Taxonomy, nomenclature and phylogeny of three cladosporium-like hyphomycetes, Sorocybe resinae, Seifertia azaleae and the Hormoconis anamorph of Amorphotheca resinae

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Abstract: Using morphological characters, cultural characters, large subunit and internal transcribed spacer rDNA (ITS) sequences, and provisions of the International Code of Botanical Nomenclature, this paper attempts to resolve the taxonomic and nomenclatural confusion surrounding three species of cladosporium-like hyphomycetes. The type specimen of Hormodendrum resinae, the basis for the use of the epithet resinae for the creosote fungus (either as Hormoconis resinae or Cladosporium resinae) represents the mononematous synanamorph of the synnematous, resinicolous fungus Sorocybe resinae. The phylogenetic relationships of the creosote fungus, which is the anamorph of Amorphotheca resinae, are with the family Myxotrichaceae, whereas S. resinae is related to Capronia (Cheetotthynales, Herpotrichiellaceae). Our data support the segregation of Pycnostysanus azaleae, the cause of bud blast of rhododendrons, in the recently described anamorph genus Seifertia, distinct from Sorocybe; this species is related to the Dothideomycetes but its exact phylogenetic placement is uncertain. To formally stabilize the name of the anamorph of the creosote fungus, conservation of Hormodendrum resinae with a new holotype should be considered. The paraphyly of the family Myxotrichaceae with the Amorphothecaceae suggested by ITS sequences should be confirmed with additional genes.

Key words: Amorphothecaceae, Cladosporium resinae, creosote fungus, Hormoconis resinae, jet fuel fungus, kerosene fungus, Myxotrichaceae, Pycnostysanus, resinicolous fungi.

INTRODUCTION

The ascomycete Amorphotheca resinae Parbery (1969) grows in hydrocarbon-rich substrates such as jet fuel, cosmetics and wood preserved with creosote or coal tar. This fungus is widely known by the anamorph name Hormoconis resinae (Lindau) Arx & G.A. de Vries or its obligate synonym Cladosporium resinae (Lindau) G.A. de Vries. It produces lightly pigmented, warty conidiophores, and branched, acropetally developing chains of lightly pigmented ameroconidia lacking conspicuous scars (Fig. 1B–E). This species is known colloquially as the “creosote fungus”, the “kerosene fungus” or the “jet fuel fungus”; to avoid confusion caused by the many heterotypic names with the epithet “resinae”, in this paper we generally will use the oldest of these informal names, “creosote fungus”, when referring to A. resinae or its anamorph. This fungus grows in jet fuel contaminated with small amounts of water, and the mycelium clogs fuel lines and corrodes metal parts. Consequently, fuel tanks in airports are monitored for this fungus by private companies using various physiological or biochemical tests.

Sorocybe resinae (Fr.) Fr. produces dark black colonies on conifer resin, comprising dark synnemata and an effuse mononematous synanamorph, both with cladosporium-like conidiogenous cells and conidia. Unlike the anamorph of the creosote fungus, the conidia of Sorocybe resinae are dark brown and the lateral walls are conspicuously thicker than the poles (Fig. 2D–G). Colonies with only the mononematous anamorph sometimes occur, and the mononematous anamorph can be sparse on colonies bearing synnemata. However, the conidia of the mononematous anamorph have identical pigmentation and lateral wall thickening to that of the synnematous anamorph. The mononematous anamorph rarely has been referred to by its own binomial name although, as we will show, there is a species epithet available. For the same reasons given above for Amorphotheca Parbery, generally we will refer to Sorocybe resinae herein as “the resin fungus”.

Despite the micromorphological differences noted above, there is disagreement about whether the creosote fungus is conspecific with the mononematous synanamorph of the resin fungus (Parbery 1969). The name for the anamorph of the creosote fungus is based on Hormodendrum resinae Lindau (1906). Christensen et al. (1942) presented a study of a cladosporium-like fungus commonly isolated from wood impregnated with creosote and coal tar and applied Lindau’s name without examining its type. A later ecological study by Marsden (1954) employed the same name for the same fungus. An extra dimension was added to the confusion when de Vries (1952, using the name Cladosporium avellaneum G.A. de Vries) described four formae for the creosote fungus (differing in the colours of their conidia, the production of setae, or the total absence of conidia), each based on single conidium isolates made from one parent culture. De Vries (1955) and Parbery (1969) examined the holotype of Hormodendrum resinae and concluded that it represented the creosote fungus. Hughes (1958), prior to the description of Amorphotheca or Hormoconis Arx & G.A. de Vries, examined the same specimen and considered it to be the mononematous synanamorph of the resin fungus. If Hughes (1958) is correct, then neither the species Hormodendrum resinae, nor the genus that it typifies, Hormoconis, can represent the creosote fungus, as intended by Parbery (1969) or von Arx and de Vries (in von Arx 1973).

