



# Exploring fungal mega-diversity: *Pseudocercospora* from Brazil

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## Key words

biodiversity  
*Capnodiales*  
cercosporoid  
*Dothideomycetes*  
multigene phylogeny  
*Mycosphaerellaceae*  
plant pathogen  
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**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-pseudocapsicola*, *P. vassobiae*, *P. wulffiae* and *P. xylopieae*. 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* ( $\equiv$  *Microcyclus ulei*, Hora Júnior et al. 2014) and leaf and fruit spot of pistachio, *P. pistacina* ( $\equiv$  *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. borrieriae* 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, Nganhom 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 NCBI 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](http://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  $\mu$ L reaction: 10–20 ng of template DNA, 1× PCR buffer, 0.63  $\mu$ L DMSO (99.9 %), 1.5 mM MgCl<sub>2</sub>, 0.5  $\mu$ M of each primer, 0.25 mM of each dNTP, 1.0 U BioTaq® DNA polymerase (Bio-line GmbH Luckenwalde, Germany). The PCRs were carried out with a MyCycler™ Thermal Cycler (Bio-Rad Laboratories B.V., Veenendaal, 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 Multi-Screen 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 <sup>1</sup>	Collector	Host	Family	Country	GenBank accession numbers <sup>2</sup>			
						LSU	ITS	tef1	actA
<i>Passalora eucalypti</i>	CBS 111318; CPC 1457 (ex-type)	P.W. Crous	<i>Eucalyptus saligna</i>	Myrtaceae	Brazil	GU253860	GU268845	GU384558	GU320548
<i>Pseudocercospora acericola</i>	CBS 122279	R. Kirschner	<i>Acer albobupurascens</i>	Acetaceae	Taiwan	GU253699	GU269650	GU384368	GU320358
<i>P. aeshynomenicola</i>	CPC 25227; COAD 1972 (ex-type)	M. Silva	<i>Aeshynomene falcata</i>	Fabaceae	Brazil	<b>KT290173</b>	<b>KT290146</b>	<b>KT290200</b>	<b>KT313501</b>
<i>P. angolensis</i>	CBS 112933; CPC 4118	M.C. Pretorius	<i>Citrus</i> sp.	Rutaceae	Zimbabwe	GU214470	AY260063/ GU269836	GU384548	JQ325010
<i>P. assamensis</i>	CBS 149.53 (ex-type)	T. de Carvalho & O. Mendes	<i>Citrus sinensis</i>	Rutaceae	Angola	JQ324941	JQ324988	JQ325011	JQ325011
	CBS 122467 (ex-type)	I. Buddenhagen	<i>Musa cultivar</i>	Musaceae	India	GU253705	GU269656	GU384374	GU320364
	CBS 114640	C.F. Hill	<i>Solanum</i> sp.	Solanaceae	New Zealand	GU253706	GU269658	GU384376	GU320365
	CBS 132010; CPC 11372	H.D. Shin	<i>Solanum nigrum</i>	Solanaceae	South Korea	GU214671	GU269657	GU384375	–
<i>P. atomarginalis</i>	CPC 25230; COAD 1975	M. Silva	<i>Solanum americanum</i>	Solanaceae	Brazil	<b>KT290176</b>	<b>KT290149</b>	<b>KT290203</b>	<b>KT313504</b>
	CBS 114664; CPC 1202 (ex-type)	M.J. Wingfield	<i>Eucalyptus grandis</i>	Myrtaceae	Colombia	GU253710/ DQ204759	DQ267600/ GU269662	DQ211675	DQ147622
	CPC 25244; COAD 1563 (ex-epitype)	R.W. Barreto	<i>Bixa orellana</i>	Bixaceae	Brazil	<b>KT290180</b>	<b>KT290153</b>	<b>KT290207</b>	<b>KT313508</b>
<i>P. bixae</i>	CPC 25243; COAD 1562	R.W. Barreto	<i>Bohemia nivea</i>	Urticaceae	Brazil	<b>KT290179</b>	<b>KT290152</b>	<b>KT290206</b>	<b>KT313507</b>
<i>P. boehmeriigena</i>	MUCC 743	C. Nakashima & I. Araki	<i>Catalpa ovata</i>	Bigoniaceae	Japan	GU253731	GU269690	GU384406	GU320395
<i>P. catalpigena</i>	CBS 132109; CPC 14481 (ex-epitype)	H.D. Shin	<i>Cercis chinensis</i>	Fabaceae	South Korea	GU253718	GU269670	GU384387	GU320376
<i>P. cercidis-chinensis</i>	CPC 25228; COAD 1973 (ex-epitype)	M. Silva	<i>Chamaecrista</i> sp.	Fabaceae	Brazil	<b>KT290174</b>	<b>KT290147</b>	<b>KT290201</b>	<b>KT313502</b>
<i>P. chamaecristae</i>	CBS 131924; CPC 10696	H.D. Shin	<i>Lycium chinense</i>	Solanaceae	South Korea	JQ324942	GU269673	GU384390	GU320379
<i>P. chengtuenis</i>	CBS 132108; CPC 14714	H.D. Shin	<i>Dioscorea quinqueloba</i>	Dioscoreaceae	South Korea	JQ324945	GU269677	GU384394	GU320385
<i>P. contraria</i>	CBS 114685; CPC 2552 (ex-type)	P.W. Crous & R.L. Benchimol	<i>Cordia goeldiana</i>	Boraginaceae	Brazil	GU214472	AF362054/ GU269681	GU384398	GU320387
<i>P. cordiana</i>									
<i>P. corylopsidis</i>	MUCC 874	T. Kobayashi & C. Nakashima	<i>Hamamelis japonica</i>	Hamamelidaceae	Japan	GU253757	GU269721	GU384437	GU320425
<i>P. cotoneastri</i>	MUCC 908 (ex-epitype)	C. Nakashima & E. Imaizumi	<i>Corylopsis spicata</i>	Hamamelidaceae	Japan	GU253727	GU269684	GU384401	GU320390
<i>P. crousii</i>	MUCC 876	T. Kobayashi & C. Nakashima	<i>Cotoneaster salicifolius</i>	Rosaceae	Japan	GU253728	GU269685	GU384402	GU320391
<i>P. cruenta</i>	CBS 119487	C.F. Hill	<i>Eucalyptus</i> sp.	Myrtaceae	New Zealand	GU253729	GU269686	GU384403	GU320392
<i>P. diplusodonii</i>	CBS 132021; CPC 10846	H. Booker	<i>Vigna</i> sp.	Fabaceae	Trinidad	GU214673	GU269688	GU384404	JQ325012
<i>P. eleocarpi</i>	MUCC 925	M. Silva	<i>Diplusodon</i> sp.	Lythraceae	Brazil	<b>KT290162</b>	<b>KT290135</b>	<b>KT290189</b>	<b>KT313490</b>
<i>P. emmotunicola</i>	CPC 25187; COAD 1491 (ex-type)	C. Nakashima	<i>Elaeocarpus</i> sp.	Elaeocarpaceae	Japan	GU253740	GU269701	GU384417	GU320405
<i>P. euphorbiacearum</i>	CPC 25179; COAD 1476 (ex-type)	M. Silva	<i>Emmotum nitens</i>	Icacinaceae	Brazil	<b>KT290163</b>	<b>KT290136</b>	<b>KT290190</b>	<b>KT313491</b>
<i>P. eustomatis</i>	CPC 25222; COAD 1537	M. Silva	<i>Dalechampia</i> sp.	Euphorbiaceae	Argentina	<b>KT290172</b>	<b>KT290145</b>	<b>KT290199</b>	<b>KT313503</b>
<i>P. exilis</i>	CBS 110822	G. Dal Bello	<i>Eustroma grandiflorum</i>	Gentianaceae	Brazil	GU253744	GU269705	GU384421	GU320409
<i>P. fijiensis</i>	CPC 25193; COAD 1501 (ex-epitype)	M. Silva	<i>Chamaecrista orbiculata</i>	Fabaceae	Brazil	<b>KT290166</b>	<b>KT290139</b>	<b>KT290193</b>	<b>KT313494</b>
<i>P. fukuokaensis</i>	CBS 120258; CIRAD 86 (ex-epitype)	J. Carlier	<i>Musa</i> sp.	Musaceae	Cameroon	JQ324952	EU514248	Genome <sup>3</sup>	Genome <sup>3</sup>
<i>P. fuligena</i>	MUCC 792	T. Kobayashi & C. Nakashima	<i>Musa cultivar</i>	Musaceae	Japan	GU253776	GU269748	JQ324994	GU320450
<i>P. glauca</i>	CBS 132111; CPC 14689	H.D. Shin	<i>Styrax japonicus</i>	Styracaceae	South Korea	GU269713	GU269713	GU384429	GU320417
<i>P. guianensis</i>	MUCC 887 (ex-epitype)	T. Kobayashi	<i>Styrax japonicus</i>	Styracaceae	Japan	GU253751	GU269714	GU384430	GU320418
<i>P. fuligena</i>	CBS 132017; CPC 12296	Z. Mersha	<i>Lycopersicon</i> sp.	Solanaceae	Thailand	JQ324953	GU269711	GU384427	GU320415
<i>P. glauca</i>	MUCC 533	C. Nakashima	<i>Lycopersicon esculentum</i>	Solanaceae	Japan	GU253749	GU269712	GU384428	GU320416
<i>P. glauca</i>	CBS 131884; CPC 10062	H.D. Shin	<i>Albizia julibrissin</i>	Fabaceae	South Korea	GU253752	GU269715	GU384431	GU320419
<i>P. guianensis</i>	MUCC 885	C. Nakashima & T. Akashi	<i>Lantana camara</i>	Verbenaceae	Japan	GU253755	GU269719	GU384435	GU320423
<i>P. latens</i>	MUCC 879	C. Nakashima	<i>Lantana camara</i>	Verbenaceae	Japan	GU253756	GU269720	GU384436	GU320424
<i>P. latens</i>	MUCC 763	C. Nakashima & T. Akashi	<i>Lespedeza wilfordii</i>	Fabaceae	Japan	GU253763	GU269732	GU384445	GU320434
<i>P. lonicericola</i>	MUCC 889 (ex-neotype)	T. Kobayashi	<i>Lonocera gracilipes</i> var. <i>glabra</i>	Caprifoliaceae	Japan	GU253766	GU269736	JQ324999	GU320438
<i>P. luzardii</i>	CPC 2556	A.C. Alfenas	<i>Hancornia speciosa</i>	Apocynaceae	Brazil	GU214477	AF362057/ GU269738	GU384450	GU320440
<i>P. lythri</i>	CPC 25196; COAD 1505 (ex-epitype)	M. Silva	<i>Hancornia speciosa</i>	Apocynaceae	Brazil	<b>KT290167</b>	<b>KT290140</b>	<b>KT290194</b>	<b>KT313495</b>
<i>P. lythri</i>	CBS 132115; CPC 14588 (ex-epitype)	H.D. Shin	<i>Lythrum salicaria</i>	Lythraceae	South Korea	GU253771	GU269742	GU384454	GU320444
<i>P. macrospora</i>	MUCC 865	I. Araki & M. Harada	<i>Lythrum salicaria</i>	Lythraceae	Japan	GU253772	GU269742	GU384455	GU320445
<i>P. macrospora</i>	CBS 114696; CPC 2553	P.W. Crous & R.L. Benchimol	<i>Bertholletia excelsa</i>	Legythidaceae	Brazil	GU214478	AF362055/ GU269745	GU384457	GU320447
<i>P. mali</i>	MUCC 886	T. Kobayashi	<i>Malus sieboldii</i>	Rosaceae	Japan	GU253773	GU269744	GU384456	GU320446



