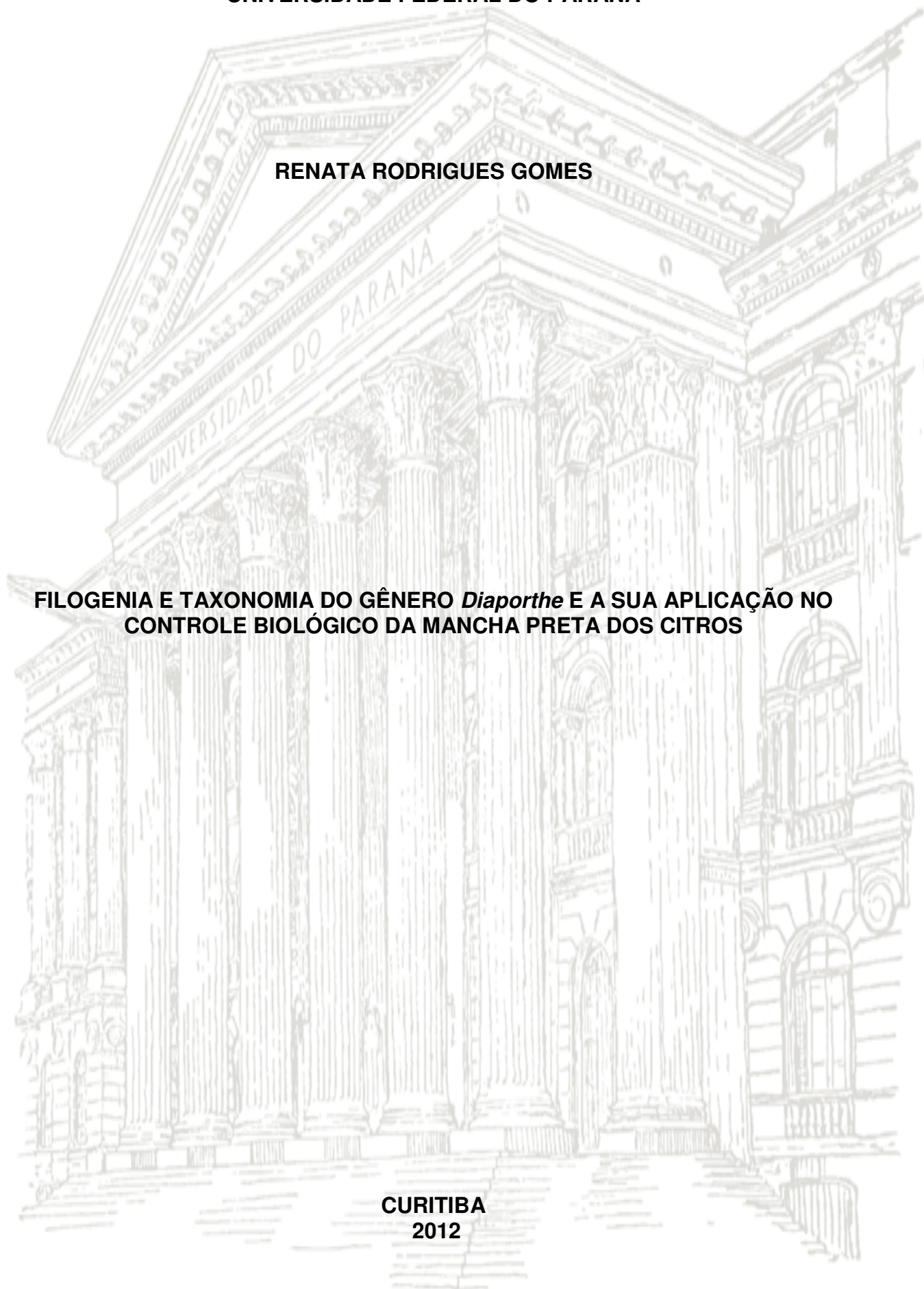


UNIVERSIDADE FEDERAL DO PARANÁ

RENATA RODRIGUES GOMES

**FILOGENIA E TAXONOMIA DO GÊNERO *Diaporthe* E A SUA APLICAÇÃO NO
CONTROLE BIOLÓGICO DA MANCHA PRETA DOS CITROS**

**CURITIBA
2012**



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CONTROLE BIOLÓGICO DA MANCHA PRETA DOS CITROS**

Tese apresentada ao Programa de Pós-graduação em Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial a obtenção do título de Doutor em Ciências Biológicas, Área de Concentração: Genética.

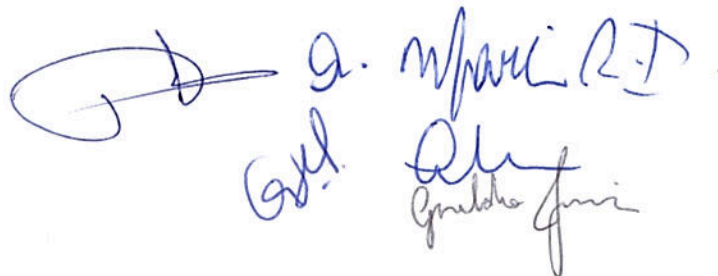
Orientadores:
Prof.^a Dr.^a Chirlei Glienke
Phd Pedro Crous

Co-Orientador:
Prof.^a Dr.^a Vanessa Kava Cordeiro

**CURITIBA
2012**

Ata da defesa de tese de doutorado de RENATA RODRIGUES GOMES

Aos trinta e um dias do mês de julho do ano de dois mil e doze, foi realizada nas dependências do Departamento de Genética, da Universidade Federal do Paraná, a defesa de tese da doutoranda **RENATA RODRIGUES GOMES**, intitulada Filogenia e Taxonomia do Complexo Diaporthe e a sua Aplicação no Controle da Mancha Preta do Citros. A abertura teve início às quatorze horas pelo Professor Doutor **Ricardo Lehtonen Rodrigues de Souza**, Coordenador do Programa de Pós-Graduação em Genética, que em seguida passou a palavra à Presidente da Banca Examinadora e Orientadora da aluna, Professora Doutora **Chirlei Glienke**. A Presidente apresentou ao público presente os membros da Banca Examinadora e passou a palavra à aluna, para que fizesse a apresentação sucinta da sua tese. Após a explanação oral, a Professora Doutora **Chirlei Glienke** passou a palavra ao primeiro examinador, Professor Doutor **Geraldo José da Silva Junior**, da Fundecitrus. Em seguida, passou a palavra ao segundo examinador, Professora Doutora **Louise Larissa May de Mio**, do Departamento de Fitotecnia e Fitossanitarismo da UFPR. Em seguida, o terceiro examinador, Professor Doutor **Marcio Roberto Pie**, do Departamento de Zoologia da UFPR, fez suas considerações. Em seguida, a Presidente da Banca passou a palavra à quarta examinadora, Professora Doutora **Danyelle Stringari**, da FAFIPAR. Findas as arguições pelos membros da banca, a Professora Doutora **Chirlei Glienke** fez uma rápida apreciação das conclusões mais importantes dos debates realizados e comunicou que a Banca Examinadora iria proceder à discussão para atribuição dos conceitos, reunindo-se em sessão secreta. Os trabalhos foram interrompidos por cinco minutos. Após, foram proclamados os conceitos atribuídos pela Banca Examinadora, a seguir descritos: Professor Doutor **Geraldo José da Silva Junior**, conceito "A"; Professora Doutora **Louise Larissa May de Mio**, conceito "A"; Professor Doutor **Marcio Roberto Pie**, conceito "A"; Professora Doutora **Danyelle Stringari**, conceito "A"; Professora Doutora **Chirlei Glienke**, conceito "A", com o conceito médio final "A". Tendo cumprido o que dita o artigo trinta e cinco das Normas Internas do Programa, a candidata cumpriu os requisitos para obtenção do grau de Doutora em Genética. Como não havia nada mais a ser tratado, a Professora Doutora **Chirlei Glienke**, após informar à candidata que ela tem, a partir desta data, até trinta dias para a entrega da versão definitiva de sua tese e comprovante de submissão de pelo menos um artigo científico para um periódico de circulação internacional com cópia do(s) artigo(s), deu por encerrada a sessão. Eu, **Ricardo Lehtonen Rodrigues de Souza**, lavrei a presente ata, a qual assino juntamente com os senhores examinadores. Curitiba, trinta e um de julho de dois mil e doze.



Handwritten signatures in blue ink, including the name "Ricardo Lehtonen Rodrigues de Souza" and "Geraldo José da Silva Junior".

Dedico

*A minha família, pelo carinho, apoio,
paciência e compreensão em todos esses
anos de distância dedicados a realização
desse trabalho.*

*“O Sertanejo é antes de tudo um forte”
Euclides da Cunha no livro Os Sertões*

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RESUMO

Filogenia e Taxonomia do Gênero *Diaporthe* e a sua Aplicação no Controle Biológico da Mancha Preta dos Citros

Espécies do gênero *Diaporthe* (*Phomopsis*) têm sido descritas em uma ampla gama de hospedeiros como fitopatogênicas, endofíticas, saprofíticas e recentemente foram descritas causando problemas de saúde em humanos e outros mamíferos. Esse trabalho teve como objetivo reavaliar o gênero *Diaporthe* através de análise taxonômica e filogenética e esclarecer a taxonomia de *Diaporthe* endofíticos com potencial antagonista contra *Phyllosticta citricarpa* agente causal da Mancha Preta dos Citros. No presente estudo nós investigamos 243 isolados usando dados de sequências de DNA em multilocus. Foram selecionados dentre nove genes os cinco melhores para resolução taxonômica. As regiões gênicas selecionadas, região espaçadora interna transcrita do rDNA (ITS1, 5.8S, ITS2), regiões parciais dos genes fator 1-alfa de alongação da tradução (TEF1), beta-tubulina (TUB), histona H3 (HIS) e calmodulina (CAL), resolveram 95 clados. Quinze novas espécies foram descritas, *Diaporthe arengae*, *D. brasiliensis*, *D. endophytica*, *D. hongkongensis*, *D. inconspicua*, *D. infecunda*, *D. mayteni*, *D. neoarctii*, *D. oxe*, *D. paranensis*, *D. pseudomangiferae*, *D. pseudophoenicicola*, *D. raonikayaporum*, *D. schini* and *D. Terebinthifolii*. Quatorze novas combinações foram introduzidas em *Diaporthe* e a espécie *D. anacardii* foi epítipificada. A taxonomia de *Diaporthe* endofitos das plantas medicinais *Spondias mombin*, *Schinus terebinthifolius*, *Aspidosperma tomentosum* e *Maytenus ilicifolia* com potencial antagonista contra *P. citricarpa* foi esclarecida. Embora as espécies de *Diaporthe* no passado tenham sido identificadas principalmente com base na associação com o hospedeiro, os resultados deste estudo confirmam que o mesmo táxon pode estar associado a diferentes hospedeiros, o que sugere que eles se movem livremente entre os mesmos. Em contrapartida, alguns patógenos de plantas e endofíticos parecem ser estritamente restritos a determinado hospedeiro. Devido ao comportamento de diversidade ecológica entre os membros do gênero *Diaporthe*, esse trabalho sugere que descrições de novas espécies ocorram apenas quando empregados dados moleculares.

Palavras-Chave: *Diaporthales*, *Diaporthe*, *Phomopsis*, Multi-Locus, Endofíticos, Controle Biológico, Mancha Preta dos Citros

ABSTRACT

Phylogeny and Taxonomy of the Genus *Diaporthe* and its Application on Biological Control of Citrus Black Spot

Species in the genus *Diaporthe* (*Phomopsis*) have been reported as plant pathogens, non-pathogenic endophytes or saprobes from a wide range of hosts and have also been recently reported as the cause of health problems in humans and other mammals. The current study aimed to resolve the taxonomy and phylogeny of a large collection of *Diaporthe* species occurring on diverse hosts, either as pathogens, saprobes, or as harmless endophytes, including endophytic *Diaporthe* with antagonistic potentials against the *Phyllosticta citricarpa* the causative agent for Citrus Black Spot. In the present study we investigated 243 isolates using multilocus DNA sequence data. Nine genes were evaluated and the five best have been selected for taxonomic resolution. The selected gene regions were the rDNA internal transcribed spacer (ITS1, 5.8S, ITS2) region, and partial translation elongation factor 1-alpha (TEF1), beta-tubulin (TUB), histone H3 (HIS) and calmodulin (CAL) genes resolved 95 clades. Fifteen new species are described, namely *Diaporthe arengae*, *D. brasiliensis*, *D. endophytica*, *D. hongkongensis*, *D. inconspicua*, *D. infecunda*, *D. mayteni*, *D. neoarctii*, *D. oxe*, *D. paranensis*, *D. pseudomangiferae*, *D. pseudophoenicicola*, *D. raonikayaporum*, *D. schini* and *D. terebinthifolii*. A further 14 new combinations are introduced in *Diaporthe*, and *D. anacardii* is epitypified. The taxonomy of endophyte *Diaporthe* from the medicinal plants *Aspidosperma tomentosum*, *Maytenus ilicifolia*, *Spondias mombin* and *Schinus terebinthifolius*, with antagonistic potential against *P. citricarpa* was clarified. Although species of *Diaporthe* have in the past chiefly been distinguished based on host association, results of this study confirm several taxa to have wide host ranges, suggesting that they move freely among hosts, frequently co-colonising diseased or dead tissue. In contrast, some plant pathogenic and endophytic taxa appear to be strictly host specific. Given this diverse ecological behaviour among members of *Diaporthe*, the current study suggests that new species descriptions should only happen when there is molecular data.

Key-Words: *Diaporthales*, *Diaporthe*, *Phomopsis*, Multi-Locus Sequence Typing (MLST), endophytic, Biological Control, Citrus Black Spot

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1. Introdução

Espécies do gênero *Diaporthe* Nitschke (fase anamórfica *Phomopsis* Sacc. Bubák) têm sido descritas em uma ampla gama de hospedeiros em todo o mundo como fitopatogênicas de importância econômica, endofíticas, saprofíticas e recentemente foram descritas causando problemas de saúde em humanos e outros mamíferos.

A identificação em nível de espécie do gênero *Diaporthe* é um desafio, principalmente quando a espécie é isolada a partir de um hospedeiro para o qual não foi descrita anteriormente. Espécies do gênero *Diaporthe* têm sido tradicionalmente descritas com base na espécie hospedeira, o que contribuiu com a proliferação de nomes. Embora o hospedeiro seja, atualmente, considerado menos importante na definição de espécies, nenhuma revisão do gênero foi realizada.

Atualmente a classificação de espécies desse gênero somente é possível quando dados moleculares são empregados (CASTLEBURY *et al.*, 2003, 2005; CROUS; GROENEWALD, 2005; SANTOS *et al.*, 2010; UDAYANGA *et al.*, 2012). Sequência da região ITS do DNA ribossomal e sequência parcial do gene que codifica o Fator $\alpha 1$ de Elongação da Tradução (EF) têm sido utilizadas para estudos taxonômicos do gênero (VAN RENSBURG *et al.*, 2006; VAN NIEKERK *et al.*, 2005; SANTOS *et al.*, 2010, 2011). Mesmo utilizando a combinação de dados morfológicos e dados moleculares nem sempre a delimitação de espécies do gênero *Diaporthe* tem se demonstrado satisfatória (CASTLEBURY; MENGISTU, 2006; RENSBURG *et al.*, 2006). Além disso, para a elucidação taxonômica desse gênero faz-se necessário o uso de isolados referência de espécimes tipo ou ex-tipo.

Para outros gêneros fúngicos com dificuldades de elucidação taxonômica análise multilocus tem sido proposta e provou ser eficaz para estudos filogenéticos e de identificação em nível de espécie como, por exemplo, em *Colletotrichum*, *Sordariales* e *Fusarium* (PRIHASTUTI *et al.*, 2009; MILLER; HUHDORF, 2005; O'DONNELL, 2004, 2009, 2010). Os nomes precisos e aceitos de *Diaporthe* fitopatogênicos são essenciais para o desenvolvimento de políticas de biossegurança, bem como, de medidas de controle eficazes.

Diaporthe tem sido relatado como um dos gêneros mais frequentes isolado como endófito em muitas plantas hospedeiras (BOTELLA; DIEZ, 2011; MURALI *et al.*, 2006). Frequentemente descrito como produtor de enzimas e metabolitos secundários (ELSAESSER *et al.*, 2005; KOBAYASHI *et al.*, 2003; ISAKA *et al.*, 2001; DAI *et al.*, 2005) com atividade antibiótica (LIN *et al.*, 2005; DETTRAKUL *et al.*, 2003; BANDRE; SASEK, 1977); fungicida (SILVA *et al.*, 2005; WU *et al.*, 2008); anticancerígena (KUMARAN, 2009) e capaz de impedir a herbivoria (BRAYFORD, 1990; WEBER, 2009; VESTERLUND *et al.*, 2011). Bem como, este gênero tem sido utilizado no controle biológico de ervas daninhas (ASH *et al.*, 2010).

Atualmente microrganismos endofíticos isolados de plantas medicinais têm sido testados quanto à atividade antimicrobiana contra o patógeno *Phyllosticta citricarpa*. Linhagens endofíticas de *Diaporthe* demonstram grande potencial *in vitro* como agente de controle da Mancha Preta dos Citros e grande potencial na produção de metabólitos secundários (GOMES *et al.*, 2012).

A Mancha Preta dos Citros (MPC) atinge a maioria das espécies cítricas especialmente laranja doce (*Citrus sinensis*) e é causada pelo fungo *Phyllosticta citricarpa* McAlpine (teleomorfo *Guignardia citricarpa* Kiely). Esta doença atinge seriamente a citricultura brasileira, pois além de dificultar a comercialização no mercado de frutas, prejudica a sua exportação devido à barreira fitossanitária imposta pela União Européia. As lesões da MPC depreciam o valor comercial dos frutos destinados ao mercado de frutas frescas e nos casos em que a doença atinge o pedicelo do fruto, pode ocorrer à queda prematura do mesmo, aumentando as perdas na produção (ROBES *et al.*, 2005).

A utilização de fungicidas é o principal método de controle da MPC, utilizado no pré e pós-colheita, elevando os custos da produção (FIALHO *et al.*, 2009). A utilização de fungicidas como controle da doença, além de onerar a produção, oferece risco para o ambiente e o ser humano. O principal problema do emprego sucessivo de fungicidas é a seleção de linhagens resistentes do agente causal, como observado na África do Sul (HERBERT; GRECH, 1985) o que levou a restrições na sua utilização (AGOSTINI *et al.*, 2006, DEISING *et al.*, 2008). Além disso, desde 2008 foi proibida nos EUA a utilização do carbendazim, agrotóxico

utilizado no controle da MPC, levando ao embargo do suco de laranja brasileiro pelo governo americano em decorrência do uso de fungicida (DURAN *et al.*, 2012;).

Estudos de bioprospecção de microrganismos endofíticos para fins farmacêuticos e biotecnológicos são fundamentais para a descoberta de novos antibióticos, agentes quimioterápicos e defensivos agrícola. Estes metabólitos produzidos pelos fungos geralmente são eficazes, possuem baixa toxicidade, e tem um menor impacto no ambiente (PEARCE, 1997; STROBEL; DAISY, 2003; SMITH *et al.*, 2008, XU *et al.*, 2010). Fungos endofíticos de plantas medicinais estão sendo amplamente estudados na busca de novos biocontroladores potencialmente úteis. Nesse contexto o gênero *Diaporthe* tem sido relatado como potencial para o uso na biotecnologia com várias descobertas importantes, incluindo novos metabólitos estruturalmente significativos e fisiologicamente ativos (UDAYANGA *et al.*, 2011).

Em função dos prejuízos causados por *P. citricarpa*, e um elevado interesse para métodos alternativos no controle da MPC são relevantes os estudos na busca de microrganismos antagonistas para o controle da doença. Nessa perspectiva Gomes *et al.*, (2012) avaliaram o potencial de isolados endofíticos de *Diaporthe* em inibir *in vitro* duas linhagens patogênicas de *P. citricarpa* isoladas de lesões da MPC, em São Paulo, Brasil, e testadas quanto a patogenicidade de acordo com Baldassarri *et al.*, (2008). Isolados de *Diaporthe* inibiram o crescimento do micélio e formação de picnídio das duas linhagens de *P. citricarpa* avaliadas, mostrando-se promissores para uso como biocontrole da MPC. Para implementação desses isolados no controle biológico de doenças de plantas faz-se necessário o estudo das relações filogenéticas entre os mesmos e espécie de *Diaporthe* fitopatogênicas.

Dessa forma, esse trabalho teve como objetivo reavaliar o gênero *Diaporthe* através de análise multilocus e identificar as espécies endofíticas de plantas medicinais com potencial antagonista contra *Phyllosticta citricarpa* agente causal da Mancha Preta dos Citros.

2. Objetivos

2.1 Objetivos Gerais

Reavaliar o gênero *Diaporthe* através de análise taxonômica e filogenética de uma grande coleção de espécies ocorrendo em diversos hospedeiros e estilos de vida e esclarecer a taxonomia de *Diaporthe* endofíticos de plantas medicinais com potencial antagonista contra *Phyllosticta citricarpa* agente causal da Mancha Preta dos Citros.

2.2 Objetivos Específicos

- ✓ Selecionar os melhores genes para delimitar espécie do gênero *Diaporthe*;
- ✓ Reavaliar o gênero *Diaporthe* através de análise multilocus, utilizando linhagens referência da coleção de culturas de fungos do CBS (Fungal Biodiversity Centre; Holanda);
- ✓ Identificar novas espécies de *Diaporthe* isoladas de diferentes hospedeiros e regiões geográficas, realizar combinações anamorfo-teleomorfo e epitipificar espécies;
- ✓ Identificar novas espécies de *Diaporthe* endófitos das plantas medicinais *Spondias mombin* (Anacardiaceae), *Schinus terebinthifolius* (Anacardiaceae), *Aspidosperma tomentosum* (Apocynaceae) e *Maytenus ilicifolia* (Celastraceae) com potencial antagonista contra *Phyllosticta citricarpa*.

Revisão de Literatura

Filogenia e taxonomia do gênero *Diaporthe* e a sua aplicação no controle biológico da Mancha Preta dos Citros

1. O Gênero *Diaporthe*

O gênero *Diaporthe*, anamorfo *Phomopsis* pertence ao filo Ascomycota, subfilo Pezizomycotina, classe Sordariomycetes, subclasse Sordariomycetidae, ordem Diaporthales, família Valsaceae. De acordo com o Código Internacional de Nomenclatura de algas, fungos e plantas (anteriormente o Código Internacional de Nomenclatura Botânica), após a eliminação do art. 59 o qual permitia a nomenclatura binomial para fungos pleiomórficos, que entra em vigor no dia 1 de janeiro de 2013, os nomes dos fungos anamorfo e teleomorfo têm o mesmo status. Neste trabalho foi utilizado o nome mais antigo *Diaporthe* Nitschke 1870 em vez de *Phomopsis* Saccardo 1905, como sugerido em trabalhos anteriores (HAWKSWORTH *et al.*, 2011; SANTOS *et al.*, 2010, 2011; CROUS *et al.*, 2011; WINGFIELD *et al.*, 2012).

Com base no conhecimento atual sobre o gênero *Diaporthe*, é um desafio identificar uma espécie isolada a partir de um hospedeiro para o qual a espécie não foi descrita anteriormente (UDAYANGA *et al.*, 2011). Muitas das espécies conhecidas têm uma ampla gama de hospedeiros e poucas características que podem diferenciá-las (UECKER, 1988).

Algumas espécies parecem ser hospedeira específica enquanto outras são capazes de infectar uma grande variedade de hospedeiros (MOSTERT *et al.*, 2001; CROUS *et al.*, 2012; SCHILDER *et al.*, 2005; SANTOS; PHILLIPS, 2009; DIOGO *et al.*, 2010). Dessa forma, isolados de *Diaporthe* provenientes de um único hospedeiro podem representar mais de um taxon (REHNER; UECKER, 1994).

Espécies desse gênero têm sido tradicionalmente descritas com base na espécie hospedeira, contribuindo para a proliferação de nomes de espécies. Por exemplo, até agora foram descritas mais de 1000 espécies para a fase anamórfica (UECKER, 1988; MycoBank 2012 - <http://www.mycobank.org>; Index Fungorum 2012 - <http://www.indexfungorum.org>). A estratégia de identificar os isolados com base no hospedeiro tem sido frequentemente questionada (REHNER; UECKER, 1994, SANTOS *et al.*, 2010). Trabalhos, incluindo estudos de cruzamentos, mostraram que esse critério não é suficiente para classificação ao nível de espécie. Além disso,

isolados provenientes do mesmo hospedeiro não necessariamente pertencem à mesma espécie, assim como uma mesma espécie de *Diaporthe* pode infectar diferentes hospedeiros (REHNER; UECKER, 1994; BRAYFORD, 1990; FARR *et al.*, 2002).

Existem vários estudos filogenéticos recentes sobre complexos de espécies de *Diaporthe* associados com um hospedeiro em particular. Quinze espécies de *Diaporthe* foram registados de uva (*Vitaceae*) (CROUS *et al.*, 2012; VAN NIEKERK *et al.*, 2005). Outros exemplos incluem a erva-doce (*Foeniculum vulgare*), que é hospedeiro de várias espécies de *Diaporthe* (SANTOS; PHILLIPS, 2009), quatro a seis espécies são conhecidas a partir de soja (*Glicine max*) de diferentes localizações geográficas (NEVENA *et al.*, 1997; ZHANG *et al.*, 1998; MENGISTU *et al.*, 2007; SANTOS *et al.*, 2011) e cinco espécies em arbusto vermelho (*Aspalathus linearis*) na África do Sul (VAN RENSBURG *et al.*, 2006). Existem várias espécies não identificadas relatadas como endófitos em teca (*Tectona grandis*), magnólia (*Magnolia liliflora*, *Manglietia garrettii*) e salgueiro (*Salix* sp.) (HORN *et al.*, 1996; PROMPUTTHA *et al.*, 2005; MURALI *et al.*, 2006; UDAYANGA *et al.*, 2011).

As conexões entre as fases teleomórfica e anamórfica foram descritas apenas para aproximadamente 20% das espécies do gênero *Diaporthe* (PHILLIPS, 2006). Essa ausência da fase sexuada em meio de cultura, principalmente quando isolado endofiticamente, torna a identificação ao nível de espécie neste gênero muito difícil (REHNER; UECKER, 1994). Várias conexões entre as fases anamórficas e teleomórficas têm sido propostas usando dados moleculares (SUTTON, 1980; REHNER; UECKER, 1994; HYDE *et al.*, 2011; UDAYANGA *et al.*, 2011).

Wehmeyer (1933) elaborou uma revisão do gênero *Diaporthe*, e considerou a associação com o hospedeiro uma característica de menor importância, tendo reduzido de 650 para 70 o número de espécies no gênero. No entanto, este estudo baseou-se unicamente em características morfológicas do teleomorfo não tendo sido consideradas as características do anamorfo. Além disso, a delimitação de espécies de *Diaporthe* baseado apenas em características morfológicas demonstrou-se inadequada (VAN DER AA, 1990). Em adição as características de morfologia do conídio e características da colônia usadas para identificar espécies, alguns autores

têm utilizado caracteres alternativos que incluem virulência (VIDIC, 1991), proteínas e sorologia (LEE; SNOW, 1992; VELICHETI *et al.*, 1991), sequências ITS do DNA ribossomal (rDNA) (ZHANG *et al.*, 1998) e enzimas e metabólitos secundários (SHIVAS *et al.*, 1991; ABREU *et al.*, 2012).

Atualmente a classificação de espécies desse gênero é baseada em dados morfológicos e principalmente em filogenias moleculares, especialmente as sequências da região ITS do DNA ribossomal e sequência parcial do gene que codifica o Fator $\alpha 1$ de Elongação da Tradução (EF). O entendimento sobre o gênero *Diaporthe* expandiu-se por meio de estudos dessas sequências, nos quais alguns dos isolados agruparam-se por regiões geográficas (REHNER; UECKER, 1994). Zhang *et al.* (1998) distinguiu cinco táxons de *Diaporthe* isolados de Soja (*Glycine max*) utilizando sequências da região ITS, porém não foram capazes de diferenciá-los com base nas características morfológicas. Farr *et al.* (2002) caracterizaram por análise molecular, com o uso de sequências ITS1, 5,8S, ITS2 e por características morfológicas, linhagens de *Diaporthe* sp. isolados de mirtilo ou “blueberry” (*Vaccinium corymbosum*) e mirtilo vermelho “cranberry” (*V. macrocarpon*), baseados nesse estudo verificaram que a maioria dos isolados tratava-se de *Phomopsis vaccinii*, uma vez que as sequências agruparam-se com uma cultura referência dessa espécie.

Para a elucidação taxonômica desse gênero faz-se necessário o uso de isolados referência de espécimes tipo ou ex-tipo. Por exemplo, Mostert *et al.* (2001) isolaram uma espécie de *Diaporthe* de videiras, que também foi isolado de plantas dos gêneros *Protea* e *Pyrus* em países como Austrália, Portugal e África do Sul. Como nenhum nome poderia ser atribuído a esta espécie acabou por ser referido como Taxon3. A mesma espécie de *Diaporthe* foi encontrada novamente em videiras por Van Niekerk *et al.* (2005), onde 15 espécies foram distinguidas, e três táxons foram referidos como *Phomopsis* sp.1. Em 2006, Rensburg *et al.* conseguiram finalmente identificar essa espécie, comparando a sequência de DNA dessa que é homóloga à da estirpe de ex-tipo de *P. theicola* Curzi (CBS 187.27), que foi originalmente descrita em *Camellia sinensis* na Itália (UECKER, 1988). Esta espécie tem, obviamente, uma ampla gama de hospedeiros e distribuição, que mais

uma vez ressalta a dificuldade que os micologistas encontram quando tentam identificar as espécies de *Diaporthe*.

Mesmo utilizando a combinação de dados morfológicos e de sequenciamento da região ITS nem sempre a delimitação de espécies do gênero *Diaporthe* tem se demonstrado satisfatória. Outros genes têm sido sequenciados na tentativa de elucidar a classificação taxonômica desse gênero. Por exemplo, Castlebury e Mengistu (2006) avaliaram *Diaporthe* isolados de soja e outros hospedeiros combinando dados de sequências de ITS do rDNA, o sequenciamento parcial dos genes EF e actina. Seis grupos de *Diaporthe* foram formados, desses, três correspondem aos táxons: *P. longicolla*, *D. melonise* *D. phaseolorum*. Os autores concluíram que a ausência de distinção morfológica e informação biológica tornam problemática a identificação de espécies aos grupos de isolados geneticamente distintos. Além disso, não está esclarecida a importância biológica e genética dos diferentes grupos, assim como, são necessários trabalhos adicionais quanto aos hospedeiros, patogenicidade e morfologia para resolver a distinção de espécies neste gênero.

Rensburg *et al.* (2006) analisaram as sequências de ITS do rDNA e parcial do gene EF, dados de morfologia e de patogenicidade e relataram que até cinco espécies de *Diaporthe* estão envolvidas com a doença “die-back” em arbusto vermelho (*Aspalathus linearis*). Os autores descreveram *D. aspalathi* (anteriormente identificados como *D. phaseolorum* ou *D. phaseolorum* var. *meridionalis*), *D. ambigua*, *Phomopsis theicola*, uma nova espécie *Phomopsis cuppatea*, e *Phomopsis* sp.9. No entanto, mais estudos são necessários, para resolver totalmente o status filogenético desse gênero envolvido com “die-back” em *A. linearis*.

Recentemente Santos *et al.* (2010) sugeriram a classificação baseada na definição biológica de espécie para o gênero *Diaporthe*. Segundo os autores esse conceito não foi aplicado a *Diaporthe*, provavelmente porque o teleomorfo foi induzido em culturas de poucas espécies. Além disso, esse critério é frágil em fungos homotáticos (autoferteis) ou fungos assexuados e dessa forma não pode ser aplicado para eles o conceito biológico de espécie (REYNOLDS, 1993; TAYLOR *et al.*, 2000). Outro problema na delimitação das espécies baseado no critério biológico

é que os métodos convencionais são trabalhosos para demonstrar *in vitro* o cruzamento para muitos fungos. Para superar essas dificuldades trabalhos têm sido realizados usando sequências do tipo de acasalamento (MAT- *mating-type*) para auxiliar na definição biológica das espécies e ou Genealógica Concordância Filogenética no Reconhecimento de Espécie (GCPSR).

Vários autores têm defendido o uso de sequências de *mating-type* na resolução filogenética de espécies de fungos, associadas a outras regiões genômicas, tais como ITS (TURGEON, 1998; PÖGGELER, 2001; DU *et al.*, 2005) e as sequências de EF (SANTOS *et al.*, 2010). Santos *et al.* (2010) utilizaram filogenias baseadas nas sequências de *mating-type*, ITS e EF para esclarecer a taxonomia de isolados de *Diaporthe* de várias plantas hospedeiras. Os testes de *mating* demonstraram que populações distintas por meio de ITS, na verdade pertenciam à mesma espécie, ou seja, pelo conceito filogenético de espécies, as sequências de ITS seriam consideradas pertencentes a espécies diferentes. Já considerando os limites do conceito biológico de espécie, esses isolados seriam considerados como uma única espécie. Os dados de cruzamento de *mating* e filogenias de *mating-type* corroboraram com as análises filogenéticas das sequências de EF. Os autores apontam a necessidade de uma ampla revisão taxonômica de isolados do gênero *Diaporthe*.

Outro critério para auxiliar na definição biológica das espécies foi desenvolvido por Taylor *et al.* (2000), baseado na Concordância Genealógica Filogenética no Reconhecimento de Espécie (GCPSR), como uma forma objetiva de definir os limites de espécies sexuais. O GCPSR usa a concordância filogenética de múltiplos genes não ligados para indicar a ausência de troca de material genético e, portanto, a independência evolutiva das linhagens. A análise dos dados multigênica provou ser eficaz na elucidação de filogenias fúngicas (PRIHASTUTI *et al.*, 2009, MILLER; HUHNDORF, 2005; O`DONNELL, 2004; 2009; 2010).

2. Estilo de vida de *Diaporthe*

Espécies do gênero *Diaporthe* têm sido descritas como patogênicas, endofíticas, saprofíticas e recentemente foi descrita causando problemas de saúde em humanos e outros mamíferos (ROSSMAN, 2007; CROUS; GROENEWALD, 2005; BODDY; GRIFFITH, 1989; REHNER; UECKER, 1994; WEBBER; GIBBS, 1984; CARROLL, 1986; GARCIA-REYNE *et al.*, 2011).

2.1 *Diaporthe* como fitopatógeno

Esse gênero é responsável por várias doenças em uma ampla gama de hospedeiros de importância econômica em todo o mundo, podendo afetar várias partes da planta causando apodrecimento de frutos e raízes, cancrios, manchas foliares, murcha entre outros (UECKER, 1988; RENSBURG *et al.*, 2006; SANTOS *et al.*, 2011; THOMPSON *et al.*, 2011). O gênero *Diaporthe* pode causar doenças em muitas plantas como na soja, uva, morango, girassol, funcho e arbusto vermelho (Figura 1) (SANTOS *et al.*, 2011; MOSTERT *et al.*, 2001; VAN NIEKERK *et al.*, 2005; MAAS, 1998; MUNTANOLA-CVETKOVIC *et al.*, 1981; THOMPSON *et al.*, 2011; SANTOS; PHILLIPS, 2009; RODEVA; GABLER, 2010; RENESBURG *et al.*, 2006).

O gênero *Diaporthe* causa várias doenças na soja, o cancro da haste da soja foi inicialmente denominado de acordo com sua incidência geográfica nos Estados Unidos, sul, “southern stem canker”, e do norte, “northern stem canker” que tinham como agente causal *D. phaseolorum* var. *meridionalis* e *D. phaseolorum* var. *caulivora*, respectivamente (MORGAN-JONES, 1989). Essas duas variedades de *D. phaseolorum* foram recentemente elevadas ao status de espécie *D. phaseolorum* var. *meridionalis* atualmente *D. aspalathi* (RENSBURG *et al.*, 2006) e *D. phaseolorum* var. *caulivora* atualmente *D. caulivora* (SANTOS *et al.*, 2011), por serem filogeneticamente distintas do complexo *D. phaseolorum*.

Além do cancro da haste outra doença da soja é a queima da haste e da vagem causada por *Diaporthe phaseolorum* (Cooke Ellis. Sacc.) var. *sojae* (S.G.

Lehman) Wehmeyer. Outra doença observada em soja é a podridão de sementes, causada por *Phomopsis longicolla* (Hobbs). *Diaporthe phaseolorum* var. *soja* e possui uma variabilidade genética maior que as outras variedades e maior proximidade genética com o *P. longicolla* além de uma ampla extensão de hospedeiros, e por isso, poderia ser considerada uma espécie distinta (ZHANG *et al.*, 1998).



FIGURA 1. DOENÇAS CAUSADAS POR *Diaporthe* EM CULTURAS DE IMPORTÂNCIA ECONÔMICA. 1 CANCRO DA HASTE DA SOJA, NECROSE DA MEDULA CAUSADA POR *D. aspalathi*= *D. phaseolorum* var. *meridionalis* (BORKERT *et al.*, 1994. EM ARQUIVO DO AGRÔNOMO, Nº 5); 2. CANCRO DO GIRASSOL CAUSADO POR *Phomopsis helianthi* (UDAYANGA *et al.*, 2011); 3. NECROSE DO CAULE EM ERVA-DOCE CAUSADO POR *Phomopsis foeniculi* (RODEVA; GABLER, 2010); 4. PODRIDÃO DA UVA CAUSADA POR *Phomopsis viticola* (www.plantmanagementnetwork.org); 5. PODRIDÃO MOLE DO MORANGO CAUSADA POR *Phomopsis obscurans* (a) ESTÁGIO INICIAL, (b) ESTÁGIO FINAL (Legard, DE: www.APSnet.org).

Além da soja, o gênero *Diaporthe* causa doenças em outras espécies de plantas no Brasil como aroeira, baru, caju do campo, mangaba (CHARCHAR *et al.*, 2003), algodão (BÉLOT; ZAMBIASI, 2007; CHARCHAR *et al.*, 2003) citros (KIMATI; GALLI, 1980), fícus (LIMA *et al.*, 2005), morango (FORTES, 2005), pêssego (LUZ, 2003), feijão (ANJOS *et al.*, 2001; SOUZA, 2011), uva (GARRIDO, 2006) cafeeiro (CHARCHAR *et al.*, 2003; ANJOS *et al.*, 2001) entre outros.

2.2 *Diaporthe* como patógeno humano

Espécies do gênero *Diaporthe* foram recentemente descritas como patógenos oportunistas em humanos. Em 1999, uma espécie de *Phomopsis* foi relatada causando uma infecção subcutânea no dedo de um agricultor imunocomprometido e o gênero foi adicionado à lista de fungos capazes de causar doença em humanos (SUTTON *et al.*, 1999). Em 2011, *Phomopsis longicolla* Hobbs, um conhecido fitopatógeno de soja, foi diagnosticado através de biópsia e sequenciamento de rDNA causando infecção cutânea em um paciente imunocomprometido após transplante renal. Os autores acreditam que o paciente tenha adquirido o fungo pelo menos cinco anos antes da infecção, quando teve contato com sementes ou plantas de soja na Guiné Equatorial (GARCIA-REYNE *et al.*, 2011).

Outra espécie fitopatogênica também descrita em soja, *Diaporthe phaseolorum*, foi relatada causando osteomielite em paciente com sorologia positiva para o vírus linfotrópico humano do tipo 1 (HTLV-1) e por isso com alterações na resposta imune. O paciente era um agricultor e a inoculação possivelmente ocorreu através de espinhos de *Amaranthus spinosus* (IRIART *et al.*, 2011).

Segundo Iriart *et al.* (2011) as plantas infectadas com *Diaporthe* são essenciais na epidemiologia da doença uma vez que as pessoas infectadas entraram em contato com plantas e o local da lesão fúngica ocorreu em partes não cobertas do corpo.

2.3 *Diaporthe* produtor de metabolitos tóxicos

O gênero *Diaporthe* além de ter sido descrito causando doenças em humanos imunocomprometidos também podem causar problemas em outros mamíferos. Algumas espécies tais como *Diaporthe toxica* é conhecida por produzir metabolitos tóxicos, phomopsins, em palha ou semente de *Lupinus*, o que pode resultar na morte de animais de pasto (PETERSON *et al.*, 1987, COWLEY *et al.*, 2010).

Van Warmelo e Marasas (1972) relataram um tipo de micotoxicose em ovelhas que ocorreu após o consumo de plantas infectadas por *P. leptostromiformis*. *Phomopsis Leptostromiformis* produz os metabolitos tóxicos Phomopsina A e B, é o fungo causador da ferrugem do caule na planta *Lupinus* spp. as quais ao ser ingerida pelos animais causa Lupinose micotóxica, a doença ocorre principalmente em ovinos, porém foi relatada ocasionalmente em bovinos, equinos e suínos (ALLAN *et al.*, 1992, MONDOLY; PONCELET, 2005).

2.4 *Diaporthe* como saprofítico

Existem vários relatos de *Diaporthe* como sapróbios em diferentes hospedeiros em decomposição, assim como endófitos e patógenos latentes são os primeiros colonizadores em uma ampla gama de hospedeiros em decomposição (PROMPUTTHA *et al.*, 2007; KUMARESAN; SURYANARAYANAN, 2002; OSONO; TAKEDA, 2002; HYDE *et al.*, 2002; HYDE *et al.*, 2007; PROMPUTTHA *et al.*, 2010).

Promputtha *et al.* (2010), compararam a microbiota endofítica e saprofítica de *Magnolia liliflora*, e dos nove isolados endofíticos obtidos três também ocorriam nas folhas no início da fase de decomposição. Outra evidencia de que isolados de *Diaporthe* endofíticos tornam-se sapróbios na senescência foliar foi demonstrada pela produção de enzimas degradantes pelos endófitos, semelhantes às produzidas por sapróbios (PROMPUTTHA *et al.*, 2010; MEENAVALLI *et al.*, 2011).

2.5 *Diaporthe* como endofítico e produtor de metabólitos secundários

As descobertas de novos metabólitos de fungos biologicamente ativos têm sido foco da produção científica em todo o mundo, incluindo novos antibióticos, agentes quimioterápicos e defensivos agrícola. Estes metabólitos produzidos pelos fungos geralmente são eficazes, possuem baixa toxicidade, e tem um menor impacto no ambiente (PEARCE, 1997; STROBEL; DAISY, 2003; SMITH *et al.*, 2008, XU *et al.*, 2010). O gênero *Diaporthe* tem sido relatado como um gênero em potencial para o uso na biotecnologia com várias descobertas importantes, incluindo novos metabolitos fúngicos estruturalmente significativos e fisiologicamente ativos.

Várias dessas substâncias são produzidas por isolados endofíticos. Microrganismos endofíticos são aqueles que vivem no interior das plantas, interagindo com outras espécies de microrganismos, sem causar dano ou prejuízo ao seu hospedeiro. Esses microrganismos podem influenciar em várias características expressas pelas plantas. Segundo Neto, Azevedo e Araujo (2002), os endófitos podem desempenhar relevante função para a sanidade vegetal, já que atuam como agentes controladores de microrganismos fitopatogênicos, no controle de insetos e até na proteção da planta contra herbívoros. Todas as plantas já estudadas são habitadas por microrganismos endofíticos, principalmente fungos e bactérias. A comunidade endofítica, cuja composição varia em função do hospedeiro e das condições ambientais, tem indivíduos que se inter-relacionam dentro da planta, em um equilíbrio harmônico. O desequilíbrio dessa harmonia afeta o comportamento de todos os integrantes da comunidade, dando condições para que fungos oportunistas manifestem seu potencial patológico contra hospedeiro (MAKI, 2006).

Os endófitos apresentam uma relação mutualística, antagonística ou neutra como hospedeiro. A interação de um fungo com seu hospedeiro vegetal variam e dependendo modo pelo qual o fungo infecta a planta e também da extensão da resposta de defesa desta. A colonização pode ser sistêmica ou local, inter ou intracelular (ARAÚJO; SILVA ;AZEVEDO, 2000). Os fungos endofíticos atuam no controle biológico de fitopatógenos, por ocuparem o mesmo nicho ecológico destes

(ARAÚJO; SILVA; AZEVEDO, 2000). Segundo Schulz e Boyle (2005), aproximadamente 80% dos fungos endofíticos produzem compostos biologicamente ativos, como antibióticos, fungicidas e herbicidas.

