The Journal of Microbiology, September 2000, p.122-131 Vol. 38, No. 3

Phylogenetic Relationships of the Aphyllophorales Inferred from Sequence Analysis of Nuclear Small Subunit Ribosomal DNA

Seon Young Kim[1] and Hack Sung Jung*

[1]Laboratory of Cell Biology, Korea Research Institute of Bioscience and Biotechnology, Taejon 305-333, Korea
**School of Biological Sciences, Seoul National University, Seoul 151-742, Korea*

(Received July 31, 2000 / Accepted September 1, 2000)

Phylogenetic classification of the Aphyllophorales was conducted based on the analysis of nuclear small subunit ribosomal RNA gene (nuc SSU rDNA) sequences. Based on phylogenetic groupings and taxonomic characters, 16 families were recognized and discussed. Although many of the characters had more or less homoplasies, microscopic characters such as the mitic system and clamp, spore amyloidity and rot type appeared to be important in the classification of the Aphyllophorales. Phylogenetically significant families were newly defined to improve the classification of the order Aphyllophorales.

Key words: Aphyllophorales, phylogeny, nuclear SSU rDNA, rot type, spore amyloidity

The order Aphyllophorales is an important group of the Hymenomycetes (Basidiomycota) possessing holobasidia but lacking gills. Many species are significant in decomposing plant materials because they can digest cellulose and/or lignin which constitute the plant cell wall (1, 25, 37). The Aphyllophorales have various forms of basidiocarps and are called corticioid fungi, pore fungi, club fungi, coral fungi, bracket fungi or shelf fungi depending on their structures. Hymenophores are also variable, and smooth, poroid, toothed or clavarioid forms occur in this order. When examining microscopic features, extremely diverse characteristics exist in this group. This large group has been taxonomically problematic and has been said to be one of the challenges for fungal taxonomists attempting to delimit monophyletic groups and determine their ancestro-descendent relationships (1).

The history of the classification of the order Aphyllophorales began with Persoon who wrote "*Synopsis methodica fungorum*" in 1801. His classification system was quite crude, because it was mainly based on macroscopic observations (22). Then appeared the works of Fries' "*Systema mycologicum*" (1821-1832), which have been used by many mushroom hunters. The principal character used by Persoon and Fries was the hymenial configuration by which Fries divided Aphyllophorales and Agaricales into five families. These families were the Clavariaceae with erect fruitbodies and amphigenous hymenium, the Thelephoraceae with smooth hymenium, the Hydnaceae, the Polyporaceae and the Agaricaceae with toothed, tubular and lamellate hymenophores, respectively.

In 1900, Patouillard introduced microscopic characters such as basidium morphology and mode of basidiospore germination having a strong impact on the natural classification of the basidiomycetes claiming that the Friesian group was polyphyletic (1, 9, 22). He divided the basidiomycetes into heterobasidiomycetes and homobasidiomycetes. The heterobasidiomycetes was characterized by septate or aseptate basidiospores and/or the capacity for two or more modes of basidiospore germination (budding, germination by repetition, formation of microconidia, or formation of germ tubes). The homobasidiomycetes was characterized by aseptate basidia and basidiospore germination by germ tubes only. He established the Aphyllophorales as opposed to the Agaricales and Gasteromycetes and suggested new families such as Serie des Igniaires (now Hymenochaetaceae) and Serie des Phylacteries (now Thelephoraceae) which consisted of genera with strongly diverse hymenial configuration (9, 22, 35). Bourdot and Galzin's magnum opus "Hym nomyc tes de France" succeeded the works of Patouillard.

Donk (8) 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 accepted Donk's work. In Donk's system, hymenial configuration were no longer accepted as familial characters, but microscopic characters such as the mitic system, spore morphology, basidium morphology, and sterile elements such as cystidia were emphasized in defining new families. Although Donk's work greatly

* To whom correspondence should be addressed.
(Tel) 82-2-880-6708; (Fax) 82-2-888-4911
(E-mail) minervas@snu.ac.kr

improved the taxonomy of the Aphyllophorales, several families remained artificial and wanted revisions. The Clavariaceae, Corticiaceae, Hydnaceae, Polyporaceae and Stereaceae were such heterogeneous families (8, 9) that many taxonomic studies tried to make those polyphyletic families more natural. The works of Reid (31), Parmasto (28), Talbot (37), Jülich (22), Jülich and Stalpers (23), Chamuris (6) and Ryvarden (32) represented important studies which contributed to the monophyletic groupings of the Aphyllophorales.

Recently, several molecular works were performed for the Aphyllophorales with a purpose of constructing natural and phylogenetic classification. Reports of Hibbett and Vilgalys (20), Hibbett and Donoghue (18), Hibbett (17), Hibbett *et al.* (19), Ko *et al.* (24) and Boidin *et al.* (3) were such examples. Among them, Hibbett and Donoghue (18), Hibbett *et al.* (19) and Boidin *et al.* (3) treated diverse genera and families of the Aphyllophorales. Through their works, many new insights into the taxonomic relationships were developed, and several monophyletic groups were newly identified. In this study, we used subunit ribosomal DNA sequences of 81 species in the Aphyllophorales and analyzed the phylogenetic relationships among them. Several new phylogenetic groups were identified and their systematic points were discussed.

