*Zelkova serrata*	Munkyong, Korea	ITS

Species name	Source	Host	Locality	Sequence
Dendrocorticum sp.(2)	SFC 990527-9	*Larix leptolepis*	Kangwha Island, Korea	ITS
Gloeoporus taxicola	SFC 990505-4	*Pinus densiflora*	Munkyong, Korea	ITS
	SFC 000111-3	*Pinus densiflora*	Worak Mt., Korea	Nuc-SSU, ITS
Haplotrichum consperum	SFC 990123-15	*Pinus densiflora*	Kangwha Island, Korea	Nuc-SSU, ITS
Hyphodontia pallidula	SFC 970926-4	*Pinus densiflora*	Mani Mt., Korea	ITS
Hyphodontia sp.	SFC 970927-3	*Quercus* sp.	Wang Mt., Korea	ITS
Hyphodontia sp.	SFC 980522-2	*Quercus* sp.	Bukhan Mt., Korea	ITS
Hypochnicium sp.	SFC 990123-16	*Pinus densiflora*	Kangwha Island, Korea	ITS
Hypochnicium sp.	SFC 990326-32	deciduous tree	Munkyong, Korea	ITS
Irpex hydnoides	SFC 971215-19	*Quercus* sp.	Chiak Mt., Korea	ITS
	SFC 971011-12	*Quercus* sp.	Baekdam Valley, Korea	ITS
I. lacteus	SFC 951007-39	*Prunus serrulata*	Kumak, Korea	ITS
	SFC 971006-12	deciduous tree	Myongji Mt., Korea	ITS
	FP[d] 105049	*Juglans* sp.	North Carolina, U.S.A.	ITS
I. vellereus	CBS 515.92	*Shorea robusta*	India	ITS
Irpex sp.	SFC 990326-32	*Pinus densiflora*	Munkyong, Korea	ITS
Laeticorticium roseum	SFC 991231-9	deciduous tree	Kwangduk Mt., Korea	Nuc-SSU, ITS
Lopharia spadicea	CBS 474.48		France	ITS
L. mirabilis	SFC 990623-11	deciduous tree	Munkyong, Korea	ITS

Species name	Source	Host	Locality	Sequence
L. mirabilis	SFC 991030-8	deciduous tree	Naezang Mt., Korea	Nuc-SSU, ITS
Meruliopsis corium	SFC 970918-1	deciduous tree	Kunwi, Korea	ITS
Mycoacia copelandii	SFC 990710-6	*Quercus mongolica*	Baekdam Valley, Korea	Nuc-SSU, ITS
M. fuscoatra	KCTC[e] 6756	*Betula papyrifera*	Ottawa, Canada	ITS
Phanerochaete calotricha	SFC 970918-2	decayed wood	Kunwi, Korea	ITS
	SFC 991008-20	*Quercus acutissima*	Kangwha Island, Korea	ITS
Pha. chrysorhiza	CBS 220.91	hardwood	Canada	ITS
	SFC 970830-21	hardwood	Daemo Mt., Korea	ITS
Pha. crassa	SFC 981212-8	*Fraxinus rhynchophylla*	Unak Mt., Korea	ITS
	SFC 980531-4	hardwood	Chiri Mt., Korea	ITS
	SFC 990326-41	*Fraxinus rhynchophylla*	Munkyong, Korea	ITS
Pha. gigantea	CBS 429.72	*Pinus sylvestris*	Soestdulinen, Nethelands	ITS
	SFC 971025-2-2	*Pinus densiflora*	Kwangduk Mt., Korea	ITS
Pha. martelliana	CBS 401.50		France	ITS
Pha. sanguinea	SFC 990617-6	*Quercus mongolica*	Kangchon, Korea	ITS
Pha. sordida	SFC 980201-11	*Quercus acutissima*	Kangwha Island, Korea	Nuc-SSU, ITS
Pha. velutina	SFC 970816-20	*Quercus* sp.	Myongji Mt., Korea	ITS
	SFC 990422-5	hardwood	Munkyong, Korea	ITS
Pha. xerophila	SFC 991008-8	*Quercus acutissima*	Kangwha Island, Korea	ITS

Species name	Source	Host	Locality	Sequence
Pha. xerophila	SFC 990326-37	deciduous tree	Munkyong, Korea	ITS
Phanerochaete sp.	SFC 910821-19	*Quercus* sp.	Naezang Mt., Korea	ITS
Phanerochaete sp.	SFC 900616-5	deciduous tree	Korea	ITS
Phanerochaete sp	SFC 990326-39	*Fraxinus rhynchophylla*	Munkyong, Korea	ITS
Phanerochaete sp.	SFC 990228-9	*Alnus* sp.	Kangwha Island, Korea	ITS
Phlebia brevispora	CBS 509.92	*Pinus elliota*	Florida, USA	ITS
Phl. chrysocrea	SFC 971231-17	*Quercus* sp.	Kwangduk Mt., Korea	ITS
	SFC 970830-16	*Quercus* sp.	Daemo Mt., Korea	ITS
	CBS 114.40	decayed wood	USA	ITS
Phl. livida	CBS 164.56		France	ITS
Phl. phlebioides	CBS 164.65	*Pinus strobus*	USA	ITS
Phl. radiata	KCTC 6759	*Pagus sylvatica*	Teuboburger, German	ITS
Phl. rufa	SFC 940702-21	hardwood	Korea	ITS
	SFC 980527-4	*Quercus serrata*	Bukhan Mt., Korea	ITS
Phl. tremellosus	KCTC 6762	*Pagus sylvatica*	Westfalen, German	ITS
	SFC 970823-7	hardwood	Myongji Mt., Korea	ITS
Plicaturopsis crispa	SFC 980102-5	*Quercus acutissima*	Kangwha Island, Korea	ITS
	SFC 990320-8	*Castanea crenata*	Munkyong, Korea	Nuc-SSU
	SFC 971215-14	*Quercus* sp.	Chiak Mt., Korea	ITS

Species name	Source	Host	Locality	Sequence
Pseudomerulius aureus	SFC 970927-4	*Pinus densiflora*	Wang Mt., Korea	Nuc-SSU, ITS
Pulchericium caeruleum	IFO 4974		Japan	ITS
Punctularia atropurpurascens	IFO 31076	*Prunus yedoensis*	Japan	ITS
P. strigoso-zonata	SFC 970816-3	*Quercus* sp.	Myongji Mt., Korea	ITS
Resinicium bicolor	HHB[f] 10103			Nuc-SSU, ITS
	CBS 253.73	*Pinus sylvestris*	Staverden, Nethelands	ITS
Schizopora flavipora	SFC 970926-4	*Quercus mongolica*	Bukhan Mt., Korea	ITS
	SFC 980125-7	*Pinus densiflora*	Ilyoung, Korea	ITS
S. paradoxa	SFC 970719-2	*Prunus leveilleana*	Bukhan Mt., Korea	ITS
	SFC 970816-2	*Castanea crenata*	Kangwha Island, Korea	ITS
	SFC 970201-10	*Quercus serrata*	Moak Mt., Korea	ITS
	DSMZ[g] 5099	*Quercus* sp.	Germany	ITS
Schizopora sp.	SFC 970924-1	*Pinus densiflora*	Bukhan Mt., Korea	ITS
Schizopora sp.	SFC 991008-27	*Pinus densiflora*	Kangwha Island, Korea	ITS
Sistotrema diademiforum	SFC 990521-13	*Quercus* sp.	Munkyong, Korea	Nuc-SSU
Stereum complicatum	CBS 415.61	*Carpinus betulus*	Maryland, USA	ITS
	CBS 451.80	*Eucalyptus* sp	Colombia, Monserrate, USA	ITS
	SFC 980201-4	*Quercus acutissima*	Kangwha Island, Korea	ITS
S. gausapatum	SFC 980929-2	*Quercus*	Kwanak Mt., Korea	ITS

Species name	Source	Host	Locality	Sequence
S. hirsutum	SFC 971011-14	deciduous wood	Kangwondo, Korea	ITS
S. lobatum	CBS 361.36			ITS
	SFC 991008-18	*Quercus acutissima*	Kangwha Island, Korea	ITS
S. ochraceo-flavum	SFC 980815-21	deciduous wood	Wui Island, Korea	ITS
S. ostrea	SFC 960726-2		USA	ITS
S. peculiare	CBS 876.84	*Quercus*	USSR, Lazo NRA[h]	ITS
	SFC 980201-6	*Quercus acutissima*	Kangwha Island, Korea	ITS
	SFC 991008-4	*Quercus acutissima*	Kangwha Island, Korea	ITS
S. rameale	CBS 119.16		Wisconsin, USA	ITS
S. reflexulum	CBS 877.84		Port-Cros, France	ITS
	CBS 878.84		Port-Cros, France	ITS
S. rugosum	CBS 474.72	angiosperm wood	Baarn, Netherlands	ITS
S. sanguinolentum	SFC 980816-9	*Pinus densiflora*	Wui Island, Korea	ITS
S. striatum	CBS 368.58		Louisiana, USA	ITS
	CBS 418.61	*Carpinus caroliniana*	Louisiana, USA	ITS
S. subtomentosum	CBS 150.79	*Alnus glutinosa*	Lelystad, Netherlands	ITS
	SFC 960316-15-2	deciduous wood	Kyoungido, Korea	Nuc-SSU, ITS
	SFC 971030-11	*Quercus* sp.	Kangwondo, Korea	ITS
	SFC 950830-10-2	*Carpinus laxiflora*	Chiri Mt., Korea	ITS

Species name	Source	Host	Locality	Sequence
S. subtomentosum	SFC 990709-12	*Carpinus laxiflora*	Kangwondo, Korea	ITS
Unidentified species	SFC 990320-18	*Lindera obtusiloba*	Munkyong, Korea	ITS
Unidentified species	SFC 990521-7	*Zanthoxylum peperium*	Munkyong, Korea	ITS
Vuilleminia comedens	SFC 990326-21	deciduous wood	Munkyong, Korea	Nuc-SSU, ITS
Xylobolus annosus	CBS 490.76	decaying wood	Japan	ITS
X. frustulatum	SFC 981217-38	*Quercus* sp.	Worak Mt., Korea	ITS
	CBS 670.85	*Quercus* sp		ITS
X. illudens	CBS 360.36			ITS
	SFC 990320-20	*Quercus* sp.	Mungyong, Korea	ITS
X. subpileatus	CBS 415.34		USA	ITS

[a] Seoul National University Fungus Collections

[b] Centraalbureau voor Schimmelcultures

[c] Institute for Fermentation, Osaka

[d] USDA Forest Products Laboratory

[e] Korean Collection for Type Cultures

[f] H. H. Burdsall

[g] Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

[h] Nature Reserve Area

an Eppendorf tube where 700 μl lysis buffer [100 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), 3 % SDS] was added. The mixture was vortexed for 30s and incubation at 70℃ for 10 min followed by freezing in liquid nitrogen for 30s. This process was repeated three times. DNA was purified through phenol-chloroform extraction. RNA was removed by RNase A treatment for 10 min before chloroform extraction. The purified DNA was precipitated with 0.5 vol of isopropanol and then centrifuged at 12000 rpm for 10 min at room temperature. The supernatant was removed and the pellet was washed with 70% ethanol, dried in air and then resuspended in 50 μl of TE.

PCR amplification and sequencing

PCR was performed at least twice to minimize the artifact. For the amplification of the 1st PCR, 20 μl PCR mixtures [10 mM Tris-HCl (pH 8.8), 150 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM dNTP mixture, each 0.2 nM of the primer, 0.2 units of DyNAzyme, about 100 ng of template DNA] were prepared using the DyNAzyme™ II DNA polymerase kit (Finnzymes). Two different primer sets [NS1, ITS2 for SSU regions and NS7, LW2 (Jung, 1999) for ITS region] were used (Fig. 3.2). For relief of amplification inhibition in PCR, 0.3% bovine serum albumin (BSA) was added (Kreader 1996). Amplifications were performed in a PTC-100 Programmable Thermal Controller (MJ Research Inc.). PCR proceeded as follows: 5 min for initial extension step at 94℃, 1 min for denaturing at 94℃, 1 min for annealing at 50℃, 2

min for extension at 72℃ and 10 min for the final extension step at 72℃ after 30 cycles. Second PCR was carried out in 50 μl reaction mixtures using nested primer sets [NS1, NS8 for SSU region and ITS5 and LW1 (Jung, 1999) for ITS region] and 1 μl of 1st PCR products as template DNA. BSA was not used in the 2nd step. The cycle reaction was as follows: 4 min for initial extension step at 94℃, 1 min for denaturing at 94℃, 1 min for annealing at 52℃, 1 min 30s for extension at 72℃ and 15 min for the final extension step at 72℃ after 35 cycles.

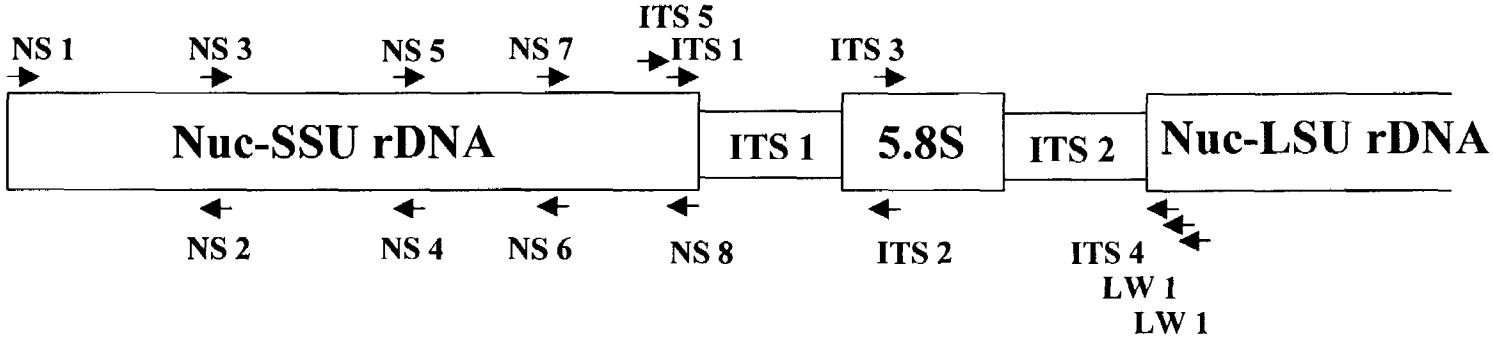
Each product was observed on 0.4% agarose gel containing EtBr in Tris-acetate EDTA (TAE) buffer. The PCR product sizes were determined by comparison to 1 kb DNA marker (GIBCO BRL). The presence of a single bright band in each lane was a check for successful amplification. Second PCR products were purified through PCR purification kit (NucleoGen) and directly sequenced by the thermal cyclic termination method with ^{35}S-labled ATP (Hillis *et al.* 1996), using Top^{TM} DNA sequencing kit (Bioneer Corp. Korea). For sequencing of SSU region, eight primers from NS1 to NS8 were used (White *et al.* 1990). For ITS regions, four primers from ITS1 to ITS4 were used (White *et al.* 1990) (Fig. 3.2).

Phylogenetic analyses

Sequences generated from materials and retrieved from GenBank were visually aligned after initial alignment by the program CLUSTAL X (Thompson *et al.*, 1997).

Ambiguously aligned regions were excluded from the following analyses. Phylogenetic relationships were estimated from the aligned sequences for each data set using PAUP*4.0b4a (Swofford, 1999), treating all alignment gaps as missing data or fifth bases. Phylogenetic analyses of data sets were done using both distance and parsimony methods. Neighbor-joining method (Saitou and Nei, 1987) with distance option and parsimony method with heuristic option were applied for phylogenetic analyses. Strengths of internal branches found in distance and parsimony analyses were statistically tested by the bootstrap analyses of 1000 replications (Felsenstein, 1985; Hillis and Bull, 1993). In order to provide additional statistical support for inferred phylogenetic relationships, a Kishino-Hasegawa test (Kishino and Hasegawa, 1989) implemented in PAUP*4.0b4a (Swofford, 1999) was performed. Constraint trees were generated using MacClade 3.04 (Maddison and Maddison, 1992) and PAUP*4.0b4a (Swofford, 1999) to depict specific phylogenetic hypotheses.

Figure 3.2. Locations and sequences of primers for the amplification and sequencing. NS and ITS primers were those designed by White *et al.*, (1990) and LW primers were designed by Jung (1999).



Forward Primers	Reverse Primers
NS 1: 5'GTAGTCATATGCTTGTCTC3'	NS 2: 5'GGCTGCTGGCACCAGACTTGC3'
NS 3: 5'GCAAGTCTGGTGCCAGCAGCC3'	NS 4: 5'CTTCCGTCAATTCCTTTAAG3'
NS 5: 5'AACTTAAAGGAATTGACGGAAG3'	NS 6: 5'GCATCACAGACCTGTTATTGCCTC3'
NS 7: 5'GAGGCAATAACAGGTCTGTGATGC3'	NS 8: 5'TCCGCAGGTTCACCTACGGA3'
ITS 5: 5'GGAAGTAAAAGTCGTAACAAGG3'	ITS 2: 5'GCTGCGTTCTTCATCGATGC3'
ITS 1: 5'TCCGTAGGTGAACCTGCGG3'	ITS 4: 5'TCCTCCGCTTATTGATATGC3'
ITS 3: 5'GCATCGATGAAGAACGCAGC3'	LW 1: 5'CCGCTTCACTCGCCGTTAC3'
	LW 2: 5'CATTCCCAAACAACTCGACTC3'

Results and Discussion

I. Phylogeny of Corticioid Fungi based on Nuclear Ribosomal DNA Sequences

In recent years, there have been many phylogenetic studies centered on specific groups of homobasidiomycetes. Phylogenetic analysis based on morphology was accomplished by Parmasto (1995) and molecular analyses basded on ITS, nuc-ssu and mt-ssu rDNA sequences for some of corticioid fungi were accomplished by Boidin *et al.* (1998) and Hibbett *et al.* (1997). Howeverver, there has been no comprehensive analysis of the entire group of corticioid fungi and restuls obtained from DNA sequencing were still only fragmentary. Most of previous results showed that there was considerable homoplasy in morphological characters. The present study aims to provide imformation on relationships between corticioid fungi and other fungi of homobasidiomycetes and to build a phylogenetic outline and perspective of the corticioid fungi.

The amplified products from the nuc-ssu rDNA of eighteen corticioid fungi were mostly 1.8 kb in length but the product from *Chondrosterum purpureum* was exceptionally 2.3 kb in size (Fig. 3.3). Sequence analysis of the PCR product for *C. purpureum* indicated the presence of an intron. The intron existed between primers NS5 and NS6 and its length was 429 bp (Fig. 3.4). This accorded with the previous

result from *C. purpurem* (CBS 427.72) by Yoon (1998). Through BLAST search of NCBI, it was predicted as Group I self-splicing intron. According to Hibbett (1996), introns were also found at a same aligned position 943 in *Letinellus omphalodes*, *L. montanus*, *Clavicorona pyxidata* and *Panellus stypticus* of the Hymenomycetes. This intron was also shown in *Schizophyllum* (Nakasone, 1996). Initially, 75 sequences belonging to the homobasidiomycetes were analysed using *Tremella foliacea* as an outgroup. Phylogenetic relationships were inferred by the neighbor-joining (NJ) method of PAUP* 4.0b4a (Swofford, 1999).

NJ tree indicated that there were ten distinct clades which mostly correspondent with the results of Hibbett and Thorn (2001). They have tentatively divided the homobasidiomycetes into eight major clades based on the results of their own and other molecular studies. However, there were taxonomically controversial groups to be studied more. The corticioid fungi were one of them. In this study, the results of Hibbett and Thorn (2001) were used as a framework for developing a preliminay phylogenetic outline of the homobasidiomycetes. Fig. 3.5 showed that the corticioid fungi were distributed into eight major clades. Five clades of them were accordant with the tentative classification system of Hibbett and Thorn (2001) but peniophoroid, laeticorticioid and botryobasidioid clades were newly recognized in this study (Fig. 3.5).

The russuloid clade was weakly supported as monophyletic but was congruent with the results of previous papers (Boidin *et al.*, 1998; Gardes and Bruns, 1996; Hibbett *et*

Figure 3.3. PCR products of corticioid fungi amplified by NS1 and NS8 primers. Lanes labeled M on both ends are 1 kb ladders. Lane 1. *Stereum subtomentosum*; Lane 2. *Resinicium bicolor*; Lane 3. *Chondrostereum purpureum*; Lane 4. *Cylindrobasidium evolvens*; Lane 5. *Mycoacia copelandii*; Lane 6. *Plicaturopsis crispa*; Lane 7. *Coniophora arida*; Lane 8. *Pseudomerulius aureus*; Lane 9. *Laeticorticium roseum*; Lane 10. *Vuilleminia comedens*; Lane 11. *Lopharia mirabilis*; Lane 12. *Columnocystis ambigua*; Lane 13. *Gloeophorus taxicola*; Lane 14. *Phanerochaete sordida*; Lane 15. *Cystidiophorus castanea*; Lane 16. *Dacryobolus karstenii*; Lane 17. *Haplotrichum consperum*; Lane 18. *Sistotrema diademiferum*. *Chondrostereum purpureum* of Lane 3 contains an intron.

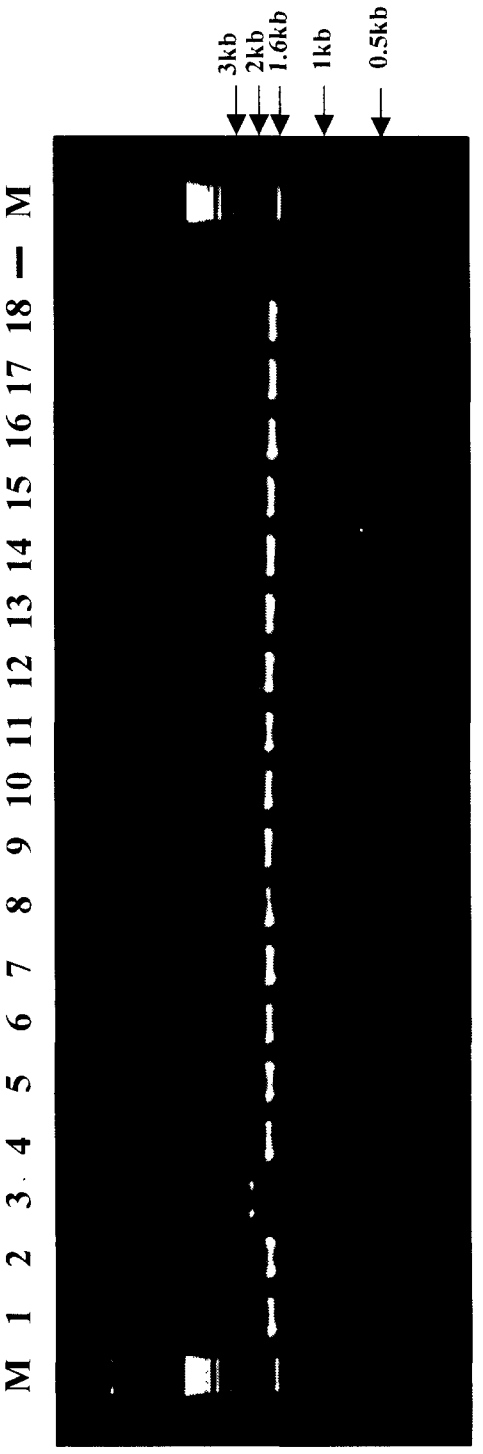
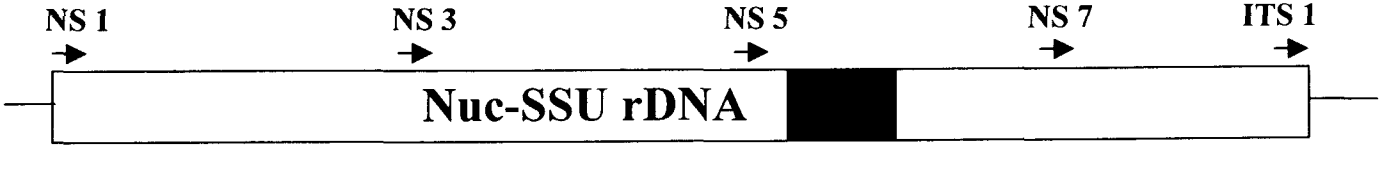

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 — M
3kb
2kb
1.6kb
1kb
0.5kb

Figure 3.4. Position and sequence of group 1 intron found in the Nuc-SSU rDNA of *Chondrostereum purpureum*



5'AAACTTTTACTAGTTTTGCCGCAGTAACTCTGCACCGAACAGCAGCCTGAAAAGGTG
AGGTGGTTCAAGCCCAGTTAGACTACTTTGTAGTGCTTTCTAAATCTAAATGCTAGTCA
TCTTTGTTAACAGAGATGGCAACACCCTCAAATTGCGGGGAGCTCCTTAGAGCTTTAAC
TACCGCCTATACATGGAAACATAGCATAGTGCACCAGGGTAATGACCTCGGGTATGGT
AAAAACGTTAAAGATTTGGACAATCCGCAGCCAAGCCTCCTTTTGTGTAGTAATATATA
AAAGGTGGAAGGTTCAGAGACTAGATGGGGGTGGGTTGCACATAACCCAAAACAAAAC
TTAATGTGTGGCTTAAGGTATAGTCCGGGCTATTAGCGAAAGCTATGTAGCATGTAAGG
TAAACCCTAATAACAAAACG3'

GROUP 1 INTRON

al., 1997). This clade contained representatives of Russulaceae and some of Aphyllophorales that had tremendous diversities of fruitbody morphology. Members of this clade are unique in their possession of amyloid spores (Eriksson and Ryvarden, 1973, 1975, 1976; Eriksson *et al.*, 1984; Ginns and Lefebvre, 1993; Hibbett and Donoghue, 1995; Hjortstam *et al.*, 1988; Maekawa, 1993, 1994). Hibbett and Thorn (2001) included *Peniophora nuda* and *Scytinostroma alutum* into the russuloid clade. However, these two species retained their own position as sister taxa to the russuloid clade in this study (Fig. 3.5) and possess non-amyloid spores. Therefore, *Peniphora* and *Scytinostroma* might have to be excluded from the russuloid clade.

The corticioid fungi in the hymenochaetoid clade contained *Resinicium* and *Hyphodontia*, which was fully supported by high bootstrap value of 100%. This clade was primarily composed of lignicolous species and appeared to be united by the possession of imperforate parenthosomes (Hibbett and Donoghue, 1995; Langer, 1994).