<i>P. manihotii</i>	CPC 25219; COAD 1534 (ex-type)	M. Silva	<i>Manihot</i> sp.	<i>Euphorbiaceae</i>	Brazil	<b>KT290171</b> GU253779	<b>KT290144</b> GU269751	<b>KT290198</b> GU384462	<b>KT313499</b> GU320453
<i>P. nephrolepidis</i>	CBS 119121	R. Kirschner	<i>Nephrolepis auriculata</i>	<i>Oleandraceae</i>	Taiwan	JQ324960	GU269752	GU384463	GU320454
<i>P. nogalesii</i>	CBS 115022	C.F. Hill	<i>Chamaecyflisus proliferus</i>	<i>Fabaceae</i>	New Zealand	GU253794	GU269772	GU384484	GU320475
<i>P. norchiensis</i>	CBS 114641	C.F. Hill	<i>Rubus</i> sp.	<i>Rosaceae</i>	New Zealand	GU253780	EF394859/	GU384464	GU320455
	CBS 120738; CPC 13049 (ex-type)	W. Gams	<i>Eucalyptus</i> sp.	<i>Myrtaceae</i>	Italy		GU269753		
<i>P. oenotherae</i>	CBS 131885; CPC 10290	H.D. Shin	<i>Oenothera odorata</i>	<i>Onagraceae</i>	South Korea	JQ324961	GU269856	GU384567	GU320559
	CBS 131920; CPC 10630	H.D. Shin	<i>Oenothera odorata</i>	<i>Onagraceae</i>	South Korea	GU253781	GU269755	GU384466	GU320457
<i>P. pallida</i>	CBS 131889; CPC 10776	H.D. Shin	<i>Campsis grandiflora</i>	<i>Bignoniaceae</i>	South Korea	GU214680	GU269758	GU384469	GU320459
<i>P. paraguayensis</i>	CBS 111286; CPC 1459	P.W. Crous	<i>Eucalyptus nitens</i>	<i>Myrtaceae</i>	Brazil	GU214479/	DQ267602	DQ211680	DQ147606
						DQ204764			
<i>P. perae</i>	CBS 111317; CPC 1458	P.W. Crous	<i>Eucalyptus nitens</i>	<i>Myrtaceae</i>	Brazil	QK852634	JQ324978	GU384522	JQ325021
<i>P. pini-densiflorae</i>	CPC 25171; COAD 1465 (ex-type)	M. Silva	<i>Pera glabrata</i>	<i>Euphorbiaceae</i>	Brazil	<b>KT290159</b> GU253785	<b>KT290132</b> GU269760	<b>KT290186</b> GU384471	<b>KT313487</b> GU320461
<i>P. pipiens</i>	MUCC 534	Y. Tokushige	<i>Pinus thunbergii</i>	<i>Pinaceae</i>	Japan	JX875063	JX875062	JX896123	–
<i>P. planaltinensis</i>	CPC 25189; COAD 1495 (ex-type)	R.E. Hanada	<i>Piper aduncum</i>	<i>Piperaceae</i>	Brazil	<b>KT290164</b> GU290131	<b>KT290137</b> GU290138	<b>KT290191</b> GU290192	<b>KT313492</b> KT313493
<i>P. plumierifolii</i>	CPC 25191; COAD 1498 (ex-epitype)	M. Silva	<i>Chamaecrista</i> sp.	<i>Fabaceae</i>	Brazil	<b>KT290165</b> GU290178	<b>KT290151</b> GU290151	<b>KT290205</b> GU290205	<b>KT313506</b> KT313506
<i>P. plunkettii</i>	CPC 26081; COAD 1548	R.W. Barreto	<i>Himantanthus obovatus</i>	<i>Apocynaceae</i>	Brazil	<b>KT290166</b> GU290158	<b>KT290131</b> GU290131	<b>KT290185</b> GU290185	<b>KT313486</b> KT313486
<i>P. pothomorphes</i>	CPC 25166; COAD 1450 (ex-type)	O.L. Pereira	<i>Mikania hirsutissima</i>	<i>Asteraceae</i>	Brazil	GU253786	GU269761	GU384472	GU320462
<i>P. pouzolziae</i>	CBS 122280	R. Kirschner	<i>Pothomorphe umbellata</i>	<i>Piperaceae</i>	Brazil	GU253723	GU269676	GU384393	GU320382
<i>P. prunicola</i>	CBS 132107; CPC 14511	H.D. Shin	<i>Gonostegia hirta</i>	<i>Urticaceae</i>	Taiwan	GU253804	GU269783	GU384494	GU320486
<i>P. purpurea</i>	CBS 114163; CPC 1664	H.D. Shin	<i>Prunus x yedoensis</i>	<i>Rosaceae</i>	South Korea	GU253792	GU269767	GU384479	GU320470
<i>P. pyracanthae</i>	MUCC 892	P.W. Crous	<i>Persea americana</i>	<i>Lauraceae</i>	Mexico	–	GU269766	GU384478	GU320469
<i>P. pyracanthigena</i>	CBS 131589; CPC 10808 (ex-type)	T. Kobayashi & C. Nakashima	<i>Pyracantha angustifolia</i>	<i>Rosaceae</i>	South Korea	GU253813	GU269770	GU384482	GU320473
<i>P. rhannellae</i>	CBS 131590; CPC 12500 (ex-type)	H.D. Shin	<i>Pyracantha angustifolia</i>	<i>Rosaceae</i>	South Korea	GU253793	GU269770	GU384482	GU320473
<i>P. rhapsicola</i>	CBS 282.66	H.D. Shin	<i>Rhamnella franguloides</i>	<i>Rhamnaceae</i>	Japan	<b>KT290154</b> GU290134	<b>KT290154</b> GU290134	<b>KT290208</b> GU290188	<b>KT313509</b> KT313489
<i>P. richardsonicola</i>	CPC 25248; COAD 1588 (ex-epitype)	K. Tubaki	<i>Rhapis flabelliformis</i>	<i>Areaceae</i>	Japan	<b>KT290161</b> GU253795	<b>KT290134</b> GU269773	<b>KT290188</b> GU384485	<b>KT313489</b> GU320476
<i>P. rigidae</i>	CPC 25175; COAD 1472 (ex-epitype)	R.W. Barreto	<i>Richardia brasiliensis</i>	<i>Rubiaceae</i>	Brazil	GU253795	GU269773	GU384485	GU320476
<i>P. rubi</i>	MUCC 875	M. Silva	<i>Palicourea rigida</i>	<i>Rubiaceae</i>	Brazil	JQ324967	GU269775	–	GU320478
<i>P. sawadae</i>	CBS 115024	T. Kobayashi & C. Nakashima	<i>Psidium guajava</i>	<i>Myrtaceae</i>	Japan	<b>KT290169</b> GU290175	<b>KT290142</b> GU290142	<b>KT290196</b> GU290196	<b>KT313497</b> KT313503
<i>P. sennae-multijugae</i>	CPC 25206; COAD 1519 (ex-type)	C.F. Hill	<i>Senna multijuga</i>	<i>Fabaceae</i>	New Zealand	<b>KT290175</b> GU253798	<b>KT290148</b> GU269777	<b>KT290202</b> GU384488	<b>KT313503</b> GU320480
<i>P. solani-pseudocapsicola</i>	CPC 25229; COAD 1974 (ex-type)	M. Silva	<i>Solanum pseudocapsicum</i>	<i>Solanaceae</i>	Brazil	GU253798	GU269777	GU384488	GU320480
<i>P. sordida</i>	MUCC 913	C. Nakashima & E. Imaizumi	<i>Campsis radicans</i>	<i>Bignoniaceae</i>	Japan	GU253799	GU269778	GU384489	GU320481
<i>Pseudocercospora</i> sp.	CBS 110998; CPC 1054	M.J. Wingfield	<i>Eucalyptus grandis</i>	<i>Myrtaceae</i>	South Africa	GU253803	GU269782	GU384493	GU320485
	CBS 111373; CPC 1493	M.J. Wingfield	<i>Eucalyptus globulus</i>	<i>Myrtaceae</i>	Uruguay	GU253754	GU269718	GU384434	GU320422
	CBS 113387	A. den Breeyen	<i>Lantana camara</i>	<i>Verbenaceae</i>	Jamaica	GU253700	GU269651	GU384369	GU320359
<i>P. stephanandrae</i>	CBS 131922; CPC 10645	P.W. Crous	–	–	Brazil	GU253831	GU269814	GU384526	GU320516
<i>P. strizolobii</i>	MUCC 914 (ex-epitype)	C. Nakashima & E. Imaizumi	<i>Stephanandra incisa</i>	<i>Rosaceae</i>	Japan	<b>KT290170</b> GU290141	<b>KT290143</b> GU269815	<b>KT290197</b> GU269815	<b>KT313498</b> KT313496
<i>P. struthanthi</i>	CPC 25217; COAD 1532	M. Silva	<i>Mucuna aterrima</i>	<i>Fabaceae</i>	Brazil	<b>KT290168</b> GU253832	<b>KT290141</b> GU269815	<b>KT290195</b> GU384527	<b>KT313496</b> GU320517
<i>P. subseisilis</i>	CPC 25199; COAD 1512 (ex-epitype)	M. Silva	<i>Struthanthus flexicaulis</i>	<i>Loranthaceae</i>	Brazil	GU253833	GU269816	GU384528	GU320518
<i>P. subtorulosa</i>	CBS 136.94	R.F. Castaneda	–	–	Cuba	<b>KT290156</b> GU290157	<b>KT290156</b> GU290157	<b>KT290209</b> GU290210	<b>KT313511</b> –
<i>P. tecomicola</i>	CBS 117230	R. Kirschner	<i>Melicope</i> sp.	<i>Rutaceae</i>	Taiwan	<b>KT290183</b> GU253833	<b>KT290157</b> GU269816	<b>KT290209</b> GU290210	<b>KT313511</b> –
<i>P. tocomicola</i>	CPC 25260; COAD 1585	R.W. Barreto	<i>Tecoma stans</i>	<i>Bignoniaceae</i>	Brazil	<b>KT290184</b> GU253830	<b>KT290157</b> GU269816	<b>KT290210</b> GU269816	<b>KT313511</b> –
<i>P. trinidadiensis</i>	CPC 26082; COAD 1756	R.W. Barreto	<i>Croton urucurana</i>	<i>Euphorbiaceae</i>	Brazil	–	GU269824	GU384537	GU320527
<i>P. udagawana</i>	CBS 131931; CPC 10799	H.D. Shin	<i>Hovenia dulcis</i>	<i>Rhamnaceae</i>	South Korea	–	GU269824	GU384537	GU320527
<i>P. varilcolor</i>	MUCC 746	C. Nakashima & I. Araki	<i>Paeonia lactiflora</i> var. <i>trichocarpa</i>	<i>Paeoniaceae</i>	Japan	GU253843	GU269826	GU384538	GU320530
<i>P. vassobiae</i>	CPC 25251; COAD 1572 (ex-type)	R.W. Barreto	<i>Vassobia breviflora</i>	<i>Solanaceae</i>	Brazil	<b>KT290182</b> GU253827	<b>KT290155</b> GU269809	–	<b>KT313510</b> GU320512
<i>P. viburnigena</i>	CBS 125998; CPC 15249 (ex-epitype)	M.K. Crous	<i>Viburnum davidii</i>	<i>Caprifoliaceae</i>	Netherlands	GU253847	GU269831	GU384520	GU320512
<i>P. weigeliae</i>	MUCC 899	T. Kobayashi & Y. Kobayashi	<i>Weigelia coraeensis</i>	<i>Caprifoliaceae</i>	Japan	<b>KT290177</b> GU290160	<b>KT290153</b> GU269834	<b>KT290204</b> GU384546	<b>KT313505</b> KT313488
<i>P. wulffiae</i>	CPC 25232; COAD 1976 (ex-type)	M. Silva	<i>Wulffia stenoglossa</i>	<i>Asteraceae</i>	Brazil	<b>KT290160</b> GU253850	<b>KT290153</b> GU269834	<b>KT290187</b> GU384546	<b>KT313505</b> JQ325028
<i>P. xyloplae</i>	CPC 25173; COAD 1469 (ex-type)	M. Silva	<i>Xyloplia aromatica</i>	<i>Annonaceae</i>	Brazil	<b>KT290177</b> GU253850	<b>KT290153</b> GU269834	<b>KT290187</b> GU384546	<b>KT313488</b> JQ325028
<i>P. zelkoveae</i>	CBS 132118; CPC 14717	H.D. Shin	<i>Zelkova serrata</i>	<i>Ulmaceae</i>	South Korea	–	GU269824	GU384537	–
	MUCC 872	T. Kobayashi & C. Nakashima	<i>Zelkova serrata</i>	<i>Ulmaceae</i>	Japan	GU253851	GU269835	GU384547	GU320537

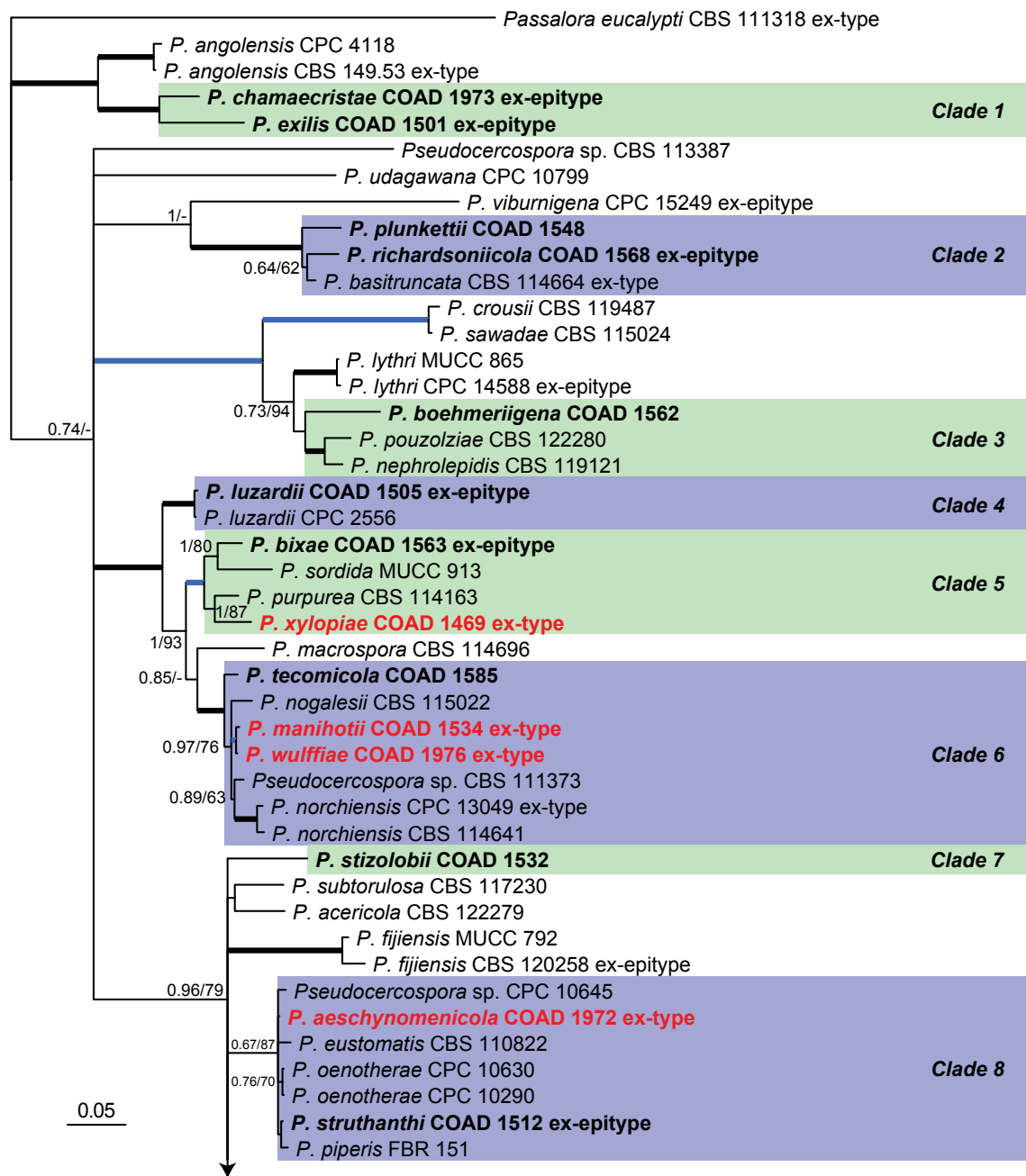
<sup>1</sup> CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; COAD: Coleção de Cultura Octávio Almeida Drummond, Universidade Federal de Viçosa, Viçosa, Brazil; CPC: Culture collection of Pedro Crous, housed at CBS; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan.  
<sup>2</sup> LSU: partial 28S mRNA gene; ITS: internal transcribed spacer regions 1 & 2 including 5.8S mRNA gene; *tefi*: partial translation elongation factor 1- $\alpha$  gene; *actA*: partial actin gene.  
<sup>3</sup> Sequence for this locus obtained from: <http://genome.jgi-psf.org/Mycfri1/Mycfri1.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 NCBI's 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 check-

