

Emerging citrus diseases in Europe caused by species of *Diaporthe*

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Abstract: Species of *Diaporthe* are considered important plant pathogens, saprobes, and endophytes on a wide range of plant hosts. Several species are well-known on citrus, either as agents of pre- or post-harvest infections, such as dieback, melanose and stem-end rot on fruit. In this study we explored the occurrence, diversity and pathogenicity of *Diaporthe* species associated with *Citrus* and allied genera in European orchards, nurseries, and gardens. Surveys were carried out during 2015 and 2016 in Greece, Italy, Malta, Portugal, and Spain. A total of 79 *Diaporthe* strains were isolated from symptomatic twigs, branches and trunks. A multi-locus phylogeny was established based on five genomic loci (ITS, *tef1*, *cal*, *his3* and *tub2*), and the morphological characters of the isolates determined. Preliminary pathogenicity tests were performed on lemon, lime, and orange plants with representative isolates. The most commonly isolated species were *D. foeniculina* and *D. baccae*, while only four isolates of *D. novem* were collected. Two new *Diaporthe* species, described here as *D. limonicola* and *D. melitensis* spp. nov. were found associated with a new devastating dieback disease of lemon plants. Furthermore, one cluster of sterile *Diaporthe* isolates was renamed as *D. infertilis*. Pathogenicity tests revealed most of the *Citrus* species as susceptible to *D. baccae*, *D. foeniculina*, and *D. novem*. Moreover, *D. limonicola* and *D. melitensis* caused serious cankers affecting all the *Citrus* species tested. This study is the first report of *D. baccae* and *D. novem* on citrus in Europe, and the first detection of a new *Diaporthe* canker disease of citrus in Europe. However, no isolates of *D. citri* were found. The study improves our understanding of the species associated with several disease symptoms on citrus plants, and provides useful information for effective disease management.

Key words:

Canker
Citrus
multi-locus sequence typing
pathogenicity

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INTRODUCTION

Diaporthe species are present worldwide as plant pathogens and endophytes in healthy leaves, stems, seeds and roots, or as saprobes on decaying tissues of a wide range of hosts (Muralli *et al.* 2006, Garcia-Reyne *et al.* 2011, Udayanga *et al.* 2011). *Diaporthe* species are well-known as the causal agents of many important plant diseases, including root and fruit rots, dieback, stem cankers, leaf spots, leaf and pod blights, and seed decay (Uecker 1988, Mostert *et al.* 2001a, b, Van Rensburg *et al.* 2006, Rehner & Uecker 1994, Santos *et al.* 2011, Udayanga *et al.* 2011, Diaz *et al.* 2017). Species of *Diaporthe* have also been extensively screened in bioassays for natural products (Isaka *et al.* 2001, Dai *et al.* 2005, Kumaran & Hur 2009, Yang *et al.* 2010), and for the biocontrol of fungal pathogens (Santos *et al.* 2016).

The generic names *Diaporthe* and *Phomopsis* are no longer used to distinguish different morphs of this genus, and recent studies (Rossman *et al.* 2015) have recommended that *Diaporthe* be adopted as the correct generic name as it has priority over *Phomopsis*.

Diaporthe was historically considered monophyletic based on the typical *Phomopsis* asexual morph and diaporthean sexual morph (Gomes *et al.* 2013). However, the paraphyletic nature was recently revealed by Gao *et al.* (2017), who demonstrated that *Ophiodiaporthe* (Fu *et al.* 2013), *Pustulomyces* (Dai *et al.* 2014), *Phaeocytostroma*, and *Stenocarpella* (Lamprecht *et al.* 2011), are embedded in *Diaporthe s. lat.* To address this issue, Senanayake *et al.* (2017) subsequently named several additional diaporthe-like clades within *Diaportheales*.

The taxonomy of *Diaporthe* species has been reviewed in several major studies (Thompson *et al.* 2011, 2014, Gomes *et al.* 2013, Udayanga *et al.* 2014a, b, 2015). Almost 2000 species names are available for both *Diaporthe* and *Phomopsis* (Index Fungorum; <http://www.indexfungorum.org>). The majority of the known species in early literature were described in relation to their host association (Uecker 1988), except for about 150 species that have been described more recently supported by molecular data (Gomes *et al.* 2013, Lombard *et al.* 2014, Udayanga *et al.* 2014a, b, 2015). However, most *Diaporthe* species can be found on

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diverse hosts, and can co-occur on the same host or lesion in different life modes (Rehner & Uecker 1994, Mostert *et al.* 2001a, Guarnaccia *et al.* 2016). This is demonstrated by *D. foeniculina*, usually known as an opportunistic pathogen of various herbaceous weeds, ornamentals, and fruit trees including citrus (Santos & Phillips 2009, Udayanga *et al.* 2014b). However, it has also been isolated from tropical trees as an endophyte, and from herbaceous plants and weeds as a pathogen or saprobe (Udayanga *et al.* 2014a). As a consequence, identification and description of species based on host association alone is no longer tenable within *Diaporthe* (Gomes *et al.* 2013, Udayanga *et al.* 2014a, b).

Before the molecular era, morphological characters such as immersed ascomata and erumpent pseudostroma with elongated perithecial necks in the sexual morph (Udayanga *et al.* 2011), and black conidiomata with dimorphic conidia in the asexual morph (Rehner & Uecker 1994), was the basis on which to study the taxonomy of *Diaporthe* (Van der Aa *et al.* 1990). Recent studies demonstrated that these characters are not always reliable for species level identification due to their variability under changing environmental conditions (Gomes *et al.* 2013).

Following the adoption of DNA sequence-based methods, the polyphasic protocols for studying the genus significantly changed the classification and species concepts, resulting in a rapid increase in the description of novelties. Therefore, genealogical concordance methods, based on multi-gene DNA sequence data, provide a much clearer approach to resolving the taxonomy for *Diaporthe*.

Recent plant pathological studies have shown several *Diaporthe* species to be particularly important on a wide range of economically significant agricultural crops, such as blueberries, citrus, grapes, oaks, sunflowers, soybeans, tea plants, tropical fruits, vegetables, and various trees (Van Rensburg *et al.* 2006, Crous *et al.* 2011a, b, 2016, Thompson *et al.* 2011, Santos & Phillips 2009, Santos *et al.* 2011, Grasso *et al.* 2012, Huang *et al.* 2013, Lombard *et al.* 2014, Gao *et al.* 2015, 2016, Udayanga *et al.* 2015, Guarnaccia *et al.* 2016). Furthermore, several *Citrus* species are colonized and/or affected by different *Diaporthe* species (Timmer *et al.* 2000, Huang *et al.* 2013), which are focussed on here.

BACKGROUND

Citrus represents one of the most important fruit industries worldwide. In the Mediterranean region, Greece, Italy, Portugal, and Spain especially are important producers of citrus fruits, and are the biggest fruit exporter after South Africa (FAO 2016). Therefore, recognizing the pathogens affecting these crops in these countries is imperative.

Diaporthe citri is a well-known pathogen causing melanose and stem-end rot disease of *Citrus* species in several regions (Timmer 2000, Mondal *et al.* 2007). Several additional *Diaporthe* species have been reported associated with *Citrus* (often as *Phomopsis*) and have previously been considered as synonyms of *D. citri*, such as *D. citrincola* described from the Philippines, *P. californica* from California, *P. caribaea* from Cuba, and *P. cytosporrella* from Italy (Rehm 1914, Fawcett 1922). Wehmeyer (1933) also considered *D. medusaea*, *D.*

californica, *P. citri*, and *P. citrincola* as synonyms of *Diaporthe citri*.

Polyphasic approaches in recent years have revealed many species associated with citrus. Huang *et al.* (2013) reported *D. citri* as the predominant species in China and described two new taxa: *D. citriasiana* and *D. citrichinensis*. In another study, Huang *et al.* (2015) identified several *Diaporthe* species as endophytes of citrus but which had previously been recovered from other hosts, such as *D. endophytica*, *D. eres*, *D. hongkongensis*, *D. sojae*, and the different taxa clustering in the *D. arecae* species complex. Moreover, they described *D. biconispora*, *D. biguttulata*, *D. discoidispora*, *D. multiguttulata*, *D. ovalispora*, *D. subclavata*, and *D. unshiuensis* as new species occurring on citrus. Several strains from China, Korea, New Zealand, and the USA have been re-assessed by Udayanga *et al.* (2014b) within *D. citri*, which was also epitypified. In the same study, *D. cytosporrella* was recovered from specimens of *Citrus limon*, *C. limonia*, and *C. sinensis* collected respectively in Spain, Italy, and the USA, and *D. foeniculina* has also been widely associated with citrus.

Diaporthe citri is generally accepted as an important pathogen of citrus, causing stem-end rot and melanose of fruits, young leaf and shoot gummosis, and blight of perennial branches and trunks (Kucharek *et al.* 1983, Timmer & Kucharek 2001, Mondal *et al.* 2007, Udayanga *et al.* 2014b). This species occurs in many citrus growing regions of the world on several *Citrus* species, including *C. limon*, *C. paradisi*, *C. reticulata*, and *C. sinensis* (Timmer *et al.* 2000).

Further infections involving twigs, perennial branches and trunks of citrus are caused by other *Diaporthe* species, such as cankers developing in woody tissues, often with a gummosis exudate, generating serious blight and dieback (Huang *et al.* 2013, Mahadevakumar *et al.* 2014). Canker diseases of citrus are also caused by other fungal genera such as *Fusarium* and *Neocosmospora* (Sandoval-Denis *et al.* 2018), and species of *Botryosphaeriaceae* and *Diatrypaceae* (Timmer *et al.* 2000, Polizzi *et al.* 2009, Mayorquin *et al.* 2016).

Although the biology and epidemiology of melanose are well studied also with a robust phylogenetic relationship of the causal organisms, genetic variability and population structure (Burnett 1962, Mondal *et al.* 2004, 2007, Udayanga *et al.* 2014b), the identification of *Diaporthe* species associated with citrus cankers and dieback has not been well resolved. Moreover, Gomes *et al.* (2013) performed a major phylogenetic and morphological study of *Diaporthe* species and grouped three isolates, one of which was collected from *Citrus sinensis* in Suriname, under *D. citri*. However, Udayanga *et al.* (2014b) re-assessed *D. citri* based on molecular phylogenetic analysis of conserved ex-type and additional strains collected exclusively from symptomatic citrus tissues in different geographic locations worldwide. Furthermore, according to this latter study, *D. citri* is unknown in Europe. Because of all these findings, changes in species concepts and poor investigation of *Diaporthe* on citrus in Europe, new surveys were required to study *Diaporthe* species diversity related to citrus and their occurrence and association with diseases.

The current study aims to investigate the major citrus production areas in Europe by employing large-scale

sampling to isolate *Diaporthe* strains, and to identify the strains obtained in the light of modern taxonomic concepts *via* morphological characterization and multi-locus DNA sequence data. In 2015 and 2016, several surveys were conducted in commercial nurseries, citrus orchards, gardens, backyards, and plant collections to determine the occurrence of *Diaporthe* species associated with *Citrus* and allied genera (e.g. *Microcitrus*). In particular the objectives of the present study were to: (1) conduct extensive surveys for sampling symptomatic plant materials; (2) cultivate as many *Diaporthe* isolates as possible; (3) subject those isolates to DNA sequence analyses combined with morphological characterization; (4) compare the obtained results with the data from other phylogenetic studies on the genus; (5) place three strains previously named as *D. citri* in the correct taxonomic context based on DNA sequence inference; and (6) evaluate the pathogenicity of the isolated *Diaporthe* species to citrus plants.

MATERIALS AND METHODS

Sampling and isolation

During 2015 and 2016 many regions of the main citrus-producing area of Europe were surveyed (Guarnaccia *et al.* 2017a, b). Twig, branch and trunk portions showing cankers and dieback were collected from more than 90 sites in: Andalusia, Valencia, and the Balearic Islands (Spain); Apulia, Calabria, Sicily, and the Aeolian Islands (Italy); Algarve (Portugal); Arta, Crete, Missolonghi, and Nafplio (Greece); and Malta and Gozo (Malta). Investigated species of *Citrus* and allied genera such as *Microcitrus* (*Rutaceae*) included Australasian lime, citrons, kumquat, mandarins, oranges, pumelo, grapefruit, limes, and lemons.

