



## ***Diaporthe* species on Rosaceae with descriptions of *D. pyracanthae* sp. nov. and *D. malorum* sp. nov.**

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### **Abstract**

The family Rosaceae includes a large number of species ranging from herbaceous (*Fragaria*) to ornamental plants (*Rosa* and *Pyracantha*) and fruit trees (*Malus* and *Pyrus*). *Diaporthe* species have been associated with twig canker, shoot blight, dieback, wood decay and fruit rot on members of the Rosaceae. In this study a collection of isolates from several Rosaceae hosts were characterised by multi-locus sequence analyses using the internal transcribed spacer, translation elongation factor 1-alpha, beta-tubulin, histone H3 and calmodulin loci. The phylogenetic analyses of the combined five loci revealed that the isolates studied were distributed among four clades, of which two correspond to *D. foeniculina* and *D. eres*. The other two clades, closely related to *D. passiflorae* and *D. leucospermi* represent two new species, *D. pyracanthae* sp. nov. and *D. malorum* sp. nov., respectively. Further, pathogenicity assays have shown that of the four species tested, *D. malorum* was the most aggressive species on apple fruit and *D. eres* was the most aggressive species on detached pear twigs. A revision of all *Diaporthe* (and *Phomopsis*) names that have been associated with Rosaceae hosts as well as their current status as pathogens of members of this family is presented.

**Key words** – *Malus* – Pathogenicity – Phylogeny – *Pyracantha* – *Pyrus*

### **Introduction**

The family Rosaceae is a large family of flowering plants that includes approximately 3000 species and 90 genera of herbs, shrubs and trees (Potter et al. 2007). This family includes herbaceous (*Fragaria*), medicinal (*Agrimonia*, *Crataegus*, *Filipendula*) and ornamental plants (*Rosa*, *Pyracantha*), shrubs (*Rubus*) and fruit trees (*Eriobotrya*, *Cydonia*, *Hesperomeles*, *Malus*, *Prunus*, *Pyrus*). Some of the species are cultivated worldwide and are economically important such as *Fragaria* (strawberry), *Malus* (apple), *Prunus* (cherry, almond, peach, and plum), *Pyrus* (pear) and *Rubus* (blackberry and raspberry) (Hummer & Janick 2009).

*Diaporthe* species are saprobes, endophytes, or plant pathogens (Webber & Gibbs 1984, Boddy & Griffith 1989, Udayanga et al. 2011). Some species of *Diaporthe* have been associated with twig canker, bud and shoot blight, dieback, wood decay and fruit rot of almond (Adaskaveg et al. 1999, Diogo et al. 2010, Gramaje et al. 2012); canker, shoot dieback, bud and shoot blight of

peach (Latham et al. 1992, Ogawa et al. 1995, Smit et al. 1996, Uddin et al. 1997, 1998, Farr et al. 1999, Thomidis & Michailides 2009); cankers and shoot blight of apple (Roberts 1913, Fujita et al. 1988, Smit et al. 1996, Abreo et al. 2012); dieback and canker of pear and plum (Sakuma et al. 1982, Nakatani et al. 1984, Kobayashi & Sakuma 1982, Ogawa et al. 1995, Uddin et al. 1998).

Identification of *Diaporthe* species was originally based on an approach that combined morphological features, cultural characteristics, and host affiliation (Udayanga et al. 2011). This resulted in an unnecessary inflation in the number of *Diaporthe* species names, which currently stands at 977 and 1099 for *Diaporthe* and 980 and 1047 for *Phomopsis* (asexual synonym of *Diaporthe*) in Index Fungorum and MycoBank, respectively (both accessed 14 November 2016). Thus, there was an urgent need to reformulate species delimitation in the genus *Diaporthe* because accurate species identification is essential for understanding epidemiology, controlling plant diseases, and to provide correct advice in the implementation of phytosanitary measures (Santos & Phillips 2009, Udayanga et al. 2011).

Over the last years, multi-loci phylogenetic analyses have routinely been used for species reassessment in *Diaporthe* (Santos & Phillips 2009, Thompson et al. 2011, Baumgartner et al. 2013, Gomes et al. 2013, Huang et al. 2013, Tan et al. 2013, Gao et al. 2014, Udayanga et al. 2014a, 2014b). The sequences most frequently used are the internal transcribed spacer (ITS) of the ribosomal DNA, translation elongation factor 1- $\alpha$  (TEF1),  $\beta$ -tubulin (TUB), histone (HIS), calmodulin (CAL), actin and DNA-lyase (Gomes et al. 2013, Huang et al. 2013, Gao et al. 2014, Udayanga et al. 2014a, 2014b, Wang et al. 2014). In general, these studies show that multi-loci phylogenies provide higher resolution for *Diaporthe* species than single locus phylogenies (Udayanga et al. 2012a, 2012b, Huang et al. 2013).

In this study a set of isolates obtained from different Rosaceae hosts was characterised based on morphology, pathogenicity and multi-loci sequence data (ITS, TEF1, HIS, TUB and CAL). In addition, a review of *Diaporthe* species occurring on Rosaceae and their current status as pathogens of members in this plant family is presented.

## Materials & Methods

### Fungal isolation and morphological characterisations

*Diaporthe* species were isolated, between 2007 and 2014, from the following Rosaceae hosts: *Malus domestica* fruits, collected in a local orchard, with post-harvest fruit rot; *Pyrus communis*, and *Pyracantha coccinea* with twig cankers in Portugal and *Prunus cerasus* with twig cankers in Russia (Table 1). Single spore isolates were obtained as described previously (Santos & Phillips 2009). In addition, isolations were made by directly plating out pieces of surface sterilized diseased tissue (5–10 mm<sup>2</sup>) on potato dextrose agar (PDA) (Merck, Germany). Plant tissue was surface sterilised in 5 % sodium hypochlorite for 1 minute followed by 96 % ethanol for 1 minute and rinsed in sterile water for 1 minute. The plates were incubated at room temperature and checked regularly for fungal growth. All *Diaporthe* isolates were transferred to half strength potato dextrose agar (½ PDA) (Merck, Germany) and pure cultures were established.

Isolates were induced to sporulate by plating them on 2 % water agar (Merck, Germany) containing sterilised fennel twigs or pine needles and incubating at room temperature (about 20–25 °C) where they received diffused daylight. Pycnidia were mounted in 100 % lactic acid and morphological characters of the conidia and mode of conidiogenesis observed with a Nikon 80i compound microscope (Nikon, Japan) and photographed with a Nikon Digital Sight DS-Ri1 camera (Nikon, Japan).

### Temperature growth studies

One plate of ½ PDA per strain of each novel species described was inoculated and incubated for 7 days at 25 °C. From these cultures, a 5-mm diam. plug for each strain was placed in the centre of PDA plates. Three replicate plates per strain were incubated at 5, 15, 20, 25, 30, 35 and 40 °C.

**Table 1** *Diaporthe* isolates from Rosaceae used in this study.

Species	Strain	Host	Symptoms	Country	Accession Number					Mating genes	
					ITS	TEF1	TUB	HIS	CAL	MAT1	MAT2
<i>D. foeniculina</i>	CAA133	<i>Pyrus communis</i>	branch canker	Portugal	KY435634	KY435624	KY435665	KY435645	KY435655	-	+
	CAA135	<i>Pyrus communis</i>	branch canker	Portugal						-	+
	CAA136	<i>Pyrus communis</i>	branch canker	Portugal						-	+
	CAA137	<i>Pyrus communis</i>	branch canker	Portugal						-	+
	CAA737	<i>Malus domestica</i>	fruit rot	Portugal	KY435641	KY435628	KY435669	KY435649	KY435659	+	-
	CAA738	<i>Malus domestica</i>	fruit rot	Portugal						+	-
	CAA739	<i>Malus domestica</i>	fruit rot	Portugal						+	-
<i>D. pyracanthae</i>	CAA481	<i>Pyracantha coccinea</i>	branch canker	Portugal						-	+
	CAA482	<i>Pyracantha coccinea</i>	branch canker	Portugal						-	+
	CAA483	<i>Pyracantha coccinea</i>	branch canker	Portugal	KY435635	KY435625	KY435666	KY435645	KY435656	-	+
	CAA484	<i>Pyracantha coccinea</i>	branch canker	Portugal						-	+
	CAA485	<i>Pyracantha coccinea</i>	branch canker	Portugal						-	+
	CAA486	<i>Pyracantha coccinea</i>	branch canker	Portugal						-	+
	CAA487	<i>Pyracantha coccinea</i>	branch canker	Portugal	KY435636	KY435626	KY435667	KY435647	KY435657	-	+
	CAA488	<i>Pyracantha coccinea</i>	branch canker	Portugal	KY435637					-	+
<i>D. malorum</i>	CAA734	<i>Malus domestica</i>	fruit rot	Portugal	KY435638	KY435627	KY435668	KY435648	KY435658	-	+
	CAA735	<i>Malus domestica</i>	fruit rot	Portugal	KY435639					-	+
	CAA736	<i>Malus domestica</i>	fruit rot	Portugal	KY435640					-	+
	CAA740	<i>Malus domestica</i>	fruit rot	Portugal	KY435642	KY435629	KY435670	KY435650	KY435660	-	+
	CAA752	<i>Malus domestica</i>	fruit rot	Portugal	KY435643	KY435630	KY435671	KY435651	KY435661	-	+
	CAA753	<i>Malus domestica</i>	fruit rot	Portugal						-	+
	CAA754	<i>Malus domestica</i>	fruit rot	Portugal						-	+
<i>D. eres</i>	CAA801	<i>Prunus cerasus</i>	branch canker	Russia	KY435644	KY435631	KY435672	KY435652	KY435662	-	+

Petri plates were examined daily for 14 days and colony diameters were measured with a caliper in two directions at right angles to each other until the colony reached the edge of the plate.

### DNA extraction and PCR fingerprinting

Isolates were grown on ½ strength PDA for 5 days at 25°C. DNA was extracted according to Möller et al. (1992). PCR fingerprinting of the isolates was performed using primer BOXA1R as described previously (Alves et al. 2007).

### PCR amplification and sequencing

For this study 5 loci (ITS, TEF1, HIS, TUB and CAL) were amplified and sequenced. The primers ITS5 and NL4 (White et al. 1990, Vilgalys & Hester 1990) were used to amplify ITS with PCR conditions of 5 min at 95 °C, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1.5 min, and a final elongation step at 72 °C for 10 min. TEF1 was amplified with the primers EF1-688F and EF1-1251R (Alves et al. 2008). The primers T1 and Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997) were used to sequence part of the TUB gene, while CYLH3F and H3-1b (Glass & Donaldson 1995, Crous et al. 2004) were used to amplify the HIS gene and CAL-228F and CAL-737R (Carbone & Kohn 1999) were used to amplify part of the CAL gene.

