

RESEARCH PAPERS

Occurrence fungi causing black foot on young grapevines and nursery rootstock plants in Italy

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Summary. Young grapevine plants with decline and wood necrosis symptoms were collected from vineyards and nurseries in the Apulia and Molise regions, Italy, from 2013 to 2015. Isolations of fungi were prepared from 45 diseased grapevine plants, and the cultures were identified. Several species commonly associated with Petri disease, Botryosphaeria dieback, and black foot disease were isolated. A detailed study was carried out, and 182 isolates resembling *Cylindrocarpon*-like asexual forms were identified through morphological characterisation and DNA analysis of internal transcribed spacer regions 1 and 2 of the rRNA gene and the partial β -tubulin gene. *Dactylonectria torrensensis* and *Ilyonectria liriodendri* were identified based on morphological features and the partial histone 3 gene, so these fungi can be defined as the causal agents of black foot on grapevine for the first time in Italy. *Thelonectria blackeriella* is also described as a new species, through morphological characterisation and multigenic analysis using sequence data for five loci (large subunit RNA, internal transcribed spacers, β -tubulin, actin, RNA polymerase II subunit 1). This new species was associated with black foot symptoms according to preliminary pathogenicity tests, with representative isolates of each of the three species. Pathogenicity tests showed that these species can cause black streaking in the wood of 1-year-old grapevine rootstock shoots. The identification of *D. torrensensis*, *I. liriodendri* and *T. blackeriella* from young grapevine plants and rooted rootstock highlights the importance of black foot disease in Italy, which has previously been overlooked.

Key words: *Dactylonectria*, *Ilyonectria*, *Thelonectria*, *Vitis vinifera*.

Introduction

Grapevine trunk diseases (GTDs) are one of the most important problems for grapevine plants worldwide, as they can result in serious economic losses. Most GTDs are caused by fungal pathogens that penetrate through vine pruning wounds and invade the wood, to cause vascular discolourations and perennial cankers, such as seen for *Botryosphaeria* spp. (Urbez-Torres *et al.*, 2006; Carlucci *et al.*, 2015b), *Phomopsis viticola* (De Guido *et al.*, 2003; van Niekerk *et al.*, 2005) and *Eutypa lata* (Larignon and Dubos, 1997). Other important GTDs include Petri

disease (PD) and esca, which are caused by vascular fungi, including *Phaeoconiella chlamydospora* and *Phaeoacremonium* spp., and parenchymatic fungi, including *Fomitiporia* spp. (Mugnai *et al.*, 1999; Armengol *et al.*, 2001; Fischer, 2002; Carlucci *et al.*, 2015a). Gramaje *et al.* (2011) and Navarrete *et al.* (2011) reported that *Cadophora melinii* and *C. luteo-olivacea* can also be associated with GTDs. Recently, another fungal species was associated with GTDs (Carlucci *et al.*, 2015a): *Pleurostomophora richardsiae* (= *Pleurostoma richardsiae*; Reblova *et al.*, 2016).

Over the last 15 years, several studies have reported the occurrence and increasing incidence of black foot disease (BFD) of grapevine in production areas around the world. Originally, the causal agents were indicated as *Cylindrocarpon* spp., which were responsible for cankers, root rot, and decay of

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woody and herbaceous plants, for which the first report was dated to 1913 by Wollenweber (Domsch *et al.*, 2007). Subsequently, genera with *Cylindrocarpon*-like asexual morphs in the Nectriaceae were subjected to substantial taxonomic revision. Booth (1966) sub-divided this genus into four groups based on the presence or absence of microconidia and chlamydospores, identified as *Cylindrocarpon magnusianum* (Sacc.) Wollenw. (an anamorph of the type species of *Neonectria*), *Cylindrocarpon cylindroides* Wollenw. (the type species of the genus *Cylindrocarpon*), *Cylindrocarpon destructans* (an anamorph of *Neonectria radicola*), and other members of *Cylindrocarpon* spp. connected with the teleomorphs of *Nectria mammoidea* (Brayford, 1993; Halleen *et al.*, 2006b). Based on this classification, reference strains of all *Nectria* groups with *Cylindrocarpon* anamorphs were transferred into the *Neonectria* genus (Rossman *et al.*, 1999).

Further studies conducted by Mantiri *et al.* (2001) and Brayford *et al.* (2004) grouped all *Neonectria*/*Cylindrocarpon* spp. into a monophyletic group. Although these authors indicated that this group included distinct sub-clades, they did not describe any new genera. Halleen *et al.* (2004) took the first formal step in the segregation from the genus *Cylindrocarpon*, with their description of the new genus of *Campylocarpon* which is morphologically similar to the *Cylindrocarpon*-like asexual morphs, but is phylogenetically not close to the *Neonectria*/*Cylindrocarpon* genus.

Subsequently, a detailed study on *Neonectria*/*Cylindrocarpon* and *Cylindrocarpon*-like anamorphs was carried out by Chaverri *et al.* (2011), who described the three new genera of *Ilyonectria*, *Rugonectria* and *Thelonectria*. Moreover, in considering *Cylindrodendrum*, which was described for first time by Bonorden (1851) with *Cylindrodendrum album* as the type species and *Cylindrocarpon*-like synasexual morphs, they suggested that this should be considered a synonym of *Cylindrocarpon* / *Neonectria*. Lombard *et al.* (2014) recently showed that *Cylindrodendrum* spp. form a well-supported monophyletic clade close to the *Ilyonectria* clade, but distant from the *Neonectria* clade.

Recent phylogenetic studies have revealed the paraphyletic nature of the genus *Ilyonectria* (Cabral *et al.*, 2012a, 2012b; Lombard *et al.*, 2013), so Lombard *et al.* (2014) described *Dactylonectria* as a new genus. Another genus such as *Cylindrocladiella* was described by Boesewinkel (1982), to accommodate five *Cylindrocladium*-like species that produced small and cylindrical conidia. This was confirmed

as a distinct clade by Lombard *et al.* (2012), who described 18 new *Cylindrocladiella* spp., based on morphological and phylogenetic studies. More recently, Salgado-Salazar *et al.* (2016) established that *Thelonectria* is also polyphyletic, and they described three new genera that are closely related to *Thelonectria*: *Cinnamomeonectria*, *Macronectria* and *Tumenectria*. *Campylocarpon*, *Dactylonectria* and *Ilyonectria* are the more common genera, that include fungal species associated with BFD of grapevine (Halleen *et al.*, 2004; Cabral *et al.*, 2012a; Lombard *et al.*, 2014). Van Coller *et al.* (2005), Agustí-Brisach *et al.* (2012) and Jones *et al.* (2012) also associated *Cylindrocladiella* spp. with BFD of grapevine. To date, 17 fungal species are known as agents that can cause BFD of grapevine worldwide. These are: *Campylocarpon fasciculare*, *Campylocarpon pseudofasciculare* (Halleen *et al.*, 2004), *Cylindrocladiella parva*, *Cylindrocladiella peruviana* (Agustí-Brisach *et al.*, 2012), *Dactylonectria alcaerensis*, *Dactylonectria estremocensis*, *Dactylonectria macrodidyma*, *Dactylonectria novozelandica*, *Dactylonectria pauciseptata*, *Dactylonectria pinicola* (= *Ilyonectria* sp.2), *Dactylonectria torresensis*, *Dactylonectria vitis* (Lombard *et al.*, 2014), *Ilyonectria europea*, *Ilyonectria liriodendri*, *Ilyonectria lusitanica*, *Ilyonectria pseudodestructans* and *Ilyonectria robusta* (Chaverri *et al.*, 2011; Cabral *et al.*, 2012a, 2012b).

These fungal pathogens have usually been isolated from older grapevines with BFD symptoms, but in more recent studies, they have also been isolated from symptomatic and asymptomatic rootstock mother-plants, rootstock cuttings, and young grafted vines. As such they have become the most common pathogenic fungi associated with young nursery vines (Rumbos and Rumbou, 2001; Halleen *et al.*, 2003, 2006a, 2007; Fourie and Halleen, 2004; Oliveira *et al.*, 2004; Dubrovsky and Fabritius, 2007; Aroca *et al.*, 2010; Cardoso *et al.*, 2012; Agustí-Brisach and Armengol, 2013). Moreover, Agustí-Brisach *et al.* (2013b, 2014) reported that BFD pathogens have also been isolated from soils in grapevine nurseries and vineyards.

Gramaje and Armengol (2011) reported that the more traditional propagation techniques used in viticulture can have significant effects on the quality of the vines produced. They stated that apparently healthy grafted nursery plants often show black discolouration and brown streaking in the wood of stems and rootstock and/or roots, from which GTD pathogens can be isolated, including those associ-

ated with PD and BFD. The most common symptoms related to BFD are sunken necrotic root lesions and reduced root biomass, under-bark black discoloration, and necrosis of xylem tissue at the base of the rootstocks. Grapevine plants affected by BFD pathogens also show reduced vigour, shortened internodes, sparse foliage, and small leaves, with interveinal chlorosis and necrosis, which the pathogens can kill affected plants (Rego *et al.*, 2000; Halleen *et al.*, 2006b; Alaniz *et al.*, 2007; Reis *et al.*, 2013). Field symptoms of BFD on vines are frequently indistinguishable from those caused by PD (Scheck *et al.*, 1998; Rego *et al.*, 2000; Halleen *et al.*, 2006b; Alaniz *et al.*, 2007, 2009; Abreo *et al.*, 2010).

The aim of the present study was to characterise a collection of fungal isolates that were obtained from diseased young grapevines and rooted rootstock in Apulia and Molise (southern and central Italy), using morphological and molecular studies. All of the *Cylindrocarpon*-like isolates were further investigated by morphological and multigenic analyses, to identify which species are involved in BFD in the Apulia and Molise regions, and to describe a new species of *Thelonectria*. In addition, through pathogenicity tests, direct correlation has been made between disease symptoms and a novel species of *Thelonectria* that is not commonly associated with BFD.

Materials and methods

Isolates

From May 2013 to October 2015, 28 young grapevines (aged from 12 to 18 months) were collected from vineyards, which included the cultivars 'Pinot grigio', 'Chardonnay' and 'Trebiano toscano'. Additionally, 17 nursery rooted rootstock vines grafted with the cultivars 'Moscato' 'Sangiovese', 'Cocociola d'Abruzzo' and 'Ciliegiolo' were collected (Table 1). Various external symptoms were observed on the plants, including stunting, reduced vine vigour, shortened internodes, shoot dieback and leaf discoloration, with interveinal chlorosis and necrosis. Moreover, sunken necrotic symptoms and reduction of root hairs were observed on young grapevines and nursery rootstock plants. The internal symptoms observed from cross-sections of young grapevines and nursery rootstock plants showed different brownish-blackish discolorations around the medullae (Figure 1). The samples transported to the laboratory for analysis consisted of root hairs and cross-sections of roots, stems from 3 cm below and above the grafted points, basal stems above the graft unions, and basal branching stems (cordons). After surface sterilisation of the symptomatic wood tissues (Fisher *et al.*, 1992), the bark of each sample was removed with a sterile

Table 1. Characteristics of young grapevine and nursery rootstock analysed.

Grapevine cultivar	Vineyard/ nursery rootstock (V/NR)	Age (Months)	Locality	Symptomatic plants in vineyard (%) ^a	Number of symptomatic samples analysed
Pinot grigio	V	18	Campomarino (CB)	38.3	8
Chardonnay	V	18	Campomarino (CB)	22.5	10
Trebiano toscano	V	18	Campomarino (CB)	11.2	10
Sangiovese	NR	12	Market nursery, Cerignola (FG)	Not estimated	5
Moscato	NR	12	Market nursery, Canosa di Puglia (BT)	Not estimated	3
Cocociola d'Abruzzo	NR	12	Market nursery, Torremaggiore (FG)	Not estimated	5
Ciliegiolo	NR	12	Market nursery, Canosa di Puglia (BT)	Not estimated	4
Total					45

^a Estimated as grapevine plants symptomatic as proportion of total plants in each vineyard.