The euagarics clade was composed mostly of Agaricales, Aphyllohorales and Gasteromycetes. The corticioid fungi in this clade included *Chondrosterum purpureum*, *Cystostereum murraii*, *Athelia bombacina*, *Cylindrobasidium evolvens*, *Mycoacia copelandii* and *Plicaturopsis crispa*. Monophyly of the euagarics clade was weakly supported in this study (bootstrap value of 60%) but strongly supported in the analysis of Hibbett *et al.* (1997). Phylogenetic position of *P. crispa* was very interesting. Although bootstrap support was weak, it was placed basal to the euagarics clade in both trees based on nuc-ssu rDNA and ITS sequences (Figs. 3.5 and 3.11). This species

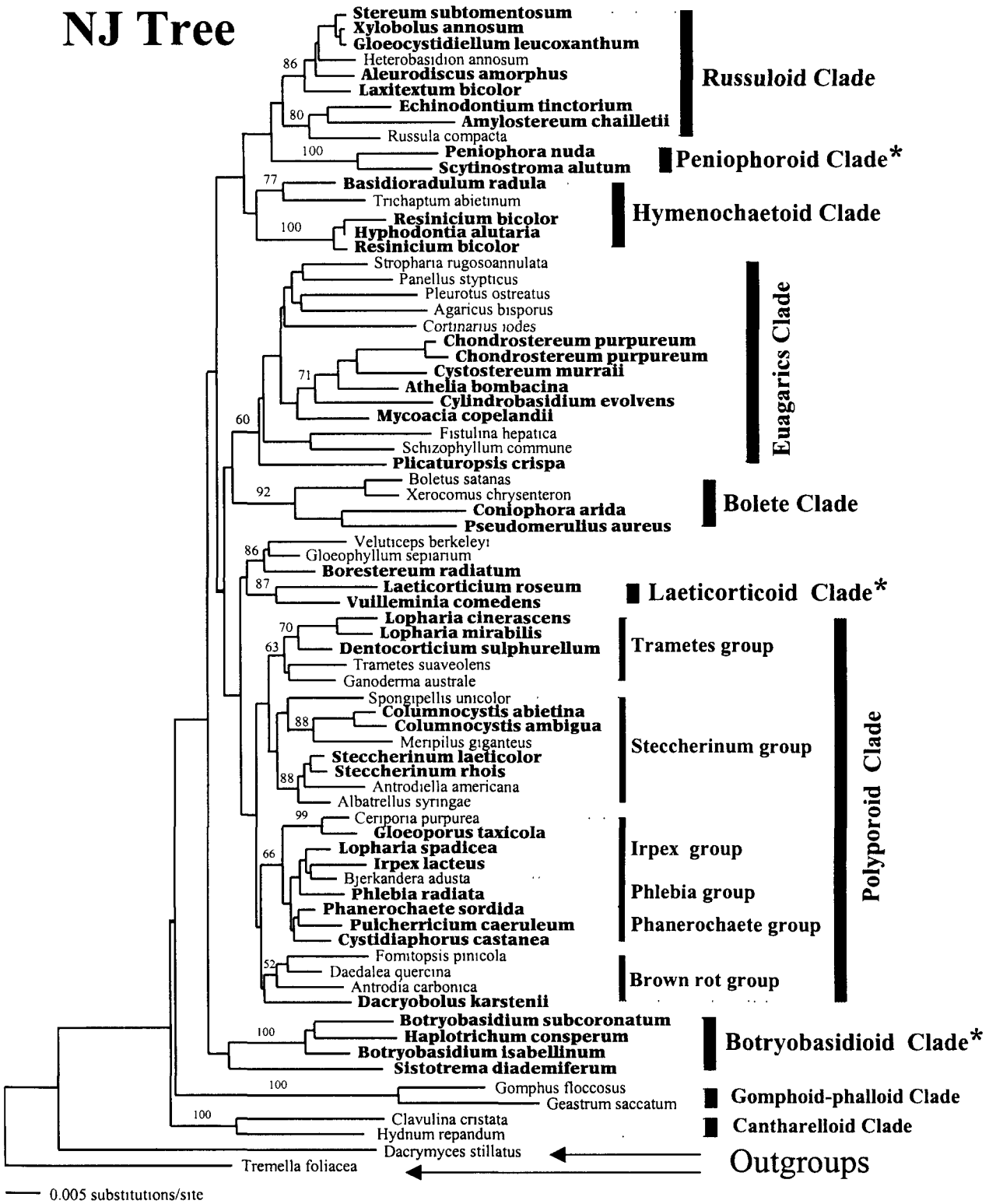
is characterized by pileate-cupulate-subresupinate fruitbodies and radial, bifurcate gill-like ridge hymenophore. This result suggested that *P. crispa* might be an ancestor of agaric lineage.

The bolete clade included members of Gomphidiaceae, Coniophoraceae, Hymenogastrales, Melanogastraceae and Calostomataceae, as well as many additional exemplars of the groups (Hibbett and Thorn, 2001) and formed the sister group of the euagarics clade. Monophyly of the bolete clade has been strongly supported at 92% confidence level. In this study, only two species were sampled, *Coniophora arida* and *Pseudomerulius aureus*, representing the family Coniophoraceae.

The laeticorticioid clade was newly found in this study. Monophyly of the laeticorticioid clade has been strongly supported at 87% confidence level. This clade was placed next to the clade including *Boreostereum, Veluticeps* and *Gloeophyllum*. This sister clade was first introduced by Yoon *et al.* (2001) who sampled only three species representing the group H (*Gloeophyllum sepiarium, Boreostereum radiatum* and *Veluticeps berkeleyi*). Phylogenetic placement of the group H was distinct outside of the polyporoid clade. Hibbett and Thorn (2001) treated members of this clade as members of the polyporoid clade according to the results of Boidin *et al.* (1998). Although the sister clade was supported by 86% confidence level, monophyly of two clades was poorly supported so that sister clade can be excluded the laeticorticioid clade.

The large clade is primarily composed of polypores and corticioid fungi, but also

Figure 3.5. Phylogenetic relationships between corticioid fungi and other members of Homobasidiomycetes inferred by neighbor-joining method. This phylogenetic tree was generated using nuclear small subunit ribosomal RNA gene sequences. Names in boldface indicate corticioid fungi. Clades indicated by asterisks were newly recognized in this study. Numbers on branches are frequencies (%) of statistical occurrence out of 1,000 bootstrap replicates (values <50% are not shown). Branch lengths correspond to numbers of nucleotide substitutions. *Dacrymyces stillatus* and *Tremella foliacea* were used as outgroups.


NJ Tree
Stereum subtomentosum
Xylobolus annosum
Gloeocystidiellum leucoxanthum
Heterobasidion annosum
Aleurodiscus amorphus
Laxitextum bicolor
Echinodontium tinctorium
Amylostereum chailletii
Russula compacta
Peniophora nuda
Scytinostroma alutum
Basidioradulum radula
Trichaptum abietinum
Resinicium bicolor
Hyphodontia alutaria
Resinicium bicolor
Stropharia rugosoannulata
Panellus stypticus
Pleurotus ostreatus
Agaricus bisporus
Cortinarius iodes
Chondrostereum purpureum
Chondrostereum purpureum
Cystostereum murraii
Athelia bombacina
Cylindrobasidium evolvens
Mycoacia copelandii
Fistulina hepatica
Schizophyllum commune
Plicaturopsis crispa
Boletus satanas
Xerocomus chrysenteron
Coniophora arida
Pseudomerulius aureus
Veluticeps berkeleyi
Gloeophyllum sepiarium
Borestereum radiatum
Laeticorticium roseum
Vuilleminia comedens
Lopharia cinerascens
Lopharia mirabilis
Dentocorticium sulphurellum
Trametes suaveolens
Ganoderma australe
Spongipellis unicolor
Columnocystis abietina
Columnocystis ambigua
Meripilus giganteus
Steccherinum laeticolor
Steccherinum rhois
Antrodiella americana
Albatrellus syringae
Ceriporia purpurea
Gloeoporus taxicola
Lopharia spadicea
Irpex lacteus
Bjerkandera adusta
Phlebia radiata
Phanerochaete sordida
Pulcherricium caeruleum
Cystidiaphorus castanea
Fomitopsis pinicola
Daedalea quercina
Antrodia carbonica
Dacryobolus karstenii
Botryobasidium subcoronatum
Haplotrichum consperum
Botryobasidium isabellinum
Sistotrema diademiferum
Gomphus floccosus
Geastrum saccatum
Clavulina cristata
Hydnum repandum
Dacrymyces stillatus
Tremella foliacea
86
80
100
77
100
60
71
92
86
87
70
63
88
88
99
66
52
100
100
100
Russuloid Clade
Peniophoroid Clade*
Hymenochaetoid Clade
Euagarics Clade
Bolete Clade
Laeticorticoid Clade*
Trametes group
Steccherinum group
Irpex group
Phlebia group
Phanerochaete group
Brown rot group
Polyporoid Clade
Botryobasidioid Clade*
Gomphoid-phalloid Clade
Cantharelloid Clade
Outgroups
0.005 substitutions/site

included the gilled mushrooms *Lentinus, Panus* and *Faerberia* (Hibbett and Thorn, 2001). The phylogenetic tree (Fig. 3.5) showed that this clade was divided largely into six groups. The first group was *Trametes* group including *Lopharia mirabilis, L. cinerascens*, *Dentocorticium sulfphurellum*, unidentified *Dentocorticium* species and other polypores. *Trametes* was a well-defined group and closely related to *Ganoderma* (Ko and Jung, 1999; Ko, 2000). Some corticioid fungi were gouped into the *Trametes* group but apparently separated from poroid *Trametes* and *Ganoderma* (Fig. 3.5). The second group consisted of *Steccherinum*, *Antrodiella*, *Albatrellus, Meripilus, Columnocystis*, *Spongipellis, Hyphoderma* and *Hypochnicium*. While *Antrodiella* is characterized by poroid light-colored basdidiocarps with small pores and absence of skeletocystidia, *Steccherinum* is characterized by odontioid hymenophores and fairly large incrusted skeletocystidia. The type of rot and basidiocarp morphology bring *Antrodiella* close to the poroid *Flaviporus* and *Junghuhnia*, which, in turn, link *Antrodiella* closely with the hydnoid *Flavodon*, *Irpex* and *Steccherinum* (Ryvarden, 1991). The absence of skeletocystidia separates *Antrodiella* from *Junghuhnia, Flaviporus* and *Steccherinum* (Dai and Niemelä, 1997). However, *Steccherinum* and *Antrodiella* formed a monophyletic group, which was congruent with the results of previous papers based on molecular and morphological data (Boidin *et al.*, 1998; Johannesson *et al.*, 2000; Kim, 2001). Although *Albatrellus* consisted of two separate lineages (Hibbett and Thorn, 2001), this taxon is closely related to *Steccherinum* and *Antrodiella*. The other group contained *Lopharia spadicea, Irpex, Phlebia,*

Phanerochaete, Pulchericium, Cystidiophorus and polypores *Ceriporia, Gloeoporus* and *Bjerkandera.* Monophyly of this group was moderately supported by 66% bootstrap value and *Irpex* group, *Phlebia* group and *Phanerochaete* group (Fig 2.3) were included here. The last group was characterized by a common character, brown rot habit, and included *Fomitopsis, Daedalea, Antrodia* and the corticioid fungus *Dacryobolus karstenii. Dacryobolus karstenii* is characterized by long and thich-walled cystidia, slender basidia and allantoid sporeses (Lim *et al.*, 2000; Maekawa, 1993). This species mainly occurs on coniferous trees such as *Abies, Picea* and *Pinus* but rarely on a deciduous tree, *Prunus sargentii* (SFC-980929-17). According to Ginns and Lefebvre (1993), *D. karstenii* is associated with a brown cubical rot that correlated with molecular data (Fig 2.3).

The botryobasidioid clade was also newly found in this study although its branch was not strongly supported by bootstrap. This clade consisted of *Botryobasidium* and *Sistotrema. Botryobasidium* and *Tulasnella* were both corticioid fungi that had imperforate parenthesomes (Wells 1994) and grouped into the cantharelloid clade by Hibbett and Thorn (2001). A distinctive feature of the cantharelloid clade was the possession of stichic basdidia, which have been observed in *Cantharellus, Clavulina, Craterellus, Hydnum* and *Multiclvula* but not in *Botryobasidium.* Due to the urniform basidia with 6-8 sterigmata as well as above results, *Botryobasidium* and *Sistotrema* have apparently close relationships

II. Molecular Phylogeny of Corticioid Fungi in the Russuloid Clade

1. Molecular Phylogeny of *Stereum* based on Nuclear Ribosomal DNA Sequences

Stereum is a white rot fungus, which has been implicated in the deterioration of forest products or contributed recycling of dead matter as a decomposer (Alexopoulos *et al.* 1996). Many species of *Stereum* occurred on corticate limbs or trunks, indicating a fairly early position in the succession of decay fungi. Some of *Stereum* have been reported to be able to decay poles (Zabel *et al.*, 1980) and showed host specificity (Chamuris 1988).

Fries adopted the generic concept of *Stereum* Hill: Pers. in 1838 as an infrageneric division of *Thelphora*. *Stereum* in that time included stipitate, pileate, effuso-relfexed and resupinate forms and such Fries' system had been widely used by the early twentieth century. The genus *Stereum* forms the core of the family Stereaceae Pilát. The family Stereaceae made by Donk (1964) and Talbot (1971, 1973) was not a natural group and some other genera distantly related to *Stereum* were usually included in this family. For many years, *Stereum* represented a heterogeneous assemblage of distantly related fungi. Between 1955 and 1968, however, the concept of *Stereum* started to become increasingly narrow. Lentz (1955) adopted a concept of *Stereum* that excluded

stipitate forms. By 1968, *Stereum* represented a homogenous cluster of species that could be interpreted as comprising a natural or monophyletic group (Chamuris, 1985b). Chamuris (1985b) proposed that only *Stereum* and *Xylobolus* probably belonged to the Stereaceae. This genus is well-defined by the combination of a monomitic hyphal system with simple-septate hyphae, oleiferous pseudocystidia, pseudoacanthohyphidia or acanthohyphidia and smooth amyloid spore (Eriksson *et al.*, 1984; Hjortstam *et al.*, 1988). It appears that the hyphal system in *Stereum* dose not fit readily into the monomitic and dimitic types proposed by Corner (1932). Talbot (1973) stated that *Stereum* had a dimitic hyphal system. Boidin *et al.* (1979) also reported a dimitic system but Eriksson *et al.* (1984) described that *Stereum* had a monomitic hyphal system.

The genus *Stereum* was once divided into three sections, *Stereum*, *Cruentata* and *Phellina* by Burt (1920). Species belonging to the section *Stereum* were *S. hirsutum*, *S. rameale*, *S. striatum*, *S. ochraceo-flavum*, *S. subtomentosum* and *S. ostrea*. Members of the section *Cruentata* had a bleeding hymenium when bruised and included *S. sanguinolentum*, *S. gausapatum* and *S. rugosum*. The section *Cruentata* was segregated under the name *Haematostereum* from *Stereum* by Pouzar at generic level, which had been rejected by those who studied the sterea in a comparative fashion (Chamuris, 1985b; Eriksson *et al.*, 1984; Jülich and Stalpers, 1980; Parmasato, 1968; Rattan, 1977; Welden, 1971). Species of the section *Phellina* were *S. frustulosum* and *S. subpileatum* and had a browinsh flesh, acanathohyphidia and small spores. This section, however,

was rearranged under the name *Xylobolus* Karst. by Boidin at generic level (Reid, 1971). Bourdot and Galzin (1921) distinguished two sections, section *Luteola* which contained the type species and section *Cruentata* which contained the species that redden upon injury. This distinction between the species was based on external appearance, while microscopic characteristics were rarely used because they display little variation under microscope (Boidin *et al.*, 1979).

Welden (1971) suggested that, based on the existence of gross morphological continua, several North American species of *Stereum* were grouped into four complexes. *Stereum striatum* complex consists of *S. striatum, S. ochraceo-flavum* and *S. sulphuratum*. *Stereum striatum* complex is characterized by relatively thin basidiocarps, a tomentum of white long hairs and almost completely absent cuticle. *Stereum frustulatum* complex characterized by hard, woody or stony, and perennial basidiocarps consists of *S. frustulatum* and *S. subpileatum*. *Stereum ostrea* complex consists of *S. ostrea, S. fasciatum, S. lobatum* and *S. australe*. The last *Stereum hirsutum* complex contains *S. hirsutum, S. complicatum, S. subtomentosum, S. versicolor* and *S. gausapatum*. Chamuris had treated *S. frustulatum* and its relatives as *Xylobulus* on the basis of their cultural and basiciocap features (Chamuris, 1988) and excluded *S. gausapatum* from the *S. hirsutum* complex (Chamuris, 1985a).

In North Europe, *Stereum* was divided into two groups based on hymenium bleeding. *Stereum rugosum* group has a bleeding hymenium and contains *S. rugosum, S. snaguinolentum* and *S. gausapatum*, and *Stereum hirsutum* group has no bleeding

hymenium and includes *S. hirsutum* and *S. subtomentosum* (Eriksson *et al.*, 1984). Boidin *et al.* (1979) divided *Stereum* into three subgenera based on hyphidium type. The subgenus *Stereum* is characterized by simple hyphidia with no prongs, the subgenus *Aculeatostereum* by pseudoacanthohyphidia with a few apical prongs and the last subgenus *Acanthostereum* by acanthohyphidia with numerous prongs. Therefore, the phylogenetic relationships of *Stereum* based on bleeding, the existence of gross morphology and hyphipidium type are not certain yet.

The aim of the present study was to evaluate the traditional taxonomic concept of the *Stereum* subgenera using the nuclear small subunit rDNA and ITS region sequences. After 2nd PCR amplifications of the nuc-ssu rDNA, PCR product of *S. subtomentosum* showed a single band of about 1.8kb in size. Phylogenetic analysis of the complete nuc-ssu rDNA data set included 1,727 characters for each of 45 taxa. The neighbor-joining tree, calculated by Kimura's (1980) two-parameter distance, is presented in Fig. 3.6A. In the tree of Fig. 3.6A, four strains of *Stereum* were clustered into a single clade and their relationships were supported by the high bootstrap value of 92%. *Stereum annosum* and *Aleurodiscus botryosus*, which were clustered together and supported by the confidence level of 96%, developed a sister group to the *Stereum* clade. *Stereum annosum*, however, is not a correct name. Its correct name is *Xylobolus annosus* (Berk.) Boid. which has been known from India, Asia, Australia, Mexico, Central America and Louisiana (Chamuris, 1988). So the genus *Stereum* inferred from the nuc-ssu rDNA formed a monophyletic group that was placed in the russuloid clade (Hibbett

and Thorn 2001). This result agreed well with the one of the previously published paper by Hibbett *et al.* (1997).

Second PCR products of all species analyzed in ITS region ranged from approximately 600 to 650 base pairs. Phylogenetic analyses were executed with unambiguously aligned sequences of 693 characters for 35 taxa. The neighbor-joining (NJ) tree based on ITS sequence calculated by Kimura's (1980) two-parameter distance is shown in Fig. 3.6B and one of 219 most parsimonious trees (tree length = 456 steps, CI = 0.7018, RI = 0.8294, RC = 0.5820) constructed by parsimony (MP) analysis in Fig. 3.7. Both trees were obtained by PAUP* 4.0b4a (Swofford, 1999). *Heterobasidium annosum* was used as an outgroup to root both phylogenetic trees. Figs. 3.6B and 3.7 also showed that the genus *Stereum* formed a monophyletic clade. Bootstrap support was excellent for this clade with values of above 99% respectively in both trees. The genus *Xylobolus* formed a sister group to the *Stererum* clade. However, *S. peculiare* was placed within the *Xylobolus* clade with 100% bootstrap value in both trees.

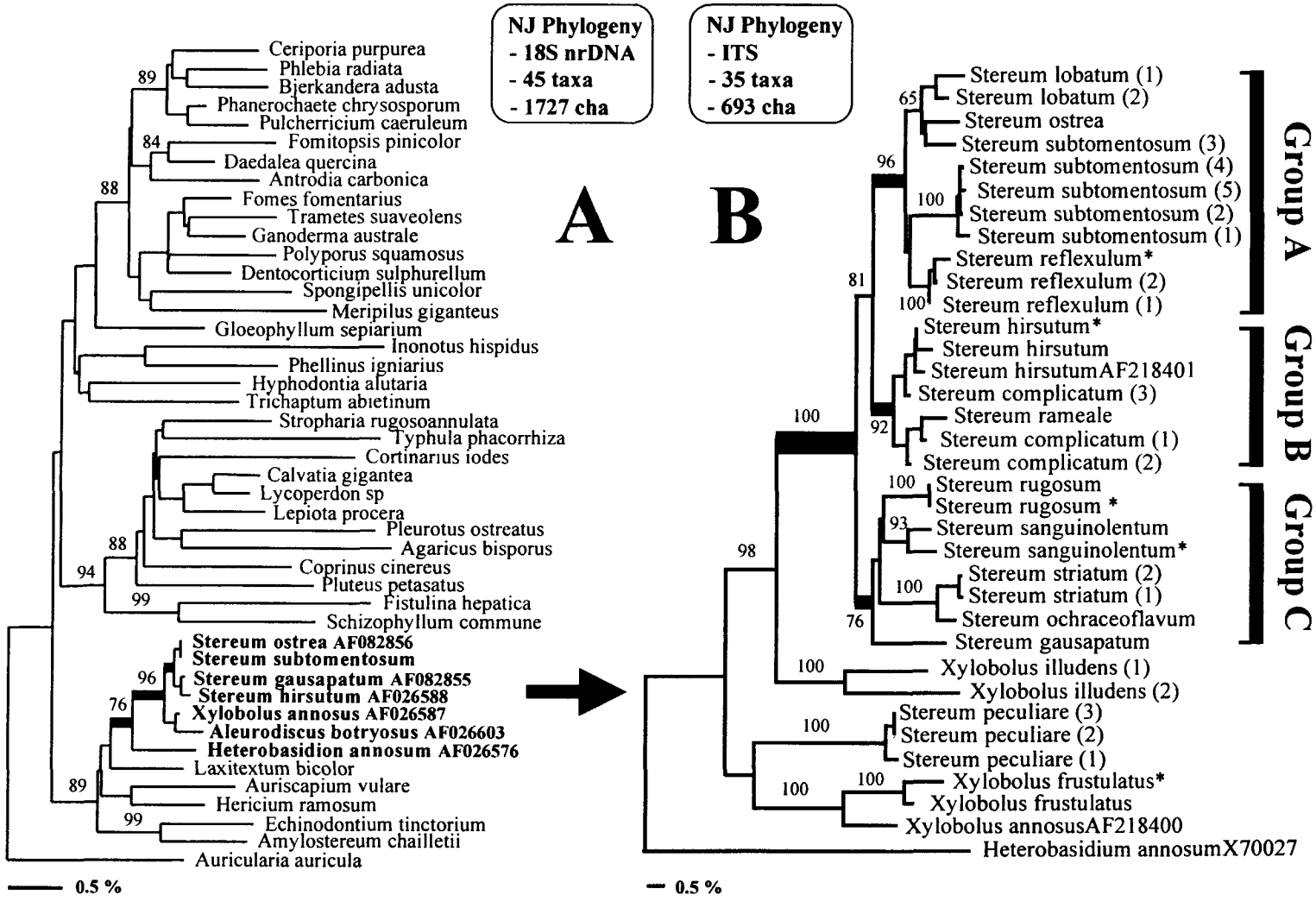
Species of the *Stereum* clade were divided into three groups (Fig. 3.6B and 3.7). Group A consisted of *S. lobatum, S. ostrea, S. subtomentosum* and *S. reflexulum* whose branch was supported by the bootstrap value of 96% in NJ phylogeny and 94% in MP phylogeny. Goup B comprised *S. hirsutum, S. rameale* and *S. complicatum* whose branch was supported by the bootstrap values of 92% in NJ phylogeny and lower than 83% in MP phylogeny. And group C included *S. rugosum, S. gausapatum, S.*

sanguinolentum, *S. striatum* and *S. ochraceo-flavum* whose branch was supported by the bootstrap values of 76% in NJ phylogeny and 77% in MP phylogeny.

Neighbor-joining analysis based on nuc-ssu rDNA showed that four *Stereum* species formed one main clade at the 92% confidence level (Fig. 3.6A). And this clade has close relationships to the *Stereum annosum-Aleurodiscus botryosus* clade. As *Stereum annosum* is now treated as *Xylobolus annosus*, the *Stereum* clade forms a monophyletic group. *Xylobolus* was treated as a satellite genus to *Stereum* and, above all, resembles the subgenus *Acanthostereum* introduced by Boidin *et al.* (1979). But *Xylobolus* was separated from *Stereum* by its vertically arranged hyphae in culture and by the pseudocystidia or oleiferous hyphae turning grayish black in sulphovanillin (Eriksson *et al.*, 1984). *Aleurodiscus botryosus* has a hyphal system with simple-septate generative hyphae, gloeocystidia and amyloid spore ornamentations. *Aleurodiscus* differs from *Stereum* in its gloeocystidia and ornamented amyloid spores (Jülich and Stalpers, 1980; Núñez and Ryvarden, 1997). *Stereum*, *Xylobolus* and *Aleurodiscus* share common characters with monomitic hyphal systems and amyloid spores and were included into the russuloid clade by Hibbett and Thorn (2001).

To evaluate the traditional taxonomic concept of the *Stereum* subgenera, ITS region sequences were used in this study. Although phylogenetic relationship of *Stereum* with related genera was previously reported using ITS sequences (Boidin *et al.*, 1998), it was not clear because of limited sampling of *Stereum* subgeneric taxa. Maximum parsimony and neighbor-joining analyses showed that phylogenetic trees were almost

Figure 3.6. Neighbor-joining (NJ) tree of *Stereum*. (A) NJ phylogeny based on 18S nuclear ribosomal DNA sequences. The sequence of *Auricularia auricula* was used as an outgroup to root the tree. Reanalyzed group is indicated in boldface. (B) NJ phylogeny based on ITS region sequences. The tree was rooted with *Heterobaidium annosum* as an outgroup. Sequences of species indicated by asterisks were donated by Mugnier. Bootstrap values more than 60% from 1,000 replications in both trees are shown at appropriate branches.


