



# Novel fungi from an ancient niche: cercosporoid and related sexual morphs on ferns

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## Key words

biodiversity  
*Cercospora*  
frond spot  
multilocus sequence typing (MLST)  
*Mycosphaerella*  
phylogeny  
*Pteridophyta*  
systematics

**Abstract** The fern flora of the world (*Pteridophyta*) has direct evolutionary links with the earliest vascular plants that appeared in the late Devonian. Knowing the mycobiota associated to this group of plants is critical for a full understanding of the Fungi. Nevertheless, perhaps because of the minor economic significance of ferns, this niche remains relatively neglected by mycologists. Cercosporoid fungi represent a large assemblage of fungi belonging to the *Mycosphaerellaceae* and *Teratosphaeriaceae* (*Ascomycota*) having cercospora-like asexual morphs. They are well-known pathogens of many important crops, occurring on a wide host range. Here, the results of a taxonomic study of cercosporoid fungi collected on ferns in Brazil are presented. Specimens were obtained from most Brazilian regions and collected over a 7-yr period (2009–2015). Forty-three isolates of cercosporoid and mycosphaerella-like species, collected from 18 host species, representing 201 localities, were studied. This resulted in a total of 21 frond-spotting taxa, which were identified based on morphology, ecology and sequence data of five genomic loci (actin, calmodulin, ITS, LSU and partial translation elongation factor 1- $\alpha$ ). One novel genus (*Clypeosphaerella*) and 15 novel species (*Cercospora samambaiae*, *Clypeosphaerella sticheri*, *Neoceratosperma alsophilae*, *N. cyatheae*, *Paramycosphaerella blechni*, *Pa. cyatheae*, *Pa. dicranopteridis-flexuosa*, *Pa. sticheri*, *Phaeopheospora pteridivora*, *Pseudocercospora brackenica*, *Ps. paranaensis*, *Ps. serpocaulonica*, *Ps. trichogena*, *Xenomycesphaerella diplazii* and *Zasmidium cyatheae*) are introduced. Furthermore, 11 new combinations (*Clypeosphaerella quasiparkii*, *Neoceratosperma yunnanensis*, *Paramycosphaerella aerothallosporum*, *Pa. dicranopteridis*, *Pa. gleicheniae*, *Pa. irregularis*, *Pa. madeirensis*, *Pa. nabiencense*, *Pa. parkii*, *Pa. pseudomarksii* and *Pa. vietnamensis*) are proposed. Finally, nine new host associations are recorded for the following known fungal species: *Cercospora coniogrammes*, *Cercospora* sp. Q, *Ps. abacopteridicola*, *Ps. lygodicola* and *Ps. telypteridis*.

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## INTRODUCTION

Cercosporoid fungi are well-known plant pathogens that are etiological agents of leaf spot diseases of many important crops (Agrios 2005). Major diseases include angular leaf spot of bean (*Pseudocercospora griseola*), black leaf streak of banana (*Ps. fijiensis*) and leaf spots on many other hosts including grapevine (*Ps. vitis*), celery (*Cercospora apii*) and sugarbeet (*C. beticola*), to name but a few (Braun et al. 2013).

Since the seminal monograph of Chupp (1954) on the genus *Cercospora*, several studies were aimed at investigating this group and dividing cercospora-like fungi into more natural genera. Of special relevance are the publications prepared with that intent (Deighton 1965, 1967, 1971, 1974, 1976, 1979, 1983, 1987, 1990, Pons & Sutton 1988, Braun 1993a–c, 1995, 1998, Crous & Braun 1996, Braun & Mel'nik 1997, Crous et al. 2000). Crous & Braun (2003) also revisited Chupp's work and, using morphological criteria, consolidated the generic circumscription of *Cercospora*, reducing the number of taxa from 3000 to 659 species names. Additionally, numerous stud-

ies dealing with cercosporoid fungi found in different countries have been published, e.g. Brazil (Viégas 1945), Japan (Katsuki 1965), Singapore and the Malay Peninsula (Yen & Lim 1980), Taiwan (Hsieh & Goh 1990), China (Guo & Hsieh 1995, Guo et al. 2003, 2005), South Africa (Crous & Braun 1996), Russia and adjacent countries (Braun & Mel'nik 1997), Korea (Shin & Kim 2001), Laos (Phengsinham et al. 2013a) and Thailand (Phengsinham et al. 2013b). Unfortunately, all of these regional studies of cercosporoids were only based on morphological, ecological and host specificity data for species delimitation, and in many instances, this has proven inadequate (Halleen et al. 2004, Lee et al. 2004, Réblová et al. 2004, Verkley et al. 2004a, b, Crous et al. 2006a, b, 2007a, b, 2009a, b, Arzanlou et al. 2007, Phillips et al. 2008, Shivas et al. 2009).

The tradition of naming fungi in the absence of molecular data remains dominant in published literature, despite the limitations of this approach rendering data-driven comparisons difficult to impossible, especially in groups with known wide host ranges. Of the fungal species described in 2013, 65 % still lacked DNA data (Crous et al. 2015a). The lack of DNA barcodes is still further complicated by the lack of ex-type cultures, which are frequently not deposited in publicly available biological resource centres. This is true for fungi in general, but in the case of the cercosporoid fungi in particular, the situation is further complicated by the fact that they are often only found as asexual morphs (Goodwin et al. 2001). When the sexual morph is present, cercosporoid taxa have traditionally been classified in entirely different genera, with few morphological characters that can be used to facilitate accurate identification (Braun et al. 2013, 2014, 2015). Moreover, many species (especially in

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the tropics and subtropics) are known only from their asexual morphs, and may exhibit considerable morphological variation due to environmental conditions, encouraging mycologists to mistakenly recognise them as distinct genera. As a result, numerous asexual genera, which may eventually prove to be artificial, have been introduced e.g. *Cercodeuterospora*, *Centrospora*, *Heterosporium* and others (Chupp 1954). On the other hand, once these groups are subjected to molecular phylogenetic comparisons, it has frequently also led to a high number of generic lineages that previously were not discernable based on morphology alone, e.g. *Paracercospora*, *Phaeocercospora* and in the *Teratosphaeriaceae* (Crous et al. 2013b, Quaedvlieg et al. 2014).

With DNA sequencing becoming widely available for use by mycologists as a reliable source of information (Taylor et al. 2000), a more concrete classification of fungi was initiated, and several studies have since been published on cercosporoid fungi (Arzanlou et al. 2007, Crous et al. 2007a, 2009b, d, 2013a, Braun et al. 2013, Groenewald et al. 2013, Bakhshi et al. 2014, 2015, Nguanhom et al. 2015). These studies have shown that some morphology-based genera were largely monophyletic, e.g. *Pseudocercospora* and *Ramularia* (Crous et al. 2013a, Groenewald et al. 2013, Bakhshi et al. 2014, 2015, Videira et al. 2015) whereas others like *Passalora* and other genera not recognised as cercosporoids, were clearly polyphyletic, e.g., *Phloeospora*, *Phoma*, *Pseudocercosporella*, *Septoria* and *Stagonospora* (Aveskamp et al. 2010, Frank et al. 2010, De Gruyter et al. 2013, Quaedvlieg et al. 2013).

Despite the intense effort by mycologists over the last two centuries at describing the world's mycobiota, this task is far from being complete (Crous et al. 2015a). Several niches harbouring unique fungi that may be of relevance for understanding fungal phylogeny, have been mostly neglected. One case in point is fungi associated with ferns. Ferns are members of the division *Pteridophyta* (= '*Monilophyta*'). In recent classifications (e.g., Smith et al. 2008) the division includes 37 families, approximately 300 genera and more than 9 000 species. Although there are presently c. 1 110 species known from Brazil, it has been estimated that this number may be far greater (Forzza et al. 2015). Approximately 60 different species of fungi have been recorded on ferns in Brazil, from which two are cercosporoid (Viégas 1961, Farr & Rossman 2015, Mendes & Urben 2015). In Brazil and elsewhere, ferns have probably been poorly collected because of the lack of economic importance of most species. One exception in the general absence of monographic treatments of fungi on ferns is the recent publications by Braun et al. (2013, 2014, 2015), a series of works aiming at congregating all cercosporoid taxa by host. Braun et al. (2013) redescribed and discussed 44 cercosporoid species occurring on 47 different fern hosts. One of these (*Pseudocercospora davallicolis*) was originally described from Brazil. Such significant morphological revisions based on previously published species, provide a solid foundation to facilitate future DNA phylogenetic studies.

Early results of the survey for plant pathogenic fungi occurring on ferns in Brazil indicated a plethora of novel taxa to exist in this niche. Two of the preliminary findings, namely two taxa in the *Parmulariaceae*, have already been published: the new genus *Rhagadolobiopsis* (Guatimosim et al. 2014a) and the new species *Inocyclus angularis* (Guatimosim et al. 2014b). Similarly, another research group in Asia has been studying fungi on ferns and have recently described the new species *Venustosynnema reniformisporum* and *Zasmidium dicranopteridis* (Kirschner & Liu 2014). Furthermore, the phylogenetic placement of the monotypic class *Mixomycetes* was recently elucidated based on the study of *Mixia osmundae*, which is an intracellular parasite of ferns (Toome et al. 2014).

The present work aims to present part of the results of a broad survey of the mycobiota of ferns in Brazil, with particular reference to the cercosporoid and related fungi which were collected in association with frond spots on members of the *Pteridophyta* collected in Brazil. Additionally, this work aims at partially supplementing the initiative of Braun et al. (2013) with robust DNA data, in order to promote a precise taxonomic classification of the cercosporoid fungi within *Mycosphaerellaceae*. In a recent study, Quaedvlieg et al. (2014) proposed employing a Consolidated Species Concept, aiming to integrate ecology, morphology, cultural characteristics and multilocus DNA phylogenetic data in order to appropriately verify species boundaries. The same approach was adopted in the present publication for the cercosporoids occurring on ferns in Brazil.

## MATERIALS AND METHODS

### *Specimens and isolates*

Frond samples bearing fungal colonies were collected in Brazil from different biomes, including natural ecosystems in the Amazon, the Atlantic rainforest, the Caatinga and the Cerrado, as well as ruderal areas and gardens between 2009 and 2015. These were dried in a plant press and later examined under a dissecting microscope to detect fungal structures. Such fungal structures, preferably spores, were scraped from a single frond spot, and whenever possible, single conidial colonies were established on potato carrot agar (PCA) (Crous et al. 2009e). In the case of ascospores-producing structures being present, excised lesions were placed in distilled water for approximately 2 h, after which they were placed at the bottom of Petri dish lids, over which the plate containing PCA was placed. Ascospore germination patterns were recognised using the different modes of ascospore germination proposed by Crous (1998). Freehand sections of fungal colonies were prepared and fungal structures mounted in clear lactic acid, lactophenol, lactofuchsin, and/or Melzer's reagent. When necessary, sections were made using a Microm HM 520 freezing microtome. Observations were made with a Nikon SMZ1500 stereo-microscope and with a Nikon Eclipse 80i light microscope using differential interference contrast (DIC) illumination and a Nikon DS-Fi1 camera and NIS-Elements imaging software. Colony descriptions were made on 2 % malt extract agar (MEA), potato dextrose agar (PDA), PCA and oatmeal agar (OA) (Crous et al. 2009e), in the dark at 25 °C and under a 12 h light/dark regime. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Representative fungarium specimens were deposited in the Fungarium of the Universidade Federal de Viçosa (VIC) and the Fungarium of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS H). Axenic cultures were deposited in the working collection of P.W. Crous (CPC), housed at CBS, and in the Coleção Octávio de Almeida Drumond (COAD), housed at the Universidade Federal de Viçosa. A complete list of the species and isolates included in this study is presented in Table 1.

### *Scanning electron microscopy*

Samples of dried material containing fungal structures were mounted on stubs with double-sided adhesive tape and gold-coated using a Balzer's FDU 010 sputter coater. A Carl-Zeiss Model LEO VP 1430 scanning electron microscope (SEM) was used to analyse and generate images from the samples.

### *DNA isolation, amplification and sequencing*

Isolates were grown on MEA plates for 20 d at 25 °C. Genomic DNA was extracted from mycelium using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions. The DNA samples were

**Table 1** Collection details and GenBank accession numbers of isolates included in this study. New generated sequences are in **bold**.



Table 1 (cont.)



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Species	Culture accession numbers <sup>1,2</sup>	Host/isolation source	Host family	Country	Collector	GenBank accession numbers <sup>3</sup>			
						ITS	tef1	act	cmdA
<i>Ps. mazandaranensis</i> (cont.)	CCTU 1146	<i>Nerium oleander</i>	Oleaceae	Iran	M. Bakhti	KM452855	KM452877	KM452832	–
	CBS 118795 <sup>ET</sup>	<i>Metrosideros collina</i>	Myrtaceae	New Zealand	C.F. Hill	GU269746	GU384458	GU320448	GU253774
<i>Ps. metrosideri</i>	CBS 111069 = CPC 1263	<i>Eucalyptus nitens</i>	Myrtaceae	South Africa	T. Coutinho	DQ325000	DQ147620	–	DQ267576
<i>Ps. natalensis</i>	CBS 119121 <sup>ET</sup>	<i>Nephrolepis auriculata</i>	Oleandraceae	Taiwan	R. Kirschner	GU269751	GU384462	GU253779	JQ324960
<i>Ps. nephrolepidis</i>	CBS 115022	<i>Chamaecytilis prolifera</i>	Fabaceae	New Zealand	C.F. Hill	GU269752	GU384463	GU320454	JQ324965
<i>Ps. nogalesii</i>	CBS 120738 <sup>ET</sup>	<i>Eucalyptus</i> sp.	Myrtaceae	Italy	W. Gams	GU269753	GU384464	GU320455	GU253780
<i>Ps. norchiensis</i>	CCTU 1009	<i>Rubus</i> sp.	Rosaceae	Iran	M. Bakhti	KM452856	KM452878	KM452833	–
<i>Ps. ocimi-basilici</i>	CCTU 1019	<i>Rubus</i> sp.	Rosaceae	Iran	M. Bakhti	KM452857	KM452879	KM452834	–
<i>Ps. oenotherae</i>	CCTU 1032	<i>Rubus</i> sp.	Rosaceae	Mexico	M. Bakhti	KM452858	KM452880	KM452835	–
<i>Ps. pallobrunnea</i>	CPC 10283 <sup>ET</sup>	<i>Ocimum basilicum</i>	Lamiaceae	South Korea	M.E. Palm	GU269754	GU384465	GU320456	JQ324961
<i>Ps. pallida</i>	CBS 124771 = CPC 10290	<i>Oenothera odorata</i>	Oenotheraceae	South Korea	F.W. Crous	GU269886	GU384567	GU320559	GQ303319
<i>Ps. panicatii</i>	CBS 131889 = CPC 10776	<i>Syzygium</i> sp.	Myrtaceae	South Korea	H.D. Shin	GU269758	GU384509	GU320450	GU214680
<i>Ps. paraguayensis</i>	CBS 137.94	<i>Campsis grandiflora</i>	–	Cuba	R.F. Castaneda	GU269759	GU384470	GU320460	GU253784
<i>Ps. paranaensis</i>	CBS 111286 = CPC 1459	<i>Eucalyptus nitens</i>	Myrtaceae	Brazil	F.W. Crous	DQ267602	DQ11680	DQ147606	GU214479
	CPC 24680 <sup>ET</sup> = COAD 1987	<i>Cyathaea atrovirens</i>	Cyatheaceae	Brazil	R.W. Barreto	<b>KT037522</b>	<b>KT037604</b>	<b>KT037563</b>	<b>KT037564</b>
<i>Ps. parapseudanthriae</i>	CODA 1180	<i>Cyathea atrovirens</i>	Cyatheaceae	Brazil	R.W. Barreto	<b>KT037523</b>	<b>KT037483</b>	<b>KT037605</b>	<b>KJ869208</b>
<i>Ps. pouzolziae</i>	CBS 137996 = CPC 23449 <sup>ET</sup>	<i>Pseudodarthria hookeri</i>	Leguminosae	South Africa	A.R. Wood	KJ869151	KJ869238	KJ869229	KJ869208
<i>Ps. profusa</i>	CBS 122280	<i>Gonostegia hirta</i>	Urticaceae	Taiwan	R. Kirschner	GU269761	GU384472	GU320462	GU253786
<i>Ps. proteae</i>	CPC 10042	<i>Acalypha australis</i>	Euphorbiaceae	South Korea	H.D. Shin	GU269762	GU384473	GU320488	GU253808
<i>Ps. prunicola</i>	CBS 132306 = CPC 10055	<i>Protea mundii</i>	Proteaceae	South Korea	F. Roets	GU269888	GU384519	GU320511	GU253826
<i>Ps. punctata</i>	CBS 131387 = CPC 15217 <sup>ET</sup>	<i>Prunus yedoensis</i>	Rosaceae	South Korea	H.D. Shin	GU269676	GU384393	GU320382	GU253723
<i>Ps. punicea</i>	CBS 132116 = CPC 14734 <sup>ET</sup>	<i>Syzygium</i> sp.	Myrtaceae	Madagascar	F.W. Crous	GU269755	GU384477	GU320468	–
	CBS 136111 = CCTU 1125	<i>Punica granatum</i>	Lythraceae	Iran	M. Bakhti	KM452859	KM452881	KM452836	–
<i>Ps. purpurea</i>	CCTU 1169	<i>Punica granatum</i>	Lythraceae	Iran	M. Bakhti	KM452860	KM452882	KM452837	–
<i>Ps. pyracantha</i>	CBS 114163 = CPC 1664	<i>Persea americana</i>	Lauraceae	Mexico	F.W. Crous	GU269783	GU384494	GU320486	GU253804
<i>Ps. rhabdothamni</i>	MUCC 892	<i>Pyracantha angustifolia</i>	Rosaceae	Japan	T. Kobayashi & C. Nakashima	GU269787	GU384479	GU320479	GU253792
<i>Ps. rhammellae</i>	CBS 114872 <sup>ET</sup>	<i>Rhadidothamnus solandri</i>	Gesneriaceae	New Zealand	M. Fletcher	GU269788	GU384480	GU320471	JQ324964
<i>Ps. rumohrae</i>	CBS 131590 = CPC 12500 <sup>ET</sup>	<i>Rhamnella franguloides</i>	Rhamnaceae	South Korea	H.D. Shin	GU269795	GU384505	GU320496	GU253813
<i>Ps. rubi</i>	CBS 117747	<i>Marrattia salicina</i>	Marattiaceae	New Zealand	C.F. Hill	GU269774	GU384486	GU320477	GU253796
<i>Ps. schizolobii</i>	MUCC 875	<i>Rubus allegheniensis</i>	Rubiaceae	Japan	T. Kobayashi & C. Nakashima	GU269773	GU384485	GU320476	KF25322
<i>Ps. serpocaulonicola</i>	CBS 120029 = CPC 12962 <sup>ET</sup>	<i>Schizolobium parahyba</i>	Polypodiales	Ecuador	M.J. Wingfield	KF253269	KF253268	KF2515826	<b>KT037566</b>
<i>Ps. sphaerocarica</i>	CPC 25077 = COAD 1866 <sup>ET</sup>	<i>Serpocaulon triseriale</i>	Polypodiales	Brazil	R.W. Barreto	<b>KT037485</b>	<b>KT037607</b>	<b>KT037566</b>	<b>KT037562</b>
<i>Ps. sordida</i>	CBS 119098 = CPC 1054	<i>Sophora alopecuroides</i>	Ebenaceae	Iran	M. Bakhti	KM452861	KM452883	KM452838	–
<i>Pseudocercospora</i> sp. A	CBS 136113 = CCTU 1165	<i>Eucalyptus grandis</i>	Bignoniaceae	South Africa	M. Bakhti	GU269777	GU384488	GU320480	GU253798
	CCTU 1166	<i>Phaeolus vulgaris</i>	Fabaceae	Iran	M. Bakhti	GU269778	GU384489	GU320481	GU253799
<i>Pseudocercospora</i> sp. B	CCTU 1066	<i>Phaseolus vulgaris</i>	Ebenaceae	Iran	M. Bakhti	KM452865	KM452887	KM452842	–
	CBS 136114 = CCTU 1206	<i>Diospyros lotus</i>	Ebenaceae	Iran	M. Bakhti	KM452866	KM452888	KM452843	–
<i>Ps. thelypteridis</i>	CPC 246764 = COAD 1985	<i>Thelypteris</i> sp.	Thelypteridaceae	Brazil	R.W. Barreto	KM452867	KM452889	KM452844	<b>KT037562</b>
<i>Ps. trichogena</i>	CPC 246760 = COAD 1088 <sup>ET</sup>	<i>Deplanter petersenii</i>	Athyriaceae	South Korea	H.D. Shin	<b>KT037521</b>	<b>KT037481</b>	<b>KT037603</b>	<b>KT037561</b>
	CBS 131931 = CPC 10799	<i>Macrothelypteris torresiana</i>	Rhamnaceae	Australia	R.W. Barreto	<b>KT037520</b>	<b>KT037480</b>	<b>KT037602</b>	<b>KT037560</b>
<i>Ps. udagawana</i>	CBS 124775 = CPC 13121 <sup>ET</sup>	<i>Hovenia dulcis</i>	Myrsinaceae	Australia	H.D. Shin	<b>KT037519</b>	<b>KT037479</b>	<b>KT037601</b>	<b>KT037561</b>
<i>Pseudoramichloridium henryi</i>	CPC 13122	<i>Corymbia henryi</i>	Corymbieae	Netherlands	A.J. Carnegie	KF901535	KF903227	KF903559	KF901857
<i>Ramularia endophylla</i>	CBS 13265 <sup>ET</sup>	dead leaf of <i>Quercus robur</i>	Fagaceae	Italy	G. Verley	KF901725	KF903246	KF902022	KF901855
<i>R. eucaalypti</i>	CBS 120726 = CPC 13043 <sup>ET</sup>	<i>Eucalyptus grandiflora</i>	Myrtaceae	India	W. Gams	KF901666	KF903241	KF902006	KF901856
<i>Septoria eucalyptorum</i>	CBS 118505 = CPC 11282 <sup>ET</sup>	leaf litter of <i>Eucalyptus</i> sp.	Myrtaceae	India	W. Gams & M. Arzanou	KF901651	KF903265	KF901991	KF901991