Figure 1. (a-b) Young grapevine showing discoloration of leaf similar to that associated with PD (Petri disease). (c-e) Black and brown wood discoloration in cross-section from which BFD (black foot disease) fungi (*Dactylonectria* spp., *Ilyonectria* spp. and *Thelonectria* sp. nov.) were mainly isolated. (f-i) Brown wood discoloration and black exudate drops in cross-section of young grapevine and rootstock from which PD and BFD fungi were mainly isolated. (j-l) Black and brown discoloration, black exudate drops, and browning streaks in cross-section of young grapevine and nursery rootstock plants from which PD, BD (*Botryosphaeria dieback*) and BFD fungi were isolated.

scalpel, and thin wood sections were cut (1 to 3 mm thick). From each portion of the samples, five small wood tissue segments were placed on potato dextrose agar (PDA; 3.9% potato dextrose agar; Oxoid Ltd.) and on malt extract agar (MEA; 2% malt extract and 2% agar Oxoid Ltd.), both supplemented with 500 mg L⁻¹ streptomycin sulphate (Oxoid Ltd.). The isolation plates were incubated at 25°C (±3°C) in the dark. After 7 to 10 d of incubation, all of the fungal cultures believed to belong to GTD genera were spread over Petri dishes of PDA, and after an overnight incubation, single germinating conidia or small pieces of hyphae were transferred to Petri dishes with fresh PDA. The morphological and culture characteristics were initially used to distinguish the fungal genera and species isolated from the symptomatic tissues (Crous and Gams, 2000; Mostert *et al.*, 2006; Essakhi *et al.*, 2008; Agusti-Brisach *et al.*, 2013a; Phillips *et al.*, 2013; Raimondo *et al.*, 2014; Carlucci *et al.*, 2015a).

A total of 450 fungal isolates were obtained, and these were grouped according to the different GTDs, and only the *Cylindrocarpon*-like isolates were used in the further analyses. The reference isolates have been deposited in the culture collection of the Department of Sciences, Agriculture, Food and Environment (SAFE) of the University of Foggia, Italy, and in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

The fungal isolation frequency (IF; %) per grapevine section sampled (young grapevines and nursery rootstock plants) was calculated as the number of tissue portions infected by a given fungus, divided by the total number of tissue segments incubated. To determine which fungal group (i.e., the causal agents of PD, Botryosphaeria dieback (BD), and BFD) was correlated to which plant organ (i.e., root, rootstock, scion, basal stem, branches), principal component analysis (PCA) was performed using XLStat 2016.1 (Addinsoft SARL, France).

To determine the syndromes (i.e., PD, BD, BFD) and the incidence with which they occurred alone or in combination on young grapevines and nursery rootstock plants, the disease incidences (DI) were calculated, as the percentage of plants infected divided by the total number of plants analysed.

Morphology

All fungal colonies morphologically attributed to BFD were subjected to detailed morphological stud-

ies, carried out according to Chaverri *et al.* (2011). Based on preliminary morphological characterisation, isolates attributed to *Thelonectria* were used to provide perithecial formation. All *Thelonectria* isolates were crossed with each other or placed alone in Petri dishes (90 mm diam.) that contained three different media: PDA, or Spezzellier Nährstoffarmer agar (Nirenberg, 1976) without and with 0.1% yeast extract (Oxoid Ltd.). The plates were incubated at 20 ± 2°C under ultraviolet light and at room temperature, for 3 to 4 weeks. For asexual morphs, if conidiation did not occur, the isolates were grown on the same media and/ or incubated under near ultraviolet light, and in the dark at 23 ± 2°C.

Fungal structures were measured from 100% lactic acid mounts by taking 30 measurements (at 400× and 1,000× magnification), using a Leica Application Suite measurement module (Leica Microsystems GmbH). Photomicrographs were recorded using a digital camera (Leica DFC320) on a microscope fitted with Normaski differential interference contrast optics (Leica DMR). The 5th and 95th percentiles were calculated for all the measurements, and the extremes are presented. Detailed measurements were conducted for six isolates per fungal species. The microscopic features of conidiophores and conidia were also determined in distilled water picking up mycelial plugs from 30-d-old cultures grown on MEA, and images taken at ×40 magnification with Leica DM5500 microscope.

Growth rates and colony characteristics of fungi were determined on plates containing 20 mL malt extract agar (MEA; 2% malt extract Oxoid agar, 1.0 L water), PDA, and oatmeal agar (30 g oats, 8 g Oxoid agar, 1.0 L water), inoculated with 5 mm diam. mycelium plugs of isolates, and then incubated at 23 ± 2°C in the dark for 16 d. Colony morphology and colour were assessed on MEA, PDA and oatmeal agar, at 23 ± 2°C after 21 d using the colour charts of Rayner (1970). Cardinal temperatures for growth were determined on MEA incubated in the dark at temperatures from 5 to 40°C, at 5°C intervals, and including 37°C. Radial growth was measured on MEA plates, after 8 d at 20 ± 2°C.

DNA extraction, amplification and sequencing

Genomic DNA of the isolates was extracted from 15-d-old cultures growing on PDA, according to Carlucci *et al.* (2013). The genera and species in the Botry-

osphaeriaceae were identified with the keys, descriptions, and sequence data provided by Phillips *et al.* (2013). *Pleurostoma* isolates were identified using the descriptions and sequence data provided by Carlucci *et al.* (2015a). *Phaeomoniella* isolates were identified according to Crous and Gams (2000). *Phaeoacremonium* isolates were identified with the keys, descriptions and sequence data provided by Mostert *et al.* (2006), Essakhi *et al.* (2008) and Raimondo *et al.* (2014).

For preliminary molecular identifications, internal transcribed spacers (ITS) 1 and 2 (including 5.8S of nuclear ribosomal DNA; ca. 600 bp) and the partial β -tubulin gene (β -tub; ca. 500 bp) were amplified for all BFD fungi (i.e., all 182 isolates). The primer pairs used were ITS1/ITS4 (White *et al.*, 1990) for the ITS region, and T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995) for β -tub. Subsequently, histone 3 (His3; ca. 500 bp) was amplified with the CYLH3F/ CYLH3R primer pair (Crous *et al.*, 2004b) for 148 isolates. Three nuclear loci were amplified for the remaining 34 isolates, as the large subunit RNA (LSU; ca. 700 bp), the α -actin gene (act; ca. 600 bp), and RNA polymerase II subunit 1 (rpb1; ca. 700 bp), with the primer pairs NL1/ NL4 (O'Donnell and Gray, 1993), FWDACT/ MIDREVACT (Weiland and Sundsbak, 2000), and CRPB1A/ RPB1-Cr (Castlebury *et al.*, 2004), respectively.

The LSU and ITS PCR reactions and conditions were performed according to Carlucci *et al.* (2012), with those for β -tub and act according to Raimondo *et al.* (2014), except for the annealing temperature of 56°C, for rpb1 according to Castlebury *et al.* (2004), and for His3 according to Crous *et al.* (2004b).

Ten microlitres of each amplicon were analysed by electrophoresis at 100 V for 30 min in 1.5% (w/v) agarose gels in 1× TAE buffer (40 mM Tris, 40 mM acetate, 2 mM EDTA, pH 8.0). The gels were stained with ethidium bromide and visualised under ultraviolet light (Gel Doc EZ System; Biorad). The PCR products were purified before DNA sequencing (Nucleo Spin Extract II purification kits; Macherey-Nagel), according to the manufacturer instructions. Both strands of the PCR products were sequenced by Eurofins Genomics Service (Milan, Italy).

Phylogenetic analysis

The nucleotide sequences obtained were manually edited using BioEdit version 7.0.9 (<http://www.mbio.ncsu.edu/BioEdit>). Consensus sequences were

compared with those available in the GenBank database, using the Basic Local Alignment Search Tool (BLAST) to verify the preliminary morphological identification, and to select and download closely related sequences for phylogenetic analyses. GenBank sequences from different species of *Cylindrodendrum*, *Dactylonectria*, *Ilyonectria*, *Neonectria* and *Thelonectria* (Table 2) were then selected and added to the sequences obtained and aligned using ClustalX, version 1.83 (Thompson *et al.*, 1997).

A selection of 47 BFD strains from the collection of 182 strains was used to perform the phylogenetic analyses. Alignment gaps were treated as missing data, and all of the characters were unordered and of equal weight. Phylogenetic analyses of the ITS and β -tub sequences was carried out using PAUP, version 4.0b10 (Swofford, 2003), using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction as the branch swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Bootstrap support values were calculated from 100 heuristic search replicates and 10 random taxon additions. The tree lengths (TL), consistency indices (CI), retention indices (RI), homoplasy indices (HI), and rescaled consistency indices (RC) were calculated, and the resulting trees were visualised with TreeView, version 1.6.6 (Page, 1996). *Campylocarpon fasciculare* (CBS 112613) and *C. pseudo-fasciculare* (CBS 112679) were used as outgroups.

Phylogenetic analyses of the *Ilyonectria* and *Dactylonectria* isolates were conducted according to single-locus alignment of the His3 gene, which has previously been shown to be a very informative locus (Cabral *et al.*, 2012b; Agustí-Brisach *et al.*, 2016). The alignment gaps were treated as fifth character, and all the characters were unordered and of equal weight. Maximum parsimony analyses were performed with PAUP, version 4.0b10 (Swofford, 2003), as described above.

Bayesian analyses were carried out with MrBayes version 3.0b (Ronquist and Huelsenbeck, 2003), using a Markov Chain Monte Carlo method. The general time-reversible model of evolution was used (Rodriguez *et al.*, 1990), which included estimation of invariable sites and assuming a discrete gamma distribution with six rate categories. Four Markov Chain Monte Carlo chains were run simultaneously, starting from random trees, for 10⁶ generations. The trees were sampled every 100th generation for a total of 10⁴ trees. The first 10³ trees were discarded as

Table 2. Sources of the fungal species and GenBank accession numbers used in the phylogenetic analyses.

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers						
					LSU	ITS	β -tub	act	rpb 1	His3	
<i>Cylindrodendrum album</i>	CBS 110655	Pine forest soil	The Netherlands	F.X. Prenafeta-Boldú	-	KM231765	KM232022	-	-	-	KM231485
<i>C. album</i>	CBS 301.83	<i>Fucus distichus</i>	Canada	R.C. Summerbell	-	KM231764	KM532021	-	-	-	KM231484
<i>C. alicantinum</i>	Cyl 11	<i>Eriobotrya japonica</i>	Spain	J. Armengol	-	KP456017	KP400581	-	-	-	KP639558
<i>C. alicantinum</i>	Cyl 3 = CBS 139518	<i>Eriobotrya japonica</i>	Spain	J. Armengol	-	KP456014	KP400578	-	-	-	KP639555
<i>C. hubiense</i>	CBS 129.97	<i>Viscum album</i>	France	W. Gams	-	KM231766	KM232023	-	-	-	KM231486
<i>C. hubiense</i>	CBS 124071	<i>Rhododendron</i>	China	W.P.Wu & W.Y. Zhuang	-	FJ560439	FJ860056	-	-	-	KP639561
<i>Campylocarpon fasciculare</i>	CBS 112613	<i>Vitis vinifera</i>	South Africa	F. Halleen	-	AY677301	AY677221	-	-	-	JF735502
<i>C. pseudofasciculare</i>	CBS 112679	<i>Vitis vinifera</i>	South Africa	F. Halleen	-	AY677306	AY677214	-	-	-	JF735503
<i>Dactylonectria alacerensis</i>	CBS 129087	<i>Vitis vinifera</i>	Portugal	A. Cabral & H. Oliveira	-	JF735333	AM419111	-	-	-	JF735630
<i>D. alacerensis</i>	Cyl134	<i>Vitis vinifera</i>	Spain	J. Armengol	-	JF735332	AM419104	-	-	-	JF735629
<i>D. anthuricola</i>	CBS 564.95	<i>Anthurium</i> sp.	The Netherlands	R. Pieters	-	JF735302	JF735430	-	-	-	JF735579
<i>D. estremocensis</i>	CBS 129085	<i>Vitis vinifera</i>	Portugal	C. Rego & T. Nascimento	-	JF735320	JF735448	-	-	-	JF735617
<i>D. estremocensis</i>	Cyl135	<i>Vitis vinifera</i>	Portugal	C. Rego & T. Nascimento	-	AM419069	AM419105	-	-	-	JF735615
<i>D. hordeicola</i>	CBS 162.89	<i>Hordeum vulgare</i>	The Netherlands	M. Barth	-	AM419060	AM419084	-	-	-	JF735610
<i>D. macrodidyma</i>	Cyl258	<i>Vitis vinifera</i>	Portugal	C. Rego	-	JF735348	JF735477	-	-	-	JF735656
<i>D. macrodidyma</i>	CBS 112615	<i>Vitis vinifera</i>	South Africa	F. Halleen	-	AY677290	AY677233	-	-	-	JF735647
<i>D. novozelandica</i>	CBS 113552	<i>Vitis</i> sp.	New Zealand	R. Bonfiglioli	-	JF735334	AY677237	-	-	-	JF735633
<i>D. novozelandica</i>	CBS 112608	<i>Vitis vinifera</i>	South Africa	F. Halleen	-	AY677288	AY677235	-	-	-	JF735632
<i>D. pauciseptata</i>	CBS 100819	<i>Erica melanthera</i>	New Zealand	H.M. Dance	-	EF607090	EF607067	-	-	-	JF735582
<i>D. pauciseptata</i>	CBS 120171	<i>Vitis</i> sp.	Slovenia	M. Žerjav	-	EF607089	EF607066	-	-	-	JF735587
<i>D. pinicola</i>	CBS 173.37	<i>Pinus laricio</i>	United Kingdom	T.R. Peace	-	JF735319	JF735447	-	-	-	JF735614
<i>D. pinicola</i>	Cyl200	<i>Vitis vinifera</i>	Portugal	N. Cruz	-	JF735317	JF735445	-	-	-	JF735612

