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Research Paper

Identification and pathogenicity of lignicolous fungi associated with grapevine trunk diseases in southern Italy

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Summary. Over the last 10 years, several fungi were isolated from grapevines with grapevine trunk disease (GTD) symptoms, in the Apulia and Molise regions of Italy. Morphological and molecular analyses allowed the identification of species belonging to Botryosphaeriaceae, *Phaeoacremonium* species, *Phaeomoniella chlamydospora*, *Pleurostoma richardsiae* and less-common fungi associated with grapevine trunk diseases, such as *Cadophora*, *Colletotrichum*, *Seimatosporium* and *Truncatella*. These last genera were isolated at significant frequencies, so they were investigated for possible involvement in GTDs. To screen the large numbers of isolates collected, microsatellite-PCR analysis was carried out with the M13 primer, and 29 strains were further studied by amplification of different genes, for multi-locus analyses. Phylogenies and morphological analyses allowed identification, for first time in Italy, of fungi associated with GTDs, including *Cadophora luteo-olivacaea*, *Colletotrichum fioriniae*, *Seimatosporium vitis-vinifera* and *Truncatella angustata*. Pathogenicity assays with these fungi and other fungi known to be pathogens for grapevines (*Lasiodiplodia citricola*, *Phaeoacremonium italicum*, *Pleurostoma richardsiae*) showed that they caused disease symptoms on two Italian grapevine cultivars ('Bombino bianco', 'Nero di Troia'), although with different degrees of severity. Among the fungi isolated for the first time in Italy, *Sei. vitis-vinifera* was the most aggressive, while *C. fioriniae* the least pathogenic. All of these fungi were re-isolated from grapevine, and thus fulfilled Koch's postulates, confirming their pathogenicity on grapevine.

Keywords. *Cadophora luteo-olivacea*, *Colletotrichum fioriniae*, *Seimatosporium vitis-vinifera*, *Truncatella angustata*, phylogenies, artificial inoculation.

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INTRODUCTION

Several diseases caused by fungi that have been associated with grapevines over the last 20 years have caused severe yield losses in other grape producing countries (Gramaje *et al.*, 2018; Guerin-Dubrana *et al.*, 2019). Grapevine trunk diseases (GTDs) are considered to be the most destruc-

tive and severe diseases of grapevine in Mediterranean countries, including Spain, France, Portugal and Italy, and also in the United States of America, Australia, and Asia (Gubler *et al*., 2005; Gramaje *et al*., 2018; Guerin-Dubrana *et al.,* 2019). The main fungi that cause GTDs are species involved in different diseases, which include Esca and Petri disease (Larignon and Dubos, 1997; Mugnai *et al.,* 1999; Gramaje *et al.,* 2011; Navarrete *et al.,* 2011; Bertsch *et al*., 2013; Carlucci *et al.,* 2015a; Travadon *et al.,* 2015), Botryosphaeria dieback (Urbez-Torres, 2011), Diaporthe and Eutypa diebacks (Larignon and Dubos, 1997; Fourie and Halleen, 2004; Urbez-Torres *et al.,* 2013), and black foot disease (Halleen *et al*., 2004; Agusti-Brisach and Armengol, 2013; Carlucci *et al*., 2017).

The main external symptom of GTDs is general decline of affected plants. The specific external symptoms consist of tiger-stripe leaves, stunted shoots and chlorotic leaves which are sometimes cupped and with necrotic margins, flattened areas of the wood without bark, cankered wood and wedge-shaped perennial cankers, black and sunken necrotic lesions on roots, and reddish brown discolouration at the bases of trunks (Gramaje *et al.,* 2018). Internal symptoms include dark-coloured xylem vessels of the grapevine trunks, with exudate from the vessels when the trunks are cut in cross-section, and dark streaks in longitudinal sections (*Phaeomoniella chlamydospora, Phaeoacremonium* spp., *Cadophora* spp.). There can also be black subcortical streaking (*Pleurostoma richardsiae*) and necrosis of the wood tissues. Other symptoms are of rootstock browning in young grapevines (due to black foot fungi). Cordon dieback can also occur, with loss of spurs and internal necrotic wedge-shaped staining in stem cross-sections (*Eutypa lata*, *Botryosphaeria* spp.), and wood white rot (caused by Basidiomycete fungi) (Gramaje *et al.,* 2018).

Grapevines can be affected by one or more GTDs at the same time, as individual plants can be infected by different pathogens, due to co-occurrence of multiple infections throughout a season, and over years. This produces overlapping of the symptoms described above, which makes their association with the specific responsible fungi particularly difficult to define, and detection of causal pathogens challenging (Gramaje *et al*., 2018).

High isolation frequency of particular fungal species involved in GTDs from mature, young and nursery grapevines in different countries can be different, due to climatic and geographic conditions, to specific pathogen aggressiveness, and to host cultivar susceptibility (Guerin-Dubrana *et al.,* 2019). Petri and black-foot diseases are mostly detected on planting material and young vines (Rego *et al.,* 2000; Agusti-Brisach and Armengol, 2013; Carlucci *et al.,* 2017). Conversely, apoplexy, Esca and grapevine leaf symptoms, and Phomopsis, Eutypa and Botryosphaeria diebacks are most frequently observed on mature grapevines (Guerin-Dubrana *et al*., 2019).

To date, up to 138 fungal species belonging to 35 genera have been reported as responsible for GTDs. However, pathogenicity towards grapevine wood has not been tested and/or confirmed for all of these fungi (Gramaje *et al.,* 2018; Berlanas *et al*., 2020; Brown *et al*., 2020). For instance, 'Pestalotioides fungi' have been frequently associated with symptomatic and asymptomatic vineyards (Farr and Rossman, 2018; Liu *et al.,* 2019), although no detailed information is available about their involvement in GTD symptoms. In Italy, incidence of Esca, grapevine leaf symptoms and apoplexy is significant and increasing in all grapevine production regions (Guerin-Dubrana *et al.,* 2019). In Apulia, Molise and Sicily, Botryosphaeria dieback has also been reported (Cristinzio, 1978; Burruano *et al.,* 2008; Carlucci *et al.,* 2009; 2015b). Eutypa and Phomopsis diebacks are known to occur, if not frequently, in Italian vineyards (Guerin-Dubrana *et al*., 2019). *Pleurostoma richardsiae*, *Dactylonectria torresensis*, *Ilyonectria liriodendri* and *Thelonectria blackeriella* were reported for the first time in Italy by Carlucci *et al*. (2015a; 2017).

A collection of fungi from a decennial survey carried out in symptomatic vineyards in the Apulia and Molise regions of Italy was subjected to identification and characterisation by morphological and molecular approaches, and pathogenicity testing of representative isolates was carried out to determine their putative involvement in GTDs. The present paper describes results from this research.

MATERIALS AND METHODS

Fungal isolates

Symptomatic grapevine samples were collected and analysed during the years 2009 to 2018. The samples were from many vineyards in the Foggia, Barletta-Trani-Andria and Campobasso provinces in Italy, and were taken from different grapevine cultivars, including 'Sangiovese', 'Montepulciano', 'Nero di Troia', 'Pinot grigio', 'Trebbiano toscano', 'Moscato bianco' and 'Chardonnay' (Table 1).

External symptoms observed on affected grapevine plants included stunting, reduced grapevine vigour, shoot dieback, and leaf discolouration with interveinal chlorosis and necrosis. Internal symptoms included black discolouration of wood under the bark, and necro-

Table 1. Information on vineyards surveyed and sampled in the Apulia and Molise regions (southern Italy).

Survey Year	Location	Vineyard			
		Cultivar	Age (year)	N. samples	GTD Incidence* (%)
2009	Cerignola (FG)	'Sangiovese'	27	8	13.4
	Lucera (FG)	'Nero di Troia'	15	3	11.5
	Lucera (FG)	'Moscato bianco'	10	3	9.8
2011	Canosa di Puglia (BT)	'Montepulciano'	21	4	16.2
	Canosa di Puglia (BT)	'Sangiovese'	12	3	11.3
	Foggia (FG)	'Nero di Troia'	19	5	13.5
	Foggia (FG)	'Pinot grigio'	13	3	9.2
2012	Foggia (FG)	'Moscato bianco'	17	4	6.7
	Campobasso (CB)	'Pinot grigio'	2	3	11.2
	Barletta (BT)	'Sangiovese'	14	3	17.3
	Barletta (BT)	'Trebbiano toscano'	11	3	16.4
2013	San Severo (FG)	'Trebbiano toscano'	29	10	21.4
	San Severo (FG)	'Nero di Troia'	21	6	18.3
	San Severo (FG)	'Pinot grigio'	12	3	16.9
	Termoli (CB)	'Pinot grigio'	5	4	8.5
	Termoli (CB)	'Chardonnay'	5	3	9.2
	Cerignola (FG)	'Sangiovese'	14	3	12.6
	Cerignola (FG)	'Trebbiano toscano'	8	6	9.3
2015	Campomarino (CB)	'Chardonnay'	2	6	11.5
	Campomarino (CB)	'Pinot grigio'	2	6	14.2
	Stornara (FG)	'Sangiovese'	31	8	19.8
	Torremaggiore (FG)	'Trebbiano toscano'	25	6	19.2
	Torremaggiore (FG)	'Nero di Troia'	15	4	14.8
2017	Foggia (FG)	'Sangiovese'	9	3	10.5
	Foggia (FG)	'Trebbiano toscano'	17	4	13.7
	Canosa di Puglia (BT)	'Montepulciano'	21	6	19.4
	Canosa di Puglia (BT)	'Sangiovese'	23	3	18.9
2018	Cerignola (FG)	'Trebbiano toscano'	19	3	13.6
	Barletta (BT)	'Chardonnay'	14	6	11.8
	Barletta (BT)	'Montepulciano'	17	6	10.3

* GTD Incidence was calculated on the basis of vines showing symptoms on 2,500 plants for each surveyed vineyard.

sis of xylem tissues. The samples included grapevine trunks, cordons and woody shoots. These were transported to the laboratory for analyses, where they initially underwent surface sterilization (Fisher *et al.*, 1992). The bark of each sample was removed with a sterile scalpel, and thin wood sections were cut (1 to 3 mm thick). From each section of each sample, five small wood tissue samples were cut and placed onto potato dextrose agar (PDA; 3.9% potato dextrose agar; Oxoid Ltd), and onto malt extract agar (MEA; 2% malt extract, 2% agar; Oxoid Ltd), both of which were supplemented with 500 mg L^{-1} streptomycin sulphate (Oxoid Ltd). After 7 to 10 d of incubation at 22±3°C in the dark, all of the fungal cultures obtained were purified by transferring single germinated conidia or small pieces of hyphae to Petri dishes containing fresh PDA.

Morphological and culture characteristics of isolated fungi were initially used to distinguish genera and species that were isolated from these symptomatic tissues (Crous and Gams, 2000; Mostert *et al.*, 2006; Essakhi *et al.*, 2008; Agustì-Brisach *et al.*, 2013; Phillips *et al.*, 2013; Raimondo *et al.*, 2014; Carlucci *et al.*, 2015a). The isolation frequency (IF; %) for each species was calculated as the number of tissue segments infected by each fungus, divided by the total number of tissue segments incubated.

