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RESEARCH PAPER

Lineages in *Nectriaceae*: re-evaluating the generic status of *Ilyonectria* and allied genera

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Summary. Genera with cylindrocarpon-like asexual morphs are important pathogens of various herbaceous and woody plant hosts globally. Recent multi-gene studies of this generic complex indicated that the genus *Ilyonectria* is paraphyletic. The present study was therefore initiated to re-evaluate the generic status of *Ilyonectria* and at the same time address some taxonomic irregularities in the genera *Cylindrodendrum* and *Neonectria*. Using multi-gene DNA data and morphological comparisons, the genus *Dactylonectria* is introduced with 10 new combinations, several of which were previously treated in *Ilyonectria*. Two new species, *D. hordeicola* and *D. pinicola*, are also described. Furthermore, one new combination is provided in the genus *Cylindrodendrum*, and three new combinations in the genus *Neonectria*, for species previously treated in the genera *Acremonium*, *Cylindrocarpon*, *Nectria* and *Neonectria*. The aquatic genus *Heliscus* is reduced to synonymy under *Neonectria*.

Key words: *Cylindrocarpon*, *Ilyonectria*, *Neonectria*, nomenclature, taxonomy.

Introduction

Genera with cylindrocarpon-like asexual morphs are cosmopolitan fungi and represent important pathogens associated with cankers, root rots and black foot disease of various woody plant hosts (Samuels and Brayford, 1994; Hirooka *et al*., 2005; Kobayashi *et al*., 2005; Castlebury *et al*., 2006; Halleen *et al*., 2006; Chaverri *et al*., 2011; Cabral *et al*., 2012b; Lombard *et al*., 2013; Salgado-Salazar *et al*., 2013; Aiello *et al*., 2014). Prior to the abolishment of dual nomenclature for fungi (Hawksworth *et al*., 2011; McNeill *et al*., 2012), the asexual genus *Cylindrocarpon* was linked to the sexual genus *Neonectria*. Booth (1966) informally classified the genus *Cylindrocarpon* into four groups based on the absence and/or presence of microconidia and chlamydospores. Additionally, the genus *Neonectria* was also informally divided into five groups based on perithecial morphology (Booth, 1959; Brayford and Samuels, 1993; Samuels and Brayford, 1994). However, Mantiri *et al*. (2001) reduced this informal division to three groups based on phylogenetic inference of mitrochondrial small subunit rDNA sequences.

Although several studies (Mantiri *et al*., 2001; Brayford *et al*., 2004; Halleen *et al*., 2004, 2006; Hirooka *et al*., 2005; Castlebury *et al*., 2006) indicated that the genus *Neonectria* and its *Cylindrocarpon* asexual morphs could represent a generic complex, they refrained from describing genera in the complex at the time. The introduction of the asexual morph genus *Campylocarpon* by Halleen *et al*. (2004), based on *C. fasciculare*, represented the first formal segregation from the genus *Cylindrocarpon*. This genus had no sexual morph, having 3–5-septate, curved macroconidia, chlamydo-

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spores, and lacking microconidia. Following this, Chaverri *et al.* (2011) were able to identify a further three new genera, namely *Ilyonectria*, *Rugonectria* and *Thelonectria*, within *Neonectria*/*Cylindrocarpon*, based on multigene phylogenetic analyses, morphological comparisons and ecological characters.

The genus *Ilyonectria*, with *I. radicicola* as type species, was introduced to accommodate *Neonectria* species belonging to the "*N. radicicola*" group (Booth, 1959) having asexual morphs and belonging to Booth's Group 3 (chlamydospores and microconidia present, Booth, 1966; Chaverri *et al*., 2011). Members of this genus are characterised by red, globose to subglobose perithecia having scaly or slightly warted perithecial walls, and producing ellipsoidal, 1-septate ascospores. The cylindrocarpon-like asexual morphs produce abundant ellipsoidal to ovoid, 0–1-septate microconidia; intercalary, globose chlamydospores and almost straight, 1–3-septate macroconidia (Chaverri *et al*., 2011). *Rugonectria*, a sexual genus typified with *R. rugulosa*, includes members of the "*N. rugulosa*" group (Samuels and Brayford, 1994) with asexual morphs belonging to Booth's Group 4 (lacking chlamydospores, Booth, 1966). They are characterised by orange to red, globose to subglobose perithecia with warted perithecial walls producing ellipsoidal to oblong, striate 1-septate ascospores. The cylindrocarpon-like asexual morphs produce ovoid to cylindrical, aseptate to 1-septate microconidia and curved, fusiform 3–9-septate macroconidia but lack chlamydospores (Chaverri *et al*., 2011). The genus *Thelonectria*, based on *T. discophora*, was established to include members of the "*N. mammoidea*" (Booth, 1959) and "*N. veuillotiana*" groups (Brayford and Samuels, 1993) with their cylindrocarpon-like asexual morphs belonging to Booth's Group 2 (microconidia and chlamydospores lacking, Booth, 1966). Members are characterised by orange to red, globose, subglobose, or pyriform to elongated perithecia with prominent, often darkened papilla and a smooth to warted perithecial wall, producing fusiform, 1-septate, warted or smooth-walled ascospores. The asexual morphs rarely produce microconidia and chlamydospores and have macroconidia that are curved and 3–9-septate (Chaverri *et al*., 2011). Chaverri *et al.* (2011) defined the genus *Neonectria* as having red, subglobose to broadly obpyriform perithecia with smooth to scurfy perithecial walls producing ellipsoidal, 1-septate ascospores. The cylindrocarpon-like asexual morphs produce either ellipsoidal to oblong 0–1-septate microconidia and sometimes globose to subglobose chlamydospores, and straight, sometimes slightly curved, cylindrical 3–9-septate macroconidia.

Recent molecular phylogenetic studies revealed that the genus *Ilyonectria* is paraphyletic (Cabral *et al*., 2012a,c; Lombard *et al*., 2013). Thus, the aim of the present study was to use multi-gene phylogeny and morphological comparisons to re-evaluate genera with cylindrocarpon-like asexual morphs, and at the same time address the paraphyletic nature of *Ilyonectria*.

Materials and methods

Isolates

Fungal strains were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands and the working collection of Pedro W. Crous (CPC) housed at the CBS (Table 1).

DNA isolation, amplification and analyses

Total genomic DNA was extracted from 7-d-old single-conidial cultures growing on 2% (w/v) potato dextrose agar (PDA) using the method of Damm *et al*. (2008). Partial gene sequences were determined for the β-tubulin gene (*tub2*), the internal transcribed spacer region with intervening 5.8S nrDNA (ITS), and the translation elongation factor 1-alpha gene (*tef1*) using the primers and protocols described by Cabral *et al.* (2012a,b). Partial 28S nrRNA gene (LSU) sequences were generated as described by Lombard *et al.* (2010). Integrity of the sequences was ensured by sequencing the amplicons in both directions using the same primer pairs used for amplification. Consensus sequences were assembled in MEGA v. 6 (Tamura *et al*., 2013), and then compared and added to representative sequences from Cabral *et al.* (2012a, b) and Lombard *et al.* (2013) (Table 1). Subsequent alignments for each locus were generated in MAFFT v. 7 (Katoh and Standley, 2013) and manually corrected where necessary. Phylogenetic congruency of the four loci was tested using a 70% reciprocal bootstrap criterion (Mason-Gamer and Kellogg, 1996).

Phylogenetic analyses were based on Bayesian inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). For both BI and ML, the evolutionary model for each partition was de-

Table 1. Strains investigated in this study.