All PCR reactions were carried out with NZYtaq 2× green Master Mix from Nzytech (Lisbon, Portugal), in a Bio-Rad C1000 touch thermal cycler (Hercules, CA, USA). PCRs were performed in 25 µl reaction mixtures containing 6.25 µl Master Mix, 15.75 µl purified water, 1 µl of each primer (10 pmol) and 1 µl of purified template DNA. The PCR conditions for *TEF*, TUB, HIS and CAL were 5 min at 95°C; followed by 30 cycles at 94°C for 30 s, 52°C, 60°C and 53° C for 30 s (for TEF/TUB, HIS and CAL, respectively), 72°C for 1 min; and then a final elongation step at 72 °C for 10 min.

Amplicons were purified with DNA Clean & Concentror™ 5 (Zymo Research, Irvine, USA) following the manufacturer's instructions. The amplicons were sequenced by GATC Biotech (Germany). The new sequences obtained in this study were deposited in GenBank (Table 1).

### Mating-type assay

The mating strategy of all isolates (Table 1) (heterothallic or homothallic) was determined by a PCR-based mating type assay using the primers DiaMAT1F/DiaMAT1R for MAT1-1 and DiaMAT2F/DiaMAT2R for MAT1-2 developed by Santos et al. (2010). Part of the alpha box domain of the MAT1-1-1 gene and part of the HMG domain from the MAT1-2-1 gene were amplified as described previously (Santos et al. 2010).

### Phylogenetic analysis

A multi-locus phylogenetic analysis based on combined sequences of 5 genes (ITS, TEF1, HIS, TUB and CAL) was performed. This analysis included all *Diaporthe* species found on Rosaceae for which there were sequences available for the 5 loci as well as *D. leucospermi* and *D. passiflorae* which were closely related to some of our isolates based on a BLASTn search (Table 2). Sequences were aligned with ClustalX v. 2.1 (Larkin et al. 2007) using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25%). The alignments were optimized manually with BioEdit (Hall 1999). MEGA v. 6 (Tamura et al. 2013) was used to create and analyse Maximum Likelihood (ML) phylogenetic trees for these alignments (Li 1997). MEGA v. 6 was also used to determine the best substitution model to be used to build the ML tree. ML analysis was performed on a NJ starting tree automatically generated by the software. Nearest-Neighbour-Interchange (NNI) was used as the heuristic method for tree inference with 1,000 bootstrap replicates. *Diaporthe toxica* was used as outgroup for the multi-locus phylogenetic analysis. Alignments and trees were deposited in TreeBase (Study Accession: S20345).

### Pathogenicity tests

One representative isolate of each *Diaporthe* species identified (CAA487 – *D. pyracanthae*, CAA737 – *D. foeniculina*, CAA740 – *D. malorum* and CAA801 – *D. eres*) were used for pathogenicity assays on detached twigs of *Pyrus communis* and fruits of *Malus domestica*. For inoculum preparation, fungi were grown on PDA ½ plates for 7 days at 25 °C.

**Table 2** *Diaporthe* isolates used in multi-locus sequence analysis. In **bold** are ex-type or ex-epitype or isotype isolates.

Species	Strain	Host	Host Family	Country	Gen Bank Accession Number				
					ITS	TEF1	TUB	HIS	CAL
<i>Diaporthe ambigua</i>	<b>CBS 114015</b>	<i>Pyrus communis</i>	Rosaceae	South Africa	KC343010	KC343736	KC343978	KC343494	KC343252
<i>Diaporthe amygdali</i>	CBS 115620	<i>Prunus persica</i>	Rosaceae	USA	KC343020	KC343746	KC343988	KC343504	KC343262
	CBS 120840	<i>Prunus salicina</i>	Rosaceae	South Africa	KC343021	KC343747	KC343989	KC343505	KC343263
	<b>CBS 126679</b>	<i>Prunus dulcis</i>	Rosaceae	Portugal	KC343022	KC343748	KC343990	KC343506	KC343264
	CBS 126680	<i>Prunus dulcis</i>	Rosaceae	Portugal	KC343023	KC343749	KC343991	KC343507	KC343265
<i>Diaporthe crataegi</i>	CBS 114435	<i>Crataegus oxyacantha</i>	Rosaceae	Sweden	KC343055	KC343781	KC344023	KC343539	KC343297
<i>Diaporthe eres</i>	AR3669	<i>Pyrus pyrifolia</i>	Rosaceae	Japan	JQ807466	JQ807415	KJ420808	KJ420859	KJ435002
	AR3670	<i>Pyrus pyrifolia</i>	Rosaceae	Japan	JQ807467	JQ807416	KJ420807	KJ420858	KJ435001
	AR3671	<i>Pyrus pyrifolia</i>	Rosaceae	Japan	JQ807468	JQ807417	KJ420814	KJ420865	KJ435017
	AR3672	<i>Pyrus pyrifolia</i>	Rosaceae	Japan	JQ807469	JQ807418	KJ420819	KJ420868	KJ435023
	AR3723	<i>Rubus fruticosus</i>	Rosaceae	Austria	JQ807428	JQ807354	KJ420793	KJ420843	KJ435024
	AR4346	<i>Prunus mume</i>	Rosaceae	Korea	JQ807429	JQ807355	KJ420823	KJ420872	KJ435003
	AR4348	<i>Prunus persici</i>	Rosaceae	Korea	JQ807431	JQ807357	KJ420811	KJ420862	KJ435004
	AR4355	<i>Prunus</i> sp.	Rosaceae	Korea	JQ807433	JQ807359	KJ420797	KJ420848	KJ435035
	AR4363	<i>Malus</i> sp.	Rosaceae	Korea	JQ807436	JQ807362	KJ420809	KJ420860	KJ435033
	AR4367	<i>Prunus</i> sp.	Rosaceae	Korea	JQ807438	JQ807364	KJ420824	KJ420873	KJ435019
	AR4369	<i>Pyrus pyrifolia</i>	Rosaceae	Korea	JQ807440	JQ807366	KJ420813	KJ420864	KJ435005
	AR4371	<i>Malus pumila</i>	Rosaceae	Korea	JQ807441	JQ807367	KJ420796	KJ420847	KJ435034
	CBS 287.74	<i>Sorbus aucuparia</i>	Rosaceae	Netherlands	KC343084	KC343810	KC344052	KC343568	KC343326
	CBS 375.61	<i>Malus sylvestris</i>	Rosaceae	-	KC343088	KC343814	KC344056	KC343572	KC343330
	<b>CBS 439.82</b>	<i>Cotoneaster</i> sp.	Rosaceae	UK	KC343090	KC343816	KC344058	KC343574	KC343332
	<b>CBS 138594</b>	<i>Ulmus laevis</i>	Ulmaceae	Germany	KJ210529	KJ210550	KJ420799	KJ420850	KJ434999
	<b>DNP128</b>	<i>Castanea mollissima</i>	Fagaceae	China	JF957786	KJ210561	KJ420801	KJ420852	KJ435040
	DP0177	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	JQ807450	JQ807381	KJ420820	KJ420869	KJ435041
	DP0179	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	JQ807452	JQ807383	KJ420803	KJ420854	KJ435028
	DP0180	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	JQ807453	JQ807384	KJ420804	KJ420855	KJ435029
	DP0590	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	JQ807464	JQ807394	KJ420810	KJ420861	KJ435037
	DP0591	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	JQ807465	JQ807395	KJ420821	KJ420870	KJ435018
	FAU483	<i>Malus</i> sp.	Rosaceae	Netherlands	KJ210537	JQ807422	KJ420827	KJ420874	KJ435022
	CBS 116953	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	KC343147	KC343873	KC344115	KC343631	KC343389
	CBS 116954	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	KC343148	KC343874	KC344116	KC343632	KC343390
	CBS 124030	<i>Malus pumila</i>	Rosaceae	New Zealand	KC343149	KC343875	KC344117	KC343633	KC343391
<i>Diaporthe foeniculina</i>	<b>CBS 123208</b>	<i>Foeniculum vulgare</i>	Apiaceae	Portugal	KC343104	KC343830	KC344072	KC343588	KC343346

	<b>CBS 123209</b>	<i>Foeniculum vulgare</i>	Apiaceae	Portugal	KC343105	KC343831	KC344073	KC343589	KC343347
	<b>CBS 187.27</b>	<i>Camellia sinensis</i>	Theaceae	Italy	KC343107	KC343833	KC344075	KC343591	KC343349
	CBS 116957	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	KC343103	KC343829	KC344071	KC343587	KC343345
	CBS 171.78	<i>Prunus amygdalus</i>	Rosaceae	Italy	KC343106	KC343832	KC344074	KC343590	KC343348
<i>Diaporthe impulsae</i>	CBS 114434	<i>Sorbus aucuparia</i>	Rosaceae	Sweden	KC343121	KC343847	KC344089	KC343605	KC343363
	CBS 141.27	<i>Sorbus americana</i>	Rosaceae	-	KC343122	KC343848	KC344090	KC343606	KC343364
<i>Diaporthe leucospermi</i>	<b>CBS 111980</b>	<i>Leucospermum</i> sp.	Proteaceae	Australia	JN712460	KY435632	KY435673	KY435653	KY435663
<i>Diaporthe neilliae</i>	CBS 144.27	<i>Spiraea</i> sp.	Rosaceae	USA	KC343144	KC343870	KC344112	KC343628	KC343386
<i>Diaporthe padi</i> var. <i>padi</i>	CBS 114200	<i>Prunus padus</i>	Rosaceae	Sweden	KC343169	KC343895	KC344137	KC343653	KC343411
<i>Diaporthe passiflorae</i>	<b>CBS 132527</b>	<i>Passiflora edulis</i>	Passifloraceae	South America	JX069860	KY435633	KY435674	KY435654	KY435664
<i>Diaporthe pustulata</i>	CBS 109784	<i>Prunus padus</i>	Rosaceae	Austria	KC343187	KC343913	KC344155	KC343671	KC343429
<i>Diaporthe rudis</i>	CBS 266.85	<i>Rosa rugosa</i>	Rosaceae	Netherlands	KC343237	KC343963	KC344205	KC343721	KC343479
	<b>CBS 113201</b>	<i>Vitis vinifera</i>	Vitaceae	Portugal	KC343234	KC343960	KC344202	KC343718	KC343476
<i>Diaporthe toxica</i>	<b>CBS 534.93</b>	<i>Lupinus angustifolius</i>	Fabaceae	Australia	KC343220	KC343946	KC344188	KC343704	KC343462

### Pathogenicity tests on fruits

Granny Smith apples were washed with water and surface disinfected with 70% ethanol prior to inoculation. A 5-mm-diameter piece of fruit tissue was removed with a cork borer and replaced with a plug of mycelium-colonized agar. Plugs of uninoculated PDA ½ were used as negative controls and the inoculation points were sealed with masking tape. Five replicate fruits for each isolate and control were incubated at room temperature for 14 days and lesion diameters were measured after 7 and 14 days. A one-way analysis of variance (ANOVA) followed by a Student test was used to evaluate the pathogenicity of isolates. Analyses were made with JMP®8.0.1 (SAS Institute Inc., NC, USA).

