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Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.)

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Abstract: Four *Cylindrocarpon* or *Cylindrocarpon*-like taxa isolated from asymptomatic or diseased *Vitis vinifera* plants in nurseries and vineyards of South Africa, New Zealand, Australia, and France were morphologically and phylogenetically compared with other *Neonectria*/*Cylindrocarpon* taxa. Sequences of the partial nuclear large subunit ribosomal DNA (LSU rDNA), internal transcribed spacers 1 and 2 of the rDNA including the 5.8S rDNA gene (ITS), and partial β-tubulin gene introns and exons were used for phylogenetic inference. *Neonectria*/*Cylindrocarpon* species clustered in mainly three groups. One monophyletic group consisted of three subclades comprising (i) members of the *Neonectria radicicola*/*Cylindrocarpon destructans* complex, which contained strains isolated from grapevines in South Africa, New Zealand, and France; (ii) a *Neonectria*/*Cylindrocarpon* species isolated from grapevines in South Africa, Canada (Ontario), Australia (Tasmania), and New Zealand, described here as *Cylindrocarpon macrodidymum*; and (iii) an assemblage of species closely related to strains identified as *Cylindrocarpon cylindroides*, the type species of *Cylindrocarpon*. This monophyletic group excluded two other groups, which comprised (i) members of the *Neonectria mammoidea* complex, with anamorphs characterised by curved macroconidia, violet or purple pigments in cultures of most of its members, and lack of microconidia and chlamydospores; and (ii) two undescribed *Cylindrocarpon*-like species, both from grapevines in South Africa. The latter two clades formed a paraphyletic group in LSU rDNA analysis but were supported as a monophyletic group in ITS and β-tubulin gene analysis. Strains of the *Neonectria radicicola*/*Cylindrocarpon destructans* complex isolated from grapevines matched *C. destructans* in morphology and DNA sequences. *Cylindrocarpon macrodidymum* formed micro- and macroconidia, but rarely formed chlamydospores. Its mostly 3-septate macroconidia were more or less straight, minutely widening towards the tip, and had an apical cell slightly bent to one side. Its teleomorph, *Neonectria macrodidyma*, was obtained in mating experiments, and was characterised by smooth to finely warted ascospores, smooth to finely warted perithecia, and moderately sized angular to subglobose cells in the outer region of the perithecial wall. The other two undescribed *Cylindrocarpon*-like species mentioned above were characterised by mostly 3–5-septate, curved macroconidia, and by the lack of microconidia. Both species differed from members of the *Neonectria mammoidea* group by brownish colonies and by brownish hyphal strands formed in the aerial mycelium. For these species a new genus, *Campylocarpon* gen. nov., is proposed. It comprises the new species *Campylocarpon fasciculare* and *Campylocarpon pseudofasciculare*, respectively. Inoculation of 6-mo-old potted grapevine rootstocks (cv. Ramsey) with selected isolates of *Cylindrocarpon destructans*, *Neonectria macrodidyma*, *Campylocarpon fasciculare*, and *Campylocarpon pseudofasciculare* resulted in a reduced root and shoot mass of inoculated plants and appearance of symptoms typical of black foot disease.

Taxonomic novelties: *Neonectria macrodidyma* Halleen, Schroers & Crous sp. nov. (anamorph *Cylindrocarpon macrodidymum* Schroers, Halleen & Crous sp. nov.), *Campylocarpon* Halleen, Schroers & Crous gen. nov., *Campylocarpon fasciculare* Schroers, Halleen & Crous sp. nov., *Campylocarpon pseudofasciculare* Halleen, Schroers & Crous sp. nov.
Key words: β-tubulin gene, black foot disease, internal transcribed spacer, *Nectriaceae*, nuclear large subunit ribosomal DNA, phylogeny, systematics.

INTRODUCTION

Species of *Cylindrocarpon* Wollenw. are common and may be isolated as soil inhabitants, saprobes on dead plant material, root colonizers or pathogens, or weak pathogens of various herbaceous and woody plants (Brayford 1993). *Cylindrocarpon destructans* (Zinnsm.) Scholten [anamorph of *Neonectria radicicola* (Gerlach & L. Nilsson) Mantiri & Samuels] and *C. obtusisporum* (Cooke & Harkn.) Wollenw. have frequently been described as the agents of root rots of various hosts (Booth 1966, Seifert *et al*. 2003b), and a black foot disease of grapevines (*Vitis vinifera* L.). The first record of *C. destructans* on grapevines was made in France in 1961 (Maluta & Larignon 1991). Since then this species has been isolated from diseased vines in Tasmania (Sweetingham 1983), Sicily (Grasso 1984), and Portugal (Rego *et al*. 2000, 2001). *Cylindrocarpon obtusisporum* has been identified as the causal agent of this disease in Sicily (Grasso &

Magnano di San Lio 1975) and California (Scheck *et al*. 1998a). Various species of *Cylindrocarpon*, preliminarily identified as "*Cylindrocarpon* sp.", have also been isolated from young vines and from vines with basal rot or root necrosis in Spain (Armengol *et al*. 2001) as well as from diseased grapevines in South Africa (Fourie *et al*. 2000, Fourie & Halleen 2001). The black foot disease of grapevines was described by Sweetingham (1983), Larignon (1999), and Fourie & Halleen (2001) as mainly affecting young vines between two and eight years of age. The symptoms described were weak or absent vegetation, drying and dying of shoots during summer, abnormal development of roots with growth parallel to the soil surface, necrotic root crowns, development of secondary root crowns, brown to black wood of rootstocks, and internal necrosis extending from the bark to the pith in diseased parts of the plants. Additional symptoms, described by Grasso & Magnano di San Lio (1975) and Scheck *et al*. (1998a) include black discoloration of the wood, gum inclusions in xylem vessels and black streaks in the vascular tissue.

Teleomorphs with *Cylindrocarpon* anamorphs were traditionally classified in *Nectria* (Fr.) Fr., but are now considered to belong to *Neonectria* Wollenw. (Rossman *et al*. 1999, Mantiri *et al*. 2001, Brayford *et al*. 2004). Wollenweber based this name on *Neon. ramulariae* Wollenw. (1916). The reintroduction of *Neonectria* resulted from the realization that *Nectria* was too broadly defined and that its segregation into numerous teleomorphic genera could be corroborated by anamorphic, phylogenetic, and ecological character patterns (Rehner & Samuels 1995, Rossman *et al*. 1999). Some pre-phylogenetic classification schemes had segregated the teleomorphs of *Cylindrocarpon* species into four infrageneric *Nectria* groups based on perithecial wall anatomy and ascospore morphology; these groups were centred on "*Nectria*" *radicicola* Gerlach & L. Nilsson, "*Nectria*" *coccinea* (Pers. : Fr.) Fr., "*Nectria*" *mammoidea* Phill. & Plowr., and "*Nectria*" *rugulosa* Pat. & Gaillard (Booth 1959, Samuels & Brayford 1990, Samuels & Brayford 1994). Wollenweber (1917, 1928) created the sections *Chlamydospora* Wollenw. and *Ditissima* Wollenw. for species with and without chlamydospores, respectively. Booth (1966) schematically segregated *Cylindrocarpon* species into four groups based on the presence or absence of microconidia and chlamydospores. *Cylindrocarpon magnusianum* (Sacc.) Wollenw., which is the anamorph of the type species of *Neonectria*, *C. cylindroides* Wollenw., which is the type species of the genus *Cylindrocarpon*, *C. destructans*, which is the anamorph of *Neonectria radicicola*, and members of *Cylindrocarpon* species predominantly connected with teleomorphs of the "*Nectria*" *mammoidea* group were core members of the anamorphic groups delineated by Booth (1966). *Cylindrocarpon obtusisporum* was originally described from the U.S.A. (California) as occurring on *Acacia* sp., where it was observed to form macroconidia and chlamydospores (Booth 1966). *Cylindrocarpon obtusisporum* strains identified by Booth (1966) originated from a broad range of host plants in Europe, New Zealand, and North America, and, at least partly, formed microconidia.

Currently, representatives of all "*Nectria*" groups with *Cylindrocarpon* anamorphs have been transferred into *Neonectria* (Rossman *et al*. 1999, Mantiri *et al*. 2001, Brayford *et al*. 2004). Mantiri *et al*. (2001) and Brayford *et al*. (2004) analysed mitochondrial small subunit (SSU) ribosomal DNA (rDNA) sequence data of some of the species and concluded that the *Neonectria*/*Cylindrocarpon* species grouped together by this reclassification were monophyletic. However, these authors also found that this overall *Neonectria*/*Cylindrocarpon* clade included distinct subclades corresponding to at least three of the four groups delineated by Booth (1966). Significant molecular variation among taxa with *Cylindrocarpon*-like anamorphs was found by Seifert *et al*. (2003b) in a study on fungi causing root rot of ginseng (*Panax quinquefolius* L.) and other hosts. The dendrograms in this study, based on partial β-tubulin gene, and nuclear ribosomal internal transcribed spacer (ITS) region sequences, suggested that subclades including (i) *Neon. radicicola*, which consisted of numerous phylogenetically distinct units, (ii) *Neon. macroconidialis* (Samuels & Brayford) Seifert, and (iii) a subclade comprising two distinct isolates, one from *Vitis vinifera* in Ontario, Canada and the other from *Picea* sp. in Quebec, Canada, were monophyletic. Other *Cylindrocarpon* species appeared to be excluded from this monophyletic group.

A great variation in cultural and morphological characters was recently observed among *Cylindrocarpon* strains isolated from grapevines in nurseries and vineyards in South Africa (Halleen *et al*. 2003, Fourie & Halleen 2004), France (Larignon 1999), New Zealand, and Australia (Halleen unpubl. data). Some of the isolates could not be identified to species level because of unusual character combinations and poorly sporulating cultures. Combining cultures of some of the strains yielded a teleomorph that also could not be identified in literature, although it was most similar to teleomorphs in the *Neon. radicicola* group (Samuels & Brayford 1990). In the present study, morphological characters and DNA sequences were used to characterise these *Cylindrocarpon*-like taxa from diseased and asymptomatic grapevines taxonomically and phylogenetically. Sequences of these taxa were compared with those of members of the *Neon. radicicola* complex published by Seifert *et al.* (2003) and various other *Neonectria*/*Cylindrocarpon* species deposited at the CBS Fungal Biodiversity Centre (CBS, Utrecht, The Netherlands). Some of these strains had earlier been deposited, studied, or identified by H.W. Wollenweber, C. Booth, W. Gams, and G.J. Samuels.

MATERIALS AND METHODS

Fungal cultures

Strains were isolated from both diseased and healthy looking, asymptomatic grapevines (Fig. 1) in nurseries and vineyards in South Africa, Australia, New Zealand, and France. They are listed in Tables 1, 2. Symptoms included various forms of decline as well as typical black foot symptoms (Table 1). The strains are stored at CBS and at the Department of Plant Pathology, University of Stellenbosch, South Africa (collection designation STE-U). Additional species of *Neonectria*/*Cylindrocarpon* were obtained from CBS (Table 2, Anonymous 2001).

Fig. 1a–i. Symptoms associated with black foot disease. a. Eight-year-old grapevines showing severe decline symptoms including absence of budding, abnormal, weak vegetation, and summer wilting. b. Cross-section through infected rootstock revealing necrosis extending from the bark to the pith. c. Part of trunk with bark removed, showing a brown, subcortical zone beginning at the base of the rootstock running up along the trunk in severely affected vines. d. Cross section of an infected root. e. Dark vascular streaking seen in longitudinal section of trunk. f. Soil compaction and poor water drainage resulting from excessive movement of farm vehicles. g. Poor root development (J-rooting) resulting from soil compaction and poor soil preparation. h. Poor root development (pothole effect) resulting from soil compaction and poor soil preparation. i. Second layer of roots, growing parallel to the soil surface, formed by the plant in order to compensate for the loss of functional roots further below. Rootstocks are also thinner below the second root layer.

Table 1. *Cylindrocarpon* strains isolated from grapevines (*Vitis vinifera*).

Taxon	CBS no [a]	Primary isolation no. [b]	STE-U no. [c]	Isolation date	Plant zone	Scion/rootstock	Origin [d]	Symptoms on host	GenBank accession no.		
									ITS1, 5.8S, ITS2 rDNA	LSU rDNA	β-tubulin
C. destructans	112606	C20	3985	9/12/1999	Roots	Semillon/unknown	South Africa, Bonnievale	Young vines die	AY677268	AY677314	AY677246
	112607	C81	3986	28/2/2000	Basal end of trunk	Merlot/101-14 Mgt	South Africa, Robertson	Black foot	AY677269	AY677316	AY677241
	112595	C17	3993	26/11/1999	Trunk	Cinsaut/101-14 Mgt	South Africa, Worcester	3-yr-old vines die	AY677263	–	AY677247
	112596	C14	3994	16/11/1999	Roots	Cabernet Sauvignon/Richter 99	South Africa, De Wet	Young vines die	AY677264	–	AY677239
	112597	C33	3995	16/11/1999	Trunk	Cabernet Sauvignon/Richter 110	South Africa, Tulbagh	Decline. Poor root development	AY677265	–	AY677240
	112602	C25	3998	26/11/1999	Roots	Chenin blanc/Richter 99	South Africa, Worcester	Old vines die	AY677267	AY677315	AY677242
	112599	F2	3672	1995	Trunk	Ugni blanc/ungrafted	France, Balan, Bordeaux	Black foot	AY677266	–	AY677243
	112591	F3	3673	1995	Trunk	Ugni blanc/ungrafted	France, Ile de Re, Bordeaux	Black foot	AY677262	–	AY677245
	112610	F4	3674	1995	Trunk	Ugni blanc/ungrafted	France, Fougerat, Bordeaux	Black foot	AY677270	–	AY677244
	113553	NZ C 59	5714	10/2/2003	Basal end of trunk	Pinot noir/101-14 Mgt	New Zealand, Canterbury	Blackening areas in wood and roots	–	–	AY677250
	113556	NZ C 65	5717	12/3/2003	Basal end of trunk	Unknown	New Zealand, Marlborough	Decline	–	–	AY677248
Campyl. fasciculare	113554	C171 (3O/C20)	5719	24/6/2003	Rootstock (within 5 cm of the basal end	Cabernet Sauvignon/ 101-14 Mgt	South Africa, Wellington (V)	Vascular streaking from base of rootstock	–	–	AY677223
	113558	C172 (310)	5720	23/6/2003	Rootstock (within 5 cm of the basal end	Cabernet Sauvignon/ 101-14 Mgt	South Africa, Wellington (V)	Vascular streaking from base of rootstock	–	–	AY677224
	113557	C173 (3I4)	5721	6/6/2003	Rootstock (within 5 cm of the basal end	Cabernet Sauvignon/ 101-14 Mgt	South Africa, Wellington (V)	Vascular streaking from base of rootstock	–	–	AY677222
	112611	C147	3965	8/3/2000	Rootstock (within 5 cm of the basal end)	Pinotage/Richter 99	South Africa, Wellington (V)	Asymptomatic nursery plant	AY677299	–	AY677225
	112612	C134	3966	14/3/2000	Roots	Sultana/Ramsey	South Africa, Wellington (L)	Asymptomatic nursery plant	AY677300	AY677307	AY677216
	112613	C76	3970	1/2/2000	Trunk	Cabernet Sauvignon/Richter 99	South Africa, Riebeeck Kasteel	Young vines die	AY677301	–	AY677221
	113560	C120	3972	2/3/2000	Roots	Pinotage/Richter 99	South Africa, Wellington (J)	Asymptomatic nursery plant	AY677304	AY677309	AY677217
	112614	C79	3973	17/2/2000	Trunk	Pinotage/Richter 99	South Africa,	Black foot	AY677302	AY677308	AY677220

