

# Cylindrocarpon root rot: multi-gene analysis reveals novel species within the *Ilyonectria radiculicola* species complex

Ana Cabral · Johannes Z. Groenewald · Cecília Rego ·  
Helena Oliveira · Pedro W. Crous

Received: 29 March 2011 / Revised: 2 July 2011 / Accepted: 11 July 2011 / Published online: 27 July 2011  
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**Abstract** *Ilyonectria radiculicola* and its *Cylindrocarpon*-like anamorph represent a species complex that is commonly associated with root rot disease symptoms on a range of hosts. During the course of this study, several species could be distinguished from *I. radiculicola* sensu stricto based on morphological and culture characteristics. DNA sequence analysis of the partial  $\beta$ -tubulin, histone H3, translation elongation factor 1- $\alpha$  and nuclear ribosomal RNA-Internal Transcribed Spacer (nrRNA-ITS) genes were employed to provide further support for the morphological species resolved among 68 isolates associated with root rot disease symptoms. Of the various loci screened, nrRNA-ITS sequences were the least informative, while histone H3 sequences were the most informative, resolving the same number of species as the combined dataset across the four genes. Within the *Ilyonectria radiculicola* species complex, 12 new taxa are delineated occurring on a diverse range of

hosts, the most common being *Cyclamen*, *Lilium*, *Panax*, *Pseudotsuga*, *Quercus* and *Vitis*.

**Keywords** *Cylindrocarpon* root rot · *Nectria*-like fungi · Phylogeny · Systematics

## Introduction

The genus *Cylindrocarpon* was introduced in 1913 by Wollenweber, with *C. cylindroides* as type. *Cylindrocarpon* and *Cylindrocarpon*-like species have since been commonly associated with root and decay of woody and herbaceous plants (Domsch et al. 2007). *Cylindrocarpon* root rot causes losses up to 30% on ginseng (*Panax quinquefolium*) (Seifert et al. 2003), and plays an important role in black foot rot of grapevines (Halleen et al. 2004, 2006), apple replant disease (Tewoldemedhin et al. 2010), and beech cankers (Castlebury et al. 2006), to name but a few hosts of economic importance.

In his taxonomic revision of *Cylindrocarpon*, Booth (1966) divided this genus into four groups based on the presence or absence of microconidia or chlamydospores. Booth's group 4 represents *Neonectria* s. str., as it accommodates the type species *N. ramulariae* (anamorph: *C. obtusiusculum*). Most of the teleomorphs of *Cylindrocarpon* species have since this date been classified in *Neonectria* (Brayford et al. 2004; Halleen et al. 2004, 2006; Mantiri et al. 2001; Rossman et al. 1999). Several phylogenetic studies have, however, revealed that *Neonectria/Cylindrocarpon* is paraphyletic (Castlebury et al. 2006; Halleen et al. 2004, 2006; Hirooka et al. 2005; Mantiri et al. 2001). The first step in resolving this issue was taken by Halleen et al. (2004), who proposed *Campylocarpon* for species resembling *Cylindrocarpon* with 3–5-septate, curved macroconidia, and

A. Cabral · C. Rego · H. Oliveira (✉)  
CEER-Biosystems Engineering, Instituto Superior de Agronomia,  
Technical University of Lisbon,  
Tapada da Ajuda,  
1349-017 Lisboa, Portugal  
e-mail: heloliveira@isa.utl.pt

J. Z. Groenewald · P. W. Crous  
CBS-KNAW Fungal Biodiversity Centre,  
P.O. Box 85167, 3508 AD Utrecht, The Netherlands

P. W. Crous  
Microbiology, Department of Biology, Utrecht University,  
Padualaan 8,  
3584 CH Utrecht, The Netherlands

P. W. Crous  
Laboratory of Phytopathology,  
Wageningen University and Research Centre (WUR),  
Droevendaalsesteeg 1,  
6708 PB Wageningen, The Netherlands

lacking microconidia. A further phylogenetic study (Chaverri et al. 2011) divided the *Neonectria* complex into four genera based on a combination of characters linked to perithecial anatomy and conidial septation: *Ilyonectria*, *Neonectria*/*Cylindrocarpon* s. str., *Rugonectria* and *Thelonectria*. In this study, a single generic name was proposed for each clade in an attempt to move towards a single nomenclature for pleomorphic fungi, meaning that the *Cylindrocarpon*-like anamorphs of *Ilyonectria*, *Rugonectria* and *Thelonectria* were placed in teleomorph genera, as recently done with other groups of pleomorphic fungi (Crous et al. 2006, 2007, 2009a; Gräfenhan et al. 2011; Lombard et al. 2010; Schroers et al. 2011).

*Cylindrocarpon* root rot is commonly associated with “*Cylindrocarpon*” *destructans* in the literature (Halleen et al. 2004; Samuels and Brayford 1990). This fungus was originally described as *Ramularia destructans* from roots of ginseng (*Panax quinquefolium*) collected in the USA (Zinssmeister 1918). Furthermore, it has been linked to the teleomorph *Ilyonectria radicola* (Booth 1966; Chaverri et al. 2011; Samuels and Brayford 1990), which Gerlach and Nilsson (1963) described from rotting bulbs of *Cyclamen persicum* collected in Sweden. Samuels and Brayford (1990) commented on the morphological variation in collections of *I. radicola* and its anamorph “*C.*” *destructans*. Seifert et al. (2003) showed that there was more than one “*C.*” *destructans*-like species occurring on *Panax*, and that none of the resolved clades correlated to the ex-type strain of *I. radicola*, leading Halleen et al. (2006) to question the purported anamorph/teleomorph link between *I. radicola* (from *Cyclamen*, Sweden) and “*C.*” *destructans* (from *Panax*, USA). Based on a phylogenetic analysis of ITS nrRNA gene sequences, Schroers et al. (2008) concluded that the *I. radicola* complex includes “*C.*” *destructans*, “*C.*” *destructans* var. *crassum*, *I. coprosmae*, *I. liriodendri*, *N. austroradicicola* and *N. macroconidialis*.

The aim of the present study was to elucidate the morphological variation present within the *I. radicola* complex, and to link fresh collections to older names introduced for species in this complex. This was addressed by combining morphological and culture characteristics with DNA sequence data derived from the Internal Transcribed Spacers (ITS) of the nrRNA gene operon, and partial  $\beta$ -tubulin (TUB), histone H3 (HIS), and translation elongation factor 1- $\alpha$  (TEF) genes.

## Materials and methods

### Isolates

This study (Table 1) included 42 “*C.*” *destructans* s. lat. isolates [including the ex-type strains of *I. radicola* (CBS

264.65) and “*C.*” *destructans* f.sp. *panacis* (CBS 124662), “*C.*” *destructans* var. *destructans* and “*C.*” *destructans* var. *crassum*], six “*C.*” *didymum* isolates, six *I. liriodendri* isolates, one *N. macroconidialis* isolate and one *I. coprosmae* isolate, all deposited at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS). Also included are two isolates that were previously identified as *Ramularia mors-panacis* (CBS 306.35) and *R. panacicola* (CBS 307.35) by Hildebrand (1935).

Besides those, 10 “*Cylindrocarpon*” spp. isolates were obtained in Portugal from grapevine plants showing decline symptoms, either 1- to 6-year-old plants in vineyards (Cy22, Cy155, Cy158, Cy190, CBS 129078, CBS 129080, CBS 129081, CBS 129082) or from rootstock nurseries (Cy23), and from a 25-year-old grapevine plant with esca symptoms (CBS 129084). Furthermore, isolates were obtained from a young *Malus domestica* (Cy164) and from the stem of a young *Quercus suber* (Cy232) plant, both showing decline symptoms, and from *Thymus* sp. (Cy231) and *Ficus* sp. (Cy228). One isolate (Cy131) was made available by P. Lecomte (Institut National de la Recherche Agronomique, Bordeaux-Aquitaine, France) and was obtained from an internal lesion of a stem of *Actinidia chinensis* ‘Hayward’. Another isolate (Cy122) was made available by W.D. Gubler (University of California, Davis, USA) and was obtained from *Vitis* sp. All of these isolates are stored in a culture collection at the Laboratório de Patologia Vegetal “Veríssimo de Almeida” (LPVVA-ISA, Lisbon, Portugal).

An additional 25 “*C.*” *destructans* isolates used during this study were made available by K.A. Seifert (Agriculture and Agri-Food, Canada), and were isolated from commercial *Panax quinquefolium* gardens (CBS 120359–120369, CBS 129079, CBS 129083, CD1666, CPC 13535, CPC 13537, NSAC-SH2, NSAC-SH2.5), *Picea glauca* (94–1628, CPC 13539), *Poa pratensis* (CPC 13534), *Pseudotsuga menziesii* (CBS 120370–120372, CPC 13536) and *Prunus cerasus* (CPC 13532) (Seifert et al. 2003).

Another 109 isolates were also included in the analysis to add phylogenetic support to this study and represent strains of the following taxa: *C. cylindroides*, *C. obtusisporum*, *C. pauciseptatum*, species 1 to 6 (Mostert et al., in preparation; Cabral et al., in preparation), *I. macrodidyma*, *N. ditissima*, *N. major*, *N. neomacrospora* and *N. ramulariae*.

### DNA isolation, sequencing and phylogenetic analysis

For each isolate, genomic DNA was isolated from mycelium following the protocol of Möller et al. (1992), adapted by Crous et al. (2009b). Sequencing of the ITS and part of the  $\beta$ -tubulin (TUB), histone H3 (HIS) and translation elongation factor 1- $\alpha$  (TEF) genes was performed after PCR amplification using 1 $\times$  PCR buffer (Bioline, London,

**Table 1** Details pertaining to isolates investigated during this study

Species	Strain number <sup>a</sup>	Collected/isolated by, year	Isolated from	Location	GenBank accession numbers				
					ITS	TUB	H3	EF1	EF2
<i>Campylocarpon fasciculare</i> , Holotype	CBS 112613; STE-U 3970; C 76	F. Halleen, 2000	<i>Vitis vinifera</i> , trunk of young grapevine showing decline symptoms; scion Cabernet Sauvignon; rootstock Richter 99	South Africa, Western Cape, Riebeck Kasteel	AY677301	AY677221	JF735502	JF735691	
<i>Campylocarpon pseudofasciculare</i> , Holotype	CBS 112679; STE-U 5472; HJS-1227	F. Halleen, 2000	<i>Vitis vinifera</i> , roots, asymptomatic nursery grapevine plant; scion Sultana; rootstock Ramsey	South Africa, Western Cape, Wellington	AY677306	AY677214	JF735503	JF735692	
<i>Neonectria macroconidialis</i>	CBS 119596; ICMP 9349; IMI 332705; GJS 85-59	G.J. Samuels, 1985	<i>Asiella</i> sp.	New Zealand, Gisborne, Urewera National Park	JF735259	JF735372	JF735504	JF735693	
<i>Ilyonectria coprosmae</i>	CBS 119606; GJS 85-39	G.J. Samuels, 1985	<i>Metrosideros</i> sp.	Canada, Ontario	JF735260	JF735373	JF735505	JF735694	
<i>Ilyonectria radicicola</i> , type strain	CBS 264.65	L. Nilsson, 1961	<i>Cyclamen persicum</i>	Sweden, Skåne, Bjärred	AY677273	AY677256	JF735506	JF735695	
<i>Ilyonectria lirioidendri</i> , type strain of "C." <i>lirioidendri</i>	CBS 110.81; IMI 303645	J.D. MacDonald & E.E. Butler, 1978	<i>Liriodendron tulipifera</i> , root	USA, California, Yolo Co., Davis	DQ178163	DQ178170	JF735507	JF735696	
<i>Ilyonectria lirioidendri</i>	CBS 117526; Cy68	C. Rego, 1999	<i>Vitis vinifera</i> , asymptomatic rootstocks; rootstock 99 R, clone 179 F	Portugal, Ribatejo e Oeste	DQ178164	DQ178171	JF735508	JF735697	
<i>Ilyonectria lirioidendri</i>	CBS 117527; Cy76	C. Rego, 1999	<i>Vitis vinifera</i> , asymptomatic rootstocks; rootstock 110 R, clone 164E	Portugal, Ribatejo e Oeste	DQ178165	DQ178172	JF735509	JF735698	
<i>Ilyonectria lirioidendri</i>	CBS 117640; IMI 357400; Cy1	C. Rego, 1992	<i>Vitis vinifera</i> , 4-year-old plant showing decline symptoms; scion Seara Nova; rootstock 99R	Portugal, Torres Vedras, Dois Portos	DQ178166	DQ178173	JF735510	JF735699	
<i>Ilyonectria lirioidendri</i>	CBS 112596; STE-U 3994; C 14	F. Halleen, 1999	<i>Vitis vinifera</i> , roots	South Africa, Western Cape, De Wet	AY677264	AY677239	JF735511	JF735700	
<i>Ilyonectria lirioidendri</i>	CBS 112607; STE-U 3986; C 81	F. Halleen, 2000	<i>Vitis vinifera</i> , basal end of trunk	South Africa, Western Cape, Robertson	AY677269	AY677241	JF735512	JF735701	
<i>Ilyonectria lirioidendri</i>	Cy164	C. Rego, 1997	<i>Malus domestica</i> ; cultivar Lysgolden; rootstock MM106	Portugal, Porto de Mós, Valboom	AM419079	AM419112	JF735513	JF735702	
<i>Ilyonectria lirioidendri</i>	Cy122	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735261	JF735374	JF735514	JF735703	
<i>Ilyonectria lirioidendri</i>	Cy190	N. Cruz, 2005	<i>Vitis vinifera</i> , basal end of 6-year-old plant; scion Alvarinho; rootstock 196-17	Portugal, Monção, Cortes	JF735262	JF735375	JF735515	JF735704	
<i>Ilyonectria lirioidendri</i>	Cy232	L. Inácio & J. Henriques, 2007	<i>Quercus suber</i> ; stem	Portugal, Macedo de Cavaleiros	JF735263	JF735376	JF735516	JF735705	
<i>Ilyonectria robusta</i>	CBS 321.34	-	<i>Loroglossum hircinum</i> , root	Tunisia, Tunis	AY677275	AY677253	JF735517	JF735706	
<i>Ilyonectria robusta</i> , type strain of <i>Ramularia robusta</i>	CBS 308.35	A.A. Hildebrand	<i>Panax quinquefolium</i>	Canada, Ontario	JF735264	JF735377	JF735518	JF735707	
<i>Ilyonectria robusta</i>	CBS 773.83	J. Hemelraad	water, in aquarium with <i>Anodonta</i>	Netherlands, Utrecht	AY677276	AY677254	JF735519	JF735708	
<i>Ilyonectria robusta</i>	CBS 605.92	R. Schröer, 1992	<i>Tilia petiolaris</i> , root	Germany, Hamburg	EF607078	EF607065	JF735520	JF735709	
<i>Ilyonectria robusta</i>	CBS 117813; IFFF 84	E. Halmschlagler, 1993	<i>Quercus robur</i> , root	Austria, Niederweiden	-	JF735378	-	-	
<i>Ilyonectria robusta</i>	CBS 117814; IFFF 85	E. Halmschlagler, 1993	<i>Quercus</i> sp., root	Austria, Patzmannsdorf	JF735265	JF735379	JF735521	JF735710	
<i>Ilyonectria robusta</i>	CBS 117815; IFFF 86	E. Halmschlagler, 1993	<i>Quercus</i> sp., root	Austria, Patzmannsdorf	JF735266	JF735380	JF735522	JF735711	
<i>Ilyonectria robusta</i>	CBS 117817; IFFF 88	E. Halmschlagler, 1993	<i>Quercus</i> sp., root	Austria, Patzmannsdorf	-	JF735381	-	-	
<i>Ilyonectria robusta</i>	CBS 117818; IFFF 89	E. Halmschlagler, 1993	<i>Quercus</i> sp., root	Austria, Patzmannsdorf	JF735267	JF735382	JF735523	JF735712	
<i>Ilyonectria robusta</i>	CBS 117819; IFFF 90	E. Halmschlagler, 1993	<i>Quercus robur</i> , root	Austria, Niederweiden	-	JF735383	-	-	
<i>Ilyonectria robusta</i>	CBS 117820; IFFF 91	E. Halmschlagler, 1993	<i>Quercus robur</i> , root	Austria, Niederweiden	JF735268	JF735384	JF735524	JF735713	
<i>Ilyonectria robusta</i>	CBS 117821; IFFF 93	E. Halmschlagler, 1993	<i>Quercus robur</i> , root	Austria, Niederweiden	JF735269	JF735385	JF735525	JF735714	

Table 1 (continued)

Species	Strain number <sup>a</sup>	Collected/isolated by, year	Isolated from	Location	GenBank accession numbers			
					ITS	TUB	H3	EFI
<i>Ilyonectria robusta</i>	CBS 117822; IFFF 94	E. Halmischlager, 1993	<i>Quercus robur</i> , root	Austria, Niederweiden	JF735270	JF735386	JF735526	JF735715
<i>Ilyonectria robusta</i>	CBS 117823; IFFF 95	E. Halmischlager, 1993	<i>Quercus robur</i> , root	Austria, Niederweiden	JF735271	JF735387	JF735527	JF735716
<i>Ilyonectria robusta</i>	CD1666	R. D. Reeleder, 1998	<i>Panax quinquefolium</i>	Canada, Nova Scotia	AY295331	JF735388	JF735528	JF735717
<i>Ilyonectria robusta</i>	CPC 13532; DAOM 139398; K 18-3A	-	<i>Prunus cerasus</i> cultivar Montmorency	Canada, Ontario	AY295330	JF735389	JF735529	JF735718
<i>Ilyonectria robusta</i>	Cy23	C. Rego, 1997	<i>Vitis</i> sp. rootstock 99R clone 179 F in nursery	Portugal, Ribatejo e Oeste	AJ875333	AM419093	JF735530	JF735719
<i>Ilyonectria robusta</i>	Cy158	C. Rego & T. Nascimento, 2004	<i>Vitis vinifera</i> , 1-year-old, died before sprouting; scion Alicante Bouschet; rootstock 1103P	Portugal, Lamego, Cambres	JF735272	JF735390	JF735531	JF735720
<i>Ilyonectria robusta</i>	CBS 129084; Cy192	N. Cruz, 2005	<i>Vitis vinifera</i> , basal end of 25-year-old plant; scion Alicante; rootstock 196-17	Portugal, Monção	JF735273	JF735391	JF735532	JF735721
<i>Ilyonectria robusta</i>	Cy231	F. Caetano, 2005	<i>Thymus</i> sp.	Portugal, Lisbon	JF735274	JF735392	JF735533	JF735722
<i>Ilyonectria crassa</i>	CBS 139.30	W.F. van Hell, 1930	<i>Lilium</i> sp., bulb	Netherlands	JF735275	JF735393	JF735534	JF735723
<i>Ilyonectria crassa</i>	CBS 158.31; IMI 061536; NRRL 6149	1930	<i>Narcissus</i> sp., root	Netherlands	JF735276	JF735394	JF735535	JF735724
<i>Ilyonectria crassa</i>	CBS 129083; NSAC-SH-1	S. Hong, 1998	<i>Panax quinquefolium</i>	Canada, Nova Scotia	AY295311	JF735395	JF735536	JF735725
<i>Ilyonectria crassa</i>	NSAC-SH-2	S. Hong, 1998	<i>Panax quinquefolium</i>	Canada, Nova Scotia	AY295313	JF735396	JF735537	JF735726
<i>Ilyonectria crassa</i>	NSAC-SH-2.5	S. Hong, 1998	<i>Panax quinquefolium</i>	Canada, Nova Scotia	AY295314	JF735397	JF735538	JF735727
<i>Ilyonectria crassa</i>	CBS 120370; CR 20	P. Axelrood, 1998	<i>Pseudotsuga menziesii</i>	Canada, British Columbia	AY295317	JF735398	JF735539	JF735728
" <i>Cylindrocarpon</i> " sp.	CBS 153.37	F. Moreau, 1937	Dune sand	France	AY677271	AY677251	JF735540	JF735729
<i>Ilyonectria rufa</i> Authentic strain of <i>Colomyces rufus</i>	CBS 156.47; IAM 14673; JCM 23100	-	<i>Azalea indica</i>	Belgium, Amandsberg	AY677272	AY677252	JF735541	JF735730
<i>Ilyonectria rufa</i>	CBS 640.77	F. Gourbière, 1977	<i>Abies alba</i>	France, Villeurbanne	JF735277	JF735399	JF735542	JF735731
<i>Ilyonectria rufa</i>	CBS 120371; CR 26	P. Axelrood, 1998	<i>Pseudotsuga menziesii</i>	Canada, British Columbia	AY295318	JF735400	JF735543	JF735732
<i>Ilyonectria rufa</i>	CBS 120372; CR 29	P. Axelrood, 1998	<i>Pseudotsuga menziesii</i>	Canada, British Columbia	JF735278	JF735401	JF735544	JF735733
<i>Ilyonectria rufa</i>	CPC 13536; DAOM 226721; CR36	P. Axelrood, 1998	<i>Pseudotsuga menziesii</i>	Canada, British Columbia	JF735279	JF735402	JF735545	JF735734
<i>Ilyonectria rufa</i>	94-1628	R.C. Hamelin, 1994	<i>Picea glauca</i>	Canada, Quebec	AY295315	JF735403	JF735546	JF735735
<i>Ilyonectria mors-panacis</i>	CBS 120359; CD1561	R. D. Reeleder, 1996	<i>Panax quinquefolium</i>	Canada, Ontario	AY295309	JF735404	JF735547	JF735736
<i>Ilyonectria mors-panacis</i>	CBS 120360; CD1567	R. D. Reeleder, 1996	<i>Panax quinquefolium</i>	Canada, Ontario	-	AY297200	-	-
<i>Ilyonectria mors-panacis</i>	CBS 120361; CD1596	R. D. Reeleder, 1996	<i>Panax quinquefolium</i>	Canada, Ontario	JF735280	JF735405	JF735548	JF735737
<i>Ilyonectria mors-panacis</i>	CBS 120362; CD1598	R. D. Reeleder, 1996	<i>Panax quinquefolium</i>	Canada, Ontario	-	AY297202	-	-
<i>Ilyonectria mors-panacis</i>	CBS 120363; CD1635	R. D. Reeleder, 1997	<i>Panax quinquefolium</i>	Canada, Ontario	-	AY297204	-	-
<i>Ilyonectria mors-panacis</i>	CBS 120364; CD1636	R. D. Reeleder, 1997	<i>Panax quinquefolium</i>	Canada, Ontario	JF735281	JF735406	JF735549	JF735738
<i>Ilyonectria mors-panacis</i>	CBS 120365; CD1637	R. D. Reeleder, 1997	<i>Panax quinquefolium</i>	Canada, Ontario	JF735282	JF735407	JF735550	JF735739
<i>Ilyonectria mors-panacis</i>	CBS 120366; CD 1639	R. D. Reeleder, 1997	<i>Panax quinquefolium</i>	Canada, Ontario	JF735283	JF735408	JF735551	JF735740
<i>Ilyonectria mors-panacis</i>	CBS 120367; CD1640	R. D. Reeleder, 1997	<i>Panax quinquefolium</i>	Canada, Ontario	AY295321	JF735409	JF735552	JF735741
<i>Ilyonectria mors-panacis</i>	CBS 120368; CD1641	R. D. Reeleder, 1997	<i>Panax quinquefolium</i>	Canada, Ontario	JF735284	JF735410	JF735553	JF735742
<i>Ilyonectria mors-panacis</i>	CBS 120369; CD1642	R. D. Reeleder, 1997	<i>Panax quinquefolium</i>	Canada, Ontario	JF735285	JF735411	JF735554	JF735743
<i>Ilyonectria mors-panacis</i>	CPC 13535; DAOM 221059; CD 0265	R. D. Reeleder, 1989	<i>Panax quinquefolium</i>	Canada, Ontario	JF735286	JF735412	JF735555	JF735744

