Non-conspecificity of *Cylindrocladium quinqueseptatum* and *Calonectria quinqueseptata* based on a β-tubulin gene phylogeny and morphology

Ji-Chuan Kang, Pedro W. Crous, Kenneth M. Old, and Mark J. Dudzinski

Abstract: *Cylindrocladium quinqueseptatum* Boedijn & Reitsma was originally described from leaf spots of *Hibiscus sabdariffa* L. from Indonesia. This fungus infects many host plants in Southeast Asia and causes severe leaf blight disease of eucalypts. *Calonectria quinqueseptata* Figueiredo & Namek., which was described from leaf spots on *Annona squamosa* L. from Brazil, has been regarded as the teleomorph of *Cy. quinqueseptatum*. Based on morphology and on the phylogeny derived from the DNA sequence of a β-tubulin gene portion spanning several phylogenetically informative introns, the two respective ex-type cultures are shown to be distinct species. Furthermore, *Calonectria reteaudii* (Bugn.) C. Booth (anamorph *Cy. reteaudii* (Bugn.) Boesew.), which was described on *Smithia bequaertii* De Wild. from Vietnam, is shown to be morphologically identical to a comprehensive selection of isolates of *Cy. quinqueseptatum* from Southeast Asia, Australia, and Madagascar. As *Cy. reteaudii* represents an older name for *Cy. quinqueseptatum*, we suggest that the fungus causing widespread damage on eucalypts and other hosts in the above regions be referred to as *Cy. reteaudii*. *Calonectria quinqueseptata* should be retained for the fungus that thus far has been found only in Brazil.

Key words: *Cylindrocladium reteaudii*, *Eucalyptus*, *Hypocreales*, phylogeny, systematics.

Résumé : Le *Cylyndrocladium quinqueseptatum* Boedijn & Reitsma a été originalement décrit à partir de taches foliaires sur l'*Hisbiscus sabdariffa* L. Ce champignon infecte plusieurs plantes hôtes du sud-est asiatique et cause une brûlure foliaire sévère chez les eucalyptus. Le *Calonectria quinqueseptata* Figueiredo & Namek., déjà décrit à partir de taches foliaires sur l'*Annona squamosa* L. au Brésil, a été considéré comme le téléomorphe du *Cy. quinquesepatatum*. Sur la base de la morphologie et de la phylogénie dérivée de la séquence ADN d'une portion de gène d'une ß-tubuline recouvrant plusieurs introns révélateurs sur la phylogénie, les auteurs montrent que les deux ex-cultures types constituent des espèces distinctes. On montre de plus, que le *Calonectria reteaudii* (Bugn.) C. Booth (anamorphe : *Cy. reteaudii* (Bugn.) Boesew.), qui a été décrit sur le *Smithia bequaertii* De Wild. au Vietnam, est morphologiquement identique à une large sélection d'isolats du *Cy. quinqueseptatum* du sud-est asiatique, de l'Australie et de Madagascar. Comme le *Cy. reteaudii* constitue un nom plus ancien pour le *Cy. quinqueseptatum*, les auteurs suggèrent que ce champignon qui cause des dommages étendus sur les eucalyptus et d'autres hôtes dans les régions mentionnées plus haut, soit considéré comme le *Cy reteaudii*. Le *Calonectria quinqueseptata* pourrait être retenu pour le champignon qui a été jusqu'ici trouvé seulement au Brésil.

Mots clés : *Cylindrocladium reteaudii*, *Eucalyptus*, *Hypocreales*, phylogénie, systématique.

[Traduit par la Rédaction]

Introduction

Cylindrocladium leaf blight (CLB) of *Eucalyptus*, caused by *Cylindrocladium quinqueseptatum* Boedijn & Reitsma, is one of the most serious leaf diseases of plantation and nursery eucalypts in India (Sharma and Mohanan 1991*b*) and Vietnam (Booth et al. 2000) and has also been regarded as important on this host in Brazil (Ferreira 1989), northern Australia (Pitkethley 1976; Bolland et al. 1985), Madagascar (Crous and Swart 1995), Indonesia, Malaysia, and Mauritius (Peerally 1974). This disease has become so significant in parts of central and southeastern Vietnam that selection for resistant provenances, families, and clones of *Eucalyptus camaldulensis* Dehnh. is necessary for successful growth of this species (Booth et al. 2000).

Studies aimed at characterizing isolates of CLB have revealed evidence for the existence of physiological strains that differ in virulence towards differential eucalypt provenances (Sharma and Mohanan 1991*b*, 1992). Although various fungicides gave good control when tested against CLB in vitro (Sharma and Mohanan 1991*a*), these control measures are costly, and the eventual solution may lie in selection and resistance breeding (Sharma and Mohanan 1992).

Received March 26, 2001. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on October 16, 2001.

J.-C. Kang and P.W. Crous.[1] Department of Plant Pathology, University of Stellenbosch, Postal Bag X1, Matieland 7602, South Africa.
K.M. Old and M.J. Dudzinski. Commonwealth Scientific and Industrial Research Organization, Forestry and Forest Products, P.O. Box E4008, Kingston, ACT 2604, Canberra, Australia.

[1]Corresponding author (e-mail: pwc@maties.sun.ac.za).

