Mycosphere 8(5) 485–511 (2017) www.mycosphere.org ISSN 2077 7019
Article (Special Issue)
Doi 10.5943/mycosphere/8/5/2

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

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Santos L, Phillips AJL, Crous P, Alves A 2017 – *Diaporthe* species on Rosaceae with descriptions of *D. pyracanthae* sp. nov. and *D. malorum* sp. nov. Mycosphere 8(5), 485–511, Doi 10.5943/mycosphere/8/5/2

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

Submitted 24 January 2017, Accepted 3 March 2017, Published 12 March 2017
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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-α (TEF1), ß-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 mm2) 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**
D. foeniculina	CAA133	*Pyrus communis*	branch canker	Portugal	KY435634	KY435624	KY435665	KY435645	KY435655	-	+
	CAA135	*Pyrus communis*	branch canker	Portugal						-	+
	CAA136	*Pyrus communis*	branch canker	Portugal						-	+
	CAA137	*Pyrus communis*	branch canker	Portugal						-	+
	CAA737	*Malus domestica*	fruit rot	Portugal	KY435641	KY435628	KY435669	KY435649	KY435659	+	-
	CAA738	*Malus domestica*	fruit rot	Portugal						+	-
	CAA739	*Malus domestica*	fruit rot	Portugal						+	-
D. pyracanthae	CAA481	*Pyracantha coccinea*	branch canker	Portugal						-	+
	CAA482	*Pyracantha coccinea*	branch canker	Portugal						-	+
	CAA483	*Pyracantha coccinea*	branch canker	Portugal	KY435635	KY435625	KY435666	KY435645	KY435656	-	+
	CAA484	*Pyracantha coccinea*	branch canker	Portugal						-	+
	CAA485	*Pyracantha coccinea*	branch canker	Portugal						-	+
	CAA486	*Pyracantha coccinea*	branch canker	Portugal						-	+
	CAA487	*Pyracantha coccinea*	branch canker	Portugal	KY435636	KY435626	KY435667	KY435647	KY435657	-	+
	CAA488	*Pyracantha coccinea*	branch canker	Portugal	KY435637					-	+
D. malorum	CAA734	*Malus domestica*	fruit rot	Portugal	KY435638	KY435627	KY435668	KY435648	KY435658	-	+
	CAA735	*Malus domestica*	fruit rot	Portugal	KY435639					-	+
	CAA736	*Malus domestica*	fruit rot	Portugal	KY435640					-	+
	CAA740	*Malus domestica*	fruit rot	Portugal	KY435642	KY435629	KY435670	KY435650	KY435660	-	+
	CAA752	*Malus domestica*	fruit rot	Portugal	KY435643	KY435630	KY435671	KY435651	KY435661	-	+
	CAA753	*Malus domestica*	fruit rot	Portugal						-	+
	CAA754	*Malus domestica*	fruit rot	Portugal						-	+
D. eres	CAA801	*Prunus cerasus*	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 25ºC. 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 ºC, followed by 30 cycles of 94 ºC for 30 s, 55 ºC for 30 s, 72 ºC for 1.5 min, and a final elongation step at 72 ºC 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 sequenced 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, 60ºC and 53º C for 30 s (for TEF/TUB, HIS and CAL, respectively), 72°C for 1 min; and then a final elongation step at 72 ºC for 10 min.

