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Characterisation and pathogenicity of *Cylindrocladiella* spp. associated with root and cutting rot symptoms of grapevines in nurseries

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Abstract. Species of *Cylindrocladiella* occur on a variety of hosts, where they are known to act as pathogens or saprobes. Eight species from this genus are currently recognised, of which five have been reported previously in South Africa: *C. camelliae*, *C. elegans*, *C. lageniformis*, *C. parva* and *C. peruviana*. Isolates of *Cylindrocladiella* were obtained from a newly established 99-Richter grapevine mother vine block exhibiting decline symptoms. The DNA phylogeny of these and additional isolates, also obtained from declining grapevines, was determined by sequencing the ITS (ITS1, ITS2 and 5.8S) as well as β-tubulin and histone H3 gene regions. This identified four species of *Cylindrocladiella* on grapevines in South Africa: *C. lageniformis*, *C. parva*, *C. peruviana* and a new species described here as *C. viticola*, which forms part of the *C. infestans* species complex. Pathogenicity trials, using stem inoculations on green and 1-year-old 99-Richter cuttings to determine the pathogenicity of selected isolates of these species, gave inconclusive results.

Additional keywords: *Cylindrocladiella viticola*, 99-Richter, 101–14 Mgt.

Introduction

Boesewinkel (1982) proposed a new hypocrealean genus, *Cylindrocladiella* Boesew., to accommodate small-spored species of *Cylindrocladium* Morgan. This decision was based on the fact that species of *Cylindrocladiella* had different conidiophore branching patterns, conidial shapes, dimensions, cultural characteristics and teleomorphs from those of *Cylindrocladium*. Although several researchers continued to consider *Cylindrocladiella* and *Cylindrocladium* as a single genus (Mandal and Dasgupta 1983; Peerally 1991; Sharma and Mohanan 1991), Crous *et al*. (1994) confirmed the two genera to be distinct, with *Cylindrocladium* having *Calonectria* de Not. teleomorphs (Rossman 1979; Crous 2002), and *Cylindrocladiella* having *Nectricladiella* Crous & C.L. Schoch teleomorphs (Schoch *et al*. 2000).

Victor *et al*. (1998) recognised seven species in the genus *Cylindrocladiella* that could be distinguished on Restriction Fragment Length Polymorphisms (RFLPs) and AT-DNA data (A + T-rich), as well as morphology. Schoch *et al*. (2000) compared phylogenies derived from the ITS regions flanking the 5.8S rRNA gene and the 5′ end of the β-tubulin gene of various *Cylindrocladiella* isolates. Based on these data, they distinguished the same seven species recognised by Victor *et al*. (1998), as well as a further species, *C. microcylindrica* Crous & D. Victor. The latter taxon was hitherto incorrectly treated as *C. infestans* Boesew. As was further noted by Schoch *et al*. (2000), and Crous (2002), significant DNA sequence variation was observed within isolates identified as *C. infestans*, and these strains were thus best treated as a species complex until their status could be resolved.

In South Africa, five species of *Cylindrocladiella* have been reported as pathogens or saprobes on various hosts. *Cylindrocladiella camelliae* (Venkataram & C.S.V. Ram) Boesew. has been associated with cutting rot of *Eucalyptus* spp., *C. elegans* Crous & M.J. Wingf. with roots of *Arachis hypogaea* L. and *Eucalyptus* leaf litter, *C. lageniformis* Crous, M.J. Wingf. & Alfenas with soil and roots of *Vitis vinifera* L., *C. parva* (P.J. Anderson) Boesew. with soil, *Eucalyptus* cuttings and roots of *Fragaria* sp., *Persea americana* Mill., *Pinus* spp., *Prunus* sp. and *V. vinifera*, and *C. peruviana* (Bat., J.L. Bezerra & M.P. Herrera) Boesew. with root and cutting rot of

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Acacia mearnsii De Wild., *Eucalyptus* spp., *Protea* sp. and *V. vinifera* (Crous and Wingfield 1993; Victor *et al*. 1998; Crous 2002). Although isolates of *C. lageniformis*, *C. parva* (P.J. Andserson) Boesew. and *C. peruviana* have over the past 10 years been isolated from roots and rootstocks of mature grapevines, cuttings and the graft union of grafted young grapevine material (S.C. Lamprecht and P.W. Crous, unpublished data), Koch's postulates have never been proven, and their role as pathogens on *Vitis* spp. still needs to be elucidated.

In a recent survey of soilborne pathogens of grapevines in nurseries, isolates of several *Cylindrocladiella* spp. were obtained from a newly established 99-Richter (*Vitis berlandieri* Planch. × *Vitis rupestris* Scheele cv. du Lot) mother vine block, showing symptoms of decline (e.g. chlorotic leaves and stunted growth), while another species of *Cylindrocladiella* was obtained from a declining grafted nursery grapevine (*Vitis vinifera* × 101–14 Mgt [*Vitis riparia* Michx. × *Vitis rupestris*]). The aims of this study were to characterise the species of *Cylindrocladiella* associated with grapevines in South Africa and to determine their pathogenicity on this host.

