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RESEARCH PAPER

Diaporthe species associated with *Vaccinium*, with specific reference to Europe

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Summary. Species of the genus *Vaccinium* are commercially cultivated in Europe for their berries, which are highly valued for dietary and pharmaceutical properties. Cultivation is severely limited due to a range of fungal diseases, especially those caused by species of *Diaporthe*. A number of *Diaporthe* isolates have been collected from *Vaccinium* growing regions in Europe, and initially identified as *D. vaccinii* based on host association. Using DNA sequence inference of the combined β-tubulin, calmodulin, translation elongation factor 1-alpha and the internal transcribed spacer region of the nuclear rDNA, along with morphological characteristics, six species were characterised. *Diaporthe eres*, *D. vaccinii* and *D. viticola* are known species and three novel taxa are described here as *D. asheicola*, *D. baccae* and *D. sterilis*. This study is the first confirmed report of *D. vaccinii* in Latvia and the Netherlands.

Key words: *Diaporthe vaccinii*, quarantine.

Introduction

The genus *Vaccinium* contains approximately 450 species of woody, perennial shrubs belonging to the family *Ericaceae* (heath) (Dierking *et al.*, 1993; Nestby *et al.*, 2012). Four *Vaccinium* spp. are regarded as economically important and indigenous to Europe, namely *V. myrtillus* (bilberry), *V. oxycoccus* (cranberry), *V. uliginosum* (bog bilberry) and *V. vitis-idaeae* (lingonberry) (Dierking *et al.*, 1993). *Vaccinium macrocarpon* (large cranberry or American cranberry) and *V. corymbosum* (highbush blueberry), which were introduced from North America, are also commercially cultivated in Europe (Naumann, 1993; Caruso and Ramsdell, 1995; Prodorutti *et al.*, 2007; Nestby *et al.*, 2012). The berries of these plants are greatly prized in the food industry based on their high content of beneficial nutrients (Nestby *et al.*, 2012), and in the pharmaceutical industry for their bioactive phytochemicals such as naturally occurring antioxidants (Faria *et al.*, 2005). However, commercial cultivation of *Vaccinium* is severely limited due to a range of fungal pathogens (Caruso and Ramsdell, 1995).

With the abolition of dual nomenclature from the International Code of Nomenclature for algae, fungi and plants (ICN), asexual and sexual names of fungi received equal status (Hawksworth *et al.*, 2011; Wingfield *et al.*, 2012), with *Phomopsis* being treated as synonym of *Diaporthe* (Santos *et al.*, 2010; 2011; Crous *et al.*, 2011; Udayanga *et al.*, 2012; Gomes

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et al., 2013; Kirk *et al*., 2013). Species of *Diaporthe* are known to cause several diseases of *Vaccinium*, including twig blight, stem cankers and fruit rot (Caruso and Ramsdell, 1995; Farr *et al*., 2002a,b; Miller *et al*., 2006; Polashock, 2006; Latorre *et al*., 2012; Tadych *et al*., 2012; Elfar *et al*., 2013). Several *Diaporthe* and two *Phomopsis* spp. have been reported from *Vaccinium* spp., including *D. ambigua* (Elfar *et al*., 2013), *D. australafricana* (Latorre *et al*., 2012; Elfar *et al*., 2013), *D. neotheicola*, *D. passiflorae* (Elfar *et al*., 2013), and *D. vaccinii* (Caruso and Ramsdell, 1995; Farr *et al*., 2002a,b; Miller *et al*., 2006; Polashock, 2006; Tadych *et al*., 2012), as well as *P. columnaris* and *P. myrtilli* (Farr *et al*., 2002b). One of these species, *D. vaccinii,* is listed as a quarantine pest for the European Union (European Union 2000). Extensive surveys to collect and identify fungal pathogens of *Vaccinium* spp. have been conducted recently in Chile (Elfar *et al*., 2013), New Zealand (Miller *et al*., 2006) and the USA (Tadych *et al*., 2012). However only a few reports have been made of *Diaporthe* and *Phomopsis* spp. isolated from *Vaccinium* spp. grown in Europe (Petrak, 1924; Wilcox and Falconer, 1961; Baker, 1972; Teodorescu *et al*., 1985; Farr *et al*., 2002b; Gabler *et al*., 2004).

Although *D. vaccinii* is globally regarded as the prominent species of *Diaporthe* on *Vaccinium* spp. (Caruso and Ramsdell, 1995; Farr *et al*., 2002a), this fungal pathogen has only been reported from Romania (Teodorescu *et al*., 1985), the United Kingdom (Wilcox and Falconer, 1961; Baker, 1972) and Lithuania (Gabler *et al*., 2004; Kačergius *et al*., 2004) in Europe, apparently introduced with American *Vaccinium* cultivars being exploited for commercial cultivation. This fungus was, however, unable to establish and spread from both these regions (Teodorescu *et al*., 1985). Generally, *D. vaccinii* is regarded as indigenous to North America as it has been reported from all *Vaccinium* growing regions in the USA and Canada (Shear *et al*., 1931; Weingartner and Klos, 1975; Chao and Glawe, 1985; Farr *et al*., 2002a,b; Tadych *et al*., 2012).

With the increase in commercial cultivation of *Vaccinium* spp. in Europe, a number of *Diaporthe* isolates have been collected from these regions and maintained in culture collections (Table 1). The majority of these isolates are treated as *D. vaccinii*, based only on host association, as they are similar to *D. phaseolorum*, but differ in host specificity (Wehmeyer, 1933; Chao and Glawe, 1985). The present study therefore aimed to place these European *Diaporthe* isolates from *Vaccinium* plants in the correct taxonomic context based on DNA sequence inference, supported by morphological and cultural data.

Materials and methods

Isolates

Strains of *D. vaccinii* and other *Diaporthe* spp. isolated from *Vaccinium* spp. were obtained from various culture collections, as indicated in Table 1. Several strains were also freshly isolated from symptomatic and asymptomatic *Vaccinium* spp. collected in the Netherlands and Italy (Table 1), as explained by Elfar *et al*. (2013). Representative strains were deposited in the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), in Utrecht, the Netherlands, and the working collection of Pedro Crous (CPC) maintained at the CBS.

DNA phylogeny

Total genomic DNA was extracted from cultures grown on potato dextrose agar (PDA) for 7 d, using UltraClean™ Microbial DNA isolation kits (Mo Bio Laboratories, Inc.) according to the manufacturer's protocol. Partial gene sequences were determined for β-tubulin (BTUB), calmodulin (CAL), translation elongation factor 1-alpha (TEF), and the internal transcribed spacer region (ITS) of the nuclear rDNA using the primers and protocols described by Gomes *et al*. (2013). Integrity of the sequences was checked by sequencing the amplicons in both directions using the same primer pairs used for amplification. Consensus sequences for loci were assembled in MEGA v. 5.1 (Tamura *et al*., 2011), then compared and added to representative sequences from Udayanga *et al*. (2012) and Gomes *et al*. (2013) (Table 1). Alignments for each locus was generated in MAFFT v. 6 (Katoh and Toh, 2010) and manually corrected where necessary.

