EDUARDO GUATIMOSIM

MICROFUNGOS FITOPATOGÊNICOS EM PTERIDÓFITAS

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

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Olinto Liparini Pereira

Pedro Bond Schwartsburd
(Coorientador)

Harold Charles Evans

José Luiz Bezerra

Robert Weingart Barreto
(Orientador)

A Luiz e Vera, querido casal que me acolheu com grande carinho, dedico.

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BIOGRAFIA

EDUARDO GUATIMOSIM, filho de Antônio Augusto Magalhães Guatimosim e Maria Aparecida Abreu Guatimosim, nasceu na cidade de Belo Horizonte – MG, no dia 08 de março de 1984, onde cursou o ensino fundamental e médio, concluindo-os em Dezembro de 2001.

Em 2002 iniciou o curso de Engenharia de Agronomia na Universidade Federal de Viçosa, graduando-se em janeiro de 2008.

Ainda em 2008, trabalhou na empresa Ambienta Soluções Ambientais, executando a função de Analista Ambiental, até fevereiro de 2009.

Em março de 2009, iniciou seus estudo no programa de Mestrado em Fitopatologia na Universidade Federal de Viçosa, onde defendeu sua dissertação em fevereiro de 2011. Ainda em 2011, iniciou o programa de Doutorado em Fitopatologia na mesma instituição.

Em 2014, desenvolveu o doutorado sanduíche no Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre na Holanda, onde realizou parte de sua pesquisa visando a obtenção do título de doutor por um ano, tendo a oportunidade de conhecer e aprofundar seus estudos com um dos grupos de pesquisa mais ativos no cenário atual – o grupo de "Fitopatologia Evolutiva" no CBS, sob liderança do Dr. Prof. Pedro W. Crous.

SUMÁRIO

RESUMO

GUATIMOSIM, Eduardo, D.Sc., Universidade Federal de Viçosa, junho de 2015. **Microfungos fitopatogênicos em pteridófitas.** Orientador: Robert Weingart Barreto. Coorientador: Pedro Bond Schwartsburd.

Um estudo sobre fungos supostamente fitopatogênicos relacionados a plantas de *Pteridophyta* no Brasil foi realizado. Plantas desta divisão, comumente conhecidas como samambaias, têm ligações evolutivas diretas com as primeiras plantas vasculares que apareceram no final do período Devoniano. Conhecer a micobiota associada a este grupo de plantas é crucial para uma compreensão completa dos fungos e sua história evolutiva. No entanto, talvez devido a baixa relevância econômica das samambaias, este nicho permanece negligenciado pelos micologistas. Espécimes oriundos de diferentes biomas brasileiros, foram coletados durante sete anos de estudos (2009–2015). O total 180 isolados fúngicos, recuperados de 40 espécies de plantas hospedeiras em 135 diferentes localidades resultaram, até o presente momento na descrição de 23 novas espécies, a saber: *Bloxamia cyatheicola, Cercospora samambaiae, Chalara cyatheae, Chalara lygodii, Inocyclus angularis, Lachnum catarinense, Lembosia abaxialis, Paramycosphaerella blechni, Paramycosphaerella cyatheae, Paramycosphaerella dicranopteridis-flexuosae, Paramycosphaerella sticheri, Phaeophleospora pteridivora, Psilachnum pteridii, Pseudocercospora brackenicola, Pseudocercospora paranaensis, Pseudocercospora trichogena, Pseudocercospora serpocaulonicola, Clypeosphaerella sticheri, Rhagadolobiopsis thelypteridis, Xenomycosphaerella alsophilae, Xenomycosphaerella cyatheae, Xenomycosphaerella diplazii* e *Zasmidium cyatheae*, e dois novos gêneros: *Clypeosphaerella* e *Rhagadolobiopsis*. Adicionalmente, durante o estudo que visou elucidar o posicionamento evolutivo da ordem *Asterinales*, uma nova família – *Asterotexiaceae* (não relacionada à fungos oriundos de samambaias) – foi proposta, bem como o posicionamento filogenético dos gêrenos *Batistinulla* e *Prillieuxina* foi elucidado. Ademais, 4 novos relatos foram realizados no Brasil para as espécies *Cercospora coniogrammes*, *Pseudocercospora abacopteridicola*, *Pseudocercospora lygodiicola* e *Pseudocercospora thelypteridis,* bem como, 6 novas associações fungo-hospedeiro para as espécies *Cercospora* sp. e *Lachnum varians*.

Desta forma, o presente trabalho permitiu uma melhor compreensão da biodiversidade de microfungos relacionada a pteridófitas no Brasil.

ABSTRACT

GUATIMOSIM, Eduardo, D.Sc., Universidade Federal de Viçosa, June, 2015. **Plant pathogenic microfungi on ferns.** Adviser: Robert Weingart Barreto. Co-Adviser: Pedro Bond Schwartsburd.

A systematic survey of supposedly pathogenic fungi associated with plants belonging to *Pteridophyta* from Brazil was carried out. These plants, commonly known as ferns or 'samambaias' in Brazil, have direct evolutionary links with the earliest vascular plants that appeared in the late Devonian period. Knowing the mycota associated with this group of plants is critical for a full understanding of the Fungi and their evolutionary history. Nevertheless, perhaps because of the minor economic significance of ferns, this niche remains neglected by mycologists. Specimens obtained from different biomes in Brazil, over seven years (2009–2015), were collected. A total amount of 180 isolates was obtained from 40 host species in 135 different localities, resulting, so far, in the description of 23 species that were or will be described as new to science, namely: *Bloxamia cyatheicola, Cercospora samambaiae, Chalara cyatheae, Chalara lygodii, Inocyclus angularis, Lachnum catarinenses, Lembosia abaxialis, Paramycosphaerella blechni, Paramycosphaerella cyatheae, Paramycosphaerella dicranopteridis-flexuosae, Paramycosphaerella sticheri, Phaeophleospora pteridivora, Psilachnum pteridii, Pseudocercospora brackenicola, Pseudocercospora paranaensis, Pseudocercospora trichogena, Pseudocercospora serpocaulonicola, Clypeosphaerella sticheri, Rhagadolobiopsis thelypteridis, Xenomycosphaerella alsophilae, Xenomycosphaerella cyatheae, Xenomycosphaerella diplazii* and *Zasmidium cyatheae,* and two novel genera, namely *Clypeosphaerella* and *Rhagadolobiopsis*. Additionally, during a study aimed at elucidating the phylogenetic placement of the *Asterinales* one new family (not including fungi on ferns) - the *Asterotexiaceae* – was proposed and the placement of the genera *Batistinulla* and *Prillieuxina* was clarified. Aditionally, 4 new records were recognised in Brazil for the following known fungal species: *Cercospora coniogrammes*, *Pseudocercospora abacopteridicola*, *Pseudocercospora lygodiicola* and *Pseudocercospora thelypteridis*, and 6 new fungus-host associations for the species *Cercospora* sp. and *Lachnum varians.* The present work allowed a better understanding

of the pathogenic microfungi biodiversity on *Pteridophyta* in Brazil and opens a new field of research for Brazilian mycologists and plant pathologists.

INTRODUÇÃO GERAL

O desafio de se estimar o tamanho da diversidade da micobiota mundial foi abordado pela primeira vez no trabalho pioneiro de Hawksworth (1991). Desde então, vários trabalhos têm lidado com estimativas do número de fungos existentes no mundo (Hawksworth & Rossman 1997, Hyde 2001, Hawksworth 2001, 2004, Bass & Richards 2011, Blackwell 2011, Fisher et al. 2012). Seja qual for o tamanho desta diversidade, desafio maior está em descrever as espécies de fungos antes que as alterações globais impostas pela atividade humana levem-nas à extinção. Estratégias e metodologias de coleta e descrição de fungos desconhecidos para a ciência foram desenvolvidas e livros inteiros foram dedicados a este tema (por exemplo, Mueller et al. 2004). Uma estratégia para a expansão do conhecimento sobre os fungos existentes é a de se estudar a micobiota associada a espécies de plantas selecionadas (Alves et al. 2010, Rocha et al. 2010) ou a grupos de plantas para as quais a micobiota é ainda pouco conhecida. Um desses grupos é composto pelas espécies brasileiras de pteridófitas, objeto deste estudo.

Na recente classificação (Smith et al. 2006), *Pteridophyta* (= *Moniliophyta*), excluindo-se as Licófitas (*Lycopodiophyta*), representa um grupo incluindo 37 famílias, aproximadamente 300 gêneros e mais de 9.000 espécies. No Brasil existem cerca de 1200 espécies conhecidas, porém estima-se que existam ainda mais (Lista de Espécies da Flora do Brasil 2012). Duas espécies se destacam por serem consideradas plantas invasoras de importância mundial: *Pteridium arachnoideum* (Kaulf.) Maxon (Dennstaedtiaceae), e *Salvinia molesta* D.S. Mitch. (Salviniaceae) (Holm et al. 1996). Outra espécie de grande destaque no Brasil é *Dicksonia sellowiana* Hook. (Dicksoniaceae), o xaxim, planta arborescente que no passado foi comum em áreas de Mata Atlântica e que, atualmente, encontra-se incluída na lista de espécies ameaçadas de extinção da flora brasileira (Biondi et al. 2009), devido à exploração excessiva para uso como substrato vegetal e fabricação de vasos.

No entanto, em sua grande maioria, espécies nativas pertencentes pteridófitas tem pouca "visibilidade", sendo desconhecidas do público e apenas referidas por nomes genéricos como samambaias, avencas e xaxins. Talvez por isso não tenham sido até

hoje objeto de qualquer estudo sistemático por micologistas e fitopatologistas. É importante ressaltar que mesmo para a micobiota das espécies citadas como mais conhecidas, sabe-se muito pouco acerca dos fungos que a colonizam.

Dentre os reinos em que são classificados os organismos que compõe a biodiversidade global, os fungos representam uma porção geralmente negligenciada pela ciência. Estima-se que existem cerca de 3 milhões de espécies fúngicas no mundo, das quais, apenas 100.000 são conhecidas (Crous et al. 2015). A grande lacuna de conhecimento existente no campo da micologia representa um notável paradoxo quando se tem amplo reconhecimento de que os fungos desempenham papel fundamental na ecologia e manutenção dos ecossistemas (Dighton 2003, Stamets 2005). Sua função não está somente relacionada ao seu papel primordial no nível trófico dos decompositores – fundamentais para processos biogeoquímicos, como a ciclagem de nutrientes, vital para a manutenção e homeostase da biosfera (Grandi 2004, Grandi & de Valois Silva 2006), mas também ocupando uma grande variedade de nichos ecológicos, mantendo relações de extrema relevância com plantas, animais e outros organismos, inclusive com outros fungos. No que tange a associação com as plantas, os fungos podem estabelecer relações simbióticas mutualísticas – como nas micorrizas, nas colonizações endofíticas e nos liquens; comensalistas – como nos epibiontes que ocorrem epifiticamente sobre plantas; ou ainda como parasitas como no caso dos fungos fitopatogênicos.

Em função das elevadas perdas impostas por fungos fitopatogênicos a plantas cultivadas, toda uma disciplina (Fitopatologia), foi construída desde meados do século XIX, com uma orientação fortemente pautada no entendimento de associações de fungos fitopatogênicos com plantas cultivadas. O estudo dos fungos fitopatogênicos teve então como foco os prejuízos causados por sua ação, e assim, tais organismos foram tratados como exclusivamente maléficos aos interesses humanos. Entretanto sob um olhar mais cauteloso e abrangente pode-se constatar que as injúrias provocadas pela ação dos fungos (doença) sobre determinada planta é um processo comum e natural e que, as devastadoras epidemias responsáveis pela perda de produção em ambientes agrícolas, é fruto da forma de agricultura baseada em monoculturas, escolhida pelo homem ou da introdução inadvertida (usualmente pelo homem) de fungos fitopatogênicos exóticos antes ausentes de regiões agrícolas ou em ecossistemas naturais. Em busca da padronização do produto final, o atual modelo de agricultura

lançou mão da uniformidade aplicada em todos seus termos: genética, ambiental, de tratos culturais, etc. Este ambiente uniforme, quando favorável a determinado patógeno, tem por consequência o desenvolvimento de doença em larga escala e consequentemente (caso nenhuma intervenção seja realizada) a perda significativa da produção.

Fungos fitopatogênicos inspiram, justificado temor, não só pelos vultosos prejuízos impostos à produção agrícola, mas também catástrofes ambientais impostas a espécies vegetais em ecossistemas naturais, resultantes geralmente da introdução de espécies fúngicas exóticas. Dentre alguns dos exemplos notáveis, podem ser citados a "doença de Jarrah" desencadeada pela introdução de *Phytophthora cinnamomi* na Austrália; a destruição da castanheira norte americana por *Cryphonectria cubensis*; a "doença holandesa do olmo" na Europa e EUA causada por *Ophiostoma ulmi* (os dois últimos introduzidos a partir da Ásia) (Money 2006); as recentes epifitias ora em progresso na Califórnia pelo avanço de *Phytophthora ramorum* originária da Europa (Rizzo et al. 2002) e a mais recente destruição de freixos (*Fraxinus excelsior*) na Inglaterra, ocasionada pelo fungo *Chalara fraxinea*, o qual já destruiu mais de 100.000 indivíduos arbóreos de novembro de 2012 a janeiro de 2013 (BBC 2013). Apesar da nocividade dos fungos fitopatogênicos permear a literatura fitopatológica, sua ampla maioria não tem qualquer relevância para a produção agrícola e florestal. Há inclusive espécies de fungos fitopatogênicos que são desejáveis, como é o caso dos fungos que vem sendo estudados ao longo dos últimos quarenta anos como agentes de controle biológico de plantas daninhas (Barreto et al. 2012).

Nesta disciplina aproveita-se o papel dos fungos fitopatogênicos como bioreguladores de espécies de plantas em ecossistemas naturais. Seja por intermédio de introduções de tais fungos em situações onde a planta hospedeira (esta sim, indesejável por algum motivo) escapou de seus inimigos naturais, seja por manipulação visando a magnificação do impacto produzido pelo fungo, almejando-se a inversão da lógica usual, fazendo pois dos fungos fitopatogênicos, espécies benéficas. Além da revisão mais recente sobre o uso de fungos para o controle biológico de plantas daninhas (Barreto et al. 2012) várias outras revisões completas já foram publicadas sobre este tema, desde a primeira experiência prática efetuada no início dos anos 70 (Hasan 1974, Huffaker 1976, Hasan 1980, Evans 1987, Adams 1988, Ayres & Paul 1990 Evans &

Ellison 1990, Charudattan 1991, TeBeest et al. 1992. Julien & White 1997, Hallett 2005, Ghosheh 2005).

O presente trabalho contempla o estudo de fungos associados a diferentes espécies pteridófitas encontradas no Brasil, incluindo tanto a já citada invasora de importância mundial *Pteridium arachnoideum*, quanto *Dicksonia sellowiana,* espécie nativa ameaçada de extinsão. Durante os levantamentos feitos na região sudeste e sul do Brasil por fungos associados às duas espécies-alvo principais, todas as oportunidades que se apresentaram de coleta de fungos associados a pteridófitas foram aproveitadas. Desta forma, espécies de gêneros ecologicamente importantes e diversos como *Adiantum, Anemia, Blechnum, Ctenitis, Cyathea, Gleichenella, Lygodium, Macrothelypteris* (um gênero exótico), *Niphidium, Pecluma, Pteris, Rumohra, Serpocaulon, Sticherus* e *Thelypteris* foram coletadas e estudadas.

Estrutura da Tese

A pesquisa apresentada nesta tese se relaciona a vários aspectos taxonômicos de microfungos que tanto possuem conhecida importância como agentes causais de doenças em plantas agrícolas – como é o caso de cercosporóides e membros da família *Mycosphaerellaceae*, (p. ex.: agentes causais de manchas foliares em cultivos agrícolas e florestais), quanto fungos de menor importância econômica, mas que ainda se apresentam como pouco conhecidos pela ciência (como os membros da ordem *Asterinales*).

Capítulos da Tese

Capítulo 1: Novidades taxonômicas na família *Parmulariaceae*

A família *Parmulariaceae* abriga fungos ascomicetos, parasitas obrigatórios de diversos hospedeiros. Apesar de ter sido tema de diversos estudos e monografias, acredita-se ainda ser pouco conhecida e haver muitos táxons da família a serem descobertos. No presente capítulo, duas novidades táxonômicas são apresentadas: a nova espécie *Inocyclus angularis* e o novo gênero e espécie, *Rhagadolobiospsis thelypteridis*, ambos encontrados em associação com pteridófitas no Brasil. Informações acerca de sua distribuição, marcadores morfológicos-chave, bem como um detalhado

estudo da ontogenia do ascoma de *Rhagadolobiopsis,* são apresentadas. Este capítulo contempla dois artigos científicos já publicados em revistas internacionais.

Capítulo 2: O posicionamento filogenético da ordem *Asterinales*

Espécies das famílias *Asterinaceae* e *Parmulariaceae* são parasitas obrigatórios que crescem em associação com os tecidos do hospedeiro e produzem ascos bitunicados em ascomas externos, na superfície do hospedeiro. Sua classificação até os dias atuais se deu através, basicamente, de características morfológicas, as quais muitas vezes geram um sistema de classificação artificial e impreciso. No presente capítulo, é apresentada uma análise filogenética baseada nas regiões genômicas ITS e LSU, das espécies tipo de *Asterinaceae* e *Parmulariaceae*, bem como de outros gêneros relacionados, permitindo elucidar o posicionamento da ordem *Asterinales* dentro da classe *Dothideomycetes*. Adicionalmente, uma nova ordem (*Asterotexiales*) foi proposta, a fim de abrigar a nova família *Asterotexiaceae*. Por fim, a nova espécie *Lembosia abaxialis*, e o posicionamento filogenético de *Bastitinula* e *Prillieuxina* (membros de *Asterinaceae*) foram apresentados. Este trabalho já se encontra disponível on line, na plataforma da revista Persoonia (http://www.ingentaconnect.com/content/nhn/pimj/pre-prints).

Capítulo 3: Espécies de cercosporóides e suas formas sexuais em pteridófitas

Fungos cercosporóides respresentam um dos mais amplos grupos de hifomicetos e pertencem às famílias *Mycosphaerellaceae* e *Teratosphaeriaceae*. Incluem agentes causais de doenças que afetam importantes culturas. No presente estudo, um levantamento sistemático deste grupo de fungos, atacando pteridófitas no Brasil é apresentado. Através de uma minuciosa análise de caracteres morfológicos, da relação patógeno-hospedeiro, dos padrões de distribuição destes organismos, bem como da análise filogenética de cinco regiões genômicas (ITS, Fator de Elongação-1α, Actina, Calmodulina e LSU), foram identificadas 21 espécies fúngicas causando doenças em 18 espécies de plantas hospedeiras. Um novo gênero, 16 novas espécies e 8 novas recombinações são propostas, revelando uma rica diversidade de fungos atacando pteridófitas, no Brasil.

Capítulo 4: Microfungos em pteridófitas

Um levantamento sistemático de fungos patogênicos a samambaias no Brasil tem sido realizado nos últimos sete anos, contemplando a maioria das regiões brasileiras do Brasil. Com base na morfologia, relação fungo-hospedeiro e filogenia molecular inferida a partir de sequências de DNA de duas regiões genômicas (ITS e LSU), espécies pertencentes ao complexo conhecido como fungos lachinóides, *Chalara* e *Bloxamia* foram investigados. No presente capítulo são descritos e ilustrados cinco novos taxa a saber: *Bloxamia cyatheicola, Chalara lygodii, Chalara cyatheae, Lachnum catarinensis* e *Psilachnum pteridimi*. Adicionalmente, a espécie *Lachum varians* é descrita como um novo relato para o Brasil.

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Capítulo 1 – Novidades taxonômicas na família *Parmulariaceae*

Artigo 1 – *Rhagadolobiopsis*, a new genus of *Parmulariaceae* from Brazil with a description of the ontogeny of its ascomata. Mycologia, 106:276–281. 2014

Artigo 2 – A new *Inocyclus* species (*Parmulariaceae*) on the neotropical fern *Pleopeltis astrolepis*. IMA Fungus 5:51–55. 2014

Mycologia, 106(2), 2014, pp. 276–281. DOI: 10.3852/106.2.276

Rhagadolobiopsis, a new genus of Parmulariaceae from Brazil with a description of the ontogeny of its ascomata

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Abstract: During a survey of the mycobiota of selected Brazilian ferns we discovered a new genus of Parmulariaceae causing tar spot-like symptoms on leaves of *Thelypteris serrata* (Thelypteridaceae). The new genus and species, decribed as *Rhagadolobiopsis thelypteridis*, differs from morphologically similar species of *Rhagadolobium* in possessing colorless, aseptate ascospores and a hymenial gel that does not become blue with iodine. In addition this is the first record of a fungus on *T. serrata*, and the first Parmulariaceae recorded on a member of the Thelypteridaceae. The ontogeny of the ascomata is described and illustrated here for the first time for the Parmulariaceae.

Key words: Ascomycota, fungal biodiversity, Neotropics, taxonomic novelties

Introduction

The challenge of describing the world's mycobiota has become a priority since the pioneering work of Hawksworth (1991). Several authors subsequently have discussed the accuracy of estimates of the numbers of fungi (Hawksworth and Rossman 1997; Hyde 2001; Hawksworth 2001, 2004; Bass and Richards 2011; Blackwell 2011; Fisher et al. 2012) and proposed strategies for collecting and describing undiscovered fungal taxa (e.g. Mueller et al. 2004). One strategy for increasing our undersanding the diversity of these fungi involves the study of the mycobiota of selected plant taxa (Alves et al. 2010, Rocha et al. 2010) or groups of plants for which the mycobiota is poorly known. Ferns represent one such group of plants.

Since 2009, a survey focused at cataloguing the fungal biodiversity of Brazilian ferns has yielded a highly diverse and taxonomically interesting array of fungi. Included among these is a novel ascomycete producing symptoms similar to tar-spot disease on *Thelypteris serrata* (Cav.) Alston (Thelypteridaceae), a widespread fern native to Brazil that occurs in the Amazon forest, Cerrado and Atlantic forest. The new fungus found on this fern is described and discussed herein.

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Materials and Methods

Specimen collection, preparation and light microscopy.—*Thelypteris serrata* with tar-spot-like disease symptoms were collected in 2010 and 2012 in an Atlantic forest conservation area belonging to the Universidade Federal de Viçosa, the Mata do Paraíso (municipality of Viçosa, state of Minas Gerais, Brazil). This material was dried in a plant press. Samples were examined with a dissecting microscope, and freehand sections of fungi and structures scraped from the plant surfaces were mounted on microscope slides in lactophenol, lactofuchsin, Lugol's solution or Melzer's reagent. When necessary, sections were made with a Microm HM 520 freezing microtome. Observations of fungal structures and measurements (at least 30 structures measured) and in preparation of line drawings and photographs were performed with an Olympus BX51 light microscope fitted with a drawing tube and an Olympus E330 digital camera.

Ascospores ejection.—Ejection of ascospores was studied on plates of potato dextrose agar (Crous et al. 2009) by attaching leaf pieces (1 cm diam) containing fertile ascomata to the inside of the upper lids of Petri dishes with Vaseline with the ascomata facing the medium. Plates were left in a growth chamber adjusted to 25 ± 2 C under a light regime of 12 h, for 2 d. The same procedure was followed with ascomata mounted over microscope slides to collect the ejected ascospores.

Scanning electron microscopy.—Samples of dried material containing ascomata were mounted on stubs with double-side 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 analyze and capture images from the samples.

Results

The ascomata of the fungus on *Thelypteris serrata* resemble those of species of *Rhagadolobium* Henn. & Lindau (Parmulariaceae). However, this taxon differs from all genera assigned to the Parmulariaceae in the multiloculate, superficial ascomata that open by radiating fissures, the connection of the ascostromata to the host at several points by columns of mycelia arising through the stomata, and a hymenial gel that does not become blue with iodine. A new genus hence is proposed to accommodate this fungus.

Rhagadolobiopsis Guatimosim & R.W. Barreto, gen. nov.

MycoBank MB802297

Etymology: derived from *Rhagadolobium*, a morphologically similar member of the Parmulariaceae.

Typus: *Rhagadolobiopsis thelypteridis* Guatimosim & R.W. Barreto.

Ascomata multiloculate, opening by radiating fissures, connected to host tissue at several points by mycelial columns formed through the stomata. Hymenial gel not reacting with iodine. Interascal tissue absent. Asci bitunicate, cylindrical-clavate to clavate, non-amyloid, eight-spored. Ascospores fusiform, aseptate, hyaline, smooth.

Rhagadolobiopsis thelypteridis Guatimosim & R. W. Barreto, sp. nov. FIGS. 1–3

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Visible as numerous, scattered, black, stromata forming superficial, ellipsoid tar spot-like colonies on the abaxial surfaces of leaves, often slightly deformed at one edge, 0.7–1.5 × 0.6–1.1 mm. External mycelium absent. Internal mycelium intracellular and intercellular, branched, subhyaline. Haustoria coralloid, one to several per host cell, hyaline. External stromata connected to the host mesophyll at multiple points by peg-like columns of aggregated, subhyaline hyphae arising through stomata. Internal stromata absent. Ascomata dark brown to black, initially circular, becoming oblong or ellipsoidal, multiloculate, composed of radiating locules, ellipsoidal in horizontal section, often slightly deformed, with undulating surface and well delimited edges, 417–811 μm, composed of brown cells, 4 × 5 μm, that form a textura prismatica, entirely superficial in vertical section and easily detached from the leaf, delimited by a covering layer above the fertile locule and by a lower layer. Covering layer 5–9 μm thick, dark brown, composed of cells 2.5 × 5 μm that form a textura prismatica organized as a layer of densely pigmented cells that overly a layer of light brown cells. Layer beneath the hymenium adjacent to the host cuticle, 10–19 μm thick, composed of brown to light brown cells, 2.5 × 4 μm, that form a textura prismatica. Hymenium a thin basal cushion with asci immersed in a non-amyloid gelatinous stratum. Asci maturing sequentially, with young and mature asci in the same locule. Young asci variably shaped, forked at the base, cylindrical to clavate. Mature asci bitunicate, cylindrical-clavate to clavate, 27–46 × 6–14 μm, non-amyloid, eight-spored, slightly forked at the base. Ascospores fusiform rounded at one end and apiculate on the other end, 7.5–13 × 2.5–5 μm, aseptate, biguttulate, hyaline, smooth-walled, uniseriate or biseriate within the asci. Interascal tissue absent. Anamorph not observed.

Ontogeny: Mature ascostromata, which are visible to the unaided eye (FIG. 2B), are multiloculate colonies composed of confluent and fused individual ascomata. Initially, a tuft of sub-hyaline to light brown hyphae grows through each stoma (FIG. 3A). Each tuft develops into a single discoid ascoma (FIG. 3D) that grows and fuses with neighboring ascomata as the larger composite structure matures (FIG. 3E). The darkly pigmented portion of the covering layer open in rows exposing fertile locules in radiating fissures through the thinner, lightly pigmented cells composing of this layer (FIG. 2C short arrows). The hyphal columns that connect the ascomata with the host tissue can be observed after removal of the ascomata (FIG. 3H, I).

Holotype: BRAZIL. MINAS GERAIS: Viçosa, Mata do Paraíso, 20°49′35″S, 42°54′27″W, 650 m. On living leaves of *Thelypteris serrata* (Cav.) Alston, 12 Feb 2012, *E. Guatimosim, EG 156*, (VIC 31939).

Additional specimens examined: BRAZIL. MINAS GERAIS: Same location as holotype, 16 Jun 2012, *R.W. Barreto, RWB 1288* (VIC 31940); Ibid., 28 Jul 2010, *R.W. Barreto, RWB 1245A*, (VIC 31941).

Etymology: referring to the host genus.

Habitat: Wet margins of a creek, on living leaves of *T. serrata*.

Distribution: Known only from the type location.

DISCUSSION

Parmulariaceae occupy an uncertain position within the Dothideomycetes (Ascomycota) (Cannon and Kirk 2007, Hibbett et al. 2007, Kirk et al. 2008). A distinctive feature of the family is the variation in shape of ascomata, both within and between genera (Inácio and Cannon 2008). In *Rhagadolobiopsis*, as in *Parmularia*, ascomata initially have irregular shape but becomes circular, with radiating locules formed within a shield-like structure. The ascomata of *Rhagadolobiopsis* are superficial as are those of species of *Cocconia* Sacc., *Cycloschizon* Henn., *Cyclostomella* Pat., *Ferrarisia* Sacc., *Hysterostomella* Speg. and *Parmularia* Lév. (Inácio and Cannon 2008).

Five genera of Parmulariaceae are common on ferns: *Inocyclus* Theiss. & Sid., *Pachypatella* Theiss. & Sid., *Polycyclus* Höhn, *Polycyclina* Theiss. & Sid. and *Rhagadolobium* Henn. & Lindau (Inácio and Cannon 2008). Differences between these genera are summarized in the key below.

DICHOTOMOUS KEY FOR THE IDENTIFICATION OF GENERA OF PARMULARIACEAE ON FERNS

1 Ascomata opening by concentric rings 2
1′ Ascomata opening by radiating fissures 5

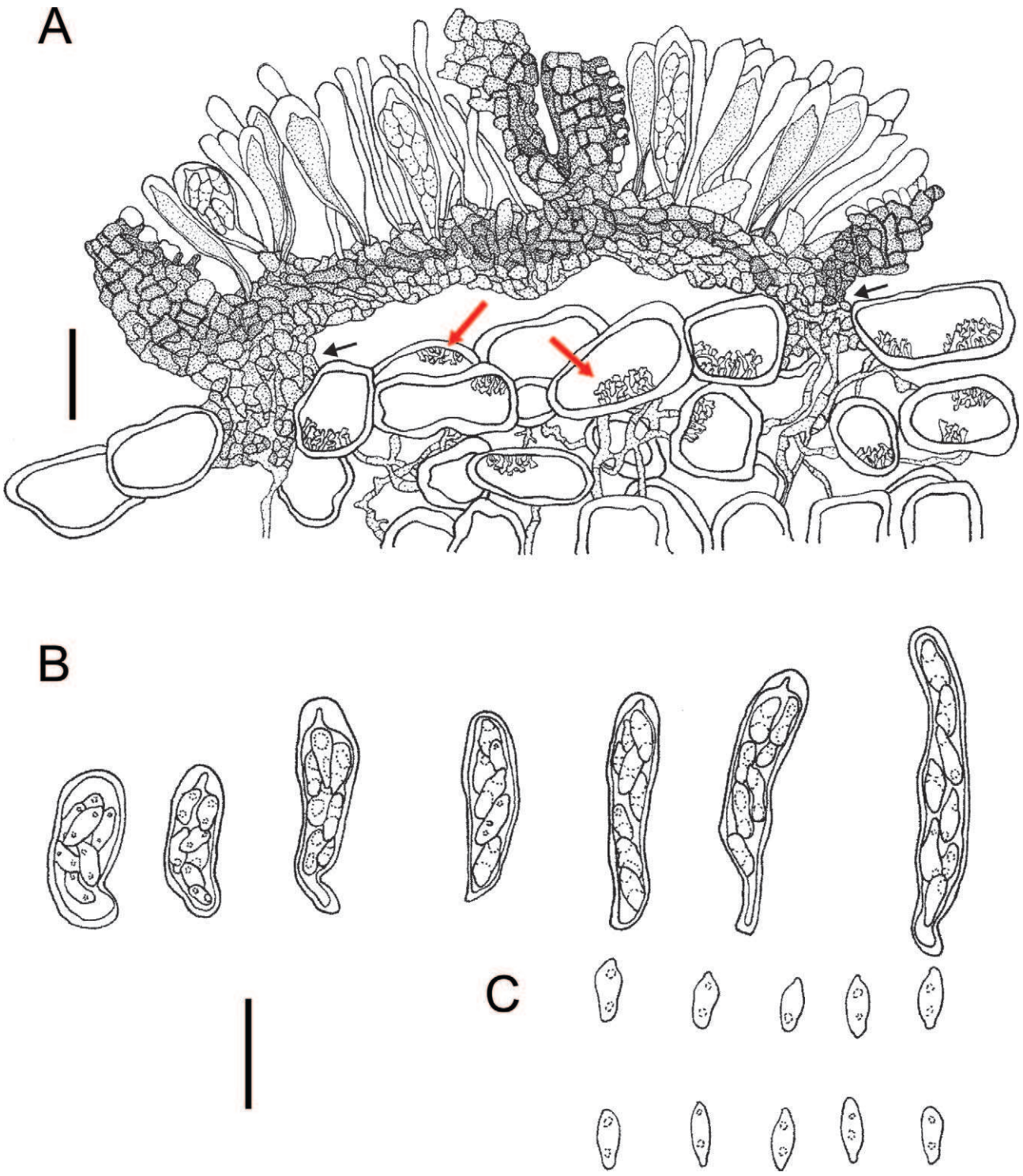

FIG. 1. *Rhagadolobiopsis thelypteridis* on *Thelypteris serrata* (VIC 31939). A. Vertical section showing superficial ascoma with fertile locules, connections to the host by peg-like columns through the stomata (short arrows), and collaroid haustoria inside the epidermal cells (long arrows). The upper layer of ascoma is not illustrated because it is fragile and very detached when sections are prepared. B. Mature bitunicate asci, with hyaline ascospores. C. Hyaline ejected ascospores. Bars = 20 μm.

2 Hymenial gel turning blue with iodine 3
2′ Hymenial gel not turning blue with iodine 4
3 Ascospores composed of cells of unequal size . . . . . *Inocyclus*
3′ Ascospores composed of cells of equal size. . . . . *Pachypatella*
4 Ascospores composed of cells of unequal size, internal stroma well developed . . . . . *Polycyclus*

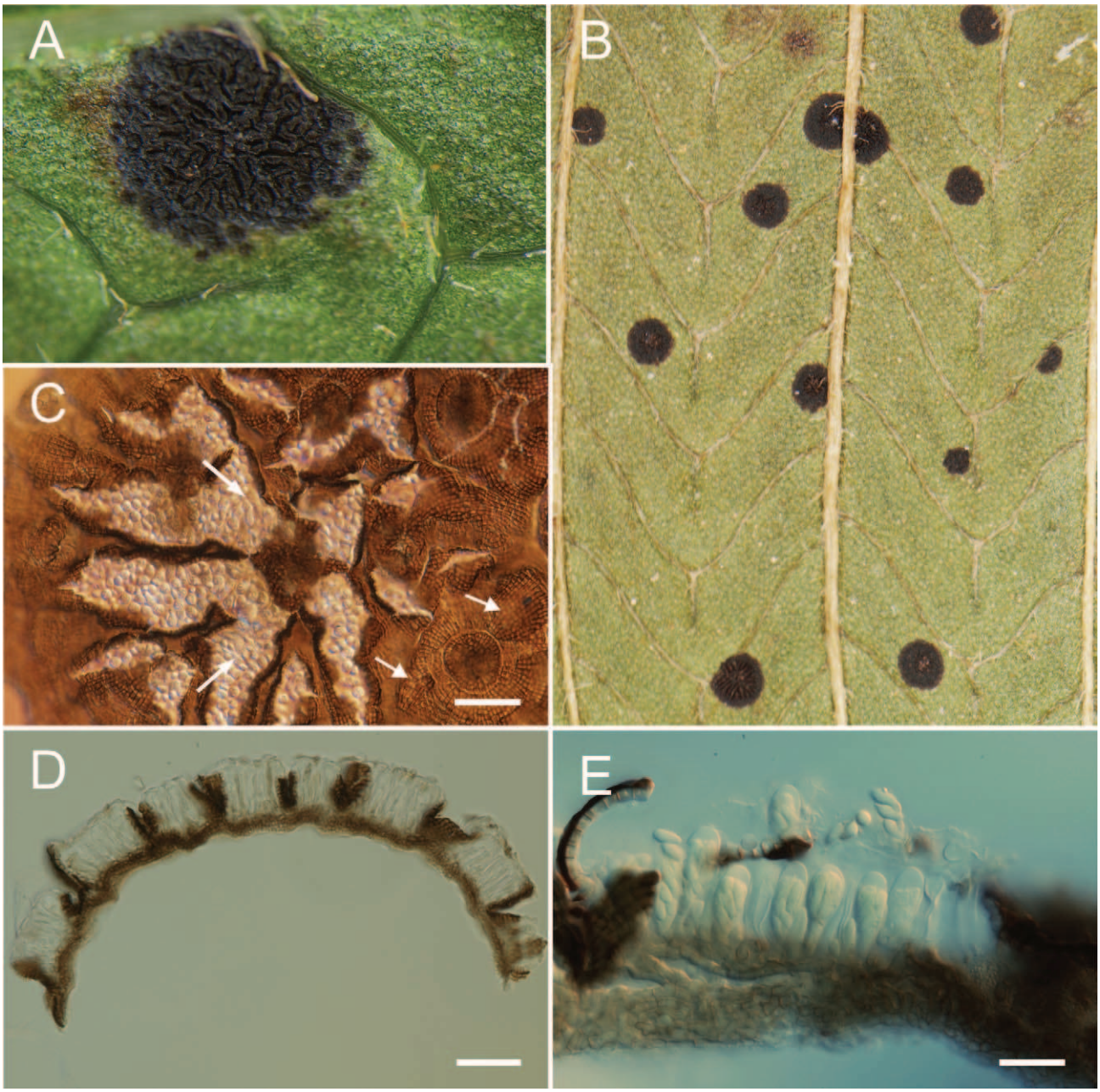

FIG. 2. *Rhagadolobiopsis thelypteridis* on *Thelypteris serrata* (VIC 31939). A, B. Stereomicroscopic images. A. Detail of the mature colony, opening by radiating fissures. B. Scattered colonies on the abaxial surface of a leaf. C–E. Light microscopic images. C. Note the thin, poorly pigmented layer of the upper layer (short arrows) that ruptures to expose the hymenium (long arrow). D. Vertical section of a detached ascoma with several fertile locules. E. Detail of an individual locule with mature asci and remnants of the covering layer. Bars: C, E = 20 μm; D = 100 μm.