 DOI: 10.1139/cjb-79-10-1241

A species that is morphologically identical to the causal agent of CLB is *Cylindrocladium reteaudii* (Bugn.) Boesew. (teleomorph *Calonectria reteaudii* (Bugn.) C. Booth). The latter species was originally described from Vietnam on leaf spots on *Smithia bequaertii* De Wild. by Bugnicourt (1939). Booth (1966) considered *Cy. reteaudii* to be representative of the genus *Cylindrocarpon* Wollenw. However, Boesewinkel (1982), who examined a dried ex-type culture of this species, found that its conidiophores had stipe extensions terminating in vesicles and, thus, correctly placed it in *Cylindrocladium* Morgan. The dried specimen, however, did not contain any teleomorph material and was generally devoid of sufficient material to make a suitable comparison with other species in the genus, so these aspects were not dealt with further. Crous and Wingfield (1992) widened the concept of the species and reduced several other species to synonymy under *Cy. reteaudii*, namely *Cylindrocladium hederae* Peerally and *Cylindrocladium leucothoes* El-Gholl, Leahy, & T.S. Schub. A recent molecular study comparing isolates of these species, however, found this concept to be too wide (Crous and Kang 2001) and again separated species such as *Cy. hederae*, *Cy. leucothoes*, and *Cylindrocladium spathulatum* El-Gholl, Kimbr., E.L. Barnard, Alfieri, & Schoult. from *Cy. reteaudii*. This study also found that most isolates treated by Crous and Wingfield (1992) as *Cy. reteaudii* were, in fact, *Cy. spathulatum*. Furthermore, a re-examination of the type of *Cy. reteaudii* found it to be morphologically identical to the species causing CLB in Southeast Asia. For the sake of clarity, the causal agent of CLB will be referred to as *Cy. reteaudii* for the remainder of this paper, which is the older and valid name of the *Cylindrocladium* species commonly occurring in Southeast Asia. *Calonectria quinqueseptata* Figueiredo & Namek., the purported teleomorph of the CLB species, was described from Brazil by Figueiredo and Namekata (1967), where it was observed to cause leaf spots of *Annona squamosa* L. and several other hosts. The *Cylindrocladium* anamorph produced by the Brazilian ex-type strain of *Ca. quinqueseptata* is, however, morphologically distinct from *Cy. reteaudii*. The aim of this study, therefore, was to compare isolates of these species, using DNA phylogeny and mating studies, to determine if these data supported our initial morphological observations. A further aim was to resolve the identity of the dominant *Cylindrocladium* species causing CLB of eucalypts in Southeast Asia and to determine if this was, in fact, the same species causing CLB in Brazil.

Materials and methods

Isolates and mating studies

Symptomatic material of CLB was collected, and single conidial cultures were obtained. Isolates (Table 1) were identified according to the methods and keys of Crous and Wingfield (1994). Ex-type strains of *Ca. quinqueseptata* (Centraalbureau voor Schimmelcultures (CBS) accession No. 728.68) and *Cy. quinqueseptatum* (CBS accession No. 582.50) were also obtained for comparison.

To determine whether the collected isolates represented the same biological species, the two ex-type strains of *Cy. quinqueseptatum* and *Ca. quinqueseptata* were mated in all combinations with 19 other isolates of *Cy. reteaudii*, resulting in 210 possible matings (Table 1). Colonized agar plugs (3 mm diameter) were removed from the periphery of actively growing cultures and mated on carnation leaf agar (CLA) (Fisher et al. 1982; Crous et al. 1992) plates as described by Crous et al. (1997). Plates were subsequently incubated for 2 months at 22°C as explained in Schoch et al. (1999). Successful matings were regarded as those isolate combinations that produced perithecia with fertile, exuding ascospores. Twenty single-ascospore cultures were obtained from one fertile perithecium and again mated against each other to determine the segregation of mating type among progeny.

DNA amplification and sequence determination

Single conidium strains were grown on malt extract agar (MEA; Biolab, Midrand, Johannesburg) plates for 7 days. Genomic DNA was isolated from fungal mycelium collected from the plates using the isolation protocol of Lee and Taylor (1990). Template DNA (20 ng) was amplified in a 25-μL polymerase chain reaction (PCR) reaction mixture consisting of 10 mM KCl; 10 mM $(NH_4)_2SO_4$; 20 mM Tris-HCl (pH 8.8); 6 mM $MgSO_4$; 500 μM each of dATP, dCTP, dGTP, and dTTP, with 60 pmol T1 (O'Donnell and Cigelnik 1997) and bt2b (Glass and Donaldson 1995) primers; and 1.5 units Biotaq (Bioline, London, U.K.) DNA polymerase. The reaction and PCR conditions and protocol were as described in Crous and Kang (2001).