Species	Isolate code [a]	Substrate	Locality	Collector	GenBank Accession No. [b]			
					ITS	LSU	*tub2*	*tef1*
Campylocarpon fasciculare	CBS 112613[T]; CPC 3970	*Vitis vinifera*	South Africa	F. Halleen	AY677301	HM364313	AY677221	JF735691
Cylindrodendrum album	CBS 301.83[T]; ATCC 46842; IMI 255534	*Fucus distichus*	Canada	R.C. Summerbell	KM231764	KM231626	KM232021	KM231889
	CBS 110655	Soil	The Netherlands	F.X. Prenafeta-Boldú	KM231765	KM231627	KM232022	KM231890
C. hubeiense	CBS 129.97	*Viscum album*	France	W. Gams	KM231766	KM231628	KM232023	KM231891
	CBS 124071[T]; HMAS 98331	*Rhododendron* sp.	China	W.P. Wu, W.Y. Zhuang, Y. Nong	FJ560439	FJ560434	FJ860056	HM054090
Dactylonectria alcacerensis	CBS 129087[T]; Cy159	*Vitis vinifera*	Portugal	A. Cabral, H. Oliveira	JF735333	KM231629	AM419111	JF735819
	Cy134	*Vitis vinifera*	Spain	J. Armengol	JF735332	–	AM419104	JF735818
D. anthuriicola	CBS 564.95[T]; PD95/1577	*Anthurium* sp.	The Netherlands	R. Pieters	JF735302	*KM515897*	JF735430	JF735768
D. estremocensis	CBS 129085[T]; Cy145	*Vitis vinifera*	Portugal	C. Rego, T. Nascimento	JF735320	KM231630	JF735448	JF735806
	CPC 13539; CCFC226730; 94-1685	*Picea glauca*	Canada	R.C. Hamelin	JF735330	–	JF735458	JF735816
D. hordeicola	CBS 162.89[T]	*Hordeum vulgare*	The Netherlands	M. Barth	AM419060	*KM515898*	AM419084	JF735799
D. macrodidyma	CBS 112601; CPC 3983	*Vitis vinifera*	South Africa	F. Halleen	AY677284	*KM515899*	AY677229	JF735833
	CBS 112615[T]; CPC 3976	*Vitis vinifera*	South Africa	F. Halleen	AY677290	*KM515900*	AY677233	JF735836
D. novozelandica	CBS 112608; CPC 3987	*Vitis vinifera*	South Africa	F. Halleen	AY677288	*KM515901*	AY677235	JF735821
	CBS 113552[T]; CPC 5713; HJS-1306; NZ C 41	*Vitis* sp.	New Zealand	R. Bonfiglioli	JF735334	–	AY677237	JF735822
D. pauciseptata	CBS 100819; LYN 16202/2	*Erica melanthera*	New Zealand	H.M. Dance	EF607090	*KM515902*	EF607067	JF735771
	CBS 120171[T]; KIS 10467	*Vitis* sp.	Slovenia	M. Žerjav	EF607089	*KM515903*	EF607066	JF735776
D. pinicola	CBS 159.34; IMI 113891; MUCL 4084; VKM F-2656	–	Germany	H.W. Wollenweber	JF735318	*KM515904*	JF735446	JF735802
	CBS 173.37[T]; IMI 090176	*Pinus laricio*	UK: England	T.R. Peace	JF735319	*KM515905*	JF735447	JF735803

(Continued)

Table 1. (Continued).

Species	Isolate code [a]	Substrate	Locality	Collector	GenBank Accession No. [b]			
					ITS	LSU	*tub2*	*tef1*
D. torresensis	CBS 119.41	*Fragaria* sp.	The Netherlands	H.C. Koning	JF735349	*KM515906*	JF735478	JF735846
	CBS 129086[T]; Cy218	*Vitis vinifera*	Portugal	A. Cabral	JF735362	KM231631	JF735492	JF735870
D. vitis	CBS 129082[T]; Cy233	*Vitis vinifera*	Portugal	C. Rego	JF735303	*KM515907*	JF735431	JF735769
Ilyonectria capensis	CBS 132815[T]; CPC 20695	*Protea* sp.	South Africa	C.M. Bezuidenhout	JX231151	*KM515908*	JX231103	JX231119
	CBS 132816; CPC 20700	*Protea* sp.	South Africa	C.M. Bezuidenhout	JX231160	*KM515909*	JX231112	JX231128
I. coprosmae	CBS 119606; GJS 85-39	*Metrosideros* sp.	Canada	G.J. Samuels	JF735260	*KM515910*	JF735373	JF735694
I. crassa	CBS 158.31; IMI 061536; NRRL 6149	*Narcissus* sp.	The Netherlands	F.H. Feekes	JF735276	*KM515911*	JF735394	JF735724
	CBS 129083; NSAC-SH-1	*Panax quinquefolium*	Canada	S. Hong	AY295311	*KM515912*	JF735395	JF735725
I. cyclaminicola	CBS 302.93	*Cyclamen* sp.	The Netherlands	M. Hooftman	JF735304	*KM515913*	JF735432	JF735770
I. europaea	CBS 537.92	*Aesculus hippocastanum*	Belgium	V. Demoulin	EF607079	*KM515914*	EF607064	JF735757
	CBS 129078; Cy241	*Vitis vinifera*	Portugal	C. Rego	JF735294	*KM515915*	JF735421	JF735756
I. gamsii	CBS 940.97	Soil	The Netherlands	J.T. Poll	AM419065	*KM515916*	AM419089	JF735766
I. leucospermi	CBS 132809[T]; CPC 20701	*Leucospermum* sp.	South Africa	C.M. Bezuidenhout	JX231161	*KM515917*	JX231113	JX231129
	CBS 132810; CPC 20703	*Protea* sp.	South Africa	C.M. Bezuidenhout	JX231162	*KM515918*	JX231114	JX231130
I. liliigena	CBS 189.49[T]; IMI 113882	*Lilium regale*	The Netherlands	M.A.A. Schippers	JF735297	*KM515919*	JF735425	JF735762
	CBS 732.74	*Lilium* sp.	The Netherlands	G.J. Bollen	JF735298	*KM515920*	JF735426	JF735763
I. liriodendri	CBS 110.81[T]; IMI 303645	*Liriodendrum tulipifera*	USA	J.D. MacDonald, E.E. Butler	DQ178163	*KM515921*	DQ178170	JF735696
	CBS 117526; Cy68	*Vitis vinifera*	Portugal	C. Rego	DQ178164	*KM515922*	DQ178171	JF735697

(Continued)

Table 1. (Continued).

Species	Isolate code [a]	Substrate	Locality	Collector	GenBank Accession No. [b]			
					ITS	LSU	*tub2*	*tef1*
I. lusitanica	CBS 129080[T]; Cy197	*Vitis vinifera*	Portugal	N. Cruz	JF735296	*KM515923*	JF735423	JF735759
I. mors-panacis	CBS 306.35[T]	*Panax quinquefolium*	Canada	A.A. Hildebrand	JF735288	–	JF735414	JF735746
	CBS 124662; NBRC 31881; SUF 811	*Panax ginseng*	Japan	Y. Myazawa	JF735290	*KM515924*	JF735416	JF735748
I. palmarum	CBS 135753; CPC 22088	*Howea forsteriana*	Italy	G. Polizzi	HF937432	–	HF922609	HF922615
	CBS 135754[T]; CPC 22087	*Howea forsteriana*	Italy	G. Polizzi	HF937431	–	HF922608	HF922614
I. panacis	CBS 129079[T]; CDC-N-9a	*Panax quinquefolium*	Canada	K.F. Chang	AY295316	*KM515925*	JF735424	JF735761
I. pseudodestructans	CBS 117824; IFFF98	*Quercus* sp.	Austria	E. Halmschlager	JF735292	–	JF735419	JF735751
	CBS 129081; Cy20	*Vitis vinifera*	Portugal	C. Rego	AJ875330	*KM515926*	AM419091	JF735752
I. radicicola	CBS 264.65[T]	*Cyclamen persicum*	Sweden	L. Nilsson	AY677273	*KM515927*	AY677256	JF735695
I. robusta	CBS 308.35[T]	*Panax quinquefolium*	Canada	A.A. Hildebrand	JF735264	*KM515928*	JF735377	JF735707
	CBS 129084; Cy192	*Vitis vinifera*	Portugal	N. Cruz	JF735273	*KM515929*	JF735391	JF735721
I. rufa	CBS 153.37[T]	Sand dune	France	F. Moreau	AY677271	*KM515930*	AY677251	JF735729
	CBS 640.77	*Abies alba*	France	F. Gourbière	JF735277	*KM515931*	JF735399	JF735731
I. venezuelensis	CBS 102032; ATCC 208837; AR2553	Bark	Venezuela	A.Y. Rossman	AM419059	*KM515932*	AY677255	JF735760
Neonectria coccinea	CBS 119156; AR 3700	*Fagus sylvatica*	Slovakia	A.Y. Rossman	–	KC660579	KC660726	KC660437
	CBS 119158; GJS 98-114	*Fagus sylvatica*	Germany	G.J. Samuels	JF268759	KC660620	KC660727	JF268734
N. confusa	CBS 127484; HMAS 99198	Twig	China	W.Y. Zhuang	*KM515889*	*KM515933*	*KM515886*	–
	CBS 127485[T]; HMAS 99197	Twig	China	W.Y. Zhuang, Y. Nong	FJ560437	*KM515934*	FJ860054	–
N. ditissima	CBS 100316	*Malus domestica* cv. Bramley	Ireland	A. McCracken	*KM515890*	*KM515935*	DQ789858	*KM515944*
	CBS 100318	*Malus domestica* cv. Bramley	Ireland	A. McCracken	KM515891	KM515936	KM515887	KM515945

