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The complex of *Diplodia* species associated with *Fraxinus* and some other woody hosts in Italy and Portugal

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Abstract Studies on the taxonomy and phylogeny of *Diplodia* have been hampered by the lack of an ex-type culture linked to the holotype of *D. mutila*, which is the type of the genus. In this study a large collection of *Diplodia* strains, obtained from ash and other woody hosts showing V-shaped cankers and branch dieback, were identified based on morphological characters and DNA sequence data from ITS and EF1-α loci. Results of combined morphological and phylogenetic analyses showed that the *Fraxinus* isolates from Italy, the Netherlands, Portugal and Spain belong to three distinct species namely *Diplodia fraxini*, *Diplodia mutila* and *Diplodia subglobosa* sp. nov. An epitype was designated for *Diplodia mutila*, with associated ex-epitype cultures. The name *D. fraxini* is re-instated and a neotype designated. Two species, *Diplodia seriata* and *Diplodia pseudoseriata* were reported for the first time on *Fraxinus* spp.

Keywords *Botryosphaeriaceae* · Epitype · Neotype · Phylogeny · Systematics · Taxonomy

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Introduction

Fraxinus L. (ash) is a tree genus native to the temperate and subtropical regions of the Northern Hemisphere. It belongs to the family *Oleaceae* and includes 43 species, most of which are large or medium-sized trees, with some shrub species widespread in dry areas (Wallander 2008). Three species, *F. angustifolia* Vahl (narrow leaved ash), *F. excelsior* L. (European ash) and *F. ornus* L. (manna ash) are widely planted as ornamentals.

Since the early 1990s severe branch dieback of *F. excelsior* was observed in different countries of Central, Eastern and Northern Europe (Przybyl 2002; Lygis et al. 2005; Pukacki and Przybyl 2005; Bakys et al. 2009). All of these studies demonstrated the occurrence of several pathogenic fungi from necrotic shoots of ash. Among these, *Diplodia mutila* (Fr.) Fr. was one of the most consistently detected species. This pathogen was also reported associated with cankers and branch dieback of *F. ornus* in Sicily (Italy) (Sidoti and Granata 2004).

In recent years, during surveys carried out in Portugal and Sardinia (Italy) aimed at clarifying the causes of a decline affecting *Fraxinus* spp. in urban and natural areas, a large collection of *Diplodia mutila*-like strains were isolated from trees showing V-shaped cankers and a progressive dieback of shoots and branches. Although morphologically similar to *D. mutila*, some of these strains differed in their colony morphology, larger conidia and DNA sequence data (ITS and EF1-α) from other known strains of *D. mutila*. This species has been reported from a wide range of hosts of agricultural and forestry importance, where it has been associated with canker, dieback and fruit rot symptoms (Farr and Rossman 2013). However, there are conflicting reports regarding its pathogenicity, and in particular, on *Fraxinus* spp. (Przybyl 2002; Sidoti and Granata 2004; Bakys et al. 2009), which may be a result of differences in strain virulence but may also be due to the existence of cryptic species. Cryptic

speciation is common in the family *Botryosphaeriaceae* and in the genus *Diplodia*, which renders species identification difficult when based solely on morphological characters (Phillips et al. 2012, 2013). Fries (1823) described the species *Sphaeria fraxini* Fr. on *Fraxinus* sp., and later (Fries 1849) he transferred it to *Diplodia* as *Diplodia fraxini* (Fr. : Fr.) Fr. Subsequently, this fungus was placed by Saccardo (1884) in the genus *Botryodiplodia* (Sacc.) Sacc. as *B. fraxini* (Fr. : Fr.) Sacc. Unfortunately, no ex-type cultures are available for this species.

Currently, 17 *Diplodia* species are known from culture (Phillips et al. 2013). These species have been recognised mainly on the basis of DNA sequence data (single or multilocus) and minor differences in conidial morphology (de Wet et al. 2003; Alves et al. 2004, 2006; Gure et al. 2005; Damm et al. 2007; Lazzizera et al. 2008; Pérez et al. 2010; Jami et al. 2012; Phillips et al. 2012, 2013; Linaldeddu et al. 2013; Lynch et al. 2013). For the majority of species there are ex-type or ex-epitype cultures deposited in publicly available culture collections that can serve as standards for the morphological and molecular characterisation of the species. The only exception is *D. mutila* for which several cultures are available but none has been linked to the type of the species. The lack of an ex-type culture, or other cultures linked to the holotype of *D. mutila* has hampered taxonomic studies on the genus *Diplodia* (which is typified by *D. mutila*), especially those based on DNA sequence data.

Therefore, the main aims of this work were: 1) to characterise collections of *D. mutila*-like and other *Diplodia* spp. isolates in terms of morphological and phylogenetic relationships to known *Diplodia* species; 2) to select a suitable epitype specimen for *D. mutila* and a neotype specimen for *D. fraxini* with related cultures that can be made available for future studies.

Materials and methods

Isolates and morphology

Diplodia isolates used in this study were obtained from branches of *F. angustifolia* and *F. ornus* showing sunken cankers and dieback. *Diplodia* species isolated from other symptomatic trees including *Populus alba* L. (white poplar), *Cupressus sempervirens* L. (Italian cypress) and *Quercus coccifera* L. (kermes oak) were also included in this study. Isolations were made from chips of inner bark and wood tissues approx. 5 mm^2 cut aseptically from the margin of necrotic lesions or directly from pycnidia. All samples were cultured on potato dextrose agar (PDA, Oxoid Ltd.) in Petri dishes. After incubation at 25 °C for 1 week, colonies were sub-cultured onto half-strength PDA (1/2 PDA) or on water agar supplemented with autoclaved poplar twigs to enhance sporulation. All colonies were kept on the laboratory bench at about 20–25 °C where they received diffused daylight.

Monoconidial cultures were obtained by spreading conidia on the surface of PDA and incubating overnight at 25 °C. Single germinating spores were transferred to fresh plates of PDA. Cardinal temperatures for growth were determined on PDA plates (90 mm) incubated at 5, 10, 15, 20, 25, 30, 35 and 40 °C (±0.5 °C) in the dark. Five replicate plates for each isolate were made and colony diameters were measured after 4 days.

For microscopy, the contents of conidiomata were dissected out and mounted in 100 % lactic acid. For observations of conidiogenesis, the conidiogenous layer was dissected out and mounted in 100 % lactic acid. Measurements of conidia were made with the Leica IM 500 measurement module from images recorded on a Leica DFC 320 digital camera. From measurements of 50 conidia the mean, standard deviation and 95 % confidence intervals were calculated. Conidial dimensions are given as the range of dimensions with extremes in parentheses. Dimensions of other structures are given as the range of at least 20 measurements.

Representative isolates were deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands and nomenclatural data in MycoBank (Crous et al. 2004). Specimens were lodged with the herbarium of Estação Agronómica Nacional, Oeiras, Portugal (LISE). Isolates used for phylogenetic analyses in this study are provided (Table 1).

DNA extraction, PCR amplification and sequencing

DNA was isolated from fungal mycelium by the method of Santos and Phillips (2009). Procedures and protocols for DNA sequencing were as described by Alves et al. (2004). PCR reactions were carried out with Taq polymerase, nucleotides and buffers supplied by MBI Fermentas (Vilnius, Lithuania) and PCR reaction mixtures were prepared according to Alves et al. (2004), with the addition of 5 % DMSO to improve the amplification of some difficult DNA templates. All primers were synthesised by MWG Biotech AG (Elbersberg, Germany). The ITS region was amplified using the primers ITS1 and ITS4 (White et al. 1990) as described by Alves et al. (2004). The primers EF1-728F and EF1-986R (Carbone and Kohn 1999) were used to amplify part of the translation elongation factor 1-α gene (EF1-α) as described by Alves et al. (2006). The amplified PCR products were purified with the JETQUICK PCR Purification Spin Kit (GENOMED, Löhne, Germany). The PCR products were sequenced by STAB Vida Lda (Portugal).