Alguns exemplos dessas substâncias produzidas endofitos são: *Phomopsis cassiae* isolado de *Cassia spectabilis* produz ethyl 2,4-dihydroxy-5,6-dimethylbenzoate e phomopsilactona com atividade antifúngica, bem como citotoxicidade contra a linhagem celular de tumor cervical humano (HeLa) (SILVA *et al.*, 2005); Phomoxanthone A, B é produzido por *Phomopsis* sp. BCC 1323 isolado de *Tectona grandis*, esse metabolito apresenta atividade *in vitro* antimalárica, antituberculose e citotóxica (ISAKA *et al.*, 2001). *Phomopsis* sp. Isolado de *Taxus cuspidata* produz taxol que apresenta atividade anticancerígena (KUMARAN, 2009); *Phomopsis* sp. H76 isolado de *Excoecaria agallocha* produz phomopsis-H76 A, B, C e apresenta *in vitro* atividade antibacteriana e citotóxica (YANG *et al.*, 2010); *Phomopsis* sp. Lz42 isolado de *Maytenus hookeri* produz um novo sesquiterpeno, esterol, e mais cinco compostos com atividade não detectada (LIN *et al.*, 2009); *Phomopsis* sp. Isolado de *Salix gracilostyla* var. *melanostachys* produz Phomopsichalasin com atividade antibacteriana e antifúngica (TAN; ZOU, 2001); *Phomopsis* sp. Isolado de *Azadirachtae indica* produz cinco lactonas com atividade antifúngica contra patógenos de plantas (Wu *et al.*, 2008); *Phomopsis longicolla* endofítico de *Dicerandra frutescens* produz dicerandrol A,B,C com atividade antibiótica e citotóxica (WAGENAAR; CLARDY, 2001); *Phomopsis* sp. Isolado de *Hydnocarpus anthelminthicus* produz derivados de mycoepoxydiene com atividade citotóxica (PRACHYA *et al.*, 2007).

Outros isolados de *Diaporthe* também foram relatados produzindo metabolitos, por exemplo: i) *Phomopsis oblonga* isolado de *Ulmus* sp. produz vários novos compostos com atividade inseticida (CLAYDON *et al.*, 1985); ii) *Phomopsis leptostromiformis* isolado de *Lupinus* sp. produz phomopsin com atividade antimicótica (YIN *et al.*, 1992, SHIVAS *et al.*, 1991); iii) *Phomopsis* sp. A123 isolado de *Kandelia candel* produz nonenolides, phomonol, phomotone, phomophene com atividade não detectada (LI *et al.*, 2010a, b); iv) *Phomopsis* sp. KS-37-2 isolado de *Prunus serotina* produz benzophomopsin A com atividade não detectada (SHINO *et*

al., 2009); v) *Phomopsis* sp. Isolado de *Erythrina cristagalli* produz mellein, nectriapyrone, 4-hydroxymellein, scytalone, tyrosol, clavatul, ácido mevinic, mevalonolactone, phomol com atividade antimicrobiana e antiinflamatória (REDKOA *et al.*, 2007; WEBER *et al.*, 2005); vi) *Phomopsis archeri* isolado de *Vanilla albidia* produz novo sesquiterpenos com atividade citotóxica contra linhagens celulares de câncer (HEMTASIN *et al.*, 2011) e vii) *Phomopsis* sp. B3 isolado de *Bischofia polycarpa* produz lacase com atividade oxidativa (DAI *et al.*, 2010).

Diaporthe tem sido relatado como um dos gêneros mais frequentes encontrados como endofíticos em muitas plantas hospedeiras (BOTELLA; DIEZ, 2011; MURALI *et al.*, 2006). Frequentemente descrito como produtor de enzimas e metabólitos secundários (ELSAESSER *et al.*, 2005; KOBAYASHI *et al.*, 2003; ISAKA *et al.*, 2001; DAI *et al.*, 2005) com potencial produtor de antibióticos (LIN *et al.*, 2005; DETTRAKUL *et al.*, 2003; BANDRE; SASEK, 1977); fungicida (SILVA *et al.*, 2005; Wu *et al.*, 2008); anticâncer (KUMARAN, 2009) e capaz de impedir a herbivoria (BRAYFORD, 1990; WEBER, 2009; VESTERLUND *et al.*, 2011).

Os fungos endofíticos têm recebido maior atenção, devido à sua diversidade o que os tem tornado candidatos interessantes para a descoberta de novos metabólitos (STROBEL; DAISY, 2003; ZHIGIANG, 2005; MITCHELL *et al.*, 2010; LIU, 2011). Isolados de *Diaporthe* endofíticos têm sido descritos principalmente como produtores de importantes metabólitos secundários, porém devido à dificuldade em identificar esse gênero em nível de espécie, a maioria desses isolados produtores de metabólitos é identificada apenas em nível de gênero.

As novas descobertas tem revelado o enorme potencial do gênero *Diaporthe*, principalmente isolados endofíticos. Esses microrganismos endofíticos têm o potencial de inibir fitopatógenos, competindo por nutrientes, parasitando e/ou produzindo metabólitos secundários como enzimas líticas e antibióticos. O resultado dessas interações antagônicas, tais como antibiose, competição, indução de defesa e parasitismo tem revelado um papel muito importante desses microrganismos na medicina, com a descoberta de novos fármacos, e na agronomia com o controle biológico de doenças de plantas.

3. *Diaporthe* como agente de controle biológico

O controle biológico de plantas daninhas por patógenos de plantas ganhou aceitação como método prático e ambientalmente seguro (TRUJILLO, 2005). Algumas espécies do gênero *Diaporthe* são micoherbicidas potenciais para controlar ervas daninhas invasivas e destrutivas, devido ao seu modo de vida hemibiotrófico a necrotrofico, extensiva esporulação e persistência no ambiente (ROSSKOPF *et al.*, 2000a, b). Tem havido notável atenção voltada para bioherbicidas ou micoherbicidas, isto é, o uso de agentes patogênicos fúngicos na promoção de estratégias de controle biológico (MORTENSEN, 1997; CHARUDATTAN, 2000; TRUJILLO, 2005).

As toxinas e enzimas envolvidas na fisiologia e funções bioquímicas dos hemibiotróficos e necrotróficos são alvos importantes para os estudos de controle biológico e entendimento da patologia de plantas contribuindo para projetar estratégias racionais para o controle da doença (VAN KAN, 2006). O conhecimento do ciclo de vida do patógeno também dirige o controle efetivo de doenças de plantas (GONZÁLEZ-FERNÁNDEZ *et al.*, 2010).

Várias plantas do gênero *Amaranthus* são ervas daninhas em sistemas de cultivo em todo o mundo, e podem reduzir a produção de algodão e outras culturas por competição. Além de apresentar rápido crescimento e abundante produção de sementes, alguns biótipos de *Amaranthus* desenvolveram resistência a um número de famílias de herbicidas e o controle biológico tem sido uma alternativa. *Phomopsis amaranthicola* pode infectar e matar *Amaranthus* spp. E portanto, tem sido utilizado como um agente bioherbicida. Esta espécie foi patenteada (Patente dos EUA n^o s 5510316 e 5393728) para o controle de *Amaranthus* spp. (ROSSKOPF; YANDOC, 2005; MORAN, 2007).

Outros exemplos de *Diaporthe* potencialmente disponíveis no controle biológico de plantas daninhas são: *Phomopsis* spp. Infecta *Carthamus lanatus* (ASH *et al.*, 2010); *P. emicis* infecta *Emex australis* (SHIVAS; SCOTT, 1993); *P. convolvulus* infecta *Convolvulus arvensis* (ORMENO-NUNEZ *et al.*, 1988; MORIN *et al.*, 1989) e *P. cirsii* infecta *Cirsium arvense* (LETH *et al.*, 2008).

A ampla gama de hospedeiros suscetíveis a *Diaporthe*, a especificidade em causar doença a determinados hospedeiros e mecanismos de infecção de algumas espécies e a sua persistência no ambiente favorece o uso de algumas espécies desse gênero no controle de plantas daninhas (ORTIZ RIBBING; WILLIAMS, 2006).

4. Mancha Preta dos Citros

A doença Mancha Preta dos Citros (MPC) causa sérios problemas fitossanitários, tem grande importância econômica e afeta a citricultura comercial em muitas regiões produtoras da África, Ásia, Austrália, América do Sul e Estados Unidos da América (FEICHTENBERGER, 1996; FIALHO *et al.*, 2010; GÓES *et al.*, 2005; AGRIANUAL, 2010). Esta doença é causada pelo fungo *Phyllosticta citricarpa* McAlpine (Teleomorfo: *Guignardia citricarpa* Kiely) e deprecia o valor comercial dos frutos destinados ao mercado de frutas frescas, reduz a produtividade devido à queda prematura dos frutos e aumenta consideravelmente o custo de produção (GÓES *et al.*, 2005). Além disso, o fruto não pode ser exportado especialmente para a Comunidade Europeia devido a restrições fitossanitárias (OEPP / EPPO 2003, 2008), causando sérias restrições na importação de frutos brasileiros nestes locais (AGUILAR-VILDOSO *et al.*, 2002, BALDASSARI *et al.*, 2008). Tal fato tem trazido sérios prejuízos ao Brasil, uma vez que o país é o maior produtor de laranjas no mundo, responsável por aproximadamente um terço da produção mundial de laranja e pelo controle de 85% do mercado internacional de suco da fruta, representando uma das principais atividades do agronegócio brasileiro (CULTIVAR, 2012).

A utilização de fungicidas é o principal método de controle utilizado no pré e pós-colheita (FIALHO *et al.*, 2011). Além disso, a utilização de fungicidas no controle da doença, além onerar a produção, oferece risco para o ambiente e o ser humano. Porém faz-se necessária quando a doença ataca de forma severa, e principalmente, quando a produção destina-se ao consumo da fruta fresca (STRINGARI, 2009). Os fungicidas inicialmente mais utilizados eram os benzimidazóis e os carbamatos (SPÓSITO, 1999). O principal problema do emprego sucessivo de fungicidas é a seleção de linhagens resistentes do agente causal, como observado na África do Sul

(HERBERT; GRECH, 1985) o que levou a restrições na sua utilização (AGOSTINI *et al.*, 2006; DEISING *et al.*, 2008).

Dessa forma, várias pesquisas têm focado na busca de medidas alternativas para o controle da MPC, principalmente através do controle biológico do fungo *P. citricarpa*. Por exemplo, o controle da MPC pela produção de metabolitos voláteis por *S. cerevisiae* para uso nos frutos pós-colheita (FIALHO *et al.*, 2010), seleção de linhagens fúngicas como potenciais agentes no controle biológico, demonstrando que algumas espécies de *Trichoderma*, são capazes de atuar *in vitro*, como biocontroladores do fitopatógeno (RODRIGUES, 2006). Porém a habilidade de *Trichoderma* spp. Em controlar a MPC *in vitro* não foi a mesma observada quando avaliado *in vivo* em pomares de laranjeira Natal, nos quais os isolados de *Trichoderma* sp. Não apresentaram potencial antagonista em condições de campo e a severidade de doença foi alta nas safras avaliadas, sendo o tratamento com fungicida mais efetivo para o controle da doença (KUPPER *et al.*, 2011).

Cardoso-Filho (2003) avaliou o uso de indutores de resistência *Saccharomyces cerevisiae*, Bion[®] e ácido salicílico ao fitopatógeno *P. citricarpa*. O autor destaca que os compostos presentes no albedo (mesocarpo) da laranja, como a celulose, carboidratos solúveis, pectinas, compostos fenólicos (flavonóides), aminoácidos e vitaminas podem exibir a ação antimicrobiana para o controle da MPC. Neste contexto, ele avaliou os extratos produzidos pelo albedo da laranja e comprovou a ação fungicida ou fungistática, inibindo a germinação, esporulação e crescimento micelial, *in vitro*, de *P. citricarpa*.

Gomes *et al.*, 2012 realizaram uma bioprospecção de fungos endofíticos do gênero *Diaporthe* das plantas medicinais *Spondias mombin*, *Myracrodruon urundeuva*, *Schinus terebinthifolius*, *Aspidosperma tomentosum* e *Maytenus ilicifolia*. Algumas espécies do gênero apresentaram potencial de biocontrole da MPC nos testes *in vitro* e em fruto. Porém para utilização de endófitos desse gênero com potencial antagonista no controle biológico de doenças de plantas faz-se necessário avaliar o potencial desses em colonizar endofiticamente plantas de citros, assim como, avaliar as relações filogenéticas desses com as espécies de *Diaporthe* fitopatogênicas.

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***Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi**

Filogenia e taxonomia do gênero *Diaporthe* e a sua aplicação no controle biológico da Mancha Preta dos Citros



Diaporthe: a genus of endophytic, saprobic and plant pathogenic fungi

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Key words

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Abstract *Diaporthe* (*Phomopsis*) species have often been reported as plant pathogens, non-pathogenic endophytes or saprobes, commonly isolated from a wide range of hosts. The primary aim of the present study was to resolve the taxonomy and phylogeny of a large collection of *Diaporthe* species occurring on diverse hosts, either as pathogens, saprobes, or as harmless endophytes. In the present study we investigated 243 isolates using multi-locus DNA sequence data. Analyses of the rDNA internal transcribed spacer (ITS1, 5.8S, ITS2) region, and partial translation elongation factor 1-alpha (TEF1), beta-tubulin (TUB), histone H3 (HIS) and calmodulin (CAL) genes resolved 95 clades. Fifteen new species are described, namely *Diaporthe arengae*, *D. brasiliensis*, *D. endophytica*, *D. hongkongensis*, *D. inconspicua*, *D. infecunda*, *D. mayteni*, *D. neoarctii*, *D. oxe*, *D. paranensis*, *D. pseudomangiferae*, *D. pseudophoenicola*, *D. raonikayaporum*, *D. schini* and *D. terebinthifolii*. A further 14 new combinations are introduced in *Diaporthe*, and *D. anacardii* is epitypified. Although species of *Diaporthe* have in the past chiefly been distinguished based on host association, results of this study confirm several taxa to have wide host ranges, suggesting that they move freely among hosts, frequently co-colonising diseased or dead tissue. In contrast, some plant pathogenic and endophytic taxa appear to be strictly host specific. Given this diverse ecological behaviour among members of *Diaporthe*, future species descriptions lacking molecular data (at least ITS and HIS or TUB) should be strongly discouraged.

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INTRODUCTION

Species of *Diaporthe* and their *Phomopsis* asexual states have broad host ranges and are widely distributed, occurring as plant pathogens, endophytes or saprobes, but also as pathogens of humans and other mammals (Webber & Gibbs 1984, Carroll 1986, Boddy & Griffith 1989, Rehner & Uecker 1994, Garcia-Reyne et al. 2011, Udayanga et al. 2011). *Diaporthe* spp. are responsible for diseases on a wide range of plants hosts, some of which are economically important worldwide, causing root and fruit rots, dieback, cankers, leaf spots, blights, decay and wilt (Uecker 1988, Mostert et al. 2001a, van Rensburg et al. 2006, Santos et al. 2011, Thompson et al. 2011).

Currently, MycoBank (accessed Sept. 2012) lists more than 1 000 names in the genus *Phomopsis*, while *Diaporthe* contains more than 860 names. In the past species have chiefly been described under the assumption they are host-specific, leading to a proliferation of names based on the hosts from which they were isolated (Uecker 1988). However, subsequent studies have found that many species are able to colonise diverse hosts as opportunists, and that several different species could even co-occur on the same host or lesion (Brayford 1990, Rehner & Uecker 1994, Mostert et al. 2001a, Farr et al. 2002, Crous & Groenewald 2005). Curiously, some species of *Diaporthe* can be either pathogenic or harmless endophytes depending on the

host and its health. For example, *D. phaseolorum* is pathogenic to soybean (Santos et al. 2011), but endophytic in mangroves (*Laguncularia racemosa*) (Sebastiane et al. 2011). With the deletion of Art. 59 from the International Code of Nomenclature for algae, fungi, and plants (ICN), asexual and sexual names of fungi receive equal status (Hawksworth et al. 2011, Wingfield et al. 2012). Because the name *Diaporthe* (1870) predates *Phomopsis* (1905), *Diaporthe* is adopted in the present study for this group of fungi (Santos et al. 2010, 2011, Crous et al. 2011, Udayanga et al. 2012).

Diaporthe (incl. its *Phomopsis* state) has been reported as one of the most frequently encountered genera of endophytic fungi in several plant hosts (Murali et al. 2006, Botella & Diez 2011). The genus has also frequently been recognised as a producer of interesting enzymes and secondary metabolites (Isaka et al. 2001, Kobayashi et al. 2003, Dai et al. 2005, Elsaesser et al. 2005) with antibiotic (Bandre & Sasek 1977, Dettrakul et al. 2003, Lin et al. 2005) or anticancer (Kumaran & Hur 2009) activity. Furthermore, species of *Diaporthe* have in the past been noted to deter herbivory (Brayford 1990, Weber 2009, Vesterlund et al. 2011), have lignocellulolytic activities (Jordaan et al. 2006), or have been applied as bioherbicides (Ash et al. 2010).

The accurate application of accepted names of plant pathogenic fungi is essential for the development of effective biosecurity and trade policies (Crous & Groenewald 2005, Wingfield et al. 2012). The taxonomy of many groups of plant pathogenic fungi has in the past been based on host association (Crous et al. 2013, Groenewald et al. 2013). Although some species of *Diaporthe* are host specific, a great number have been noted to occur on more than one host (Brayford 1990, Rehner & Uecker 1994, Farr et al. 2002). Similar observations led Wehmeyer

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Table 1 Host / substrate, locality, collector and GenBank accession numbers of strains included in the study.

Species	Original name	Strain ¹	Isolation source	Host family	Locality	Collector	GenBank Accession numbers (ITS, CAL, HIS, TEF1, TUB) ²
<i>Diaporthe acaotigena</i>	<i>D. acaotigena</i>	CBS 129521; CPC 17622 (ex-type)	<i>Acacia retinodes</i> , leaves	Mimosaceae	Australia	P.W. Crous, I.G. Pascoe & J. Edwards	KC343489 KC343731 KC343973
<i>Diaporthe acerina</i>	<i>D. acerina</i>	CBS 137.27	<i>Acer saccharum</i>	Aceraceae	–	L.E. Wehmeyer	KC343248 KC343732 KC343974
<i>Diaporthe alleghaniensis</i>	<i>D. alleghaniensis</i>	CBS 495.72; ATCC 24097 (ex-type)	<i>Betula alleghaniensis</i> , branches	Betulaceae	Canada	R.H. Arnold	KC343249 KC343733 KC343975
<i>Diaporthe alnea</i>	<i>D. alnea</i>	CBS 146.46	<i>Alnus</i> sp.	Betulaceae	–	S. Truter	KC343250 KC343734 KC343976
	<i>D. alnea</i>	CBS 159.47	<i>Alnus</i> sp.	Betulaceae	–	S. Truter	KC343251 KC343735 KC343977
<i>Diaporthe ambigua</i>	<i>D. ambigua</i>	CBS 114015; STE-U 2657; CPC 2657 (ex-epitype)	<i>Pyrus communis</i>	Rosaceae	South Africa	S. Denman	KC343252 KC343736 KC343978
	<i>D. ambigua</i>	CBS 117167; STE-U 5414; CPC 5414	<i>Aspalathus linearis</i> , crown	Fabaceae	South Africa	J.C. Janse van Rensburg	KC343495 KC343737 KC343979
	<i>D. ambigua</i>	CBS 123210; Di-C003/10	<i>Foeniculum vulgare</i>	Apiaceae	Portugal	J.M. Santos	KC343254 KC343738 KC343980
	<i>D. ambigua</i>	CBS 123211; Di-C002/9	<i>Foeniculum vulgare</i>	Apiaceae	Portugal	J.M. Santos	KC343255 KC343739 KC343981
	<i>D. scabra</i>	CBS 127746; IMI 395956	<i>Platanus acerifolia</i>	Platanaceae	Italy	G. Granata	KC343256 KC343740 KC343982
	<i>D. helianthi</i>	CBS 187.87	<i>Helianthus annuus</i>	Asteraceae	Italy	A. Zizzerini	KC343257 KC343741 KC343983
<i>Diaporthe ampelina</i> , comb. nov.	<i>P. viticola</i>	CBS 111888; ATCC 48153; STE-U 2673; CPC 2673	<i>Vitis vinifera</i>	Vitaceae	USA; California	J.D. Cuccuzza	KC343258 KC343742 KC343984
	<i>P. viticola</i>	CBS 114016; STE-U 2660; CPC 2660; PV F98-1 (ex-neotype)	<i>Vitis vinifera</i>	Vitaceae	France	P. Laignon	AY745026 – AY745056 JX275452
	<i>P. viticola</i>	CBS 114867; STE-U 4708; CPC 4708	<i>Vitis vinifera</i>	Vitaceae	Turkey	M. Erkan	KC343259 KC343743 KC343985
	<i>P. viticola</i>	CBS 267.80; STE-U 2671; CPC 2671	<i>Vitis vinifera</i>	Vitaceae	Italy	A. Zizzerini	KC343260 KC343744 KC343986
<i>Diaporthe amygdali</i>	<i>P. amygdali</i>	CBS 111811; STE-U 2632; CPC 2632	<i>Vitis vinifera</i>	Vitaceae	South Africa	L. Mostert	KC343261 KC343745 KC343987
	<i>P. amygdali</i>	CBS 115620; FAU 1005	<i>Prunus persica</i> , cankers	Rosaceae	USA; Georgia	W. Uddin	KC343262 KC343746 KC343988
	<i>P. amygdali</i>	CBS 120840; STE-U 5833; CPC 5833	<i>Prunus salicina</i> , wood	Rosaceae	South Africa	U. Damm	KC343263 KC343747 KC343989
	<i>P. amygdali</i> 3B	CBS 126679 (ex-epitype)	<i>Prunus dulcis</i>	Rosaceae	Portugal	E. Diogo	KC343264 KC343748 KC343990
	<i>P. amygdali</i> 55A	CBS 126680	<i>Prunus dulcis</i>	Rosaceae	Portugal	E. Diogo	KC343265 KC343749 KC343991
<i>Diaporthe anacardii</i> , comb. nov.	<i>P. anacardii</i>	CBS 720.97 (ex-epitype)	<i>Anacardium occidentale</i>	Anacardiaceae	East Africa	M. Puccioni	KC343266 KC343750 KC343992
<i>Diaporthe angelicae</i>	<i>P. foeniculi</i>	CBS 100871	<i>Foeniculum vulgare</i> , dying twig	Apiaceae	Italy	L. Mignai	KC343267 KC343751 KC343993
	<i>D. angelicae</i>	CBS 111591; AP 3724	<i>Heracleum sphondylium</i> , decaying stems	Apiaceae	Austria	A.Y. Rossman	KC343268 KC343752 KC343994
	<i>D. angelicae</i>	CBS 111592; AR3776 (ex-epitype)	<i>Heracleum sphondylium</i> , decaying stems	Apiaceae	Austria	A.Y. Rossman	KC343269 KC343753 KC343995
	<i>D. angelicae</i>	CBS 123215; Ph-C133/1	<i>Foeniculum vulgare</i>	Apiaceae	Portugal	A.J.L. Phillips	KC343270 KC343754 KC343996
	<i>D. asteriscus</i>	CBS 344.86	<i>Eryngium maritimum</i> , leaf spots	Apiaceae	France	H.A. van der Aa	KC343271 KC343755 KC343997
<i>Diaporthe arctii</i>	<i>D. arctii</i>	CBS 501.90	<i>Heracleum sphondylium</i> , seeds	Apiaceae	France	H.A. van der Aa	KC343272 KC343756 KC343998
	<i>D. arctii</i>	CBS 136.25	<i>Arctium</i> sp.	Asteraceae	–	A.W. Archer	KC343273 KC343757 KC343999
<i>Diaporthe arecae</i> , comb. nov.	<i>P. phoenicicola</i>	CBS 161.64 (ex-istotype)	<i>Areca catechu</i> , fruit	Areaceae	India	H.C. Sivastava	KC343274 KC343758 KC344000
	<i>D. citri</i>	CBS 535.75	<i>Citrus</i> sp., fruits	Rutaceae	Suriname	I. Block	KC343275 KC343759 KC344001
<i>Diaporthe arengae</i> , sp. nov.	<i>P. pittospori</i>	CBS 114979; HKUCC 5527 (ex-type)	<i>Arenga engleri</i>	Areaceae	Hong Kong	K.D. Hyde	KC343276 KC343760 KC344002
<i>Diaporthe aspalathi</i>	<i>D. aspalathi</i>	CBS 117168; STE-U 5420; CPC 5420	<i>Aspalathus linearis</i> , crown	Fabaceae	South Africa	J.C. Janse van Rensburg	KC343277 KC343761 KC344003
	<i>D. aspalathi</i>	CBS 117169; STE-U 5428; CPC 5428 (ex-type)	<i>Aspalathus linearis</i> , branch	Fabaceae	South Africa	J.C. Janse van Rensburg	KC343278 KC343762 KC344004
	<i>D. aspalathi</i>	CBS 117500; STE-U 5408; CPC 5408	<i>Aspalathus linearis</i>	Fabaceae	South Africa	S. Lamprecht	KC343279 KC343763 KC344005
<i>Diaporthe australafricana</i>	<i>D. australafricana</i>	CBS 111886; STE-U 2676; CPC 2676 (ex-type)	<i>Vitis vinifera</i>	Vitaceae	Australia	R.W.A. Schepers	KC343280 KC343764 KC344006
	<i>D. australafricana</i>	CBS 113487; STE-U 2655; CPC 2655 (ex-type)	<i>Vitis vinifera</i>	Vitaceae	South Africa	L. Mostert	KC343281 KC343765 KC344007
<i>Diaporthe batatas</i>	<i>D. batatas</i>	CBS 122.21	<i>Ipomoea batatas</i>	Convolvulaceae	USA	L.L. Harter	KC343282 KC343766 KC344008
<i>Diaporthe beckhausii</i>	<i>D. beckhausii</i>	CBS 138.27	<i>Viburnum</i> sp.	Caprifoliaceae	–	L.E. Wehmeyer	KC343283 KC343767 KC344009
<i>Diaporthe brasiliensis</i> , sp. nov.	–	CBS 133183; LGMF924; CPC 20300 (ex-type)	<i>Aspidosperma tomentosum</i> , endophytic in leaf	Apocynaceae	Brazil	K. Rodriguez	KC343284 KC343768 KC344010
	–	LGMF926; CPC 20302	<i>Aspidosperma tomentosum</i> , endophytic in leaf	Apocynaceae	Brazil	K. Rodriguez	KC343285 KC343769 KC344011
<i>Diaporthe carpini</i>	<i>D. carpini</i>	CBS 114437; UPSC 2980	<i>Carpinus betulus</i>	Corylaceae	Sweden	K. & L. Holm	KC343286 KC343770 KC344012
<i>Diaporthe caulivora</i>	<i>D. caulivora</i>	CBS 127268; Dpc1 (ex-neotype)	<i>Glycine max</i> , stem	Fabaceae	Croatia	K. Vrandečić	KC343287 KC343771 KC344013
	<i>D. phaseolorum</i> var. <i>caulivora</i>	CBS 178.55; ATCC 12048; Altaro 243	<i>Glycine soja</i> , mature stem	Fabaceae	Canada	A.A. Hildebrand	KC343288 KC343772 KC344014
<i>Diaporthe celastrina</i>	<i>D. celastrina</i>	CBS 139.27	<i>Celastrus scandens</i>	Celastraceae	–	L.E. Wehmeyer	KC343289 KC343773 KC344015
<i>Diaporthe chamaerops</i> , comb. nov.	<i>P. phoenicicola</i>	CBS 454.81	<i>Chamaerops humilis</i> , dead part of leaf	Areaceae	Greece	H.A. van der Aa	KC343290 KC343774 KC344016
	<i>D. cinerascens</i>	CBS 753.70	<i>Spartium junceum</i> , dead branch	Fabaceae	Croatia	J.A. von Arx	KC343291 KC343775 KC344017
<i>Diaporthe cinerascens</i>	<i>P. cinerascens</i>	CBS 719.96	<i>Ficus carica</i> , branch	Moraceae	Bulgaria	E. Ilieva	KC343292 KC343776 KC344018
<i>Diaporthe citri</i>	<i>D. conorum</i>	CBS 199.39	–	–	Italy	G. Goldanich	KC343293 KC343777 KC344019
	<i>D. citri</i>	CBS 230.52	<i>Citrus sinensis</i> , decaying fruit	Rutaceae	Suriname	N.J. van Suchtelen	KC343294 KC343778 KC344020

<i>Diaporthe</i> sp.	LGMF946; CPC 20322	<i>Glycine max</i> , seed	Brazil	A. Almeida	KC343779	KC343537	KC343779	KC344021
<i>P. convolvuli</i>	CBS 124654; DP 0727	<i>Convolvulus arvensis</i> , leaves	Turkey	D. Berner	KC343780	KC343538	KC343780	KC344022
<i>Diaporthe crataegi</i>	CBS 114435; UPSC 2938	<i>Crataegus oxyacantha</i>	Sweden	K. & L. Holm	KC343781	KC343539	KC343781	KC344023
<i>Diaporthe croatalariae</i>	CBS 162.33 (ex-type)	<i>Crotalaria spectabilis</i>	USA	G.F. Weber	KC343782	KC343540	KC343782	KC344024
<i>Diaporthe cuppatea</i>	CBS 117499; STE-U 5431; CPC 5431 (ex-type)	<i>Aspalathus linearis</i>	South Africa	J.C. Jansen van Rensburg	KC343783	KC343541	KC343783	KC344025
<i>Diaporthe cynaroidis</i>	CBS 122676; CMW 22190; CPC 13180 (ex-type)	<i>Protea cynaroides</i> , leaf litter	South Africa	S. Marinowitz	KC343784	KC343542	KC343784	KC344026
<i>D. decedens</i>	CBS 109772; AR 3459	<i>Corylus avellana</i>	Austria	W. Jaklitsch	KC343785	KC343543	KC343785	KC344027
<i>D. decedens</i>	CBS 114281; UPSC 2957	<i>Corylus avellana</i>	Sweden	K. & L. Holm	KC343786	KC343544	KC343786	KC344028
<i>D. detrusa</i>	CBS 109770; AR 3424	<i>Berberis vulgaris</i>	Austria	A.Y. Rossman	KC343787	KC343545	KC343787	KC344029
<i>D. detrusa</i>	CBS 114652; UPSC 3371	<i>Berberis vulgaris</i>	Sweden	K. & L. Holm	KC343788	KC343546	KC343788	KC344030
<i>D. detrusa</i>	CBS 140.27	<i>Berberis vulgaris</i>	—	L.E. Wehmeyer	KC343789	KC343547	KC343789	KC344031
<i>P. eleagni</i>	CBS 504.72	<i>Eleaagnus</i> sp., twig	Netherlands	J. Gremmen	KC343790	KC343548	KC343790	KC344032
—	CBS 133811; LGMF916; CPC 20292 (ex-type)	<i>Schinus terebinthifolius</i> , endophytic in leaf	Brazil	J. Lima	KC343791	KC343549	KC343791	KC344033
—	LGMF911; CPC 20287	<i>Schinus terebinthifolius</i> , endophytic in leaf	Brazil	J. Lima	KC343792	KC343550	KC343792	KC344034
—	LGMF919; CPC 20295	<i>Schinus terebinthifolius</i> , endophytic in leaf	Brazil	J. Lima	KC343793	KC343551	KC343793	KC344035
—	LGMF928; CPC 20304	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343794	KC343552	KC343794	KC344036
—	LGMF934; CPC 20310	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343795	KC343553	KC343795	KC344037
—	LGMF935; CPC 20311	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343796	KC343554	KC343796	KC344038
—	LGMF937; CPC 20313	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343797	KC343555	KC343797	KC344039
—	LGMF948; CPC 20324	<i>Glycine max</i> , seed	Brazil	A. Almeida	KC343798	KC343556	KC343798	KC344040
<i>D. eres</i>	CBS 101742	<i>Fraxinus</i> sp., fallen fruit	Netherlands	G.J.M. Verkleij	KC343799	KC343557	KC343799	KC344041
<i>D. medusaea</i>	CBS 102.81	<i>Juglans regia</i> , twig	Italy	M. Bisiach	KC343800	KC343558	KC343800	KC344042
<i>D. eres</i>	CBS 109767; AR 3538; WJ 1643	<i>Acer campestre</i>	Austria	W. Jaklitsch	KC343801	KC343559	KC343801	KC344043
<i>D. arctii</i>	CBS 110.85	<i>Arctium</i> sp., dead stems	Netherlands	M. de Noij	KC343802	KC343560	KC343802	KC344044
<i>P. skirrmiae</i>	CBS 122.82	<i>Skirrmia japonica</i> , dying twigs	Netherlands	H.A. v. Kesteren	KC343803	KC343561	KC343803	KC344045
<i>Phomopsis</i> sp. no. 23	CBS 129168	<i>Rhododendron</i> sp.	Latvia	I. Apine	KC343804	KC343562	KC343804	KC344046
<i>D. conorum</i>	CBS 186.37	<i>Picea abies</i> , seedling	UK	T.R. Peace	KC343805	KC343563	KC343805	KC344047
<i>P. controversa</i>	CBS 250.38	<i>Fraxinus excelsior</i> , living and dead twig	UK; Scotland	J.A. MacDonald	KC343806	KC343564	KC343806	KC344048
<i>P. stictica</i>	CBS 267.32	—	—	W.G. Hutchinsonson	KC343807	KC343565	KC343807	KC344049
<i>P. rudis</i>	CBS 267.55	<i>Laburnum</i> × <i>watereri</i> 'Vossii'	Netherlands	I. de Boer	KC343808	KC343566	KC343808	KC344050
<i>P. rangjevicii</i>	CBS 283.85	<i>Allium giganteum</i> , dead stem	Netherlands	H.A. van der Aa	KC343809	KC343567	KC343809	KC344051
<i>D. eres</i>	CBS 287.74	<i>Sorbus aucuparia</i> , dead branch	Netherlands	W.M. Loerakker	KC343810	KC343568	KC343810	KC344052
<i>P. osmanthi</i>	CBS 297.77	<i>Osmanthus aquifolium</i> , leaf tip	Netherlands	H.A. van der Aa	KC343811	KC343569	KC343811	KC344053
<i>P. cacti</i>	CBS 365.97	<i>Opuntia</i> sp., cladodes	Netherlands	H.A. van der Aa	KC343812	KC343570	KC343812	KC344054
<i>P. crustosa</i>	CBS 370.67; MUOL 9931	<i>Ilex aquifolium</i> , dead leaf	Netherlands	H.A. van der Aa	KC343813	KC343571	KC343813	KC344055
<i>D. pennicosa</i>	CBS 375.61	<i>Malus sylvestris</i> , rotten fruit	Netherlands	Geigy	KC343814	KC343572	KC343814	KC344056
<i>P. phaseoli</i>	CBS 422.50	<i>Phaseolus vulgaris</i>	Netherlands	Goossens	KC343815	KC343573	KC343815	KC344057
<i>P. cotoneastris</i>	CBS 439.82; BBA P-407; IMI 162181a (isotype of <i>Phomopsis cotoneastris</i>)	<i>Cotoneaster</i> sp.	UK; Scotland	H. Butin	KC343816	KC343574	KC343816	KC344058
<i>P. cruciferae</i>	CBS 445.62	<i>Alliaria officinalis</i>	Netherlands	G.H. Boerema	KC343817	KC343575	KC343817	KC344059
<i>P. durandiana</i>	CBS 485.96	<i>Rumex hydrolapathum</i> , dead stem	Netherlands	H.A. van der Aa	KC343818	KC343576	KC343818	KC344060
<i>D. seposita</i>	CBS 528.83	<i>Wisteria sinensis</i> , dead branch	Netherlands	H.A. van der Aa	KC343819	KC343577	KC343819	KC344061
<i>P. abutilonis</i>	CBS 688.97	<i>Abutilon</i> sp.	Netherlands	A. Aptroot	KC343820	KC343578	KC343820	KC344062
<i>P. crustosa</i>	CBS 694.94	<i>Ilex aquifolium</i> , twigs suffering from dieback	Netherlands	G.J.M. Verkleij	KC343821	KC343579	KC343821	KC344063
<i>P. magnolicola</i>	CBS 791.68	<i>Magnolia</i> × <i>soulangeana</i> , withering leaf	Netherlands	H.A. van der Aa	KC343822	KC343580	KC343822	KC344064
<i>P. tritici</i>	CBS 841.84	<i>Hordeum</i> sp., leaf spot	Germany	M. Hosfeld	KC343823	KC343581	KC343823	KC344065
<i>P. eugeniae</i>	CBS 444.82	<i>Eugenia aromatica</i> , leaf	West Sumatra	R. Kasim	KC343824	KC343582	KC343824	KC344066
<i>D. fibrosa</i>	CBS 109751; AR 3425	<i>Rhamnus cathartica</i>	Austria	A.Y. Rossman	KC343825	KC343583	KC343825	KC344067
<i>D. fibrosa</i>	CBS 113830; UPSC 2117	<i>Rhamnus cathartica</i>	Sweden	K. & L. Holm	KC343826	KC343584	KC343826	KC344068
<i>D. foeniculacea</i>	CBS 111553	<i>Foeniculum vulgare</i> , base of senescent stem	Spain	A.J.L. Phillips	KC343827	KC343585	KC343827	KC344069
<i>D. foeniculacea</i>	CBS 111554	<i>Foeniculum vulgare</i> , base of senescent stem	Portugal	A.J.L. Phillips	KC343828	KC343586	KC343828	KC344070
<i>P. theicola</i>	CBS 116957; NZ-37	<i>Pyrus pyrifolia</i>	New Zealand	W. Kandula	KC343829	KC343587	KC343829	KC344071
<i>D. neotheicola</i>	CBS 123208; Di-C004/5 (ex-type of <i>D. neotheicola</i>)	<i>Foeniculum vulgare</i>	Portugal	A.J.L. Phillips	KC343830	KC343588	KC343830	KC344072
<i>D. neotheicola</i>	CBS 123209; Di-C004/4 (ex-type of <i>D. neotheicola</i>)	<i>Foeniculum vulgare</i>	Portugal	A.J.L. Phillips	KC343831	KC343589	KC343831	KC344073
<i>P. mali</i> f.sp. <i>amygdali</i>	CBS 171.78	<i>Prunus amygdalus</i> , dried fruit	Italy	A. Ciccarone	KC343832	KC343590	KC343832	KC344074

Table 1 (cont.)