The 70% reciprocal bootstrap method of Mason-Gamer and Kellogg (1996) was used to determine whether the four loci sequenced were congruent. Evolutionary models were estimated in MrModeltest (Nylander, 2004) using the Akaike Information Criterion (AIC) for each locus, and implemented in the bootstrap analysis runs in PAUP (Phylogeny Analysis Using Parsimony, v. 4.0b10; Swofford, 2003) for 10,000 replicates. The inferred tree topologies

were visually compared for conflicts between the separate loci.

Phylogenetic analyses were based both on Bayesian inference (BI) and Maximum Likelihood (ML). For both methods, the best evolutionary models for each partition were determined using MrModeltest (Nylander, 2004) and incorporated into the analyses. For the BI analysis, MrBayes v. 3.1.1 (Ronquist and Huelsenbeck, 2003) was used to generate phylogenetic trees under optimal criteria per partition. A Markov Chain Monte Carlo (MCMC) algorithm of four chains was started in parallel from a random tree topology with the heating parameter set at 0.3. The MCMC analysis lasted until the average standard deviation of split frequencies came below 0.01 with trees saved each 1,000 generations. The first 25% of saved trees were discarded as the "burn-in" phase and posterior probabilities (PP) determined from the remaining trees.

The ML analysis was carried out for the same partitions using RAxML software (Stamatakis *et al*., 2005) through the CIPRES Website (http://www.phylo.org), to obtain a second measure of branch support. The robustness of the analysis was evaluated by bootstrap support (BS) with the bootstrap replicates automatically determined by the software (Stamatakis *et al*., 2008). Novel sequences generated in this study were deposited in GenBank (Table 1), and the alignments and phylogenetic tree were deposited in TreeBASE (S15189).

Sterile species (see species notes) were characterised using unique fixed single nucleotide polymorphisms (SNP's). For each sterile species treated, the closest phylogenetic neighbour(s) were selected and subjected to SNP analyses using DnaSP v.5.00.07 (Librado and Rozas, 2009).

Taxonomy

Axenic cultures were sub-cultured onto 2% tap water agar supplemented with sterile pine needles (PNA; Smith *et al*., 1996), PDA, oatmeal agar (OA) and 2% malt extract agar (MEA) (Crous *et al*., 2009), and incubated at 18–20°C under a 12 h near-ultraviolet light/12 h dark cycle to induce sporulation. Fungal structures were mounted in clear lactic acid, and 30 measurements were made for all structures including the conidia. The 95% confidence levels were determined, and extreme dimensions determined (recorded here in parentheses). Colony characteristics were noted after 10 d of growth on PDA, OA and MEA at 24°C in the dark, and colony colours were determined using the colour charts of Rayner (1970). Descriptions, nomenclature and illustrations were deposited in MycoBank (Crous *et al*., 2004).

Results

Isolates

A total of 38 isolates representing *Diaporthe* spp. isolated from *Vaccinium* spp. were obtained from various culture collections, of which 28 isolates originated from Europe (Table 1). Seventeen of these were obtained from the symptomatic and asymptomatic plant material collected in Italy and the Netherlands (Table 1). Typical symptoms observed on infected *Vaccinium* plants included dark red-brown cankers on the stems with internal discolouration of the vascular tissues beneath these cankers radiating up and down the stems. Red-brown leaf spots were also observed.

DNA phylogeny

The 70% reciprocal bootstrap tree topologies showed no conflicts for the BTUB, CAL and TEF gene regions. However, the ITS gene region revealed a conflicting tree topology to the other three gene regions included. This was ignored, based on the argument of Cunningham (1997) that combining incongruent partitions could increase phylogenetic accuracy. Therefore, the four gene regions were combined, as was done by Gomes *et al*., (2013) for the same loci.

The combined alignment of BTUB, CAL, ITS and TEF used for both Bayesian and ML analyses contained 1621 characters from 76 isolates (including outgroup). Of the ingroup isolates, 37 were selected based on comparisons to representative sequences from Udayanga *et al*. (2012) and Gomes *et al*. (2013), and subsequently retrieved from GenBank (Table 1). The numbers of unique site patterns per data partition were 286 from 537 for BTUB, 165 from 334 for CAL, 136 from 474 for ITS and 199 from 276 for TEF which included alignment gaps. Based on the results of MrModeltest all partitions had dirichlet base frequencies and a GTR+I+G model with inverse gamma-distributed rates was used for ITS and HKY+I+G with inverse gamma-distributed rates were implemented for BTUB, CAL and TEF. The Bayesian anal-

Table 1. Diaporthe isolates used in this study.

Species	Strain[1]	Host	Country	Collector	Genbank accession numbers[2]			
					BTUB	CAL	ITS	TEF
Diaporthe alleghaniensis	**CBS 495.72** = ATCC 24097	*Acer saccharum*	–	R.H. Arnold	KC343975	KC343249	KC343007	KC343733
D. ampelina	**CBS 114016** = CPC 2660 = PV F98-1	*Vitis vinifera*	France	P. Larignon	JX275452	AY745026	AF230751	AY745056
	CBS 114867 = CPC 4708	*V. vinifera*	Turkey	M. Erkan	KC343985	KC343259	KC343017	KC343743
D. amygdali	CBS 115620 = FAU 1005	*Prunus persica*	USA	W. Uddin	KC343988	KC343262	KC343020	KC343746
	CBS 126679	*P. dulcis*	Portugal	E. Diogo	KC343990	KC343264	KC343022	KC343748
D. angelicae	CBS 111591 = AR 3724	*Heraleum sphondylium*	Austria	A.Y. Rossman	KC343994	KC343268	KC343026	KC343752
D.arctii	CBS 136.25	*Arctium* sp.	–	A.W. Archer	KC343999	KC343273	KC343031	KC343757
D. asheicola	**CBS 136967** = CPC 16508	*Vaccinium ashei*	Chile	A. Schilder	*KJ160518*	*KJ160542*	*KJ160562*	*KJ160594*
	CBS 136968 = CPC 16511	*V. ashei*	Chile	A. Schilder	*KJ160519*	*KJ160543*	*KJ160563*	*KJ160595*
D. australafricana	**CBS 111886** = CPC 2676	*Vitis vinifera*	Australia	R.W.A. Schepers	KC344006	KC343280	KC343038	KC343764
	CBS 113487 = CPC 2655	*V. vinifera*	South Africa	L. Mostert	KC344007	KC343281	KC343039	KC343765
D. baccae	CBS 136971 = CPC 20587[a]	*Vaccinium corymbosum*	Italy	G. Polizzi	–	–	*KJ160564*	*KJ160596*
	CBS 136972 = CPC 20584[a]	*V. corymbosum*	Italy	G. Polizzi	–	–	*KJ160565*	*KJ160597*
	CPC 20583[a]	*V. corymbosum*	Italy	G. Polizzi	–	–	*KJ160566*	*KJ160598*
	CPC 20585[a]	*V. corymbosum*	Italy	G. Polizzi	–	–	*KJ160567*	*KJ160599*
	CPC 20586[a]	*V. corymbosum*	Italy	G. Polizzi	–	–	*KJ160568*	*KJ160600*
D. celastrina	CBS 139.27	*Celastrus scandens*	–	L.E. Wehmeyer	KC344015	KC343289	KC343047	KC343773
D.chamaeropsis	CBS 753.70	*Spartium junceum*	Croatia	J.A. von Arx	KC344017	KC343291	KC343049	KC343775
D. cinerascens	CBS 719.96	*Ficus carica*	Bulgaria	E. Ilieva	KC344018	KC343292	KC343050	KC343776
D. cuppatea	**CBS 117499** = CPC 5431	*Aspalathus linearis*	South Africa	J.C. Janse van Rensburg	KC344025	KC343299	KC343057	KC343783
D. cynaroidis	**CBS 122676** = CPC 13180 = CMW 22190	*Protea cynaroides*	South Africa	S. Marincowitz	KC344026	KC343300	KC343058	KC343784