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers					
					LSU	ITS	β -tub	act	rpb 1	His3
<i>D. torresensis</i>	CBS 129086	<i>Vitis vinifera</i>	Portugal	A. Cabral	-	JF735362	JF735492	-	-	JF735681
<i>D. torresensis</i>	CBS 119.41	<i>Fragaria</i> sp.	The Netherlands	H.C. Koning	-	JF735349	JF735478	-	-	-
<i>D. torresensis</i>	BF1	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	KX778715	KX778697	-	-	KX778706
<i>D. torresensis</i>	BF3	<i>Vitis vinifera</i> cv. Pinot grigio	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF9	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF22	<i>Vitis vinifera</i> cv. Ciliegolo	Canosa di Puglia (BT), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF33	<i>Vitis vinifera</i> cv. Coccochiola d'Abruzzo	Torremaggiore (FG), Italy	M.L. Raimondo	-	-	-	-	-	-
<i>D. torresensis</i>	BF44	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	KX778716	KX778698	-	-	KX778707
<i>D. torresensis</i>	BF56	<i>Vitis vinifera</i> cv. Moscato	Canosa di Puglia (BT), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF71	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF76	<i>Vitis vinifera</i> cv. Coccochiola d'Abruzzo	Torremaggiore (FG), Italy	M.L. Raimondo	-	-	-	-	-	-
<i>D. torresensis</i>	BF98	<i>Vitis vinifera</i> cv. Ciliegolo	Canosa di Puglia (BT), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF130	<i>Vitis vinifera</i> cv. Moscato	Canosa di Puglia (BT), Italy	A. Carlucci	-	KX778714	KX778696	-	-	KX778705
<i>D. torresensis</i>	BF131	<i>Vitis vinifera</i> cv. Trebbiano toscano	Torremaggiore (FG), Italy	M.L. Raimondo	-	-	-	-	-	-
<i>D. torresensis</i>	BF132	<i>Vitis vinifera</i> cv. Chardonnay	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF134	<i>Vitis vinifera</i> cv. Pinot grigio	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF135	<i>Vitis vinifera</i> cv. Trebbiano toscano	Canosa di Puglia (BT), Italy	A. Carlucci	-	-	-	-	-	-

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers					
					LSU	ITS	β -tub	act	rpb 1	His3
<i>D. torresensis</i>	BF136	<i>Vitis vinifera</i> cv. Pinot grigio	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF138	<i>Vitis vinifera</i> cv. Pinot grigio	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-
<i>D. torresensis</i>	BF143	<i>Vitis vinifera</i> cv. Trebbiano toscano	Torremaggiore (FG), Italy	M.L. Raimondo	-	KX778715	KX778697	-	-	KX778706
<i>D. vitis</i>	CBS 129082	<i>Vitis vinifera</i>	Portugal	C. Rego	-	JF735303	JF735431	-	-	JF735580
<i>Ilyonectria capensis</i>	CBS 132816	<i>Protea</i> sp.	South Africa	C.M. Bezuidehouth	-	JX231160	JX231112	-	-	JX231144
<i>I. capensis</i>	CBS 132815	<i>Protea</i> sp.	South Africa	C.M. Bezuidehouth	-	JX231151	JX231103	-	-	JX231135
<i>I. coprosmae</i>	CBS 119606	<i>Metrosideros</i> sp.	Canada	G.J. Samuels	-	JF735260	JF735373	-	-	JF735505
<i>I. crassa</i>	CBS 158.31	<i>Narcissus</i> sp.	The Netherlands	W.F. van Hell	-	JF735276	JF735394	-	-	JF735535
<i>I. crassa</i>	CBS 139.30	<i>Lilium</i> sp.	The Netherlands	W.F. van Hell	-	JF735275	JF735393	-	-	JF735534
<i>I. cyclaminicola</i>	CBS 302.93	<i>Cyclamen</i> sp.	The Netherlands	M. Hooftman	-	JF735304	JF735432	-	-	JF735581
<i>I. europaea</i>	CBS 102892	Stem	Germany	W.Leibinger	-	JF735295	JF735422	-	-	JF735569
<i>I. europaea</i>	CBS 129078	<i>Vitis vinifera</i>	Portugal	C. Rego	-	JF735294	JF735421	-	-	JF735567
<i>I. gamsii</i>	CBS 940.97	Soil	The Netherlands	J.T. Poll	-	AM419065	AM419089	-	-	JF735577
<i>I. leucospermi</i>	CBS 132810	<i>Protea</i> sp.	South Africa	C.M. Bezuidehouth	-	JX231162	JX231114	-	-	JX231146
<i>I. leucospermi</i>	CBS 132809	<i>Leucospermum</i> sp.	South Africa	C.M. Bezuidehouth	-	JX231161	JX231113	-	-	JX231145
<i>I. liliigena</i>	CBS 732.74	<i>Lilium</i> sp.	The Netherlands	G.J. Bollen	-	JF735298	JF735426	-	-	JF735574
<i>I. liliigena</i>	CBS 189.49	<i>Lilium regale</i>	The Netherlands	M.A.A. Schippers	-	JF735297	JF735425	-	-	JF735573
<i>I. lirioidendri</i>	CBS 110.81	<i>Liriiodendron tulipifera</i>	USA	J.D. MacDonald & E.E.	-	DQ178163	DQ178170	-	-	JF735507
<i>I. lirioidendri</i>	BF6	<i>Vitis vinifera</i> cv. Ciliegolo	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-
<i>I. lirioidendri</i>	BF12	<i>Vitis vinifera</i> cv. Ciliegolo	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-
<i>I. lirioidendri</i>	BF30	<i>Vitis vinifera</i> cv. Chardonnay	Campomarino (CB), Italy	M.L. Raimondo	-	-	-	-	-	-
<i>I. lirioidendri</i>	BF31	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers						
					LSU	ITS	β -tub	act	rpb 1	His3	
<i>I. liriodendri</i>	BF41	<i>Vitis vinifera</i> cv. Cococciola d'Abruzzo	Torremaggiore (FG), Italy	M.L. Raimondo	-	KX778718	KX778700	-	-	-	KX778709
<i>I. liriodendri</i>	BF47	<i>Vitis vinifera</i> cv. Trebbiano toscano	Campomarino (CB), Italy	M.L. Raimondo	-	-	-	-	-	-	-
<i>I. liriodendri</i>	BF50	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>I. liriodendri</i>	BF59	<i>Vitis vinifera</i> cv. Moscato	Cerignola (FG), Italy	A. Carlucci	-	KX778717	KX778699	-	-	-	KX778708
<i>I. liriodendri</i>	BF61	<i>Vitis vinifera</i> cv. Trebbiano toscano	Campomarino (CB), Italy	M.L. Raimondo	-	-	-	-	-	-	-
<i>I. liriodendri</i>	BF66	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>I. liriodendri</i>	BF68	<i>Vitis vinifera</i> cv. Cococciola d'Abruzzo	Torremaggiore (FG), Italy	M.L. Raimondo	-	-	-	-	-	-	-
<i>I. liriodendri</i>	BF74	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	KX778719	KX778701	-	-	-	KX778710
<i>I. liriodendri</i>	BF144	<i>Vitis vinifera</i> cv. Pinot grigio	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>I. Iusitanica</i>	CBS 129080	<i>Vitis vinifera</i>	Portugal	N. Cruz	-	JF735296	JF735423	-	-	-	JF735570
<i>I. mors-panacis</i>	CBS 124662	<i>Panax ginseng</i>	Japan	Y. Miyazawa	-	JF735290	JF735416	-	-	-	JF735559
<i>I. mors-panacis</i>	CBS 306.35	<i>Panax quinquefolium</i>	Canada	A.A. Hildebrand	-	JF735288	JF735414	-	-	-	JF735557
<i>I. palmarum</i>	CBS 135754	<i>Howea forsteriana</i>	Italy	G. Polizzi	-	HF937431	HF922608	-	-	-	HF922620
<i>I. palmarum</i>	CBS 135753	<i>Howea forsteriana</i>	Italy	G. Polizzi	-	HF937432	HF922609	-	-	-	HF922621
<i>I. panacis</i>	CBS 129079	<i>Panax quinquefolium</i>	Canada	K.F. Chang	-	AY295316	JF735424	-	-	-	JF735572
<i>I. protearum</i>	CBS 132811	<i>Protea</i> sp.	South Africa	C.M. Bezuidehouth	-	JX231157	JX231109	-	-	-	JX231141
<i>I. protearum</i>	CBS 132812	<i>Protea</i> sp.	South Africa	C.M. Bezuidehouth	-	JX231165	JX231117	-	-	-	JX231149
<i>I. pseudodestructans</i>	CBS 117824	<i>Quercus</i> sp.	Austria	E. Halmshlager	-	JF735292	JF735419	-	-	-	JF735562
<i>I. pseudodestructans</i>	CBS 129081	<i>Vitis vinifera</i>	Portugal	C. Rego	-	AJ875330	AM419091	-	-	-	JF735563

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers					
					LSU	ITS	β -tub	act	rpb 1	His3
<i>I. radicola</i>	CBS 264.65	<i>Cyclamen persicum</i>	Sweden	L. Nilsson	-	AY677273	AY677256	-	-	JF735506
<i>I. robusta</i>	CBS 117815	<i>Quercus</i> sp.	Austria	E. Halmschlager	-	JF735266	JF735380	-	-	JF735522
<i>I. robusta</i>	CBS 308.35	<i>Panax quinquefolium</i>	Canada	A.A. Hildebrand	-	JF735264	JF735377	-	-	JF735518
<i>I. rufa</i>	CBS 156.47	<i>Azalea indica</i>	-	Belgium	-	AY677272	AY677252	-	-	JF735541
<i>I. rufa</i>	CBS 153.37	Dune sand	France	F. Moreau	-	AY677271	AY677251	-	-	JF735540
<i>I. venezuelensis</i>	CBS 102032	Bark	Venezuela	A. Rossman	-	AM419059	AY677255	-	-	JF735571
<i>I. vredehoekensis</i>	CBS 132807	<i>Protea</i> sp.	South Africa	C.M. Bezuïdenhout	-	JX231155	JX231107	-	-	JX231139
<i>I. vredehoekensis</i>	CBS 132808	<i>Protea</i> sp.	South Africa	C.M. Bezuïdenhout	-	JX231159	JX231111	-	-	JX231143
<i>Neonectria coccinea</i>	CBS 119158	<i>Fagus sylvatica</i>	Germany	G.J. Samuels	-	JF268759	KC660727	-	-	-
<i>N. confusa</i>	CBS 127484	Twig	China	W.Y. Zhuang	-	KM515889	KM515886	-	-	-
<i>N. confusa</i>	CBS 127485	Twig	China	W.Y. Zhuang	-	FJ560437	FJ860054	-	-	-
<i>N. ditissima</i>	CBS 100316	<i>Malus domestica</i>	Ireland	A. McCracken	-	HM364298	DQ789858	-	-	-
<i>N. ditissima</i>	CBS 835.97	<i>Salix cinerea</i>	Belgium	W. Gams	-	JF735310	DQ789880	-	-	-
<i>N. faginata</i>	CBS 217.67	<i>Cryptococcus fagi</i> nymph on <i>Fagus grandifolia</i>	Canada	G.L. Stone	-	HQ840385	JF268730	-	-	-
<i>N. faginata</i>	CBS 119160	Unknown	USA	Unknown	-	HQ840384	DQ789883	-	-	-
<i>N. fuckeliana</i>	CBS 119200	<i>Picea abies</i>	Austria	W. Jaklitsch	-	HQ840387	JF268731	-	-	-
<i>N. fuckeliana</i>	CBS 239.29	<i>Picea sitchensis</i>	Scotland	Unknown	-	HQ840386	DQ789871	-	-	-
<i>N. lugdunensis</i>	CBS 125485	<i>Populus fremontii</i>	USA	T. Gräfenhan	-	KM231762	KM232019	-	-	-
<i>N. lugdunensis</i>	CBS 127475	Twig	China	X.M. Zhang	-	KM515896	KM515888	-	-	-
<i>N. major</i>	CBS 240.29	<i>Alnus incana</i>	Norway	H.W. Wollenweber	-	JF735308	DQ789872	-	-	-
<i>N. major</i>	HMAS 183183	-	China	Rossmann	-	JF268766	JF268732	-	-	-
<i>N. neomacrospora</i>	CBS 198.62	<i>Abies concolor</i>	-	W. Gerlach	-	AJ009255	HM352865	-	-	-
<i>N. neomacrospora</i>	CBS 118984	<i>Abies balsamea</i>	Canada	-	-	JF735311	DQ789882	-	-	-
<i>N. obtusispora</i>	CBS 183.36	<i>Solanum tuberosum</i>	Germany	H.W. Wollenweber	-	AM419061	AM419085	-	-	-