NJ Phylogeny
- 18S nrDNA
- 45 taxa
- 1727 cha
NJ Phylogeny
- ITS
- 35 taxa
- 693 cha
A
B
Ceriporia purpurea
Phlebia radiata
Bjerkandera adusta
Phanerochaete chrysosporum
Pulcherricium caeruleum
Fomitopsis pinicolor
Daedalea quercina
Antrodia carbonica
Fomes fomentarius
Trametes suaveolens
Ganoderma australe
Polyporus squamosus
Dentocorticium sulphurellum
Spongipellis unicolor
Meripilus giganteus
Gloeophyllum sepiarium
Inonotus hispidus
Phellinus igniarius
Hyphodontia alutaria
Trichaptum abietinum
Stropharia rugosoannulata
Typhula phacorrhiza
Cortinarius iodes
Calvatia gigantea
Lycoperdon sp
Lepiota procera
Pleurotus ostreatus
Agaricus bisporus
Coprinus cinereus
Pluteus petasatus
Fistulina hepatica
Schizophyllum commune
Stereum ostrea AF082856
Stereum subtomentosum
Stereum gausapatum AF082855
Stereum hirsutum AF026588
Xylobolus annosus AF026587
Aleurodiscus botryosus AF026603
Heterobasidion annosum AF026576
Laxitextum bicolor
Auriscapium vulare
Hericium ramosum
Echinodontium tinctorium
Amylostereum chailletii
Auricularia auricula
0.5 %
Stereum lobatum (1)
Stereum lobatum (2)
Stereum ostrea
Stereum subtomentosum (3)
Stereum subtomentosum (4)
Stereum subtomentosum (5)
Stereum subtomentosum (2)
Stereum subtomentosum (1)
Stereum reflexulum*
Stereum reflexulum (2)
Stereum reflexulum (1)
Stereum hirsutum*
Stereum hirsutum
Stereum hirsutumAF218401
Stereum complicatum (3)
Stereum rameale
Stereum complicatum (1)
Stereum complicatum (2)
Stereum rugosum
Stereum rugosum *
Stereum sanguinolentum
Stereum sanguinolentum*
Stereum striatum (2)
Stereum striatum (1)
Stereum ochraceoflavum
Stereum gausapatum
Xylobolus illudens (1)
Xylobolus illudens (2)
Stereum peculiare (3)
Stereum peculiare (2)
Stereum peculiare (1)
Xylobolus frustulatus*
Xylobolus frustulatus
Xylobolus annosusAF218400
Heterobasidium annosumX70027
Group A
Group B
Group C
0.5 %

same and most species of *Stereum* formed one main clade at above 99% confidence level and were divided into three subgroups (Figs. 3.6A and 3.7). These grouping were not exactly congruent with the traditional taxonomy based on hyphidium type, external appearance and shape of gross morphology.

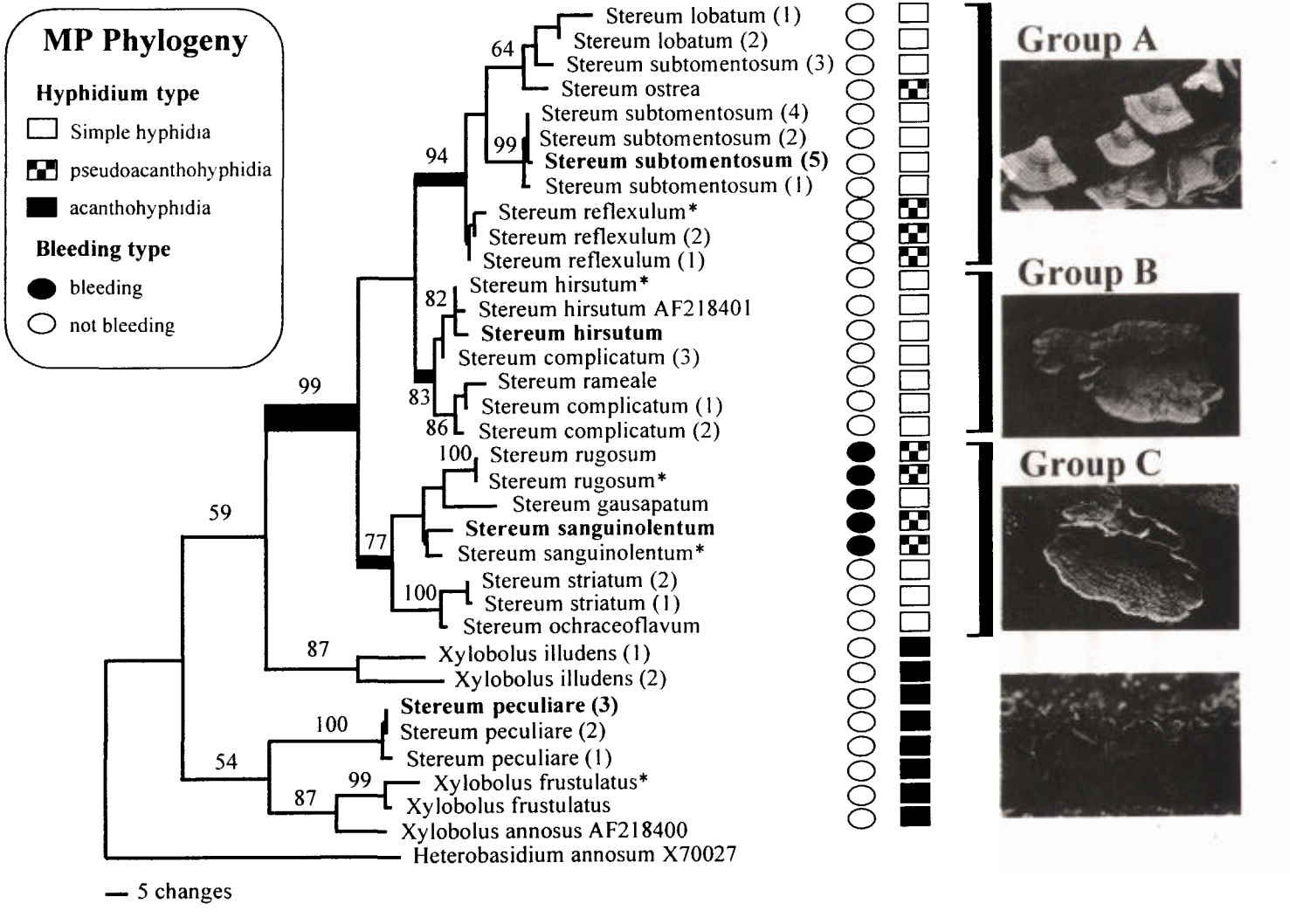
Stereum peculiare and *S. illudens* were included in the *Stereum* subgenus *Acanthostereum* because they had characters like white rot, multiple clamps in culture and positive oxidase (Boidin *et al.*, 1979). However, these species did not belong to the main clade of *Stereum* and proved to be a basal group. Refshauge and Proctor (1935) described *S. illudens* as bearing single sparse clamps at many septa and forming ochraceous-salmon to salmon colonies. Boidin *et al.* (1979) showed that one culture of *S. illudens* kept in Baarn made verticillated clamps but above character was not observed in CBS 360.36 sample they used (Stalpers, 1978).

Stereum peculiare was one of frequently collected species in Korea (Lim and Jung, 1999; Lim *et al.*, 2000) and was rather easy to recognize in *Quercus* forests because of its scattered irregular fine teeth, brown color of resupinate hymenial surface, acanthohyphidia and large cylindrical spores (Lim and Jung, 1999). In contrast to the mycelium of *Xylobolus* species, the mycelium of *S. peculiare* had a strong positive reaction with guaiacol and did not blacken in sulphuric acid (Boidin *et al*, 1979). Although *S. peculiare* and *X. illudens* with distinct acanthohyphidia were excluded from the main *Stereum*, their phylogenetic relationship with other species of *Stereum* and *Xylobolus*, therefore, remained ambiguous.

Group A contained species with all types of hyphidia (Fig. 3.7). *Stereum subtomentosum* is rather easy to recognize in the field because of large and distinctly fan-shaped to spathulate fruitbodies (Eriksson *et al.*, 1984; Lim *et al.*, 2000). Because of morphological similarity, *S. subtomentosum* has been misidentified as *S. ostrea* in Korea. However, the former has simple hyphidia and a temperate distribution but the latter has pseudoacanthohyphidia and a trophical or subtropical distribution. *Stereum ostrea*, originally described from Java, fits well into the combined concepts of *S. fasciatum* and *S. lobatum* in North America. *Stereum lobatum* has lobate, sessile basidiomata, and is more prevalent in the southern region whereas *S. fasciatum* in northern region in North America (Chamuris, 1988). *S. ostrea, S. fasciatum* and *S. lobatum* were known to take place between same individuals by fusion of basidiomata as well as fusion of mycelia in population structure (Rayner and Todd, 1982). *Stereum ostrea* was the oldest sanctioned name and has been treated as the correct name to use for this species (Chamuris, 1988). However, *S. lobatum* collected from Kangwha Island in Korea was slightly different from *S. ostrea* by smaller basidia (23-26 × 3.6-4.1 μm), basidiospores (4.7-5.3 × 1.7-1.8 μm) and the absence of pseudoacanthohyphidia.

Reid's original description of *S. reflexulum* was based on a hardly reflexed specimen. Its basidiocarp was effused-reflexed to sessile and the upper surface of the concentrically furrowed reflexed parts had hirsute by grayish tufts (Boidin *et al.*, 1979). Hyphidium of *S. reflexulum* had numerous apical prongs (Boidin *et al.*, 1979) and was similar to the one of *Aculeatostereum* than *Acanthostereum*. Our phylogenetic analyses

Figure 3.7. Phylogenetic relationships of *Stereum* subgenera inferred from ITS sequences. This tree deduced by parsimony method showed almost the same topology as that of neighbor-joining method. Sequences of species indicated by asterisks were donated by Mugnier. Photographed species were indicated by boldface names.


MP Phylogeny
Hyphidium type
Simple hyphidia
pseudoacanthohyphidia
acanthohyphidia
Bleeding type
bleeding
not bleeding
Stereum lobatum (1)
Stereum lobatum (2)
Stereum subtomentosum (3)
Stereum ostrea
Stereum subtomentosum (4)
Stereum subtomentosum (2)
Stereum subtomentosum (5)
Stereum subtomentosum (1)
Stereum reflexulum*
Stereum reflexulum (2)
Stereum reflexulum (1)
Stereum hirsutum*
Stereum hirsutum AF218401
Stereum hirsutum
Stereum complicatum (3)
Stereum rameale
Stereum complicatum (1)
Stereum complicatum (2)
Stereum rugosum
Stereum rugosum*
Stereum gausapatum
Stereum sanguinolentum
Stereum sanguinolentum*
Stereum striatum (2)
Stereum striatum (1)
Stereum ochraceoflavum
Xylobolus illudens (1)
Xylobolus illudens (2)
Stereum peculiare (3)
Stereum peculiare (2)
Stereum peculiare (1)
Xylobolus frustulatus*
Xylobolus frustulatus
Xylobolus annosus AF218400
Heterobasidium annosum X70027
64
94
99
82
99
83
86
100
59
77
100
87
100
54
99
87
5 changes
Group A
Group B
Group C

supported the reduction trend that a species with simple hyphida was as a derivative reduced taxon (Chamuris, 1988).

Group B consisted of three species. *Stereum rameale* (Schw.) Burt was a synonym for *S. hirsutum* and *S. rameale* (Berk.) Messee was a synonym for *S. ohraceoflavum* (Schw.) Ellis. But *S. rameale* (Berk.) Messee from CBS used in this study was closely related to *S. complicatum* and *S. hirsutum*. This result suggests that *S. rameale* (Schw.) Burt might have been misidentified or mislabled as *S. remeale* (Berk.) Messee at CBS. *Stereum hirsutum, S. complicatum* and *S. subtomentosum* were treated as a single, polytypic species and this aggregate was also treated as *S. hirsutum* complex (Chamuris, 1988; Welden, 1971). But *S. subtomentosum* grouped into group A separately from the group B. The basidiocarp in this group is effused-reflexed to sessile, becoming laterally extended, and have matted-tomentose, strigose-hirsute to hispid, zonate, often furrowed surface. Its size is similar to that of group B and larger than that of group C.

Group C contained species showing hymenial bleeding except for two species, *S. ochraceo-flavum* and *S. striatum*. *Stereum ochraceo-flavum* is similar to *S. striatum* but differs in possessing a well-developed tomentum and in its absence on *Carpinus caroliniana*. And the others in group C have a common character of hymenial bleeding. So it was treated as an important character that was used in dividing *Stereum* into subdivisions by Burt, Boutdot and Galzin, and Eriksson. Pouzar (1959) placed them into *Haematostereum*. The bleeding is brought about by the great amount of phenolic

substances and phenoloxidases, released on rupturing the extremely brittle tips of pseudocystidia, and was not taxonomically useful at generic or subgeneric levels (Boidin *et al.*, 1979; Chamuris, 1985b). As pigments have been traditionally given taxonomic weight in Polyporaceae, *Pycnoporus* Fr. was separated from *Trametes* Fr. only by the pigment. This preference is not used in other taxonomic groups. For example, pigments are completely neglected at generic level in Agaricales (Ko, 2000). Molecular phylogeny based on ITS sequences (Fig. 3.7), however, showed that hymenial bleeding is a derived character in group C and an important character which should not be neglected at the subgeneric level of *Stereum*. Most members of group C have developed various degrees of substratum specialization. *Stereum ochraceo-flavum* inhabits small space usually on attached limbs of hardwood trees, *S. gausapatum* on *Quercus* species, *S. striatum* on *Carpinus caroliniana* and *S. sanguinolentum* on coniferous wood (Chamuris, 1988). *Stereum sanguinolentum* is common throughout coniferous forests as a primary saprotrophic decay fungus and infects living trees often throught broken tops and attached wood (Eriksson *et al.*, 1984).

Molecular study clearly indicates that three groupings were not exactly congruent with the traditional taxonomy based on hyphidium type, external appearance and the shape of gross morphology in *Stereum* subdivisions and that *Acanthostereum* might be more closely related to *Xylobolus* than to *Stereum*. These results showed that members of the subgenera *Aculeatostereum* and *Stereum* have lost the tendency to form hyphidial prongs. And grouping based on ITS sequences supported the hypothetical

evolutionary direction from resupinate or effused-reflexed to distinctly fanshaped or spathulate fruitbodies. While *X. frustulatum* has a resupinate fruitbody and *S. peculiare* has a resupinate or slightly reflexed fruitbody, most species of groups B and C in *Stereum* have effused-reflexed forms or become laterally extended and most species of group B have distinctly fan-shaped forms.

In summary, the genus *Stereum* formed a monophyletic clade based on nuc-ssu rDNA (Fig. 3.6A) and ITS (Fig. 3.6B and 3.7) sequences. In this study, evidence is provided that hymenium bleeding was is important defining character in group C but hyphidium type and the shape of gross morphology are not taxonomically useful at the subgeneric level of *Stereum*. The subgenus *Acanthostereum* containing *S. peculiare* and *X, illudes*, therefore, should be excluded from the genus *Stereum*. Our results also supported the reduction trend of hyphidium type in *Stereum* and suggest that the evolutionary direction might be proceeding from resupinate to distinctly fan-shaped or spathulate fruitbodies in the genus *Stereum*.

2. Phylogenetic Relationships of *Xylobolus* and Allied Genera based on ITS1-5.8S-ITS2 Sequences

Xylobolus Karst. is a basidiomycete of the Stereaceae and is capable of causing a white pocket rot in corticate oaks. This causes many localized pockets of degradation within wood that are composed of cellulosic white tissues and are virtually free of lignin. *Xylobolus* species first delignify and then remove the exposed cellulose fibrils, so they have been treated as an ecologically important fungus (Otjen and Blanchette, 1984).

In taxonomy, *Xylobolus* is closely related to *Stereum*, so called a satellite genus to *Stereum*, and resembles the *Stereum* subgenus *Acanathostereum* (Boidin *et al.*, 1979; Chamuris, 1985b). However, it differs from *Stereum* in its hard perennial basidiomata, the production of a white pocket rot, lack of detectable extracellular phenoloxidase system, lack of multiple clamp connections in culture, vertically arranged hyphae and pseudocystidia turninig grayish black in sulphovanillin (Chamuris, 1988; Eriksson *et al.*, 1984). As *X. subpileatus* has horizontally arranged hyphae and pseudocystidia that seems to lack the positive reaction in sulphovanillin, Eriksson and his colleagues suggested that this species fitted better in *Stereum* (Eriksson *et al.*, 1984). *Stereum reflexulum, S. peculiare* and *X. illudens* were inluded into the *Stereum* subgenus *Acanthostereum* due to acanthohyphidia, multiple clamps in culture and oxidase-positive reaction (Boidin *et al.*, 1979; Chamuris, 1988).

All species of *Xylobous* have acanthohyphidia that are a prominent character and might be used for generic splitting (Núñez and Ryvarden, 1997). This character also occurred in *Acanthophysium, Acanthobasidium* and *Stereum* subgenus *Acnathostereum.* However, its importance is neglected in *Stereum* that contains species without acanthohyphidia (subgenus *Stereum*) or pseudoacanthohyphidia (subgenus *Aculeatostereum*) as well as species with acanthohyphidia (subgenus *Acanthostereum*). Therefore, acanthohyphida seemed to have arisen several times in response to environmental stress (Núñez and Ryvarden, 1997).

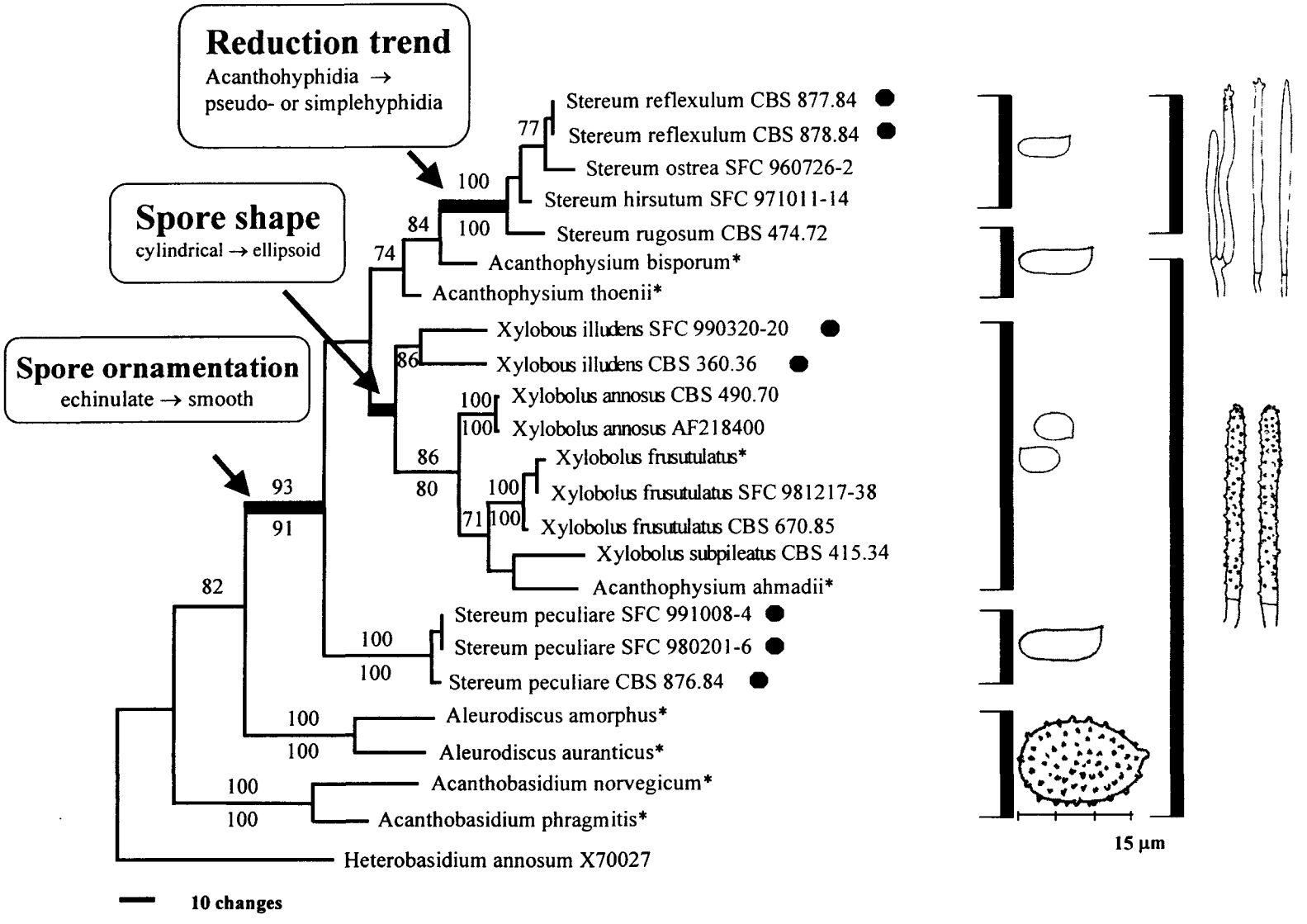
As *Xylobolus* shares many common characters with *Acanthophysium* as well as *Stereum*, phylogenetic relationships of these genera based on morphology are problematic. To access the natural classification of *Xylobous* and allied genera, intensive phylogenetic studies as well as morphological information are definitely needed. According to the study of Boidin *et al.* based on ITS sequences, *Xylobolus frustulatus,* the only species of *Xylobolus* included, was merged into the genus *Acanthophysiume* instead of *Stereum* (Boidin *et al.*, 1998). Acanthohyphidum strongly supported this relationship, so it might be an important character in grouping *Xylobous* and *Acanthohyphisum.* That result suggests a question to think over whether the phylogenetic position of the *Stereum* subgenus *Acanthostereum* is right or not. To test whether *Xylobolus* is monophyletic or not and to know its phylogenetic relationship, inclusion of additional *Xylobolus* species seems necessary.

Phylogenetic analyses using molecular sequence data show much promise for

resolving phylogenetic relationships for many problematic species complexes in basidiomycetes (Boidin *et al.*, 1998; Moncalvo *et al.*, 1995; Redecker *et al.*, 2000; Zambino and Szabo, 1993). The purpose of this study was to investigate relationships in the genus *Xylobolus* and its allies and to investigate phylogenetic position of the *Stereum* subgenus *Acanthostereum* through phylogenetic analyses of sequence information from the two internal transcribed spacer (ITS1 and 2) regions and 5.8S ribosomal DNA. In order to provide additional statistical support for inferred phylogenetic relationships, the Kishino-Hasegawa test (1989) implemented in PAUP*4.0b4a (Swofford, 1999) was performed. Constraint trees were generated using MacClade 3.04 (Maddison and Maddison, 1992) and PAUP*4.0b4a (Swofford, 1999) to depict specific phylogenetic hypotheses. Three distinct phylogenetic hypotheses (constrained trees) were tested. In Hypothesis I, *Xylobolus* was constrained to form a monophyletic clade. In Hypothesis II, *Xylobolus* was constrained to form a sister clade with *Stereum.* For Hypothesis III, the *Stereum* subgenus *Acanthostereum, S. peculiare, S. reflexulum* and X. *illudens* were constrained to form a monophyletic clade within the genus *Stereum.*

Phylogenetic analyses of the ITS regions included 541 characters for each of 24 taxa. Of these characters, 308 characters were constant and 173 were potentially parsimony-informative. Maximum parsimony analyses performed with gaps treated as fifth bases yielded one most parsimonious tree (Fig. 3.8). This phylogenetic tree required 587 steps. The tree was rooted with sequence of *Heterobasidium annosum* as

Figure 3.8. Phylogenetic tree of *Xylobolus* inferred from the analysis of nuclear ribosomal ITS1-5.8S-ITS2 sequences of 24 strains. A single most parsimonious tree (tree lengths = 587 steps, CI = 0.6491, RI = 0.7379 and RC = 0.4790) was obtained by the heuristic search using TBR of PAUP*4.0b4a. Names in bold type represent *Xylobous* taxa. *Heterobasidium annosum* was used as an outgroup. Numbers at branches indicate the percentage of 1,000 maximum parsimony (neighbor-joining) bootstrap values supported by more than 60%. Sequences of asterisked taxa were donated by Mugnier (Rhône-Poulenc, France)


Reduction trend
Acanthohyphidia →
pseudo- or simplehyphidia
Spore shape
cylindrical → ellipsoid
Spore ornamentation
echinulate → smooth
Stereum reflexulum CBS 877.84
Stereum reflexulum CBS 878.84
Stereum ostrea SFC 960726-2
Stereum hirsutum SFC 971011-14
Stereum rugosum CBS 474.72
Acanthophysium bisporum*
Acanthophysium thoenii*
Xylobous illudens SFC 990320-20
Xylobous illudens CBS 360.36
Xylobolus annosus CBS 490.70
Xylobolus annosus AF218400
Xylobolus frusutulatus*
Xylobolus frusutulatus SFC 981217-38
Xylobolus frusutulatus CBS 670.85
Xylobolus subpileatus CBS 415.34
Acanthophysium ahmadii*
Stereum peculiare SFC 991008-4
Stereum peculiare SFC 980201-6
Stereum peculiare CBS 876.84
Aleurodiscus amorphus*
Aleurodiscus auranticus*
Acanthobasidium norvegicum*
Acanthobasidium phragmitis*
Heterobasidium annosum X70027
10 changes
15 µm

an outgroup that was deduced by previously published papers (Boidin *et al.*, 1998; Hibbett *et al.*, 1997; Hibbett and Thorn, 2001; Ko *et al.*, 1997). The tree constructed by the neighbor-joining method (Saitou and Nei, 1987) was topologically similar to the parsimony tree except for branch supporting values and branching patterns between *Aleurodiscus* and *Acanthobasidium* clades. Parsimony and distance analyses of the whole ITS region sequences revealed a reasonable degree of agreement between morphological criteria classically used for delimiting genera *Xylobolus, Stereum* and *Acanthophysium* and molecular data of DNA sequences.

Both analyses showed that *Acanthophysium* was inmermingled with *Stereum* and *Xylobolus*. This group was supported by bootstrap values of 93% and 91% in parsimony and neighbor-joining trees respectively. *Acanthophysium* is recognized as an independent genus by Boidin *et al.* (1985) and Parmasto (1995), but not by Jülich and Stalpers (1980) and Núñez and Ryvarden (1997). According to the cladistic analyses using morphological data, *Acanthophysium* formed a distinct clade separated from *Stereum* and *Xylobolus* (Parmasto, 1995), but Boidin *et al.* (1998) have shown micromorphological similarities between *Stereum* subgenus *Acanthostereum* and *Acanthophysium*. According to the molecular data, it formed a sister group with *Stereum* (Boidin *et al.*, 1998; Hallenberg and Parmasto, 1998). Present phylogenetic analyses based on molecular data support that relationship. The group containing *Acanthophysium*, *Stereum* and *Xylobolus* was also nested in the russuloid clade (Hibbett and Thorn, 2001) and had distinctive characters of smooth amyloid spores and

Table 3.2. Statistical results on three hypotheses using the Kishino and Hasegawa test implemented in PAUP* 4.0b4a.

Tree	-ln Likelihood	Differences[a]	s.d.[b]	T[c]	P[d]
MP tree	2866.39544	best	-	-	-
Hypothesis I	2894.66889	28.27345	18.42612	1.5344	0.1255
Hypothesis II	2925.69587	59.30043	29.57604	3.0292	0.0026*
Hypothesis III	2963.58467	97.18923	25.50126	3.8112	0.0002*

[a] Difference in log likelihood compared to that of the best tree.

[b] The standard deviation of log likelihood.

[a] Value that Differences were divided by s.d.

[b] Probability of getting a more extreme T-value under the null hypothesis of no difference between the two trees (two-tailed test). Asterisked values indicate significant difference at $P < 0.05$.

acanthohyphidia. These characters are also present in outgroup taxa, *Aleurodiscus* and *Acanthobasdium*, but their spores are very large and ornamented. Although ornamented spores were treated as a derived feature in many taxonomic groups (Hibbett and Thorn, 2001), present results suggest that the large and ornamented spore is a plesiomorhic feature and transitions to small smooth forms occur in basal branch taxa of this group (Fig. 3.8).