Materials and Methods

Materials and DNA extraction

Taxa of cultures and specimens used in this study are listed in Table 1 together with the taxa of retrieved sequences from GenBank. Sequences with designated sources are those analyzed for the present study. Strains from CBS, ATCC, or IFO were mycelial cultures and the others were dried herbarium specimens. Cultures were provided by KCTC (Korean Collection for Type Cultures), KRIBB (Korea Research Institute for Bioscience and Biotech-

Table 1. List of taxa used in the analysis of nuclear SSU rDNA sequences

Species	Family	Sources	GenBank Accession
Albatrellus syringae (Parm.) Pouz.	Albatrellaceae		AF026632
Aleurodiscus botryosus Burt	Corticiaceae		AF026603
Amylostereum areolatum (Fr.: Fr.) Boidin	Stereaceae	CBS[a] 334.66 = KCTC[b] 6818	AF082845
Amylostereum chailletii (Pers.: Pers.) Boidin	Stereaceae	CBS 480.83 = KCTC 6855	AF082846
Anomoporia albolutescens (Bull.: Fr.) Sing.	Hericiaceae	CBS 337.63 = KCTC 6867	AF082675
Antrodia carbonica (Overh.) Ryv. & Gilbn.	Polyporaceae		U59059
Antrodiella americana Ryv.	Polyporaceae	CBS 386.51 = KCTC 6877	AF082677
Auriscalpium vulgare S. F. Gray	Auriscalpiaceae		U59060
Basidioradulum radula (Fr.: Fr.) Nobles	Hyphodermataceae		AF026611
Bjerkandera adusta (Willd.: Fr.) Karst.	Polyporaceae		U59061
Bondarzewia berkeleyi (Fr.) Bond. & Sing.	Bondarzewiaceae		U59062
Boreostereum radiatum (Peck) Parm.	Stereaceae	CBS 417.61 = KCTC 6860	AF082847
Botryobasidium isabellinum (Bres.) Eriksson	Botryobasidiaceae		AF026610
Botryobasidium subcoronatum (v. Höhn & Litsch.) Donk	Botryobasidiaceae		AF026609
Cantharellus tubaeformis Fr.	Cantharellaceae		AF026636
Ceriporia purpurea (Fr.) Donk	Polyporaceae		U59065
Ceriporiopsis subvermispora (Pil.) Gilbn. & Ryv.	Polyporaceae	CBS 525.92	AF082678
Chondrostereum purpureum (Pers.: Fr.) Pouzar	Stereaceae	CBS 427.72 = KCTC 6839	AF082851
Clavariadelphus pistillaris (L.) Donk	Gomphaceae		AF026639
Clavicorona pyxidata (Fr.: Fr.) Doty	Auriscalpiaceae		U59066
Clavulina cristata (Holmsk.: Fr.) Schroet.	Clavulinaceae		AF026640
Coltricia perennis (L.: Fr.) Murr.	Hymenochaetaceae		U59064
Columnocystis abietina (Pers.: Fr.) Pouzar	Stereaceae	HHB[c]-12622-Sp	AF082848
Cymatoderma caperatum (Berk. & Mont.) Reid	Stereaceae	CBS 201.62 = KCTC 6858	AF082849
Cystostereum murraii (Berk. & Curt.) Pouzar	Stereaceae	CBS 257.73 = KCTC 16003	AF082850
Daedalea quercina Fr.	Polyporaceae		U59067
Datronia mollis (Sommerf.: Fr.) Donk	Polyporaceae	SFC[d] 941028-38	AF082669
Dentocorticium sulphurellum (Peck) Larsen & Gilbn.	Corticiaceae		AF026604
Diplomitoporus crustulinus (Bres.) Dom.	Polyporaceae	CBS 443.48 = KCTC 16021	p.c.[e]
Donkiporia expansa (Desm.) Kotl. & Pouz.	Polyporaceae	CBS 299.93 = KCTC 6999	AF082679
Echinodontium tinctorium (Ell. & Everh.) Ell. & Everh.	Echinodontiaceae		AF026578
Fistulina hepatica Schaeff.: Fr.	Fistulinaceae		U59070
Fomes fomentarius (L.: Fr.) Kickx.	Polyporaceae		U59069

Table 1. Continued

Species	Family	Sources	GenBank Accession
Fomitopsis pinicola (Swartz: Fr.) Karst.	Polyporaceae		U59071
Ganoderma australe (Fr.) Pil.	Ganodermataceae		AF026629
Gloeocystidiellum leucoxanthum (Bres.) Boidin	Gloeocystidiellaceae		AF026602
Gloeophyllum sepiarium (Fr.) Karst.	Polyporaceae		AF026608
Gloeoporus taxicola (Pers.: Fr.) Gilbn. & Ryv.	Corticiaceae	SFC 950815-16	AF082682
Glomphus floccosus Schw.	Gomphaceae		AF026637
Hericium ramosum (Bull.: Mérat) Lat.	Hericiaceae		U59073
Heterobasidion annosum (Fr.) Bref.	Polyporaceae		U59072
Hydnellum sp.	Thelephoraceae		AF026626
Hydnum repandum (L.) Fr.	Hydnaceae		AF026641
Hyphodontia alutaria (Burt) Eriksson	Hyphodermataceae		AF026615
Inonotus hispidus (Bull.: Fr.) Karst.	Hymenochaetaceae		U59074
Irpex lacteus (Fr.: Fr.) Fr.	Polyporaceae	IFO[f] 5367 = KCTC 6718	AF082683
Junghuhnia nitida (Pers.: Fr.) Ryv.	Polyporaceae	SFC 940903-7	AF082685
Laetiporus sulphureus (Bull.: Fr.) Murr.	Polyporaceae		U59079
Laxitextum bicolor (Pers.: Fr.) Lentz	Gloeocystidiellaceae		AF026605
Lentinellus montanus (Fr.: Fr.) Kühner	Auriscalpiaceae		U59076
Lentinula edodes (Berk.) Pegler	Tricholomataceae		AF082686
Lentinula lateritia (Berk.) Pegler	Tricholomataceae		U59075
Lentinus tigrinus (Bull.: Fr.) Fr.	Polyporaceae		U59098
Lopharia cinerascens (Schw.) Cunn.	Stereaceae	CBS 486.62 = KCTC 6836	AF082852
Lopharia spadicea (Per.: Fr.) Boidin	Stereaceae	CBS 474.48 = KCTC 6710	AF082853
Melanoporia nigra (Berk.) Murr.	Polyporaceae	CBS 341.63 = KCTC 6848	AF082674
Meripilus giganteus (Fr.) Karst.	Polyporaceae		U59082
Multiclavula mucida (Pers.) Petersen	Clavariaceae		AF026613
Oligoporus balsameus (Pk.) Gilbn. & Ryv.	Polyporaceae	SFC 910803-6	AF082684
Oxyporus latemariginata (Dur. & Mont.: Mont.) Donk	Polyporaceae	ATCC[s] 9408 = KCTC 6661	AF082670
Oxyporus sp.	Polyporaceae		AF026616
Panus rudis (schw.) Fr.	Polyporaceae		U59086
Peniophora nuda (Fr.) Bres.	Corticiaceae		U59085
Perenniporia subacida (Peck) Donk	Polyporaceae		U59087
Phanerochaete chrysosporium Burds.	Corticiaceae		U59084
Phaeolus schweinitzii (Fr.) Pat.	Hymenochaetaceae		U59087
Phellinus igniarius (L.: Fr.) Quél.	Hymenochaetaceae		AF026614
Phlebia radiata Fr.	Corticiaceae		AF026649
Pleurotus ostreatus (Jacq.: Fr.) Kummer	Pleurotaceae		U59091
Podoscypha elegans (Meyer: Fr.) Pat.	Stereaceae	CBS 322.66 = KCTC 6838	AF082854
Polyporus squamosus (Hud.: Fr.) Fr.	Polyporaceae		U59089
Pulcherricium caeruleum (Fr.) Parm.	Corticiaceae		U59083
Ramaria stricta (Fr.: Fr.) Quél.	Ramariaceae		AF026638
Resinicium bicolor (Fr.) Parm.	Corticiaceae	CBS 253.73 = KCTC 6769	p.c.
Rigidoporus vinctus (Berk.) Ryv.	Polyporaceae	ATCC 32575 = KCTC 6672	AF082673
Russula compacta Frost et Peck	Russulaceae		U59093
Schizophyllum commune Fr.	Schizophyllaceae		X54865
Schizopora paradoxa (Schrad.: Fr.) Donk	Polyporaceae		AF026612
Sparassis spathulata (Schw.: Fr.) Fr.	Sparassidaceae		U59096
Spongipellis unicolor (Schw.: Fr.) Murr.	Polyporaceae		M59760
Steccherinum rhois S. (Schw.) Banker	Steccherinaceae	SFC 941015-47	AF082680
Stereum gausaparum (Fr.: Fr.) Fr.	Stereaceae	CBS 348.39 = KCTC 6709	AF082855
Stereum hirsutum (Willd.: Fr.) S. F. Gray	Stereaceae		U59095
Stereum ostrea (Bl. & Nees) Fr.	Stereaceae	SFC 960921-8	AF082856
Thelephora sp.	Thelephoraceae		AF026627
Tremella foliacea Fr.	Tremellaceae		L22262
Trichaptum abietinum (Dicks.: Fr.) Ryv.	Polyporaceae		U59097

