ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/40104638

Diplodia seriata, the anamorph of "Botryosphaeria" obtusa

Article *in* Fungal diversity · April 2007

Source: OAI

CITATIONS
89

READS
385

3 authors:

Alan John Lander Phillips
University of Lisbon
438 PUBLICATIONS **5,277** CITATIONS
SEE PROFILE

Pedro W Crous
Westerdijk Fungal Biodiversity Institute
1,987 PUBLICATIONS **34,014** CITATIONS
SEE PROFILE

Artur Alves
University of Aveiro
383 PUBLICATIONS **2,853** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

COST Action FP1406 PINESTRENGTH "Pine pitch canker - strategies for management of Gibberella Circinata in greenhouses and forests" View project

Citizen Science Project: Searching for new fungal taxa from soil samples all over the Netherlands View project

All content following this page was uploaded by Artur Alves on 20 May 2014.

The user has requested enhancement of the downloaded file.

Diplodia seriata, the anamorph of "*Botryosphaeria*" *obtusa*

Alan J.L. Phillips[1*], Pedro W. Crous[2] and Artur Alves[3]

[1]Centro de Recursos Microbiológicos, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[2]CBS Fungal Biodiversity Centre, P.O. Box 85167, 5508 AD Utrecht, The Netherlands
[3]Centro de Estudos do Ambiente e do Mar, Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

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

The correct name to use for the anamorph of *Botryosphaeria obtusa* has been uncertain for many years. Since the genus name *Botryophaeria* is no longer available for this fungus the question of the correct *Diplodia* anamorph name must be resolved. Therefore, the aims of this work were to determine the correct name to apply to the anamorph of "*B.*" *obtusa* through a study of relevant type specimens. The phylogenetic relationship of the species to its nearest relatives, and phylogenetic variation within the species were determined through a study of ITS sequence data. The species was shown to be relatively homogenous, cosmopolitan and plurivorous. The name *Diplodia seriata* was shown to be the oldest suitable name available. An epitype specimen is designated.

Key words: *Botryosphaeria obtusa*, *Diplodia*, ITS, nomenclature, phylogeny, *Sphaeropsis*, taxonomy

Introduction

The fungus known for many years as *Botryosphaeria obtusa* (Schwein.) Shoemaker is an important pathogen of apples causing frog-eye spot, black rot, canker and shoot dieback. In addition to apples, *B. obtusa* has been isolated from at least 34 different hosts (Punithalingam and Walker, 1973). In recent years it has been recognized as a pathogen of *Vitis vinifera* in Portugal (Phillips, 1998, 2002), Australia (Castillo-Pando *et al*., 2001) and South Africa (van Niekerk *et al*., 2004).

The fungus was first described by Schweinitz (1832) as *Sphaeria obtusa* Schwein., and later Cooke (1892) transferred it to *Physalospora obtusa* (Schwein.) Cooke. Shoemaker (1964) considered this to be a species of *Botryosphaeria* Ces. & De Not., and introduced the new combination *Botryosphaeria obtusa*. The connection between the teleomorph and anamorph

[*]Corresponding author: Alan Philips; e-mail: alp@fct.unl.pt

was established by Hesler (1916) and confirmed by Shear *et al*. (1925) and Stevens (1936).

On account of its brown, aseptate conidia formed on phialides, proliferating via periclinal thickening or annellations, and lining the inner wall of pycnidial conidiomata, the anamorph of *Botryosphaeria obtusa* clearly belongs in *Diplodia* Fr. However, the correct name for the species has been the subject of much confusion and discussion. In the past the debate has mainly revolved around the names *Sphaeropsis malorum* (Berk.) Berk. and *S. malorum* Peck.

Peck (1880) found what he considered to be the conidial state of *B. obtusa* in New York, and reported it as *Sphaeropsis malorum* (Berk.) Berk. According to Stevens (1933), *S. malorum* (Berk.) Berk. is a synonym of *Diplodia mutila* Fr., which is the anamorph of *Botryosphaeria stevensii* Shoemaker and has hyaline conidia. Stevens (1933) studied Peck's collection and found dark aseptate conidia, thus showing that Peck did in fact have the conidial state of *B. obtusa*.

The anamorph has also been referred to as *S. malorum* Peck. This name came about when Saccardo (1884) transferred *S. malorum* (Berk.) Berk. to the genus *Phoma* on account of its hyaline conidia. Because Peck's collection had brown conidia, Saccardo considered it not the same as Berkley's collection, and used the name *S. malorum* Peck. Thus, Peck did not name a new species and even if he had proposed the name *S. malorum* in 1880, it would be an illegitimate later homonym of *S. malorum* (Berk.) Berk. (1860). Since *S. malorum* Peck is illegitimate and *S. malorum* (Berk.) Berk. is a synonym of *D. mutila*, neither of these names can be used for the anamorph of "*Botryosphaeria*" *obtusa*.

