RESEARCH ARTICLE

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

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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 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.

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	GQ281531	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 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
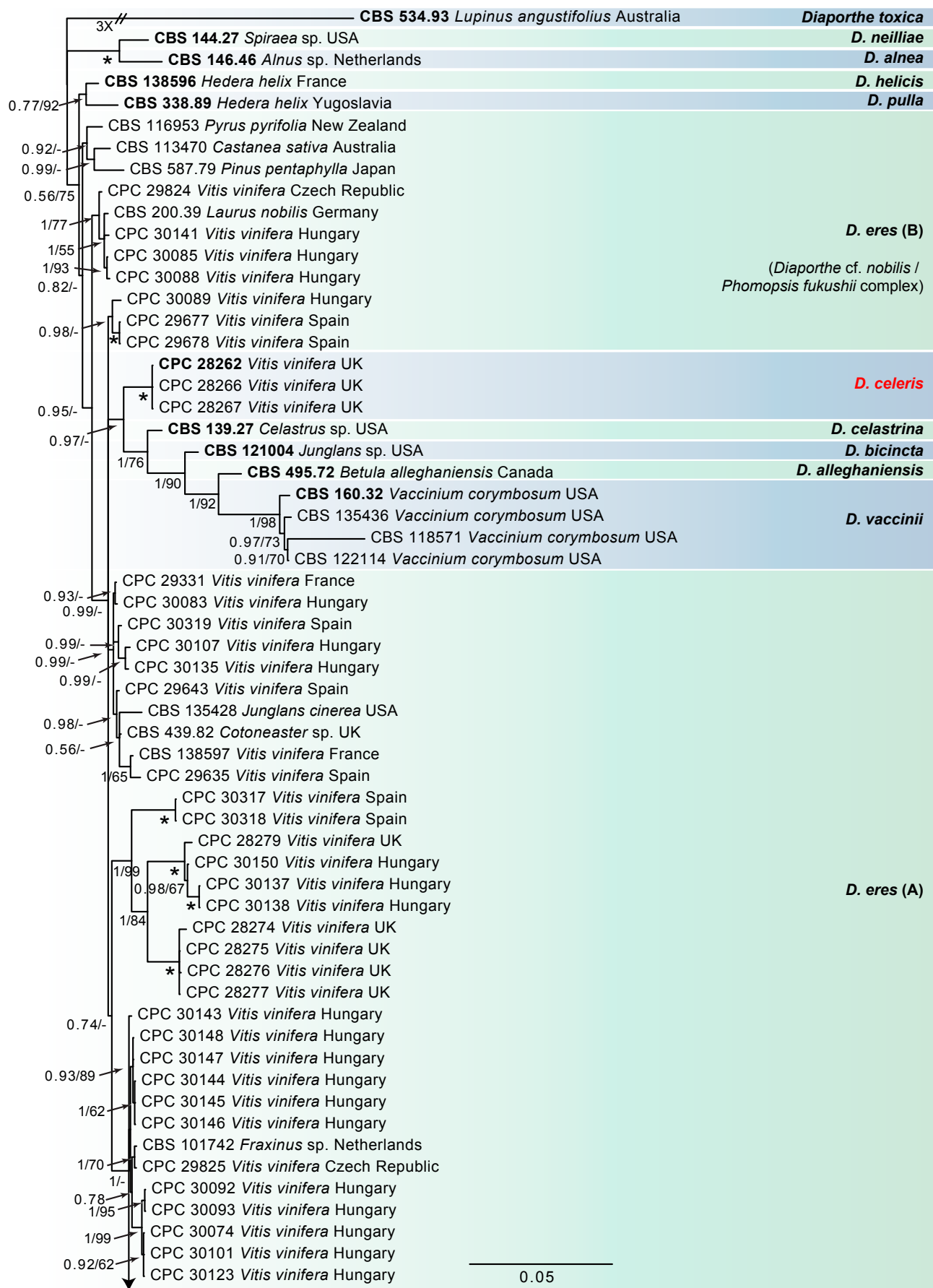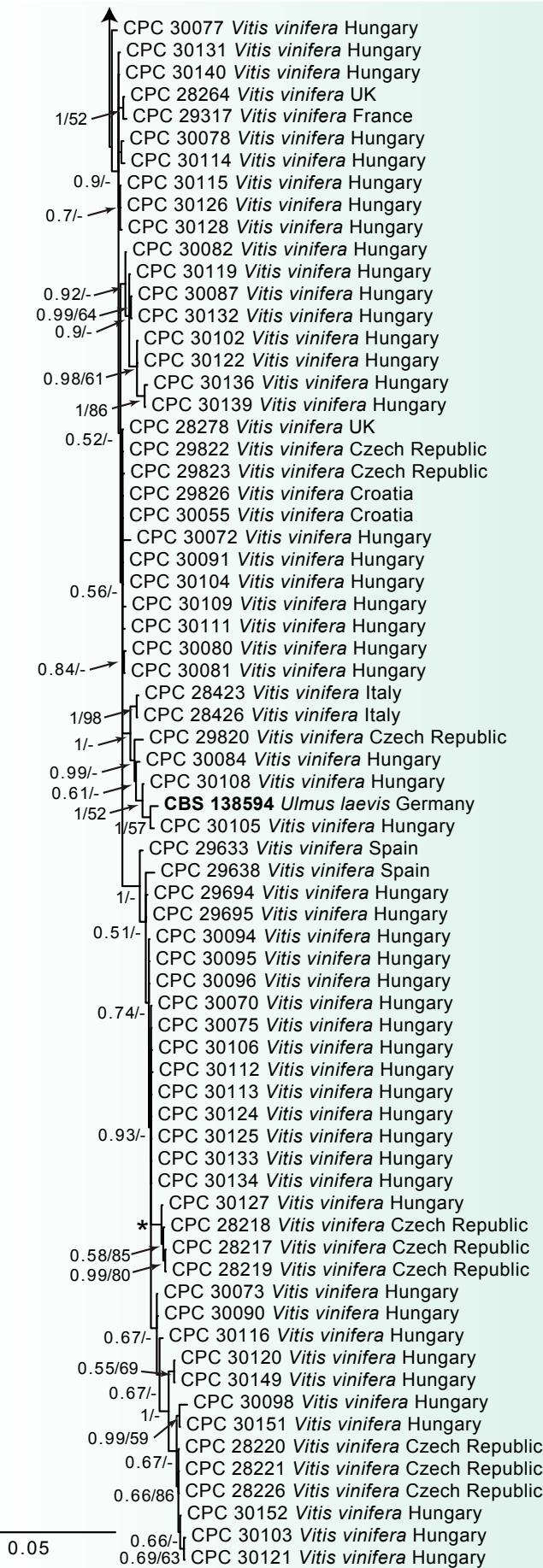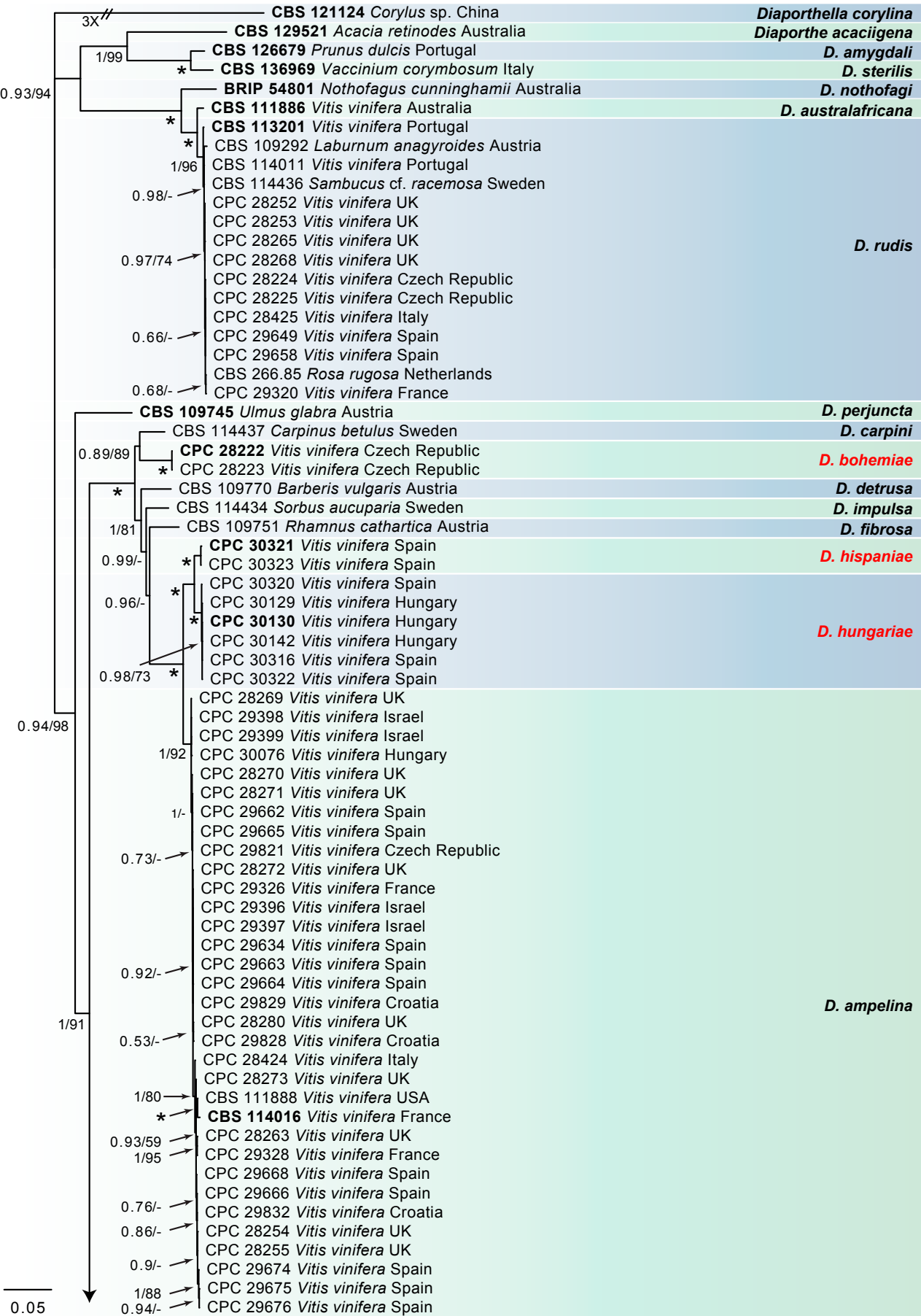
Fig. 1 Consensus phylogram of 86 082 trees resulting from