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

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

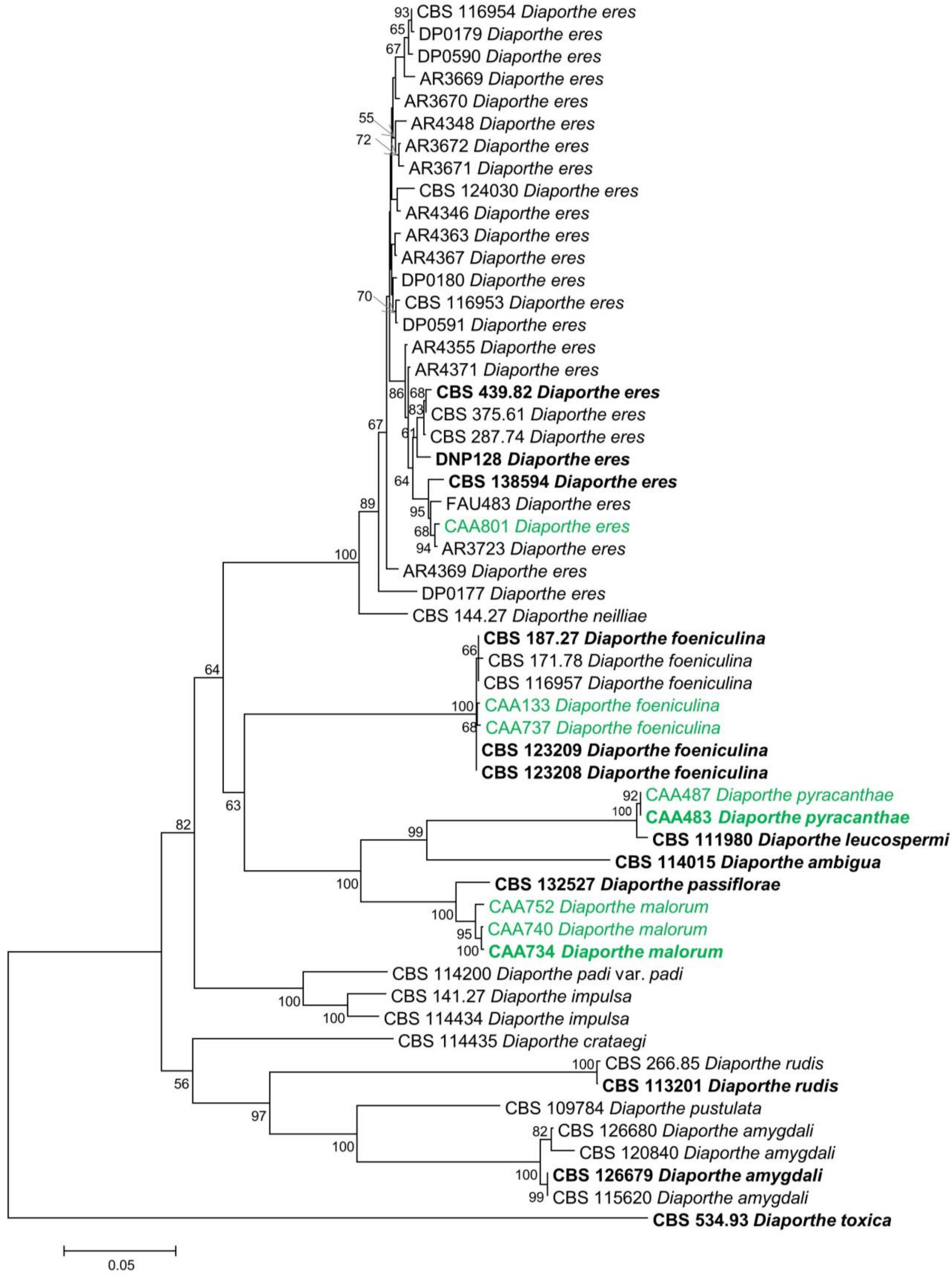

Figure 1 – ML tree built using the five loci ITS-TEF1-TUB- HIS-CAL for the *Diaporthe* species found in Rosaceae. Bootstrap values are shown next to the branches. Ex-type, ex-epitype, or isotype isolates are given in **bold**. The studied isolates are shown in green. The tree was rooted to *D. toxica*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