(Continued)

Table 1. (Continued).

Species	Isolate code [a]	Substrate	Locality	Collector	GenBank Accession No. [b]			
					ITS	LSU	*tub2*	*tef1*
N. faginata	CBS 217.67[T]; ATCC 16547; IMI 105738	*Cryptococcus fagi* nymph on *Fagus grandifolia*	Canada	G.L. Stone	HQ840385	HQ840382	JF268730	JF268746
	CBS 119160; GJS 04-159	*Fagus grandifolia*	USA	G.J. Samuels, R. Baird	HQ840384	HQ840383	DQ789883	DQ789740
N. fuckeliana	CBS 239.29; IMI 039700	*Picea sitchensis*	UK: Scotland	H.W. Wollenweber	HQ840386	HQ840377	DQ789871	JF268748
	CBS 119200; AR 4110; WJ 2652	*Picea abies*	Austria	W. Jaklitsch	HQ840387	HQ840381	JF268731	JF268706
N. hederae	CBS 714.97; PD 97/1932	*Hedera helix*	The Netherlands	J.W. Veenbaas-Rijks	–	KC660616	DQ789878	KC660461
	IMI 058770a[T]; ATCC 16543	*Hedera helix*	UK: England	–	–	KC660617	DQ789895	DQ789752
N. lugdunensis	CBS 222.84	Soil	The Netherlands	–	*KM515892*	*KM515937*	–	–
	CBS 250.58[T]	*Ilex aquifolium*	UK	J. Webster	*KM515893*	*KM515938*	–	–
	CBS 251.58	*Ilex aquifolium*	UK	J. Webster	*KM515894*	*KM515939*	–	–
	CBS 270.53	–	France	F. Moreau	*KM515895*	*KM515940*	–	–
	CBS 125485; DAOM 235831; TG 2008-07	*Populus fremontii*	USA	T. Gräfenhan	KM231762	KM231625	KM232019	KM231887
	CBS 127475; HMAS 173254	Twig	China	X.M. Zhang	KM515896	KM515941	KM515888	KM515946
N. major	CBS 240.29[T]; IMI 113909	*Alnus incana*	Norway	H.W. Wollenweber	JF735308	*KM515942*	DQ789872	JF735782
N. neomacrospora	CBS 198.62; BBA 9628; IMI 113890	*Abies concolor*	–	W. Gerlach	AJ009255	HM364316	HM352865	HM364351
	CBS 324.61; DSM 62489; IMB 9628	*Abies concolor*	The Netherlands	J.A. von Arx	JF735312	HM364318	DQ789875	HM364335
N. obtusispora	CBS 183.36; IMI 113895	*Solanum tuberosum*	Germany	H.W. Wollenweber	AM419061	*KM515943*	AM419085	JF735796
	CPC 13544; DAOM 182772; JAT 1366	*Prunus armenica*	Canada	J.A. Traquair	AY295306	–	JF735443	JF735797

(Continued)

Table 1. (Continued).

Species	Isolate code [a]	Substrate	Locality	Collector	GenBank Accession No. [b]			
					ITS	LSU	*tub2*	*tef1*
N. punicea	CBS 242.29	*Rhamnus* sp.	Germany	H.W. Wollenweber	KC660522	KC660565	DQ789873	DQ789730
	CBS 119724; AR 3102; WJ 1383	*Frangula alnus*	Austria	W. Jaklitsch	KC660496	KC660568	DQ789824	KC660431
N. ramulariae	CBS 151.29; IMI 113894; MUCL 28094	*Malus sylvestris*	UK: England	H.W. Wollenweber	JF735313	HM042436	JF735438	JF735791
	CBS 182.36; IMI 113893; UPSC 1903	*Malus sylvestris*	–	H.W. Wollenweber	JF735314	HM042435	JF735439	JF735792
N. tsugae	CBS 788.69[T]	*Tsuga heterophylla*	Canada	J.E. Bier	KM231763	HQ232146	KM232020	DQ789720

[a] AR: Collection of A.Y. Rossman; ATCC: American Type Culture Collection, U.S.A.; BBA: Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CCFC: Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; CPC: Collection of P.W. Crous, housed at CBS; Cy: *Cylindrocarpon* collection housed at Laboratório de Patologia Vegetal "Veríssimo de Almeida" - ISA, Lisbon, Portugal; DAOM: Agriculture and Agri-Food Canada National Mycological Herbarium, Canada; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; GJS: Collection of G.J. Samuels; HJS: Collection of H.-J. Schroers; HMAS: Mycological Herbarium, Institute of Microbiology, Chinese Academy of Science; IFFF: Institute of Forest Entomology, Forest Pathology and Forest Protection, Austria; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; JAT: collection of J.A. Traquair; KIS: Agricultural Institute of Slovenia, Ljubljana, Slovenia; LYN: Lynchburg College, Biology Department, USA; MUCL: Mycothèque de l'Université Catholique de Louvain, Belgium; NBRC: NITE Biological Resource Center, Japan; NRRL: Agricultural Research Service Culture Collection, USA; NZ: Collection of L. Castlebury. PD: Collection of the Dutch National Plant Protection Organization (NPPO-NL), Wageningen, The Netherlands; UPSC: Uppsala University Culture Collection of Fungi, Botanical Museum University of Uppsala, Uppsala, Sweden; VKM: All-Russian Collection of Microorganisms, Russia; WJ: Collection of W. Jaklitsch.

[b] ITS: internal transcribed spacer; LSU: 28S large subunite; *tub2*: beta-tubulin; *tef1*: translation elongation factor 1-alpha.

[T] Ex-type cultures.

termined using MrModeltest (Nylander, 2004) and incorporated into the analyses. For the BI analysis, MrBayes v. 3.1.1 (Ronquist and Huelsenbeck, 2003) was used to generate phylogenetic trees under the optimal model per partition. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was started in parallel from a random tree topology with the heating parameter set to 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies decreased below 0.01 with trees saved each 1,000 generations. The first 25% of saved trees were discarded as the "burn-in" phase and posterior probabilities determined from the remaining trees.

The ML analysis was done using RAxML (randomised axelerated (sic) maximum likelihood for high performance computing, Stamatakis *et al*., 2005, 2008) through the CIPRES website (http://www.phylo.org) to obtain a second measure of branch support. The robustness of the analysis was evaluated by bootstrap support (BS) analysis with the bootstrap replicates automatically determined by the software.

The MP analysis was done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10, Swofford, 2003) with phylogenetic relationships estimated by heuristic searches with 1,000 random sequence-additions. Tree bisection-reconnection was implemented, with the branch swapping option set on "best tree" only. All characters were weighted equally and alignment gaps were treated as "fifth state". Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC). The bootstrap support analysis was based on 1,000 replications. Novel sequences generated in this study were deposited in GenBank (Table 1) and the alignments and phylogenetic tree in TreeBASE (S16355).