**Table 1** (continued)

Species	Strain number <sup>a</sup>	Collected/isolated by, year	Isolated from	Location	GenBank accession numbers			
					ITS	TUB	H3	EFI
<i>Ilyonectria mors-panacis</i>	CPC 13537; DAOM 226727; CD 1570	R. D. Reeleder, 1996	<i>Panax quinquefolium</i>	Canada, Ontario	JF735287	JF735413	JF735556	JF735745
<i>Ilyonectria mors-panacis</i> , type of <i>Ramularia mors-panacis</i>	CBS 306.35	A.A. Hildebrand	<i>Panax quinquefolium</i>	Canada, Ontario	JF735288	JF735414	JF735557	JF735746
<i>Ilyonectria mors-panacis</i>	CBS 307.35	A.A. Hildebrand	<i>Panax quinquefolium</i>	Canada, Ontario	JF735289	JF735415	JF735558	JF735747
<i>Ilyonectria mors-panacis</i> , type of “C.” <i>destructans</i> f.sp. <i>panacis</i>	CBS 124662; NBRC 31881; SUF 811	Y. Miyazawa	<i>Panax ginseng</i>	Japan, Nagano, Kitasakugun	JF735290	JF735416	JF735559	JF735748
<i>Ilyonectria pseudodestructans</i>	CPC 13534; DAOM 150670; Berkenkamp 1	B. Berkenkamp, 1974	<i>Poa pratensis</i>	Canada, Alberta, Lacombe	AY295319	JF735417	JF735560	JF735749
<i>Ilyonectria pseudodestructans</i>	CBS 117812; IFFF 83	E. Halmshlager, 1993	<i>Quercus</i> sp., root	Austria, Patzmannsdorf	JF735291	JF735418	JF735561	JF735750
<i>Ilyonectria pseudodestructans</i>	CBS 117824; IFFF 98	E. Halmshlager, 1993	<i>Quercus</i> sp., root	Austria, Patzmannsdorf	JF735292	JF735419	JF735562	JF735751
<i>Ilyonectria pseudodestructans</i>	CBS 129081; Cy20	C. Rego, 1996	<i>Vitis vinifera</i> , 4-year-old, showing decline symptoms, scion Malvasia Finai; rootstock 1103P	Portugal, Gouveia, São Paio	AJ875330	AM419091	JF735563	JF735752
<i>Ilyonectria pseudodestructans</i>	Cy22	C. Rego, 1996	<i>Vitis vinifera</i> , 5-year-old, showing decline symptoms, scion Aragonéz; rootstock 99R	Portugal, Viseu, Silgueiros	AJ875331	AM419092	JF735564	JF735753
<i>Ilyonectria europaea</i>	Cy131	P. Lecomte & S. Chamont, 2000	<i>Actinidia chinensis</i> ‘Hayward’, internal lesion of stem	France, St. Chieq-du-Gaue	AM419067	AM419103	JF735565	JF735754
<i>Ilyonectria europaea</i>	Cy155	C. Rego & H. Oliveira, 2004	<i>Vitis vinifera</i> , 2-year-old, showing decline symptoms, scion Alfrocheiro; rootstock SO4	Portugal, Alter do Chão	JF735293	JF735420	JF735566	JF735755
<i>Ilyonectria europaea</i>	CBS 129078; Cy241	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Petit Verdoy; rootstock 110R	Portugal, Vidigueira	JF735294	JF735421	JF735567	JF735756
<i>Ilyonectria europaea</i>	CBS 537.92	V. Demoulin, 1992	<i>Aesculus hippocastanum</i> , wood	Belgium, Liège	EF607079	EF607064	JF735568	JF735757
<i>Ilyonectria europaea</i>	CBS 102892; No.5/97-12	W. Leibinger, 1997	<i>Phragmites australis</i> , stem	Germany, Lake Constance	JF735295	JF735422	JF735569	JF735758
<i>Ilyonectria lusitanica</i>	CBS 129080; Cy197	N. Cruz, 2005	<i>Vitis vinifera</i> , below grafting zone, 6-year-old plant; scion Alvarinho; rootstock 196-17	Portugal, Melgaço, Alvaredo	JF735296	JF735423	JF735570	JF735759
<i>Ilyonectria venezuelensis</i>	CBS 102032; ATCC 208837; AR2553	A. Rossman, 1985	Bark	Venezuela, Amazonas, Cerro de la Neblina	AM419059	AY677255	JF735571	JF735760
<i>Ilyonectria panacis</i>	CBS 129079; CDC-N-9a	K. F. Chang, 1998	<i>Panax quinquefolium</i>	Canada, Alberta	AY295316	JF735424	JF735572	JF735761
<i>Ilyonectria liliigena</i>	CBS 189.49; IMI 113882	M.A.A. Schipper	<i>Lilium regale</i> , bulb	Netherlands, Hoom	JF735297	JF735425	JF735573	JF735762
<i>Ilyonectria liliigena</i>	CBS 732.74	G.J. Bollen, 1973	<i>Lilium</i> sp.	Netherlands, Heemskerk	JF735298	JF735426	JF735574	JF735763
<i>Ilyonectria liliigena</i>	CBS 304.85	G.J. Bollen, 1985	<i>Lilium</i> sp., bulb	Netherlands	JF735299	JF735427	JF735575	JF735764
<i>Ilyonectria liliigena</i>	CBS 305.85	G.J. Bollen, 1985	<i>Lilium</i> sp., bulb	Netherlands	JF735300	JF735428	JF735576	JF735765
<i>Ilyonectria gamsii</i>	CBS 940.97	J.T. Poll, 1997	Soil	Netherlands, Lelystad	AM419065	AM419089	JF735577	JF735766
“ <i>Cylindrocarpum</i> ” sp.	Cy228	F. Caetano, 2003	<i>Ficus</i> sp.	Portugal, Lisbon	JF735301	JF735429	JF735578	JF735767
<i>Ilyonectria anthracicola</i>	CBS 564.95; PD 95/1577	R. Pieters, 1995	<i>Anthurium</i> sp., root	Netherlands, Bleiswijk	JF735302	JF735430	JF735579	JF735768
<i>Ilyonectria vitis</i>	CBS 129082; Cy233	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Touriga Nacional; rootstock 110R	Portugal, Vidigueira	JF735303	JF735431	JF735580	JF735769

Table 1 (continued)

Species	Strain number <sup>a</sup>	Collected/isolated by, year	Isolated from	Location	GenBank accession numbers			
					ITS	TUB	H3	
<i>Ilyonectria cyclaminicola</i>	CBS 302.93	M. Hooftman, 1993	<i>Cyclamen</i> sp., bulb	Netherlands, Roslofarendsveen	JF735304	JF735432	JF735581	JF735770
<i>Cylindrocarpon pauciseptatum</i>	CBS 100819; LYN 16202/2	H.M. Dance, 1998	<i>Erica melanthera</i> , root	New Zealand, Tauranga	EF607090	EF607067	JF735582	JF735771
<i>Cylindrocarpon pauciseptatum</i>	CBS 113550	2003	<i>Irits</i> sp., blackening areas in wood and base of trunk	New Zealand, Keesbury Estate	EF607080	EF607069	JF735583	JF735772
<i>Cylindrocarpon pauciseptatum</i>	CBS 120497; KIS 10763	H.-J. Schroers, 2006	<i>Irits</i> sp. brownish spots of healthy looking root of ca. 12-year-old, possibly dead, in vineyard	Slovenia, Mrzljak	EF607085	EF607071	JF735584	JF735773
<i>Cylindrocarpon pauciseptatum</i>	CBS 120498; KIS 10775	M. Žerjav, 2006	<i>Irits</i> sp., decayed secondary roots with black areas of 3-year-old, dead	Slovenia, Ljutomer	EF607087	EF607072	JF735585	JF735774
<i>Cylindrocarpon pauciseptatum</i>	CBS 120499; KIS 10780	M. Žerjav, 2006	<i>Irits</i> sp., decayed secondary roots with black areas of 3-year-old, dead	Slovenia, Ljutomer	EF607084	EF607074	JF735586	JF735775
<i>Cylindrocarpon pauciseptatum</i> , type	CBS 120171; KIS 10467	M. Žerjav, 2005	<i>Irits</i> sp., partly decayed roots of 4-year-old plant, still living but badly shooting; in vineyard	Slovenia, Krško	EF607089	EF607066	JF735587	JF735776
<i>Cylindrocarpon pauciseptatum</i>	CBS 120172; KIS 10729	M. Žerjav, 2006	<i>Irits</i> sp., strongly decayed, blackish brown root of ca. 9-year-old plant, possibly dead; in vineyard	Slovenia, Žužemberk	EF607086	EF607070	JF735588	JF735777
<i>Cylindrocarpon pauciseptatum</i>	CBS 120173; KIS 10468	M. Žerjav, 2005	<i>Irits</i> sp., partly decayed roots of 4-year-old plant, still living but badly shooting; in vineyard	Slovenia, Krško	EF607088	EF607068	JF735589	JF735778
<i>Cylindrocarpon pauciseptatum</i>	Cy196	N. Cruz, 2005	<i>Irits vinifera</i> , basal end of 4-year-old plant; scion Alvarinho; rootstock 196-17	Portugal, Melgaço/Monção	JF735305	JF735433	JF735590	JF735779
<i>Cylindrocarpon pauciseptatum</i>	Cy217	A. Cabral, 2007	<i>Irits vinifera</i> , asymptomatic; scion Gouveio	Portugal, Torres Vedras	JF735306	JF735434	JF735591	JF735780
<i>Cylindrocarpon pauciseptatum</i>	Cy238	C. Rego, 2008	<i>Irits vinifera</i> , basal end of a 2-year-old plant; scion Petit Verdor; rootstock 110R	Portugal, Vidigueira	JF735307	JF735435	JF735592	JF735781
<i>“Cylindrocarpon”</i> sp 1	CBS 162.89	M. Barth, 1988	<i>Hordeum vulgare</i> , root	Netherlands, Noordoostpolder, Marknesse, Lovinkhoeve	AM419060	AM419084	JF735610	JF735799
<i>“Cylindrocarpon”</i> sp 2	Cy108	C. Rego, 1999	<i>Irits vinifera</i> , basal end of a 4-year-old plant showing decline symptoms; scion Aragonez; rootstock SO4	Portugal, Nelas	JF735316	AM419100	JF735611	JF735800
<i>“Cylindrocarpon”</i> sp 2	Cy200	N. Cruz, 2005	<i>Irits vinifera</i> , basal end of a 16-year-old plant; scion Alvarinho; rootstock 196-17	Portugal, Melgaço	JF735317	JF735445	JF735612	JF735801
<i>“Cylindrocarpon”</i> sp 2	CBS 159.34; IMI 113891; MUCL 4084; VKM F-2656	H.W. Wollenweber, 1934		Germany	JF735318	JF735446	JF735613	JF735802
<i>“Cylindrocarpon”</i> sp 2	CBS 173.37; IMI 090176	T.R. Peace, 1937	<i>Pinus laricio</i> , associated with dieback	UK, England, Devon, Haldon	JF735319	JF735447	JF735614	JF735803
<i>“Cylindrocarpon”</i> sp. 3	Cy135	C. Rego & T. Nascimento, 2003	<i>Irits vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	AM419069	AM419105	JF735615	JF735804
<i>“Cylindrocarpon”</i> sp. 3	Cy144	C. Rego & T. Nascimento, 2003	<i>Irits vinifera</i> , grafting zone of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	AM419074	AM419107	JF735616	JF735805
<i>“Cylindrocarpon”</i> sp. 3	CBS 129085; Cy145	C. Rego & T. Nascimento, 2003	<i>Irits vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735320	JF735448	JF735617	JF735806
<i>“Cylindrocarpon”</i> sp. 3	Cy146	C. Rego & T. Nascimento, 2003	<i>Irits vinifera</i> , grafting zone of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735321	JF735449	JF735618	JF735807

**Table 1** (continued)

Species	Strain number <sup>a</sup>	Collected/isolated by, year	Isolated from	Location	GenBank accession numbers			
					ITS	TUB	H3	EFI
" <i>Cylindrocarpum</i> " sp. 3	Cy147	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , grafting zone of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735322	JF735450	JF735619	JF735808
" <i>Cylindrocarpum</i> " sp. 3	Cy148	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735323	JF735451	JF735620	JF735809
" <i>Cylindrocarpum</i> " sp. 3	Cy149	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735324	JF735452	JF735621	JF735810
" <i>Cylindrocarpum</i> " sp. 3	Cy150	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735325	JF735453	JF735622	JF735811
" <i>Cylindrocarpum</i> " sp. 3	Cy151	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , asymptomatic 1.5-year-old plant; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735326	JF735454	JF735623	JF735812
" <i>Cylindrocarpum</i> " sp. 3	Cy152	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , asymptomatic 1.5-year-old plant; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735327	JF735455	JF735624	JF735813
" <i>Cylindrocarpum</i> " sp. 3	Cy153	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , asymptomatic 1.5-year-old plant; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735328	JF735456	JF735625	JF735814
" <i>Cylindrocarpum</i> " sp. 3	Cy243	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old-plant; scion Touriga Nacional; rootstock 110R	Portugal, Vidigueira	JF735329	JF735457	JF735626	JF735815
" <i>Cylindrocarpum</i> " sp. 3	CPC 13539; 94-1685; CFC226730	R. C. Hamelin, 1994	<i>Picea glauca</i>	Canada, Quebec	JF735330	JF735458	JF735627	JF735816
" <i>Cylindrocarpum</i> " sp. 5	Cy133; IAFM Cy9-1	J. Armengol	<i>Vitis vinifera</i>	Spain, Valencia, L'Aleudia	JF735331	JF735459	JF735628	JF735817
" <i>Cylindrocarpum</i> " sp. 5	Cy134; IAFM Cy20-1	J. Armengol	<i>Vitis vinifera</i>	Spain, Ciudad Real, Villanueva de los Ojos	JF735332	AM419104	JF735629	JF735818
" <i>Cylindrocarpum</i> " sp. 5	CBS 129087; Cy159	A. Cabral & H. Oliveira, 2004	<i>Vitis vinifera</i> , basal end of a 3-year-old plant with root discoloration and decline symptoms; scion Sangiovese; rootstock 1103P	Portugal, Alcácer do Sal, Torrão	JF735333	AM419111	JF735630	JF735819
" <i>Cylindrocarpum</i> " sp. 6	CBS 112593; STE-U 3990; C.107	F. Halleen, 2000	<i>Vitis vinifera</i> , roots of an asymptomatic nursery plant; scion Pinotage; rootstock 101-14 Mgt	South Africa, Western Cape, Wellington, Voortrekkers	AY677281	AY677236	JF735631	JF735820
" <i>Cylindrocarpum</i> " sp. 6	CBS 112608; STE-U 3987; C.62	F. Halleen, 2000	<i>Vitis vinifera</i> , roots, scion Chardonnay; rootstock 101-14 Mgt	South Africa, Western Cape, Citrusdal	AY677288	AY677235	JF735632	JF735821
" <i>Cylindrocarpum</i> " sp. 6	CBS 113552; STE-U 5713; HUS-1306; NZ C.41	R. Bonfiglioli, 2003	<i>Vitis</i> sp. decline of nursery plants dead rootstocks	New Zealand, Candy P New Ground	JF735334	AY677237	JF735633	JF735822
" <i>Cylindrocarpum</i> " sp. 6	Cy115	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735335	JF735460	JF735634	JF735823
" <i>Cylindrocarpum</i> " sp. 6	Cy116	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	AJ875322	JF735461	JF735635	JF735824
" <i>Cylindrocarpum</i> " sp. 6	Cy117	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	AJ875321	JF735462	JF735636	JF735825
" <i>Cylindrocarpum</i> " sp. 6	Cy119	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735336	JF735463	JF735637	JF735826
" <i>Cylindrocarpum</i> " sp. 6	Cy124	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735337	JF735464	JF735638	JF735827
" <i>Cylindrocarpum</i> " sp. 6	Cy125	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	AM419066	JF735465	JF735639	JF735828
" <i>Cylindrocarpum</i> " sp. 6	Cy129	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735338	JF735466	JF735640	JF735829
" <i>Cylindrocarpum</i> " sp. 6	Cy130	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735339	JF735467	JF735641	JF735830
" <i>Cylindrocarpum</i> " sp. 6	Cy230	F. Caetano, 2005	<i>Festuca duriuscula</i>	Portugal, Lisbon	JF735340	JF735468	JF735642	JF735831

Table 1 (continued)

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					ITS	TUB	H3	EFI
<i>Ilyonectria macrodidyma</i>	CBS 112594; STE-U 3991; C 111	F. Halleen, 2000	<i>Vitis vinifera</i> , roots of an asymptomatic nursery plant; scion Pinotage; rootstock Richter 99	South Africa, Western Cape, Malmesbury, Jakkalsfontein	AY677282	AY677231	JF735643	JF735852
<i>Ilyonectria macrodidyma</i>	CBS 112601; STE-U 3983; C 82	F. Halleen, 1999	<i>Vitis vinifera</i> , roots with black foot symptoms; scion Pinotage; rootstock US 8-7	South Africa, Western Cape, Tulbagh	AY677284	AY677229	JF735644	JF735833
<i>Ilyonectria macrodidyma</i>	CBS 112603; STE-U 4007; C 8	F. Halleen, 1999	<i>Vitis vinifera</i> , trunk of a plant showing decline symptoms, scion Sauvignon blanc; rootstock Richter 110	South Africa, Western Cape, Darling	AY677285	JF735469	JF735645	JF735834
<i>Ilyonectria macrodidyma</i>	CBS 112605; STE-U 3984; C 106	F. Halleen, 2000	<i>Vitis vinifera</i> , basal end of an asymptomatic nursery plant; scion Sultana; rootstock 143-B Mgt	South Africa, Western Cape, Malmesbury, Jakkalsfontein	AY677287	AY677230	JF735646	JF735835
<i>Ilyonectria macrodidyma</i> , holotype of <i>C. macrodidymum</i>	CBS 112615; STE-U 3976; C 98	F. Halleen, 2000	grapevine plant scion Sultana; rootstock 143-B Mgt	South Africa, Western Cape, Malmesbury, Jakkalsfontein	AY677290	AY677233	JF735647	JF735836
<i>Ilyonectria macrodidyma</i>	Cy123	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735341	JF735470	JF735648	JF735837
<i>Ilyonectria macrodidyma</i>	Cy128	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735342	JF735471	JF735649	JF735838
<i>Ilyonectria macrodidyma</i>	Cy139	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	AM419071	AM419106	JF735650	JF735839
<i>Ilyonectria macrodidyma</i>	Cy140	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , grafting zone of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735343	JF735472	JF735651	JF735840
<i>Ilyonectria macrodidyma</i>	Cy175	C. Rego, 2004	<i>Vitis vinifera</i> , basal discoloration in rootstocks; scion Touriga Nacional; rootstock 1103P	Portugal, Torre de Moncorvo	JF735344	JF735473	JF735652	JF735841
<i>Ilyonectria macrodidyma</i>	Cy181	C. Rego, 2005	<i>Vitis vinifera</i> , scion 140-Ru; rootstock Aragonês	Portugal, Alcácer do Sal	JF735345	JF735474	JF735653	JF735842
<i>Ilyonectria macrodidyma</i>	Cy216	A. Cabral, 2007	<i>Vitis vinifera</i> , asymptomatic; scion Marssanne	Portugal, Torres Vedras	JF735346	JF735475	JF735654	JF735843
<i>Ilyonectria macrodidyma</i>	Cy244	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Petit Verdot; rootstock 110R	Portugal, Vidigueira	JF735347	JF735476	JF735655	JF735844
<i>Ilyonectria macrodidyma</i>	Cy258	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Cabernet Sauvignon; rootstock 110R	Portugal, Vidigueira	JF735348	JF735477	JF735656	JF735845
" <i>Cylindrocarpum</i> " sp.4	CBS 119.41	H.C. Koning	<i>Fragaria</i> sp., root	Netherlands, Baam	JF735349	JF735478	JF735657	JF735846
" <i>Cylindrocarpum</i> " sp.4	CBS 188.49	J.A. von Arx	<i>Abies nordmanniana</i> , root	Netherlands, Egmond	AM419063	AM419087	JF735658	JF735847
" <i>Cylindrocarpum</i> " sp.4	CBS 112604; STE-U 4004; C 10	F. Halleen, 1999	<i>Vitis vinifera</i> , roots; scion Cabernet Sauvignon; rootstock 101-14 Mgt	South Africa, Western Cape, Paarl	AY677286	AY677227	JF735659	JF735848
" <i>Cylindrocarpum</i> " sp.4	CBS 112609; STE-U 3969; HUS-1217	M. Sweetingham, 1979	<i>Vitis</i> sp., dark brown discoloration in trunk; scion Cabernet Sauvignon	Australia, Tasmania, Bream Creek	AY677289	AY677226	JF735660	JF735849
" <i>Cylindrocarpum</i> " sp.4	CBS 113555; STE-U 5715; HUS-1309; NZ C 60	R. Bonfiglioli, 2003	<i>Vitis</i> sp., blackening areas in wood and roots; scion Pinot Noir; rootstock 101-14	New Zealand, Fiddlers Green	JF735350	AY677234	JF735661	JF735850
" <i>Cylindrocarpum</i> " sp.4	CBS 112598; STE-U 3997; C 115	F. Halleen, 2000	<i>Vitis vinifera</i> , roots of an asymptomatic plant; scion Sultana; rootstock Ramsey	South Africa, Western Cape, Wellington, Lellenfontein	JF735351	JF735479	JF735662	JF735851
" <i>Cylindrocarpum</i> " sp.4	CPC 13533; CCFC 144524; Dias 2B	H.F. Dias, 1972	<i>Vitis vinifera</i> , Concord Bratt grapes, roots and stems	Canada, Ontario	AY295332	JF735480	JF735663	JF735852
" <i>Cylindrocarpum</i> " sp.4	Cy69	C. Rego, 1999	<i>Vitis vinifera</i> , asymptomatic rootstocks; rootstock SO4, clone 102 F	Portugal, Ribatejo e Oeste	AJ875332	AM419095	JF735664	JF735853
" <i>Cylindrocarpum</i> " sp.4	Cy71	C. Rego, 1999	<i>Vitis vinifera</i> , asymptomatic rootstocks; rootstock 99R, clone 96 F	Portugal, Ribatejo e Oeste	AJ875335	AM419096	JF735665	JF735854