DNA extraction and microsatellite PCR profiles

Genomic DNA was extracted from the 420 isolates obtained, from 15-d-old cultures grown on PDA (Carlucci *et al.*, 2013). Many of the isolates (339) belonged to Botryosphaeriaceae and *Phaeoacremonium*, *Phaeomoniella*, *Pleurostoma*, *Cadophora*, *Colletotrichum*, *Seimatosporium* and *Truncatella*, so preliminary screening was carried out for each genus based on the M13 minisatellite primers (5'-GAGGGTGGCGGTTCT-3') (Meyer *et al.,* 1993). Microsatellite (MSP)-PCR profiles were generated according to Santos and Phillips (2009). The DNA banding patterns were analysed using the Bionumerics v. 5.1 software (Applied Maths), with calculation of Pearson's correlation coefficients according to the unweighted pair group method with arithmetic means. The reproducibility levels were calculated by comparisons of the banding profiles obtained for the M13 primer. For this purpose, from any cluster, 10% of the strains were chosen at random, and their profiles were repeat.

Molecular characterisation

The MSP dendrogram generated for each genus produced different clades from which representative isolates were chosen for phylogenetic analysis data not shown). Eighty-four representative isolates of Botryosphaeriaceae, *Phaeoacremonium* spp., *Phaeomoniella* spp. and *Pleurostoma* spp. were identified using the keys, descriptions and sequence data from Phillips *et al.* (2013), Mostert *et al.* (2006), Essakhi *et al.* (2008), Raimondo *et al.* (2014), Crous and Gams (2000) and Carlucci *et al.* (2015a). For 41 Botryosphaeriaceae strains, ITS1-5.8S-ITS2 were amplified using the universal primers ITS1 and ITS4 (White *et al.*, 1990), and part of EF1-a was amplified using the primers EF1-688F and EF1-1251R (Alves *et al.*, 2008), according to Carlucci *et al.* (2015b). For 27 *Phaeoacremonium* strains, partial β-tubulin and partial actin genes were amplified using the universal primers T1 (O'Donnell and Cigelnik, 1997), Bt2b (Glass and Donaldson, 1995) and ACT-512F/ACT-783R (Carbone and Kohn, 1999), according to Raimondo *et al.* (2014). For seven *Phaeomoniella* and *Pleurostoma* strains, ITS1-5.8S-ITS2 were amplified using the universal primers ITS1 and ITS4 (White *et al.* 1990), according to Damm *et al.* (2010) and Carlucci *et al.* (2015a).

The other 29 representative strains that belonged to the *Seimatosporium*, *Truncatella*, *Cadophora* or *Colletotrichum* were further studied using molecular and morphological tools. Five loci were amplified for seven *Seimatosporium* and 11 *Truncatella* strains that were representative of the MSP-PCR groups. For large subunit RNA (LSU; ca. 500 bp) were used NL1/NL4 primer pairs (O'Donnell and Gray, 1993); for internal transcribed spacers (ITS) 1 and 2 (including 5.8S of nuclear ribosomal DNA; ca. 500 bp) were used ITS5/ITS4 (White *et al.*, 1990); for the partial β-tubulin gene (*tub*; ca. 680 bp) were used T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995); for the partial translation elongation factor 1-alpha (*tef-1α*; ca. 300 bp) were used EF1-688F and EF1-1251R (Alves *et al.,* 2008); and for the second-largest subunit of DNA-directed RNA polymerase II (*rpb2*; ca. 500 bp) were used RPB2-5f2/RPB2-7cr (Liu *et al.,* 1999; Sung *et al.*, 2007).

The LSU and ITS PCR reactions and conditions were performed according to Carlucci *et al.* (2012), with those for β-tubulin, *tef-1α* and *rpb2* according to Liu *et al.* (2019).

Three loci including ITS (ca. 550 bp), the partial translation elongation factor 1-alpha (*tef-1α*; ca. 420bp) and the partial β-tubulin gene (*tub*; ca. 500 bp) were amplified from six *Cadophora* strains, as representative of the MSP-PCR groups. These amplifications used the following primer pairs: ITS5/ITS4 (White *et al.*, 1990) for internal transcribed spacers (ITS) 1 and 2; EF1-728F and EF1-986R (Carbone and Kohn, 1999) for the partial translation elongation factor 1-alpha; and BTCadF 5' and BTCadR 5' (Travadon *et al.*, 2015) for the partial β-tubulin gene. The ITS PCR reactions and conditions were performed as described above, while those for *tef-1α* and β-tubulin according to Travadon *et al.* (2015).

Six loci were amplified for five *Colletotrichum* strains, as representative of the MSP-PCR groups. These included: the 5.8S nuclear ribosomal gene with the two flanking ITS (ca. 538 bp); β-tubulin (*tub*; ca. 500 bp); partial actin (*act*; ca. 250 bp); the intron of glyceraldehyde-3-phosphate dehydrogenase (*gapdh*; ca. 250 bp), and chitin synthase (*chs-1*; ca. 280 bp). The primer pairs used were ITS5/ITS4 (White *et al.*, 1990) for internal transcribed spacers (ITS) 1 and 2; T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995)) for β-tubulin; ACT-512F/ACT-783R (Carbone and Kohn, 1999) for partial actin; GDF1/GDR1 (Guerber *et al.*, 2003) for the intron of glyceraldehyde-3-phosphate dehydrogenase; and CHS-79F/CHS-345R (Carbone and Kohn, 1999) for chitin synthase. The PCR amplifications and conditions were performed according to Fu *et al.* (2019).

Five microlitres of each amplicon was analysed by electrophoresis, using 1.5% (w/v) agarose gels in 1× TAE buffer (40 mM Tris, 40 mM acetate, 2 mM EDTA, pH 8.0) at 100 V for 30 min. 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 analyses

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 confirm the preliminary morphological identification, and to select and download closely related sequences for phylogenetic analyses. GenBank sequences from different species of *Seimatosporium*, *Truncatella*, *Cadophora* and *Colletotrichum* were then selected and added to the sequences dataset obtained (Tables 2, 3).

The sequences were manually concatenated and aligned using the online multiple alignment programme MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley, 2013). The alignments were visually checked and manually improved where necessary. Multilocus analyses according to maximum parsimony and maximum likelihood were carried out for the LSU, ITS, β-tubulin, *tef-1α* and *rpb2* genes of the *Seimatosporium* and *Truncatella* sequence data.

The maximum parsimony analyses were performed using PAUP, version 4.0b10 (Swofford, 2003), with 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 1,000 heuristic search replicates and ten random taxon additions. The tree length (TL), consistency index (CI), retention index (RI), homoplasy index (HI), and rescaled consistency index (RC) were calculated for each, and the resulting trees were visualised with TreeView, version 1.6.6 (Page, 1996). Alignment gaps were treated as missing data for *Seimatosporium* strains, and as fifth characters for *Truncatella* strains.

The maximum likelihood analysis was carried out using RAxML-HPC v.8.2.12 (Stamatakis, 2006; Stamatakis *et al*., 2008) on the XSEDE Teragrid of the CIPRES Science Gateway (https://www.phylo.org) (Miller *et al*., 2010), with rapid bootstrap analysis, followed by 1,000 bootstrap replicates. The final trees were selected among the suboptimal trees from each run by comparing the likelihood and bootstrap scores. The outgroups in the *Seimatosporium* multigenic analysis were *Synnemapestaloides juniperi* (CBS 447.77) and *Discosia artocreas* (CBS 124848), and those for *Truncatella* were *Phlogicylindrium eucalypti* (CBS 120080) and *Beltrania pseudorhombica* (CBS 138003).

Multilocus alignment of the *Cadophora* (ITS, *tef-1α*, β-tubulin genes) and *Colletotrichum* (ITS, β-tubulin, *act*, *gapdh*, *chs-1* genes) strains was performed as described above with alignment gaps treated as missing data. *Hyaloscypha finlandica* (CBS 444.86) was used as outgroup in the *Cadophora* analysis, and *Colletotrichum gloeosporioides* (ICMP 17821) for the *Colletotrichum* analysis.

Morphological analyses

For each species identified using molecular tools (as described above), three isolates were used for morphological studies. To enhance sexual sporulation or conidiation, these fungi were grown on MEA in Petri dishes for 10 to 21 d under UV light at 23±2°C. Fungal structures were observed and measured from 100% lactic acid microscope slide mounts by making 30 measurements (at ×400 or ×1,000 magnification), using a measurement module (Leica Application Suite; Leica Microsystems GmbH). Photomicrographs were recorded using a digital camera (DFC320; Leica) on a microscope fitted with Nomarski differential interference contrast optics (DMR; Leica). The morphological features of conidiogenous cells and conidia were also determined in distilled water, by picking mycelium plugs from 30-d-old cultures grown on MEA, with images captured using a microscope (DM5500; Leica) at ×40 magnification.

Pathogenicity tests

Three isolates of each species were used in pathogenicity tests, to determine the infection of grapevine wood tissues by the less-known GTD fungi, and to compare their aggressiveness with the most common and previously determined GTD fungi. The previously determined GTD fungi used were: *Lasiodiplodia citricola* (Carlucci *et al*., 2015b), *Phaeoacremonium italicum* (Raimondo *et al*., 2014), and *Pleurostoma richardsiae* (Carlucci *et al*., 2015a).

Inoculations were carried out in June 2018, on 1-year-old canes (diam. 1.0-2.5 cm) from 10-y-old grapevines of the cultivars 'Nero di Troia' and 'Bombino bianco' in vineyards in an open field. The canes were inoculated at the internodes by wounding, as described by Carlucci *et al*. (2013). The wounds (each 1.0-2.0 cm long) were made on the cane surfaces with a sterile scalpel.

Table 2. Isolate identification numbers, locations, hosts and GenBank accession numbers of the strains of *Seimatosporium* spp., *Truncatella* spp. and *Cadophora* spp. used in the multigenic analyses.

