Mycoscience 42: 51–57, 2001

Phylogenetic confirmation of *Calonectria spathulata* and *Cylindrocladium leucothoes* based on morphology, and sequence data of the β-tubulin and ITS rRNA genes

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Accepted for publication 30 October 2000

A *Calonectria* sp. forming a *Cylindrocladium* anamorph was found to be commonly associated with leaf spot and cutting rot of *Eucalyptus* in Brazil, Colombia, Ecuador and Argentina. Based on morphology, isolates of this species resembled *Cal. hederae*, *Cal. reteaudii* and *Cy. leucothoes*, taxa that are commonly referred to as the *Cal. reteaudii*-complex. Using DNA sequence data of the ITS1, 5.8S and ITS2 of the rRNA gene, as well as the β-tubulin gene, this species was shown to represent a previously described, but rather poorly known species, *Cal. spathulata*, for which an emended description is provided. Furthermore, the recently described *Cy. perseae* was also shown to be synonymous with *Cy. leucothoes*, a species shown to be distinct from *Cy. spathulatum*. Based on vesicle and conidium morphology, as well as perithecial colour and ascospore morphology, it appears that *Cal. hederae* and *Cal. reteaudii* should also be retained as separate species. Additional collections and cultures would be required, however, to suitably characterise the latter two species in future studies.

Key Words——*Calonectria*; *Cylindrocladium*; fungal plant pathogen; Hypocreales; *Eucalyptus*.

Since 1993, a devastating *Cylindrocladium* leaf spot disease was noticed to occur in eucalypt plantations in South America, particularly in Brazil, Ecuador, Colombia, and recently also in Argentina. Morphologically this species closely resembled others placed in the *Calonectria reteaudii* (Bugnic.) C. Booth species complex, into which the Brazilian collections of this species were provisionally placed by Crous and Wingfield (1994). Recently, however, Schubert et al. (1999) described yet another species that resembled this complex, namely *Cylindrocladium perseae* T. S. Schub., Leahy & El-Gholl. The *Cal. reteaudii*-complex was reviewed by Crous and Wingfield (1992), who found that, in the absence of cultures, there was insufficient evidence to distinguish the species occurring in this complex, namely *Cal. reteaudii* [anamorph: *Cylindrocladium reteaudii* (Bugnic.) Boesew.], *Cal. hederae* C. Booth & J.S. Murray (anamorph: *Cy. hederae* Peerally) and *Cy. leucothoes* El-Gholl, Leahy & T.S. Schub. (teleomorph: unknown). A further species that resembles this complex is *Cal. spathulata* El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult. (anamorph: *Cy. spathulatum* El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult.), though this species generally has smaller, 1(–3) septate conidia (Crous and Wingfield, 1994).

Species of *Cylindrocladium* are mostly distinguished based upon their conidium and phialide morphology, as well as width and shape of their terminal vesicles. Those species that form *Calonectria* teleomorphs, also have features such as ascus and ascospore morphology, as well as their perithecial morphology and anatomy to aid in their identification. Species in the *Cy. reteaudii*-complex are broadly characterised by having clavate to ellipsoidal or ovoid to subglobose vesicles, (1–)3(–6)-septate conidia, and orange to red perithecia with (1–)3-septate ascospores. The aim of the present study was to identify the *Cylindrocladium* species that was associated with the prominent leaf spot disease in South America, and to re-examine the *Cy. reteaudii*-complex based on the new collections and cultures that have been obtained in the last few years.

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Materials and Methods

Isolates and morphology The *Cylindrocladium* isolates studied are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa (STE-U). Isolates were cultured on 2% malt extract agar (MEA) (Oxoid), plated onto carnation-leaf agar (CLA) (Fisher et al., 1982; Crous et al., 1992), incubated at 25°C under near-ultraviolet light, and examined after 7 d. Observations were made from conidiophores sporulating on carnation leaves. Mounts were prepared in lactophenol, and measurements made at × 1000. Perithecia were re-hydrated in 3% KOH, and sectioned with a Leica CM 1100 cryostat freezing microtome. Sections (10 μm thick) were mounted in distilled water, and examined microscopically. The 95% confidence intervals were derived from 30 observations, and the minimum and maximum values given in parentheses.