**Table 1** (continued)

Species	Strain number <sup>a</sup>	Collected/isolated by, year	Isolated from	Location	GenBank accession numbers			
					ITS	TUB	H3	EFI
" <i>Cylindrocarpum</i> " sp.4	Cy72	C. Rego, 1999	<i>Vitis vinifera</i> , asymptomatic rootstocks; rootstock clone 113 F	Portugal, Ribatejo e Oeste	AJ875336	AM419097	JF735666	JF735855
" <i>Cylindrocarpum</i> " sp.4	Cy75	C. Rego, 1999	<i>Vitis vinifera</i> , asymptomatic rootstocks; rootstock 99R	Portugal, Ribatejo e Oeste	AJ875334	AM419098	JF735667	JF735856
" <i>Cylindrocarpum</i> " sp.4	Cy96	E. Halm-schlager	<i>Quercus</i> sp., root	Austria, Patzmannsdorf	JF735352	JF735481	JF735668	JF735857
" <i>Cylindrocarpum</i> " sp.4	Cy97	E. Halm-schlager	<i>Quercus</i> sp., root	Austria, Patzmannsdorf	JF735353	JF735482	JF735669	JF735858
" <i>Cylindrocarpum</i> " sp.4	Cy118	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	JF735354	JF735483	JF735670	JF735859
" <i>Cylindrocarpum</i> " sp.4	Cy120	W.D. Gubler	<i>Vitis vinifera</i>	USA, California	AJ875320	AM419101	JF735671	JF735860
" <i>Cylindrocarpum</i> " sp.4	Cy132; IAFM Cy1-1	J. Armengol	<i>Vitis vinifera</i>	Spain, Alicante	JF735355	JF735484	JF735672	JF735861
" <i>Cylindrocarpum</i> " sp.4	Cy136	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735356	JF735485	JF735673	JF735862
" <i>Cylindrocarpum</i> " sp.4	Cy137	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	AM419070	JF735486	JF735674	JF735863
" <i>Cylindrocarpum</i> " sp.4	Cy138	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735357	JF735487	JF735675	JF735864
" <i>Cylindrocarpum</i> " sp.4	Cy141	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735358	JF735488	JF735676	JF735865
" <i>Cylindrocarpum</i> " sp.4	Cy142	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , grafting zone of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735359	JF735489	JF735677	JF735866
" <i>Cylindrocarpum</i> " sp.4	Cy143	C. Rego & T. Nascimento, 2003	<i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309 C	Portugal, Estremoz	JF735360	JF735490	JF735678	JF735867
" <i>Cylindrocarpum</i> " sp.4	Cy157	H. Oliveira, 2004	<i>Vitis vinifera</i> , scion Touriga Nacional; rootstock 99R	Portugal, Alenquer	AM419077	AM419110	JF735679	JF735868
" <i>Cylindrocarpum</i> " sp.4	Cy214	A. Cabral, 2007	<i>Vitis vinifera</i> , asymptomatic; scion Grenache	Portugal, Torres Vedras	JF735361	JF735491	JF735680	JF735869
" <i>Cylindrocarpum</i> " sp.4	CBS 129086; Cy218	A. Cabral, 2007	<i>Vitis vinifera</i> , asymptomatic; scion Chenin	Portugal, Torres Vedras	JF735362	JF735492	JF735681	JF735870
" <i>Cylindrocarpum</i> " sp.4	Cy221	L. Leandro	<i>Fragaria x ananassa</i>	USA, North Caroline, Asheville	JF735363	JF735493	JF735682	JF735871
" <i>Cylindrocarpum</i> " sp.4	Cy222	L. Leandro	<i>Fragaria x ananassa</i>	USA, North Caroline, Asheville	JF735364	JF735494	JF735683	JF735872
" <i>Cylindrocarpum</i> " sp.4	Cy223	L. Leandro	<i>Fragaria x ananassa</i>	USA, North Caroline, Asheville	JF735365	JF735495	JF735684	JF735873
" <i>Cylindrocarpum</i> " sp.4	Cy235	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Cabernet Sauvignon; rootstock 110R	Portugal, Vidigueira	JF735366	JF735496	JF735685	JF735874
" <i>Cylindrocarpum</i> " sp.4	Cy237	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Chardonnay; rootstock 110R	Portugal, Vidigueira	JF735367	JF735497	JF735686	JF735875
" <i>Cylindrocarpum</i> " sp.4	Cy240	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Touriga Nacional; rootstock 140RU	Portugal, Vidigueira	JF735368	JF735498	JF735687	JF735876
" <i>Cylindrocarpum</i> " sp.4	Cy246	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Antão Váz; rootstock 110R	Portugal, Vidigueira	JF735369	JF735499	JF735688	JF735877
" <i>Cylindrocarpum</i> " sp.4	Cy260	C. Rego, 2008	<i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Cabernet Sauvignon; rootstock 110R	Portugal, Vidigueira	JF735370	JF735500	JF735689	JF735878

Table 1 (continued)

Species	Strain number <sup>a</sup>	Collected/isolated by, year	Isolated from	Location	GenBank accession numbers			
					ITS	TUB	H3	
" <i>Cylindrocarpon</i> " sp.4	Cy262	C. Rego, 2008	<i>Itis vinifera</i> , basal end of a 2-year-old plant; scion Cabernet Sauvignon; rootstock 110R	Portugal, Vidigueira	JF735371	JF735501	JF735690	JF735879
<i>Neonectria major</i> , type strain	CBS 240.29; IMI 113909	H.W. Wollenweber	<i>Alnus incana</i> , canker	Norway	JF735308	DQ789872	JF735593	JF735782
<i>Neonectria ditissima</i> , authentic strain of	CBS 226.31; IMI 113922	H.W. Wollenweber	<i>Fagus sylvatica</i>	Germany, Tharandt	JF735309	DQ789869	JF735594	JF735783
<i>C. willkommii</i>								
<i>Neonectria ditissima</i> , representative strain of <i>N. galligena</i>	CBS 835.97	W. Gams, 1997	<i>Salix cinerea</i> , dead branch of still living tree	Belgium, Marais de Sampant	JF735310	DQ789880	JF735595	JF735784
<i>Neonectria ditissima</i>	Cy169	H. Oliveira, 1997	<i>Malus domestica</i>	Portugal, Alcobaça	AM419080	AM419113	JF735596	JF735785
<i>Neonectria ditissima</i>	Cy172	T. Nascimento, 2004	<i>Malus domestica</i> ; scion Oregon; rootstock MM107	Portugal, Caldas da Rainha	AM419081	AM419114	JF735597	JF735786
<i>Neonectria neomacrospora</i> , representative strain	CBS 118984; GJS 03-28	L. Reitman, 2005	<i>Arcuthobium tsugense</i> , parasitizing <i>Abies balsams</i>	Canada, British Columbia, Vancouver Island, Spider Lake	JF735311	DQ789882	JF735598	JF735787
<i>Cylindrocarpon cylindroides</i> , representative strain	CBS 324.61; DSM 62489; IMB 9628	J.A. von Arx	<i>Abies concolor</i>	Netherlands, Zwolle	JF735312	DQ789875	JF735599	JF735788
<i>Cylindrocarpon cylindroides</i>	CBS 503.67	F. Roll-Hansen	<i>Abies alba</i> , wood	Norway, Hordaland, Fana	AY677261	JF735436	JF735600	JF735789
<i>Cylindrocarpon</i> sp.	CPC 13545; DAOM 185212; # 5	J.A. Traquair & B. Harrison, 1982	<i>Pyrus</i> sp.	Canada, Ontario, Harrow	AY295303	JF735437	JF735601	JF735790
<i>Neonectria ramulariae</i> , authentic strain of <i>C. obtusiusculum</i> (= <i>C. magnusianum</i> )	CBS 151.29; IMI 113894; MUCL 28083; MUCL 28094	H.W. Wollenweber	<i>Malus sylvestris</i> , fruit	UK, England, Cambridge	JF735313	JF735438	JF735602	JF735791
<i>Neonectria ramulariae</i>	CBS 182.36; IMI 113893; UPSC 1903	H.W. Wollenweber	<i>Malus sylvestris</i> , fruit	-	JF735314	JF735439	JF735603	JF735792
<i>Cylindrocarpon</i> sp.	CR21	P. Axelrood	<i>Pseudotsuga menziesii</i>	Canada, British Columbia	JF735315	JF735440	JF735604	JF735793
<i>Cylindrocarpon</i> sp.	CPC 13530; DAOM 185722; JAT 1591	J.A. Traquair, 1983	<i>Pyrus</i> sp., lesions on seedlings	Canada, Ontario, Harrow	AY295302	JF735441	JF735605	JF735794
<i>Cylindrocarpon</i> sp.	CPC 13531; CCFC 226722; DAOM 226722; CR6	P. Axelrood	<i>Pseudotsuga menziesii</i> , root	Canada, British Columbia	AY295301	JF735442	JF735606	JF735795
<i>Cylindrocarpon obtusisporum</i>	CBS 183.36; IMI 113895	H.W. Wollenweber, 1936	<i>Solanum tuberosum</i> , tuber	Germany	AM419061	AM419085	JF735607	JF735796
<i>Cylindrocarpon obtusisporum</i>	CPC 13544; DAOM 182772; JAT 1366	J.A. Traquair, 1982	<i>Prunus armenica</i> , twigs	Canada, Ontario, Ruthven	AY295306	JF735443	JF735608	JF735797
<i>Cylindrocarpon obtusisporum</i>	94-1356	R. C. Hamelin, 1994	<i>Picea mariana</i>	Canada, Quebec	AY295304	JF735444	JF735609	JF735798

<sup>a</sup> ATCC American Type Culture Collection, USA; CBS CBS-KNAW Fungal Biodiversity Centre (Centraalbureau voor Schimmelcultures), Utrecht, The Netherlands; CCFC Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; CPC Culture collection of Pedro 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 Gary J. Samuels collection; HJS Hans-Josef Schroers collection; IAFM Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Spain; IAM Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan; ICMP International Collection of Microorganisms from Plants, Auckland, New Zealand; IFFF Institute of Forest Entomology, Forest Pathology and Forest Protection, Austria; IMI International Mycological Institute, CABI-Bioscience, Egham, Boreham Lane, U.K.; JAT J. A. Traquair collection; JCM Japan Collection of Microorganisms, Japan; 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; STE-U Stellenbosch University, South Africa; UPSC Fungal Culture Collection at the Botanical Museum, Uppsala University, Uppsala, Sweden; VKM All-Russian Collection of Microorganisms, Russia.

UK), 1.5 mM MgCl<sub>2</sub>, 32 μM of each dNTPs, 0.24 μM of each primer, 0.5 units *Taq* DNA Polymerase (Bioline), and 1 μl of diluted gDNA in a final volume of 12.5 μl. The cycle conditions in a iCycler thermocycler (BioRad, Hercules, USA) were 94°C for 5 min, followed by 40 cycles at 94°C for 30 s, 52°C for 30 s and 72°C for 80 s, and a final elongation at 72°C for 10 min. Primers were V9G (de Hoog and Gerrits van den Ende 1998) and ITS4 (White et al. 1990) for ITS, T1 (O'Donnell and Cigelnik 1997) and Bt-2b (Glass and Donaldson 1995) for TUB, CYLH3F and CYLH3R (Crous et al. 2004b) for HIS, and EF1 and EF2 (O'Donnell et al. 1998) or CyleF-1 (5'- ATG GGT AAG GAV GAV AAG AC-3'; J.Z. Groenewald, unpublished) and CyleF-R2 (Crous et al. 2004b) for TEF. For TEF, the following modifications were made to the amplification protocol: 2.0 mM of MgCl<sub>2</sub>, 40 μM of each dNTPs and addition of 5% of Dimethyl sulfoxide (DMSO; Sigma-Aldrich, Zwijndrecht, Netherlands).

After confirmation by agarose gel electrophoresis, amplicons were sequenced in both directions with the corresponding PCR primers and a DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Diegem, Belgium) according to the manufacturer's recommendations. The products were analysed on an ABI Prism 3700 DNA Sequencer (Perkin-Elmer, Waltham, USA).

Sequences were assembled and edited to resolve ambiguities, using the EditSeq and SeqMan modules of the Lasergene software package (DNASTar, Madison, USA). Consensus sequences for all isolates were compiled into a single file (Fasta format) and aligned using CLUSTAL X v. 2.0.11 (Larkin et al. 2007). Following manual adjustment of the alignment by eye where necessary, the alignment was subjected to phylogenetic analyses as described by Crous et al. (2004b). Novel sequences were lodged in GenBank (Table 1), taxonomic novelties in MycoBank (Crous et al. 2004a), and the alignments and phylogenetic trees in TreeBASE (<http://www.treebase.org>).

### Morphology

Isolates were grown for up to 5 weeks at 20°C on synthetic nutrient poor agar (SNA; Nirenberg 1976) with and without two 1-cm<sup>2</sup> filter paper pieces, carnation leaf agar (CLA; Crous et al. 2009b), potato-dextrose agar (PDA; Difco, Detroit, USA) and oatmeal agar (OA; Crous et al. 2009b) under continuous n-UV light (NUV, 400–315 nm; Blacklight-Blue; Sylvania, Capelle a/d IJssel, Netherlands).

Measurements were done on a 1-cm<sup>2</sup> agar plug removed from the colony margin, placed on a microscope slide, to which a drop of water and coverslip were added. For each isolate, 30 measurements were obtained for each structure. Measurements were done at ×1,000 magnification using a Nikon Eclipse 80i microscope, or a Leica DM2500. Images

were captured using a Nikon DS-Fi1 digital camera with NIS-Elements Software, or a Leica DFC295 digital camera with the Leica Application Suite. Measurements for length and width of conidia and ascospores are given as (Minimum) Lower Limit of a 95% Confidence Interval – Upper Limit of a 95% Confidence Interval (Maximum). For other measurements, only the extreme values are given.

Culture characteristics (texture, density, colour, growth front, transparency and zonation) were described on PDA after incubation at 20°C in the dark for 14 days. Colour (surface and reverse) was described using the colour chart of Rayner (1970). Cardinal temperatures for growth were assessed by inoculating 90-mm-diam PDA dishes with a 3-mm-diam plug cut from the edge of an actively growing colony. Growth was determined after 7 days in two orthogonal directions. Trials were conducted at various temperatures (4, 10, 15, 18, 20, 22, 25, 30 and 35°C) with three replicate plates per strain at each temperature.

To induce the formation of perithecia, isolates were crossed in 60-mm-diam Petri dishes containing a minimal salts medium supplemented with two sterile birch toothpicks (Guerber and Correll 2001). The plates were incubated at 20°C under n-UV light for 8–20 weeks. Two strains were considered sexually compatible if perithecia were formed that exuded masses of viable ascospores. The colour reaction of the perithecia was checked in 3% KOH and in lactic acid. For sectioning, perithecia were mounted in Jung Tissue Freezing Medium (Leica) or in Arabian Gum, and cut in 10- to 15-μm-thick sections using a Leica cryostat CM3050 S or CM1850 at –20°C.

## Results

### Phylogeny

Amplification products of approximately 700 bases (ITS), 650 bases (TUB), 500 bases (HIS) and 600–800 bases (TEF) were obtained for the isolates listed in Table 1. The manually adjusted combined alignment contains 189 sequences (including the two outgroup sequences) and the statistical parameters for the combined and individual analyses are presented in Table 2. For the combined analysis, only a maximum of 1,000 equally most parsimonious trees were saved, the first of which is presented as Fig. 1. Phylogenetic trees derived from the individual loci are available in TreeBASE. The combined analysis of the four genes enabled the identification of 37 species. However, the analysis of HIS data alone was enough to resolve these taxa. Sequences of TEF could not distinguish species 6, *I. robusta*, *I. europaea*, *I. lusitanica*, *I. rufa* and *N. ditissima*; whereas sequences of TUB could not separate *I. robusta*, species 4, and 6, while "*I.*" *macro-*

**Table 2** Statistical information on the individual datasets and number of equally most parsimonious trees for each locus [Internal Transcribed Spacers (ITS) of the nuclear ribosomal RNA gene operon, andpartial  $\beta$ -tubulin (*TUB*), histone H3 (*HIS*) and translation elongation factor 1- $\alpha$  (*TEF*) genes]

	ITS	TUB	HIS	TEF	Combined
Aligned characters (including gaps)	475	502	440	696	2,113
Parsimony-informative characters	122	212	215	364	913
Variable and parsimony-uninformative characters	31	38	11	43	123
Constant characters	322	252	214	289	1,077
Equally most parsimonious trees obtained	136	384	1	60	1,000
Tree length	294	603	1,095	1,149	3,259
Consistency index (CI)	0.718	0.660	0.468	0.611	0.559
Retention index (RI)	0.978	0.972	0.946	0.966	0.959
Rescaled Consistency index (RC)	0.702	0.642	0.442	0.590	0.537

*didyma*, species 5, *I. liliigena* and *I. pseudodestructans* were supported by low bootstrap values, and CBS 120370 clustered apart from the remaining isolates of *I. crassa*. Of all loci screened, ITS proved to be the least informative, being unable to resolve 22 of the species in this study. Neighbour-Joining (NJ) analyses using the three substitution models, as well as the parsimony analysis, yielded trees with similar topology and bootstrap support values for the individual and combined gene analyses. The trees obtained supported the same clades, sometimes with rearrangements in the order of these clades between the different analyses (data not shown). The results of the phylogenetic analyses are highlighted below under the taxonomic notes or in the Discussion, where applicable.

### Taxonomy

The present study treats isolates that have been freshly collected, or previously identified and maintained in culture collections as “*Cylindrocarpon destructans*”, meaning cylindrical, rarely curved, 3-septate macroconidia with obtuse apices, abundant microconidia and chlamydo-spores (Samuels and Brayford 1990). The latter species has in the past been acknowledged as anamorph of *I. radicola* (Booth 1966; Chaverri et al. 2011; Samuels and Brayford 1990). However, an examination of the neotype of “*C.*” *destructans* in this study [CUP-011985, conidia (18.0)23.0–30.0(35.0)  $\times$  (6.0)6.5(7.0)  $\mu$ m], found conidia to be considerably smaller than those of *I. radicola* (24.0)33.1 (47.0)  $\times$  (4.9)6.4(7.8)  $\mu$ m (Gerlach and Nilsson 1963) (also confirmed in the present study by examination of CBS 264.65, ex-type), revealing them to represent two distinct species. Furthermore, based on the phylogenetic and morphological data obtained in the present study, several novel species could be distinguished that are phylogenetically distinct from *I. radicola*, and morphologically distinct based on a range of characters linked to culture

characteristics, conidiophores, macro- and microconidium morphology. Some of these could be linked to older names, or taxa long regarded as potential synonyms of “*destructans*”, which could now be resurrected. These taxa are treated below:

### *Ilyonectria anthuriicola* A. Cabral & Crous, sp. nov. (Fig. 2)

Mycobank 560108.

*Etymology*: Named after its host, *Anthurium*.

*Cylindrocarpi destructantis* morphologicis simile, sed longitudine media conidiorum longiore, 29.5–32.2  $\mu$ m, distinguitur.

*Conidiophores* simple or complex to sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched bearing up to three phialides, 1–3-septate, 40–95  $\mu$ m long; phialides monophialidic, more or less cylindrical but slightly tapering towards the tip, 10.5–20.5  $\mu$ m long, 2.5–3.5  $\mu$ m wide at the base, 3.0–4.5  $\mu$ m at widest point, 1.5–2.5  $\mu$ m near the aperture. Conidiophores giving rise to microconidia, formed on mycelium at agar surface, penicillately mono- or bi-verticillate; phialides monophialidic, narrowly flask-shaped, typically with widest point near the middle, 8–15  $\mu$ m long, 2.0–3.0  $\mu$ m wide at the base, 2.5–4.5  $\mu$ m at widest point, 1.0–2.0  $\mu$ m near the apex. *Sporodochial conidiophores* irregularly branched; phialides cylindrical, mostly widest near the middle. *Macroconidia* formed in flat domes of slimy masses, (1–)

**Fig. 1** The first of 1,000 equally most parsimonious trees obtained from the combined ITS, TUB, HIS and TEF sequence alignment of *Cylindrocarpon* isolates and relatives with a heuristic search using PAUP v. 4.0b10. The tree was rooted using *Campylocaupon* isolates as outgroup sequences and bootstrap support values are indicated near the nodes, where “ns” designates not supported. Ex-type strains are indicated in *bold*. Newly described species are indicated by blue boxes. Scale bar shows 10 changes

