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Phylogeny of *Calonectria* and selected hypocrealean genera with cylindrical macroconidia

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Abstract: This study employs DNA sequence analysis to infer the phylogeny of *Calonectria* species and species of other hypocrealean genera with cylindrical macroconidia. The taxonomy of *Cylindrocladiella* species was also investigated. *Calonectria* forms a monophyletic lineage, as do the anamorph genera *Cylindrocladiella, Cylindrocarpon, Curvicladium, Gliocephalotrichum, Gliocladiopsis* and *Xenocylindrocladium*. Based on molecular data and distinctive anamorph morphology, new holomorph genera are proposed for the teleomorphs of *Cylindrocladiella* (*Nectricladiella*), *Gliocladiopsis* (*Glionectria*) and *Xenocylindrocladium* (*Xenocalonectria*). The data also support the recognition of previously proposed holomorph genera for *Cylindrocarpon* (*Neonectria*) and *Gliocephalotrichum* (*Leuconectria*). To date, no teleomorph has been reported for *Curvicladium cigneum*, although our results suggest that *C. cigneum* is closely related to *Xenocalonectria*. Eight species of *Cylindrocladiella* are recognized, with two having known teleomorphs in *Nectricladiella*, namely *N. camelliae* (*Cylindrocladiella microcylindrica*) and *N. infestans* (*Ce. infestans*). A key to holomorph genera, based primarily on anamorphic characters, is appended.

Key words: *Cylindrocarpon*, *Cylindrocladiella*, *Cylindrocladium*, *Gliocladiopsis*, *Hypocreales*, *Nectriaceae*, systematics

Introduction

The ascomycete order *Hypocreales* includes fungi of agricultural, medical and industrial importance that are found in a variety of ecological niches. These fungi are characterized by unitunicate asci produced in typically ostiolate, brightly or lightly coloured perithecia, hyaline ascospores and a hamathecium of apical paraphyses that disintegrate at maturity (Rogerson, 1970; Rossman *et al.*, 1999). A large number of anamorph genera are associated with the *Hypocreales* (Samuels & Seifert, 1987; Rossman, this volume). These can be described as moniliaceous and typically phialidic; conidia are held in lightly to brightly coloured slime (Samuels & Seifert, 1987). The importance of anamorph morphology in the *Hypocreales* is emphasized by the fact that in many economically important species this form is more frequently encountered than the teleomorph, and is thus often the only way to identify a species.

In the *Hypocreales*, *Nectria* (Fr.) Fr. has included more species than any other genus in the order, with more than 600 described. Traditionally, all species having fleshy, uniloculate ascocarps with a hypocrealean centrum, hyaline, non-apiculate, bicellular ascospores, and phialidic anamorphs have been included in *Nectria* (Rossman, 1993). In this traditional generic definition, ascospore morphology and septation dominated. Other genera were segregated from *Nectria* on the basis of single characters, including ascospore septation and pigmentation, and synnematous anamorphs. For example, *Calonectria* De Not. (*Ca.*), one of the segregate genera of special interest here, was described for species having multiseptate ascospores (Saccardo, 1883).

Booth (1959) used a combination of characters, including anatomy of the perithecial wall, ecology and anamorphs, to describe informal taxonomic groups within *Nectria sensu lato*. Subsequent authors (e.g. Samuels, 1976; Samuels *et al*., 1991; Brayford & Samuels, 1993; and Samuels & Brayford, 1993) followed Booth in recognizing informal groups within the large genus *Nectria.* In a recent revision of genera of the *Hypocreales* (Rossman *et al*., 1999), many of these groups were given generic status and additional genera were described. *Nectria sensu stricto* was restricted to the type species, *Nectria cinnabarina* (Tode) Fr., and species similar to it. Rossman *et al.* (1999) split the large, polyphyletic genus *Nectria* into several smaller genera within two families, the *Nectriaceae* and the *Bionectriaceae*. *Calonectria* was included in the *Nectriaceae*, but was differentiated from *Nectria sensu stricto* on the basis of ascocarp morphology and anatomy, the occurrence of *Cylindrocladium* Morgan (*Cy.*) anamorphs, and basic differences in biology. Although the singular character of ascospore morphology was regarded as less important (Rossman, 1983; Crous & Wingfield, 1994), ascospores of *Calonectria* are distinct from those of *Nectria.*

In a study based on sequence alignments of nuclear large-subunit ribosomal DNA from several genera in the *Hypocreales*, Rehner & Samuels (1995) identified some close neighbours to *Calonectria.* These authors showed that species of *Calonectria* grouped closely to *Leuconectria clusiae* Rossman *et al.* (anamorph: *Gliocephalotrichum bulbilium* J.J. Ellis & Hesselt.), as well as to *'Nectria' radicicola* Gerlach & L. Nilsson [anamorph: *Cylindrocarpon destructans* (Zinssm.) Scholten], with two typical species of *Nectria*, *N. pseudotrichia* Berk. & M.A. Curtis [anamorph: *Tubercularia lateritia* (Berk.) Seifert] and *N. cinnabarina*, forming part of this subclade, but grouping more distantly. This phylogeny generally confirmed morphological observations, where similarities were found between the *Gliocephalotrichum* and *Cylindrocladium* anamorphs of *Leuconectria* and *Calonectria* species (Rossman, Samuels & Lowen, 1993). The most notable similarities were the formation of cylindrical conidia and brown pigment diffusing in the agar.

In addition to *Gliocephalotrichum*, several other anamorph form-genera are similar to *Cylindrocladium* in producing cylindrical macroconidia, phialidic conidiogenous cells and slimy conidia. Among these are *Cylindrocladiella* Boesew. (*Ce.*), *Gliocladiopsis* S.B. Saksena, *Xenocylindrocladium* Decock *et al.* and *Curvicladium* Decock & Crous. Of these, only *Cylindrocladiella* (Boesewinkel, 1982) and *Xenocylindrocladium* (Decock *et al.*, 1997) have species linked to teleomorphs, both included in *Nectria sensu lato.* We have included representatives of these genera in the present evaluation of holomorphs having cylindrical conidia.

Anamorphs have assumed an increasingly important role in the delimitation of genera of the *Hypocreales* (Rossman *et al*., 1999), to the extent that they have replaced ascospores as the most important phylogenetically informative characters. Ribosomal rDNA gene sequence data has provided independent support for the phylogenetic significance of anamorphs. These data have indicated that some anamorphs with the 'hypocrealean phenotype' do, in fact, cluster with sexually reproducing genera of the *Hypocreales* (e.g. Spatafora & Blackwell, 1993; Rehner & Samuels, 1994, 1995; Glenn *et al*., 1996; O'Donnell *et al*., 1998). Moreover, individual anamorph species that are either unknown to reproduce sexually, or that are encountered frequently in the absence of sexual reproduction (ie. perithecia) can be phylogenetically related to sexually reproducing holomorphs (Kuhls *et al.*, 1996, 1997).

Additional anamorph genera and species are likely to be linked to the *Hypocreales* as additional DNA sequence data continues to accumulate. Considering this and recent trends in favour of discarding the phenetically based form-genera of the deuteromycetes (Sutton, 1993), Rossman (1993, this volume) proposed that each hypocrealean teleomorph genus ideally should be linked to only one anamorph genus. This is in step with a more holomorphic approach, encompassing both teleomorph and anamorph (Hawksworth, 1993). However, current generic concepts still have a strong influence from Saccardo's original taxonomic system (Rossman, 1996, this volume), and detailed cultural and molecular studies are required to clarify anamorph/teleomorph relationships and attain a genus for genus phylogeny as far as possible.

The revision of genera of the *Hypocreales* proposed by Rossman *et al.* (1999) was acknowledged by the authors as a 'starting point' rather than a final statement on the *Hypocreales.* They acknowledged that many of the genera that they delimited are still poly- or paraphyletic, and that new genera remain to be described as new species are discovered. Most genera recognized by Rossman *et al.* (1999) have not been assessed using DNA characters. In the present work, we consider holomorphs of nectriaceous ascomycetes that have cylindrical conidia whose anamorphs are classified in several genera. These ascomycetes are united by the formation of small, red perithecia that are situated on a small basal stroma, occur singly or in clusters, and have pigments that change colour in 3% KOH. Species of *Calonectria* are characterized by warted perithecia, and clavate, long-stemmed asci without a visible apical discharge mechanism, and large (> 25 μm), 1- to multiseptate, hyaline, smooth, fusiform ascospores with obtuse ends that aggregate in the upper third of the ascus. Based on teleomorph morphology alone, however, species of *Calonectria* can only be identified to species complexes, and the anamorph is required for identification at species level. The perithecial wall anatomy of *Calonectria* is not unique, but is also shared by teleomorphs of some *Cylindrocarpon* (*destructans*-complex), *Xenocylindrocladium* and *Gliocladiopsis* species. The latter are primarily distinguished from *Calonectria* species by their ascus and ascospore morphologies. That said, teleomorphs of the latter three genera would be difficult if not impossible to distinguish without knowledge of their respective anamorphs. In contrast, the teleomorphs of *Cylindrocladiella* species are quite distinct from those discussed above, because they have smooth, relatively thin-walled *Cosmospora*-like perithecia that collapse laterally when dry, a less well-developed basal stroma, and smaller ascospores.

