Dematiocladium celtidis gen. sp. nov. (Nectriaceae, Hypocreales), a new genus from Celtis leaf litter in Argentina

Pedro W. CROUS1*, Natalia ALLEGRUCCI1, Angélica Margarita ARAMBARRI1, María Cecilia CAZAU1, Johannes Z. GROENEWALD2 and Michael J. WINGFIELD3

1 Instituto Spegazzini, 53 N° 477, La Plata, Argentina.
2 Centraalbureau voor Schimmelcultures, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands.
3 Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.
E-mail: crous@cbs.knaw.nl

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A Cylindrocladium-like hyphomycete collected on leaf litter of Celtis tala in Argentina had rDNA sequence data (ITS and LSU) that showed it resides in the Hypocreales, and is a member of the Nectriaceae, closely related to, but distinct from Cylindrocladium. A new genus, Dematiocladium and species, D. celtidis gen. sp. nov. is, therefore, introduced to accommodate this fungus. Based on morphology, it can be distinguished from other conidial hypocrealean genera with hyaline, penicillate conidiophores and cylindrical conidia by lacking stipe extensions and vesicles, and by the presence of brown to dark brown, thick-walled setae.

INTRODUCTION

A study on fungi associated with leaves of Celtis tala (Ulmaceae) in Argentina has revealed a Cylindrocladium-like fungus sporulating profusely on leaf litter of this tree. The fungus resembles other species currently accommodated in Cylindrocladium (Crous 2002, Crous et al. 2004), except that it lacks sterile stipe extensions that terminate in vesicles of characteristic shape. In contrast, penicillate conidiophores were found to occur adjacent to brown setae. In some cases, however, the setae tend to become fertile, and develop branches with phialides and conidia, as found on typical penicillate conidiophores of this fungus.

The genus Cylindrocladium is restricted to anamorphs of Calonectria as defined by Rossman (1979). Most species of Cylindrocladium have stipe extensions with terminal vesicles (Crous 2002). One exception is C. avesiculatum, which has stipe extensions that are thick-walled, pale brown, and frequently avesiculate. C. avesiculatum also has a Calonectria teleomorph, and this variation is thus acceptable in the genus. This has also been supported in a recent phylogenetic analysis including most of the species of Cylindrocladium (Schoch et al. 2001, Crous et al. 2004).

The aim of this study was to describe the species collected from Celtis in Argentina, and to elucidate its phylogenetic position in the Hypocreales. This was achieved using DNA sequence data for the large subunit rDNA gene.

MATERIALS AND METHODS

Isolates derived from conidia, lifted from conidiophores on Celtis leaves, were grown on 2% malt extract agar (MEA; Oxoid), plated onto carnation leaf agar (CLA; Crous, Phillips & Wingfield 1992), incubated at 25 °C under n-uv light, and examined after 7 d. The 95% confidence intervals of conidial measurements were derived from 30 observations of structures formed on carnation leaves. Growth rates and cultural characteristics were determined after 6 d on MEA at 25 °C in the dark, using procedures described by Crous & Wingfield (1994). Colony colours were coded according to Rayner (1970). Isolates are maintained in the Centraalbureau voor Schimmelcultures (CBS, Utrecht).

Genomic DNA isolation, amplification and sequencing of the internal transcribed spacer (ITS) region and partial 28S rDNA gene (LSU) were done following the protocol described by Lee, Groenewald & Crous (2004). Phylogenetic analyses of the alignments were performed...
as described by Lee et al. (2004) and the alignments have been lodged in TreeBASE (accession no. SN2083) and the sequences with GenBank (Table 1).

RESULTS

For the LSU gene, approximately 900 bases were determined for the isolates studied (Table 1). The manually adjusted alignments contain 28 taxa (including the two outgroups) and 850 characters including alignment gaps. Of the 850 characters used in the phylogenetic analysis, 95 were parsimony-informative, 27 were variable and parsimony-uninformative and 728 were constant. Neighbour-joining analysis using three substitution models on the sequence data, yielded trees identical and did not differ from the topologies obtained using a parsimony analysis (data not shown). Parsimony analysis of the aligned sequences yielded four most parsimonious trees, one of which is shown (Fig. 2). In both the neighbour-joining and parsimony analyses, the fungus from Celtis is placed as a sister taxon to the clade containing the Glionecladium/Gliocladiopsis isolates. However, contrary to the results obtained for the LSU sequence phylogeny, this association is supported by a bootstrap value of 82% in the ITS phylogeny (a value of 88% was obtained using neighbour joining, results not shown). In order to test the robustness of the placement of the fungus from Celtis, different outgroups and taxon samplings were subjected to the different analysis methods. Little effect was observed, however, on the placement of this fungus (data not shown).