ed 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

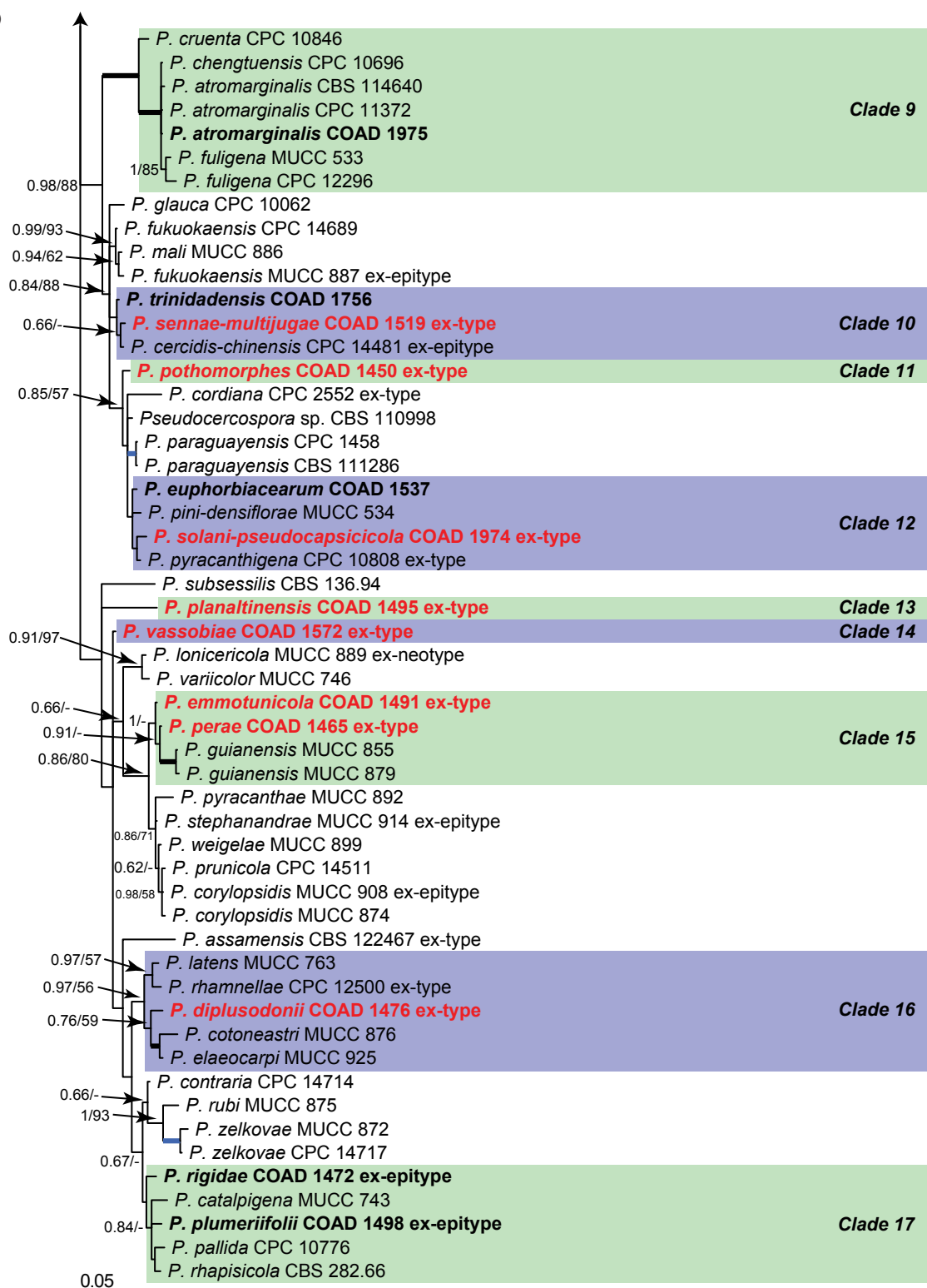


**Fig. 1** The Bayesian phylogenetic tree inferred from DNA sequence data from the multigenic alignment (ITS, *actA* and *tef1*) of *Pseudocercospora* species. Species from Brazil are in **bold** face and in coloured blocks with clade numbers for reference in the species notes. Novel species are indicated 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](http://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 out-group 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). *Pas-salora 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.

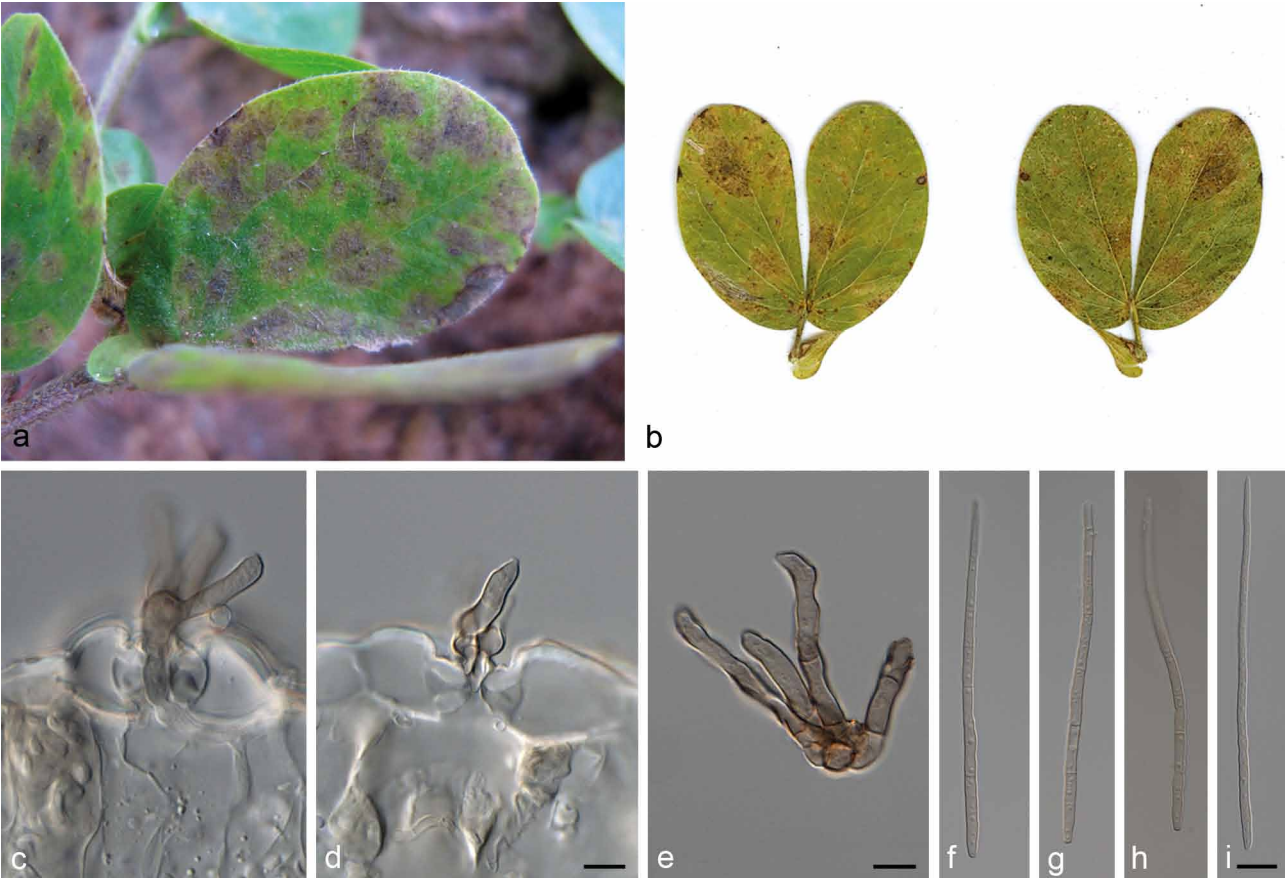


Fig. 2 *Pseudocercospora aeshynomenicola* (VIC 42805). a. *Aeschynomene falcata* with leaf spots; b. leaf spots on upper and lower leaf surface; c, d. conidiophores emerging through stomata; e. conidiogenous cells; f–i. conidia. — Scale bars: c–i = 10 µm.



## 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. diplosodonii*, *P. emmotunicola*, *P. manihotii*, *P. perae*, *P. planaltinensis*, *P. pothomorphes*, *P. sennae-multijugae*, *P. solani-pseudocapsicicola*, *P. vassobiae*, *P. wulfiae* and *P. xylopieae* 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-sinuuous, 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 *Eustoma 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. chengtzensis*, 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. chengtzensis*, *P. fuligena* or *P. stizolobii* based solely on ITS data, or from *P. chengtzensis*, *P. cruenta* or *P. fuligena* based solely on a *tef1* phylogeny. In the *actA* phylogeny it cannot be distinguished from *P. chengtzensis*, 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 Agrônomo 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

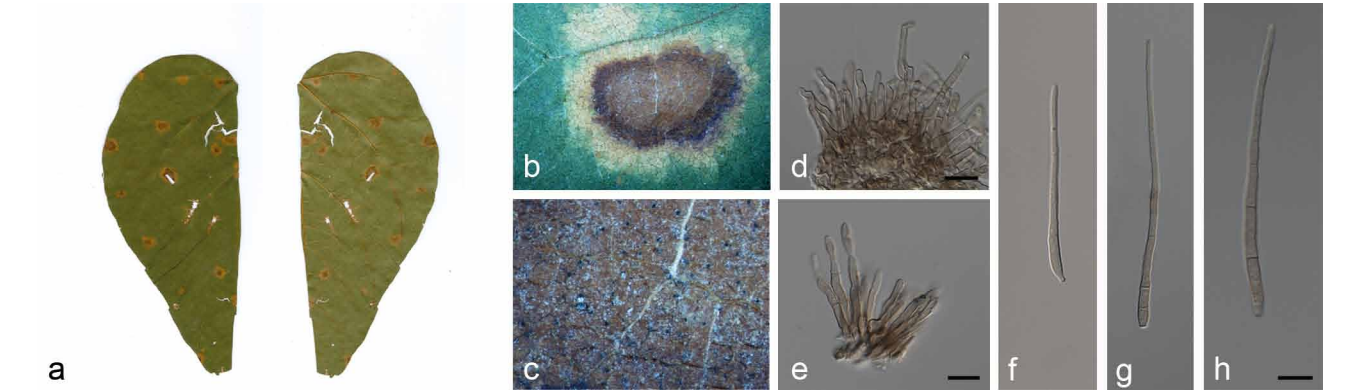
*Basionym.* *Cercospora boehmeriae* Peck, Ann. Rep. N.Y. State Mus. Nat. Hist. 34: 48. 1881.

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

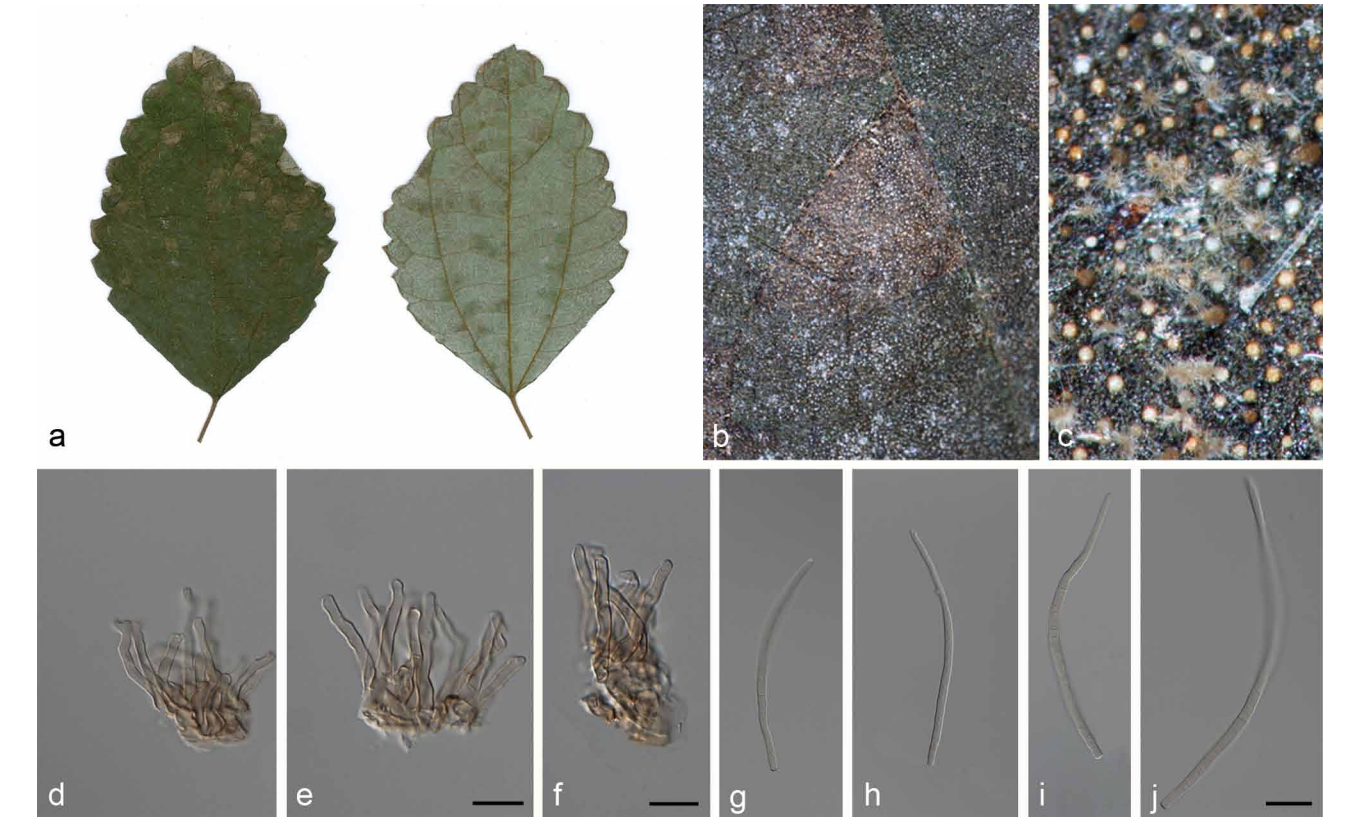
*Leaf spots* amphigenous, irregular to angular, pale brown to brown, 4–13 mm diam, vein-delimited. *Internal mycelium* indistinct. *External mycelium* absent. *Stromata* poorly developed, consisting of a few brown cells. *Conidiophores* epiphyllous, aggregated in loose fascicles, cylindrical, 13–26.5 × 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 subobtusely 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 synnematus 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; isoeotype CBS H-22165, culture ex-isoeotype 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. synnematus 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.