	112600	C132	3981	1/3/2000	Roots	Pinotage/101-14 Mgt	Stellenbosch South Africa, Wellington (J)	Asympto-matic nursery plant	AY677298	–	AY677219
	113559	C119	4006	9/3/2000	Roots	Sultana/ 143-B Mgt	South Africa, Wellington (V)	Asympto-matic nursery plant	AY677303	–	AY677218
Campyl. pseudofas-ciculare	112679	C108	5472	3/3/2000	Roots	Sultana/Ramsey	South Africa, Wellington (J)	Asympto-matic nursery plant	AY677306	–	AY677214
	112592	C89	3988	2/3/2000	Roots	Pinotage/Richter 99	South Africa, Wellington (J)	Asympto-matic nursery plant	AY677305	AY677310	AY677215
Neon. macrodi-dyma	112604	C10	4004	2/12/1999	Roots	Cabernet Sauvi-gnon/101-14 Mgt	South Africa, Paarl	Decline	AY677286	–	AY677227
	112615	C98	3976	2/3/2000	Roots	Sultana/143-B Mgt	South Africa, Wellington (J)	Asympto-matic nursery plant	AY677290	AY677322	AY677233
	112601	C82	3983	24/2/2000	Roots	Pinotage/US 8-7	South Africa, Tulbagh	Black foot	AY677284	–	AY677229
	112605	C106	3984	2/3/2000	Rootstock (within 5 cm of the basal end)	Sultana/143-B Mgt	South Africa, Wellington (J)	Asympto-matic nursery plant	AY677287	–	AY677230
	112608	C62	3987	27/1/2000	Roots	Chardonnay/101-14 Mgt	South Africa, Citrusdal	Black foot	AY677288	AY677325	AY677235
	112593	C107	3990	7/3/2000	Roots	Pinotage/101-14 Mgt	South Africa, Wellington (V)	Asympto-matic nursery plant	AY677281	AY677324	AY677236
	112594	C111	3991	2/3/2000	Roots	Pinotage/Richter 99	South Africa, Wellington (J)	Asympto-matic nursery plant	AY677282	–	AY677231
	112598	C115	3997	14/3/2000	Roots	Sultana/Ramsey	South Africa, Wellington (L)	Asympto-matic nursery plant	AY677283	–	AY677228
	112603	C8	4007	20/12/1999	Trunk	Sauvignon blanc/Richter 110	South Africa, Darling	Decline of young vines	AY677285	AY677323	AY677232
	112609	A	3969	1979	Trunk	Cabernet Sauvi-gnon/ungrafted	Australia, Bream Creek, Tasmania	Dark brown discoloration in trunk	AY677289	–	AY677226
	113552	NZ C 41	5713	31/1/2003	Rootstock	Unknown	New Zealand, Gisborne	Declining of nursery plants (dead rootstocks)	–	–	AY677237
	113555	NZ C 60	5715	10/2/2003	Basal end of trunk	Pinot noir/101-14 Mgt	New Zealand, Canterbury	Blackening areas in wood and roots	–	–	AY677234

[a] CBS strain numbers, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; [b] strains F2, F3, and F4 collected by Philippe Larignon (INRA, France); strain A collected by Mark Sweetingham (Australia); strains NZ C 41, 59, 60, 64, 65, and 72 collected by Rod Bonfiglioli (New Zealand); all other strains collected by Francois Halleen (F.H.); [c] STE-U strain numbers, University of Stellenbosch, South Africa; [d] (J), (L) and (V) = three grapevine nurseries, Western Cape Province, South Africa.

Table 2. Other taxa with *Cylindrocarpon*-like anamorphs, of which sequences were newly generated.

Taxon	Strain no. [a]	Location/host, substrate	GenBank accession no.		
			ITS1, 5.8S, ITS2 rDNA	LSU rDNA	β-tubulin
"*Neonectria*" *trachosa* Samuels & Brayford	CBS 112467 (= G.J.S. 92-45)	Scotland/conifer bark	AY677297	AY677337	AY677258
C. album (teleomorph: "*Nectria*" *punicea* (Schmidt : Fr.) Fr.	CBS 242.29	Germany/*Rhamnus* sp.	–	AY677326	–
	CBS 152.29	Germany/*Solanum tuberosum* tuber	AY677260	–	–
C. candidum	CBS 237.29	Norway/*Ulmus* sp.	–	AY677327	–
C. cylindroides (teleomorph: *Neon. neomacrospora* (C. Booth & Samuels) Mantiri & Samuels	CBS 189.61	France/*Abies alba*	–	AY677331	–
	CBS 503.67	Norway/*Abies alba*	AY677261	–	–
C. faginatum (teleomorph: "*Nectria*" *coccinea* var. *faginata* Lohman *et al*.)	CBS 217.67	Canada/*Cryptococcus fagi* nymph on *Fagus grandifolia*	AY677277	AY677328	–
C. heteronema (teleomorph: *Neonectria galligena*	CBS 316.34	Canada/*Betula lutea*	AY677278	–	–
	CBS 232.31	Germany/*Fraxinus excelsior*	–	AY677329	–
C. willkommii	CBS 226.31	Germany/*Fagus sylvatica*	–	AY677330	–
"*Nectria*" *fuckeliana* C. Booth	CBS 112466 (= G.J.S. 90-31)	Switzerland/*Picea* sp.	–	AY677334	AY677238
"*Neonectria*" *discophora* (Mont.) Mantiri & Samuels (anamorph: *C. ianthothele* var. *majus* Wollenw.)	CBS 328.81	Switzerland/unknown	AY677279	–	–
	CBS 112460 (= G.J.S. 85-45)	New Zealand/root of indet. tree	–	AY677338	AY677257
"*Neonectria*" *lucida* (Höhnel) Samuels & Brayford (anamorph: *Cylindrocarpon lucidum* Booth)	CBS 112455 (C.T.R. 72-71)	Venezuela/bark	–	AY677336	AY677259
	CBS 112457 (= G.J.S. 85-27)	New Zealand/bark	–	AY677335	–
	CBS 112456 (= G.J.S. 96-35)	U.S.A., Puerto Rico/bark of recently dead *Cecropia* sp.	AY677296	–	–
C. ianthothele var. *minus* Reinking	CBS 266.36	Germany/unknown	AY677280	–	–
C. victoriae (Henn. ex Wollenw.) Wollenw.	CBS 174.37	Germany/unknown	–	AY677339	–
C. destructans	CBS 264.65	Sweden/*Cyclamen persicum*	AY677273	AY677318	AY677256
	CBS 321.34	Tunisia/*Loroglossum hircinum*	AY677275	AY677313	AY677253
	CBS 102032	Venezuela/bark		AY677311	AY677255
	CBS 156.47	Belgium/*Azalea indica*	AY677272	AY677320	AY677252
	CBS 153.37	France/dune sand	AY677271		AY677251
	CBS 301.93	Netherlands/*Cyclamen* sp.	AY677274	AY677317	AY677249
C. destructans var. *crassum* (Wollenw.) C. Booth	CBS 773.83	Netherlands/water, in aquarium with *Anodonta*	AY677276	AY677312	AY677254
C. didymum	CBS 640.77	France/*Abies alba*	–	AY677319	–
	CBS 305.85	Netherlands/*Lilium* sp.	–	AY677321	–
C. obtusisporum (teleomorph: *Neonectria tawa* Dingley)	CBS 183.36	Germany/*Solanum tuberosum*	AY677292	AY677332	–
C. magnusianum (teleomorph: *Neonectria ramulariae* Wollenw.	CBS 151.29	UK England/*Malus sylvestris*	AY677291	AY677333	–
C. olidum var. *crassum* Gerlach	CBS 216.67	Germany/*Zygocactus* sp.	AY677294	–	–
C. olidum var. *olidum* (Wollenw.) Wollenw.	CBS 215.67	Germany/Rotting rhizome of *Asparagus officinalis*	AY677293	–	–

[a] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; G.J.S.: G.J. Samuels; C.T.R.: C.T. Rogerson.

DNA isolation, sequencing and phylogenetic analyses

Mycelium was grown in tubes with 2 mL of complete medium (Raper & Raper 1972) and DNA was extracted using the FastDNA® Kit (Bio 101, Carlsbad, CA, U.S.A.). PCR for the partial β-tubulin gene introns and exons was performed as described by Schroers *et al*. (2004). ITS and partial large subunit (LSU) rDNA was amplified using the primer pair V9G/LR5 (de Hoog & Gerrits van den Ende 1998, Vilgalys & Hester 1990). The same PCR system was used as for the partial β-tubulin gene and the following PCR program: an initial denaturation step at 94 °C for 2 min, 35 cycles of 94 °C for 35 s, 55 °C for 50 s, 72 °C for 2 min, and a final extension at 72 °C for 6 min. The vials of 50 µL contained 1 µL genomic DNA extract, 25 pmol of each of the primers, 200 µmol of each of the dNTPs (Amersham Biosciences Europe GmbH, Freiburg, Germany), 1 U of Taq polymerase (Super Taq, HT Biotechnology Ltd, Cambridge, UK), and 1× standard PCR buffer supplied together with the Taq polymerase.

PCR fragments were purified using GFX™ purification kit (Amersham Pharmacia Biotech Inc., Roosendaal, The Netherlands). Sequencing was done on an ABI Prism 3700 instrument (Applied Biosystems, Foster City CA, U.S.A.) with a BigDye terminator cycle sequencing kit (Applied Biosystems) or DYEnamicET dye terminator (Amersham Biosciences) following the conditions recommended by the vendors. PCR products were sequenced using the primers ITS1 and ITS4 (White *et al*. 1990) for the ITS; LR5, NL1, and NL4 (O'Donnell 1993) for the LSU rDNA; and T1 and T2 (O'Donnell & Cigelnik 1997) for the partial region of the β-tubulin gene.

Strains of which sequences were newly generated and their host and origin are listed in Tables 1, 2. Newly generated sequences have been deposited in GenBank (Tables 1, 2). The following taxa and published (i) ITS, (ii) LSU, and (iii) β-tubulin sequences, indicated by GenBank accession numbers, were included in the analyses: (i) *C. cylindroides*: CR6 (AY295301) (Seifert *et al*. 2003b), *C. destructans*: UAMH 4907 (University of Alberta Microfungus Collection, Edmonton, AB, Canada) (AF172261) (Iwen *et al*. unpubl.), *Cylindrocarpon* sp.: 94-1356 (AY295304), 94-1685 (= CCFC 226730 [Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, ON, Canada]) (AY295334), CD1401 (AY295335) (Seifert *et al*. 2003b), *Nectria cinnabarina* (Tode : Fr.) Fr.: CBS 279.48 (AF163025) (Lee *et al*. 2000), *Neon. coprosmae* (Dingley) Seifert: G.J.S. 85-182 (AF220971), CTR 73-152 (AF220970) (Schoch *et al*. 2000), G.J.S. 85-39 (AY295326) (Seifert *et al*. 2003b), *Neon. macroconidialis*: G.J.S. 83-162 (AY295327) (Seifert *et al.* 2003b), *Neon. radicicola*: IMI 061536 (CABI Biosciences, Egham, U.K.) (AJ007354), IMI 375717 (AJ007355), IMI 375719 (AJ007356), IMI 376403 (AJ007351), IMI 376404 (AJ007357), IMI 376408 (AJ007352), IMI 376409 (AJ007353) (Langrell unpubl.), AR 2553 (AF220968), CTR 71-322 (AF220969) (Schoch *et al*. 2000), CCFC 139398 (AY295330), CCFC 144524 (AY295332), CCFC 150670 (AY295319), CD1557 (AY295329), CD1666 (AY295331), CD999 (AY295312), CR20 (AY295317), CY9801 (AY295310), IMI 3133237 (AY295333), JAT1378 (AY295328), NSAC-SH-1 (AY295311), NSAC-SH-2 (AY295313), NSAC-SH-2.5 (AY295314) (Seifert *et al*. 2003b); (ii) *Albonectria albosuccinea* (Pat.) Rossman & Samuels: NRRL 20459 (National Center for Agricultural Utilization Research, U.S. Dept. of Agriculture, Peoria, IL, USA) (U34554) (O'Donnell & Cigelnik 1997), *Albonectria rigidiuscula* (Berk. & Broome) Rossman & Samuels: NRRL 13412 (U88104) (O'Donnell 1993), *Calonectria morganii* Crous *et al*.: ATCC 11614 (American Type Culture Collection, Bethesda, MD, U.S.A.) (U17409) (Rehner & Samuels 1995), *Cosmospora episphaeria* (Tode : Fr.) Rossman & Samuels: NRRL 20687 (U88100) (O'Donnell 1993), *Cosmospora vilior* (Starbäck) Rossman & Samuels: ATCC 16217 (U57348) (Glenn & Bacon unpubl.), *C. cylindroides*: CCFC 226722 (AY283551) (Seifert *et al*. 2003b), *Cylindrocladium floridanum* Sobers & C.P. Seymour: ATCC 22677 (U17408) (Rehner & Samuels 1995), *Fusarium culmorum* (W.G. Smith) Sacc.: NRRL 25475 (AF006322) (O'Donnell *et al*. 1998), *Fusarium fujikuroi* Nirenberg: NRRL 13566 (U34528) (O'Donnell & Cigelnik 1997), *Fusarium oxysporum* Schlecht. : Fr.: NRRL 26409 (AF060383) (Cigelnik unpubl.), *Fusarium solani* (Mart.) Sacc.: NRRL 22292 (L36629) (O'Donnell & Gray 1995), *Fusarium verticilloides* (Sacc.) Nirenberg: NRRL 22172 (U34526), (O'Donnell & Cigelnik 1997), *Haematonectria haematococca* (Berk. & Broome) Samuels & Nirenberg: NRRL 22141 (L36623) (O'Donnell & Gray 1995), *Leuconectria clusiae* (Samuels & Rogerson) Rossman, Samuels & Lowen: AR2706 (U17412), (Rehner & Samuels 1995), *Nectria cinnabarina*: G.J.S. 89-107 (U00748) (Rehner & Samuels 1994), *Nectria pseudotrichia* Berk. & M.A. Curtis: AR1755 (U17410) (Rehner & Samuels 1995), "*Nectria*" *mariannaeae* Samuels & Seifert: CCFC 226709 (AY283553) (Seifert *et al*. 2003a), "*Nectria*" *ventricosa* Appel & Wollenw.: NRRL 20846 (L36613) (O'Donnell 1993), *Neocosmospora endophytica* Polishook *et al*.: AR2674 (U17411) (Rehner & Samuels 1995), *Neon. coccinea*: NRRL 20485 (U88124) (O'Donnell 1993), *Neon. galligena* (Bres.) Rossman & Samuels: NRRL 20487 (U88126) (O'Donnell 1993), *Neon. radicicola*: AR 2553 (U17415) (Schoch *et al*. 2000), CCFC 226721 (AY283552), (Seifert *et al*. 2003b), *Verticillium dahliae* Kleb.: ATCC 16535 (U17425) (Rehner & Samuels 1995), *Viridispora diparietispora* (J.H.

