Mycol. Res. **109** (7): 833–840 (July 2005). © The British Mycological Society
doi:10.1017/S0953756205002911 *Printed in the United Kingdom.*

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

Pedro W. CROUS[2]*, Natalia ALLEGRUCCI[1], Angélica Margarita ARAMBARRI[1], María Cecilia CAZAU[1], Johannes Z. GROENEWALD[2] and Michael J. WINGFIELD[3]

[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*

Received 2 November 2004; accepted 28 March 2005.

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).

* Corresponding author.

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 ° 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

Table 1. Isolates sequenced for the present study.

Species	Accession no.[1]	Host	Country	Collector	GenBank accession no.[2] ITS	GenBank accession no.[2] LSU
Curvicladium cigneum	CPC 1595[3]	Leaf litter	French Guiana	C. Decock	AF220973	AY793431
Cylindrocladiella microcylindrica (*Nectricladiella camelliae*)	ATCC 38571/CPC 2375/CBS 111794[3]	Fruit of tree	Australia	W. A. Shipton	AF220960	AY793432
Cylindrocladiella parva	CPC 373	*Pinus radiata*	South Africa	P. W. Crous	AF220965	AY793433
Cylindrocladiella peruviana	CPC 2404/IMUR 1843[3]	Ants	Brazil	M. P. Herrera	AF220966	AY793434
Cylindrocladium floridanum (*Calonectria kyotensis*)	ATCC 18834[3]	*Robinia pseudoacacia*	Japan	T. Terashita	AY793429	
Cylindrocladium multiphialidicum	CBS 112678[3]	*Musa* sp.	Cameroon	Abadie	AF493961	AY793435
Cylindrocladium reteaudii	CPC 516	*Eucalyptus* sp.	Thailand	M. J. Wingfield	AF231970	AY793436
(*Calonectria reteaudii*)	CPC 759	*Eucalyptus* sp.	Madagascar	P. W. Crous	AF231969	AY793437
Dematiocladium celtidis	CBS 115994	*Celtis tala*	Argentina	N. Allegrucci	AY793430	AY793438
Gliocladiopsis sp.	CBS 111038/CPC 1157	Soil	Colombia	M. J. Wingfield		AY793439
	CBS 112365/CPC 10491	*Archontophoenix purpurea*	Australia	F. Hill		AY793440
	CBS 111142/CPC1279	*Araucaria* sp.	Malaysia	M. J. Wingfield		AY793441
Gliocladiopsis sumatrensis	CBS 111198/CPC1352[3]	Soil	Indonesia	M. J. Wingfield		AY793442
Xenocylindrocladium guianense	CBS 112180/MUCL 41976/CPC 3497[3]	Leaf litter	French Guiana	C. Decock	AF317349	AY793443

[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.

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 with similar topology and bootstrap values, but differed from the parsimony analysis in the placement of the branches within the clade containing the *Cylindrocladiella*, *Cylindrocarpon*, *Cylindrocladium* and *Gliocladiopsis* isolates (data not shown). Parsimony analysis of the alignment yielded 20 most parsimonious trees, one of which is Fig. 1. In both the neighbour-joining and parsimony analyses, the fungus from *Celtis* occupies a position as a sister taxon to the clade containing the *Cylindrocarpon* isolates. This relationship, however, does not have strong bootstrap support in any of the analyses. As the phylogenetic placement of the taxa included in the LSU alignment did not provide a high level of resolution for intergeneric placement, the ITS region was also sequenced. For the ITS sequence, approximately 600 bases were determined for the isolates noted in Table 1. The manually adjusted alignment contained 27 taxa (including the two outgroups) and 474 characters including alignment gaps, of which 134 were parsimony-informative, 44 were variable and parsimony-uninformative, and 296 were constant. The topology of the trees generated using neighbour-joining analysis with different substitution models were 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 *Glionectria*/*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