Presently sterile isolates (see Taxonomy section below) were characterised using unique fixed single nucleotide polymorphisms (SNP's). For each sterile isolate treated, the closest phylogenetic neighbour was selected and subjected to SNP analysis using MEGA v. 6.

Morphology

Axenic cultures were grown on synthetic nutrient-poor agar (SNA, Nirenberg, 1981) with two 1-cm^2 sterile filter paper pieces and potato-dextrose agar (PDA) as described by Cabral *et al.* (2012a). For known aquatic isolates, 5–10 mL sterile water was poured onto plates prior to incubation. Inoculated plates were incubated at room temperature (22–25 °C) under ambient light conditions and examined after 1–3 wks. Observations were made with a Zeiss Axioscope 2 microscope with interference contrast (DIC) illumination. Morphological descriptions and taxonomic novelties and metadata were deposited in MycoBank (http://www.MycoBank.org; Crous *et al.*, 2004).

Results

Phylogeny

The 70% reciprocal bootstrap tree topologies showed no conflicts for the *tub2*, ITS and *tef1* gene regions. However, the LSU gene region revealed a conflicting tree topology (insufficiently resolved the *Neonectria* clade as a basal polytomy) compared to the other three gene regions, which was ignored based on the argument of Cunningham, (1997) that combining incongruent partitions could increase phylogenetic accuracy. Therefore, the four gene regions were combined.

The combined alignment of ITS, LSU, *tub2* and *tef1* used for BI, ML and MP analyses contained 2,463 characters from 79 taxa (including outgroup). The number of unique site patterns per data partition, including alignment gaps, was 145 from 513 characters for ITS, 72 from 830 characters for LSU, 264 from 552 characters for *tub2*, and 358 from 568 characters for *tef1*. MrModeltest revealed that all four partitions had dirichlet base frequencies. A GTR+I+G model with inverse gamma-distributed rates was used for ITS, LSU and *tef1* while HKY+I+G with inverse gamma-distributed rates was implemented for *tub2*. The Bayesian analysis lasted 440,000 generations, and the consensus tree, with posterior probabilities, was calculated from 662 trees left after 220 trees were discarded as the burn-in phase. For the MP and ML analyses, the combined alignment consisted of 716 parsimony-informative, 1,529 constant, and 218 parsimony-uninformative characters. MP analysis yielded 15,062 equally most parsimonious trees (TL = 2,667; CI = 0.540; RI = 0.876; RC = 0.473) and a single best ML tree with –lnL = -13910.342139. The Bayesian consensus tree confirmed the tree topologies obtained from the ML and MP analyses, and therefore only the Bayesian consensus tree is presented.

In the phylogenetic tree (Figure 1), strains of the genera *Cylindrodendrum*, *Ilyonectria*, the new *Dactylonectria* and *Neonectria* formed four well-supported clades. The *Neonectria* clade (ML-bootstrap (ML-BS) and MP-bootstrap (MP-BS) = 100; posterior probability (PP) < 0.95) incorporated the ex-type of *Acremonium tsugae* (CBS 788.69) as well as representatives of *Heliscus lugdunensis* (as *N. lugdunensis* in the tree; CBS 250.58, CBS 251.58, CBS 222.84, CBS 270.53, CBS 125485) which included the ex-type of *N. shennongjiana* (CBS 127475). Two isolates (CBS 183.36 and CPC 13544) so far known as *Cylindrocarpon obtusisporum* formed a basal sister clade to *N. lugdunensis* in the *Neonectria* clade. The *Neonectria* clade also includes strains of the generic type species, *N. ramulariae* (CBS 151.29 and CBS 182.36, authentic for *Cylindorcarpon magnusianum*, now *C. obtusiusculum*). Strains of *Ilyonectria* clustered into two separate well-supported clades, indicating that this genus is paraphyletic. The first *Ilyonectria* clade (ML-BS and MP-BS = 100; PP = 1.0), which includes *I. macrodidyma* (ex-type CBS 112615), incorporates several important pathogens of grapevine (*Vitis vinifera*) from various localities (Halleen *et al*., 2004; Cabral *et al*., 2012b,c) and is introduced as a new genus, *Dactylonectria*, below. The second *Ilyonectria* clade (ML-BS and MP-BS = 100; PP = 1.0), includes the type species of the genus, *I. radicicola* (ex-type CBS 264.65). Strains of the monotypic genus *Cylindrodendrum* formed the fourth well-supported clade (ML-BS and MP-BS = 100; PP = 1.0), nested between *Dactylonectria* and *Ilyonectria*. In this clade, two smaller clades could be resolved, one of which (ML-BS and MP-BS = 100; PP = 1.0) represents the generic type species, *C. album* (CBS 301.83 and CBS 110655). The other smaller clade (ML-BS and MP-BS = 100; PP = 1.0) included the ex-type of *Neonectria hubeiensis* (CBS 124071), which is combined into *Cylindrodendrum* below.

Taxonomy

Based on phylogenetic inference in this study, the classification and nomenclature of some members in the genera *Cylindrodendrum*, *Ilyonectria* and *Neonectria* are re-considered. To address the paraphyletic nature of *Ilyonectria*, a new genus, *Dactylonectria*, is introduced here, with associated new combinations. Furthermore, two new species in the genus *Dactylonectria*, which are sterile, are described here based on DNA sequence data, following the approach of Gomes *et al.* (2013) and Lombard *et al.* (2014).

Cylindrodendrum hubeiense (W.Y. Zhuang, Y. Nong & J. Luo) L. Lombard & Crous, **comb. nov.**
MycoBank MB810141
(Figure 2)

Basionym: *Neonectria hubeiensis* W.Y. Zhuang, Y. Nong & J. Luo, *Fungal Diversity* 24, 351 (2007).

Material examined: **France**: Dép. Jura, Châtelneuf near St. Laurent, on *Viscum album*, 26 Sept. 1996, *W. Gams* (CBS H-5723; culture CBS 129.97, previously as *C. album*). **China**: Hubei, Wufeng County, Houhe Nature reserve, on fruit of *Rhododendron* sp., 13 Sept. 2004, *W.P. Wu, W.Y. Zhuang & Y. Nong* (CBS 124071 = HMAS 98331).

Notes: Zhuang *et al.* (2007) introduced this new species, isolated from fruits of a *Rhododendron* sp., in the genus *Neonectria* based on minimal morphological similarities with the sexual morph of *N. ramulariae*. Their study did not include any DNA sequence data of the ex-type (CBS 124071); based on phylogenetic inference in this study, this species belongs to the genus *Cylindrodendrum*, for which we provide a new combination.

Dactylonectria L. Lombard & Crous, **gen. nov.**
MycoBank MB810142.
(Figure 3)

Etymology: Name refers to "foot" as members of this genus are associated with black foot disease of grapevine.

Diagnosis: *Perithecia* ovoid to obpyriform, smooth to finely warted, dark-red with papillate ostiolar region at the apex. Asexual morph producing abundant macro- and microconidia, but rarely chlamydospores in culture.

Type species: *Dactylonectria macrodidyma* (Halleen, Schroers & Crous) L. Lombard & Crous.

