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Phylogenetic analyses of *Aleurodiscus* s.l. and allied genera

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Abstract: The limits and possible subdivision of *Aleurodiscus* s.l. into *Acanthobasidium, Acanthofungus, Acanthophysellum, Aleurobotrys, Aleurocystidiellum, Aleurodiscus* s.s., and *Gloeosoma* were evaluated. Molecular characters were obtained from an approximately 980 base pair fragment at the 5′ end of the nuclear large subunit ribosomal DNA, in 33 strains representing 23 species of *Aleurodiscus* s.l., *Stereum, Xylobolus,* and *Megalocystidium leucoxanthum.* Published sequences of 20 additional species of the russuloid clade were also included. Phylogenetic analyses suggest that *Aleurodiscus* s.l., *Megalocystidium leucoxanthum, Stereum* and *Xylobolus* form a monophyletic group, which may be classified as the family Stereaceae. *Corticium roseum,* which is the type species of the Corticiaceae, is not in this group, thus Stereaceae is not synonymous with Corticiaceae. *Aleurocystidiellum* is supported as a monophyletic group. *Acanthobasidium,* which is characterized by pleurobasidia, is also monophyletic. *Aleurodiscus* s.s. is supported as monophyletic, but *Gloeosoma* is not, and the two are not congeneric. The importance of amyloid acanthophyses for recognizing *Aleurobotrys* is suspect, and its generic status should be further studied. Most of the smooth-spored species form a monophyletic group. Phylogenetic analyses suggest that there has been homoplasy in most of the characters that have been used to subdivide *Aleurodiscus* s.l., including spore ornamentation, hymenial color, hyphal septation, clamp connections, acanthophyses, and phenoloxidase reactions.

Key Words: Basidiomycota, corticioid fungi, molecular systematics, russuloid clade

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INTRODUCTION

Aleurodiscus Rabenh. ex J. Schröt. is a member of the corticioid homobasidiomycetes. Seventy one species world-wide have been accepted in *Aleurodiscus* (Núñez and Ryvarden 1997). The limits of *Aleurodiscus* have been controversial. Lemke (1964) excluded taxa with inamyloid spores from *Aleurodiscus* sensu stricto. In contrast, Núñez and Ryvarden (1997) adopted a broad concept of *Aleurodiscus.* These two publications and the work of Boidin (1985) offer a comprehensive compilation of current taxonomy and species concepts of this group. In this paper, *Aleurodiscus* sensu lato is essentially equivalent to *Aleurodiscus* sensu Núñez and Ryvarden (1997). An overview of character combinations used to discriminate *Aleurodiscus* s.s. and segregate genera of *Aleurodiscus* s.l. is provided in TABLE I.

Mycologists recognize *Aleurodiscus* using either a broad or a narrow generic concept (compare Boidin et al 1985, and Núñez and Ryvarden 1997). Several narrowly defined genera have segregated from *Aleurodiscus* s.l., including *Acanthobasidium* Oberw., *Acanthofungus* Sheng H. Wu et al, *Acanthophysellum* Parmasto, *Aleurobotrys* Boidin, *Aleurocystidiellum* P.A. Lemke, and *Gloeosoma* Bres. TABLE I summarizes characters used to separate these groups. Most species of *Aleurodiscus* s.l. have acanthophyses, whereas *Aleurodiscus* s.s. and *Aleurocystidiellum* lack them. Species of *Aleurodiscus* s.s. have hyphidia, which may be occasionally branched. Dendrohyphidia, present in a few species, could be considered as an intermediate form between acanthophyses and occasionally branched hyphidia. Most species of *Aleurodiscus* s.l. are associated with a uniform white rot in wood, whereas *Acanthofungus* and *Xylobolus* are associated with a white-pocket rot in wood. In *Aleurodiscus* s.l., the mycelia of uniform white rot-causing species usually show a positive phenoloxidase reaction, while this reaction is usually negative for the white pocket rot-causing species (S.-H. Wu, unpubl).

Macroscopically, cupulate basidiocarps were generally recognized as the typical form, but this character varies among *Aleurodiscus* s.l., and effuse, effused-reflexed, or pulvinate forms are present. A pinkish- or orange-tinted hymenial surfaces was regarded as typical for *Aleurodiscus,* but white, gray, or cream-colored hymenial surfaces occur in many spe-

TABLE I. Main characters used for separating segregate genera in *Aleurodiscus* s.l., as *Stereum* and *Xylobolus*[a]

	Ornamented spores	Acanthophyses	Clamp connections in basidiocarps	Phenoloxidase reaction
Acanthobasidium	+	+	+	+
Acanthofungus	−	+	+	−
Acanthophysellum	−	+	+	+
Aleurobotrys	+	+	−	+
Aleurodiscus s.s.	+	−	±	+
Aleurocystidiellum	+	−	+	+
Gloeosoma	+	+	+	+
Stereum	−	±[b]	−	+
xylobolus	−	+	−	−

[a] Data from Boidin et al 1985, Núñez and Ryvarden 1997, and Wu et al 2000.

[b] Some species of *Stereum* lack acanthophyses but have acutophyses, which might be a reduced form of acanthophyses.

cies. Boidin et al (1985) suggested that the pink or orange spore print is an important feature of *Aleurodiscus* s.s. (including *Gloeosoma*), which differs from the white spore print of other genera in *Aleurodiscus* s.l. This character may be important, but is not known for many species.

Microscopically, gloeocystidia and amyloid basidiospores are uniformly present in all species. Basidia and basidiospores of many species are large, relative to other species of corticioid fungi. Acanthophyses characterize the majority of the species in *Aleurodiscus* s.l. Lemke (1964) regarded *Aleurodiscus* as having a catahymenium, but Eriksson and Ryvarden (1973) considered this character to be questionable, especially for the members in *Acanthophysellum*. The catahymenium is more typically present in *Aleurodiscus* s.s. The distinction between a euhymenium and a catahymenium is hard to define, however. For instance, Lemke (1964) described all species in his concept of *Aleurodiscus* s.s. as having a catahymenium, but this is not correct. As a result, this character is not considered in this study. Some other important characters vary among species of *Aleurodiscus* s.l. For instance, basidiospores can be ornamented or smooth and the hyphae can be nodose or simple-septate. In addition, nuclear behavior and mating systems vary among species of *Aleurodiscus* s.l. (Boidin and Lanquetin 1984b).

Aleurodiscus s.l. includes species with highly varied characters, which is why many mycologists are not willing to accept *Aleurodiscus* as a single genus. Moreover, the characters used for separating different groups in *Aleurodiscus* s.l. are not congruent. For example, some groups with ornamented spores have acanthophyses whereas other do not, and some have nodose hyphae whereas other have simple-septate hyphae. Further, some species still do not fit any genus as listed in TABLE I. For example, *A. farlowii* has smooth spores, acanthophyses, and simple-septate hyphae; *A. monilifer* Malençon has smooth spores, lacks acanthophyses, and has nodose-septate hyphae; and *A. limonisporus* D.A. Reid has smooth spores, lacks acanthophyses, and has simple-septate hyphae. These three species cannot be placed in any of the segregated genera of *Aleurodiscus* s.l. as currently circumscribed In fact, the known species in *Aleurodiscus* s.l. have satisfied all possible combinations for the three important characters of spore surface (ornamented or smooth), acanthophyses (present or not), and hyphal septation (nodose or simple-septate). Thus, some mycologists (Hjortstam 1997, Núñez and Ryvarden 1997) consider the subdivision of this group impossible.