Materials and Methods

CULTURES

Strains were either obtained from other culture collections or isolated from infected plant material or soil samples (Crous *et al.*, 1997) and deposited in the culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa (acronym STE-U, Table 1). Hypocrealean genera are **abbreviated** as follows: *Calonectria – Ca.*; *Cylindrocladium – Cy.*; *Cylindrocladiella – Ce.*, and *Cylindrocarpon – Co.*

Acronyms used to denote culture collections of institutions and individuals from which isolates were obtained include: ATCC – American Type Culture Collection, Virginia, U.S.A.; A.R. – A.R. (A. Y. Rossman), C. T. R. (C. T. Rogerson) and G. J. S. (G. J. Samuels), United States Department of Agriculture, A.R.S., Beltsville, Maryland, U.S.A.; IMI – CABI Bioscience, Bakeham Lane, Egham, U.K.; IMUR – Institute of Mycology, University of Recife, Brazil; MUCL – Mycothèque, Laboratoire de Mycologie Systématique et Appliqée, Université Louvain-la-Neuve, Belgium; STE-U – (see above); and UFV – (A. C. Alfenas), Department of Plant Pathology, University of Viçosa, Viçosa, Minas Gerais, Brazil.

MORPHOLOGICAL COMPARISONS

Strains were cultured on 2% malt extract agar (MEA) (Biolab, Midrand, South Africa), 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. Only material growing on carnation leaves was examined microscopically. Mounts were prepared in lactophenol, examined using Nomarski interference contrast, bright-field and phase contrast microscopy, and measurements made at 1000 × magnification. The 95% confidence intervals were determined from at least 30 observations and the minimum and maximum ranges given in parentheses. Cardinal temperature requirements for growth and cultural characteristics were determined after 6 d on MEA, using procedures described by Crous & Wingfield (1994). Colony colours were coded according to Rayner (1970). Sections of perithecia were cut at 10 μm thickness on a CM1100 Cryostat microtome (Leica, Heidelberg, Germany).

DNA EXTRACTION AND SEQUENCING

Single conidium isolates were grown on MEA plates. Mycelial mats were removed and ground to powder in liquid nitrogen using a mortar and pestle. Approximately 40 mg of ground mycelium was added to 2 ml microtubes containing 600 μl of extraction buffer. The extraction buffer consisted of 1% SDS, 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 100 mM EDTA. The protocol for the Wizard Genomic DNA Purification kit (Promega, Madison, U.S.A.) was subsequently followed.

PCR Reactions (total volume 25 μl) comprised 1.5 units Biotaq (Bioline, London, U.K.) with the buffer as recommended by the manufacturer, 1 mM deoxynucleoside triphosphates, 4 mM $MgCl_2$, 0.5 μM primer oligonucleotide and approximately 10 to 30 ng of fungal genomic DNA as template. Reactions were performed on a Rapidcycler (Idaho Technology Idaho, U.S.A.). Reaction conditions consisted of an initial denaturation for 2 min at 96°C, followed by 30 cycles of 15 s at 96°C, 30 s at 55°C and 35 s at 75°C with a slope of 1.0. A final elongation step of 2 min at 75°C was included. The regions amplified were the 5.8S ribosomal gene with the two flanking internal transcribed spacers (ITS-1 and ITS-2), as well as the 5' end of the β-tubulin gene. DNA was amplified using the primers ITS-1 and ITS-4 (White *et al.*, 1990) as well as T1 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995), yielding products of approximately 540 bp and 600 bp, respectively. The PCR products were sequenced using the ABI Prism 377 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). Sequencing conditions were as described by Schoch *et al.* (1999). Sequence data are deposited at GenBank (AF059281, 210876, 210881, 210882, 210887, 210888, 220952–220982).

PHYLOGENETIC ANALYSIS

Sequences were aligned with the computer package Malign version 2.7 (Wheeler & Gladstein, 1991) and adjusted manually. Phylogenetic analyses of aligned DNA sequences were performed using PAUP* Version 4.0b1 (Swofford, 1998) and printed with the help of TreeView Version 1.5 (Page, 1996). To limit the influence of large gaps consisting of several characters, only the first character of a multi-character gap was coded. Subsequent gap characters were coded as missing data. Having done this, analyses were made treating single character gaps as fifth characters. A number of strains representing different species in each genus were selected for the generic analysis (Fig. 1). In this instance, the heuristic search option with 1000 random addition sequences was used. The subsequent analysis for species with *Cylindrocladiella* anamorphs was performed using a similar strategy as for the ITS data set, while the branch and bound search option was used for the β-tubulin and combined data sets. Confidence intervals were determined using 1000 bootstrap replications in all cases. Decay indices were determined with AutoDecay Version 4.0 (Eriksson, 1998). A partition homogeneity test was performed in PAUP* Version 4.0b1 in order to test whether phylogenies obtained from the ITS and β-tubulin data sets differed significantly. Data sets were also analyzed by using Neighbor-Joining with uncorrected ('p') and maximum-likelihood distance methods in PAUP* Version 4.0b1.

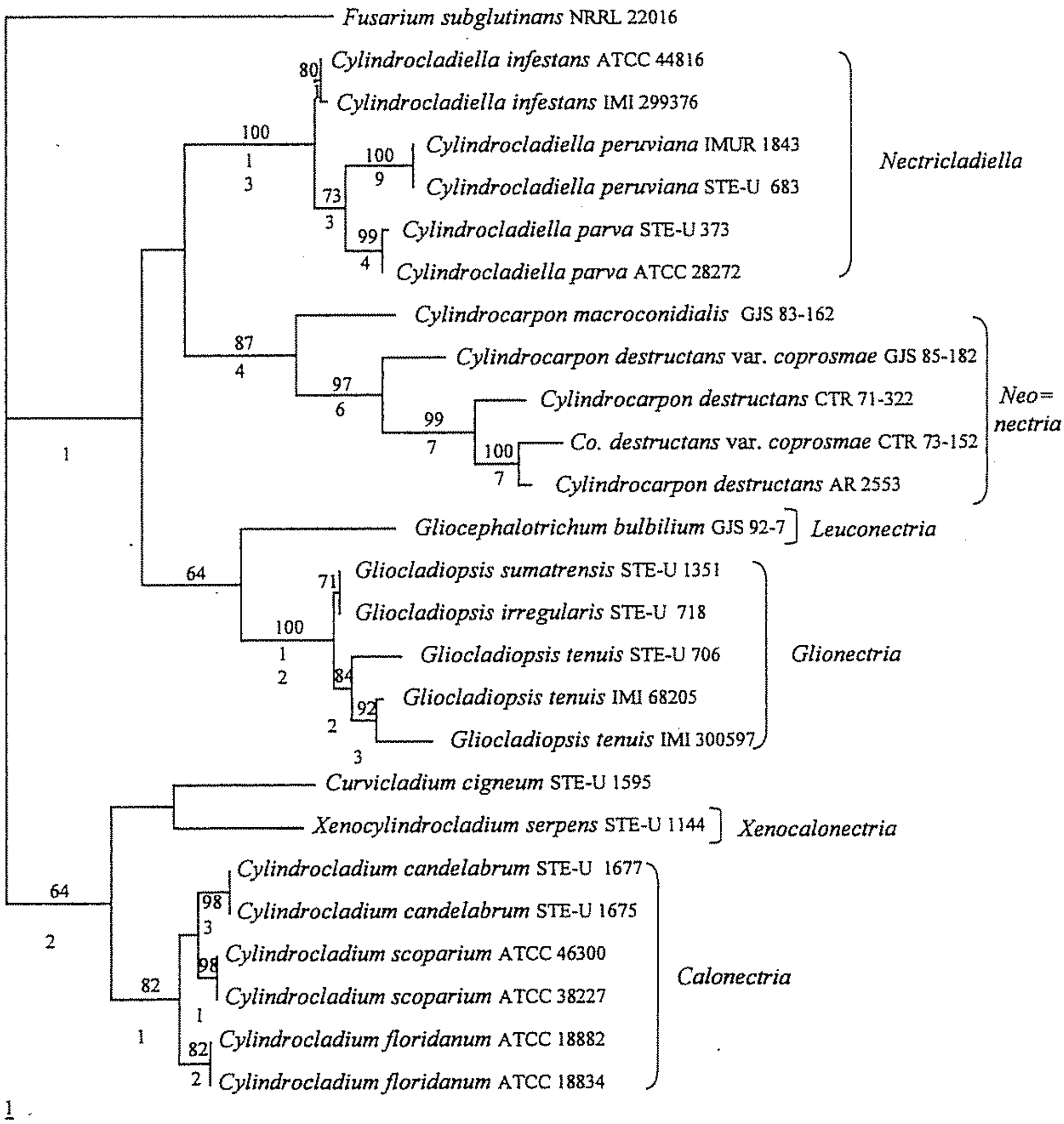


Fig. 1. Phylogenetic relationships among hypocrealean anamorphs with cylindrical macroconidia. One of four most parsimonious trees obtained with a heuristic search in PAUP* version 4.0b1 and 1000 random addition sequences (405 steps, CI = 0.681, RC = 0.554, RI = 0.812). Bootstrap values are shown above branches and decay indices below. Characters used were based on a data set comprising of ITS-1 and -2 as well as the 5.8S ribosomal gene DNA sequences. The bar represents one step.

Table 1. Cultures used for sequencing in this study.