4′ Ascospores composed of cells of equal size, internal stroma poorly developed. . . . . *Polycyclina*
5 Hymenial gel turning blue with iodine, ascomata dimorphic (one type buried in the host tissue and the other becoming erumpent or totally superficial) . . . . . *Rhagadolobium*
5′ Hymenial gel not turning blue with iodine, ascomata monomorphic and entirely superficial . . . . . *Rhagadolobiopsis*

In addition to the distinctive characteristics noted in the key, *Rhagadolobiopsis thelypteridis* differs from other Parmulariaceae on ferns in possessing ascospores that remain hyaline and aseptate when mature. The peg-like connection between the ascomata and the host also is characteristic of *Parmularia*, but *Rhagadolobiopsis* differs from *Parmularia* in lacking an internal stroma. Members of the morphologically

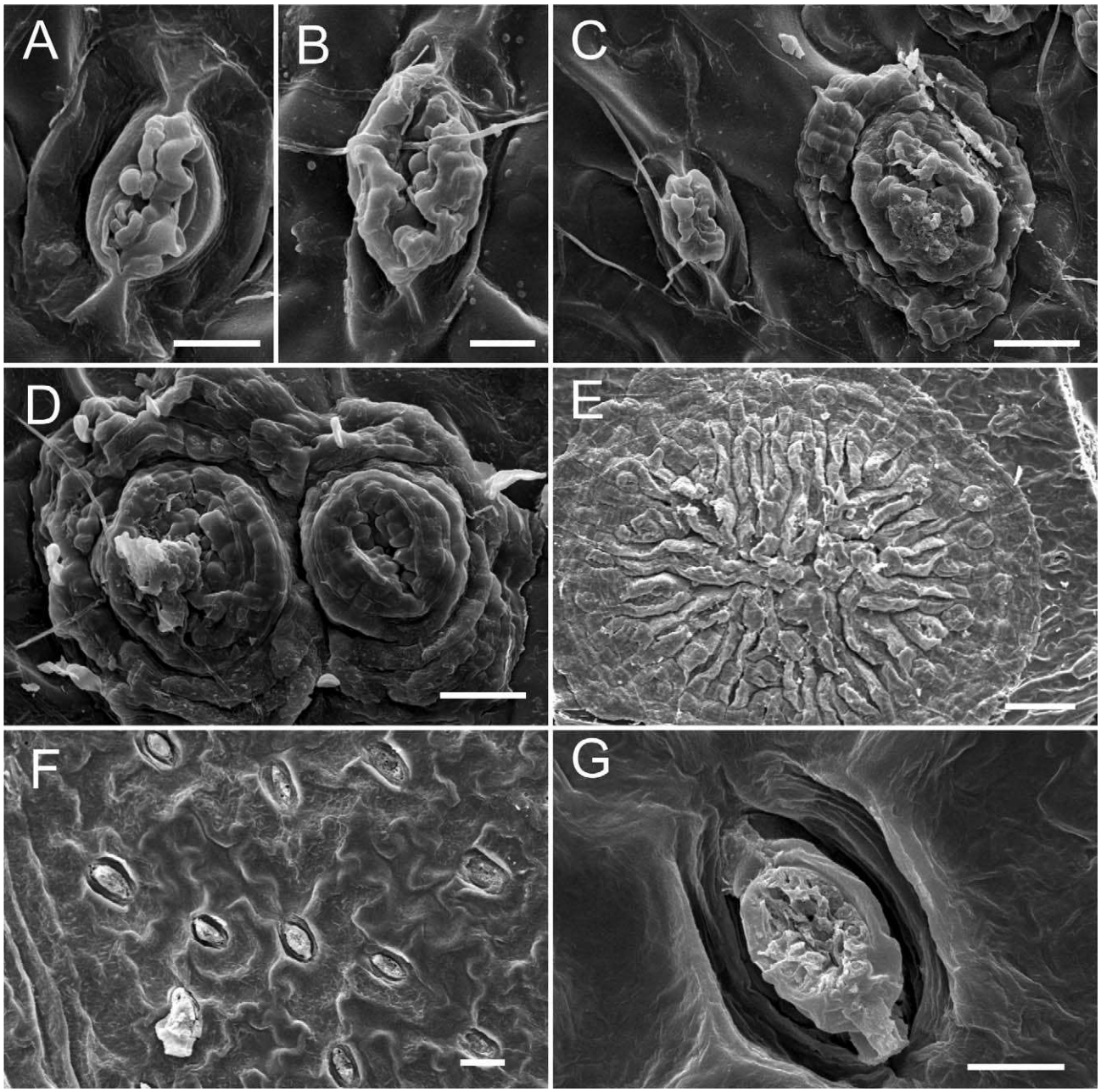

FIG. 3. Ontogeny of ascomata of *Rhagalodobiopsis thelypteridis*; SEM images. A. Emergence of a hyphal tuft through a stoma. B, C. Early stages of the development of the ascoma. Note the ellipsoidal shape of the stroma, following the shape of the stoma. D. Two coalescing stromata. E. Mature colony with radiating fissures exposing the fertile ascomata. F. Leaf surface after the removal of a colony. Note the multiple stomatal openings with remnants of the hyphal columns that connected the colony to the host. G. Individual peg-like column emerging through the stoma after the removal of ascomata. Bars: A, B–G = 10 μm; C–D = 20 μm; E = 100 μm; F = 30 μm.

similar genus *Rhagadolobium* are distinguished from *Rhagadolobiopsis thelypteridis* in producing aseptate, colorless ascospores that become light brown to brown and one-septate when mature; these species also possess a hymenial gel that becomes blue in iodine (Inácio and Cannon 2008). In material collected during two seasons in 2010 and 2012, *R. thelypteridis* always presented colorless aseptate ascospores, and a hymenial gel did not turn blue with iodine. Ejected ascospores, which we interpreted as mature, were colorless and aseptate. These ascospores did not germinate, but this is not unexpected inthat Parmulariaceae are considered to be obligate biotrophs (Inácio and Cannon 2008).

Current generic concepts in the Parmulariaceae are likely artificial, and the family includes numerous monotypic genera (Inácio and Cannon 2008). Present concepts for genera of Parmulariaceae are very narrow, and *R. thelypteridis* could not be placed into any existing genus without a questionable emendation. There are no other published records of fungi on both *T. serrata* and on Thelypteridaceae, confirming the lack of attention given to the mycobiota of ferns in general and of tropical ferns in particular.

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IMA FUNGUS · VOLUME 5 · NO 1: 51–55

ARTICLE

A new *Inocyclus* species (*Parmulariaceae*) on the neotropical fern *Pleopeltis astrolepis*

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Abstract: During a survey for fungal pathogens associated with ferns in Brazil, a tar spot-causing fungus was found on fronds of *Pleopeltis astrolepis*. This was recognised as belonging to *Inocyclus* (*Parmulariaceae*). After comparison with other species in the genus, it was concluded that the fungus on *P. astrolepis* is a new species, described here as *Inocyclus angularis* sp. nov.

Key words:
Ascomycota
Brazil
Neotropics
tropical ferns

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INTRODUCTION

The mycodiversity in Brazil is very rich, and numerous novel records of known and new fungal taxa have recently been published, as mycological activity appears to be gaining momentum in this country. Poorly exploited biomes, such as the semi-arid Caatinga (Isabel *et al.* 2013, Leão-Ferreira *et al.* 2013) and the savannah-like Cerrado, are having their mycobiota surveyed and described (Hernández-Gutiérrez & Dianese 2014, Soares & Dianese 2014), and host-plant focused fungal surveys (such as of native weeds and endangered plant species) have been conducted. Since 2009, a survey of fungi occurring on ferns (also a group of host-plants poorly studied by mycologists) is being conducted in southern and south-eastern Brazil. Numerous mycological findings have resulted and publications are in preparation to describe these. The first mycological novelty to be published as a result of this intensive study was a new genus of *Parmulariaceae, Rhagadolobiopsis* described on *Thelypteris serrata* (*Thelypteridaceae*) (Guatimosim *et al.* 2014). *Parmulariaceae* includes 59 genera of foliicolous biotrophic fungi, occurring mainly in the Neotropics and Paleotropics (Kirk *et al.* 2008). The family was recently reviewed (Inácio & Cannon 2008). Although numerous publications have covered this fungal family (Sivanesan 1970, Müller & von Arx 1973, von Arx & Müller 1975, Barr 1987, Sivanesan & Hsieh 1989, Sivanesan & Sinha 1989, Sivanesan *et al.* 1998, Inácio & Minter 2002a-i, Inácio 2003, Inácio & Cannon 2003a, b, Inácio 2005, Inácio *et al.* 2011a, b, Inácio *et al.* 2012) it is acknowledged to be still poorly known and many taxa in the family are still awaiting discovery. Delimitation of existing taxa is purely morphology-based, and a serious limitation to improving our understanding of *Parmulariaceae* is the absence of any molecular data for fungi in the family. Although a general molecular-based reappraisal of the family is desirable, technical difficulties for dealing with such biotrophic parasites still frustrates progress. Nevertheless the description of novel taxa of *Parmulariaceae* should not be interrupted awaiting for adequate methodologies to become available for molecular studies. Herein, a new member of the family, found on a fern in Brazil during our ongoing surveys, is described based on its distinct morphology, as compared to related species. The host plant is *Pleopeltis astrolepis,* a member of a genus containing approximately 90 species and occurring primarily in the Americas, but also having species in Africa, India, and Sri Lanka (Mickel & Smith 2004, Otto *et al.* 2009, Smith & Tejero-Díez 2014). *Pleopeltis astrolepis* is a widespread fern occurring throughout the Neotropics and extending into Mexico and Florida in North America (Mickel & Smith 2004).

MATERIALS AND METHODS

Samples of leaves of the epiphytic fern *Pleopeltis astrolepis* (*Polypodiaceae*) bearing minute black (tar-spot-like) colonies were collected in a private garden and also on a fallen tree in an Atlantic forest area in the municipality of Nova Friburgo, state of Rio de Janeiro (Brazil), in 2013. These were dried in a plant press and later examined under a dissecting microscope. Freehand sections of fungal colonies on leaves were prepared and also fungal structures scraped from the plant surface were mounted in lactophenol, lactofuchsin, Lugol's solution, and Melzer's reagent. When necessary, sections were made using a Microm HM 520 freezing microtome. Fungal structures were observed, measured (at least 30 structures), and line drawings and photographs were prepared, with an Olympus BX51 light microscope fitted with a drawing tube and an Olympus E330 digital camera.

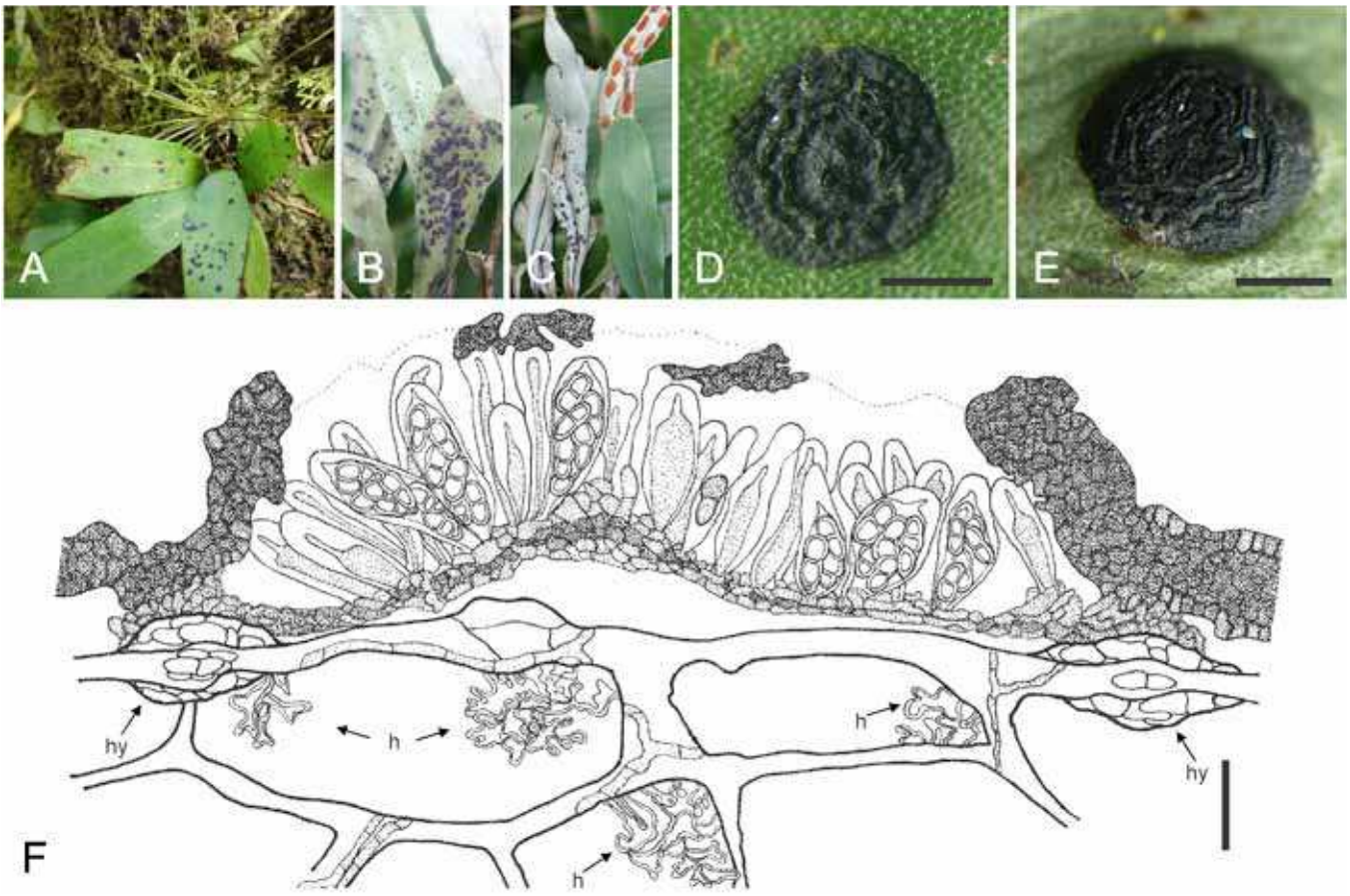

Fig. 1. *Inocyclus angularis* (VIC 39747). **A–E.** Colonies on *P. astrolepis*. **F.** Ascoma with asci and ascospores. h = coralloid haustoria, hy = hypostroma that connects the ascoma with the host tissue. Bars: D–E = 4 mm, F = 20 µm.

Representative specimens were deposited at the herbarium of the Universidade Federal de Viçosa (VIC).

In order to observe details of ascospore germination and to investigate the possibility of obtaining pure cultures of the fungus, ascospores were ejected onto the surface of PDA agar in Petri plates (Crous *et al.* 2009). This was done by attaching 1cm^2 frond pieces bearing fertile ascomata to the inside of the upper lids of Petri dishes, using vaseline, with the ascomata facing the medium. Plates were left in a growth chamber adjusted to 25 ± 2 ºC under a light regime of 12 h for 2 d. Additionally, ascospores were also directly ejected onto sterile microscope slides under similar conditions with an equivalent apparatus, but using a Petri dish lined with sterile filter paper soaked with sterile water over which a sterile microscope slide was kept suspended on sterile glass rods.

TAXONOMY

Inocyclus angularis Guatimosim & R.W. Barreto, **sp. nov.**
MycoBank MB805976
(Figs 1–2)

Etymology: *angularis,* derived from the angle formed by the germ-tube during ascospore germination.

Diagnosis: Differs from *Inocyclus discoideus* by having amphigenous ascomata and roughened versicoloured ascospores (i.e. with one brown and one subhyaline cell).

Type: **Brazil**: *Rio de Janeiro*: Nova Friburgo, Mury, Sítio Colonial, on living leaves of *Pleopeltis astrolepis*, 30 Mar. 2013, *R.W. Barreto* (VIC 39747 – **holotype**).

Description: Symptoms visible as superficial amphigenous black tar-spot-like colonies, numerous and scattered over leaves, not associated with necrosis, occasionally confluent, mostly ellipsoid to discoid, 3–10 × 7–8 mm. *External mycelium* absent. *Internal mycelium* intra- and intercellular, deeply penetrating the mesophyll, branched, 2–3 µm, sub-hyaline, smooth. *Haustoria* coralloid, several per host cell, hyaline. *Internal stromata* absent. *External stromata* superficial with radiating cells, amphigenous, ellipsoid to discoid, opening in circumferentially arranged locules connected to the host mesophyll at multiple points by discrete pulley wheel-like (in section) pads composed of internal and external aggregations of pale brown hyphae connected by a peg emerging through the cuticle. *Ascomata* black, initially circular, becoming ellipsoidal, producing locules arranged in one-two rings with undulated surface, 800–980 × 650–670 µm, composed of dark brown *textura prismatica* (cells 9 × 4 µm). In vertical section: stromata entirely superficial, strongly connected to the leaf, delimited

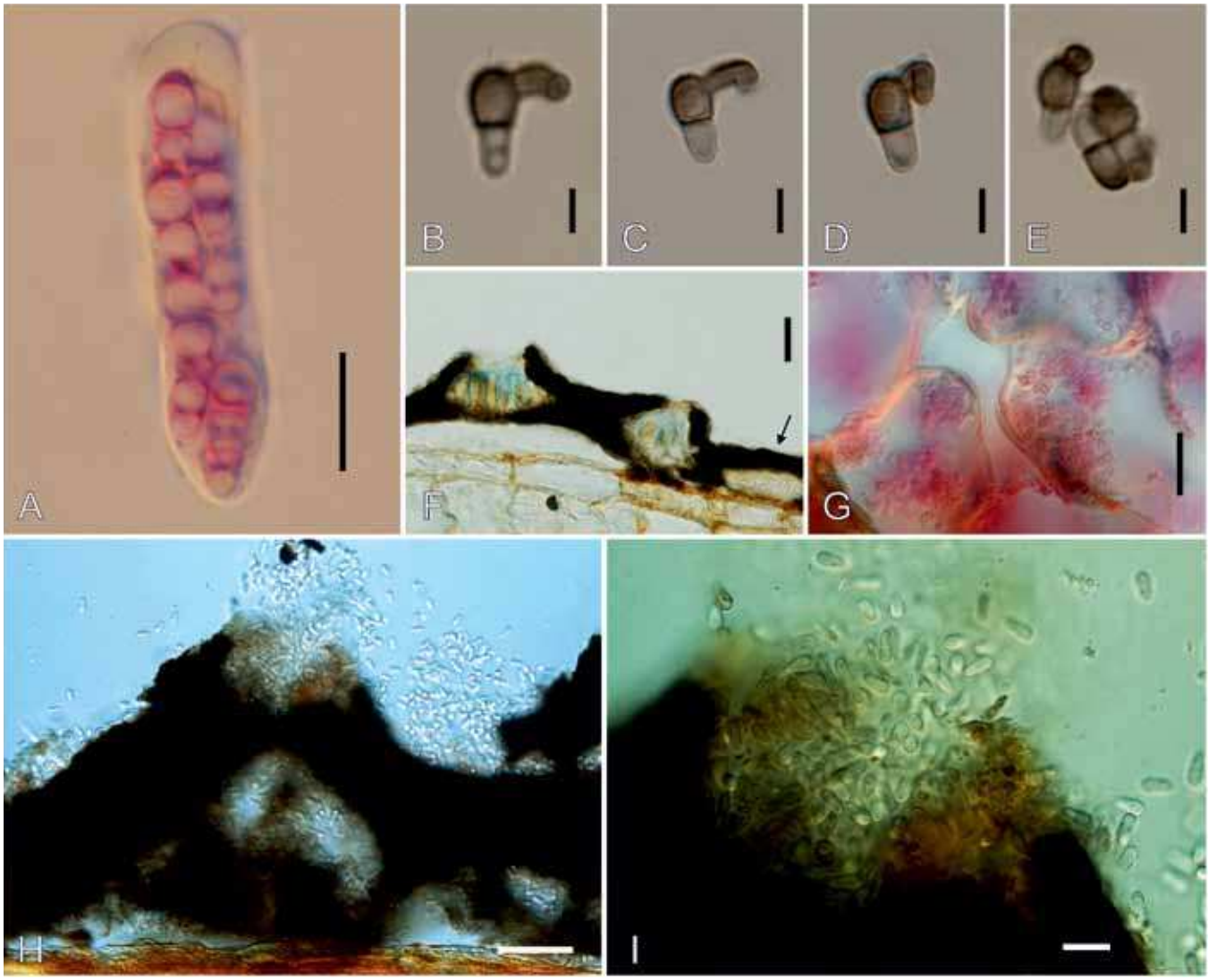

Fig. 2. *Inocyclus angularis* (VIC 39747). **A.** Ascus with hyaline ascospores (mounted on lactofuchsin). **B–E.** Germinated conidia, showing the nearly right angle formed by the germ tube. **F.** Amyloid reaction of the asci matrix on IKI. **G.** Detail of coralloid haustoria. Note the presence of the asexual morph (arrowed) intermixed with the sexual morph. **H–I.** Asexual morph with conidia. Bars: A = 10 µm, B–E = 5 µm, F = 100 µm, G, I = 20 µm, H = 50 µm.

by a covering layer (above the fertile locules) and a lower layer. Covering layer 12–16.5 µm thick, black, consisting of dense dark brown-pigmented radiating cells of *textura angularis* (cells 4 × 5 µm). Lower layer underneath the hymenium adjacent to the host cuticle, 5–12 µm thick, composed of brown to light brown *textura angularis* (cells 2–3 × 5 µm). Locule composed of a thin basal cushion above the lower layer, with asci, immersed in amyloid gelatinous stratum, 35–192 × 45–65 µm. *Hamathecium* not seen, possibly evanescent. *Asci* maturing sequentially, with young and mature asci in the same locule; young asci variable in shape before spores can be distinguished, truncated at the base, subcylindrical; mature asci bitunicate in structure, dehiscence not observed, subcylindrical, 31–45 × 10–12 µm, non-amyloid, 8-spored, biseriate or inordinate becoming uniseriate at maturity. *Ascospores* ellipsoidal to clavate, initially hyaline, becoming versicoloured, 1-septate, constricted at the septum, with unequal cells (apiospores), the upper cell larger, darker and rounded and the lower cell smaller and acute, 10–13 × 3–4 µm, roughened; only versicoloured ascospores were ejected; germination through the upper cell only, germ tubes readily folding at approximate right angles to the main ascospore axis. *Asexual morph* intermixed with the ascomata, occupying the same stromata located in the central region of the colonies. *Conidia* hyaline, aseptate, smooth, fusiform to clavate, with one large guttule at the rounded side, 7–10 × 3–4 µm.

Host: *Pleopeltis astrolepis* (*Polypodiaceae*), an epiphytic fern from the tropical and subtropical Americas (Florida to Southern Brazil).

Additional specimens examined: **Brazil**: *Rio de Janeiro*: Nova Friburgo, Mury, Sítio Colonial, on living leaves of *P. astrolepis*, 8 June 2013, *R.W. Barreto* (VIC 39748); Nova Friburgo, Riograndina, Fazenda Barreto, on living leaves of *P. astrolepis*, 9 June 2013, *R.W. Barreto* (VIC 39749).

DISCUSSION

Inocyclus angularis, as other *Parmulariaceae*, seems to be unculturable on artificial media. When ascospores were ejected onto culture medium, they readily germinated but, shortly afterwards ceased to grow.

In *Parmulariaceae*, five genera (besides *Inocylus*) are known on ferns: *Pachypatella*, *Polycyclus*, *Polycyclina*, *Rhagadolobium*, and *Rhagadolobiopsis* (Inácio & Cannon 2008, Guatimosim *et al.* 2014). Except for *Polycyclus*, all are easily separated from *Inocyclus* through observation of morphological features, as indicated in the dichotomous key for the identification of genera of *Parmulariaceae* on ferns provided by Guatimosim *et al.* (2014).

Separation between *Polycyclus* and *Inocyclus* is somewhat tenuous. The most relevant differences between these two genera, according to Inácio & Cannon (2008), are as follows. In *Inocyclus* the ascal gelatinous layer has a strong amyloid reaction and the locules are irregularly or radially arranged, whereas in *Polycyclus* no amyloid reaction is observed and the locules are circumferentially arranged.

The current generic delimitations in *Parmulariaceae* are highly artificial and this status will remain unchanged until molecular information becomes available for fungi in this family. The new species has circumferentially arranged locules as in *Polycyclus*. However we preferred to place it in *Inocyclus* because of the highly intense amyloid reaction observed in its hymenial gel.

The genus *Inocyclus* includes seven accepted species, namely the type species *I. psychotriae*, and *I. blechni, I. calotheus*, *I. myrtacearum*, *I. discoideus*, *I. australiensis*, and *I. dovyalidis* (http://nt.ars-grin.gov/fungaldatabases).

Among all *Inocyclus* species, only *I. discoideus* is known from the host family *Polypodiaceae*. It has been recorded on different species of *Polypodium* from Indonesia and the Philippines, and differs from *I. angularis* in having hypophyllous ascomata (while *I. angularis* has amphigenous ascomata) and smooth, pigmented ascospores (roughened and versicoloured in *I. angularis*).

Inocyclus angularis is the first pathogenic fungus recorded on a species of *Pleopeltis* worldwide.

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Capítulo 2 – O posicionamento filogenético da ordem *Asterinales*

Artigo – Towards a phylogenetic reappraisal of *Parmulariaceae* and *Asterinaceae* (*Dothideomycetes*). Persoonia 35:230–241. 2015

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RESEARCH ARTICLE

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Towards a phylogenetic reappraisal of *Parmulariaceae* and *Asterinaceae* (*Dothideomycetes*)

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Key words

Asterinales
epitype
Neotropical fungi
taxonomic novelties
type species

Abstract Members of the *Asterinaceae* and *Parmulariaceae* are obligate biotrophic fungi with a pantropical distribution that grow in direct association with living plant tissues and produce external ascomata and bitunicate asci. These fungi are poorly known, with limited information about their taxonomic position in the *Dothideomycetes*. Much of what is known is conjectural and based on observation of morphological characters. An assessment of the phylogenetic position of the *Asterinaceae* and *Parmulariaceae* is provided based on a phylogenetic analysis of the nrDNA operon (ITS) and the large subunit rDNA (LSU) sequence data obtained from fresh material of selected species collected in Brazil. Three key species were included and epitypified, namely *Asterina melastomatis*, which is the type species for the type genus of the *Asterinaceae*; *Prillieuxina baccharidincola* (*Asterinaceae*); and *Parmularia styracis*, which is the type species for the type genus of the *Parmulariaceae*. An LSU rDNA phylogenetic analysis was performed indicating the correct phylogenetic placement of the *Asterinales* within the *Dothideomycetes*. From this initial analysis it is clear that the *Parmulariaceae* as currently circumscribed is polyphyletic, and that the *Asterinaceae* and *Parmulariaceae* are related, which justifies the maintenance of the order *Asterinales*. *Asterotexis cucurbitacearum* is recognised as distinct from other *Dothideomycetes* and placed in the newly proposed family and order (*Asterotexiaceae*, *Asterotexiales*), while the higher order phylogeny of *Inocyclus angularis* remains unresolved. Additionally, *Lembosia abaxialis* is introduced as a novel species and the phylogenetic placement of the genera *Batistinula* and *Prillieuxina* is clarified.

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INTRODUCTION

The *Parmulariaceae* (*Ascomycota*) was informally proposed by Müller & von Arx (1962) to accommodate plant parasitic fungi with superficial, dimidiate shield-shaped or crust-like, pulvinate stromata, strongly flattened ascomata that open by irregular disintegration, or by lateral to radial, or ring-like splits. The externally visible stromata usually originate from internal hyphae or internal hypostroma (von Arx & Müller 1975). Asci in this family are ovoid to clavate, with fissitunicate or rostrate dehiscence with a hamathecium composed of pseudoparaphyses. Ascospores of members of this family are hyaline or brown, usually septate and, with or without a mucilaginous sheath. Asexual morphs of fungi in this group are poorly known. The family was formally described by Barr (1979). A more detailed account of the *Parmulariaceae* was provided in the monograph published by Inácio & Cannon (2008).

The *Parmulariaceae* together with families of foliicolous ascomycetes such as *Asterinaceae* and *Aulographaceae*, has traditionally been treated as a group with uncertain placement (*incertae sedis*) in the *Dothideomycetes* (Hyde et al. 2013). The *Parmulariaceae* differs from the supposedly closely related *Asterinaceae*, by having an apical stroma formed by several layers of pigmented cells, and a basal hypostroma formed by fungal hyphae, as well as by the absence of appressoria (Inácio et al. 2012a, Hongsanan et al. 2014). Superficial hyphae are absent in species of *Parmulariaceae* with the exception of *Antoniomyces*, *Aulacostroma*, *Mintera and Symphaeophyma*, although commonly found in the *Asterinaceae* (Inácio et al. 2012a). The taxonomic value of this feature was considered an artificial criterion for distinguishing the two families (von Arx & Müller 1975). Nevertheless as a matter of convenience, this morphological feature is still widely used to recognise whether a taxon belongs to one family or the other. The hypothesis of affinity between these two families has never been tested with modern molecular tools.

Léveillé (1845) described eight species in two genera, *Asterina* and *Lembosia*. In 1899, *Asterina* was included in *Microthyriaceae* and the family was divided into two subfamilies, *Asterineae* and *Microthyrieae*, based on the presence or absence of superficial mycelium (Theissen 1913a, b). Subsequently, the family *Asterinaceae* was described and 18 genera were included (Hansford 1946).

Currently the *Asterinaceae* includes species that are either epiphytic or obligate biotrophs. Fungi in this family have dimidiate ascomata that open irregularly at maturity by means of stellar, longitudinal or irregular slits. Ascomata contain bitunicate upright asci, which are globose to oval or cylindrical. Colonies are formed on the surface of leaves or green stems of plants. When present, superficial mycelium is composed of hyphae that have opposite, alternate or irregular branches with uni- or

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bi-cellular appressoria that are either alternate, unilateral or a mixture of these forms and with shapes that vary between oval, ampulliform, lobate or variable. Haustoria are present in many genera (von Arx & Müller 1975, Eriksson 1981, Bezerra 2004, Hofmann et al. 2010, Hofmann & Piepenbring 2011, Hosagoudar 2012).

Recent studies have shown that morphological features alone are not a reliable basis for a natural classification that reflects true phylogenetic relationships. Some examples are found at the generic level in taxa such as *Cladosporium*, *Microcyclosporella*, *Phaeomoniella*, *Radulidium*, *Ramichloridium* and *Septoria*, among others (Arzanlou et al. 2007, Schubert et al. 2007, Frank et al. 2010, Quaedvlieg et al. 2013) and at the family level in *Botryosphaeriaceae* and *Teratosphaeriaceae* (Slippers et al. 2013, Quaedvlieg et al. 2014). Delimitation and affiliation of both the *Asterinaceae* and *Parmulariaceae* and the genera they contain have relied entirely on morphological features such as ascospore septation, hamathecium reaction to iodine, presence and shape of internal stromata, plectenchyma texture and colour, ascomata and ascus dehiscence.

Morphological features are often combined with conjectured host specificity. However, the host specificity of fungi in these families has never been experimentally tested (Hofmann et al. 2010). The *Asterinaceae* and *Parmulariaceae* were regarded as probably polyphyletic both by Inácio & Cannon (2008) and Hongsanan et al. (2014), respectively. Practical difficulties related to DNA extraction from old herbarium material and difficulties with recollection of type specimens have hampered a reappraisal of these two families.

Inácio & Cannon (2008) included 35 genera as members of the *Parmulariaceae*, while Lumbsch & Huhndorf (2010) recognised 34 genera, with the inclusion of *Hemigrapha* and exclusion of *Apoa* and *Parmulariella*. The latest publication mentioning this family (Hyde et al. 2013) added *Antoniomyces* and excluded four genera (*Coccodothis*, *Dothidasteroma*, *Englerodothis* and *Perischizon*) from the *Parmulariaceae* based on the shape of the ascomata, reducing the total number to 31 genera. Now, with the addition of the recently described genus *Rhagadolobiopsis* (Guatimosim et al. 2014a), the *Parmulariaceae* include 32 genera and 114 synonyms (Inácio & Cannon 2008, Lumbsch & Huhndorf 2010, Inácio et al. 2012b, Hyde et al. 2013, Guatimosim et al. 2014a, b).

Lumbsch & Huhndorf (2010) included 38 genera in the *Asterinaceae* but, more recently, Hongsanan et al. (2014) revised the *Asterinaceae*, and recognised only 17 genera and 42 synonyms as belonging to the family. These revisions were mostly based on morphological observations, and were not substantiated by molecular data.

Molecular phylogenetic studies of the *Parmulariaceae* are difficult because of their biotrophic nature as well as the difficulties involved in DNA extraction from herbarium specimens. The pioneering study of the phylogenetic placement of *Asterinaceae* (Hofmann et al. 2010) and recent successful DNA extraction from the *Meliolales* (Pinho et al. 2012, 2014), shows that phylogenetic approaches can be applied to obligate biotrophs, even when only old herbarium material is available.

The aim of this study was to assess the phylogenetic placement of the *Asterinaceae* and *Parmulariaceae* based on the study of newly collected epitype materials of *Parmularia styracis* (the type species of *Parmulariacae*), *Asterina melastomatis* (the type species of *Asterinaceae*) and *Prillieuxina baccharidincola* (*Asterinaceae*). *Asterotexis cucurbitacearum*, formerly placed in the *Asterinaceae*, was re-examined and found to represent a separate family, described here as new. Additionally, a new species of *Lembosia* is introduced and the phylogenetic placement of *B. gallesiae* and *P. baccharidincola* is elucidated.

MATERIALS AND METHODS

Sample collection and morphology

Leaf samples bearing black fungal colonies were collected in Brazil in different biomes between 2009 and 2014. These were dried in a plant press and later examined under a stereo microscope. 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 Zeiss V20 Discovery stereo microscope and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an MRc5 camera and ZEN imaging software. Representative specimens were deposited at the herbarium of the Universidade Federal de Viçosa (VIC) and CBS Herbarium (CBS H).

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

Leaves harbouring fertile ascomata were examined under a stereo-microscope to check for possible contamination by other fungi, including yeasts. The leaves were then soaked in sterile water for 1 h in order to hydrate and remove the ascomata. Thirty fertile ascomata were removed from the leaves with a sterile fine pointed needle, and placed into a microcentrifuge tube (1.5 mL). Total genomic DNA was extracted by using Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions and the steps described by Pinho et al. (2012).

