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Relationships among *Amylostereum* species associated with siricid woodwasps inferred from mitochondrial ribosomal DNA sequences

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Abstract: The genus *Amylostereum* currently includes four species, namely *A. areolatum*, *A. chailletii*, *A. laevigatum* and *A. ferreum*. Two of these species, *A. areolatum* and *A. chailletii*, are well known for their association with siricid woodwasps. Despite much interest in these fungus-woodwasp symbioses, the taxonomy and phylogeny of this genus received little attention in the past. The aim of this study was to investigate the phylogenetic relationship between the four species of *Amylostereum*. The placement of *Amylostereum* spp. among the Basidiomycetes was also investigated based on mt-SSU-rDNA sequence analyses. These data also clarify the taxonomic status of previously unidentified isolates. In this study, we have shown that *A. areolatum* is more distantly related to the three other species of *Amylostereum*, than they are to each other. Of the remaining three species, *A. ferreum* and *A. laevigatum* are more closely related to each other. One isolate that was collected from *Sirex areolatus*, and, therefore, expected to be *A. chailletii*, was more closely related to *A. laevigatum* and *A. ferreum*. As neither of the latter species have been implicated in associations with woodwasps, this finding warrants further investigation. Our data show that *Amylostereum* spp. group with neither *Stereum* nor *Peniophora*, as has been previously hypothesised, but rather with *Echinodontium tinctorium*. From this and other studies there was also an obvious relationship between *Amylostereum/Echinodontium* and *Russula*.

Key Words: mt-SSU-rDNA, phylogeny, symbiosis

INTRODUCTION

Members of the genus *Amylostereum* are best known for their mutualistic association with Siricidae, a family of woodwasps with a woodboring larval state (Talbot 1977). These woodwasps and their associated fungi have the potential to cause serious damage and mortality to various conifers such as *Pinus*, *Abies*, *Picea*, *Pseudotsuga* (Spradbery and Kirk 1978, 1981). In the Northern Hemisphere where woodwasps originate, a natural balance exists between them, their natural parasites and their host trees, such that they are generally considered as secondary invaders (Hall 1978, Spradbery and Kirk 1978).

The *Sirex noctilio-Amylostereum areolatum* complex has been introduced into a number of countries in the Southern Hemisphere where it causes severe damage to exotic pine plantations (Neumann and Marks 1990, Chou 1991, Bedding 1995). In these regions, this pest complex is considered a primary problem. A combination of the environmental stresses on pine trees, the genetic uniformity of these plantations and the absence of natural enemies of *Sirex* have all contributed to the increase in pathogenicity of this wasp-fungus association in the Southern Hemisphere (Spradbery 1973, Spradbery and Kirk 1978).

Boidin (1958) first described the genus *Amylostereum* as distinct from species of *Stereum* and *Peniophora*. General morphological characters include smooth amyloid basidiospores, brown encrusted cystidia and regular simple clamps. *Amylostereum chailletii* (Pers.:Fr.) Boid., the type species, and *A. areolatum* (Fr.) Boid. are the only two species of *Amylostereum* implicated in associations with woodwasps (Gaut 1970, Boidin and Lanquetin 1984). Both species were initially included in the genus *Stereum* as *S. chailletii* (Pers.:Fr. as *Thelophora*) Fr. and *S. areolatum* (Fr.:Fr. as *Thelephora*) Fr. respectively (Boidin 1958). *Amylostereum chailletii* and *A. areolatum* are morphologically very similar, but can be distinguished in culture based on the fact that only *A. areolatum* forms arthrospores in culture (Thomsen 1998).

The third species described by Boidin (1958) in the genus *Amylostereum*, *A. laevigatum* (Fr.) Boid., was known as *Peniophora laevigata* Fr. (as *Thelephora*) Karst. and later as *S. juniperi* (Karst.) Boid. *Amylostereum laevigatum* is also found in softwood trees, predominantly species of *Juniperus*. This species differs from *A. chailletii* and *A. areolatum* in the absence of horizontal hyphae in the fruiting structures, as well

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as in the fact that it has a monomitic hyphal system (Breitenbach and Kränzlin 1986).

Boidin and Lanquetin (1984) described *A. ferreum* (Berk. & Curt.) Boid. & Lanq. (= *Stereum ferreum*) as a fourth species in the genus *Amylostereum*. A major difference between *A. ferreum* and the three other species of *Amylostereum* is its occurrence exclusively in *Podocarpus* species. Unlike the other three species that are known from the Northern Hemisphere, *A. ferreum*, has been found only in South America (Boidin and Lanquetin 1984).