Phylogenetic analysis

Sequence data of the β-tubulin gene of isolates studied (Table 1) were assembled using the Tex-Edit Plus programme (available from tombb@aol.com). *Fusarium nygamai* L.W. Burgess & Trimboli (GenBank accession No. U34426) was used as an outgroup. Additional sequences of *Cylindrocladium multiseptatum* Crous & M.J. Wingf.; *Cylindrocladium rumohrae* El-Gholl & Alfenas; *Cylindrocladium heptaseptatum* Sobers, Alfieri, & Knauss; and *Cylindrocladium spathiphylli* Schoult., El-Gholl, & Alfieri (Table 1) were also included for comparison purposes. Sequences were primarily aligned with CLUSTAL W (Thompson et al. 1994) and optimized manually. Alignment gaps were treated as missing data in the analysis. Phylogenetic analysis of aligned DNA sequences was performed using PAUP* version 4.0b2a (Swofford 1999). The most parsimonious trees were determined from the data set using the branch and bound and the heuristic search options with 1000 random sequence input orders with MULPARS on, and TBR branch swapping for, the exact solution. The tree stability was evaluated by 1000 parsimony bootstrap replicates with 1000 random sequence input orders accommodated in PAUP*. The decay indices were also calculated using AutoDecay (Eriksson 1998) to further test the robustness of the branches of the tree. Other measures including tree length, consistency, retention, rescaled consistency, and homoplasy indexes were also calculated. The best-fit maximum-likelihood tree was also calculated with 10 random sequence input orders and global rearrangement to test the parsimonious tree topology. The substitution model was selected for the likelihood setting with a transition/transversion ratio of 2.

Results

Phylogenetic analysis

A portion of approximately 560 base pairs (bp) from the 5′ end of the β-tubulin gene, which includes three phylogenetically informative intron regions, was sequenced. The optimized nucleotide sequence alignment of the β-tubulin gene portion spans 610 sites and contains 108 parsimony-informative characters. The sequences of the isolates studied (Table 1) were deposited at GenBank (accession Nos. AF389829–AF389847), and the alignment at TreeBase (available from http://www.herbaria.harvard.edu/treebase/index.html) with accession Nos. S624 and M963.

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

Species[a]	Accession No.	Substrate	Origin	Mating strategy[d]	Reference
Ca. multiseptata	STE-U 1589[b,e]	*Eucalyptus*	Indonesia	Homothallic	Crous et al. (1998)
(*Cy. multiseptatum*)	STE-U 1602[e]	*Eucalyptus*	Indonesia	Homothallic	Crous et al. (1998)
Ca. quinqueseptata	CBS 728.68[b,e]	*Annona squamosa*	Brazil	Presumed homothallic	Figueiredo and Namekata (1967)
(*Cylindrocladium* sp.)					
Ca. reteaudii	ATCC 16550[e]	*Asplenium* sp.	Solomon Is	Undetermined	Present study
(*Cy. reteaudii*)	CBS 582.50[c,e]	*Hibiscus sabdariffa*	Indonesia	Undetermined	Present study
	STE-U 516[e]	*Eucalyptus* sp.	Thailand	Heterothallic (+)	Present study
	STE-U 759[e]	*Eucalyptus* sp.	Madagascar	Undetermined	Present study
	STE-U 3200	*Eucalyptus camaldulensis*	Vietnam	Undetermined	Present study
	STE-U 3201[e]	*Eucalyptus camaldulensis*	Vietnam	Heterothallic (+)	Present study
	STE-U 3202	*Eucalyptus urophylla*	Australia	Heterothallic (–)	Present study
	STE-U 3203	*Eucalyptus camaldulensis*	Vietnam	Heterothallic (+)	Present study
	STE-U 3204	*Eucalyptus camaldulensis*	Vietnam	Heterothallic (–)	Present study
	STE-U 3205[e]	*Eucalyptus camaldulensis*	Vietnam	Heterothallic (+)	Present study
	STE-U 3208	*Eucalyptus grandis*	Australia	Heterothallic (–)	Present study
	STE-U 3209	*Eucalyptus camaldulensis*	Vietnam	Heterothallic (+)	Present study
	STE-U 3210[e]	*Eucalyptus pellita*	Australia	Heterothallic (–)	Present study
	STE-U 3212	*Eucalyptus camaldulensis*	Australia	Heterothallic (–)	Present study
	STE-U 3213[e]	*Eucalyptus urophylla*	Australia	Heterothallic (–)	Present study
	STE-U 3214[e]	*Eucalyptus camaldulensis*	Vietnam	Heterothallic (–)	Present study
	STE-U 3215	*Eucalyptus urophylla*	Australia	Heterothallic (+)	Present study
	STE-U 3216[e]	*Eucalyptus urophylla*	Australia	Heterothallic (+)	Present study
	STE-U 3217[e]	*Eucalyptus camaldulensis*	Vietnam	Heterothallic (+)	Present study
	STE-U 3218	*Eucalyptus pellita*	Australia	Undetermined	Present study
Ca. rumohrae	UFV 215[e]	*Rumohra adiantiformis*	Panama	Homothallic	El-Gholl et al. (1997)
(*Cy. rumohrae*)	UFV 218[b,e]	*Rumohra adiantiformis*	Panama	Homothallic	Crous et al. (1999)
	STE-U 1603[e]	*Adianthum* sp.	Netherlands	Homothallic	Crous et al. (1999)
Ca. spathiphylli	ATCC 44730[e]	*Spathiphyllum* sp.	Florida, U.S.A.	Heterothallic	Crous and Peerally (1996)
Cy. heptaseptatum	FTCC 1002[e]	*Rumohra adiantiformis*	Florida, U.S.A.	Teleomorph unknown	Crous and Wingfield (1994)
	FTCC 1003[e]	*Rumohra adiantiformis*	Florida, U.S.A.	Teleomorph unknown	Crous and Wingfield (1994)

[a]Species of *Calonectria* with *Cylindrocladium* anamorphs listed in parentheses.
[b]Ex-type strains.
[c]Ex-type strain of *Cy. quinqueseptatum*.
[d]Heterothallic strains of *Cy. reteaudii* indicated by (–) or (+) to designate mating type.
[e]Strains subjected to DNA analysis.