PCR amplification

The LSU region of each fungus included in the study was sequenced with the primers LR0R + LR5 (Vilgalys & Hester 1990). For the *Parmulariaceae*, two additional loci, including the internal transcribed spacer regions and intervening 5.8S rDNA (ITS) and the translation elongation factor 1-alpha (EF-1α) were amplified and sequenced with the primer pairs ITS1-F (Gardes & Bruns 1993) + ITS4 (White et al. 1990), EF2-Fd (Groenewald et al. 2013) or EF1-728F (Carbone & Kohn 1999) + EF-2 (O'Donnell et al. 1998). PCR amplifications were performed in a total volume of 12.5 µL solution containing 10–20 ng of template DNA, 1× PCR buffer, 0.63 µL DMSO (99.9 %), 1.5 mM $MgCl_2$, 0.5 µM of each primer, 0.25 mM of each dNTP, 1.0 U BioTaq DNA polymerase (Bioline GmbH Luckenwalde, Germany). PCR conditions for ITS and LSU were set as follows: an initial denaturation temperature of 95 °C for 5 min, followed by 35 cycles of denaturation temperature of 95 °C for 30 s, primer annealing at 52 °C for 30 s, primer extension at 72 °C for 1 min and a final extension step at 72 °C for 1 min. PCR conditions for EF-1α were set as follows: an initial denaturation temperature of 94 °C for 5 min, followed by 45 cycles of denaturation temperature of 94 °C for 45 s, primer annealing at 52 °C for 30 s, primer extension at 72 °C for 90 s and a final extension step at 72 °C for 6 min.

DNA sequencing and phylogenetic inference

PCR amplicons of the regions targeted in this study served as templates for DNA sequencing reactions with the BigDye® Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA, USA) following the protocol of the manufacturer. DNA sequencing reactions used the same

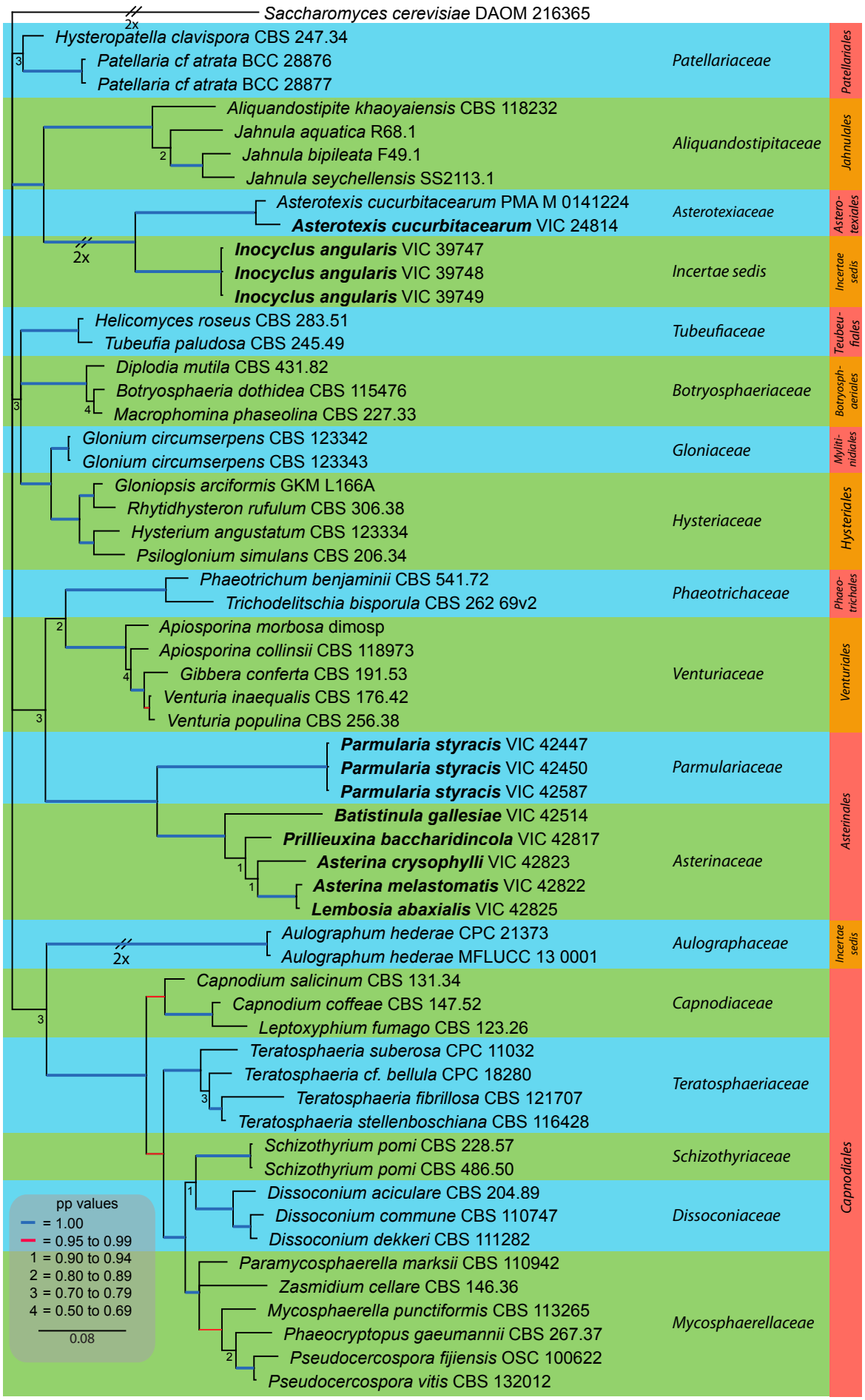

Fig. 1 A Bayesian 50 % majority rule tree based on a full length LSU alignment, containing all strains generated in this study. Bayesian posterior probabilities support values for the respective nodes are displayed in the tree. The tree was rooted to *Saccharomyces cerevisiae*. The scale bar indicates 0.08 expected changes per site. New sequence data are in **bold**.

primers as those for the PCR reactions. 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 Sequencer (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 for initial alignment and the construction of sequence datasets. Sequences obtained from Schoch et al. (2009), TreeBASE study S10245, and from GenBank (www.ncbi.nlm.nih.gov) and the novel sequences generated on this study were aligned using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; Katoh et al. 2002) and manually improved in MEGA as indicated.

Phylogenetic analysis

Appropriate gene models were selected using MrModeltest v. 2.3 (Nylander 2004) and applied to the gene partition. Based on the results of MrModeltest, a Bayesian phylogenetic analysis was performed with MrBayes v. 3.1.2 applying a general time-reversible (GTR+I+G) substitution model with inverse gamma rates and dirichlet base frequencies and a heating parameter set at 0.01. *Saccharomyces cerevisiae* DAOM 216365 (JN938921) served as outgroup for the phylogenetic analyses. Posterior probabilities were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.1 (Ronquist et al. 2012). Six simultaneous Markov chains were run for 10 000 000 generations and trees were sampled every 100th generation and 10 000 trees were obtained. The first 2 000 trees, representing the burn-in phase were discarded, while the remaining 8 000 trees were used for calculating posterior probabilities. Bayesian posterior probabilities are presented on the left of each node (Fig. 1). Sequences derived in this study were lodged in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1), the alignment and tree in TreeBASE (http://www.treebase.org) (study number 17355) and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004).

RESULTS

Taxonomy

Parmulariaceae M.E. Barr, Mycologia 71: 944. 1979

Type species. Parmularia styracis Lév., Ann. Sci. Nat., Bot. 5: 286. 1846.

This family includes fungi forming foliicolous or lichenicolous, superficial, dark brown to black colonies. *Haustoria* coralloid, hyaline, numerous in each host-cell. *Ascomata* solitary to gregarious, superficial (or rarely immersed), shield-like, star-shaped, ellipsoidal or boat-shaped, strongly flattened, membranaceous to carbonaceous, originating from emerging hyphae or from an erumpent hypostroma, covered by a dark wall composed of often radiating rows of cells and opening by fissure or by deliquescence, containing numerous asci, dark brown to black. *Asci* 8-spored, thick-walled, fissitunicate, variously shaped, short stalked, with a distinct ocular chamber. *Ascospores* oblong, ellipsoidal or ovoid, ends rounded, 1-septate, constricted or not at the septum, hyaline to dark brown, smooth to verrucose (Inácio & Cannon 2008, Hyde et al. 2013).

Parmularia styracis Lév., Ann. Sci. Nat., Bot. 5: 286. 1846 — Fig. 2

= *Schneepia guaranitica* Speg., Anales Soc. Ci. Argent. 19: 259. 1885.
≡ *Parmularia guaranitica* (Speg.) Henn., Hedwigia 36: 230. 1897.
= *Schneepia arechavaletae* Speg., Bol. Acad. Nac. Ci. 11: 581. 1889.
≡ *Parmularia arechavaletae* (Speg.) G. Arnaud, Ann. Ecole Natl. Agric. Montpellier 16: 116. 1918.
= *Parmularia styracis* var. *minor* Henn., Hedwigia 34: 112. 1895.

Colonies visible as superficial, epiphyllous, black discoid structures, numerous and scattered over leaves, not associated with necrosis, 2–3 mm diam. *External mycelium* absent. *Internal mycelium* intra- and intercellular, deeply penetrating the mesophyll, branched, 1.5–3.5 µm diam, sub-hyaline to dark brown, smooth. *Haustoria* coralloid, hyaline, several per host cell occupying both the subcuticular and the lacunar parenchymal cells. *Internal stromata* globular, 27–67 µm diam, located at the central portion of the colony, erupting through the cuticle, cells composed of a combination of *textura angularis* and *textura prismatica*, 3–8 × 2–5 µm. *External stromata* epiphyllous, superficial, discoid, laciniate at the edges, 1–3 mm diam, cells composed of *textura prismatica*, 3–7 × 1.5–3.5 µm. *Ascomata*, producing locules arranged in radiating lirellae-like slits, with undulated surface. In vertical section: ascomata entirely superficial, loosely connected to the leaf, delimited by a covering layer (above the fertile locules) and a lower layer. Covering layer black, 27–63 µm thick, consisting of dense dark brown radiating cells of *textura angularis*, 3–7 × 2–4 µm. Lower layer beneath the hymenium adjacent to the host cuticle, colourless to pale brown, intimately mingled with hyphal cells of the basal cushion, 13–46 µm thick, composed of pale brown to brown *textura angularis* (cells 2–7 × 1.5–4 µm). Locules with a thin basal cushion above the lower layer, asci and hamathecium immersed in a non-amyloid gelatinous stratum, 76–353 µm diam, 100–320 µm high. *Pseudoparaphyses* mostly colourless and pale brown at the rounded and slightly swollen, slightly verrucose tips, sometimes with brown to dark brown external material adhering, 49–115 × 1.5–3 µm, septate, thin-walled, filiform, sometimes dichotomously branched near the base. *Asci* bitunicate, maturing sequentially, with young and mature asci in the same locule; young asci variable in shape before

Table 1 Strains and NCBI GenBank accessions generated in this study. Type specimens are in **bold**.

Species	Accession number	Host / Substrate	Locality	Collector	GenBank accessions		
					LSU	ITS	EF-1α
Asterina crysophylli	VIC 42823	*Henriettea succosa*	Brazil	A.L. Firmino	KP143738	–	–
A. melastomatis	**VIC 42822**	*Miconia* sp.	Brazil	A.L. Firmino	KP143739	–	–
Asterotexis cucurbitacearum	**VIC 24814**	*Cucurbita pepo*	Brazil	O.L. Pereira & A.L. Firmino	KP143734	–	–
Batistinula gallesiae	VIC 42514	*Caesalpinia echinata*	Brazil	A.L. Firmino, D.B. Pinho & O.L. Pereira	KP143736	–	–
Inocyclus angularis	**VIC 39747**	*Pleopeltis astrolepis*	Brazil	R.W. Barreto	KP143731	KP273233	KP289328
	VIC 39748	*Pleopeltis astrolepis*	Brazil	R.W. Barreto	KP143732	KP273234	KP289329
	VIC 39749	*Pleopeltis astrolepis*	Brazil	R.W. Barreto	KP143733	KP273235	KP289330
Lembosia abaxialis	**VIC 42825**	*Miconia jucunda*	Brazil	R.W. Barreto	KP143737	–	–
Parmularia styracis	**VIC 42447**	*Styrax ferrugineus*	Brazil	M.S. Silva & O.L. Pereira	KP143728	KP273230	KP289325
	VIC 42450	*Styrax ferrugineus*	Brazil	M.S. Silva & O.L. Pereira	KP143729	KP273231	KP289326
	VIC 42587	*Styrax ferrugineus*	Brazil	R.W. Barreto	KP143730	KP273232	KP289327
Prillieuxina baccharidincola	**VIC 42817**	*Baccharis* sp.	Brazil	O.L. Pereira	KP143735	–	–

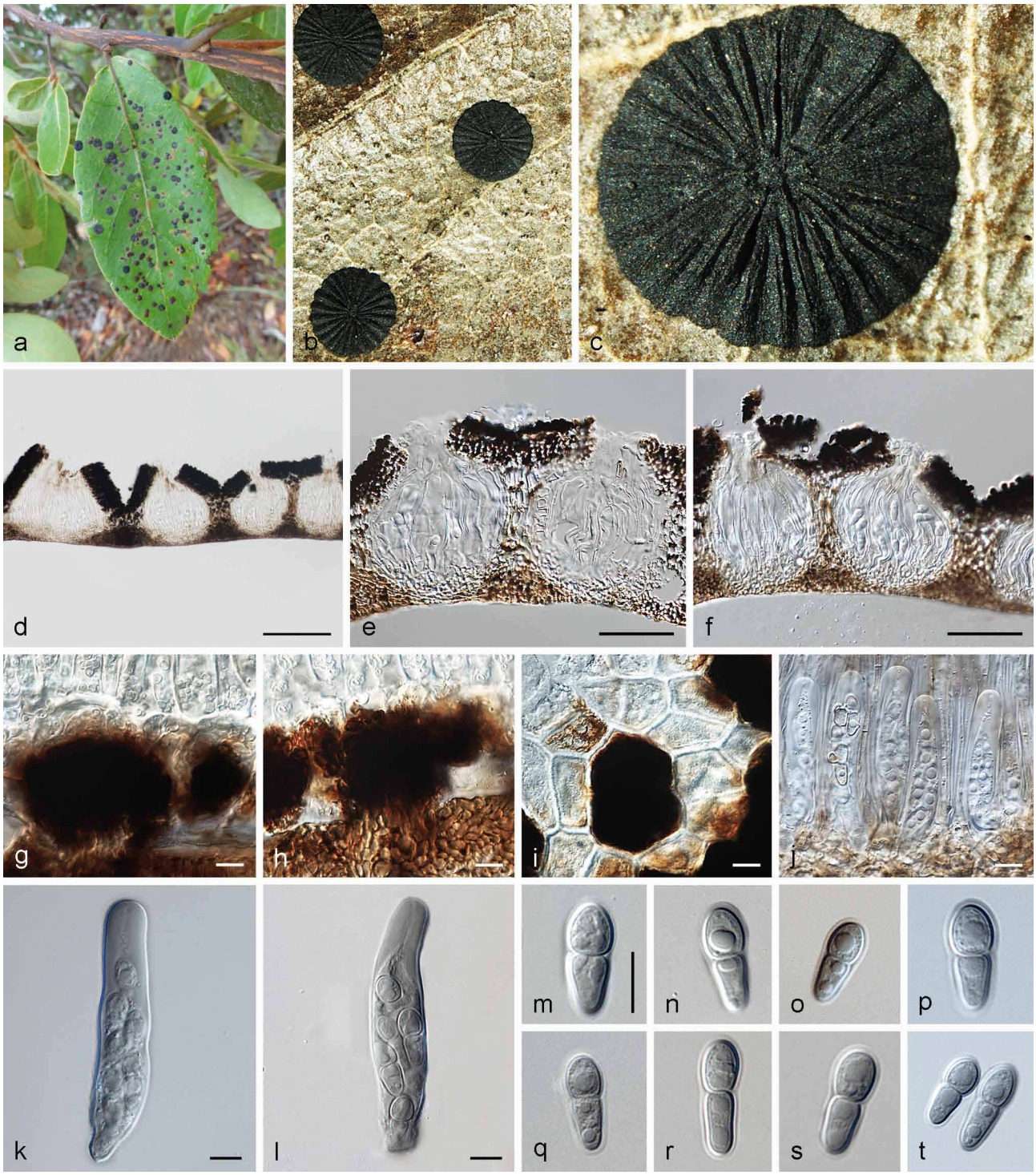

Fig. 2 *Parmularia styracis* VIC 42447. a. Living leaves of *Styrax ferrugineus* with epiphyllous colonies; b, c. detail of the mature colony, opening by radiating fissures; d. vertical section showing entirely superficial ascoma with fertile locules; e, f. detail of the fertile locules; g, h. hyphal columns which connect the colony with the host tissue; i. horizontal section showing the detail of a tuft of internal mycelium that ruptures the cuticle and produce the initial stages of the ascostromata; j. detail of the fertile locule with fully developed asci and pseudoparaphyses; k, l. asci; m–t. ascospores. — Scale bars: d = 100 µm; e, f = 50 µm; g–m = 10 µm.

spores can be distinguished, truncated at the base, subcylindrical; mature asci thick-walled (particularly in the upper portion), cylindric-clavate to clavate, 47–81 × 9–18 µm, non-amyloid, 6–8-spored, biseriate (with colourless hyaline ascospores) or unordered but becoming uniseriate at maturity (the stage containing pale brown ascospores), dehiscence through a large apical fracture in the outer wall, with the inner layer extending through it. *Ascospores* ellipsoidal to clavate, mostly hyaline to pale brown, thin-walled, verrucose, 1-septate, constricted at the septum, the upper cell broader and rounded, and the lower cell tappering towards a rounded end, 14–20 × 5–7 µm, smooth. *Asexual morph* unknown.

Type material. Brazil, Planaltina, on living leaves of *Styrax*, *Clauseen*, 1846 (PC!, holotype); on living leaves of *Styrax ferrugineus*, vicinities of the Estação Ecológica de Águas Emendadas, Cerrado biome, 16 Apr. 2013, *M. Silva* & *O.L. Pereira* (VIC 42447 = CBS H-22026, epitype designated here, MBT200333).

Additional materials examined. Brazil, Planaltina, on living leaves of *Styrax ferrugineus*, vicinities of the Estação Ecológica de Águas Emendadas, Cerrado biome, 18 Apr. 2013, *M. Silva* & *O.L. Pereira*, VIC 42450 = CBS H-22025; Minas Gerais, Capitólio, Furnas, on living leaves of *S. ferrugineus*, S20°38'54.5" W46°13'36.8", 9 Nov. 2012, *R.W. Barreto*, VIC 42587 = CBS H-22027.

Notes — The ontogeny of ascomata of *P. styracis* resembles that recently described for the genus *Rhagadolobiopsis*, in that mature ascostromata are produced from several ascostromatal primordia that coalesce to form a multiloculate structure (Guatimosim et al. 2014a) (Fig. 2b, c). In contrast, *Parmularia* produces a column of internal mycelium in the centre of the colony that ruptures through the cuticle (Fig. 2i). When the ascomatal disk is removed, the hyphal columns are limited to the central portion of the area below the colony (Fig. 2g, h).

Asterinaceae Hansf., Mycol. Pap. 15: 188. 1946

Type species. Asterina melastomatis Lév., Ann. Sci. Nat., Bot. 3: 59. 1845.

Foliicolous, epiphytic, obligately biotrophic. *Sexual morph*: *External mycelium* usually with or without appressoria, opposite, alternate or irregular branches, blackened. *Appressoria* uni- or bi-cellular, lateral and/or intercalary, and opposite, alternate or alternate and opposite, oval, ampulliform, lobate or variable, brown to dark brown, with penetration peg piercing through cuticle and invading the epidermic cells or on top of guard cells, forming stomatopodia. *Haustoria* present in various genera. *Ascomata* dimidiate, superficial, growing on the surface of plant leaves or stems, circular, elongate or linear, dehiscence non-ostiolate, opening by radiating star-like, longitudinal or irregular slits. *Scutellum* radiate, composed of isodiametric to cylindrical cells, with straight to dichotomously branched hyphae. *Hypostroma* (internal stroma or internal hyphae) present in some members. *Pseudoparaphyses* present or not, cylindrical, septate, branched or unbranched, hyaline to yellowish. *Asci* fissitunicate, upright and parallel, globose, ovoid or cylindrical, 4–8-spored, usually lacking a stalk, hyaline. *Ascospores* ellipsoidal, occasionally cylindrical, 2–6-celled, yellowish to brown (mostly brown when mature), walls smooth or with capitate ornamentation. *Setae* present or not on the ascomata and/or mycelium. *Asexual morph* hyphomycetous or coelomycetous states with pycnothyria. *Conidiophores* solitary, unbranched, brown. *Conidiogenous cells* monoblastic or proliferating percurrently, hyaline or brown. *Conidia* ovoid, cylindrical, conical or staurosporous, brown (von Arx & Müller 1975, Eriksson 1981, Bezerra 2004, Hofmann et al. 2010, Hofmann & Piepenbring 2011, Hosagoudar 2012, Hyde et al. 2013, Hongsanan et al. 2014).

Asterina melastomatis Lév., Ann. Sci. Nat., Bot. 3: 59. 1845. — Fig. 3

≡ *Parasterina melastomatis* (Lév.) Theiss., Syd. & P. Syd., Ann. Mycol. 15: 246. 1917.

Colonies epiphyllous, irregular to circular, single to confluent, black, 2–6 mm diam. *External mycelium* straight to flexuous, branching alternate to unilateral, rarely opposed, pale brown to brown, septate, hyphal cells cylindrical, 4–5 µm diam, smooth. *Appressoria* numerous, entire, sessile, straight to angular, rarely crooked, rectangular to long-ovate, unicellular, alternate to unilateral, never opposed, 6–7.5 × 7–8 µm, brown, penetration peg in middle part of appressorial cell. *Ascomata* thyriothecia, dimidiate, superficial, developing below external mycelium, circular, single to confluent, in small clusters, fringed at margins, 165–220 µm diam, dark brown to blackish, opening by a central star-shaped fissure. *Pseudoparaphyses* cylindrical, septate, unbranched, hyaline to yellowish. *Scutellum* radiate, composed of isodiametric to cylindrical cells. *Asci* bitunicate, ovoid to slightly clavate, 8-spored, 47.5–57.5 × 27.5–30 µm, hyaline. *Ascospores* 2-celled, cylindrical, straight, constricted at the septum, hyaline initially, pale brown to brown at maturity, smooth, 19.5–21 × 9.5–11 µm. *Asexual morph* absent.

Type material. Brazil, locality unknown, on living leaves of *Miconia* sp., date unknown, Guillemin, (herbarium specimen not preserved); Minas Gerais, Lavras Novas, on living leaves of *Miconia* sp., on the track of the Cachoeira das Três Quedas, S20°28'39.63" W43°29'42.27", 26 Oct. 2013, A.L. Firmino (VIC 42822, neotype designated here MBT200348). – French Guiana, Cayene, on leaves of *Melastomataceae*, Nov. 1800, *Leprieur* (herb. *Montagne* 1133, Crypt. Guyan. 582); PC0084477. Referred to by Hongsanan et al. (2014) as a neotype designated by Theissen (1912 – actually 1913), but that author only referred to species being represented by that collection.

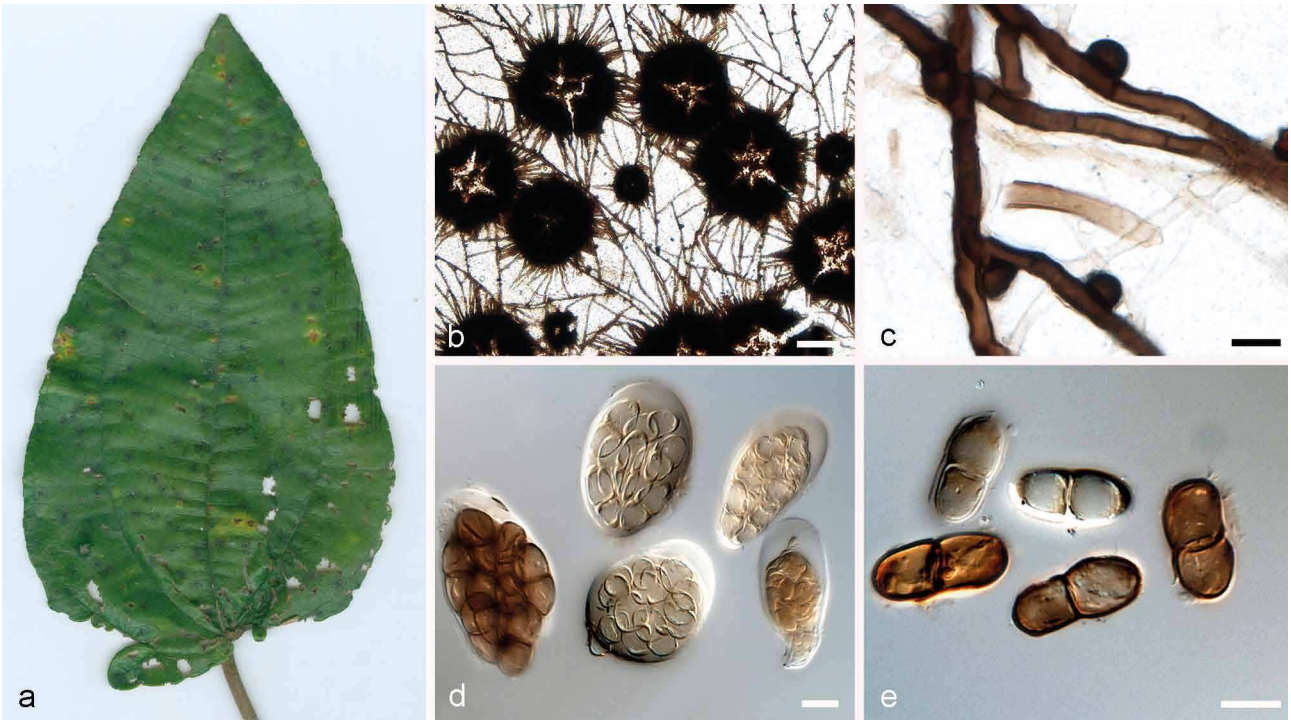

Fig. 3 *Asterina melastomatis* VIC 42822. a. Living leaves of *Miconia* sp. with epiphyllous colonies; b. colony with open thyriothecia and external mycelium; c. appressoria cylindrical to long-ovate, unicellular; d. asci ovoid to slightly clavate; e. ascospores hyaline, becoming pale brown to brown at maturity. — Scale bars = 10 µm.

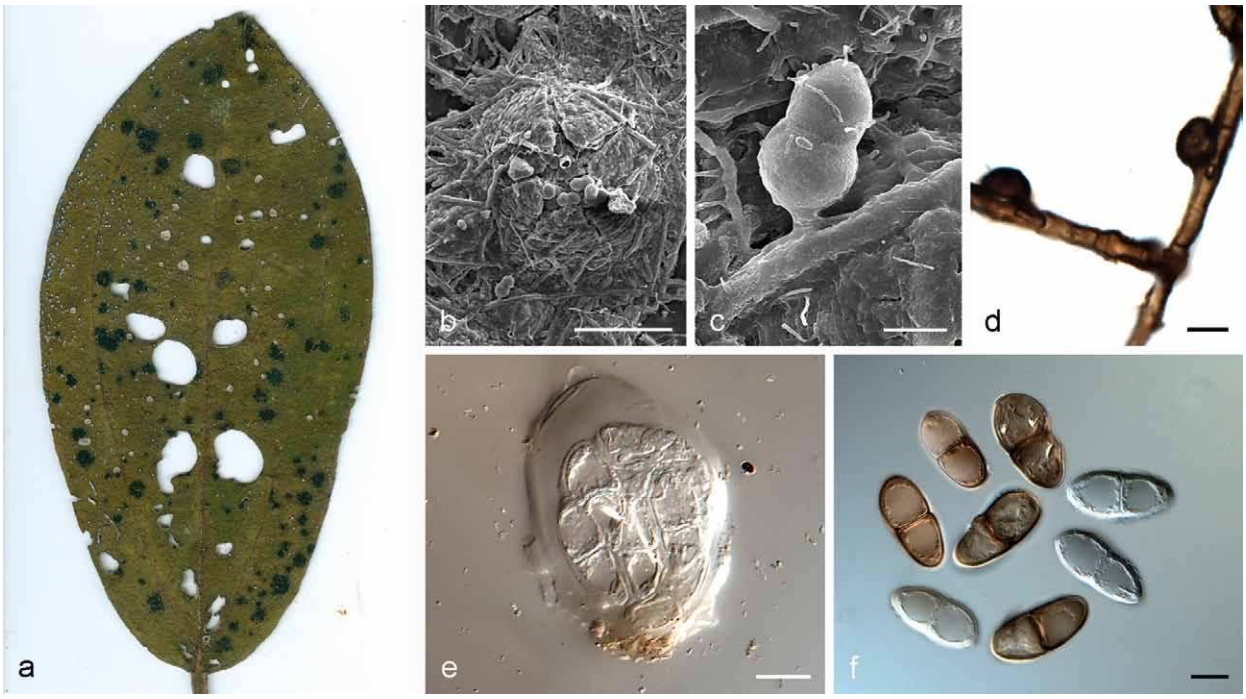

Fig. 4 *Asterina chrysophylli* VIC 42823. a. Living leaves of *Henriettea succosa* with epiphyllous colonies; b, c. SEM images: b. thyriothecium opened by a central star-shaped fissure; c. ascospore oblong, smooth, constricted at the septum; d. appressoria straight, globose to pyriform, unicellular; e. asci globose to ovoid; f. ascospores hyaline, becoming brown at maturity. — Scale bars = 10 µm.

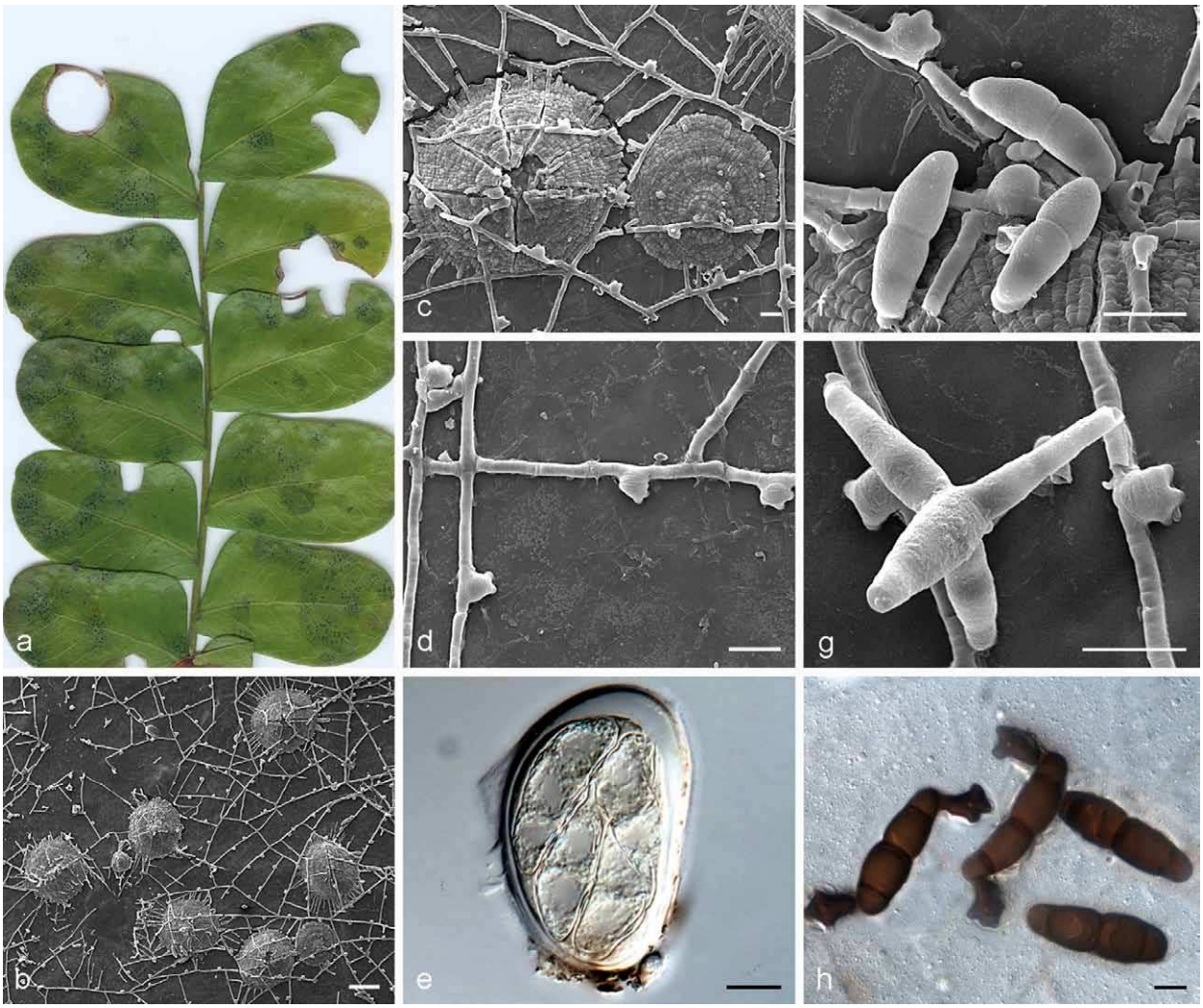

Fig. 5 *Batistinula gallesiae* VIC 42514. a. Living leaves of *Caesalpinia echinata* with epiphyllous colonies; b–d, f, g. SEM images: b. colony with open thyriothecia and external mycelium; c. thyriothecium opened by a central star-shaped fissure; d. appressoria straight, lobate, cylindrical, unicellular; e. asci ovoid, showing immature ascospores; f. ascospores oblong, with ends broadly rounded, constricted at the septum; g. conidia of *Triposporium* (asexual morph) and erect conidiophore; h. ascospores with lobate appressoria. — Scale bars: b = 100 µm; c, d, f, g = 20 µm; e, h = 10 µm.

Asterina chrysophylli Henn., Hedwigia 48: 12. 1908. — Fig. 4

Colonies epiphyllous, irregular to circular, solitary to confluent, black 0.5–6 mm diam. *External mycelium* straight to slightly flexuous, branching irregularly, pale brown to brown, septate, hyphal cells cylindrical, 4.5–5 µm diam, smooth. *Appressoria* numerous, entire, sessile, straight, globose to pyriform, unicellular, alternate to unilateral, never opposed, 7.5–9.5 × 11–12.5 µm, brown, penetration peg in the middle portion of the appressorial cell. *Ascomata* superficial, thyriothecioid dimidiate, developing below external mycelium, circular, solitary to confluent, fringed at margins, 162–253 µm diam, opening through central star-shaped fissures, dark brown to black. *Scutellum* radiate, composed of somewhat isodiametric to cylindrical cells, straight. *Asci* bitunicate, globose to ovoid, 8-spored, 52.5–57.5 × 32.5–35 µm, hyaline, smooth. *Ascospores* oblong to slightly fusiform, straight to slightly curved, constricted at the septum, 27–30 × 14–15 µm, 2-celled, hyaline, becoming brown at maturity, smooth. *Asexual morph* absent.

Material examined. Brazil, Espírito Santo, Sooretama, Reserva Natural Vale, on living leaves of *Henriettea succosa*, 19 June 2012, *A.L. Firmino*, VIC 42823.

Batistinula gallesiae Arx, Publicações Inst. Micol. Univ. Recife 287: 6. 1960. — Fig. 5

Colonies amphigenous, irregular to circular, solitary becoming confluent, black, 1–7 mm diam. *External mycelium* straight, branching alternate, unilateral or opposite, pale brown to brown, septate, composed of cylindrical hyphal cells, 4.5–5 µm diam, smooth. *Appressoria* numerous, sessile, straight, cylindrical, 2–3 lobate, 9.5–15 × 9.5–14 µm, unicellular, alternate or unilateral, never opposed, brown, penetration peg centrally on the appressorial cell. *Ascomata* thyriothecioid dimidiate, isolated, superficial, developed below external mycelium, circular, fringed at margins, 152–213 µm diam, opening by a central star-shaped fissure, dark brown to black. *Scutellum* radiated, composed of isodiametric to cylindrical cells, straight. *Asci* bitunicate, globose, 50–67.5 × 32.5–47.5 µm, 4–8-spored, smooth, hyaline. *Ascospores* oblong, straight to slightly curved, 40–48 × 11–15 µm, base and apex broadly rounded, 4-celled, constricted at median septum, pale brown to brown, smooth. *Asexual morph*: *Colonies* superficial, developing above the external mycelium, brown to dark brown. *Conidiophores* arising from the hyphae, monoblastic, erect, cylindrical, unbranched, 33–60 × 9–13.5 µm, septate, brown. *Conidia* solitary, staurospores with three arms, 31–42.5 × 9.5–14 µm, brown, smooth, germinating at the ends of arms.