Table 1. Continued

Species	Family	Sources	GenBank Accession
Veluticeps berkeleyi (Berk. & Curt.) Cooke	Stereaceae	CBS 725.68 = KCTC 6859	AF082857
Wolfiporia cocos (Schw.) Ryv. & Gilbn.	Polyporaceae	ATCC 13490	AF082671
Xylobolus annosus (Berk. & Br.) Boid.	Stereaceae		U59089

[a]Centraalbureau voor Schimmelcultures
[b]Korean Collection for Type Cultures
[c]H. H. Burdsall, Jr., Center for Forest Mycology Research
[d]Seoul National University Fungus Collection, Institute of Microbiology, Seoul National University
[e]personal communication
[f]Institue of Fermentation, Osaka
[g]American Type Culture Collection

nology) and grown in ME broth (malt extract 2%, peptone 0.5%) at 24°C, harvested, lyophilized and ground to a fine powder. Herbarium specimens of SFC (Seoul National University Fungus Collection), IMSNU (Institute of Microbiology, Seoul National University), were visually examined and the internal portion of specimens was removed using a blade after external surfaces were scraped off. The sample was then ground to fine powder with a mill. DNA extraction was performed after Bruns *et al.* (4). Samples of 5~30 mg (both lyophilized cultures and herbarium specimens) were placed in 1.5 ml microcentrifuge tubes, suspended in 700 μl of buffer [50 mM EDTA, 50 mM Tris (pH 7.5), 3% SDS], incubated for 30~60 minutes at 65°C, extracted with phenol-chloroform, precipitated by addition of 50 μl of 3 M ammonium acetate and 500 μl of isopropanol, washed with 70% ethanol, and resuspended in 250 μl of TE buffer. Extracted DNA was stored at -20°C.

Polymerase chain reaction

Nuclear small subunit rRNA gene (nuc SSU rDNA) was amplified from extracted total genomic DNA, using NS1 and NS8 primers (39). PCR amplification of DNA was performed in 10 mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl, 0.1% Triton X-100, 2 mM $MgSO_4$, 0.2 mM of each dATP, dGTP, dCTP and dTTP, 0.5 μM of each primer with about 0.2 μg of template DNA which was diluted 1/100 fold by sterilized DDW, and 1 unit of *Taq* DNA polymerase (Poscochem) one PCR sample. Total volume was adjusted to 50 μl. PCR reactions were performed in a programmable PCR machine (DNA Thermal Cycler of Perkin Elmer) with the following parameters: an initial extension step for 3 min at 94°C, 30 s at 94°C for denaturation, 30 s at 50°C for annealing, and 2 min at 72°C for extension. This process was cycled 30 times, and then a final extension step for 30 min at 72°C was added.

PCR products were observed by electrophoresing 3 μl of PCR product solution on a 1.5% agarose gel containing EtBr in Tris-acetate EDTA (TAE) buffer. The presence of a single bright band in each lane was checked for successful amplification. A negative control that contained all the components except for the DNA template was always included to detect any possible contamination during PCR. After successful amplification, 150 μl of sterilized DDW was added to each PCR tube, extracted with an equal volume of chloroform, and then 0.1 vol. of 3 M sodium acetate and 2.5 vol. of ice-cold ethanol were added, precipitated by centrifugation at 4°C for 15 min at 12,000 rpm, washed with 70% ethanol, and evaporated using a vacuum evaporator.

