Phylogeny and morphology of *Diplodia* species on olives in southern Italy and description of *Diplodia olivarum* sp. nov.

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During a recent study of *Botryosphaeria* and *Neofusicoccum* species on olives, a number of *Diplodia* species were isolated. Most of these were *Diplodia seriata* while others resembled *Diplodia mutila* in their hyaline, aseptate, thick-walled conidia. These latter isolates were morphologically (conidial dimensions) and phylogenetically (ITS and EF1-α sequences) distinct from other *Diplodia* species and are described here as *Diplodia olivarum* sp. nov.

Key words: *Botryosphaeriaceae*, ITS, *Olea europaea*, phylogeny, taxonomy

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Introduction

Species of *Diplodia* have a worldwide distribution and are known to be pathogens, endophytes and latent pathogens of a variety of woody hosts (Barr, 1987; von Arx, 1987; Crous *et al*., 2006; Slippers and Wingfield, 2007). They are associated with various symptoms including shoot blights, dieback, cankers and fruit rots. Some *Diplodia* species have wide host ranges, for example *Diplodia seriata* De Not. (="*Botryosphaeria*" *obtusa* (Schwein.) Shoemaker) (Phillips *et al*., 2007), which has been recorded on more than 250 hosts (Farr *et al*., 2008) On the other hand, some species appear to be restricted to a single host genus, or to closely related genera. For example, *Diplodia pinea* (Desm.) J. Kickx fil. (= *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton) and *D. scrobiculata* J. de Wet, Slippers & M.J. Wingf. occur only on conifers, *D. corticola* A.J.L. Phillips, A. Alves & J. Luque has been found only on *Quercus* species (Alves *et al*., 2004) while *Diplodia cupressi* A.J.L. Phillips & A. Alves has been recorded only on *Cupressus* and *Juniperus* species (Alves *et al*., 2006). However, *Diplodia mutila* Fr., the type species of the genus, has been associated with a variety of hosts (Farr *et al*., 2008).

Based on the type species (*D. mutila*) *Diplodia* is circumscribed by having uni- or multilocular conidiomata lined with conidiogenous cells that form hyaline, aseptate, thick-walled conidia at their tips (Phillips *et al*., 2005). Typically the conidia remain hyaline for a long time before they become brown and septate, but in *D. seriata*, *D. pinea* and *D. scrobiculata* the conidia become brown before discharge from the pycnidia.

For many years species in *Diplodia* were described on the basis of host association with the result that more than 1000 species have now been described. A search of MycoBank (January 2008; www.mycobank.org) revealed 1176 names while Species Fungorum (January 2008; www.speciesfungorum.org) lists 1236 names. Since host is no longer considered to be of primary importance in species differentiation in the *Botryosphaeriaceae* (Slippers *et al*., 2004) it is likely that many of these names are

synonyms. At present, species are defined according to the dimensions and colour of their conidia.

Species of *Diplodia* that occur on olives have not been studied in detail and only six reports of *Diplodia* on olives are listed by Farr *et al*. (2008). Recently, *D. seriata* was reported on olive drupes in Spain (Moral *et al*., 2008). A search of the literature showed that *D. oleae* De Not. and *D. elaeophila* Sacc. & Roum. are the only other *Diplodia* species that have been reported from this host. However, *D. oleae* was recently shown to be a synonym of *Coleonaema oleae* (DC.) Höhn. (= *Coleophoma oleae* (DC.) Petr. & Syd.) (Duan *et al*., 2007).

In the present paper, *Diplodia* species on rotting olive drupes were surveyed in the main olive producing regions of southern Italy. Phylogenetic relationships between the isolates were studied through analysis of nucleotide sequences of the 5.8S ribosomal gene and it flanking internal transcribed spacers, ITS1 and ITS2 (ITS) and partial sequences of the translation elongation factor gene (EF1-α).