Wood fragments (5 × 5 mm) were cut from the margin between affected and healthy tissues and washed in running tap water. Then, each fragment was surface sterilised by soaking in 70 % ethanol for 5 s, 4 % sodium hypochlorite for 90 s, sterile water for 60 s (Kumaresan & Suryanarayanan 2001) and then dried on sterile filter paper. The fragments were placed on malt extract agar (MEA; Crous *et al.* 2009) amended with 100 µg / mL penicillin and 100 µg / mL streptomycin (MEA-PS) and incubated at 25 °C until characteristic *Diaporthe* colonies were observed. In a second procedure, plant material was incubated in moist chambers at room temperature (20 ± 3 °C) for up to 10 d and inspected daily for fungal sporulation. Sporulating conidiomata obtained through both procedures were collected and crushed in a drop of sterile water and then spread over the surface of MEA-PS plates. After 24 h germinating spores were individually transferred onto MEA plates. The isolates used in this study are maintained in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands, and in the working collection of Pedro Crous (CPC), housed at the Westerdijk Institute.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega, WI) following the manufacturer's

instructions. Partial regions of six loci were amplified. The primers ITS5 and ITS4 (White *et al.* 1990) were used to amplify the ITS region of the nuclear ribosomal RNA operon, including the 3' end of the 18S rRNA, the first internal transcribed spacer region, the 5.8S rRNA gene; the second internal transcribed spacer region and the 5' end of the 28S rRNA gene. The primers EF1-728F and EF1-986R (Carbone & Kohn 1999) were used to amplify part of the translation elongation factor 1- α gene (*tef1*). Primers CAL-228F and CAL-737R (Carbone & Kohn 1999) or CL1/ CL2A (O'Donnell *et al.* 2000) were used to amplify part of the calmodulin (*cal*) gene. The partial histone H3 (*his3*) region was amplified using CYLH3F and H3-1b primer sets (Glass & Donaldson 1995, Crous *et al.* 2004a), and the beta-tubulin (*tub2*) region was amplified using Bt2a and Bt2b primer sets (Glass & Donaldson 1995). The PCR products were sequenced in both directions using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems Life Technologies, Carlsbad, CA), after which amplicons were purified through Sephadex G-50 Fine columns (GE Healthcare, Freiburg) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were analysed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA). The DNA sequences generated were analysed and consensus sequences were computed using SeqMan Pro (DNASTAR, Madison, WI).

Phylogenetic analyses

New sequences generated in this study were blasted against the NCBI's GenBank nucleotide database to determine the closest relatives for a taxonomic framework of the studied isolates. Alignments of different gene regions, including sequences obtained from this study and sequences downloaded from GenBank, were initially performed with the MAFFT v. 7 online server (<http://mafft.cbrc.jp/alignment/server/index.html>) (Kato & Standley 2013), and then manually adjusted in MEGA v. 7 (Kumar *et al.* 2016).

To establish the identity of the isolates at species level, phylogenetic analyses were conducted first individually for each locus (data not shown) and then as combined analyses of five loci. One analysis was performed for all the *Diaporthe* isolates recovered from samples collected during the surveys conducted for this study. Additional reference sequences were selected based on recent studies of *Diaporthe* species (Gomes *et al.* 2013, Huang *et al.* 2013, Udayanga *et al.* 2014a, b). Phylogenetic analyses were based on Maximum Parsimony (MP) for all the individual loci and on both MP and Bayesian Inference (BI) for the multi-locus analyses. For BI, the best evolutionary model for each partition was determined using MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses. MrBayes v. 3.2.5 (Ronquist *et al.* 2012) was used to generate phylogenetic trees under optimal criteria per partition. The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The heating parameter was set to 0.2 and trees were sampled every 1000 generations. Analyses stopped once the average standard deviation of split frequencies was below 0.01. The MP analyses were done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003). Phylogenetic relationships were estimated by heuristic searches with 100

random addition sequences. Tree bisection-reconnection was used, with the branch swapping option set on “best trees” only with all characters weighted equally and alignment gaps treated as fifth state. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC) were calculated for parsimony and the bootstrap analyses (Hillis & Bull 1993) were based on 1000 replications. Sequences generated in this study are deposited in GenBank (Table 1) and alignments and phylogenetic trees in TreeBASE (www.treebase.org).

Morphological analyses

Agar plugs (6 mm diam) were taken from the edge of actively growing cultures on MEA and transferred onto the centre of 9 cm diam Petri dishes containing 2 % tap water agar supplemented with sterile pine needles (PNA; Smith *et al.* 1996), potato dextrose agar (PDA), oatmeal agar (OA) and MEA (Crous *et al.* 2009), and incubated at 20–21 °C under a 12 h near-ultraviolet light/12 h dark cycle to induce sporulation as described in recent studies (Gomes *et al.* 2013, Lombard *et al.* 2014). Colony characters and pigment production on MEA, OA and PDA were noted after 10 d. Colony colours were rated according to Rayner (1970). Cultures were examined periodically for the development of ascomata and conidiomata. Colony diameters were measured after 7 and 10 d. The morphological characteristics were examined by mounting fungal structures in clear lactic acid and 30 measurements at $\times 1000$ magnification were determined for each isolate using a Zeiss Axioscope 2 microscope with interference contrast (DIC) optics. Descriptions, nomenclature and illustrations of taxonomic novelties are deposited in MycoBank (www.MycoBank.org; Crous *et al.* 2004b).

Pathogenicity

Pathogenicity tests with five *Diaporthe* species isolated from the European citrus samples were performed to satisfy Koch's postulates.

Two isolates of each of the five species (*D. baccae*: CPC 26170, CPC 27831; *D. foeniculina*: CPC 28033, CPC 28081; *D. limonicola*: CPC 28200, CPC 31137; *D. melitensis*: CPC 27873, CPC 27875; and *D. novem*: CPC 26188, CPC 28165), were inoculated onto potted 2-yr-old healthy plants of lemon (*Citrus limon*), lime (*C. aurantiifolia*), mandarin (*C. reticulata*), and two clones ('New Hall' and 'Tarocco Meli') of sweet orange (*C. sinensis*). Three plants per replicate for each isolate were inoculated, each having five wounds on twigs made using a sterile blade. Mycelial plugs (6 mm diam), taken from the margin of actively growing colonies on MEA, were placed on the wound sites on each plant. An equivalent number of plants and inoculation sites were inoculated with sterile MEA plugs and served as controls. The inoculation sites were covered with Parafilm® (American National Can, Chicago, IL). The inoculated plants were incubated with a 16 h photoperiod in a growth chamber at 100 % relative humidity and 25 ± 1 °C. After 2 mo external symptoms were assessed. Twigs were cut and the bark peeled off to check for any internal discoloration.

Small sections (0.5 cm) of symptomatic tissue from the edge of twig lesions were placed on MEA to re-isolate the fungal species, and were identified based on *tef1* and *tub2* sequencing to fulfil Koch's postulates.

RESULTS

Isolates

Several shoot blight and canker infections on woody tissue were frequently observed on multiple *Citrus* species in all countries investigated. Some orchards presented blight of vigorously growing branches and cankers involving both scion branches and rootstock trunks, resulting in a general dieback and tree death (Fig. 1A). Affected trunks and branches appeared cracked, darkly discoloured and/or slightly sunken. Abundant gummosis was frequently associated with the affected tissues (Fig. 1B–D). Twigs showed wilting, typical dieback and wither-tip, and occasionally gummosis (Fig. 1E–F). Under the bark, cankers were reddish brown and variable in shape. Pycnidial formation on dead twig tissue was observed (Fig. 1G). A total of 79 monosporic isolates resembling those of the genus *Diaporthe* were collected. The *Diaporthe* isolates were recovered from 10 species of *Citrus* at 31 sites in different locations of Greece, Italy, Malta, Spain, and Portugal. Among them, 27 isolates were obtained from branch infections, 13 were associated with trunk cankers, and 39 from twig dieback (Table 1).

Phylogenetic analyses

Six alignments were analysed representing single gene analyses of ITS, *tub2*, *his3*, *tef1*, *cal* and a combined alignment of the five genes. The alignments produced topologically similar trees. The combined species phylogeny of the *Diaporthe* isolates consisted of 123 sequences, including the outgroup sequences of *Diaporthe corylina* (culture CBS 121124). A total of 3026 characters (ITS: 1–582, *tef1*: 589–1052, *tub2*: 1059–1 862, *cal*: 1869–2484, *his3*: 2 491–3026) were included in the phylogenetic analysis, 1355 characters were parsimony-informative, 468 were variable and parsimony-uninformative, and 1161 were constant. A maximum of 1000 equally most parsimonious trees were saved (Tree length = 5528, CI = 0.584, RI = 0.868 and RC = 0.507). Bootstrap support values from the parsimony analysis are plotted on the Bayesian phylogenies in Fig. 2. For the Bayesian analyses, MrModeltest suggested that all partitions should be analysed with dirichlet state frequency distributions. The following models were recommended by MrModeltest and used: GTR+I+G for ITS, *tef1* and *cal*, HKY+G for *tub2* and GTR+G for *his3*. In the Bayesian analysis, the ITS partition had 188 unique site patterns, the *tef1* partition had 357 unique site patterns, the *tub2* partition had 510 unique site patterns, the *cal* partition had 364 unique site patterns, the *his3* partition had 239 unique site patterns and the analysis ran for 1 880 000 generations, resulting in 3762 trees of which 2822 trees were used to calculate the posterior probabilities.

In the combined analysis, 54 *Citrus* isolates clustered with five reference strains and the ex-type of *D. foeniculina*, whilst 14 isolates clustered with the ex-type of *D. baccae*. Four isolates clustered with the ex-type strain of *D. novem*. Moreover, five isolates identified as *D. limonicola* and a further two as *D. melitensis*, formed two highly supported subclades (1.00/100) embedded in the *D. arecae* species complex.

The individual alignments and trees of the five single loci used in the analyses, were also compared with respect to their performance in species recognition. *D. novem* was differentiated by each gene used. Moreover, *tef1* and *tub2* separated both *D. limonicola* and *D. melitensis* from the other species belonging to the *D. arecae* species complex.



Fig. 1. Symptoms on citrus tissues with associated *Diaporthe* species. **A.** Commercial lemon orchard infected by *D. limonicola* and *D. melitensis* (Malta). **B–C.** Trunk canker with gummosis of *Citrus limon* and *C. sinensis* plants (Malta). **D.** Branch canker of *C. sinensis* (Portugal). **E–F.** Twigs dieback of lemon (Italy). **G.** Orange twigs wither-tip with *Diaporthe* pycnidial formation (Italy).

Table 1. Collection details and GenBank accession numbers of isolates included in this study.