Anamorph	Teleomorph	Accession no.	Collector	Substratum	Origin
Cylindrocladium scoparium	*Calonectria morganii*	ATCC 38227	S.A. Alfieri	*Mahonia bealei*	Florida, U.S.A.
		ATCC 46300	D.M. Benson	*Leucothoë catesbaei*	North Carolina, U.S.A.
Cylindrocladium floridanum	*Calonectria kyotensis*	ATCC 18882	R.H. Morrison	Peach roots	Florida, U.S.A.
		ATCC 18834	T. Terashita	*Robinia pseudoacacia*	Japan
Cylindrocladium candelabrum	*Calonectria scoparia*	STE-U 1677	A.C. Alfenas	*Eucalyptus* sp.	Amazonas, Brazil
		STE-U 1674	A.C. Alfenas	*Eucalyptus* sp.	Bahia, Brazil
Cylindrocladium multiseptatum	*Calonectria multiseptata*	STE-U 1589	M.J. Wingfield	*Eucalyptus* sp.	Sumatra, Indonesia
		STE-U 1602	M.J. Wingfield	*Eucalyptus* sp.	Sumatra, Indonesia
Cylindrocladiella novaezelandiae	None described	ATCC 44815	H.J. Boesewinkel	*Rhododendron indicum*	New Zealand
Cylindrocladiella elegans	None described	STE-U 518	P.W. Crous	Litter	Western Cape, South Africa
Cylindrocladiella parva	None described	ATCC 28272	H.J. Boesewinkel	*Telopea speciosissima*	New Zealand
		STE-U 373	P.W. Crous	*Pinus radiata*	Western Cape, South Africa
Cylindrocladiella peruviana	None described	IMUR 1843	M.P. Herrera	Ants	Peru
		STE-U 395	P.W. Crous	*Acacia mearnsii*	KwaZulu-Natal, South Africa
		STE-U 683	M.J. Wingfield	Soil	Thailand
		STE-U 918	Unknown	Soil	Salta, Argentina
Cylindrocladiella lageniformis	None described	UFV 115	A.C. Alfenas	*Eucalyptus* sp.	Brazil
Cylindrocladiella infestans	*Nectricladiella infestans*	ATCC 44816	H.J. Boesewinkel	*Pinus pinea*	New Zealand
		IMI 299376	K.B. Boedijn & J. Reitsma	*Arenga pinnata*	Indonesia
		STE-U 708	M.J. Wingfield	Soil	Hong Kong
		STE-U 2319	J.E. Taylor	Soil	Madagascar
Cylindrocladiella microcylindrica	*Nectricladiella camelliae*	ATCC 38571	W.A. Shipton	*Pinus pinea*	Australia
Cylindrocladiella camelliae	None described	STE-U 234	P.W. Crous	*Eucalyptus grandis*	Northern Province, South Africa
		STE-U 277	P.W. Crous	*Eucalyptus grandis*	Northern Province, South Africa
Cylindrocarpon macroconidiale	'*Nectria*' *radicicola* var. *macroconidialis*	G.J.S. 83-162	G.J. Samuels	*Astelia* sp.	New Zealand
Cylindrocarpon destructans	'*Nectria*' *radicicola* var. *radicicola*	A.R. 2553	A.Y. Rossman	Bark	Venezuela
		C.T.R. 71-322	G.J. Samuels	Host unknown	Venezuela
Cylindrocarpon destructans var. *coprosmae*	'*Nectria*' *radicicola* var. *coprosmae*	C.T.R. 73-152	G.J. Samuels	*Cosmospora* sp.	New Zealand
		G.J.S. 85-182	G.J. Samuels	Unknown	New Zealand
Gliocephalotrichum bulbilium	*Leuconectria clusiae*	G.J.S. 92-7	W.R. Buck	*Clusia* sp.	Puerto Rico
Gliocladiopsis tenuis	*Glionectria tenuis*	IMI 68205	F. Bugnicourt	*Indigofera* sp.	Indo-China
		IMI 300597	Unknown	*Psidium guajava*	India
		STE-U 706	M.J. Wingfield	Soil	Hong Kong
Gliocladiopsis sumatrensis	None described	STE-U 1351	M.J. Wingfield	Soil	Sumatra, Indonesia
Gliocladiopsis irregularis	None described	STE-U 718	A.C. Alfenas	Soil	Sumatra, Indonesia
Curvicladium cigneum	None described	STE-U 1595	C. Decock	Leaf of angiosperm	French Guiana
Xenocylindrocladium serpens	*Xenocalonectria serpens*	STE-U 1144	G.L. Hennebert	Bark of unknown tree	Ecuador

Taxonomy

A phylogenetic analysis of all species included in this study, based on the DNA sequence of the two flanking internally transcribed spacers (ITS-1 and ITS-2) and the 5.8S ribosomal RNA gene, is shown in Fig. 1. When gaps were coded as missing, the number of possible most parsimonious trees exceeded 1000. With gaps treated as a fifth character, only one most parsimonious tree was found. No difference in the number of most parsimonious trees was found when all subsequent gap characters after the first gap character was coded as missing. However, this reduced the number of parsimony informative sites from 163 to 139. All species clustered in accordance with their respective anamorphs and these groupings are discussed in more detail below.

Calonectria/Cylindrocladium, Curvicladium, Nectria/Xenocylindrocladium

The type species of *Calonectria* is *Ca. daldiniana* De Not., now considered a synonym of *Ca. pyrochroa* (Desm.) Sacc. (Rossman, 1979a). *Calonectria* encompasses species with brightly coloured perithecia that become red in 3% KOH (KOH+), with a thick perithecial wall consisting of large cells, arising from a darkened, stromatic base. Ascospores of *Calonectria* species tend to be longer than 25 μm, fusiform, and usually phragmosporous. *Cylindrocladium* species have been linked exclusively to *Calonectria* teleomorphs (Rossman, 1993). Rossman (1979b) redisposed many species incorrectly ascribed to *Calonectria*.

The anamorph genus *Cylindrocladium* was originally based on *Cy. scoparium* Morgan, a species collected from a dead pod of honey locust (*Gleditsia triacanthos*) in Ohio, U.S.A. (Morgan, 1892). Species of this genus are well-known plant pathogens and have been isolated from all continents in tropical and subtropical zones world-wide (Crous & Wingfield, 1994). Species concepts in *Cylindrocladium* have been based on the dimensions and septation of conidia, phialide shape, stipe length, cultural characteristics, as well as the shape and diameter of the terminal vesicle found on stipes emanating from the conidiophores (Figs. 2–5) (Crous & Wingfield, 1994).

Previous studies in this genus showed that concordant phylogenies could be derived from the gene trees based on sequences of ITS, β-tubulin and the HMG box of *MAT-2* (Crous *et al.*, 1999; Schoch *et al.*, 1999). Some of these species were also shown to have interfertility barriers, thus complying with a biological species concept (Schoch *et al.*, 1999). Although these results generally coincided with morphological species concepts, in some cases several phylogenetic species (based on DNA sequence data) and biological species could be described within the parameters of a single morphological species.

Two hyphomycete genera with penicillate conidiophores and unique stipe elongations were recently described that also appeared to be morphologically closely related to *Cylindrocladium* (Decock *et al.*, 1997; Decock & Crous, 1998). *Xenocylindrocladium serpens* was described from Ecuador as the type species of this genus, while its teleomorph, distinct from *Calonectria*, was '*Nectria*' *serpens* Decock *et al.* (Decock *et al.*, 1997) (Figs. 6–11). A similar fungus, *Curvicladium cigneum* Decock & Crous, was later described in this complex, characterized by curved, rough, sparsely septate stipe extensions (Decock & Crous, 1998). No teleomorph has yet been reported for *Curvicladium* (Fig. 12).

The species of *Calonectria* included in this study all produced *Cylindrocladium* anamorphs characteristic of this genus, and formed a clearly distinct clade, strongly supported by high bootstrap values (Fig. 1). The *Calonectria* clade was shown to be closely related to *Xenocylindrocladium* and *Curvicladium* (Fig. 1). Their close proximity to *Calonectria* suggests a shared ancestor. This hypothesis will still have to be tested further, however, using additional gene trees. Based on the phylogenetic distance shown in Fig. 1, as well as distinct morphological differences in the anamorph of *Xenocylindrocladium serpens*, we propose the following new holomorph genus:

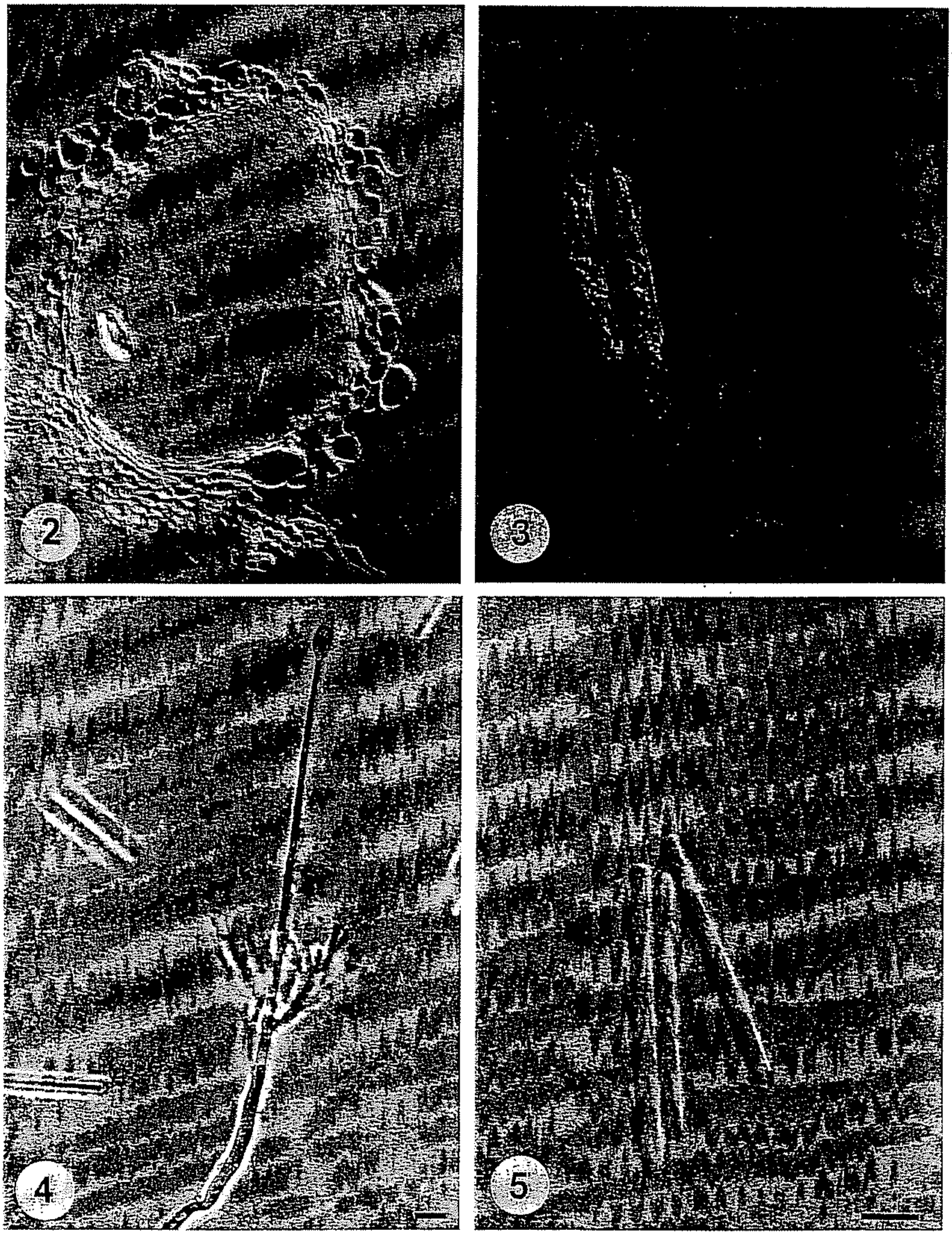
XENOCALONECTRIA Crous & C.L. Schoch, *gen. nov.*

Anamorph: *Xenocylindrocladium* Decock, Hennebert & Crous

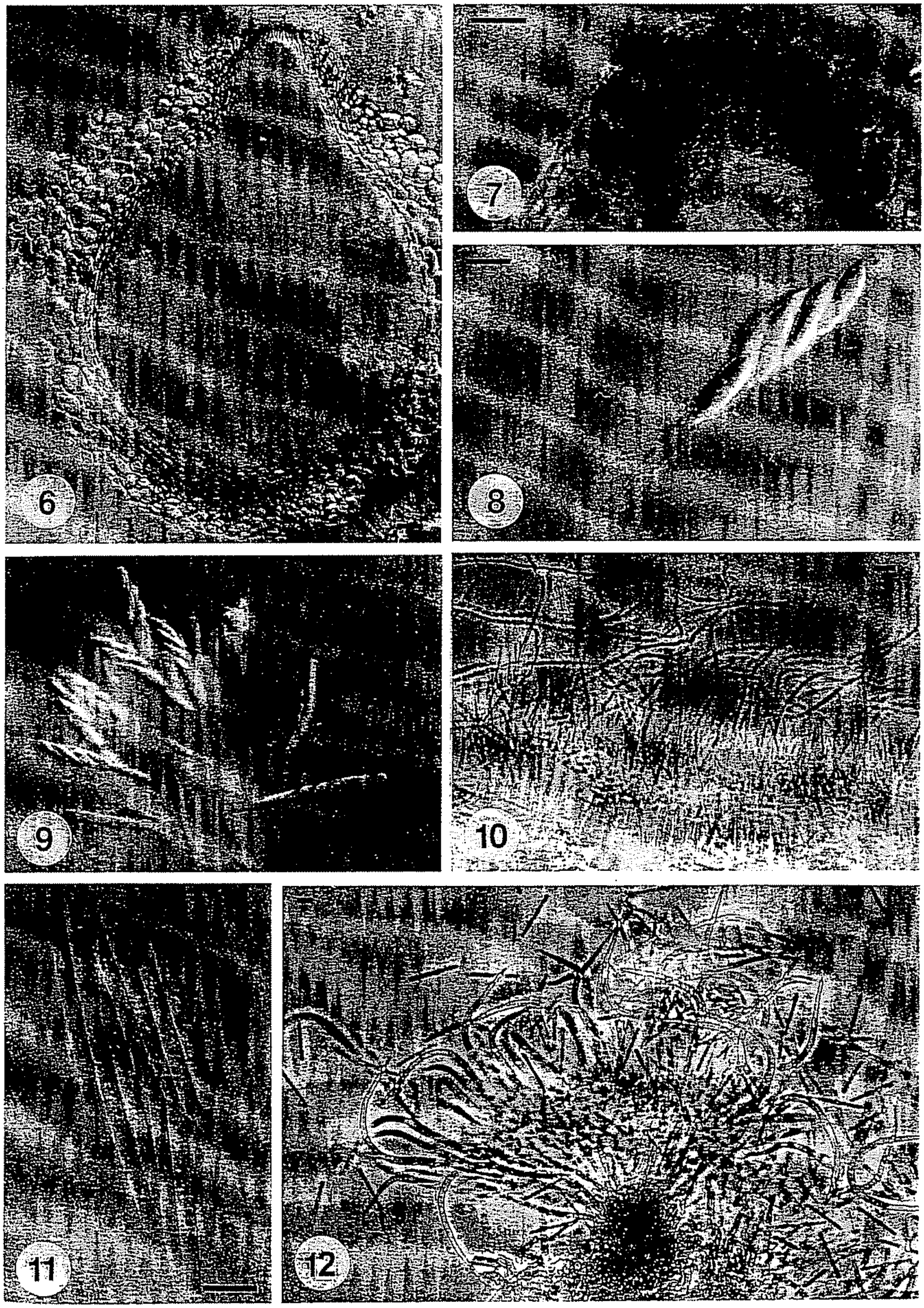
Typus: *Xenocalonectria serpens* (Decock, Hennebert & Crous) Crous & C.L. Schoch

Perithecia superficialia, solitaria vel aggregata, globosa ad subglobosa, verrucosa, lutea usque ad rubra, basi obscure rubra stromatica, KOH+; pariete perithecii ex duabus regionibus composito: strato exteriore *textura globulosa* crassitunicata, strato interiore ex cellulis compressis *texturae angularis* constante; periphyses ostioli hyalinae, tubulares cum apicibus rotundatis. Asci unitunicati, octospori, cylindrici, basi elongata, apice applanato et apparatu apicali refringente. Ascosporae in parte superiore asci aggregatae, hyalinae, late vel anguste ellipsoideae, leves, medio uniseptatae.

Perithecia superficial, solitary or in clusters, globose to subglobose, warted, yellow to red and with a dark red stromatic base, KOH+; perithecial wall consisting of two regions: outer layer of thick-walled *textura globulosa*, inner layer of compressed



Figs 2–5. *Calonectria mexicana* and its anamorph, *Cylindrocladium mexicanum*. 2. Vertical section through a perithecium. 3. Asci with ascospores. 4. Conidiophore with extending stipe and terminal vesicle. 5. One-septate conidia. Bars = 10 μm.



Figs 6–12. *Xenocalonectria serpens* and *Curvicladium cigneum*. 6–11. *Xenocalonectria serpens* and its anamorph *Xenocylindrocladium serpens*. 6. Vertical section through perithecium. 7. Ostiolar region of perithecium. 8–9. Cylindrical asci with apical apparatus. 10. Conidiophores with stipe extensions (bar = 20 μm). 11. One-septate conidia. 12. *Curvicladium cigneum*, conidiophores and conidia. Bars = 10 μm.

cells of *textura angularis*; ostiolar periphyses hyaline, tubular with rounded ends. Asci unitunicate, 8-spored, cylindrical, with long basal stalks, a flattened apex, and a refractive apical apparatus. Ascospores aggregated in the upper third of the ascus, hyaline, broadly to narrowly ellipsoidal, smooth, medianly 1-septate. Anamorph *Xenocylindrocladium*.

Xenocalonectria serpens (Decock, Hennebert & Crous) Crous & C.L. Schoch, *comb. nov.* — (Figs 6–11).

≡ *Nectria serpens* Decock, Hennebert & Crous, Mycol. Res. 101: 788. 1997.

Anamorph: *Xenocylindrocladium serpens* Decock, Hennebert & Crous, Mycol. Res. 101: 788. 1997.

This species was described in full by Decock *et al.* (1997). Ascospores aggregated in the upper third of the ascus, hyaline, broadly to narrowly ellipsoidal, smooth, with granular contents, (8–)12–20(–25) × 4–5(–6) μm, medianly 1-septate, becoming constricted at the septum, and developing up to 2 septa with age. Macroconidia cylindrical, hyaline, straight with rounded ends, 1-septate, (24–)27–33(–36) × 2.5–3 (–3.5) μm.

COLONY COLOUR.— Amber brown (reverse) (13K, Rayner, 1970). Chlamydospores in extensive numbers with medium to extensive sporulation on the aerial mycelium.

CARDINAL TEMPERATURE REQUIREMENTS FOR GROWTH.— Minimum above 5°C, optimum 25–30°C, maximum below 35°C.

HABITAT.— Bark of fallen trees.

KNOWN DISTRIBUTION.— Ecuador.

HOLOTYPES.— ECUADOR. SUCUMBIOS: Reserva de Producción Faunística, Cuyabeno, Tierra firme, bark of a fallen tree trunk, G.L. Hennebert, July 1993, MUCL 39315a, holotype of teleomorph. MUCL 39315b, holotype of anamorph (culture ex-type: MUCL 39315 = STE-U 1144).