PCR amplification and sequencing Genomic DNA was

isolated from fungal mycelia collected from the plates using the isolation protocol of Lee and Taylor (1990). Template DNA (20 ng) was amplified in a 25 μl PCR reaction mixture consisting of 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 6 mM $MgCl_2$, and 500 μM each of dATP, dCTP, dGTP, and dTTP, with 60 pmols ITS1 and ITS4 primers (White et al., 1990) or T1 (O'Donnell and Cigelnik, 1997) and bt2b (Glass and Donaldson, 1995) primers, and 2.5 units Biotaq (Bioline, London, UK) DNA polymerase. The reaction was set up as follows: initial denaturation at 96°C for 2 min, followed by 30 cycles of denaturation at 96°C for 15 s, annealing at 55°C for 30 s, extension at 75°C for 35 s, and final extension at 75°C for 2 min in a Rapidcycler (Idaho Technology Idaho, USA). A negative control using water instead of template DNA was set up for each experiment. PCR products were analysed by electrophoresis at 75 V for 2 h in a 0.8% (w/v) agarose gel in 0.5× TAE buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light in a transilluminator (TFX-35C,Vilber Lourmat) following ethidium bromide staining.

PCR products were purified by using a QIAquick PCR Purification Kit (Qiagen GmbH, Germany). The purified PCR products were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). The cycle sequencing reaction with 20 to 40 ng of DNA template and 3.2 pmol primer in a total volume of 10 μl was carried out with a Dye Terminator Cycle Sequencing Ready Reaction Kit containing AmpliTaq DNA Polymerase (Perkin-Elmer). The reaction was set up as 25 cycles of 96°C for 30 s, 50°C for 15 s, and 60°C for 4 min. The resulting fragments were finally purified using Centri-Sep Spin columns (Princeton Separations, Adelphia, New Jersey) and loaded onto the sequencing gel.

Phylogenetic analysis The nucleotide sequences of the ITS and β-tubulin genes of this study and those of the outgroup, *Fusarium proliferatum* (Matsush.) Nirenberg (GenBank accession numbers: X94171 and U34558) were assembled using Tex-Edit Plus (Bender, 1995; TomBB@aol.com). Alignments of the sequence files were conducted using the CLUSTAL W software (Thompson et al., 1994). Adjustments for improvement were made by eye where necessary. Alignment gaps were coded as missing data in the analysis. Phylogenetic analyses were performed with PAUP* version 4.0b2a (Swofford, 1999). The most parsimonious trees were determined from the original sequence data set using the branch and bound option. The robustness of the trees was evaluated by 1000 bootstrap replications (Hillis and Bull, 1993). Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) were also calculated.

Results

Morphology A re-examination of the representative type specimens revealed that *Cal. hederae* would be best treated as distinct from *Cal. reteaudii*, as the former tends to have ellipsoid to obpyriform vesicles and orange-red perithecia, whereas *Cal. reteaudii* has dark red perithecia (Bugnicourt, 1939), and predominantly clavate vesicles. Cultures representing the *Eucalyptus* pathogen from South America were similar to others of *Cal. spathulata* in perithecium, conidium and vesicle morphology. The exception in this regard was the type culture of *Cal. spathulata*, which rarely formed 3-septate conidia. Cultures of *Cy. perseae* were indistinguishable from those of *Cy. leucothoes* in vesicle morphology, conidium septation and dimensions. They differed, however, in that *Cy. perseae* primarily formed curved conidia, as well as a microconidial state which were both absent in *Cy. leucothoes*.