Species	Original name	Strain ¹	Isolation source	Host family	Locality	Collector	GenBank Accession numbers (ITS, CAL, HIS, TEF1, TUB) ²
	<i>P. theicola</i>	CBS 187.27 (ex-type of <i>P. theicola</i>)	<i>Camellia sinensis</i> , leaves and branches	Thesaceae	Italy	M. Curzi	KC343349 KC343591 KC343833 KC344075
	<i>P. diospyri</i>	CBS 287.56	<i>Diospyros kaki</i> , twig, after frost damage	Ebenaceae	Italy	M. Ribaldi	KC343592 KC343834 KC344076
	<i>D. seposita</i>	CBS 357.69	<i>Wisteria sinensis</i> , dead twigs	Fabaceae	Netherlands	H.A. van der Aa	KC343351 KC343594 KC344077
	<i>P. casuarinae</i>	CBS 400.48	–	–	India	S.R. Bose	KC343352 KC343595 KC343836 KC344078
	<i>P. bougainvilleae</i>	CBS 603.88	<i>Bougainvillea spectabilis</i> , peduncles of flowers	Nyctaginaceae	Portugal	H.A. van der Aa	KC343353 KC343595 KC343837 KC344079
	<i>P. ganjae</i>	CBS 180.91; ILLS 43621 (ex-type)	<i>Cannabis sativa</i> , dead leaf	Cannabaceae	USA: Illinois	J.M. McPartland	KC343354 KC343596 KC343838 KC344080
	<i>Diaporthe gardeniae</i> , comb. nov.	CBS 288.56	<i>Gardenia florida</i> , stem	Rubiaceae	Italy	M. Ribaldi	KC343355 KC343597 KC343839 KC344081
	<i>Diaporthe helianthi</i>	CBS 344.94	<i>Helianthus annuus</i> , seed	Asteraceae	–	–	KC343356 KC343598 KC343840 KC344082
	<i>D. helianthi</i>	CBS 592.81 (ex-type)	<i>Helianthus annuus</i> , overwintering stem	Asteraceae	Serbia	M. Muntaniola-Cvetkovic	KC343357 KC343599 KC343841 KC344083
	<i>Diaporthe cf. heveae</i> 1	CBS 852.97	<i>Hevea brasiliensis</i>	Euphorbiaceae	Brazil	K.C. Jayarathnam	KC343358 KC343600 KC343842 KC344084
	<i>Diaporthe cf. heveae</i> 2	CBS 681.84	<i>Hevea brasiliensis</i> , leaf	Euphorbiaceae	India	K. Jayarathnam	KC343359 KC343601 KC343843 KC344085
	<i>Diaporthe hickoriae</i>	CBS 145.26 (ex-type)	<i>Carya glabra</i>	Juglandaceae	USA: Michigan	L.E. Wehmeyer	KC343360 KC343602 KC343844 KC344086
	<i>Diaporthe hongkongensis</i> , sp. nov.	CBS 115448; HKUCC 9104; AT 646.DF 24 (ex-type)	<i>Dichroa febrifuga</i> , fruit	Hydrangeaceae	Hong Kong	K.D. Hyde	KC343361 KC343603 KC343845 KC344087
	<i>Diaporthe hordei</i> , comb. nov.	CBS 481.92	<i>Hordeum vulgare</i> , root	Poaceae	Norway	L. Sundheim	KC343362 KC343604 KC343846 KC344088
	<i>Diaporthe impulsae</i>	CBS 114434; UFGS 3052	<i>Sorbus aucuparia</i>	Rosaceae	Sweden	K. & L. Holm	KC343363 KC343605 KC343847 KC344089
	<i>D. impulsae</i>	CBS 141.27	<i>Sorbus americana</i>	Rosaceae	–	L.E. Wehmeyer	KC343364 KC343606 KC343848 KC344090
	<i>Diaporthe inconspicua</i> , sp. nov.	CBS 133813; LGMF930; CPC 20306 (ex-type)	<i>Maytenus ilicifolia</i> , endophytic in petiole	Celastraceae	Brazil	R.R. Gomes	KC343365 KC343607 KC343849 KC344091
	<i>Diaporthe</i> sp.	LGMF922; CPC 20298	<i>Spondias mombin</i>	Anacardiaceae	Brazil	K. Rodriguez	KC343366 KC343608 KC343850 KC344092
	<i>Diaporthe</i> sp.	LGMF931; CPC 20307	<i>Maytenus ilicifolia</i> , endophytic in petiole	Celastraceae	Brazil	R.R. Gomes	KC343367 KC343609 KC343851 KC344093
	<i>Diaporthe</i> sp.	CBS 133812; LGMF906; CPC 20282 (ex-type)	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343368 KC343610 KC343852 KC344094
	<i>Diaporthe</i> sp.	LGMF908; CPC 20284	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343369 KC343611 KC343853 KC344095
	<i>Diaporthe</i> sp.	LGMF912; CPC 20288	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343370 KC343612 KC343854 KC344096
	<i>Diaporthe</i> sp.	LGMF917; CPC 20293	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343371 KC343613 KC343855 KC344097
	<i>Diaporthe</i> sp.	LGMF918; CPC 20294	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343372 KC343614 KC343856 KC344098
	<i>Diaporthe</i> sp.	LGMF920; CPC 20296	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343373 KC343615 KC343857 KC344099
	<i>Diaporthe</i> sp.	LGMF933; CPC 20309	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	R.R. Gomes	KC343374 KC343616 KC343858 KC344100
	<i>Diaporthe</i> sp.	LGMF940; CPC 20316	<i>Maytenus ilicifolia</i> , endophytic in petiole	Celastraceae	Brazil	R.R. Gomes	KC343375 KC343617 KC343859 KC344101
	<i>Diaporthe juglandina</i>	CBS 121004; DP 0659	<i>Maytenus ilicifolia</i> , endophytic in petiole	Juglandaceae	USA: Tennessee	L. Vasilyeva	KC343376 KC343618 KC343860 KC344102
	<i>Diaporthe longispora</i> , comb. nov.	CBS 194.36 (ex-type)	<i>Juglans</i> sp., dead wood	Juglandaceae	USA: Tennessee	L. Vasilyeva	KC343377 KC343619 KC343861 KC344103
	<i>Diaporthe longispora</i> var. <i>longispora</i>		<i>Ribes</i> sp.	Grossulariaceae	Canada	L.E. Wehmeyer	KC343377 KC343619 KC343861 KC344103
	<i>Diaporthe lusitanicae</i>	CBS 123212; Di-C001/5 (ex-type)	<i>Foeniculum vulgare</i> , stem	Apiaceae	Portugal	J.M. Santos	KC343378 KC343620 KC343862 KC344104
	<i>D. lusitanicae</i>	CBS 123213; Di-C001/3	<i>Foeniculum vulgare</i> , stem	Apiaceae	Portugal	J.M. Santos	KC343379 KC343621 KC343863 KC344105
	<i>Diaporthe manihoti</i>	CBS 505.76	<i>Manihot utilissima</i> , leaves	Euphorbiaceae	Rwanda	J. Semal	KC343380 KC343622 KC343864 KC344106
	<i>Diaporthe mayteni</i> , sp. nov.	CBS 133185; LGMF938; CPC 20314 (ex-type)	<i>Maytenus ilicifolia</i> , endophytic in petiole	Celastraceae	Brazil	R.R. Gomes	KC343381 KC343623 KC343865 KC344107
	<i>Diaporthe megalospora</i>	CBS 143.27	<i>Sambucus canadensis</i>	Caprifoliaceae	–	L.E. Wehmeyer	KC343382 KC343624 KC343866 KC344108
	<i>Diaporthe melonis</i> var. <i>sojae</i>	CBS 435.87	<i>Glycine soja</i>	Fabaceae	Indonesia	H. Vermeulen	KC343383 KC343625 KC343867 KC344109
	<i>Diaporthe musigena</i>	CBS 507.78 (ex-isotype)	<i>Cucumis melo</i>	Cucurbitaceae	USA: Texas	L. Beraha & M.J. O'Brien	KC343384 KC343626 KC343868 KC344110
	<i>Diaporthe neilliae</i>	CBS 129519; CPC 17026 (ex-type)	<i>Musa</i> sp., leaves	Musaceae	Australia	P.W. Crous & R.G. Shivas	KC343385 KC343627 KC343869 KC344111
	<i>Diaporthe neoarctii</i> , sp. nov.	CBS 144.27	<i>Spiraea</i> sp.	Rosaceae	–	L.E. Wehmeyer	KC343386 KC343628 KC343870 KC344112
		CBS 109490; GB 6421; AR 3450 (ex-type)	<i>Ambrosia trifida</i>	Asteraceae	USA:	G. Bills	KC343387 KC343629 KC343871 KC344113
	<i>Diaporthe nobilis</i>	CBS 113470; DAOM 226800	<i>Castanea sativa</i> , chestnuts collected in grocery store	Fagaceae	New Jersey	K.A. Seifert	KC343388 KC343630 KC343872 KC344114
	<i>P. fukushii</i>	CBS 116953; NZ-26	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	W. Kandlea & L. Castlebury	KC343389 KC343631 KC343873 KC344115
	<i>P. fukushii</i>	CBS 116954; NZ-27	<i>Pyrus pyrifolia</i>	Rosaceae	New Zealand	W. Kandlea & L. Castlebury	KC343390 KC343632 KC343874 KC344116
	<i>D. perniciosa</i>	CBS 124030; GUS 77-49	<i>Malus pumila</i> , bark	Rosaceae	New Zealand	G.J. Samuels	KC343391 KC343633 KC343875 KC344117
	<i>Phnomopsis</i> sp. no. 22	CBS 129167	<i>Rhododendron</i> sp.	Ericaceae	Latvia	I. Apine	KC343392 KC343634 KC343876 KC344118
	<i>D. nobilis</i>	CBS 200.39	<i>Laurus nobilis</i> , stem	Lauraceae	Germany	Kothhoff	KC343393 KC343635 KC343877 KC344119
	<i>D. pulla</i>	CBS 338.89	<i>Hedera helix</i>	Araliaceae	Yugoslavia	M. Muntaniola-Cvetkovic	KC343394 KC343636 KC343878 KC344120

<i>P. conorum</i>	CBS 587.79	<i>Pinus pentaphylla</i> , bonzai imported from Japan into Netherlands	Japan	G.H. Boerema	KC343153	KC343395	KC343637	KC343879	KC344121
<i>D. nomurai</i>	CBS 157.29	<i>Morus</i> sp.	Japan	T. Togashi	KC343154	KC343396	KC343638	KC343880	KC344122
<i>D. novem</i>	CBS 127269; 5-27/3-1	<i>Glycine max</i> , seed	Croatia	K. Duvnjak	KC343155	KC343397	KC343639	KC343881	KC344123
<i>D. novem</i>	CBS 127270; 4-27/3-1 (ex-type)	<i>Glycine max</i> , seed	Croatia	T. Duvnjak	KC343156	KC343398	KC343640	KC343882	KC344124
<i>D. novem</i>	CBS 127271; 5/27/3-3	<i>Glycine max</i> , seed	Croatia	T. Duvnjak	KC343157	KC343399	KC343641	KC343883	KC344125
<i>D. parafloata</i>	CBS 354.71	<i>Polygonatum odoratum</i> , leaves	Romania	O. Constantinescu	KC343158	KC343400	KC343642	KC343884	KC344126
<i>Diaporthe</i> sp.	LGMF943; CPC 20319	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343159	KC343401	KC343643	KC343885	KC344127
<i>D. oncostoma</i>	CBS 100454	<i>Robinia pseudoacacia</i> , leaf spot	Germany	H. Butlin	KC343160	KC343402	KC343644	KC343886	KC344128
<i>D. oncostoma</i>	CBS 109741; AR 3445	<i>Robinia pseudoacacia</i> , dead branches	Russia	L. Vasilyeva	KC343161	KC343403	KC343645	KC343887	KC344129
<i>D. oncostoma</i>	CBS 589.78	<i>Robinia pseudoacacia</i> , leaf	France	H.A. van der Aa	KC343162	KC343404	KC343646	KC343888	KC344130
<i>P. crustosa</i>	CBS 809.85	<i>Ilex aquifolium</i> , leaf	Germany	M. Hesse	KC343163	KC343405	KC343647	KC343889	KC344131
–	CBS 133186; LGMF942; CPC 20318 (ex-type)	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343164	KC343406	KC343648	KC343890	KC344132
–	CBS 133187; LGMF936; CPC 20312	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343165	KC343407	KC343649	KC343891	KC344133
–	LGMF915; CPC 20291	<i>Schinus terebinthifolius</i> , endophytic in leaf	Brazil	J. Lima	KC343166	KC343650	KC343892	KC344134	
–	LGMF939; CPC 20315	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	S.A.V. Pileggi	KC343167	KC343409	KC343651	KC344135	
–	LGMF945; CPC 20321	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343168	KC343410	KC343652	KC343894	KC344136
<i>Diaporthe pedf</i> var. <i>padf</i>	CBS 114200; UPSC 2569	<i>Prunus padus</i>	Sweden	K. & L. Holm	KC343169	KC343411	KC343653	KC343895	KC344137
<i>D. valsiformis</i>	CBS 114649; UPSC 3496	<i>Alnus glutinosa</i>	Sweden	K. & L. Holm	KC343170	KC343412	KC343654	KC343896	KC344138
–	CBS 133184; LGMF929; CPC 20305 (ex-type)	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343171	KC343413	KC343655	KC343897	KC344139
<i>Diaporthe perijuncta</i>	CBS 109745; ARSEF 3461; AR 3461 (ex-epitype)	<i>Ulmus glabra</i>	Austria	A.Y. Rossman	KC343172	KC343414	KC343656	KC343898	KC344140
<i>P. perseae</i>	CBS 151.73	<i>Persea gratissima</i> , young fruit	Netherlands Antilles	E. Laville	KC343173	KC343415	KC343657	KC343899	KC344141
<i>P. oleariae</i>	CBS 113425	<i>Oleaia cf. rani</i>	New Zealand	G.J.M. Verkleij	KC343174	KC343416	KC343658	KC343900	KC344142
<i>D. phaseolorum</i>	CBS 116019; STAM 30	<i>Caperonia palustris</i>	USA, Mississippi	A. Mengistu	KC343175	KC343417	KC343659	KC343901	KC344143
<i>D. phaseolorum</i>	CBS 116020; STAM 31	<i>Aster exilis</i>	USA, Mississippi	A. Mengistu	KC343176	KC343418	KC343660	KC343902	KC344144
<i>Diaporthe</i> sp.	CBS 127465; GJS 83-379	<i>Actinidia chinensis</i> , rotting fruit	New Zealand	S.R. Pennycook	KC343177	KC343419	KC343661	KC343903	KC344145
<i>D. melonis</i>	CBS 257.80	–	–	L. Beraha	KC343178	KC343420	KC343662	KC343904	KC344146
<i>Diaporthe</i> sp.	LGMF927; CPC 20303	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343179	KC343421	KC343663	KC343905	KC344147
<i>Diaporthe</i> sp.	LGMF941; CPC 20317	<i>Maytenus ilicifolia</i> , endophytic in petiole	Brazil	R.R. Gomes	KC343180	KC343422	KC343664	KC343906	KC344148
<i>P. mangiferae</i>	CBS 101339 (ex-type)	<i>Mangifera indica</i>	Dominican Republic	P. de Leeuw	KC343181	KC343423	KC343665	KC343907	KC344149
<i>P. mangiferae</i>	CBS 388.89	<i>Mangifera indica</i> , peel of fruit	Mexico	–	KC343182	KC343424	KC343666	KC343908	KC344150
<i>Diaporthe pseudophoenicicola</i> , sp. nov.	CBS 176.77	<i>Mangifera indica</i> , showing dieback	Iraq	M.S.A. Al-Momen	KC343183	KC343425	KC343667	KC343909	KC344151
<i>P. phoenicicola</i>	CBS 462.69 (ex-type)	<i>Phoenix dactylifera</i> , dead tops of green leaves	Spain	H.A. van der Aa	KC343184	KC343426	KC343668	KC343910	KC344152
<i>D. pustulata</i>	CBS 109742; AR 3430	<i>Acer pseudoplatanus</i>	Austria	A.Y. Rossman	KC343185	KC343427	KC343669	KC343911	KC344153
<i>D. pustulata</i>	CBS 109760; AR 3535	<i>Acer pseudoplatanus</i>	Austria	A.Y. Rossman	KC343186	KC343428	KC343670	KC343912	KC344154
<i>D. padf</i>	CBS 109784; AR 3419	<i>Prunus padus</i>	Austria	A.Y. Rossman	KC343187	KC343429	KC343671	KC343913	KC344155
<i>Diaporthe reonikayaporum</i> , sp. nov.	CBS 133182; LGMF923; CPC 20289 (ex-type)	<i>Spondias mombin</i> , endophytic in leaf	Brazil	K. Rodriguez	KC343188	KC343430	KC343672	KC343914	KC344156
<i>D. rhoina</i>	CBS 146.27	<i>Rhus toxicodendron</i>	–	L.E. Wehmeyer	KC343189	KC343431	KC343673	KC343915	KC344157
<i>P. saccarata</i>	CBS 116311; STE-U 3743; CPC 3743 (ex-type)	<i>Protea repens</i> , cankers	South Africa	S. Denman	KC343190	KC343432	KC343674	KC343916	KC344158
–	CBS 133181; LGMF921; CPC 20297 (ex-type)	<i>Schinus terebinthifolius</i> , endophytic in leaf	Brazil	J. Lima	KC343191	KC343433	KC343675	KC343917	KC344159
–	LGMF910; CPC 20286	<i>Schinus terebinthifolius</i> , endophytic in leaf	Brazil	J. Lima	KC343192	KC343434	KC343676	KC343918	KC344160
<i>P. sclerotoides</i>	CBS 296.67; ATCC 18585; IMI 151828 (ex-type)	<i>Cucumis sativus</i> , root	Netherlands	H.A. van der Kesteren	KC343193	KC343435	KC343677	KC343919	KC344161
<i>P. sclerotoides</i>	CBS 710.76; PD 76/674	<i>Cucumis sativus</i> , root	Netherlands	–	KC343194	KC343436	KC343678	KC343920	KC344162
<i>P. scobina</i>	CBS 251.38	<i>Fraxinus excelsior</i> , living and dead twig	UK; Scotland	J.A. MacDonald	KC343195	KC343437	KC343679	KC343921	KC344163
<i>P. longicolla</i>	CBS 100.87	<i>Glycine soja</i>	Italy	P. Glunghi	KC343196	KC343438	KC343680	KC343922	KC344164
<i>P. longicolla</i>	CBS 116017; DP 0508; STAM 28	<i>Euphorbia nutans</i>	USA; Mississippi	A. Mengistu	KC343197	KC343439	KC343681	KC343923	KC344165
<i>P. longicolla</i>	CBS 116023; STAM 35	<i>Glycine max</i>	USA; Mississippi	A. Mengistu	KC343198	KC343440	KC343682	KC343924	KC344166
<i>P. longicolla</i>	CBS 127267; PL4	<i>Glycine max</i> , stem	Croatia	K. Vrandečić	KC343199	KC343441	KC343683	KC343925	KC344167
<i>D. phaseolorum</i> var. <i>sojae</i>	CBS 180.55; ATCC 12050; CECT 2024; Allaro 245	<i>Glycine soja</i> , mature stem	–	A.A. Hildebrand	KC343200	KC343442	KC343684	KC343926	KC344168
<i>D. phaseolorum</i> var. <i>sojae</i>	CBS 659.78; NRRL 13656	<i>Glycine soja</i> , seedling	USA	J. Marcinkowska	KC343201	KC343443	KC343685	KC343927	KC344169

Table 1 (cont.)

Species	Original name	Strain ¹	Isolation source	Host family	Locality	Collector	GenBank Accession numbers (ITS, CAL, HIS, TEF1, TUB) ²
<i>Diaporthe</i> sp. 1	<i>D. phaseolorum</i>	CBS 119639; B 11861	Man, abscess	–	Germany	K. Plechulla	KC343686 KC343444 KC343928 KC344170
<i>Diaporthe</i> sp. 2	<i>Diaporthe</i> sp.	LGMF947; CPC 20323	<i>Glycine max</i> , seed	Fabaceae	Brazil	A. Almeida	KC343687 KC343445 KC343929 KC344171
<i>Diaporthe</i> sp. 3	<i>Diaporthe</i> sp.	LGMF932; CPC 20308	<i>Marylenus ilicifolia</i> , endophytic in petiole	Celastraceae	Brazil	R.R. Gomes	KC343688 KC343446 KC343930 KC344172
<i>Diaporthe</i> sp. 4	<i>P. conorum</i>	CBS 287.29	<i>Pseudotsuga menziesii</i>	Pinaceae	UK; Scotland	G.G. Hahn	KC343689 KC343447 KC343931 KC344173
<i>Diaporthe</i> sp. 5	<i>Diaporthe</i> sp.	LGMF944; CPC 20320	<i>Marylenus ilicifolia</i> , endophytic in petiole	Celastraceae	Brazil	R.R. Gomes	KC343690 KC343448 KC343933 KC344174
<i>Diaporthe</i> sp. 6	–	CBS 125575	<i>Acer opalus</i>	Aceraceae	Italy	W. Jaklitsch	KC343691 KC343449 KC343934 KC344175
<i>Diaporthe</i> sp. 7	<i>P. pitcospori</i>	CBS 115584; HKUCC 7784; AT 7	<i>Maesa perlaris</i> , fruit	Myrsinaceae	Hong Kong	K.D. Hyde	KC343692 KC343450 KC343934 KC344176
<i>Diaporthe</i> sp. 8	<i>P. pitcospori</i>	CBS 115595; HKUCC 10129	<i>Maesa perlaris</i> , fruit	Myrsinaceae	Hong Kong	K.D. Hyde	KC343693 KC343451 KC343935 KC344177
<i>Diaporthe</i> sp. 9	<i>P. anacardii</i>	CBS 458.78	<i>Anacardium occidentale</i>	Anacardiaceae	India	H.C. Govindu	KC343694 KC343452 KC343936 KC344178
<i>Diaporthe</i> sp. 10	<i>Diaporthe</i> sp.	LGMF925; CPC 20301	<i>Aspidosperma tomentosum</i>	Apocynaceae	Brazil	K. Rodriguez	KC343695 KC343453 KC343937 KC344179
<i>Diaporthe</i> sp. 11	<i>P. stricta</i>	CBS 370.54	<i>Buxus sempervirens</i> , dead twig	Buxaceae	Italy	M. Ribaldi	KC343696 KC343454 KC343938 KC344180
<i>Diaporthe</i> sp. 12	<i>P. subordinaria</i>	CBS 101711	<i>Plantago lanceolata</i> , blackened seed	Plantaginaceae	New Zealand	B. Alexander	KC343697 KC343455 KC343939 KC344181
<i>Diaporthe</i> sp. 13	<i>P. subordinaria</i>	CBS 464.90	<i>Plantago lanceolata</i> , stalk	Plantaginaceae	South Africa	R. Shivas	KC343698 KC343456 KC343940 KC344182
<i>Diaporthe</i> sp. 14	<i>P. tecomae</i>	CBS 100547	<i>Tabebuia</i> sp., mycocecidium caused by <i>Prosopodium tecomicola</i>	Biognoniaceae	Brazil	A. Aptroot	KC343699 KC343457 KC343941 KC344183
<i>Diaporthe</i> sp. 15	–	CBS 133180; LGMF914; CPC 20290 (ex-type)	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343700 KC343458 KC343942 KC344184
<i>Diaporthe</i> sp. 16	–	LGMF907; CPC 20283	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343701 KC343459 KC343943 KC344185
<i>Diaporthe</i> sp. 17	–	LGMF909; CPC 20285	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343702 KC343460 KC343944 KC344186
<i>Diaporthe</i> sp. 18	–	LGMF913; CPC 20289	<i>Schinus terebinthifolius</i> , endophytic in leaf	Anacardiaceae	Brazil	J. Lima	KC343703 KC343461 KC343945 KC344187
<i>Diaporthe</i> sp. 19	<i>D. toxica</i>	CBS 534.93; ATCC 96741 (ex-type)	<i>Lupinus angustifolius</i> , stem	Fabaceae	Western Australia	J.B. Nunn	KC343704 KC343462 KC343946 KC344188
<i>Diaporthe</i> sp. 20	<i>D. toxica</i>	CBS 535.93	<i>Lupinus</i> sp.	Fabaceae	Western Australia	P.M. Williamson	KC343705 KC343463 KC343947 KC344189
<i>Diaporthe</i> sp. 21	<i>D. toxica</i>	CBS 546.93	<i>Lupinus</i> sp., stem	Fabaceae	Western Australia	P.M. Williamson	KC343706 KC343464 KC343948 KC344190
<i>Diaporthe</i> sp. 22	<i>D. vaccinii</i>	CBS 118571; G.C.A.Dvacc	<i>Vaccinium corymbosum</i>	Ericaceae	USA; Michigan	G.C. Adams	KC343707 KC343465 KC343949 KC344191
<i>Diaporthe</i> sp. 23	<i>P. vaccinii</i>	CBS 122112; FAU 474	<i>Vaccinium macrocarpon</i>	Ericaceae	USA; New Jersey	L. Carnis	KC343708 KC343466 KC343950 KC344192
<i>Diaporthe</i> sp. 24	<i>P. vaccinii</i>	CBS 122114; FAU 634	<i>Vaccinium corymbosum</i>	Ericaceae	USA; Michigan	D.C. Ramsdell	KC343709 KC343467 KC343951 KC344193
<i>Diaporthe</i> sp. 25	<i>P. vaccinii</i>	CBS 122115; FAU 590	<i>Vaccinium corymbosum</i>	Ericaceae	USA; Michigan	D.C. Ramsdell	KC343710 KC343468 KC343952 KC344194
<i>Diaporthe</i> sp. 26	<i>P. vaccinii</i>	CBS 122116; DF 5022	<i>Vaccinium corymbosum</i>	Ericaceae	USA; North Carolina	D.F. Farr	KC343711 KC343469 KC343953 KC344195
<i>Diaporthe</i> sp. 27	<i>D. vaccinii</i>	CBS 160.32; IFO 32646 (ex-type)	<i>Oxyccoccus macrocarpos</i>	Ericaceae	USA; Massachusetts	C.L. Shear	KC343712 KC343470 KC343954 KC344196
<i>Diaporthe</i> sp. 28	<i>P. vexans</i>	CBS 127.14	<i>Solanum melongena</i>	Solanaceae	USA	L.L. Harter	KC343713 KC343471 KC343955 KC344197
<i>Diaporthe</i> sp. 29	<i>P. controversa</i>	CBS 100170	<i>Fraxinus excelsior</i> , leaf spot	Oleaceae	Netherlands	H.A. van der Aa	KC343714 KC343472 KC343956 KC344198
<i>Diaporthe</i> sp. 30	<i>D. aucubae</i>	CBS 106.95	<i>Aucuba japonica</i> , branches and twigs	Aucubaceae	Netherlands	G.J.M. Verkley	KC343715 KC343473 KC343957 KC344199
<i>Diaporthe</i> sp. 31	<i>D. medusaea</i>	CBS 109492	<i>Laburnum anagyroides</i>	Fabaceae	Austria	A.Y. Rossmann	KC343716 KC343474 KC343958 KC344200
<i>Diaporthe</i> sp. 32	<i>D. pardalota</i>	CBS 109768; AR 3478	<i>Epilobium angustifolium</i>	Onagraceae	Canada	M. Barr	KC343717 KC343475 KC343959 KC344201
<i>Diaporthe</i> sp. 33	<i>D. viticola</i>	CBS 113201; STE-U 5683; CPC 5683 (ex-epitype)	<i>Vitis vinifera</i>	Vitaceae	Portugal	A.J.L. Phillips	KC343718 KC343476 KC343960 KC344202
<i>Diaporthe</i> sp. 34	<i>D. viticola</i>	CBS 114011; CPC 2677	<i>Vitis vinifera</i>	Vitaceae	Portugal	A.J.L. Phillips	KC343719 KC343477 KC343961 KC344203
<i>Diaporthe</i> sp. 35	<i>D. circumscripta</i>	CBS 114436; UPSC 2960	<i>Sambucus cf. racemosa</i>	Caprifoliaceae	Sweden	K. & L. Holm	KC343720 KC343478 KC343962 KC344204
<i>Diaporthe</i> sp. 36	<i>D. medusaea</i>	CBS 266.85; PD 85/25	<i>Rosa rugosa</i>	Rosaceae	Netherlands	G.H. Boerema	KC343721 KC343479 KC343963 KC344205
<i>Diaporthe</i> sp. 37	<i>D. woodii</i>	CBS 312.91	<i>Lupinus arboreus</i> , dead stem	Fabaceae	Netherlands	H.A. van der Aa & F. Meurs	KC343722 KC343480 KC343964 KC344206
<i>Diaporthe</i> sp. 38	<i>P. salicina</i>	CBS 446.62	<i>Salix</i> sp., twig	Salicaceae	Netherlands	G.H. Boerema	KC343723 KC343481 KC343965 KC344207
<i>Diaporthe</i> sp. 39	<i>D. woodii</i>	CBS 449.82	<i>Lupinus</i> sp., dead stem	Fabaceae	Netherlands	H.A. van der Aa	KC343724 KC343482 KC343966 KC344208
<i>Diaporthe</i> sp. 40	<i>P. dipsaci</i>	CBS 502.85	<i>Dipsacus fullonum</i> , dead stem	Dipsacaceae	Netherlands	H.A. van der Aa	KC343725 KC343483 KC343967 KC344209
<i>Diaporthe</i> sp. 41	<i>P. asphodelina</i>	CBS 759.95	<i>Asphodelus albus</i> , 1-yr-old stems	Asphodelaceae	France	G.J.M. Verkley	KC343726 KC343484 KC343968 KC344210
<i>Diaporthe</i> sp. 42	<i>D. aucubae</i>	CBS 794.96	<i>Aucuba japonica</i>	Aucubaceae	UK	G.J.M. Verkley	KC343727 KC343485 KC343969 KC344211
<i>Diaporthe</i> sp. 43	<i>D. woodii</i>	CBS 558.93	<i>Lupinus</i> sp., stem	Fabaceae	Western Australia	P.M. Williamson	KC343728 KC343486 KC343970 KC344212
<i>Diaporthe</i> sp. 44	<i>D. woolworthii</i>	CBS 148.27	<i>Ulmus americana</i>	Ulmaceae	–	L.E. Wehmeyer	KC343729 KC343487 KC343971 KC344213
<i>Diaporthe</i> sp. 45	<i>Diaporthe corylina</i>	CBS 121124; AR 4131	<i>Corylus</i> sp., dying stems	Corylaceae	China; Fuyuan	L.N. Vassiljeva	KC343730 KC343488 KC343972 KC344214

¹ AR: Collection of A.Y. Rossmann; ATCC: American type culture collection; CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CECT: Colección Española de Cultivos Tipo, University of Valencia, Valencia, Spain; CPC: Collection Pedro Crous, housed at CBS; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Oltawa, Canada; HKUCC: University of Hong Kong Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; IFO: Institute for Fungal Culture, Osaka, Japan; IMI: International Mycological Institute, CAB-Bioscience, Egham, Bakenham Lane, U.K.; LGMF: Culture collection of Laboratory of Genetics of Microorganisms, Federal University of Paraná, Curitiba, Brazil; MUCL: Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NRRL: National Center for Agricultural Utilization Research, Peoria, Illinois, U.S.A.; PD: Plant Protection Service, Wageningen, The Netherlands; STE-U: Stellanbosch University culture collections, South Africa; UPSC: Fungal Culture Collection at the Botanical Museum, Uppsala University, Uppsala, Sweden

² TUB: partial beta-tubulin gene; CAL: partial calmodulin gene; HIS: partial histone H3 gene; ITS: internal transcribed spacer regions of the nrDNA and intervening 5.8S nrDNA; TEF1: partial translation elongation factor 1-alpha gene.

(1933) to the conclusion that host-association was not informative enough in *Diaporthe*, thereby reducing the number of species from 650 to only 70 in the genus. However, this revision was based strictly on morphological characters of the *Diaporthe* sexual state, and connections to the *Phomopsis* asexual states (prior to molecular analyses) had been identified only in 20 % of the species (Wehmeyer 1933).

Although the classification of *Diaporthe* has been on-going, species are presently being redefined based on a combination of morphological, cultural, phytopathological, mating type and DNA sequence data (Rehner & Uecker 1994, Zhang et al. 1998, Mostert et al. 2001a, Farr et al. 2002, Santos et al. 2010). However, even when using a combination of morphological and molecular data, the delimitation of species within the genus *Diaporthe* only proved satisfactory once multi-gene DNA sequence data were generated (Castlebury & Mengistu 2006, van Rensburg et al. 2006, Santos et al. 2010, Udayanga et al. 2012), since this adds valuable information in the resolution of complex evolutionary relationships. The aims of the present study were thus to: 1) provide a multi-gene phylogeny for the genus *Diaporthe* based on a large set of well-identified cultures deposited in the CBS culture collection; 2) to identify potential isolates for epitypification, thereby fixing the application of previously established names; 3) to link *Diaporthe* names to their *Phomopsis* asexual states; and 4) to identify a collection of mostly sterile endophytic *Diaporthe* strains isolated from several medicinal hosts in Brazil.

MATERIALS AND METHODS

Isolates

In the present study we analysed 243 *Diaporthe* isolates (Table 1), as well as the outgroup *Diaporthe corylina*. Isolates were obtained from several sources, including 40 endophytic strains isolated from medicinal plants in Brazil (LabGeM/UFPR collection, Curitiba, Brazil), and three isolates from the EMBRAPA-SOJA collection, Londrina, Brazil. A further 199 isolates were obtained from the CBS-KNAW Fungal Biodiversity Centre (CBS), or the working collection of P.W. Crous (CPC) housed at CBS.

DNA isolation, amplification and phylogenetic analysis

Colonies were cultivated on 2 % potato-dextrose agar (PDA), and genomic DNA extraction was undertaken using the Ultra-Clean™ Microbial DNA Kit (MO Bio, Carlsbad, CA, USA) according to manufacturer's instructions. Using 20 isolates, we screened nine loci, of which the five more informative loci were selected for multi-gene analyses.

The primers ITS5 and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA gene operon, including the 3' end of the 18S nrRNA, the first internal transcribed spacer region, the 5.8S nrRNA gene; the second internal transcribed spacer region and the 5' end of the 28S nrRNA gene. The primers EF1-728F and EF1-986R (Carbone & Kohn 1999) were used to amplify part of the translation elongation factor 1- α gene (TEF1) and the primers ACT-512F and ACT-783R (Carbone & Kohn 1999) were used to amplify part of the actin gene (ACT). The primers Gpd1-LM and Gpd2-LM (Myllys et al. 2002) were used to amplify part of the glyceraldehyde-3-phosphate dehydrogenase (GPDH) gene, and part of the calmodulin (CAL) gene was sequenced using the primers CAL-228F and CAL-737R (Carbone & Kohn 1999). The primers CYLH3F (Crous et al. 2004b) and H3-1b (Glass & Donaldson 1995) were used to amplify part of the histone H3 (HIS) gene, and the primers T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995) to amplify

part of the β -tubulin gene (TUB). The primers NMS1 and NMS2 (Li et al. 1994) were used to amplify an internal region of the mitochondrial SSU (mtSSU). The partial large subunit nrDNA (LSU) was sequenced using the primers LSU1Fd (Crous et al. 2009a) and LR5 (Vilgalys & Hester 1990).

Amplification reactions had a total reaction volume of 12.5 μ L which was composed of 1 \times PCR buffer (Bioline GmbH, Luckenwalde, Germany), 5.6 % DMSO (v/v), 20 μ M dNTPs, 0.2 μ M of each forward and reverse primers, 0.25 U of BioTaq Taq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), and 10 ng of genomic DNA. PCR conditions were the same for all loci, except for the MgCl₂ concentration: 2 mM MgCl₂ for the genes LSU and TEF1, 1.5 mM MgCl₂ for the genes ACT, GPDH, mtSSU, ITS and TUB, and 1 mM MgCl₂ for CAL and HIS genes. The PCR conditions were: start step of 2 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 1 min at adequate annealing temperature, and 1 min at 72 °C, followed by a finishing step of 3 min at 72 °C and a cool down step to 4 °C. The annealing temperature varied for each gene: 61 °C (ACT, GPDH, mtSSU); 58 °C (CAL, ITS, HIS); 55 °C (TEF1, TUB) and 48 °C (LSU).

However, some of these primer pairs failed to amplify with some isolates included in this study, and therefore additional combinations were used. The amplification reaction and cycle conditions were the same except the annealing temperature and MgCl₂ concentration. For the amplification of TEF1 with primers EF1-728F and EF2 (O'Donnell et al. 1998), 52 °C and 2 mM MgCl₂; TUB with primers T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous et al. 2004b), 50 °C and 1 mM MgCl₂; CAL with primers CAL-228F and CAL2Rd (Quaedvlieg et al. 2011, Groenewald et al. 2013), 58 °C and 1 mM MgCl₂.

Amplicons were sequenced using both PCR primers with a BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, and sequences were analysed on an ABI Prism 3700 DNA Sequencer (Perkin-Elmer, Norwalk, Foster City, CA, USA). The consensus sequences were visually inspected using MEGA v. 5 software (Tamura et al. 2011). The alignment of obtained sequences was performed using the online MAFFT interface (Kato & Toh 2008; <http://mafft.cbrc.jp/alignment/server>).

For the phylogenetic analyses based on Maximum Likelihood and Bayesian inference, we chose the best evolutionary models for each data partition using the software MrModelTest v. 2.3 (Nylander 2004). MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003) was used to generate the phylogenetic trees under optimal criteria per data partition. The heating parameter was set at 0.3 and the Markov Chain Monte Carlo (MCMC) analysis of four chains was started in parallel from a random tree topology and lasted until the average standard deviation of split frequencies came below 0.01. Trees were saved each 10 000 generations and the resulting phylogenetic tree (Fig. 1) was printed with Geneious v. 5.5.4 (Drummond et al. 2011) and the layout of the tree was done in Adobe Illustrator v. CS5.1. *Diaporthe corylina* (CBS 121124) was used as outgroup in the phylogenetic analyses based on its position as sister family in *Diaporthales* (Vasilyeva et al. 2007). New sequences generated in this study were deposited in NCBI's GenBank nucleotide database (www.ncbi.nlm.nih.gov; Table 1) and the alignment and phylogenetic tree in TreeBASE (study S13943; www.treebase.org).

Locus resolution and SNP detection

Neighbour-joining analyses using the general time-reversible substitution model were applied to each data partition individually to check the stability and robustness of each species clade under each dataset using PAUP v. 4.0b10 (Swofford 2003) (TreeBASE study S13943). Alignment gaps were treated as missing data and all characters were unordered and of equal

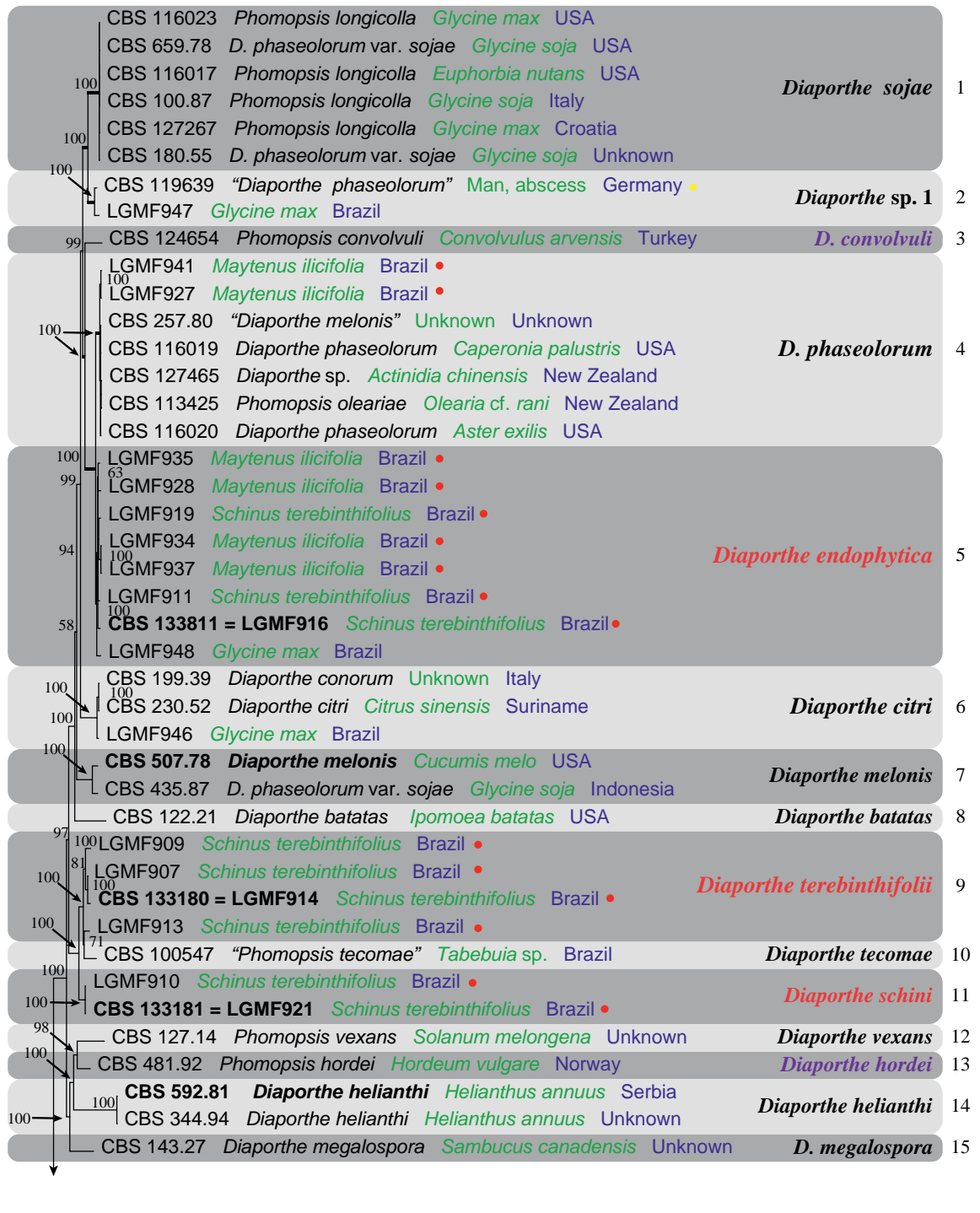


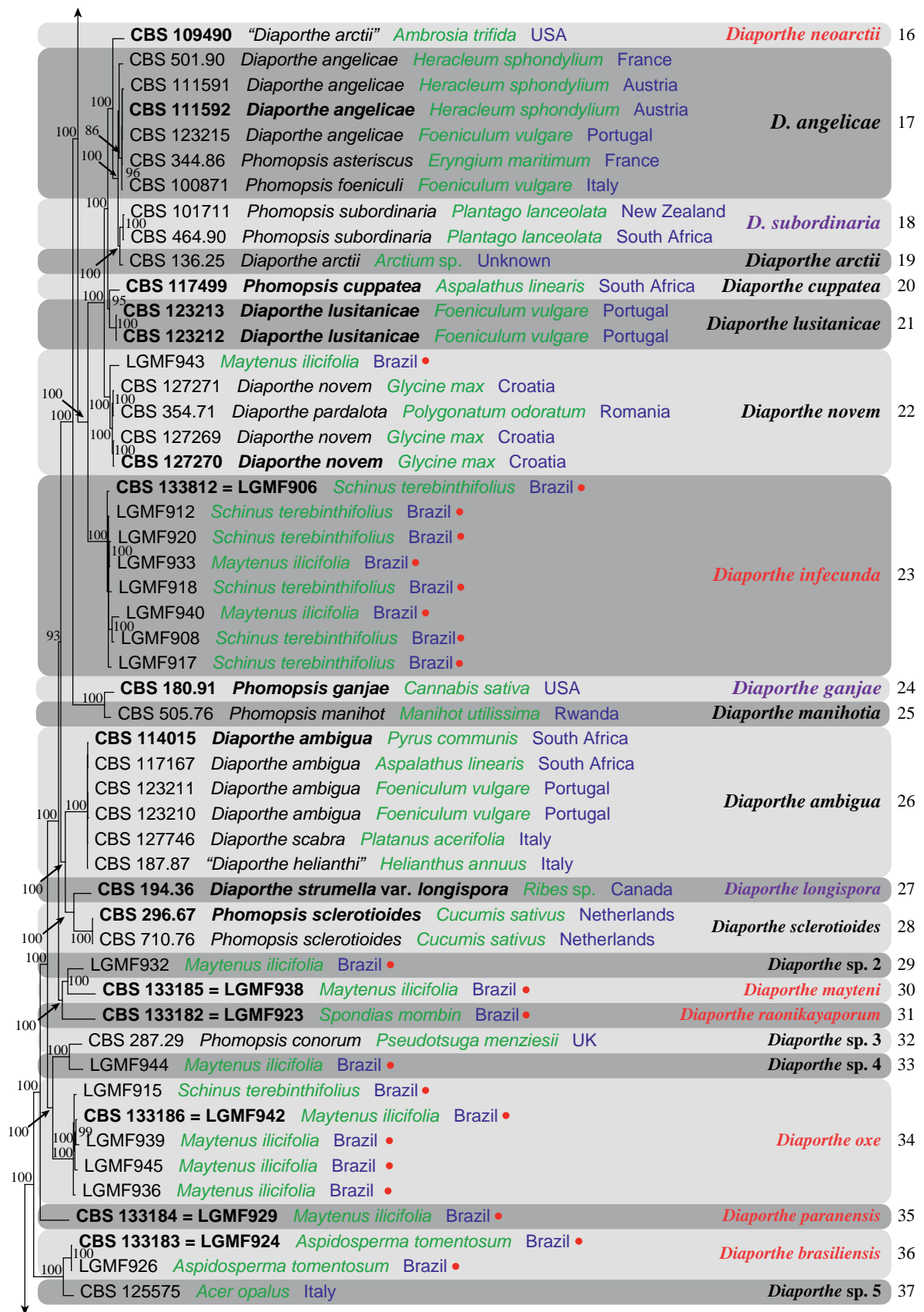
Fig. 1 Consensus phylogram of 22 104 trees resulting from a Bayesian analysis of the combined 5-gene sequence alignment. Clades are numbered on the right of the boxes and *Diaporthe* species names in purple reflect new combinations and in red new species. Strain accession numbers are followed by the original species name (black, when applicable), the isolation source (green) and country of origin (blue). Accession numbers and names in **bold** represent strains known to be ex-type strains or are considered to be authentic for the species. Red dots indicate strains from medicinal plants and yellow dots from humans. Bayesian posterior probabilities are shown at the nodes and the scale bar represents the expected changes per site. The tree was rooted to *Diaporthe corylina* (strain CBS 121124).

weight. Any ties were broken randomly when encountered. The robustness of the trees obtained was evaluated by 1 000 bootstrap replications (Hillis & Bull 1993). In the present study, both the analysis of the combined alignment (Fig. 1) and of the individual loci were used to determine the species boundaries. For each clade in the combined analysis, the position of the members of that clade was determined in the phylogenetic tree obtained from each of the individual loci to check whether these members still represent a single clade in the individual gene tree. In this way the robustness of a given clade could

be evaluated together with the posterior probability value of that clade. A species was only counted if it was distinct from its closest relatives and the species clade contained all the associated strains.

Unique fixed nucleotide positions are used to characterise and describe several sterile species (see applicable species notes). For each sterile species that was described, the closest phylogenetic neighbour(s) were selected from Fig. 1 and this focused dataset was subjected to SNP analyses. These single nucleotide polymorphisms (SNPs) were determined for

Fig. 1 (cont.)



0.2

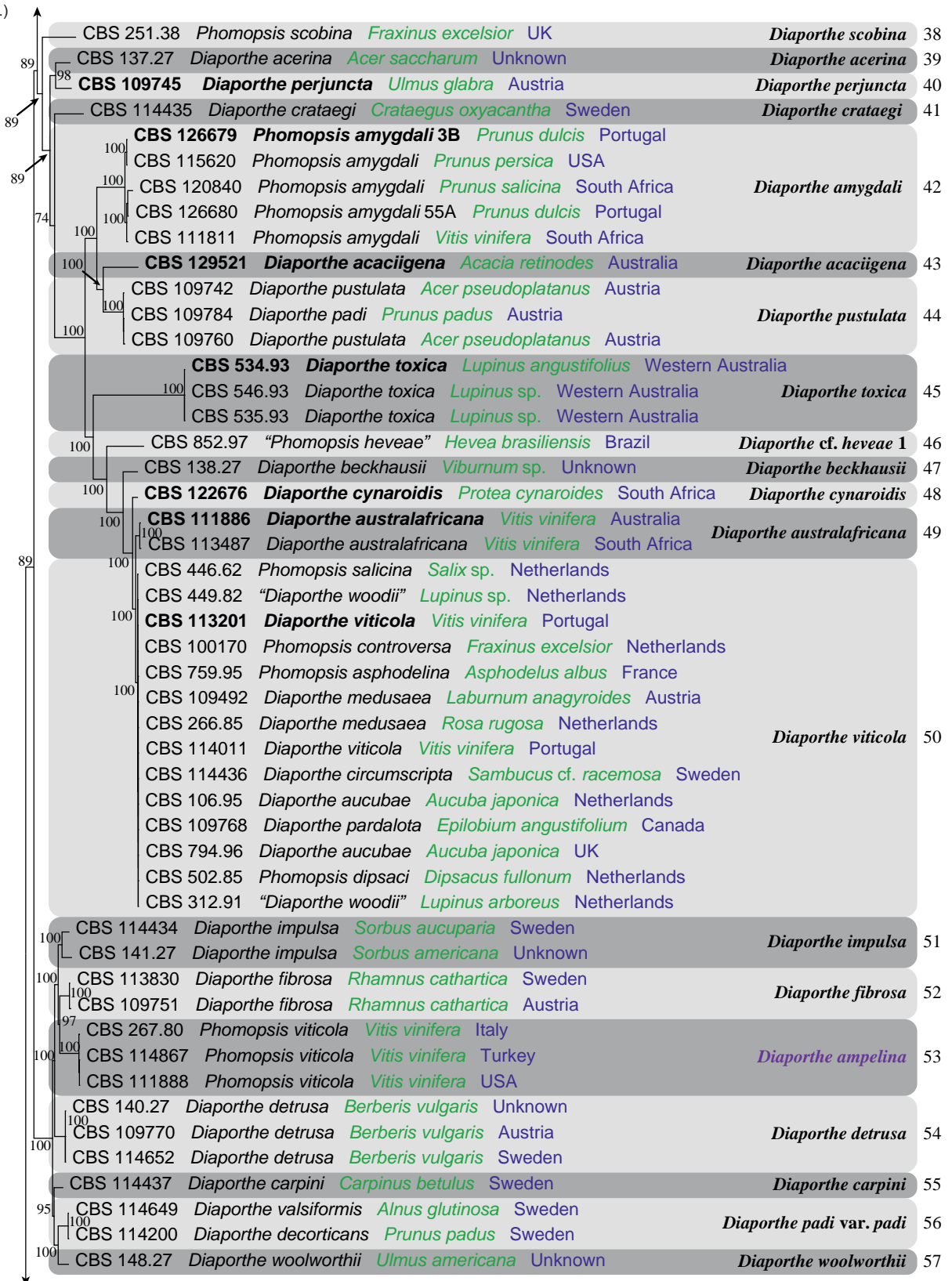
each aligned data partition using DnaSP v. 5.00.07 (Librado & Rozas 2009).

