MEIRIELE DA SILVA

EXPLORING FUNGAL DIVERSITY: PASSALORA, PSEUDOCERCOSPORA, SIROSPORIUM AND ZASMIDIUM ON BRAZILIAN PLANTS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de Doctor Scientiae.

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_____	_____
Tiago de Souza Leite	Davi Mesquita de Macedo
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Antônio Hernández-Gutiérrez	Gleiber Quintão Furtado

_____

Prof. Robert Weingart Barreto
(Orientador)

A Deus,
A minha mãe,
Por serem o meu apoio.

Dedico!

"Contudo, seja qual for o grau a que chegamos o que importa é prosseguir decididamente" Fi 3,16

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BIOGRAFIA

MEIRIELE DA SILVA, filha de Maria Raimunda, nasceu na cidade de Corinto, Minas Gerais, no dia 15 de setembro de 1981.

Realizou os estudos básicos na cidade de Paraopeba, no mesmo estado.

Em 2005 iniciou o curso de graduação em Agronomia na Universidade Federal de Viçosa, UFV, graduando-se em janeiro de 2010.

Em março de 2010, iniciou o programa de Mestrado em Fitopatologia na UFV, concentrando seus estudos nas áreas de micologia (taxonomia de fungos fitopatogênicos), defendendo sua dissertação em fevereiro de 2012.

Em março de 2012, iniciou o programa de doutorado em Fitopatologia na UFV. Em 2014 desenvolveu parte de seu doutorado (doutorado sanduíche), no Centraalbureal voor Schimmelcultures (CBS) Fungal Biodiversity Centre na Holanda, sob orientação do Dr. Prof. Pedro W. Crous.

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RESUMO

SILVA, Meiriele da, D.Sc., Universidade Federal de Viçosa, fevereiro de 2016. **Explorando a diversidade fúngica: Passalora, Pseudocercospora, Sirosporium e Zasmidium em plantas brasileiras**. Orientador: Robert Weingart Barreto. Coorientador: Olinto Liparini Pereira.

Os fungos conhecidos pela denominação informal de cercosporóides são um dos maiores grupos de microfungos, compreendendo mais de 2000 espécies e englobando diversos gêneros de formas assexuais tendo como correspondente fase assexuada formas ascosporogênicas que eram tradicionalmente classificadas no gênero Mycosphaerella e equivalentes. Possuem distribuição cosmopolita e são altamente diversos especialmente nos países tropicais e subtropicais. Fungos cercosporóides podem ser encontrados como saprófitas, hiperparasitas sendo muito comuns como patógenos de plantas – causando, sobretudo manchas foliares. Diversas doenças importantes de plantas cultivadas são causadas por fungos cercosporóides. Nos últimos anos, a aplicação de técnicas moleculares ao estudo dos cercosporóides gerou novas informações que estão auxiliando o melhor entendimento das relações filogenéticas dentro desse grupo levando a uma re-estruturação do sistema de classificação deste grupo de fungos com rearranjos, fusão de gêneros e reconhecimento de novos gêneros. O presente estudo pretendeu contribuir para estender essa abordagem para cercosporóides já conhecidos no Brasil e novos taxa coletados em trabalhos de campo. Um total de 27 Pseudocercospora spp., 7 Passalora spp., 4 Zasmidium spp. e 1 Sirosporium foram coletados, isolados em cultura pura, sequenciados e submetidos a análises filogenéticas multigênicas. Quatro regiões genômicas (LSU, ITS, tef1 e actA) foram utilizadas para realização de uma análise Bayesiana com o alinhamento das regiões combinadas ITS, actA e tef1. Os resultados obtidos associando filogenia, morfologia e características da cultura revelaram uma rica diversidade incluindo dezoito novas espécies a serem propostas, a saber: Pseudocercospora aeschynomenicola, Ps. diplusodonii, Ps. emmotunicola, Ps. manihotii, Ps. perae, Ps. planaltinensis, Ps. pothomorphes, Ps. sennae-multijugae, Ps. solani-pseudocapsicicola, Ps. vassobiae, Ps. wulffiae, Ps. xylopiae, Passalora dasyphyllii, Sirosporium tocoyenae, Zasmidium aspidospermae, Z. brosimii, Z. peixotoana e Z. roupalina. Onze epitipos foram designados, para as espécies: Pseudocercospora bixae, P. chamaecristae, P.exilis, P. luzardii, P. plumeriifolii, P. richardsoniicola, P. rigidae, P. struthanthi, Passalora schefflerae, Pa. rubida e Pa.

vicosae. Três dentre os taxa encontrados representam novos relatos para o Brasil, respectivamente: Ps. euphorbiacearum, Ps. tecomicola, Ps. trinidadensis e vários hospedeiros foram relatados como representando novas associações com cercosporóides de ocorrência já conhecida anteriormente no Brasil. Adicionalmente, o posicionamento filogenético de Camptomeris leucaenae foi investigado pela primeira vez baseado em sequências da região LSU. Confirmou-se que C. leucaenae pertence à família Mycospaerellaceae (Capnodiales, Dothideomycetes), situando-se próximo de Cymadothea trifolii, patógeno de uma leguminosa nativa da Europa. O presente estudo é uma contribuição para uma abordagem moderna para a compreensão com base em dados moleculares da sistemática de cercosporóides do Brasil. Foram geradas informações morfológicas e moleculares para 40 taxa, representando apenas uma pequena fração da diversidade de espécies de cercosporóides conhecida no país. Muitas espécies de cercosporóides descritas ou relatadas por micologistas no Brasil no passado precisam ser recoletadas para se consolidar um entendimento mais preciso desse importante grupo de fungos.

ABSTRACT

SILVA, Meiriele da, D.Sc., Universidade Federal de Viçosa, February, 2016. **Exploring fungal diversity: Passalora, Pseudocercospora, Sirosporium and Zasmidium on Brazilian plants**. Adviser: Robert Weingart Barreto. Co-adviser: Olinto Liparini Pereira.

Fungi cercosporoid are one of the largest groups of microfungi, with over than 2000 associated names, including several genera of Mycosphaerella and Mycosphaerella-like sexual morph. The cercosporoids are cosmopolitan fungi and are highly diverse especially in tropical and subtropical countries. Cercoporoid fungi vary from being saprobic, hyperparasitic and plant pathogenic, in the last case they are known to causes several important diseases in several crops. In the last years, the taxonomy of cercosporoid fungi has undergone significant changes. The application of molecular techniques have generated new informations that are helping the understanding of the phylogenetic relationships leading to a re-structuration of the classification system of this group of fungi with rearrangements, fusion of genera and recognition of new genera as distinct. In this study, a total of 27 Pseudocercospora spp., 7 Passalora spp., 4 Zasmidium spp. and one Sirosporium were collected, cultivated and subjected to a multigene analysis. Four genomic regions (LSU, ITS, tef1 and actA) were used to performed a Bayesian analysis with combined ITS, actA and tef1 sequence alignment. Our results based on DNA phylogeny integrated with morphology, revealed a rich diversity with eighteen new species to be described, namely: Pseudocercospora aeschynomenicola, Ps. diplusodonii, Ps. emmotunicola, Ps. manihotii, Ps. perae, Ps. planaltinensis, Ps. pothomorphes, Ps. sennae-multijugae, Ps. solani-pseudocapsicicola, Ps. vassobiae, Ps. wulffiae, Ps. xylopiae, Passalora dasyphyllii, Sirosporium tocoyenae, Zasmidium aspidospermae, Z. brosimii, Z. peixotoana and Z. roupalina. Eleven epitype specimens were designated, Pseudocercospora bixae, P. chamaecristae, P.exilis, P. luzardii, P. plumeriifolii, P. richardsoniicola, P. rigidae, P. struthanthi, Passalora schefflerae, Pa. rubida and Pa. vicosae, three species newly reported, Ps. euphorbiacearum, Ps. tecomicola, Ps. trinidadensis and several new host records linked to known cercosporoid in Brazil. Additionally, the phylogenetic position of Camptomeris leucaenae was investigated for the first time based in sequences of the large subunit ribosomal (LSU). This study confirmed that C. leucaenae belongs to Mycosphaerellaceae s. str. (Capnodiales, Dothideomycetes) and is closely related to

Cymadothea trifolii a pathogen a native leguminous plant from Europe. The present study represents the first organized effort towards generating molecular data to support the taxonomy of cercosporoid from Brazil. It yielded information for 40 taxa, representing only a small fraction of yet unknown species diversity in the country. Many additional species still need to be collected and recollected to enable a better understanding of systematic of cercosporoid fungi in Brazil.

INTRODUÇÃO GERAL

Os fungos conhecidos pela denominação informal de cercosporóides são um dos maiores grupos de microfungos, compreendendo mais de 2000 espécies e englobando diversos gêneros de formas assexuais tendo como correspondente fase sexuada formas ascosporogênicas que eram tradicionalmente classificadas no gênero Mycosphaerella e relacionados (Crous & Braun 2003). Os cercosporóides encontram-se amplamente distribuídos em todos os continentes, sendo altamente diversificados especialmente nos países tropicais e subtropicais, causando doenças (cercosporioses) em uma ampla gama de hospedeiros. Estimam-se cerca de 2500 gêneros de plantas hospedeiras, distribuídos em 155 famílias, incluindo pteridófitas, monocotiledôneas e dicotiledôneas (Chupp 1954, Crous & Braun, 2003, Braun et al. 2013, 2014, 2015).

Os fungos cercosporóides são agentes etiológicos de diversas doenças em grandes culturas, fruteiras, essências florestais e plantas ornamentais de importância econômica e estão comumente associados a manchas foliares necróticas, mas podem também causar lesões em outras partes das plantas como pecíolos, frutos, brácteas e até mesmo sementes (Chupp 1954, Crous & Braun 2003). Alguns exemplos de doenças imporantes causadas por cercosporóides são, mancha angular do feijoeiro causada por Pseudocercospora griseola, mancha púrpura da soja causada por Cercospora kikuchii, mal das folhas da seringueira causada por Pseudocercospora ulei, mancha foliar de cercospora em milho causado por Cercospora zea-maydis, sigatoga-negra e sigatoka amarela em bananeira causada por Pseudocercospora fijiensis e P. musae respectivamente e a mancha de olho pardo causada por Cercospora coffeicola em cafeeiro (Kimati et al. 2005).

Em outra perspectiva, algumas espécies de cercosporóides podem ser empregados como agentes de biocontrole de plantas daninhas (Morris & Crous 1994) a exemplo de Cercospora rodmanii e Cercospora caricis investigadas para o desenvolvimento de bioherbicida para o biocontrole de Eichornia crassipes e para Cyperus rotundus, respectivamente (Barreto & Evans 1995a,b). Outras espécies foram relatadas em plantas daninhas e poderiam ter potencial para o uso em controle biológico como por exemplo: Cercospora appi em Solanum

glaucophyllum e Xanthium strumarium (Rocha et al. 2007), Pseudocercospora pereskiae em Pereskia aculeata (Pereira et al. 2007), Cercospora mitracarpi-hirti, Pseudocercospora borreriae, Passalora pseudocapnodioides, e Passalora mitaracarpi-hirti, encontradas associadas a Mitracarpus hirtus (Pereira & Barreto 2005), Pseudocercospora palicoureae encontrada atacando Palicourea marcgravii (Pereira & Barreto 2006) e Pseudocercospora cryptostegiae-madagascariensis em Cryptostegia madagascariensis (Silva et al. 2008).

Nos últimos anos, a taxonomia dos cercosporóides tem sofrido alterações significativas. Fries em 1849, introduziu o primeiro gênero de hifomiceto cercosporóide, Passalora, seguido pelo gênero Cercospora, introduzido por Fresenius (em Fuckel 1863). Entretanto, o gênero Cercospora foi pela ultima vez monografado por Chupp (1954). Este autor aceitou mais de 1500 espécies como pertencendo ao gênero Cercospora. Ao rever as espécies anteriormente incluídas no gênero, Chupp observou que o uso do nome Cercospora havia sido utilizado inadequadamente ao longo das décadas anteriores por micologistas que erroneamente descreveram como Cercospora espécies que na verdade pertenciam a gêneros muito diferentes morfologicamente tais como taxa pertencentes a Fusarium, Alternaria e outros. Embora excluindo tais taxa do gênero Cercospora, o conceito de Chupp para Cercospora ainda era demasiado abrangente. Ele rejeitou os sistemas de classificação que segregavam o gênero Cercospora em várias sessões ou gêneros, reunindo todos esses táxons em Cercospora (Chupp 1954), aproximando o gênero do que hoje denominamos informalmente como "fungos cercosporóides" e que inclui dezenas de gêneros diferentes.

Autores posteriores, particularmente Deighton, Ellis e Braun dividiram o complexo Cercospora dentro de unidades morfologicamente menores, baseado em uma combinação de caracteres como estrutura do conidioma (esporodóquio, sinêmio, conidióforo livre, fascículo, etc.), do micélio (presença ou ausência de micélio superficial e sua textura), do conidióforos (arranjo, ramificação, pigmentação e ornamentação), das células conidiogênicas (localização, proliferação e tipo de cicatriz) e do conídio (formação, forma, septação, ornamentação, pigmentação e catenulação) (Deighton 1965, 1967, 1971, 1973, 1974, 1976, 1979, 1983, 1987, 1990; Ellis 1971, 1976; Braun 1995, 1998). Crous & Braun (2003) também revisaram os gêneros de cercosporóides utilizando

critérios morfológicos introduzidos por autores posteriores a Chupp tais como: estrutura do locus conidiogênico, hilo e presença ou ausência de pigmentação no conidióforo e no conídio.

A revisão de Crous & Braun (2003) e os primeiros dados filogenéticos baseado em sequências de DNA para os cercosporóides (Crous et al. 2000, 2001), ocasionou uma considerável redução dos gêneros desse grupo de fungos. Nos últimos anos, a taxonomia dos cercosporóides tem sofrido alterações significativas (Crous et al. 2009). A aplicação de técnicas moleculares gerou novas informações que estão auxiliando o entendimento das relações filogenéticas levando a uma re-estruturação do sistema de classificação deste grupo de fungos com rearranjos, fusão de gêneros e reconhecimento de novos gêneros.

Vários outros estudos subsequentes baseados na combinação de informação morfologica e advindas de sequências de DNA confirmaram o reconhecimento de gêneros segregados de Cercospora (Minnis et al. 2011, Braun et al. 2013, 2014, 2015, Crous et al. 2013, Groenewald et al. 2013, Quaedvlieg et al. 2014 e Bakhshi et al. 2015).

Levantamentos da biodiversidade de cercosporóides no Brasil, associados a plantas nativas e cultivadas iniciaram-se em 1929, quando A.S. Muller coletou e descreveu diversas espécies de “Cercospora" no Estado de Minas Gerais (Muller & Chupp 1934, 1936). Posteriormente, A.P. Viégas dedicou atenção especial a este grupo de fungos no Brasil, descrevendo mais de 90 espécies neste gênero em uma única publicação (Viégas 1945). Augusto Chaves Batista também investigou e descreveu várias espécies adicionais de cercosporóides (Batista et al. 1960). Mais tarde, o re-exame das espécies descritas por Viégas resultou em combinações em outros gêneros de cercosporóides (Crous et al. 1997, 1999). Durante os últimos anos, numerosas espécies de cercosporóides foram descritas no Brasil (Braun et al. 1999, Furlanetto & Dianese 1999, Braun & Freire 2002, 2004, 2006, Pereira & Barreto 2005, Silva & Pereira 2007, Rocha et al. 2008, Soares & Barreto 2008, Silva et al. 2012, Firmino et al. 2013, Parreira et al. 2014, Hernández-Gutiérrez & Dianese 2008, 2009, 2014a,b, Hernández-Gutiérrez et al. 2014, 2015, Guatimosim et al. 2016). Com apenas algumas exceções (a exemplo de Guatimosim 2016, Parreira et al. 2014 e Rocha & Soares 2013), mesmo as descrições mais recentes de cercosporóides no Brasil foram baseadas apenas em

dados morfológicos, sem nenhuma informação molecular que permita a comparação e análise filogenética com outras espécies no mundo. Muitas vezes presumiu-se haver uma especificidade em relação à espécie ou gênero de hospedeiro, uma opção hoje reconhecida como equivocada para cercosporóides de alguns gêneros que apresentam espécies polífagas, como é o caso de taxa no complexo Cercospora apii (Groenewald et al. 2006, 2007). A disponibilização de sequências para fungos pertencentes a este megadiverso e importante grupo de fungos no Brasil é de fundamental importância para o entendimento global da sistemática de cercosporóides.

O objetivo do presente estudo foi, portanto, iniciar uma reavaliação taxonômica de cercosporóides no Brasil, com base em uma combinação de caracteres morfológicos e moleculares, conforme proposto por Quaedvlieg et al. (2014).

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Capítulo 1

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Exploring fungal mega-diversity: *Pseudocercospora* from Brazil

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Key words

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Capnodiales
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systematics

Abstract Although the genus *Pseudocercospora* has a worldwide distribution, it is especially diverse in tropical and subtropical countries. Species of this genus are associated with a wide range of plant species, including several economically relevant hosts. Preliminary studies of cercosporoid fungi from Brazil allocated most taxa to *Cercospora*, but with the progressive refinement of the taxonomy of cercosporoid fungi, many species were relocated to or described in *Pseudocercospora*. Initially, species identification relied mostly on morphological features, and thus no cultures were preserved for later phylogenetic comparisons. In this study, a total of 27 *Pseudocercospora* spp. were collected, cultured, and subjected to a multigene analysis. Four genomic regions (LSU, ITS, *tef1* and *actA*) were amplified and sequenced. A multigene Bayesian analysis was performed on the combined ITS, *actA* and *tef1* sequence alignment. Our results based on DNA phylogeny, integrated with ecology, morphology and cultural characteristics revealed a rich diversity of *Pseudocercospora* species in Brazil. Twelve taxa were newly described, namely *P. aeschynomenicola*, *P. diplusodonii*, *P. emmotunicola*, *P. manihotii*, *P. perae*, *P. planaltinensis*, *P. pothomorphes*, *P. sennae-multijugae*, *P. solani-pseudocapsicicola*, *P. vassobiae*, *P. wulffiae* and *P. xylopiae*. Additionally, eight epitype specimens were designated, three species newly reported, and several new host records linked to known *Pseudocercospora* spp.

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INTRODUCTION

The genus *Pseudocercospora* was described by Spegazzini (1910) with *P. vitis* as type species. *Pseudocercospora* belongs to the *Mycosphaerellaceae* (*Capnodiales*, *Dothideomycetes*), and several species have mycosphaerella-like sexual morphs (Crous et al. 2013a). With the amendment of Article 59 of the International Code of Nomenclature for algae, fungi and plants (ICN), a single generic name is now used for *Pseudocercospora* spp. (Hawksworth et al. 2011, Wingfield et al. 2012, Crous et al. 2015). This has led to changes in the holomorphic name of some important fungal pathogens such as the etiological agent of South American leaf blight of rubber, *P. ulei* (≡ *Microcyclus ulei*, Hora Júnior et al. 2014) and leaf and fruit spot of pistachio, *P. pistacina* (≡ *Septoria pistacina*, Crous et al. 2013b).

Pseudocercospora is a cosmopolitan genus of phytopathogenic fungi that is associated with a wide range of plant species, including several economically relevant hosts (Crous et al. 2013a, Bakhshi et al. 2014). Furthermore, some of the species, e.g. *P. angolensis* and *P. fijiensis* are regarded as being of quarantine significance (Churchill 2011, Crous et al. 2013a).

Several important plant pathogenic *Pseudocercospora* spp. are known from Brazil. Besides *P. fijiensis* (black leaf streak of *Musa*), *P. griseola* (angular leaf spot of *Phaseolus vulgaris*) and *P. ulei* (South American leaf blight of *Hevea brasiliensis*), other economically relevant species include *P. abelmoschi* (leaf spot of *Abelmoschus esculentus*), *P. anacardii* (leaf spot of *Anacardium occidentale*), *P. bixae* (leaf spot of *Bixa orellana*), *P. cruenta* (leaf spot of *Vigna unguiculata* ssp. *sesquipedalis*), *P. kaki* (leaf spot of *Diospyros kaki*), *P. musae* (yellow Sigatoka of *Musa*), *P. paraguayensis* (leaf spot of *Eucalyptus*) and *P. vitis* (leaf spot of *Vitis*) (Chupp 1954, Crous & Braun 2003, Kimati et al. 2005, Hunter et al. 2006, Crous et al. 2006, 2013a, Arzanlou et al. 2007, 2008, 2010, Churchill 2011, Braun et al. 2013, Kirschner 2014).

Among the *Pseudocercospora* spp. described from Brazil, several have also been recognised as having potential for use as biological control agents of invasive weeds. For example, *P. borreriae* could be used for the biocontrol of *Mitracarpus hirtus* (Pereira & Barreto 2005), *P. cryptostegiae-madagascariensis* for *Cryptostegia madagascariensis* (Silva et al. 2008), *P. palicourea* for *Palicourea marcgravii* (Pereira & Barreto 2006), *P. pereskiae* as a classical biocontrol agent against *Pereskia aculeata* (Pereira & Barreto 2007) and *P. subsynnematosa* for *Tibouchina herbacea* (Parreira et al. 2014).

Surveys of the biodiversity of Brazilian cercosporoid fungi in native and cultivated plants date back as far as 1929, when A.S. Muller collected and described many species from the State of Minas Gerais (Muller & Chupp 1934). Later, A.P. Viégas dedicated particular attention to this group of fungi in Brazil, describing more than 90 species in a single publication (Viégas 1945). A.C. Batista also investigated and described several additional species (Batista et al. 1960). Some publications have dealt with the re-examination of the species described by Viégas (Crous et al. 1997, 1999); these studies resulted in several cercosporoid fungi being allocated to other genera, including *Pseudocercospora*. During the last decades numerous *Pseudocercospora* spp. have been described from Brazilian biomes such as the Caatinga (semi-arid) (Braun et al. 1999, Braun & Freire 2002, 2004, 2006), the Atlantic rainforest - Mata Atlântica (Rocha et al. 2008, Soares & Barreto 2008, Parreira et al. 2014), and especially from the Cerrado (Furlanetto & Dianese 1999, Hernández-Gutiérrez & Dianese 2009, 2014, Hernández-Gutiérrez et al. 2014). With a few exceptions (e.g.,

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Crous et al. 2013a, Rocha et al. 2013, Parreira et al. 2014), publications dealing with Brazilian *Pseudocercospora* spp. lack molecular data and rely solely on morphological characteristics, making phylogenetic comparisons to species from other countries impossible. The genus *Pseudocercospora* accommodates several synnematal and non-synnematal cercospora-like species that produce pigmented conidiophores and conidia with unthickened (or slightly thickened), non-darkened conidial scars and hila (Deighton 1976, Braun 1995). However, the application of DNA phylogenetic analyses to species in the *Mycosphaerella* complex (Stewart et al. 1999, Crous et al. 2000, 2001) demonstrated that *Pseudocercospora* is heterogeneous. Indeed, Crous et al. (2001) regarded the unthickened (or slightly thickened) conidial scars to be a synapomorphy shared among several cercosporoid genera. Recently, multigene DNA analyses revealed that the morphological characteristics previously ascribed solely to *Pseudocercospora* evolved more than once within the *Mycosphaerellaceae* (Frank et al. 2010, Crous et al. 2013a).

Pseudocercospora s.str. was circumscribed as having species with conidiophores that are solitary, fasciculate, synnematal, or arranged in sporodochia, giving rise to conidia that are pigmented with unthickened or slightly thickened and darkened scars (Braun et al. 2013, Crous et al. 2013a). However, some species with characteristics that are not typical of *Pseudocercospora* s.str. were placed in *Pseudocercospora* until more sequences became available, and the clades these species belong to become better resolved (Minnis et al. 2011, Crous et al. 2013a). Additionally, Crous et al. (2013b) recently included *Septoria pistacina*, which only has pycnidial conidiomata, in *Pseudocercospora* s.str., highlighting the morphological plasticity occurring within this genus. Hora Júnior et al. (2014) employed multigene DNA data to reconstruct the molecular phylogeny of the fungus causing South American leaf blight of rubber (*P. ulei*), and showed that it was firmly located within *Pseudocercospora* s.str. Moreover, the associated conidiomatal *Aposphaeria* morph was shown to possess a spermatial function. All of these cases suggest that the present generic circumscription of *Pseudocercospora* s.str. has changed with time as more DNA phylogenetic data became available (Crous et al. 2013a, Bakhshi et al. 2014, Nguanhom et al. 2015), and may continue to be further refined in future years.

The aim of the present study was therefore to initiate a re-evaluation of *Pseudocercospora* spp. occurring in Brazil, based on a combination of morphological, cultural and molecular data using the Consolidated Species Concept proposed by Quaedvlieg et al. (2014). Whenever possible, epitypes for known species were designated and DNA sequences deposited in NCBIs GenBank nucleotide database.

MATERIAL AND METHODS

Sample collection and isolates

Surveys were conducted between 2013 and 2014 in the Reserva Florestal Mata do Paraíso (Viçosa, Minas Gerais), the campus of the Universidade Federal de Viçosa (Viçosa, Minas Gerais) and neighbouring areas in the municipality of Viçosa, Floresta Nacional de Paraopeba (Paraopeba, Minas Gerais), Estação Ecológica de Águas Emendadas (Distrito Federal, Brasília), Parque Nacional da Chapada dos Veadeiros (Alto Paraíso de Goiás, Goiás), Instituto Agronômico de Campinas (Campinas, São Paulo), municipality of Lavras (Minas Gerais) and Nova Friburgo (Rio de Janeiro). Samples with cercosporoid leaf spot symptoms were collected, dried in a plant press, and taken to the laboratory. Fungal isolations were performed by direct transfer of fungal structures onto plates containing vegetable broth agar (VBA) as described by Pereira et al. (2003) or 2 % potato-dextrose agar (PDA; HiMedia). Axenic cultures were preserved on potato-carrot agar (PCA) slants or on silica gel and were deposited in the culture collection of the Universidade Federal de Viçosa, Coleção Oswaldo Almeida Drummond (COAD). Representative specimens were deposited at the Fungarium of the Universidade Federal de Viçosa (VIC) and CBS Fungarium (CBS H).

Morphology

Taxonomic descriptions were based on observations of fungal structures present on plant specimens. Samples with cercosporoid leaf spot symptoms were viewed under a Nikon® SMZ 1 000 dissecting microscope. Morphological structures were removed from the lesions with a sterile dissecting needle and mounted in clear lactic acid. Measurements were made at 1 000× magnification using a Carl Zeiss® Axioskop 2 compound microscope. High-resolution photographic images of diseased material, leaf lesions and microscopic fungal structures were captured with a Nikon® digital sight DS-fi1 high definition colour camera. Images of fungal structures were captured and measurements were taken using the Nikon® software NIS-Elements v. 2.34. Adobe Photoshop CS5 was used for the final editing of the acquired images and photographic preparations. Culture descriptions were based on observations of colonies formed in plates containing 2 % malt extract agar (MEA) following incubation at 24 °C for 2–4 wk in the dark in duplicate. Colour terminology followed Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org, Crous et al. 2004).

DNA isolation, PCR amplification and sequencing

Genomic DNA was extracted from mycelium growing on MEA plates at 25 °C for up to 4 wk depending on their growth rate, using the CTAB extraction protocol as outlined by Crous et al. (2009). Four nuclear gene regions were targeted for Polymerase Chain Reaction (PCR) amplification and subsequent sequencing. The Internal Transcribed Spacer (ITS) region was amplified using primers ITS-5 and ITS-4 (White et al. 1990), the Large Subunit (28S nrDNA, LSU) with LR0R (Rehner & Samuels 1994) and LR5 (Vilgalys & Hester 1990), the translation elongation factor 1-alpha (*tef1*) with EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell et al. 1998) and actin (*actA*) with ACT-512F and ACT-783R (Carbone & Kohn 1999). PCR mixtures included the following ingredients for each 12.5 µL reaction: 10–20 ng of template DNA, 1× PCR buffer, 0.63 µL DMSO (99.9 %), 1.5 mM $MgCl_2$, 0.5 µM of each primer, 0.25 mM of each dNTP, 1.0 U BioTaq® DNA polymerase (Bioline GmbH Luckenwalde, Germany). The PCRs were carried out with a MyCycler™ Thermal Cycler (Bio-Rad Laboratories B.V., Veenendal, The Netherlands). Conditions for the PCR amplification consisted of an initial denaturation at 95 °C for 5 min; followed by 40 cycles of denaturation at 95 °C for 30 s; annealing at 52 °C for ITS and LSU, 54 °C for *tef1* or 55 °C for *actA* for 30 s; extension at 72 °C for 1 min and a final extension step at 72 °C for 7 min. Following PCR amplification, amplicons were visualised on 1 % agarose gels to check for product size and purity. The PCR products were sequenced in both directions using the PCR primers and the BigDye® Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA, USA), following the protocol of the manufacturer. DNA sequencing amplicons were purified through Sephadex® G-50 Superfine columns (Sigma Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were run on an ABI Prism 3730xl DNA Analyser (Life Technologies, Carlsbad, CA, USA). The consensus sequences were generated using MEGA v. 6.0.6 (Molecular Evolutionary

Table 1 Collection details and GenBank accession numbers of isolates included in this study.

Species	Culture accession numbers[1]	Collector	Host	Family	Country	GenBank accession numbers[2]			
						LSU	ITS	tef1	actA
Passalora eucalypti	CBS 111318; CPC 1457 (ex-type)	P.W. Crous	*Eucalyptus saligna*	*Myrtaceae*	Brazil	GU253860	GU269845	GU384558	GU320548
Pseudocercospora acericola	CBS 122279	R. Kirschner	*Acer albopurpurascens*	*Aceraceae*	Taiwan	GU253699	GU269650	GU384368	GU320358
P. aeschynomenicola	CPC 25227; COAD 1972 (ex-type)	M. Silva	*Aeschynomene falcata*	*Fabaceae*	Brazil	**KT290173**	**KT290146**	**KT290200**	**KT313501**
P. angolensis	CBS 112933; CPC 4118	M.C. Pretorius	*Citrus* sp.	*Rutaceae*	Zimbabwe	GU214470	AY260063/ GU269836	GU384548	JQ325010
	CBS 149.53 (ex-type)	T. de Carvalho & O. Mendes	*Citrus sinensis*	*Rutaceae*	Angola	JQ324941	JQ324975	JQ324988	JQ325011
P. assamensis	CBS 122467 (ex-type)	I. Buddenhagen	*Musa* cultivar	*Musaceae*	India	GU253705	GU269656	GU384374	GU320364
P. atromarginalis	CBS 114640	C.F. Hill	*Solanum* sp.	*Solanaceae*	New Zealand	GU253706	GU269658	GU384376	GU320365
	CBS 132010; CPC 11372	H.D. Shin	*Solanum nigrum*	*Solanaceae*	South Korea	GU214671	GU269657	GU384375	–
	CPC 25230; COAD 1975	M. Silva	*Solanum americanum*	*Solanaceae*	Brazil	**KT290176**	**KT290149**	**KT290203**	**KT313504**
P. basitruncata	CBS 114664; CPC 1202 (ex-type)	M.J. Wingfield	*Eucalyptus grandis*	*Myrtaceae*	Colombia	GU253710/ DQ204759	DQ267600/ GU269662	DQ211675	DQ147622
P. bixae	CPC 25244; COAD 1563 (ex-epitype)	R.W. Barreto	*Bixa orellana*	*Bixaceae*	Brazil	**KT290180**	**KT290153**	**KT290207**	**KT313508**
P. boehmeriigena	CPC 25243; COAD 1562	R.W. Barreto	*Bohemia nivea*	*Urticaceae*	Brazil	**KT290179**	**KT290152**	**KT290206**	**KT313507**
P. catalpigena	MUCC 743	C. Nakashima & I. Araki	*Catalpa ovata*	*Bignoniaceae*	Japan	GU253731	GU269690	GU384406	GU320395
P. cercidis-chinensis	CBS 132109; CPC 14481 (ex-epitype)	H.D. Shin	*Cercis chinensis*	*Fabaceae*	South Korea	GU253718	GU269670	GU384387	GU320376
P. chamaecristae	CPC 25228; COAD 1973 (ex-epitype)	M. Silva	*Chamaecrista* sp.	*Fabaceae*	Brazil	**KT290174**	**KT290147**	**KT290201**	**KT313502**
P. chengtuensis	CBS 131924; CPC 10696	H.D. Shin	*Lycium chinense*	*Solanaceae*	South Korea	JQ324942	GU269673	GU384390	GU320379
P. contraria	CBS 132108; CPC 14714	H.D. Shin	*Dioscorea quinqueloba*	*Dioscoreaceae*	South Korea	JQ324945	GU269677	GU384394	GU320385
P. cordiana	CBS 114685; CPC 2552 (ex-type)	P.W. Crous & R.L. Benchimol	*Cordia goeldiana*	*Boraginaceae*	Brazil	GU214472	AF362054/ GU269681	GU384398	GU320387
P. corylopsidis	MUCC 874	T. Kobayashi & C. Nakashima	*Hamamelis japonica*	*Hamamelidaceae*	Japan	GU253757	GU269721	GU384437	GU320425
	MUCC 908 (ex-epitype)	C. Nakashima & E. Imaizumi	*Corylopsis spicata*	*Hamamelidaceae*	Japan	GU253727	GU269684	GU384401	GU320390
P. cotoneastri	MUCC 876	T. Kobayashi & C. Nakashima	*Cotoneaster salicifolius*	*Rosaceae*	Japan	GU253728	GU269685	GU384402	GU320391
P. crousii	CBS 119487	C.F. Hill	*Eucalyptus* sp.	*Myrtaceae*	New Zealand	GU253729	GU269686	GU384403	GU320392
P. cruenta	CBS 132021; CPC 10846	H. Booker	*Vigna* sp.	*Fabaceae*	Trinidad	GU214673	GU269688	GU384404	JQ325012
P. diplusodonii	CPC 25179; COAD 1476 (ex-type)	M. Silva	*Diplusodon* sp.	*Lythraceae*	Brazil	**KT290162**	**KT290135**	**KT290189**	**KT313490**
P. elaeocarpi	MUCC 925	C. Nakashima	*Elaeocarpus* sp.	*Elaeocarpaceae*	Japan	GU253740	GU269701	GU384417	GU320405
P. emmotunicola	CPC 25187; COAD 1491 (ex-type)	M. Silva	*Emmotum nitens*	*Icacinaceae*	Brazil	**KT290163**	**KT290136**	**KT290190**	**KT313491**
P. euphorbiacearum	CPC 25222; COAD 1537	M. Silva	*Dalechampia* sp.	*Euphorbiaceae*	Brazil	**KT290172**	**KT290145**	**KT290199**	**KT313503**
P. eustomatis	CBS 110822	G. Dal Bello	*Eustroma grandiflorum*	*Gentianaceae*	Argentina	GU253744	GU269705	GU384421	GU320409
P. exilis	CPC 25193; COAD 1501 (ex-epitype)	M. Silva	*Chamaecrista orbiculata*	*Fabaceae*	Brazil	**KT290166**	**KT290139**	**KT290193**	**KT313494**
P. fijiensis	CBS 120258; CIRAD 86 (ex-epitype)	J. Carlier	*Musa* sp.	*Musaceae*	Cameroon	JQ324952	EU514248	Genome[3]	Genome[3]
	MUCC 792	T. Kobayashi & C. Nakashima	*Musa* cultivar	*Musaceae*	Japan	GU253776	GU269748	JQ324994	GU320450
P. fukuokaensis	CBS 132111; CPC 14689	H.D. Shin	*Styrax japonicus*	*Styracaceae*	South Korea	GU253750	GU269713	GU384429	GU320417
	MUCC 887 (ex-epitype)	T. Kobayashi	*Styrax japonicus*	*Styracaceae*	Japan	GU253751	GU269714	GU384430	GU320418
P. fuligena	CBS 132017; CPC 12296	Z. Mersha	*Lycopersicon* sp.	*Solanaceae*	Thailand	JQ324953	GU269711	GU384427	GU320415
	MUCC 533	C. Nakashima	*Lycopersicon esculentum*	*Solanaceae*	Japan	GU253749	GU269712	GU384428	GU320416
P. glauca	CBS 131884; CPC 10062	H.D. Shin	*Albizzia julibrissin*	*Fabaceae*	South Korea	GU253752	GU269715	GU384431	GU320419
P. guianensis	MUCC 855	C. Nakashima & T. Akashi	*Lantana camara*	*Verbenaceae*	Japan	GU253755	GU269719	GU384435	GU320423
	MUCC 879	C. Nakashima	*Lantana camara*	*Verbenaceae*	Japan	GU253756	GU269720	GU384436	GU320424
P. latens	MUCC 763	C. Nakashima & T. Akashi	*Lespedeza wilfordii*	*Fabaceae*	Japan	GU253763	GU269732	GU384445	GU320434
P. lonicericola	MUCC 889 (ex-neotype)	T. Kobayashi	*Lonicera gracilipes* var. *glabra*	*Caprifoliaceae*	Japan	GU253766	GU269736	JQ324999	GU320438
P. luzardii	CPC 2556	A.C. Alfenas	*Hancornia speciosa*	*Apocynaceae*	Brazil	GU214477	AF362057/ GU269738	GU384450	GU320440
	CPC 25196; COAD 1505 (ex-epitype)	M. Silva	*Harcornia speciosa*	*Apocynaceae*	Brazil	**KT290167**	**KT290140**	**KT290194**	**KT313495**
P. lythri	CBS 132115; CPC 14588 (ex-epitype)	H.D. Shin	*Lythrum salicaria*	*Lythraceae*	South Korea	GU253771	GU269742	GU384454	GU320444
	MUCC 865	I. Araki & M. Harada	*Lythrum salicaria*	*Lythraceae*	Japan	GU253772	GU269743	GU384455	GU320445
P. macrospora	CBS 114696; CPC 2553	P.W. Crous & R.L. Benchimol	*Bertholletia excelsa*	*Lecythidaceae*	Brazil	GU214478	AF362055/ GU269745	GU384457	GU320447
P. mali	MUCC 886	T. Kobayashi	*Malus sieboldii*	*Rosaceae*	Japan	GU253773	GU269744	GU384456	GU320446