(Continued)

Table 1. Continued

Species	Strain[1]	Host	Country	Collector	Genbank accession numbers[2]			
					BTUB	CAL	ITS	TEF
D. eres	CBS 287.74	*Sorbus aucuparia*	The Netherlands	W.M. Loerakker	KC344052	KC343326	KC343084	KC343810
	CBS 439.82 = BBA P-407 =IMI 162181a	*Cotoneaster* sp.	Scotland	H. Butin	KC344058	KC343332	KC343090	KC343816
	CBS 524.82	*V. vitis-idea*	Poland	H.A. van der Aa	*KJ160520*	–	JQ807448	JQ807377
	CBS 109767 = AR 3538 = WJ 1643	*Acer campestre*	Austria	W. Jaklitsch	KC344043	KC343317	KC343075	KC343801
	CBS 134736 = PD02125472	*Vaccinium* sp.	The Netherlands	–	–	*KJ160544*	*KJ160569*	*KJ160601*
	CBS 134739 = PD05035464	*V. corymbosum*	The Netherlands	L. Colon	–	–	*KJ160570*	*KJ160602*
	CBS 134742 = PD02026319	*V. oxycoccus*	Lithuania	Z. Jovaišienė	*KJ160521*	–	*KJ160571*	*KJ160603*
	CPC 16510	*V. corymbosum*	Chile	A. Schilder	*KJ160522*	*KJ160545*	*KJ160572*	*KJ160604*
	CPC 23802 = PD04995578-2[a]	*V. myrtillus*	The Netherlands	G.C.M van Leeuwen	*KJ160523*	*KJ160546*	*KJ160573*	*KJ160605*
	CPC 23803 = PD04995551[a]	*V. myrtillus*	The Netherlands	G.C.M van Leeuwen	*KJ160524*	–	*KJ160574*	*KJ160606*
	CPC 23804 = PD04328898[a]	*V. corymbosum*	The Netherlands	G.C.M van Leeuwen	*KJ160525*	–	*KJ160575*	*KJ160607*
	CPC 23806	*Vaccinium* sp.	Germany	–	*KJ160526*	–	*KJ160576*	*KJ160608*
	CPC 23808 = PD03173021	*V. corymbosum*	USA	–	*KJ160527*	*KJ160547*	*KJ160577*	*KJ160609*
	CPC 23809	*Vaccinium* sp.	Lithuania	–	–	–	*KJ160578*	*KJ160610*
D. foeniculacea	CBS 187.27	*Camellia sinensis*	Italy	M. Cruzi	KC344075	KC343349	KC343107	KC343833
	CBS 117501 = CPC 5422	*Aspalathus linearis*	South Africa	J.C. Janse van Rensburg	–	–	DQ286287	DQ286261
	CBS 123208 = Di-C004/5	*Foeniculum vulgare*	Portugal	A.J.L. Phillips	KC344072	KC343346	KC343104	KC343830
	CBS 123209 = Di-C004/4	*F. vulgare*	Portugal	A.J.L. Phillips	KC344073	KC343347	KC343105	KC343831
D. juglandina	CBS 121004 = DP 0659	*Juglans* sp.	USA	L. Vasilyeva	KC344102	KC343376	KC343134	KC343860
D. lusitanicae	**CBS 123212** = Di-C001/5	*F. vulgare*	Portugal	J.M. Santos	KC344104	KC343378	KC343136	KC343862
	CBS 123213 = Di-C001/3	*F. vulgare*	Portugal	J.M. Santos	KC344105	KC343379	KC343137	KC343863
D. nobilis	CBS 200.39	*Laurus nobilis*	Germany	Kotthoff	KC344119	KC343393	KC343151	KC343877

(Continued)

