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Phylogeny, morphology and pathogenicity of *Diaporthe* and *Phomopsis* species on almond in Portugal

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Abstract The ascomycete genus *Diaporthe* includes plant pathogens and endophytes on a wide range of hosts including economically important crops. Anamorphs are coelomycetous and reside in the genus *Phomopsis*. *Phomopsis amygdali* is the causal agent of twig canker and blight of almonds. In a recent survey of dieback of almonds in Portugal, the most frequent fungi detected were *Diaporthe*/*Phomopsis* species. Isolates from almond and other *Prunus* species were characterised and grouped according to their microsatellite-primed PCR (MSP-PCR) profiles and representatives of the different groups were selected for a phylogenetic study based on the ITS rDNA region (ITS1–5.8S–ITS2). Combining morphological, cultural, molecular and pathogenicity data, three species were distinguished. *Phomopsis amygdali* was shown to be the main pathogen on almond and is epitypified in the present work. *Diaporthe neotheicola* is reported for the first time on this host. A third species represented by a single isolate could not be unequivocally identified.

Keywords Almond · *Diaporthe* · Epitype · ITS · *Phomopsis* · Systematics

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Introduction

The ascomycete genus *Diaporthe* Nitschke includes plant pathogens and endophytes on a wide range of hosts including economically important crops (Uecker 1988). Species in this genus and their *Phomopsis* (Sacc.) Bubák anamorphs were characterized largely by host association, which resulted in a proliferation of names (Uecker 1988). Recent studies have revealed that host association is not a reliable character for species definition since the same species can be found on different hosts and several species can occur on the same host (Rehner and Uecker 1994; Mostert et al. 2001). For example, in grapevine (*Vitis vinifera*), 15 different species of *Phomopsis* have been reported (Mostert et al. 2001; van Niekerk et al. 2005). Molecular data, such as phylogenies derived from sequences of the internal transcribed spacer (ITS) and other genes have helped to redefine species in this genus (Mostert et al. 2001; Santos et al. 2010; Santos and Phillips 2009; van Niekerk et al. 2005; van Rensburg et al. 2006).

Phomopsis amygdali is the causal agent of twig canker and blight of almonds (*Prunus dulcis*) and peach (*Prunus persica*) wherever these hosts are grown. It was first described as *Fusicoccum amygdali* Delacr. causing cankers on almonds in France (Delacroix 1905). Tuset and Portilla (1989) re-examined the type specimen of *F. amygdali* and, based on morphology and symptomatology, they considered that it would be best accommodated in *Phomopsis* as *Phomopsis amygdali* (Delacr.) J.J Tuset and M.T. Portilla. They also considered *Phomopsis amygdalina* Canonaco to be a synonym of *P. amygdali*. Unfortunately, no cultures linked unequivocally to the type exist.

In a recent survey of dieback of almonds in Portugal, the fungi most frequently detected were *Diaporthe*/*Phomopsis* species. Although most of the isolates corresponded morphologically with *P. amygdali*, some were clearly different species. Therefore, the aim of this work was to use morphological, molecular and pathogenicity data to clarify the identity of the *Diaporthe*/*Phomopsis* species that occur on almond in Portugal, and to select a suitable specimen as epitype of *P. amygdali*.

Materials and methods

Isolations

Branches from almond trees showing symptoms of cankers or dieback were collected in the two major production areas of Portugal. Six orchards in the northern region of Trás-os-Montes were sampled while in the south of the country two orchards in Algarve and one in Alentejo were sampled. Isolations were made from small pieces of host tissue taken from the margin of the cankers. Tissue pieces of about 3 mm^2 were surface disinfected in 3% sodium hypochlorite for 5 min, rinsed in sterile distilled water and plated on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA.) amended with 100 ppm of streptomycin sulphate (Sigma-Aldrich, St. Louis, MO, USA) to suppress bacterial growth. The resulting cultures were induced to sporulate and single spore isolations were made for all isolates. Isolates were maintained on 2% water agar (WA) slants with a piece of sterile alfalfa stem at 4°C (Farr et al. 1999). The isolates used in this study are summarised in Table 1. For comparison, isolates from other *Prunus* hosts were included. Reference isolates were deposited in the public culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

DNA isolation, MSP-PCR and sequence analysis

DNA was isolated from mycelia scraped from the surface of a PDA plate. DNA was extracted with the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, USA), following the manufacturer's instructions. Instead of using ground lyophilized mycelia, fresh mycelium was used and was disrupted by adding approx. 50 µl of glass beads (425–600 µm diam.) to the extraction buffer and vortexing for 2 min. MSP-PCR profiles were generated following the protocol of Uddin and Stevenson (1997) using the primers $(GTG)_5$, $(GGA)_7$, $(ACAC)_5$ and phage M13 core sequence. (Meyer et al. 1993). Amplicons were separated by electrophoresis at 5 $V.cm^{-1}$ and visualised as described in Santos and Phillips (2009). The isolates were clustered on the basis of their profiles in a consensus dendrogram built with GelCompar™ version 4.1 (Applied Maths BVBA, Kortrijk, Belgium) using Pearson's correlation coefficient and UPGMA (Fig. 1). These experiments were repeated once.