The purpose of this study is to assess the limits of *Aleurodiscus* s.l. and the monophyly of its generic segregates. The taxa chosen represent various segregate genera of *Aleurodiscus* s.l., plus the allied genera *Stereum* and *Xylobolus*. Mycologists have traditionally regarded *Aleurodiscus* s.l. as a member of the Corticiaceae Heter in the broad sense (Donk 1964, Parmasto 1986). Parmasto's (1995) analysis based on morphological characters suggested that this group is in the Corticiaceae s.s. *Stereum* and *Xylobolus* are generally placed in the Stereaceae Pilát (e.g., Ginns and Lefebvre 1993). Stereaceae has been customarily used to accommodate genera with stereoid basidiocarps and dimitic hyphal systems (Donk 1964, Parmasto 1986). Wu (1996) stressed the importance of amyloid basidiospores and gloeoplerous hyphae and limited the Stereceae (in his concept of the subfamily Stereoideae Ulbr.) to *Stereum* and *Xylobolus*, excluding all other genera that lack these two characteristics. *Stereum* and *Xylobolus* share an important character, the acanthophyses, with *Aleurodiscus* s.l., which suggests that they are closely related (Lemke 1964, Boidin et al 1979). This view has been upheld by

molecular phylogenies (Hibbett et al 1997, Hibbett and Donoghue in press), which suggest that *Aleurodiscus botryosus, A. cerrusatus, Stereum hirsutum, S. rugosum, Xylobolus frustulatus,* and *Megalocystidium leucoxanthum* form a monophyletic group that is nested in the russuloid clade of the homobasidiomycetes (Hibbett and Thorn 2000). The rDNA internal transcribed spacer (ITS) analysis of Boidin et al (1998) also suggested that species of *Aleurodicus* s.l. are closely related to *Gloeocystidiellum* s.l., *Stereum, Xylobolus,* and other members of the russuloid clade. Interpretation of the phylogenetic tree of Boidin et al (1998, FIG. 6) is problematical, however, because there are no bootstrap values or other measures of robustness. Therefore, it is not possible to know what aspects of their tree are strongly supported by the data.

MATERIALS AND METHODS

Molecular characters were obtained from an approximately 980 base pair (bp) fragment of the nuclear large subunit ribosomal DNA (nuc-lsu rDNA), which was amplified using primers LR0R and LR5 (Moncalvo et al 2000). Sequences were generated from 33 strains representing 19 species of *Aleurodiscus* s.l., plus two species of *Stereum* and *Xylobolus* (TABLE II). Sequences of ten other species in the russuloid clade (Hibbett and Thorn 2000) that were published previously, including *A. cerrusatus* and *M. leucoxanthum* (Hibbett et al 2000), were also included. A published sequence of *Hyphodontia alutaria* was included for rooting purposes (Hibbett et al 2000). Based on previous studies, *H. alutaria,* a member of the hymenochaetoid clade, is an appropriate outgroup for an analysis of the russuloid clade (Hibbett and Thorn 2000). Some analyses (see below) also included 28 partial nuc-lsu rDNA sequences of wood-decaying species of the russuloid clade published by Hallenberg and Parmasto (1998), including 7 species of *Aleurodiscus* s.l., 2 species of *Stereum,* 13 species of *Peniophora,* and others. The sequences of Hallenberg and Parmasto (1998) cover 477–606 bp (49–62%) of the LR0R-LR5 fragment.

Cultures were grown on malt extract agar and liquid malt-yeast-glucose media. Isolates of *A. abietis* (T330), *A. farlowii* (HHB 14354), *A. lapponicus* (FP100753-R), and *A. grantii* (T541) were tested for phenoloxidase activity, as described by Wu (1996).

Freeze-dried or fresh liquid-cultured mycelium was ground in a 1% SDS, 0.15 M NaCl, 50 mM EDTA extraction buffer, extracted twice with phenol-chloroform-isoamyl alcohol (24:24:1), extracted once with chloroform, and precipitated with ethanol and sodium acetate. DNA was diluted up to 1000-fold with deionized water for use as PCR template. PCR products were generated using Promega (Madison) reagents in an MJ Research thermal cycler, purified using GeneClean (BIO 101, La Jolla, California), and cycle sequenced using dye terminator sequencing reagents (Applied Biosystems, Foster City, California), with primers LR0R, LR22, LR3, LR3R, and LR5 (www.botany.duke.edu/fungi/mycolab/primers.htm). Sequencing reactions were purified using Pellet Paint (Calbiochem, San Diego, California), and run on an Applied Biosystems 377XL automated DNA sequencer with a 5% Long Ranger acrylamide gel (FMC BioProducts). Sequences were edited and assembled using Sequencher (GeneCodes Corp., Ann Arbor, Michigan), and have been deposited in GenBank (accession numbers AY039305–AY039336).

Sequences were manually aligned in the PAUP*4.0b4a (Swofford 1999) data editor. The dataset has been deposited in TreeBASE (accession number S626). Three sets of analyses were performed. Analysis 1 included 42 complete LR0R-LR5 sequences (31 new sequences, plus 11 published sequences). Analysis 2 included the same sequences as analysis 1, except the sequence from *A. farlowii,* which was found to be highly divergent (see Results). Analysis 3 included all 42 complete LR0R-LR5 sequences, plus the 28 partial sequences of Hallenberg and Parmasto (1998).

Phylogenetic analyses were performed in PAUP* using equally weighted parsimony. In analyses 1 and 2, 1000 replicate heuristic searches were performed, each with a random taxon addition sequence, MAXTREES set to autoincrease, and TBR branch swapping. Bootstrapping in analyses 1 and 2 used 1000 replicates, each with 100 heuristic searches, random taxon addition sequences, MAXTREES set to 100, and TBR branch swapping. The baseline parsimony search in analysis 3 used a two-step search protocol, with 1000 replicate searches in the first step, with random taxon addition sequences, TBR branch swapping, and keeping up to ten trees per replicate. The second step used the shortest trees found in the first step as starting trees for TBR branch swapping with MAXTREES set to autoincrease. Bootstrapping in analysis 3 used the same settings as analyses 1 and 2, but only 100 replicates were performed.

Two constrained analyses were performed using the same taxa and settings as analysis 2. One constrained analysis forced the monophyly of *Aleurodiscus* s.l., including *Acanthofungus* (i.e., excluding *Stereum, Xylobolus, M. leucoxanthum,* and all other members of the russuloid clade). The other constrained analysis forced the monophyly of *Aleurodiscus* s.l., *Stereum, Xylobolus, M. leucoxanthum, Peniophora nuda,* and *Amylostereum laevigatum* (FIG. 1). This analysis was done to evaluate the results of Hallenberg and Parmasto (1998), which suggested that *Peniophora* and *Amylostereum* are nested in *Aleurodiscus* s.l. The constrained trees were compared to unconstrained trees from analysis 2 using the Kishino-Hasegawa maximum likelihood ratio test (HKY85 model, transition/transversion bias = 2, empirical base frequencies, 4 rate classes with "discrete gamma" distribution) and the Templeton non-parametric test, both implemented in PAUP*.