Materials and methods

Isolates

Isolations were made by directly plating out pieces of diseased olive drupes on PDA after surface sterilization in 70% ethanol for 2 minutes. Isolates were cultured on half-strength PDA (1/2 PDA) or on water agar supplemented with autoclaved pine needles placed on the agar surface. Cultures were kept on the laboratory bench at about 20-25°C where they received diffused daylight. Growth rates were determined on PDA plates incubated in the dark at 25°C. Representative cultures and specimens were deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

DNA isolation and amplification

DNA was isolated from fungal mycelium by the method of Möller *et al*. (1992). Procedures and protocols for DNA sequencing were as described in 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 (Ebersberg, 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 EF1-688F and EF1-986R (Alves *et al*., 2008) were used to amplify part of the translation elongation factor 1-alpha (EF1-α) as described by Phillips *et al*. (2005). The amplified PCR fragments were purified with the JETQUICK PCR Purification Spin Kit (GENOMED, Löhne, Germany). Both strands of the PCR products were sequenced by STAB Vida Lda (Portugal). The nucleotide sequences were read and edited with FinchTV 1.4.0 (http://www.geospiza.com/finchtv). Sequences were checked manually and nucleotide arrangements at ambiguous positions were clarified using both primer direction sequences. Sequences were deposited in GenBank (Table 1) and the alignments in TreeBase. Nucleotide sequences of additional isolates were retrieved from GenBank (Table 1).

Phylogenetic analyses

The sequences were aligned with ClustalX version 1.83 (Thompson *et al*., 1997) using the following parameters: pairwise alignment gap opening = 10, gap extension = 0.1 and multiple alignment gap-opening = 10, gap extension =0.2, delay divergent sequences = 25% and transition weight = 0.5. Alignments were checked and manual adjustments were made where necessary.

Phylogenetic analyses were done using PAUP* v.4.0b10 (Swofford, 2003). The trees were rooted to *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. and *Neofusicoccum luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips and visualized with TreeView (Page, 1996).

The HKY85 nucleotide substitution model (Hasegawa *et al*., 1985) was used for distance analysis. All characters were unordered and of equal weight. Bootstrap values were obtained from 1000 NJ bootstrap replicates. MP analyses were performed using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the tree swapping algorithm. All characters

Table 1. Isolates included in the phylogenetic study.

Isolate number[1]	Species	Host	Collector	Locality	GenBank[2]	
					ITS	EF
CBS119049	*D. seriata*	*Vitis vinifera*	L. Mugnai	Italy	*DQ458889*	*DQ458874*
CAP154	*D. seriata*	*Vitis vinifera*	P. Larignon	France	EU392303	EU392280
CAP160	*D. seriata*	*Vitis vinifera*	L. Mugnai	Italy,	EU392304	EU392281
CAP171	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Ruffano	EU392305	EU392282
CAP172	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Ruffano	EU392287	EU392264
CBS121884	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Lucugnano	EU392288	EU392265
CBS121885	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Casarano	EU392289	EU392266
CAP206	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Ruffano	EU392290	EU392267
CAP207	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Matino	EU392291	EU392268
CAP208	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Ugento	EU392292	EU392269
CAP217	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Ruffano	EU392293	EU392270
CAP220	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Felline	EU392294	EU392271
CAP228	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Supersano	EU392298	EU392275
CAP229	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Brindisi, Brindisi	EU392299	EU392276
CAP230	*D. seriata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Spongano	EU392300	EU392277
CBS112555	*D. seriata*	*Vitis vinifera*	A.J.L. Phillips	Portugal, Alentejo, Montemor-o-Novo	*AY259093*	*AY573219*
CAP166	*D. pinea*	*Olea europaea*	S. Frisullo	Italy, Basilicata, Matera, Scanzano	EU392284	EU392261
CAP168	*D. pinea*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Scorano	EU392285	EU392262
CAP169	*D. pinea*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Cutrofiano	EU392286	EU392263
CBS393.84	*D. pinea* "A"	*Pinus nigra*	H.A. van der Aa	Netherlands, Putten	*DQ458895*	*DQ458880*
CBS109727	*D. pinea* "A"	*Pinus radiata*	W.J. Swart	South Africa, Stellenbosch	*DQ458897*	*DQ458882*
CBS109725	*D. pinea* "C"	*Pinus patula*	M.J. Wingfield	Indonesia, Habinsaran	*DQ458896*	*DQ458881*
CBS109943	*D. pinea* "C"	*Pinus patula*	M.J. Wingfield	Indonesia	*DQ458898*	*DQ458883*
CAP163	*D. scrobiculata*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Supersano	EU392283	EU392260
CBS109944	*D. scrobiculata*	*Pinus greggii*	M.J. Wingfield	Mexico	*DQ458899*	*DQ458884*
CBS113423	*D. scrobiculata*	*Pinus greggii*	M.J. Wingfield	Mexico	*DQ458900*	*DQ458885*
CAP222	*D. olivarum*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Cutrofiano	EU392295	EU392272
CAP224	*D. olivarum*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Salice Salentino	EU392296	EU392273
CAP225	*D. olivarum*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Campi Salentino	EU392297	EU392274
CBS121886	*D. olivarum*	*Olea europaea*	S. Frisullo	Italy, Puglia, Brindisi, San Pietro Vernotico	EU392301	EU392278
CBS121887	*D. olivarum*	*Olea europaea*	S. Frisullo	Italy, Puglia, Lecce, Bosco Belvedere, Scorrano	EU392302	EU392279
CBS230.30	*D. mutila*	*Phoenix dactylifera*	L.L. Huilllier	U.S.A., California	*DQ458886*	*DQ458869*
CBS112553	*D. mutila*	*Vitis vinifera*	A.J.L. Phillips	Portugal, Alentejo, Montemor-o-Novo	*AY259093*	*AY573219*
CBS168.87	*D. cupressi*	*Cupressus sempervirens*	Z. Solel	Israel, Bet Dagan	*DQ458893*	*DQ458878*
CBS261.85	*D. cupressi*	*Cupressus sempervirens*	Z. Solel	Israel, Bet Dagan	*DQ458894*	*DQ458879*
CBS418.64	*B. tsugae*	*Tsuga heterophylla*	A. Funk	Canada, British Columbia	*DQ458888*	*DQ458873*
CBS112549	*D. corticola*	*Quercus suber*	A. Alves	Portugal, Aveiro	*AY259100*	*AY573227*

