Persoonia 40, 2018: 135–153
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

ISSN (Online) 1878-9080
https://doi.org/10.3767/persoonia.2018.40.06

Diaporthe diversity and pathogenicity revealed from a broad survey of grapevine diseases in Europe

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Key words

canker
multi-locus sequence typing
pathogenicity
Vitis

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 grapevines, either as agents of pre- or post-harvest infections, including Phomopsis cane and leaf spot, cane bleaching, swelling arm and trunk cankers. In this study we explore the occurrence, diversity and pathogenicity of *Diaporthe* spp. associated with *Vitis vinifera* in major grape production areas of Europe and Israel, focusing on nurseries and vineyards. Surveys were conducted in Croatia, Czech Republic, France, Hungary, Israel, Italy, Spain and the UK. A total of 175 *Diaporthe* strains were isolated from asymptomatic and symptomatic shoots, 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 were determined. Preliminary pathogenicity tests were performed on green grapevine shoots with representative isolates. The most commonly isolated species were *D. eres* and *D. ampelina*. Four new *Diaporthe* species described here as *D. bohemiae*, *D. celeris*, *D. hispaniae* and *D. hungariae* were found associated with affected vines. Pathogenicity tests revealed *D. baccae*, *D. celeris*, *D. hispaniae* and *D. hungariae* as pathogens of grapevines. No symptoms were caused by *D. bohemiae*. This study represents the first report of *D. ambigua* and *D. baccae* on grapevines in Europe. The present study improves our understanding of the species associated with several disease symptoms on *V. vinifera* plants, and provides useful information for effective disease management.

Article info Received: 30 October 2017; Accepted: 5 January 2018; Published: 19 February 2018.

INTRODUCTION

Diaporthe species are endophytes in asymptomatic plants, plant pathogens, or saprobes on decaying tissues of a wide range of hosts (Carroll 1986, Muralli et al. 2006, Garcia-Reyne et al. 2011, Udayanga et al. 2011). *Diaporthe* species are widespread, and well-known as 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, Tan et al. 2013). Species of the genus have also been used in secondary metabolite research due to their production of a large number of polyketides and a variety of unique low- and high-molecular-weight metabolites with different antibacterial, anticancer, antifungal, antimalarial, antiviral, cytotoxic and herbicidal activities (Corsaro et al. 1998, Isaka et al. 2001, Dai et al. 2005, Kumaran & Hur 2009, Yang et al. 2010, Gomes et al. 2013, Chepkirui & Stadler 2017), and for biological control of fungal pathogens (Santos et al. 2016).

Following the abolishment of dual nomenclature for fungi, the generic names *Diaporthe* and *Phomopsis* are no longer used

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to distinguish different morphs of this genus, and Rossman et al. (2015) proposed that the genus name *Diaporthe* should be retained over *Phomopsis* because it was introduced first, represents the majority of species, and therefore has priority.

Diaporthe was historically considered as monophyletic based on its typical sexual morph and *Phomopsis* asexual morph (Gomes et al. 2013). However, Gao et al. (2017) recently revealed its paraphyletic nature, showing that *Mazzantia* (Wehmeyer 1926), *Ophiodiaporthe* (Fu et al. 2013), *Pustulomyces* (Dai et al. 2014), *Phaeocytostroma* and *Stenocarpella* (Lamprecht et al. 2011), are embedded in *Diaporthe* s.lat. Furthermore, Senanayake et al. (2017) recently showed additional genera included in *Diaporthe* s.lat., such as *Paradiaporthe* and *Chiangraiomyces*.

The initial species concept of *Diaporthe* based on the assumption of host-specificity (Uecker 1988), resulted in the introduction of almost 2 000 species names available for both *Diaporthe* and *Phomopsis* (www.MycoBank.org). Most *Diaporthe* species can be found on 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, Guarnaccia & Crous 2017). Thus, identification and description of species based on host association is not reliable within *Diaporthe* (Gomes et al. 2013, Udayanga et al. 2014a, b).

Before the molecular era, morphological characters such as size and shape of ascomata (Udayanga et al. 2011) and conidiomata (Rehner & Uecker 1994), were the basis on which to study the taxonomy of *Diaporthe* (Van der Aa et al. 1990). Recent studies demonstrated how these characters are not always informative 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 *Diaporthe* changed the taxonomy and species concepts in this genus, 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*. Several major recent studies revealed ± 170 species supported by molecular data (Gomes et al. 2013, Lombard et al. 2014, Udayanga et al. 2014a, b, 2015, Gao et al. 2017, Dissanayake et al. 2017). *Diaporthe* taxonomy is actively changing, with numerous species being described each year mostly based on molecular phylogenetic approaches and morphological characterisation (Gao et al. 2017, Guarnaccia & Crous 2017).

Recent plant pathology studies confirmed *Diaporthe* species to be associated with several diseases on a broad range of economically significant agricultural crops such as *Camellia*, *Citrus*, *Glycine*, *Helianthus*, *Persea*, *Vaccinium*, *Vitis*, vegetables, fruit crops and forest plants (Van Rensburg et al. 2006, Santos & Phillips 2009, Crous et al. 2011a, b, 2016, Santos et al. 2011, Thompson 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, Guarnaccia & Crous 2017).

Diaporthe species are commonly found associated with *V. vinifera* and have been reported to be associated with several major diseases of grapevines. Important studies described *Diaporthe* species associated with grapevines using morphology, pathogenicity and molecular data (Merrin et al. 1995, Kuo & Leu 1998, Phillips 1999, Scheper et al. 2000, Mostert et al. 2001a, Van Niekerk et al. 2005, Dissanayake et al. 2015, Cinelli et al. 2016). One of the most significant studies (Van Niekerk et al. 2005) used ITS sequence data combined with morphology to examine South African strains and additional isolates obtained from worldwide collections to reveal several species associated with grapevine, such as *D. ambigua*, *D. ampelina* (as *P. viticola*), *D. amygdali* (as *P. amygdali*), *D. australafricana*, *D. helianthi*, *D. kyushuensis* (as *P. vitimegaspora*), *D. perjuncta* and *D. rudis* (as *D. viticola*). Moreover, they distinguished eight undescribed distinct species (as *Phomopsis* spp. 1–8) from grapevines. Schilder et al. (2005) confirmed *D. ampelina* (as *P. viticola*) to be a widespread pathogen in the Great Lakes Region of North America on the basis of DNA sequences from *tef1* and *cal* gene regions. *Diaporthe ampelina* was also the most prevalent species isolated from grapevine cankers in California, where the occurrence of *D. ambigua*, *D. eres* and *D. foeniculina* (as *D. neotheicola*) was also reported in vineyards (Úrbez-Torres et al. 2013). Similarly, Baumgartner et al. (2013) identified *D. ampelina* and *D. eres* (as *P. fukushii*) in eastern North America. In Europe, *D. eres* was reported by Kaliterna et al. (2012) in Croatia and by Cinelli et al. (2016) in Italy. Four species of *Diaporthe* were identified after surveys in China, which included *D. eres*, *D. hongkongensis*, *D. phaseolorum* and *D. sojae*, and their pathogenicity was confirmed through artificial inoculation on detached grapevine twigs (Dissanayake et al. 2015).

Phomopsis cane and leaf spot is a major disease of grapevines, causing serious losses due to shoots breaking off at the base, stunting, dieback, loss of vigour, reduced bunch set and fruit rot (Pine 1958, 1959, Pscheidt & Pearson 1989, Pearson & Goheen 1994, Wilcox et al. 2015). Canes show brown to black necrotic irregular-shaped lesions, and clusters show rachis necrosis and brown, shrivelled berries close to harvest (Pearson & Goheen 1994). *Diaporthe ampelina* is historically the most common species known to cause this disease, which, together with *D. amygdali*, have been confirmed as severe pathogen of grapevines (Mostert et al. 2001a, Van Niekerk et al. 2005). Phomopsis cane and leaf spot is more severe in humid temperate climate regions, occurring throughout the growing season (Erincik et al. 2001). Recently, Úrbez-Torres et al. (2013) provided strong evidence for the role of *P. viticola* as a canker-causing organism, and suggested its addition to the fungi involved in the grapevine trunk diseases complex. Moreover, *D. ampelina* is the causal agent of grapevine swelling arm, induced also by *D. kyushuensis* (as *P. vitimegaspora*) (Kajitani & Kanematsu 2000, Van Niekerk et al. 2005). Cane bleaching is another grapevine symptom caused by *D. perjuncta* and *D. ampelina* (Kuo & Leu 1998, Kajitani & Kanematsu 2000, Mostert et al. 2001a, Rawnsley et al. 2004, Van Niekerk et al. 2005). *Diaporthe eres* was found as a weak to moderate pathogen causing wood-canker of vine (Kaliterna et al. 2012, Baumgartner et al. 2013).

Several diseases are often reported as caused by more than one *Diaporthe* species, or frequently, one *Diaporthe* species may cause various plant diseases (Santos & Phillips 2009, Diogo et al. 2010, Santos et al. 2011, Thompson et al. 2011, 2015). For example, *D. caulivora*, *D. longicolla, D. novem* and *D. phaseolorum* cause disease on soybean in Croatia (Santos et al. 2011). Sunflower stem blight is caused by *D. gulyae*, *D. helianthi*, *D. kochmanii* and *D. kongii* (Says-Lesage et al. 2002, Thompson et al. 2011). Devastating cankers caused by *D. limonicola* and *D. melitensis* were reported on lemon trees (Guarnaccia & Crous 2017). Moreover, *D. novem* has been reported as pathogen on *Aspalathus linearis*, *Citrus* spp., *Glycine max*, *Helianthus annuus* and *Hydrangea macrophylla* (Santos et al. 2011). Similarly, multiple *Diaporthe* species have been found associated with Phomopsis cane and leaf spot disease as well as cankers and swelling arm of grapevine (Phillips 1999, Kajitani & Kanematsu 2000, Mostert et al. 2001a, Rawnsley et al. 2004, Van Niekerk et al. 2005).

Only a few studies have dealt with the distribution of *Diaporthe* spp. on grapevine in Europe and other countries from the Mediterranean basin. Considering also the recent findings of *Diaporthe* species in different major grape production areas, and the changes in the species concepts, new surveys are required to study the occurrence and diversity of *Diaporthe* species related to grapevines and their association with diseases.