In this paper, we present micromorphological, cultural and molecular evidence that the resin fungus is a different species from the creosote fungus. Combined with re-examination of the holotype of Hormodendrum resinae, this information is used to provide a revised taxonomy and nomenclature for these two species. A third cladosporium-like fungus, Seifertia azaleae, is also considered in our discussion of generic concepts.

Historical review

The history of the fungus now known as Sorocybe resinae began with Fries (1815), who described Racodium resinae Fr. as follows:

“310. Racodium resinae, expansum molluscum dense contextum nignis, filis inaequilibus.

In resina Pini Aleatis in silvis Suecia passim.

Habitu et loco natali distinctum. Fila divaricato-ramosa; alia rigidula apice capituli sera, sub micsroco. Coremio Link similia, Demat. villosum Schleich. huic simile; sed sub microsc. fila maxime differunt.”
The comparison with Coremium Link indicates the probability of a synnematous fungus, and an authentic specimen of Fries’ fungus, which as the only known authentic material we interpret as the holotype, is preserved in Link’s herbarium (see below). It represents the synnematous form of the resin fungus.  

Fries (1832) later transferred his species to Sporocybe Fr. (1825), a genus then used for relatively conspicuous dark hyphomycetes with dry spores (Mason & Ellis 1953). The 1832 description explicitly stated... “capitulo rotundato inaequali, sporidiis seriatis, stipite aequali simplici.” The use of “capitulo” and “stipite” imply what would now be recognised as a synnematous fungus. Fries (1832) further characterised the habit of the fungus as “habitu stipitum Calicii,” a further comparison to a group of black, stipitate lichenized fungi classified in Calicium Pers., which under a hand lens look similar to a dark synnematous fungus.

Fries (1849) next described the genus Sorocybe Fr. for this fungus, as follows:

Fig. 1. Amorphotheca resinae, colony characters and anamorph micromorphology. A. 10-d-old colony on PDA. B, D–E. Micromorphology of conidiophores, showing acropetal conidal chains, ramoconidia, and conidia. C. Conidia. DAOM 170427; for C, E see scale bar in D.

1Persoon (1822) described a form of R. resinae “β piceum”. Hughes (1968) examined the holotype of this form, and it represents the mycelium of the ascomycete Strigopodia resinae (Sacc. & Bres.) S.J. Hughes. This taxon is thus not relevant to the three species that are the focus of this paper.
Hormoconis resinae and Morphologically Similar Taxa

Sorocybe Fr.
Habitus prioris. sed mycelium floccosum densum, stroma comeo-carbonaceum, sporis moniliformi-concatenatis basi excipulum incompletum praebens.
1. S. resinae. Fr. 1–4. at raro fructif. Klotzsch exs. C. 2.

Because this description explicitly referred to the Systema, Fries presumably was segregating the fungus, originally described as Racodium resinae, into a new monotypic genus (McNeill et al. 2007; Art. 33.3) and this interpretation of R. resinae as the basionym generally has been followed in subsequent treatments of Sorocybe resinae.

As noted in Table 1, Fries’ Racodium resinae was placed in several other hyphomycete genera by eighteenth century authors. These diversions need not be reviewed in detail here because the modern status of these other genera, and their lack of similarity with Sorocybe, is clear.

Bonorden (1851) described Hormodendrum Bonord., with four species originally placed in Penicillium Link by Corda (1839); H. olivaceum (Corda) Bonord. (≡ Penicillum olivaceum Corda 1839) was designated as lectotype by Clements & Shear (1931). This genus was frequently, but incorrectly, spelled “Hormodendron”. Bonorden’s descriptions and illustrations are of variable quality by modern standards, and his herbarium is unknown (Stafleu et al. 1995). Consequently the actual identities of the species Bonorden placed in Hormodendrum are unknown and Corda’s Cladosporium olivaceum (Corda) Bonord. was dismissed in Penicillium monographs because the drawing shows branched conidial chains (Thom 1930), although the specimen has apparently not been re-examined. The generic name was used as a segregate for Cladosporium Link by some authors (e.g. Kendrick 1961), in particular for species with ameroconidia (de Vries 1952). Although it sometimes has been considered a synonym of Cladosporium, it will remain a nomen dubium until the type species is properly typified.