The *Xylobolus* clade containing *A. ahmadii* is weakly supported by bootstrapping, but it appears to be monophyletic by the Kishino-Hasegawa test which accepted the null hypothesis I that the genus *Xylobous* was monophyletic (Table 3.2). The character that strongly supports this clade is short-ellipsoid spores (Fig. 3.8). The spores in species of *Stereum* and *Acanthophysium* are almost cylindrical. Although *X. subpileatus* has been treated as the species within the genus *Stereum* by hyphal arrangement and pseudocystidia by Eriksson *et al.* (1984), the thickness, corky to hard texture, and tuberculate cracked hymenial surface distinguish it from *Stereum* (Chamuris, 1988). In phylogenetic analyses, this species formed a group with *A. ahmadii* and was connected with other *Xylobous* species sequentially (Fig. 3.8). The microstructure of *A. ahmadii* is also *Xylobolus*-like with acanthohyphidia and ellipsoid amyloid spores, but the clamed generative hyphae differ from those of *Xylobolus*. Phylogenetic analysis of the ITS data set demonstrates the spore transition from cylindrical to elliptic forms and occurs at the basal branch of the *Xylobolus* clade (Fig. 3.8).

Four *Xylobolus* species have close relationships to the *Stereum*, but three species of *Acanthophysium* were merged into them, which was congruent with result of Boidin *et al.* (1998). Kishino-Hasegawa test results (Table 3.2) of the ITS data set reject the null hypothesis II that *Xylobolus* was treated as a satellite genus to *Stereum* (Chamuris, 1988). Common characters between *Xylobolus* and *Acanthophysium* are acanthohyphidia and smooth spores but gloeocystidia make a difference between them. *Acanthophysium bisporum* and *A. thoenii* were placed at the base of the *Stereum* clade. The latter species like *A. ahmadii* possessed generative hyphae with clamps (Núñez and Ryvarden, 1997; Rattan, 1977). However, the spores of *A. bisporum* and *A. thoenii* are cylindrical and larger than those of *A. ahmadii* (Núñez and Ryvarden, 1997).

Strict enforcement of topological constraints (Hypothesis III) corresponding to the systems of Boidin *et al.* (1979) and Chamuris (1985b) for the infrageneric subdivision of *Stereum* yielded longer trees (Table 3.2). Chamuris (1988) grouped *X. illudens* (Berk) Boid. into the *Stereum* subgenus *Acanthostereum* due to acanthohyphidum, multiple clamps in culture and oxidase-positive reaction. Refshauge and Proctor (1935) described *X. illudens* (Berk.) Boid. to have clamps at many septa and to develop ochraceous salmon to salmon colonies. However, *X. illudens* (CBS 360.36) used in this study no longer showed above characters (Stalpers, 1978) and thus formed a monophyletic group to other *Xylobolus* (Fig. 3.8). All species of *Stereum* except for *S. peculiare* form a complete monophyletic clade with bootstrap support of 100% in both parsimony and neighbor-joining trees. The three strains of *S. peculiare* presented in the

study showed identical sequences across the whole region and the only species of *Stereum* that fell outside the main *Stereum* clade. *Stereum peculiare* occupies a basal position within the ingroup containing *Acanthphysium, Stereum* and *Xylobolus*. *Stereum peculiare* is one of frequently collected species in *Quercus* forests of Korea (Lim *et al.*, 2000) and is rather easy to recognize in the field because of its scattered irregular fine teeth, brown color of resupinate hymenial surface, acanthohyphidia and large cylidrical spores (Lim and Jung, 1999). Also Boidin *et al.* (1979) showed that there were transitions from pseudocystidia to the obpyriform gloeocystidia in *S. peculiare*. Above characters of *S. peculiare* was very similar to those of *Acanthphysium*. *Stereum peculiare* and other species of *Stereum* are thus considered to have different lineages. Our results based on parsimony, distance analyses and Kishino-Hasegawa test (Hypothesis III) suggested that the subdivision of *Stereum* with hyphidium type (Boidin, *et al.*, 1979; Chamuris, 1985b) was artificial and that hyphidium type appeared to have undergone simplification at the basal branch of the *Stereum* clade (Chamuris, 1988). In contrast to mycelium of *Xylobolus* species, the mycelium of *S. peculiare* had a strong positive reaction with guaiacol and did not blacken in sulphuric acid (Boidin *et al.*, 1979). Phylogenetic analyses based on ITS sequences and taxonomic considerations on morphological characters showed that *S. peculiare* and *Acanthphysium* are closely related each other and it is proposed here that *S. peculiare* be transferred into *Acanthophysium*.

Acanthophysium peculiare (Boidin, Parmasto & Dhingra) Y. W. Lim & H. S. Jung, comb. nov.

Basionym: *Stereum peculiare* Boidin, Parmasto & Dhingra, *Persoonia* 10; 311-324, 1979.

III. Molecular Phylogeny of Corticioid Fungi in the Hymenochaetoid Clade

The corticioid fungi of the Hymenochaetoid clade included *Basidioradulum*, *Hyphodontia* and *Resinicium*. The polyporoid genera *Schizopora, Trichaptum, Inonotus* and *Phellinus* are also included in this clade. These results except for *Resinicium* were supported by the previous papers based on mt-ssu rDNA (Hibbett and Donoghue 1995) and nuc-ssu rDNA sequences (Hibbett *et al.*, 1997; Ko *et al.*, 1997). Members of the hymenochaetoid clade are composed of lignicolous species and appeared to be united by the possession of imperforate parenthosomes and

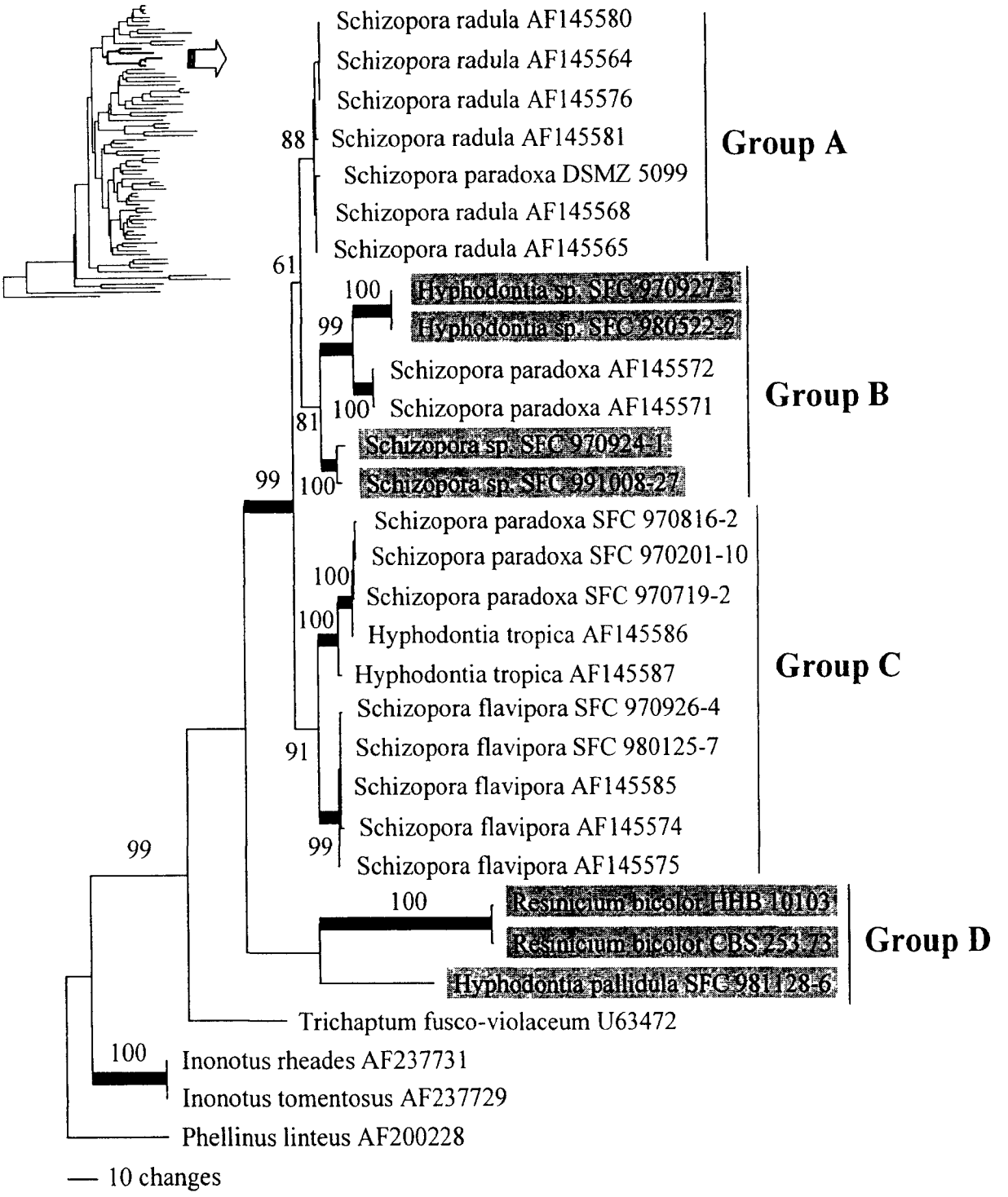
Schizopora causing a white rot on dead hardwood has variable hymenophores from poroid, lacerate, to more or less irpicoid forms. Due to its variable hymenophores, it was once treated as *Irpex* or *Steccherinum* but its microscopic details, except for skeletal hyphae, were almost same as those of *Hyphodontia* of the family Corticiaceae (Jung, 1987). The close affinity between *Schizopora* and *Hyphodontia* was highlighted by Eriksson *et al.* (1984), Hassan and David (1983) and Langer and Oberwinkler (1993). Langer (1994) has treated these two genera all together in his monograph on *Hyphodontia* and proposed to conserve the latter genus against *Schizopora* (Langer *et al.*, 1996). *Schizopora* has nomenclatural priority over *Hyphodontia*, but a disadvantageous change of nomenclature, in the sense of Art. 14. 1-2 of the Code (ICBN reference), would result if both genera were combined as *Schizopora*.

Investigations of micromorphological, cultural and ultrastructural characters of the genera *Hyphodontia* and *Schizopora* have shown that it is not possible to clearly distinguish *Schizopora* and *Hyphodontia* on the basis of either macro- or micromorphology. Phylogenetic study based on morphological characters (Langer, 1994), ITS sequence (Paulus *et al.*, 2000) and mt-ssu rDNA sequence (Langer 1998) showed that *Schizopora* species were nested inside one of the main clades of *Hyphodontia*.

Hyphodontia is a natural group and is marked by a combination of characters. This genus is primarily characterized by its hyphal branch pattern and suburniform basidia (Maekawa, 1994). Its fruitbody is fully resupinate, effused, adnate but never pileate or detachable. The hymenial surface possesses various forms from smooth or finely tuberculate to odontioid with aculei. The presence of cystidia or, at least, cystidioid capitate hyphal end is constant for the species hitherto referred to the genus (Fig. 2.1). All species grow on dead conifer or deciduous wood, in which they cause a white decay. As few morphological characters are available for telling their relationships, molecular techniques were used as means to study taxonomic and phylogenetic relationships between them.

Sizes of DNA amplified by PCR using ITS5 and LW1 primers varied from 570 to 600 bp and the multiple sequence alignment showed that the homology among isolates ranged from 70-90%. The sizes of ITS region (covering ITS 1 region, 5.8S rDNA and ITS 2 region) are listed in Table 3.3. It was regularly observed that the ITS1 region was

Figure 3.9. Phylogenetic tree showing the relationships among corticioid fungi in hymenochaetoid clade. This tree based on ITS sequences depicts one of six most parsimonious trees. Significant bootstrap support values (above 60%) are given at corresponding branches. Species of *Phellinus*, *Inonotus* and *Trichaptum* were used as outgroup species. Odontioid hymenophores are indicated by shaded boxes.


Schizopora radula AF145580
Schizopora radula AF145564
Schizopora radula AF145576
88
Schizopora radula AF145581
Schizopora paradoxa DSMZ 5099
Schizopora radula AF145568
Schizopora radula AF145565
Group A
61
100
Hyphodontia sp. SFC 970927-3
99
Hyphodontia sp. SFC 980522-2
Schizopora paradoxa AF145572
100
Schizopora paradoxa AF145571
81
Schizopora sp. SFC 970924-1
99
100
Schizopora sp. SFC 991008-27
Group B
Schizopora paradoxa SFC 970816-2
Schizopora paradoxa SFC 970201-10
100
Schizopora paradoxa SFC 970719-2
100
Hyphodontia tropica AF145586
Hyphodontia tropica AF145587
Group C
Schizopora flavipora SFC 970926-4
91
Schizopora flavipora SFC 980125-7
Schizopora flavipora AF145585
Schizopora flavipora AF145574
99
Schizopora flavipora AF145575
99
100
Resinicium bicolor HHB 10103
Resinicium bicolor CBS 253.73
Group D
Hyphodontia pallidula SFC 981128-6
Trichaptum fusco-violaceum U63472
100
Inonotus rheades AF237731
Inonotus tomentosus AF237729
Phellinus linteus AF200228
— 10 changes

longer than the ITS 2 for all the strains selected except for *R. bicolor*. From the aligned sites by ClustalX, unalignable internal sites were excluded. The resultant data matrix comprised of 30 taxa by 591 characters. Parsimony analysis with the data set produced 6 equally parsimonious trees of 641 steps. Through log likelihood test (Kishino and Hasegawa, 1989) implemented in PAUP* 4.0b4a (Swofford, 1999), one of 6 six most parsimonious trees was selected as a best tree (Fig. 3.9; CI = 0.7036, RI = 0.8708, RC = 0.5704). Strengths of internal branches found in parsimony analyses were statistically tested by the bootstrap analyses of 1,000 replications (Felsenstein 1985). The tree was rooted with the sequence of *Phellinus linteus* that was deduced by nuc-ssu rDNA sequences analysis (Fig. 3.5).

In the tree of Fig. 3.9, *Hyphodontia, Schizopora* and *Resinicium* formed a monophyletic lineage although its bootstrap support dropped below 60%. Parsimony analysis showed that these genera were intermingled with each other. The genera *Hyphodontia* and *Schizopora* seemed to be a natural group although they crossed the borderlines between corticioid forms and polypores. This showed that it was rather less difficult to define strictly artificial families during the transition period of history in the classification of the Hymenomycetes (Donk, 1971).

Species in the phylogenetic tree were divided into four groups (Fig. 3.9). Group A contained *S. radula* and *S. paradoxa* (DSM 5099) and its branch was supported by 88% bootstrap values. Genetic distances among group A ranged from 0 to 1.12% indicating lower levels of dissimilarity (Table 3.4). *Schizopora radula* was segregated

Table 3.3. Comparisons of sequence lengths and morphological characters.

	Sequence lengths (bp)			Morphological characters		
	ITS1	ITS2	ITS + 5.8S	basidia size (μm)	spore size (μm)	pore/mm
Schizopora radula	228	193	605	-	4-5 X 2.8-3.8	1-3
S. paradoxa DSMZ 5099	227	191	602	12-20 X 4-5	5-6 X 3.5-4	1-3
Hyphodontia sp. SFC 970927-3	232	196	612	16-21 X 3.5-4.5	4.5-5.5 X 2.5-3	odontoid
Hyphodontia sp. SFC 980522-2	232	196	612	16-21 X 3.5-4.5	4.5-5.5 X 2.5-3	odontoid
Schizopora sp. SFC 970924-1	231	192	607	20-25 X 3-3.5	5-6 X 3-4	hydnate 1mm
Schizopora sp. SFC 991008-27	230	192	606	25-30 X 4.5	5-6 X 3-4	hydnate to pore 1-3
S. paradoxa SFC 970719-2	233	190	607	14-17 X 4.5-5	4-5 X 2.5-3 (3.5)	3-4
S. paradoxa SFC 970816-2	233	190	607	14-17 X 4.5-5	4.5-6 X 3.5-4.5	3-4
S. paradoxa SFC 970201-10	233	190	607	14-17 X 4.5-5	4-5.5 X 3-4	3-4
S. flavipora SFC 970926-4	229	191	604	12-15 X 3-5	3.5-4.5 X 2-3	5-6
S. flavipora SFC 980125-7	230	191	605	12-15 X 3-5	3.5-4.5 X 3-3.5	5-6
S. flavipora AF145585	230	191	605	12-15 X 5-6	3.5-5 X 2.5-3.5	3-5
H. pallidula SFC 981128-6	227	199	610	12-16 X 3-3.5	3.5-4 X 1.8-2.1	odontoid
Resinicium bicolor	175	204	563	12-23 X 5-9	6-7.5 X 2.5-3.5	odontoid

from *S. paradoxa* complex on the basis of incompatibility (Hallenberg, 1983) and ITS sequences analyses (Paulus *et al.*, 2000). However, it was in many cases difficult or even impossible to keep them separate with certainty (Eriksson *et al.*, 1984).

Group B consisted of unidentified *Hyphodontia* species, *Schizopora paradoxa* and two unidentified *Schizpora* species and and its basal branch was supported by 81% bootstrap values. Distances among them ranged from 0.0 to 9.05%. Two *Hyphodontia* spp. have an odontioid hymenial surface and two strains of *Schizopora* have poroid and irregular teeth forms on hymenial surface.

Group C comprised *S. paradoxa*, *S. flavipora* and *H. tropica* and its basal branch was supported by 91% bootstrap values. Distances among them ranged from 0.0 to 6.52%. *Schizopora paradoxa* is a very common species and has been regarded as a variable species. In the microstructure, there are variations in the size of basidiospores, in the occurrence of very thick-walled skeletals with a narrow lumen, capitate hyphal ending (allocystis) and incrustations on the hyphal tips in pore mouths (Hallenberg, 1983). In ITS sequence analysis, *S. paradoxa* occurs in each of three groups A, B and C (Fig. 3.9). The morphological similarity of *S. paradoxa* with other *Schizopora* species has led to a contradictory result. Three *S. paradoxa* from Korea and *H. tropica* formed a sister clade with the *S. flavipora* clade. The genetic differences were 5.52-6.52% observed between *S. paradoxa* from Korea and *S. flavipora*. Five strains of *S. flavipora* clustered with bootstrapping values of 99% in phylogenetic tree (Fig. 3.9). *Schizopora flavipora* is distinguished by having smaller pores and spores (Table 3.3)

and its pore portion looks like oppressed form of *S. paradoxa*. This species is perhaps the most common and conspicuous polypore in Korea but often has been confused with *S. paradoxa*.

The last group D contained the type species of *Hyphodontia, H. pallidula*, and *Resinicium bicolor*. But their branch support was very low. Considering the morphological differences and the relatively high genetic distance (43.0-43.5%) between *H. pallidula* and *R. bicolor*, it was possible that the grouping of two species might not reflect a true phylogenetic relationship but could be an artifact generated during the process of analysis. Phylogenetic reconstruction has a number of potential sources of error (Swofford *et al*, 1996). One such possible source is long branch attraction where two long branches in a tree attract each other when they are linked by a short internal branch (Hendy and Penny, 1989). Although the phylogenetic placement of *R. bicolor* in the tree (Fig. 3.9) was uncertain, this species and *Hyphodontia alutaria* were connected together with 100% bootstrap support in nuc-ssu rDNA sequence analysis (Fig. 3.5).

The fruitbody of *Resinicium bicolor* gets greenish tints because its basal layer is usually associated with living cells of green algae. Whether there is a symbiotic connection between this species and green algae is unclear yet and the phylogenetic significance of green algal association is still questionable. The distinguishing characteristic of the genus *Resinicium* is the presence of asterocystidia as well as halocystidia (Lee and Jung, 1998). In other respects, the genus showed affinities to

Table 3.4. Genetic distances between pairs of fungal strains calculated by the Kimura two-parameter model.

	Group A	Group B	Group C	Group D
Group A	0.00 - 1.12			
Group B	6.11 - 11.96	0.00 - 9.05		
Group C	5.40 - 7.38	7.57 - 12.26	0.00 - 6.52	
Group D	26.02 -33.64	25.13 - 34.87	26.07 - 36.87	0.39 - 43.54

Phlebia (Eriksson *et al.*, 1981). However, macromorphology such as totally resupinate fruitbodies, hymenium of granular, odontioid or hydnoid forms, and micromorphology of *Resinicium* resemble those of *Hyphodontia* rather than those of *Phlebia*. Their relationship was supported by nuc-ssu rDNA and ITS sequence analyses.

IV. Molecular Phylogeny of Corticioid Fungi in the Euagarics Clade

The euagaics clade was a very large one mostly composed of Agaricales and some of Aphyllophorales and Gasteromycetes. Aphyllophorales occupied a relatively small part of this clade. Fistulinaceae, Schizophyllaceae and a few of Corticiaceae were included in this clade. *Athelia bombacina* (Gargas *et al.*, 1995) and the corticioid bark beetle symbiont *Gloeocystidium ipidophilum* appeared to be in the euagarics clade (Hsiau, 1996). Another members of the corticioid fungi in the euagarics clade have been investigated in phylogenetic studies (Kim and Jung, 2000; Yoon *et al.*, 2001). In their papers, *Chondrostereum* and *Cystostereum* of Stereaceae were included in this clade and showed a close relationship to *A. bombacina*. In the study of Boidin *et al.* (1998) based on ITS sequences analyses, however, *Cystostereum* was grouped in the Phanerochaetales, represented in the polyporoid clade, instead of the euagarics clade. In spite of no common characters, Kim and Jung (2000) established an independent family Cystosteraceae sensu Jülich, which consisted of *Cystostereum, Chondrostereum, Athleia, Lentinula, Pleurotus*, *Schizophyllum* and *Fistulina*. But it would be premature to conclude because they didn't consider many other agaricales.

In this study, six other corticioid fungi were included in the euagarics clade; *Mycoacia copelandii, Cylindrobasidium evolvens* and *Plicaturopsis crispa* for the analysis of nuc-ssu rDNA sequences and, those three species, *Dentipellis fragillis*, and

two *Hypochnicium* species for the ITS sequence analysis. The amplified size by PCR using NS1 and NS8 primers was about 2.3kb for *Chondrosterum purpurem* collected in Korea and about 1.8 kb for the rest of the species (Fig. 3.3). This difference resulted from the presence of an intron. The intron existed between primers NS5 and NS6 and its length was 429 bp (Fig. 3.4). This accorded with the previous result studied for *C. purpurem* strain purchased from CBS (Yoon, 1998). It was identified as Group I self-splicing intron through BLAST Search of NCBI. Such introns were also found at the aligned sequence position 943 in *Lentinellus omphalodes, L. montanus, Clavicorona pyxidata* and *Panellus stypticus* of the Hymenomycetes (Hibbett, 1996).

PCR products of ITS region using primer ITS5 and LW1 ranged from approximately 600 to 650 base pairs. Ambiguously aligned sequences were removed from phylogenetic analyses. The NJ tree based on 18S rDNA and ITS sequences was constructed using Kimura's (1980) two-parameter distance (Figs. 3.10 and 3.11). Statistical supports from 1,000 bootstrap resamplings were numbered on appropriate branches. *Coniophora puteana* belonging to the bolete clade was used as an outgroup. Two NJ trees based on ITS and nuc-ssu rDNA sequence considerably differed each other. This topological difference might have resulted from different evolution rates of two regions.

In the nuc-ssu rDNA analyses (Fig. 3.10), five species of the corticioid fungi were clustered into a single clade although their relationships were weakly supported by the bootstrap value of 60%. However, this clade was clearly differentiated from other

Figure 3.10. Phylogenetic relationships between agarics and corticioid fungi. This tree was generated by neighbor-joning method and based on small subunit ribosomal DNA sequences. The bolete clade was used as an outgroup.