RESULTS

LR0R-LR5 PCR products were all approximately 980 bp long; there were no major insertions or deletions. Sequences generated for this analysis were 892–967 bp long. The aligned sequences included 997 positions, with 372 variable and 202 parsimony-informa-

TABLE II. Strains used for obtaining new sequences

Taxon	Alternative genera	Source[a]
Acanthofungus rimosus Sheng H. Wu et al.		Wu9601-1; Taiwan, Taichung, on *Calocedrus formosana*
Aleurodiscus abietis H.S. Jacks. & P.A. Lemke		T330; Canada, Nova Scotia; on *Abies balsamea*
Aleurodiscus amorphous (Pers.:Fr.) J. Schröt.		HHB15282; USA, Alaska; on *Picea glauca*
Aleurodiscus aurantius (Pers.Fr.) J. Schröt.		T621 (= LY734, CBS), France, Rhone
Aleurodiscus bisporus (Boidin & Lanq.) Núñez & Ryvarden	*?Stereum*	T627 (= LY7666, CBS; as *Acanthophysium bisporum*); Guadeloupe, Cascade du Carbet; on branch
		T614 (= LY7664, CBS; as *A. bisporum*); Guadeloupe, Cascade du Carbet; on dead twig
Aleurodiscus botryosus Burt	*Aleurobotrys*	CBS195.91 (as *Aleurodiscus botryosus*); Canada, Ontario; on *Thuja occidentalis*
		Wu9302-61; Taiwan, Nantou; on angiosperm
Aleurodiscus cerussatus (Bres.) Höhn. & Litsch.	*Acanthophysellum*	HHB11294 (as *Acanthophysium cerussatum*); USA, Minnesota, on *Pinus banksiana*
		FPL11572 (as *Acanthophysium cerussatum*)
		HHB11235 (as *Aleurodiscus laurentianus*); USA, Minnesota; on *Abies balsameus*
Aleurodiscus disciformis (DC.:Fr.) Pat.	*Aleurocystidiellum*	T529; USA, California; on *Quercus chrysolepsis*
		T629; USA, California; on *Quercus ilex*
Aleurodiscus farlowii Burt	*Acanthobasidium*	HHB14354; USA, Wisconsin; on *Tsuga Canadensis*
Aleurodiscus grantii Lloyd		T541; Canada, British Columbia; on *Abies lasiocarpa*
		T569; USA, California; on *Abies magnifica* var. *shastensis*
Aleurodiscus lapponicus Litsch.	*Acanthophysellum*	FP100753; USA, Minnesota, on down branch of hardwood
Aleurodiscus lividocoeruleus (P. Karst.) P.A. Lemke	*Acanthophysellum*	MB1825 (as *Acanthophysium lividocaeruleum*); USA, Colorado; on *Abies lasiocarpa*
		FP100292 (as *Acanthophysium lividocaeruleum*); USA, Colorado; on *Pinus contorta*
Aleurodiscus mirabilis (Berk. & M.A. Curtis) Höhn.	*Gloeosoma*	Wu9304-105; Taiwan; Nantou; on angiosperm
Aleurodiscus norvegicus J. Erikss. & Ryvarden	*Acanthobasidium*	T623 (as *Acanthobasidium norvegicum*); France, near Calluna; on *Rubus*
Aleurodiscus oakesii (Berk. & M.A. Curtis) Cooke		HHB9243; USA, Michigan; on *Ostrya virginiana*
		FP101813; USA, Wisconsin, on *Quercus rubra*
Aleurodiscus penicillatus Burt	*Gloeosoma*	322; Canada, Nova Scotia; on *Picea sp.*
Aleurodiscus phragmitis (Boidin et al.) Núñez & Ryvarden	*Acanthobasidium*	CBS233.86 (as *Acanthobasidium phragmitis*); France, Landes; on *Phragmites australis*
Aleurodiscus weirii Burt	*Acanthobasidium*	FP134813 (as *Acanthophysium weirii*); USA, Idaho; on *Thuja plicata*
		HHB12678 (as *Acanthophysium weirii*); USA, Alaska; on *Picea stichensis*
Megalocystidium leucoxanthum (Bres.) Jülich		CBS454.86 (as *Gloeocystidiellum leucoxanthum*)
Stereum hirsutum (Willd.:Fr.) Gray		TJV93-161; USA, Washington; on *Alnus rubra*
		Wu9711-20; Taiwan, Taichung; on angiosperm
S. rugosum (Pers.:Fr.) Fr.		HHB13390; USA, Alaska; on *Alnus*
Xylobolus frustulatus (Pers.:Fr.) Boidin		FP106073-T; USA, Mississippi; on *Quercus lyrata*
X. subpileatus (Berk. & M.A. Curtis) Boidin		FP106735; USA, Mississippi; on *Quercus* stump

[a] CBS = Centraalbureau voor Schimmelcutures, Baarn, Netherlands; HHB, FP, T, MB, TJV = Center for Forest Mycology Research, USDA, Madison, WI, USA; Wu = National Museum of Natural Science, Taichung, ROC.

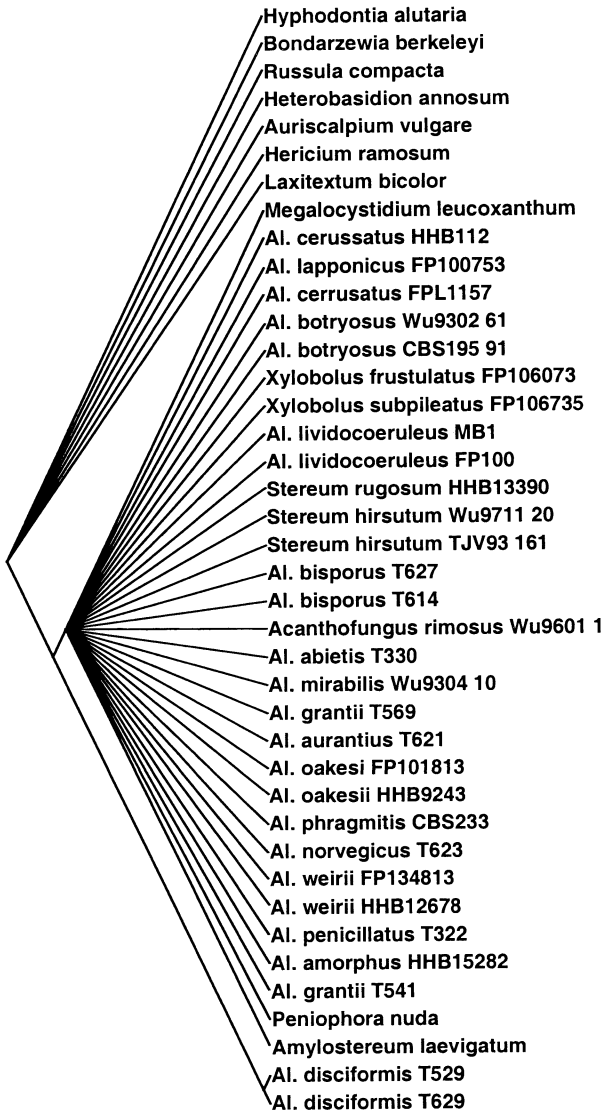

FIG. 1. Constraint tree used to evaluate results of Hallenberg and Parmasto (1998).