Methods

Cultures

Strains used in previous studies (Crous and Wingfield 1993; Victor *et al*. 1998; Schoch *et al*. 2000; Crous 2002) and deposited at the culture collection of the Department of Plant Pathology at the University of Stellenbosch (STE-U) were included. Freshly obtained isolates from diseased grapevines encountered during surveys at a grapevine nursery in the Western Cape province of South Africa were also included. Strains were isolated using the methods explained in Crous (2002). Representative strains were deposited at the Centraalbureau voor Schimmelcultures (CBS) at Utrecht, the Netherlands (Table 1), and the nomenclature and species description in MycoBank (www.MycoBank.org).

DNA extraction, sequencing and phylogeny

Genomic DNA was isolated from fungal mycelium following the protocol of Lee and Taylor (1990), after which the ITS and β-tubulin regions (Kang *et al*. 2001), as well as the histone H3 region, were amplified (Crous *et al*. 2004). The primers ITS1 and ITS4 (White *et al*. 1990) were used to amplify the ITS1 region, 5.8S rRNA gene and the ITS2 region of the nuclear-encoded rRNA gene. Part of the β-tubulin gene was amplified with the primers T1 (O'Donnell and Cigelnik 1997) and Bt-2b (Glass and Donaldson 1995). Part of the histone H3 gene was amplified and sequenced using the CYLH3F and CYLH3R primers described by Crous *et al*. (2004). The amplification products were visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining. The amplified fragments were purified using a commercial kit (NucleoSpin Extract 2 in 1 Purification Kit, Macherey-Nagel GmbH, Germany) and the PCR primers were used to sequence both strands of the purified products using an ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut).

The sequences generated, together with BLASTn retrievals from GenBank, were assembled and aligned using Sequence Alignment Editor v2.0a11 (Rambaut 2002). Sequences of *Cylindrocladium floridanum* Sobers & C.P. Seym. were used as outgroup for the sequence alignments. The phylogenetic analysis of the sequence alignment was done using PAUP (Phylogenetic Analysis Using Parsimony) v4.0b10 (Swofford 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analyses were performed for all datasets using the heuristic search option with 100 random taxon additions and tree bisection and reconstruction (TBR) as the branch swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The resulting trees were evaluated with 1000 bootstrap replications to test the clade stability (Hillis and Bull 1993). Other measures calculated included tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC). The resulting trees were printed with TreeView Version 1.6.6 (Page 1996). A partition homogeneity test (Farris *et al*. 1994) was conducted in PAUP (Swofford 2000) to examine the possibility of a joint analysis of the three datasets.

Morphological and cultural comparisons

Strains were cultured on 2% malt-extract agar (MEA) (Biolab Diagnostics, 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 days. Only material growing on carnation leaves from CLA was examined. Mounts were prepared in lactophenol, examined using Nomarski interference phase contrast and brightfield phase contrast microscopy, and measurements made at ×1000 magnification. The 95% confidence intervals of conidial measurements were determined from at least 30 observations and the minimum and maximum ranges given in parentheses. Temperature requirements for growth and cultural characteristics were determined after 7 days on MEA in the dark at 25°C, using procedures described by Crous and Wingfield (1994). Colony colours were determined using the colour charts of Rayner (1970).

Stem inoculations

The response of grapevine rootstocks to isolates of the different *Cylindrocladiella* spp. was determined with stem inoculations in two different trials, which were both repeated in two experiments, using the same methodology. In the first experiment, the isolates were inoculated onto green shoots (7–15 mm diameter, 30 cm long), and in the second experiment, 1-year-old shoots (10–20 mm diameter, 30 cm long) were inoculated. Eighteen isolates of *Cylindrocladiella*, obtained from declining grapevines, were used, six of *C. lageniformis*, one of *C. parva*, nine of *C. peruviana*, and two of an unknown *Cylindrocladiella* species. Shoots were pruned from a visually healthy 99-Richter mother vine block. The shoots were placed in 500 mL flasks and 50 mL municipal tap water was applied to each flask, in order to establish sap flow in the shoots. One day later, the grapevines were subjected to one of two different inoculation treatments, wounded and unwounded inoculation. Shoots were wounded 5 cm from the apical end using a 4-mm-diameter cork borer to remove the cortex tissue (one wound per shoot). Colonised mycelial plugs from 2-week-old cultures of the different *Cylindrocladiella* cultures, grown on 2% potato-dextrose agar (PDA; Biolab Diagnostics, Midrand, South Africa) were inserted into the wounds and sealed with Parafilm. Two control treatments were used, non-colonised PDA plugs, and a negative (non-pathogen) control, which consisted of mycelial plugs colonised with *Clonostachys rosea*. For the unwounded treatment, the mycelial plugs were placed on the outside of the shoot, 5 cm from the apical end and sealed with Parafilm. All treatments were replicated three times. The shoots were placed in a dew chamber in a completely