a Bayesian analysis of the combined ITS, *tub2*, *his3*, *tef1* and *cal* sequence alignments of the *D. eres* complex. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. The asterisk symbol (*) represents 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).
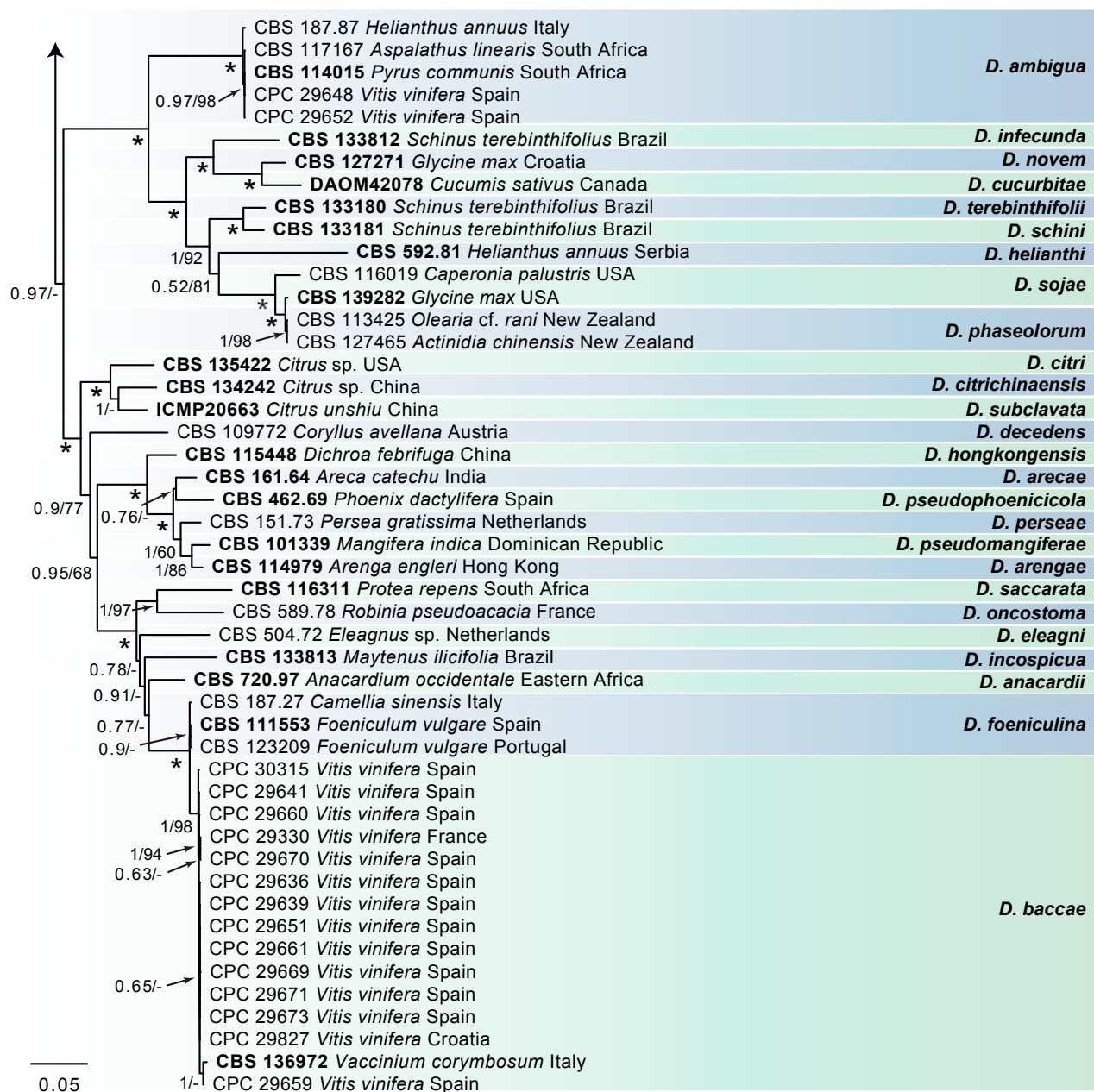