Boidin and Lanquetin (1984) evaluated the genus *Amylostereum* based on mating studies and the Buller phenomenon (Buller 1931). They concluded that *A. chailletii*, *A. laevigatum* and *A. ferreum* are more closely related to each other than they are to *A. areolatum*. No positive mating reactions were observed between *A. areolatum* and the other three species. No compatible mating was observed between *A. chailletii* and *A. laevigatum*, but *A. ferreum* gave partially compatible crosses with both these species. Boidin and Lanquetin (1984) also hypothesised that, based on morphological evidence, the genus *Amylostereum* could be more closely related to *Peniophora* than to *Stereum*.

Morphological studies of the Basidiomycetes are complicated by the limited number of available characters, as well as the influence of convergent and parallel evolution (Hibbett et al 1997). For example, in a study of 89 Basidiomycete species, using sequence data from the nuclear and mitochondrial small subunit rRNA operon, Hibbett et al (1997) showed that a major character such as gills might have evolved six times. Similarly various researchers have used the combined features of conserved and less conserved regions in the rRNA genes to resolve problematic phylogenetic and taxonomic questions in the Basidiomycetes, often in conjunction with morphological data (Hibbett and Vilgalys 1991, 1993, Hibbett 1992, Swann and Taylor 1993, 1995, Zambino and Szabo 1993, Hibbett and Donoghue 1995, Hsiau 1996).

The aim of this study was to test the hypotheses of Boidin and Lanquetin (1984) as well as other researchers regarding the phylogenetic relationships among the different species of *Amylostereum*, based on part of the mitochondrial ribosomal gene complex. In addition, relationships among species of *Amylostereum* and other Basidiomycetes are also considered. The taxonomic status of isolates of unknown or uncertain identity is also investigated using these data.

MATERIALS AND METHODS

Fungal isolates.—Isolates used in this study were obtained from a variety of sources (TABLE I). These include those made from *S. noctilio* collected in South Africa and Brazil, those from cultures of the parasitic nematode *Deladenus siricidicola*, isolates from Europe supplied by Dr. I. M. Thomson (Danish Forest and Landscape Research Institute, Hoersholm, Denmark), Dr. R. Vasiliauskas (Swedish University of Agricultural Sciences, Uppsala, Sweden), those from culture collections CBS (Centraal Bureau voor Schimmelcultures, Baarn, Netherlands) and DAOM (Centre for Land and Biological Resources Research, Canada). Isolates were maintained on MYA (2% malt extract, 0.2% yeast extract and 1.5% agar) at 25 C and stored in McCartney bottles containing MYA at 4 C.

DNA techniques.—Mycelium from actively growing cultures on MYA was used to inoculate liquid MY (2% malt extract and 0.2% yeast extract) medium (100 mL in 250 mL Erlenmeyer flasks). These were incubated at 25 C on a shaker for ca 2 wk. A modification of the method of Raeder and Broda (1985) was used for isolating DNA from mycelium. Unlike the Raeder and Broda (1985) method, each sample was divided into two equal amounts for the whole extraction procedure, after cell debris had been removed. Furthermore, the phenol chloroform extraction (1:1 phenol to chloroform) step was repeated several times until the interphase between the aqueous and upper phases was clean from contaminating proteins and cell debris. Precipitation of the nucleic acids was done using 3 M NaAc (0.1 v/v) and isopropanol (0.6 v/v) and was incubated overnight at −20 C. After centrifugation, to harvest the nucleic acids, and washing with 70% ethanol, the pellet was resuspended in 200 µL sterile water. The two samples of each isolate were then combined. One µL RNaseA (10 mg/mL) was added to the resuspended sample and left at 37 C overnight to degrade all RNA in the sample. DNA concentrations were subsequently determined using an UV spectrophotometer (Beckman Du Series 7500) (Maniatis et al 1982).

PCR amplification and purification. A portion of the mitochondrial small sub-unit ribosomal RNA gene (mt-SSU-rDNA) was amplified with the primers MS1 and MS2 (White et al 1990) using the Polymerase Chain Reaction (PCR). PCR was performed using the Expand® High Fidelity Polymerase System (Roche Diagnostics, South Africa). Total volumes of the reaction mixtures varied among 50 µL, 75 µL, and 100 µL. The reaction mixture consisted of a final concentration of 2.65 mM $MgCl_2$, 200 µM of each of the four dNTP, Expand High Fidelity buffer, 0.375 µM of each of the two primers and 2.6 U Expand® High Fidelity Taq polymerase mixture. Extracted genomic DNA (50–80 ng) was used as template for the amplification reactions.