Phylogenetic analysis

The ITS and EF1-α sequences were combined and the dataset, including sequences of other *Diplodia* species downloaded

Table 1 Isolates used in this study

Isolate number[a]	Species	Host	Locality	Collector	GenBank	
					ITS[b]	EF
CBS 120835[c]	*Diplodia africana*	*Prunus persica*	Paarl, South Africa	U. Damm	*EF445343*	*EF445382*
CBS 121104	*D. africana*	*Prunus persica*	Paarl, South Africa	U. Damm	*EF445344*	*EF445383*
DA1	*D. africana*	*Juniperus phoenicea*	Caprera, Sardinia, Italy	B.T. Linaldeddu	*JF302648*	*JN157807*
CBS 132777	*D. agrifolia*	*Quercus agrifolia*	San Diego Co. CA, USA	S.C. Lynch & A. Eskalen	*JN693507*	*JQ517317*
UCROK1429	*D. agrifolia*	*Quercus agrifolia*	San Diego Co. CA, USA	S.C. Lynch & A. Eskalen	*JQ411412*	*JQ512121*
CBS 124931	*D. alatafructa*	*Pterocarpus angolensis*	Sudwala Caves area, South Africa	J. Mehl & J. Roux	*FJ888460*	*FJ888444*
CBS 124933	*D. alatafructa*	*Pterocarpus angolensis*	Sudwala Caves area, South Africa	J. Mehl & J. Roux	*FJ888478*	*FJ888446*
CBS 130408	*D. allocellula*	*Acacia karroo*	Pretoria, South Africa	F. Jami & M. Gryzenhout	*JQ239397*	*JQ239384*
CBS 130410	*D. allocellula*	*Acacia karroo*	Pretoria, South Africa	F. Jami & M. Gryzenhout	*JQ239399*	*JQ239386*
CBS 124254	*D. bulgarica*	*Malus sylvestris*	Plovdiv, Bulgaria	S. Bobev	*GQ923853*	*GQ923821*
CBS 124135	*D. bulgarica*	*Malus sylvestris*	Plovdiv, Bulgaria	S. Bobev	*GQ923852*	*GQ923820*
CBS 112549	*D. corticola*	*Quercus suber*	Aveiro, Portugal	A. Alves	*AY259100*	*AY573227*
CBS 112547	*D. corticola*	*Quercus ilex*	Córdoba, Spain	M. E. Sanchez, A. Trapero	*AY259110*	*DQ458872*
BL7	*D. corticola*	*Quercus afares*	Aïn Draham, Tunisia	B.T. Linaldeddu	*JX894190*	*JX894209*
BL11	*D. corticola*	*Quercus ilex*	Caprera, Sardinia, Italy	B.T. Linaldeddu	*JX894192*	*JX894211*
CBS 168.87	*D. cupressi*	*Cupressus sempervirens*	Bet Dagan, Israel	Z. Solel	*DQ458893*	*DQ458878*
CBS 261.85	*D. cupressi*	*Cupressus sempervirens*	Bet Dagan, Israel	Z. Solel	*DQ458894*	*DQ458879*
BL102	*D. cupressi*	*Cupressus sempervirens*	Carthage, Tunisia	B.T. Linaldeddu	KF307722	KF318769
CBS 431.82	*D. fraxini*	*Fraxinus excelsior*	Maarseveen, Netherlands	H.A. van der Aa	*AY236955*	*AY236904*
CBS 136013	*D. fraxini*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307710	KF318757
CBS 136011	*D. fraxini*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307711	KF318758
BL135	*D. fraxini*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307712	KF318759
BL136	*D. fraxini*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307713	KF318760
BL137	*D. fraxini*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307714	KF318761
BL138	*D. fraxini*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307715	KF318762
CBS 136010	*D. fraxini*	*Fraxinus angustifolia*	Monte da Caparica, Portugal	A. Deidda	KF307700	KF318747
CAD002	*D. fraxini*	*Fraxinus angustifolia*	Monte da Caparica, Portugal	A. Deidda	KF307701	KF318748
CAD003	*D. fraxini*	*Fraxinus angustifolia*	Monte da Caparica, Portugal	A. Deidda	KF307702	KF318749
CAD004	*D. fraxini*	*Fraxinus angustifolia*	Monte da Caparica, Portugal	A. Deidda	KF307703	KF318750
CAD005	*D. fraxini*	*Fraxinus angustifolia*	Lisbon, Portugal	A. Deidda	KF307704	KF318751
CAD006	*D. fraxini*	*Fraxinus angustifolia*	Lisbon, Portugal	A. Deidda	KF307705	KF318752
CAD007	*D. fraxini*	*Fraxinus angustifolia*	Beja, Portugal	A. Deidda	KF307706	KF318753
CAD008	*D. fraxini*	*Fraxinus angustifolia*	Beja, Portugal	A. Deidda	KF307707	KF318754
CAD009	*D. fraxini*	*Fraxinus angustifolia*	Cascais, Portugal	A. Deidda	KF307708	KF318755
CBS 136012	*D. fraxini*	*Fraxinus angustifolia*	Cascais, Portugal	A. Deidda	KF307709	KF318756
CBS 112556	*D. intermedia*	*Malus sylvestris*	Monte da Caparica, Portugal	A.J.L. Phillips	*AY259096*	*GQ923851*
CAA147	*D. intermedia*	*Malus domestica* (fruit rot)	Aveiro, Portugal	A. Alves	*GQ923857*	*GQ923825*
CBS 124130	*D. malorum*	*Malus sylvestris*	Monte da Caparica, Portugal	A.J.L. Phillips	*GQ923865*	*GQ923833*
CBS 112554	*D. malorum*	*Malus sylvestris*	Monte da Caparica, Portugal	A.J.L. Phillips	*AY259095*	*DQ458870*
BL126	*D. malorum*	*Populus alba*	Sardinia, Italy	B.T. Linaldeddu	KF307716	KF318763
BL127	*D. malorum*	*Populus alba*	Sardinia, Italy	B.T. Linaldeddu	KF307717	KF318764
CBS 302.36	*D. mutila* (as *Physalospora mutila*)	*Fraxinus excelsior*	Saltash, England	N.E. Stevens	KJ361841	KJ361833
CBS 112553	*D. mutila*	*Vitis vinifera*	Montemor-o-Novo, Portugal	A.J.L. Phillips	*AY259093*	*AY573219*
CAA096	*D. mutila*	*Taxus baccata*	Aveiro, Portugal	A. Alves	*JX878523*	*KJ361834*
CAA115	*D. mutila*	*Chamaecyparis lawsoniana*	Aveiro, Portugal	A. Alves	*JX878524*	KJ361835
CBS 136014	*D. mutila*	*Populus alba*	Aveiro, Portugal	A. Alves	KJ361837	KJ361829
CBS 136015	*D. mutila*	*Populus alba*	Aveiro, Portugal	A. Alves	KJ361838	KJ361830
CBS 136016	*D. mutila*	*Fraxinus ornus*	Aveiro, Portugal	A. Alves	KJ361839	KJ361831

Table 1 (continued)