Alignment of nucleotide sequences For each isolate, approx 530 bases of the 5.8S rRNA gene and the flanking internal transcribed spacers (ITS1 and ITS2) and 550 bases of the 5' end of the β-tubulin gene were determined. The manually adjusted alignments of the nucleotide sequences contained 504 and 575 sites for the two data sets, respectively (data not shown). Of the 504 aligned nucleotide sites for the ITS data set, only 5 parsimony-informative characters were found. The β-tubulin gene data set, on the other hand, contained 120 parsimony-informative characters out of the total 575 aligned sites.

Phylogenetic relationships The manually aligned 11 sequences of the ITS and β-tubulin genes were subjected to maximum parsimony analysis using branch and bound option in PAUP (Swofford, 1999). The majority consensus tree of 3 equally most parsimonious trees obtained from the branch and bound search was evaluated with 1000 bootstrap replications. The result of a partition-homogeneity test showed that the ITS and β-tubulin gene data sets were combinable (P=0.928). The maximum parsimony analysis of the joint data set generated 33 equally most parsimonious trees using branch and bound option in PAUP (Swofford, 1999). The topology of the majority consensus tree (Fig. 1), which was concordant with the β-tubulin gene tree, clearly segregated the *Cal. spathulata* and *Cy. leucothoes* with 100% bootstrap support. Neighbor-joining and Maximum-likelihood programmes in PAUP were also used for the analyses of the joint data sets, which produced identical topologies to that of the parsimonious trees.

Discussion

As stated previously (Crous and Wingfield, 1992) there is little to choose between the species occurring in the *Cal. reteaudii*-complex based on their anamorphs. All these taxa are known to produce conidia that are (1–)3(–6)-septate, 40–110× 5–7 μm in size. Vesicles were reported to range from clavate to subglobose (*Cy. reteaudii*), clavate to oval (*Cy. hederae*), oval to ellipsoid (*Cy. leucothoes*) or umbonate (*Cy. perseae*) (Boesewinkel, 1982; El-Gholl et al., 1989; Peerally, 1991; Schubert et al., 1999). The exception is *Cy. spathulatum*, which is known to have 1(–3)-septate conidia, 48–75× 4–6 μm, and clavate to spathulate vesicles (El-Gholl et al., 1986; Crous and Wingfield, 1994). Although the vesicles