PCR reactions were performed on a Hybaid TouchDown PCR machine (Hybaid Limited, UK). Reaction conditions included an initial denaturation step of 3 min at 94 C followed by 10 cycles of denaturation at 94 C for 15 s, primer annealing at 55 C for 45 s and elongation at 72 C for 1 min. This was followed by 20 cycles using the same reaction conditions, but with an increase of 20 s elongation time per cycle. A final elongation step at 72 C for 7 min ensured complete elongation of the amplification product. PCR products were subjected to electrophoresis on an 1% (wt/v) ethidium bromide stained agarose gel and visualised un-

TABLE I. Isolates of *Amylostereum* used in this study

CMW[a]	Original No.	Identity	Host or source of isolation	Origin	Date	Isolator
2905	CBS 483.83	*Amylostereum chailletii*	Mycangium of woodwasp *Urocerus gigas*	Scotland, UK	1981	D. B. Redfern
3309	CBS 305.82	*A. areolatum*	Unknown	France	1964	J. Boiden
3310	CBS 334.66	*A. areolatum*	From *Picea abies*	Germany	1967	Dimitri
3311	CBS 624.84	*A. laevigatum*	*Juniperus nana*	France	1978	P. Lanquetin
3045	CBS 633.84	*A. ferreum*	*Podocarpus lambertii*	Brazil	1978	R. T. Guerrero
3295	DAOM 21327	*A. chailletii*	Sporophore on *Abies balsamea*	Ontario, Canada	1948	R. F. Cain
3296	Stillwell 309(3)	*Amylostereum* sp.	Mycangium of *Sirex areolatus*	California, U.S.A.	Unknown	Stillwell
3298	Waite Inst. 6195	*Amylostereum* sp.	Mycangium of *S. noctilio*	Tasmania	1962	Unknown
3301	DAOM 21785	*Amylostereum* sp.	Wood of *P. radiata* around oviposition bores of *S. noctilio*	New Zealand	Unknown	G. B. Rawlings
3302	54-95	*A. chailletii*	Sporophore on fallen log in stand of hemlock conifers	Ontario, Canada	1954	A. Hill and S. Gibson
4629	Sc 62.8	*A. chailletii*	Fruiting body on *Picea sitchensis*	Scotland, U.K.	1981	D. B. Redfern
4631	L234	*A. chailletii*	Wood of wounded *P. abies*	Lithuania	1995	R. Vasiliauskas
4632	L204	*A. areolatum*	Wood of wounded *P. abies*	Lithuania	1994	R. Vasiliauskas
4636	DK37	*A. areolatum*	Fruiting body on *P. abies*	Denmark	1993	I. M. Thomson
4641	S225	*A. areolatum*	Wood of wounded *P. abies*	Sweden	1994	R. Vasiliauskas
4644	A3	*Amylostereum* sp.	Isolates from nematode cultures from CSIRO	Australia	1995	B. Slippers
4650	Br 38	*Amylostereum* sp.	Mycangia of *S. noctilio*	Brazil	1997	B. Slippers
4658	M5W	*Amylostereum* sp.	Wood around *S. noctilio* in *P. radiata*	South Africa	1994	M. J. Wingfield
4659	SN19A	*Amylostereum* sp.	Mycangia of *S. noctilio*	South Africa	1996	B. Slippers

[a] Culture collection of the Tree Pathology Co-operative Programme at the Forestry and Agricultural Biotechnology Institute, University of Pretoria.

der UV illumination. Size estimates of the PCR fragments were done using a 100 bp ladder (Promega, Madison, Wisconsin) as a molecular weight marker.

DNA sequencing and sequence data analysis. DNA sequencing of the amplified mt-SSU-rDNA was performed on an ABI PRISM® 377 automated DNA sequencer. PCR products were purified prior to sequencing, using a Nucleon®QC PCR/OLIGO clean up kit (Amersham Life Science Inc.). Thermo Sequenase® dye terminator cycle sequencing pre-mix kit (Amersham Life Science Inc.) was used for all sequencing reactions. The primers MS1 and MS2 were used to sequence both DNA strands.

To determine the phylogenetic relationships amongst *Amylostereum* species, mt-SSU-rDNA sequences of all isolates (TABLE I, GenBank AF238446-AF238464) were manually aligned by inserting gaps. Alignments are deposited in TreeBASE (SN448). All characters were given equal weight and gaps were coded as newstate (fifth character). Analysis of the data was done using PAUP (Phylogenetic Analysis Using Parsimony) version 3.1.1 (Swofford 1993). Heuristic searches using TBR (Tree Bisection Reconstruction) branch swapping and MULPAR on, were done to determine the most parsimonious relationships between the taxa. Strict and semistrict consensus trees were obtained in PAUP for all equally parsimonious trees saved. Trees were not rooted to an outgroup taxon. Branch supports and confidence intervals were determined using BOOTSTRAP analysis (1000 replicates) (Felsenstein 1993).