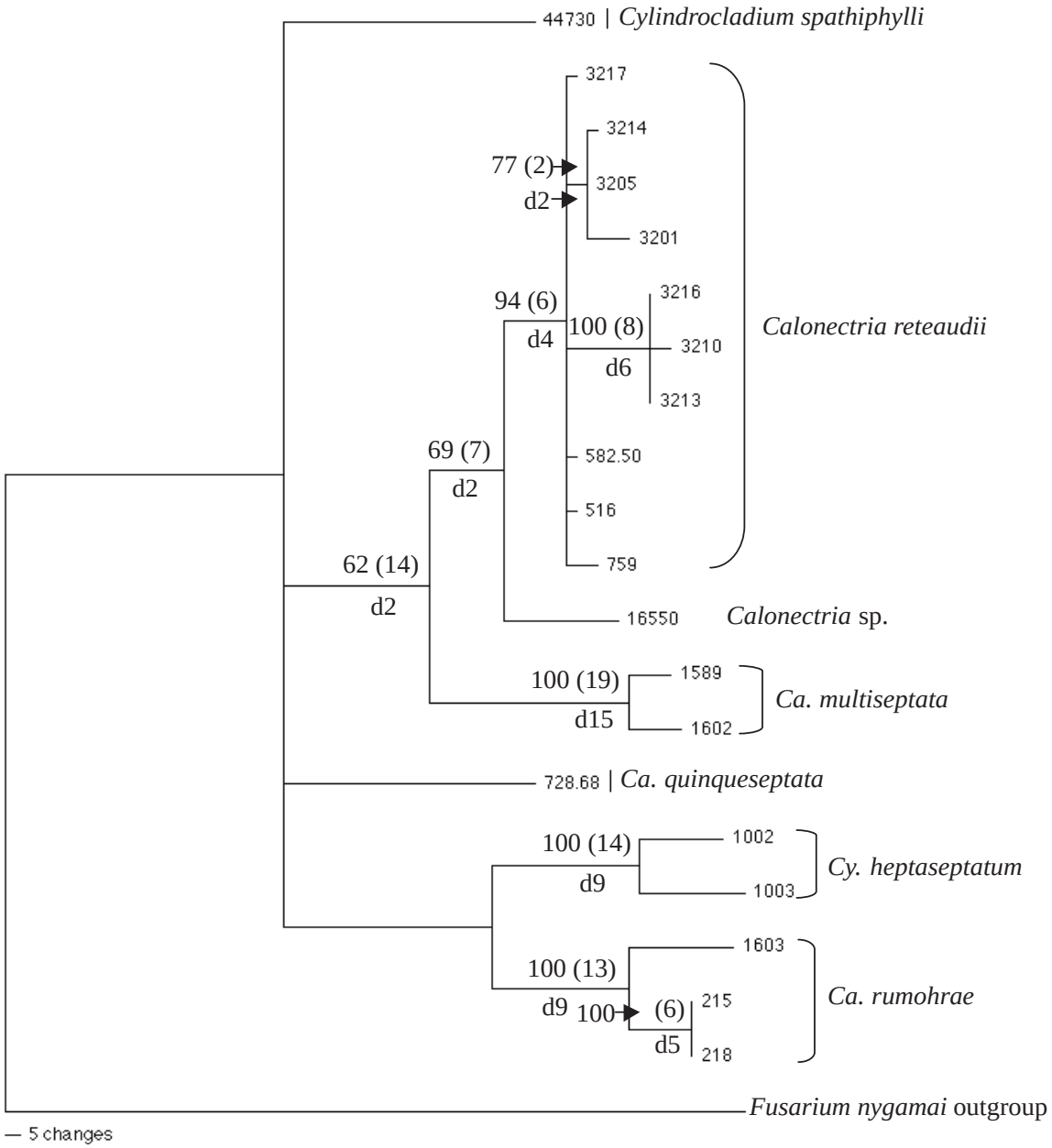
The alignment was subjected to maximum-parsimony analysis using the branch and bound search for an exact solution. Two equally most parsimonious trees (MPTs) were generated. One of the MPTs was selected as the phylogenetic tree (Fig. 1) and evaluated using 1000 bootstrap replications in a branch and bound search for clade stability. The robustness of the branches of the tree was further tested by decay indices calculated using AutoDecay (Eriksson 1998). Maximum-likelihood analyses were also performed in PAUP for the data set, which produced an identical tree topology as in the phylogenetic tree (Fig. 1).

In the phylogenetic tree, four clades representing *Cy. reteaudii*, *Cy. multiseptatum*, *Cy. rumohrae*, and *Cy. heptaseptatum* were resolved with strong bootstrap support (94–100%) and decay indices (d4–d15). The ex-type strain of *Cy. quinqueseptatum* (CBS 582.50) clustered within the *Cy. reteaudii* clade. Although morphologically indistinguishable from other isolates of *Cy. reteaudii*, isolate 16550 from the American Type Culture Collection (ATCC) was basal to the *Cy. reteaudii* clade. The node assembling it with the clade only received low bootstrap support (69%) and decay indices (d2). This isolate probably evolved from a recent common ancestor of all the *Cy. reteaudii* isolates. *Cylindrocladium multiseptatum* is the sister taxon to a clade including ATCC isolate No. 16550 and all the other *Cy. reteaudii* isolates. *Cylindrocladium spathiphylli* (ATCC isolate No. 44730) and *Ca. quinqueseptata* (CBS accession No. 728.68) segregated as two distinct phylogenetic species. The node joining the clades of *Cy. rumohrae* and *Cy. heptaseptatum* obtained high bootstrap value (93%) and decay indices (d4), which indicates that the evolving common ancestor of the two species in the clade accumulated a number of substitutions.

