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Botryosphaeriaceae species associated with diseased loquat trees in Italy and description of *Diplodia rosacearum* sp. nov.

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Abstract

Loquat (*Eriobotrya japonica*) is a fruit tree cultivated in several countries in the Mediterranean region. A survey of a loquat orchard in Sicily (Italy) revealed the presence of plants showing dieback symptoms and cankers with wedge-shaped necrotic sectors. Fungi from the genera *Diplodia* and *Neofusicoccum* were isolated from symptomatic plants. On the basis of morphological characters and DNA sequence data four species were identified, *Neofusicoccum parvum, N. vitifusiforme, Diplodia seriata* and a novel *Diplodia* species, which is here described as *D. rosacearum* sp. nov. Inoculation trials of loquat plants cv Sanfilipparo showed that *N. parvum, D. seriata* and *D. rosacearum* were pathogenic to this host. Although variability was observed between isolates, *N. parvum* and *D. rosacearum* were the most aggressive species.

Key words – *Diplodia* – *Neofusicoccum* – phylogeny – pathogenicity

Introduction

Loquat (*Eriobotrya japonica*), a species in the family Rosaceae, is a fruit tree that apparently originates from China, which is the world's largest producer. This plant has been spread worldwide and is currently cultivated in many countries. In the Mediterranean region it is cultivated in countries such as Spain, Portugal, Greece and Italy. In the latter almost its entire production is located in Sicily (Caballero & Fernandez 2002).

This crop is affected by a number of diseases of which the most important are loquat scab, caused by *Fusicladium eriobotryae* (Sánchez-Torres et al. 2009), and loquat decline caused by several soilborne pathogens including *Armillaria mellea, Rosellinia necatrix, Phytopththora* spp. and Cylindrocarpon-like spp. (González-Domínguez et al. 2009, Agustí-Brisach et al. 2016). Also, branch canker and dieback associated with *Botryosphaeriaceae* species has been recently reported in the main loquat producing areas in Spain (González-Domínguez et al. 2016). Twelve different species of *Botryosphaeriaceae* were identified from plants with branch canker and dieback symptoms and all species were shown to be pathogenic to loquat plants (González-Domínguez et al. 2016).

Species in the family *Botryosphaeriaceae* are well known as important pathogens of fruit crops including, among many others, almond (Gramaje et al. 2012), olive (Lazzizera et al. 2008a),

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apple (Phillips et al. 2012) and mango (Rodríguez-Gálvez et al. 2016). However, until very recently their status as pathogens of loquat was poorly known.

In Sicily, the most important loquat producing area in Italy, plants with symptoms of branch cankers and dieback identical to those reported by González-Domínguez et al. (2016) and caused by species of *Botryosphaeriaceae* were observed. Thus, the aim of this work was to identify the *Botryosphaeriaceae* species associated with diseased loquat trees using a combination of morphological and DNA sequence data, and to evaluate the pathogenicity of the identified species to loquat plants.

Materials & Methods

Field surveys, sampling and fungal isolation

In 2014 (May) and 2015 (March) observations were carried out in a loquat orchard located in Palermo province at 150 m a.s.l. (S. Maria di Gesù, 38°04'N; 13°22'E). The 25 year-old orchard extends over 10000 m^2 and includes nine autochthonous cultivars (BRT20, Claudia, Fiore, La Mantia, Marcenò, Nespolone Bianco Dolce, Nespolone di Trabia, Sanfilipparo and Virticchiara) and seven allochthonous (Algerie, Bueno, El Buenet, Golden Nugget, Magdall, Peluche and Tanaka). After the preliminary observation, within three plots of 150 m^2 randomly selected in March 2015, the trees with cankers were counted to determine disease incidence. During each field survey, four samples from symptomatic branches were collected from four plants as representative diseased loquats.

Symptomatic branch samples were taken to the laboratory and the outer bark was removed. To observe internal symptoms longitudinal and transversal cuts were made from samples. Wood portions were flame sterilized and fungal isolations were made from small chips cut from the margin of necrotic lesions with a sterile scalpel.

Wood chips were placed on potato dextrose agar (PDA Oxoid Ltd., Basingstoke, UK) supplemented with 0.5 g l^{-1} of streptomycin sulphate (Sigma-Aldrich, St. Louis, MO, USA). After incubation at 25 °C in the dark for 5–7 days, hyphal tips from the margin of every putative *Botryosphaeriaceae* colony were sub-cultured on PDA. Isolates were stored in 15 % glycerol at -80°C and maintained in the fungal culture collection of the Dipartimento di Scienze Agrarie e Forestali (University of Palermo). The ex-type culture of the new *Diplodia* species was deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands and nomenclatural data in MycoBank (Crous et al. 2004) database. The holotype was deposited in the herbarium of Instituto Nacional de Investigação Agrária e Veterinária I.P., Oeiras, Portugal (LISE).