Fig. 2 (cont.)

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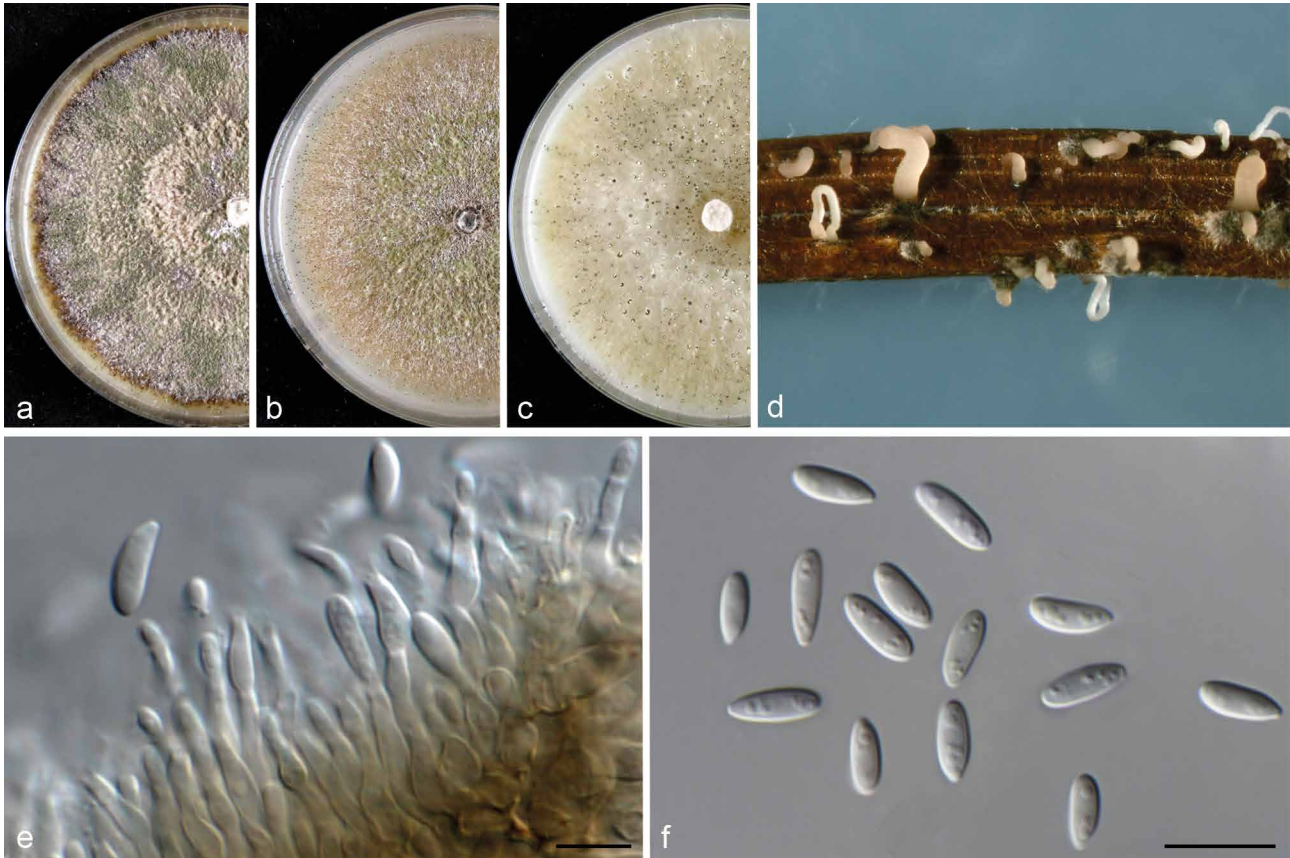
Fig. 3   *Diaporthe bohemiae* (CBS 143347). a–c. Colonies on MEA, PDA and OA, respectively; d. conidiomata sporulating on PNA; e. conidiogenous cells; f. alpha conidia. — Scale bars = 10 µm.