Species	Culture no. ¹	Host	Locality	Associated symptoms	GenBank no. ²				
					ITS	tub2	his3	tef1	cal
<i>D. angelicae</i>	CBS 111592	<i>Heracleum sphondylium</i>	Austria	-	KC343026	KC343994	KC343511	KC343752	KC343268
<i>D. arecae</i>	CBS 161.64	<i>Areca catechu</i>	India	-	KC343032	KC344000	KC343516	KC343758	KC343274
	CBS 535.75	<i>Citrus</i> sp.	Suriname	-	KC343033	KC344001	KC343517	KC343759	KC343275
<i>D. arengae</i>	CBS 114979	<i>Arenga engleri</i>	Hong Kong	-	KC343034	KC344002	KC343518	KC343760	KC343276
<i>D. baccae</i>	CBS 136972	<i>Vaccinium corymbosum</i>	Italy	-	KJ160565	MF418509	MF418264	KJ160597	-
	CPC 26170 = CBS 142545	<i>Citrus sinensis</i> 'Tarocco Tapi'	Italy, Catania	Twig dieback	MF418351	MF418510	MF418265	MF418430	MF418185
	CPC 26465	<i>Citrus limon</i>	Italy, Catania	Branch canker	MF418352	MF418511	MF418266	MF418431	MF418186
	CPC 26963	<i>Citrus paradisi</i>	Italy, Vibo Valentia	Branch canker	MF418353	MF418512	MF418267	MF418432	MF418187
	CPC 27029	<i>Citrus sinensis</i>	Italy, Vibo Valentia	Twig dieback	MF418354	MF418513	MF418268	MF418433	MF418188
	CPC 27075	<i>Citrus limon</i>	Italy, Vibo Valentia	Twig dieback	MF418355	MF418514	MF418269	MF418434	MF418189
	CPC 27079	<i>Citrus limon</i>	Italy, Vibo Valentia	Twig dieback	MF418356	MF418515	MF418270	MF418435	MF418190
	CPC 27821	<i>Citrus reticulata</i> 'Caffin'	Italy, Cosenza	Trunk canker	MF418357	MF418516	MF418271	MF418436	MF418191
	CPC 27831 = CBS 142546	<i>Citrus sinensis</i>	Italy, Catania	Trunk canker	MF418358	MF418517	MF418272	MF418437	MF418192
	CPC 27834	<i>Citrus sinensis</i>	Italy, Catania	Trunk canker	MF418359	MF418518	MF418273	MF418438	MF418193
	CPC 27835	<i>Citrus sinensis</i>	Italy, Catania	Trunk canker	MF418360	MF418519	MF418274	MF418439	MF418194
	CPC 27836	<i>Citrus sinensis</i>	Italy, Catania	Trunk canker	MF418361	MF418520	MF418275	MF418440	MF418195
	CPC 27837	<i>Citrus sinensis</i>	Italy, Catania	Trunk canker	MF418362	MF418521	MF418276	MF418441	MF418196
	CPC 27850	<i>Citrus sinensis</i>	Italy, Catania	Twig dieback	MF418363	MF418522	MF418277	MF418442	MF418197
	CPC 27852	<i>Citrus sinensis</i>	Italy, Catania	Twig dieback	MF418364	MF418523	MF418278	MF418443	MF418198
<i>D. biconispora</i>	ICMP20654	<i>Citrus grandis</i>	China	-	KJ490597	KJ490418	KJ490539	KJ490476	-
<i>D. biguttulata</i>	ICMP20657	<i>Citrus limon</i>	China	-	KJ490582	KJ490403	KJ490524	KJ490461	-
<i>D. citri</i>	CBS 134237	<i>Citrus reticulata</i>	China	-	JQ954660	KC357426	MF418279	JQ954676	KC357465
	CBS 134239	<i>Citrus sinensis</i>	Florida, USA	-	KC357553	KC357456	MF418280	KC357522	KC357488
	CBS 135422	<i>Citrus</i> sp.	USA	-	KC843311	KC843187	MF418281	KC843071	KC843157
<i>D. citriasiana</i>	CBS 134240	<i>Citrus unshiu</i>	China	-	JQ954645	KC357459	MF418282	JQ954663	KC357491
<i>D. citrichinensis</i>	CBS 134242	<i>Citrus</i> sp.	China	-	JQ954648	MF418524	KJ420880	JQ954666	KC357494
<i>D. cuppatea</i>	CBS 117499	<i>Aspalathus linearis</i>	South Africa	-	AY339322	JX275420	KC343541	AY339354	JX197414
<i>D. cytosporella</i>	CBS 137020	<i>Citrus limon</i>	Spain	-	KC843307	KC843221	MF418283	KC843116	KC843141
<i>D. discoidispora</i>	ICMP20662	<i>Citrus unshiu</i>	China	-	KJ490624	KJ490445	KJ490566	KJ490503	-
<i>D. endophytica</i>	ZJUD73	<i>Citrus unshiu</i>	China	-	KJ490608	KJ490429	KJ490550	KJ490487	-
<i>D. eres</i>	CBS 439.82	<i>Cotoneaster</i> sp.	Scotland	-	KC343090	KC344058	KC343574	KC343816	KC343332
<i>D. foeniculina</i>	CBS 187.27	<i>Camellia sinensis</i>	Italy	-	KC343107	KC344075	KC343591	KC343833	KC343349
	CBS 111553	<i>Foeniculum vulgare</i>	Spain	-	KC343101	KC344069	KC343585	KC343827	KC343343

Table 1. (Continued).

Species	Culture no. ¹	Host	Locality	Associated symptoms	GenBank no. ²				
					ITS	tub2	his3	tef1	cal
	CBS 111554	<i>Foeniculum vulgare</i>	Portugal	-	KC343102	KC344070	KC343586	KC343828	KC343344
	CBS 123208	<i>Foeniculum vulgare</i>	Portugal	-	KC343104	KC344072	KC343588	KC343830	KC343346
	CBS 123209	<i>Foeniculum vulgare</i>	Portugal	-	KC343105	KC344073	KC343589	KC343831	KC343347
	CBS 135430	<i>Citrus limon</i>	USA	-	KC843301	KC843215	MF418284	KC843110	KC843135
	CPC 26184	<i>Citrus maxima</i>	Italy, Messina	Branch canker	MF418365	MF418525	MF418285	MF418444	MF418199
	CPC 26194	<i>Citrus sinensis</i> 'Sanguinello'	Italy, Messina	Branch canker	MF418366	MF418526	MF418286	MF418445	MF418200
	CPC 26365	<i>Citrus limon</i>	Italy, Catania	Twig dieback	MF418367	MF418527	MF418287	MF418446	MF418201
	CPC 26439	<i>Citrus reticulata</i>	Italy, Catania	Twig dieback	MF418368	MF418528	MF418288	MF418447	MF418202
	CPC 26441	<i>Citrus reticulata</i>	Italy, Catania	Twig dieback	MF418369	MF418529	MF418289	MF418448	MF418203
	CPC 26461	<i>Citrus reticulata</i>	Italy, Catania	Twig dieback	MF418370	MF418530	MF418290	MF418449	MF418204
	CPC 26863	<i>Citrus maxima</i>	Greece, Missolonghi	Branch canker	MF418371	MF418531	MF418291	MF418450	MF418205
	CPC 26873	<i>Citrus reticulata</i>	Greece, Arta	Twig dieback	MF418372	MF418532	MF418292	MF418451	MF418206
	CPC 26883	<i>Citrus maxima</i>	Greece, Missolonghi	Branch canker	MF418373	MF418533	MF418293	MF418452	MF418207
	CPC 26885	<i>Citrus bergamia</i>	Greece, Missolonghi	Branch canker	MF418374	MF418534	MF418294	MF418453	MF418208
	CPC 26913	<i>Citrus limon</i>	Greece, Missolonghi	Branch canker	MF418375	MF418535	MF418295	MF418454	MF418209
	CPC 26923	<i>Citrus maxima</i>	Greece, Missolonghi	Branch canker	MF418376	MF418536	MF418296	MF418455	MF418210
	CPC 26927	<i>Citrus maxima</i>	Greece, Missolonghi	Branch canker	MF418377	MF418537	MF418297	MF418456	MF418211
	CPC 26953	<i>Citrus bergamia</i>	Greece, Missolonghi	Branch canker	MF418378	MF418538	MF418298	MF418457	MF418212
	CPC 26967	<i>Citrus mitis</i>	Italy, Messina	Twig dieback	MF418379	MF418539	MF418299	MF418458	MF418213
	CPC 26971	<i>Citrus mitis</i>	Italy, Messina	Twig dieback	MF418380	MF418540	MF418300	MF418459	MF418214
	CPC 27027	<i>Citrus limon</i>	Italy, Cosenza	Branch canker	MF418381	MF418541	MF418301	MF418460	MF418215
	CPC 27033	<i>Citrus mitis</i>	Italy, Messina	Twig dieback	MF418382	MF418542	MF418302	MF418461	MF418216
	CPC 27037	<i>Citrus paradisi</i>	Italy, Vibo Valentia	Branch canker	MF418383	MF418543	MF418303	MF418462	MF418217
	CPC 27041	<i>Citrus sinensis</i>	Italy, Cosenza	Branch canker	MF418384	MF418544	MF418304	MF418463	MF418218
	CPC 27167	<i>Citrus paradisi</i>	Italy, Vibo Valentia	Branch canker	MF418385	MF418545	MF418305	MF418464	MF418219
	CPC 27756	<i>Citrus limon</i>	Italy, Catania	Trunk canker	MF418386	MF418546	MF418306	MF418465	MF418220
	CPC 27832	<i>Citrus sinensis</i>	Italy, Catania	Trunk canker	MF418387	MF418547	MF418307	MF418466	MF418221
	CPC 27833	<i>Citrus sinensis</i>	Italy, Catania	Trunk canker	MF418388	MF418548	MF418308	MF418467	MF418222
	CPC 27859	<i>Citrus paradisi</i>	Malta, Gozo	Trunk canker	MF418389	MF418549	MF418309	MF418468	MF418223
	CPC 27877	<i>Citrus limon</i>	Malta, Gozo	Trunk canker	MF418390	MF418550	MF418310	MF418469	MF418224
	CPC 27895	<i>Citrus japonica</i>	Malta, Gozo	Twig dieback	MF418391	MF418551	MF418311	MF418470	MF418225
	CPC 27896	<i>Citrus japonica</i>	Malta, Gozo	Twig dieback	MF418392	MF418552	MF418312	MF418471	MF418226

Table 1. (Continued).

Species	Culture no. ¹	Host	Locality	Associated symptoms	GenBank no. ²				
					ITS	tub2	his3	tef1	cal
	CPC 27897	<i>Citrus japonica</i>	Malta, Gozo	Twig dieback	MF418393	MF418553	MF418313	MF418472	MF418227
	CPC 27898	<i>Citrus japonica</i>	Malta, Gozo	Twig dieback	MF418394	MF418554	MF418314	MF418473	MF418228
	CPC 27901	<i>Citrus limon</i>	Malta, Gozo	Branch canker	MF418395	MF418555	MF418315	MF418474	MF418229
	CPC 27903	<i>Citrus limon</i>	Malta, Gozo	Branch canker	MF418396	MF418556	MF418316	MF418475	MF418230
	CPC 27945	<i>Citrus paradisi</i>	Portugal, Faro	Branch canker	MF418397	MF418557	MF418317	MF418476	MF418231
	CPC 27947	<i>Citrus sinensis</i>	Portugal, Faro	Branch canker	MF418398	MF418558	MF418318	MF418477	MF418232
	CPC 27949	<i>Citrus sinensis</i>	Portugal, Faro	Branch canker	MF418399	MF418559	MF418319	MF418478	MF418233
	CPC 27950	<i>Citrus sinensis</i>	Portugal, Faro	Twig dieback	MF418400	MF418560	MF418320	MF418479	MF418234
	CPC 27959	<i>Citrus sinensis</i>	Portugal, Faro	Twig dieback	MF418401	MF418561	MF418321	MF418480	MF418235
	CPC 28033 = CBS 142547	<i>Citrus sinensis</i> 'Valencia'	Portugal, Mesquita	Twig dieback	MF418402	MF418562	MF418322	MF418481	MF418236
	CPC 28035	<i>Citrus paradisi</i>	Portugal, Faro	Twig dieback	MF418403	MF418563	MF418323	MF418482	MF418237
	CPC 28039	<i>Citrus limon</i>	Portugal, Monchique	Twig dieback	MF418404	MF418564	MF418324	MF418483	MF418238
	CPC 28041	<i>Citrus limon</i>	Portugal, Monchique	Twig dieback	MF418405	MF418565	MF418325	MF418484	MF418239
	CPC 28043	<i>Citrus limon</i>	Portugal, Monchique	Twig dieback	MF418406	MF418566	MF418326	MF418485	MF418240
	CPC 28045	<i>Citrus limon</i>	Portugal, Monchique	Twig dieback	MF418407	MF418567	MF418327	MF418486	MF418241
	CPC 28047	<i>Citrus limon</i>	Portugal, Monchique	Twig dieback	MF418408	MF418568	MF418328	MF418487	MF418242
	CPC 28071	<i>Citrus limon</i>	Spain, Algemesi	Twig dieback	MF418409	MF418569	MF418329	MF418488	MF418243
	CPC 28072	<i>Citrus limon</i>	Spain, Algemesi	Twig dieback	MF418410	MF418570	MF418330	MF418489	MF418244
	CPC 28073	<i>Citrus reticulata</i>	Spain, Algemesi	Twig dieback	MF418411	MF418571	MF418331	MF418490	MF418245
	CPC 28074	<i>Citrus reticulata</i>	Spain, Algemesi	Twig dieback	MF418412	MF418572	MF418332	MF418491	MF418246
	CPC 28077	<i>Citrus limon</i>	Spain, Algemesi	Twig dieback	MF418413	MF418573	MF418333	MF418492	MF418247
	CPC 28079	<i>Citrus reticulata</i>	Spain, Algemesi	Twig dieback	MF418414	MF418574	MF418334	MF418493	MF418248
	CPC 28081 = CBS 142548	<i>Citrus reticulata</i>	Spain, Algemesi	Twig dieback	MF418415	MF418575	MF418335	MF418494	MF418249
	CPC 28163	<i>Microcitrus australasica</i>	Italy, Catania	Twig dieback	MF418416	MF418576	MF418336	MF418495	MF418250
	CPC 31135	<i>Citrus limon</i>	Malta, Gozo	Branch canker	MF418417	MF418577	MF418337	MF418496	MF418251
	CPC 31159	<i>Citrus sinensis</i>	Malta, Zurrieq	Branch canker	MF418418	MF418578	MF418338	MF418497	MF418252
<i>D. helianthi</i>	CBS 344.94	<i>Helianthus annuus</i>	-	-	KC343114	KC344082	KC343598	KC343840	KC343356
	CBS 592.81	<i>Helianthus annuus</i>	Serbia	-	KC343115	KC344083	KC343599	KC343841	JX197454
<i>D. hongkongensis</i>	CBS 115448	<i>Dichroa febrifuga</i>	China	-	KC343119	KC344087	KC343603	KC343845	KC343361
<i>D. inconspicua</i>	CBS 133813	<i>Maytenus ilicifolia</i>	Brazil	-	KC343123	KC344091	KC343607	KC343849	KC343365
<i>D. infertilis</i>	CBS 199.39	Unknown	Italy	-	KC343051	KC344019	KC343535	KC343777	KC343293
	CBS 230.52	<i>Citrus sinensis</i>	Suriname	-	KC343052	KC344020	KC343536	KC343778	KC343294

Table 1. (Continued).