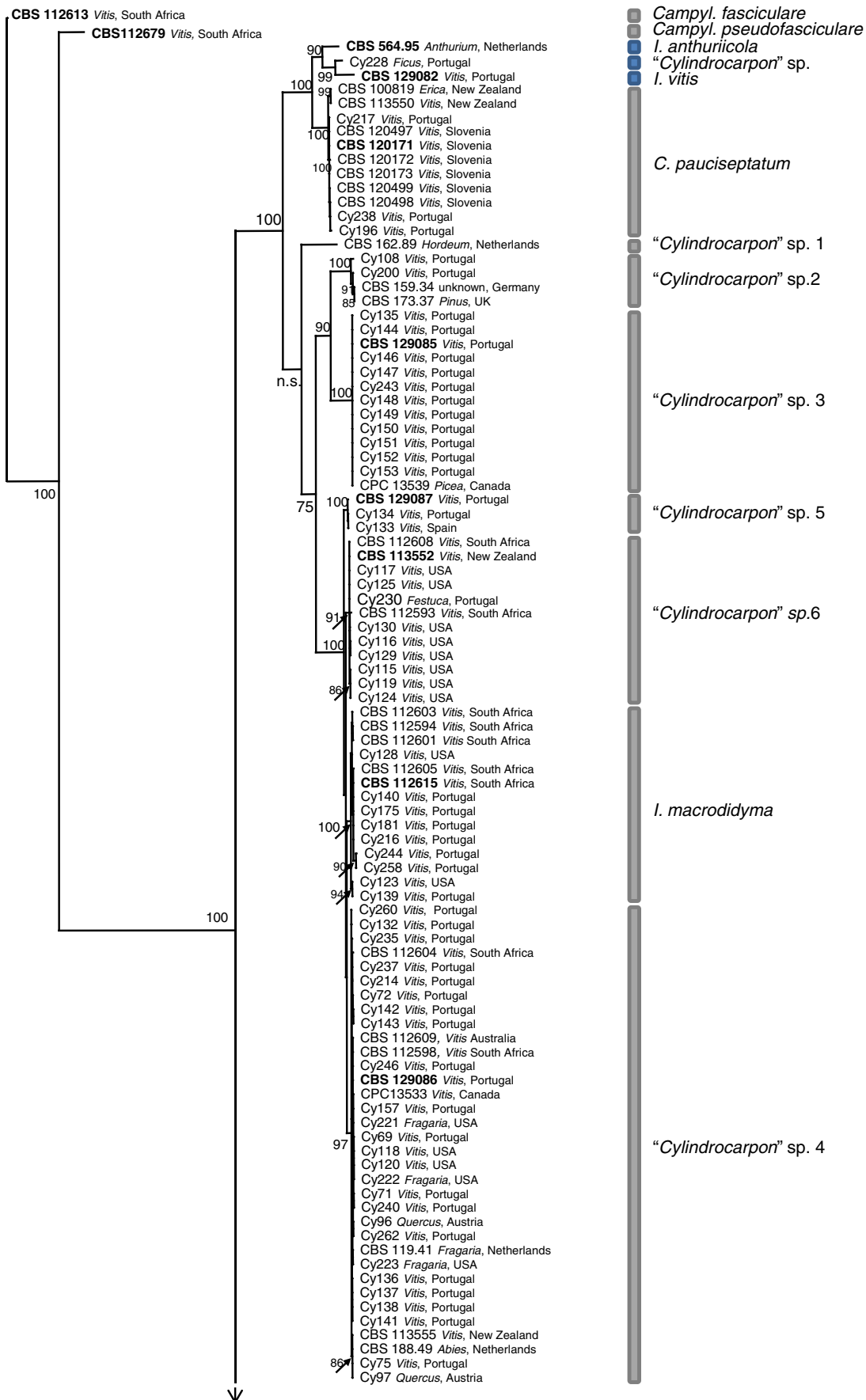
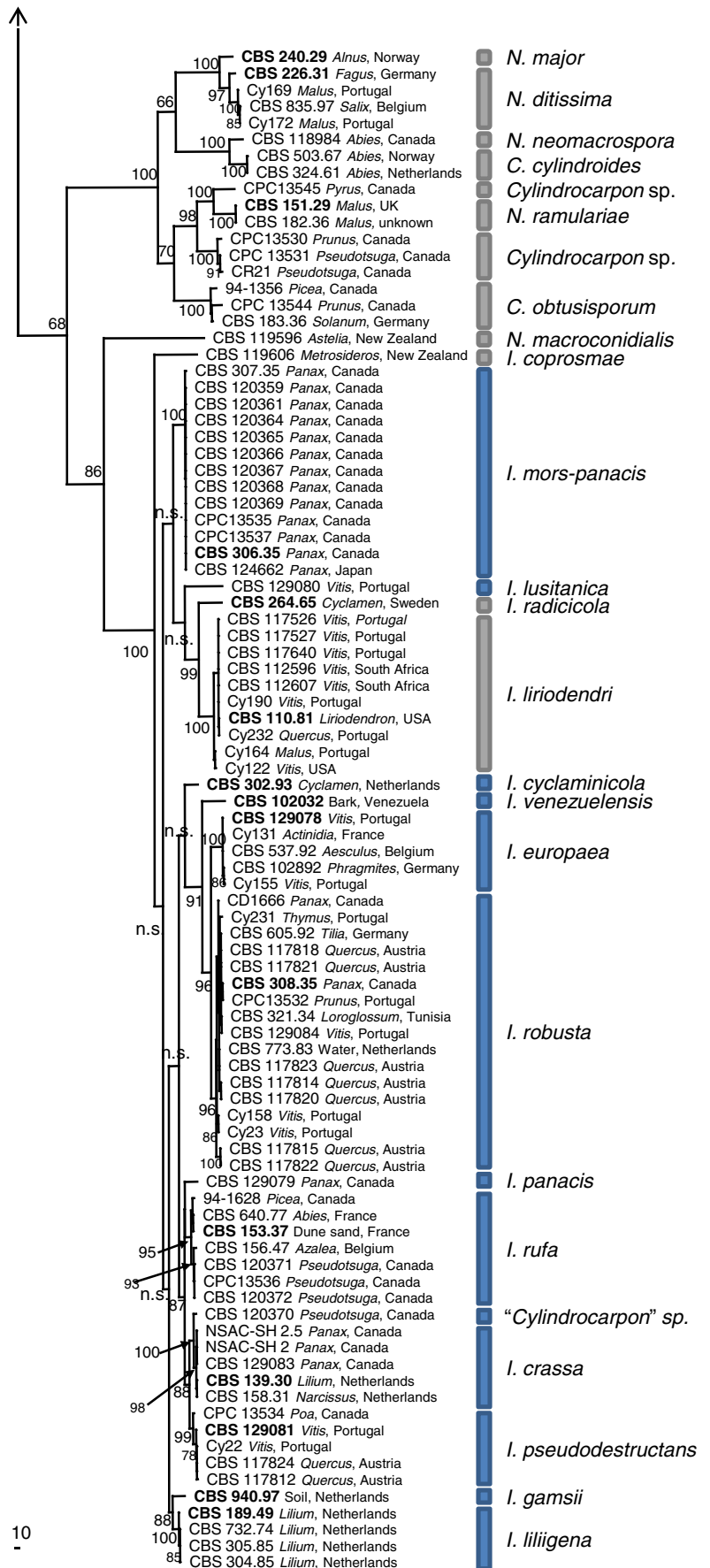
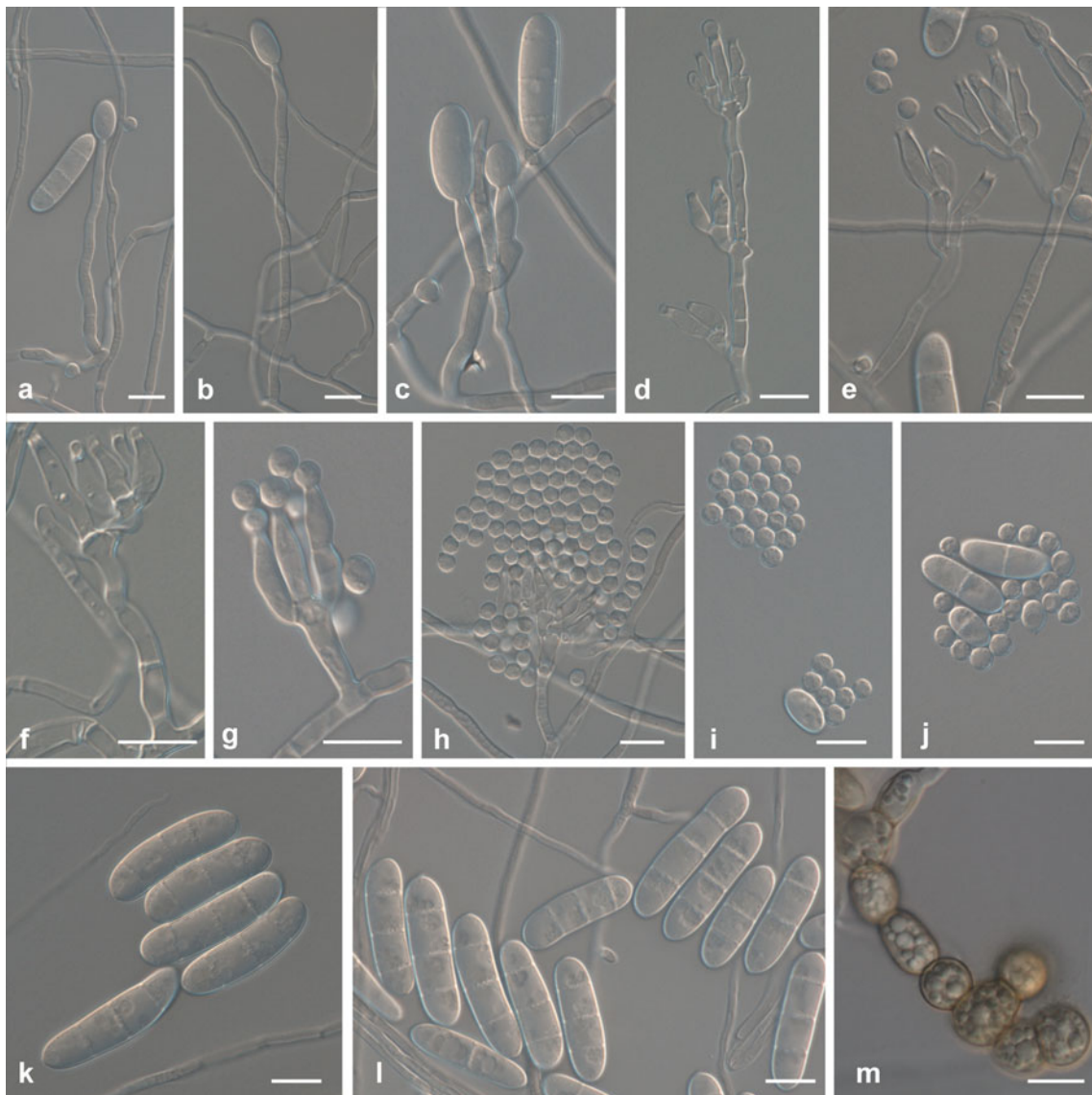


Fig. 1 (continued)





**Fig. 2** *Ilyonectria anthuriicola* (CBS 564.95). **a–c** Simple conidiophores on aerial mycelium. **d–g** Conidiophores giving rise to microconidia, formed on mycelium at agar surface, penicillately mono- or

bi-verticillate. **h–l** Micro- and macroconidia. **m** Chlamydospores in mycelium. Bars 10  $\mu\text{m}$

3-septate, straight or minutely curved, cylindrical with both ends more or less obtusely rounded, mostly without a visible hilum; 1-septate, (20.0)23.5–26.7(29.0)  $\times$  (5.5)5.9–6.8(7.0)  $\mu\text{m}$  (average = 25.1  $\times$  6.4  $\mu\text{m}$ ), with a length:width ratio of 3.6–4.8; 2-septate, (25.0)26.6–29.3(32.0)  $\times$  (6.5)6.8–7.8(8.5)  $\mu\text{m}$  (av. = 27.9  $\times$  7.3  $\mu\text{m}$ ), with a length:width ratio of 3.2–4.8; 3-septate, (25.0)29.5–32.2(38.0)  $\times$  (6.0)7.5–8.1(9.0)  $\mu\text{m}$  (av. = 30.8  $\times$  7.8  $\mu\text{m}$ ) with a length:width ratio of 3.1–5.2. *Microconidia* 0(–1)-septate, subglobose to ovoid, rarely ellipsoid, mostly with a visible centrally located or slightly laterally displaced hilum; aseptate microconidia, (4.9)5.0–8.1(12.0)  $\times$  (4.0)4.3–5.5(6.5)  $\mu\text{m}$  (av. = 6.5  $\times$  4.9  $\mu\text{m}$ ), with a length:width ratio of 1.0–1.8; 1-septate, (11.0)11.6–16.7(18.0)  $\times$  (5.0)5.4–6.1(6.0)  $\mu\text{m}$

(av. = 14.1  $\times$  5.8  $\mu\text{m}$ ), with a length:width ratio 1.8–3.0. *Chlamydospores* globose to subglobose to ellipsoid, 8–14  $\times$  7–12  $\mu\text{m}$ , smooth, but often appearing rough due to deposits, thick-walled, formed intercalary in chains or in clumps and also in the cells of macroconidia, hyaline, becoming golden-brown.

*Holotype*: Netherlands, Bleiswijk, root rot of *Anthurium* sp., 1995, coll./isol. R. Pieters, holotype CBS H-20555, culture ex-type CBS 564.95.

*Culture characteristics*: Mycelium felty with average density. Surface on OA chestnut, with aerial mycelium sparse, saffron; margin pure yellow to orange. Surface on PDA, chestnut with saffron aerial mycelium, growth at margin luteous; zonation absent, transparency homogeneous, margin

even; reverse similar to surface, but chestnut to cinnamon on OA, and chestnut on PDA. Colonies on PDA do not grow at 4°C after 7 days. Optimum temperature 20°C when colonies reach 25–27 mm, after 7 days. Colony diam was 20–22 mm at 25°C, after 7 days. Hardly grows at 30°C (2 mm colony diam after 7 days).

*Isolate studied*: CBS 564.95 (Table 1).

*Host and distribution*: Roots of *Anthurium* sp. (Netherlands).

***Ilyonectria crassa*** (Wollenw.) A. Cabral & Crous, *comb. et stat. nov.* (Fig. 3)

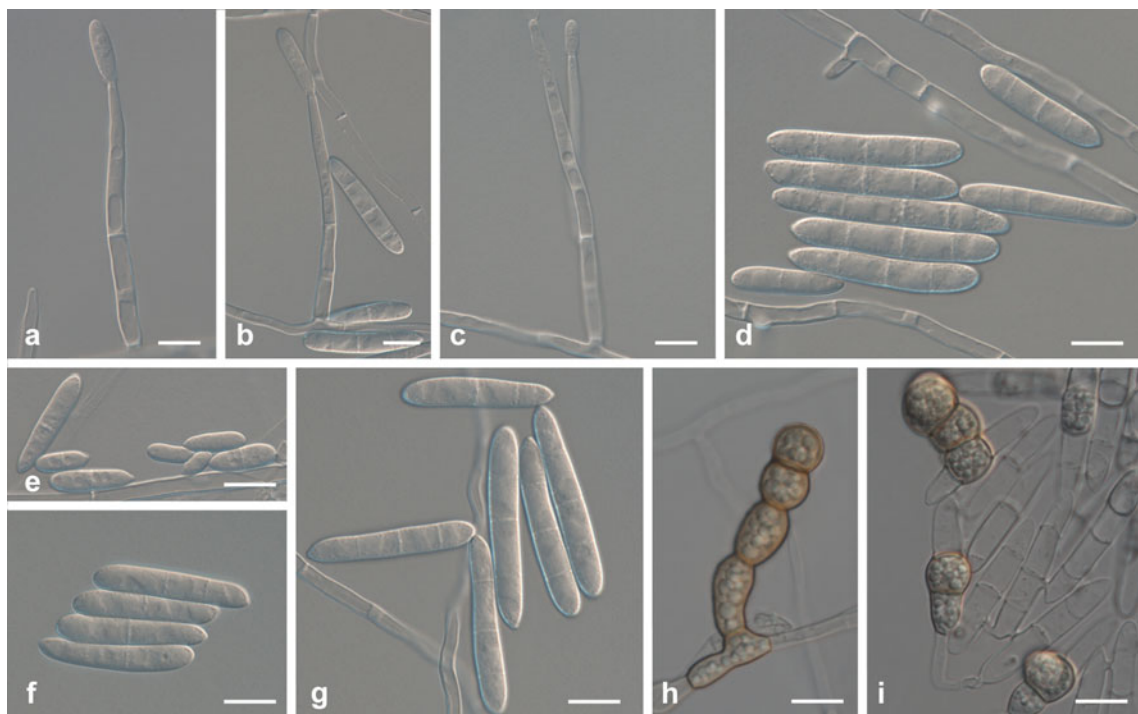
Mycobank 560109.

*Basionym*: *Cylindrocarpon radicolica* var. *crassum* Wollenw., Z. Parasitenkunde 3: 495. 1931.

≡ *Cylindrocarpon destructans* var. *crassum* (Wollenw.) C. Booth, Mycol. Pap. 104: 37. 1966.

*Conidiophores* simple or complex, to sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched bearing up to two phialides, rarely consisting only of phialides, 1–4-septate, 40–180 µm long; phialides monophialidic, cylindrical to subulate, 20–55 µm long, 2.5–4.0 µm wide at the base, 1.5–2.0 µm near the apex. *Complex conidiophores* aggregated in small sporodochia (on carnation leaf), repeatedly and irregularly

branched; phialides more or less cylindrical, but tapering slightly in the upper part towards the apex, or narrowly flask-shaped, mostly with widest point near the middle, 17–24 µm long, 2.0–3.0 µm wide at the base, 2.5–3.5 µm at the widest point, and 1.5–2.5 µm wide near the apex. *Macroconidia* predominating, formed on both type of conidiophores, on SNA formed in flat domes of slimy masses, 1–3-septate, straight, cylindrical, but may narrow towards the tip, more or less broadly rounded, and the base appearing somewhat acute due to the presence of the hilum, mostly centrally located; 1-septate, (21.0)25.7–27.3(34.0) × (4.5)5.0–5.3(6.5) µm (av. = 26.5 × 5.1 µm), with a length:width ratio of 3.8–6.7; 2-septate, (23.0)28.5–30.3(37.0) × (4.5)5.3–5.6(6.5) µm (av. = 29.4 × 5.4 µm) with a length:width ratio of 4.2–6.7; 3-septate, (29.0)34.1–36.0(49.0) × (5.0)5.6–5.8(7.0) µm (av. = 35.1 × 5.7 µm), with a length:width ratio of 4.8–8.9. *Microconidia* 0–1-septate, ellipsoid to subcylindrical, more or less straight, with a visible, truncate hilum; aseptate microconidia, (7.0)9.7–10.9 (15.0) × (3.0)3.3–3.6(4.5) µm (av. = 10.3 × 3.5 µm), with a length:width ratio of 1.8–4.3; 1-septate, (12.0)14.2–15.2 (19.0) × (3.0)3.8–4.2(5.0) µm (av. = 14.7 × 4.0 µm), with a length:width ratio 2.7–5.0. *Conidia* formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses. *Chlamydospores* globose to subglobose to cylindrical, 7–15 × 6–10 µm, smooth, but often appearing rough due to deposits, thick-walled, terminal on short



**Fig. 3** *Ilyonectria crassa* (CBS 129083). **a–c** Simple conidiophores on aerial mycelium. **d–g** Micro- and macroconidia. **h–i** Chlamydospores and macroconidia. Bars 10 µm



lateral branches, rarely intercalary, single, in chains or in clumps, and also in the cells of the macroconidia, hyaline, becoming pale brown.

**Lectotype:** The Netherlands, on *Lilium* bulbs, Dec. 1930, coll./isol. W.F. van Hell, lectotype designated here CBS H-20556, culture ex-lectotype CBS 139.30.

**Culture characteristics:** Mycelium cottony to felty with average to strong density. Surface on OA cinnamon, with aerial mycelium sparse, buff. Surface on PDA saffron with aerial mycelium sparse buff to saffron to pale luteous. No zonation was observed, transparency was homogeneous and growth at margin even. Reverse similar to surface, except in colour, saffron to cinnamon on OA, and chestnut to sienna on PDA. Colonies on PDA grow 5–8 mm diam at 4°C after 7 days. Optimum temperature at 20°C, when colonies reach 31–46 mm diam, after 7 days. Colony diam was 19–34 mm at 25°C, after 7 days. No growth was observed at 30°C.

**Isolates studied:** CBS 139.30; CBS 158.31; CBS 129083; NSAC-SH-2; NSAC-SH-2.5 (Table 1).

**Hosts and distribution:** *Lilium* sp. (bulbs), *Narcissus* sp. (roots) (Netherlands), *Panax quinquefolium* (roots) (Canada).

**Notes:** In the original description, Wollenweber (1931) cites *Cylindrocarpon radicolica* var. *crassum* as occurring on roots of *Ulmus*, *Taxus* and *Lilium* in Europe (Germany and the Netherlands). He did not designate any type specimen. However, he specifically refers to a culture sent to him by Prof. J. Westerdijk on *Lilium* from the CBS in the

Netherlands in 1930, which was regarded as authentic for the species. This culture is represented by CBS 139.30 (accessioned in 1930, from *Lilium*, the Netherlands), and thus we designate a dried, sporulating culture as lectotype for the species.

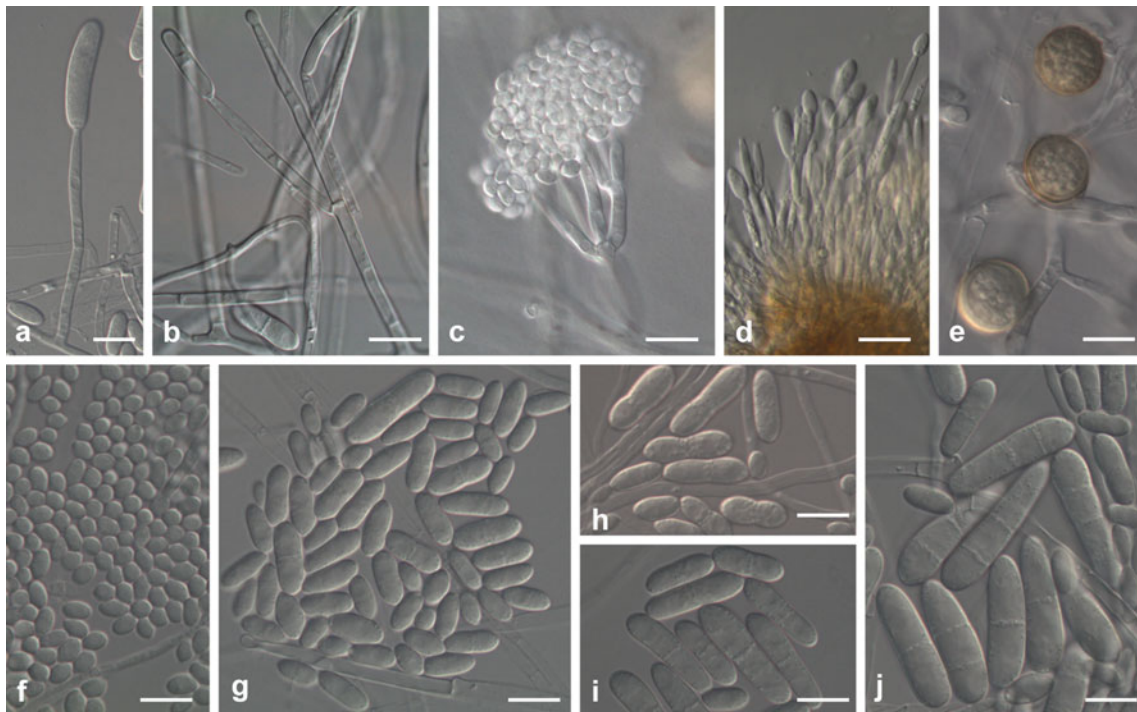
***Ilyonectria cyclaminicola* A. Cabral & Crous, sp. nov.**  
(Fig. 4)

Mycobank 560110.

**Etymology:** Named after the host from which it was isolated, *Cyclamen* sp.

*Cylindrocarpi destructantis* morphologicis simile, sed longitudine media conidiorum longiore, 26.9–31.9 µm, distinguitur.

**Conidiophores** simple or complex to sporodochial. **Simple conidiophores** arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to two phialides, 1–3-septate, 60–120 µm long; phialides monophialidic, more or less cylindrical but slightly tapering towards the tip, 20–60 µm long, 2.0–4.0 µm wide at the base, 3.0–4.5 µm at widest point, 1.5–2.5 µm near the aperture. Conidiophores giving rise to microconidia formed by mycelium at agar surface, penicillate to mono-verticillate; phialides monophialidic, more or less cylindrical, but with slight taper towards the tip, 19–34 µm long, 1.5–2.5 µm wide at the base, 2.0–3.0 µm



**Fig. 4** *Ilyonectria cyclaminicola* (CBS302.93). **a, b** Simple conidiophores on aerial mycelium. **c** Pennicillate conidiophores with aseptate microconidia. **d** Sporodochial conidiophore on carnation leaf agar. **e**

Isolated chlamydospores formed in lateral branches. **f–j** Micro- and macroconidia. Bars 10 µm

at widest point, 1.0–2.0  $\mu\text{m}$  near the apex. *Sporodochial conidiophores* irregularly branched; phialides more or less cylindrical, but slightly tapering towards the tip, or narrowly flask-shaped, with widest point near the base, 14–26  $\mu\text{m}$  long, 2.5–3.5  $\mu\text{m}$  wide at the base 3.0–4.0  $\mu\text{m}$  at widest point, 1.0–2.0  $\mu\text{m}$  near the apex. *Macroconidia* formed in flat domes of slimy masses, 1(–3)-septate, straight or minutely curved, cylindrical with both ends more or less broadly rounded, sometimes with a constriction at the septa, mostly without a visible hilum; 1-septate, (19.2)21.3–23.6 (29.8)  $\times$  (4.4)5.4–6.0(7.3)  $\mu\text{m}$  (av. = 22.5  $\times$  5.7  $\mu\text{m}$ ), with a length:width ratio of 3.4–5.5; 2-septate, (23.8)24.0–28.4 (29.8)  $\times$  (5.0)5.5–7.3(8.0)  $\mu\text{m}$  (av. = 26.2  $\times$  6.4  $\mu\text{m}$ ), with a length:width ratio of 3.1–5.1; 3-septate, (25.3)26.9–31.9 (33.6)  $\times$  (5.8)5.9–6.5(6.9)  $\mu\text{m}$  (av. = 29.4  $\times$  6.2  $\mu\text{m}$ ), with a length:width ratio of 3.7–5.6. *Microconidia* formed in heads or on the agar surface, 0–1-septate, subglobose to ovoid to subcylindrical, mostly with a visible, centrally located or slightly laterally displaced hilum; aseptate microconidia, (3.9)7.6–8.9(12.9)  $\times$  (2.2)3.6–3.9(5.4)  $\mu\text{m}$  (av. = 8.2  $\times$  3.7  $\mu\text{m}$ ), with a length:width ratio of 1.2–3.4; 1-septate, (11.5)13.8–15.2(17.5)  $\times$  (3.7)4.6–4.9(5.5)  $\mu\text{m}$  (av. = 14.5  $\times$  4.7  $\mu\text{m}$ ), with a length:width ratio of 2.3–3.9. *Chlamydospores* globose to subglobose, 10–18  $\times$  10–16  $\mu\text{m}$ , smooth, but often appearing rough due to deposits, thick-walled,

formed in lateral branches, rarely intercalary, mostly isolated, hyaline, becoming medium brown.

*Holotype*: Netherlands, Roelofarendsveen, NAKS laboratory, *Cyclamen* bulb, May 1993, coll./isol. M. Hooftman, iden. E.J. Hermanides-Nijhof, holotype CBS H-20557, culture ex-type CBS 302.93.

