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

Bernard Slippers¹
Michael J. Wingfield
Brenda D. Wingfield
Teresa A. Coutinho

*Forresty and Agricultural Biotechnology Institute, Department of Microbiology and Plant Pathology, Faculty of Biological and Agricultural Sciences, University of Pretoria, Pretoria, 0002, South Africa*

Abstract: The genus *Amylostereum* currently includes four species, namely *A. areolatum*, *A. chailletii*, *A. laevisatum* 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. laevisatum* 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. laevisatum* and *A. ferreum*. As neither of the latter species have been implicated in associations with woodwasp, 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 woodwasp 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 woodwasp 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 woodwasp (Gaut 1970, Boidin and Lanquetin 1984). Both species were initially included in the genus *Stereum* as *S. chailletii* (Pers.:Fr. as *Thelephora*) 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. laevisatum* (Fr.) Boid., was known as *Peniophora laevisata* Fr. (as *Thelephora*) Karst. and later as *S. juniperi* (Karst.) Boid. *Amylostereum laevisatum* 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

Accepted for publication April 20, 2000.

¹ Email: bernard.slippers@fabi.up.ac.za
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 hypothesized 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 1995, 1995, Zambino and Szabo 1993, Hibbett and Donoghue 1995, Hsiu 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. nodiflora* collected in South Africa and Brazil, those from cultures of the parasitic nematode *Deladenus sricicola*, isolates from Europe supplied by Dr. I. M. Thomson (Danish Forest and Landscape Research Institute, Hoersholm, Denmark), Dr. R. Vassilias (Swedish University of Agricultural Sciences, Uppsala, Sweden), those from culture collections CBS (Centraal Bureau voor Schimmelcultures, Baarn, Netherlands) and DAOAM (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 inter-phase 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 (0.8 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 subunit 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 MgCl₂, 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 0.5 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>
<thead>
<tr>
<th>CMW*</th>
<th>Original No.</th>
<th>Identity</th>
<th>Host or source of isolation</th>
<th>Origin</th>
<th>Date</th>
<th>Isolator</th>
</tr>
</thead>
<tbody>
<tr>
<td>2905</td>
<td>CBS 483.83</td>
<td><em>Amylostereum chailettii</em></td>
<td>Mycangium of woodwasp <em>Urocerus gigas</em></td>
<td>Scotland, UK</td>
<td>1981</td>
<td>D. B. Redfern</td>
</tr>
<tr>
<td>3309</td>
<td>CBS 305.82</td>
<td><em>A. areolatum</em></td>
<td>Unknown</td>
<td>France</td>
<td>1964</td>
<td>J. Boiden</td>
</tr>
<tr>
<td>3310</td>
<td>CBS 334.66</td>
<td><em>A. areolatum</em></td>
<td>From <em>Picea abies</em></td>
<td>Germany</td>
<td>1967</td>
<td>Dimitri</td>
</tr>
<tr>
<td>3311</td>
<td>CBS 924.84</td>
<td><em>A. laevigatum</em></td>
<td><em>Juniperus nana</em></td>
<td>France</td>
<td>1978</td>
<td>P. Lanquetin</td>
</tr>
<tr>
<td>3045</td>
<td>CBS 633.84</td>
<td><em>A. ferreum</em></td>
<td><em>Podosarpus lambertii</em></td>
<td>Brazil</td>
<td>1978</td>
<td>R. T. Guerrero</td>
</tr>
<tr>
<td>3295</td>
<td>DAOM 21327</td>
<td><em>A. chailettii</em></td>
<td>Sporophore on <em>Abies balsamea</em></td>
<td>Ontario, Canada</td>
<td>1948</td>
<td>R. F. Guin</td>
</tr>
<tr>
<td>3296</td>
<td>Stillwell 309(3)</td>
<td><em>Amylostereum sp.</em></td>
<td>Mycangium of <em>Sirex aralidus</em></td>
<td>California, U.S.A.</td>
<td>Unknown</td>
<td>Stillwell</td>
</tr>
<tr>
<td>3298</td>
<td>White Inst. 6195</td>
<td><em>Amylostereum sp.</em></td>
<td>Mycangium of <em>S. noctilio</em></td>
<td>Tasmania</td>
<td>1962</td>
<td>Unknown</td>
</tr>
<tr>
<td>3301</td>
<td>DAOM 21785</td>
<td><em>Amylostereum sp.</em></td>
<td>Wood of <em>P. radiata</em> around oviposition holes of <em>S. noctilio</em></td>
<td>New Zealand</td>
<td>Unknown</td>
<td>G. B. Rawlings</td>
</tr>
<tr>
<td>3302</td>
<td>54-95</td>
<td><em>A. chailettii</em></td>
<td>Sporophore on fallen log in stand of hemlock conifers</td>
<td>Ontario, Canada</td>
<td>1954</td>
<td>A. Hill and S. Gibson</td>
</tr>
<tr>
<td>4629</td>
<td>Sc 62.8</td>
<td><em>A. chailettii</em></td>
<td>Fruiting body on <em>Picea stichensis</em></td>
<td>Scotland, U.K.</td>
<td>1981</td>
<td>D. B. Redfern</td>
</tr>
<tr>
<td>4631</td>
<td>L234</td>
<td><em>A. chailettii</em></td>
<td>Wood of wounded <em>P. abies</em></td>
<td>Lithuania</td>
<td>1996</td>
<td>R. Vasiliuksas</td>
</tr>
<tr>
<td>4632</td>
<td>L204</td>
<td><em>A. areolatum</em></td>
<td>Wood of wounded <em>P. abies</em></td>
<td>Lithuania</td>
<td>1994</td>
<td>R. Vasiliuksas</td>
</tr>
<tr>
<td>4636</td>
<td>DR37</td>
<td><em>A. areolatum</em></td>
<td>Fruiting body on <em>P. abies</em></td>
<td>Denmark</td>
<td>1993</td>
<td>I. M. Thomson</td>
</tr>
<tr>
<td>4641</td>
<td>S225</td>
<td><em>A. areolatum</em></td>
<td>Wood of wounded <em>P. abies</em></td>
<td>Sweden</td>
<td>1994</td>
<td>R. Vasiliuksas</td>
</tr>
<tr>
<td>4644</td>
<td>A3</td>
<td><em>Amylostereum sp.</em></td>
<td>Isolates from nematode cultures from CSIRO</td>
<td>Australia</td>
<td>1995</td>
<td>B. Slippers</td>
</tr>
<tr>
<td>4650</td>
<td>Br 38</td>
<td><em>Amylostereum sp.</em></td>
<td>Mycangium of <em>S. noctilio</em></td>
<td>Brazil</td>
<td>1997</td>
<td>B. Slippers</td>
</tr>
<tr>
<td>4658</td>
<td>MSW</td>
<td><em>Amylostereum sp.</em></td>
<td>Wood around <em>S. noctilio</em> in <em>P. radiata</em></td>
<td>South Africa</td>
<td>1994</td>
<td>M. J. Wingfield</td>
</tr>
<tr>
<td>4659</td>
<td>SN19A</td>
<td><em>Amylostereum sp.</em></td>
<td>Mycangium of <em>S. noctilio</em></td>
<td>South Africa</td>
<td>1996</td>
<td>B. Slippers</td>
</tr>
</tbody>
</table>