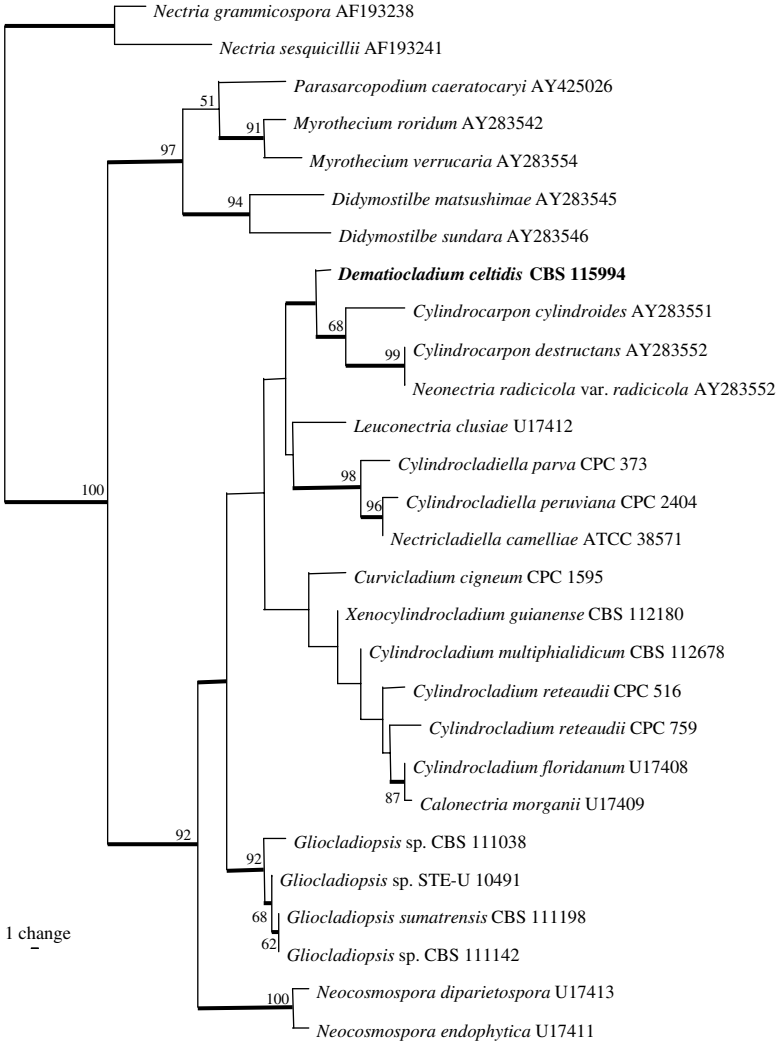


Fig. 1. One of 20 most parsimonious trees obtained from large subunit sequence data (TL = 249 steps, CI = 0.590, RI = 0.766, RC = 0.452). The scale bar indicates a single change and the numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. The GenBank sequences of *Nectria grammicospora* and *N. sesquicilii* (AF193238 and AF210678, respectively) were included as outgroups.