Isolate number[a]	Species	Host	Locality	Collector	GenBank	
					ITS[b]	EF
CBS 136017	*D. mutila*	*Fraxinus ornus*	Aveiro, Portugal	A. Alves	KJ361840	KJ361832
CBS 230.30	*D. mutila*	*Phoenix dactylifera*	California, USA	L. L. Huillier	*DQ458886*	*DQ458869*
BL98	*D. mutila*	*Vitis vinifera*	Sardinia, Italy	A. Deidda	KF307718	KF318765
PD46	*D. mutila*	unknown	OR, USA	T.J. Michailides	*GU251116*	*GU251248*
PD61	*D. mutila*	*Persea americana*	Ventura Co., CA, USA	T.J. Michailides	*GU251117*	*GU251249*
PD73	*D. mutila*	*Ilex* sp.	CA, USA	T.J. Michailides	*GU251118*	*GU251250*
PD75	*D. mutila*	*Ilex* sp.	CA, USA	T.J. Michailides	*GU251119*	*GU251251*
STE-U5038	*D. mutila*	*Vitis vinifera*	Portugal	A.J.L. Phillips	*AY343484*	*AY343370*
STE-U5824	*D. mutila*	*Prunus salicina*	Paarl, South Africa	U. Damm	*EF445346*	*EF445381*
UCD288Ma	*D. mutila*	*Vitis vinifera*	Madera Co., CA, USA	J.R. Úrbez-Torres	*DQ008313*	*EU012411*
CBS 121887	*D. olivarum*	*Olea europaea*	Puglia, Italy	S. Frisullo	*EU392302*	*EU392279*
CAP301	*D. olivarum*	*Ceratonia siliqua*	Sicily, Italy	A. Sidoti	*GQ923873*	*GQ923841*
BL97	*D. olivarum*	*Quercus coccifera*	Hammamet, Tunisia	B.T. Linaldeddu	KF307719	KF318766
CBS 109725	*D. sapinea*	*Pinus patula*	Habinsaran, Indonesia	M.J. Wingfield	*DQ458896*	*DQ458881*
CBS 109727	*D. sapinea*	*Pinus radiata*	Stellenbosch, South Africa	W.J. Swart	*DQ458897*	*DQ458882*
CBS 393.84	*D. sapinea*	*Pinus nigra*	Putten, Netherlands	H.A. van der Aa	*DQ458895*	*DQ458880*
CBS 109943	*D. sapinea*	*Pinus patula*	Indonesia	M.J. Wingfield	*DQ458898*	*DQ458883*
CBS 124906	*D. pseudoseriata*	*Blepharocalyx salicifolius*	Uruguay	C. Pérez	*EU080927*	*EU863181*
UY1263	*D. pseudoseriata*	*Myrciaria tenella*	Uruguay	C. Pérez	*EU080933*	*EU863182*
BL132	*D. pseudoseriata*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307720	KF318767
BL133	*D. pseudoseriata*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307721	KF318768
CBS 133852	*D. quercivora*	*Quercus canariensis*	Tabarka, Tunisia	B.T. Linaldeddu	*JX894205*	*JX894229*
CBS 133853	*D. quercivora*	*Quercus canariensis*	Tabarka, Tunisia	B.T. Linaldeddu	*JX894206*	*JX894230*
CBS 116470	*D. rosulata*	*Prunus africana*	Gambo, Ethiopia	A. Gure	*EU430265*	*EU430267*
CBS 116472	*D. rosulata*	*Prunus africana*	Gambo, Ethiopia	A. Gure	*EU430266*	*EU430268*
CBS 109944	*D. scrobiculata*	*Pinus greggii*	Mexico	M.J. Wingfield	*DQ458899*	*DQ458884*
CBS 113423	*D. scrobiculata*	*Pinus greggii*	Mexico	M.J. Wingfield	*DQ458900*	*DQ458885*
CAP163	*D. scrobiculata*	*Olea europaea*	Puglia, Italy	S. Frisullo	*EU392283*	*EU392260*
BL5	*D. scrobiculata*	*Arbutus unedo*	Sardinia, Italy	B.T. Linaldeddu	*GU722102*	*JX894231*
CBS 112555	*D. seriata*	*Vitis vinifera*	Montemor-o-Novo, Portugal	A.J.L. Phillips	*AY259093*	*AY573219*
CBS 119049	*D. seriata*	*Vitis vinifera*	Italy	L. Mugnai	*DQ458889*	*DQ458874*
CAA502	*D. seriata*	*Fraxinus ornus*	Aveiro, Portugal	A. Alves	KJ361842	KJ361836
BL130	*D. seriata*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307723	KF318770
BL131	*D. seriata*	*Fraxinus angustifolia*	Sardinia, Italy	B.T. Linaldeddu	KF307724	KF318771
CBS 124131	*D. subglobosa*	*Fraxinus ornus*	Sicily, Italy	A. Sidoti	*GQ923855*	*GQ923823*
CBS 124132	*D. subglobosa*	*Fraxinus excelsior*	Cataluna, Spain	J. Luque	*DQ458887*	*DQ458871*
CBS 124133	*D. subglobosa*	*Lonicera nigra*	Cataluna, Spain	J. Luque	*GQ923856*	*GQ923824*
CMW7776	*D. subglobosa*	*Fraxinus excelsior*	Italy	B. Slippers	*AY972106*	*DQ280420*
CBS 418.64	*D. tsugae*	*Tsuga heterophylla*	British Columbia, Canada	A. Funk	*DQ458888*	*DQ458873*
CBS 124.13	*Lasiodiplodia theobromae*	unknown	USA	J.J. Taubenhaus	*DQ458890*	*DQ458875*
CBS 164.96	*L. theobromae*	Fruit along coral reef coast	New Guinea	A. Aptroot	*AY640255*	*AY640258*

[a] Acronyms of culture collections: BL, B.T. Linaldeddu, Università degli Studi di Sassari, Italy; CAA, A. Alves, Universidade de Aveiro, Portugal; CAD, A. Deidda, Università degli Studi di Sassari, Italy; CAP, A.J.L. Phillips, Universidade Nova de Lisboa, Portugal; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW, M.J. Wingfield, FABI, University of Pretoria, South Africa; PD, Department of Plant Pathology, University of California, Davis; STE-U, University of Stellenbosch, South Africa; UCD, Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California, Davis, USA; UCROK, Department of Plant Pathology and Microbiology, University of California, Riverside; UY, Department of Plant Pathology, University of Minnesota

[b] Sequence numbers in italics were retrieved from GenBank. All others were obtained in the present study

[c] Ex-type strains in bold face

from GenBank, was compiled with the outgroup *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (Table 1). Sequences were aligned with ClustalX v. 1.83 (Thompson et al. 1997), 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 %). Alignments were checked and manual adjustments were made where necessary.

Phylogenetic analyses of sequence data were done using PAUP* v.4.0b10 (Swofford 2003) for Maximum-parsimony (MP) analyses and Mr Bayes v.3.0b4 (Ronquist and Huelsenbeck 2003) for Bayesian Inference (BI) analyses. The general time-reversible model of evolution (Rodriguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+Γ+G) was used for BI analyses. Trees were visualized with TreeView (Page 1996).

Maximum-parsimony analyses were performed using the heuristic search option with 1,000 random taxon additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as fifth character. Maxtrees were set to 500, branches of zero length were collapsed, and all multiple equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1,000 bootstrap replications (Hillis and Bull 1993). Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI).

Bayesian analyses employing a Markov Chain Monte Carlo method were performed. Four MCMC chains were run simultaneously, starting from random trees for 1,000,000 generations. Trees were sampled every 100th generation for a total of 10,000 trees. The first 1,000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 9,000 trees. This analysis was repeated three times starting from different random trees to ensure trees from the same tree space were sampled during each analysis.

A comparison of highly supported clades (bootstrap support values≥70 %) among trees generated from MP analyses of individual data sets was performed in order to detect conflict between individual phylogenies (Alves et al. 2008).

Results

DNA phylogeny

Approximately 550 and 300 bases were determined for ITS and EF1-α respectively. New sequences were deposited in GenBank (Table 1) and the alignment in TreeBase (14460). Individual gene phylogenies revealed no major conflicts thus indicating that the two loci could be combined. The combined ITS and EF1-α dataset consisted of 853 characters (including alignment gaps) for 87 ingroup and 2 outgroup taxa. Of the 853 characters 165 were excluded due to ambiguous alignment, 538 were constant and 9 were variable and parsimony-uninformative. Maximum parsimony analysis of the remaining 141 parsimony-informative characters resulted in 279 most parsimonious trees of 302 steps (CI=0.717, RI=0.945, HI=0.283) and one is shown in Fig. 1.

In the phylogenetic analysis four main clades (labeled 1 to 4) were resolved in the ingroup (Fig. 1). These clades are characterized by distinct conidial morphological features as explained in detail by Phillips et al. (2012, 2013). Within these four main clades, 19 sub-clades corresponding to species were recognized; of which 17 represent known *Diplodia* species (Fig. 1). The *D. mutila*-like isolates studied were distributed into 5 sub-clades within clade 1. Thus, two isolates from *P. alba* were identified as *Diplodia malorum* Fuckel; one isolate from *Q. coccifera* was identified as *Diplodia olivarum* A.J.L. Phillips, Frisullo & Lazzizera and a set of isolates from *P. alba*, *F. ornus* and *Vitis vinifera* L. (grapevine) clustered in a larger group identified as *D. mutila*. For one of the remaining two clades the name *Diplodia fraxini* (Fr. : Fr.) Fr. was deemed to be appropriate and this name is reinstated for a set of isolates obtained from *F. excelsior* and *F. angustifolia*. The second clade containing isolates from *F. excelsior*, *F. ornus* and *Lonicera nigra* L. (black-berried honeysuckle) represents a previously unrecognized species, which is described here as *Diplodia subglobosa* sp. nov.