Description: *Ascomata* perithecial, superficial, solitary or aggregated in groups, ovoid to obpyriform, dark red, becoming purple-red in 3% KOH, smooth to finely warted, with papillate apex; without recognisable stroma; perithecial wall consisting of two poorly distinguishable regions; outer region com-

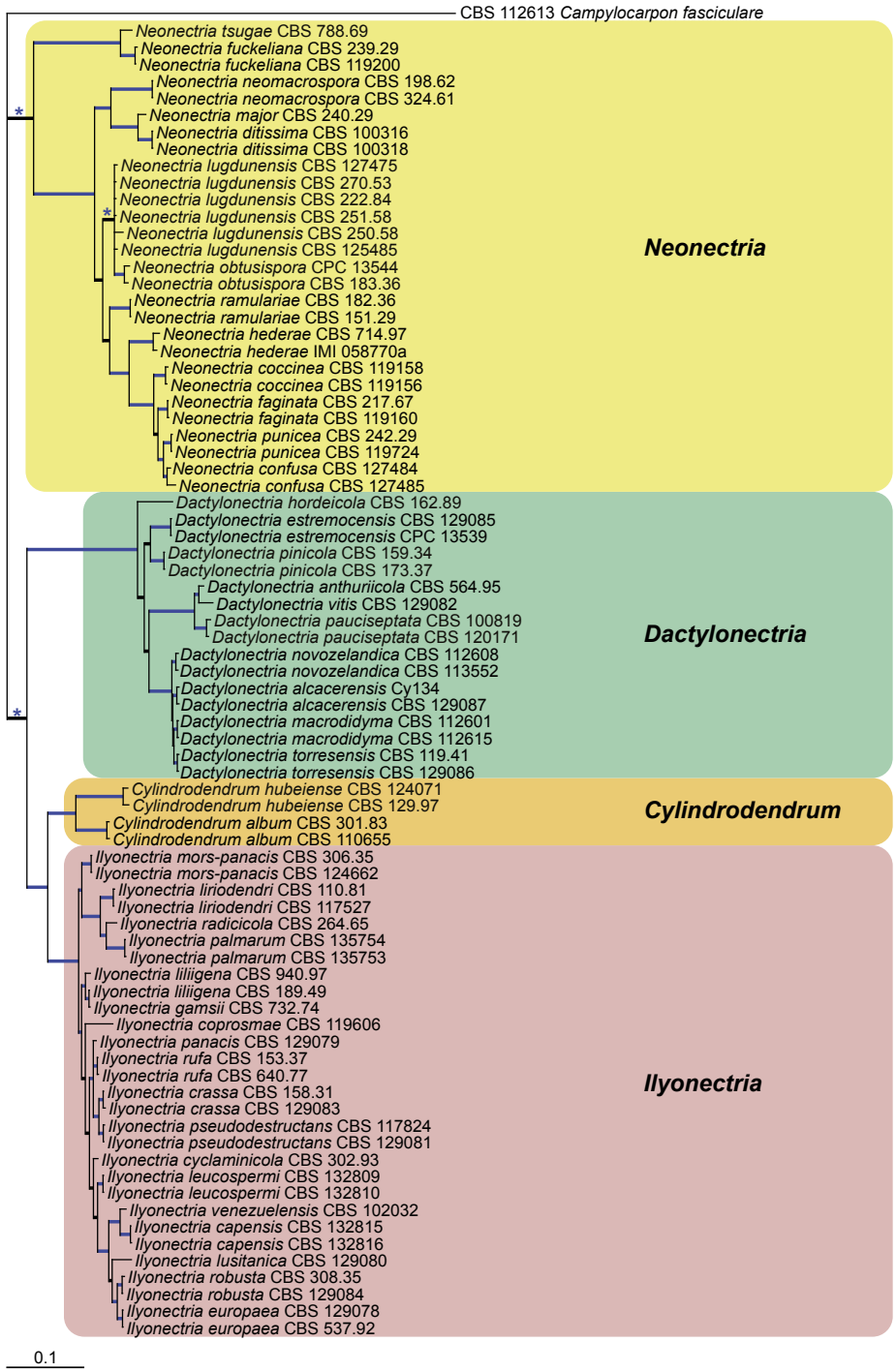

Figure 1. Consensus phylogram of 662 trees resulting from a Bayesian analysis of the combined four-gene sequence alignment. Genera are indicated in coloured blocks. Thickened lines represents branches also present in the Maximum Likelihood (ML) and Maximum Parsimony (MP) consensus trees. Blue lines indicate Bayesian posterior probabilities (PP) ≥ 0.95 and bootstrap support (BS) values for both ML and MP ≥ 95% and blue stars indicate ML-BS and MP-BS ≥ 95% and PP < 0.95. The scale bar represents the expected number of changes per site. The tree was rooted to *Campylocarpon fasciculare* (CBS 112613).

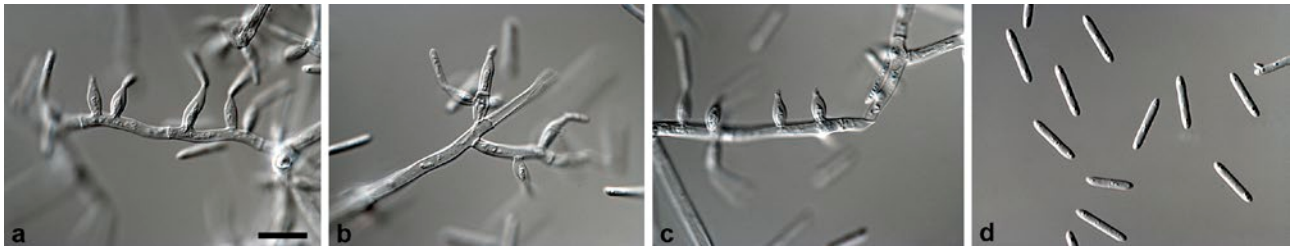

Figure 2. *Cylindrodendrum hubeiense* (CBS 129.97). a–c. Conidiophores on somatic hyphae. d. Conidia. Scale bar: a = 10 μm (apply to b–d).

posed of 1–3 layers of angular to subglobose cells; inner region 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. *Asci* clavate to narrowly clavate, 8-spored; apex rounded, with a minutely visible ring. *Ascospores* ellipsoidal to oblong-ellipsoidal, somewhat tapering towards the ends, medianly septate, smooth to finely warted. *Conidiophores* simple or aggregated to form sporodochia; simple conidiophores arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, septate, bearing up to three phialides; phialides monophialidic, more or less cylindrical, tapering slightly in the upper part towards the apex. *Macroconidia* cylindrical, hyaline, straight to slightly curved, 1–4-septate, apex or apical cell typically slightly bent to one side and minutely beaked, base with visible, centrally located or laterally displaced hilum. *Microconidia* ellipsoid to ovoid, hyaline, straight, aseptate to 1-septate, with a minutely or clearly laterally displaced hilum. *Chlamydospores* rarely formed, globose to subglobose, smooth but often appearing rough due to deposits, thick-walled, mostly occurring in chains.

Notes: *Dactylonectria* shares several morphological features with *Ilyonectria* and *Neonectria* but can be distinguished by their characteristic ovoid to obpyriform, smooth to finely warted, dark-red perithecia with papillate ostiolar region at the apex. Members of *Ilyonectria* have globose to subglobose, scaly to slightly warted, orange to red perithecia whereas *Neonectria* is characterised by globose to broadly obpyriform, smooth to scurfy, yellow to orange to red perithecia (Chaverri *et al*., 2011). Isolates of *Dactylonectria* produce abundant macro- and microconidia, but rarely chlamydospores in culture (Halleen *et al*., 2004; Cabral *et al*., 2012c). Isolates of *Ilyonectria* produce abundant macro-, microconidia and chlamydospores in culture (Chaverri *et al*., 2011; Cabral *et al*., 2012a; Lombard *et al*., 2013), while those of *Neonectria* produce abundant macroconidia but rarely any chlamydospores (Chaverri *et al*., 2011). All members of *Dactylonectria,* with the exception of *D. anthuriicola* and *D. hordeicola* (Cabral *et al*., 2012a), have thus far been associated with black foot disease of grapevine in Australia, Europe, New Zealand, South Africa and USA (Halleen *et al*., 2004; Cabral *et al*., 2012a–c).

Dactylonectria alcacerensis (A. Cabral, Oliveira & Crous) L. Lombard & Crous, **comb. nov.**
MycoBank MB810143

Basionym: *Ilyonectria alcacerensis* A. Cabral, Oliveira & Crous, *Fungal Biology* 116, 71 (2012).

Description and illustrations: Cabral *et al.* (2012c).