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

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	*tub2*	*his3*	*tef1*	*cal*
Diaporthe acaciigena	**CBS 129521**	*Acacia retinodes*	Australia	KC343005	KC343973	KC343489	KC343731	KC343247
D. alleghaniensis	**CBS 495.72**	*Betula alleghaniensis*	Canada	FJ889444	KC843228	KC343491	GQ250298	KC343249
D. alnea	**CBS 146.46**	*Alnus* sp.	Netherlands	KC343008	KC343976	KC343492	KC343734	KC343250
D. ambigua	CBS 187.87	*Helianthus annuus*	Italy	KC343015	KC343983	KC343499	KC343741	KC343257
	CBS 114015	*Pyrus communis*	South Africa	KC343010	KC343978	KC343494	KC343736	KC343252
	CBS 117167	*Aspalathus linearis*	South Africa	KC343011	KC343979	KC343495	KC343737	KC343253
	CBS 143342 = CPC 29648	*Vitis vinifera*	Spain	*MG280968*	*MG281141*	*MG281314*	*MG281489*	*MG281662*
	CPC 29652	*V. vinifera*	Spain	*MG280969*	*MG281142*	*MG281315*	*MG281490*	*MG281663*
D. ampelina	CBS 111888	*V. vinifera*	USA	KC343016	KC343984	KC343500	KC343742	KC343258
	CBS 114016	*V. vinifera*	France	AF230751	JX275452	–	GQ250351	JX197443
	CPC 28254	*V. vinifera*	UK	*MG280970*	*MG281143*	*MG281316*	*MG281491*	*MG281664*
	CPC 28255	*V. vinifera*	UK	*MG280971*	*MG281144*	*MG281317*	*MG281492*	*MG281665*
	CPC 28263	*V. vinifera*	UK	*MG280972*	*MG281145*	*MG281318*	*MG281493*	*MG281666*
	CPC 28269	*V. vinifera*	UK	*MG280973*	*MG281146*	*MG281319*	*MG281494*	*MG281667*
	CPC 28270	*V. vinifera*	UK	*MG280974*	*MG281147*	*MG281320*	*MG281495*	*MG281668*
	CPC 28271	*V. vinifera*	UK	*MG280975*	*MG281148*	*MG281321*	*MG281496*	*MG281669*
	CPC 28272	*V. vinifera*	UK	*MG280976*	*MG281149*	*MG281322*	*MG281497*	*MG281670*
	CPC 28273	*V. vinifera*	UK	*MG280977*	*MG281150*	*MG281323*	*MG281498*	*MG281671*
	CPC 28280	*V. vinifera*	UK	*MG280978*	*MG281151*	*MG281324*	*MG281499*	*MG281672*
	CBS 143345 = CPC 28424	*V. vinifera*	Italy	*MG280979*	*MG281152*	*MG281325*	*MG281500*	*MG281673*
	CPC 29326	*V. vinifera*	France	*MG280980*	*MG281153*	*MG281326*	*MG281501*	*MG281674*
	CPC 29328	*V. vinifera*	France	*MG280981*	*MG281154*	*MG281327*	*MG281502*	*MG281675*
	CPC 29396	*V. vinifera*	Israel	*MG280982*	*MG281155*	*MG281328*	*MG281503*	*MG281676*
	CPC 29397	*V. vinifera*	Israel	*MG280983*	*MG281156*	*MG281329*	*MG281504*	*MG281677*
	CPC 29398	*V. vinifera*	Israel	*MG280984*	*MG281157*	*MG281330*	*MG281505*	*MG281678*
	CPC 29399	*V. vinifera*	Israel	*MG280985*	*MG281158*	*MG281331*	*MG281506*	*MG281679*
	CPC 29634	*V. vinifera*	Spain	*MG280986*	*MG281159*	*MG281332*	*MG281507*	*MG281680*
	CPC 29662	*V. vinifera*	Spain	*MG280987*	*MG281160*	*MG281333*	*MG281508*	*MG281681*
	CPC 29663	*V. vinifera*	Spain	*MG280988*	*MG281161*	*MG281334*	*MG281509*	*MG281682*
	CPC 29664	*V. vinifera*	Spain	*MG280989*	*MG281162*	*MG281335*	*MG281510*	*MG281683*
	CPC 29665	*V. vinifera*	Spain	*MG280990*	*MG281163*	*MG281336*	*MG281511*	*MG281684*
	CPC 29666	*V. vinifera*	Spain	*MG280991*	*MG281164*	*MG281337*	*MG281512*	*MG281685*
	CPC 29668	*V. vinifera*	Spain	*MG280992*	*MG281165*	*MG281338*	*MG281513*	*MG281686*
	CPC 29674	*V. vinifera*	Spain	*MG280993*	*MG281166*	*MG281339*	*MG281514*	*MG281687*
	CPC 29675	*V. vinifera*	Spain	*MG280994*	*MG281167*	*MG281340*	*MG281515*	*MG281688*
	CPC 29676	*V. vinifera*	Spain	*MG280995*	*MG281168*	*MG281341*	*MG281516*	*MG281689*
	CPC 29821	*V. vinifera*	Czech Republic	*MG280996*	*MG281169*	*MG281342*	*MG281517*	*MG281690*
	CPC 29828	*V. vinifera*	Croatia	*MG280997*	*MG281170*	*MG281343*	*MG281518*	*MG281691*
	CPC 29829	*V. vinifera*	Croatia	*MG280998*	*MG281171*	*MG281344*	*MG281519*	*MG281692*
	CPC 29832	*V. vinifera*	Croatia	*MG280999*	*MG281172*	*MG281345*	*MG281520*	*MG281693*
	CPC 30076	*V. vinifera*	Hungary	*MG281000*	*MG281173*	*MG281346*	*MG281521*	*MG281694*
D. amygdali	**CBS 126679**	*Prunus dulcis*	Portugal	KC343022	KC343990	KC343506	KC343748	KC343264
D. anacardii	**CBS 720.97**	*Anacardium occidentale*	East Africa	KC343024	KC343992	KC343508	KC343750	KC343266
D. arecae	**CBS 161.64**	*Areca catechu*	India	KC343032	KC344000	KC343516	KC343758	KC343274
D. arengae	**CBS 114979**	*Arenga engleri*	Hong Kong	KC343034	KC344002	KC343518	KC343760	KC343276
D. australafricana	**CBS 111886**	*V. vinifera*	Australia	KC343038	KC344006	KC343522	KC343764	KC343280
D. baccae	**CBS 136972**	*Vaccinium corymbosum*	Italy	KJ160565	MF418509	MF418264	KJ160597	*MG281695*
	CBS 143343 = CPC 29330[3]	*V. vinifera*	France	*MG281001*	*MG281174*	*MG281347*	*MG281522*	*MG281696*
	CPC 29636	*V. vinifera*	Spain	*MG281002*	*MG281175*	*MG281348*	*MG281523*	*MG281697*
	CPC 29639	*V. vinifera*	Spain	*MG281003*	*MG281176*	*MG281349*	*MG281524*	*MG281698*
	CPC 29641[3]	*V. vinifera*	Spain	*MG281004*	*MG281177*	*MG281350*	*MG281525*	*MG281699*
	CPC 29651	*V. vinifera*	Spain	*MG281005*	*MG281178*	*MG281351*	*MG281526*	*MG281700*
	CPC 29659	*V. vinifera*	Spain	*MG281006*	*MG281179*	*MG281352*	*MG281527*	*MG281701*
	CPC 29660	*V. vinifera*	Spain	*MG281007*	*MG281180*	*MG281353*	*MG281528*	*MG281702*
	CPC 29661	*V. vinifera*	Spain	*MG281008*	*MG281181*	*MG281354*	*MG281529*	*MG281703*
	CPC 29669	*V. vinifera*	Spain	*MG281009*	*MG281182*	*MG281355*	*MG281530*	*MG281704*
	CPC 29670	*V. vinifera*	Spain	*MG281010*	*MG281183*	*MG281356*	*MG281531*	*MG281705*
	CPC 29671	*V. vinifera*	Spain	*MG281011*	*MG281184*	*MG281357*	*MG281532*	*MG281706*
	CPC 29673	*V. vinifera*	Spain	*MG281012*	*MG281185*	*MG281358*	*MG281533*	*MG281707*
	CPC 29827	*V. vinifera*	Croatia	*MG281013*	*MG281186*	*MG281359*	*MG281534*	*MG281708*
	CPC 30315	*V. vinifera*	Spain	*MG281014*	*MG281187*	*MG281360*	*MG281535*	*MG281709*
D. bicincta	**CBS 121004**	*Juglans* sp.	USA	KC343134	KC344102	KC343618	KC343860	KC343376
D. bohemiae	**CBS 143347 = CPC 28222**[3]	*Vitis* spp.	Czech Republic	*MG281015*	*MG281188*	*MG281361*	*MG281536*	*MG281710*
	CBS 143348 = CPC 28223[3]	*Vitis* spp.	Czech Republic	*MG281016*	*MG281189*	*MG281362*	*MG281537*	*MG281711*
D. carpini	CBS 114437	*Carpinus betulus*	Sweden	KC343044	KC344012	KC343528	KC343770	KC343286
D. celastrina	**CBS 139.27**	*Celastrus* sp.	USA	KC343047	KC344015	KC343531	KC343773	KC343289
D. celeris	**CBS 143349 = CPC 28262**[3]	*V. vinifera*	UK	*MG281017*	*MG281190*	*MG281363*	*MG281538*	*MG281712*
	CBS 143350 = CPC 28266[3]	*V. vinifera*	UK	*MG281018*	*MG281191*	*MG281364*	*MG281539*	*MG281713*
	CPC 28267	*V. vinifera*	UK	*MG281019*	*MG281192*	*MG281365*	*MG281540*	*MG281714*
D. citri	**CBS 135422**	*Citrus* sp.	USA	KC843311	KC843187	MF418281	KC843071	KC843157
D. citrichinensis	**CBS 134242**	*Citrus* sp.	China	JQ954648	MF418524	KJ420880	JQ954666	KC357494
D. cucurbitae	**DAOM42078**	*Cucumis sativus*	Canada	KM453210	KP118848	KM453212	KM453211	–
D. decedens	CBS 109772	*Corylus avellana*	Austria	KC343059	KC344027	KC343543	KC343785	KC343301
D. detrusa	CBS 109770	*Berberis vulgaris*	Austria	KC343061	KC344029	KC343545	KC343787	KC343303
D. eleagni	CBS 504.72	*Eleagnus* sp.	Netherlands	KC343064	KC344032	KC343548	KC343790	KC343306
D. eres	CBS 200.39	*Laurus nobilis*	Germany	KC343151	KC344119	KC343635	KC343877	KC343393
	CBS 439.82	*Cotoneaster* sp.	Scotland	KC343090	KC344058	KC343574	KC343816	KC343332

Table 1 (cont.)

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	*tub2*	*his3*	*tef1*	*cal*
D. eres (cont.)	CBS 587.79	*Pinus pentaphylla*	Japan	KC343153	KC344121	KC343637	KC343879	KC343395
	CBS 101742	*Fraxinus* sp.	Netherlands	KC343073	KC344041	KC343557	KC343799	KC343315
	CBS 113470	*Castanea sativa*	Australia	KC343146	KC344114	KC343630	KC343872	KC343388
	CBS 116953	*Pyrus pyrifolia*	New Zealand	KC343147	KC344115	KC343631	KC343873	KC343389
	CBS 135428	*Juglans cinerea*	USA	KC843328	KC843229	KJ420840	KC843121	KC843155
	CBS 138594	*Ulmus laevis*	Germany	KJ210529	KJ420799	KJ420850	KJ210550	KJ434999
	CBS 138597	*V. vinifera*	France	KJ210518	KJ420783	KJ420833	KJ210542	KJ434996
	CBS 143344 = CPC 28217	*V. vinifera*	Czech Republic	*MG281020*	*MG281193*	*MG281366*	*MG281541*	*MG281715*
	CPC 28218	*V. vinifera*	Czech Republic	*MG281021*	*MG281194*	*MG281367*	*MG281542*	*MG281716*
	CPC 28219	*V. vinifera*	Czech Republic	*MG281022*	*MG281195*	*MG281368*	*MG281543*	*MG281717*
	CPC 28220	*V. vinifera*	Czech Republic	*MG281023*	*MG281196*	*MG281369*	*MG281544*	*MG281718*
	CPC 28221	*V. vinifera*	Czech Republic	*MG281024*	*MG281197*	*MG281370*	*MG281545*	*MG281719*
	CPC 28226	*V. vinifera*	Czech Republic	*MG281025*	*MG281198*	*MG281371*	*MG281546*	*MG281720*
	CPC 28264	*V. vinifera*	UK	*MG281026*	*MG281199*	*MG281372*	*MG281547*	*MG281721*
	CPC 28274	*V. vinifera*	UK	*MG281027*	*MG281200*	*MG281373*	*MG281548*	*MG281722*
	CPC 28275	*V. vinifera*	UK	*MG281028*	*MG281201*	*MG281374*	*MG281549*	*MG281723*
	CPC 28276	*V. vinifera*	UK	*MG281029*	*MG281202*	*MG281375*	*MG281550*	*MG281724*
	CPC 28277	*V. vinifera*	UK	*MG281030*	*MG281203*	*MG281376*	*MG281551*	*MG281725*
	CPC 28278	*V. vinifera*	UK	*MG281031*	*MG281204*	*MG281377*	*MG281552*	*MG281726*
	CPC 28279	*V. vinifera*	UK	*MG281032*	*MG281205*	*MG281378*	*MG281553*	*MG281727*
	CPC 28423	*V. vinifera*	Italy	KT369109	KT369113	*MG281379*	KT369111	*MG281728*
	CPC 28426	*V. vinifera*	Italy	KT369110	KT369114	*MG281380*	KT369112	*MG281729*
	CPC 29317	*V. vinifera*	France	*MG281033*	*MG281206*	*MG281381*	*MG281554*	*MG281730*
	CPC 29331	*V. vinifera*	France	*MG281034*	*MG281207*	*MG281382*	*MG281555*	*MG281731*
	CPC 29633	*V. vinifera*	Spain	*MG281035*	*MG281208*	*MG281383*	*MG281556*	*MG281732*
	CPC 29635	*V. vinifera*	Spain	*MG281036*	*MG281209*	*MG281384*	*MG281557*	*MG281733*
	CPC 29638	*V. vinifera*	Spain	*MG281037*	*MG281210*	*MG281385*	*MG281558*	*MG281734*
	CPC 29643	*V. vinifera*	Spain	*MG281038*	*MG281211*	*MG281386*	*MG281559*	*MG281735*
	CPC 29677	*V. vinifera*	Spain	*MG281039*	*MG281212*	*MG281387*	*MG281560*	*MG281736*
	CPC 29678	*V. vinifera*	Spain	*MG281040*	*MG281213*	*MG281388*	*MG281561*	*MG281737*
	CPC 29694	*V. vinifera*	Hungary	*MG281041*	*MG281214*	*MG281389*	*MG281562*	*MG281738*
	CPC 29695	*V. vinifera*	Hungary	*MG281042*	*MG281215*	*MG281390*	*MG281563*	*MG281739*
	CPC 29820	*V. vinifera*	Czech Republic	*MG281043*	*MG281216*	*MG281391*	*MG281564*	*MG281740*
	CPC 29822	*V. vinifera*	Czech Republic	*MG281044*	*MG281217*	*MG281392*	*MG281565*	*MG281741*
	CPC 29823	*V. vinifera*	Czech Republic	*MG281045*	*MG281218*	*MG281393*	*MG281566*	*MG281742*
	CPC 29824	*V. vinifera*	Czech Republic	*MG281046*	*MG281219*	*MG281394*	*MG281567*	*MG281743*
	CPC 29825	*V. vinifera*	Czech Republic	*MG281047*	*MG281220*	*MG281395*	*MG281568*	*MG281744*
	CPC 29826	*V. vinifera*	Croatia	*MG281048*	*MG281221*	*MG281396*	*MG281569*	*MG281745*
	CPC 30055	*V. vinifera*	Croatia	*MG281049*	*MG281222*	*MG281397*	*MG281570*	*MG281746*
	CPC 30070	*V. vinifera*	Hungary	*MG281050*	*MG281223*	*MG281398*	*MG281571*	*MG281747*
	CPC 30072	*V. vinifera*	Hungary	*MG281051*	*MG281224*	*MG281399*	*MG281572*	*MG281748*
	CPC 30073	*V. vinifera*	Hungary	*MG281052*	*MG281225*	*MG281400*	*MG281573*	*MG281749*
	CPC 30074	*V. vinifera*	Hungary	*MG281053*	*MG281226*	*MG281401*	*MG281574*	*MG281750*
	CPC 30075	*V. vinifera*	Hungary	*MG281054*	*MG281227*	*MG281402*	*MG281575*	*MG281751*
	CPC 30077	*V. vinifera*	Hungary	*MG281055*	*MG281228*	*MG281403*	*MG281576*	*MG281752*
	CPC 30078	*V. vinifera*	Hungary	*MG281056*	*MG281229*	*MG281404*	*MG281577*	*MG281753*
	CPC 30080	*V. vinifera*	Hungary	*MG281057*	*MG281230*	*MG281405*	*MG281578*	*MG281754*
	CPC 30081	*V. vinifera*	Hungary	*MG281058*	*MG281231*	*MG281406*	*MG281579*	*MG281755*
	CPC 30082	*V. vinifera*	Hungary	*MG281059*	*MG281232*	*MG281407*	*MG281580*	*MG281756*
	CPC 30083	*V. vinifera*	Hungary	*MG281060*	*MG281233*	*MG281408*	*MG281581*	*MG281757*
	CPC 30084	*V. vinifera*	Hungary	*MG281061*	*MG281234*	*MG281409*	*MG281582*	*MG281758*
	CPC 30085	*V. vinifera*	Hungary	*MG281062*	*MG281235*	*MG281410*	*MG281583*	*MG281759*
	CPC 30087	*V. vinifera*	Hungary	*MG281063*	*MG281236*	*MG281411*	*MG281584*	*MG281760*
	CPC 30088	*V. vinifera*	Hungary	*MG281064*	*MG281237*	*MG281412*	*MG281585*	*MG281761*
	CPC 30089	*V. vinifera*	Hungary	*MG281065*	*MG281238*	*MG281413*	*MG281586*	*MG281762*
	CPC 30090	*V. vinifera*	Hungary	*MG281066*	*MG281239*	*MG281414*	*MG281587*	*MG281763*
	CPC 30091	*V. vinifera*	Hungary	*MG281067*	*MG281240*	*MG281415*	*MG281588*	*MG281764*
	CPC 30092	*V. vinifera*	Hungary	*MG281068*	*MG281241*	*MG281416*	*MG281589*	*MG281765*
	CPC 30093	*V. vinifera*	Hungary	*MG281069*	*MG281242*	*MG281417*	*MG281590*	*MG281766*
	CPC 30094	*V. vinifera*	Hungary	*MG281070*	*MG281243*	*MG281418*	*MG281591*	*MG281767*
	CPC 30095	*V. vinifera*	Hungary	*MG281071*	*MG281244*	*MG281419*	*MG281592*	*MG281768*
	CPC 30096	*V. vinifera*	Hungary	*MG281072*	*MG281245*	*MG281420*	*MG281593*	*MG281769*
	CPC 30098	*V. vinifera*	Hungary	*MG281073*	*MG281246*	*MG281421*	*MG281594*	*MG281770*
	CPC 30101	*V. vinifera*	Hungary	*MG281074*	*MG281247*	*MG281422*	*MG281595*	*MG281771*
	CPC 30102	*V. vinifera*	Hungary	*MG281075*	*MG281248*	*MG281423*	*MG281596*	*MG281772*
	CPC 30103	*V. vinifera*	Hungary	*MG281076*	*MG281249*	*MG281424*	*MG281597*	*MG281773*
	CPC 30104	*V. vinifera*	Hungary	*MG281077*	*MG281250*	*MG281425*	*MG281598*	*MG281774*
	CPC 30105	*V. vinifera*	Hungary	*MG281078*	*MG281251*	*MG281426*	*MG281599*	*MG281775*
	CPC 30106	*V. vinifera*	Hungary	*MG281079*	*MG281252*	*MG281427*	*MG281600*	*MG281776*
	CPC 30107	*V. vinifera*	Hungary	*MG281080*	*MG281253*	*MG281428*	*MG281601*	*MG281777*
	CPC 30108	*V. vinifera*	Hungary	*MG281081*	*MG281254*	*MG281429*	*MG281602*	*MG281778*
	CPC 30109	*V. vinifera*	Hungary	*MG281082*	*MG281255*	*MG281430*	*MG281603*	*MG281779*
	CPC 30111	*V. vinifera*	Hungary	*MG281083*	*MG281256*	*MG281431*	*MG281604*	*MG281780*
	CPC 30112	*V. vinifera*	Hungary	*MG281084*	*MG281257*	*MG281432*	*MG281605*	*MG281781*
	CPC 30113	*V. vinifera*	Hungary	*MG281085*	*MG281258*	*MG281433*	*MG281606*	*MG281782*
	CPC 30114	*V. vinifera*	Hungary	*MG281086*	*MG281259*	*MG281434*	*MG281607*	*MG281783*
	CPC 30115	*V. vinifera*	Hungary	*MG281087*	*MG281260*	*MG281435*	*MG281608*	*MG281784*