*Culture characteristics*: Mycelium felty with average density. Surface on OA sepia to chestnut. Surface on PDA sepia to chestnut, with sparse, rust, aerial mycelium; no zonation was observed, and transparency was homogeneous; margins predominantly even. Reverse similar to surface, except in colour, sepia to dark brick on OA and chestnut on PDA. Colonies on PDA do not grow at 4°C after 7 days. Optimum temperature at 22°C, when colonies reach 68–70 mm diam, after 7 days. Colony diam was 63–64 mm at 25°C, after 7 days. No growth was observed at 30°C.

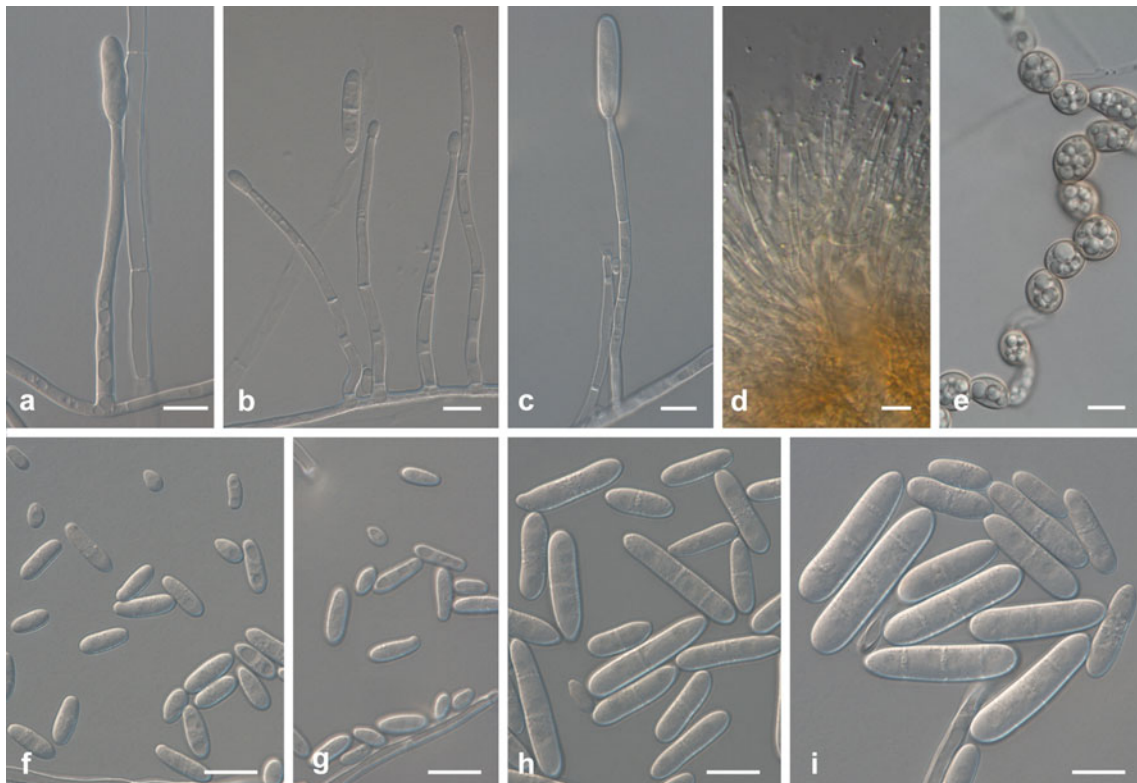
*Isolate studied*: CBS 302.93 (Table 1).

*Host and distribution*: Bulb of *Cyclamen* sp. (Netherlands).

*Ilyonectria europaea* A. Cabral, Rego & Crous, sp. nov. (Fig. 5)

MycoBank 560103.

*Etymology*: Named after the European continent, where this fungus appears to be widely distributed.



**Fig. 5** *Ilyonectria europaea* (CBS 129078). **a–c** Simple conidiophores on aerial mycelium. **d** Sporodochial conidiophore on carnation leaf agar. **e** Chlamydospores in aerial mycelium. **f–i** Micro- and macroconidia. Bars 10  $\mu\text{m}$

*Ilyonectria robustae* morphologicis similis, sed longitudine media macroconidiorum brevior, 29.7–31.5  $\mu\text{m}$ , distinguitur.

*Conidiophores* simple or complex to sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to three phialides, 1–3-septate, 50–120  $\mu\text{m}$  long; phialides monophialidic, cylindrical to subulate, 26–60  $\mu\text{m}$  long, 2.5–3.5  $\mu\text{m}$  wide at the base, 1.5–2.5  $\mu\text{m}$  near the apex. *Complex conidiophores* aggregated in small sporodochia (on carnation leaf), repeatedly and irregularly branched. *Macroconidia* predominating, formed on both type of conidiophores, on SNA formed in flat domes of slimy masses, 1(–3)-septate, straight or minutely curved, cylindrical with both ends more or less broadly rounded, but may narrow towards the tip, mostly without a visible hilum; 1-septate, (16.4)21.9–23.4(34.0)  $\times$  (4.0)5.2–5.6(7.8)  $\mu\text{m}$  (av. = 22.7  $\times$  5.4  $\mu\text{m}$ ), with a length:width ratio of 3.2–5.4; 2-septate, (22.0)26.4–28.1(34.0)  $\times$  (4.4)5.9–6.4(8.0)  $\mu\text{m}$  (av. = 27.2  $\times$  6.1  $\mu\text{m}$ ), with a length:width ratio of 3.4–6.4; 3-septate, (22.0)29.7–31.5(40.0)  $\times$  (5.0)6.5–6.9 (8.6)  $\mu\text{m}$  (av. = 30.6  $\times$  6.7  $\mu\text{m}$ ), with a length:width ratio of 3.5–6.0. *Microconidia* 0–1-septate, ellipsoid to ovoid, more or less straight, without a visible hilum; aseptate microconidia sometimes curved towards one end, (3.0)8.5–9.8(17.0)  $\times$  (1.7)3.3–3.5(5.0)  $\mu\text{m}$  (av. = 9.1  $\times$  3.4  $\mu\text{m}$ ), with a length:width ratio of 1.5–3.4; 1-septate, (9.2)13.4–14.6(18.9)  $\times$  (3.0)4.0–4.4(5.9)  $\mu\text{m}$  (av. = 14.0  $\times$  4.2  $\mu\text{m}$ ), with a length:width ratio 2.6–4.0. *Conidia* formed in heads or on simple conidiophores as white (OA) or unpigmented (SNA) masses. *Chlamydospores* globose to subglobose, 9–14  $\times$  7–14  $\mu\text{m}$ , smooth, but often appearing rough due to deposits, thick-walled, terminal on short or long lateral branches or intercalary, single, in chains or in clumps, golden-brown.

*Holotype*: Portugal, Vidigueira, at basal end of a 2-year-old *Vitis vinifera* plant; scion Petit Verdor, rootstock 110R, 2008, coll./isol. C. Rego, holotype CBS H-20558, culture ex-type CBS 129078=Cy241=CPC 19165.

*Culture characteristics*: Mycelium felty with average density. Surface on OA chestnut, with saffron aerial mycelium. Sienna to saffron on PDA, with luteous aerial mycelium. Concentric zonation, with homogeneous transparency, margins predominantly even. Reverse similar to surface, except in the colour; sepia on OA, and chestnut to umber on PDA. Colonies on PDA grow poorly, 1–5 mm diam at 4°C after 7 days. Optimum temperature for growth is 22°C, when colonies reach 43–57 mm diam, after 7 days. Colony diam was 37–47 mm at 25°C, after 7 days. No growth was observed at 30°C.

*Isolates studied*: Cy131; Cy155; CBS 537.92; CBS 102892; CBS 129078 (Table 1).

*Hosts and distribution*: *Actinidia chinensis* ‘Hayward’ (internal lesion of stem) (France), *Aesculus hippocastanum* (wood) (Belgium), *Phragmites australis* (stem) (Germany), *Vitis vinifera* (Portugal).

*Ilyonectria gamsii* A. Cabral & Crous, sp. nov. (Fig. 6)

MycoBank 560112.

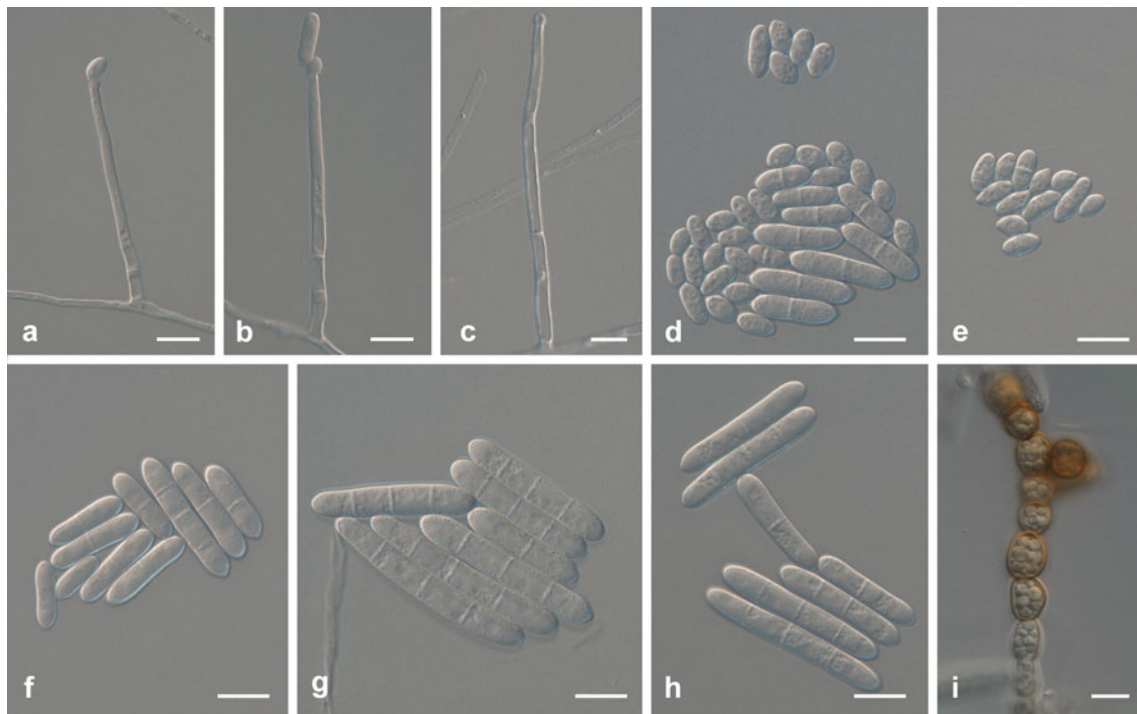
*Etymology*: Named after Prof. dr. Walter Gams, who has made a major contribution to our knowledge of Hypocrealean soil fungi.

*Ilyonectria panacis* morphologicis similis, sed longitudine media macroconidiorum brevior, 34.3–38.5  $\mu\text{m}$ , distinguitur.

*Conidiophores* simple or complex to sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to two phialides, 1–3-septate, 50–150  $\mu\text{m}$  long; phialides monophialidic, cylindrical to subulate, 30–60  $\mu\text{m}$  long, 2.5–3.5  $\mu\text{m}$  wide at the base, 1.5–2.0  $\mu\text{m}$  near the aperture. *Sporodochial conidiophores* irregularly branched; phialides cylindrical, mostly widest near the base. *Macroconidia* predominating, formed on simple conidiophores, on SNA formed in flat domes of slimy masses, 1–3-septate, straight, cylindrical with both ends broadly rounded, with mostly visible, centrally located hilum; 1-septate, (22.0)25.7–27.9(33.0)  $\times$  (4.0)5.1–5.5(6.0)  $\mu\text{m}$  (av. = 26.8  $\times$  5.3  $\mu\text{m}$ ), with a length:width ratio of 4.3–6.2; 2-septate, (25.0)28.2–31.7(39.0)  $\times$  (5.0)5.5–5.9(6.5)  $\mu\text{m}$  (av. = 29.9  $\times$  5.7  $\mu\text{m}$ ), with a length:width ratio of 4.2–7.1; 3-septate, (24.0)34.3–38.5(44.0)  $\times$  (5.0)5.9–6.3(7.0)  $\mu\text{m}$  (av. = 36.4  $\times$  6.1  $\mu\text{m}$ ), with a length:width ratio of 4.3–7.3. *Microconidia* 0–1-septate, ellipsoid to subcylindrical, more or less straight, mostly with a visible hilum; aseptate microconidia (4.0)6.9–8.0(10.0)  $\times$  (3.0)4.0–4.5(5.0)  $\mu\text{m}$  (av. = 7.4  $\times$  4.3  $\mu\text{m}$ ), with a length:width ratio of 1.3–2.9; 1-septate, (8.0)12.9–15.7(18.0)  $\times$  (4.0)4.2–4.7(5.5)  $\mu\text{m}$  (av. = 14.3  $\times$  4.4  $\mu\text{m}$ ), with a length:width ratio 1.8–4.0. *Chlamydospores* globose to subglobose to ellipsoidal, 8–14  $\times$  7–12  $\mu\text{m}$ , smooth, but often appearing rough due to deposits, thick-walled, mostly intercalary, rarely terminal on short lateral branches, single, in chains or in clumps, hyaline, becoming medium brown.

*Holotype*: Netherlands, Lelystad, soil, June 1997, coll./isol. J.T. Poll, iden. W. Gams, holotype CBS H-20559, culture ex-type CBS 940.97.

*Culture characteristics*: Mycelium cottony, dense. Surface on OA cinnamon, with sparse, buff aerial mycelium, on PDA umber to chestnut, with buff to saffron aerial mycelium; zonation absent, transparency homogeneous, margin even; reverse similar to surface, but chestnut on PDA. Colonies on PDA grow 6–7 mm diam at 4°C after 7 days. Optimum temperature at 22°C when colonies reach



**Fig. 6** *Ilyonectria gamsii* (CBS 940.97). **a–c** Simple conidiophores on aerial mycelium. **d–h** Micro- and macroconidia. **i** Chlamydospores on mycelium. Bars 10 µm

44–45 mm diam, after 7 days. Colony diam is 22–24 mm at 25°C, after 7 days. No growth observed at 30°C.

*Isolate studied*: CBS 940.97 (Table 1).

*Habitat and distribution*: Soil (Netherlands).

***Ilyonectria liliigena* A. Cabral & Crous, sp. nov. (Fig. 7)**

Mycobank 560114.

*Etymology*: Named after its host, *Lilium regale*.

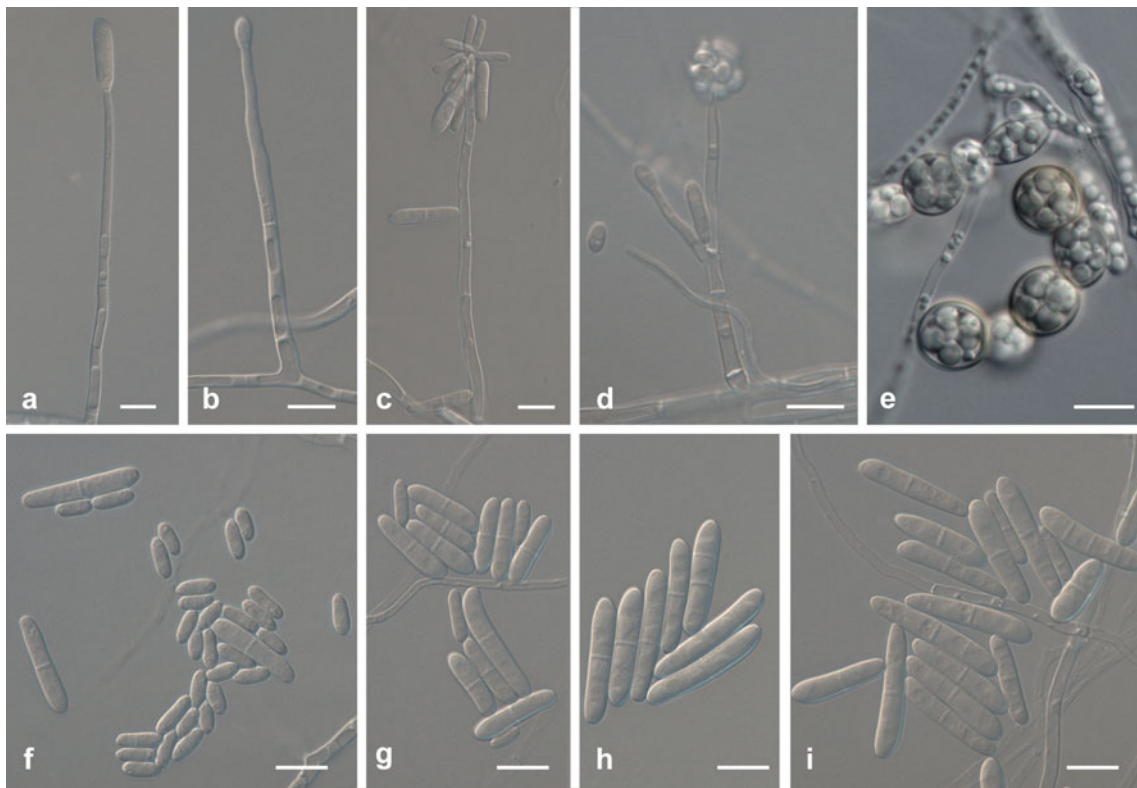
*Ilyonectriae panacis* morphologicè similis, sed longitudine media macroconidiorum 3-septatorum breviorè, 27.9–29.8 µm, distinguitur.

*Conidiophores* simple or complex or sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to two phialides, 1–4-septate, 50–170 µm long; phialides monophialidic, cylindrical to subulate, 30–65 µm long, 2.0–3.5 µm wide at the base, 1.5–2.0 µm near the apex. *Sporodochial conidiophores* irregularly branched; phialides cylindrical, mostly widest near the base. *Macroconidia* predominating, formed on simple conidiophores, on SNA formed in flat domes of slimy masses, 1(–3)-septate, straight or frequently minutely curved, cylindrical or sometimes typically minutely widening towards the tip, therefore appearing somewhat clavate, mostly without a visible hilum; 1-septate, (19.0)22.9–24.6 (30.0)×(3.3)4.2–4.5(5.2) µm (av. = 23.8×4.3 µm), with a

length:width ratio of 4.0–7.0; 2-septate, (21.0)26.1–27.7 (32.1)×(4.0)4.7–5(5.7) µm (av. = 26.9×4.9 µm) with a length:width ratio of 3.8–7.0; 3-septate, (23.9)27.9–29.8 (35.0)×(3.9)4.7–5.1(6.0) µm (av. = 28.9×4.9 µm), with a length:width ratio of 4.0–8.3. *Microconidia* 0–1-septate, ellipsoidal to subcylindrical, more or less straight, mostly with a visible hilum; aseptate, microconidia (5.9)8.9–10.3 (17.0)×(2.5)3.0–3.2(4.4) µm (av. = 9.6×3.1 µm), with a length:width ratio of 2.0–4.6; 1-septate, (10.0)12.9–14.3 (18.0)×(2.5)3.3–3.6(4.5) µm (av. = 13.6×3.4 µm), with a length:width ratio 2.8–5.6. *Conidia* formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses. *Chlamydospores* globose to subglobose, 6–14×5–12 µm, smooth but often appearing rough due to deposits, thick-walled, mostly in terminal on short lateral branches or rarely intercalary, single, in chains or in clumps, hyaline, becoming slightly brown at margins.

*Holotype*: Netherlands, Hoorn, bulb rot of *Lilium regale*, 1949, coll./isol. M.A.A. Schipper, holotype CBS H-20560, culture ex-type CBS 189.49.

*Culture characteristics*: Mycelium felty, with an average to strong density. Surface on OA sienna, with sparse, saffron, aerial mycelium. Surface on PDA sepia to cinnamon, with saffron to buff aerial mycelium. Zonation absent or concentric, with homogeneous transparency. Margins were even, or sometimes slightly uneven. Reverse similar to surface, except in colour; on OA pale vinaceous



**Fig. 7** *Ilyonectria liliigena* (CBS 189.49). **a–d** Simple conidiophores on aerial mycelium. **e** Chlamydospores on mycelium. **f–i** Micro- and macroconidia. Bars 10  $\mu\text{m}$

to cinnamon; on PDA buff to saffron to chestnut. Colonies on PDA grew poorly (1–4 mm diam) at 4°C after 7 days. Optimum temperature at 22°C, when colonies reach 34–45 mm diam, after 7 days. Colony diam was 16–29 mm at 25°C, after 7 days. No growth was observed at 30°C.

*Isolates studied:* CBS 189.49; CBS 732.74; CBS 304.85; CBS 305.85 (Table 1).

*Host and distribution:* *Lilium regale* bulbs (Netherlands).

***Ilyonectria lusitanica*** A. Cabral, Rego & Crous, *sp. nov.* (Fig. 8)

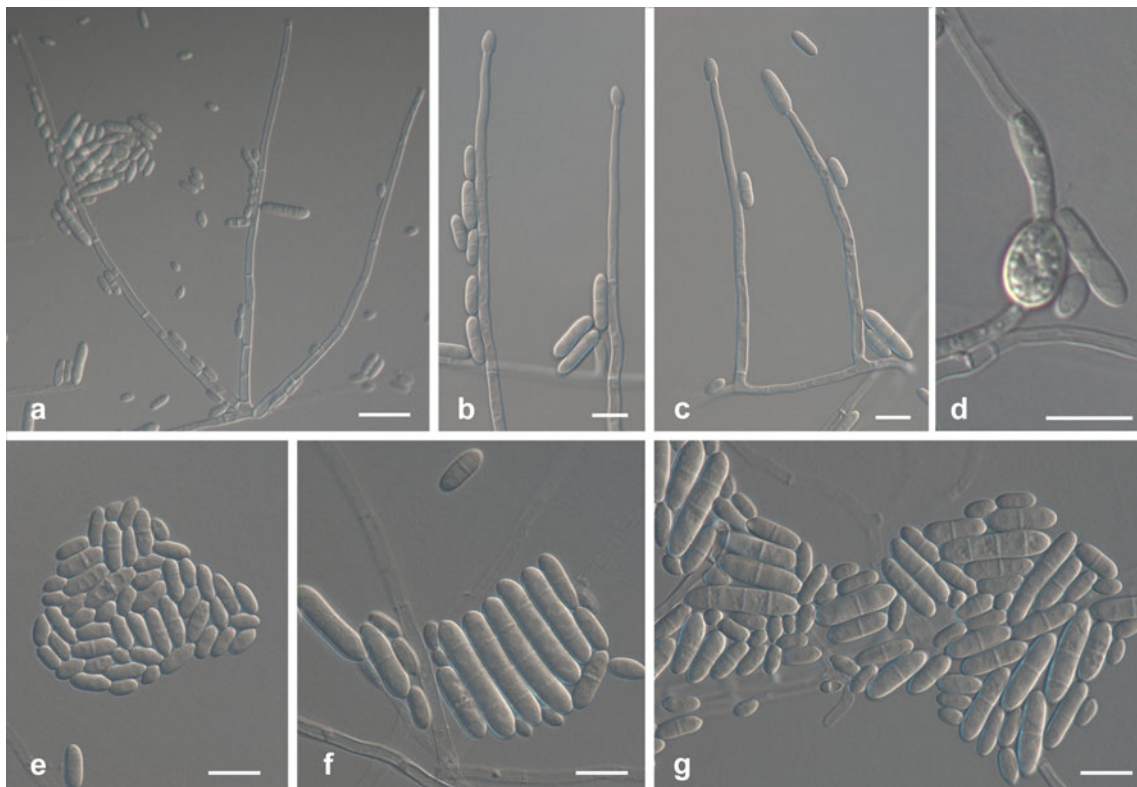
Mycobank 560105.