Nectria/Cylindrocarpon

Perithecial anatomy in *'Nectria' radicicola* and its relatives is similar to that occurring in *Calonectria* species (Samuels & Brayford, 1990). Samuels and Seifert (1987) commented on the similarity between *Cylindrocladium* and the *Cylindrocarpon* Wollenw. anamorphs of *N. radicicola* and closely related species. *Calonectria* and the nectriaceous species centred around *N. radicicola* are distinguished primarily by the respective occurrence of *Cylindrocladium* and *Cylindrocarpon* anamorphs, as well as on their distinctive ascus and ascospore morphology (Samuels & Brayford, 1990). Ascospores of the *N. radicicola*-group are, however, much smaller than those of *Calonectria* species. Rossman *et al.* (1999) referred many holomorphs having *Cylindrocarpon* anamorphs to *Neonectria* Wr. *'Nectria' radicicola* (which was not transferred to *Neonectria*) and its relatives, all of which have *Cylindrocarpon* anamorphs, cluster in a clade (Fig. 1) that is sister to *Cylindrocladiella*. Whether *N. radicicola* is representative of all holomorphs having *Cylindrocarpon* anamorphs (*Neonectria*) is currently being evaluated (F. Mantiri & G. Samuels, pers. comm.).

Nectria/Cylindrocladiella

The anamorph genus *Cylindrocladiella* Boesewinkel (1982) was proposed for five small-spored species of *Cylindrocladium sensu lato* having different conidiophore branching patterns, conidial shapes and dimensions, as well as cultural characteristics. The recognition of *'Nectria' camelliae* Shipton as the teleomorph for one of these species strengthened the case for the delimitation of the new anamorph genus. More recent studies have confirmed the genera *Cylindrocladium* and *Cylindrocladiella* to be distinct (Crous & Wingfield, 1993; Crous *et al.*, 1994; Victor *et al.*, 1998). Samuels *et al.* (1991) allocated *N. camelliae* (anamorph: *Ce. infestans*) to *Nectria* subg. *Dialonectria*, while Rossman *et al.* (1999), in a re-evaluation of the group, placed the species in *Cosmospora* as *C. camelliae* (Shipton) Rossman & Samuels, based on its perithecial morphology and anatomy. As presently defined by Rossman *et al.* (1999), *Cosmospora* is heterogeneous in having diverse anamorphs, including *Cylindrocladiella*. In comparison to *Calonectria* species, the perithecial wall of *Cosmospora camelliae* is smooth, narrow, and its ascospores are much smaller.

Victor *et al.* (1998) recognized seven species in *Cylindrocladiella*. All these species could be distinguished based on RFLP and AT-DNA data (A+T-rich), as well as morphology. The AT-DNA data showed differences in the profiles of the ex-type isolates of *Cosmospora camelliae* (ATCC 38571; teleomorph) and *Ce. infestans* (ATCC 44816; anamorph). One restriction enzyme also showed differences in the RFLP profiles, but cultural and morphological characters exhibited little variation other than in conidium length (Victor *et al.*, 1998).

The *Nectria/Cylindrocladiella* clade has strong bootstrap support (Fig. 1). Relationships between the *Nectria/Cylindrocladiella* and *Neonectria* clades are equivocal because the clade that includes these two groups received only weak bootstrap support. However, both groups are strongly supported as separate entities corresponding with their different anamorphs. Two areas of the genome were utilized to investigate relationships among species with *Cylindrocladiella* anamorphs. When the phylogenies derived from data sets obtained from the ITS regions flanking the 5.8S ribosomal RNA gene as well as the 5' end of the β-tubulin gene were compared in a partition homogeneity test, they were found not to differ significantly ($P = 0.33$, where $P < 0.05$ denotes significance) (Fig. 13). The number of parsimony-informative sites in the ITS data set (25) was lower than in the β-tubulin data set (109). A similar trend occurred in *Calonectria* species (Schoch *et al.*, 2000). An analysis of the combined data set of ITS and β-tubulin sequences had higher bootstrap values supporting branches. A branch and bound search yielded four most parsimonious trees (Fig. 14).

The DNA sequence data of both ITS and β-tubulin loci showed clear differences between two groups of isolates identified as *Ce. infestans* (Fig. 13). One group is characterized by the ex-type culture of *Cosmospora camelliae,* while the other is characterized by the ex-type culture of *Ce. infestans.* Two additional clades in the β-tubulin gene tree were observed within the group of *Ce. infestans* strains. Furthermore, a strain from an 'anamorph type group' recently obtained from Madagascar, produced a teleomorph in culture. The clear differences shown in the molecular data, based on two DNA sequence data sets and the previous characters described by Victor *et al.* (1998), suggest that *Ce. infestans* contains more than one genetically distinct taxon. These are described as new below.

Cylindrocladiella microcylindrica, *Ce. peruviana* (Bat., J.L. Bezerra & S. Herrera) Boesew. and *Ce. camelliae* (Venkataram & C.S.V. Ram) Boesew. clustered together in the ITS tree (Fig. 13). Likewise, the β-tubulin data set supported a similar grouping of these species, but could differentiate between them (Fig. 13). Previously, Crous & Wingfield (1993) synonymized *Ce. peruviana* with *Ce. camelliae* based on similarities in morphology. Conidiophores of both species have ellipsoidal to lanceolate vesicles and similar conidium dimensions, as well as similar temperature–growth relationships, but Victor *et al.* (1998) separated them based on differences in RFLP profiles as well as vesicle width and taper. The data in Figs 1, 13 and 14 show that this close relationship is also reflected in the molecular characters used here. The β-tubulin data set supported their separation. Further variation in this clade was also evident from the β-tubulin sequences. Although molecular data confirmed *Ce. camelliae* and *Ce. peruviana* to be different, strains of the third species, *Ce. microcylindrica*, exhibited similarities to these two taxa, and more isolates will have to be added to clearly resolve the boundaries among these species.

The relationships of the other species in the genus, *Ce. elegans* Crous & M.J. Wingf., *Ce. novaezelandiae* (Boesew.) Boesew., *Ce. lageniformis* Crous *et al.* and *Ce. parva* (P.J. Anderson) Boesew. were equivocal (Figs 13, 14). Each species was distinct and separated from other species, but there was no clear indication of interspecies relationships. A close relationship between *Ce. novaezelandiae* and *Ce. elegans* is only supported by the β-tubulin data set.

Based on the distinct clade of *Cylindrocladiella* species identified here, as well as their unique morphological traits, supported by molecular data, a new holomorphic genus is proposed below.

NECTRICLADIELLA Crous & C.L. Schoch, *gen. nov.* Anamorph: *Cylindrocladiella* Boesew.

Typus: *Nectricladiella camelliae* (Shipton) Crous & C.L. Schoch

Perithecia superficialia, solitaria, stromate basilari egentes, globosa ad obpyriformia, collabentia ubi arida, levia, numerosis setis parvis ex pariete laterali perithecii orientibus; apice et corpore perithecii rubro, basi brunnea, KOH+, ostiolum ex cellulis columnaribus compositum, periphysibus hyalinis inconspicuis indutum; pariete perithecii ex 3–4 stratis texturae angularis composito, cellulis compressis, hyalinis. Asci unitunicati, octospori, cylindrici, sessiles, tenuitunicati, apice applanato. Ascosporae uniseriatae, superpositae, hyalinae, ellipsoideae ad fusiformes, apicibus obtusis, uniseptatae.

Perithecia superficial, solitary, basal stroma absent, globose to obpyriform, collapsing laterally when dry, smooth, with several minute, brown setae arising from the perithecial wall surface; red, KOH+; ostiole consisting of clavate cells, lined with inconspicuous periphyses; perithecial wall consisting of a single region of 3–4 cell layers of *textura angularis*, which become hyaline and slightly flattened towards the centre. Asci unitunicate, 8-spored, cylindrical, sessile, thin-walled, with a flattened apex, and a refractive apical apparatus. *Ascospores* uniseriate, overlapping, hyaline, ellipsoidal to fusoid with obtuse ends, smooth, 1-septate. Anamorph *Cylindrocladiella.*

Nectricladiella camelliae (Shipton) Crous & C.L. Schoch, *comb. nov.*

≡ *Calonectria camelliae* Shipton & C. Booth, Trans. Br. Mycol. Soc. 69: 59. 1977 (*nom. nud.*).

≡ *Calonectria camelliae* Shipton, Trans. Br. Mycol Soc. 72: 163. 1979.

≡ *Nectria camelliae* (Shipton) Boesew., Canad. J. Bot. 60: 2293. 1982.

≡ *Cosmospora camelliae* (Shipton) Rossman & Samuels, Stud. Mycol. 42: 118. 1999.