Taxonomy

All descriptions provided are based on colonies sporulating in culture, which for the most part only formed the asexual morph. Colonies were subcultured onto 2 % tap water agar supplemented with sterile pine needles (PNA; Smith et al. 1996), or

autoclaved leaf pieces of *Ilex aquifolium*, *Maytenus ilicifolia* or *Schinus terebinthifolius*, PDA, oatmeal agar (OA), and 2 % malt extract agar (MEA) (according to Crous et al. 2009b), and incubated at 20 °C under a 12 h near-ultraviolet light (400–315 nm) (Sylvania Blacklight-Blue, Osram Nederland B.V., Alphen aan den Rijn, The Netherlands), 12 h dark cycle to promote sporulation. Structures were mounted in clear lactic acid, and 50 measurements determined for conidia, and 30 for other

Fig. 1 (cont.)



0.2

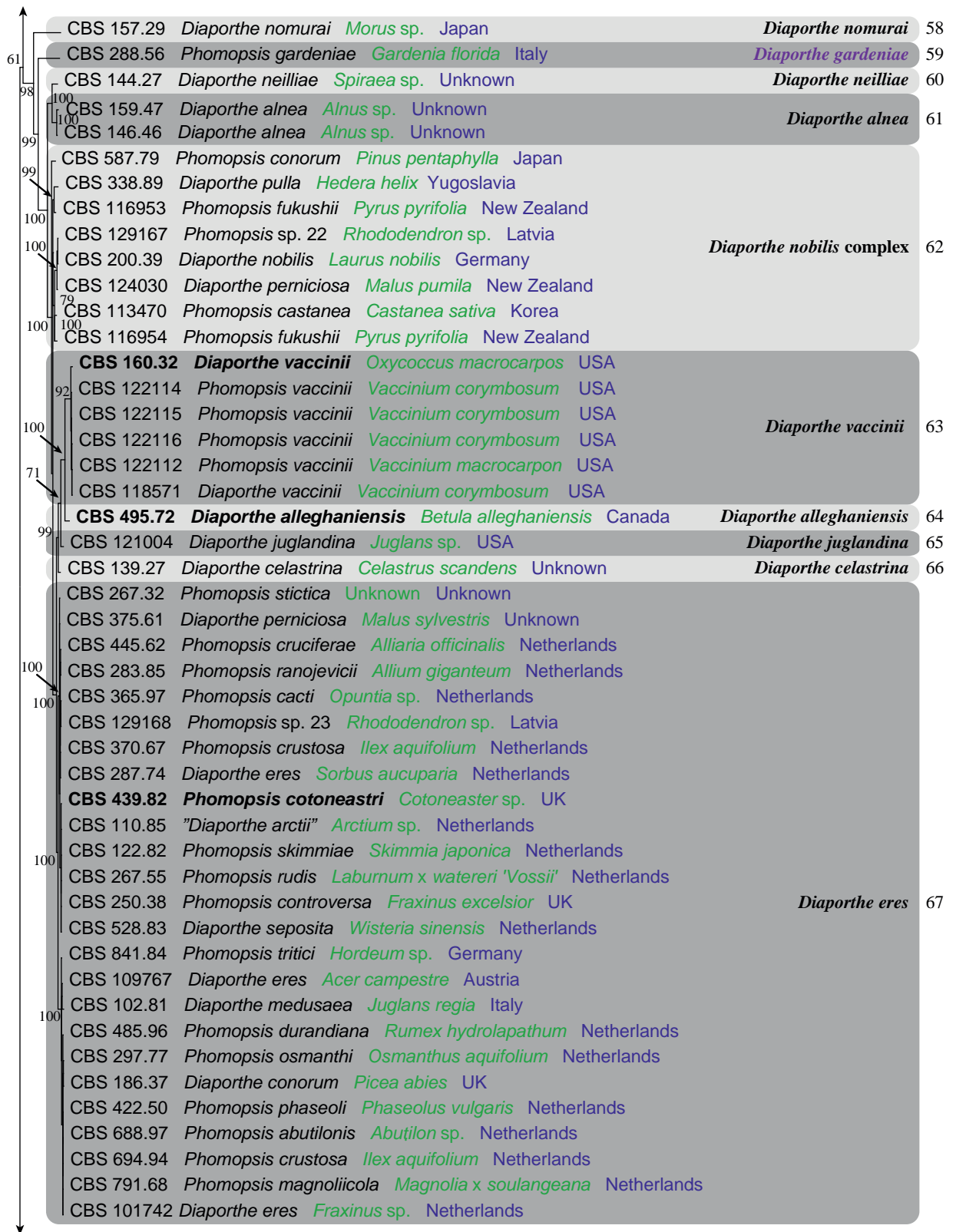
structures. The 95 % confidence levels were determined, and the extremes given in parentheses. Colony diameters were determined at 25 °C in darkness on PDA, OA and MEA. Colony colours (surface and reverse) were described after 14 d using the colour charts of Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (www.Mycobank.org; Crous et al. 2004a).

RESULTS

DNA sequencing and phylogenetic analysis

The most suitable genes for *Diaporthe* species delimitation in this study were found to be CAL, HIS, ITS, TEF1 and TUB. The amplified genomic regions of these genes were more informative, and the combined analysis provided a more robust species identification, from which phylogenetic relationships could be inferred.

Fig. 1 (cont.)

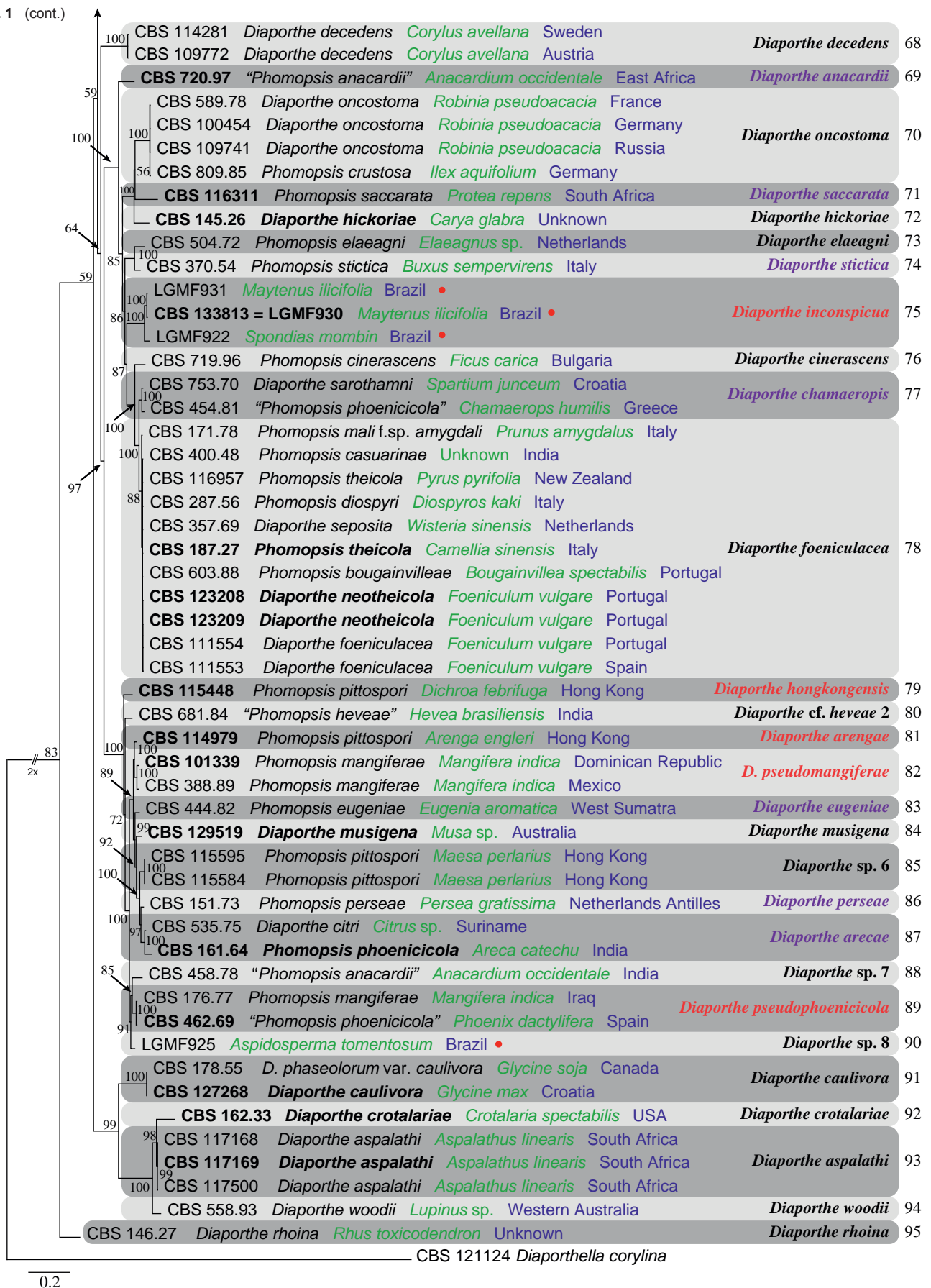


0.2

The manually adjusted, combined (ITS, TUB, CAL, TEF1 and HIS) alignment for the Bayesian analysis contained 243 isolates (including the outgroup sequences) and 2 435 characters were used in the phylogenetic analysis. The number of unique site patterns per data partition were 210, 616, 242, 281 and 183, respectively and were based on 466, 874, 355, 316 and 424 alignment positions, respectively. Based on the results of MrModeltest, the following priors were set in MrBayes for the different data partitions: all partitions had dirichlet base frequen-

cies and GTR+I+G models with inverse gamma-distributed rates were implemented for ITS and HIS, and HKY+I+G with inverse gamma-distributed rates for TUB, CAL and TEF1. The Bayesian analysis lasted 14 735 000 generations and the consensus trees and posterior probabilities were calculated from the 22 104 trees left after discarding 7 368 trees (the first 25 % of generations) for burn-in (Fig. 1). Ninety-five clades are recognised and discussed here.

Fig. 1 (cont.)



Locus resolution and SNP detection

The mtSSU and LSU regions had very few informative sites for the tested strains and were therefore not selected as good markers at species level. The ACT and GPDH regions were also discarded as suitable candidates for the multi-gene analyses because of their long branch lengths which made unambigu-

ous alignments impossible. These four loci were therefore not used for further amplification and sequencing on the complete dataset. The remaining five loci had varied success for species identification and some phylogenetic lineages were more prone to less variability than others. Fifty-eight of the 95 spe-

cies could be identified by all five loci. The loci are treated individually below:

CAL – The locus could distinguish 74 of the 95 species (78 % success). It had difficulty separating: *D. endophytica* and *D. phaseolorum* (clades 4, 5); *D. angelicae*, *D. arctii* and *D. subordinaria* (clades 17–19); *D. alleghaniensis*, *D. alnea*, *D. celsastrina*, *D. eres*, *D. juglandina*, *D. neilliae* and *D. nobilis* (clades 60–62, 64–67); and *D. eugeniae*, *D. musigena*, *D. perseae*, *D. pseudomangiferae*, *D. pseudophoenicicola*, *Diaporthe* sp. 6 and *Diaporthe* sp. 7 (clades 82–86, 88, 89). A single strain each of *D. angelicae* (clade 17), *D. novem* (clade 22) and *D. terebinthifolii* (clade 9) clustered separate from the other strains of the species.

HIS – The locus could distinguish 84 of the 95 species (88 % success). It had difficulty separating: *D. australafricana* and *D. viticola* (clades 49, 50); *D. celsastrina*, *D. eres* and *D. nobilis* (clades 66, 67, 62); *D. arecae* and *D. perseae* (clades 86, 87); and *D. pseudophoenicicola* and *Diaporthe* sp. 8 (clades 89, 90). A single strain each of *D. endophytica* (clade 5) and *D. terebinthifolii* (clade 9) clustered separate from the other strains of the species. This is the only locus that can distinguish *D. angelicae* (clade 17).

ITS – The locus could distinguish 75 of the 95 species (79 % success). It had difficulty separating: *D. angelicae*, *D. arctii* and *D. subordinaria* (clades 17–19); *D. cynaroides* and *D. viticola* (clades 48, 50); *D. alnea*, *D. neilliae* and *D. nobilis* (clades 60–62); *D. arengae*, *D. eugeniae* and *D. pseudomangiferae* (clades 81–83); *D. arecae* and *D. perseae* (clades 86, 87); and *D. aspalathi* and *D. woodii* (clades 93, 94). A single strain each of *D. arecae* (clade 87), *D. inconspicua* (clade 75), *D. novem* (clade 22) and *D. terebinthifolii* (clade 9), and two strains each of *D. impulsiva* (clade 51) and *D. infecunda* (clade 23), clustered separate from the other strains of the species. This is the only locus that can distinguish *D. celsastrina* (clade 17) and *D. eres* (clade 67).

TEF1 – The locus could distinguish 72 of the 95 species (76 % success). It had difficulty separating: *D. tecomae*, *D. terebinthifolii* (clades 9, 10); *D. angelicae*, *D. arctii* and *D. subordinaria* (clades 17–19); *D. australafricana* and *D. viticola* (clades 49, 50); *D. celsastrina* and *D. juglandina* (clades 65, 66); *D. eres* and *D. nobilis* (clades 67, 62); *D. chamaeropsis*, *D. cinerascens* and *D. foeniculaceae* (clades 76–78); and *D. arengae*, *D. arecae*, *D. eugeniae*, *D. musigena*, *D. perseae*, *D. pseudomangiferae*, *D. pseudophoenicicola*, *Diaporthe* sp. 6 and *Diaporthe* sp. 8 (clades 81–87, 89, 90).

TUB – The locus could distinguish 84 of the 95 species (88 % success). It had difficulty separating: *D. endophytica* and *D. phaseolorum* (clades 4, 5); *D. alleghaniensis*, *D. celsastrina*, *D. eres*, *D. juglandina*, *D. nobilis* and *D. vaccinii* (clades 62–67); and *D. aspalathi* and *D. woodii* (clades 93, 94). A single strain of *D. angelicae* (clade 17) clustered separate from the other strains of the species. This is the only locus that can distinguish *D. perseae* (clade 86).

Descriptions based on DNA characters are provided for three species in the Taxonomy section, namely *D. endophytica* (clade 5), *D. inconspicua* (clade 75) and *D. infecunda* (clade 23). *Diaporthe endophytica* (clade 5) was compared to *D. phaseolorum* (clade 4); *D. inconspicua* (clade 75) to *D. anacardii* (clade 69), *D. chamaeropsis* (clade 77), *D. cinerascens* (clade 76), *D. elaeagni* (clade 73), *D. foeniculaceae* (clade 78), *D. hickoriae* (clade 72), *D. oncostoma* (clade 70), *D. saccharata* (clade 71) and *D. stictica* (clade 74); and *D. infecunda* (clade 23) to *D. angelicae* (clade 17), *D. arctii* (clade 19), *D. cuppatea* (clade 20), *D. lusitanicae* (clade 21), *D. neoarctii* (clade 16), *D. novem* (clade 22) and *D. subordinaria* (clade 18).

Taxonomy

The multigene analyses resulted in 95 well-supported clades correlating to 243 isolates of *Diaporthe* (Table 1, Fig. 1). Fifteen new species are described, nine of which were isolated from medicinal plants (*Aspidosperma tomentosum*, *Maytenus ilicifolia*, *Schinus terebinthifolius*, *Spondias mombin*) in Brazil (clades 5, 9, 11, 23, 30, 31, 34, 35 and 36). Twenty-eight clades contain ex-type strains of presently known species, or strains accepted as authentic for the species name or which could be designated as epitypes in the present study, and were therefore well-resolved (7, 8, 12, 14, 17, 20–22, 24, 26–28, 40, 42, 43, 45, 48–50, 63, 64, 69, 71, 72, 84 and 91–93). The sexual-asexual relationship was resolved for several taxa, and is reported below. New combinations in *Diaporthe* are introduced below for several *Phomopsis* names that represented well-resolved taxa. Several potential epitypes were identified during this study, which are discussed below.

Diaporthe acaciigena Crous, Pascoe & Jacq. Edwards, *Perseonia* 26: 123. 2011

Specimen examined. AUSTRALIA, Victoria, Otway Ranges, Anglesea, S38°23'21.7" E144°11'12.7", on leaves of *Acacia retinodes*, 16 Oct. 2009, P.W. Crous, I.G. Pascoe & J. Edwards (holotype CBS H-20581, ex-type culture CPC 17622 = CBS 129521).

Notes — Clade 43 contains the ex-type culture of *D. acaciigena* isolated from *Acacia retinodes* in Australia. This species is morphologically similar to *D. amygdali* (clade 42) (Crous et al. 2011), and closely related to *D. pustulata* (clade 44).

Diaporthe acerina (Peck) Sacc., Syll. Fung. (Abellini) 1: 611. 1882

Basionym. *Valsa acerina* Peck, Ann. Rep. N.Y. State Mus. Nat. Hist. 28: 73. 1876. 1874.

Specimen examined. UNKNOWN, from *Acer saccharum*, Sept. 1927, L.E. Wehmeyer (CBS 137.27).

Notes — Clade 39 is represented by *D. acerina*, isolated from *Acer saccharum*. This species is genetically similar to *D. perijuncta* (clade 40). It is known to occur in Europe and North America on dead limbs and trunks of *Acer pseudoplatanus*, *A. saccharinum*, *A. saccharum*, *A. spicatum*, and *Acer* sp. (*Aceraceae*) (Spielman 1985, Farr et al. 1989).

Diaporthe alleghaniensis R.H. Arnold, *Canad. J. Bot.* 45: 787. 1967

Specimen examined. CANADA, Ontario, on branches of *Betula alleghaniensis*, June 1972, R.H. Arnold (ex-type culture CBS 495.72 = ATCC 24097 = DAOM 45776).

Notes — Clade 64 contains the ex-type strain of *D. alleghaniensis*, isolated from *Betula alleghaniensis* in Canada. *Diaporthe alleghaniensis* causes canker and dieback of *B. alleghaniensis*, *B. lenta*, *B. papyrifera* and *B. pendula* in Canada (Arnold 1975), but has also been reported from Japan (Farr & Rossman 2012).

Diaporthe alnea Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 207. 1870 (1869–1870)

= *Phomopsis alnea* Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 115: 681 (33 of repr.). 1906.

Specimens examined. UNKNOWN, on *Alnus* sp., June 1946, S. Truter (CBS 146.46); on *Alnus* sp., Aug. 1947, S. Truter (CBS 159.47).

Notes — Clade 61 consists of two isolates from *Alnus* (presumably collected in the Netherlands). *Diaporthe alnea* causes dieback of *Alnus glutinosa* (alder) and *A. incana* (grey alder).

It has been reported from Europe, Russia and the USA (Munk 1957, Oak & Dorset 1983, Moricca 2002, Mel'nik et al. 2008, Farr & Rossman 2012).

Diaporthe ambigua Nitschke, Pyrenomyces Germanici 2: 311. 1870

Specimens examined. ITALY, Sicily, Catania, on *Platanus acerifolia*, G. Granata (CBS 127746 = IMI 395956); Perugia, on *Helianthus annuus*, Mar. 1987, A. Zizzerini (CBS 187.87). – PORTUGAL, Vale Andeiro, on *Foeniculum vulgare*, J.M. Santos (CBS 123210 = Di-C003/10, CBS 123211 = Di-C002/9). – SOUTH AFRICA, Western Cape Province, from *Pyrus communis*, deposited 2002, S. Denman (ex-epitype culture CBS 114015 = CPC 2657); Western Cape Province, on crown of *Aspalathus linearis*, 15 May 1997, J.C. Janse van Rensburg (CBS 117167 = CPC 5414).

Notes — Clade 26 represents *D. ambigua*, which contains two isolates previously misidentified as *D. scabra* (CBS 127746) and *D. helianthi* (CBS 187.87), and four isolates of *D. ambigua*, including the ex-epitype culture. *Diaporthe ambigua* is an important pathogen of *Malus domestica*, *Prunus salicina* and *Pyrus communis* in South African fruit orchards. Infection by *D. ambigua* is associated with sunken lesions with longitudinal cracks on affected fruit trees. The fungus rapidly kills nursery rootstocks, but also kills mature rootstocks over a longer period of time (Smit et al. 1996). This species is also found as saprobe on wild fennel (Santos & Phillips 2009). It has been reported on *Aspalathus linearis* (van Rensburg et al. 2006), *Foeniculum vulgare*, *Malus domestica* (Smit et al. 1996, Santos & Phillips 2009), *Malus sylvestris* (Crous et al. 2000), *Prunus* spp. (Smit et al. 1996, Mostert et al. 2001a), *Pyrus communis* (Nitschke 1867), *Pyrus ussuriensis* (Tai 1979) and *Vitis vinifera* (van Niekerk et al. 2005). It is widely distributed, and is known from China, Cuba (Tai 1979), Germany (Nitschke 1867), South Africa (Smit et al. 1996), UK (Dennis 1986) and the USA (Washington) (Shaw 1973).

Diaporthe ampelina (Berk. & M.A. Curtis) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802922

Basionym. *Phoma ampelina* Berk. & M.A. Curtis, Grevillea 2, 18: 81. 1873.

≡ *Phomopsis ampelina* (Berk. & M.A. Curt.) Grove, Bull. Misc. Inform. Kew 4: 184. 1919.

= *Phoma viticola* Sacc., Michelia 2: 92. 1880.

≡ *Phomopsis viticola* (Sacc.) Sacc., Ann. Mycol. 13: 118. 1915.

= *Fusicoccum viticulum* Reddick, Cornell Univ. Agric. Exp. Sta. Bull. 263: 331. 1909.

≡ *Phomopsis viticola* (Reddick) Goid., Atti Reale Accad. Naz. Lincei 26: 107. 1937.

= *Phomopsis viticola* Sacc. var. *ampelopsisidis* Grove, Bull. Misc. Inform. Kew 4: 183. 1919.

= *Diaporthe neoviticola* Udayanga, Crous & K.D. Hyde, Fung. Diversity 56: 166. 2012 (a nom. nov. based on *Phoma viticola* Sacc.).

Conidiomata pycnidial, eustromatic, subepidermal, brown to black, scattered or aggregated, globose, flask-like to conical, outer surface smooth, convoluted to unilocular, singly ostiolate, up to 430 µm wide and 190–300 µm tall, including short necks which rarely occur. Pycnidial wall consisting out of two regions of *textura angularis*; the outer region brown, 2–3 cells thick, 5–7 µm wide, inner region brown, 3–4 cells thick, 7–15 µm wide, with the outside cells compressed. *Conidial mass* globose or in cirrhi, white, pale-yellow to yellow, but predominantly pale-yellow. *Alpha conidiophores* cylindrical, some filiform, rarely septate and branched, 5–35 × 1–3 µm (av. = 25 × 2 µm). *Alpha conidiogenous cells* subcylindrical, tapering towards the apex, collarettes and periclinal thickening present, 3–19 × 1–2 µm (av. = 10 × 1.5 µm). *Alpha conidia* commonly found, fusoid-ellipsoidal, apex acutely rounded, base obtuse to subtruncate, multi-guttulate with guttules grouped at the polar ends, rarely biguttulate, (7–)9.5–10.5(–13) × (1.5–)2–3(–3.5) µm (av. = 10 × 2.5 µm). *Beta conidiophores* ampulliform to subcylindrical, rarely branched, 10–34 × 1–2 µm (av. = 26 × 1.5 µm). *Beta conidiogenous cells* subcylindrical, tapering towards the apex, collarette and periclinal thickening present, 7–14 × 1–2 µm (av. = 11–1.5 µm). *Beta conidia* less common than alpha conidia, straight, curved or hamate, 20–25 × 0.5–1 µm (av. = 23–1 µm). *Gamma conidia* rarely observed, fusoid to subcylindrical, apex acutely rounded, base subtruncate, multi-guttulate, 12–18 × 1.5–2 µm (av. = 15 × 2 µm). Description adapted from Mostert et al. (2001a).

Specimens examined. FRANCE, Bordeaux, Naujan-et-Postiac, on *Vitis vinifera* (Cabernet Sauvignon grapevine), May 1998, P. Larignon (PREM 56460 neotype, ex-neotype culture CBS 114016). – ITALY, Perugia, on *Vitis vinifera*, May 1980, A. Zizzerini (CBS 267.80 = CPC 2671). – TURKEY, from *Vitis vinifera*, 1 Dec. 2001, M. Erkan (CBS 114867 = CPC 4708). – USA, California, on *Vitis vinifera*, J.D. Cucuzza (CBS 111888 = ATCC 48153 = CPC 2673).

Notes — Grove (1919) distinguished *P. ampelina* (K 58408) from *P. viticola* by its external appearance on the host. However, Mostert et al. (2001a) re-examined the type specimen, and found alpha conidia to be ellipsoid-fusoid, 8–12 × 2.5–3.5 µm, within the range of *P. viticola* (Mostert et al. 2001a: f. 29), and thus considered them to be synonymous. Udayanga et al. (2012) proposed *D. neoviticola* as a nom. nov. for *P. viticola*, but this name is superfluous, as the older epithet '*ampelina*' has precedence and should be adopted.

Diaporthe ampelina (clade 53) is a well-resolved species. It causes cane and leaf spot and infections of pruning wounds of *Vitis* and *Ampelopsis* spp. (*Vitaceae*). Several species of *Diaporthe* can infect the host and cause variable symptoms in different parts of the vine (canes, leaves and fruits) causing considerable confusion in the taxonomy of these species on grapevine (Phillips 1999, Scheper et al. 2000, Mostert et al.

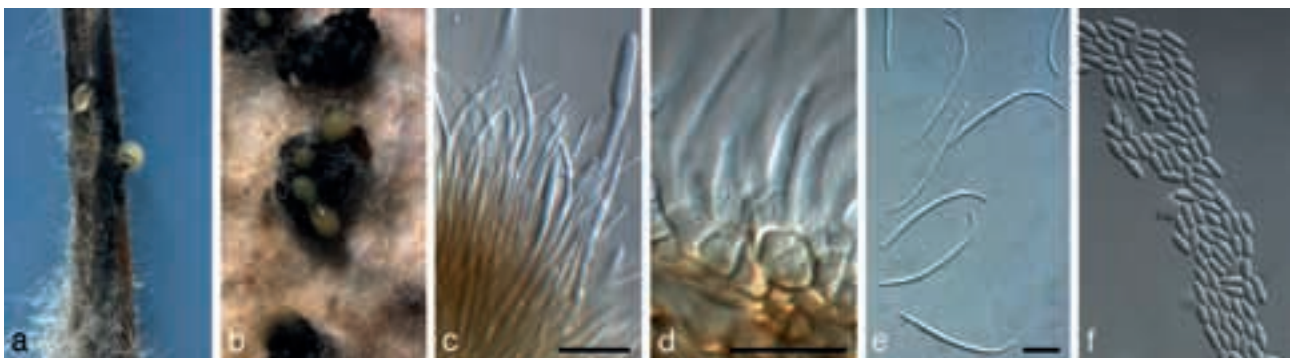


Fig. 2 *Diaporthe anacardii* (CBS 720.97). a. Conidiomata sporulating on PNA; b. conidiomata sporulating on PDA; c, d. conidiogenous cells; e. beta conidia; f. alpha conidia. — Scale bars = 10 µm.

2001a). Merrin et al. (1995) studied the variation of *Diaporthe* in Australia using morphology. They identified two taxa (*Phomopsis* taxon 1 and taxon 2), which cause cane and leaf blight of *Vitis* spp.; and taxon 2 was identified as showing more resemblance to *P. viticola*. Mostert et al. (2001a) studied the species occurring on grapevines in South Africa using morphological, cultural, molecular and pathological characterisation and clarified the taxonomy of this complex. *Diaporthe ampelina* (= *Phomopsis viticola*, *D. neoviticola*, *Phomopsis* taxon 2 from Australia) was found to be the cause of cane and leaf spot disease, and was neotypified. Although the sexual morph has never been reported, Santos et al. (2010) found both MAT loci to be present in this species, and showed that it is heterothallic. However, the sexual morph could not be induced in culture by crossing opposing mating types.

Diaporthe amygdali (Delacr.) Udayanga, Crous & K.D. Hyde, Fung. Diversity 56: 166. 2012

Basionym. *Fusicoccum amygdali* Delacr., Bull. Soc. Mycol. France 21: 280. 1905.

= *Phomopsis amygdali* (Delacr.) J.J. Tuset & M.T. Portilla, Canad. J. Bot. 67, 5: 1280. 1989.

Specimens examined. PORTUGAL, Mirandela, from *Prunus dulcis*, 2010, *E. Diogo* (ex-epitype culture CBS 126679); Tavira, on *Prunus dulcis*, 2010, *E. Diogo* (CBS 126680). — SOUTH AFRICA, Western Cape Province, on *Vitis vinifera*, 1 Mar. 1997, *L. Mostert* (CBS 111811 = CPC 2632); Western Cape Province, in wood on *Prunus salicina*, 2008, *U. Damm* (CBS 120840 = CPC 5833). — USA, Georgia, cankers on *Prunus persica*, Mar. 1994, *W. Uddin* (CBS 115620 = FAU 1005).

Notes — *Diaporthe amygdali* (clade 42) is the causal agent of twig canker and blight of almonds (*Prunus dulcis*) and peach (*P. persica*) wherever these hosts are grown (Diogo et al. 2010). It was first described as *Fusicoccum amygdali* causing cankers on almonds in France (Delacroix 1905). Tuset & Portilla (1989) re-examined the type specimen of *F. amygdali* and, based on morphology and symptomatology, they considered that it would be best accommodated in the genus *Phomopsis*. Clade 42 contains the ex-epitype strain (CBS 126679), five *Phomopsis amygdali* isolates from *Prunus dulcis* in Portugal, from *P. persica* in USA, *P. salicina* in South Africa, and from *Vitis vinifera* in South Africa.

Diaporthe anacardii (Early & Punith.) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802923; Fig. 2

Basionym. *Phomopsis anacardii* Early & Punith., Trans. Brit. Mycol. Soc. 59, 2: 345. 1972.

Conidiomata pycnidial, sporulating profusely on OA, globose, up to 600 µm diam, multilocular, black, erumpent; cream conidial droplets exuding from central ostioles; walls consisting of 3–6 layers of medium brown *textura angularis*. *Conidiophores* hyaline, smooth, 1–3-septate, branched, densely aggregated, cylindrical, straight to sinuous, 10–25 × 2–3 µm. *Conidiogenous cells* 9–16 × 1.5–2 µm, phialidic, cylindrical to cymbiform, terminal and lateral, with slight taper towards apex, 1–1.5 µm diam, with visible periclinal thickening; collarette slightly flared, up to 2 µm long when present. *Paraphyses* rarely present, hyaline, smooth, 1–3-septate, cylindrical with obtuse ends, extending above conidiophores. *Alpha conidia* aseptate, hyaline, smooth, guttulate, fusoid to ellipsoid, tapering towards both ends, straight, apex subobtuse, base bluntly rounded with flattened hilum, (6.5–)7–8(–9) × (2–)3(–3.5) µm. *Gamma conidia* not observed. *Beta conidia* spindle-shaped, aseptate, smooth, hyaline, apex subacutely rounded, base truncate, tapering from lower third towards apex, curved, (15–)20–25 × 1.5(–2) µm.

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C. On OA dirty white with moderate aerial mycelium and patches of iron-grey. On PDA having patches of dirty white and umber, reverse bay with patches of umber. On MEA having patches of dirty white and olivaceous-grey, reverse umber with patches of olivaceous-grey.

Specimens examined. EAST AFRICA, on *Anacardium occidentale*, Apr. 1997, *M. Puccioni* (epitype designated here CBS H-21101, culture ex-epitype CBS 720.97). — KENYA, on *Anacardium occidentale*, 4 Dec. 1969, *M.P. Early* (holotype IMI 144866).

Notes — *Phomopsis anacardii* (clade 69 as *D. anacardii*) was described from *Anacardi occidentalis* in Kenya, and also recorded from Nigeria, Guinea and Cuba (Early & Punithal-ingam 1972).

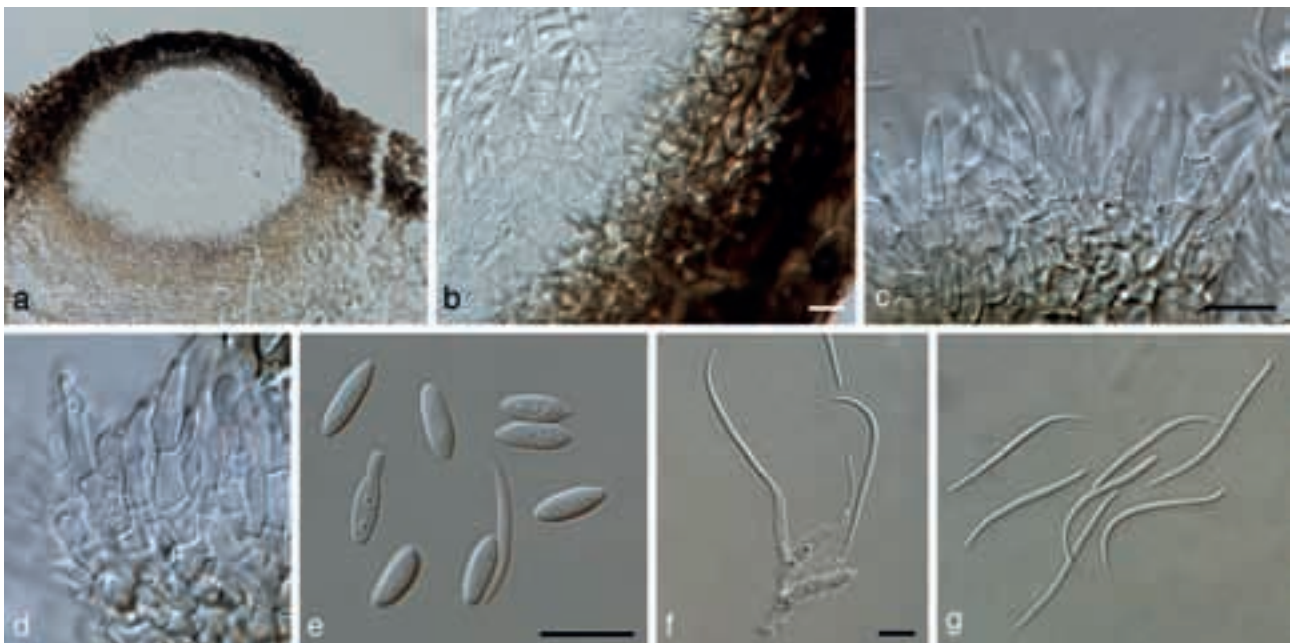


Fig. 3 *Diaporthe angelicae* (CBS 111591). a, b. Transverse section through conidiomata, showing conidiomatal wall; c, d. conidiogenous cells; e. alpha and beta conidia; f. conidiogenous cells giving rise to beta conidia; g. beta conidia. — Scale bars: a = 140 µm, all others = 10 µm.

Diaporthe angelicae (Berk.) D.F. Farr & Castl., Mycoscience 44: 204. 2003. — Fig. 3

Basionym. *Sphaeria angelicae* Berk., Mag. Zool. Bot.: 28. 1837.

≡ *Diaporthopsis angelicae* (Berk.) Wehm., The genus *Diaporthe* Nitschke: 228. 1933.

≡ *Mazzantia angelicae* (Berk.) Lar. N. Vassiljeva, Pyrenomyces of the Russia Far East. I. Gnomoniaceae: 49. 1993.

= *Leptosphaeria nigrella* Auersw., Mycol. Eur. Pyr. 5/6, pl. 12, f. 163. 1869.

≡ *Diaporthe nigrella* (Auersw.) Niessl, Beitr.: 51. 1872.

≡ *Diaporthopsis nigrella* (Auersw.) Fabre, Ann. Sci. Nat., Bot. 6 15: 35. 1883.

Conidiomata pycnidial, globose to ellipsoidal, aggregated or scattered, dark brown to black, immersed, ostiolate, 100–281 µm wide, 70–200 µm tall, lacking necks, with outer surface covered in hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose or exuding in cirrhi, white to pale luteous or pale yellow. *Conidiophores* hyaline, subcylindrical, rarely branched, tapering towards the apex, aseptate, (12–)13–16(–18) × 3(–4) µm. *Conidiogenous cells* hyaline, subcylindrical, straight to curved, tapering towards the apex, collarette not flared, periclinal thickening inconspicuous, 8–10(–11) × 3(–3.5) µm. *Alpha conidia* hyaline, oblong to ellipsoid, apex bluntly rounded, base obtuse to subtruncate, bi- to multi-guttulate, (7–)8–10(–11) × 3(–4) µm. *Beta conidia* hyaline, smooth, spindle shaped, slightly curved, (19–)22–26(–28) × (1–)2 µm. *Gamma conidia* not observed (CBS 111592).

Culture characteristics — See Castlebury et al. (2003).

Specimens examined. AUSTRIA, Karnten, St. Margareten, decaying stems of *Heracleum sphondylium*, Aug. 2001, A.Y. Rossman (CBS 111591 = AR 3724); Niederosterreich, Ottenstein, decaying stems of *Heracleum sphondylium*, Aug. 2001, A.Y. Rossman (ex-epitype culture CBS 111592 = AR3776). — FRANCE, Bretagne, La Ville Borée, near Quessoy, on seeds of *Heracleum sphondylium*, 27 July 1990, H.A. van der Aa (CBS 501.90); sea dunes near Seignose le Penon, on *Eryngium maritimum*, leaf spots, 10 June 1986, H.A. van der Aa (CBS 344.86). — ITALY, San Casciano, Prov., Florence, twig blight of *Foeniculum vulgare*, July 1996, L. Mugnai (CBS 100871). — PORTUGAL, Malveira da Serra, Sintra, on *Foeniculum vulgare*, A.J.L. Phillips (CBS 123215 = Ph-C133/1).

Notes — *Diaporthe angelicae* (clade 17) is known to cause stem decay in several hosts including *Heracleum sphondylium* (*Apiaceae*) and *Foeniculum vulgare* (*Apiaceae*) in Europe and North America (Santos & Phillips 2009). Wehmeyer (1933) not only linked the conidial form of *Phomopsis asteriscus* to the sexual state *Diaporthopsis angelicae*, but also stated that *Diaporthe berkeleyi* was a synonym of *Diaporthopsis angelicae*. However, Castlebury et al. (2003) showed that *Diaporthopsis* is a synonym of *Diaporthe*, and also designated an epitype for *D. angelicae*.

Diaporthe arctii (Lasch) Nitschke, Pyrenomyces Germanici 2: 268. 1870

Basionym. *Sphaeria arctii* Lasch, in Rabenh., Klotzsch. Herb. Vivum Mycol.: no. 1046. 1846.

≡ *Phomopsis arctii* (Lasch) Traverso, Fl. Ital. Crypt., Pars 1: Fungi. Pyrenomycetaceae, Xylariaceae, Valsaceae, Ceratostomataceae: 226. 1906.

Specimen examined. UNKNOWN, from *Arctium* sp., Sept. 1925, A.W. Archer (CBS 136.25).

Notes — There are several clades that contain isolates previously identified as *D. arctii* (clades 16, 19, part 2 and 67, part 4). We suspect that clade 19 may represent the real *D. arctii*, as it is basal to *D. subordinaria*, and Wehmeyer (1933) regarded the latter (from *Plantago lanceolata*) as synonym of *D. arctii* (from *Arctium*).

Diaporthe arecae (H.C. Srivast., Zakia & Govindar.) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802924

Basionym. *Subramanella arecae* H.C. Srivast., Zakia & Govindar., Mycologia 54, 1: 7. 1962.

Specimens examined. INDIA, on fruit of *Areca catechu*, Feb. 1964, H.C. Srivastava (isotype CBS H-7808, ex-isotype culture CBS 161.64). — SURINAME, on fruits of *Citrus* sp., Oct. 1975, I. Block (CBS 535.75).

Notes — The *Diaporthe* isolate from citrus (CBS 535.75) could well be distinct, but more strains are required to resolve this clade (clade 87).

Diaporthe arengae R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802925; Fig. 4

Etymology. Named after the host genus from which it was collected, *Arenga*.

Pycnidia in culture on PNA sporulating poorly, subglobose, up to 250 µm diam, black, erumpent; cream conidial droplets exuding from central ostiole; walls consisting of 3–6 layers of medium brown *textura angularis*. *Conidiophores* hyaline in upper region, pale brown at base, smooth, 0–6-septate, branched, densely aggregated, cylindrical, straight to sinuous, 10–60 × 2.5–4 µm. *Conidiogenous cells* 8–15 × 1.5–2.5 µm, phialidic, cylindrical, terminal and lateral, with slight taper towards apex, 1–1.5 µm diam, with visible periclinal thickening; collarette not flared, up to 2 µm long when present. *Paraphyses* not observed. *Alpha conidia* aseptate, hyaline, guttulate, fusoid-ellipsoid, tapering towards both ends, apex subobtuse, base with flattened hilum, (5–)6–7(–9) × (2–)2.5(–3) µm. *Gamma conidia* not observed. *Beta conidia* rarely observed, subcylindrical, aseptate, smooth, hyaline, apex bluntly rounded, base truncate, tapering absent to very slight, curved, 20–25 × 1.5 µm.

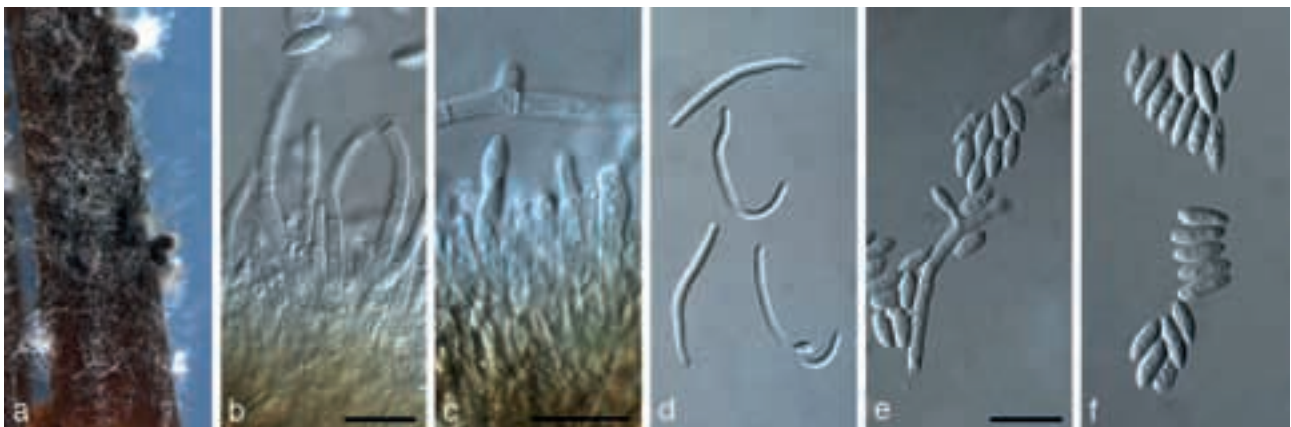


Fig. 4 *Diaporthe arengae* (CBS 114979). a. Conidiomata sporulating on PNA; b, c. conidiogenous cells; d. beta conidia; e, f. alpha conidia. — Scale bars = 10 µm.