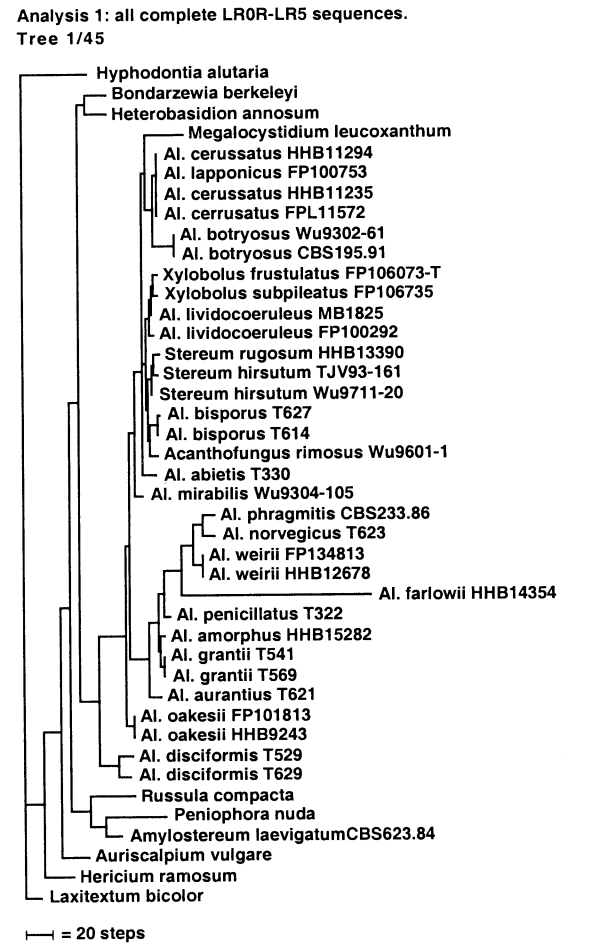

FIG. 2. Analysis 1: phylogenetic relationships of *Aleurodiscus* s.l. inferred from analysis of all complete LR0R-LR5 sequences. Phylogram showing one of 45 equally parsimonious trees (841 steps, CI = 0.577, RI = 0.670). Note long terminal branch leading to *A. farlowii*.

tive positions (calculated with all sequences included).

Analysis 1 (with all complete LR0R-LR5 sequences) recovered 45 trees of 841 steps (CI = 0.577, RI = 0.670) in two islands (Maddison 1991; FIG. 2). Analysis 2 (with all complete LR0R-LR5 sequences, except *A. farlowii*) recovered 42 trees of 675 steps (CI = 0.538, RI = 0.697) in two islands (FIG. 3). In both analyses 1 and 2, every replicate in the heuristic searches found one of the two islands. There is no positive conflict among the strict consensus trees of analyses 1 and 2; the only difference is that one branch in the strict consensus tree of analysis 2 collapses in the strict consensus of analysis 1. Overall, levels of bootstrap support in analysis 1 are lower than those in analysis 2. Five nodes received equal support in analyses 1 and 2 (including four nodes supported at 100%), but no nodes that received stronger bootstrap support in analysis 1 than analysis 2 (FIG. 3).

Strict consensus trees from both analyses 1 and 2 show a basal polytomy in the russuloid clade, with eight unresolved lineages leading to *Amylostereum, Auriscalpium, Bondarzewia/Heterobasidion, Hericium, Laxitextum, Peniophora, Russula,* and a large clade consisting of *Aleurodiscus* s.l., *Stereum, Xylobolus,* and *M. leucoxanthum,* which we hereafter refer to as the Stereaceae (FIG. 3).

The Stereaceae is supported by 87% of the bootstrap replicates in analysis 1 and 91% of the bootstrap replicates in analysis 2. The first group branching off in the Stereaceae is a strongly supported lineage composed of two isolates of *A. disciformis* (bootstrap = 100% in both analyses 1 and 2). The remaining taxa in the Stereaceae form a strongly supported