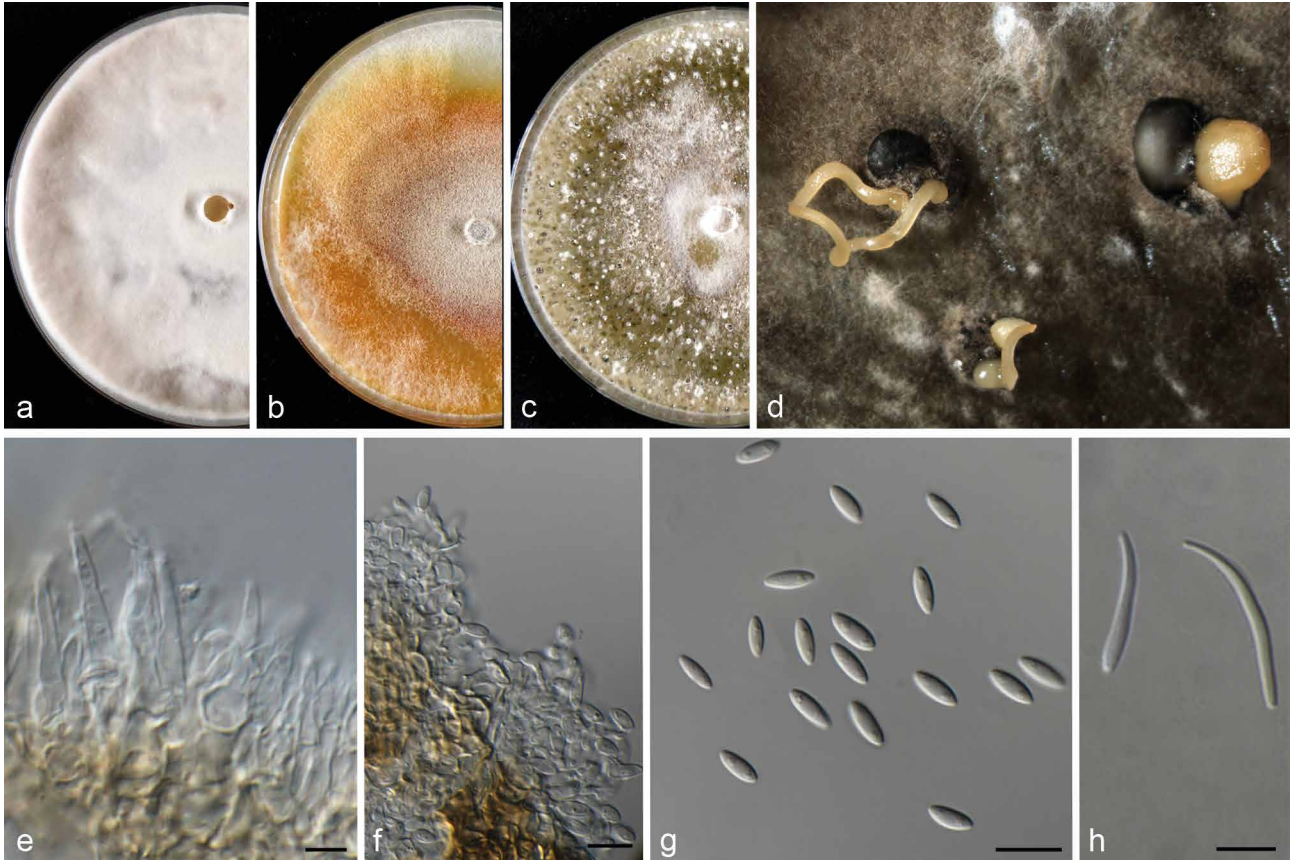

Fig. 4   *Diaporthe celeris* (CBS 143349). a–c. Colonies on MEA, PDA and OA, respectively; d. conidiomata sporulating on OA; e. conidiophores; f. conidiogenous cells; g. alpha conidia; h. beta conidia. — Scale bars = 10 µm.