A set of 19 isolates representing each MSP-PCR cluster was selected for a phylogenetic analysis of the ITS region following the protocol of Alves et al. (2004) and increasing the amount of primers to 25 pmol each and of *Taq* polymerase to 1.25 U. The cycling conditions were: initial denaturation of 10 min at 95°C, 35 cycles of 1 min at 95°C, 1 min at 55°C and 30 sec at 72°C, and a final extension step of 10 min at 72°C. The amplicons were purified and sequenced as in Santos and Phillips (2009). Sequences were edited and used in a phylogenetic analysis following the protocol of Santos and Phillips (2009). The sequence of *Phomopsis theicola* CBS 187.27 was retrieved from TreeBASE (S1506, M2708, van Rensburg et al. 2006). Sequences obtained from GenBank are listed by their taxon names and accession numbers in the tree (Fig. 2), while newly generated sequences are listed by their isolate number. Newly generated sequences have been deposited in GenBank (Table 1) and the alignment and phylogeny in TreeBASE (S10334, M5013).

Morphology

Cultures were grown on 2% WA with a sterile alfalfa stem (Farr et al. 1999) at 22°C with 12 h of near UV light per day. Morphological characters were studied and recorded as in Santos and Phillips (2009). Growth rates were determined on PDA plates as described by Santos and Phillips (2009). Colony diameters were measured after 3 days of incubation.

Pathogenicity

One year old almond twigs cv. Ferragnès, about 30 cm long, were inoculated with 4 isolates of *P. amygdali*, 4 of *D. neotheicola* and the single isolate of the unidentified *Phomopsis* species. A 3 mm^2 flap of epidermis was lifted, colonised agar plugs of about the same size were placed underneath the epidermis and the wounds sealed with parafilm. The twigs were kept in an upright position with their lower ends immersed in jars of water in a controlled environment at 25°C with 14 h of light per day. The twigs were covered with a plastic bag during the first 7 days to keep a moist environment. The water was changed every 3 days. Ten twigs per isolate were used. A negative control was treated in the same way with a sterile agar plug. Lesion lengths were measured 24 days after inoculation and analysed by analysis of variance. Means were compared by least significant difference test (LSD) (Table 2).

Table 1 List of isolates used in this study

Species	Isolate no.	Host	Origin	MSP-PCR Group	GenBank accession no. (ITS)[a]
Phomopsis amygdali	Pes	*Prunus persica*	Alentejo	I	
	2B	*Prunus dulcis*	Trás-os Montes	I	
	2C	*Prunus dulcis*	Trás-os Montes	I	
	3A	*Prunus dulcis*	Trás-os Montes	I	
	3B = CBS 126679[b]	*Prunus dulcis*	Trás-os Montes	I	GQ281791
	4B	*Prunus dulcis*	Trás-os Montes	I	
	5A	*Prunus dulcis*	Trás-os Montes	I	
	5C	*Prunus dulcis*	Trás-os Montes	I	
	6A	*Prunus dulcis*	Trás-os Montes	I	
	7B	*Prunus dulcis*	Trás-os Montes	I	
	8B	*Prunus dulcis*	Trás-os Montes	I	
	10A	*Prunus dulcis*	Trás-os Montes	I	
	10B	*Prunus dulcis*	Trás-os Montes	I	
	10C	*Prunus dulcis*	Trás-os Montes	I	
	11A	*Prunus dulcis*	Trás-os Montes	I	GQ281792
	11B	*Prunus dulcis*	Trás-os Montes	I	
	11C	*Prunus dulcis*	Trás-os Montes	I	
	12B	*Prunus dulcis*	Trás-os Montes	I	
	16A	*Prunus dulcis*	Trás-os Montes	I	GQ281793
	16C	*Prunus dulcis*	Trás-os Montes	I	
	17B	*Prunus dulcis*	Trás-os Montes	I	GQ281794
	18B	*Prunus dulcis*	Trás-os Montes	I	
	20C	*Prunus dulcis*	Trás-os Montes	I	
	21A	*Prunus dulcis*	Trás-os Montes	I	
	25A	*Prunus dulcis*	Alentejo	I	GQ281795
	25B	*Prunus dulcis*	Alentejo	I	
	29E	*Prunus dulcis*	Alentejo	I	GQ281797
	36A	*Prunus persica*	Alentejo	I	
	49A1	*Prunus dulcis*	Algarve	I	GQ281806
	49A2	*Prunus dulcis*	Algarve	I	
	51B	*Prunus dulcis*	Algarve	I	
	55A = CBS 126680	*Prunus dulcis*	Algarve	I	GQ281805
	56A	*Prunus dulcis*	Algarve	I	
	59A	*Prunus dulcis*	Algarve	I	
	59B	*Prunus dulcis*	Algarve	I	
	61A	*Prunus dulcis*	Algarve	I	
	62C1	*Prunus dulcis*	Algarve	I	GQ281802
	62C2	*Prunus dulcis*	Algarve	I	
	64A	*Prunus dulcis*	Algarve	I	GQ281803
	66A	*Prunus dulcis*	Algarve	I	
	67B	*Prunus dulcis*	Algarve	I	GQ281801
	68A	*Prunus dulcis*	Algarve	I	
	68B	*Prunus dulcis*	Algarve	I	
Diaporthe neotheicola	29C	*Prunus dulcis*	Algarve	II	GQ281796
	43B	*Prunus dulcis*	Algarve	II	GQ281798
	44A	*Prunus dulcis*	Algarve	II	GQ281799
	44B	*Prunus dulcis*	Algarve	II	GQ281800
	45B	*Prunus dulcis*	Algarve	II	GQ281809
	46A	*Prunus dulcis*	Algarve	II	GQ281808