Species	Isolate number[a]	Location	Host	GenBank accession number				
				LSU	ITS	*tub*	*tef-1α*	*rpb2*
Discosia artocreas	CBS 124848 ET[b]	Germany	*Fagus sylvatica*	MH554213	MH553994	MH554662	MH554420	MH554903
Seimatosporium botan	NBRC 104200 HT	Japan	*Paeonia suffruticosa*	AB593731	AB594799	LC047770	–	–
Sei. germanicum	CBS 437.87 HT	Germany	Unknown	MH554259	MH554047	MH554723	MH554482	MH554957
Sei. luteosporum	CBS 142599 HT	USA	*Vitis vinifera*	KY706309	KY706284	KY706259	KY706334	–
Sei. physocarpi	CBS 139968 HT	Russia	*Physocarpus opulifolius*	KT198723	KT198722	MH554676	MH554434	MH554917
	CBS 789.68	The Netherlands	*Physocarpus amurensis*	MH554278	MH554066	MH554742	MH554502	MH554979
Sei. pistaciae	CBS 138865 HT	Iran	*Pistacia vera*	KP004491	KP004463	MH554674	MH554432	MH554915
	CPC 24457	Iran	*Pistacia vera*	MH554331	MH554126	MH554799	MH554561	MH555035
Sei. rosae	CBS 139823 ET	Russia	*Rosa kalmiussica*	KT198727	LT853105	LT853253	LT853203	LT853153
Sei. vitifusiforme	CBS 142600 HT	USA	*Vitis vinifera*	KY706321	KY706296	KY706271	KY706346	–
Sei. vitis-viniferae	CBS 123004 HT	Spain	*Vitis vinifera*	MH554211	MH553992	MH554660	MH554418	MH554901
	CBS 116499	Iran	*Vitis vinifera*	MH554201	MH553984	MH554643	MH554402	MH554884
	CRCC 212[c]	Italy	*Vitis vinifera*	**MN862466**	**MN862459**	**MN862452**	**MN862445**	**MN862473**
	CRCC 213	Italy	*Vitis vinifera*	**MN862467**	**MN862460**	**MN862453**	**MN862446**	**MN862474**
	CRCC 214	Italy	*Vitis vinifera*	**MN862468**	**MN862461**	**MN862454**	**MN862447**	**MN862475**
	CRCC 229	Italy	*Vitis vinifera*	**MN862472**	**MN862465**	**MN862458**	**MN862451**	**MN862479**
	CRCC 247	Italy	*Vitis vinifera*	**MN862469**	**MN862462**	**MN862455**	**MN862448**	**MN862476**
	CRCC 248	Italy	*Vitis vinifera*	**MN862470**	**MN862463**	**MN862456**	**MN862449**	**MN862477**
	CRCC 251	Italy	*Vitis vinifera*	**MN862471**	**MN862464**	**MN862457**	**MN862450**	**MN862478**
Sei. vitis	MFLUCC 14-0051	Italy	*Vitis vinifera*	KR920362	KR920363	–	–	–
	Napa774	Napa County, USA	*Vitis vinifera*	KY706276	KY706301	KY706251	KY706326	–
	Napa772	Napa County, USA	*Vitis vinifera*	KY706275	KY706300	KY706250	KY706325	–
	Napa782	Napa County, USA	*Vitis vinifera*	KY706278	KY706303	KY706253	KY706328	–
	Napa764	Napa County, USA	*Vitis vinifera*	KY706273	KY706298	KY706248	KY706323	–
	Napa759	Napa County, USA	*Vitis vinifera*	KY706282	KY706307	KY706257	KY706332	–
	VMT2_1	Italy	*Vitis vinifera*	–	LS991528	LS997596	LS999502	–
Sporocadus biseptatus	CBS 110324 HT	Unknown	Unknown	MH554179	MH553956	MH554615	MH554374	MH554853
Spo. cornicola	CBS 143889	Germany	*Cornus sanguinea*	MH554326	MH554121	MH554794	MH554555	MH555029
Spo. incanus	CBS 123003 HT	Spain	*Prunus dulcis*	MH554210	MH553991	MH554659	MH554417	MH554900
Spo. lichenicola	CBS 354.90	Germany	*Fagus sylvatica*	MH554252	MH554035	MH554711	MH554470	MH554948
	CPC 24528	Germany	*Juniperus communis*	MH554332	MH554127	MH554800	MH554562	MH555036
	NBRC 32625; IMI 079706 ET	UK	*Rosa canina*	MH883646	MH883643	MH883645	MH883644	MH883647
Spo. mali	CBS 446.70 HT	The Netherlands	*Malus sylvestris*	MH554261	MH554049	MH554725	MH554484	MH554960
Spo. microcyclus	CBS 424.95 HT	Germany	*Sorbus aria*	MH554258	MH554045	MH554721	MH554480	MH554956

(Continued)

Table 2. (Continued).

Species	Isolate number[a]	Location	Host	GenBank accession number				
				LSU	ITS	*tub*	*tef-1α*	*rpb2*
	CBS 887.68	The Netherlands	*Ribes* sp.	MH554280	MH554068	MH554744	MH554504	MH554981
Spo. multiseptatus	CBS 143899 HT	Serbia	*Viburnum* sp.	MH554343	MH554141	MH554814	MH554576	MH555047
Spo. rosarum	CBS 113832	Sweden	*Rosa canina*	MH554189	MH553970	MH554629	MH554388	MH554864
Spo. rosigena	CBS 116498	Iran	*Vitis vinifera*	MH554200	MH553983	MH554642	MH554401	MH554883
	CBS 129166	Latvia	*Rhododendron*	MH554215	MH553996	MH554665	MH554423	MH554905
	CBS 182.50	The Netherlands	*Pyrus communis*	MH554233	MH554013	MH554689	MH554447	MH554926
	CBS 250.49	The Netherlands	*Rubus fruticosus*	MH554245	MH554023	MH554699	MH554457	MH554934
	CBS 466.96	The Netherlands	*Rubus* sp.	MH554265	MH554052	MH554728	MH554487	MH554965
Spo. rotundatus	CBS 616.83 HT	Canada	*Arceuthobium pussilum*	MH554273	MH554060	MH554737	MH554496	MH554974
Spo. sorbi	CBS 160.25	Unknown	Unknown	MH554229	MH554008	MH554684	MH554442	MH554924
Sporocadus sp. 1	CBS 506.71	Italy	*Euphorbia* sp.	MH554268	MH554055	MH554731	MH554490	MH554968
Spo. trimorphus	CBS 114203 HT	Sweden	*Rosa canina*	MH554196	MH553977	MH554636	MH554395	MH554876
Synnemapestaloides juniperi	CBS 477.77 HT	France	*Juniperus phoenicea*	MH554266	MH554053	MH554729	MH554488	MH554966
Bartalinia bella	CBS 464.61 HT	Brazil	Air	MH554264	MH554051	MH554727	MH554486	MH554964
Bar. robillardoides	CBS 122615	South Africa	*Cupressus lusitanica*	MH554207	MH553989	MH554657	MH554415	MH554897
	CBS 122705 ET	Italy	*Leptoglossus occidentalis*	KJ710438	LT853104	LT853252	LT853202	LT853152
Bar. pini	CBS 143891 HT	Uganda	*Pinus patula*	MH554330	MH554125	MH554797	MH554559	MH555033
	CBS 144141	USA	*Acacia koa*	MH554364	MH554170	MH554843	MH554605	MH555067
Beltrania pseudorhombica	CBS 138003	China	*Pinus tabulaeformis*	KJ869215	MH554124	–	MH554558	MH555032
Broomella vitalbae	HPC 1154	Unknown	Unknown	MH554367	MH554173	MH554846	MH554608	MH555069
Diversimediispora humicola	CBS 302.86 HT	USA	Soil	MH554247	MH554028	MH554705	MH554463	MH554941
Heterotruncatella proteicola	CBS 144020 HT	South Africa	*Protea acaulos*	MH554288	MH554077	MH554751	MH554512	MH554989
Het. quercicola	CBS 143895 HT	USA	*Quercus walshii*	MH554337	MH554135	MH554808	MH554570	MH555041
Het. restionacearum	CBS 118150	South Africa	*Restio filiformis*	MH554203	DQ278914	MH554649	MH554407	MH554889
	CBS 119210 HT	South Africa	*Ischyrolepis cf. gaudichaudiana*	DQ278929	DQ278915	MH554653	MH554411	MH554892
Het. spadicea	CBS 118144	South Africa	*Ischyrolepis* sp.	DQ278926	DQ278921	MH554646	MH554404	MH554886
	CBS 118145 ET	South Africa	*Cannomois virgata*	DQ278927	DQ278912	MH554647	MH554405	MH554887
	CBS 118148	South Africa	*Rhodocoma capensis*	DQ278928	DQ278913	MH554648	MH554406	MH554888
	CPC 17911; CMW 22206	South Africa	*Elegia filacea*	MH554308	MH554098	MH554771	MH554532	MH555012
	CPC 28956	Australia	*Sorghum halepense*	MH554353	MH554157	MH554830	MH554592	MH555056
Hymenopleella austroafricana	CBS 143886 HT	South Africa	*Gleditsia triacanthos*	MH554320	MH554115	MH554788	MH554549	MH555023
	CBS 144026	South Africa	*Bridelia mollis*	MH554322	MH554117	MH554790	MH554551	MH555025
	CBS 144027	Zambia	*Combretum hereroense*	MH554324	MH554119	MH554792	MH554553	MH555027

(Continued)

Table 2. (Continued).

Species	Isolate number[a]	Location	Host	GenBank accession number				
				LSU	ITS	*tub*	*tef-1α*	*rpb2*
Hym. polyseptata	CBS 143887 HT	South Africa	*Combretum* sp.	MH554321	MH554116	MH554789	MH554550	MH555024
Hym. hippophaeicola	CBS 113687	Sweden	*Hippophae rhamnoides*	MH554188	MH553969	MH554628	MH554387	MH554863
	CBS 140410 ET	Austria	*Hippophae rhamnoides*	MH554224	KT949901	MH554678	MH554436	MH554919
Hym. subcylindrica	CBS 164.77	India	*Cocos nucifera*	MH554230	MH554009	MH554685	MH554443	MH554925
	CBS 647.74 HT	India	*Gypsophilla* seeds	MH554275	MH554062	MH554739	MH554498	MH554976
Morinia acaciae	CBS 100230	New Zealand	*Prunus salicina* Omega	MH554174	MH553950	MH554609	MH554368	MH554847
	CBS 137994 HT	France	*Acacia melanoxylon*	MH554221	MH554002	MH554673	MH554431	MH554914
Mor. crini	CBS 143888 HT	South Africa	*Crinum bulbispermum*	MH554323	MH554118	MH554791	MH554552	MH555026
Mor. longiappendiculata	CBS 117603 HT	Spain	*Calluna vulgaris*	MH554202	AY929324	MH554644	AY929316	MH554885
Parabartalinia lateralis	CBS 399.71 HT	South Africa	*Acacia karroo*	MH554256	MH554043	MH554719	MH554478	MH554954
Pseudosarcostroma osyridicola	CBS 103.76 HT	France	*Osyris alba*	MH554177	MH553954	MH554613	MH554372	MH554851
Truncatella angustata	CBS 113.11	Germany	*Picea abies*	MH554185	MH553966	MH554625	MH554384	MH554860
	CBS 135.97	Spain	Decaying bark	MH554220	MH554001	MH554671	MH554429	MH554912
	CBS 165.25	Unknown	*Prunus armeniaca*	MH554231	MH554010	MH554686	MH554444	–
	CBS 231.77 = CBS 296.77	Turkey	*Gossypium* sp.	MH554243	MH554021	MH554697	MH554455	MH554932
	CBS 338.32	The Netherlands	*Lupinus* sp.	MH554250	MH554033	MH554709	MH554467	MH554945
	CBS 398.71	Turkey	Soil	MH554255	MH554042	MH554718	MH554477	MH554953
	CBS 144025 NT	France	*Vitis vinifera* Prunelard	MH554318	MH554112	MH554785	MH554546	MH555021
	CBS 449.51	Unknown	*Salix* sp. or *Thuja* sp.	MH554262	MH554050	MH554726	MH554485	MH554961
	CBS 938.70	The Netherlands	*Prunus laurocerasus*	MH554281	MH554070	MH554746	MH554506	MH554982
	CPC 21366	France	*Vitis vinifera* Prunelard	MH554319	MH554113	MH554786	MH554547	MH555022
	CBS 208.80	The Netherlands	Food	MH554239	MH554020	MH554696	MH554454	–
	CBS 443.54	UK	*Picea abies*	MH554260	MH554048	MH554724	MH554483	MH554959
	CPC 21354	France	*Vitis vinifera* Prunelard	MH554317	MH554111	MH554784	MH554545	MH555020
	CBS 642.97	Switzerland	*Heterodera carotae* cyst egg mass, on *Daucus carota*	MH554274	MH554061	MH554738	MH554497	MH554975
	CBS 564.76	Switzerland	*Pyrus malus*	MH554271	MH554057	MH554733	MH554492	MH554970
	CRCC 147	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 165	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 188	Italy	*Vitis vinifera*	**MN862441**	**MN862439**	**MN862437**	**MN862435**	**MN862443**
	CRCC 189	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 195	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 199	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 201	Italy	*Vitis vinifera*	–	–	–	–	–

(Continued)

Table 2. (Continued).