Species	Culture no. ¹	Host	Locality	Associated symptoms	GenBank no. ²					
					ITS	tub2	his3	tef1	cal	
<i>D. limonicola</i>	CPC 20322	<i>Glycine max</i>	Brazil	-	KC343053	KC344021	KC343537	KC343779	KC343295	
	CPC 27869	<i>Citrus limon</i>	Malta, Gozo	Trunk canker	MF418419	MF418579	MF418339	MF418498	MF418253	
	CPC 27871	<i>Citrus limon</i>	Malta, Gozo	Trunk canker	MF418420	MF418580	MF418340	MF418499	MF418254	
	CPC 27879	<i>Citrus limon</i>	Malta, Gozo	Branch canker	MF418421	MF418581	MF418341	MF418500	MF418255	
	CPC 28200 = CBS 142549	<i>Citrus limon</i>	Malta, Gozo	Branch canker	MF418422	MF418582	MF418342	MF418501	MF418256	
	CPC 31137 = CBS 142550	<i>Citrus limon</i>	Malta, Zurrifq	Branch canker	MF418423	MF418583	MF418343	MF418502	MF418257	
	CPC 27873 = CBS 142551	<i>Citrus limon</i>	Malta, Gozo	Branch canker	MF418424	MF418584	MF418344	MF418503	MF418258	
	CPC 27875 = CBS 142552	<i>Citrus limon</i>	Malta, Gozo	Branch canker	MF418425	MF418585	MF418345	MF418504	MF418259	
	ICMP20656	<i>Citrus grandis</i>	China	-	KJ490633	KJ490454	KJ490575	KJ490512	-	
	<i>D. novem</i>	CBS 127270	<i>Glycine max</i>	Croatia	-	KC343156	KC344124	KC343640	KC343882	KC343398
<i>D. ovalispora</i>	CBS 127271	<i>Glycine max</i>	Croatia	-	KC343157	KC344125	KC343641	KC343883	KC343399	
	CPC 26188 = CBS 142553	<i>Citrus japonica</i>	Italy, Messina	Twig dieback	MF418426	MF418586	MF418346	MF418505	MF418260	
	CPC 28165 = CBS 142554	<i>Citrus aurantifolia</i>	Italy, Catania	Twig dieback	MF418427	MF418587	MF418347	MF418506	MF418261	
	CPC 28167	<i>Citrus aurantifolia</i>	Italy, Catania	Twig dieback	MF418428	MF418588	MF418348	MF418507	MF418262	
	CPC 28169	<i>Citrus aurantifolia</i>	Italy, Catania	Twig dieback	MF418429	MF418589	MF418349	MF418508	MF418263	
	ICMP20659	<i>Citrus limon</i>	China	-	KJ490628	KJ490449	KJ490570	KJ490507	-	
	CBS 101339	<i>Mangifera indica</i>	Dominican Republic	-	KC343181	KC344149	KC343665	KC343907	KC343423	
	CBS 462.69	<i>Phoenix dactylifera</i>	Spain	-	KC343184	KC344152	KC343668	KC343910	KC343426	
	CBS 113201	<i>Vitis vinifera</i>	Portugal	-	KC343234	KC344202	KC343718	KC343960	KC343476	
	CBS 116311	<i>Protea repens</i>	South Africa	-	KC343190	KC344158	KC343674	KC343916	KC343432	
<i>D. subclavata</i>	FAU 635	<i>Glycine max</i>	USA	-	KJ590719	KJ610875	KJ659208	KJ590762	-	
	ZJUD68	<i>Citrus unshiu</i>	China	-	KJ490603	KJ490424	KJ490545	KJ490482	-	
	CBS 136969	<i>Vaccinium corymbosum</i>	Italy	-	KJ160579	KJ160528	MF418350	KJ160611	KJ160548	
	ICMP20663	<i>Citrus unshiu</i>	China	-	KJ490630	KJ490451	KJ490572	KJ490509	-	
	CGMCC3.17569	<i>Citrus unshiu</i>	China	-	KJ490587	KJ490408	KJ490529	KJ490466	-	
	CBS 121124	<i>Corylus sp.</i>	China	-	KC343004	KC343972	KC343488	KC343730	KC343246	

¹ CPC: Culture collection of P.W. Crous, housed at Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC: China, General Microbiological Culture Collection, Beijing, China; FAU: Isolates in culture collection of Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, MD, USA; ICMP: International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand; ZJUD, *Diaporthe* strains in Zhejiang University, China. Ex-type and ex-epitype cultures are indicated in **bold**.

² ITS: internal transcribed spacers 1 and 2 together with 5.8S rDNA; tub2: partial beta-tubulin gene; his3: histone3; tef1: partial translation elongation factor 1- α gene; cal: partial calmodulin gene. Sequences generated in this study indicated in *italics*.

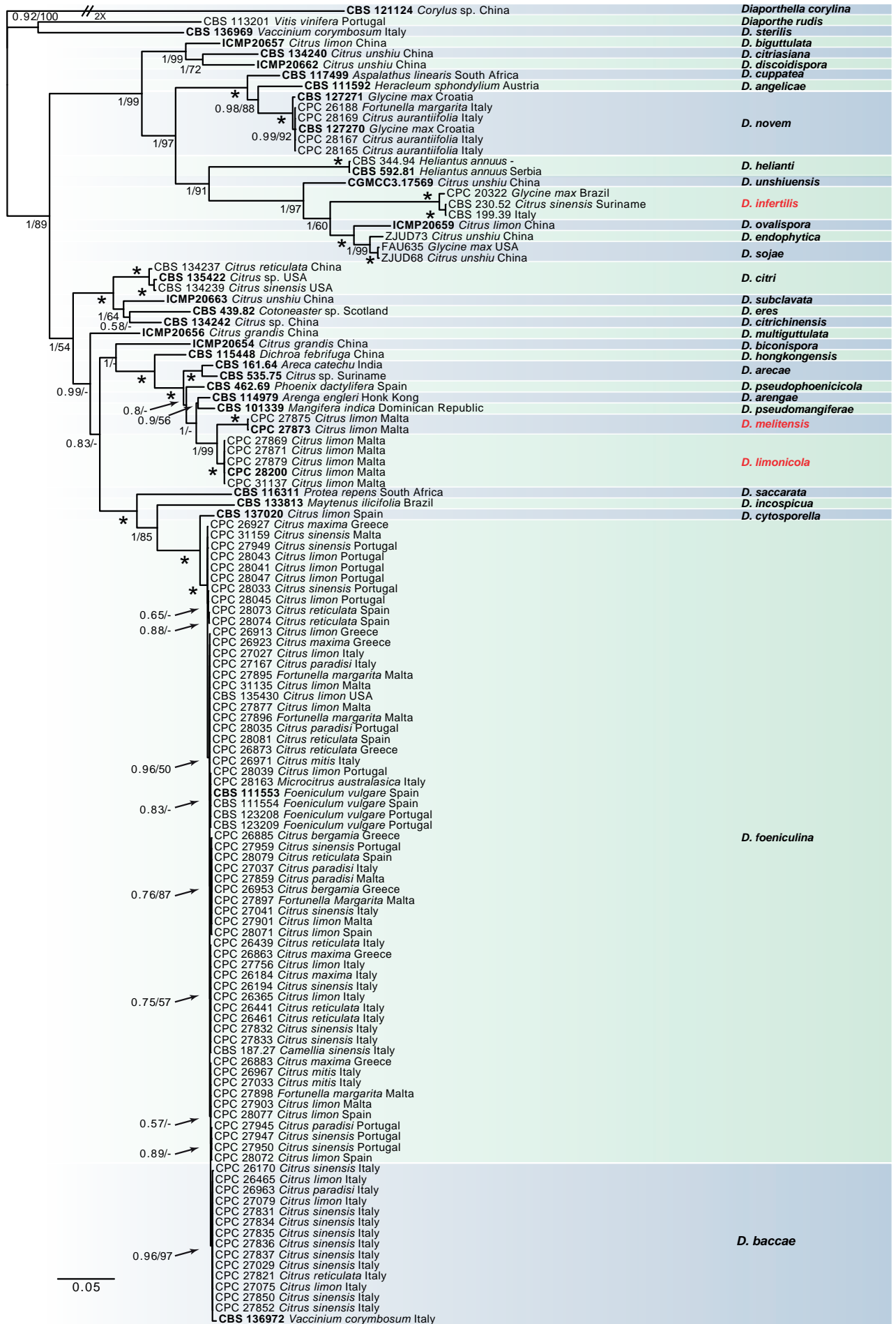


Table 2. *Diaporthe* species associated with citrus and their morphological characteristics.

Species	Conidiomata (µm)	Conidiophores (µm)	Alpha conidia (µm)	Beta conidia (µm)	References
<i>D. arecae</i>	up to 400	15–40 × 1.5–3	6–10 × 2–3	-	Gomes <i>et al.</i> (2013)
<i>D. baccae</i>	up to 650	20–57 × 2–3	7–9 × 2–3	20–24 × 1–2	Lombard <i>et al.</i> (2014)
<i>D. biconispora</i>	145–185	12–35.5 × 1.6–2.6	6–10.5 × 2–3.5	-	Huang <i>et al.</i> (2015)
<i>D. biguttulata</i>	up to 300	5.8–16.9 × 1.3–2.3	5.7–7.8 × 2.5–2.9	23.7–31.6 × 0.9–1.6	Huang <i>et al.</i> (2015)
<i>D. citri</i>	200–250	10–15 × 1–2	7.6–10.2 × 3–4.2	-	Udayanga <i>et al.</i> (2014b)
<i>D. citriasiana</i>	up to 627	3.5–10.5 × 1–2	10.5–15 × 4–6.5	24–42 × 1–2	Huang <i>et al.</i> (2013)
<i>D. citrichinensis</i>	up to 435	9–19.5 × 1.5–3	5.5–9 × 1.5–2.5	27.5–40 × 1–1.5	Huang <i>et al.</i> (2013)
<i>D. cytospora</i>	150–200	7–18 × 1–2	8–9 × 2.6–3.2	-	Udayanga <i>et al.</i> (2014b)
<i>D. discoidispora</i>	200–118	8.9–23.4 × 1.3–2.7	5.6–8 × 2.1–3.2	21.2–38.7 × 0.9–1.6	Huang <i>et al.</i> (2015)
<i>D. endophytica</i> (sterile)	-	-	-	-	Gomes <i>et al.</i> (2013)
<i>D. eres</i>	200–250	10–15 × 2–3	6.5–8.5 × 3–4	22–28 × 1–1.5	Udayanga <i>et al.</i> (2014a)
<i>D. foeniculina</i>	400–700	9–15(–18) × 1–2	8.5–9 × 2.3–2.5	22–28 × 1.4–1.6	Udayanga <i>et al.</i> (2014b)
<i>D. hongkongensis</i>	up to 200	5–12 × 2–4	6–7 × 2.5	18–22 × 1.5–2	Gomes <i>et al.</i> (2013)
<i>D. infertilis</i> (sterile)	-	-	-	-	This study
<i>D. limonicola</i>	up to 670	5–20 × 1.5–4	5.5–8.5 × 1.5–2.5	15–26.5 × 1–2	This study
<i>D. melitensis</i>	up to 650	5–15 × 1.5–5.5	4.5–7 × 1.5–3	-	This study
<i>D. multiguttulata</i>	up to 358	9.8–14.8 × 1.3–3.6	8–12.6 × 4.2–6	-	Huang <i>et al.</i> (2015)
<i>D. novem</i>	up to 580	5.3–10.4 × 1.9–3.2	6.3–8.9 × 1.9–2.5	26.4–37.7 × 1–1.3	Santos <i>et al.</i> (2011)
<i>D. ovalispora</i>	up to 242	9.5–21.6 × 1.6–3.6	6.1–7.9 × 2.7–3.8	-	Huang <i>et al.</i> (2015)
<i>D. sojiae</i>	200–250	12–16 × 2–4	5.3–7.3 × 2–3	-	Udayanga <i>et al.</i> (2015)
<i>D. subclavata</i>	-	14.2–27.3 × 1.6–2.6	5.5–7.2 × 2.2–2.9	-	Huang <i>et al.</i> (2015)
<i>D. unshiuensis</i>	up to 152	14.3–24.2 × 1.4–2.6	5.2–7.5 × 2–3.9	-	Huang <i>et al.</i> (2015)