Slippers *et al*. (2007) initially regarded *Diplodia malorum* Fuckel to be a more appropriate name. However, after studying the type specimen in G (Fungi rhenani 1706) they rejected this possibility. Apparently the two specimens of the exsiccata in G contain spores of two species of *Diplodia*, but the morphology of neither corresponds to the anamorph of "*Botryosphaeria*" *obtusa*. Therefore *D. malorum* is not the anamorph of "*Botryosphaeria*" *obtusa*.

The uncertainty over the correct name for the anamorph was never resolved mainly because the teleomorph name had priority and was used for both states of the fungus. However, the genus *Botryosphaeria* was re-evaluated recently and determined to be composed of a number of phylogenetic lineages that represent individual genera (Crous *et al*., 2006). As a result, *Botryosphaeria* is now recognised to be a relatively small genus consisting only of *B. dothidea* (Moug. : Fr.) Ces. & De Not. (the type species of the

genus) and *B. corticis* (Demaree & M.S. Wilcox) Arx & Müll. (a species restricted to *Vaccinium* spp., Phillips *et al*., 2006). Consequently, the name *Botryosphaeria* is no longer available for most of the species of "*Botryosphaeria*", including those with *Diplodia* anamorphs. When Crous *et al*. (2006) proposed the various genera within what was known as *Botryosphaeria*, they anticipated a system of a single name for species of fungi as proposed by Rossman and Samuels (2005). For this reason, new teleomorph genera were not proposed in instances where acceptable anamorph generic names were available, as in the case of *Diplodia* and "*Botryosphaeria*" *obtusa*. For most species this was perfectly acceptable because anamorph names are available. However, as explained above, no acceptable name is available for the anamorph "*Botryosphaeria*" *obtusa*.

We have recently epitypified *Botryosphaeria corticis*, the cause of blueberry cane canker (Phillips *et al*., 2006) as this will better help us to understand the biology of the species. The aim of the present work was to determine, through a study of appropriate herbarium specimens, the correct name for the anamorph of "*Botryosphaeria*" *obtusa*. Phylogenetic relationships among isolates identified as "*B.*" *obtusa* were determined through a study of ITS sequence data.

Materials and methods

Isolates

Single conidial isolates were prepared according to the methods described by Alves *et al*. (2004).

DNA isolation, PCR amplification and sequencing

Procedures and protocols for DNA isolation and sequencing were as explained in Alves *et al*. (2004). Nucleotide sequences of additional *Botryosphaeria* species were retrieved from GenBank (Table 1).

Phylogenetic analyses

ITS sequences of 40 isolates identified as "*Botryosphaeria*" *obtusa* and 14 isolates representing 6 species of *Diplodia* were retrieved from GenBank (Table 1). The ITS 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

Table 1. Isolates used in the phylogenetic analysis.

Isolate number	Identity	Host	Locality	Collector	ITS GenBank
5-16-1DEX1	*"Botryosphaeria" obtusa*	*Vitis vinifera*	California, U.S.A.	L. Epstein	AY662399
5-17-31EX2	*"Botryosphaeria" obtusa*	*Vitis vinifera*	California, U.S.A.	L. Epstein	AY662401
CBS200.49	*"Botryosphaeria" obtusa*	*Alnus* sp.	Germany	J.A. von Arx	EF127892
CBS112555	*"Botryosphaeria" obtusa*	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY259094
CBS112876	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343434
CBS119049	*"Botryosphaeria" obtusa*	*Vitis vinifera*	Italy	L. Mugnai	DQ458889
CMW568	*"Botryosphaeria" obtusa*	*Malus* sp.	Ceres, South Africa	W.A. Smit	DQ836726
CMW918	*"Botryosphaeria" obtusa*	*Pyrus communis*	Villiersdorp, South Africa	W.A. Smit	DQ836721
CMW986	*"Botryosphaeria" obtusa*	*Pyrus communis*	Hermanus, South Africa	W.A. Smit	DQ836722
CMW1050	*"Botryosphaeria" obtusa*	*Pyrus communis*	Koue Bokkeveld, South Africa	W.A. Smit	DQ836723
CMW1069	*"Botryosphaeria" obtusa*	*Prunus persica*	Robertson, South Africa	W.A. Smit	DQ836724
CMW1159	*"Botryosphaeria" obtusa*	*Prunus salicina*	Swellendam, South Africa	W.A. Smit	DQ836720
CMW1179	*"Botryosphaeria" obtusa*	*Populus* sp.	Ceres, South Africa	W.A. Smit	DQ836725
CMW7774	*"Botryosphaeria" obtusa*	*Ribes* sp.	New York, U.S.A.	B. Slippers/G. Hudler	AY236953
CMW7775	*"Botryosphaeria" obtusa*	*Ribes* sp.	New York, U.S.A.	B. Slippers/G. Hudler	AY236954
CMW8230	*"Botryosphaeria" obtusa*	*Picea glauca*	Canada	J. Reid	AY972104
CMW8232	*"Botryosphaeria" obtusa*	*Malus domestica*	South Africa	W.A. Smit	AY972105
KJ93.56	*"Botryosphaeria" obtusa*	Hardwood shrub	New York, U.S.A.	G. Samuels	AF027759
ATCC60851	*"Botryosphaeria" obtusa*	*Prunus persica*	Georgia, U.S.A.	K. Britton	AF243409
STE-U4440	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343420
STE-U4442	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343422
STE-U4444	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343424
STE-U4538	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343431
STE-U4539	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343432

Table 1. Isolates used in the phylogenetic analysis.