Cloning

Extracted PCR product was cloned using the Promega T-vector system (Promega). The ligation condition was as follows: 25 ng of pGEM T-vector, 100 ng of PCR products and 3 Weiss unit of T4 ligase in 10 μl of reaction mixture. Ligation reaction was performed overnight at 14°C. Ligation mixture was transformed according to the manufacturer's instruction. Initially, 50 μl of efficient competent cells was added to the microcentrifuge tube containing ligation mixture, incubated on ice for 20 min, heat-shocked for 2 min at 42°C, then 300 μl of LB broth was added, incubated for 45 min at 37°C in a shaking incubator. Then, 4 μl of IPTG, 40 μl of X-gal, and 20 μl of ampicillin were added, and total mixture was spread onto a LBA plate, and incubated overnight in a 37°C incubator.

Sequencing

Plasmid templates were prepared by the alkaline lysis methods and then sequenced using a Sequenase II kit (United States Biochemical) according to the manufacturer's instructions. Initially, plasmids were denatured by the alkaline denaturation method (0.2 M NaOH, 0.2 mM EDTA, 37°C, 30 min), and were sequenced using NS1 to NS8 primers (39) and NS9 which was one of the primers designed by Gargas and Taylor (14) on the basis of small subunit ribosomal DNA gene sequences of lichenized fungi, and an NS19SNU primer which was designed in the authors' laboratory.

Data Analysis

Sequences were initially aligned using Clustal W, checked by eye, and relocated to allow maximal alignment. Align-

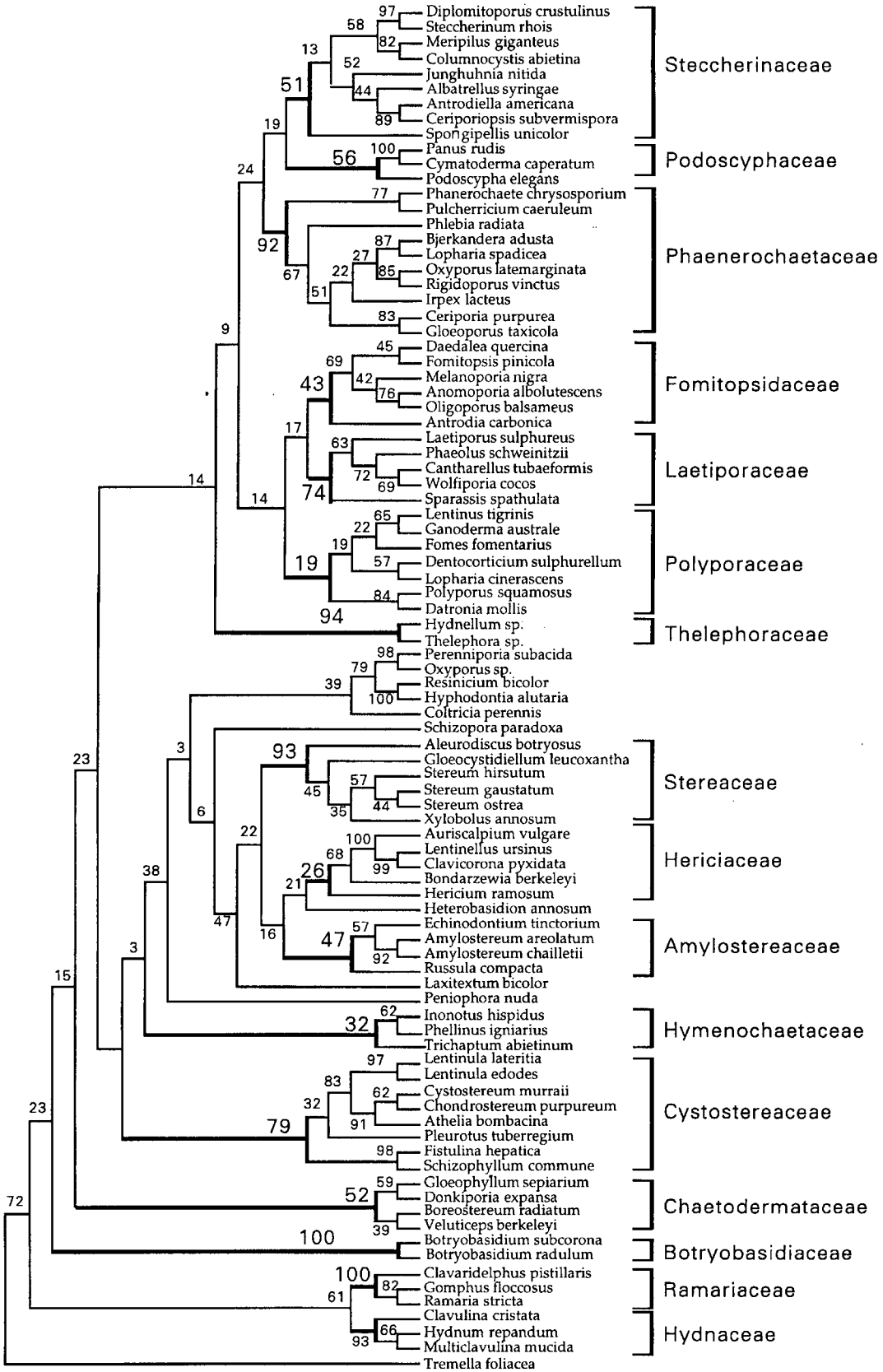

Fig. 1. One of 100 most parsimonious trees inferred from nuclear small subunit ribosomal RNA gene sequences (tree length = 2297 steps, CI = 0.435, RI = 0.553, RC = 0.241, HI = 0.565). Statistical supports from 500 bootstrap resampling are shown on corresponding branches. *Tremella foliacea* was used as an outgroup taxon to root the tree.

ment parameters were 10.0 for gap opening penalty, 0.05 for gap extension penalty, 40% for delay divergent sequences, and weighted for transitions. To analyze data, the most parsImonious trees were sought using PAUP 4.0* (36) on a MacIntosh computer. Gaps were treated as missing data. Due to the size of taxa, the search was limited to heuristic searches using a simple addition sequence, TBR branch swapping, MAXTREES unrestricted, and MULPARS on. To evaluate the strength of support for branches in most parsimonious trees, 500 replicates of bootstrap resampling (simple additions sequence, TBR swapping, MAXTREES 10) were performed (13).