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C. On MEA surface with fluffy aerial mycelium, pale luteous, in reverse orange with patches of sienna. On OA umber with patches of sienna and saffron, in reverse umber with patches of saffron. On PDA surface with fluffy white aerial mycelium, umber with patches of saffron, in reverse umber with patches of pale luteous to luteous.

Specimen examined. HONG KONG, Victoria Peak, from *Arenga engleri*, 7 Oct. 1999, K.D. Hyde (holotype CBS H-21104, culture ex-type CBS 114979 = HKUCC 5527).

Notes — The *Diaporthe* species occurring on palms are summarised by Fröhlich et al. (1997). *Diaporthe arengae* (clade 81) is distinguished from known species based on a combination of its conidial morphology and host.

Diaporthe aspalathi E. Jansen, Castl. & Crous, *Stud. Mycol.* 55: 71. 2006

Basionym. *Diaporthe phaseolorum* var. *meridionalis* F.A. Fernández, *Mycologia* 88: 438. 1996 (non *D. meridionalis* Sacc., *Syll. Fung.* 1: 638. 1878).

Specimens examined. SOUTH AFRICA, Western Cape Province, Clanwilliam, Langebergpunt, in branch on *Aspalathus linearis*, J.C. Janse van Rensburg (ex-type culture CBS 117169 = CPC 5428); in crown on *Aspalathus linearis*, 17 Oct. 1997, J.C. Janse van Rensburg (CBS 117168 = CPC 5420); on *Aspalathus linearis*, 2 Dec. 1996, S. Lamprecht (CBS 117500 = CPC 5408).

Notes — *Diaporthe aspalathi* (clade 93) causes soybean stem canker in the South-eastern USA (Fernández & Hanlin 1996), and is not closely related to *D. phaseolorum* as might be expected. Although morphologically similar, this species clustered apart from the reference strain of *D. phaseolorum* (clade 4). *Diaporthe aspalathi* is also the main causal organism of canker and dieback of rooibos (*Aspalathus linearis*), and not *D. phaseolorum* as reported earlier (Smit & Knox-Davies 1989a, b, van Rensburg et al. 2006).

Diaporthe australafricana Crous & Van Niekerk, *Australas. Pl. Pathol.* 34: 33. 2005

Specimens examined. AUSTRALIA, on *Vitis vinifera*, 1 July 1995, R.W.A. Schepers (ex-type culture CBS 111886 = CPC 2676). — SOUTH AFRICA, on *V. vinifera*, 1 Nov. 1997, L. Mostert (CBS 113487 = CPC 2655).

Notes — Clade 49 contains two isolates of *D. australafricana*, one of them being the ex-type strain (CBS 111886), which is a sibling species of *D. viticola* in clade 50 (van Niekerk et al. 2005). Both species were described from *Vitis vinifera*, but *D. australafricana* is thus far only known from grapevines in Australia and South Africa.

Diaporthe batatas Harter & E.C. Field, *Phytopathology* 2: 121. 1912

Specimen examined. USA, on *Ipomoea batatas*, Feb. 1921, L.L. Harter (CBS 122.21).

Notes — Clade 8 consists of a single strain of *D. batatas* isolated from *Ipomoea batatas* in the USA. This species and *D. phaseolorum* have in the past been considered as varieties, namely *D. phaseolorum* var. *batatatis* and *D. phaseolorum* var. *batatae*. However, the genetic data revealed no homology between the two species. Although it is not certain if CBS 122.21 (culture sterile) is an ex-type strain of *D. batatas*, it is regarded as authentic for the name.

Diaporthe beckhausii Nitschke, *Pyrenomyces Germanici* 2: 295. 1870

Specimen examined. UNKNOWN, from *Viburnum* sp., Sept. 1927, L.E. Wehmeyer (CBS 138.27).

Notes — Clade 47 is represented by *D. beckhausii*, which was isolated from *Viburnum* sp. (origin unknown, presumably North America, whereas the species was originally described from *Viburnum* collected in Germany). *Diaporthe beckhausii* is known from woody stems of *Betula* sp., *Cydonia japonica*, *Elaeagnus angustifolia*, *Halesia* sp., *Menispermum canadense*, *Menispermum* sp., *V. opulus*, *Viburnum* sp. and *V. tinus* in temperate North America and Europe (Farr & Rossman 2012).

Diaporthe brasiliensis R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802926; Fig. 5

Etymology. Named after the country where it was collected, Brazil.

Conidiomata pycnidial, globose to conical, immersed, scattered or aggregated, brown to black, ostiolate, 70–160 µm wide,

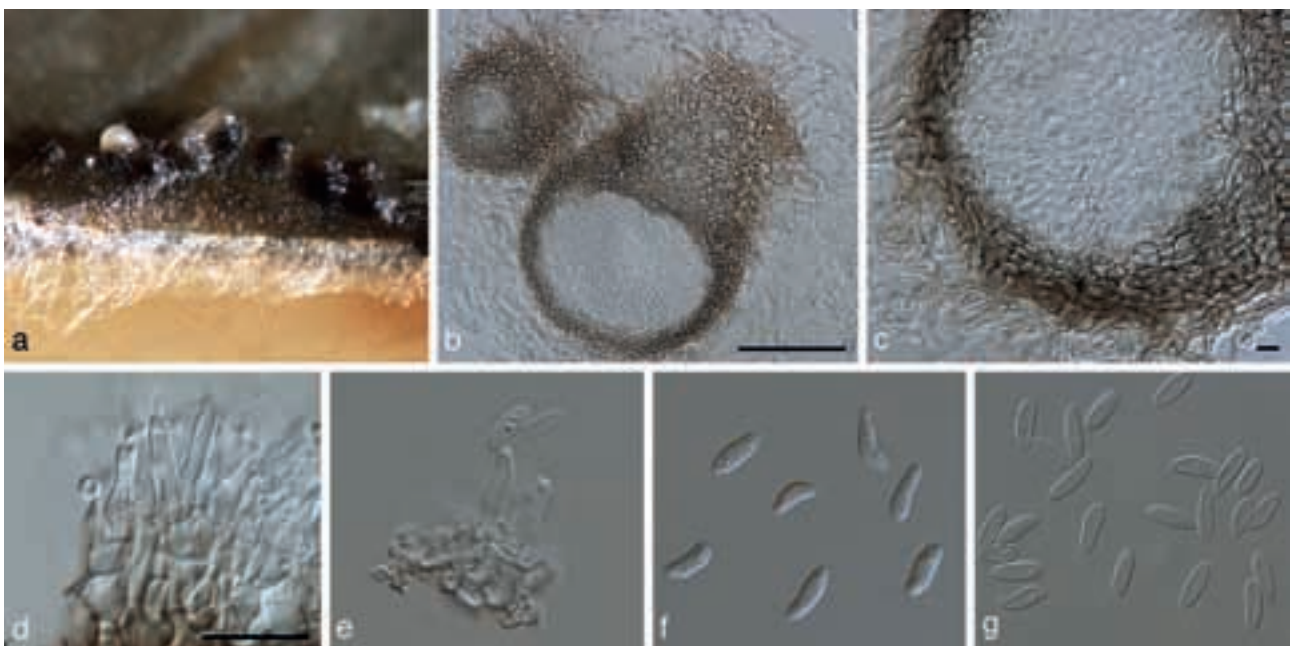


Fig. 5 *Diaporthe brasiliensis* (CBS 133183). a. Conidiomata sporulating on PDA; b, c. transverse section through conidiomata, showing conidiomatal wall; d, e. conidiogenous cells; f, g. alpha conidia. — Scale bars: b = 80 µm, all others = 10 µm.

60–140 µm tall, necks 60–130 µm tall, outer surface smooth; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose, white to pale-luteous. *Conidiophores* hyaline, cylindrical, filiform, straight to curved, 1–3-septate, (17–)20–27(–30) × 2(–4) µm. *Alpha conidiogenous cells* hyaline, cylindrical, filiform, straight to curved, collarette flared, with slight periclinal thickening, (7–)8–12(–14) × 2(–3) µm. *Alpha conidia* hyaline, ellipsoid to irregular, apex bluntly rounded, base obtuse to subtruncate, bi- to multi-guttulate, 6–7(–8) × 2–3 µm. *Beta* and *gamma conidia* not observed.

Culture characteristics — Colonies on PDA flat, with an entire edge, surface mycelium dense and felty, buff, grey-olivaceous or olivaceous-grey; colonies covering dish after 2 wk at 25 °C in the dark; reverse olivaceous, dull green, olivaceous-buff. On OA raised, entire edge, surface mycelium dense felty, smoke-grey to grey-olivaceous; reverse purplish grey to pale purplish grey, grey olivaceous or olivaceous buff. On MEA raised, with an entire edge, buff, smoke-grey, with patches of olivaceous-grey and vinaceous-buff; reverse dark mouse-grey, buff.

Specimens examined. BRAZIL, Rio de Janeiro, endophytic species isolated from leaf of *Aspidosperma tomentosum* (popular name Peroba-do-campo), July 2007, K. Rodriguez (holotype CBS H-21100, ex-type culture CBS 133183 = LGMF 924 = CPC 20300); same collection details (LGMF 926 = CPC 20302).

Notes — Endophytic isolates (clade 36) from a medicinal plant in Brazil.

Diaporthe carpini (Pers.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 205. 1870 (1869–1870)

Basionym. *Sphaeria carpini* Pers., Syn. Meth. Fung. (Göttingen) 1: 39. 1801.

Specimen examined. SWEDEN, Skåne, S. Mellby par., Stenshuvud, on *Carpinus betulus*, 14 Apr. 1989, K. & L. Holm (CBS 114437 = UPSC 2980).

Notes — *Diaporthe carpini* (clade 55) is known from several European countries, where it occurs on *Carpinus* spp.

Diaporthe caulivora (Athow & Caldwell) J.M. Santos, Vrandečić & A.J.L. Phillips, Persoonia 27: 13. 2011

Basionym. *Diaporthe phaseolorum* var. *caulivora* Athow & Caldwell, Phytopathology 44: 323. 1954.

Specimens examined. CANADA, Ontario, in mature stem on *Glycine soja*, Mar. 1955, A.A. Hildebrand (CBS 178.55 = ATCC 12048 = CECT 2023). — CROATIA, in stem on *Glycine max*, K. Vrandečić (ex-neotype culture CBS 127268).

Notes — Clade 91 is represented by two isolates of *D. caulivora* on *Glycine soja* and *G. max*, respectively obtained from Canada (CBS 178.55) and Croatia (ex-neotype: CBS 127268). The soybean canker species complex was recently treated by Santos et al. (2011).

Diaporthe celastrina Ellis & Barthol., J. Mycol. 8, 4: 173. 1902

Specimen examined. UNKNOWN, on *Celastrus scandens*, Sept. 1927, L.E. Wehmeyer (CBS 139.27).

Notes — Strains from the USA are required to confirm the identity of this culture (clade 66).

Diaporthe chamaeropsis (Cooke) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802927; Fig. 6

Basionym. *Phoma chamaeropsis* Cooke, Grevillea 13 (no. 68): 95. 1885. ≡ *Phomopsis chamaeropsis* (Cooke) Petr., as '*Phomopsis chamaeropsis*', Ann. Mycol. 17, 2/6: 83. 1920 (1919).

Conidiomata pycnidial in culture on PNA, globose, up to 400 µm diam (up to 600 µm diam on OA), black, erumpent; cream conidial droplets exuding from central ostioles; walls consisting of 3–6 layers of medium brown *textura angularis*. *Conidiophores* hyaline, smooth, 1–5-septate, branched, densely aggregated, cylindrical, straight to sinuous, 10–50 × 2–2.5 µm. *Conidiogenous cells* 10–20 × 1.5–2 µm, phialidic, cylindrical, terminal and lateral, with slight taper towards apex, 1–1.5 µm diam, with visible periclinal thickening; collarette not observed. *Paraphyses* not observed. *Alpha conidia* aseptate, hyaline, smooth, guttulate, fusoid to ellipsoid, tapering towards both ends, straight, apex subobtuse, base subtruncate, (5–)6–8(–9) × 2(–2.5) µm. *Gamma conidia* not observed. *Beta conidia* spindle-shaped, aseptate, smooth, hyaline, apex acutely rounded, base truncate, tapering from lower third towards apex, curved, (20–)22–27(–30) × 1.5(–2) µm.

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C. On OA with moderate aerial mycelium, surface dirty white with patches of pale olivaceous-grey, reverse with patches of dirty white and sienna. On MEA surface

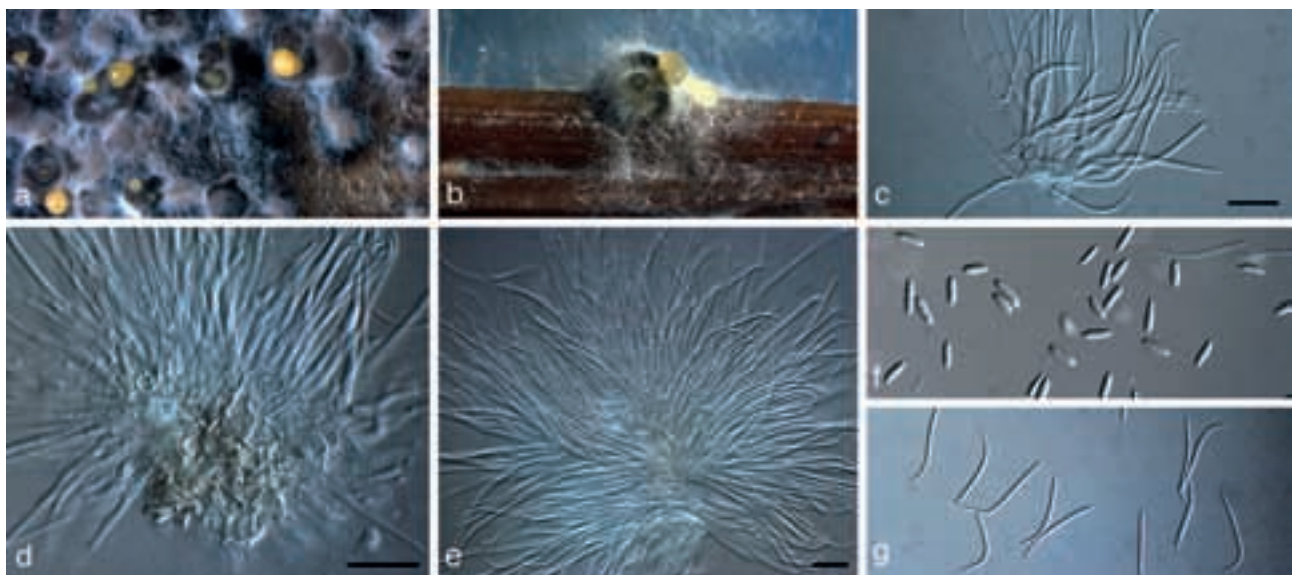


Fig. 6 *Diaporthe chamaeropsis* (CBS 454.81). a. Conidiomata sporulating on PDA; b. conidiomata sporulating on PNA; c–e. conidiogenous cells; f. alpha conidia; g. beta conidia. — Scale bars = 10 µm.

dirty white with patches of olivaceous-grey, reverse sienna, with patches of luteous. On PDA surface olivaceous-grey with patches of dirty white, reverse iron-grey.

Specimens examined. CROATIA, Rab, slope behind Hotel 'Imperial', on dead branch of *Spartium junceum*, July 1970, J.A. von Arx (CBS 753.70). — GREECE, Thessaloniki, dead part of leaf of *Chamaerops humilis*, Aug. 1981, H.A. van der Aa (CBS 454.81).

Notes — Conidial dimensions closely fit those provided in the original description (on *Chamaerops humilis* from Czechoslovakia; Uecker 1988), suggesting that these cultures (clade 77) could be authentic for the name.

Diaporthe cinerascens Sacc., Syll. Fung. (Abellini) 1: 679. 1882. — Fig. 7

= *Phoma cinerescens* Sacc., *Michelia* 1 (no. 5): 521. 1879.

≡ *Phomopsis cinerascens* (Sacc.) Traverso, Fl. Ital. Crypt. Pyrenomycetae 2, 1: 278. 1906.

Conidiomata pycnidial, sporulating poorly on MEA, globose, up to 300 µm diam, black, erumpent; creamy-luteous conidial droplets exuding from central ostioles; walls consisting of 3–6 layers of medium brown *textura angularis*. *Conidiophores* hyaline, smooth, 1–3-septate, branched, densely aggregated, cylindrical, straight to sinuous, 17–30 × 2–3 µm. *Conidiogenous cells* 8–18 × 2–3 µm, phialidic, cylindrical, terminal and lateral, with slight taper towards apex, 1.5–2 µm diam, with visible periclinal thickening; collarette mostly absent, slightly flared when present, up to 2 µm long. *Paraphyses* not observed. *Alpha conidia* aseptate, hyaline, smooth, guttulate, fusoid to ellipsoid, tapering towards both ends, straight, apex subobtuse, base subtruncate, 7–8(–9) × (2.5–)3 µm. *Gamma conidia* aseptate, hyaline, smooth, ellipsoid-fusoid, apex acutely rounded, base subtruncate, 8–12 × 3 µm. *Beta conidia* not observed.

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C. On MEA with profuse aerial mycelium, surface dirty white, reverse ochreous with patches of umber. On PDA with sparse aerial mycelium, surface olivaceous-grey, reverse iron-grey. On OA surface with moderate aerial mycelium, olivaceous-grey to pale olivaceous-grey.

Specimen examined. BULGARIA, Kostinbrod, Plant Protection Institute, on branch of *Ficus carica*, 1995, E. Ilieva (CBS 719.96).

Notes — *Diaporthe cinerascens* (clade 76) represents a European species occurring on *Ficus*, so the present culture could be authentic for the name, as the conidial dimensions match those provided in the original description. This species was originally associated with canker and dieback of *Ficus* spp. in Italy (Saccardo 1879), and the causal organism identified as *Phomopsis cinerascens* (sexual morph: *Diaporthe cinerascens*) by Grove (1935). *Diaporthe cinerascens* affects all commercial figs in California (Ogawa & English 1991), and is found in several geographical locations of the world (Hampson 1981,

Anderson & Hartman 1983, Benschop et al. 1984, Banihashemi & Javadi 2009). *Ficus* spp. are important exotic garden ornamentals across the USA and Canada as well as in the tropics.

Diaporthe citri F.A. Wolf, J. Agric. Res. 33, 7: 625. 1926

= *Phomopsis citri* H.S. Fawc., *Phytopathology* 2, 3: 109. 1912.

Specimens examined. BRAZIL, on seed of *Glycine max*, A. Almeida EM-BRAPA/PR (LGMF 946 = CPC 20322). — ITALY, unknown host, June 1939, G. Goidánich (CBS 199.39). — SURINAME, Paramaribo, on decaying fruit of *Citrus sinensis*, Apr. 1932, N.J. van Suchtelen (CBS 230.52).

Notes — Clade 6 is represented by three isolates. One isolate (CBS 199.39) was previously identified as *D. conorum* from Italy, while another originates from soybean seed collected in Brazil (LGMF 946), and the third isolate is from *Citrus sinensis* in Suriname (CBS 230.52). Because *D. conorum* is regarded as synonym of *D. eres* (clade 67), we tentatively refer to this clade as *D. citri*, awaiting more isolates from *Citrus*. *Diaporthe citri* is a serious pathogen that is widely distributed, and associated with melanosis and stem-end rot of citrus fruits (Punithalingam & Holliday 1973, McKenzie 1992, Mondal et al. 2007, Farr & Rossman 2012).

Diaporthe convolvuli (Ormeno-Núñez, Reeleder & A.K. Watson) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802928

Basionym. *Phomopsis convolvuli* Ormeno-Núñez, Reeleder & A.K. Watson, *Canad. J. Bot.* 66, 11: 2232. 1988.

Specimen examined. TURKEY, isolated from leaves with anthracnose on *Convolvulus arvensis*, D. Berner (CBS 124654 = DP 0727).

Notes — *Phomopsis convolvuli* (clade 3) was originally described from diseased leaves of *Convolvulus arvensis* in Québec (Ormeno-Núñez et al. 1988). The isolate of *Phomopsis convolvuli* studied here (CBS 124654), was found causing anthracnose on field bindweed (*Convolvulus arvensis*), a troublesome perennial weed to many important agricultural crops in the world, and was considered potentially useful as biological control agent (Kuleci et al. 2009).

Diaporthe crataegi (Curr.) Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 204. 1870

Basionym. *Valsa crataegi* Curr., *Trans. Linn. Soc. London* 22: 278. 1858.

Specimen examined. SWEDEN, Skåne, Trolle-Ljungby par., Tosteberga, on *Crataegus oxyacantha*, 15 Apr. 1989, K. & L. Holm (CBS 114435 = UPSC 2938).

Notes — Clade 41 is represented by *D. crataegi* isolated from *Crataegus oxyacantha* in Sweden. The species is common on *C. chrysoarpa*, *C. laevigata* and *C. oxyacantha* in Canada and Europe (Farr & Rossman 2012).

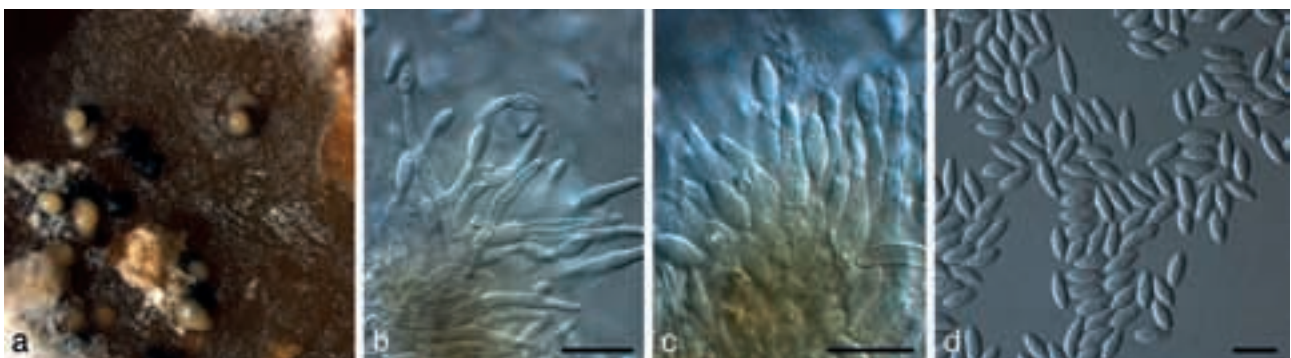


Fig. 7 *Diaporthe cinerascens* (CBS 719.96). a. Conidiomata sporulating on PDA; b, c. conidiogenous cells; d. alpha conidia. — Scale bars = 10 µm.

Diaporthe crotalariae G.F. Weber, *Phytopathology* 23: 602. 1933

= *Phomopsis crotalariae* G.F. Weber, *Phytopathology* 23: 602. 1933.

Specimen examined. USA, on *Crotalaria spectabilis*, Oct. 1933, G.F. Weber (ex-type culture CBS 162.33).

Notes — Clade 92 contains the ex-type strain (CBS 162.33) of *D. crotalariae* isolated from *Crotalaria spectabilis* in the USA.

Diaporthe cuppatea (E. Jansen, Lampr. & Crous) Udayanga, Crous & K.D. Hyde, *Fung. Diversity* 56: 166. 2012

Basionym. *Phomopsis cuppatea* E. Jansen, Lampr. & Crous, *Stud. Mycol.* 55: 72. 2006.

Specimen examined. SOUTH AFRICA, Western Cape Province, on *Aspalathus linearis*, 2006, J. Janse van Rensburg (holotype CBS H-19687, ex-type culture CBS 117499 = STE-U 5431 = CPC 5431).

Notes — *Diaporthe cuppatea* (clade 20) is known only from the original collection made from dying branches of *Aspalathus linearis* in South Africa (van Rensburg et al. 2006).

Diaporthe cynaroidis Marinč., M.J. Wingf. & Crous, *CBS Biodiversity Ser. (Utrecht)* 7: 39. 2008

Specimen examined. SOUTH AFRICA, Western Cape Province, on leaf litter of *Protea cynaroides*, 26 June 2000, S. Marincowitz (ex-type culture CBS 122676 = CMW 22190 = CPC 13180).

Notes — Clade 48 contains the ex-type culture of *D. cynaroidis* (CBS 122676), which was isolated from *Protea cynaroides* in South Africa (Marincowitz et al. 2008). This species is closely related to *D. australafricana* and *D. viticola* (clades 49 and 50, respectively).

Diaporthe decedens (Pers.) Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 30. 1871

Basionym. *Sphaeria tessella* var. *decedens* Pers., *Syn. Meth. Fung. (Göttingen)* 1: 48. 1801.

Specimens examined. AUSTRIA, on *Corylus avellana*, Oct. 2001, W. Jaklitsch (CBS 109772 = AR 3459). – SWEDEN, Öland, Kastlösa par., on *Corylus avellana*, 7 June 1989, K. & L. Holm (CBS 114281 = UPSC 2957).

Notes — *Diaporthe decedens* represents a European species on *Corylus*. Clade 68 consists of two isolates obtained on *Corylus avellana* from Austria and Sweden.

Diaporthe detrusa (Fr.) Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 205. 1870 (1869–1870)

Basionym. *Sphaeria detrusa* Fr., in Kunze & Schmidt, *Mykologische Hefte (Leipzig)* 2: 43. 1823.

= *Phoma detrusa* Sacc., *Michelia* 2: 96. 1880.

≡ *Phomopsis detrusa* (Sacc.) Traverso, *Fl. Ital. Crypt. Pars 1: Fungi. Pyrenomycetae. Xylariaceae, Valsaceae, Ceratostomataceae* 1, 1: 195. 1906.

Specimens examined. AUSTRIA, on *Berberis vulgaris*, Oct. 2001, A.Y. Rossman (CBS 109770 = AR 3424). – SWEDEN, Uppland, Hällnäs par., on *Berberis vulgaris*, 14 May 1991, K. & L. Holm (CBS 114652 = UPSC 3371). – UNKNOWN, on *Berberis vulgaris*, Sept. 1927, L.E. Wehmeyer (CBS 140.27).

Notes — Clade 54 contains three isolates of *D. detrusa* obtained from *Berberis vulgaris* in Austria, Sweden and one of them with an unknown origin (presumably North America). This European species is known to also occur in the USA (Farr & Rossman 2012).

Diaporthe elaeagni Rehm, *Syll. Fung.* 14: 546. 1899. — Fig. 8

?= *Phoma elaeagni* Sacc., *Michelia* 1, 3: 354. 1878.

≡ *Phomopsis elaeagni* (Sacc.) Petr., *Ann. Mycol.* 19, 1–2: 48. 1921.

Specimen examined. NETHERLANDS, Maassluis, on twig of *Elaeagnus* sp., May 1972, J. Gremmen (CBS 504.72).

Notes — In culture CBS 504.72 (clade 73) primarily produces beta conidia (spindle shaped, 16–22 × 2 µm, thus wider than seen on average in most other species); alpha conidia rarely observed, fusoid-ellipsoidal, 7–10 × 2–3 µm, thus correlating with dimensions of *Phomopsis elaeagni* (Sacc.) Petr., which is a homonym of *P. elaeagni* Sacc. Furthermore, conidial dimensions of the asexual state of *D. elaeagni* are not known. Additional collections and type studies are thus required to resolve the complex occurring on *Elaeagnus*.

Diaporthe endophytica R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802929

Etymology. Named after its endophytic growth habit.

Cultures sterile. *Diaporthe endophytica* (clade 5) differs from its closest phylogenetic neighbour, *D. phaseolorum* (clade 4), by unique fixed alleles in five loci based on alignments of the separate loci deposited in TreeBase as study S13943: ITS positions 357 (C), 359 (G), 360 (T), 368 (A), 369 (A), 371 (A), 372 (G) and 373 (G); TUB positions 135 (C) and 592 (T); CAL position 145 (G); TEF1 positions 18 (G), 26 (T), 40 (T), 42 (A), 63 (A), 124 (A), 175 (A) and 343 (A); HIS position 369 (C).

Culture characteristics — Colonies with sparse aerial mycelium, covering the dish after 2 wk at 25 °C. On PDA buff, honey to isabelline; reverse smoke-grey. On OA smoke-grey to olivaceous-grey. On MEA buff with umber patches; reverse dark mouse-grey, with patches of isabelline.

Specimens examined. BRAZIL, endophytic in leaf on *Schinus terebinthifolius*, July 2007, J. Lima (LGMF 911 = CPC 20287, LGMF 919 = CPC 20295), (holotype CBS H-21107, culture ex-type LGMF 916 = CPC 20292)

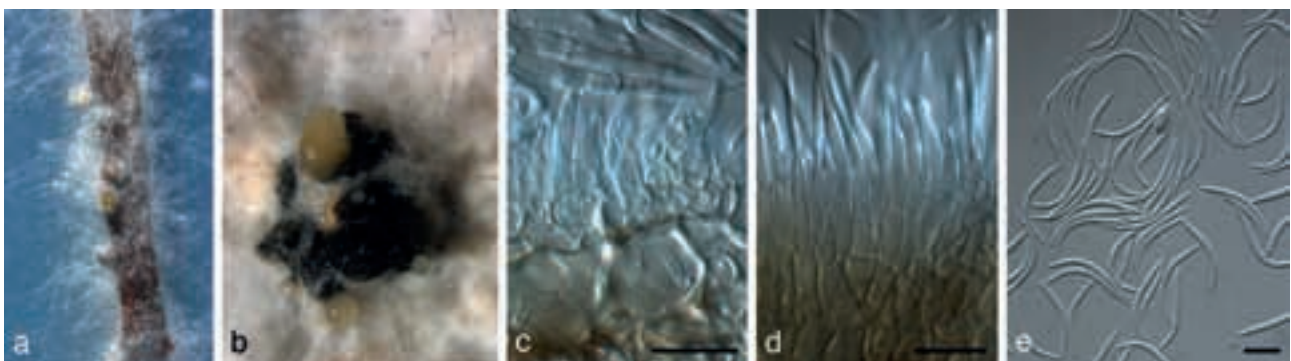


Fig. 8 *Diaporthe elaeagni* (CBS 504.72). a. Conidiomata sporulating on PNA; b. conidiomata sporulating on PDA; c, d. conidiogenous cells; e. beta conidia. — Scale bars = 10 µm.

= CBS 133811); endophytic in petiole on *Maytenus ilicifolia*, July 2007, R.R. Gomes (LGMF 928 = CPC 20304, LGMF 934 = CPC 20310, LGMF 935 = CPC 20311, LGMF 937 = CPC 20313); in seed on *Glycine max*, A. Almeida EMBRAPA/PR (LGMF 948 = CPC 20324).

Notes — Clade 5 represents a distinct lineage, containing eight sterile isolates originating from Brazil. Four of them were isolated from *Maytenus ilicifolia*, three from *S. terebinthifolius* and one from soybean seeds. Isolates could not be induced to sporulate on any of the media defined in this study, nor on sterilised plant host tissue placed on WA.

Diaporthe eres Nitschke, Pyrenomycetes Germanici 2: 245. 1870

= *Phomopsis cotoneastri* Punith., Trans. Brit. Mycol. Soc. 60, 1: 157. 1973.
 = *Phoma oblonga* Desm., Ann. Nat. Sci. Bot. 20: 218. 1853.
 ≡ *Phomopsis oblonga* (Desm.) Traverso, Fl. Ital. Crypt. Pars 1: Fungi. Pyrenomycetae. Xylariaceae, Valsaceae, Ceratostomataceae: 248. 1906.

Specimens examined. AUSTRIA, on *Acer campestre*, Oct. 2001, W. Jaklitsch (CBS 109767 = AR 3538 = WJ 1643). — GERMANY, Monheim, on leaf spot of *Hordeum* sp., 5 Aug. 1984, M. Hossfeld (CBS 841.84). — ITALY, Milano, on twig of *Juglans regia*, Dec. 1980, M. Bisiach (CBS 102.81). — LATVIA, on *Rhododendron* sp., I. Apine (CBS 129168). — NETHERLANDS, Oostvoorne, on dead stems of *Arctium* sp., 13 Dec. 1984, M. de Nooij (CBS 110.85); Soest, Dalweg, on fallen fruit of *Fraxinus* sp., 21 Feb. 1999, G. Verkley (CBS 101742); Veldhoven, on dead branch of *Sorbus aucuparia*, Nov. 1973, W.M. Loerakker (CBS 287.74); Baarn, garden Chopinlaan, on dead branch of *Wisteria sinensis*, 6 June 1983, H.A. van der Aa (CBS 528.83); Soest, inside house, on *Abutilon* sp., 26 Mar. 1997, A. Aptroot (CBS 688.97); Baarn, potted plant, on cladodes of *Opuntia* sp., 23 Sept. 1996, H.A. van der Aa (CBS 365.97); on *Alliaria officinalis*, Feb. 1962, G.H. Boerema (CBS 445.62); Baarn, on dead leaf of *Ilex aquifolium*, 11 June 1967, H.A. van der Aa (CBS 370.67 = MUCL 9931); Prov. Zuid-Holland, Huize Oud-Poelgeest, Oegstgeest, dieback of *Ilex aquifolium*, 21 Nov. 1994, G.J.M. Verkley (CBS 694.94); Baarn, garden Eemnesserweg 90, on dead stem of *Rumex hydrolapathum*, 19 Mar. 1996, H.A. van der Aa (CBS 485.96); Baarn, Cantonspark, on withering leaf of *Magnolia × soulangeana*, 23 Oct. 1968, H.A. van der Aa (CBS 791.68); Zuid-Holland, Ridderkerk, Huys ten Donck, on leaf tip of *Osmanthus aquifolium*, 7 May 1977, H.A. van der Aa (CBS 297.77); on *Phaseolus vulgaris*, Sept. 1950, Goossens (CBS 422.50); Baarn, on dead stem of *Allium giganteum*, May 1985, H.A. van der Aa (CBS 283.85); from *Laburnum × watereri* 'Vossii', Apr. 1935, I. de Boer (CBS 267.55); Boskoop, nursery, dying twigs of *Skimmia japonica*, Nov. 1981, H.A. v. Kesteren (CBS 122.82). — UK, Scotland, on living and dead twig of *Fraxinus excelsior*, Feb. 1938, J.A. MacDonald

(CBS 250.38); on *Cotoneaster* sp., 1971, H. Butin (ex-type culture of *P. cotoneaster* CBS 439.82 = BBAP-407 = IMI 162181a); Oxford, on *Picea abies* seedling, Nov. 1937, T.R. Peace (CBS 186.37). — UNKNOWN, on rotten fruit of *Malus sylvestris*, May 1961, Geigy (CBS 375.61); May 1932, W.G. Hutchinson (CBS 267.32).

Notes — *Diaporthe eres* (clade 67) is the type species of the genus *Diaporthe*, and is present in several hosts, though it is known to be morphologically highly variable (Castlebury et al. 2002). Wehmeyer (1933) described this species on more than 60 hosts, and listed several synonymies based on morphological data. A detailed morphological study is required to designate a suitable epitype strain for *D. eres*, and to resolve the status of all its purported synonyms.

Diaporthe eugeniae (Punith.) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802930

Basionym. *Phomopsis eugeniae* Punith., Trans. Brit. Mycol. Soc. 63, 2: 232. 1974.

Specimens examined. WEST SUMATRA, on *Eugenia aromatica*, May 1973, J. Waller (holotype IMI 177560); Lampung, on leaf of *Eugenia aromatica*, July 1982, R. Kasim (CBS 444.82).

Notes — *Diaporthe eugeniae* (clade 83) was originally described on *Eugenia aromatica* from West Sumatra. Although the present isolate could be authentic for the name, it unfortunately proved to be sterile.

Diaporthe fibrosa (Pers.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 204. 1870 (1869–1870)

Basionym. *Sphaeria fibrosa* Pers., Syn. Meth. Fung. (Göttingen) 1: 40. 1801.

Specimens examined. AUSTRIA, Vienna, on *Rhamnus cathartica*, Oct. 2001, A.Y. Rossmann (CBS 109751 = AR 3425). — SWEDEN, Uppland, Dalby par., Hässleborg, on *Rhamnus cathartica*, 10 Mar. 1987, K. & L. Holm (CBS 113830 = UPSC 2117).

Notes — Clade 52 consists of two isolates from *Rhamnus cathartica* collected in Sweden and Austria. *Diaporthe fibrosa* was originally described from Europe on *Rhamnus*, so these cultures may well prove to be authentic for the name.

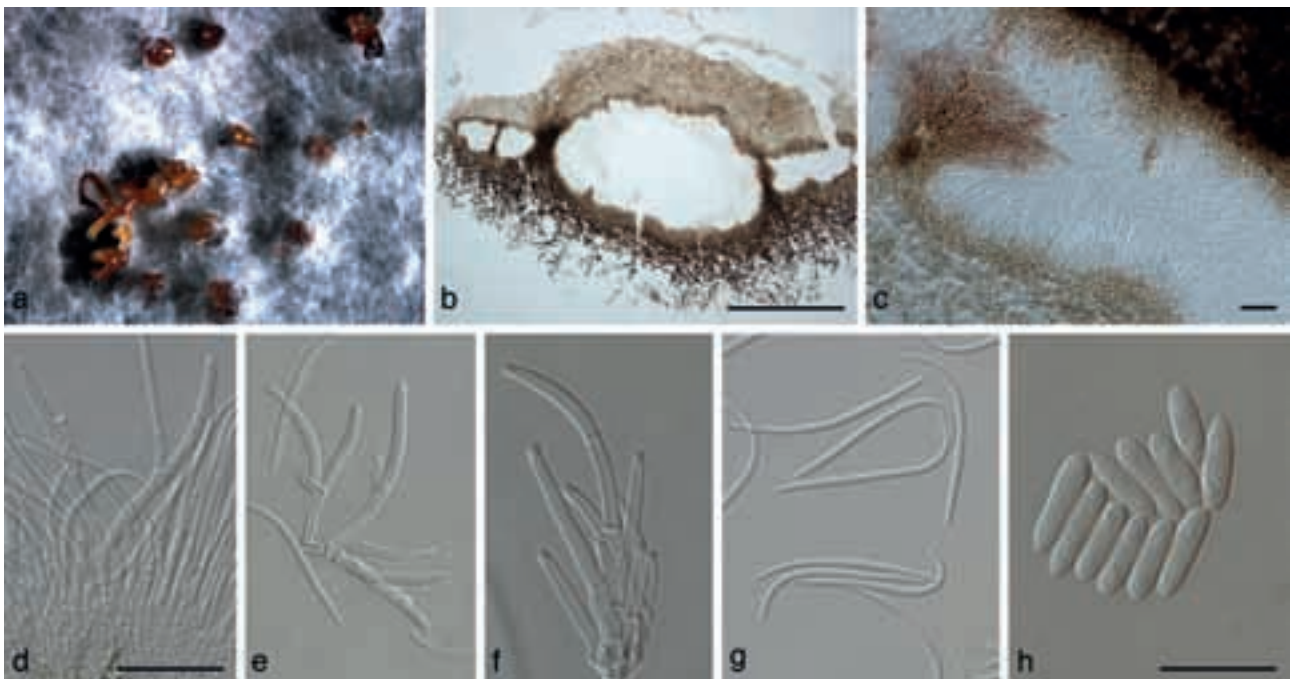


Fig. 9 *Diaporthe foeniculacea* (CBS 111554). a. Conidiomata sporulating on PDA; b, c. transverse section through conidiomata, showing conidiomatal wall; d–f. conidiogenous cells; g. beta conidia; h. alpha conidia. — Scale bars: b = 250 µm, all others = 10 µm.

Diaporthe foeniculacea Niessl, in von Thümen, Contr. Ad. Fl. Myc. Lusit. 2: 30. 1880. — Fig. 9

= *Phoma foeniculina* Sacc., Syll. Fung. 3: 125. 1884.

≡ *Phomopsis foeniculina* (Sacc.) Câmara, Agron. Lusit. 9: 104. 1947.

= *Phomopsis theicola* Curzi, Atti Ist. Bot. Univ. Pavia, 3 sér., 3: 65. 1927.

= *Diaporthe neotheicola* A.J.L. Phillips & J.M. Santos, Fung. Diversity 34: 120. 2009.

Conidiomata pycnidial, eustromatic, multilocular, immersed, ostiolate, dark brown, scattered or aggregated, 350–890 µm wide, 160–320 µm tall, necks absent, outer surface covered with hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose to conical and exuding in cirrhi, yellow to reddish brown. *Conidiophores* hyaline, sub-cylindrical and cylindrical, filiform, branched above the septa, tapering towards the apex, 1–3-septate, (19–)20–28(–32) × 2(–3) µm. *Conidiogenous cells* hyaline, subcylindrical and filiform, straight, slightly tapering towards the apex, collarette not flared, prominent periclinal thickening, (10–)11–15(–17) × 2(–3) µm. *Alpha conidia* hyaline, oblong to ellipsoidal, apex bluntly rounded, base obtuse to subtruncate, bi- to multi-guttulate (6–)7–9 × 2(–3) µm. *Beta conidia* hyaline, smooth, slightly curved, (26–)28–32(–34) × 1(–2) µm. *Gamma conidia* not observed (based on isolate CBS 111554).

Specimens examined. INDIA, Calcutta, unknown host, Feb. 1948, S.R. Bose (CBS 400.48). — ITALY, on leaves and branches of *Camellia sinensis*, Oct. 1927, M. Curzi (ex-type culture of *P. theicola* CBS 187.27); Perugia, on *Diospyros kaki*, June 1956, M. Ribaldi (CBS 287.56); Apulia, near Bari, on *Prunus amygdalus*, winter 1974/75, A. Ciccarone (CBS 171.78). — NETHERLANDS, Baarn, 'Madoera', back frond, on *Wisteria sinensis*, 24 Apr. 1969, H.A. van der Aa (CBS 357.69). — NEW ZEALAND, Waikato region, on *Pyrus pyrifolia*, 2001, W. Kandula (CBS 116957). — PORTUGAL, near Lisbon, São Marcos, base of senescent stem of *Foeniculum vulgare*, Apr. 2002, A.J.L. Phillips (CBS 111554); Évora, *Foeniculum vulgare*, 1 Nov. 2007, A.J.L. Phillips (ex-type cultures of *Diaporthe neotheicola* CBS 123209, CBS 123208); Pedras del Rei, near Tavira, on *Bougainvillea spectabilis*, 15 June 1988, H.A. van der Aa (CBS 603.88); Madeira, Serra da Agua, base of senescent stem of *Foeniculum vulgare*, Aug. 2001, A.J.L. Phillips (CBS 111553).

Notes — *Diaporthe foeniculacea* (clade 78) was originally described from *Foeniculum vulgare* in Portugal, and represents an older name for *D. theicola* and *D. neotheicola*. There are many described species that occur on *Foeniculum vulgare* (wild fennel). Among them, *P. theicola* and its teleomorph *D. neotheicola* (Santos & Phillips 2009), and *D. foeniculacea*, the causal agent of stem necrosis of fennel. Phillips (2003) redescribed *D. foeniculacea*, and established the sexual-asexual connection between *D. foeniculacea* and *Phomopsis foeniculina*. The synonymy of *D. neotheicola* under *D. foeniculacea* is based on the fact that the cultures matching the original descriptions are in fact genetically identical. However, as there are no ex-type strains of *D. foeniculacea*, this synonymy strongly relies on the earlier opinion of Phillips (2003). Either way, this matter can only be resolved once an epitype has been designated for *D. foeniculacea*, fixing the application of the name. We recommend that additional collections linked to stem necrosis of fennel in Portugal are obtained, before this decision is made.

Diaporthe ganjae (McPartl.) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802932

Basionym. *Phomopsis ganjae* McPartl., Mycotaxon 18, 2: 527. 1983.

Specimen examined. USA, Illinois, Hannah City, dead leaf of *Cannabis sativa*, deposited Mar. 1991, J.M. McPartland (holotype HA 10987, ex-type culture ILLS 43621 = CBS 180.91).