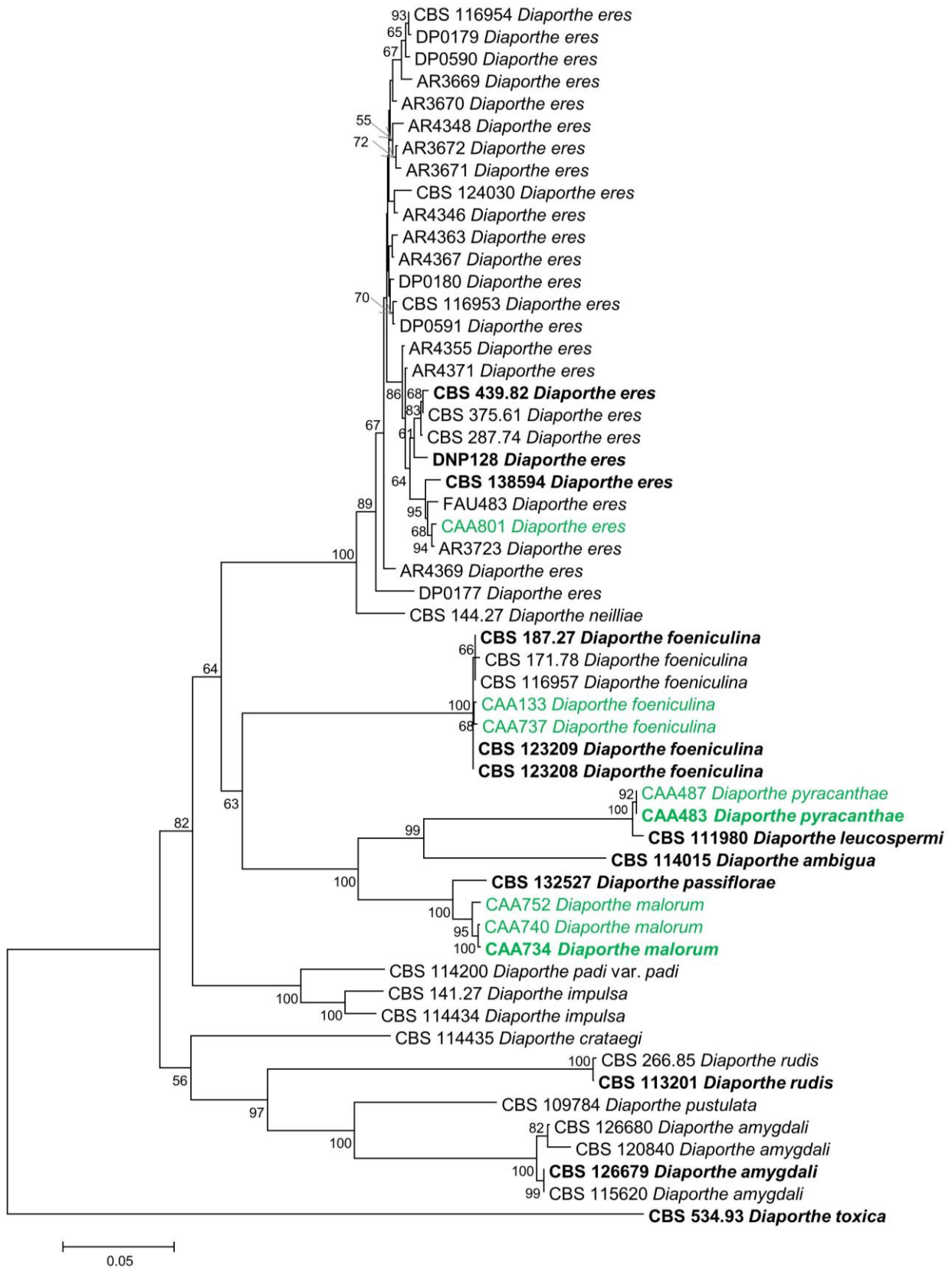
### Pathogenicity tests on twigs

Healthy twigs of *Pyrus communis* were surface disinfected with 70% ethanol and inoculated by making a hole with a 5-mm-diameter cork borer exposing the cambium. A mycelial plug was applied, with the mycelium side facing inward, and sealed with Parafilm®. Five replicate twigs per isolate and controls were incubated at room temperature in a humid chamber for 28 days. Plugs of uninoculated ½ PDA were used as negative controls. Lesion lengths were measured after 28 days. The normality of the data was checked with the Shapiro-Wilk test. A one-way analysis of variance (ANOVA) followed by a Student test was used to determine the significance of differences between means. Analyses were done with JMP®8.0.1 (SAS Institute Inc., NC, USA).

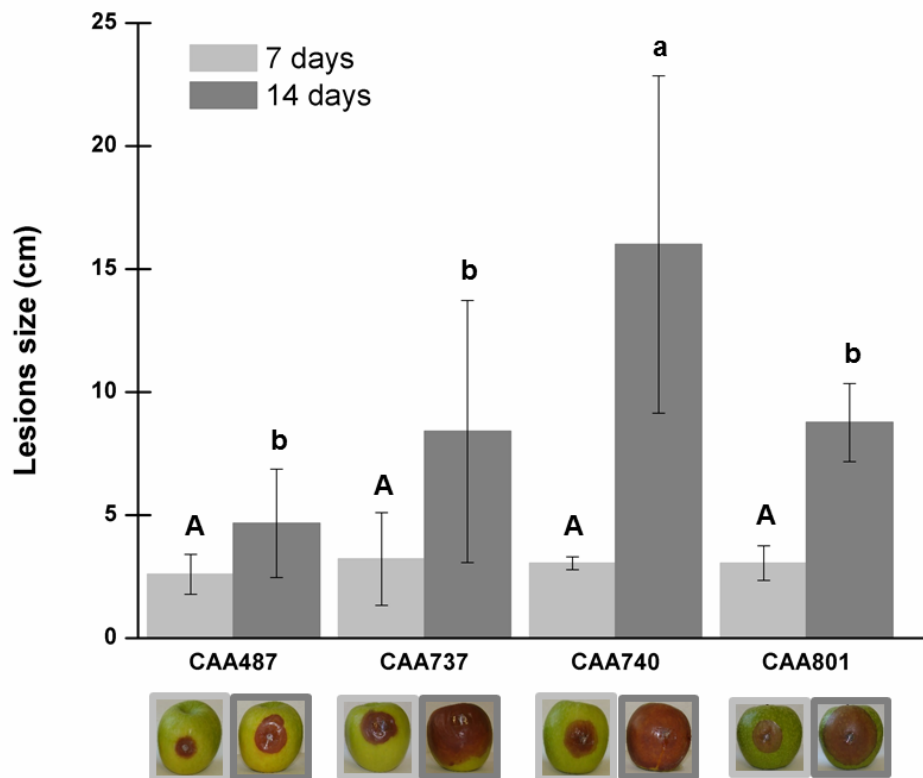
### Fungal isolation

Ten isolates were obtained from 10 apple fruits exhibiting post-harvest rot, and 10 isolates from shoot cankers, namely 1 isolate from *Prunus cerasus*, 1 isolate from *Pyrus communis* and 8 isolates from *Pyracantha coccinea*. From BOX-PCR fingerprinting analysis 8 isolates representative of the overall genetic diversity were selected for further molecular identification by sequencing five loci (ITS, TEF1, HIS, TUB and CAL).

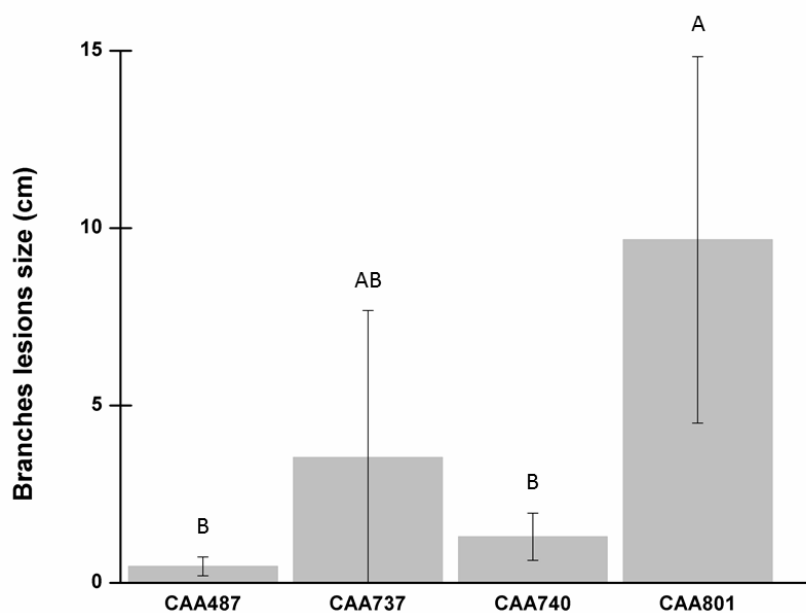
## Results



**Figure 1** – ML tree built using the five loci ITS-TEF1-TUB- HIS-CAL for the *Diaporthe* species found in Rosaceae. Bootstrap values are shown next to the branches. Ex-type, ex-epitype, or isotype isolates are given in **bold**. The studied isolates are shown in green. The tree was rooted to *D. toxica* (CBS 534.93).



**Figure 2** – Lesion size in apple fruit after 7 and 14 days. The vertical lines indicate standard deviations. Bars with the same letter are not significantly different.



**Figure 3** – Lesion lengths on pear twigs after 28 days. The vertical lines indicate standard deviations. Bars with the same letter are not significantly different.

### Phylogenetic analysis

For the multi-loci (ITS, TEF1, HIS, TUB and CAL) phylogenetic analysis, apart from our isolates we considered 10 *Diaporthe* species that have been found in Rosaceae and for which sequences from all the five loci were available. Additionally, two *Diaporthe* species relevant for this study (*D. leucospermi* and *D. passiflorae*) were also included (Tables 1 and 2). ML analysis was based on the Tamura-Nei's model assuming a gamma distribution (Tamura & Nei 1993) as determined by MEGA6. Fig. 1 shows the ML tree for the 5 concatenated loci.



In the ML phylogenetic tree 15 clades could be identified of which 13 correspond to known *Diaporthe* species: *D. ambigua*, *D. amygdali*, *D. crataegi*, *D. eres*, *D. foeniculina*, *D. impuls*a, *D. leucospermi*, *D. neilliae*, *D. padi* var. *padi*, *D. passiflorae*, *D. pustulata*, *D. rudis* and *D. toxica*. The remaining two clades include isolates obtained in this study and represent previously undescribed species, closely related to *D. leucospermi* (CAA 483 and CAA487) and *D. passiflorae* (CAA734, CAA740 and CAA752) which are here described as *D. pyracanthae* sp. nov. and *D. malorum* sp. nov. respectively. The other isolates obtained in this study clustered within the clades corresponding to *D. eres* (CAA801) and *D. foeniculina* (CAA 133 and CAA 737). Isolates CBS 116953, CBS 116954 and CBS 124030 were initially identified as belonging in the *Diaporthe nobilis* complex by Gomes et al. (2013), but in this study, we show them to reside within the *D. eres* clade.