Dactylonectria anthuriicola (A. Cabral & Crous) L. Lombard & Crous, **comb. nov.**
MycoBank MB810144

Basionym: *Ilyonectria anthuriicola* A. Cabral & Crous, *Mycological Progress* 11, 666 (2012).

Description and illustrations: Cabral *et al.* (2012a).

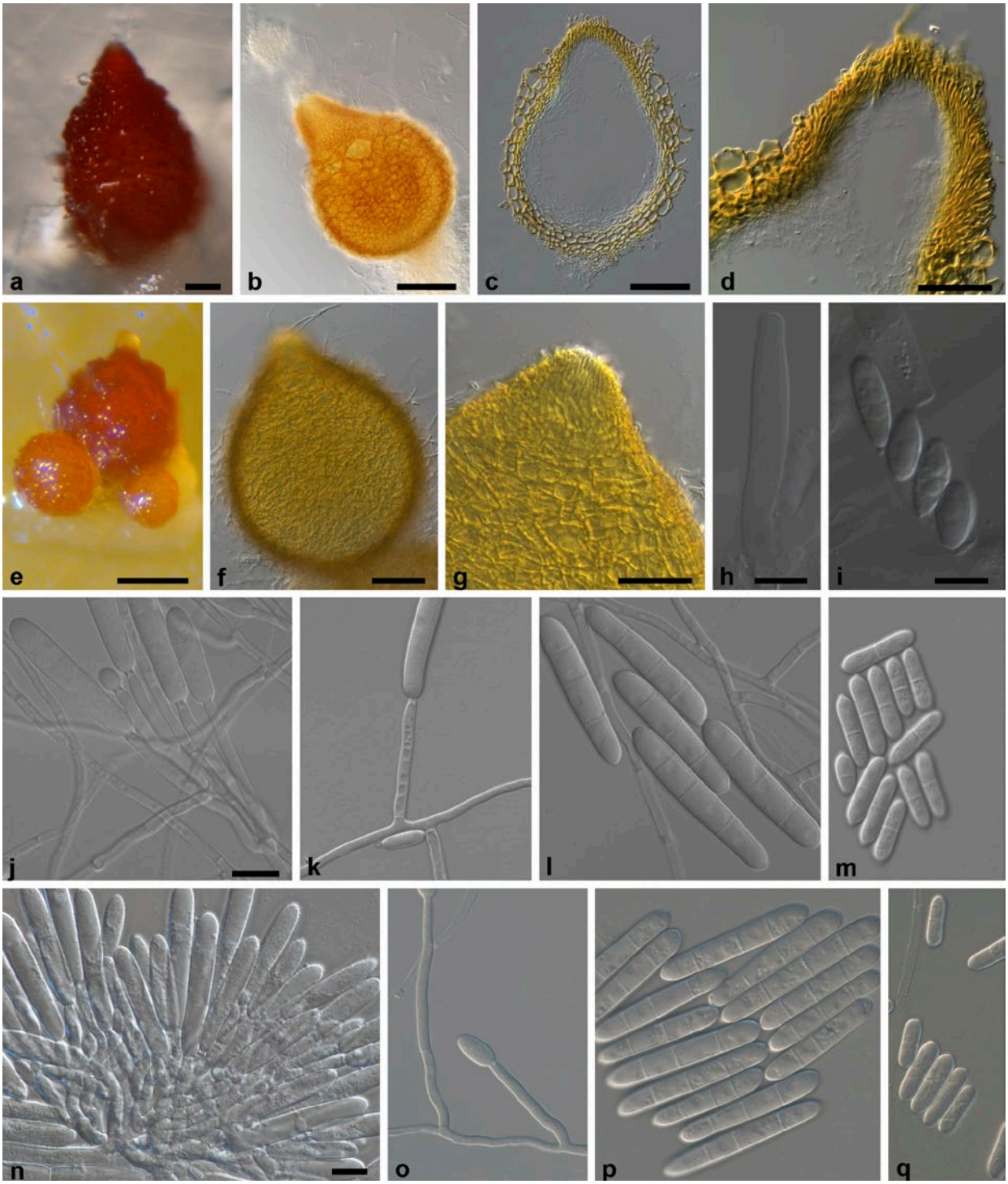

Figure 3. *Dactylonectria* (Adapted from Cabral *et al.* (2012c) Figs. 4–7). a–d, j–m. *D. novozelandica*. e–i, n–q. *D. torresensis*. a–b, e–f. Perithecial ascomata. c–d. Longitudinal sections of ascomata showing details of the papillate ostiolar region of *D. novozelandica*. g. Ostiolar region of *D. torresensis*. h. Ascus. i. Ascospores. j, n. Complex conidiophore. k, o. Simple conidiophores. l, p. Macroconidia. m, q. Microconidia. Bars: a–c, e = 100 µm, d, f–g = 50 µm, h–i = 10 µm, j = 10 µm (apply to k–m), n = 10 µm (apply to o–q).

Dactylonectria estremocensis (A. Cabral, Nascimento & Crous) L. Lombard & Crous, **comb. nov.**
MycoBank MB810145

Basionym: *Ilyonectria estremocensis* A. Cabral, Nascimento & Crous, *Fungal Biology* 116, 73 (2012).

Description and illustrations: Cabral *et al.* (2012c).

Dactylonectria hordeicola L. Lombard & Crous, **sp. nov.**
MycoBank MB810146

Etymology: Name derived from the host, *Hordeum vulgare*, from which this fungus was isolated.

Diagnosis: Culture now sterile, differing from other species in this genus by unique fixed alleles in three loci based on alignments of the separate loci.

Type: **The Netherlands**: Flevoland, Noordoost Polder, Marknesse, Lovinkhoeve, isolated from washed roots of *Hordeum vulgare*, 1988, *M. Barth* (CBS 162.89, as *Cylindrocarpon obtusisporum*, preserved as metabolically inactive culture – holotype; CBS 162.89 – ex-type culture).

Description: *Dactylonectria hordeicola* differs from the other species in this genus by unique fixed alleles in three loci based on alignments of the separate loci deposited in TreeBASE (S16355): *tub2* positions 26(T), 84(T), 113(A), 121(G), 203(A), 205(A), 210(A), 220(A), 237(T), 238(A), 242(C), 250(C), 349(T), 409(T), 434(T), 436(T), 437(T), 451(G), 525(T) and 549(T); ITS positions 113(T), 124(G), 159(A) and 165(T); *tef1* positions 25(T), 34(T), 69(T), 90(A), 112(T), 114(T), 169(C), 199(G), 293(T), 295(T), 332(T), 421(T), 481(A), 488(A), 490(C), 494(C), 506(T), 507(T), 520(C) and 535(C).

Culture characteristics: Colonies covering the medium within 10 d at 24°C. Colonies on PDA with abundant white aerial mycelium and white in reverse. Colonies on SNA with semi-immersed aerial mycelium and no sporulation on or next to the sterile filter paper.

Notes: The isolate representing *Dactylonectria hordeicola* could not be induced to sporulate on any of the media used in this study. Phylogenetic inference and SNP analysis in this study showed that this species is clearly distinct from other species in this genus.

Dactylonectria macrodidyma (Halleen, Schroers & Crous) L. Lombard & Crous, **comb. nov.**
MycoBank MB810148

Basionym: *Neonectria macrodidyma* Halleen, Schroers & Crous, *Studies in Mycology* 50, 445 (2004).
≡ *Ilyonectria macrodidyma* (Halleen, Schroers & Crous) P. Chaverri & C. Salgado, *Studies in Mycology* 68, 71 (2011).
= *Cylindrocarpon macrodidymum* Halleen, Schroers & Crous, *Studies in Mycology* 50, 446 (2004).

Description and illustrations: Halleen *et al.* (2004).

Dactylonectria novozelandica (A. Cabral & Crous) L. Lombard & Crous, **comb. nov.**
MycoBank MB810150

Basionym: *Ilyonectria novozelandica* A. Cabral & Crous, *Fungal Biology* 116, 74 (2012).