Notes — *Diaporthe ganjae* (clade 24) is known only from the original collection taken from wilted, dead leaves of *Cannabis sativa* in Illinois, USA (McPartland 1983). Phylogenetically *D. ganjae* is closely related to an isolate identified as *D. mani-*

hotia (CBS 505.76), isolated from *Manihot utilissima* in Rwanda (clade 25).

Diaporthe gardeniae (Buddin & Wakef.) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802933

Basionym. *Phomopsis gardeniae* Buddin & Wakef., Gard. Chron., ser. 3 103: 45. 1938.

= *Phomopsis gardeniae* H.N. Hansen & Barrett, Mycologia 30, 1: 18. 1938 (homonym).

Specimen examined. ITALY, on stem of *Gardenia florida*, June 1956, M. Ribaldi (CBS 288.56).

Notes — *Diaporthe gardeniae* (clade 59) causes gardenia canker in *Gardenia jasminoides*, *G. lucida* and *Gardenia* sp. (Farr & Rossman 2012). This disease is considered as serious (Tilford 1934, Huber 1936, Miller 1961). It was originally observed in 1894 in England (Cooke 1894), and has since been reported from the USA (Preston 1945) and India (Mathur 1979). All parts of the plant are susceptible to infection, including roots, stems and leaves (McKenzie et al. 1940), although cankered stems are the most diagnostic symptoms for this disease.

Diaporthe helianthi Munt.-Cvetk., Mihaljč. & M. Petrov, Nova Hedwigia 34: 433. 1981

= *Phomopsis helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov, Nova Hedwigia 34: 433. 1981.

Specimens examined. SERBIA, Vojvodina, overwintering stem on *Helianthus annuus*, 1980, M. Muntañola-Cvetkovic (ex-type culture CBS 592.81 = CBS H-1540). — UNKNOWN, on seed of *H. annuus*, June 1994, Vanderhave Res., Rilland, Netherlands (CBS 344.94).

Notes — *Diaporthe helianthi* (clade 14) is associated worldwide with stem canker and grey spot disease of sunflower (*Helianthus annuus*) (Muntañola-Cvetkovic et al. 1981). Yield reductions of up to 40 % have been recorded in Europe (Masirevic & Gulya 1992) including the former Yugoslavia as well as France where it was considered a major pathogen of sunflower (Battilani et al. 2003, Debaeke et al. 2003). *Diaporthe helianthi* is also widespread in the sunflower growing regions of the USA (Gulya et al. 1997). The wide geographic distribution, and high genetic variability of the pathogen lead to the evolution of new strains that could be more aggressive, causing large yield losses and a decline in disease control (Pecchia et al. 2004, Rekab et al. 2004).

***Diaporthe cf. heveae* 1**

Specimen examined. BRAZIL, São Paulo, from *Hevea brasiliensis*, Apr. 1997, D.S. Attili (CBS 852.97) (originally identified as *Phomopsis heveae*).

Notes — *Diaporthe heveae* and *Phomopsis heveae* were both described from *Hevea* in Sri Lanka, and could represent the same species. Two isolates deposited in CBS under this name, CBS 852.97 (from *Hevea brasiliensis* in Brazil) and CBS 681.84 (from *Hevea brasiliensis* in India) were shown to represent two distinct species (clades 46 and 80, respectively). However, as both were found to be sterile, their taxonomy could not be resolved.

***Diaporthe cf. heveae* 2**

Specimen examined. INDIA, Kerala, Kottayam, in leaf on *Hevea brasiliensis*, Sept. 1984, K. Jayarathnam (CBS 681.84).

Notes — Isolate CBS 681.84 (clade 80, *P. heveae* from *Hevea brasiliensis* in India) is sterile, and thus its taxonomy could not be resolved. *Diaporthe heveae* has been reported from Brazil, China, India, Indonesia, Malaysia, Sri Lanka and Thailand (Holliday 1980, Zhuang 2001, Udayanga et al. 2011).

Diaporthe hickoriae Wehm., Monogr. Gen. Diaporthe Nitschke & Segreg., Univ. Michigan Stud., Sci. Ser. 9: 149. 1933

Specimen examined. USA, Michigan, on *Carya glabra*, June 1926, L.E. Wehmeyer (ex-type culture CBS 145.26).

Notes — *Diaporthe hickoriae* (clade 72) occurs on the bark of *Carya glabra* in the USA (Wehmeyer 1933).

Diaporthe hongkongensis R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802934; Fig. 10

Etymology. Named after the location where it was collected, Hong Kong.

Conidiomata pycnidial, superficial to embedded on PDA, solitary to aggregated, globose with central ostiole, exuding a creamy conidial cirrus; pycnidial up to 200 µm diam; wall of 3–6 layers of brown *textura angularis*. *Conidiophores* lining the inner cavity, reduced to conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to subcylindrical with prominent apical taper, 5–12 × 2–4 µm; apex with periclinal thickening and minute collarette, 1 µm long. *Paraphyses* intermingled among conidiophores, hyaline, smooth, frequently branched below, up to 4-septate, with clavate terminal cell, up to 80 µm long, apex 2–8 µm diam. *Alpha conidia* hyaline, smooth, granular to guttulate, aseptate, fusiform, tapering towards both ends, mostly straight, apex acutely rounded, base truncate, (5–)6–7(–8) × (2–)2.5(–3) µm. *Gamma conidia* aseptate, hyaline, smooth, ellipsoid-fusoid, apex subobtuse, base truncate, 10–13 × 2 µm. *Beta conidia* aseptate, hyaline, smooth, spindle-shaped, apex acutely rounded, base truncate, widest in mid region, mostly curved in upper part, 18–22 × 1.5–2 µm.

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C, with moderate aerial mycelium. On OA surface dirty white with patches of pale olivaceous-grey, reverse dirty white with patches of olivaceous-grey and iron-grey. On PDA surface iron-grey, with patches of dirty white, reverse iron-grey. On MEA surface dirty white with patches of olivaceous-grey, reverse iron-grey with patches of dirty white.

Specimen examined. HONG KONG, Tai Po Kau, on fruit of *Dichroa febrifuga*, 20 Feb. 2002, K.D. Hyde (holotype CBS H-21103, culture ex-type CBS 115448 = HKUCC 9104).

Notes — Isolate CBS 115448 (clade 79; reported as *Phomopsis pittospori* on *Dichroa febrifuga* from Hong Kong) is morphologically distinct from *P. pittospori* (from *Pittosporum* twigs in California; alpha conidia 6–8 × 1.5 µm, beta conidia 18–20 × 1 µm), with wider alpha and beta conidia.

Diaporthe hordei (Punith.) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802935

Basionym. *Phomopsis hordei* Punith., Trans. Brit. Mycol. Soc. 64, 3: 428. 1975.

Specimen examined. NORWAY, Fellesbygget, As, on root of *Hordeum vulgare*, Oct. 1992, L. Sundheim (CBS 481.92).

Notes — *Diaporthe hordei* (clade 13) was described from *Hordeum vulgare* in the UK. Although the present culture could be authentic (from *Hordeum* collected in Norway), it proved to be sterile, so its morphology could not be confirmed.

Diaporthe impulsa (Cooke & Peck) Sacc., Syll. Fung. (Abellini) 1: 618. 1882

Basionym. *Valsa impuls*a Cooke & Peck, Ann. Rep. N.Y. State Mus. Nat. Hist. 27: 109. 1875 (1874).

Specimens examined. SWEDEN, Uppland, Dalby par., Jerusalem, on *Sorbus aucuparia*, 24 Oct. 1989, K. & L. Holm (CBS 114434 = UPSC 3052). — UNKNOWN, on *Sorbus americana*, Sept. 1927, L.E. Wehmeyer (CBS 141.27).

Notes — Clade 51 is represented by two isolates of *D. impuls*a occurring on *Sorbus* spp. *Diaporthe impuls*a is a known pathogen of *Sorbus* spp., and has a wide geographic distribution (Farr & Rossman 2012). It was originally described from *Sorbus* in the USA, thus CBS 141.27 may well prove to be a good reference strain for the species.

Diaporthe inconspicua R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802936

Etymology. Referring to its inconspicuous nature, growing as endophyte in host tissue.

Cultures sterile. *Diaporthe inconspicua* (clade 75) differs from its closest phylogenetic neighbours, clade 68–74 and 76–78, by unique fixed alleles in four loci based on alignments of the separate loci deposited in TreeBase as study S13943: TUB positions 33 (A), 102–104 and 106–111 (indels), 127 (G), 149 (C), 151 (A), 195 (C), 204 (T), 357 (G), 446 (G), 449 (C), 465 (T), 484 (T), 559 (A), 592 (A), 629 (T), 653 (T), 708 (C), 732 (C), 754 (A), 763 (C), 784 (A) and 787 (G); CAL positions 28 (C), 102 (G), 114 (T), 148 (T), 152 (T), 153 (A), 157 (C), 170 (G), 199 (C) and 281 (C); TEF1 positions 9 (T), 16 (A), 22 (A), 29 (G), 30 (G), 81 (C), 86 (C), 87 (A), 88 (A), 89 (T), 131 (A), 275 (A), 298 (C) and 315 (T); HIS positions 139 (T), 211 (T), 244 (T) and 408 (T).

Culture characteristics — Colonies covering the dish after 2 wk in the dark at 25 °C. On OA spreading, flat with sparse aerial mycelium, surface cream in centre, umber in outer region.

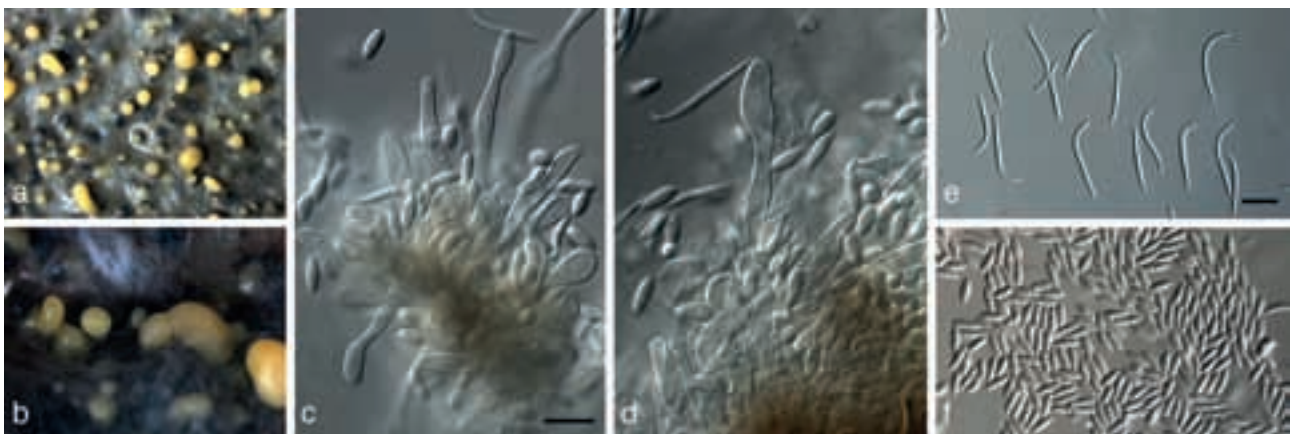


Fig. 10 *Diaporthe hongkongensis* (CBS 115448). a, b. Conidiomata sporulating on PDA; c, d. conidiogenous cells; e. beta conidia; f. alpha conidia. — Scale bars = 10 µm.

On PDA surface and reverse cream to dirty white with sparse aerial mycelium. On MEA with sparse aerial mycelium, surface becoming folded, dirty white in centre, sienna in outer region, and luteous in reverse.

Specimens examined. BRAZIL, on petiole of *Maytenus ilicifolia*, July 2007, R.R. Gomes (holotype CBS H-21102, ex-type culture LGMF 930 = CPC 20306 = CBS 133813); same collection details (LGMF 931 = CPC 20307); on *Spondias mombin*, 2007, K. Rodriguez (LGMF 922 = CPC 20298).

Notes — Sterile endophytic isolates (clade 75) from medicinal plants in Brazil.

Diaporthe infecunda R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802937

Etymology. Named after its sterile growth in culture.

Cultures sterile. *Diaporthe infecunda* (clade 23) differs from its closest phylogenetic neighbours, clade 16–22, by unique fixed alleles in five loci based on alignments of the separate loci deposited in TreeBase as study S13943: ITS positions 108 (T), 279 (C), 292 (G), 359 (C) and 360 (G); TUB positions 11 (indel), 106 (G), 138 (T), 140 (A), 153 (G), 155 (T), 184 (A), 197 (G), 202 (C), 302 (A), 354 (A), 369–374 (indels), 398 (G), 407 (indel), 414 (C), 422 (T), 424 (G), 425 (C), 432 (G), 452 (C), 454 (C), 458 (C), 461 (G), 479 (T), 482 (T), 486 (C), 540 (T), 572 (C), 622 (A), 694 (T), 696 (T), 697 (G), 716 (C), 728 (C), 776 (G), 778 (G) and 796 (C); CAL positions 64 (T), 83 (T), 104 (G), 146 (C), 151 (C), 155 (G), 159 (C), 172 (C), 176 (T), 179 (A), 184 (G), 197 (T), 206 (T), 212 (C) and 221 (T); TEF1 positions 6 (A), 9 (G), 13 (G), 16 (C), 21 (A), 30 (G), 32 (indel), 39 (A), 40 (A), 41 (G), 42 (T), 43 (A), 79 (G), 83 (T), 90 (T), 92 (T), 96 (A), 97 (C), 106 (C), 116 (A), 120 (C), 123 (A), 127 (A), 132 (A), 135 (G), 173 (G), 255 (T), 284 (A), 294 (C), 299 (C) and 309 (A); HIS positions 173 (T), 196 (T), 197 (G), 199 (C/T), 221 (C), 222 (C), 230 (G), 263 (C), 264 (T), 268 (C), 273 (T) and 279 (C).

Culture characteristics — Colonies covering the dish after 2 wk in the dark at 25 °C. On PDA surface umber with patches of white, reverse chestnut. On MEA surface dirty white, reverse umber. On OA surface with patches of dirty white and umber.

Specimens examined. BRAZIL, on leaf of *Schinus terebinthifolius*, July 2007, J. Lima (holotype CBS H-21095, ex-type culture LGMF 906 = CPC 20282 = CBS 133812); additional isolates with same collection details (LGMF 908 = CPC 20284, LGMF 912 = CPC 20288, LGMF 917 = CPC 20293, LGMF 918 = CPC 20294, LGMF 920 = CPC 20296); in petiole of *Maytenus ilicifolia*, July 2007, R.R. Gomes (LGMF 933 = CPC 20309, LGMF 940 = CPC 20316).

Notes — Clade 23 represents endophytic isolates from leaves of medicinal plants growing in Brazil. It consists of eight isolates, two from *Maytenus ilicifolia*, and six from *Schinus terebinthifolius*.

Diaporthe juglandina (Fuckel) Nitschke, *Pyrenomyces Germanici* 2: 281. 1870

Basionym. *Aglaospora juglandina* Fuckel, *Fungi Rhenani Exsicc.*, suppl. 7 (no. 2101–2200): no. 2159. 1868.

Specimen examined. USA, Tennessee, Great Smoky Mts National Park, dead wood of *Juglans* sp., *L. Vasiljeva* (CBS 121004).

Notes — *Diaporthe juglandina* (clade 65) represents a European taxon described from *Juglans*. European collections are required to confirm whether this name can be applied to the clade.

Diaporthe longispora (Wehm.) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802938

Basionym. *Diaporthe strumella* var. *longispora* Wehm., *Mycologia* 28, 1: 46. 1936.

Specimen examined. CANADA, Ontario, Toronto, on *Ribes* sp., May 1936, L.E. Wehmeyer (ex-type culture CBS 194.36).

Notes — Clade 27 comprises the ex-type culture of *D. strumella* var. *longispora* isolated from *Ribes* sp., and forms a sister clade with *D. sclerotoides* (clade 28). *Diaporthe strumella* is found on woody limbs, especially of *Ribes* spp. in temperate North America and Europe (Farr & Rossman 2012). As *D. strumella* var. *longispora* is morphologically clearly a distinct species, we elevate this variety to species status.

Diaporthe lusitanicae A.J.L. Phillips & J.M. Santos, *Fung. Diversity* 34: 118. 2009

Specimen examined. PORTUGAL, Lisbon, Oeiras, Estação Agronómica Nacional, stem of *Foeniculum vulgare*, 14 Aug. 2007, J.M. Santos (ex-type cultures CBS 123212 = Di-C001/5, CBS 123213 = Di-C001/3).

Notes — This species (clade 21) was described in 2009 on senescent stems of *Foeniculum vulgare* (wild fennel) in Portugal by Santos & Phillips (2009).

Diaporthe manihotia Punith., *Kavaka* 3: 29. 1976 (1975)

= *Phomopsis manihotis* Swarup, L.S. Chauhan & Tripathi, *Mycopathol. Mycol. Appl.* 28, 4: 345. 1966.

Specimen examined. RWANDA, on leaves of *Manihot utilissima*, 9 July 1976, J. Semal (CBS 505.76).

Notes — *Phomopsis manihotis* (clade 25 as *D. manihotia*) causes leaf spot of cassava (*Manihot esculenta*), though the disease is also referred to as *Phomopsis* blight of tapioca. Severe infection leads to defoliation and stem lesions. Affected areas become shrivelled with numerous pycnidia embedded in the tissue. On severely infected stems the bark starts to gradually peel off, leading to partial or total girdling. The disease is known from Africa (Ethiopia, Nigeria), Asia (India), Central America and West Indies (S.E. Dominica), and South America (Colombia) (Sarbhoy et al. 1971, Mathur 1979, Farr & Rossman 2012).

Diaporthe mayteni R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802939; Fig. 11

Etymology. Named after the host genus from which it was collected, *Maytenus*.

Conidiomata pycnidial, globose, immersed, scattered and aggregated, brown to black, ostiolate, 70–230 µm wide, 40–150 µm tall, with short necks, 40–140 µm; outer surface smooth or covered in hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose or exuding in cirrhi; predominantly yellow, pale luteous to cream. *Conidiophores* hyaline, subcylindrical to cylindrical, rarely branched above the septa, tapering towards the apex, 1–3-septate, (10–)13–27(–36) × (2–)3(–4) µm. *Conidiogenous cells* hyaline, subcylindrical, rarely tapering towards the apex, collarete present and not flared, with prominent periclinal thickening, (5–)6–10(–13) × 2(–3) µm. *Alpha conidia* hyaline, oblong to ellipsoid, apex bluntly rounded, base obtuse; biguttulate, (5–)6(–7) × (2–)3 µm. *Beta* and *gamma conidia* absent.

Culture characteristics — Colonies on PDA flat, with entire edge, cottony, olivaceous buff, with primrose aerial mycelium in concentric rings, with olivaceous patches; colonies reaching 66 mm diam after 2 wk at 25 °C; reverse olivaceous buff and greenish olivaceous. On OA flat, with entire edge, cottony

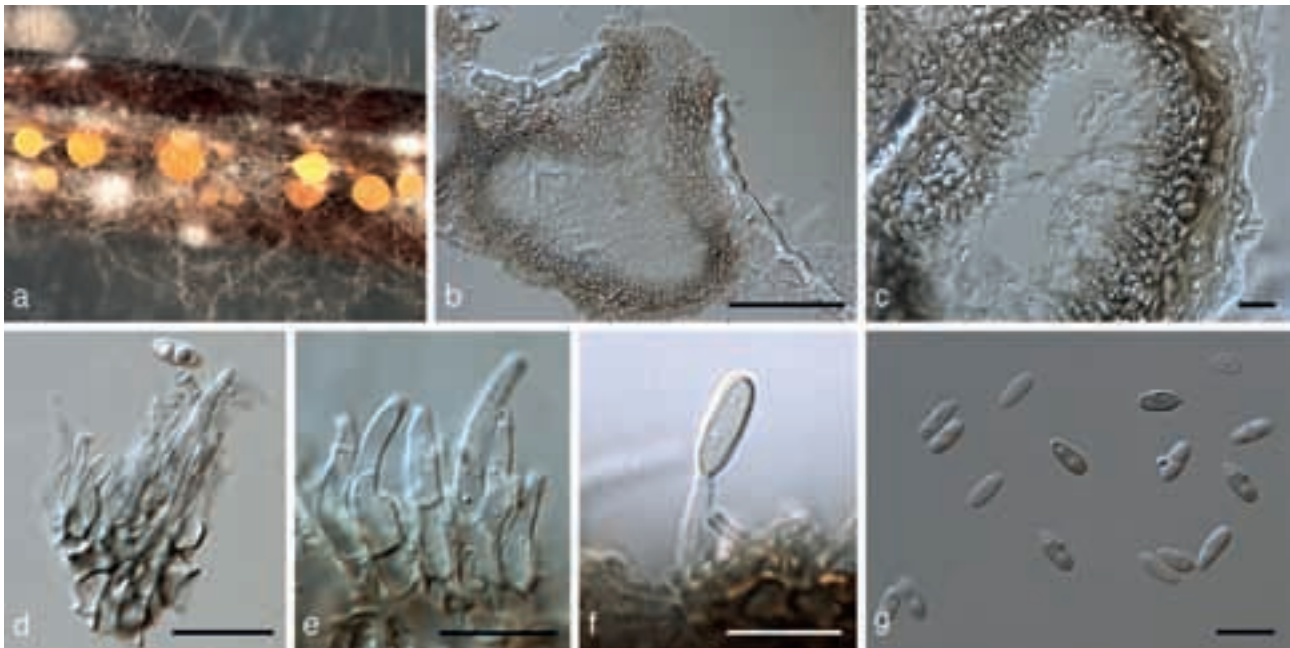


Fig. 11 *Diaporthe mayteni* (CBS 133185). a. Conidiomata sporulating on PNA; b, c. transverse section through conidiomata, showing conidiomatal wall; d, e. conidiogenous cells; f. beta conidia; g. alpha conidia. — Scale bars: b = 85 μ m, all others = 10 μ m.

appressed, buff, white, the center of the colony pale olivaceous-grey, patches isabelline and luteous; colonies reaching 56 mm diam; reverse buff and pale olivaceous grey. On MEA flat, with entire edge, aerial mycelium cottony, white to pale olivaceous grey or olivaceous buff; colonies reaching 37 mm diam; reverse hazel, ochreous, with patches greenish black and olivaceous black.

Specimen examined. BRAZIL, Paran, Colombo, endophytic species isolated from petiole of *Maytenus ilicifolia* (popular name Espinheira Santa), July 2007, R.R. Gomes (holotype CBS H-21096, ex-type culture CBS 133185 = LGMF 938 = CPC 20314).

Notes — *Diaporthe mayteni* (clade 30) grows endophytically in *Maytenus ilicifolia* in Brazil.

Diaporthe megalospora Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 42: 235. 1890

Specimen examined. UNKNOWN, from *Sambucus canadensis*, Sept. 1927, L.E. Wehmeyer (CBS 143.27).

Notes — *Diaporthe megalospora* (clade 15) is known on *Sambucus canadensis* from North America (Wehmeyer 1933, Hanlin 1963, Farr & Rossman 2012). Fresh collections are required to designate an epitype, and fix the genetic application of the name.

Diaporthe melonis Beraha & M.J. O'Brien, Phytopathol. Z. 94, 3: 205. 1979

= *Phomopsis cucurbitae* McKeen, Canad. J. Bot. 35: 46. 1957.

Specimens examined. INDONESIA, Java, Muneng, Exp. Station, on *Glycine soja*, Sept. 1987, H. Vermeulen (CBS 435.87). — USA, Texas, Rio Grande Valley, on *Cucumis melo*, 1978, L. Beraha & M.J. O'Brien (ex-isotype culture CBS 507.78, specimen derived from culture CBS H-891).

Notes — Clade 7 represents *D. melonis* (Beraha & O'Brien 1979), and contains the ex-isotype culture, and one isolate previously identified as *D. phaseolorum* var. *sojae* (though the two isolates are not identical). *Diaporthe melonis* is frequently reported on soybean (Santos et al. 2011). *Phomopsis cucurbitae* (treated here as synonym) is reported to have a cosmopolitan distribution, and to cause black rot disease of greenhouse

cucumbers (McKeen 1957, Punithalingam & Holliday 1975, Ohsawa & Kobayashi 1989).

Diaporthe musigena Crous & R.G. Shivas, Persoonia 26: 119. 2011

Specimen examined. AUSTRALIA, Queensland, Brisbane Botanical Garden, on leaves of *Musa* sp., 14 July 2009, P.W. Crous & R.G. Shivas (ex-type culture CBS 129519 = CPC 17026).

Notes — Clade 84 represents *D. musigena*, isolated from *Musa* sp. in Australia (Crous et al. 2011).

Diaporthe neilliae Peck, Ann. Rep. N.Y. State Mus. Nat. Hist. 39: 52. 1887 (1886)

Specimen examined. UNKNOWN, on *Spiraea* sp., Sep. 1927, L.E. Wehmeyer (CBS 144.27).

Notes — *Diaporthe neilliae* (clade 60) was originally described from *Spiraea* sp. from North America. The origin of the present isolate, however, remains unclear (presumably North America).

Diaporthe neoarctii R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802940, Fig. 12

Etymology. Named after its superficial resemblance to *Diaporthe arctii*.

Conidiomata pycnidial, ampulliform to finger-like, aggregated, dark brown to black, immersed, ostiolate, 300–450 μ m wide, 200–670 μ m tall, with prominent necks 240–560 μ m long, outer surface covered with hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose, pale yellow. *Conidiophores* hyaline, ampulliform to subcylindrical, filiform, branched above the septa, tapering towards the apex, rarely septate, (12–)13–17(–18) \times (2–)3 μ m. *Conidiogenous cells* hyaline, subcylindrical, filiform, straight, tapering towards the apex, collarette flared, periclinal thickening prominent, (10–)11–13(–14) \times (1.5–)2(–3) μ m. *Alpha conidia* hyaline, fusoid, apex acute, base obtusely rounded to subtruncate, bi- to multi-guttulate, (9–)11–13(–14) \times 3(–4) μ m. *Beta* and *gamma conidia* not observed.

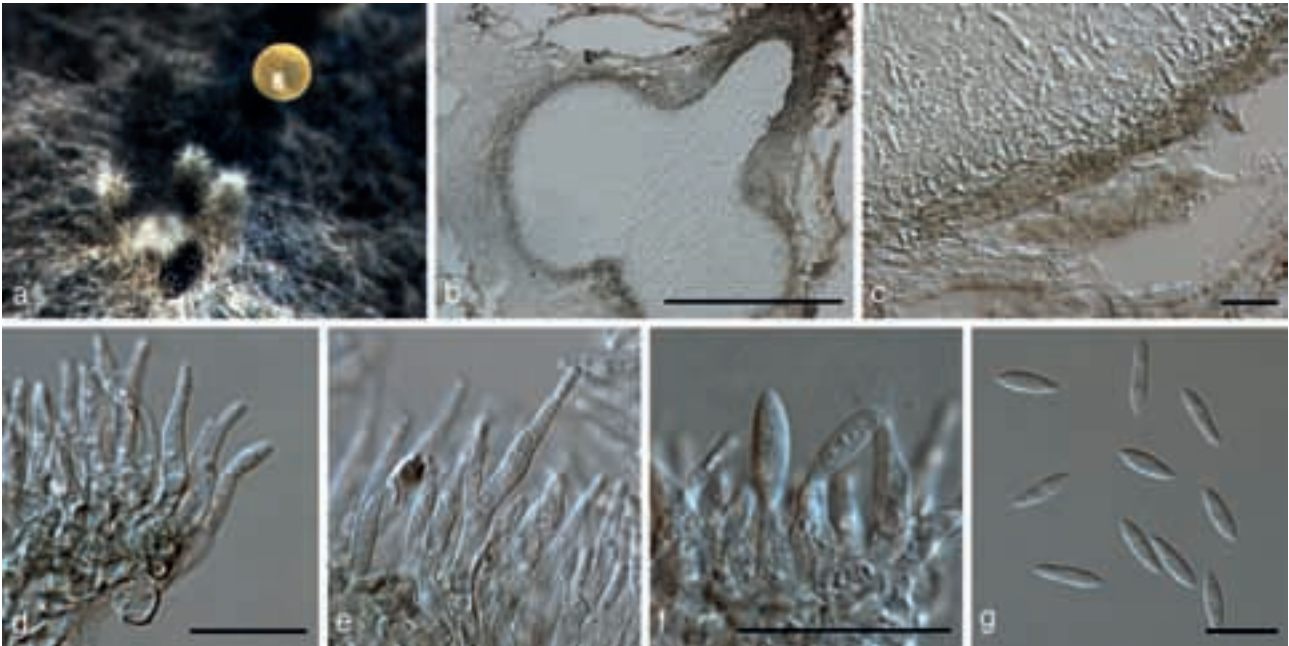


Fig. 12 *Diaporthe neoarctii* (CBS 109490). a. Conidiomata sporulating on PDA; b, c. transverse section through conidiomata, showing conidiomatal wall; d–f. conidiogenous cells; g. alpha conidia. — Scale bars: b = 225 μ m, all others = 10 μ m.

Culture characteristics — Colonies with sparse aerial mycelium, covering the dish after 2 wk in the dark at 25 °C. On MEA umber with patches of greyish sepi, umber in reverse. On PDA fuscous-black on surface and in reverse.

Specimen examined. USA, New Jersey, isolated from *Ambrosia trifida*, May 2001, G. Bills (holotype CBS H-21094, ex-type culture CBS 109490 = GB 6421 = AR 3450).

Notes — Isolates originally identified as *D. arctii* cluster in clades 19 and 67 (Fig. 1). *Diaporthe neoarctii* (clade 16) was isolated from *Ambrosia trifida* in New Jersey, USA, and differs morphologically from the ex-type culture of *D. arctii* (alpha conidia 7×3 – 3.5μ m) (clade 19). Based on these differences *D. neoarctii* is described as a novel species.

Diaporthe nobilis complex

Specimens examined. GERMANY, Münster, on stem of *Laurus nobilis*, Feb. 1939, Kotthoff (CBS 200.39). — JAPAN, isolate from *Pinus pentaphylla* bonzai plant imported from Japan into the Netherlands, May 1979, G.H. Boerema (CBS H-16732, culture CBS 587.79). — KOREA, on imported chestnuts (*Castanea sativa*), collected in grocery store in Sydney, Australia, 5 July 1999, K.A. Seifert (CBS 113470 = DAOM 226800). — LATVIA, *Rhododendron* sp., I. Apine (CBS 129167). — NEW ZEALAND, on bark of *Malus pumila*, G.J. Samuels (CBS 124030 = GJS 77-49); Waikato region, on *Pyrus pyrifolia*, 2001, isol. W. Kandula, det. L. Castlebury (CBS 116953 = NZ-26, CBS 116954 = NZ-27). — YUGOSLAVIA, on *Hedera helix*, July 1989, M. Muntaniola-Cvetkovic (CBS 338.89).

Notes — Clade 62 is poorly resolved in this dataset, but has some internal structure, suggesting that it contains several potentially distinct species. More isolates would be required to resolve their taxonomy. Isolates in this clade were originally identified as *Phomopsis fukushii* (on *Pyrus pyrifolia*, New Zealand), *P. conorum* (on *Pinus pentaphylla*, the Netherlands), *P. castanea* (on *Castanea sativa*, UK), *Diaporthe perniciosa* (*Malus pumila*, New Zealand), *D. pulla* (on *Hedera helix*, Yugoslavia) and *D. nobilis* (on *Laurus nobilis*, Germany).

Diaporthe nomurai Hara, in Hara, Diseases of cultivated plants: 140. 1925. — Fig. 13

Conidiomata in culture on OA sporulating poorly, globose, up to 300 μ m diam, black, erumpent; cream conidial droplets exuding from central ostioles; walls consisting of 3–6 layers of medium brown *textura angularis*. **Conidiophores** hyaline, smooth, 0–1-septate, rarely branched, densely aggregated, cylindrical, straight to sinuous, 10 – 20×2 – 3μ m. **Conidiogenous cells** 6 – 10×1.5 – 3μ m, phialidic, cylindrical, terminal, with slight taper towards apex, 1 – 1.5μ m diam, with visible periclinal thickening; collarette not flared, minute. **Paraphyses** not observed. **Alpha conidia** aseptate, hyaline, smooth, guttulate, fusoid-ellipsoid to clavate, straight to variously curved, tapering towards both ends, straight, apex subobtuse, base truncate, (7) – 9 – 11 – $(13) \times (2.5)$ – 3μ m. **Gamma conidia** not observed. **Beta conidia** spindle-

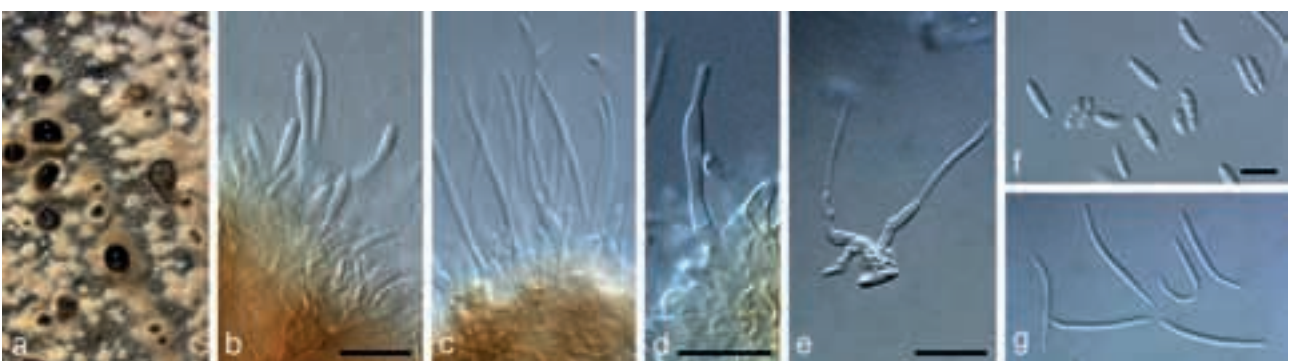


Fig. 13 *Diaporthe nomurai* (CBS 157.29). a. Conidiomata sporulating on PDA; b–e. conidiogenous cells; f. alpha conidia; g. beta conidia. — Scale bars = 10 μ m.

shaped, aseptate, smooth, hyaline, apex acutely rounded, base truncate, tapering from lower third towards apex, gently curved, $(20\text{--}25\text{--}27\text{--}30) \times 1.5\text{--}(2) \mu\text{m}$.

Culture characteristics — Colonies reaching up to 8 cm diam after 2 wk in the dark at 25 °C. On MEA surface isabelline, reverse sepia. On OA surface pale mouse grey with concentric rings of mouse grey; reverse mouse grey. On PDA surface and reverse fuscous-black, with sparse aerial mycelium.

Specimen examined. JAPAN, on *Morus* sp., Dec. 1929, K. Togashi (CBS 157.29).

Notes — Clade 58 represents *D. nomurai* from *Morus* sp. in Japan. *Diaporthe nomurai* is known from hosts such as *Morus alba*, *M. bombycis*, *M. latifolia* and *Morus* sp. (Farr & Rossman 2012).

Diaporthe novem J.M. Santos, Vrandečić & A.J.L. Phillips, *Persoonia* 27: 14. 2011

= *Phomopsis* sp. 9 van Rensburg et al., *Stud. Mycol.* 55: 65. 2006.

Specimens examined. BRAZIL, endophytic in petiole on *Maytenus ilicifolia*, July 2007, R.R. Gomes (LGMF 943 = CPC 20319). — CROATIA, Slavonija, in seed on *Glycine max*, Sept. 2008, T. Duvnjak (holotype CBS H-20462, ex-type cultures CBS 127270 = 4-27/3-1, CBS 127271 = 5/27/3-3, CBS 127269 = 5-27/3-1). — ROMANIA, Calugareni, Distr. Mizil, living leaves on *Polygonatum odoratum*, 31 July 1970, O. Constantinescu (CBS 354.71).

Notes — Clade 22 represents *D. novem* (Santos et al. 2011), and contains an endophytic isolate (LGMF 43) from *Maytenus ilicifolia*, one isolate previously identified as *Diaporthe pardalota* on *Polygonatum odoratum* from Romania, and three isolates of *D. novem* which includes the ex-type isolate. Isolate LGMF 943 represents higher genetic variation than the other isolates, and appears to represent a different species. Since this isolate did not sporulate, further morphological characterisation was not possible and we refrain from excluding it from the species pending collection of more strains to clarify its status.

Diaporthe novem was reported as pathogen of *Aspalathus linearis* (van Rensburg et al. 2006) as *Phomopsis* sp. 9. It was recently described as pathogen of *Glycine max* (Santos et al. 2011). This species was also reported on *Hydrangea macrophylla* (Santos et al. 2010), *Helianthus annuus* and *Vitis vinifera*

(Santos et al. 2011). It is known to occur in Brazil, Romania, Croatia, Italy (Rekab et al. 2004), Portugal (Santos et al. 2010) and South Africa (van Niekerk et al. 2005, van Rensburg et al. 2006).

Diaporthe oncostoma (Duby) Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 205. 1870. (1869–1870). — Fig. 14

Basionym. *Sphaeria oncostoma* Duby, in Rabenh., Klotzsch. *Herb. Vivum Mycol.*: no. 253. 1854.

Conidiomata pycnidial, globose to ellipsoidal, aggregated as well as scattered, dark brown to black, immersed, ostiolate, 430–1170 μm wide, 370–790 μm tall, lacking necks, with outer surface covered in brown hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose or exuding in cirrhi, white to pale luteous or pale yellow. *Conidiophores* hyaline, subcylindrical, branched above the septa, tapering towards the apex, 1–2-septate, $(10\text{--})11\text{--}19\text{--}(22) \times 3\text{--}(4) \mu\text{m}$. *Conidiogenous cells* hyaline, subcylindrical, straight or curved, tapering towards the apex, collarette not flared, periclinal thickening prominent, $(6\text{--})7\text{--}9\text{--}(10) \times (2\text{--})3 \mu\text{m}$. *Alpha conidia* hyaline, fusoid to ellipsoidal, straight to slightly curved, acute at apex, subobtuse at base, bi- or multi-guttulate, $(7.5\text{--})9\text{--}11\text{--}(12) \times (2\text{--})3\text{--}(4) \mu\text{m}$. *Gamma conidia* hyaline, smooth, ellipsoid-fusoid, apex acutely rounded, and tapering towards truncate base, $(11\text{--})12\text{--}16 \times 3\text{--}(3.5) \mu\text{m}$. *Beta conidia* and sexual morph not observed in culture.

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C. On MEA surface dirty white with profuse aerial mycelium, reverse umber. On OA surface dirty white with patches of umber, same in reverse. On PDA surface and reverse sienna, with sparse aerial mycelium.

Specimens examined. FRANCE, Hte Savoie, Aigueblanche-Bellecombe, outlet of river Morel in Isère, on dead branches of *Robinia pseudoacacia*, 17 July 1978, H.A. van der Aa (CBS 589.78). — GERMANY, Wolfenbüttel, on leaf spot of *Robinia pseudoacacia*, 15 Nov. 1996, H. Butin (CBS 100454); Berlin, on leaf of *Ilex aquifolium*, Nov. 1985, M. Hesse (CBS 809.85). — RUSSIA, on *Robinia pseudoacacia*, June 2000, L. Vasilyeva (CBS 109741 = AR 3445).

Notes — *Diaporthe oncostoma* (clade 70) has been considered to be a saprobic, or low virulence pathogen, which plays some role in natural pruning and self-thinning of black locust

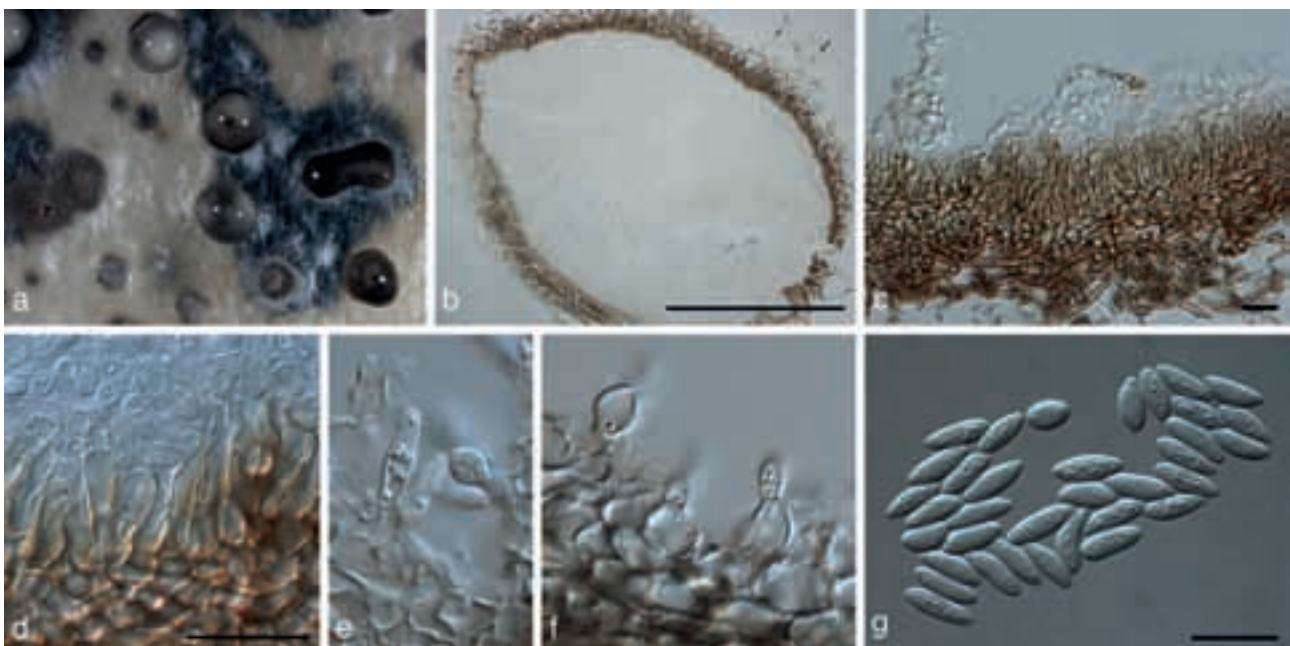


Fig. 14 *Diaporthe oncostoma* (CBS 100454). a. Conidiomata sporulating on OA; b, c. transverse section through conidiomata, showing conidiomatal wall; d–f. conidiogenous cells; g. alpha conidia. — Scale bars: b = 225 μm , all others = 10 μm .

forests (*Robinia pseudoacacia*) (Vajna 2002). However, this fungus has been reported as a causal agent of canker and severe dieback disease of black locust in Russia (Scerbin-Parfenenko 1953) and in Greece (Michalopoulos-Skarmoutsos & Skarmoutsos 1999).

Although isolate CBS 809.85 was obtained from *Ilex aquifolium* in Germany, we treat it as belonging to *D. oncostoma*, as it matches the other strains phylogenetically as well as morphologically.

Diaporthe oxo R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802941; Fig. 15

Etymology. The word 'oxe' is an expression used in northeastern Brazil that means amazement or surprise, in relation to the number of novel species isolated as endophytes from medicinal plants in Brazil.

Conidiomata pycnidial ampulliform to finger-like, eustromatic, convoluted to unilocular, semi-immersed, scattered, dark brown to black, ostiolate, 60–170 µm wide, 60–220 µm tall; necks variable in length, 20–150 µm, outer surface covered with hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose or exuding in cirrhi, pale-luteous to cream or pale-yellow. *Conidiophores* hyaline, ampulliform to subcylindrical, branched above the septa, tapering towards the apex, 1–2-septate, (14–)17–25(–27) × (2–)3 µm. *Conidiogenous cells* hyaline, subcylindrical, filiform, straight to curved, tapering towards the apex, collarette flared, periclinal thickening prominent, (5–)6–10(–12) × 2(–3) µm. *Alpha conidia* hyaline, oblong to ellipsoid, apex bluntly rounded, base obtuse to subtruncate, bi- to multi-guttulate, (5–)6–7(–8) × (2–)3 µm. *Beta conidia* hyaline, smooth, curved or hamate, (17–)22–30(–33) × 2–3 µm. *Gamma conidia* not observed.