*Etymology:* Named after the Latin name for the country from where it was collected, Portugal.

*Ilyonectriae europaeae* morphologicice similis, sed longitudine media macroconidiorum brevior, 25–28.4  $\mu\text{m}$ , distinguitur.

*Conidiophores* simple or complex, sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to two phialides, 1–4-septate, 60–220  $\mu\text{m}$  long; phialides monophialidic, cylindrical to subulate, 20–70  $\mu\text{m}$  long, 2.5–3.5  $\mu\text{m}$  wide at the base, 1.5–

2.5  $\mu\text{m}$  near the aperture. *Complex conidiophores* aggregated in small sporodochia, repeatedly and irregularly branched. *Macroconidia* predominating, formed by both type of conidiophores, on SNA formed in flat domes of slimy masses, 1(–3)-septate, straight or minutely curved, cylindrical with both ends more or less broadly rounded, but may narrow towards the tip, without a visible hilum, and may have a constriction on the septa in older cultures; 1-septate, (14.0) 17.3–18.8(21.0)  $\times$  (4.0) 4.6–5(5.5)  $\mu\text{m}$  (av. = 18.1  $\times$  4.8  $\mu\text{m}$ ), with a length:width ratio of 2.8–4.8; 2-septate, (18.0) 20.5–22.1(27.0)  $\times$  (4.0) 4.9–5.2(6.0)  $\mu\text{m}$  (av. = 21.3  $\times$  5.1  $\mu\text{m}$ ), with a length:width ratio of 3.5–5.4; 3-septate, (18.0) 25.0–28.4 (38.0)  $\times$  (4.5) 5.2–5.5(6.0)  $\mu\text{m}$  (av. = 26.7  $\times$  5.4  $\mu\text{m}$ ), with a length:width ratio of 3.6–6.8. *Microconidia* 0–1-septate, ellipsoid to ovoid, more or less straight, without a visible hilum, and may have a constriction at the septum; aseptate, (5.0) 6.9–8.2(10.0)  $\times$  (2.5) 3.0–3.3(4.0)  $\mu\text{m}$  (av. = 7.6  $\times$  3.2  $\mu\text{m}$ ), with a length:width ratio of 1.7–3.3; 1-septate, (8.0) 10.0–11.0 (14.0)  $\times$  (3.0) 3.4–3.7(4.0)  $\mu\text{m}$  (av. = 10.5  $\times$  3.6  $\mu\text{m}$ ), with a length:width ratio 2.0–3.7. *Conidia* formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses. *Chlamydospores* rarely observed, globose to subglobose to cylindrical, 9–13  $\times$  7–11  $\mu\text{m}$ , smooth, but often appearing rough due to deposits, thick-walled, intercalary, hyaline, becoming slightly brown at the margin.



**Fig. 8** *Ilyonectria lusitanica* (CBS 129080). **a–c** Simple conidiophores of the aerial mycelium. **d** Chlamydospores on mycelium. **e–g** Micro- and macroconidia. Bars (**a**) 20  $\mu\text{m}$ , (**b–g**) 10  $\mu\text{m}$

*Holotype*: Portugal, Melgaço, Alvaredo, on *Vitis vinifera*, below grafting zone, 6-year-old plant; scion Alvarinho on rootstock 196–17, 2005, coll./isol. N. Cruz, holotype CBS H-20563, culture ex-type CBS 129080=Cy197=CPC 19166.

*Culture characteristics*: Mycelium felty with average density. Surface on OA cinnamon, with aerial mycelium sparse, buff. Surface on PDA, cinnamon, with sparse, ochreous to buff aerial mycelium. Zonation absent, transparency homogeneous, margin even. Reverse similar to surface but buff to cinnamon on OA, and chestnut to cinnamon on PDA. Colonies on PDA grow 5–6 mm at 4°C after 7 days. Optimum temperature between 20 and 22°C, with colonies reaching 42–46 mm and 43–46 mm, respectively, after 7 days. Colony diam was 31–32 mm at 25°C, after 7 days. No growth observed at 30°C.

*Isolate studied*: CBS 129080 (Table 1).

*Host and distribution*: *Vitis vinifera* (Portugal).

*Ilyonectria mors-panacis* (A.A. Hildebr.) A. Cabral & Crous, *comb. nov.* (Fig. 9)

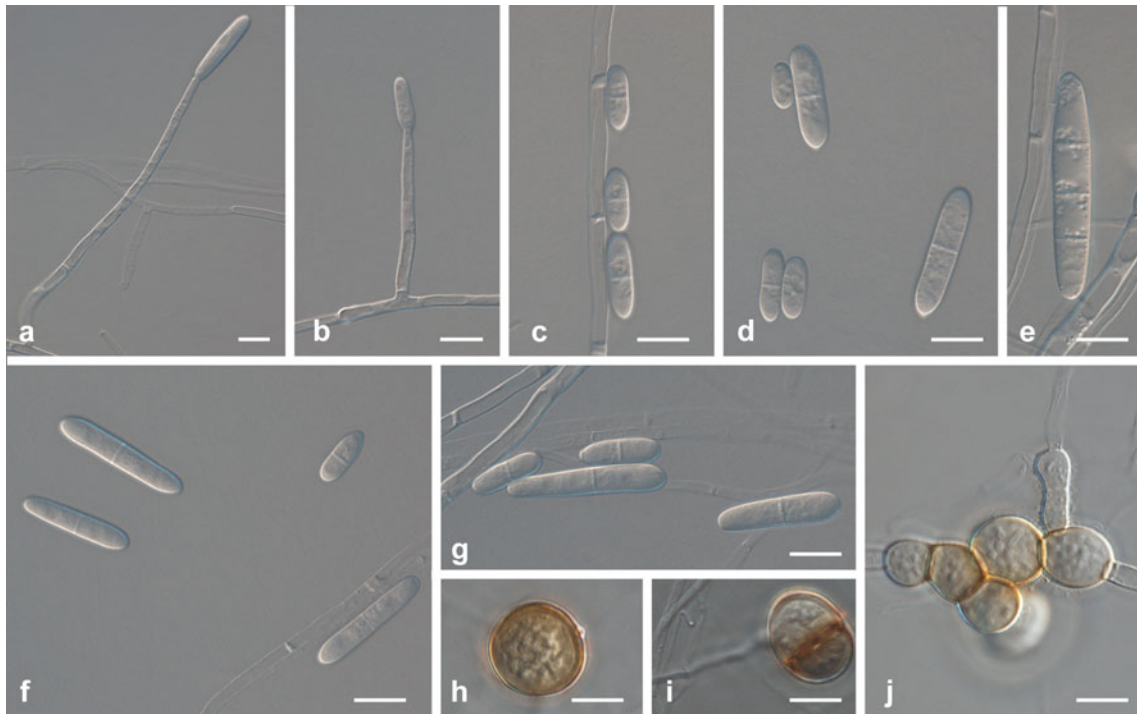
Mycobank 560115.

*Basionym*: *Ramularia mors-panacis* A.A. Hildebr., Can. J. Res. 12: 101. 1935.

= *Cylindrocarpon panacis* Matuo & Miyaz., Trans. Mycol. Soc. Japan 9: 111. 1969.

= *Cylindrocarpon destructans* f.sp. *panacis* Matuo & Miyaz., Ann. Phytopath. Soc. Japan 50: 390. 1984.

*Conidiophores* simple or complex, sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, rarely consisting only of phialides, 1–3-septate, 45–170  $\mu\text{m}$  long; phialides monophialidic, cylindrical to subulate, 23–55  $\mu\text{m}$  long, 2.0–3.0  $\mu\text{m}$  wide at the base, 1.5–3.0  $\mu\text{m}$  near the apex. *Complex conidiophores* aggregated in small sporodochia, repeatedly and irregularly branched. *Macroconidia* predominating, formed on simple conidiophores, on SNA formed in flat domes of slimy masses, 1(–3)-septate, straight, cylindrical with both ends more or less broadly rounded, mostly without a hilum; 1-septate, (21.0)28.2–31.6(40.0)  $\times$  (5.0)5.8–6.3(7.5)  $\mu\text{m}$  (av. = 29.9  $\times$  6.1  $\mu\text{m}$ ), with a length:width ratio of 3.3–7.0; 2-septate, (28.0)30.5–38.4(42.0)  $\times$  (5.0)5.9–6.4–7.0(7.1)  $\mu\text{m}$  (av. = 34.4  $\times$  6.4  $\mu\text{m}$ ), with a length:width ratio of 4.0–6.0; 3-septate, (37.8)39.0–44.2(45.0)  $\times$  (6.9)7.0–7.5(7.5)  $\mu\text{m}$  (av. = 41.0  $\times$  7.2  $\mu\text{m}$ ), with a length:width ratio of 5.3–6.0. *Microconidia* 0–1-septate, ellipsoid to subcylindrical, more or less straight, without a visible hilum; aseptate, (5.0)8.9–10.4(17.0)  $\times$  (2.5)3.6–3.9(5.0)  $\mu\text{m}$  (av. = 9.6  $\times$  3.8  $\mu\text{m}$ ), with a length:width ratio of 1.3–3.4; 1-septate, (9.0)12.5–14.1(19.0)  $\times$  (3.5)4.4–4.8(5.5)  $\mu\text{m}$  (av. = 13.3  $\times$  4.6  $\mu\text{m}$ ), with a length:width ratio 2.0–4.0. *Conidia*



**Fig. 9** *Ilyonectria mors-panacis* (CBS120363). **a, b** Simple conidiophores on aerial mycelium. **c–g** Micro- and macroconidia. **h–j** Chlamydospores on mycelium. Bars 10  $\mu\text{m}$

formed in heads on simple conidiophores or as white, creamy (OA) or hyaline (SNA) masses. *Chlamydospores* globose to subglobose, 8–16 $\times$ 7–15  $\mu\text{m}$ , smooth, but often appearing rough due to deposits, thick-walled, terminal on short lateral branches or intercalary, single, in chains or in clumps, hyaline, becoming medium brown.

**Lectotype:** Canada, Ontario, on living roots of *Panax quinquefolium*, June 1935, A.A. Hildebrand, lectotype designated here CBS H-20561, culture ex-lectotype CBS 306.35.

**Culture characteristics:** Mycelium felty with an average density. Surface on OA and PDA chestnut, with sparse, buff to rosy-buff to cinnamon or saffron aerial mycelium. Concentric zonation, with homogeneous transparency, and even margins. Reverse similar to surface, ochreous to fulvous, or sepia to dark vinaceous on OA, and chestnut to sienna on PDA. Colonies on PDA grow 3–9 mm diam at 4 $^{\circ}\text{C}$  after 7 days. Optimum temperature for growth is 18 $^{\circ}\text{C}$ , when colonies reach 22–40 mm diam, after 7 days. Colony diam was 31–40 mm at 25 $^{\circ}\text{C}$  after 7 days. No growth was observed at 30 $^{\circ}\text{C}$ .

**Isolates studied:** CBS 306.35; CBS 307.35; CBS 120359; CBS 120360; CBS 120361; CBS 120362; CBS 120363; CBS 120364; CBS 120365; CBS 120366; CBS 120367; CBS 120368; CBS 120369; CBS 124662; CPC 13535; CPC 13537 (Table 1).

**Hosts and distribution:** *Panax ginseng* (Japan), *P. quinquefolium* (Canada).

**Notes:** *Ilyonectria mors-panacis* is distinct from “*C.*” *destructans* (anamorph: “*C.*” *destructans*, neotype CUP-

011985, conidia (18.0)23.0–30.0(35.0)  $\times$  (6.0)6.5(7.0)  $\mu\text{m}$ ) in having larger conidia, and indistinct hila (being prominent, flat, 2  $\mu\text{m}$  diam in *I. radicularis*; see also Samuels and Brayford 1990, Fig. 1). “*Ramularia*” *panacicola* is distinct by also having shorter conidia than *I. mors-panacis*, 5.5–34.2  $\times$  2.5–7.2  $\mu\text{m}$  (Zinssmeister 1918), and appears to be another potential synonym of “*C.*” *destructans*. However, no authentic material could be located of “*R.*” *panacicola*, and the only isolate deposited under this name was a Canadian strain collected by Hildebrand (1935), which in fact represented *I. mors-panacis* (Fig. 1). The oldest name for the species on *Panax* treated here, therefore, is *R. mors-panacis* (CBS 306.35), with the Japanese collections (“*C.*” *panacis*  $\equiv$  “*C.*” *destructans* f.sp. *panacis*, CBS 124662 = NBRC 31881) being later synonyms (see Fig. 1).

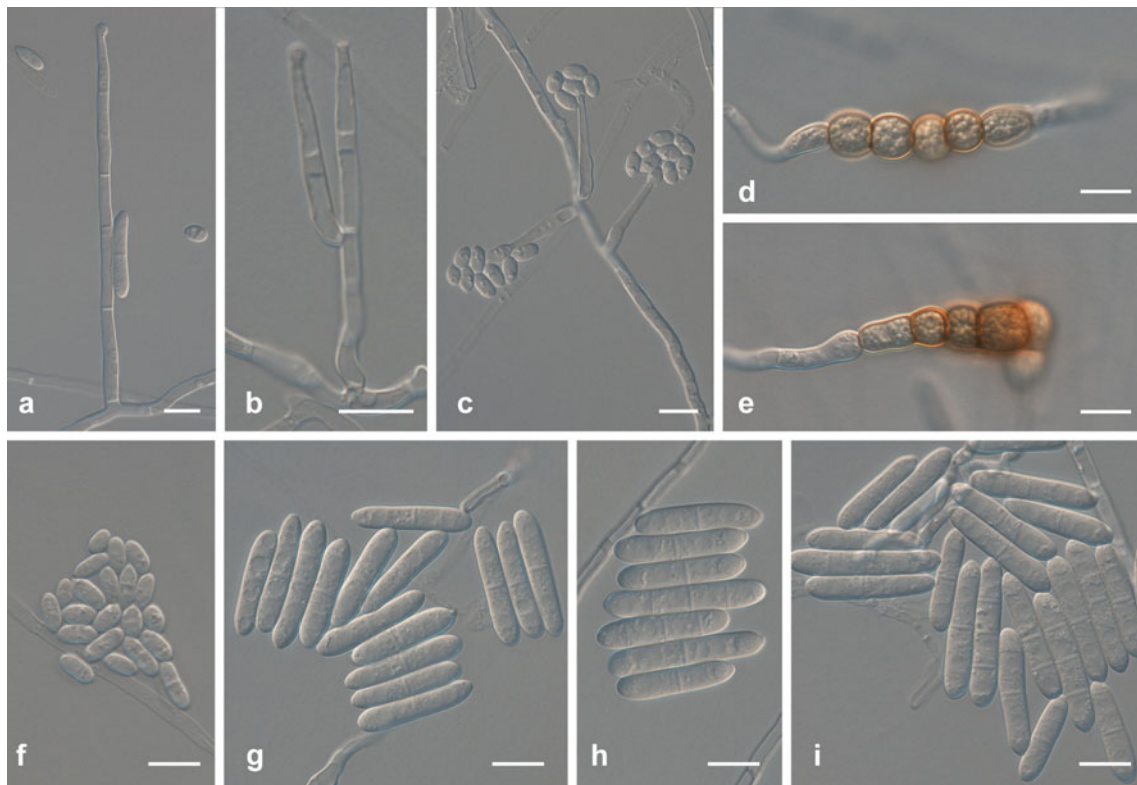
#### *Ilyonectria panacis* A. Cabral & Crous, sp. nov. (Fig. 10)

MycoBank 560104.

**Etymology:** Named after its host, *Panax quinquefolium*.

*Ilyonectriae liliigenae* morphologicis similis, sed longitudine media macroconidiorum longiore, 31–35  $\mu\text{m}$ , distinguitur.

**Conidiophores** simple or complex, sporodochial. **Simple conidiophores** arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched bearing up to three phialides, 1–5-septate, 60–220  $\mu\text{m}$  long; phialides monophialidic, cylindrical to



**Fig. 10** *Ilyonectria panacis* (CBS 129079). **a–c** Simple, unbranched or sparsely branched conidiophores on aerial mycelium. **d, e** Chlamydospores on mycelium. **f–i** Micro- and macroconidia. Bars 10  $\mu\text{m}$

subulate, 20–65  $\mu\text{m}$  long, 2.5–3.0  $\mu\text{m}$  wide at the base, 1.5–2.0  $\mu\text{m}$  near the aperture. *Complex conidiophores* aggregated in small sporodochia, repeatedly and irregularly branched. *Macroconidia* predominating, formed on both type of conidiophores, on SNA formed in flat domes of slimy masses, 1(–3)-septate, straight, cylindrical with both ends more or less broadly rounded, mostly with a visible centrally located hilum; 1-septate, (20.0)23.7–25.9(32.0)  $\times$  (4.0)4.7–5.0(5.5)  $\mu\text{m}$  (av. = 24.8  $\times$  4.8  $\mu\text{m}$ ), with a length:width ratio of 4.0–6.0; 2-septate, (23.0)27.0–30.3(37.0)  $\times$  (4.8)5.0–5.4(6.0)  $\mu\text{m}$  (av. = 28.7  $\times$  5.2  $\mu\text{m}$ ), with a length:width ratio of 4.6–6.7; 3-septate, (27.0)31.2–35.0(49.0)  $\times$  (5.0)5.4–5.8(6.0)  $\mu\text{m}$  (av. = 33.1  $\times$  5.6  $\mu\text{m}$ ), with a length:width ratio of 4.9–8.2. *Microconidia* 0–1-septate, ellipsoid to ovoid to subcylindrical, more or less straight, mostly with a visible hilum; aseptate, (6.0)8.0–9.8(13.0)  $\times$  (3.5)3.7–3.9(4.0)  $\mu\text{m}$  (av. = 8.9  $\times$  3.8  $\mu\text{m}$ ), with a length:width ratio of 1.7–3.3; 1-septate, (8.0)11.3–13.7(16.0)  $\times$  (3.5)3.8–4.2(4.5)  $\mu\text{m}$  (av. = 12.5  $\times$  4.0  $\mu\text{m}$ ), with a length:width ratio 1.8–4.3. *Conidia* formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses. *Chlamydospores* globose to subglobose to ellipsoidal, 8–14  $\times$  6–10  $\mu\text{m}$ , smooth, but often appearing rough due to deposits, thick-walled, terminal on short lateral branches or interca-

lary, single, in chains or in clumps, hyaline, becoming medium brown.

*Holotype*: Canada, Alberta, *Panax quinquefolium*, 1998, coll./isol. K. F. Chang, holotype CBS H-20562, culture ex-type CBS 129079=CDC-N-9A=CPC 19167.

*Culture characteristics*: Mycelium felty with strong density. Surface on OA chestnut to sienna, with aerial mycelium sparse, vinaceous-buff. Surface on PDA chestnut to cinnamon, with aerial mycelium sparse, buff to saffron. No zonation was observed, and transparency was homogeneous; margins predominantly even. Reverse similar to surface, except in the colour, fawn to cinnamon on OA, and chestnut on PDA. Colonies on PDA grow 5 mm diam at 4°C after 7 days. Optimum temperature at 20°C, with colonies reaching 40–42 mm diam, after 7 days. Colony diam was 15 mm at 25°C after 7 days. No growth observed at 30°C.

*Isolate studied*: CBS 129079 (Table 1).

*Host and distribution*: *Panax quinquefolium* (Canada).

*Notes*: Several species have in the past been described on *Panax* in the genera *Ramularia* and *Cylindrocarpon*. The only unresolved species is “*C.*” *destructans* (and its potential synonym, “*Ramularia*” *panacicola*, see above). “*Cylindrocarpon*” *destructans* is clearly different from *I.*



*panacis*, which has larger conidia, (27.0)31.2–33.1–35.0 (49.0)×(5.0)5.4–5.6–5.8(6.0) μm.

***Ilyonectria pseudodestructans* A. Cabral, Rego & Crous, sp. nov. (Fig. 11)**

Mycobank 560106.

**Etymology:** Named after its morphological similarity to “*Cylindrocarpon*” *destructans*.

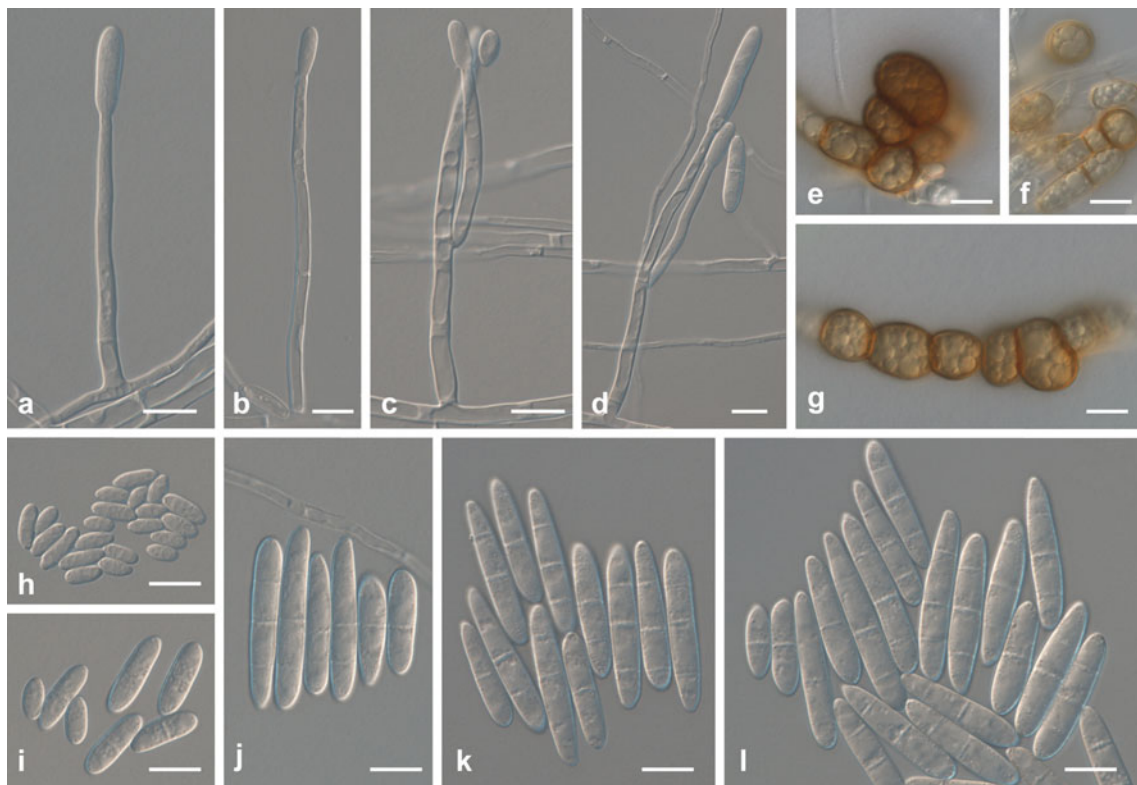
*Ilyonectriae crassae* morphologicis similis, sed macroconidiis clavatis distinguitur.