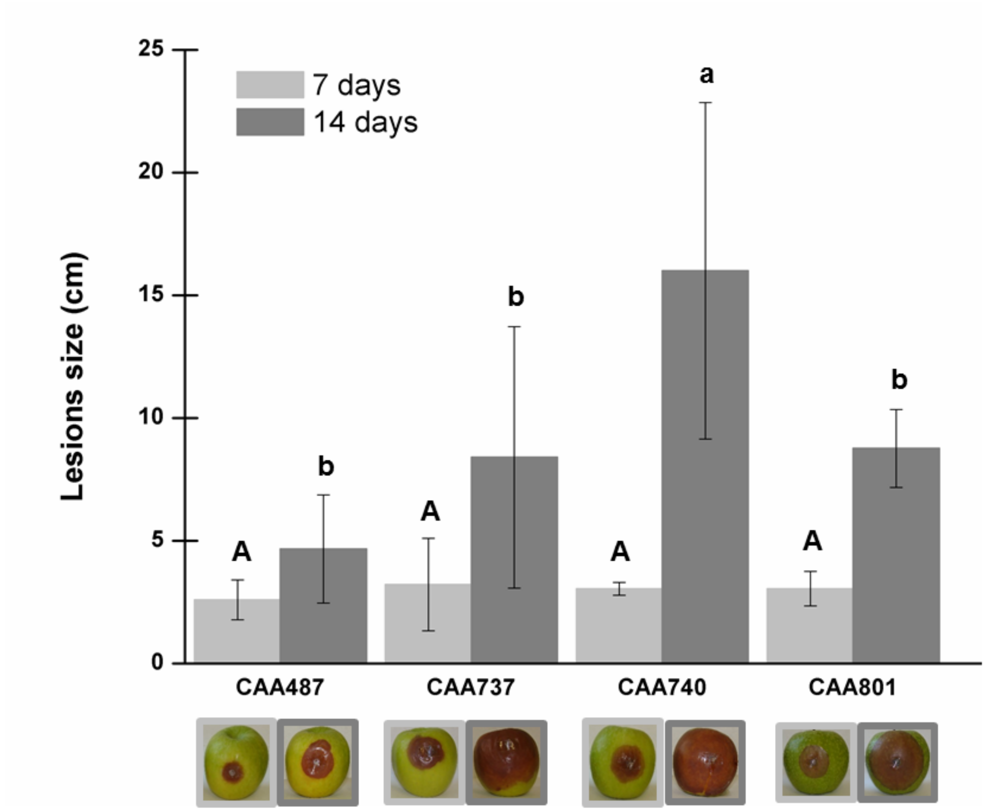

Figure 2 – Lesion size in apple fruit after 7 and 14 days. The vertical lines indicate standard deviations. Bars with the same letter are not significantly different.

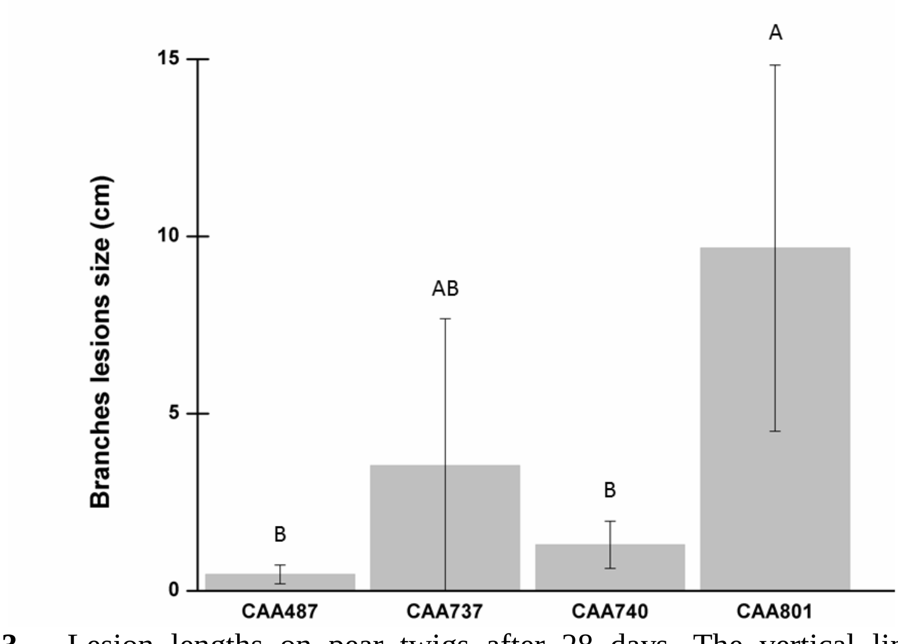

Figure 3 – Lesion lengths on pear twigs after 28 days. The vertical lines indicate standard deviations. Bars with the same letter are not significantly different.

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. impulsa*, *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 cirrhus. 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) × (1.6)–2.4– (3.0) µm (mean ± S.D. = 6.7 ± 0.6 × 2.4 ± 0.2 µm, n = 100), on fennel twigs (6.0)–6.8– (7.9) × (1.6)–2.2–(2.9) µm (mean ± S.D. = 6.8 ± 0.4 × 2.2 ± 0.2 µm, 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) × (0.8)–1.3–(1.9) µm (mean ± S.D. = 30.0 ± 2.7 × 1.3 ± 0.8 µm, n = 100), on fennel twigs (15.8)–26.8–(33.6) × (0.8)–1.3–(2.0) µm (mean ± S.D. = 26.8 ± 4.2 × 1.3 ± 0.2 µm, 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 25ºC; 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 40ºC ($p<0.05$), minimum temperature for growth between 4 and 9 ºC ($p<0.05$) and optimum temperature between 21 and 27 º C ($p<0.05$).