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
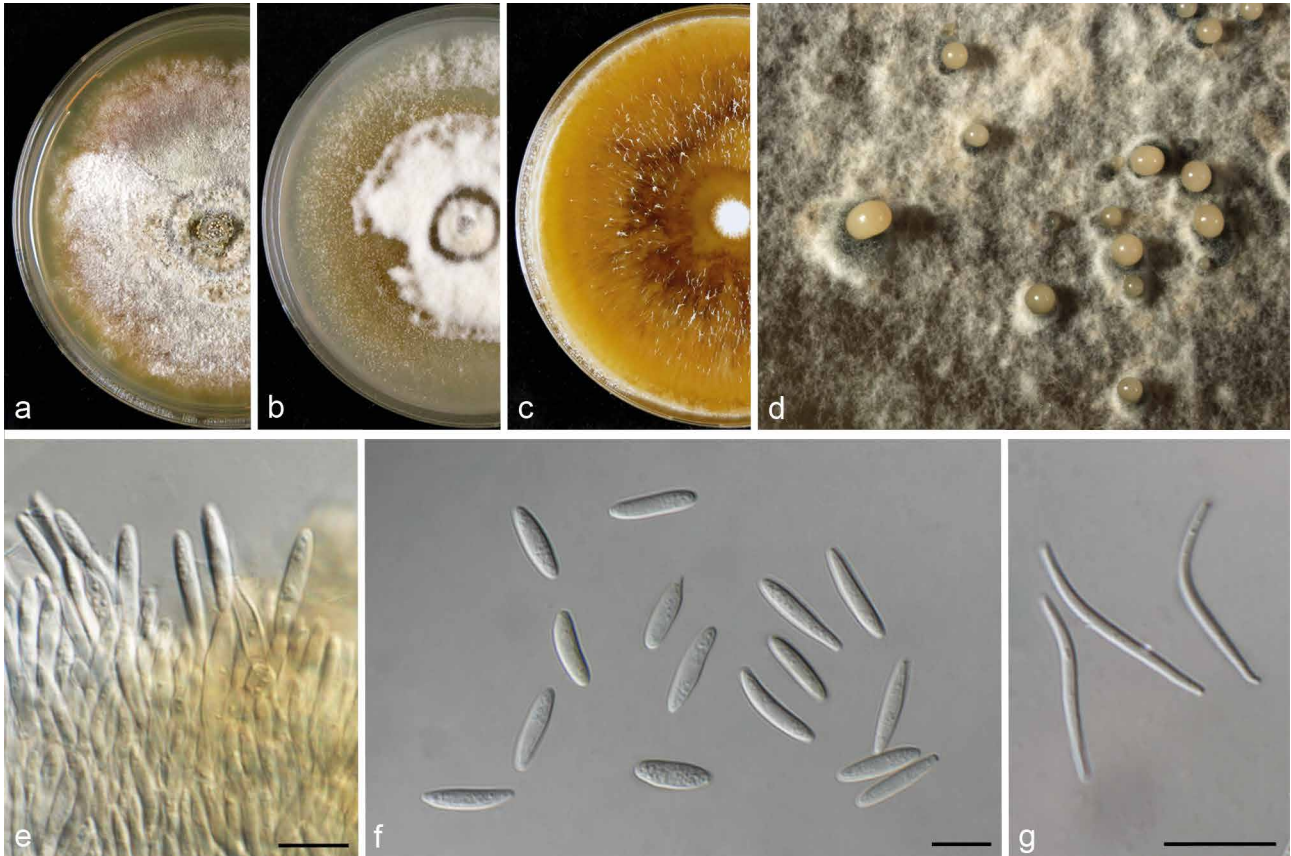

Fig. 5 *Diaporthe hispaniae* (CBS 143351). a−c. Colonies on MEA, PDA and OA, respectively; d. conidiomata sporulating on PDA; e. conidiogenous cells; f. alpha conidia; g. beta conidia. — Scale bars = 10 µm.