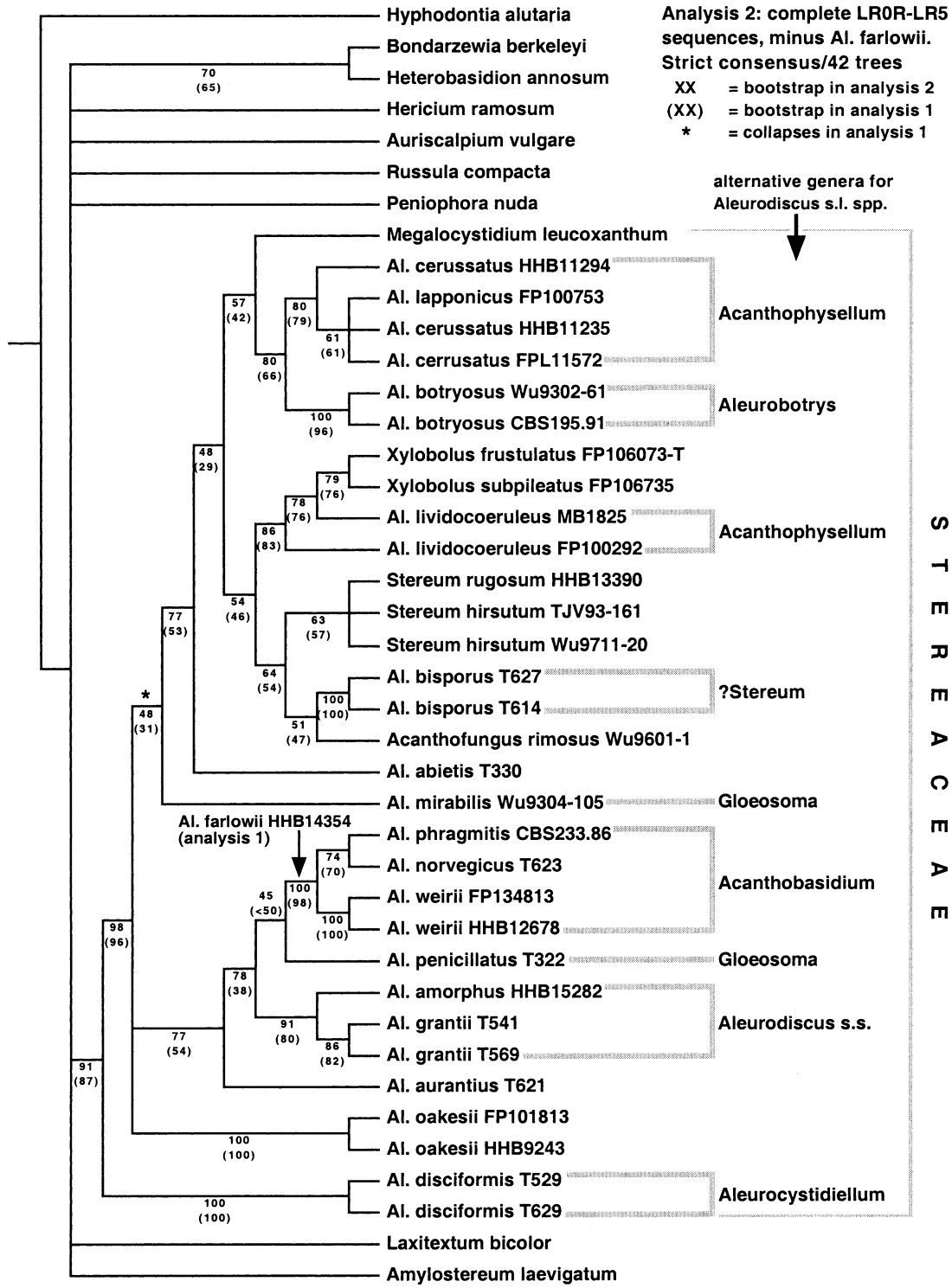

FIG. 3. Analysis 2: phylogenetic relationships of *Aleurodiscus* s.l. inferred from analysis of all complete LR0R-LR5 sequences, except that of *A. farlowii*. Strict consensus of 42 trees (675 steps, CI = 0.538, RI = 0.697). Segregate genera of *Aleurodiscus* s.l. are indicated with brackets. Numbers by nodes indicate bootstrap support in analysis 2 and analysis 1 (in parentheses). The placement of *A. farlowii* in analysis 1 is indicated with an arrow. The one branch that collapses in the strict consensus tree of analysis 1 is indicated with an asterisk.

monophyletic group (bootstrap = 96% in analysis 1, 98% in analysis 2; FIG. 3).

Most pairs of putatively conspecific isolates were grouped together, including *A. disciformis, A. botryosus, A. oakesii, A. weirii,* and *A. bisporus* (FIGS. 2, 3). However, the two isolates of *A. lividocoeruleus* form a paraphyletic group, in which *Xylobolus* is nested, the three isolates of *A. cerrusatus* form a paraphyletic group in which *A. lapponicus* is nested, and the two isolates of *S. hirsutum* cannot be resolved from an isolate of *S. rugosum.*

Analysis 3 (with all sequences, including 28 partial sequences of Hallenberg and Parmasto [1998]) recovered 511 trees of 1034 steps (CI = 0.526, RI = 0.741), which were found in 54 replicate heuristic searches in the first step of the analysis. TBR branch swapping on these trees in the second step of the analysis recovered an additional 312 trees of the same length (FIG. 4). The Stereaceae is still supported as monophyletic in analysis 3, but the bootstrap support is only 40%, vs 87–91% in analyses 1 and 2 (FIGS. 3, 4). As in analyses 1 and 2, a lineage including *A. disciformis* is the sister group of the rest of the Stereaceae. Two sequences of *A. subcruentatum* and *A. disciformis* from Hallenberg and Parmasto (1998) are also in this group in analysis 3. The bootstrap support for the remaining taxa of the Stereaceae is only 68%, vs 96–98% in analyses 1 and 2 (FIGS. 3, 4).

Several isolates from Hallenberg and Parmasto's (1998) dataset are placed close to putatively conspecific sequences generated in the present study, including *A. cerrusatus, S. hirsutum,* and *A. lividocoeruleus* (FIG. 4). However, in each case, the species in question is not supported as monophyletic (FIG. 4). There are also two anomalous results. The sequences of *A. aurantius* and *A. weirii* published by Hallenberg and Parmasto (1998) form a monophyletic group that is outside of the Stereaceae and far removed from the sequences generated for the present study. The sequence of *A. aurantius* and *A. weirii* published by Hallenberg and Parmasto (1998) differ at 56 and 40 positions, respectively, from the sequences of these species generated in the present study. Therefore, we suspect that the sequences published for *A. aurantius* and *A. weirii* by Hallenberg and Parmasto (1998) actually represent taxa that are outside of *Aleurodiscus* s.l.

The constrained analysis that forced the monophyly of *Aleurodiscus* s.l. recovered 125 trees of 702 steps (27 steps longer than the unconstrained trees obtained in analysis 2), all of which were rejected by the Kishino-Hasegawa test (P = 0.0015–0.0066) and the Templeton test (P = 0.0056–0.0191). The constrained analysis designed to test support for inclusion of *Peniophora* and *Amylostereum* in the Stereaceae recovered nine trees of 684 steps (nine steps longer than the unconstrained trees). The Kishino-Hasegawa test rejected all of the constrained trees (P = 0.0073–0.0126), and the Templeton test rejected three of the trees (P = 0.0495–0.1282).

Aleurodiscus abietis (T330), *A. farlowii* (HHB 14354), *A. lapponicus* (FP100753-R), and *A. grantii* (T541) gave positive phenoloxidase reactions.

DISCUSSION

The results of this study suggest that *Aleurodiscus* s.l., *Stereum, Xylobolus,* and *M. leucoxanthum* are in a single clade (FIGS. 2–4), which we suggest should be recognized as the Stereaceae. Bootstrap support for the Stereaceae is strong in the analyses that include only the full-length LR0R-LR5 sequences (87–91%) but it is weak (40%) in the analysis that includes the partial sequences of Hallenberg and Parmasto (1998). Thus, future analyses aimed at understanding relationships of Stereaceae with nuc-lsu rDNA should use full-length LR0R-LR5 sequences. Results of constrained analyses reject the view that *Peniophora* and *Amylostereum* are nested in the Stereaceae (Hallenberg and Parmasto 1998). However, additional studies are needed to estimate what other taxa are in the Stereaceae. In particular, more species of *Gloeocystidiellum* s.l. should be sampled. At present, *Gloeocystidiellum* s.l. is represented in our dataset only by *M. leucoxanthum.* As a result, we can not say whether all of *Gloeocystidiellum* s.l. belongs to the Stereaceae. The analysis of Boidin et al (1998) suggests that species of *Gloeocystidiellum* s.l. are found in several lineages in the russuloid clade.

Gloeocystidiellum s.l. appears to be allied to *Aleurodiscus* s.l. (Ginns and Lefebvre 1993, Hibbett et al 1997, Boidin et al 1998). However, the subdivision of *Gloeocystidiellum* s.l. has challenged mycologists for many years. Analysis of several characters for *Gloeocystidiellum* s.l. allows separation of various genera in this group (Ginns and Freeman 1994, Wu 1996, Boidin et al 1997). In the future, it will be necessary to perform analyses including representatives of all the segregates of *Gloeocystidiellum* s.l. and *Aleurodiscus* s.l., as well as *Stereum* and *Xylobolus.*

The type species of *Corticium, C. roseum,* is in a separate clade from the Stereaceae (FIG. 4). Thus, the Stereacae is not a synonym of Corticiaceae. These findings conflict with those of Parmasto (1995), who suggested that *Aleurodiscus* is in the Corticiaceae s.s., based on an analysis of morphological characters.