Anamorph: **Cylindrocladiella microcylindrica** Crous & D. Victor, *sp. nov.*

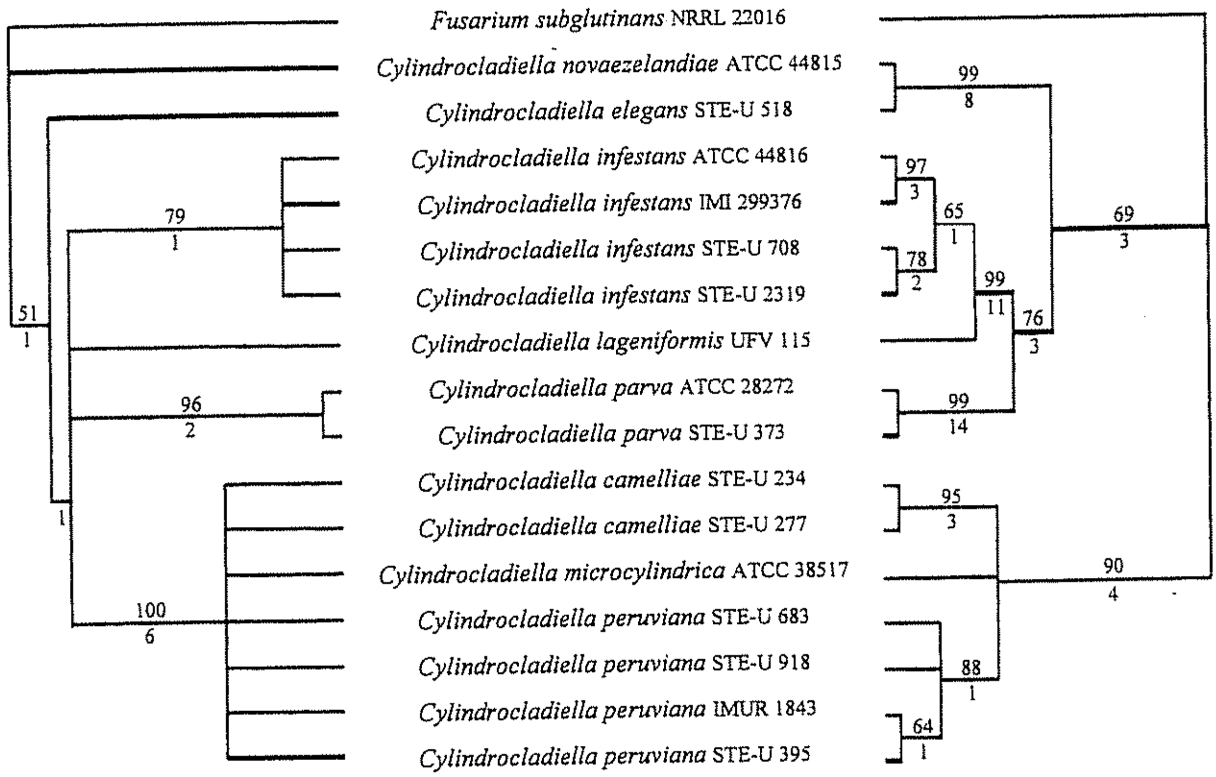


Fig. 13. Phylogenetic relationships among *Cylindrocladiella* species. Concordance of two most parsimonious trees obtained from the ITS (left) and β-tubulin (right) data sets. The ITS data set yielded three most parsimonious trees (118 steps, CI = 0.924, RI = 0.917, RC = 0.847) and β-tubulin yielded four (301 steps, CI = 0.847, RI = 0.897, RC = 0.760). Trees from the ITS data set were obtained with a heuristic search and 1000 random addition sequences, while a branch and bound search was done for the β-tubulin data set in PAUP* version 4.0b1. Bootstrap values are shown above branches and decay indices below.

Characteribus culturae, morphologia et reactione temperaturae *Ce. infestanti* similis sed conidiis minoribus distincta. Conidia hyalina, 1-septata, cylindrica, apicibus obtusis, (10–)12–14(–15) × 2(–3) μm.

Perithecia described in full by Shipton (1979). Ascospores hyaline, medianly septate, unconstricted, oval to ellipsoidal, 6.5–10.5 × 2.5–4 μm. Anamorph morphology and cultural characteristics similar to those of *Ce. infestans*, but conidia shorter (10–)12–14(–15) × 2(–3) μm, than those of the former (10–) 14–16(–20) × 2(–3) μm.

ETYMOLOGY.— *Micro* + *cylindrica*, referring to its smaller conidia and cylindrical vesicles.

COLONY COLOUR.— Buff yellow (reverse) (19D, Rayner, 1970). Chlamydospores in moderate numbers, arranged in chains.

CARDINAL TEMPERATURE REQUIREMENTS FOR GROWTH.— Minimum above 5°C, optimum 25°C, maximum below 35°C.

HABITAT.— Soil.

KNOWN DISTRIBUTION.— Australia, Argentina, Brazil, Thailand.

HOLOTYPES.— AUSTRALIA. QUEENSLAND: Fruit of a rainforest tree, W.A. Shipton, 1973, IMI 174836, holotype of the teleomorph; PREM 51724, holotype of the anamorph (ex-type culture: ATCC 38571 = STE-U 2375).

Nectricladiella infestans Crous & C.L. Schoch, *sp. nov.* — Figs. 15–19.

Anamorph: *Cylindrocladiella infestans* Boesew., Canad. J. Bot. 60: 2290. 1982.

Nectricladiellae camelliae simillima, sed anamorphe conidiis majoribus differens.

Perithecia superficial, solitary, basal stroma absent, globose to obpyriform, 150–200 μm high and diam, collapsing laterally when dry, smooth, with several minute, brown setae arising from the perithecial wall surface; apex and perithecial body red, base brown, changing colour in KOH, upper part turning red-brown, base becoming brown-red; ostiole consisting of columnar cells, lined with inconspicuous, hyaline periphyses; perithecial wall consisting of one region 6–8 cells thick; outer 3–4 layers of *textura angularis*, 10–15 μm thick; inner 3–4 cell layers becoming more flattened, thin-walled, hyaline, and disappearing with maturity. Asci

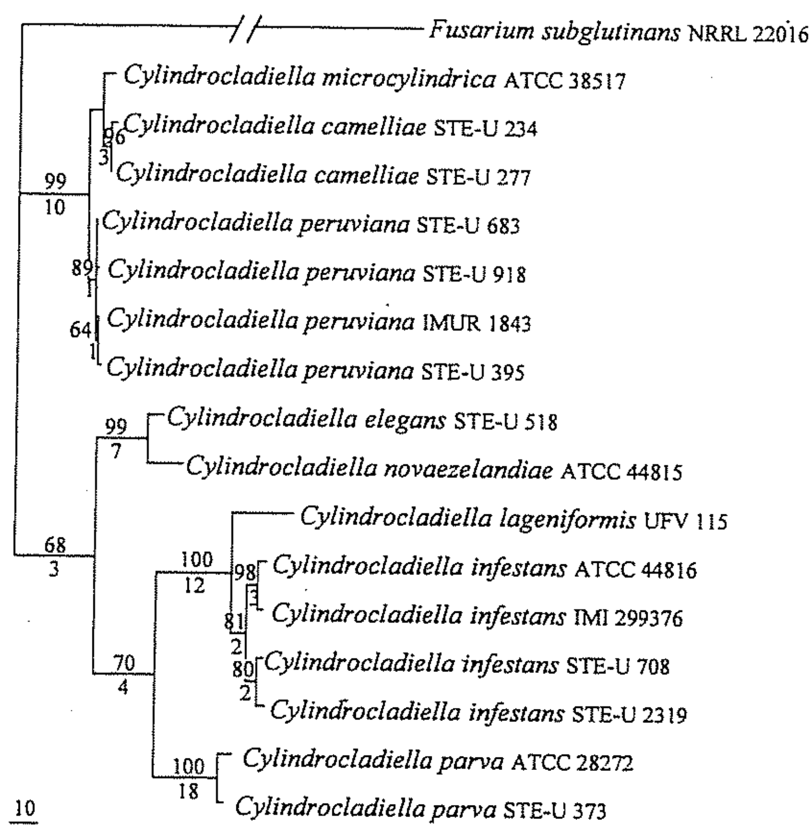


Fig. 14. Phylogenetic relationships among *Cylindrocladiella* species. One of four most parsimonious trees (422 steps, CI = 0.863, RI = 0.896, RC = 0.773) obtained from a branch and bound analysis of the combined ITS and β-tubulin data sets. Bootstrap values are shown above branches and decay indices below. The bar represents 10 steps.

unitunicate, 8-spored, cylindrical, becoming slightly clavate at maturity, sessile, thin-walled, with a flattened apex, and a refractive apical apparatus, 35–60 × 4–6 μm. Ascospores 8 per ascus, uniseriate, overlapping, hyaline, ellipsoidal to fusoid with obtuse ends, smooth, widest at the median septum or slightly above, unconstricted, 8–10(–12) × 3–3.5 μm; extruding from perithecia in a yellow mass. Anamorph morphology and cultural characteristics similar to those of *Ce. microcylindrica*, but conidia longer, (10–)14–16(–20) × 2(–3) μm (see Crous & Wingfield, 1993; Victor *et al.*, 1998).

HABITAT.— *Arenga pinnata*, *Pinus pinea*, soil.