TAXONOMY

The fungus isolated from Celtis leaves in Argentina is morphologically distinct from species of

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**Table 1.** Isolates sequenced for the present study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession no.¹</th>
<th>Host</th>
<th>Country</th>
<th>Collector</th>
<th>GenBank accession no.²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylindrocladiella microclyndrica</td>
<td>ATCC 38571/CPC 2575/CBS 111794²</td>
<td>Fruit of tree</td>
<td>Australia</td>
<td>W. A. Shipton</td>
<td>AY793432</td>
</tr>
<tr>
<td>Cylindrocladiella parviana</td>
<td>CPC 373</td>
<td>Pinus radiata</td>
<td>South Africa</td>
<td>P. W. Crous</td>
<td>AY793433</td>
</tr>
<tr>
<td>Cylindrocladium floridumum</td>
<td>ATCC 18834²</td>
<td>Ants</td>
<td>Brazil</td>
<td>M. P. Herrera</td>
<td>AY793434</td>
</tr>
<tr>
<td>Cylindrocladium multiphialdicum</td>
<td>CBS 112678³</td>
<td>Musa sp.</td>
<td>Cameroon</td>
<td>Abadie</td>
<td>AY793435</td>
</tr>
<tr>
<td>Cylindrocladium retaeulii</td>
<td>CPC 516</td>
<td>Eucalyptus sp.</td>
<td>Thailand</td>
<td>M. J. Wingfield</td>
<td>AY793436</td>
</tr>
<tr>
<td>Cylindrocladium reteaudii</td>
<td>CPC 759</td>
<td>Eucalyptus sp.</td>
<td>Madagascar</td>
<td>P. W. Crous</td>
<td>AY793437</td>
</tr>
<tr>
<td>Cylindrocarpon celtidis</td>
<td>CBS 111357/CPC 1157</td>
<td>Soil</td>
<td>Argentina</td>
<td>N. Allegrucci</td>
<td>AY793438</td>
</tr>
<tr>
<td>Gliocladiopsis sp.</td>
<td>CBS 111265/CPC 10491</td>
<td>Archontophoenix purpurea</td>
<td>Australia</td>
<td>F. Hill</td>
<td>AY793440</td>
</tr>
<tr>
<td>Gliocladiopsis sumatensis</td>
<td>CBS 111142/CPC 1279</td>
<td>Aracanaria sp.</td>
<td>Malaysia</td>
<td>M. J. Wingfield</td>
<td>AY793441</td>
</tr>
<tr>
<td>Xenocylindrocladium guianense</td>
<td>CBS 112180/MUCL 41976/CPC 3497³</td>
<td>Leaf litter</td>
<td>French Guiana</td>
<td>C. Decock</td>
<td>AY793442</td>
</tr>
</tbody>
</table>

¹ CBS, Centraalbureau voor Schimmelcultures, Utrecht; CPC, Culture collection of Pedro Crous, at CBS; ATCC, American Type Culture Collection, Virginia; IMUR, Instituto de Micologia Universidade, Recife; MUCL, Mycoteque de l’Universite Catholique de Louvain, Louvain-la-Neuve.  
² ITS, internal transcribed spacer region; LSU, partial 28S rDNA gene sequence.  
³ Ex-type cultures.
Fig. 1. One of 20 most parsimonious trees obtained from large subunit sequence data (TL = 249 steps, CI = 0.590, RI = 0.766, RC = 0.452). The scale bar indicates a single change and the numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. The GenBank sequences of Nectria grammicospora and N. sesquicilium (AF193238 and AF193241, respectively) were included as outgroups.

Fig. 2. One of four most parsimonious trees obtained from ITS sequence data (TL = 373 steps, CI = 0.735, RI = 0.877, RC = 0.644). The scale bar indicates a single change and the numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. The GenBank sequences of Bionectria grammicospora and B. sesquicilium (AF210678 and AF210666, respectively) were included as outgroups.
Cylindrocladium, Cylindrocarpon and Gliocladiopsis. Notable differences include the lack of stipe extensions (extending from the stipe that gives rise to the conidiogenous apparatus), and with age, the presence of dark brown setae (forming from cells arranged in a basal stroma), that occur among separate penicillate conidiophores. These morphological features are unique, and this fungus can, therefore, not be accommodated in Cylindrocladium or any Cylindrocladium-like genus (Crous 2002). The unique morphology of this fungus is also supported by its phylogenetic position in the Hypocreales (Fig. 1). A new genus is, therefore, proposed to accommodate this fungus as follows:

Dematiocladium Allegr., Aramb., Cazau & Crous, gen. nov.

Etym.: Dematio-, dematiaceous (pigmented); -cladium, cladus (branch), the root of the similar genus Cylindrocladium.

Cylindrocladio similis sed projectionibus sterilibus vesiculatis carens, setas pigmentatas ferens.


Morphologically similar to Cylindrocladium, but different in lacking stipe extensions with vesicles, and having dark brown setae.

Dematiocladium celtidis Allegr., Aramb., Cazau & Crous, sp. nov. (Figs 3–17)

Etym.: Named after the host on which it was isolated.