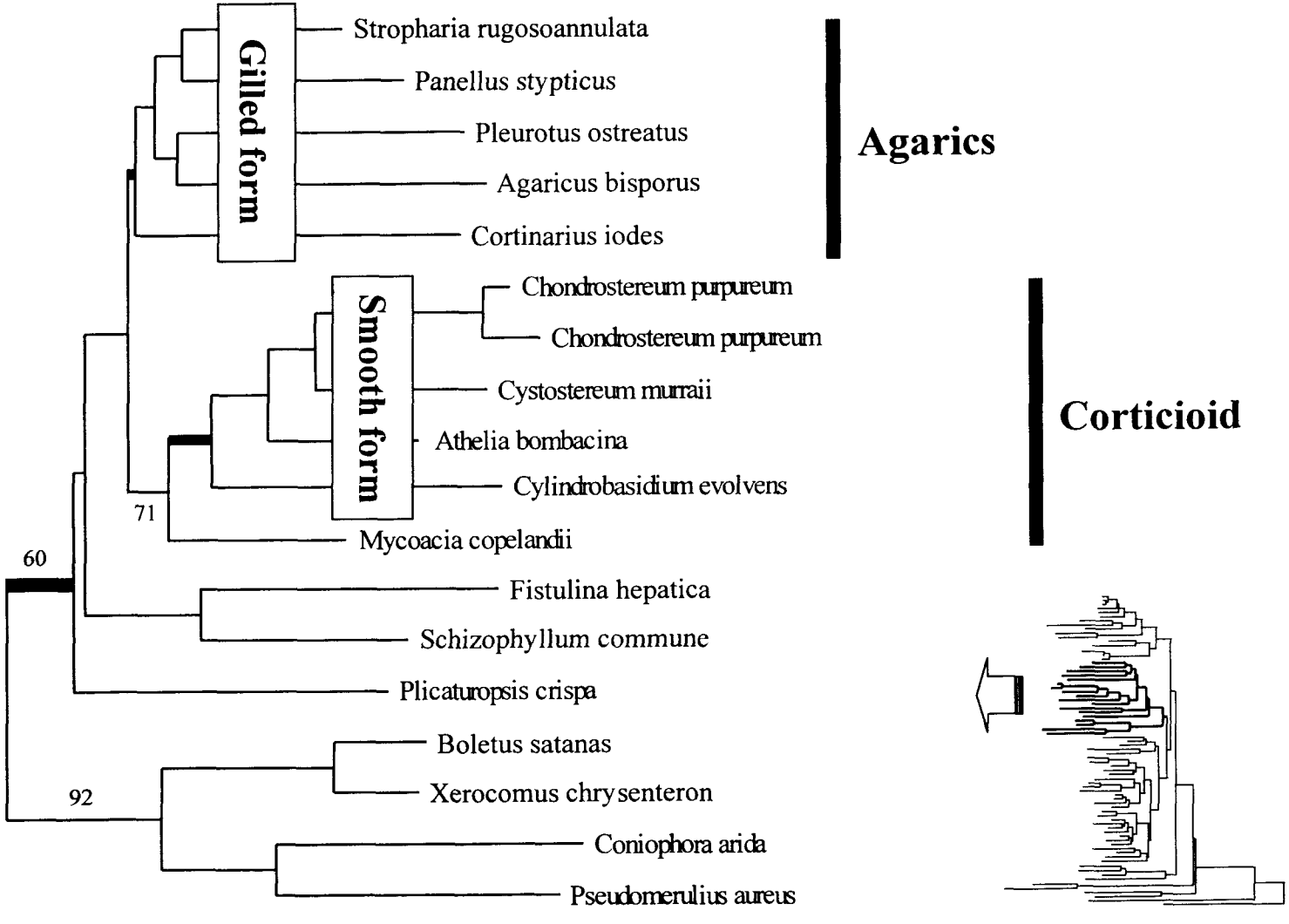
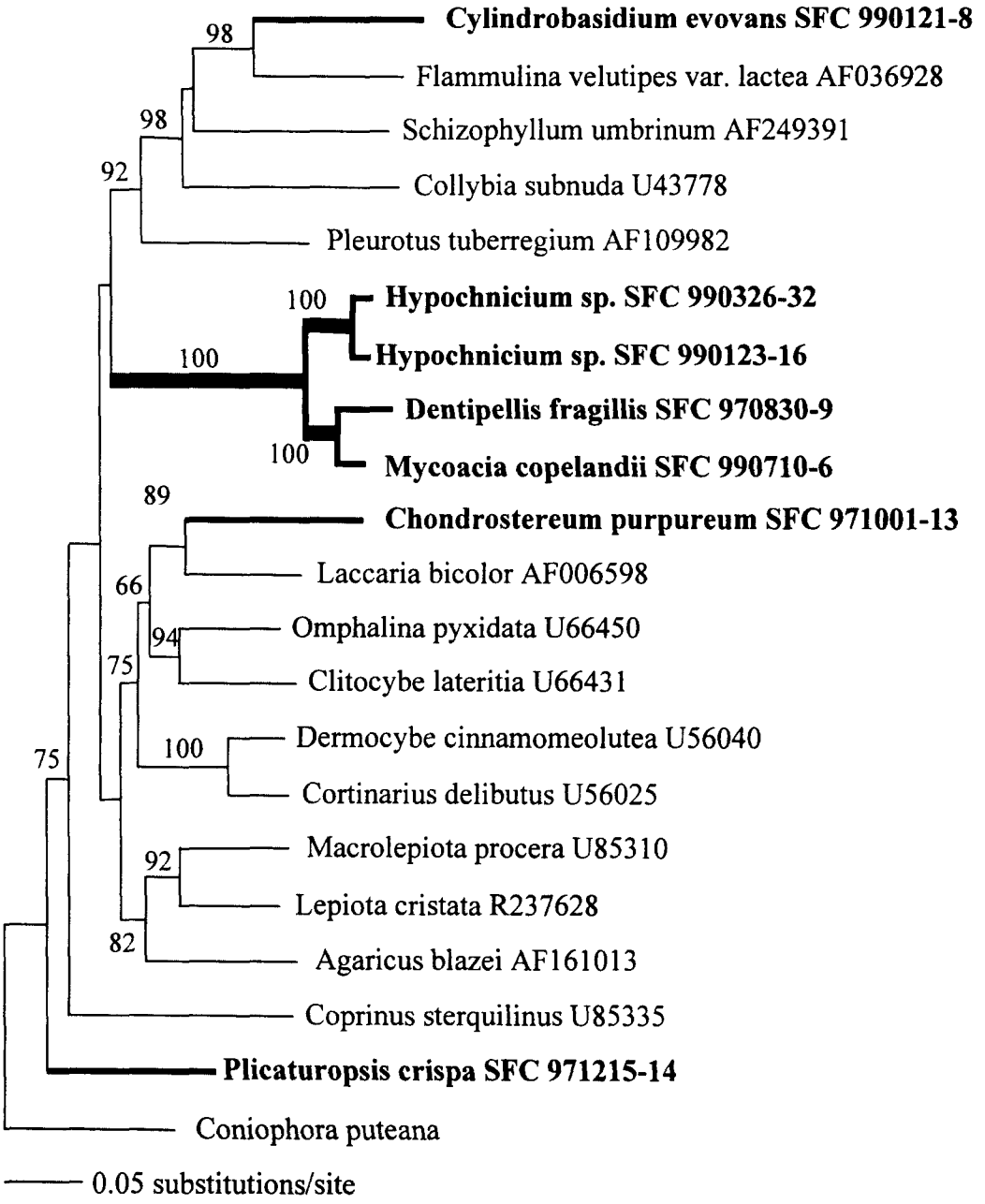

Gilled form
Stropharia rugosoannulata
Panellus stypticus
Pleurotus ostreatus
Agaricus bisporus
Cortinarius iodes
Agarics
Smooth form
Chondrostereum purpureum
Chondrostereum purpureum
Cystostereum murraii
Athelia bombacina
Cylindrobasidium evolvens
71
Mycoacia copelandii
Corticioid
60
Fistulina hepatica
Schizophyllum commune
Plicaturopsis crispa
Boletus satanas
92
Xerocomus chrysenteron
Coniophora arida
Pseudomerulius aureus

Figure 3.11. Phylogenetic relationships of corticioid fungi in the euagarics clade inferred from ITS sequences. Distance matrix was produced using Kimura's two parameter model with the transition/transvertion ratio of two. The tree was reconstructed by neighbor-joining algorithm using *Coniophora puteana* as an outgroup. The bootstrap analysis based on 1,000 resamplings was performed and values over 60% were indicated at corresponding branches.


Cylindrobasidium evovans SFC 990121-8
Flammulina velutipes var. lactea AF036928
Schizophyllum umbrinum AF249391
Collybia subnuda U43778
Pleurotus tuberregium AF109982
Hypochnicium sp. SFC 990326-32
Hypochnicium sp. SFC 990123-16
Dentipellis fragillis SFC 970830-9
Mycoacia copelandii SFC 990710-6
Chondrostereum purpureum SFC 971001-13
Laccaria bicolor AF006598
Omphalina pyxidata U66450
Clitocybe lateritia U66431
Dermocybe cinnamomeolutea U56040
Cortinarius delibutus U56025
Macrolepiota procera U85310
Lepiota cristata R237628
Agaricus blazei AF161013
Coprinus sterquilinus U85335
Plicaturopsis crispa SFC 971215-14
Coniophora puteana
0.05 substitutions/site
98
98
92
100
100
100
89
66
94
75
75
100
92
82

agarics of the euagarics clade. This clade consisted of *Cho. purpureum*, *Cys. muraii*, *A. bombacina*, *Cyl. evolvens* and *M. copelandii.* Common features of this clade were resupinate or effused-reflexed basidiomata, clamped generative hyphae and smooth non-amyloid basidiospores (Chamuris, 1988; Hjortstam *et al.*, 1987). While *A. bombacina* and *Cho. purpureum* have monomitic hyphal systems, *Cys. murraii* has a dimitic hyphal system with skeletal hyphae and numerous vesicles with yellow oily or resinous contents (Eriksson and Ryvarden, 1973; Chamuris, 1988). Although *Cho. purpureum* and *Cys. murraii* differ in the miticity, they have important common characters such as white rot and abundant vesicles in zones throughout the thickened hymenium (Reid, 1971). However, in the study of Boidin *et al.* (1998), *Cystostereum* was separated and grouped in the Phanerochaetales. Therefore, exact phylogenetic position is not still clear. Adding more sequence data or taxa might be necessary to resolve this problem.

Cylindrobasidium evolvens (Fr.) Jülich is a pioneer corticioid fungus among those that colonize various kinds of recently dead deciduous and coniferous wood, especially on fresh cut surfaces (Rimvydas and Stenlid, 1998). This species is known to be very common throughout all forest parts of central and northern Europe (Eriksson and Ryvarden, 1976) and is an invader of injuries on living Norway spruce in Scandinavia (Rimvydas and Stenlid, 1998). *Cylindrobasidium evolvens* was more often found in spruces infected by a serious pathogen *Stereum sanguinolentum* than would be expected by chance (Roll-Hansen and Roll-Hansen, 1980). In Korea, however, it was

found on recently dead deciduous wood but not on dead coniferous wood (Lim and Jung, 1999; Lim *et al.*, 2000).

Phylogenetic position of *M. copelandii* occured on a basal region of corticioid fungi in the euagarics clade of homobasidiomycetes based on nuc-ssu rDNA analyses (Fig. 3.10). However, its phylogenetic placement was more ambiguous in the ITS sequences analyses (Fig. 3.11). *Dentipellis fragilis* and two *Hypochnicium* species represented a close relationship to *M. copelandii*. *Dentipellis fragilis* was treated as a member of the order Hericales, which belongs to the russuloid clade, in the study of Boidin *et al.* (1998). *Hypochnicium* consisted of two separate lineages: one group of species in the polyporoid clade, along with *Hyphoderma* (Boidin *et al.*, 1998; Kim, 2001), and the other group in the euagarics clade, along with *M. copelandii* and *D. fragilis* (Fig. 3.11). *Mycoacia copelandii* and *D. fragilis* were primarily characterized by its hydnoid hymenial surface with long aculei (1-8 mm in length) and globose basidiospores and, on the other hand, two *Hypochnicium* species by smooth hymenial surface. Common characters among them were globose basidiospores, clavate basidia and monomitic hyphal systems with clamp connections (see Chapter 2).

The species name, *Mycoacia copelandii* (Patouillard) Aoshima & Furukawa, was firstly described by Aoshima and Furukawa (1966) and has been used in Japan and Korea. However, *M. copelandii* distinctly differs from *M. fuscoatra* (Fr.: Fr.) Donk, the type species of the genus, in morphology of basidiospores and basidia. The difference between *M. copelandii* and other *Mycoacia* species was supported by molecular data.

In the ITS sequences analysis, *M. nothofagi* and *M. nuda* were grouped into Phlebiales (Boidin *et al.*, 1998) and *M. fuscoatra* was also grouped into the *Phlebia* clade (VII-2, Chapter 3), which are all included in the polyporoid clade. Hydnoid hymenial surface and globose spores also characterize the genus *Radulodon* as well as *Mycoacia*. So Maekawa (1993) proposed a new combination *Radulodon copelandii* (Pat.) Maekawa for *M. copelandii*. *Sarcodontia copelandii* suggested by Imazeki and Hongo (1965) was another synonym of *M. copelandii* and a legitimated name. But the relationship between *M. copelandii* and *Radulodon* or *Sarcodontia* species is unclear because there are no published reports about *Radulodon* and *Sarcodontia* based on molecualr phylogeny.

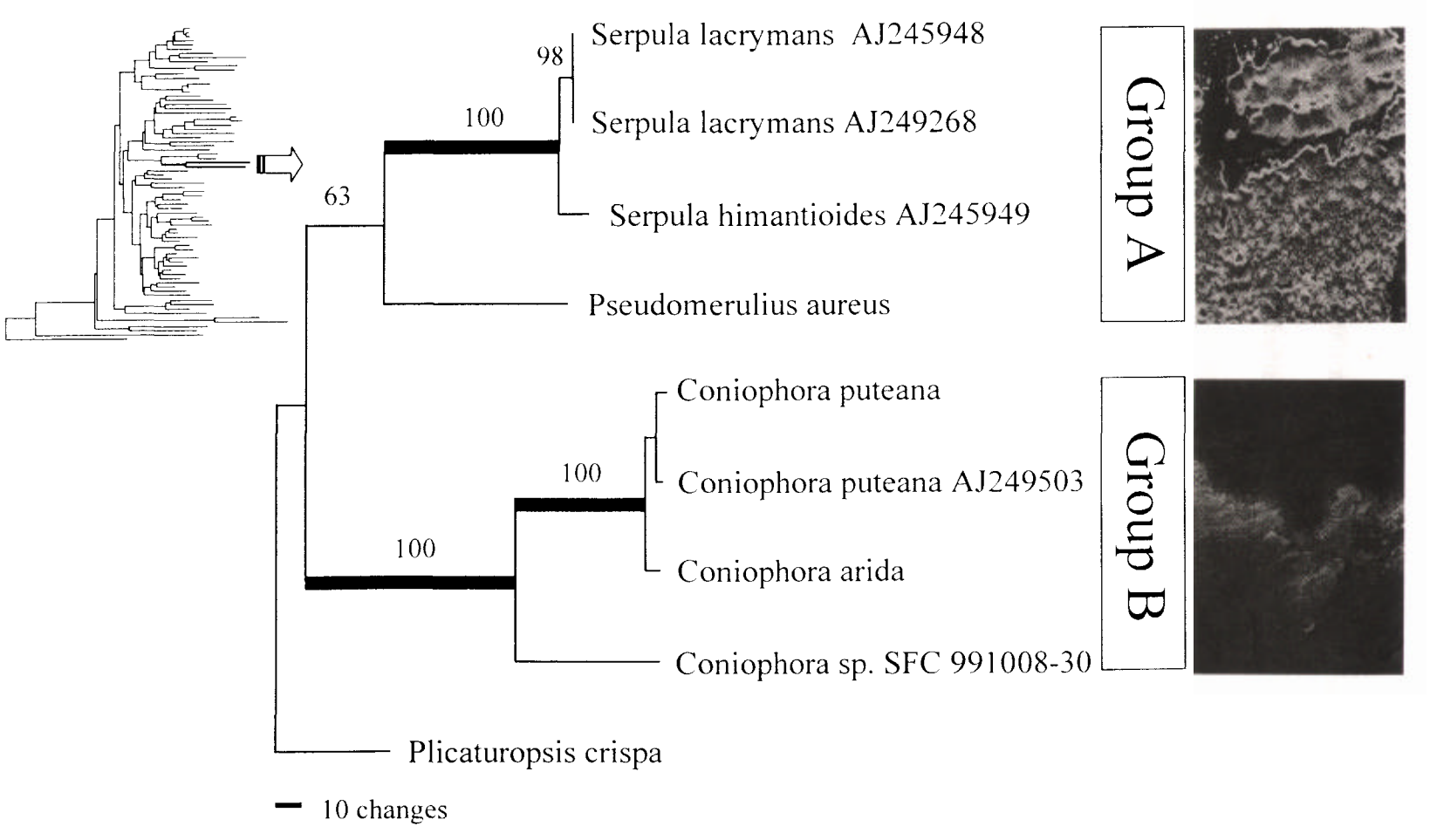
Phylogenetic position of *Plicaturopsis crispa* was very interesting. Although bootstrap support is weak, it was placed at the basal branch of the euagaric clade in both trees based on nuc-ssu rDNA and ITS sequences (Figs. 3.10 and 3.11). This species was characterized by pileate, cupulate, or subresupinate fruitbodies and radially bifurcating gill-like ridge hymenophores. Gilled mushrooms have probably been derived from morphologically diverse precursors. For example, it is inferred that *Lentinus* was derived from stipitate polypores (Hibbett and Vilgalys 1993: Hibbett *et al.*, 1997). Thus, the above result suggested a possibility that *P. crispa* might have been an ancestor of agarics.

V. Molecular Phylogeny of Corticioid Fungi in the Bolete Clade

The bolete clade inluded members of the Gomphidiaceae, Coniophoraceae, Hymenogastrales, Melanogastraceae, and Calostomataceae (Bruns *et al.*, 1998; Hibbett *et al.*, 1997). Monophyly of the bolete clade has been strongly supported in analyses based on nuc-ssu rDNA at the 92% confidence level (Fig. 3.5). Taken together with previous studies, close relationships have been suspected among poroid boletes (e.g. *Boletus* and *Suillus*), certain gilled (e.g. *Phylloporus*), resupinate (e.g. *Coniophora*) and gasteroid (e.g. *Rhizopogon* and *Melanogster*) forms based on anatomy, spore morphology, wood decay chemistry, susceptibility to certain fungal pathogens and pigments (Hibbett and Thorn, 2001). Analyses of nuc-ssu rDNA sequences show a basal bifurcating in the bolete clade (Fig. 3.5). One was mostly poroid bolete lineage that includes *Boletus satanas* and *Xerocomus chrysenteron* and the other was the lineage of resupinate corticioid fungi such as *Coniophora* and *Pseudomerulius*.

The data set based on ITS sequences was 688 aligned interleaved nucleotides (195 informative sites). Heuristic analysis gave two most parsimonious trees (tree length = 431 steps, CI = 0.9118, RI = 0.9110, RC = 0.8307). Of these, Fig. 3.12 was selected as a best tree through log likelihood test (Kishino and Hasegawa, 1989) implemented in PAUP* 4.0b4a (Swofford, 1999). Parsimony analyses formed two separate groups. Group A consisted of *Serpula lacrymans, S. himantioides* and *P. aureus* and group B *Coniopora puteana, C. aureus* and unidentified *Coniophora* species. Group A is

Figure 3.12. Phylogram of corticioid fungi in the bolete clade showing one of two most parsimonious trees resulted from phylogenetic analysis. Tree length = 431 steps, consistency index = 0.9118, retention index = 0.9110 and rescaled consistency index = 0.8307. The best tree was selected through the log likelihood test implemented in PAUP*4.0b4a. Statistical support for each clade by bootstrap analysis was indicated by on the corresponding branch. *Plicaturopsis crispa* was used as an outgroup.


Serpula lacrymans AJ245948
Serpula lacrymans AJ249268
Serpula himantioides AJ245949
Pseudomerulius aureus
Coniophora puteana
Coniophora puteana AJ249503
Coniophora arida
Coniophora sp. SFC 991008-30
Plicaturopsis crispa
98
100
63
100
100
10 changes
Group A
Group B

supported at 63% confidence level and group B at 100% confidence level.

All species within groups A and B were classified into the family Coniophoraceae. This family is characterized by the nature of yellowish brown spores under microscope, oblong in shape, smooth, double-walled, cyanophilous and with an apical germ pore. The hyphae of the family Coniophoraceae shows a considerable variation from monomitic to dimitic (trimitic) systems combined with an advanced polymorphism. Almost all species in Coniophoraceae are wood-rotting fungi and associated with brown rot and many of them cause severe decay in wooden constructions (Hallenberg and Eriksson, 1985). They selectively remove cellulose and other polysaccharides from wood cell walls and leave a residue of slightly modified lignin. This brown rot residue is highly stable in nature and becomes important soil component with significant ecological functions in forest ecosystems (Gilbertson and Hemmes, 1997). Brown rot fungi have been reported to occur mainly in temperate conifer forests (Gilbertson and Ryvarden, 1986). Based on basidiospore characters, this group has been suggested as a relative of the boletes and the family was placed in Boletales by Ginns and Lefebvre (1993).

The dry-rot fungus, *Serpula lacrymans*, was a major cause of timber decay in buildings in Europe. *Serpula lacrymans* posed an enigma for a long time because it was unknown from any natural environment but only from buildings. However, it is exceptionally found in forests of northern India. Dry rot has been recorded in Europe since about 1765, before there was any export of timber from India. So this rare fungus

seems to have arrived in Europe as air-borne basidiospores and then flourished in buildings where the climatic conditions are similar to those in its natural habitat (Deacon, 1997). During the days of wooden warships, this fungus was a major cause of serious and widespread decay of sailing vessels. Even today, *S. lacyrmans* is still important, primarily in Europe, where the dreaded dry rot causes tremendous damage to wooden structural elements and floors in houses and other buildings. The cost associated with the efforts to protect wood products from decay was staggering. The destructive effects were comparable to termite damage done to wooden structures in the United States (Alexopoulos *et al.*, 1996). So the accurate identifications were accomplished by various molecular techniques: SDS-PAGE of intracellular proteins, immunological methods and species-specific PCR methods (Schmidt and Moreth, 2000).

The *Serpula* clade in group A is supported by whole bootstrap value (100%) and its genetic distance ranged from 0.00-2.46% (Table 3.5). *Serpula himantioides* and *S. lacrymans* were characterized by merulioid, dark brown hymenium, large ellipsoid spores with yellowish or brownish thick walls. Their spore sizes are about 9-12 × 4.5-6.5 μm. *Pseudomerulius* classified in the Coniophoraceae has an irregularly netted hymenophore and is closely related to *Leucogyrophana*. Considering cyanophilous spores and hymenophore morphology, there are too small differences to differentiate from the genus *Leucogyrophana* (Eriksson *et al.*, 1981). However, the nature of spores, such as shape, size and thin wall, differentiates *P. aureus* from *Leucogyropha.*

Table 3.5. Genetic distances calculated by the uncorrected ("p") distance model.

	1	2	3	4	5	6	7	8
1 *S. lacrymans* AJ245948	-							
2 *S. lacrymans* AJ249268	0.00000	-						
3 *S. himantioides* AJ245949	0.02458	0.02458	-					
4 *P. aureus* SFC 970927-4	0.20058	0.20058	0.20090	-				
5 *C. puteana* IFO 6275	0.29104	0.29104	0.28609	0.28984	-			
6 *C. arida* SFC 990911-57	0.28535	0.28535	0.28040	0.28575	0.01265	-		
7 *C. puteana* AJ249503	0.28038	0.28038	0.27552	0.27939	0.01109	0.01743	-	
8 *Coniophora* sp. SFC 991008-30	0.26443	0.26443	0.26177	0.28178	0.15879	0.15519	0.15637	-

Pseudomerulius is easily recognized in the field because of its brightly golden fruitbody, irregularly composed merulioid hymenophore and host specificity (Lim and Jung, 1998b). *Pseudomerulius aureus* occurred next to *Serpular* and clustered within the group A. Although their genetic distance was long (20.1%), meulioid hymenophore strongly supported their relationship.

Coniophora is found in most of coniferous woods but sometimes cause brown heartwood rot in citrus (Demetriou *et al.*, 2000). Its morphological characters are very similar to *Serpula*. However, yellowish hymenophore of *Coniophora* is smooth or tuberculate. The molecular identification of ectomycorrhizal basidiomycetes by Bruns and his colleagues (1998) demonstrated that *Coniophora* is located in the root of the clade clustering *Calostoma, Gyroporus, Scleroderma, Pisolithus, Boletinellus* and *Phaeogyroporus*. The phylogenetic analysis provided a tree topology that placed *C. arida* as a close relative of *C. puteana*. The genetic distance within *C. puteana* strains was very short with only 1.11% dissimilarity, while there was 1.27 – 1.74% dissimilarity found between *C. arida* and *C. puteana*. These two species represent a close relationship along with morphological features. *Coniophora puteana* differs, however, by having a fleshy, membranaceous, tuberculate furitbody and brown spores. Another unidentified *Coniophora* species occurs in the basal branch of group B (Fig. 3.12). This species showed a relatively long distance to other *Coniophora* species and its genetic distance ranged from 15.52 to 15.88%. Unidentified *Coniophora* species was macroscopically similar to *C. arida* but distinguishable from *C. arida* by its short

basidia (see Chapter 2). It differed from *C. puteana* in having a thin, membranous fruitbody and a smooth hymenial surface. The results of the sequence analysis coupled with the morphological characteristics indicated that the unidentified *Coniophora* species might be an unrecorded species in Korea or a new species.

VI. Molecular Phylogeny of Corticioid Fungi in the Laeticorticioid Clade

The laeticorticioid clade has been generally accepted as the family Vuilleminaceae (Jülich, 1981) or the order Vuillemiales (Boidin *et al.*, 1998) based on the following suite of characters: basidiocarps with monomitic hyphal system, catahymenia with richly branched dendrohyphidia, large basdidia with or without vesicular probasidia and large, hyaline, smooth, inamylid spores. Larsen and Gilbertson (1974) divided *Laeticorticium* into three genera, *Laeticorticium, Dendrocorticum* and *Dentocorticium*, emphasizing the nature and position of the probasidial bladder.

In the ITS sequence analyses, Boidin *et al.* (1998) tried to evaluate the phylogenetic relationships within the order Aphyllophorales using ribosomal internal transcribed spacer (ITS) sequences. They classified and revised the Aphyllophorales into twenty orders, creating five new orders. The order Vuilleminiales was one of them. The order Vuilleminiales consisted of *Corticium* (=*Laeticorticium*), *Dendrocorticium*, *Punctularia* and *Vuilleminia*. ITS regions are known to evolve rapidly, so they are not thought to be appropriate in the study of higher level relationships of fungi. The results of Boidin and colleagues (1998) didn't show their phylogenetic positions within the homobasidiomycetes but only the result that those genera were closely related to each other. Therefore, in the study of Hibbett and Thorn (2001), above four genera were grouped into the polyporoid clade according to the result of Boidin *et al.* (1998).

Phylogenetic analysis based on nuc-ssu rDNA apparently showed where the position of the laeticorticioid clade was (Fig. 3.5). The laeticorticioid clade based on nuc-ssu rDNAs and ITS sequences included *Cytidia, Dendrocorticium, Laeticorticium, Punctularia* and *Vuilleminia* in the family Corticiaceae. Monophyly of the laeticorticioid clade based on nuc-ssu rDNA analysis has been strongly supported. This clade was placed next to the clade including *Boreostereum*, *Veluticeps* and *Gloeophyllum*. This sister clade was first described by Yoon *et al.* (2001) who sampled only three species representing the group H (*Gloeophyllum sepiarium, Boreostereum radiatum* and *Veluticeps berkeleyi*). Phylogenetic placement of the group H was distinct outside the polyporoid clade. Yoon and his colleagues (2001) indicated that the brown rot and brown-colored hyphae apparently play an important role in phylogenetic characterization of the clade but these characters have nothing to do with the laeticorticioid clade. Although the sister clade has been supported by 86% confidence level, monophyly of two clades has been poorly supported so that the sister clade needs to be excluded from the laeticorticioid clade.

PCR products of ITS region using ITS5 and LW1 primers ranged from 530 to 570 base pairs. A single band was detected in each amplification reaction when analyzed by agarose gel electrophoresis. Only the sequences of the total ITS regions (including 5.8S) were used for phylogenetic analysis. ClustalX alignment was used with gap open penalty of 10.00 and gap extension penalty of 2.00. The total length of the obtained alignment was 558 of which 188 were parsimony informative. Heuristic analysis gave

two most parsimonious trees (tree length = 490 steps, CI = 0.7367, RI = 0.8307, RC = 0.6120). One of the most parsimonious trees is shown in Fig. 3.13. The percentages representing a measure of statistical confidence are greater than 93% in most cases (Fig. 3.13). Horizontal lengths of the branches indicate the relative genetic distance between the isolates, corresponding to the number of nucleotide substitutions. There was 0-23.81% dissimilarity among the sequences of total ITS of all isolates used. Sequence dissimilarities among groups are summarized in Table 3.6.

Parsimony analysis showed that this clade was congruent with the results of previously papers (Boidin *et al.*, 1998; Hallenberg and Parmasto, 1998). Species in the phylogenetic tree were divided into five groups in this stduy (Fig. 3.6). Group A consisting of the genus *Punctularia* was supported by 98% bootstrap values. Genetic distances in group A ranged from 4.330 to 6.275%. The genus *Punctularia* has resupinate or reflexed fruitbodies. Due to subgelatinous hymenium, it was provisionally placed in Thelephoraceae subfamily Merulioideae by Talbot. The genus *Punctularia* showed great resemblance to *Laeticorticium*, especially in the presence of dendrohyphidia, nature of basidia and shape of spores. Also the nature of the hyphae was essentially the same. The remaining difference is the pigmentation of the dendrohyphidia, the subgelatinious consistency of the hymenial layer and the ability to form reflexed pilei of *Punctulaira* (Eriksson *et al.*, 1981). According to Maekawa (1994), the genus *Punctularia* is microscopically similar to *Dentocorticium* in hymenial structures. However, the former forms mainly effuso-reflexed fruitbodies

Table 3.6. Genetic distances between pairs of fungal strains calculated by the Kimura two-parameter model.