Table 1 (cont.)

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	*tub2*	*his3*	*tef1*	*cal*
D. eres (cont.)	CPC 30116	*V. vinifera*	Hungary	*MG281088*	*MG281261*	*MG281436*	*MG281609*	*MG281785*
	CPC 30119	*V. vinifera*	Hungary	*MG281089*	*MG281262*	*MG281437*	*MG281610*	*MG281786*
	CPC 30120	*V. vinifera*	Hungary	*MG281090*	*MG281263*	*MG281438*	*MG281611*	*MG281787*
	CPC 30121	*V. vinifera*	Hungary	*MG281091*	*MG281264*	*MG281439*	*MG281612*	*MG281788*
	CPC 30122	*V. vinifera*	Hungary	*MG281092*	*MG281265*	*MG281440*	*MG281613*	*MG281789*
	CPC 30123	*V. vinifera*	Hungary	*MG281093*	*MG281266*	*MG281441*	*MG281614*	*MG281790*
	CPC 30124	*V. vinifera*	Hungary	*MG281094*	*MG281267*	*MG281442*	*MG281615*	*MG281791*
	CPC 30125	*V. vinifera*	Hungary	*MG281095*	*MG281268*	*MG281443*	*MG281616*	*MG281792*
	CPC 30126	*V. vinifera*	Hungary	*MG281096*	*MG281269*	*MG281444*	*MG281617*	*MG281793*
	CPC 30127	*V. vinifera*	Hungary	*MG281097*	*MG281270*	*MG281445*	*MG281618*	*MG281794*
	CPC 30128	*V. vinifera*	Hungary	*MG281098*	*MG281271*	*MG281446*	*MG281619*	*MG281795*
	CPC 30131	*V. vinifera*	Hungary	*MG281099*	*MG281272*	*MG281447*	*MG281620*	*MG281796*
	CPC 30132	*V. vinifera*	Hungary	*MG281100*	*MG281273*	*MG281448*	*MG281621*	*MG281797*
	CPC 30133	*V. vinifera*	Hungary	*MG281101*	*MG281274*	*MG281449*	*MG281622*	*MG281798*
	CPC 30134	*V. vinifera*	Hungary	*MG281102*	*MG281275*	*MG281450*	*MG281623*	*MG281799*
	CPC 30135	*V. vinifera*	Hungary	*MG281103*	*MG281276*	*MG281451*	*MG281624*	*MG281800*
	CPC 30136	*V. vinifera*	Hungary	*MG281104*	*MG281277*	*MG281452*	*MG281625*	*MG281801*
	CPC 30137	*V. vinifera*	Hungary	*MG281105*	*MG281278*	*MG281453*	*MG281626*	*MG281802*
	CPC 30138	*V. vinifera*	Hungary	*MG281106*	*MG281279*	*MG281454*	*MG281627*	*MG281803*
	CPC 30139	*V. vinifera*	Hungary	*MG281107*	*MG281280*	*MG281455*	*MG281628*	*MG281804*
	CPC 30140	*V. vinifera*	Hungary	*MG281108*	*MG281281*	*MG281456*	*MG281629*	*MG281805*
	CPC 30141	*V. vinifera*	Hungary	*MG281109*	*MG281282*	*MG281457*	*MG281630*	*MG281806*
	CPC 30143	*V. vinifera*	Hungary	*MG281110*	*MG281283*	*MG281458*	*MG281631*	*MG281807*
	CPC 30144	*V. vinifera*	Hungary	*MG281111*	*MG281284*	*MG281459*	*MG281632*	*MG281808*
	CPC 30145	*V. vinifera*	Hungary	*MG281112*	*MG281285*	*MG281460*	*MG281633*	*MG281809*
	CPC 30146	*V. vinifera*	Hungary	*MG281113*	*MG281286*	*MG281461*	*MG281634*	*MG281810*
	CPC 30147	*V. vinifera*	Hungary	*MG281114*	*MG281287*	*MG281462*	*MG281635*	*MG281811*
	CPC 30148	*V. vinifera*	Hungary	*MG281115*	*MG281288*	*MG281463*	*MG281636*	*MG281812*
	CPC 30149	*V. vinifera*	Hungary	*MG281116*	*MG281289*	*MG281464*	*MG281637*	*MG281813*
	CPC 30150	*V. vinifera*	Hungary	*MG281117*	*MG281290*	*MG281465*	*MG281638*	*MG281814*
	CPC 30151	*V. vinifera*	Hungary	*MG281118*	*MG281291*	*MG281466*	*MG281639*	*MG281815*
	CPC 30152	*V. vinifera*	Hungary	*MG281119*	*MG281292*	*MG281467*	*MG281640*	*MG281816*
	CPC 30317	*V. vinifera*	Spain	*MG281120*	*MG281293*	*MG281468*	*MG281641*	*MG281817*
	CPC 30318	*V. vinifera*	Spain	*MG281121*	*MG281294*	*MG281469*	*MG281642*	*MG281818*
	CPC 30319	*V. vinifera*	Spain	*MG281122*	*MG281295*	*MG281470*	*MG281643*	*MG281819*
D. fibrosa	CBS 109751	*Rhamnus cathartica*	Austria	KC343099	KC344067	KC343583	KC343825	KC343341
D. foeniculina	CBS 187.27	*Camellia sinensis*	Italy	KC343107	KC344075	KC343591	KC343833	KC343349
	CBS 111553	*Foeniculum vulgare*	Spain	KC343101	KC344069	KC343585	KC343827	KC343343
	CBS 123209	*Foeniculum vulgare*	Portugal	KC343105	KC344073	KC343589	KC343831	KC343347
D. helianthi	**CBS 592.81**	*Helianthus annuus*	Serbia	KC343115	KC344083	KC343599	KC343841	JX197454
D. helicis	**CBS 138596**	*Hedera helix*	France	KJ210538	KJ420828	KJ420875	KJ210559	KJ435043
D. hispaniae	**CBS 143351 = CPC 30321**[3]	*V. vinifera*	Spain	*MG281123*	*MG281296*	*MG281471*	*MG281644*	*MG281820*
	CBS 143352 = CPC 30323[3]	*V. vinifera*	Spain	*MG281124*	*MG281297*	*MG281472*	*MG281645*	*MG281821*
D. hongkongensis	**CBS 115448**	*Dichroa febrifuga*	China	KC343119	KC344087	KC343603	KC343845	KC343361
D. hungariae	CPC 30129	*V. vinifera*	Hungary	*MG281125*	*MG281298*	*MG281473*	*MG281646*	*MG281822*
	CBS 143353 = CPC 30130[3]	*V. vinifera*	Hungary	*MG281126*	*MG281299*	*MG281474*	*MG281647*	*MG281823*
	CBS 143354 = CPC 30142[3]	*V. vinifera*	Hungary	*MG281127*	*MG281300*	*MG281475*	*MG281648*	*MG281824*
	CPC 30316	*V. vinifera*	Spain	*MG281128*	*MG281301*	*MG281476*	*MG281649*	*MG281825*
	CPC 30320	*V. vinifera*	Spain	*MG281129*	*MG281302*	*MG281477*	*MG281650*	*MG281826*
	CPC 30322	*V. vinifera*	Spain	*MG281130*	*MG281303*	*MG281478*	*MG281651*	*MG281827*
D. impulsa	CBS 114434	*Sorbus aucuparia*	Sweden	KC343121	KC344089	KC343605	KC343847	KC343363
D. inconspicua	**CBS 133813**	*Maytenus ilicifolia*	Brazil	KC343123	KC344091	KC343607	KC343849	KC343365
D. infecunda	**CBS 133812**	*Schinus terebinthifolius*	Brazil	KC343126	KC344094	KC343610	KC343852	KC343368
D. neilliae	**CBS 144.27**	*Spiraea* sp.	USA	KC343144	KC344112	KC343628	KC343870	KC343386
D. nothofagi	**BRIP 54801**	*Nothofagus cunninghamii*	Australia	JX862530	KF170922	–	JX862536	–
D. novem	**CBS 127271**	*Glycine max*	Croatia	KC343157	KC344125	KC343641	KC343883	KC343399
D. oncostoma	CBS 589.78	*Robinia pseudoacacia*	France	KC343162	KC344130	KC343646	KC343888	KC343404
D. perjuncta	**CBS 109745**	*Ulmus glabra*	Austria	KC343172	KC344140	KC343656	KC343898	KC343414
D. perseae	CBS 151.73	*Persea gratissima*	Netherlands	KC343173	KC344141	KC343657	KC343899	KC343415
D. phaseolorum	CBS 113425	*Olearia* cf. *rani*	New Zealand	KC343174	KC344142	KC343658	KC343900	KC343416
	CBS 127465	*Actinidia chinensis*	New Zealand	KC343177	KC344145	KC343661	KC343903	KC343419
D. pseudomangiferae	**CBS 101339**	*Mangifera indica*	Dominican Republic	KC343181	KC344149	KC343665	KC343907	KC343423
D. pseudophoenicicola	**CBS 462.69**	*Phoenix dactylifera*	Spain	KC343184	KC344152	KC343668	KC343910	KC343426
D. pulla	**CBS 338.89**	*Hedera helix*	Yugoslavia	KC343152	KC344120	KC343636	KC343878	KC343394
D. rudis	CBS 266.85	*Rosa rugosa*	Netherlands	KC343237	KC344205	KC343721	KC343963	KC343479
	CBS 109292	*Laburnum anagyroides*	Austria	KC843331	KC843177	–	KC843090	KC843146
	CBS 113201	*V. vinifera*	Portugal	KC343234	KC344202	KC343718	KC343960	KC343476
	CBS 114011	*V. vinifera*	Portugal	KC343235	KC344203	KC343719	KC343961	KC343477
	CBS 114436	*Sambucus* cf. *racemosa*	Sweden	KC343236	KC344204	KC343720	KC343962	KC343478
	CBS 143346 = CPC 28224	*V. vinifera*	Czech Republic	*MG281131*	*MG281304*	*MG281479*	*MG281652*	*MG281828*
	CPC 28225	*V. vinifera*	Czech Republic	*MG281132*	*MG281305*	*MG281480*	*MG281653*	*MG281829*
	CPC 28252	*V. vinifera*	UK	*MG281133*	*MG281306*	*MG281481*	*MG281654*	*MG281830*
	CPC 28253	*V. vinifera*	UK	*MG281134*	*MG281307*	*MG281482*	*MG281655*	*MG281831*
	CPC 28265	*V. vinifera*	UK	*MG281135*	*MG281308*	*MG281483*	*MG281656*	*MG281832*
	CPC 28268	*V. vinifera*	UK	*MG281136*	*MG281309*	*MG281484*	*MG281657*	*MG281833*

Table 1 (cont.)