Unaware of the resinicolous fungus described by Fries, Lindau described two species growing on conifer resin, Pycnostysanus resinae Lindau (1904), the type of this anamorph generic name, and Hormodendrum resinae Lindau (1906). The former was clearly illustrated and described as a synnematous species. The protologue of the latter concludes with, “Mit Pycnostysanus resinae hat die Art nichts zu tun.” Clearly, Lindau observed no synnemata on the specimen of the mononematous fungus and he believed it was a different fungus, rather than what would now be called a synanamorph of the synnematous fungus that he had described previously. Lindau (1910) reproduced the 1904 illustration of Pycnostysanus resinae as Stysanus resinae (Fr.) Sacc. (1906), thus accepting its identity with the species originally described as Racodium resinae Fr. Lindau (1910) made no mention of Hormodendrum resinae, indicating he still made no association between the synnematous and mononematous fungi on resin.

De Vries (1952) described a new species, Cladosporium avellaneum G.A. de Vries, isolated from cosmetics. Later, he noted the similarities between his C. avellaneum and the creosote fungus, and suggested that they were the same species (de Vries 1955), replacing the name of one of his previously described formae, i.e. viride, with the forma name resinae. He examined Lindau’s type of Hormodendrum resinae and decided that it provided an earlier epithet for C. avellaneum. He transferred the species into Cladosporium as C. resinae (Lindau) G.A. de Vries, and this name was widely used for the creosote fungus until 1973. This binomial is still commonly employed in non-taxonomic literature, especially commercial publications dealing with the creosote fungus.

Fig. 2. Sorocybe resinae, synnematous form. A. Colony on bark of living, standing conifer. B. Synnemata. C. Four-month-old colony on DG18. D–G. Acropetally developing chains of conidia. Note that the lateral walls are conspicuously thickened; compare with Fig. 3. A, C. DAOM 239134. B, D–G. DAOM 11381.

Sorocybe Fr.
Habitus prioris. sed mycelium floccosum densum, stroma comeo-carbonaceum, sporis moniliformi-concatenatis basi excipulum incompletum praebens.
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Creosote fungus

eanomorph:

Hormodendrum resinae Lindau, in Rabenh. Krypt.-Fl., 2, 1 (Pilze) 8: 699. 1906 (B!)

≡ Cladosporium resinae (Lindau) G.A. de Vries, Antonie van Leeuwenhoek 21: 167. 1955.

≡ Hormoconis resinae (Lindau) von Arx & G.A. de Vries, in von Arx, Verh. K. Ned. Akad. Wet., Afd. Natuurk. 61: 62. 1973.

resin fungus

Mononematous synanamorph:

Hormodendrum resinae Lindau, in Rabenh. Krypt.-Fl., 2, 1 (Pilze) 8: 699. 1906 (B!)

≡ Cladosporium resinae (Lindau) G.A. de Vries, Antonie van Leeuwenhoek 21: 167. 1955.

≡ Hormoconis resinae (Lindau) von Arx & G.A. de Vries, in von Arx, Verh. K. Ned. Akad. Wet., Afd. Natuurk. 61: 62. 1973.

Synnematous anamorph:

Sorocybe resinae (Fr.) Fr., Summa Veg. Scan. 2: 468. 1849.

≡ Raciocodium resinae Fr., Obs. Mycol. 1: 216. 1815 (basionym) (Bl).

≡ Sorocybe resinae (Fr.) Fr., Syst. Mycol. 3: 341. 1832.

≡ Dendryphion resinae (Fr.) Corda, Icon. Fung. 6: 11. 1854.

≡ Stysanopsis resinae (Fr.) Ferr., Flora Ital. Crypt., 1 (Fungi, Hyphales), p. 167. 1910.

≡ Sporothrix resinae (Fr.) Link, Mag. Ges. naturf. Fr. 3: 21. 1809 (Bl).

≡ Sorothricum resinae (Link) Link, Mag. Ges. naturf. Fr. Berlin 7: 35. 1815.

≡ Pycnostysanus resinae Lindau, Verh. Bot. Ver. Brandenb. 45 : 160. 1904 (Bl).

≡ Stysanus resinae (Lindau) Sacc., Syll. Fung. 18: 651. 1906.