Table 1. ***Cylindrocladiella*** **isolates included in this study for sequence analysis and/or morphological comparison**

Anamorph	Teleomorph	Accession number[A]	Substrate	Country	Collector	Areas sequenced		
						ITS	β-tubulin	Histone
Cylindrocladiella camelliae	Unknown	STE-U 234	*Eucalyptus grandis*	Northern Province, South Africa	P. W. Crous	AF220952	AY793471	AY793509
C. camelliae	Unknown	STE-U 277	*Eucalyptus grandis*	Northern Province, South Africa	P. W. Crous	AF220953	AY793472	AY793510
C. elegans	Unknown	STE-U 518	Litter	Western Cape, South Africa	P. W. Crous	AF220954	AY793473	AY793511
C. elegans[B]	Unknown	CBS 338.92	Leaf litter	KwaZulu-Natal, South Africa	I. Rong	AY793444	AY793474	AY793512
C. infestans[B]	*Nectricladiella* sp.	ATCC 44816	*Pinus pinea*	New Zealand	H. J. Boesewinkel	AF220955	AF320190	AY793513
C. infestans	*Nectricladiella* sp.	CBS 191.50 / IMI 299376	*Arenga pinnata*	Indonesia	K. B. Boedijn & J. Reitsma	AF220956	AY793475	AY793514
C. lageniformis	Unknown	STE-U 5607	*Vitis vinifera* cv. 99-Richter[C]	Western Cape, South Africa	G. J. van Coller	AY793445	AY725652	AY725699
C. lageniformis	Unknown	STE-U 5608	*Vitis vinifera* cv. 99-Richter[C]	Western Cape, South Africa	G. J. van Coller	AY793446	AY793476	AY793515
C. lageniformis	Unknown	STE-U 5609	*Vitis vinifera* cv. 99-Richter[C]	Western Cape, South Africa	G. J. van Coller	AY793447	AY793477	AY793516
C. lageniformis	Unknown	STE-U 5610	*Vitis vinifera* cv. 99-Richter[C]	Western Cape, South Africa	G. J. van Coller	AY793448	AY793478	AY793517
C. lageniformis	Unknown	STE-U 5611	*Vitis vinifera* cv. 99-Richter[C]	Western Cape, South Africa	G. J. van Coller	AY793449	AY793479	AY793518
C. lageniformis	Unknown	STE-U 5736	*Vitis vinifera* cv. 99-Richter[C]	Western Cape, South Africa	G. J. van Coller	AY793450	AY793480	AY793519
C. lageniformis[B]	Unknown	UFV115 / STE-U 2519	*Eucalyptus* sp.	Brazil	A. C. Alfenas	AF220959	AY793481	AY793520
C. lageniformis		STE-U 11300	*Vitis vinifera*	Western Cape, South Africa	G. J. van Coller	AY793451	AY793482	AY793521
C. microcylindrica[B]	*Nectricladiella camelliae*	ATCC 38571	*Pinus pinea*	Australia	W. A. Shipton	AF220960	AF320191	AY793522
C. microcylindrica	Unknown	STE-U 10451	*Echeveria elegans*	Indonesia	C. F. Hill	AY793452	AY793483	AY793523
C. microcylindrica	Unknown	STE-U 10452	*Agalonema commutatum*	USA	C. F. Hill	AY793453	AY793484	AY793524
C. novaezelandiae[B]	Unknown	ATCC 44815	*Rhododendron indicum*	New Zealand	H. J. Boesewinkel	AF220963	AY793485	AY793525
C. parva[B]	Unknown	ATCC 28272	*Telopea speciosissima*	New Zealand	H. J. Boesewinkel	AF220964	AY793486	AY793526
C. parva	Unknown	STE-U 373	*Pinus radiata*	Western Cape, South Africa	P. W. Crous	AF220965	AY793487	AY793527
C. parva	Unknown	STE-U 5735	*Vitis vinifera* cv. 101–14 Mgt[D]	Western Cape, South Africa	G. J. van Coller	AY793454	AY793488	AY793528
C. parva	Unknown	STE-U 10956	*Quercus robur*	Italy	S. Mutto Accordi	AY793455	AY793489	AY793529
C. peruviana	Unknown	STE-U 395	*Acacia mearnsii*	KwaZulu-Natal, South Africa	P. W. Crous	AF220967	AY793490	AY793530
C. peruviana	Unknown	STE-U 683	Soil	Thailand	M. J. Wingfield	AF220961	AY793491	AY793531

(*Continued next page*)