$\mu\text{m}$ , subhyaline to pale brown, subcylindrical, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, guttulate, pale brown, smooth, subcylindrical, straight to curved,  $24\text{--}99 \times 2\text{--}3.5 \mu\text{m}$ , apex obtuse, base truncate,  $1.5\text{--}2.5 \mu\text{m}$  wide, 1–12-septate; hila unthickened, not darkened,  $1.5\text{--}2 \mu\text{m}$  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* (Icacinaeae), 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* (Icacinaeae) (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

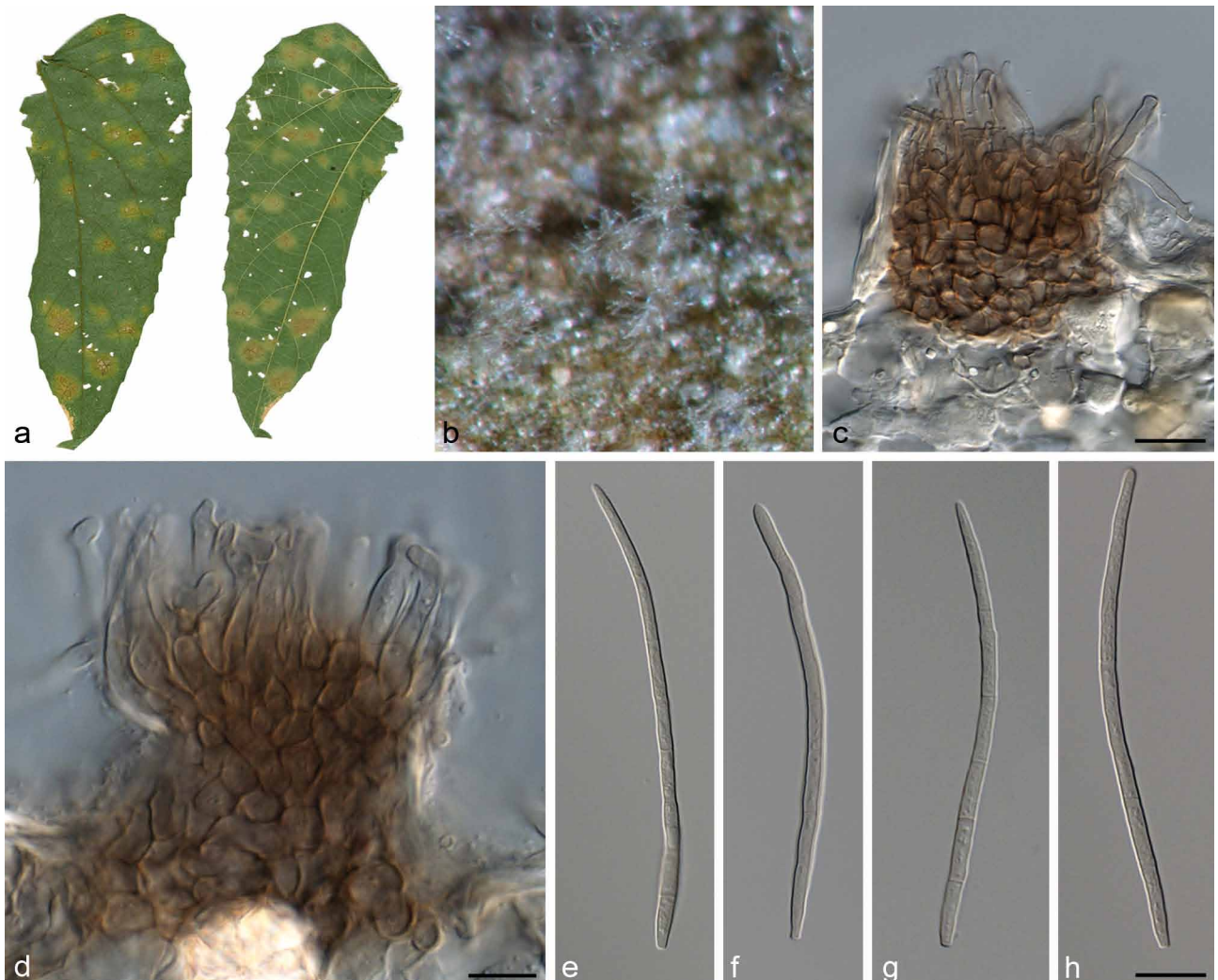
*Leaf spots* amphigenous, circular to irregular, chlorotic with a white centre, 4–12 mm diam. *Internal mycelium* intercellular,

$2\text{--}3.5 \mu\text{m}$ , branched, subhyaline, septate, smooth. *External mycelium* absent. *Stromata* hypophyllous, erumpent, well-developed, erumpent,  $17\text{--}31.5 \times 17\text{--}47 \mu\text{m}$ , composed of brown *textura angularis*. *Conidiophores* aggregated in dense fascicles arising from the upper cells of the stromata, subcylindrical,  $17\text{--}42 \times 2.5\text{--}4 \mu\text{m}$ , 0–4-septate, straight to geniculate-sinuous, unbranched, pale olivaceous to olivaceous brown, smooth. *Conidiogenous cells* terminal, integrated, subcylindrical, proliferating sympodially,  $10\text{--}27 \times 2.5\text{--}4 \mu\text{m}$ , brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, finely guttulate, subhyaline to pale olivaceous, smooth, subcylindrical, straight to curved,  $49\text{--}94 \times 3\text{--}4 \mu\text{m}$ , apex obtuse, base obconically to truncate,  $2.5\text{--}3.5 \mu\text{m}$  wide, 3–14-septate; hila unthickened, not darkened,  $1\text{--}2 \mu\text{m}$  diam.

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

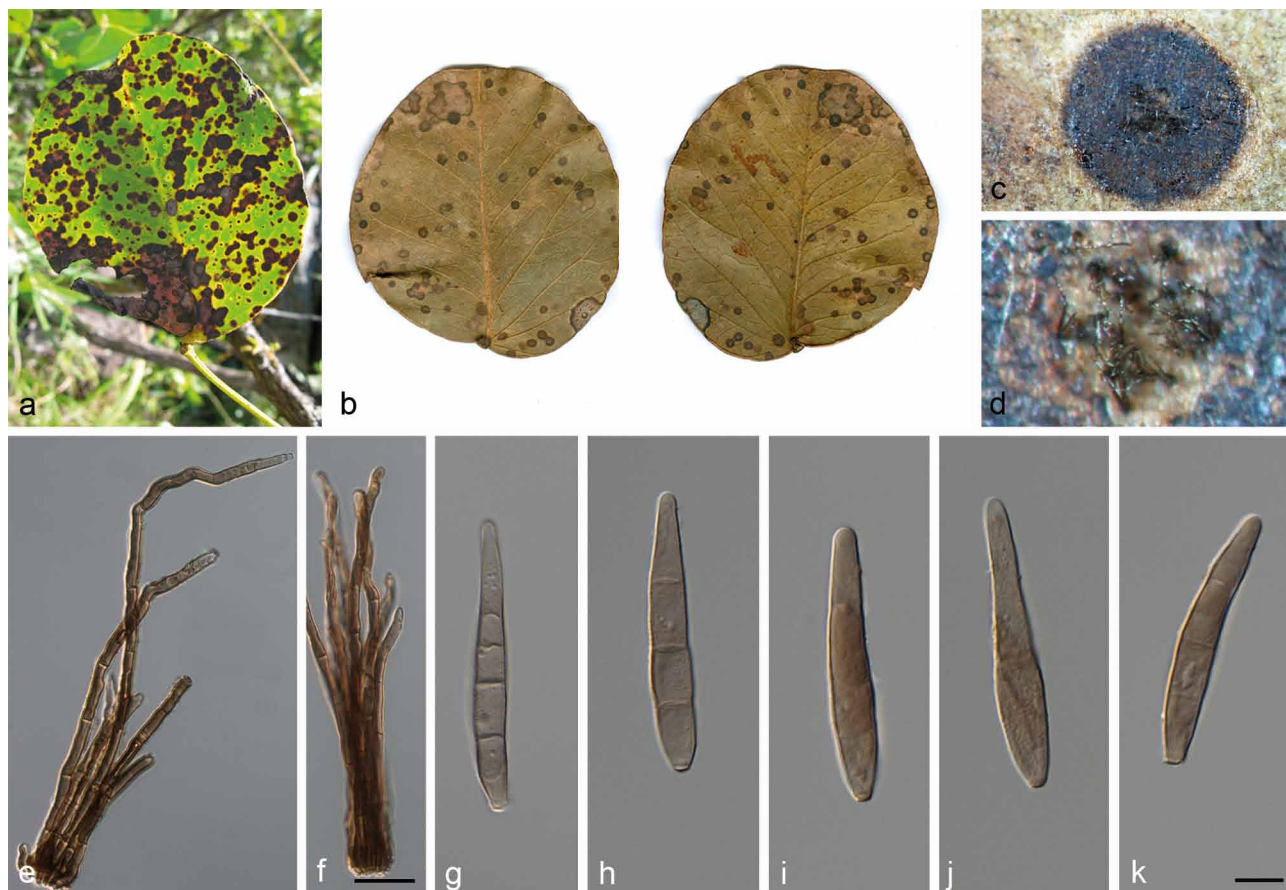
*Specimen examined.* BRAZIL, Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of *Dalechampia* sp. (Euphorbiaceae), 5 Aug. 2013, M. Silva (CBS H-22163, VIC 42797, cultures COAD 1537, CPC 25222).

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  $\mu\text{m}$ .





**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. synnematus conidiophores; g–k. conidia. — Scale bars: e–k = 10  $\mu$ m.

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  $\mu$ m diam, composed of brown *textura globosa*. **Conidiophores** amphigenous, aggregated in synnemata, subcylindrical, 115–306  $\times$  5–6.5  $\mu$ m, 4–15-septate, straight, curved or geniculate-sinuous at the upper portion, unbranched, brown, smooth. **Conidiogenous cells** integrated, terminal, proliferating percurrently, 18–32  $\times$  5–6.5  $\mu$ m, 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  $\times$  5–6.5  $\mu$ m, apex rounded, base obconically truncate, 4.5–6  $\mu$ m wide, 1–7-septate; hila unthickened, not darkened, 2.5–4  $\mu$ m 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).

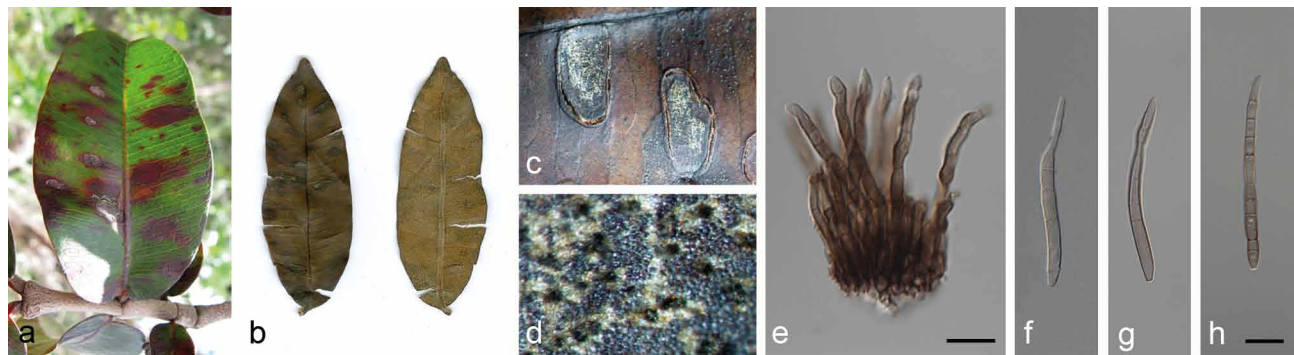
**Notes** — The epitype of *P. exilis*, designated here, is morphologically similar to the holotype, particularly in morphology of conidiophores and conidia, and originates from the same biome and country as the holotype. Also see the notes under *P. chamaecristae*. In the multigene phylogenetic analysis, *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  $\times$  43–82  $\mu$ m, compose of dark brown *textura angularis*. **Conidiophores** aggregated in dense fascicles, cylindrical, 19–84  $\times$  3–6  $\mu$ m, 1–6-septate, straight or sinuous, unbranched, brown, smooth. **Conidiogenous cells** integrated, terminal, polyblastic, proliferating percurrently, 6–25  $\times$  3–6  $\mu$ m, 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  $\times$  3–5  $\mu$ m, apex subobtuse, base obconic to subtruncate, 3–4.5  $\mu$ m wide, 1–8-septate; hila neither thickened nor darkened, 1.5–2  $\mu$ m 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 *Harconia speciosa* (Apocynaceae), 10 Apr. 1993, J.C. Dianese (holotype, UB Mycol. Col. 4149); Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of *Harconia 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.