Species	Culture no.[1]	Host	Country	GenBank no.[2]				
				ITS	*tub2*	*his3*	*tef1*	*cal*
D. rudis (cont.)	CPC 28425	*V. vinifera*	Italy	*MG281137*	*MG281310*	*MG281485*	*MG281658*	*MG281834*
	CPC 29320	*V. vinifera*	France	*MG281138*	*MG281311*	*MG281486*	*MG281659*	*MG281835*
	CPC 29649	*V. vinifera*	Spain	*MG281139*	*MG281312*	*MG281487*	*MG281660*	*MG281836*
	CPC 29658	*V. vinifera*	Spain	*MG281140*	*MG281313*	*MG281488*	*MG281661*	*MG281837*
D. saccarata	**CBS 116311**	*Protea repens*	South Africa	KC343190	KC344158	KC343674	KC343916	KC343432
D. schini	**CBS 133181**	*Schinus terebinthifolius*	Brazil	KC343191	KC344159	KC343675	KC343917	KC343433
D. sojae	CBS 116019	*Caperonia palustris*	USA	KC343175	KC344143	KC343659	KC343901	KC343417
	CBS 139282	*Glycine max*	USA	KJ590719	KJ610875	KJ659208	KJ590762	KJ612116
D. sterilis	**CBS 136969**	*Vaccinium corymbosum*	Italy	KJ160579	KJ160528	MF418350	KJ160611	KJ160548
D. subclavata	**ICMP20663**	*Citrus unshiu*	China	KJ490630	KJ490451	KJ490572	KJ490509	–
D. terebinthifolii	**CBS 133180**	*Schinus terebinthifolius*	Brazil	KC343216	KC344184	KC343700	KC343942	KC343458
D. toxica	**CBS 534.93**	*Lupinus angustifolius*	Western Australia	KC343220	KC344188	KC343704	KC343946	KC343462
D. vaccinii	**CBS 160.32**	*Vaccinium macrocarpon*	USA	AF317578	KC344196	KC343712	GQ250326	KC343470
	CBS 118571	*Va. corymbosum*	USA	KC343223	KC344191	KC343718	KC343949	KC343465
	CBS 122114	*Va. corymbosum*	USA	KC343225	KC344193	KC343709	KC343951	KC343467
	CBS 135436	*Va. corymbosum*	USA	AF317570	KC843225	KJ420877	JQ807380	KC849457
Diaporthella corylina	**CBS 121124**	*Corylus* sp.	China	KC343004	KC343972	KC343488	KC343730	KC343246

[1] BRIP: Plant Pathology Herbarium, Department of Primary Industries, Dutton Park, Queensland, Australia; CPC: Culture collection of P.W. Crous, housed at Westerdijk Fungal Biodiversity Institute; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; DAOM: Canadian Collection of Fungal Cultures or the National Mycological Herbarium, Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; ICMP: International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand.
Ex-type and ex-epitype cultures are indicated in **bold**.
[2] ITS: internal transcribed spacers 1 and 2 together with 5.8S nrDNA; *tub2*: partial beta-tubulin gene; *his3*: partial histone H3 gene; *tef1*: partial translation elongation factor 1-α gene; *cal*: partial calmodulin gene. Sequences generated in this study are indicated in *italics*.
[3] Isolates used for pathogenicity test.

Therefore, several surveys were performed in European countries and Israel to collect grapevine specimens for *Diaporthe* isolations. This study was conducted in order to fully characterise these strains using morphological characters and multi-locus phylogenetic inference based on modern taxonomic concepts. In particular, the objectives of the present study were:

i. to conduct extensive surveys for sampling *V. vinifera*;
ii. to cultivate *Diaporthe* isolates;
iii. to subject those isolates to DNA sequence analyses combined with morphological characterisation;
iv. to compare the obtained results with the data from other phylogenetic studies on the genus; and
v. to evaluate the pathogenicity of the *Diaporthe* strains.

MATERIALS AND METHODS

Sampling and isolation

Pure cultures of *Diaporthe* were collected in seven European countries (Croatia, Czech Republic, France, Hungary, Italy, Spain and the UK) and Israel from asymptomatic and symptomatic *Vitis vinifera* plants, in both nursery and vineyard environments. Several samples showed multiple symptoms such as cane and leaf spot, cane bleaching, and additionally vascular browning and sectorial necrosis in grapevine wood. Isolations were performed from different plant organs such as canes, cordons and trunks. 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 (Table 1).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following manufacturer's instructions. Partial regions of five loci were amplified. The primers ITS5 and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA operon, including the 3' end of the 18S nrRNA, the first internal transcribed spacer region, the 5.8S nrRNA gene; the second internal transcribed spacer region and the 5' end of the 28S nrRNA 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*). The 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 the CYLH3F and H3-1b primer set (Glass & Donaldson 1995, Crous et al. 2004a) and the beta-tubulin (*tub2*) region was amplified using the Bt2a and Bt2b primer set (Glass & Donaldson 1995) or Tub2FD (Aveskamp et al. 2009) and T22 (O'Donnell & Cigelnik 1997). The PCR products were sequenced in both directions using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems Life Technologies, Carlsbad, CA, USA), after which amplicons were purified through Sephadex G-50 Fine columns (GE Healthcare, Freiburg, Germany) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were analyzed on an Applied Biosystems 3730xl DNA Analyser (Life Technologies, Carlsbad, CA, USA). The DNA sequences generated were analysed and consensus sequences were computed using the program SeqMan Pro (DNASTAR, Madison, WI, USA).

Phylogenetic analyses

Novel sequences generated in this study were blasted against the NCBIs 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 by using the MAFFT v. 7 online server (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh & 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. Two separate analyses were conducted for the *D. eres* species complex and the remainder of the *Diaporthe* spp. included in this study, as similarly performed in a recent study about *Colletotrichum* taxonomy (Guarnaccia et al. 2017). Additional reference sequences were selected based on recent

Table 2 Number of isolates collected for each *Diaporthe* sp. identified and country investigated.

	Croatia	Czech Republic	France	Hungary	Israel	Italy	Spain	UK	Total
D. ambigua	–	–	–	–	–	–	2	–	2
D. ampelina	3	1	2	1	4	1	10	9	31
D. baccae	1	–	1	–	–	–	12	–	14
D. bohemiae	–	2	–	–	–	–	–	–	2
D. celeris	–	–	–	–	–	–	–	3	3
D. eres	2	11	2	72	–	2	9	7	105
D. hispaniae	–	–	–	–	–	–	2	–	2
D. hungariae	–	–	–	3	–	–	3	–	6
D. rudis	–	2	1	–	–	1	2	4	10
Total	6	16	6	76	4	4	40	23	175

studies on *Diaporthe* species (Gomes 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 1 000 generations. Analyses stopped once the average standard deviation of split frequencies was below 0.01. The MP analyses were performed 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 1 000 replications. Sequences generated in this study are deposited in GenBank (Table 1) and alignments and phylogenetic trees in TreeBASE (www.treebase.org).

Taxonomy

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 malt extract agar (MEA) (Crous et al. 2009), and incubated at 21–22 °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 15 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 ×1 000 magnification were determined for each isolate using a Zeiss Axioscope 2 microscope with interference contrast (DIC) optics. Descriptions, nomenclature and illustrations of taxonomic novelties were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004b).

Pathogenicity

Pathogenicity testing was conducted using a proven inoculation method for *Diaporthe* (Mostert et al. 2001a, Úrbez-Torres et al. 2009, Dissanayake et al. 2015). Green shoots (6–8 mm diam, 15–30 cm long), cut from healthy mature grapevine cv. 'Riesling', were artificially inoculated to determine the pathogenicity of the five *Diaporthe* species not previously reported to be associated with *Vitis* spp.

Ten different isolates representing *D. baccae*, *D. bohemiae*, *D. celeris*, *D. hispaniae* and *D. hungariae*, were selected (Table 1). Green canes were collected in July 2017 and were brought to the laboratory. All the leaves, lateral branches, and tendrils were removed. Canes were inoculated the same day they were sampled. Canes were surface-sterilized in 10 % sodium hypochlorite for 10 min. After air drying, five canes were inoculated with each *Diaporthe* isolate. Canes were superficially wounded in between two nodes forming a slit using a sterile blade. Inoculations were conducted by placing a 1-wk-old, 6 mm diam agar plug from each fungal culture on a wound. Wounds were then wrapped with Parafilm® (American National Can, Chicago, IL, USA). Ten shoots were inoculated as described above with 6-mm-diam non-colonised MEA plugs as negative controls. Inoculated canes were immediately placed in 6 L transparent plastic containers with a tight-fitting lid containing wet paper towels with 400 mL distilled water to maintain a humid environment. Five canes per plastic container including controls were arranged in a completely randomized design. Inoculated canes were collected after 21 d of incubation at room temperature and inspected for lesion development. Each cane was cut longitudinally through the inoculation point to evaluate the type of symptom developed. In order to demonstrate pathogenicity, the inoculated fungi were re-isolated from canes showing lesions, and the identity of the re-isolated fungi was confirmed by sequencing the *tef1* and *tub2* loci as described above.

RESULTS

Sampling and isolation

Symptoms caused by *Diaporthe* spp. were frequently observed on *Vitis* spp., including Phomopsis cane and leaf spot, cane bleaching, and additionally vascular internal browning, sectorial necrosis, and other necrotic lesions on grapevine wood. Symptoms were observed on rootstock and scion grapevine plants. A total of 175 monosporic isolates resembling those of the genus *Diaporthe* were collected. The *Diaporthe* isolates were recovered from multiple locations of all the countries investigated (Table 1, 2). Based on preliminary ITS sequencing, all 175 isolates were selected (Table 1) for phylogenetic analyses and further taxonomic study.

Phylogenetic analyses

The 10 MP trees derived from the single gene sequence alignments (ITS, *tef1*, *cal*, *his3* and *tub2*) for both the *D. eres* species

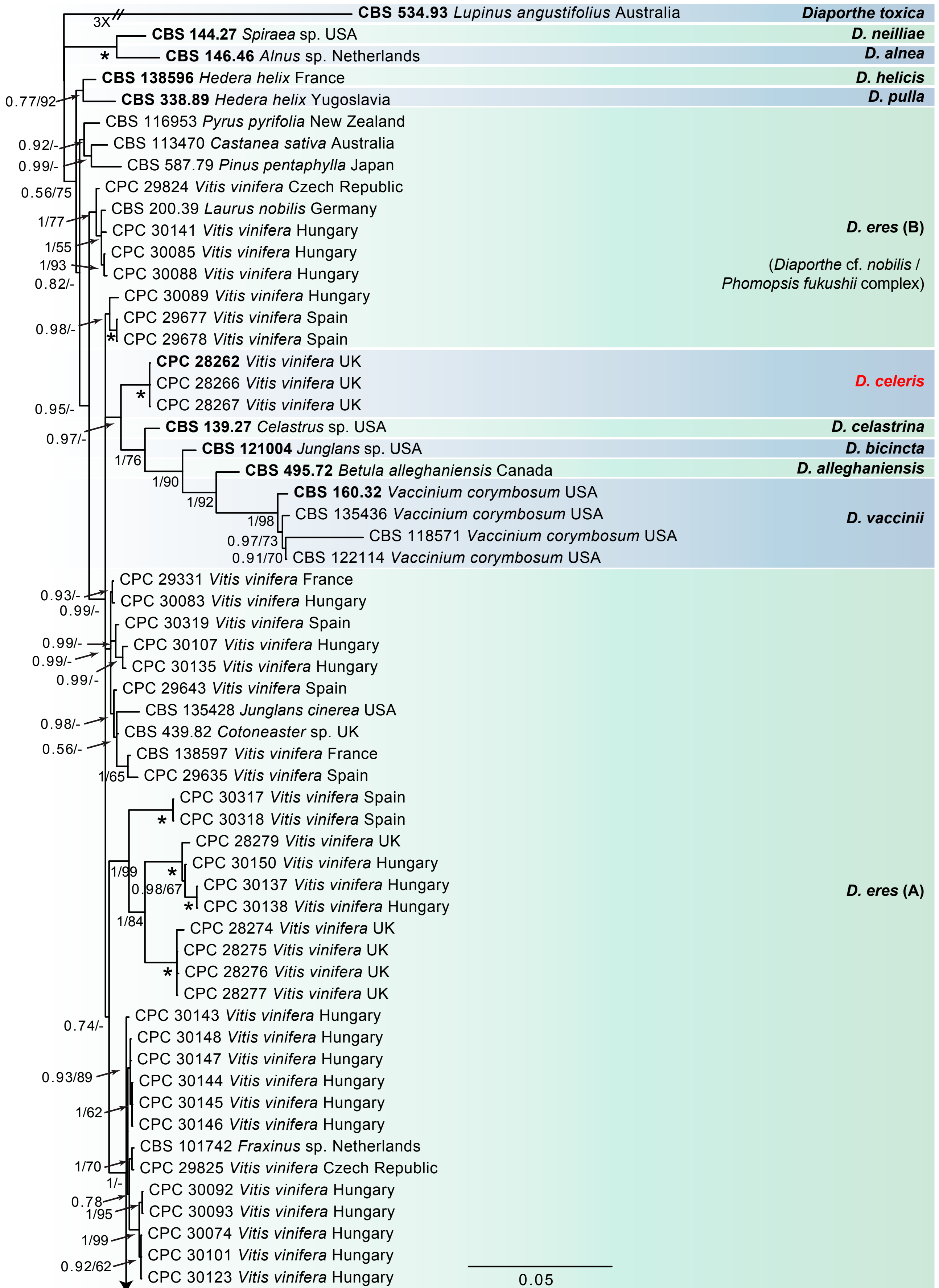

Fig. 1 Consensus phylogram of 86 082 trees resulting from a Bayesian analysis of the combined ITS, *tub2*, *his3*, *tef1* and *cal* sequence alignments of the *D. eres* complex. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. The asterisk symbol (*) represents full support (1/100). Substrate and country of origin are listed next to the strain numbers. Ex-type isolates are indicated in **bold**. The novel species are shown in red text. The tree was rooted to *Diaporthe toxica* (CBS 534.93).