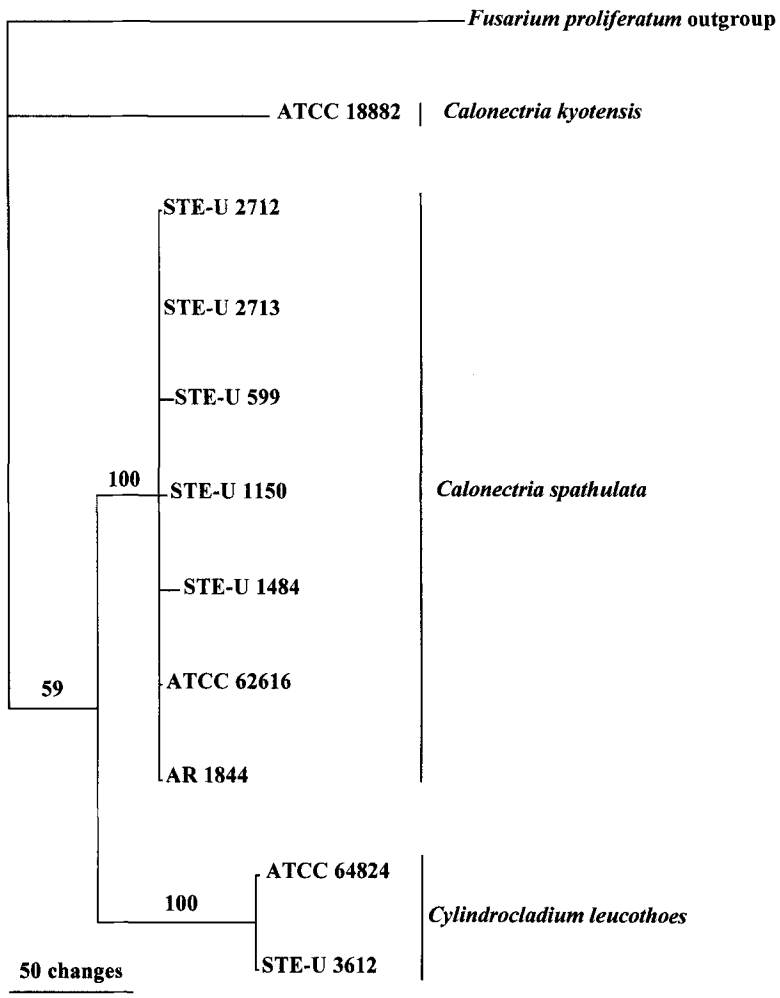

Fig. 1. The majority consensus tree of 33 most parsimonious trees derived from the alignment of approx 530 bases of the ITS1, 5.8S and ITS2 rDNA and 550 bases of the 5′ end of the β-tubulin gene of 10 *Cylindrocladium* isolates using parsimony analysis with the branch and bound option and 1000 bootstrap replicates. The tree is rooted with the outgroup *Fusarium proliferatum* (GenBank Accession: X94171). The tree length = 503, CI = 0.932, RI = 0.785, RC = 0.732.

resemble those of other species in this complex, this taxon would normally not be compared to taxa in the *Cal. reteaudii*-complex due to its smaller, primarily 1-septate conidia.

The absence of cultures for most of these species, as well as depleted type specimens, have made it impossible to determine the range of variation present in the vesicles of *Cy. reteaudii* and *Cy. hederae*. In our re-examination of these type specimens, the few vesicles observed for *Cy. hederae* (IMI 39232, type of anamorph) were ellipsoid. The teleomorph type specimen of *Cal. hederae* (IMI 75300), which was collected by Booth and Murray (1960), had vesicles which were clavate to ovoid or ellipsoid. It must be noted, however, that this specimen consists of dried down agar cultures, which usually results in vesicles having wider diameters than that observed on CLA.

The type material of *Cal. reteaudii*, which was deposited at Herb. Paris (PC), has been discarded (J. Mouchacca, pers. comm.), and the only remaining material is a dried down agar culture at IMI. No previous examinations of this material were successful in locating any vesicles (Bugnicourt, 1939; Booth, 1966; Peerally, 1991; Crous and Wingfield, 1992). When Boesewinkel (1982) transferred this species into *Cylindrocladium*, however, he noted that vesicles were clavate to subglobose, 5–7 μm wide. The exact status of this species is uncertain at present and further collections and cultures would be required to suitably characterise this pathogen.

No *Calonectria* teleomorphs are known for *Cy. leucothoes* and *Cy. perseae*. *Cal. reteaudii* is reported to have deep red perithecia that become darker, red-brown, with asci that are (88–)130(–154) × (7–)11(–15), and ascospores that are 1(–3)-septate, (28–)57(–69) × (4–)5(–6) μm (Bugnicourt, 1939). *Cal. hederae* (IMI 75300) was reported to have yellowish red to red perithecia, asci 160–180 × 24–40 μm, and ascospores (1–)3-septate, 45–65 × 6–8 μm (Booth and Murray, 1960). Peerally (1974) reported ascospores to be 33.5–69 × 4.5–7 μm. A re-examination of IMI 75300 found perithecia to be

Table 1. Isolates of species of *Calonectria* and *Cylindrocladium* studied.