Culture characteristics — Colonies on PDA flat, with an entire edge, surface mycelium dense and felty, ochreous to fulvous, dark brick, honey, buff, exudates rarely present as colourless drops; colonies reaching 49 mm diam after 2 wk at 25 °C; reverse umber, ochreous to fulvous. On OA flat, with an entire edge, surface mycelium dense and felty, rosy buff, pale olivaceous-grey, iron-grey, with patches olivaceous buff, exudates in colourless and pale luteous drops; colonies reaching 40 mm diam; reverse dark brick, olivaceous. On MEA raised, with an entire edge, surface mycelium dense and felty, buff,

rosy-buff, with chestnut coloured exudates in the centre of the colony, and pale luteous at the periphery; colonies reaching 49 mm diam; reverse chestnut and bay.

Specimens examined. BRAZIL, on petiole of *Maytenus ilicifolia*, July 2007, R.R. Gomes (holotype CBS H-21098, ex-type culture CBS 133186 = LGMF 942 = CPC 20318); same collection details (CBS 133187 = LGMF 936 = CPC 20312); on leaf of *Schinus terebinthifolius*, July 2007, J. Lima (LGMF 915 = CPC 20291); on petiole of *M. ilicifolia*, S.A.V. Pileggi (LGMF 939 = CPC 20315); on petiole of *M. ilicifolia*, July 2007, R.R. Gomes (LGMF 945 = CPC 20321).

Notes — Endophytic isolates (clade 34) from medicinal plants in Brazil.

Diaporthe padi* var. *padi G.H. Otth, Mitth. Naturf. Ges. Bern: 99. 1871 (1870)

Specimens examined. SWEDEN, Uppland, Dalby par., Tuna, on *Prunus padus*, 17 Apr. 1988, K. & L. Holm (CBS 114200 = UPSC 2569); Dalarna, Folkärna par., Sonnbo, on *Alnus glutinosa*, Dec. 1992, K. & L. Holm (CBS 114649 = UPSC 3496).

Notes — *Diaporthe padi* var. *padi* (clade 56) represents a European taxon occurring on *Prunus*. We chose the name *D. padi* over *D. decorticans*, as the basionym of the latter, *Sphaeria decorticans*, is an illegitimate homonym.

Diaporthe paranensis R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802942, Fig. 16

Etymology. Named after Paraná, the state in Brazil from where it was collected.

Conidiomata pycnidial, ampulliform, semi-immersed, scattered, brown to black, ostiolate, 130–220 µm wide, 60–130 µm tall; prominent necks 50–210 µm long, outer surface smooth or covered in hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose, predominantly pale-luteous to yellow and some cases green-olivaceous. *Conidiophores* hyaline, subcylindrical to cylindrical, filiform, branched above the septa on a globose cell, not tapering towards the apex, 2–3-septate, (14–)15–22(–26) × (2–)3(–4) µm. *Conidiogenous cells* hyaline, subcylindrical, filiform, rarely tapering towards the apex, collarette present and flared, slight periclinal thickening, (5–)8–14(–15) × 2(–3) µm.

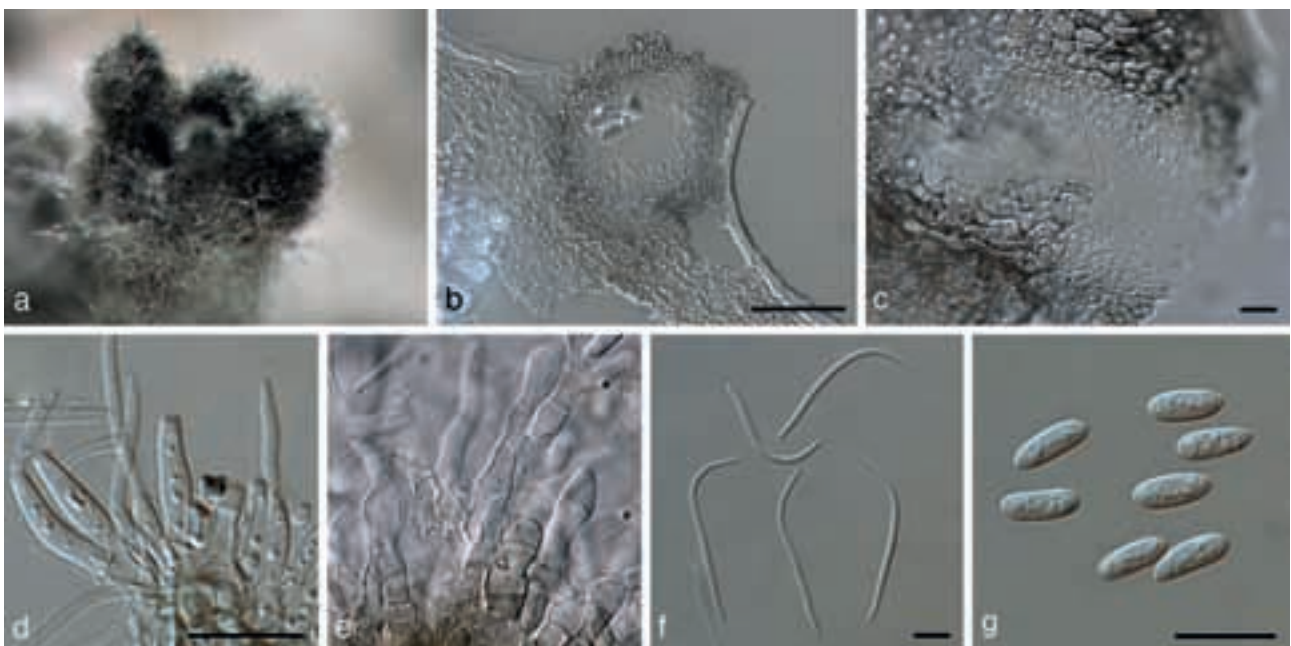


Fig. 15 *Diaporthe oxo* (CBS 133186). a. Conidiomata sporulating on PDA; b, c. transverse section through conidiomata, showing conidiomatal wall; d, e. conidiogenous cells; f. beta conidia; g. alpha conidia. — Scale bars: b = 100 µm, all others = 10 µm.

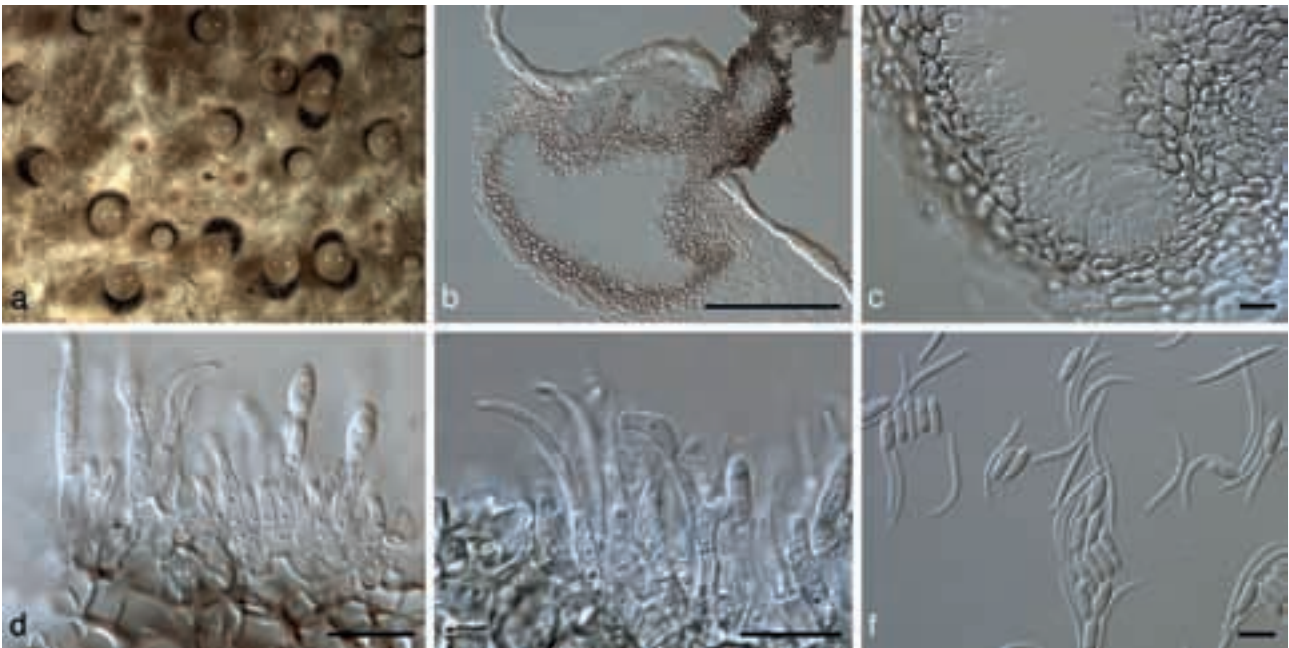


Fig. 16 *Diaporthe paranensis* (CBS 133184). a. Conidiomata sporulating on PDA; b, c. transverse section through conidiomata, showing conidiomatal wall; d, e. conidiogenous cells; f. alpha and beta conidia. — Scale bars: b = 100 µm, all others = 10 µm.

Alpha conidia hyaline, fusoid-ellipsoidal, apex bluntly rounded, base obtuse to subtruncate, bi- to multi-guttulate, (6–)7–8(–9) × (2–)3 µm. *Beta conidia* hyaline, smooth, curved or hamate and slightly curved, (16–)17–21(–23) × (1–)2 µm. *Gamma conidia* not observed.

Culture characteristics — Colonies on PDA flat, with an entire edge, mycelium growing in concentric rings, cottony texture, white to smoke-grey; colonies reaching up to 64 mm diam after 2 wk at 25 °C; reverse buff and isabelline. On OA flat, with an entire edge, aerial mycelium in concentric rings, ranging in colour from smoke-grey to grey-olivaceous and white in the centre; colonies reaching 44 mm diam; reverse iron-grey, grey-olivaceous to olivaceous-buff. On MEA flat, with an entire edge, aerial mycelium growing in concentric rings, with cottony texture, pale olivaceous-grey to grey-olivaceous and buff; colonies reaching 56 mm diam; reverse umber, fulvous with patches of greenish black.

Specimen examined. BRAZIL, Paraná, Colombo, endophytic species isolated from petiole of *Maytenus ilicifolia* (popular name Espinheira Santa), July 2007, R.R. Gomes (holotype CBS H-21099, ex-type culture CBS 133184 = LGMF 929 = CPC 20305).

Notes — Endophytic isolate (clade 35) from medicinal plant in Brazil.

Diaporthe perijuncta Niessl, Hedwigia 15: 153. 1876

Specimen examined. AUSTRIA, from *Ulmus glabra*, Oct. 2001, A.Y. Rossman (ex-epitype culture CBS 109745 = ARSEF 3461 = AR 3461).

Notes — *Diaporthe perijuncta* (clade 40) is associated with fallen branches of *Ulmus campestris* and *U. glabra* (*Ulmaceae*). This species is found in Austria, Germany and Portugal. *Diaporthe perijuncta* is distinguished from *D. viticola* and *D. australafricana* based on morphology and DNA sequence data (van Niekerk et al. 2005). Pathogenicity studies and endophytic isolation of ‘*D. perijuncta*’ from grapevines in Australia and South Africa in fact represent isolates of *D. australafricana* (Mostert et al. 2001a, Rawnsley et al. 2004, van Niekerk et al. 2005).

Diaporthe perseae (Zerova) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802944; Fig. 17

Basionym. *Phomopsis perseae* Zerova, J. Bot. Acad. Sci. RSS Ukraine 1, 1–2: 307. 1940.

Conidiomata pycnidial in culture on MEA, globose, up to 400 µm diam, black, erumpent; cream conidial droplets exuding from central ostioles; walls consisting of 3–6 layers of medium brown *textura angularis*. **Conidiophores** hyaline, smooth, 1–3-septate, branched, densely aggregated, cylindrical, straight to sinuous, 15–35 × 3–4 µm. **Conidiogenous cells** 8–17 × 1.5–2.5 µm,

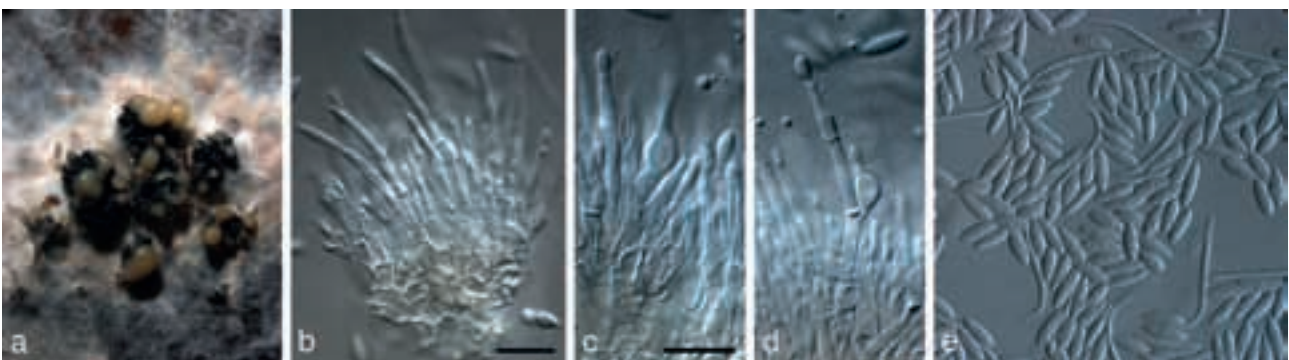


Fig. 17 *Diaporthe perseae* (CBS 151.73). a. Conidiomata sporulating on PDA; b–d. conidiogenous cells; e. alpha and beta conidia. — Scale bars = 10 µm.

phialidic, cylindrical, terminal and lateral, with slight taper towards apex, 1–1.5 µm diam, with visible periclinal thickening; collarette prominent, up to 5 µm long. *Paraphyses* hyaline, smooth, subcylindrical with obtuse ends, 2–4-septate, up to 60 µm long, 3 µm diam. *Alpha conidia* aseptate, hyaline, smooth, guttulate, fusoid to ellipsoid, tapering towards both ends, straight, apex subobtuse, base subtruncate, (6–)7–8(–9) × 2(–2.5) µm. *Gamma conidia* aseptate, hyaline, smooth, ellipsoid-fusoid, apex acutely rounded, base subtruncate, 9–14 × 1.5–2 µm. *Beta conidia* spindle-shaped, aseptate, smooth, hyaline, apex acutely rounded, base truncate, tapering from lower third towards apex, curved, (15–)22–25(–28) × 1.5(–2) µm.

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C, with moderate aerial mycelium. On OA surface ochreous, with patches of dirty white and iron-grey. On PDA surface dirty white with patches of sienna, reverse sienna with patches of umber. On MEA surface sienna, with patches of umber, reverse umber with patches of sienna.

Specimen examined. NETHERLANDS ANTILLES, Martinique, on young fruit of *Persea gratissima*, 10 July 1972, E. Laville (CBS 151.73).

Notes — *Diaporthe perseae* (clade 86) was originally described from branches of dying *Persea gratissima* trees in Russia. Based on the morphology (alpha conidia 7–10.2 × 2.3–2.5 µm; Uecker 1988), this strain could be authentic for the name.

Diaporthe phaseolorum (Cooke & Ellis) Sacc., Syll. Fung. 1: 692. 1882

Basionym. *Sphaeria phaseolorum* Cooke & Ellis, Grevillea 6, 39: 93. 1878.

Specimens examined. BRAZIL, endophytic in petiole on *Maytenus ilicifolia*, July 2007, R.R. Gomes (LGMF 927 = CPC 20303, LGMF 941 = CPC 20317). — NEW ZEALAND, from *Olearia cf. rani*, 22 Jan. 2003, G.J.M. Verkley (CBS 113425); *Actinidia chinensis*, rotting fruit, kiwifruit orchard, S.R. Pennycook (CBS 127465 = GJS 83-379). — UNKNOWN, Apr. 1980, L. Beraha (CBS 257.80). — USA, Mississippi, from *Caperonia palustris*, Oct. 2003, A. Mengistu (CBS 116019); Mississippi, from *Aster exilis*, Oct. 2003, A. Mengistu (CBS 116020).

Notes — Clade 4 represents isolates of *D. phaseolorum*. It includes two endophytic isolates from *Maytenus ilicifolia* collected in Brazil, one isolate previously misidentified as *D. melonis* (CBS 257.80), two isolates respectively from *Caperonia palustris* and *Aster exilis* in the USA (Mengistu et al. 2007), one isolate from *Olearia cf. rami*, and one from *Actinidia chinensis*. The ITS and TEF1 sequences of this clade are similar to sequences (GenBank U11323, U11373 and EU222020, respectively) of a well-characterised isolate of *D. phaseolorum* (ATCC 64802 = FAU458). By accepting this clade as authentic for

D. phaseolorum, we follow the precedent set by van Rensburg et al. (2006), Mengistu et al. (2007) and Santos et al. (2011).

Diaporthe pseudomangiferae R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802945; Fig. 18

Etymology. Named after its morphological similarity to *Phomopsis mangiferae*.

Conidiomata pycnidial, erumpent to superficial on PDA, globose, up to 300 µm diam with elongated necks with central ostioles that exude yellow-orange to cream conidial droplets; walls of 6–8 layers of brown *textura angularis*. *Conidiophores* hyaline, smooth, 1–3-septate, branched, densely aggregated, cylindrical, straight to sinuous, 20–30 × 2–2.5 µm. *Conidiogenous cells* phialidic, cylindrical, terminal and lateral with slight apical taper, 10–15 × 2–3 µm; collarette flared, up to 3 µm long. *Paraphyses* hyaline, smooth, cylindrical, septate, extending above conidiophores, straight to flexuous, unbranched or branched below, up to 80 µm long, 2–3 µm wide at base. *Alpha conidia* aseptate, hyaline, smooth, guttulate to granular, fusiform, tapering towards both ends, apex acutely rounded, base truncate, (6–)7–9(–10) × (2–)2.5(–3) µm. *Beta* and *gamma conidia* not seen (description based on CBS 101339).

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C, with moderate aerial mycelium. On OA surface and reverse dirty white with patches of iron-grey. On PDA surface dirty white to ochreous, reverse umber. On MEA surface greyish sepia with patches of iron-grey, reverse greyish sepia with patches of iron-grey.

Specimens examined. DOMINICAN REPUBLIC, from *Mangifera indica*, P. de Leeuw, ATO-DLO, Wageningen (holotype CBS H-21105, culture ex-type CBS 101339). — MEXICO, on fruit peel of *Mangifera indica* (CBS 388.89).

Notes — Although these isolates (clade 82) were originally described as representative of *P. mangiferae* (dead leaves of *Mangifera indica*, Pakistan), they differ in having larger conidiomata, longer conidiophores and larger alpha conidia.

Diaporthe pseudophoenicicola R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB803839; Fig. 19

Etymology. Named after its morphological similarity to *Diaporthe phoenicicola*.

Conidiomata pycnidial on MEA, up to 400 µm diam, erumpent, globose with neck; ostiole exuding yellow-orange conidial droplets; walls consisting of 3–6 layers of medium brown *textura angularis*. *Conidiophores* hyaline, smooth, densely aggregated, 1–3-septate, branched, cylindrical, straight to curved, 12–45 × 1.5–3 µm. *Conidiogenous cells* phialidic, cylindrical, terminal

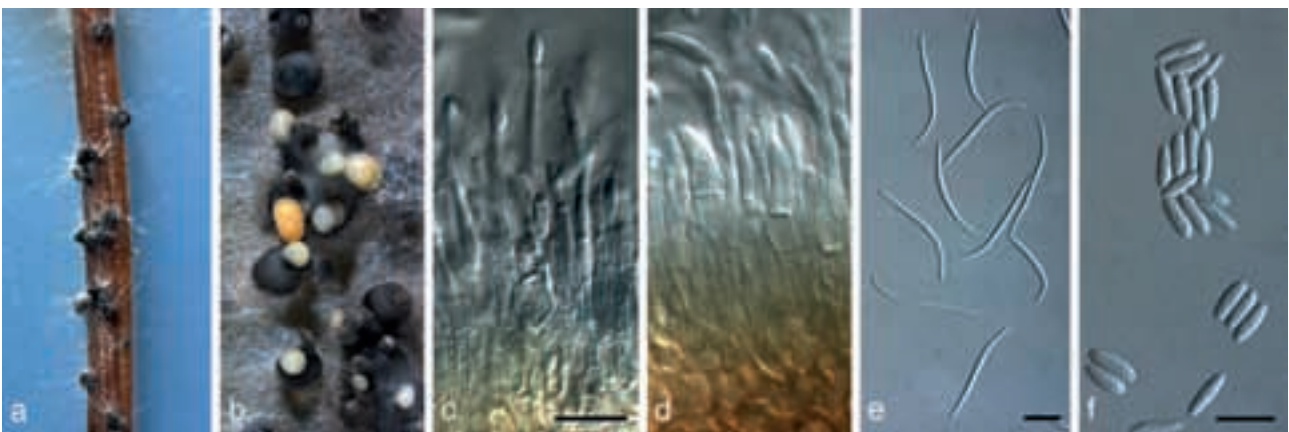


Fig. 18 *Diaporthe pseudomangiferae* (CBS 101339). a. Conidiomata sporulating on PNA; b. conidiomata sporulating on PDA; c, d. conidiogenous cells; e. beta conidia; f. alpha conidia. — Scale bars = 10 µm.

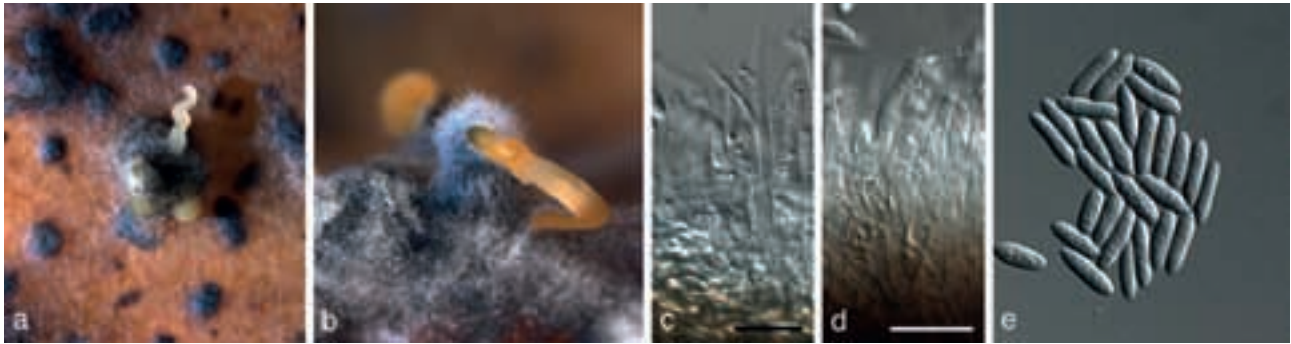


Fig. 19 *Diaporthe pseudophoenicicola* (CBS 462.69). a, b. Conidiomata sporulating on PDA; c, d. conidiogenous cells; e. alpha conidia. — Scale bars = 10 µm.

and lateral with slight apical taper, 12–20 × 1.5–2 µm, with visible periclinal thickening; collarete flared, 2–5 µm long. *Paraphyses* hyaline, smooth, cylindrical, 1–3-septate, extending above conidiophores, straight to flexuous, unbranched or branched, up to 100 µm long, and 3 µm wide at base. *Alpha conidia* aseptate, hyaline, granular, smooth, fusiform, tapering towards both ends, straight, acutely rounded apex, and truncate base, (6–)7–8(–9) × (2–)2.5(–3) µm. *Beta* and *gamma conidia* not seen (description based on CBS 462.69).

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C, with sparse aerial mycelium. On MEA surface dirty white with patches of sienna, reverse umber with patches of sienna. On OA surface dirty white with patches of sienna. On PDA surface ochreous with patches of olivaceous-grey, reverse iron-grey with patches of ochreous.

Specimens examined. IRAQ, Prov. Basrah, Shalt El Arab, showing dieback on *Mangifera indica*, 1976, M.S.A. Al-Momen (CBS 176.77). — SPAIN, Mallorca, Can Pastilla, dead tops of green leaves on *Phoenix dactylifera*, 27 May 1969, H.A. van der Aa (holotype CBS H-21106, culture ex-type CBS 462.69).

Notes — *Diaporthe pseudophoenicicola* (clade 89) is distinct from *D. phoenicicola* (conidia 8–12 × 2–2.5 µm; Uecker 1988) by having shorter, and wider alpha conidia. A similar strain was isolated from *Mangifera indica* in Iraq (CBS 176.77), suggesting that this species has a wider host range.

***Diaporthe pustulata* Sacc., Syll. Fung. (Abellini) 1: 610. 1882**

Specimens examined. AUSTRIA, on *Acer pseudoplatanus*, Oct. 2001, A.Y. Rossman (CBS 109742 = AR 3430 and CBS 109760 = AR 3535); Raab, Au Wald, on *Prunus padus*, Oct. 2001, A.Y. Rossman (CBS 109784 = AR 3419).

Notes — Clade 44 contains one isolate from *Prunus padus* and two isolates from *Acer pseudoplatanus*, all isolated from Austria. Clade 56 contains another isolate on *Prunus padus* from Sweden. Clearly there are two different species from *Prunus*, one isolated in Austria and another in Sweden. Because *D. pustulata* was originally described on *Acer pseudoplatanus*, we tentatively apply this name to isolates in clade 44. To clarify the status of isolates in clades 44 and 56, however, additional isolates and a comparison with type materials would be required.

***Diaporthe raonikayaporum* R.R. Gomes, C. Glienke & Crous, sp. nov. — MycoBank MB802947; Fig. 20**

Etymology. *Raoni* + *Kayapo* = after the name of a leader (Raoni) of the indigenous Kayapo ethnic tribe in Brazil. The Kayapos are inhabitants of the Amazon region in Brazil. They use the medicinal plant *Spondias mombin*, from which this species was isolated, as adornment or ornament, and for its medicinal properties.

Conidiomata pycnidial, globose to conical or ampulliform, eustromatic and convoluted or unilocular, scattered, dark brown to black, immersed, ostiolate, 110–200 µm wide, 50–130 µm tall,

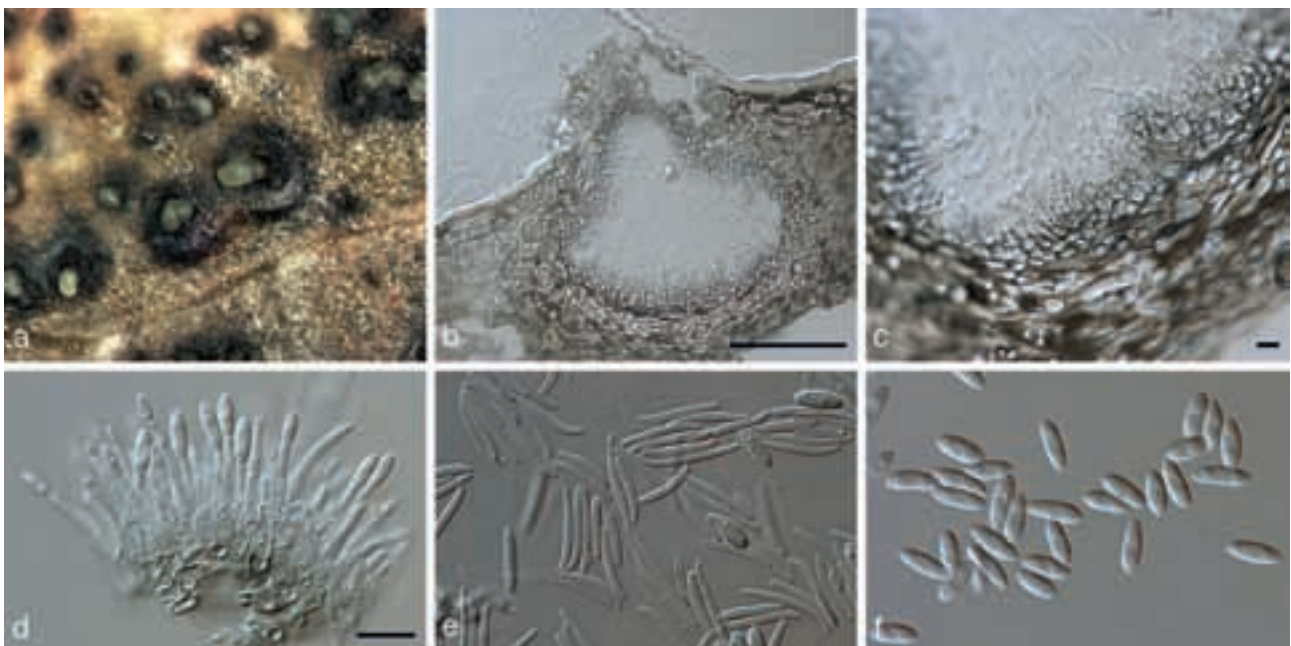


Fig. 20 *Diaporthe raonikayaporum* (CBS 133182). a. Conidiomata sporulating on PNA; b, c. transverse section through conidiomata, showing conidiomatal wall; d. conidiogenous cells; e. beta with a few alpha conidia; f. alpha conidia. — Scale bars: b = 100 µm, all others = 10 µm.

with prominent necks 40–140 μm long, outer surface smooth or covered in hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose or exuding in cirrhi, white to pale-luteous. *Conidiophores* hyaline, ampulliform to subcylindrical, filiform, branched above the septa, tapering towards the apex, 1–3-septate, (16–)17–22(–26) \times (2–)3 μm . *Conidiogenous cells* hyaline, subcylindrical, filiform, straight to curved, tapering towards the apex, collarette not flared, periclinal thickening prominent, (5–)7–9(–10) \times (2–)3 μm . *Alpha* and *gamma* conidia are formed in the same conidiogenous cells. *Alpha* conidia hyaline, oblong to ellipsoid, apex bluntly rounded, base obtuse to subtruncate, bi- to multi-guttulate, (6–)7(–8) \times (2–)3 μm . *Beta* conidia not observed. *Gamma* conidia hyaline, fusoid to subcylindrical, slightly curved, apex bluntly rounded, base obtuse to subtruncate, bi- to multi-guttulate, or eguttulate, (7–)9–11(–13) \times (1–)2 μm .

Culture characteristics — Colonies on PDA flat, with an entire edge, aerial mycelium forming concentric rings with cottony texture, olivaceous-buff, isabelline to honey on surface; colonies reaching 63 mm diam after 2 wk at 25 °C; reverse pale purplish grey to smoke-grey. On OA flat, with an entire edge, aerial mycelium forming concentric rings, white, olivaceous on surface, colonies reaching 31 mm diam; reverse buff and greenish olivaceous. On MEA flat, with a lobate edge, aerial mycelium forming wooly concentric rings, olivaceous-grey, greenish olivaceous and patches of amber on surface, colonies reaching 51 mm diam; reverse brown-vinaceous.

Specimen examined. BRAZIL, Pará, Redenção, endophytic species isolated from leaf of *Spondias mombin* (popular name Cajazeira and Taperebá), July 2007, K. Rodriguez (holotype CBS H-21097, ex-type culture CBS 133182 = LGMF 923 = CPC 20299).

Notes — Endophytic isolate (clade 31) from medicinal plant in Brazil.

Diaporthe rhoina Feltgen, Vorstud. Pilzfl. Luxemb., Nachtr. III: 145. 1903

Specimen examined. UNKNOWN, on *Rhus toxicodendron*, Sept. 1927, L.E. Wehmeyer (CBS 146.27).

Notes — This species (clade 95) was originally described on *Rhus typhina* from Luxembourg. European isolates of this

pathogen will need to be collected to confirm the identity of CBS 146.27, which is presumably of North American origin.

Diaporthe saccharata (J.C. Kang, L. Mostert & Crous) Crous, *comb. nov.* — MB802948

Basionym. *Phomopsis saccharata* J.C. Kang, L. Mostert & Crous, *Sydowia* 53, 2: 230. 2001.

Specimen examined. SOUTH AFRICA, Western Cape Province, Jonkershoek Mountains, Stellenbosch, on cankers of *Protea repens*, Mar. 1999, S. Denman (ex-type culture CBS 116311 = CPC 3743).

Note — *Diaporthe saccharata* (clade 71) is known to cause a canker disease on shoots of *Protea repens* in South Africa (Mostert et al. 2001b).

Diaporthe schini R.R. Gomes, C. Glienke & Crous, *sp. nov.* — MycoBank MB802949; Fig. 21

Etymology. Named after the host genus from which it was isolated, *Schinus*.

Conidiomata pycnidial, eustromatic, multilocular, immersed to erumpent, ostiolate, dark brown to black, scattered or aggregated, 80–270 μm wide, 70–240 μm tall, prominent necks 70–220 μm long, outer surface covered with hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; conidial mass globose, pale-luteous to cream. *Conidiophores* hyaline, subcylindrical, filiform, rarely branched, tapering towards the apex, 0–1-septate, (11–)12–17(–20) \times (2–)3(–4) μm . *Conidiogenous cells* hyaline, subcylindrical and filiform, straight, tapering towards the apex, collarette not observed, with prominent periclinal thickening 5–6(–7) \times (1–)2 μm . *Beta* conidia hyaline, smooth, curved or hamate (14–)22–28(–30) \times (1–)2 μm . *Alpha* and *gamma* conidia not observed.

Culture characteristics — Colonies on PDA flat, with a lobate margin, surface mycelium sparse, felty and appressed, buff, honey to isabelline; colonies reaching 30 mm diam after 2 wk at 25 °C; reverse greyish sepia, smoke-grey. On OA with a lobate margin, surface mycelium flat, sparse, felty and appressed, smoke-grey, olivaceous-grey, or olivaceous buff; colonies reaching 21 mm diam; reverse pale mouse-grey to

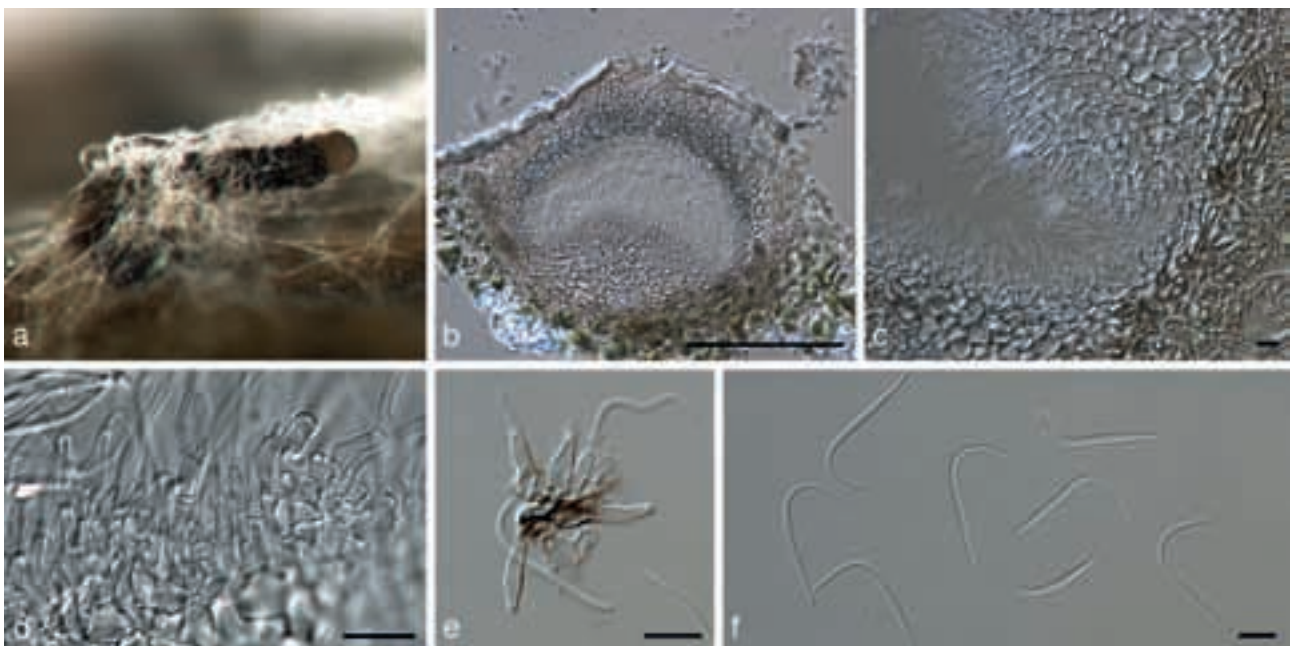


Fig. 21 *Diaporthe schini* (CBS 133181). a. Conidiomata sporulating on PDA; b, c. transverse section through conidiomata, showing conidiomatal wall; d, e. conidiogenous cells; f. beta conidia. — Scale bars: b = 135 μm , all others = 10 μm .

olivaceous-grey or buff. On MEA with a lobate margin, surface mycelium flat, dense, felty and appressed, buff with umber patches; colonies reaching 30 mm diam; reverse dark mouse-grey, umber, with patches of isabelline or luteous.

Specimen examined. BRAZIL, Paraná, Curitiba, endophytic species isolated from leaf of *Schinus terebinthifolius* (popular name Aroeira), July 2007, J. Lima (holotype CBS H-21093, culture ex-type CBS 133181 = LGMF 921 = CPC 20297); same collection details (LGMF 910).

Notes — Other than *D. schini* (clade 11), additional endophytic isolates were also obtained from *Schinus terebinthifolius* in Brazil, but these are morphologically different and cluster in clades 5 and 9 (*D. endophytica* and *D. terebinthifolii*).

Diaporthe sclerotioides (Kesteren) Udayanga, Crous & K.D. Hyde, Fung. Diversity 56: 166. 2012

Basionym. *Phomopsis sclerotioides* Kesteren, Neth. J. Pl. Path. 73: 115. 1967.

Specimens examined. NETHERLANDS, Maarssen, on root of *Cucumis sativus*, June 1967, H.A. van der Kesteren (ex-type culture CBS 296.67 = ATCC 18585 = IMI 151828 = PD 68/690); Roermond, on root of *C. sativus*, Dec. 1976 (CBS 710.76 = PD 76/674).

Notes — *Diaporthe sclerotioides* (clade 28) was originally described from roots of *Cucumis sativus* in the Netherlands. This species has subsequently been reported to cause black root rot of *Citrullus lanatus*, *Cucumis sativus*, *C. ficifolia*, *C. maxima* and *C. moschata* in various countries in the world (Udayanga et al. 2011).

Diaporthe scobina Nitschke, Pyrenomyces Germanici 2: 293. 1870

= *Phomopsis scobina* Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 115: 681 (33 of repr.). 1906.

Specimen examined. SCOTLAND, living and dead twig of *Fraxinus excelsior*, Feb. 1938, J.A. MacDonald (CBS 251.38).

Notes — Clade 38 is represented by *D. scobina* isolated from *Fraxinus excelsior* in Scotland. The fungus is known on this host from Scotland and Poland (Mulenko et al. 2008, Farr & Rossman 2012).

Diaporthe sojae Lehman, Ann. Missouri Bot. Gard. 10: 128. 1923

= *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehm., The genus *Diaporthe* Nitschke and its segregates 47: 1933.

= *Phomopsis longicolla* Hobbs, Mycologia 77: 542. 1985.

= *Diaporthe longicolla* (Hobbs) J.M. Santos, Vrandečić & A.J.L. Phillips, Persoonia 27: 13. 2011.

Specimens examined. CROATIA, on *Glycine max* stem, Sept. 2005, K. Vrandečić (specimen CBS H-20460, culture CBS 127267). — ITALY, Bologna, from *Glycine soja*, 1986, P. Giunchi (specimen CBS H-16776, culture CBS 100.87). — UNKNOWN, on *Glycine soja* ('Blackhawk') mature stem, A.A. Hildebrand (CBS 180.55 = ATCC 12050 = CECT 2024). — USA, Mississippi, from *Euphorbia nutans*, A. Mengistu (CBS 116017 = DP 0508) and from *Glycine max*, Oct. 2003, A. Mengistu (CBS 116023); on *Glycine soja* seedling, J. Marcinkowska (CBS 659.78 = NRRL 13656).

Notes — Isolates of *D. phaseolorum* var. *sojae* clustered in two distinct clades (clade 1, *D. sojae*; clade 7, *D. melonis*). *Diaporthe sojae* causes pod and stem blight of soybean, while *P. longicolla* is known to cause seed decay (Santos et al. 2011). Several authors have found it difficult to distinguish them based on disease symptoms alone, and usually report them together (Almeida & Seixas 2010). Hobbs et al. (1985) described *P. longicolla* as a different species to *D. sojae* (*Diaporthe phaseolorum* var. *sojae*) based on morphological characters. Both symptom types, however, have also been linked to the same species

(Kulik 1984, Morgan-Jones 1989, Kulik & Sinclair 1999). Considering their genetic similarity based on the five genes studied here, disease etiology and common host, it appears that these isolates belong to the same species, which is distinct from *D. phaseolorum* (clade 4). *Diaporthe sojae* (clade 1) is an older name than *D. longicolla*, and is therefore applied to this clade.

***Diaporthe* sp. 1**

Specimens examined. BRAZIL, EMBRAPA/PR, on *Glycine max* seed, A. Almeida (LGMF 947 = CPC 20323). — GERMANY, Bielefeld, human abscess, K. Plechulla (CBS 119639 = B 11861).

Notes — Isolates from clade 2 appear to represent a novel species, *Diaporthe* sp. 1 (sterile). It is represented by CBS 119639, isolated from an abscess of a male patient in Germany, and isolate LGMF 947, obtained from soybean seeds in Brazil. Isolates from this clade share a low genetic homology to isolates of the clade 4 (*D. phaseolorum*; Fig. 1, part 1).

Diaporthe species commonly described from soybean were also reported as opportunistic human pathogens. In 1999, a species of *Phomopsis* was reported as etiological agent of a subcutaneous infection on the finger of an immunosuppressed farmer and this genus was added to the list of fungi capable to cause human disease (Sutton et al. 1999). In 2011, *D. sojae* (as *Phomopsis longicolla*), a known pathogen of soybean, was identified as causing skin infection in an immunocompromised patient after kidney transplantation. The authors believed that this patient acquired the fungus at least 5 yr before, when he had contact with seeds or soybean plants in Equatorial Guinea (Garcia-Reyne et al. 2011).

Another phytopathogenic species also described in soybean, *Diaporthe phaseolorum*, was reported causing osteomyelitis in patients with positive serology for human lymphotropic virus type 1 (HTLV-1), disturbing the immune response. The patient was a farmer and inoculation occurred possibly through injury with *Amaranthus spinosus* thorns (Iriart et al. 2011).

***Diaporthe* sp. 2**

Specimen examined. BRAZIL, on petiole of *Maytenus ilicifolia*, July 2007, R.R. Gomes (LGMF 932 = CPC 20308).

Notes — Sterile, endophytic isolate from medicinal plant in Brazil, which appears to represent a novel species (clade 29).

***Diaporthe* sp. 3**

Specimen examined. SCOTLAND, on *Pseudotsuga menziesii*, Mar. 1929, G.G. Hahn (CBS 287.29).

Notes — Clade 32 was tentatively named *Diaporthe* sp. 3, and is represented by a single isolate previously identified as *Phomopsis conorum*, and obtained from *Pseudotsuga menziesii* in Scotland. This clade was not resolved, because there are at least eight different conifer species without any ex-type cultures (Udayanga et al. 2011).

***Diaporthe* sp. 4**

Specimen examined. BRAZIL, endophytic in petiole on *Maytenus ilicifolia*, July 2007, R.R. Gomes (LGMF 944 = CPC 20320).

Notes — Sterile endophytic isolate (clade 33) from a medicinal plant in Brazil, appearing to represent an undescribed species.

Diaporthe sp. 5

Specimen examined. ITALY, from *Acer opalus*, W. Jaklitsch (CBS 125575).

Notes — This isolate (clade 37) represents a novel species occurring on *Acer*, which will be treated separately as part of another study (W. Jaklitsch, pers comm.).

Diaporthe sp. 6

Specimens examined. HONG KONG, University Drive, on fruit of *Maesa perlaris*, 18 Dec. 2000, K.D. Hyde (CBS 115595 = HKUCC 10129, CBS 115584 = HKUCC 7784).

Notes — The two strains (clade 85) studied here were originally identified as *P. pittospori* (described from *Pittosporum* twigs, USA, California), which seems highly unlikely, as they were isolated from fruit of *Maesa perlaris* in Hong Kong. Unfortunately both strains proved to be sterile, so their identity could not be confirmed.