In order to consider the relationship of *Amylostereum* spp. with other Basidiomycetes, sequence data of the mt-ssu-rDNA for 89 species of Basidiomycetes (Hibbett and Donoghue 1995, Hibbett et al 1997) were obtained from TreeBASE. Sequence data for *A. chailletii* were initially compared to all 89 species using PAUP to resolve a clade of maximum relationship. Sequence data from the most closely related taxa determined using this analysis, were then compared to DNA sequence data of all four described species of *Amylostereum*. Sequence analysis was done using PAUP, as described above, except that all resulting trees were rooted to an outgroup taxon. Here, *Laxitextum bicolor* (Fr.) Lentz. was chosen as an outgroup because of its basal relationship to the taxa selected as closely related to *Amylostereum* in the analysis of Hibbett et al (1997).

RESULTS

The region of the mt-SSU-rRNA gene targeted with the MS1 and MS2 primers was highly conserved in all the species of *Amylostereum*, based on the size of the amplified PCR fragments. Fragments of ca 570 bp were amplified from all but three isolates used in this study. The three exceptions, isolates Stillwell 309(3), CBS 624.84 (*A. laevigatum*) and CBS 633.84 (*A. ferreum*), produced PCR amplification fragments of ca 590 bp.

Manual alignment of sequences representing the amplified region of the mt-SSU-rDNA of the different species of *Amylostereum* resulted in the total alignment of 538 characters. Absolute lengths of the sequences ranged from 518 bp to 537 bp. Sequences of the above-mentioned region were highly conserved for all the species of *Amylostereum*. One variable region was observed between 190 and 226 bp (aligned length) of the fragment.

Heuristic searches using PAUP of these sequences resulted in 18 equally parsimonious trees (CI = 0.968, HI = 0.032, RI = 0.986) of 31 steps each (FIG. 1). The topology of these trees was similar and differences were due to variations in branch length and the arrangement among isolates CBS624.84 (*A. laevigatum*), CBS633.84 (*A. ferreum*) and Stillwell 309(3) (isolated from *S. areolatus*).

The main feature of the trees obtained from heuristic searches of sequence data of the different *Amylostereum* spp., was the appearance of two major groups supported by a 100% confidence interval at the branching point. The one group contained representative isolates of *A. areolatum*. Within the *A. areolatum* group only one branch was retained in consensus trees that was weakly supported by bootstrap analysis (65%). The second main group was comprised of representative isolates of *A. chailletii*, *A. laevigatum*, *A. ferreum* and isolate Stillwell 309(3). *A. chailletii* grouped on a separate branch (93% confidence interval) within this second group from *A. laevigatum*, *A. ferreum* and isolate Stillwell 309(3). *A. ferreum*, Stillwell 309(3) and *A. laevigatum* were grouped together and basal to *A. chailletii* in strict and semistrict consensus trees, as well as by bootstrap analysis. Therefore, a revised form of the evolutionary tree of decent reported by Boidin and Lanquetin (1984) (FIG. 2a), is proposed (FIG. 2b).

Manual alignment of sequence data of 16 selected species from the data set of Hibbett et al (1997) and the four species of *Amylostereum* resulted in a total aligned data set of 771 characters. Absolute values varied from 513 bp for *Russula compacta* Frost to 674 bp for *Peniophora nuda* (Fr.) Bres. Sequences could be divided into four relatively conserved regions, interspersed with three hypervariable regions, as was reported by other researchers (Hibbett and Donoghue 1995, Hsiau 1996, Hibbett et al 1997). The three hypervariable regions were located between bases 55 and 128, bases 266 and 400 and bases 623 and 671 (based on aligned values).

Alignment in these hypervariable regions was difficult and often impossible. This resulted in a large amount of ambiguity in their alignment. Analysis of the data was thus performed with and without these hypervariable regions. In the latter case, this resulted in the exclusion of 258 bp (aligned values). The general topology of the trees showed some variation compared to the trees resulting from analysis of the