Teleomorph	Anamorph	No.[a]	Collector	Substrate	Origin	Date isolated	GenBank (ITS)	GenBank (β-tubulin)
Cal. kyotensis	*Cy. floridanum*	ATCC 18882[b]	R.H. Morrison	Peach roots	Florida, U.S.A.	1967	AF307343	AF308457
Unknown	*Cy. leucothoes*	ATCC 64824[b]	N.E. El-Gholl	*Leucothoe axillaris*	Florida, U.S.A.	1988	AF307351	AF308465
Unknown	*Cy. perseae*[c]	STE-U 3612[b]	N.E. El-Gholl	*Leucothoe* sp.	Florida, U.S.A.	1997	AF307352	AF308466
Cal. spathulata	*Cy. spathulatum*	ATCC 62616[b]	N.E. El-Gholl	*Eucalyptus viminalis*	Brazil	1985	AF307349	AF308463
		STE-U 2806	A.C. Alfenas	*Eucalyptus grandis*	Argentina	1999	–	–
		AR 1844	C.S. Hodges	*Eucalyptus grandis*	Brazil	1974	AF307350	AF308464
		PPRI 4742	C.S. Hodges	*Eucalyptus* sp.	Brazil	1974	–	–
		PPRI 4743	C.S. Hodges	*Pteridium* sp.	Brazil	1974	–	–
		PPRI 4744	C.S. Hodges	*Araucaria angustifolia*	Brazil	1973	–	–
		STE-U 599	P.W. Crous	*Eucalyptus saligna*	Brazil	1993	AF307346	AF308460
		STE-U 598	C. Auer	*Eucalyptus dunnii*	Brazil	1993	–	–
		STE-U 611	C. Auer	*Ilex paraguariensis*	Brazil	1993	–	–
		STE-U 2712–2717	M.J. Wingfield	*Eucalyptus grandis*	Colombia	1998	AF307344, 307345	AF308458, 308459
		STE-U 1150, 1069–1072, 1142–1150, 1165–1168	M.J. Wingfield	*Eucalyptus grandis*	Colombia	1995	AF307347	AF308461
		STE-U 1151, 1152, 1155, 1156, 1158	M.J. Wingfield	*Eucalyptus* sp.	Colombia	1996	–	–
		STE-U 2846–2851	M.J. Wingfield	*Eucalyptus grandis*	Colombia	2000	–	–
		STE-U 1484, 1631	M.J. Wingfield	Soil	Ecuador	1997	AF307348	AF308462

[a] ATCC – American Type Culture Collection, Virginia, U.S.A.;
A.R. – A.Y. Rossman, United States Department of Agriculture, A.R.S., Beltsville, Maryland, U.S.A.;
STE-U – Department of Plant Pathology, Univ. of Stellenbosch, Stellenbosch, South Africa, and
PPRI – National Collection of Fungi, Pretoria, South Africa (PREM).
[b] Ex–type culture.
[c] A synonym of *Cy. leucothoes*.

Table 2. Morphological comparison of species of *Calonectria* and *Cylindrocladium* studied.

Teleomorph	Anamorph	Ascospores		Vesicles	Conidia		Reference
		Septation	Length × Width (μm)		Septation	Length × Width (μm)	
Cal. hederae	*Cy. hederae*	(1–)3	33.5–69 × 5–7	Clavate to ovoid or ellipsoid	(1–)3(–5)	44–102 × 3.5–9	Peerally (1974)
Unknown	*Cy. leucothoes*		—	Ellipsoid to obpyriform	(1–)3(–6)	(45–)68–78(–97) × (4–)5–5.5(–6.5)	Present study
Cal. spathulata	*Cy. spathulatum*	1(–3)	(38–)45–55(–60) × (4.5–)5–6(–7)	Ellipsoid to obpyriform or clavate	(1–)3(–6)	(60–)75–90(–100) × 5–6	Present study
Cal. reteaudii	*Cy. reteaudii*	1(–3)	28–69 × 4–6	Not observed	(1–)3(–6)	36–68 × 4.5–7.5	Bugnicourt (1939)