Type material. Brazil, Pernambuco, Recife, Poço das Maçãs, on living leaves of *Gallesiae gorazemae*, 7 Aug. 1960, *O.S. Silva* (URM 19988, holotype).

Additional material examined. Brazil, Espírito Santo, Sooretama, Reserva Natural Vale, on living leaves of *Caesalpinia echinata*, S19°19'03.28" W40°05'42.10", 15 July 2012, *A.L. Firmino*, *D.B. Pinho* & *O.L. Pereira* VIC 42514.

Notes — *Batistinula gallesiae* was originally described from living leaves of *Gallesia gorazema* (*Phytolaccaceae*) in the state of Pernambuco (Brazil). The present collection was from living leaves of *Caesalpinia echinata* (*Fabaceae*) collected in the state of Espírito Santo (Brazil). This specimen has the same morphological and biometric characteristics of the type. *Caesalpinia echinata* is a new host of *B. gallesiae* and the genus remains monotypic, with distribution restricted to Brazil.

Lembosia abaxialis Firmino & R.W. Barreto, *sp. nov.* — MycoBank MB812000; Fig. 6

Etymology. Name derived from the observation that colonies of this taxon are only formed abaxially.

Colonies hypophyllous, irregular to circular, solitary to confluent, black, 2–6 mm diam. *External mycelium* straight to flexuous, branching irregularly, septate, composed of cylindrical hyphal cells, 3–5 µm diam, brown, smooth. *Appressoria* numerous, entire to irregularly lobate, sessile, straight to angular, 7–10 × 10–10.5 µm, unicellular, unilateral to alternate, never opposed, brown, penetration peg centrally on the appressorial cell. *Ascomata* hysterothecioid, superficial, developed below external mycelium, mostly linear, rarely Y-shaped, solitary to grouped, fringed at margins, 340–550 × 160–250 µm, dark brown to black, opening by longitudinal fissures. *Scutellum* radiated, composed of isodiametric to cylindrical cells, straight. *Asci* bitunicate, slightly clavate, 52.5–57.5 × 25–37.5 µm, 8-spored, hyaline. *Pseudoparaphyses* cylindrical, septate, unbranched, hyaline. *Ascospores* oblong to cylindrical, 25–29 × 12.5–15 µm, 2-celled, constricted at the septum, hyaline, becoming pale brown to brown at maturity, smooth. *Asexual morph* absent.

Type material. Brazil, Rio de Janeiro, Bosque da Barra, Barra da Tijuca, on living leaves of *Miconia jucunda*, 22 Mar. 2014, *R.W. Barreto* (VIC 42825, holotype).

Notes — Twelve species of *Lembosia* have been recorded on *Melastomataceae* (Montagne 1855, 1856, Hennings 1904, Theissen 1913c, Arnaud 1918, Petrak & Ciferri 1930, Petrak & Sydow 1931, Song & Hosagoudar 2003, Hosagoudar & Appaiah 2005, Hosagoudar 2012, Farr & Rossman 2014). Only three of these have been reported from Brazil, namely, *L. catervaria*, *L. melastomatum* and *L. miconiicola*. All are distinct from *L. abaxialis* (Table 2).

Based on morphological characters, *L. domingensis* shows similarities with *L. abaxialis*, but differs by epiphyllous colonies, few, sparse, entire and conic appressoria, hysterothecia that are Y–X-shaped, with scarce, smaller asci, and slightly clavate

Table 2 Morphological characteristics of *Lembosia* spp. from *Melastomataceae*[1].

Taxon	Appressoria (µm)	Ascomata (µm)	Asci (µm)	Ascospores (µm)
Lembosia abaxialis[2]	7–10 × 10–10.5	340–550 × 160–250	52.5–57.5 × 25–37.5	25–29 × 12.5–15
Lembosia catervaria	6–8 diam	500–700 × 70–100	40 × 70	30–38 × 15–19
Lembosia domingensis	5–6 × 7–9	300–800 × 150–250	40–52 × 28–35	25–33 × 11–15
Lembosia gigantea	12–17 × 9	784–1064 × 302–504	84–96 × 33–41	26–29 × 14
Lembosia melastomacearum	14 × 9	784 × 336	55–72 × 41–48	26–29 × 12
Lembosia melastomatum	6–8 diam	700 × 250	70–96 × 42–52	35–40 × 16–20
Lembosia memecyli	–	200–450 × 120–150	35–55 × 26–35	20–23 × 8–10
Lembosia memecylicola	4–12 × 6–8	294–882 × 176–300	up to 45 diam	22–26 × 11–13
Lembosia miconiae-prasinae	7 wide	470–860 × 313–448	69–84 × 33–43	24–29 × 12
Lembosia miconiicola	–	500–800 high	22 × 11.5	23–28 × 11–13
Lembosia rolliniae	5–7 wide	300–350 × 100	50–60 × 30	24–26 × 10–11
Lembosia ryanii	7–17 × 5	235–425 × 145–168	36–46 × 21–31	20–21 × 9–12
Lembosia sclerolobii	–	up to 1000 × 140–180	35–50 × 30–40	17–23 × 6–9

[1] Montagne (1855), Montagne (1856), Hennings (1904), Theissen (1913c), Arnaud (1918), Petrak & Ciferri (1930), Petrak & Sydow (1931), Song & Hosagoudar (2003), Hosagoudar & Appaiah (2005), Hosagoudar (2012).
[2] This publication.

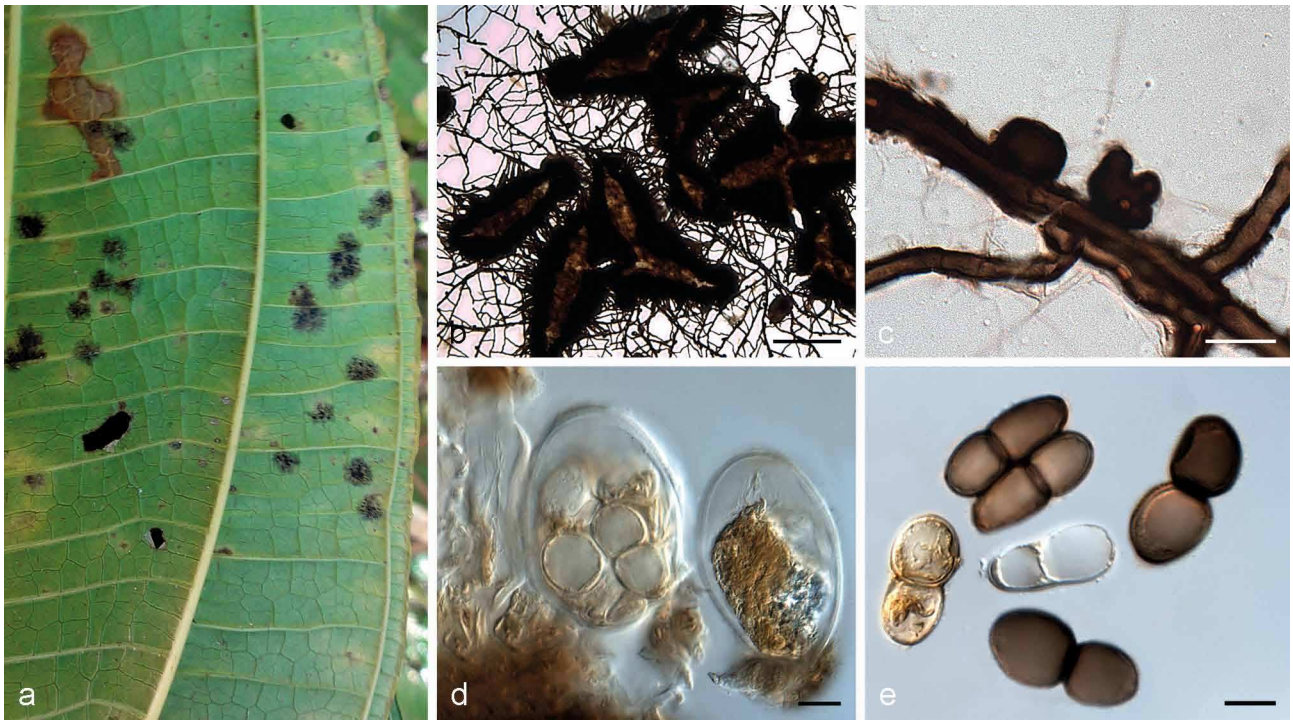

Fig. 6 *Lembosia abaxialis* VIC 42825. a. Living leaves of *Miconia jucunda* with hypophyllous colonies; b. colony with open hysterothecia and external mycelium; c. appressoria straight to angular, entire to irregularly lobate, unicellular; d. asci ovoid to slightly clavate; e. ascospores hyaline becoming pale brown to brown at maturity. — Scale bars: b = 20 µm; c–e = 10 µm.

ascospores (Petrak & Ciferri 1930). Additionally, *L. catervaria* differs from *L. abaxialis* by epiphyllous colonies, thicker hyphae, smaller appressoria, longer and narrower hysterothecia, wider asci and larger ascospores (Montagne 1855). *Lembosia melastomatum* differs from *L. abaxialis* by epiphyllous colonies, smaller appressoria, larger asci and ascospores (Montagne 1856). Finally, *L. miconiicola* differs from *L. abaxialis*, by epiphyllous colonies, larger hysterothecia and smaller asci (Arnaud 1918). *Lembosia abaxialis* is the first asterinaceous fungus reported on *Miconia jucunda* (*Melastomataceae*).

Prillieuxina baccharidincola (Rehm) Petr., Sydowia 4: 536. 1950. — Fig. 7

Basionym. *Lembosia drimydis* var. *baccharidincola* Rehm, Ann. Mycol. 5: 532. 1907.

≡ *Echidnodes baccharidincola* (Rehm) Theiss. & Syd., Ann. Mycol. 15: 422. 1926.

Colonies epiphyllous, irregular to circular, solitary becoming confluent, black, 1–6.5 mm diam. *External mycelium* straight to flexuous, branching irregularly, septate, hyphal cells cylindrical, 3–4 µm diam, pale brown, smooth. *Appressoria* absent. *Ascomata* thyriothecioid, single to confluent, superficial, developed below external mycelium, circular to ellipsoid, 102–160 µm diam, dark brown to blackish, opening by a central star-shaped fissure. *Asci* bitunicate, ovoid to subclavate, 37.5–50 × 20–30 µm, 8-spored, hyaline. *Ascospores* cylindrical to oblong, straight, 15–22 × 9–11.5 µm, base and apex broadly rounded, 2-celled, constricted at the septum, brown, smooth. *Asexual morph* absent.

Type materials. Brazil, São Paulo, on living leaves of *Baccharis* sp., unknown date, *A. Usteri* 8 (Z+ZT, syntype, here designated lectotype MBT200871); São Paulo, on living leaves of *Baccharis* sp., 5 July 1907, *Usteri* 41 (Z+ZT, syntype); ibid., 24 July 1907, *Usteri* 5 (Z+ZT, syntype); Minas Gerais, Nova Lima, on living leaves of *Baccharis* sp., 18 July 2012, *O.L. Pereira* (VIC 42817, epitype designated here MBT200345).

Additional material examined. Brazil, Minas Gerais, Lavras Novas, on living leaves of *Baccharis* sp., 10 Sept. 2012, *A.L. Firmino*, VIC 42818.

Asterotexiales Firmino, O.L. Pereira & Crous, *ord. nov.* — MycoBank MB812001

Type family. *Asterotexiaceae* Firmino, O.L. Pereira & Crous, *fam. nov.*

Description as for the constituent family *Asterotexiaceae* (see below).

Notes — Representative sequences of the major orders in the *Dothideomycetes* support *Asterotexiales* as a separate entity (Fig. 1). Within *Asterotexiales*, two lineages can be defined, one that contains the *Asterotexiaceae*, and another that contains *I. angularis*, which is maintained as *incertae sedis* at the family level. The type species of *Inocyclus* needs to be recollected and its phylogenetic position resolved.

Asterotexiaceae Firmino, O.L. Pereira & Crous, *fam. nov.* — MycoBank MB812002

Type genus. *Asterotexis* Arx, Fungus 28: 6. 1958.

Type species. *Asterotexis cucurbitacearum* (Rehm) Arx (as '*cucurbitarum*'), Fungus 28: 6. 1958.

Foliar pathogens, asterinaceae-like, obligately biotrophic, colonies irregular to star-shaped, solitary to confluent, sometimes extending along the veins, dark brown to black. *External mycelium* growing through ascomatal cavity towards the host epidermis, connecting the neighbouring ascomata, septate, hyaline (unlike members of *Asterinaceae*), smooth. *Appressoria* formed underneath the ascomata, solitary or forming in small clusters, globose, cone-shaped, ovoid to elongate, brown, with a central, hyaline penetration peg. *Ascomata* superficial, scutellate, dimidiate, brown to blackish. *Scutellum* formed by radially arranged rows of cells, opening by numerous irregular fissures, smooth. *Asci* bitunicate, fissitunicate, clavate to cylindrical, 8-spored, hyaline, numerous, parallel, vertically oriented within ascomata. *Ascospores* ellipsoidal to slipper-shaped, unequally 2-celled, slightly constricted at the septum, upper cell subglobose, lower cell smaller, subcylindrical to subcuneate, hyaline to slightly yellowish (unlike members of the *Asterinaceae*), smooth. *Asexual morph* unknown.

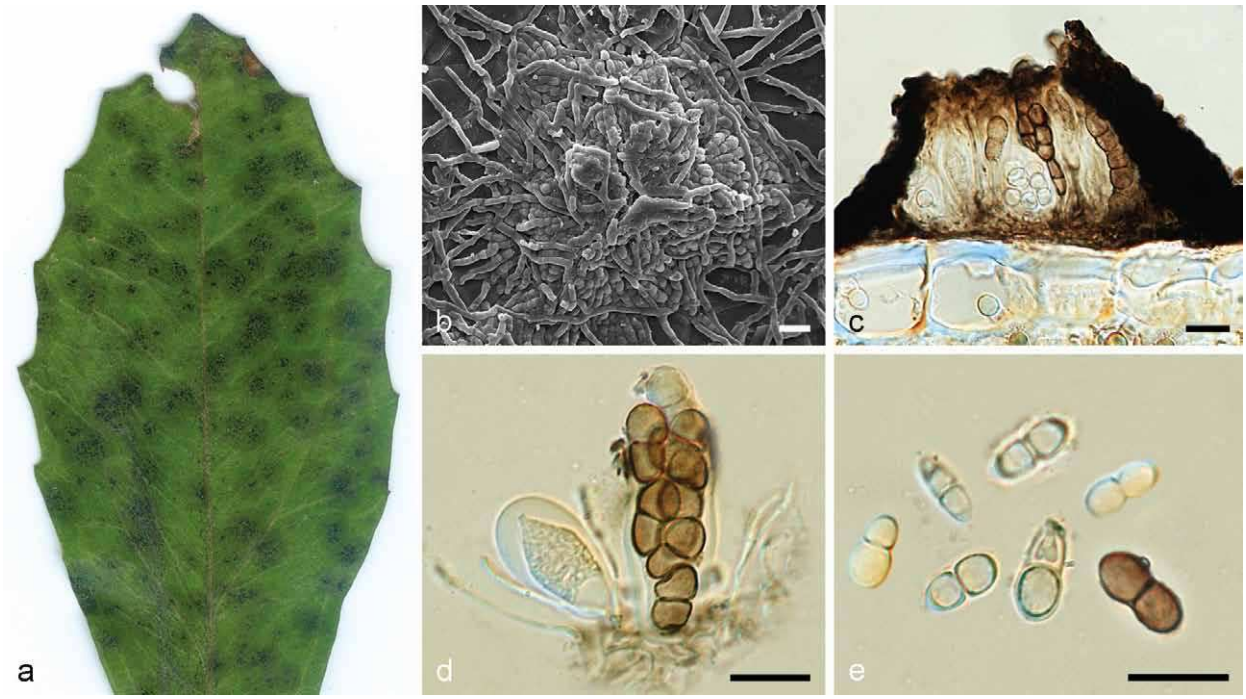

Fig. 7 *Prillieuxina baccharidincola* VIC 42817. a. Living leaves of *Baccharis* sp. with epiphyllous colonies; b. SEM image; thyriothecium opened by a central star-shaped fissure; c. vertical section of the ascoma; d. asci ovoid to subclavate showing pseudoparaphyses; e. ascospores hyaline becoming pale brown to brown at maturity. — Scale bars = 20 µm.

Fig. 8 *Asterotexis cucurbitacearum* VIC 42814. a, b. Symptoms on leaves of *Cucurbita pepo*: a. adaxial side; b. abaxial side, showing the hypophyllous colonies; c. external mycelium hyaline, connecting the ascomata in formation; d. immature ascomata in formation; e. fertile locules exposed on irregular fissures; f, g. vertical section of the ascomata, showing the appressoria with a central hyaline penetration peg, covered by the mature ascomata; h. vertical section of a fully developed ascoma, showing parallel and vertically orientated asci; i. asci; j. ascospores. — Scale bars: c–i = 10 µm; j = 5 µm.

Asterotexis cucurbitacearum (Rehm) Arx, Fungus 28: 6. 1958. — Fig. 8

Basionym. *Dothidella cucurbitacearum* Rehm, Hedwigia 36: 376. 1897. ≡ *Rhagadolobium cucurbitacearum* (Rehm) Theiss. & Syd., Ann. Mycol. 12: 275 .1914.

Colonies hypophyllous, irregular to star-shaped, solitary to confluent, sometimes extending along the veins, dark brown to black, 1–3 mm. *External mycelium* growing through ascomatal cavity towards the host epidermis, connecting the neighbouring ascomata, 3–4 µm diam, hyaline, septate, smooth. *Appressoria* formed underneath the ascomata, solitary or forming small groups, globose, cone-shaped, ovoid to elongate, 8–10 × 5–7 µm, brown, with a hyaline central penetration peg. *Ascomata* superficial, solitary to confluent, sometimes growing to surround the basis of individual trichomes of the host, scutellate, dimidiate, circular to irregular, 1–3 mm diam, upper cells irregularly shaped and thin-walled, brown to black. *Scutellum* formed by radially arranged rows of cells, opening by numerous irregular fissures, pale brown, smooth. *Asci* bitunicate, fissitunicate, clavate to cylindrical, 40–45 × 9.5–12.5 µm, 8-spored, numerous, parallel, vertically orientated within ascomata, hyaline, smooth. *Ascospores* ellipsoidal to slipper-shaped, 10–14 × 4–5 µm, unequally 2-celled, slightly constricted at the septum, upper cell subglobose, lower cell smaller, subcylindrical to subcuneate, hyaline to slightly yellowish, smooth. *Asexual morph* unknown.

Type materials. Brazil, Blumenau, on living leaves of *Cucurbita pepo*, May 1887, *E. Ule* 1415 (S F47805 syntype, here designated lectotype MBT200872); Rio de Janeiro, on living leaves of *Cucurbita pepo*, May 1887, *E. Ule* 676 (S F7565, syntype); Bahia, Igrapiúna, Reserva Ecológica Michelin, on living leaves of *Cucurbita pepo*, 15 July 2010, *O.L. Pereira* & *A.L. Firmino*, S13°49'17.90" W39°10'16.31" (VIC 42814, epitype designated here MBT200349).

Notes — *Asterotexis cucurbitacearum* has been recorded on living leaves of *Cayaponia americana* in the Dominican Republic and West Indies; on *Cucurbita moschata* in Venezuela and West Indies; on *Cucurbita pepo* in Brazil, Panama, Trinidad & Tobago and West Indies; on *Cucurbita* sp. in Brazil and Grenada; on *Gurania* sp. in the Dominican Republic; on *Trichosanthes* sp. in the Dominican Republic and on *Sechium edule* in Costa Rica; *Asterotexis quercina* has been recorded on *Quercus glauca* in Nepal (Guerrero et al. 2011, Farr & Rossman 2014).

INCERTAE SEDIS

Inocyclus angularis Guatimosim & R.W. Barreto, IMA Fungus 5: 52. 2014. — MB805976

Description and illustrations — Guatimosim et al. (2014b).

Materials examined. Brazil, Rio de Janeiro, Nova Friburgo, Mury, Sítio Colonial, on living leaves of *Pleopeltis astrolepis*, 30 Mar. 2013, *R.W. Barreto* (VIC 39747, holotype; CBS H-22028, isotype); ibid., 8 June 2013, *R.W. Barreto* VIC 39748, CBS H-22029; Rio de Janeiro, Nova Friburgo, Riograndina, Fazenda Barreto, on living leaves of *P. astrolepis*, 9 June 2013, *R.W. Barreto* VIC 39749, CBS H-22030.

Notes — Although *I. angularis* is not the type species of the genus *Inocyclus*, it is presently the only species from which DNA is available. A fresh collection of the type species, *I. psychotriae*, is required to clarify the correct placement of this genus.

DISCUSSION

The order *Asterinales* was included within *Dothideomycetes* based on the SSU and LSU analyses of five species of *Asterina* and a related asexual morph (Hofmann et al. 2010). In recent years, *Asterinales* was thought to comprise the families *Asterinaceae*, *Parmulariaceae* and *Aulographaceae* (Wu et al. 2011, Hyde et al. 2013). Recently, Hongsanan et al. (2014) provided a reassessment of the order. Based on LSU maximum likelihood and Bayesian analysis, and, despite the absence of molecular data for the *Parmulariaceae*, the authors concluded that only *Asterinaceae* should be included within *Asterinales*.

In the present study, we provide a robust molecular dataset that includes the type species of *Asterina*, as well as three other genera of *Asterinaceae*, the type species of the *Parmulariaceae* and a genus formerly assigned to the *Parmulariaceae*. The resulting LSU rDNA tree (Fig. 1) agrees in general with recent multigene analysis of the *Dothideomycetes* (Schoch et al. 2009) and demonstrated that the *Asterinales* comprises both *Asterinaceae* and *Parmulariaceae* as proposed by Barr & Huhndorf (2001), clustering with *Phaeotrichiaceae* and *Venturiaceae*.

A second analysis (available in TreeBASE), was done aiming at verifying if the former molecular studies involving species of *Asterina* and *Lembosia* (Hofmann et al. 2010, Hongsanan et al. 2014) correctly assigned the taxa included to the *Asterinaceae*. Based on these studies we conclude that these taxa, although considered by the authors as representative of species in the *Asterinaceae*, are in fact misplaced, and should be treated as *incertae sedis*, since they do not group with *A. melastomatis* – the type species of this family. The *Asterinaceae*, including the genera *Asterina*, *Batistinula*, *Lembosia* and *Prillieuxina* may, therefore, be polyphyletic, requiring a thorough reassessment. Nevertheless, it is important to note that all studies performed until now (Hofmann et al. 2010, Hongsanan et al. 2014), used relatively short LSU sequences (c. 490 bp) that may not provide the necessary resolution needed.

Asterotexis cucurbitacearum was initially classified in the *Parmulariaceae* (Theissen & Sydow 1914) and then transferred to *Asterinaceae* (Inácio & Cannon 2008, Kirk et al. 2008, Guerrero et al. 2011). This species is clearly not a member of the *Asterinaceae* (contradictory to what was shown by Hongsanan et al. 2014) and is transferred here to the newly proposed family *Asterotexiaceae*. This new family grouped (Fig. 1) with *Inocylus angularis* (originally described as a member of the *Parmulariaceae*).

Nuclear DNA of *P. styracis*, the type species of the *Parmulariaceae* was isolated and studied for the first time here. DNA was successfully isolated from *I. angularis*, allowing a preliminary assessment of the *Parmulariaceae*. Although involving only two taxa, the finding that *I. angularis* does not group with the type of *Parmulariaceae*, confirm that the *Parmulariaceae* is polyphyletic (Inácio & Cannon 2008, Hongsanan et al. 2014). The status of *I. angularis* within the genus *Inocyclus* requires confirmation, ideally with a molecular assessment of the type species of *Inocyclus*.

The molecular phylogenetic analysis presented here clearly indicates that both the *Parmulariaceae* and *Asterinaceae* are polyphyletic. Only the epitypification of the taxa in these and other families of thyriothecioid ascomycetes, followed by molecular phylogenetic analysis will resolve their taxonomic placement and produce a more natural classification for these neglected tropical fungi.

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Capítulo 3 – Espécies de cercosporóides e suas formas sexuais em pteridófitas

Artigo – Novel fungi from an old niche: Cercosporoid and related sexual morphs on ferns.

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

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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 is 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 were studied, collected from 18 host species representing 201 localities. This resulted in a total of 21 frond-spotting taxa, which were identified based on morphology, ecology and sequence data of five genomic loci (ITS, partial transcription-elongation factor 1-α, actin, calmodulin and LSU). One novel genus (*Clypeosphaerella*) and 17 novel species (*Cercospora samambaiae, Paramycosphaerella blechni, Pa. cyatheae, Pa. dicranopteridis-flexuosae, Pa. gleicheniae, Pa. sticheri, Phaeophlospora pteridivora, Pseudocercospora brackenicola, Ps. paranaensis, Ps. trichogena, Ps. serpocaulonicola, Clypeosphaerella sticheri, Xenomycosphaerella alsophilae, X. cyatheae, X. diplazii,* and *Zasmidium cyatheae*) are introduced. Furthermore, ten new combinations (*Clypeosphaerella quasiparkii, Paramycosphaerella aerohyalinosporum, Pa. dicranopteridis, Pa. gleicheniae, Pa. irregularis, Pa. madeirensis, Pa. nabiacense, 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. lygodiicola* and *Ps. thelypteridis*.
Key words: *Cercospora*, frond spot, multilocus sequence, *Mycosphaerella*, *Pteridophyta*, systematics

INTRODUCTION

Cercosporoid fungi are well-known plant pathogens that are etiological agents of leaf spot diseases of several important crops, such as angular leaf spot of bean (*Pseudocercospora griseola*), black leaf streak of banana (*P. fijiensis*), leaf spots of gravevines (*P. vitis*), celery (*Cercospora apii*), sugarbeet (*Cercospora beticola*), and many other hosts (Braun et al. 2013).

Since the seminal monograph of Chupp (1954) on the genus *Cercospora*, several studies were carried out aiming at investigate 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, b, c, 1995, 1998, Crous & Braun 1996, Braun and Melnik 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 studies 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 & Hsieh1995), South Africa (Crous & Braun 1996), Russia and adjacent countries (Braun & Melńik 1997), Korea (Shin & Kim 2001), China (Guo et al. 2003, 2005), Laos (Phengsintham et al. 2013a) and Thailand (Phengsintham 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. 2006b, c, 2007a, b, Arzanlou et al. 2007, Phillips et al. 2008, Crous et al. 2009a, b, 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 research 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. 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 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 recognize them as distinct genera. As a result, numerous asexual genera, which may eventually prove to be artificial, have been introduced. 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. in the *Teratosphaeriaceae* (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. These studies have shown that some morphology-based genera were largely monophyletic, e.g. *Cercospora*, *Pseudocercospora* and *Ramularia* (Crous et al. 2013b, Groenewald et al. 2013, Bakshi et al. 2014, 2015, Videira et al. 2015) whereas others were clearly polyphyletic, e.g. *Passalora, Phloeospora, Phoma, Pseudocercosporella, Septoria, Stagonospora* (Frank et al. 2010, Aveskamp et al. 2010, 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 completed (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. 1110 species known from Brazil, it has been estimated that this number may be far greater (Forzza et al. 2015). Approximatly 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 economical importance of most species. One exception in the general absence of monographic treatments of fungi on ferns are 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 davalliicola*) 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 that a plethora of novel taxa to exist in this niche. Two of the preliminaries 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). Additionally, the phylogenetic placement of the monotypic class *Mixomycetes* was 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. 2009c). 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 underneath of Petri dish lids, over which the plate containing PCA was placed. Ascospores germination patterns were recognized 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 Zeiss V20 Discovery stereo-microscope and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and a MRc5 camera and ZEN imaging software. Colony descriptions were made on malt extract agar (MEA), potato dextrose agar (PDA), PCA and oatmeal agar (OA) (Crous et al. 2009c), 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 Herbarium of the Universidade Federal de Viçosa (VIC) and the Herbarium 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 doublesided 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 Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions. The DNA samples were subsequently diluted 50–100 times in preparation for further DNA amplification reactions. All strains were screened for different loci. 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-α (Tef1-α). Additionally, for the *Cercospora* strains, a part of the calmodulin gene (CAL) was amplified. The primers employed are listed in Table 2. The PCR amplifications were performed in a total volume of 12.5 µL solution containing 10–20 ng of template DNA, 1× PCR buffer, 0.63 µL DMSO (99.9 %), 1.5 mM MgCl2, 0.5 µM 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, 1 min). PCR conditions for Tef-1α 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 CAL, the PCR conditions were set as an initial denaturation (94 °C, 5 min) 45 cycles aplification (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 Sequencer (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 for initial alignment and the construction of sequence datasets. Sequences obtained from Schoch et al. (2009), TreeBASE study S10245, Groenewald et al. (2013), TreeBASE study number S13645, Crous et

al. (2013b) TreeBASE study number S12805, from GenBank (www.ncbi.nlm.nih.gov), and the novel sequences generated on this study, were aligned using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; (Katoh et al. 2002) and whenever indicated, manually improved in MEGA.

Phylogenetic analysis

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. 3.1.2 applying different substitution models for each locus as listed in Table 3. *Septoria provencalis* CBS 118910 served as outgroup for the phylogenetic analyses of *Cercospora* species, *Passalora eucalypti* CBS 111318 for *Pseudocercospora* species and *Staninwardia suttonii* HT 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 (Ronquist et al. 2012). Six simultaneous Markov chains were run for 10.000.000 generations and trees were sampled every 100th generation, and 10.000 trees were obtained. The first 2.000 trees, representing the burn-in phase were discarded, while the remaining 8.000 trees were used for calculating posterior probabilities. Bayesian posterior probabilities (PP) are presented on the left of each node, on each tree. Sequences derived in this study were lodged in GenBank, the alignment and tree in TreeBASE (http://www.treebase.org) and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004a).

RESULTS

Phylogenetic results

The three datasets consisted of 1 273 characters for the *Cercospora* tree (184 characters for ACT, 322 for Tef1-α, 476 for ITS, and 291 for CAL), 1 111 characters for the *Pseudocercospora* tree (216 characters for ACT, 392 for Tef1-α, and 503 for ITS), and 1 944 characters for the mycosphaerella-like tree (232 characters for ACT, 435 for Tef1-α, 507 for ITS, and 758 for LSU).

The respective alignments included 348 parsimony-informative characters for the *Cercospora* tree (74 for ACT, 125 for Tef1-α, 41 for ITS, and 108 for CAL), 333 parsimony-informative characters for the *Pseudocercospora* tree (75 for ACT, 200 for Tef1-α, and 58 for ITS), and 723 parsimony-informative characters for the mycosphaerella-like tree (127 for ACT, 226 for Tef1-α, 221 for ITS, and 149 for LSU).

After topological convergence of the Bayesian runs at 0.30 for *Cercospora*, and 0.15 for *Pseudocercospora* and mycosphaerella-like taxa, 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: 2675/2140 for *Cercospora*, 7213/5770 for *Pseudocercospora*, and 1710/1368 for mycosphaerella-like taxa. 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.

TAXONOMY

Cercospora Fresen., Beiträge zur Mykologie 3: 91. 1863.

Cercospora coniogrammes Crous & R.G. Shivas, Stud. Mycol. 75: 151. 2013. — MycoBank MB800653; Fig. 4

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.

Frond spots irregular, starting on the edges of the pinnulets, extending to ecompass whole pinnulets and sometimes leading to the necrosis of the entire pinnule. Starting centrally, pale brown, becoming to pale brown to reddish at the edges, coalescing, turning dark-brown to black. *Caespituli* hypophyllous, abundant. *External hyphae* absent. *Internal hyphae* indistinct. *Stromata* rudimentary, irregular, composed of *textura globulosa*, dark-brown. *Conidiophores* rising through the stomata, hypohyllous, forming fascicles (6–11 stalks per fascicle), sub-cylindrical, straight to curved, geniculate, 92–509 × 5–6 µm, unbranched, 3–15-septate, guttulate, pale-brown becoming paler at the apex, smooth. *Conidiogenous cells* terminal, integrated, holoblastic, sub-cylindrical, 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, 134–320 × 2–3 µm, apex acute, base subtruncate, 2.5–4.5 µm diam at the base, 14–34-septate, guttulate, hyaline, smooth.

Culture characteristics — Colonies on PCA slow-growing, 80 mm diam after 28 d; flat, with scarce aerial mycelium, mouse grey centrally, lavender grey to white at periphery, pigmenting the medium in 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); 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 — In the Tef-1α, and CAL 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. coniogammes* (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 thinner conidiophores (15–120 × 4–5 µm), 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 (50–110 × 3–4 µm) and shorter and narrower conidiophores (25–160 × 4–5 µm) (Braun et al. 2013).

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

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 pinnulet. *Caespitulli* hypophyllous, abundant. *Internal hyphae* septate, intra- and intercellular, frequently branched, 2–4 µm wide, pale-brown, smooth. *Stromata* rudimentary, globular, composed of *textura globulosa*, dark brown. *Conidiophores* rising through the stomata, hypophylous, forming loose fascicles (3–7 stalks per fascicle), sub-cylindrical, straight or slightly curved to sinuose, geniculate, 96–326 × 4–5 µm, unbranched, 3–9-septate, olivaceous brown, thin-walled, smooth. *Conidiogenous cells* terminal, rarely integrated, holoblastic, sub-cylindrical, tapering to a flat-tipped apex, with numerous tightly aggregated apical conidiogenous loci, proliferating sympodially, 26–102 × 4–5 µm, pale brown, smooth, scars conspicuous, protruding, 2.5–4 µm diam, thickened, darkened. *Conidia* solitary, acicular, sinuous to slightly curved, 142–303 × 2–3

µm, apex acute, base subtruncate, 2.5–4 µm diam at the base, 10–31-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 Bomsucesso, 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. To resolve their taxonomy, fresh collections authentic for the names, based on host and country, such as *C. acaciae-mangii* from Thailand and *C. dioscoreae-pyrifoliae* from India, need to be recollected and included in future studies (Groenewald et al. 2013, Bakhshi et al. 2015). Morphologically, the isolates from Brazil are not different from *C. apii s.l.,* but the hosts on which they cause disease are significantly different, e.g. all isolates included in *Cercospora* sp. Q, were obtained from angiosperms, while the Brazilian isolates are from three different orders of *Pteridophyta*, namely *Cyatheales, Polypodiales* and *Schizaeales*. Phylogenetically, the species included in this clade are different from the other species by their position in the CAL and Tef-1α phylogeny; while in the ACT phylogeny they cannot be distinguished from *Cercospora* spp. F (data not shown). In the combined tree (Fig. 1), *Cercospora* sp. Q. has *Cercospora* cf. *zinniae* as a sister taxon.

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 ascomata, ressembling a *pseudoclypeus*.

Frondiicolous, plant pathogenic. *Ascomata* pseudothecial, epiphyllous, solitary, sub-cuticular to erumpent, globose, walls of 2–3 layers of brown to dark brown *textura angularis*, ostiole central. *Asci* bitunicate, aparaphysate, 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.l.*, differing by having the thicker upper wall of the ascomata, resembling a *pseudoclypeus*. Additionally, the former genus is phylogenetically distinct from other mycosphaerella-like fungi.

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

Basionym. Mycosphaerella quasiparkii Cheewangkoon, K.D. Hyde & Crous, Persoonia 21: 85. 2008.

Description and illustration – Cheewangkoon et al. 2008.