	Group A	Group B	Group C	Group D	Group E
Group A	4.33-6.28				
Group B	10.98-12.11	0.00-0.20			
Group C	16.64-16.75	14.97-15.18	0.00		
Group D	21.38-23.81	22.18-22.56	22.06-22.50	0.59-5.17	
Gruup E	15.04-16.51	14.53-15.25	15.08-16.43	18.46-20.95	1.05-12.61

with dark brown abhymenium whereas the latter has resupinate basidiomata and a close relationship to *Trametes* and *Ganoderma* in the polyporoid clade (I, Chapter 3). This genus was mainly distributed in tropical and subtropical regions but *P. strigoso-zonata* is distributed widely in temperate to subtropical regions.

The two or three species of this remarkable genus, *Punctularia*, have been placed in various Friesian genera. Patouillard was the first to discover that the knobs and folds were not really what they appeared to be but were distinct cushions covered by the hymenium and separated by narrow sterile tough hyphae. Talbot also detected the great similarity between *Punctularia* and *Phaeophlebia* and firstly recognized that there might be a good case for the proposal of a new family to accommodate *Punctularia*. So Donk (1964) proposed the new family *Punctulariaceae*. Jülich (1981) included the family Punctulariaceae into the order Aleurodiscales that also included the family Vuilleminiaceae. For a long time, their relationships were acknowledged by many mycologists.

Group B contained the genus *Dendrocorticium* and its branch was fully supported by 100% bootstrap values. Genetic distances among group B was very short and ranged from 0.00 to 0.204%. Larsen and Gilbertson (1974) segregated the genus *Dendrocorticium* from *Laeticorticium* based on the mode of basidial ontogeny. The main difference between *Dendrocorticium* and *Laeticorticium* is the probasidial bladders, which is located in the subhymenial layer in the former genus and situated in or on the subicular layer in the latter genus. In very young fruitbodies of *L. roseum*

where the hymenial layer is very thin, the probasidial bladders have the described position but are found all over the subhymenium in more mature furitbodies with a thickened subhymenium. However, many mycologists have treated the genus *Dendrocorticium* as a synonym of *Laeticorticium* until now. And *Dendrocorticum* has been treated as an artificial taxon rather than a natural one because *D. lundellii* is clearly more close to *L. roseum* than to the generic type. In this study, the genus *Dendrocorticum* was apparently different from the *Laeticorticium* group whose distance ranged 22.183 to 22.561%. Due to the basidiocarp of species in group B was thick and tough when fresh, *D. violaceum* sometimes was misidentified as *Phanaerochaete crassa*. Unidentified *Dendrocorticum* species (KD-971231-7) in group B deviated clearly in some respects (see Chapter 2) but coincided with other members of *Dendrocorticium*. Therefore, the unidentified species is judged to be a new species or phylogenetic species of *D. violaceum*.

Group C included one unidentified *Dendrocorticium* species. Group C formed a distinct clade supported by 100% confidence level and its relationship with other *Dendrocorticium* and *Laeticorticium* correlated with morphological and molecular data. The mature basidiocarp of this species was not confirmed because the examined specimen was once collected as a premature one. This species had apparent deviations in microscopic characters (see Chapter 1). Molecualr and morphological data suggested that this one might be a new species.

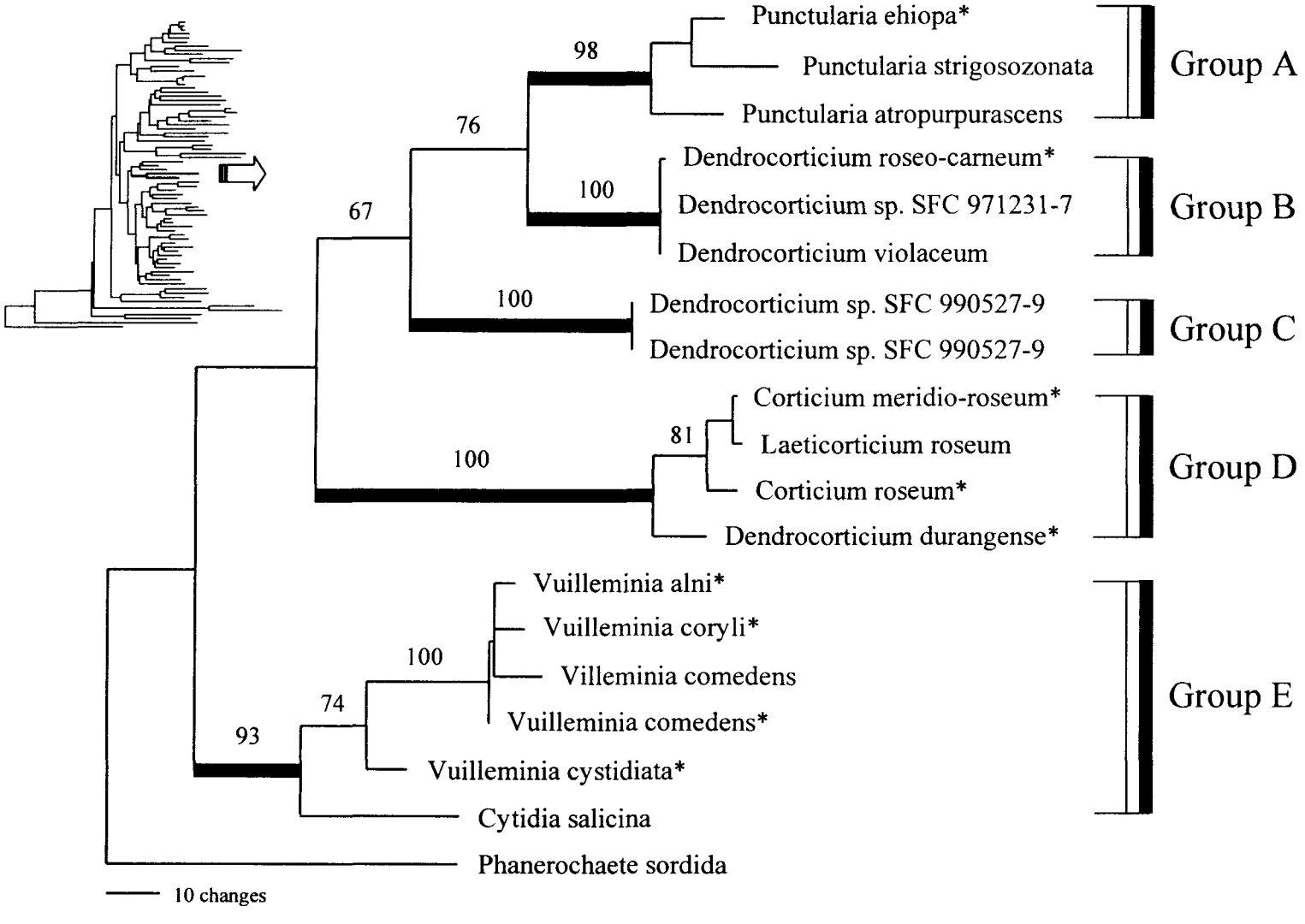
Group D consisted of *Laeticorticium* species and *D. durangense* and was supported

by 100% bootstrap values. Distance among them ranged from 0.539 to 5.168%. The genus *Corticium* had long been regarded as a receptacle for acystidiate corticioid species for which no suitable genus was available until Donk (1956) erected the new genus *Laeticorticium* on the basis of *C. reseum*. *Laeticorticium* has a narrower generic concept than *Corticium* and encompasses those corticioid taxa that possess catahymenia, nodose-septate hyphae, probasidia and non-amyloid, smooth basdiospores. However, Jülich (1982) revived the generic name *Corticium* which had nomenclatural priority over *Laeticorticium* and reduced the generic concept of *Corticium* Pers., which was accepted by some mycologists (Maekawa, 1994). Although *Corticium* had nomenclatural priority over *Laeticorticium*, many mycologists have used the name *Laeticorticium* until now. In the present study, the name *Laeticorticium* is used. The presence of basidial state is, in *Laeticorticium*, an adaptation for delayed ripening of the basidium. In *Laeticorticium*, the basidium starts as a round or angular bladder in the autumn but does not mature until spring. It survives the winter in the resting state (Eriksson and Ryvarden, 1976).

The third genus in the arrangement of Larsen and Gilbertson, *Dentocorticum*, is certainly a more or less founded one. Its two species, *D. sulfurellum* and *D. ussuricum*, decidedly differ from *Laeticorticium*. They are both odontioid, have allantoid spores and lack the red or violaceous colors, which characterize most species of *Laeticorticium*.

The genus *Vuilleminia* is characterized by the long, tubular basidia and large

Figure 3.13. One of two most parsimonious trees inferred from ITS regions and 5.8S rDNA gene sequences. The tree was rooted by the outgroup sequence of *Phanerochaete sordida*. Bootstrap values more than 60% from 1,000 replications are indicated above internodes.


Punctularia ehiopa*
Punctularia strigosozonata
Punctularia atropurpurascens
98
76
Dendrocorticium roseo-carneum*
Dendrocorticium sp. SFC 971231-7
Dendrocorticium violaceum
100
67
100
Dendrocorticium sp. SFC 990527-9
Dendrocorticium sp. SFC 990527-9
Corticium meridio-roseum*
Laeticorticium roseum
Corticium roseum*
Dendrocorticium durangense*
81
100
Vuilleminia alni*
Vuilleminia coryli*
Villeminia comedens
Vuilleminia comedens*
Vuilleminia cystidiata*
Cytidia salicina
Phanerochaete sordida
100
74
93
10 changes
Group A
Group B
Group C
Group D
Group E

basidiospores and shown in group E. Group E formed a monophyletic group supported by high bootstrap value (93%). Using the partial nuc-lsu rDNA sequence, morphological data and combined data analyses, Hallenberg and Parmasto (1998) revealed that *Vuilleminia* was paraphyletic and *Dentocorticium* was intermingled with *Corticium*. However, they showed that *Corticium*, *Dendrocoticium*, *Cytidia*, *Vuilleminia* and *Duportella* belonged to one monophyletic suprageneric taxon. According to Boidin *et al.*, (1998), *Duportella* was closely related to *Peniophora* species. *Cytidia salicina* was easily distinguished from other genera of the corticioid fungi by its dark red, discoid, and coriaceous to gelatinous basidiomata and by possessing pale brownish dendrohyphidia, large basidia and large basidiospores. The microscopic features of this species resemble very much other members in the laeticorticioid clade but the nature of the fruitbody as well as the reaction to sulfovanilline made them quite different (Eriksson and Ryvarden, 1975). Considering phylogenetic results, micromorphology was taxonomically useful but the reaction to sulfovanilline did not. These results were also found in the study of the russuloid clade. Phylogenetic analysis also showed that evolutionary direction from resupinate form to distinctly fan-shaped form of fruibody occurred in this clade.

VII. Molecular Phylogeny of Corticioid Fungi in the Polyporoid Clade

1. A new species of *Irpex* based on morphological, cultural and molecular data

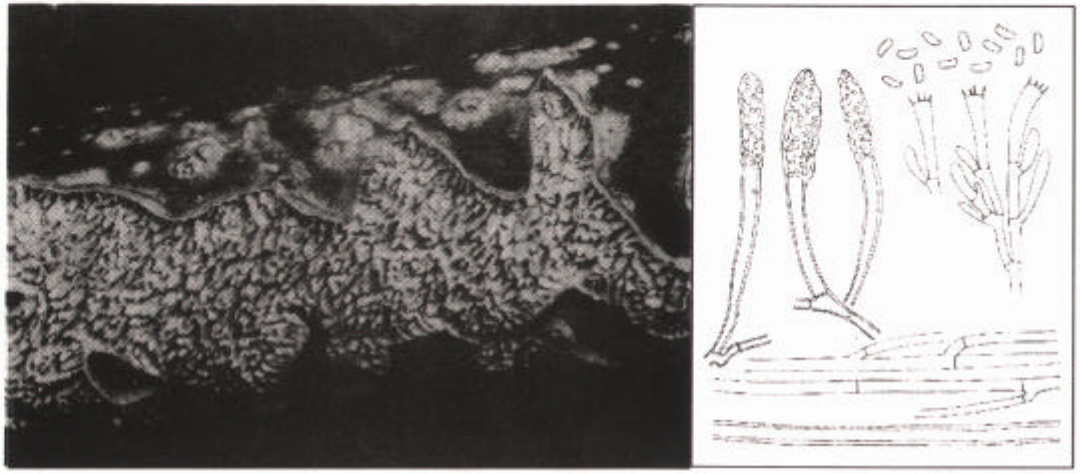
Irpex Fr. is one of the frequently collected genus in Korea and is easily identified by its strongly hydnaceous hymenophore, conspicuously encrusted cystidia, tomentose pileus and simple-septate hyphae, but its morphology is various depending on environments. Sometimes it is vigorously growing and measures up to 3 m long along fallen twigs of various hardwoods (Jung, 1987). Because of its various morphology, numerous other species have been described in or transferred to the genus *Irpex*. But Maas Geesteranus (1974) concluded that these were all either synonyms of *I. lacteus* or needed to be properly placed in other genera, maintaining *Irpex* as a monotypic genus.

During a mycological excursion at Kangwon province, some fresh *Irpex* species were collected from a fallen branch of various angiosperms. But its morphological characteristics and cultural characteristics differentiated this taxon clearly from *I. lacteus*. To further examine the fitness of the new species based on morphology and culture, the sequence analyses corresponding to the ITS1, ITS2 region and the 5.8S rRNA gene were performed. This new species was named *I. hydnoides*, numbered 18 and described in Latin in Chaper 2. The genus *Irpex* is characterized by the apparently

Figure 3.14. Comparison of macro- and microscopic features between *Irpex hydnoides* and *I. lacteus*. Their descriptions are presented in the annotated list no. 18.

A: *Irpex hydnoides*

B: *Irpex lacteus*



irpicoid, white to cream hymenophore, effused-reflexed or resupinate basidiocarps, a dimitic hyphal system, generative hyphae with simple-septate, and conspicuously encrusted cystidia. But, because of its various morphology, many *Irpex* species have been described in relation with other genera, especially *Steccherinum* and *Junghuhnia*. The generative hyphae with simple-septate, however, clearly differentiate the genus *Irpex* from *Steccherinum* and *Junghuhnia* which have clamp connections.

When *Irpex hydnoides* was collected, it was mistaken for *I. lacteus*. However *Irpex hydnoides* is distinguished by the apparently long teeth (up to 4 mm), deep yellowish (3A3, 3A5) hymenophore, subglobose spores and densely tomentose upper surface (Fig. 3.14). Although the teeth of *Irpex lacteus* measure up to 3 mm long when it is vigorously growing (Jung, 1987), its hymenophore always represents white to cream color. The basidia morphologies of *I. lacteus* and *I. hydnoides* are similar, but those of *I. hydnoides* are larger (28-32 × 6-7 μm) than those of *I. lacteus* (20-25 × 4-5 μm). While the spores of *I. lacteus* are oblong to cylindric, straight to slightly curved, and 5-6 × 2-3 μm in size, those of *I. hydnoides* are subglobose to ellipsoid, 5.7-6.5 × 3.7-3.8 μm in size (Fig. 3.14).

The cultural characteristics and the results of phylogenetic analyses of the new species are as follows:

Cultural characteristics

Growth on MEA slightly late, forming mats of 11.2 mm diam in 7 days at 25℃; advancing zone even, hyaline and slightly raised; hyphae distant, branched, 2-5.7 μm thick; aerial mycelium at first downy, becoming low cottony, white, 2-3 μm thick; reverse bleached.

Species code of Stalpers (1978): 1, 2, 9, 12, 15, 17, 30, 31, 37, 39, 46, 50, 52, 53

Extracellular oxidase reactions: α-naphthol + : p-cresol ++

Culture examined: SFC971215-19 isolated from basidiocarp (holotype) tissue

Cultural characteristics of *I. hydnoides* were also different from those of *I. lacteus* in growth rate and extracellula oxidase reaction. The growth rate of *I. lacteus* is 4.9-5.3 mm/day and is correspondent to the study of Stalpers (1978), but the one of *I. hydnoides* is quite slow and increases just 1.6 mm per day (Fig. 3.15). In the *p*-cresol test to see the presence of tyrosinase activity, strong color change occurred in *I. hydnoides* but there was no such a reaction in *I. lacteus* at all.

DNA amplification and Sequence analyses

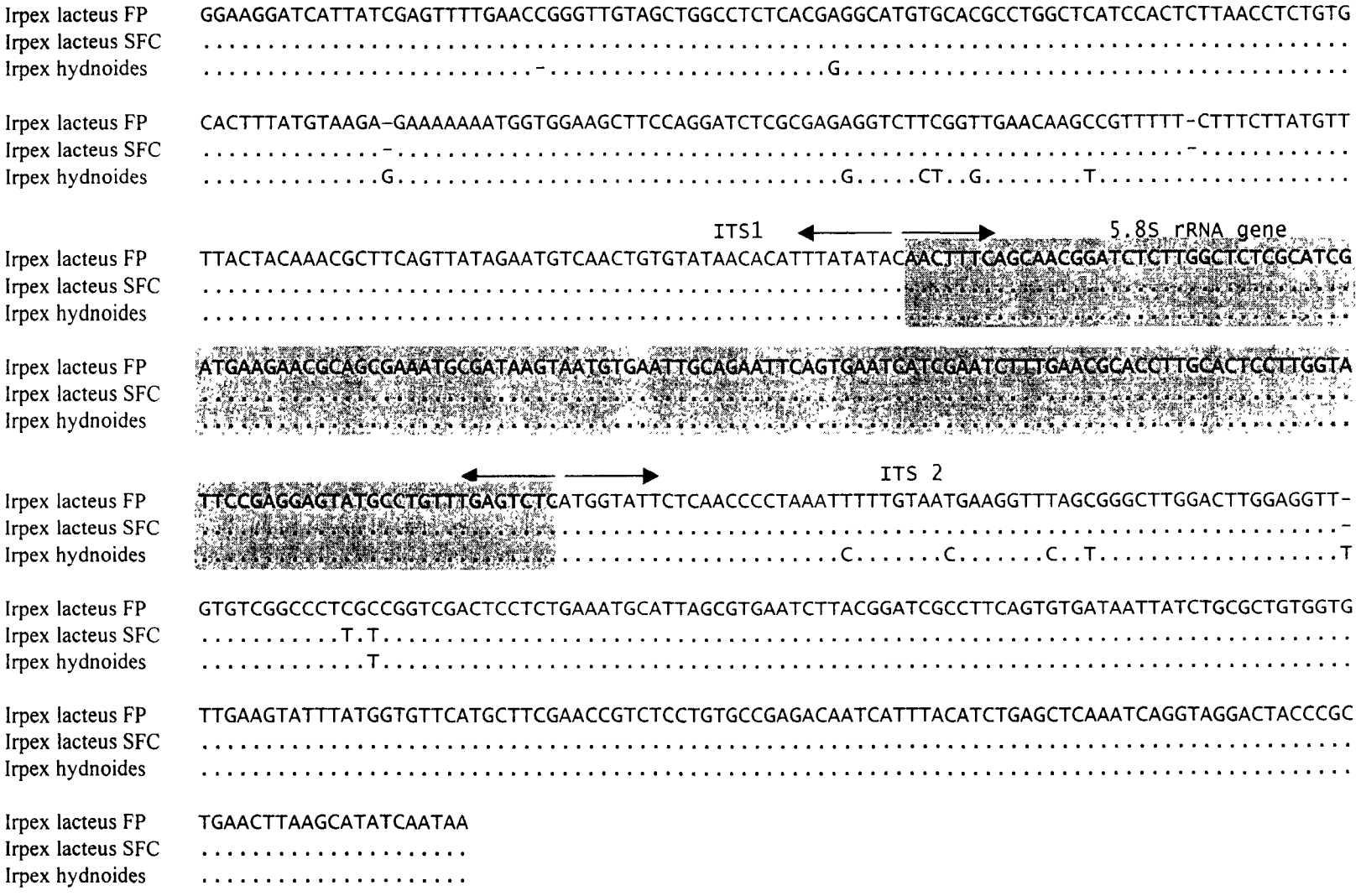
The amplification products using primers NS7 and ITS4 were approximately 1 kb in size. Total sequence length of ITS1-5.8S rDNA-ITS2 region ranged from 630 bp to 650 bp (primer pair ITS5-ITS4). There was no intraspecific variation in the ITS region of *I. lacteus* and *I. hydnoides* from Korea. Two strains of *I. lacteus* from Korea and

Figure 3.15. Macroscopic mats in culture showing the growth on MEA in 5 days at 25℃ (bar = 2 cm)

A) *Irpex hydnoides* (isolated from holotype) B) *Irpex lacteus*

A
B

Figure 3.16. Aligned sequences of two internal transcribed spacer regions ITS1 and ITS2, and the 5.8S rRNA gene. Dots (.) in the sequences indicate conserved bases, and dashes (-) indicate gaps. Shaded box represents 5.8S region. *Irpex lacteus* FP represents a species donated by the USDA Forest Products Laboratory from U.S.A., *Irpex lacteus* SFC represents isolates from South Korea (see Table 3.1.).


Irpex lacteus FP GGAAGGATCATTATCGAGTTTTGAACCGGGTTGTAGCTGGCCTCTCACGAGGCATGTGCACGCCTGGCTCATCCACTCTTAACCTCTGTG
Irpex lacteus SFC
Irpex hydnoides
Irpex lacteus FP CACTTTATGTAAGA-GAAAAAAATGGTGGAAGCTTCCAGGATCTCGCGAGAGGTCTTCGGTTGAACAAGCCGTTTTT-CTTTCTTATGTT
Irpex lacteus SFC
Irpex hydnoides
ITS1
5.8S rRNA gene
Irpex lacteus FP TTACTACAAACGCTTCAGTTATAGAATGTCAACTGTGTATAACACATTTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCG
Irpex lacteus SFC
Irpex hydnoides
Irpex lacteus FP ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCACTCCTTGGTA
Irpex lacteus SFC
Irpex hydnoides
ITS 2
Irpex lacteus FP TTCCGAGGAGTATGCCTGTTTGAGTCTCATGGTATTCTCAACCCCTAAATTTTTTGTAATGAAGGTTTAGCGGGCTTGGACTTGGAGGTT-
Irpex lacteus SFC
Irpex hydnoides
Irpex lacteus FP GTGTCGGCCCTCGCCGGTCGACTCCTCTGAAATGCATTAGCGTGAATCTTACGGATCGCCTTCAGTGTGATAATTATCTGCGCTGTGGTG
Irpex lacteus SFC
Irpex hydnoides
Irpex lacteus FP TTGAAGTATTTATGGTGTTCATGCTTCGAACCGTCTCCTGTGCCGAGACAATCATTTACATCTGAGCTCAAATCAGGTAGGACTACCCGC
Irpex lacteus SFC
Irpex hydnoides
Irpex lacteus FP TGAACTTAAGCATATCAATAA
Irpex lacteus SFC
Irpex hydnoides

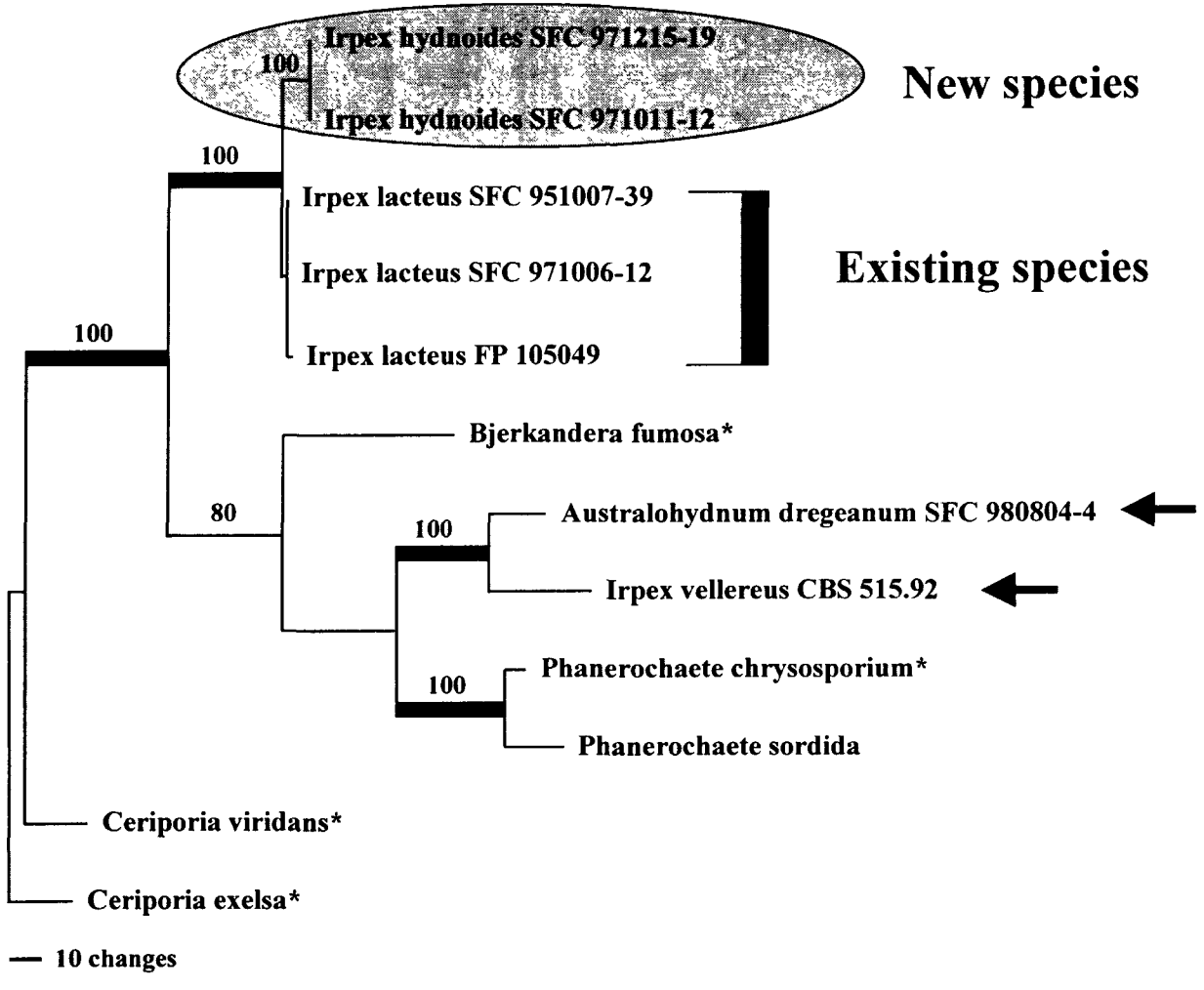
North America differed by 0.3% in sequences. Dissimilarity between *I. lacteus* and *I. hydnoides* ranged from 2.15 to 2.31% and variable sites of ITS region are shown in Fig. 3.16. In *Phytophthora*, intraspecific variability in the ITS among several taxa showed no intraspecific variation or was 2.4 to 4.6% divergent (Lee and Taylor, 1992). In *Laccaria bicolor*, three strains differed by 1-2% in sequences and were 3.5-5% divergent from other *Laccaria* species (Gardes *et al.*, 1991). Anderson and Stasovski (1992) showed extremely low levels of diversity (less than 0.5%) in the ITS region among closely related taxa and intersterility groups in *Armillaria* species. Within *Amylostereum chailletii* and *A. laevigatum* isolates, no intraspecific variation was also found. The sequence divergences among three *Amylostereum* species was very low (< 3%) (Vasiliauskas *et al.*, 1999). Therefore, the results of sequence divergence of two *Irpex* species supported that *I. hydnoides* distinctly differed from *I. lacteus*.