Table 1. Continued

Anamorph	Teleomorph	Accession number[A]	Substrate	Country	Collector	Areas sequenced		
						ITS	β-tubulin	Histone
C. peruviana	Unknown	STE-U 4210	*Vitis vinifera*	California, USA		AY793456	AY793492	AY793532
C. peruviana	Unknown	STE-U 5612	*Vitis vinifera*	Western Cape, South Africa	S. C. Lamprecht	AY793457	AY793493	AY793533
C. peruviana	Unknown	STE-U 5613	*Vitis vinifera*	Western Cape, South Africa	S. C. Lamprecht	AY793458	AY793494	AY793534
C. peruviana	Unknown	STE-U 5614	*Vitis vinifera*	Western Cape, South Africa	S. C. Lamprecht	AY793459	AY725653	AY725700
C. peruviana	Unknown	STE-U 5615	*Vitis vinifera*	Western Cape, South Africa	S. C. Lamprecht	AY793460	AY793495	AY793535
C. peruviana	Unknown	STE-U 5616	*Vitis vinifera*	Western Cape, South Africa	S. C. Lamprecht	AY793461	AY793496	AY793536
C. peruviana	Unknown	STE-U 5617	*Vitis vinifera*	Western Cape, South Africa	S. C. Lamprecht	AY793462	AY793497	AY793537
C. peruviana	Unknown	STE-U 5618	*Vitis vinifera*	Western Cape, South Africa	S. C. Lamprecht	AY793463	AY793498	AY793538
C. peruviana	Unknown	STE-U 5619	*Vitis vinifera*	Western Cape, South Africa	S. C. Lamprecht	AY793464	AY793499	AY793539
C. peruviana[B]	Unknown	IMUR 1843	Ants	Peru	M. P. Herrera	AF220966	AY793500	AY793540
C. peruviana	Unknown	IMI 384951	*Oryza sativa* ex rice field	India		AY793465	AY793501	AY793541
C. peruviana	Unknown	CBS 115673 / STE-U 917	Soil	South America	P. W. Crous	AY793466	AY793502	AY793542
C. peruviana	Unknown	CBS 115675	Soil	South America	P. W. Crous	AY793467	AY793503	AY793543
C. viticola[B]	Unknown	STE-U 5606 / CBS 112897	*Vitis vinifera* cv. 99-Richter[C]	Western Cape, South Africa	G. J. van Coller	AY793468	AY793504	AY793544
C. viticola	Unknown	STE-U 5620	*Vitis vinifera* cv. 99-Richter[C]	Western Cape, South Africa	F. Halleen	AY793469	AY793505	AY793545
Cylindrocladiella sp.	Unknown	STE-U 708	Soil	Hong Kong	M. J. Wingfield	AF220958	AY793506	AY793546
Cylindrocladiella sp.	*Nectricladiella infestans*	CBS 112364 / Lynfield 791-A / STE-U 10490	*Archontophoenix purpurea*	Australia	F. Hill	AY793470	AY793507	AY793547
Cylindrocladiella sp.	*Nectricladiella infestans*[B]	STE-U 2319	Soil	Madagascar	J. E. Taylor	AF220957	AY793508	AY793548

[A]ATCC: American Type Culture Collection, Virginia, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; IMUR: Institute of Mycology, University of Recife, Brazil; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; UFV: (A.C. Alfenas), Department of Plant Pathology, University of Viçosa, Viçosa, Minas Gerais, Brazil.
[B]Ex-type cultures.
[C]Richter 99 = *Vitis berlandieri* × *Vitis rupestris* cv. du Lot.
[D]101–14 Mgt = *Vitis riparia* × *Vitis rupestris*.

randomised design. The dew chamber temperature was maintained at 22°C, with a 12 h fluorescent white light/dark regime. After 14 days, lesion lengths were determined, and re-isolations were made from lesion margins onto PDA to confirm Koch's postulates. Lesion lengths were calculated by subtracting the diameter of the cork-borer from the lesion formed.

Statistical analysis

Results of wounded and unwounded treatments of the two experiments were analysed separately. To test for homogeneity of variances between repeats of the pathogenicity experiments, Levene's test was performed (Levene 1960). In cases where not enough evidence for homogeneity existed, an analysis of combined data was performed using the variances of the repeats as weights (John and Quenouille 1977). To test for non-normality, Shapiro-Wilk's test was performed (Shapiro and Wilk 1965). Data obtained from the experiments were then subjected to analysis of variance (ANOVA) using SAS statistical software version 8.02 (SAS Institute Inc. 1999). Student's t least significant differences (l.s.d.) pairwise comparisons (Snedecor and Cochran 1989) were calculated at the 5% level of significance to establish differences between treatment means.

Results

DNA phylogeny

DNA amplification products of ~550 bp were obtained for both ITS and β-tubulin and of ~475 bp for histone. The manually adjusted alignment of the three loci contained 44 taxa (including the two outgroup taxa) and 1501 characters including alignment gaps. Of the aligned nucleotide sites, 490 characters were parsimony-informative, 53 variable characters were parsimony-uninformative and 958 were constant. The result of the partition homogeneity test ($P = 0.684$; where $P \geq 0.05$ was taken as significantly incongruent) showed that the three datasets were combinable. Sixteen equally most parsimonious trees (TL = 993 steps, CI = 0.802, RI = 0.951, RC = 0.762) were obtained from maximum parsimony analysis of the combined sequence data, one of which is shown in Fig. 1. New sequences were deposited in GenBank (Table 1), and the alignments in TreeBASE (accession number SN2082).