Table 1 (continued)

Species	Isolate no.	Host	Origin	MSP-PCR Group	GenBank accession no. (ITS)[a]
	48B	*Prunus dulcis*	Algarve	II	GQ281807
	211-IF-06	*Prunus armeniaca*	Unknown	II	
	212-IF-06	*Prunus armeniaca*	Unknown	II	
Phomopsis sp.	0C	*Prunus domestica*	Açores	III	
	0N	*Prunus domestica*	Açores	III	
Phomopsis sp.	58A	*Prunus dulcis*	Algarve	IV	GQ281804

All isolates were collected and isolated by E. Diogo

[a] ITS: Internal Transcribed Spacer

[b] Ex-epitype isolate of *Phomopsis amygdali*

Results

MSP-PCR and sequence analysis

Based on the MSP-PCR profiles the isolates clustered in four groups (Fig. 1). Group I included isolates from almond and peach, obtained from all the regions sampled. Group II included isolates from almond and apricot, all from orchards in Alentejo and Algarve except those from apricot with unknown origin. The two isolates in group III were obtained from plum leaves, and isolate 58A, from almond in Algarve, was the sole isolate in group IV. Almond isolates representative of each group and geographic origin within each group were selected for phylogenetic analysis.

Amplification products and sequences ranged from 578 to 590 bp. Sequences selected from a BLAST search in GenBank were added to the sequences generated in this study together with additional GenBank sequence of *Phomopsis amygdali* and *P. theicola*. The dataset consisted of 39 ingroup taxa and two outgroup taxa and the alignment contained 504 characters including alignment gaps. Of the 504 characters, 113 were parsimony informative and included in the analysis resulting in seven equally parsimonious trees. One of these trees is represented in Fig. 2. Neighbour-Joining analysis resulted in a tree with the same topology as the MP tree except for three branches on terminal clades indicated by * in Fig. 2. Four major clades could be distinguished. Clade A (with 100% support in both NJ and MP) included sequences from *P. amygdali* isolates. Within this clade a subclade (with bootstrap values of 99% for NJ and 95% for MP) included isolates from almond in the three sampled geographic regions. Sequences of *P. amygdali* from almonds in Portugal were identical to sequences of isolates from almonds in Italy (AF102994) and Spain (AF102997) and peach in USA (U86406, AF102995 and AF102996). Clade B (with bootstrap values of 100% for NJ and 99% for MP) included the ex-epitype isolate of *Diaporthe viticola* and the ex-type isolate of *Diaporthe australafricana*. Clade C (with 100% bootstrap support for both NJ and MP) included almond isolates collected only in the South of Portugal that clustered with ex-type isolates of *P. theicola* and *Diaporthe neotheicola*. Clade D (with bootstrap values of 100% for NJ and 99% for MP) included only one almond isolate that clustered close to a reference isolate of *Diaporthe eres*. This clade also included sequences from other isolates assigned to different species.

Morphology

According to Tuset and Portilla (1989) the type specimen of *Fusicoccum amygdali* (basionym of *P. amygdali*) was deposited in herbarium PC. In the present study, we attempted to study this specimen in detail. Unfortunately, the herbarium PC was closed for renovation during the time period of this work. Therefore, we based our morphological comparisons between *P. amygdali* and the Portuguese isolates found on almond on published descriptions of this species.