Heuristic searches based on the sequences of the ITS1-5.8S rDNA-ITS2 region were performed with a character matrix of 614 sites from 12 species. Of these characters, 356 characters were constant and 164 were potentially parsimony-informative. Maximum parsimony analyses performed with gaps treated as missing data yielded 5 equally most parsimonious trees (Fig. 3.17; tree length = 405 steps, CI = 0.8444, RI = 0.8433, RC = 0.7121). The tree was rooted with sequences of *Ceriporia* which was deduced by previously published papers (Boidin *et al.*, 1998; Hibbett *et al.*, 1997; Thorn *et al.*, 2000). Parsimony analyses showed that two new *Irpex* species formed a distinct clade at the 100 % confidence level which differed from the *Irpex lacteus* clade

(Fig. 3.17).

Australohydnum dregeanum has a dimitic hyphal system and both metuloids and skeletocystidia. Microscopic characteristics of *A. dregeanum* are very similar to *I. hydnoides*. While the hymenophore of *I. hydnoides* is distinctly toothed, the one of *A. dregeanum* is irpicoid to daedaleoid-labyrinthine. *Australohydnum dregeanum* is somewhat similar to *Phlebiopsis* that is monomitic and lacks skeletocystidia (Hjortstam and Ryvarden, 1990). Phylogenetic analyses based on ITS region sequences show that *Australohydnum dregeanum,* clustered with its synonym taxon *Irpex vellereus* by 100% level, formed a sister group with the genus *Phanerochaete* and was placed in a distant lineage from the *Irpex lacteus* group. The results of the sequence analysis, coupled with the morphological and cultural characteristics, indicate that *Irpex hydnoides* is not a kind of existing species. Therefore, it is concluded that *I. hydnoides* is a new species.

Figure 3.17. The most parsimonious tree of *Irpex* and related taxa based on sequence data of the ITS region (tree length of 405 steps, CI of 0.8444, RI of 0.8433, and RC of 0.7121). Numbers above branches are bootstrap percentage from 1,000 replicates. The sequences from *Ceriporia* spp. were used as an outgroup. Species with arrows have similar microscopic features to those of *I. hydnoides* and with asterisks were donated by Mugnier.


Irpex hydnoides SFC 971215-19
100
Irpex hydnoides SFC 971011-12
New species
100
Irpex lacteus SFC 951007-39
Irpex lacteus SFC 971006-12
Irpex lacteus FP 105049
Existing species
100
Bjerkandera fumosa*
80
100
Australohydnum dregeanum SFC 980804-4
Irpex vellereus CBS 515.92
100
Phanerochaete chrysosporium*
Phanerochaete sordida
Ceriporia viridans*
Ceriporia exelsa*
10 changes

2. Phylogenetic analyses of *Irpex* group and *Phlebia* group

Total length of the alignment was 514 bp. Of the characters, 254 were constant, 57 were variable but parsimony-uninformative, and 203 were parsimony-informative. With the heuristic search option and gaps treated as missing data, one most parsimonious tree was obtained (tree length = 739, CI = 0.5494, RI = 0.7063, RC = 0.3881). The best tree represented two main groups, *Irpex* group and *Phlebia* group (Fig. 3.18).

Irpex group of the polyporoid clade included *Irpex lacteus, I. hydnoides*, *Irpex* sp, *Oxyporus latemarginatus*, *Hexagonia hydnoides*, *Meruliopsis corium*, *Gloeoporus dichrous*, *G. taxicolor*, *Phanerochaete tuberculata*, *Ceriporia excelsa*, *C. viridans* and two unidentified species (MG-990320-18 and MG-990521-07). Its basal branch was supported by bootstrap value of 73% (Fig. 3.18) and genetic distance in the *Irpex* group was represented in Table 3.7. Most species of the *Irpex* group were characterized by the simple-sepate hyphal system and lamprocystidia with apical incrustation. But there were some exceptions. *Hexagonia hydnoides* and *G. dichrous* had always clamp connections and hyphae of *M. corium* were mostly simple-septate with rarely observed clamp connections. *Hexagonia hydnoides*, *M. corium*, two *Gloeoporus* and *two Ceriporia* do not have lamprocystidia.

Irpex lacteus is one of the most widely distributed wood rot fungi. And it is an industrially important mushroom. It has been reported to produce a commercial

Table 3.7. Genetic distances between pairs of fungal strains calculated by the uncorrected ("p") distance model.

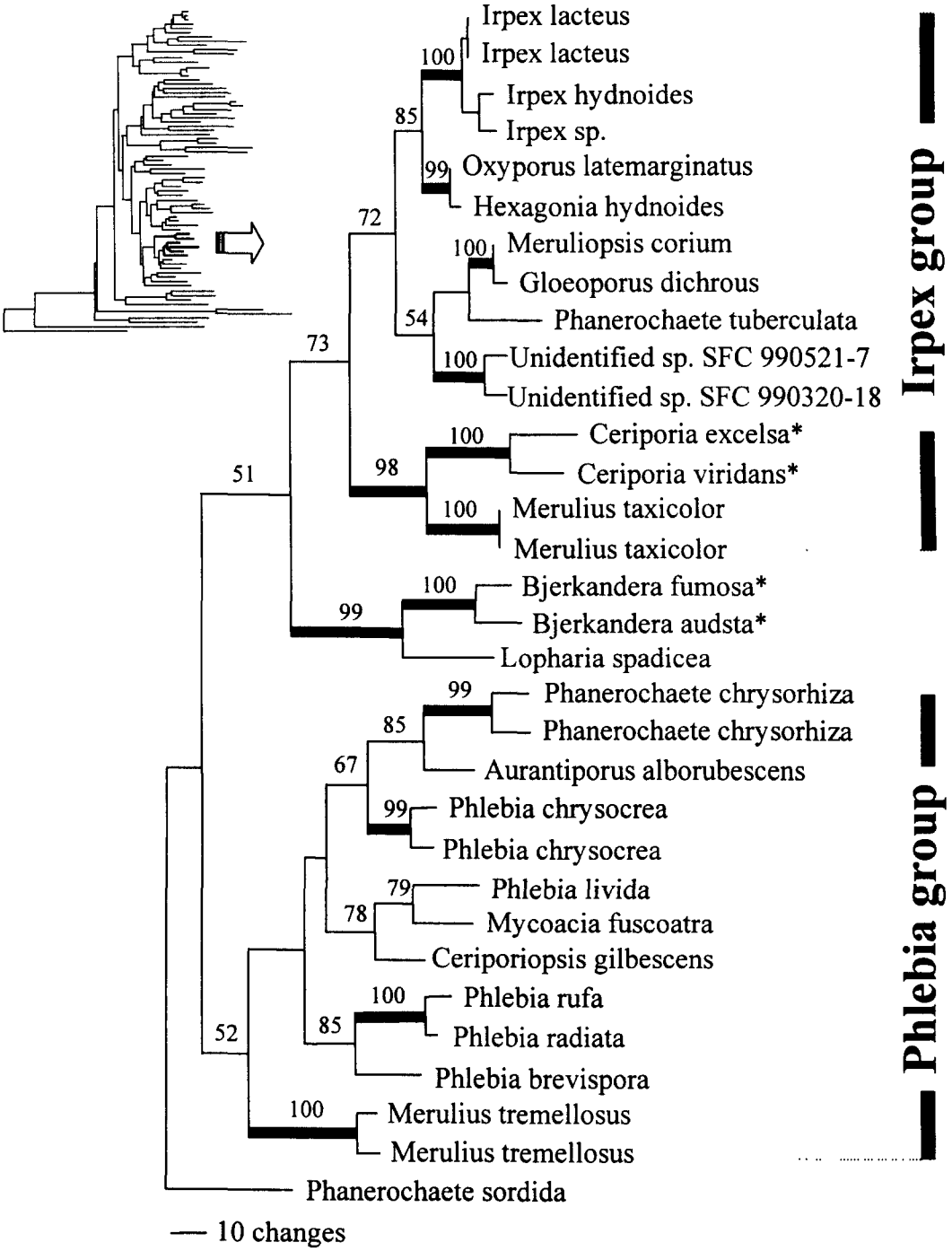
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*Irpex. lacteus* FP[a]	-														
2	*Irpex lacteus* 1[a]	0 0000	-													
3	*Irpex lacteus* 3[a]	0 0183	0.0183	-												
4	*Irpex hydnoides*	0.0204	0 0203	0.0183	-											
5	*Oxyporus latemarginatus*	0.0422	0.0422	0 0486	0.0526	-										
6	*Hexagonia hydnoides*	0.0485	0.0485	0.0550	0 0590	0 0064	-									
7	*Meruliopsis corium*	0.0810	0 0810	0.0958	0 0955	0.0587	0 0633	-								
8	*Gloeoporus dichrous*	0.0873	0.0873	0 1021	0 1018	0.0652	0 0699	0.0085	-							
9	SFC 990521-7[b]	0.0873	0 0873	0.0956	0.1000	0.0712	0.0759	0.0647	0.0711	-						
10	SFC 990320-18[b]	0 0954	0.0954	0.1034	0 1079	0 0774	0.0822	0.0729	0.0793	0.0190	-					
11	*Phanerochaete tuberculata*	0.0891	0.0891	0 0957	0.0980	0 0744	0.0796	0.0634	0.0703	0.0879	0.0942	-				
12	Ceriporia *excelsa*	0 1534	0 1534	0.1577	0.1642	0.1524	0 1580	0.1546	0.1612	0.1722	0.1863	0.1681	-			
13	*Ceriporia viridans*	0.1487	0.1487	0.1529	0 1571	0 1499	0 1533	0 1531	0 1596	0.1707	0.1783	0.1656	0 0725	-		
14	*Gloeoporus taxicola* 1[c]	0.1276	0.1276	0 1298	0.1361	0 1227	0.1258	0.1069	0.1112	0.1249	0.1287	0.1282	0.1318	0.1231	-	
15	*Gloeoporus taxicola* 2[c]	0.1276	0.1276	0.1298	0.1361	0.1227	0.1258	0.1069	0.1112	0 1249	0.1287	0 1282	0.1318	0.1231	0.0000	-

[a] *Irpex lacteus* FP = FP 105049, *Irpex lacteus* 1 = SFC 971006-12, *Irpex lacteus* 3 = SFC 971011-12

[b] unidentified species

[c] *Gloeoporus taxicola* 1 = SFC 990505-4, *Gloeoporus taxicola* 2 = SFC 000111-3

Figure 3.18. Phylogenetic relationship of *Irpex* group and *Phlebia* group. This most parsimonious tree with 739 steps (CI = 0.5494, RI = 0.5616 and RC = 0.3881), was inferred from ITS sequences. *Phanerochaete sordida* was selected as an outgroup. Bootstrap values greater than 50% were on respective branches.


Irpex lacteus
Irpex lacteus
Irpex hydnoides
Irpex sp.
Oxyporus latemarginatus
Hexagonia hydnoides
Meruliopsis corium
Gloeoporus dichrous
Phanerochaete tuberculata
Unidentified sp. SFC 990521-7
Unidentified sp. SFC 990320-18
Ceriporia excelsa*
Ceriporia viridans*
Merulius taxicolor
Merulius taxicolor
Bjerkandera fumosa*
Bjerkandera audsta*
Lopharia spadicea
Phanerochaete chrysorhiza
Phanerochaete chrysorhiza
Aurantiporus alborubescens
Phlebia chrysocrea
Phlebia chrysocrea
Phlebia livida
Mycoacia fuscoatra
Ceriporiopsis gilbescens
Phlebia rufa
Phlebia radiata
Phlebia brevispora
Merulius tremellosus
Merulius tremellosus
Phanerochaete sordida
10 changes
Irpex group
Phlebia group

enzyme, Driselase. Endo- and exo-type cellulases have geen isolated from this species (Ko, 2000). *Irpex lacteus* has been known to be closely related to *Junghuhnia* and *Steccherinum* (Ryvarden, 1991) and is separated from *Junghuhnia* only by the simple-septate generative hyphae. And its separation from *Steccherinum* is more problematic because *S. cremeoalbum* and *S. subcrinales* have simple-septae hyphae just like *I. lacteus* (Ryvarden, 1991). According to Boidin *et al.* (1998) and Kim (2001), *J. fimbriata, Antrodiella* spp. and *Steccherinum* spp. were intermingled into a signle clade. *Junghuhnia nidita* was also disposed in the family Steccherinaceae (Kim and Jung, 2000).

Surprisingly enough, *Irepx*, *Oxyporus* and *Hexagonia* showed a close relationship with each other although they had different characteristics and belonged to different subgroups of the family Polyporaceae. *Oxyporus* is a poroid genus with a monomitic hyphal system (Ryvarden and Gilbertson, 1994), which has been classified into the *Rigidoporus* group by Ryvarden (1991). *Hexagonia* has been included into the *Trametes* group, with a trimitic hyphal system and white rot habit. On the other hand, *Irpex* of the *Junghuhnia* group has hydnoid hymenophore and its hyphal system is dimitic with skeletals. *Oxyporus latemarginatus* and *H. hydnoides* were different in many respects. Hyphal system of the former species is monomitic, whereas that of the latter species is trimitic. Metuloid cystidia are present in *Irpex, Oxyporus latemarginatus*, *Phanerochaete tuberculata* and two unidentified species. These cystidia are also observed in *Lopharia spacicea*, which formed a sister clade of the

Irpex group and *Phanerochaete*. Therefore, metuloid cystidia seemed to be a convergent character.

The two strains of *Gloeoporus* came out into two different locations in the *Irpex* group (Fig. 3.18). While *G. dichrous* has clamped generative hyphae and occurs on both deciduous and coniferous woods, *G. taxicola* has simple-septate hyphae and occurs on only coniferous wood. In this study, *G. taxicola* was colsely related to *Ceriporia* species, which result was supported by morphological features like the reddish color and distinct poroid hymenophore.

The other group, *Phlebia* group, consisted of *Phlebia, Mycoacia, Ceriporiopsis, Merulius tremellosus, Aurantiporus* and *Phanerochaete chrysorhiza. Phlebia* as well as *Phanerochaete* are very efficient wood decomposers in nature, produce all three types of enzymes, lignin peroxidases (LiPs), manganase peroxidases (MnPs) and laccase (Hatakka, 1994; Vares *et al.*, 1995). Most of them are able to degrade lignin, cellulose and hemicellulose. *Phlebia* represents a classic example of a polythetic genus because taxa within the genus have many characters (Nakasone, 1996) but no phenotypic traits are common to all. Species included in this genus are variable in appearance, but their texture is more or less ceraceous to gelatinous, combined with a dense palisade of narrowly clavate basidia. Most species of *Phlebia* sensus stricto have a unifactorial mating type system.

Sizes of DNA amplified by PCR using ITS5 and LW1 primers varied from 600 to 650 base pairs and the multiple seuqence alignment showed that the genetic distance

among them ranged from 1.4 to 16.6% (Table 3.8). The *Phlebia* clade was weakly supported as a monophyletic group by the bootstrap value of 52% but was accordant with previously published results (Boidin *et al.*, 1998; Dresler-Nurmi *et al.*, 1999). *Merulius tremellosus* occurred as a basal branch to this group. *Merulius* was treated to be synonymous with *Phlebia* by Nakasone and Burdsall (1984). Although bootstrap support was poor (52%), *M. tremellosus* was included in the *Phlebia* clade. In culture studies, however, *M. tremellosus* developed nodose-septate hyphae but most of *Phlebia* formed simple-setate hyphae. *Phlebia radiata, P. rufa* and *P. brevispora* formed one clade and their branch was supported by 85% conficdence level. *Phlebia radiata* is mircoscopically similar to *P. rufa* but is macroscopically distinguishable from the latter. *Phlebia radiata* forms radially plicate hymenial surface, while that of *P. rufa* is raduloid, merulioid to irregularly poroid (Maekawa, 1993). And their close relationship was also supported by genetic diatance, which was 2.194%.

Phlebia livida is characterized by smooth to tuberculate hymenial surface and subulate leptocystidia. Its fusiform leptocystidia are similar to *Mycoacia fuscoatra*. Genetic distance between two species was short, which was 8.074%. According to Eriksson and Ryvarden (1976), *Mycoacia* can be described as a genus comprising species with a distinctly hydnoid hymenial surface, but its microscopical features totally agree with those of *Phlebia*. Although hymenial surface is slightly different, *Phlebia* was closely related to *Mycoacia*, which was supported by micromophology and molecular data analyses (Boidin *et al.*, 1998; Eriksson and Ryvarden, 1976).

Table 3.8. Genetic distances between pairs of fungal strains calculated by the uncorrected ("p") distance model.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Merulius tremellosus* (1)	-													
2	*Merulius tremellosus* (2)	0 01448	-												
3	*Phanerochaete chrysorhiza* CBS	0 14391	0 14588	-											
4	*Phanerochaete chrysorhiza* SFC	0.14835	0.15705	0 06129	-										
5	*Aurantiporus* AJ006683	0.13911	0 14882	0 10564	0.10069	-									
6	*Phlebia livida*	0.12082	0.12474	0.15507	0 15329	0 13311	-								
7	*Mycoacia copelandii*	0 12335	0 13213	0.13674	0 14489	0 12631	0.08074	-							
8	*Ceriporiopsis* AJ006684	0.12374	0.13452	0.13879	0.12174	0 12610	0 08547	0.08286	-						
9	*Phlebia chyrosocrea* CBS	0.13209	0 14022	0 12652	0 12261	0 09671	0.11178	0 12152	0.10277	-					
10	*Phlebia chrysocrea* SFC	0.13067	0 13642	0.12171	0 11338	0 09746	0.10056	0 12456	0 08989	0.03077	-				
11	*Phlebia rufa*	0 15593	0.16640	0.15409	0 15547	0 14876	0.13207	0.12075	0 11828	0 13130	0 12757	-			
12	*Phlebia radiata*	0 15262	0 16341	0.14706	0 15216	0 13963	0 13600	0 12455	0 11983	0.12640	0.11613	0 02194	-		
13	*Phlebia brevispora*	0.12754	0 14214	0.12633	0.12823	0 12029	0.13866	0.12358	0.09871	0.11181	0.09714	0 09575	0.08672	-	
14	*Phanerochaete sordida*	0.15158	0.16043	0.16327	0.16352	0.15641	0 16535	0 15776	0.14017	0 14278	0.15660	0 16017	0.15454	0.14723	-

Phanerochaete chrysorhiza can be recognized by the hydnaceous and orange hymenial surface, the concolorous hyphal strands and fusiform leptocystia. Therefore, this species was described as a member of the genus *Mycoacia* by some mycologists. The hyphal system of *P. chrysorhiza* is monomitic usually without clamps but sometimes with single, double or multiple clamps at septa (Maekawa, 1993). *Phlebia chrysocrea* is distinguishable from other species of *Phlebia* in that its hymenial surface is thin, smooth to tuberculate and yellowish brown, becoming wine-red in 3% KOH. Except for smooth hymenial surface and hyphae with clamp, all other characters of *Phanerochaete chrysorhiza* are congruent with *Phlebia chrysocrea.* In the present study, *Phlebia*, *Mycoacia* and *Pha. chrysorhiza* formed a mnophyletic group. It is interesting to note that *M. fuscoatra*, *P. chrysorhiza, Phl. chrysocrea* and *Phl. livida* were also characterised by fusiform leptocystidia. Close relationships of *Mycoacia, Phlebia* and *P. chrysorhiza* can be found in this study and the previous paper by Boidin *et al.* (1998).

3. Phylogenic relationship of *Phanerochaete* and related genera

White rot fungus, *Phanerochaete,* is very efficient wood decomposer. Most species of the genus *Phanerochaete* are able to degrade lignin, cellulose and hemicellulose. They usually cause white rot and produce three types of extracellular enzymes like laccase, lignin peroxidases (LiPs) and manganase peroxidases (MnPs), which all are involved in lignin biodegradation. Different white-rot fungi produce various combinations of these enzymes. Hatakka (1994) proposed three taxonomically heterogenous groups of fungi based on the patterns of ligninolytic enzymes. *Phanerochaete chrysosporum* is a typical representative of Lip-MnP group. But there are some differences in enzymatic profiles among these fungi. *P. chrysosporium* (Tien and Kirk, 1983) and *P. sanguinea* produce variable amount of LiP and MnP isoenzymes. LiP activity was not detected in *P. sordida* cultures, although Lip-like genes were amplified using reverse transcription-coupled PCR (Rajakumar *et al.*, 1996). Laccase is apparently not characteristic for *Phanerochaete*. Due to enzymatic abilities of the genus *Phanerochaete, P. sordida* (Harazono *et al.*, 1996) and *P. gigantea* (synonym of *Phlebia gigantea* and *Phlebiopsis gigantea*) (Behrendt and Blanchette, 1997) have been applicated in a variety of industrial processes like biopulping and pulp bleaching, as well as in bioremediation of chemically contaminated soils (Lamar *et al.*, 1990).

The only common character of *Phanerochaete* members is lack of clamp

connection. Identification of *Phanerochaete* species is generally based on morphological characteristics following the keys by Eriksson and Ryvarden (1976) and Eriksson and his colleagues (1978; 1981). *Phanerochaete* is in most cases easily recognized. There are still some difficulties and uncertain species delimitation in *Phanerochaete*, but in spite of that, the genus is considered to be homogenous (Eriksson *et al.*, 1978).

The ITS region amplification products ranged from approximately 600 to 650 base pairs in length. Sixty best trees (tree length = 1035 steps, CI = 0.5130, RI = 0.7243, RC = 0.3716) were found (Fig. 3.19). Through the log likelihood test (Kishino and Hasegawa, 1989) implemented in PAUP*4.0b4a (Swofford, 1999), the second tree among 60 most parsimonious trees was chosen because of having the highest log likelihood value (Ln = - 6351.37756). Sister groups and outgroup taxa were deduced by previously published papers (Boidin *et al.*, 1998; Hibbett and Thorn, 2001) and nuc-ssu rDNA sequence analyses.

Phanerochaete and its sister group (Fig. 3.19) which are traditionally classified in the Corticiaceae have also been segregated into other families or orders. Hibbett and Donoghue (1995) designated *Phanerochaete, Pulcherricium* and *Bjerkandera* as group 5 that was strongly supported by the molecular characters of mt rDNA. All three have smooth, cylindric, non-amyloid spores, monomitic hyphal systems and white rot habit. In the nuc-ssu rDNA analyses containing more taxa, *Bjerkandera* showed more close relationship to *Irpex* and *Phlebia* groups, which are located next to the *Phanerochaete*

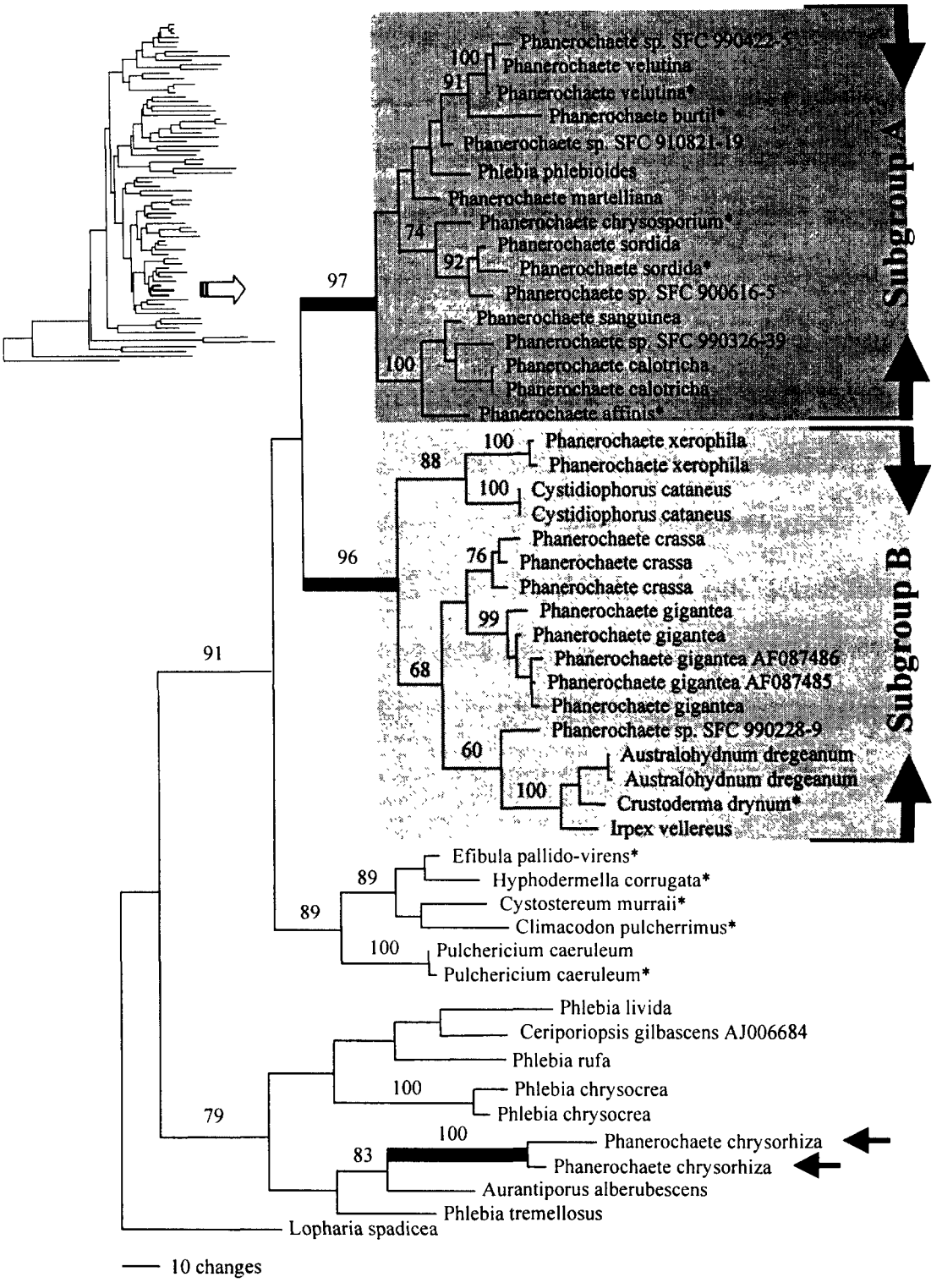
group.