orange to red, and ascospores to be (1–)3-septate, 34–50 × 5.5–6.5 μm. Although *Cal. reteaudii* was reported to have predominantly 1-septate ascospores, in contrast to those of *Cal. hederae* that are 3-septate, there is considerable overlap in their ascospore dimensions. The biggest distinguishing factor, however, is the predominantly orange-red perithecia of *Cal. hederae*, compared to the dark red perithecia of *Cal. reteaudii*. Perithecia of the *Eucalyptus* pathogen are orange at maturity, becoming dark orange-red to red-brown with age. Ascospores are (1–)3-septate, (38–)45–55(–60) × (4.5–)5–6(–7) μm, thus closely resembling those of *Cal. hederae*. However, conidia are (60–)75–90(–100) × 5–6 μm, and vesicles are clavate or ellipsoid to obpyriform, thus being distinct from that of *Cy. hederae*, which are 42–102 × 5.5–8 μm, and ovoid to clavate or ellipsoid in shape, respectively. Based on these differences, we were of the opinion that the *Eucalyptus* pathogen from South America represented a new species. Much to our surprise, however, this species had an identical sequence alignment (ITS and β-tubulin) to other sequences obtained for *Cy. spathulatum*. In a previous study (Crous et al., 1993) we delineated several isolates of *Cy. spathulatum* based on isozymes and A+T-rich DNA profiles. In the present study, the identity of these isolates was once again confirmed based on DNA sequence analysis. However, although most isolates could be induced to form larger conidia resembling those of the present *Eucalyptus* pathogen, the type of *Cy. spathulatum* (ATCC 62616) rarely formed 3-septate conidia, and if formed, these were still significantly smaller than that observed in the present study. Based on molecular data, *Cy. spathulatum* is clearly the name applicable to our pathogen. In agreement with this study, *Cy. spathulatum* was originally described as a leaf spot pathogen of *Eucalyptus viminalis*, *E. cloeziana* and *E. grandis* from Brazil (El-Gholl et al., 1986). Furthermore, several isolates have recently been collected from eucalypts in Brazil that lack the ability to express the full range of morphological variation now known to exist for this species. Our molecular data suggest that the type culture of *Cy. spathulatum* is merely a representative from the edge of the population, and for some reason it has lost the ability to form large macroconidia and microconidia. Based on the molecular data derived from this study, we therefore propose an emended description for this species below:

Calonectria spathulata El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult., Mycotaxon **26**: 159. 1986, emend. Crous

Anamorph: ***Cylindrocladium spathulatum*** El-Gholl, Kimbr., E.L. Barnard, Alfieri & Schoult., Mycotaxon **26**: 159. 1986, emend. Crous

Perithecia orange, becoming orange-red to red-brown once dried down, subglobose to ovoid, 300–500 μm high, 200–350 μm wide, base red-brown, situated on a stroma of pseudoparenchymatal cells; body becoming red in 3% KOH, base becoming dark red-brown; ostiole papillate, same color as perithecial body; perithecia rough walled, wall consisting of two layers; outside layer of thick-walled textura globosa, 20–50 μm wide, cells 10–30 × 10–25 μm; inner layer consisting of flattened, thick-walled cells of textura prismatica, hyaline, 10–20 μm wide, cells 10–20 × 3–6 μm; perithecial base up to 200 μm wide, consisting of dark red-brown, angular cells. Asci 8-spored, clavate, 90–150 × 13–17 μm, tapering to a long thin stalk. Ascospores aggregated in the upper third of the ascus, hyaline, fusoid with rounded ends, becoming finely guttulate with age, straight to slightly curved, (1–)3-septate, not constricted at septa, or slightly constricted at median septum, becoming constricted once discharged, (38–)45–55(–60) × (4.5–)5–6(–7) μm. Macroconidiophores comprised of a stipe, a terminal vesicle and a penicillate arrangement of fertile branches. Stipe septate, 150–300 μm long, terminating in an ellipsoid to obpyriform or clavate vesicle, 6–7(–10) μm wide; primary branches 0–1-septate, 15–35 × 5–6 μm; secondary branches aseptate, 15–25 × 4–5 μm; tertiary branches aseptate, 15–20 × 4–5 μm, each terminal branch producing 2–6 phialides; phialides cylindrical, or doliiform to reniform, hyaline, aseptate, 10–15 × 3.5–5 μm, apex with minute periclinal thickening, and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, (60–)75–90(–100) × 5–6 μm, (1–)3(–6)-septate, lacking a visible abscission scar, held in cylindrical clusters with colourless slime. Microconidiophores comprised of a stipe, a terminal vesicle and penicillate arrangement of fertile branches. Stipe septate, hyaline, terminating in an ellipsoidal to obpyriform vesicle. Conidiophore branches: primary branches non-septate, 10–20 × 4–6 μm; secondary branches non-septate, 10–15 × 4–5 μm. Phialides arising singly from the ends of branches, or arranged in groups of 2–4, cylindrical to doliiform, hyaline, non-septate, 10(–15) × 3(–4) μm, collarettes minute. Microconidia cylindrical, hyaline, 1-septate with obtuse, frequently slightly swollen ends, (40–)50–58 × (3–)4–5 μm. Chlamydospores dark brown, thick-walled, formed in extensive numbers throughout the medium, and aggregated to form microsclerotia. Megaconidia not observed.