Table 1 (continued). Isolates included in the phylogenetic study.

Isolate number[1]	Species	Host	Collector	Locality	GenBank[2]	
					ITS	EF
CBS112547	*D. corticola*	*Quercus ilex*	M.E. Sánchez, A. Trapero	Spain, Córdoba	*AY259110*	*DQ458872*
CBS116470	*D. rosulata*	*Prunus africana*	A. Gure	Ethiopia, Gambo	EU430265	EU430267
CBS116472	*D. rosulata*	*Prunus africana*	A. Gure	Ethiopia,Gambo	EU430266	EU430268
CBS110299	*N. luteum*	*Vitis vinifera*	A.J.L. Phillips	Portugal, Oeiras	*AY259091*	*AY573217*
CBS110302	*B. dothidea*	*Vitis vinifera*	A.J.L. Phillips	Portugal, Alentejo, Montemor-o-Novo	*AY259092*	*AY573218*
CBS120835	*D. africana*	*Prunus persica*	U. Damm	South Africa, Western Cape, Paarl	*EF445343*	*EF445382*
CBS121104	*D.africana*	*Prunus persica*	U. Damm	South Africa, Western Cape, Paarl	*EF445344*	*EF445383*

[1]Acronyms of culture collections: CAP – A.J.L. Phillips, Centro de Recursos Microbiológicos, Portugal; CBS – Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. Cultures in bold type are ex-type.
[2]Sequences in italics were retrieved from GenBank. All others were obtained in this study.

were unordered and of equal weight and gaps were treated as fifth character. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis and Bull, 1993). Other measures used were consistency index (CI), retention index (RI), and homoplasy index (HI). A partition homogeneity test was done in PAUP to assess the validity of combining the ITS and EF1-α data.

Results

Phylogenetic analyses

Approximately 550 and 300 bases were determined for the ITS and EF1-α genes, respectively. New sequences were deposited in GenBank (Table 1) and the alignments in TreeBase (SN3760). The sequence alignment of 44 isolates (Table 1), including the two outgroup isolates, consisted of 552 characters for the ITS region and 332 for the EF1-α gene including alignment gaps. A partition homogeneity test showed no significant differences (P = 0.330) between the data from the two gene regions indicating that they could be combined in a single dataset.