P. manihotii	CPC 25219; COAD 1534 (ex-type)	M. Silva	*Manihot* sp.	*Euphorbiaceae*	Brazil	**KT290171**	**KT290144**	**KT290198**	**KT313499**
P. nephrolepidis	CBS 119121	R. Kirschner	*Nephrolepis auriculata*	*Oleandraceae*	Taiwan	GU253779	GU269751	GU384462	GU320453
P. nogalesii	CBS 115022	C.F. Hill	*Chamaecytisus proliferus*	*Fabaceae*	New Zealand	JQ324960	GU269752	GU384463	GU320454
P. norchiensis	CBS 114641	C.F. Hill	*Rubus* sp.	*Rosaceae*	New Zealand	GU253794	GU269772	GU384484	GU320475
	CBS 120738; CPC 13049 (ex-type)	W. Gams	*Eucalyptus* sp.	*Myrtaceae*	Italy	GU253780	EF394859/ GU269753	GU384464	GU320455
P. oenotherae	CBS 131885; CPC 10290	H.D. Shin	*Oenothera odorata*	*Onagraceae*	South Korea	JQ324961	GU269856	GU384567	GU320559
	CBS 131920; CPC 10630	H.D. Shin	*Oenothera odorata*	*Onagraceae*	South Korea	GU253781	GU269755	GU384466	GU320457
P. pallida	CBS 131889; CPC 10776	H.D. Shin	*Campsis grandiflora*	*Bignoniaceae*	South Korea	GU214680	GU269758	GU384469	GU320459
P. paraguayensis	CBS 111286; CPC 1459	P.W. Crous	*Eucalyptus nitens*	*Myrtaceae*	Brazil	GU214479/ DQ204764	DQ267602	DQ211680	DQ147606
	CBS 111317; CPC 1458	P.W. Crous	*Eucalyptus nitens*	*Myrtaceae*	Brazil	GQ852634	JQ324978	GU384522	JQ325021
P. perae	CPC 25171, COAD 1465 (ex-type)	M. Silva	*Pera glabrata*	*Euphorbiaceae*	Brazil	**KT290159**	**KT290132**	**KT290186**	**KT313487**
P. pini-densiflorae	MUCC 534	Y. Tokushige	*Pinus thunbergii*	*Pinaceae*	Japan	GU253785	GU269760	GU384471	GU320461
P. piperis	FBR 151	R.E. Hanada	*Piper aduncum*	*Piperaceae*	Brazil	JX875063	JX875062	JX896123	–
P. planaltinensis	CPC 25189; COAD 1495 (ex-type)	M. Silva	*Chamaecrista* sp.	*Fabaceae*	Brazil	**KT290164**	**KT290137**	**KT290191**	**KT313492**
P. plumeriifolii	CPC 25191; COAD 1498 (ex-epitype)	M. Silva	*Himatanthus obovatus*	*Apocynaceae*	Brazil	**KT290165**	**KT290138**	**KT290192**	**KT313493**
P. plunkettii	CPC 26081; COAD 1548	R.W. Barreto	*Mikania hirsutissima*	*Asteraceae*	Brazil	**KT290178**	**KT290151**	**KT290205**	**KT313506**
P. pothomorphes	CPC 25166; COAD 1450 (ex-type)	O.L. Pereira	*Pothomorphe umbellata*	*Piperaceae*	Brazil	**KT290158**	**KT290131**	**KT290185**	**KT313486**
P. pouzolziae	CBS 122280	R. Kirschner	*Gonostegia hirta*	*Urticaceae*	Taiwan	GU253786	GU269761	GU384472	GU320462
P. prunicola	CBS 132107; CPC 14511	H.D. Shin	*Prunus* x *yedoensis*	*Rosaceae*	South Korea	GU253723	GU269676	GU384393	GU320382
P. purpurea	CBS 114163; CPC 1664	P.W. Crous	*Persea americana*	*Lauraceae*	Mexico	GU253804	GU269783	GU384494	GU320486
P. pyracanthae	MUCC 892	T. Kobayashi & C. Nakashima	*Pyracantha angustifolia*	*Rosaceae*	Japan	GU253792	GU269767	GU384479	GU320470
P. pyracanthigena	CBS 131589; CPC 10808 (ex-type)	H.D. Shin	*Pyracantha angustifolia*	*Rosaceae*	South Korea	–	GU269766	GU384478	GU320469
P. rhamnellae	CBS 131590; CPC 12500 (ex-type)	H.D. Shin	*Rhamnella frangulioides*	*Rhamnaceae*	South Korea	GU253813	GU269795	GU384505	GU320496
P. rhapisicola	CBS 282.66	K. Tubaki	*Rhapis flabellifornis*	*Arecaceae*	Japan	GU253793	GU269770	GU384482	GU320473
P. richardsoniicola	CPC 25248; COAD 1568 (ex-epitype)	R.W. Barreto	*Richardia brasiliensis*	*Rubiaceae*	Brazil	**KT290181**	**KT290154**	**KT290208**	**KT313509**
P. rigidae	CPC 25175; COAD 1472 (ex-epitype)	M. Silva	*Palicourea rigida*	*Rubiaceae*	Brazil	**KT290161**	**KT290134**	**KT290188**	**KT313489**
P. rubi	MUCC 875	T. Kobayashi & C. Nakashima	*Rubus allegheniensis*	*Rosaceae*	Japan	GU253795	GU269773	GU384485	GU320476
P. sawadae	CBS 115024	C.F. Hill	*Psidium guajava*	*Myrtaceae*	New Zealand	JQ324967	GU269775	–	GU320478
P. sennae-multijugae	CPC 25206; COAD 1519 (ex-type)	M. Silva	*Senna multijuga*	*Fabaceae*	Brazil	**KT290169**	**KT290142**	**KT290196**	**KT313497**
P. solani-pseudocapsicicola	CPC 25229; COAD 1974 (ex-type)	M. Silva	*Solanum pseudocapsicum*	*Solanaceae*	Brazil	**KT290175**	**KT290148**	**KT290202**	**KT313503**
P. sordida	MUCC 913	C. Nakashima & E. Imaizumi	*Campsis radicans*	*Bignoniaceae*	Japan	GU253798	GU269777	GU384488	GU320480
Pseudocercospora sp.	CBS 110998; CPC 1054	M.J. Wingfield	*Eucalyptus grandis*	*Myrtaceae*	South Africa	GU253799	GU269778	GU384489	GU320481
	CBS 111373; CPC 1493	M.J. Wingfield	*Eucalyptus globulus*	*Myrtaceae*	Uruguay	GU253803	GU269782	GU384493	GU320485
	CBS 113387	A. den Breeyen	*Lantana camara*	*Verbenaceae*	Jamaica	GU253754	GU269718	GU384434	GU320422
	CBS 131922; CPC 10645	P.W. Crous	–	–	Brazil	GU253700	GU269651	GU384369	GU320359
P. stephanandrae	MUCC 914 (ex-epitype)	C. Nakashima & E. Imaizumi	*Stephanandra incisa*	*Rosaceae*	Japan	GU253831	GU269814	GU384526	GU320516
P. stizolobii	CPC 25217; COAD 1532	M. Silva	*Mucuna aterrima*	*Fabaceae*	Brazil	**KT290170**	**KT290143**	**KT290197**	**KT313498**
P. struthanthi	CPC 25199; COAD 1512 (ex-epitype)	M. Silva	*Struthanthus flexicaulis*	*Loranthaceae*	Brazil	**KT290168**	**KT290141**	**KT290195**	**KT313496**
P. subsessilis	CBS 136.94	R.F. Castaneda	–	–	Cuba	GU253832	GU269815	GU384527	GU320517
P. subtorulosa	CBS 117230	R. Kirschner	*Melicope* sp.	*Rutaceae*	Taiwan	GU253833	GU269816	GU384528	GU320518
P. tecomicola	CPC 25260; COAD 1585	R.W. Barreto	*Tecoma stans*	*Bignoniaceae*	Brazil	**KT290183**	**KT290156**	**KT290209**	**KT313511**
P. trinidadensis	CPC 26082; COAD 1756	R.W. Barreto	*Croton urucurana*	*Euphorbiacea*	Brazil	**KT290184**	**KT290157**	**KT290210**	–
P. udagawana	CBS 131931; CPC 10799	H.D. Shin	*Hovenia dulcis*	*Rhamnaceae*	South Korea	–	GU269824	GU384537	GU320527
P. variicolor	MUCC 746	C. Nakashima & I. Araki	*Paeonia lactiflora* var. *trichocarpa*	*Paeoniaceae*	Japan	GU253843	GU269826	GU384538	GU320530
P. vassobiae	CPC 25251; COAD 1572 (ex-type)	R.W. Barreto	*Vassobia breviflora*	*Solanaceae*	Brazil	**KT290182**	**KT290155**	–	**KT313510**
P. viburnigena	CBS 125998; CPC 15249 (ex-epitype)	M.K. Crous	*Viburnum davidii*	*Caprifoliaceae*	Netherlands	GU253827	GU269809	GU384520	GU320512
P. weigelae	MUCC 899	T. Kobayashi & Y. Kobayashi	*Weigela coraeensis*	*Caprifoliaceae*	Japan	GU253847	GU269831	GU384543	GU320535
P. wulffiae	CPC 25232; COAD 1976 (ex-type)	M. Silva	*Wulffia stenoglossa*	*Asteraceae*	Brazil	**KT290177**	**KT290150**	**KT290204**	**KT313505**
P. xylopiae	CPC 25173; COAD 1469 (ex-type)	M. Silva	*Xylopia aromatica*	*Annonaceae*	Brazil	**KT290160**	**KT290133**	**KT290187**	**KT313488**
P. zelkovae	CBS 132118; CPC 14717	H.D. Shin	*Zelkova serrata*	*Ulmaceae*	South Korea	GU253850	GU269834	GU384546	JQ325028
	MUCC 872	T. Kobayashi & C. Nakashima	*Zelkova serrata*	*Ulmaceae*	Japan	GU253851	GU269835	GU384547	GU320537

[1] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; COAD: Coleção de Cultura Octávio Almeida Drummond, Universidade Ferderal de Viçosa, Viçosa, Brazil; CPC: Culture collection of Pedro Crous, housed at CBS; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan.

[2] LSU: partial 28S nrRNA gene; ITS: internal transcribed spacer regions 1 & 2 including 5.8S nrRNA gene; *tef1*: partial translation elongation factor 1-alpha gene; *actA*: partial actin gene.

[3] Sequence for this locus obtained from: http://genome.jgi-psf.org/Mycfi1/Mycfi1.home.html.

Genetics Analyses) (Tamura et al. 2013). All sequences were checked manually, and nucleotides with ambiguous positions were clarified using both primer direction sequences.

Phylogenetic analyses

Consensus sequences were compared against NCBIs GenBank nucleotide database using their megaBLAST algorithm. The most similar sequences were downloaded in FASTA format and the sequence datasets for the four genomic loci were aligned individually using the MAFFT v. 7 online portal (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh & Standley 2013). In addition, the combined sequence alignment of Crous et al. (2013a) was downloaded from TreeBASE (Study S12805) and used as an initial reference alignment for species identification. Resulting sequence alignments were manually checked and adjusted in MEGA v. 6.06 and were concatenated with Mesquite v. 2.75 (Maddison & Maddison 2011). A phylogenetic re-construction was conducted on the aligned LSU dataset to determine generic relationships. For the LSU alignment, MrModeltest v. 2.2 (Nylander 2004) was used to select the optimal model of nucleotide substitution prior to the Bayesian Inference (BI) analysis using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003). The general time-reversible model of evolution (Rodriguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) was used. Subsequently, a species-level phylogeny was derived from a concatenated ITS (alignment position 1–482), *actA* (alignment position 510–714) and *tef1* (alignment position 720–1270) dataset using MrModeltest v. 2.2 to select the optimal model of nucleotide substitution for each

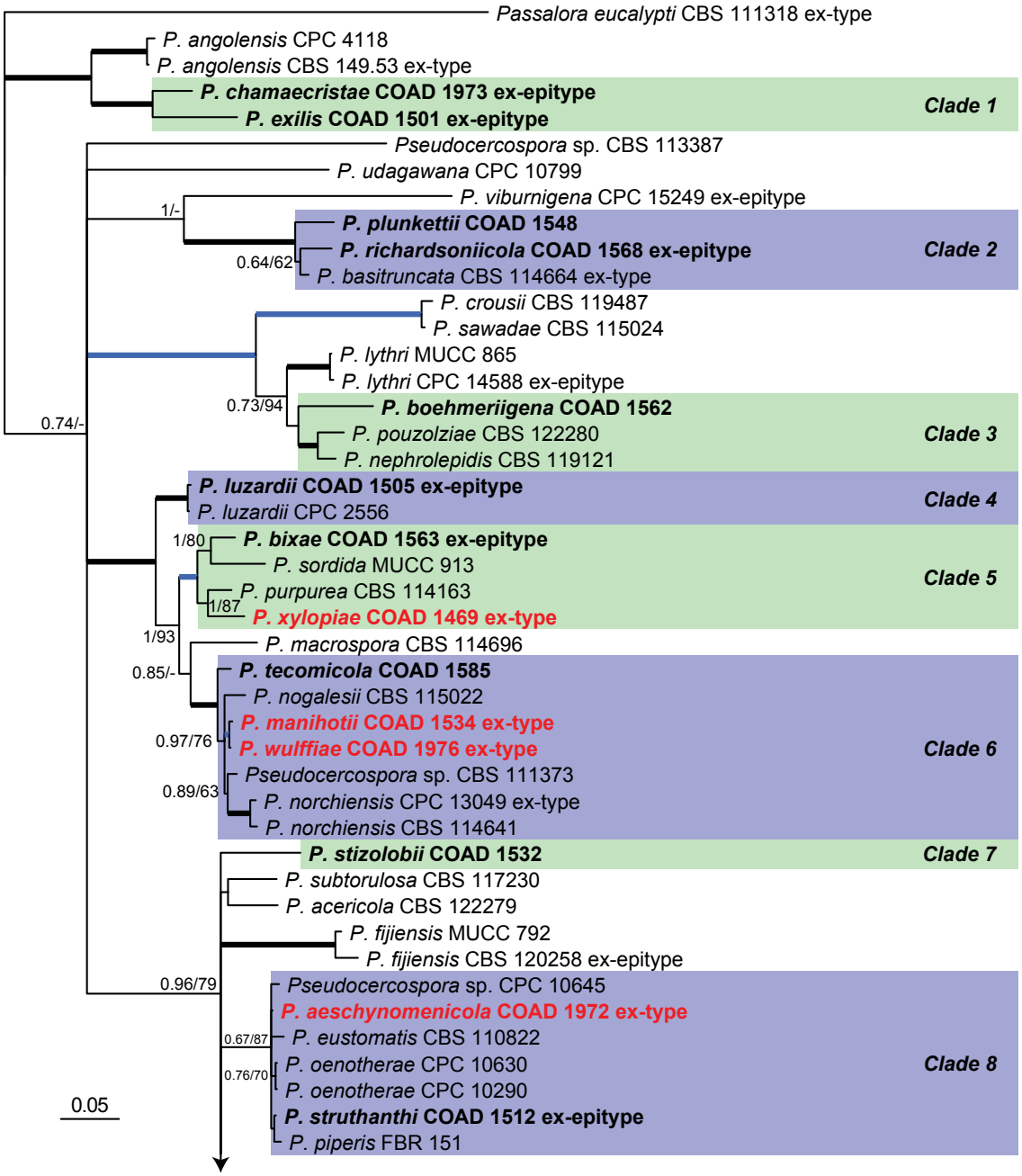
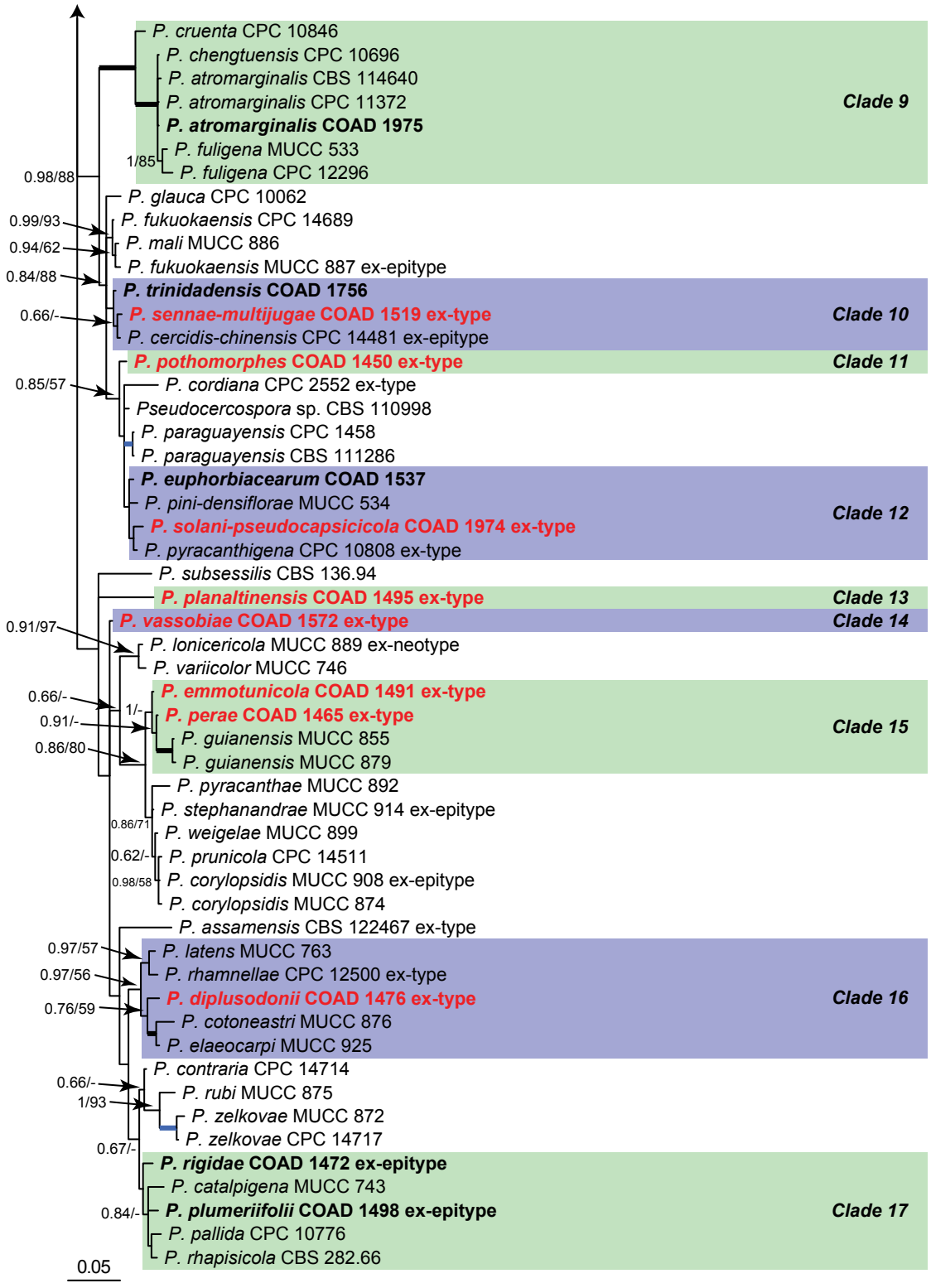


Fig. 1 The Bayesian phylogenetic tree inferred from DNA sequence data from the multigene alignment (ITS, *actA* and *tef1*) of *Pseudocercospora* species. Species from Brazil are in **bold** face and in coloured blocks with clade numbers for reference in the species notes. Novel species are indicated in red colour and the type status of strains are indicated next to the culture collection number. Bayesian posterior probabilities (BPP, > 0.60) and parsimony bootstrap support (PBS, > 60) values are indicated at the nodes (BPP/PBS). Thickened black branches represent nodes which are fully supported in both analyses (BPP = 1.00 / PBS = 100), while thickened blue branches were highly supported in both analyses (BPP = > 0.94 / PBS = > 94). The tree was rooted to *Passalora eucalypti* CBS 111318.

locus based on the Akaike Information Criterion prior to the BI analysis. Gaps longer than 10 nucleotides were excluded from the analyses (*tef1* only, see alignment in TreeBASE). The results of MrModeltest recommended a HKY85 model for *tef1*, and a GTR model for ITS and *actA*. For *actA* and *tef1*, a dirichlet (1,1,1,1) state frequency distribution was set and for ITS a fixed (equal) state frequency distribution, and for all three loci an inverse gamma distributed rate variation. Two sets of four MCMC (Markov Chain Monte Carlo) chains were run simultaneously, starting from random trees and lasting until the critical value for the topological convergence diagnostic reached 0.01. Trees were sampled every 1 000 generations and the first 25 % of the trees were discarded as the burn-in phase for each analysis and posterior probabilities (Rannala & Yang 1996) were determined from the remaining trees and are presented on the left of each node (Fig. 1). Sequences derived from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1), the alignments and trees in TreeBASE (www.treebase.org) (S17995). A parsimony analysis was also performed on the combined alignment as described by Arzanlou et al. (2008). The resulting phylogenetic tree was printed with Geneious v. 7.1.8 (http://www.geneious.com, Kearse et al. 2012), and the layout of the tree for publication was carried out using Adobe Illustrator v. CS5.

Fig. 1 (cont.)



RESULTS

Isolates

A total of 42 specimens bearing *Pseudocercospora* colonies were obtained in the surveys. Twenty-seven species of *Pseudocercospora* were recognised as being present in these samples. Hosts belonged to the following families: *Annonaceae*, *Apocynaceae*, *Asteraceae*, *Bignoniaceae*, *Bixaceae*, *Euphorbiaceae*, *Fabaceae*, *Icacinaceae*, *Loranthaceae*, *Lythraceae*, *Piperaceae*, *Rubiaceae*, *Solanaceae* and *Urticaceae*. These hosts included weeds, agricultural species, forestry species and native plants from the Mata Alântica and the Cerrado.

Phylogeny

The LSU alignment consisted of 69 strains (including the outgroup sequence) and 713 characters were included in the analysis. The alignment had 97 unique site patterns. The LSU phylogeny (TreeBASE S17995), revealed that all strains obtained from the survey and recognised as having the morphological features of members of *Pseudocercospora* clustered within *Pseudocercospora* s.str. (data not shown, see TreeBASE). These were subsequently included in the combined *actA*, *tef1* and ITS alignment for species level identification (Fig. 1).

For the species level analysis of the 27 *Pseudocercospora* isolates from Brazil, DNA sequence data from the *actA*, *tef1* and ITS gene regions were combined for the Bayesian analyses. The concatenated alignment contained a total of 97 strains (70 strains from NCBI and 27 strains from this study) (Table 1). *Passalora eucalypti* (CBS 111318) served as the outgroup taxon. The final aligned sequences of the ITS (482 characters), *actA* (205 characters) and *tef1* (373 characters) gene regions had a total length of 1 060 characters (including alignment gaps) which were included in the analyses. The gaps in the alignment were treated as fifth base for the parsimony analyses and from the analysed characters 504 were constant (ITS: 335, *actA*: 90, *tef1*: 79), 167 were variable and parsimony-uninformative (ITS: 72, *actA*: 23, *tef1*: 72) and 389 were parsimony informative (ITS: 75, *actA*: 92, *tef1*: 222). All genes were also assessed individually using Bayesian analyses (data not shown, see TreeBASE). The Bayesian analysis of the combined alignment, based on 543 unique site patterns (ITS: 141, *actA*: 120, *tef1*: 282) lasted 7 055 000 generations and the consensus trees and posterior probabilities (PP) were calculated from the 10 584 trees left after discarding 3 528 trees (the first 25 % of the generations) for burn-in (Fig. 1). A maximum of 1 000 equally most parsimonious trees (Tree Length = 2 288, CI = 0.481, RI = 0.817, RC = 0.393) were saved from the parsimony analysis (data not shown, see TreeBASE). Overall, the same terminal clades were found and the biggest differences between the parsimony tree and Bayesian tree were observed as rearrangements in the backbone of the tree, affecting the order of clades and not the species delimitation. Parsimony bootstrap support values (PBS) are plotted at the nodes, which are congruent between the parsimony bootstrap tree and the Bayesian phylogeny (Fig. 1).

The ITS region had limited resolution for differentiating species, resolving only 12 of the included 82 species, whereas the Bayesian trees based on the *actA* and *tef1* regions resolved 41 and 38 out of 80 (for two species of each locus sequence data were missing) species respectively (data not shown, see TreeBASE). Only 11 species were supported as being distinct by all three loci in the individual Bayesian analyses, whereas 32 species were not distinct based on any of the individual loci. Details about the performance of the different loci are provided under the species notes below.

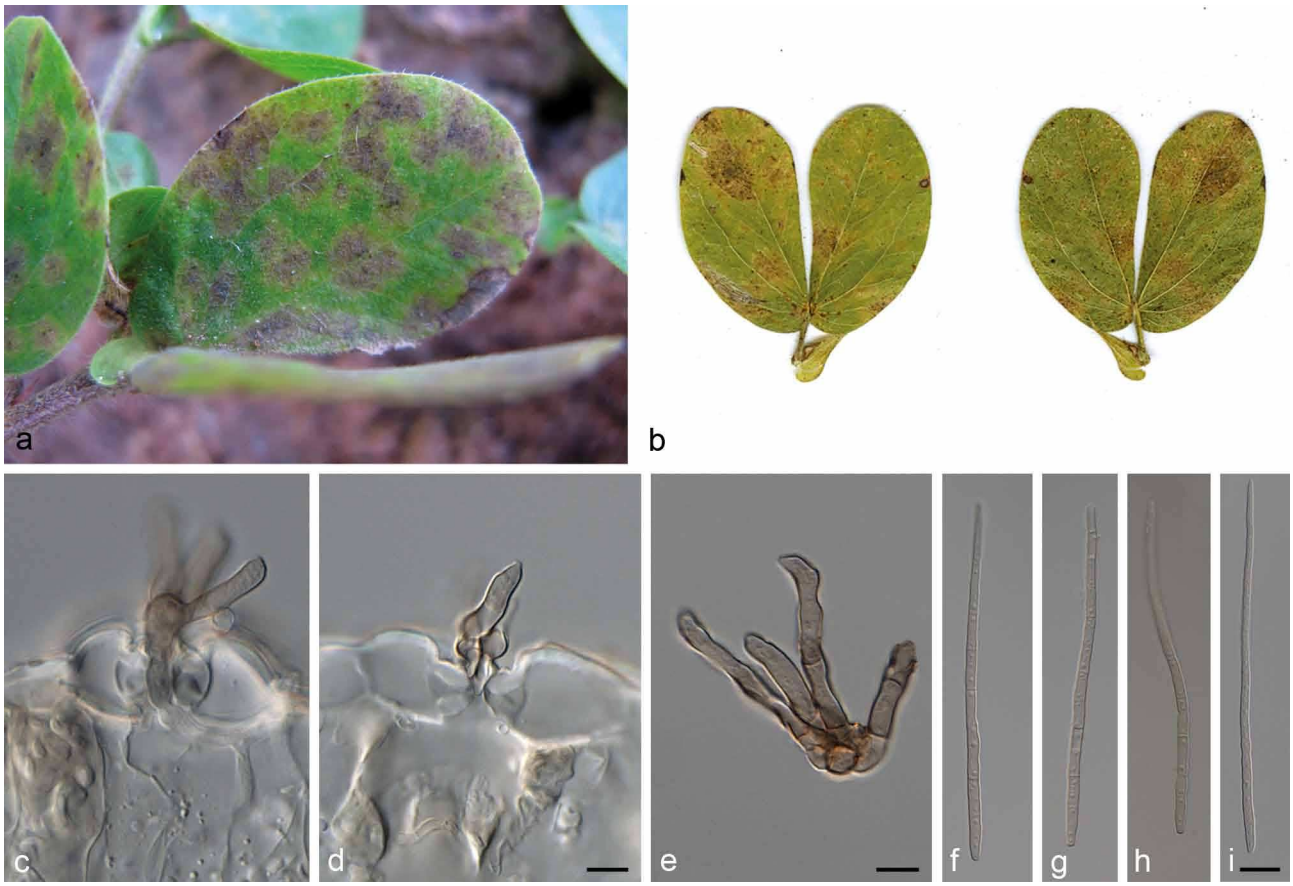


Fig. 2 *Pseudocercospora aeschynomenicola* (VIC 42805). a. *Aeschynomene falcata* with leaf spots; b. leaf spots on upper and lower leaf surface; c, d. conidiophores emerging through stomata; e. conidiogenous cells; f–i. conidia. — Scale bars: c–i = 10 µm.

Taxonomy

Based on phylogenetic analyses, host data and morphological comparisons (Consolidated Species Concept), the *Pseudocercospora* isolates from Brazil could be assigned to 27 different taxa (Fig. 1), revealing a rich diversity among the *Pseudocercospora* spp. in this country. Among these, 12 species namely *P. aeschynomenicola*, *P. diplusodonii*, *P. emmotunicola*, *P. manihotii*, *P. perae*, *P. planaltinensis*, *P. pothomorphes*, *P. sennae-multijugae*, *P. solani-pseudocapsicicola*, *P. vassobiae*, *P. wulffiae* and *P. xylopiae* were treated as new and are described below. Epitypes were designated for a further eight species namely *P. bixae*, *P. chamaecristae*, *P. exilis*, *P. luzardii*, *P. plumeriifolii*, *P. richardsoniicola*, *P. rigidae* and *P. struthanthi*, and three species namely *P. boehmeriigena*, *P. euphorbiacearum* and *P. tecomicola* were found to represent new reports for Brazil, and three species represented new host associations. Additionally four isolates were shown to belong to known species. Brazilian isolates were distributed across the whole phylogeny and therefore did not cluster following a common geographic origin. The clades containing the Brazilian *Pseudocercospora* isolates are highlighted in the phylogenetic tree (Fig. 1). The phylogenetic relation of the various isolates is discussed in the species notes, where applicable.

Pseudocercospora aeschynomenicola Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813624; Fig. 2

Etymology. Name derived from the plant host genus *Aeschynomene*, from which it was collected.

Leaf spots amphigenous, irregular, scattered, grey-brown surrounded by a chlorotic halo, 1–5 mm diam. *Internal mycelium*, subhyaline, branched, septate, smooth, 2–2.5 µm diam. *External mycelium* absent. *Stromata* absent or small, substomatal, composed of brown *textura angularis*. *Conidiophores* hypophyllous, solitary or in small fascicles, loose, emerging through stomata, cylindrical, 12–42.5 × 3–5 µm, 0–4-septate, straight to geniculate-sinuous, unbranched, pale to medium brown, smooth. *Conidiogenous cells* terminal, integrated, proliferating sympodially and percurrently, subcylindrical, 8–21 × 3–5 µm, pale brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, finely guttulate, brown, smooth, subcylindrical-filiform, straight to sigmoid, 35–167 × 2–3.5 µm, apex obtuse to subacute, base obconically truncate, 2.5–3 µm wide, 4–14-septate; hila unthickened, not darkened, 1–2 µm diam.

Culture characteristics — Very slow-growing (16–18 mm diam after 20 d), convex with smooth to slightly irregularly lobate margins, aerial mycelium velvety, olivaceous grey centrally, olivaceous black periphery, iron-grey to green-black reverse, sterile.

Specimens examined. Brazil, Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of *Aeschynomene falcata* (*Fabaceae*), 22 Jan. 2014, *M. Silva* (holotype VIC 42805, culture ex-type COAD 1972; isotype CBS H-22164, culture ex-isotype CPC 25227).

Notes — Only one cercosporoid fungus is thus far known to occur on *Aeschynomene falcata*, namely *Semipseudocercospora aeschynomenes* from Brazil (Crous & Braun 2003). The genus *Semipseudocercospora* is distinguished from *Pseudocercospora* by having "short cylindrical pegs on which the conidia are borne, aggregated towards the tip of the conidiophores" (Yen 1983) and having ellipsoid-ovoid, short conidia with attenuated bases (Yen 1983, Crous & Braun 2003). The morphology of the fungus collected on *A. falcata* clearly places it in *Pseudocercospora*. Phylogenetically, *P. aeschynomenicola* clustered between *Pseudocercospora* sp. from an unknown host (CPC 10645) and *P. eustomatis* on *Eustroma glandiflorum* (*Gentianaceae*) (Fig. 1, clade 8). It is not possible to distinguish *P. aeschynomenicola* from numerous other *Pseudocercospora* spp. based solely on an ITS or *actA* phylogeny, and in the *tef1* phylogeny it cannot be distinguished from *Pseudocercospora* sp. CPC 10645, *P. piperis* (strain FBR 151) and *P. struthanthi*.

Pseudocercospora atromarginalis (G.F. Atk.) Deighton, Mycol. Pap. 140: 139. 1976

Basionym. *Cercospora atromarginalis* G.F. Atk., J. Elisha Mitchell Sci. Soc. 8: 59. 1892.

Descriptions & Illustrations — Deighton (1976: 139, f. 237), Hsieh & Goh (1990: 313, f. 237).

Specimen examined. Brazil, Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of *Solanum americanum* (*Solanaceae*), 23 Jan. 2014, *M. Silva* (CBS H-22167, VIC 42808, cultures COAD 1975, CPC 25230).

Notes — *Pseudocercospora atromarginalis* and *P. chengtuensis*, both described on *Solanaceae*, could not be distinguished based on the phylogenetic analysis of the combined alignment (Fig. 1, clade 9). This was also observed by Crous et al. (2013a) and Bakhshi et al. (2014). Furthermore, these species are morphologically similar (Crous et al. 2013a). To confirm whether they are synonymous or distinct species it is necessary to re-collect samples from the type localities of both species. It is not possible to distinguish *P. atromarginalis* from *P. chengtuensis*, *P. fuligena* or *P. stizolobii* based solely on ITS data, or from *P. chengtuensis*, *P. cruenta* or *P. fuligena* based solely on a *tef1* phylogeny. In the *actA* phylogeny it cannot be distinguished from *P. chengtuensis*, and is it very closely related to *P. fuligena*.

Pseudocercospora bixae (Allesch. & F. Noack) Crous et al., Mycotaxon 64: 418. 1997 — Fig. 3

Basionym. *Cercospora bixae* Allesch. & F. Noack, Bol. Inst. Agron. São Paulo 85. 1898.

Leaf spots amphigenous, irregular, pale brown surrounded by an ill-defined black margin followed by a chlorotic halo, 4–12 mm diam. *Internal mycelium*, subhyaline, septate, branched, smooth, 3–4 µm diam. *External mycelium* absent. *Stromata* well-developed, semi-immersed, 12–32 × 22–50 µm, composed of medium brown *textura angularis*. *Conidiophores* amphigenous, in loose to dense fascicles arising from the upper cells of the stroma, subcylindrical, 12–50 × 2.5–4 µm, 0–3-septate, straight to variously curved, unbranched, medium brown, smooth. *Conidiogenous cells* terminal, integrated, subcylindrical, proliferating sympodially and percurrently, 5–31 × 2.5–4 µm. *Conidiogenous loci* inconspicuous, unthickened, not darkened, somewhat refractive. *Conidia* solitary, finely guttulate, pale brown, smooth, obclavate, straight to slightly curved, 34–99 × 3–4 µm, apex subobtuse, base obconically truncate, 2–3.5 µm wide, 2–7-septate; hila unthickened, not darkened, 1.5–2.5 µm diam.

Culture characteristics — Slow-growing (23–26 mm diam after 20 d); circular, raised, convex, margin smooth, irregular, aerial mycelium velvety, olivaceous grey, reverse olivaceous black, sterile.

Specimens examined. Brazil, São Paulo, Instituto Agronômico de Campinas, on leaves of *Bixa orellana* (*Bixaceae*), Sept. 1897, *F. Noack* (holotype IACM); Minas Gerais, Viçosa, Universidade Federal de Viçosa, on leaves of *Bixa orellana*, 21 May 2013, *R.W. Barreto* (epitype designated here VIC 41563, MBT202072, culture ex-epitype COAD 1563; iso-epitype CBS H-22171, culture ex-isoepitype CPC 25244).

Notes — The epitype of *P. bixae*, designated here, is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same country as the type. No DNA sequence data were available

for *P. bixae* until now. Phylogenetically, *P. bixae* is most similar to *P. sordida* (Fig. 1, clade 5). *Pseudocercospora sordida* occurs on hosts in the *Bignoniaceae*, while *P. bixae* occurs on hosts in the *Bixaceae* (Crous & Braun 2003). Morphologically, the two species are quite distinct. *Pseudocercospora sordida* has longer and wider conidiophores (20–120 × 3.5–5 µm) and longer and wider conidia (20–200 × 3–5.5 µm) than those of *P. bixae* (Deighton 1976). It is not possible to distinguish *P. bixae* from *P. sordida* and *P. luzardii* based solely on ITS data, and it is close to, but distinct from, *P. purpurea* based on the *tef1* phylogeny. In the *actA* phylogeny it is distinct from all other species.

Pseudocercospora boehmeriigena U. Braun, Trudy Bot. Inst. Komarova 20: 42. 1997 — Fig. 4

Basionym. *Cercospora boehmeriae* Peck, Ann. Rep. N.Y. State Mus. Nat. Hist. 34: 48. 1881.

≡ *Pseudocercospora boehmeriae* (Peck) Y.L. Guo & X.L. Liu, Mycosystema 2: 229. 1989. Nom. Illegit., Art. 53.1.

Leaf spots amphigenous, irregular to angular, pale brown to brown, 4–13 mm diam, vein-delimited. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* poorly developed, consisting of a few brown cells. *Conidiophores* epiphyllous, aggregated in loose fascicles, cylindrical, 13–26.5 × 2.5–3.5 µm, 0–2-septate, straight or variously curved, unbranched, pale to brown, smooth. *Conidiogenous cells* terminal, subcylindrical, proliferating sympodially, 6–20 × 2.5–3 µm, brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, guttulate, pale to pale brown, smooth, cylindrical, straight to curved, 50–102 × 3–4.5 µm, apex subobtuse or bluntly rounded, base truncate, 2–4 µm wide, 3–12-septate; hila neither thickened nor darkened, 2–3 µm diam.

Culture characteristics — Very slow-growing (12–14 mm diam after 20 d); corrugated, compressing the medium, raised, erumpent, aerial mycelium sparse, irregularly lobate margins, white and grey, reverse iron-grey, sterile.

Specimen examined. Brazil, Minas Gerais, Viçosa, Universidade Federal de Viçosa (Avicultura), on leaves of *Boehmeria nivea* (*Urticaceae*), 21 May

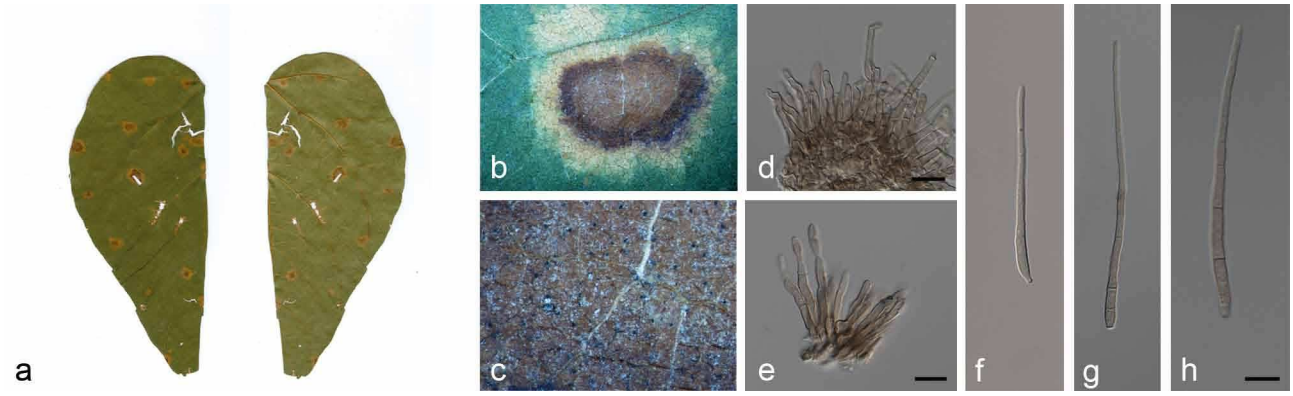


Fig. 3 *Pseudocercospora bixae* (VIC 41563). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. close-up of leaf spot with fruiting; d, e. fasciculate conidiophores and conidiogenous cells; f–h. conidia. — Scale bars: d–h = 10 µm.