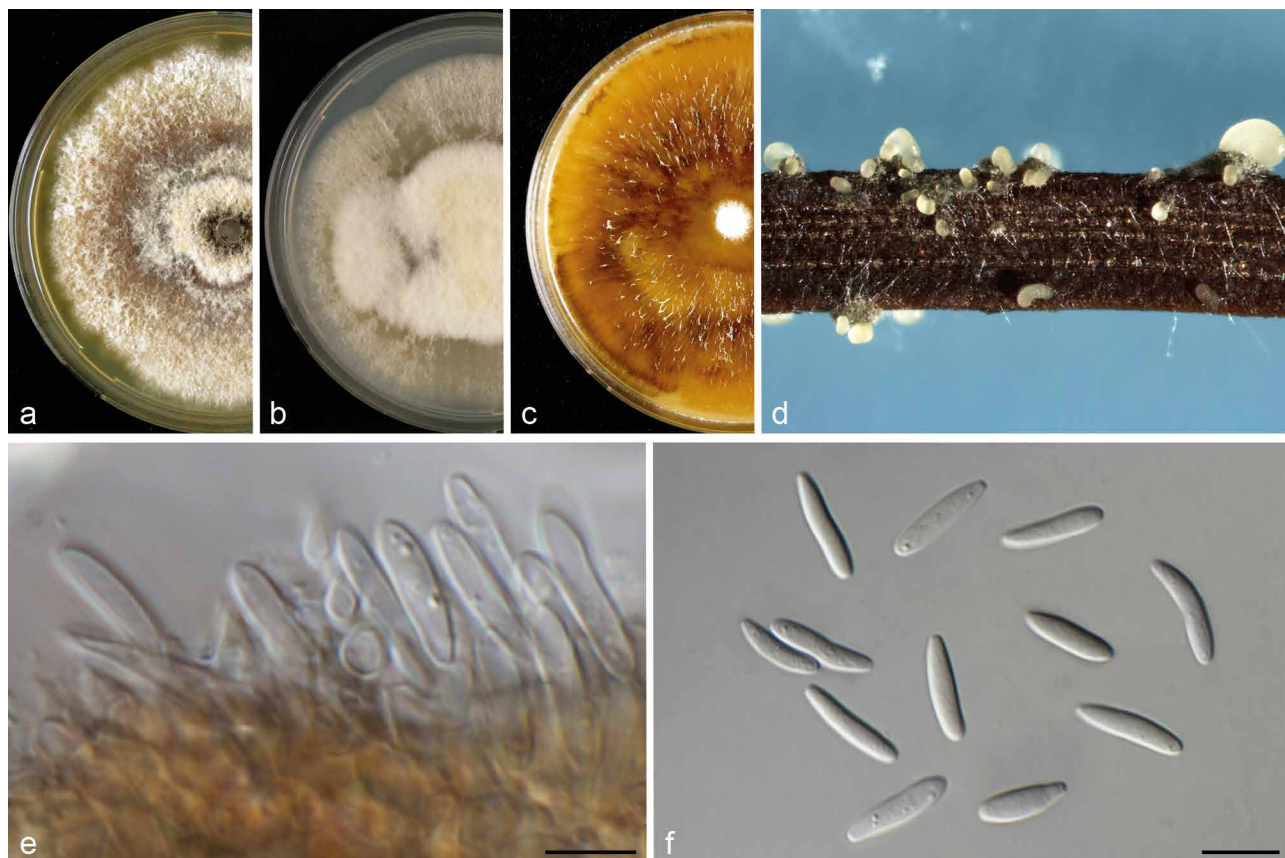
Fig. 6 *Diaporthe hungariae* (CBS 143353). a–c. Colonies on MEA, PDA and OA, respectively; d. conidiomata sporulating on PNA; e. conidiogenous cells; f. alpha conidia. — Scale bars = 10 μm.