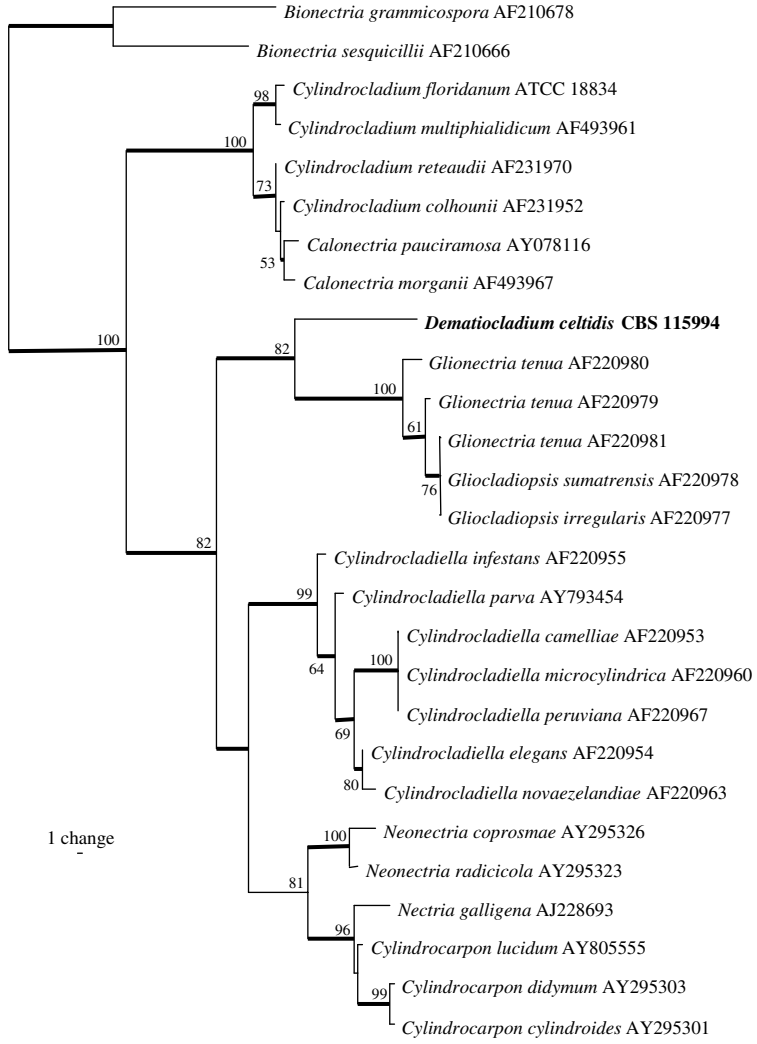


Fig. 2. One of four most parsimonious trees obtained from ITS sequence data (TL = 373 steps, CI = 0.735, RI = 0.877, RC = 0.644). The scale bar indicates a single change and the numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. The GenBank sequences of *Bionectria grammicospora* and *B. sesquicilii* (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 simile sed projectionibus sterilibus vesiculatis carens, setas pigmentatas ferens.

Typus: *Dematiocladium celtidis* Allegr., Aramb., Cazau & Crous 2005.

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 acuto vel subobtuso. Conidiophora e stipite et penicillo ramorum fertilium composita; pars penicillata 40–80 μm longa, 40–60 μm diam; rami hyalini, leves, 0–2-septati; rami distales 1–6 phialides ferentes. Phialides elongatae, doliiformes vel reniformes vel subcylindricae, continuae, 10–20 × 3–4 μm; apex exigue periclinaliter inspissatus et collari inconspicuo praeditus. Conidia cylindrica, utrinque rotundata, recta, hyalina, (30–)38–45(–58) × (3–)3.5(–4) μm (in medio 40 × 3.5 μm), 1(–2)-septata, fasciculis parallelis conglobata.

Typus: **Argentina**: *Buenos Aires*: Punta Indio, on leaf litter of *Celtis tala*, May 2004, *N. Allegrucci* (LPS 47255 – holotypus; CBS 9921 – isotypus, cultura viva CBS 115994).

Setae unbranched, straight to flexuous, 150–400 × 8–10 μm, arising from pseudoparenchymatous cells in a basal stroma, adjacent to cells that give rise to conidiophore stipes; setae brown, verruculose, thick-walled; basal cell initially smooth, becoming medium brown with age, tapering from a base which is either rounded and well-defined, or cylindrical and continuous with the cells in the pseudoparenchymatous stroma, to an acutely or subobtusely rounded apex, which is pale brown, thin-walled towards the apex; apical cell sometimes becoming fertile with age, forming an apical penicillate conidiophore; setae extending beyond the conidiophores, and could thus be mistaken for aesiculate stipe extensions. *Conidiophores* consisting of a stipe, a penicillate arrangement of fertile branches, and rarely, an extension of the stipe, signifying continued growth and eventual branching of the stipe and secondary penicillate conidiophores. *Stipe* septate, hyaline, smooth, brown at the base, arising from tightly arranged pale to medium brown pseudoparenchymatous cells in a basal stroma, 80–350 × 5–6 μm; frequently terminating in a swollen, globose apical cell, 10–15 μm wide, that gives rise to 1–6 primary branches. *Conidiogenous apparatus* 40–80 μm long, 40–60 μm wide; branches hyaline, smooth, 0–2-septate; primary branches subcylindrical to more swollen and doliiform to ellipsoid, 12–50 × 5–7 μm; additional branches (to 4) 10–30 × 5–7 μm; terminal branches producing 1–6 phialides. *Phialides* elongate doliiform to reniform or subcylindrical, straight to slightly curved, aseptate, 10–20 × 3–4 μm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, hyaline, (30–)38–45(–58) × (3–)3.5(–4) μm (av. 40 × 3.5 μm), 1(–2)-septate, lacking a visible abscission scar, held in parallel clusters by colourless slime. *Chlamydospores* globose, 12–17 μm wide, thick-walled, medium brown, arranged in intercalary mycelial chains.