Morphology and mating studies

Cultures of *Cy. reteaudii* were studied on CLA as explained above and found to have conidia that were (1–)5(–6)-septate, (50–)75–95(–120) × (5–)6–7 μm, and penicillate conidiophores

Fig. 1. One of the two MPTs obtained from the alignment of the β-tubulin data set using the branch and bound search of maximum parsimony. The tree is rooted with the outgroup *Fusarium nygamai* (tree length = 315 steps, consistency index = 0.806, retention index = 0.818, rescaled consistency index = 0.660, homoplasy index = 0.194). Bootstrap values and branch lengths (in parentheses) are indicated above the branches, while decay indices are indicated below.



that terminated in clavate vesicles, (3–)5(–6) µm. Ascospores were (1–)3-septate, (30–)50–80(–100) × (4–)5–7(–8) µm. It must be noted that several isolates produced conidia and ascospores that were either in the lower or higher size range, indicating that there was considerable variation in spore dimension for this species. The same had also been observed by Sharma and Mohanan (1991*b*), who compared conidial dimensions of 10 isolates of *Cy. reteaudii*. Of the 20 isolates subjected to mating studies, 15 isolates produced perithecia with viable progeny and were classed as either positive or negative (Tables 1 and 2). The mating type of 20 single ascospore progeny derived from a single perithecium also segregated in a Mendelian fashion, thereby indicating that *Cy. reteaudii* has a biallelic heterothallic mating system. Perithecia with exuding ascospores were generally observed after 1 month of incubation on CLA. Plate dilutions made of exuding ascospore masses confirmed that all matings were fertile.

Although Figueiredo and Namekata (1967) reported the teleomorph, *Ca. quinqueseptata* to form in culture, the ex-type strain (CBS 728.68) only formed the anamorph in the present study, and thus, its mating strategy could not be confirmed. Conidiophores of *Ca. quinqueseptata* produced stipe extensions terminating in narrowly clavate vesicles, 2–3 µm diameter. Macroconidia were straight to slightly

Table 2. Results of mating studies between isolates of *Calonectria reteaudii* and *Ca. quinqueseptata* (CBS 728.68).

	728.68	16550	582.50	516	759	3201	3202	3203	3204	3205	3208	3209	3210	3212	3213	3214	3215	3216	3217
728.68	–																		
16550	–	–																	
582.50	–	–	–																
516	–	–	–	–															
759	–	–	–	–	–														
3201	–	–	–	–	–	–													
3202	–	–	–	+	–	+	–												
3203	–	–	–	–	–	–	+	–											
3204	–	–	–	+	–	+	–	+	–										
3205	–	–	–	–	–	–	+	–	+	–									
3208	–	–	–	+	–	+	–	+	–	+	–								
3209	–	–	–	–	–	–	+	–	+	–	+	–							
3210	–	–	–	+	–	+	–	+	–	+	–	+	–						
3212	–	–	–	+	–	+	–	+	–	–	–	+	–	–					
3213	–	–	–	+	–	+	–	+	–	+	–	+	–	–	–				
3214	–	–	–	+	–	+	–	+	–	+	–	+	–	–	–	–			
3215	–	–	–	–	–	–	+	–	+	–	+	–	+	+	+	+	–		
3216	–	–	–	–	–	–	+	–	+	–	+	–	+	+	+	+	–	–	
3217	–	–	–	+	–	+	+	+	–	+	–	+	–	–	–	–	+	+	–

curved, (1–)3–5(–7)-septate, (45–)55–65(–70) × (4–)5(–6) μm in size, thus significantly smaller than those of *Cy. reteaudii*.

An examination of a dried agar culture (IMI 55922) derived from the ex-type of *Cy. reteaudii*, found stipe extensions to terminate in clavate vesicles, 4–5 μm diameter, while conidia were 1–6-septate, 36–110 × 4.5–7.5 μm. No teleomorph material was found, but Bugnicourt (1939) reported ascospores to be 1–3-septate, 28–69 × 3.5–6 μm. Phialide, conidium, and vesicle morphology of *Cy. reteaudii* were indistinguishable from those of *Cy. quinqueseptatum*. The morphology of the teleomorph (Bugnicourt 1939) also correlated with the heterothallic teleomorph produced by mating isolates of *Cy. reteaudii*. However, the fungus described as *Ca. quinqueseptata* is morphologically sufficiently distinct to be considered a species. The latter taxon is presently known only from Brazil. Based on these findings, it is clear that the much confused name, *Cy. reteaudii* (Crous and Wingfield 1992), represents the older name for *Cy. quinqueseptatum*, a fungus that occurs prominently throughout Southeast Asia.

Calonectria reteaudii (Bugn.) C. Booth. Mycol. Pap. **104**: 41 (1966).

≡ *Neonectria reteaudii* Bugn. Encycl. Mycol. **11**: 189 (1939) (as *reteaudi*).