In his study of type collections of classical hypomycetes, Hughes (1958) included Pycnostysanus resinae Lindau and Hormodendrum resinae Lindau as facultative synonyms of Sorocybe resinae (Fr.) Fr., with Racodium resinae Fr. and several other nomenclatural variants as obligate synonyms (Table 1). The synnematous Pycnostysanus resinae was cited as “Pycnostysanus state [i.e. synanamorph] of Sorocybe resinae”. Hormodendrum resinae thus remained to represent the mononematous synanamorph of what was interpreted as a single species.

Parberry (1969) described a cleistothecial ascomycete, Amorphotheca resinae, for the teleomorph of the creosote fungus. He also examined the holotype of Hormodendrum resinae and agreed with the conclusions of de Vries (1955). He used the epithet resinae for the teleomorph to correspond with that of the anamorph. He discounted the possibility that the synnematous Sorocybe resinae could be the same fungus as Hormodendrum resinae because synnemata never developed in his cultures of the creosote fungus.

Von Arx and de Vries (in von Arx 1973) described the genus Hormoconis, typified by Hormodendrum resinae, with the new combination Hormoconis resinae (Lindau) Arx & G.A. de Vries. Their intention was to erect an anamorph genus for the anamorph of the creosote fungus, which they suggested was improperly classified in Cladosporium because it lacked darkened, thickened secession scars on the conidia.

A third cladosporium-like fungus is relevant to this story. Seifertia azaleae (Peck) Partridge & Morgan-Jones [until recently known as Pycnostysanus azaleae (Peck) E.W. Mason] is a cosmopolitan fungus causing bud blast and twig blight of azaleas and rhododendrons. This species is morphologically similar to Sorocybe resinae, but the conidia are paler and lack laterally thickened walls. Sorocybe and Pycnostysanus have often been considered taxonomic synonyms (Ellis 1976, Carmichael et al. 1980); as shown above, both are based on the synnematous form of the resin fungus. Partridge and Morgan-Jones (2002) argued that Sorocybe resinae and “Pycnostysanus azaleae” are not congeneric, and described the new genus Seifertia Part. & Morgan-Jones for the Rhododendron fungus. They observed that the connection between conidia in Seifertia azaleae is much narrower than in Sorocybe resinae, and that minute denticles are visible on the conidiogenous cells of the former fungus. The broader connections between conidia of Sorocybe resinae result in broadly protuberant conidiogenous loci on the conidiogenous cells, and more truncate detached conidia.

**MATERIALS AND METHODS**

**Herbarium material and fungal strains**

Full details of herbarium material examined are listed below. Cultures and dried herbarium specimens were studied in 90 % lactic acid without stains; preparations of some exsiccate and types were mounted in glycerin jelly. Cultures were grown on potato-dextrose agar (PDA, Difco), oatmeal agar (OA, Samson et al. 2004), Blakeslee’s malt extract agar (MEA, Samson et al. 2004) and dichloran-18 % glycerol agar (DG-18, Samson et al. 2004). Colony characters were taken from cultures grown at 25 °C in darkness. Cultures are maintained in the Canadian Collection of Fungal Cultures (DAOM), Agriculture & Agri-Food Canada, Ottawa.
Exsiccati and types

**Dematium nigrum** [scr. Link]. E. Hbr. Link (23) = Sorocybe resinae ill. 341 [scr. ?] [herb. Link, B].

**Hormodendrum resinae** Lindau, n. sp. F. v. Hamburg 206, auf Harz an Picea excelsa, Sachsenwald, leg. O. Jaap, 29-4-1906. [scr. Lindau]. (DAOM 41888, slide prepared from the holotype preserved in B.)

**Pycnostysanthes resinae** Lindau nov. gen. et nov. spec., Kabat et Bubák: Fungi imperfecti exsiccati no. 99. Auf erhärteten Fichtenharz an Brockenweg, am Dreiieckigen Pfahl in Harz, Deutschland, leg. G. Lindau, 13.VIII. 1903 (holotype, B).

**Racodium resinae** Fries. E. Hbr. Link, Fries legi, Smol. [scr. Fries]. (DAOM 41890, slide prepared from herb. Link, B). This is the presumed holotype of *R. resinae*, the basionym for the resin fungus, *Sorocybe resinae*. The specimen includes dark, decapitated synnemata, brown conidia with laterally thickened walls, and acropetal conidial chains, allowing it to be recognised as the fungus we now know as *S. resinae*. Fries perhaps sent this fungus to Link to see if it could be differentiated from *Coremium Link*. The minimal details, that the fungus was collected by Fries, presumably in Småland (a province of Sweden), match the details in the protologue of *Sorocybe resinae*.