Sexual morph – not observed

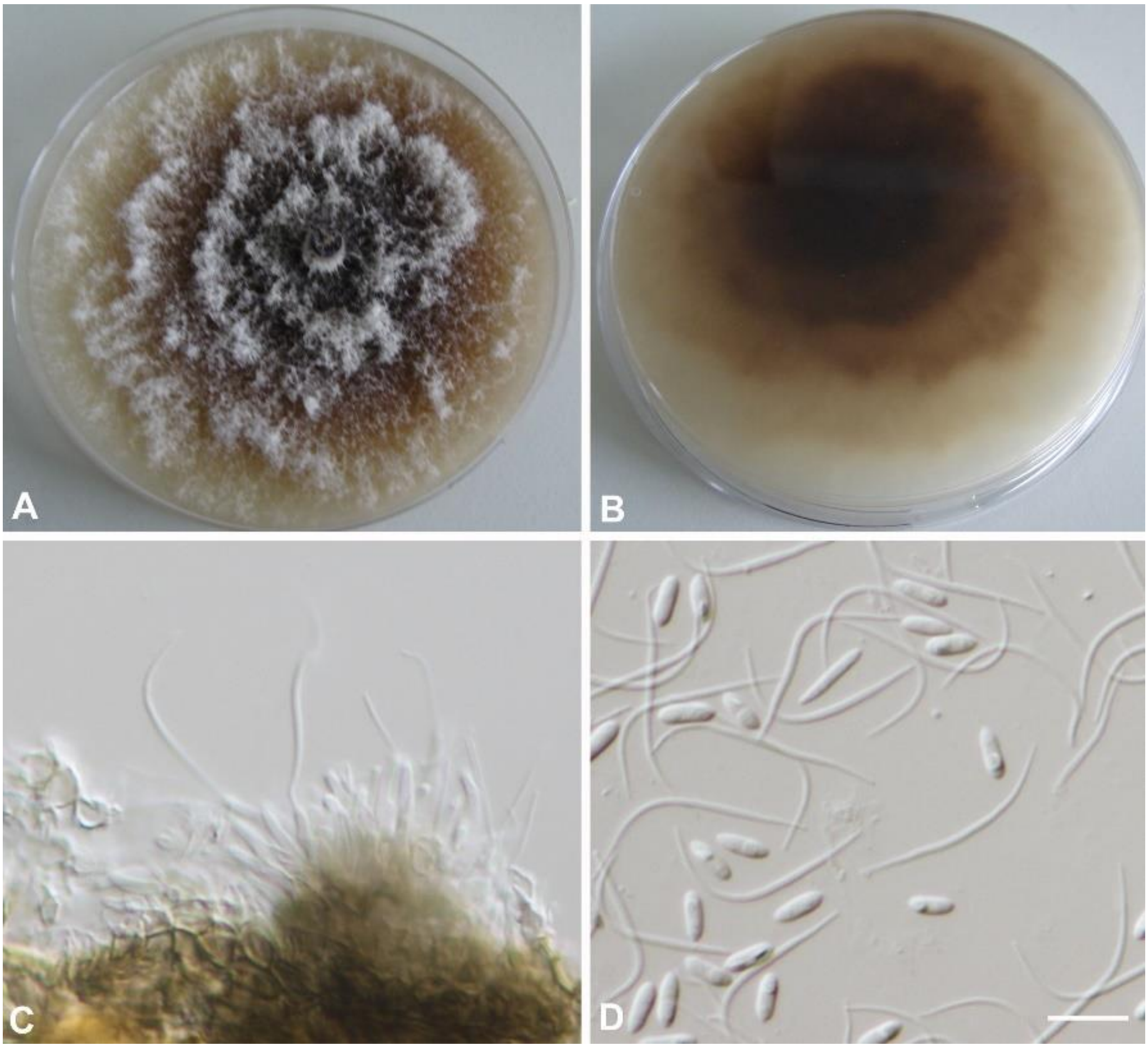

Figure 4 – *Diaporthe pyracanthae*. A. Upper culture surface on PDA, 25ºC and 7 days. B. Reverse culture surface on PDA, 25 ºC and 7 days. C. Conidiogenous cells. D. Alpha, beta and gamma conidia. Scale bar: C–D = 10 µm.

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.* Fig. 5

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 cirrhus. 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,

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

Locus		Isolates		
		Diaporthe leucospermi	CAA483	CAA487
ITS (537 bp)	61	C	T	T
	450	T	C	C
	467	T	C	C
TEF1 (332 bp)	16	C	T	T
	27	T	C	C
TUB (497 bp)	45	A	G	G
	89	T	C	C
	161	T	C	C
	298	A	C	C
	339	T	C	C
	347	T	C	C
	452	T	C	C
HIS (457 bp)	188	G	A	A
	189	G	A	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 25ºC, 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 40ºC ($p<0.05$), minimum temperature between 2 and 6 ºC ($p<0.05$) and optimum temperature between 13 and 20 ºC ($p<0.05$).

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).