The *Cylindrocladiella* isolates group in three main clades each with 100% bootstrap support. The first main clade contains isolates of *C. camelliae* (100% bootstrap support), *C. microcylindrica* (99% bootstrap support) and *C. peruviana* (100% bootstrap support). The second main clade contains *C. novaezelandiae* (Boesew.) Boesew. and *C. elegans*. The third main clade contains a subclade (99% bootstrap support) containing two isolates of *C. infestans* that clusters with a bootstrap support value of 83%, and a second cluster containing a *Cylindrocladiella* sp. (84% bootstrap support), a single *Nectricladiella infestans* Crous & C.L. Schoch isolate that sits as a basal polytomy, and a clade containing *C. viticola* Crous & G.J. van Coller (100% bootstrap support). Subclades containing isolates of *C. lageniformis* and *C. parva* both received high bootstrap support.

Morphology and taxonomy

Four species of *Cylindrocladiella* were found on grapevines in South Africa. The single isolate of *C. parva* obtained (STE-U 5736), had conidia that were 12–20 × 2–3 µm in size, thus similar to that reported for the species (Crous 2002). Vesicles were subcylindrical to pyriform, or clavate with an acutely rounded apex, as shown in Fig. 296 in Crous (2002), thus closely agreeing with the description of the species. Isolates of *C. lageniformis* had ovoid to ellipsoidal, or lageniform to pyriform vesicles, which agreed with the species (Crous 2002), but were generally narrower (7–9 µm wide) than reported for the ex-type strain. Conidia were 9–16 × 1.5–2 µm, closely matching that of the species (Crous 2002). Isolates of *C. peruviana* were characterised by their ellipsoidal to lanceolate vesicles, and conidia which were 9–15 × 2–3 µm in size, also closely matching those of the species (Crous 2002).

In addition to confirming that these three species occur on grapevines in South Africa, a previously undescribed species was also revealed in the *C. infestans* species complex. Although this complex has been acknowledged as being variable, based on sequence data obtained in previous studies (Schoch *et al*. 2000; Crous 2002), the inclusion of additional isolates have shown this complex to consist of at least four taxa (Fig. 1): *C. infestans* (ATCC 44816, ex-type), *Nectricladiella infestans* (STE-U 2319, ex-type), a *Cylindrocladiella* sp. (STE-U 708, 10490), and a new species which is described here.

Cylindrocladiella viticola Crous & G.J. van Coller, *sp. nov.* MB500187 (Fig. 2)

Characteribus culturae et morphologia *C. infestanti* similis sed distincta propter conidia minoria. Conidia hyalina 1-septata, cylindracea apicibus obtusis, (8–)9–14(–15) × 2–2.5(–3) µm.

Teleomorph unknown. *Conidiophores* monomorphic, penicillate, or dimorphic, penicillate and subverticillate, mononematous, hyaline; penicillate conidiophores comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; subverticillate conidiophores comprising a stipe, and one or two series of phialides; stipe septate, hyaline, smooth; stipe extensions aseptate, straight, 80–145 µm long, thick-walled, with one basal septum, terminating in thin-walled, irregularly ellipsoid to clavate vesicles, 4–8 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate or 1-septate, 13–25 × 3–4 µm; secondary branches aseptate, 9–13 × 2.5–3 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–15 × 2.5–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* in moderate numbers, phialides cymbiform to subcylindrical, 10–25 × 2.5–3 µm. *Conidia* cylindrical, rounded at