Isolate number	Identity	Host	Locality	Collector	ITS GenBank
STE-U4586	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343440
STE-U5031	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343443
STE-U5037	*"Botryosphaeria" obtusa*	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY343446
STE-U5052	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	J.M. van Niekerk	AY343449
STE-U5147	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	F. Halleen	AY343456
STE-U5162	*"Botryosphaeria" obtusa*	*Vitis vinifera*	South Africa	J.M. van Niekerk	AY343458
Thv1-9B	*"Botryosphaeria" obtusa*	*Vitis vinifera*	California, U.S.A.	L. Epstein	AY662400
UCD645So	*"Botryosphaeria" obtusa*	*Vitis vinifera*	California, U.S.A.	J.R. Urbez Torres	DQ008319
UCD710SJ	*"Botryosphaeria" obtusa*	*Vitis vinifera*	California, U.S.A.	J.R. Urbez Torres	DQ008321
UCDC343Spa	*"Botryosphaeria" obtusa*	*Vitis vinifera*	California, U.S.A.	J.R. Urbez Torres	DQ356354
V16	*"Botryosphaeria" obtusa*	*Vitis vinifera*	California, U.S.A.	L. Epstein	AY662398
WAC10196	*"Botryosphaeria" obtusa*	*Vitis vinifera*	W. Australia	T. Burgess	AY727844
WAC11070	*"Botryosphaeria" obtusa*	*Vitis vinifera*	W. Australia	T. Burgess	AY727840
WAC11071	*"Botryosphaeria" obtusa*	*Vitis vinifera*	W. Australia	T. Burgess	AY727845
WAC11073	*"Botryosphaeria" obtusa*	*Vitis vinifera*	W. Australia	T. Burgess	AY727842
WAC11342	*"Botryosphaeria" obtusa*	*Vitis vinifera*	W. Australia	T. Burgess	AY727839
CBS418.64	*"Botryosphaeria" tsugae*	*Tsuga heterophylla*	BC, Canada	A. Funk	DQ458888
CBS112545	*Diplodia corticola*	*Quercus suber*	Cádiz, Spain	M.E. Sánchez	AY259089
CBS112549	*Diplodia corticola*	*Quercus suber*	Aveiro, Portugal	A. Alves	AY259100
CBS168.87	*Diplodia cupressi*	*Cupressus sempervirens*	Bet Dagan, Israel	Z. Solel	DQ458893
CBS230.30	*Diplodia mutila*	*Phoenix dactylifera*	California, U.S.A.	L.L. Huillier	DQ458886
CBS261.85	*Diplodia cupressi*	*Cupressus sempervirens*	Bet Dagan, Israel	Z. Solel	DQ458894
CBS393.84	*Diplodia pinea* A	*Pinus nigra*	Putten, Netherlands	H.A. van der Aa	DQ458895
CBS112553	*Diplodia mutila*	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY259093
CBS109727	*Diplodia pinea* A	*Pinus radiata*	Stellenbosch, SA	W.J. Swart	DQ458897
CBS109725	*Diplodia pinea* C	*Pinus patula*	Habinsaran, Indonesia	M.J. Wingfield	DQ458896
CBS109943	*Diplodia pinea* C	*Pinus patula*	Indonesia	M.J. Wingfield	DQ458898
CBS109944	*Diplodia scrobiculata*	*Pinus greggii*	Mexico	M.J. Wingfield	DQ458899
CBS113423	*Diplodia scrobiculata*	*Pinus greggii*	Mexico	M.J. Wingfield	DQ458900

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 information contained in indels (gaps) was incorporated into the phylogenetic analysis using simple indel coding as implemented by GapCoder (Young and Healy, 2003).

Phylogenetic analyses of sequence data were done using PAUP* v.4.0b10 (Swofford, 2003) for Maximum-parsimony (MP) and Neighbour-joining (NJ) analyses and Mr Bayes v.3.0b4 (Ronquist and Huelsenbeck, 2003) for Bayesian analyses. The trees were rooted to *D. corticola* 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.

Maximum-parsimony analyses were performed using the heuristic search option with 1000 simple taxa additions and nearest-neighbour interchange (NNI) as the branch-swapping algorithm. All characters were unordered and of equal weight and alignment gaps were treated as missing data. 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 tree length (TL) consistency index (CI), retention index (RI) and homoplasy index (HI).