Specimen examined. THAILAND, Burirum, 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. 19

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

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, becomming fertile, confluent and necrotic. *Internal hyphae* intra- and intercellular, 1.5–3.5 µm wide, branched, septate, sub-hyaline, smooth. *Ascomata* pseudothecial, epiphyllous, mostly congregated at the basis of the pinnae, solitary, sub-cuticular 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* bitunicate, aparaphysate, 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, lavender grey centrally, and pale vinaceous at periphery, vinaceous buff reverse. On OA, aerial mycelium scarce, 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, *C. sticheri* is most similar to *C. 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 later), 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 *C. sticheri* (Type F, Crous 1998) whereas in *C. quasiparkii* germ spores arise from the polar ends develop firstly parallel to the main axis, and later grow perpendicularly becomes distorted (Type D, Crous 1998) (Cheewangkoon et al. 2008). Additionally, it is also phylogenetically distinct (Fig. 3).

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

Notes — The genus *Paramycosphaerella* is based on *P. brachystegia*, which occurs on *Brachystegia* sp. (*Fabaceae*) from Zimbabwe (Crous et al. 2013a). So 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 and illustration – Crous et al. 2009d.

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. 7

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

Frond spots amphigenous, starting on the pinnule as pale brown random spots, vein-delimited, with a pale brown central area, coalescencing 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, sub-hyaline to pale brown, smooth. *Ascomata* pseudothecial, epiphyllous, solitary, subcuticular to erumpent, globose to subglobose, 92–90 × 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. *Asci* 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 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, *P. blechni* is rather similar to *P. dicranopteridis-flexuosae* described on *Dicranopteris flexuosa* from Brazil (this study), but can be distinguished from it by having thinner obpyriform to ovoid asci (pyriform to narrowly ellipsoid, 10–18 µm wide in *P. dicranopteridis-flexuosae*). Phylogenetically, *P. blechni* is related to *P. 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. 8

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

Frond spots randomly affecting individual pinnules, irregular, initially necrotic along the main vein of the pinnulet, pale brown, with a cream central area where ascomata are formed, becomming dark brown. *Internal hyphae* branched, septate, intra- and intercellular, 2.5–4.5 µm wide, sub-hyaline, smooth. *Ascomata* pseudothecial, epiphyllous, solitary, sub-cuticular to erumpent, globose, 36–101 × 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, aparaphysate, 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 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 and phylogenetically, *P. cyatheae* is rather similar to *P. madeirae* described on *Eucalyptus* sp. from Madeira (Crous et al. 2004b), and to *P. sticheri*, described on *Sticherus penninger* from Brazil (this study), but can be distinguished by having wider asci (8–12 µm wide in *P. madeirae*), and smaller ascospores (14–20 × 3–5.5 µm in *P. sticheri*). Phylogenetically, *P. cyatheae* has *P. madeirae* as sister clade (Fig. 3). However it differs from it by having the following number of variable sites for each locus: 23 bp for ACT, and 17 bp for ITS. *Paramycosphaerella madeirae* is likely to have a pseudocercospora-like asexual morph, but as no cultures of the conidial stage were obtained, the connection remains uncertain (Crous et al. 2004b).

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

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

Description and 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. 9

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

Frond spots amphigenous, irregular, starting as small dark brown spots, with a white centre adaxially, leading to the chlorosis of the pinnulet (particularly of 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, sub-hyaline to pale brown, smooth. *Ascomata* pseudothecial, epiphyllous, solitary, sub-cuticular to erumpent, globose, 46–114 × 55–109 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. *Asci* bitunicate, aparaphysate, fasciculate, subsessile, 8-spored, obclatave 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 towards 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. Schwartsburd & A.P. Fortuna* (holotype CBS H-22091, isotype VIC 43118, culture ex-type CPC 24743); *ibid.,* vicinities of the Parque Estadual do Itacolomi, on fronds of *Dicranopteris flexuosa*, 8 June 2013, *E. Guatimosim*, VIC 42475.

Notes — Morphologically and phylogenetically, *P. dicranopteridis-flexuosae* is rather similar to *P. gleicheniae*, recorded on *D. linearis* from India, Malasya and Taiwan (Kirschner & Liu 2014), but can be distinguished from the latter by having longer and wider asci (18–33 × 9–15 µm in the latter) and ascospores (12–15 × 3 µm in the latter) (Ramakrishnan & Ramakrishnan 1950). Only ITS sequence data is available for *P. gleicheniae* (Kirschner & Liu 2014). *Paramycosphaerella dicranopteridis-flexuosae* differs from *P. gleicheniae* by 5 bp only. Nevertheless the tree produced in this study (Fig. 3) demonstrated that *P. gleicheniae* is quite distinct from *P. dicranopteridis-flexuosae* with a high support value (PP = 1). Additional loci should be sequenced for the former species, aiming at clarifying the true species boundaries. At present, based on host difference and with the lack of additional data, we decided to maintain them as distinct taxa. An asexual *Pseudocercospora* morph was observed on different specimens, collected in different seasons at the same place, being associated with similar symptoms like the ones caused by *P. dicranopteridis-*flexuosae. 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. Sect. B 32: 205. 1950.

Specimens examined. INDIA, Coonoor, Nilgiris, Tamil Nadu, on fronds of *Dicranopteris linearis* (=*Gleichenia linearis*), 29 May 1948, *T. S. Ramakrishnan* and *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, Weiliao Old Trail, 29 Sept. 2013, *R. Kirschner* (TNM 3945, culture RoKi 3945).

Notes — The type of *P. gleicheniae* was described from India and it is holotype is presumably 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 (Cheewangkoon, K.D. Hyde & Crous) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812824

Basionym. *Mycosphaerella irregularis* Cheewangkoon, K.D. Hyde & Crous, Persoonia 21: 82. 2008, as "*irregulari*".

Description and 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 and 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 and illustration – Crous et al. 2009d.

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 & Alfenas) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812810

Basionym. *Mycosphaerella parkii* Crous, Wingf., Ferreira & Alfenas, Mycol. Res. 97: 582. 1993.

≡ *Stenella parkii* Crous & Alfenas, Mycologia 87: 121. 1995.

≡ *Zasmidium parkii* (Crous & Alfenas) Crous & U. Braun, Schlechtendalia 20: 102. 2010.

Description and illustration – 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).

Paramycosphaerella pseudomarksii (Cheewangkoon, K.D. Hyde & Crous) Guatimosim, R.W. Barreto & Crous, *comb. nov.* — MycoBank MB812811

Basionym. *Mycosphaerella pseudomarksii* Cheewangkoon, K.D. Hyde & Crous, Persoonia 21: 83. 2008.

Description and 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. 10

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

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 pinnule. *Internal hyphae* branched, septate, intra- and intercellular, 2–2.5 µm wide, sub-hyaline to pale brown, smooth. *Ascomata* pseudothecial, amphigenous, more abundant abaxially, solitary, sub-cuticular to erumpent, globose, 51–106 × 45–94 µm, walls of 2–3 layers of brown to dark brown *textura angularis*, cells 2.5–4 × 2–3 µm, black, ostiole central, 16–30 µm diam. *Asci* bitunicate, aparaphysate, fasciculate, subsessile, 8-spored, obpyriform, straight or slightly curved, 24–58 × 11–20 µm, hyaline, smooth. *Ascospores* inordinate, overlapping, fusoid, straight, 14–20 × 3–5.5 µm, 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 direction of spore long axis, germ tubes irregular in width, slightly distorting, spores 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 *P. sticheri* is rather similar to *P. dicranopteridis-flexuosae*, recorded on *Dicranopteris flexuosa* from Brazil (this study). Nevertheless, it can be distinguished from the latter by having thinner ascospores (2–4.5 µm in the latter) and the following number of variable sites for each locus: 28 bp for ACT, 43 bp for ITS, 101 bp for Tef-1α and 8 bp for LSU. Additionally, based on phylogenetic inference (Fig. 3), *P. sticheri* grouped basal to other taxa in the genus, having *P. bracystegia* as sister clade, differing from the latter by a high support value (PP = 1).

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

Phaeophleospora pteridivora Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812826; Fig. 11

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

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 becomming 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, smooth. *Ascomata* pseudothecial, hypophyllous, solitary, sub-cuticular 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. *Asci* 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, formed

next to where sexual fruiting structures are formed, 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, sub-cylindrical to ampuliform, straight, 5–25 × 2–5 µm, unbranched, aseptate, sub-hyaline to pale brown, smooth. *Conidiogenous cells* terminal, determined, unbranched, tapering to the apex, sub-hyaline to pale brown, smooth, scars inconspicuous, one per cell, not thickened, nor darkened. *Conidia* solitary, sub-cylindrical, 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, feathery 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 sterile.

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*, and its type species *P. eugeniae*, were described on *Eugenia uniflora* (*Myrtaceae*) from 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 having 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 becoming rather morphologically diverse.

Pseudocercospora Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires, Ser. 3, 13: 437. 1911. — MycoBank MB9559

Pseudocercospora abacopteridicola J.M. Yen & Lim, Cah. Pacifique 17: 97. 1973. — MycoBank MB113053; Fig. 12

Frond spots amphigenous, starting as minute, vein-delimited, pale brown spots, affecting random pinnules, leading to an extensive necrosis of the entire pinnae, which then becomes dark brown to black, with a central area whitish 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, sub-cylindrical, straight, geniculate, 5–5.5 × 2–2.5 µm, unbranched, aseptate, pale brown, smooth, scars indistinct. *Conidia* solitary, sub-cylindrical, straight or curved, 4.5–77 × 1.8–3 µm, rounded apex, base truncate, 1–3 µm diam at the base, 2–8-septate, guttulate, pale brown, smooth, hila not thickened, not darkened.

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 center, 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 – *Adiathum* 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 *P. abacopteridicola* until DNA from the fungus from Singapore becomes available for a molecular comparison.

Pseudocercospora brackenicola Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812813; Fig. 13

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

Frond spots, amphigenous, irregular, starting as small, dark brown vein delimited spots at pinnulet margins, spreading and becoming black with age and occasionally reaching the entire pinnulet. *Caespituli* hypophylous, 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, sub-hyaline 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, sub-cylindrical, 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, sub-cylindrical, 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 sub-cylindrical, straight, curved, or sinuous, 20–77 × 1–2 µm, rounded apex, base truncate, 1–2 µm diam at the base, 1–6-septate, guttulate, pale brown, smooth, hilum not thickened, nor darkened.

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; olivacecous 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, *P. brackenicola* clusters with *P. purpurea* and *P. sordida* as sister clade, differing from them by a highly supported branch (PP = 0.89) (Fig. 2). Both species are clearly different from *P. brackenicola* by having larger conidiophores (20–200 × 3.5–4.5 µm, in *P. purpurea*, and 20–90 × 3.5–5 µm, in *P. sordida*), and larger conidia (20–100 × 2–4.5 µm, in *P. purpurea*, and 20–165 × 3–5.5 µm, in *P. sordida*) (Chupp 1954, Guo & Hsieh 1995). Adittionally, the hosts of *P. purpurea* and *P. sordida* are higher plant families in the *Perseaceae* and *Bignoneaceae*, respectively (Farr & Rossman 2015). Morphologically, *P. brackenicola* is similar to *P. davallicola* (described on *Davallia fejeensis* from Brazil) and to *P. 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 *P. davallicola* can be formed in small chains (completely absent in *P. brackenicola*), and the conidiophores of *P. davallicola* are isolated, whereas on *P. brackenicola* it forms fascicles emerging from a stroma, through stomata (Braun et al. 2013). Secondly, *P lonchitidis,* has erumpent, well-developed stromata (loosely dense, emerging through the stoma in *P. brackenicola*), straight and thicker conidiophores, 3–5 µm wide in *P. davallicola*, (curved to sinuous, 2–3 µm wide in *P. brackenicola*), and conidiogenous loci are subdenticulate, (inconspicuous in *P. brackenicola*) (Braun et al. 2013). This is the first record of a *Pseudocercospora* on the genus *Pteridium*. The fungus causes a damaging disease on its host (bracken), which is a highly noxious weed. This fungus should be further investigated as a potential biological control agent.

Pseudocercospora lygodiicola Y.L. Guo & U. Braun, IMA Fungus 4: 317. 2013. — MycoBank MB805526; Fig. 14

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, sub-globose, 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), sub-cylindrical, sinuous or curved, geniculate towards the apex, 26–80 × 3–5 µm, unbranched, 3–6-septate, eguttulate, pale brown, smooth. *Conidiogenous cells* terminal, holoblastic, sub-cylindrical, attenuated at the tip, 3–18 × 2–4 µm sub-hyaline, 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 truncate, 2.5–4 µm diam at the base, 6–12-septate, guttulate, pale brown, smooth.

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 olivaceous grey areas centrally, olivaceous grey towards periphery; reverse olicavecous 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 *P. lygodii* (on *L. japonicum* from Taiwan), *P. lygodiicola* (on *L. japonicum* from China), *P. lygodiigena* and *P. polypodiacearum* (both on *Lygodium* sp. from India) (Braun et al. 2013). The species boundaries among these taxa is based on morphological and biometric characters, which could be considered as tentative, as the host range and distribution range of these taxa are quite similar. There are no records of ex-type cultures or DNA information on any of them.

The fungus isolated from *L. volubile* in Brazil has morphological and biometric data similar to *P. lygodiicola*, and until epitipification of this taxon has been carried out, we decided to extend its host range, rather than propose a new name for it. Phylogenetically, *P. lygodiicola* clusters in the same clade of

three other species isolated from ferns, namely *P. cyatheicola*, *P. rumohrae,* and *P. thelypteridis* (Fig. 2).

Pseudocercospora paranaensis Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812814; Fig. 15

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 greyish 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, aparaphysate, fasciculate, subssesile, 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, 1-septate, slightly constricted at the septum, unequally, tappering towards rounded ends, with two large opposed guttules, hyaline, thin-walled, smooth. *Ascospore germination* not observed. *Asexual morph*: *Caespituli* hypophyllous, abundant. *Stromata* sub-superficial, 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, ampuliform, swollen at the base, 7–11 × 1.5–2 µm, unbranched, aseptate, eguttulate, pale brown, smooth, scars, truncate, 2 µm wide, neither thickened, nor darkened. *Conidia* solitary, sub-cylindrical or obclavate, curved or rarely straight, 79–99 × 2–3 µm, rounded apex, base truncate, 2–3 µm diam at the base, 3–9-septate, guttulate, pale brown, smooth, hilum not thickened, nor darkened.

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 reverse; cultures sterile.

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

Notes — *Pseudocercospora paranaensis* clusters at a basal position to the other taxa compared with it (Fig. 2), having *P. basitruncata* as sister clade, but differing from it by a highly support value (PP = 1). Besides *P. basitruncata* is known to be an extremely variable species, some features staying relatively constant such as the irregular annellations on the conidiogenous cells, and the conidial shape: when smaller conidia tend to be cylindrical, whereas larger conidia have tapering to more obtuse apices (Crous 1998). *Pseudocercospora paranaensis* does not have any annellations on its conidiogenous cells, which proliferate sympodially instead. Additonally *P. paranaensis* differs from *P. basitruncata* 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, *P. basitruncata* is only known from an unrelated species of *Eucalyptus* (Hunter et al. 2011, Crous et al. 2013b).

Two other species of *Pseudocercospora* have already been recorded on members of *Cyatheaceae*, namely: *P. cyatheae* described on *Cyathea* sp. from Japan, and *P. cyatheicola* on *Cyathea australis* from Australia (Braun et al. 2013). With regards to *P. cyatheae*, the only sequence available in GenBank for this species is of the ITS region. *Pseudocercospora paranaensis* differs from *P. cyatheae* in ITS and clusters in a separate and highly supported clade (data not shown). However, this result should be regarded as weak evidence for species separation since the ITS locus does not provide the necessary resolution needed for separating *Pseudocercospora* species (Crous et al. 2013b). Nevertheless, morphological criteria clearly separate the two species. *Pseudocercopora cyatheae*, in contrast to *P. paranaensis,* has epiphyllous caespitulli. Its conidiogenous cells have a rim-like thickening at the tip, and it also has thicker, cylindrical to obclavate conidia (30–50 × 3.7–5.5 µm), with a rounded base (Nakashima et al. 2006). *Pseudocercospora cyatheicola* is different from *P. paranaensis* both phylogenetically – grouping at the bottom 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 thelypteridis Goh & W.H. Hsieh, Trans. Mycol. Soc. Republ. China 4: 30. 1989. — MycoBank MB355103; Fig. 16

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

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 with *P. cyatheicola* and *P. rumohrae* as sister clade, differing from it by a branch with low support (PP = 0.67, Fig. 2). However, *P. cyatheicola* is different from *P. thelypteridis* by having erumpent and amphigenous stromata, longer and narrower conidiophores (30–70 × 2–3 µm), percurrent proliferating conidiogenous cells, and pale brown conidia (Crous et al. 2011). On the other hand, *P. rumohrae* differs from the new species by the absence of stromata, with conidiophores arising directly from the hyphae, longer and thinner 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 to describe the fungus found in Brazil as *P. thelypteridis*. This is the first time that *P. thelypteridis* is described from Brazil.

Pseudocercospora trichogena Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812827; Fig. 17

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

Frond spots on *Deparia petersenii*, amphigenous, evident adaxially, irregular, pale brown with necrotic fertile centre and distinctive black halo. *Ascomata* pseudothecial, epyphyllous, 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. *Asci* bitunicate, aparaphysate, sessile, 8-spored, fusoid-ellipsoidal when immature, pyriform at maturity, curved, 26–42 × 8–14 µm, hyaline, smooth. *Ascospores* biseriate to inordinate, overlapping, fusoid, straight, 9–15 × 2–4 µm, 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 pinnulet, and spreading towards the, initially pale brown becoming dark and necrotic. *Caespituli* hypophylous, abundant on the trichoma. *External hyphae* hypophyllous abundant, often erupting through the cuticle, rarely arising through the stoma, and climbing the trichoma, spreading and covering the entire lesion, 2–3 µm wide, branched, septate, pale brown, smooth. *Internal hyphae* intra- and intercellular, abundant, 1–3 µm wide, prominently branched, septate, sub-hyaline, smooth. *Stromata* absent. *Conidiophores* arising from the external hyphae, hypophyllous, often restricted to the conidiogenous cells, formed in groups on trichoma, sub-cylindrical, attenuated at the tip, straight or sinuous, 19–74 × 5–6 µm, often branched, 1–5-septate, eguttulate, pale brown to brown, smooth. *Conidiogenous cells* terminal, integrated, holoblastic, sub-cylindrical, determined, 10–35 × 5–6 µm, pale brown to brown, smooth, scars inconspicuous, 1 per cell, 1–2 µm, not darkened, nor thickened. *Conidia* solitary, obclavate, straight or curved, 72–147 × 3–5 µm, apex rounded, base truncate, 4–5 µm diam at the base, 4–13-septate, guttulate, pale brown, smooth, hilum not darkened, nor thickened.

Culture characteristics — Colonies on MEA slow-growing, 10–23 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 *P. 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, *P. trichogena* clusters with *P. araliae*, *P. dendrobii*, and *P. jussiaeae* as sister clade, separating from them by a highly support value (PP = 1, Fig. 2). Those three species are different from the former by having fasciculate conidiophores, arising from a stroma and emerging through the stomata. Stromata are not well developed, but still present, in *P. araliae* (Braun et al. 2013) but completely absent in *P. trichogena*. Additionally, these species were described colonising higher plants and are seemingly absent from Brazil. *Pseudocercospora araliae* infects *Aralia* spp. in China, Japan, and Taiwan, *P. dendrobii* on *Dendrobium* sp. from China, Japan, Korea, Taiwan and USA, and *P. jussiaeae* infects *Jussiaea* and *Ludwigia* spp. in a number of countries around the world (Deighton 1976, Hsieh & Goh 1990, Farr & Rossman 2015). Morphologically, *P. trichogena* is similar to three other species recorded on *Thelypteridaceae*, namely, *P. abacopteridicola* on *Abacopteris urophylla* from Singapore, *P. pteridophytophila* on *Cyclosorus acuminatus* from Asia, and *P. thelypteridis* on *Nephrolepis* sp. and *Thelypteris laxa* from Asia (Braun et al. 2013, Farr & Rossman 2015). Among those, *P*. *pteridophytophila* is the only species for which there is molecular data available in GenBank (Kirschner & Liu 2014), though the ITS region lacks the necessary resolution needed to distinguish species of *Pseudocercospora* at the species level (Crous et al. 2013b). Additionally, *P. pteridophytophila* and *P. thelypteris* differ from *P. 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 *P. 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.

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

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

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, sub-hyaline to pale brown, smooth. *Stromata* rudimentary, sub-cuticular, composed of pale brown *textura angularis,* 15–36.5 µm wide, pale brown, smooth. *Conidiophores* restricted to the conidiogenous cell, arising from the stromata, epiphyllous, forming loose fascicles with up to 15 stalks, sub-cylindrical, attenuated at the tip, sinuous, often geniculate, 7–22 × 2–3.5 µm, unbranched, 0–1-septate, eguttulate, sub-hyaline to pale brown, smooth, scars inconspicuous, 1 per cell, not thickened, nor darkened. *Conidia* solitary, sub-cylindrical to obclavate, straight or curved, 31–75 × 2–3.5 µm, apex attenuated, base obconically truncate, 1.5–3.5 µm diam at the base, 2–7-septate, guttulate, pale brown, smooth.

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; olicavecous black centrally and olivaceous grey at periphery 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). SOUTH AFRICA, Kwazulu Natal, on leaves of *Eucalyptus grandis*, 15 May 1995, *M.J. Wingfield*, culture CBS 110998.

Notes — *Pseudocercospora serpocaulonicola* clustered within a new clade, together with an isolate recorded on *Eucalyptus grandis* from South Africa, having *P. pyracanthae* and *P. schizolobii* as sister clade (Fig. 2). It was not possible to compare with the fungus from *Eucalyptus* as the herbarium

specimen was in poor condition, and neither conidiophores nor conidia were seen. Moreover, the cultures proved to be sterile. Two other *Pseudocercospora* species (for which no DNA data is available in GenBank) have a similar morphology to *P. serpocaulonicola*, namely, *P. microsori* on *Microsorum pustulatum* from Australia and *P. phyllitidis,* from various ferns belonging to different families, having a cosmopolitan distribution (Shivas et al. 2010, Braun et al. 2013). *Pseudocercospora microsori* differs from the new species found on *S. tritseriale* by having well-developed stromata (20–60 µm wide), longer (30–65 × 3–5 µm), densely fasciculate (5–30 stalks per fascicle), reddish brown conidiophores, and moderately wide (2.5–4 µm), curved to flexuous conidia (Shivas et al. 2010). On the other hand, *P. phyllitidis* is known to be an extremely variable species, and probably polyphyletic. However, one distinctive feature that remains relatively constant for specimens belonging to this species is the persistency of the conidia, which remains attached to the conidiogenous cells for a long time (Braun et al. 2013). This feature is absent in *P. serpocaulinicola*. Additionally, *P. phyllitidis* has immersed stromata (ill-formed and sub-cuticular in *P. serpocaulinicola*) and moderately wider conidiophores (1.5–4 µm), than in *P. serpocaulinicola* (2–3.5 µm) (Braun et al. 2013). This is the first record of a fungus causing disease on *S. tritseriale*.

Xenomycosphaerella Quaedvlieg & Crous, Persoonia 33: 24. 2014 — MycoBank MB807787

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 were known from the genus, and once they are morphologically typical as mycosphaerella-like fungi, they were allocated on the genus based solely in the phylogenetic inference (Quaedvlieg et al. 2014). The taxa allocated to *Xenomycosphaerella* here, contain also asexual morphs, which are zasmidium-like in morphology.

Xenomycosphaerella alsophilae Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812816; Fig. 20

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

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, sub-hyaline, smooth. *External hyphae* absent. *Ascomata* pseudothecial, epiphyllous, solitary, sub-cuticular 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* bitunicate, aparaphysate, 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, OA and PDA slow-growing, 26 mm diam after 24 d; centrally raised, with lobate, smooth margins, aerial mycelium velvety, pale mouse grey centrally, and mouse grey in the outer region; leaden black reverse; cultures sterile.

Specimen 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, *X. alsophilae* is close to *X. yunnanensis* described on *Eucalyptus urophylla,* restricted to the southwest of China (Burgess et al. 2007). It can be distinguished from *X. yunnanensis* by having smaller and narrower, obclavate to broadly ellipsoidal asci (ovoid to obclavate, 27–38 x 7–11 µm in *X. yunnanensis*), and smaller and narrower ascospores (10–12.5 × 2.5–3 µm in *X. yunnanensis*). Moreover, *X. yunnanenis* is phylogenetically distinct from *X. alsophilae* (Fig. 3).

Xenomycosphaerella cyatheae Guatimosim, R.W. Barreto & Crous, *sp. nov.* — MycoBank MB812817; Fig. 21

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

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 pinnulet 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 cynamon to yellowish brown areas, appearing at the pinnae bases. *Internal hyphae* intra- and intercellular, 2–3 µm wide, septate, branched, sub-hyaline 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*, limited to the conidiogenous cells, obcuneiform, straight, proliferating sympodially, 4–19 × 2–6 µm, unbranched, aseptate, pale brown, smooth, scars conspicuous, several per cell, terminal, crowded, darkened, thickened. *Conidia* solitary, sub-cylindrical, straight, curved or sinuous, 40–280 × 3–5 µm, apex obtuse, base subtruncate, 3–5 µm diam at the base, indistinctly 5–19-septate, guttulate, pale to dark brown, strongly verruculose, hilum 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; greenish black reverse; cultures sterile.

Specimen examined. BRAZIL, Rio de Janeiro, Fazenda Barreto II, Riograndina, 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, vicitinities of 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 Bitancourt, 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 — *Xenomycosphaerella cyatheae* is phylogenetically different from all other species in this clade (Fig. 3). It was not possible to compare the new species with *X. diplazii, X. elongata* or *X. yunnanensis*, since all of these species are only known from their sexual morphs (Burgess et al. 2007, Crous et al. 2007b). Whereas for *X. cyatheae,* only the asexual stage was found, which ressembles the morphology of zasmidium-like fungi, which is known to be polyphyletic (Crous et al. 2009a, b). This is the first record of an asexual morph of *Xenomycosphaerella*.

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.*

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, sub-hyaline, smooth. *Ascomata* pseudothecial, epiphyllous, solitary, sub-cuticular 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, aparaphysate, 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 scarce, olivaceous grey centrally, buff to rosy buff periphery; cinnamon reverse. On PDA, raised, yeast-like, rosy buff centrally, buff 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 — *Xenomycosphaerella diplazii* is morphologically similar to *X. alsophilae* isolated from *Asophila* sp. in Brazil (this study), but differs from the latter by having narrower and shorter ascospores (10–17 × 2–4 µm in *X. alsophilae*). Phylogenetically, *X. diplazii* is different from all other species in this clade (Fig. 3). All the attempts to induce sporulation in *X. diplazii* have thus far proven unsuccessful.

Zasmidium Fr., Summa Veg. Scand., section Post. (Stockholm): 407. 1849 — MycoBank MB22396

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 *Stnella* clusters within *Teratosphaeriaceae* while *Zasmidium* clusters within *Mycosphaerellaceae* (Arzanlou et al. 2007, 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 — Herbarium 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). Up to date, there are no sequences or knowing 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*.

Frond spots amphigenous, irregular, affecting random pinnulets, starting at the apex of the pinnulets leading firstly to dark brown to black necrosis of the pinnulet apex, then spreading to the base, where a cream area appears causing the necrosis of the entire pinnulets, and occasionally of the pinnae. *External hyphae* absent. *Internal hyphae* intra- and intercellular, 1.5–2 µm wide, branched, septate, sub-hyaline to pale brown, smooth. *Ascomata* pseudothecial, epiphyllous, solitary, sub-cuticular 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, aparaphysate, 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, vicitinities 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).

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. 2006a). *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 Tef-1α, and 23 bp for ITS. The sexual morph (ressembling mycosphaerella-like structures) is known for only two among the six species of *Zasmidium* included in this study. These are *Z citri* (found on *Citrus paradisa* from the USA), and *Z. eucalyptorum* (collected on *Eucalyptus* sp. from Indonesia) (Whiteside 1972, Quaedvlieg et al. 2014). However, the ascospores of *Z. cyatheae* (14–22 x 3–6 µm) are larger 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. 2006a).

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 this work, the authors focused on pathogens of *Eucalyptus,* which makes it interesting to compare the Brazilian fern fungi with these taxa, to determine if the fungi occurring on ferns are somehow related,to those attacking higher plants such as *Eucalyptus*, or if they evolved independently with the fern hosts.

Forty four cercosporoid species are known causing frond spots in *Pterydophyta* worldwide: 13 *Cercospora* spp., two *Passalora* spp., 28 *Pseudocercospora* spp., and one *Zasmidium* spp. (Braun et al. 2013).

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 plants in two additional families. Plant hosts from *Pteridophyta* represent one of the oldest ancestral of evolved plants like those classified within *Angiospermae* and *Gymnospermae* (Smith et al. 2008). It is interesting to note that *C. coniogrammes* is on the one hand proving to have a wide host range within the *Pteridophyta,* and is 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 understanding of the taxonomy of the genus. Recollecting and epitypifying these numerous species is a challenging but important task for mycologists dealing with cercosporoid fungi. Three examples of taxonomic decisions that remain unresolved in this publication are *P. abacopteridicola*, *P. lygodiicola* and *P. thelypteridis* collected in Brazil. Although we suspect that these collections may in fact represent novel species, this can only be resolved following the recollection of fresh materials from the type localities (Singapore, China and Taiwan, respectively – Yen & Lim 1980, Braun et al. 2013), followed by epipypification and a phylogenetic comparison.

Historically, the taxonomy of cercosporoid fungi has been based on morphological and ecological features, including assumed host specificity (Chupp 1954, Deighton 1965, 1971, 1973, 1974, 1976, Pons & Sutton 1988, Braun 1993a, b, c, 1995, 1998, Crous & Braun 1996, Braun and Mel'nik 1997, Crous et al. 2000, Braun et al. 2013, 2014, 2015). It is now widely accepted that this was an inadequate base for the taxonomy of this complex plethora of fungi. A case in point emerging from the present study was that of the two novel species: *Zasmidium cyatheae* (only asexual morph found) and *Xeomycosphaerella cyatheae* (only sexual morph found), that co-occurred on the same frond spot, on the fern *Cyathea delgadii*. In the past, taxonomists would be led to the mistaken conclusion that *Z. cyatheae* was the asexual morph of *X. cyathea*. A similar situation occurred for *Paramycosphaerella sticheri* and *Clypeosphaerella sticheri*. Both were found attacking two different species in the same host genus *Sticherus* causing identical disease symptoms. It is likely that, in the past, without DNA data being available, many conjectured conections between these two genera have mistakenly been made, and efforts towards testing these conections with modern criteria should be continued in order to generate an appropriate and consolidated taxonomy of cercosporoid fungi (Taylor et al. 2000, Quaedvlieg et al. 2014).

The present study has expands sigfnificantly our knowledge of cercosporoid and mycosphaerella-like fungi associated with frond spots in Brazilian *Pteridophyta*. Thus far, only one cercosporoid and one mycosphaerella-like species (*Pseudocercospora davalliicola* and *Mycosphaerella tocoyenae*,

respectively) were known causing diseases on ferns from Brazil (Farr & Rossman 2015, Mendes & Urben 2015). The present work has expanded this number significantly and added one new genus (*Clypeosphaerella*) and 17 new species to this list. It provides 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 these cultures will in future contribute to a more robust phylogeny of these fungi across various families of host plants, to help us gain 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 part of a highly diverse mycobiota that still awaits discovery.