The remaining isolates, morphologically distinct from *D. mutila*, were distributed amongst clades 2 and 3 and were identified as *Diplodia cupressi* A.J.L. Phillips & A. Alves (one isolate from *C. sempervirens*), *Diplodia seriata* De Not. (one isolate from *F. ornus* and two isolates from *F. excelsior*) and *Diplodia pseudoseriata* C.A. Pérez, Blanchette, Slippers & M.J. Wingf. (two isolates from *F. angustifolia*).

Taxonomy

Diplodia Fr., in Montagne, Ann. Sci. Nat., Bot., 2e Sér., 1: 302. 1834.

MycoBank: MB8047

Diplodia fraxini (Fr. : Fr.) Fr., Summa Veg. Scand. 2: 417. 1849.

MycoBank: MB247549 (Fig. 2)

≡ *Sphaeria fraxini* Fr. : Fr., Syst. Mycol. 2: 493. 1823.

≡ *Botryodiplodia fraxini* (Fr. : Fr.) Sacc., Syll. Fung. 3: 378. 1884.

Ascomata not seen. *Conidiomata* stromatic, pycnidial, solitary to aggregated, immersed, partially erumpent when mature, dark brown to black, globose, up to 600 μm diam., wall

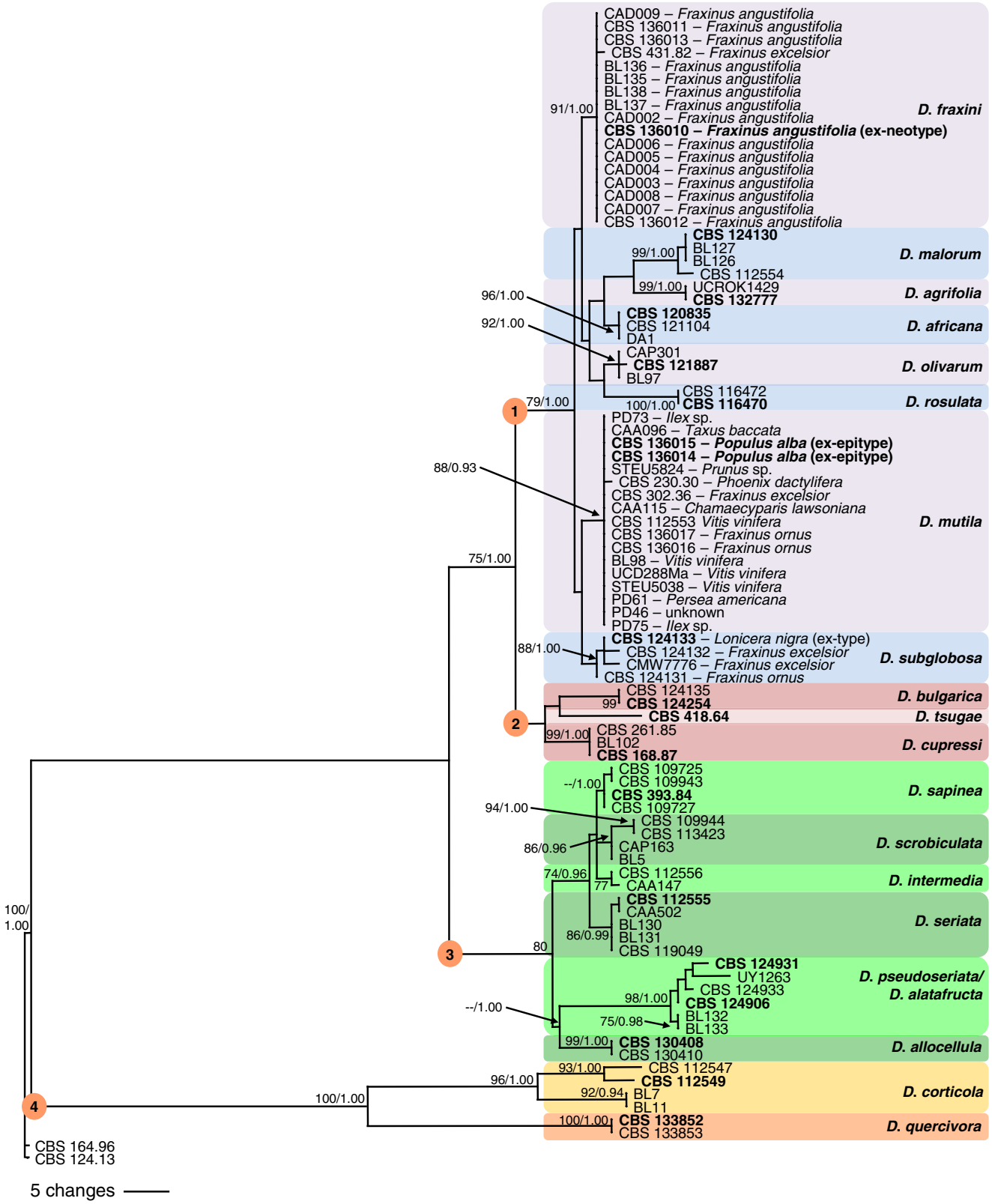

Fig. 1 One of the 279 most parsimonious trees resulting from the combined analysis of ITS and EF1-α sequence data. Bootstrap support and posterior probability values are given at the nodes. The tree was rooted to *Lasiodiplodia theobromae*. Ex-type isolates are in *bold*. The *bar* shows five changes