Overall, the morphology of the Portuguese *P. amygdali* isolates, especially that of isolate CBS 126679 (= 3B), correlated very well with the original description of *F. amygdali* given by Delacroix (1905) as well as with the detailed description of its type specimen provided by Tuset and Portilla (1989). The pycnidial diameter range of our isolates falls within the range given by Tuset and Portilla (1989). Moreover, conidiophores have the same shape, are rarely branched and exhibit the same dimensions as described by the same authors. Alpha conidia are ovoid-ellipsoid, matching the shape and dimensions given by Delacroix (1905) and Tuset and Portilla (1989). The absence of beta conidia in the Portuguese isolates correlates with their absence in Delacroix (1905) and their scarcity reported by Tuset and Portilla (1989). In fact, these last authors did not see beta conidia in the type specimen of *F. amygdali* nor in any *in vitro* culture. These spores were only seen in a pycnidium on an almond leaf.

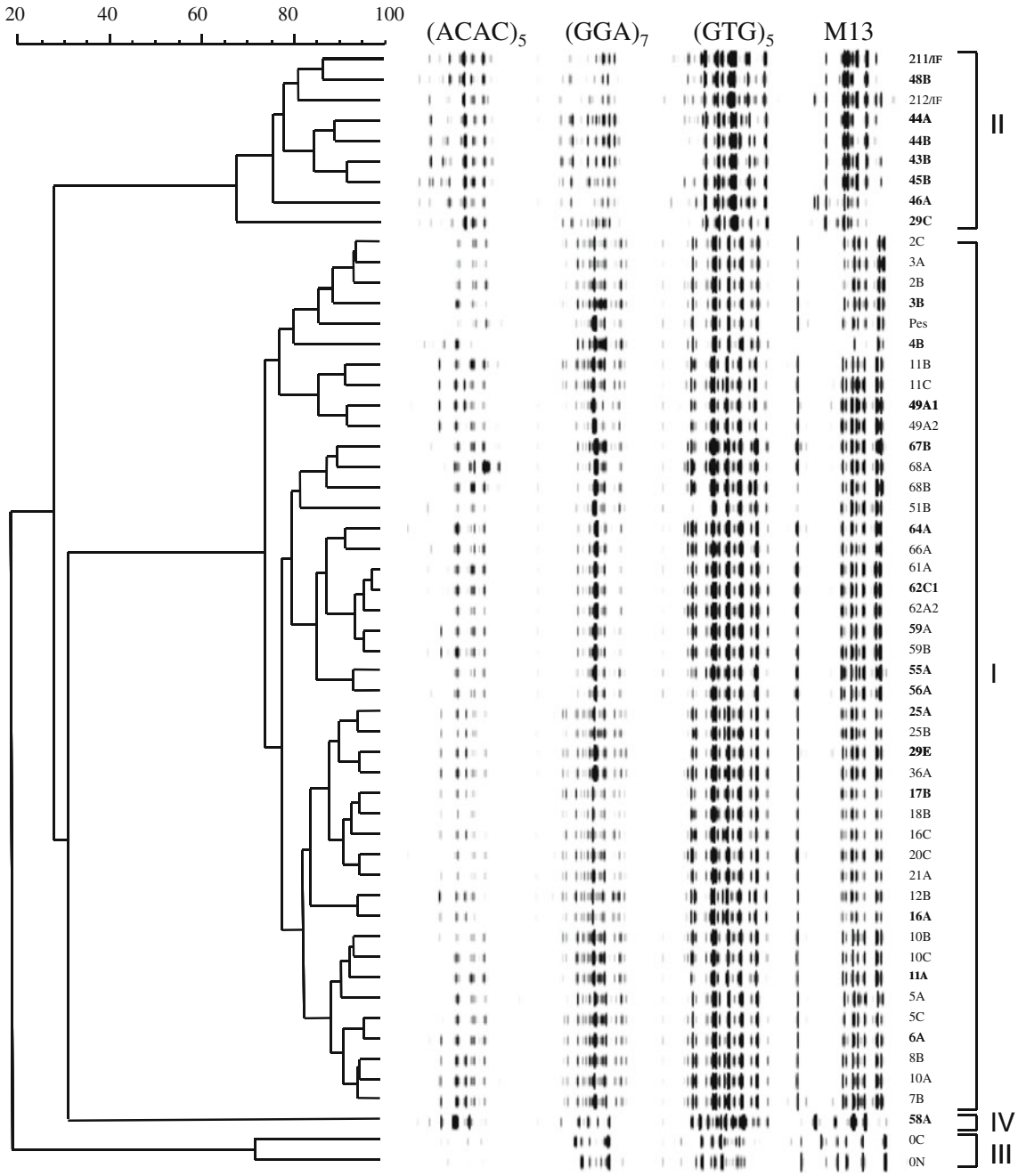

Fig. 1 Consensus dendrogram from M13, $(GTG)_5$, $(GGA)_7$ and $(ACAC)_5$ MSP-PCR profiles performed in GelCompar™ using Pearson's correlation coefficient and UPGMA. Isolates shown in bold were selected for the phylogenetic analysis. Scale bar represents percentage of similarity. Groups I, II, III and IV are referred to in the text