Setae e strato parenchymatico oriundae, simplices, flexuosae, 150–400 × 8–10 μm; cellula basilari dilute brunnea, levi, sursum brunnea et verruculosa, crassitunicata; apice acute vel subobtuso. Conidiophora e stipite et penicillo ramorum fertilium composita; pars penicillata 40–80 μm; rami hyalini, leves; terminal branches producing 1–6 phialides. Phialides elongatae, doliiformes vel reniformes vel subcylindricae, continuae, 10–20 × 3–4 μm; apex acute vel subobtusum, carens, setas pigmentatas ferens. Cylindrocladio similis sed projectionibus sterilibus vesiculatis carens, setas pigmentatas ferens.

Host: Celtis tala. Distribution: Argentina.

DISCUSSION

Dematiocladium represents a distinct genus residing in the Hypocreales, and one of a complex of nine genera characterised by hyaline, separate, penicillate conidiophores, and cylindrical conidia (Crous 2002). Although no teleomorph has been found for D. celtidis, its phylogenetic position suggests that if this occurs, it will be a member of the Nectriaceae, morphologically similar to that of Neoneuctria (Cylindrocarpon), Leuconeuctria (Gliocladaliotrichum), Caloneuctria (Cylindrocladium) and Glioneuctria (Gliocladiopsis) (Figs 1–2) (Crous 2002, Halleen et al. 2004).

Colonies of D. celtidis produce significantly fewer chlamydospores and microsclerotia than species of Cylindrocladium. They have colonies that are more distinctly ochreous to umber in colour on MEA, in contrast to those of Cylindrocladium spp., which tend to be more red-brown to cinnamon-buff or sienna. Several genera in this complex are presented in the key.
Figs 3–12. *Dematiocladium celtidis* (CBS 115994). Fig. 3. Colony on MEA. Fig. 4. Hyaline conidiophores on MEA. Fig. 5. Squash mount showing brown setae, and hyaline conidiophores. Figs 6–7. Apices and bases of brown setae. Figs 8–11. Conidiophores, frequently with a single swollen apical cell (Fig. 10), or several swollen primary branches (Fig. 11), that give rise to conidiophore branches. Fig. 12. Conidia. Bars: Figs 5, 8 = 40 μm; Figs 6–7, 9–10, 12 = 13 μm; and Fig. 11 = 10 μm.
of Crous (2002). Conidiophores of D. celtidis appear most similar to those of Cylindrocladium, but they tend to have shorter and more dense branches, which are also distinct in frequently having swollen, globose apical cells. These give rise to up to four levels of branches, terminating in 1–6 phialides. Furthermore, on CLA colonies sporulate within the agar medium. Although some species of Cylindrocladium can form megaconidia (Crous & Seifert 1998) in the agar, none are known to form penicillate conidiophores in the agar as is found in D. celtidis. D. celtidis can be distinguished from other hypocrealean genera by its stromata of pseudo-parenchymatous tissue that give rise to individual, hyaline penicillate conidiophores with cylindrical conidia, as well as by its thick-walled, dark-brown setae. It can readily be distinguished from Cylindrocarpon by its more penicillate conidiophores, and the absence of unbranched conidiophores with a single conidiogenous cell, and abscission scars on micro- and macroconidia (Halleen et al. 2004). Dematiocladium is morphologically also similar to Gliocladiopsis (Schoch et al. 2000), but can be distinguished based on the presence of swollen cells in its conidiogenous apparatus, and dark-brown setae.

The collection of D. celtidis on leaf litter in Argentina represents one of a number of distinct, Cylindrocladium-like genera. This group of hypocrealean genera appears to be well-represented on litter. For example, Curvicoladium was recently collected on leaf litter in French Guinea (Decock & Crous 1998), Gliocladiopsis and Gliocephalotrichum are known from litter of various hosts in tropical regions of the world (Rossman et al. 1999, Schoch et al. 2000), Cylindrocladiella, although plant-pathogenic, is also common on litter (Crous & Wingfield 1993), and Xenocylindrocladium is known from litter collected in Ecuador and Singapore (Decock, Hennebert & Crous 1997, Crous, Decock & Schoch 2001). Like Dematiocladium, most of these genera are only known from one or a few collections. The morphology of these fungi is peripherally similar and suggests convergent evolution of a morphological theme that must facilitate spore dispersal in different but related fungi. The discovery of D. celtidis also suggest that more attention should be given to collecting saprobic microflora occurring on leaf litter in the tropics, as it clearly represents an untapped wealth of new hypocrealean fungi and an important element of mycological biodiversity.
Fig. 17. *Dematiocladium celtidis* (CBS 115994). (A) Penicillate conidiophores exhibiting swollen, apical cells that give rise to conidiophore branches. (B) One-septate conidia. (C) Brown, verruculose setae with obtuse apices from a stroma of pseudoparenchymatous cells. Bars = 10 μm.
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REFERENCES


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