The combined dataset consisted of 844 characters of which 562 were constant and 76 were parsimony-uninformative. Maximum parsimony analysis of the remaining 206 parsimony-informative characters resulted in two trees of 428 steps with low levels of homoplasy (HI = 0.150). NJ analysis resulted in a tree with the same topology as the MP tree. The MP tree (Fig. 1) consists of two major clades, one that corresponds to species with conidia that darken before discharge and remain mostly aseptate (clade A), and another (clade B) that corresponds to species with conidia that remain hyaline for a considerable time before darkening and becoming one-septate only after discharge. Clade A was composed of three sub-clades that correspond to *Diplodia seriata*, *D. pinea* and *D. scrobiculata*. Most of the isolates from olives (12 isolates) fell within the *D. seriata* clade but three isolates grouped with *D. pinea* morphotype A and one in a clade sister to *D. scrobiculata*. This latter isolate was separated from *D. scrobiculata* by one bp in ITS and four bp in EF.

Clade B was composed of six sub-clades corresponding to "*B*". *tsugae*, *D. cupressi*, *D. rosulata*, *D. mutila*, *D. africana* Damm & Crous and five isolates from olives. All six clades were supported by moderately high NJ and MP bootstrap values. Since the isolates from olives were phylogenetically and morphologically distinct from the other species in this clade they are described here as new.

Morphology

Diplodia olivarum A.J.L. Phillips, Frisullo & Lazzizera, **sp. nov.** (Figs 2-7)
MycoBank 511402

Etymology: Named after its host the European olive.

Diplodiae mutilae similis sed conidiis minoribus (21.5-)22-27.5(-28.5) × (10-)11-13.5(-14.5), in medio 24.4 × 12.4 µm.

Conidiomata pycnidial, produced on pine needles on WA after 7-14 days, solitary, globose to ovoid, dark brown to black, up to 150 µm wide, wall composed of dark brown, thick-walled *textura angularis*, becoming thin-walled and hyaline towards the inner region, semi-immersed to erumpent, unilocular, with a short neck. *Ostiole* circular, central. *Conidiophores* hyaline, cylindrical, 10-15 × 3.5-5 µm. *Conidiogenous cells* 8-12 × 3-6 µm, hyaline, cylindrical, holoblastic forming a single conidium at the tip, proliferating internally to form periclinal thickenings or proliferating percurrently giving rise to 2-3 annellations. *Conidia* hyaline, aseptate, smooth, thick-walled, oblong to oval, widest in the middle, apex broadly rounded, base rounded or truncate, rarely becoming pale brown, internally verruculose, one septate after discharge from the pycnidia, (21.5-)22-27.5(-28.5) × (10-)11-13.5(-14.5) µm, 95% confidence intervals = 23.9-24.8 × 12.2-12.7 µm, $\bar{x}$ ± S.D. = 24.4 ± 1.6 × 12.4 ± 1 µm, L/W = 1.97 ± 0.17.

Teleomorph: not seen.

Habitat: On drupes of *Olea europaea*.

Known distribution: Italy.

Material examined: ITALY, Puglia, Lecce, Scorrano, Basco Belvedere, on rotting drupes of *Olea europaea*, December 2004, S. Frisullo (CBS-H 19914; **holotype designated here**, culture ex-type CBS 121887 = CAP254). Other isolates are given in Table 1.

Notes: This species is similar to *D. mutila* but the two species can be distinguished on