<i>Sonderhenia eucalypticola</i>	CPC 11251 CPC 11252 CBS 112502 = CPC 3749 CBS 118910 = CPC 12226 <sup>ET</sup> CBS 120061 = CPC 13055 <sup>ET</sup> CPC 24691 <sup>ET</sup> = COAD 1990 CBS 120735 = CPC 13378 <sup>ET</sup>	Eucalyptus <i>globulus</i> Eucalyptus <i>globulus</i> Eucalyptus sp. Eucalyptus sp. Eucalyptus <i>robusta</i> Eucalyptus sp. Diplazium sp. Eucalyptus <i>camaldulensis</i> × <i>urophylla</i> wine cellar	Spain Spain Spain Myrtaceae Myrtaceae Myrtaceae France Australia Brazil Venezuela	M.J. Wingfield M.J. Wingfield F.W. Crous F.W. Crous E.A. Summerell R.W. Barreto M.J. Wingfield	KF901746 KF901747 KF901677 KF901649 KF901552 <b>KT037542</b> KF901808	KF903266 KF903268 KF903267 KF903269 KF903270 <b>KT037501</b> KF903374	KF903596 KF903597 KF903454 KF901988 KF901874 <b>KT037584</b> KF9033628
<i>Sphaerulina cercidis</i>	CBS 116366 = CPC 10522 = CMW 11730	<i>Zasmidium cellare</i> <i>Z. citri</i>	CBS 146.36 <sup>ET</sup> CBS 116366 = CPC 10522 = CMW 11730	<i>Acacia mangium</i> —	— Thailand	EU041821 KF901780	EU041878 KF902138
<i>Statinwardia suttonii</i>	CPC 15291	<i>Z. cyathaeae</i> <i>Z. eucalyptigenum</i> <i>Z. eucalyptorum</i> <i>Z. pseudoparkii</i>	Citrus sp. Cyathaea <i>delgadii</i> Eucalyptus <i>urophylla</i> Eucalyptus sp. Eucalyptus <i>grandis</i> Eucalyptus <i>grandis</i> Eucalyptus <i>grandis</i> Blechnaceae Eucalyptus <i>grandis</i>	USA Brazil Mozambique Indonesia Colombia Colombia Colombia Brazil Indonesia	— E. Guatimosim M.J. Wingfield M.J. Wingfield M.J. Wingfield M.J. Wingfield M.J. Wingfield M.J. Wingfield R.W. Barreto M.J. Wingfield	KF901793 <b>KT037530</b> KP04458 KF901652 KF901642 KF903273 KF901640 KF901641 <b>KT037540</b> KF901663	KF903676 <b>KT037529</b> KP044486 — KF903101 KF903495 KF903273 KF903271 KF903418 KF903272 <b>KT037528</b> KF903274
<i>Xenomycosphaerella diplozii</i>	CPC 24725 = COAD 1425 <sup>ET</sup> CBS 138860 = CPC 24251 <sup>ET</sup> CBS 118500 = CPC 11174 <sup>ET</sup> CBS 110999 = CPC 1087 <sup>ET</sup> CBS 110988 = CPC 1090 CBS 111049 = CPC 1089 CPC 24679 = COAD 1178 CBS 111185 = CPC 1300 <sup>ET</sup>	<i>Z. elongata</i>	— — — — — — — — —	— — — — — — — — —	— — — — — — — — —	KF902152 <b>KT037571</b> KP044486 — KF901977 KF901975 KF901976 <b>KT037581</b> KF902002	KF902152 <b>KT037571</b> KP044486 — KF901977 KF901975 KF901976 <b>KT037581</b> KF902002

<sup>1</sup> BCRC: Bioresource Collection and Research Center, Hsinchu, Taiwan; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CCTU: Culture Collection of Tabriz University, Tabriz, Iran; CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute, Pretoria, South Africa; COAD: Coleção Octávio de Almeida Drumond, Viçosa, Minas Gerais, Brazil; CPC: Culture collection of Pedro Crous, housed at CBS; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie Prefecture, Japan; RoKI: R. Kirschner, dried specimen deposited in National Museum of Natural Science, Taichung, Taiwan; WAC: Department of Agriculture Western Australia Plant Pathogen Collection, Perth, Australia.

<sup>2</sup> ET: ex-type; EET: ex-epitype.

<sup>3</sup> ITS: internal transcribed spacers and intervening 5.8S rRNA; *tef1*: translation elongation factor 1alpha; *act*: actin; *cmdA*: calmodulin; *LSU*: 28S rRNA gene.

subsequently diluted 50–100 times in preparation for further DNA amplification reactions. Four partial nuclear genes were initially targeted for PCR amplification and sequencing, namely 28S nrRNA gene (LSU), internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrDNA operon, actin (*act*), and translation elongation factor 1- $\alpha$  (*tef1*). Additionally, for the *Cercospora* strains, a part of the calmodulin gene (*cmdA*) was amplified. The primers employed are listed in Table 2. The PCR amplifications were performed in a total volume of 12.5  $\mu$ L solution containing 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 (Bioline GmbH Luckenwalde, Germany). PCR conditions for ITS and LSU were set as follows: an initial denaturation (95 °C; 5 min), 35 cycles amplification (95 °C, 30 s; annealing (Table 2), 30 s; 72 °C, 1 min) and a final extension (72 °C, 6 min). PCR conditions for *tef1* were set as an initial denaturation (94 °C, 5 min), 45 cycles amplification (94 °C, 45 s; annealing (Table 2), 30 s; 72 °C, 90 s) and a final extension (72 °C, 6 min). For *cmdA*, the PCR conditions were set as an initial denaturation (94 °C, 5 min) 45 cycles amplification (94 °C, 24 s; annealing (Table 2) 40 s; 72 °C, 40 s) and a final extension (72 °C, 5 min).

For *act*, a touchdown protocol was used and set as an initial denaturation (94 °C, 5 min), 13 amplification cycles (94 °C, 30 s; 65 °C, 30 s; 72 °C, 30 s); 25 amplification cycles (94 °C, 30 s; 56 °C, 30 s; 72 °C, 30 s) and a final extension (72 °C, 7 min). The resulting fragments were sequenced using the PCR primers and the BigDye® Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems™, Foster City, CA, USA) following the protocol of the manufacturer. DNA sequencing amplicons were purified through Sephadex® G-50 Superfine columns (Sigma Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were run on an ABI Prism 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA, USA).

DNA sequence data were analysed in MEGA (Molecular Evolutionary Genetics Analysis) v. 6.0 (Tamura et al. 2013). Consensus sequences were generated and imported into MEGA v. 6.0 for initial alignment and the construction of sequence datasets. Initially, sequences obtained from the datasets of Schoch et al. (2009, TreeBASE S10245), Groenewald et al. (2013, TreeBASE S13645), Crous et al. (2013a, TreeBASE S12805), from GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and the novel sequences generated during this study, were aligned using MAFFT v. 7 ([mafft.cbrc.jp/alignment/server/index.html](http://mafft.cbrc.jp/alignment/server/index.html); Katoh & Standley 2013) and whenever necessary, manually improved in MEGA v. 6.0. After a preliminary analysis, the datasets were trimmed down to Brazilian isolates and the direct neighbours.

## Phylogenetic analyses

Appropriate gene models were selected using MrModeltest v. 2.3 (Nylander 2004) and applied to each gene partition. Based on the results of MrModeltest, a Bayesian phylogenetic analysis was performed with MrBayes v. 3.2.1 (Ronquist et al. 2012) applying different substitution models for each locus as listed in Table 3. *Sphaerulina cercidis* (CBS 118910) served as outgroup for the phylogenetic analyses of *Cercospora* species, *Passalora eucalypti* (CBS 111318) for *Pseudocercospora* species and *Staninwardia suttonii* (CBS 120061) served as outgroup for the mycosphaerella-like species. Posterior probabilities were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.1. Six simultaneous Markov chains were run for 10 000 000 generations and trees were sampled every 100th generation, until convergence (stopval = 0.01) was reached. A heating parameter ('temp') of 0.30 was used for the *Cercospora* analysis and 0.15 for the *Pseudocercospora* and mycosphaerella-like taxa analyses. Sequences derived in this study were lodged in GenBank, the alignments and trees in

**Table 2** Details of primers used in this study for the PCR amplification and sequencing of different genes.

Gene <sup>1</sup>	Primer name	Sequence 5'→3'	Annealing temperature (°C)	Orientation	Reference
<i>act</i>	ACT-512F	ATG TGC AAG GCC GGT TTC GC	65→56	Forward	Carbon & Kohn 1999
	ACT-783 R	TAC GAG TCC TTC TGG CCC AT	65→56	Reverse	Carbon & Kohn 1999
<i>cmdA</i>	CAL-228F	GAG TTC AAG GAG GCC TTC TCC C	58	Forward	Carbon & Kohn 1999
	CAL-737R	CAT CTT TCT GGC CAT CAT GG	58	Reverse	Carbon & Kohn 1999
ITS	ITS5	GGA AGT AAA AGT CGT AAC AAG G	52	Forward	White et al. 1990
	ITS4	TCC TCC GCT TAT TGA TAT GC	52	Reverse	White et al. 1990
LSU	LR0R	ACC CGC TGA ACT TAA GC	52	Forward	Vilgalys & Hester 1990
	LR5	TCC TGA GGG AAA CTT CG	52	Reverse	Vilgalys & Hester 1990
<i>tef1</i>	EF-728F	CAT CGA GAA GTT CGA GAA GG	52	Forward	Carbon & Kohn 1999
	EF2Fd	GAT CTA CCA GTG CGG TGG	52	Forward	Groenewald et al. 2013
	EF-2	GGA RGT ACC AGT SAT CAT GTT	52	Reverse	O'Donnell et al. 1998

<sup>1</sup> *act*: actin gene; *cmdA*: calmodulin gene; ITS: internal transcribed spacer regions and intervening 5.8S nrRNA gene of the nrDNA operon; LSU: 28S nrRNA gene; *tef1*: translation elongation factor 1-α.

**Table 3** Substitution models applied to the different phylogenetic analyses performed in this study.

	Locus <sup>1</sup>				
	ITS	<i>tef1</i>	<i>act</i>	<i>cmdA</i>	LSU
<i>Cercospora</i> spp.	SYM+I	HKY+G	K80+G	HKY+I+G	
<i>Pseudocercospora</i> spp.	SYM+G	HKY+I+G	SYM+I+G		
mycosphaerella-like spp.	GTR+I+G	HKY+I+G	HKY+I+G		GTR+I+G

<sup>1</sup> Substitution models used in the studies. GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; K80: Kimura 2-parameter; SYM: symmetrical model; Non-uniformity of evolutionary rates among sites were modeled by using a discrete Gamma distribution (+G) alone and with five rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+I).

TreeBASE (<http://www.treebase.org>; S17948), and taxonomic novelties in MycoBank ([www.Mycobank.org](http://www.Mycobank.org); Crous et al. 2004a).

## RESULTS

### Phylogenetic analyses

The three datasets consisted of 1 265 characters, representing 92 taxa for the *Cercospora* tree, including the outgroup (*act*: 183, *tef1*: 315, ITS: 476 and *cmdA*: 291), 1 114 characters, representing 94 taxa for the *Pseudocercospora* tree, including the outgroup (*act*: 217, *tef1*: 394 and ITS: 503) and 1 944 characters, representing 84 taxa for the mycosphaerella-like tree, including the outgroup (*act*: 232, *tef1*: 435, ITS: 507 and LSU: 758).

The respective alignments included 351 unique site patterns for the *Cercospora* tree (*act*: 76, *tef1*: 125, ITS: 41 and *cmdA*: 109), 351 unique site patterns for the *Pseudocercospora* tree (*act*: 79, *tef1*: 200 and ITS: 72) and 723 unique site patterns for the mycosphaerella-like tree (*act*: 127, *tef1*: 226, ITS: 221 and LSU: 149).

After topological convergence of the Bayesian runs, the following numbers of trees were generated and subsequently sampled (using a burn-in fraction of 0.25 and indicated after the slash) in order to generate the three Bayesian phylogenies: 2948/2140 for *Cercospora* (Fig. 1), 4465/3572 for *Pseudocercospora* (Fig. 2) and 1710/1368 for mycosphaerella-like taxa (Fig. 3). The resulting phylogenetic trees of all three individual combined datasets showed consistent clustering of all taxa over each one of the trees, and the results are treated below. Bayesian posterior probabilities (PP) are presented on the left of each node, on each tree.

## TAXONOMY

The Consolidated Species Concept was employed in this study to distinguish species, revealing a rich diversity among the cercosporoid fungi on ferns in Brazil. Forty-three isolates of cer-

cosporoid and mycosphaerella-like species, collected from 18 host species representing 201 localities, were studied. The Bayesian analysis resulted in a total of 20 frond-spotting taxa, which belong to eight genera including *Cercospora*, *Clypeosphaerella*, *Neoceratosperma*, *Paramycosphaerella*, *Phaeophleospora*, *Pseudocercospora*, *Xenomycosphaerella* and *Zasmidium*. Three of these were assigned to an existing species name, one more could not be named unequivocally, a further 15 were described as new, and one novel species, as well as one new genus, are introduced below for the remaining taxon.

### *Cercospora* Fresen., Beitr. Mykol. 3: 91. 1863

#### *Cercospora coniogrammes* Crous & R.G. Shivas, Stud. Mycol. 75: 151. 2013 — Fig. 4

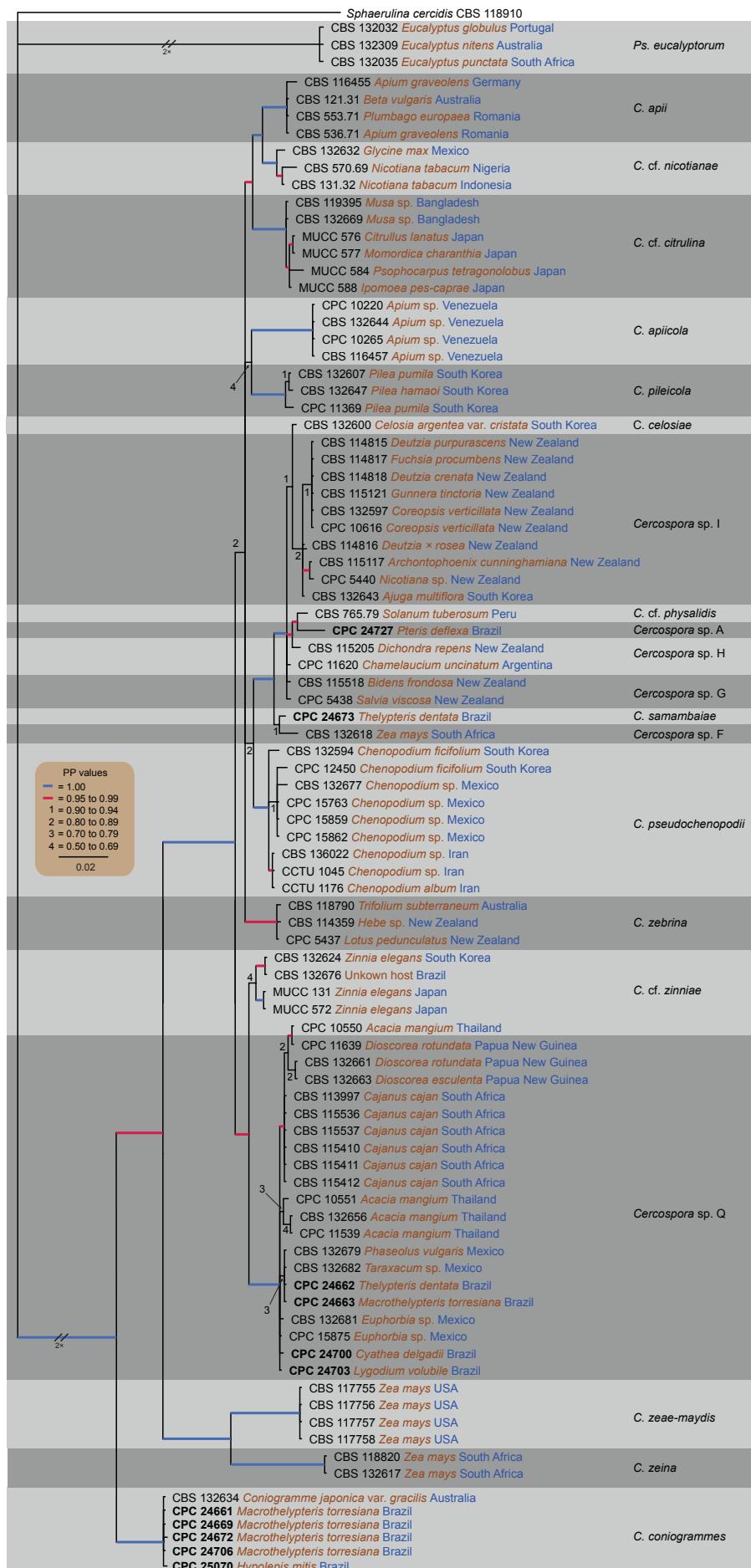
Description & Illustration — Groenewald et al. (2013).

*Specimens examined.* BRAZIL, Rio de Janeiro, Nova Friburgo, Fazenda Barreto II, garden, on fronds of *Macrothelypteris torresiana*, 7 Aug. 2010, R.W. Barreto (VIC 42537, CBS H-22063, cultures CPC 24661, COAD 1067); Rio de Janeiro, Nova Friburgo, Alto do Micheis, Riograndina, reforestation area, on fronds of *M. torresiana*, 13 June 2011, R.W. Barreto (VIC 42545, CBS H-22064, cultures CPC 24669, COAD 1093); Rio de Janeiro, Gávea, Atlantic rainforest, on fronds of *M. torresiana*, 12 Oct. 2011, R.W. Barreto (VIC 42554, CBS H-22065, cultures CPC 24672, COAD 1089); Minas Gerais, Araponga, Pedra Dourada, Atlantic rainforest, on fronds of *M. torresiana*, 19 Nov. 2011, E. Guatimosim (VIC 42464, CBS H-22073, cultures CPC 24706); Rio de Janeiro, Nova Friburgo, Macaé de Cima, roadside, on fronds of *Hypolepis mitis*, 10 May 2014, R.W. Barreto (cultures CPC 25070, COAD 1769).