TAXONOMY

Morphological observations, supported by phylogenetic inference, were used to identify three known species (*D. baccae*, *D. foeniculina*, and *D. novem*), and to recognize three new species described here (Table 2). One species (represented by three isolates) was sterile in culture, and is therefore characterized by DNA sequence data (Gomes *et al.* 2013).

Diaporthe infertilis Guarnaccia & Crous, **sp. nov.**

Mycobank MB821727

(Fig. 3)

Etymology: Named after its sterile growth in culture.

Diagnosis: *Diaporthe infertilis* differs from its closest phylogenetic neighbour, *D. ovalispora*, in 26 unique fixed alleles in ITS locus, 68 in *tef1*, 30 in *tub2* and 48 in *his3* based on the alignments deposited in TreeBASE.

Type: Suriname: Paramaribo, from decaying fruit of *Citrus sinensis*, Apr. 1932, *N.J. van Suchtelen* (CBS H-23179 – holotype; CBS 230.52 – culture ex-type).

Description: Culture characteristics: Colony on MEA covering the entire plate after 10 d, pale luteous with abundant white compact aerial mycelium in fluctuating rings. On OA and PDA at first white, becoming cream to yellowish, flat, with dense and felted mycelium, reverse pale brown with brownish dots with age. Cultures sterile.

Notes: Three isolates clustered in a clade distinct from species of *Diaporthe* known from DNA sequence data. One strain (CPC 20322) was differentiated from the other two (CBS 199.39, CBS 230.52) by unique fixed alleles in four loci based on alignments of the separate loci deposited in TreeBASE: *tef1* positions 115 (C), 261 (indel), 314 (G), 395 (C); *tub2* positions 123 (C), 631 (G); *cal* positions 132 (T), 207 (A), 210 (T), 256 (T), 259 (T), 262 (A), 364 (G), 366 (A), 438 (G), 439 (G), 448 (C); *his3* positions 201 (A), 438 (A), 448 (T), 450 (A). Gomes *et al.* (2013) tentatively referred to this clade as *D. citri*. However, after a molecular re-assessment of many *Diaporthe* species, *D. citri* is restricted to a different clade of citrus isolates (Udayanga *et al.* 2014b). We therefore describe *D. infertilis* as a new species for this clade.

Fig. 2. Consensus phylogram of 3 762 trees resulting from a Bayesian analysis of the combined ITS, *tub2*, *his3*, *tef1* and *cal* sequence. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. The asterisk symbol (*) represents full support (1/100). Substrate and country of origin are listed next to the strain numbers. The newly recognized species are in red. The tree was rooted to *Diaporthe corylina* (CBS 121124).

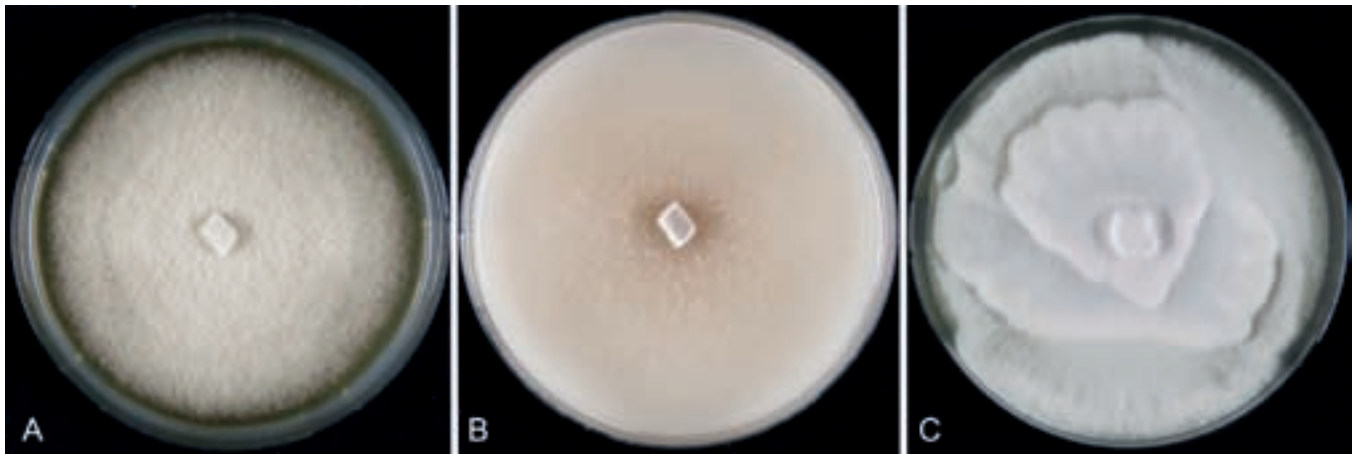


Fig. 3. *Diaporthe infertilis* (CBS 230.52). A–C. Colonies after 7 d at 21 °C on MEA, OA and PDA, respectively.

Additional material examined: **Brazil:** from seeds of *Glycine max*, A. Almeida (culture LGMF946 = CPC 20322). – **Italy:** from unknown host, G. Goidanich (CBS 199.39).

***Diaporthe limonicola* Guarnaccia & Crous, sp. nov.**
Mycobank MB821731
(Fig. 4)

Etymology: In reference to the occurrence on *Citrus limon*.

Diagnosis: *Diaporthe limonicola* can be distinguished from the closely related *D. pseudomangiferae* based on *tef1*,

tub2, *his3* and *cal* loci (96 % in *tef1*, 96 % in *tub2*, 97 % in *his3*, and 96 % in *cal*). *Diaporthe limonicola* differs from *D. pseudomangiferae* in the shorter alpha conidia and in producing beta and gamma conidia.

Type: **Malta:** Gozo, from branch canker of *Citrus limon*, 11 Jul. 2016, V. Guarnaccia (CBS H-23126 – holotype; CBS 142549 = CPC 28200 – culture ex-type).

Description: *Conidiomata* pycnidial in culture on PNA, PDA, OA and MEA, solitary or aggregated, deeply embedded in

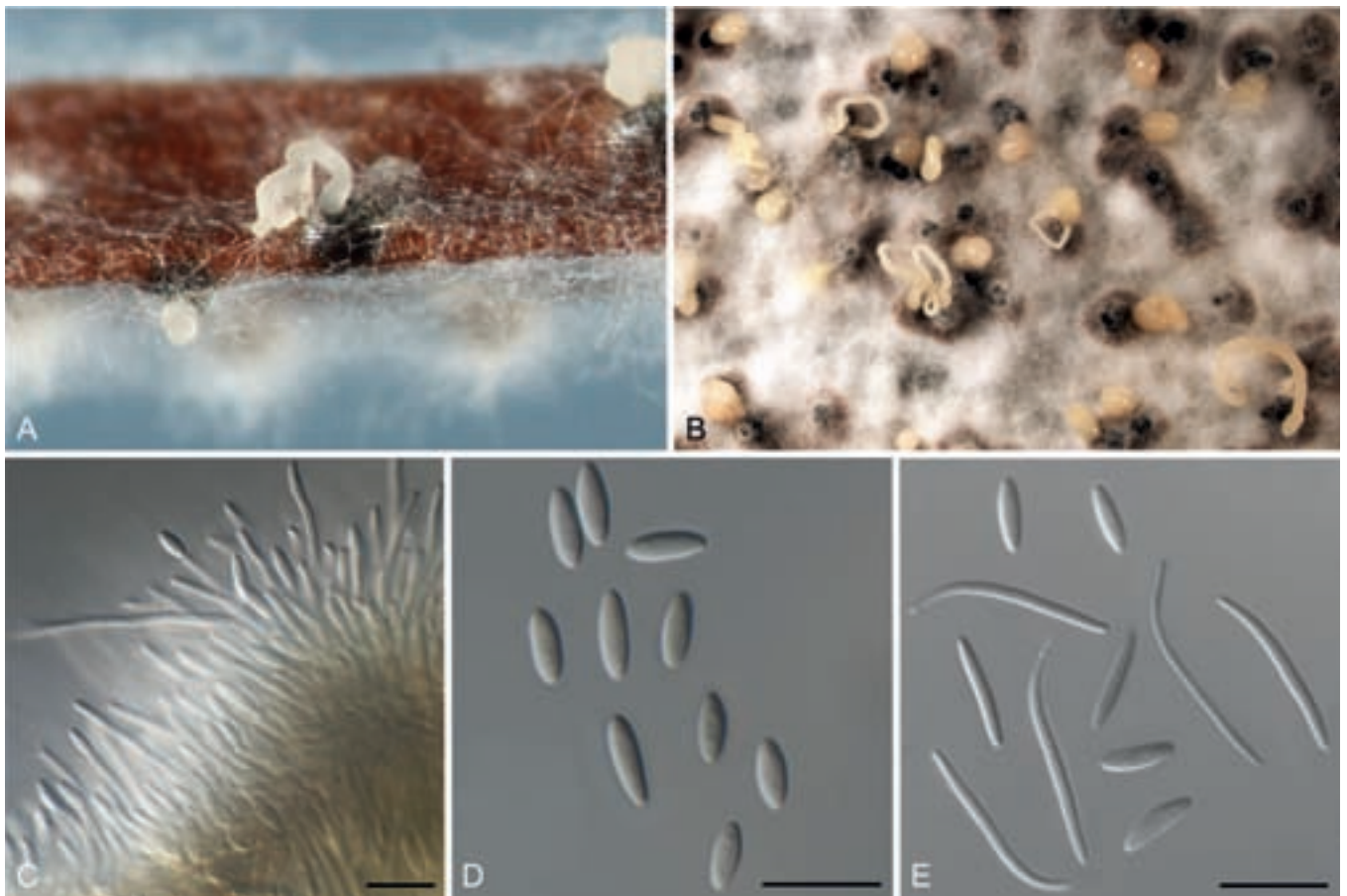


Fig. 4. *Diaporthe limonicola* (CBS 142549). A. Conidiomata sporulating on PNA. B. Conidiomata sporulating on OA. C. Conidiogenous cells. D. Alpha conidia. E. Alpha, beta and gamma conidia. Bars = 10 µm.

PDA, erumpent, dark brown to black, 250–670 μm diam, whitish translucent to cream conidial drops exuded from the ostioles. *Conidiophores* hyaline, smooth, 1-septate, densely aggregated, cylindrical, straight, 5–20 \times 1.5–4 μm . *Conidiogenous cells* phialidic, hyaline, terminal, cylindrical, 5–12 \times 1–2 μm , tapered towards the apex. *Paraphyses* intermingled among conidiophores, hyaline, smooth, 1–3-septate, to 90 μm long, apex 1–2 μm diam. *Alpha conidia* unicellular, aseptate, fusiform, hyaline, mono- to biguttulate and acute at both ends, 5.5–8.5 \times 1.5–2.5 μm , mean \pm SD = 6.8 \pm 0.6 \times 2.1 \pm 0.3 μm , L/B ratio = 2.8. *Beta conidia* hyaline, aseptate, eguttulate, filiform, curved, tapering towards both ends, 15–26.5 \times 1–2 μm , mean \pm SD = 22.7 \pm 2.6 \times 1.4 \pm 0.3 μm , L/B ratio = 16.2. *Gamma conidia* hyaline, multiguttulate, fusiform to subcylindrical with an acute or rounded apex, 9–15.5 \times 1–2 μm , mean \pm SD = 10.7 \pm 1.6 \times 1.4 \pm 0.2 μm , L/B ratio 7.6.