Morphological characterization

Sporulation was induced by sub-culturing the isolates onto quarter-strength PDA (¼ PDA) and water agar (WA) supplemented with double-autoclaved pine needles and poplar twigs. Cultures were incubated at 23°C with a 12 h photoperiod of fluorescent lighting until pycnidia developed. Morphological identifications were based on conidial characters as described by Phillips et al. (2013).

For each isolate, cardinal temperatures for growth were determined on PDA plates incubated at 5, 10, 15, 20, 25, 30, 35 and 40°C in the dark. Three replicates for each isolate and temperature combination were used. Colony diameters were measured along two perpendicular axes when the colonies had covered almost the entire diameter of the plate and data converted to daily radial growth (millimeters per day). Plates incubated at temperatures in which there was no growth, were transferred to 25°C to determine if those temperatures were fungistatic or fungicidal. For microscopy pycnidia were cut and mounted in 100% lactic acid and morphological characters of the conidia and conidiogenesis were observed with an Axioskop (Zeiss, Germany) microscope. Images were captured with an AxioCam MRc5 camera (Zeiss, Germany) and measurements made with the software AxioVision 4.6. Conidial dimensions are given as mean values of a minimum of 50 conidia with extreme values in parentheses. For other structures at least 20 measurements were made.

DNA extraction, PCR amplification and sequencing

Genomic DNA was isolated from 1-week-old fungal cultures grown on PDA at 25°C in the dark using a standard CTAB-based protocol (O'Donnell et al. 1998). The internal transcribed spacer (ITS) region of the ribosomal DNA and part of the translation elongation factor 1 alpha gene (*tef1*-α) were amplified and sequenced with primers ITS1/ITS4 (White et al. 1990) and EF1-728F/EF1-986R (Carbone and Kohn 1999), respectively. PCR amplification and sequencing of amplicons was carried out as described previously (Phillips et al. 2013). Sequences were edited with FinchTV v1.4.0 (Geospiza, Inc., Seattle, Washington, USA; http://www.geospiza.com/finchtv) and compared with sequences deposited in GenBank through BLASTn searches. New sequences were deposited in GenBank (Table 1) and alignments in TreeBase (S19915).

Phylogenetic analyses

Sequences of all *Diplodia* species currently known from culture were retrieved from GenBank and aligned with sequences of the isolates obtained in this study. Alignments were done 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 made if necessary using BioEdit v. 7.2.5 (Hall 1999). Maximum likelihood (ML) analyses were performed using MEGA6 (Tamura et al. 2013). The best fitting DNA evolution model was determined by MEGA6. ML analyses were performed on a Neighbour-Joining starting tree automatically generated by the software. Nearest-Neighbour-Interchange (NNI) was used as the heuristic method for tree inference and 1000 bootstrap replicates were performed. The robustness of the trees was evaluated by 1000 bootstrap replications. Trees were visualized with TreeView v. 1.6.6 (Page 1996). An ITS only phylogenetic analysis was carried out because there are no *tef1-α* sequences available for the species *D. huaxii*, *D. italica* and *D. pseudoplatani*. To adequately resolve *Diplodia* species a combined ITS plus *tef1-α* phylogenetic analysis was performed.

Pathogenicity test

Pathogenicity of all isolates investigated was tested in June 2015 on 2-year-old loquat plants cv. Sanfilipparo, grown in pots. For each fungal isolate and control 10 plants were used in a randomized block experimental design. The inoculated loquat plants were kept under environmental conditions in the field.

For inoculation the bark of twigs was surface-disinfected with 70% ethanol and a piece of bark was removed with a sterile cork borer (diam. 5 mm). An agar plug cut from the margin of a 6-day-old colony grown on PDA at 25°C in the dark was placed on the wound and covered with Parafilm. Non-colonized agar plugs were used to inoculate control plants. After 3 months, the outer bark of the twigs was removed with a scalpel and the length of necrotic lesion upward and downward from the point of inoculation was measured.

To fulfil Koch's postulates attempts were made to re-isolate the inoculated fungi by transferring to PDA pieces of wood taken from the margins of each lesion. Plates were incubated at 25ºC until fungal colonies developed. The re-isolated fungi were identified from their micromorphological characters as explained above.

Statistical analyses

Data from pathogenicity experiments were checked for normality using the Shapiro-Wilk test and subjected to one-way analysis of variance (ANOVA). Significance of differences between mean values was determined by Fisher's least significant difference (LSD) multiple range test at $P = 0.05$ using SAS version 9.0 (SAS Institute, Cary, NC, USA).