### Pathogenicity test

All isolates tested caused apple rot (Fig. 2). At day 14, isolate CAA740 (isolated from *Malus domestica*) produced significantly larger lesions than the other isolates tested ( $F_{3,20} = 6.508$ ,  $p < 0.003$ ), almost completely rotting the entire fruit and with partial liquefaction. Regarding the pathogenicity assay on detached pear twigs isolate CAA801 (*D. eres* isolated from *Prunus cerasus*) produced lesions significantly longer than the other isolates tested ( $F_{3,8} = 4.6713$ ,  $p < 0.036$ ) (Fig. 3).

### Mating-type test

The mating strategy was determined for all 20 isolates (Table 1). All the tested isolates were heterothallic. Within *D. foeniculina* isolates both mating types were identified, namely MAT1-2-1 (CAA133) and others with MAT1-1-1 genes (CAA737, CAA738 and CAA739). For *D. pyracanthae*, *D. malorum* and *D. eres* isolates only MAT1-2-1 gene was detected.

### Taxonomy

*Diaporthe pyracanthae* L. Santos & A. Alves, sp. nov.

Fig. 4

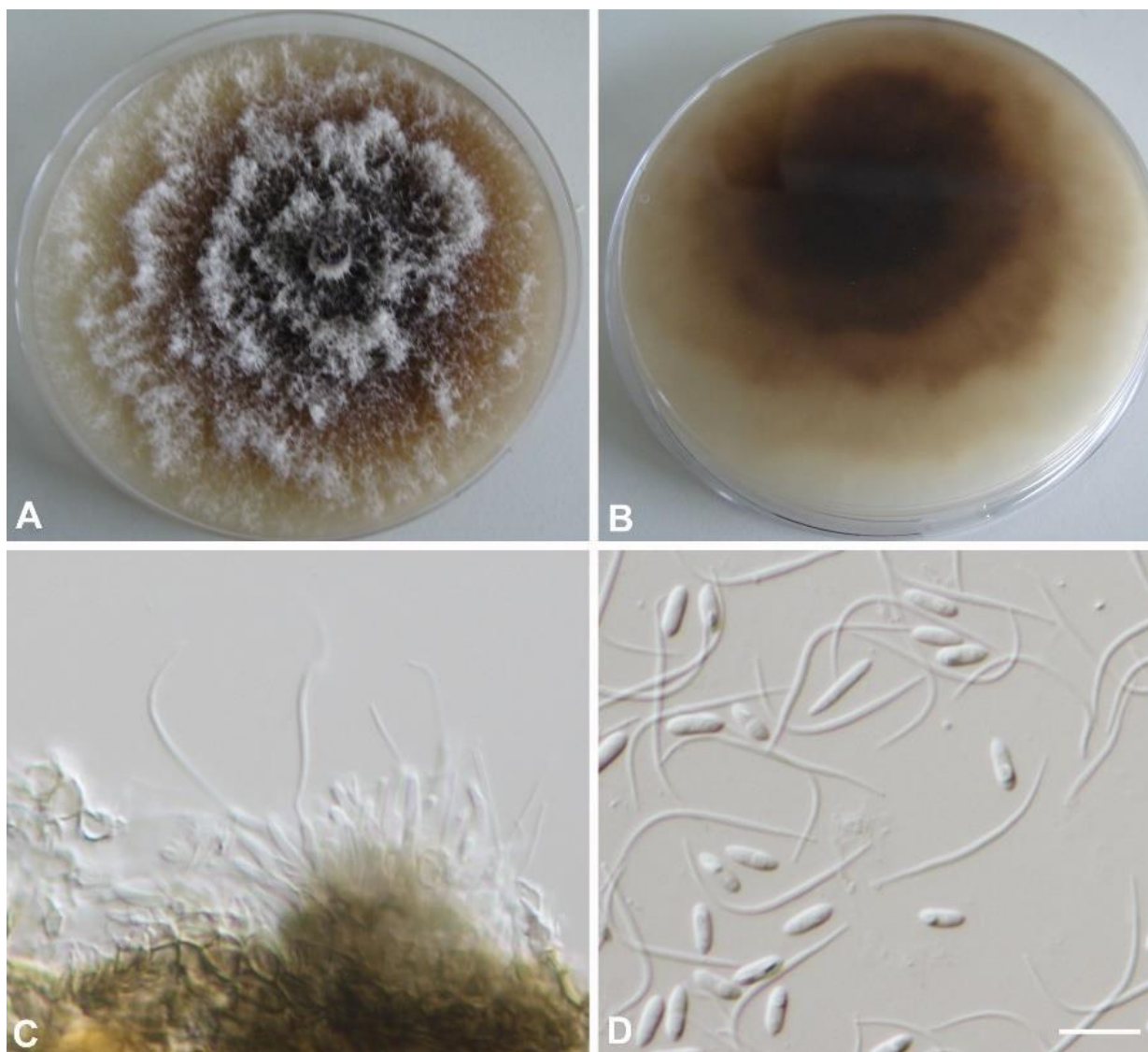
MycoBank MB820224

Etymology – named for the host it was first isolated from, namely *Pyracantha coccinea*.

Conidiomata pycnidial, dark brown, superficial, solitary to aggregated, opening via a central ostiole, exuding a creamy to white conidial cirrhous. Conidiophores lining the inner cavity, subcylindrical, hyaline, smooth, reduced to conidiogenous cells. Conidiogenous cells phialidic, hyaline, smooth and subcylindrical with apical taper. Alpha conidia hyaline, aseptate, smooth, fusiform, frequently biguttulate, ellipsoid, rounded apex, and obtuse to truncate at base, on pine needles (5.2)–6.7–(8.8)  $\times$  (1.6)–2.4–(3.0)  $\mu\text{m}$  (mean  $\pm$  S.D. =  $6.7 \pm 0.6 \times 2.4 \pm 0.2 \mu\text{m}$ ,  $n = 100$ ), on fennel twigs (6.0)–6.8–(7.9)  $\times$  (1.6)–2.2–(2.9)  $\mu\text{m}$  (mean  $\pm$  S.D. =  $6.8 \pm 0.4 \times 2.2 \pm 0.2 \mu\text{m}$ ,  $n = 100$ ). Beta conidia hyaline, aseptate, smooth, filiform, frequently hooked in apical part, apex acute, base truncate, on pine needles (20.8)–30.0–(36.8)  $\times$  (0.8)–1.3–(1.9)  $\mu\text{m}$  (mean  $\pm$  S.D. =  $30.0 \pm 2.7 \times 1.3 \pm 0.8 \mu\text{m}$ ,  $n = 100$ ), on fennel twigs (15.8)–26.8–(33.6)  $\times$  (0.8)–1.3–(2.0)  $\mu\text{m}$  (mean  $\pm$  S.D. =  $26.8 \pm 4.2 \times 1.3 \pm 0.2 \mu\text{m}$ ,  $n = 100$ ). Gamma conidia infrequent, aseptate, hyaline, smooth, fusoid, apex acutely rounded, base subtruncate.

Culture characteristics – Colonies spreading, flat, with sparse to moderate aerial mycelium, covering a Petri dish in 7 days at 25°C; on PDA growing with concentric zones, pale brown to smoke-grey, reverse pale brown to smoke-grey; optimal growth rate between 5 and 9 mm/day ( $p < 0.05$ ), maximum temperature for growth between 37 and 40°C ( $p < 0.05$ ), minimum temperature for growth between 4 and 9 °C ( $p < 0.05$ ) and optimum temperature between 21 and 27 °C ( $p < 0.05$ ).

Sexual morph – not observed



**Figure 4** – *Diaporthe pyracanthae*. A. Upper culture surface on PDA, 25°C and 7 days. B. Reverse culture surface on PDA, 25 °C and 7 days. C. Conidiogenous cells. D. Alpha, beta and gamma conidia. Scale bar: C–D = 10 µm.

Known distribution – Portugal.

Material examined – Portugal, Aveiro, from branch canker of *Pyracantha coccinea*, March 2012, A. Alves, (LISE 96313 **holotype**), a dried culture sporulating on pine needles, ex-type living culture, CBS142384 = CAA483. Other isolates studied are listed in Table 1.

Notes – *Diaporthe pyracanthae* is phylogenetically closely related but distinct from *D. leucospermi*. Although conidial dimensions of both species are similar they differ in several nucleotide positions in the following loci: ITS (3 nt), TEF1 (1 nt), TUB (8 nt), and HIS (2 nt) (Table 3).

***Diaporthe malorum*** L. Santos & A. Alves, *sp. nov.*

Mycobank MB820226

Etymology – named for the host it was first isolated from, namely *Malus domestica*.

Conidiomata pycnidial, dark brown, superficial, solitary or more frequently aggregated, opening via a central ostiole, exuding a creamy to white conidial cirrhous. Conidiophores lining the inner cavity, subcylindrical, hyaline, smooth, reduced to conidiogenous cells. Conidiogenous cells phialidic, hyaline, and smooth, subcylindrical with apical taper Alpha conidia hyaline, aseptate,

Fig. 5

**Table 3** Nucleotide differences between *D. leucospermi* and *D. pyracanthae* (CAA483 and CAA487).

Locus	Isolates		
	<i>Diaporthe leucospermi</i>	CAA483	CAA487
ITS (537 bp)	61	C	T
	450	T	C
	467	T	C
TEF1 (332 bp)	16	C	T
	27	T	C
	45	A	G
TUB (497 bp)	89	T	C
	161	T	C
	298	A	C
	339	T	C
	347	T	C
	452	T	C
HIS (457 bp)	188	G	A
	189	G	A
CAL (492 bp)	-		

smooth, fusiform, rarely biguttulate, ellipsoid, rounded apex and obtuse to truncate base, on pine needles (5.0)–6.3–(7.5) × (1.5)–2.2–(3.2) μm (mean ± S.D. = 6.3 ± 0.5 × 2.2 ± 0.3 μm, n = 100), on fennel twigs (5.6)–7.0–(8.7) × 2.2–3.4 μm (mean ± S.D. = 7.0 ± 0.6 × 2.8 ± 0.3 μm, n = 100). Gamma conidia infrequent, aseptate, hyaline, smooth, fusoid, apex acutely rounded, base subtruncate, on pine needles (7.1)–9.7–(12.4) × (1.3)–1.8–(2.3) μm (mean ± S.D. = 9.7 ± 1.3 × 1.8 ± 0.2 μm, n = 40), on fennel twigs (7.2)–10.6–(17.0) × (1.2)–1.9–(2.6) μm (mean ± S.D. = 10.6 ± 1.8 × 1.9 ± 0.3 μm, n = 100). Beta conidia infrequent, hyaline, aseptate, smooth, filiform, frequently hooked in apical part, apex acute, base truncate, on pine needles very infrequent, on fennel twigs (17.4)–21.5–(26.6) × (0.8)–1.3–(2.0) μm (mean ± S.D. = 21.5 ± 2.1 × 1.3 ± 0.3 μm, n = 50).