"Fungi Rhenani Fasc. II, 1863, L. Fuckel, no. 129, ad Abietis resinae, rarum Hieme, in sylva Hochtichiensis" (as *Myxotrichum resinae* Fr., DAOM 55543 ex FH). “Flora Suecica, 2956, Ad resinam piceae, Småland: Femsjö, Prostadiagshogen, 6 Aug. 1929, leg. J.A. Nannfeldt, s.n." (as *Stysanus resinae* (Fr.) Sacc., DAOM 41891 ex UPS). “Flora Suecica, 4709, Ad resinam abietinum, Upland: Bondkyss gen Valsätta, 9 May 1932, leg. J.A. Nannfeldt" (as *Hormodendrum resinae* Lindau, DAOM 41889 ex UPS). 

Other material examined

**Sorocybe resinae**. Canada. British Columbia: Burnaby, Central Park, on resin of Tsuga heterophylla, leg. S. & L. Hughes, 17 Aug. 2000 (DAOM 228572a, 228573a); Cameron Lake, Cathedral Grove, on Pseudotsuga menziesii, leg. sis. J.S. Hughes, 21 Aug. 1957 (DAOM 56088a). Ladvismith, Ivy Green Park, on resinous exudates, leg. R.J. Bandoni no. BC-978, 18 Apr. 1960, det. S.J. Hughes (DAOM 70462). North Vancouver, Lynn Valley Conservation Area, on bark of living Tsuga heterophylla, leg. det. S.J. Hughes, 1 Jul. 1975 (DAOM 134614); Vancouver, Stanley Park, leg. K.A. Seifert no. 1571, 11 May 2002 (culture and specimen, DAOM 239136, Lsu GenBank EU030327). Ireland, Munter, Kerry, near Glenbeigh (ca. N 52° 03’ W 95° 94’), leg. K.A. Seifert no. 3197, 26 Sep. 2006 (culture and specimen, DAOM 239135, ITS GenBank EU030273). Netherlands, Gelderland, Krijt-Müller Museum, leg. K.A. Seifert no. 1235, 12 May 2000 (DAOM 227136). United Kingdom, Wales, Hafod Estate (ca. N 52° 22’ W 3° 51’), leg. K.A. Seifert no. 3198, 1 Oct. 2006 (culture and specimen, DAOM 239137, ITS GenBank EU030274).

DNA extraction, amplification and sequencing

DNA was isolated using a FastDNA™ Kit and the FastPrep™ FP120 (BIO 101 Inc.) or an UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, U.S.A.) using mycelium removed from agar cultures. PCR and cycle sequencing reactions were performed on a Tecne Genius™ thermocycler (Technne Cambridge Ltd.). PCR reactions were performed using Ready-To-Go™ Beads (Amersham Canada Ltd.), in 25 µL volumes, each containing 20–100 ng of genomic DNA, 2.5 units pure Taq DNA Polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 200 µM of each dNTP, 0.2 µL of each primer (50 µM), and stabilizers including bovine serum albumin. The reaction profile included an initial denaturation for 4 min at 94 °C, followed by 30 cycles of 1.5 min denaturation at 95 °C, 1 min annealing at 55 °C, and 1 min extension at 72 °C. A final extension of 10 min at 72 °C. Amplicons were purified by ethidium/sodium acetate precipitation and resuspended as recommended for processing on an ABI PRISM 3100 DNA Analyzer or an ABI 373 Stretch DNA Sequencer (Applied Biosystems, Foster, CA). Amplification products were sequenced using the BigDye v. 2.0™ Terminator Cycle Sequencing Ready reaction Kit (ABI Prism/Applied Biosystems) following the manufacturer's directions. An approximately 1 000 bp portion of the large subunit (LSU) ribosomal DNA was amplified and sequenced using primers LR0R and LR6, and cycle-sequenced using primers LR0R, LR3R, LR16 and LR6 (Vilgalys & Hester 1990, Rehner & Samuels 1995; www.biology.duke.edu/fungi/mycolab/primers. html). The complete ITS and 5.8S rRNA genes were amplified and sequenced using the primers ITS5 and ITS4, with ITS2 and ITS3 primers used for cycle sequencing when necessary (White et al. 1990). Some sequences were derived from single PCR amplifications of the ITS-5′-LSR region. Data matrices were subjected to parsimony analysis using heuristic searches in PAUP* v. 4.0b10 (Swofford 2002) with simple stepwise addition of taxa, and tree bisection-reconnection (TBR) branch swapping. Uninformative characters were removed for all analyses. Strict consensus trees were calculated, and the robustness of the phylogenies was tested using full bootstrap analyses (1 000 replications). For all analyses, GenBank accession numbers are given on the tree figures, and the sequences generated in this study are indicated in bold.
Fig. 3. Hormodendrum resinae. A–B. Conidiophores and acropetally developing chains of conidia. C. Conidia. Note that the lateral walls are conspicuously thickened compared to the walls at the poles. From a slide (DAOM 41888) prepared from the holotype (S).