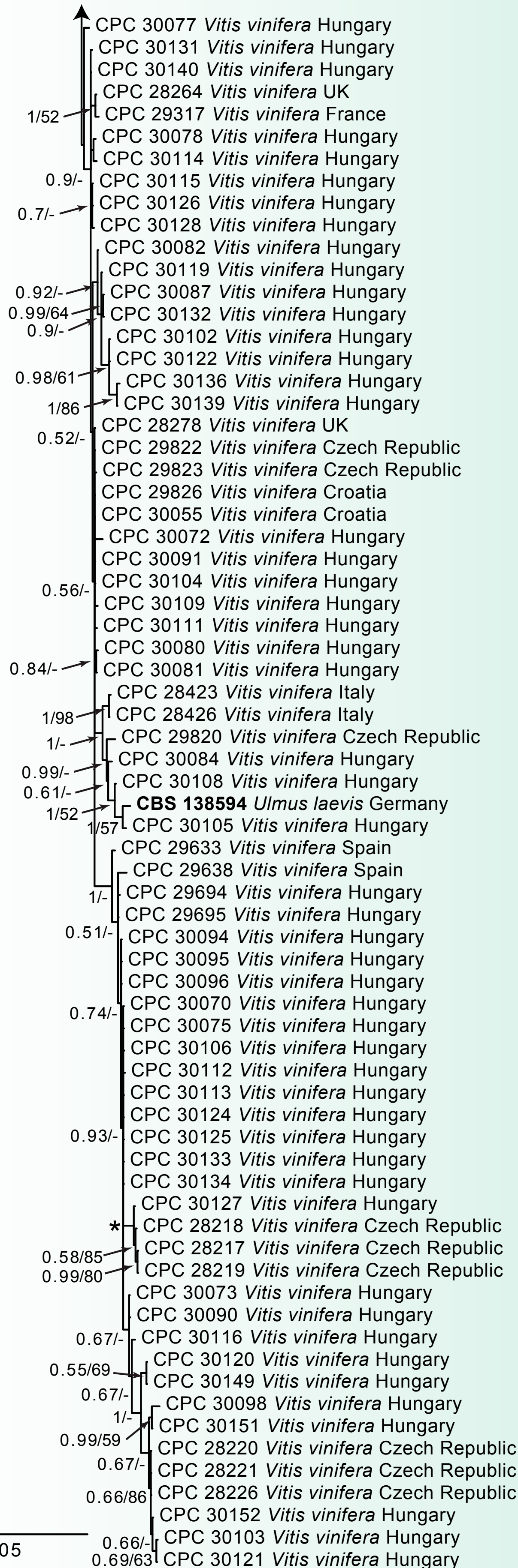

Fig. 1 (cont.)

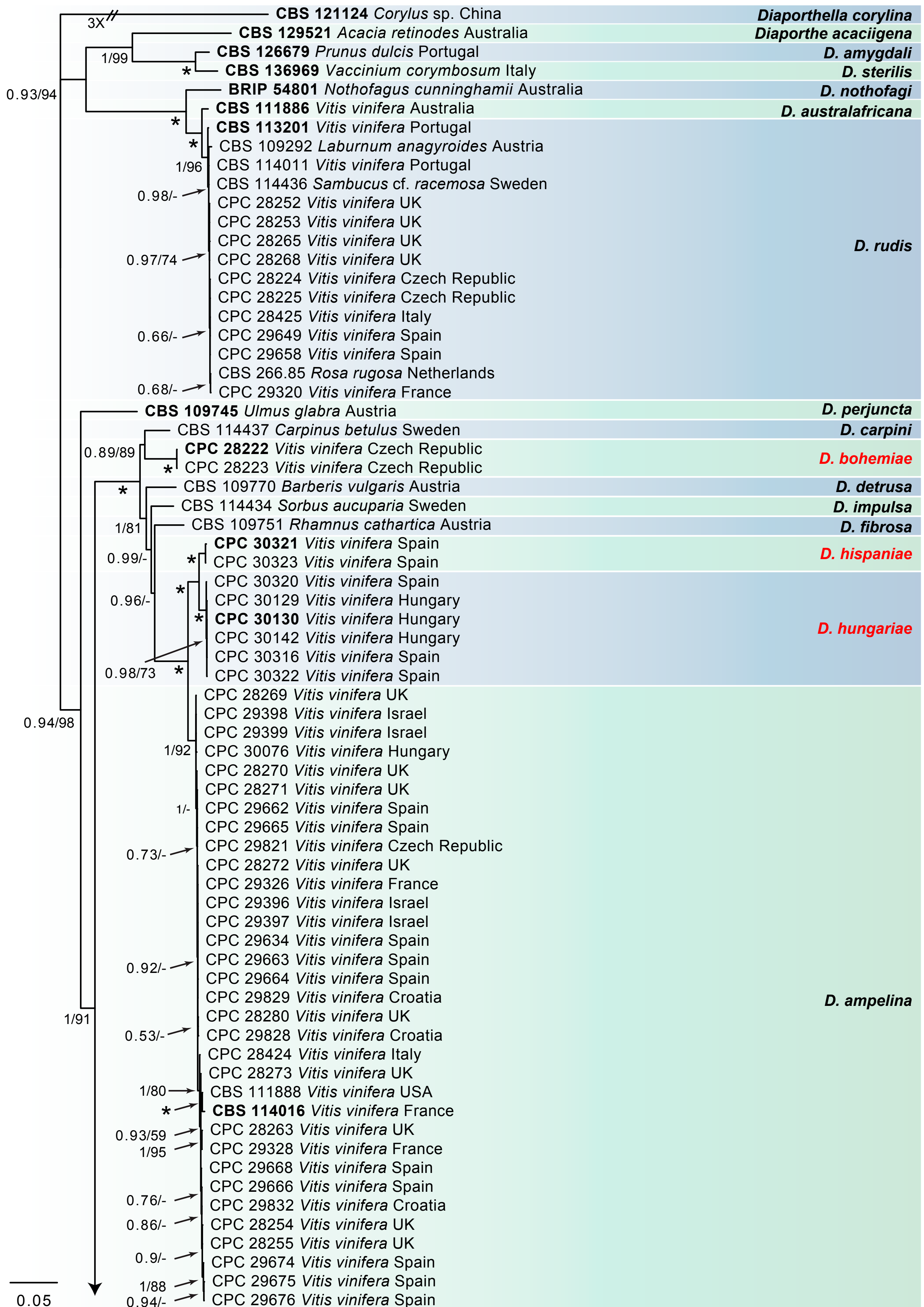

Fig. 2 Consensus phylogram of 3 862 trees resulting from a Bayesian analysis of the combined ITS, *tub2*, *his3*, *tef1* and *cal* sequence alignments of *Diaporthe* spp. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. The asterisk symbol (*) represents full support (1/100). Substrate and country of origin are listed next to the strain numbers. Ex-type isolates are indicated in **bold**. The novel species are shown in red text. The tree was rooted to *Diaporthella corylina* (CBS 121124).

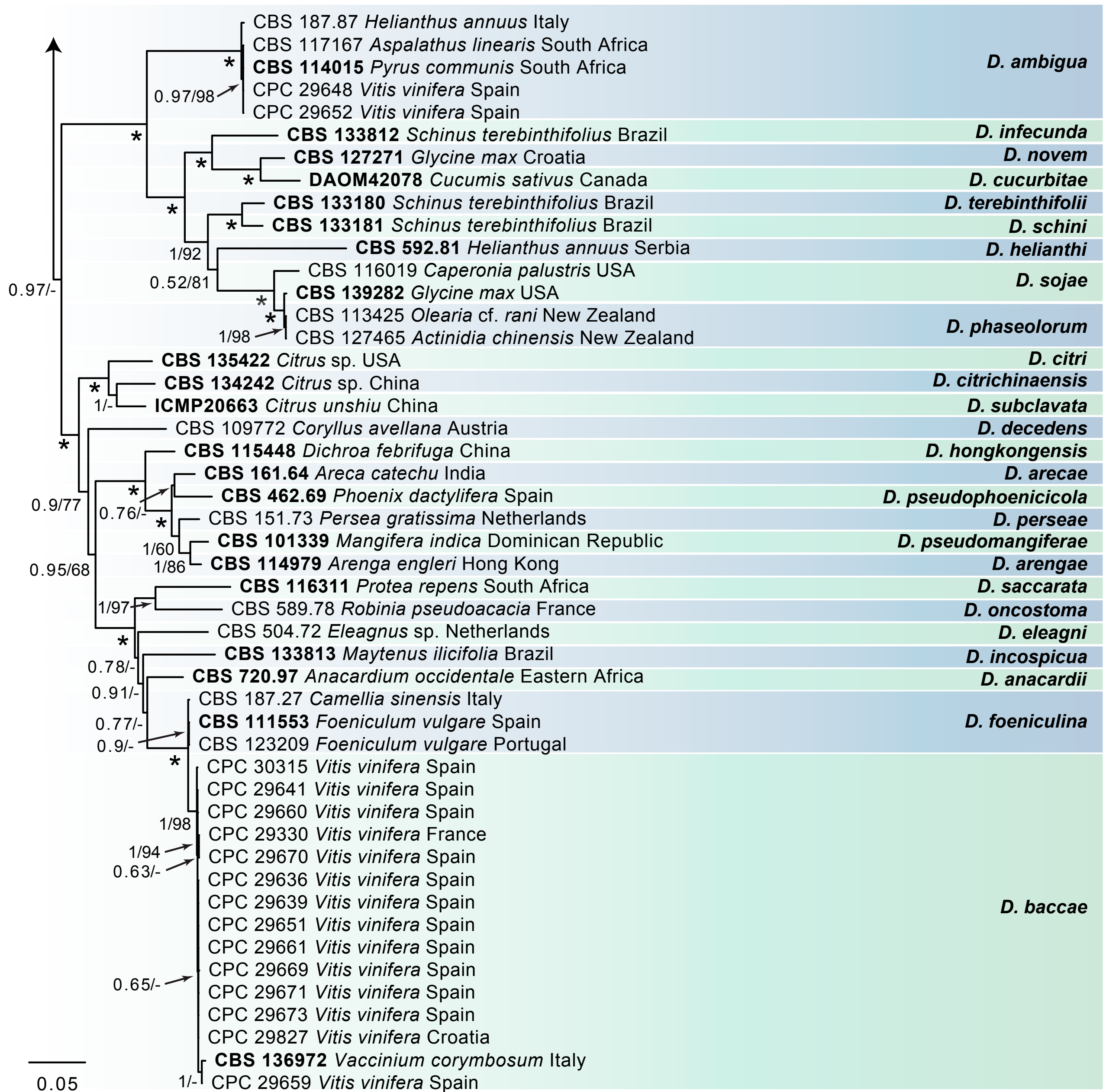

Fig. 2 (cont.)

complex and the remaining *Diaporthe* spp. produced topologically similar trees, and confirmed that 108 isolates recovered in this study belong to the *D. eres* species complex. The remaining 67 isolates were identified as various *Diaporthe* species. The combined species phylogeny of the *D. eres* species complex (TreeBASE: S21957) consisted of 129 sequences, including the outgroup sequences of *D. toxica* (culture CBS 534.93). The remaining species were included in a combined phylogeny (TreeBASE: S21958) consisting of 117 sequences, including the outgroup sequences of *Diaporthella corylina* (CBS 121124). A total of 3 805 characters (ITS: 1–583, *tef*: 590–1 232, *tub2*: 1 239–2 574, *cal*: 2 581–3 305, *his3*: 3 312–3 805) were included in the *D. eres* complex phylogenetic analyses, of which 423 characters were parsimony-informative, 543 were variable and parsimony-uninformative and 2 815 characters were constant. A maximum of 1 000 equally most parsimonious trees were saved (Tree length = 1 858, CI = 0.625, RI = 0.840 and RC = 0.525). Regarding the remainder of *Diaporthe* species, a total of 4 220 characters were included in the phylogenetic analyses (ITS: 1–640, *tef*: 647–1 360, *tub2*: 1 367–2 807, *cal*: 2 814–3 625, *his3*: 3 632–4 220), of which 1 524 characters were parsimony-informative, 909 were variable and parsimony-uninformative and 1 763 characters were constant. A maximum of 1 000 equally most parsimonious trees were saved (Tree length = 8 303, CI = 0.530, RI = 0.877 and RC = 0.465). Bootstrap support values from the parsimony analysis were plotted on the Bayesian phylogenies presented in Fig. 1 and 2. For both of the Bayesian analyses, MrModeltest suggested that all partitions should be analysed with dirichlet state frequency distributions, except for the ITS partition in the *D. eres* species complex analysis, which was analysed with a fixed state frequency distribution. The following models were recommended by MrModeltest and used in the Bayesian analysis of the *D. eres* species complex: SYM+I+G for ITS, HKY+G for *tef1*, *tub2* and *his3* and GTR+G for *cal*. The ITS partition had 90 unique site patterns, the *tef1* partition 164, the *tub2* partition 256, the *cal* partition 182, the *his3* partition 147, and the analysis ran for 43 040 000 generations, resulting in 86 082 trees of which 64 562 trees were used to calculate the posterior probabilities. Regarding the Bayesian analysis of the remaining *Diaporthe* species, the following models were used according to MrModeltest: GTR+I+G for ITS, *tef1* and *cal*, HKY+I+G for *tub2* and GTR+I+G for *cal*. The ITS partition had 217 unique site patterns, the *tef1* partition 501, the *tub2* partition 560, the

Table 3 *Diaporthe* spp. associated with grapevines and their morphological characteristics.