Diaporthe sp. 7

Specimen examined. INDIA, Bangalore, on *Anacardium occidentale*, Aug. 1978, H.C. Govindu (CBS 458.78).

Notes — The identity of the present isolate (identified as *Phomopsis anacardii*) could not be confirmed, as the culture proved to be sterile. However, phylogenetically (clade 88) it represents a distinct taxon from *D. anacardii* (clade 69), and when recollected, should be described as new.

Diaporthe sp. 8

Specimen examined. BRAZIL, from *Aspidosperma tomentosum*, K. Rodriguez (LGMF 925 = CPC 20301).

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C, with moderate aerial mycelium. On PDA surface ochreous, reverse pale luteous. On OA surface and reverse luteous. On MEA surface pale luteous, reverse orange to apricot.

Notes — Although this isolate (clade 90) appears to represent an undescribed species based on phylogenetic data, it proved to be sterile. As we presently only have a single strain of this taxon, its treatment will have to await further collections.

Diaporthe stictica (Berk. & Broome) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802950

Basionym. *Phoma stictica* Berk. & Broome, Ann. Mag. Nat. Hist., ser. II 5: 370. 1850.

≡ *Phomopsis stictica* (Berk. & Broome) Traverso, Fl. Ital. Crypt. 2, 1: 276. 1906.

Specimen examined. ITALY, Perugia, on dead twig of *Buxus sempervirens*, Dec. 1954, M. Ribaldi (CBS 370.54).

Notes — *Diaporthe stictica* (clade 74) represents a European species occurring on *Buxus sempervirens* (Italy, Germany). Although the present isolate could be authentic for the name, this could not be confirmed based on morphology, as the isolate proved to be sterile.

Diaporthe subordinaria (Desm.) R.R. Gomes, C. Glienke & Crous, *comb. nov.* — MycoBank MB802951

Basionym. *Phoma subordinaria* Desm., Ann. Sci. Nat., Bot. ser. 3, 9: 284. 1849.

≡ *Phomopsis subordinaria* (Desm.) Traverso, Fl. Ital. Crypt. Pars 1: Fungi. Pyrenomycetaceae. Xylariaceae, Valsaceae, Ceratostomataceae: 232. 1906.

Specimens examined. NEW ZEALAND, blackened seed of *Plantago lanceolata*, Apr. 1999, B. Alexander (CBS 101711). — SOUTH AFRICA, Eastern Cape Province, Grahamstown, on stalks of *Plantago lanceolata*, 2 Dec. 1989, R. Shivas (CBS 464.90).

Notes — *Diaporthe subordinaria* (clade 18) has a global distribution on *Plantago lanceolata*, on which it causes a stalk disease (de Nooij & van der Aa 1987). It is possible that the disease relates to several different species occurring on *Plantago*, but this matter can only be resolved following further collections and correlation with type material.

Diaporthe tecomae Sacc. & P. Syd., Syll. Fung. 14: 550. 1899 (nom. nov. for *D. interrupta* Niessl). — Fig. 22

?= *Phoma tecomae* Sacc., Nuovo Giorn. Bot. Ital. 8: 201. 1876.

≡ *Phomopsis tecomae* (Sacc.) Traverso & Spessa, Bol. Soc. Brot. Coimbra, sér. 1, 25: 124. 1910.

Conidiomata pycnidial, sporulating poorly on OA, globose, up to 1 mm diam, black, erumpent, multilocular; cream conidial droplets exuding from central ostioles; walls consisting of 3–6 layers of medium brown *textura angularis*. **Conidiophores** hyaline in upper region, pale brown at base, smooth, 1–3-septate, branched, densely aggregated, cylindrical, straight to sinuous, 20–30 × 2–3 µm. **Conidiogenous cells** 8–15 × 1.5–3 µm, phialidic, cylindrical, terminal and lateral, with slight taper towards apex, 1 µm diam, with visible periclinal thickening; collarette not flared, minute. **Paraphyses** not observed. **Beta conidia** spindle-shaped, aseptate, smooth, hyaline, apex acutely rounded, base truncate, tapering from lower third towards apex, apex strongly curved, (17–)22–24(–26) × 1.5(–2) µm. **Alpha and gamma conidia** not observed.

Culture characteristics — Colonies covering dish after 2 wk in the dark at 25 °C. On OA fluffy, dirty white with patches of grey olivaceous. On PDA dirty white with patches of olivaceous grey and isabelline, reverse with patches of dirty white, brown vinaceous and dark brick. On MEA dirty white with patches of isabelline and olivaceous grey, reverse brown vinaceous with patches of dark brick.

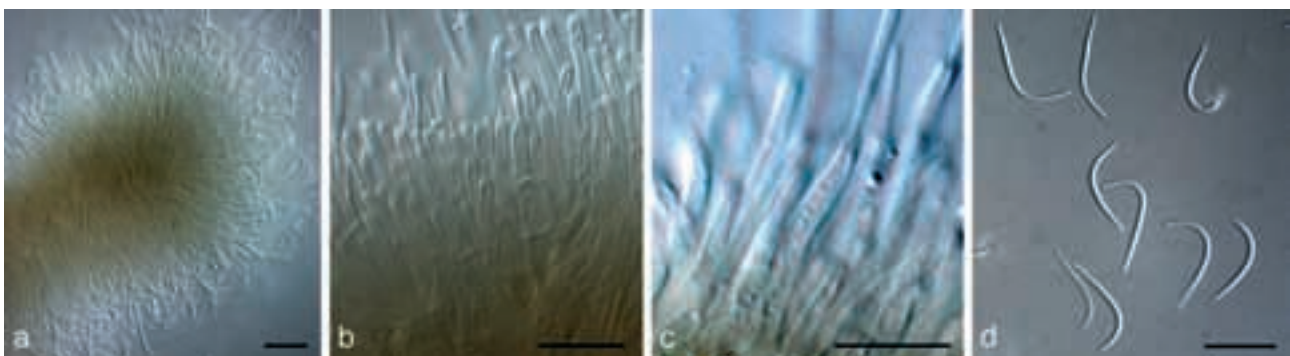


Fig. 22 *Diaporthe tecomae* (CBS 100547). a. Conidiomata forming on PNA; b, c. conidiogenous cells; d. beta conidia. — Scale bars = 10 µm.

Specimen examined. BRAZIL, Sao Paulo, Serra da Mantiqueira, mycocecidium caused by *Prosopodium tecomicola* on living young branch of *Tabebuia* sp., 27 Sept. 1997, coll. A. Aptroot, isol. H.A. van der Aa (specimen CBS H-16834, culture CBS 100547).

Notes — *Diaporthe tecomae* was a new name proposed for *D. interrupta* Niessl (on *Tecoma radicans*, Portugal), as the epithet was already occupied. The link between the *Diaporthe* and *Phomopsis* state remains to be proven. The asexual morph was originally described as *Phoma tecomae* (from Italy on *Tecoma radicans*, conidiophores $20 \times 1 \mu\text{m}$, conidia $8 \times 3 \mu\text{m}$; Saccardo 1878), and is probably distinct from the fungus represented by CBS 100547, which occurs on *Tabebuia* sp. in Brazil. However, as no ex-type strains are available of *D. tecomae* (clade 10), and no alpha conidia were observed in culture, this could not be confirmed, and is pending fresh collections.

Diaporthe terebinthifolii R.R. Gomes, C. Glienke & Crous, sp. nov. — MycoBank MB802952; Fig. 23

Etymology. Named after the host species from which it was isolated, *Schinus terebinthifolius*.

Conidiomata pycnidial, globose to conical, immersed, ostiolate, brown to black, scattered or aggregated, 95–110 μm wide, 140–160 μm tall, rarely forms necks, but when present, they are short and covered with hyphae; pycnidial wall consisting of brown, thick-walled cells of *textura angularis*; *conidial mass* globose, white or pale-luteous to cream. *Conidiophores* hyaline, subcylindrical, filiform, branched above septa, tapering towards the apex, 1–2-septate, $(13\text{--}15\text{--}21\text{--}22) \times 2\text{--}(3) \mu\text{m}$. *Beta conidiogenous cells* hyaline, ampulliform to subcylindrical and filiform, tapering towards the apex, collarette present and not flared, slight periclinal thickening, $(3\text{--}6\text{--}10\text{--}14) \times 2\text{--}(3) \mu\text{m}$. *Beta conidia* hyaline, smooth, curved or hamate, $(18\text{--})20\text{--}24\text{--}26) \times 1\text{--}(2) \mu\text{m}$. *Alfa* and *gamma conidia* not observed.

Culture characteristics — Colonies on PDA flat, with an entire edge, aerial mycelium cottony, greyish white, colonies reaching 64 mm diam after 2 wk in the dark at 25 °C; reverse buff. On OA flat, entire edge, aerial mycelium cottony, with concentric rings, pale olivaceous-grey, smoke-grey and greyish white, colonies

reaching 48 mm diam; reverse olivaceous-grey and olivaceous buff. On MEA flat, with an entire edge; aerial mycelium cottony, smoke-grey, colonies reaching 60 mm diam; reverse umber with patches of fuscous-black.

Specimens examined. BRAZIL, Paraná, Curitiba, endophytic species isolated from leaf of *Schinus terebinthifolius* (popular name Aroeira), July 2007, J. Lima (holotype CBS H-21097, ex-type culture CBS 133180 = LGMF 914 = CPC 20290); same collection details (LGMF 909 = CPC 20285, LGMF 907 = CPC 20283, LGMF 913 = CPC 20289).

Notes — The multigene analysis of isolates in clade 9 exhibited insignificant homology to sequences found in GenBank. An isolate previously identified as *Phomopsis tecomae* (CBS 100547) also resides in this clade, but is morphologically distinct. No morphologically similar isolates are known from *S. terebinthifolius*, and thus we designate these isolates as representative of a new taxon.

Diaporthe toxica P.M. Will., Highet, W. Gams & Sivasith., Mycol. Res. 98: 1367. 1994

Specimens examined. WESTERN AUSTRALIA, Morawa, on stem of *Lupinus angustifolius*, 6 May 1991, J.B. Nunn (ex-type culture CBS 534.93 = ATCC 96741); Serpentine, on *Lupinus* sp., 8 June 1993, P.M. Williamson (CBS 535.93); Medina, on *Lupinus* sp., 8 June 1993, P.M. Williamson (CBS 546.93).

Notes — Clade 45 contains three isolates of *D. toxica*, including the ex-type culture (CBS 534.93), isolated from *Lupinus angustifolius* in Western Australia. Two varieties of *Phomopsis* (*P. leptostromiformis* var. *leptostromiformis* and *P. leptostromiformis* var. *occidentalis*) were identified as causing disease in *Lupinus* sp. *Diaporthe woodii* was later recognised as the sexual state of *P. leptostromiformis* var. *occidentalis* (Punithalingam 1974), while Williamson et al. (1994) designated the name *D. toxica* for the sexual state of the toxicogenic variety, *P. leptostromiformis* var. *leptostromiformis*.

Lupins (*Lupinus* spp.) are grown in many parts of the world as a grain legume crop. The seeds are used for animal feed and increasingly as flour for human consumption. The plants increase soil nitrogen and are grown in rotation with other crops.

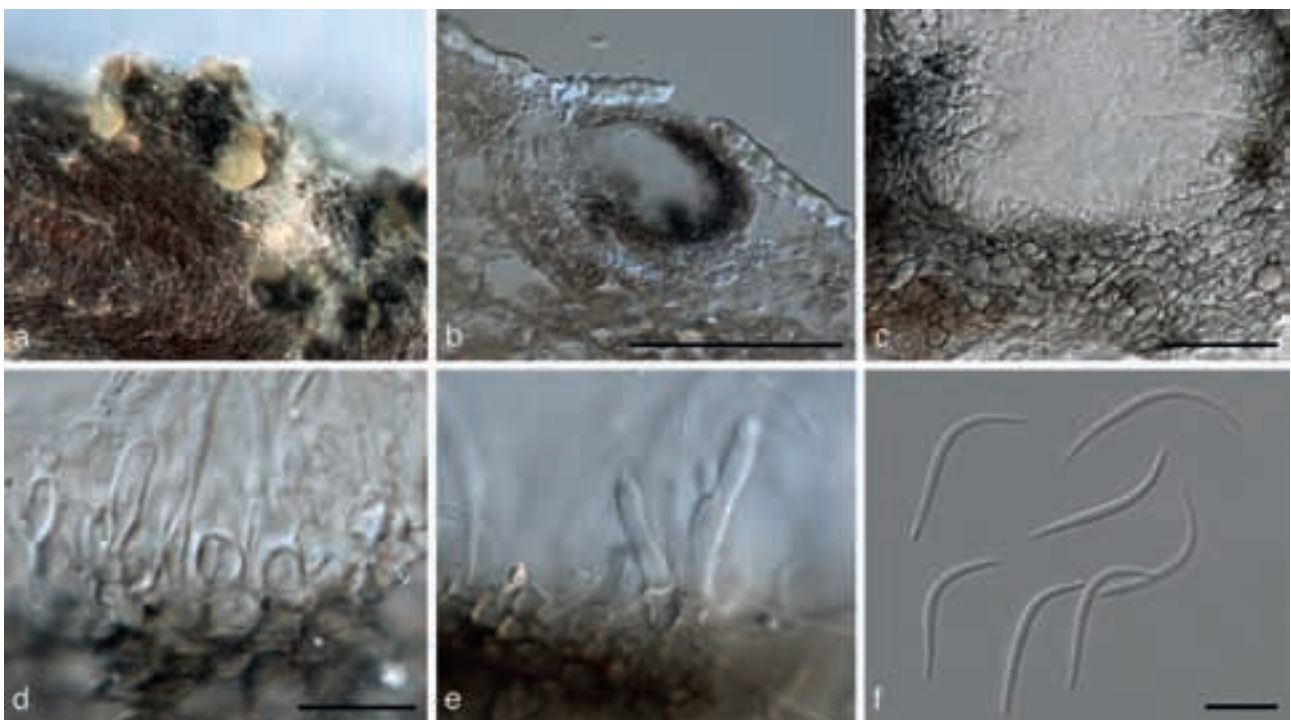


Fig. 23 *Diaporthe terebinthifolii* (CBS 133180). a. Conidiomata sporulating on PNA; b, c. transverse section through conidiomata, showing conidiomatal wall; d, e. conidiogenous cells; f. beta conidia. — Scale bars: b = 100 μm , c = 25 μm , all others = 10 μm .

In Australia the stubble left after harvesting aids soil conservation and is a valuable summer feed for livestock. *Diaporthe toxica* is considered to be an important limiting factor to more extensive sowing of lupins. This organism has been reported to cause stem blight in young lupins (*Lupinus luteus*) (Ostazeski & Wells 1960) and to produce phomopsins (Culvenor et al. 1977). These mycotoxins cause the animal liver disease known as lupinosis (Gardiner 1975, Allen & Wood 1979).

Diaporthe vaccinii Shear, U.S. Dept. Agric. Tech. Bull. 258: 7. 1931

= *Phomopsis vaccinii* Shear, U.S. Dept. Agric. Tech. Bull. 258: 7. 1931.

Specimens examined. USA, Massachusetts, on *Oxycoccus macrocarpos*, Mar. 1932, C.L. Shear (ex-type culture CBS 160.32 = IFO 32646); Michigan, on *Vaccinium corymbosum*, G.C. Adams (CBS 118571); New Jersey, on *V. macrocarpon*, 1988, L. Carris (CBS 122112 = FAU 474); Michigan, on *V. corymbosum*, 1992, D.C. Ramsdell (CBS 122114 = FAU 634, CBS 122115 = FAU 590); North Carolina, from *V. corymbosum*, pre-1999, D.F. Farr (CBS 122116 = DF 5022).

Notes — Clade 63 consists of six isolates of *D. vaccinii*, including the ex-type strain (CBS 160.32) isolated on *Vaccinium corymbosum* from the USA. *Diaporthe vaccinii* causes fruit rot and twig blight and leaf spots of *Vaccinium* spp. (blueberries) in the USA (Alfieri et al. 1984, Farr et al. 2002, Farr & Rossman 2012). The principal hosts are American and European cranberries (*Vaccinium macrocarpon*, *V. oxycoccus*, *V. oxycoccus* var. *intermedium*), highbush blueberry (*V. corymbosum*) and rabbiteye blueberry (*V. ashei*). *Diaporthe vaccinii* is restricted to cultivated *Vaccinium* species. The wild European species, *V. oxycoccus*, which usually occurs in mountain bogs, could be a potential reservoir for the pest. In the EPPO region it has been reported from Romania (found in experimental plots of introduced American cultivars, but did not establish (Teodorescu et al. 1985)), UK (found in plants originally imported from the Netherlands and USA, but did not establish (Wilcox & Falconer 1961, Baker 1972)).

Symptoms in susceptible blueberry cultivars include blighting of 1-yr-old woody stems with flower buds. Infected succulent, current-year shoots wilt in 4 d and become covered with minute lesions. The fungus continues to travel downward through the stem, killing major branches, and often entire plants (Wilcox 1939, Daykin & Milholland 1990). Infected fruits turn reddish-brown, soft, mushy, often splitting and causing leakage of juice (Milholland & Daykin 1983).

Diaporthe vexans (Sacc. & P. Syd.) Gratz, Phytopathology 32: 542. 1942

Basionym. *Phoma vexans* Sacc. & P. Syd., Syll. Fung. (Abellini) 14, 2: 889. 1899.

= *Phomopsis vexans* (Sacc. & P. Syd.) Harter, J. Agric. Res. 2, 5: 338. 1914.

Specimen examined. USA, from *Solanum melongena*, Dec. 1914, L.L. Harter (CBS 127.14).

Notes — *Diaporthe vexans* (clade 12) causes fruit rot, leaf spot, stem and tip blight disease of eggplants (*Solanum melongena* and *S. wendlandii*) and other solanaceous species, *Acacia* sp. (*Fabaceae*), *Prunus* sp. (*Rosaceae*) and *Sorghum bicolor* (*Poaceae*), *Capsicum annuum* and *Lycopersicon esculentum* (*Solanaceae*). The disease is widespread in North America, the West Indies, and Eastern and Central Asia, also in Africa (Senegal, Tanzania, Zambia) and Mauritius (Punithalingam & Holliday 1972). Additional records include Brunei, Haiti, Iran, Iraq and Romania (Harter 1914, Farr & Rossman 2012).

Diaporthe viticola Nitschke, Pyrenomycetes Germanici 2: 264. 1870

Specimens examined. AUSTRIA, Vienna, Risenbergbach-Weg, on *Laburnum anagyroides*, May 2001, A.R. Rossman (CBS 109492). — CANADA, British Columbia, Sidney, on *Epilobium angustifolium*, Oct. 2001, M. Barr (CBS 109768 = AR 3478). — FRANCE, Dordogne, near Sarlat la Canéda, 1-yr-old stems on *Asphodelus albus*, 20 May 1995, G. Verkley (CBS 759.95). — NETHERLANDS, Utrecht, Baarn, in branches and twigs of *Aucuba japonica*, Jan. 1995, G. Verkley (CBS 106.95); on *Rosa rugosa*, 18 Mar. 1985, G.H. Boerema (CBS 266.85 = PD 85/25); Lelystad, in dead stem on *Lupinus* sp., May 1982, H.A. van der Aa (CBS 449.82); Wieringermeer, Robbenoordbos, in dead stem on *Lupinus arboreus*, 12 Mar. 1991, H.A. van der Aa & F. Meurs (CBS 312.91); Flevoland, trees in front of Info Centre Lepelaarsplassen, in leaf spot on *Fraxinus excelsior*, 31 Aug. 1997, H.A. van der Aa (CBS 100170); Baarn, in dead stem on *Dipsacus fullonum*, 14 June 1985, H.A. van der Aa (CBS 502.85); on twig on *Salix* sp., Apr. 1962, G.H. Boerema (CBS 446.62). — PORTUGAL, on *Vitis vinifera* (Galego durado), 1 Jan. 1998, A.J.L. Phillips (CBS 114011 = CPC 2677); Burgaes, Santo Tirso, on *Vitis vinifera*, 16 Feb. 1998, A.J.L. Phillips (ex-type culture CBS 113201 = CPC 5683). — SWEDEN, Skåne, Maglehem par., on *Sambucus* cf. *racemosa*, 14 Apr. 1989, K. Holm & L. Holm (CBS 114436 = UPSC 2960). — UK, Sheffield, on *A. japonica*, July 1996, G. Verkley (CBS 794.96).

Notes — *Diaporthe viticola* (clade 50) is known from several hosts, but especially from grapevines, on which it causes a cane spot disease in Europe (Portugal, Germany). Merrin et al. (1995) referred to several Australian isolates from grapevines as *Phomopsis* taxon 1. The same species was reported by Phillips (1999) as *D. perijuncta* and by Scheper et al. (2000) as *D. viticola*. In a subsequent study, Mostert et al. (2001a) chose to follow Phillips (1999) and applied the name *D. perijuncta* to taxon 1. However, they also noted that minor morphological differences existed in perithecia and ascospores between the European and Southern Hemisphere material, which led to the description of a novel taxon, *D. australafricana*, for isolates from Australia and South Africa (van Niekerk et al. 2005), and the epitypification of *D. viticola* based on European material. Based on the results obtained here, *D. viticola* (clade 50) is closely related to *D. australafricana* (clade 49), and is clearly distinguishable from *D. perijuncta* (clade 40).

Diaporthe woodii Punith., Mycol. Pap. 136: 51. 1974

= *Phomopsis leptostromiformis* var. *occidentalis*, R.G. Shivas, J.G. Allen & P.M. Will., Mycol. Res. 95: 322. 1991.

Specimen examined. WESTERN AUSTRALIA, Medina, stems of *Lupinus* sp., 8 July 1993, P.M. Williamson (CBS H-5319, culture CBS 558.93).

Notes — Clade 94 represents *Diaporthe woodii* (CBS 558.93), which was characterised by Williamson et al. (1994), based on the ex-type strain (IMI 166508). *Diaporthe crotalariae* (clade 92), *D. aspalathi* (clade 93) and *D. woodii* are closely related species. *Diaporthe woodii* causes stem rot, stem cankers, leaf infections and seed decay of *Lupinus angustifolius* and *L. cosentini*, and blight and seed discoloration of *L. albus*, *L. angustifolius*, *L. cosentini*, *L. luteus*, *L. pilosus* and *Trifolium subterraneum* (subterranean clover). The fungus is known to occur in Brazil, South Africa, USA (Florida), and Western Australia (Williamson et al. 1994).

Diaporthe woolworthii (Peck) Sacc., Syll. Fung. (Abellini) 1: 615. 1882

Basionym. *Valsa woolworthii* Peck, Ann. Rep. N.Y. State Mus. Nat. Hist. 28: 73. 1876 (1875).

Specimen examined. UNKNOWN, on *Ulmus americana*, Sept. 1927, L.E. Wehmeyer (CBS 148.27).

Notes — Clade 57 contains a single isolate of *D. woolworthii* from *Ulmus americana*. This taxon represents an American species occurring on *Ulmus*, so this culture (presumably from North America), could prove to be authentic for the name.

DISCUSSION

A major aim of the present study was to resolve the taxonomy of *Diaporthe* species occurring on diverse hosts, either as pathogens, saprobes, or as harmless endophytes. To delimitate these taxa, nine genes were screened, from which the best five were selected to conduct a multi-gene phylogenetic analysis (ITS, TEF1, ACT, HIS and CAL). *Diaporthe* represents a highly complex genus containing numerous cryptic species, several of which are newly described in the present study, while others remain unclear, awaiting fresh collections and type studies. Many *Diaporthe* species that are morphologically similar proved to be genetically distinct, and several isolates that were formerly identified based on their host, were shown to represent different taxa.

Although the genera *Diaporthe* and *Phomopsis* have received much taxonomic attention, few phylogenetic studies have thus far been conducted, and hence the taxonomy of this group is still problematic. Due to the lack of reference strains, and the fact that few gene loci other than ITS have in the past been used for DNA analysis, most of the conclusions reached thus far have been incorrect, meaning that published literature will have to be interpreted with care.

In this study we studied 15 endophytic *Diaporthe* species from Brazil. Three were not identified to species level, two were identified as *D. novem* and *D. phaseolorum*, while a further 10 were described as new. High genetic diversity was found amongst the analysed isolates from medicinal plants. Species of *Diaporthe* are commonly isolated as endophytes from several hosts in temperate and tropical regions (Bussaban et al. 2001, Murali et al. 2006, Rossmann et al. 2007, Botella & Diez 2011, González & Tello 2011). Skaltsas et al. (2011) isolated 108 *Diaporthe* isolates from asymptomatic leaves and bark of three different hosts (*Hevea brasiliensis*, *H. guianensis* and *Micandra* spp.) from Cameroon, Mexico and Peru. Using a multigene approach, the authors found more than 40 phylogenetic species, of which several appeared to represent novel taxa (Skaltsas et al. 2011).

Despite members of *Diaporthe* commonly being described as phytopathogenic, an increasing number of reports link this genus to endophytic studies, focusing on its potential as a producer of enzymes and novel secondary metabolites, with antibiotic, fungicide and anticancer activity (Dai et al. 2005, Elsaesser et al. 2005, Lin et al. 2005, Silva et al. 2005, Wu et al. 2008, Kumaran & Hur 2009, Weber 2009, Vesterlund et al. 2011).

The ecology of species of *Diaporthe* remains poorly understood, as some endophytes isolated from the medicinal plant *Maytenus ilicifolia* were identified as *D. phaseolorum* (clade 4) and *D. novem* (clade 22), respectively known as pathogen of soybean (Santos et al. 2011) and *Aspalathus linearis* (van Rensburg et al. 2006). *Diaporthe novem* is also reported from hosts such as *Hydrangea macrophylla* (Santos et al. 2010), *Helianthus annuus* and *Vitis vinifera* (Santos et al. 2011). These reports agree with the pogo stick hypothesis, postulating that host-specific fungal plant pathogens frequently exhibit the ability to colonise non-host tissue, enabling them to disperse further, in an attempt to find the host on which they are pathogenic (Crous & Groenewald 2005).

The taxonomy of *Diaporthe* (incl. *Phomopsis*) has traditionally been based on host association, with species being described on the assumption that they are host-specific. In the present study the taxonomy of all *Diaporthe* isolates deposited in the CBS culture collection over time were reviewed, based on this assumption. The employment of this criterion, has led to an exponential growth in the number of taxa described in *Diaporthe* thus far (Uecker 1988). However, in spite of the

apparent synonymies outlined in this study, there was evidence for a huge proliferation of cryptic taxa that were formerly overlooked based on a morphological approach in the absence of molecular data. Species delimitation in *Diaporthe* based on morphological characters is challenging, as most taxa in culture do not produce all spore states of the asexual (alpha, beta and gamma conidia) or the sexual morph. The description of novel taxa in *Diaporthe* in the absence of molecular data (at least ITS and HIS or TUB; see discussion in next section) should thus be strongly discouraged in the future.

In conclusion thus, it seems that in spite of the fact that these taxa readily colonise or co-colonise non-hosts (see also Rehner & Uecker 1994, Mostert et al. 2001a, Farr et al. 2002, Diogo et al. 2010), there is still a multitude of undescribed taxa awaiting further study in this complex. It is thus hoped that the phylogenetic backbone generated here provides a stable platform to enable future studies by others interested in the biology of *Diaporthe*.

Phylogenetic species recognition by genealogical concordance

Taylor et al. (2000) developed the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept to define the limits of sexual species, using the phylogenetic concordance of multiple unlinked genes. This concept has proved greatly useful in fungi, because it is more finely discriminating than other species concepts, as several species are unable to be crossed, or cannot be recognised due to the lack of distinguishing morphological characters or sterility (Reynolds 1993, Taylor et al. 2000, Cai et al. 2011). The adoption of genealogical concordance for species recognition in *Diaporthe* enabled us to distinguish species that were otherwise not possible to identify due to either sterility, or the loss of specific character states. For instance, *D. viticola* and *D. australafricana* are two closely related species (clades 50 and 49, respectively) associated with grapevines. They are morphologically similar, but occur on different continents (van Niekerk et al. 2005). These species have probably accumulated genetic differences due to their geographical isolation. Several cryptic species were recently described in other genera using the GCPSR criterion, some of which are consistent with allopatric divergence, because these species occupy non-overlapping areas separated by geographic barriers, e.g. in *Cladosporium* (Bensch et al. 2012), *Colletotrichum* (Damm et al. 2012a, b), *Harknessia* (Crous et al. 2012), *Ilyonectria* (Cabral et al. 2012a, b) and *Phyllosticta* (Glienke et al. 2011), to name but a few. Using the GCPSR concept it is possible to define the genetic variation observed in some species, but still insufficient to establish them as distinct species, since genetic flow still occurs between them. For example, isolates of clades 79–90 clustered differently based on analyses of the different genes, probably because of recent gene flow among them.

We have compared the location and monophyly of the strains in each clade in the phylogenetic tree of the combined alignment (Fig. 1) to those phylogenetic trees obtained from the individual loci to determine the species boundaries and species resolution. The five loci selected for the Bayesian phylogeny have a similar resolution for species discrimination, ranging from TEF1 resolving 72 out of the 95 species, to HIS and TUB resolving 84 of the 95 species.

The ITS region, which is often considered to be less than optimal for closely related species, was not much better or worse (resolving 75 of the 95 species) than the other included loci. However, given the recent acceptance of the ITS region as official fungal barcode (Schoch et al. 2012) and its intermediate resolving power in the present study, this locus should not

be discarded from future studies. Also, TEF1, which has in the past been used as additional locus for phylogenetic studies of *Diaporthe*, performed the worst in this study (resolving 72 of the 95 species), although this was not much worse than ITS and CAL (resolving 75 and 74 of the 95 species, respectively). The HIS and TUB regions appear to have the best resolution for species discrimination in the present study and therefore are good candidates as secondary markers to the commonly used ITS region. Similar results were also reported for ITS, CAL, TEF1 and TUB by Udayanga et al. (2012), who suggested that TUB be considered as secondary phylogenetic marker for *Diaporthe*.

The importance of epitypification in *Diaporthe*

The best option to supplement poor type material is via epitypification (Cannon et al. 2012). To employ the GCPSR concept in fungi, DNA is mostly extracted from poorly preserved, ancient herbarium specimens with difficulty, and in many cases it only results in short sequences of the ITS region (Quaedvlieg et al. 2011, Cheewangkoon et al. 2012). Therefore, epitypification of living material, and its preservation and deposit in publically available collections and databases, are important steps to provide a stable platform to enable others to test future hypotheses. Although it is not a prerequisite, it is strongly recommended that the chosen epitype should originate from the same geographical region and host, and have morphological, cultural and pathological characteristics similar to those described in the original publication (see Damm et al. 2012a, b, Cannon et al. 2012, Weir et al. 2012).

Despite the fact that close to 2 000 species of *Diaporthe* (incl. *Phomopsis*) have been described in literature, hardly any ex-type strains are available today, the majority of which were included in the present study. Due to the lack of ex-type strains, the taxonomy of several species continue to be unresolved, some of which are important plant pathogens. A serious effort will thus be called for to recollect and redescribe all these old names. An alternative approach would be to simply start over, ensuring that all newly described names are based not only on morphology, but also supplemented by DNA barcodes. However, as long as fungal nomenclature is governed by the ICN, this seems unobtainable. Eventually though, all mycologists will realise that a stable fungal nomenclature must incorporate DNA data, and that this is only achievable if mycology follows a code of nomenclature that incorporates this requirement.

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Discussão Geral

Filogenia e taxonomia do gênero *Diaporthe* e a sua aplicação no controle biológico da Mancha Preta dos Citros

Discussão Geral

O gênero *Diaporthe* têm sido encontrado em diversos hospedeiros em todo o mundo, apesar das espécies mais estudadas serem as fitopatogênicas de importância econômica (MOSTERT *et al.*, 2001; VAN NIEKERK *et al.*, 2005; RENESBURG *et al.*, 2006; SANTOS; PHILLIPS, 2009; RODEVA; GABLER, 2010; SANTOS *et al.*, 2011; THOMPSON *et al.*, 2011), o gênero também tem sido descrito como um dos mais frequente isolado como endófito com potencial biotecnológico (BOTELLA; DIEZ, 2011; MURALI *et al.*, 2006; ELSAESSER *et al.*, 2005; KOBAYASHI *et al.*, 2003; ISAKA *et al.*, 2001; DAI *et al.*, 2005; LIN *et al.*, 2005; DETTRAKUL *et al.*, 2003; BANDRE; SASEK, 1977; SILVA *et al.*, 2005; WU *et al.*, 2008; KUMARAN, 2009; BRAYFORD, 1990; WEBER, 2009; VESTERLUND *et al.*, 2011).

Em geral isolados de *Diaporthe* quando obtidos como endofíticos não são identificados em nível de espécie. Isso ocorre, especialmente, pela dificuldade em identificar espécies de *Diaporthe* isoladas a partir de um hospedeiro do qual essa não tenha sido descrita anteriormente; por terem sido tradicionalmente descritas com base na espécie hospedeira, o que contribuiu com a proliferação de nomes sem isolados referência ou depositados em herbários e não mais viáveis para estudos biológicos e moleculares.

A elucidação taxonômica do gênero *Diaporthe* Nitschke (*Phomopsis* Sacc. Bubák) foi realizada através da análise filogenética dos genes ITS, TEF1, ACT, HIS e CAL (CAPÍTULO 2; GOMES *et al.*, 2013). A análise multilocus resultou em 95 clados bem suportados nos quais as relações filogenéticas de 243 isolados, entre eles espécimes tipo ou ex-tipo disponíveis na coleção de culturas de fungos CBS (Fungal Biodiversity Centre), pôde ser inferido. Quinze novas espécies foram descritas, novas combinações foram introduzidas em *Diaporthe* e a espécie *D. anacardii* foi epitipificada. A análise multilocus tem sido proposta e provou ser eficaz para estudos filogenéticos e de identificação em nível de espécie para outros gêneros com dificuldades de elucidação taxonômica como, por exemplo, em

Colletotrichum, *Sordariales* e *Fusarium* (PRIHASTUTI *et al.*, 2009; MILLER; HUHDORF, 2005; O`DONNELL, 2004, 2009, 2010).

Microrganismos endofíticos isolados das plantas medicinais, *Spondias mombin*, *Myracrodruon urundeuva*, *Schinus terebinthifolius*, *Aspidosperma tomentosum* e *Maytenus ilicifolia*, foram testados quanto à atividade antimicrobiana contra o patógeno *Phyllosticta citricarpa*. Sendo os isolados identificados como, *Diaporthe* spp., os que apresentaram maior potencial *in vitro* e em fruto como agente de controle da Mancha Preta dos Citros, além de grande potencial para produção de metabólitos secundários (GOMES *et al.*, 2012). Entretanto somente depois da revisão do gênero *Diaporthe* realizada nesse trabalho, foi possível a identificação dessas espécies com potencial de controle biológico da Mancha Preta dos Citros (MPC).

A Mancha Preta dos Citros atinge a maioria das espécies cítricas especialmente laranja doce (*Citrus sinensis*) e é causada pelo fungo *Phyllosticta citricarpa* McAlpine (teleomorfo *Guignardia citricarpa* Kiely). Esta doença atinge seriamente a citricultura brasileira, dificultando a comercialização no mercado de frutas, a sua exportação devido à barreira fitossanitária imposta pela União Européia. Além disso, as lesões da MPC depreciam o valor comercial dos frutos destinados ao mercado de frutas frescas e nos casos em que a doença atinge o pedicelo do fruto, pode ocorrer à queda prematura do mesmo, aumentando as perdas na produção (ROBES *et al.*, 2005).

A utilização de fungicidas como principal método de controle da MPC, utilizado no pré e pós-colheita, além de onerar os custos da produção (FIALHO *et al.*, 2009) oferece risco para o ambiente e o ser humano. Por essa razão, desde 2008, foi proibida nos EUA a utilização do carbendazim, um dos agrotóxico utilizado no controle da MPC, levando ao embargo do suco de laranja brasileiro pelo governo americano em decorrência do uso desse fungicida (DURAN *et al.*, 2012). Outro problema no uso de fungicidas e o emprego sucessivo dos mesmos é a seleção de linhagens resistentes do agente causal, como observado na África do Sul

(HERBERT; GRECH, 1985) o que levou a restrições na sua utilização (AGOSTINI *et al.*, 2006, DEISING *et al.*, 2008).

Estudos de bioprospecção de microrganismos endofíticos para descobrir novos antibióticos, agentes quimioterápicos e defensivos agrícola são fundamentais para fins biotecnológicos e como alternativa a utilização de fungicida no controle de doenças de plantas. Estes metabólitos produzidos pelos fungos geralmente são eficazes, possuem baixa toxicidade, e tem um menor impacto no ambiente (PEARCE, 1997; STROBEL; DAISY, 2003; SMITH *et al.*, 2008, XU *et al.*, 2010). Fungos endofíticos de plantas medicinais estão sendo amplamente estudados na busca de novos biocontroladores potencialmente úteis. Nesse contexto o gênero *Diaporthe* tem sido relatado como potencial para o uso na biotecnologia com várias descobertas importantes, incluindo novos metabólitos estruturalmente significativos e fisiologicamente ativos (UDAYANGA *et al.*, 2011; GOMES *et al.*, 2012).

Em função dos prejuízos causados por *P. citricarpa*, e um elevado interesse para métodos alternativos no controle da MPC são relevantes os estudos na busca de microrganismos antagonistas para o controle da doença. Nessa perspectiva Gomes *et al.*, (2012) avaliaram o potencial de isolados endofíticos de *Diaporthe* em inibir *in vitro* duas linhagens patogênicas de *P. citricarpa* isoladas de lesão da MPC, em São Paulo, Brasil, e testadas quanto a patogenicidade de acordo com Baldassari *et al.*, (2008). Isolados de *Diaporthe* inibiram o crescimento do micélio e formação de picnídio das duas linhagens de *P. citricarpa* avaliadas, mostrando-se promissores para uso como biocontrole da MPC. Para implementação desses isolados no controle biológico de doenças de plantas faz-se necessário o estudo das relações filogenéticas entre os mesmos e espécie de *Diaporthe* fitopatogênicas.

A revisão do gênero *Diaporthe*, realizado nesse trabalho, permitiram a identificação de 40 isolados com potencial antimicrobiano contra o patógeno *Phyllosticta citricarpa*, obtidos endofiticamente de plantas medicinais no Brasil. Esses isolados foram agrupados em 15 espécies filogeneticamente distintas, dessas, treze pertenciam a espécies novas; dez foram descritas: *D. brasiliensis*, *D. endophytica*, *D. inconspicua*, *D. infecunda*, *D. mayteni*, *D. oxe*, *D. paranensis*, *D. raonikayaporum*, *D. schini* e *D. terebinthifolii*; três não puderam ser identificadas ao

nível de espécie por não produzirem estruturas de reprodução *in vitro*, assim como, pela ausência de mais isolados para confirmar as diferenças genéticas encontradas nos cinco loci analisados; duas foram identificadas como *D. phaseolorum* e *D. novem*.

Os taxons *D. phaseolorum* e *D. novem* são conhecidos como patógenos de soja (SANTOS *et al.*, 2011) e *Aspalathus linearis* (VAN RENSBURG *et al.*, 2006). *Diaporthe novem* também é relatado a partir de hospedeiros, como *Hydrangea macrophylla*, *Helianthus annuus* e *Vitis vinifera* (SANTOS *et al.*, 2010; 2011). Estas espécies mesmo tendo sido isoladas colonizando plantas medicinais sem causar dano são potenciais fitopatógenos. Do contrário, as espécies endofíticas descritas nesse trabalho não agruparam com nenhuma espécie fitopatogênica (CAPÍTULO 2; GOMES *et al.*, 2013).

Trinta e nove isoaldos, das quinze espécies de *Diaporthe* avaliados por Gomes *et al.*, 2013, inibiram significativamente o crescimento de duas linhagens de *Phyllosticta citricarpa*, PC13/96 e a linhagem tipo LGMF06 isolada de lesão característica da MPC em São Paulo, Brasil com patogenicidade confirmada (BALDASSARI *et al.*, 2008; GOMES *et al.*, 2012), pela técnica de cultura pareada, apresentando inibição superior ao fungicida derosal (19%). As interações observadas sugerem que as relações de inibição do fitopatógeno pelo endofítico são mediadas através da produção de metabólitos secundários e ou por parasitismo (GOMES *et al.*, 2012).

A inibição do crescimento tanto do micélio quanto do picnídio são importantes para o controle da mancha preta dos citros uma vez que a doença pode ser causada por duas fontes de inóculo, pelos picnidiósporos e pelos ascósporos, sendo estes produzidos em folhas caídas no solo (BALDASSARI *et al.*, 2008). Compostos orgânicos voláteis ou as misturas artificiais têm sido sugeridos como promissores no controle da mancha preta ou outras doenças pós-colheita (FIALHO *et al.*, 2010). Gomes *et al.*, 2012 avaliaram também os isolados quanto ao potencial de inibir o crescimento de *P. citricarpa* pela produção de metabólitos voláteis (MV) e não voláteis (MNV), assim com, à capacidade de inibir a formação de picnídios do fitopatógeno em folhas de citros. Isolados das espécies *Diaporthe endophytica*, *D.*

infecunda e *D. teribinthifolii*, inibiram o crescimento em 50% de *P. citricarpa* pelo teste de MV. Isolados dessas mesmas especies além de *D. inconspicua*, *D. mayteni*, *D. oxe* e *D. schini* apresentaram os melhores resultados tanto na inibicao por MNV quanto na capacidade de inibir a formação de picnídios do fitopatógeno em folhas de citros.

Santos *et al.*, 2012, relatam o potencial de *Diaporthe endophytica* e *D. terebinthifolii* de inibir *P. citricarpa* tanto *in vitro* quanto nas frutas de laranja, assim como, a capacidade dessas espécies de *Diaporthe* em colonizar endofiticamente plantas cítricas através da transformação mediada por *Agrobacterium tumefaciens* do gene *DsRed* que expressa uma proteína flourescente vermelha. As novas descobertas tem revelado o enorme potencial do gênero *Diaporthe*. Além da produção de metabólitos secundários os microrganismos endofíticos tem o potencial de inibirem fitopatógenos, competindo por nutrientes, e parasitando-os. Os resultados dessas interações antagônicas, tais como antibiose, competição, indução de defesa e parasitismo tem revelado um papel muito importante desses microrganismos na medicina com a descoberta de novos fármacos, assim como leva ao controle biológico de doenças de plantas. Nesse contexto os dados apresentados aqui revelam que os isolados endofíticos de *Diaporthe* são promissores no controle biológico da MPC.

Em conclusão, ainda há um grande número de táxons não descritos, especialmente quando isolados endofiticamente. Dessa forma, espera-se que a base filogenética gerada nesse trabalho forneça uma plataforma estável para permitir novos estudos na biologia do gênero *Diaporthe*.

Conclusões Finais

- ✓ Os melhores genes para análise taxonômica e filogenética do gênero *Diaporthe* selecionados foram ITS, TEF1, ACT, HIS e CAL;
- ✓ A reavaliação do gênero *Diaporthe* através de análise multilocus de 243, entre eles espécimes tipo ou ex-tipo disponíveis na coleção de culturas de fungos CBS (Fungal Biodiversity Centre) resultou em 95 clados bem suportados;
- ✓ Quinze novas espécies de diferentes hospedeiros e regiões geográficas foram descritas, novas combinações foram introduzidas em *Diaporthe* e a espécie *D. anacardii* foi epítipificada;
- ✓ A base filogenética gerada nesse trabalho fornece uma plataforma estável para permitir a descrição de táxons do Gênero *Diaporthe* que ainda estão para ser descritos, assim como, para estudos da biologia do Gênero;
- ✓ A taxonomia de espécies de *Diaporthe* endófitos das plantas medicinais *Aspidosperma tomentosum*, *Maytenus ilicifolia*, *Spondias mombin* e *Schinus terebinthifolius*, com potencial antagonista contra *Phyllosticta citricarpa* foi esclarecida;
- ✓ As relações filogenéticas confirmaram que o mesmo táxon pode estar associado a diferentes hospedeiros, o que sugere que eles se movem livremente entre os mesmos. Em contrapartida, alguns patógenos de plantas e endófitos parecem ser estritamente restritos a determinado hospedeiro.

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