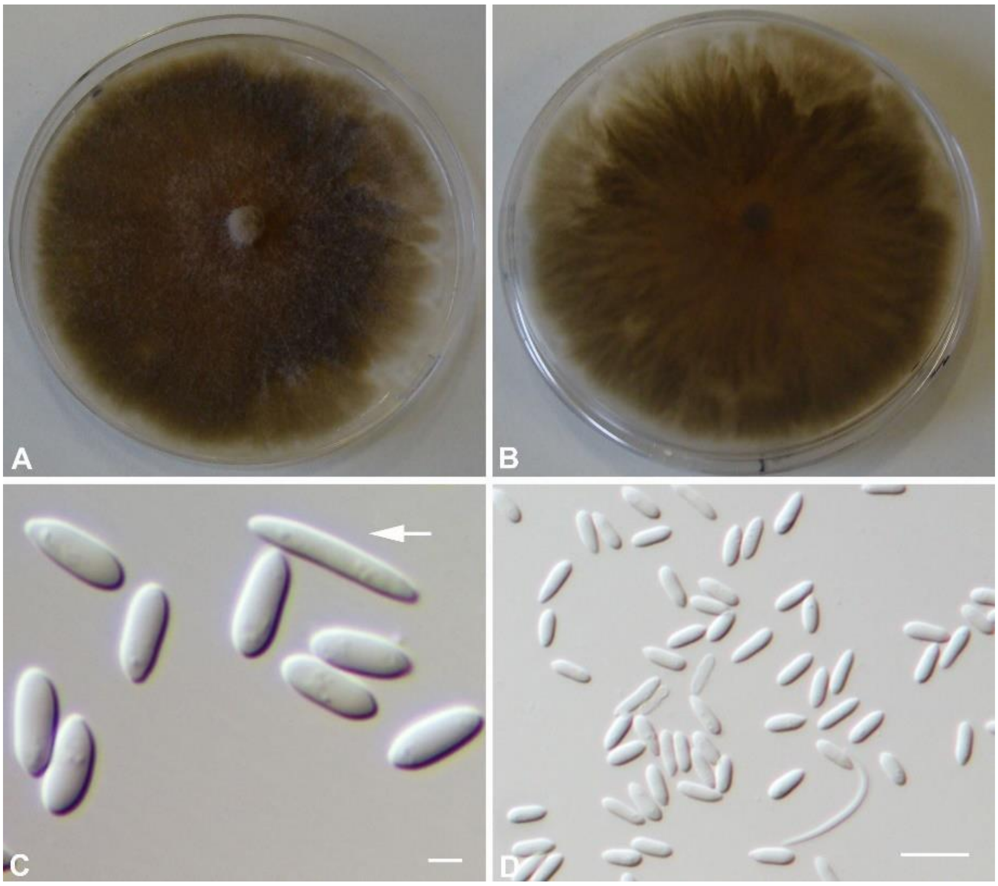

Figure 5 – *Diaporthe malorum*. A. Upper culture surface on PDA, 20 ºC and 9 days. B. Reverse culture surface on PDA, 20 ºC and 9 days. C. Gamma conidia. D. alpha and beta conidia. – Scale bars: C = 2 µm, D = 10 µm.

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

Locus		Isolates			
		Diaporthe passiflorae	**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
TEF1 (346 bp)	27	G	A	A	A
	50	A	-	-	-
	51	C	-	-	-
	93	C	A	A	A
	96	A	G	G	G
	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	-	-	-
TUB (502 bp)	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
	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
HIS (425 bp)	62	C	G	G	G
	150	A	G	G	G
	158	A	C	C	C
	164	T	G	G	G
	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
CAL (486 bp)	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
	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
Diaporthe actinidiae **N.F. Sommer & Beraha**		*Malus domestica*	New Zealand	Farr & Rossman 2016
Diaporthe ambigua **Nitschke**	*Phoma ambigua* (Nitschke) Sacc.	*Malus domestica*	South Africa	Farr & Rossman, 2016
	Phomopsis ambigua Traverso	*Malus sylvestris*	Netherlands	Murali et al. 2006
			South Africa	Farr & Rossman 2016
		Malus sp.	Armenia	Farr & Rossman 2016
			United Kingdom	
		Prunus salicina	South Africa	Farr & Rossman 2016
		Prunus sp.	South Africa	Farr & Rossman 2016
				van Niekerk et al. 2005
		Pyrus communis	Canada	Farr & Rossman 2016
			Cuba	Gomes et al. 2013
			Germany	
			South Africa	
			USA	
		Pyrus ussuriensis	China	Farr & Rossman 2016
Diaporthe amygdali **(Delacr.) Udayanga, Crous & K.D. Hyde**	*Fusicoccum amygdali* Delacr.	*Amygdalus persica*	Japan	Farr & Rossman 2016
	Phomopsis amygdali (Delacr.) J.J. Tuset & M.T. Portilla	*Prunus amygdalus*	China	Farr & Rossman 2016
		Prunus armeniaca	China	Farr & Rossman 2016
	Phomopsis amygdalina Canonaco	*Prunus dulcis*	Italy	Farr & Rossman 2016
			Portugal	Santos et al. 2010
			USA	Diogo et al. 2010
			World wide	Gomes et al. 2013
		Prunus persica	China	Farr & Rossman 2016
			France	Gomes et al. 2013
			Greece	
			Japan	
			Portugal	
			South Africa	
			USA	
			World Wide	
		Prunus persica var. *vulgaris*	Japan	Farr & Rossman 2016
		Prunus salicina	China	Farr & Rossman 2016
			South Africa	Gomes et al. 2013
		Prunus salicina var. *corlata*	China	Farr & Rossman 2016
		Prunus sp.	USA	Murali et al. 2006
Diaporthe australafricana **Crous & Van Niekerk**		*Prunus dulcis*	USA	Farr & Rossman 2016