Table 1 Isolates included in this study. The newly generated sequences are indicated in italics and ex-type strains in bold face.

Species	Isolate number	Host	Country	GenBank	
				ITS	*tef1-α*
D. africana	**CBS 120835**	*Prunus persica*	South Africa	EF445343	EF445382
	CBS 121104	*P. persica*	South Africa	EF445344	EF445383
D. agrifolia	**CBS 132777**	*Quercus agrifolia*	USA	JN693507	JQ517317
	UCROK1429	*Q. agrifolia*	USA	JQ411412	JQ512121
D. alatafructa	**CBS 124931**	*Pterocarpus angolensis*	South Africa	FJ888460	FJ888444
D. allocellula	**CBS 130408**	*Acacia karroo*	South Africa	JQ239397	JQ239384
	CBS 130410	*A. karroo*	South Africa	JQ239399	JQ239386
D. bulgarica	**CBS 124254**	*Malus sylvestris*	Bulgaria	GQ923853	GQ923821
	CBS 124135	*M. sylvestris*	Bulgaria	GQ923852	GQ923820
D. corticola	**CBS 112549**	*Quercus suber*	Portugal	AY259100	AY573227
	BL10	*Quercus ilex*	Italy	JX894191	JX894210
D. crataegicola	**MFLUCC 15-0648**	*Crataegus* sp.	Italy	KT290244	KT290248
D. cupressi	**CBS 168.87**	*Cupressus sempervirens*	Israel	DQ458893	DQ458878
	BL102	*C. sempervirens*	Tunisia	KF307722	KF318769
D. eriobotryicola	**CBS 140851**	*Eriobotrya japonica*	Spain	KT240355	KT240193
D. fraxini	**CBS 136010**	*Fraxinus angustifolia*	Portugal	KF307700	KF318747
	CBS 136013	*F. angustifolia*	Italy	KF307710	KF318757
D. galiicola	**MFLUCC 15-0647**	*Galium* sp.	Italy	KT290245	KT290249
D. huaxii	**GUCC 0922-1**	*Platanus* sp.	China	KU848201	-
D. italica	**MFLUCC 14-1007**	*Crataegus* sp.	Italy	KU848202	-
D. intermedia	**CBS 124462**	*M. sylvestris*	Portugal	GQ923858	GQ923826
	CBS 112556	*M. sylvestris*	Portugal	AY259096	GQ923851
D. rosacearum	**NB7 = CBS 141915**	*E. japonica*	Italy	*KT956270*	*KU378605*
	NB8	*E. japonica*	Italy	*KT956271*	*KU378606*
	NB9	*E. japonica*	Italy	*KT956272*	*KU378607*
	NB10	*E. japonica*	Italy	*KT956273*	*KU378608*
	BN-67	*E. japonica*	Spain	KT240354	KT240192
	CAP330	*Pyracantha coccinea*	Bulgaria	GQ923881	GQ923849
D. insularis	**CBS 140350**	*Pistacia lentiscus*	Italy	KX833072	KX833073
	BN-55	*E. japonica*	Spain	KT240361	KT240275
D. malorum	**CBS 124130**	*M. sylvestris*	Portugal	GQ923865	GQ923833
	BL127	*Populus alba*	Italy	KF307717	KF318764
D. mutila	**CBS 136014**	*P. alba*	Portugal	KJ361837	KJ361829
	CBS122553	*Vitis vinifera*	Portugal	AY259093	AY573219
D. neojuniperi	**CBS 138652**	*Juniperus chinensis*	Thailand	KM006431	KM006462
	CPC 22754	*J. chinensis*	Thailand	KM006432	KM006463
D. olivarum	**CBS 121887**	*Olea europaea*	Italy	EU392302	EU392279
	CAP 257	*O. europaea*	Italy	GQ923874	GQ923842
D. pseudoseriata	**CBS 124906**	*B. salicifolius*	Uruguay	EU080927	EU863181
D. pseudoplatani	**GUCC G603-1**	*Platanus* sp.	China	KU848200	-
D. quercivora	**CBS 133852**	*Quercus canariensis*	Tunisia	JX894205	JX894229
	CBS 133853	*Q. canariensis*	Tunisia	JX894206	JX894230
D. rosulata	**CBS 116470**	*Prunus africana*	Ethiopia	EU430265	EU430267
	CBS 116472	*P. africana*	Ethiopia	EU430266	EU430268
D. sapinea	**CBS 393.84**	*Pinus nigra*	Netherlands	DQ458895	DQ458880