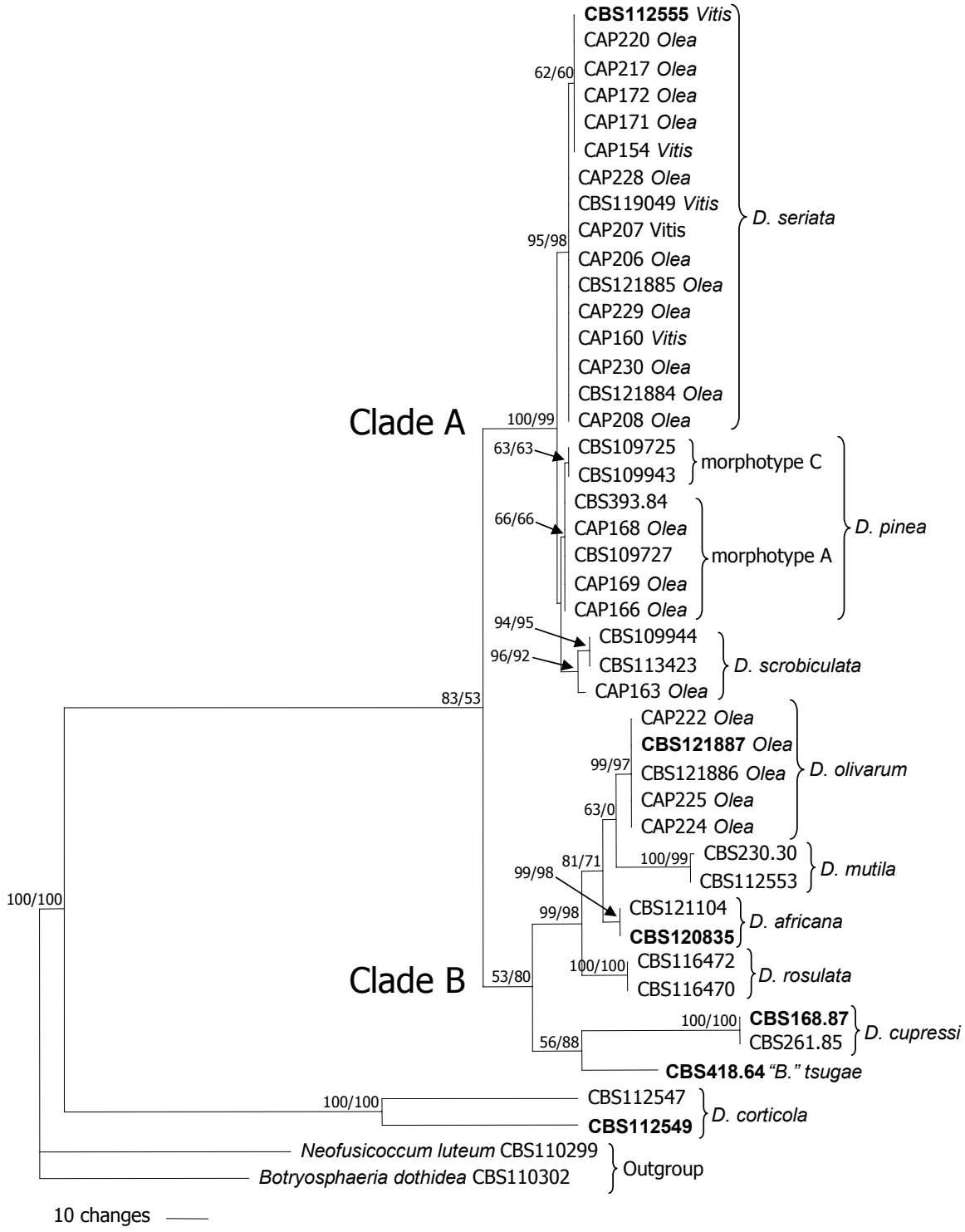