Bayesian analyses employing a Markov Chain Monte Carlo method with 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. 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 1000 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 done three times starting from different random trees to ensure that trees from the same tree space were being sampled during each analysis.

Morphology

Isolates were cultured on half strength PDA and incubated at 25°C for determination of cultural characters. For sporulation, isolates were grown on 2% water agar bearing autoclaved poplar twigs and kept in the laboratory at about 20 ± 5°C where they received indirect daylight. Conidia oozing from the

ostioles were mounted in 100% lactic acid. The conidiogenous layer was excised from pycnidia and mounted in 100% lactic acid. Herbarium specimens were rehydrated in 3% KOH and then rinsed with distilled water before mounting in 100% lactic acid. Microscope slides were prepared in the same way as described for the fresh cultures. Digital images were recorded with a Leica DCF320 camera with the ×100 objective lens. Dimensions were determined with the Leica IM 500 measurement module and the mean, standard deviation and 95% confidence intervals were calculated from measurements of 50 conidia.

Results

Phylogenetic analysis

The ITS dataset consisted of 48 ingroup and 2 outgroup taxa. Alignments were deposited in TreeBase (SN3147). The alignment contained 565 characters including coded alignment gaps. Of these 564 characters 493 were constant, 13 were variable and parsimony uninformative and 58 were parsimony informative.

The phylogenetic trees generated by Neighbour-joining and Maximum-parsimony analyses were identical (TL = 89 steps, CI = 0.899, HI = 0.101, RI = 0.945, RC = 0.850). The Bayesian analysis was done three times and the resulting trees in each run were identical. Bayesian analyses produced trees with the same topology as the NJ and MP trees. The tree resulting from the Bayesian analysis is shown in Fig. 1 with NJ bootstrap supports above and posterior probabilities below the branches.

The purpose of the phylogenetic analysis was to determine the degree of variation found amongst isolates of "*B.*" *obtusa* and to determine the relationship of this species to its nearest relatives. Therefore the trees were rooted to *D. corticola* because previous analyses have shown that this species occupies a basal position within the clade containing *Diplodia* species (Alves *et al*., 2004).

Seven clades corresponding to seven *Diplodia* species were resolved. All clades with the exception of the *D. pinea* clade received high bootstrap and posterior probabilities support. A large clade containing several isolates from a variety of hosts and geographic regions and previously identified as "*B.*" *obtusa* (*Diplodia* sp.) was readily identified. Although this clade was further differentiated into a number of sub-clades, none of them (apart from the clade containing two isolates from Australia) received significant bootstrap support.