Species	Isolate number[a]	Location	Host	GenBank accession number				
				LSU	ITS	*tub*	*tef-1α*	*rpb2*
	CRCC 240	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 241	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 243	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 245	Italy	*Vitis vinifera*	**MN862442**	**MN862440**	**MN862438**	**MN862436**	**MN862444**
Phlogicylindrium eucalypti	CBS 120080 HT	*Australia*	*Eucalyptus globulus*	DQ923534	NR_132813	MH704633	MH704607	MH554893
Robillarda africana	CBS 122.75 HT	South Africa	Unknown	KR873281	KR873253	MH554656	MH554414	MH554896
Rob. australiana	CBS 143882 HT	Australia	Unknown	MH554301	MH554091	MH554764	MH554525	MH555005
Rob. terrae	CBS 587.71 HT	India	Soil	KJ710459	KJ710484	MH554734	MH554493	MH554971
Rob. roystoneae	CBS 115445 HT	Hong Kong	*Roystonea regia*	KR873282	KR873254	KR873317	KR873310	MH554880
Strickeria kochii	CBS 140411 ET	Austria	*Robinia pseudoacacia*	KT949918	NR_154423	MH554679	MH554437	MH554920
Cad. gregata	ATCC11073 HT	Unknown	Soybean root	–	U66731	Mf677920	Mf979586	–
Cad. helianthii	CBS 144752 HT	Ukraine	*Helianthus annuus*	–	MF962601	MH733391	MH719029	–
Cad. interclivum	BAP37	Banff, Canada	*Picea glauca,* root	–	MF677930	MF677919	MF979585	–
	BAP33	Banff, Canada	*Picea glauca*, root	–	MF677929	MF677918	MF979584	–
	CBS143323 HT	Banff, Canada	*Carex sprengelii*, root	–	MF677928	MF677917	MF979583	–
Cad. luteo-olivacea	CBS 141.41 HT	Sweden	Unknown	–	AY249066	KM497133	KM497089	–
	A19	California, USA	*Vitis viniferae*	–	KM497038	KM497119	KM497075	–
	A41	California, USA	*Vitis vinifera* 'Chardonnay'	–	KM497039	KM497120	KM497076	–
	A42	California, USA	*Vitis vinifera* 'Chardonnay'	–	KM497040	KM497121	KM497077	–
	U5	California, USA	*Vitis vinifera* 'Sangiovese'	–	KM497041	KM497122	KM497078	–
	U7	California, USA	*Olea europa*	–	KM497044	KM497125	KM497081	–
	U8	California, USA	*Vitis vinifera* 'Semillon'	–	KM497042	KM497123	KM497079	–
	U17	California, USA	*Vitis vinifera* 'Chardonnay'	–	KM497043	KM497124	KM497080	–
	U21	California, USA	*Vitis vinifera*	–	KM497045	KM497126	KM497082	–
	U22	California, USA	*Vitis vinifera* 'Chardonnay'	–	KM497046	KM497127	KM497083	–
	U53	California, USA	*Vitis vinifera* 'Chardonnay'	–	KM497047	KM497128	KM497084	–
	U56	California, USA	*Vitis vinifera* 'Syrah'	–	KM497048	KM497129	KM497085	–
	CRCC 11B	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 113A	Italy	*Vitis vinifera*	–	**MN871929**	**MN871925**	**MN871927**	–
	CRCC 122	Italy	*Vitis vinifera*	–	**MN871930**	**MN871926**	**MN871928**	–
	CRCC 131	Italy	*Vitis vinifera*	–	–	–	–	–
Cad. malorum	CBS 165.42 HT	The Netherlands	*Amblystoma mexicanum*	–	AY249059	KM497134	KM497090	–
Cad. melinii	CBS 268.33 HT	Unknown	Unknown	–	AY249072	KM497132	KM497088	–
	U11	California, USA	*Vitis vinifera* 'Sangiovese'	–	KM497032	KM497113	KM497069	–

(Continued)

Table 2. (Continued).

Species	Isolate number[a]	Location	Host	GenBank accession number				
				LSU	ITS	*tub*	*tef-1α*	*rpb2*
	ONC1	Ontario, Canada	*Vitis vinifera* 'Cabernet Franc'	–	KM497033	KM497114	KM497070	–
Cad. meredithiae	CBS143322 HT	Banff, Canada	*Carexsprengelii,* root	–	MF677925	MF677914	MF979580	–
	BAP6	Banff, Canada	*Picea glauca*, root	–	MF677926	MF677915	MF979581	–
	BAP13	Banff, Canada	*Picea glauca*, root	–	MF677927	MF677916	MF979582	–
Cad. novi-eboraci	NYC14 HT	New York, USA	*Vitis labruscana* 'Concord'	–	KM497037	KM497118	KM497074	–
	NYC2	New York, USA	*Vitis labruscana* 'Concord'	–	KM497034	KM497115	KM497071	–
	NYC13	New York, USA	*Vitis vulpina*	–	KM497036	KM497117	KM497073	–
	NYC1	New York, USA	*Vitis vinifera* 'Cabernet' Sauvignon	–	KM497035	KM497116	KM497072	–
Cad. orchidicola	UAMH8152	Alberta, Canada	Northern green orchid, root	–	AF214576	MF677921	MF979587	–
Cad. orientoamericana	NHC1 HT	New Hampshire, USA	*Vitis* hybrid 'Niagara'	–	KM497018	KM497099	KM497055	–
	CTC1	Connecticut, USA	*Vitis vinifera* 'Chardonnay'	–	KM497012	KM497093	KM497049	–
Cad. spadicis	RIC1	Rhode Island, USA	*Vitis vinifera* 'Cabernet' Sauvignon	–	KM497029	KM497110	KM497066	–
	RIC3	Rhode Island, USA	*Vitis hybrid* 'Vidal'	–	KM497030	KM497111	KM497067	–
	QCC1	Quebec, Canada	*Vitis vinifera* 'Gamay'	–	KM497031	KM497112	KM497068	–
	CBS 111743 HT	Italy	*Actinidia chinensis*	–	DQ404351	KM497136	KM497091	–
Cad. viticola	CBS 139517 HT	Spain	*Vitis vinifera* 'Syrah'	–	HQ661096	–	HQ661081	–
	Cme-1	Spain	*Vitis vinifera* 'Syrah'	–	HQ661096	–	HQ661081	–
	Cme-3	Spain	*Vitis vinifera* 'Syrah'	–	HQ661098	–	HQ661083	–
Hyaloscypha finlandica	CBS 444.86 HT	Finland	Unknown	–	AF486119	KM497130	KM497086	–

[a] **ATCC**: American Type Culture Collection, Virginia, USA; BCC: BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Khlong Luang, Pathumthani, Thailand. **CBS**: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. **CMW**: Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. **CPC**: Culture collection of Pedro Crous, housed at the Westerdijk Institute. **CRCC**: Carlucci and Raimondo Culture Collection, housed at Dept. SAFE of University of Foggia. **HPC**: Herbarium of Pedro Crous, housed at the Westerdijk Institute. **IMI**: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, United Kingdom. **MFLU(CC)**: Mae Fah Luang University Culture Collection. **NBRC**: Biological Resource Center.
[b] Status: status of the strains. ET: ex-epitype. NT: ex-neotype. HT: ex-Holotype.
[c] Strain numbers and newly generated sequences are indicated in bold font.

Table 3. Isolate identification numbers, locations, hosts and GenBank accession numbers of the strains of *Colletotrichum* spp. used in the multigenic analyses.

Species	Isolate number[a]	Location	Host	GenBank accession number				
				ITS	*gapdh*	*chs-1*	*act*	*tub*
C. acerbum	CBS 128530 HT[b]	New Zealand	*Malus domestica*, bitter rot of fruit	JQ948459	JQ948790	JQ949120	JQ949780	JQ950110
C. acutatum	CBS 112996 HT	Australia	*Carica papaya*	JQ005776	JQ948677	JQ005797	JQ005839	JQ005860
C. australe	CBS 116478 HT	South Africa	*Trachycarpus fortunei*	JQ948455	JQ948786	JQ949116	JQ949776	JQ950106
C. brisbanense	CBS 292.67 HT	Australia	*Capsicum annuum*	JQ948291	JQ948621	JQ948952	JQ949612	JQ949942
C. chrysanthemi	IMI 364540, CPC 18930	China	*Chrysanthemum coronarium,* leaf spot	JQ948273	JQ948603	JQ948934	JQ949594	JQ949924
C. cosmi	CBS 853.73 HT	The Netherlands	*Cosmos* sp., seed	JQ948274	JQ948604	JQ948935	JQ949595	JQ949925
C. costaricense	CBS 330.75 HT	Costa Rica	*Coffea arabica,* cv. 'Typica', berry	JQ948180	JQ948510	JQ948841	JQ949501	JQ949831
C. cuscutae	IMI 304802, CPC 18873 HT	Dominica	*Cuscuta* sp.	JQ948195	JQ948525	JQ948856	JQ949516	JQ949846
C. fioriniae	CBS 128517 HT	USA	*Fiorinia externa* (elongate hemlock scale, insect)	JQ948292	JQ948622	JQ948953	JQ949613	JQ949943
	CBS 125396	USA	*Malus domestica*, fruit lesion	JQ948299	JQ948629	JQ948960	JQ949620	JQ949950
	CBS 124958	USA	*Pyrus* sp., fruit rot	JQ948306	JQ948636	JQ948967	JQ949627	JQ949957
	CBS 126526	The Netherlands	*Primula* sp., leaf spots	JQ948323	JQ948653	JQ948984	JQ949644	JQ949974
	IMI 324996, CPC 18880	USA	*Malus pumila*	JQ948301	JQ948631	JQ948962	JQ949622	JQ949952
	CRCC 104[c]	Italy	*Vitis vinifera*	**MN871933**	**MN871939**	**MN871937**	**MN871931**	**MN871935**
	CRCC 140	Italy	*Vitis vinifera*	**MN871934**	**MN871940**	**MN871938**	**MN871932**	**MN871936**
	CRCC 144	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 154	Italy	*Vitis vinifera*	–	–	–	–	–
	CRCC 160	Italy	*Vitis vinifera*	–	–	–	–	–
C. godetiae	CBS 133.44 HT	Denmark	*Clarkia hybrida*, cv. 'Kelvon Glory', seed	JQ948407	JQ948738	JQ949068	JQ949728	JQ950058
C. guajavae	IMI 350839, CPC 18893 HT	India	*Psidium guajava*, fruit	JQ948270	JQ948600	JQ948931	JQ949591	JQ949921
C. indonesiense	CBS 127551 HT	Indonesia	*Eucalyptus* sp.	JQ948288	JQ948618	JQ948949	JQ949609	JQ949939
C. johnstonii	CBS 128532 HT	New Zealand	*Solanum lycopersicum*, fruit rot	JQ948444	JQ948775	JQ949105	JQ949765	JQ950095
C. kinghornii	CBS 198.35 HT	UK	*Phormium* sp.	JQ948454	JQ948785	JQ949115	JQ949775	JQ950105
C. laticiphilum	CBS 112989 HT	India	*Hevea brasiliensis*	JQ948289	JQ948619	JQ948950	JQ949610	JQ949940
C. lauri	MFLUCC 17-0205 HT	Italy	*Laurus nobilis*	KY514347	KY514344	KY514341	KY514338	KY514350
C. limetticola	CBS 114.14 HT	USA, Florida	*Citrus aurantifolia*, young twig	JQ948193	JQ948523	JQ948854	JQ949514	JQ949844
C. lupini	CBS 109225 HT	Ukraine	*Lupinus albus*	JQ948155	JQ948485	JQ948816	JQ949476	JQ949806
C. melonis	CBS 159.84 HT	Brazil	*Cucumis melo*, peel of fruit	JQ948194	JQ948524	JQ948855	JQ949515	JQ949845
C. nymphaeae	CBS 515.78 HT	The Netherlands	*Nymphaea alba*, leaf spot	JQ948197	JQ948527	JQ948858	JQ949518	JQ949848
C. orchidophilum	CBS 632.80 HT	USA	*Ascocenda* sp.	JQ948152	JQ948482	JQ948813	JQ949473	JQ949803
C. paxtonii	IMI 165753, CPC 18868 HT	Saint Lucia	*Musa* sp.	JQ948285	JQ948615	JQ948946	JQ949606	JQ949936
C. phormii	CBS 118194 HT	Germany	*Phormium* sp.	JQ948446	JQ948777	JQ949107	JQ949767	JQ950097
C. pyricola	CBS 128531 HT	New Zealand	*Pyrus communis*, fruit rot	JQ948445	JQ948776	JQ949106	JQ949766	JQ950096

(Continued)

Table 3. (Continued).