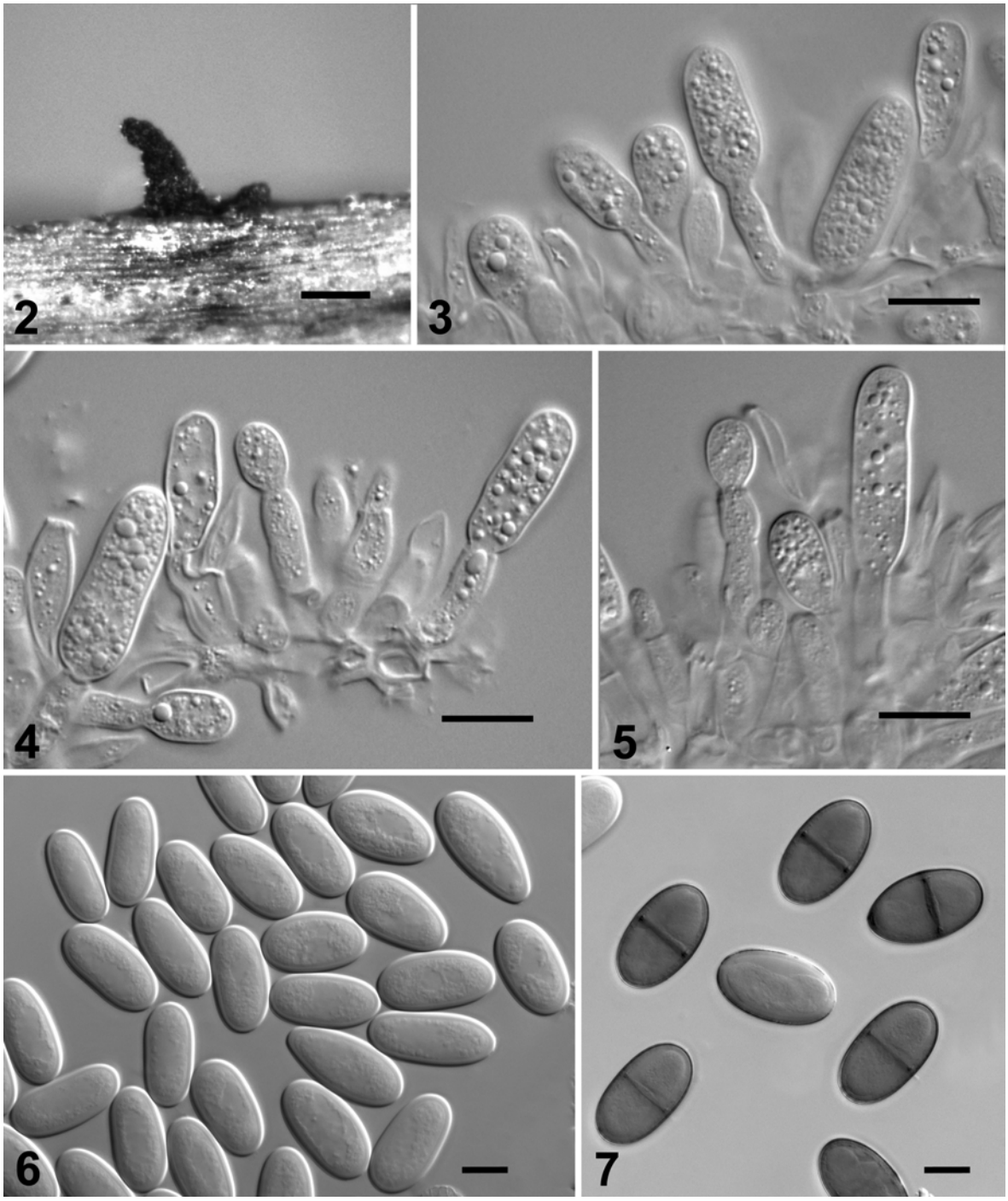