*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 (Ameraham Life Science Inc.) Thermo Sequenase® dye terminator cycle sequencing pre-mix kit (Ameraham 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 1, GenBank AF236446-AF236464) 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 1998)). Heuristic searches using TBR (Tree Bisection Recontruction) branch swapping and MULPAR, on were done to determine the most parsimonius relationships between the taxa. Strict and semisstrict 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 1983).

In order to consider the relationship of *Amylostereum* spp. with other Basidiomycetes, sequence data of the mt-susy-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 clad 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. ferrum*), 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, RI = 0.932, 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. ferrum*) 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. areolatus*. Within the *A. areolatus* group only one branch was retained in consensus trees that was weakly supported by bootstrap analysis (65%). The second major group was comprised of representative isolates of *A. chailletii*, *A. laevigatum*, *A. ferrum* and isolate Stillwell 309(3). *A. chailletii* grouped on a separate branch (93% confidence interval) within this second group from *A. laevigatum*, *A. ferrum* and isolate Stillwell 309(3). *A. ferrum*, Stillwell 309(3) and *A. laevigatum* were grouped together and basal to *A. chailletii* in strict and semisstrict consensus trees, as well as by bootstrap analysis. Therefore, a revised form of the evolutionary tree of decent reported by Boidin and Lannucetin (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 *Pneumophora 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, Hsiu 1996, Hibbett et al 1997). The three hypervariable regions were located between bases 55 and 128, bases 266 and 400 and bases 625 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
SLIPPERS ET AL: AMYLOSTereum PHYLOGENY