Aleurodiscus s.l. is paraphyletic (FIGS. 2–4), and therefore should not be recognized as a formal taxon. Two of the segregate genera, *Aleurocystidiellum* and *Acanthobasidium,* are supported as monophylet-

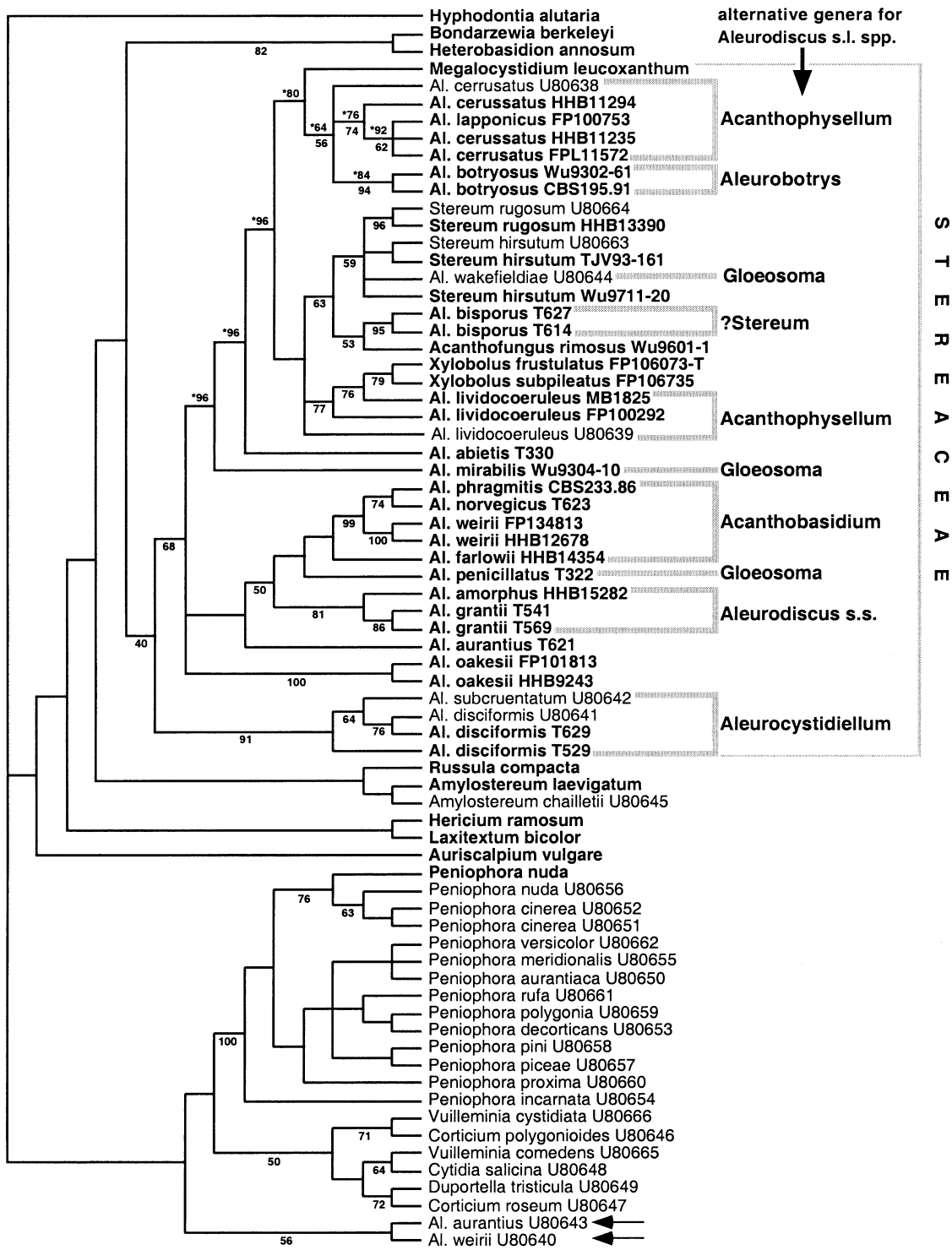

FIG. 4. Analysis 3: phylogenetic relationships of *Aleurodiscus* s.l. inferred from analysis of all complete LR0R-LR5 sequences (written in bold font) and partial sequences of Hallenberg and Parmasto (1998, written in plain font). Problematical sequences of *A. aurantius* and *A. weirii* from Hallenberg and Parmasto (1998) are indicated with arrows. Other symbols as in FIG. 3.

ic, but *Acanthophysellum* and *Gloeosoma* are polyphyletic (FIGS. 2–4). *Aleurobotrys* and *Acanthofungus* were represented in our analyses by only a single species each. The taxonomic implications of our results, emphasizing the segregate genera, are discussed below.

Aleurodiscus amorphus, which is the type species of *Aleurodiscus,* and *A. grantii* are supported as a monophyletic group in this study (FIGS. 2–4). These two species represent *Aleurodiscus* s.s., with occasionally branched hyphidia, but lacking acanthophyses and dendrohyphidia. *Aleurodiscus aurantius* resembles *Aleurodiscus* s.s. morphologically, but it has dendrohyphidia and lacks the sparsely branched hyphidia. The molecular results suggest that this species is basal to the clade that includes *Aleurodiscus* s.s., *A. penicillatus,* and *Acanthobasidium* (FIGS. 2–4). Further studies are needed to verify whether *Aleurodiscus* s.s. only includes species with occasionally branched hyphidia, not dendrohyphidia.

Aleurocystidiellum, proposed by Lemke (1964) for a single species, *Stereum subcruentatum* Berk. & M.A. Curtis, was originally characterized by a dimitic hyphal system and encrusted skeletal cystidia (pseudocystidia). Later, *A. disciformis* (DC.: Fr.) Boidin et al (1968) was transferred to this genus. Unlike the type species, *A. disciformis* is monomitic with gloeocystidia. Nevertheless, both species occur on bark of living trees (Boidin et al 1985). In addition, Hallenberg and Parmasto (1998) observed similar wart-like ornamentations on the spore surfaces of both species, suggesting that they are closely related. The monophyly of *Aleurocystidiellum* is strongly supported in this study (FIG. 4), as well as the analysis of Hallenberg and Parmasto (1998). Thus, the skeletal cystidia in *A. subcruentatum* and the gloeocystidia in *A. disciformis* may be homologous, both representing modifications of the gloeoplerous hyphae that characterize other taxa in the russuloid clade (Donk 1964, Hibbett and Thorn 2000). A similar case is present in *Stereum wakullum* (Burds et al) Sheng H. Wu. Most species of *Stereum* have distinct skeletal-like cystidia (pseudocystidia), but they are lacking in *S. wakullum,* which has gloeocystidia with basally thickened walls. The skeletal-like cystidia of *Stereum* probably represent a modification of the gloeoplerous hyphae.

Acanthobasidium is also strongly supported as monophyletic in this study (FIGS. 2–4). This genus is characterized chiefly by the pleurobasidia (and ornamented spores and clamp connections). A feature of secondary importance is the lateral protuberances produced on the basidia (acanthobasidia). Acanthobasidia are not unique to *Acanthobasidium,* but are also found in several other groups of *Aleurodiscus* s.l. and some species of *Stereum. Aleurodiscus phragmitis, A. norvegicus* and *A. weirii* are strongly supported as monophyletic (FIGS. 3, 4). Pleurobasidia are clearly present in *A. phragmitis* and *A. norvegicus.* Lemke (1964) suggested that the basidioles of *A. weirii* resemble pleural basidia. Thus, molecular and anatomical features suggest that *A. weirii* may be properly classified as a member of *Acanthobasidium.*

Aleurodiscus farlowii has a close relationship with *Acanthobasidium,* according to analyses 2 and 3 (FIGS. 2–4). *Aleurodiscus farlowii* differs from the three other species of *Acanthobasidium* in having smooth spores and simple-septate hyphae (FIG. 5). The position of *A. farlowii* based on molecular characters is problematical because its rDNA sequence is highly divergent from other sequences of *Aleurodiscus* s.l. (FIG. 2), which raises the possibility that its placement is an artifact due to long branch attraction, and the monophyly of *A. farlowii, A. phragmitis, A. norvegicus,* and *A. weirii* was supported by only 45% of the bootstrap replicates in analysis 1 (FIG. 3). Nevertheless, the present molecular phylogeny suggests that this species is probably an *Acanthobasidium.*

Species representing the genera *Acanthophysellum, Aleurobotrys, Acanthofungus, Stereum,* and *Xylobolus,* and the species *A. bisporus, A. abietis,* and *Megalocystidium leucoxanthum* form a moderately supported monophyletic group (bootstrap = 77%; FIG. 3). This group is characterized by having smooth basidiospores (except *A. botryosus*; FIG. 5). The segregate genera of *Aleurodiscus* s.l. represented in this group are discussed below.