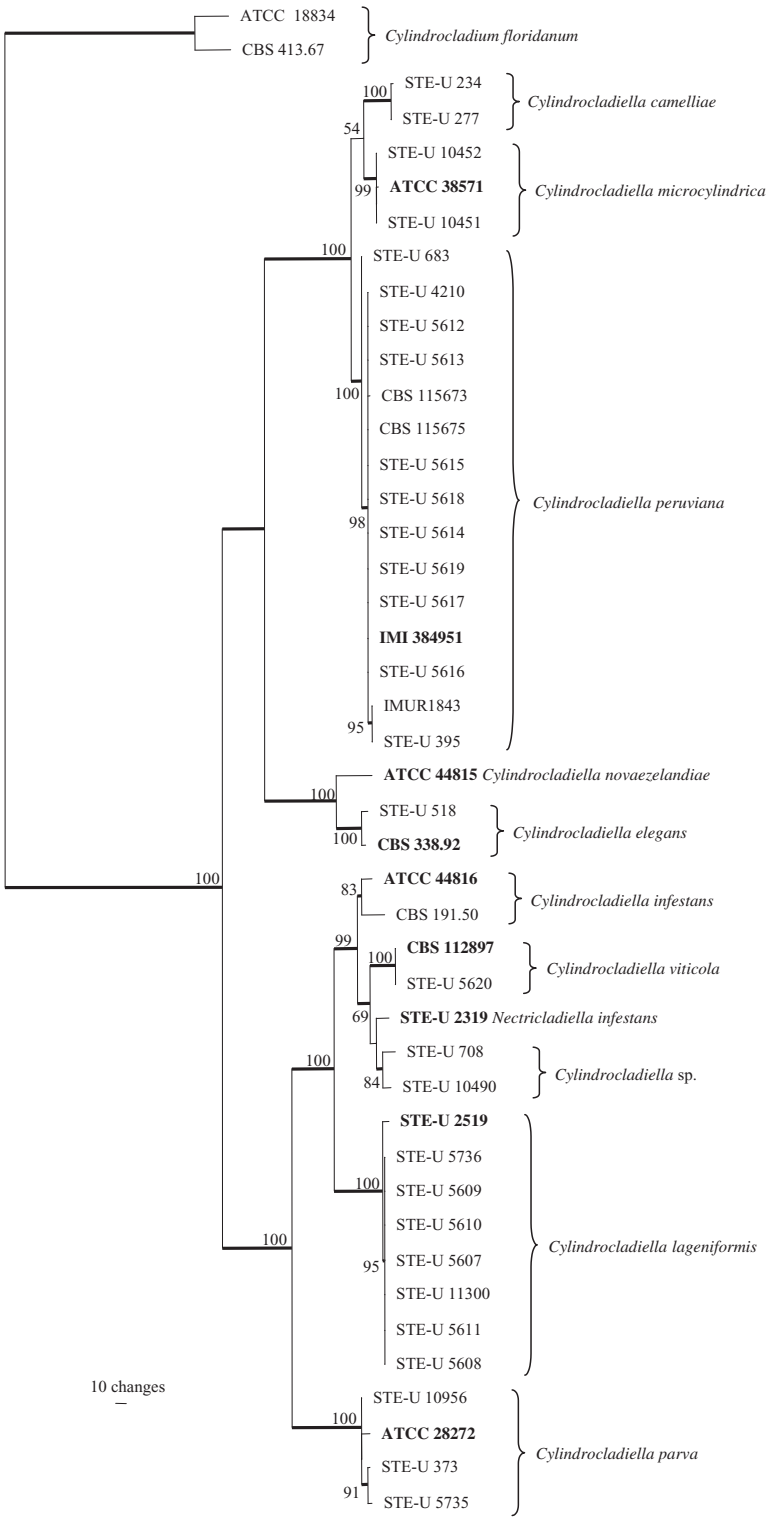

Fig. 1. One of 16 most parsimonious trees obtained from the combined ITS, β-tubulin and histone sequence data (TL = 993 steps, CI = 0.802, RI = 0.951, RC = 0.762). The scale bar indicates ten changes and the numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. Ex-type cultures are indicated in bold. The GenBank sequences of *Cylindrocladium floridanum* (AF348247, AF348215, AF348231 and AF348251, AF348219, AF348235, respectively for ITS, β-tubulin and histone) were included as outgroup.