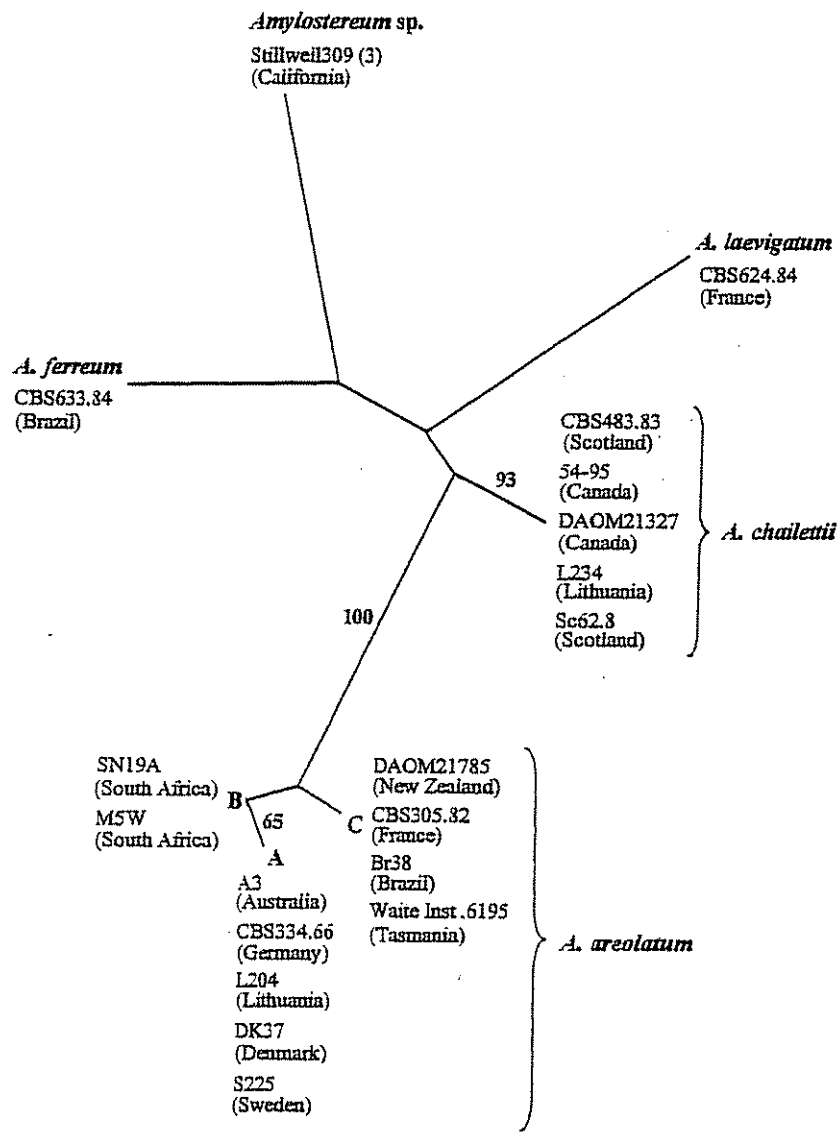

FIG. 1. One of the most parsimonious trees obtained by heuristic searches of the sequence data of the mt-SSU-rDNA for isolates representing the different species of *Amylostereum* (TABLE I). The length of the tree = 31 steps, CI = 0.968, HI = 0.032 and RI = 0.986. Bootstrap values (1000 replicates) are given at the branching points.

full sequence, but most of the species groupings were not affected.

Heuristic searches of the full sequence data set resulted in three equally parsimonious trees of 1495 steps (CI = 0.601, HI = 0.399, RI = 0.522) (FIG. 3). The topology of the trees were the same except for variations in branch lengths and whether *A. laevigatum* and *A. ferreum* were put on separate branches or not. Seven most parsimonious trees of 639 steps (CI = 0.604, HI = 0.396, RI = 0.595) were obtained when analysis were conducted on the DNA sequences with the variable regions excluded (FIG. 4). Differences in the seven trees could again be ascribed to variation in branch lengths.

The four species of *Amylostereum* formed a monophyletic clade that is the sister group of *Echinodontium tinctorium* Ell. & Ev. Monophyly of *Amylostereum* was supported by a 98% bootstrap value irrespective of the inclusion or exclusion of the hypervariable regions. The bootstrap branch support for the *Echinodontium–Amylostereum* grouping was 70% when the hypervariable regions were included and 94% when they were excluded. *Heterobasidion annosum* (Fr.) Bref. and *R. compacta* grouped together and neighboring the group that contained *Echinodontium* and *Amylostereum* spp. *Lentinellus omphalodes* (Fr.) Kar. and *L. ursinus* (Fr.) Kühn., *Auriscalpium vulgare* S. F. Gray, *Clavicorona pyxidata* (Fr.) Doty and *Hericium ramosum* (Bull. ex Mér) Let. were also grouped close to *Echinodontium*, *Amylostereum*, *Heterobasidion* and