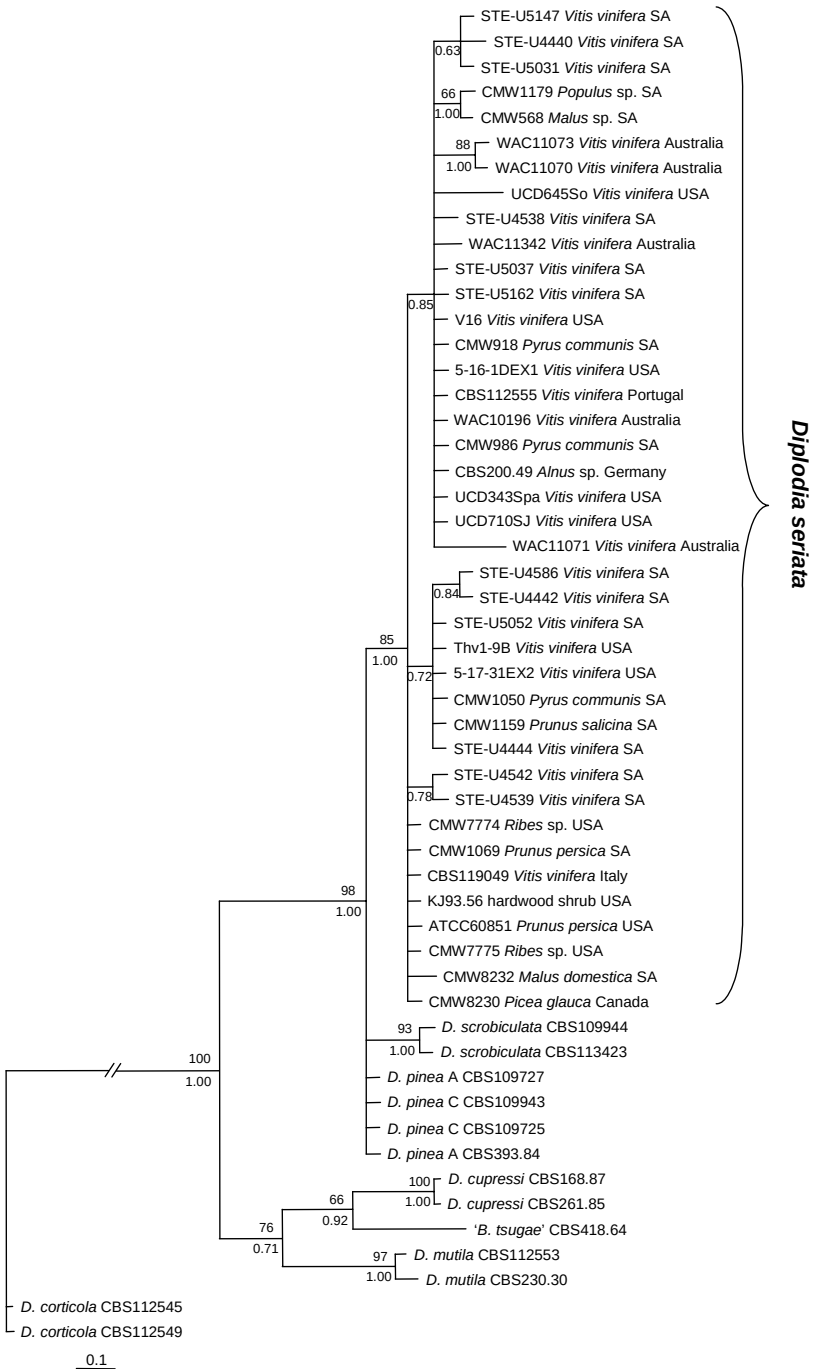


Fig. 1. Phylogenetic tree resulting from a Bayesian analysis of ITS sequence data. Bootstrap values are shown above the nodes with pooled posterior probabilities from three independent Bayesian analyses below the nodes. The tree was rooted to *Diplodia corticola*.

In phylogenetic terms this group of isolates is closely related to *D. pinea* and *D. scrobiculata* but clearly distinct from both.

Morphology

The isolates studied in detail could not be separated from one another on the morphology of their pycnidia, conidia and mode of conidiogenesis. Conidia were initially hyaline but soon became brown before they were released from the pycnidia and remained aseptate. The wall was moderately thick (about 0.5 µm), with a smooth outer surface and roughened on the inner surface. They measured (18–)21–26(–28.5) × (10–)11–14(–16) µm. These characters correspond to those reported for the anamorph of "*Botryosphaeria*" *obtusa* (Shoemaker, 1964; Slippers *et al*., 2007). A specimen on *V. vinifera* collected in Portugal (CBS-H 19809) is herein proposed as epitype with a corresponding culture (CBS 112555).

A number of herbarium specimens were studied. Of these *Diplodia seriata* De Not., *D. profusa* De Not., and *D. pseudodiplodia* Fuckel all corrresponded morphologically to the anamorph of "*B.*" *obtusa* as reported by Shoemaker (1964), Slippers *et al*. (2007) and in this study. Since *D. seriata* is the oldest name available it is proposed that this is a suitable name for the anamorph of "*B.*" *obtusa*.

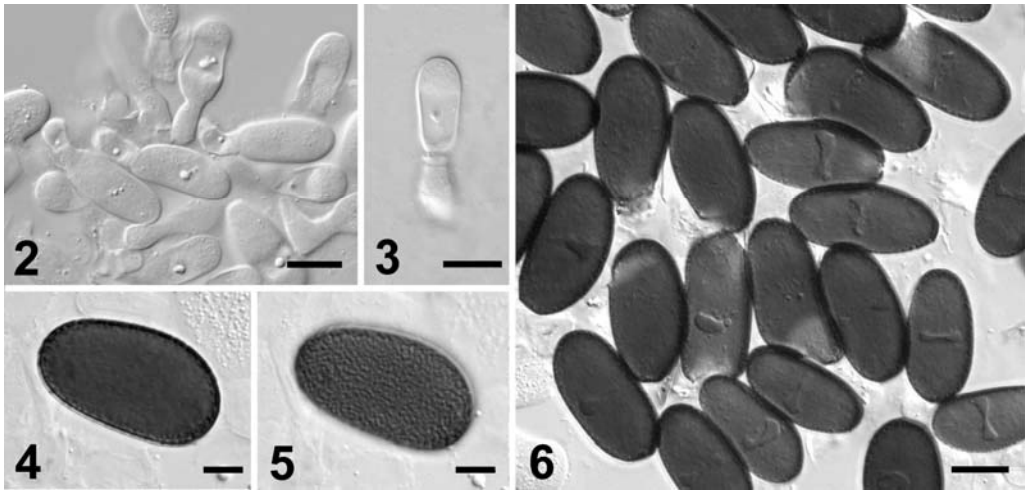
Taxonomy

Diplodia seriata De Not., *Mirom. Ital. Dec*. IV No **6**. 1842. (Figs 2-14)

= *Diplodia profusa* De Not., *Microm. Ital. Dec*. IV, no 8. 1842.

= *Diplodia pseudodiplodia* Fuckel, *Jb. Nassau. Ver. Naturk*. 23-24: 393. 1870.