Fig. 4. Parsimony analysis of large subunit sequences, demonstrating the phylogenetic positions of Amorphotheca resinae, Sorocybe resinae and Seifertia azaleae (all shown in bold) in the Ascomycota. One of 12 equally parsimonious trees (1 888 steps, CI = 0.390, RI = 0.554, RC = 0.216, HI = 0.610) with Golovinomyces cichoracearum as the outgroup. Bootstrap values above 70 % are shown at the relevant nodes, with an asterisk representing 100 % bootstrap support; branches with thick lines occurred in all equally parsimonious trees.
The large subunit matrix was assembled from the closest BLAST matches using our sequences for the three fungi of interest, S. resinae, A. resinae and S. azaleae; Golovinomyces cichoracearum was added as an out-group to root the tree. Although these sequences were put into a single matrix, there is no implication that this data set represents the diversity of the Ascomycota. The alignment was calculated using MAFFT (Katoh & Kuma 2008) and adjusted using Se-Al. (Sequence Alignment Program v. 1.d1; http://evolve.zoo.ox.ac.uk/software/Se-Al/main.html) to maximise homology.

The internal transcribed spacers alignment including Sorocybe resinae was derived from an alignment of Capronia and related anamorphs used by Davey & Currea (2007), originally produced using MAFFT. This data set was modified considerably using Se-Al to maximise homology, but still included several areas where the homology of aligned sequences was difficult to evaluate. ITS sequences of Amorphotheca resinae were used to retrieve closely related sequences using a BLAST search of GenBank, and these relevant sequences were added to an alignment of Oidiodendron Robak sequences from the study of Hambleton et al. (1998), and then adjusted using Se-Al.

We attempted direct PCR from two specimens containing only the putative mononematous synanamorph of Sorocybe resinae (DAOM 228772a, 228573a), to allow comparison of sequences obtained from cultures of the synnematous synanamorph. These attempts, using the same methods outlined above, were unsuccessful.

RESULTS

Cultural characters and micromorphology

Most micromorphological characters of the resin fungus Sorocybe resinae (Partridge & Morgan-Jones 2002), the creosote fungus Amorphotheca resinae (Parbery 1969, de Vries 1952, 1955, Ho et al. 1999) and the rhododendron fungus Seifertia azaleae (Ellis 1976, Partridge & Morgan-Jones 2002, Glawe & Hummel 2006) are well-described in the literature and will not be repeated here.

The three species are readily distinguished based on growth rates and overall cultural phenotypes. Agar colonies of Sorocybe resinae are coal-black, wrinkled, and restricted in growth, no matter what agar medium is employed; even after 3 mo, the colonies are unsuccessful.

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Phylogeny

The large subunit alignment (LSU) was used to demonstrate the general phylogenetic relationships of the resin fungus Sorocybe resinae (DAOM239134), the creosote fungus Amorphotheca resinae (DAOM 170427, 194228) and the rhododendron fungus Seifertia azaleae (DAOM 239136), and subsequent analyses of the internal transcribed spacers were used to estimate more precise affinities. Fig. 4 shows the LSU analysis and demonstrates that Sorocybe resinae appears to be a member of the Herpotrichiellaceae, Chaetothyriales, A. resinae is related to the inoperculate discomycetes (Leotiomycetes) and Seifertia azaleae is most closely related to a sequence labelled Mycosphaerella mycophalli A. Funk & Dorworth, which is unrelated to Mycosphaerella s. str.