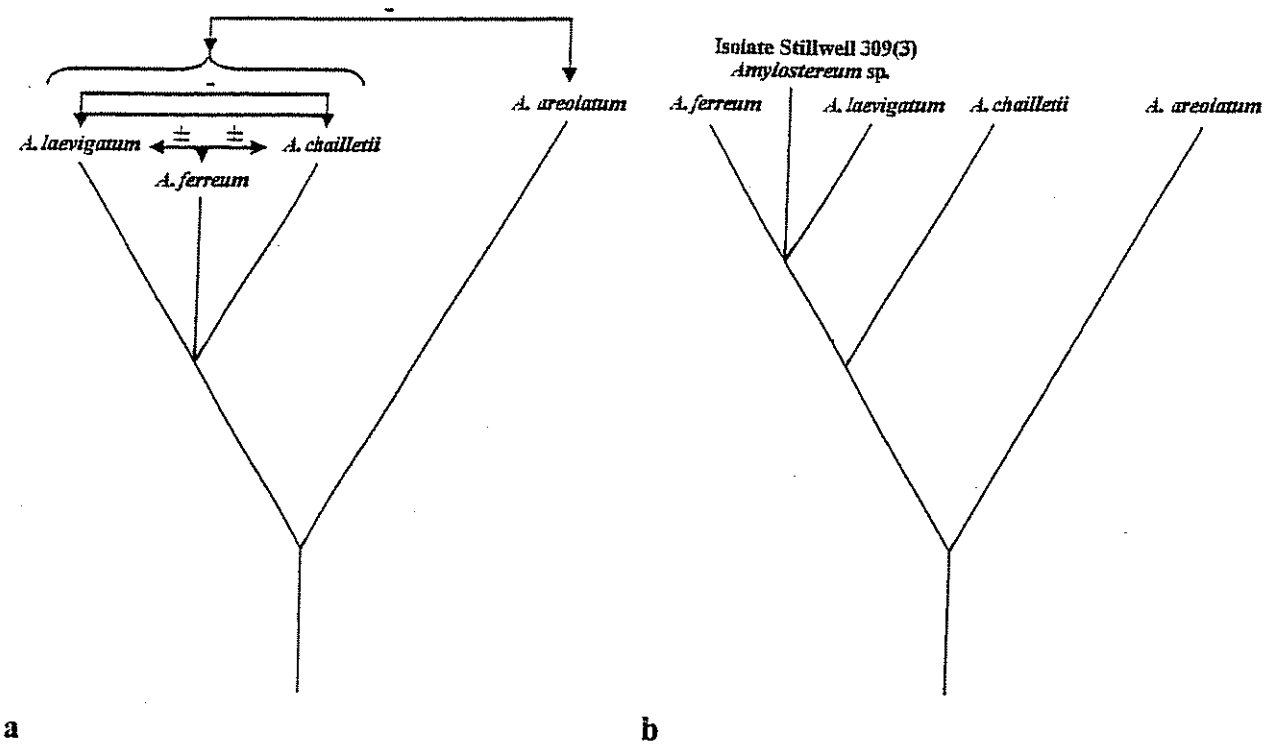

FIG. 2. Trees of descent of *Amylostereum* spp. a. Tree reported by Boidin and Lanquetin (1984). This tree is based on mating behavior between the four *Amylostereum* spp. The results of their mating studies between the *Amylostereum* spp. are indicated here as sexually incompatible (−) or partially compatible (±). b. Tree indicated by the results from the present study based on mt-SSU-rDNA sequence data analysis.

Russula in analysis of the data set without exclusion of the hypervariable regions. In analyses ignoring the sequence of the hypervariable regions, *Hericium* and *Clavicorona* were removed from this group. *Heterobasidion* and *Russula* also grouped closer to *Lentinellus* and *Auriscalpium* spp. than to the *Echinodontium–Amylostereum* group in this analysis. Neither *Stereum* nor *Peniophora* spp. were in the above-mentioned groups in any of the analyses. Instead, *Stereum* spp. were grouped with *Gloeocystidiellum leucoxantha* (Bres.) Boid. and *P. nuda* with *Scitinostroma alutum* Lanq. in all trees.

Differences in the topologies of trees derived when including and excluding hypervariable data occurred at branches that were not supported or only weakly supported by Bootstrap values. Well supported branches were unaffected by different analyses. Unsupported branches also accounted for the topological variation between trees derived in this study and those reported by Hibbett et al (1997), from which some of the sequences were obtained.

DISCUSSION

The phylogenetic relationships of the four species of *Amylostereum* could be resolved in this study using sequence data of the mt-ssu-rDNA. Isolates representing *A. areolatum* clustered on a well supported branch, separate from all the other species in the genus. Vasiliauskas et al (1999), using internal transcribed spacer sequences of the ribosomal DNA, also report that *A. laevigatum* and *A. chailletii* are more closely related to each other than to *A. areolatum*. This is consistent with the hypothesis of Boidin and Lanquetin (1984) that *A. areolatum* is the most clearly defined species in the genus. In their study, no mating compatibility was observed between isolates of *A. areolatum* and any of the other *Amylostereum* species in this group, whereas partial compatibility was observed between some of the other species of *Amylostereum*.