Species	Isolate number[a]	Location	Host	GenBank accession number				
				ITS	*gapdh*	*chs-1*	*act*	*tub*
C. rhombiforme	CBS 129953 HT	Portugal	*Olea europaea*	JQ948457	JQ948788	JQ949118	JQ949778	JQ950108
C. salicis	CBS 607.94 HT	The Netherlands	*Salix* sp., leaf, spot	JQ948460	JQ948791	JQ949121	JQ949781	JQ950111
C. scovillei	CBS 126529 HT	Indonesia	*Capsicum* sp.	JQ948267	JQ948597	JQ948928	JQ949588	JQ949918
C. simmondsii	CBS 122122 HT	Australia	*Carica papaya*, fruit	JQ948276	JQ948606	JQ948937	JQ949597	JQ949927
C. sloanei	IMI 364297, CPC 18929 HT	Malaysia	*Theobroma cacao*, leaf	JQ948287	JQ948617	JQ948948	JQ949608	JQ949938
C. tamarilloi	CBS 129814 HT	Colombia	*Solanum betaceum*, fruit, anthracnose	JQ948184	JQ948514	JQ948845	JQ949505	JQ949835
C. walleri	CBS 125472 HT	Vietnam	*Coffea* sp., leaf tissue	JQ948275	JQ948605	JQ948936	JQ949596	JQ949926
C. paranaense	CBS 134729 HT	–	–	KC204992	KC205026	KC205043	KC205077	KC205060

[a] **CBS**: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. **CPC**: Culture collection of Pedro Crous, housed at the Westerdijk Institute. **CRCC**: Carlucci and Raimondo Culture Collection, housed at Dept. SAFE of University of Foggia. **IMI**: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, United Kingdom. **MFLU(CC)**: Mae Fah Luang University Culture Collection.

[b] Status: status of the strains. ET: ex-epitype. NT: ex-neotype. HT: ex-Holotype.

[c] Strain numbers and newly generated sequences are indicated in bold font.

Agar plugs (diam. 0.5 cm) were taken from 7-d-old fungal cultures grown on water agar at 23±2°C, and the plugs were placed under the cane bark. Wounds were then wrapped with wet sterile cotton wool and sealed with Parafilm. The experimental control canes were inoculated with sterile agar plugs. Each experiment included 18 replicates per treatment.

The canes were examined at 240 d after inoculation, and the lengths of any visible necrotic wood lesions, after removal of the bark, were measured and subjected to mycological analyses. Ten tissue pieces from each inoculated cane were placed on MEA supplemented with streptomycin sulphate at 300 mg L^{-1}, and incubated at 23±2°C in the dark. Resulting fungal colonies were identified to fulfil the Koch's postulates, and the proportions of re-isolation (%) were calculated.

Shapiro-Wilk (W) tests were used to determine whether the data obtained followed normal distributions. Homogeneity of the variances of the dataset was assessed using Levene tests. Statistical analyses were performed using Statistica version 6 (StatSoft). Factorial ANOVA analyses were performed to define the significance of any differences in mean lesion lengths caused by the isolates of each fungal species and the different fungal species, and to detect any interactions between these factors (i.e., isolate × fungal species). One-way ANOVA analyses were performed to evaluate statistically significant differences in the mean 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

Fungal isolates

The data related to grapevine trunk disease incidence, recorded during surveys carried out through 10 y in vineyards of different cultivars, in the Apulia and Molise regions, are summarized in Table 1. Isolation frequencies of the fungal taxa isolated from symptomatic grapevine samples affected by GTDs and collected during the 10 y are shown in Figure 1.

The Botryosphaeriaceae (IF = 29.3%) and *Phaeoacremonium* spp. (IF = 19.3%) were the most frequently isolated fungi. *Phaeomoniella chlamydospora* (IF = 5.0%) and *Pleurostoma richardsiae* (IF = 6.4%) were responsible for vascular and subcortical streaking discolouration. The fungal taxa considered as less-known, including *Seimatosporium vitis-vinifera*,

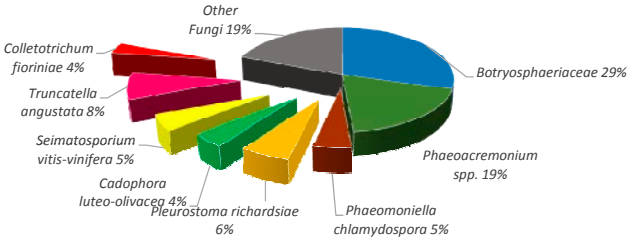

Figure 1. Isolation frequencies of fungal species obtained from symptomatic grapevines during a 10 year survey in the Apulia and Molise regions of Italy.

Truncatella angustata, *Cadophora luteo-olivacea* and *Colletotrichum fioriniae*, were isolated at IFs of 3.6% to 7.9%. The other group denoted here as 'other fungi' had IF of 19.0%, and included several fungal species, including *Alternaria* spp., *Aspergillus* spp., *Epicoccum nigrum*, *Fusarium* spp., *Penicillium* spp. and *Phoma*-like. These were not considered to be the causes of the disease symptoms observed, because these fungi are known common saprophytes.

Molecular identification of representative isolated fungi

Based on the keys, descriptions and sequence of Phillips *et al.* (2013), Mostert *et al.* (2006), Essakhi *et al.* (2008), Raimondo *et al.* (2014), Crous and Gams (2000) and Carlucci *et al.* (2015b), the 84 isolates selected as representative MSP-PCR clades were identified as follows (number of isolates): *Botryosphaeria dothidea* (four); *Diplodia corticola* (one); *D. mutila* (three); *D. seriata* (15); *Lasiodiplodia citricola* (eight); *L. theobromae* (five); *Neofusicoccum parvum* (five); *Phaeoacremonium iranium* (five); *P. italicum* (11); *P. minimum* (six); *P. scolyti* (three); *P. sicilianum* (two); *Phaeomoniella chlamydospora* (seven); and *Pleurostoma richardsiae* (nine) (data not shown).

The data obtained from the phylogenetic studies carried out on the 29 strains that were considered less well-known pathogens, and were representative of MSP-PCR clades related to the *Seimatosporium*, *Truncatella*, *Cadophora* and *Colletotrichum*, are summarized below.

The LSU, ITS, β-tub*ulin, tef-1α* and *rpb2* sequences were generated for seven *Seimatosporium* strains selected from the MSP-PCR profiles, and were aligned with 41 sequences retrieved from GenBank (Table 2). The dataset consisted of 48 taxa, which included the outgroup taxa *Synnemapestaloides juniperi* and *Discosia artocreas*. After alignment and exclusion of incomplete portions at either end, the dataset consisted of 3,344 characters (including alignment gaps), of which 2,276 were constant, while 279 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 789 parsimony-informative characters resulted in the 100 most-parsimonious trees (TL = 2,274; CI = 0.576; RI = 0.826; RC = 0.476; HI = 0.424). The maximum likelihood analysis produced a tree with similar topology (TreeBASE S25531; Figure 2). All of the *Seimatosporium* strains obtained clustered as a single clade with the type sequences of *Sei. vitis-viniferae* (CBS 123004) and *Sei. vitis* (MFLUCC 14-0051) (Figure 2). For the type strain of *Sei. vitis*, only the LSU and ITS sequences were available in GenBank, which were identical to those of *Sei. vitis-viniferae*. However, the isolates analysed here showed β-tubulin, *tef-1α* and *rpb2* sequences identical to those of *Sei. vitis-vinifera*, and therefore the morphological features (conidium dimensions and basal appendages) were used to discriminate between these two species, according to Liu *et al.* (2019).

The LSU, ITS, β-tub*ulin, tef-1α* and *rpb2* sequences were generated for 11 *Truncatella* isolates selected from the MSP-PCR profiles, which were aligned with 53 sequences retrieved from GenBank (Table 2). The dataset consisted of 64 taxa, which included two outgroup taxa, *Beltrania pseudorhombica* and *Phlogicylindrium eucalypti*. After alignment and exclusion of incomplete portions at either end, the dataset consisted of 3,983 characters (including alignment gaps), of which 1,124 were constant and 511 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 2,348 parsimony-informative characters resulted in 35 most-parsimonious trees (TL = 10,415; CI = 0.522; RI = 0.798; RC = 0.417; HI = 0.478). Maximum likelihood analysis produced a tree with similar topology (TreeBASE S25532; Figure 3). All of the *Truncatella* strains obtained in this study clustered with the *ex-neotype* sequences of *T. angustata* (*Stilbospora angustata* CBS 114025) (Figure 3).

The ITS, *tef-1α* and β-tubulin sequences generated for six *Cadophora* strains selected from the MSP-PCR profiles were aligned with 44 sequences retrieved from GenBank (Table 2). The dataset consisted of 60 taxa, which included the outgroup taxon, *Hyaloscypha finlandica*. After alignment and exclusion of incomplete portions at either end, the dataset consisted of 1,613 characters (including alignment gaps), of which 952 were constant, while 167 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 494 parsimony-informative characters resulted in 100 most-parsimonious trees (TL = 1,255; CI = 0.735; RI = 0.932; RC = 0.686; HI = 0.265). Maximum likelihood analysis produced a tree with similar topology (TreeBASE S25533; Figure 4). All of the *Cadophora* isolates obtained in this study clustered with the type sequences of *Cadophora luteo-olivacea* (CBS 141.41) (Figure 4).