Results and Discussion

Parsimony analysis produced over 100 most parsimonious trees with 2297 steps and consistency index of 0.435. As those trees showed similar topologies one another with only minor differences of terminal branches, one of most parsimonious trees was taken for the discussion. Statistical supports from 500 bootstrap resamplings were calculated and shown on branches of the phylogenetic tree (Fig. 1). Based on parsimony analysis, at least sixteen phylogenetic groups were recognized and taxonomically significant groups were newly defined at the family level to improve the current classification system of the order Aphyllophorales.

The family grouping was first done according to the topology of the phylogenetic tree, and the taxonomic emendation for the grouping was principally based on the phylogenetic significances of characters emphasized by taxonomists rather than simply on the bootstrap values of statistical support for tree branches. The present grouping is actually a result that came from combination of taxonomic characters and molecular data. References on such recent results of phylogenetic studies are cited in the text of paragraphs for groupings wherever they were needed.

Steccherinaceae Parm. 1968 emend. S.Y. Kim and H.S. Jung

In this family, *Diplomitoporus, Steccherinum, Meripilus, Columnocystis, Junghuhnia, Albatrellus, Antrodiella, Ceriporiopsis* and *Spongipellis* were included. Among them, the relatedness of *Antrodiella, Junghuhnia* and *Steccherinum* was repeatedly stressed (10, 22, 32, 40) because they share a dimitic hyphal system with clamped generative hyphae, small ellipsoid inamyloid spores and white rot habit. *Diplomitoporus* also has similar microscopic characters to those of three genera and white rot habit (15, 33). According to Ryvarden (32) and Yu and Niemëla (40), *Flaviporus* and *Irpex* are also closely related to this group. In the analysis of Boidin *et al.* (3), *Meripilus* and *Steccherinum* formed a monophyletic group together with *Antrodiella, Junghuhnia, Galzinia, Spongipellis, Physisporinus, Hypochnicium* and *Rigidoporus* in the order Hyphodermatales.

As *Ceriporiopsis* has a monomitic hyphal system composed of generative hyphae only, it differs from other genera in the hyphal system. Except for miticity, *Ceriporiopsis* has similar clamped generative hyphae, inamyloid spores, and white rot habit (7, 15, 33). *Meripilus giganteus* deviates from others having simple-septate generative hyphae. For *Columnocystis*, Nakasone (26) suggested that *Veluticeps* and *Columnocystis* are congeneric and *Columnocystis* should be included into *Veluticeps*. Current sequence data contradicted her view, but more data are needed to clarify this point.

Albatrellus syringae has a monomitic hyphal system with clamps, gloeoplerous hyphae and inamyloid spores. On the type of rot, *Albatrellus syringae* is suggested to be a white rotter (33). However, the taxonomic position of *Albatrellus* is not certain, and more species in the genus should be investigated before we can decisively determine the taxonomic position of *Albatrellus*. Morphological and molecular characters showed that *Antrodiella, Ceriporiopsis, Diplomitoporus, Junghuhnia* and *Steccherinum* are closely related genera one another. However, the taxonomic relations of *Albatrellus, Columnocystis* and *Meripilus* are not certain at present.

Podoscyphaceae Reid 1965 emend. S.Y. Kim and H.S. Jung

Considerably low bootstrap support of 56% was found in this group. Podoscyphaceae Reid was suggested from the Stereaceae including only stipitate fungi and later approved by many authors. Common characters of this group were a dimitic hyphal system with skeletal hyphae and clamped generative hyphae. At present, sequences of only three species (*Panus rudis, Cymatoderma caperatum, Podoscypha elegans*) are available. According to the result of Boidin *et al.* (3), *Cymatoderma, Podoscypha, Hypochnicium* and *Sarcodontia* form the order Podoscyphales.

Phanerochaetaceae Jülich 1981 emend. S.Y. Kim and H.S. Jung

In this family, poroid genera (*Bjerkandera, Ceriporia, Gloeoporus, Oxyporus, Rigidoporus*), corticioid genera (*Phanerochaete, Pulcherricium, Phlebia, Lopharia*) and irpicoid genera (*Irpex*) were included. The relatedness of genera in this group was suggested by many authors (22, 23, 29, 32, 38), and relationships of some genera were verified by molecular data (3, 18). In the Phanerochaetales of Boidin *et al.* (3), *Phanerochaete, Pulcherricium, Phlebiopsis, Candelabrochaete, Ceriporia, Leptoporus, Bjerkandera, Climacodon* and *Cotylidia* were included. *Oxyporus* and *Phlebia* were included in a different order Phlebiales.

Common characters of this family are monomitic to dimitic hyphal systems with simple-septate or clamped

generative hyphae, hyaline thin-walled inamyloid basidiospores, and white rot. Although many taxonomists considered *Irpex lacteus* to be related to the Steccherinaceae (10, 22, 32, 40), sequence data did not support their views. *Irpex lacteus* (without clamps) differs from *Steccherinum* and related genera (with clamps) in the septation of generative hyphae. Inclusion of *Irpex lacteus* in the family Phanerochaetaceae is also supported by the analysis using ITS regions (unpublished data).

Fomitopsidaceae Jülich 1981 emend. S.Y. Kim and H.S. Jung

In this group, *Daedalea quercina, Fomitopsis pinicola, Melanoporia nigra, Anomoporia albolutescens, Oligoporus balsameus* and *Antrodia carbonica* are included. Common characters of this family are clamped generative hyphae and brown rot. The monophyly of *Fomitopsis, Daedalea* and *Piptoporus* was once verified by Hibbett and Donoghue (18). In the analysis of Boidin *et al.* (3), *Antrodia, Oligoporus* and *Fomitopsis* were grouped in the order Fomitopsidales together with *Ischnoderma* and *Skeletocutis* which are white rotters. According to the taxonomy of Ryvarden (32), the *Daedalea* group has dimitic to trimitic hyphal systems with clamped generative hyphae and cause brown rot. His *Daedalea* group consists of *Amylocystis, Antrodia, Daedalea, Auriporia, Fomitopsis, Gloeophyllum, Oligoporus, Piptoporus* and *Stiptophyllum*. Among them, no sequence data are available for *Auriporia* and *Stiptophyllum* at present. *Gloeophyllum* deviates from the other genera of the group, judging from their current molecular phylogenetic analyses (3, 18).