Cultures: Colonies 18–19 mm diam on MEA after 6 d in the dark at 25 °. Colonies even with smooth, regular margins; aerial mycelium mostly sparse, but colonies appearing fluffy due to long stipes, which develop terminal conidiophores, forming a raised slimy layer above the colony surface. Colonies cream to white (surface), and ochreous (15′b) to umber (15″i) or cinnamon (15″b) (reverse).

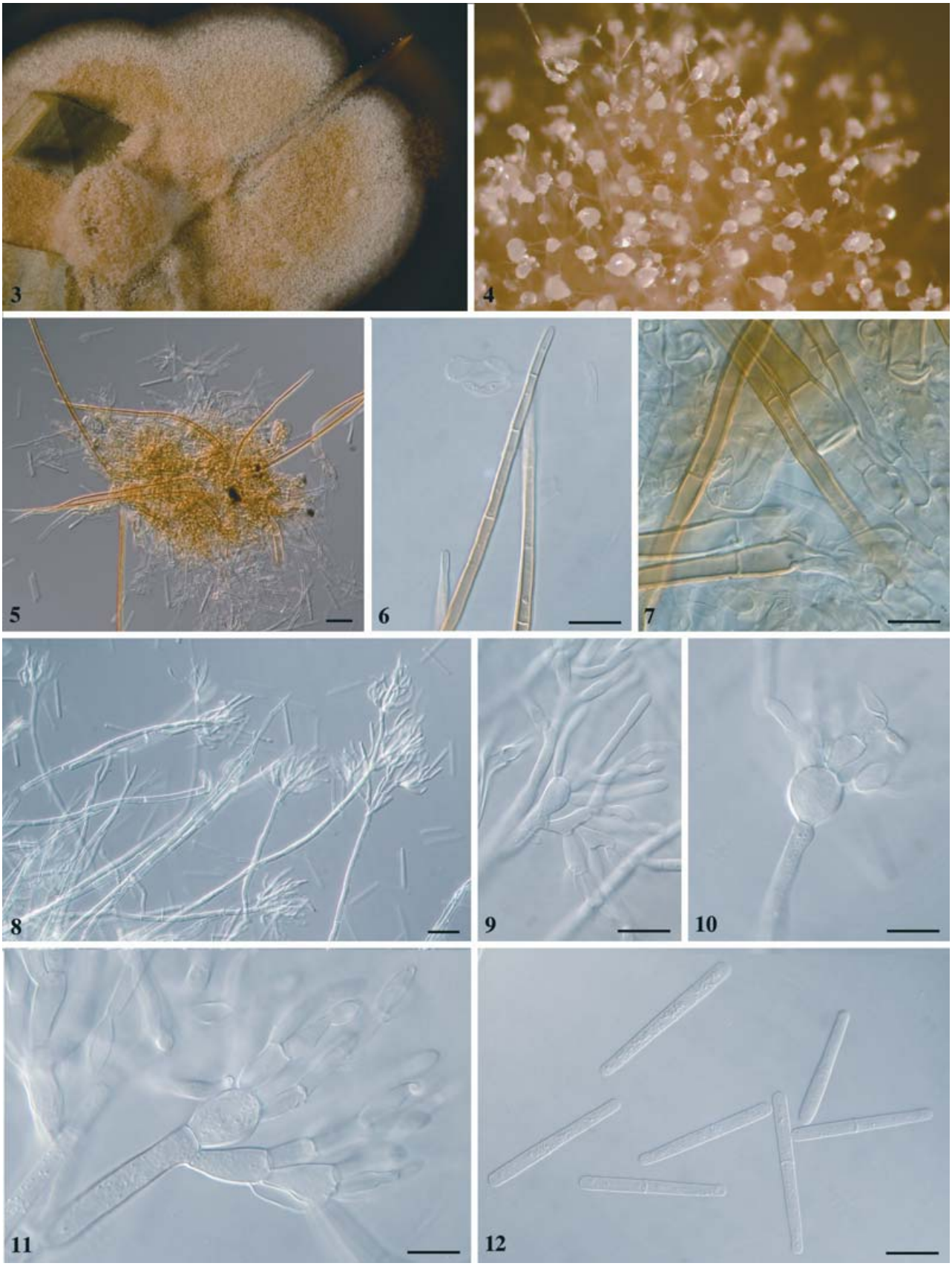
Host: *Celtis tala*.