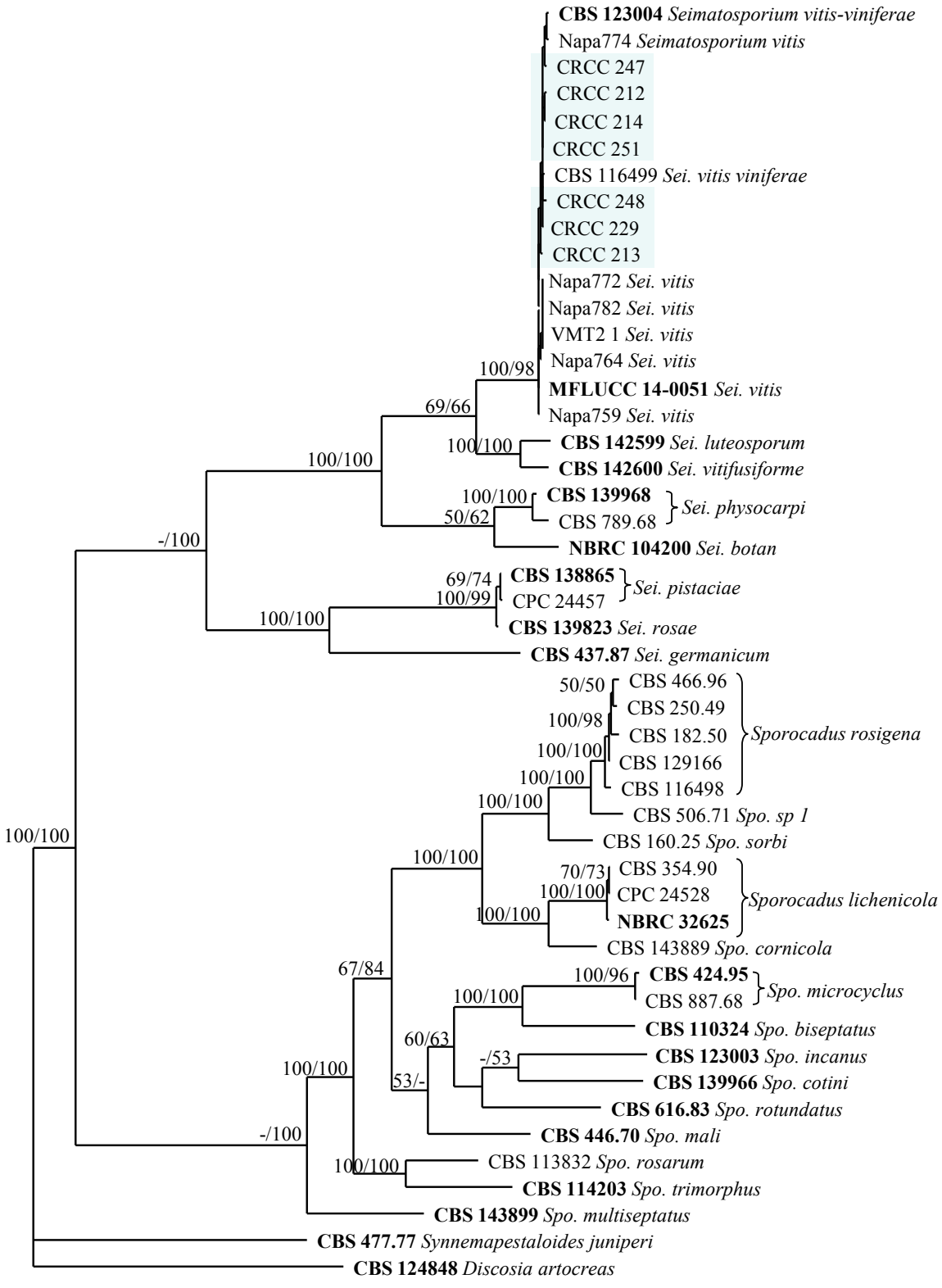

Figure 2. One of the most parsimonious trees obtained from the combined alignment of the LSU, ITS, *tub*, *tef-1*α and *rpb2* sequence datasets of *Seimatosporium* isolates, with bootstrap support values from maximum parsimony/maximum likelihood analyses. Isolates obtained in this study are indicated by blue rectangles. *Ex-type* sequences are given in bold. *Synnemapestaloides juniperi* and *Discosia artocreas* were used as outgroups.

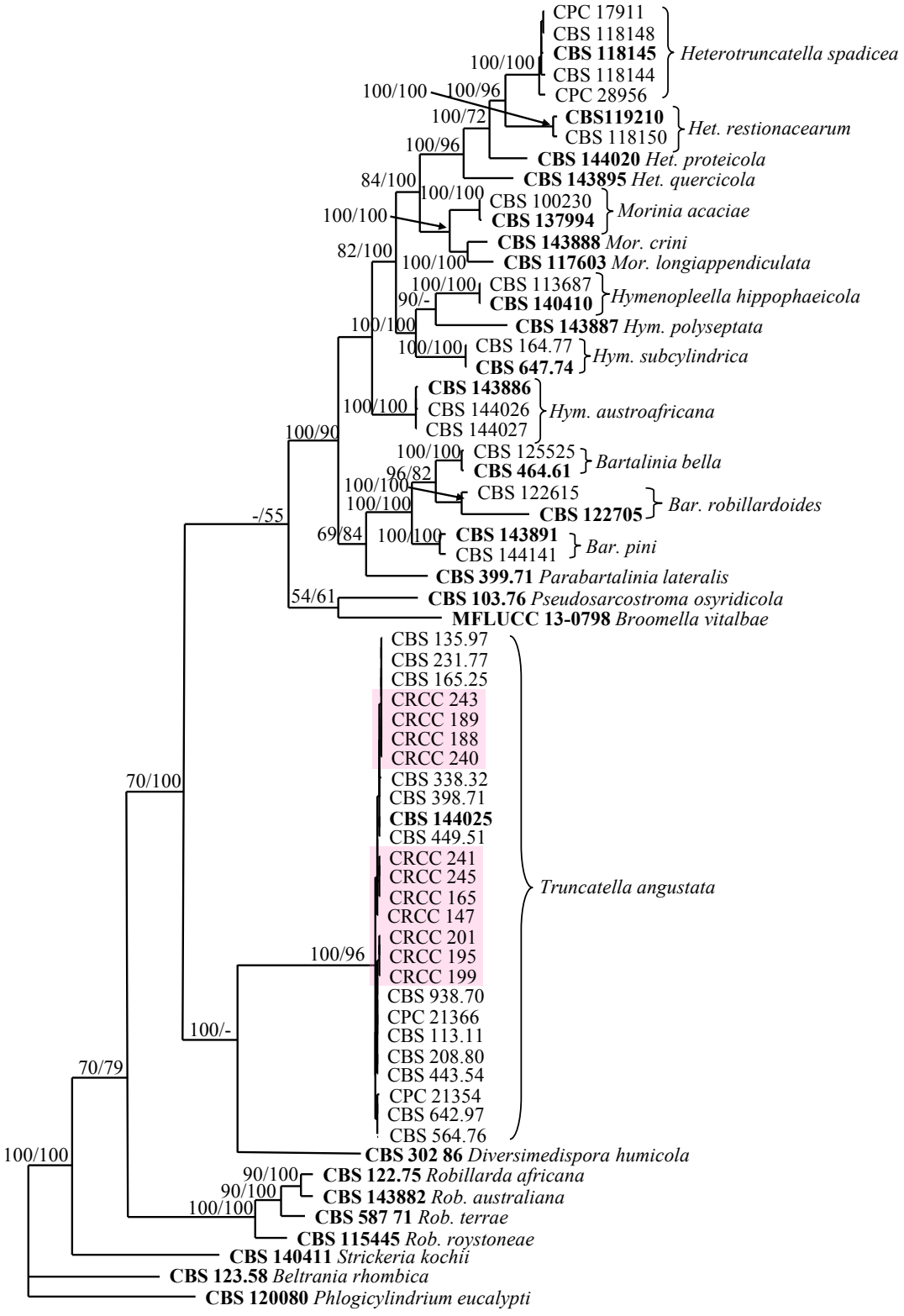

Figure 3. One of the most parsimonious trees obtained from combined alignment of the LSU, ITS, *tub*, *tef-1*α and *rpb2* sequence datasets of *Truncatella* isolates, with bootstrap support values from maximum parsimony/maximum likelihood analyses. Isolates obtained in this study are indicated by pink rectangles. *Ex-type* sequences are indicated in bold. *Beltrania pseudorhombica* and *Phlogicylindrium eucalypti* were used as outgroups.

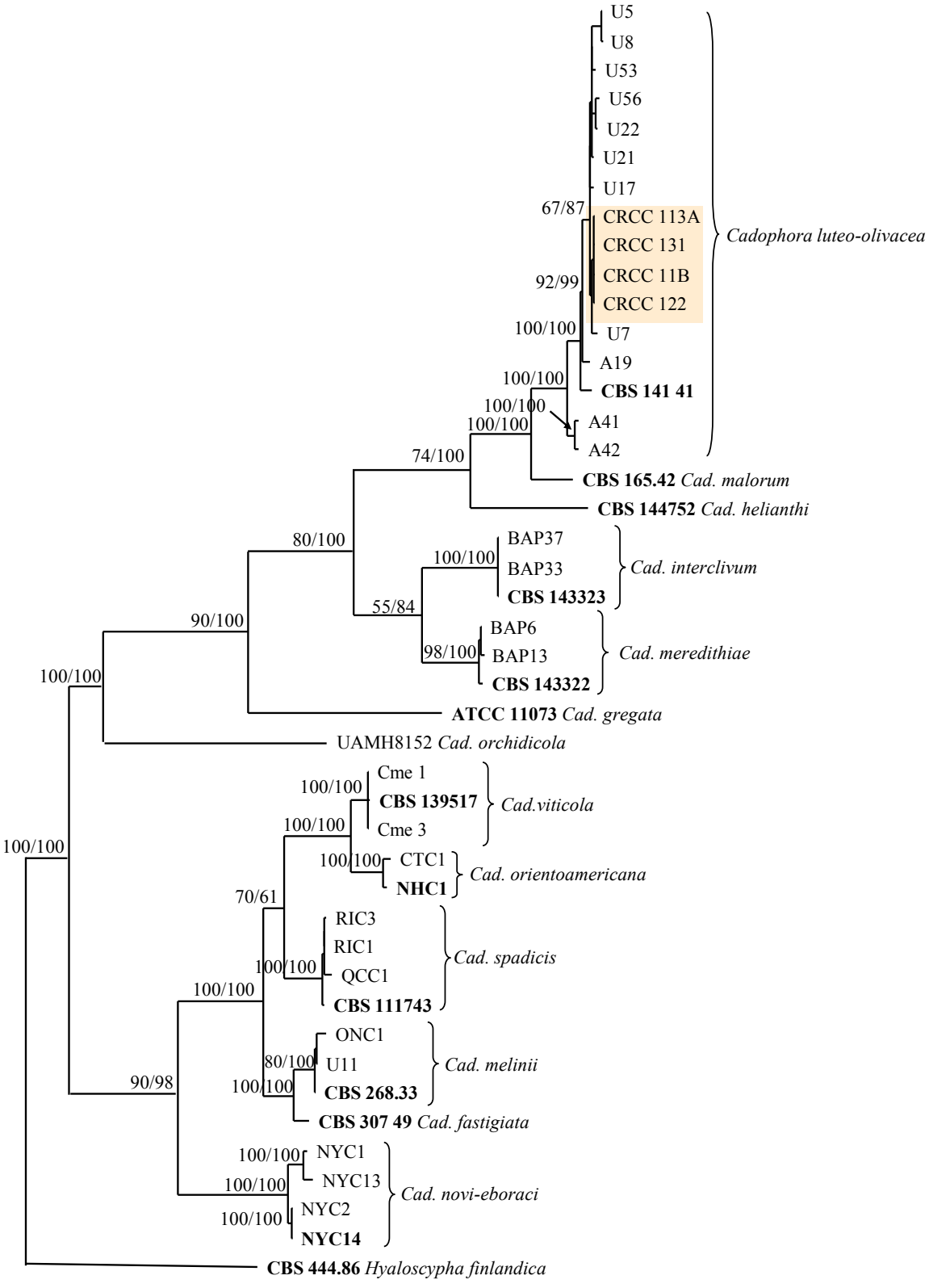

Figure 4. One of the most parsimonious trees obtained from combined ITS, *tef-1*α and *tub* sequence datasets of *Cadophora* isolates, with bootstrap support values from maximum parsimony/maximum likelihood analyses. Isolates obtained in this study are indicated by an orange rectangle. *Ex-type* sequences are indicated in bold. *Hyaloscypha finlandica* was used as outgroup.