Miller *et al*.) Samuels & Rossman: ATCC 13214 (U17413) (Rehner & Samuels 1995); (iii) *C. cylindroides*: CR21 (AY297211), CR6 (AY297172) (Seifert *et al*. 2003b), *Cylindrocarpon* sp.: 94-1356 (AY297175), 94-2057 (= CCFC 226735) (AY297176) (Seifert *et al*. 2003b), *Fusarium* sp.: NRRL 22900 (U34422) (O'Donnell & Cigelnik 1997), *Neon. coprosmae*: G.J.S. 85-39 (AY297192) (Seifert *et al*. 2003b), *Neon. galligena*: KAS1224 (AY297216) (Seifert *et al*. 2003b), *Neon. macroconidialis*: G.J.S. 83-162 (AY297193) (Seifert *et al*. 2003b), *Neon. radicicola*: 20M15 (AY297215), 94-1628 (AY297185), CCFC 139398 (AY297196), CCFC 144524 (AY297198), CD1557 (AY297195), CD1561 (AY297179), CD1596 (AY297217), CD1598 (AY297202), CD1636 (AY297205), CD1666 (AY297197), CD842 (AY297214), CD999 (AY297184), CR20 (AY297187), CR26 (AY297188), CR29 (AY297212), CR36 (AY297213), IFO 31882 (NITE Biological Resources Centre, Chiba, Japan) (AY297191), IMI 3133237 (AY297194), NSAC-SH-1 (AY297181), NSAC-SH-2 (AY297182) (Seifert *et al*. 2003b).

The sequences were aligned automatically using the software ClustalX 1.81 (Jeannmougin *et al*. 1998). Alignments were adjusted manually using the software Bioedit (http://www.mbio.ncsu.edu/BioEdit/bioedit) (TreeBASE S1207, M2082–6). Sequences of strains from grapevines were compared with those of hypocrealean taxa characterised by multiseptate macroconidia typical of the genera *Fusarium* Link and *Cylindrocarpon*. A few other hypocrealean taxa such as "*Nectria*" *mariannaeae* Samuels & Seifert, *Leuconectria clusiae* (Samuels & Rogerson) Rossman, Samuels & Lowen, *Calonectria* De Not., and *Nectria sensu stricto* were also included. In phylogenetic trees, downloaded sequences are indicated by their GenBank accession numbers; newly generated sequences are indicated by CBS strain numbers. Five datasets were created and analysed separately (Figs 2–6). Phylogenetic relationships were estimated from the aligned sequences by the maximum parsimony criterion as implemented in PAUP 4.0b10 (Swofford 2002). Heuristic searches were performed using parsimony-informative, unordered, and equally weighted characters. Gaps were treated as missing characters. A maximum of 1000 trees was allowed. Branch robustness in the analyses was tested by 1000 heuristic search replications, each on bootstrapped data sets. Simple sequence addition was used.

Morphological examination

Strains were grown in darkness or under continuous near-UV light (400–315 nm) (Sylvania Blacklight-Blue, Osram Nederland B.V., Alphen aan den Rijn, The Netherlands) at 20 °C. Media used were synthetic nutrient-poor agar (SNA) with and without the addition of a 1 × 3 cm piece of filter-paper to the colony surface (Nirenberg 1976), potato-dextrose agar (Difco PDA, Becton Dickinson, Sparks, MD, U.S.A.), oatmeal agar (OA, Gams *et al*. 1998), and a diluted V8-juice agar ($V8_{50}$, as described in Gams *et al*. 1998, diluted 1:1 with water agar), and malt extract agar (MEA) (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) using 9 cm diam Petri dishes. Growth rates and colony diameters of cultures incubated in darkness were measured on SNA and PDA. Characters such as size and shape of conidia, phialides, and chlamydospores were measured from strains grown on SNA, OA, PDA, or $V8_{50}$ after 14–21 d. For measurements, water was used as mounting medium in microscopic slides. Images were taken from slides mounted in water or lactic acid. Measurements in the description are given as described by Schroers (2001). Macroscopic characters of colonies were described after 14 d; colour names are from Kornerup & Wanscher (1978). Cardinal temperatures for growth were assessed on PDA incubated for 7 d in the dark at 4, 10, 15, 20, 25, 30, and 35 °C. Mating experiments were performed on carnation leaf agar (CLA) at 23 °C using the methods and conditions outlined by Schoch *et al*. (1999). Three replicates were done for each cross. Petri dishes were sealed with Parafilm (Pechiney Plastic Packaging, Menasha, WI, U.S.A.) and observed at weekly intervals for a total period of 60 d. Two strains were considered sexually compatible if they produced perithecia with viable, exuding masses of ascospores within this time.

Pathogenicity

A pathogenicity study was conducted with 6-mo-old potted grapevine rootstocks (cv. Ramsey) in a glasshouse. Due to the lack of "disease-free" nursery plants, tissue culture plants were prepared by the Breeding and Evaluation Division, ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council, Stellenbosch, South Africa). Four isolates, CBS 112597 (F.H. c33), CBS 112604 (F.H. c10), CBS 112614 (F.H. c79) and CBS 112679 (F.H. c108), which represented the four *Cylindrocarpon*-like species from grapevines delineated in phylogenetic analyses, were used. The isolates were selected based on the results of preliminary pathogenicity screenings (results not shown). The plants, 20 per isolate, were inoculated by dipping the roots (Scheck *et al*. 1998b) in a 1×10^6 conidial suspension for 30 min. Control plants were dipped in sterile water. Inoculated plants were planted individually in pots containing sterilised potting mixture and placed in a glasshouse at 24 °C in a completely randomised design. Evaluation was conducted after 4.5 mo by counting dead plants, as well as by making isolations from the stem bases of the remaining plants to confirm the continuing presence of the inoculated fungus. The experiment was repeated once.

The data was subjected to analysis of variance using SAS version 8.2 (SAS 1999). Levene's test for homogeneity of variance was performed to test if the experimental variability in observations was of comparable magnitude. A Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk 1965). The Student's t-Least Significant Difference was calculated at the 5 % confidence level to compare treatment means. Levene's test for homogeneity of variance ($P > 0.05$) indicated that the experimental variability in observations was of comparable magnitude, and hence a combined analysis could be conducted. There was no significant ($P > 0.05$) evidence for any experiment × isolate interactions, and therefore the means of the main effects were studied.

RESULTS

Phylogenetic analyses

Heuristic parsimony analyses of aligned partial LSU sequences of representatives of selected hypocrealean taxa and *Verticillium dahliae* (*Phyllachorales*), which was used as outgroup (**dataset 1**; 529 bp alignment) yielded four equally most parsimonious trees, of which one is shown in Fig. 2. The four trees, based on 98 parsimony-informative characters (PIC), were 371 steps long and had a consistency index (CI) of 0.380 and a retention index (RI) of 0.749. The trees differed by minor changes mainly within the clade that included *Haematonectria*, *Neocosmospora*, *Cosmospora*, and "*Nectria*" *ventricosa*. Taxa with *Cylindrocarpon*-like anamorphs were found in three distinct clades. The largest of these clades consisted of a group of species centred around *Cylindrocarpon cylindroides*, including strains originally studied by Wollenweber (Anonymous 2001), such as CBS 151.29 (*C. magnusianum*), CBS 183.36 (*C. obtusisporum*), CBS 226.31 [*C. willkommii* (Lindau) Wollenw.], CBS 232.31 [*C. heteronema* (Berk. & Broome) Wollenw.], CBS 237.29 [*C. candidum* (Link : Fr.) Wollenw.], and CBS 242.29 [*C. album* (Sacc.) Wollenw.]. Also included were CBS 217.67, which is the ex-type strain of *C. faginatum* C. Booth, as well as additional strains identified as *Neon. coccinea* (NRRL 20485, as U88124), "*Nectria*" *fuckeliana* C. Booth (CBS 112466), *Neon. galligena* (NRRL 20487, as U88126), and *C. cylindroides* (CBS 189.61; CCFC 226722, as AY283551). Additional fungi associated with the same clade were *Neon. macrodidyma*, a species newly recognised in this paper, and members of the *Neonectria radicicola*/*Cylindrocarpon destructans* complex including the ex-type strain of *Neon. radicicola*, CBS 264.65 (Tables 1, 2). Neither the overall *Neonectria*/*Cylindrocarpon* clade nor any of its three subclades, however, received support in bootstrap analyses. *Neonectria*/*Cylindrocarpon* formed the sister group to several other, phenotypically dissimilar genera of the *Nectriaceae* (*Hypocreales*) such as *Nectria* (anamorph: *Tubercularia* Tode), *Calonectria* (anamorph: *Cylindrocladium* Morgan), "*Nectria*" (anamorph: *Mariannaea* Arnaud ex Samson), and *Leuconectria* Rossmann, Samuels & Lowen, and was also the sister group of a large clade comprising taxa with *Fusarium*-like anamorphs. This last clade included *Albonectria* Rossman & Samuels, *Gibberella* Sacc., *Neocosmospora* E.F. Sm., *Haematonectria* Samuels & Nirenberg, and *Cosmospora* Rabenh. *Neonectria*/*Cylindrocarpon* appeared to be unrelated to two other clades with *Cylindrocarpon*-like anamorphs that were found near the base of the tree. One of these two clades comprised taxa of the "*Neon.*" *mammoidea* group (Samuels *et al*. 1990, as *Nectria discophora* group; Samuels & Brayford 1993, Brayford & Samuels 1993, Rossman *et al*. 1999, Brayford *et al*. 2004), while the other clade contained the genus *Campylocarpon*, which is newly described in this paper.

In ITS analysis, it was found that the lengths of the included sequences differed by up to 80 bp, which necessitated the introduction of multiple gaps in the alignment. Three regions with these multiple gaps, alignment positions 85–110, 399–419, and 439–461, were excluded from the analyses of **dataset 2**, which included representatives of the three major clades with *Cylindrocarpon*-like fungi analysed in Fig. 2, as well as *Nectria cinnabarina* and *Fusarium solani*, of which the latter was used as outgroup. Dataset 2 formed a 588 bp alignment. Heuristic parsimony analyses of aligned included regions yielded 10 equally most parsimonious trees, of which one is shown in Fig. 3, based on 143 PIC 479 steps in length with a CI of 0.547 and a RI of 0.838. The 10 trees differed by minor changes within the *Neon. radicicola* complex and the clade referred to as "*Cylindrocarpon cylindroides* and other species". The analyses supported the existence of three clades containing taxa with *Cylindrocarpon*-like anamorphs.

The monophyly of species of the "*Neon.*" *mammoidea* group was moderately supported (bootstrap value = 78 %). The monophyly of *Neonectria*/*Cylindrocarpon* was strongly supported with a bootstrap value of 96 %, as was that of *Campylocarpon* with 100 % bootstrap support. *Neonectria*/*Cylindrocarpon* appeared excluded from the *Campylocarpon* and the "*Neon.*" *mammoidea* group but the phylogeny of these three groups remained unresolved. Relatedness of the "*Neon.*" *mammoidea* group to *Campylocarpon* was strongly supported in bootstrap analyses (bootstrap value = 96 %), a result contradicting that obtained in LSU analysis. Monophyly of members of the *Neon. radicicola* complex, including *Neon. macrodidyma*, was relatively strongly supported (bootstrap value = 87 %).

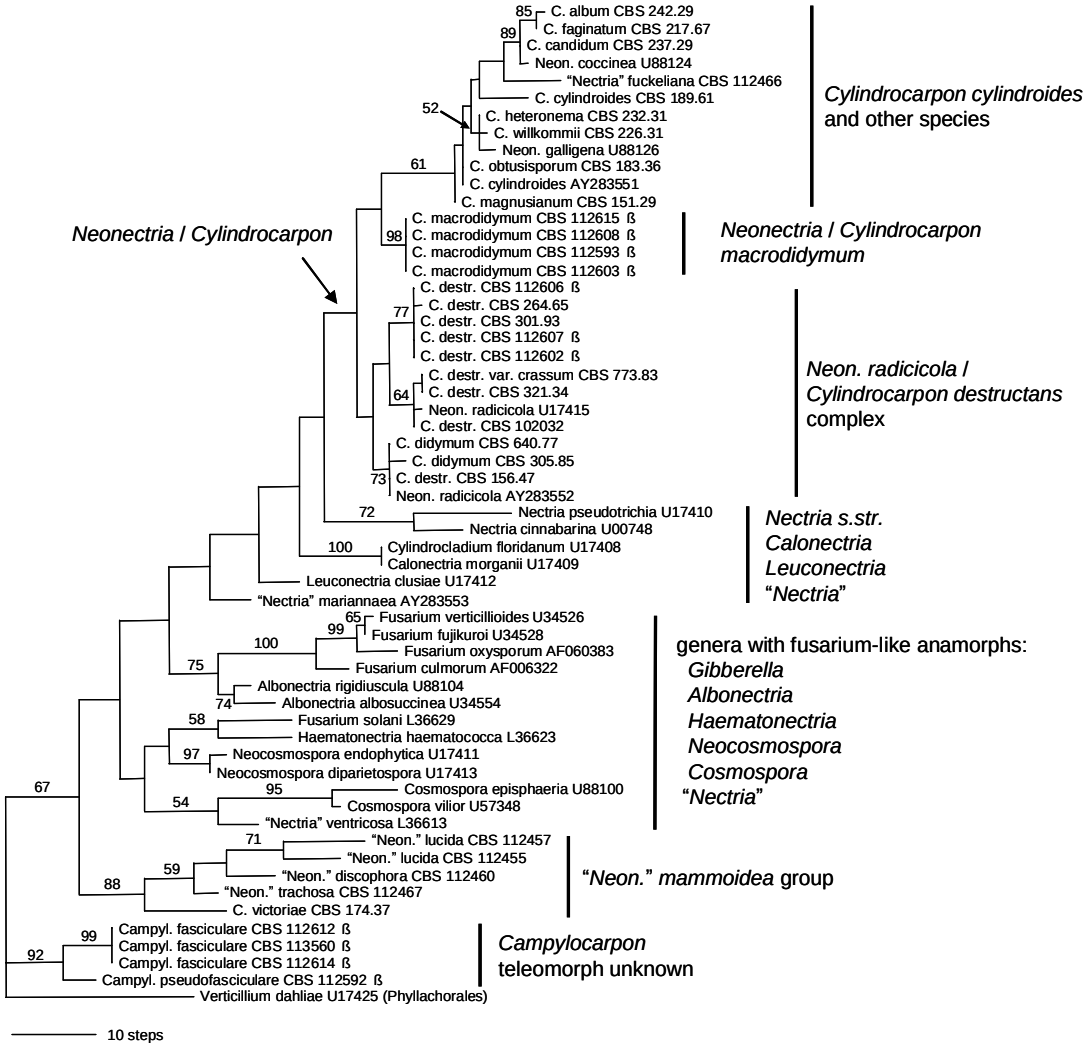

Fig. 2. One of four equally parsimonious phylograms (dataset 1) inferred from partial LSU rDNA sequences. Bootstrap values are indicated near nodes. Strains isolated from grapevines are marked by arrows.