The *D. neotheicola* isolates obtained in the present study were morphologically identical to the type isolates and type specimen of this species as described by Santos and Phillips (2009). Pycnidia are globose to subglobose, having the same dimensions as in Santos and Phillips (2009). Conidiophores are cylindrical, septate, ranging from 9 to 30 μm in length. Conidiogenous cells are phialidic, 7–21× 1.5–3 μm. Alpha conidia are fusoid, 5.5–10.5×2–3 μm, while beta conidia are filiform, 21–32×1–1.5 μm.

Pathogenicity

The mean length of lesions observed on almond twigs inoculated with the isolates in this study are given in Table 2. The lesions observed on twigs inoculated with isolates identified as *P. amygdali* and isolate 58A were significantly longer than those caused by *D. neotheicola*, except for isolate 17B, as determined by the LSD test. All isolates identified as *P. amygdali* produced lesions in all

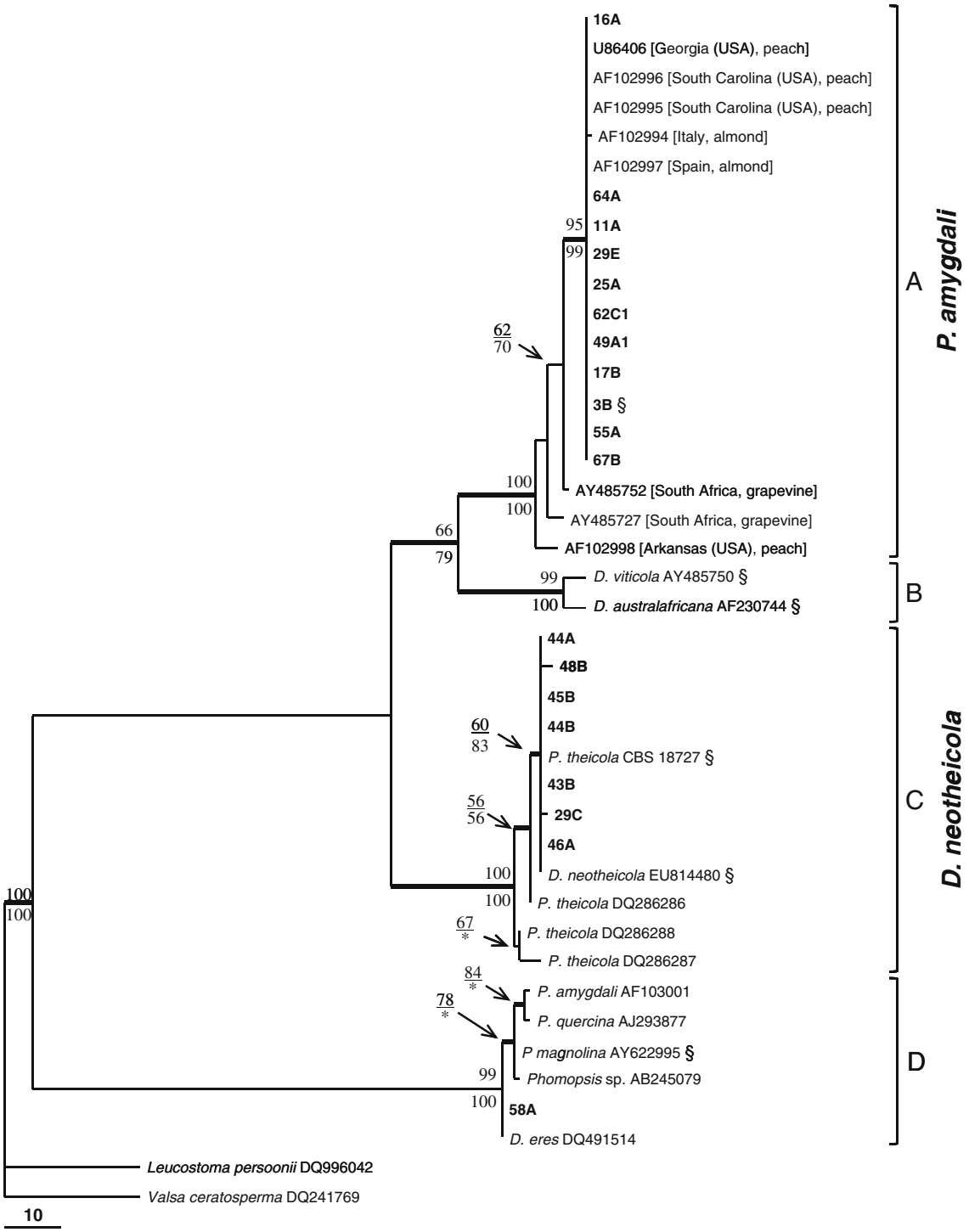

Fig. 2 One of seven equally parsimonious trees resulting from the alignment of 504 characters of the ITS rDNA region. Length=256; consistency index (CI)=0.813; retention index (RI)=0.952; homoplasy index (HI)=0.188; rescaled consistency index (RC)=0.774. Newly generated sequences are listed in bold. Bootstrap values with 1000 replications are shown above the branches for Maximum Parsimony and below the