ANAMORPH: *Cylindrocladium reteaudii* (Bugn.) Boesew. Trans. Br. Mycol. Soc. **78**: 554 (1982).

≡ *Cylindrocarpon reteaudii* Bugn. Encycl. Mycol. **11**: 189 (1939) (as *reteaudi*).

= *Cylindrocladium quinqueseptatum* Boedijn & Reitsma. Reinwardtia **1**: 59 (1950).

Perithecia solitary or in groups, orange to red-brown under dissection microscope, subglobose to ovoid, 350–450 μm high, 250–350 μm wide, apex and body turning dark red, and base dark red-brown (KOH +); perithecial apex consisting of flattened, thick-walled hyphal elements with rounded tips forming a palisade, discontinuous with warty outer layer, gradually becoming thinner towards the ostiolar canal, and merging with outer periphyses. Perithecia rough walled, wall consisting of two thick-walled layers: outside layer of textura globulosa, 20–50 μm wide, becoming more compressed towards inner layer of textura angularis, 10–15 μm wide, becoming thin walled and hyaline towards center, outer cells 20–45 × 10–25 μm, inner cells 10–20 × 3–6 μm; perithecial base up to 210 μm wide, consisting of dark red-brown, angular cells, merging with an erumpent stroma, cells of the outer wall layer continue into the pseudoparenchymatous cells of the erumpent stroma. Asci 8-spored, clavate, 70–150 × 7–20 μm, tapering to a long thin stalk. Ascospores aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, slightly curved, sigmoid, (1–)3(–5)-septate, not or slightly constricted at the septum, (50–)65–85(–100) × (4–)5–6(–7) μm (mean 70 × 5.5 μm). Macroconidiophores comprised of a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle. Stipe septate, hyaline, smooth, 70–370 × 6–7 μm. Conidiogenous apparatus with aseptate or 1-septate primary branches, 20–55 × 3–6 μm; secondary branches aseptate, 15–20 × 3–5 μm, tertiary and additional branches (–6), aseptate, 12–19 × 3–5 μm, each terminal branch producing 2–4 phialides; phialides cylindrical to allantoid, hyaline, aseptate, 13–26 × 4–5 μm, apex with minute periclinal thickening and inconspicuous collarette; stipe extensions septate, straight to flexuous, 150–380 μm long, 2.5–3.5 μm wide at apical septum, terminating in a clavate vesicle, (3–)5(–6) μm diameter. Conidia cylindrical, rounded at both ends, straight, (50–)75–95(–120) × (5–)6–7 μm, (mean = 84 × 6.5 μm), (1–)5(–6)-septate, lacking a visible abscission scar, held in cylindrical clusters by colorless slime. Microconidiophores comprising a stipe, a stipe elongation and a penicillate or subverticillate arrangement of fertile branches. Stipe elongation septate, thin walled, and terminating in a narrowly clavate vesicle. Primary branches aseptate, rarely 1-septate, subcylindrical, straight to curved, 15–24 × 3–4 μm; secondary and additional branches aseptate, rarely 1-septate, 8–18 × 2–4 μm, terminating in 1–4 phialides that are cylindrical, straight to slightly curved, 11–15 × 2–3 μm; apex with minute periclinal thickening and collarette. Microconidia cylindrical, straight or curved, rounded at apex, flattened at

base, (20–)30–45(–50) × 2.5–3(–5) μm (mean 30 × 3 μm), 1(–3)-septate, held in packets by colorless slime. Megaconidiophores unknown.

CULTURAL CHARACTERISTICS: Colonies ochraceous buff (reverse), 15′b (Rayner 1970); chlamydospores extensive, arranged in fine chains, forming microsclerotia.

CARDINAL TEMPERATURES FOR GROWTH: Minimum above 10°C, maximum below 35°C, optimum 25°C. This is a high-temperature species, with no or only sparse sporulation on aerial mycelium.

TYPES: VIETNAM: leaves of *Smithia bequaertii*, *F. Bugnicourt*, IMI 55922 (dried ex-type culture of *Ca. reteaudii*, depauperate); Binh Phuoc Province, Chon Thanh, living leaves of *Eucalyptus camaldulensis*, Oct. 1998, *M.J. Dudzinski & P.Q. Thu*, neotypes designated here for, respectively, *Ca. reteaudii* (PREM 57211) and *Cy. reteaudii* (PREM 57212), cultures ex-type STE-U 3201 (+) and STE-U 3204 (–).

SYMPTOMS: Leaf spots, damping-off, seedling blight, root-rot, stem infection, leaf and shoot blights, tip blight, dieback (Sharma and Mohanan 1982; Mohanan and Sharma 1985).

SUBSTRATE: *Acacia auriculiformis* A. Cunn. ex Benth., *Acacia mangium* Willd., *Ageratum conyzoides* L., *Anacardium occidentale* L., *Asplenium* sp., *Camellia sinensis* (L.) Kuntze, *Citrus* sp., *Clerodendron* sp., *Eucalyptus* spp., *Eugenia* sp., *Euphorbia hirta* L., *Hevea* sp., *Hibiscus sabdariffa* L., *Malpighia punicifolia* L., *Manihot esculenta* Crantz, *Manilkara* sp., *Monodora* sp., *Pimenta* sp., *Psidium guajava* L., *Pterocarpus indicus* Willd., *Sida* sp., *Smithia bequaertii*, soil, *Synedrella nodiflora* (L.) Gaertn., *Syzygium aromaticum* (L.) Merrill & Perry, *Terminalia paniculata* Roth (Peerally 1974; Sarma and Nambiar 1978; Sulochana et al. 1982; Mohanan and Sharma 1985; Mohanan and Sharma 1988; Sankaran et al. 1988).