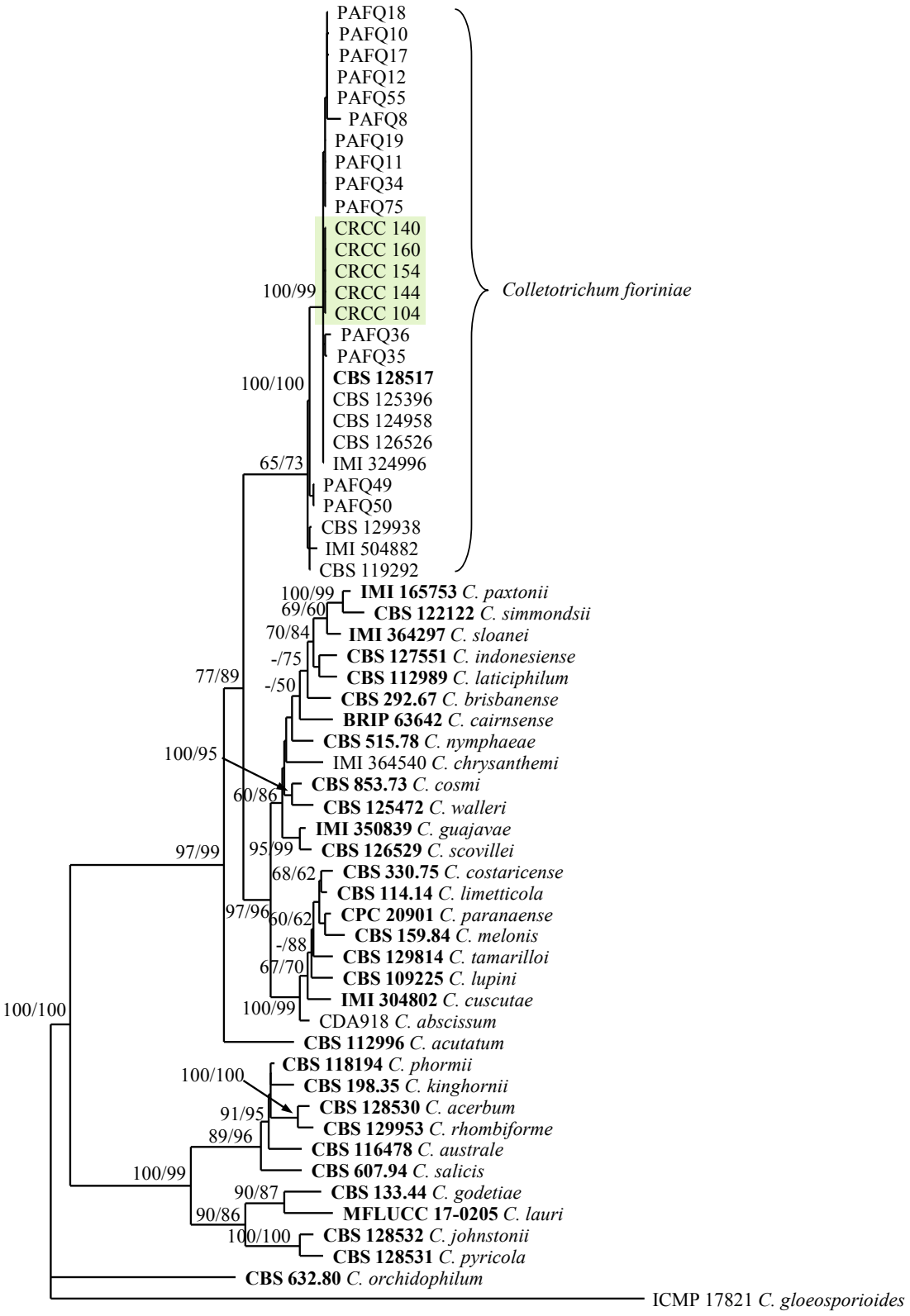

Figure 5. One of the most parsimonious trees obtained from the combined alignment of the ITS, *gapdh*, *chs-1*, *act* and *tub* sequence datasets of *Colletotrichum* isolates, with bootstrap support values from maximum parsimony/maximum likelihood analyses. Isolates obtained in this study are indicated by green rectangles. *Ex-type* sequences are indicated in bold. *Colletotrichum orchidophilum* and *C. gloeosporioides* were used as outgroups.

The ITS, β-tubulin, *act*, *gapdh* and *chs*-1 sequences generated for five *Colletotrichum* strains selected from the MSP-PCR profiles were aligned with 44 sequences retrieved from GenBank (Table 3). The dataset consisted of 61 taxa, which included the two outgroup taxa, *Colletotrichum gloeosporioides* and *C. orchidophilum*). After alignment and exclusion of incomplete portions at either end, the dataset consisted of 1,879 characters (including alignment gaps), of which 1,304 were constant, while 339 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 236 parsimony-informative characters resulted in 100 most-parsimonious trees (TL = 977; CI = 0.736; RI = 0.872; RC = 0.642; HI = 0.264). Maximum likelihood analysis produced a tree with similar topology (TreeBASE S25534; Figure 5). The *Colletotrichum* isolates obtained in this study clustered in the clade of *Colletotrichum fioriniae* with the *holotype* sequences of *C. fioriniae* (CBS 128517) (Figure 5).

Morphological characterisation of representative isolates

Colonies of the *Seimatosporium* isolates on MEA had entire edges, with brown to purplish grey mycelia, and reached mean diameter of 6.9 cm after 21 d at 23°C. The conidiomata were black and immersed. Conidia were fusoid, 3(-6)-septate, with measurements of 13.8-24.0 × 4.1-5.9 µm. They had truncated basal cells 2.3-3.8 µm long, similar to that of median cells. The median cells (2 -4) were each 3.3-5.1 µm long, and the conidium apical cells were 1.3-4.2 µm long. The majority of conidia each had a single unbranched appendage at both ends (apical appendage, 3.9-11.5 µm long; basal appendage, 3.6-10.3 µm long). On the basis of these culture and morphological features, all of the *Seimatosporium* strains had characteristics similar to those reported by Liu *et al.* (2019) for *Sei. vitis-viniferae*.

Colonies of the *Truncatella* isolates on MEA had entire edges, with white to pale grey mycelia, and reached mean diameter of 7.1 cm after 21 d at 23°C. Conidiomata were black, gregarious, semi-immersed and stromatic. Conidia were fusoid, occasionally slightly curved, mostly 3-septate, and not constricted at the septa (mean, 18.3 ± 1.69 × 6.8 ± 0.50 µm). The basal cells of the conidia had truncate bases, were hyaline to pale brown, 1.3-3.6 µm long, each with two pale to mid-brown doliiform median cells which were pale to mid-brown, each 5.3-7.7 µm long, and the apical cells were conic, hyaline, and 1.9-4.9 µm long. Each conidium had 2 to 4 apical appendages, which were centric, flexuous and branched, 0.6-22 µm long, and were without basal appendages. On the basis of these culture and morphological features, all of the *Truncatella* isolates studied had characteristics similar to those reported by Liu *et al.* (2019) for *Truncatella angustata*, which confirmed the data obtained in the molecular analysis.

Colonies of the *Cadophora* isolates on MEA had entire edges, and the mycelia were white to olivaceous green to grey. Mean colony diameter reached 4.5 cm after 21 d at 23°C. The conidiophores were mostly short, usually unbranched, up to 7-septate and measuring (-11.5) 26-63.90 × 1.78-1.94 (-2.5) µm. The conidiogenous cells were monophialidic, hyaline, terminal or lateral, mostly cylindrical, sometimes elongated ampulliform and attenuated at the base or navicular and tapering towards the apex. These cells measured 7.9-27.3 × 1.4-3.1 µm. The conidia were hyaline, mostly biguttulate, ovoid and aseptate, and measured 3.7-7.3 × 2.1-3.6 µm. On the basis of these culture and morphological features, all of the *Cadophora* strains studied had characteristics similar to those reported by Gramaje *et al.* (2011) and Travadon *et al.* (2015) for *Cadophora luteo-olivacea*, which confirmed the data obtained in the molecular analysis.

Colonies of the *Colletotrichum* isolates on MEA had entire edges, with aerial cottony pink to vinaceous mycelia. Mean colony diameter reached 4.5 cm after 21 d at 23°C. The conidiomata were sparse, with masses of orange conidia. Conidiophores were hyaline to pale brown, septate, branched, and up to 33 µm long. Conidiogenous cells were hyaline to pale brown, cylindrical to elongate ampulliform, monophialic and measured 3.8-11.9 × 2.2-3.9 µm. Conidia were elliptical, hyaline, with both ends acute, and measured 8.0-15.3 × 3.2-4.6 µm. On the basis of these culture and morphological features, all of the *Colletotrichum* isolates studied had characteristics similar to those reported by Damm *et al.* (2012) for *C. fioriniae*, which confirmed the data obtained in the molecular analysis.

Pathogenicity tests

According to Shapiro-Wilk tests, the data from the pathogenicity tests carried out on the grapevine cultivars 'Nero di Troia' and 'Bombino bianco' 240 d after, inoculations followed a normal distribution, with W values, respectively for the cultivars, of 0.96 ($P < 0.01$) and 0.97 ($P < 0.01$). The Levene tests determined for the two cultivars showed that the homogeneity of the variance was significant for 'Nero di Troia' ($F = 7.04$; $P < 0.01$) and 'Bombino bianco' ($F = 4.93$; $P < 0.01$). Factorial ANOVA demonstrated that significant differences in pathogenicity were detected among the fungal species inoculated on both 'Nero di Troia' ($F = 44.5$; $P < 0.01$) and 'Bombino bianco' ($F = 83.40$; $P < 0.01$). There were no significant

Table 4. Mean lesion lengths from the pathogenicity assays carried out for isolates of seven fungal species on two grapevine cultivars (one-way ANOVA).

Cultivar	Fungal species	Length of brown wood discolouration (cm)			Re-isolation (%)
		Mean	SD	Min–Max[a]	
'Nero di Troia'	Control	0.63 A	0.24	0.30–1.10	0.00
	Colletotrichum fiorinae	8.83 B	2.57	5.00–16.00	73.33
	Cadophora luteo-olivacaea	12.97 C	5.01	11.00–34.00	88.33
	Seimatosporium vitis-vinifera	16.98 D	3.13	13.00–24.70	80.00
	Truncatella angustata	18.01 D	7.31	3.40–29.50	91.67
	Pleurostoma richardsiae	18.53 DE	3.87	6.00–19.90	76.67
	Phaeoacremonium italicum	19.25 DE	3.41	13.60–27.00	93.33
	Lasiodiplodia citricola	21.77 E	4.59	12.00–27.00	86.67
'Bombino bianco'	Control	0.62 A	0.29	0.30–1.10	0.00
	Colletotrichum fiorinae	8.43 B	2.34	4.40–12.40	78.33
	Cadophora luteo-olivacaea	14.13 C	7.08	3.30–38.20	91.67
	Truncatella angustata	15.71 CD	3.20	9.50–19.50	80.00
	Phaeoacremonium italicum	18.41 DE	4.47	14.00–29.60	86.67
	Pleurostoma richardsiae	20.11 E	5.47	9.20–30.50	78.33
	Seimatosporium vitis-vinifera	23.63 F	3.23	13.00–23.00	91.67
	Lasiodiplodia citricola	29.20 G	2.00	24.40–32.00	95.00

[a] Minimum and maximum values detected (18 observations).
Data within each cultivar followed by different capital letters within the column are significantly different ($P < 0.01$; Fischer's tests).

differences in aggressiveness among the isolates of each fungal species used in the artificial inoculations of 'Nero di Troia' (F = 0.12; $P = 0.89$) or 'Bombino bianco' (F = 0.99; $P = 0.37$).