Species	Isolate number	Host	Country	GenBank	
				ITS	*tef1-α*
	CBS 109725	*Pinus patula*	Indonesia	DQ458896	DQ458881
D. scrobiculata	**CBS 118110**	*Pinus banksiana*	USA	KF766160	KF766399
	CBS 109944	*Pinus greggii*	Mexico	DQ458899	DQ458884
	CBS 113423	*P. greggii*	Mexico	DQ458900	DQ458885
D. seriata	**CBS 112555**	*V. vinifera*	Portugal	AY259094	AY573220
	CBS 119049	*V. vinifera*	Italy	DQ458889	DQ458874
	NB4	*E. japonica*	Italy	*KT956267*	*KU310680*
	CAA502	*Fraxinus ornus*	Portugal	KJ361842	KJ361836
D. subglobosa	**CBS 124133**	*Lonicera nigra*	Spain	GQ923856	GQ923824
	CBS 124131	*F. ornus*	Italy	GQ923855	GQ923823
D. tsugae	**CBS 418.64**	*Tsuga heterophylla*	Canada	DQ458888	DQ458873
N. parvum	NB5	*E. japonica*	Italy	*KT956268*	*KU310681*
	NB6	*E. japonica*	Italy	*KT956269*	*KU310682*
N. vitifusiforme	NB1	*E. japonica*	Italy	*KT956264*	*KU310677*
	NB2	*E. japonica*	Italy	*KT956265*	*KU310678*
	NB3	*E. japonica*	Italy	*KT956266*	*KU310679*

Acronyms of culture collections: BL: B.T. Linaldeddu culture collection housed at Dipartimento di Agraria, Università di Sassari, Italy; CAA: Collection of Artur Alves housed at Department of Biology, University of Aveiro, Portugal; CAP, A.J.L. Phillips, Universidade Nova de Lisboa, Portugal; CBS: Centraalbureau voor Schimmelcultures, The Netherlands; CPC: Collection of Pedro Crous housed at CBS; UCROK, Department of Plant Pathology and Microbiology, University of California, Riverside; GUCC: Guizhou University Culture Collection (GUCC); MFLUCC: Mae Fah Luang University Culture Collection.

Results

Field surveys, fungal isolation and identification

Surveys of the loquat orchard revealed the presence of plants showing dieback of the branches, and cankered areas of variable size, both on stems and trunks (Fig. 1). All symptomatic plants belonged to cultivars Algerie and Bueno. No symptoms were observed on plants of other cultivars present in the orchard surveyed. Disease incidence on both cultivars was 48.5%. Samples collected from diseased loquat plants showed beneath the bark brown to black vascular longitudinal discoloration and wedge-shaped necrotic sectors visible in cross sections (Fig. 1).

From the samples collected 10 botryosphaeriaceous fungi were isolated. Apart from these, the only other fungi isolated were *Aspergillus* spp. Identification based on morphological characters and DNA sequence from the ITS and *tef1*-α regions revealed four distinct species: *Neofusicoccum parvum* (2 isolates: NB5, NB6), *N. vitifusiforme* (3 isolates: NB1, NB2, NB3), *Diplodia seriata* (1 isolate: NB4) and a *Diplodia* sp. (4 isolates: NB7, NB8, NB9, NB10) that could not be assigned to any of the currently known species. For all species, BLAST searches showed 99–100% similarity with reference sequences of representative strains including ex-type strains.

Diplodia species were obtained from cultivar Bueno plants only whereas *Neofusicoccum* species were obtained from cultivar Algerie plants only. However, symptoms caused by *Neofusicoccum* and *Diplodia* species were similar.

Phylogenetic analyses

ML analysis based on ITS sequences, although not able to discriminate clearly all species, showed that the isolates of *Diplodia* species from loquat were closely related to *D. sapinea* and *D. intermedia* (data not shown). Combined ITS and *tef1-α* ML analysis (Fig. 2) differentiated all *Diplodia* species although not all clades received high bootstrap support. The *Diplodia* sp. isolates in this study (NB7, NB8, NB9 and NB10) clustered with another isolate from loquat from Spain

Fig. 1 – Symptoms observed on loquat plants cvs. Algerie and Bueno. **a**. Dieback of twigs and branches. **b,c**. cankers in the trunk and stems. **d**. cross section of a branch showing a necrotic area.

(BN-67) and an isolate from firethorn (*Pyracantha coccinea*) from Bulgaria (CAP330). These isolates formed a separate sub-clade, with moderate bootstrap support (62%), within a larger clade containing the species *D. sapinea, D. scrobiculata, D. seriata, D. crataegicola* and *D. intermedia*.