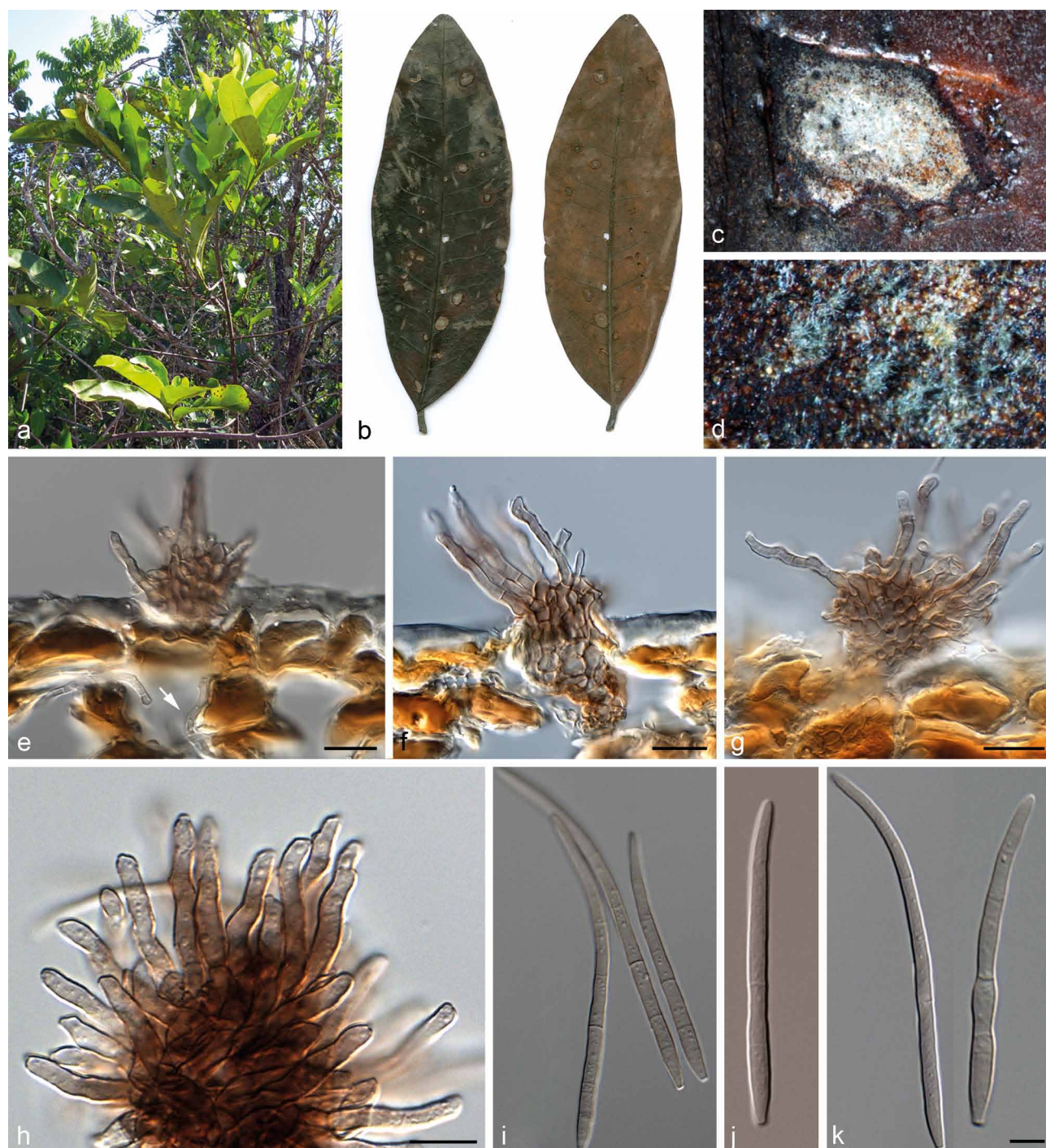
pale brown, smooth. *Conidiogenous loci* slightly conspicuous, slightly thickened, not darkened. *Conidia* solitary, finely guttulate, pale brown, smooth, cylindrical to narrowly obclavate, straight to curved,  $19\text{--}97 \times 2\text{--}4\text{ }\mu\text{m}$ , apex rounded to subacute, base obconically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

**Culture characteristics** — Very slow-growing ( $15\text{--}18\text{ mm}$  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 ( $5\text{--}40 \times 3\text{--}4.5\text{ }\mu\text{m}$ ) (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 =  $10\text{ }\mu\text{m}$ .



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*.

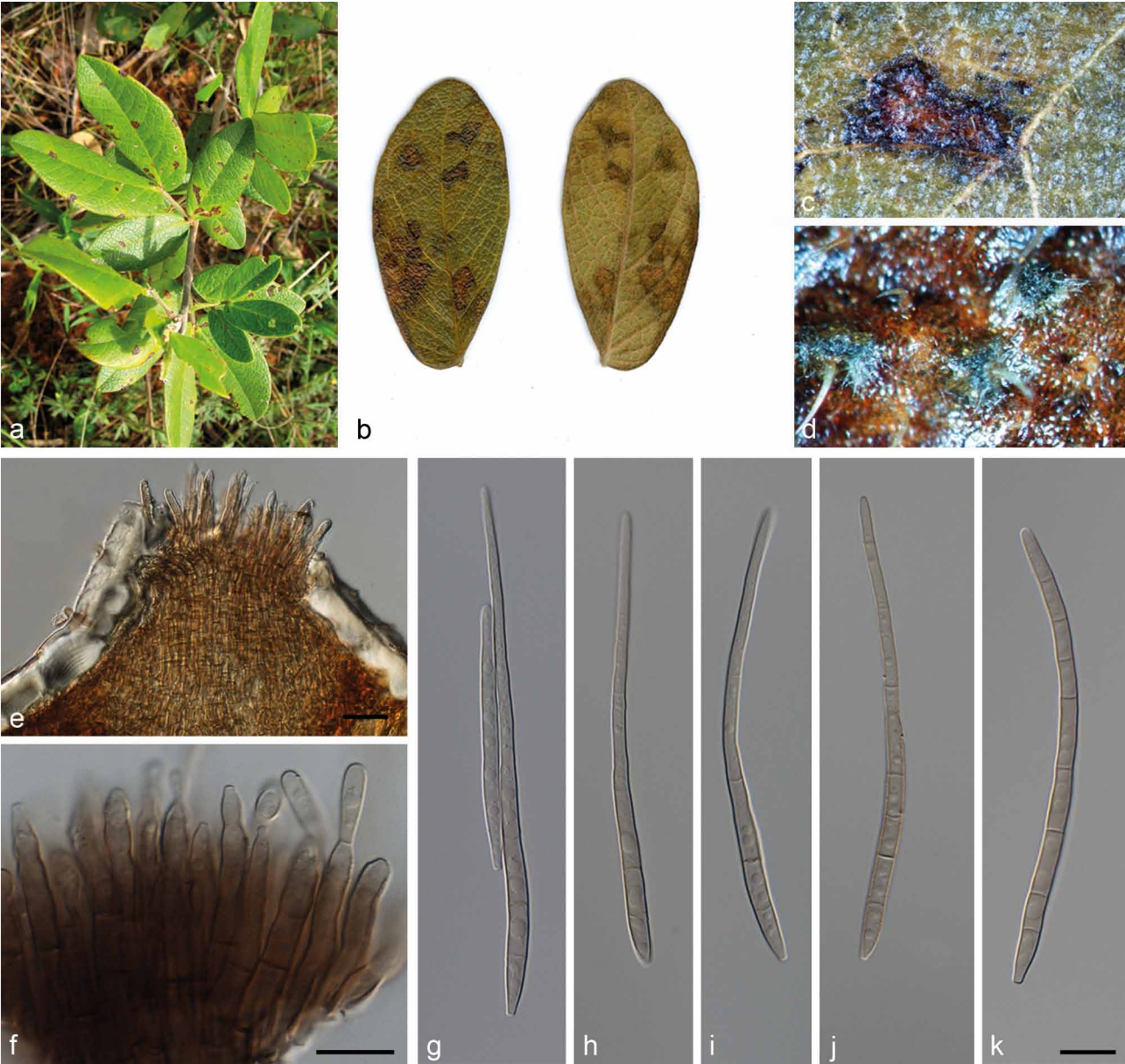
**Leaf spots** amphigenous, circular to irregular, pale brown to brown, on upper surface white centrally, 3–6 mm diam, surrounded by a black margin. **Internal mycelium**, subhyaline, septate, branched, smooth, 3.5–4 µm diam. **External mycelium** absent. **Stromata** well-developed, 14–35 × 23–42 µm, 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.

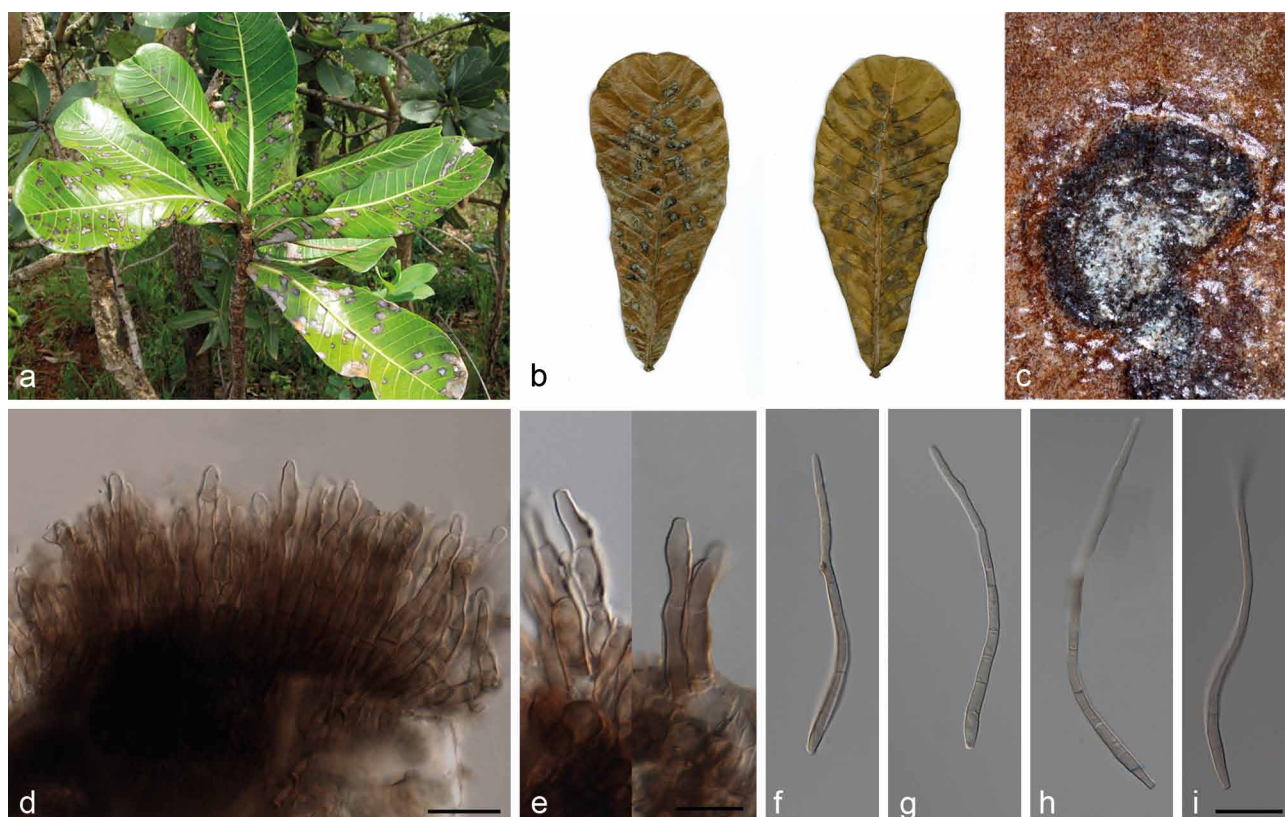
**Specimen examined.** BRAZIL, Distrito Federal, Brasília, Estação Ecológica de Águas Emendadas, on leaves of *Chamaecrista* sp. (*Fabaceae*), 17 Apr. 2013, M. Silva (holotype VIC 42748, culture ex-type COAD 1495; isotype CBS H-22153, culture ex-isotype CPC 25189).

**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 synnematus 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,  $13\text{--}45 \times 2.5\text{--}4\ \mu\text{m}$ , 0–4-septate, straight to geniculate-sinuous, unbranched, brown, smooth. *Conidiogenous cells* terminal, proliferating sympodially,  $7\text{--}19 \times 3\text{--}4\ \mu\text{m}$ , subcylindrical to sinuous, brown, smooth. *Conidia* solitary, guttulate, olivaceous to olivaceous brown, smooth, obclavate, straight to curved,  $25\text{--}110 \times 3\text{--}5\ \mu\text{m}$ , apex obtuse, base obconically truncate,  $2.5\text{--}4.5\ \mu\text{m}$  wide, 2–9-septate; hila unthickened, not darkened,  $1.5\text{--}2.5\ \mu\text{m}$  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; isoeotype CBS H-22154, culture ex-isoeotype 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. rhapsicola* and *P. rigidae* (Fig. 1, clade 17). *Pseudocercospora catalpigena* differs from *P. plumeriifolii* by having shorter and wider conidiophores ( $5\text{--}35 \times 3\text{--}6\ \mu\text{m}$ ) (Braun et al. 2003), while *P. rigidae* has longer and wider conidiophores ( $21\text{--}85 \times 3\text{--}5\ \mu\text{m}$ ). *Pseudocercospora pallida* and *P. rhapsicola* 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. rhapsicola* in the *tef1* phylogeny.

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

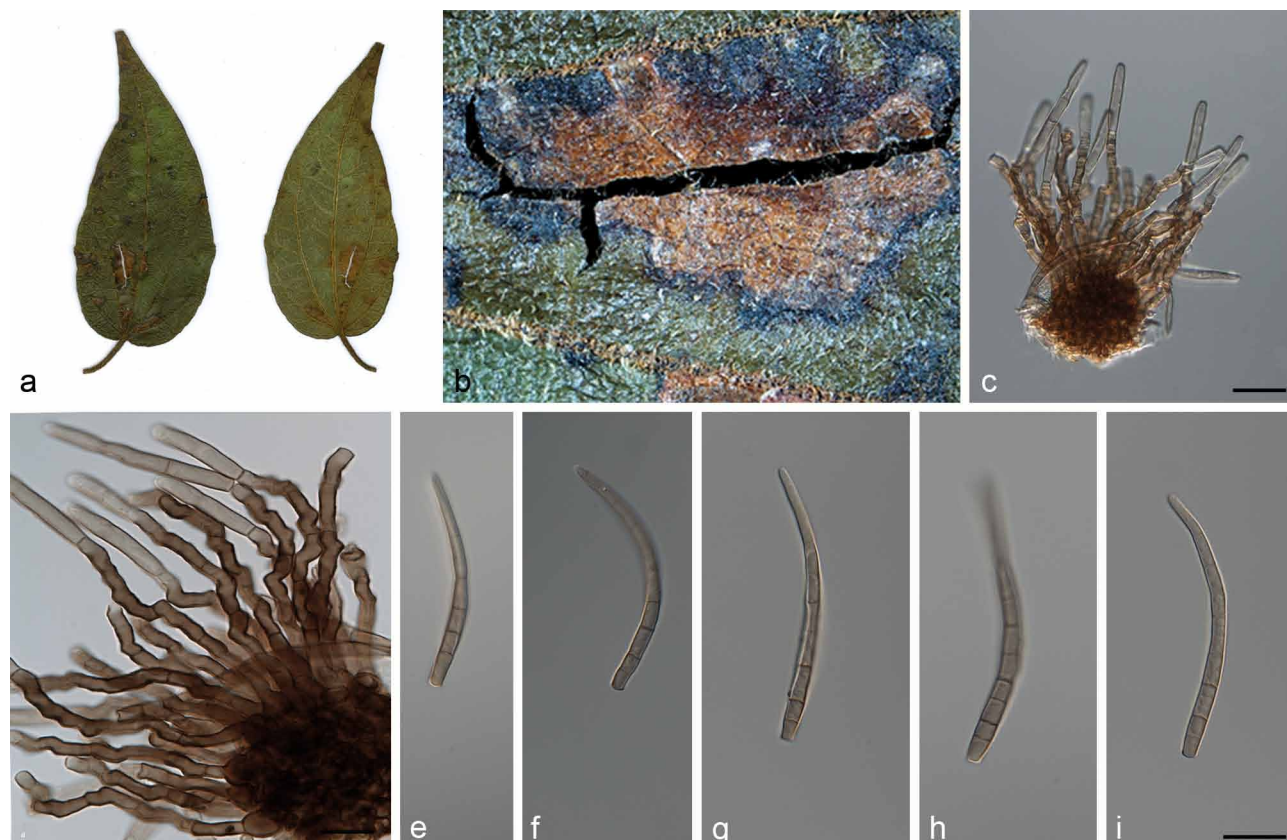
**Basionym.** *Cercospora plunkettii* Chupp, A monograph of the fungus *Cercospora*: 154. 1954.