Description and illustrations: Cabral *et al.* (2012c).

Dactylonectria pauciseptata (Schroers & Crous) L. Lombard & Crous, **comb. nov.**
MycoBank MB810151

Basionym: *Cylindrocarpon pauciseptatum* Schroers & Crous, *Mycological Research* 112, 86 (2008).

Description and illustrations: Schroers *et al.* (2008).

Dactylonectria pinicola L. Lombard & Crous, **sp. nov.**
MycoBank MB810152

Etymology: Name derived from the host, *Pinus laricio*, from which the ex-type of this fungus was isolated.

Diagnosis: Culture now sterile, differing from other species in this genus by unique fixed alleles in two loci based on alignments of the separate loci.

Type: **UK**: England, Devon, Haldon, from *Pinus laricio*, Feb. 1937, T.R. Peace (CBS 173.37, as *Cylindorcarpon obtusisporum*, preserved as metabolically inactive culture – holotype; CBS 173.37 = IMI 090176 – ex-type culture).

Description: *Dactylonectria pinicola* differs from the other species in this genus by unique fixed alleles in two loci based on alignments of the separate loci deposited in TreeBASE (S16355): *tub2* positions 193(A), 200(T), 202(T), 232(G), 241(C), 298(C), 388(A) and 402(G); *tef1* positions 60(A), 89(T), 265(C), 266(T), 322(T), 323(C), 487(T), 516(A) and 519(A).

Culture characteristics: Colonies covering the medium within 10 d at 24°C. Colonies on PDA with abundant white aerial mycelium and white in reverse. Colonies on SNA with semi-immersed aerial mycelium and no sporulation on or next to the sterile filter paper.

Additional culture sequenced: **Germany**: details and host unknown, Oct. 1934, *H.W. Wollenweber* (CBS 159.34 = IMI 113891 = MUCL 4084 – culture).

Notes: The isolates representing *Dactylonectria pinicola* could not be induced to sporulate on any of the media used in this study, nor on sterilised pine needles placed on both SNA and PDA. This species is closely related to but distinct from *D. estremocensis* based on phylogenetic inference and SNP analysis done in this study.

Dactylonectria torresensis (A. Cabral, Rego & Crous) L. Lombard & Crous, **comb. nov.**
MycoBank MB810153

Basionym: *Ilyonectria torresensis* A. Cabral, Rego and Crous, *Fungal Biology* 116, 75 (2012).

Description and illustrations: Cabral *et al.* (2012c).

Dactylonectria vitis (A. Cabral, Rego & Crous) L. Lombard & Crous, **comb. nov.**
MycoBank MB810154

Basionym: *Ilyonectria vitis* A. Cabral, Rego & Crous, *Mycological Progress* 11, 684 (2012).

Description and illustrations: Cabral *et al.* (2012a).

Neonectria lugdunensis (Sacc. & Therry) L. Lombard & Crous, **comb. nov.**
MycoBank MB810155
(Figure 4)

Basionym: *Heliscus lugdunensis* Sacc. & Therry, *Michelia* 2, 132 (1880).

= *Heliscus aquaticus* Ingold, *Transactions of the British Mycological Society* 25, 360 (1942).

= *Nectria lugdunensis* J. Webster, *Transactions of the British Mycological Society* 42, 325 (1959).

= *Neonectria shennongjiana* J. Luo & W.Y. Zhuang, *Mycologia* 102, 145 (2010).

Description and illustrations: Saccardo (1880), Ingold (1942, 1944), Webster (1959).

Type of teleomorph: **UK**: England, Sheffield, River Porter near Forge Dam, on submerged decayed leaf of *Ilex aquifolium*, Jun. 1958, *J. Webster* (IMI 7495 – holotype; CBS 250.58 ex-type culture).

Additional cultures examined: **China**: Hubei, Shennongjia, 1 700 m alt., submerged twig of unknown dicotyledonous tree, 13 Sept. 2003, *X.M. Zhang* (CBS 127475 = HMAS 173254); **The Netherlands**: Flevoland, De Schreef, from potato-field soil, Apr. 1984, *unknown* (CBS 222.84); **USA**: Arizona, Huachuca Mountains, Miller Canyon, on submerged twig of *Populus fremontii*, Jan. 2008, *T. Gräfenhan* (CBS 125485 = DAOM 235831 = T.G. 2008-07).

Notes: The genus *Heliscus* has been used for aquatic or Ingoldian hyphomycetes with straight, apically bifurcate phialoconidia. The intensity of this bifurcation is variable and depends on cultural conditions and age of the isolate. It can be much more pronounced than shown in Fig. 4f or almost absent, so that the conidia look like those of *Cylindrocarpon.*

Based on phylogenetic inference in this study, all isolates previously known as *Heliscus lugdunensis* (Webster, 1959; Gräfenhan *et al.*, 2011) clustered together within the *Neonectria* clade and therefore a new combination is provided here. The ex-type of *N. shennongjiana* (CBS 127475; Luo & Zhuang, 2010) also grouped with these isolates and therefore we consider this species a synonym of *N. lugdunensis*.

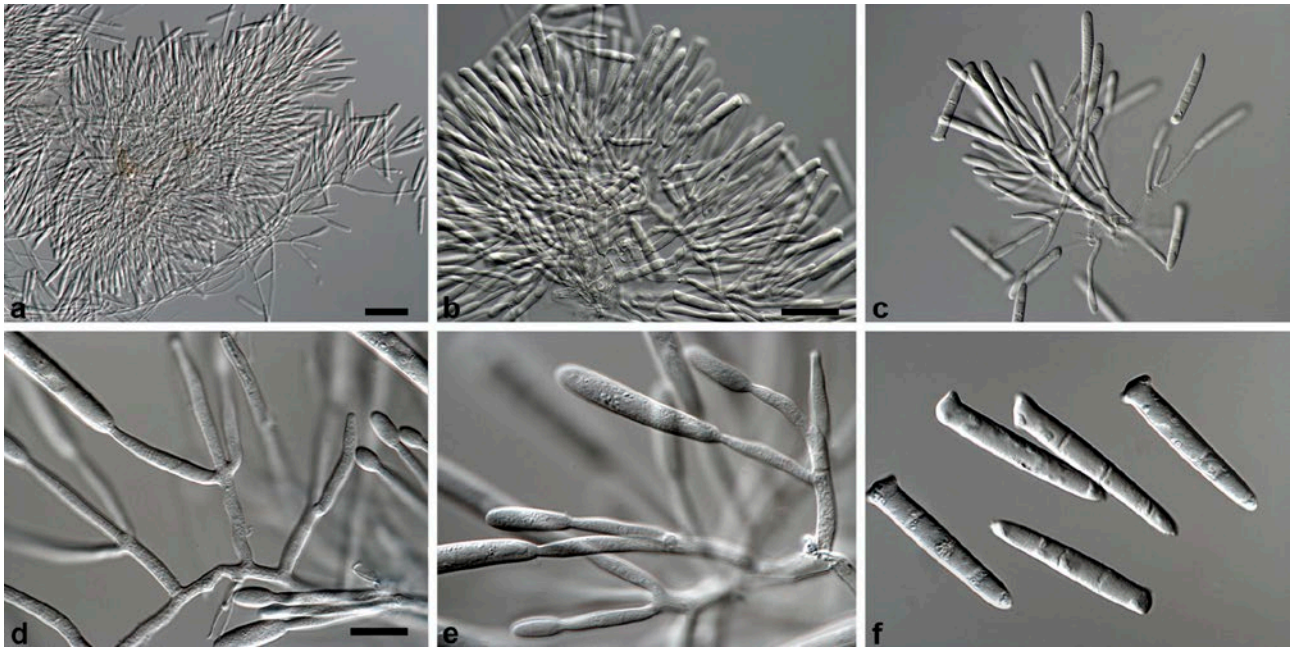

Figure 4. *Neonectria lugdunensis* (ex-type CBS 250.58). a–e. Complex conidiophores. f. Macroconidia. Scale bars: a = 50 µm, b = 20 µm (apply to c), d = 10 µm (apply to e–f).