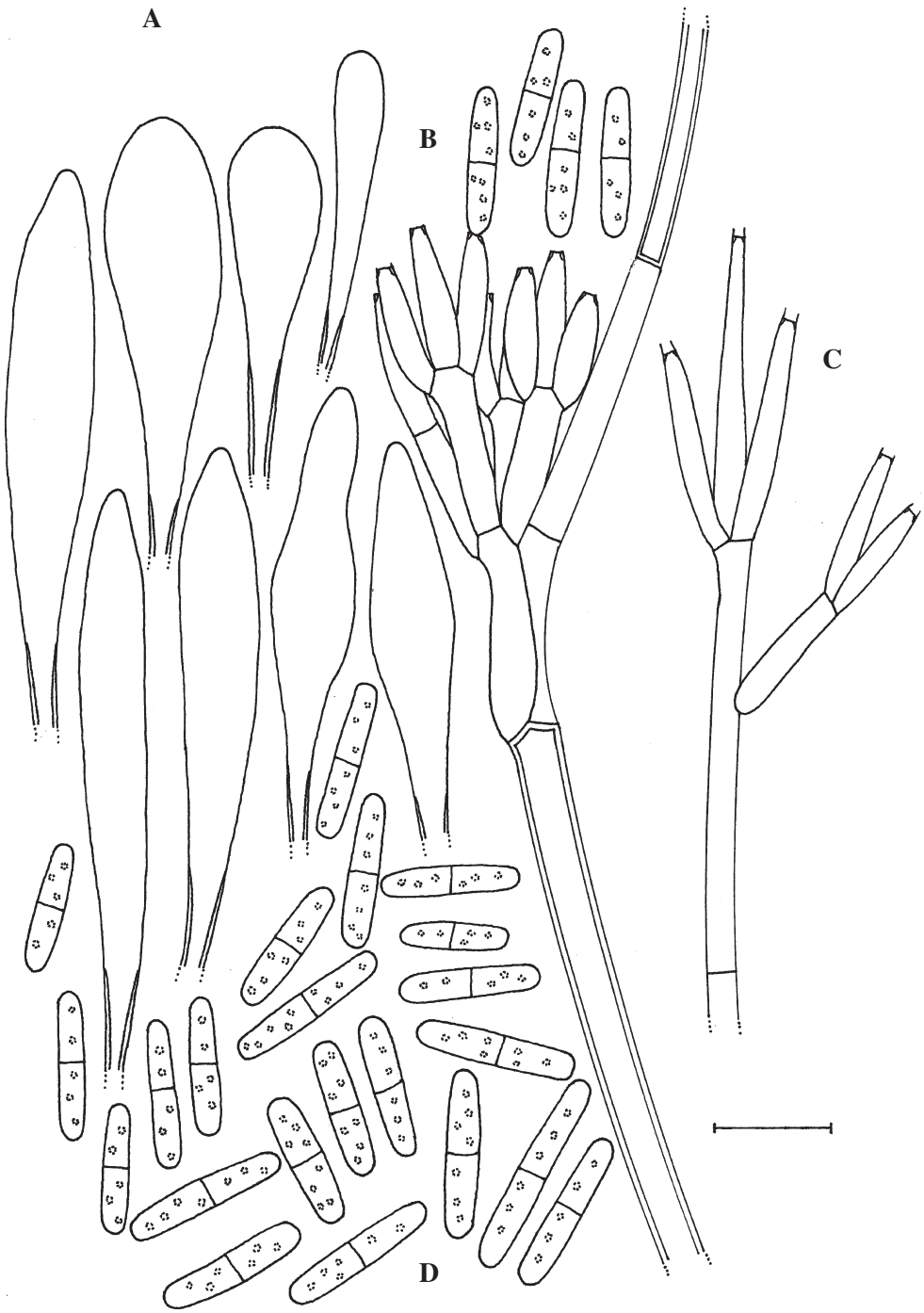

Fig. 2. *Cylindrocladiella viticola*. (*A*) Ellipsoidal to clavate vesicles. (*B*) Penicillate conidiophore. (*C*) Subverticillate conidiophore. (*D*) 1-Septate conidia. Bar = 10 µm.

both ends, straight, (8–)9–14(–15) × 2–2.5(–3) µm (mean = 13 × 2.5 µm), (0–)1-septate, frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Cultural characteristics

Colonies raised, cottony, with smooth margins, white with straw (21'd) tint in patches (surface), umber (13'i) (reverse); chlamydospores extensive throughout medium, occurring in chains; reaching 29 mm after 6 days on MEA at 25°C in the dark.

Type

SOUTH AFRICA. Western Cape province, Wellington, *Vitis vinifera*, Mar. 2001, *G. J. van Coller*, herb. CBS 7951 (holotype), ex-type culture STE-U 5606 = CBS 112897.

Symptoms

Cutting rot.

Additional culture

SOUTH AFRICA. Western Cape province, Wellington, *Vitis vinifera*, Aug. 2002, *F. Halleen*, STE-U 5620.

Notes

Cylindrocladiella viticola is presently known only from grapevines in South Africa. It has smaller conidia (8–)9–14(–15) × 2–2.5(–3) µm than those of *C. infestans* and the morphologically similar anamorph of *Nectricladiella infestans*, which are (10–)14–16(–20) × 2(–3) µm. Furthermore, *C. viticola* has irregularly ellipsoidal to clavate vesicles, in contrast to the cylindrical vesicles of *C. infestans*, and the cylindrical to lanceolate vesicles of *N. infestans*.

Stem inoculations

Green shoots. Since Levene's test for homogeneity (Levene 1960) indicated that the data of the two repeats were of comparable magnitude ($P = 0.074$), an analysis of combined data was performed. Evidence against normality was due to kurtosis and not skewness and, therefore, the results were further analysed with ANOVA and Student's t l.s.d. pairwise comparisons.

Cylindrocladiella parva and *C. peruviana* were the only species that caused lesions on unwounded green shoots, although lesions obtained were not significantly ($P = 0.05$) longer than either of the control treatments (Table 2). Isolates of *C. peruviana* differed in their ability to induce lesions on unwounded green shoots, since the isolate STE-U 5613 caused significantly ($P = 0.05$) longer lesions than isolates STE-U 5616, STE-U 5618 and STE-U 5619 (Table 3).

For the wounded treatment, only *C. lageniformis* (isolate STE-U 5611) caused lesions that were significantly ($P = 0.05$) longer than the controls (Table 3). The other *Cylindrocladiella* isolates induced a range of different lesion lengths on wounded green shoots, but these did not differ significantly ($P = 0.05$) from the controls (Table 3).