Culture characteristics: Colonies covering the medium within 1 wk at 21 $^{\circ}\text{C}$, surface mycelium flattened, dense and felt-like. Colony on MEA and OA at first white, becoming cream to yellowish, flat, with dense and felted mycelium, reverse pale brown with brownish dots with age, with visible solitary or aggregated conidiomata at maturity. On PDA cream to smoke-grey, reverse pale brown.

Notes: *Diaporthe limonicola* was isolated from *Citrus limon* trunk cankers in two different islands of the Malta archipelago, where all the plants were affected. Five strains representing *D. limonicola* cluster in a well-supported clade, and appear most closely related to *D. pseudomangiferae* and *D. arengae*. *Diaporthe limonicola* can be distinguished based on *tef1*, *tub2*, *his3* and *cal* loci from *D. pseudomangiferae* (96 % in *tef1*, 96 % in *tub2*, 97 % in *his3*, and 96 % in *cal*), and from *D. arengae* (97 % in *tef1*, 98 % in *tub2*, 98 % in *his3*, and 96 % in *cal*). This species is phylogenetically close to but clearly differentiated from *D. melitensis* (described below) by 22 unique fixed alleles in ITS locus, 2 in *tef1* and 47 in *tub2*.

Morphologically, *D. limonicola* differs from *D. pseudomangiferae* in the shorter alpha conidia (5.5–8.5 vs. 7–9 μm) (Gomes *et al.* 2013) and the production of beta and gamma conidia, which are not known in *D. pseudomangiferae* (Gomes *et al.* 2013).

Additional material examined: **Malta:** Zurrieq, from branch canker of *Citrus limon*, 11 Jul. 2016, V. Guarnaccia (culture CBS 142550 = CPC 31137).

***Diaporthe melitensis* Guarnaccia & Crous, sp. nov.**
Mycobank MB821732
(Fig. 5)

Etymology: Named after the country where it was collected, Malta (ancient Latin name, *Melita*).

Diagnosis: *Diaporthe melitensis* can be distinguished from the closely related *D. pseudomangiferae* by the ITS, *tef1*, *tub2*, *his3* and *cal* loci (98 % in ITS, 96 % in *tef1*, 97 % in *tub2*, 97 % in *his3*, and 96 % in *cal*). *Diaporthe melitensis* also differs from *D. pseudomangiferae* in the shorter alpha conidia.

Type: **Malta:** Gozo, from branch canker of *Citrus limon*, 22 Sep. 2015, V. Guarnaccia (CBS H-23127 – holotype; CBS 142551 = CPC 27873 – culture ex-type).

Description: *Conidiomata* pycnidial in culture on PNA, PDA, OA and MEA, solitary or aggregated, deeply embedded in the PDA, erumpent, dark brown to black, 250–650 μm diam, whitish translucent to yellowish conidial drops exuded from the ostioles. *Conidiophores* hyaline, smooth, 1-septate, densely aggregated, cylindrical, straight, 5–15 \times 1.5–5.5 μm . *Conidiogenous cells* phialidic, hyaline, terminal, cylindrical, 6–12 \times 1–3 μm , tapered towards the apex. *Paraphyses* not observed. *Alpha conidia* unicellular, aseptate, fusiform, hyaline, 1–4-guttulate with acute ends, 4.5–7 \times 1.5–3 μm , mean \pm SD = 5.9 \pm 0.6 \times 2.2 \pm 0.4 μm , L/B ratio = 2.7. *Beta conidia* and *Gamma conidia* not observed.

Culture characteristics: Colonies covering the dish within 1 wk at 21 $^{\circ}\text{C}$, surface mycelium flattened, dense and felt-like. Colony on MEA and OA at first white, becoming yellowish, flat, with dense and felted mycelium, reverse pale sepia with brownish dots with age, with visible solitary or aggregated conidiomata at maturity. On PDA cream to smoke-grey, reverse pale brown.

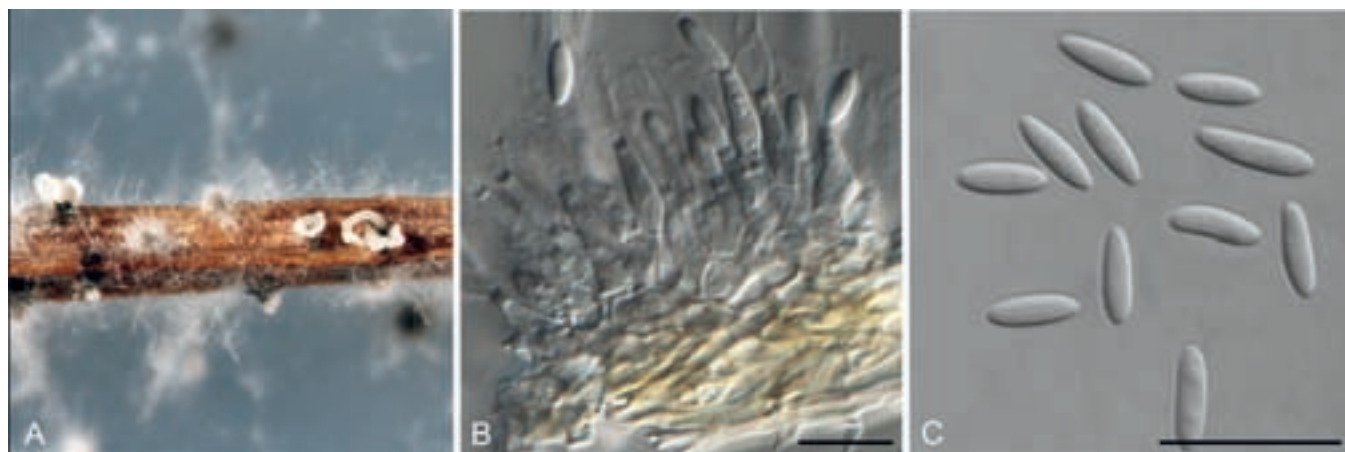


Fig. 5. *Diaporthe melitensis* (CBS 142551). **A.** Conidiomata sporulating on PNA. **B.** Conidiogenous cells. **C.** Alpha conidia. Bars = 10 μm .

Notes: *Diaporthe melitensis* was isolated from trunk samples of *Citrus limon* showing serious cankers in Gozo (Malta). The two strains representing *D. melitensis* cluster in a well-supported clade, and appear closely related to *D. pseudomangiferae* and *D. arengae*. This species is phylogenetically closely related to, but clearly differentiated from, *D. limonicola* (described above) by 22 different unique fixed alleles in ITS, *tef1* and *tub2* loci (22, 2, and 47 respectively) based on the alignments deposited in TreeBASE.

Morphologically *D. melitensis* differs from *D. pseudomangiferae* in the shorter alpha conidia (4.5–7 vs. 7–9 µm) (Gomes *et al.* 2013).

Additional material examined: **Malta:** Gozo, from branch canker of *Citrus limon*, 22 Sep. 2015, V. Guarnaccia (culture CBS 142552 = CPC 27875).

PATHOGENICITY

After 30 d all the isolates of the inoculated species induced lesions on most of the *Citrus* species tested. The inoculated

twigs developed cankers similar to those detected in the field, and the fungi were successfully re-isolated, fulfilling Koch's postulates (Fig. 6). Cankers and internal discoloration were observed in correspondence to inoculation points. On the contrary, no symptoms were observed on the control plants. Clear differences in aggressiveness among the isolates and susceptibility of the *Citrus* species were observed: *D. limonicola* and *D. melitensis* caused the most serious symptoms with no difference among the hosts. *Diaporthe foeniculina* was weakly aggressive to each *Citrus* species. Similarly, *D. novem* was weakly aggressive on all the hosts except the orange clones, whilst *D. baccae* caused disease symptoms only on mandarin.

DISCUSSION

After a major screening of fungal diseases of citrus in Europe (Guarnaccia *et al.* 2017a, b, Sandoval-Denis *et al.* 2018), molecular phylogenetic and morphological analyses were used to evaluate the diversity of *Diaporthe* species in the Mediterranean basin, focusing on symptomatic plants.

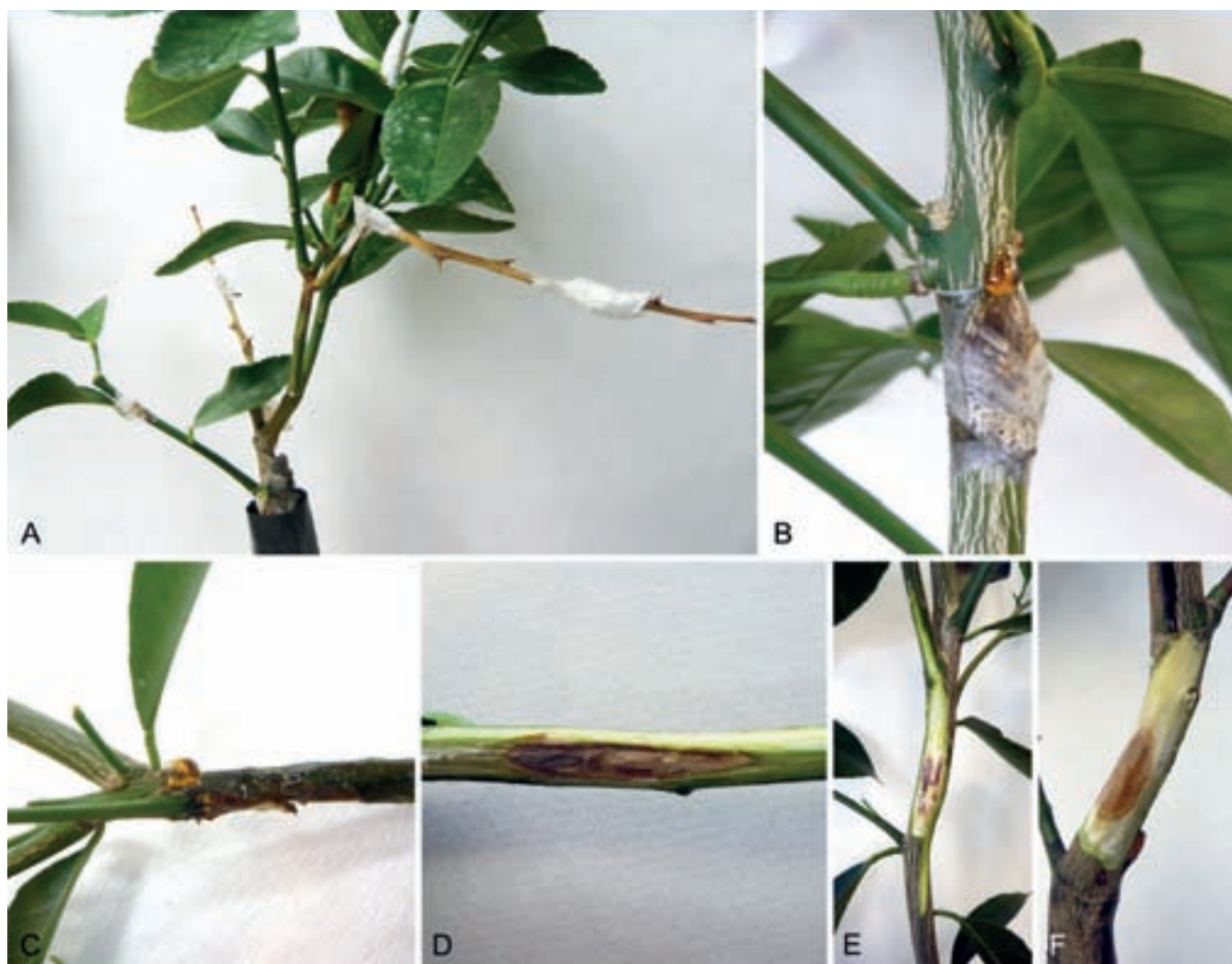


Fig. 6. Pathogenicity test of selected *Diaporthe* isolates on citrus plants after 30 d. **A.** Shoot blight of lime plants inoculated with *D. novem* (CPC 26188). **B–C.** Cankers with gummosis of lemon plants caused by *D. limonicola* and *D. melitensis* (CPC 28200, CPC 27873). **D–E.** Internal discoloration of mandarin twigs inoculated respectively with *D. melitensis* and *D. baccae* (CPC 27873, CPC 26170). **F.** Internal lesion of orange branch caused by *D. foeniculina* (CPC 28081).