Neonectria obtusispora (Cooke & Harkn.) Rossman, L. Lombard & Crous, **comb. nov.**
MycoBank MB810156

Basionym: Fusarium obtusisporum Cooke & Harkn., *Grevillea* 12, 97 (1884).

≡ *Ramularia obtusispora* (Cooke & Harkn.) Wollenw., Fusaria Autographice Delineata 1, 465 (1916).

≡ *Cylindrocarpon obtusisporum* (Cooke & Harkn.) Wollenw., Fusaria Autographice Delineata 1, 465 (1916).

= *Nectria tawa* Dingley, *Transactions of the Royal Society of New Zealand* 79, 199 (1951).

Description and illustrations: Dingley (1951), Booth (1966).

Notes: Dingley (1956) provided the first link between *Nectria tawa* and a cylindrocarpon-like asexual morph, which Booth (1966) later confirmed as *Cylindrocarpon obtusisporum*. Samuels & Brayford (1990), however, questioned this link and synonymised *N. tawa* along with *N. coprosmae* under *N. radicicola* var. *coprosmae* (now *Ilyonectria coprosmae*; Chaverri *et al*., 2011) even though they recognised morphological differences between these species. Although there are no DNA sequence data presently available to confirm the link between *N. tawa* and *C. obtusisporum*, we elect to provide a new combination for Dingley's fungus in the genus *Neonectria*, pending recollection of fresh material from the type localities.

Neonectria tsugae (W. Gams) L. Lombard & Crous, **comb. nov.**
MycoBank MB810157

Basionym: *Acremonium tsugae* W. Gams, *Cephalosporium-artige Schimmelpilze*: 117 (1971).

Description and illustrations: Gams (1971).

Notes: The ex-type of *Acremonium tsugae* (CBS 788.69; Gams, 1971), so far only known as asexual morph, clustered within the *Neonectria* clade, closely related to but distinct from *N. fuckeliana* (CBS 239.29 & CBS 119200); therefore a new combination is provided for this species in the genus *Neonectria*.

Discussion

This study emerged as a result of taxonomic discrepancies noted in the genera *Cylindrodendrum*, *Ilyonectria* and *Neonectria*. The latter two genera comprise important pathogens associated with basal stem and root diseases of various woody plant hosts (see Introduction), whereas little information is available in the literature on the pathogenicity of species in the genus *Cylindrodendrum*.

The genus *Cylindrodendrum*, first erected by Bonorden, (1851) with *C. album* as type species, is regarded as a semi-aquatic saprobe (Buffin and Hennebert, 1984; Summerbell *et al*., 1989) able to grow on decaying plant material in marine, fresh-water and terrestrial environments. This genus is characterised by conidiomata consisting of lateral phialides on thick, erect somatic hyphae, sometimes becoming verticillate, with the terminal part having a swollen tip and producing straight, cylindrical, 0–1-septate conidia (Buffin and Hennebert, 1984; Summerbell *et al*., 1989). Both Buffin and Hennebert (1984) and Summerbell *et al.* (1989) noted the presence of a cylindrocarpon-like synasexual morph formed by *C. album* in culture, which Buffin and Hennebert (1984) named *Cylindrocarpon hydrophilum* but which has since not been used in literature. In their treatment of *Cylindrodendrum hubeiense* (as *Neonectria hubeiensis*), Zhuang *et al.* (2007) also illustrated a synasexual morph, which they provisionally indicated as *Cylindrocarpon* cf. *orthosporum*. However, this synasexual morph was not formally described and no DNA sequence data were available to confirm their treatment of this species in the genera *Cylindrocarpon* and *Neonectria*. Although Chaverri *et al.* (2011) suggested that *Cylindrodendrum* should be considered a synonym of *Cylindrocarpon*/*Neonectria*, phylogenetic inference in the current study showed that species of *Cylindrodendrum* form a well-supported monophyletic sister clade to the *Ilyonectria* clade, distant from the *Neonectria* clade.

The new genus *Dactylonectria* is introduced here for a group of species previously treated in the genus *Ilyonectria* (Chaverri *et al*., 2011; Cabral *et al*., 2012a,b,c). Multi-gene studies of the genus *Ilyonectria*, Cabral *et al.* (2012a,c) and Lombard *et al.* (2013) revealed that this genus is paraphyletic, but the authors did not contemplate this fact at that time. This genus now includes 10 species: *D. alcacerensis*, *D. anthuriicola*, *D. estremocensis*, *D. hordeicola*, *D. macrodidyma*, *D. novozelandica*, *D. pauciseptata*, *D. pinicola*, *D. torresensis* and *D. vitis*. Of these, only *D. anthuriicola* and *D. hordeicola* are not associated with black foot disease of grapevine (Cabral *et al*., 2012a). *Dactylonectria torresensis* appears to have the largest host range, having been reported from plant hosts in the genera *Abies*, *Fragaria*, *Quercus* and *Vitis*, whereas *D. alcacerensis, D. macrodidyma, D. novozelandica*, *D. pauciseptata* and *D. vitis* are only known from grapevines (Halleen *et al*., 2004, 2006; Cabral *et al*., 2012a, c). *Dactylonectria estremocensis* and *D. pinicola* are also known from grapevines in Europe, with the former also reported from *Picea* in Canada and the latter from *Pinus* in the UK (Cabral *et al*., 2012a,c).

Species of *Ilyonectria* are important soil-borne pathogens of various woody and herbaceous plant hosts, mostly associated with stem cankers and root diseases (Seifert *et al*., 2003; Halleen *et al*., 2004, 2006; Chaverri *et al*., 2011; Cabral *et al*., 2012a,b,c; Vitale *et al*., 2012; Lombard *et al*., 2013; Aiello *et al*., 2014). Presently 18 species (Chaverri *et al*., 2011; Cabral *et al*., 2012a,c; Lombard *et al*., 2013; Aiello *et al*., 2014) are recognised in this genus, all associated with disease symptoms of their respective plant hosts.

Three new combinations are provided in the genus *Neonectria* for species previously treated in the genera *Acremonium*, *Cylindrocarpon* and *Heliscus*. Gams (1971) distinguished *Neonectria tsugae* (as *A. tsugae*), isolated from *Tsuga heterophylla*, from *Neonectria fuckeliana* (as *Nectria fuckeliana*) based on its conidial morphology, a distinction that was supported by DNA sequence data in the present study. Gräfenhan *et al.* (2011) also illustrated this close relationship but did not treat this taxon at that time. The type species of the aquatic genus *Heliscus*, *H. lugdunensis* (Ingold, 1942), is also relocated to the generally terrestrial genus *Neonectria,* based on phylogenetic inference in the present study. The genus *Heliscus* included six aquatic species, four of which were later placed in the aquatic genus *Clavatospora* (Nilsson, 1964). The taxonomic status of the only remaining species in *Heliscus*, *H. submersus*, is still uncertain and needs to be investigated further, and therefore is left in limbo at present. Following the new International Code of Nomenclature for algae, fungi and plants (ICN; McNeill *et al*., 2012), the generic name *Heliscus* (1880) should take priority over the generic name *Neonectria* (1917). However, based on the number of name changes required and the familiarity of the generic name *Neonectria* among plant pathologists and other applied biologists, we agree with the

decision of Chaverri *et al.* (2011) to synonymise *Heliscus* under *Neonectria*.

Black foot rot of grapevines is a well-documented disease in various countries, now associated with fungal species in the four genera treated here, namely *Campylocarpon*, *Dactylonectria*, *Ilyonectria* and *Neonectria* (Halleen *et al*., 2003, 2004, 2006; Chaverri *et al*., 2011; Cabral *et al*., 2012a,b,c). This finding highlights the importance of correct fungal pathogen identification, which could have significant impact on the quality of grapevine rootstocks and control measures implemented for disease control. Morphologically it is very difficult to distinguish not only between species within these genera, but also between genera, and therefore DNA sequence data are essential when working with these fungi.

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