Acanthophysellum is polyphyletic (FIGS. 2–4). The type species, *A. lividocoeruleum,* appears to be closely related to *Xylobolus,* but the remaining species, *A. cerussatus* and *A. lapponicus,* appear to be the sister group of *Aleurobotrys* (*A. botryosus*; FIGS. 2–4). *Aleurodiscus lapponicus* appears to be nested in *A. cerussatus,* and it is possible that the two are conspecific (FIGS. 2–5). The close relationship of *A. lividocoeruleus* and *Xylobolus* is surprising because *A. lividocoeruleus* produces a white rot (positive phenoloxidase reaction) and has clamped hyphae in the basidiocarps, whereas *Xylobolus* produces a white pocket rot (negative phenoloxidase reaction) and has clampless hyphae in the basidiocarps (FIG. 5).

Acanthofungus rimosus is closely related to *A. bisporus* and *Stereum* s.s. (FIGS. 2–4). *Acanthofungus* differs from *Stereum* in that it produces a white pocket rot and has clamped septa, whereas *Stereum* produces a white rot and has unclamped septa in the basidiocarp (FIG. 5). Taken together, the results for *Acanthophysellum, Acanthofungus, Stereum,* and *Xylobolus* suggest that both decay type (uniform white rot vs white pocket rot) and hyphal septation (clamped vs clampless) are evolutionarily labile (FIG. 5).

Aleurodiscus bisporus is closely related to *Stereum*

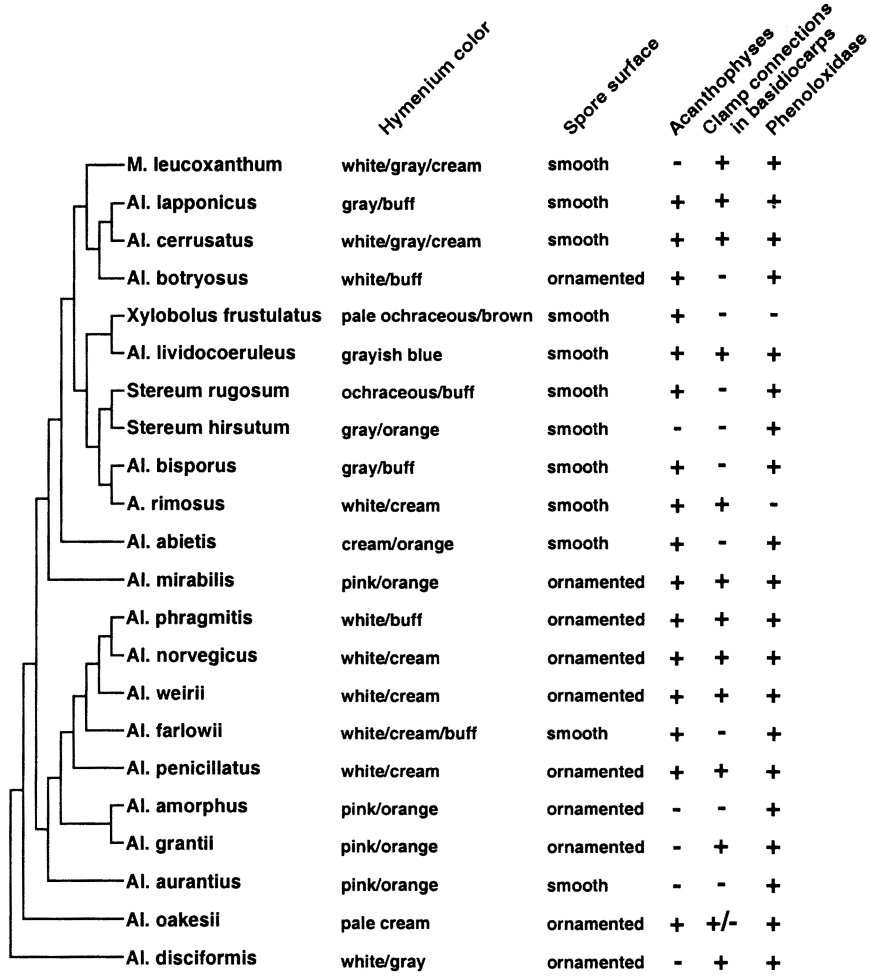

FIG. 5. Simplified tree showing taxa sampled in this study, with character states for hymenium color, spore ornamentation, acanthophyses, clamp connections in the basidiocarp, and phenoloxidase reactions, from personal observations and literature sources (Boidin 1950, 1958, Boidin et al 1968, Coates et al 1981, Rayner and Turton 1982, Boidin and Lanquetin 1984, Nakasone 1990, Wu 1996, Wu et al 2000).

and *Acanthofungus* (FIGS. 2–4), but this clade is not strongly supported (bootstrap = 54–64%; FIGS. 3, 4). Nevertheless, this result is not so surprising because *A. bisporus* has morphological characters that resemble those of *S. wakullum*. Basidiocarps of *S. wakullum* and *A. bisporus* are effuse, not stereoid, as is typical in *Stereum*. These two species also have gloeocystidia that are thick-walled except at the apex, which may be a modification of the skeletal-like cystidia that are common in *Stereum*. *Acanthofungus* and *A. bisporus* are weakly supported as the sister group of *Stereum* (FIGS. 3, 4). If *S. wakullum* is in this clade, then *Stereum*, as presently circumscribed, is polyphyletic (cf. Boidin et al 1998).

Analyses including sequences of Hallenberg and Parmasto (1998) suggest that *A. wakefieldae* is nested in *Stereum* (FIG. 4). A few other species in *Aleurodiscus* s.l. probably have a close relationship with *Stereum*, including *A. antarcticus* (Speg.) Ryvarden and *A. parmuliformis* G. Cunn., as suggested by Núñez and Ryvarden (1997).

Acanthophysellum appears to be polyphyletic, and *Stereum* is paraphyletic or polyphyletic (FIGS. 2–4), suggesting that considerable taxonomic changes are necessary. However, insufficient sampling of *Stereum*, *Acanthofungus*, and *Aleurodiscus* s.l., and a lack of topological robustness preclude making taxonomic changes. Therefore, for practical purposes, we will continue to recognize *Acanthophysellum* and *Acanthofungus*.