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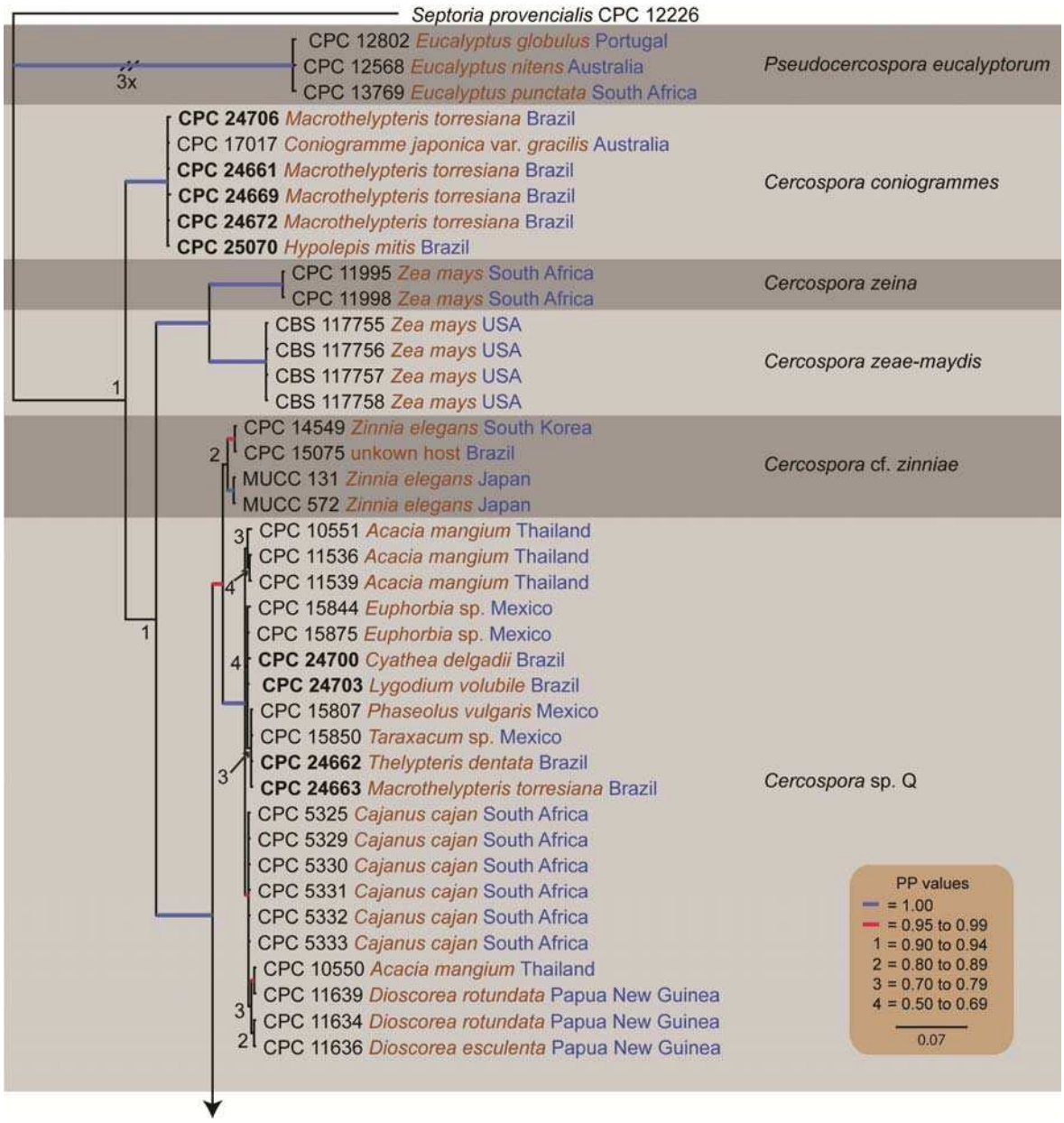
FIGURES


Septoria provencialis CPC 12226
CPC 12802 Eucalyptus globulus Portugal
CPC 12568 Eucalyptus niteris Australia
CPC 13769 Eucalyptus punctata South Africa
3x
Pseudocercospora eucalyptorum
CPC 24706 Macrothelypteris torresiana Brazil
CPC 17017 Coniogramme japonica var. gracilis Australia
CPC 24661 Macrothelypteris torresiana Brazil
CPC 24669 Macrothelypteris torresiana Brazil
CPC 24672 Macrothelypteris torresiana Brazil
CPC 25070 Hypolepis mitis Brazil
Cercospora coniogrammes
CPC 11995 Zea mays South Africa
CPC 11998 Zea mays South Africa
Cercospora zeina
CBS 117755 Zea mays USA
CBS 117756 Zea mays USA
CBS 117757 Zea mays USA
CBS 117758 Zea mays USA
Cercospora zeae-maydis
CPC 14549 Zinnia elegans South Korea
CPC 15075 unkown host Brazil
MUCC 131 Zinnia elegans Japan
MUCC 572 Zinnia elegans Japan
Cercospora cf. zinniae
CPC 10551 Acacia mangium Thailand
CPC 11536 Acacia mangium Thailand
CPC 11539 Acacia mangium Thailand
CPC 15844 Euphorbia sp. Mexico
CPC 15875 Euphorbia sp. Mexico
CPC 24700 Cyathea delgadii Brazil
CPC 24703 Lygodium volubile Brazil
CPC 15807 Phaseolus vulgaris Mexico
CPC 15850 Taraxacum sp. Mexico
CPC 24662 Thelypteris dentata Brazil
CPC 24663 Macrothelypteris torresiana Brazil
CPC 5325 Cajanus cajan South Africa
CPC 5329 Cajanus cajan South Africa
CPC 5330 Cajanus cajan South Africa
CPC 5331 Cajanus cajan South Africa
CPC 5332 Cajanus cajan South Africa
CPC 5333 Cajanus cajan South Africa
CPC 10550 Acacia mangium Thailand
CPC 11639 Dioscorea rotundata Papua New Guinea
CPC 11634 Dioscorea rotundata Papua New Guinea
CPC 11636 Dioscorea esculenta Papua New Guinea
Cercospora sp. Q
PP values
= 1.00
= 0.95 to 0.99
1 = 0.90 to 0.94
2 = 0.80 to 0.89
3 = 0.70 to 0.79
4 = 0.50 to 0.69
0.07

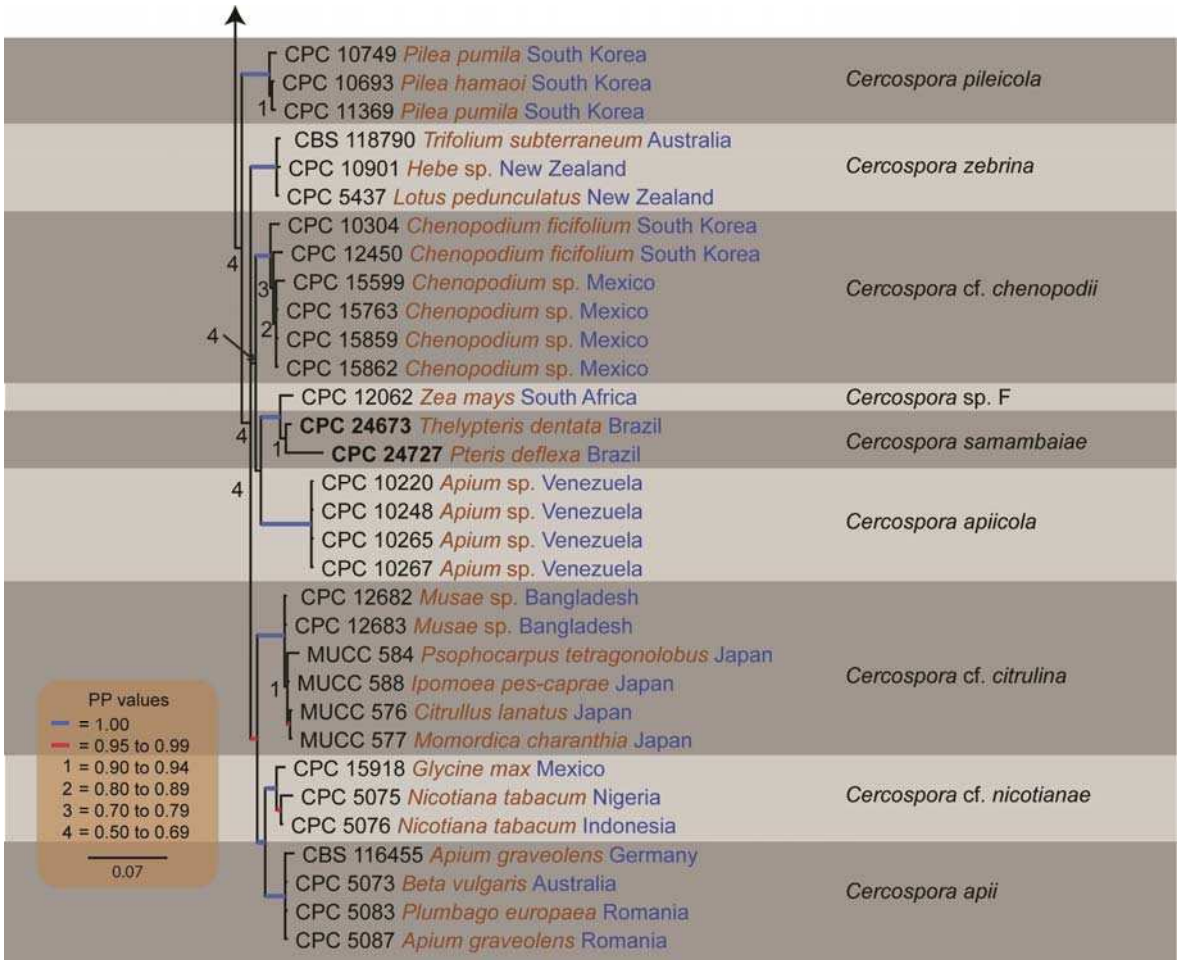
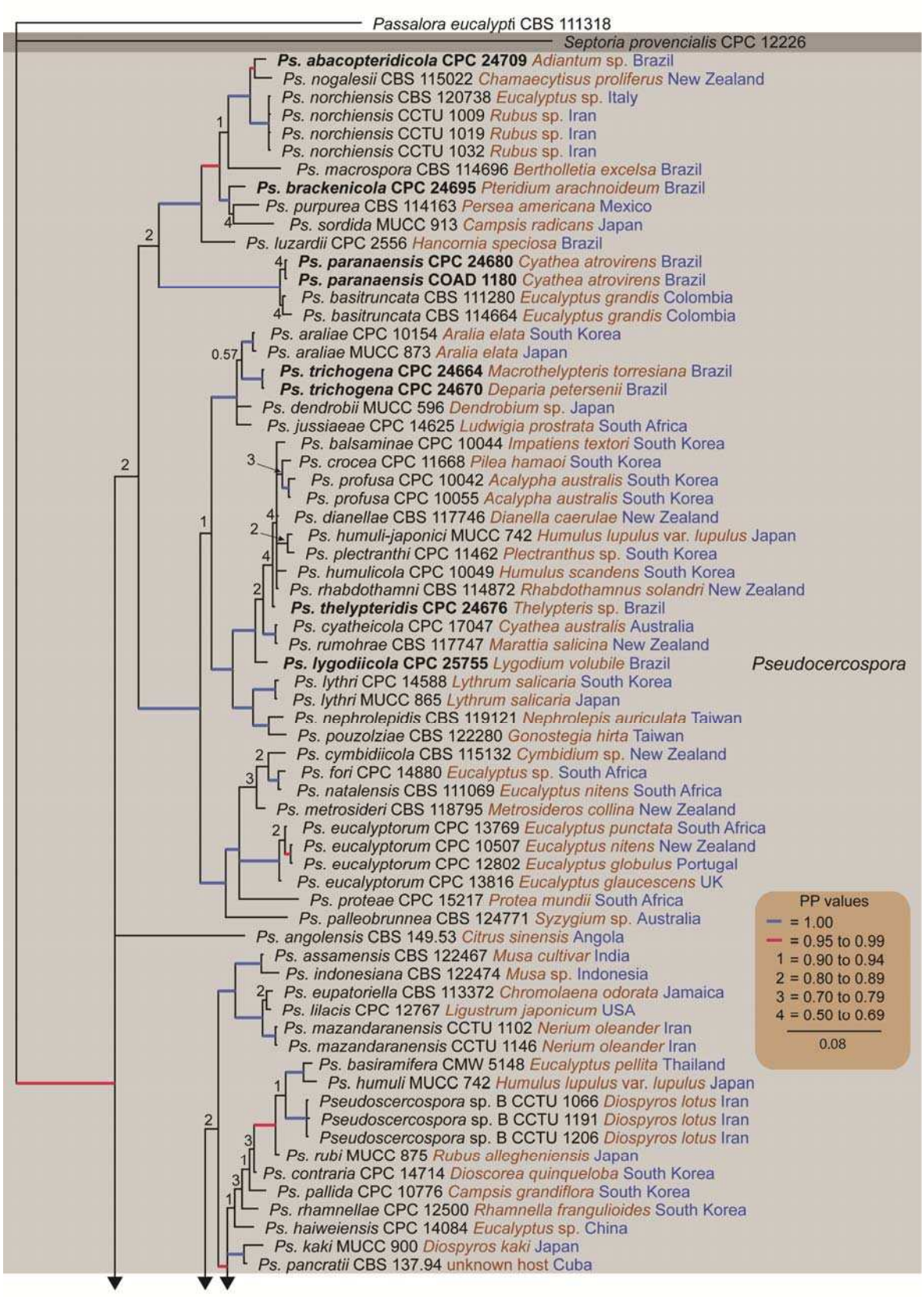


Fig. 1. Consensus phylogram (50 % majority rule) of *Cercospora* species, from a Bayesian analysis of the combined 4-gene sequence alignment (ITS, Tef1-α, ACT, CAL). Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend) and the scale bar indicates 0.07 expected 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 *Septoria provencialis* (isolate CPC 12226).


Passalora eucalypti CBS 111318
Septoria provencialis CPC 12226
Ps. abacopteridicola CPC 24709 Adiantum sp. Brazil
Ps. nogalesii CBS 115022 Chamaecytisus proliferus New Zealand
Ps. norchiensis CBS 120738 Eucalyptus sp. Italy
Ps. norchiensis CCTU 1009 Rubus sp. Iran
Ps. norchiensis CCTU 1019 Rubus sp. Iran
Ps. norchiensis CCTU 1032 Rubus sp. Iran
Ps. macrospora CBS 114696 Bertholletia excelsa Brazil
Ps. brackenicola CPC 24695 Pteridium arachnoideum Brazil
Ps. purpurea CBS 114163 Persea americana Mexico
Ps. sordida MUCC 913 Campsis radicans Japan
Ps. luzardii CPC 2556 Hancornia speciosa Brazil
Ps. paranaensis CPC 24680 Cyathea atrovirens Brazil
Ps. paranaensis COAD 1180 Cyathea atrovirens Brazil
Ps. basitruncata CBS 111280 Eucalyptus grandis Colombia
Ps. basitruncata CBS 114664 Eucalyptus grandis Colombia
Ps. araliae CPC 10154 Aralia elata South Korea
Ps. araliae MUCC 873 Aralia elata Japan
Ps. trichogena CPC 24664 Macrothelypteris torresiana Brazil
Ps. trichogena CPC 24670 Deparia petersenii Brazil
Ps. dendrobii MUCC 596 Dendrobium sp. Japan
Ps. jussiaeae CPC 14625 Ludwigia prostrata South Africa
Ps. balsaminae CPC 10044 Impatiens textori South Korea
Ps. crocea CPC 11668 Pilea hamaoi South Korea
Ps. profusa CPC 10042 Acalypha australis South Korea
Ps. profusa CPC 10055 Acalypha australis South Korea
Ps. dianellae CBS 117746 Dianella caerulae New Zealand
Ps. humuli-japonici MUCC 742 Humulus lupulus var. lupulus Japan
Ps. plectranthi CPC 11462 Plectranthus sp. South Korea
Ps. humulicola CPC 10049 Humulus scandens South Korea
Ps. rhabdothamni CBS 114872 Rhabdothamnus solandri New Zealand
Ps. thelypteridis CPC 24676 Thelypteris sp. Brazil
Ps. cyatheicola CPC 17047 Cyathea australis Australia
Ps. rumohrae CBS 117747 Marattia salicina New Zealand
Ps. lygodiicola CPC 25755 Lygodium volubile Brazil
Ps. lythri CPC 14588 Lythrum salicaria South Korea
Ps. lythri MUCC 865 Lythrum salicaria Japan
Ps. nephrolepidis CBS 119121 Nephrolepis auriculata Taiwan
Ps. pouzolziae CBS 122280 Gonostegia hirta Taiwan
Ps. cymbidiicola CBS 115132 Cymbidium sp. New Zealand
Ps. fori CPC 14880 Eucalyptus sp. South Africa
Ps. natalensis CBS 111069 Eucalyptus nitens South Africa
Ps. metrosideri CBS 118795 Metrosideros collina New Zealand
Ps. eucalyptorum CPC 13769 Eucalyptus punctata South Africa
Ps. eucalyptorum CPC 10507 Eucalyptus nitens New Zealand
Ps. eucalyptorum CPC 12802 Eucalyptus globulus Portugal
Ps. eucalyptorum CPC 13816 Eucalyptus glaucescens UK
Ps. proteae CPC 15217 Protea mundii South Africa
Ps. palleobrunnea CBS 124771 Syzygium sp. Australia
Ps. angolensis CBS 149.53 Citrus sinensis Angola
Ps. assamensis CBS 122467 Musa cultivar India
Ps. indonesiana CBS 122474 Musa sp. Indonesia
Ps. eupatoriella CBS 113372 Chromolaena odorata Jamaica
Ps. lilacis CPC 12767 Ligustrum japonicum USA
Ps. mazandaranensis CCTU 1102 Nerium oleander Iran
Ps. mazandaranensis CCTU 1146 Nerium oleander Iran
Ps. basiramifera CMW 5148 Eucalyptus pellita Thailand
Ps. humuli MUCC 742 Humulus lupulus var. lupulus Japan
Pseudoscercospora sp. B CCTU 1066 Diospyros lotus Iran
Pseudoscercospora sp. B CCTU 1191 Diospyros lotus Iran
Pseudoscercospora sp. B CCTU 1206 Diospyros lotus Iran
Ps. rubi MUCC 875 Rubus allegheniensis Japan
Ps. contraria CPC 14714 Dioscorea quinqueloba South Korea
Ps. pallida CPC 10776 Campsis grandiflora South Korea
Ps. rhamnellae CPC 12500 Rhamnella frangulioides South Korea
Ps. haiweiensis CPC 14084 Eucalyptus sp. China
Ps. kaki MUCC 900 Diospyros kaki Japan
Ps. pancratii CBS 137.94 unknown host Cuba
Pseudocercospora
PP values
= 1.00
= 0.95 to 0.99
1 = 0.90 to 0.94
2 = 0.80 to 0.89
3 = 0.70 to 0.79
4 = 0.50 to 0.69
0.08

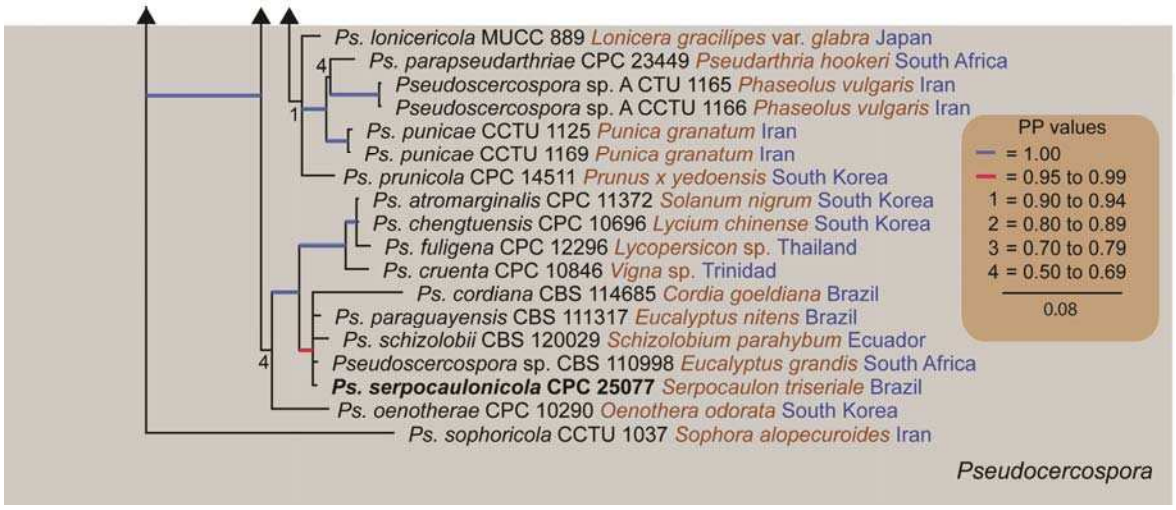


Fig. 2. Consensus phylogram (50 % majority rule) of *Pseudocercospora* species, from a Bayesian analysis of the combined 3-gene sequence alignment (ITS, ACT, Tef-1α). Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend) and the scale bar indicates 0.07 expected 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).

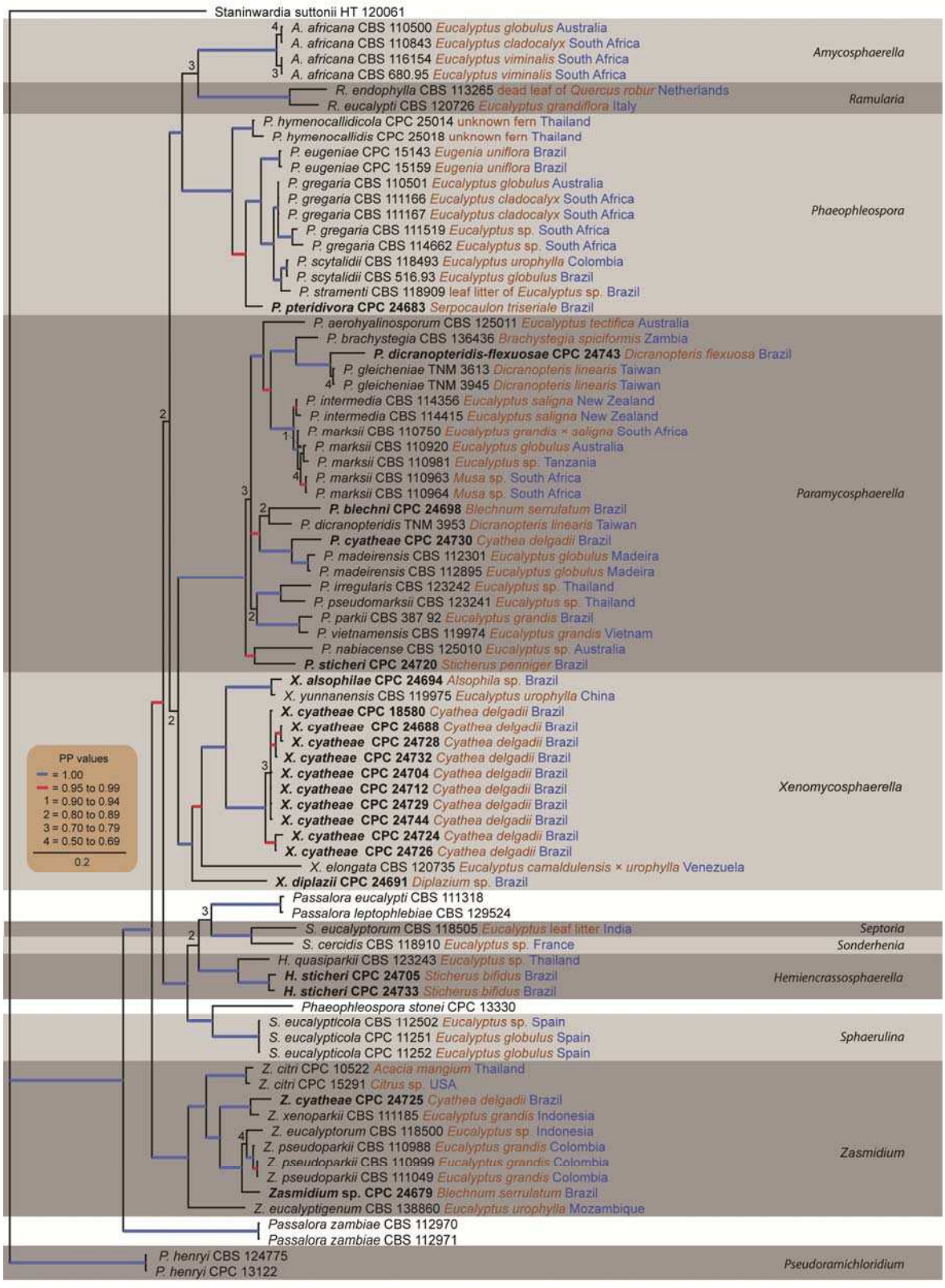


Fig. 3. Consensus phylogram (50 % majority rule) of mycosphaerella-like species, from a Bayesian analysis of the combined 4-gene sequence alignment (ACT, Tef1-α, ITS, LSU). Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend) and the scale bar indicates 0.2 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 HT 120061).

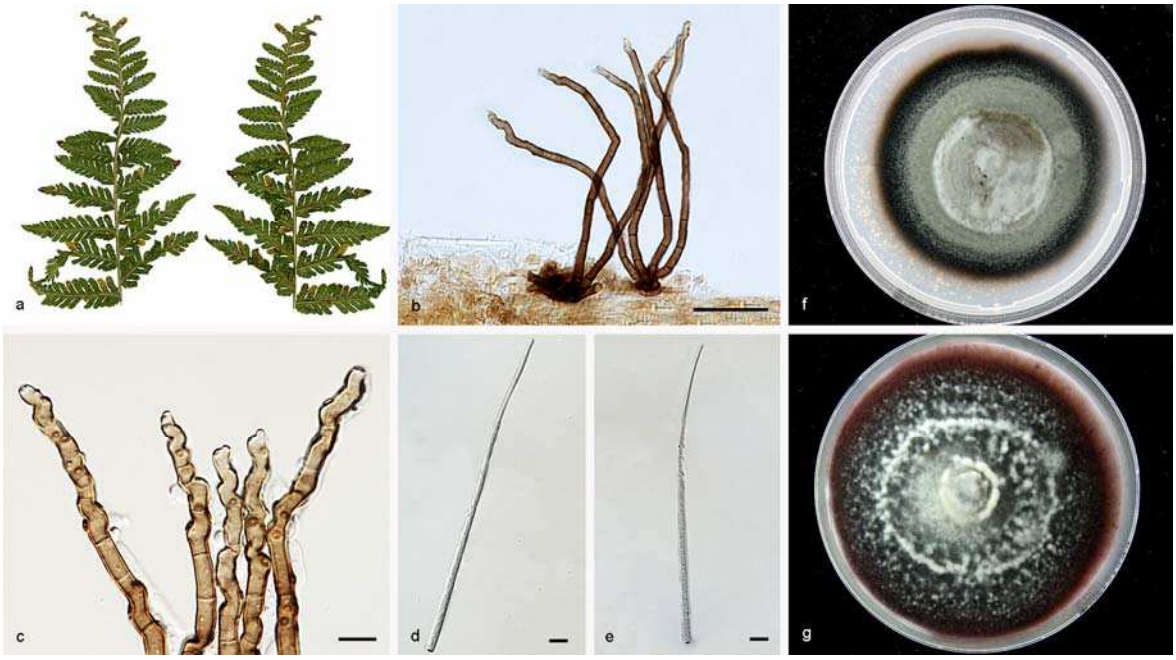


Fig. 4. *Cercospora coniogrammes* (CPC 24661). a. Frond spots on *Marcothelypteris torresiana*; b–c. conidiophores; d–e. conidia; f. culture on PDA; g. culture on PCA. — Scale bars b = 50 µm; c–e = 10 µm.

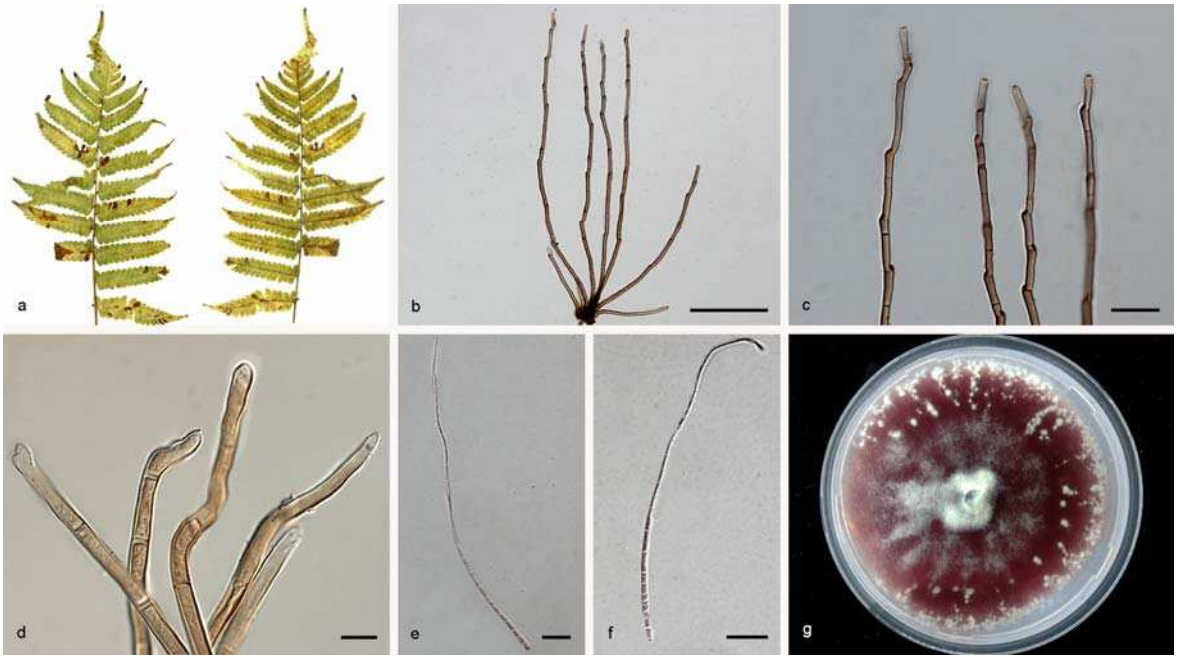


Fig. 5. *Cercospora samambaiae* (CPC 24673). a Frond spots on *Thelypteris dentata*; b–d. conidiophores; e–f. conidia; g. culture on PCA. — Scale bars b = 100 µm; c–e = 50 µm; f = 10 µm.

Fig. 6. *Cercospora* Q (CPC 24662). a. Frond spots on *Lygodium volubile*; b. frond spots on *Cyathea delgadii*; c. frond spots *Thelypteris dentata*; d–e. sporulation on the pinnule; f–h. conidiophores; i–m. conidia. — Scale bars f = 10 µm; g, i–k = 25 µm; l–m = 50 µm.

Fig. 7. *Paramycosphaerella blechni* (CPC 24698). a–c. Frond spots on *Blechnum serrulatum*; d–e. vertical section of the ascoma; f. asci; g. ascospores; h. culture on MEA; i. culture on OA; j. culture on PDA. — Scale bars = 10 µm.

Fig. 8. *Paramycosphaerella cyatheae* (CPC 24730). a–c. Frond spots on *Cyathea delgadii*; 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.

Fig. 9. *Paramycosphaerella dicranopteridis-flexuosae* (CPC 24743). a–c. Frond spots on *Dicranopteris flexuosa*; 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.

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

Fig. 11. *Phaeophleospora pteridivora* (CPC 24683). a–b. Frond spots on *Serpocaulon triseriale*; c. erumpent subcuticular ascomata, fruiting epiphyllous; d–e. vertical section of the ascoma; f. conidiophores arising from the stroma; g. conidia; h. culture on MEA; i. culture on OA; j. culture on PDA. — Scale bars = 10 µm.

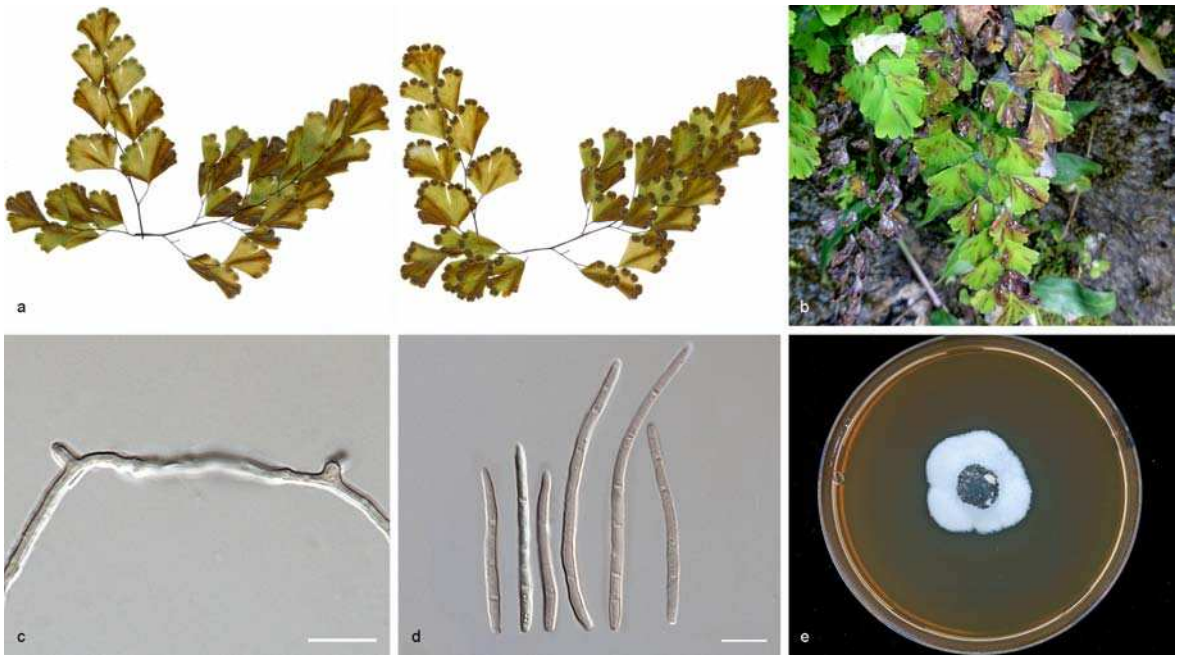


Fig. 12. *Pseudocercospora abacopteridicola* (CPC 24709). a–b. Frond spots on *Adiathum* sp.; c. conidiophores restricted to the conidiogenous cells, arising from the hyphae; d. conidia; e. culture on MEA. — Scale bars = 10 µm.

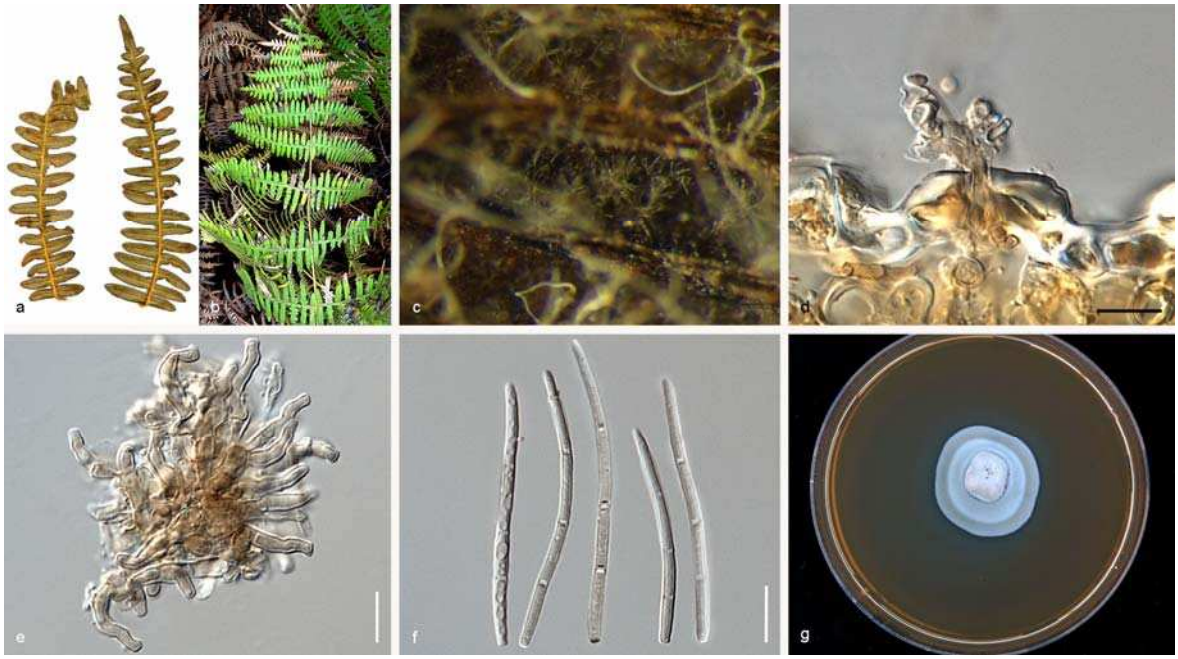


Fig. 13. *Pseudocercospora brackenicola* (CPC 24709). a–b. Frond spots on *Pteridium arachnoideum*; c. conidiophores sporulating abaxially; d. detail of conidiophores arising through the stoma; e. conidiophores; f. conidia; g. culture on MEA. — Scale bars = 10 µm.

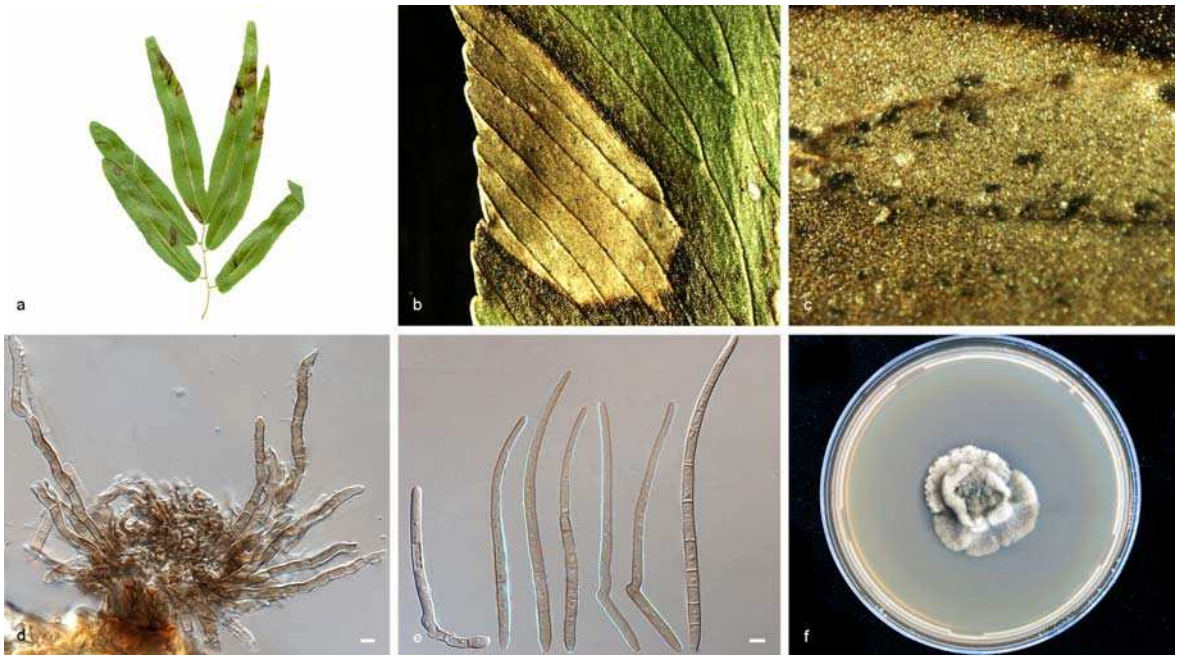


Fig. 14. *Pseudocercospora lygodiicola* (CPC 25755). a–c. Frond spots on *Lygodium volubile*; d. conidiophores arising from the stroma through the stoma; e. conidia; f. culture on MEA. — Scale bars = 10 µm.

Fig. 15. *Pseudocercospora paranaensis* (CPC 24680). a–b. Frond spots on *Cyathea atrovirens*; c. conidiophores 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.

Fig. 16. *Pseudocercospora thelypteridis* (CPC 24676). a–d. Frond spots on *Thelypteris* sp.; e. conidiophores arising from the stroma; f. conidia; g. culture on MEA. — Scale bars = 10 µm.

Fig. 17. *Pseudocercospora trichogena* (asexual 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. conidiophores sporulating on a trichoma, hypophyllous; e. asci; f. ascospores; g. detail of the external hyphae arising through the stoma, and climbing the thichoma; h. conidiophores; i–j. conidia; k. culture on MEA. — Scale bars = 10 µm.

Fig. 18. *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.

Fig. 19. *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, ressembling a *pseudoclypeus*; g–h. asci; i. ascospores; j. germinating ascospores; k. culture on MEA; l. culture on OA; m. culture on PDA. — Scale bars = 10 µm.

Fig. 20. *Xenomycosphaerella 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. asci; h. ascospores; i. culture on MEA; j. culture on OA; k. culture on PDA. — Scale bars = 10 µm.