Several *Diaporthe* species are well established in Europe (Thomidis & Michailides 2009, Santos *et al.* 2011, Lombard *et al.* 2014, Guarnaccia *et al.* 2016). *Diaporthe* species are also frequently associated with citrus diseases worldwide (Timmer *et al.* 2000, Huang *et al.* 2013), such as melanose and stem-end rot. Since the late 18th century these diseases have affected different citrus organs and also cause a sort of wood gummosis (Fawcett 1936, Timmer *et al.* 2000, Mondal *et al.* 2007). *Diaporthe citri* is considered a key pathogen of *Citrus* species and has been confirmed from Brazil, China, Korea, and New Zealand, and is also reported as widely spread throughout Asia, Australasia, and South America (Timmer *et al.* 2000, Mondal *et al.* 2007, Udayanga *et al.* 2014b). However, *D. citri* has never been reported from Europe, whilst *D. cytospora* and *D. foeniculina* have been recently isolated from citrus in Spain (Udayanga *et al.* 2014b).

DNA sequence data are essential in resolving taxonomic questions, redefining species boundaries, and the accurate naming of species required for effective communication about plant pathogens. Thus, during the past decade, a polyphasic approach was used in several *Diaporthe* studies, revealing new species involved with citrus diseases and as endophytes and plant pathogens (Huang *et al.* 2013, 2015). Santos *et al.* (2017a) showed that species separation is better when five loci (ITS, *tef1*, *tub2*, *his3*, and *cal*) are simultaneously used to build the phylogeny of *Diaporthe* isolates.

Citrus crops are already compromised by a range of fungal pathogens other than *Diaporthe* (Vicent *et al.* 2007, Aiello *et al.* 2015, Guarnaccia *et al.* 2017a, Sandoval-Denis *et al.* 2018). Considering that no surveys for citrus diseases caused by *Diaporthe* had been performed in Europe, a large-scale investigation of *Diaporthe* species associated with citrus infections in Europe was needed. This study provides the first molecular characterization of *Diaporthe* diversity related to citrus production in Europe, combined with morphological characterisation.

Several citrus orchards, plant nurseries, private gardens and collections in five Mediterranean European countries were investigated. We further investigated different host plants in *Citrus*-allied genera such as *Microcitrus*, which is also economically important for fruit production.

Canker symptoms were frequently observed on several *Citrus* species in all countries investigated. Twigs showed wilting, dieback, wither-tip, and gummosis. Some orchards presented branch blight and trunk cankers associated with abundant gummosis. The most critical situation seen was in different lemon orchards in Malta, where the infections led to tree death. Melanose and stem-end rot were never observed.

We collected 79 *Diaporthe* strains. Phylogenetic analyses based on single and the combined five loci (ITS, *tef1*, *tub2*, *his3*, and *cal*), as well as morphological characters, revealed five *Diaporthe* species associated with infections on several *Citrus* species in Europe. We included in the analysis the closest taxa to the five *Diaporthe* species recovered in this study, based on BLAST searches of NCBI's GenBank nucleotide database. The final phylogenetic tree distinguished two newly described species (*D. limonicola* and *D. melitensis*) and three known species (*D. baccae*, *D. foeniculina*, and *D. novem*). Moreover, a known clade represented by three strains (CBS 199.39, CBS 230.52, CPC 20322), previously

named *D. citri*, appeared in our final tree. However, this clade also required a separate name as *D. citri* s. str. is restricted to the pathogen causing melanose and stem-end rot of citrus fruit (Udayanga *et al.* 2014b). Thus, in this study we have described these three isolates as *D. infertilis*. Based on sampling in this study, *D. citri* appears to be absent in Europe as previously reported by Udayanga *et al.* (2014b).

Huang *et al.* (2015) obtained two separate groups of citrus isolates within the *D. arecae* complex, which were either not well supported or non-monophyletic based on a four-locus phylogenetic analysis. However, our analysis based on five loci, combined with morphological observations, clearly separated both *D. limonicola* and *D. melitensis* from *D. pseudomangiferae* and *D. arengae*, the most closely related species, and from other species in the *D. arecae* complex such as *D. podocarpimacrophylli* and *D. xishuangbanica* (Gao *et al.* 2017). Morphologically, *D. limonicola* and *D. melitensis* differ from *D. pseudomangiferae* in the shorter alpha conidia. Moreover, *D. limonicola* is the only taxon among these species to produce beta and gamma conidia.

Diaporthe foeniculina was the predominant species found in all the Mediterranean countries sampled, but its pathogenicity on *Citrus* was unknown (Udayanga *et al.* 2014b). Recently, Lombard *et al.* (2014) described *D. baccae* as a new species associated with *Vaccinium corymbosum* cankers in Italy. Similarly, we found this species associated with twig, branch and trunk cankers of citrus in Italy. *Diaporthe novem* was isolated for the first time from infected citrus plants in our study, where it was found associated with twig dieback of *C. japonica* (kumquat) and *C. aurantiifolia* (lime) in Italy. Moreover, the newly described species were isolated from devastated lemon plants in several orchards on Malta: *D. limonicola* was recovered from symptomatic trunks and branches, whilst *D. melitensis* was isolated only from branches. They were isolated separately and from the same affected sample. Colonization of the same host plant by diverse *Diaporthe* species appears to be frequent as previously reported (Crous & Groenewald 2005, Van Niekerk *et al.* 2005, Thompson *et al.* 2011).

Our results reveal a large diversity of *Diaporthe* species spanning several clades and species complexes, associated with citrus wood cankers in European countries. These include *D. baccae*, *D. infertilis*, *D. novem*, and the two newly described species. In total, 22 *Diaporthe* species are now confirmed as associated with citrus.

Pathogenicity of the species isolated from citrus samples collected in Europe was tested on healthy plants of lemon, lime, mandarin, and two clones of *Citrus sinensis* ('New Hall' and 'Tarocco Meli'). All of the *Diaporthe* species tested caused lesions to develop on twigs. Recently, *D. foeniculina* (syn. *D. neotheicola*) has been reported as causing disease in many other hosts: shoot blight of persimmon in Australia (Golzar *et al.* 2012), kiwi-fruit disease in Greece (Thomidis *et al.* 2013), and avocado branch cankers (Guarnaccia *et al.* 2016). This species evidently has the ability to infect a wide range of fruits and plant hosts as an opportunistic pathogen. *Diaporthe foeniculina* (as "*D. foeniculacea*" in Gomes *et al.* 2013) proves to be a pathogen with a broad host range amongst temperate woody plants and fruit trees. In our study, *D. foeniculina* was

isolated from symptomatic plants of eight *Citrus* species (*C. bergamia*, *C. japonica*, *C. limon*, *C. maxima*, *C. mitis*, *C. paradisi*, *C. reticulata*, and *C. sinensis*) and also *Microcitrus australasica*. In the pathogenicity tests, it was weakly aggressive, but produced lesions on each species tested.

These results demonstrate a cross-infection potential of multiple *Diaporthe* species on different *Citrus* species, as previously reported (Lombard *et al.* 2014, Guarnaccia *et al.* 2016). *Diaporthe limonicola* and *D. melitensis* caused prominent symptoms in all the citrus species inoculated, and because they were isolated from plants with severe disease symptoms, these species can be considered as potentially major new pathogens of *Citrus limon*. *Diaporthe baccae* caused symptoms only on mandarin, while *D. novem* infected lime, lemon, and mandarin plants. Both of these species seemed to be weakly aggressive, with different host susceptibility and known distribution. These fungi merit adding to the list of fungal taxa causing citrus cankers worldwide (Adesemoye *et al.* 2014, Mayorquin *et al.* 2016, Sandoval-Denis *et al.* 2018).

This study provides the first overview of *Diaporthe* diversity associated with cankers of citrus plants in Europe, and includes information on their pathogenicity. Two of the new species described were established as causal agents of a devastating disease of lemon plants, inducing branch and trunk cankers that lead to plant death. The present study also appears to represent the first reports of *D. baccae* and *D. novem* associated with citrus disease in Europe. Despite the worldwide distribution and economical importance of citrus, knowledge of the fungal species associated with *Citrus* species is still incomplete. Further studies are required in order to fully elucidate the host range, specificity, and global distribution of *Diaporthe* species, as well as other fungi causing cankers of citrus plants.

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REFERENCES

- Adesemoye AO, Mayorquin JS, Wang DH, Twizeyimana S, Lynch C, *et al.* (2014) Identification of species of *Botryosphaeriaceae* causing bot gummosis in citrus in California. *Plant Disease* **98**: 55–61.
- Aiello D, Carrieri R, Guarnaccia V, Vitale A, Lahoz E, *et al.* (2015) Characterization and pathogenicity of *Colletotrichum gloeosporioides* and *C. karstii* causing preharvest disease on *Citrus sinensis* in Italy. *Journal of Phytopathology* **163**: 168–177.
- Burnett HC (1962) *Melanose: Diaporthe citri* (Fawc) Wolf. [Plant Pathology Circular no. 2.] Gainesville: Florida Department of Agriculture, Division of Plant Industry.
- Carbone I, Kohn LM (1999) A method for designing primer sets for the speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553–556.
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004b) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.
- Crous PW, Groenewald JZ (2005) Hosts, species and genotypes: opinions versus data. *Australasian Plant Pathology* **34**: 463–470.
- Crous PW, Groenewald JZ, Risede JM, *et al.* (2004a) *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Studies in Mycology* **50**: 415–430.
- Crous PW, Groenewald JZ, Shivas RG, Edwards J, Seifert KA, *et al.* (2011a) Fungal Planet description sheets: 69–91. *Persoonia* **26**: 108–156.
- Crous PW, Summerell BA, Swart L, Denman S, Taylor JE, *et al.* (2011b) Fungal pathogens of *Proteaceae*. *Persoonia* **27**: 20–45.
- Crous PW, Verkley GJM, Groenewald JZ, *et al.* (2009) *Fungal Biodiversity*. [CBS Laboratory Manual Series no. 1]. Utrecht: CBS-KNAW Fungal Biodiversity Institute.
- Crous PW, Wingfield MJ, Richardson DM, Le Roux JJ, Strasberg D, *et al.* (2016) Fungal planet description sheets: 400–468. *Persoonia* **36**: 316–458.
- Dai J, Krohn K, Floerke U, Gehle D, Aust HJ, *et al.* (2005) Novel highly substituted biaryl ethers, phomopsines D-G, isolated from endophytic fungus *Phomopsis* sp. from *Adenocarpus foliolosus*. *European Journal of Organic Chemistry* **23**: 5100–5105.
- Dai DQ, Wijayawardene NN, Bhat DJ, Chuokeatirote E, Bahkali AH, *et al.* (2014) *Pustulomyces* gen. nov. accommodated in *Diaporthaceae*, *Diaporthales*, as revealed by morphology and molecular analyses. *Cryptogamie, Mycologie* **35**: 63–72.
- Díaz GA, Latorre BA, Lolos M, Ferrada E, Naranjo P, *et al.* (2017) Identification and characterization of *Diaporthe ambigua*, *D. australafricana*, *D. novem*, and *D. rudis* causing a postharvest fruit rot in kiwifruit. *Plant Disease* **101**: 1402–1410.
- Diogo ELF, Santos JM, Phillips AJL (2010) Phylogeny, morphology and pathogenicity of *Diaporthe* and *Phomopsis* species on almond in Portugal. *Fungal Diversity* **44**: 107–115.
- Fawcett HS (1912) The cause of stem-end rot of Citrus fruits (*Phomopsis citri* n. sp.). *Phytopathology* **2**: 109–113.
- Fawcett HS (1922) A *Phomopsis* of citrus in California. *Phytopathology* **12**: 107.
- Fawcett HS (1936) *Citrus Diseases and their Control*. New York: McGraw Hill.
- Food and Agricultural Organization (2016) *Citrus fruits fresh and processed: annual statistics*. Rome: Food and Agricultural Organization; <http://www.fao.org/3/a-i5558e.pdf>
- Fu CH, Hsieh HM, Chen CY, Chang TT, Huang YM, *et al.* (2013) *Ophiodiaporthe cyatheae* gen. et sp. nov., a diaporthalean pathogen causing a devastating wilt disease of *Cyathea lepifera* in Taiwan. *Mycologia* **105**: 861–872.
- Gao Y, Su YY, Sun W, Cai L (2015) *Diaporthe* species occurring on *Lithocarpus glabra* in china, with descriptions of five new species. *Fungal Biology* **119**: 295–309.
- Gao YH, Liu F, Cai L (2016) Unravelling *Diaporthe* species associated with *Camellia*. *Systematics and Biodiversity* **14**: 102–117.
- Gao YH, Lui F, Duan W, Crous PW, Cai L (2017) *Diaporthe* is paraphyletic. *IMA Fungus* **8**: 153–187.
- García-Reyne A, López-Medrano F, Morales JM, Esteban CG, Martín I, *et al.* (2011) Cutaneous infection by *Phomopsis longicolla* in a renal transplant recipient from Guinea: first report of human infection by this fungus. *Transplant Infectious Disease* **13**: 204–207.
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes