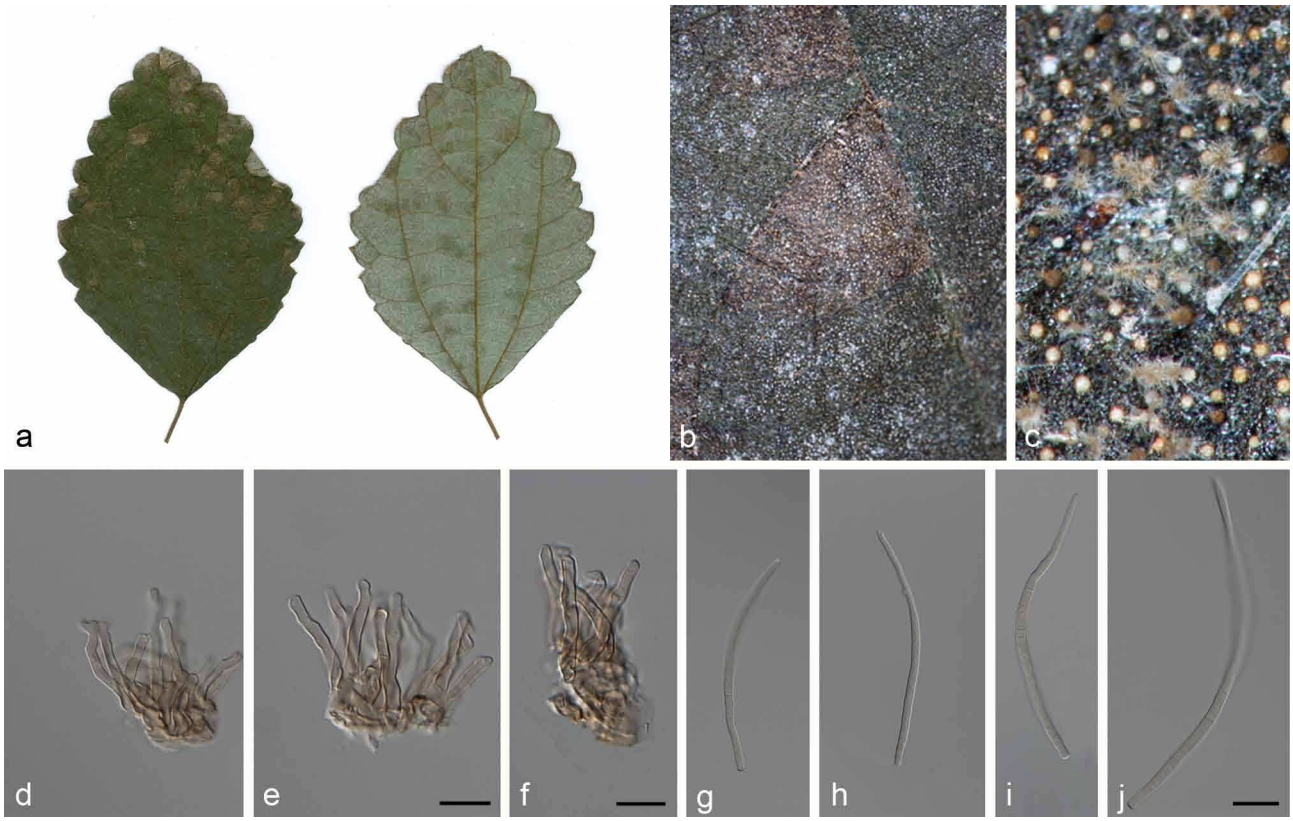


Fig. 4 *Pseudocercospora boehmeriigena* (VIC 41562). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. close-up of leaf spot with fruiting; d, e. conidiophores in a loose fascicle; f. conidiogenous cells; g–j. conidia. — Scale bars: d–j = 10 µm.

2013, *R.W. Barreto* (CBS H-22170, VIC 1562, cultures COAD 41562, CPC 25243).

Notes — The morphology of the Brazilian collection on *Boehmeria nivea* (ramie) fits well with the description of *P. boehmeriigena* (Braun & Mel'nik 1997). This species was previously reported from several countries, including Cambodia, China, Cuba, India and Indonesia (Crous & Braun 2003). This is the first report of *P. boehmeriigena* associated with leaf spots of *B. nivea* in Brazil. Phylogenetically, *P. boehmeriigena* is distinct from other species (Fig. 1, clade 3) and it has a position basal to a clade containing *P. nephrolepidis* and *P. pouzolziae*. It is not possible to distinguish *P. boehmeriigena* from *P. nephrolepidis* and *P. pouzolziae* based solely on ITS data. In the *actA* and *tef1* phylogenies it is distinct from all other species.

Pseudocercospora chamaecristae U. Braun & F.O. Freire, Cryptog. Mycol. 23: 305. 2002 — Fig. 5

Leaf spots amphigenous, irregular, scattered, reddish centrally surrounded by a dark brown border, 1–3 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* immersed, substomatal, 24–46 µm diam, composed of dark brown *textura angularis*. *Conidiophores* hypophyllous, aggregated in dense synnematous conidiomata, subcylindrical, 126–278.5 × 3–4 µm, multiseptate, straight, variously curved or geniculate-sinuous, unbranched, individual conidiophores, brown to medium brown, smooth. *Conidiogenous cells* integrated, terminal, subcylindrical, proliferating sympodially and percurrently, 21–34 × 3–4 µm, pale brown, smooth. *Conidiogenous loci* inconspicuous to subinconspicuous, somewhat refractive. *Conidia* solitary, guttulate, pale brown, smooth, subcylindrical to ellipsoid-fusoid, obclavate, straight to curved, 30–38 × 4–6 µm, apex obtuse, base obconically truncate, 4–5 µm wide, 0–4-septate; hila unthickened, not darkened, 2–3 µm diam.

Culture characteristics — Very slow-growing (6 mm diam after 20 d), raised, stromatic, compressing and cracking the medium, iron-grey, reverse olivaceous black, sterile.

Specimens examined. Brazil, Ceará, Preaoca, Cascavel, on leaves of *Chamaecrista setosa* (*Fabaceae*), 9 Nov. 2000, *F. Freire* (holotype HAL 1718); Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of *Chamaecrista* sp. (*Fabaceae*), 22 Jan. 2014, *M. Silva* (epitype designated here VIC 42806, MBT202015, culture ex-epitype COAD 1973; isoepitype CBS H-22165, culture ex-isoepitype CPC 25228).

Notes — The epitype of *P. chamaecristae* designated here is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same country as the type. Phylogenetically, *Pseudocercospora chamaecristae* described from *Chamaecrista* sp. clustered in the same clade with *P. exilis* described from *Chamaecrista orbiculata* (Fig. 1, clade 1). Although both species form synnemata and occur on the same host genus, they were considered to be morphologically distinct by Hernández-Gutiérrez & Dianese (2009). *Pseudocercospora exilis* has percurrently proliferating conidiogenous cells, longer conidiophores (149–332 µm) and longer conidia (38–103 µm) (Hernández-Gutiérrez & Dianese 2009). Our molecular data support their view and confirm that *P. chamaecristae* and *P. exilis* are in fact distinct species. In the ITS and *tef1* phylogenies *P. chamaecristae* is distinct from all other species, while it is distinct from but related to *P. exilis* in the *actA* phylogeny.

Fig. 5 *Pseudocercospora chamaecristae* (VIC 42806). a, b. Leaf spots on upper and lower leaf surface; c. close-up of lesion with fruiting; d. synnematous conidiophores; e. conidiogenous cells; f–h. conidia. — Scale bars: d–h = 10 µm.

Fig. 6 *Pseudocercospora diplusodonii* (VIC 42730). a. *Diplusodon* sp. with leaf spots on field; b. leaf spots on upper and lower leaf surface; c. close-up of lesion; d. cross-section showing the internal mycelium; e. conidiophore in a small fascicle; f. conidiogenous cells; g–j. conidia. — Scale bars: d–j = 10 µm.

Pseudocercospora diplusodonii Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813581; Fig. 6

Etymology. Name derived from the plant host genus *Diplusodon*.

Leaf spots amphigenous, irregular, scattered, initially chlorotic, becoming brown with age, angular and vein-delimited, 3–8 mm diam. *Internal mycelium*, intra- and intercellular, 2.5–4.5 µm diam, branched, subhyaline, septate, smooth. *External mycelium* absent. *Stromata* well-developed, emerging through stomata, subglobose to irregular, brown, 17–27 × 17–39 µm, composed of dark brown *textura subglobosa*. *Conidiophores* hypophyllous, aggregated in fascicles arising from the upper cells of the stroma, subcylindrical, 12–39 × 3–5 µm, 0–4-septate, straight or geniculate, unbranched, brown, smooth. *Conidiogenous cells* terminal, subcylindrical, proliferating sympodially, 7.5–25 × 3.0–4.5 µm, brown, smooth to finely verruculose. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, guttulate, subhyaline to pale brown, smooth, subcylindrical, straight to gently curved, 46–105 × 3–4 µm, apex obtuse, base truncate, 2.5–3 µm wide, 3–8-septate; hila unthickened, neither darkened nor refractive, 1.5–2 µm diam.

Culture characteristics — Slow-growing (18–20 mm diam after 20 d), raised, convex, corrugate, margins lobate, with aerial mycelium sparse, pale olivaceous grey, reverse iron-grey, sterile.

Specimen examined. Brazil, Minas Gerais, Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of *Diplusodon* sp. (*Lythraceae*), 31 Mar. 2013, *M. Silva* (holotype VIC 42730, culture ex-type COAD 1476; isotype CBS H-22151, culture ex-isotype CPC 25179).

Notes — No species of *Pseudocercospora* seem to have been recorded on *Diplusodon* (Crous & Braun 2003, Farr & Rossman 2015). Among the *Pseudocercospora* spp. described on plants in the *Lythraceae*, only *P. cupheae*, *P. lagerstroemiae-lanceolatae* and *P. lythri* are morphologically similar to *P. diplusodonii*. *Pseudocercospora cupheae* has shorter and narrower conidiophores (5–15 × 2–3 µm) and longer conidia (40–130 µm) than the newly described species (Braun 1999). In contrast to *P. lagerstroemiae-lanceolatae*, *P. diplusodonii* has no external mycelium with solitary conidiophores and longer and wider fasciculate conidiophores (10–100 × 3–6 µm) (Crous & Braun 2003), and is also distinguished from *P. lythri* by lacking external mycelium, longer conidiophores (10–90 × 2.5–5.5 µm), and wider conidia (20–110 × 3–5 µm) (Shin & Braun 2000). *Pseudocercospora diplusodonii* is clearly distinct from all other species of *Pseudocercospora* included in the phylogenetic analysis (Fig. 1, clade 16), including *P. lythri* (which is located between clades 2 and 3 in Fig. 1), which is also associated with a member of the *Lythraceae*. It is not possible to distinguish *P. diplusodonii* from numerous other *Pseudocercospora* spp. based solely on an ITS or *actA* phylogeny, but it is distinct in the *tef1* phylogeny.

Pseudocercospora emmotunicola Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813583; Fig. 7

Etymology. Name derived from the host genus *Emmotum*.

Leaf spots amphigenous, scattered, chlorotic becoming ochraceous-yellow, poorly delimited, diffuse, 5–15 mm diam. *Internal mycelium*, subhyaline, septate, smooth, 2–2.5 µm diam. *External mycelium* absent. *Stromata* well-developed, 12–22 × 20–38 µm, erumpent, angular, composed of dark brown *textura angularis*. *Conidiophores* hypophyllous, sporodochial arising from the stroma, subcylindrical, 8–29 × 2–3 µm, 0–1-septate, straight or geniculate, pale brown, unbranched, becoming subhyaline towards the apex, smooth. *Conidiogenous cells* terminal, integrated, proliferating sympodially, 9–16 × 2–3.5

Fig. 7 *Pseudocercospora emmotunicola* (VIC 42744). a. *Emmotum nitens* with leaf spots; b. leaf spots on upper and lower leaf surface; c. cross-section showing the internal mycelium; d. sporodochial conidiophores; e. conidiogenous cells; f. conidia. — Scale bars: c–f = 10 µm.

µm, subhyaline to pale brown, subcylindrical, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, guttulate, pale brown, smooth, subcylindrical, straight to curved, 24–99 × 2–3.5 µm, apex obtuse, base truncate, 1.5–2.5 µm wide, 1–12-septate; hila unthickened, not darkened, 1.5–2 µm diam.

Culture characteristics — Slow-growing (21–24 mm diam after 20 d), raised with smooth to slightly irregular margins, aerial mycelium velvety, olivaceous grey, grey-sepia centrally, olivaceous black periphery, reverse iron-grey to greenish black, sterile.

Specimen examined. Brazil, Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of *Emmotum nitens* (*Icacinaceae*), 16 Apr. 2013, *M. Silva* (holotype VIC 42744, culture ex-type COAD 1491; isotype CBS H-22152, culture ex-isotype CPC 25187).

Notes — No species of *Pseudocercospora* are known to occur on *Emmotum* (*Icacinaceae*) (Farr & Rossman 2015). In the multigene phylogenetic analysis, *P. emmotunicola* is basal in a clade containing *P. perae* and *P. guianensis* (Fig. 1, clade 15). It is not possible to distinguish *P. emmotunicola* from numerous other *Pseudocercospora* spp. based solely on an ITS or *actA* phylogeny, and it cannot be distinguished from *P. perae* in the *tef1* phylogeny.

Pseudocercospora euphorbiacearum U. Braun, Biblioth. Lichenol. 86: 89. 2003 — Fig. 8

Leaf spots amphigenous, circular to irregular, chlorotic with a white centre, 4–12 mm diam. *Internal mycelium* intercellular, 2–3.5 µm, branched, subhyaline, septate, smooth. *External mycelium* absent. *Stromata* hypophyllous, erumpent, well-developed, erumpent, 17–31.5 × 17–47 µm, composed of brown *textura angularis*. *Conidiophores* aggregated in dense fascicles arising from the upper cells of the stromata, subcylindrical, 17–42 × 2.5–4 µm, 0–4-septate, straight to geniculate-sinuous, unbranched, pale olivaceous to olivaceous brown, smooth. *Conidiogenous cells* terminal, integrated, subcylindrical, proliferating sympodially, 10–27 × 2.5–4 µm, brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, finely guttulate, subhyaline to pale olivaceous, smooth, subcylindrical, straight to curved, 49–94 × 3–4 µm, apex obtuse, base obconically to truncate, 2.5–3.5 µm wide, 3–14-septate; hila unthickened, not darkened, 1–2 µm diam.

Culture characteristics — Slow-growing (25–28 mm diam after 20 d), convex, circular with smooth to slightly irregularly lobate margins, aerial mycelium velvety, pale olivaceous grey, reverse olivaceous black, sterile.

Specimen examined. Brazil, Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of *Dalechampia* sp. (*Euphorbiacea*), 5 Aug. 2013, *M. Silva* (CBS H-22163, VIC 42797, cultures COAD 1537, CPC 25222).

Notes — The morphology of the Brazilian specimen fits well within the original description of *P. euphorbiacearum* described on *Dalechampia scandens* from the Dominican Republic (Braun 2003). This is the first report of *P. euphorbiacearum* in Brazil, and the first time molecular data is generated for this species. Phylogenetically, *P. euphorbiacearum* (on *Euphorbiaceae*) is closely related to *P. pini-densiflorae* (on *Pinaceae*) based on

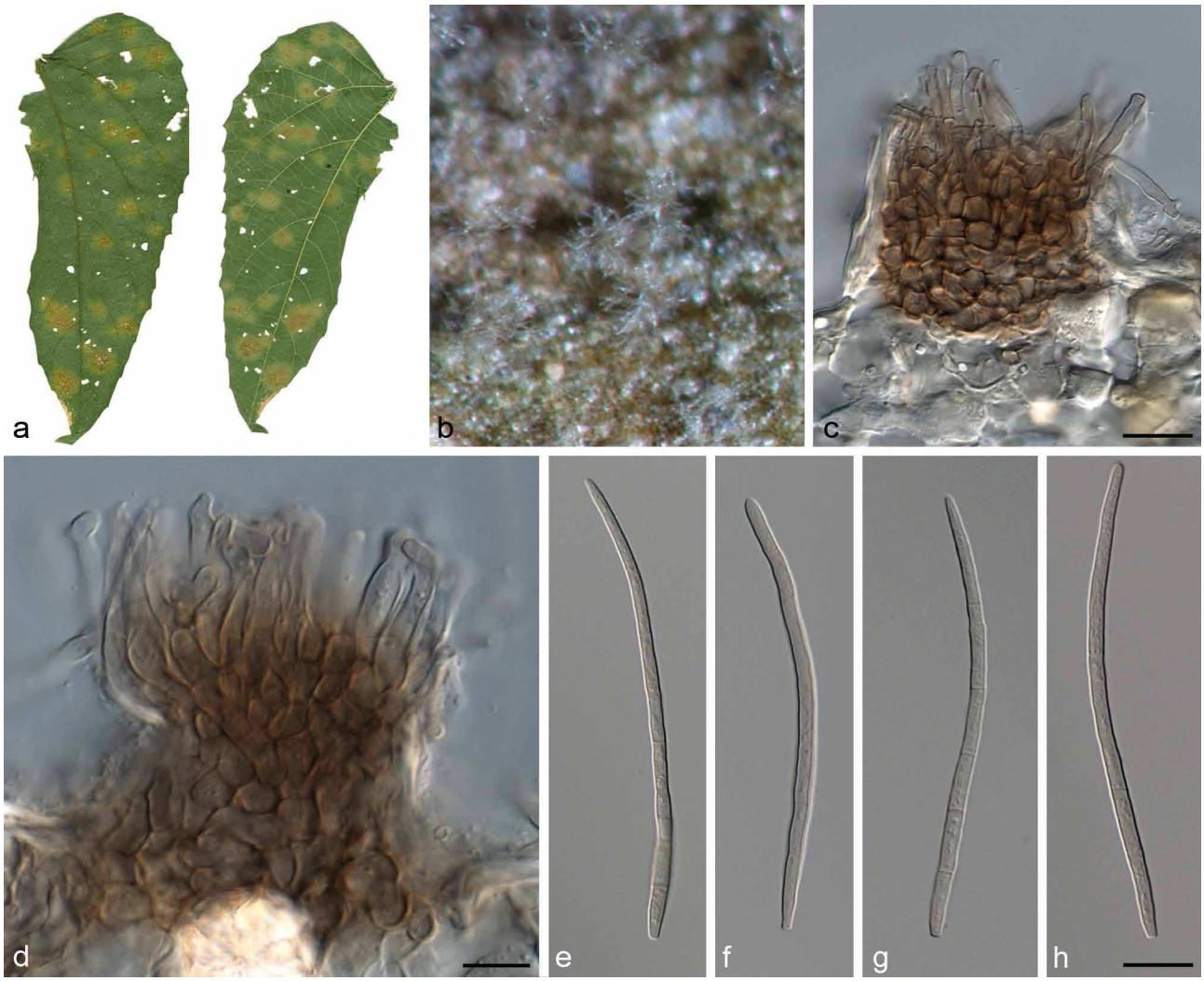


Fig. 8 *Pseudocercospora euphorbiacearum* (VIC 42797). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion with fruiting; c. fasciculate conidiophores; d. conidiogenous cells; e–h. conidia. — Scale bars: c–h = 10 µm.

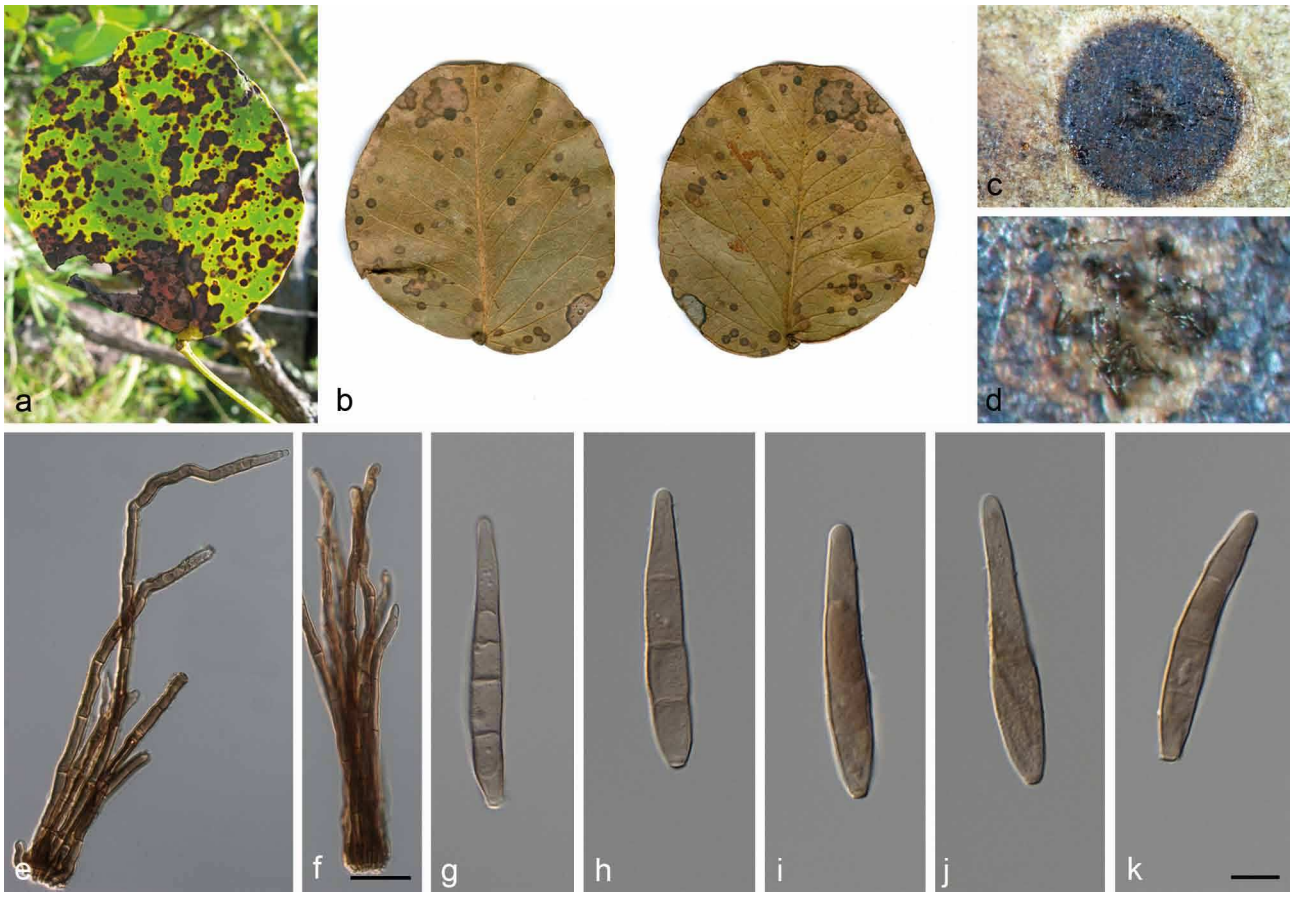


Fig. 9 *Pseudocercospora exilis* (VIC 42754). a. *Chamaecrista orbiculata* with leaf spots; b. leaf spots on upper and lower leaf surface; c. close-up of circular lesion; d. close-up of lesion with fruiting; e, f. synnematous conidiophores; g–k. conidia. — Scale bars: e–k = 10 µm.

the multigene alignment (Fig. 1, clade 12). *Pseudocercospora pini-densiflorae* is a pathogen of a distantly related host family (*Pinaceae*) and is morphologically distinct from *P. euphorbiacearum* (Chupp 1954, Crous & Braun 2003). It is not possible to distinguish *P. euphorbiacearum* from numerous other *Pseudocercospora* spp. based solely on an ITS or *actA* phylogeny, and it can barely be distinguished from *P. pini-densiflorae* and *P. trinidadensis* in the *tef1* phylogeny.

Pseudocercospora exilis A. Hern.-Gut. & Dianese, Mycotaxon 108: 17. 2009 — Fig. 9

Leaf spots amphigenous, circular or irregular, scattered, grey-brown centrally with a dark brown to black margin, 1–6 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* small to well-developed, substomatal, 15–42 µm diam, composed of brown *textura globosa*. *Conidiophores* amphigenous, aggregated in synnemata, subcylindrical, 115–306 × 5–6.5 µm, 4–15-septate, straight, curved or geniculate-sinuous at the upper portion, unbranched, brown, smooth. *Conidiogenous cells* integrated, terminal, proliferating percurrently, 18–32 × 5–6.5 µm, pale brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened, somewhat refractive. *Conidia* solitary, finely guttulate, pale brown, smooth, obclavate or fusoid, straight to slightly curved, 42–78.5 × 5–6.5 µm, apex rounded, base obconically truncate, 4.5–6 µm wide, 1–7-septate; hila unthickened, not darkened, 2.5–4 µm diam.

Culture characteristics — Very slow-growing (12–15 mm diam after 20 d), raised, corrugated, with smooth, irregular margins, green-black centrally with shiny black margins, reverse olivaceous black, sterile.

Specimens examined. Brazil, Distrito Federal, Brasília, on leaves of *Chamaecrista orbiculata* (*Fabaceae*), 9 Aug. 1992, *J.C. Dianese* (holotype UB Mycol. Col. 1477); Estação Ecológica de Águas Emendadas, on leaves of *Chamaecrista orbiculata*, 21 Apr. 2013, *M. Silva* (epitype designated here VIC 42754, MBT202016, culture ex-epitype COAD 1501; isoepitype CBS H-22155, culture ex-isoepitype CPC 25193).

Notes — The epitype of *P. exilis*, designated here, is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same biome and country as the holotype. Also see the notes under *P. chamaecristae*. In the multigene phylogenetic analysis, *P. exilis* groups with *P. chamaecristae* (Fig. 1, clade 1). In the *ITS* and *tef1* phylogenies *P. exilis* is distinct from all other species, while it is distinct from but related to *P. chamaecristae* in the *actA* phylogeny.

Pseudocercospora luzardii Furlan. & Dianese, Mycol. Res. 103: 1207. 1999 — Fig. 10

Leaf spots amphigenous, distinct, oval to irregular, pale grey in the centre surrounded by a purple brown to dark brown margin, 2–7 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* epiphyllous, well-developed, subimmersed, 34–53.5 × 43–82 µm, compose of dark brown *textura angularis*. *Conidiophores* aggregated in dense fascicles, cylindrical, 19–84 × 3–6 µm, 1–6-septate, straight or sinuous, unbranched, brown, smooth. *Conidiogenous cells* integrated, terminal, polyblastic, proliferating percurrently, 6–25 × 3–6 µm, pale brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, finely guttulate, pale brown to brown, smooth, cylindrical, straight to variously curved, 19–84 × 3–5 µm, apex subobtuse, base obconic to subtruncate, 3–4.5 µm wide, 1–8-septate; hila neither thickened nor darkened, 1.5–2 µm diam.

Culture characteristics — Very slow-growing (18 mm diam after 20 d), raised, corrugated, with smooth, lobate margins,

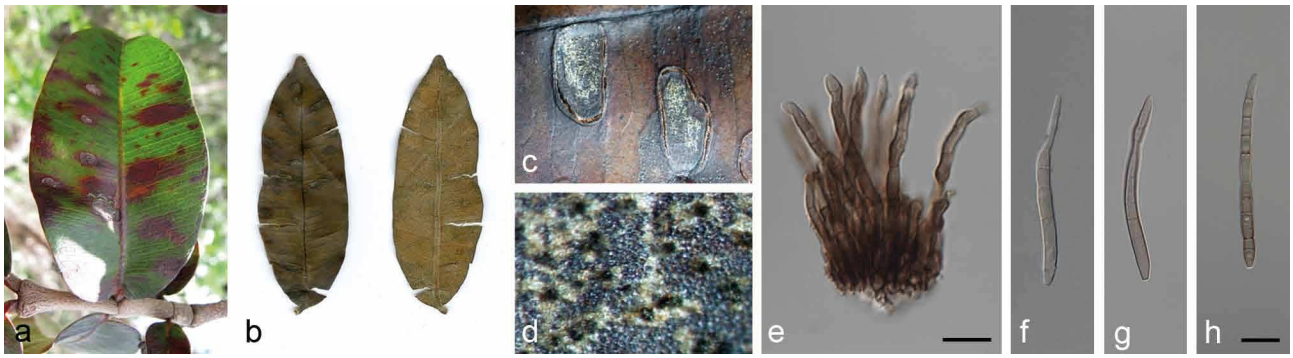


Fig. 10 *Pseudocercospora luzardii* (VIC 42758). a. *Harconia speciosa* with leaf spots; b. leaf spots on upper and lower leaf surface; c. close-up of lesion; d. close-up of lesion with fruiting; e. fasciculate conidiophores; f–h. conidia. — Scale bars: e–h = 10 µm.

aerial mycelium sparse, velvety, grey with patches of olivaceous grey, reverse iron-grey, sterile.

Specimens examined. Brazil, Goiás, Cristalina, Fazenda Nova Índia, on leaves of *Harcornia speciosa* (*Apocynaceae*), 10 Apr. 1993, *J.C. Dianese* (holotype, UB Mycol. Col. 4149); Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of *Harcornia speciosa*, 19 Apr. 2013, *M. Silva* (epitype designated here VIC 42758, MBT202017, culture ex-epitype COAD 1505; isoepitype CBS H-22156, culture ex-isoepitype CPC 25196).

Notes — The epitype of *P. luzardii*, designated here, is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same locality as the holotype. The DNA sequence data place the culture from this study together with strain CPC 2556, identified by Crous et al. (2013a) as *P. luzardii* (Fig. 1, clade 4). The phylogenetic placement is in agreement with the morphological data, confirming this species as *P. luzardii*. It is not possible to distinguish *P. luzardii* from *P. bixae* and *P. sordida* based solely on an ITS phylogeny, but it can be distinguished from all other *Pseudocercospora* spp. based on the individual *tef1* and *actA* phylogenies.

Pseudocercospora manihotii Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813584; Fig. 11

Etymology. Name derived from the plant host genus *Manihot*.

Leaf spots amphigenous, irregular, scattered, reddish brown surrounded by a dark brown to black margin, 10–35 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* well-developed, subimmersed or erumpent, 23–46 × 38–64 µm, composed of brown *textura angularis*. *Conidiophores* epiphyllous, aggregated in dense fascicles arising from the upper cells of the stroma, cylindrical, 15–56 × 3–6 µm, 0–3-septate, straight to slightly geniculate-sinuous, unbranched, pale brown, smooth. *Conidiogenous cells* terminal, sometimes intercalary, cylindrical, proliferating sympodially, 12.5–29 × 3–5.5 µm,

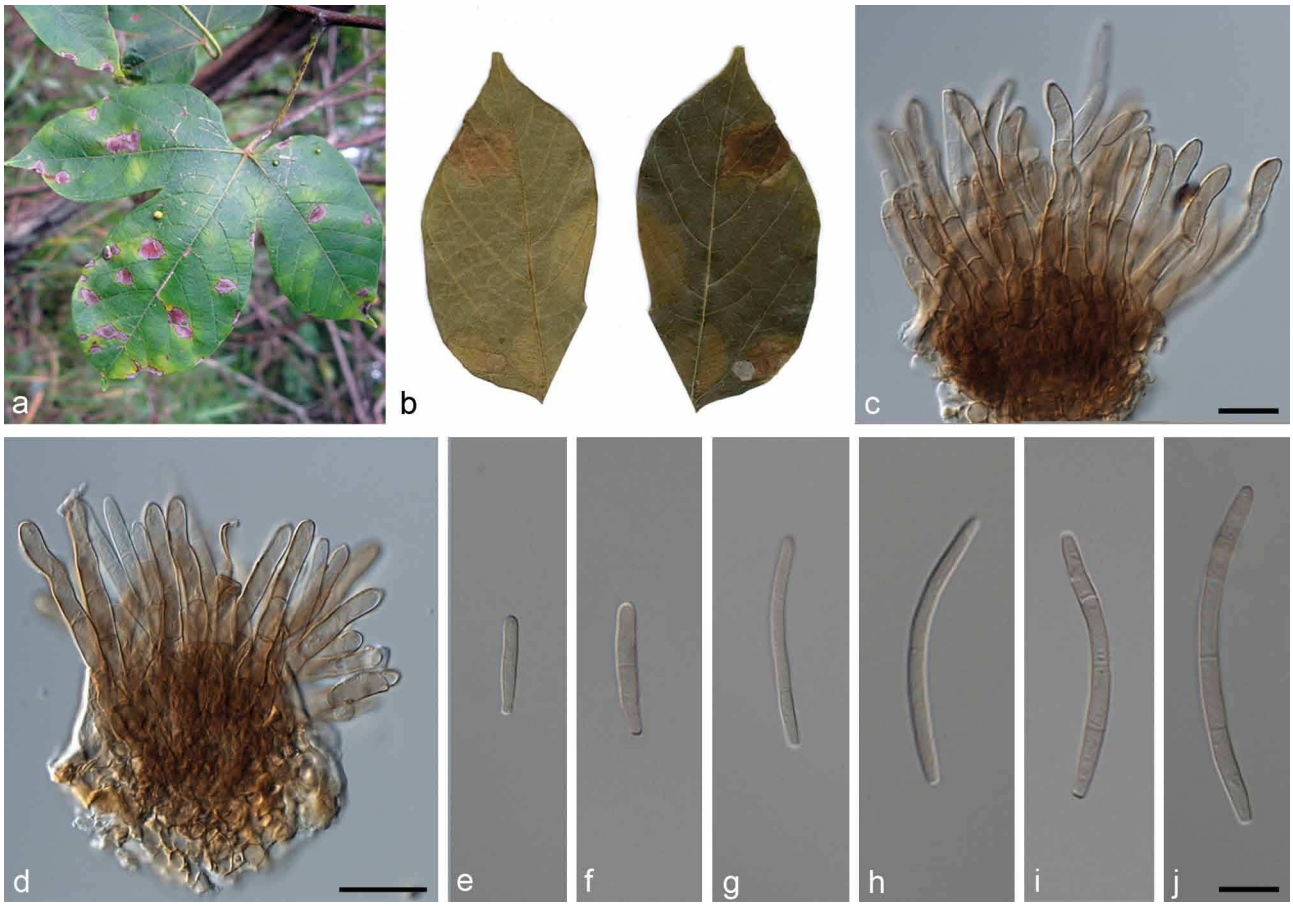


Fig. 11 *Pseudocercospora manihotii* (VIC 42793). a. *Manihot* sp. with leaf spots; b. leaf spots on upper and lower leaf surface; c, d. fasciculate conidiophores; e–j. conidia. — Scale bars: c–e = 10 µm.

pale brown, smooth. *Conidiogenous loci* slightly conspicuous, slightly thickened, not darkened. *Conidia* solitary, finely guttulate, pale brown, smooth, cylindrical to narrowly obclavate, straight to curved, 19–97 × 2–4 µm, apex rounded to subacute, base obconically truncate, 2–3 µm wide, 0–10-septate; hila unthickened, not darkened, 1.5–2.5 µm diam.

Culture characteristics — Very slow-growing (15–18 mm diam after 20 d); convex, with smooth, lobate margins, and sparse aerial mycelium, olivaceous grey, reverse iron-grey, sterile.

Specimen examined. Brazil, Minas Gerais, Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of *Manihot* sp. (*Euphorbiaceae*), 29 Apr. 2013, *M. Silva* (holotype VIC 42793, culture ex-type COAD 1534; isotype CBS H-22161, culture ex-isotype CPC 25219).