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers					
					LSU	ITS	β -tub	act	rpb 1	His3
<i>N. obtusispora</i>	CPC 13544	<i>Prunus armenica</i>	Canada	J.A. Traquair	-	AY295306	JF735443	-	-	-
<i>N. punicea</i>	CBS 242.29	<i>Rhamnus</i> sp.	Germany	H.W. Wollenweber	-	KC660522	DQ789873	-	-	-
<i>N. punicea</i>	CBS 119724	<i>Frangula alnus</i>	Austria	W. Jaklitsch	-	KC660496	DQ789824	-	-	-
<i>N. ramulariae</i>	CBS 151.29	<i>Malus sylvestris</i>	England	H.W. Wollenweber	-	AY677291	JF735438	-	-	-
<i>N. ramulariae</i>	CBS 182.36	<i>Malus sylvestris</i>	-	H.W. Wollenweber	-	HM054157	JF735439	-	-	-
<i>N. shennongjiana</i>	HMAS 183185	-	China	J. Luo & W.Y. Zhuang	-	FJ560440	FJ860057	-	-	-
<i>N. tsugae</i>	CBS 788.69	<i>Tsuga heterophylla</i>	Canada	J.E. Bier	-	KM231763	KM232020	-	-	-
<i>Rugonectria rugulosa</i>	TPPH-32	<i>Myrica rubra</i>	Japan	-	-	AB233176	AB237526	-	-	-
<i>R. sinica</i>	HMAS 76865	Bark	China	W.Y. Zhuang,	-	HM054142	HM054120	-	-	-
<i>R. sinica</i>	HMAS 183542	Dead twigs	China	W.Y. Zhuang	-	HM054141	HM054119	-	-	-
<i>Thelonectria acrotyla</i>	G.J.S. 90-171 = CBS 123766	Unknown	Venezuela	-	JQ403368	JQ403329	JQ394720	JQ365047	JQ403407	
<i>T. ananimensis</i>	MAFF 239819	<i>Pinus luchuensis</i>	Japan	-	JQ403375	JQ403337	JQ394727	JQ365054	KJ022408	
<i>T. ananimensis</i>	MAFF 239820	<i>Pinus luchuensis</i>	Japan	-	JQ403376	JQ403338	JQ394728	JQ365055	JQ403413	
<i>T. asiatica</i>	MAFF 241576	Bark	Japan	Y. Hirooka	-	KC153774	KC153839	-	-	-
<i>T. asiatica</i>	G.J.S. 88-84 = IM1348190	Bark	China	R.P. Korf	-	KC153741	KC153806	-	-	-
<i>T. beijungensis</i>	HMAS 188498	Bark	China	Z.Q. Zeng, J. Luo & W.Y. Zhuang	-	JQ836656	JQ836658	-	-	-
<i>T. blackerella</i>	BF5	<i>Vitis vinifera</i> cv. Cocociola d'Abruzzo	Torremaggiore (FG), Italy	M.L. Raimondo	KX778692	KX778713	KX778704	KX778689	KX778695	-
<i>T. blackerella</i>	BF10	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-
<i>T. blackerella</i>	BF21	<i>Vitis vinifera</i> cv. Trebbiano toscano	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-
<i>T. blackerella</i>	BF29	<i>Vitis vinifera</i> cv. Cocociola d'Abruzzo	Torremaggiore (FG), Italy	M.L. Raimondo	-	-	-	-	-	-

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers						
					LSU	ITS	β -tub	act	rpb 1	His3	
<i>T. blackerriella</i>	BF48	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF65	<i>Vitis vinifera</i> cv Chardonnay	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF79	<i>Vitis vinifera</i> cv. Trebbiano toscano	Torremaggiore (FG), Italy	M.L. Raimondo	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF82	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF88	<i>Vitis vinifera</i> cv. Pinot grigio	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF99	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF106	<i>Vitis vinifera</i> cv. Trebbiano toscano	Torremaggiore (FG), Italy	M.L. Raimondo	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF109	<i>Vitis vinifera</i> cv Chardonnay	Campomarino (CB), Italy	M.L. Raimondo	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF120	<i>Vitis vinifera</i> cv Chardonnay	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF125	<i>Vitis vinifera</i> cv. Pinot grigio	Campomarino (CB), Italy	A. Carlucci	-	-	-	-	-	-	-
<i>T. blackerriella</i>	BF133 = CBS142201	<i>Vitis vinifera</i> cv. Sangiovese	Cerignola (FG), Italy	A. Carlucci	KX778691	KX778712	KX778703	KX778688	KX778694	-	-
<i>T. blackerriella</i>	BF142 = CBS142200	<i>Vitis vinifera</i> cv. Pinot grigio	Campomarino (CB), Italy	A. Carlucci	KX778690	KX778711	KX778702	KX778687	KX778693	-	-
<i>T. blattea</i>	CBS 95268	Wheat fiel soil	Germany	W. Gams	-	KC153725	KC153790	-	-	-	-
<i>T. blattea</i>	CBS 14277	Soil	The Netherlands	J.W. Veenbaas-Rijks	-	KC153720	KC153785	-	-	-	-
<i>T. brayfordii</i>	CBS 118612	<i>Quercus robur</i>	New Zealand	C.F. Hill	-	KC153719	KC153784	-	-	-	-
<i>T. brayfordii</i>	ICMP 14105	Root	New Zealand	C.F. Hill	-	KC153758	KC153823	-	-	-	-
<i>T. cidaria</i>	G.J.S. 10.135 = CBS 132323	Twigs of dead shrub	Costa Rica	C. Salgado	JQ403316	JQ403324	JQ394714	KJ022239	JQ403401	-	-
<i>T. cidaria</i>	C.T.R. 71.79 = IM1325844	Twigs of dead shrub	Jamaica	C. Salgado	KJ022027	JQ403315	JQ394707	JQ365035	JQ403392	-	-
<i>T. conchylia</i>	G.J.S. 87-45 = IM1325855	Wood	Guyana	G.J. Samuels	-	KC153739	KC153804	-	-	-	-

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers					
					LSU	ITS	β -tub	act	rpb 1	His3
<i>T. conchyliaata</i>	G.J.S. 87-49 = CBS 112461	Branchlets of dead tree	Guyana	G.J. Samuels	-	KC153740	KC153805	-	-	-
<i>T. coronalis</i>	93082102 = CBS 132337	Bark	Taiwan	J.-R. Guu	JQ403380	JQ403343	JQ394732	KJ022240	JQ403418	-
<i>T. coronalis</i>	94043006 = CBS 132338	Bark	Taiwan	J.-R. Guu	JQ403381	JQ403344	JQ394733	KJ022241	JQ403419	-
<i>T. coronata</i>	G.J.S. 10-108 = CBS 132322	Bark of decaying shrub	Costa Rica	C. Salgado	JQ403360	JQ403320	JQ394711	JQ365040	JQ403397	-
<i>T. coronata</i>	G.J.S. 85-207 = IMI325241	Herbaceous stem	Indonesia	G.J. Samuels	JQ403365	JQ403326	JQ394717	JQ365044	JQ403404	-
<i>T. diademata</i>	A.R. 4765 = CBS 132331	Bark of a fallen tree	Argentina	C. Salgado	JQ403348	JQ403308	JQ394700	JQ365029	JQ403384	-
<i>T. diademata</i>	A.R. 4787 = CBS 132332	Bark of a fallen tree	Argentina	C. Salgado	JQ403351	JQ403311	JQ394703	JQ365032	JQ403387	-
<i>T. diademata</i>	C.T.R. 71.52 = CBS 132333	<i>Pinus patula</i>	Jamaica	C.T. Rogerson	JQ403354	JQ403314	JQ394706	KJ022242	JQ403391	-
<i>T. diademata</i>	G.J.S. 10-137 = CBS 132321	Bark of decaying shrub	Costa Rica	C. Salgado	JQ403364	JQ403325	JQ394716	KJ022243	JQ403403	-
<i>T. discophora</i>	A.R. 4742 = CBS 134034	<i>Tepuallia stipularis</i>	Chile	A. de Errasti	-	KC153714	KC153779	-	-	-
<i>T. discophora</i>	G.J.S. 92-48 = CBS 134031	<i>Aesculus</i> sp. dead branchlets	Scotland	G.J. Samuels	-	KC153753	KC153818	-	-	-
<i>T. gibba</i>	G.J.S. 96-35 = CBS 112456	Bark	Puerto Rico	G.J. Samuels, H.J. Schroers	-	KC153757	KC153822	-	-	-
<i>T. gibba</i>	G.J.S. 96-10 = CBS 112469	Bark of <i>Casearia arborea</i>	Puerto Rico	G. J. Samuels, H. J. Schroers	-	KC153754	KC153819	-	-	-
<i>T. gongylodes</i>	G.J.S. 89-131 = IMI336160	<i>Acer rubrum</i>	USA	G.J. Samuels	JQ403374	JQ403336	JQ394726	JQ365053	JQ403412	-
<i>T. gongylodes</i>	G.J.S. 90-50 = IMI343571	Bark of dead <i>Fragis</i> sp.	USA	G.J. Samuels	JQ403370	JQ403331	JQ394721	JQ365048	JQ403408	-
<i>T. gongylodes</i>	G.J.S. 04.171 = CBS 124611	Bark of <i>Acer</i> sp.	USA	G.J. Samuels	JQ403358	JQ403318	JQ394710	JQ365038	JQ403395	-
<i>T. ianthina</i>	G.J.S. 10-118 = CBS 134023	Bark	Costa Rica	C. Salgado	-	KC153731	KC153796	-	-	-

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers						
					LSU	ITS	β -tub	act	rpb 1	His3	
<i>T. inthina</i>	92122107 = CBS 134038	Bark	Taiwan	J.-R. Guu	-	KC153711	KC153775	-	-	-	-
<i>T. japonica</i>	MAFF 241524	Twigs	Japan	Y. Hirooka	-	KC153766	KC153831	-	-	-	-
<i>T. japonica</i>	MAFF 241543	Twigs	Japan	Y. Hirooka	-	KC153769	KC153834	-	-	-	-
<i>T. japonica</i>	MAFF 241554	Bark	Japan	Y. Hirooka	-	KC153770	KC153835	-	-	-	-
<i>T. mamma</i>	94043002 = CBS 136787	Bark	Taiwan	J.R. Guu	-	KF569839	KF569866	-	-	-	-
<i>T. mamma</i>	G.J.S. 86-249 = IMI 325261	Stem of <i>Philodendron</i> sp.	French Guiana	G.J. Samuels	-	KF569840	KF569867	-	-	-	-
<i>T. mamma</i>	92112704	Bark	Taiwan	J.R. Guu	-	KF569838	KF569865	-	-	-	-
<i>T. mammoidea</i>	IMI169361	<i>Smyxium olusatrum</i>	England	E.A. Ellis	-	KC153763	KC153828	-	-	-	-
<i>T. mammoidea</i>	CBS 32881	Bark	Switzerland	O. Petrini	-	KF569836	KF569863	-	-	-	-
<i>T. nodosa</i>	G.J.S. 90-66 = CBS 124352	Bark of <i>Acer</i> sp.	USA	G.J. Samuels	JQ403371	JQ403332	JQ394722	JQ365049	JQ403409	-	-
<i>T. nodosa</i>	G.J.S. 04-155 = CBS 132327	Bark of <i>Thuja canadensis</i>	USA	G.J. Samuels	JQ403357	JQ403317	JQ394709	JQ365037	JQ403394	-	-
<i>T. nodosa</i>	G.J.S. 91-105 = IMI351445	<i>Rhododendron</i> sp.	USA	G.J. Samuels	JQ403372	JQ403333	JQ394723	JQ365050	JQ403410	-	-
<i>T. ostrina</i>	G.J.S. 96-23 = IMI370947	Bark	Puerto Rico	G.J. Samuels	-	KC153756	KC153821	-	-	-	-
<i>T. ostrina</i>	MAFF 241564	Bark	Japan	Y. Hirooka	-	KC153772	KC153837	-	-	-	-
<i>T. ostrina</i>	G.J.S. 09-1327 = CBS 134022	Wood	Venezuela	C. Salgado	-	KC153729	KC153794	-	-	-	-
<i>T. papillata</i>	G.J.S. 90-146 = CBS 136788	Bark	Costa Rica	C. Salgado	-	KC153746	KC153811	-	-	-	-
<i>T. papillata</i>	G.J.S. 90-166 = CBS 126099	Bark	Venezuela	Samuels, G.J	-	KC153748	KC153813	-	-	-	-
<i>T. papillata</i>	A.R. 4781 = CBS 134036	Bark of a rotting fallen tree	Argentina	C. Salgado, A.Y. Rossmann, A. Romero	-	KC153716	KC153781	-	-	-	-
<i>T. phoenicea</i>	G.J.S. 85-179 = IMI329113	Twig	Indonesia	G.J. Samuels	-	KC153736	KC153801	-	-	-	-