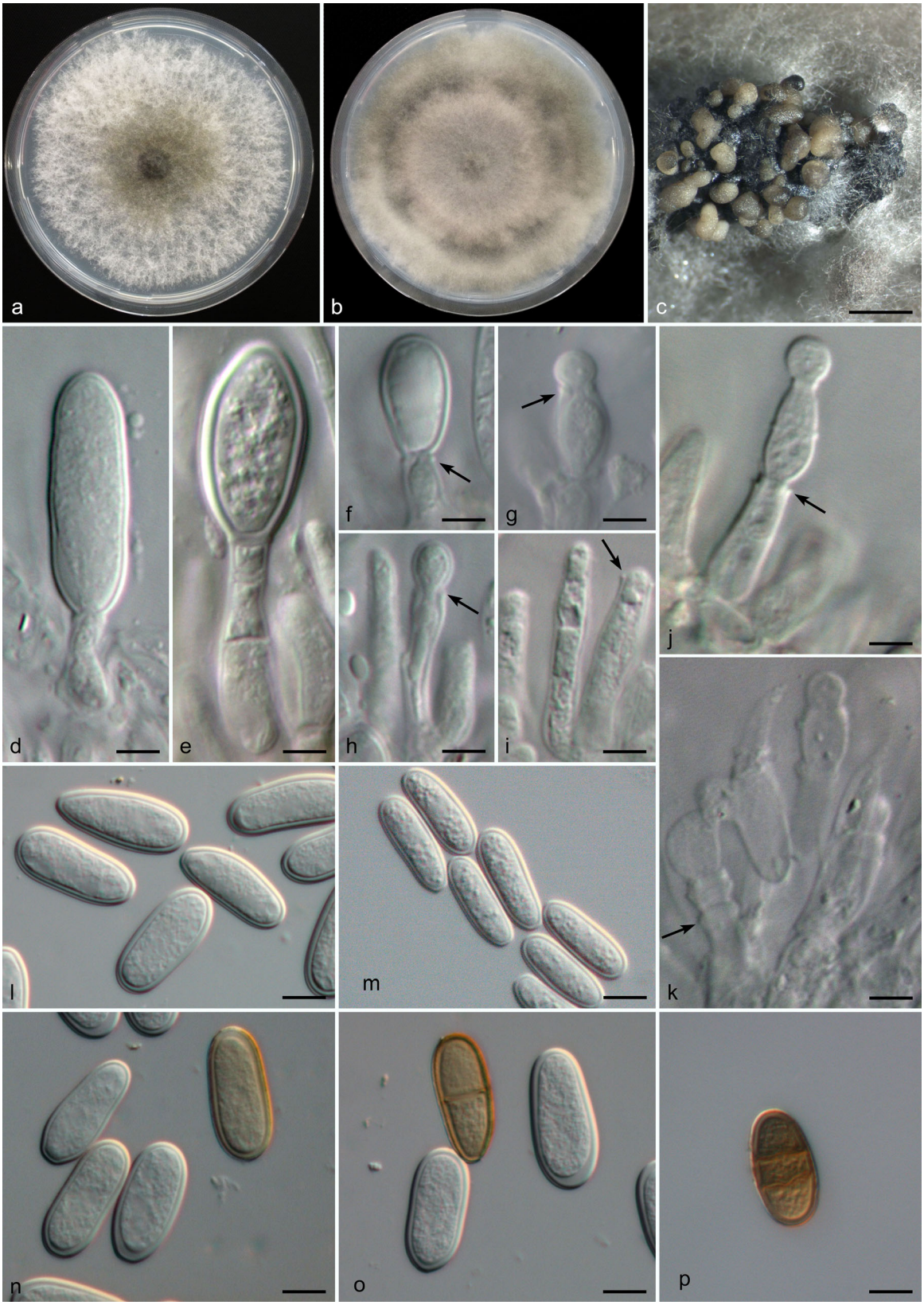

Fig. 2 *Diplodia fraxini*. **a** Colony of typical *D. fraxini* after 7 days growth at 25 °C on PDA. **b** colony morphology of *D. fraxini* morphotype A growing on PDA. **c** conidia oozing from picnidia. **d–e** conidia developing on conidiogenous cells. **f–k** conidiogenous cells with periclinal thickenings (*arrowed*). **l** hyaline conidia of *D. fraxini* morphotype A. **m** hyaline conidia of *D. fraxini*. **n** hyaline and one pale brown aseptate conidia. **o** pale brown one septate conidium. **p** mature, brown conidium showing two septa. *Bars*: **c**=1 mm; **d–p**=10 μm

composed of three layers, an outer of dark brown, thick-walled *textura angularis*, a middle layer of dark brown thin-walled cells, an inner layer of thin-walled hyaline cells. *Ostiole* central, circular, papillate. *Conidiophores* absent. *Conidiogenous cells* (11–)12–15(–16.5)×(2.5–)3.5–4.5(–5) μm, holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate, proliferating at the same level giving rise to periclinal thickenings, or proliferating percurrently to form one or two indistinct annellations. *Conidia* hyaline, aseptate, smooth, thick-walled, oblong to ovoid, straight, both ends broadly rounded, becoming pale brown to brown and one-two septate with age.

Significant differences in conidial dimensions were observed among the *D. fraxini* isolates considered in this work. In most of the isolates the conidia were (23.5−)25–27(–30)×(11−)12.5–13.5(−15) μm, 95 % confidence limits=25.72–26.45×12.87–13.33 μm (mean ± S.D. of 50 conidia=26.08±1.31×13.10±0.83 μm, L/W ratio=2.00±0.14), which correspond to the conidial dimensions reported by Saccardo (1884) and for this reason these isolates are here considered as typical of the species. Two isolates, one from Portugal and one from Sardinia, consistently produced longer conidia measuring (26.5−)29–31.5(–33)×(11−)12.5–14(−17.5) μm, 95 % confidence limits=29.28–30.23×13.12–13.83 μm (mean ± S.D. of 50 conidia=29.76±1.72×13.47±1.28 μm, L/W ratio=2.22±0.19). Since these isolates are phylogenetically indistinguishable from the typical ones we regard these as a morphological variant and report them as *Diplodia fraxini* morphotype A.

Cultural characteristics: Colonies on PDA grew moderately, reaching 90 mm diameter or less in 7 days at 25 °C, the mycelium was sparsely to moderately aerial, surface white at first and later turned pale to dark grey and greyish to brown in reverse (Fig. 3). Colonies of morphotype A on PDA grey-brown with dense aerial mycelium.

Cardinal temperatures for growth: minimum 5 °C, maximum <35 °C and optimum 25 °C.

Habitat: On branches of *F. angustifolia* and *F. excelsior*.

Known distribution: Italy, Portugal and the Netherlands (this paper), Sweden, Germany, Italy, France (Saccardo 1884).

Specimens examined: PORTUGAL, Monte da Caparica, on dead twigs of *Fraxinus angustifolia*, 14 March 2013, Antonio Deidda (LISE 96134, neotype designated herein), MBT176183, culture ex-neotype CBS 136010 = CAD001. Cascais, on dead twigs of *F. angustifolia*, 13 April 2013, Antonio Deidda, designated morphotype A, culture CBS 136012 = CAD010. ITALY, Bortigiadas, isolated from a branch canker of *F. angustifolia*, 03 June 2011, Benedetto T. Linaldeddu, culture CBS 136011 = BL70. ITALY, Siliqua, isolated from a branch canker of *F. angustifolia*, 11 November 2009, Benedetto T. Linaldeddu, CBS 136013 = BL16 (morphotype A). Additional isolates are given in Table 1.

Notes: Fries (1849) did not refer specifically to any previous description and gave only a brief Latin comment "Vidi triloc." (= "I have seen trilocular [pycnidia]"). Nevertheless, the binomial *Diplodia fraxini* should be interpreted as a recombination based on *Sphaeria fraxini* as suggested by Saccardo (1884). The holotype of neither *S. fraxini* nor *D. fraxini* (on a branch of *Fraxinus* sp. collected in Sweden by Fries) could be located and are presumed lost. For this reason a neotype (LISE 96134) is designated here. All except two of the isolates studied here conformed morphologically to Saccardo's (1884) description of the species. The morphotype A isolates, with large conidia, were phylogenetically indistinguishable from typical *D. fraxini* isolates and thus were considered to be a morphological variant of *D. fraxini*.

Diplodia mutila (Fr. : Fr.) Fr., Summa Veg. Scand. 2: 417. 1849.

MycoBank: MB201741 (Fig. 3)

≡ *Sphaeria mutila* Fr. : Fr., Syst. Mycol. 2: 424. 1823.

≡ *Physalospora mutila* (Fr. : Fr.) N.E. Stevens, Mycologia 28: 333. 1936.

≡ *Botryosphaeria stevensii* Shoemaker, Can. J. Bot. 42: 1299. 1964.

Ascomata unilocular, solitary or clustered, immersed, partially erumpent when mature, globose, up to 300 μm diam., dark brown to black, thick-walled, wall composed of outer layers of thick-walled, dark-brown *textura angularis*, inner layers of thin-walled, hyaline, *textura angularis*. *Ostiole* central, circular, papillate, periphysate. *Pseudoparaphyses* hyaline, branched, septate, 2–3 μm wide, constricted at septa. *Asci* clavate, stipitate, bitunicate with a thick endotunica and well-developed apical chamber, 100–160×14–22 μm (including stipe), containing eight, biseriate ascospores. *Ascospores* (25–)28–35(–36)×(9.5–)10–12.5(–13.5) μm; 95 % confidence limits=30.8–32.1×11.2–11.7 μm (mean ± S.D. of 50 ascospores=31.5±2.3×11.4±0.9 μm) with L/W of 2.8±0.3, fusiform to oval, widest in the middle, both ends obtuse, hyaline, thin-walled, smooth, aseptate, rarely becoming light brown with age. *Conidiomata* solitary or aggregated in clusters of up to five or more, immersed, partially erumpent when mature, dark brown to black, more or less globose, up to 600 μm diam., wall composed of three layers, an outer of dark brown, thick-walled *textura angularis*, a middle layer of dark brown thin-walled cells, an inner layer of thin-walled hyaline cells. *Ostiole* central, circular, papillate. *Conidiophores* absent. *Conidiogenous cells* (11−)12–15×4–5 μm, holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate, proliferating at the same level giving rise to periclinal thickenings, or proliferating percurrently to form one or two indistinct annellations. *Conidia* hyaline, aseptate, smooth, thick-walled, oblong to ovoid, straight, both ends broadly rounded, (20−)21.5–25.5(–27.5)×(9.5−)12–14(−15.5) μm, 95 % confidence limits=24.69–25.73×13.26–13.78 μm (mean ± S.D. of 50 conidia=25.4±1.0×