Species	Conidiomata (µm)	Conidiophores (µm)	Alpha conidia (µm)	Beta conidia (µm)	References
D. ambigua	–	15–45 × 2–3	6–8 × 2–3	–	Van Rensburg et al. (2006)
D. ampelina	up to 430	5–35 × 1–3	9.5–10.5 × 2–3	20–25 × 0.5–1	Gomes et al. (2013)
D. amygdali	up to 800	6–25 × 1–2	4.5–8 × 1–2	12–20 × 0.5–1	Mostert et al. (2001a)
D. australafricana	–	–	5–6 × 1.5–2	–	Van Niekerk et al. (2005)
D. baccae	up to 650	20–57 × 2–3	7–9 × 2–3	20–24 × 1–2	Lombard et al. (2014)
D. bohemiae	up to 400	5–20 × 1.5–4	7.5–8.5 × 1.5–3	–	This study
D. celeris	up to 650	5–18 × 1–3	5.5–7.5 × 2–3	16–22.5 × 1–2	This study
D. eres	200–250	10–15 × 2–3	6.5–8.5 × 3–4	22–28 × 1–1.5	Udayanga et al. (2014a)
D. foeniculina	400–700	9–15(–18) × 1–2	8.5–9 × 2.3–2.5	22–28 × 1.4–1.6	Udayanga et al. (2014b)
D. helianthi	up to 380	11.5–23.5 × 1.8–3.5	–	11.5–32 × 0.5–2	Gao et al. (2017)
D. hispaniae	up to 400	5–30 × 1–4	9–14.5 × 2–4	18–24 × 1–2	This study
D. hongkongensis	up to 200	5–12 × 2–4	6–7 × 2.5	18–22 × 1.5–2	Gomes et al. (2013)
D. hungariae	up to 650	5–25 × 1–3.5	9.5–16 × 2–3.5	–	This study
D. kyushuensis	up to 860	–	15.5–24 × 4.5–8	25–55 × 1–2	Kajitani & Kanematsu (2000)
D. perjuncta	–	17–23 × 1.5–2.5	5–7 × 2–2.5	12–20 × 0.5–1	Mostert et al. (2001a)
D. phaseolorum	up to 300	7–12 × 2–3	7.3–10.3 × 2.8–3.5	–	Udayanga et al. (2015)
D. rudis	up to 500	20–45 × 2–2.4	6.3–8.7 × 2–2.5	27–35.2 × 3–4.2	Udayanga et al. (2014b)
D. sojae	200–250	12–16 × 2–4	5.3–7.3 × 2–3	–	Udayanga et al. (2015)

cal partition 510, the *his3* partition 259, and the analysis ran for 1 930 000 generations, resulting in 3 862 trees of which 2 898 trees were used to calculate the posterior probabilities.

In the *D. eres* complex analysis (Fig. 1), 98 *V. vinifera* isolates clustered with five reference strains of *D. eres* (A), whilst seven isolates clustered with four reference strains of *D. eres* (B), the clade previously known as the *Diaporthe* cf. *nobilis*/*Phomopsis fukushii* complex (Gomes et al. 2013). Moreover, three isolates were identified as *D. celeris*, forming a highly-supported subclade (1.00/100) in the complex. In the other analyses, 10 isolates clustered with the ex-type strain of *D. rudis*, 31 isolates with the ex-type strain and other reference strains of *D. ampelina*, 2 with the ex-type and other reference strains of *D. ambigua* and 14 isolates with the ex-type strain of *D. baccae* (Fig. 2). Furthermore, two isolates were identified as *D. bohemiae* (closely related to *D. carpini*), two isolates as *D. hispaniae* and six as *D. hungariae* (close to *D. ampelina*). The individual alignments and resulting trees of the five single genes in both analyses were compared with respect to their performance in species recognition. In the *D. eres* complex analysis, *D. celeris* was differentiated with *tef1*, *his3* and *cal*, whilst in the other analysis *D. bohemiae* was differentiated by every single gene used. Moreover, the single locus *tub2*, was informative enough to distinguish *D. hispaniae*, *D. hungariae* and *D. ampelina*.

Taxonomy

Morphological observations, supported by phylogenetic inference, were used to identify five known species (*D. ambigua*, *D. ampelina*, *D. baccae*, *D. eres* and *D. rudis*), and to describe four new species (Table 3). Culture characteristics were assessed, and the colour of upper and lower surfaces on different media determined as shown in Fig. 3–6. Based on the results of both the phylogenetic and morphological analyses, the four distinct novel species are described below.

Diaporthe bohemiae Guarnaccia, Eichmeier & Crous, *sp. nov.* — MycoBank MB823244; Fig. 3

Etymology. Named after the country where it was collected, Czech Republic (ancient Latin name, *Bohemia*).

Conidiomata pycnidial on PNA, globose or irregular, solitary, deeply embedded in PDA, erumpent, dark brown to black, 250–400 µm diam, whitish translucent to yellow conidial drops exuded from the ostioles. *Conidiophores* hyaline, smooth, 1-septate, densely aggregated, cylindrical, straight, 5–20 × 1.5–4 µm. *Conidiogenous cells* phialidic, hyaline, terminal, cylindrical, 6–8 × 1–2 µm, tapered towards the apex. *Paraphyses* intermingled among conidiophores, hyaline, smooth, 1–3-septate, up to 70 µm long, apex 1–2 µm diam. *Alpha conidia* produced on all the tested media, aseptate, fusiform, hyaline, multi-guttulate and acute at both ends, 7.5–8.5 × 1.5–3 µm, mean ± SD = 7.6 ± 0.6 × 2.3 ± 0.3 µm, L/W ratio = 3.3. *Beta conidia* and *gamma conidia* not observed.

Culture characteristics — Colonies covering the medium within 9 d at 21 °C, with surface mycelium flattened, dense and felty. Colony on MEA, PDA and OA at first white, becoming cream to yellowish, flat on PDA and OA, and dark brown on MEA, with dense and felted mycelium. Reverse pale brown with brownish dots with age, with visible solitary conidiomata at maturity on MEA and PDA. On OA visible solitary conidiomata within 10 d.

Materials examined. Czech Republic, Znojmo, Dyjákovičky, from root of *Vitis* spp., 30 Mar. 2015, *A. Eichmeier* (CBS H-23236 – holotype; CBS 143347 = CPC 28222 – culture ex-type); from root of *Vitis* spp., 30 Mar. 2015, *A. Eichmeier* (culture CBS 143348 = CPC 28223).

Notes — *Diaporthe bohemiae* was collected from roots of *Vitis* spp. used as rootstock, in the Czech Republic. This species is phylogenetically close but clearly differentiated from *D. carpini* based on ITS, *tef1*, *tub2*, *his3* and *cal* sequence similarity (98 % in ITS, 91 % in *tef1*, 96 % in *tub2*, 94 % in *his3*, and 94 % in *cal*). Morphologically, *D. bohemiae* differs from *D. carpini* in its shorter alpha conidia (5.5–8.5 vs 7–9 µm) (Gomes et al. 2013) and the shape of its alpha conidia having acute ends, not observed in *D. carpini* which has conidia with rounded ends (Wehmeyer 1933).

Diaporthe celeris Guarnaccia, Woodhall & Crous, *sp. nov.* — MycoBank MB823245; Fig. 4

Etymology. From Latin *celere* 'fast', referring to the fast growth rate on different media.

Conidiomata pycnidial on PNA, globose or irregular, solitary, deeply embedded in OA, erumpent, dark brown to black, 350–650 µm diam, yellowish translucent to brown conidial cirrus or drops exuded from the ostioles. *Conidiophores* hya-

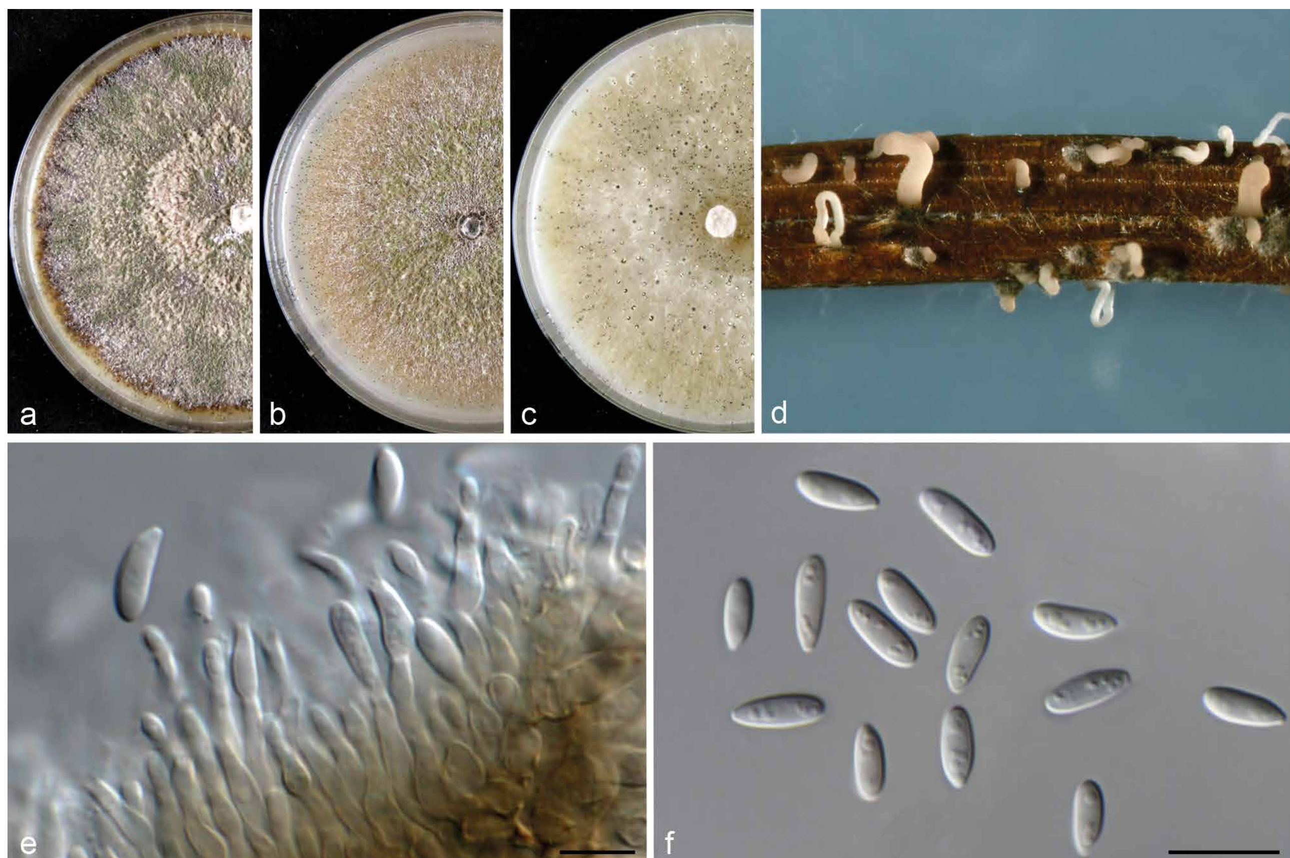

Fig. 3 *Diaporthe bohemiae* (CBS 143347). a–c. Colonies on MEA, PDA and OA, respectively; d. conidiomata sporulating on PNA; e. conidiogenous cells; f. alpha conidia. — Scale bars = 10 μm.