The order Fomitopsidales of Jülich (22) contained both brown rot families (Daedaleaceae, Fomitopsidaceae, Gloeophyllaceae) and white rot families (Fomitaceae, Haploporaceae, Heterobasidiaceae, Ischnodermataceae, Laricifomitaceae). However, available sequence data suggest that the type of rot is just as important as amyloidity of spores in the higher level classification of the Aphyllophorales. In fact, differences in the type of rot reflect fundamental differences in the enzymatic system involved in the degradation of wood (1).

Laetiporaceae Jülich 1981 emend. S.Y. Kim and H.S. Jung

This group consists of taxa from several families and *Laetiporus, Wolfiporia* (Polyporaceae), *Phaeolus* (Hymenochaetaceae), *Cantharellus* (Cantharellaceae) and *Sparassis* (Sparassidaceae) are included here. The position of *Cantharellus* was quite different from that of the analysis by Hibbett *et al.* (19) where *Cantharellus* was grouped together with *Clavulina* and *Hydnum*. The nuc SSU rRNA gene sequence of *Cantharellus* differed greatly from other sequences, rendering the alignment quite difficult and, for that reason, the nuc SSU rRNA gene sequences were once excluded from the analysis of Hibbett *et al.* (19).

The authors excluded *Cantharellus* from the present discussion because its taxonomic position is quite problematic. In this emended group, common characters are brown rot and monomitic to dimitic hyphal systems. The generative hyphae may be simple-septate (*Laetiporus, Wolfiporia, Phaeolus*) or clamped (*Sparassis, Cantharellus*). The monophyly of *Laetiporus* and *Phaeolus* was discussed at length by Ryvarden (32) and Hibbett and Donoghue (18). Ryvarden (32) suggested that the *Laetiporus* group shares simple septa and brown rot and is composed of *Laetiporus, Leptoporus, Phaeolus, Pycnoporellus* and *Wolfiporia*. This group also proves the importance of rot type in the higher classification level of the Aphyllophorales.

Polyporaceae Corda 1839 emend. S.Y. Kim and H.S. Jung

This emended family is restricted to poroid taxa having dimitic to trimitic hyphal systems with clamped generative hyphae, inamyloid smooth cylindrical basidiospores, tetrapolar sexuality and white rot. In the analysis of Hibbett and Donoghue (18), *Cryptoporus, Daedaleopsis, Datronia, Fomes, Ganoderma, Lentinus, Lenzites, Polyporus, Pycnoporus* and *Trametes* formed a strongly supported group. Likewise, in the analysis of Boidin *et al.* (3), *Lenzites* and *Trametes* formed a monophyletic clade together with *Lopharia*. Ryvarden (32) classified *Polyporus* (Polyporaceae s. s.) and *Trametes* groups to accommodate the above listed genera. *Lopharia cinerascens* has a dimitic hyphal system with clamped generative hyphae and skeletal hyphae, and encrusted hymenial cystidia (38), thus differing from the characters of the Polyporaceae s. s. However, *Lopharia cinerascens* was included in the Polyporaceae s. s. by Boidin *et al.* (3), but Corner (7) put this group in synonymy with *Trametes* s. l.

Stereaceae Pilát 1930 emend. S.I. Yoon, S.Y. Kim, Y.W. Lim & H.S. Jung

This family contained *Stereum, Xylobolus, Gloeocystidiellum* and *Aleurodiscus* with a high bootstrap support value of 93%. They have monomitic to dimitic hyphal systems with skeletal hyphae, gloeocystidia and amyloid spores in common. Clamps may be absent or present, and spores may be ornamented or not. *Xylobolus* and *Stereum* have a dimitic hyphal system with simple-septate generative hyphae and hyaline to brown skeletal hyphae, and hyaline amyloid spores (5, 6, 12, 22). *Gloeocystidiellum leucoxanthum* has a monomitic hyphal system with clamps, gloeoplerous hyphae, and smooth amyloid spores (11). *Aleurodiscus botryosus* has a monomitic hyphal system with simple-septate generative hyphae, gloeocystidia, and ornamented amyloid spores (23, 27). The monophyly of this group was already verified by Hibbett and Donoghue (18) and Boidin *et al.* (3).

Hericiaceae Donk 1964

This unique group has been characterized by the amy-

loidity of spores and monomitic to dimitic hyphal systems with skeletal hyphae but was poorly supported by bootstrap analysis. *Auriscalpium* used to be treated in the family Auriscalpiaceae. There have been controversies over the taxonomic position of *Heterobasidion* and *Bondarzewia* (18, 30, 34), however, the former species is connected outside the present group.

Amylostereaceae Boidin, Mugnier & Canales 1998

This group has a dimitic hyphal system with skeletal hyphae, clamped generative hyphae, smooth amyloid spores, and colored encrusted cystidia (6, 23) but was weakly supported by bootstrap analysis. *Echinodontium* and *Amylostereum* are included in this family. *Russula*, which is one of the typical species of the Agaricales, has a monomitic hyphal system and amyloid basidiospores. The result of Boidin *et al.* (3) indicated that *Amylostereum, Echinodontium, Boidinia* and *Gloeodontia* formed a monophyletic group.

Hymenochaetaceae Donk 1948 emend. S.Y. Kim and H.S. Jung

This family is formed by *Inonotus, Phellinus* and *Trichaptum*. Traditionally, the Hymenochaetaceae has been regarded as a unique monophyletic group in the order Aphyllophorales (8, 32). In the study of Hibbett and Donoghue (18), *Coltricia, Inonotus, Phellinus, Phylloporia* and *Trichaptum* formed a monophyletic clade having imperforate parenthosomes in common. Based on molecular data of nuc SSU rDNA sequences (24) and ITS sequences (3), the monophyly of the Hymenochaetaceae and *Trichaptum* was again verified. Ultrastructures such as parenthosomes and spindle pole bodies have been proved to be important as molecular and ultrastructural data are being accumulated (2).