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

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

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

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

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

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

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

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

Dearn.) Wehm.		*Prunus × yedoensis*	Japan	Farr & Rossman 2016
		Prunus sp.	World Wide	Farr & Rossman 2016
***Phomopsis rhodophila* (Sacc.) N.F. Buchw.**	*Phoma rhodophila* Sacc.	*Rosa* sp.	China	Farr & Rossman 2016
***Phomopsis ribatejana* Sousa da Câmara**		*Prunus persica*	Portugal	Sousa da Câmara 1948
***Phomopsis rubiseda* Fairm.**		*Rubus* sp.	USA	Farr & Rossman 2016
***Phomopsis sorbariae* (Sacc.) Höhn.**	*Phoma sorbariae* Sacc.	*Spiraea chamaedryfolia*	Armenia	Farr & Rossman 2016
***Phomopsis sorbicola* Grove**		*Sorbus aucuparia*	Poland	Farr & Rossman 2016
		Sorbus sp.	Canada	Farr & Rossman 2016
***Phomopsis spiraeae* (Desm.) Grove**	*Phoma spiraeae* Desm.	*Spiraea nipponica*	Poland	Farr & Rossman 2016
		Spiraea sp.	USA	Farr & Rossman 2016
***Phomopsis strictosoma* Grove**		*Cydonia oblonga*	Zimbabwe	Farr & Rossman 2016
***Phomopsis truncicola* Miura**		*Malus prunifolia*	China	Farr & Rossman 2016
		Malus pumila	China	Farr & Rossman 2016
		Malus pumila var. *domestica*	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., *Rhaphiolepis 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-Jóźwick & 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 10ºC 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 others 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 speces.

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/neotypificaton 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.

Acknowledgements

This work was partially financed by European Funds through COMPETE and by National Funds through the Portuguese Foundation for Science and Technology (FCT) within project PANDORA (PTDC/AGR-FOR/3807/2012 – FCOMP-01-0124-FEDER-027979). The authors acknowledge financing from FCT to CESAM (UID/AMB/50017/2013 – POCI-01- 0145-FEDER-007638), Artur Alves (FCT Investigator Programme – IF/00835/2013) and Liliana Santos (post-doctoral grant –SFRH/BPD/90684/2012). Alan JL Phillips acknowledges the support from Biosystems and Integrative Sciences Institute (BioISI, FCT/UID/ Multi/04046/2013).

References

Abreo E, Martínez S, Sessa L, Bettucci L, Lupo S. 2012 – *Phomopsis cotoneastri* as a pathogen associated with trunk cankers and death of young apple trees cv. Cripps Pink. Journal of Phytopathology 160, 434–436.

Adaskaveg JE, Forster H, Connell JH. 1999 – First report of fruit rot and associated branch dieback of almond in California caused by a *Phomopsis* species tentatively identified as *P. amygdali*. Plant Disease 83, 1073.

Alves A, Crous PW, Correia A, Phillips AJL. 2008 – Morphological and molecular data reveal cryptic species in *Lasiodiplodia theobromae*. Fungal Diversity 28, 1–13.

Alves A, Phillips AJL, Henriques I, Correia A. 2007 – Rapid differentiation of species of Botryosphaeriaceae by PCR fingerprinting. Research in Microbiology 158, 112–121.