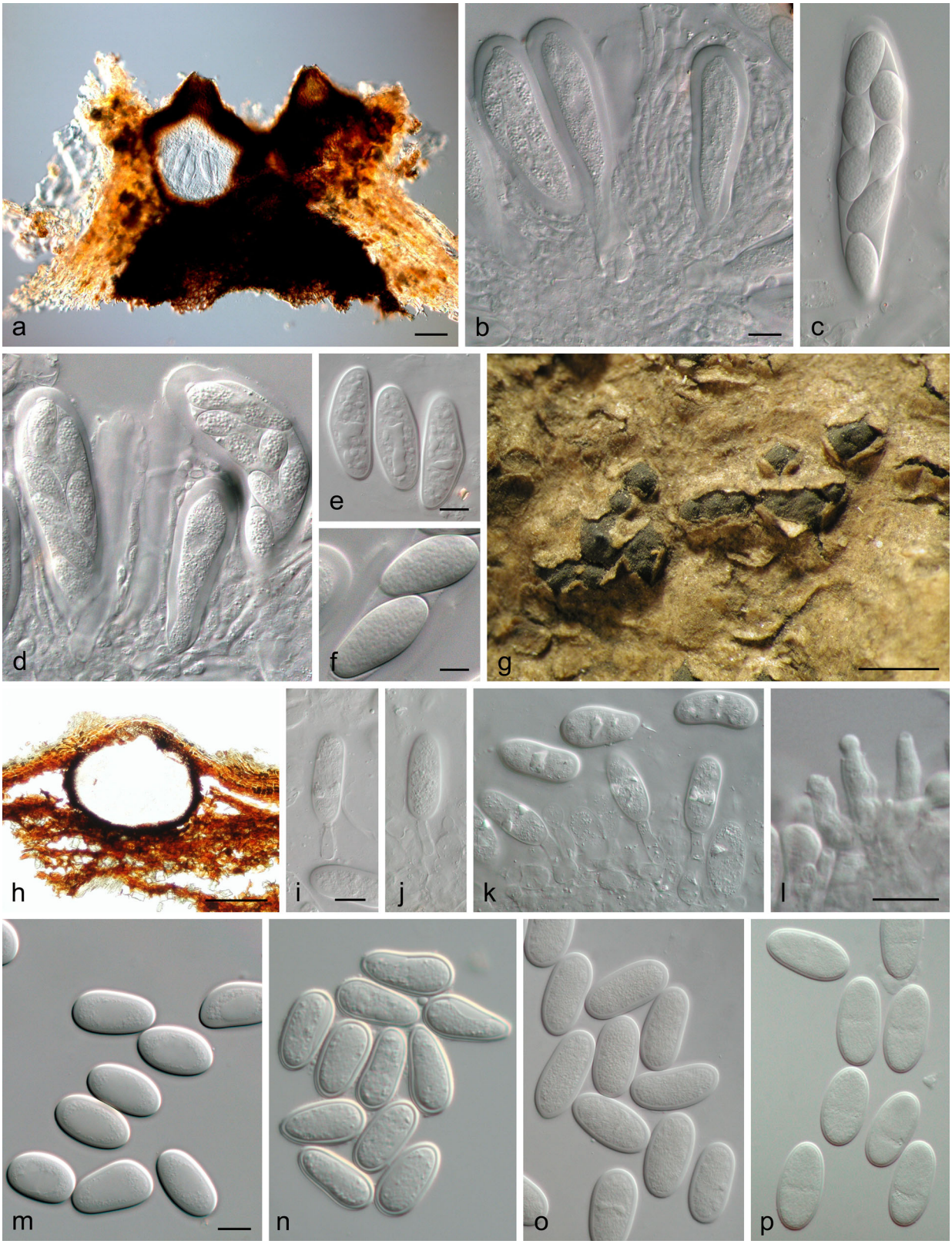

Fig. 3 *Diplodia mutila*. **a** Sectioned ascoma. **b** Immature asci and pseudoparaphyses. **c**, **d** Asci with ascospores. **e**, **f** Ascospores. **g** Conidiomata partially erumpent through host. **h** Sectioned conidioma. **i**–**l** Conidiogenous cells. **m**–**p** Conidia. **m** Hyaline, aseptate conidia of CBS 112553. **n** Hyaline, aseptate conidia of CBS 136014. **o** Hyaline, aseptate conidia of BPI 599153. **p** Hyaline, aseptate conidia of K(M) 99664. *Scale bars* **a**=100 μm, **b**=10 μm, **e**, **f**=10 μm, **g**=500 μm, **h**=100 μm, **i**, **l**=10 μm, **m**=10 μm. *Scale bar* in **b** applies to **c**, **d**. Scale bar in **i** applies to **j**, **k**. Scale bar in **m** applies to **n**, **o**, **p**

13.4±0.5 μm, L/W ratio=1.9±0.1), rarely becoming pale brown and one-septate with age. Table 2 illustrate conidial sizes for all species of *Diplodia* belonging to clade 1.

Habitat: While Farr and Rossman (2013) list 55 hosts for *D. mutila* it is now clear that many of the earlier reports of this fungus could be misidentifications (Alves et al. 2004, 2006; Lazzizera et al. 2008; Phillips et al. 2012). The following are confirmed hosts: *Chamaecyparis lawsoniana*, *Fraxinus* spp., *Malus* spp., *Populus* spp., *Taxus baccata*, *Vitis vinifera* (Phillips et al. 2013).

Known distribution: England, France, Italy, Portugal, South Africa, USA (California) (Phillips et al. 2013), New Zealand (Dingley 1969; Laundon 1973).

Specimens examined: *Diplodia mutila*: FRANCE, Ardenne, Sedan, on bark of *Populus nigra*, date unknown, Montagne, (K 99664, isotype). PORTUGAL, Alentejo, Montemor-o-Novo, *Vitis vinifera,* 1996, A.J.L. Phillips (CBS H-20187), living culture CBS 112553. PORTUGAL, Beira Litoral, Aveiro, *Populus alba,* 2012, A. Alves, (LISE 96136, epitype of *Diplodia mutila* designated herein), MBT176182, culture ex-epitype CBS 136014. *Sphaeria mutila*: Scler. Suec. 164 (STR); Scler. Suec. 385 (STR). *Physalospora mutila*: ENGLAND, Cornwall, Saltash, on bark of *Malus* sp., 22 Aug. 1935, N.E. Stevens (BPI 599153, lectotype); Surrey, Ranmore Common, on *Fraxinus* sp., 19 Apr. 1957, C. Booth (BPI 599150 ex IMI 69064).

Notes: Although Montagne (1834) indicated *Sphaeria mutila* as the type of the new genus *Diplodia*, the 1834 protologue did not make any definite association of "*mutila*" with "*Diplodia*", as required for a valid comb. nov. Therefore, the frequently cited date of 1834 for publication of the combination *Diplodia mutila* is incorrect. Fries (1823) described *Sphaeria mutila* and distributed two exsiccati under that name as Scler. Suec. 164 and 385. Stevens (1933) and Sutton (1980) reported that these two exsiccati in BPI and K had no spores. Alves et al. (2004) examined material of the same two exsiccati in STR and also found no spores. Montagne sent Fries a fungus that was identified as *S. mutila*. The record was listed under *S. mutila* Fr. by Montagne (1834) with the note that this would become the type of a new genus, *Diplodia*, later characterized by Fries (1849). Thus, the binomial *Diplodia mutila* was first introduced by Fries (1849). Montagne distributed this fungus in his exsiccatus No. 498. According to Alves et al. (2004) no material of this exsiccatus could be found in STR. Alves et al. (2004) examined Montagne's specimen of *D. mutila* in Kew, K(M) 99664 (presumed to be an isotype) and found it to agree in all aspects with Stevens' (1933) account of Montagne's exs. 498. Stevens (1933) described *Physalospora mutila* as the teleomorph of *D. mutila* referring to BPI 599151, but this name was invalid because it lacked a Latin description. Alves et al. (2004) examined this specimen and could find no teleomorph, but they did find ample material of the telomorph on BPI 599153, which is a specimen on apple collected by Stevens from the same locality at same time he collected BPI 599151. Shoemaker (1964) considered the teleomorph to be a species of *Botryosphaeria* and since the name *B. mutila* was already taken, he proposed the name *Botryosphaeria stevensii*. After Crous et al. (2006) revised *Botryosphaeria* reducing it to *B. dothidea* (Moug.) Ces. & De Not. and *B. corticis* (Demaree & Wilcox) Arx & E. Müll., the fungus known as *B. stevensii* was referred to only by its anamorphic name *D. mutila*. The epitype designated here conforms in all ways with the isotype of *D. mutila* and with the asexual morph on BPI 599153 as described by Alves et al. (2004).