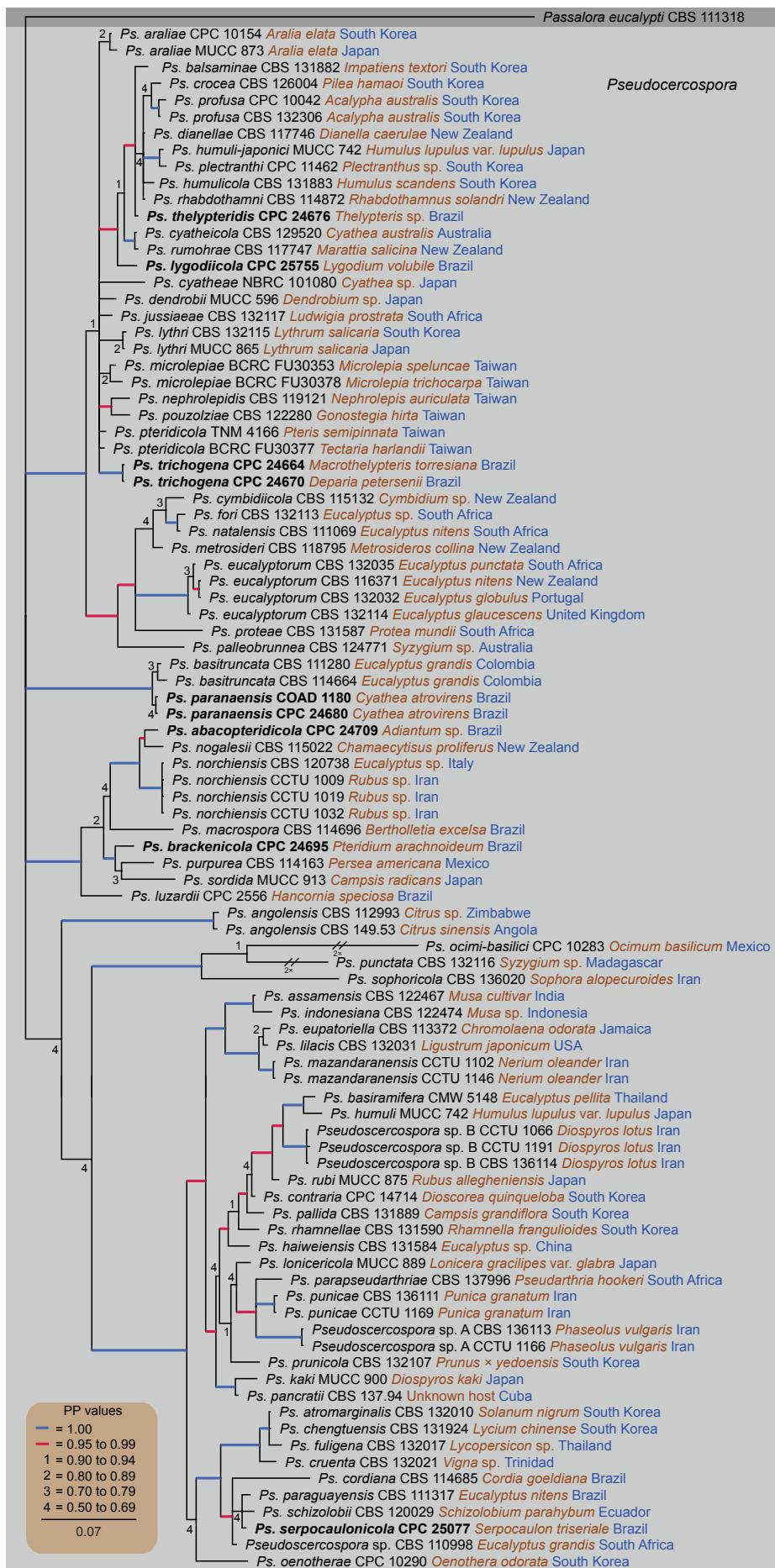
#### *Cercospora samambaiae* Guatimosim, R.W. Barreto & Crous, sp. nov. — MycoBank MB812771; Fig. 5

*Etymology.* Name refers to the common name used for ferns in Brazil, or of native Indian Tupi language origin – samambaia.

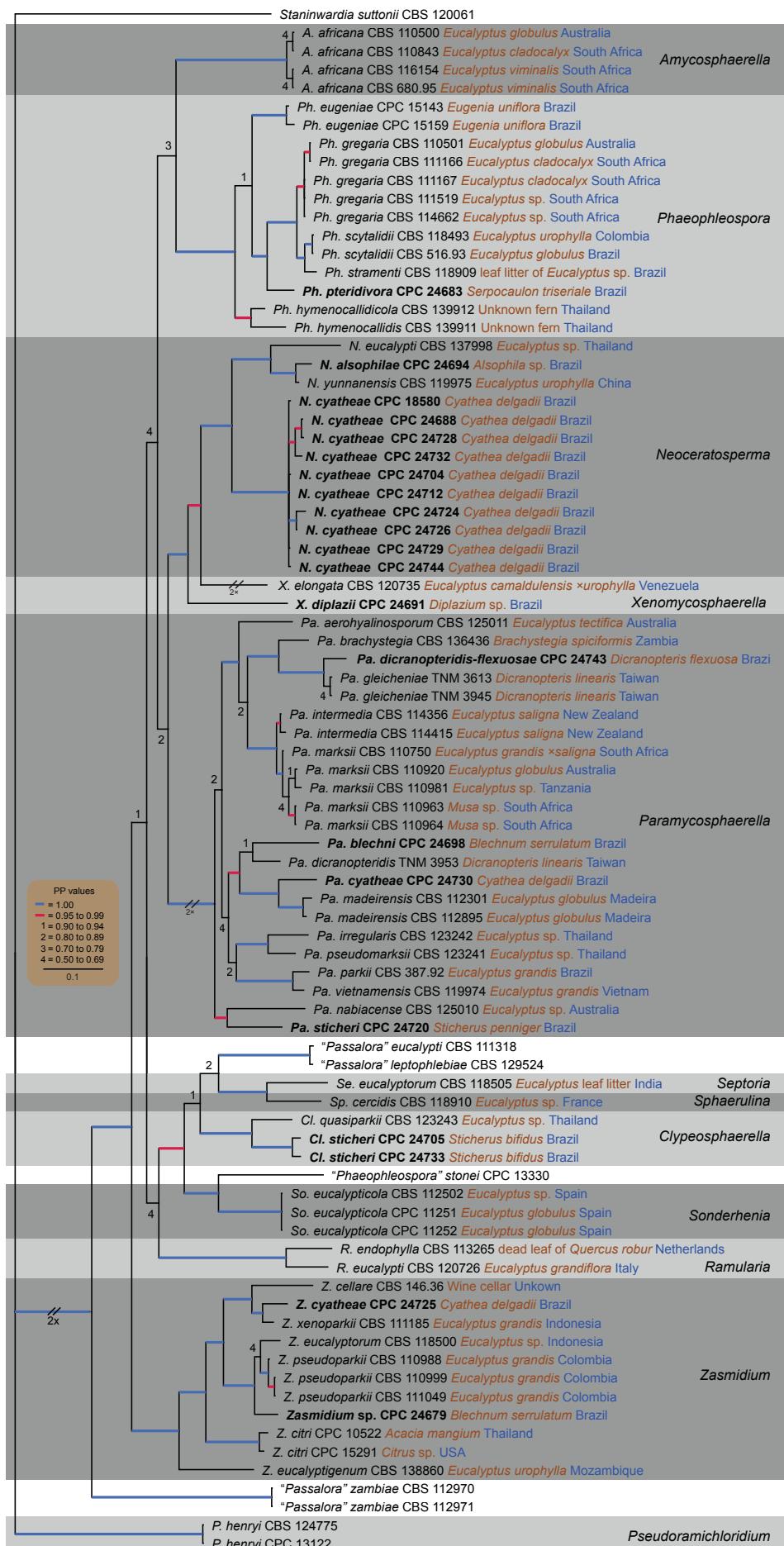
Description in planta — *Frond spots* irregular, starting on the edges of the pinnulets, extending to encompass whole pinnulets and sometimes leading to the necrosis of the entire pinnule. Starting centrally, pale brown, becoming pale brown to red at the edges, coalescing, turning dark brown to black. *Caespituli*



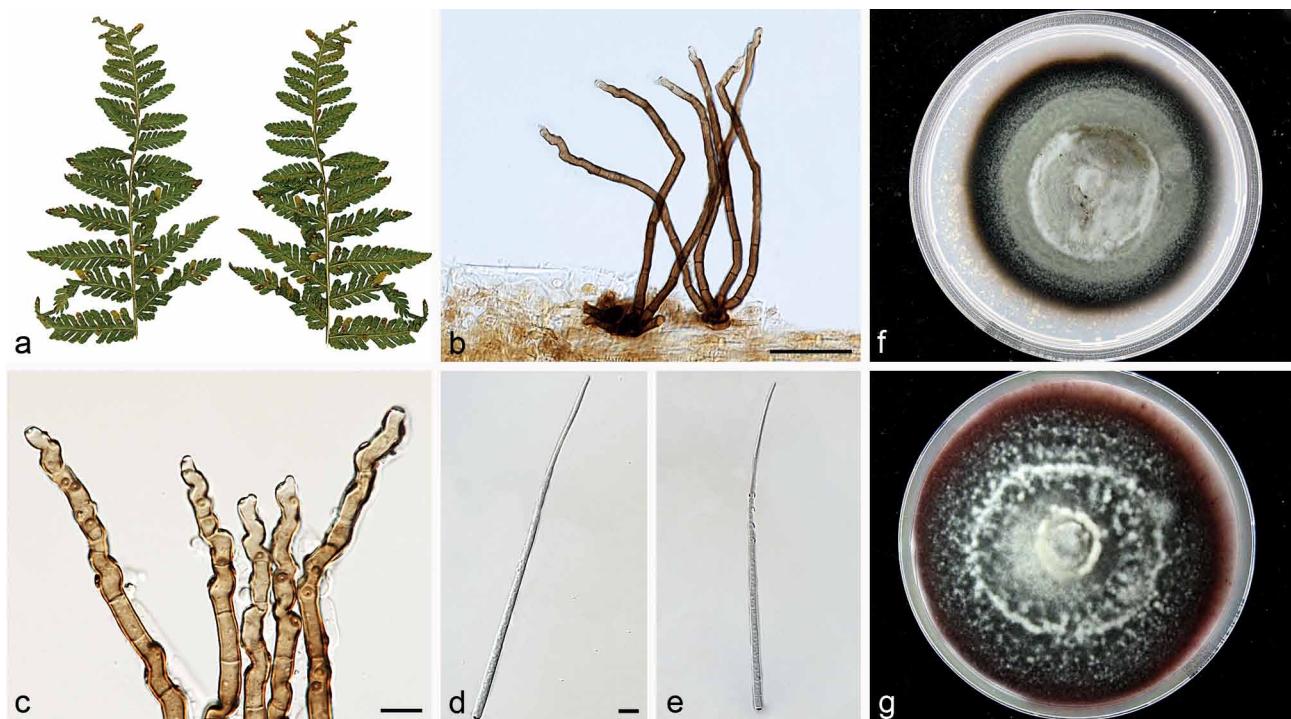
**Fig. 1** Consensus phylogram (50 % majority rule) of *Cercospora* species, from a Bayesian analysis of the combined 4-gene sequence alignment (ITS, *tef1*, *act*, *cmdA*). Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend) and the scale bar indicates 0.02 expected changes per site. Isolates from Brazil are indicated in **bold**. Hosts and countries of origin are indicated in brown and blue text, respectively. The tree was rooted to *Sphaerulina cercidis* (isolate CBS 118910).



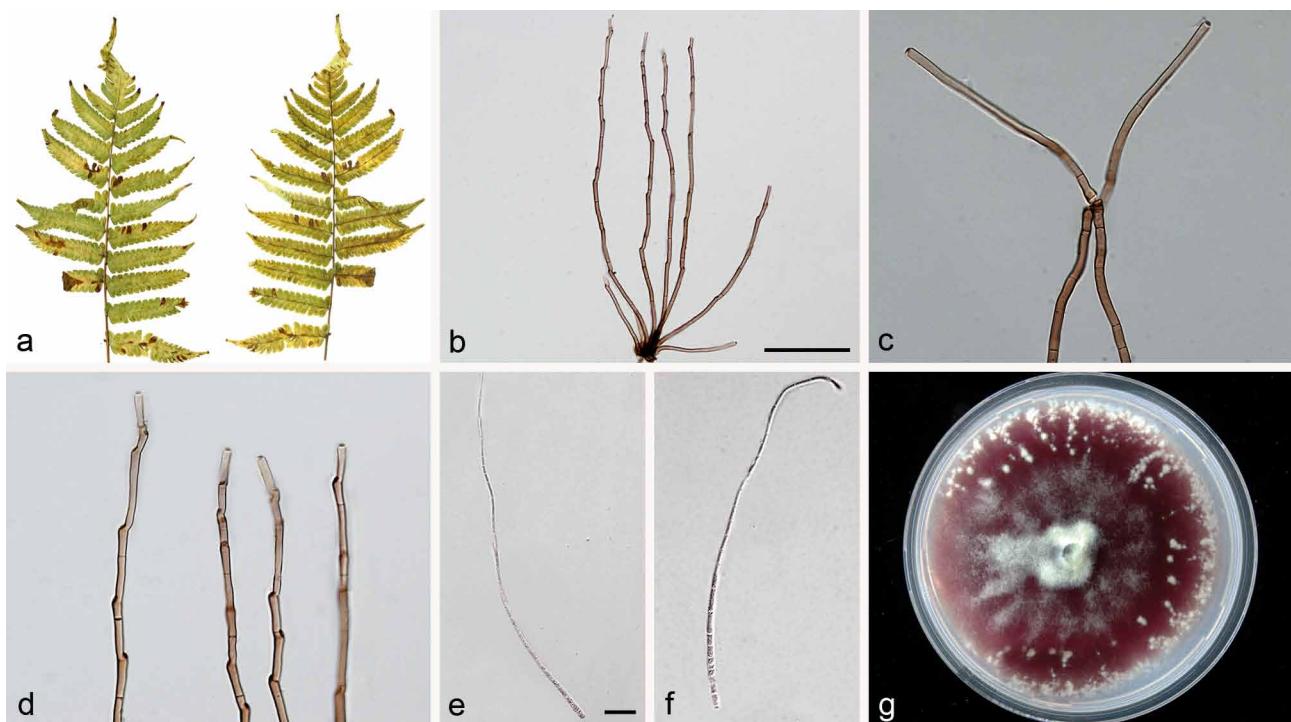
**Fig. 2** Consensus phylogram (50 % majority rule) of *Pseudocercospora* species, from a Bayesian analysis of the combined 3-gene sequence alignment (ITS, *act*, *tef1*). Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend). The scale bar indicates 0.07 expected changes per site. Isolates from Brazil are indicated in **bold**. Hosts and countries of origin are indicated in brown and blue text, respectively. The tree was rooted to *Passalora eucalypti* (isolate CBS 111318).



**Fig. 3** Consensus phylogram (50 % majority rule) of mycosphaerella-like species, from a Bayesian analysis of the combined 4-gene sequence alignment (*act*, *tef1*, ITS, LSU). Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend). The scale bar indicates 0.1 expected changes per site. Isolates from Brazil are indicated in **bold**. Hosts and countries of origin are indicated in brown and blue text, respectively. The tree was rooted to *Staninwardia suttonii* (isolate CBS 120061).



**Fig. 4** *Cercospora coniogrammes* (CPC 24661). a. Frond spots on *Marcothelypteris torresiana*; b. c. conidiophores; d. e. conidia; f. culture on PDA; g. culture on PCA. — Scale bars: b = 50 µm; c, d = 10 µm.



**Fig. 5** *Cercospora samambaiae* (CPC 24673). a. Frond spots on *Thelypteris dentata*; b-d. conidiophores; e. f. conidia; g. culture on PCA. — Scale bars: b = 100 µm; e = 50 µm.

hypophylloous, abundant. External hyphae absent. Internal hyphae indistinct. Stromata rudimentary, irregular, composed of *textura globulosa*, dark brown. Conidiophores rising through the stomata, hypophylloous, forming fascicles (6–11 stalks per fascicle), subcylindrical, straight to curved, geniculate, (92) 140–320(–509) × 5–6 µm, unbranched, 3–15-septate, guttulate, pale brown becoming paler at the apex, smooth. Conidigenous cells terminal, integrated, holoblastic, subcylindrical, predominantly sympodial, 40–95 × 4–6 µm, pale to olivaceous brown, scars conspicuous, 1–3 per cell, 1.5–4 µm, thickened, darkened. Conidia solitary, acicular, straight to slightly curved, (134)–218–282(–320) × 2–3 µm, apex acute, base subtrun-

cate, 2.5–4.5 µm diam at the base, (13)–16–21(–34)-septate, guttulate, hyaline, smooth; hila thickened, darkened, refractive, 2–4 µm diam.

Culture characteristics — Colonies on PCA slow-growing, 80 mm diam after 28 d; flat, with sparse aerial mycelium, mouse grey centrally, lavender grey to white at periphery, pigmenting the medium to livid red; reverse livid red.

Specimens examined. BRAZIL, Minas Gerais, Itabirito, Posto Esperança, garden, on fronds of *Thelypteris dentata*, 23 Oct. 2011, R.W. Barreto (holotype CBS H-22071, isotype VIC 42555, cultures ex-type CPC 24673, COAD 1090).

**Notes** — In the *tef1*, and *cmdA* phylogeny, isolates of *C. samambaiae* and *Cercospora* sp. F (sensu Groenewald et al. 2013) cluster together in a distinct well-supported clade. In the *act* phylogeny, *C. samambaiae* forms a distinct clade, whereas *Cercospora* sp. F cannot be distinguished from *Cercospora* sp. Q (sensu Groenewald et al. 2013), nor from *C. coniogrammes* (data not shown). The different *act* sequences explain the basal position of *Cercospora* sp. F to the *C. samambaiae* clade in the combined phylogeny (Fig. 1). Two *Cercospora* species are known to cause frond spots on species of *Thelypteridaceae*, namely *C. abacopteridis* and *C. cyclosori*. *Cercospora abacopteridis* is morphologically quite distinct from *C. samambaiae* in having much smaller and narrower conidiophores ( $15\text{--}120 \times 4\text{--}5 \mu\text{m}$ ), rising directly from the internal hyphae. Additionally, *C. abacopteridis* is only known from Singapore, causing leaf spots on *Abacopteris urophylla* (Braun et al. 2013). *Cercospora cyclosori*, described on *Cyclosorus* spp. from India and Taiwan, is even more distinct from *C. samambaiae* in having shorter and wider conidia ( $50\text{--}110 \times 3\text{--}4 \mu\text{m}$ ) and shorter and narrower conidiophores ( $25\text{--}160 \times 4\text{--}5 \mu\text{m}$ ) (Braun et al. 2013).

### *Cercospora* sp. A

**Culture characteristics** — Colonies on PCA slow-growing, 60 mm diam after 28 d; flat, with sparse aerial mycelium, pale mouse grey centrally, mouse grey to olivaceous grey at periphery; reverse leaden black.

**Specimens examined.** BRAZIL, Paraná, Curitiba, BR 116 road to Rio Negro, roadside, on fronds of *Pteris deflexa*, 14 Apr. 2013, E. Guatimosim (CBS H-22070, VIC 42529, cultures CPC 24727, COAD 1427).

**Notes** — Fungarium specimens of this fungus were in poor condition and no conidia were seen. Isolation was performed by conidiophore transfer only. Phylogenetically, this specimen has *C. cf. physalidis* (CBS 765.79) as sister clade (Fig. 1), but differs from the latter by having the following number of variable sites: 11 for *act*, 5 for *cmdA* and 1 for *tef1*. Once no conidia were seen and all attempts to promote sporulation in vitro proved to be unsuccessful, it is not possible to determine the species boundaries of this isolate.

### *Cercospora* sp. Q sensu Groenewald et al. (2013) — Fig. 6

**Description in planta** — *Frond spots* amphigenous, irregular, starting at the apex of the pinnulets, spreading to the base of the pinnule, coalescing, leading to complete necrosis of the pinnule. *Caespituli* hypophylloous, abundant. *Internal hyphae* septate, intra- and intercellular, frequently branched, 2–4  $\mu\text{m}$  wide, pale brown, smooth. *Stromata* rudimentary, globular, composed of *textura globulosa*, dark brown. *Conidiophores* rising through the stomata, hypophylloous, forming loose fascicles (3–7 stalks per fascicle), subcylindrical, straight or slightly curved to sinuose, geniculate, (96–)141–230(–326)  $\times$  4–5  $\mu\text{m}$ , unbranched, 3–9-septate, olivaceous brown, thin-walled, smooth. *Conidiogenous cells* terminal, rarely integrated, holo-



**Fig. 6** *Cercospora* sp. Q (CPC 24662). a. Frond spots on *Lygodium volubile*; b. frond spots on *Cyathea delgadilii*; c. frond spots on *Thelypteris dentata*; d. e. sporulation on the pinnule; f–h. conidiophores; i–m. conidia. — Scale bars: f = 10  $\mu\text{m}$ ; h = 50  $\mu\text{m}$ ; i = 15  $\mu\text{m}$ .

blastic, subcylindrical, tapering to a flat-tipped apex, with numerous tightly aggregated apical conidiogenous loci, proliferating sympodially, (26–)38–71(–102) × 4–5 µm, pale brown, smooth, scars conspicuous, protruding, 2.5–4 µm diam, thickened, darkened. *Conidia* solitary, acicular, sinuous to slightly curved, (142–)192–256(–303) × 2–3 µm, apex acute, base subtruncate, (10–)18–28(–31)-septate, rarely guttulate, hyaline, thin-walled, smooth; hila thickened, darkened, refractive, 2–4 µm diam.

*Specimens examined.* BRAZIL, Minas Gerais, Viçosa, Sítio Cristais, from a garden, on fronds of *Thelypteris dentata*, 10 May 2011, R.W. Barreto (CBS H-22067, VIC 42538, cultures CPC 24662, COAD 630); Rio de Janeiro, Nova Friburgo, Alto do Micheis, Riograndina, reforestation area, on fronds of *M. torresiana*, 13 June 2011, R.W. Barreto (CBS H-22068, VIC 42540, cultures CPC 24663, COAD 322); Goiás, Pirenópolis, Fazenda Bom Sucesso, Cerrado biome, on fronds of *Cyathea delgadii*, 26 Sept. 2013, R.W. Barreto

(CBS H-22069, VIC 42601, cultures CPC 24700, COAD 1418); Minas Gerais, Viçosa, Sítio Cristais, from a garden, on fronds of *Lygodium volubile*, 4 Feb. 2014, R.W. Barreto (CBS H-22066, culture CPC 24703).