For the ITS alignment of Sorocybe resinae, two preliminary parsimony analyses were conducted, one with informative characters from the full alignment, the second with a subset with 179 characters excluded from seven ambiguously aligned regions. The consistency indices (full 0.301, partial 0.324), tree topologies, and bootstrap supports for the two analyses were relatively similar. Therefore, the complete alignment was used for the tree presented here (Fig. 5). The data matrix included 57 taxa, with 352 of 752 characters phylogenetically informative. Sorocybe resinae clearly is related to Capronia and allied anamorph genera, as suggested by the LSU analysis. In the ITS analysis (Fig. 6) it forms a well-supported clade with C. villosa Samuels, that is a well-supported sister group to species now in three different anamorph genera, Phaeococcomyces nigricans (M.A. Rich & A.M. Stem) de Hoog, Ramichloridium cerophilum, and an undescribed species of Heteroconium Petr.

The ITS matrix for A. resinae included 42 taxa, with 171 phylogenetically informative characters in the 530 base alignment. The phylogenetic analysis confirmed the relationship of this species with the Leotiomycetes, and provided a more precise hypothesis of its family-level relationships (Fig. 6). Amorphotheca resinae DAOM 170427 and 194228 had identical ITS sequences to another strain of the same species reported in GenBank (AY251067, from Braun et al. 2003), and one bp substitution from a second strain (AF393726 based on the isotype ATCC 200942 = CBS 406.68). These four sequences formed a sister group to two sequences of “Cladosporium” breviramulos Morgan-Jones (AF393683, AF393684). The well-supported clade of A. resinae and C. breviramulos, which represent the proposed family Amorphothecaceae, was previously noted by Braun et al. (2003). The nesting of this clade within two well-supported...
clades of *Myxotrichum* spp. and the associated anamorph genus *Oidiodendron*, which comprise the family *Myxotrichaceae*, has not been documented previously.

The ITS sequences of two strains of *Seifertia azaleae* were 474 bp and differed by one bp. BLAST searches with these sequences revealed significant homologies only with unidentified fungi, and lower probability matches with various members of the Dothideomycetes. Therefore, no taxonomically meaningful phylogenetic analysis can be presented with these ITS sequences. The species does seem to have affinities with the Dothideomycetes, but the putative relationship with *Mycosphaerella* suggests by the LSU analysis, could not be confirmed with the ITS analysis.

**DISCUSSION**

Micromorphological comparisons, differences in culture characters, and phylogenetic analysis all support the conclusion that the mononematous synanamorph of *Sorocybe resinae*, the resin fungus, is different from the anamorph of *Amorphotheca resinae*, the creosote fungus. Based on ribosomal DNA sequences, the creosote fungus is related to the family *Myxotrichaceae*, the genus *Myxotrichum* and its *Oidiodendron* anamorphs (Fig. 5). In this gene tree, *Myxotrichum* and the *Myxotrichaceae* are paraphyletic, with *Amorphotheca* and the *Amorphothecaceae* nested within them. *Sorocybe* appears to be an additional anamorph genus phylogenetically associated with *Capronia* (*Herpotrichiellaceae, Chaetothyriales*, Fig. 6). The genetic connection between the synnematous and mononematous morphs of *S. resinae* was verified by morphological comparison of polyspore isolates derived from the two synanamorphs. However, the living cultures are no longer available and the connection was not confirmed with single conidium isolations. The type specimen of *Hormodendrum resinae* (Fig. 3) is the basis for the application of the most frequently used anamorph epithet for the creosote fungus. This specimen represents the mononematous synanamorph of *Sorocybe resinae*, not the anamorph of *Amorphotheca resinae*.

It is difficult to understand how these two fungi were confused when their micromorphologies are so different. The conidia are of the same general size and shape, but in both morphs of *Sorocybe resinae* (Figs 2D–G, 3C), the lateral walls are conspicuously thickened, a condition not present in the creosote fungus (Fig. 1C), and the conidia are much darker. In his monograph of *Cladosporium*, de Vries (1952) noted that single conidium isolates of *C. avellaneum* gave rise to four different colony types. In 1955, he extended these observations and decided that the much darker resin fungus was
the same as one of his mutant forms of the creosote fungus, despite never having isolated such a dark spored form from any of his cultures. Parbery (1969) implied that the demonstrated ability of the creosote fungus to grow on a diversity of hydrocarbon-rich substrates favoured the thought that it would be able to grow on conifer resin. If cultures of the true Sorocybe resinae had been available, it is unlikely that this confusion would have persisted for so long. In vivo, the creosote fungus and the resin fungus are so different (Figs 1A, 2C) that it would be difficult to defend the idea that they were mutants of the same fungus. These differences in the cultures are reflected by the disparate phylogenetic affinities of what now are clearly demonstrated to be two different species.