Inclusion of *Neon. macrodidyma* within this clade was mainly due to the sequence of strain IMI 376409 (AJ007353) (S. Langrell unpubl., referred to as *Neon. radicicola*), which contained apomorphic characters typical of the *Neon. radicicola* as well as other apomorphies seen in *Neon. macrodidyma*. Analyses done without this sequence placed *Neon. macrodidyma* as a sister to the *Neon. radicicola* complex (not shown). A long branch of 16 steps (Fig. 3) indicated that both *Neon. macrodidyma* and IMI 376409 differed from members of the *Neon. radicicola* complex. A subclade referred to as "*Cylindrocarpon cylindroides* and other species" contained strains identified as *Cylindrocarpon cylindroides*, *C. magnusianum*, *C. obtusisporum*, *C. faginatum*, *C. album*, *C. heteronema*, and *Cylindrocarpon* sp.; its monophyly was moderately supported (bootstrap value = 70 %).

Heuristic parsimony analyses of aligned ITS sequences of **dataset 3** (533 bp alignment), which was restricted to members of the *Neon. radicicola* complex and closely related species as well as *Nectria cinnabarina* and *Fusarium solani*, of which *Fusarium solani* was used as outgroup, yielded 14 equally most-parsimonious trees, of which one is shown in Fig. 4, based on 75 PIC 174 steps in length with a CI of 0.626 and a RI of 0.925. The trees differed by minor changes within the *Neon. radicicola* complex and the clade referred to as "*Cylindrocarpon cylindroides* and other species". All trees placed *Neon. macrodidyma* as a sister taxon to members of the *Neon. radicicola* complex. Relatedness of *Neon. macrodidyma* and members of the *Neon. radicicola* complex was moderately supported in bootstrap analyses (bootstrap value = 78 %). Both these clades together formed the sister group to a clade including representatives of *C. magnusianum*, *C. cylindroides*, *C. album*, *C. faginatum*, *C. heteronema*, and *C. obtusisporum*. Monophyly of members of the latter group was weakly supported (bootstrap value = 70 %). Monophyly of the conjunction of this clade, *Neon. macrodidyma*, and the *Neon. radicicola* complex was strongly supported (bootstrap value = 99 %). In these analyses, however, *Neon. macroconidialis* (G.J.S. 83-162, AY295327) and the above-mentioned unusual *Neon. radicicola* isolate IMI 376409 (AJ007353) were excluded.

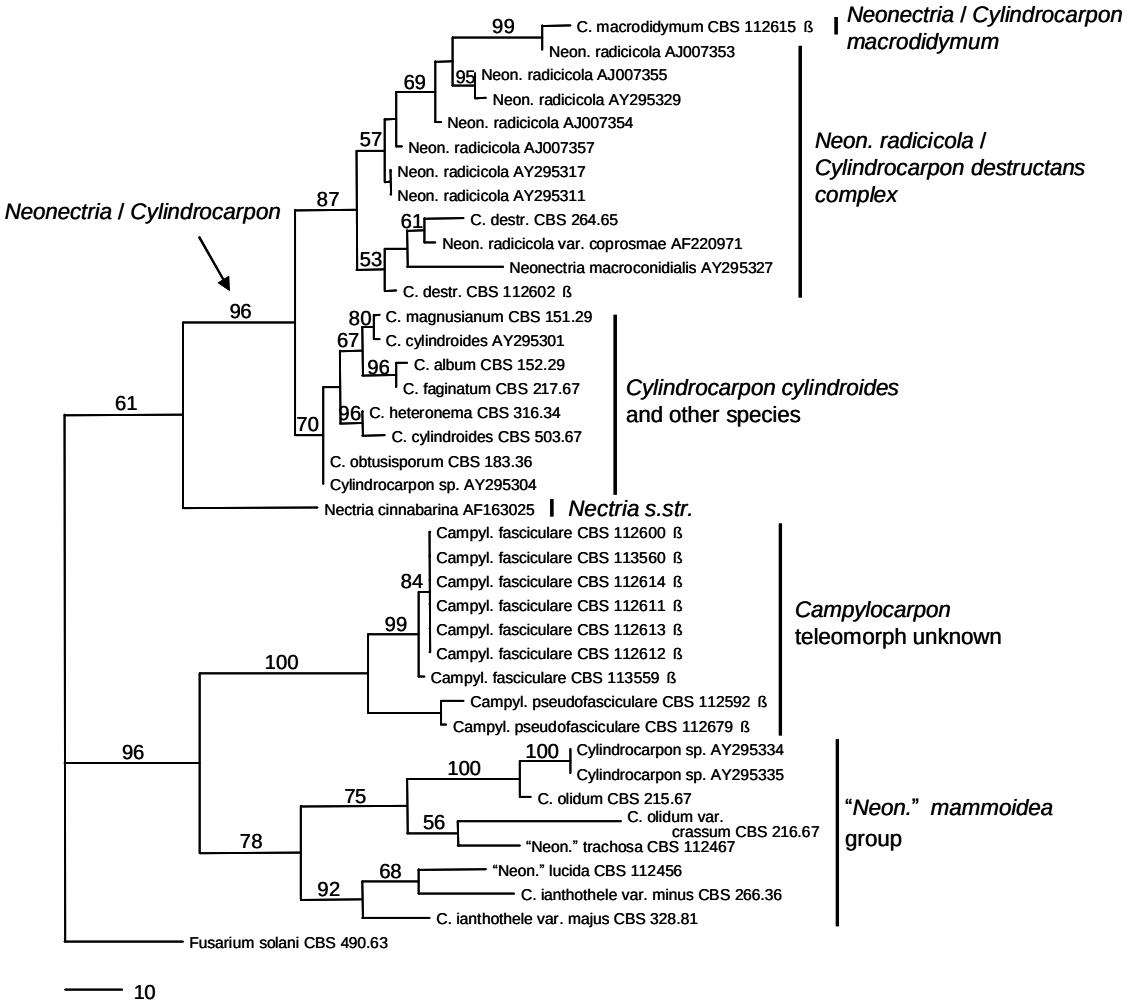

Fig. 3. One of 10 equally parsimonious phylograms (dataset 2) inferred from sequences of the internal transcribed spacer region 1, 5.8S rDNA and internal transcribed spacer region 2. Bootstrap intervals are indicated above nodes. Strains isolated from grapevines are marked by arrows.

Inclusion of these two taxa resulted in an inconclusive analysis yielding more than 1000 equally parsimonious trees, in which a clade containing *Neon. macrodidyma* and IMI 376409 was placed on a long branch of 18 steps among members of the *Neon. radicicola* complex, similar to the arrangement shown in Fig. 3. Strain DAOM 144524 (Seifert *et al*. 2003b), isolated from grapevine in Ontario, Canada, had ITS sequences identical to those of *Neon. macrodidyma* strains originating from South African, Australian, and New Zealand grapevines. All *Neon. macrodidyma* strains had identical ITS sequences. The species clade for *Neon. macrodidyma* was strongly supported (bootstrap value = 100 %). Members of *Neon. radicicola* formed a weakly supported monophyletic clade (bootstrap value = 66 %) that comprised relatively variable sequences. Forty percent of the PIC encountered in the dataset applied only to this clade. Analysis of the variation resulted in several, moderately to strongly supported subclades (see also Seifert *et al*. 2003b). One of the moderately supported subclades (bootstrap value = 74 %) contained strains from grapevines in South Africa, France, and New Zealand. However, this clade also contained a strain isolated from *Cyclamen* in the Netherlands (CBS 301.93). The variation within this clade did not appear to correlate with geographical patterns or host diversity.

Heuristic parsimony analyses of 500–550 bp long sequences of the β-tubulin gene, flanked by primers T1 and T2 (**dataset 4**), resulted in a 572 bp alignment and yielded one most parsimonious tree (Fig. 5) based on 167 PIC 404 steps in length with a CI of 0.681 and a RI of 0.918. Taxa analysed in this dataset include those isolated from vines as well as additional *Cylindrocarpon*-like strains. A *Fusarium* sp. isolate was used as outgroup. Members of the "*Neon.*" *mammoidea* group were excluded from the analyses. *Neonectria*/*Cylindrocarpon*, represented by members of the *Neon. radicicola* complex, *Neon. macrodidyma*, and "*Nectria*" *fuckeliana*, fell into one strongly supported monophyletic clade (bootstrap value = 96 %).

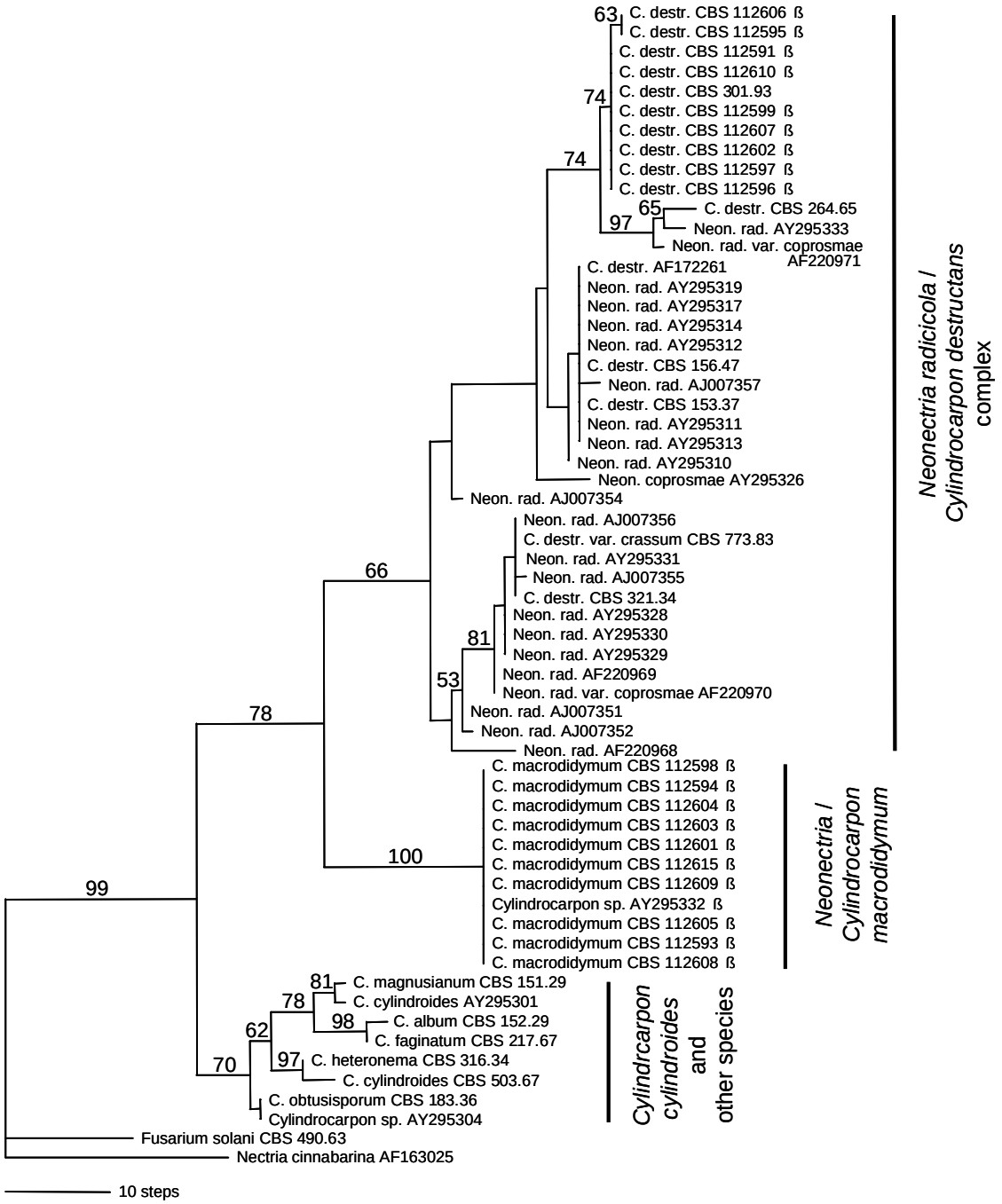

Fig. 4. One of 14 equally parsimonious phylograms of a wider range of *Cylindrocarpon* isolates (dataset 3) inferred from sequences of the internal transcribed spacer region 1, 5.8S rDNA and internal transcribed spacer region 2. Bootstrap values are indicated above nodes. Strains isolated from grapevines are marked by arrows.

Unlike what was seen in LSU and ITS analyses, members of the *Neon. radicicola* complex formed a strongly supported monophyletic group (bootstrap value = 100 %). Strains of *C. destructans* from grapevines in South Africa, France, and New Zealand, as well as CBS 301.93 from *Cyclamen* in the Netherlands, had identical partial β-tubulin sequences. Among the South African strains of *C. macrodidymum* that originated from seven different locations, five different scions, and six different rootstocks (Table 1), four variable sites were encountered in the partial β-tubulin gene. The variation within these strains did not appear to correlate with geographical patterns or host diversity.

A major, strongly supported clade (bootstrap value = 95 %) comprised representatives of the "*Neon.*" *mammoidea* group and *Campylocarpon*. This clade was only remotely related to *Neonectria*/*Cylindrocarpon*. Within this clade the "*Neon.*" *mammoidea* group and *Campylocarpon* were accommodated in different subclades, with the former obtaining 84 % and the latter 100 % bootstrap support. Within the three representatives of the "*Neon.*" *mammoidea* complex, considerable variation was encountered, consisting of numerous substitutions and indels.

The isolates of *Campyl. fasciculare* had identical partial β-tubulin sequences. Between the two strains of *Campyl. pseudofasciculare* 1 indel and 9 substitutions were observed.

Dataset 5 comprised β-tubulin gene sequences of grapevine isolates as well as of selected *Cylindrocarpon*-like isolates published by Seifert *et al.* (2003b) that were not included in dataset 4. Heuristic parsimony analyses of this dataset was based on *ca*. 350–380 bp long sequences (386 bp alignment), flanked by primers T10 (O'Donnell & Cigelnik 1997) and T2. They yielded 360 equally most parsimonious trees, of which one is shown in Fig. 6, based on 118 PIC 286 steps in length with a CI of 0.640 and a RI of 0.942. The trees differed by minor changes mainly within the *Neon. radicicola* complex. The highly supported monophyletic *Neonectria*/*Cylindrocarpon* clade (bootstrap value = 100 %) contained three well supported subclades. One, supported by a 94 % bootstrap value, contained *Neon. galligena* and *C. cylindroides* (note that various species shown to be related to *C. cylindroides* in ITS analysis were not included in β-tubulin analysis). A second clade consisted of *Neon. macrodidyma* (bootstrap value = 100 %), and the third contained the *Neon. radicicola* complex (bootstrap value = 99 %). Single sequences of *Neon. macroconidialis* and "*Nectria*" *fuckeliana* included in the analysis also belonged to *Neonectria*/*Cylindrocarpon*, but within this group their phylogenetic positions remained unresolved. In contrast to the ITS analysis (Fig. 3), the present analysis only weakly supported close relatedness of *Neon. macroconidialis* to the *Neon. radicicola* complex.