**Notes** — Four Brazilian isolates, from different hosts and families, cluster within this clade, to which different names can be applied. As stated by Groenewald et al. (2013) and Bakhshi et al. (2015), to resolve their taxonomy, fresh collections authentic for the names, based on host and country, need to be recollected and included in future studies. Morphologically, the isolates from Brazil are indistinguishable from *C. apii*, but the hosts on which they cause disease are significantly different, e.g. all isolates included in *Cercospora* sp. Q so far, were obtained from angiosperms, while the Brazilian isolates in this study, are from three different orders of *Pteridophyta*, (*Cyatheales*, *Polypodiales* and *Schizaeales*). Phylogenetically,



**Fig. 7** *Clypeosphaerella sticheri* (CPC 24705). a–c. Frond spots on *Sticherus bifidus*; d. erumpent subcuticular ascomata, fruiting epiphyllous; e, f. vertical section of the ascoma, note the thicker upper part of the ascoma, resembling a pseudoclypeus; g, h. ascospores; i. germinating ascospores; k. culture on MEA; l. culture on OA; m. culture on PDA. — Scale bars = 10 µm.

the isolates included in *Cercospora* sp. Q clade differ from the other species by their position in the *cmdA* and *tef1* phylogeny; while in the *act* phylogeny they cannot be distinguished from *Cercospora* sp. F (data not shown). Based on the genes studied here, and five other different loci studied by Groenewald et al. (2013), the species boundaries of all isolates included in this clade could not be clarified.

***Clypeosphaerella*** Guatimosim, R.W. Barreto & Crous, *gen. nov.*  
— MycoBank MB812820

Type species. *Clypeosphaerella sticheri* Guatimosim, R.W. Barreto & Crous.

**Etymology.** Named after the thickened wall of the ascocarps, resembling a *pseudoclypeus*.

Frondicolous, plant pathogenic. *Ascomata* pseudothelial, epiphyllous, solitary, subcuticular to erumpent, globose, walls of 2–3 layers of brown to dark brown *textura angularis*, ostiole central. Asci bituncate, apophysate, fasciculate, subsessile, 8-spored, obpyriform to ovoid, hyaline, smooth. Ascospores inordinate, overlapping, fusoid, straight, 1-septate, slightly constricted at the septum, biguttulate, hyaline, thin-walled, smooth. Ascospores germinating at both ends, remaining hyaline, germ tubes following the main axis of the spore.

Notes — *Clypeosphaerella* is morphologically similar to species of *Mycosphaerella* s.lat., differing by having the thicker upper wall of the ascocarps, resembling a *pseudoclypeus*. Additionally, the former genus is phylogenetically distinct from other mycosphaerella-like fungi (Fig. 3).

***Clypeosphaerella quasiparkii*** (Cheew. et al.) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812821

Basionym. *Mycosphaerella quasiparkii* Cheew. et al., Persoonia 21: 85. 2008.

Description & Illustration — Cheewangkoon et al. (2008).

Specimen examined. THAILAND, Buriram, on leaves of *Eucalyptus* sp., July 2007, P. Suwannawong (holotype CBS H-20132, cultures ex-type CBS 123243, CPC 15433, CPC 15434).

***Clypeosphaerella sticheri*** Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812822; Fig. 7

**Etymology.** Name refers to the host genus from which it was isolated, *Sticherus*.

Description in planta — *Frond spots* epiphyllous, affecting almost all the pinnulets, starting as small dark brown areas, irregular, usually close to the main vein of the pinnae, spreading through the pinnulet, becoming fertile, confluent and necrotic. Internal hyphae intra- and intercellular, 1.5–3 µm wide, branched, septate, subhyaline, smooth. *Ascomata* pseudothelial, epiphyllous, mostly congregated at the basis of the pinnae, solitary, subcuticular to erumpent, globose, 40–71 × 43–83 µm, walls of 2–3 layers of brown to dark brown *textura angularis*, cells 4–8 × 1.5–5 µm, ostiole central, 10–24 µm diam. Asci bituncate, apophysate, fasciculate, subsessile, 8-spored, obpyriform to ovoid, straight or slightly curved, 20–34 × 10–14 µm, hyaline, smooth. Ascospores inordinate, overlapping, fusoid, straight, 9–13 × 2–4 µm, 1-septate, slightly constricted at the septum, tapering towards rounded ends, narrower towards the lower end, biguttulate, hyaline, thin-walled, smooth. Ascospores germinating at both ends, remaining hyaline, germ tubes following the main axis of the spore, while the spore becomes distorted and constricted at the septum (Type F, Crous 1998). Asexual morph not known.

Culture characteristics — Colonies on MEA slow-growing, 22 mm diam after 24 d; raised, aerial mycelium velvety, laven-

der grey centrally and pale vinaceous at periphery, vinaceous buff reverse. On OA, aerial mycelium sparse, mouse grey centrally, buff periphery; dark mouse grey with rosy buff periphery reverse. On PDA pale mouse grey centrally, white periphery; smoke with rosy buff periphery reverse; cultures sterile.

**Specimens examined.** BRAZIL, Rio de Janeiro, Nova Friburgo, Fazenda Barreto II, Riograndina, ruderal, on fronds of *Sticherus bifidus*, 11 Feb. 2014, R.W. Barreto (holotype CBS H-22088, isotype VIC 42607, culture ex-type CPC 24705); Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, path to Pico do Pato, Atlantic rainforest, on fronds of *S. bifidus*, 21 Feb. 2014, E. Guatimosim (CBS H-22089, VIC 42516, culture CPC 24733).

Notes — Morphologically, *Cl. sticheri* is most similar to *Cl. quasiparkii* described on *Eucalyptus* sp. from Thailand (Cheewangkoon et al. 2008), but can be distinguished from it by having smaller and wider asci (45–50 × 8.5–9 µm in the latter), larger ascospores (10–11 × 3–3.5 µm in the latter) and by the germination of the ascospores – following the main axis, regular in width, not distorted in *Cl. sticheri* (Type F, Crous 1998) whereas in *Cl. quasiparkii* germ tubes arise from the polar ends, develop firstly parallel to the main axis, and later grow perpendicularly, becoming distorted (Type D, Crous 1998) (Cheewangkoon et al. 2008). Additionally, it is also phylogenetically distinct (Fig. 3).

***Neoceratosperma*** Crous & Cheew., Persoonia 32: 255. 2014  
— MycoBank MB808935

Notes — *Neoceratosperma* has thus far been known only from its type species, *N. eucalypti*, isolated on *Eucalyptus* sp. (Myrtaceae) from Thailand (Crous et al. 2014). *Neoceratosperma eucalypti* is asexual and zasmidium-like in morphology. In the present study, we expanded the generic concept by including three additional species, two of which are known from their sexual morphs, being mycosphaerella-like in morphology.

***Neoceratosperma alsophilae*** Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812816; Fig. 8

**Etymology.** Name refers to the host genus from which it was isolated, *Alsophila*.

Description in planta — *Frond spots* random on pinnules, amphigenous, irregular, initially pale brown with cream central area at the tips the pinnulets, spreading through the base of the pinnulet, becoming necrotic with a fertile cream to pale brown centre and distinct dark brown to black halo. Internal hyphae intra- and intercellular, 1.5–3 µm wide, septate, branched, subhyaline, smooth. External hyphae absent. *Ascomata* pseudothelial, epiphyllous, solitary, subcuticular to erumpent, globose, 61–91 × 64–112 µm, walls of 2–3 layers of pale to dark brown *textura angularis*, cells 5–8 × 3–5 µm, ostiole central, 17–32 µm diam. Asci bituncate, apophysate, fasciculate, subsessile, 8-spored, obovoid to broadly ellipsoidal, straight or slightly curved, 29–42 × 9–18 µm, hyaline, smooth. Ascospores inordinate, overlapping, fusoid, straight or slightly curved, 10–17 × 2–4 µm, medianly 1-septate, wider in middle of apical cell, tapering toward rounded ends, biguttulate, hyaline, thin-walled, smooth. Asexual morph not known.

Culture characteristics — Colonies on MEA, slow-growing, 26 mm diam after 24 d; centrally raised, with lobate, smooth margins, aerial mycelium velvety, olivaceous grey centrally, and mouse grey in the outer region; leaden black in reverse. On OA, colony radially striate with lobate margins, aerial mycelium cottony, pale mouse grey centrally and mouse greenish grey in the outer region; leaden black in reverse. On PDA colony centrally elevated, aerial mycelium sparse to absent, mouse grey centrally and producing a black halo in the outer region; leaden black in reverse; cultures sterile.



**Fig. 8** *Neoceratosperma alsophilae* (CPC 24694). a, b. Frond spots on *Alsophila* sp.; c, d. erumpent subcuticular ascomata, fruiting epiphyllous; e, f. vertical section of the ascoma; g. ascospores; h. ascospores; i. culture on MEA; j. culture on OA; k. culture on PDA. — Scale bars = 10 µm.

**Specimens examined.** BRAZIL, Minas Gerais, Capitólio, Furnas, roadside next to Rio do Turvo Inn, on fronds of *Alsophila* sp., 9 Nov. 2012, E. Guatimosim (holotype CBS H-22075, isotype VIC 42586, cultures ex-type CPC 24694, COAD 1181).

**Notes** — Morphologically and phylogenetically, *N. alsophilae* is closely related to *N. yunnanensis* described on *Eucalyptus urophylla*, restricted to the southwest of China (Burgess et al. 2007). It can be distinguished from *N. yunnanensis* by having narrower, obclavate to broadly ellipsoidal ascospores (10–12.5 × 2.5–3 µm in *N. yunnanensis*) and ascospores (10–12.5 × 2.5–3 µm in *N. yunnanensis*). Moreover, *N. yunnanensis* is phylogenetically distinct from *N. alsophilae* (Fig. 3).

#### ***Neoceratosperma cyatheae* Guatimosim, R.W. Barreto & Crous, sp. nov. — MycoBank MB812817; Fig. 9**

**Etymology.** Name refers to the host genus from which it was isolated, *Cyathea*.

**Description in planta** — *Frond spots* random on pinnulets, amphigenous, irregular to angular, starting on the edges of the pinnulets and spreading along the centre, 3–9 × 3–5 mm, leading to entire pinnule necrosis and, at the final stages, the entire pinnae being affected. Becoming chlorotic (under high humidity conditions), sometimes leading to complete necrosis of the pinnae tip, together with distinct cinnamon to yellow-brown areas, appearing at the pinnae bases. *Internal hyphae* intra- and intercellular, 2–3 µm wide, septate, branched, subhyaline

to pale brown, smooth. *External hyphae* hypophyllous, arising through stomata and covering the entire lesion, 2–3 µm wide, septate, branched, pale brown to brown, strongly verruculose. *Conidiophores* arising singly from superficial hyphae, reduced to conidiogenous cells obtuse, straight, proliferating sympodially, 4–19 × 2–6 µm, unbranched, aseptate, pale brown, smooth, scars conspicuous, several per cell, terminal, crowded, darkened, thickened. *Conidia* solitary, subcylindrical, straight, curved or sinuous, (40)–95–160(–280) × 3–5 µm, apex obtuse, base subtruncate, distoseptate when young, indistinctly 5–19-septate at maturity, strongly guttulate, pale to dark brown, strongly verruculose; hila 1–3 µm wide, thickened, darkened and refractive. *Sexual morph* not known.

**Culture characteristics** — Colonies on MEA and OA slow-growing, 20 mm diam after 24 d; raised, with lobate, feathery margins and velvety aerial mycelium, lavender grey centrally, leaden black mixed with lavender grey areas at periphery; iron-grey reverse. On PDA, colony humid centrally, pale mouse grey centrally, mouse grey periphery; green-black reverse; cultures sterile.

**Specimens examined.** BRAZIL, Rio de Janeiro, Fazenda Barreto II, Rio-grandina, on fronds of *C. delgadii*, 11 Feb. 2014, R.W. Barreto (holotype CBS H-22074, isotype VIC 42605, culture ex-type CPC 24704); Rio de Janeiro, Nova Friburgo, Macaé de Cima, on fronds of *C. delgadii*, 11 July 2009, R.W. Barreto (CBS H-22078, VIC 42533, cultures CPC 18580, COAD 573); Rio Grande do Sul, Ituporanga, highway to Alfredo Wagner, roadside, on fronds of *C. delgadii*, 15 Apr. 2013, E. Guatimosim (CBS H-22083, VIC 42520, cultures CPC 24729, COAD 1428); São Paulo, Eldorado, vicinities of



**Fig. 9** *Neoceratosperma cyatheae* (CPC 24704). a, b. Frond spots on *Cyathea delgadii*; c. SEM of the conidia and conidiophore, note the smooth conidiophore reduced to conidiogenous cell; d. detail of the external hyphae arising through the stoma; e. conidiophores arising through hyphae, reduced to conidiogenous cells; f–k. conidia; l. culture on MEA; m. culture on OA; n. culture on PDA. — Scale bars = 10 µm.

Parque Caverna do Diabo, Atlantic rainforest, on fronds of *C. delgadii*, 13 Apr. 2013, E. Guatimosim (CBS H-22084, culture CPC 24724); São Paulo, Barra do Turvo, highway Regis Bitencourt, roadside, on fronds of *C. delgadii*, 13 Apr. 2013, E. Guatimosim (CBS H-22081, VIC 42527, culture CPC 24726); São Paulo, Iporanga, highway to Barra do Turvo, roadside, 13 Apr. 2013, E. Guatimosim (CBS H-22082, VIC 42530, cultures CPC 24728); Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, Atlantic rainforest, on fronds of *C. delgadii*, 21 Feb. 2014, E. Guatimosim (CBS H-22080, VIC 42524, culture CPC 24732); ibid., 23 Feb. 2014, E. Guatimosim (CBS H-22079, VIC 42461, culture CPC 24744); Rio de Janeiro, road between Macaé de Cima and Lumiar, riverside, on fronds of *C. delgadii*, 29 Apr. 2012, R.W. Barreto (CBS H-22077, VIC 42578, cultures CPC 24688, COAD 1238); Rio Grande do Sul, Ituporanga, highway to Rio do Sul, roadside, on fronds of *C. delgadii*, 15 Apr. 2013, E. Guatimosim (CBS H-22085, VIC 42477, culture CPC 24712).

**Notes** — *Neoceratosperma cyatheae* is phylogenetically different from all other species in this clade (Fig. 3). It was not possible to compare *N. cyatheae* with *N. alsophilae* and *N. yunnanensis* since the latter species are only known from their sexual morphs (Burgess et al. 2007, this study). In contrast for *N. cyatheae* only the asexual morph was found, which resembles zasmidium-like fungi, which are known to be polyphyletic (Crous et al. 2009a, b). Morphologically, *N. cyatheae* is similar to *N. eucalypti*, but differs from the latter by having smooth conidiophores reduced to conidiogenous cells (1–15-septate, verruculose, up to 100 µm long in *N. eucalypti*) and solitary conidia (solitary to catenate in *N. eucalypti*) (Crous et al. 2014). The distoseptation in young conidia, a characteristic

feature for *Neoceratosperma*, can easily be overlooked due to the abundant, large guttules.

***Neoceratosperma yunnanensis* (Barber & T.I. Burgess) Guatimosim, R.W. Barreto & Crous, comb. nov. — MycoBank MB813444**

**Basionym.** *Mycosphaerella yunnanensis* Barber & T.I. Burgess, Fung. Diversity 24: 150. 2007.

= *Xenomycosphaerella yunnanensis* Quaedvlieg & Crous, Persoonia 33: 24. 2014.

**Description & Illustration** — Burgess et al. (2007).

**Specimen examined.** CHINA, Yunnan, Lancang, leaves of *Eucalyptus urophylla*, May 2005, B. Dell (holotype MURU 407, culture ex-type CBS 119975 = CMW 23443).

***Paramycosphaerella* Crous, Persoonia 31: 245. 2013. — MycoBank MB805850**

**Notes** — The genus *Paramycosphaerella* is based on *Pa. brachystegia*, which occurs on *Brachystegia* sp. (Fabaceae) from Zimbabwe (Crous et al. 2013b). Thus far, only sexual morphs were known from this genus, which contains mycosphaerella-like species. In a previous study, Quaedvlieg et al. (2014) restricted their analyses to two species of *Paramycosphaerella*, relying on phylogenetic inferences to allocate species to this

genus. In the present study, we expanded the genus by also including additional phylogenetically related taxa.

**Paramycosphaerella aerohyalinosporum** (Crous & Summerell) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB509762

*Basionym.* *Zasmidium aerohyalinosporum* Crous & Summerell, Persoonia 23: 142. 2009.

Description & Illustration — Crous et al. (2009c).

*Specimen examined.* AUSTRALIA, New South Wales, road to Robin Falls, on leaves of *Eucalyptus tectifica*, 23 Sept. 2007, B.A. Summerell (holotype CBS H-20274, cultures ex-type CBS 125011, CPC 14636, CPC 14637).

**Paramycosphaerella blechni** Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812773; Fig. 10

*Etymology.* Name refers to the host genus from which it was isolated, *Blechnum*.

Description in planta — *Frond spots* amphigenous, starting on the pinnule as pale brown random spots, vein-delimited, with a pale brown central area, coalescing with age, becoming irregular, with a central pale brown necrotic area surrounded with a distinct dark brown halo where ascomata are produced. *Internal hyphae* branched, septate, intra- and intercellular, 1.5–3.5 µm wide, subhyaline to pale brown, smooth. *Ascomata*

pseudothelial, epiphyllous, solitary, subcuticular to erumpent, globose to subglobose, 52–90 × 58–76 µm, walls of 2–3 layers of brown to dark brown *textura angularis*, cells 3.5–7 × 2–3.5 µm, black, ostiole central, 17–28 µm diam. *Ascii* bitunicate, aparaphysate, fasciculate, subsessile, 8-spored, obpyriform to ovoid, straight or slightly curved, 22–52 × 7.5–14 µm, hyaline, smooth. *Ascospores* inordinate, overlapping, fusoid, straight to slightly curved, 12.5–19 × 2–4.5 µm, medianly 1-septate, apical cell wider, tapering towards both ends, but more prominently towards the upper end, guttulate, hyaline, thin-walled, smooth. *Ascospore germination* not seen. *Asexual morph* not known.

Culture characteristics — Colonies on MEA and PDA slow-growing, 42 mm diam after 24 d; raised with lobate margins, sparse feathery aerial mycelium in centre, immersed mycelium at periphery, humid, lavender grey to white in centre, iron-grey at periphery; reverse iron-grey. On OA, colony entirely lavender grey; leaden grey with amber zones in reverse; cultures sterile.

*Specimen examined.* BRAZIL, Paraná, Curitiba, highway to Joinville, roadside, on fronds of *Blechnum serrulatum*, 14 Nov. 2012, E. Guatimosim (holotype CBS H-22090, isotype VIC 42593, culture ex-type CPC 24698, COAD 1183).

Notes — Morphologically, *Pa. blechni* is rather similar to *Pa. dicranopteridis-flexuosa* described on *Dicranopteris flexuosa* from Brazil (this study), but can be distinguished from it by having narrower obpyriform to ovoid ascii (pyriform to narrowly ellipsoid, 10–18 µm wide in *Pa. dicranopteridis-flexuosa*).



Fig. 10 *Paramycosphaerella blechni* (CPC 24698). a–c. Frond spots on *Blechnum serrulatum*; d. e. vertical section of the ascoma; f. ascospores; g. ascospores; h. culture on MEA; i. culture on OA; j. culture on PDA. — Scale bars = 10 µm.

Phylogenetically, *Pa. blechni* is related to *Pa. dicranopteridis*, which is only known from its asexual morph. Both species differ from other species within this clade (Fig. 3). *Paramycosphaerella dicranopteridis* is presently only known from its ITS DNA sequence data (Kirschner & Liu 2014). Nevertheless, the two species differ on 33 bp for the ITS region.

***Paramycosphaerella cyatheae*** Guatimosim, R.W. Barreto & Crous, sp. nov. — MycoBank MB812775; Fig. 11

**Etymology.** Name refers to the host genus from which it was isolated, *Cyathea*.

**Description in planta** — *Frond spots* randomly affecting individual pinnules, irregular, initially necrotic along the main vein of the pinnulet, pale brown, with a cream central area where ascocarps are formed, becoming dark brown. *Internal hyphae* branched, septate, intra- and intercellular, 2.5–4.5 µm wide, subhyaline, smooth. *Ascomata* pseudothecial, epiphyllous, solitary, subcuticular to erumpent, globose, (36–)50–82(–101) × 62–90 µm, walls of 2–3 layers of brown to dark brown *textura angularis*, cells 5–10 × 2–6 µm, black, ostiole central, 11–23 µm diam. *Asci* bitunicate, apophysate, fasciculate, subsessile, 8-spored, obpyriform, straight or slightly curved, 26–54 × 9–20 µm, hyaline, smooth. *Ascospores* inordinate, overlapping, fusoid, straight, 10–15 × 2.5–4 µm, unequally 1-septate, constricted at the septum, upper cell shorter, tapering towards

rounded ends, with two large opposed guttules, hyaline, thin-walled, smooth. *Ascospores germinating* from both ends, remaining hyaline after germination, germ tubes growing along the main axis of ascospore, germ tubes irregular in width, not to slightly distorted, spores becoming slightly constricted at the septum (Type C, Crous 1998). *Asexual morph* not known.