Unfortunately, the name Hormodendrum resinae has been misapplied to the creosote fungus, a species of economic importance. Also unfortunately, this species is the type of Hormoconis, a generic name that the community concerned with this fungus has been slow to adapt to in the 30 years since its introduction. There are several possible solutions to this problem. The conventional solution would be to apply names based strictly on the type specimens and accept Hormoconis as a synonym of Sorocybe, or to use it as a generic name for the mononematous synanamorph of the resin fungus. A new anamorph genus would allow for conservation of a name with a different type from that of the basionym for its type. However, the International Code of Botanical Nomenclature (McNeill et al. 2006) allows for conservation of a name with a different type from that

Fig. 6. Parsimony analysis of internal transcribed spacers sequences, demonstrating the position of Sorocybe resinae (shown in bold) among species of Capronia (Herpotrichiellaceae, Chaetothyriales) and its associated anamorph genera. One of 34 equally parsimonious trees (2 607 steps, CI = 0.301, RI = 0.506, RC = 0.153, HI = 0.699), with mid-point rooting. Bootstrap values above 70 % are shown at the relevant nodes; branches with thick lines occurred in all equally parsimonious trees.
The name *Hormodendrum resinae* is not otherwise needed because the mononomatous synanamorph of the resin fungus is rarely referred to by a Latin binomial, and because *Sorocybe resinae* is based on a different type. Therefore, a new type specimen could be proposed and conserved for *Hormodendrum resinae* (MELU 7130). This would make the anamorph-teleomorph connection unequivocal, maintain current species epithets and taxonomic authorities, and ensure that most of the historical literature can be interpreted easily without the need to consult complicated nomenclators (Table 1). However, by perpetuating the use of the epithet “*resinae*”, this change would also perpetuate the misunderstanding that resin is a possible substrate for the creosote fungus. In any case, the use of this epithet for the teleomorph of the creosote fungus, *Amorphotheca resinae*, is legitimate and valid, and unlikely ever to be changed.

A third option would be an intermediate one. The application of the name *Cladosporium avellaneum* G.A. de Vries has never been in doubt, and it would be possible to conserve this species as the type of *Hormoconis*. This has the advantage of maintaining the familiar generic name *Hormoconis*, in combination with a species epithet that has been consistently applied. Furthermore, this solution would allow the confusion about the application and correct author citation around the epithet “*resinae*” for the anamorph of creosote fungus to recede.

The second and third solutions require formal taxonomic proposals to be published in Taxon. We will argue the merits of these possible solutions at more length in that venue.

The phylogenetic position of *A. resinae* raises additional taxonomic problems. This fungus typifies the monotypic family *Amorphothecaceae*, which has been considered *incertae sedis* since its description by Parbery (1969). Our phylogenetic analysis suggests that this family sits within the *Myxotrichaceae*. *Amorphothecaceae* (1969) is the older name, but *Myxotrichaceae* (1985) is well-entrenched in the mycological literature. As a consequence, the *Myxotrichaceae* are paraphyletic with respect to the *Amorphothecaceae*. The peridium of *A. resinae*, the only species presently placed in this family, lacks the thick-walled appendages that characterise most species of the *Myxotrichaceae*. Furthermore, the acropetal-blastic features of the anamorph of *A. resinae* differ from the thallic-arthic conidia of the other anamorphs associated with the *Myxotrichaceae*, principally *Oidiodendron*. These morphological differences explain why the affinity of *A. resinae* with the *Myxotheca* was not noted before. A formal proposal to conserve *Myxothecaceae* as the name for this family might be prudent eventually, but this should await analysis of additional genes to confirm the phylogenetic relationship.

Whether *Cladosporium breviramosum*, originally isolated from discoloured wallpaper, is actually a distinct species from the creosote fungus, *Amorphotheca resinae*, is not otherwise needed because the mononomatous synanamorph of the resin fungus is rarely referred to by a Latin binomial, and because *Sorocybe resinae* is based on a different type. Therefore, a new type specimen could be proposed and conserved for *Cladosporium breviramosum* (ICMU 1149, 1150), but this sequence does not cluster with others representing the family *Mycosphaerellaceae* (data not shown). Similarly, the ITS sequences of the rhododendron fungus did not cluster with the many ITS sequences of *Mycosphaerella* available. Presently, it seems that *Seiftertia azaleae* fungus is allied with the *Dothydeomyces*, but its precise affinities are uncertain. It is clear that this fungus should not be classified in *Pycnostysanus* (a taxonomic synonym of *Sorocybe*), and continued recognition of the monotypic genus *Seiftertia* seems justified.

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