Boidin and Lanquetin (1984) could not clearly define the relationship between *A. chailletii, A. laevigatum* and *A. ferreum*. In their study, European isolates of *A. chailletii* and *A. laevigatum* showed no mating compatibility, but both these species showed partial mating compatibility with *A. ferreum*. Our analysis showed that *A. chailletii, A. laevigatum* and *A. ferreum* formed a cluster separate from *A. areolatum*, which is in agreement with their mating studies. Isolates of *A. chailletii* formed a separate group within the latter group, while *A. laevigatum* and *A. ferreum* could not

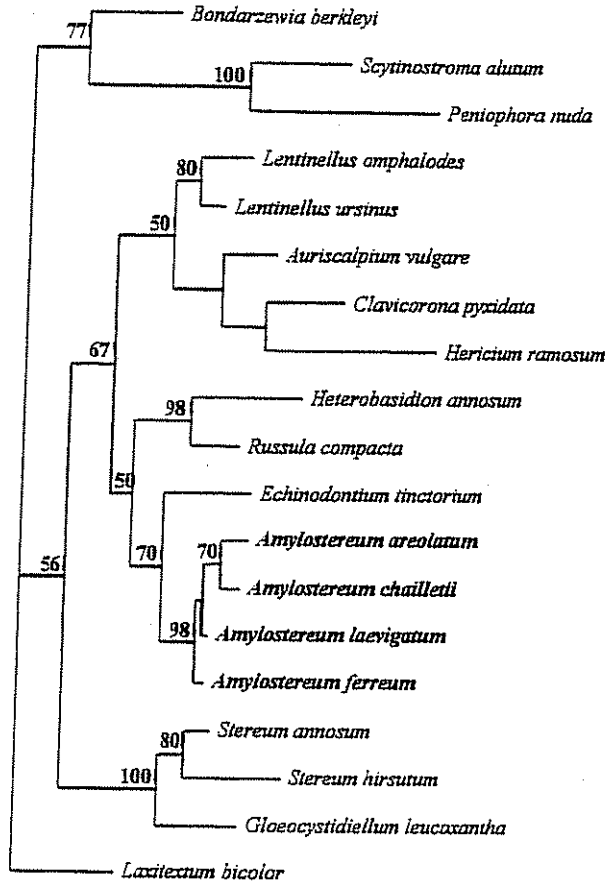

FIG. 3. One of the most parsimonious trees obtained by heuristic searches of the full sequence data set (including the hypervariable regions) (tree length = 1495 steps, CI = 0.601, HI = 0.399 and RI = 0.522).

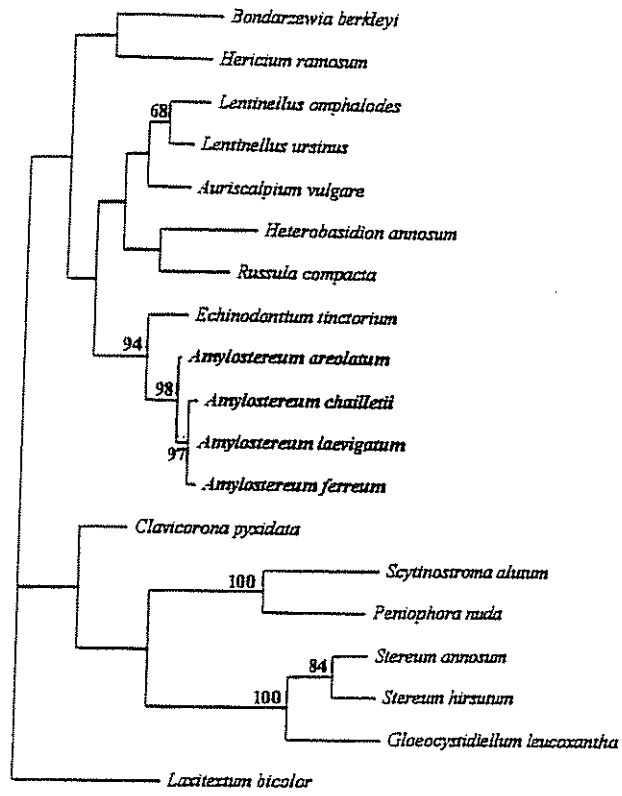

FIG. 4. One of the most parsimonious trees where the hypervariable regions were excluded (tree length = 639 steps, CI = 0.604, HI = 0.396 and RI = 0.595) of the mt-SSU-rDNA for 19 different Basidiomycetes spp, including the four *Amylostereum* spp. Bootstrap values (1000 replicates) are given at the branching points.

be separated in strict analyses of the data. These results suggest a closer relationship between *A. ferreum* and *A. laevigatum* than between either of these species and *A. chailletii*.