Notes — No *Pseudocercospora* spp. are known to be associated with the genus *Manihot*. Several species of *Pseudocercospora* are known to occur on *Euphorbiaceae*, but all are dissimilar to the fungus collected on *Manihot* (Crous & Braun 2003, Farr & Rossman 2015). *Pseudocercospora hurae* is the species having the most similar morphology to that of *P. manihotii* among those described on members of the *Euphorbiaceae* (Deighton 1976). It also has well-developed stromata with conidiophores forming dense fascicles, but differs from the newly proposed species in having smaller and narrower conidiophores (5–40 × 3–4.5 µm) (Deighton 1976). *Pseudocercospora manihotii* clusters together with *P. wulffiae* in the phylogeny derived from the combined alignment (Fig. 1, clade 6). The DNA sequences generated here (ITS, *actA* and *tef1*) did not allow for a clear distinction between *P. manihotii* and *P. wulffiae* (Fig. 1, clade 6). However, *P. wulffiae* is a pathogen of plants belonging to a different host family (*Asteraceae*), and it has a clearly distinct mor-

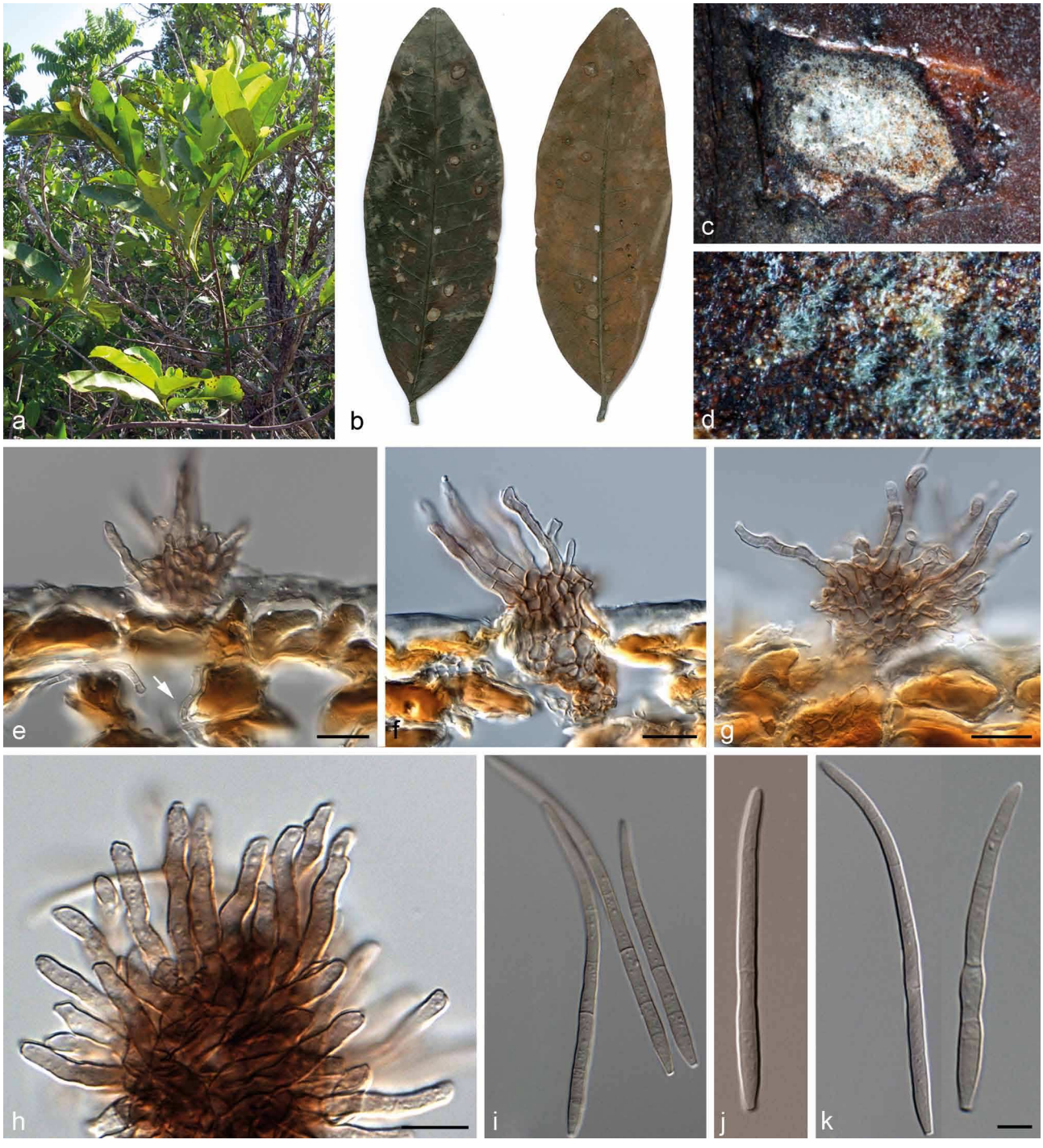


Fig. 12 *Pseudocercospora perae* (VIC 42721). a. *Pera glabrata* with leaf spots; b. leaf spots on upper and lower leaf surface; c. close-up of lesion; d. close-up of lesion with fruiting; e. cross-section showing the internal mycelium; f, g. conidiophores in sporodochial; h. conidiogenous cells; i–k. conidia. — Scale bars: e–k = 10 µm.

phology (shorter and narrower conidiophores (14–21 × 2–3 µm) and shorter conidia (37.5–87 µm) indicating that these are distinct taxa for which additional gene regions will be required to resolve the species boundaries. It is not possible to distinguish *P. manihotii* from several other *Pseudocercospora* spp. based solely on an ITS phylogeny, and it cannot be distinguished from *P. wulffiae* in the *tef1* phylogeny. In the *actA* phylogeny it is more distinct from closely related species.

Pseudocercospora perae Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813589; Fig. 12

Etymology. Name derived from the plant host genus *Pera*.

Leaf spots amphigenous, circular to irregular, pale brown to brown, on upper surface white centrally, 3–6 mm diam, surrounded by a black margin. *Internal mycelium*, subhyaline, septate, branched, smooth, 3.5–4 µm diam. *External mycelium* absent. *Stromata* well-developed, 14–35 × 23–42 µm, subimmersed or erumpent, brown, composed of dark brown *textura angularis*. *Conidiophores* hypophyllous, aggregated in loose to dense fascicles, arising from the upper cells of the stroma, cylindrical, 9–68.5 × 3–4 µm, 0–3-septate, straight or geniculate, unbranched, brown, smooth. *Conidiogenous cells* terminal, integrated, subcylindrical, proliferating percurrently, 7–17 × 3–3.5 µm, brown, smooth to finely verruculose. *Conidiogenous loci* inconspicuous, slightly thickened, not darkened. *Conidia* solitary, finely guttulate, subhyaline to pale brown, smooth, subcylindrical, straight to curved at the apex, 27–102 × 3–5 µm, apex obtuse, base truncate, 2.5–3.5 µm wide, 5–6-septate; hila unthickened, neither darkened nor refractive, 1.5–2 µm diam.

Culture characteristics — Slow-growing (25–28 mm diam after 20 d), raised, circular with smooth to slightly irregular margins, aerial mycelium velvety, pale olivaceous grey with olivaceous black periphery, reverse greenish black, sterile.

Specimen examined. Brazil, Minas Gerais, Paraopeba, Floresta Nacional (FLONA), on leaves of *Pera glabrata* (*Euphorbiaceae*), 3 Jan. 2013, *M. Silva* (holotype VIC 42721, culture ex-type COAD 1465; isotype CBS H-22148, culture ex-isotype CPC 25171).

Notes — No species of *Pseudocercospora* or other cercosporoid fungi and mycosphaerella-like sexual morphs are presently known to occur on species of *Pera*, but numerous *Pseudocercospora* spp. have been described from hosts in the *Euphorbiaceae* (Farr & Rossman 2015). Among these *P. crotoniphila* is morphologically similar but distinguishable from *P. perae* by having shorter and wider conidiophores (20–40 × 4–5 µm) and shorter conidia (20–90 µm) (Crous et al. 1999). Another

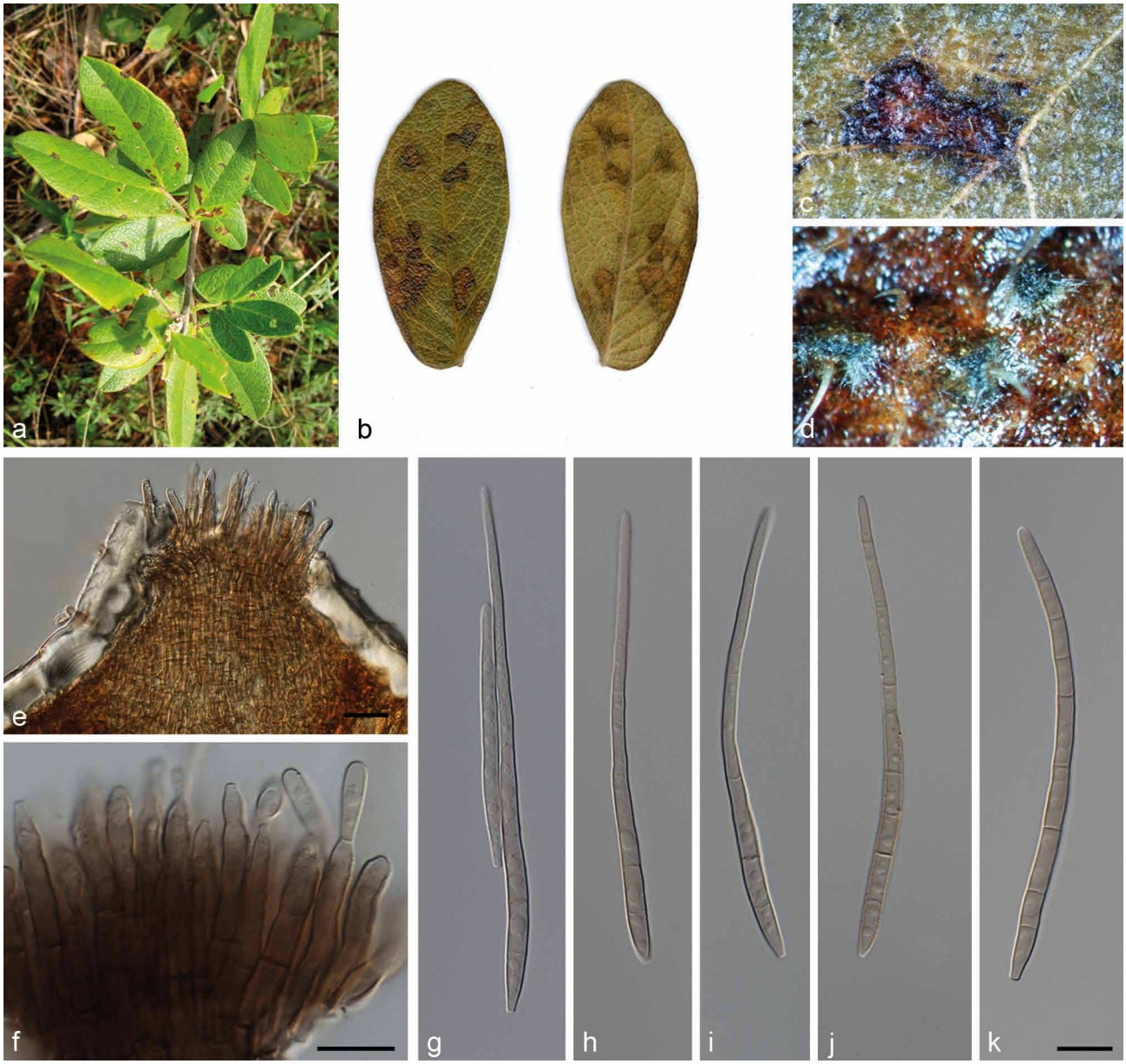


Fig. 13 *Pseudocercospora planaltinensis* (VIC 42748). a. *Chamaecrista* sp. with leaf spots; b. leaf spots on upper and lower leaf surface; c. close-up of lesion; d. close-up of lesion with fruiting; e. cross-section showing the sporodochial conidioma; f. conidiogenous cells; g–k. conidia. — Scale bars: e–k = 10 µm.

species similar to *P. perae* is *P. hieronymae* that differs by having narrower conidia (2.5–4 µm) (Chupp 1954, Crous & Braun 2003), while *P. hurae* has shorter conidiophores (5–40 × 3–4.5 µm) and narrower conidia (2–4.5 µm) (Chupp 1954). In the multigene phylogenetic analysis, *P. perae* is in a clade containing *P. emmotunicola* and *P. guianensis* (Fig. 1, clade 15). It is not possible to distinguish *P. perae* from numerous other *Pseudocercospora* spp. based solely on an ITS or *actA* phylogeny, and it cannot be distinguished from *P. emmotunicola* in the *tef1* phylogeny.

Pseudocercospora planaltinensis Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813591; Fig. 13

Etymology. Name derived from Planaltina, the Brazilian municipality where the fungus was first found.

Leaf spots amphigenous, brown, surrounded by a dark brown to black defined margin, irregular, 2–11 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* well-developed, immersed, 128–147.5 µm diam, composed of brown *textura porrecta*. *Conidiophores* amphigenous, mostly epiphyllous, sporodochial, arising from the stromata, cylindrical, 11–68 × 3–5.5 µm, 0–3-septate, straight, unbranched, brown, smooth. *Conidiogenous cells* terminal, cylindrical, proliferating percurrently, 5–31 × 3–5 µm, brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, guttulate, pale brown, smooth, cylindrical to obclavate, straight to curved, 49–129 × 3–5 µm, apex obtuse or acute, base obconically truncate, 2.5–4.5 µm wide, 1–8-septate; hila not thickened, not darkened, 1.5–2.5 µm diam.

Culture characteristics — Very slow-growing (16–18 mm diam after 20 d), raised, margins lobate, aerial mycelium velvety, pale olivaceous grey, reverse iron-grey, sterile.

Specimen examined. Brazil, Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of *Chamaecrista* sp. (*Fabaceae*), 17 Apr. 2013, *M. Silva* (holotype VIC 42748, culture ex-type COAD 1495; isotype CBS H-22153, culture ex-isotype CPC 25189).

Notes — There are five *Pseudocercospora* spp. known to occur on the host genus *Chamaecrista*, namely *P. chamaecristae*, *P. chamaecristigena*, *P. exilis*, *P. luzianiensis* and *P. nigricans* (Farr & Rossman 2015). *Pseudocercospora chamaecristae*, *P. chamaecristigena*, *P. exilis* and *P. luzianiensis* are easily separated on morphological basis from *P. planaltinensis* by having different conidial shapes and wider conidia with longer synnematous conidiophores (Braun & Freire 2002, Hernández-Gutiérrez & Dianese 2009). *Pseudocercospora nigricans* has conidia similar to those of *P. planaltinensis*. However, conidia of *P. nigricans* are smaller (18–80 × 3–5 µm), its conidiophores are not arranged in sporodochia and the stromata are either absent or reduced to a few cells (Chupp 1954, Brown & Morgan-Jones 1977). Genetically, *P. planaltinensis* is very distinct from all other species of *Pseudocercospora* included in the phylogenetic analysis (Fig. 1, clade 13), and is somewhat related to *P. subsessilis*, a species known to cause leaf spots on *Azadirachta indica*, *Melia azadirachta* and *Swietenia macrophylla* (*Meliaceae*) (Braun & Castañeda-Ruiz 1991, Braun & Freire 2006, Farr & Rossman 2015). Morphologically, *P. subsessilis* differs from *P. planaltinensis* by having smaller and narrower conidia (25–80 × 2–4 µm) (Chupp 1954). The species is distinct from all other included *Pseudocercospora* spp. based on individual gene trees of all three loci, ITS, *actA* and *tef1*.

Pseudocercospora plumeriifolii (Bat. & Peres) U. Braun et al., Cryptog. Mycol. 20: 102. 1999 — Fig. 14

Basionym. *Cercospora plumeriifolii* Bat. & Peres, Pub. Inst. Micol. Recife 262: 23. 1960.

Leaf spots amphigenous, scattered, irregular, greyish, delimited by a dark brown to black margin, 4–12 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* amphigenous, well-developed, 55–92 × 99–121 µm, immersed to partly erumpent, angular to globose, composed of dark brown *textura angularis*. *Conidiophores* sporodochial, arising from a

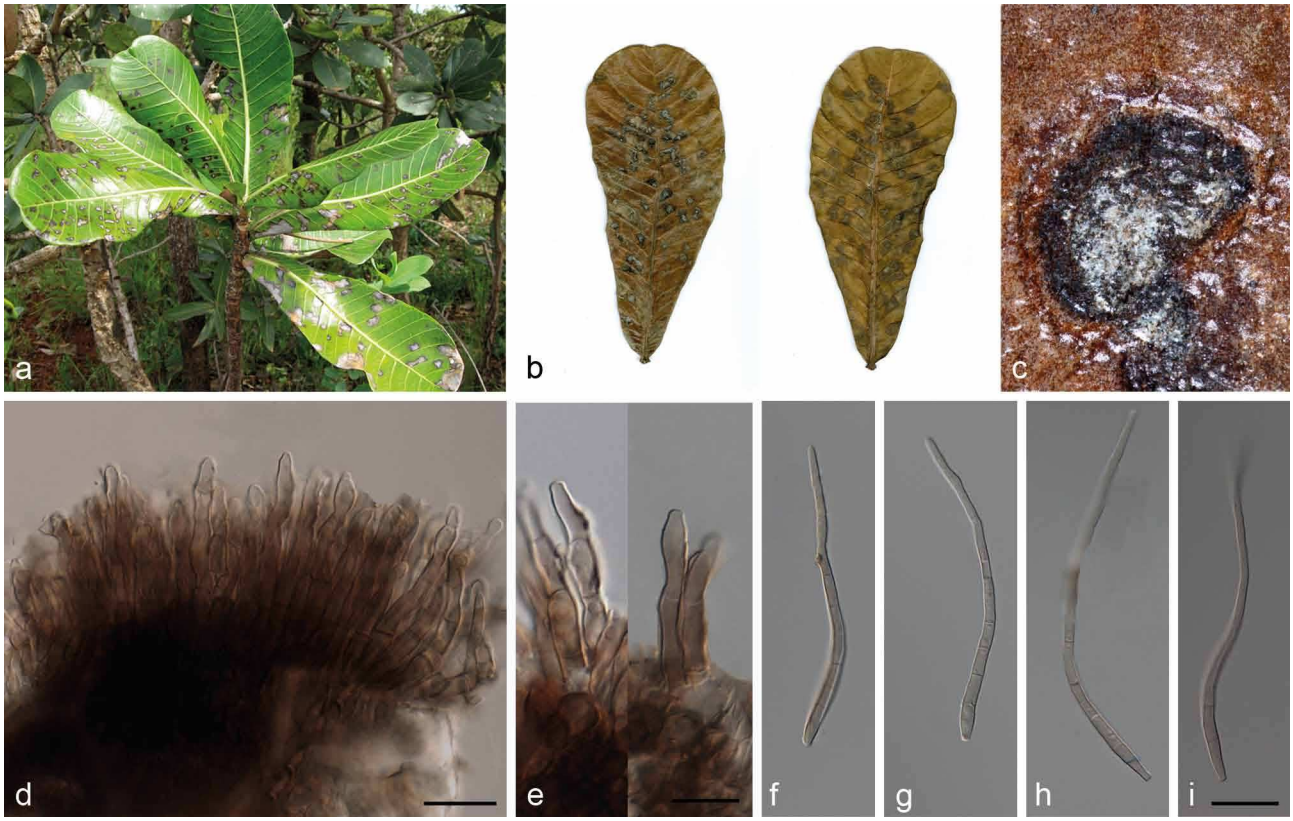


Fig. 14 *Pseudocercospora plumeriifolii* (VIC 42751). a. *Himatanthus obovatus* with leaf spots; b. leaf spots on upper and lower leaf surface; c. close-up of lesion; d. sporodochial conidioma; e. conidiogenous cells; f–i. conidia. — Scale bars: d–i = 10 µm.

stroma, cylindrical, 13–45 × 2.5–4 µm, 0–4-septate, straight to geniculate-sinuous, unbranched, brown, smooth. *Conidiogenous cells* terminal, proliferating sympodially, 7–19 × 3–4 µm, subcylindrical to sinuous, brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, guttulate, olivaceous to olivaceous brown, smooth, obclavate, straight to curved, 25–110 × 3–5 µm, apex obtuse, base obconically truncate, 2.5–4.5 µm wide, 2–9-septate; hila unthickened, not darkened, 1.5–2.5 µm diam.

Culture characteristics — Very slow-growing (20 mm diam after 20 d), raised with smooth margins, aerial mycelium velvety, centre olivaceous grey, olivaceous black periphery, reverse green-black, sterile.

Specimens examined. Brazil, Minas Gerais, Paraopeba, Horto Florestal, on leaves of *Himatanthus obovatus* (*Apocynaceae*), 1960, *Batista* (holotype, IMUR 19074); Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of *Himatanthus obovatus*, 19 Apr. 2013, *M. Silva* (epitype designated here VIC 42751, MBT202067, culture ex-epitype COAD 1498; isoepitype CBS H-22154, culture ex-isoepitype CPC 25191).

Notes — The epitype of *P. plumeriifolii*, designated here, is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same biome and country as the type. No DNA sequence data were available for *P. plumeriifolii* until now. Phylogenetically, *P. plumeriifolii* clusters in a clade with *P. catalpigena*, *P. pallida*, *P. rhapisicola* and *P. rigidae* (Fig. 1, clade 17). *Pseudocercospora catalpigena* differs from *P. plumeriifolii* by having shorter and wider conidiophores (5–35 × 3–6 µm) (Braun et al. 2003), while *P. rigidae* has longer and wider conidiophores (21–85 × 3–5 µm). *Pseudocercospora pallida* and *P. rhapisicola* are morphologically similar, but they are described from hosts in different families, *Bignoniaceae* and *Arecaceae*, respectively (Goh & Hsieh 1989, Shin & Braun 2000). It is not possible to distinguish *P. plumeriifolii* from numerous other *Pseudocercospora* spp. based solely on an ITS or *actA* phylogeny, and it can barely be distinguished from *P. catalpigena*, *P. pallida* and *P. rhapisicola* in the *tef1* phylogeny.

Pseudocercospora plunkettii (Chupp) R.F. Castañeda & U. Braun, Cryptog. Bot. 2: 295. 1991 — Fig. 15

Basionym. *Cercospora plunkettii* Chupp, A monograph of the fungus *Cercospora*: 154. 1954.

Leaf spots amphigenous, irregular, grey-brown surrounded by a black border, 3–12 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* amphigenous, well-developed, 32–39 × 48–53 µm, angular to irregular, composed of dark brown *textura angularis*. *Conidiophores* aggregated in dense fascicles, emerging through stromata, 20–85 × 3.5–5 µm, 3–8-septate, straight to strongly geniculate-sinuous, unbranched, pale brown, smooth. *Conidiogenous cells* terminal, 6–31 × 3.5–5 µm, pale brown, proliferating sympodially, rarely percurrently, smooth. *Conidia* solitary, guttulate, pale brown, smooth, subcylindrical to obclavate, straight to curved, 49–81 × 3–5 µm, apex obtuse to subacute, base obconically truncate, 3–5 µm, 6–10-septate; hila unthickened, not darkened, 2.5–5 µm diam.

Culture characteristics — Slow-growing (23 mm diam after 20 d), raised with smooth to slightly irregular margins, aerial mycelium velvety, olivaceous grey, reverse olivaceous black, sterile.

Specimen examined. Brazil, Rio de Janeiro, Nova Friburgo, Fazenda Barreto II, on leaves of *Mikania* sp. (*Asteraceae*), 10 Feb. 2013, *R.W. Barreto* (CBS H-22169, VIC 42644, COAD 1548, CPC 26081).

Notes — *Pseudocercospora plunkettii* was previously recorded on *Mikania cordifolia* in Cuba and Mexico (Chupp 1954, Braun & Castañeda-Ruiz 1991) and on *Mikania micrantha* in Venezuela and Brazil (Barreto & Evans 1995, Crous & Braun 2003). Our fungus compared well with the description of *P. plunkettii*, and the present study represents the first sequence data

Fig. 15 *Pseudocercospora plunkettii* (VIC 42644). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. sporodochial conidiophores; d. close-up of conidiophores and conidiogenous cells; e–i. conidia. — Scale bars: c, e–i = 10 µm, d = 20 µm.

for this species. The species clusters with *P. basitruncata* and *P. richardsoniicola* (Fig. 1, clade 2). *Pseudocercospora basitruncata* is morphologically distinct from *P. plunkettii* by having shorter conidiophores (12–60 µm) and longer conidia (25–90 µm), while *P. richardsoniicola* has longer conidiophores and conidia (90–192 µm, 36–97 µm, respectively) (Crous 1998, Crous & Câmara 1998). *Pseudocercospora plunkettii* is distinct from other species in the ITS phylogeny, and closely related to *P. basitruncata* and *P. richardsoniicola* in the *tef1* and *actA* phylogenies.

Pseudocercospora pothomorphes Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB814904; Fig. 16

Etymology. Name derived from the plant host genus *Pothomorphe*.

Leaf spots amphigenous, irregular or angular, scattered, brown, vein-delimited, 1–8.5 mm diam. *Internal mycelium* subhyaline, septate, branched, smooth, 2.5–4 µm diam. *External mycelium* absent. *Stromata* lacking or reduced to only a few cells. *Conidiophores* hypophyllous, aggregated in small to moderately large fascicles, loose, arising from stromata, emerging through stomata, cylindrical, 15–90 × 3.5–6 µm, 0–5-septate, straight or sinuous, rarely branched, brown, becoming paler towards the apex, smooth. *Conidiogenous cells* terminal, pale brown, subcylindrical, smooth, proliferating sympodially and percurrently, 7–19 × 3–5.5 µm, apical loci indistinct, unthickened and not darkened. *Conidia* solitary, guttulate, subhyaline to pale brown, smooth, subcylindrical to narrowly obclavate, straight to curved, 26–68.5 × 3.5–5 µm, apex rounded to subacute, base truncate, 2.5–4 µm wide, 1–7-septate; hila neither thickened nor darkened, 2–2.5 µm diam.

Culture characteristics — Slow-growing (19–22 mm diam after 20 d), convex, somewhat folded, with smooth to slightly irregular margins, aerial mycelium velvety, olivaceous grey, green-black reverse, sterile.

Specimen examined. Brazil, Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of *Pothomorphe umbellata* (*Piperaceae*), 15 Nov. 2012, *O.L. Pereira* (holotype VIC 42705, culture ex-type COAD 1450; isotype CBS H-22147, culture ex-isotype CPC 25166).

Notes — One species of *Pseudocercospora* is known on *Pothomorphe*, namely *Pseudocercospora piperis* reported on *Pothomorphe peltata* in Panama and on *Po. umbellata* in Brazil (Crous & Braun 2003, Farr & Rossman 2015). Morphologically, *P. piperis* differ from *P. pothomorphii* by having conidiophores that are branched and shorter (20–80 µm), as well as longer conidia (25–130 µm) (Deighton 1976). Rocha et al. (2013) deposited sequences in GenBank for *P. piperis* on *Piper aduncum* (*tef1*: JX896123; ITS: JX875062) that differ from the sequences generated for *P. pothomorphes* on *Pothomorphe umbellata* collected during this study (Table 1). Based on DNA sequence data, these species possess only 87 % similarity in the partial gene region of *tef1*; unfortunately no *actA* sequences of strain FBR1 are available for comparison. In the molecular phylogeny derived from the multigene alignment, the two isolates cluster in two different clades (Fig. 1, clade 8 for strain FBR 151 and clade 11 for *P. pothomorphes*). It is not possible to distinguish strains FBR 151 and COAD 1450 from numerous other *Pseudocercospora* spp. based solely on an ITS phylogeny. In the *tef1* phylogeny, *P. pothomorphes* cannot be distinguished from *Pseudocercospora* sp. CBS 110998 and *P. cordiana*, whereas strain FBR 151 cannot be distinguished from *Pseudocercospora* sp. CPC 10645, *P. aeschynomenicola* and *P. struthanthi*. In the *actA* phylogeny, *P. pothomorphes* is close to but distinct from *Pseudocercospora* sp. CPC 10645.

Pseudocercospora richardsoniicola Crous & M.P.S. Câmara, Mycotaxon 68: 307. 1998 — Fig. 17

Basionym. *Cercospora richardsoniae* Henn., Hedwigia 41: 117. 1902 (non *C. richardsoniae* Ellis & Everh.).

Leaf spots amphigenous, irregular to circular, scattered, pale brown, surrounded by a dark brown border, 4–14 mm diam. *Internal* and *external mycelium* pale brown, 3–4 µm diam. *Stromata* amphigenous, well-developed, 45–61 × 54–70 µm subimmersed, angular, composed of brown *textura angularis*. *Conidiophores* arising from stromata aggregated in dense fascicles, cylindrical, 90–192 × 3–5 µm, 4–15-septate, straight to slightly curved, unbranched, medium brown, becoming paler

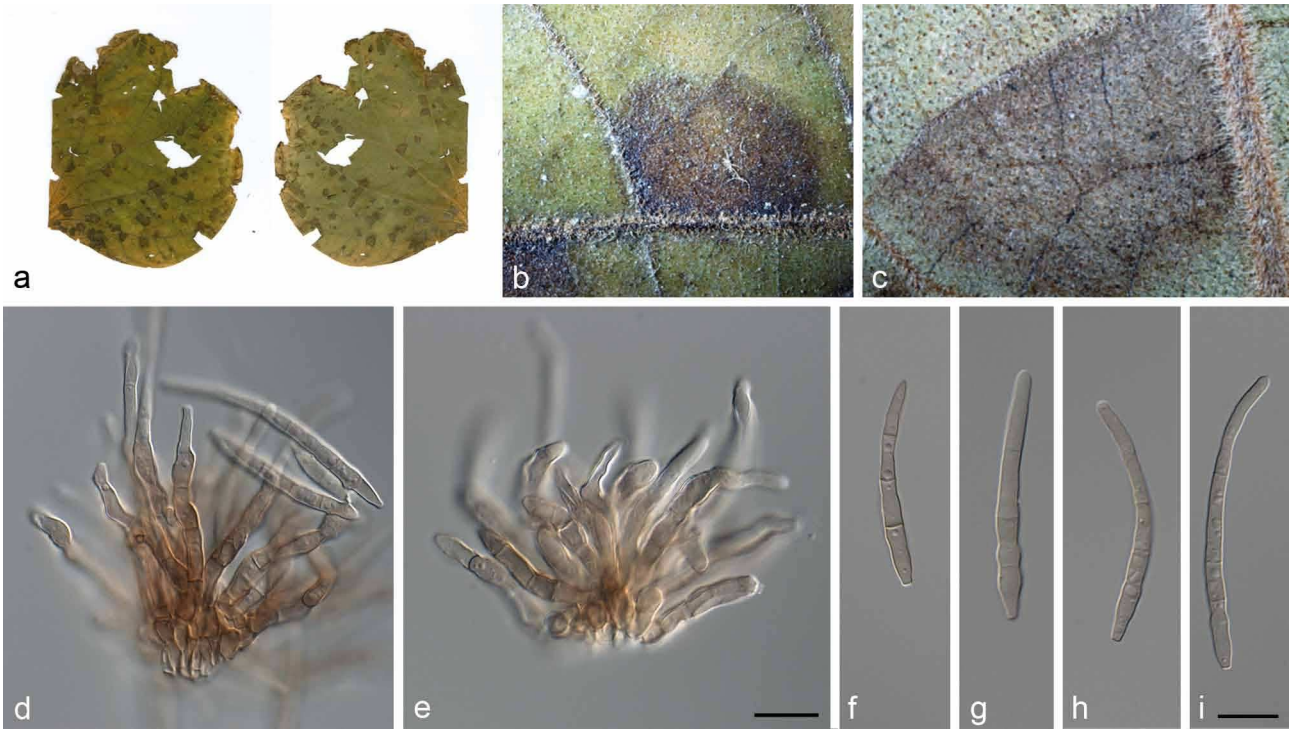


Fig. 16 *Pseudocercospora pothomorphes* (VIC 42705). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. close-up of leaf spot with fruiting; d, e. fasciculate conidiophores; f–i. conidia. — Scale bars: d–i = 10 µm.

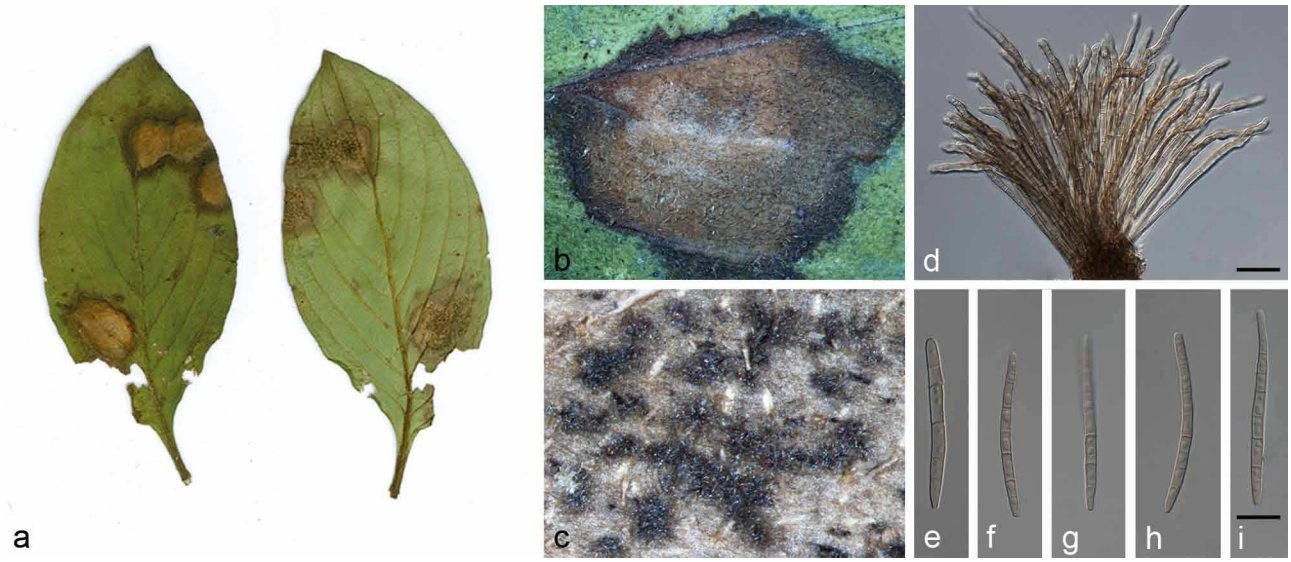


Fig. 17 *Pseudocercospora richardsoniicola* (VIC 42661). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. close-up of leaf spot with fruiting; d. fasciculate conidiophores; e–i. conidia. — Scale bars: d–i = 10 µm.

toward the apex, smooth. *Conidiogenous cells* terminal, proliferating sympodially, 9–71 × 2.5–5 µm, pale brown, cylindrical, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, cylindrical to obclavate, straight to slightly curved, 36–97 × 3–5 µm, apex rounded to obtuse, base obconically truncate, 3–8-septate, guttulate, pale brown, smooth, 2.5–5 µm wide; hila neither thickened nor darkened, 1.5–2.5 µm diam.

Culture characteristics — Very slow-growing (12–14 mm diam after 20 d), raised with smooth, lobate margins, aerial mycelium sparse, white and greyish, reverse black, sterile.

Specimens examined. Brazil, São Paulo, Botanic Garden, on leaves of *Richardsonia* sp. (*Rubiaceae*), 4 Feb. 1901, *A. Puttemans* (holotype BPI 440387); Rio de Janeiro, Nova Friburgo, Mury, on leaves of *Richardia brasiliensis*, 9 June 2013, *R.W. Barreto* (epitype designated here VIC 42661, MBT202068, culture ex-epitype COAD 1568; isoepitype CBS H-22172, culture ex-isoepitype CPC 25248).

Notes — The epitype of *P. richardsoniicola*, designated here, is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same country as the type. *Pseudocercospora richardsoniicola* is phylogenetically closely related to *P. basitruncata*, and sister to *P. plunkettii* (Fig. 1, clade 2). *Pseudocercospora basitruncata* occurs on a distantly related host (*Eucalyptus* sp.) belonging to a different host family (*Myrtaceae*) and has a clearly distinct morphology – shorter conidiophores (12–60 µm) and narrower conidia (2.5–3.5 µm) (Crous 1998). For *P. plunkettii* see notes above. *Pseudocercospora richardsoniicola* is distinct from other species in the ITS phylogeny, and closely related to *P. plunkettii* and *P. richardsoniicola* in the *tef1* and *actA* phylogenies.

Pseudocercospora rigidae Meir. Silva & O.L. Pereira, Mycotaxon 102: 261. 2007 — Fig. 18

Leaf spots amphigenous, irregular or vein delimited, pale brown, surrounded by a dark brown to black border, confluent, covering large areas of the leaf surface, 2–15.5 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* well-developed, subepidermal, erumpent, dark brown, 16–27 × 19–53 µm, composed of brown *textura globosa*. *Conidiophores* amphigenous, fasciculate, arising from the subepidermal stromata, 21–85 × 3–5 µm, 3–9-septate, straight to geniculate-sinuous, rarely branched below, dark brown, smooth. *Conidiogenous cells* terminal or lateral, proliferation percurrently and sometimes sympodially, 12–23 × 3–4 µm, brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, pale brown to brown, smooth, guttulate, obclavate-cylindrical, straight to slightly curved, 25–99 × 3–5 µm, apex obtuse to subacute, 2–2.5 µm wide, 0–7-septate; hila slightly thickened, slightly darkened not refractive, 1.5–2 µm diam.

Culture characteristics — Slow-growing (19–22 mm diam after 20 d), raised, corrugated with smooth, lobate margins, aerial mycelium velvety, olivaceous grey, reverse olivaceous black, sterile.

Specimens examined. Brazil, Minas Gerais, Carrancas, on leaves of *Palicourea rigida* (*Rubiaceae*), Mar. 2007, *O.L. Pereira* (holotype VIC 30472); Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of *Palicourea rigida*, 30 Mar. 2013, *M. Silva* (epitype designated here VIC 42726, MBT202069, culture ex-epitype COAD 1472; isoepitype CBS H-22150, culture ex-isoepitype CPC 25175).

Notes — The epitype of *P. rigidae*, designated here, is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same locality as the type. This study represents the first phylogenetic data available for this species, showing that it is basal to a clade containing *P. catalpigena*, *P. pallida*, *P. plumeriifolii* and *P. rhapisicola* (see morphological differences of these species in the above notes under *P. plumeriifolii*) (Fig. 1, clade 17). It is not possible to distinguish *P. rigidae* from numerous other *Pseudocercospora* spp. based solely on an ITS or *actA* phylogeny, and it is closely related to *P. zelkovae* in the *tef1* phylogeny.

Pseudocercospora sennae-multijugae Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB814905; Fig. 19

Etymology. Name derived from the plant host *Senna multijuga.*

Leaf spots amphigenous, grey-brown in the centre, surrounded by a dark brown to black margin, mostly in the border of leaves, irregular, 2–18 mm diam. *Mycelium* internal, subhyaline, consisting of septate, smooth hyphae, 2.5–3 µm diam wide. *External mycelium* subhyaline, consisting of septate, smooth hyphae, 2.5–4 µm diam. *Stromata* well-developed, substomatal, 25–67 µm diam, brown, composed of brown *textura angularis*. *Conidiophores* hypophyllous, sporodochial, arising from stroma, emerging through stomata, 8–14 × 2–4.5 µm, 0–2-septate, straight to sinuous, unbranched, medium brown to brown, smooth. *Conidiogenous cells* terminal, or conidiophores

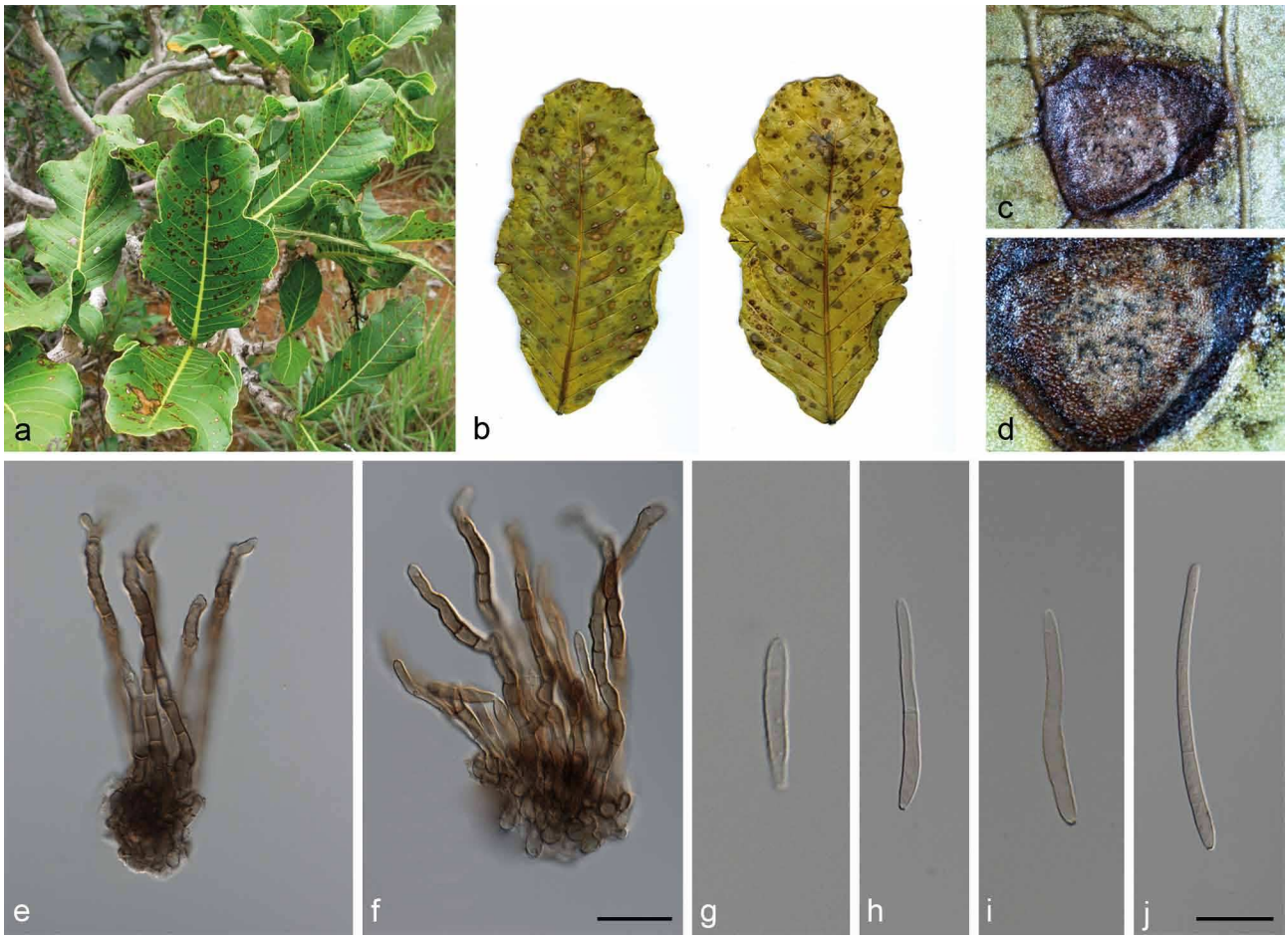


Fig. 18 *Pseudocercospora rigidae* (VIC 42726). a. *Palicourea rigida* with leaf spots; b. leaf spots on upper and lower leaf surface; c. close-up of lesion; d. close-up of lesion with fruiting; e, f. fasciculate conidiophores; g–j. conidia. — Scale bars: e, f = 10 µm.