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers						
					LSU	ITS	β-tub	act	rpb 1	His3	
<i>T. phoenicea</i>	G.J.S. 85-187 = AICC 76478	<i>Acacia ceisa</i>	Australia	A.Y. Rossman	-	KC153737	KC153802	-	-	-	-
<i>T. pinea</i>	A.R. 4324 = CBS 125153	<i>Pinus radiata</i>	New Zealand	G.B. Rawlings	-	HM364294	HM352860	-	-	-	-
<i>T. pinea</i>	A.R. 4321 = CBS 134033	<i>Pinus radiata</i>	New Zealand	M. Dick	-	KC153713	KC153777	-	-	-	-
<i>T. porphyria</i>	MAFF 241515	Bark	Japan	Y. Hirooka	-	KC153764	KC153829	-	-	-	-
<i>T. porphyria</i>	MAFF 241539	Twigs	Japan	Y. Hirooka	-	KC153768	KC153833	-	-	-	-
<i>T. porphyria</i>	MAFF 241517	<i>Cryptomeria japonica</i>	Japan	Y. Hirooka	-	KC153765	KC153830	-	-	-	-
<i>T. purpurea</i>	C.T.R. 71-281 = CBS 112458	Wood	Aragua	K.P. Dumont	-	KC153726	KC153791	-	-	-	-
<i>T. purpurea</i>	G.J.S. 10-131 = CBS 134024	Bark	Costa Rica	C. Salgado	-	KC153732	KC153797	-	-	-	-
<i>T. rubi</i>	CBS 177-27	Roots of <i>Rubus idaeus</i>	England	R.M. Nattrass	-	KC153721	KC153786	-	-	-	-
<i>T. rubi</i>	CBS 113-12	Roots of <i>Rubus idaeus</i>	Switzerland	A. Osterwalder	-	KC153718	KC153783	-	-	-	-
<i>T. sinensis</i>	HMAS 183186	Bark of a coniferous tree	China	Luo and Zhuang	-	FJ560441	FJ860058	-	-	-	-
<i>T. stemmata</i>	C.T.R. 71.19 = CBS 112468	Wood	Jamaica	G.J. Samuels	JQ403352	JQ403312	JQ394704	JQ365033	JQ403388	-	-
<i>T. stemmata</i>	C.T.R. 71.21 = CBS 132336	<i>Cecropia</i> sp.	Jamaica	A.Y. Rossman	JQ403353	JQ403313	JQ394705	JQ365034	JQ403389	-	-
<i>T. torulosa</i>	A.R. 4764 = CBS 132339	Bark of a fallen tree	Argentina	C. Salgado, A.Y. Rossman	JQ403349	JQ403309	JQ394701	JQ365030	JQ403385	-	-
<i>T. torulosa</i>	A.R. 4768A = CBS 132340	Bark	Argentina	C. Salgado, A.Y. Rossman	JQ403350	JQ403310	JQ394702	JQ365031	JQ403386	-	-
<i>T. trachosa</i>	G.J.S. 85-50 = CBS 119608	Bark of <i>Phyllocladus</i> sp.	New Zealand	P.R. Johnston, L.M. Kohn	-	KF569841	KF569868	-	-	-	-
<i>T. trachosa</i>	G.J.S. 92-45 = CBS 112467	Bark of conifer	Scotland	D. Brayford, G.J. Samuels	-	KF569842	KF569869	-	-	-	-
<i>T. truncata</i>	G.J.S. 04-357 = CBS 132329	Bark of decaying tree	USA	G.J. Samuels	JQ403359	JQ403319	KJ022324	JQ365039	JQ403396	-	-

(Continued)

Table 2. (Continued).

Species	Isolate number ^a	Host	Location	Collector	GenBank accession numbers						
					LSU	ITS	β -tub	act	rpb 1	His3	
<i>T. truncata</i>	MAFF 241521	Twigs	Japan	Y. Hirooka	JQ403377	JQ403339	KJ022325	JQ365056	JQ403414	-	
<i>T. tyrus</i>	G.J.S. 90-46 = CBS 134029	<i>Quercus</i> sp.	USA	G.J. Samuels	-	KC153751	KC153816	-	-	-	
<i>T. tyrus</i>	A.R. 4499 = CBS 125172	<i>Fagus grandifolia</i>	USA	R. Marra	-	HM364296	KC153778	-	-	-	
<i>T. veuillotiana</i>	G.J.S. 92-24 = CBS 125114	Bark of <i>Fagus sylvatica</i>	France	G.J. Samuels	GQ506005	JQ403335	JQ394725	GQ505980	GQ506034	-	
<i>T. veuillotiana</i>	A.R. 1751 = CBS 132341	Bark of <i>Eucalyptus</i> sp.	Azores Island	A.Y. Rossman	JQ403345	JQ403305	JQ394698	KJ022273	JQ403382	-	
<i>T. violaria</i>	A.R. 4766 = CBS 134035	Bark	Argentina	C. Salgado	-	KC153715	KC153780	-	-	-	
<i>T. violaria</i>	C.T.R. 72-188 = CBS 134040	Bark	USA	C. Salgado	-	KC153727	KC153792	-	-	-	
<i>T. westlandica</i>	IMI255610	Bark of <i>Metrosideros robusta</i>	New Zealand	P.R. Johnston, G.J. Samuels	KF569852	KF569843	KF569870	KF569833	KF569880	-	
<i>T. westlandica</i>	G.J.S. 83-156 = CBS 112464	Bark of <i>Dacryocarpus cupressinum</i>	New Zealand	T. Matsushima, A. Rossman, G.J. Samuels	-	HM484559	HM352868	-	-	-	
<i>T. westlandica</i>	ICMP10387	<i>Rosa</i> sp.	New Zealand	G. Laundon	KF569853	KF569844	KF569871	KF569834	KF569881	-	
<i>T. yunnanica</i>	HMAS 183564	Bark	China	Z.Q. Zeng	-	FJ560438	JQ836660	-	-	-	

^a Ex-type strains are shown in bold. CBS, collection of Centraalbureau voor Schimmelcultures, The Netherlands; HMAS, Herbarium Mycologicum Academiae Sinicae; CPC, personal collection of Pedro Crous; GJS, collection of Gary J. Samuels maintained at the USDA-ARS Beltsville collection; MAFF, Ministry of Agriculture, Forestry and Fisheries; ICMP, International Collection of Microorganisms from Plants; IMI, CAB International Microorganism Resource Collection.

the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang, 1996) were determined from the 50% majority-rule consensus tree generated from the remaining 9,000 trees. The analysis was repeated three times starting from different random trees, to ensure trees from the same tree space were being sampled during each analysis. *Campylocarpon fasciculare* (CBS 112613) and *C. pseudofasciculare* (CBS 112679) were used as the outgroups.

The combined alignment of the five loci (i.e., LSU, ITS, β -tub, act, rpb1) was created and analysed to infer the multigenic analysis of the *Thelonectria* isolates. Alignment gaps were treated as missing data, and all the characters were unordered and of equal weight. Maximum parsimony analysis and Bayesian analyses were performed as described above.

Maximum likelihood analyses were carried out using RAxML on the web-server (Stamatakis *et al.*, 2008) at <http://phylobench.vital-it.ch/raxml-bb/index.php>, using the gamma model of rate heterogeneity and maximum likelihood search. *Thelonectria westlandica* (IMI255610, ICMP10387) was used as the outgroup.

The sequences generated in this study have been submitted to GenBank, and the alignment to TreeBASE (www.treebase.org), and the taxonomic novelties to MycoBank (www.Mycobank.org) (Crous *et al.*, 2004a). The GenBank accession numbers of the strains collected during the present study are listed in Table 2.

The sequences of *C. fasciculare* (CBS 112613) and *C. pseudofasciculare* (CBS 112679) were used as the outgroups in the phylogenetic analysis.

Pathogenicity testing

To assess the infection of grapevine wood tissues by *Thelonectria* sp., and to compare its aggressiveness with *I. liriodendri* and *D. torresensis* isolated from young grapevines and nursery rootstock plants, three isolates of each (i.e., BF109, BF133, BF142; BF12, BF47, BF144; BF33, BF130; BF135) were used in the pathogenicity tests carried out in October 2016, on 1-year-old shoots (0.8–1.2 cm diam.) cut from 4-year-old 1103 Paulsen rootstock from mother plants. Before artificial inoculation, the shoots were subjected to hot water treatment at 53°C for 30 min to ensure that the plants were pathogen free. Once the shoots were recognized as pathogen free, a mycelial plug from a 10-d-old colony of candidate isolate grown on

water agar was artificially inserted into a wound by removing the bark of the shoot. Inoculated wounds were wrapped with wet sterile cotton-wool for about 2 d and then placed in a plastic box sealed with cellophane film for another 13 d. The controls were mock inoculated with sterile distilled water. Each experiment included five replicates per isolate. After incubation at 23 ± 2 °C for 15 d, the inoculated shoots were examined by removing the bark and measuring the lengths of brown streaking. All the inoculated shoots were subjected to re-isolation, to fulfill Koch's postulates.

To determine whether the data obtained followed a normal distribution, Shapiro-Wilk test (W test) was used. The homogeneity of the variance of the dataset was assessed using Levene test. Statistical analyses were performed using Statistica, version 6 (StatSoft, Hamburg, Germany).

Factorial ANOVA analysis was performed to define the significance of any differences in lesion lengths caused by the isolates of the same fungal species and different fungal species, and to detect any interactions between these factors (i.e., isolate \times fungal species). One-way ANOVA analysis was performed to evaluate the significant differences in the brown wood streaking lengths caused by each fungal species inoculated. Fischer's tests were used for the comparisons of the treatment means, at $P < 0.01$.

Results

Isolates

The fungi isolated from symptomatic grapevine samples are shown in Table 3. The fungi commonly associated with PD, which included *Phaeoacremonium* spp., *Ph. chlamydospora* and *Pleurostoma richardisiae*, were isolated with an IF of 12.0%, which ranged from 0.2 to 3.0% for each species. The most common species isolated were *Pm. minimum* (IF, 3.0%), *Pm. italicum* (IF, 2.3%) and *Ph. chlamydospora* (IF, 1.9%). Fungi belonging to Botryosphaeriaceae spp. were isolated with an IF of 11.8%, individually ranging from 1.5 to 4.7%. In this group, *Diplodia seriata* was the most frequently isolated species (IF, 4.7%). Fungi associated with BFD were the most frequently isolated, with an IF of 16.2%. The fungal species *D. torresensis*, *I. liriodendri* and *Thelonectria* sp. were isolated with IFs, respectively, of 7.4, 5.8, and 3.0%. According to PCA (Figure 2), the five variables related to the

Table 3. Fungal species isolated from symptomatic grapevine samples.