Fig. 21. *Xenomycosphaerella cyatheae* (CPC 24704). a–b. Frond spots on *Cyathea delgadii*; c. external hyphae and conidia, covering the frond spot, epiphyllous; d. SEM of the conidia and conidiophore, note the smooth conidiophore restricted to the conidiogenous cell; e. detail of the external hyphae arising through the stoma; f. conidiophores arising through the hyphae, restricted to the conidiogenous cells; g–m conidia; n. culture on MEA; o. culture on OA; p. culture on PDA. — Scale bars = 10 µm.

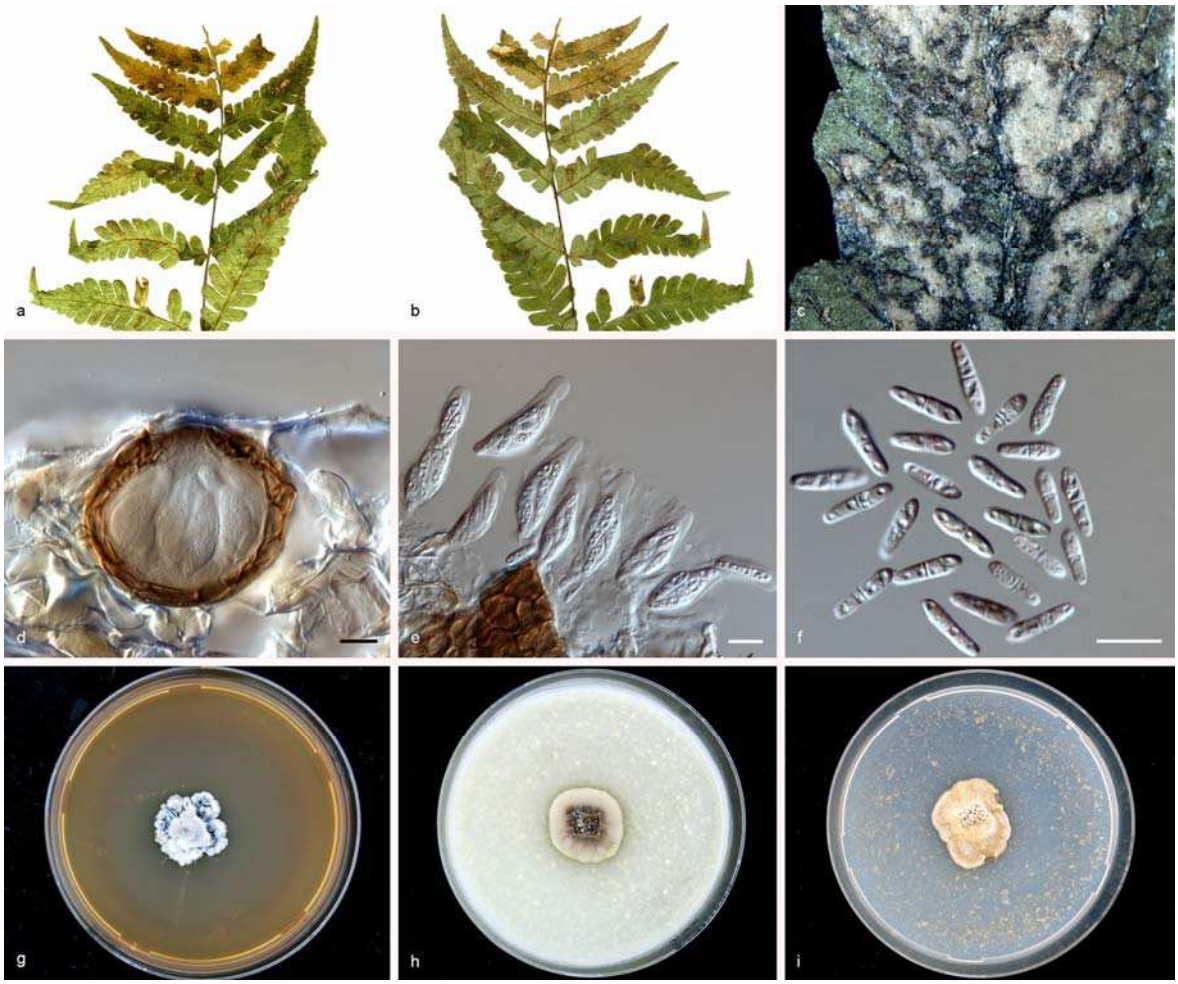


Fig. 22. *Xenomycosphaerella diplazii* (CPC 24691). a–b. Frond spots on *Diplazium* sp.; c. erumpent subcuticular ascomata, fruiting epiphyllous; d. vertical section of the ascoma; e. asci; f. ascospores; g. culture on MEA; h. culture on OA; i. culture on PDA. — Scale bars = 10 µm.

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. asci; f. ascospores; g. culture on MEA; h. culture on OA; i. culture on PDA. — Scale bars = 10 µm.

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

Species	Culture accession numbers[1,2]	Host/isolation source	Host family	Country	Collector	GenBank accession numbers[3]				
						ITS	TEF1α	ACT	CAL	LSU
Amycosphaerella africana	CBS 110500^{ET}	*Eucalyptus globulus*	*Myrtaceae*	Australia	A. Maxwell	KF901516	KF903115	KF903395	—	KF901837
	CBS 110843 = CPC 850^{ET}	*Eucalyptus cladocalyx*	*Myrtaceae*	South Africa	P.W. Crous	KF901702	KF903118	KF903407	—	KF902049
	CBS 116154 = CPC 794^{ET}	*Eucalyptus viminalis*	*Myrtaceae*	South Africa	P.W. Crous	KF901700	KF903116	KF903480	—	KF902047
	CBS 680.95 = CPC 796^{ET}	*Eucalyptus viminalis*	*Myrtaceae*	South Africa	P.W. Crous	KF901701	KF903117	KF903589	—	KF902048
Cercospora apii	CBS 116455 = CPC 11556^{ET}	*Apium graveolens*	*Apiaceae*	Germany	K. Schrameyer	AY840519	AY840486	AY840450	AY840417	—
	CBS 121.31 = CPC 5073	*Beta vulgaris*	*Chenopodiaceae*	Austria	E.W. Schmidt	AY343371	AY343334	AY840444	AY840411	—
	CBS 536.71 = CPC 5087	*Apium graveolens*	*Apiaceae*	Romania	O. Constantinescu	AY752133	AY752166	AY752194	AY752225	—
	CBS 553.71 = CPC 5083	*Plumbago europaea*	*Plumbaginaceae*	Romania	O. Constantinescu	DQ233320	DQ233344	DQ233370	DQ233396	—
Cercospora apiicola	CBS 116457 = CPC 10267^{ET}	*Apium* sp.	*Apiaceae*	Venezuela	N. Pons	AY840536	AY840503	AY840467	AY840434	—
	CBS 132644 = CPC 10248	*Apium* sp.	*Apiaceae*	Venezuela	N. Pons	AY840539	AY840506	AY840470	AY840437	—
	CPC 10220	*Apium* sp.	*Apiaceae*	Venezuela	N. Pons	AY840538	AY840505	AY840469	AY840436	—
	CPC 10265	*Apium* sp.	*Apiaceae*	Venezuela	N. Pons	AY840540	AY840507	AY840471	AY840438	—
Cercospora cf. *chenopodii*	CBS 132594 = CPC 10304^{ET}	*Chenopodium ficifolium*	*Chenopodiaceae*	South Korea	H.D. Shin	JX143572	JX143328	JX143082	JX142836	—
	CBS 132677 = CPC 15599	*Chenopodium* sp.	*Chenopodiaceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143573	JX143329	JX143083	JX142837	—
	CPC 12450	*Chenopodium ficifolium*	*Chenopodiaceae*	South Korea	H.D. Shin	JX143574	JX143330	JX143084	JX142838	—
	CPC 15763	*Chenopodium* sp.	*Chenopodiaceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143575	JX143331	JX143085	JX142839	—
	CPC 15859	*Chenopodium* sp.	*Chenopodiaceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143576	JX143332	JX143086	JX142840	—
	CPC 15862	*Chenopodium* sp.	*Chenopodiaceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143577	JX143333	JX143087	JX142841	—
Cercospora cf. *citrulina*	CBS 119395 = CPC 12682	*Musa* sp.	*Musaceae*	Bangladesh	I. Buddenhagen	EU514222	JX143335	JX143089	JX142843	—
	CBS 132669 = CPC 12683	*Musa* sp.	*Musaceae*	Bangladesh	I. Buddenhagen	EU514223	JX143336	JX143090	JX142844	—
	MUCC 576 = MAFF 237913	*Citrullus lanatus*	*Cucurbitaceae*	Japan	T. Kobayashion et al.	JX143579	JX143337	JX143091	JX142845	—
	MUCC 577 = MAFF 238205	*Momordica charanthia*	*Cucurbitaceae*	Japan	E. Imaizumi & C. Nomi	JX143580	JX143338	JX143092	JX142846	—
	MUCC 584 = MAFF 305757	*Psophocarpus tetragonolobus*	*Fabaceae*	Japan	—	JX143581	JX143339	JX143093	JX142847	—
	MUCC 588 = MAFF 239409	*Ipomoea pescaprae*	*Convolvulaceae*	Japan	—	JX143582	JX143340	JX143094	JX142848	—
Cercospora coniogrammes	CBS 132634 = CPC 17017^{ET}	*Coniogramme japonica*	*Cryptogrammaceae*	Australia	P.W. Crous	JX143583	JX143341	JX143095	JX142849	—
	CPC 24661 = COAD 1067	*Macrothelypteris torresiana*	*Thelypteridaceae*	Brazil	R.W. Barreto	**KT037509**	**KT037469**	**KT037591**	**KT037458**	**KT037550**
	CPC 24669 = COAD 1093	*Macrothelypteris torresiana*	*Thelypteridaceae*	Brazil	R.W. Barreto	**KT037512**	**KT037472**	**KT037594**	**KT037461**	**KT037553**
	CPC 24672 = COAD 1089	*Macrothelypteris torresiana*	*Thelypteridaceae*	Brazil	R.W. Barreto	**KT037513**	**KT037473**	**KT037595**	**KT037462**	**KT037554**
	CPC 24706	*Macrothelypteris torresiana*	*Thelypteridaceae*	Brazil	E. Guatimosim	**KT037507**	**KT037467**	**KT037589**	**KT037456**	**KT037548**
	CPC 25070 = COAD 1769	*Hypolepis mitis*	*Dennstaedtiaceae*	Brazil	R.W. Barreto	**KT037517**	**KT037477**	**KT037599**	**KT037466**	**KT037558**
Cercospora cf. *nicotianae*	CBS 131.32 = CPC 5076	*Nicotiana tabacum*	*Solanaceae*	Indonesia	H. Diddens and A. Jaarsveld	DQ835073	DQ835099	DQ835119	DQ835146	—
	CBS 132632 = CPC 15918	*Glycine max*	*Fabaceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143631	JX143390	JX143144	JX142898	—
	CBS 570.69 = CPC 5075	*Nicotiana tabacum*	*Solanaceae*	Nigeria	S.O. Alasoadura	DQ835074	DQ835100	DQ835120	DQ835147	—
Cercospora pileicola	CBS 132607 = CPC 10749^{ET}	*Pilea pumila*	*Urticaceae*	South Korea	H.D. Shin	JX143634	JX143393	JX143147	JX142901	—
	CBS 132647 = CPC 10693	*Pilea hamaoi*	*Urticaceae*	South Korea	H.D. Shin	JX143635	JX143394	JX143148	JX142902	—
	CPC 11369	*Pilea pumila*	*Urticaceae*	South Korea	H.D. Shin	JX143636	JX143395	JX143149	JX142903	—
Cercospora sp. F	CBS 132618 = CPC 12062	*Zea mays*	*Poaceae*	South Africa	P. Caldwell	DQ185071	DQ185083	DQ185095	DQ185107	—
Cercospora sp. Q	CBS 132656 = CPC 11536	*Acacia mangium*	*Fabaceae*	Thailand	K. Pongpanich	JX143723	JX143482	JX143236	JX142990	—
	CPC 10551	*Acacia mangium*	*Fabaceae*	Thailand	K. Pongpanich	AY752140	AY752173	AY752201	AY752232	—
	CPC 11539	*Acacia mangium*	*Fabaceae*	Thailand	K. Pongpanich	JX143729	JX143488	JX143242	JX142996	—
	CPC 10550	*Acacia mangium*	*Fabaceae*	Thailand	K. Pongpanich	AY752139	AY752172	AY752200	AY752231	—
	CBS 113997 = CPC 5325	*Cajanus cajan*	*Fabaceae*	South Africa	L. van Jaarsveld	JX143717	JX143476	JX143230	JX142984	—

Species	Culture accession numbers[1,2]	Host/isolation source	Host family	Country	Collector	GenBank accession numbers[3]				
						ITS	TEF1α	ACT	CAL	LSU
Cercospora sp. Q	CBS 115410 = CPC 5331	*Cajanus cajan*	*Fabaceae*	South Africa	L. van Jaarsveld	JX143718	JX143477	JX143231	JX142985	—
	CBS 115411 = CPC 5332	*Cajanus cajan*	*Fabaceae*	South Africa	L. van Jaarsveld	JX143719	JX143478	JX143232	JX142986	—
	CBS 115412 = CPC 5333	*Cajanus cajan*	*Fabaceae*	South Africa	L. van Jaarsveld	JX143720	JX143479	JX143233	JX142987	—
	CBS 115536 = CPC 5329	*Cajanus cajan*	*Fabaceae*	South Africa	L. van Jaarsveld	JX143721	JX143480	JX143234	JX142988	—
	CBS 115537 = CPC 5330	*Cajanus cajan*	*Fabaceae*	South Africa	L. van Jaarsveld	JX143722	JX143481	JX143235	JX142989	—
	CBS 132663 = CPC 11636	*Dioscorea esculenta*	*Dioscoreaceae*	Papua New Guinea	J. Peters & A.N. Jama	JX143725	JX143484	JX143238	JX142992	—
	CBS 132661 = CPC 11634	*Dioscorea rotundata*	*Dioscoreaceae*	Papua New Guinea	J. Peters & A.N. Jama	JX143724	JX143483	JX143237	JX142991	—
	CPC 11639	*Dioscorea rotundata*	*Dioscoreaceae*	Papua New Guinea	J. Peters & A.N. Jama	JX143730	JX143489	JX143243	JX142997	—
	CBS 132681 = CPC 15844	*Euphorbia* sp.	*Euphorbiaceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143727	JX143486	JX143240	JX142994	—
	CPC 15875	*Euphorbia* sp.	*Euphorbiaceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143731	JX143490	JX143244	JX142998	—
	CBS 132679 = CPC 15807	*Phaseolus vulgaris*	*Fabaceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143726	JX143485	JX143239	JX142993	—
	CBS 132682 = CPC 15850	*Taraxacum* sp.	*Asteraceae*	Mexico	Ma. de Jesús Yáñez-Morales	JX143728	JX143487	JX143241	JX142995	—
	CPC 24662 = COAD 630	*Thelypteris dentata*	*Thelypteridaceae*	Brazil	R.W. Barreto	**KT037510**	**KT037470**	**KT037592**	**KT037459**	**KT037551**
	CPC 24663 = COAD 322	*Macrothelypteris torresiana*	*Thelypteridaceae*	Brazil	R.W. Barreto	**KT037511**	**KT037471**	**KT037593**	**KT037460**	**KT037552**
	CPC 24700 = COAD 1418	*Cyathea delgadii*	*Cyatheaceae*	Brazil	R.W. Barreto	**KT037515**	**KT037475**	**KT037597**	**KT037464**	**KT037556**
	CPC 24703	*Lygodium volubile*	*Lygodiaceae*	Brazil	R.W. Barreto	**KT037516**	**KT037476**	**KT037598**	**KT037465**	**KT037557**
Cercospora samambaiae	CPC 24673 = COAD 1090[ET]	*Thelypteris dentata*	*Thelypteridaceae*	Brazil	R.W. Barreto	**KT037514**	**KT037474**	**KT037596**	**KT037463**	**KT037555**
	CPC 24727 = COAD 1427	*Pteris deflexa*	*Pteridaceae*	Brazil	E. Guatimosim	**KT037508**	**KT037468**	**KT037590**	**KT037456**	**KT037549**
Cercospora zeae-maydis	CBS 117757[ET]	*Zea mays*	*Poaceae*	U.S.A.	B. Fleener	DQ185074	DQ185086	DQ185098	DQ185110	—
	CBS 117755	*Zea mays*	*Poaceae*	U.S.A.	B. Fleener	DQ185072	DQ185084	DQ185096	DQ185108	—
	CBS 117756	*Zea mays*	*Poaceae*	U.S.A.	B. Fleener	DQ185073	DQ185085	DQ185097	DQ185109	—
	CBS 117758	*Zea mays*	*Poaceae*	U.S.A.	B. Fleener	DQ185075	DQ185087	DQ185099	DQ185111	—
Cercospora zebrina	CBS 114359 = CPC 10901	*Hebe* sp.	*Scrophulariaceae*	New Zealand	C.F. Hill	JX143746	JX143508	JX143262	JX143016	—
	CBS 118790	*Trifolium subterraneum*	*Fabaceae*	Australia	M.J. Barbetti	JX143748	JX143510	JX143264	JX143018	—
	CPC 5437	*Lotus pedunculatus*	*Fabaceae*	New Zealand	C.F. Hill	JX143754	JX143516	JX143270	JX143024	—
Cercospora zeina	CBS 118820 = CPC 11995[ET]	*Zea mays*	*Poaceae*	South Africa	P. Caldwell	DQ185081	DQ185093	DQ185105	DQ185117	—
	CBS 132617 = CPC 11998	*Zea mays*	*Poaceae*	South Africa	P. Caldwell	DQ185082	DQ185094	DQ185106	DQ185118	—
Cercospora cf. *zinniae*	CBS 132624 = CPC 14549	*Zinnia elegans*	*Asteraceae*	South Africa	H.D. Shin	JX143756	JX143518	JX143272	JX143026	—
	CBS 132676 = CPC 15075	—	—	Brazil	A.C. Alfenas	JX143757	JX143519	JX143273	JX143027	—
	MUCC 131	*Zinnia elegans*	*Asteraceae*	Japan	J. Nishikawa	JX143758	JX143520	JX143274	JX143028	—
	MUCC 572 = MUCNS 215 = MAFF 237718	*Zinnia elegans*	*Asteraceae*	Japan	S. Uematsu	JX143759	JX143521	JX143275	JX143029	—
Clypeosphaerella quasiparkii	CBS 123243 = CPC 15409[ET] of *mycosphaerella quasiparkii*	*Eucalyptus* sp.	*Myrtaceae*	Thailand	P. Suwannawong	KF901771	KF903113	KF903543	—	KF902128
C. sticheri	CPC 24705[ET]	*Sticherus bifidus*	*Gleicheniaceae*	Brazil	R.W. Barreto	**KT037546**	**KT037505**	**KT037610**	—	**KT037588**
	CPC 24733	*Sticherus bifidus*	*Gleicheniaceae*	Brazil	E. Guatimosim	**KT037536**	**KT037495**	**KT037609**	—	**KT037577**
Paramycosphaerella aerohyalinosporum	CBS 125011 = CPC 14636[ET]	*Eucalyptus tectifica*	*Myrtaceae*	Australia	B.A. Summerell	KF901605	KF903376	KF903576	KF902788	KF901930
Pa. blechni	CPC 24698 = COAD 1183[ET]	*Blechnum serrulatum*	*Blechnaceae*	Brazil	R.W. Barreto	**KT037544**	**KT037503**	**KT037611**	—	**KT037586**
Pa. brachystegia	CBS 136436 = CPC 21137, CPC 21136[ET]	*Brachystegia* sp.	*Fabaceae*	Zimbabwe	J. Roux	KF777178	**KT037506**	**KT037612**	—	**KF777230**
Pa. cyatheae	CPC 24730[ET]	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037534**	—	**KT037613**	—	**KT037575**
Pa. dicranopteridis	BCRC FU30234[ET] of *Zasmidium dicranopteridis*	*Dicranopteris linearis*	*Gleicheniaceae*	Taiwan	*R. Kirschner*	**KJ201941**	—	—	—	—
Pa. dicranopteridis-flexuosae	CPC 24743[ET]	*Dicranopteris flexuosa*	*Gleicheniaceae*	Brazil	P.B. Schwatzburd & A.P. Fortuna	**KT037538**	**KT037497**	**KT037614**	—	**KT037579**
Pa. gleicheniae	RoKi 3613	*Dicranopteris linearis*	*Gleicheniaceae*	Taiwan	*R. Kirschner*	KJ201929	—	—	—	—
	RoKi 3945	*Dicranopteris linearis*	*Gleicheniaceae*	Taiwan	*R. Kirschner*	KJ201930	—	—	—	—
Pa. intermedia	CBS 114356 = CPC 10902	*Eucalyptus saligna*	*Myrtaceae*	New Zealand	M. Dick	KF901681	KF903142	KF903466	—	KF902026
	CBS 114415 = CPC 10922	*Eucalyptus saligna*	*Myrtaceae*	New Zealand	M. Dick	KF901682	KF903143	KF903468	—	KF902027
Pa. irregularis	CBS 123242 = CPC 15408[ET]	*Eucalyptus globulus*	*Myrtaceae*	Thailand	R. Cheewangkoon	KF901769	KF903107	KF903542	—	KF902126

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						ITS	TEF1α	ACT	CAL	LSU
Pa. madeirensis	CBS 112301 = CPC 3747^{ET}	*Eucalyptus globulus*	*Myrtaceae*	Portugal	S. Denman	KF901688	KF903108	KF903453	—	KF902033
	CBS 112895 = CPC 3745 = CMW 14458	*Eucalyptus globulus*	*Myrtaceae*	Portugal	S. Denman	KF901675	KF903109	—	—	KF902017
Pa. marksii	CBS 110750 = CPC 822 = CMW 14778	*Eucalyptus grandis*	*Myrtaceae*	South Africa	G. Kemp	KF901709	KF903149	KF903404	—	KF902056
	CBS 110920 = CPC 935	*Eucalyptus botryoides*	*Myrtaceae*	Australia	A.J. Carnegie	KF901520	KF903145	KF903410	—	KF901842
	CBS 110963 = CPC 4632	*Musa* sp.	*Musaceae*	South Africa	K. Surridge	KF901707	KF903146	KF903411	—	KF902054
	CBS 110964 = CPC 4633	*Musa* sp.	*Musaceae*	South Africa	K. Surridge	KF901708	KF903147	KF903412	—	KF902055
	CBS 110981 = CPC 1073	*Eucalyptus* sp.	*Myrtaceae*	Tanzania	M.J. Wingfield	KF901749	KF903148	KF903417	—	KF902103
Pa. nabiacense	CBS 125010 = CPC 12748^{ET} of *Zasmidium nabiacense*	*Eucalyptus* sp.	*Myrtaceae*	Australia	A.J. Carnegie	KF901608	KF903391	KF903575	—	KF901933
Pa. parkii	CBS 387.92 = CPC 353^{ET} of *Zasmidium parkii*	*Eucalyptus grandis*	*Myrtaceae*	Brazil	M.J. Wingfield	KF901785	KF903392	KF903585	—	KF902143
Pa. pseudomarksii	CBS 123241 = CPC 15410^{ET} of *Mycosphaerella pseudomarksii*	*Eucalyptus* sp.	*Myrtaceae*	Thailand	R. Cheewangkoon	KF901770	KF903111	KF903541	—	KF902127
Pa. sticheri	CPC 24720 = COAD 1422^{ET}	*Sticherus penniger*	*Gleicheniaceae*	Brazil	E. Guatimosim	**KT037528**	**KT037488**	**KT037615**	—	**KT037569**
Pa. vietnamensis	CBS 119974 = CMW 23441 = MUCC 66^{ET} of *Mycosphaerella vietnamensis*	*Eucalyptus grandis* hybrid	*Myrtaceae*	Vietnam	T.I. Burgess	KF901809	KF903114	KF903514	—	KF902171
Passalora eucalypti	CBS 111318 = CPC 1457^{ET}	*Eucalyptus saligna*	*Myrtaceae*	Brazil	P.W. Crous & A.C. Alfenas	KF901613	KF903153	KF903445	—	KF901938
Pas. leptophlebiae	CBS 129524 = CPC 18480^{ET}	*Eucalyptus leptophlebia*	*Myrtaceae*	Brazil	P.W. Crous, A.C. Alfenas, R. Alfenas & O.L. Pereira	KF901614	KF903155	KF903580	—	KF901939
Pas.zambiae	CBS 112970 = CPC 1228^{ET}	*Eucalyptus globulus*	*Myrtaceae*	Zambia	T. Coutinho	KF901811	KF903157	KF903458	—	KF902175
	CBS 112971 = CPC 1227^{ET}	*Eucalyptus globulus*	*Myrtaceae*	Zambia	T. Coutinho	KF901810	KF903156	KF903459	—	KF902174
Phaeophleospora eugeniae	CPC 15143	*Eugenia uniflora*	*Myrtaceae*	Brazil	A.C. Alfenas	KF901615	KF903160	KF903674	—	KF901940
	CPC 15159	*Eugenia uniflora*	*Myrtaceae*	Brazil	A.C. Alfenas	KF901742	KF903159	KF903675	—	KF902095
Ph. gregaria	CBS 110501	*Eucalyptus globulus*	*Myrtaceae*	Australia	A. Maxwell	KF901524	KF903161	KF903396	—	KF901846
	CBS 111166 = CPC 1224	*Eucalyptus cladocalyx*	*Myrtaceae*	South Africa	A.R. Wood	KF901710	KF903162	KF903433	—	KF902057
	CBS 111167 = CPC 1225	*Eucalyptus cladocalyx*	*Myrtaceae*	South Africa	A.R. Wood	KF901711	KF903163	KF903434	—	KF902058
	CBS 111519 = CPC 1191	*Eucalyptus* sp.	*Myrtaceae*	South Africa	P.W. Crous	KF901712	KF903164	KF903448	—	KF902059
	CBS 114662 = CPC 1193^{ET}	*Eucalyptus* sp.	*Myrtaceae*	South Africa	P.W. Crous	KF901713	KF903165	KF903470	—	KF902060
Ph. hymenocallidis	CBS 139911 = CPC 25018^{ET}	unkown fern	*Polypodiaceae*	Thailand	P.W. Crous	KR476740	—	—	—	KR476773
Ph. hymenocallidicola	CBS 139912 = CPC 25014^{ET}	unkown fern	*Polypodiaceae*	Thailand	P.W. Crous	KR476739	—	—	—	KR476772
Ph. pteridivora	CPC 24683 = COAD 1182^{ET}	*Serpocaulon triseriale*	*Polypodiaceae*	Brazil	R.W. Barreto	**KT037547**	**KT037499**	**KT037631**	—	**KT037582**
Ph. scytalidii	CBS 118493 = CPC 10998^{ET}	*Eucalyptus urophylla*	*Myrtaceae*	Colombia	M.J. Wingfield	KF901631	KF903167	KF903493	—	KF901966
	CBS 516.93 = CPC 653	*Eucalyptus globulus*	*Myrtaceae*	Brazil	F.A. Ferreira	KF901616	KF903166	KF903588	—	KF901941
Ph. stonei	CBS 120830 = CPC 13330^{ET}	*Eucalyptus* sp.	*Myrtaceae*	Australia	P.W. Crous & J. Stone	KF901525	KF903168	KF903645	—	KF901847
Ph. stramenti	CBS 118909 = CPC 11545^{ET}	Leaf litter of *Eucalyptus* sp.	*Myrtaceae*	Brazil	A.C. Alfenas	KF901617	KF903169	KF903506	—	KF901942
Pseudocercospora abacopteridicola	CPC 24709	*Adianthum* sp.	*Pteridaceae*	Brazil	E. Guatimosim	**KT037518**	**KT037478**	**KT037600**	—	**KT037559**
Ps. angolensis	CBS 149.53	*Citrus sinensis*	*Rutaceae*	Angola	T. de Carvalho & O. Mendes	JQ324975	JQ324988	JQ325011	—	JQ324941
Ps. araliae	CPC 10154	*Aralia elata*	*Araliaceae*	South Korea	H.D. Shin	GU269652	GU384370	GU320360	—	GU253701
	MUCC 873^{ET}	*Aralia elata*	*Araliaceae*	Japan	T. Kobayashi & C. Nakashima	GU269653	GU384371	GU320361	—	GU253702
Ps. assamensis	CBS 122467^{ET}	*Musa* cultivar	*Musaceae*	India	I. Buddenhagen	GU269656	GU384374	GU320364	—	GU253705
Ps. atromarginalis	CBS 114640	*Solanum* sp.	*Solanaceae*	New Zealand	C.F. Hill	GU269658	GU384376	GU320365	—	GU253706
Ps. balsaminae	CBS 131882 = CPC 10044	*Impatiens textori*	*Balsaminaceae*	South Korea	H.D. Shin	GU269660	GU384379	GU320367	—	GU253708
Ps. basiramifera	CMW 5148	*Eucalyptus pellita*	*Myrtaceae*	Thailand	M.J. Wingfield	AF309595	DQ211677	DQ147607	—	DQ204761
Ps. basitruncata	CBS 114664 = CPC 1202	*Eucalyptus grandis*	*Myrtaceae*	Colombia	M.J. Wingfield	GU269662	DQ211675	DQ147622	—	GU253710
	CBS 111280 = CMW 14785	*Eucalyptus grandis*	*Myrtaceae*	Colombia	M.J. Wingfield	DQ267601	DQ211676	DQ147621	—	DQ204760

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Ps. brackenicola	CPC 24695[ET]	*Pteridium arachnoideum*	*Dennstaedtiaceae*	Brazil	R.W. Barreto	**KT037524**	**KT037484**	**KT037606**	—	**KT037565**
Ps. chengtuensis	CBS 131924 = CPC 10696	*Lycium chinense*	*Solanaceae*	South Korea	H.D. Shin	GU269673	GU384390	GU320379	—	JQ324942
Ps. contraria	CBS 132108 = CPC 14714	*Dioscorea quinqueloba*	*Dioscoreaceae*	South Korea	H.D. Shin	GU269677	GU384394	GU320385	—	JQ324945
Ps. cordiana	CBS 114685 = CPC 2552[ET]	*Cordia goeldiana*	*Boraginaceae*	Brazil	P.W. Crous & R.L. Benchimol	GU269681	GU384398	GU320387	—	GU214472
Ps. crocea	CBS 126004 = CPC 11668[ET]	*Pilea hamaoi*	*Urticaceae*	South Korea	H.D. Shin	GU269792	GU384502	GU320493	—	JQ324947
Ps. cruenta	CBS 132021 = CPC 10846	*Vigna* sp.	*Fabaceae*	Trinidad	H. Booker	GU269688	GU384404	JQ325012	—	GU214673
Ps. cyatheicola	CBS 129520 = CPC 17047 = CPC 17048[ET]	*Cyathea australis*	*Cyatheaceae*	Australia	P.W. Crous & R.G. Shivas	JF951139	**KT072761**	**KT072760**	—	JF951159
Ps. cymbidiicola	CBS 115132[ET]	*Cymbidium* sp.	*Orchidaceae*	New Zealand	C.F. Hill	GU269692	GU384408	GU320397	—	GU253733
Ps. dendrobii	MUCC 596	*Dendrobium* sp.	*Orchidaceae*	Japan	C. Nakashima & K. Motohashi	GU269696	GU384412	GU320401	—	GU253737
Ps. dianellae	CBS 117746	*Dianella caerulae*	*Liliaceae*	New Zealand	C.F. Hill	GU269695	GU384411	GU320400	—	GU253736
Ps. eucalyptorum	CBS 116371 = CPC 10507	*Eucalyptus nitens*	*Myrtaceae*	New Zealand	P.W.Crous	GU269687	JQ324989	GU320393	—	JQ324950
	CBS 132309 = CPC 12568	*Eucalyptus nitens*	*Myrtaceae*	Australia	C. Mohammed	GU269796	GU384506	GU320497	—	GU253814
	CBS 132032 = CPC 12802	*Eucalyptus globulus*	*Myrtaceae*	Portugal	A. Phillips	JQ324976	JQ324990	GU320466	—	GU253789
	CBS 132035 = CPC 13769	*Eucalyptus punctata*	*Myrtaceae*	South Africa	P.W. Crous	GU269659	GU384378	GU320366	—	GU253707
	CBS 132114 = CPC 13816	*Eucalyptus glaucescens*	*Myrtaceae*	United Kingdom	S. Denman	GU269801	JQ324992	GU320504	—	GU253819
Ps. eupatoriella	CBS 113372	*Chromolaena odorata*	*Asteraceae*	Jamaica	M.J. Morris	GU269704	GU384420	GU320408	—	GU253743
Ps. fori	CBS 132113 = CPC 14880	*Eucalyptus* sp.	*Myrtaceae*	South Africa	P.W. Crous	GU269806	GU384517	GU320509	—	GU253824
Ps. fuligena	CBS 132017 = CPC 12296	*Lycopersicon* sp.	*Solanaceae*	Thailand	Z. Mersha	GU269711	GU384427	GU320415	—	JQ324953
Ps. haiweiensis	CBS 131584 = CPC 14084[ET]	*Eucalyptus* sp.	*Myrtaceae*	China	X. Zhou	GU269803	GU384514	GU320506	—	GU253821
Ps. humuli	MUCC 742[ET]	*Humulus lupulus* var. *lupulus*	*Cannabaceae*	Japan	C. Nakashima & I. Araki	GU269725	GU384439	GU320428	—	GU253758
Ps. humuli-japonici	CPC 11462[ET]	*Plectranthus* sp.		Republic of Korea	H.D. Shin	JX901784	JX901682	JX902139	—	JX901892
Ps. humulicola	CBS 131883 = CPC 10049	*Humulus scandens*	*Cannabaceae*	South Korea	H.D. Shin	GU269724	JQ324996	JQ325018	—	JQ324955
Ps. indonesiana	CBS 122474	*Musa cultivar*	*Musaceae*	Indonesia	I.W. Buddenhagen	EU514283	JQ324997	JQ325019	—	JQ324957
Ps. jussiaeae	CBS 132117 = CPC 14625	*Ludwigia prostrata*	*Onagraceae*	South Korea	H.D. Shin	JQ324977	JQ324998	JQ325020	—	JQ324958
Ps. kaki	MUCC 900	*Diospyros kaki*	*Ebenaceae*	Japan	S. Uematsu & C. Nakashima	GU269729	GU384442	GU320431	—	GU253761
Ps. lilacis	CBS 132031 = CPC 12767	*Ligustrum japonicum*	*Oleaceae*	U.S.A	C. Hodges	GU269737	GU384449	GU320439	—	GU253767
Ps. lonicericola	MUCC 889[ET]	*Lonicera gracilipes* var. *glabra*	*Caprifoliaceae*	Japan	T. Kobayashi	GU269736	JQ324999	GU320438	—	GU253766
Ps. luzardii	CPC 2556[ET]	*Hancornia speciosa*	*Apocynaceae*	Brazil	A.C. Alfenas	GU269738	GU384450	GU320440	—	GU214477
Ps. lygodiicola	CPC 25755 = COAD 1745	*Lygodium volubile*	*Lygodiaceae*	Brazil	R.W. Barreto	**KT037526**	**KT037486**	**KT037608**	—	**KT037567**
Ps. lythri	CBS 132115 = CPC 14588[ET]	*Lythrum salicaria*	*Lythraceae*	South Korea	H.D. Shin	GU269742	GU384454	GU320444	—	GU253771
	MUCC 865	*Lythrum salicaria*	*Lythraceae*	Japan	I. Araki & M. Harada	GU269743	GU384455	GU320445	—	GU253772
Ps. macrospora	CBS 114696 = CPC 2553[ET]	*Bertholletia excelsa*	*Lecythidaceae*	Brazil	P.W. Crous & R.L. Benchimol	GU269745	GU384457	GU320447	—	GU214478
Ps. mazandaranensis	CCTU 1102 = CBS 136115[ET]	*Nerium oleander*	*Oleaceae*	Iran	M. Bakhshi	KM452854	KM452876	KM452831	—	—
	CCTU 1146	*Nerium oleander*	*Oleaceae*	Iran	M. Bakhshi	KM452855	KM452877	KM452832	—	—
Ps. metrosideri	CBS 118795[ET]	*Metrosideros collina*	*Myrtaceae*	New Zealand	C.F. Hill	GU269746	GU384458	GU320448	—	GU253774
Ps. natalensis	CBS 111069 = CPC 1263	*Eucalyptus nitens*	*Myrtaceae*	South Africa	T. Coutinho	DQ303077	JQ325000	DQ147620	—	DQ267576
Ps. nephrolepidis	CBS 119121[ET]	*Nephrolepis auriculata*	*Oleandraceae*	Taiwan	R. Kirschner	GU269751	GU384462	GU320453	—	GU253779
Ps. nogalesii	CBS 115022	*Chamaecytisus proliferus*	*Fabaceae*	New Zealand	C.F. Hill	GU269752	GU384463	GU320454	—	JQ324960
Ps. norchiensis	CBS 120738[ET]	*Eucalyptus* sp.	*Myrtaceae*	Italy	W. Gams	GU269753	GU384464	GU320455	—	GU253780
	CCTU 1009	*Rubus* sp.	*Rosaceae*	Iran	M. Bakhshi	KM452856	KM452878	KM452833	—	—
	CCTU 1019	*Rubus* sp.	*Rosaceae*	Iran	M. Bakhshi	KM452857	KM452879	KM452834	—	—
	CCTU 1032	*Rubus* sp.	*Rosaceae*	Iran	M. Bakhshi	KM452858	KM452880	KM452835	—	—
Ps. oenotherae	CBS 131885 = CPC 10290	*Oenothera odorata*	*Onagraceae*	South Korea	H.D. Shin	GU269856	GU384567	GU320559	—	JQ324961
Ps. palleobrunnea	CBS 124771 = CPC 13387[ET]	*Syzygium* sp.	*Myrtaceae*	Australia	P.W. Crous	GQ303288	GU384509	GU320500	—	GQ303319