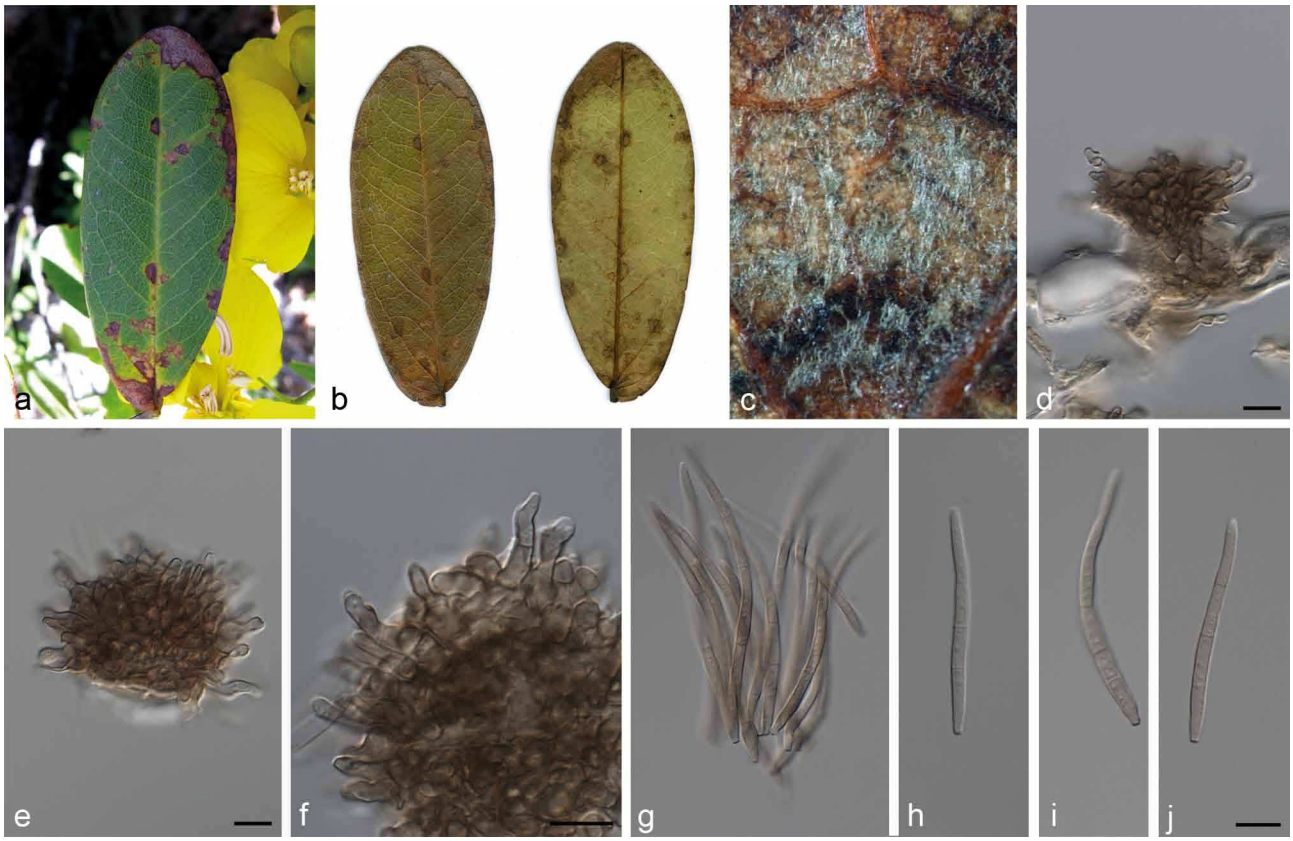


Fig. 19 *Pseudocercospora sennae-multijugae* (VIC 42775). a. *Senna multijuga* with leaf spots; b. leaf spots on upper and lower leaf surface; c. close-up of lesion with fruiting; d. cross-section showing the internal mycelium; e. fasciculate conidiophores; f. conidiogenous cells; g–j. conidia. — Scale bars: d–j = 10 µm.

reduced to conidiogenous cells, 8–11 µm long, medium brown, subcylindrical, smooth, proliferating sympodially. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, olivaceous brown, finely guttulate, smooth, cylindrical to narrowly obclavate, straight to curved, 11–81 × 3–4 µm, apex obtuse, base obconically truncate, 2.5–4 µm wide, 2–7-septate; hila neither thickened nor darkened, 2–2.5 µm diam.

Culture characteristics — Slow-growing (18–20 mm diam after 20 d), raised, corrugated with irregular margins, aerial mycelium sparse, olivaceous grey, reverse green-black, sterile.

Specimen examined. Brazil, Goiás, Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, on leaves of *Senna multijuga* (*Fabaceae*), 23 Apr. 2013, *M. Silva* (holotype VIC 42775; culture ex-type COAD 1519, isotype CBS H-22158, culture ex-isotype CPC 25206).

Notes — Nine species of *Pseudocercospora* have previously been recorded on members of *Senna*, namely *P. angustata*, *P. cassiae-alatae*, *P. cassiae-fistulae*, *P. cassiae-occidentalis*, *P. cassiae-siameae*, *P. nigricans*, *P. simulate*, *P. singaporensis* and *P. taichugensis* (Farr & Rossman 2015). Two *Pseudocercospora* species known on *Senna* have a similar morphology to *P. sennae-multijugae*, namely *P. nigricans*, which occurs on different hosts on *Fabaceae*, and *P. taichungensis* reported on *Senna atomataria* and *Cassia fistula* (Farr & Rossman 2015). *Pseudocercospora nigricans* differs from *P. sennae-multijugae* by having well-developed stromata (25–67 µm diam) and branched, longer conidiophores (30–100 µm) (Brown & Morgan-Jones 1977), while *P. taichungensis* has longer and narrower conidiophores (10–25 × 1–3 µm) and shorter and narrower conidia (20–55 × 1.5–3 µm) (Hsieh & Goh 1990). Phylogenetically, *P. sennae-multijugae* clustered in the same clade with *P. cercidis-chinensis*, a species described on another member of the *Fabaceae*, *Cersis chinensis* (Fig. 1, clade 10). It is not possible to distinguish *P. sennae-multijugae* from numerous other *Pseudocercospora* spp. based solely on an ITS phylogeny, or from *P. cercidis-chinensis*, *P. solani-pseudocapsicicola* and *P. pyracanthigena* in the *tef1* phylogeny. In the *actA* phylogeny it cannot be distinguished from *P. acericola*, *P. cercidis-chinensis*, *P. fukuokaensis* and *P. mali*. Morphologically, all species above differ from *P. sennae-multijugae*. *Pseudocercospora cercidis-chinensis* differs by having longer and narrower conidiophores (10–40 × 3–3.5 µm) (Shin & Braun 2000). *Pseudocercospora pyracanthigena* has narrower conidiophores (2–3 µm) and shorter conidia (30–45 µm) (Crous et al. 2013a), whereas *P. acericola* differs by having longer and wider conidiophores (10–65 × 4–5.5 µm) and longer and wider conidia (35–145 × 4–6 µm) (Chupp 1954). *Pseudocercospora fukuokaensis* has longer conidiophores (5–30 µm) and shorter and narrower conidia (30–70 × 2–3.5 µm) (Chupp 1954), while *P. mali* differs by having longer conidiophores (8–40 µm) and narrower conidia (1.5–3 µm) (Deighton 1976).

Pseudocercospora solani-pseudocapsicicola Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB814906; Fig. 20

Etymology. Name derived from the plant host *Solanum pseudocapsicum*.

Leaf spots amphigenous, elliptical to irregular, scattered, with pale yellow areas on upper surface, 2–12 mm diam. *Internal mycelium* subhyaline, septate, branched, smooth, 3–5 µm diam. Stromata lacking. *Conidiophores* hypophyllous, in loose fascicles, arising from internal hyphae, through stomata, subcylindrical, 10–35 × 3–5 µm, 0–3-septate, straight to geniculate-sinuous, unbranched or rarely branched, pale olivaceous to pale brown, smooth. *Conidiogenous cells* terminal, unbranched, pale brown, subcylindrical, smooth, proliferating sympodially and percurrently, 10–27 × 3–4.5 µm. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, guttulate, olivaceous to pale brown, smooth, obclavate-cylindrical, straight to curved, 42–128 × 2–3.5 µm, apex obtuse, base obconically truncate, 2–3 µm wide, 2–6-septate; hila not thickened, not darkened, 1–2.5 µm diam.

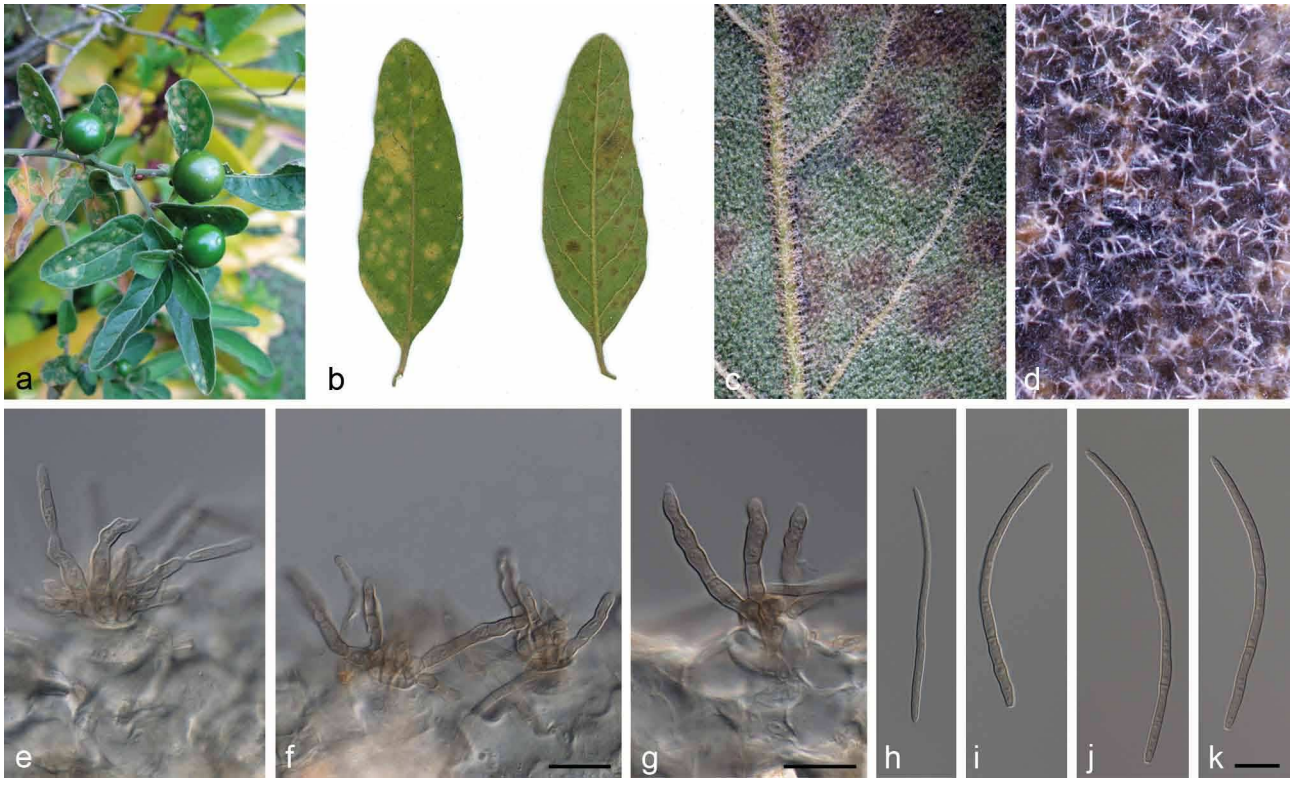


Fig. 20 *Pseudocercospora solani-pseudocapsicicola* (VIC 42807). a. *Solanum pseudocapsicum* with leaf spots; b. leaf spots on upper and lower leaf surface; c. close-up of lesion; d. close-up of lesion with fruiting; e, f. conidiophores emerging through stomata; g. conidiogenous cells; h–k. conidia. — Scale bars: e, f, h–k = 10 µm, g = 20 µm.

Culture characteristics — Very slow-growing (13–16 mm diam after 20 d), raised, with smooth to slightly irregularly lobate margins, aerial mycelium sparse, olivaceous grey, reverse iron-grey to green-black, sterile.

Specimen examined. Brazil, Minas Gerais, Viçosa, Sítio Criciúma, on leaves of *Solanum pseudocapsicum* (*Solanaceae*), 23 Jan. 2014, *M. Silva* (holotype VIC 42807, culture ex-type COAD 1974; isotype CBS H-22166, culture ex-isotype CPC 25229).

Notes — There are 21 species of *Pseudocercospora* known to occur on *Solanaceae* (Chupp 1954, Crous & Braun 2003). Only one species is described on *Solanum pseudocapsicum*, namely *P. fasciculata* described from Argentina (Deighton 1976). *Pseudocercospora fasciculata* is quite different from *P. solani-pseudocapsicicola* by having well-developed stroma, and longer and narrower conidiophores (80–110 × 2.5–3 µm). Two other species described on *Solanaceae* are morphologically more similar to *P. solani-pseudocapsicicola*, namely *P. marcelinae* described on *Solanum micranthum* in Argentina (Crous & Braun 2003) and *P. venezuelae* on *Solanum argenteum* in Venezuela and Brazil (Crous & Braun 2003). The former species differs from *P. solani-pseudocapsicicola* by having well-developed stromata, conidiophores which are shorter and narrower (5–25 × 2–4 µm) and shorter conidia (15–70 µm) (Chupp 1954), while *P. venezuelae* has well-developed stromata, conidiophores which are longer, arranged in dense fascicles (10–60 µm) and shorter conidia (2–4 µm) (Deighton 1976). *Pseudocercospora solani-pseudocapsicicola* grouped closely, but with poor support, with *P. pyracanthigena* (Fig. 1, clade 12), a species known to cause leaf spots on *Pyracantha angustifolia* (*Rosaceae*). Nevertheless, it is both morphologically and phylogenetically distinct from *P. pyracanthigena*. *Pseudocercospora pyracanthigena* is morphologically distinct from *P. fasciculata* in having shorter and narrower conidiophores (7–15 × 2–3 µm) and shorter conidia (30–45 µm) (Crous et al. 2013a). Deighton (1976) examined the original material of *P. fasciculata* and mentioned that “the type material is in very poor condition” and suggested that “further collections of this species are much to be desired”. An epitype therefore needs to be designated for this species. It is not possible to distinguish *P. solani-pseudocapsicicola* from numerous other *Pseudocercospora* spp. based solely on an ITS phylogeny, and it cannot be distinguished from *P. cercidis-chinensis*, *P. sennae-multijugae* and *P. trinidadensis* in the *tef1* phylogeny. In the *actA* phylogeny it is closely related to *P. pothomorphii* (COAD 1450) and *Pseudocercospora* sp. (CPC 10645).

Pseudocercospora stizolobii (Syd. & P. Syd.) Deighton, Mycol. Pap. 140: 153. 1976 — Fig. 21

Basionym. *Cercospora stizolobii* Syd. & P. Syd., Ann. Mycol. 11: 270. 1913.

Descriptions & Illustrations — Chupp (1954: 335), Hsieh & Goh (1990: 204, f. 157).

Culture characteristics — Very slow-growing (16 mm diam after 20 d); colonies erumpent, surface folded, moderate aerial mycelium, smooth to slightly irregular lobate margins darker than the rest of the colony. Surface olivaceous grey; reverse olivaceous black.

Specimen examined. Brazil, Goiás, Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, on leaves of *Mucuna aterrima* (*Fabaceae*), 26 Apr. 2013, *M. Silva* (CBS H-22160, VIC 42791, COAD 1532, CPC 25217).

Notes — Although this species was previously reported from Brazil (Crous & Braun 2003), this study represents the first phylogenetic data for this taxon (Fig. 1, clade 7). *Pseudocercospora stizolobii* is distinct from other species in the *tef1* and *actA* phylogenies, and slightly different from *P. atromarginalis*, *P. chengtuensis* and *P. fuligena* in the ITS phylogeny.

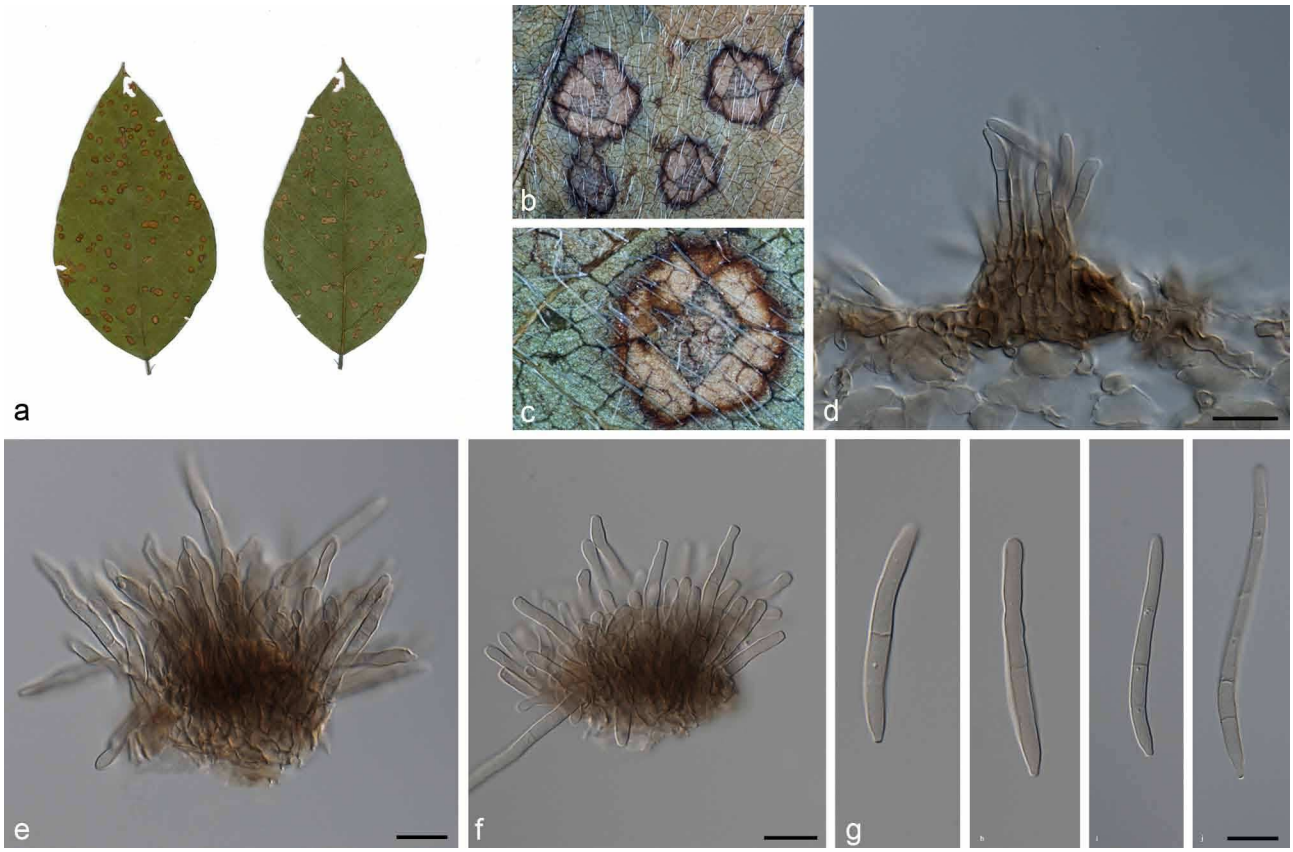


Fig. 21 *Pseudocercospora stizolobii* (VIC 42791). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. close-up of lesion with fruiting; d, e. fasciculate conidiophores; f. conidiogenous cells; g–j. conidia. — Scale bars: d–j = 10 µm.

Pseudocercospora struthanthi U. Braun et al., Cryptog. Mycol. 23: 316. 2002 — Fig. 22

Leaf spots amphigenous, circular, 4–10 mm diam, dark brown, margin poorly defined, sometimes with the chlorotic halo. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* small or well-developed, 21–43 × 32–63 µm, subimmersed or erumpent, angular, brown, composed of brown *textura angularis*. *Conidiophores* amphigenous, predominantly hypophyllous, aggregated in dense fascicles, cylindrical to subcylindrical, 7.5–31 × 3–5.5 µm, 0–3-septate, straight, unbranched, brown, smooth. *Conidiogenous cells* terminal, 7.5–17× 3–5 µm brown, smooth, conidiophores usually reduced to conidiogenous cells. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, finely guttulate, pale brown to brown, smooth, obclavate to cylindrical, straight to curved, 41–83.5 × 3–4 µm, apex obtuse to subacute, base obconically truncate to truncate, 2.5–3 µm wide, 1–10-septate; hila unthickened, not darkened, 1–2 µm diam.

Culture characteristics — Slow-growing (20 mm diam after 20 d); colonies erumpent, surface folded with moderate aerial mycelium and smooth, lobate margins. Surface olivaceous grey surrounded by a pale olivaceous grey margin; reverse iron-grey.

Specimens examined. Brazil, Ceará, Fortaleza, on leaves of *Struthanthus* sp. (*Loranthaceae*), 20 June 2000, *F. Freire* (paratype HAL 1719); Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of *Struthanthus flexicaulis*, 19 Apr. 2013, *M. Silva* (epitype designated here VIC 42766, MBT202070, culture ex-epitype COAD 1512; isoepitype CBS H-22157, culture ex-isoepitype CPC 25199).

Notes — The epitype of *P. struthanthi* designated here is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same country as the type. *Pseudocercospora struthanthi* clusters closely together with *P. pipers* (Fig. 1, clade 8). It is not possible to distinguish *P. struthanthi* from numerous other *Pseudocercospora* spp. based solely on an ITS or *actA* phylogeny, and it cannot be distinguished from *P. aeschynomenicola*, *P. piperis* and *Pseudocercospora* sp. CPC 10645 in the *tef1* phylogeny.

Pseudocercospora tecomicola (J.M. Yen) U. Braun & Bagyan., Sydowia 51: 12. 1999 — Fig. 23

Basionym. *Cercospora tecomicola* J.M. Yen, Rev. Mycol. 196. 1967.
≡ *Cercoseptoria tecomicola* (J.M. Yen) J.M. Yen, Gard. Bull. Singapore 33: 154. 1980.

Leaf spots amphigenous, irregular, brown, 2–10 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* almost lacking or 14–35 µm diam, subimmersed, globular, brown, composed of brown *textura globosa*. *Conidiophores* amphigenous, in small fascicles, mostly reduced to conidiogenous cells, emerging through stomata, cylindrical, 8–20 × 2–3.5 µm, 0–1-septate, straight to sinuous, unbranched, pale brown, smooth. *Conidiogenous cells* terminal, pale brown, cylindrical, smooth, proliferating sympodially. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, finely guttulate, pale brown, smooth, cylindrical to narrowly obclavate, straight to slightly curved, 21.5–63 × 2–4 µm, apex rounded to subacute, base truncate, 2–4 µm wide, 0–7-septate; hila neither thickened nor darkened, 1.5–2.5 µm diam.

Culture characteristics — Slow-growing (28 mm diam after 20 d); colonies circular, erumpent, surface velvety, with moderate aerial mycelium, smooth to slightly irregular margins. Surface olivaceous grey surrounded by pale olivaceous grey margin; reverse iron-grey.

Specimen examined. Brazil, Minas Gerais, Universidade Federal de Viçosa, on leaves of *Tecoma stans* (*Bignoniaceae*), 31 July 2013, *R.W. Barreto* (CBS H-22175, VIC 42687, COAD 1585, CPC 25260).

Notes — Three *Pseudocercospora* spp. are known to occur on species of the host genus *Tecoma*, viz. *P. sordida* on *Tecoma*

Fig. 22 *Pseudocercospora struthanthi* (VIC 42766). a. *Struthanthus flexicaulis* with leaf spots; b. leaf spots on upper and lower leaf surface; c. fasciculate conidiophores; d–g. conidia. — Scale bars: c–g = 10 µm.

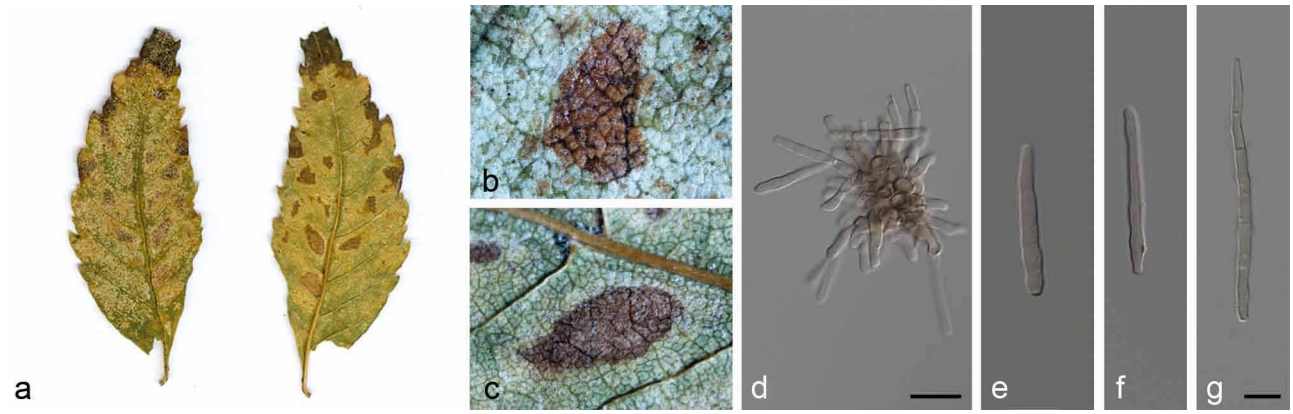


Fig. 23 *Pseudocercospora tecomicola* (VIC 42687). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. close-up of lesion with fruiting; d. conidiophores in small fascicle; e–g. conidia. — Scale bars: d–g = 10 µm.

stans, *T. radicans* and *Tecoma* sp., *P. tecomicola* on *T. stans* and *P. tecomae-heterophyllae* on *T. heterophylla* and *T. undulata* (Crous & Braun 2003, Farr & Rossman 2015). *Pseudocercospora sordida* has been previously described from Brazil on *Tecoma* sp. (Viégas 1945, Hanlin 1992, Crous & Braun 2003), but is morphologically and phylogenetically (Fig. 1, clade 5) quite distinct from *P. tecomicola* (Fig. 1, clade 6). The present *Pseudocercospora* collection closely matches the morphological features of *P. tecomicola* (Yen 1967, Bagyanarayana & Braun 1999) previously reported from Barbados and Singapore. This is the first report of *P. tecomicola* associated with *T. stans* in Brazil. It is not possible to distinguish *P. tecomicola* from several other *Pseudocercospora* spp. based solely on the ITS phylogeny, but it is distinct in the *tef1* phylogeny. In the *actA* phylogeny it is closely related to *P. nogalesii* and *P. wulffiae*.

Pseudocercospora trinidadensis (F. Stevens & Solheim) Crous et al., Mycotaxon 72: 179. 1999 — Fig. 24

Basionym. *Cercospora trinidadensis* F. Stevens & Solheim, Mycologia 23: 376. 1931.

Leaf spots amphigenous, grey-brown in the centre, surrounded by a dark brown to black margin, irregular, 3–11 mm diam. *Mycelium* internal, subhyaline, consisting of septate, smooth hyphae, 2.5–4 µm diam. *External mycelium* absent. *Stromata* small substomatal, globular, 9–13 µm diam, composed of brown *textura globosa*. *Conidiophores* amphigenous, sporodochial, mostly reduced to conidiogenous cells, 10–22 × 3–5 µm, 0–2-septate, straight to sinuous, unbranched, pale to medium brown, smooth. *Conidiogenous cells* terminal, pale to medium brown, subcylindrical, smooth, proliferating sympodially, 7–15 × 3–5 µm. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, olivaceous, finely guttulate, smooth, cylindrical to narrowly obclavate, straight to slightly curved, 29–88 × 3–5 µm, apex obtuse, base obconically truncate, 3–5 µm wide, 0–14-septate; hila neither thickened nor darkened, 2–2.5 µm diam.

Culture characteristics — Slow-growing (26 mm diam after 20 d); colonies erumpent, surface velvety, with sparse aerial mycelium, smooth to slightly irregular margins, margin of colony darker than colony interior. Surface olivaceous grey; reverse olivaceous black.

Specimens examined. Brazil, Rio de Janeiro, Nova Friburgo, Fazenda Barreto II, on leaves of *Croton urucurana* (*Euphorbiaceae*), 1 June 2014, *R.W. Barreto* (CBS H-22174, VIC 42851, COAD 1756, CPC 26082).

Notes — *Pseudocercospora trinidadensis* was reported from Trinidad and Tobago on leaves of *Croton gossypiifolius* (Crous & Braun 2003). The morphology of our specimen is in agreement with the description by Crous et al. (1999), and is reported here for the first time on *Croton urucurana* and from Brazil. Based on the multigene phylogenetic analysis it is closely related to *P. cercidis-chinensis* and *P. sennae-multijugae* (Fig. 1, clade 10). It is not possible to distinguish *P. trinidadensis* from numerous other *Pseudocercospora* spp. based solely on the ITS phylogeny, and it could barely be distinguished from *P. euphorbiacearum* and *P. pini-densiflorae* in the *tef1* phylogeny. No *actA* sequence of *P. trinidadensis* was available for comparison.

Pseudocercospora vassobiae Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813592; Fig. 25

Etymology. Name derived from host genus *Vassobia*.

Leaf spots amphigenous, irregular, becoming vein-delimited, brown to red, 3–8 mm diam. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* absent. *Conidiophores* hypophyllous, single or in small fascicles, emerging through stomata, 20–65 × 3–4 µm, 1–5-septate, straight to slightly curved, unbranched, brown, smooth. *Conidiogenous cells* terminal, integrated, cylindrical, proliferating percurrently, 10–43 × 3–4 µm, brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, finely guttulate, brown, smooth, cylindrical to obclavate, straight to curved, 27–108 × 3–5 µm, apex subacute to subobtuse, base obconically truncate, 2.5–4.5 µm wide, 2–10-septate; hila neither thickened nor darkened, 1–2.5 µm diam.

Culture characteristics — Slow-growing (17–20 mm diam after 20 d); raised, corrugated, aerial mycelium sparse, margins lobate, olivaceous grey, reverse olivaceous black, sterile.

Specimen examined. Brazil, Rio de Janeiro, Nova Friburgo, on leaves of *Vassobia breviflora* (*Solanaceae*), 9 June 2013, *R.W. Barreto* (holotype VIC 42676, culture ex-type COAD 1572; isotype CBS H-22173, culture ex-isotype CPC 25251).

Notes — No species of *Pseudocercospora* have previously been described on *Vassobia breviflora*. *Pseudocercospora*

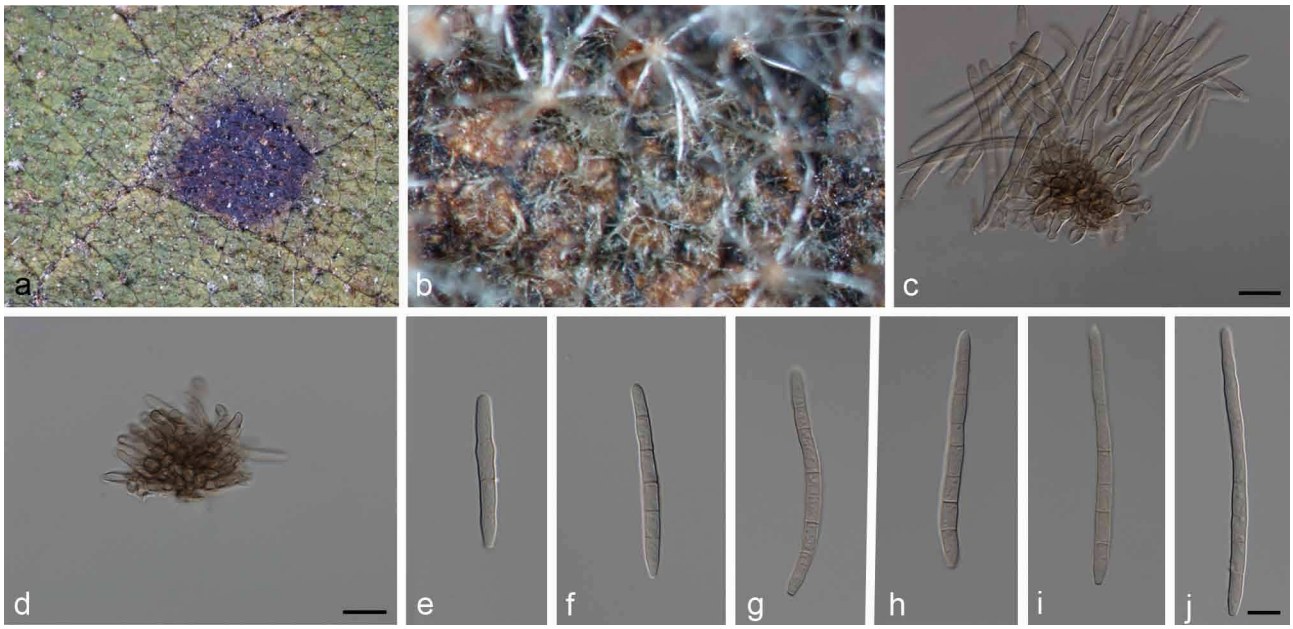


Fig. 24 *Pseudocercospora trinidadensis* (VIC 42851). a. Close-up of lesion; b. close-up of leaf spot with fruiting; c. sporodochial conidiophores; d. conidiogenous cells; e–j. conidia. — Scale bars: c–j = 10 µm.

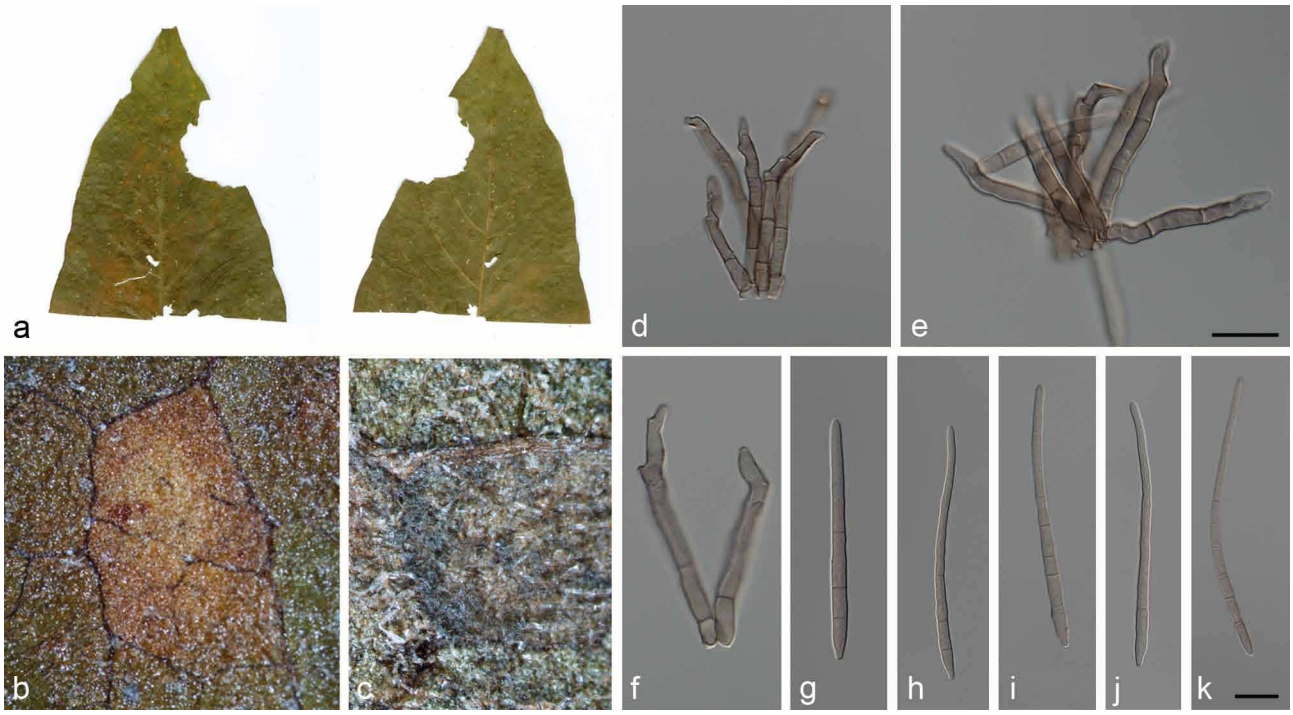


Fig. 25 *Pseudocercospora vassobiae* (VIC 42676). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. close-up of lesion with fruiting; d, e. conidiophores in a loose fascicle; f. conidiogenous cells; g–k. conidia. — Scale bars: d–k = 10 µm.

vassobiae is morphologically similar to *P. solani-asperi* and *P. daturina*. *Pseudocercospora solani-asperi* is distinct from *P. vassobiae* by having shorter and wider conidiophores (10–60 × 3–5 µm) and shorter and narrower conidia (30–80 × 3–4 µm) (Baker & Dale 1951, Deighton 1976) and *P. daturina* differs from *P. vassobiae* by having longer and wider conidiophores (30–80 × 4–6 µm) and longer conidia (51–123 µm) (Yen 1965, Deighton 1976). Phylogenetically, *P. vassobiae* clusters separate from other species of *Pseudocercospora* for which comparison of DNA sequence data is presently available (Fig. 1, clade 14). It is not possible to distinguish *P. vassobiae* from numerous other *Pseudocercospora* spp. based solely on the ITS or *actA* phylogenies. No *tef1* sequence of *P. vassobiae* was available for comparison.