Table 1. Continued

Species	Strain[1]	Host	Country	Collector	Genbank accession numbers[2]			
					BTUB	CAL	ITS	TEF
D. novem	**CBS 127270** = 3-27/3-1	*Glycine max*	Croatia	T. Duvnjak	KC344124	KC343398	KC343156	KC343882
	CBS 127271 = 5/27/3-3	*G. max*	Croatia	T. Duvnjak	KC344125	KC343399	KC343157	KC343883
D. perniciosa	CBS 124030 = GJS 77-49	*Malus pumila*	New Zealand	G.J. Samuels	KC344117	KC343391	KC343149	KC343875
D. pulla	CBS 338.89	*Hedera helix*	Yugoslavia	M. Muntañola-Cvetkovic	KC344120	KC343394	KC343152	KC343878
D. sterilis	**CBS 136969** = CPC 20563[a]	*V. corymbosum*	Italy	G. Polizzi	*KJ160528*	*KJ160548*	*KJ160579*	*KJ160611*
	CBS 136970 = CPC 20577[a]	*V. corymbosum*	Italy	G. Polizzi	*KJ160529*	*KJ160549*	*KJ160580*	*KJ160612*
	CPC 20575[a]	*V. corymbosum*	Italy	G. Polizzi	*KJ160530*	*KJ160550*	*KJ160581*	*KJ160613*
	CPC 20580[a]	*V. corymbosum*	Italy	G. Polizzi	*KJ160531*	*KJ160551*	*KJ160582*	*KJ160614*
	CPC 20582[a]	*V. corymbosum*	Italy	G. Polizzi	*KJ160532*	*KJ160552*	*KJ160583*	*KJ160615*
D. subordinaria	CBS 101711	*Plantago lanceolata*	New Zealand	B. Alexander	KC344181	KC343455	KC343213	KC343939
D. vaccinii	**CBS 160.32** = IFO 32646	*V. macrocarpon*	USA	C.L. Shear	KC344196	KC343470	KC343228	KC343954
	CBS 118571	*V. corymbosum*	USA	G.C. Adams	KC344191	KC343465	KC343223	KC343949
	CBS 122112 = FAU 474	*V. macrocarpon*	USA	L. Carris	KC344192	KC343466	KC343224	KC343950
	CBS 122113 = FAU 446	*V. macrocarpon*	USA	F. Caruso	*KJ160532*	*KJ160553*	*KJ160584*	*KJ160616*
	CBS 122114 = FAU 634	*V. corymbosum*	USA	D.C. Ramsdell	KC344193	KC343467	KC343225	KC343951
	CBS 122115 = FAU 590	*V. corymbosum*	USA	D.C. Ramsdell	KC344194	KC343468	KC343226	KC343952
	CBS 122116 = DF 5022	*V. corymbosum*	USA	D.F. Farr	KC344195	KC343469	KC343227	KC343953
	CBS 134741 = PD03276619[a]	*V. corymbosum*	The Netherlands	G.C.M van Leeuwen	*KJ160534*	*KJ160554*	*KJ160585*	*KJ160617*
	CPC 23811 = PD02026335	*V. oxycoccus*	Lithuania	Z. Jovaišienė	–	–	*KJ160586*	*KJ160618*
	CPC 23812 = PD04067579	*V. macrocarpon*	Lithuania	K Paruma	*KJ160535*	*KJ160555*	*KJ160587*	*KJ160619*
	CPC 23813 = PD05317242	*V. macrocarpon*	Latvia	K Paruma	*KJ160536*	*KJ160556*	*KJ160588*	*KJ160620*
D. viticola	**CBS 113201** = CPC 5683	*Vitis vinifera*	Portugal	A.J.L. Phillips	KC344202	KC343476	KC343234	KC343960
	CBS 114011 = CPC 2677	*V. vinifera*	Portugal	A.J.L. Phillips	KC344203	KC343477	KC343235	KC343961
	CPC 23799 = PD04995578-1[a]	*Vaccinium myrtillus*	The Netherlands	G.C.M van Leeuwen	*KJ160537*	*KJ160557*	*KJ160589*	*KJ160621*

(Continued)

Table 1. Continued

Species	Strain[1]	Host	Country	Collector	Genbank accession numbers[2]			
					BTUB	CAL	ITS	TEF
	CPC 23800 = PD03279491	*V. corymbosum*	The Netherlands	–	*KJ160538*	*KJ160558*	*KJ160590*	*KJ160622*
	CPC 23801 = PD04995586[a]	*V. myrtillus*	The Netherlands	G.C.M van Leeuwen	*KJ160539*	*KJ160559*	*KJ160591*	*KJ160623*
Diaporthe sp.1	CBS 134743[a]	*V. myrtillus*	The Netherlands	L. Lombard	*KJ160540*	*KJ160560*	*KJ160592*	*KJ160624*
Diaporthe sp. 2	CBS 101574	*Vaccinium* sp.	Italy	G. Mancini	*KJ160541*	*KJ160561*	*KJ160593*	–
"Phomopsis castanea"	CBS 113470 = DAOM 226800	*Castanea sativa*	Korea	K.A. Seifert	KC344114	KC343388	KC343146	KC343872
"Phomopsis conorum"	CBS 587.79	*Pinus pentaphylla*	Japan	G.H. Boerema	KC344121	KC343395	KC343153	KC343879
"Phomopsis fukushii"	CBS 116953 = NZ-26	*Pyrus pyrifolia*	New Zealand	W. Kandula & L. Castlebury	KC344115	KC343389	KC343147	KC343873
	CBS 116954 = NZ-27	*P. pyrifolia*	New Zealand	W. Kandula & L. Castlebury	KC344116	KC343390	KC343148	KC343874
Diaporthella corylina	CBS 121124 = AR 4131	*Corylus* sp.	China	L.N. Vassiljeva	KC343972	KC343246	KC343004	KC343730

[1] AR: Collection of A.Y. Rossman; ATCC: American type culture collection; BBA: Institut für Mikrobiologie culture collection, Berlin, Germany; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW: Collection of M.J. Wingfield housed at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC: Collection of P.W. Crous housed at CBS; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; DF: Collection of D.F. Farr, University of California, USA; Di: Collection of A.J.L. Phillips, Centro de Recursos Microbiológicos, Universidade Nova Lisboa, Lisbon, Portugal; FAU: Biological Science Department, Florida Atlantic University, Florida, USA; GJS: Collection of G.J. Samuels; IFO: institute for Fermentation, 17-85, Juso-honmachi, 2-chome, Yodogawa-ku, Osaka 532, Japan; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; PD: Collection of the Dutch National Plant Protection Organization (NPPO-NL), Wageningen, The Netherlands; NZ: Collection of L. Castlebury. Ex-type cultures indicated in bold.

[2] BTUB: partial beta-tubulin gene; CAL: partial calmodulin gene; ITS: internal transcribed spacer regions of the nrDNA and 5.6S nrDNA; TEF: partial translation elongation factor 1-alpha gene; sequences generated in this study indicated in italics.

[a] Isolates obtained from symptomatic and asympomatic *Vaccinium* plants during this study.

ysis lasted 10,000'000 generations, and the consensus tree with posterior probability was calculated from 15,002 trees left after 5,000 trees were discarded as the burn-in phase. The tree topology and bootstrap values of the ML supported the trees obtained from the Bayesian analysis. The tree was rooted to *Diaporthella corylina* (CBS 121124) (Figure 1).

In the phylogenetic tree the *Diaporthe* isolates from *Vaccinium* spp. clustered within eight clades, of which three clades and two single lineages represent possible new phylogenetic species (Figure 1). Two of these clades represent isolates originating from Italy. The first of these Italian clades (containing CBS 136969 and CBS 136970), with high BS (100%) and PP (1.00) values, is closely related to, but separate from, *D. amygdali* (ex-type CBS 126679). The second clade of Italian isolates (containing CBS 136971 and CBS 136972) is closely related to isolates representing *D. foeniculacea*. A third isolate originating from Italy, CBS 101574, formed a sister taxon to the clade containing *D. lusitanicea* (ex-type CBS 123212) and *D. cuppatea* (ex-type CBS 117499). Isolates from Germany (CPC 23806), Poland (CBS 524.82) and Lithuania (CPC 23809 and CBS 134742) grouped within the *Diaporthe eres* (CBS 439.82, CBS 109767; Gomes *et al*., 2013) clade along with several other isolates collected in the Netherlands (CPC 23802, CPC 23803, CPC 23804, CBS 134736 and CBS 134739). The isolates CPC 23804, CPC 23803 and CPC 23802 were initially identified as *Diaporthe vaccinii* (NPPO, 2013), however, the present study demonstrates that these isolates belong to the *D. eres* clade. The remaining isolates from Lithuania (CPC 23811 and CPC 23812) grouped within the *D. vaccinii* clade along with an isolate from Latvia (CPC 23813) and one from the Netherlands (CBS 134741). The phylogenetic position of one isolate from the Netherlands (CBS 134743) remains unresolved. Two isolates (CBS 136967 and CBS 136968) originating from Chile, grouped together in a well-supported clade (BS = 100%; PP = 1.00), closely related to, but separate from, *D. australafricana* (ex-type CBS 111886), *D. cynaroidis* (ex-type CBS 122676) and *D. viticola* (ex-type CBS 113201). Three isolates from the Netherlands (CPC 23799, CPC 23780 and CPC 23801) grouped within the *D. viticola* (ex-type CBS 113201) clade.