We confirmed the identity of isolates of *Amylostereum* that could previously not be assigned species names. The two CLBBR cultures identified only as *Amylostereum* sp. (Waite Inst 6195 and DAOM 21785) from Tasmania and New Zealand, clearly resided in the group containing identified isolates of *A. areolatum* (CBS 305.82, CBS 334.66 and isolates from Europe that were identified by Drs. Thomsen and Vasiliauskas). Also represented in this group are isolates from South Africa, Brazil and isolates obtained from nematode (*Deladenus siricidicola*) cultures imported to South Africa from Australia. Furthermore, the two Canadian isolates of *A. chailletii* (DAOM 21327 and 54-95) clearly clustered with other identified isolates of *A. chailletii* (L234, Sc62.8 and CBS 483.83). Boidin and Lanquiten (1984) found partial mating compatibility between two Canadian *Amylostereum* isolates and authentic isolates of *A. chailletii*, *A. laevigatum* and *A. ferreum*. According to our data this mating behavior, therefore, only supports the close relationship between these three species.

Isolate Stillwell 309(3) is reported to have been isolated from the mycangium of *S. areolatus*. Therefore, we would expect it to be *A. chailletii* as was suggested by Gaut (1970). This isolate was deposited in DAOM as an *Amylostereum* sp. Results of this study show that the isolate is most closely related to *A. laevigatum* and *A. ferreum*. Neither of these species have previously been implicated in associations with woodwasps. If this isolate is an actual sub-culture of the isolate collected from *S. areolatus*, it might represent a link between the species associated with woodwasps (*A. areolatum* and *A. chailletii*) and the other two species (*A. laevigatum* and *A. ferreum*). It might also represent an undescribed species of *Amylostereum*. Further study of this isolate is clearly warranted.

Various hypotheses have been proposed for the placement of *Amylostereum* amongst the Basidiomycetes. Boidin and Lanquetin (1984) speculated that *Amylostereum* might be more closely related to *Peniophora* based on the presence of gloeocystidia posi-

tive in sulfuric-aldehyde, normal nuclear behavior and the tetrapolarity in all four species. In a cladistic study using 86 morphological characters, Parmasto (1995) reduced the Stereaceae to synonymy with the Peniophoraceae. In this analysis, *A. chailletii* groups sister to *Stereum* and *Xylobolus* (P. Karst.) and the former three genera form a group basal to the group that contains the genus *Peniophora*. Hallenberg and Parmasto (1998), however, conclude from a parsimony analysis of morphological and molecular rDNA data that *Amylostereum* is a sister genus of *Peniophora*. In a study by Hsiau (1996) using mt-ssu-rDNA, *A. chailletii* grouped sister to *Stereum* and further away from *Peniophora*. Boidin (1998), using ITS rDNA sequence data, however, proposed a new family, Amylostereaceae, which groups sister to *Echinodontium tinctorium* (Echinodontiaceae), a genus that was not included in any previous analyses.

In the present study the four *Amylostereum* spp. formed a monophyletic group that is sister to neither *Stereum* nor *Peniophora*, but to *E. tinctorium*. This observation was supported by strong bootstrap values for this grouping in all analyses. It is interesting to note that *E. tinctorium* is also characterised by amyloid basidiospores and encrusted cystidia, such as those formed by *Amylostereum* spp.

Echinodontium tinctorium has been described as closely related to *Stereum* (Gross 1964, Stalpers 1978). Hibbett et al (1997) and Hibbett and Donghue (pers comm), however, found that *E. tinctorium* is more closely related to *Peniophora nuda* than to any of the *Stereum* spp. included in their analyses. Boidin (1998) found that *Amylostereum* and *Echinodontium* grouped most closely to *Boidinia* and *Gloeocystidiellum* and that this group is more closely related to *Stereum* than to *Peniophora*. In the present study, the *Amylostereum-Echinodontium* group was, however, most closely related to *Russula, Heterobasidion, Lentinellus* and *Auriscalpium* in all analyses. These genera grouped more closely to *Stereum* and *Gleocystidiellum* than to *Peniophora* when the hypervariable region was included, but were separated from both *Stereum* and *Peniophora* when these regions were excluded. Our data thus support the hypothesis of Hibbett and Donoghue (pers comm) that places these genera, including *Amylostereum*, together in a 'russuloid clade,' but could not infer the ancestral relationship of *Amylostereum* to *Stereum* and *Peniophora*.

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