Taxonomy

Diplodia rosacearum S. Giambra, A. Alves, J. Armengol & S. Burruano, **sp. nov.** Fig. 3
MycoBank: MB818575

Etymology – the epithet refers to the fact that the species has, so far, been found only in hosts from the family Rosaceae.

Sexual morph not seen. *Conidiomata* pycnidial, produced on poplar twigs and pine needles on ¼ strength PDA within 2 weeks, solitary or aggregated, black, globose and uniloculate. *Conidiophores* absent. *Conidiogenous cells* hyaline, smooth, cylindrical, sometimes slightly swollen at the base, holoblastic, proliferating percurrently to form distinct annellations or proliferating internally giving rise to periclinal thickenings, (10.3–)14.6(–19.9) × (2.5–)4.0(–7.80) µm; n=20. *Conidia* ovoid to ellipsoid, apex obtuse, base truncate or rounded, initially hyaline becoming pigmented even while still attached to the conidiogenous cell, dark brown when mature, unicellular, but may become 1-septate when mature or more rarely when still hyaline, ellipsoid to ovoid, wall finely roughened on the inner surface, (16.1)–23.7–(31.9) × (9.8)–12.8–(16.7) µm, ($\bar{x}$ ± S.D. = 23.7 ± 2.4 × 12.8 ± 1.1 µm, L/W 1.9 ± 0.2; n = 200).

Culture characteristics – Colonies on PDA filling a 90 mm diameter Petri dish before 7 days in the dark at 25 °C. Mycelium moderately aerial initially white becoming pale grey to dark and dark in reverse.

Cardinal temperatures – min. < 5 °C, max. > 35 °C, opt. 25 °C.

Known distribution – Sicily (Italy), Valencia (Spain) and Plovdiv (Bulgaria).

Habitat – On cankered branches of *Eriobotrya japonica* and *Pyracantha coccinea*.