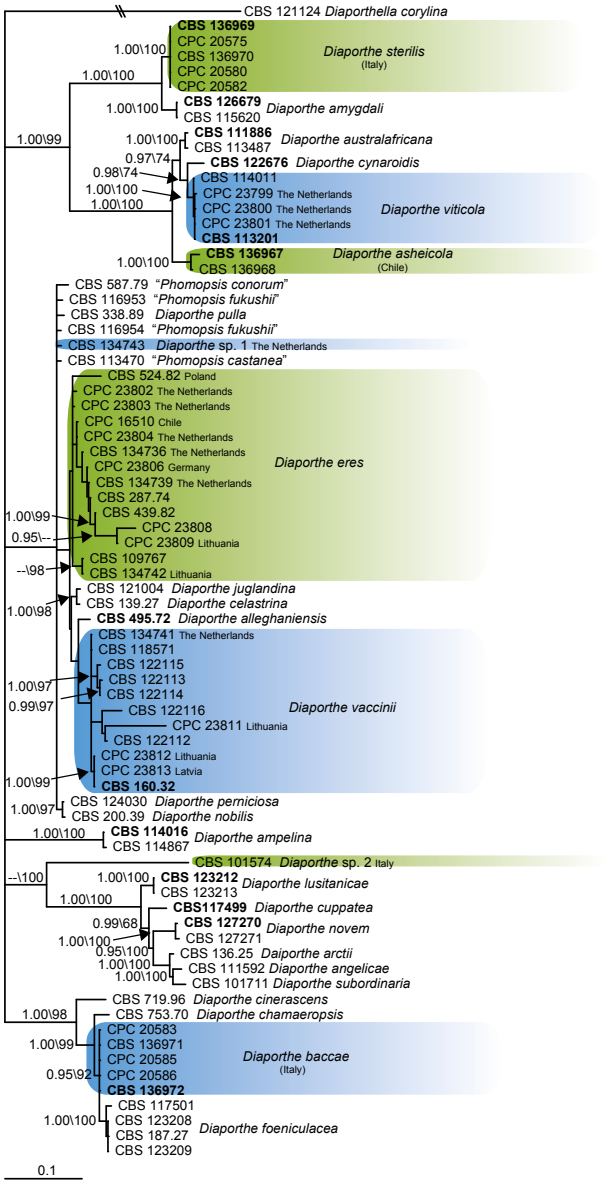

Figure 1. Consensus phylogram of 15,002 trees resulting from a Bayesian analysis of the combined four gene sequence alignment. European strains of *Diaporthe* spp. from *Vaccinium* spp. are indicated in the coloured blocks with country of origin next to the strain accession number. Accession numbers in **bold** represents ex-type strains. Bayesian posterior probabilities and Maximum Likelihood bootstrap support values are indicated at the nodes and the scale bar represents the expected changes per site. The tree was rooted to *Diaporthella corylina* (CBS 121124).

Taxonomy

Based on DNA sequence analysis and morphological features, three new species of *Diaporthe* are

recognised. Two of these species were sterile in culture and are therefore described based on DNA sequence, following the approach of Gomes *et al*. (2013). Isolate CBS 134743 is not considered here, as its phylogenetic position could not be resolved. Isolate CBS 101574 is also excluded as this strain is sterile and possibly represents a single lineage. More isolates of this strain are needed to confirm the positions of unique fixed alleles in the four loci studied here.

Diaporthe asheicola L. Lombard & Crous, **sp. nov.**
MycoBank MB807598

Etymology. Named after the host species from which it was isolated, *Vaccinium ashei*.

Cultures sterile. *Diaporthe asheicola* differs from its closest phylogenetic neighbours, *D. australafricana*, *D. cynaroidis* and *D. viticola* by unique fixed alleles in two loci based on alignments of the separate loci deposited in TreeBASE (S15189): BTUB positions 1 (T) and 12 (A); ITS positions 8 (indel), 25 (indel), 27 (T), 28 (A), 47 (T), 49 (G), 50 (indel), 51 (indel), 52 (indel), 56 (C), 57 (C), 60 (T), 61 (C), 62 (A), 63 (C), 65 (indel), 66 (indel), 67 (C), 68 (T), 69 (T), 70 (G), 75 (T), 76 (T), 80 (A), 81 (C), 99 (C), 100 (C), 150 (T), 364 (C), 365 (C), 449 (C), 451 (C), 457 (C), 459 (G) and 460 (T).

Culture characteristics. Colonies covering the medium within 2 weeks at 24°C, with sparse aerial mycelium. On PDA, honey (19″) to isabelline (19″i) with salmon (9′d) patches; reverse buff (19d) to honey (19″). On OA, buff (19d) to smoke-grey (21‴d). On MEA, white to buff (19d); reverse buff (19d).

Specimens examined: **Chile**: near Gorbea, on *Vaccinium ashei*, Feb. 2009, *A. Schilder*, holotype CBS H-21513, culture ex-type CBS 136967 = CPC 16508; CBS 136968 = CPC 16511.

Notes. Both isolates representing *D. asheicola* could not be induced to sporulate on any of the media used in this study, nor on sterilised *V. myrtillus* tissue placed on WA.

Diaporthe baccae L. Lombard, G. Polizzi & Crous, **sp. nov.**
MycoBank MB807599
(Figure 2)

Etymology. Name refers to the berries of the host plant, *Vaccinium corymbosum*, from which this fungus was isolated.