**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,  $32\text{--}39 \times 48\text{--}53\ \mu\text{m}$ , angular to irregular, composed of dark brown *textura angularis*. **Conidiophores** aggregated in dense fascicles, emerging through stromata,  $20\text{--}85 \times 3.5\text{--}5\ \mu\text{m}$ , 3–8-septate, straight to strongly geniculate-sinuous, unbranched, pale brown, smooth. **Conidiogenous cells** terminal,  $6\text{--}31 \times 3.5\text{--}5\ \mu\text{m}$ , pale brown, proliferating sympodially, rarely percurrently, smooth. **Conidia** solitary, guttulate, pale brown, smooth, subcylindrical to obclavate, straight to curved,  $49\text{--}81 \times 3\text{--}5\ \mu\text{m}$ , apex obtuse to subacute, base obconically truncate,  $3\text{--}5\ \mu\text{m}$ , 6–10-septate; hila unthickened, not darkened,  $2.5\text{--}5\ \mu\text{m}$  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 =  $10\ \mu\text{m}$ , d =  $20\ \mu\text{m}$ .



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  $\mu\text{m}$ ) and longer conidia (25–90  $\mu\text{m}$ ), while *P. richardsoniicola* has longer conidiophores and conidia (90–192  $\mu\text{m}$ , 36–97  $\mu\text{m}$ , 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  $\mu\text{m}$  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  $\times$  3.5–6  $\mu\text{m}$ , 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  $\times$  3–5.5  $\mu\text{m}$ , 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  $\times$  3.5–5  $\mu\text{m}$ , apex rounded to subacute, base truncate, 2.5–4  $\mu\text{m}$  wide, 1–7-septate; hila neither thickened nor darkened, 2–2.5  $\mu\text{m}$  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.

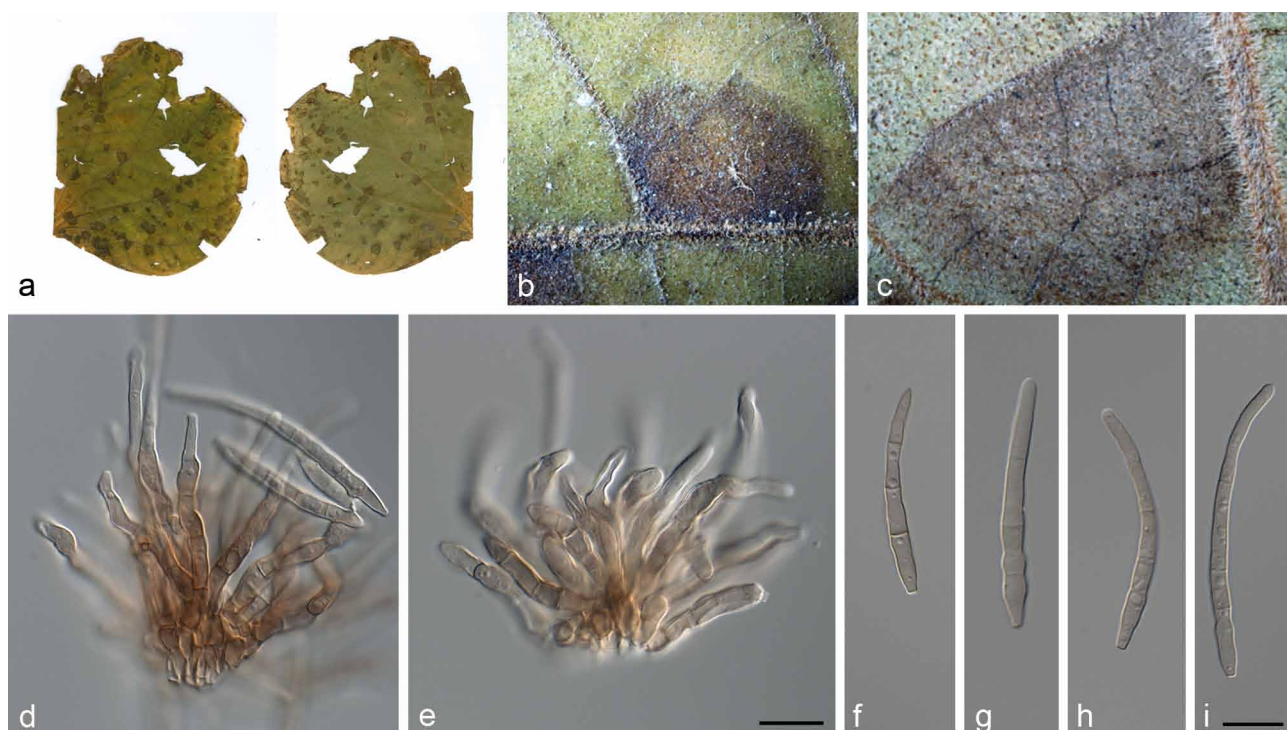
**Specimen examined.** BRAZIL, Minas Gerais, Viçosa, Reserva Florestal Mata do Paraíso, on leaves of *Pothomorphe umbellata* (Piperaceae), 15 Nov. 2012, O.L. Pereira (holotype VIC 42705, culture ex-type COAD 1450; isotype CBS H-22147, culture ex-isotype CPC 25166).

**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  $\mu\text{m}$ ), as well as longer conidia (25–130  $\mu\text{m}$ ) (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. aeshynomenicola* and *P. struthanthii*. 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

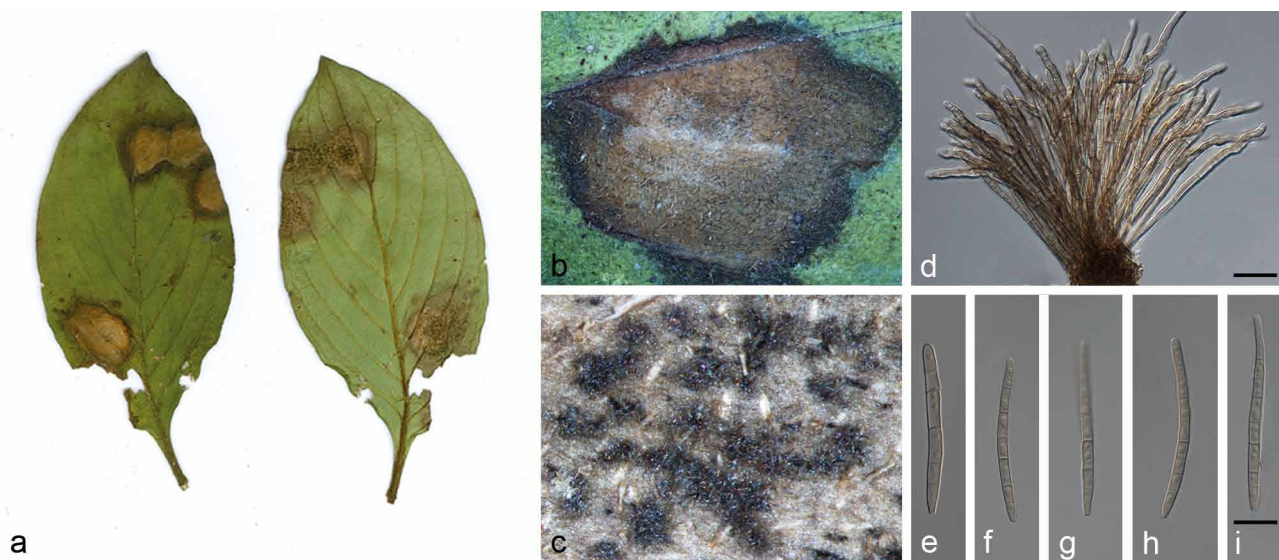
**Basionym.** *Cercospora richardsoniae* Henn., Hedwigia 41: 117. 1902 (non *C. richardsoniae* Ellis & Everh.).

**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  $\mu\text{m}$  diam. **Stromata** amphigenous, well-developed, 45–61  $\times$  54–70  $\mu\text{m}$  subimmersed, angular, composed of brown *textura angularis*. **Conidiophores** arising from stromata aggregated in dense fascicles, cylindrical, 90–192  $\times$  3–5  $\mu\text{m}$ , 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  $\mu\text{m}$ .





**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  $\mu$ m.

toward the apex, smooth. *Conidiogenous cells* terminal, proliferating sympodially,  $9\text{--}71 \times 2.5\text{--}5$   $\mu$ m, pale brown, cylindrical, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, cylindrical to obclavate, straight to slightly curved,  $36\text{--}97 \times 3\text{--}5$   $\mu$ m, apex rounded to obtuse, base obconically truncate, 3–8-septate, guttulate, pale brown, smooth, 2.5–5  $\mu$ m wide; hila neither thickened nor darkened, 1.5–2.5  $\mu$ m 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; isoeotype CBS H-22172, culture ex-isoeotype 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  $\mu$ m) and narrower conidia (2.5–3.5  $\mu$ m) (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,  $16\text{--}27 \times 19\text{--}53$   $\mu$ m, composed of brown *textura globosa*. **Conidiophores** amphigenous, fasciculate, arising from the subepidermal stromata,  $21\text{--}85 \times 3\text{--}5$   $\mu$ m, 3–9-septate, straight to geniculate-sinuous, rarely branched below, dark brown, smooth. **Conidiogenous cells** terminal or lateral, proliferation percurrently and sometimes

sympodially,  $12\text{--}23 \times 3\text{--}4$   $\mu$ m, brown, smooth. *Conidiogenous loci* inconspicuous, unthickened, not darkened. *Conidia* solitary, pale brown to brown, smooth, guttulate, obclavate-cylindrical, straight to slightly curved,  $25\text{--}99 \times 3\text{--}5$   $\mu$ m, apex obtuse to subacute, 2–2.5  $\mu$ m wide, 0–7-septate; hila slightly thickened, slightly darkened not refractive, 1.5–2  $\mu$ m 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.

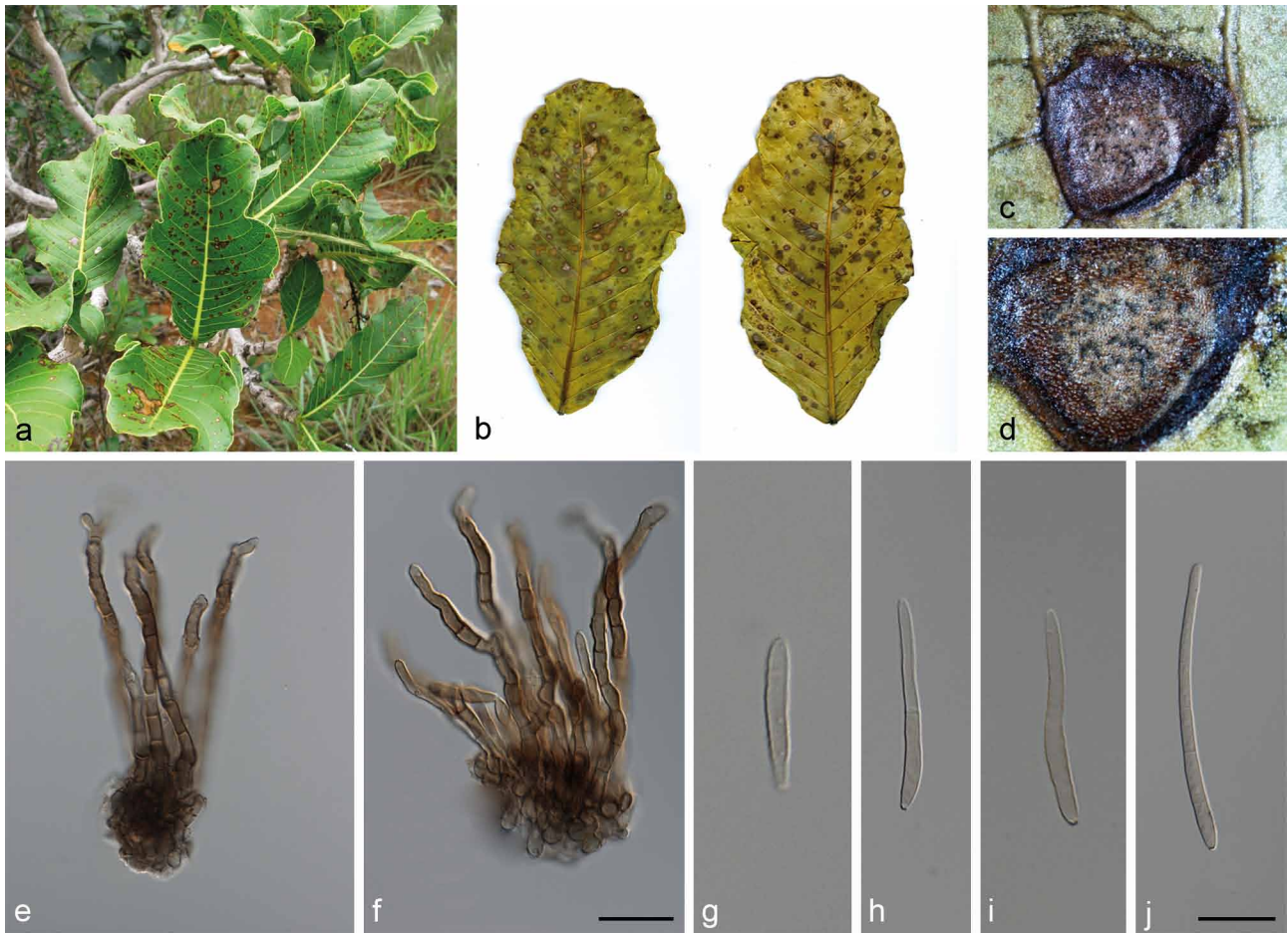
**Specimens examined.** BRAZIL, Minas Gerais, Carrancas, on leaves of *Palicourea rigida* (Rubiaceae), Mar. 2007, O.L. Pereira (holotype VIC 30472); Paraopeba, Floresta Nacional de Paraopeba (FLONA), on leaves of *Palicourea rigida*, 30 Mar. 2013, M. Silva (epitype designated here VIC 42726, MBT202069, culture ex-epitype COAD 1472; isoeotype CBS H-22150, culture ex-isoeotype 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. rhapsicola* (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. zelvovae* in the *tef1* phylogeny.