Pseudocercospora wulffiae Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813623; Fig. 26

Etymology. Name derived from the plant host genus *Wulffia*, from which it was collected.

Leaf spots amphigenous, irregular, grey-brown surrounded by a dark brown margin, on lower surface medium brown, with poorly defined margin, 8–20 mm diam. *Internal mycelium* subhyaline, consisting of septate, branched, smooth, 3–4 µm diam hyphae. *External mycelium* absent. *Stromata* well-developed, 14–41 × 21–39 µm, immersed in the substomatal chamber, angular to irregular, medium brown, composed of brown *textura angularis*. *Conidiophores* hypophyllous, sporodochial, cylindrical, emerging through stomata, mostly reduced to conidiogenous cells, 14–21 × 2–3 µm, 0–2-septate, straight, unbranched, pale to medium brown, becoming paler toward the apex, smooth. *Conidiogenous cells* terminal, integrated, subcylindrical, proliferating percurrently, 8–21 × 2–3 µm, pale brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, cylindrical, apex rounded to subobtuse, straight to curved, 37.5–87 × 2–3.5 µm, base obconically truncate, 2.5–3 µm wide, 2–6-septate, pale brown, finely guttulate, smooth; hila unthickened, not darkened, 1.5–2.5 µm diam.

Culture characteristics — Slow-growing (22 mm diam after 20 d); colonies erumpent, surface folded with sparse aerial mycelium and smooth, lobate margins. Surface olivaceous grey with patches of pale olivaceous grey; reverse iron-grey to greenish black.

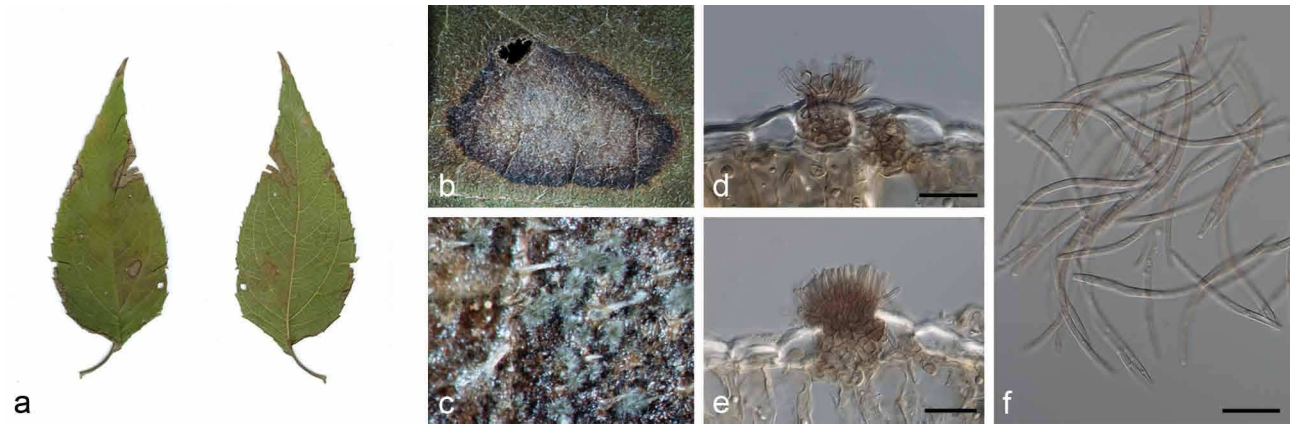


Fig. 26 *Pseudocercospora wulffia* (VIC 42810). a. Leaf spots on upper and lower leaf surface; b. close-up of lesion; c. close-up of lesion with fruiting; d. cross-section showing internal mycelium; e. conidiophore emerging through stomata; f. conidia. — Scale bars: d–f = 10 µm.

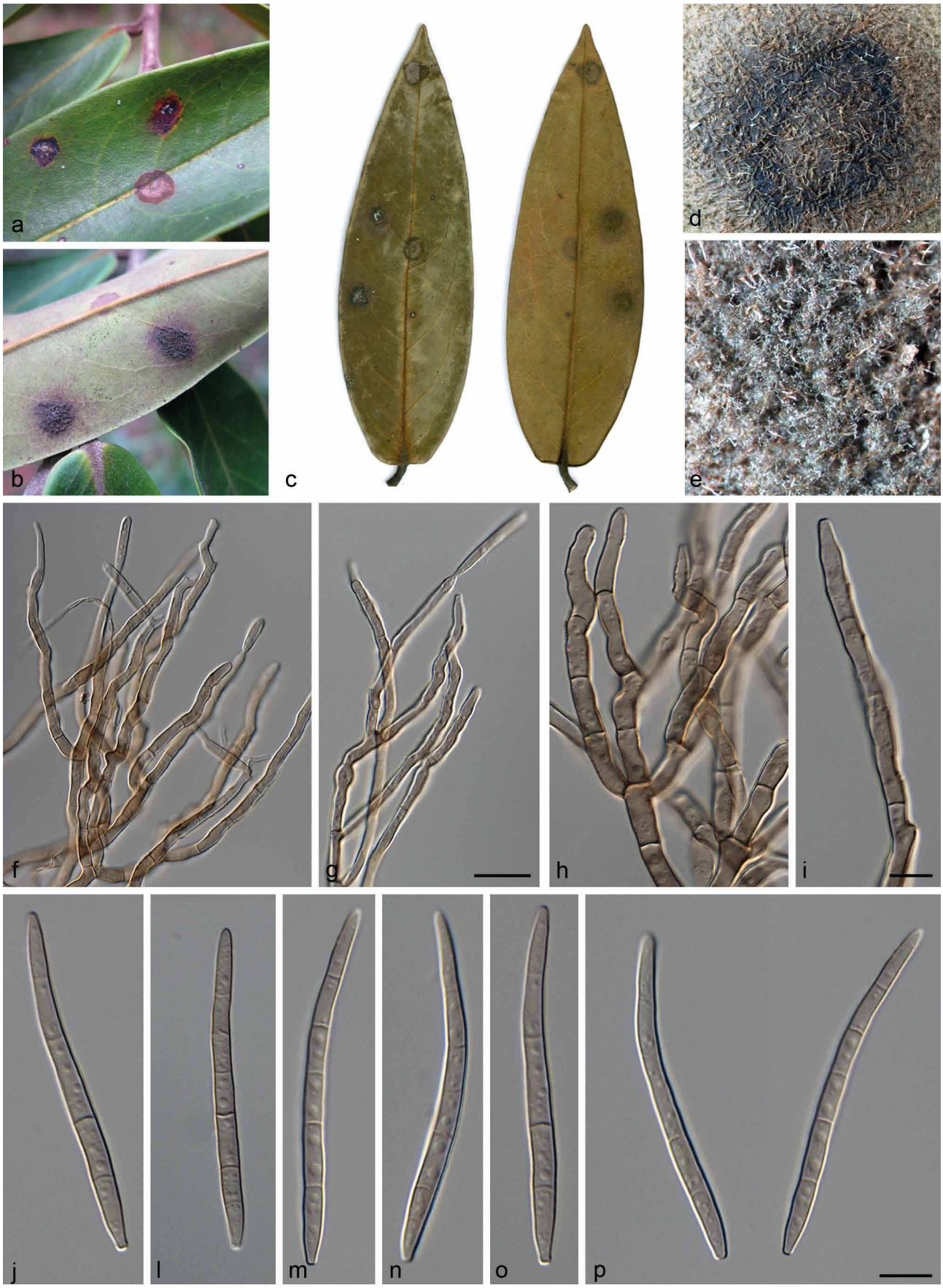


Fig. 27 *Pseudocercospora xylopiae* (VIC 42723). a, b. *Xylopia aromatica* with leaf spots; c. leaf spots on upper and lower leaf surface; d. close-up of lesion; e. close-up of lesion with fruiting; f, g. conidiophores in loose fascicles; h, i. conidiogenous cells; j–p. conidia. — Scale bars: f–p= 10 µm.

Specimen examined. Brazil, Minas Gerais, Lavras, on leaves of *Wulffia stenoglossa* (*Asteraceae*), 29 Jan. 2014, *M. Silva* (holotype VIC 42810, culture ex-type COAD 1976; isotype CBS H-22168, culture ex-isotype CPC 25232).

Notes — The description of Muller & Chupp (1936) of a new species of *Cercospora* (*C. wulffiae*) on *Wulffia stenoglossa* from Viçosa, Brazil, was invalid because it lacked a Latin diagnosis (Crous & Braun 2003). Currently, *C. wulffiae* is regarded as synonym of *P. wedeliae* (≡ *Cercospora wedeliae*), which occurs on different *Wedelia* spp. (Deighton 1976, Crous & Braun 2003). Although they have different host genera, "the morphological characteristics are nearly alike that they are considered identical" (Chupp 1954). We recollected the *Pseudocercospora* on *Wulffia stenoglossa*, and based on our phylogenetic data, we show that the species of *Pseudocercospora* described on *Wulffia* and *Wedelia* are different taxa. A sequence of the ITS region of *P. wulffia* (GenBank KT290150) possesses only 96 % similarity with the ITS sequence of *P. wedeliae* (GenBank KJ201940) (Kirschner & Liu 2014), confirming that they represent different species. Also see notes under *P. manihotii*, to which it is phylogenetically almost identical (Fig. 1, clade 6). It is not possible to distinguish *P. wulffiae* from several other *Pseudocercospora* spp. based solely on an ITS phylogeny, and it cannot be distinguished from *P. manihotii* in the *tef1* phylogeny. In the *actA* phylogeny it is closely related to *P. nogalesii* and *P. tecomicola*.

Pseudocercospora xylopiae Meir. Silva, R.W. Barreto & Crous, *sp. nov*. — MycoBank MB813622; Fig. 27

Etymology. Name derived from the plant host genus *Xylopia*.

Leaf spots amphigenous, circular to irregular, sparse, brown to red-brown, white in the centre, sometimes surrounded by a reddish chlorotic halo, 4–7 mm diam. *Internal mycelium* indistinct. *External mycelium* abundant, brown, septate, forming conidiophores. *Stromata* absent. *Conidiophores* hypophyllous, in loose fascicles, forming a dense network, climbing leaf trichomes, 5–7-septate, 15–187 × 3–5 µm, branched, brown, smooth. *Conidiogenous cells* terminal or intercalary, subcylindrical, proliferating sympodially, 8–20 × 2.5–4 µm, geniculate, brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, guttulate, pale brown, smooth, subcylindrical, straight to gently curved, 30–86.5 × 3–4.5 µm, apex obtuse, base truncate, 2.5–4 µm wide, 3–10-septate; hila unthickened, neither darkened nor refractive, 1.5–2.5 µm.

Culture characteristics — Slow-growing (16 mm diam after 20 d); colonies erumpent, surface velvety, convex, with smooth to slightly irregular margins. Surface olivaceous grey with olivaceous black border; reverse iron-grey to green-black.

Specimen examined. Brazil, Minas Gerais, Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of *Xylopia aromatica* (*Annonaceae*), 3 Jan. 2013, *M. Silva* (holotype VIC 42723, culture ex-type COAD 1469; isotype CBS H-22149, culture ex-isotype CPC 25173).

Notes — Only one species of *Pseudocercospora* was known to occur on a member of *Xylopia* (Farr & Rossman 2015), namely *P. aethiopicae* on *Xylopia aethiopica* from Sierra Leone (Deighton 1976). *Pseudocercospora aethiopicae* clearly differs from *P. xylopiae* by having shorter and narrower conidiophores (10–40 × 2.5–4 µm), arranged in dense fascicles, and not forming on external mycelium, and having smaller conidia, 32–65 × 2.5–3 µm (Deighton 1976). Additionally, *P. xylopiae* does not correspond to any sequences available in GenBank at present, and is phylogenetically related to *P. purpurea* (Fig. 1, clade 5). Hence, it is described here as a new species. It is not possible to distinguish *P. xylopiae* from several other *Pseudocercospora* spp. based solely on an ITS phylogeny, but it is distinct in the *tef1* and *actA* phylogenies.

DISCUSSION

This publication provides a multigene (ITS, *actA* and *tef1*) phylogenetic comparison of *Pseudocercospora* spp. collected from 15 host families occurring in Brazil. Currently, *Pseudocercospora* is recognised as genus name for the fungal holomorph, although its biology and morphological diversity are still under investigation (Braun et al. 2013, 2014, 2015, Crous et al. 2013a, Hora Júnior et al. 2014). Crous et al. (2013a) noted that significant ramifications pertaining to plant health and quarantine will only be resolved once critical taxa occurring in the Americas and Europe have been recollected from their original hosts and localities, isolated and epitypified, allowing for DNA sequence-based comparisons. This study is part of a broader project aimed at recollecting and providing molecular data for cercosporoid fungi occurring in Brazil, while also contemplating the description of newly collected species of cercosporoid fungi.

Several biomes in Brazil remain underexplored and entire plant families have never been investigated by mycologists. A recent example of the extent of the mycodiversity in Brazil awaiting discovery was provided by Guatimosim et al. (2016) who surveyed cercosporoid fungi on ferns in Brazil. These collections resulted in a significant increase in the known fern mycobiota in Brazil. Additionally, there is a complete lack of molecular information in public databases for the majority of Brazilian cercosporoid species.

The ITS barcode region (Schoch et al. 2012) was not able to differentiate many taxa at species level, resolving only 12 out of the 82 species included in the Bayesian analysis based only on the ITS alignment (data not shown, see TreeBASE). The lack of resolution of this region for *Pseudocercospora* was already commented on by Crous et al. (2013a) and Bakhshi et al. (2014), and is further confirmed here. The partial gene sequences of the protein-coding regions *actA* and *tef1* were individually better (resolving each approximately half of all included species) for the identification of *Pseudocercospora* spp. from Brazil, as was also reported by Crous et al. (2013a) and observed for other cercosporoid genera, such as *Cercospora* (Groenewald et al. 2013, Bakhshi et al. 2015) and *Ramularia* (Videira et al. 2015). The combined phylogeny presented in Fig. 1 allows for better species discrimination than a phylogeny derived from any individual locus. Most species could be resolved, although the resolving power of the combined analysis failed for species in some clades, such as clades 8 and 9. For many of the examined species, any given locus alone is insufficient for species recognition, and requires the inclusion of at least one additional locus to resolve the species. The low resolution per individual locus also adds up in the combined alignment, ranging from low to no support values for clades containing closely related species (for example in clades 8, 9, 12 and 17). In the present study, only 11 species (*P. angolensis*, *P. chamaecristae*, *P. exilis*, *P. fijiensis*, *P. guianensis*, *P. macrospora*, *P. planaltinensis*, *P. plunkettii*, *P. richardsoniicola*, *Pseudocercospora* sp. CBS 113387 and *P. udagawana*) were supported as distinct by all three loci in the Bayesian phylogenies. Future work on identifying a more robust molecular marker for species discrimination in *Pseudocercospora* is therefore essential.

Fungi included in *Pseudocercospora* have been regarded as host-specific (Crous et al. 2013a, Bakhshi et al. 2014). However the same authors also reported species occurring on more than one host. There is a great need for studies involving inoculation experiments to address questions regarding host specificity of *Pseudocercospora* and pseudocercospora-like taxa. Furthermore, the general view of *Pseudocercospora* spp. being host-specific may change as molecular confirmation of species identity becomes available for more strains of a given species. The generation and public availability of phyloge-

netically informative gene regions of *Pseudocercospora* spp. is of great phytopathological importance for understanding the epidemiology of many important plant diseases. One among many examples is provided by a 'pending enigma', involving *P. fijiensis* (the aetiological agent of black Sigatoka of banana – a devastating disease of bananas and plantains). Gasparotto et al. (2005) reported this fungus as occurring on the ornamental plant *Heliconia psittacorum*, a member of a distinct plant family (*Heliconiaceae*) in Brazil. That study was based on symptomatology, fungus morphology and cross inoculations. However, the use of DNA data could lead to more conclusive evidence of the status of the fungus on *H. psittacorum*, which could have consequences for black Sigatoka management, including proper treatment and quarantine regulations.

The present study represents the first organized effort towards generating molecular data to support the taxonomy of *Pseudocercospora* spp. from Brazil. It yielded information for 27 taxa, representing only a small fraction of yet unknown species diversity in this and other genera of cercosporoid fungi. Twelve taxa found in this study represented novel species. Additionally, a further eight epitype specimens were designated, while three species were newly reported from Brazil. One of the purposes of this study was to recollect Brazilian cercosporoids described by pioneers of the discipline such as A.S. Muller and A.P. Viégas. Other cercosporoid fungi described by these authors were also recollected, and they will be treated in future publications. Many additional species still need to be recollected to enable a better understanding of what may be the largest known genus of cercosporoid fungi.

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Capítulo 2

According to the guidelines of Persoonia

Artigo — Exploring fungal mega-diversity: multi-gene analyses of some Passalora, Sirosporium and Zasmidium from Brazil

Exploring fungal mega-diversity: multi-gene analyses of some Passalora, Sirosporium and Zasmidium from Brazil

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Abstract Differently from cercosporoid fungi belonging to the genera Cercospora and Pseudocercospora, there are very few DNA sequences available on public databases for most of the other genera in this group hampering the phylogenetic studies that may allow a better understanding of relationship between these taxa. There is a recognized lack of such sequences for members of Passalora, Zasmidium and Sirosporium, although there is ongoing work that may contribute to improve this situation in the near future. Although these genera have a worldwide distribution, they are especially diverse in tropical and subtropical countries. Many species are endemic in Brazil, however most of them are known only by morphological features, and no cultures are presently available for use in phylogenetic analysis. In this study, a total of seven members of Passalora, four species of Zasmidium and one of Sirosporium were collected, cultured, and subjected to multigene analysis. Four genomic regions (LSU, ITS, tef1 and actA) were amplified and sequenced. A multigene Bayesian analysis was performed on the combined ITS, actA and tef1 sequence alignment. Six taxa were newly described, namely: P. dasyphyllii, S. tocoyenae, Z. aspidospermae, Z. brosimii, Z. peixotoana and Z. roupalina. Additionally, three epitype specimens were designated, Passalora rubida, P. schefflerae and P. vicosae.

Key words: biodiversity, Capnodiales, cercosporoid, Dothideomycetes, Mycosphaerellaceae, plant pathogen, systematics.

INTRODUCTION

Fungi known by the informal denomination of “cercosporoids” or “Cercospora-like fungi” are one of the largest known groups of microfungi, including over 2000 species and including several genera of asexual morphs of ascomycetes traditionally placed in Mycosphaerella and Mycosphaerella-like sexual morphs (Crous & Braun 2003). These cercosporoid are cosmopolitan fungi and are highly diverse especially in tropical and subtropical countries (Crous & Braun 2003, Braun & Freire 2002, 2004, 2006, Hernández-Gutiérrez & Dianese 2008, 2009, 2014a,b, Hernández-Gutiérrez 2015). Cercoporoid fungi vary from being saprobic, hyperparasitic and plant pathogens. Numerous cercosporoids are plant parasites causing mostly leaf spots but also several other plant diseases, including attacking some of the major crop plants and causing major losses (Shin & Kin 2001, Goodwin et al. 2001, Jackson et al. 2004, Arzanlou et al. 2007). Examples of economically relevant hosts are: soybean (Cercospora kikuchii), common bean (Pseudocercospora griseola), banana (Pseudocercospora fijiensis) and citrus (Pseudocercospora angolensis) (Agrios 2005).

The taxonomy of cercosporoid fungi have rapidly changed in recent years due to the application of molecular tools to verify the delimitation of taxa and their true affinities. The monograph of cercosporoid by Chupp (1954) provided a starting point after a long period of rather loose treatment of Cercospora-like fungi. In his monograph, Chupp re-examined a large amount of specimens placed in Cercospora since the emergence of the genus and concluded that, along the years, the name had served as a “dumping ground” for taxa that belonged to unrelated and dissimilar genera such as species of Fusarium and Alternaria. Although he excluded such discrepant taxa from Cercospora Chupp’s concept for the genus Cercospora remained far too broad and he rejected previous separations

in sessions (Chupp 1954). For Chupp, all the diverse assemblage now called “cercosporoids” belonged to the single genus Cercospora. Deighton (1965, 1967, 1971, 1973, 1974, 1976, 1979, 1983, 1987,1990), Ellis (1971, 1976) and Braun (1995, 1998) either proposed or resurrected cercosporoid genera ignored or rejected by Chupp and divided the Cercospora-complex in tens of morphologically-similar genera. The first phylogenetic studies based on sequences data for cercosporoids (Crous et al. 2000) and the review of Crous & Braun 2003 led to a later reduction recognized genera of cercosporoids. Subsequently, several others studies based on morphology and DNA sequence data have been published and confirmed most of the changes in generic circumscriptions proposed in the past (Crous & Braun 2003, Arzanlou 2007, Minnis et al. 2011, Braun et al. 2013, 2014, 2015, Crous et al. 2013, Groenewald et al. 2013) but also revealed the existence of overlooked genera that were not evidently distinct based on morphology studies alone (Crous et al. 2013, Amaradasa et al. 2014, Bakhshi et al. 2015a). With the abolishment of Article 59 of the International Code of Nomenclature for Algae, Fungi and Plants (ICN), a single generic name is now used for sexual and asexual morphs (Hawkworth et al. 2011, Wingfield et al. 2012, Crous et al. 2015). Mycosphaerella s. st. was recognized as a name that should be applied only to taxa having Ramularia asexual morphs and the name Mycosphaerella is now the facultative synonym of Ramularia. The others Mycosphaerella-like species were better placed in the other genera (Verkley et al. 2004), however the generic circumscription of most of these genera are dubious, since few DNA sequences are available for most of them. Even for genera such as Cercospora and Pseudocercospora for which a considerable number o sequences are available on public sequence databases and detailed phylogenetic analysis have been published the generic circumscription has changed with time as more DNA phylogenetic data has become available (Crous et al. 2013, Bakhshi et al. 2014, 2015a,b, Groenewald et al. 2013, Silva et al. 2016). On the other hand, for the great majority of other cercosporoid genera, particularly Passalora and Passalora-like genera, very few sequences are available on

public sequences databases, making it very difficult (or even impossible) to properly establish generic delimitations for them. Fortunately, this situation is likely to change with the approaching publication of a reappraisal of Passalora (Videira et al., in prep.). It is known that the Brazilian mycobiota is very rich in Passalora-like fungi (Braun & Freire 2002, 2004, 2006, Pereira & Barreto 2005, Hernández-Gutiérrez & Dianese 2008, 2009, 2014a,b, Firmino et al. 2013, Parreira et al. 2014, Hernández-Gutiérrez et al. 2015) but, unfortunately, most of the publications dealing with these fungi do not contain any molecular information. The aim of the present study was therefore to initiate a reevaluation of Passalora and Passalora-like fungi occurring in Brazil, similarly to what has recently been accomplished for Brazilian Pseudocercopora spp. (Silva et al. 2016).

MATERIAL AND METHODS

Sample collection and isolates

Surveys were conducted between 2013 and 2014 in the Reserva Florestal Mata do Paraíso (Viçosa, Minas Gerais), the campus of the Universidade Federal de Viçosa (Viçosa, Minas Gerais) and neighbouring areas in the municipality of Viçosa, Floresta Nacional de Paraopeba (Paraopeba, Minas Gerais), Estação Ecológica de Águas Emendadas (Distrito Federal, Brasília), Parque Nacional da Chapada dos Veadeiros (Alto Paraíso de Goiás, Goiás), Instituto Agronômico de Campinas (Campinas, São Paulo), municipality of Lavras (Minas Gerais) and Nova Friburgo (Rio de Janeiro). Samples with cercosporoid leaf spot symptoms were collected, dried in a plant press, and taken to the laboratory. Fungal isolations were performed by direct transfer of fungal structures onto plates containing vegetable broth agar (VBA) as described by Pereira et al. (2003) or 2 % potato-dextrose agar (PDA; HiMedia). Axenic cultures were preserved in potato-carrot agar (PCA) slants or on silica gel and were deposited in the culture collection of the Universidade Federal de Viçosa, Coleção Oswaldo Almeida Drummond (COAD). Representative specimens were deposited at the Fungarium of the Universidade Federal de Viçosa (VIC).

Morphology

Taxonomic descriptions were based on observations of fungal structures formed on the tissue of plant specimens. Samples with cercosporoid leaf spot symptoms were examined under a stereomicroscope Olympus SZ X7. Fungal structures were removed from the lesions with a sterile dissecting needle and mounted in lactophenol. Observations, measurements and high-resolution photographic images of microscopic fungal structures were taken with an Olympus BX 53 light microscope with an Olympus Q-Color5™ digital high definition colour camera. Adobe Photoshop CS5 was used for the final editing of the acquired images and photographic preparations. Culture descriptions were based on observations of colonies formed in plates containing potato dextrose agar (PDA) following incubation at 24 ℃ under a 12 h light/dark regime for 2 –4 wk in duplicate. Colour terminology followed Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org, Crous et al. 2004).

DNA isolation, PCR amplification and sequencing

Genomic DNA was extracted from mycelium growing on malt extract agar (MEA) plates at 25 ºC for up to 4 wk depending on their growth rate, using the CTAB extraction protocol as outlined by Crous et al. (2009). Four nuclear gene regions were targeted for Polymerase Chain Reaction (PCR) amplification and subsequent sequencing. The Internal Transcribed Spacer (ITS) region was amplified using primers ITS-5 and ITS-4 (White et al. 1990), the Large Subunit (28S nrDNA, LSU) with LR0R (Rehner & Samuels 1994) and LR5 (Vilgalys & Hester 1990), the translation elongation factor 1-alpha (tef1) with EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell et al. 1998) and actin (actA) with ACT-512F and ACT-783R (Carbone & Kohn 1999). PCR mixtures included the following ingredients for each 12.5 µL reaction: 10–20 ng of template DNA, 1× PCR buffer, 0.63 µL DMSO (99.9 %), 1.5 mM $MgCl_2$, 0.5 µM of each primer, 0.25 mM of each dNTP, 1.0 U BioTaq® DNA polymerase (Bioline GmbH

Luckenwalde, Germany). The PCRs were carried out with a MyCycler™ Thermal Cycler (Bio-Rad Laboratories B.V., Veenendal, The Netherlands). Conditions for the PCR amplification consisted of an initial denaturation at 95 ºC for 5 min; followed by 40 cycles of denaturation at 95 ºC for 30 s; annealing at 52 ºC for ITS and LSU, 54 ºC for tef1 or 55 ºC for actA for 30 s; extension at 72 ºC for 1 min and a final extension step at 72 ºC for 7 min. Following PCR amplification, amplicons were visualised on 1 % agarose gels to check for product size and purity. The PCR products were sequenced in both directions using the PCR primers and the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA, USA), following the protocol of the manufacturer. DNA sequencing amplicons were purified through Sephadex® G-50 Superfine columns (Sigma Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were run on an ABI Prism 3730xl DNA Analyser (Life Tecnologies, Carlsbad, CA, USA). The consensus sequences were generated using the MEGA v. 6.0.6 (Molecular Evolutionary Genetics Analyses) (Tamura et al. 2013). All sequences were checked manually, and nucleotides with ambiguous positions were clarified using both primer direction sequences.

Phylogenetic analyses

Consensus sequences were compared against NCBI's GenBank nucleotide database using their mega BLAST algorithm. The most similar sequences were downloaded in FASTA format and the sequence datasets for the four genomic loci were aligned individually using the MAFFT v. 7 online portal (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh & Standley 2013). In addition, the combined sequence alignment of Quadvilieg et al. (2014) was downloaded from TreeBASE (Study S16145) and used as an initial reference alignment for species identification. Resulting sequence alignments were manually checked and adjusted in MEGA v. 6.06 and were concatenated with Mesquite v. 2.75 (Maddison & Maddison 2011). A phylogenetic reconstruction was conducted on the aligned LSU data set to determine generic relationships. For the LSU

alignment, MrModeltest v. 2.2 (Nylander 2004) was used to select the optimal model of nucleotide substitution prior to the Bayesian Inference (BI) analysis using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003). The general time-reversible model of evolution (Rodriguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) was used. Subsequently, a species-level phylogeny was derived from a concatenated ITS (alignment position 1–532), tef1 (alignment position 538–1103) and actA (alignment position 1109–1340) dataset using MrModeltest v. 2.2 to select the optimal model of nucleotide substitution for each locus based on the Akaike Information Criterion prior to the BI analysis. For ITS, tef1 and actA, a dirichlet (1,1,1,1) state frequency distribution was set and for all three loci an inverse gamma distributed rate variation. Two sets of four MCMC (Markov Chain Monte Carlo) chains were run simultaneously, starting from random trees and lasting until the critical value for the topological convergence diagnostic reached 0.01. Trees were sampled every 1000 generations and the first 25 % of the trees were discarded as the burn-in phase for each analysis and posterior probabilities (Rannala & Yang 1996) were determined from the remaining trees and are presented on the left of each node (Fig. 1). Sequences derived from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1), the alignments and trees in TreeBASE (www.treebase.org/treebase/index.html). The resulting phylogenetic tree was printed with Geneious v. 7.1.8 (http://www.geneious.com, Kearse et al. 2012), and the layout of the tree for publication was carried out using in Adobe Illustrator v. CS5.

RESULTS

Phylogenetic analyses

The LSU alignment consisted of 78 OTU's (including the outgroup sequence) and 522 characters were included in the analysis. The alignment had 191 unique site patterns. The LSU phylogeny, revealed that all strains obtained from the survey were recognised as members of

Mycosphaerellaceae (data not shown, see TreeBASE). These were subsequently included in the combined actA, tef1 and ITS alignment for species level identification (Fig. 1).

For the species level analysis of the 13 isolates from Brazil, DNA sequence data from the actA, tef1 and ITS gene regions were combined for the Bayesian analyses. The concatenated alignment contained a total of 56 strains (43 strains from NCBI, and 13 strains from this study) (Table 1). Staninwardia suttonii (CBS 120061) served as the outgroup taxon. The results of MrModeltest recommended a GTR+I+G model for ITS, HKY+I+G model for tef1 and actA.The final aligned sequences of the ITS (532 characters), actA (231 characters) and tef1 (565 characters) gene regions had a total length of 1340 characters (including alignment gaps) which were included in the analyses. The Bayesian analysis of the combined alignment, based on 602 unique site patterns (ITS: 203, actA: 116, tef1: 283), posterior probabilities (PP) were calculated from the 1896 trees left after discarding 474 trees (the first 25 % of the generations) for burn-in (Fig. 1). Bayesian posterior probabilities (PP) are presented on the left of each node, on each tree. Details about the performance of the different loci are provided under the species notes below.

Taxonomy

Passalora bougainvilleae (Munt.-Cvetk) R.F. Castañeda & U. Braun. Cryptogamic Botany 2 (2-3): 291 (1991)

Description and illustration – Castañeda & Braun 1991.

Specimens examined. BRAZIL, Minas Gerais, Viçosa, on leaves of Bougainvillea sp. (Nyctaginaceae), 01 Dec. 2013, R.W.Barreto (VIC 42677, COAD 1574).

Passalora calotropidis (Ellis & Everh.) U. Braun. Schlechtendalia 5: 60 (2000) — Fig. 2

Lesions on living leaves amphigenous, large circular or irregular blotches, dark brown to black, 5–17 mm diam, coalescing. Internal mycelium

indistinct. External mycelium absent. Stromata lacking or only with small stromatic aggregations of swollen hyphal cells, substomatal, 7–25 µm diam, brown. Conidiophores amphigenous, sporodochial, mostly restricted to conidiogenous cells, arising from stomata, cylindrical, straight to curved, geniculate, 10–50 × 3–5 µm, 0–4 septate, rarely branched, pale to variously medium olivaceous, smooth. Conidiogenous cells integrated, terminal or intercalary, proliferation sympodial, polyblastic, cylindrical, 6–50 × 3–5 µm pale olivaceous. Conidiogenous loci thickened, darkened. Conidia dry, solitary, cylindrical to cylindro-obclavate, straight to slightly curved, 10–85 × 3–5 µm, obconically truncate to truncate base, rounded apex, 0–7 septate, sometimes constricted at septae, pale to medium dark olivaceous brown, eguttulate, smooth, hila thickened and darkened.

Culture characteristics — Very slow-growing (11–13 mm diam after 23 d), raised with smooth, irregular lobate margins, aerial mycelium sparse, greyish; reverse black; not sporulating.

Specimens examined. BRAZIL, Ceará, Jericoacoara, on leaves of Calotropis procera (Apocynaceae), 01 Dec. 2013, R.W.Barreto (VIC 42691, COAD 2032), Ceará, Marco, on leaves of Calotropis procera (Apocynaceae), 01 Dec. 2013, R.W.Barreto (VIC 42692, COAD 2033).

Notes — The morphology of the Brazilian collection on Calotropis procera fits well with the description of Passalora calotropidis (Braun 2000), although conidia and conidiophores being slightly wider than in Braun's description. Such differences are regarded here as having no taxonomic relevance. This species was previously reported from several countries, including Brazil, Cuba, Dominican Republic, Egypt, Ethiopia and others (Crous & Braun 2003). This is the first time molecular data is generated for this species. Phylogenetically, Passalora calotropidis clusters with Mycosphaerella quasiparkii that is reported in Eucalyptus sp. as sister clade, however differing by a highly supported branch (PP = 1.0) (Fig. 1, clade 1).

Passalora dasyphyllii Meir. Silva, R.W. Barreto & Crous, sp. nov. — Fig. 3

Etymology. Name derived from the plant host genus Dasyphyllum.

Leaf spots amphigenous, starting as small black dots that become vein-delimited brown spot surrounded by a chlorotic halo, coalescing, 3–13 mm diam. Internal mycelium indistinct. External mycelium absent. Stromata small or well-developed, erumpent, 32–54 × 25–75 µm, composed of brown textura globosa. Conidiophores amphigenous, mostly hypophyllous, sporodochial, arising from the upper cells of the stroma, mostly restricted to conidiogenous cells, cylindrical, 51−10 × 4–5 µm, 0–3 septate, straight, unbranched, brown, smooth. Conidiogenous cells terminal or intercalary, integrated, proliferation sympodial, cylindrical, 10–21 × 3–5 µm brown, smooth. Conidiogenous loci thickened, darkened. Conidia solitary, subcylindrical, straight to curved, 15–98 × 3–5 µm, apex obtuse, base obconically truncate, 5–7 septate, pale brown, smooth; hila thickened and darkened.

Culture characteristics — Very slow-growing (12–14 mm diam after 23 d), raised, lobate margins, aerial mycelium sparse, olivaceous grey; reverse olivaceous black; not sporulating.

Specimens examined. BRAZIL, Minas Gerais, Carrancas, trilha da Cachoeira Esmeralda, on leaves of Dasyphyllum sp. (Asteraceae), 23 Apr. 2013, M. Silva (holotype VIC 42812, culture ex-type COAD 2031).

Notes — No species of Passalora is known to occur on Dasyphyllum (Farr & Rossman 2015). In the multigene phylogenetic analysis, P. dasyphyllii is basal in a clade containing P. vicosae, P. rubidae and Sirosporium tocoyenae (Fig. 1, clade 2).

Passalora delamonicae A. Hern. Gut. & Dianese. Mycological Progress 2013; Fig. 4

Lesions on living leaves amphigenous, circular or irregular, pale grey centrally inside of dark greyish brown lesion surrounded by a dark brown well-defined margin, 2–15 mm diam, coalescing. Internal mycelium indistinct. External mycelium absent. Stromata erumpent, well-developed, subcuticular, 36–108 µm, composed of brown textura globosa. Conidiophores epiphyllous, aggregated in dense fascicles, arising from

stromata, cylindrical, straight to curved, geniculate, 44–110 × 5–6 µm, 3–7 septate, unbranched, ligth brown, paler at the apex. Conidiogenous cells integrated, terminal or intercalary, proliferation sympodial, polyblastic, cylindrical, 15–33 × 5–6 µm pale brown.Conidiogenous loci prominent dark and thickened. Conidia dry, solitary, cylindrical or narrowly obclavate, straight to curved, 55–83 × 4–5 µm, obconically truncate at the base, rounded to subobtuse apex, 5–11 septate, olivaceous brown, eguttulate, smooth; hila thickened and darkened.

Culture characteristics — Very slow-growing (12–14 mm diam after 23 d), raised, irregular, aerial mycelium sparse, greyish; reverse black; not sporulating.

Specimens examined. BRAZIL, Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of Banisteriopsis oxyclata (Malpighiaceae), 17 Apr. 2013, M. Silva (VIC 42747, COAD 1494).

Notes — This study provides the first phylogenetic data available for this species. In the multigene phylogenetic analysis, P. delamonicae clusters separately from other species of Passalora for which comparison of DNA sequence data is presently available (Fig. 1, clade 3).

Passalora schefflerae A. Hernández-Gutiérrez & Dianese, Mycotaxon 106: 47 (2008) — Fig. 5

Lesions on leaves amphigenous, localized scorched-like necrotic areas of tissue, circular or irregular, dark brown surrounded by a well-defined raised rim adaxially, 5–9 mm diam. Internal mycelium branched, septate, 3–5 µm diam. External mycelium abundant, brown, septate, bearing secondary conidiophores. Stromata well-developped, substomatal, erumpent, globular, 19–73 µm diam, composed of brown textura globosa. Conidiophores hypophyllous, aggregated in loose fascicles, cylindrical, straight to geniculate-sinuous, 25–263 × 4–6 µm, multiseptate, branched, brown, smooth. Conidiogenous cells terminal or intercalary, integrated, polyblastic, 10–30 × 3–6 µm, subcylindrical, light brown to brown. Conidiogenous loci darkened and thickened. Conidia dry, solitary, cylindrical to obclavate, straight to slightly curved, 22–84 × 6–10 µm, base

obconically truncate, apex obtuse, 1–7 septate, with a thicker, darker septum in the middle, olivaceous to pale brown, eguttulate, smooth; hilum thickened and darkened.