DISTRIBUTION: Australia, Hong Kong, India, Indonesia, Laos, Madagascar, Malaysia, Mauritius, Papua New Guinea, Solomon Islands, Sri Lanka, Thailand, and Vietnam (Peerally 1974; Pitkethley 1976; Sharma and Mohanan 1982; Arentz 1991).

ADDITIONAL SPECIMENS EXAMINED: AUSTRALIA: *Eucalyptus phoenicea*, 1976, *Pitketh.*, IMI 201927; SOLOMON ISLANDS; *Asplenium* sp. (= *Scolopendrium* sp.), Aug. 1965, *R.W.G. Dennis*, IMI 114953.

Discussion

This study has revealed that the fungus described from Vietnam by Bugnicourt (1939) as *Cylindrocarpon reteaudii* is, in fact, the same as *Cy. quinqueseptatum*, a major causal organism of CLB of *Eucalyptus*. The main reason that the name *Cylindrocarpon reteaudii* has largely escaped detection is that the original description was incomplete (Bugnicourt 1939). When Boesewinkel (1982) treated this species, the type of the teleomorph could not be located, and the anamorph specimen was depauperate, making any comparisons difficult. Boesewinkel (1982) noted that vesicles were clavate to subglobose, 5–7 μm wide. Based on the assumption of a clavate to subglobose vesicle, the name *Cy. reteaudii* was applied to a complex of species (Crous and Wingfield 1992). It was only in a recent molecular study (Crous and Kang 2001), that the type of *Cy. reteaudii* was re-examined, and its true identity was discovered.

A re-examination of the dried ex-type culture of *Cy. reteaudii* revealed a few vesicles to be present, which were clavate, 4–5 μm wide. Conidia were 36–110 × 4.5–7.5 μm, 1–6-septate. Perithecia of *Ca. reteaudii* were reported to be deep red, becoming red-brown (Bugnicourt 1939), while ascospores were 28–69 × 3.5–6 μm. As can be expected from a heterothallic species, the single conidial subculture studied by Booth (1966) and Boesewinkel (1982) did not produce perithecia, and hence, the only teleomorph information available is the description of Bugnicourt (1939). Thus, the present study represents the first record of this species having a biallelic heterothallic mating strategy.

Although the names *Ca. quinqueseptata* and its purported anamorph, *Cy. quinqueseptatum*, are well known in the literature, the fact that these names represent different species with different distributions, and that there is an older name available for the collections from Southeast Asia, further supports the synonymy of *Cy. quinqueseptatum* under *Cy. reteaudii*. *Cylindrocladium reteaudii* (teleomorph: *Ca. reteaudii*), which was originally associated with leaf spots of *Smithia bequaertii* in Vietnam, is well distributed throughout Southeast Asia (Booth et al. 2000), where it causes CLB of eucalypts and several other hosts. One species not yet clearly defined is *Ca. quinqueseptata*, which was originally described from Brazil, where it caused leaf spot symptoms on *Annona squamosa*, *Eucalyptus* spp., and *Syzygium aromaticum* (Figueiredo and Namekata 1967). Further collections would be required, therefore, to fully clarify the host range and distribution of *Ca. quinqueseptata*, and to determine if *Cy. reteaudii* also occurs in South America.

References

Arentz, F. 1991. Forest nursery diseases in Papua New Guinea. *In* Diseases and Insects in Forest Nurseries. Proceedings of the First Meeting of IUFRO Working Party S2.07.09, 22–30 Aug. 1990, Victoria, B.C. *Edited by* J.R. Sutherland and S.G. Glover. Forestry Canada, Pacific Forestry Centre, Victoria, B.C. pp. 97–99.

Boesewinkel, H.J. 1982. Heterogeneity within *Cylindrocladium* and its teleomorphs. Trans. Br. Mycol. Soc. **78**: 553–556.

Bolland, L., Tierney, J.W., and Tierney, B.J. 1985. Studies on leaf spot and shoot blight of *Eucalyptus* caused by *Cylindrocladium quinqueseptatum*. Eur. J. For. Pathol. **15**: 385–397.

Booth, C. 1966. The genus *Cylindrocarpon*. Mycol. Pap. **104**: 1–56.

Booth, T.H., Jovanovic, T., Old, K.M., and Dudzinski, M.J. 2000. Climatic mapping to identify high-risk areas for *Cylindrocladium quinqueseptatum* leaf blight on eucalypts in mainland South East Asia and around the world. Environ. Pollut. **108**: 365–372.

Bugnicourt, F. 1939. Les *Fusarium* et *Cylindrocarpon* de l'Indochine. Encycl. Mycol. **11**: 1–206.

Crous, P.W., and Kang, J.-C. 2001. Phylogenetic confirmation of *Calonectria spathulata* and *Cylindrocladium leucothoes* based on morphology, β-tubulin and ITS rDNA sequence data. Mycoscience, **42**: 51–57.

Crous, P.W., and Peerally, A. 1996. *Gliocladiopsis irregulare* sp. nov., and notes on *Cylindrocladium spathiphylli*. Mycotaxon, **58**: 119–128.