Holotype: BRAZIL. Santa Catarina: living leaves of *Eucalyptus viminalis*, N.E. El-Gholl, 1983, FTCC 1001, FLAS F54257. Culture ex-type ATCC 62616, STE-U 2384.

Substrates: *Araucaria angustifolia*, *Eucalyptus cloeziana*, *E. grandis*, *E. saligna*, *E. viminalis*, *Pteridium* sp., soil.

Geographic distribution: Argentina, Brazil, Colombia, Ecuador.

Another aspect that this study has clearly shown is the fact that the absence of a microconidial state (i.e. in the ex-type culture of *Cy. leucothoes*) is not informative as an intraspecific character, as many strains of species known to produce these conidial forms (i.e. *Cy. pteridis* F.A. Wolf, *Cy. ovatum* El-Gholl, Alfenas, Crous & T.S. Schub., *Cy. spathulatum* and *Cy. spathiphylli* Schoult., El-Gholl & Alfieri) sometimes do not. The specific cultural conditions required to induce the production of microconidial states is poorly understood, and hence optimis-

ing conditions for their production still needs to be further investigated. As shown in this study with *Cy. spathulatum*, describing species from a single collection can also create taxonomic problems if that collection represents a strain from the outer limits of the population, or has lost the ability to form all the structures characteristic of the specific taxon in question.

Similarily, curved conidia is also an intra- and not interspecific character, and this feature alone is insufficient for justifying the description of new species (i.e. in the ex-type culture of *Cy. perseae*). Recent studies aimed at characterising *Cy. ovatum* populations with large, curved and smaller, straight conidia, respectively, have shown that they still cross to produce fertile progeny, and are also similar based on isozyme analysis (Crous et al., 1998). In species such as *Cy. hawksworthii* Peerally, *Cy. curvatum* Boedijn & Reitsma and *Cy. curvisporum* Crous & D. Victor, however, curved conidia appear to be the norm. Still, it is possible that additional collections of these species will be obtained that have straight conidia, as noted for the other species discussed above. Based on the morphological similarities between *C. leucothoes* and *C. perseae*, as well as the sequence data, the following synonymy is proposed:

Cylindrocladium leucothoes El-Gholl, Leahy & Schubert Can. J. Bot. **67**: 2530. 1989. (as *leucothoeae*).
=*Cylindrocladium perseae* Schubert, Leahy & El-Gholl, Mycotaxon **73**: 474. 1999.