Culture characteristics — Very slow-growing (15–17 mm diam after 23 d), raised, irregular margins, aerial mycelium cotony, olivaceous grey; reverse olivaceous black; not sporulating.

Specimens examined. BRAZIL, Goiás, Fazenda Nova Índia, on leaves of Schefflera macrocarpa (Araliaceae), 19 Apr. 1993, J.C. Dianese 1058; UB mycol. Col. 4464 (Holotype); Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of Schefflera macrocarpa, 03 Jan. 2013, M. Silva (epitype designated here VIC 42722, MBTxxx, culture ex-epitype COAD 1468).

Notes — The epitype of P. schefflerae, designated here, is morphologically equivalent to the holotype, particularly in morphology of conidiophores and conidia, and originated from the same biome as the holotype. In the multigene phylogenetic analysis, P. schefflerae clustered separately from other species of Passalora for which comparison of DNA sequence data is presently available (Fig. 1, clade 5).

Passalora rubida Crous, Alfenas & R.W. Barreto, Mycotaxon 64: 425 (1997) — Fig. 6

Lesions on living leaves amphigenous, starting as chlorotic areas that later become brown, irregular, 1–7 mm diam, coalescing with age. Internal mycelium branched, septate, light brown, 3–6 µm diam. External mycelium branched, septate, light brown, 3–6 µm diam, climbing trichomes and forming conidiophores and conidia. Stromata absent. Conidiophores hypophyllous, arising singly from superficial hyphae, cylindrical, straight to curved, 16–80 × 4–6 µm, 1–6 septate, branched, medium brown, finely verruculose. Conidiogenous cells terminal or intercalary, subcylindrical, proliferation sympodial or sometimes percurrent, 9–33 × 4–6 µm, medium to light brown, thickened. Conidiogenous loci darkened and thickened. Conidia dry, catenate, chains simple or branched, cylindrical, 25–112 × 4–6 µm, obconically truncate or rounded at the base, subobtuse apex, 0–8

septate, light to medium brown, guttulate, smooth; hila thickened and darkened

Culture characteristics — Slow-growing (11–14 mm diam after 23 d); raised, convex, irregular, aerial mycelium velvety, redish; reverse redish; not sporulating.

Specimens examined. BRAZIL, Minas Gerais, Viçosa, on leaves of Croton floribundus (Euphorbiaceae), 19 Apr. 1933, A.S. Muller; IACM 3742 (holotype), CUP-MG 3742 Isotype; Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of Croton floribundus, 22 Jan. 2014, M. Silva (epitype designated here VIC 42712, MBTxxx, culture ex-epitype COAD 1262).

Notes — The epitype of P. rubida, designated here, is morphologically equivalent to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same region as the holotype. Phylogenetically, P. rubidae clusters with P. vicosae and Sirosporium tocoyenae in a sister clade (Fig. 1, clade 2).

Passalora vicosae (A.S. Mull. & Chupp) Crous, Alfenas & R.W. Barreto Mycotaxon 64: 414 (1997) — Fig. 7

Basionym. Cercospora vicosae A.S. Mull. & Chupp, Arquivos do Instituto de Biologia Vegetal do Rio de Janeiro 1(3): 220 (1935).

Lesions on leaves none or indistinct, with black effuse abaxial colonies. Internal mycelium branched, septate, hyaline, 3–5 µm diam. External mycelium absent. Stromata small or absent, globular, 9–13 mm diam, composed of brown textura globosa. Conidiophores hypophyllous, aggregated in loose to dense fascicles, subcylindrical, straight becoming pronouncedly geniculate towards the apex, 56–189 × 4–6 µm, multiseptate, rarely branched, brown, smooth. Conidiogenous cells terminal and intercalary, integrated, proliferating sympodially, 12–30 × 4–6 µm, subcylindrical, light brown. Conidiogenous loci darkened and thickened Conidia dry, solitary, cylindrical to obclavate, straight to slightly curved, 27–93 × 4–6 µm, base obconically truncate, apex obtuse, 1–8 septate, olivaceous to pale brown, guttulate, smooth; hilum thickened, darkened, refractive.

Culture characteristics — Slow-growing (13–16 mm diam after 23 d); circular, raised, convex, irregular lobate margins, aerial mycelium sparse, olivaceous grey; reverse olivaceous black; not sporulating.

Specimens examined. BRAZIL, Minas Gerais, Viçosa-Escola, on leaves of Manihot sp. (Euphorbiaceae), 16 Apr. 1933, A.S. Muller; IACM 468 (Holotype); Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of Manihot sp. (Euphorbiaceae), 22 Jan. 2014, M. Silva (epitype designated here VIC 42800, MBTxxx, culture ex-epitype COAD 2030).

Notes — Notes — The epitype of P. vicosae, designated here, is morphologically equivalent to the holotype and originates from the same area as the holotype. No DNA sequence data was available for Passalora vicosae until now. In the multigene phylogenetic analysis, Passalora vicosae described on Manihot sp. clustered in the same clade with Sirosporium tocoyenae described from Tocoyena formosa (Fig. 1, clade 2). The phylogenetic position of the genera Sirosporium and Passalora remain unresolved, since no sequences are available for the type or epitype of these genera until present.

Sirosporium tocoyenae Meir. Silva, R.W. Barreto & Crous, sp. nov. — Fig. 8

Etymology. Name derived from the plant host genus Tocoyena.

Colonies hypophyllous, effuse, vein delimited, black. Internal mycelium indistinct. External mycelium, 2.5–5 µm diam, branched, septate, brown, smooth. Stromata absent. Conidiophores hypophyllous, arising singly from superficial hyphae, lateral or terminal, cylindrical, straight to geniculate-sinuous, 9–58 × 5–7 µm, 0–3 septate, branched, light brown, smooth. Conidiogenous cells monoblastic or poliblastic, terminal or intercalary, 13–21 × 4–7 µm, cylindrical, light brown, bearing thick scars. Conidia dry, solitary, cylindrical when immature to obclavate when mature, straight to slightly flexuous, 32–104 × 6–10 µm, apex rounded, base protruding, 3–21 transverse and occasionally 1–3 longitudinal and oblique septa, constricted at the septae when mature, pale brown to brown, thick-walled, eguttulate, smooth; hilum sometimes protruding.

Culture characteristics — Slow-growing (9–12 mm diam after 23 d); raised, circular, margin smooth, aerial mycelium sparse, velvety, dark reddish; reverse dark reddish; not sporulating.

Specimens examined. BRAZIL, Minas Gerais, Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of Tocoyena formosa (Rubiaceae), 30 Mar. 2013, M. Silva (holotype VIC 42733, culture ex-type COAD 1479).

Notes — Two species of Sirosporium have been described as having members of the Rubiaceae as hosts: Sirosporium morindina on Morinda tomentosa and S. morindinum on Morinda tinctiria (Farr & Rossman 2015). Sirosporium morindina differs from the newly proposed species by having longer and narrower conidiophores (60–90 × 4–6 µm) and longer, wider conidia (80–120 × 6–12 µm) (Agarwal 2002), whereas S. morindinum has longer and narrower conidiophores and conidia (14–138 × 3.5–5 µm, 13–127 × 4–7 µm; respectively) (Kamal & Morgan-Jones 1985). This is the first species of Sirosporium reported on a member of the genus Tocoyena (Rubiaceae). Phylogenetically, Sirosporium tocoyenae clusters with Passalora vicosae in the same clade with a strong support (PP = 0.98) (Fig. 1, clade 2). The phylogenetic position of the genera Sirosporium and Passalora remain unresolved, since no sequences are available for the type or epitype of these genera until present.

Zasmidium aspidospermae Meir. Silva, R.W. Barreto & Crous, sp. nov. — Fig. 9

Etymology. Name derived from the plant host genus Aspidosperma.

Lesions on leaves none or indistinct, with black caespituli hypophyllous, small dots. Internal mycelium indistinct. External mycelium scarse, verruculose, 2.5–3 µm wide, pale brown, septate. Stromata well developed, erumpent, 47–75 × 40–68 µm, composed of dark brown textura angularis. Conidiophores hypophyllous, aggregated in loose to dense fascicles, cylindrical, straight to geniculate-sinuous, 181–389 × 4–6 µm, multiseptate, unbranched, light brown, smooth. Conidiogenous cells terminal or intercalary, integrated, proliferation sympodial, 7.5–61 × 3–5 µm, cylindrical, light brown to brown; conidiogenous scars, non

protuberant, thickened and darkened. Conidia dry, solitary, obclavate or fusoid, straight to curved, 38–93 × 4–7 µm, apex rounded to subacute, base obconically truncate to truncate, 1–6 septate, pale brown, thick walled with darkened septae, guttulate, smooth; hilum slightly thickened and darkened.

Culture characteristics — Very slow-growing (22–25 mm diam after 23 d), raised, with smooth, feathery margins, aerial mycelium velvety, greyish centrally; reverse olivaceous grey; not sporulating.

Specimens examined. BRAZIL, Minas Gerais, Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of Aspidosperma tomentosum (Apocynaceae), 30 Mar. 2013, M. Silva (holotype VIC 42727, culture ex-type COAD 1473).

Notes — Two cercosporoid fungi are known to occur on Aspidosperma (Apocynaceae), Pseudocercospora aspidospermatis on Aspidosperma tomentosum, A. doricarpon and A. macrocarpon and Cercospora aspidospermatis on A. dasycarpon (Farr & Rossman 2015). Two species of Zasmidium are known to occur on members of the Apocynaceae: Zasmidium ichnocarpicola on Ichnocarpi frurescentis and P. plumeriae on Plumeria acutifolia (Farr & Rossman 2015, Singh et al. 2001). Zasmidium plumeriae has the closest morphology to that of Zasmidium aspidospermae but differs from the newly described species by having shorter and narrower conidiophores (33–82.5 × 3–4.5 µm) and narrower conidia (3.5–5 µm) (Sarbajna & Chattopadhyay 1991). Zasmidium ichnocarpicola is rather different from P. aspidospermae. It has much shorter and narrower conidiophores (7–61 × 2.5–3.5 µm) arising single from superficial hyphae or in small fascicle and catenate, shorter conidia (36–60 µm) (Kamal 2010). Phylogenetically, Z. aspidospermae groups with Z. peixotoana but their morphology is quite different, Z. peixotoana has conidiophores which are solitary, never forming fascicles as in Z. aspidospermae and which are smaller and narrower (42–172 × 3–5 µm) than in the new species. It also has shorter and narrower conidia (21–80 × 4–5 µm). Our molecular data (Fig. 1 clade 4) support the morphological data and confirm that Z. aspidospermae and Z. peixotoana are in fact distinct species.

Zasmidium brosimii Meir. Silva, R.W. Barreto & Crous, sp. nov. — Fig. 10

Etymology. Name derived from the plant host genus Brosimum.

Leaf spots amphigenous, irregular, reddish to brown surrounded by well-defined borders with a chlorotic halo surrounding the spots, 3–10 mm diam. Internal mycelium indistinct. External mycelium hypophyllous, climbing the trichomes, abundant, verruculose, Selacking. Conidiophores hypophyllous, solitary, arising from superficial hyphae, lateral, occasionally terminal, erect, straight, restricted to the conidiogenous cells, subcylindrical, 2.5–7 × 2.5–3 µm unbranched, brown, smooth; conidiogenous loci inconspicuous to somewhat conspicuous, slightly darkned-refractive, slightly thickened. Conidia dry, solitary, cylindrical to subobclavate, straight to slightly curved, 15–85 × 2.5–4.5 µm, base obconically truncate, apex rounded to subacute, 1–7 septate, pale brown to brown, guttulate, verrucolose; hilum slightly thickened, darkened.

Culture characteristics — Very slow-growing (21–23 mm diam after 23 d), raised, with irregular lobate margins, slightly corrugated, aerial mycelium sparse, grey centrally, iron-grey periphery; reverse iron-grey;not sporulating.

Specimens examined. BRAZIL, Minas Gerais, Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of Brosimum gaudichaudii (Moraceae), 30 Mar. 2013, M. Silva (holotype VIC 42724, culture ex-type COAD 1470).

Notes — No species of Zasmidium are known to occur on Brosimum (Moraceae), but two Zasmidium species have been described from hosts in the Moraceae (Farr & Rossman 2015), namely: Z. macluricola on Maclura cochinchinensis and Z. ficinum on Ficus benghalensis. Morphologically, Z. macluricola is distinct from Z. brosimii by having well developed stromata, sporodochial conidiomata, longer and wider conidiophores (15–45 × 4–6 µm) and conidia forming branched chains and which are longer and wider (15–45 × 4–5 µm) (Shivas et al. 2009). Shivas et al. (2009) deposited sequences in GenBank for their new species Z.

macluricola (strain BRIP52143) on Maclura cochinchinensis (ITS: GU108499). These were compared with those generated from Z. brosimii and found to only have a 86 % similarity. Zasmidium ficinum is distinguishable from Z. brosimii by having longer and wider conidiophores (29–43 × 3.75–4.5 µm) and shorter and narrower conidia (21.5–36 × 2.9–3.6 µm) (Kamal et al. 1981). Phylogenetically, Zasmidium brosimii did not cluster in the same clade of the type species, Z. cellare. Instead, it grouped with Xenomycosphaerella yunnanensis described on Eucalyptus urophylla (Burgess et al. 2007, Quaedvlieg et al. 2014). According Quaedvlieg et al. 2014, “Xenomycosphaerella morphologically is a typical species of Mycosphaerella s.l. but phylogenetically distinct” and no asexual morphs are known until now. (Fig. 1, clade 6). It is possible that Xenomycosphaerella has a Zasmidium-like asexual morph but this requires confirmation. Zasmidium is paraphyletic in Mycosphaerellaceae and remains poorly resolved (Crous et al. 2009b).

Zasmidium peixotoana Meir. Silva, R.W. Barreto & Crous, sp. nov. — Fig. 11

Etymology. Name derived from the plant host genus Peixotoa.

Colonies hypophyllous, effuse, irregular, dark brown to black. Internal mycelium indistinct. External mycelium dense, climbing trichomes branched, 3–5 mm diam, septate, mostly straight, olivaceous brown, verruculose. Stromata lacking. Conidiophores abundant, hypophyllous, solitary, arising from superficial hyphae, lateral, occasionally terminal, subcylindrical, straight to curved, 42–172 × 3–5 µm, branched, light brown to brown, smooth. Conidiogenous cells terminal or intercalary, proliferation sympodial, cylindrical, 7–35 × 2.5–5 µm, light brown. Conidia dry, solitary, cylindrical to subobclavate, straight to curvate, 21–80 × 4–5 µm, base obconically truncate, apex rounded to subacute, 1–10 septate, pale brown to brown, eguttulate, verrucolose; hilum thickened and darkened.

Culture characteristics — Very slow-growing (22–25 mm diam after 23 d), raised, surface folded with sparse aerial mycelium, lobate margins, olivaceous grey; reverse iron-grey; not sporulating.

Specimens examined. BRAZIL, Goiás, Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, on leaves of Peixotoa sp. (Malpighiaceae), 23 Apr. 2013, M. Silva (holotype VIC 42760, culture ex-type COAD 1507).

Notes — No species of Zasmidium or Stenella are known to occur on Peixotoa or on any member of the Malpighiaceae (Farr & Rossman 2015). Zasmidium peixotoana is recognized here as a new addition to Zasmidium. In the multigene phylogenetic analysis, Z. peixotoana clustered with Z. aspidospermae (see notes under Z. aspidospermae) (Fig. 1, clade 4).

Zasmidium roupalina Meir. Silva, R.W. Barreto & Crous, sp. nov. — Fig. 12

Etymology. Name derived from the plant host genus Roupala.

Colonies hypophyllous, effuse colonies, spread over the surface of the leaves, velvety. Internal mycelium indistinct. External mycelium 2–3 µm diam, branched, septate, pale brown, verruculose, climbing the trichomes. Stromata lacking. Conidiophores hypophyllous, monomematous, solitary, verruculose, cylindrical, straight to curved, 150–450 × 4–5.5 µm, multiseptate, rarely constrict at septa, straight to curved, geniculate, unbranched, dark brown, smooth. Conidiogenous cells terminal or intercalary, proliferation sympodial, subcylindrical, 7–33 × 4–5.5 µm, brown; conidiogenous loci non-protuberant, somewhat thickened and darkened Conidia dry, solitary, cylindrical to subacute, straight to slightly curved, 22–133 × 2.5–5 µm, base obconically truncate, apex rounded to subacute, 1–10 septate, brown, eguttulate, verrucolose; hilum thickened and darkened.

Specimens examined. BRAZIL, Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of Roupala montana (Proteaceae), 16 Apr. 2013, M. Silva (holotype VIC 42734, culture ex-type COAD 1480).

Culture characteristics — Very slow-growing (22–25 mm diam after 23 d), raised, with irregular lobate margins, aerial mycelium sparse, surface olivaceous grey with olivaceous black border; reverse iron-grey; not sporulating.

Notes — No species of Zasmidium has been recorded on Roupala or any other member of the Proteaceae (Farr & Rossman 2015). The morphological boundaries of Zasmidium coincide with those of Stenella. The sole significant morphological difference between fungi in these genera is that conidiogenous loci (mirrored by conidial hila) of Zasmidium are planate, somewhat thickened and darkened whereas in Stenella these are pileate (Crous et al. 2009). Zasmidium is presently recognized as belonging to the Mycosphaerellaceae whereas Stenella fits into the Teratosphaeriaceae (Crous et al. 2009, Braun et al. 2013). One species of Stenella was reported on Lomata silaifolia (Proteaceae), namely S. lomatiae. This species has not been reexamined in recent times and it is unclear if its conidiogenous loci are typical of Stenella or whether it is phylogenetically connected with Stenella or Zasmidium. Nevertheless Stenella lomatiae is different from Z. roupalina by having shorter and wider conidiophores and conidia (up to 150 × 5–7 µm, 14–55 × 5–9 µm; respectively) (Priest 1991). In our study, Z. roupalina is the first species of Zasmidium grouping in the clade where the type species of Zasmidium, Z. cellare, sits. This is a clade sister with Z. eucalyptorum described on Eucalyptus sp. (Crous et al. 2006) and Z. pseudoparkii described in Eucalyptus grandis (Quaedvlieg et al. 2014) (Fig. 1, clade 7).

DISCUSSION

In this publication a multi-gene (ITS, actA and tef1) phylogenetic comparation of Passalora, Zasmidium and Sirosporium collected from 9 different host families occurring in Brazil is provided. Except for P. rubida and P. vicosa (collected at Atlantic Rain Forest sites) and P. calotropidis (found in the Brazilian semi-arid northeast) all other specimens were

collected in savanah-like Cerrado sites. A great diversity of Passalora and Passalora-like fungi has already been reported from the Cerrado on host belonging to numerous families (Hernández-Gutiérrez & Dianese 2008, 2009, 2014a,b, Hernández-Gutiérrez et al. 2015). However, until now, knowledge about most of these species is based only on morphology, without any preserved cultures being available for much needed phylogenetic analysis (Hernández-Gutiérrez & Dianese 2008, 2009, 2014a,b, Hernandez-Gutiérrez et al. 2015). With a few exceptions (e.g. Guatimosim in press), publications dealing with Brazilian Passalora and Passalora-like fungi lack molecular data and rely solely on morphological characteristics, making phylogenetic comparisons with species from other countries impossible. For the great majority of cercosporoid fungi from Brazil, there is a great need for recollecting, culturing and performing phylogenetic evaluations, preferably through multigene analysis.

Surveys of the biodiversity of Brazilian cercosporoid fungi in native and cultivated plants date back to 1929, when A.S. Muller collected and described several species from the state of Minas Gerais (Muller & Chupp 1934, 1936). Two of these species originally collected by Muller were recollected and isolated during our survey, P. rubida (≡ Cercospora rubida) and P. vicosae (≡ Cercospora vicosae). Epitypification is provided here and sequences of these species will now become available on public databases for phylogenetic analysis. Another taxon which was epitypified is Passalora schefflerae. Hopefully these will represent only the first of numerous cercosporoid taxa to be epitypified by present and future generations of mycologists working on Brazilian material.

The Passalora spp. collected in our study were found to belong to several different clades when subjected by a multigene phylogenetic analysis, similarly to what has been observed by other authors (Crous et al. 2009b). The concept of Passalora introduced by Braun (1995) and expanded by Crous & Braun (2003) appears to be excessively wide when preliminar phylogenetic analised were conducted (Crous et al. 2000, 2001, 2009b). Analysis based on ITS and other genomic regions indicate that Passalora s. lat. is not monophyletic (Crous et al. 2000, 2001, 2009b, c,

2013). At least for the present, Braun et al. (2013) pointed out that Passalora has to be considered as a para- or polyphyletic genus. For a better circumscription of Passalora, the type (P. bacilligera) needs to be recollected and analyzed molecularly and a much broader sampling of species need to be performed (Crous et al. 2009b, Braun et al. 2013).

The new species of Sirosporium collected in our study, Sirosporium tocoyenae, is a typical Sirosporium species with thick walled conidia and oblique to longitudinal septa (dictyosporous) (Braun 1995, Braun et al. 2013). Sirosporium tocoyenae clustered in a well supported clade with P. vicosae, P. rubida and P. dasyphyllii. This result support the idea that maybe the thick conidial walls and oblique to longitudinal septa as distinguishing characters between Sirosporium and Passalora having no phylogenetic meaning. Other authors support the idea that Sirosporium and Passalora should be treated as separate genera (Braun 1995, Crous & Braun 2003). However, our result is only preliminary and, as for Passalora, it is also necessary to recollect the type of the genus Sirosporium (S. antenniforme), as also recollect and epitypify a range of species belonging to this genus, allowing a better understanding of phylogenetic affinities in this genus.

The four Zasmidium species collected in our study showed a great morphological diversity. Zasmidium aspidospermatis and Z. peixotoana clustered together in a well supported clade, however the two other fell in separate clades. Zasmidium roupalina belonged to the same clade of the type of Zasmidium, Zasmidium cellare. The genus Zasmidium is known to be paraphyletic in the Mycosphaerellaceae (Crous et al. 2009b). Since the Zasmidium-like morphology exist in separate lineages in the Mycosphaerellaceae (Crous et al. 2009b), it is not surprising that our species fell into different clades. Except for Z. roupalina, the other Zasmidium discussed herein may prove to belong to distinct genera in the future. Nevertheless, based on the available evidence we decided that it would be premature to propose new genera for these fungi and maintained them in Zasmidium (Crous et al. 2009b).

It is hoped that this work will encourage other mycologists working with Brazilian fungi to expand this study aiming at generating molecular data and elucidating the taxonomy of Passalora and Passalora-like species together with other taxa representing the mega-diverse assemblage of cercosporoid from Brazil.

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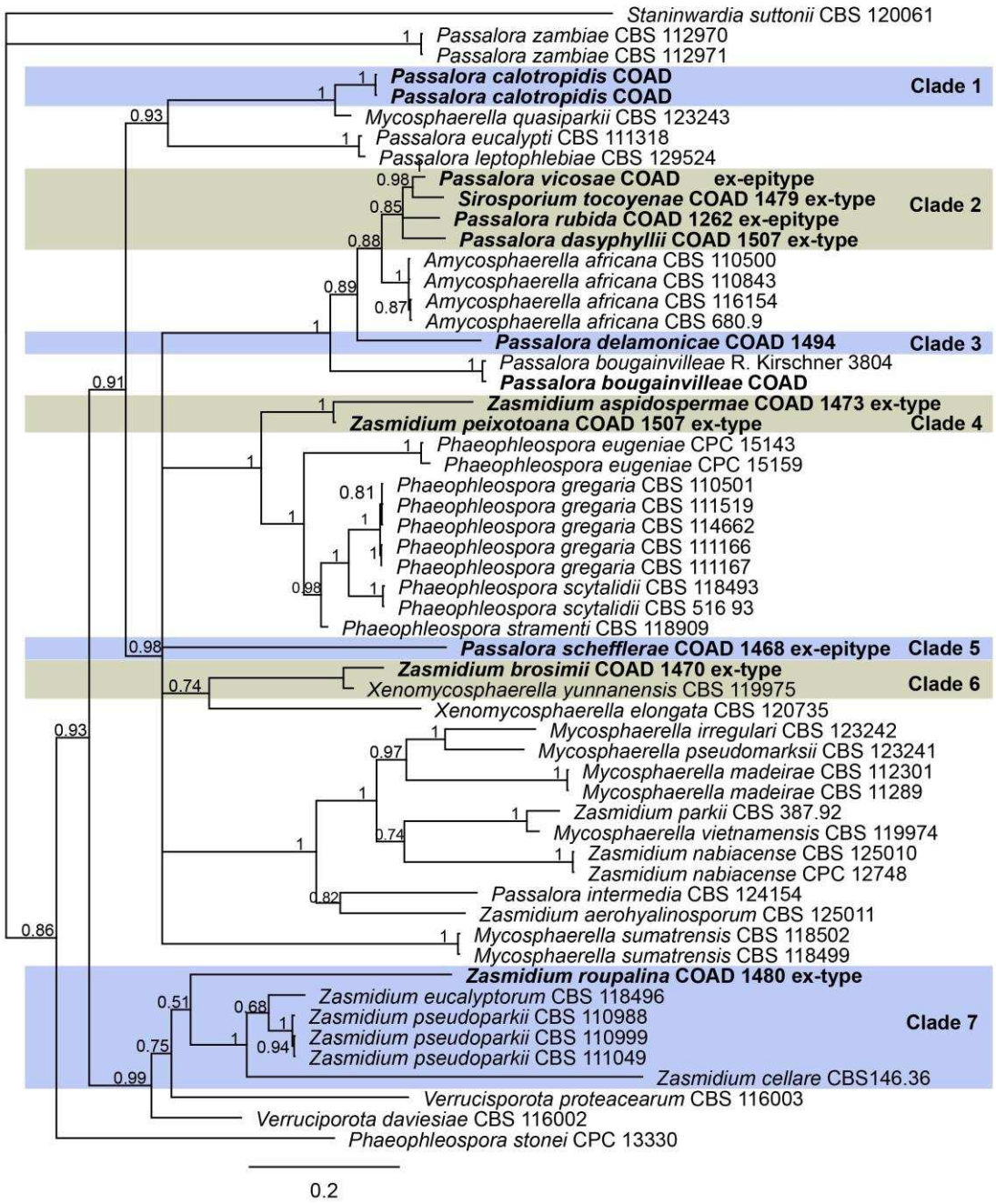

Fig. 1 Bayesian phylogenetic tree inferred from DNA sequence data from the multigene alignment (ITS, actA and tef1) of cercosporoid species. Species from Brazil are in **bold** face and in coloured blocks with clade numbers for reference in the species notes. The type status of strains is indicated next to the culture collection number. Bayesian posterior probabilities are indicated at the nodes. The tree was rooted with Staniwardia suttonii (isolate CBS 120061).

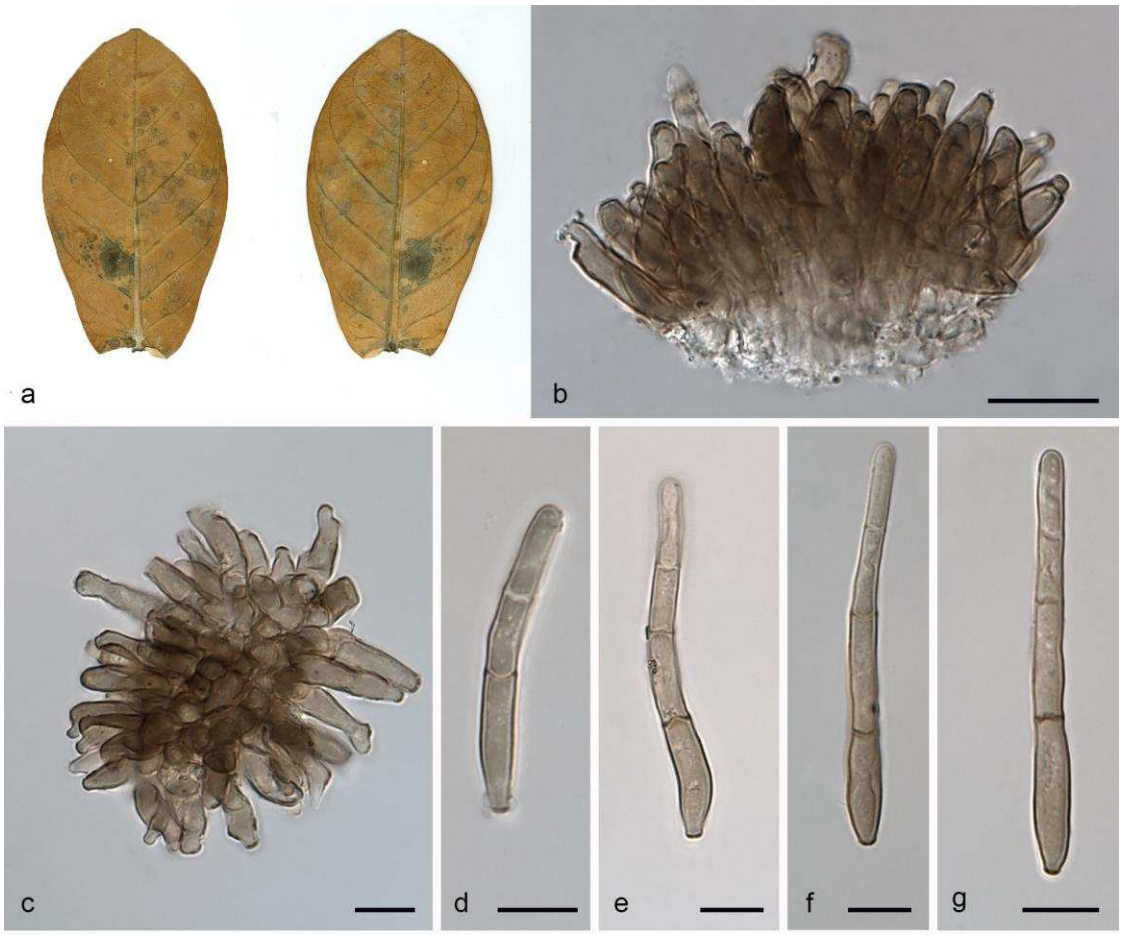

Fig. 2 Passalora calotropidis (VIC 42692) on Calotropis procera. a. leaf spots on upper and lower leaf surface; b–c. sporodochial conidiophores and conidiogenous cells; d–g. conidia. — Scale bars: b–g= 10 µm.

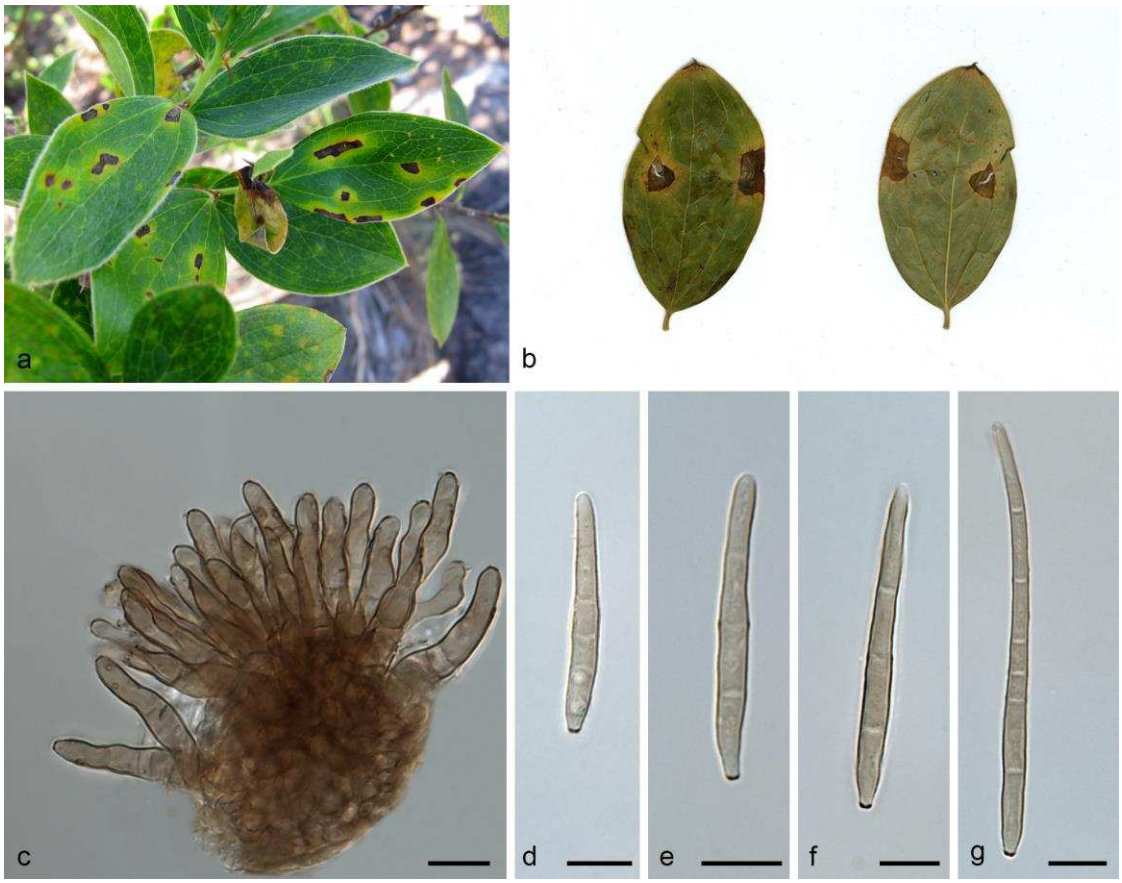

Fig. 3 Passalora dasyphyllii (VIC 48812) on Dasyphyllum sp. a. foliage bearing leaf spots in the field; b. leaf spots on upper and lower leaf surface; c. stroma and sporodochium; d–g. conidia. — Scale bars: c–g = 10 µm.

Fig. 4 Passalora delamonicae (VIC 42747) on Banisteriopsis oxyclata. a. leaf bearing spots; b. Leaf spots on upper and lower leaf surface; c. conidiophores forming dense fascicles; d. conidiogenous cell with attached conidium; e–f. conidia. — Scale bars: c–f= 10 µm.

Fig. 5 Passalora schefflerae (VIC 42722) on Schefflera macrocarpa. a. adaxial and abaxial view of colonized leaf; b. conidiophores; c–d. conidiophore and conidiogenous cells; e–h. conidia. — Scale bars: b–h = 10 µm.

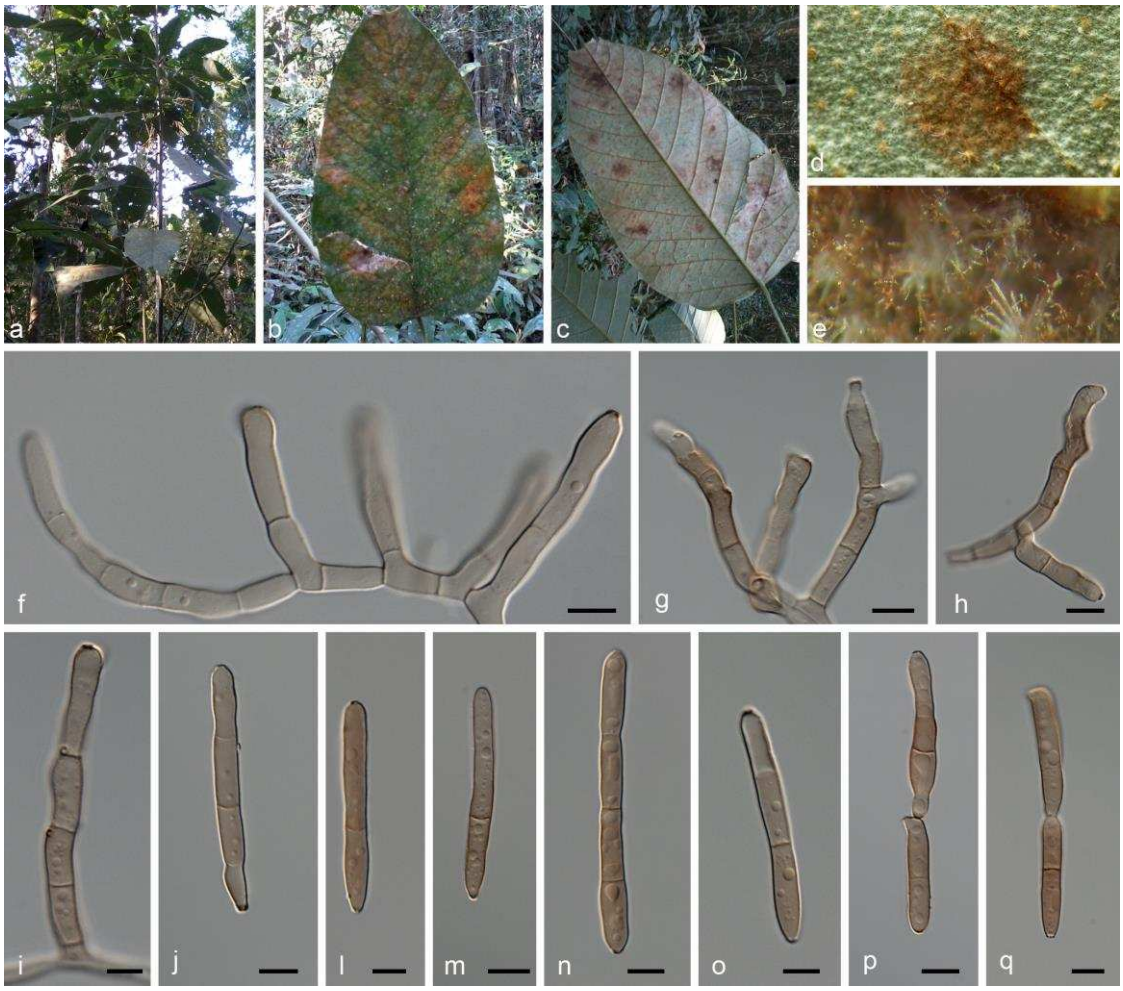

Fig. 6 Passalora rubida (VIC 42712) on Croton floribundus. a. Croton floribundus in the field; b–c. leaf spots on upper and lower leaf surface; d. close-up of circular lesion; e. close-up of sporulating colony on leaf; f–i conidiophores; j–o. conidia; p–q. catenate conidia— Scale bars: f–q = 10 µm.

Fig. 7 Passalora vicosae (VIC 42800) on Manihot sp. a. Leaf spots on upper and lower leaf surface; b–c. fasciculate conidiophores; d–g. conidia. — Scale bars: b–g = 10 µm.

Fig. 8 Sirosporium tocoyenae (VIC 42733) on Tocoyena formosa. a. foliage bearing leaf spots in the field; b. upper and lower leaf surface; c. close-up of sporulating colony on leaf surface; d–i. conidiophores; j–l. imature conidia; m–q. mature conidia. — Scale bars: d–q= 10 µm.