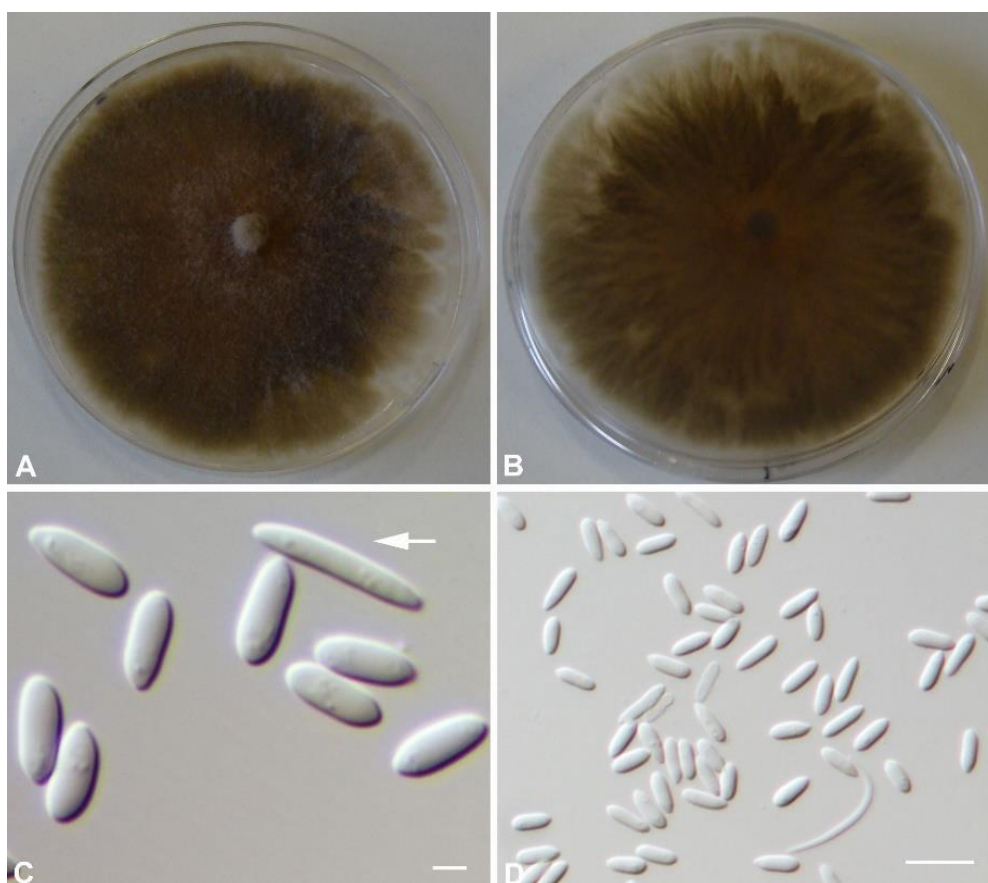
Culture characteristics – Colonies spreading, flat, with sparse to moderate aerial mycelium, not covering a Petri dish in 7 days at 25°C, sometimes with a reddish exudate; on PDA growing with pale brown to brown, reverse pale brown to dark reddish brown mycelia at 14 days; optimal growth rate between 3 and 7 mm/day (p<0.05), maximum temperature between 34 and 40°C (p<0.05), minimum temperature between 2 and 6 °C (p<0.05) and optimum temperature between 13 and 20 °C (p<0.05).

Sexual morph – not observed

Known distribution – Portugal.

Material examined – Portugal, Felgueiras, from *Malus domestica* fruit with rot symptoms, January 2014, A. Alves, (LISE 96314 **holotype**), a dried culture sporulating on pine needles, ex-type living culture, CBS142383 = CAA734. Other isolates studied are listed in Table 1.

Notes – *Diaporthe malorum* is phylogenetically closely related but distinct from *D. passiflorae*. Although conidial sizes of both species are similar they differ in several nucleotide positions in the following loci: ITS (5 nt), TEF1 (21 nt), TUB (12 nt), HIS (10 nt), and CAL (13 nt) (Table 4).



**Figure 5** – *Diaporthe malorum*. A. Upper culture surface on PDA, 20 °C and 9 days. B. Reverse culture surface on PDA, 20 °C and 9 days. C. Gamma conidia. D. alpha and beta conidia. – Scale bars: C = 2  $\mu$ m, D = 10  $\mu$ m.

**Table 4** Nucleotide differences between *D. passiflorae* and *D. malorum*.

Locus	<i>Diaporthe passiflorae</i>	Isolates			
		CAA734	CAA740	CAA752	
ITS (542 bp)	92	G	A	A	A
	383	C	G	G	G
	384	G	-	-	-
	385	C	-	-	-
	388	G	A	A	A
	27	G	A	A	A
	50	A	-	-	-
	51	C	-	-	-
	93	C	A	A	A
	96	A	G	G	G
TEF1 (346 bp)	103	-	C	C	C
	172	T	G	G	G
	212	C	T	T	T
	236	C	T	T	T
	238	G	-	-	-
	239	C	-	-	-
	240	A	-	-	-
	241	C	-	-	-
	242	C	-	-	-
	243	A	-	-	-

	244	T	-	-	-
	245	C	-	-	-
	246	A	-	-	-
	247	C	-	-	-
	248	C	-	-	-
	249	A	-	-	-
	12	A	G	G	G
	18	G	A	A	A
	41	G	T	T	T
	48	A	G	G	G
	84	C	T	T	T
TUB (502 bp)	86	A	C	C	C
	88	C	T	T	T
	90	C	T	T	T
	201	C	G	G	G
	292	C	T	T	T
	298	G	A	A	A
	421	C	T	T	T
	62	C	G	G	G
	150	A	G	G	G
	158	A	C	C	C
	164	T	G	G	G
HIS (425 bp)	175	A	G	G	G
	181	C	G	G	G
	191	C	T	T	T
	376	C	T	T	T
	409	T	C	C	C
	421	C	T	T	T
	69	C	G	G	G
	143	C	A	A	A
	184	T	G	G	G
	191	A	T	T	T
	210	G	A	A	A
CAL (486 bp)	226	A	C	C	C
	229	T	A	A	A
	293	A	C	C	C
	323	C	T	T	T
	385	G	C	C	C
	419	G	T	T	T
	421	G	C	C	C
	458	C	T	T	T

### Review of *Diaporthe* names reported from Rosaceae

A search of the Systematic Mycology and Microbiology Laboratory Fungus-Host Database (Farr & Rossman 2016) revealed 91 species of *Diaporthe/Phomopsis* associated with hosts in the family Rosaceae. These names were verified against the Index Fungorum and MycoBank databases as well as the available published literature, especially the most recent treatments of the genus *Diaporthe* (e.g. Gomes et al. 2013. Udayanga et al. 2014a, 2014b), which reduced the number to 53 *Diaporthe* species. Table 5 lists all current names of the *Diaporthe/Phomopsis* species associated with Rosaceae, their currently accepted synonymies and respective hosts.

**Table 5** – List of *Diaporthe* and *Phomopsis* names associated with Rosaceae.

Species	Synonyms	Host	Country	Reference
<i>Diaporthe actinidiae</i> N.F. Sommer & Beraha		<i>Malus domestica</i>	New Zealand	Farr & Rossman 2016
<i>Diaporthe ambigua</i> Nitschke	<i>Phoma ambigua</i> (Nitschke) Sacc. <i>Phomopsis ambigua</i> Traverso	<i>Malus domestica</i> <i>Malus sylvestris</i>  <i>Malus</i> sp.  <i>Prunus salicina</i> <i>Prunus</i> sp.  <i>Pyrus communis</i>	South Africa Netherlands South Africa Armenia United Kingdom South Africa South Africa  Canada Cuba Germany South Africa USA	Farr & Rossman, 2016 Murali et al. 2006 Farr & Rossman 2016 Farr & Rossman 2016  Farr & Rossman 2016 Farr & Rossman 2016 van Niekerk et al. 2005 Farr & Rossman 2016 Gomes et al. 2013
<i>Diaporthe amygdali</i> (Delacr.) Udayanga, Crous & K.D. Hyde	<i>Fusicoccum amygdali</i> Delacr. <i>Phomopsis amygdali</i> (Delacr.) J.J. Tuset & M.T. Portilla <i>Phomopsis amygdalina</i> Canonaco	<i>Pyrus ussuriensis</i> <i>Amygdalus persica</i> <i>Prunus amygdalus</i> <i>Prunus armeniaca</i> <i>Prunus dulcis</i>  <i>Prunus persica</i>	China Japan China China Italy Portugal USA World wide China France Greece Japan Portugal South Africa USA World Wide	Farr & Rossman 2016 Farr & Rossman 2016 Farr & Rossman 2016 Farr & Rossman 2016 Farr & Rossman 2016 Santos et al. 2010 Diogo et al. 2010 Gomes et al. 2013 Farr & Rossman 2016 Gomes et al. 2013
<i>Diaporthe australafricana</i> Crous & Van Niekerk		<i>Prunus persica</i> var. <i>vulgaris</i> <i>Prunus salicina</i>  <i>Prunus salicina</i> var. <i>corlata</i> <i>Prunus</i> sp. <i>Prunus dulcis</i>	Japan China South Africa China USA USA	Farr & Rossman 2016 Farr & Rossman 2016 Gomes et al. 2013 Farr & Rossman 2016 Murali et al. 2006 Farr & Rossman 2016

<b><i>Diaporthe beckhausii</i> Nitschke</b>	<i>Lophiosphaera beckhausii</i> (Nitschke) Berl. & Voglino <i>Lophiostoma beckhausii</i> Nitschke <i>Valsa beckhausii</i> (Nitschke) Cooke <i>Phomopsis beckhausii</i> (Cooke) Traverso	<i>Cydonia japonica</i>	Czech Republic	Farr & Rossman 2016
<b><i>Diaporthe cerasi</i> Fuckel <i>Diaporthe congesta</i> Ellis &amp; Everh. <i>Diaporthe crataegi</i> (Curr.) Fuckel</b>	<i>Valsa crataegi</i> Curr.	<i>Cerasus avium</i> <i>Pyrus americana</i>  <i>Crataegus chrysocarpa</i> <i>Crataegus laevigata</i> <i>Crataegus oxyacantha</i>  <i>Crataegus</i> sp.	Denmark USA  Canada Poland Austria United Kindom France Germany Italy Poland Sweden Bulgaria Denmark Poland Sweden United Kingdom Denmark Ukraine Ukraine Japan Poland Russia USA United Kingdom USA USA Austria Germany Poland United Kingdom Sweden	Farr & Rossman 2016 Farr & Rossman 2016  Farr & Rossman 2016 Farr & Rossman 2016 Farr & Rossman 2016 Gomes et al. 2013           Farr & Rossman 2016
<b><i>Diaporthe decorticans</i> (Lib.) Sacc. &amp; Roum.</b>	<i>Diaporthe padi</i> G.H. Otth <i>Diaporthe padi</i> var. <i>padi</i> G.H. Otth <i>Diaporthe padi</i> var. <i>patria</i> (Speg.) Wehm. <i>Diaporthe patria</i> Speg. <i>Sphaeria decorticans</i> Lib. <i>Phomopsis padina</i> (Sacc.) Dietel	<i>Cerasus padus</i> <i>Laurocerasus officinalis</i> <i>Laurocerasus officinalis</i> var. <i>zabeliana</i> <i>Malus sieboldii</i> <i>Padus avium</i>  <i>Prunus cerasus</i>  <i>Prunus hortulana</i> <i>Prunus munsoniana</i> <i>Prunus padus</i>	Denmark Ukraine Ukraine Japan Poland Russia USA United Kingdom USA USA Austria Germany Poland United Kingdom Sweden	Farr & Rossman 2016 Farr & Rossman 2016 Farr & Rossman 2016 Farr & Rossman 2016 Farr & Rossman 2016  Farr & Rossman 2016  Farr & Rossman 2016 Farr & Rossman 2016 Farr & Rossman 2016  Gomes et al. 2013, Farr