Macroconidiophores comprised of a stipe, a terminal vesicle and a penicillate arrangement of fertile branches. Stipe septate, 160–250 μm long, terminating in an ellipsoid to obpyriform vesicle, 6–11.5 μm wide; primary branches 0–1-septate, 16–55 × 3–7 μm; secondary branches aseptate, 14–36 × 3–6 μm; tertiary branches aseptate, 12–21 × 3–5.5 μm, additional branches (up to 6), 10–19 × 3–5 μm, each terminal branch producing 2–4 phialides; phialides straight cylindrical, or doliiform to reniform, hyaline, aseptate, 10–20 × 3–6 μm, apex with minute periclinal thickening, and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight or curved, (45–)68–78(–97) × (4–)5–5.5(–6.5) μm, (1–)3(–6)-septate, lacking a visible abscission scar, held in cylindrical clusters with colourless slime. Microconidiophores comprised of a stipe and penicillate arrangement of fertile branches. Conidiophore branches: primary branches non-septate, 10–20 × 4–6 μm; secondary branches non-septate, 10–15 × 4–5 μm. Phialides arising singly from the ends of branches, or arranged in groups of 2–4, cylindrical hyaline, non-septate, 10(–15) × 3(–4) μm, collarettes minute. Microconidia cylindrical, hyaline, straight or curved, 1(–3)-septate, (14–)20–30(–43) × (2–)3–4 μm. Chlamydospores dark brown, thick-walled, formed in extensive numbers throughout the medium, and aggregated to form microsclerotia. Megaconidia not observed.
Holotype: U.S.A. Florida: Leaves of *Leucothoeae axillaris*, El-Gholl, Feb. 1988, ATCC 64824, STE-U 2385, P88-490 (ex-type culture).

One of the features used to distinguish species in the present study has been perithecial colour. This feature should also be interpreted with care, however, as it was recently shown that in heterothallic species of *Calonectria* such as *Cal. morganii*, perithecia could range from yellow to orange or red-brown when mated with outliers of the population (Schoch et al., 2000). Such dramatic variation has not yet been observed to occur in homothallic species of *Calonectria*, and more collections and detailed studies are needed to address this issue. Some variation has been reported in *Cy. floridanum* Sobers & Seymour, where perithecia range from orange to red. However, subsequent studies (Victor et al., 1997) have shown that this species is polyphyletic, and that several biological species have in the past been treated under this epithet.

Calonectria spathulata causes a prominent leaf spot and cutting rot disease in *Eucalyptus* plantations and nurseries in Brazil and Colombia. The leaf spot disease, which causes severe defoliation of susceptible clones, is apparently influenced by climate. Furthermore, eucalypt stands in which it proved dominant the one year, would not necessarily have the same *Cylindrocladium* species the following year, but could be dominated by another, namely *C. parasiticum* Crous, M. J. Wingf. & Alfenas, *C. candelabrum* Viégas or *C. pteridis*. This interaction, and the specific climatic conditions that favour one species more than the other, is poorly understood. One obvious difference is the mating system, with some being homothallic, and others being biallelic heterothallic (Crous and Wingfield, 1994). Furthermore, the species that proved to be dominant in the canopy of that specific year, also proved, in most cases, to be the taxon most commonly recovered when soil under the canopies were baited (Crous et al., 1997) during the disease outbreak for *Cylindrocladium* spp. The latter could be explained by spores (sexual and asexual) washed and blown down from the canopy filled with inoculum, landing on the soil, germinating and forming chlamydospores and microsclerotia, which are again easily recovered once these soils are baited. Further research now needs to be directed towards the interaction of the various *Cylindrocladium* spp. occurring in eucalypt plantations, as well as their epidemiology, and the climatic conditions that favour some above others.

Acknowledgements——The authors gratefully acknowledge Dr N.E. El-Gholl (Florida Dept. of Agric., U.S.A.), Profs M.J. Wingfield (Univ. of Pretoria, South Africa) and A.C. Alfenas (Univ. of Viçosa, MG, Brazil) for providing many of the cultures studied, and C.L. Schoch who made β-tubulin gene sequence of *Cy. spathulatum* available for inclusion in this study, and for bringing the similarity in alignment to our attention. The National Research Foundation of South Africa is also acknowledged for financial support.

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