Conidiomata pycnidial in culture on PDA, PNA and OA, globose to conical, eustromatic, multilocular, occasionally with ostiolate necks, scattered or ag-

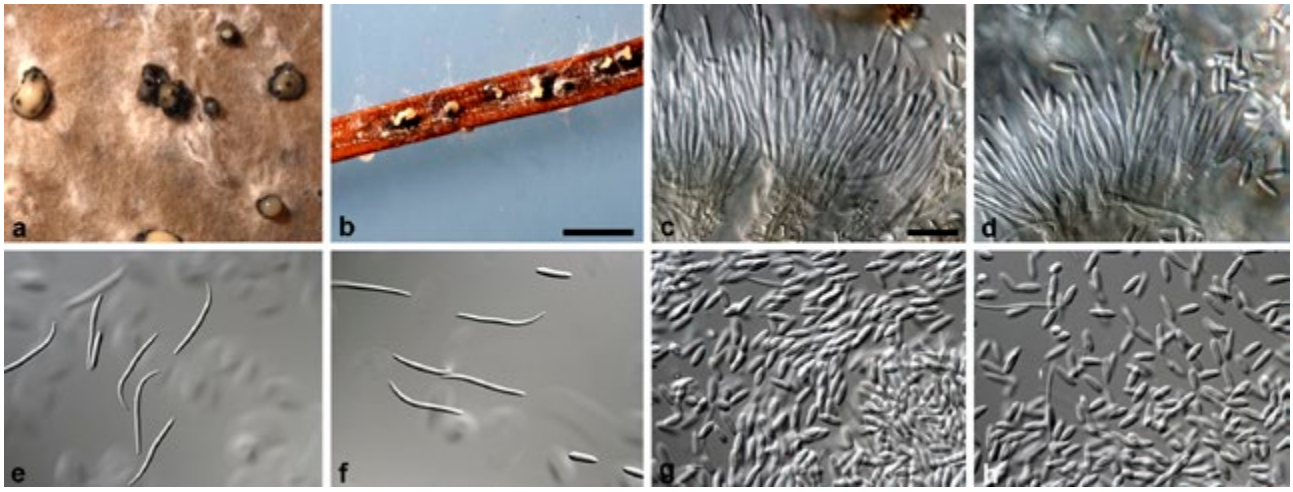

Figure 2. *Diaporthe baccae* (ex-type CBS 136972). a. Conidiomata sporulating on OA; b. Conidiomata sporulating on PNA; c, d. Conidiogenous cells. e, f. beta conidia; g, h. alpha conidia. Scale bars b = 500 μm; c = 10 μm (apply to d–h).

gregated, up to 650 µm diam, brown to black, surface covered with hyphae; cream to pale luteous conidial droplets exuding from the central ostioles; walls consisting of light brown, thick walled *textura angularis*. *Conidiophores* hyaline, smooth, 1–3-septate, branched, densely aggregated, cylindrical, straight to sinuous, 20–57 × 2–3 µm. *Conidiogenous cells* phialidic, cylindrical, terminal and lateral, 9–23 × 1–2 µm, slightly tapering towards the apex, with visible periclinal thickening; collarette not observed. *Paraphyses* not observed. *Alpha conidia* aseptate, hyaline smooth, guttulate, fusoid to ellipsoid, straight, tapering towards both ends, apex subobtuse, base subtruncate, (6–)7–9 × 2–3 µm (av. 8 × 2 µm). *Gamma conidia* not observed. *Beta conidia* spindle-shaped, aseptate, smooth, hyaline, apex acutely rounded, base truncate, tapering from lower third towards apex, curved, (17–)20–24(–26) × 1–2 µm (av. 22 × 2 µm).

Culture characteristics. Colonies covering the medium within 2 weeks at 24°C, with surface mycelium flattened, dense and felty. On PDA, cream (19′f) to smoke-grey (21′′′d); reverse greyish sepia (17″m). On OA, white to greyish sepia (17″m).

Specimens examined. **Italy**: Sicily, Catania Province, Valverde, on *Vaccinium corymbosum*, June 2012, *G. Polizzi*, holotype CBS H-21514, cultures ex-type CBS 136972 = CPC 20584; CPC 20583; CPC 20585; CPC 20586; CPC 20587.

Notes. Based on the phylogenetic inference in this study, *D. baccae* is closely related to *D. chamaeropsis* and *D. foeniculacea*. The beta conidia of *D. baccae* [(17–)20–24(–26) × 1–2 µm (av. 22 × 2 µm)] are smaller than those of *D. chamaeropsis* [(20–)22–27(–30) × 1.5(–2) µm; Gomes *et al*., 2013] and *D. foeniculacea* [(26–)28–32(–34) × 1(–2) µm; Gomes *et al*., 2013]. Moreover, the conidiophores of *D. baccae* are longer (up to 57 µm) than those of *D. chamaeropsis* (up to 50 µm; Gomes *et al*., 2013) and *D. foeniculacea* (up to 32 µm; Gomes *et al*., 2013).

Diaporthe sterilis L. Lombard, G. Polizzi & Crous, **sp. nov.**
MycoBank MB807600

Etymology. Named after its sterile growth in culture.

Cultures sterile. *Diaporthe sterilis* differs from its closest phylogenetic neighbour, *D. amygdali*, by unique fixed alleles in three loci based on alignments of the separate loci deposited in TreeBASE (S15189): BTUB positions 12 (A), 254 (G), 272 (G), 273 (A), 283 (A), 377 (A), 382 (A), 384 (T), 393 (G), 400 (T), 406 (G), 409 (A), 413 (T), 427 (G), 441 (G), 442 (A), 474 (indel), 479 (T) and 500 (T); ITS positions 44 (T), 48 (C), 50 (T), 51 (G), 68 (C), 159 (T), 358 (indel), 370 (indel), 371 (G) and 372 (G); TEF positions 16 (G), 178 (C), 179 (A), 180 (C), 181 (T), 182 (A), 183 (C), 184 (A), 185 (T), 186 (A), 187 (C), 194 (C), 195 (A), 196 (C), 197 (C), 198 (A), 199 (C), 236 (C), 238 (C) and 270 (A).

Culture characteristics. Colonies covering the medium within 2 weeks at 24°C, with sparse aerial mycelium. On PDA, buff (19d), honey (19″) to isabelline (19″i); reverse greyish sepia (17″m). On OA, white to greyish sepia (17″m). On MEA, white to vinaceous buff (17′′′d); reverse olivaceous buff (21′′′d).

Specimens examined. **Italy**: Sicily, Catania Province, Valverde, on *Vaccinium corymbosum*, June 2012, *G. Polizzi*, holotype CBS H-21515, cultures ex-type CBS 136969 = CPC 20563; CBS 136970 = CPC 20577; CPC 20575; CPC 20580; CPC 20582.

Notes. All five isolates representing *D. sterilis* could not be induced to sporulate on any of the media used in this study, nor on sterilised *V. myrtillus* tissue placed on WA.

Discussion

Three new *Diaporthe* spp. are here described as *D. asheicola*, *D. baccae*, and *D. sterilis*, based on phylogenetic inference, morphological and cultural features. *Diaporthe baccae* and *D. sterilis* originated from Europe and *D. asheicola* originated from Chile. Both *D. asheicola* and *D. sterilis* were found to be sterile, and are therefore described based on single nucleotide polymorphisms.