Fig. 4 *Diaporthe celeris* (CBS 143349). a–c. Colonies on MEA, PDA and OA, respectively; d. conidiomata sporulating on OA; e. conidiophores; f. conidiogenous cells; g. alpha conidia; h. beta conidia. — Scale bars = 10 μm.

line, smooth, 1-septate, unbranched, ampulliform, cylindrical, straight, 5–18 × 1–3 µm. *Conidiogenous cells* phialidic, hyaline, terminal, cylindrical, 5–8 × 1–2 µm, tapered towards the apex. *Paraphyses* not observed. *Alpha conidia* aseptate, fusiform, hyaline, mono- to biguttulate and acutely rounded at both ends, 5.5–7.5 × 2–3 µm, mean ± SD = 6.6 ± 0.5 × 2.5 ± 0.3 µm, L/W ratio = 2.6. *Beta conidia* hyaline, aseptate, eguttulate, filiform, curved, tapering towards both ends, 16–22.5 × 1–2 µm, mean ± SD = 19.7 ± 2.1 × 1.4 ± 0.3 µm, L/W ratio = 14. *Gamma conidia* not observed.

Culture characteristics — Colonies covering the medium within 6 d at 21 °C, with surface mycelium flattened, dense and felty. Colony on MEA with white floccose mycelium. On PDA and OA at first white, becoming cream to brown and grey, respectively, flat on PDA and OA, and dark brown on MEA, with abundant production of conidiomata only on OA. Reverse pale brown on MEA and whitish to cream on PDA and OA.

Materials examined. UK, Sussex, from trunk of *Vitis vinifera*, 12 Nov. 2013, *J. Woodhall* (CBS H-23237 – holotype; CBS 143349 = CPC 28262 – culture ex-type); from trunk of *Vitis vinifera*, 12 Nov. 2013, *J. Woodhall* (culture CBS 143350 = CPC 28266).

Notes — *Diaporthe celeris* was isolated from *V. vinifera* in the UK. Three strains representing this species cluster in a well-supported clade embedded in the *D. eres* species complex. This species is phylogenetically close but clearly differentiated from *D. celastrina* based on *tef1*, *his3* and *cal* sequence similarity (96 % in *tef1*, 96 % in *his3*, and 98 % in *cal*) and from *D. eres* based on *tef1* sequence similarity (97 %). Morphologically, *D. celeris* differs from *D. celastrina* in the production of beta conidia not observed in *D. celastrina*, and from *D. eres* in its fast growth rate in culture and shorter alpha conidia (Udayanga et al. 2014a).

Diaporthe hispaniae Guarnaccia, Armengol & Crous, *sp. nov.* — MycoBank MB823246; Fig. 5

Etymology. Named after the country where it was collected, Spain (ancient Latin name, *Hispania*).

Conidiomata pycnidial in culture on PNA, globose or irregular, scattered or solitary, deeply embedded in MEA and PDA, erumpent, dark brown to black, 150–400 µm diam, cream translucent to orange conidial drops exuded from the ostioles. *Conidiophores* hyaline, some filiform, smooth, aseptate, densely aggregated, cylindrical, straight, 5–30 × 1–4 µm. *Conidiogenous cells* phialidic, hyaline, terminal, cylindrical, 6–10 × 1–2 µm, tapered towards the apex. *Paraphyses* not observed. *Alpha conidia* common, fusiform, hyaline, rarely curved, apex acutely rounded, base obtuse to subtruncate, multi-guttulate, aseptate, 9–14.5 × 2–4 µm, mean ± SD = 11.4 ± 1.3 × 2.7 ± 0.4 µm, L/W ratio = 4.2. *Beta conidia* less common, straight or curved, 18–24 × 1–2 µm, mean ± SD = 22.7 ± 2.3 × 1.6 ± 0.3 µm, L/W ratio = 14.2. *Gamma conidia* not observed.

Culture characteristics — Colonies covering the medium within 12 d at 21 °C, with surface mycelium flattened, dense and felty. Colony on MEA and PDA at first white becoming pale brown to grey with abundant production of sporulating conidiomata. On OA cream to dark brown. Reverse pale brown to cream on MEA and PDA, dark brown on OA.

Materials examined. Spain, Valencia, Aielo de Malferit, from necrotic scion of *Vitis vinifera*, 2016, *J. Armengol* (CBS H-23238 – holotype; CBS 143351 = CPC 30321 – culture ex-type); from necrotic wood of *Vitis vinifera*, 2016, *J. Armengol* (culture CBS 143352 = CPC 30323).

Notes — *Diaporthe hispaniae* was isolated from *V. vinifera* samples collected in Spain. Two strains representing this species cluster separately in a well-supported clade, and appear most closely related to *D. ampelina* based on the *tub2* sequence similarity (93 %). This species is phylogenetically close but clearly differentiated from *D. hungariae* (described below) by

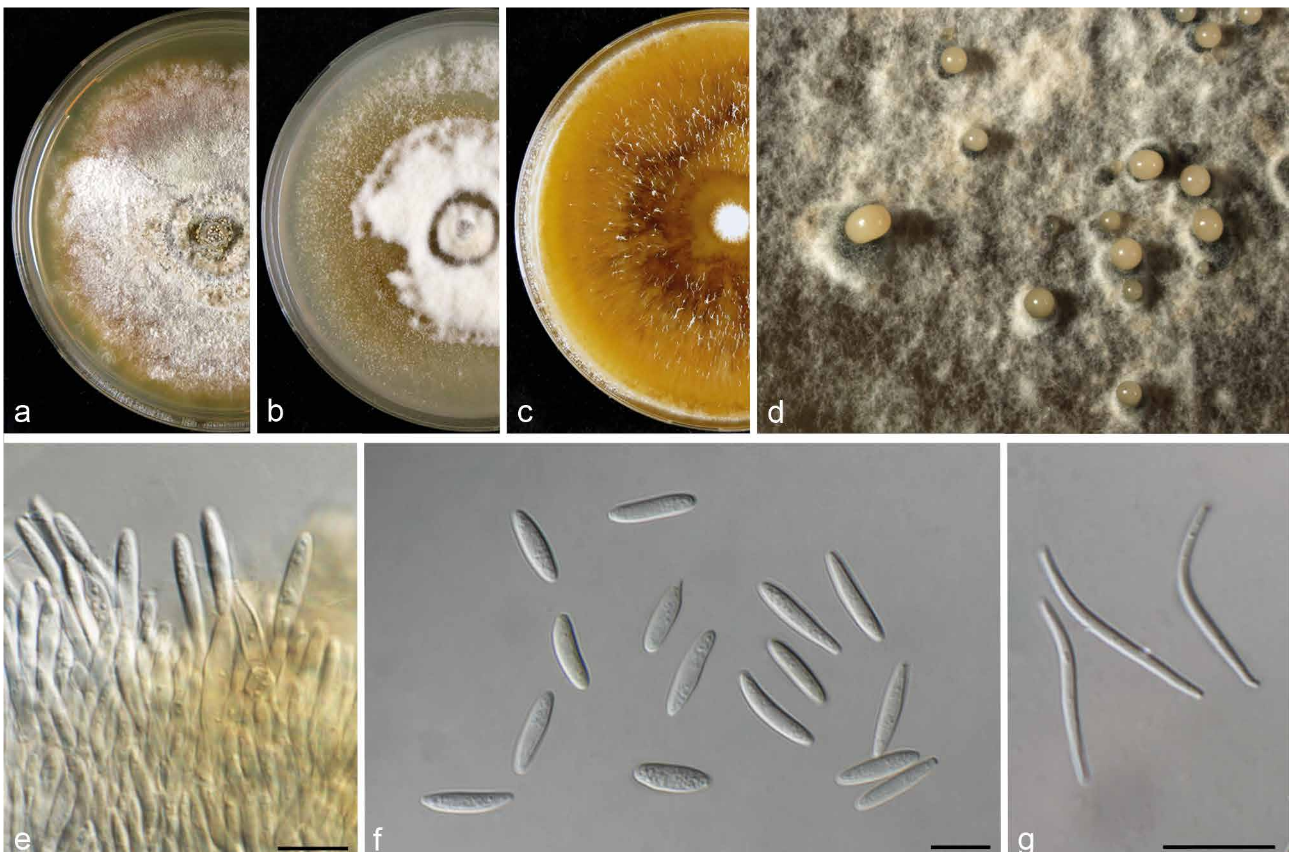

Fig. 5 *Diaporthe hispaniae* (CBS 143351). a–c. Colonies on MEA, PDA and OA, respectively; d. conidiomata sporulating on PDA; e. conidiogenous cells; f. alpha conidia; g. beta conidia. — Scale bars = 10 µm.

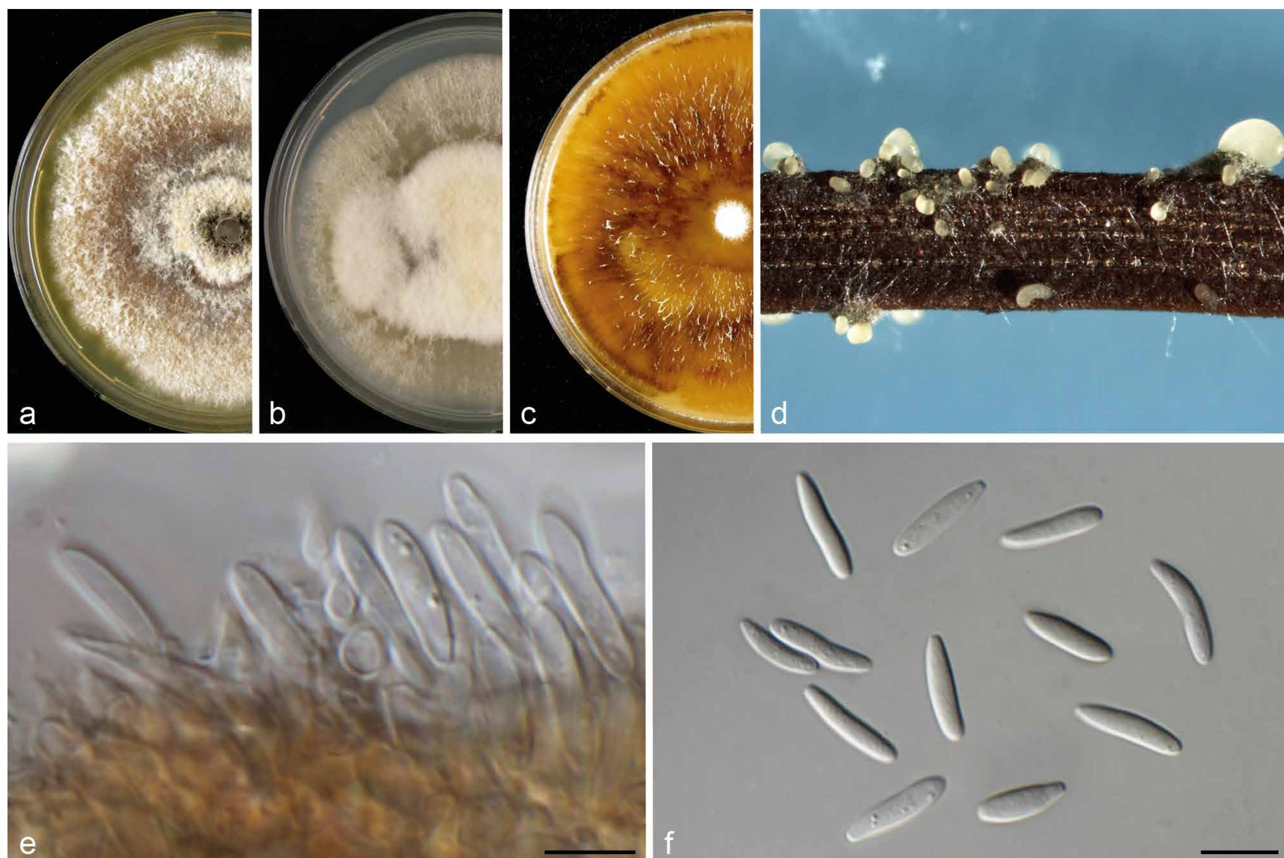

Fig. 6 *Diaporthe hungariae* (CBS 143353). a–c. Colonies on MEA, PDA and OA, respectively; d. conidiomata sporulating on PNA; e. conidiogenous cells; f. alpha conidia. — Scale bars = 10 μm.

53 unique fixed alleles in *tub2*. Morphologically, *D. hispaniae* differs from *D. ampelina* in its longer alpha conidia and larger beta conidia (Gomes et al. 2013). This species differs from *D. hungariae* in the production of beta conidia.

Diaporthe hungariae Guarnaccia, Armengol & K.Z. Váczy, *sp. nov*. — MycoBank MB823247; Fig. 6

Etymology. Named after the country where the ex-type strain was collected, Hungary (ancient Latin name, *Hungaria*).

Conidiomata pycnidial in culture on PNA, globose or irregular, solitary, aggregated or solitary, deeply embedded in MEA, PDA and OA, erumpent, dark brown to black, 150–650 μm diam, white translucent to cream conidial cirrus or drops exuded from the ostioles. *Conidiophores* hyaline, acute, smooth, aseptate, densely aggregated, cylindrical, straight, 5–25 × 1–3.5 μm. *Conidiogenous cells* phialidic, hyaline, terminal, cylindrical, 6–9 × 1–2 μm, tapered towards the apex. *Paraphyses* not observed. *Alpha conidia* commonly found, fusiform, hyaline, rarely curved, apex acutely rounded, base obtuse to subtruncate, mono- to multi-guttulate, aseptate, 9.5–16 × 2–3.5 μm, mean ± SD = 11.7 ± 1.4 × 2.6 ± 0.4 μm, L/W ratio = 4.5. *Beta* and *gamma conidia* not observed.

Culture characteristics — Colonies covering the medium within 15 d at 21 °C, with surface mycelium flattened, dense and felty. Colony on MEA and PDA at first white becoming pale brown to grey. On OA cream to dark brown showing sectorial areas with abundant production of sporulating conidiomata. Reverse pale brown to cream on MEA and PDA, dark brown on OA.