Diplodia subglobosa **A.J.L. Phillips, Deidda & Linaldeddu sp. nov.**

MycoBank: MB806049 (Fig. 4)

Etymology: Named for the sub-globose conidia.

Ascomata not seen. *Conidiomata* solitary, immersed in the host, dark brown to black, globose to ovoid, up to 560 μm diam and 400 μm high, wall composed of three layers, an

Table 2 Conidial sizes and L/W ratios

Species	Conidia		References
	Size (μm)	L/W ratio	
D. africana	(17–)25.5–33(–34)×(10–)12–14(–15)	2.2	Damm et al. (2007)
D. agrifolia	(21.3–)27.0–36.5×(12.1–)14.5–17.8	1.9	Lynch et al. (2013)
D. fraxini	20–25×10	–	Saccardo (1884)
D. fraxini	(23.5−)25–27(–30)×(11−)12.5–13.5(−15)	2.0	This study
D. fraxini morphotype A	(26.5−)29–31.5(–33)×(11−)12.5–14(−17.5)	2.2	This study
D. malorum	(24–)26–32(–36)×(12–)13–17.5(–18.5)	1.9	Phillips et al. (2012)
D. mutila	(23.5–)25.1–25.7(–27.4)×(12.4–)13.2–13.5(–14.3)	1.9	Alves et al. (2004)
D. olivarum	(21.5–)22–27.5(–28.5)×(10–)11–13.5(–14.5)	2.0	Lazzizera et al. (2008)
D. rosulata	(21–)25–32(–36)×(10–)11–17.5(–19.5)	1.9	Gure et al. (2005)
D. subglobosa	(24.0−)24.5–27.0(−32)×(15.5−)16.5–19.0(−22)	1.5	This study

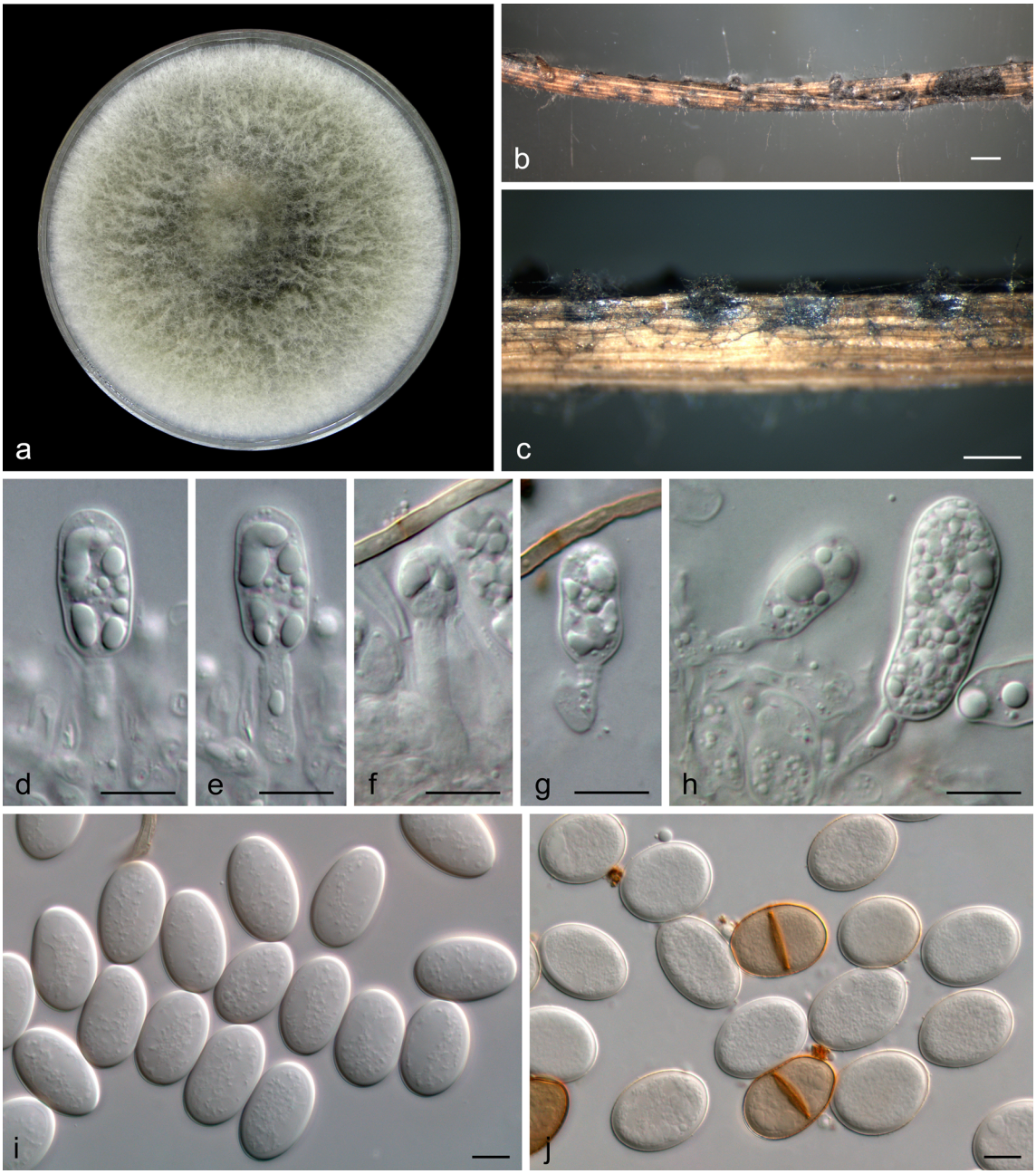

Fig. 4 *Diplodia subglobosa*. **a** Colony on PDA after 7 days at 25 °C. **b**, **c** Conidiomata formed in culture on pine needles. **d–h** Conidiogenous cells with developing conidia. **i** Hyaline, aseptate conidia. **j** Hyaline, aseptate and coloured, 1-septate conidia. *Bars*: **b, c**=500 μm; **d–j**=10 μm

outer of dark brown, thick-walled *textura angularis*, a middle layer of dark brown thin-walled cells, an inner layer of thin-walled hyaline cells. *Ostiole* central, circular, papillate. *Conidiogenous cells* 11–25×3–9 μm, holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate, proliferating at the same level giving rise to periclinal thickenings, or proliferating percurrently to form one or two indistinct annellations. *Conidia* hyaline, aseptate, smooth, thick-walled, oblong to ovoid, straight, both ends broadly rounded, (24.0−)24.5–27.0(−32)×(15.5−)16.5–19.0(−22) μm, 95 % confidence limits=27.7–28.0×18.1–18.5 μm (mean ± S.D. of 150 conidia=27.7±1.8×18.3±1.2 μm, L/W ratio=1.5±0.1), becoming pale brown and septate when aged.

Habitat: Twigs and branches of *Fraxinus* spp. and *Lonicera nigra*.

Known geographic distribution: Italy and Spain.

Specimens examined: ITALY: Sicily, *Fraxinus ornus*, 2006, A. Sidoti (CBS 124131). SPAIN: Cataluña, *Fraxinus excelsior*, (no date), J. Luque (CBS 124132); *Lonicera nigra*, (no date), J. Luque Holotype LISE 96135 (culture ex-type CBS 124133). ITALY, *Fraxinus excelsior*, (no date) B. Slippers (CMW7776).