**Culture characteristics** — Colonies on MEA, OA and PDA slow-growing, 14 mm diam after 24 d; raised, with discrete margins, and dense cottony aerial mycelium, smoke grey centrally, iron at periphery, humid; iron-grey in reverse. On OA, slightly pigmenting the media, olivaceous grey; cultures sterile.

**Specimen examined.** BRAZIL, Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, path to Pico do Pato, on fronds of *Cyathea delgadii*, 22 Feb. 2014, E. Guatimosim (holotype CBS H-22092, isotype VIC 42519, culture ex-type CPC 24730).

**Notes** — Morphologically, *Pa. cyatheae* is rather similar to *Pa. madeirae* described on *Eucalyptus* sp. from Madeira (Crous et al. 2004b) and to *Pa. sticheri*, described on *Sticherus penniger* from Brazil (this study), but can be distinguished by having wider asci (8–12 µm wide in *Pa. madeirae*) and smaller ascospores (14–20 × 3–5.5 µm in *Pa. sticheri*). Phylogenetically, *Pa. cyatheae* has *Pa. madeirae* as sister clade (Fig. 3). These two species, however, differ from each other by having the following number of variable sites for each locus: 23 bp for *act* and 17 bp for ITS.



**Fig. 11** *Paramycosphaerella cyatheae* (CPC 24730). a, b. Frond spots on *Cyathea delgadii*; c. erumpent subcuticular ascomata, fruiting epiphyllous; d. vertical section of the ascoma; e. asci; f. ascospores; g. germinating ascospores; h. culture on MEA; i. culture on OA; j. culture on PDA. — Scale bars = 10 µm.

**Paramycosphaerella dicranopteridis** (R. Kirschner) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812807

Basionym. *Zasmidium dicranopteridis* R. Kirschner, Phytotaxa 176: 319. 2014.

Description & Illustration — Kirschner & Liu (2014).

Specimen examined. TAIWAN, Taipei City, Wenshan District, Maokong, on fronds of *Dicranopteris linearis* var. *linearis*, 20 Oct. 2013, R. Kirschner (holotype TNM 3953, culture ex-type RoKi 3953).

**Paramycosphaerella dicranopteridis-flexuosae** Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812776; Fig. 12

*Etymology.* Name refers to the host species epithet, *Dicranopteris flexuosa*.

Description in planta — *Frond spots* amphigenous, irregular, starting as small dark brown spots, with a white centre adaxially, leading to the chlorosis of the pinnule (particularly at the apex), and subsequently its necrosis, which become entirely brown to black, deformed, and often brittle, ascomata produced adaxially in a grey well-delimited area, coalescing and leading to the blight of entire pinnae. *Internal hyphae* branched, septate, intra- and intercellular, 1.5–5 µm wide, subhyaline to pale brown, smooth. *Ascomata* pseudothelial, epiphyllous, solitary,

subcuticular to erumpent, globose, (46–)74–98(–114) × (55–)84–95(–109) µm, walls of 3–4 layers of pale to dark brown *textura angularis*, cells 4–11.5 × 1.5–3.5 µm, ostiole central, 9–17 µm diam. *Ascii* bitunicate, aplanospore, fasciculate, subsessile, 8-spored, obclavate to narrowly ellipsoid, straight or slightly curved, 24–51 × 10–18 µm, hyaline, smooth. *Ascospores* inordinate, overlapping, fusoid, straight, 10–19 × 2–4.5 µm, medianly 1-septate, tapering toward both rounded ends, guttulate, hyaline, thin-walled, smooth. *Ascospore germination* mostly from both ends, remaining hyaline, extending at an angle in reference to main ascospore apex, irregular in width, slightly distorted (mixture of Type G and K, Crous 1998).

Culture characteristics — Colonies on MEA, OA and PDA slow-growing, 23 mm diam after 24 d; raised, with lobate, undulate, feathery margins, and cottony aerial mycelium, iron-grey centrally, lavender grey at periphery; leaden black in reverse; On OA and PDA, slightly pigmenting the media, rosy vinaceous; cultures sterile.

Specimens examined. BRAZIL, Minas Gerais, Ouro Preto, Parque Municipal das Andorinhas, on fronds of *Dicranopteris flexuosa*, 25 Jan. 2014, P.B. Schwartsburg (holotype CBS H-22091, isotype VIC 43118, culture ex-type CPC 24743); ibid., vicinity of the Parque Estadual do Itacolomi, on fronds of *Dicranopteris flexuosa*, 8 June 2013, E. Guatimosim, VIC 42475.

Notes — Morphologically, *Pa. dicranopteridis-flexuosa* is quite similar to *Pa. gleicheniae*, recorded on *D. linearis* from India, Malaysia and Taiwan (Kirschner & Liu 2014), but can



Fig. 12 *Paramycosphaerella dicranopteridis-flexuosa* (CPC 24743). a–c. Frond spots on *Dicranopteris flexuosa*; d. vertical section of the ascoma; e. ascospores; f. germinating ascospores; g. culture on MEA; i. culture on OA; j. culture on PDA. — Scale bars = 10 µm.

be distinguished from the latter by having longer and wider ascospores ( $24\text{--}51 \times 10\text{--}18 \mu\text{m}$  in *Pa. dicranopteridis-flexuosa* and  $18\text{--}33 \times 9\text{--}15 \mu\text{m}$  in *Pa. gleicheniae*) (Ramakrishnan & Ramakrishnan 1950). In fact, the two hosts, *D. flexuosa* and *D. linearis*, are also very similar and retained as two geographical entities: the former occurring only in the Neotropics, and the latter in the Paleotropics (Mickel & Smith 2004, Bingyang et al. 2013). Phylogenetically, only ITS sequence data is available for *Pa. gleicheniae* (Kirschner & Liu 2014), from which only 5 bp are different from *Pa. dicranopteridis-flexuosa*. Nevertheless, the tree produced in this study (Fig. 3) demonstrated that *Pa. gleicheniae* is quite distinct from *Pa. dicranopteridis-flexuosa*. Additional loci should be sequenced for the former species, aiming at clarifying the true species boundaries. At present, based on the host species, geographical distribution, and until additional loci have been studied, we decided to maintain them as distinct taxa. An asexual stigmina-like morph was observed on different specimens, collected in different seasons at the same place, being associated with similar symptoms to those caused by *Pa. dicranopteridis-flexuosa*. However, no cultures were obtained from this fungus and the connection between these two morphs needs to be confirmed.

***Paramycosphaerella gleicheniae* (T.S. Ramakr. & K. Ramakr.)**

Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812808

*Basionym.* *Mycosphaerella gleicheniae* T.S. Ramakr. & K. Ramakr., Proc. Indian Acad. Sci., B 32: 205. 1950.

*Specimens examined.* INDIA, Coonoor, Nilgiris, Tamil Nadu, on fronds of *Dicranopteris linearis* (= *Gleichenia linearis*), 29 May 1948, T.S. Ramakrishnan & K. Ramakrishnan (holotype presumably lost). — TAIWAN, New Taipei City, Yingge, trail to Yingge Rock, on fronds of *D. linearis*, 11 Apr. 2012, R. Kirschner (TNM 3613, culture RoKi 3613); Taoyuan County, Dasi (Daxi) Township, Weiliiao Old Trail, 29 Sept. 2013, R. Kirschner (TNM 3945, culture RoKi 3945).

*Notes* — *Paramycosphaerella gleicheniae* was described from India, the holotype of which has presumably been lost (Aptroot 2006). The specimens examined here are from the same host, but from a different country (Taiwan), therefore inadequate to be used as neotype. However, despite the ascospores from the Taiwanese material being somewhat different from the type (Kirschner & Liu 2014), it is probable that they are conspecific. *Paramycosphaerella gleicheniae* still awaits neotypification.

***Paramycosphaerella irregularis* (Cheew. et al.) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812824**

*Basionym.* *Mycosphaerella irregularis* Cheew. et al., Persoonia 21: 82. 2008, as ‘irregulari’.

*Description & Illustration* — Cheewangkoon et al. (2008).

*Specimen examined.* THAILAND, Udonthani, on leaves of *Eucalyptus* sp., July 2007, R. Cheewangkoon (holotype CBS H-20135, culture ex-type CBS 123242).

***Paramycosphaerella madeirensis* (Crous & Denman) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812825**

*Basionym.* *Mycosphaerella madeirensis* Crous & Denman, Stud. Mycol. 50: 204. 2004, as ‘madeirae’.

*Description & Illustration* — Crous et al. (2004b).

*Specimen examined.* MADEIRA, Party Farm, on leaves of *Eucalyptus globulus*, Apr. 2000, S. Denman (holotype CBS H-9898, cultures ex-type CBS 112895, CBS 112301).

***Paramycosphaerella nabiacense* (Crous & Carnegie) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812809**

*Basionym.* *Zasmidium nabiacense* Crous & Carnegie, Persoonia 23: 142. 2009.

*Description & Illustration* — Crous et al. (2009c).

*Specimen examined.* AUSTRALIA, New South Wales, Nabiac, on leaves of *Eucalyptus* sp., 30 Nov. 2005, A.J. Carnegie (holotype CBS H-20273, cultures ex-type CBS 125010, CPC 12749, 12750).

***Paramycosphaerella parkii* (Crous et al.) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812810**

*Basionym.* *Mycosphaerella parkii* Crous et al., Mycol. Res. 97: 582. 1993.  
= *Stenella parkii* Crous & Alfenas, Mycologia 87: 121. 1995.  
≡ *Zasmidium parkii* (Crous & Alfenas) Crous & U. Braun, Schlechtendalia 20: 102. 2010.

*Descriptions & Illustrations* — Crous et al. (1993), Crous & Alfenas (1995).

*Specimen examined.* BRAZIL, Aracruz Florestal nursery, on leaves of *Eucalyptus grandis*, 24 Feb. 1990, M.J. Wingfield (holotype PREM 50668, culture ex-type CBS 387.92, CMW 14775, STE-U 353).

*Notes* — The link between the sexual (PREM 50668, culture CBS 387.92) and asexual morph (PREM 51713) was based on morphology, and never corroborated by DNA sequence data. Because subsequent studies have revealed ‘*Mycosphaerella parkii*’ to be a species complex (Crous et al. 2006b, Cheewangkoon et al. 2008), fresh collections are required to resolve the status of *Zasmidium parkii*.

***Paramycosphaerella pseudomarksii* (Cheew. et al.) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812811**

*Basionym.* *Mycosphaerella pseudomarksii* Cheew. et al., Persoonia 21: 83. 2008.

*Description & Illustration* — Cheewangkoon et al. (2008).

*Specimen examined.* THAILAND, Chiang Mai, Mae Tang, on leaves of *Eucalyptus* sp., June 2007, R. Cheewangkoon (holotype CBS H-20134, culture ex-type CBS 123241).

***Paramycosphaerella sticheri* Guatimosim, R.W. Barreto & Crous, sp. nov.** — MycoBank MB812777; Fig. 13

*Etymology.* Name refers to the host genus from which it was isolated, *Sticherus*.

*Description in planta* — *Frond spots* amphigenous, irregular, initially small and vein delimited along the pinnulets, black and dark brown intermixed areas, growing and leading to complete necrosis of the pinnula, sometimes causing blight of entire pinna. *Internal hyphae* branched, septate, intra- and intercellular, 2–2.5  $\mu\text{m}$  wide, subhyaline to pale brown, smooth. *Ascomata* pseudothecial, amphigenous, more abundant abaxially, solitary, subcuticular to erumpent, globose, (51)–60–96(–106)  $\times$  45–94  $\mu\text{m}$ , walls of 2–3 layers of brown to dark brown *textura angularis*, cells 2.5–4  $\times$  2–3  $\mu\text{m}$ , black, ostiole central, 16–30  $\mu\text{m}$  diam. *Asci* bitunicate, apotheciate, fasciculate, subsessile, 8-spored, obpyriform, straight or slightly curved, 24–58  $\times$  11–20  $\mu\text{m}$ , hyaline, smooth. *Ascospores* inordinate, overlapping, fusoid, straight, 14–20  $\times$  3–5.5  $\mu\text{m}$ , medianly 1-septate, not to slightly constricted at the septum, tapering towards rounded ends, but more prominently towards the lower end, guttulate, hyaline, thin-walled, smooth. *Ascospores* germinating from both ends, remaining hyaline, germ tubes following the long axis of the spore, germ tubes irregular in width, slightly distorting, spores



**Fig. 13** *Paramycosphaerella sticheri* (CPC 24720). a. Frond spots on *Sticherus penniger*; b. erumpent subcuticular ascomata, fruiting epiphyllous; c. vertical section of the ascoma; d. e. ascospores; f. ascospores; g. germinating ascospores; h. culture on MEA; i. culture on OA; j. culture on PDA. — Scale bars = 10 µm.

becoming constricted at the septum (Type C, Crous 1998). Asexual morph not known.

Culture characteristics — Colonies on MEA and PDA slow-growing, 19 mm diam after 24 d; dome-shaped, lobate, with sharp margins and velvety aerial mycelium, pale mouse grey centrally, mouse grey at periphery; olivaceous grey reverse. On OA, surface pale mouse grey centrally, outer region lavender grey, with a distinct leaden black margin; greenish grey reverse; cultures sterile.

**Specimen examined.** BRAZIL, Santa Catarina, São Pedro de Alcântara, roadside, on fronds of *Sticherus penniger*, 17 Apr. 2013, E. Guatimosim (holotype CBS H-22093, isotype VIC 42498, culture ex-type CPC 24720, COAD 1422).

**Notes** — Morphologically, *Pa. sticheri* is rather similar to *Pa. dicranopteridis-flexuosa*, recorded on *Dicranopteris flexuosa* from Brazil (this study). Nevertheless, it can be distinguished from the latter species by having slightly narrower ascospores (2–4.5 µm in the latter). Moreover, they are phylogenetically quite distinct from each other according to the following number of variable sites for each locus: 28 bp for *act*, 43 bp for ITS, 101 bp for *tef1* and 8 bp for LSU. Additionally, based on multi-gene phylogenetic inference (Fig. 3), *Pa. sticheri* grouped basal to other taxa in the genus, having *Pa. nabiacense* as sister clade.

***Paramycosphaerella vietnamensis* (Barber & T.I. Burgess)**  
Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812812

**Basionym.** *Mycosphaerella vietnamensis* Barber & T.I. Burgess, Fung. Diversity 24: 148. 2007.

**Description & Illustration** — Burgess et al. (2007).

**Specimen examined.** VIETNAM, South East Forestry Institute nursery, from leaves of *Eucalyptus grandis*, 6 July 2004, T.I. Burgess (holotype MURU411, ex-culture CBS 119974, CMW 23441).

***Phaeophleospora pteridivora* Rangel, Arq. Mus. Nac. Rio de Janeiro 18: 162. 1916. — MycoBank MB9311**

***Phaeophleospora pteridivora* Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812826; Fig. 14**

**Etymology.** Name refers to the high degree of damage caused by the fungus on infected fronds.

**Description in planta** — *Frond spots* amphigenous, irregular, affecting almost all the pinnulets. Starting as small pale brown areas, usually close to the apex of the pinnulets, affecting the edges, which becomes distorted and brittle, spreading and becoming confluent, necrotic, leading to complete necrosis of the pinnulet. *External hyphae* absent. *Internal hyphae* branched, septate, intra- and intercellular, 1.5–3 µm wide, dark brown,



**Fig. 14** *Phaeophleospora pteridivora* (CPC 24683). a, b. Frond spots on *Serpocaulon triseriale*; c. erumpent subcuticular ascomata, fruiting epiphyllous; d. e. vertical section of the ascus; f. conidiophores arising from the stroma; g. conidia; h. culture on MEA; i. culture on OA; j. culture on PDA. — Scale bars = 10 µm.

smooth. **Ascomata** pseudothecial, hypophyllous, solitary, subcuticular to erumpent, globose, 44–64 × 42–61 µm, wall of 3–4 layers of brown to dark brown *textura angularis* cells, 2–11 × 2–8 µm, black, ostiole central, 10–22 µm diam. Ascii bitunicate, aparaphysate, fasciculate, subsessile, 8-spored, ellipsoidal to ovoid, straight or slightly curved, 15–25 × 6–8 µm, hyaline, smooth. **Ascospores** inordinate, overlapping, fusoid, straight, 1.5–12 × 1–8 µm, medianly 1-septate, not constricted at the septum, tapering towards rounded ends, with two large opposed guttules, hyaline, thin-walled, smooth. **Ascospore germination** not seen. **Asexual morph** cercosporoid, hypophyllous. **Stromata** subcuticular, erumpent, globose, 40–46 × 50–54 µm, composed of an aggregation of *textura angularis*, cells 4–5 × 2–5 µm, brown to dark brown, smooth. **Conidiophores** sporodochial, arising from the stroma, restricted to the conidiogenous cells, subcylindrical to ampuliform, straight, 5–25 × 2–5 µm, unbranched, aseptate, subhyaline to pale brown, smooth. **Conidiogenous cells** terminal, determined, unbranched, tapering to the apex, subhyaline to pale brown, smooth, scars inconspicuous, one per cell, not thickened, nor darkened. **Conidia** solitary, subcylindrical, curved to sinuous, 70–107 × 2–3 µm, tapering toward the acute apex, base truncate, 1.5–2.5 µm diam at the base, 6–9-septate, guttulate, pale brown to olivaceous brown, smooth, scars not thickened, nor darkened.

**Culture characteristics** — Colonies on MEA slow-growing, 46 mm diam after 24 d; undulated, spreading, with lobate, fea-

thery margins and sparse aerial mycelium, mouse grey centrally, pale mouse grey at periphery with a distinct narrow white external rim; greenish grey reverse. On OA, cream with a honey to buff periphery; iron-grey centrally with amber periphery reverse. On PDA, mouse grey with lavender grey periphery; mouse grey reverse centrally, amber periphery; cultures sporulating moderately on OA, producing conidia.

**Specimen examined.** BRAZIL, Rio de Janeiro, Cláudio Coutinho path, Praia Vermelha, Urca, humid rocks, on fronds of *Serpocaulon triseriale*, 3 Feb. 2012, R.W. Barreto (holotype CBS H-22097, isotype VIC 42559, culture ex-type CPC 24683, COAD 1182).

**Notes** — The genus *Phaeophleospora*, which is based on *Ph. eugeniae*, was collected from *Eugenia uniflora* (Myrtaceae) in Brazil (Crous et al. 1997) and clusters within Mycosphaerellaceae (Crous et al. 2007a). In the past, this genus included species that are presently accommodated in *Teratosphaeria* (= *Kirramyces*) and have pycnidial asexual morphs (Walker et al. 1992, Andjic et al. 2007). The new species described on *Serpocaulon triseriale* (Polypodiaceae) was based on material producing both the sexual and asexual morphs. Surprisingly, its asexual morph is a sporodochial hyphomycete (Fig. 3). Given the recent conidiomatal species with aseptate conidia described from ferns collected in Thailand (Crous et al. 2015b), the genus *Phaeophleospora* as presently defined based on DNA phylogeny, is morphologically rather diverse.

**Pseudocercospora** Spieg., Anales Mus. Nac. Hist. Nat. Buenos Aires, Ser. 3, 13: 437. 1911

**Pseudocercospora abacopteridicola** J.M. Yen & Lim, Cah. Pacifique 17: 97. 1973. — Fig. 15

Description in planta — *Frond spots* amphigenous, starting as minute, vein-delimited, pale brown spots, affecting random pinnales, leading to an extensive necrosis of entire pinnae, which then become dark brown to black, with a central area white to grey. *Caespituli* hypophyllous, abundant. *External hyphae* branched, septate, arising from the stomata, 1.6–2.5 µm wide, pale to medium brown, smooth. *Internal hyphae* indistinct. *Stromata* absent. *Conidiophores* arising from the hyphae, hypophyllous, restricted to the conidiogenous cells. *Conidiogenous cells* terminal, holoblastic, subcylindrical, straight, geniculate, 5–5.5 × 2–2.5 µm, unbranched, aseptate, pale brown, smooth, scars indistinct. *Conidia* solitary, subcylindrical, straight or curved, (25–)45–66(–77) × 1.8–3 µm, rounded apex, base subtruncate, 2–8-septate, guttulate, pale brown, smooth; hila not thickened, nor darkened, 1–3 µm diam.