Materials examined. Hungary, Pécs, from trunk of *Vitis vinifera*, 28 Aug. 2014, *K.Z. Váczy* (CBS H-23239 – holotype; CBS 143353 = CPC 30130 – culture ex-type); from trunk of *Vitis vinifera*, 28 Aug. 2014, *K.Z. Váczy* (culture CBS 143354 = CPC 30142).

Notes — *Diaporthe hungariae* was isolated from *V. vinifera* samples collected in Hungary and Spain. Two isolates from Hungary were used for the species description. Six strains representing this species cluster separately in a well-supported clade, and appear most closely related to *D. ampelina* based on *tub2* sequence similarity (93 %). This species is phylogenetically close but clearly differentiated from *D. hispaniae* (described above) by 53 unique fixed alleles in *tub2*. Morphologically, *D. hungariae* differs from *D. ampelina* in its larger conidiomata, longer alpha conidia and the absence of beta conidia, normally observed in *D. ampelina* and also in *D. hispaniae* (Gomes et al. 2013).

Pathogenicity

After 21 d, all the *Diaporthe* isolates induced necrotic lesions on the inoculated grapevines shoots except for the isolates of *D. bohemiae*, and the fungi were successfully re-isolated (Fig. 7f, g). Cankers and internal discolourations were observed in correspondence to inoculation points. No symptoms were observed on the control shoots. Preliminary differences in aggressiveness among the isolates and susceptibility of *V. vinifera* were observed: *D. hispaniae* and *D. hungariae* caused larger cankers and necrotic lesions than *D. baccae* and *D. celeris*, whilst *D. bohemiae* caused no symptoms.

DISCUSSION

We collected 175 *Diaporthe* strains from eight countries. Single gene and multilocus DNA sequence analyses were performed using five loci (ITS, *tef1*, *tub2*, *his3*, and *cal*) commonly used in previous phylogenetic studies of *Diaporthe* species (Gomes et al. 2013, Udayanga et al. 2014a, b, Santos et al. 2017). Only the closest taxa to the nine *Diaporthe* species recovered in

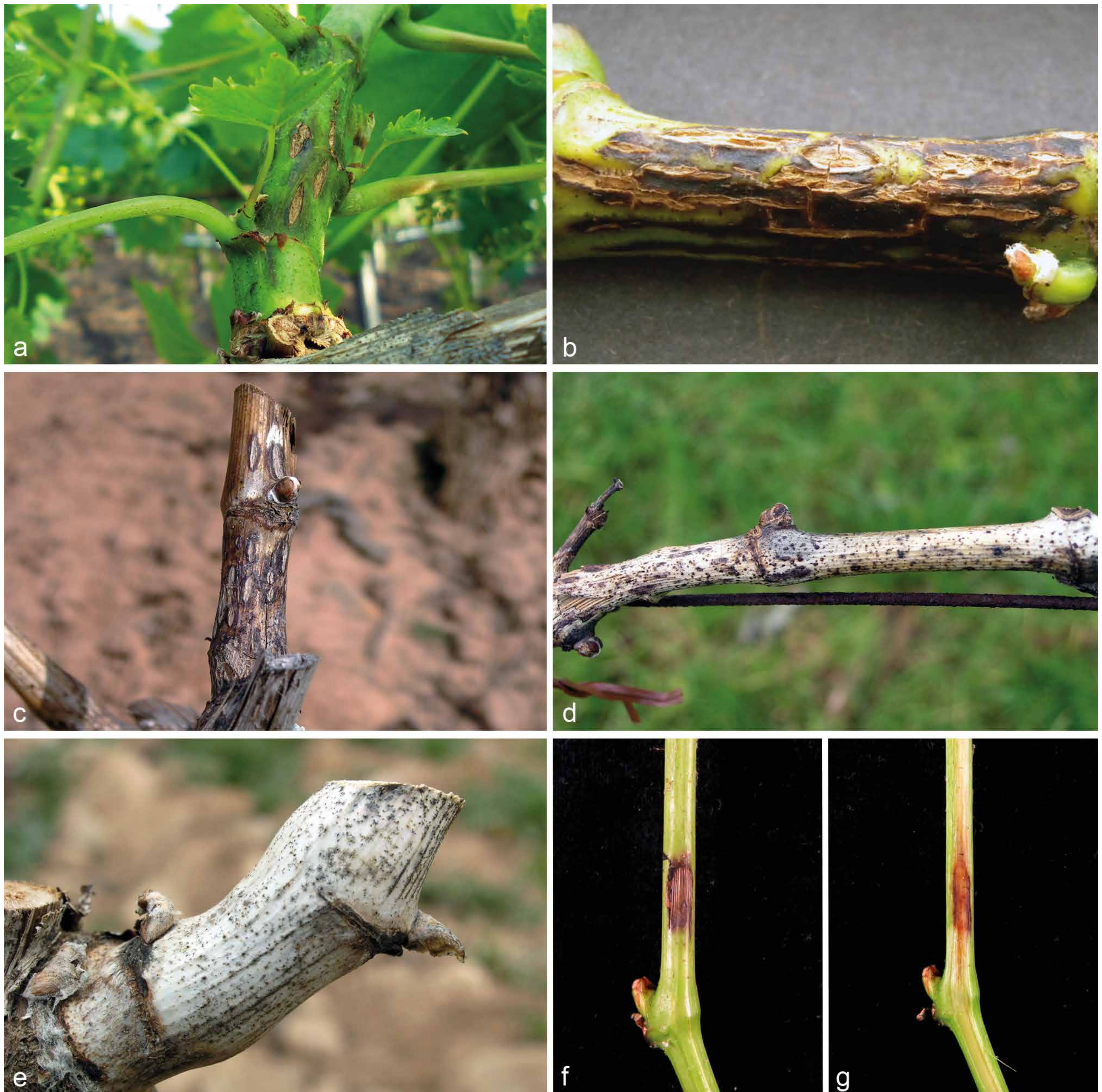

Fig. 7 a–e. Natural and f–g. artificial symptoms on *V. vinifera* with associated *Diaporthe* species. a–c. Lesions of Phomopsis cane and leaf spot on shoot: a. initial symptoms (courtesy Alessandro Vitale); b. severe symptoms on green; c. dead shoot (courtesy José Luis Ramos Sáez de Ojer). — d–e. Cane bleaching (courtesy José Luis Ramos Sáez de Ojer). — f–g. External and internal discoloration of shoot inoculated with *D. hispaniae* (CPC 30323).

this study, were selected based on BLAST searches of NCBIs GenBank nucleotide database and included in the phylogenetic analyses. The final phylogenetic trees clearly distinguished four species newly described here (*D. bohemiae*, *D. celeris*, *D. hispaniae* and *D. hungariae*) and five known species (*D. ambigua*, *D. ampelina*, *D. baccae*, *D. eres* and *D. rudis*).

After sampling grapevine plants in several European countries and in Israel, molecular phylogenetic and morphological analyses were used to evaluate the diversity of *Diaporthe* species associated with this host. Several *Diaporthe* species are well-established in Europe in association with important diseases affecting agricultural crops such as peach, soybean, blueberry, citrus and avocado (Santos et al. 2011, Lombard et al. 2014, Guarnaccia et al. 2016, Prencipe et al. 2017, Guarnaccia & Crous 2017).

Diaporthe spp. are also frequently associated with grapevine diseases worldwide (Mostert et al. 2001a, Van Niekerk et al. 2005), such as Phomopsis cane and leaf spot, consisting of shoots breaking off, stunting, dieback and fruit rot. Moreover, cankers, swelling arms, and cane bleaching are serious diseases caused by *Diaporthe* spp. (Rawnsley et al. 2004, Úrbez-Torres et al. 2013). *Diaporthe ampelina* (= *Phomopsis viticola*) is known to affect all green parts of grapevines and is the main *Diaporthe* species causing Phomopsis cane and leaf spot. This species has been studied since 1958 (Pine 1958, 1959, Pscheidt & Pearson 1989), and recently, its ability to also cause wood cankers was demonstrated (Úrbez-Torres et al. 2013). *Diaporthe kyushuensis* and *D. perjuncta* are respectively known for causing swelling arm and dormant cane bleaching (Kajitani & Kanematsu 2000). *Diaporthe ambigua*, *D. eres* and *D. foeniculina* occurred in Californian vineyards (Úrbez-Torres et al. 2013). *Diaporthe eres* was also reported as causing diseases in Croatia and Italy (Kaliterna et al. 2012, Cinelli et al. 2016), whilst *D. eres*, *D. hongkongensis*, *D. phaseolorum* and *D. sojae* were reported as pathogens in China (Dissanayake et al. 2015).

DNA sequence data are essential in resolving taxonomic questions, redefining species boundaries, and accurate naming of

species as required for the effective communication about plant pathogens. Regarding *Diaporthe*, Santos et al. (2017) showed that species separation is better when five loci (ITS, *tef1*, *tub2*, *his3* and *cal*) are simultaneously used to build the resulting phylogenies. Recent phylogenetic analyses of the genus *Diaporthe* studied more than 170 species, and grouped some of those in species complexes, such as *D. arecae*, *D. eres* and *D. sojae*, which include important plant pathogenic species (Huang et al. 2013, Udayanga et al. 2014a, 2015). Moreover, a polyphasic approach has substantially reshaped the taxonomy of *Diaporthe* species involved with grapevine diseases (Mostert et al. 2001a, Van Niekerk et al. 2005, Dissanayake et al. 2015).

Although several studies on the presence of *Diaporthe* in major grapevine production areas were conducted in the past, this was never the case in Europe, and thus a large-scale investigation of *Diaporthe* spp. associated with grapevine was needed. This study provides the first molecular characterisation of *Diaporthe* diversity related to *Vitis* spp. in Europe and Israel, combined with morphological characterisation.

A combined alignment of seven genes (*act*, *Apn*2, *cal*, *tef1*, *his3*, FG1093 and *tub2*) was incorporated in a recent revision of the *D. eres* complex, among which the *tef1*, *Apn2* and *his3* genes were considered as the most informative loci for defining species in this complex (Udayanga et al. 2014a). The ITS region was excluded from their phylogenetic analysis and the authors stated that a poorly supported non-monophyletic grouping was observed when ITS sequences were included in the combined analysis. This problem was detected in our phylogenetic analysis of the *D. eres* complex and in other studies (Gomes et al. 2013, Dissanayake et al. 2017, Gao et al. 2017) where two separate clades of *D. eres* are observed (*D. eres* (A) and *D. eres* (B), Fig. 1). The *D. eres* (A) clade included the ex-epitype culture CBS 138594, several other known taxa in the *D. eres* complex and 98 strains collected from grapevines in the present (Fig. 1), and a previous study (Cinelli et al. 2016). Several highly-supported subclades clustered with *D. eres* (A). However, they were not clearly differentiated based on both single-locus and morphological similarity. Thus, they are not considered as new species. The *D. eres* (B) clade, previously known as the *Diaporthe* cf. *nobilis*/*Phomopsis fukushii* complex (Gomes et al. 2013), grouped four reference strains of *D. eres*, according to the seven-gene analysis from Udayanga et al. (2014b), and seven isolates from grapevines. *Diaporthe eres* was recovered from grapevines in all the countries investigated except Israel. A further three strains collected in the UK was revealed to represent a new species (*D. celeris*) in the *D. eres* complex, clearly differentiated from the closest species (*D. celastrina* and *D. eres*) based on multi-locus phylogenetic analyses and morphology.

Another two new species, *D. hungariae* (reported from Hungary and Spain) and *D. hispaniae* (from Spain), were closely related, but clearly separated based on morphological and molecular characteristics from *D. ampelina*, historically known as the most aggressive *Diaporthe* species of grapevine and found in all the countries sampled in this study. The final species described in this study as new is *D. bohemiae*, that was collected in the Czech Republic. *Diaporthe rudis* was isolated from samples collected in Czech Republic, France, Italy, Spain and UK, confirming its role as key pathogen of grapevine. Two isolates of *D. ambigua* were recovered in Spain, and for the first time after its description by Lombard et al. (2014), *D. baccae* was found in Croatia, France and Spain. *Diaporthe baccae* was previously found in Croatia by Kaliterna et al. (2012) but wrongly identified as closely related *D. foeniculina* (as *D. neotheicola*).

Preliminary pathogenicity tests of the species found associated with grapevine for the first time in the current study focused on green shoots (Phillips 1999, Mostert et al. 2001a, Van Niekerk et al. 2005). Inoculation of green shoots in growth chambers with *D. celeris* and *D. baccae* resulted in the development of lesions. The most severe symptoms were detected on stems inoculated with *D. hispaniae* and *D. hungariae*. Therefore, this study provides results about the ability from these species to cause disease of grapevines, together with the well-known key pathogen *D. ampelina*. The other inoculated species, *D. bohemiae*, was not able to induce lesions, appearing to be an endophyte in grapevines.

The present study is the first evaluation of *Diaporthe* species associated with grapevines in Europe and Israel, combining morphology and molecular data, providing useful information for evaluating pathogenicity of the various species. To our knowledge, this study represents also the first report of *D. baccae* associated with grapevines, and of *D. ambigua* on grapevines in Europe.

Acknowledgements This research was supported by the European COST Action FA1303 on Sustainable control of grapevine trunk diseases (Mana-GTD). Surveys and fungal collection performed in Hungary were supported by Szechenyi 2020 programme, the European Regional Development Fund, the Hungarian Government (GINOP-2.3.2-15-2016-00061) and EU H2020 project no. 652601. We are grateful to Arien van Iperen (cultures), Mieke Starink-Willemse and Elias Jonk (DNA isolation, amplification, and sequencing) for their technical assistance.

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