branches for Neighbour-Joining (NJ). Branches marked with * where not present in NJ tree. Thickened lines indicate branches that were present in the strict consensus tree. Bar represent 10 changes. Isolates followed by § are ex-type/ex-epitype isolates. *Valsa ceratosperma* (DQ241769) and *Leucostoma persoonii* (DQ996042) were included as outgroups. Clades A, B, C and D are referred to in the text. Phylogeny deposited in TreeBASE (S10334)

Table 2 Mean lesion length caused by *Diaporthe* and *Phomopsis* isolates on detached almond twigs cv. Ferragnès

	Isolate	Mean
P. amygdali	67B	68,2
P. amygdali	11A	60,7
P. amygdali	3B	46,8
Phomopsis sp.	58A	44,2
P. amygdali	17B	26,3
D. neotheicola	46A	24,5
D. neotheicola	45B	23,3
D. neotheicola	43B	15,7
D. neotheicola	48B	4,9
Control		0,0
LSD (P=0.05)		17,42

inoculated almond twigs. Some of the twigs inoculated with *D. neotheicola* isolates did not show any lesions.

Taxonomy

The isolates in clade A (Fig. 2) were morphologically identical to *F. amygdali* as described by Delacroix (1905) and *P. amygdali* as described by Tuset and Portilla (1989). Furthermore, they were phylogenetically indistinguishable from isolates from Italy, Spain and USA. One specimen (CBS-H 20420) was selected and designated herein as epitype.

Phomopsis amygdali (Delacr.) J.J. Tuset and M.T. Portilla, Can J Bot 67: 1280 (1989).

(Fig. 3)