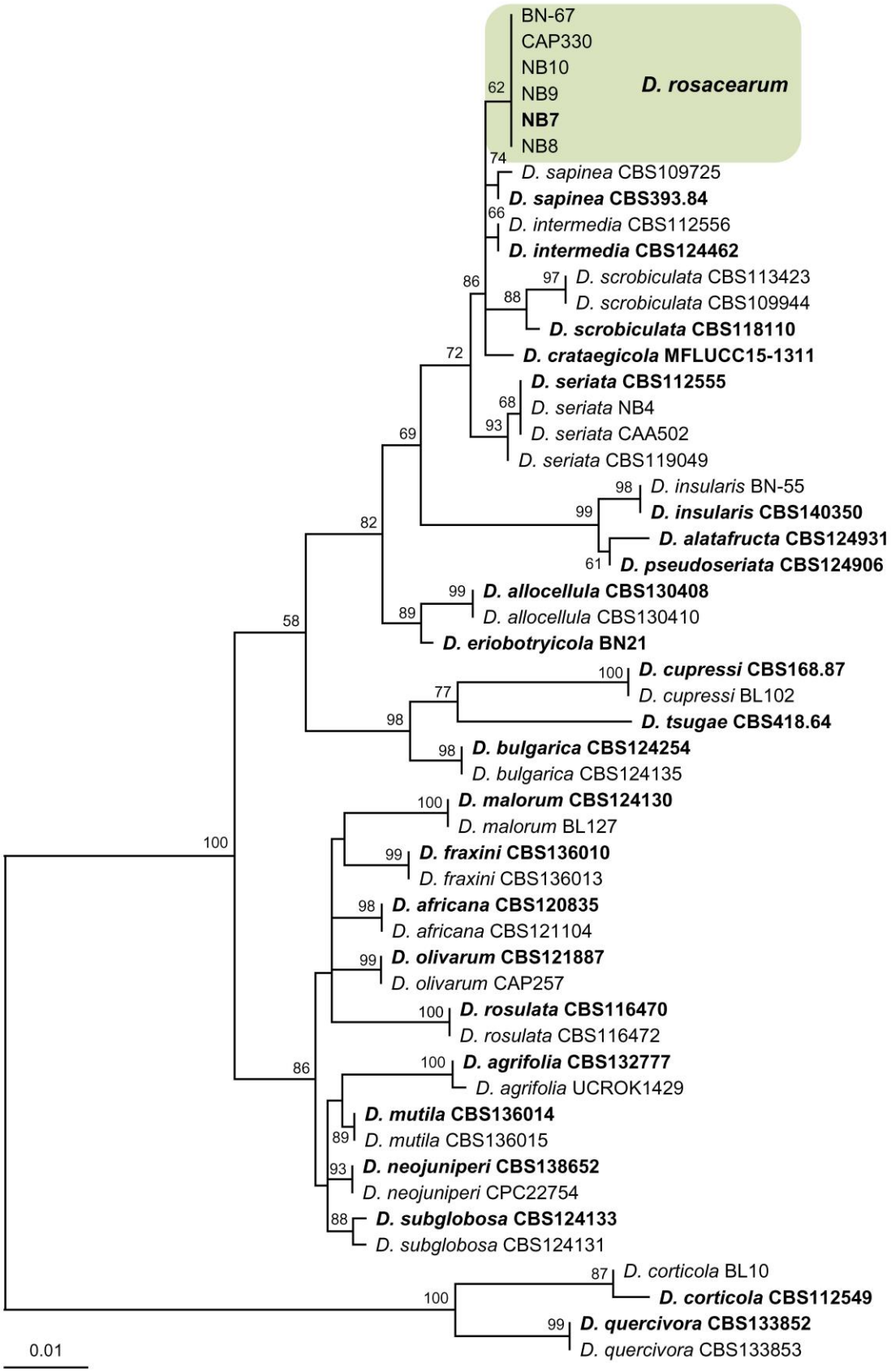

Fig. 2 – Maximum Likelihood tree of combined ITS and *tef1-α* sequence data and based on the Hasegawa-Kishino-Yano model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap support values in percentage (1000 replicates) are given at the nodes.

Material examined – Italy, Sicily (S. Maria di Gesù, 38°04'N; 13°22'E), isolated from a branch canker of *Eriobotrya japonica*, March 2015, Selene Giambra, HOLOTYPE LISE 96310, a dried culture sporulating on *Populus tremula* twigs, culture ex-holotype CBS 141915 = NB7. Other isolates are listed in Table 1.

Notes – *Diplodia rosaceraum* is closely related to *D. intermedia* but can be distinguished by DNA sequence data and the size of conidia, which are on average larger in *D. intermedia*.

Pathogenicity test

Three months after inoculation stems showed external cankers and subcortical dark-brown discoloration spreading upward and downward from the inoculation point. Moreover, some of the symptomatic plants also reacted by issuing new shoots below the point of inoculation. No discoloration was observed in the stems of control plants as well as in plants inoculated with *N. vitifusiforme* isolates NB2 and NB3.

Average lesion lengths differed significantly ($F_{7, 28} = 14.02$; $P < 0.001$) between species and isolates tested (Fig. 4). The largest lesions were caused by *N. parvum* isolate NB5 (74.1 mm), followed by *D. rosacearum* NB8 and NB10 isolates (59.9 mm and 45.6 mm, respectively). The smallest lesions, ranging between 12.2 mm and 22.3 mm, were caused by *N. vitifusiforme* (isolate NB1) and *D. seriata* (isolate NB4). All fungal species tested were successfully re-isolated from inoculated plants, thus fulfilling Koch's postulates. No fungal pathogens were isolated from control plants.

Discussion

The aetiology of canker and dieback symptoms on loquat plants was studied in an orchard in Sicily, the main Italian loquat producing area. Four *Botryosphaeriaceae* species belonging to the genera *Diplodia* and *Neofusicoccum* were identified. These included *N. parvum*, *N. vitifusiforme*, *D. seriata* and a novel species here described as *Diplodia rosacearum.*

In a previous study (González-Domínguez et al. 2016) twelve species of *Botryosphaeriaceae* belonging to the genera *Diplodia*, *Dothiorella*, *Neofusicoccum*, and *Spencermartinsia* were identified from loquat plants showing canker and dieback symptoms in Spain. This represents a much greater diversity than the one presented here. However, in that previous study, field surveys covered 36 loquat orchards located in six different provinces of Spain. It is possible that a wider survey of a larger number of orchards in Sicily would reveal more species of *Botryosphaeriaceae*.

Of the four species found *N. vitifusiforme* is reported for the first time on loquat. This species has been previously found in Italy on olive (Lazzizera et al. 2008b) and grapevine (Mondello et al. 2013). *Neofusicoccum parvum* and *D. seriata* are cosmopolitan species known to occur on a very wide range of hosts (Phillips et al. 2013) and have been previously reported on loquat in Spain (González-Domínguez et al. 2016). In the survey performed by González-Domínguez et al. (2016) *D. seriata* was the most prevalent species isolated from loquat cankers. On the contrary, notwithstanding the small number of isolates obtained in this study, *D. seriata* was not the most frequent species. Nevertheless, *D. seriata* appears to be an important pathogen on loquat. This is further reinforced by the recent report of this fungus as a postharvest pathogen of loquat fruit (Palou et al. 2013).