Conidiomata pycnidial, separate or aggregated and confluent, immersed in the host, partially emergent at maturity, dark brown to black, ostiolate, non-papillate, thick-walled, outer layers composed of dark brown *textura angularis*, inner layers of thin-walled hyaline *textura angularis*. *Conidiogenous cells* 3-5.5 × 7-10(-15) µm, hyaline, thin-walled, smooth, cylindrical, swollen at the base, discrete, producing a single conidium at the tip, indeterminate, proliferating internally giving rise to periclinal thickenings or proliferating percurrently forming 2-3 annelations. *Conidia* (21.5-)22-27(-28) × (11-)11.5-14.5(-15.5) µm, 95% confidence limits = 24.3-25.4 × 12-6-13.2 µm ($\bar{x}$ ± S.D. of 50 = 24.9 ± 1.9 × 12.9 ± 1.1 µm, L/W = 1.9 ± 0.1) initially hyaline, becoming dark brown, moderately thick-walled (ca. 0.5 µm thick), wall externally smooth, roughened on the inner surface, aseptate, ovoid, widest in the middle, apex obtuse, base truncate or rounded.



Figs 2-6. *Diplodia seriata* from holotype. **2.** Conidiogenous cells. **3.** Conidiogenous cell with two annellations. **4, 5.** Conidium photographed at two different levels of focus to show the smooth outer surface (Fig. 4) and the roughened inner surface (Fig. 5) of the conidium wall. **6.** Conidia. Bars 2, 3, 6 = 10 µm; 4, 5 = 5 µm.

Teleomorph: *Botryosphaeria obtusa* (Schwein.) Shoemaker, *Can. J. Bot.* 42: 1298. 1964.

= *Physalospora obtusa* (Schwein.) Cooke, *Grevillea* 20: 86. 1892.

≡ *Sphaeria obtusa* Schwein., *Trans. Amer. Phil. Soc. II*, 4: 220. 1832.

= *Melanops quercum* (Schwein.) Rehm. forma *vitis* Sacc. Sec. Shear, *Science* 31: 748. 1910.

= *Physalospora cydoniae* Arnaud, Sec. Hesler, *Bull Cornell Univ. Agr. Exp. Sta.* 379: 101. 1916.

= *Physalospora malorum* (Peck) Shear, *Mycologia* 17: 100. 1925.

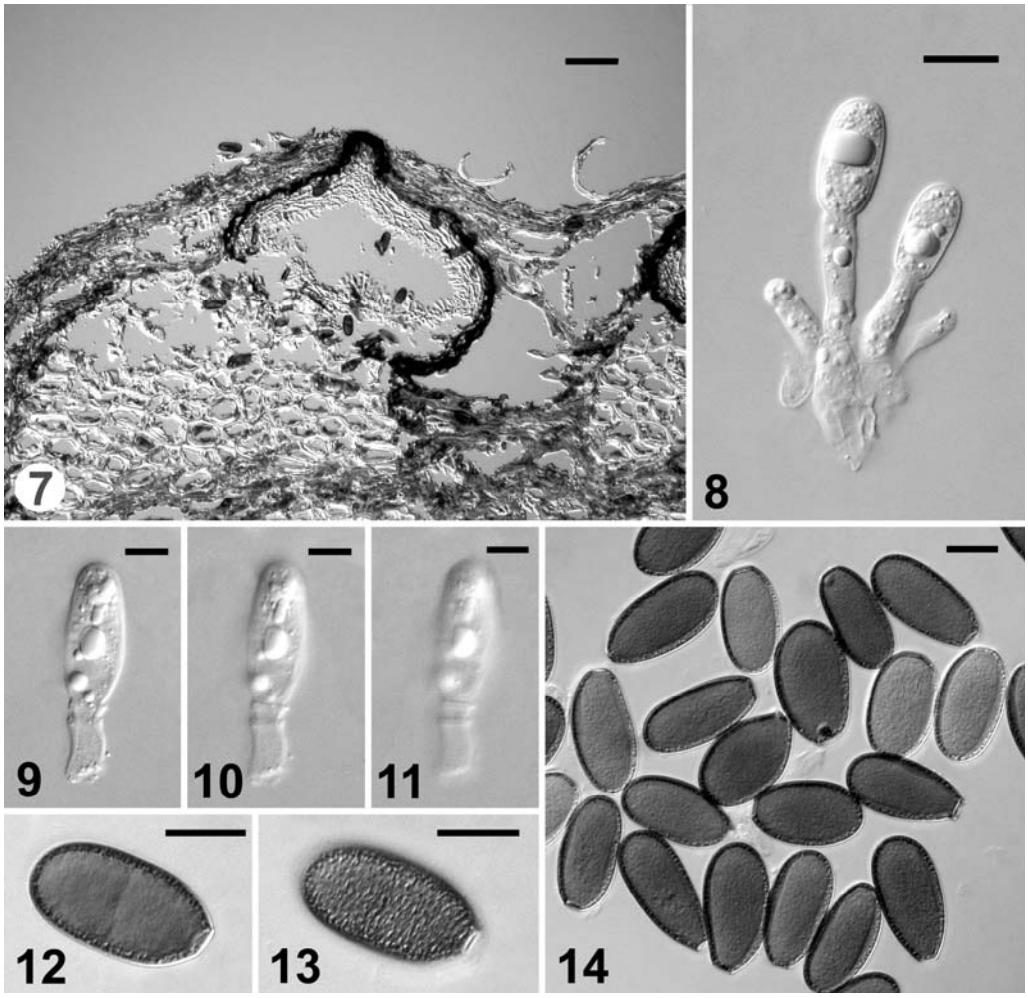
Habitat: On wood of a wide range of plants