Distribution: Argentina.

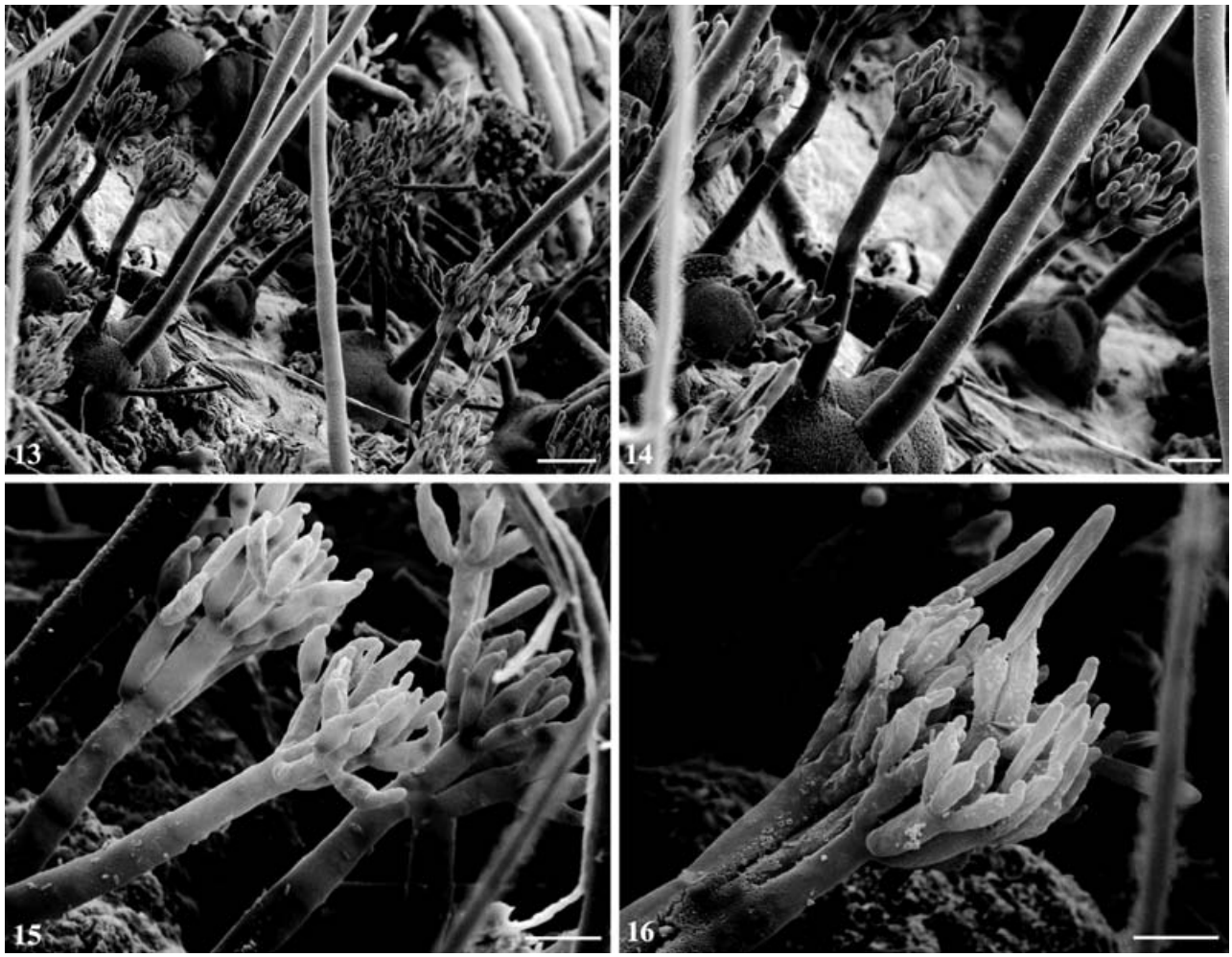
DISCUSSION

Dematiocladium represents a distinct genus residing in the *Hypocreaceae*, 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 *Neonectria* (*Cylindrocarpon*), *Leuconectria* (*Gliocephalotrichum*), *Calonectria* (*Cylindrocladium*) and *Glionectria* (*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.