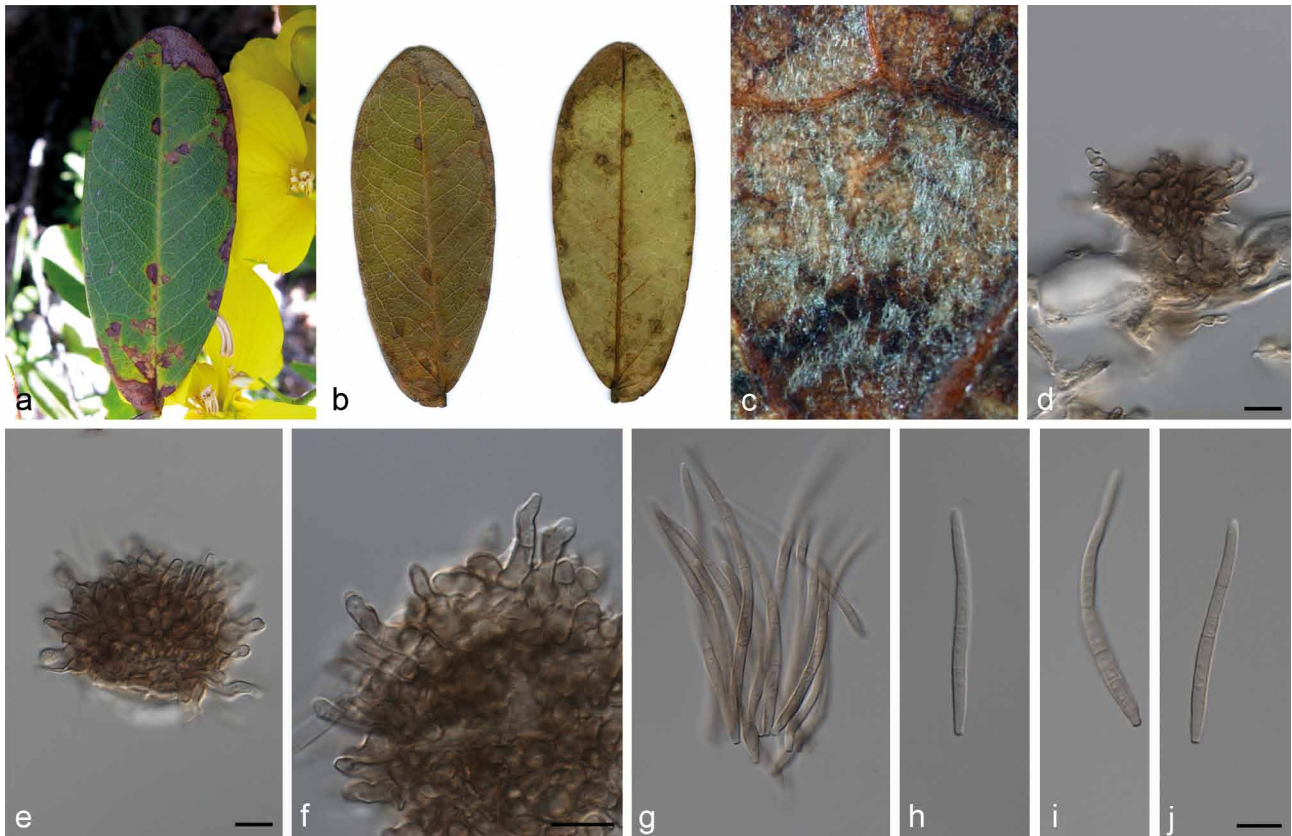
***Pseudocercospora sennae-multijugae*** Meir. Silva, R.W. Barreto & Crous, sp. nov. — MycoBank MB814905; Fig. 19

**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  $\mu$ m diam wide. **External mycelium** subhyaline, consisting of septate, smooth hyphae, 2.5–4  $\mu$ m diam. **Stromata** well-developed, substomatal, 25–67  $\mu$ m diam, brown, composed of brown *textura angularis*. **Conidiophores** hypophyllous, sporodochial, arising from stroma, emerging through stomata,  $8\text{--}14 \times 2\text{--}4.5$   $\mu$ m, 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  $\mu$ m.



**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  $\mu$ m.



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).

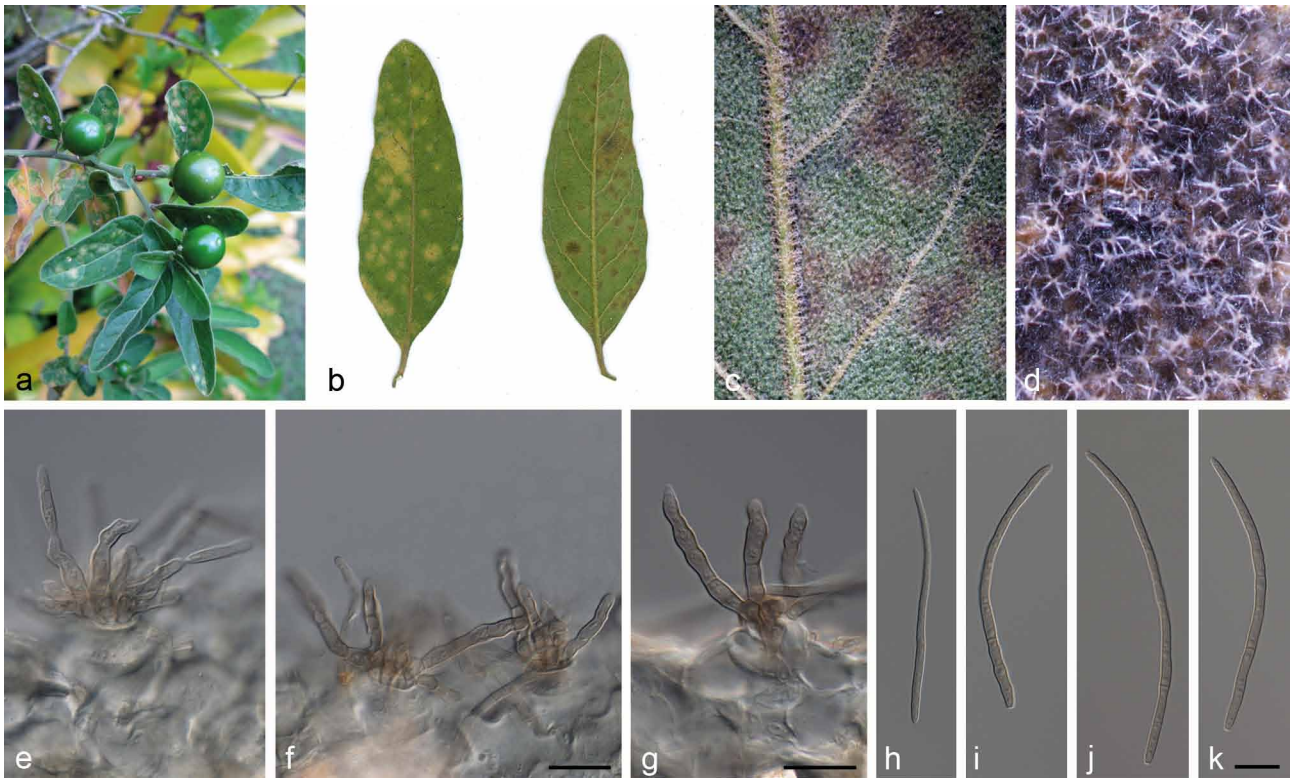
Notes — Nine species of *Pseudocercospora* have previously been recorded on members of *Senna*, namely *P. angustata*, *P. cassiae-alatae*, *P. cassiae-fistulae*, *P. cassiae-occidentalis*, *P. cassiae-siameae*, *P. nigricans*, *P. simulate*, *P. singaporensis* and *P. taichungensis* (Farr & Rossman 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-pseudocapsicola* 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-pseudocapsicola*** 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-pseudocapsicola* (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-pseudocapsicola* 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-pseudocapsicola*, 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-pseudocapsicola* 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-pseudocapsicola* 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-pseudocapsicola* 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

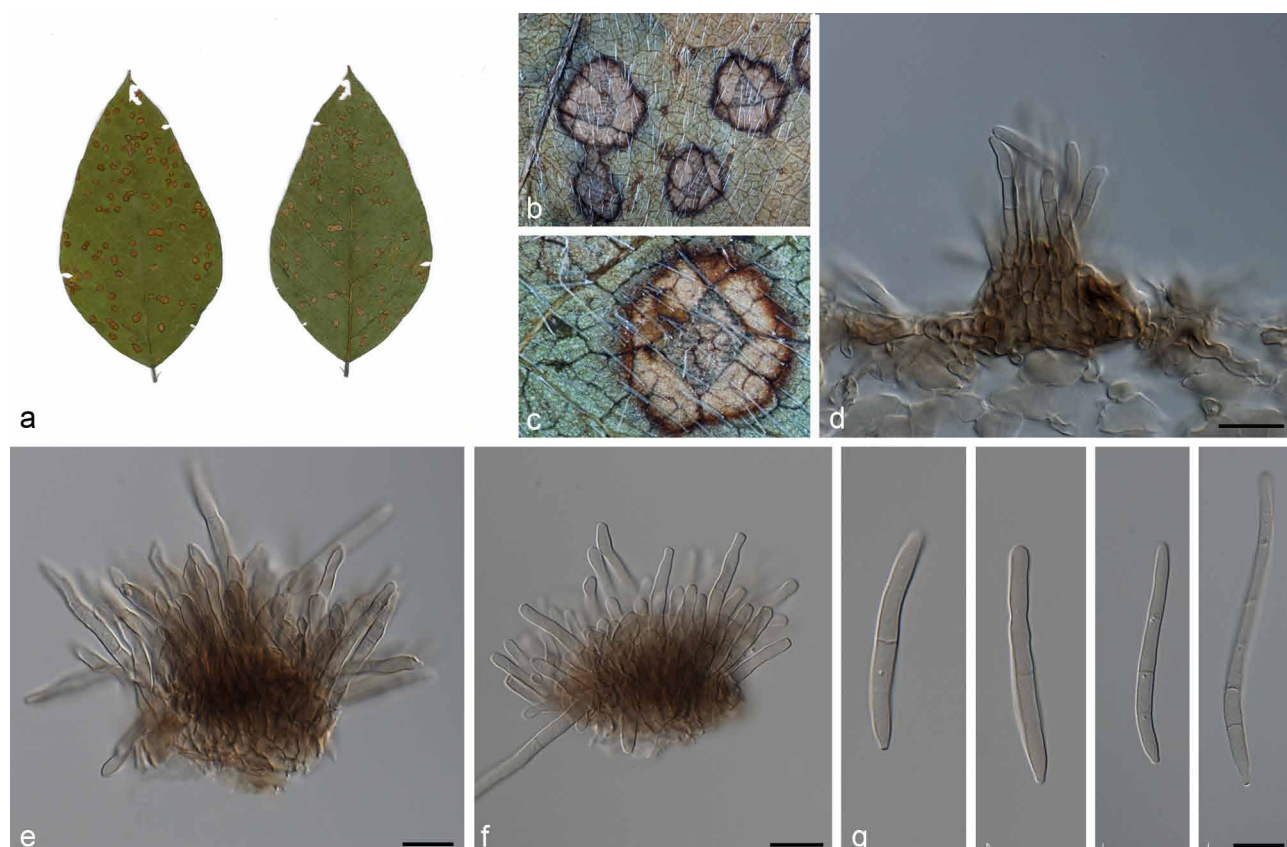
**Basionym.** *Cercospora stizolobii* Syd. & P. Syd., Ann. Mycol. 11: 270. 1913.

**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. piperis* (Fig. 1, clade 8). It is not possible to distinguish *P. struthanthi* from numerous other *Pseudocer-*

*cospora* 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 & Bag-  
yan., 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.

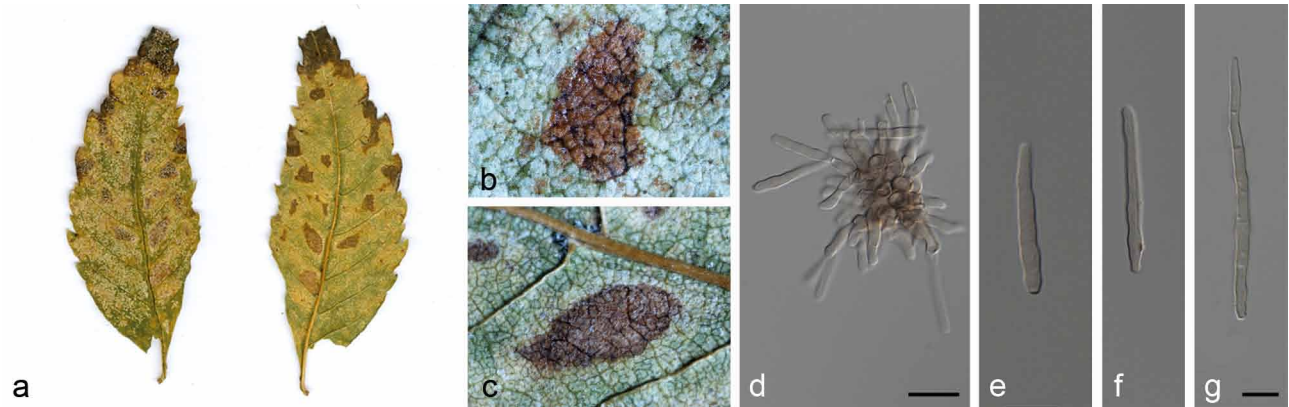
*Culture characteristics* — Slow-growing (28 mm diam after 20 d); colonies circular, erumpent, surface velvety, with moderate aerial mycelium, smooth to slightly irregular margins. Surface olivaceous grey surrounded by pale olivaceous grey margin; reverse iron-grey.