The mean lengths of vascular discolouration caused by the inoculated, fungal species used in the pathogenicity tests, and examined for one-way analysis of variance, are reported in the Table 4. All of the fungi produced brown wood discolourations on canes of both grapevine cultivars. The most aggressive species was *Lasiodiplodia citricola* towards 'Nero di Troia' and 'Bombino bianco', which produced the longest brown wood discolourations (respective mean lengths = 21.77 and 29.20 cm). *Phaeoacremonium italicum* and *Pleurostoma richardsiae* were pathogenic for both grapevine cultivars, which confirmed their aggressiveness reported by Carlucci *et al.* (2015a) and Raimondo *et al.* (2014). These fungi produced discolourations with mean lengths from 18.41 to 20.11 cm. Among the reference grapevine pathogens used, *Cadophora luteo-olivacea* was less pathogenic than *P. italicum* and *L. citricola*, as it produced mean discolouration lengths of 14.13 and 12.97 cm, respectively, on 'Nero di Troia' and 'Bombino bianco'. *Seimatosporium vitis-vinifera* and *Truncatella angustata* produced variable significant discolouration lengths on both grapevine cultivars, similar to those produced by *Cad. luteo-olivacea*. *Sei. vitis-vinifera* was less aggressive on 'Nero di Troia' (mean discolouration length = 16.98 cm) than on 'Bombino bianco' (mean length = 23.63 cm). *Truncatella angustata* produced different and variable discolouration lengths on 'Nero di Troia' and 'Bombino bianco' of 18.01 and 15.71 cm, respectively. *Colletotrichum fioriniae* was less aggressive, as it produced the least mean discolouration lengths on 'Nero di Troia' and 'Bombino bianco', which were, respectively, 8.83 and 8.43 cm. All of these fungi were re-isolated from the inoculated grapevines, which fulfilled Koch postulates (Table 4).

DISCUSSION

The data obtained in the present study show that vineyards in southern Italy were affected by different fungal species, some of which are known to be responsible for GTDs, such as Esca and Petri disease, and Botryosphaeria dieback. During the survey carried out on symptomatic vineyards over a 10 year period, different fungal species were among the samples collected, including *Botryosphaeria* spp., *Phaeoacremonium* spp. *Phaeomoniella chlamydospora* and *Pleurostoma richardsiae* as the most frequently isolated, and less frequently isolated taxa included *Seimatosporium*, *Truncatella*, *Cadophora* and *Colletotrichum*. The fungi of the first group are spread in most world grape-growing regions, and their

pathogenicities and involvement in diseases associated with grapevines are known (Raimondo *et al*., 2014; Carlucci *et al*., 2015a; 2015b).

The molecular analysis used in the present study allowed identification of the second group of fungi as *Seimatosporium vitis-viniferae*, *Truncatella angustata*, *Cadophora luteo-olivacea* and *Colletotrichum fioriniae*. The morphological characterisation confirmed the molecular data, and helped in the identification of isolates of *Sei. vitis-viniferae*, for which molecular identification was not discriminatory.

To date, many studies have reported the isolation of "pestalotioides fungi", such as *Seimatosporium* species, from symptomatic grapevines or from dead stems in different countries, initially including Australia (Shivas**,** 1989), England and France (Sutton**,** 1980), England and Germany (Nag Raj, 1993) and Pakistan (Ahmad, 1969; Ahmad *et al*., 1997). More recent reports also include Chile, Hungary, Iran, Italy, Spain and the USA (Castillo-Pando *et al*., 2001; Sergeeva *et al.,* 2005; Dìaz *et al.,* 2012; Senanayake *et al.,* 2015; Mehrabi *et al*., 2017; Vàczy, 2017; Lawrence *et al.,* 2018, Camele and Mang, 2019, Liu *et al.,* 2019). However, little information has been provided about their involvement in specific grapevine diseases.

Nine *Seimatosporium* species have been associated with grapevines, including *Sei. botan*, *Sei. hysterioides*, *Sei. lonicerae*, *Sei. luteosporum*, *Sei. macrospermum*, *Sei. parasiticum*, *Sei. vitifusiforme*, *Sei. vitis* and *Sei. vitis-viniferae* (Farr and Rossman, 2018; Liu *et al.,* 2019). However, only four of these have been assessed in standard pathogenicity trials on trunks and canes of vineyard grapevines, to confirm their pathogenicity roles and involvement in GTDs. *Seimatosporium botan* was isolated from symptomatic grapevines in Chile and was reported to be pathogenic on woody canes and trunks of potted grapevines (Dìaz *et al.,* 2012). *Seimatosporium vitis* strains were isolated from symptomatic grapevines in Hungary (Vàczy, 2017), North Carolina, USA (Lawrence *et al*., 2018) and Italy (Camele and Mang, 2019), and were demonstrated to be pathogenic on green shoots and woody stems of potted grapevines. *Seimatosporium luteosporum* and *Sei. vitifusiforme* were reported as pathogens on woody stems of grapevines in North Carolina, USA (Lawrence *et al*. 2018).

In the present study, the pathogenicity of *Sei. vitis-viniferae* was tested for the first time, which increased the number of *Seimatosporium* species that have been confirmed to be associated with GTDs to five. Based on molecular and morphological studies on the pestalotioides fungi reported by Liu *et al*. (2019), the identification of *Sei. vitis* in some studies appears to have been incorrect. The multilocus analyses performed with LSU, ITS, *tef-1α*, β-tubulin and *rpb2* sequences in the present study demonstrated that the strains of *Sei. vitis* reported by Lawrence *et al*. (2018) and Camele and Mang (2019) all clustered in the clade of *Sei. vitis-viniferae*. The morphological description provided by Lawrence *et al*. (2018) for *Sei. vitis* strains, including conidium dimensions and the presence of appendages at both ends of conidia does not agree with the description of *Sei. vitis* by Senanayake *et al.* (2015), although it does agree with that of Liu *et al*. (2019) for *Sei. vitis-viniferae*. Although the *tef-1α* and β-tubulin sequences of *Sei. vitis* reported by Camele and Mang (2019) were identical to those of *ex-type Sei. vitis-viniferae* described by Liu *et al*. (2019), no detailed morphological information was reported. Therefore, to the best of our knowledge, the present study provides the first report of *Sei. vitis-viniferae* associated with GTD symptoms in Italy.

The genus *Truncatella* is closely related to *Seimatosporium*, which belongs to the pestalotioides fungi, and it has wide distribution and occurs in many hosts, including grapevines (Sutton, 1980). Few reports are available about the association of *Truncatella* with grapevine, and its involvement in GTDs. Nag Raj (1993) reported *T. angustata* and *T. pitospora* (now *Pestalotia pitospora*) on grapevine, but did not include any information on their pathogenicity. Some years later, Casieri *et al*. (2009), in Switzerland, and Gonzalez and Tello (2011), in Spain, reported *T. angustata* as endophytes that were collected from different grapevine cultivars. Urbez-Torrez *et al*. (2009) also isolated *T. angustata* from cankers on grapevines in Texas, and performed pathogenicity tests to demonstrate that this fungus can be a weak and/or opportunistic pathogen on lignified grapevine canes. The pathogenicity of *T. angustata* and its involvement in GTD symptoms were also confirmed by Arzanlou *et al*. (2013) in Iran. Maharachchikumbura *et al*. (2016) and Pintos *et al*. (2018) reported *T. angustata* associated with GTD symptoms on grapevines in France, but no pathogenicity trials were performed. Based on a recent taxonomic revision of the genus *Truncatella* by Liu *et al*. (2019), there is now just one accepted species, as *T. angustata*, while other *Truncatella* species were transferred to different genera, including *Bartalinia*, *Heterotruncatella* and *Morinia*, due to the polyphyletic nature of this genus or to synonymy with *T. angustata*. The pathogenicity tests performed in the present study confirmed the pathogenic behaviour of *T. angustata* and its involvement in GTDs (Arzanlou *et al*. 2013). This is the first report of *T. angustata* associated with GTD symptoms on grapevines in Italy.

To date, seven *Cadophora* species have been reported from grapevines, including *Cad. fastigiata*, *Cad. luteo-*

olivacea, *Cad. melinii*, *Cad. novi-eboraci*, *Cad. orientoamericana*, *Cad. spadicis* and *Cad. viticola* (Overton *et al*., 2005; Halleen *et al.* 2007; Crous *et al*., 2015; Travadon *et al.,* 2015). Halleen *et al.* (2007) reported *Cad. luteo-olivacea* from grapevines showing decline symptoms, and from apparently healthy plants in commercial nurseries in South Africa. Pathogenicity tests demonstrated that *Cad. luteo-olivacea* caused significant lesions on the trunks and pruned wood of 15-year-old grapevines.

Casieri *et al.* (2009), in Switzerland, and Fischer *et al*. (2016), in Germany, reported *Cad. fastigiata* and *Cad. luteo-olivacea* as fungal species that can cause grapevine diseases. Gramaje *et al.* (2011) reported *Cad. luteo-olivacea* and *Cad. melinii* from nursery grapevines, although pathogenicity tests demonstrated that only *Cad. luteo-olivacea* caused grapevine disease on 1-year-old grapevine cutting rootstock. Travadon *et al.* (2015) confirmed the involvement of *Cad. luteo-olivacea* in GTDs, and associated four other *Cadophora* species with wood decay of grapevines in North America (*Cad. melinii*, and three new species, *Cad. orientoamericana*, *Cad. novi-eboraci* and *Cad. spadicis*). In 2015, Crous *et al.* (2015) described a new species of *Cad. viticola* (previously identified as *Cad. melinii* by Gramaje *et al*., 2011), which was isolated from grapevine shoots that showed black streaks. *Cad. luteo-olivacea* is the most frequently isolated *Cadophora* species associated with GTD symptoms in different countries, including the USA, France, Germany, New Zealand, South Africa, Spain, Switzerland and Uruguay (Casieri *et al.,* 2009; Manning and Munday, 2009, Gramaje *et al.,* 2011; Travadon *et al*., 2015; Fischer *et al*., 2016; Pintos *et al*., 2018). Isolation of *Cad. luteo-olivacea* in the present study confirms the wide distribution of this species, while the pathogenicity tests performed here confirm the pathogenic behaviour of *Cad. luteo olivacea* and its involvement in GTDs. This is the first report of *Cadophora luteo-olivacea* associated with GTD symptoms in Italy.

Colletotrichum fioriniae was also less frequently isolated that other fungi, and this species is in the *C. acutatum* species complex. The role of *Colletrotrichum* on grapevines is not clear; there have been few reports of species of the *C. acutatum* complex that have described their behaviour on grapevines. *Colletotrichum fioriniae* (Kepner and Swett 2018) and *C. godetiae* (Zapparata *et al.,* 2017) have been associated with grape berry rot, respectively, in the USA and Italy. *Colletotrichum godetiae* has also been reported as a leaf anthracnose agent in the United Kingdom (Baroncelli *et al.,* 2014), and as a saprophyte in China, Italy, Russia and Thailand (Jayawardena *et al*., 2018). In 2016, Liu *et al.* (2016) reported the first association of species in the *C. acutatum* complex with the wood of grapevines when they described *C. nymphaeae* from twig anthracnose in China.

To date, there has only been one report of *Colletotrichum* spp. associated with GTDs, from a grapevine nursery in France (Pintos *et al*., 2018), although no specific identification was carried out. In the present study, as *C. fioriniae* produced wood discolouration on both of the grapevine cultivars included, and although this was less severe (shorter discolouration) than for the other fungi inoculated. This fungus can now be considered as a weak pathogen on grapevine wood. This is, therefore, the first report of *C. fioriniae* associated with GTD symptoms.

The study reported in the present paper has demonstrated the presence of *Cadophora luteo-olivacea*, and *Truncatella angustata*, as well as their virulence, also on grapevine in Italy. *Seimatosporium vitis-vinifera*, isolated from grapevine for first time in Italy, when artificially inoculated, was the most aggressive fungus among the less-common fungi assayed here, indicating its involvement in GTDs. *Colletotrichum fioriniae*, although less aggressive among the fungi assayed, was also shown to be another fungus involved in GTDs. These results add to knowledge on the expanding group of fungi involved in the GTD complex.

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