Known distribution: Cosmopolitan.

Material examined: *Diplodia seriata*. ITALY. On dead stems of *Jasminium* sp., 18 Aug 1837, *De Notaris* (HERB RO, **holotype**). PORTUGAL. Montemor-o-Novo, on dead stems of *Vitis vinifera*, 31 July 1997, *A.J.L. Phillips* (CBS-H 19809; **epitype designated here**; culture ex-epitype CBS 112555). *Diplodia pseudodiplodia*. AUSTRIA. Hattenheim. On bark of *Pyrus malus*, *Fuckel* (Fungi rhenani 535, in G, **holotype**). *Diplodia profusa*. ITALY. On twigs of *Robinia pseudoacacia*, Genoa, May 1842, *De Notaris* (Herb. RO, **holotype**). *Diplodia tecta*. On leaf of *Prunus laurocerasi*. (Herb. M.J. Berkeley, K(M) 142319, **holotype**, K(M) 142318).

Discussion

In this study, ITS sequences of 40 isolates identified as *B. obtusa* from seven different host genera collected in the USA, Canada, Europe, Australia



Figs. 7-14. "*Botryosphaeria" obtusa* anamorph (from epitype). **7.** Vertical section through a pycnidium. **8.** Conidiogenous cells and developing conidia. Thickening of the periclinal wall of the cell on the right can be seen. **9-11.** A conidiogenous cell photographed at three different levels of focus to show the annellations resulting from percurrent proliferation. **12, 13.** Conidium photographed at two different levels of focus to show the smooth outer surface (Fig. 6) and the roughened inner surface (Fig. 7) of the conidium wall. **14.** Conidia. Bars: 7 = 50 µm; 8, 12, 13, 14 = 10 µm; 9, 10 = 5 µm.

and South Africa were included in a phylogenetic analysis. Phylogenetically this species appears to be relatively homogenous in terms of ITS sequence data. Nevertheless, several minor sub-clades were detected within the major clade. However, bootstrap support for these sub-clades was low and the sub-clades probably reflect the normal degree of variation within a species.

Furthermore, there does not seem to be any correlation between hosts and sub-clades, since various hosts occur within each sub-clade.

The morphology of representative isolates was studied, and together with morphological data previously reported for other isolates that were included in the phylogenetic study (Slippers *et al*., 2007) they corresponded well with published descriptions of the anamorph of "*B.*" *obtusa* (Stevens, 1926; Shoemaker, 1964; Punithalingam and Walker, 1973). Therefore, all the isolates included in this study are accepted as representing a single species, "*B." obtusa*. This species can thus be regarded as a cosmopolitan, plurivorous fungus. A single, well-studied isolate (CBS 112555) derived from the epitype specimen, was subsequently chosen to represent this species. Although the epitype is on *V. vinifera* from Portugal, while the type of *D. seriata* was found on *Jasminium* sp. in Italy, the cosmopolitan and pluriverous nature of this fungus means that the host and geographic origin is of minor importance.

A number of type specimens of *Diplodia* spp. were examined that according to the protologue are morphologically similar to the anamorph of "*B.*" *obtusa*, or are amongst the earliest names known in the genus, namely *D. seriata*, *D. profusa* and *D. pseudodiplodia*. Of these, the type specimen of *Diplodia seriata* in RO was found to conform in all details with descriptions of the anamorph of "*B.*" *obtusa*. This name was published in 1842 and as far as we could ascertain, it is the second oldest name in *Diplodia*, the only older name being *Diplodia mutila* (1834), which is the type species of the genus. Since this is the oldest name applied to a fungus with these characteristics, we consider *D. seriata* to be the correct name for the fungus known as "*B.*" *obtusa*.

All isolates identified as "*B.*" *obtusa* that were included in the phylogenetic study fell within a single well-supported clade sister to a less well-defined clade consisting of *D. scrobiculata* and *D. pinea*. All three species in this major clade have conidia that become brown and do not form septa while within the conidioma. All other species in *Diplodia* lay within the other major clade of this tree, with *D. corticola* at the root. The species in this other major clade, including *D. corticola*, have conidia that remain hyaline for a long period and become coloured only after they have been discharged from the pycnidia.