MycoBank MB 518643

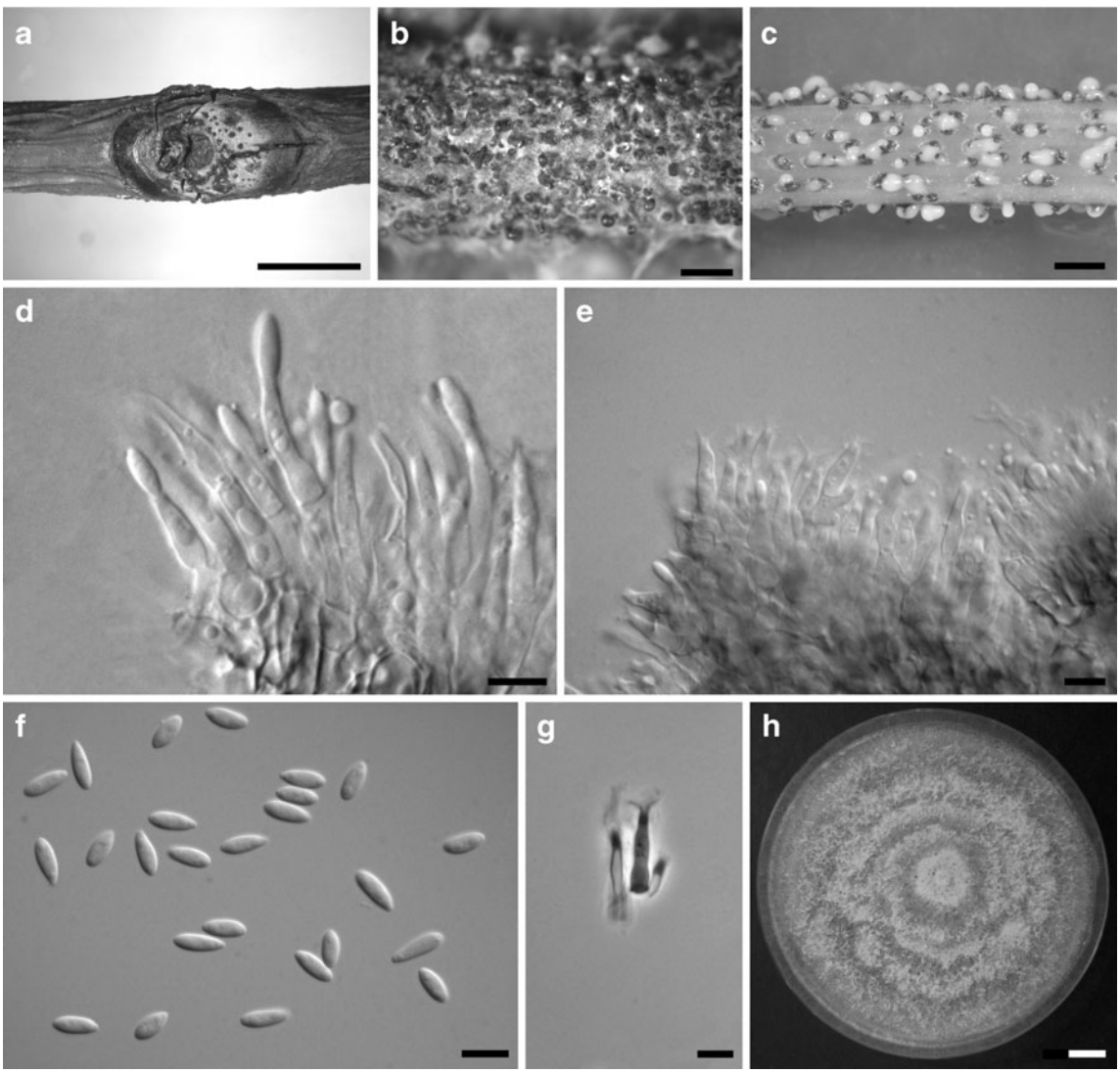

Fig. 3 *Phomopsis amygdali* CBS 126679. Pycnidia on almond twig *in vivo* (**a**) and in culture (**b**). **c**, Pycnida on alfalfa stem in culture. **d** and **e**, conidiophores. **f,** α conidia, **g**, conidiophore with prominent collarette. **h**, 10 days old culture on PDA. Bars: **a**=5 mm; **b**, **c**=1 mm; **d**, **e**, **f** , **g**=5 μm; **h**=1 cm

Basionym: *Fusicoccum amygdali* Delacr., Bull Trimest Soc Mycol Fr, 21 (3): 184 (1905).

≡ *Phomopsis amygdalina* Canonaco, Riv Pat Veget 26: 157 (1936).

Teleomorph: Unknown.

Conidiomata eustromatic, subglobose to ampuliform, subepidermal, erumpent, dark brown to black, 240–390 μm wide× 140–160 μm tall on host. In culture 160–220 μm wide×120–300 μm tall. Conidia exuding from the pycnidia in white to cream drops. *Conidiophores* subcylindrical, hyaline, seldom branched, 7.4–36.3×1.5–3.2 μm, $\bar{x}$ ± S.D.=14.5±4.16×2.3± 0.36 μm (*n*=380). *Conidiogenous cells* phialidic, cylindrical, tapering toward the apex, periclinal thickening and collarette present, 5.0–20.0×1.5–3.2 μm, $\bar{x}$ ± S.D.=9.8±2.29×2.3± 0.36 μm (*n*=380). *Alpha conidia* ovoid-ellipsoid, mostly with one end obtuse and the other acute, 0–2 guttulate, (4.18–)6.27–6.32(–9.64)×(1.63–)2.36–2.38(–3.31) μm, $\bar{x}$ ± S.D.=6.3± 0.63×2.37±0.22 μm (*n*=2100). *Beta conidia* not seen.

Colonies on PDA reaching a diameter of 43.5 mm after 3 days at 25°C in the dark. Colonies white, cottony, with raised margins, becoming pale brown, reverse pale brown with brown patches.

Habitat On branches and fruits of *Prunus armeniaca* (Garofalo 1973), *P. dulcis* (Adaskaveg et al. 1999), *P. persica* (Farr et al. 1999) and *Vitis vinifera* (Mostert et al. 2001).

Known distribution France (Delacroix 1905), Greece (Pantidou 1973), Italy (Canonaco 1936; Garofalo 1973), Japan (Kanematsu et al. 1999), Portugal (Dias et al. 1982), South Africa (Mostert et al. 2001), Spain (Tuset and Portilla 1989), Tunisia (Trigui 1968) and USA (Adaskaveg et al. 1999; Farr et al. 1999).