Boidin and his colleagues (1998) based on ITS sequence analyses created the new order Phanerochaetales containing 13 genera; *Bjerkandera, Candelabrochaete, Ceriporia, Climacodon, Cotylidia, Crustoderma, Cystostereum, Efifula, Hyphodermella, Leptoporus, Phanerochaete, Phlebiopsis* and *Pulcherricium*. In the nuc-ssu rDNA sequence analyses, Kim and Jung (2000) suggested the emendation of family Phanerochaetaceae that was somewhat congruent with the results of Boidin *et al.* (1998). It is not easy to find the common characters for this order or family.

Although the branch was weakly supported (bootstrap value below 50%), the genus *Phanerochaete* formed a monophyletic group and composed of two distinct groups whose main branch was strongly supported by bootstrap analysis. Sources of error in the present estimate for the phylogeny may come from incorrect hypotheses of homology for individual nucleotide positions owing to uncertainty in sequence alignment. To facilitate discussion, above two groups were designated as subgroups A and B (Fig. 3.19).

Subgroup A included 16 strains traditionally classified in the *Phanerochaete* characterized by resupinate, white to reddish or yellowish color, smooth or tuberculated fruitbodies, monomitic hyphal system without clamp connection, with or without metuloid cystidia, and narrowly ellipsoid non-amyloid, non-cyanophilous basidiospores. Burdsall (1985) divided the genus *Phanerochaete* into three subgenera on the basis of cystidia (presence or absence), consistency and construction of

Figure 3.19. One of 60 most parsimonious trees based on ITS region sequences. This tree was selected by the log likelihood test of equally parsimonious trees (tree length = 1035 steps, CI = 0.5130, RI = 0.7243 and RC = 0.3716). *Lopharia spadicea* was used as an outgroup to root the tree. Bootstap percentage (1000 replicates) is numbered on appropriate branches supported by more than 60% and bold lines represent clades including *Phanerochaete*.


Phanerochaete velutina
Phanerochaete velutina*
Phanerochaete burtii*
Phanerochaete sp. SFC 910821-19
Phlebia phlebioides
Phanerochaete martelliana
Phanerochaete chrysosporium*
Phanerochaete sordida
Phanerochaete sordida*
Phanerochaete sp. SFC 900616-5
Phanerochaete sanguinea
Phanerochaete sp. SFC 990326-39
Phanerochaete calotricha
Phanerochaete calotricha
Phanerochaete affinis*
Subgroup A
Phanerochaete xerophila
Phanerochaete xerophila
Cystidiophorus cataneus
Cystidiophorus cataneus
Phanerochaete crassa
Phanerochaete crassa
Phanerochaete crassa
Phanerochaete gigantea
Phanerochaete gigantea
Phanerochaete gigantea AF087486
Phanerochaete gigantea AF087485
Phanerochaete gigantea
Phanerochaete sp. SFC 990228-9
Australohydnum dregeanum
Australohydnum dregeanum
Crustoderma drynum*
Irpex vellereus
Subgroup B
97
96
91
Efibula pallido-virens*
Hyphodermella corrugata*
Cystostereum murraii*
Climacodon pulcherrimus*
Pulchericium caeruleum
Pulchericium caeruleum*
89
89
100
Phlebia livida
Ceriporiopsis gilbascens AJ006684
Phlebia rufa
Phlebia chrysocrea
Phlebia chrysocrea
100
79
100
83
Phanerochaete chrysorhiza
Phanerochaete chrysorhiza
Aurantiporus alberubescens
Phlebia tremellosus
Lopharia spadicea
10 changes

basidiocarps. Subgroup A coincided with the subgenus *Phanerochaete* by Burdsall and the *Velutina* group by Eriksson *et al.* (1978).

Phanerochaete velutina is easily recognized but the variation is very great in the macroscopic aspect. Hymenium color and extent of cordon development are particularly variable. Therefore, it was most likely to be confused with *Pha. laevis* and occasionally with *Pha. sordida*. However, *Pha. velutina* microscopically differs from both *Pha. laevis* and *Pha. sordida*, which was supported by molecular evidence. Phylogenetic analysis showed that *Pha. velutina* is closely related to *Pha. burtii* and unidentified *Phanerochaete* species (NZ-910821-19). Genetic distance among those species ranged from 0.502 to 5.027%.

Phanerochaete chrysosporium has been most extensively studied in lignin degradation. Hatakka (1994) proposed three taxonomically heterogenous groups of fungi based on the patterns of ligninolytic enzymes. *Phanerochaete chrysosporum* is a typical representative of Lip-MnP group although there are some differences in enzymatic profiles. Phylogenetically, *Pha. chrysosporium* was very close to *Pha. sordida* and their relationship was supported by 72% bootstrap value (Fig. 3.19). *Phanerochaete chrysosporium* belonged to the form complex around *Pha. sordida* from which it may be distinguished in the longer, thin-walled and more or less inclosed cystidia. It produces in culture a conidial state that has been referred to the imperfect genus *Chrysoporium* (Eriksson *et al.*, 1978). But Nakasone (1990) reported that the anamorphic state of *Pha. chrysosporium* was *Sporotrichum pulveruletum* and formed

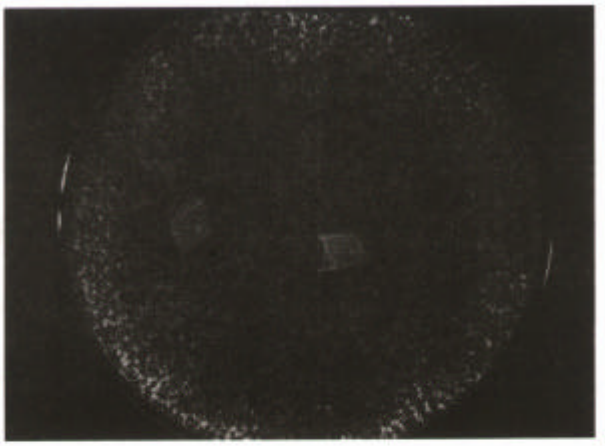
2.5-7 μm thick, thin to thick-walled, simple-septate and moderately branched merged hyphae in culture. In this study, *Pha. chrysosporium* (IMSNU 30074) formed clamped septa and many subglobose to ellipsoid conidia (Fig. 3.20). Genetic distance among those species ranged from 1.681 to 4.703%. Most species in subgroup A have metuloid cystidia except for *Pha. calotricha* that has smooth cystida (fusiform leptocystidia) and close relationships to *Pha. affinis, Pha. sanguinea* and *Phanerochaete* sp. (MG-990326-39). Genetic distance among them ranged up to 4.537%.

Species in subgroup B were morphologically heterogeneous but there were certain anatomical features which suggested that these species were related to subgroup A. Subgroup B contained *Australohydnum dregeanum, Crustoderma dryinum, Cystidiophorus castaneus, Irpex vellereus, Pha. crassa, Pha. gigantea, Pha. xerophila, and Phanerochaete* sp. (BR-990228-09).

The relationship between *Pha. xerophila* and *C. castaneus* was supported by the morpholgy and molecular data. The branch confidence was supported by bootstrapping (88%) and their genetic distance ranged up to 5.592%. However, these two species had a remarkable diversity in fruiting body morphologies. The basidiocarp of *Pha. xerophila* is fully resupinate, ochre-colored, membranous, adherent and cracking extensively. This species has been reported frequently as *Phlebia chrysocrea* for its morphological similarity. However, it differs from *Phl. chrysocrea* in its hyphal system without clamp connections and the phylogenetic position of the latter species was in the *Phlebia* group (Fig. 3.18). *Cystidiophorus castaneus* has a fully resupinate

Figure 3.20. Cultural features of *Phanerochaete chrysosporium* (IMSNU 30074). (A) macroscopic mats in culture showing the growth on MEA in 6 days at 25℃ (bar = 2 cm). (B) Microscopic feature of conidia and hyphae. Conidia were indicated by arrow (a) and the hypha with clamp connection by arrow (b) (bar = 10 μm).

A: Aerial mycelium



B: Conidia and hypha

basidiocarp with folded wrinkles and bright golden brown hymenophore. Its hymenophore apparently differs from *Phaneorochaete* but microscopic features of this species are congruent with *Phaenrochaete* (see Chapter 2).

Phanerochaete crassa, formerly placed in *Stereum*, *Laxitextum* or *Lopharia*, is easily recoginized in the field by its narrowly reflexed margin, soft texture and violaceous brown hymenial surface. Because of dimitic hyphal systems and brown cystidia, Hjortstam and Ryvaden (1990) transferred this species into *Porostereum*. However, molecular characters provided evidence that *Pha. crassa* was related to *Pha. gigantea* rather than to *Porosterum*. *Phanerochaete gigantea*, formerly placed in *Phlebiopsis*, differs from *Phanerochaete* in the consistency of the subhymenium that is very firm or hard when dry, a result of thick hyphal walls and close hyphal junctions (Eriksson *et al.*, 1981). But many mycologists treated *Phlebiopsis giganteum* as *Phanerochaete gigantea* because of cystidia and simple-septate hyphal system. Present molecular analysis showed that this species was grouped into the *Phanerochaete* group and closely related to *Pha. crassa*.

Australohydnum dregeanum has a dimitic hyphal system and both metuloids and skeletocystidia (Hjortstam and Ryvarden, 1990). Microscopic characteristics of *A. dregeanum* are very similar to *Irpex* species. While the hymenophore of *Irpex* species has a distinctly teeth form, *A. dregeanum* has an irpicoid to daedaleoid-labyrinthine form. Phylogenetic analyses based on ITS region sequences showed that *A. dregeanum* clustered with its synonym taxon *Irpex vellereus* by 100% confidence level and was

placed in a distant lineage from the *Irpex* group. *Crustoderma dryinum* intermingled with *A. dregeanum* and its phylogenetic position was uncertain. The nature of hyphae with clamps and cystidia differentiated *C. dryinum* from *A. dregeanum*. Genetic distance among them was considerably short and ranged 0.164 to 3.807%.

Phanerochaete chrysorhiza was treated as a member of the genus *Phanerochaete* because of mostly simple-septate hyphae. However, molecular data showed that this species clustered within the *Phlebia* group and closely related to *Aurantioporus alborubescens*. *Aurantioporus* was classified within the family Polyporaceae and its types species is a taxonomic synonym of *Polyorus croceus*, now commonly placed in *Hapalopilus* (Ryvarden, 1991). *Phanerochaete chrysorhiza* was exceptional and was found to be closely related to *Au. alborubescens*. However, hyphal system and fusiform leptocystidia strongly supported that *Pha. chrysorhiza* was closely related to *Phlebia* and *Mycoacia*.

CHAPTER 4

Conclusions

The corticioid fungi (Hymenomycetes, Basidiomycota) are characterized by simple fruitbodies in resupinate, crust-like or closely attached forms. The ecological roles of these species are more diverse than saprophytism. Some are parasitic, causing a variety of diseases from root rot to leaf blights. Several species are excellent examples of how fungi can be used by man; biological control agent, antibiotic, biopulping of wood, bioremediation of contaminated water and soil, and enzymatic application.

The corticioid fungi were very heterogeneous, for which reason it could not be possible to place them at a single stage in a phylogenetic scheme. At present, there is no generally acceptable taxonomic system for corticioid fungi. In most papers dealing with this group, the genera are in alphabetical order and any attempt at hierarchical classification is avoided for the present. As there have been no comprehensive studies of the entire corticioid group, there is no generally accepted system of higher ranks for this group. Phylogenetic studies on corticioid fungi were performed by morphology for a very long time. Nowadays, however, phylogenetic methods were focused mainly on molecular methods. In the present thesis, fairly diverse taxa of corticioid fungi were included and their phylogenetic relationships were discussed, based on morphological and molecular characteristics.

From morphological studies, cultural characters and molecular phylogenies, this study has drawn several considerations on corticioid fungi. Firstly, many corticioid fungi were collected from various regions of Korea and then observed under microscope. When the identified fungi were listed according to taxa, total of 40

corticioid fungi were confirmed as new or unrecorded species to Korea. They corresponded to 1 order, 6 families and 27 genera. An annotated list of the new corticioid fungi was presented in alphabetical order of species and phylogenetic placement for each taxon was explained in each remark.

To infer both higher and lower level relationships of corticioid fungi, nuclear small subunit rDNA and ITS sequences were used in this study. Phylogenetic analysis based on nuc-ssu rDNA showed that Homobasidiomycetes was divided into ten major clades and that the corticioid fungi were distributed in eight major clades. Five clades of them were accordant with the tentatively classification system of Hibbett and Thorn (2001) but three of them, peniophoroid clade, laeticorticioid clade and botryobasidioid clade, were newly recognized in this study.

The russuloid clade contained representatives of Russulaceae and some of Aphyllophorales that had a tremendous diversity of fruiting body morphology. Members of this clade are unique in their possession of amyloid spores. In this clade, two genera, *Stereum* and *Xylobolus*, were mainly studied. Molecular study clearly indicated that grouping of *Stereum* was not congruent with the traditional taxonomy based on hyphidium type, external appearance and the shape of gross morphology. However, it showed that hymenium bleeding was an important defining character. The reduction trend of hyphidum type and the evolutionary direction from resupinate to distinctly fan-shaped or spathulate fruitbodies were suggested in *Stereum* and *Xylobolus* phylogeny.

The peniophoroid clade included *Peniophora, Duportella* and *Scytinostroma.* These taxa were grouped into the russuloid clade by Hibbett and Thorn (2001) but they possessed non-amyloid spores and retained their position as sister taxa to the russuloid clade. Therefore, it is suggested that the peniophoroid clade needs to be separated from the russuloid clade.

The corticioid fungi in the hymenochaetoid clade contained *Hyphodontia* and *Resinicium.* Macro- and micromorphology of *Resinicium* resembled those of *Hyphodontia* rather than *Phlebia.* Their relationship was supported by nuc-ssu rDNA and ITS sequence analyses. This clade was primarily composed of lignicolous species and appeared to be united by the possession of imperforate parenthosomes.

The euagarics clade was composed mostly of Agaricales but some corticioid fungi were included. Phylogenetic position of *Plicaturopsis crispa* was very interesting and the phylogeny based on morphological and molecular data suggested that this species might be an ancestor of agarics. Another interesting taxa were *Mycoacia copelandii, Dentipellis fragilis* and two unidentified *Hypochnicium* species that presented a close relationship one another and their common characters were globose basidiospores, clavate basidia and monomitic hyphal systems with clamp connections.

The bolete clade containing the Coniophoraceae is wood rot fungi and is associated with brown rot. This clade was divided into two groups by hymenophoral type. Group A was characterized by merulioid hymenium and group B by smooth hymenium.

The laeticorticioid clade was newly formed in this study although Hibbett and

Thorn (2001) treated the members of this clade in the polyporoid clade in agreement with the ITS phylogeny of Boidin and his colleagues (1998). Large basidia, basidiospores and dendrohyphidia characterize this clade.

The polyporoid clade is very large and is composed of many polypores and corticioid fungi. The phylogenetic analysis divided the clade largely into six groups, *Trametes* group, *Steccherinum* group, *Irpex* group, *Phlebia* group, *Phanerochaete* group and brown rot group. The corticioid fungi of each group have distinctive characters, e.g., dendrohyphidia in *Trametes* group, simple-septate hyphal systems in *Irpex* group, capitate leptocystida in *Phlebia* group, incrusted lamprocystidia and simple-septate hyphal systems in *Phanerochaete* group and brown rot habit in brown rot group.

The last clade botryobasidioid clade was also newly found. This clade consisted of *Botryobasidium* and *Sistotrema*. Due to the urniform basidia with 6-8 sterigmata as well as molecular data-based results, *Botryobasidium* and *Sistotrema* have apparently close relationships each other.

Considering above results, basidiocarp morphology of corticioid fungi is considered to have evolved convergently. Therefore, corticioid members could be a plesiomorphic paraphyletic group that has given rise to multiple lineages of complex forms or a polyphyletic group that has been repeatedly derived by reduction. Further studies that include more taxa from various taxonomic groups are needed to understand the comprehensive total phylogeny of corticioid fungi. This study is expected to be

utilized to provide a phylogenetic outline for such a future phylogenetic scheme.

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국문초록

배착성의 단순한 자실체를 지닌 고약버섯은 분류학적으로 담자균문의 균심강에 속한다. 삼림에서 섬유소와 리그닌을 분해하는 부후활성으로인해 이들 버섯은 삼림생태의 중요한 역할을 담당하고 있다. 이러한 중요성에도 불구하고 몇몇 작은 분류군의 단편적인 연구를 제외하고는 고약버섯류의 종합적인 계통연구가 되어있지 않았고 이로 인해 이들 그룹의 일반적인 분류체계가 없는 실정이다. 본 논문에서는 고약버섯류의 계통관계를 추론하기 위하여 다양한 분류군을 첨가하고 형태적, 분자적 자료에 기초한 계통분석을 시도하였다.

최근까지 한국의 고약버섯류는 대부분 고약버섯과에 속하는 종을 포함해서 7 과의 39 속에 속하는 총 83 종이 알려져 왔다. 형태학적 연구를 통해 신종 및 한국의 미기록종으로 6 과 27 속에 분포하는 40 종의 고약버섯류를 확인하였고 이들의 형태학적 특징을 학명의 알파벳순으로 기술하였다. 분자자료를 이용한 계통연구에서는 고약버섯의 상위계급 관계와 하위계급 관계를 추론하기 위해 핵의 소형 소단위 리보좀 DNA (nuc-ssu rDNA)와 ITS 염기서열을 이용하였다. 핵의 소형 소단위 리보좀 DNA 염기서열에 기초한 계통분석은 담자균류에서 10 개의 주된 clade 가 있음을 보여주고 있다. 이러한 결과는 최근의 분자계통연구의 결과들과 일치하고 있다. 고약버섯류는 10 개의 주된 clade 에서 8 개의 clade 에서 나타나고 있다. 이들 clade 는 다음과 같다. (1) russuloid clade, (2) peniophoroid clade, (3) hymenochaetoid clade, (4)

euagarics clade, (5) bolete clade, (6) laeticorticioid clade, (7) polyporoid clade, (8) botryobasidioid clade. 이들중 3 clade (peniophoroid clade, laeticorticioid clade, botryobasidioid clade)는 본 논문에서 새롭게 추가되었다. Peniophoroid clade 는 비아밀로이드성 포자를 가지며 russuloid clade 와 가까운 유연관계를 갖고 있다. Laeticorticioid clade 는 큰 담자기, 담자포자와 dendrohyphidia 를 지니며 botryobasidioid clade 는 6-8 개의 담자병을 지닌 단지형의 담자기에 의한 강한 지지를 받는다.

매우 가까운 속간의 계통분석은 ITS 부분의 염기서열을 이용하여 분석하였다. Russuloid clade 에서는 주로 꽃구름버섯(*Stereum*)과 거북꽃구름버섯(*Xylobolus*)의 계통관계가 연구되었다. 꽃구름버섯의 계통연구에서는 자실층의 상처시 붉은색의 유액이 중요한 특징으로 인식되었고, 꽃구름버섯과 거북꽃구름버섯의 계통연구에서 hyphidia 의 복잡한 구조에서 단순한 구조로의 형태적 진화방향과 배착성 자실체에서 반배착성 및 중생의 자실체형태로 진화가 일어나고 있음을 보여주고 있다. Hymenochaetoid clade 는 돌기고약버섯(*Hyphodontia*)이 좀구멍버섯(*Schizopora*)과 수지고약버섯(*Resinicium*)과 가까운 유연관계를 가지고 있음을 확인하였고 수지고약버섯이 이들 그룹에 속하는 것은 현미경적 형태자료에서도 잘 반영하고 있다. Euagarics clade 는 자색꽃구름버섯(*Chondrosterum purpureum*), 담자고약버섯(*Cylindrobasidium evolvens*), 털침버섯(*Dentipellis fragilis*), 긴송곳버섯(*Mycoacia copellandii*), 주름고약버섯(*Plicaturopsis crispa*)과 종 동정이 안된 후막고약버섯(*Hypochnicium*)의 2 종을 포함하고 있다. 이 clade 의 조상종이 주름고약버섯(*Plicaturopsis crispa*)일 수

도 있다라는 것을 본 계통분석에서 보여주고 있다. 또한 침형의 자실층과 구형의 담자포자는 털침버섯(*Dentipellis fragilis*)과 긴송곳버섯(*Mycoacia copellandii*)의 관계를 잘 반영하고 있으며 또한 이들은 후막고약버섯(*Hypochnicium*)과도 가까운 관계를 나타내고 있다.

Bolete clade 는 자실층의 형태에 의해 두 group 으로 나누어진다. Group A 는 자실층이 merulioid 모양이며 *Serpula* 와 *Pseudomerulius* 를 포함하며 group B 는 밋밋한 자실층을 갖는 *Coniophora* 를 포함한다. 배착성의 자실체를 가지는 새재고약버섯(*Vuilleminia*), 장미고약버섯(*Laeticorticium*), *Dendrocorticium* 과 반배착성 및 중생이 형태를 지닌 *Punctularia* 가 Laeticorticioid clade 에 속한다. 이 clade 에서도 russuloid clade 서와 같이 지면에서 발생하는 배착성에서 점차 발생하는 위치가 기주의 윗쪽으로 갈수록 포자의 크기는 작아지고 자실체는 반배착성으로 진화한다는 것을 계통도에서는 잘 보여주고 있다. Polyporoid clade 는 6 group 으로 나누어지는데 이들은 *Trametes* group, *Steccherinum* group, *Irpex* group, *Phlebia* group, *Phanerochaete* group, brown rot group 등이다. 이 clade 의 고약버섯류는 독특한 형태적 및 다른 특징을 가진다. *Trametes* group 의 고약버섯류는 dendrohyphidia 를 지니며, *Irpex* group 에서는 단순격막의 균사체제, *Phlebia* group 에서의 capitate leptocystidia, *Phanerochaete* group 의 incrusted lamprocystidia 와 단순격막 균사체제, brown rot group 의 갈색부후성질등이 그것이다. 또한 형태적, 배양적 특징과 함께 염기서열분석으로 *Irpex* group 의 *Irpex hydnoides* 가 신종임을 확인하였다.

Hyphidia 의 유형, 자실층과 자실체의 형태, 현미경적미세구조 및 균사체

제, clamp connection 의 유무, 포자의 아밀로이드성질, 부후형태등과 같은 계통적 의의를 갖는 다른 관련된 형태에 고려하여 볼 때 고약버섯류는 진화가 일어난 동안 다양한 방향에서 수렴적으로 발전하여 왔다고 볼 수 있으나 그들의 목재의 ligno-cellulose 를 이용할 수 있는 양분이용 방식이나 현미경적 미세구조는 매우 다양하고 복잡한 양상을 보이고 있다.

■주요어: 고약버섯과, 고약버섯류, 분류학, 분자계통, ITS, 소형 소단위리보솜 DNA.

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감사의 글

버섯 분류를 처음 공부할때는 우리가 지닌 새로운 방법의 잣대를 이용하여 버섯을 이리재고 저리재는 일이라고 단정을 지었고 단지 이름을 지어주고 새롭게 재편성하여 의미를 부여하는 것이 분류인줄 알았습니다. 하지만 지난 몇 년동안 버섯을 찾아다니느라 전국의 산야를 헤매고 이들을 분류한다고 밤낮을 안가려가며 현미경과 많은 분류서적들을 뒤지고 또 그들을 여러 방법을 이용하여 연구할 결과 존재하는 모든 것에는 분명한 이유가 있는 것처럼 무성한 숲풀속에 또는 썩어 가는 고목 밑에 실오라기처럼 자라고 있는 균사도 그 의미와 중요한 존재가치를 가지고 있다는 결론을 내릴 수 있었습니다. 버섯은 무수한 산림생태중 아주 작은 하나에 불과하고 나의 연구는 그 작은 것 중에서도 작은 부분을 연구한지라 아주 보잘것없이 생각되지만 그래도 이러한 연구를 통해 도와준 많은 사람들에게 감사를 드릴 명분을 찾을 수 있는 것이 매우 다행스럽게 생각됩니다. 또 다른 시작을 위하여 하나의 과정을 마무리하고 조그만 논문을 펴내기에 앞서 결코 짧지 않은 지난 시간들 동안 저에게 여러 가지 도움을 주신 많은 분들께 깊은 고마움을 전하고 싶습니다.

지난 육여년동안 참으로 많은 가르침을 주시고 균학자로의 길을 갈 수 있도록 자상한 배려와 세심한 지도를 아끼지 않으신 정학성 선생님께 깊은 감사를 드립니다. 깊은 미소와 자상함으로 격려해주신 김상종 선생님과 정가진 선생님께 감사를 드립니다. 논문의 전체적인 방향과 중요한 점들을 지

적해 주신 최형태선생님과 신광수선생님께도 감사를 드립니다. 아울러 18동에서 따스한 미소로 격려해 주신 미생물학과 교수님들께도 감사를 드립니다.

내 몸에 밴 실험 설계 및 방법에 있어서는 실험실 선배인 홍순규박사님과 정원진박사님의 영향이 아니었나 생각이 듭니다. 자상하지만 날카롭게 논쟁을 때로는 조언을 해주었던 김선영박사, 무척이나 논문과 정보에 밝았던 그래서 연구결과를 논문화하는데 많은 도움을 주었던 고관수 박사에게도 고마움을 드립니다. 온지 얼마 안되었지만 몇 년을 같이 한 사람처럼 편한 함을 주신 유박사님, 옆에 있는 것만으로도 푸근하고 곰팡내를 내는 진성이, 뭐든지 열심히 하는 하영이, 컴퓨터 닮은꼴의 경모, 좋은 엄마와 부인역을 하느라 바쁜 미선누나, 변리사일로 바쁜 와중에도 늘 긍정적인 사고를 주었던 여강이형, 지금은 멀리 있는 소연과 양희에게도 큰 감사를 드립니다.

병마와 싸우며 힘겹게 계신 어머님께 공부한다는 핑계로 소홀히 한 것이 끝내 아쉽고 죄송스럽지만 박사가 되었다는 그 말에 웃음을 지어주시던 어머님께 머리를 숙여 감사를 드립니다. 형님내외분, 누님, 여동생에게도 감사를 드리고 어려움이 있을 때 늘 기도로써 큰 힘을 주신 장인어른과 장모님께도 깊은 감사를 드립니다.

두 아이와 어머니를 모시느라 고생하면서도 불평 없이 뒤에서 오늘의 내가 있도록 큰 힘이되준 아내 성숙에겐 어떠한 감사의 표현을 해야 할지 모르겠습니다. 늘 장난치는 것만으로도 나에게 큰 힘이 되었던 두 아들, 진현과 예원 그리고 아내에게 이 논문의 한귀퉁이를 통해 사랑을 전하고 싶습니다.