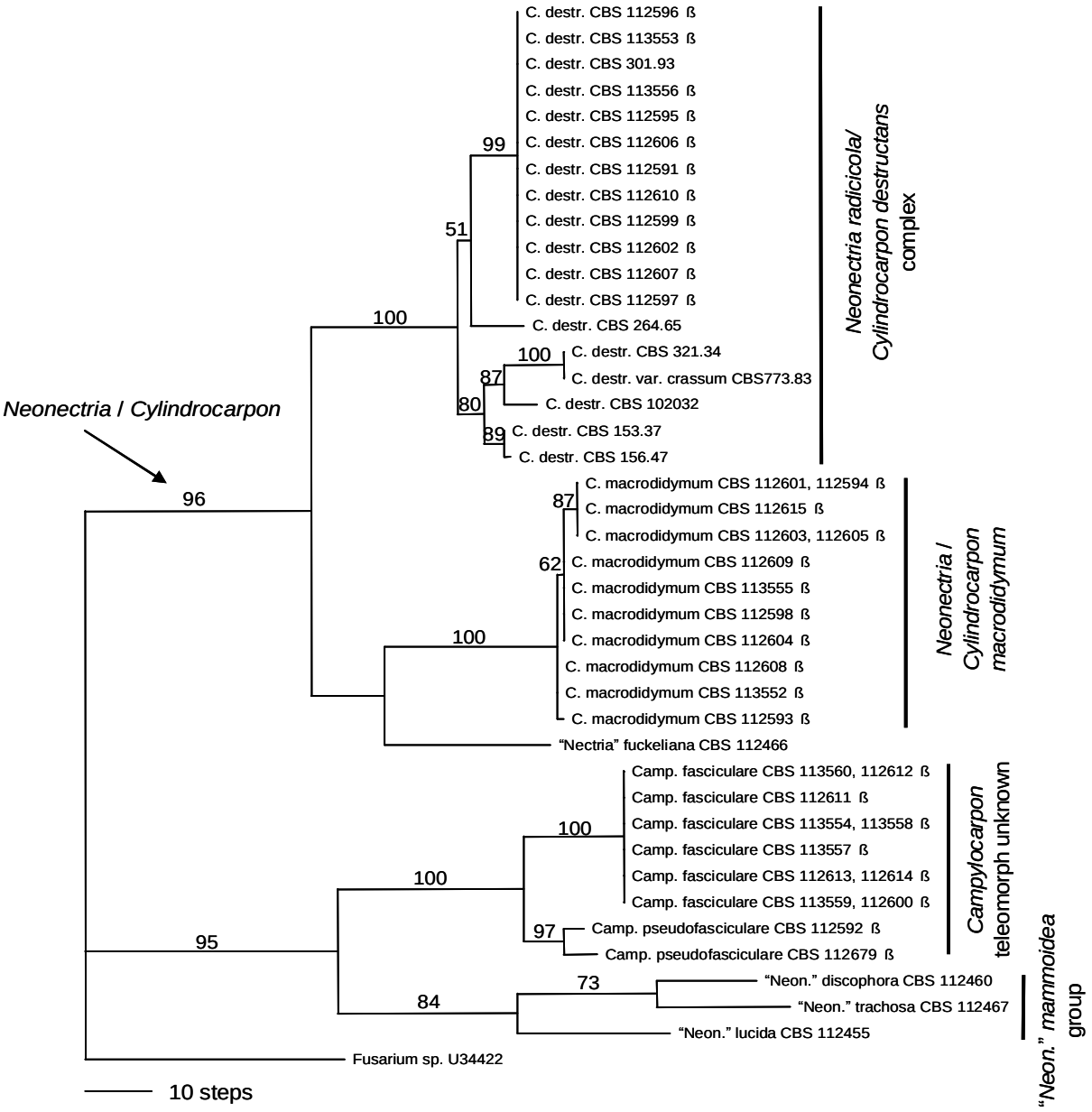

Fig. 5. Single most parsimonious phylogram for *Cylindrocarpon* and *Campylocarpon* isolates (dataset 4) inferred from sequences of a 572 bp alignment of the β-tubulin gene. Bootstrap values are indicated above the nodes. Strains isolated from grapevines are marked by arrows.

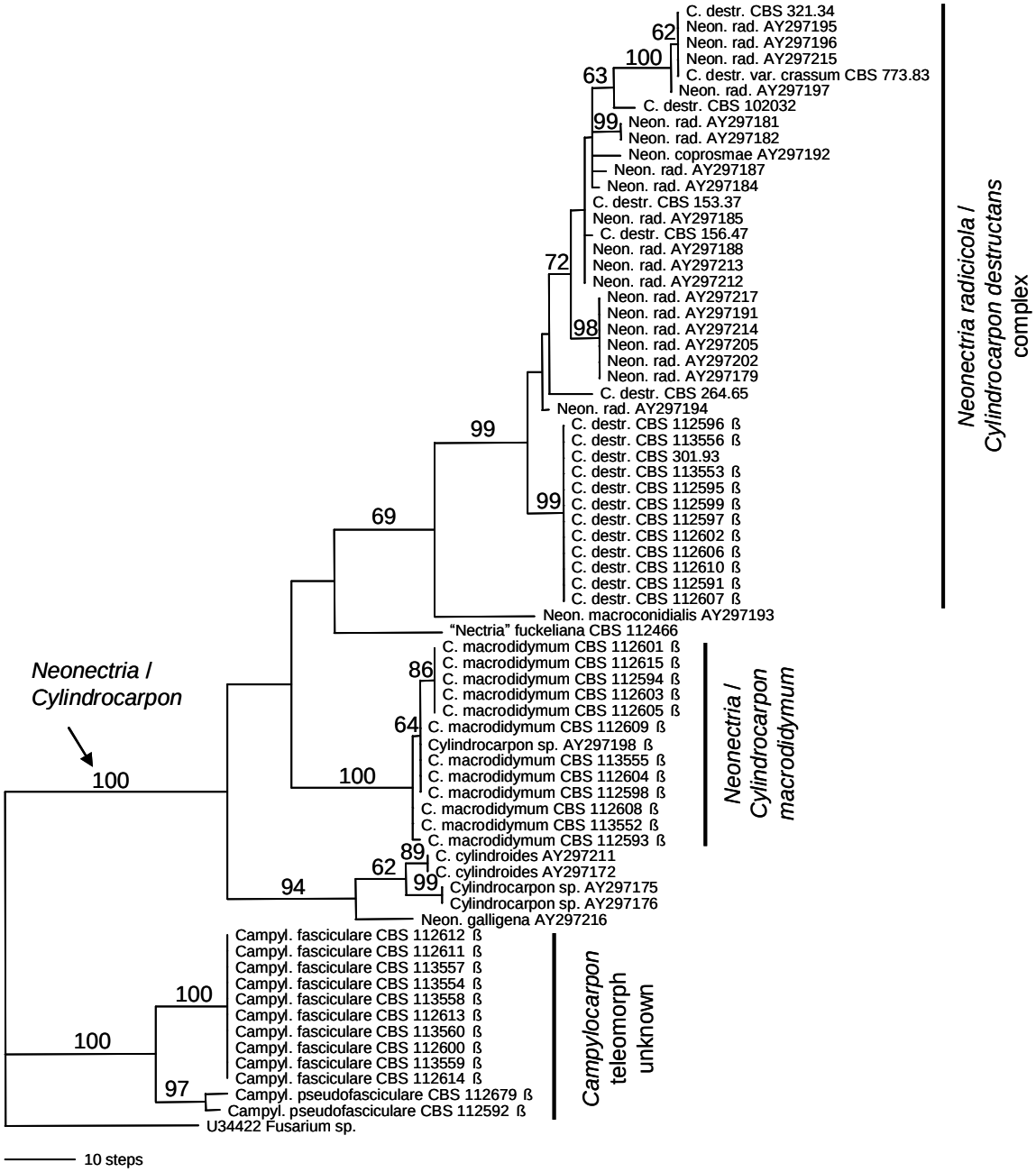

Fig. 6. One of 360 equally parsimonious phylograms for mainly grapevines isolates of *Cylindrocarpon* and *Campylocarpon* isolates (dataset 5) inferred from sequences of a 386 bp alignment of the β-tubulin gene. Bootstrap values are indicated above the nodes. Strains isolated from grapevines are marked by arrows.

Morphology

Isolates from grapevines in South Africa, France, and New Zealand identified as *C. destructans* showed micro- and macromorphological characters that were consistent overall. Conidiophores on SNA were mostly free-standing, unbranched or sparsely branched, and several times septate, as well as sporodochial, relatively short, and irregularly branched. Phialides borne on unbranched conidiophores had well-developed collarettes. Other characters included aseptate microconidia; predominantly 3-septate, mostly straight, sometimes slightly curved, apically rounded macroconidia; and chlamydospores abundantly formed on SNA within 14 d. Following Booth (1966) and Samuels & Brayford (1990), these strains were judged to be morphologically indistinguishable from *Neon. radicicola*/*C. destructans*.

Neonectria macrodidyma/*C. macrodidymum* is characterised by details of the shape of its macroconidia, the apex or apical cells, which are typically slightly bent to one side. Similar apical cells have also been described for *C. didymum* (Hartig) Wollenw., which differs from *Neon. macrodidyma* by forming smaller, 0–2-septate conidia (Domsch *et al*. 1980). Macroconidia of *C. destructans* and *C. obtusisporum* typically have an obtuse apex (Samuels & Brayford 1990, Booth 1966). *Neonectria macrodidyma* forms free-standing conidiophores that are mostly unbranched and several times septate, as well as sporodochial conidiophores that are relatively short and irregularly branched. Macroconidia were produced by

both kinds of conidiophores. *Neon. macrodidyma* typically formed brown colonies on PDA and OA. In contrast to what is observed in some *Cylindrocarpon* species, however, it frequently shows a yellow pigmentation at the margin of PDA and OA colonies and below the filter paper of SNA colonies. The presence of micro- and macroconidia and the rare formation of chlamydospores would tend to place *Neon. macrodidyma* in groups 1 or 3 in Booth (1966). In *Neon. macrodidyma*, teleomorphic characters are reminiscent of those seen in the *Neon. radicicola* complex (Samuels & Brayford 1990). As with members of that complex, this species has perithecial walls that are at least slightly verruculose, and also has a principal perithecial wall region composed of angular to globose cells. *Neonectria macrodidyma* differs from *Neon. radicicola* in narrower perithecial walls [mostly less than 30 µm in *Neon. macrodidyma*, (20–)35–50 (–60) µm in *Neon. radicicola* (Samuels & Brayford 1990)], smaller cells in the outer region of the perithecial wall [mostly less than 20 × 15 µm diam in *Neon. macrodidyma*, (10–)20–50(–60) µm diam in *Neon. radicicola* (Samuels & Brayford 1990)], and in longer ascospores [mostly longer than 14 µm in *Neon. macrodidyma*, mostly shorter than 13 µm in *Neon. radicicola* (Samuels & Brayford 1990)]. Perithecia of *Neon. macrodidyma* are solitary or formed in loose aggregates, as in the *Neon. radicicola* complex, whereas *Neon. galligena* and *Neon. coccinea* typically form crowded perithecia in large numbers on a well developed stroma.

Campylocarpon fasciculare and *Campyl. pseudofasciculare*, both isolated from grapevines in South Africa, form only multi-septate macroconidia and are therefore reminiscent of members of Booth's group 2 (1966), which accommodates most anamorphs of the "*Neon.*" *mammoidea* group (Brayford *et al*. 2004). In *Campyl. pseudofasciculare*, aerial chlamydospores have been observed; any formation of such structures is atypical for Booth's group 2. *Campylocarpon fasciculare* is particularly distinguished by fascicles of aggregated conidiophores formed on brownish strands of aerial hyphae and on unusually broad hyphae near the agar surface. A similar morphology has been described for the anamorph of *Neon. phaeodisca* (Rossman) Samuels & Brayford (Samuels & Brayford 1993), which Brayford *et al*. (2004) placed among other members of the "*Neon.*" *mammoidea* species group. The two species differ in their colony pigment, which is violaceous-brown or pale violaceous in *C. phaeodiscum* (Samuels & Brayford 1993) but rather dark brown in *Campyl. fasciculare*, and in the length ranges of their macroconidia [62.5–91 µm in *C. phaeodiscum* (Samuels & Brayford 1993) but typically less than 60 µm long in *Campyl. fasciculare*]. Brownish strands of aerial hyphae have also been observed in *Campyl. pseudofasciculare*. In this species, however, only solitary, mostly branched conidiophores have been seen.

TAXONOMY

Neonectria macrodidyma Halleen, Schroers & Crous, **sp. nov.** MycoBank MB500113. Figs 7a–l, 8.

Anamorph: *Cylindrocarpon macrodidymum* Schroers, Halleen & Crous, sp. nov.

Etymology: *Makrós* (Greek), large, referring to the macroconidia, which resemble those of *Cylindrocarpon didymum* but are significantly longer.

Neonectriae radicicolae similis sed ascosporis levibus vel verruculosis, et peritheciis levibus vel verruculosis distincta.

Perithecia formed heterothallically *in vitro*, disposed solitarily or in groups, developing directly on the agar surface or on sterile pieces of carnation leaf, ovoid to obpyriform, dark red, becoming purple red in 3 % KOH (positive colour reaction), smooth to finely warted, 200–250 µm diam, up to 270–300 µm high when rehydrated; without recognizable stroma; perithecial wall consisting of two poorly distinguishable regions; outer region 20–30 µm thick, composed of 1–3 layers of angular to subglobose cells, 9–20 × 7–15 µm (n = 15); cell walls up to 1 µm thick; inner region around 5 µm thick, composed of cells that are flat in transverse optical section and angular to oval in subsurface optical face view; walls in the outer and inner region sometimes locally thinning to form pseudopores in conjunction with matching structures in adjacent cells; perithecial warts consisting of globose to subglobose cells, 7–16.5 × 4.5–13.5 µm (n = 20), that have walls up to 2.5 µm thick. *Asci* clavate to narrowly clavate, *ca.* 65 × 10 µm, 8-spored; apex rounded, with a minutely visible ring. *Ascospores* divided into two cells of equal size, ellipsoidal to oblong ellipsoidal, somewhat tapering towards the ends, smooth to finely warted, (12–)14–15–16(–18) × (3.5–)4(–4.5) µm (n = 37).