<b><i>Diaporthe eres</i> Nitschke</b>	<i>Phoma oblonga</i> Desm.	<i>Prunus persica</i>	World Wide	& Rossman 2016
	<i>Phomopsis oblonga</i> (Desm.) Traverso	<i>Sorbus aria</i>	Germany	Farr & Rossman 2016
	<i>Phomopsis cotoneastri</i> Punith.	<i>Chaenomeles speciosa</i>	Ukraine	Farr & Rossman 2016
	<i>Diaporthe cotoneastri</i> (Punith.) Udayanga, Crous & K.D. Hyde	<i>Cotoneaster adpressus</i>	Poland	Farr & Rossman 2016
	<i>Phomopsis castaneae-mollisimae</i> S.X. Jiang & H.B. Ma		Ukraine	
	<i>Diaporthe castaneae-mollisimae</i> (S.X. Jiang & H.B. Ma) Udayanga, Crous & K.D. Hyde	<i>Cotoneaster buxifolius</i>	Ukraine	Farr & Rossman 2016
	<i>Phomopsis fukushii</i> Tanaka & S. Endô	<i>Cotoneaster dammeri</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster divaricatus</i>	Poland	Farr & Rossman 2016
			Ukraine	
		<i>Cotoneaster foveolatus</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster franchetii</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster glaucophyllus</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster microphyllus</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster moupinensis</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster praecox</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster rhytidophyllus</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster simonsii</i>	Ukraine	Farr & Rossman 2016
		<i>Cotoneaster</i> sp.	United Kingdom	Farr & Rossman 2016
			Canada	Udayanga et al. 2014b
		<i>Crataegus oxyacantha</i>	Czech Republic	Farr & Rossman 2016
			Germany	
		<i>Crataegus pojarkovae</i>	Ukraine	Farr & Rossman 2016
		<i>Crataegus</i> sp.	Canada	Farr & Rossman 2016
		<i>Kerria japonica</i>	Germany	Farr & Rossman 2016
			Japan	
		<i>Malus domestica</i>	New Zealand	Farr & Rossman 2016
			Uruguay	
		USA		
	<i>Malus sylvestris</i>	Zimbabwe	Farr & Rossman 2016	
		-	Gomes et al. 2013	
	<i>Malus pumila</i>	Korea	Udayanga et al. 2014b	
	<i>Malus pumila</i> var. <i>domestica</i>	China	Farr & Rossman 2016	
	<i>Malus</i> sp.	Korea	Udayanga et al. 2014b	
		Netherlands		
	<i>Physocarpus opulifolius</i>	USA	Farr & Rossman 2016	
	<i>Physocarpus</i> spp.	USA	Farr & Rossman 2016	
	<i>Prunus avium</i>	China	Farr & Rossman 2016	
		Japan		
	<i>Prunus cerasus</i>	Bulgaria	Farr & Rossman 2016	



<i>Prunus cornuta</i>	Pakistan	Farr & Rossman 2016
<i>Prunus davidiana</i>	Japan	Farr & Rossman 2016
<i>Prunus domestica</i>	Bulgaria	Farr & Rossman 2016
<i>Prunus dulcis</i>	Portugal	Diogo et al. 2010
<i>Prunus lannesiana</i> f. <i>sekiyama</i>	Japan	Farr & Rossman 2016
<i>Prunus mume</i>	Korea	Udayanga et al. 2014b
<i>Prunus persica</i>	Australia	Farr & Rossman 2016
	Greece	
	USA	
	Korea	Udayanga et al. 2014b
<i>Prunus persica</i> var. <i>vulgaris</i>	Japan	Farr & Rossman 2016
<i>Prunus sargentii</i>	Japan	Farr & Rossman 2016
<i>Prunus</i> sp.	Japan	Farr & Rossman 2016
	New Zealand	
	USA	
	Korea	Udayanga et al. 2014b
<i>Pyracantha crenatoserrata</i>	Ukraine	Farr & Rossman 2016
<i>Pyracantha rogersiana</i>	Ukraine	Farr & Rossman 2016
<i>Pyracantha</i> sp.	Ukraine	Farr & Rossman 2016
<i>Pyrus communis</i>	USA	Farr & Rossman 2016
	New Zealand	
<i>Pyrus pyrifolia</i>	China	Farr & Rossman 2016
	Japan	
<i>Pyrus pyrifolia</i> var. <i>culta</i>	China	Farr & Rossman 2016
<i>Pyrus serotina</i>	Japan	Farr & Rossman 2016
	Korea	
<i>Pyrus pyrifolia</i>	Japan	Murali et al. 2006
	Korea	Udayanga et al. 2014b
	New Zealand	
<i>Pyrus serotina</i> var. <i>culta</i>	Japan	Farr & Rossman 2016
<i>Pyrus ussuriensis</i>	China	Farr & Rossman 2016
<i>Pyrus</i> sp.	China	Farr & Rossman 2016
<i>Rhaphiolepis indica</i>	Ukraine	Farr & Rossman 2016
<i>Rosa canina</i>	Belgium	Farr & Rossman 2016
	Czech Republic	
	United Kingdom	
	USA	
	Germany	
<i>Rosa</i> sp.	USA	Farr & Rossman 2016
	Italy	

			New Zealand	
		<i>Rubus fruticosus</i>	Ireland	Farr & Rossman 2016
			Austria	Udayanga et al. 2014b
		<i>Rubus idaeus</i>	Germany	Farr & Rossman 2016
		<i>Rubus</i> sp.	Croatia	Farr & Rossman 2016
			France	
		<i>Sorbus aucuparia</i>	Netherlands	Farr & Rossman 2016
			USA	Gomes et al. 2013
		<i>Spiraea cantoniensis</i>	Ukraine	Farr & Rossman 2016
		<i>Spiraea chamaedryfolia</i>	Ukraine	Farr & Rossman 2016
		<i>Spiraea</i> sp.	Ukraine	Farr & Rossman 2016
		<i>Prunus cerasifera</i>	Bulgaria	Farr & Rossman 2016
		<i>Prunus spinosa</i>	Poland	Farr & Rossman 2016
		<i>Malus domestica</i>	New Zealand	Udayanga et al. 2014a
		<i>Prunus amygdalus</i>	Italy	Gomes et al. 2013
				Farr & Rossman 2016
		<i>Prunus dulcis</i>	Portugal	Diogo et al. 2010
				Farr & Rossman 2016
		<i>Prunus spinosa</i>	Poland	Farr & Rossman 2016
		<i>Pyrus pyrifolia</i>	New Zealand	Gomes et al. 2013
		<i>Spiraea ulmifolia</i>	Sweden	Farr & Rossman 2016
		<i>Sorbus americana</i>	-	Gomes et al. 2013
			Canada	Farr & Rossman 2016
			USA	
		<i>Sorbus aria</i>	Austria	Farr & Rossman 2016
		<i>Sorbus aucuparia</i>	Austria	Gomes et al. 2013
			Czech Republic	Farr & Rossman 2016
			Poland	
			Sweden	
			United Kingdom	
		<i>Sorbus aucuparia</i> subsp. <i>glabrata</i>	Poland	Farr & Rossman 2016
		<i>Sorbus commixta</i>	Japan	Farr & Rossman 2016
		<i>Sorbus sitchensis</i>	USA	Farr & Rossman 2016
		<i>Sorbus</i> sp.	USA	Farr & Rossman 2016
		<i>Rosa canina</i>	Poland	Farr & Rossman 2016
		<i>Rosa indica</i>	India	Farr & Rossman 2016
<b><i>Diaporthe fibrosa</i> (Pers.) Fuckel</b>	<i>Sphaeria fibrosa</i> Pers.			
<b><i>Diaporthe foeniculina</i> (Sacc.) Udayanga &amp; Castl.</b>	<i>Hercospora fibrosa</i> (Pers.) Petr			
	<i>Phoma foeniculina</i> Sacc.			
	<i>Phoma foeniculina</i> Sacc.			
	<i>Phomopsis foeniculina</i> (Sacc.) Câmara			
	<i>Phomopsis theicola</i> Curzi			
	<i>Diaporthe neotheicola</i> A.J.L. Phillips & J.M. Santos			
	<i>Diaporthe foeniculacea</i> Niessl,			
	<i>Diaporthe theicola</i> Curzi			
	<i>Phomopsis theicola</i> Curzi			
	<i>Phomopsis californica</i> H.S. Fawc.			
	<i>Diaporthe rhusicola</i> Crous			
<b><i>Diaporthe fuckelii</i> J. Kunze</b>				
<b><i>Diaporthe impuls</i> (Cooke &amp; Peck) Sacc.</b>	<i>Valsa impuls</i> Cooke & Peck			
<b><i>Diaporthe incarcerata</i> (Berk. &amp; Broome) Nitschke</b>	<i>Diatrype incarcerata</i> Berk. & Broome			
	<i>Phoma incarcerata</i> (Nitschke) Sacc.			