The genus *Sphaeropsis* Sacc. was introduced to accommodate species of *Diplodia* with brown conidia that remain aseptate (Saccardo 1880). Sutton (1980) considered that in addition to conidial colour and septation, conidiogenesis in *Sphaeropsis* differs from that in *Diplodia*. Thus, he was of the opinion that while conidiogenous cells in *Sphaeropsis* proliferate percurrently and internally, conidiogenous cells in *Diplodia* proliferate internally only. Denman *et al*. (2000) questioned the taxonomic value and

reliability of using the timing of septation and colouration of conidia to separate *Sphaeropsis* and *Diplodia*. They also suggested that conidiogenous cells in *Diplodia* can proliferate percurrently, and thus the two genera cannot be distinguished and should be regarded as synonyms. Phillips (2002) and Alves *et al*. (2004, 2006) provided photographic evidence of percurrent proliferation in conidiogenous cells of *Diplodia* species thus confirming the suggested synonymy proposed by Denman *et al*. (2000). Nevertheless, conidia of *D. scrobiculata*, *D. pinea* and the anamorph "*B.*" *obtusa* are distinct in that their conidia become dark-walled whilst within the pycnidia but conidia of other *Diplodia* species become coloured only after discharge from the pycnidia. This morphological distinction is supported by ITS phylogeny. However, phylogenetic distinctions based on ITS sequence data are not suitable to support taxa at the genus level.

Acknowledgements

Cynthia Goodwin and Judith Harrison of the British Library kindly provided information on the publication date of *Diplodia tecta*. This work was financed by the European Regional Development Fund and Fundação para a Ciência e a Tecnologia (FCT) under project POCI/AGR/56140/2004. A. Alves was supported by a grant No. SFRH/BPD/24509/2005 and A. Phillips by grant No. SFRH/BCC/15810/2006 from FCT. The curators of K and RO are thanked for letting us study specimens in their care.

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). Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. Fungal Diversity 23: 1-15.

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

Cooke, M.C. (1892). Neglected diagnoses. Grevillea 20: 81-87.

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.

Denman, S., Crous, P.W., Taylor, J.E., Kang, J-C, Pascoe, I., Wingfield, M.J. (2000). An overview of the taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Studies in Mycology 45: 129-140.

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.

Hesler, L.R. (1916). Black rot, leaf spot, and canker of pomaceous fruits. Cornel University Agricultural Bulletin 379.
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.
Page, R.D. (1996). TreeView: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.
Peck, C.H. (1880). Report of the Botanist. New York State Museum of Natural History Annual Report 34: 22-58.
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: 3-18.
Phillips, A.J.L., Oudemans, P.V., Correia, A. and Alves, A. (2006). Characterisation and epitypification of *Botryosphaeria corticis*, the cause of blueberry cane canker. Fungal Diversity 21: 141-155.
Punithalingam E and Walker JM. (1973). *Botryosphaeria obtusa*. Descriptions of pathogenic fungi and bacteria, No. 394. Commonwealth Mycological institute, Kew, Surrey, England.
Rannala, B. and Yang, Z. (1996). Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of Molecular Evolution 43: 304-311.
Rodriguez, F., Oliver, J.F., Marin, A. and Medina, J.R. (1990). The general stochastic model of nucleotide substitutions. Journal of Theoretical Biology 142: 485-501.
Ronquist, F. and Huelsenbeck, J.P. (2003). MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.
Rossman, A.Y. and Samuels, G.J. (2005). Towards a single scientific name for species of fungi. Inoculum 56: 3-6.
Saccardo, P.A. (1880). Fungi gallici, ser, II. Michelia 2(6): 38-135.
Saccardo, P.A. (1884). Sylloge Fungorum Omnium Hucusque Cognitorum 3: 1-860.
Schweinitz, L.D. (1832. Synopsis Fungorum in America boreali media degentium. Transactions of the American Philosophical Society. 4 141-316.
Shear, C.L., Stevens, N.E. and Wilcox, M.S. (1925). *Botryosphaeria* and *Physalospora* in the Eastern United States. Mycologia 17: 98-107.
Shoemaker RA. 1964. Conidial states of some *Botryosphaeria* species on *Vitis* and *Quercus*. Canadian Journal of Botany 42: 1297-1301.
Slippers, B., Smit, W.A., Crous, P.W., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2007). Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruits in South Africa and other regions of the world. Plant Pathology 56: 128-139.
Stevens, N.E. (1933). Two apple black rot fungi in the United States. Mycologia 25: 536-548.
Stevens, N.E. (1936). Two species of *Physalospora* in England. Mycologia 28: 330-336.
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.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols*: *A Guide to Methods and Applications* (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White). Academic Press San Diego, California: 315-322.

Young, N.D. and Healy, J. (2003). GapCoder automates the use of indel characters in phylogenetic analysis. BMC Bioinformatics 4: art. 6.

(Received 1 December 2006; accepted 17 January 2007)

View publication stats