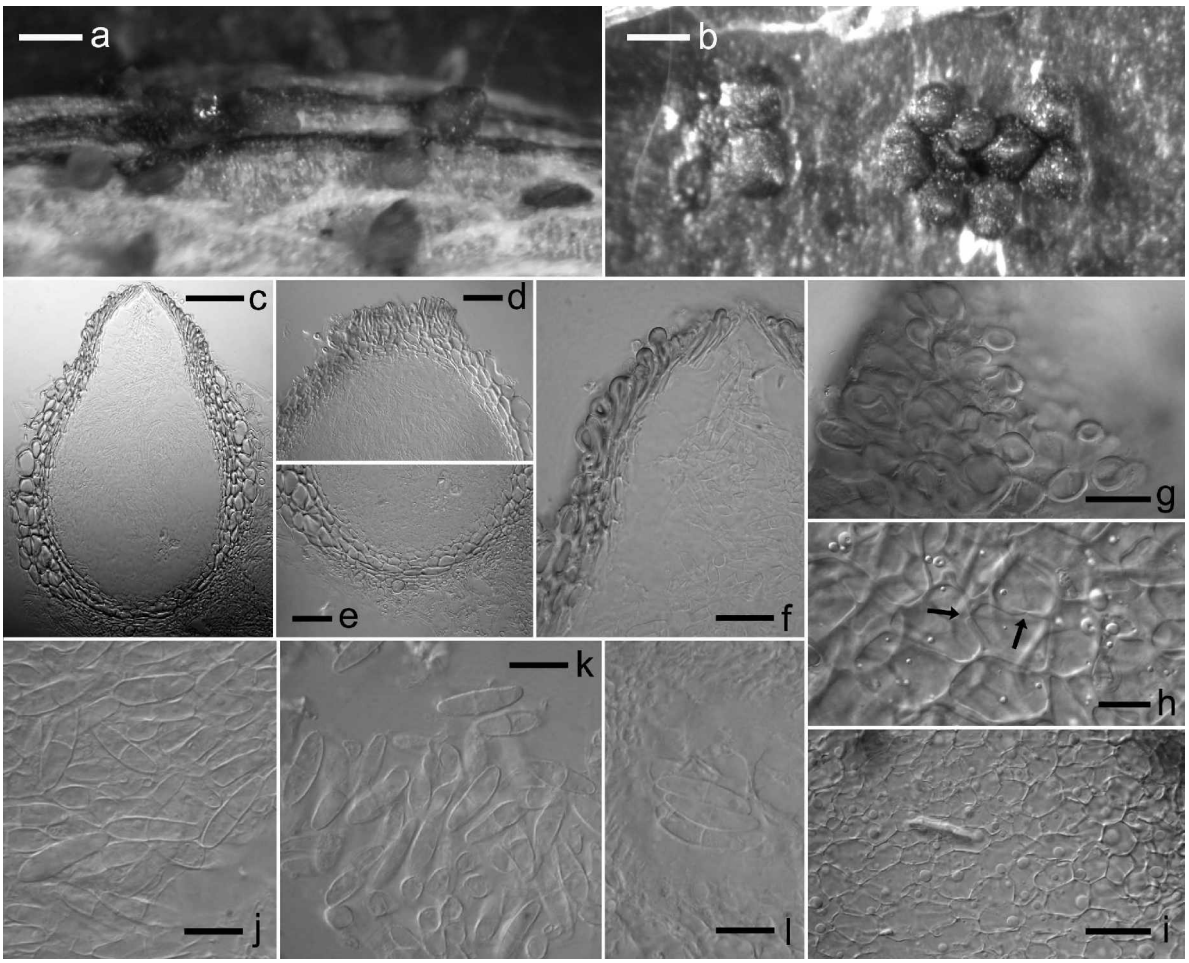

Fig. 7a–l. *Neonectria macrodidyma*/*Cylindrocarpon macrodidymum*. a, b. Solitary to aggregated development of perithecia. c–f. Longitudinal sections of perithecia showing details of wall, apex and base. g–i. Surface (g) and subsurface (h) optical face view of outer perithecial wall region; subsurface optical face view of inner perithecial wall region (i); main perithecial wall region consisting of cells with walls showing "pseudopores" (arrows in h). j–l. Ascospores. All from a crossing of CBS 112594 with CBS 112603 on CLA. Scale bars: a, b = 200 µm; c = 50 µm; d, e = 30 µm; f, g, i = 20 µm, h, j–l = 10 µm.

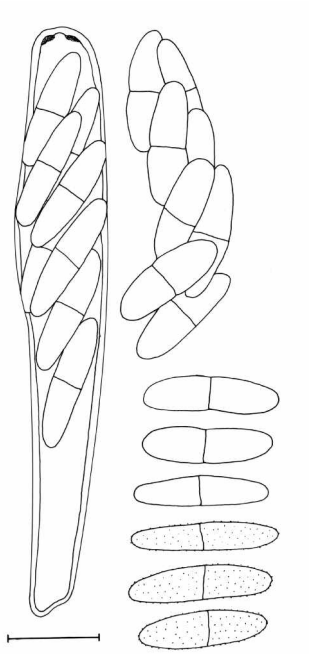

Fig. 8. Ascus and ascospores of *Neonectria macrodidyma*/*Cylindrocarpon macrodidymum*. All from a crossing of CBS 112594 with CBS 112603 on CLA. Scale bar = 10 µm.

Cylindrocarpon macrodidymum Schroers, Halleen & Crous, **sp. nov.** MycoBank MB500114. Fig. 9a–y.

Anamorphe a *Cylindrocarpo didymo* conidiis plerumque 3-septatis distincta, a *C. destructante* chlamydosporis paucissimis differt. Microconidia 0–1-septata, ellipsoidea vel ovoidea, plus minusve recta, hilo plus minusve laterali, (5.5–)8–9.5–10.5(–12.5) × (3.5–)4–4–4.5(–4.5) µm; macroconidia copiosiora, 1–3(–4)-septata, plus minusve recta, cylindrica vel sursum paulo expansa, cellula apicali modice unilateraliter curvata et exigue rostrata; macroconidia 3-septata (26–)34–36–38(–45) × (4–)5.5–6–6.5(–8) µm.

Conidiophores simple or complex, sporodochial. Simple conidiophores arising laterally or terminally from the aerial mycelium or erect, arising from the agar surface, solitary to loosely aggregated, unbranched or sparsely branched, 1–4-septate, rarely consisting only of the phialide, 55–120 µm long; phialides monophialidic, cylindrical, slightly tapering toward the base, 16.5–30 µm long, 2.5–3.5 µm wide at base, 2–2.5 µm near aperture (n = number of measurements = 28). Complex, sporodochial conidiophores aggregated in pionnote sporodochia, repeatedly,

irregularly branched; phialides cylindrical but slightly tapering towards the tip or narrowly flask-shaped, mostly with widest point near the middle, (15.5–) 17.5–20–21(–28.5) µm long, (2.5–)3(–3.5) µm wide at the base, (2.5–)3.5(–4) µm at the widest point, and (1.5–)2(–2.5) µm near the aperture (n = 58). *Micro-* and *macroconidia* present on both types of conidiophores. *Macroconidia* predominating, formed by both types of conidiophores, predominantly 1–3(–4)-septate, straight or sometimes slightly curved, cylindrical or typically minutely widening towards the tip, therefore appearing somewhat clavate, particularly when still attached to the phialide, with apex or apical cell typically slightly bent to one side and minutely beaked, mostly with a visible, slightly laterally displaced hilum; 1-, 2-, and 3-septate macroconidia of similar size range; 1-septate macroconidia 24–32 × 5–7 µm (n = 10); 3-septate macroconidia (26–)34–36–38(–45) × (4–)5.5–6–6.5(–8) µm (n = 116). *Microconidia* sparsely produced on SNA, moderately common on OA, 0–1-septate, ellipsoidal to ovoidal, more or less straight, with a minutely or clearly laterally displaced hilum; aseptate microconidia (5.5–)8–9.5–10.5(–12.5) × (3.5–)4(–4.5) µm (n = 43). *Conidia* formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses on simple as well as complex conidiophores. *Chlamydospores* sometimes occurring, mostly in short, intercalary chains, 7–12.5 × 6–10 µm (n = 25).

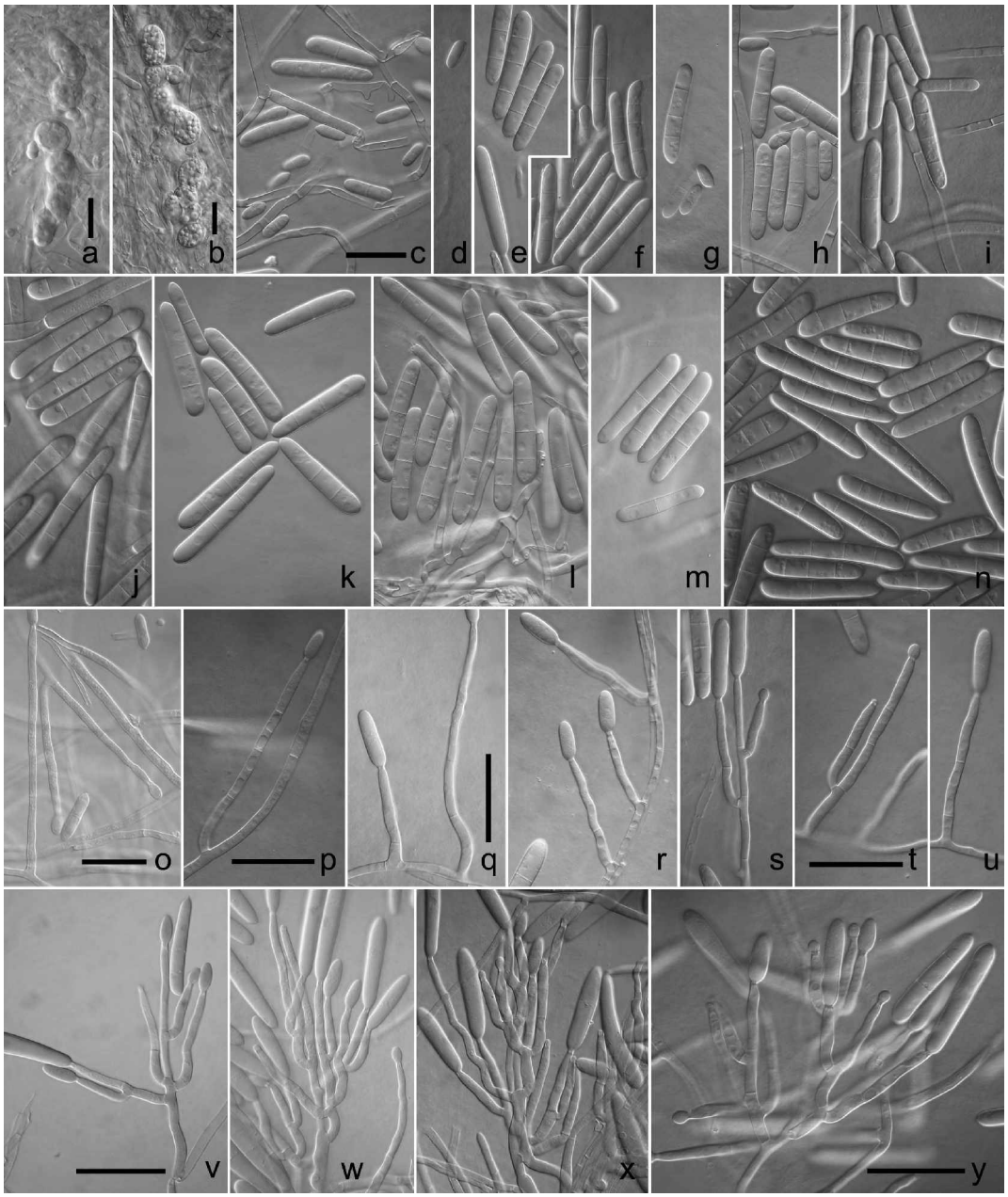

Fig. 9a–y. *Neonectria macrodidyma*/*Cylindrocarpon macrodidymum*. a, b. Chlamydospores. c–i. Micro- and macroconidia formed on SNA. j–n. Macroconidia formed on sporodochial conidiophores on OA. o–u. Simple, unbranched or sparsely branched, septate conidiophores on SNA. v–y. Complex, sporodochial conidiophores. a, b from CBS 112609, c–y from CBS 112615; a–i, o–y from 10-d-old SNA culture; j–n all 10-d-old OA culture. Scale bars: a, b = 10 µm; c–n = 20 µm, o–y = 30 µm.

Holotypes: For the anamorph: dried SNA colony of CBS 112615 (herb. CBS 6588); for the teleomorph: dried CLA colony with perithecia formed by crossing CBS 112594 and CBS 112603 (herb. CBS. 6589).

Cardinal temperatures for growth: Minimum temperature not determined, < 4 °C; optimum temperature 20–25 °C, at which PDA colonies reach 30–45 mm diam after 7 d in the dark; maximum temperature ≤ 30 °C. *Colonies* at 20 °C reaching 10–20 mm diam on SNA and 10–17 mm diam on PDA after 4 d, 30–45 mm diam on SNA and 25–35 mm diam on PDA after 7 d. *Aerial mycelium* on SNA only developed near or on the filter paper, highly diffuse, consisting of single hyphae, overall white, or, if accumulating on the filter paper yellowish; on OA and PDA abundantly formed, covering the whole colony or sectors thereof, resulting in a felty appearance; on OA white to yellowish, on PDA predominately yellowish, strongly vacuolated. *Colony reverse* on SNA not pigmented; on OA mustard-brown to cinnamon-brown (5E6–6E6) or Pompaeian to honey-yellow (5C6–5D6), sometimes with a pale yellow to mustard-yellow (3A5–3B6) margin; on PDA in central parts of the colony burnt umber (6E7–6F7), towards the margin raw Sienna (6D7), reddish golden (6C7), or brownish yellow (5C7), typically with a pale yellow to amber-yellow (3A5–4B6) margin; pigmentation similar on PDA incubated in darkness or under continuous near UV-light.

Strains studied: CBS 112615 (STE-U 3976, F.H. c98), CBS 112593 (STE-U 3990, F.H. c107), CBS 112603 (STE-U 4007, F.H. c8), CBS 112609 (STE-U 3969, F.H. a), CBS 112608 (STE-U 3987, F.H. c62), CBS 112594 (STE-U 3991, F.H. c111), CBS 112598 (STE-U 3997, F.H. c115), CBS 112605 (STE-U 3984, F.H. c106) (Table 1).

Fertile matings: Perithecia observed mostly within 3 or 4 wks in crossings of strains CBS 112603 × CBS 112615, CBS 112603 × CBS 112609, CBS 112603 × CBS 112605, CBS 112603 × CBS 112598, CBS 112601 × CBS 112605, CBS 112594 × CBS 112601, CBS 112594 × CBS 112603.

Distribution: Australia, Canada, New Zealand, South Africa.

Habitat: Roots and rootstocks of grapevines, causing black foot disease.

Phylogenetic affinity: *Nectriaceae, Hypocreales*.

Campylocarpon Halleen, Schroers & Crous, **gen. nov.** MycoBank MB500115.

Etymology: *Kampylos* (Greek), referring to the curved macroconidia, the second part matching *Cylindrocarpon*.