	<i>Sphaeropsis depressa</i> Lév. <i>Phomopsis incarcerationata</i> Höhn. <i>Phomopsis depressa</i> (Lév.) Traverso	<i>Rosa</i> sp.	Denmark South Africa United Kingdom Zimbabwe	Farr & Rossman 2016
<b><i>Diaporthe insignis</i> Fuckel.</b>		<i>Rubus fruticosus</i>	Denmark Poland	Farr & Rossman 2016
		<i>Rubus idaeus</i>	Denmark	Farr & Rossman 2016
<b><i>Diaporthe japonica</i> Sacc.</b>	<i>Phoma japonica</i> (Sacc.) Sacc., <i>Michelia</i> 1 (5): 521. 1879 <i>Phomopsis japonica</i> (Sacc.) Traverso, <i>Flora Italica Cryptogama. Pars 1: Fungi. Pyrenomycetae. Xylariaceae, Valsaceae, Ceratostomataceae</i> 1(1): 241. 1906	<i>Kerria japonica</i>	Poland USA	Farr & Rossman 2016 Farr & Rossman 2016
		<i>Kerria japonica</i> var. <i>pleniflora</i>	Portugal	Farr & Rossman 2016
<b><i>Diaporthe mali</i> Bres.</b>		<i>Malus pumila</i>	Japan	Farr & Rossman 2016
<b><i>Diaporthe neilliae</i> Peck</b>		<i>Spiraea</i> sp.	USA	Udayanga et al., 2014b
<b><i>Diaporthe nobilis</i> complex</b>		<i>Malus pumila</i>	New Zealand	Gomes et al. 2013 Farr & Rossman 2016
		<i>Pyrus pyrifolia</i>	New Zealand	Gomes et al. 2013 Farr & Rossman 2016
<b><i>Diaporthe novem</i> J.M. Santos, Vrandečić &amp; A.J.L. Phillips</b>		<i>Prunus dulcis</i>	USA	Farr & Rossman 2016
<b><i>Diaporthe parabolica</i> Fuckel</b>		<i>Prunus spinosa</i>	Denmark	Farr & Rossman 2016
<b><i>Diaporthe pardalota</i> (Mont.) Nitschke ex Fuckel</b>	<i>Sphaeria pardalota</i> Mont. <i>Phomopsis pardalota</i> Died.	<i>Prunus divaricata</i>	Ukraine	Farr & Rossman 2016
		<i>Prunus laurocerasus</i>	France	Farr & Rossman 2016
		<i>Rubus fruticosus</i>	Germany	Farr & Rossman 2016
<b><i>Diaporthe pennsylvanica</i> (Berk. &amp; M.A. Curtis) Wehm.</b>	<i>Valsa pennsylvanica</i> Berk. & M.A. Curtis <i>Calospora pennsylvanica</i> (Berk. & M.A. Curtis) Sacc.	<i>Prunus pensylvanica</i>	USA	Farr & Rossman 2016
<b><i>Diaporthe perniciosa</i> Marchal &amp; É.J. Marchal</b>	<i>Phomopsis prunorum</i> (Cooke) Grove <i>Phomopsis mali</i> Roberts <i>Phomopsis mali</i> (Schulzer & Sacc.) Died.	<i>Prunus serotina</i>	USA	Farr & Rossman 2016
		<i>Prunus virginiana</i>	USA	Farr & Rossman 2016
		<i>Cydonia oblonga</i>	Greece	Farr & Rossman 2016
		<i>Malus domestica</i>	Brazil Greece Japan New Zealand	Farr & Rossman 2016
		<i>Malus melliana</i>	United Kingdom	Farr & Rossman 2016
		<i>Malus pumila</i>	China	Farr & Rossman 2016
		<i>Malus pumila</i> var. <i>dulcissima</i>	Chile	Farr & Rossman 2016
		<i>Malus sylvestris</i>	Korea	Farr & Rossman 2016
			Australia	Farr & Rossman 2016

		<i>Malus</i> sp.	USA	
		<i>Prunus cerasus</i>	Canada	Farr & Rossman 2016
		<i>Prunus domestica</i>	Bulgaria	Farr & Rossman 2016
			Bulgaria	Farr & Rossman 2016
			Central Asia	
			USA	
		<i>Prunus dulcis</i>	World Wide	Farr & Rossman 2016
		<i>Prunus mahaleb</i>	Canada	Farr & Rossman 2016
		<i>Prunus persica</i>	USA	Farr & Rossman 2016
			World Wide	
		<i>Prunus</i> sp.	Cyprus	Farr & Rossman 2016
			Lithuania	
			New Zealand	
			USA	
			World Wide	
		<i>Pyrus communis</i>	Australia	Farr & Rossman 2016
			Greece	
			Japan	
			New Zealand	
			Poland	
			USA	
		<i>Pyrus malus</i>	USA	Farr & Rossman 2016
		<i>Prunus angustifolia</i>	USA	Farr & Rossman 2016
		<i>Prunus hortulana</i>	USA	Farr & Rossman 2016
		<i>Prunus munsoniana</i>	USA	Farr & Rossman 2016
		<i>Prunus serotina</i>	USA	Farr & Rossman 2016
		<i>Prunus virginiana</i>	Canada	Farr & Rossman 2016
			USA	
		<i>Prunus</i> sp.	Canada	Farr & Rossman 2016
			USA	
		<i>Prunus americana</i>	USA	Farr & Rossman 2016
		<i>Prunus divaricata</i>	Ukraine	Farr & Rossman 2016
		<i>Prunus pensylvanica</i>	Canada	Farr & Rossman 2016
			USA	
		<i>Prunus serotina</i>	Canada	Farr & Rossman 2016
			USA	
		<i>Prunus virginiana</i>	Canada	Farr & Rossman 2016
		<i>Prunus</i> sp.	Canada	Farr & Rossman 2016
			USA	
		<i>Prunus padus</i>	Austria	Farr & Rossman 2016
		<i>Sorbus aucuparia</i>	United	Farr & Rossman 2016
<b><i>Diaporthe pruni</i> Ellis &amp; Everh.</b>				
<b><i>Diaporthe prunicola</i> (Peck) Wehm.</b>	<i>Valsa prunicola</i> Peck <i>Engizostoma prunicola</i> (Peck) Kuntze			
<b><i>Diaporthe pustulata</i> Sacc.</b>				
<b><i>Diaporthe rehmii</i> Nitschke</b>				

<b><i>Diaporthe rudis</i> (Fr.) Nitschke</b>	<i>Sphaeria rudis</i> Fr.	<i>Malus pumila</i> var. <i>domestica</i>	Kingdom	
	<i>Rabenhorstia rudis</i> (Fr.) Fr.	<i>Pyrus communis</i>	Japan	Farr & Rossman 2016
	<i>Aglaospora rudis</i> (Fr.) Tul. & C. Tul.	<i>Pyrus serotina</i> var. <i>culta</i>	Japan	Farr & Rossman 2016
	<i>Phoma rudis</i> Sacc.	<i>Pyrus ussuriensis</i> var. <i>sinensis</i>	Japan	Farr & Rossman 2016
	<i>Phomopsis rudis</i> (Sacc.) Höhn.	<i>Pyrus</i> sp.	New Zealand	Udayanga et al. 2014a
	<i>Diaporthe faginea</i> Sacc.	<i>Rosa canina</i>	Austria	Udayanga et al. 2014a
	<i>Diaporthe medusaea</i> Nitschke			Farr & Rossman 2016
	<i>Diaporthe viticola</i> Nitschke	<i>Rosa rugosa</i>	Netherlands	Gomes et al. 2013
	<i>Diaporthe silvestris</i> Sacc. & Berl.			Farr & Rossman 2016
		<i>Spiraea</i> sp.	USA	Murali et al. 2006
<b><i>Diaporthe sorbariae</i> Nitschke</b>	<i>Spiraea salicifolia</i>	Poland	Farr & Rossman 2016	
<b><i>Diaporthe spiculosa</i> (Pers.) Nitschke</b>	<i>Sorbus aucuparia</i>	Switzerland	Farr & Rossman 2016	
<b><i>Diaporthe tanakae</i> Ts. Kobay. &amp; Sakuma</b>				
<b><i>Diaporthe vexans</i> (Sacc. &amp; P. Syd.) Gratz</b>	<i>Phoma vexans</i> Sacc. & P. Syd.	<i>Malus pumila</i> var. <i>domestica</i>	Japan	Farr & Rossman 2016
	<i>Phomopsis vexans</i> (Sacc. & P. Syd.) Harter	<i>Pyrus communis</i>	Japan	Farr & Rossman 2016
		<i>Prunus armeniaca</i>	Argentina	Farr & Rossman 2016
			Korea	
<b><i>Diaporthe viburni</i> Dearn. &amp; Bisby, in Bisby</b>	<i>Diaporthe viburni</i> var. <i>spiraeicola</i> Wehm.	<i>Prunus mume</i>	Korea	Farr & Rossman 2016
		<i>Spiraea tomentosa</i>	Canada	Farr & Rossman 2016
			USA	
		<i>Spiraea</i> sp.	Canada	Farr & Rossman 2016
			USA	
<b><i>Phomopsis biwa</i> Hara</b>		<i>Eriobotrya japonica</i>	Japan	Farr & Rossman 2016
<b><i>Phomopsis corticis</i> (Fuckel) Grove</b>	<i>Phoma corticis</i> Fuckel	<i>Rubus</i> sp.	Poland	Farr & Rossman 2016
<b><i>Phomopsis hughesii</i> N.D. Sharma</b>	<i>Macrophoma corticis</i> (Fuckel) Berl. & Voglino			
<b><i>Phomopsis muelleri</i> (Cooke) Grove</b>	<i>Phoma muelleri</i> Cooke	<i>Eriobotrya japonica</i>	China	Farr & Rossman 2016
<b><i>Phomopsis obscurans</i> (Ellis &amp; Everh.) B. Sutton</b>	<i>Phoma obscurans</i> Ellis & Everh.	<i>Rubus giraldianus</i>	India	
	<i>Sphaeropsis obscurans</i> (Ellis & Everh.) Kuntze	<i>Rubus idaeus</i>	Poland	Farr & Rossman 2016
	<i>Phyllosticta obscurans</i> (Ellis & Everh.) Tassi	<i>Fragaria ananassa</i>	Russia	Farr & Rossman 2016
	<i>Dendrophoma obscurans</i> (Ellis & Everh.) H.W. Anderson		Bulgaria	Farr & Rossman 2016
		<i>Fragaria chiloensis</i>	Tonga	
		<i>Fragaria vesca</i>	USA	Farr & Rossman 2016
			Brazil	Farr & Rossman 2016
			Brunei	
			Darussalam	
			Malawi	
			Myanmar	
		<i>Fragaria</i> × <i>ananassa</i>	Australia	Farr & Rossman 2016
			Canada	