Culture characteristics — Colonies on MEA slow-growing, 26 mm diam after 20 d in the dark; surface smooth, raised with dense aerial mycelium and even margins, olivaceous grey in the centre, followed by a pale olivaceous grey ring and greenish black periphery; iron-grey reverse; cultures sterile.

Specimen examined. BRAZIL, Minas Gerais, Cachoeira do Campo, Café Retiro Novo, on fronds of *Adiantum* sp., 12 Nov. 2012, E. Guatimosim (CBS H-22098, culture CPC 24709).

Notes — *Pseudocercospora abacopteridicola* was only known from the type specimen, collected on *Abacopteris urophylla* (*Thelypteridaceae*) from Singapore (Yen & Lim 1980, Braun et al. 2013). The specimen collected in Brazil was found on a distantly related host — *Adiantum* sp. (*Pteridaceae*). However, as morphology and biometric data are indistinguishable, instead of describing the fungus from Brazil as new, we prefer to place it in *Ps. abacopteridicola* until DNA of the fungus from Singapore becomes available for a molecular comparison.

**Pseudocercospora brackenicola** Guatimosim, R.W. Barreto & Crous, sp. nov. — MycoBank MB812813; Fig. 16

*Etymology.* Name refers to bracken — the common English name for species of *Pteridium*.

Description in planta — *Frond spots*, amphigenous, irregular, starting as small, dark brown vein delimited spots at pinnule margins, spreading and becoming black with age and occasionally reaching the entire pinnule. *Caespituli* hypophyllous, abundant. *External hyphae* hypophyllous, arising from a tuft through the stomata and spreading, slightly branched, septate, pale brown, smooth. *Internal hyphae* intra- and intercellular, septate, branched, 1.4–3.5 µm, subhyaline to pale brown, smooth. *Stromata* rudimentary, inside the stomatal cavity, irregular, 24.5–56.5 × 11.5–25.5 µm, composed of a few globose cells, pale brown. *Conidiophores* hypogenous, arising through the stomata, producing dense fascicles, up to 20 conidiophores per fascicle, subcylindrical, straight to curved, often geniculate at the tip, 11–29.5 × 2–3 µm, branched, mostly aseptate, rarely 1–2-septate, eguttulate, pale brown, smooth. *Conidiogenous cells* terminal, integrated, holoblastic, subcylindrical, sympodial, 4.5–17 × 2–3 µm, pale brown, smooth, scars indistinct, 1 per cell, discoid, c. 2 µm diam, not thickened, nor darkened. *Conidia* solitary, obclavate to subcylindrical, straight, curved, or sinuous, 20–77 × 1–2 µm, rounded apex, base truncate, 1–6-septate, guttulate, pale brown, smooth; hila not thickened, nor darkened, 1–2 µm diam.

Culture characteristics — Colonies on MEA slow-growing, 30 mm diam after 20 d in the dark; raised with velvety aerial mycelium, pale greenish grey centrally, and mouse grey at periphery; olivaceous grey in reverse; cultures sterile.

Specimens examined. BRAZIL, Minas Gerais, Capitólio, Furnas, Rio do Turvo Inn, in front of the announcement board of Clube Náutico, on fronds of *Pteridium arachnoideum*, 9 Nov. 2012, R.W. Barreto (holotype CBS H-22101, isotype VIC 42588, culture ex-type CPC 24695).

Notes — Phylogenetically, *Ps. brackenicola* clusters with *Ps. purpurea* and *Ps. sordida* as sister clade (Fig. 2), but differs from them by having the following number of variable sites for each locus: *Ps. purpurea* (7 bp for ITS, 9 bp for act, 24 bp

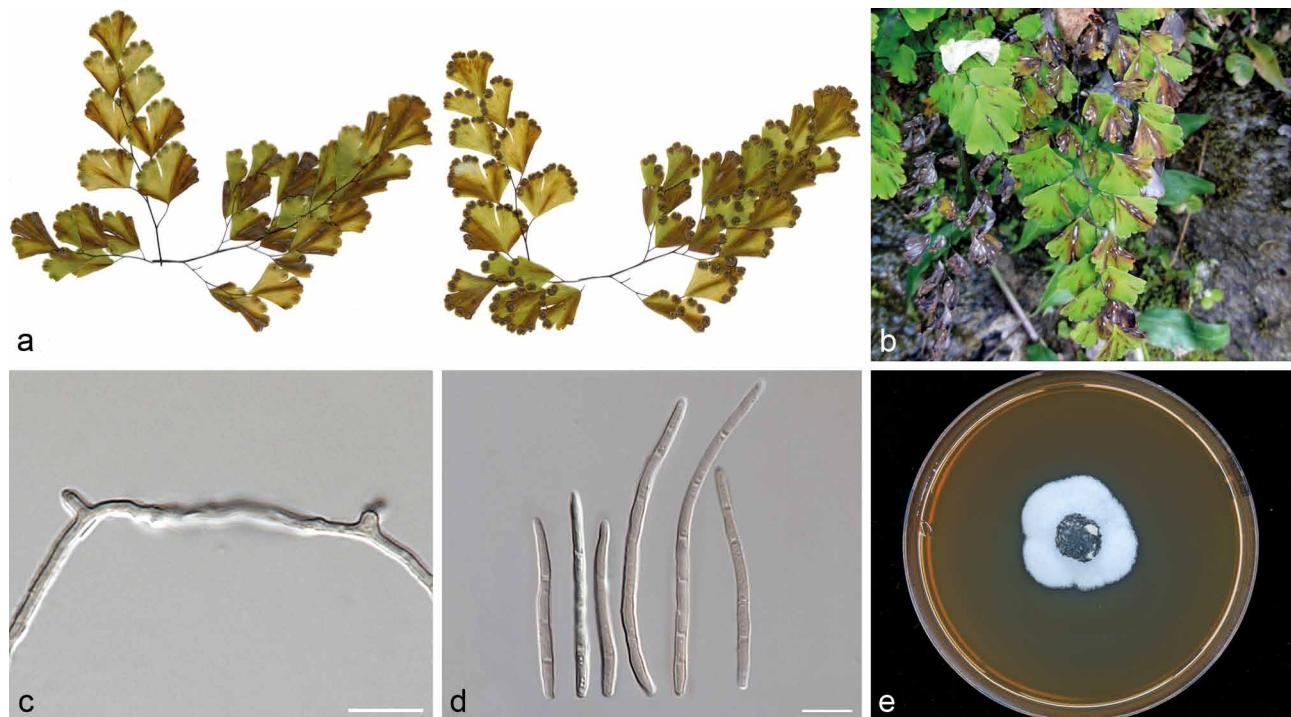
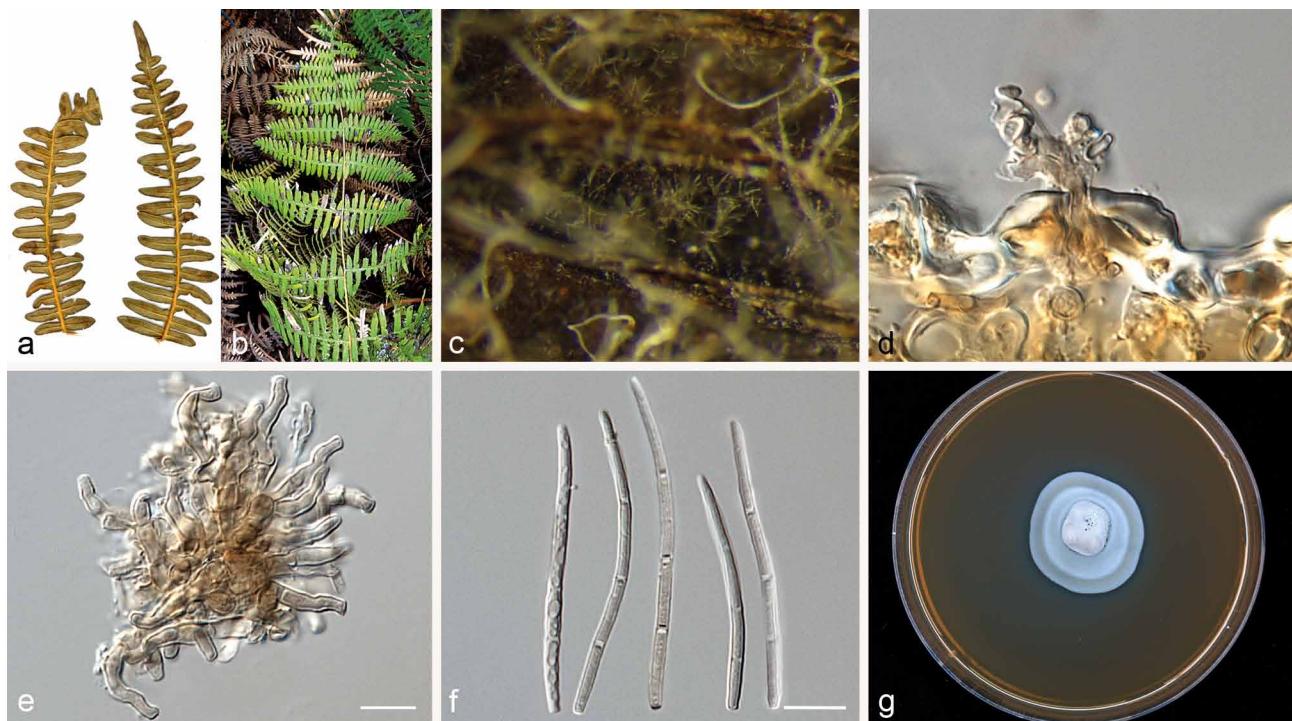


Fig. 15 *Pseudocercospora abacopteridicola* (CPC 24709). a, b. Frond spots on *Adiantum* sp.; c. conidiophores restricted to the conidiogenous cells, arising from the hyphae; d. conidia; e. culture on MEA. — Scale bars = 10 µm.



**Fig. 16** *Pseudocercospora brackenicola* (CPC 24709). a, b. Frond spots on *Pteridium arachnoideum*; c. conidia sporulating abaxially; d. detail of conidiophores arising through the stoma; e. conidiophores; f. conidia; g. culture on MEA. — Scale bars = 10 µm.

for *tef1*) and *Ps. sordida* (8 bp for ITS, 14 bp for *act*, 33 bp for *tef1*). Morphologically, both species are clearly different from *Ps. brackenicola* by having larger conidiophores (20–200 × 3.5–4.5 µm in *Ps. purpurea* and 20–90 × 3.5–5 µm in *Ps. sordida*) and larger conidia (20–100 × 2–4.5 µm in *Ps. purpurea* and 20–165 × 3–5.5 µm in *Ps. sordida*) (Chupp 1954, Guo & Hsieh 1995). Additionally, the hosts of *Ps. purpurea* and *Ps. sordida* are higher plant families in the *Perseaceae* and *Bignoneaceae*, respectively (Farr & Rossman 2015). *Pseudocercospora brackenicola* is similar to *Ps. davallicola* (described on *Davallia fejeensis* from Brazil) and to *Ps. lonchitidis* (described on *Lonchitis hirsuta* from Venezuela) (Braun et al. 2013). Molecular data are lacking for both species, but there are various morphological differences that distinguish them. Firstly, the conidia in *Ps. davallicola* can be formed in short chains (absent in *Ps. brackenicola*), and the conidiophores of *Ps. davallicola* are solitary, whereas on *Ps. brackenicola* they form fascicles emerging from stromata, through stomata (Braun et al. 2013). Secondly, *Ps. lonchitidis* has erumpent, well-developed stromata (loosely dense, emerging through the stoma in *Ps. brackenicola*), straight and thicker conidiophores, 3–5 µm wide in *Ps. davallicola* (curved to sinuous, 2–3 µm wide in *Ps. brackenicola*), and conidiogenous loci are subdenticulate (inconspicuous in *Ps. brackenicola*) (Braun et al. 2013). This is the first record of a *Pseudocercospora* sp. on the genus *Pteridium*. *Pseudocercospora brackenicola* causes a damaging disease on its host (bracken), which is a highly noxious weed. Further investigations are required to determine its potential role as biological control agent.

***Pseudocercospora lygodiicola* Y.L. Guo & U. Braun, IMA Fungus 4: 317. 2013. — Fig. 17**

Description in planta — *Frond spots* amphigenous, irregular, starting from the main vein and spreading until the edges of the pinnulets, becoming centrally cream and necrotic, with a distinct dark brown to black halo. *Caespituli* hypophyllous, abundant. *External hyphae* absent. *Internal hyphae* intra- and intercellular, 1.5–3.5 µm wide, septate, branched, pale brown, smooth. *Stromata* rudimentary, arising from the stomatal cavity, subglobose,

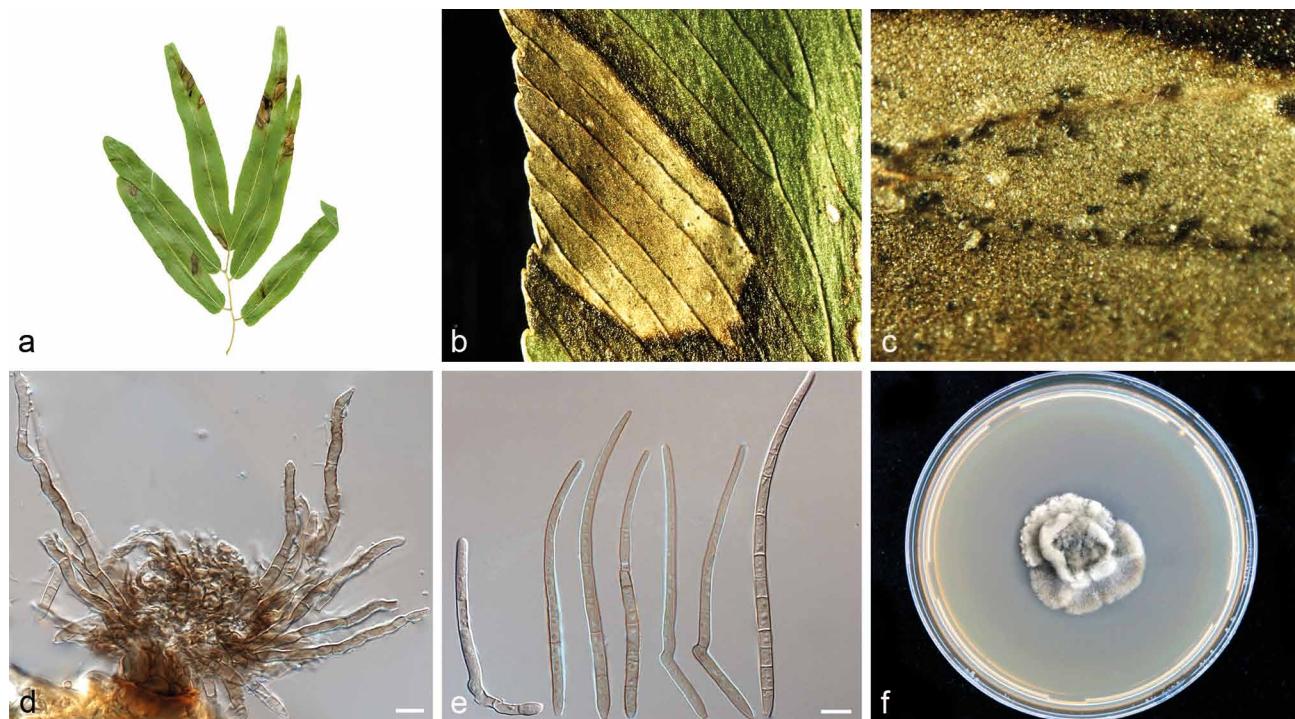
composed of *textura angularis*, 22–70 µm diam, dark brown, cells 3–7 × 2.5–3 µm. *Conidiophores* arising from stromata, hypophyllous, forming small fascicles (up to 15), subcylindrical, sinuous or curved, geniculate towards the apex, 26–80 × 3–5 µm, unbranched, 3–6-septate, eguttulate, pale brown, smooth. *Conidiogenous cells* terminal, holoblastic, subcylindrical, attenuated at the tip, 3–18 × 2–4 µm, subhyaline, smooth, scars inconspicuous, 1 per cell, subdenticulate, 1–3.5 µm, not thickened, nor darkened. *Conidia* solitary, obclavate, curved or sinuous, 43–117 × 2.5–4.5 µm, tapering toward rounded apex, base obconically truncate, 6–12-septate, guttulate, pale brown, smooth; hila not thickened, nor darkened, 1–4 µm diam.

Culture characteristics — Colonies on MEA slow-growing, reaching 32 mm diam after 20 d in the dark; centrally raised, and flat at periphery, aerial mycelium cottony, dry, iron-grey combined with olivaceous grey areas centrally, olivaceous grey towards periphery; reverse olivaceous black centrally and olivaceous grey at periphery; cultures sterile.

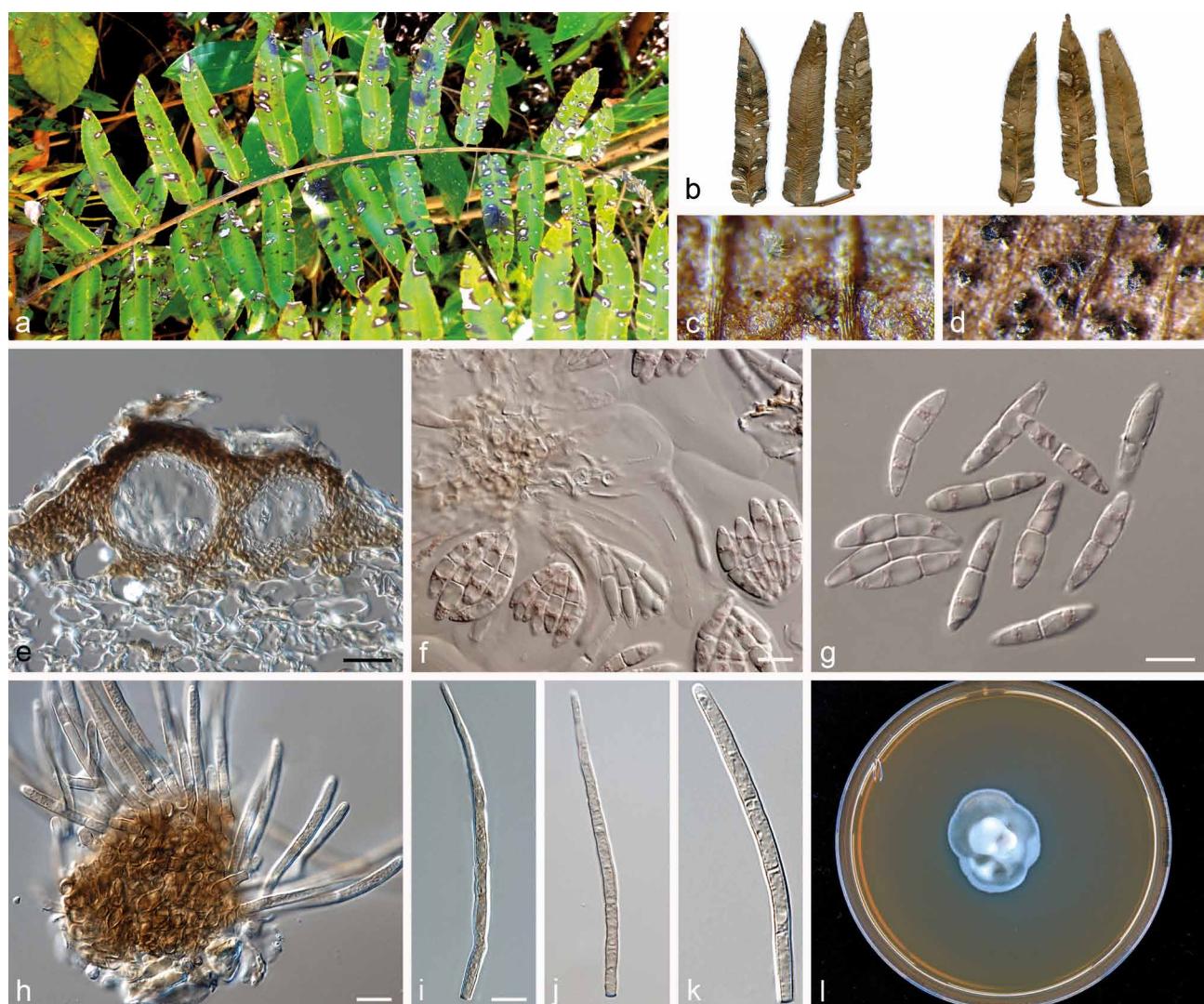
Specimen examined. BRAZIL, Rio de Janeiro, BR-116 Highway, near to Parque Nacional Serra dos Órgãos, roadside, on fronds of *Lygodium volubile*, 14 June 2014, R.W. Barreto (VIC 42917, cultures CPC 25755, COAD 1745).