Cylindrocarpo simile, microconidiis carens, chlamydosporis raris vel absentibus, macroconidiis vulgo curvatis. Conidiophora e latere hypharum aeriarum singularium vel fasciculatarum vel ex hyphis in superficie substrati crescentium oriunda, singula vel laxe vel arcte aggregata, capitula conidiorum mucida pionnoti similia formantia. Cellulae basilares stipitis conidiophori ad 16 µm latae, nonnullas phialides vel penicillum irregularem ramorum brevium portantes; rami ultimi 1 vel complures phialides portantes. Macroconidia sicut in *Cylindrocarpo*, sed typice curvata, ad 6-septata; cellula apicalis obtusa, basilaris obtusa vel hilo inconspicuo praedita.

Type: *Campylocarpon fasciculare* Schroers, Halleen & Crous, sp. nov.

Similar to *Cylindrocarpon*. *Microconidia* absent, *chlamydospores* rare or absent, macroconidia mostly curved. *Conidiophores* arising laterally from single or fasciculate aerial hyphae or from creeping substrate hyphae, singly or in loose or dense aggregates; conidial heads forming pionnotes-like aggregates. *Conidiophore* stipe base to 16 um wide, bearing several phialides or a penicillus of irregular branches; terminal branches bearing 1 or several phialides. *Macroconidia* as in *Cylindrocarpon*, but typically curved, up to 6-septate; apical cell obtuse, basal cell obtuse or with inconspicuous hilum.

Phylogenetic affinity: Apparently a sister taxon of the "*Neonectria*" *mammoidea* group (*Nectriaceae, Hypocreales*).

Campylocarpon fasciculare Schroers, Halleen & Crous, **sp. nov.** MycoBank MB500116. Fig. 10a–o.

Teleomorph: Not known.

Etymology: *Fasciculus* (Latin), a little bundle, referring to the compact, densely branched conidiophores that arise from short supporting cells.

Cylindrocarpo phaeodisco simile sed conidiis paulo minoribus et coloniis brunneis, pigmento purpureo carentibus distinctum. Conidiophora compacta, ex una vel compluribus cellulis stipitariis et semel vel compluries irregulariter ramoso penicillo composita, ex hyphis aeriis saepe brunneis, fasciculatis vel ex hyphis in substrato repentibus ad 16 µm latis oriunda. Microconidia nulla, macroconidia cylindrica, recta vel modice curvata, (1–)3–4(–5)-septata, utrinque angustata et obtusa, hilo vix visibili; 3-septata (29–)38–41.5–44.5(–53) × (5.5–)6.5–7.5–8(–9) µm, 4-septata (39–)47–49–51.5(–58) × (6.5–)7.5–8–8.5 (–9). Chlamydosporae absentes.

Conidiophores initially simple, consisting of single phialides, or phialides in whorls of up to three members, that are formed on short supporting cells situated laterally on hyphae of the aerial mycelium; later arranged in dense fascicles, 40–60 µm high and 80–140 µm diam, arising laterally from unpigmented hyphae of the aerial mycelium, unpigmented hyphae growing near the agar surface, or brownish pigmented hyphal strands of the aerial mycelium. Hyphal cells supporting the conidiophore fascicles 15–40 × 6–16 µm (n = 12); fascicles 1–3 times branched, with basal cells 8–35 × 5.5–16 µm and metulae 7.5–13.5 × 3.5–5.5 µm (n = 10).

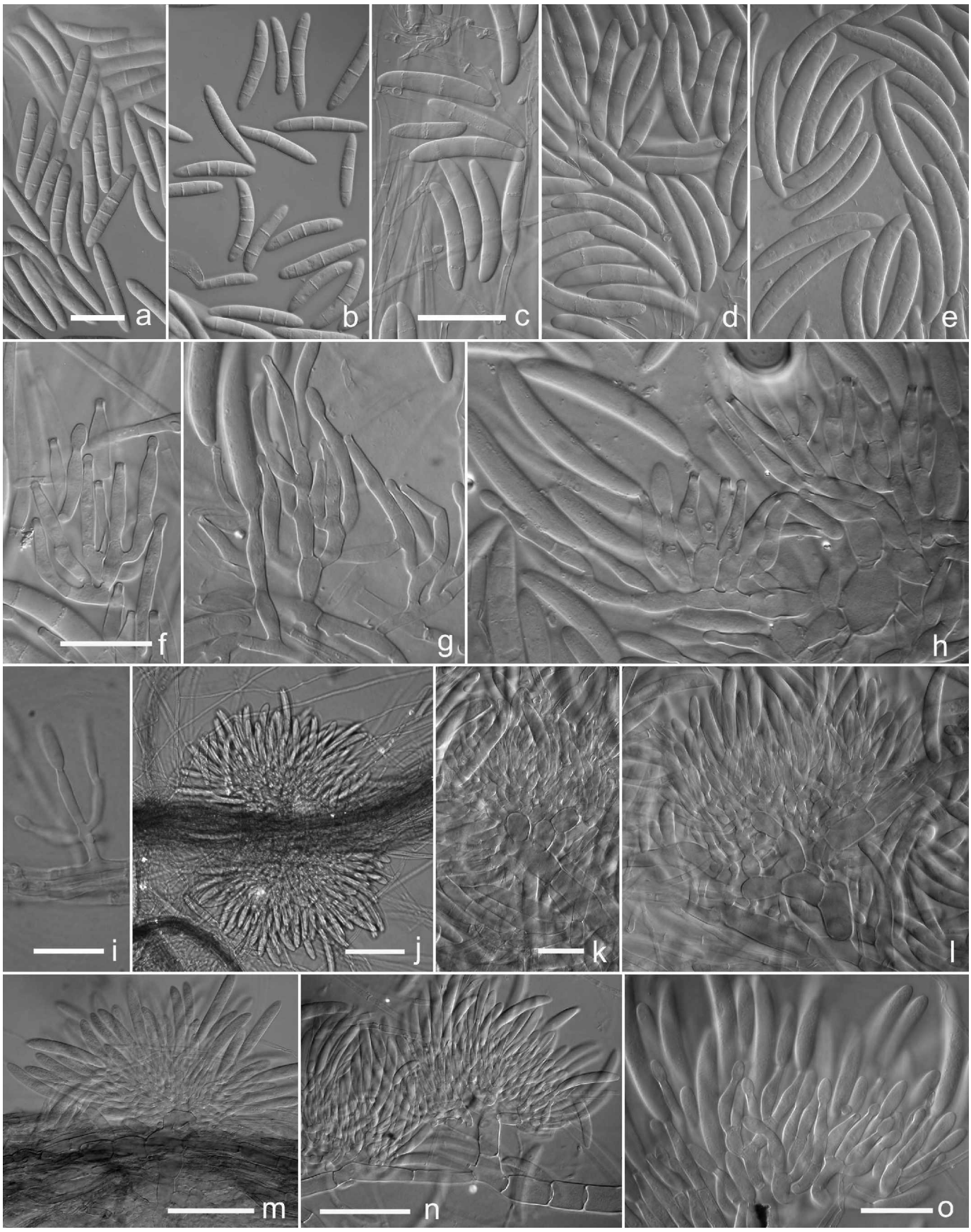

Fig. 10a–o. *Campylocarpon fasciculare*. a–e. Macroconidia. f–h. Branched conidiophores. i. Branched conidiophore arising from apical part of hyphal strand. j–n. Fascicles of branched conidiophores arising from brownish hyphal strands (j, m) or from the agar surface (k, l, n). o. Whorls of phialides of a fascicle of conidiophores. All from CBS 112613; a–o from 21-d-old $V8_{50}$ cultures. Scale bars: a–e = 30 µm, f–i, k, l, o = 20 µm, j, m, n = 50 µm.

Phialides narrowly flask-shaped, mostly with widest point near the middle, (13–)16–18–19(–29) µm long, (2–)2.5–3(–3.5) µm wide at the base, (2–)3.5–4(–4.5) µm at the widest point, and 2(–2.5) µm near the aperture (n = 52). *Macroconidia* mostly 3–4-septate, also 1-, 2-, and 5-septate, cylindrical, slightly to moderately curved, with minutely tapering, obtuse ends, sometimes somewhat more strongly tapering at the base; base with or without an obscure hilum; when 1-septate 24–32 × 5–7 µm (n = 5), when 2-septate (28.5–)35–38–43.5(–47) × (6–)6.5–7–7.5(–9) µm (n = 34), when 3-septate (29–)38–41.5–44.5(–53) × (5.5–)6.5–7.5–8(–9) µm (n = 126), when 4-septate (39–)47–49–51.5(–58) × (6.5–)7.5–8–8.5(–9) µm (n = 33), and when 5-septate 44.5–54 × 7.5–9 µm (n = 5). *Microconidia* not observed. *Conidial masses* off-white, hemispherical, with shape arising from formation on fascicles borne on hyphal strands; or alternatively pionnote-like, covering the agar surface. *Chlamydospores* not observed.

Holotype: Dried MEA colony of CBS 112613 (herb. CBS 6590).

Cardinal temperatures for growth: Minimum temperature 10 °C; optimum temperature 30 °C, at which PDA colonies reach *ca.* 30–45 mm diam after 7 d in the dark; maximum temperature not determined, ≥ 35 °C. *Colonies* reaching 10–18 mm diam on SNA and 8–16 mm diam on PDA after 4 d, 20–30 mm diam on SNA and PDA after 7 d. *Aerial mycelium* on SNA essentially absent; on OA and PDA abundantly formed, covering the whole of colony or sectors thereof, white, thick, cottony to felty, intermingled with or giving rise to erect, white or brown hyphal strands up to 8 mm long and 20–70 µm thick; these strands sometimes partly covered by off-white slime. Aerial mycelium on $V8_{50}$ sparsely formed or absent; surface on $V8_{50}$ smooth to waxy or covered by slimy domes up to 300 µm in diam consisting of conidial masses. *Colony reverse* on SNA not pigmented; on OA and PDA chocolate-brown (6F4) to dark brown (7F4–7F8) or, in some sectors, camel (6D4); pigmentation similar on PDA incubated in darkness or under continuous near-UV light.

Strains studied: CBS 113560 (STE-U 3972, F.H. c120), CBS 113559 (STE-U 4006, F.H. c119), CBS 112614 (STE-U 3973, F.H. c79), CBS 112613 (STE-U 3970, F.H. c76), CBS 112612 (STE-U 3966, F.H. c134), CBS 112611 (STE-U 3965, F.H. c147), CBS 112600 (STE-U 3981, F.H. c132) (Table 1).

Distribution: South Africa.

Habitat: Roots, rootstock and trunk of grapevines, causing black foot disease.

Campylocarpon pseudofasciculare Halleen, Schroers & Crous, **sp. nov.** MycoBank MB500117. Fig. 11a–m.
Teleomorph: Not known.

Etymology: *Pseudo* (Greek), false, referring to its similarities to *Campyl. fasciculare*.

Cylindrocarpo fasciculari simile sed conidiophoris singulis, simplicibus vel irregulariter ramosis, e latere hypharum aeriarum singularium vel fasciculatarum saepe brunnearum oriundis distinctum. Macroconidia cylindrica, plus minusve curvata, plerumque magis curvata in parte distali, utrinque exigue angustata, obtusa, hilo vix visibili; (2–)3–5(–6)-septata, 3-septata (29–)37.5–44–48.5(–68.5) × (6–)6.5–7–7.5(–9.5) µm; 4-septata (40.5–)46.5–51–53.5(–62) × (6.5–)7–8–8.5(–9.5); 5-septata (36.5–)51–55–59(–68) × (6.5–)7.5–8–8.9(–10) µm. Chlamydosporae rarae, plerumque 3–5 aggregatae, intercalares vel in ramis brevibus lateralibus, rotundatae vel modice angulares, (6–)8–9.5–11(–13) × (4.5–)7–7.5–8.5(–11) µm.

Conidiophores rarely simple, consisting of single phialides, or phialides in whorls of up to 3 members, that are formed on short supporting cells or on more highly branched structures that develop laterally on hyphae or hyphal strands. Most basal cells of conidiophores 12–19 × 5–7 µm; metulae 10–17 × 3–5.5 µm (n = 10). Phialides narrowly flask-shaped, mostly with widest point near the middle, (14–)16.5–19–20.5 (–24.5) µm long, (2–)3(–3.5) µm wide at the base, (3.5–)4(–5) µm at the widest point, and (1.5–)2(–2.5) µm near the aperture (n = 18). *Macroconidia* cylindrical, slightly to moderately curved; typically somewhat more curved at the tip than at the base; with minutely tapering, obtuse ends, somewhat more strongly tapering at the base than at the apex, and with or without an obscure hilum; 3–5-septate, also 2- and 6-septate; when 2-septate 24–36 × 6–7 µm (n = 2), when 3-septate (29–)37.5–44–48.5(–68.5) × (6–)6.5–7–7.5 (–9.5) µm (n = 59), when 4-septate (40.5–)46.5–51–53.5(–62) × (6.5–)7–8–8.5(–9.5) µm (n = 29), when 5-septate (36.5–)51–55–59(–68) × (6.5–)7.5–8–8.9(–10) µm (n = 119), and when 6-septate 59.8–61.45 × 8.5–9 µm (n = 2). Chlamydospores sparse, typically in clusters of 3–5, intercalary or borne on short side-branches, round to somewhat angular, (6–)8–9.5–11 (–13) × (4.5–)7–7.5–8.5(–11) µm.

Holotype: Dried PDA colony of CBS 112679 (herb. CBS 6591).