**Conidiophores** simple or complex, sporodochial. **Simple conidiophores** arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to two phialides, 1–3-septate, 50–180 μm long; phialides monophialidic, cylindrical to subulate, 30–58 μm long, 2.5–3.5 μm wide at the base, 1.5–2.0 μm near the aperture. **Complex conidiophores** aggregated in small sporodochia, repeatedly and irregularly branched. **Macroconidia** predominating, formed by simple conidiophores, on SNA formed in flat domes of slimy masses, 1–3(–4)-septate, straight, typically clavate, mostly centrally located hilum; 1-septate, (19.0)25.8–27.5(35.0)×(4.0)5.0–5.3(6.5) μm (av. = 26.7×5.2 μm), with a length:

width ratio of 3.8–6.6; 2-septate, (23.0)30.0–31.7(38.0)×(5.0)5.3–5.5(6.0) μm (av. = 30.9×5.4 μm), with a length:width ratio of 4.3–7.4; 3-septate, (28.0)34.2–36.2(48.0)×(5.0)5.9–6.2(7.0) μm (av. = 35.2×6.0 μm), with a length:width ratio of 4.6–7.4. **Microconidia** 0–1-septate, ellipsoid to ovoid to subcylindrical, more or less straight, with a visible, centrally located hilum; aseptate (6.0)10.5–11.8 (15.0)×(3.0)3.6–3.8(4.5) μm (av. = 11.2×3.7 μm), with a length:width ratio of 1.5–4.3; 1-septate, (10.0)14.6–15.6 (18.0)×(3.0)4.1–4.4(5.0) μm (av. = 15.1×4.2 μm), with a length:width ratio of 2.4–5.0. **Conidia** formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses. **Chlamydospores** globose to subglobose to ellipsoid, 9–18×8–14 μm, smooth but often appearing rough due to deposits, thick-walled, terminal on short lateral branches or intercalary, in chains or in clumps, and also in the cells of macroconidia, hyaline, becoming medium brown.

**Holotype:** Portugal, São Paio, Gouveia, *Vitis vinifera*, 4-year-old, showing decline symptoms, scion Malvasia fina; rootstock 1103P, 1996, coll./isol. C. Rego, holotype CBS H-20564, culture ex-type CBS 129081=Cy20=CPC 19164.

**Culture characteristics:** Mycelium felty, with average to strong density. Surface on OA cinnamon, with sparse, buff



**Fig. 11** *Ilyonectria pseudodestructans* (all from CBS 129081, except **g** and **e** from CBS117824). **a–d** Simple, unbranched or sparsely branched conidiophores on aerial mycelium. **e–g** Chlamydospores on mycelium and macroconidia. **h–l** Micro- and macroconidia. Bars 10 μm

to saffron or chestnut to sienna aerial mycelium. Surface on PDA cinnamon to vinaceous, with sparse, saffron to buff or chestnut to sienna aerial mycelium. Zonation absent, with homogeneous transparency; margins even. Reverse similar to surface, except in colour, sepia to cinnamon on OA and chestnut to cinnamon on PDA. Colonies on PDA grow poorly (4–6 mm diam), at 4°C after 7 days. Optimum temperature between 20–22°C, when colonies reach 32–44 mm and 37–41 mm diam, respectively, after 7 days. Colony diam was 22–29 mm at 25°C after 7 days. No growth was observed at 30°C.

*Isolates studied*: CPC 13534; CBS 117812; CBS 117824; CBS 129081; Cy22 (Table 1).

*Hosts and distribution*: *Poa pratensis* (Canada), *Quercus* sp. (Austria), *Vitis vinifera* (Portugal).

*Notes*: *Ilyonectria pseudodestructans* is reminiscent of “*Cylindrocarpon*” *destructans*, in having a similar conidial morphology (3-septate, with central, truncate hilum). However, conidia of *I. pseudodestructans* are somewhat longer than those of *I. radicola*.

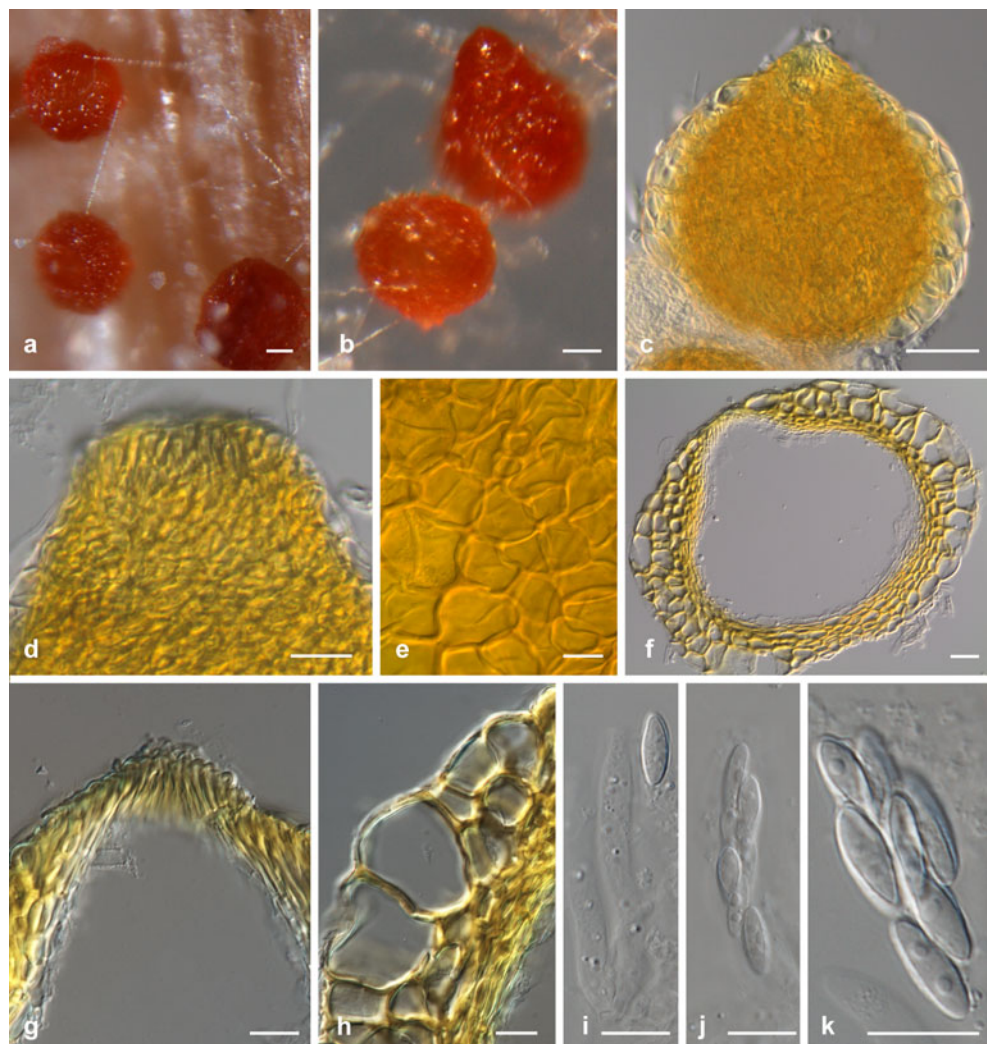
***Ilyonectria robusta*** (A.A. Hildebr.) A. Cabral & Crous, *comb. nov.* (Figs. 12 and 13)

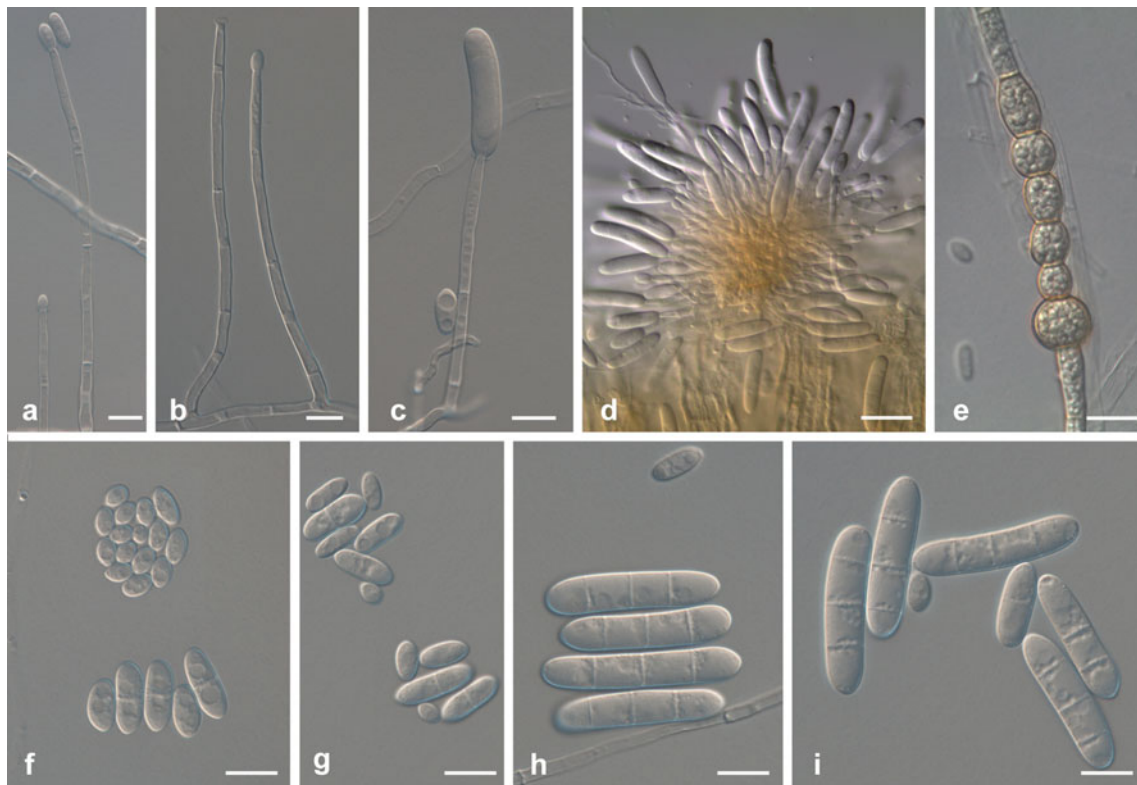
Mycobank 560113.

*Basionym*: *Ramularia robusta* A.A. Hildebr. Can. J. Res. 12: 102. 1935.

*Perithecia* formed heterothallically in vitro, disposed solitarily or in groups, developing directly on the agar surface or on sterile pieces of birch wood, ovoid to obpyriform, with a flattened apex, up to 70 µm wide, orange to red, becoming purple-red in 3 % KOH (positive colour reaction), smooth to warted, up to 250 µm diam and high; *perithecial wall* consisting of two regions; outer region 11–36 µm thick, composed of 1–3 layers of angular to subglobose cells, 10–30×6–24 µm; cell walls up to 1 µm thick; inner region 8–14 µm thick, composed of cells that are flat in transverse optical section and angular to oval in subsurface optical face view, 5–11×2.5–5 µm; *Asci* narrowly clavate to cylindrical, 40–50×4.5–6 µm, 8-spored; apex subtruncate, with a minutely visible ring.

**Fig. 12** *Ilyonectria robusta* (a, b from CPC 13532×CBS 308.35; c–k from CPC 13532×CBS 117813). a, b Development of perithecia on the surface of a birch toothpick or agar. c–e Perithecium mounted in lactic acid. d Ostiolar area. e Surface view of perithecium wall region. f–h Longitudinal sections of perithecia showing details of ostiole and wall. i–k Asci and ascospores. Bars (a–c) 50 µm; (d, f) 20 µm; (e, g–k) 10 µm





**Fig. 13** *Ilyonectria robusta* (All from CBS 129084, except **f** from CBS 605.92). **a–c** Simple conidiophores on aerial mycelium. **d** Sporodochial conidiophore on carnation leaf agar. **e** Chlamydospores on mycelium **f–i** Micro- and macroconidia. Bars 10  $\mu\text{m}$

*Ascospores* medianly 1-septate, ellipsoid to oblong-ellipsoid, somewhat tapering towards both ends, smooth to finely warted, frequently guttulate, hyaline,  $(8.2)9.4\text{--}9.7\text{--}10.0(11.5)\times(2.5)2.9\text{--}3.0\text{--}3.1(3.7)\ \mu\text{m}$ . *Conidiophores* simple or complex or sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to three phialides, 1–4-septate, 55–160  $\mu\text{m}$  long; phialides monophialidic, cylindrical to subulate, 20–60  $\mu\text{m}$  long, 2.0–3.0  $\mu\text{m}$  wide at the base, 1.5–2.0  $\mu\text{m}$  near the apex. *Complex conidiophores* aggregated in small sporodochia (on carnation leaf agar; Crous et al. 2009b), repeatedly and irregularly branched; phialides more or less cylindrical, but tapering slightly in the upper part towards the apex, or narrowly flask-shaped, mostly with widest point near the middle, 15–20  $\mu\text{m}$  long, 2.5–3.5  $\mu\text{m}$  wide at the base, 3.0–4.0  $\mu\text{m}$  at the widest point, and 1.0–2.0  $\mu\text{m}$  wide near the apex. *Macroconidia* predominating, formed on simple conidiophores, on SNA formed in flat domes of slimy masses, 1–3-septate, straight, minutely curved or sometimes distorted, cylindrical with both ends more or less broadly rounded, but may narrow towards the tip, mostly without a visible hilum; 1-septate,  $(15.0)22.8\text{--}24.6(35.0)\times(4.5)6.3\text{--}6.7(8.0)\ \mu\text{m}$  (av. = 23.7 $\times$ 6.5  $\mu\text{m}$ ), with a length:width ratio of 2.7–5.2; 2-septate,  $(20.0)26.2\text{--}28.1(38.0)\times$

$(5.0)6.9\text{--}7.2(8.0)\ \mu\text{m}$  (av. = 27.2 $\times$ 7.0  $\mu\text{m}$ ), with a length:width ratio of 2.9–5.2; 3-septate,  $(24)32.3\text{--}34.7(58)\times(6.0)7.2\text{--}7.5(9.0)\ \mu\text{m}$  (av. = 33.5 $\times$ 7.4  $\mu\text{m}$ ), with a length:width ratio of 3.1–7.3. *Microconidia* 0–1-septate, ellipsoid to ovoid to subcylindrical, more or less straight, without a visible hilum; aseptate,  $(4.0)8.0\text{--}9.3(14.0)\times(2.5)3.6\text{--}4.0(5.5)\ \mu\text{m}$  (av. = 8.7 $\times$ 3.8  $\mu\text{m}$ ), with a length:width ratio of 1.3–4.0; 1-septate,  $(9.0)13.5\text{--}14.7(18.0)\times(3.5)4.7\text{--}5.1(6.0)\ \mu\text{m}$  (av. = 14.1 $\times$ 4.9  $\mu\text{m}$ ), with a length:width ratio 1.5–4.5. *Conidia* formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses. *Chlamydospores* globose to subglobose, 7–14 $\times$ 6–13  $\mu\text{m}$ , smooth, but often appearing rough due to deposits, thick-walled, mostly occurring intercalary in chains, hyaline, becoming golden-brown.

*Lecto- and teleotype*: Canada, Ontario, on living roots of *Panax quinquefolium*, 1935, A.A. Hildebrand, lectotype designated here CBS H-20565, as dried culture of CBS 308.35; teleotype designated here CBS H-20566, including fertile perithecia of the teleomorph (CPC 13532 $\times$ CBS 308.35), culture ex-lectotype CBS 308.35.

*Fertile matings*: Perithecia observed after 4 wk in crossings of strains: CPC 13532 $\times$ CBS 308.35, CPC 13532 $\times$ CBS 773.83, CPC 13532 $\times$ CBS 605.92, CPC 13532 $\times$ CBS 117813, CBS 129084 $\times$ CBS 308.35, CBS 129084 $\times$ CBS 605.92, CBS 129084 $\times$ CBS 117813.

**Culture characteristics:** Mycelium felty with an average density. Surface on OA sienna to sepia with aerial mycelium sparse, buff. Surface on PDA cinnamon, with aerial mycelium buff to cinnamon, or rosy buff on PDA. Zonation absent to concentric, with homogeneous transparency; margins predominantly even, but sometimes uneven. Reverse similar to surface, except in the colour, sienna on OA and chestnut at the centre, and sienna to orange towards the margin on PDA. Colonies on PDA grow 4–7 mm at 4°C after 7 days. Optimum temperature at 22°C when colonies reach 40–52 mm diam, after 7 days. Colony diam was 35–48 mm at 25°C after 7 days. No growth to slight growth (0–2 mm) was observed at 30°C.

**Isolates studied:** CBS 321.34; CBS 308.35; CBS 773.83; CBS 605.92; CBS 117813; CBS 117814; CBS 117815; CBS 117817; CBS 117818; CBS 117819; CBS 117820; CBS 117821; CBS 117822; CBS 117823; CBS 129084; CD1666; CPC 13532; Cy23; Cy158; Cy231 (Table 1).

**Hosts and distribution:** *Loroglossum hircinum* (root) (Tunisia), *Panax quinquefolium* (root) (Canada), *Prunus cerasus*, *Thymus* sp., *Vitis vinifera* (basal end of rootstock) (Portugal), *Quercus robur* (root), *Quercus* sp. (root) (Austria), *Tilia petiolaris* (rootstock) (Germany), water (in aquarium with *Anodonta* sp.) (Netherlands).

**Notes:** When Hildebrand (1935) described *Ramularia robusta* from living roots of *Panax quinquefolium* in

Ontario, Canada, he did not indicate a type specimen. However, he deposited an original culture in the CBS. A sporulating, dried-down culture is thus herewith designated as lectotype, and a new name proposed in *Ilyonectria*, with a teleotype represented by a fertile mating between CPC 13532 × CBS 308.35.

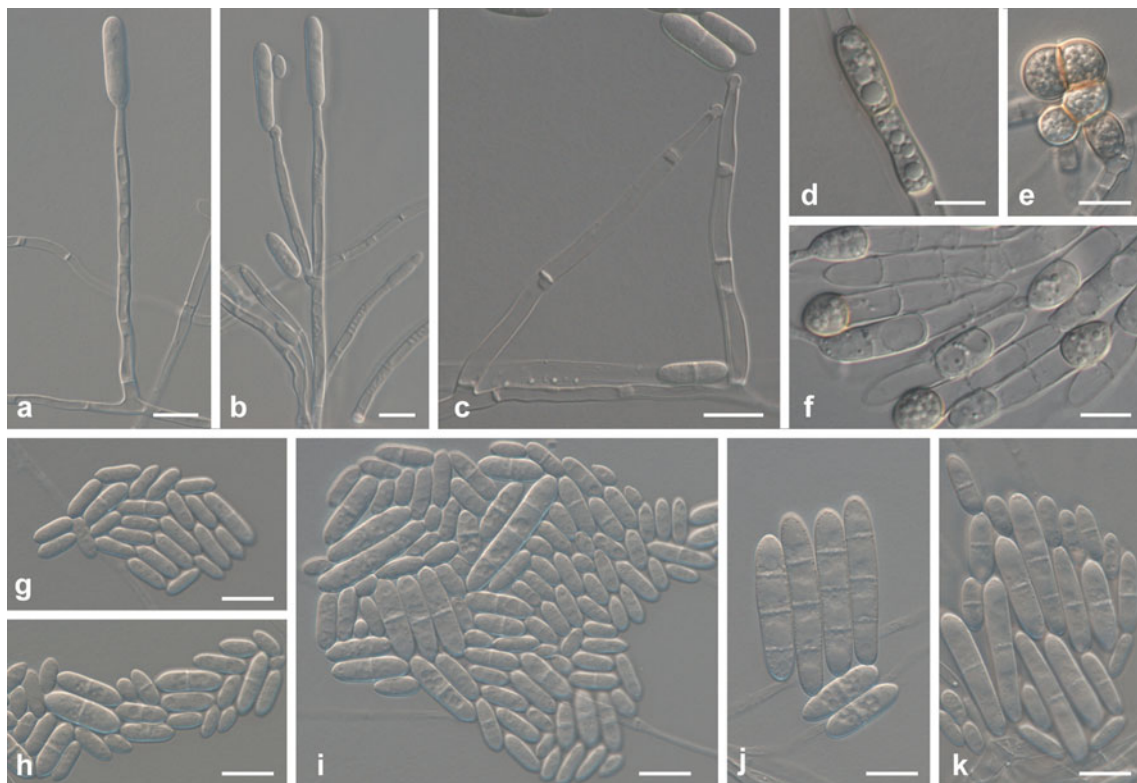
***Ilyonectria rufa* A. Cabral & Crous, sp. nov. (Fig. 14)**

Mycobank 560116.

**Etymology:** The epithet “*rufa*” referring to “*Coleomyces rufus*”, a provisional name proposed for this species by Moreau and Moreau (1937).

*Ilyonectriae crassae* morphologicis similis, sed macroconidiis brevioribus, 28–31.2 µm longis, distinguitur.

**Conidiophores** simple or complex, sporodochial. **Simple conidiophores** arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to two phialides, 1–5-septate, 55–210 µm long; phialides monophialidic, cylindrical to subulate, 20–57 µm long, 2.5–3.5 µm wide at the base, 1.5–2.0 µm near the aperture. **Complex conidiophores** aggregated in small sporodochia, repeatedly and irregularly branched. **Macroconidia** predominating, formed on both types of conidiophores, on SNA formed in flat domes of slimy masses, 1(–3)-septate, straight, cylindrical with both



**Fig. 14** *Ilyonectria rufa* (All from CBS 156.47, except c from CBS 120372). **a–c** Simple, sparsely branched conidiophores on aerial mycelium. **d–f** Chlamydospores in mycelium and in macroconidia. **g–k** Micro- and macroconidia. Bars 10 µm

ends broadly round, mostly centrally located hilum; 1-septate, (17.0)22.3–23.8(29.0)×(4.0)5.1–5.4(6.0) μm (av. = 23.1×5.3 μm), with a length:width ratio of 3.1–5.6; 2-septate, (19.0)24.5–26.6(32.0)×(4.0)5.2–5.5(6.5) μm (av. = 25.5×5.4 μm), with a length:width ratio of 3.4–6.0; 3-septate, (23.0)28.6–31.2(37.0)×(5.0)5.5–5.9(7.0) μm (av. = 29.9×5.7 μm), with a length:width ratio of 3.4–7.2. *Microconidia* 0–1-septate, ellipsoid to subcylindrical, more or less straight, with a visible, centrally located hilum; aseptate, (4.0)8.4–9.8(15.0)×(3.0)3.5–3.8(5.0) μm (av. = 9.1×3.6 μm), with a length:width ratio of 1.3–4.0; 1-septate, (9.0)12.1–13.3(17.0)×(3.0)4.2–4.6(5.5) μm (av. = 12.7×4.4 μm), with a length:width ratio 2.2–3.8. *Conidia* formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses. *Chlamydospores* globose to subglobose to cylindrical, 7–12×6–9 μm, smooth, but often appearing rough due to deposits, thick-walled, terminal on short, lateral branches, or intercalary, single, in chains or in clumps, and also in the cells of the macroconidia, hyaline, becoming slightly brown in the outer wall.

*Holotype*: France, dune sand, Feb. 1937, coll./isol. F. Moreau, holotype CBS H-20567, culture ex-type CBS 153.37.

*Culture characteristics*: For CBS 153.37, CBS 156.47, CPC 13536 and 94–1628: Mycelium felty with average to strong density. Surface on OA buff to saffron, aerial mycelium sparse, buff. On PDA rosy-buff to cinnamon, with aerial mycelium sparse, buff to rosy-buff or pale luteus in the centre. For CBS 640.77, CBS 120371 and CBS 120372: Mycelium felty, with low to average density. Surface on OA cinnamon to sienna, aerial mycelium sparse, saffron to cinnamon. On PDA saffron to cinnamon, with aerial mycelium cinnamon to rust. Zonation absent or concentric, with homogeneous transparency; margins even or sometimes uneven. Reverse similar, except in colour, saffron on OA, and cinnamon to rosy-buff on PDA, or sienna with pigments, pale vinaceous in OA and umber to chestnut on PDA. Colonies on PDA grow poorly, (2–7 mm diam) at 4°C, after 7 days. Optimum temperature between 20–22°C, when colonies reach 28–42 mm, 31–46 mm diam, respectively, after 7 days. Colony diam was 19–24 mm at 25°C after 7 days. No growth observed at 30°C.