- from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323–1330.
- Golzar H, Tan YP, Shivas RG, Wang C (2012) First report of shoot blight of persimmon caused by *Diaporthe neotheicola* in Australia. *Australasian Plant Disease Notes* **7**: 115–117.
- Gomes R, Glienke C, Videira S, Lombard L, Groenewald J, *et al.* (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia* **31**: 1–41.
- Grasso FM, Marini M, Vitale A, Firrao G, Granata G (2012) Canker and dieback on *Platanus* × *acerifolia* caused by *Diaporthe scabra*. *Forest Pathology* **42**: 510–513.
- Guarnaccia V, Groenewald JZ, Polizzi G, Crous PW (2017a) High species diversity in *Colletotrichum* associated with citrus diseases in Europe. *Persoonia* **39**: 32–50.
- Guarnaccia V, Groenewald JZ, Li H, Glienke C, Carstens E, *et al.* (2017b) First report of *Phyllosticta citricarpa* and description of two new species, *P. paracapitalensis* and *P. paracitricarpa*, from citrus in Europe. *Studies in Mycology* **87**: 161–185.
- Guarnaccia V, Vitale A, Cirvilleri G, Aiello D, Susca A, *et al.* (2016) Characterisation and pathogenicity of fungal species associated with branch cankers and stem-end rot of avocado in Italy. *European Journal of Plant Pathology* **146**: 963–976.
- Habib W, Gerges E, Antelmi I, Baroudy F, Choueiri E, *et al.* (2015) *Diaporthe foeniculina* associated with severe shoot blight of lemon in Lebanon. *Phytopathologia Mediterranea* **54**: 149–150.
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182–192.
- Huang F, Hou X, Dewdney MM, Fu YS, Chen GQ, *et al.* (2013) *Diaporthe* species occurring on citrus in China. *Fungal Diversity* **61**: 237–250.
- Huang F, Udayanga D, Wang X, Hou X, Mei X, *et al.* (2015) Endophytic *Diaporthe* associated with *Citrus*: A phylogenetic reassessment with seven new species from China. *Fungal Biology* **119**: 331–347.
- Isaka M, Jaturapat A, Rukseree K, Danwisetkanjana K, Tanticharoen M, *et al.* (2001) Phomoxanthonones A and B, novel xanthone dimers from the endophytic fungus *Phomopsis* species. *Journal of Natural Products* **64**: 1015–1018.
- Katoh K, Standley DM (2013) MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Kucharek T, Whiteside J, Brown E (1983) *Melanose and Stem end rot of Citrus*. [Plant pathology fact sheet.] Gainesville: Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**: 1870–1874.
- Kumaran RS, Hur B (2009) Screening of species of the endophytic fungus *Phomopsis* for the production of the anticancer drug taxol. *Biotechnology and Applied Biochemistry* **54**: 21–30.
- Kumaresan V, Suryanarayanan TS (2001) Occurrence and distribution of endophytic fungi in a mangrove community. *Mycological Research* **105**: 1388–1391.
- Lamprecht SC, Crous PW, Groenewald JZ, Tewoldemedhin YT, Marasas WF (2011) *Diaportheaceae* associated with root and crown rot of maize. *IMA Fungus* **2**: 13–24.
- Lombard L, Van Leeuwen GCM, Guarnaccia V, Polizzi G, Van Rijswijk PC, *et al.* (2014) *Diaporthe* species associated with *Vaccinium*, with specific reference to Europe. *Phytopathologia Mediterranea* **53**: 287–299.
- Mahadevakumar S, Yadav V, Tejaswini GS, Sandeep SN, Janardhana GR (2014) First report of *Phomopsis citri* associated with dieback of *Citrus lemon* in India. *Plant Disease* **98**: 1281–1281.
- Mayorquin JS, Wang DH, Twizeyimana M, Eskalen A (2016) Identification, distribution, and pathogenicity of *Diatrypaceae* and *Botryosphaeriaceae* associated with citrus branch canker in the southern California desert. *Plant Disease* **100**: 2402–2413.
- Mondal SN, Agostini JP, Zhang L, Timmer LW. (2004) Factors affecting pycnidium production of *Diaporthe citri* on detached citrus twigs. *Plant Disease* **88**: 379–382.
- Mondal SN, Vicent A, Reis RF, Timmer LW (2007) Saprophytic colonization of citrus twigs by *Diaporthe citri* and factors affecting pycnidial production and conidial survival. *Plant Disease* **91**: 387–392.
- Mostert L, Crous PW, Kang JC, Phillips AJL (2001a) Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. *Mycologia* **93**: 146–167.
- Mostert L, Kang JC, Crous PW, Denman S (2001b) *Phomopsis saccharata* sp. nov., causing a canker and die-back disease of *Protea repens* in South Africa. *Sydowia* **53**: 227–235.
- Muralli TS, Suryanarayanan TS, Geeta R (2006) Endophytic *Phomopsis* species: host range and implications for diversity estimates. *Canadian Journal of Microbiology* **52**: 673–680.
- Nylander JAA (2004) *MrModeltest v. 2*. Programme distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.
- O'Donnell K, Nirenberg HI, Aoki T, Cigelnik E (2000) A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. *Mycoscience* **41**: 61–78.
- Polizzi G, Aiello D, Vitale A, Giuffrida F, Groenewald JZ, *et al.* (2009) First report of shoot blight, canker, and gummosis caused by *Neoscytalidium dimidiatum* on citrus in Italy. *Plant Disease* **93**: 1215.
- Rayner RW (1970) *A Mycological Colour Chart*. Kew: Commonwealth Mycological Institute.
- Rehm H (1914) Ascomycetes Philippinenses VI. *Leaflets of Philippine Botany* **6**: 2258–2281.
- Rehner SA, Uecker FA (1994) Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. *Canadian Journal of Botany* **72**: 1666–1674.
- Ronquist F, Teslenko M, Mark P van der, *et al.* (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- Rossmann AY, Adams GC, Cannon PF, Castlebury LA, Crous PW *et al.* (2015) Recommendations of generic names in *Diaporthales* competing for protection or use. *IMA Fungus* **6**: 145–154.
- Sandoval-Denis M, Guarnaccia V, Polizzi G, Crous PW (2018) Symptomatic citrus trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species. *Persoonia* **40**: 1–25.
- Santos L, Alves A, Alves R (2017a) Evaluating multi-locus phylogenies for species boundaries determination in the genus *Diaporthe*. *PeerJ* **5**: e3120; DOI 10.7717/peerj.3120.
- Santos JM, Phillips AJL, Crous PW, Alves A (2017b) *Diaporthe* species on *Rosaceae* with descriptions of *D. pyracanthae* sp. nov. and *D. malorum* sp. nov. *Mycosphere* **8**: 485–511.

- Santos JM, Phillips AJL (2009) Resolving the complex of *Diaporthe* (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. *Fungal Diversity* **34**: 111–125.
- Santos JM, Vrandečić K, Čosić J, Duvnjak T, Phillips AJL (2011) Resolving the *Diaporthe* species occurring on soybean in Croatia. *Persoonia* **27**: 9–19.
- Santos PJC, Savi DC, Gomes RR, Goulin EH, da Senkiv C, et al. (2016) *Diaporthe endophytica* and *D. terebinthifolii* from medicinal plants for biological control of *Phyllosticta citricarpa*. *Microbiological Research* **186–187**: 153–160.
- Senanayake IC, Crous PW, Groenewald JZ, Maharachchikumbura SSN, Jeewon R, et al. (2017) Families of *Diaporthales* based on morphological and phylogenetic evidence. *Studies in Mycology*. doi: 10.1016/j.simyco.2017.07.003.
- Smith H, Wingfield MJ, Crous PW, Coutinho TA (1996) *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86–88.
- Swofford DL (2003) *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, v. 4.0b10. Sunderland, MA: Sinauer Associates.
- Thomidis T, Exadaktylou E, Chen S (2013) *Diaporthe neotheicola*, a new threat for kiwi fruit in Greece. *Crop Protection* **47**: 35–40.
- Thomidis T, Michailides TJ (2009) Studies on *Diaporthe eres* as a new pathogen of peach trees in Greece. *Plant Disease* **93**: 1293–1297.
- Timmer LW, Garnsey SM, Graham JH (2000) *Compendium of Citrus Diseases*. 2nd edn. St Paul, MN: American Phytopathological Society Press.
- Timmer LW, Kucharek TA (2001) *Melanose (revised)*. [Fact Sheet PP-150.] Gainesville: Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.
- Thompson S, Tan Y, Young A, Neate S, Aitken E, et al. (2011) Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe* (*Phomopsis*) species. *Persoonia* **27**: 80–89.
- Thompson S, Tan Y, Shivas R, Neate S, Morin L, et al. (2014) Green and brown bridges between weeds and crops reveal novel *Diaporthe* species in Australia. *Persoonia* **35**: 39–49.
- Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD (2015) The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. *Fungal Biology* **119**: 383–407.
- Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD (2014a) Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. *Fungal Diversity* **67**: 203–229.
- Udayanga D, Castlebury LA, Rossman AY, Hyde KD (2014b) Species limits in *Diaporthe*: molecular re-assessment of *D. citri*, *D. cytospora*, *D. foeniculina* and *D. rudis*. *Persoonia* **32**: 83–101.
- Udayanga D, Liu X, McKenzie EHC, Chukeatirote E, Bahkali AHA, et al. (2011) The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. *Fungal Diversity* **50**: 189–225.
- Udayanga D, Xingzhong L, Crous PW, McKenzie EHC, Chukeatirote E, et al. (2012) A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). *Fungal Diversity* **56**: 157–171.
- Uecker FA (1988) A world list of *Phomopsis* names with notes on nomenclature, morphology and biology. *Mycological Memoirs* **13**: 1–231.
- Van der Aa HA, Noordeloos ME, De Gruyter J (1990) Species concepts in some larger genera of the coelomycetes. *Studies in Mycology* **32**: 3–19.
- Van Niekerk JM, Groenewald JZ, Farr DF, Fourie PH, Halleen F, et al. (2005) Reassessment of *Phomopsis* species on grapevines. *Australasian Plant Pathology* **34**: 27–39.
- Van Rensburg JCJ, Lamprecht SC, Groenewald JZ, Castlebury LA, Crous PW (2006) Characterization of *Phomopsis* spp. associated with die-back of rooibos (*Aspalathus linearis*) in South Africa. *Studies in Mycology* **55**: 65–74.
- Vicent A, Armengol J, García-Jiménez J (2007) Rain fastness and persistence of fungicides for control of *Alternaria* brown spot of citrus. *Plant Disease* **91**: 393–399.
- Wehmeyer LE (1933) The genus *Diaporthe* Nitschke and its segregates. *University of Michigan Studies, Science Series* **9**: 1–349.
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds): 315–322. San Diego: Academic Press.
- Yang J, Xu F, Huang C, Li J, She Z, et al. (2010) Metabolites from the mangrove endophytic fungus *Phomopsis* sp. (#zsu-H76). *European Journal of Organic Chemistry* **19**: 3692–3695.