A new *Diplodia* species was described here. This species was first isolated from firethorn by Phillips et al. (2012) who recognized differences between it (*Diplodia* sp. isolate CAP330) and *D. intermedia*, but chose not to introduce a new species until more isolates were available. More recently, González-Domínguez et al. (2016) isolated a *Diplodia* sp. (isolate BN-67) from loquat plants in Spain that was closely related to the one reported by Phillips et al. (2012) but did not provide a formal description of the species. Our study confirms that the *Diplodia* sp. obtained here and those previously reported on firethorn in Bulgaria and loquat in Spain represent a distinct species for which the name *D. rosacearum* is introduced.

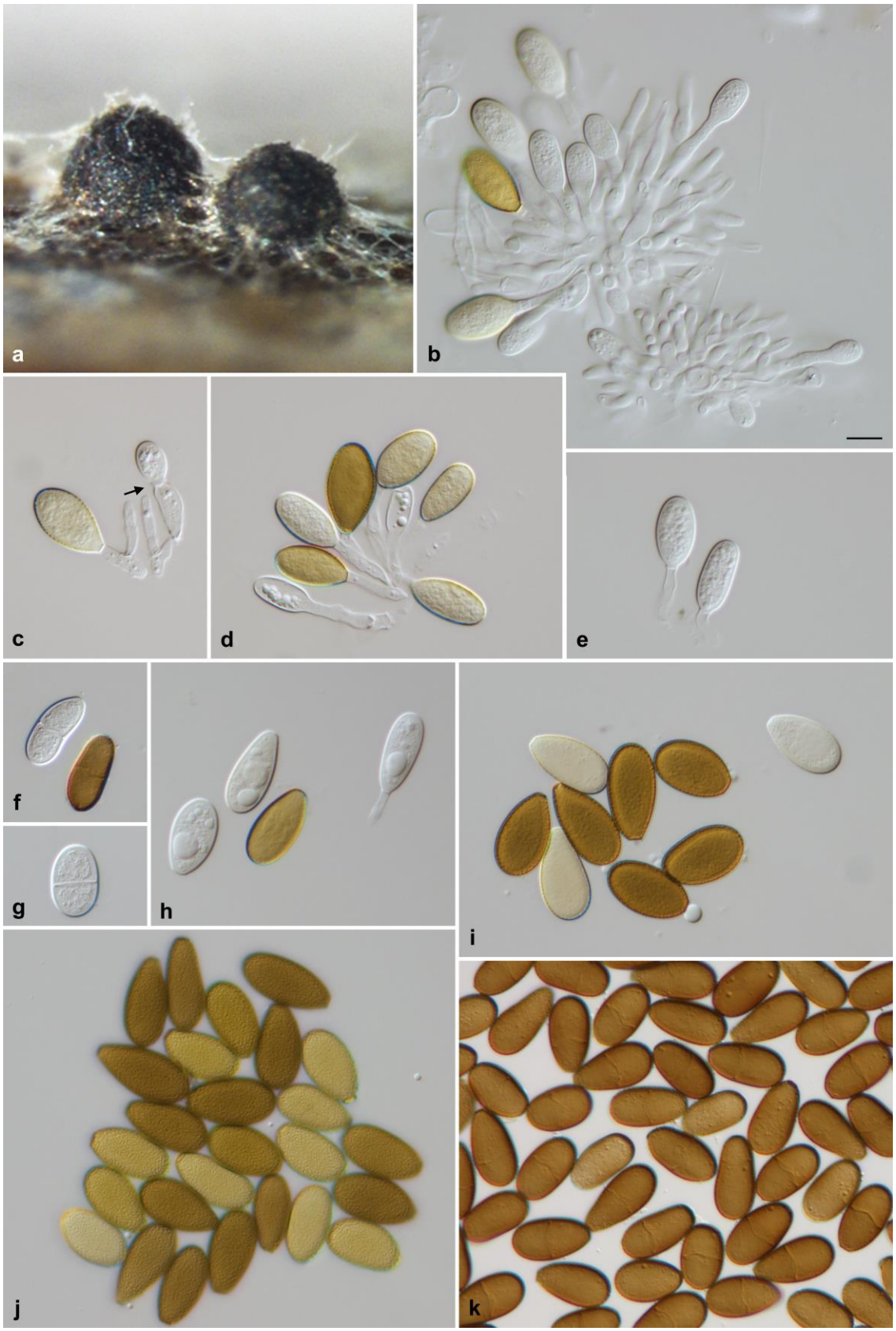

Fig. 3 – *Diplodia rosacearum* NB7 (CBS 141915). **a**. pycnidia formed on poplar twigs. **b**. conidia developing on conidiogenous cells. **c,d,e**. conidia attached to conidiogenous cell, arrow indicates annellations. **f,g**. hyaline septate and brown septate conidia. **h,i**. conidia showing several stages of colour development. **j**. conidia showing verruculose inner surface of the wall. **k**. mature brown aseptate and 1-septate conidia. Scale bar: b–k = 10 μm.