Figs 13–16. *Dematiocladium celtidis* (CBS 115994): penicillate conidiophores and setae *in vivo* on *Celtis tala*. Bars: Fig. 13 = 10 μm; and Figs 14–16 = 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 pseudoparenchymatous 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, *Curvicladium* 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. celtis* 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.

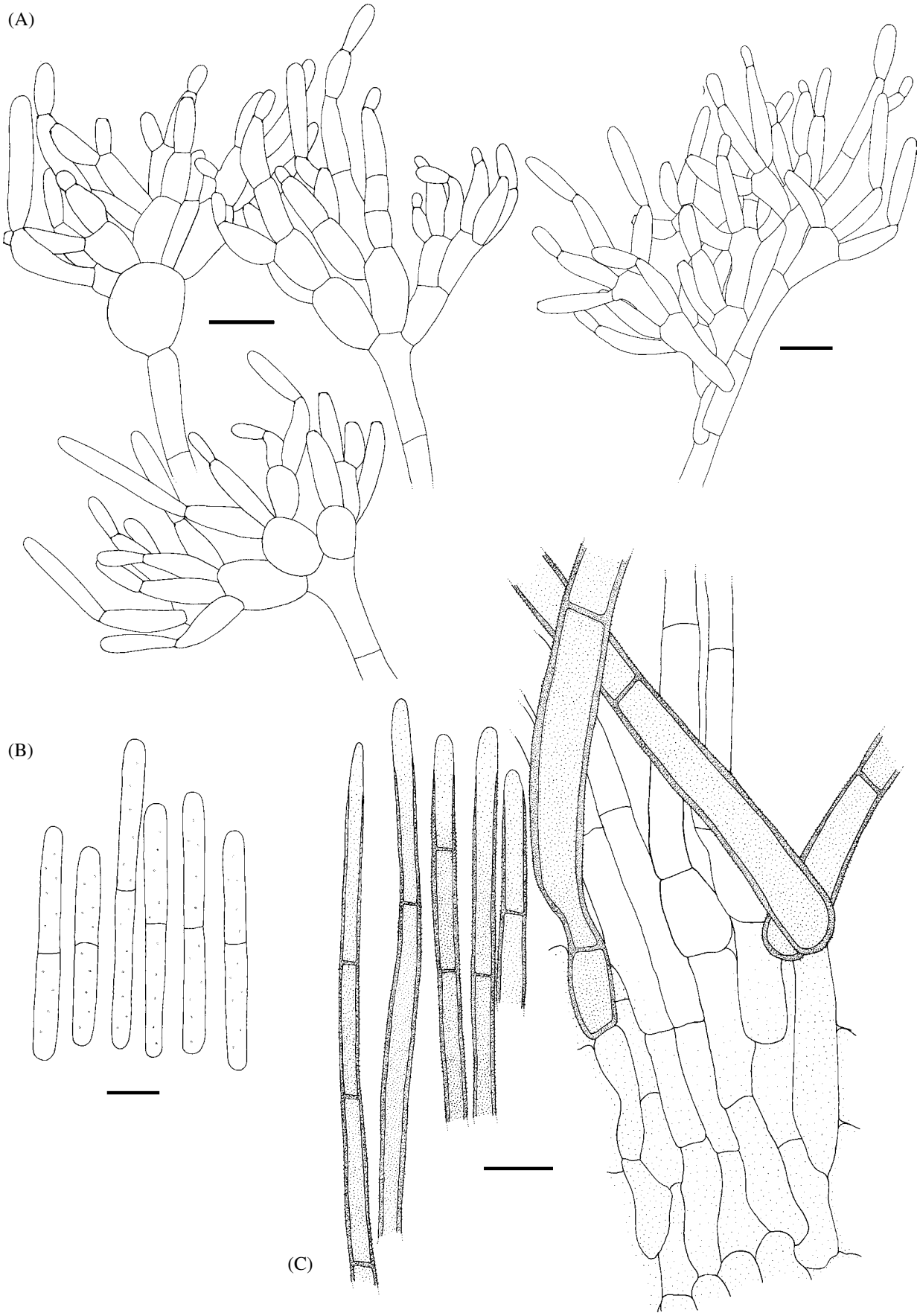


Fig. 17. *Dematiocladium celtidis* (CBS 115994). (A) Penicillate conidiophores exhibiting swollen, apical cells that give rise to conidiophore branches. (B) One-septate conidia. (C) Brown, verruculose setae with obtuse apices from a stroma of pseudoparenchymatal cells. Bars = 10 μm.

ACKNOWLEDGEMENT

Walter Gams is thanked for providing the Latin diagnosis.

REFERENCES

Crous, P. W. (2002) *Taxonomy and Pathology of Cylindrocladium* (*Calonectria*) *and allied genera*. American Phytopathological Society Press, St Paul, MN.

Crous, P. W., Decock, C. & Schoch, C. L. (2001) *Xenocylindrocladium guianense* and *X. subverticillatum*, two new species of hyphomycetes from plant debris in the tropics. *Mycoscience* **42**: 559–566.

Crous, P. W., Groenewald, J. Z., Risède, J.-M., Simoneau, P. & Hywel-Jones, N. L. (2004) *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Studies in Mycology* **50**: 415–430.

Crous, P. W., Phillips, A. J. L. & Wingfield, M. J. (1992) Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. *Mycologia* **84**: 497–504.

Crous, P. W. & Seifert, K. A. (1998) Megaconidia as an additional taxonomic character in *Cylindrocladium*, with a note on *Cylindrocladiopsis*. *Fungal Diversity* **1**: 51–62.

Crous, P. W. & Wingfield, M. J. (1993) A re-evaluation of *Cylindrocladiella*, and a comparison with allied genera. *Mycological Research* **97**: 433–448.