*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 gossypifolius* (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

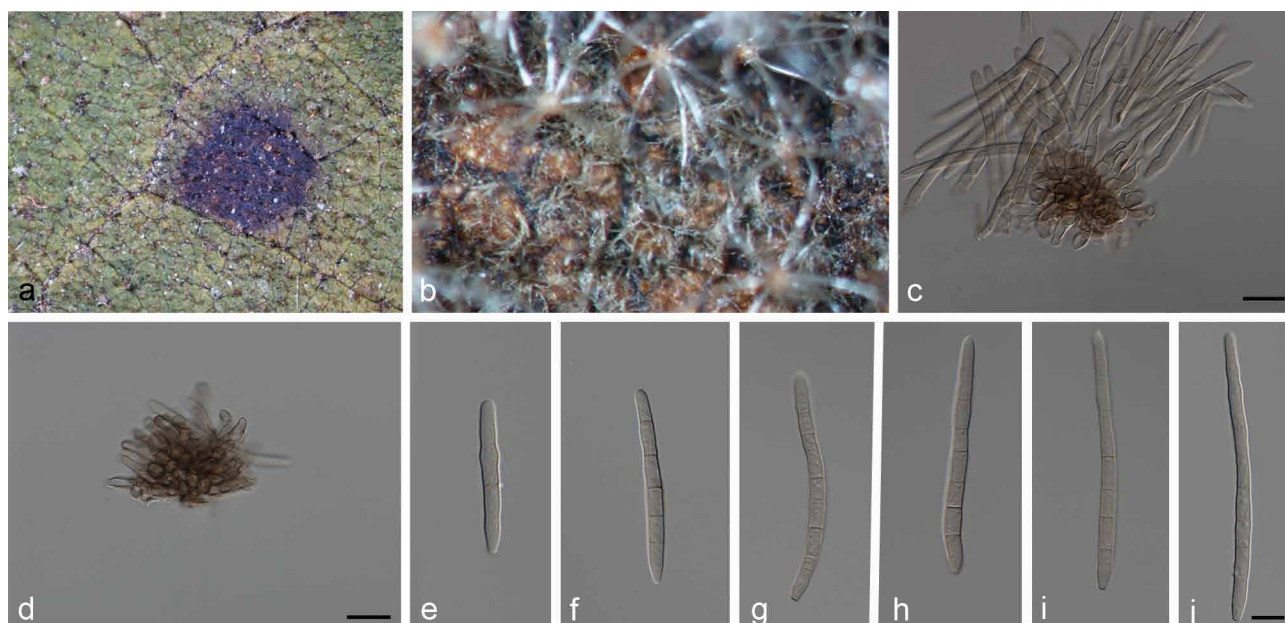
**Etymology.** Name derived from host genus *Vassobia*.

**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.

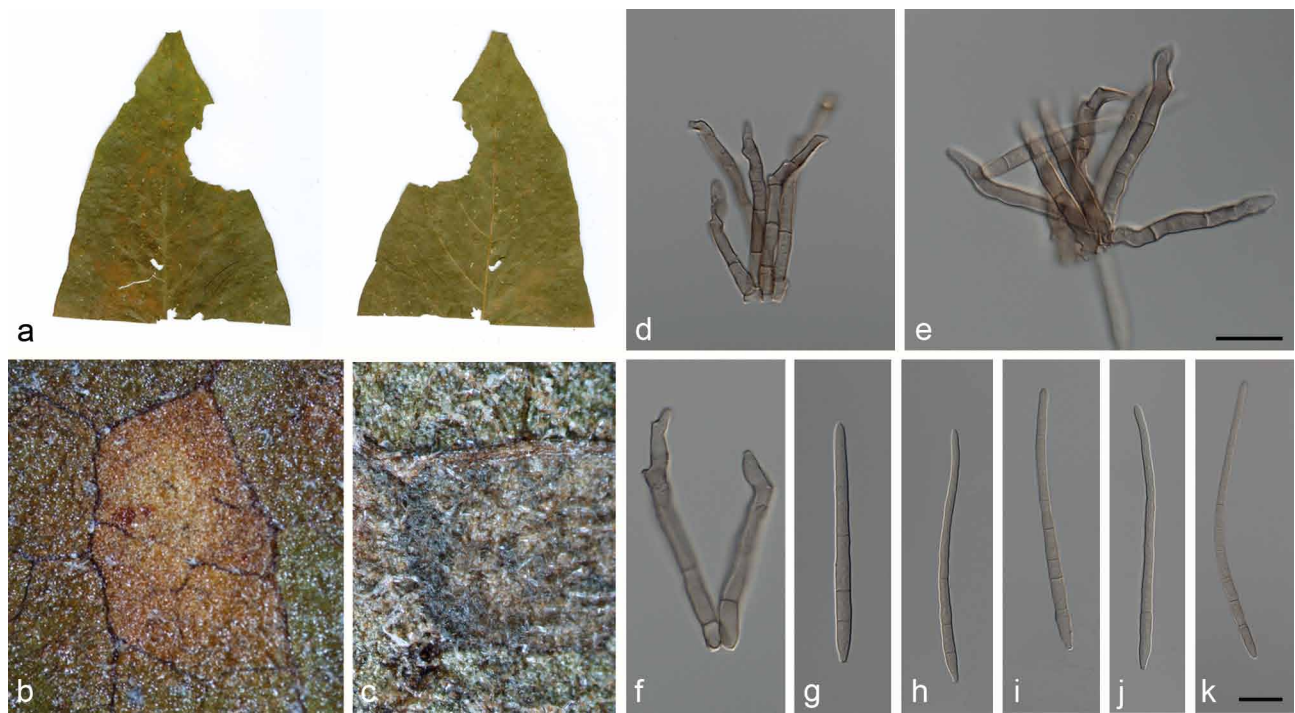
**Specimen examined.** BRAZIL, Rio de Janeiro, Nova Friburgo, on leaves of *Vassobia breviflora* (Solanaceae), 9 June 2013, R.W. Barreto (holotype VIC 42676, culture ex-type COAD 1572; isotype CBS H-22173, culture ex-isotype CPC 25251).

**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.

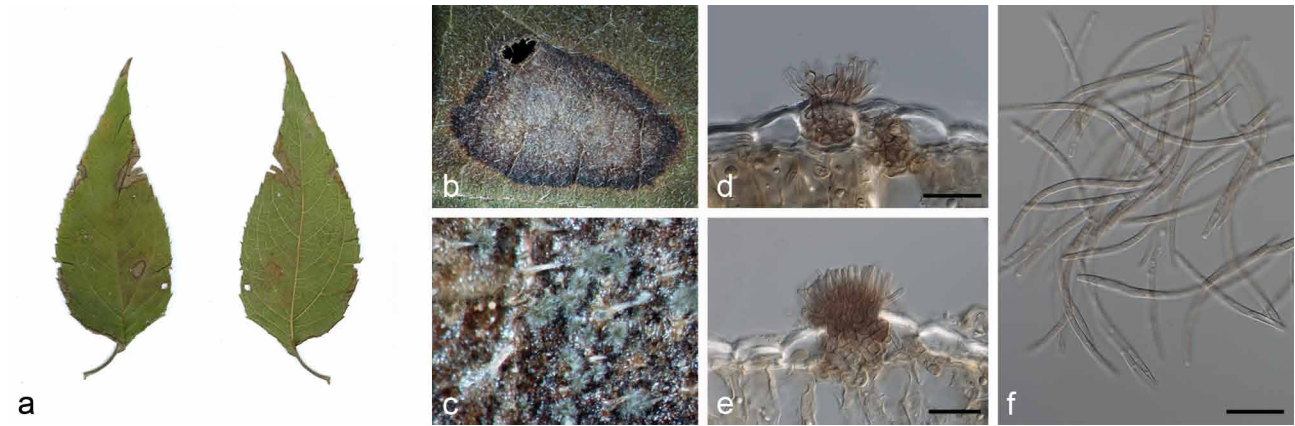
***Pseudocercospora wulfiae*** Meir. Silva, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB813623; Fig. 26

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

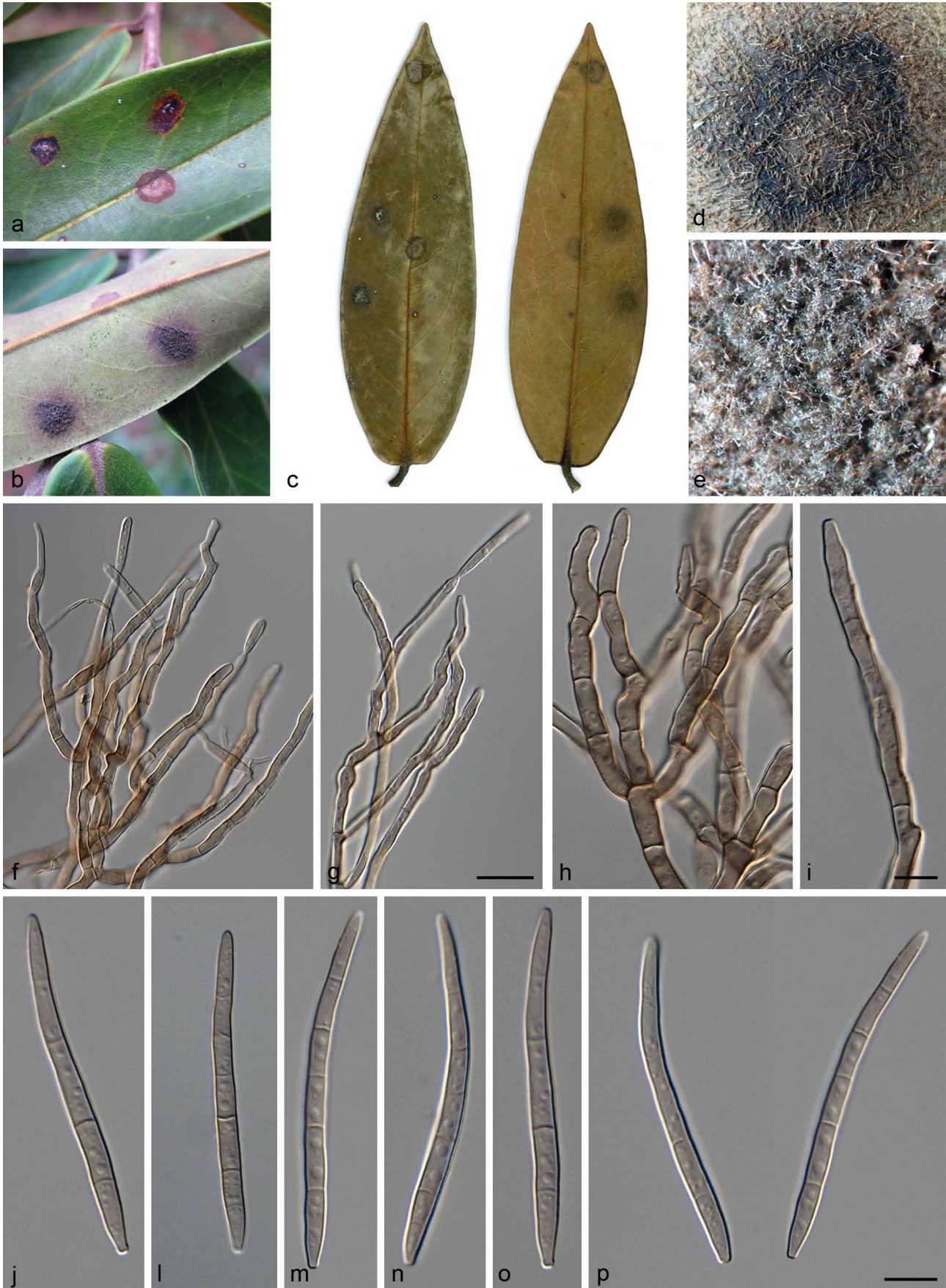
*Leaf spots* amphigenous, irregular, grey-brown surrounded by a dark brown margin, on lower surface medium brown, with poorly

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 wulfia* (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 xylopiiae* (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 fructing; f, g. conidiophores in loose fascicles; h, i. conidiogenous cells; j–p. conidia. — Scale bars: f–p= 10  $\mu$ m.



**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* ( $\equiv$  *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 xylopiæ*** Meir. Silva, R.W. Barreto & Crous, sp. nov. — MycoBank MB813622; Fig. 27

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

**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  $\times$  3–5  $\mu$ m, branched, brown, smooth. **Conidiogenous cells** terminal or intercalary, subcylindrical, proliferating sympodially, 8–20  $\times$  2.5–4  $\mu$ m, geniculate, brown, smooth. **Conidiogenous loci** inconspicuous, unthickened, not darkened. **Conidia** solitary, guttulate, pale brown, smooth, subcylindrical, straight to gently curved, 30–86.5  $\times$  3–4.5  $\mu$ m, apex obtuse, base truncate, 2.5–4  $\mu$ m wide, 3–10-septate; hila unthickened, neither darkened nor refractive, 1.5–2.5  $\mu$ m.

**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 *Xylopiæ 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 *Xylopiæ* (Farr & Rossman 2015), namely *P. aethiopicae* on *Xylopiæ aethiopica* from Sierra Leone (Deighton 1976). *Pseudocercospora aethiopicae* clearly differs from *P. xylopiæ* by having shorter and narrower conidiophores (10–40  $\times$  2.5–4  $\mu$ m), arranged in dense fascicles, and not forming on external mycelium, and having smaller conidia, 32–65  $\times$  2.5–3  $\mu$ m (Deighton 1976). Additionally, *P. xylopiæ* 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. xylopiæ* 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 mycodyversity 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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