Table 2. Mean lesion lengths after inoculation of green shoots of 99-Richter with different *Cylindrocladiella* species

Species	Lesion length (mm)[A]	
	Unwounded	Wounded
C. lageniformis	0.00a	5.21a
C. parva	0.50a	4.75a
C. peruviana	0.66a	3.45a
C. viticola	0.00a	4.20a
Negative control: *Clonostachys rosea*	0.00a	4.25a
Positive control: Agar	0.00a	4.50a

[A]Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

One-year-old-shoots. One-year-old shoots subjected to unwounded inoculation yielded no reaction and, therefore, only results from the wounded inoculation were subjected to statistical analysis. Since not enough evidence for homogeneity existed, an analysis of combined data was performed using the variances of the repeats as weights (John and Quenouille 1977). Evidence against normality was due to kurtosis and not skewness and, therefore, the results were further analysed with ANOVA and Student's t l.s.d. pairwise comparisons. There were no significant differences in lesion length caused by the various species of *Cylindrocladiella* tested compared with the control treatments ($P = 0.8626$). Neither were there any significant differences in lesion lengths caused by the various isolates ($P = 0.9726$).

Discussion

Four species of *Cylindrocladiella* were found associated with grapevines in South Africa: *C. lageniformis*, *C. parva*, *C. peruviana* and *C. viticola*, a new species within the *C. infestans* complex.

Although the present study has confirmed earlier records that *C. lageniformis*, *C. parva* and *C. peruviana* occur on grapevines in South Africa, it has also added valuable information to our understanding of the *C. infestans* species complex, and distinguished a new species within this complex. Schoch *et al*. (2000) provided ITS and β-tubulin sequence data to corroborate the RFLP data of

Table 3. Mean lesion lengths after inoculation of green shoots of 99-Richter with isolates of different *Cylindrocladiella* spp.

Species	Isolate no.	Lesion length (mm)[A]	
		Unwounded	Wounded
C. lageniformis	STE-U 5607	0.00b	4.67a–d
	STE-U 5608	0.00b	5.83ab
	STE-U 5609	0.00b	5.00a–c
	STE-U 5610	0.00b	4.50b–f
	STE-U 5611	0.00b	6.60a
	STE-U 5736	0.00b	4.83a–c
C. parva	STE-U 5735	0.50ab	4.75a–d
C. peruviana	STE-U 4210	0.00b	4.17b–g
	STE-U 5612	0.80ab	4.00b–g
	STE-U 5613	1.60a	2.60e–g
	STE-U 5614	1.25ab	2.50fg
	STE-U 5615	1.33ab	2.17g
	STE-U 5616	0.00b	4.40b–f
	STE-U 5617	1.20ab	3.60c–g
	STE-U 5618	0.00b	4.60a–e
	STE-U 5619	0.00b	2.80d–g
C. viticola	STE-U 5606	0.00b	5.80ab
	STE-U 5620	0.00b	2.60e–g
Clonostachys rosea	Negative control	0.00b	4.25b–f
Control	Control	0.00b	4.50b–f

[A]Means within a column followed by the same letter do not differ significantly ($P = 0.05$).

Victor *et al*. (1998), proving that *Nectricladiella camelliae* was not the teleomorph of *C. infestans*. Furthermore, a newly collected isolate from Madagascar (Table 1) proved to be morphologically and phylogenetically similar to *C. infestans*, and produced a teleomorph in culture which was subsequently described as *N. infestans*. With the inclusion of additional sequence data and isolates in the present study, however, it appears that the connection proposed by Schoch *et al*. (2000) is probably incorrect, and that *C. infestans* could be a cryptic species closely related to *N. infestans*. Although vesicle morphology is a reliable feature aiding in the identification of *Cylindrocladium* spp. (Crous 2002), it seems considerably less reliable in *Cylindrocladiella*. This is possibly due to *Cylindrocladiella* stipe extensions being aseptate, which results in vesicles that are more prone to variation depending on the osmotic potential of the medium used (Crous *et al*. 1992). Furthermore, DNA sequence data from additional isolates lead us to conclude that the taxonomy of *Cylindrocladiella* is much more complicated than currently assumed, and that many more species await formal description. Their potential role as plant pathogens, however, remains to be resolved.

Although the *Cylindrocladiella* isolates used for inoculations in this study were all obtained from diseased grapevines exhibiting symptoms of dieback, chlorotic leaves and a general unthrifty appearance, results obtained from the pathogenicity trials gave inconclusive results. As was mentioned earlier, five species of *Cylindrocladiella* have been reported from South Africa on various hosts, three of which were also recorded on declining *V. vinifera* (Crous and Wingfield 1993; Victor *et al*. 1998; Crous 2002). Although these reports clearly confirm the pathogenic ability of *Cylindrocladiella* to various hosts, we were not able to prove Koch's postulates on grapevines. Further studies would, therefore, have to be conducted testing different plant parts (e.g. roots and canes), under different environmental conditions (e.g. high moisture regimes), and also using different methods of inoculation, to resolve the pathogenic status of *Cylindrocladiella* spp. to grapevines.

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