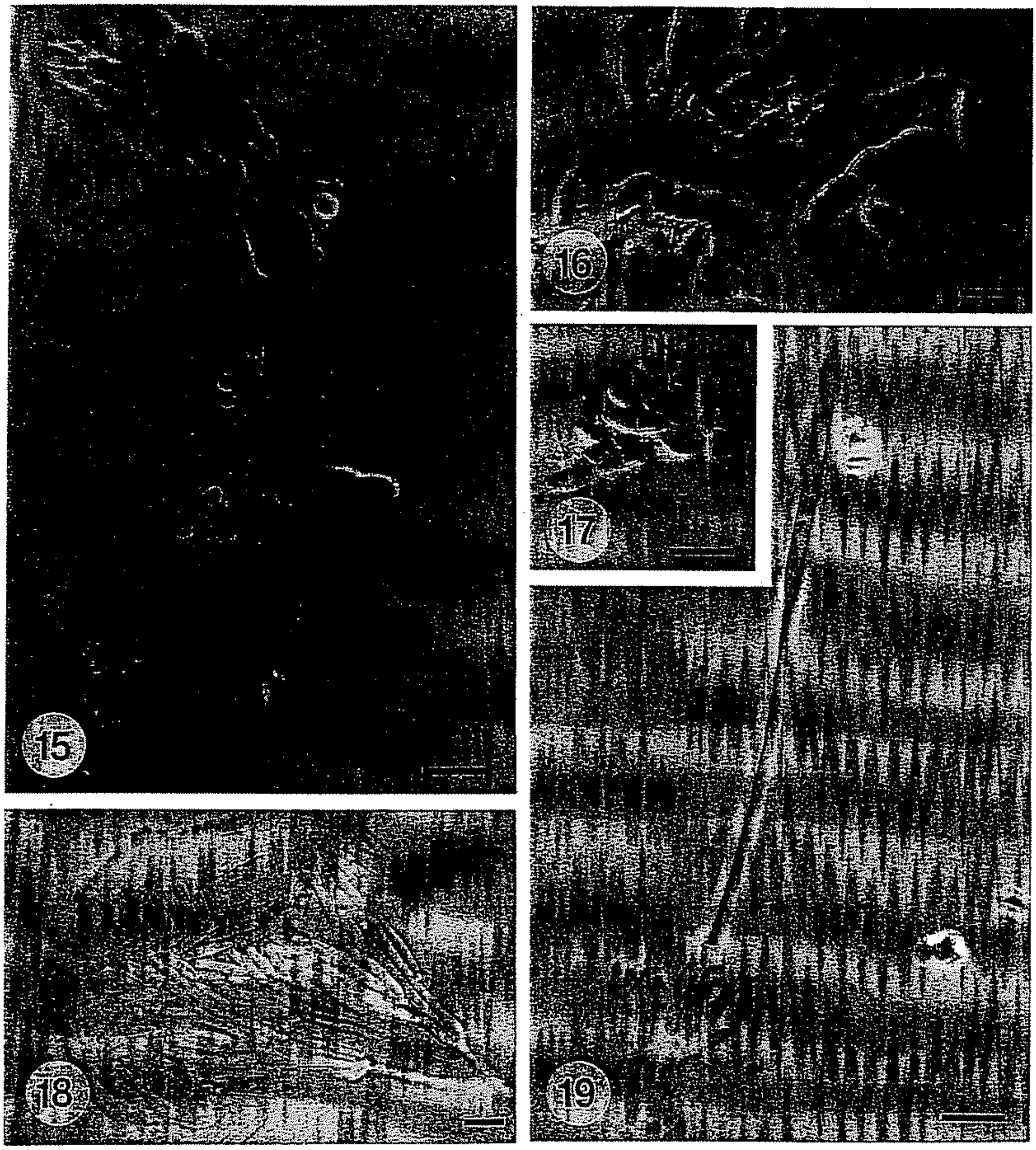
KNOWN DISTRIBUTION.— New Zealand, Madagascar, Hong Kong, Indonesia.

HOLOTYPES.— MADAGASCAR. Rana, isolated from soil, J.E. Taylor, 1998, PREM 56380, holotype of teleomorph (culture ex-type: STE-U 2319). NEW ZEALAND. Isolated from *Pinus pinea*, H.J. Boesewinkel, CBS 487.76, holotype of anamorph (culture ex-type: ATCC 44816 = STE-U 2380).

Leuconectria/Gliocephalotrichum

The similarities in perithecial anatomy between *Leuconectria* and *Calonectria* species have been noted before (Rossman *et al.*, 1993). Their *Gliocephalotrichum* and *Cylindrocladium* anamorphs also share several characteristics. Besides having penicillate conidiophores, cylindrical conidia, and forming chlamydospores in culture, the species of both anamorph genera have stipe extensions, even though they originate from different areas on the conidiophores. Cultural characteristics are also similar. Furthermore, both teleomorphs have KOH+, solitary, red perithecia. Perithecia of *Leuconectria* are distinctive, however, in having a white covering that is absent in species of *Calonectria*. Thus far, isolates of *Leuconectria* have been obtained only from decaying leaves, fruits, or from soil, and nothing is known about their potential status as plant pathogens. *Leuconectria* is similar to the other taxa in this paper in that the species occupy similar habitats, all basically being soil fungi that form more or less similar, small, red perithecia. This is in contrast to *Cylindrocarpon sensu stricto* (exclusive of the *N. radicicola* complex), which are primarily lignicolous and canker-forming.

The DNA sequence data employed here support the separation of *Leuconectria* from other related genera (Fig. 1). The data were ambiguous about the relationship of *Leuconectria* to other genera with cylindrical conidia, while at the same time confirming a close relationship with *Calonectria* (see also Rehner & Samuels, 1995).



Figs 15–19. *Nectricladiella infestans* and its *Cylindrocladiella infestans* anamorph. 15. Vertical section through a perithecium, showing smooth wall and small, brown hyphal seta. 16. Broken asci and ascospores 17. Ascospores. 18. Conidiophore and conidia. 19. Conidiophore with stipe extension and terminal cylindrical vesicle. Bars = 10 μm.

Gliocladiopsis

The anamorph genus *Gliocladiopsis* S.B. Saksena (Saksena, 1954; Crous & Peerally, 1996) closely resembles *Cylindrocladium*. The type species of the genus, *G. sagariensis* S.B. Saksena, was shown to be synonymous with *Cylindrocarpon tenue* Bugn. (Barron, 1968). Although it had been suggested previously that *Gliocladiopsis* should be retained for species lacking stipe extensions (Crous & Wingfield, 1993), Watanabe (1994) synonymized it with *Cylindrocladium* because he felt that the presence of stipe extensions was not a stable character. However, studies on *Cylindrocladium* and *Cylindrocladiella* have shown that species of both genera regularly produce stipe extensions on their conidiophores under controlled conditions (Crous & Wingfield, 1993, 1994), suggesting that *Gliocladiopsis*, with its multi-branched, penicillate conidiophores, should be retained. In our analysis, *Gliocladiopsis* is also represented by a separate clade. However, as with *Leuconectria*, the relationship of this genus to the other genera selected for this study is still equivocal, because of the low bootstrap support for the phylogeny (Fig. 1). The three described species of *Gliocladiopsis* have not been known previously to have teleomorphs (Saksena, 1954; Crous & Wingfield, 1993; Crous & Peerally, 1996). The present study describes the first teleomorph associated with this genus, which was produced by homothallic cultures obtained from single conidia of *G. tenuis* (Bugn.) Crous & M.J. Wingf. (STE-U 706) on CLA after 2 mo of incubation at 22°C with a 12 h fluorescent white light / dark regime.

Herewith, we propose a new holomorph genus for *Gliocladiopsis*. The new genus is based on the distances observed between other genera in the ITS DNA sequence-based tree, as well as the distinctive anamorph, *Gliocladiopsis*.

GLIONECTRIA Crous & C.L. Schoch, *gen. nov.*

Anamorph: *Gliocladiopsis* S.B. Saksena

Typus: *Glionectria tenuis* Crous & C.L. Schoch

Perithecia superficialia, dense gregaria, stromati tenui basilari insidentia, obovoidea ad late obpyriformia, collabentia ubi arida, verrucosa, rubrobrunnea, basi stromatica atro-rubra, KOH+, pariete perithecii ex duabus regionibus composito: exteriore strato ex *textura globulosa* crassitunicata, interiore strato ex cellulis compressis texturae angularis; periphyses ostioli cylindricae, apicibus rotundatis. Asci unitunicati, octospori, cylindrici, sessiles, apice applanato et apparatu apicali refringente. Ascosporae uniseriatae, superpositae, hyalinae, ellipsoidae, leves, medio uniseptatae.

Perithecia superficial, densely gregarious, seated on a thin basal stroma, obovoid to broadly obpyriform, collapsing laterally when dry, warted, red-brown with a dark red stromatic base, changing color in KOH; perithecial wall consisting of two regions: outer region of thick-walled *textura globulosa*, inner region of compressed cells of *textura angularis*; ostiolar periphyses tubular with rounded ends. Asci unitunicate, 8-spored, cylindrical, sessile, with a flattened apex, and a refractive apical apparatus. Ascospores uniseriate, overlapping, hyaline, ellipsoidal, smooth, medianly 1-septate. Anamorph *Gliocladiopsis*.

Glionectria tenuis Crous & C.L. Schoch, *sp. nov.* — Figs. 20–25.

Anamorph: *Gliocladiopsis tenuis* (Bugn.) Crous & M.J. Wingf., Mycol. Res. 97: 446. 1993.