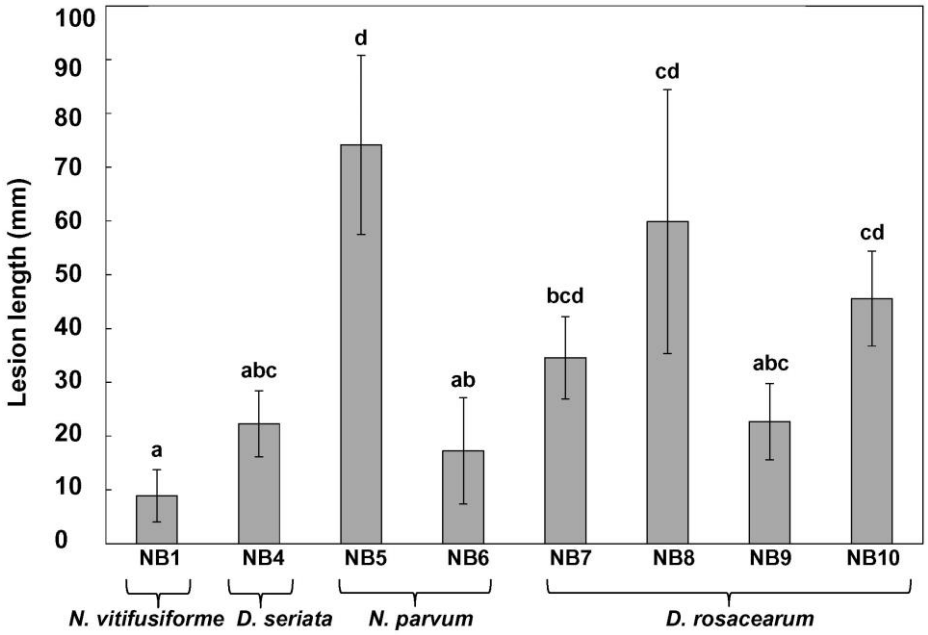

Fig. 4 – Mean internal lesion length (mm) in stems of loquat plants cv. Sanfilipparo inoculated with isolates of *D. rosacearum*, *D. seriata*, *N. parvum* and *N. vitifusiforme*. The vertical lines indicate standard errors. Bars with the same letter do not differ significantly according to Fisher's LSD test (P <0.05).

Although some differences in aggressiveness were detected between isolates, *N. parvum*, *D. seriata* and *D. rosacearum* caused larger necrotic lesions on inoculated plants than *N. vitifusiforme*. In this last species two of the three isolates tested caused no lesions on inoculated plants, thus suggesting that *N. vitifusiforme* is non-pathogenic or weakly pathogenic to loquat. However, future studies with more isolates should be performed to confirm this. *Diplodia rosacearum* was the most aggressive of the species tested, which is in agreement with the report of González-Domínguez et al. (2016). In their study, *D. rosacearum* (as *Diplodia* sp.) was the most aggressive species causing death of all inoculated plants.

In the orchard surveyed only plants from cultivars Algerie and Bueno showed disease symptoms, although several different cultivars were present. This suggests that there may be some variation in susceptibility among cultivars, with cultivars Algerie and Bueno being apparently more susceptible. However, more studies are needed to confirm this hypothesis. Nevertheless, all species were shown to be pathogenic to cultivar Sanfilipparo in artificial inoculation assays.

Additionally there was some apparent cultivar preference among the fungal species found with *Neofusicoccum* species occurring on cultivar Algerie plants only and *Diplodia* species occurring on cultivar Bueno plants only. However, the isolates studied were obtained from a small number of plants and it is thus necessary to carry out a wider sampling in order to evaluate if this cultivar preference is not an artifact. Besides, *Diplodia* species have been shown to be pathogenic to cultivar Algerie plants with *D. rosacearum* being highly aggressive (González-Domínguez et al. 2016).

It is interesting to notice that although loquat is a native and important crop in Asia there are no reports of *Botryosphaeriaceae* species affecting loquat from that part of the world. It is not known if these fungi have passed unnoticed in previous studies or if they are emerging as pathogens of this host. Nevertheless, this study and previous ones (González-Domínguez et al. 2016, Palou et al. 2013) show that species in the *Botryosphaeriaceae* should be considered as important pathogens of loquat. More studies should be carried out in other loquat growing regions to estimate the overall diversity and pathogenicity of these fungi. In particular, the new species *D. rosacearum* due to its apparent high aggressiveness deserves more attention on loquat as well as other hosts in the Rosaceae.

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