Cystostereaceae Jülich 1981

This group is moderately supported by bootstrap analysis and consists of taxa from several families. It includes *Cystostereum, Chondrostereum* (Stereaceae), *Athelia* (Corticiaceae), *Lentinula, Pleurotus* (Pleurotaceae), *Schizophyllum* (Schizophyllaceae) and *Fistulina* (Fistulinaceae) together. No common characters exist for this group. *Chondrostereum purpureum* has a monomitic hyphal system with clamped generative hyphae and smooth or encrusted cystidia. *Cystostereum murraii* has a dimitic hyphal system with skeletal hyphae and clamped generative hyphae, and numerous vesicles with yellow-oily or resinous vesicles. According to Boidin *et al.* (3), *Cystostereum* was grouped in the order Phanerochaetales.

Chaetodermataceae Jülich 1981 emend. S.Y. Kim and H.S. Jung

This group is formed by *Gloeophyllum, Donkiporia* (Polyporaceae), *Veluticeps* and *Boreostereum* (Stereaceae). Among them, *Gloeophyllum, Veluticeps* and *Boreostereum* cause brown rot, while *Donkiporia* causes white rot. Microscopically, *Gloeophyllum* and *Donkiporia* have a trimitic hyphal system with clamped generative hyphae, *Veluticeps* a dimitic hyphal system with clamped generative hyphae (or monomitic hyphal system with sclerofied generative hyphae), and *Boreostereum* a dimitic hyphal system with simple-septate generative hyphae, respectively. However, common character uniting those four genera is brown colored skeletal hyphae which give brown color to the basidiocarp. Nakasone (26) suggested Chaetodermataceae Jülich emend. Nakasone and included *Gloeophyllum* as a possible relative of the family. Ryvarden (32) compared *Donkiporia* (white rot, clamped generative hyphae) with *Gloeophyllum* (brown rot, clamped generative hyphae) or *Phellinus* (white rot, simple-septate generative hyphae, setae). Both Hibbett and Donoghue (18) and Boidin *et al.* (3) suggested no close relatives to *Gloeophyllum* in their phylogenetic studies.

Botryobasidiaceae (Parm.) Jülich 1981

In the study of Boidin *et al.* (3), *Botryobasidium* formed an order Botryobasidiales. Specific characters of this family are 4 to 8 sterigmata on basidia, cyanophilicity of spores and basidia, and imperforate parenthosomes (21, 28). *Botryobasidium* is known to have a close relationship to *Uthatobasidium, Thanatephorus, Ceratobasidium* and *Cejpomyces* which are looked on as heterobasidiomycetes.

Ramariaceae Corner 1970

In this group *Clavariadelphus* (Clavariaceae), *Gomphus* and *Ramaria* (Gomphaceae) were included. *Gomphus* and *Ramaria* have cyanophilic spore ornamentation and gloeoplerous hyphal contents. *Clavariadelphus* and *Gomphus* show similar chemical reactions (22). However, according to current taxonomic systems, Clavariadelphaceae and Gomphaceae are separated from the Ramariaceae.

Hydnaceae Chev. 1826 emend. S.Y. Kim and H.S. Jung

This group consists of taxa from three families strongly supported by bootstrap analysis and is composed of *Clavulina* (Clavulinaceae), *Hydnum* (Hydnaceae) and *Multiclavulina* (Clavariaceae). The main character of the family *Clavulina* was stichic basidia. The definition of *Hydnum* was formerly very large but only a few taxa currently remain here. Common characters of those genera are not always clear and further investigation of this group is needed to make the situation clear.

Miscellaneous families

Among families which were excluded in this phylogenetic study are Lachnocladiaceae and Coniophoraceae. In the analysis of Boidin *et al.* (3), the Lachnocladiales formed a monophyletic group with *Dichostereum, Scy-*

tinostroma and *Vararia*. This family was monographed at length by Hallenberg (16). On the Coniophoraceae, no appropriate sequence data are available at present. Its phylogenetic position is a matter of great interest because the family has a unique ensemble of characters of brown rot, dimitic hyphal system with dextrinoid hyphae, and amyloid spores (1, 16).

General conclusions

Recent molecular works have been helpful in distinguishing homologies from homoplasies in many fungal groups, and current works proved to be the same. Through the phylogenetic analysis using nuc ssu rDNA sequences, sixteen taxonomic groups were identified in spite of some limited number of tested taxa to improve the current classification system of the order Aphyllophorales. Although each group has one or a few exceptional cases, sixteen families were characterized based on parsimony analysis mainly by taxonomically significant microscopic characters such as the hyphal system, colors of hyphae, type of rot, presence or absence of clamps, and ultrastructure such as parenthosome structures.

Among those families, ten families were emended by the authors. They were Steccherinaceae Parm. 1968, Podoscyphaceae Reid 1965, Phanerochaetaceae Jülich 1981, Fomitopsidaceae Jülich 1981, Laetiporaceae Jülich 1981, Polyporaceae Corda 1839, Stereaceae Pilát 1930, Hymenochaetaceae Donk 1948, Chaetodermataceae J lich 1981 and Hydnaceae Chev. 1826. On the contrary to microscopic structures, macromorphological characters such as the shape of the basidiocarp or hymenophores proved to be less reliable and insufficient in revealing phylogenetic relationships among the species of the Aphyllophorales.

Acknowledgments

The authors are grateful to Dr. K.S. Bae of KCTC (Korean Collection for Type Cultures), KRIBB (Korea Research Institute for Bioscience and Biotechnology), who kindly provided strains for research collaboration. This work was supported by the Brain Korea 21 Project.