Fig. 1. One of two most parsimonious trees obtained from the combined analysis of ITS and EF1-α sequence data. Bootstrap values from 1000 pseudoreplicates are given at the nodes followed by NJ bootstrap values.



Figs 2-7. *Diplodia olivarum* (**from holotype**). **2.** Conidioma formed on pine needles in culture. **3-5.** Conidiogenous cells with developing conidia. **6.** Hyaline, aseptate conidia. **7.** Brown, one septate conidia and one hyaline, aseptate conidium. Bars: 2 = 100 µm; 3-7 = 10 µm.

minor differences in the dimensions of their conidia. Although the ranges of dimensions overlap considerably, mean dimensions of conidia of *D. olivarum* are smaller than *D. mutila*.

Discussion

This study revealed four *Diplodia* species, *D. seriata*, *D. pinea*, *D. scrobiculata* and *D. olivarum*, associated with diseased olive drupes in southern Italy. These four species could be distinguished on DNA sequence data (ITS and EF1-α) and unique morphological characteristics.

Most of the isolates were *Diplodia seriata*. This fungus was known for many years as

Botryosphaeria obtusa. However, in a study of phylogenetic lineages in *Botryosphaeria*, Crous *et al*. (2006) showed that *Botryosphaeria* is restricted to two species, namely *B*. *dothidea* and *B*. *corticis*. As a result, the name *B*. *obtusa* was no longer suitable and, furthermore, no valid anamorph name was available for this species. Subsequently, Phillips *et al*. (2007) determined that *Diplodia seriata* was the oldest suitable name available. This fungus is known to occur on a wide range of hosts (Punithalingam and Waller, 1973) and to cause significant disease in some, such as apple. Farr *et al*. (2008) list 264 hosts under its former name of *B*. *obtusa*. In recent years *D*. *seriata* has been recognized as a pathogen of *Vitis vinifera* in Portugal (Phillips, 1998, 2002), Australia (Castilho-Pando *et al*., 2001) and South Africa (van Niekerk *et al*., 2004). More recently it has been reported from olive drupes in Spain and has been proved to be a pathogen on this host (Moral *et al*., 2008). To our knowledge, this is the first report, of *D*. *seriata* on *O*. *europaea* in Italy.

Three isolates grouped closely with *Diplodia pinea*. This species is a well known pathogen of *Pinus* spp. worldwide (Punithalingam and Waterston, 1970). It has also been reported on *Abies*, *Araucaria*, *Chamaecyparis*, *Cupressus*, *Larix*, *Picea*, *Pseudotsuga* and *Thuja* (Punithalingam and Waterston, 1970; Sutton, 1980). There are also unconfirmed reports of it on *Eucalyptus* spp. in Uruguay (Bettucci *et al*., 1999; 2004), and recently it was reported on *Prunus* spp. in South Africa (Damm *et al*., 2007). The present study is the first report of *D*. *pinea* on *O*. *europaea*. The olive orchards sampled were surrounded by pine trees and it is likely that high inoculum pressure resulted in a few infections of the olives by *D*. *pinea*. For this reason we suspect that *D*. *pinea* is an opportunist on *Olea*, which should not be regarded as a major host. In a similar way, Damm *et al*. (2007) noted that *D*. *pinea* was isolated from pycnidia on the bark of pruning debris of *Prunus* and was not associated with necrosis within the host tissue.

A single isolate clustered close to *D*. *scrobiculata*, from which it differed by one bp in ITS and four in EF1-α. These differences were not considered to be significant and probably represent normal variation within *D*. *scrobiculata*. This is the first time that *D*. *scrobiculata* has been reported on a host other than *Pinus*. As with *D*. *pinea*, it is probable that the high levels of inoculum from the surrounding pine trees resulted in this infection and it is unlikely that *D*. *scrobiculata* is a primary pathogen of olives.

Diplodia olivarum is recognized as a new species closely related to *D*. *mutila*, *D*. *rosulata* and *D*. *africana*. It has morphological features typical of the genus, namely hyaline, aseptate, thick-walled conidia that ultimately turn brown and become one-septate (Phillips *et al*., 2005). Although *D*. *olivarum* resembles *D*. *mutila* and *D*. *rosulata*, the three species can be separated on the size of their conidia and colony characters. Thus, colonies of *D*. *rosulata* typically have a rosulate margin, which is not seen in the other two species. Conidia of *D*. *olivarum* are smaller than those of *D*. *africana*. Although the range of conidial dimensions of *D*. *olivarum* and *D*. *mutila* overlap, these two species can be distinguished on the mean dimensions, which are smaller in *D*. *olivarum*.

Diplodia olivarum was isolated from several different olive groves in southern Italy and was associated with rotting drupes. However, pathogenicity has not been tested and it is not clear if *D*. *olivarum* was the primary cause of the disease. Furthermore, it is not known if this fungus occurs on other hosts or if it is restricted to olives.

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References

Alves, A., Correia, A., Luque, J. and Phillips, A.J.L. (2004). *Botryosphaeria corticola* sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph *Diplodia mutila*. Mycologia 96: 598-613.

Alves, A., Correia, A. and Phillips, A.J.L. (2006). Multigene genealogies and morphological data

support *Diplodia cupressi* sp. nov., previously recognized as *Diplodia pinea* f. sp. *cupressi* as a distinct species. Fungal Diversity 23: 1-15.

Alves, A., Crous, P.W., Correia, A. and Phillips, A.J.L. (2008). Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity 28: 1-13.