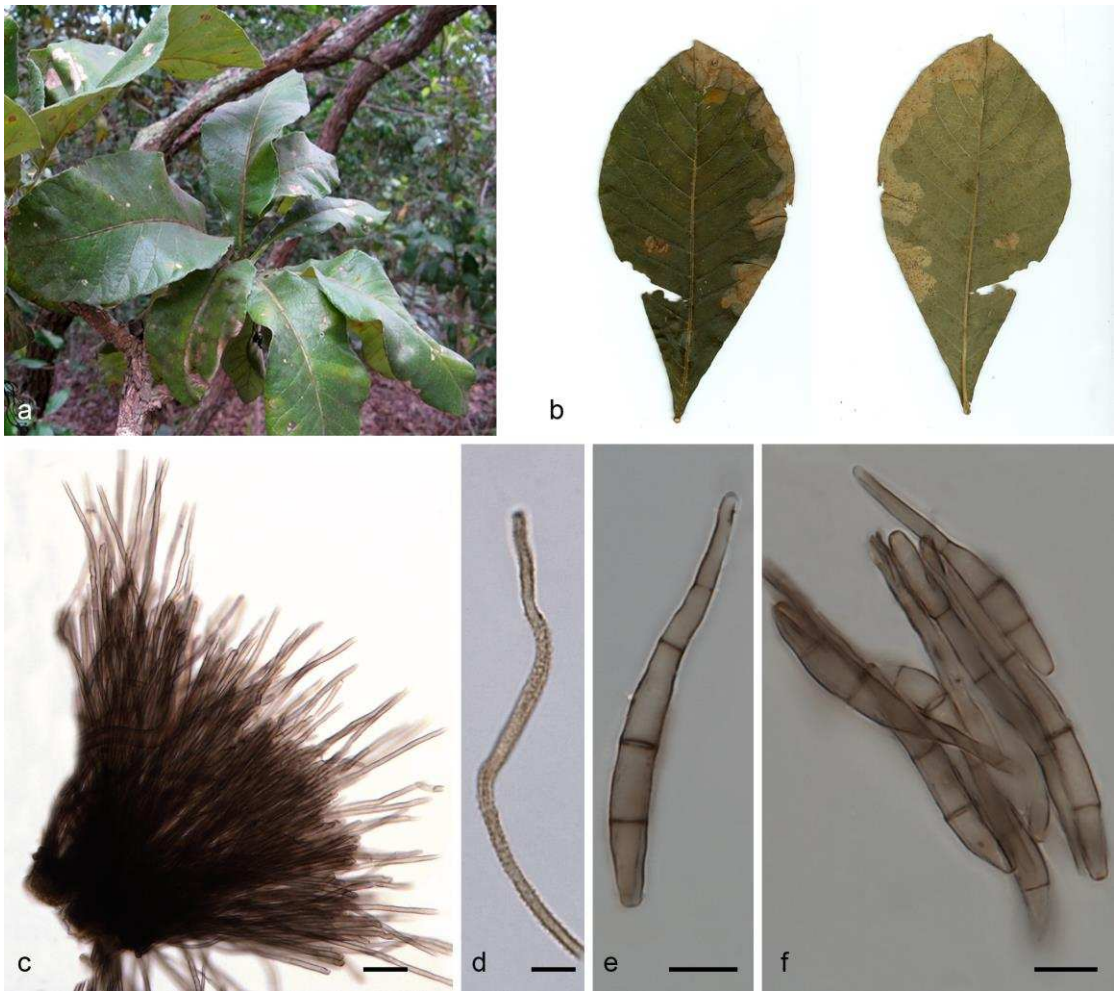

Fig. 9 Zasmidium aspidospermae (VIC 42727) on Aspidosperma tomentosum a. foliage bearing leaf spots in the field; b. leaf spots on upper and lower leaf surface; c. conidiophores in dense fascicles; d. verruculose external mycelium; e–f. conidia. — Scale bars: c–e= 10 µm.

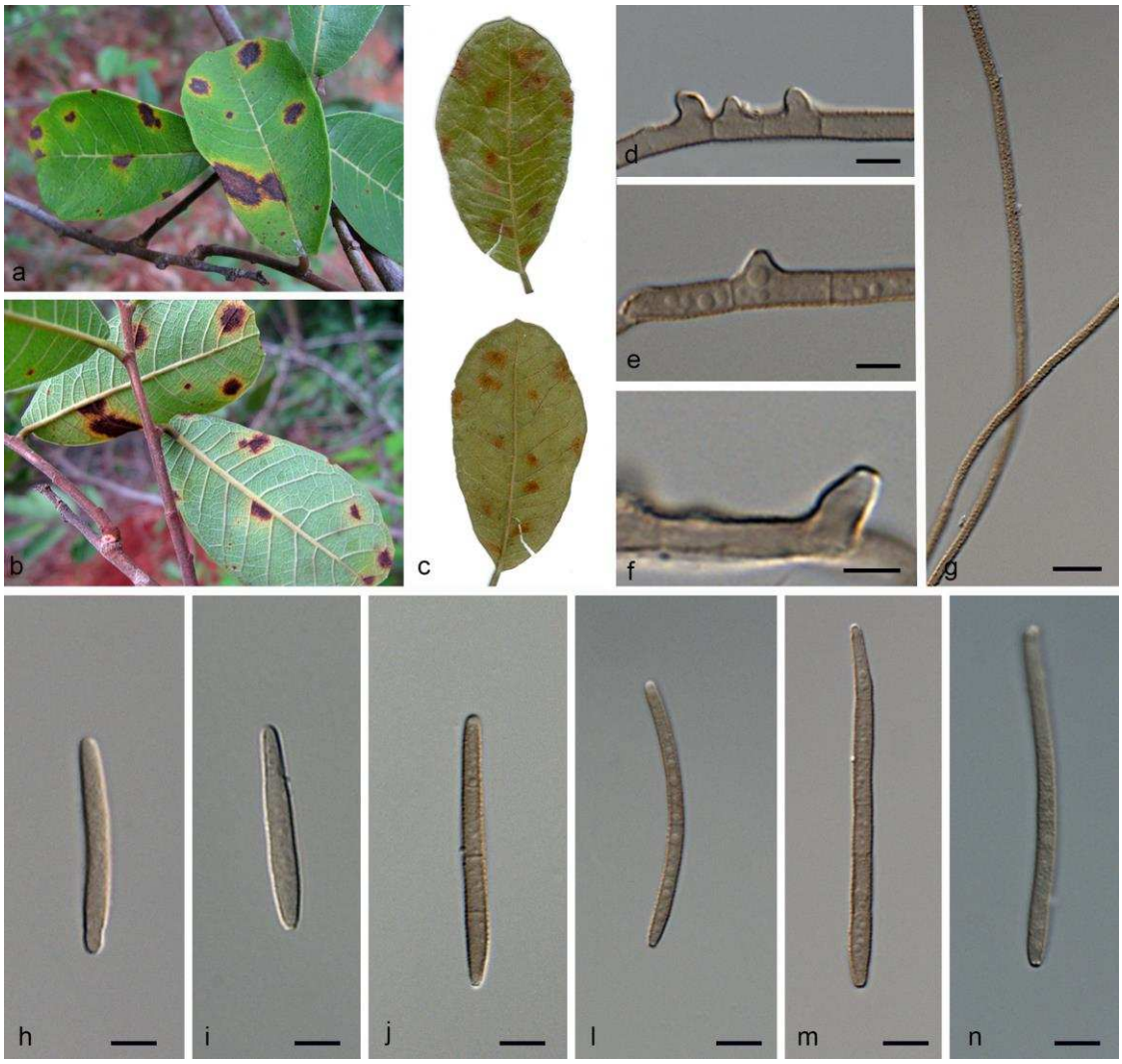

Fig. 10 Zasmidium brosimii (VIC 42724) on Brosimum gaudichaudii. a–b. foliage bearing leaf spots in the field; c. leaf spots on upper and lower leaf surface; d–f. conidiophores; g. verruculose external mycelium; h–n. verruculose conidia. — Scale bars: d–n= 10 µm.

Fig. 11 Zasmidium peixotoana (VIC 42760) on Peixotoa sp. a. Foliage leaf in the field; b. adaxial and abaxial view of colonized leaf; c–d. branched conidiophores arising from external hyphae; e. verruculose external mycelium; f–h. verruculose conidia. — Scale bars: c–h = 10 µm.

Fig. 12 Zasmidium roupalina (VIC 42734) on Roupala montana. a. leaf bearing spots in the field; b. leaf spots on upper and lower leaf surface; c–d. solitary conidiophores; e. verruculose external mycelium; f–h. verruculose conidia. — Scale bars: c–h = 10 µm.

Capítulo 3

Artigo — Camptomeris leucaenae belongs to Mycospharellaceae and is related to Cymadothea trifolii

Camptomeris leucaenae belongs to Mycospharellaceae and is related to Cymadothea trifolii

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Abstract: Despite the taxonomy of the cercosporoid fungi having received great attention along the recent years and its reappraisal based on the application of molecular phylogeny having contributed to a significant refinement of the classification of this important group some genera included in this fungal complex have been left behind. This is the case of the genus Camptomeris. Here, a study of one of the most widespread species in this genus - Camptomeris leucaenae – a foliage pathogen of the central American tree legume leucena, was performed based on the combination of molecular and morphological data aimed at clarifying its taxonomy and phylogenetic affinities. The phylogenetic position of Camptomeris was investigated for the first time based in sequences of the large subunit ribosomal (LSU). The study confirmed that C. leucaenae belongs to Mycosphaerellaceae s. str. (Capnodiales, Dothideomycetes) and is closely related to Cymadothea trifolii a pathogen of another leguminous plant in Europe.

Keywords: Capnodiales, Leucaena leucocephala, phylogeny, taxonomy.

INTRODUCTION

Despite the taxonomy of the cercosporoid fungi having received great attention along the recent years and its reappraisal based on the application of molecular phylogeny having contributed to a significant refinement of the

classification of this important group some genera as included in this fungal complex have been left behind (Arzanlou et al 2007, Crous et al. 2013, Groenewald et al. 2013, Quaedvlieg et al. 2014, Bakhshi et al 2015).

The genus Camptomeris Sydow was described by Sydow in 1927 and the majority species belonging to this genus are confined to living leaves of woody Fabaceae. Hosts belong to the following genera: Leucaena Benth., Calliandra Benth., Acacia Mill. Desmanthus Willd., Pithecolobium Benth. and Albizzia Benth. (Sydow 1927, Bessey 1953, Ellis 1971). Camptomeris spp. are Cercospora-like fungi characterized by having subhyaline swollen cells (vesicle-like), emerging from an hypostroma and bearing cylindrical brown conidiophores organized in pulvinate sporodochia and producing oblong, light brown, verruculose conidia (Sydow 1927).

Although the existing knowledge of the morphological characteristics of Camptomeris spp. (Sydow 1927, Ellis, 1971) no molecular data is available for any Camptomeris spp. that might be used to better clarify its phylogenetic position within the Ascomycota. Camptomeris leucaenae (F. Stevens & Dalbey) Syd. – the etiologic agent of leaf spots on Leucaena leucocephala (=Leucaena glauca) was reported for the first time in Leucaena glauca by Stevens & Dalbey (1919) in Porto Rico. This fungus causes severe defoliation on its host, L. leucocephala and is well distributed in Brazil.

The aim of this study was to obtain preliminary indications of the phylogenetic position of the genus Camptomeris by molecular DNA sequences analyses taking as model C. leucaenae (F. Stevens & Dalbey) Syd. – the etiologic agent of leaf spots on L. leucocephala.

MATERIALS AND METHODS

Samples of L. leucocephala plants colonized by C. leucaenae were collected on the campus of the Universidade Federal de Viçosa, (state of Minas Gerais, Brazil) in March 2013, dried in a plant press and deposited in the herbarium of the Universidade Federal de Viçosa (VIC 39753).

DNA extraction and amplification

Several attempts to isolate this fungus in pure culture failed and conidia were not found to germinate on the following culture media: Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), Vegetable Broth Agar (VBA) (Pereira et al. 2003) and Melin Norkrans Modified (MNM) (Marx 1969). This reinforced the existing suspicion that this fungus is a biotrophic parasite. DNA was then directly extracted by removing fungal structures from carefully selected colonies (free from contaminants or mycoparasites) from the plant tissue with a fine glass needle and caring for excluding any plant material from the sample. Fungal material was placed in a sterile 1.5 mL microcentrifuge tube and ground into a fine powder using liquid nitrogen. Genomic DNA was extracted using the Wizard® Genomic DNA Purification kit (Promega) according to the manufacturer's instructions and the steps described by Pinho et al. (2012).

The nuclear gene regions targeted for PCR amplification was the partial large subunit ribosomal (LSU) and the Internal Trancribed Spacer (ITS). These regions were amplified and sequenced using the primer pair LR0R (Rehner & Samuels 1994) and LR5 (Vilgalys & Hester 1990) and using the primers ITS-5 and ITS-4 (White et al. 1990), respectively.

PCR conditions for each 25µL reaction were as follows: 4.0 µL of genomic DNA (25 ng/ µL), 12.5 µL of Dream Taq TM PCR Master Mix 2X (MBI Fermentas), 1 µL of each primer synthesized by Invitrogen, 1 µL of dimethyl sulfixide (DMSO, Sigma-Aldrich), 5 µL of 100x (10mg/ml) bovine serum albumin (BSA, Sigma-Aldrich) and nuclease-free water to complete the volume. The amplifications were carried out starting with a BIO RAD C1000 (Thermal Cycler) with initial denaturation at 95 ºC for 5 min, followed by 40 cycles of 94 ºC for 60s, annealing at 53 ºC for 45 s, extension at 72 ºC for 2 min and a final extension at72 ºC for 7 min. Amplified products were visualized on 1% agarose gel stained with GelRed^{TM} and viewed under UV light to check for product size and purity. PCR products were purified and sequenced by Macrogen Inc., South Corea (http://www.macrogen.com). PCR generated fragments were purified using Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, UK) according to the manufacturer's recommendations. The purified fragment was ligated into the attached to the plasmid vector pGEM T Easy Vector System I (Promega, USA) for 12 hours at 4°C and transferred to Escherichia coli DH5α

competent cells, through the transformation process by the use of heat shock (Sambrook & Russel2001). After cloning, the plasmidial DNA was extracted from the E. coli transformed cells using the Illustra Kit Plasmidprep Mini Spin Kit (GE Healthcare, UK), according to the manufacturer's recommendations. The cloned fragments were sent for sequencing at Macrogen Inc. (Korea).

The DNA sequences obtained from forward and reverse primers were used to obtain consensus sequences using DNA Dragon software (Hepperle 2011). The sequences obtained in this study were deposited in GenBank and compared against others LSU sequences in the NCBI nucleotide collection database using the Mega BLAST program to identify their closest species (Table 1). The closest sequences were then downloaded in FASTA format and aligned using the multiple sequence alignment program MUSCLE (Edgar 2004). Alignments were manually adjusted when necessary in MEGA v.5 software (Tamura et al. 2011).

LSU Bayesian inference analyses (BI) were performed. MrMODELTEST 2.3 (Posada and Buckley 2004) was used to select the model of nucleotide substitution for gene region and included in the BI analysis. Once the likelihood scores were calculated, the model was selected according to the Akaike Information Criterion (AIC) applying GTR+I+G. A phylogenenetic analysis of the alignment was performed on CIPRES webportal (Miller et al. 2010) using MrBayes v.3.1.2 (Huelsenbeck et al. 2002). Analyses of four Markov chain Monte Carlo (MCMC) were run from a random trees for 10.000.000 generations and sampled every 1000 generations, resulting in 10000 saved trees. The first 2500 trees, which represented the burn-in phase of the analyses, were discarded and posterior probabilities (Rannala and Yang 1996) determined from the remaining tress. Phylogenetic trees were visualized in FigTree (Rambaut 2009). New sequences generated in this study were deposited in NCBI’s GenBank nucleotide database (www.ncbi.nlm.nih.gov).

Morphological studies

Samples of infected leaves were examined under an Olympus SZ40 stereomicroscope. Hands free sections containing the fungal structures and fungal structures scraped with a scalpel from the plant surfaces were mounted in lactophenol. Observations were carried out using an Olympus BX 51 light microscope fitted with a drawing tube and an Olympus E330 camera.

RESULTS

Amplification of the partial LSU was selected for the molecular phylogenetic study of Camptomeris leucaenae. The manually adjusted alignment included 60 taxa including the outgroup sequence (Asteroma alneum CBS 109840; GenBank Acession: EU167609). Of the 522 characters used in the aligment, 188 were parsimony informative, 227 were variable and 292 were conserved. The result of the tree from the Bayesian analysis showed that C. leucaenae grouped in a clade together with Cymadothea trifolii (Pers.) F.A. Wolf with a strong posterior probability value (1,00) (Fig. 1). Cymadothea trifolii is an obligate biotrophic ascomycete described on species of Trifolium sp. (Fabaceae) from Europe, which has an anamorph, Polythrincium trifolii (Simon et al. 2009). Such results clearly demonstrate that C. leucaenae belongs to Mycosphaerellaceae s. str. (Capnodiales, Dothideomycetes).

Unfortunately, all attempts to sequence the ITS region failed. An attempt was then made of cloning after sequencing the ITS region for this species. Although the ITS region was sequenced, it was not used in the phylogenetic analysis due to the lack of available ITS sequences for C. trifolii. Attempts should be made of cloning the ITS sequence of C. trifolii with the same methodology successfully utilized here for C. leucaenae. Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the ITS sequence of C. leucaenae are Passalora bougainvilleae [GenBank KF 539412; Identities 347/377 (92%)].

Camptomeris leucaenae (F. Stevens & Dalbey) Syd., Annales Mycologici 28: 222 (1930) (Fig. 2-3)

Synonym. Exosporium leucaenae F. Stevens & Dalbey, Mycologia 11: 5 (1919)

Lesions on living leaves amphigenous, starting as chlorosis that later develop into necrosis in the oldest parts of leaves, irregular, brown. Internal mycelium indistinct. External mycelium absent. Conidiophores grouped in sporodochia 87–176 µm diam., cylindrical, straight to curved, 35–52 × 7.5–10 µm, unbranched, brown, smooth, mostly restricted to the conidiogenous cells, on subhyaline swollen cells (vesicle-like), 31–45 × 5.0–12 µm. Conidiogenous cells

terminal, holoblastic, integrated, dark brown. Conidiogenous loci terminal, conspicuous, 1–3.0 µm diam, thickened and darkened. Conidia solitary, obclavate with rounded ends, straight to slightly curved, 40–59 × 9–10 µm, 2–3 septate, hilum thickened and darkened, pale brown, guttulate, verruculose.

Specimens examined: On living leaves of Leucaena leucocephala. BRAZIL: Minas Gerais: Viçosa, Campus UFV, 14 Jul 2012, M. Silva (VIC 33975).

DISCUSSION

Although sequence data for the majority of the representative genera in the Mycosphaerellaceae are currently available (Crous et al. 2000, 2007, 2009a, 2009b, 2013; Simon et al. 2009; Goodwin et al. 2001, Taylor et al. 2003; Groenewald 2013), some genera were "left behind". Camptomeris is a case in point. Until this work, no molecular data was available for any species in the genus that might indicate its true phylogenetic position. Our analyses based upon partial LSU sequence data show that C. leucaenae belongs to Mycosphaerellaceae (Capnodiales, Dothideomycetes). According to the phylogenetic analyses, the closest relative to C. leucaenae is Cymathodea trifolii, a sooty/black blotch leaf fungus reported on Trifolium spp. Camptomeris leucaenae and C. trifolii are ecologically, morphologically and phylogenetically similar. Both are pathogens on members of the Fabaceae and are biotrophic.The asexual state of C. trifolii, Polythrincium trifolii, is a Passalora-like genus (Crous and Braun 2003), characterized by pigmented conidiophores borne on one foot-cell, with conspicuous scars, producing pigmented 2-celled conidia (although occasionally 1 or 3-celled conidia are also produced) (Wolf 1935). Camptomeris species differ from Polytrichium by having pulvinate sporodochia formed over vesicle-like cells but such morphological difference may not be of major phylogenetic significance and inadequate for separation at the generic level. Phylogenetically, C. leucaenae formed a well-supported sub-clade (PP = 1.0) close to C. trifolii with 98% of nucleotide homology for LSU. It is possible that these two taxa will be recognized as belonging to the same genus. Nevertheless, we prefer to await until additional

sequences are obtained for other members of Camptomeris and other sequences of C. trifolii become available for a synonymization to be proposed. The first sequences obtained for an obligate biotrophic member of Mycosphaerellaceae was from C. trifolii (Simon et al. 2009). To our knowledge, this is the second report of sequence data for an obligate biotrophic member of Mycosphaerellaceae and these sequences can be further utilized on future works on the phylogeny of Passalora-like Mycosphaerellaceae.

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TABLES AND FIGURES

Table 1 - Genbank acession numbers of the partial 28S rDNA sequence from strains used in the phylogenetic analysis, unless otherwise indicated.

Spicies	**Isolate**	**Genbank acession numbers**
Ascochyta fabae	CBS 114.36	EU167566
Ascochyta pisi var. pisi	CBS 108.26	EU167557
Ascochyta viciapannonicae	CBS 254.92	EU167559
Ascochyta viciaevillosae	CBS 255.92	EU167560
Asteroma alneum	CBS 109840	EU167609
Bagnisiella examinans	CBS 551.66	EU167562
Camptomeris leucaenae	VIC 39753	
Cercospora beticola	CBS 116456	AY840527
Cladosporium sp. 1	CBS 280.49	EU167574
Cladosporium sp. 2	CBS 282.49	EU167586
Cladosporium sp. 3	CBS 266.53	EU167592
Cymadothea trifolii	CBS H-20110	EU167610
Cymadothea trifolii	CBS H-20110	EU167611
Davidiella macrospora	CBS 138.40	EU167591
Davidiella tassiana	CBS 723.79	EU167558
Didymella bryoniae	CBS 233.52	EU167573
Didymella exitialis	CBS 446.82	EU167564
Didymella phacae	CBS 184.55	EU167570
Didymella rabiei	CBS 237.37	EU167600
Dothidea berberidis	CBS 186.58	EU167601
Dothidea muelleri	CBS 191.58	EU167593
Guignardia vaccinii	CBS 114751	EU167584
Kabatiella caulivora	CBS 242.64	EU167576
Kabatiella microsticta	CBS 342.66	EU167608
Mycosphaerella aleuritidis	CBS 282.62	EU167594
Mycosphaerella arbuticola	CBS 355.86	EU167571
Mycosphaerella berberidis	CBS 324.52	EU167603
Mycosphaerella brassicicola	CBS 174.88	EU167607
Mycosphaerella coacervata	CBS 113391	EU167596
Mycosphaerella crystallina	CBS 681.95	EU167579
Mycosphaerella flageoletiana	CBS 114302	EU167597
Mycosphaerella fragariae	CBS 719.84	EU167605
Mycosphaerella gregaria	CBS 110501	EU167580
Mycosphaerella handelii	CBS 113302	EU167581
Mycosphaerella harthensis	CBS 325.52	EU167602
Mycosphaerella laricina	CBS 326.52	EU167595
Mycosphaerella linorum	CBS 261.39	EU167590
Mycosphaerella microsora	CBS 100352	EU167599
Mycosphaerella milleri	CBS 541.63	EU167577

Mycosphaerella punctata	CBS 113315	EU167582
Mycosphaerella populicola	CBS 100042	EU167578
Mycosphaerella pseudoellipsoidea	CBS 114709	EU167585
Mycosphaerella punctiformis	CBS 113265	EU167569
Mycosphaerella pyri	CBS 100.86	EU167606
Mycosphaerella grossulariae	CBS 235.37	EU167588
Mycosphaerella rosigena	CBS 330.51	EU167587
Mycosphaerella rubi	CBS 238.37	EU167589
Mycosphaerella stromatosa	CBS 101953	EU167598
Phaeosphaeria rousseliana	CBS 580.86	EU167604
Phoma exigua var. exigua	CBS 118.94	EU167567
Phoma medicaginis var. medicaginis	CBS 533.66	EU167575
Phoma pinodella	CBS 110.32	EU167565
Phoma sojicola	CBS 567.97	EU167568
Pleiochaeta ghindensis	CBS 552.92	EU167561
Pleiochaeta setosa	CBS 496.63	EU167563
Pseudocercospora vitis	CPC 11595	DQ073923
Ramichloridium cerophilum	CBS 103.59	EU041798
Schizothyrium pomi	CBS 486.50	EF134948
Schizothyrium pomi	CBS 406.61	EF134949
Teratosphaeria fibrillosa	CPC 1876	EU019282
Teratosphaeria microspora	CBS 101951	EU167572
Teratosphaeria molleriana	CBS 118359	EU167583

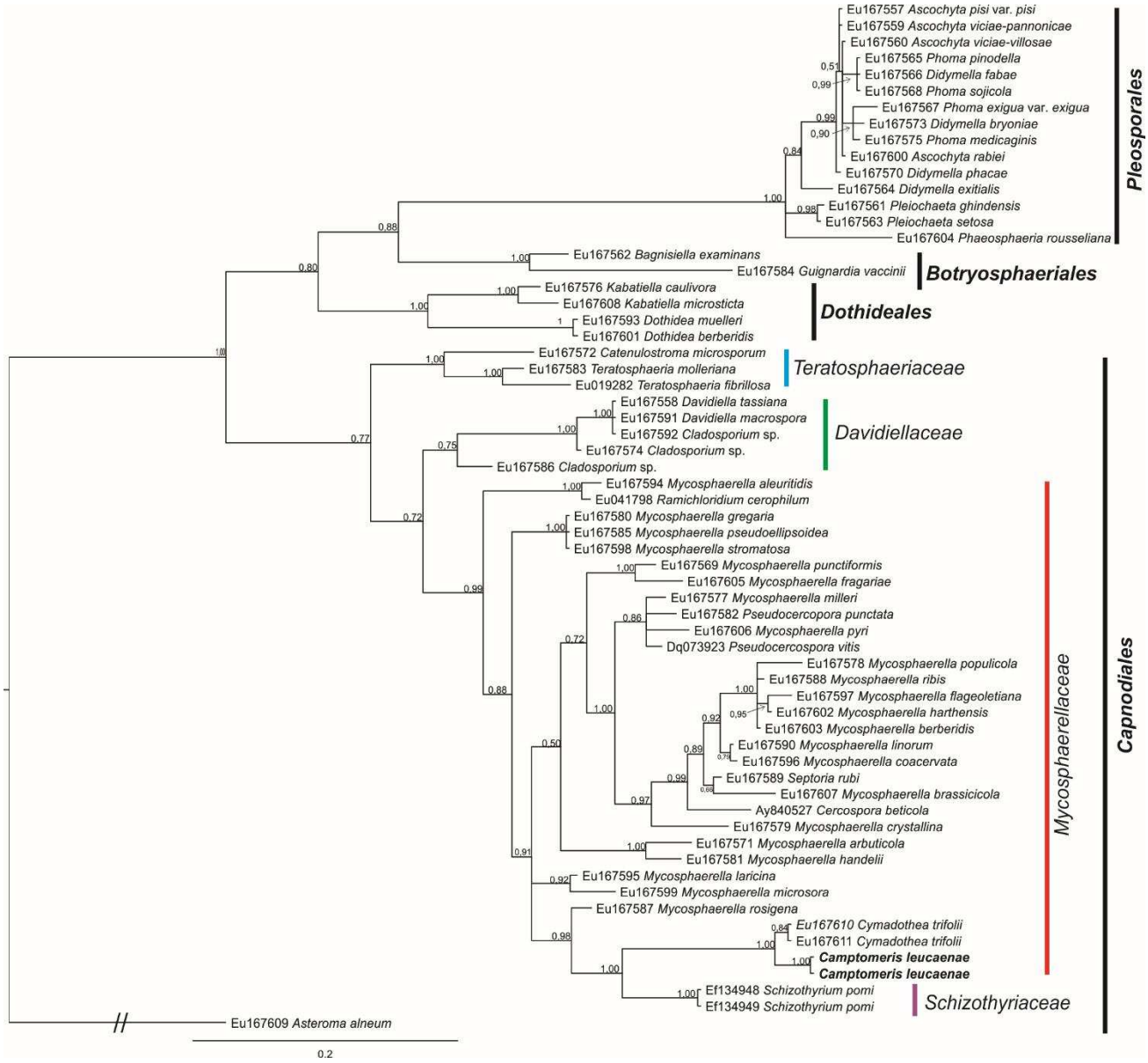

Fig. 1 Determination of the phylogenetic placement of Camptomeris leucaenae with regards to related fungi derived from Bayesian analysis of the partial nuclear large subunit 28 S rRNA gene sequences. Bayesian posterior probabilities are indicated at the nodes. The tree was rooted with Asteroma alneum.

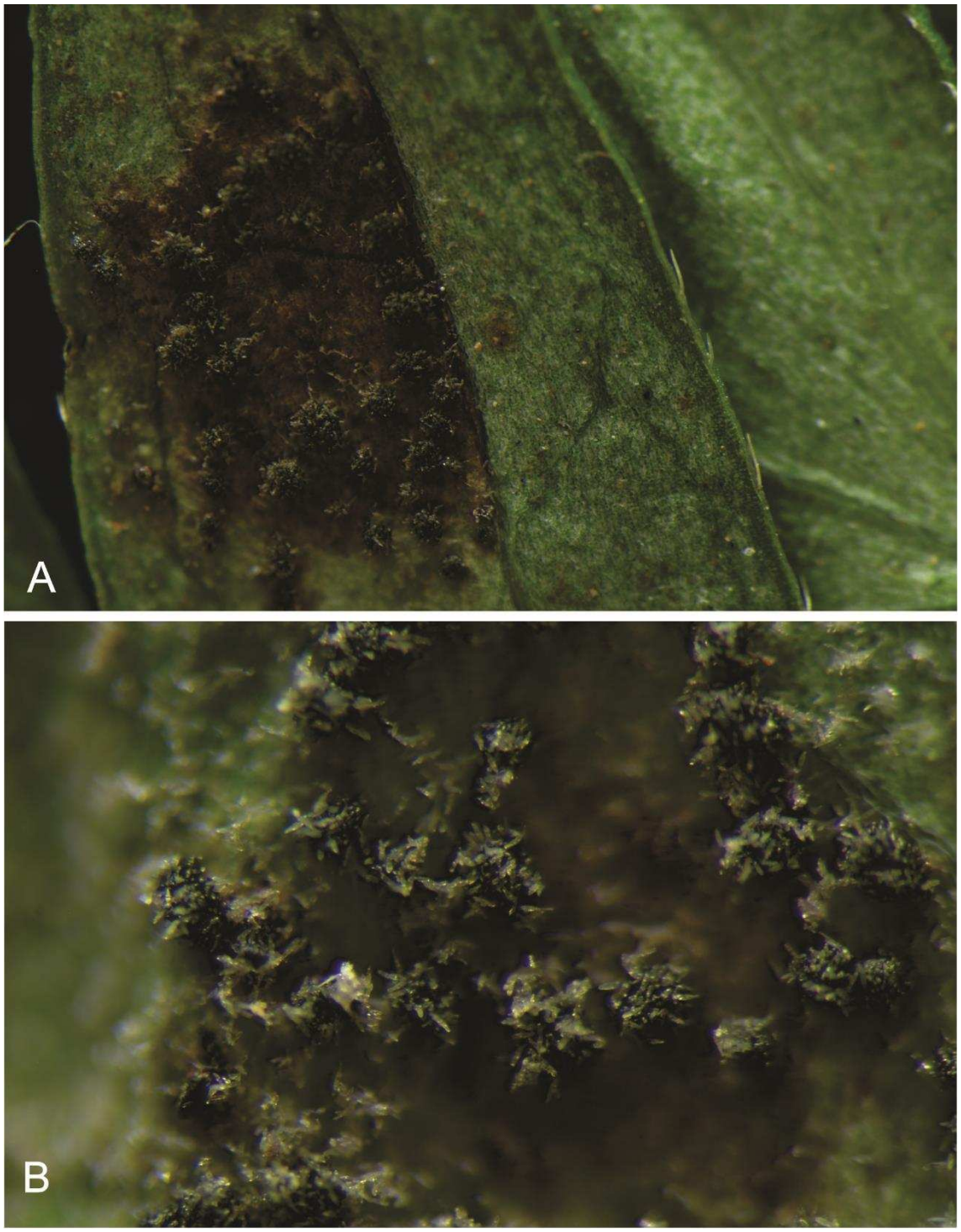

Fig. 2 Colony of Camptomeris leucaenae on a leaf of Leucaena leucocephala. (A) Conidiophores. (B) Close-up of conidia on tufts of conidiophores.

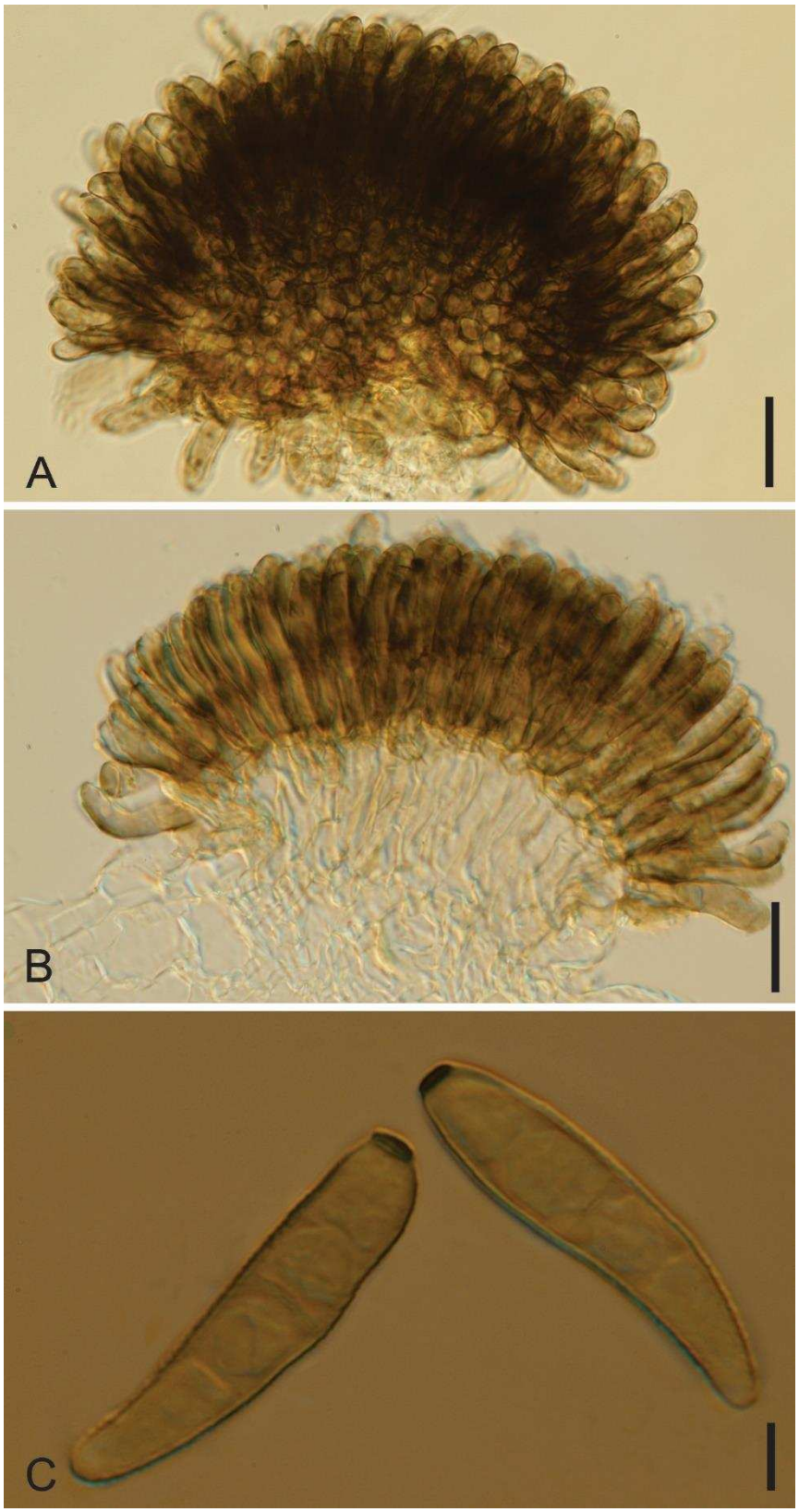

Fig. 3 Camptomeris leucaenae on Leucaena leucocephala. (A) Sporodochium. (B) Cross section of sporodochium. (C) Close-up of conidia. Bars: 20 µm.

CONCLUSÕES GERAIS

O presente estudo representou uma contribuição para a sistemática polifásica de cercosporóides do Brasil e teve como base a combinação de dados moleculares, morfológicos e culturais. Quarenta taxa foram coletados ou recoletados e investigados.

Diversas novidades taxonômicas foram obtidas. Dezoito novas espécies foram reconhecidas, a saber: Pseudocercospora aeschynomenicola, Ps. diplusodonii, Ps. emmotunicola, Ps. manihotii, Ps. perae, Ps. planaltinensis, Ps. pothomorphes, Ps. sennae-multijugae, Ps. solani-pseudocapsicicola, Ps. vassobiae, Ps. wulffiae, Ps. xylopiae, Passalora dasyphyllii, Sirosporium tocoyenae, Zasmidium aspidospermae, Z. brosimii, Z. peixotoana e Z. roupalina. Onze epitipos serão designados: Pseudocercospora bixae, P. chamaecristae, P.exilis, P. luzardii, P. plumeriifolii, P. richardsoniicola, P. rigidae, P. struthanthi, Passalora schefflerae, Pa. rubida e Pa. vicosae. Três dentre os taxa encontrados representam novos relatos para o Brasil: Pseudocercospora euphorbiacearum, P. tecomicola, P. trinidadensis e novos relatos de associação de Pseudocercospora spp. de ocorrência já conhecida anteriormente no Brasil em novos hospedeiros. O posicionamento filogenético, ainda incerto, de Camptomeris leucaenae foi investigado pela primeira vez baseado em sequências da região LSU e finalmente esclarecido, confirmando-se que C. leucaenae pertence à família Mycospaerellaceae (Capnodiales, Dothideomycetes), situando-se próximo de Cymadothea trifolii.

A grande diversidade micológica em ambientes tropicais é desafiadora para os micologistas devido ao enorme volume de novidades taxonômicas gerado nos trabalhos de campo. O presente trabalho, que pretendia, originalmente contemplar principalmente a recoleta, reavaliação e epitificação de cercosporóides descritos por micologistas que trataram desse grupo no Brasil, em bases mais modernas, produziu um resultado diferente do originalmente pretendido e expandiu ainda mais o número de espécies de cercoporóides brasilerias reconhecidas. Permanece como desafio “em aberto” para a comunidade micológica revisitar os taxa descritos por A. P. Viégas, A. S. Muller, F.C.O. Freire e outros para melhor consolidar o conhecimento sobre esse megadiverso e importante grupo de fungos fitopatogênicos.