Notes — There are four species of *Pseudocercospora* known from *Lygodium*, namely *Ps. lygodii* (on *L. japonicum* from Taiwan), *Ps. lygodiicola* (on *L. japonicum* from China), *Ps. lygodigena* and *Ps. polypodiacearum* (both on *Lygodium* sp. from India) (Braun et al. 2013). Species boundaries among these taxa are based on morphological and biometric characters, which could be considered as tentative, as the host and distribution range of these taxa are quite similar. Currently there are no records of ex-type cultures or DNA information on any of these taxa.

The fungus isolated from *L. volubile* in Brazil has morphological and biometric data similar to *P. lygodiicola*, but until the latter has been epitypified, we decided to extend its host range, rather than propose a new name for the Brazilian collection. Phylogenetically, *Ps. lygodiicola* clusters in the same clade with three other species isolated from ferns, namely *Ps. cyatheicola*, *Ps. rumohrae* and *Ps. thelypteridis* (Fig. 2).



**Fig. 17** *Pseudocercospora lygodicola* (CPC 25755). a, b. Frond spots on *Lygodium volubile*; c. conidiophores sporulating adaxially; d. conidiophores arising from the stroma through the stoma; e. conidia; f. culture on MEA. — Scale bars = 10 µm.



**Fig. 18** *Pseudocercospora paranaensis* (aseexual morph COAD 1180, sexual morph CPC 24680). a, b. Frond spots on *Cyathea atrovirens*; c. conidia sporulating abaxially; d. erumpent subcuticular ascomata, fruiting epiphyllous; e. vertical section of the ascoma; f. asci; g. ascospores; h. conidiophores arising from the stroma; i–k. conidia; l. culture on MEA. — Scale bars = 10 µm.

**Pseudocercospora paranaensis** Guatimosim, R.W. Barreto & Crous, sp. nov. — MycoBank MB812814; Fig. 18

**Etymology.** Name refers to the state in Brazil from where the fungus was collected, Paraná.

**Fronds spots** amphigenous, firstly irregular, vein delimited, pale brown to black, distributed along the pinnules, becoming circular, white to grey at the centre, with a brown to black halo sometimes perforated centrally leading to necrosis of the whole pinnule, and occasionally whole pinnae. **External hyphae** absent. **Internal hyphae** intra- and intercellular, septate, branched, 1–2 µm wide, hyaline, smooth. **Ascomata** pseudothecial, hypophyllous, solitary to confluent, subepidermal to erumpent, globose to subglobose, 40–80 × 45–73.5 µm, walls of 2–3-layers of *textura angularis*, medium brown to dark, 9.5–32 µm thick, ostiole central, c. 39 µm diam. **Asci** bitunicate, aplanospore, fasciculate, subsessile, 8-spored, fusoid-ellipsoidal when immature and pyriform at maturity, straight or slightly curved, 40–75 × 13–30 µm, hyaline, smooth. **Ascospores** biseriate to inordinate, overlapping, fusoid, straight, 18–27 × 3.5–6 µm, unequally 1-septate, slightly constricted at the septum, tapering towards rounded ends, with two large opposed guttules, hyaline, thin-walled, smooth. **Ascospore germination** not observed. **Asexual morph:** *Caespituli* hypophyllous, abundant. **Stromata** subsuperficial, globose, composed of dark brown *textura globulosa*, 26–39 × 15–31.5 µm. **Conidiophores** arising from the stroma, hypophyllous, sporodochial, restricted to the conidiogenous cells, ampulliform, swollen at the base, 7–11 × 1.5–2 µm, unbranched, aseptate, eguttulate, pale brown, smooth; scars, 2 µm wide, neither thickened, nor darkened. **Conidia** solitary, subcylindrical or obclavate, curved or rarely straight, 79–99 × 2–3 µm, rounded to obtuse apex, base truncate, 3–9-septate, guttulate, pale brown, smooth; hila not thickened, nor darkened, sometimes slightly darkened and slightly refractive, 1–2 µm diam.

**Culture characteristics** — Colonies on MEA slow-growing, 28 mm diam after 20 d in the dark; smooth with even margins, raised, aerial mycelium velvety, surface olivaceous grey, mixed with pale olivaceous grey; iron-grey in reverse; cultures sterile.

**Specimens examined.** BRAZIL, Paraná, Piraquara, Mananciais da Serra, on fronds of *Cyathea atrovirens*, sexual morph, 2 Feb. 2012, R.W. Barreto (holotype CBS H-22099, isotype VIC 42559, culture ex-type CPC 24680); ibid, asexual morph, 2 Feb. 2012, R.W. Barreto (VIC 42558, culture COAD 1180).

**Notes** — Both morphs (sexual and asexual) were found occurring hypophyllously, on different fronds from the same host. *Pseudocercospora paranaensis* clusters in an isolated clade (Fig. 2), having *Ps. basitrunca* as sister clade. Besides, *Ps. basitrunca* is known to be an extremely variable species, some features remaining relatively constant such as the irregular annellations on the conidiogenous cells, and the conidial shape. Smaller conidia tend to be cylindrical, whereas larger conidia are tapered to more obtuse apices (Crous 1998). *Pseudocercospora paranaensis* does not have any annellations on its conidiogenous cells, which proliferate sympodially instead. Additionally, *Ps. paranaensis* differs from *Ps. basitrunca* by having significantly smaller conidiophores (7–11 µm in the former and 12–60 µm in the latter) and longer conidia (79–99 µm in the former and 45–70 µm in the latter). Finally, *Ps. basitrunca* is only known from an unrelated species of *Eucalyptus* (Hunter et al. 2011, Crous et al. 2013a).

Two other species of *Pseudocercospora* have already been recorded on members of Cyatheaceae, namely *Ps. cyatheae* described on *Cyathea* sp. from Japan and *Ps. cyatheicola* on *Cyathea australis* from Australia (Braun et al. 2013). With regards to *Ps. cyatheae*, the only sequence available in GenBank for this species is of the ITS region. *Pseudocercospora paranaensis*

differs from *Ps. cyatheae* in ITS and clusters in a separate and highly supported clade (data not shown). Nevertheless, morphological criteria alone clearly separate the two species. *Pseudocercopora cyatheae*, in contrast to *Ps. paranaensis*, has epiphyllous caespituli, its conidiogenous cells have a rim-like thickening at the scars, and it also has thicker, cylindrical to obclavate conidia (30–50 × 3.7–5.5 µm) with rounded bases (Nakashima et al. 2006). *Pseudocercospora cyatheicola* is different from *Ps. paranaensis* both phylogenetically – grouping in a different clade of the tree (Fig. 2) – and morphologically – having amphigenous stromata, larger conidiophores (30–70 × 2–3 µm), and percurrently proliferating conidiogenous cells (Crous et al. 2011).

**Pseudocercospora serpocaulonicola** Guatimosim, R.W. Barreto & Crous, sp. nov. — MycoBank MB812815; Fig. 19

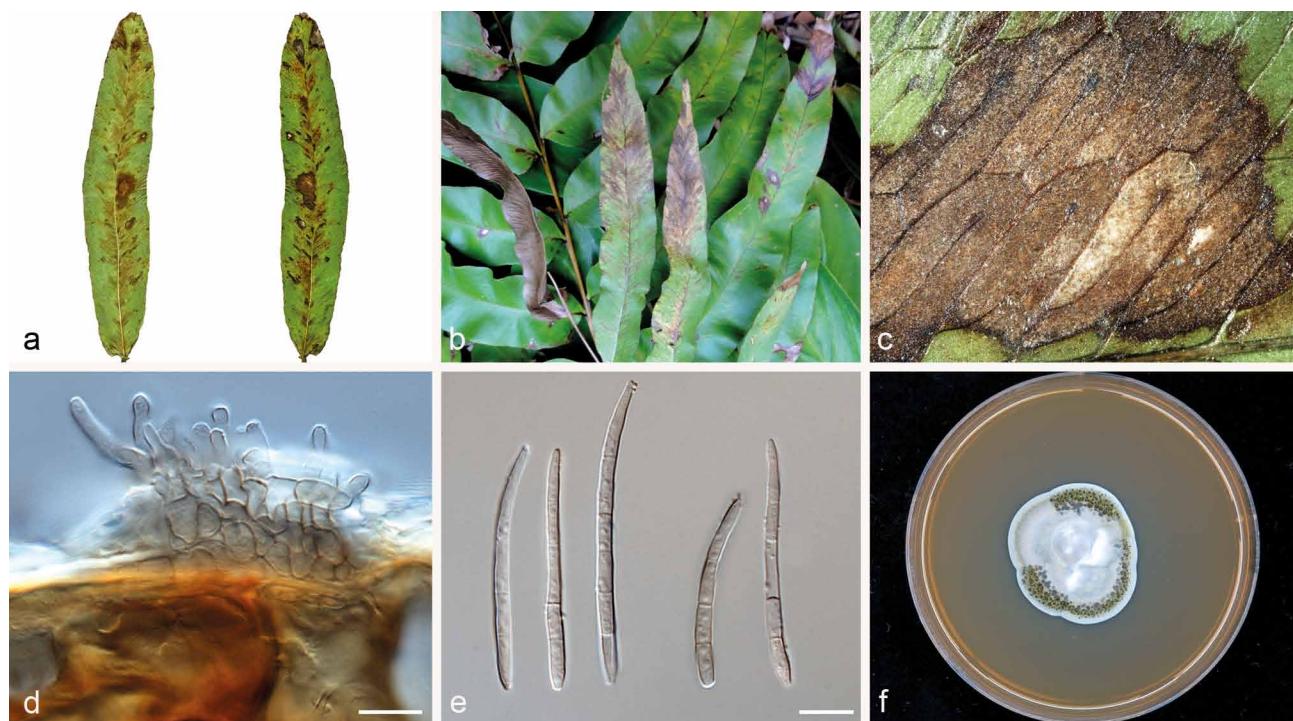
**Etymology.** Name refers to the host genus from which it was isolated, *Serpocaulon*.

**Description in planta** — **Frond spots** amphigenous, irregular, firstly concentrated next to the main vein and progressively spreading towards the margins of the pinnule, centrally pale brown, becoming dark brown towards the periphery. **Caespituli** epiphyllous, abundant. **External hyphae** absent. **Internal hyphae** intra- and intercellular, 1–2.5 µm wide, branched, septate, subhyaline to pale brown, smooth. **Stromata** rudimentary, subcuticular, composed of pale brown *textura angularis*, 15–36.5 µm wide, pale brown, smooth. **Conidiophores** restricted to the conidiogenous cell, arising from the stroma, epiphyllous, forming loose fascicles with up to 15 stalks, subcylindrical, attenuated at the tip, sinuous, often geniculate, 7–22 × 2–3.5 µm, unbranched, 0–1-septate, eguttulate, subhyaline to pale brown, smooth, scars inconspicuous, 1 per cell, not thickened, nor darkened. **Conidia** solitary, subcylindrical to obclavate, straight or curved, 31–75 × 2–3.5 µm, apex attenuated, base obconically truncate, 2–7-septate, guttulate, pale brown, smooth; hila not thickened, nor darkened, 2–4 µm diam.

**Culture characteristics** — Colonies on MEA slow-growing, 31 mm diam after 20 d in the dark; flat, aerial mycelium cottony, with water droplets at periphery, pale olivaceous-grey combined lavender grey areas centrally, greenish grey towards periphery; olivaceous black centrally and olivaceous grey at periphery in reverse; cultures sterile.

**Specimens examined.** BRAZIL, Rio de Janeiro, Gávea, Parque da Cidade, on fronds of *Serpocaulon triseriale*, 14 June 2014, R.W. Barreto (holotype CBS H-22105, cultures ex-type CPC 25077, COAD 1866).

**Notes** — *Pseudocercospora serpocaulonicola* clustered within a new clade, together with an isolate recorded on *Eucalyptus grandis* from South Africa (CBS 110998), *Ps. cordiana*, *Ps. paraguayensis* and *Ps. schizolobii* (Fig. 2), but differs from them by having the following variable sites for each locus: *Pseudocercospora* sp. (9 bp for ITS), *Ps. cordiana* (30 bp for ITS, 1 bp for act, 1 bp for tef1), *Ps. paraguayensis* (6 bp for ITS, 1 bp for act, 4 bp for tef1) and *Ps. schizolobii* (5 bp for ITS, 5 bp for act, 4 bp for tef1). Morphologically, it was not possible to compare the present collection to *Pseudocercospora* sp. (CBS 110998), as the fungarium specimen was in poor condition, and neither conidiophores nor conidia were seen. Moreover, the cultures proved to be sterile. Two other *Pseudocercospora* species known on ferns (for which no DNA data are available in GenBank) have a similar morphology to *Ps. serpocaulonicola*. These are *Ps. microsori* on *Microsorum pustulatum* from Australia, and *Ps. phyllitidis*, which occurs on various ferns belonging to different families, and has a cosmopolitan distribution (Shivas et al. 2010, Braun et al. 2013). *Pseudocercospora microsori* differs from *Ps. serpocaulonicola* by having well-developed stromata (20–60 µm wide), longer (30–65 × 3–5



**Fig. 19** *Pseudocercospora serpocaulonicola* (CPC 25077). a–c. Frond spots on *Serpocaulon triseriale*; d. conidiophores arising through the stoma; e. conidia; f. culture on MEA. — Scale bars = 10 µm.

µm), densely fasciculate (5–30 stalks per fascicle), red-brown conidiophores, and moderately wide (2.5–4 µm), curved to flexuous conidia (Shivas et al. 2010). On the other hand, *Ps. phyllitidis* is known to be an extremely variable species and probably is polyphyletic. However, one distinctive feature that remains relatively constant for specimens belonging to this species is the persistency of the conidia, which remain attached to the conidiogenous cells for a long time (Braun et al. 2013). This feature is absent in *Ps. serpocaulonicola*. Additionally, *Ps. phyllitidis* has immersed stromata (ill-formed and subcuticular in *Ps. serpocaulonicola*) and moderately wider conidiophores

(1.5–4 µm), compared to *Ps. serpocaulonicola* (2–3.5 µm) (Braun et al. 2013). This is the first record of a fungus causing disease on *S. triseriale*.

***Pseudocercospora thelypteridis*** Goh & W.H. Hsieh, Trans. Mycol. Soc. Repub. China 4: 30. 1989. — Fig. 20

Description in planta — *Frond spots* amphigenous, irregular, starting from the main vein and spreading until the edges of the pinnulets, dark brown to black, sometimes reaching the entire pinnule. *Caespituli* hypophyllous, abundant. *External*



**Fig. 20** *Pseudocercospora thelypteridis* (CPC 24676). a–d. Frond spots on *Thelypteris* sp.; e. conidiophores arising from the stroma; f–h. conidia; i. culture on MEA. — Scale bars = 10 µm.

*hyphae* absent. *Internal hyphae* intra- and intercellular, septate, branched, subhyaline, smooth. *Stromata* subepidermal, discoid, composed of *textura angularis*, 19 × 44.5 µm, pale to dark brown. *Conidiophores* arising from stromata, reduced to the conidiogenous cells, hypophylloous, forming dense fascicles (more than 40 stalks per fascicle), subcylindrical, attenuated at the tip, straight, 14–23 × 2.5–4 µm, unbranched, aseptate, eguttulate, subhyaline, smooth, scars inconspicuous, 1 per cell, 2–2.5 µm, not thickened, nor darkened. *Conidia* solitary, subcylindrical to acicular, straight or slightly curved, 65–96 × 2.5–4 µm, obtuse to round apex, base truncate, 5–8-septate, guttulate, subhyaline, smooth; hila not thickened, nor darkened, 2–2.5 µm diam.

Culture characteristics — Colonies on MEA slow-growing, 41 mm diam after 20 d in the dark; surface smooth with even margins, flat, cottony aerial mycelium, surface olivaceous grey mixed with zones of pale olivaceous grey; iron-grey reverse; cultures sterile.

Specimen examined. BRAZIL, Rio de Janeiro, Nova Friburgo, Mury, near a waterfall, growing over humid rocks, on fronds of *Thelypteris* sp., 5 Nov. 2011, R.W. Barreto (VIC 42569, CBS H-22102, culture CPC 24676).

Notes — *Pseudocercospora thelypteridis* clusters basal to a clade including several species of *Pseudocercospora*, e.g. *Ps. basilaminae*, *Ps. crocea*, *Ps. dianellae*, *Ps. humuli-japonici*, *Ps. humulicola*, *Ps. plectranthi*, *Ps. profusa* and *Ps. rhabdothamni*, while *Ps. cyatheicola* and *Ps. rumohrae* clusters basal to *Ps. thely-*

*teridis* (Fig. 2). However, *Ps. cyatheicola* is different from *Ps. thelypteridis* by having erumpent and amphigenous stromata, longer and narrower conidiophores (30–70 × 2–3 µm), percurrently proliferating conidiogenous cells, and pale brown conidia (Crous et al. 2011). *Pseudocercospora rumohrae* differs from the new species by the absence of stromata, with conidiophores arising directly from the hyphae, as well as longer and narrower conidia (60–120 × 3–3.5 µm) (Braun et al. 2013).

*Pseudocercospora thelypteridis* is known from the type material on *Thelypteris laxa* from Taiwan and China, and on *Nephrolepis* sp. from Brunei (Braun et al. 2013). However, as the morphology and biometric data are quite similar, we chose not to introduce a novel species for the fungus found in Brazil. This is the first record of *P. thelypteridis* from Brazil.

***Pseudocercospora trichogena* Guatimosim, R.W. Barreto & Crous, sp. nov.** — MycoBank MB812827; Fig. 21

*Etymology.* Name derived from the trichomata habit of the species.

Description in planta — *Frond spots* on *Deparia petersenii*, amphigenous, evident adaxially, irregular, pale brown with necrotic fertile centre and distinctive black halo. *Ascomata* pseudothecial, epiphyllous, solitary, subepidermal to erumpent, globose to subglobose, 42–81 × 37–60 µm, walls of 2–3 layers of brown to dark brown *textura angularis*, cells 3–4 × 2–3 µm, black, ostiole central, 12–25 µm diam. *Ascii* bitunicate, apophysate,



**Fig. 21** *Pseudocercospora trichogena* (aseexual morph CPC 24664, sexual morph CPC 24670). a. Frond spots on *Deparia petersenii*; b. frond spots on *Macrothelypteris torresiana*; c. erumpent subcuticular ascomata, fruiting epiphyllous; d. conidia sporulating on a trichoma, hypophylloous; e. ascii; f. ascospores; g. detail of the external hyphae arising through the stoma, and growing along the trichoma; h. conidiophores; i. j. conidia; k. culture on MEA. — Scale bars = 10 µm.

sessile, 8-spored, fusoid-ellipsoidal when immature, pyriform at maturity, curved,  $26–42 \times 8–14 \mu\text{m}$ , hyaline, smooth. Ascospores biseriate to inordinate, overlapping, fusoid, straight,  $9–15 \times 2–4 \mu\text{m}$ , 1-septate, with one cell larger than the other, tapering towards rounded ends, guttulate, hyaline, thin-walled, smooth. Ascospore germination not observed. Asexual morph: Frond spots on *Macrothelypteris torresiana*, amphigenous, irregular, starting from the main vein of the pinnule, and spreading towards the edge, initially pale brown, becoming dark and necrotic. *Caespituli* hypophyllous, abundant on trichomata. External hyphae hypophyllous, abundant, often erupting through the cuticle, rarely arising through the stoma, and growing along the trichoma, spreading and covering the entire lesion,  $2–3 \mu\text{m}$  wide, branched, septate, pale brown, smooth. Internal hyphae intra- and intercellular, abundant,  $1–3 \mu\text{m}$  wide, prominently branched, septate, subhyaline, smooth. Stromata absent. Conidiophores arising from external hyphae, hypophyllous, often reduced to conidiogenous cells, formed in groups on trichomata, subcylindrical, attenuated at the tip, straight or sinuous,  $19–74 \times 5–6 \mu\text{m}$ , often branched, 1–5-septate, eguttulate, pale brown to brown, smooth. Conidiogenous cells terminal, integrated, holoblastic, subcylindrical, determinate,  $10–35 \times 5–6 \mu\text{m}$ , pale brown to brown, smooth, scars inconspicuous, 1 per cell,  $1–2 \mu\text{m}$ , not thickened, nor darkened. Conidia solitary, obclavate, straight or curved,  $72–147 \times 3–5 \mu\text{m}$ , apex rounded, base truncate, 4–13-septate, guttulate, pale brown, smooth; hila not thickened, nor darkened,  $1–2 \mu\text{m}$  diam.

Culture characteristics — Colonies on MEA slow-growing,  $10–23 \text{ mm diam}$  after 20 d in the dark; smooth to folded or concentrically folded, raised, aerial mycelium cottony or velvety, mouse grey, pale olivaceous grey or lavender grey; purplish grey or iron-grey in reverse; cultures sterile.

Specimens examined. BRAZIL, Rio de Janeiro, Nova Friburgo, Limeira, on fronds of *Macrothelypteris torresiana*, asexual morph, 13 June 2011, R.W. Barreto (holotype CBS H-22104, isotype VIC 42542, cultures ex-type CPC 24664, COAD 1087); Rio de Janeiro, Faz. Barreto II, Alto do Micheis, Riograndina, reforestation area, on fronds of *Deparia petersenii*, sexual morph, 13 June 2011, R.W. Barreto, (CBS H-22103, VIC 42546, cultures CPC 24670, COAD 1088).