Although several *Diaporthe* spp. have been recorded on *Vaccinium* spp. in various countries, *D. vaccinii* remains the main concern in Europe. This fungal pathogen is listed in both the EPPO A2 (http://www.eppo.org) and EU Council Directive 2000/29/EC (annex IIAI) (http://eur-lex.europa.eu) as an organism recommended for regulation as a quarantine pest, therefore restricting movement of infected

plants and plant products into and within Europe. To date, *D. vaccinii* has only been reported sporadically in Europe, occurring in Lithuania (Gabler *et al*., 2004; Kačergius *et al*., 2004), Romania (Teodorescu *et al*., 1985), and the United Kingdom (Wilcox and Falconer, 1961; Baker, 1972). In the present study, isolates originating from Latvia, Lithuania and the Netherlands were identified as *D. vaccinii*. The study therefore confirms an earlier report of the presence of *D. vaccinii* in the Netherlands (NPPO, 2009), and represents the first report of this pathogen in Latvia.

Several isolates from the Netherlands, Germany, Lithuania and Poland were identified as *D.eres*. Although *Diaporthe eres*, the type species of the genus *Diaporthe*, has been reported on a large number of plant hosts (Wehmeyer, 1933; Gomes *et al*., 2013), no record could be found on *Vaccinium* spp. The present record therefore also represents the first report of *D. eres* on *V. corymbosum*, *V. myrtillus* and *V. vitis-idea* (Table 1). The phylogenetic position of a single isolate from the Netherlands (CBS 134743) could not be resolved. The closest phylogenetic neighbours to *D. eres* belong to the *D. nobilis* complex (Gomes *et al*., 2013), which is poorly resolved at present, awaiting examination of further collections.

A disease survey of *V. corymbosum* in Sicily, Italy in 2012, yielded several *Diaporthe* isolates, resulting in the identification of two new *Diaporthe* spp., *D. baccae* and *D. sterilis* in the present study. Infected plants displayed characteristic cankers at their bases, with brown lesions developing on the green stems and twigs, which resulted in twig blight. Plant mortality was observed when these cankers were present in the crowns of these plants. Preliminary pathogenicity tests on four *V. corymbosum* cultivars (Darrow, Berkeley, Legacy, Elliot) induced identical symptoms to those observed in the field. Host specificity of both these new *Diaporthe* spp., needs to be further investigated.

Three isolates from the Netherlands were identified as *D. viticola*. *Diaporthe viticola* is a well-known fungal pathogen in Europe (Phillips, 1999; Schepers *et al*., 2000; Mostert *et al*., 2001; van Niekerk *et al*., 2005), associated with cane spot of grapevines. This fungus has also been found on several other woody hosts in the Netherlands (Gomes *et al*., 2013). However, this is the first report of *D. viticola* on *V. corymbosum* and *V. myrtillus* in Europe. The description of *D. asheicola* from Chile was serendipitous, as these isolates were originally thought to represent *D. australafricana*. Although both isolates representing *D. asheicola* were sterile, either due to age, repeated subculturing or as an intrinsic feature of this species, they could be distinguished from *D. australafricana* and *D. viticola* based on single nucleotide polymorphisms following the approach of Gomes *et al*. (2013). As with *D. baccae* and *D. sterilis*, the pathogenicity and host specificity of *D. asheicola* needs to be further investigated.

This study has resulted in the identification of several *Diaporthe* spp. associated with *Vaccinium* spp. in Europe. Both *D. eres* and *D. viticola* are known to be host non-specific in Europe (Gomes *et al*., 2013), and therefore it is not surprising that they are also newly reported from various *Vaccinium* spp. *Diaporthe vaccinii* is known to be host-specific (Chao and Glawe, 1985) and is possibly indigenous to North America (Shear *et al*., 1931; Weingartner and Klos, 1975; Chao and Glawe, 1985; Farr *et al*., 2002a,b; Tadych *et al*., 2012), but is now shown to be present in Europe on *V. corymbosum, V. macrocarpon* and *V. oxycoccus*. However, large scale surveys on *Vaccinium* spp. grown commercially and in public areas need to be conducted to determine the distribution of *D. vaccinii* and other *Diaporthe* spp. in Europe. This information would contribute to understanding of the distribution of these fungi, and the extent to which quarantine measures need to be applied for these pathogens occurring on *Vaccinium* plants and plant products moved into and within Europe.

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Literature cited

Baker J.J. 1972. Report on diseases of cultivated plants in England and Wales for the years 1957–1968. *Ministry of Agriculture, Fisheries and Food Technical Bulletin* 25, 148.

Caruso F.L. and D.C. Ramsdell (ed.), 1995. *Compendium of Blueberry and Cranberry Diseases*. APS Press, The American Phytopathological Society, St. Paul, U.S.A.

Chao C.P. and D.A. Glawe, 1985. Studies on the taxonomy of *Diaporthe vaccinii*. *Mycotaxon* 23, 371–381.

Crous P.W., W. Gams, J.A. Stalpers, V. Robert and G. Stegehuis, 2004. MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* 50, 19–22.

Crous P.W., G.J.M Verkleij, J.Z. Groenewald and R.A. Samson (ed.), 2009. *Fungal Biodiversity. CBS Laboratory Manual Series 1.* Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.

Cunningham C.W., 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14, 733–740.

Dierking W., S. Dierking and W. Dierking, 1993. European *Vaccinium* species. *Acta Horticulturae* 346, 299–304.

Elfar K., R. Torres, G.A. Díaz and B.A. Latorre, 2013. Characterization of *Diaporthe australafricana* and *Diaporthe* spp. associated with stem canker of blueberry in Chile. *Plant Disease* http://dx.doi.org/10.1094/PDIS-11-12-1030-RE

European Union 2000. Council Directive 2000/29/EC, 8 May 2000, Brussels. http://eur-lex.europa.eu.

Faria A., J. Oliveira, P. Neves, P. Gameiro, C. Santos-Buelga, V. de Freitas and N. Mateus, 2005. Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. *Journal of Agricultural and Food Chemistry* 53, 6896–6902.

Farr D.F., L.A. Castlebury and A.Y. Rossman, 2002a. Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of *Phomopsis* from blueberry and cranberry in the Eastern United States. *Mycologia* 94, 494–504.

Farr D.F., L.A. Castlebury, A.Y. Rossman and M.L. Putman, 2002b. A new species of *Phomopsis* causing twig dieback of *Vaccinium vitis-idaea* (lingonberry). *Mycological Research* 106, 745–752.

Gabler J., A. Kačergius and Z. Jovaišienė, 2004. Detection of *Phomopsis vaccinii* on blueberry and cranberry in Europe by Direct Tissue Blot Immunoassay and Plate-Trapped Antigen ELISA. *Journal of Phytopathology* 152, 630–632.