Fungal disease	Fungal species isolated	Number of fungal isolates (% fungal isolation frequency)					
		Roots	Rootstock (below grafted union)	Scions (above grafted union)	Basal stems	Branches	Total
Petri disease	<i>Phaeoconiella chlamydospora</i>	12 (1.1)	7 (0.6)	2 (0.2)	0 (0.0)	0 (0.0)	21 (1.9)
	<i>Phaeoacremonium croatiense</i>	4 (0.4)	1 (0.1)	4 (0.4)	2 (0.2)	0 (0.0)	11 (1.0)
	<i>Pm. iranianum</i>	4 (0.4)	3 (0.3)	3 (0.3)	3 (0.3)	0 (0.0)	13 (1.2)
	<i>Pm. italicum</i>	7 (0.6)	5 (0.4)	10 (0.9)	4 (0.4)	0 (0.0)	26 (2.3)
	<i>Pm. minimum</i>	11 (1.0)	6 (0.5)	12 (1.1)	5 (0.4)	0 (0.0)	34 (3.0)
	<i>Pm. parasiticum</i>	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.2)
	<i>Pm. scolyti</i>	5 (0.4)	2 (0.2)	6 (0.5)	2 (0.2)	0 (0.0)	15 (1.3)
	<i>Pm. sicilianum</i>	2 (0.2)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.3)
	<i>Pleurostoma richardsiae</i>	4 (0.4)	0 (0.0)	5 (0.4)	0 (0.0)	0 (0.0)	9 (0.8)
	Subtotal	47 (4.7)	25 (2.2)	37 (3.8)	16 (1.4)	0 (0.0)	125 (12.0)
Botryosphaeria dieback	<i>Diplodia seriata</i>	16 (1.4)	13 (1.2)	11 (1.0)	8 (0.7)	5 (0.4)	53 (4.7)
	<i>Lasiodiplodia citricola</i>	8 (0.7)	4 (0.4)	4 (0.4)	1 (0.1)	0 (0.0)	17 (1.5)
	<i>L. theobromae</i>	9 (0.8)	6 (0.5)	5 (0.4)	1 (0.1)	0 (0.0)	21 (1.9)
	<i>Neoufusisporium parvum</i>	11 (1.0)	6 (0.5)	5 (0.5)	3 (0.3)	0 (0.0)	25 (2.2)
	<i>N. vitifusiforme</i>	6 (0.5)	2 (0.2)	7 (0.6)	3 (0.3)	0 (0.0)	18 (1.6)
	Subtotal	54 (4.4)	31 (2.8)	37 (2.8)	16 (1.4)	5 (0.4)	143 (11.8)
Black foot disease	<i>Dactylonectria torresensis</i>	57 (5.2)	26 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	83 (7.4)
	<i>Ilyonectria liriodendri</i>	43 (3.8)	22 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	65 (5.8)
	<i>Thelonectria</i> sp.	19 (1.7)	15 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	34 (3.0)
	Subtotal	119 (10.6)	63 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	182 (16.2)
	No fungal growth	4 (0.4)	92 (8.2)	136 (12.1)	187 (16.6)	207 (18.4)	626 (55.7)
	Bacteria	0 (0.0)	6 (0.5)	8 (0.7)	4 (0.4)	5 (0.5)	23 (2.1)
	Saprophytic fungi*	1 (0.1)	8 (0.7)	7 (0.6)	2 (0.2)	8 (0.7)	26 (2.3)
	Subtotal	4 (0.3)	106 (9.4)	151 (13.4)	193 (17.2)	220 (19.6)	675 (60.0)
Total number tissue portions		225 (20.0)	225 (20.0)	225 (20.0)	225 (20.0)	225 (20.0)	1125 (100.0)

* *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp.

plant organs (i.e., Var1-5: root, rootstock, scion, basal stem, branches) of the young grapevines and nursery rootstock plants were reduced to two factors, which explained 100.00% of the total variability (factor 1,

83.37%; factor 2, 16.63%) (Figure 2). The variables Var1 (root), Var2 (rootstock), Var3 (scion) and Var4 (basal stem) were related to factor 1, whereas Var5 (branches) was mostly related to factor 2. The projec-

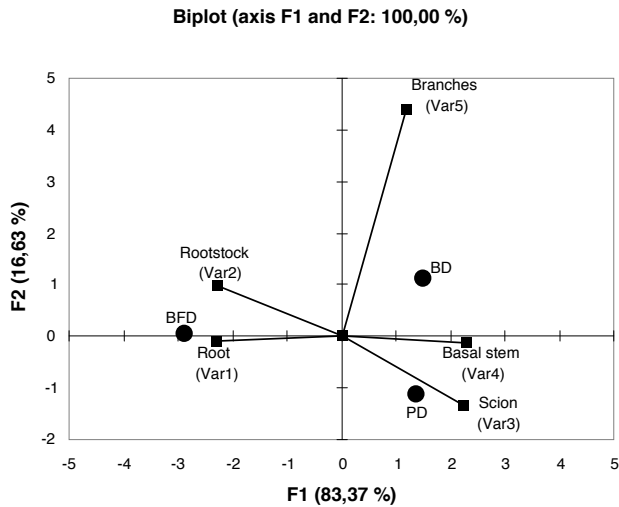


Figure 2. Principal component analysis based on isolation frequencies (IFs).

tion of the variables and cases (i.e., biplot analysis; Figure 2) showed that from root and rootstock (i.e., Var1, Var2, respectively), the main species isolated were those associated with BFD, with IFs of 10.6 and 5.6%, respectively. From scion (Var3), the species with the greater IFs (3.7%) were those associated

with PD. From basal stem (Var4), only fungi associated with PD and BD were isolated, each with IFs of 1.4%. Finally, from branches (Var5), the only fungi isolated were associated with BD (Table 3; Figure 2).

In addition, all the young grapevines (28) and nursery rootstock plants (17) analysed had BFD fungi. Of these, 13 plants (i.e., 11 young grapevines, two nursery rootstock plants) had only BFD infections, 19 plants (eight young grapevines, 11 nursery rootstock plants) had both BFD and BD infections, and 13 plants (nine young grapevines, four nursery rootstock plants) had mixed infections of BFD, BD and PD (Table 4).

Molecular identification, phylogenetic analysis, and morphological characterisation

Phylogenetic analyses were performed for the ITS and β -tub sequences of 47 strains aligned with 160 sequences retrieved from GenBank. The dataset consisted of 207 taxa, which included the outgroup taxa (*Campylocarpon fasciculare* and *C. pseudofasciculare*). After alignment and exclusion of incomplete portions at either end, the dataset consisted of 1,083 characters (including alignment gaps). Of these characters, 525 were constant, while 34 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 524 parsimony-informa-

Table 4. Grapevine samples affected by fungal diseases associated with vineyards, based on the fungal species isolated.

Plant	Cultivar	Number of infected plants (% disease incidence)			
		BFD ^a	BFD + BD ^b	BFD + BD + PD ^c	Total
Young grapevine	Chardonnay	5 (17.9)	2 (7.1)	3 (10.7)	10 (35.7)
	Pinot grigio	3 (10.7)	3 (10.7)	4 (14.4)	10 (35.7)
	Trebbiano toscano	3 (10.7)	3 (10.7)	2 (7.1)	8 (28.6)
	Subtotal	11 (24.4)	8 (17.8)	9 (20.0)	28 (62.2)
Nursery rootstock	Ciliegiolo	1 (5.9)	2 (11.7)	1 (5.9)	4 (23.5)
	Cocociola d’Abruzzo	1 (5.9)	3 (17.6)	1 (5.9)	5 (29.4)
	Moscato	-	2 (11.7)	1 (5.9)	3 (17.6)
	Sangiovese	-	4 (23.6)	1 (5.9)	5 (29.4)
	Subtotal	2 (4.4)	11 (24.4)	4 (8.9)	17 (37.8)
Total		13 (28.9)	19 (42.2)	13 (28.9)	45 (100.0)

^aBlack foot disease; ^bBotryosphaeria dieback; ^cPetri disease.

tive characters resulted in 1,000 most-parsimonious trees (TL = 2,378; CI = 0.439; RI = 0.935; RC = 0.410; HI = 0.561). Bayesian analysis resulted in a tree with essentially the same topology as the maximum parsimony trees (TreeBASE S19784) (Figure 3).

Phylogenetic analyses of the His3 single-locus alignment were generated for 31 strains, and these were aligned with 56 sequences retrieved from GenBank. The dataset consisted of 87 taxa, which included the outgroup taxa (*Campylocarpon fasciculare* and *C. pseudofasciculare*). After alignment and exclusion of incomplete portions at either end, the dataset consisted of 453 characters (including alignment gaps). Of these, 225 were constant, while 23 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 205 parsimony-informative characters resulted in 18 most-parsimonious trees (TL = 945; CI = 0.512; RI = 0.904; RC = 0.463; HI = 0.488). Bayesian analysis resulted in a tree with essentially the same topology as the maximum parsimony trees (TreeBASE S19785) (Figure 4). The *Dactylonectria* and *Ilyonectria* isolates obtained in this study clustered into two groups with the sequences of *Dactylonectria* and *Ilyonectria* spp. retrieved from GenBank. Eighteen isolates clustered with the ex-type of *D. torresensis*, while 13 isolates clustered with the ex-type of *I. liriodendri*.

All of the isolates that belonged to these two species produced aerial and cottony mycelia, with the colony colours variable from white to dark yellow or slightly brown, with a strong density of texture of the mycelia. No ascomata were seen in culture. Based on microscopic observations, all these isolates produced macroconidia, microconidia and chlamydospores, with sizes similar to those described by Cabral *et al.* (2012a) and Halleen *et al.* (2006a).

The combined dataset of the five loci (i.e., LSU, ITS, β -tub, act, rpb1) of the *Thelonectria* isolates consisted of 45 taxa, which included the outgroup taxa (*Thelonectria westlandica* IMI255610, ICMP10387). After alignment and exclusion of incomplete portions at either end, the dataset consisted of 2,641 characters (including alignment gaps). Of these characters, 2,125 were constant, while 59 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 457 parsimony-informative characters resulted in two most-parsimonious trees (TL = 834; CI = 0.783; RI = 0.941; RC = 0.737; HI = 0.217). Maximum likelihood and Bayesian analyses resulted in a tree with essentially the same topology as the maximum parsimony trees (TreeBASE S19786)

(Figure 5). The *Thelonectria* isolates obtained in the present study clustered in the *Thelonectria coronata* complex, but did not match any of the *Thelonectria* spp. belonging to this complex.

Taxonomy

Based on DNA sequence analyses of the *Thelonectria* isolates, six species fell in the *T. coronata* complex, of which five belonged to known species. However, one was distinct from all known species, and is described below as a new species. The description includes the culture characteristics and the characteristics of the asexual morph, as sexual compatibility tests failed to induce perithecia.

Thelonectria blackeriella M.L. Raimondo & A. Carlucci sp. nov. Figure 6,

MycoBank MB374246.

Holotype: Italy, Campomarino (CB), on rootstock of *Vitis vinifera* cv. 'Pinot grigio', September 2014, A. Carlucci, isolate number BF142 (holotype CBS H-22939, dried PDA colony). Ex-type culture CBS142200, GenBank accession numbers for LSU/ITS/ β -tub/ act/ rpb1: KX778690/ KX778711/ KX778702/ KX778687/ KX778693.

Etymology: Named after the black from BFD, because this species was isolated for the first time from grapevine plants affected by BFD.

Description: Mycelia not visible on host. No perithecia formation under laboratory conditions. Colonies on MEA reaching 50 to 55 mm diam. after 16 d at $25 \pm 2^\circ\text{C}$. Minimum temperature for growth in culture 8°C , optimum 20°C , and maximum 36°C . After 21 d, colonies on MEA cottony, with irregular margins, cream (19''f) on top, xanthine orange (13i) and marocco red (5k) sectors on the underside; colonies on oatmeal agar aerial, with concentric circles and entire margins, tilleul buff (17'''f) on top, vinaecous fawn (13'''b) to tilleul buff (17'''f) on the underside; colonies on PDA cottony, with not entire margins, pinkish buff (17''d) on the top, pinkish cinnamon (15''b) to cartridge buff (19''f) on the underside. No pigment produced at $> 25^\circ\text{C}$.

Mycelia composed of branched septate, sometimes fasciculate, hyphae that occur singly or in bundles of up to five; *hyphae* hyaline to dark yellow to pale brown, smooth, 2.55 to 10.30 μm wide.