Crous, P. W. & Wingfield, M. J. (1994) A monograph of *Cylindrocladium*, including anamorphs of *Calonectria*. *Mycotaxon* **51**: 341–435.

Decock, C. & Crous, P. W. (1998) *Curvicladium* gen. nov., a new hyphomycete genus from French Guiana. *Mycologia* **90**: 276–281.

Decock, C., Hennebert, G. L. & Crous, P. W. (1997) *Nectria serpens* sp. nov. and its hyphomycetous anamorph *Xenocylindrocladium* gen. nov. *Mycological Research* **101**: 786–790.

Halleen, F., Schroers, H.-J., Groenewald, J. Z. & Crous, P. W. (2004) Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). *Studies in Mycology* **50**: 431–455.

Lee, S., Groenewald, J. Z. & Crous, P. W. (2004) Phylogenetic reassessment of the coelomycete genus *Harknessia* and its teleomorph *Wuestneia* (*Diaporthales*), and the introduction of *Apoharknessia* gen. nov. *Studies in Mycology* **50**: 235–252.

Rayner, A. W. (1970) *A Mycological Colour Chart*. Commonwealth Mycological Institute, Kew.

Rossman, A. Y. (1979) *Calonectria* and its type species, *C. daldiniana*, a later synonym of *C. pyrochroa*. *Mycotaxon* **8**: 321–328.

Rossman, A. Y., Samuels, G. J., Rogerson, C. T. & Lowen, R. (1999) Genera of *Bionectriaceae*, *Hypocreaceae* and *Nectriaceae* (*Hypocreales*, *Ascomycetes*). *Studies in Mycology* **42**: 1–248.

Schoch, C. L., Crous, P. W., Wingfield, B. D. & Wingfield, M. J. (2001) Phylogeny of *Calonectria* based on β-tubulin DNA sequence comparisons. *Mycological Research* **105**: 1045–1052.

Schoch, C. L., Crous, P. W., Wingfield, M. J. & Wingfield, B. D. (2000) Phylogeny of *Calonectria* and selected hypocrealean genera with cylindrical macroconidia. *Studies in Mycology* **45**: 45–62.

Corresponding Editor: D. L. Hawksworth