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 1). The length of the tree = 31 steps, CI = 0.966, HI = 0.932 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 = 0.599, RI = 0.522) (Fig. 3). The topology of the trees was 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 = 0.596, 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.) Küh., Auriscalpium vulgare S. F. Gray, Clavicipita pyzidata (Fr.) Dusy and Hericium ramulosum (Bull. ex Mér) Let. were also grouped close to Echinodontium, Amylostereum, Heterobasidion and
Russula in analysis of the data set without exclusion of the hypervariable regions. In analyses ignoring the sequence of the hypervariable regions, Hericium and Clavicipita were removed from this group. Heterobasidion and Russula also grouped closer to Lentinelus and Auviscalpium 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 leucoxanthen (Bres.) Boid. and P. nuda with Sciaenostroma 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 mtsu-rDNA. Isolates representing A. arrolatum 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. arrolatum. This is consistent with the hypothesis of Boidin and Lanquetin (1984) that A. arrolatum is the most clearly defined species in the genus. In their study, no mating compatibility was observed between isolates of A. arrolatum 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. arrolatum, 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
be separated in strict analyses of the data. These results suggest a closer relationship between \textit{A. ferreum} and \textit{A. laevigatum} than between either of these species and \textit{A. chaillietii}.

We confirmed the identity of isolates of \textit{Amylostereum} that could previously not be assigned species names. The two CLBBR cultures identified only as \textit{Amylostereum} sp. (Waite Inst 6195 and DAOM 21785) from Australia and New Zealand, clearly resided in the group containing identified isolates of \textit{A. areolatum} (CBS 305.82, CBS 394.66 and isolates from Europe that were identified by Drs. Thomsen and Vassilikas). Also represented in this group are isolates from South Africa, Brazil and isolates obtained from nematode (\textit{Deladenus striatitola}) cultures imported to South Africa from Australia. Furthermore, the two Canadian isolates of \textit{A. chaillietii} (DAOM 21327 and 54-95) clearly clustered with other identified isolates of \textit{A. chaillietii} (L.254, Sc.62.8 and CBS 483.83). Bothin and Lanquetin (1984) found partial mating compatibility between two Canadian \textit{Amylostereum} isolates and authentic isolates of \textit{A. chaillietii}, \textit{A. laevigatum} and \textit{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 \textit{S. areolatus}. Therefore, we would expect it to be \textit{A. chaillietii} as suggested by Gaut (1970). This isolate was deposited in DAOM as an \textit{Amylostereum} sp. Results of this study show that the isolate is most closely related to \textit{A. laevigatum} and \textit{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 \textit{S. areolatus}, it might represent a link between the species associated with woodwasps (\textit{A. areolatum} and \textit{A. chaillietii}) and the other two species (\textit{A. laevigatum} and \textit{A. ferreum}). It might also represent an undescribed species of \textit{Amylostereum}. Further study of this isolate is clearly warranted.

Various hypotheses have been proposed for the placement of \textit{Amylostereum} amongst the Basidiomycetes. Bothin and Lanquetin (1984) speculated that \textit{Amylostereum} might be more closely related to \textit{Pentaphora} based on the presence of gloeosclerotia posi-
tive in sulfuric-aldehyde, normal nuclear behavior and the tennapolarity 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. chattelisi groups sister to Stereum and Xylobothus (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 Hisaia (1996) using me-su-rDNA, A. chattelisi 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 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 Donoghue (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 Gloeocystidium 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 Gloeocystidium 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.

ACKNOWLEDGMENTS

We thank Drs. Geoff Tribe, Ilten Thomas, Rinvis Vasiliauskas and Mr. Erich Scharia who provided cultures or wasps from which the fungi were isolated and for fruitful discussion regarding the project. We also thank Dr. David Hibbett for helpful comments on an earlier version of the manuscript and for providing unpublished results. The CBS and DAOM culture collections kindly provided isolates and this work was financially supported by the National Research Foundation (NRF) and members of the Tree Pathology Cooperative Programme (TFCP), South Africa.

LITERATURE CITED


_____1993. Phylogenetic relationships of Lentin-
us (Basidiomycotina) inferred from molecular and morphological characters. Sys Bot 18:409–433.