Crous, P.W., and Swart, W.J. 1995. Foliicolous fungi of *Eucalyptus* spp. from eastern Madagascar: implications for South Africa. S. Afr. For. J. **172**: 1–5.

Crous, P.W., and Wingfield, M.J. 1992. *Cylindrocladium leucothoes* and *C. hederae*, synonyms of *C. reteaudii*. S. Afr. J. Bot. **58**: 397–400.

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

Crous, P.W., Phillips A.J.L., and 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., Mchau, G.R.A., Van Zyl, W.H., and Wingfield, M.J. 1997. New species of *Calonectria* and *Cylindrocladium* isolated from soil in the tropics. Mycologia, **89**: 653–660. Crous, P.W., Wingfield, M.J., Mohammed, C., and Yuan, Z.Q. 1998. New foliar pathogens of *Eucalyptus* from Australia and Indonesia. Mycol. Res. **102**: 527–532.

Crous, P.W., Kang, J.-C., Schoch C.L., and Mchau, G.R.A. 1999. Phylogenetic relationships of *Cylindrocladium pseudogracile* and *Cylindrocladium rumohrae* with morphologically similar taxa, based on morphology and DNA sequences of internal transcribed spacers and β-tubulin. Can. J. Bot. **77**: 1813–1820.

El-Gholl, N.E., Alfenas, A.C., Junghans, D.T., Schubert, T.S., Miller, J.W., and Leahy, R.M. 1997. Description of *Calonectria rumohrae* sp. nov. (anamorph = *Cylindrocladium rumohrae* sp. nov.). Mycotaxon, **64**: 467–484.

Eriksson, T. 1998. AutoDecay, version 4.0 edited (program distributed by the author). Department of Botany, Stockholm University, Stockholm.

Ferreira, F.A. 1989. Patologia florestal. Principais doenças florestais no Brasil. Sociedade de Investigaçoes Florestais, Viçosa, Brasil.

Figueiredo, M.B., and Namekata, T. 1967. Record of *Calonectria quinqueseptata* n. sp., perfect state of *Cylindrocladium quinqueseptatum* Boedijn & Reitsma on *Annona squamosa* and *Eucalyptus* sp. Arq. Inst. Biol. Sao Paulo, **34**: 91–96.

Fisher, N.L., Burgess, L.W., Toussoun, T.A., and Nelson, P.E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology, **72**: 151–153.

Glass, N.L., and Donaldson, G. 1995. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. **61**: 1323–1330.

Lee, S.B., and Taylor, J.W. 1990. Isolation of DNA from fungal mycelia and single spores. *In* PCR protocols: a guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.S. Sninsky, and T.J. White. Academic Press, New York. pp. 282–287.

Mohanan, C., and Sharma, J.K. 1985. Shot-hole disease of *Terminalia paniculata* caused by *Cylindrocladium quinqueseptataum*—a new record. Eur. J. For. Pathol. **15**: 157–159.

Mohanan, C., and Sharma, J.K. 1988. Diseases of exotic acacias in India. J. Trop. For. **4**: 357–361.

O'Donnell, K., and Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phylogenet. Evol. **7**: 103–116.

Peerally, A. 1974. *Calonectria quinqueseptata*. CMI (Commonw. Mycol. Inst.) Descr. Pathog. Fungi Bact. No. 423.

Pitkethley, R.N. 1976. *Cylindrocladium quinqueseptatum* on myrtaceous tree seedlings. Aust. Plant Pathol. Soc. Newsl. **5**: 57.

Rayner, R.W. 1970. A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey, England.

Sankaran, K.V., Florence, E.J.M., and Sharma, J.K. 1988. Foliar diseases of some forest trees in Kerala, India: new records. Ind. J. For. **11**: 104–107.

Sarma, Y.R., and Nambiar, K.K.N. 1978. *Cylindrocladium* leaf rot of clove. Plant Dis. Rep. **62**: 562–564.

Schoch, C.L., Crous, P.W., Wingfield, B.D., and Wingfield, M.J. 1999. The *Cylindrocladium candelabrum* species complex includes four distinct mating populations. Mycologia, **91**: 286–298.

Sharma, J.K., and Mohanan, C. 1982. *Cylindrocladium* spp. associated with various diseases of *Eucalyptus* in Kerala. Eur. J. For. Pathol. **12**: 129–136.

Sharma, J.K., and Mohanan, C. 1991*a*. In vitro evaluation of fungicides against *Cylindrocladium* spp. causing diseases of *Eucalyptus* in Kerala, India. Eur. J. For. Pathol. **21**: 17–26.

Sharma, J.K., and Mohanan, C. 1991*b*. Pathogenic variation in *Cylindrocladium quinqueseptatum* causing leaf blight of *Eucalyptus*. Eur. J. For. Pathol. **21**: 210–217.

Sharma, J.K., and Mohanan, C. 1992. Relative susceptibility of *Eucalyptus* provenances to Cylindrocladium leaf blight in Kerala, India. Eur. J. For. Pathol. **22**: 257–265.

Sulochana, K.K., Wilson, K.I., and Nair, M.C. 1982. Some new host records for *Cylindrocladium quinqueseptatum* from India. Agric. Res. J. Kerala, **20**: 106–108.

Swofford, D.L. 1999. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4 edition. Sinauer Associates, Sunderland, Mass.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**: 4673–4680.