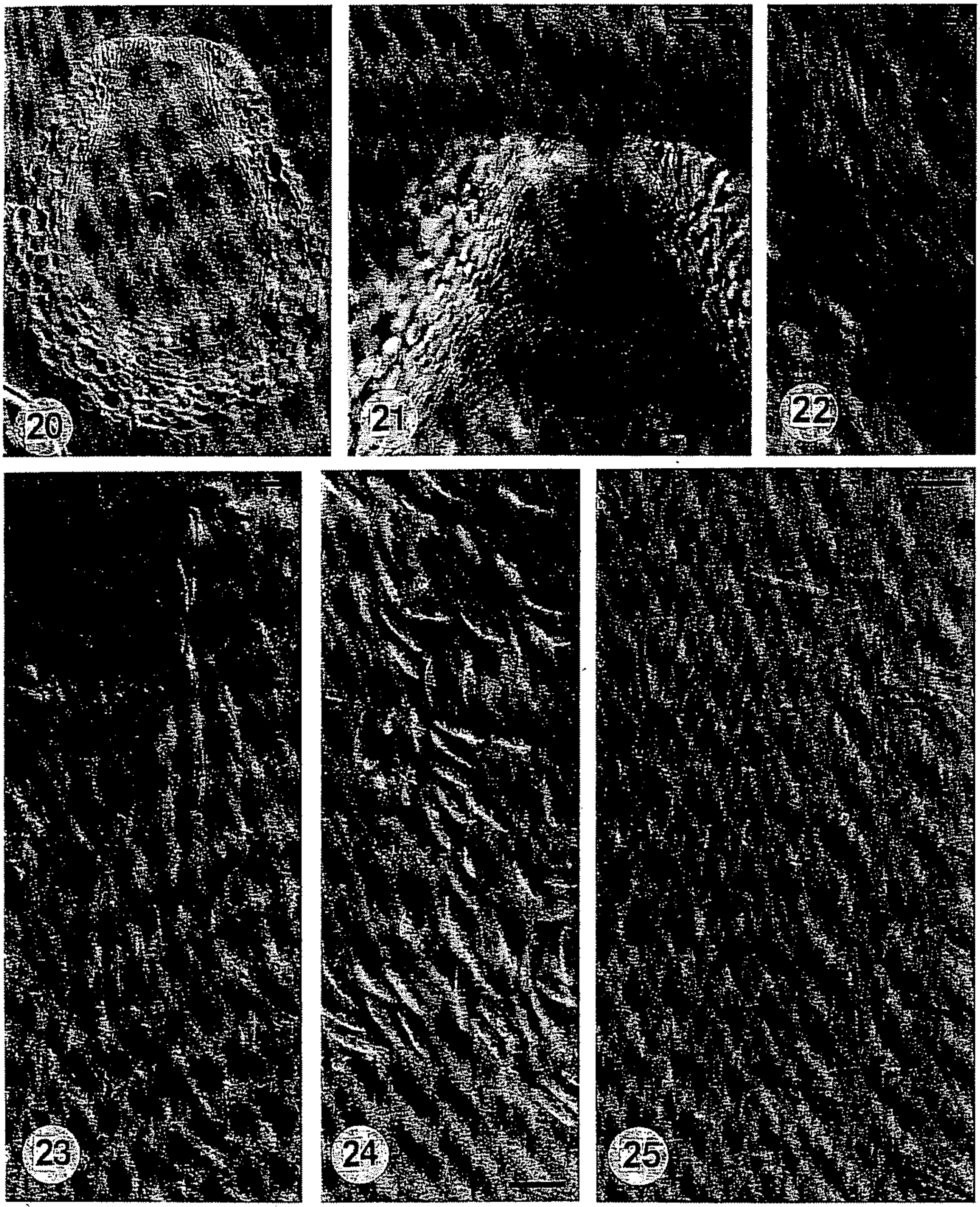
≡ *Cylindrocarpon tenue* Bugn., Encycl. Mycol. 11: 178. 1939.

≡ *Cylindrocladium tenue* (Bugn.) T. Watan., Mycologia 86: 155. 1994.

= *Gliocladiopsis sagariensis* Saksena, Mycologia 46: 663. 1954.

Perithecia superficialia, dense gregaria, stromati basilari tenui insidentia, obovidea ad late obpyriformia, collabentia ubi arida, usque ad 400 µm alta et 350 µm diam, verrucosa, apice leviter applanato, aurantiaca, corpore et basi rubro-brunnea, KOH+, apice aurantio-rubro, perithecii purpureo-rubro et base atro-rubro brunnea. Regione ostiolari usque ad 180 µm diam. Pariete perithecii ex duabus regionibus composito: regione exteriore ex 4–5 stratis texturae globulosae crassitunicatae composita, usque ad 60 µm crassa, compressa ad centrum, regione interiore ex 3–4 stratis texturae angularis composita, usque ad 20 µm crassa. Asci unitunicati, octospori, cylindrici, apice obtuse rotundato, sessiles, apparatu apicali refringente, 50–80 × 4–5 µm. Ascosporae uniseriatae, superpositae, hyalinae, leves, ellipsoideae, apicibus rotundatis, 9–12 × 2.5–3 µm, latissimae ad septum medianum, non constrictae.

Perithecia superficial, densely gregarious, seated on a thin basal stroma, obovoid to broadly obpyriform, collapsing laterally when dry, up to 400 µm high and 350 µm diam, warted, apex slightly flattened, orange, body and base red-brown, KOH+, apex becoming orange-red, perithecial body purple-red and base dark red-brown. Ostiolar region up to 180 µm thick. Perithecial wall consisting of two regions: outer region of 4–5 layers of thick-walled *textura globulosa* up to 60 µm thick, becoming compressed towards the centrum, inner region consisting of 3–4 layers of *textura angularis* up to 20 µm thick. Asci unitunicate, 8-spored, cylindrical, with a bluntly rounded apex, sessile, with a refractive apical apparatus, 50–80 × 4–5 µm. Ascospores uniseriate, overlapping, hyaline, smooth, ellipsoidal with rounded ends, 9–12 × 2.5–3 µm, widest at the median septum, not constricted. Conidiophores penicillate, without stipe extensions and terminal vesicles. Conidiophore branches aseptate: primary branches 9–23 × 3–5 µm, secondary branches 10–18 × 2.5–4 µm, tertiary branches 9–14 × 2.5–3.5 µm, quaternary branches rare to absent, 9–12 × 2.5–3 µm. Phialides doliiform to cymbiform to cylindrical, 10–25 × 2.5–3 µm, arranged in terminal whorls of up to 7 per branch, with minute collarettes. Conidia cylindrical, hyaline, smooth, with rounded ends, medianly 1-septate, (12–)16–19(–23) × 1.5–2(–2.5) µm.
COLONY COLOUR.— Sayal brown (reverse) (15''I, Rayner, 1970). Chlamydospores abundant, in clearly delimited, mostly unbranched chains.
CARDINAL TEMPERATURE REQUIREMENTS FOR GROWTH.— Minimum above 5°C, optimum 25–30°C, maximum above 35°C.



Figs 20–25. *Glionectria tenuis* and its anamorph *Gliocladiopsis tenuis*. 20. Vertical section through a perithecium. 21. Section through ostiolar region. 22. Paraphyses. 23. Cylindrical asci with apical mechanism. 24. One-septate ascospores. 25. Conidiophore with cylindrical, 1-septate condia. Bars = 10 μm.

HABITAT.— *Indigofera* sp., *Psidium guajava*, *Shorea robusta*, *Camellia sinensis*, *Chamaedorea elegans*, soil.
KNOWN DISTRIBUTION.— Brazil, Colombia, Hong Kong, India, Indonesia, Thailand, U.S.A.

HOLOTYPES.— HONG KONG. Soil, M.J. Wingfield, 1993, PREM 56381, holotype of teleomorph (culture ex-type: STE-U 706). INDOCHINA (country unknown). *Indigofera* sp., F. Bugnicourt, Nov. 1936, PC 540, holotype of anamorph (ex-type culture: IMI 68205 = STE-U 2403).

KEY TO GENERA OF THE *NECTRIACEAE* HAVING CYLINDRICAL CONIDIA BORNE IN HYALINE OR PALE YELLOW MASSES

1. Conidiophores penicillate or nearly so, conidiomata sporodochial or synnematous 2
1. Conidiophores penicillate, mononematous 4

2. Stipe extensions on conidiophores absent; conidia in hyaline or pale yellow slime; perithecia solitary to gregarious, the warted wall consisting of two layers; asci cylindrical, sessile, with apical apparatus; ascospores smooth, hyaline, 1-septate 3
2. Stipe extensions present on conidiophores; conidia in hyaline slime; extensions with one apical and basal septum, apical cell curved, pigmented, verruculose .... *Curvicladium*

3. Conidiophores always penicillate with more than 2 series of branches, rarely solitary, mostly gregarious; macroconidia cylindrical with rounded ends, 1-septate, straight or curved, abscission scar inconspicuous; microconidia absent .... *Glionectria* (*Gliocladiopsis*)
3. Conidiophores frequently divergent, or unbranched with a single conidiogenous cell; macroconidia cylindrical, straight or curved, 1-multiseptate, attenuating to rounded ends with a basal abscission scar; microconidia (when present) fusoid to ellipsoidal, 0–1-septate .... *Neonectria* (*Cylindrocarpon*)

4. Stipe extensions of conidiophores hyaline, arising above the apical penicillus, perithecia not as below 5
4. Stipe extensions of conidiophores slightly pigmented, forming below the apical penicillus; perithecia warted, wall consisting of two layers, perithecial wall with a white outer coat; asci narrowly clavate, sessile, with apical apparatus; ascospores smooth, hyaline, 1-septate .... *Leuconectria* (*Gliocephalotrichum*)

5. Perithecium wall warted, consisting of two layers; asci with long basal stalk; stipe extensions of conidiophores multi-septate, thin-walled; conidia longer than 25 µm; phialide collarettes divergent 6
5. Perithecium wall smooth, 1-layered, frequently with a few reduced hyphal setae, body collapsing at maturity; asci cylindrical, sessile, with apical apparatus; ascospores smooth, hyaline, 1-septate; stipe extensions aseptate, thick-walled; conidia shorter than 25 µm; phialide collarettes convergent .... *Nectricladiella* (*Cylindrocladiella*)

6. Asci clavate without an apical apparatus; ascospores 1–6-septate; stipe extensions of conidiophores straight, terminating in a swollen vesicle of characteristic shape . *Calonectria* (*Cylindrocladium*)
6. Asci cylindrical with apical apparatus; ascospores 1-septate; stipe extensions spirally twisted, hyaline, smooth, avesiculate; 1-septate .... *Xenocalonectria* (*Xenocylindrocladium*)

Acknowledgements

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