			China	
			Korea	
			New Zealand	
			USA	
		<i>Fragaria</i> sp.	Australia	Farr & Rossman 2016
			Brazil	
			South Africa	
			USA	
		<i>Photinia serrulata</i>	China	Farr & Rossman 2016
<b><i>Phomopsis padina</i> (Sacc.) Dietel</b>	<i>Phoma padina</i> Sacc.	<i>Laurocerasus officinalis</i>	Ukraine	Farr & Rossman 2016
		<i>Laurocerasus officinalis</i> var. <i>zabeliana</i>	Ukraine	Farr & Rossman 2016
		<i>Prunus avium</i>	USA	Farr & Rossman 2016
		<i>Prunus cerasus</i>	USA	Farr & Rossman 2016
		<i>Prunus dulcis</i>	World Wide	Farr & Rossman 2016
		<i>Prunus padus</i>	United Kingdom	Farr & Rossman 2016
		<i>Prunus persica</i>	World Wide	Farr & Rossman 2016
<b><i>Phomopsis parabolica</i> Petr.</b>		<i>Prunus dulcis</i>	World Wide	Farr & Rossman 2016
		<i>Prunus persica</i>	World Wide	Farr & Rossman 2016
<b><i>Phomopsis perniciosa</i> Grove</b>		<i>Cerasus avium</i>	Poland	Farr & Rossman 2016
		<i>Crataegus</i> sp.	Poland	Farr & Rossman 2016
		<i>Laurocerasus ciliospinosa</i>	China	Farr & Rossman 2016
		<i>Malus domestica</i>	Portugal	Farr & Rossman 2016
		<i>Malus pumila</i>	Poland	Farr & Rossman 2016
		<i>Malus purpurea</i>	Poland	Farr & Rossman 2016
		<i>Malus sylvestris</i>	Kenya	Farr & Rossman 2016
		<i>Malus</i> sp.	Poland	Farr & Rossman 2016
		<i>Padus avium</i>	Russia	Farr & Rossman 2016
		<i>Prunus dulcis</i>	World Wide	Farr & Rossman 2016
		<i>Prunus persica</i>	Portugal	Farr & Rossman 2016
			World Wide	
		<i>Prunus</i> sp.	Canada	Farr & Rossman 2016
			Lithuania	
			Poland	
			Yugoslavia	
		<i>Pyrus communis</i>	India	Farr & Rossman 2016
		<i>Pyrus malus</i>	Southern Africa	Farr & Rossman 2016
<b><i>Phomopsis pyrorum</i> Sacc. &amp; Trotter</b>	<i>Phomopsis pyrorum</i> Sacc. & Trotter	<i>Pyrus pyrifolia</i>	China	Farr & Rossman 2016
<b><i>Phomopsis pruni</i> (Ellis &amp;</b>	<i>Cytospora pruni</i> Ellis & Dearn	<i>Prunus dulcis</i>	World Wide	Farr & Rossman 2016

<b>Dearn.) Wehm.</b>		<i>Prunus × yedoensis</i>	Japan	Farr & Rossman 2016
		<i>Prunus</i> sp.	World Wide	Farr & Rossman 2016
<i>Phomopsis rhodophila</i> (Sacc.) N.F. Buchw.	<i>Phoma rhodophila</i> Sacc.	<i>Rosa</i> sp.	China	Farr & Rossman 2016
<i>Phomopsis ribatejana</i> Sousa da Câmara		<i>Prunus persica</i>	Portugal	Sousa da Câmara 1948
<i>Phomopsis rubiseda</i> Fairm.		<i>Rubus</i> sp.	USA	Farr & Rossman 2016
<i>Phomopsis sorbariae</i> (Sacc.) Höhn.	<i>Phoma sorbariae</i> Sacc.	<i>Spiraea chamaedryfolia</i>	Armenia	Farr & Rossman 2016
<i>Phomopsis sorbicola</i> Grove		<i>Sorbus aucuparia</i>	Poland	Farr & Rossman 2016
		<i>Sorbus</i> sp.	Canada	Farr & Rossman 2016
<i>Phomopsis spiraeae</i> (Desm.) Grove	<i>Phoma spiraeae</i> Desm.	<i>Spiraea nipponica</i>	Poland	Farr & Rossman 2016
<i>Phomopsis strictosoma</i> Grove		<i>Spiraea</i> sp.	USA	Farr & Rossman 2016
<i>Phomopsis truncicola</i> Miura		<i>Cydonia oblonga</i>	Zimbabwe	Farr & Rossman 2016
		<i>Malus prunifolia</i>	China	Farr & Rossman 2016
		<i>Malus pumila</i>	China	Farr & Rossman 2016
		<i>Malus pumila</i> var. <i>domestica</i>	Japan	Farr & Rossman 2016

## Discussion

In the present study four *Diaporthe* species were identified from Rosaceae hosts. Of these, two were described as new (*D. pyracanthae* associated with canker of firethorn and *D. malorum* associated with post-harvest fruit rot of apple). These two species are closely related to *D. leucospermi* and *D. passiflorae*, respectively, but clearly distinct phylogenetically. Within *D. malorum* isolate CAA752 clustered on a separated branch from CAA734 and CAA740 with high bootstrap support, but this was considered as intraspecific genetic variability. This isolate differs in 7 nucleotide positions in the sequence of one locus (CAL) but the sequences from the remaining loci are 100% identical to other isolates in the species. We also identified *D. eres* from canker of *Prunus cerasus* in Russia and *D. foeniculina* from canker of pear tree and post-harvest fruit rot of apple in Portugal.

*Diaporthe eres* (syn. *Phomopsis oblonga*) is the type species of the genus and one of the most studied species of *Diaporthe*. Despite this, the delimitation of the species and its many synonyms has been complicated by the absence of ex-type cultures. Recently, Udayanga et al. (2014b) addressed the issue of species delimitation in the *D. eres* complex using a multi-gene genealogical approach and clearly resolved nine distinct phylogenetic species. Moreover, they designated epitypes for several species, including for *D. eres*, thus clarifying the status of *D. eres* and closely related species.

*Diaporthe eres* is a cosmopolitan species and has been found on the following members of Rosaceae: *Chaenomeles speciosa*, *Cotoneaster* spp., *Crataegus* spp., *Kerria japonica*, *Malus* spp., *Physocarpus* spp., *Prunus* spp., *Pyracantha* spp., *Pyrus* spp., *Raphiolepis indica*, *Rosa* spp., *Rubus* spp., *Sorbus aucuparia*, and *Spiraea* spp. (Farr & Rossman 2016, Vrandečić et al. 2011). As far as we know *D. eres* has never been reported from *Prunus cerasus* in Russia.

Although it is a well-known species there are relatively few studies on pathogenicity of *D. eres* on Rosaceae, although it is known to cause shoot blight and canker in peaches (Thomidis & Michailides 2009); cane blight in blackberry (Vrandečić et al. 2011); trunk canker and death of young apple trees (Abreo et al. 2012) and wilting of shoots of *Cotoneaster* species (Frużyńska-Józwick & Jerzak 2006). Vrandečić et al. (2011) showed that *D. eres* can produce lesions on long green shoots of potted blackberry plants. Thomidis & Michailides (2009) showed that *D. eres* is able to produce necrosis in peach and nectarine fruits, but when the fruits were stored at 10°C or lower the fungus was unable to cause fruit rot. They also showed that this species is aggressive when tested on peach shoots in the field.

Here we showed that in artificial inoculation trials *D. eres* caused rotting of apple fruits and lesions on detached pear twigs. In the detached pear twigs inoculation assay, it was the most aggressive species tested and caused lesions with a mean of 6.9 cm. Surprisingly, *D. eres* is considered a weak to moderate pathogen of woody plants (Udayanga et al. 2014b).

Another well-known species associated with hosts in Rosaceae, but less common than *D. eres*, is *D. foeniculina*. This species has been found on *Malus domestica*, *Prunus amygdalus*, *Prunus dulcis*, *Pyrus bretschneideri* and *Pyrus pyrifolia* (Cloete et al. 2011, Diogo et al. 2010, Farr & Rossman 2016). The present study represents the first report of the species on *Pyrus communis* and also the first report on *Malus domestica* in Portugal. There is only one other report from *M. domestica* and that was from New Zealand (Udayanga et al. 2014b). In Portugal, until now, *D. foeniculina* (as *D. neotheicola*) has been reported on *Prunus dulcis* and *Prunus armeniaca* (Diogo et al. 2010) as well as several other hosts outside the Rosaceae such as *Acer negundo*, *Euphorbia pulcherrima*, *Foeniculum vulgare*, and *Hydrangea macrophylla* (Santos & Phillips 2009, Santos et al. 2010).

In our pathogenicity trials, *D. foeniculina* caused rot on apple fruits and lesions on detached pear twigs being the second most aggressive species in both tests. However, Cloete et al. (2011) observed that *D. foeniculina* (as *Phomopsis theicola*) did not form lesions significantly different from controls on detached woody shoots of apple and pear. Also, Diogo et al. (2010) inoculated detached almond twigs with *D. foeniculina* and considered it as a weak pathogen of *Prunus dulcis*. These differences in aggressiveness may be a reflection of variation in the aggressiveness of different isolates within the species.

*Diaporthe ambigua* and *D. amygdali*, although not found in this study, are known pathogens of several Rosaceae hosts with worldwide distribution. *Diaporthe ambigua* has been found on *Malus domestica*, *M. sylvestris*, *Prunus armeniaca*, *Prunus salicina*, *Pyrus communis* and *Pyrus ussuriensis* (Gomes et al. 2013, Farr & Rossman 2016). *Diaporthe ambigua* is an important pathogen causing canker of apple (*Malus domestica*), pear (*Pyrus communis*) and plum (*Prunus salicina*) rootstocks in South Africa (Smit et al. 1996). The species was shown to kill nursery rootstocks quickly while mature rootstocks were killed over a longer period of time (Smit et al. 1996).

*Diaporthe amygdali* has been reported on *Prunus armeniaca*, *Prunus dulcis*, *Prunus persica*, *Prunus salicina*, and *Pyrus pyrifolia* (Farr & Rossman 2016). This species is well known as the causal agent of twig canker and blight of almond (*Prunus dulcis*) and peach (*Prunus persica*) in all areas where these hosts are cultivated (Diogo et al. 2010). It has also been associated with wood decay of almonds, fruit rot of peaches and fruit rot and branch dieback of almond (Adaskaveg et al. 1999, Kanematsu et al. 1999, Michailides & Thomidis 2006, Carlier et al. 2011, Gramaje et al. 2012). When inoculated on peach twigs and young almond twigs or apple twigs this species produced lesions, sometimes resulting in constriction canker (Dai et al. 2012, Diogo et al. 2010). When inoculated on mature and immature peaches, almonds and Japanese pears it caused fruit rot (Adaskaveg et al. 1999, Kanematsu et al. 1999, Michailides & Thomidis 2006).

More than 50 *Diaporthe* (and its asexual morph *Phomopsis*) species names have been associated with hosts in the family Rosaceae. However, apart from the above-mentioned species, *D. ambigua*, *D. amygdali*, *D. eres*, *D. foeniculina*, and the two newly described species, there is a scarcity of information regarding the taxonomic and pathogenic status of those taxa. For most of



them there is no other information available apart from the original description of the species. To complicate matters even further, often there are no ex-type cultures from which phenotypical, phytopathological and molecular data can be obtained. In the past *Diaporthe/Phomopsis* species have mostly been described assuming they were host-specific (Udayanga et al. 2011). However, it is now clear that although some species appear to be host specific, many are not and can be found on diverse plant hosts. Currently, the circumscription of species within *Diaporthe* can be accomplished only by use of multi-gene DNA sequence data (Gomes et al. 2013, Udayanga et al. 2012b, 2014a, 2014b, 2014c). Thus, in the absence of ex-type cultures it is impossible to carry out multi-locus phylogenetic analyses to assess the validity of these older species names and their relationship to currently accepted species in *Diaporthe*.

In recent years, a revision of the genus *Diaporthe* has been initiated and considerable progress has been made towards resolving species complexes and the epitypification/neotypification of species (Gomes et al. 2013, Udayanga et al. 2012b, 2014a, 2014b, 2014c). However, considering the large number of species described in *Diaporthe/Phomopsis* there is still much to be done.

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