Notes — Sexual and asexual morphs of *Ps. trichogena* were found in the same region but on different hosts. However, based on DNA phylogenetic analyses, there is no doubt that they belong to the same species. Phylogenetically, *Ps. trichogena* clusters in a highly diverse clade, differing from all species within it (Fig. 2). Morphologically, *Ps. trichogena* is similar to three other species recorded on *Theleppteridaceae*, namely *Ps. abacopteridicola* on *Abacopteris urophylla* from Singapore, *Ps. pteridophytophila* on *Cyclosorus acuminatus* from Asia and *Ps. thelypteridis* on *Nephrolepis* sp. and *Theleppteris laxa* from Asia (Braun et al. 2013, Farr & Rossman 2015). Among those, *Ps. pteridophytophila* is the only species for which there is molecular data available in GenBank (Kirschner & Liu 2014), though the ITS region differs from *Ps. trichogena* by 8 bp. Additionally,

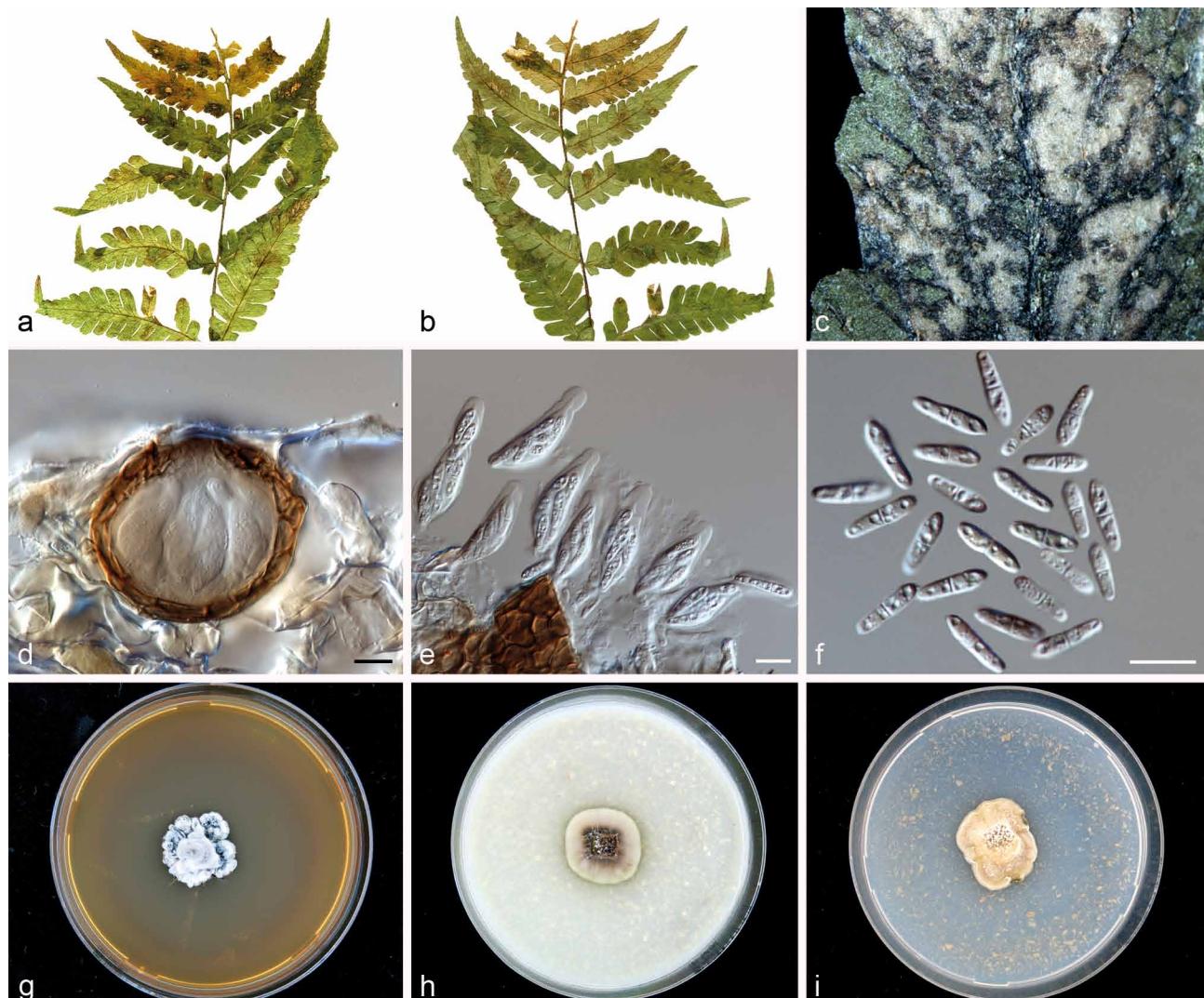


Fig. 22 *Xenomycesphaerella diplazii* (CPC 24691). a, b. Frond spots on *Diplazium* sp.; c. erumpent subcuticular ascomata, fruiting epiphyllous; d. vertical section of the ascoma; e. ascii; f. ascospores; g. culture on MEA; h. culture on OA; i. culture on PDA. — Scale bars =  $10 \mu\text{m}$ .

*Ps. pteridophytophila* and *Ps. thelypteris* differ from *Ps. trichogena* by having well-developed stromata, arising from the stomata with narrower conidiophores, 2–5 µm and 2–3 µm, respectively (Hsieh & Goh 1990), while *Ps. abacopteridicola* has narrower and smaller conidia (30–80 × 2–3 µm) and conidiophores (5–15 × 2.5–3 µm) (Yen & Lim 1980). *Pseudocercospora trichogena* is the first species of *Pseudocercospora* with a trichomatose habit recorded on ferns.

**Xenomycosphaerella** Quaedvlieg & Crous, Persoonia 33: 24. 2014

Notes — The genus *Xenomycosphaerella* is based on *X. elongata*, which occurs on *Eucalyptus camaldulensis* × *urophylla* from Venezuela (Crous et al. 2007b). So far, only sexual morphs are known for the genus, and because they are morphologically similar to *Mycosphaerella*, they were allocated to *Xenomycosphaerella* based solely on phylogenetic inference (Quaedvlieg et al. 2014).

**Xenomycosphaerella diplazii** Guatimosim, R.W. Barreto & Crous, sp. nov. — MycoBank MB812818; Fig. 22

*Etymology.* Name refers to the host genus from which it was isolated, *Diplazium*.

Description in planta — *Frond spots* random on pinnulets, but more intense on the pinnule apices, amphigenous, irregular, starting as a dark brown spot at the main vein of the pinnule, expanding towards the margins of the pinnulets, becoming centrally necrotic, with a fertile cream central area with a distinct dark brown to black halo. *External hyphae* absent. *Internal hyphae* intra- and intercellular, 2–4 µm wide, septate, branched, subhyaline, smooth. *Ascomata* pseudothelial, epiphyllous, solitary, subcuticular to erumpent, globose, 50–55 × 55–128 µm, walls of 1–2 layers of pale to dark brown *textura angularis*, cells 7–12 × 4–7 µm, ostiole central, 9–22 µm diam. *Asci* bitunicate, apophysate, fasciculate, subsessile, 8-spored, obovoid to broadly ellipsoidal, straight or slightly curved, 28–42 × 9–13 µm, hyaline, smooth. *Ascospores* inordinate, overlapping, fusoid, straight or slightly curved, 7–13 × 1.5–3 µm, medianly 1-septate, tapering towards rounded ends, narrower towards the lower end, guttulate, hyaline, thin-walled, smooth. *Asexual morph* not known.

Culture characteristics — Colonies on MEA slow-growing, 25 mm diam after 24 d; raised, crustose, with lobate, feathery margins and cottony aerial mycelium at periphery, lavender grey centrally, and lavender grey mixed with leaden grey at periphery; leaden black reverse. On OA flat, aerial mycelium sparse, olivaceous grey centrally, buff to rosy buff periphery; cinnamon reverse. On PDA raised, yeast-like, rosy buff centrally, buff at the periphery; buff reverse; cultures sterile.

*Specimen examined.* BRAZIL, Rio de Janeiro, Macaé de Cima, road to Fazenda Ouro Verde, on fronds of *Diplazium* sp., 29 Apr. 2012, R.W. Barreto (holotype CBS H-22076, isotype VIC 42565, culture ex-type CPC 24691).

Notes — Based on morphology, *X. diplazii* is similar to *X. elongata*, but differs from the latter by having smaller asci (45–60 µm long in *X. elongata*) and smaller and narrower ascospores, not constricted at the septum (20–25 × 4–5 µm, constricted at the septum in *X. elongata*) (Crous et al. 2007b). Phylogenetically (Fig. 3), *X. diplazii* differs from *X. elongata* by 51 bp for *act*, 69 bp for *ITS*, 26 bp for *LSU* and 96 bp for *tef1*. All attempts to induce sporulation of *X. diplazii* have thus far proven unsuccessful. Currently, members of the genus *Xenomycosphaerella* are restricted to South America (Brazil and Venezuela).

**Zasmidium** Fr., Summa Veg. Scand., section Post. (Stockholm): 407. 1849

Notes — The genus *Zasmidium*, based on *Z. cellare*, comprises species with conspicuously thickened, darkened conidiogenous loci and hila, as typical of *Stenella* (Braun et al. 2013). However, *Stenella* has wide, flat conidial hila and scars, and clusters within *Teratosphaeriaceae*, while *Zasmidium* has planate and somewhat thickened and darkened conidial hila and scars, and clusters within *Mycosphaerellaceae* (Arzanlou et al. 2007, Braun et al. 2013, Quaedvlieg et al. 2014).

**Zasmidium** sp.

Culture characteristics — Colonies on MEA slow-growing, 53 mm diam after 24 d; flat, with undulate, lobate, feathery margins, mycelium centrally immersed, and velvety aerial mycelium periphery, vinaceous buff centrally, pale mouse grey periphery; isabelline centrally and iron-grey periphery reverse. On OA and PDA lavender grey with iron-grey periphery; olivaceous grey reverse; cultures sterile.

*Specimen examined.* BRAZIL, Paraná, Guaraguaçu, sand dune area, on fronds of *Blechnum serrulatum*, 1 Feb. 2012, R.W. Barreto (CBS H-22087, culture CPC 24679, COAD 1178).

Notes — Fungarium specimens of this fungus were in poor condition and no conidia were seen. Isolation was performed by conidiophore transfer only. All attempts to promote sporulation in vitro proved to be unsuccessful. It appears that this taxon is a cryptic lineage closely related to *Zasmidium australiensis*, described on the same host, *Blechnum serrulatum*, from Australia (Mulder 1989, Braun et al. 2013). Presently, there are no sequences or known cultures available for *Z. australiensis*.

**Zasmidium cyatheae** Guatimosim, R.W. Barreto & Crous, sp. nov. — MycoBank MB812819; Fig. 23

*Etymology.* Name refers to the host genus from which it was isolated, *Cyathea*.

Description in planta — *Frond spots* amphigenous, irregular, affecting random pinnulets, starting at the apex of the pinnulets leading firstly to dark brown to black necrosis of the pinnule apex, then spreading to the base, where a cream area appears causing a necrosis of entire pinnulets, and occasionally of the pinnae. *External hyphae* absent. *Internal hyphae* intra- and intercellular, 1.5–2 µm wide, branched, septate, subhyaline to pale brown, smooth. *Ascomata* pseudothelial, epiphyllous, solitary, subcuticular to erumpent, globose, 33–59 × 21–52 µm, walls of 2–3 layers of brown to dark brown *textura angularis*, cells 5–9 × 3–7 µm, ostiole central, 10–18 µm diam. *Asci* bitunicate, apophysate, fasciculate, subsessile, 8-spored, obpyriform, straight, 30–46 × 12–16 µm, hyaline, smooth. *Ascospores* inordinate, overlapping, fusoid, straight, 14–22 × 3–6 µm, medianly 1-septate, tapering towards both rounded ends, narrower towards the lower end, guttulate, hyaline, thin-walled, smooth. *Ascospore germination* not seen. *Asexual morph* not observed.

Culture characteristics — Colonies on MEA and PDA slow-growing, 31 mm diam after 24 d; raised, with smooth, feathery margins, aerial mycelium velvety, pale mouse grey centrally, iron-grey periphery, iron-grey reverse. On OA, aerial mycelium absent, centrally black, periphery of velvety mouse grey aerial mycelium, olivaceous grey reverse; cultures sterile.

*Specimen examined.* BRAZIL, São Paulo, Eldorado, vicinities of Parque Caverna do Diabo, Atlantic rainforest, on fronds of *Cyathea delgadii*, 13 Apr. 2013, E. Guatimosim (holotype CBS H-22086, isotype VIC 42526, cultures ex-type CPC 24725, COAD 1425).



**Fig. 23** *Zasmidium cyatheae* (CPC 24725). a, b. Frond spots on *Cyathea delgadii*; c. erumpent subcuticular ascomata, fruiting epiphyllous; d. vertical section of the ascoma; e. ascii; f. ascospores; g. culture on MEA; h. culture on OA; i. culture on PDA. — Scale bars = 10 µm.

**Notes** — Phylogenetically, *Z. cyatheae* clustered with *Z. xenoparkii* as sister clade (Fig. 3). *Zasmidium xenoparkii* was described on *Eucalyptus grandis* from Indonesia (Crous et al. 2006b). *Zasmidium cyatheae* is clearly different from *Z. xenoparkii* by having the following number of variable sites for each locus: 11 bp for *act*, 24 bp for *tef1* and 23 bp for ITS. The sexual morph (having mycosphaerella-like structures) is known for only two of the seven species of *Zasmidium* included in this study. These are *Z. citri* (described on *Citrus* sp. from USA) (Huang et al. 2015) and *Z. eucalyptorum* (collected on *Eucalyptus* sp. from Indonesia) (Whiteside 1972, Quaedvlieg et al. 2014). However, the ascospores of *Z. cyatheae* (14–22 × 3–6 µm) are longer and wider than those of *Z. citri* (6–11 × 2–3 µm) and *Z. eucalyptorum* (12–17 × 3.5–4.5 µm) (Whiteside 1972, Crous et al. 2006b). This is the first record of a *Zasmidium* species from *Cyatheaceae*.

## DISCUSSION

The present survey presents a phylogenetic overview of the cercosporoid taxa and related sexual morphs that were collected during a systematic survey of fern fungi from Brazil. Quaedvlieg et al. (2014) recently provided a phylogenetic overview of fungi clustering in the *Teratosphaeriaceae*. In the latter study, the authors focused on pathogens of *Eucalyptus*, which makes it interesting to compare to the Brazilian fern fungi, as this could provide an insight into the question if the fungi occurring on

ferns are somehow related to those attacking distant related taxa, such as *Eucalyptus*, or if they evolved independently with their fern hosts.

Forty-four cercosporoid species are known causing frond spots of *Pteridophyta* worldwide: 13 *Cercospora* spp., two *Passalora* spp., 28 *Pseudocercospora* spp. and one *Zasmidium* sp. (Braun et al. 2013). Although no pathogenicity tests were done, all species described on the present study were found associated with frond spot symptoms, indicating their probable habit as pathogens. However, further studies are necessary to clarify the pathogenicity of these fungi on ferns.

Most *Cercospora* species are morphologically very similar to taxa occurring in the *C. apii* species complex (Braun et al. 2013). In the present study, we were able to identify one new *Cercospora* species, and demonstrate that the host range of *C. coniogrammes* is wider than previously known, including ferns belonging to two additional families. Plant hosts from *Pteridophyta* represent some of the oldest lineages of vascular plants (Smith et al. 2008). It is interesting to note that *C. coniogrammes* is on one hand proving to have a wider host range within the *Pteridophyta* and, on the other hand, found to be basal in the phylogeny of the genus *Cercospora* (Groenewald et al. 2013; Fig. 1).

As for *Pseudocercospora*, a long list of names have been published for which there are no DNA data and ex-type cultures available (Braun et al. 2013), complicating a better understand-

ing of the taxonomy of the genus. Recollecting and epitypifying all these species is a challenging, but necessary task for mycologists dealing with cercosporoid fungi. Three examples of taxonomic decisions that are still pending even after the present study involve *Ps. abacopteridicola*, *Ps. lygodiicola* and *Ps. thelypteridis*. Although we suspect that these Brazilian collections may in fact represent novel species, this can only be confirmed after the recollection of fresh materials from the type localities (Singapore, China and Taiwan, respectively – Yen & Lim 1980, Braun et al. 2013), followed by epitypification and a phylogenetic comparison.

Historically, the taxonomy of cercosporoid fungi has been based upon morphological and ecological features, including conjectured host specificity (Chupp 1954, Deighton 1965, 1971, 1973, 1974, 1976, Pons & Sutton 1988, Braun 1993a, b, c, 1995, 1998, Crous & Braun 1996, Braun & Mel'nik 1997, Crous et al. 2000, Braun et al. 2013, 2014, 2015). It is now widely accepted that this was an inadequate basis for the taxonomy of this complex plethora of fungi. Two examples of potentially mistaken conclusions based on morphology, symptoms and host-association alone were provided by our results:

1. *Zasmidium cyatheae* (only sexual morph found) and *Neoceratosperma cyatheae* (only asexual morph found), co-occurred on the same frond spot, on the fern *Cyathea delgadii*. Without pure cultures and access to molecular data the mistaken conclusion would be that *Z. cyatheae* was the sexual morph of *N. cyatheae*.
2. A similar situation occurred for *Paramycosphaerella sticheri* and *Clypeosphaerella sticheri*. Both were found attacking two different species in the same host genus *Sticherus* causing similar disease symptoms. It is likely that many conjectured connections between asexual and sexual morphs have been mistakenly made for cercosporoids and other fungal groups. Efforts towards clarifying these connections with modern criteria should be continued in order to generate an appropriate and consolidated taxonomy of cercosporoids and other fungal groups (Taylor et al. 2000, Crous & Groenewald 2005, Crous et al. 2009f, 2015b, Quaedvlieg et al. 2014).

In the past, mycologists have hypothesized that plant pathogenic fungi associated with primitive plants were also evolutionarily basal to the evolution of fungi. Thus, Savile (1971) proposed that primitive plant hosts, such as ferns, would have primitive rust genera. Later, phylogenetic studies involving rust species in different genera have proven this hypothesis wrong. For example, *Hemileia* and *Maravalia* – sister genera at the base of the *Pucciniales* phylogenetic tree (Wingfield et al. 2004, Aime 2006) – are pathogens of higher plant taxa, especially in the *Rubiaceae* and *Asclepiadaceae*, respectively.

As for the cercosporoid and mycosphaerella-like species documented here, there is some evidence that the fungal species associated with ferns are evolutionarily basal to the evolution of their relatives. In the *Cercospora* phylogeny (Fig. 1), *C. coniogrammes* (recorded only from ferns) is basal to the evolution of all other *Cercospora* species, whilst the same pattern is reproduced in the *Pseudocercospora* phylogeny (Fig. 2), where *Ps. cyatheicola*, *Ps. lygodiicola*, *Ps. rumohrae* and *Ps. thelypteridis*, all isolated from ferns, appear to be evolutionarily basal in the clade where they cluster; in the phylogeny of mycosphaerella-like taxa (Fig. 3), a basal position was observed for *Phaeophleospora hymenocallidis*, *Ps. hymenocallidicola* and *Ps. pteridivora* (all from ferns), appearing evolutionarily basal to all other species in the genus for which sequence data were available.

As more sequence data become available for cercosporoids associated with ferns, this preliminary evidence may become

stronger and allow for an elucidation of further cercosporoid genealogies and, hence, should permit a better understanding of the co-evolutionary history of this fungal group and its association with host plants.

The present study has significantly expanded our knowledge of cercosporoid and mycosphaerella-like fungi associated with frond spots in Brazilian *Pteridophyta*. Previously, only one cercosporoid and one mycosphaerella-like species (*Ps. davallicola* and '*Mycosphaerella*' *tocoyenae*, respectively) were known to be associated with diseases on ferns in Brazil (Farr & Rossman 2015, Mendes & Urben 2015). The present work has expanded this number significantly by adding one new genus (*Clypeosphaerella*) and 15 new species to this list. Here we also provide novel molecular information that may be useful to obtain a better understanding of the evolution of cercosporoid and mycosphaerella-like fungi. We also hope that further exploration of these cultures will contribute in the future to a more robust phylogeny of these fungi across various families of host plants, and help establishing a better understanding of their host specificity and evolution. The clear abundance of novel taxa collected on ferns in Brazil, also underlines the scientific value of host or host-group based surveys as a source of mycological novelties. Finally, our findings confirm that mycologists in the tropics have thus far given little attention to fungi occurring on plant hosts with apparent limited economic relevance, such as ferns. Fern fungi in Brazil and other tropical regions are likely to represent an important source of a highly diverse mycoflora that still awaits discovery.

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