Baumgartner K, Fujiyoshi F, Travadon R, Castlebury LA, Wilcox WF, Rolshausen PE. 2013 – Characterization of species of *Diaporthe* from wood cankers of grape in Eastern North American vineyards. Plant Disease 97, 912–920.

Boddy L, Griffith GS. 1989 – Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees. Sydowia 41, 41–73.

Carbone I, Kohn LM. 1999 – A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553–556.

Carlier J, Cabrita L, Leitão J, Sousa RM, Sousa AT. 2011 – ISSR and AFLP characterization of *Phomopsis amygdali* (Del.) Tuset & Portilla accessions. Acta Horticulturae 912, 645–651.

Cloete M, Fourie PH, Damm U, Crous PW, Mostert L. 2011 – Fungi associated with die-back symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease pathogens. Phytopathologia Mediterranea 50, 176–190.

Crous PW, Groenewald JZ, Risede JM, Hywel-Jones NL. 2004 – *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Studies in Mycology 50, 415–430.

Dai FM, Zeng R, Lu JP. 2012 – First report of twig canker on peach caused by *Phomopsis amygdali* in China. Plant Disease 96, 288.

Diogo ELF, Santos JM, Phillips AJL. 2010 – Phylogeny, morphology and pathogenicity of *Diaporthe* and *Phomopsis* species on almond in Portugal. Fungal Diversity 44, 107–115.

Farr DF, Castlebury LA, Pardo-Schultheiss RA. 1999 – *Phomopsis amygdali* causes peach shoot blight of cultivated peach trees in the southeastern United States. Mycologia 91, 1008–1015.

Farr DF, Rossman AY. 2016 – Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. http://nt.ars-grin.gov/fungaldatabases/ (accessed 15 February 2016).

Frużyńska-Jóźwick D, Jerzak E. 2006 – Fungi on the collection of cotoneaster plants in the poznań botanical garden in 2003. Phytopathologia Polonica 40, 63–68.

Fujita K, Sugiki T, Matsunaka K. 1988 – Apple blight caused by *Diaporthe tanakae* in Aomori Prefecture. Bulletin of the Aomori Field Crops and Horticultural Experiment Station 6, 17–35.

Gao YH, Sun W, Su Y-Y, Cai L. 2014 – Three new species of *Phomopsis* in Gutianshan Nature Reserve in China. Mycological Progress 13, 111–121.

Glass NL, Donaldson G. 1995 – Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61, 1323–1330.

Gomes RR, Glienke C, Videira SIR, Lombard L et al. 2013 – *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia 31, 1–41.

Gramaje D, Agustí-Brisach C, Pérez-Sierra A, Moralejo E et al. 2012 – Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). Persoonia 28, 1–13.

Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium 41, 95–98.

Huang F, Hou X, Dewdney MM, Fu Y et al. 2013 – *Diaporthe* species occurring on citrus in China. Fungal Diversity 61, 237–250.

Hummer KE, Janick J. 2009 – Rosaceae: taxonomy, economic importance, genomics. In: Genetics and Genomics of Rosaceae. Folta KM, Gardiner SE, Springer, New York, 1–17.

Kanematsu S, Yokoyama Y, Kobayashi T, Kudo A, Ohtsu Y. 1999 –Taxonomic reassessment of the causal fungus of peach *Fusicoccum* canker in Japan. Annals of the Phytopathological Society of Japan 65, 531–536.

Kobayashi T, Sakuma T. 1982 – Materials for the fungus flora of Japan. Transactions of the Mycological Society of Japan 23, 37–40.

Larkin MA, Blackshields G, Brown NP, Chenna R et al. 2007 – Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Latham AJ, Morgan-Jones G, Campbell HL. 1992 – *Phomopsis* dieback of peach shoots in Alabama. Plant Disease 76, 426.

Li W. 1997 – Molecular Evolution. Sinauer and Associates, Sunderland, Massachusetts, USA.

Michailides TJ, Thomidis T. 2006 – First report of *Phomopsis amygdali* causing fruit rot on peaches in Greece. Plant Disease 90, 1551.

Möller EM, Bahnweg G, Sandermann H, Geiger HH. 1992 – A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Research 20, 6115–6116.

Murali T, Suryanarayanan T, Geeta R. 2006 – Endophytic *Phomopsis* species: host range and implications for diversity estimates. Canadian Journal of Microbiology 52, 673–680.

Nakatani F, Hiraragi T, Sekizawa H. 1984 – Studies on the canker of pear caused by *Diaporthe tanakae* Kobayashi et Sakuma. Bulletin of the Iwate Horticultural Experiment Station 5, 47–70.

O'Donnell K, Cigelnik E. 1997 – Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7, 103–116.