Conidiophores unbranched, enlarged at the bases, erect, up to 3 to 4-septate, each ending in a single

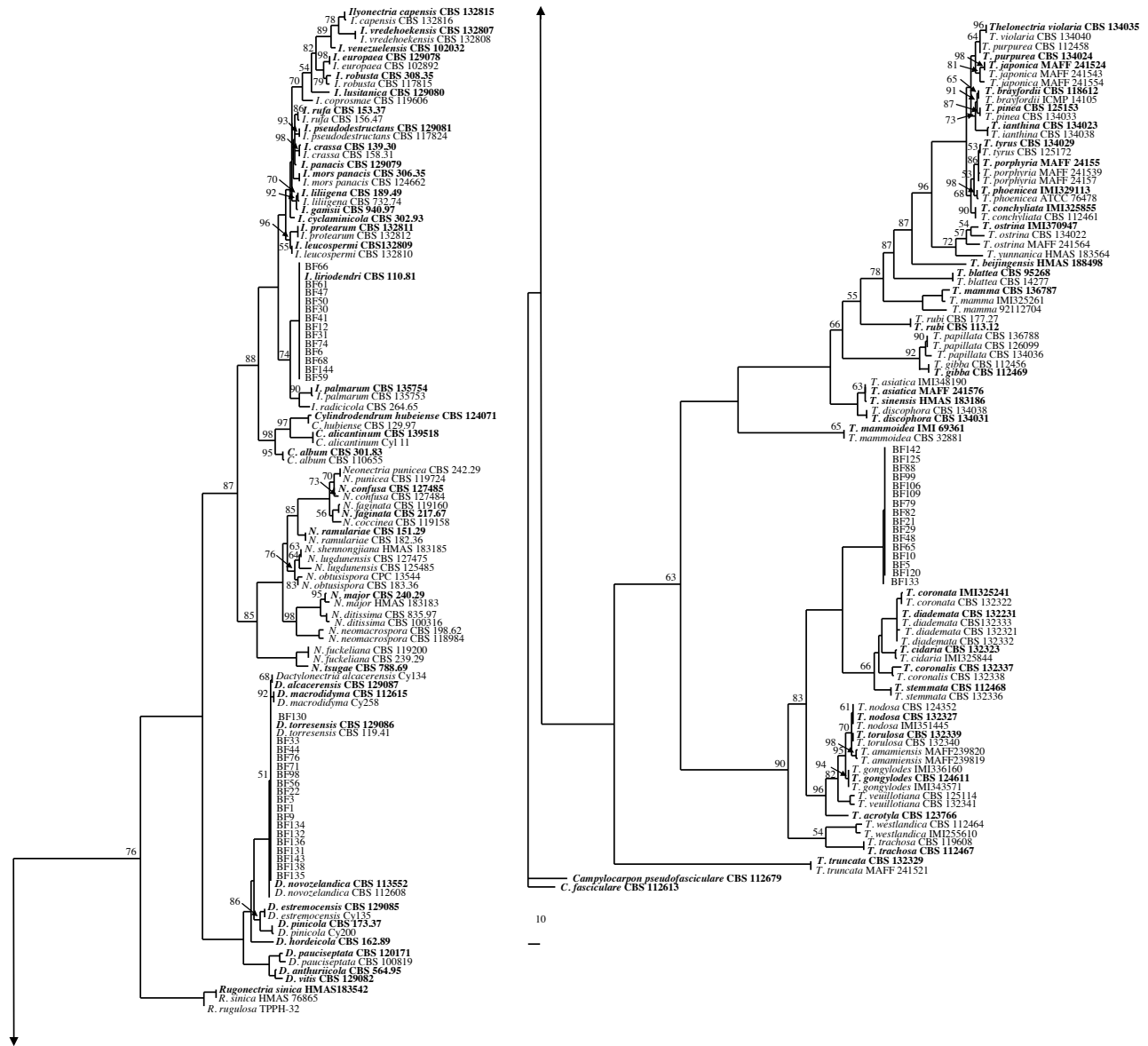


Figure 3. One of the 1,000 most parsimonious trees obtained from alignment of the ITS and β -tub sequence data, with bootstrap values from 100 replicates from maximum parsimony. Only bootstrap values <99 are shown at the internodes. Ex-type sequences are highlighted in bold. *Campylocarpon fasciculare* and *C. pseudofasciculare* were included as outgroups.

terminal phialide, often bearing one or two lateral phialides next to the terminal phialide, sub-hyaline to pale brown (16.41–) 19.58–34.76 (–44.72) \times (1.99–) 2.27–3.47 (–3.62) μm (mean 27.17 \times 2.87 μm). *Phialides* borne apically on irregularly branched clusters of cells, cylindrical or slightly swollen (7.25–) 8.16–13.39 (–15.21) \times (–2.07) 1.52–5.85 (–7.99) μm (mean 10.78

\times 3.69 μm), with periclinal thickening and collarets. *Macroconidia* formed in yellow, slimy droplets in aerial mycelia or on agar surface; cylindrical or slightly fusiform, curved with round ends, 1–4-septate: 1-septate (21.97–) 23.36–25.34 (–28.22) \times (2.44–) 3.81–4.48 (–4.66) μm (mean 24.35 \times 4.15 μm); 2-septate (24.75–) 27.84–29.15 (–33.13) \times (2.80–) 4.11–4.56 (–6.09) μm

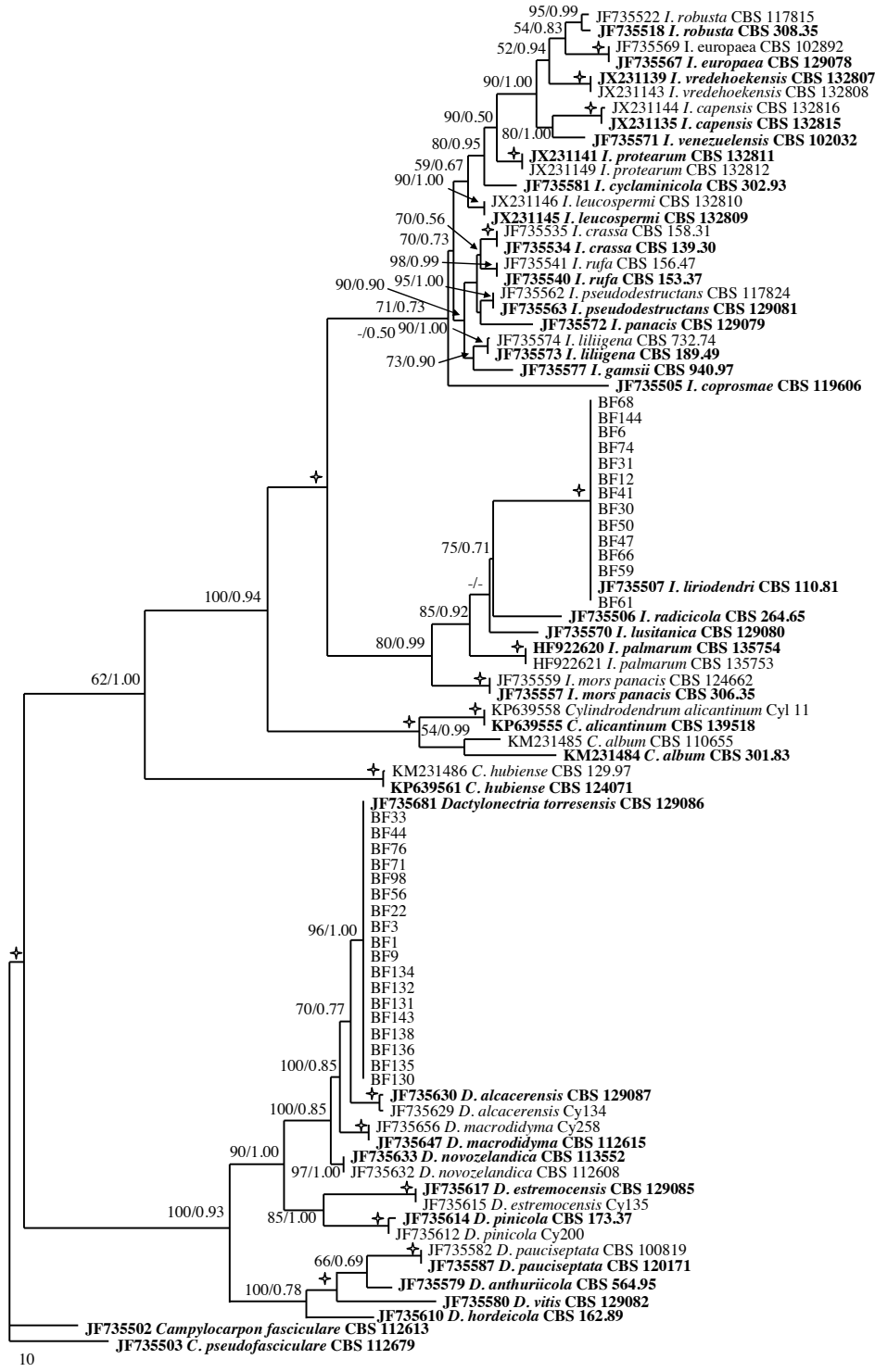


Figure 4. One of the 18 most parsimonious trees obtained from alignment of the His3 sequence data, with bootstrap values from 1,000 replicates from maximum parsimony / Bayesian posterior probability shown at the internodes. Bootstrap values of 100% are indicated with star symbols. Ex-type sequences are highlighted in bold. *Campylocarpon fasciculare* and *C. pseudofasciculare* were included as outgroups.

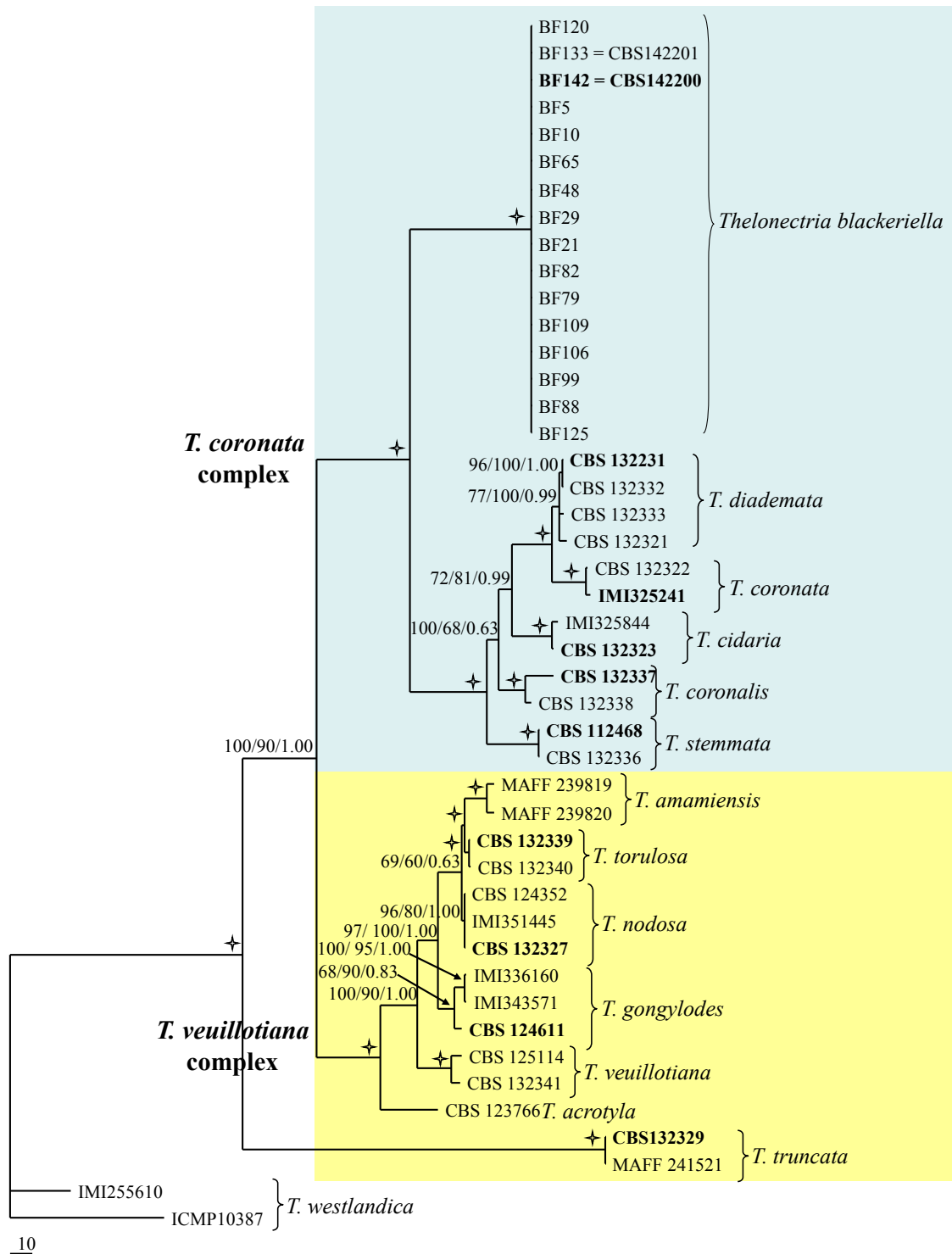


Figure 5. One of the two most parsimonious trees obtained from multiple alignment of the five genes (LSU, ITS, β -tub, act, rpb1) with bootstrap values from 1,000 replicates from maximum likelihood/maximum parsimony/Bayesian posterior probability shown at the internodes. Bootstrap values of 100% are indicated with star symbols. Ex-type sequences are highlighted in bold. *Thelephora westlandica* was included as outgroup.