Aleurobotrys is characterized by having acanthophyses with coralloid and amyloid apical branches. So far, this genus includes only the type species *A. botryosus*. Núñez and Ryvarden (1997) mentioned that some other species of *Aleurodiscus* s.l. have acanthophyses with amyloid reactions to varying extents. Three species with amyloid acanthophyses, *A. botryosus*, *A. abietis*, and *A. farlowii*, included in this study

did not form a monophyletic group (FIGS. 2–4), which suggests that amyloid acanthophyses have evolved more than once. *Aleurodiscus botryosus* is the only species with ornamented spores among the monophyletic smooth-spored group. The distribution of character states on the tree (FIG. 5) suggests that the presence of ornamented spores in *A. botryosus* is due to a reversal.

Aleurodiscus mirabilis, A. penicillatus, and *A. wakefieldiae* have been placed in *Gloeosoma,* but they do not form a monophyletic group (FIGS. 2–4). The apparent close relationship between *A. wakefieldiae* and *Stereum,* based on the analysis including Hallenberg and Parmasto's (1998) sequence data (FIG. 4), is surprising because *A. wakefieldiae* differs from *Stereum* in having clamp connections in the basidiocarp and large, ornamented spores. Additional representatives of *A. wakefieldiae* are needed to confirm this result. *Aleurodiscus oakesii* resembles *Gloeosoma* but has both clamped and clampless septa. This species showed no distinct close relationship with any other studied species. *Aleurodiscus* s.s. probably represents a monophyletic group, but *Gloeosoma* does not.

Species with pinkish or orange-tinted hymenial surfaces occur in several different clades (FIG. 5). With our present sample of taxa, it appears that the plesiomorphic condition of the Stereaceae is to have gray to white hymenial surfaces, but that there have been multiple gains and (or) losses of pink to orange hymenial surfaces (FIG. 5). Other characters that have undergone multiple transformations include spore ornamentation, presence/absence of clamp connections, decay type (phenoloxidase reactions), and presence/absence of acanthophyses (FIG. 5). Data regarding nuclear behavior and sexuality in *Aleurodiscus* s.l. are lacking for many species, so they are not presented here. Nevertheless, previous reports, interpreted in the context of our results, suggest that these features also exhibit homoplasy in *Aleurodiscus* s.l. (e.g., Boidin 1958, Boidin et al 1968, Rayner and Turton 1982, Wu et al 2000).

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LITERATURE CITED

Boidin J. 1950. Sur la cytologie des *Stereum* Fr. en culture pure. C R Acad S. Paris 230:1096–1098.

———. 1958. Essai biotaxonomique sur les Hydnés résupinés et les Corticiés. Rev Mycol Mém Hors-Sér 6:1–388.

———. 1985. Contribution á la connaissance des Aleurodiscoideae á spores amyloïdes (Basidiomycotina, Corticiaceae) I. Introduction. Bull Soc Mycol France 101: 334–340.

———, Lanquetin P. 1984a. Répertoire des données utiles pour effectuer les tests d'intercompatibilité chez les Basidiomycètes. I. Introduction. Crypt Mycol 5:33–45.

———, ———. 1984b. Répertoire des données utiles pour effectuer les tests d'intercompatibilité chez les Basidiomycètes. III. Aphyllophorales non porées. Crypt Mycol 5:193–245.

———, ———, Gilles G. 1997. Le genre *Gloeocystidiellum* sensu lato (Basidiomycotina). Bull Soc Mycol France 113:1–80.

———, ———, ———, Candoussau F, Hugueney R. 1985. Contribution a la connaissance des Aleurodiscoideae a spores amyloides (Basidiomycotina, Corticiaceae). Bull Soc Mycol France 101:333–367.

———, Mugnier J, Canales R. 1998. Taxonomie moleculaire des Aphyllophorales. Mycotaxon 66:445–491.

———, Parmasto E, Dhingra GS, Languetin P. 1979. Stereums with acanthophyses, their positions and affinities. Persoonia 10:311–324.

———, Terra JP, Lanquetin P. 1968. Contribution á la connaissance des caractères mycéliens et sexuels des genres *Aleurodiscus, Dendrothele, Laeticorticium* et *Vuilleminia* (Basidiomycètes, Corticiaceae). Bull Soc Mycol France 84:53–84.

Coates D, Rayner ADM, Todd NK. 1981. Mating behaviour, mycelial antagonism and the establishment of individuals in *Stereum hirsutum.* Trans Br Mycol Soc 76:41–51.

Donk MA. 1964. A conspectus of the families of Aphyllophorales. Persoonia 3:199–324.

Eriksson J, Ryvarden L. 1973. The Corticiaceae of North Europe. Vol. 2. *Aleurodiscus-Confertobasidium.* Oslo: Fungiflora. 546 p.

Ginns J, Freeman GL. 1994. The Gloeocystidiellaceae (Basidiomycota: Hericiales) of North America. Bibl Mycol 157:1–120.

———, Lefebvre MNL. 1993. Lignicolous corticioid fungi (Basidiomycota) of North America. Mycol Mem 19:1–248.

Hallenberg N, Parmasto E. 1998. Phylogenetic studies in species of Corticiaceae growing on branches. Mycologia 90:640–654.

Hibbett DS, Donoghue MJ. 2000. Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. Syst Biol 49: (In press).

———, Gilbert L-B, Donoghue MJ. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature 407:506–508.

———, Pine EM, Langer E, Langer G, Donoghue MJ. 1997. Evolution of gilled mushrooms and puffballs inferred

from ribosomal DNA sequences. Proc Nat Acad Sci USA 94:12002–12006.

———, Thorn RG. 2001. Basidiomycota: Homobasidiomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The mycota. Vol. VII. Systematics and evolution. Berlin, Germany: Springer-Verlag. p 121–168.

Hjortstam K. 1997. A checklist to genera and species of corticioid fungi (Basidiomycotina, Aphyllophorales). Windahlia 23:1–54.

Lemke A. 1964. The genus *Aleurodiscus* (sensu stricto) in North America. Can J Bot 42:213–281.

Maddison DR. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Syst Zool 40: 315–328.

Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst Biol 49:278–305.

Nakasone KK. 1990. Cultural studies and identification of wood-inhabiting Corticiaceae and selected Hymenomycetes from North America. Mycol Mem 15:1–412.

Núñez M, Ryvarden L. 1997. The genus *Aleurodiscus* (Basidiomycotina). Synop Fung 12:1–164.

Parmasto E. 1986. On the origin of the Hymenomycetes (what are corticioid fungi?) Windahlia 16:3–19.

———. 1995. Corticioid fungi: a cladistic study of a paraphyletic group. Can J Bot 73:S843–S852.

Rayner ADM, Turton MN. 1982. Mycelial interactions and population structure on the genus *Stereum*: *S. rugosum, S. sanguinolentum* and *S. rameale*. Trans Br Mycol Soc 78:483–493.

Swofford DL. 1999. PAUP* 4.0 Phylogenetic analysis using parsimony, version 4.0b. Sunderland, Massachusetts: Sinauer Associates, Inc.

Wu SH. 1996. Studies on *Gloeocystidiellum* sensu lato (Basidiomycotina) in Taiwan. Mycotaxon 58:1–68.

———, Boidin J, Chien CY. 2000. *Acanthofungus rimosus* gen. et sp. nov., with reevaluation of the related genera. Mycotaxon 76:153–161.