Arx, J.A. von (1987). *Plant Pathogenic Fungi*. J. Cramer, Berlin Germany.

Barr, M.E. (1987). Prodromus to Class *Loculoascomycetes*. Amherst, Massachusetts: Published by the author.

Bettucci, L., Alonso, R. and Tiscornia, S. (1999). Endophytic mycobiota of healthy twigs and the assemblage of species associated with twig lesions of *Eucalyptus globulus* and *E. grandis* in Uruguay. Mycological Research 103: 468-472.

Bettucci, L., Simento, S., Alonso, R. and Lupo, S. (2004). Endophytic fungi of twigs and leaves of three native species of Myrtaceae in Uruguay. Sydowia 56: 8-23.

Castilho-Pando, M., Somers, A., Green, C.D., Priest, M. and Srisjathades, M. (2001). Fungi associated with a dieback of Semillon gravines in the Hunter Valley of New South Wales. Australasian Plant Pathology 30: 59-63.

Crous, P.W., Slippers, B., Wingfield, M.J., Reeder, J., Marasas, W.F.O., Phillips, A.J.L., Alves A., Burgess, T., Barber, P. and Groenewald, J.Z. (2006). Phylogenetic lineages in the *Botryosphaeriaceae*. Studies in Mycology 55: 235-253.

Damm, U., Crous, P.W. and Fourie, P.H. (2007). *Botryosphaeriaceae* as potential pathogens of Prunus species in South Africa with desciptions of *Diplodia africana* and *Lasiodiplodia plurivora* spp. nov. Mycologia 99: 664-680.

Duan, J., Liu, X. and Wu, W. (2007). Reinstatement of *Coleonaema* for *Coleophoma oleae* and notes on *Coleophoma*. Fungal Diversity 26: 187-204.

Farr, D.F., Rossman, A.Y., Palm, M.E., and McCray, E.B. (2008) Fungal Databases, Systematic Botany & Mycology Laboratory, ARS, USDA. Retrieved January 17, 2008, from http://nt.ars-grin.gov/fungaldatabases/.

Hasegawa, M., Kishino, H. and Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22: 160-174.

Hillis, D.M. and Bull, J.J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182-192.

Möller, E.M., Bahnweg, G., Sndermann, H. and Geiger, H.H. (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Research 20: 6115-6116.

Moral, J., Luque, F., and Trapero A. (2008). First report of *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*, causing fruit rot of olive in Spain. Plant Disease 92: 311.

Page, R.D. (1996). TreeView: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.

Phillips, A.J.L. (1998). *Botryosphaeria dothidea* and other fungi associated with excoriose and dieback of grapevines in Portugal. Journal of Phytopathology 146: 327-332.

Phillips, A.J.L. (2002). *Botryosphaeria* species associated with diseases of grapevines in Portugal. Phytopathologia Mediterranea 41: 318.

Phillips, A.J.L., Alves, A., Correia, A. and Luque, J. (2005). Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. Mycologia 97: 513-529.

Phillips, A.J.L., Crous, P.W. and Alves, A. (2007). *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*. Fungal Diversity 25: 141-155.

Punithalingam, E, and Waterston, J.M. (1970). *Diplodia pinea*. Desciptions of pathogenic fungi and bacteria, No. 273. Commonwealth Mycological Institute, Kew, Surrey, England.

Punithalingam, E. and Waller, J.M. (1973). *Botryosphaeria obtusa*. *Descriptions of pathogenic fungi and bacteria*, *No. 394*. Commonwealth Mycological Institute, Kew, Surrey, England.

Slippers, B., Crous, P.W., Denman, S., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2004). Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96: 83-101.

Slippers, B. and Wingfield, M.J. (2007). *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. Fungal Biology Reviews 21: 90-106.

Sutton, B.C. (1980). *The Coelomycetes*. Kew, UK: Commonwealth Mycological Institute.

Swofford, D.L. (2003). *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods) Version 4*. Sunderland, Massachusetts: Sinauer Associates.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876-4882.

Van Niekerk, J.M., Crous, P.W., Groenewald, J.Z., Fourie, P.H. and Halleen, F. (2004). DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. Mycologia 96: 781-798.