Notes: The relatively wide conidia and L/W ratio of 1.5 of this species are distinctive amongst *Diplodia* species with hyaline conidia belonging to clade 1.

Discussion

Results of phylogenetic analyses provide robust evidence that the *D. mutila*-like isolates from *Fraxinus* spp. in Italy, the Netherlands, Portugal and Spain belong to three separate sub-clades corresponding to three distinct species within *Diplodia* clade 1. For one clade, which includes several isolates from *F. angustifolia* in Italy and Portugal, and an isolate from *F. excelsior* in the Netherlands (CBS 431.82), the name *D. fraxini* was considered appropriate. The type of neither *S. fraxini* nor *D. fraxini* could be located, but morphologically the isolates we studied agree in all ways with Saccardo's (1884) description of the species and therefore the name is re-instated and a neotype designated. Two isolates, one from Italy and one from Portugal, differed in cultural characteristics and conidia dimensions from typical isolates and since they were phylogenetically indistinguishable from the typical isolates they were considered to be morphological variants and are designated as morphotype A. The descriptions of distinct morphological forms has been reported for another species in *Diplodia*, namely *D. sapinea* (morphotype A and C) and the C morphotype is considered to be the most virulent (de Wet et al. 2002).

The second clade includes isolates from *F. ornus* in Italy, *F. excelsior* in Italy and Spain and one isolate from *Lonicera nigra* in Spain and represents a previously unrecognized *Diplodia* species, which we describe here as *D. subglobosa* sp. nov. Conidia of this new species are sub-globose with an average L/W ratio of 1.5. In this respect it resembles the anamorph of "*Botryosphaeria*" *quercuum* (Shoemaker 1964) but it can be distinguished on account of its larger conidia (24.0−)24.5–27.0(−32)×(15.5−)16.5–19.0(−22) μm. In "*Botryosphaeria*" *quercuum* the conidia are (18–)21–24(–25)×(12–)15–16(–17).

The third group of isolates obtained from *F. ornus* in Portugal were considered to be *D. mutila*, the type species of the genus *Diplodia* (Montagne 1834; Fries 1849). Unfortunately, no live cultures linked to the holotype of *D. mutila* are extant and this has severely hampered studies on the taxonomy and phylogeny of *Diplodia*. Alves et al. (2004) provided a detailed description of this species based on one isolate from grapevines in Portugal (CBS 112553), an isotype of *D. mutila* (K99664) and one of Stevens' (1936) specimens of *Physalospora mutila* (BPI 99153). They showed that CBS 112553 correlated closely with the morphology of *D. mutila* and this was confirmed in the present study. This culture has subsequently been cited as typical of *D. mutila* and has been regarded as a standard isolate for this species (Alves et al. 2006; Damm et al. 2007; Lazzizera et al. 2008; Phillips et al. 2012; Lynch et al. 2013). Although it is possible to use as epitype a specimen collected from a different host it is preferable, whenever possible, to obtain the epitype from the same host as the type specimen.

In an effort to obtain a suitable specimen that can be used as epitype for *D. mutila* we collected samples from *Populus* (type host of *D. mutila*) and *Fraxinus* (type host of the sexual morph). No sexual morph was found on either of the hosts, but several isolates with typical morphological features of *D. mutila* were obtained. Isolates obtained from *P. alba* and *F. ornus* in Portugal clustered with isolates from several other hosts including *V. vinifera*, *Persea americana* Mill., *Taxus baccata* L. and *Chamaecyparis lawsoniana* (A. Murray bis) Parl. CBS 302.36, deposited by N. E. Stevens as *Physalospora mutila* N.E. Stevens also clustered in this group. Since the culture is no longer sporulating, most likely due to the fact that it has been in culture for many years now, we could not study its morphology. This culture is not linked in any way to the type specimen but given that it was obtained by Stevens it can be regarded as representative of his concept of the sexual morph of *D. mutila*. Thus, this clade is regarded as representing true *D. mutila*. The isolates from *P. alba* and *F. ornus* conformed in all ways with the morphological characters of the isotype of *D. mutila* (K99664), the asexual morph of *P. mutila* on BPI 599153 (Alves et al. 2004) and the isolate from grapevines in Portugal (CBS 112553) that has been used as a standard. Furthermore, the type host of *D. mutila* is a *Populus* species. Therefore, the specimen on *P. alba* is herein designated as epitype for *D. mutila*. The data presented here supports the plurivorous nature of this pathogen since isolates from several different hosts clustered together in the clade corresponding to this species.

Diplodia mutila clustered in the *Diplodia* clade 1, which includes species with hyaline conidia that become brown and one-septate some time after discharge from the pycnidia. This clade comprises eight species that are morphologically similar and can be difficult to separate on morphology alone. Nonetheless, they can be differentiated on slight differences in conidial dimensions (Table 2). Thus, conidia of *Diplodia africana* Damm & Crous are the largest in the clade 1, while *D. olivarum* has the smallest conidia. Although conidia of *D. malorum* are morphologically similar to those of *Diplodia rosulata* Gure, Slippers & Stenlid this last species forms distinctive rosulate colonies (Gure et al. 2005). *Diplodia agrifolia* S.C. Lynch & A. Eskalen differs from *D. mutila* by its longer and wider conidia. Moreover, conidia of *D. agrifolia* are hyaline and aseptate, but most become dark brown and one-septate before discharge from pycnidia (Lynch et al. 2013).

Another group of isolates obtained in this study from cankered branches of declining *P. alba* trees in Italy clustered in another clade together with *D. malorum*. The name

D. malorum was reinstated by Phillips et al. (2012) for isolates obtained from *Malus* spp. Although this species is morphologically similar to *D. mutila* it can be distinguished by its larger conidia and from DNA sequence data (Phillips et al. 2012, 2013). Until now *D. malorum* has been reported only from *Malus* spp. and other *Rosaceae* and this represents the first report of the species in a different host (*Populus*) and a different family. In addition, it is reported for the first time in Italy.

A single isolate obtained from a cankered branch of *Quercus coccifera* in Tunisia clustered within the *D. olivarum* clade. This species was initially described from *Olea europaea* L. (olive tree) in Italy where it was associated with diseased olive drupes (Lazzizera et al. 2008). Since then, it has been found associated with cankers on *Ceratonia siliqua* L. (carob tree) in Italy (Granata et al. 2011) and trunk disease of *Prunus dulcis* (Mill.) D.A. Webb (almond) in Spain (Gramaje et al. 2012). This represents the first report of the species in Tunisia and first report on *Q. coccifera*.

Additional *Diplodia* isolates morphologically different from *D. mutila* were included in this study. One isolate from *F. ornus* in Portugal and two isolates from *F. angustifolia* in Italy clustered within *D. seriata*. This is the first report of the species on *Fraxinus* spp. in Europe. The only other known report is on *Fraxinus americana* L. (green ash) in the USA under the name *Physalospora obtusa* (=*Botryosphaeria obtusa*) (Farr et al. 2013) which is the sexual morph of *D. seriata* (Phillips et al. 2007). This species is well known by its cosmopolitan and plurivorous nature (Punithalingam and Walker 1973; Phillips et al. 2007).

Another two isolates from *F. angustifolia* in Italy clustered with *D. pseudoseriata* and *D. alatafructa*. *Diplodia pseudoseriata* was described from several species of native *Myrtaceae* trees in Uruguay (Pérez et al. 2010) while *Diplodia alatafructa* Mehl & Slippers was first reported on *Pterocarpus angolensis* DC. (wild teak) in South Africa (Mehl et al. 2011). Interestingly, the two Italian isolates form a distinct sub-clade within *D. pseudoseriata*/*D. alatafructa* clade with 75 % bootstrap support. Further morphological and phylogenetic analysis are currently in progress to clarify the status of these two isolates.

In this study we have shown that the name *D. mutila* has been applied to a number of cryptic species. In order to stabilize the name and allow its unambiguous application an epitype specimen with associated ex-epitype cultures was selected. At the same time the name *D. fraxini* is re-instated and a neotype designated. In the future more studies should be done in order to verify the pathogenicity of *D. mutila*, *D. fraxini* and *D. subglobosa* on *Fraxinus* spp. and establish their role in the etiology of ash dieback.

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