*Isolates studied*: CBS 153.37; CBS 156.47; CBS 640.77; CBS 120371; CBS 120372; CPC 13536; 94–1628 (Table 1).

*Hosts and distribution*: *Azalea indica* (Belgium), dune sand (France), *Picea glauca*, *Pseudotsuga menziesii* (Canada).

*Notes*: The genus *Coleomyces* represents a later synonym of *Cylindrocarpon* (Booth 1966). However, *Coleomyces*, which is based on *C. rufus* (Moreau and Moreau 1937), was published as “*ad interim*”, suggesting that Moreau and Moreau were planning to validate the name later, which was

not the case. Based on the International Code of Botanical Nomenclature (Art. 34.1, Ex. 6), Chaverri et al. (2011) correctly chose to ignore the name. However, an original strain of *C. rufus* was deposited in the CBS (CBS 153.37), and the species epithet is herewith validated for the species.

*Ilyonectria venezuelensis* A. Cabral & Crous, sp. nov.  
(Fig. 15)

Mycobank 560117.

*Etymology*: Named after the country from where it was collected, Venezuela.

*Ilyonectriae robustae* morphologicis similis, sed conidiophoris cum verticillo terminali phialidum distinguitur.

*Conidiophores* simple or complex, sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium or from agar surface, solitary to loosely aggregated, unbranched, or bearing terminal, penicillate phialides, 1–4-septate, 35–200 μm long; phialides monopodial, cylindrical to subulate, 40–60 μm long, 2.5–3.5 μm wide at the base, 1.5–2.5 μm near the apex, or narrowly flask-shaped, 16–40 μm long, 2.0–3.0 μm wide at the base, 3.0–4.0 μm at the widest point, 1.5–2.5 μm near the apex. *Complex conidiophores* aggregated in small sporodochia, repeatedly and irregularly branched. *Macroconidia* predominating, formed by both types of conidiophores, on SNA formed in flat domes of slimy masses, 1–3-septate, straight or minutely curved, cylindrical with both ends more or less broadly rounded, but may narrow towards the tip, mostly without a visible hilum; 1-septate, (22.0)24.6–27.3(35.0)×(5.0)5.3–5.7(6.5) μm (av. = 26.0×5.5 μm), with a length:width ratio of 3.8–7.0; 2-septate, (25.0)26.3–37.4(44.0)×(5.9)6.0–6.6(7.0) μm (av. = 31.9×6.3 μm), with a length:width ratio of 4.2–6.8; 3-septate, (28.0)36.5–41.7(50.0)×(6.0)6.9–7.5(8.0) μm (av. = 39.1×7.2 μm), with a length:width ratio of 4.0–6.7. *Microconidia* 0–1-septate, ellipsoid to ovoid, more or less straight, without a visible hilum; aseptate, (5.0)8.4–10.5(13.0)×(3.0)3.3–3.7(4.0) μm (av. = 9.5×3.5 μm), with a length:width ratio of 1.7–3.4; 1-septate, (11.0)14.5–16.3(19.0)×(3.5)3.9–4.3(5.0) μm (av. = 15.4×4.1 μm), with a length:width ratio 2.8–4.8. *Conidia* formed in heads on simple conidiophores or as white (OA) or unpigmented (SNA) masses. *Chlamydospores* ovoid to ellipsoidal, 6–13×5–7 μm, smooth, but often appearing rough due to deposits, thick-walled, terminal on short lateral branches or intercalary, single, in chains or in clumps, hyaline, becoming slightly brown at the margin.

*Holotype*: Venezuela, Amazonas, Cerro de la Neblina, tree bark, 1985, coll./isol. A. Rossman, holotype CBS H-20568, culture ex-type CBS 102032.

*Culture characteristics*: Mycelium cottony with average to strong density. Surface on OA saffron, with aerial



**Fig. 15** *Ilyonectria venezuelensis* (CBS 102032). **a, b** Simple conidiophores on aerial mycelium. **c–e** Conidiophores bearing terminal, penicillate phialides. **f–j** Micro- and macroconidia. Bars 10  $\mu\text{m}$

mycelium sparse, buff, on PDA buff to saffron, with aerial mycelium saffron to pale luteous; zonation absent, transparency homogeneous, margin even; reverse similar to surface, but saffron to cinnamon on PDA. Colonies on PDA grow poorly (2–3 mm) at 4°C, after 7 days. Optimum temperature at 20°C, with colonies reaching 49 mm diam, after 7 days. Colony diam was 35–36 mm at 25°C after 7 days. No growth was observed at 30°C.

*Isolate studied*: CBS 102032 (Table 1).

*Host and distribution*: Tree bark (Venezuela).

***Ilyonectria vitis*** A. Cabral, Rego & Crous, *sp. nov.*

(Fig. 16)

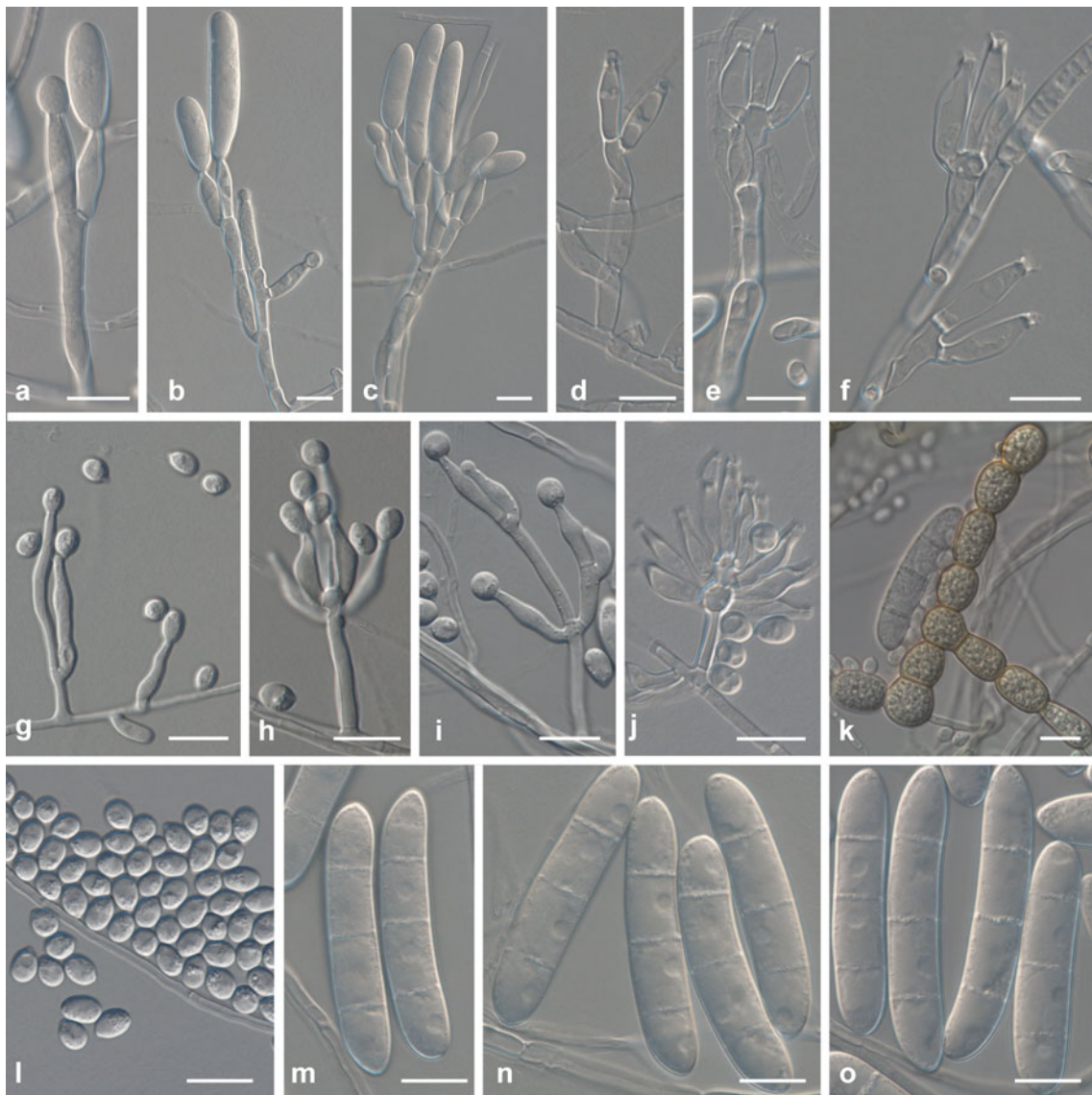
Mycobank 560107.

*Etymology*: Named after the host from which it was collected, *Vitis vinifera*.

*Ilyonectriae anthuriicolae* morphologicis similis, sed longitudine media macroconidiorum longiore, 41.6–43.5  $\mu\text{m}$ , distinguitur.

*Conidiophores* simple or complex or sporodochial. *Simple conidiophores* arising laterally or terminally from aerial mycelium, solitary to loosely aggregated, unbranched or sparsely branched, bearing up to three phialides, 1–3 septate, 30–70  $\mu\text{m}$  long; monophialides more or less cylindrical, but tapering slightly towards the tip, 11–

21  $\mu\text{m}$  long, 2.0–3.0  $\mu\text{m}$  wide at the base, 3.0–4.5  $\mu\text{m}$  at widest point, 1.5–2.5  $\mu\text{m}$  near the apex. *Conidiophores* forming microconidia arising from mycelium at agar surface, reduced to monophialides, or a stipe with a terminal arrangement of phialides, ranging from 2 to a dense cluster; sparsely branched or penicillate; monophialides narrowly flask-shaped, typically with widest point near the middle, 10–17  $\mu\text{m}$  long, 1.5–3.0  $\mu\text{m}$  wide at the base, 2.5–4.0  $\mu\text{m}$  at widest point, 1.0–2.0  $\mu\text{m}$  near the apex. *Sporodochial conidiophores* irregularly branched; phialides more or less cylindrical but slightly tapering towards the tip, or narrowly flask-shaped, with widest point near the middle, 14–20  $\mu\text{m}$  long, 2.5–3.5  $\mu\text{m}$  wide at the base, 3.0–4.5  $\mu\text{m}$  at widest point, 1.5–2.5  $\mu\text{m}$  near the apex. *Macroconidia* formed in flat domes of slimy masses, predominantly 3-septate, rarely 1–2- or 4-septate, straight or minutely curved, cylindrical with both ends more or less broadly rounded, mostly without a visible hilum; 3-septate conidia (34.9)41.6–43.5(51.6)  $\times$  (6.2)7.9–8.2(9.5)  $\mu\text{m}$  (av. = 42.5  $\times$  8.0  $\mu\text{m}$ ), with a length:width ratio of 3.9–6.7. *Microconidia* on SNA formed in heads, aseptate, subglobose to ovoid, rarely ellipsoid, mostly with a visible, centrally located or slightly laterally displaced hilum, (3.7)4.9–5.4(6.7)  $\times$  (3.2) 3.7–4.0(4.6)  $\mu\text{m}$  (av. = 5.1  $\times$  3.9  $\mu\text{m}$ ), with a length:width ratio of 1.1–1.7. *Chlamydospores* globose to subglobose to ellipsoid, 9–18  $\times$  6–13  $\mu\text{m}$ , smooth, but often appearing



**Fig. 16** *Ilyonectria vitis* (CBS 129082). **a–c** Simple conidiophores on aerial mycelium. **d–g** Conidiophores forming microconidia arising from mycelium at agar surface, reduced to a stipe with a terminal

arrangement of phialides, ranging from 2 to a dense cluster; sparsely branched or penicillate. **i–l** Micro- and macroconidia. **m** Chlamydo-spores on mycelium. Bars 10  $\mu$ m

rough due to deposits, thick-walled, formed intercalary in chains or in clumps, and also in the cells of macroconidia, hyaline, becoming golden-brown.

**Holotype:** Portugal, Vidigueira, *Vitis vinifera*, basal end of a 2-year-old plant; scion Touriga Nacional; rootstock 110R, 2008, coll./isol. C. Rego, holotype CBS H-20569, culture ex-type CBS 129082=Cy233=CPC 19168.

**Culture characteristics:** Mycelium felty with density low to average. Surface on OA sienna, with sparse, saffron aerial mycelium, and luteous growth at margin. Surface on PDA chestnut, with sienna aerial mycelium, with luteous margin. Zonation was absent (OA) or concentric (PDA), transparency was homogeneous (PDA) or not (OA). Growth at margin even to uneven. Reverse similar to

surface, except in colour, sienna to saffron on OA, and chestnut to umber on PDA. Colonies on PDA do not grow at 4°C after 7 days. Optimum temperature at 20°C, when colonies reach 29–30 mm diam, after 7 days. Colony diam was 39–40 mm at 25°C and 8–9 mm at 30°C after 7 days. No growth was observed at 35°C.

**Isolate studied:** CBS 129082 (Table 1).

**Host and distribution:** *Vitis vinifera* (Portugal).

#### Key to species treated

- 1 Growth at margin on OA after 14 days at 20°C, lacking yellow pigmentation
- 2 Colony diameter on PDA after 7 days at 25°C < 30 mm

- 3 Macroconidia forming chlamydospores
- 4 Macroconidia 1–3-septate, 3-septate macroconidia mean range 34.1–36.2  $\mu\text{m}$  long
- 5 Macroconidia cylindrical, with the base appearing somewhat acute *I. crassa*
- 5\* Macroconidia clavate *I. pseudodestructans*
- 4\* Macroconidia predominantly 1-septate; 3-septate macroconidia smaller, mean range 28–31.2  $\mu\text{m}$  long *I. rufa*
- 3\* Macroconidia lacking chlamydospores
- 6 Macroconidia predominantly curved *I. liliigena*
- 6\* Macroconidia straight
- 7 Macroconidia lacking visible hilum *I. mors-panacis*
- 7\* Macroconidia with a visible, centrally located hilum
- 8 Three-septate macroconidia mean range 31.0–35.0  $\mu\text{m}$  long *I. panacis*
- 8\* Three-septate macroconidia mean range 34.3–38.5  $\mu\text{m}$  long *I. gamsii*
- 2\* Colony diameter after 7 days at 25°C >30 mm
- 9 Colony diameter after 7 days at 25°C, >50 mm *I. cyclaminicola*
- 9\* Colony diameter after 7 days at 25°C, 30–50 mm
- 10 Conidiophores bearing a terminal whorl of phialides *I. venezuelensis*
- 10\* Conidiophores unbranched, or different from above
- 11 Teleomorph known, and can be induced in culture
- 12 Three-septate macroconidia mean range 32.3–34.7  $\mu\text{m}$  long; ascospores mean range 9.4–10.0  $\mu\text{m}$  long *I. robusta*
- 12\* Three-septate macroconidia mean range 30.0–36.0  $\mu\text{m}$  long; ascospores mean range longer, 10–13  $\mu\text{m}$  long *I. radicola*<sup>a</sup>
- 11\* Teleomorph unknown
- 13 Mean range of 3-septate macroconidia, 29.7–31.5  $\times$  6.5–6.9  $\mu\text{m}$  *I. europaea*
- 13\* Mean range of 3-septate macroconidia smaller, 25.0–28.4  $\times$  5.2–5.5  $\mu\text{m}$  *I. lusitana*
- 1\* Yellow pigmentation present at margin
- 14 Macroconidia 3-septate, mean range 29.5–32.2  $\mu\text{m}$  long *I. anthuriicola*
- 14\* Macroconidia 3-septate, mean range 41.6–43.5  $\mu\text{m}$  long *I. vitis*

<sup>a</sup>No authentic cultures of “*C.*” *destructans*, conidia (18.0–)23.0–30.0(–35.0)  $\times$  (6.0–)6.5(–7.0)  $\mu\text{m}$ , are presently available.

## Discussion

“*Cylindrocarpon*” *destructans* is a cosmopolitan soil-borne pathogen causing disease on a wide number of herbaceous and woody plant species (Samuels and Brayford 1990). The wide morphological and pathogenic amplitude of “*C.*”

*destructans* makes it a commonly identified species, with many diseases from the *Cylindrocarpon*-complex being attributed to it, and ranking at the top of all “*Cylindrocarpon*” spp. deposited in the NCBI nucleotide database.

In this study, “*C.*” *destructans* isolates from the CBS culture collection (deposited under the wider concept of the species) were analysed using a multigene approach in order to clarify taxonomic aspects of this species complex. Molecular analyses show that these isolates cluster in various clades supported by high bootstrap support values. A previous study (Seifert et al. 2003) included a subset of the strains used here, and already highlighted the existence of unexpected divergence in “*C.*” *destructans*, as opposed to a large homogeneity in e.g. *Neonectria ditissima*. Several species have in recent years been separated from the “*C.*” *destructans* complex, including “*C.*” *macroconidialis*, “*C.*” *coprosmae* and “*C.*” *austroradicicola* based on morphological (Samuels and Brayford 1990) and molecular characters (Schroers et al. 2008; Seifert et al. 2003). Furthermore, several isolates causing black foot disease of grapevine, previously considered as “*C.*” *destructans*, were recently identified as *I. liriodendri* (Chaverri et al. 2011; Halleen et al. 2006), along with the ex-type strain from *Liriodendron tulipifera* (CBS 110.81) and a strain from *Cyclamen* (CBS 301.93). In this study, two further strains isolated from young *Malus domestica* and *Quercus suber* trees showing decline symptoms were also identified as *I. liriodendri*.

Altogether, we analysed 68 strains putatively belonging to “*C.*” *destructans*, but none of them clustered together with the ex-type culture of *I. radicola* (CBS 264.65), suggesting that this species may not be as common as previously accepted. Halleen et al. (2006) identified a single strain (IMI 313237, isolated from arecoid palm) clustering with CBS 264.65. This also raises questions relating to the correlation between the anamorph, “*C.*” *destructans*, and its purported teleomorph, *I. radicola*.

“*Nectria*” *radicola* was described by Gerlach and Nilson (1963) from decayed leaves, flowers stalks and corms of *Cyclamen persicum* collected in Sweden, with a “*Cylindrocarpon*” anamorph they identified as “*C.*” *radicola*.

In 1924, Wollenweber introduced “*C.*” *radicola* (McAlpine) Wollenw. as a new combination, based on *Septocylindrium radicola* McAlpine (1899), described from *Citrus* trees in Australia. Later, Wollenweber (1928) noted that *Septocylindrium radicola*, with catenulate conidia, was different from “*C.*” *radicola*, and the name was therefore based on Wollenweber’s (1928) description. Because of this confusion in names, Booth (1966) suggested that “*C.*” *radicola* should be dropped, and that the name to be used as anamorph for *I. radicola* should be “*C.*” *destructans* [originally described by Zinssmeister (1918) on *Panax quinquefolia* from Wisconsin, USA].



Furthermore, Booth (1966) designated a neotype for “*C.*” *destructans*, obtained from *P. quinquefolia* in USA, KY, Washington Co., Springfield; collected by W.B. Edwards in 1922, available as herbarium material in Cornell Plant Pathology Herbarium, CUP-011985. This specimen was re-examined in the present study [conidia (18.0)23.0–30.0(35.0) × (6.0)6.5(7.0) μm] [original description by Zinssmeister (1918), 9.0–32.4 × 3.2–8.1 μm], thus revealing conidia to be smaller than those formed by *I. radicolica*, which are (24.0)33.1(47.0) × (4.9)6.4(7.8) μm. From these observations, we conclude that “*C.*” *destructans*, which occurs on *P. quinquefolia* in the USA, represents yet another species distinct from *I. radicolica*, which is not yet represented in our phylogenetic tree (Fig. 1).

A strain deposited in CBS culture collection by Hildebrand in 1935, as “*Ramularia*” *mors-panacis*, was found to represent original material (ex-type CBS 306.35), collected from living roots of *Panax quinquefolium* in Ontario, Canada. The epithet “*mors-panacis*” is therefore resurrected for this clade, while the Japanese collection identified as “*Cylindrocarpon destructans*” f. sp. *panacis* (ex-type CBS 124662=NBRC 31881), isolated from *Panax ginseng* in Japan, is treated as synonym.

The ex-type strain of “*Ramularia*” *robusta* (CBS 308.35), isolated from living roots of *Panax quinquefolium* in Ontario, Canada, can be resurrected for a large clade representing isolates from a range of hosts and continents. Similarly, an authentic strain of “*C.*” *destructans* var. *crassum* (Booth 1966; Wollenweber 1931) is available for a species occurring on *Panax* and *Pseudotsuga* in Canada, *Lilium* and *Narcissus* in the Netherlands, and can thus be resurrected as *I. crassa*. Although strain CBS 120370 clustered together with other strains of *I. crassa* for most genes (no nucleotide differences in ITS and TEF, two nucleotide differences in HIS), this strain was not included in that species because of an 8-bp difference in TUB, a slower growth rate (e.g., 21 mm colony diam at 20°C for 7 days, as opposed to 31–46 mm for other isolates), a lower conidial length:width ratio [e.g. for 3-septate conidia 4.0–6.5, as opposed to 4.8–8.9 and smaller conidia (e.g. 3-septate conidia ranging from (26.0)31.2–34.0(40.0) × (6.0) 6.6–7.1(8.0) μm (av. 32.6 × 6.9 μm), as opposed to (29.0) 34.1–36(49.0) × (5.0)5.6–5.8(7.0) μm (av. 35.1 × 5.7 μm)]. Further studies should thus be conducted in order to clarify the taxonomy of this strain.

*Ilyonectria anthuriicola* and *I. vitis* are very similar in morphology to “*C.*” *pauciseptatum*. These species all have predominantly 3-septate macroconidia after 10 days in culture. *Ilyonectria anthuriicola* is easier to distinguish than “*C.*” *pauciseptatum* as the 3-septate conidia are smaller and narrower, (25.0)29.5–32.2(38.0) × (6.0)7.5–8.1(9.0) μm, while in “*C.*” *pauciseptatum* they are (37.0)42.0–47.0

(54.0) × (7.0)8.5–9.5(10.0) (Schroers et al. 2008). For *I. vitis* 3-septate macroconidia are of similar size to those of “*C.*” *pauciseptatum*, (34.9)41.6–43.5(51.6) × (6.2)7.9–8.2 (9.5), making it difficult to distinguish them based on this character. Growth rate at 20°C is slower in *I. vitis* than in “*C.*” *pauciseptatum*, and they also differ regarding colony characteristics and colours. Morphologically, however, they remain difficult to distinguish.

In this study, the genetic structure of the *I. radicolica* complex was analysed using a multi-locus approach along with morphological and culture characters. Three major groups were identified based on this approach, each group containing several species. Although we have been able to clarify several aspects related to the host range and distribution of taxa in the *I. radicolica* species complex, further collections, especially from *Panax* in the USA, will be essential to elucidate the status of “*C.*” *destructans*.

**Acknowledgements** The first author would like to thank Dr M.C. Silva (Coffee Rust Research Centre / Tropical Research Institute, Portugal) for making equipment available, and for assisting with the use of the cryostat for perithecial sectioning. Drs F. Caetano (Instituto Superior de Agronomia, Portugal), J. Armengol (Univ. Politécnica de Valencia, Spain), K.A. Seifert (Agriculture and Agri-Food, Canada), M.L. Inácio (Instituto Nacional de Recursos Biológicos/INIA, Portugal) P. Lecomte (Institut National de la Recherche Agronomique, Bordeaux-Aquitaine, France) and W.D. Gubler (Univ. California, Davis, USA) are thanked for making several strains available, without which this study would not have been possible. The curator of CUP is acknowledged for making the neotype “*C.*” *destructans* available for examination. This article is part of a PhD dissertation (Instituto Superior de Agronomia, Technical University of Lisbon, Portugal). This work was partially supported by Fundação para a Ciência e a Tecnologia, Portugal (grant number SFRH/BD/24790/2005; project PTDC/AGR-AAM/099324/2008).

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