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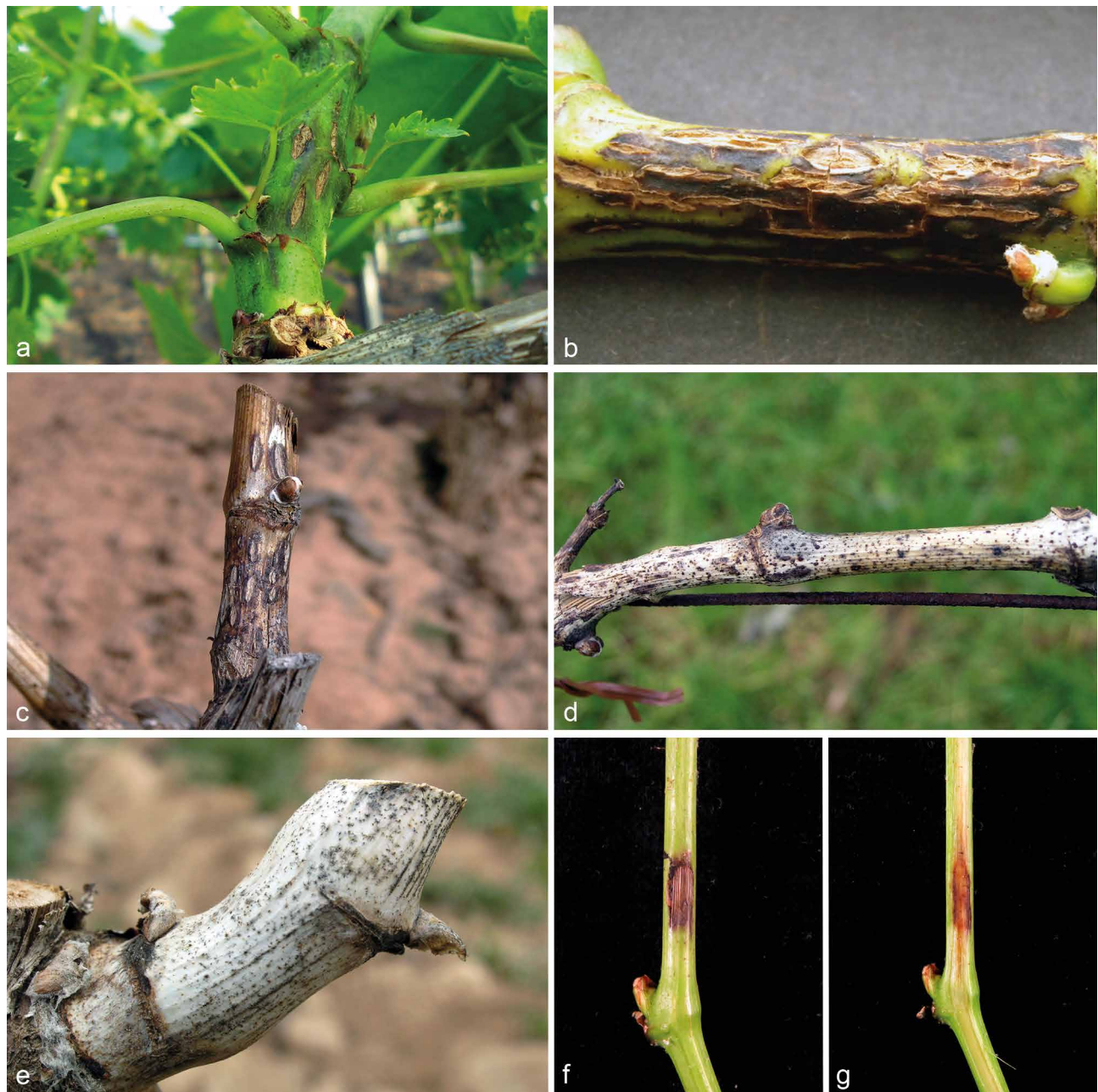
Fig. 7   a–e. Natural and f–g. artificial symptoms on *V. vinifera* with associated *Diaporthe* species. a–c. Lesions of Phomopsis cane and leaf spot on shoot: a. initial symptoms (courtesy Alessandro Vitale); b. severe symptoms on green; c. dead shoot (courtesy José Luis Ramos Sáez de Ojer). — d–e.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

over, 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.

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