Gomes R.R., C. Glienke, S.I.R. Videira, L. Lombard, J.Z. Groenewald and P.W. Crous, 2013. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia* 31, 1–41.

Hawksworth D.L., P.W. Crous, S.A. Redhead, D.R. Reynolds, R.A. Samson, K.A. Seifert, J.W. Taylor, M.J. Wingfield, Ö. Abaci, C. Aime, J.C. Frisvad, 2011. The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* 2, 105–112.

Kačergius A., Z. Jovaišienė and A. Valuškaitė, 2004. *Phomopsis vaccinii* on *Vaccinium corymbosum* in Lithuania. *Botanica Lithuanica* 10, 75–80.

Katoh K. and H. Toh, 2010. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* 26, 1889–1900.

Kirk P.M., J.A. Stalpers, U. Braun, P.W. Crous, K. Hansen, D.L. Hawksworth, K.D. Hyde, R. Lücking, T.H. Lumbsch, A.Y. Rossman, K.A. Seifert and M. Stadler, 2013. A without-prejudice list of generic names of fungi for protection under the International Code of Nomenclature for algae, fungi, and plants. *IMA Fungus* 4, 381–443.

Latorre B.A., K. Elfar, J.G. Espinoza, R. Torres and G.A. Díaz, 2012. First report of *Diaporthe australafricana* associated with stem canker on blueberry in Chile. *Plant Disease* 96, 768.

Librado P. and J. Rozas, 2009. DnaSP v. 5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452.

Mason-Gamer R. and E. Kellogg, 1996. Testing for phylogenetic conflict among molecular datasets in the tribe *Triticeae* (*Graminae*). *Systematic Biology* 45, 524–545.

Miller S.A., N. Patel and C.J. Stanley, 2006. Cranberry pests and diseases in New Zealand. *Acta Horticulturae* 715, 509–512.

Mostert L., P.W. Crous, J.-C. Kang and A.J.L. Philips, 2001. Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: Morphological, cultural, molecular and pathological characterization. *Mycologia* 93, 146–167.

National Plant Protection Organization (NPPO), 2009. *Diaporthe vaccinii* – Blueberry twig blight – on one blueberry plant at one fruit production facility in The Netherlands. April 2009 PEST Report – The Netherlands.

National Plant Protection Organization (NPPO), 2013. Follow-up: *Diaporthe vaccinii* – Blueberry twig blight – on one blueberry plant at one fruit production facility in The Netherlands. April 2013 PEST Report – The Netherlands.

Naumann W.D., 1993. Overview of the *Vaccinium* industry in Western Europe. *Acta Horticulturae* 346, 53–58.

Nestby R., D. Percival, I. Martinussen, N. Opstad and J. Rohloff, 2012. The European blueberry (*Vaccinium myrtillus* L.) and the potential for cultivation. A review. *The European Journal of Plant Science and Biotechnology* 5, 5–16.

Niekerk J.M. van, J.Z. Groenewald, D.F. Farr, P.H. Fourie, F. Halleen and P.W. Crous, 2005. Reassessment of *Phomopsis* species on grapevines. *Australasian Plant Pathology* 34, 27–39.

Nylander J.A.A., 2004. MrModeltest v. 2. Programme distributed by the author. Evolutionary Biology Centre, Uppsala University.

Petrak F., 1924. Mycologische Notizen. VII. *Annales Mycologici* 22, 1–182.

Philips A.J.L., 1999. The relationship between *Diaporthe perjuncta* and *Phomopsis viticola* on grapevines. *Mycologia* 91, 1001–1007.

Prodorutti D., I. Pertot, L. Giongo and C. Gessler, 2007. Highbush Blueberry: Cultivation, protection, breeding and biotechnology. *The European Journal of Plant Science and Biotechnology* 1, 44–56.

Polashock J.J., 2006. Screening for resistance to *Botryosphaeria* stem blight and *Phomopsis* twig blight in blueberry. *Acta Horticulturae* 715, 493–495.

Rayner R.W.(ed.), 1970. *A mycological colour chart*. Commonwealth Mycological Institute, Kew, Surrey. British Mycological Society.

Ronquist F. and J.P. Huelsenbeck, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.

Schepers R.W.A., D.C. Crane, D.L. Whisson and E.S. Scott, 2000. The *Diaporthe* teleomorph of *Phomopsis* Taxon 1 on grapevine. *Mycological Research* 104, 226–231.

Shear C.L., N.E. Stevens and H.F. Bain, 1931. Fungus diseases of the cultivated cranberry. *Technical Bulletin, United States Department of Agriculture* 258, 7–8.

Smith H., M.J. Wingfield, P.W. Crous and T.A. Coutinho, 1996. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* 62, 86–88.

Stamatakis A., P. Hoover and J. Rougemont, 2008.A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* 75, 758–771.

Stamatakis A., T. Ludwig and H. Meier, 2005. RaxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* 21, 456–463.

Swofford D.L., 2003. PAUP*. *Phylogenetic analysis using parsimony (*and other methods), v. 4.0b10*. Computer programme. Sinauer Associates, Sunderland, Massachusetts, USA..

Tadych M., M.S. Bergen, J. Johnson-Cicalese, J.J. Polashock, N. Vorsa and J.F. White Jr., 2012. Endophytic and pathogenic fungi of developing cranberry ovaries from flowers to mature fruit: diversity and succession. *Fungal Diversity* 54, 101–116.

Tamura K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony methods. *Molecular Biology and Evolution* 28, 2731–2739.

Teodorescu G., V. Copaescu and S. Florea, 1985. The behaviour of some blueberry cultivars to the main mycoses in Romania. *Acta Horticulturae* 165, 159–165.

Udayanga D., X.Z. Liu, P.W. Crous, E.H.C. Mckenzie, E. Chukeatirote and K.D. Hyde, 2012. A multilocus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). *Fungal Diversity* 56, 157–171.

Wehmeyer L.E., 1933. The genus *Diaporthe* Nitschke and its segregates. *University of Michigan Studies Scientific Series* 9, 1–349.

Weingartner D.P. and E.J. Klos, 1975. Etiology and symptomatology of canker and dieback diseases on highbush blueberries caused by *Godronia* (*Fusicoccum*) *cassandrae* and *Diaporthe* (*Phomopsis*) *vaccinii*. *Phytopathology* 65, 105–110.

Wilcox H.J. and M.A. Falconer, 1961. New or uncommon plant pests. *Plant Pathology* 10, 123–124.

Wingfield M.J., Z.W. de Beer, B. Slippers, B.D. Wingfield, J.Z. Groenewald, L. Lombard and P.W. Crous, 2012. One fungus one name promotes progressive plant pathology. *Molecular Plant Pathology* 13, 604–613.

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