Material examined PORTUGAL: Trás-os-Montes, Mirandela, September 2005, *E. Diogo*, (CBS-H 20420, **EPITYPE** designated herein; culture ex-epitype 3B = CBS 126679). See Table 1 for other isolates studied.

Notes Beta conidia were not seen in any of the isolates. Tuset and Portilla (1989) observed beta conidia in pycnidia on the host but never saw them in culture. However, these authors were unable to find beta conidia in the type specimen. This species differs from *Diaporthe neotheicola* in having consistently shorter alpha conidia and white colonies becoming pale brown instead of olivaceous green.

Discussion

Sequence analyses based on the ITS rDNA region revealed three species of *Diaporthe* and *Phomopsis* on almonds in Portugal. These three species correlated perfectly with the groups revealed in the MSP-PCR profiles. Although ITS is the locus most commonly used to infer phylogenies in fungi (Nilsson et al. 2008) it was recently demonstrated that this region of the ribosomal gene cluster shows high within species variation in some *Diaporthe*/*Phomopsis* species (Santos et al. 2010). Nevertheless, ITS can still be used to distinguish species in this genus provided the phylogenies are interpreted with care. Indeed, as shown in this paper, there was little within species variation in *P. amygdali* (group I in Fig. 1 and clade A in Fig. 2).

By far the largest number of isolates from almonds resided in a clade with isolates previously identified as *P. amygdali*. These isolates were collected from widely separate geographical regions including Georgia and South Carolina (Southern USA), Italy and Spain. Isolates in this clade were the most virulent ones in the pathogenicity tests and morphologically all the isolates in this clade that we studied corresponded with the original description of *F. amygdali* (Delacroix 1905) and the detailed description of the type specimen given by Tuset and Portilla (1989). Although the type of *F. amygdali* is housed in PC, it was not available during the course of this work. However, this specimen was examined by Tuset and Portilla (1989) who provided a detailed description. Since no ex-type cultures of *P. amygdali* exist we proposed CBS-H 20420 as epitype with CBS 126679 as ex-epitype culture. Ideally an epitype should be derived from the same host and same locality as the holotype (Hyde & Zhang 2008). However, since ITS sequences of *P. amygdali* from almonds in Italy, Portugal and Spain are identical, and the isolates from Portugal are indistinguishable morphologically from the type we felt justified in proposing the specimen from Portugal as epitype. In a similar way, Phillips et al. (2006) found that ITS sequences of two isolates of *Botryosphaeria corticis* (Demaree & M.S. Wilcox) Arx & E. Müll. from North Carolina (the type locality of this species) were identical to isolates from New Jersey. Since this latter collection from New Jersey was morphologically identical to the type it was designated epitype.

Phomopsis amygdali is not restricted to peach and almond but has been isolated, albeit infrequently, from grapevines in South Africa (van Niekerk et al. 2005). ITS sequences of the two isolates from grapevines, and a further isolate from peach from Arkanas, USA, difffered slightly from the typical isolates of *P. amygdali*. These differences would suggest that recombination had ocurred at some time further suggesting that a sexual state of this species may exist although none has been reported for *P. amygdali* in nature. However, Kanematsu et al. (2000) reported succesful mating between *Diaporthe* G-type and *P. amygdali* isolates in culture. Furthermore, Santos et al. (2010) showed that these two taxa may be the same

phylogenetic species. Taking together these two observations it seems that compatible isolates of *P. amygdali* are able to mate, although, for some reason, this rarely happens in nature.

Diaporthe neotheicola (group II in Fig. 1 and clade C in Fig. 2) was identified by association with the ex-type isolate CBS 123208 as well as with the ex-type isolate of *Phomopsis theicola* (CBS 187.27). As far as we know, this is the first report of *D. neotheicola* on *Prunus dulcis* and *Prunus armeniaca*. This species was first described as *P. theicola* on *Camellia sinensis* in Italy (Curzi 1927) but has since been isolated from several different hosts including *Vitis vinifera, Protea*, *Pyrus* (Mostert et al. 2001; van Niekerk et al. 2005), *Aspalathus linearis* (van Rensburg et al. 2006) and recently from *Foeniculum vulgare* in Portugal (Santos and Phillips 2009). The pathogenicity results showed that *D. neotheicola* is probably a weak pathogen on *P. dulcis*, which confirms previous studies with different hosts (van Niekerk et al. 2005; van Rensburg et al. 2006; Santos and Phillips 2009). The results obtained in the present work further confirm the previous findings that host affiliation is an unreliable character for identification of *Diaporthe*/*Phomopsis* species (Farr et al. 2002; Mostert et al. 2001; Rehner and Uecker 1994).

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