Ogawa JM, Zehr EI, Bird GW, Ritchie DF et al. 1995 – Compendium of Stone Fruit Diseases. APS Press, the American Phytopathological Society, St. Paul, USA.
Potter D, Eriksson T, Evans RC, Oh S et al. 2007 – Phylogeny and classification of Rosaceae. Plant Systematics and Evolution 266, 5–43.
Roberts JW. 1913 – The “rough bark” disease of Yellow Newtown apple. U. S. Department of Agriculture – Bureau of Plant Industry – Bulletin 280.
Sakuma T, Batra LR, Nakatani F, Sawamura K. 1982 – European pear die-back (*Diaporthe* sp.) in Japan and its comparison with pear fire blight (*Erwinia amylovora*). Bulletin of the Fruit Tree Research Station Series C, 9, 79–89.
Santos J, Phillips A. 2009 – Resolving the complex of *Diaporthe* (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. Fungal Diversity 34, 111–125.
Santos JM, Correia VG, Phillips AJL. 2010 – Primers for mating-type diagnosis in *Diaporthe* and *Phomopsis*: their use in teleomorph induction in vitro and biological species definition. Fungal Biology 114, 255–270.
Smit WA, Viljoen CD, Wingfield BD, Wingfield MJ, Calitz FJ. 1996 – A new canker disease of apple, pear, and plum rootstocks caused by *Diaporthe ambigua* in South Africa. Plant Disease 80, 1331–1335.
Sousa da Câmara M. 1948 – Mycetes aliquot Lusitaniae VIII. Agronomia Lusitana 10, 279–320.
Tamura K, Nei M. 1993 – Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10, 512–526.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 – MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. Doi: 10.1093/molbev/mst197
Tan YP, Edwards J, Grice KRE, Shivas RG, 2013 – Molecular phylogenetic analysis reveals six new species of *Diaporthe* from Australia. Fungal Diversity 61, 251–260.
Thomidis T, Michailides TJ. 2009 – Studies on *Diaporthe eres* as a new pathogen of peach trees in Greece. Plant Disease 93, 1293–1297.
Thompson SM, Tan YP, Young AJ, Neate SM et al. 2011 – Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe* (*Phomopsis*) species. Persoonia 27, 80–89.
Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD. 2014b – Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. Fungal Diversity 67, 203–229.
Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD. 2014c – The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. Fungal Biology 119, 383–407.
Udayanga D, Castlebury LA, Rossman AY, Hyde KD. 2014a – Species limits in *Diaporthe*: molecular re-assessment of *D. citri*, *D. cytosporella*, *D. foeniculina* and *D. rudis*. Persoonia 32, 83–101.
Udayanga D, Liu X, Crous PW, McKenzie EHC et al. 2012b – A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). Fungal Diversity 56, 157–171.
Udayanga D, Liu X, McKenzie EHC, Chukeatirote E et al. 2011– The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Diversity 50, 189–225.
Udayanga D, Liua X, Mckenzie EHC, Chukeatirote E, Hyde KD. 2012a – Multi-locus phylogeny reveals three new species of *Diaporthe* from Thailand. Cryptogamie, Mycologie 33, 295–309.
Uddin W, Stevenson KL, Pardo-Schultheiss RA, Rehner SA. 1998 – Pathogenic and molecular characterization of three *Phomopsis* isolates from peach, plum, and Asian pear. Plant Disease 82, 732–737.

Uddin W, Stevenson KL, Pardo-Schultheiss RA. 1997 – Pathogenicity of a species of *Phomopsis* causing a shoot blight on peach in Georgia and evaluation of possible infection courts. Plant Disease 81, 983–989.

Van Niekerk JM, Groenewald JZ, Farr DF, Fourie PH et al. 2005 – Reassessment of *Phomopsis* species on grapevines. Australasian Plant Pathology 34, 27–39.

Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172, 4239–4246.

Vrandečić K, Jurković D, Ćosić J, Postic J, Riccioni L. 2011 – First report of cane blight on blackberry caused by *Diaporthe eres* in Croatia. Plant Disease 95, 612.

Wang J, Xu X, Mao L, Lao J et al. 2014 – Endophytic *Diaporthe* from Southeast China are genetically diverse based on multi-locus phylogeny analyses. World journal of microbiology & biotechnology 30, 237–243.

Webber J, Gibbs JN. 1984 – Colonization of elm bark by *Phomopsis oblonga*. - Transactions of the British Mycological Society 82, 348–352.

White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. Innis MA, Gelfand DH, Sninsky JJ, White TJ, Academic Press, San Diego, USA, 315–322.