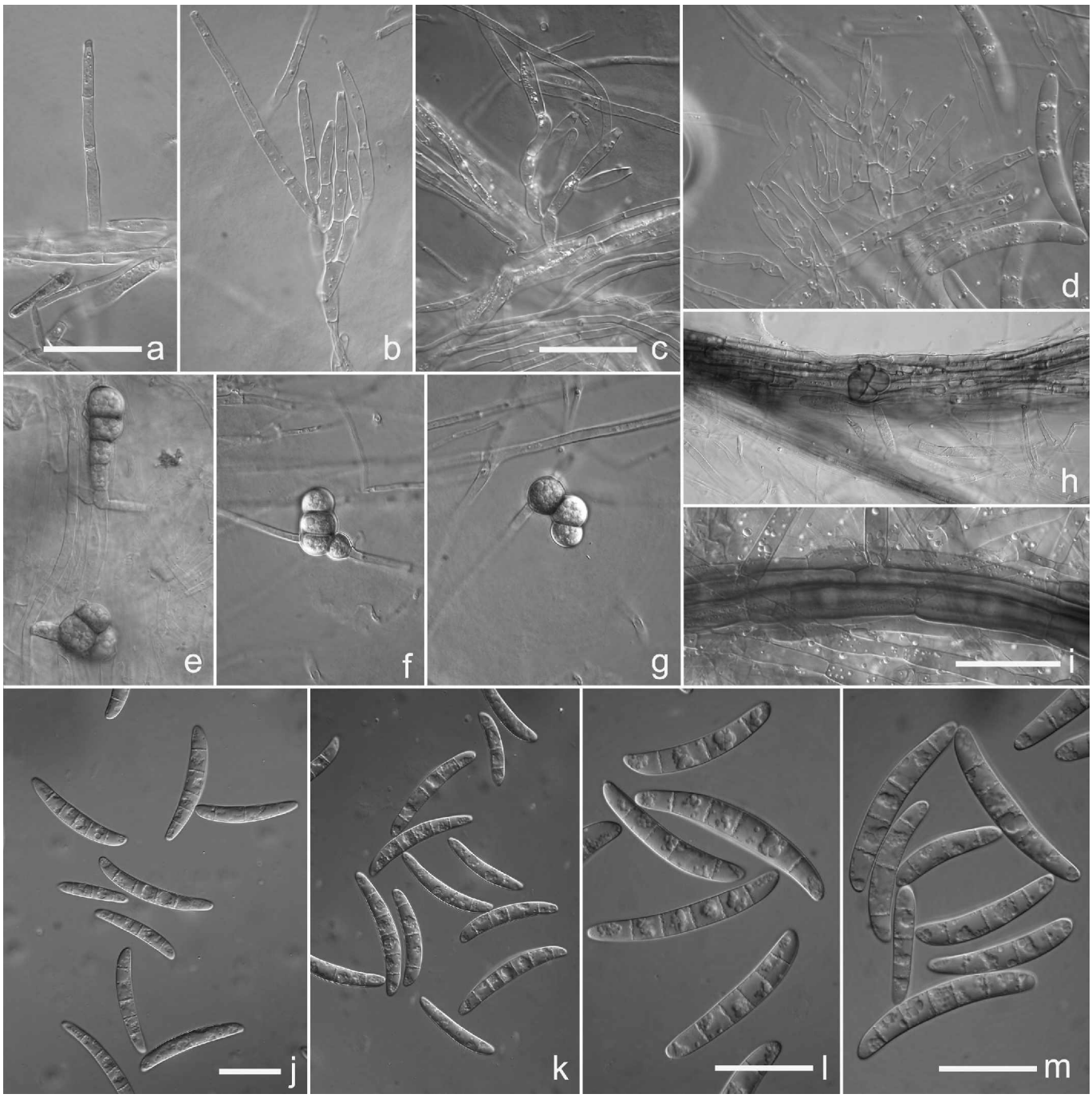

Fig. 11a–m. *Campylocarpon pseudofasciculare*. a. Simple, unbranched, septate conidiophore. b–d. Branched conidiophores. e–g. Chlamydospores. h–i. Brownish hyphal strand formed in the aerial mycelium. j–m. Macroconidia. All from CBS 112679; a–d, h–m from 21-d-old PDA culture; e from 21-d-old OA culture; f, g from 21-d-old SNA culture. Scale bars: a–h, j–m = 30 µm, i = 20 µm.

Cardinal temperatures for growth: Minimum temperature 10 °C; optimum temperature 30 °C, at which PDA colonies reach 45 mm diam after 7 d in the dark; maximum temperature not determined, ≥ 35 °C. *Colonies* reaching 10–15 mm diam on SNA and around 10 mm diam on PDA after 4 d, 25 mm diam on SNA and PDA after 7 d. *Aerial mycelium* on SNA essentially absent; on $V8_{50}$ sparsely formed or absent; on OA and PDA abundant, covering the whole or sectors of the colony, white to off-white or slightly brownish, thickly cottony to felty, intermingled with or giving rise to erect white or brown hyphal strands up to 10 mm long and 10–80 µm thick. *Colony reverse* on SNA not pigmented; on OA and PDA chocolate-brown (6F4) to dark brown (7F4–7F8); pigmentation similar on PDA whether incubated in darkness or continuous near-UV light.

Isolates studied: CBS 112592 (STE-U 3988, F.H. c89), CBS 112679 (STE-U 5472, F.H. c108) (Table 1).

Distribution: South Africa.

Habitat: Roots of asymptomatic nursery grapevines.

Pathogenicity

None of the control plants died, while 35 %, 27.5 %, 22.5 % and 17.5 % of the plants inoculated with CBS 112604 (F.H. c10) (*Neon. macrodidyma*), CBS 112597 (F.H. c33) (*Cy. destructans*), CBS 112614 (F.H. c79) (*Ca. fasciculare*) and CBS 112679 (F.H. c108) (*Ca. pseudofasciculare*) died, respectively. The corresponding isolates were re-isolated from 81–100 % of the remaining plants. No *Cylindrocarpon* or *Campylocarpon* species were isolated from any of the control plants. Inoculations resulted in a dramatic reduction of root ($P < 0.0001$) and shoot mass ($P = 0.0087$) of the potted grapevines (ANOVA table not shown). Compared to the uninoculated control plants, CBS 112597, CBS 112604, CBS 112614 and CBS 112679 reduced root mass by 57.5 %, 48 %, 42.1 %, and 40.7 %, respectively. Statistically, however, there was no difference between the isolates. Compared to the uninoculated control plants, CBS 112597, CBS 112614, CBS 112679 and CBS 112604, reduced shoot mass by 39.4 %, 27.5 %, 22.5 %, and 17.8 %, respectively.

DISCUSSION

Our phylogenetic analyses suggest that the circumscription of *Cylindrocarpon* must be restricted to anamorphs within the *Neon. radicicola* complex, the anamorph of *Neon. macrodidyma*, and an assemblage of species including *C. cylindroides* (the type species of *Cylindrocarpon*), *C. magnusianum*, *C. obtusisporum*, *C. willkommii*, *C. heteronema*, *C. candidum*, *C. album*, and *C. faginatum*, as well as the anamorphs of *Neon. coccinea*, "*Nectria*" *fuckeliana*, *Neon. galligena*, and *Neon. macroconidialis*, plus some additional species not included in these analyses. The connection of *Neonectria* as the holomorphic genus corresponding to species related to *Cylindrocarpon sensu stricto* is based on the work of Wollenweber (1928), who identified *C. magnusianum* as the anamorph of *Neon. ramulariae*, the type species of *Neonectria*. Among the species of *Neonectria*/*Cylindrocarpon*, "*Nectria*" *fuckeliana* and *Neon. macroconidialis* deviated most strongly in sequence from the other included species.

The analyses exclude *Campylocarpon* species and members of the former "*Nectria*" *mammoidea* group (Booth 1959, Samuels & Brayford 1993) from *Neonectria/Cylindrocarpon*, contradicting the recent transfer of the latter group to *Neonectria* (Brayford *et al*. 2004). Because these species are phylogenetically not closely related to *Neonectria*/*Cylindrocarpon*, the generic designation of the "*Neon*." *mammoidea* group has been rendered in quotation marks throughout this paper.

The perithecial wall of species within the *Neon. radicicola* complex, as well as that of *Neon. macrodidyma*, consists mainly of angular to subglobose cells (Samuels & Brayford 1990; this study). This anatomy differs from that of the perithecial wall of teleomorphs within the "*Neon*." *mammoidea* group; walls of this group are characterised by a region of elongate cells perpendicularly oriented to the surface of the perithecial wall (Samuels & Brayford 1993, Brayford *et al*. 2004). However, a similar perithecial wall region has also been described for *Neon. ramulariae* (Rossman *et al*. 1999) and "*Nectria*" *fuckeliana* (Brayford *et al*. 2004). As the former species is, as mentioned previously, the type species of *Neonectria*, this deprives this character of significance at the generic level.

Species here accepted for *Neonectria*/*Cylindrocarpon* represent Booth's groups 1, 3, and 4 (Booth 1966). They feature cylindrical to slightly curved, multi-septate macroconidia but only some of them form microconidia or chlamydospores. Microconidia and chlamydospores therefore appear not to be important in the distinction of *Neonectria*/*Cylindrocarpon* from related taxa. The excluded taxa consist of some members of Booth's group 4 such as *C. olidum* (Wollenw.) Wollenw., which clusters among members of the "*Neon*." *mammoidea* group (Fig. 3), most taxa of Booth's group 2, which includes anamorphs of species of the "*Neon*." *mammoidea* group, and the two *Campylocarpon* species. These species are characterised by typically curved, rarely straight macroconidia, and they lack microconidia. Although chlamydospores have not been described in Booth's group 2 (Booth 1966, Brayford *et al*. 2004), they are known to be formed in one species of *Campylocarpon* and in *C. olidum* (Booth 1966). Monophyly of *Campylocarpon* and the "*Neon*." *mammoidea* group is supported in ITS and partial β-tubulin analysis. However, there is considerable genetic variation within the "*Neon*." *mammoidea* group, giving rise to long terminal and subterminal branches for species such as *C. olidum* var. *crassum* (CBS 216.67), "*Neon*." *trachosa* (CBS 112467), "*Neon*." *lucida* (CBS 112456), and *C. ianthothele* var. *majus* (CBS 328.81). *Campylocarpon* species, though similar in macroconidial morphology to members of the "*Neon*." *mammoidea* complex, can be distinguished by the formation of typically brownish rather than violaceous cultures, as well as by production of brownish hyphae, often in strands, and, in *Campyl. pseudofasciculare*, by formation of chlamydospores.

To address their strong molecular as well as morphological differences from anamorphs classified in *Cylindrocarpon*, a new genus, *Campylocarpon*, is proposed for *Campyl. fasciculare* and *Campyl. pseudofasciculare*. The fasciculate conidiophore aggregates formed by *Campyl. fasciculare* have also been described for *C. phaeodiscum* Samuels & Brayford (Samuels & Brayford 1993), the anamorph of "*Neon*." *phaeodisca*, but not for any other *Cylindrocarpon* species. These two species differ in their

colony pigment and in the length of their macroconidia. *Cylindrocarpon phaeodiscum* is phylogenetically closely related to "*Neon.*" *discophora*, "*Neon.*" *lucida*, and other species of the "*Neon*." *mammoidea* group (Brayford *et al*. 2004), and therefore could also be related to *Campylocarpon*, as can be inferred from ITS and β-tubulin analyses. It is possible that *Campylocarpon* and members of the "*Neon*." *mammoidea* group should be considered congeneric. The conidiophores of *Campyl. fasciculare* are similar to those of *C. phaeodiscum*, and *Campylocarpon* macroconidia resemble those formed by anamorphs of the "*Neon*." *mammoidea* group (Brayford *et al*. 2004). In addition no microconidia are formed in either of these groups. *Campylocarpon fasciculare* and *Campyl. pseudofasciculare* are similar in forming conidiophores from sometimes brownish mycelial strands, as well as curved, mostly 3–5-septate macroconidia, and brownish pigmented cultures. Because they are phylogenetically closely related (Figs 2, 3, 5, 6), we initially expected also to find conidiophore fascicles in *Campyl. pseudofasciculare*, but instead, only separate, mostly branched conidiophores (Fig. 11b–d) were observed. *Campylocarpon pseudofasciculare*, however, sporulated poorly in cultures, and the absence of fascicles might have been an artifact of suboptimal culture conditions.

Ostensible *C. destructans* isolates from diseased grapevines on different continents were found to contain an additional, readily phylogenetically distinguished member of the *Neon. radicicola* complex *sensu* Seifert *et al*. (2003b). No morphological character was found to segregate these grapevine isolates from *C. destructans* as described by Samuels & Brayford (1990). This cryptic taxon is commonly associated with the typical black foot symptoms as described by Larignon (1999), and particularly affects young grapevines and frequently causes decline during the first year after planting. While this species seems to be particularly aggressive to grapevines and has mainly been isolated from that source, it is interesting that one strain originated from a *Cyclamen* sp. in the Netherlands.

Neonectria macrodidyma, though forming conidiophores like those of *C. destructans*, forms relatively fewer chlamydospores. Also, *Neon. macrodidyma* macroconidia have a slightly bent apical cell, while the corresponding cells in *C. destructans* are rather broadly rounded (Samuels & Brayford 1990). Slightly bent apical cells have also been described particularly for *C. didymum* (Domsch *et al*. 1980), but this species has 0–2-septate conidia that are smaller than those of *Neon. macrodidyma*. Similar conidia have been illustrated for a *C. obtusisporum* isolate from a *Tilia* branch (Wollenweber 1916, no. 465). Macroconidia of this isolate, however, were 20–40 × 3.5–5.75 µm, while those of *Neon. macrodidyma* appeared wider, measuring (26–)34–36–38(–45) × (4–)5.5–6–6.5(–8) µm. The shape of the macroconidia also distinguishes *Neon. macrodidyma* from the type of *C. obtusisporum*, macroconidia of which were described by Cooke (1884) as obtuse at both ends and measuring 30–35 × 4–5 µm. Booth (1966) described macroconidia of similar shape in *C. obtusisporum*. According to Booth, however, 2–3-septate macroconidia of C. *obtusisporum* measure 34–50 × 6–7.5 µm. *Cylindrocarpon obtusisporum* has been identified as the cause of black foot disease on grapevines (Grasso & Magnano di San Lio 1975, Scheck *et al*. 1998a) and on some other hosts (Booth 1966). It is not known if these *C. obtusisporum* were correctly identified or if they were actually *Neon. macrodidyma* isolates.

Isolates of *Neon. macrodidyma* were derived from asymptomatic grapevines, and from vines displaying a range of decline symptoms, including typical black foot symptoms. The fact that many of these isolates were derived from nursery plants may be of great concern to the South African grapevine propagation industry, since a previous study (Halleen *et al*. 2003) has already shown that these infections occur in nurseries. Isolates of *Campylocarpon* were mostly derived from asymptomatic nursery plants, although they were also obtained from some plants with decline and black foot symptoms. A significant conclusion of our investigations is that black foot symptoms are caused by a cryptic species in the *C. destructans* complex, as well as *Neon. macrodidyma*, and possibly some *Campylocarpon* strains. This information might be useful in the improvement of control methods, since the fungi involved may differ in their susceptibility to selective fungicides as well as in epidemiology. Studies are currently underway to investigate fungicide responses among these isolates. The present study also impacts on the development of molecular detection strategies, since most of the primers currently used would not detect the two species of *Campylocarpon*.

Most of the isolates from grapevines included in this study were derived from diseased plants brought in by individual farmers or consultants, and therefore we make no claim to have adequately documented the geographic distribution of these taxa. However, it was noted that the South African strains of the *C. destructans* genotype that were aggressively pathogenic to grapevines originated from relatively warm and dry viticultural areas, which normally have relatively dry soils. This pattern of occurrence might be explained by the use in such areas of a drip-irrigation practice whereby the roots and the belowground region of the stem are kept moist for long periods, creating conditions favourable for disease development.

Most South African nurseries are confined within a few limited areas where conditions are suitable, and the same soils have been used for many years. These "old" soils are hardly ever replaced by fresh soil, and fumigation practices are not a viable option. Standard

nursery practice includes a 2-year rotation system, whereby cuttings are planted every second year and alternated with a cover crop. This might also favour the build-up of soilborne pathogens including the pathogens studied here. Future research needs to be focused on the distribution of these taxa in local nurseries, with an eye to establishing the extent to which cultivation practices such as rotation systems and cover crops, play a role in disease prevalence. The susceptibility of different grapevine rootstocks towards the four taxa also needs to be investigated.

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