References

1. Alexopoulos, C.J., C.W. Mims, and M. Blackwell. 1996. Introductory mycology, 4th ed. John Wiley & Sons, New York.
2. Berbee, M. and J.W. Taylor. 1995. From 18S ribosomal sequence data to evolution of morphology among the fungi. *Can. J. Bot.* 73(S), S677-S683.
3. Boidin, J., J. Mugnier, and R. Canales. 1998. Taxonomie moleculaire des aphyllophorales. Mycotaxon 66, 445-491.
4. Bruns, T.D., R. Fogel, and T.W. 1990. Amplification and sequencing of DNA from fungal herbarium specimens. *Mycologia* 82, 175-184.
5. Chamuris, G.P. 1985. Infrageneric taxa in *Stereum* and key to North American species. *Mycotaxon* 22, 105-117.
6. Chamuris, G.P. 1988. The non-stipitate stereoid fungi in the Northern United States and adjacent Canada. *Mycol. Mem.* 14, 1-247.
7. Corner, E.J.H. 1989. Ad Polyporaceas VI. The genus *Trametes*. *Nova Hedw.* 97, 1-197.
8. Donk, M.A. 1964. The conspectus of the families of the Aphyllophorales, *Persoonia* 3, 199-324.
9. Donk, M.A. 1971a. Progress in the study of classification of the higher Basidiomycetes, p. 3-25. *In* R.H. Petersen (ed.), Evolution in the higher Basidiomycetes. The University of Tennessee Press, Knoxville, Tennessee.
10. Donk, M.A. 1971b. Multiple convergence in the polyporaceous fungi, p. 393-422. *In* R.H. Petersen (ed.), Evolution in the higher Basidiomycetes. The University of Tennessee Press, Knoxville, Tennessee.
11. Eriksson, J. and L. Ryvarden. 1975. The Corticiaceae of North Europe, Vol. 3. *Coronicium-Hyphoderma*. Fungiflora, Oslo, Norway.
12. Eriksson, J., K. Hjortstam, and L. Ryvarden. 1984. The Corticiaceae of North Europe, Vol. 7. *Schizopora-Suillosporium*. Fungiflora, Oslo, Norway.
13. Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783-791.
14. Gargas, A and J.W. Taylor. 1992. Polymerase chain reaction (PCR) primers for amplifying and sequencing 18S rDNA from lichenized fungi. *Mycologia* 84, 589-592.
15. Gilbertson, R.L. and L. Ryvarden. 1986. North American polypores, Vol. 1. *Abortiporus-Lindtneria*. Fungiflora, Oslo, Norway.
16. Hallenberg, N. 1985. The Lachnocladiaceae and Coniophoraceae of North Europe. Fungiflora, Oslo, Norway.
17. Hibbett, D.S. 1996. Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom forming fungi. *Mol. Biol. Evol.* 13, 903-917.
18. Hibbett, D.S. and M.J. Donoghue. 1995. Progress toward a phylogenetic analysis of the Polyporaceae through parsimony analysis of mitochondrial ribosomal DNA sequences. *Can. J. Bot.* 73(S), S853-S861.
19. Hibbett, D.S., E.M. Pine, E. Langer, G. Langer, and M.J. Donoghue. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proc. Natl. Acad. Sci. USA.* 94, 12002-12006.
20. Hibbett, D.S. and R. Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. *Sys. Bot.* 18, 409-433.
21. Hjortstam, K., K.H. Larsson, and L. Ryvarden. 1987. The Corticiaceae of North Europe, Vol. 1. Introductions and keys. Fungiflora, Oslo, Norway.
22. Jülich, W. 1981. Higher taxa of Basidiomycetes. *Bibl. Mycol.* 85, 1-485.
23. Jülich, W. and J.A. Stalpers. 1980. The resupinate non-poroid Aphyllophorales of the temperate northern hemisphere. North-Holland Publishing Company, Amsterdam, Netherlands.
24. Ko K.S., S.G. Hong, and H.S. Jung. 1997. Phylogenetic analysis of *Trichaptum* based on nuclear 18S, 5.8S, and ITS ribosomal DNA sequences. *Mycologia* 89, 727-734.
25. Moore-Landecker, E. 1990. Fundamentals of the fungi, 3rd ed. Prentice Hall, Englewood Cliffs, New Jersey.
26. Nakasone, K.K. 1990. Taxonomic studies of *Veluticeps* (Aphyllophorales). *Mycologia* 82, 622-641.

27. Nunez, M. and L. Ryvarden. 1997. The genus *Aleurodiscus* (Basidiomycotina). *Syn. Fung.* 12, 1-164.
28. Parmasto, E. 1995. Corticioid fungi: a cladistic study of a paraphyletic group. *Can J. Bot.* 73(S), S843-S852.
29. Pouzar, Z. 1967. Studies in the taxonomy of the polypores 3. *Ceska Mykol.* 21, 205-212.
30. Readhead, S.A. and L.L. Norvell. 1993. Notes on *Bondarzewia, Heterobasidion* and *Pleurogala. Mycotaxon* 38, 371-380.
31. Reid, D.A. 1971. Intermediate generic complexes between the Thelephoraceae and other families, p. 331-344. *In* R.H. Petersen (ed.), Evolution in the higher Basidiomycetes. The University of Tennessee Press, Knoxville, Tennessee.
32. Ryvarden, L. 1991. Genera of polypores: nomenclature and taxonomy. *Syn. Fung.* 5, 1-363.
33. Ryvarden, L. and R.L. Gilbertson. 1993. European polypores, Part 1. *Abortiporus-Lindtneria. Syn. Fung.* 6, 1-387.
34. Stalpers, J.A. 1979. *Heterobasidion* (*Fomes*) *annosum* and the Bondarzewiaceae. *Taxon* 28, 414-417.
35. Swann, E.C. and J.W. Taylor. 1993. Higher taxa of basidiomycetes: an 18S rDNA perspective. *Mycologia* 85, 923-936.
36. Swofford, D.L. 1999. PAUP*: phylogenetic analysis using parsimony (* and other methods), Version 4. Sinaur Associates, Sunderland, Massachusetts.
37. Talbot, P.H.B. 1973. Aphyllophorales I: general characteristics; thelephoroid and cupuloid families, p. 327-349. *In* G.C. Ainsworth, F.K. Sparrow, and A.F. Sussman (eds.), The Fungi, Vol. IVB. Academic Press, New York.
38. Welden, A.L. 1975. *Lopharia, Mycologia* 67, 530-551.
39. White, T.J., T. Bruns, S. Lee, and J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315-322, *In* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White (eds.), PCR protocols: a guide to methods and application. Academic Press, San Diego, California.
40. Yu, C.D. and T. Niemëla. 1997. Changbai wood rotting fungi 6. Study on *Antrodiella*, two new species and notes on some other species. *Mycotaxon* 64, 67-81.