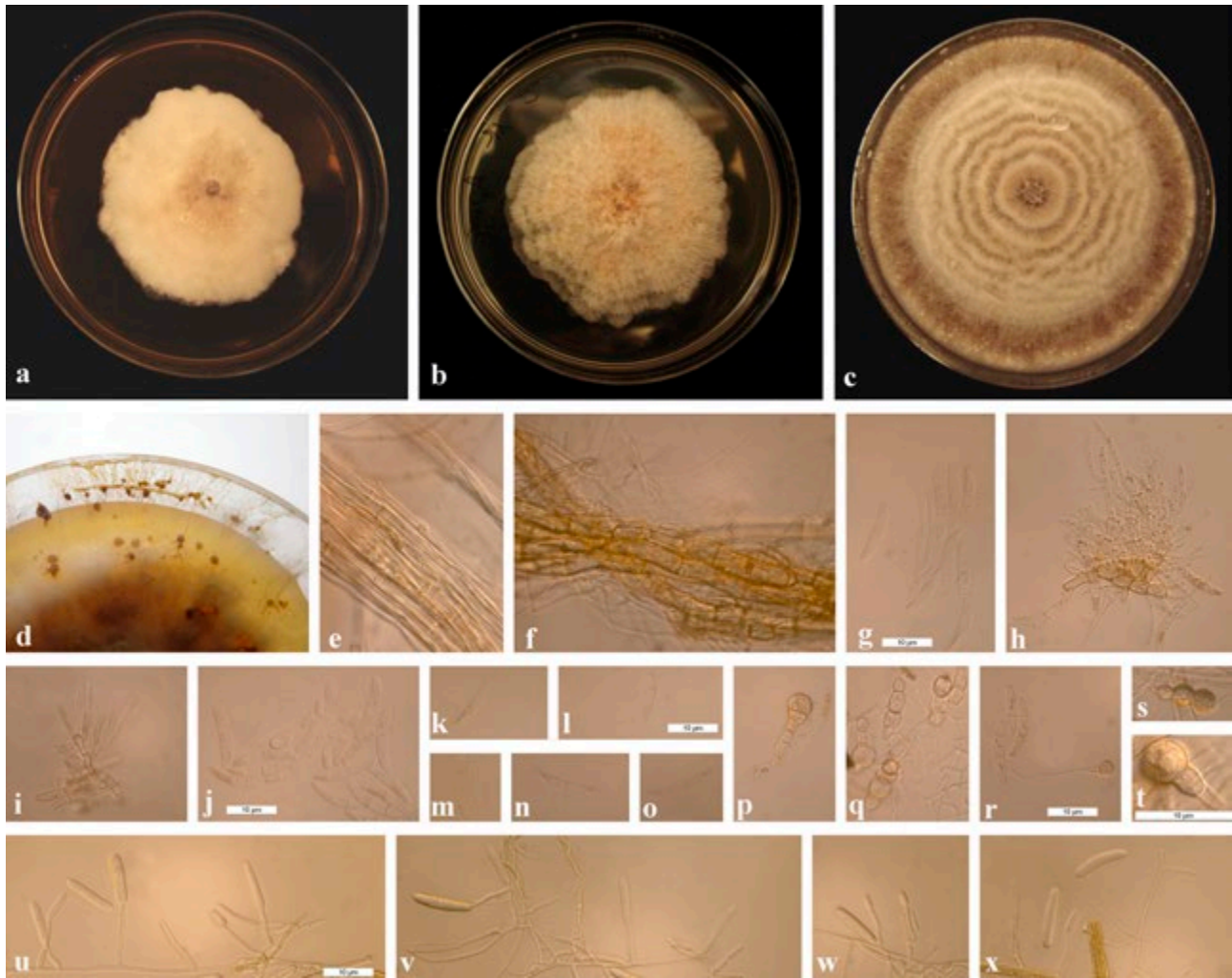


Figure 6. *Thelonectria blackleriella* sp. nov. (a-c) Sixteen-d-old colonies on MEA (a), PDA (b) and oatmeal agar (c), at 25°C. (d) Detail of exudates in Petri dishes containing conidia. (e, f) Mycelia in bundles of up to five. (g-i) Conidiophores and phialides. (j-o) Macroconidia most frequently observed in culture; (j), Different septate macroconidia; (k), 1-septate macroconidia; (l), 2-septate macroconidia; (m), 3-septate macroconidia; (n), 4-septate macroconidia; (o), 5-septate macroconidia. (p-r) *Chlamydospores* formed by macroconidia. (s-t) *Chlamydospores* formed on hyphae. (u-x) Conidiophores and phialides in distilled water.

(mean $28.50 \times 4.33 \mu\text{m}$); 3-septate (24.75–) 27.84–29.15 (–33.13) \times (2.80–) 4.11–4.56 (–6.09) μm (mean $28.50 \times 4.33 \mu\text{m}$); 4-septate (27.14–) 29.54–30.88 (–32.64) \times (3.62–) 4.50–5.19 (–6.80) μm (mean $30.21 \times 4.84 \mu\text{m}$).

Microconidia not produced in culture. *Chlamydospores* formed in culture, globose to subglobose (mean $8.62 \times 4.69 \mu\text{m}$).

Habitat and distribution: Isolated from grapevine in agricultural settings. Known from Italy.

Additional specimens examined: Italy, Cerignola (FG), on rootstock of *Vitis vinifera* cv. 'Sangiovese', September 2014, A. Carlucci, isolate number (BF133) CBS142201.

Pathogenicity tests

According to the Shapiro-Wilk test data from the pathogenicity tests 15 d after inoculations, these

followed a normal distribution, with a *W* value of 0.93 ($P < 0.012$). The Levene test revealed that the homogeneity of the variance was significant ($F = 2.91$, $P = 0.013$).

Factorial ANOVA demonstrated that significant differences in pathogenicity were detected among the inoculated fungal species ($F = 8.92$, $P = 0.00071$), while no significant differences in aggressiveness were observed among the isolates of the same fungal species used in the artificial inoculation ($F = 1.22$, $P = 0.30512$).

The mean lengths of vascular discolouration caused by *D. torresensis*, *I. liriodendri* and *T. blackeriella* (one-way analysis of variance) are reported in Table 5. All the fungal species produced brown wood discolouration on young shoots. The most aggressive species was *T. blackeriella*. At 15 d after inoculation, *T. blackeriella* produced the longest brown wood discolouration, which ranged from means of 14.80 to 18.40 mm. *Ilyonectria liriodendri* produced shorter brown wood discolouration on young shoots, compared to *D. torresensis* and *T. blackeriella*, which ranged from mean lengths of 8.20 mm to 11.80 mm at 15 d after inoculation. The fungal species were re-isolated from discoloured tissues of all of the inoculated shoots, which fulfilled Koch's postulates (Table 5).

Discussion

The present study confirms the presence of the fungal species *Ph. chlamydospora*, *Phaeoacremonium* spp., *Pl. richardsiae*, Botryosphaeriaceae spp. and *Cylindrocarpon*-like anamorphs commonly associated with the severe grapevine diseases of PD, BD and BFD. The simultaneous presence of different fungal species that are associated with other severe diseases that occur on grapevines, such as PD and other trunk diseases, is not new, as similar scenarios were also reported by Sofia *et al.* (2013) and Carlucci *et al.* (2015b) on grapevines, respectively, in Portugal and Italy. The PD complex is one of the most widespread grapevine diseases in Italy, where it has been known since 1998 (Bertelli *et al.*, 1998). Among the main fungi associated with these diseases, *Phaeoacremonium* spp. have been the most isolated, together with *Ph. chlamydospora* (Mugnai *et al.*, 1999; Raimondo *et al.*, 2014), *Fomitiporia mediterranea* (Fisher *et al.*, 2002), and more recently, *Pleurostomophora richardsiae* (Carlucci *et al.*, 2015a), with this last renamed as *Pleurostoma richardsiae* by Réblová *et al.* (2016). To date, ten species of *Phaeoacremonium* have been reported as fungal pathogens for esca disease and PD of vineyards in Italy (Raimondo *et al.*, 2014), which here included *Phaeoacremonium croatiense*, isolated from

Table 5. Mean lesion lengths from pathogenicity assays carried out for three fungal species on grapevines (one-way ANOVA).

Fungal species	Id Isolate	Length of brown wood discolouration (mm)		
		Mean	SD	Min-Max ^a
Control	-	1.0 A ^b	1.0	0-2
<i>Ilyonectria liriodendri</i>	BF144	8.2 B	3.1	5-13
	BF47	10.8 B	1.1	9-12
	BF12	11.8 BC	1.9	9-14
<i>Dactylonectria torresensis</i>	BF130	11.8 BC	2.8	9-15
	BF33	12.0 BC	2.9	8-16
	BF135	12.4 BC	4.6	7-17
<i>Thelonectria blackeriella</i>	BF142	14.8 C	6.0	9-23
	BF109	16.8 CD	6.1	8-24
	BF133	18.4 D	6.3	11-26

^a Minimum and maximum values detected, (12 observations).

^b Data followed by different capital letters within the column are significantly different (Fischer's tests; $P < 0.01$)

grapevines in Italy for first time. BFD has been another very important problem in viticulture in Italy since the end of the 1970s (Cristinzio, 1978; Rovesti and Montermini, 1987; Mondello *et al.*, 2013; Carlucci *et al.*, 2015b). The present study confirms the common presence of *D. seriata* in Italian vineyards, compared to other Botryosphaeriaceae spp. like those previously reported by Mohammadi *et al.* (2013) and Carlucci *et al.* (2015b).

Black foot disease of grapevines is widespread in Portugal, Spain, South Africa and California, USA (Rego *et al.*, 2000; Halleen *et al.*, 2004; Petit and Gubler, 2005; Alaniz *et al.*, 2007). There have been no previous reports of BFD for Italy, except for Grasso and Magnano di San Lio (1975) and Grasso (1984), who respectively associated *Cylindrocarpon obtusisporum* and *C. destructans* with infections in Sicilian vineyards.

The analysis of the young grapevines and nursery rootstock plants that showed sunken necrotic root lesions, reduced root biomass, black sub-cortical discoloration, and necrosis of xylem tissues also led to a high number of *Cylindrocarpon*-like asexual morphs (182) being isolated. These high isolation frequencies induced us to further investigate this fungal group. The preliminary phylogenetic study carried out here was based on the ITS and β -tub sequences, and this allowed the division of the collected isolates into three main clades. The first of these clustered close to *Ilyonectria*, the second to *Dactylonectria*, and the third to *Thelonectria*. The subsequent phylogenetic analysis based on His3 data allowed the attribution of 83 isolates to *D. torresensis* and 65 to *I. liriodendri*. Thirty-four isolates of the third group that resembled *Thelonectria* were further studied using multigenic DNA analyses, which resulted in all of these grouped together in a monophyletic clade of *Thelonectria* spp., which is described here as *T. blackeriella* sp. nov.

Dactylonectria torresensis and *I. liriodendri* have been commonly associated with BFD throughout the world (Halleen *et al.*, 2006a; Cabral *et al.*, 2012a; Agustí-Brisach *et al.*, 2013a; Reis *et al.*, 2013), although to date there have been no reports that have described these species from Italy. The present study reports these for the first time from grapevines in Italy. For the *Thelonectria* genus, this was mainly considered to be a group of saprobic fungi that can live on decaying plant material of different hosts. This group has been isolated from different substrates, such as the bark of twigs and branches, or trunks

of recently dead or dying trees (Samuels *et al.*, 1990; Brayford *et al.*, 2004; Guu *et al.*, 2007), with a cosmopolitan distribution (Brayford, *et al.*, 2004). To date, there have been few reports that have described the pathogenic role of *Thelonectria* spp. For instance, Salgado-Salazar *et al.* (2012, 2015) reported *T. coronata*, *T. diademata* and *T. stemmata* in association with small cankers on shrubs and trees, and *T. rubi* as a plant pathogen on several species of *Rubus*.

To date, *Thelonectria* spp. have also not been reported as pathogens of grapevines and have not been associated with BFD, except for one report of two strains of *Thelonectria*, previously mis-identified as *Cylindrocarpon* sp./*Neonectria mammoidea* group (Cyl11, ITS/ β -tub, accession number HQ338494/HQ338503; Cyl19, ITS/ β -tub accession number HQ338502/HQ338511), from BFD symptomatic grapevines in north-eastern USA and south-eastern Canada (Petit *et al.*, 2011), although no pathogenicity tests were carried out for these reports.

To determine the pathogenic role of *T. blackeriella* and its involvement in BFD, preliminary pathogenicity tests were carried out here, as also for *I. liriodendri* and *D. torresensis*. These tests demonstrated that *T. blackeriella*, *I. liriodendri* and *D. torresensis* can infect young grapevine rootstock shoots. The statistical analyses also showed that *T. blackeriella* produces longer wood discoloration than the other two species. To the best of our knowledge, this is the first association of *T. blackeriella* with BFD on grapevines worldwide. Based on these preliminary results, further pathogenicity studies are needed to understand the level of severity of disease caused by *T. blackeriella*.

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