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Ps. pallida	CBS 131889 = CPC 10776	*Campsis grandiflora*	*Bignoniaceae*	South Korea	H.D. Shin	GU269758	GU384469	GU320459	—	GU214680
Ps. pancratii	CBS 137.94	—	—	Cuba	R.F. Castaneda	GU269759	GU384470	GU320460	—	GU253784
Ps. paraguayensis	CBS 111286 = CPC 1459	*Eucalyptus nitens*	*Myrtaceae*	Brazil	P.W. Crous	DQ267602	DQ211680	DQ147606	—	GU214479
Ps. paranaensis	CPC 24680^{ET}	*Cyathea atrovirens*	*Cyatheaceae*	Brazil	R.W. Barreto	**KT037522**	**KT037482**	**KT037604**	—	**KT037563**
	COAD 1180	*Cyathea atrovirens*	*Cyatheaceae*	Brazil	R.W. Barreto	**KT037523**	**KT037483**	**KT037605**	—	**KT037564**
Ps. parapseudarthriae	CBS 137996 = CPC 23449^{ET}	*Pseudarthria hookeri*	*Leguminosae*	South Africa	A.R. Wood	KJ869151	KJ869238	KJ869229	—	KJ869208
Ps. pouzolziae	CBS 122280	*Gonostegia hirta*	*Urticaceae*	Taiwan	R. Kirschner	GU269761	GU384472	GU320462	—	GU253786
Ps. profusa	CPC 10042	*Acalypha australis*	*Euphorbiaceae*	South Korea	H.D. Shin	GU269787	GU384497	GU320488	—	GU253808
	CBS 132306 = CPC 10055	*Acalypha australis*	*Euphorbiaceae*	South Korea	H.D. Shin	GU269762	GU384473	GU320463	—	GU253787
Ps. proteae	CBS 131587 = CPC 15217^{ET}	*Protea mundii*	*Proteaceae*	South Africa	F. Roets	GU269808	GU384519	GU320511	—	GU253826
Ps. prunicola	CBS 132107 = CPC 14511	*Prunus yedoensis*	*Rosaceae*	South Korea	H.D. Shin	GU269676	GU384393	GU320382	—	GU253723
Ps. punicae	CCTU 1125 = CBS 136111	*Punica granatum*	*Lythraceae*	Iran	M. Bakhshi	KM452859	KM452881	KM452836	—	—
				Iran	M. Bakhshi	KM452860	KM452882	KM452837	—	—
Ps. purpurea	CBS 114163 = CPC 1664	*Persea americana*	*Lauraceae*	Mexico	P.W. Crous	GU269783	GU384494	GU320486	—	GU253804
Ps. pyracanthae	MUCC 892	*Pyracantha angustifolia*	*Rosaceae*	Japan	T. Kobayashi & C. Nakashima	GU269767	GU384479	GU320470	—	GU253792
Ps. rhabdothamni	CBS 114872^{ET}	*Rhabdothamnus solandri*	*Gesneriaceae*	New Zealand	M. Fletcher	GU269768	GU384480	GU320471	—	JQ324964
Ps. rhamnellae	CBS 131590 = CPC 12500^{ET}	*Rhamnella frangulioides*	*Rhamnaceae*	South Korea	H.D. Shin	GU269795	GU384505	GU320496	—	GU253813
Ps. rumohrae	CBS 117747	*Marattia salicina*	*Marattiaceae*	New Zealand	C.F. Hill	GU269774	GU384486	GU320477	—	GU253796
Ps. rubi	MUCC 875	*Rubus allegheniensis*	*Rosaceae*	Japan	T. Kobayashi & C. Nakashima	GU269773	GU384485	GU320476	—	GU253795
Ps. schizolobii	CBS 120029 = CPC 12962^{ET}	*Schizolobium parahyba*	*Fabaceae*	Ecuador	M.J. Wingfield	KF251322	KF253269	KF253628	—	KF251826
Ps. sophoricola	CBS 136020 = CCTU 1037^{ET}	*Sophora alopecuroides*	*Fabaceae*	Iran	M. Bakhshi	KM452861	KM452883	KM452838	—	—
Ps. sordida	MUCC 913	*Campsis radicans*	*Bignoniaceae*	Japan	C. Nakashima & E. Imaizumi	GU269777	GU384488	GU320480	—	GU253798
Pseudocercospora sp. A	CCTU 1165 = CBS 136113	*Phaseolus vulgaris*	*Fabaceae*	Iran	M. Bakhshi	KM452863	KM452885	KM452840	—	—
	CCTU 1166	*Phaseolus vulgaris*	*Fabaceae*	Iran	M. Bakhshi	KM452864	KM452886	KM452841	—	—
Pseudocercospora sp. B	CCTU 1066	*Phaseolus vulgaris*	*Ebenaceae*	Iran	M. Bakhshi	KM452865	KM452887	KM452842	—	—
	CCTU 1191	*Diospyros lotus*	*Ebenaceae*	Iran	M. Bakhshi	KM452866	KM452888	KM452843	—	—
	CCTU 1206 = CBS 136114	*Diospyros lotus*	*Ebenaceae*	Iran	M. Bakhshi	KM452867	KM452889	KM452844	—	—
Pseudocercospora sp.	CBS 110998 = CPC 1054	*Eucalyptus grandis*	*Myrtaceae*	South Africa	M.J. Wingfield	GU269778	GU384489	GU320481	—	GU253799
Ps. thelypteridis	CPC 24676^{ET}	*Thelypteris* sp.	*Thelypteridaceae*	Brazil	R.W. Barreto	**KT037521**	**KT037481**	**KT037603**	—	**KT037562**
Ps. trichogena	CPC 24670 = COAD 1088^{ET}	*Deparia petersenii*	*Athyriaceae*	Brazil	R.W. Barreto	**KT037520**	**KT037480**	**KT037602**	—	**KT037561**
	CPC 24664 = COAD 1087	*Macrothelypteris torresiana*	*Thelypteridaceae*	Brazil	R.W. Barreto	**KT037519**	**KT037479**	**KT037601**	—	**KT037560**
Ps. serpocaulonicola	CPC 25077 = COAD 1866^{ET}	*Serpocaulon triseriale*	*Polypodiaceae*	Brazil	R.W. Barreto	**KT037525**	**KT037485**	**KT037607**	—	**KT037566**
Pseudoramichloridium henryi	CBS 124775 = CPC 13121^{ET}	*Corymbia henryi*		Australia	A.J. Carnegie	KF901535	KF903227	KF903559	—	KF901857
	CPC 13122	*Corymbia henryi*		Australia	A.J. Carnegie	KF901533	KF903226	KF903639	—	KF901855
Ramularia endophylla	CBS 113265^{EET}	Dead leaf of *Quercus robur*		Netherlands	G. Verkley	KF901725	KF903240	KF903461	—	KF902072
R. eucalypti	CBS 120726 = CPC 13043^{ET}	*Eucalyptus grandiflora*	*Myrtaceae*	Italy	W. Gams	KF901666	KF903241	KF903525	—	KF902006
Septoria eucalyptorum	CBS 118505 = CPC 11282^{ET}	Leaf litter of *Eucalyptus* sp.	*Myrtaceae*	India	W. Gams & M. Arzanlou	KF901651	KF903265	KF903501	—	KF901991
Sonderhenia eucalypticola	CPC 11251	*Eucalyptus globulus*	*Myrtaceae*	Spain	M.J. Wingfield	KF901746	KF903266	KF903596	—	KF902099
	CPC 11252	*Eucalyptus globulus*	*Myrtaceae*	Spain	M.J. Wingfield	KF901747	KF903268	KF903597	—	KF902100
	CBS 112502 = CPC 3749	*Eucalyptus* sp.	*Myrtaceae*	Spain	P.W. Crous	KF901677	KF903267	KF903454	—	KF902019
Sphaerulina cercidis	CBS 118910 = CPC 12226^{ET}	*Eucalyptus* sp.	*Myrtaceae*	France	P.W. Crous	KF901649	KF903269	KF903507	—	KF901988
Staninwardia suttonii	CBS 120061 = CPC 13055^{ET}	*Eucalyptus robusta*	*Myrtaceae*	Australia	B.A. Summerell	KF901552	KF903270	KF903517	KF902693	KF901874
Xenomycosphaerella alsophilae	CPC 24694 = COAD 1181^{ET}	*Alsophila* sp.	*Cyatheaceae*	Brazil	R.W. Barreto	**KT037543**	**KT037502**	**KT037616**	—	**KT037585**
X. cyatheae	CPC 18580 = COAD 573	*Cyathea delgadii*	*Cyatheaceae*	Brazil	R.W. Barreto	**KT037539**	**KT037498**	**KT037624**	—	**KT037580**

Species	Culture accession numbers[1,2]	Host/isolation source	Host family	Country	Collector	GenBank accession numbers[3]				
						ITS	TEF1α	ACT	CAL	LSU
X. cyatheae	CPC 24688 = COAD 1238	*Cyathea delgadii*	*Cyatheaceae*	Brazil	R.W. Barreto	**KT037541**	**KT037500**	**KT037625**	—	**KT037583**
	CPC 24704[ET]	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037545**	**KT037504**	**KT037626**	—	**KT037587**
	CPC 24712	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037527**	**KT037487**	**KT037617**	—	**KT037568**
	CPC 24724	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037529**	**KT037489**	**KT037618**	—	**KT037570**
	CPC 24726 = COAD 1426	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037531**	**KT037491**	**KT037619**	—	**KT037572**
	CPC 24728	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037532**	**KT037492**	**KT037620**	—	**KT037573**
	CPC 24732	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037535**	**KT037494**	**KT037622**	—	**KT037576**
	CPC 24744	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037537**	**KT037496**	**KT037623**	—	**KT037578**
	CPC 24729 = COAD 1428	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037533**	**KT037493**	**KT037621**	—	**KT037574**
X. diplazii	CPC 24691[ET]	*Diplazium* sp.	*Athyriaceae*	Brazil	R.W. Barreto	**KT037542**	**KT037501**	**KT037627**	—	**KT037584**
X. elongata	CBS 120735 = CPC 13378[ET]	*Eucalyptus camaldulensis* × *urophylla*	*Myrtaceae*	Venezuela	M.J. Wingfield	KF901808	KF903374	KF903528	—	KF902170
X. yunnanensis	CBS 119975 = CMW 23443 = MUCC 410[ET]	*Eucalyptus urophylla*	*Myrtaceae*	China	B. Dell	KF901628	KF903375	KF903515	—	KF901962
Z. citri	CBS 116366 = CPC 10522 = CMW 11730	*Acacia mangium*	*Fabaceae*	Thailand	K. Pongpanich	KF901780	KF903386	–	—	KF902138
	CPC 15291	*Citrus* sp.	*Rutaceae*	USA	–	KF901793	KF903382	KF903676	—	KF902152
Z. cyatheae	CPC 24725 = COAD 1425[ET]	*Cyathea delgadii*	*Cyatheaceae*	Brazil	E. Guatimosim	**KT037530**	**KT037490**	**KT037629**	—	**KT037571**
Z. eucalyptigenum	CBS 138860 = CPC 24251[ET]	*Eucalyptus urophylla*	*Myrtaceae*	Mozambique	M.J. Wingfield	KP004458	—	**KT037630**	—	KP004486
Z. eucalyptorum	CBS 118500 = CPC 11174[ET]	*Eucalyptus* sp.	*Myrtaceae*	Indonesia	M.J. Wingfield	KF901652	KF903101	KF903495	—	–
Z. pseudoparkii	CBS 110999 = CPC 1087[ET]	*Eucalyptus grandis*	*Myrtaceae*	Colombia	M.J. Wingfield	KF901642	KF903273	KF903419	—	KF901977
	CBS 110988 = CPC 1090	*Eucalyptus grandis*	*Myrtaceae*	Colombia	M.J. Wingfield	KF901640	KF903271	KF903418	—	KF901975
	CBS 111049 = CPC 1089	*Eucalyptus grandis*	*Myrtaceae*	Colombia	M.J. Wingfield	KF901641	KF903272	KF903426	—	KF901976
Zasmidium sp.	CPC 24679 = COAD 1178	*Blechnum serrulatum*	*Blechnaceae*	Brazil	R.W. Barreto	**KT037540**	—	**KT037628**	—	**KT037581**
Z. xenoparkii	CBS 111185 = CPC 1300[ET]	*Eucalyptus grandis*	*Myrtaceae*	Indonesia	M.J. Wingfield	KF901663	KF903274	KF903438	—	KF902002

[1] 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.

[2] ET: ex-type; EET - ex-epitype.

[3] ITS: internal transcribed spacers and intervening 5.8S nrDNA, TEF1α: translation elongation factor 1alpha, ACT: actin, CAL: calmodulin, LSU: 28S nrRNA gene.

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

Gene	Primer Name	Sequence 5'→3'	Annealing temperature (°C)	Orientation	Reference
ACT	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
CAL	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
Tef1-α	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

Table 3 Substitution models applied to the different phyllogenetic analysis performed in this study.

	Locus				
	ITS	Tef-1α	ACT	CAL	LSU
Cercospora spp.	SYM+I	HKY+G	K80+G	HKY+I+G	
Pseudocercospora spp.	SYM+G	HKY+I+G	SYM+I+G		
mycosphaerella-like spp.	GTR+I+G	HKY+I+G	HKY+I+G		GTR+I+G

Capítulo 4 – Microfungos em pteridófitas

Artigo – Novel fungi from an ancient niche: lachnoid and chalara-like fungi on ferns

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Novel fungi from an ancient niche: lachnoid and chalara-like fungi on ferns

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Abstract A systematic survey of fungi on ferns in various localities in Brazil, was conducted over seven years (2009–2015). A significant diversity of fungi have been collected belonging to fungal groups such as cercosporoids, members of the *Parmulariaceae* and others that will be covered in separate publications. Here lachnoid and chalara-like fungi found during the survey are described and discussed. Based on morphology and inferred phylogeny from DNA sequences of two loci, namely the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (LSU), species belonging to the lachnoid species complex, *Chalara* and *Bloxamia* have been recognized. Eighteen isolates recovered from five host species from ten different localities are included. An analysis of the morphology and molecular data resulted on six fern-related fungi taxa, of which five are new to science whose are described and illustrated herein, namely: *Bloxamia cyatheicola*, *Chalara lygodii, Chalara cyatheae, Lachnum catarinense* and *Psilachnum pteridimi*. *Lachum varians* is recorded for the first time in Brazil in association with a novel host.

Keywords *Bloxamia, Chalara*, *Lachnum*, phylogenetic analysis, *Psilachnum,* tropical ferns

Introduction

Lachnoid fungi are members of *Hyaloscyphaceae* Nannf, which is considered the largest family in *Helotiales*, comprising about 930–940 species organized in 74 genera (Kirk et al. 2008). Species in this family are characterized as smaller discomycetes, with brightly colored apothecia that are ornamented with conspicuous hairs along the margins and lower surface (Han et al. 2014). The cladistics work of Cantrell and Hanlin (1997) suggested the family as probably monophyletic, and based on this premise, most mycologists have considered the presence of hairs as a synapomorphic character.

Based on morphology, *Hyaloscyphaceae* was subdivided into three tribes: *Arachnopezizeae*, *Hyaloscypheae*, and *Lachneae*. *Arachnopezizeae* included species with an apothecium seated on a well-developed subiculum or in a false subiculum-like hyphae; *Hyaloscypheae* contained species with minute apothecia presenting hairs with highly diverse shapes, and mostly cylindric paraphyses; and *Lachneae* included species with relatively large apothecia, multiseptate granulate hairs, and lanceolate paraphyses (Nannfeldt 1932).

Raitviir (2004) elevated *Lachneae* to the familial rank, *Lachnaceae,* and Hosoya et al. (2010) based on morphology and multi-locus DNA analysis, confirmed this hypothesis. However, the latter authors concluded that the paucity of species sampling is a barrier to discuss the taxonomy of lachnoid fungi. In the recent work dealing with taxonomy of *Hyaloscyphaceae*, Han et al. (2014) examined the morphological characteristics in the context of multi-locus molecular phylogeny, and based on 70 species included in all three former tribes, the authors showed *Hyaloscyphaceae* as to be polyphyletic, and rejected the presence of hairs as a synapomorphic feature for the family. Additionally, *Hyaloscyphaceae* sensu stricto was tentatively restricted to the genus *Hyaloscypha*, but the limited sampling within this family is still an on-going problem (Han et al. 2014).

Since DNA sequencing became available to properly evaluate evolutionary relationships among fungi, the genus *Chalara* (Corda) Rabenh and allied species have been intensely addressed (Réblová 1999; Coetsee et al. 2000; Paulin and Harrington 2000; Paulin-Mahady et al. 2002). *Chalara* is known as a paraphyletic genus, occupying different positions within *Helotiales*, with some species closely related to *Hyaloscyphaceae* (Cai et al. 2004). The problem to assign *Chalara* and allied genera to a specific family of *Helotiales* is worsened by the fact that most helotiaceous *Chalara* species lack a known sexual morph, and probably might have lost their ability to reproduce sexually (Nag Raj and Kendrick 1975).

Brazilian's biodiversity is very rich and numerous novel fungal taxa have recently been published (Machado et al. 2014, Guatimosim et al. 2014a, 2014b, Crous et al. 2015). This scenario is more evident, when a systematic approach is carried out for group of host-plants poorly studied by mycologists, like the tropical ferns (*Pteridophyta*). Currently, there are around 48 fungal species recorded on ferns from Brazil (Farr and Rossman 2015, Mendes and Urben 2015). A recent survey, focused on cercosporoid and their sexual related stages, causing frond diseases in Brazilian ferns, has fungi yielded 17 new species, three new host-diseases records, and one novel genus (Guatimosim et al. 2015), indicating this group of plants, as an important part of a highly diverse mycobiota.

Based on morphological characters and phylogenetic inference of two DNA regions (ITS and LSU), 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 lachnoid fungi, *Chalara* and allied genera.

Materials and Methods

Specimens and isolates

Frond samples bearing fungal colonies were collected in Brazil in different biomes, including the Amazon, Atlantic rainforest, Caatinga and Cerrado between 2011 and 2015. These were dried in a plant press and later examined under a Nikon SMZ1500 stereo-microscope (Nikon Instruments, Tokyo, Japan) to observe sporulation. Conidia were scraped from a single frond spot, and single conidial colonies were established on potato carrot agar – PCA (Crous et al. 2009). To obtain ascospores isolates, excised lesions were placed in distilled water for approximately 2 h, after which they were placed on the bottom of Petri dish lids, over which the plate containing PCA was inverted. Freehand sections of fungal colonies were prepared and fungal structures mounted in clear lactic acid, lactic acid, lactofuchsin, and/or Melzer's reagent. When necessary, sections were made using a Microm HM520 freezing microtome (Microm, Hellersbergstraße, Germany). Observations were made with a Nikon Eclipse 80i (Nikon Instruments, Tokyo, Japan) light microscope with differential interference contrast (DIC) illumination and a Nikon DS-Fi1 camera and NIS-Elements imaging software (Nikon Instruments, Tokyo, Japan). Colonies descriptions were made on potato dextrose agar – PDA (Crous et al. 2009) and PCA, in the dark and under a 12 h light regime (noted in taxonomic descriptions). After 30 d, the colony diameter was measured and the colony color was described according to the mycological color charts of Rayner (1970). Representative herbaria specimens were deposited at the Herbarium of the Universidade Federal de Viçosa (VIC) and the Herbarium of the CBS-KNAW

Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS H). Axenic cultures were deposited at CBS, 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 isolates used in this study is presented in Table 1.

Scanning electron microscopy

Samples of dried material containing fungal structures were mounted on stubs with doublesided adhesive tape and gold-coated using a Balzer's FDU 010 sputter coater (Optics Balzers, Neugrüt, Liechtenstein). A LEO VP 1430 scanning electron microscope – SEM (Carl-Zeiss, Jena, Germany) was used to analyze and generate images from the samples.

DNA isolation, amplification and sequencing

Isolates were grown on 2 % malt extract agar – MEA (Crous et al. 2009) for 20 d at 25 °C, over the bench. Genomic DNA was extracted from mycelium using Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions. For *Bloxamia* species, leaves harboring fertile stromata were examined under a stereo-microscope to check for possible contamination by other fungi, including yeasts. The leaves were then soaked in sterile water for 1 h in order to hydrate and facilitate to remove the stromata. Thirty fertile stromata were removed from the leaves with a sterile fine pointed needle, and placed into a microcentrifuge tube (1.5 mL). Total genomic DNA was extracted by using Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions and the steps described by Pinho et al. (2012). The DNA samples were subsequently diluted 50–100 times in preparation for further DNA amplification reactions. All strains were screened for different loci. Two partial nuclear genes were targeted for PCR amplification and sequencing, namely, the 28S nrRNA gene (LSU) and the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrDNA operon were amplified. The primers LR0R + LR5 (Vilgalys and Hester 1990) were used to amplify and sequence the LSU locus while the ITS locus was amplified and sequenced with the primer pairs ITS5 + ITS4 (White et al. 1990). The PCR amplifications were performed in a total volume of 12.5 µL solution containing 10–20 ng of template DNA, 1× PCR buffer, 0.63 µL DMSO (99.9 %), 1.5 mM MgCl2, 0.5 µM of each primer, 0.25 mM of each dNTP, 1.0 U BioTaq DNA polymerase (Bioline GmbH Luckenwalde, Germany). PCR conditions were set as follows: an initial denaturation temperature of 95 °C for 5 min, followed by 35 cycles of denaturation temperature of 95 °C for 30 s, primer annealing at 52 °C for 30 s, primer extension at 72 °C for 1 min and a final extension step at 72 °C for 1 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, USA) in MultiScreen HV plates (Millipore, Billerica, MA, USA). Purified sequence reactions were run on an ABI Prism 3730xl DNA Sequencer (Life Technologies, Carlsbad, CA, USA).

DNA sequence data were analyzed in MEGA (Molecular Evolutionary Genetics Analysis) v. 6.0 (Tamura et al. 2013). Consensus sequences were generated and imported into MEGA for initial alignment and the construction of sequence datasets. Sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov), and the novel sequences generated on this study, were aligned using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; (Katoh et al. 2002) and whenever indicated, manually improved in MEGA.

Phylogenetic analysis

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. 3.2.3 applying the GTR+I+G substitution model for ITS and LSU, through Cipres Gateway (Miller et al. 2010). *Saccharomyces cerevisiae* DAOM 216365 served as outgroup for both analyses. Posterior probabilities were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.3 (Ronquist et al. 2012). Six simultaneous Markov chains were run for 10.000.000 generations and trees were sampled every 100th generation, and 10.000 trees were obtained. The first 2.000 trees, representing the burn-in phase were discarded, while the remaining 8.000 trees were used for calculating posterior probabilities. Bayesian posterior probabilities (PP) are presented on the left of each node, on each tree. Sequences derived in this study were lodged in GenBank, the alignment and tree in TreeBASE (http://www.treebase.org), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004a).

Results

Phylogenetic results

The two datasets consisted of 476 characters for ITS and 795 for LSU. The respective alignments included 251 parsimony-informative characters for ITS and 214 for LSU. After topological convergence of the Bayesian runs at 0.15 for both studies, 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 two Bayesian phylogenies, 2440/1952 for ITS and 4063/3250 for LSU. The resulting phylogenetic trees of the individual datasets could not be

concatenated, once several isolates have the DNA data available exclusively only for ITS or LSU. The results are treated below.

Taxonomy

Bloxamia cyatheicola Guatimosim, R.W. Barreto & Crous **sp. nov.** (Fig. 3).

MycoBank: MB 813045

Etymology: Refers to the generic name of the host species, *Cyathea*

Frond spots randomly affecting individual pinnules, irregular, chlorotic. Asexual morph: *Conidioma* sporodochial, hypophyllous, erumpent, scattered on the edge of the pinnulet, discoid, up to 1000 × 2000 µm, solitary, when wet pulvinate, slimy, amber-coloured, when dry flattened, contracted and of a horny consistency, black. In vertical section, sporodochia with a basal stroma of *textura intricata*, 190–205 µm deep in the center of the conidioma, composed of cells 4–5 µm diam, pale brown. *Stroma* brown lower down, becoming paler, gradually hyaline towards the top. *Phialophores* often reduced to phialides, rarely 1-septated. *Phialides* arising from the stroma surface in a densely packed palisade, discrete, terminal, branched, subcylindrical, 17–41 × 1.5–3.5 µm, light brown, becoming paler towards the apex, smooth-walled. *Phialoconida* endogenous, basipetal, extruded in easily dispersible chains, cylindrical, truncate at both ends, 2.5–8 × 1–3 µm, non-septate, hyaline, with small guttules, smooth-walled. Sexual morph: *Apothecia* hypophyllous, sometimes associated with the conidioma on the same pinnulet, erumpent, scattered on the edge of the pinnulet, discoid, becoming cupulate when dried, up to 500 × 1900 µm, solitary, sessile, black, horny to the touch. In vertical section, apothecia with a basal stroma of *textura intricata*, 103–198 µm deep, composed of cells 3 µm diam, *Medullary excipulum* of textura epidermoidea, up to 250 µm high, thin-walled, composed of hyphae 1–1.5 µm diam, sub-hyaline to hyaline. *Paraphyses* filiform, swollen at the tip, 1–2.5 µm, septate, hyaline, smooth. *Asci* unitunicate, sub-cylindric to clavate, straight to curved, 68–113 × 6.5–14 µm, 8-spored, non-amyloid, hyaline, smooth. *Ascospores* fusoid, with one cell slightly bigger, 10–18 × 4–7 µm, uniseriate, rarely biseriate, hyaline, with two opposite guttules, smooth.

Holotype: Brazil. Rio de Janeiro, Macaé de Cima, on fronds of *Cyathea delgadii* (*Cyatheaceae*), 29 Apr 2012, *R.W. Barreto*, (VIC 42563), sexual moprh. Rio de Janeiro, Nova Fribrugo, on fronds of *Cyathea delgadii* (*Cyatheaceae*), 29 Apr 2012, *R.W. Barreto*, (VIC 42579) asexual morph.

Habitat/Distribution: Known from *Cyathea delgadii* and *Cyathea atrovirens* (*Cyatheaceae*) in the states of Minas Gerais, Paraná and Rio de Janeiro, Brazil.

Additional specimens examined: Brazil, Paraná, Quatro Barras, on fronds of *C. atrovirens*, 01 Feb 2012, *R.W. Barreto*, (VIC 42574), sexual morph. Rio de Janeiro, Nova Friburgo, on fronds of *C. delgadii*, 29 Jul 2012, *R.W. Barreto*, (VIC 42584), assexual morph. Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, on fronds of *C. delgadii*, 23 Feb 2014, *E. Guatimosim*, (VIC 42460), assexual morph.

Notes: The genus *Bloxamia* Berk. & Broome includes seven species, and among them, only *B. foliicola* is known as a pathogen, causing disease on *Oxyspora paniculata* from China, which is different from *B. cyatheicola* by having the phialophores organized in synnema (Liu and Zhang 1998). The other species, which has sporodochial conidioma, are not known from ferns (Table 2). Based on morphology, *B. cyatheicola* is should be compared with *B. cremea* recorded on dead wood from Argentina (Arambarri et al. 1992) and *B. truncata* recorded on decorticated wood of *Ulmus* sp. from England (Pirozynski and Morgan-Jones 1968). The fungus from Brazil is different from *B. cremea* by having an amber-coloured to black conidioma (white to creamy in the latter), bigger and broader pale brown phialophores (24–26 × 2.5–3 µm, dark brown in the later) and phialoconidia, extruded on easily dispersible chains in the former (in long and slimy chains in the later) (Arambarri et al. 1992). On the other hand, *B. truncata* is different from *B. cyatheicola* by having more or less cuboid phialoconidia produced endogenously in basipetal sucession, where up to six conidia can be visualized within the phialophore (Minter and Holubová-Jechová 1981, Pirozynski and Morgan-Jones 1968), which does not occur in the latter. All attempts to isolate the fungus have failed.

Chalara cyatheae Guatimosim, R.W. Barreto & Crous **sp. nov.** (Fig. 4).

MycoBank: MB 813046

Etymology: Refers to the generic name of the host species, *Cyathea delgadii*

Frond spots amphigenous, 2.5–4 × 1.5–3 mm, somewhat angular, starting as small necrotic areas along the margins of the pinnulets and spreading. Affecting random pinnules. Sporulating abundantly. *Internal hyphae* not observed. *External hyphae* absent. *Stroma* absent. *Phialophores* reduced to phialides. *Phialides* erumpent through the cuticle, lageniform to subcylindrical, brown to cinnamon brown, paler above, smooth-walled, scattered, hypophylous, solitary, 32–50 µm long, 5–8.5 µm wide at the base; venter subcylindrical to ellipsoid, 12–26 × 3–7 µm; collarette cylindrical, 15–23 × 2–3.5 µm, transition from ventre

to collarette gradual. *Phialoconidia* endogenous, basipetal, extruded singly or in easily dispersible chains, cylindrical, truncate at both ends, unicellular, hyaline, guttulated with two large opposal guttules, 6–10 × 1.5–3 µm, smooth-walled.

Holotype: Brazil. Rio de Janeiro, Nova Friburgo, on fronds of *Cyathea delgadii* (*Cyatheaceae*), 13 Jun 2011, *R.W. Barreto*, (VIC 42543, culture ex-type CPC 24665, COAD 1092).

Culture characteristics: Colonies on PDA reaching 3–3.5 cm diam after 30 d at 25 °C in 12 h of light regime; circular, flat, centrally with felty aerial mycelia, surface centrally rosy buff, passing to white, periphery buff, dry, diurnal zonation absent, sporulation absent; reverse centrally hazel, passing to honey, passing to buff. Colonies on PCA reaching 2.2–2.6 cm diam after 30 d at 25 °C in 12 h of light regime; circular, flat, entirely yeast-like, surface white, with some central random tiny dots of aerial mycelia dark mouse grey, dry, diurnal zonation absent, sporulation abundant; reverse as similar as the superior view.

Habitat/Distribution: Known from *Cyathea delgadii* (*Cyatheaceae*) in the states of Minas Gerais and Rio de Janeiro, Brazil.

Additional specimens examined: Brazil, Rio de Janeiro, Macaé de Cima, on fronds of *C. delgadii*, 29 Apr 2012, *R.W. Barreto*, (VIC 42562, culture CPC 24690). Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, on fronds of *C. delgadii*, 23 Feb 2014, *E. Guatimosim*, (VIC 42518, VIC 42462, cultures CPC 24735, CPC 24736). Rio de Janeiro, Macaé de Cima, on fronds of *C. delgadii*, 01 Jun 2014, *R.W. Barreto*, (culture CPC 25072, COAD 1758).

Notes: See the notes for *Chalara lygodii*.

Chalara lygodii Guatimosim, R.W. Barreto & Crous **sp. nov.** (Fig. 5).

MycoBank: MB 813046

Etymology: Refers to the generic name of the host species, *Lygodium volubile*

Frond spots amphigenous, irregular, starting as small, vein delimited, pale brown to cinnamon-brown areas, close to the mid vein of the pinnulets and spreading towards the apex. At later stages, becoming dark, necrotic and distorting the pinnulets, sometimes causing necrosis of the entire pinnulet. Affecting mostly the upper pinnulets. Sporulating abundantly. *Internal hyphae* not observed. *External hyphae* absent. *Stroma* absent. *Phialophores* reduced to phialides, *Phialides* erumpent through the cuticle, lageniform, brown to cinnamon-brown, paler above, smooth-walled, scattered, hypophylous, solitary, 29–38 µm long, 5.5–9 µm wide at the base; venter subcylindrical to ellipsoid, pedicellate, 13–16 × 5–6.5 µm; collarette cylindrical, 16–21 × 3–4 µm, transition

from venter to collarette gradual. *Phialoconidia* extruded in easily dispersible chains, cylindrical, truncated at the base and with rounded apex, unicellular, hyaline, guttulated with two large opposal guttules, 6.5–12 × 1.5–3 µm, smooth.

Culture characteristics: in preparation

Holotype: Brazil, Minas Gerais, Viçosa, on fronds of *Lygodium volubile* (*Lygodiaceae*), 06 Mar 2013, *E. Guatimosim*, (VIC 42470, culture ex-type CPC 24710).

Habitat/Distribution: Known from *L. volubile* in the states of Minas Gerais and Rio de Janeiro, Brazil.

Additional specimens examined: Brazil, Rio de Janeiro, Lumiar, on fronds of *L. volubile*, 02 Mai 2013, *R.W. Barreto*, (VIC 42600, culture CPC 24699).

Notes: Morphologically, *C. lygodii* can be compared with *C. fungorum*, but differs from it by having a wider base of the phialides (5.5–9 µm in the former and 3–6.5 µm in the latter) and bigger phialoconidia (6.5–12 µm in the former and up to 8 µm in the latter) (Nag Raj and Kendrick 1975). Additionally, *C. fungorum* is only known attacking angiosperms from Canada, Italy and the United Kingdom (Farr and Rossman 2015, Nag Raj and Kendrick 1975), while *C. lygodii* is only known causing diseases on the Neotropical fern *Lygodium volubile* from Brazil.

Besides the different host range, *C. lygodii* is different from *C. cyatheae* by having by having 15 different bp. of variable sites for the locus ITS and 10 bp. of variable sites for the locus LSU.

Lachnum catarinense Guatimosim, R.W. Barreto & Crous **sp. nov.** (Fig. 6).

MycoBank: MB 813047

Etymology: Refers to the state in Brazil from where the fungus was collected, Santa Catarina.

Frond spots amphigenous affecting the apex of pinnules, irregular, pale brown becoming necrotic, where ascomata are formed. *Apothecia* scattered, hypophyllous, short-stipitate, disc closed, cupulate, 230–248 × 300–315 µm, stipe 52 × 48 µm, white. *Receptacle* concolorous with the disc, densely clothed with hyaline hairs. *Ectal excipulum* of *textura prismatica,* composed of cells 8–10 × 4–5 µm, thin-walled, oriented at low angle, more intricate towards the base, hyaline, smooth. *Hairs* obclavate, straight or curved, 56–94 × 2.5–10 µm, 3–4-septate, tapering toward the rounded apex, hyaline, thin-walled, roughened with hyaline, rod-shaped granules, non-amyloid. *Asci* unitunicate, 8-spored,

clavate, straight or curved, 45–58 × 7–14 µm, short-pediculate, not arising from croziers, hyaline, thin-walled, smooth, pore non-amyloid. *Ascospores* uniseriate, overlapping, sub-cylindrical to fusoid, curved, 32–46 × 1–2.5 µm, 3-septate, tapering towards both ends, guttulate, hyaline, smooth. *Ascospores germinating* from both ends. *Paraphyses* clavate, 55–60 µm long, 4–5 µm wide at the widest point, apex hemispherical, exceeding the asci, straight or curved, unbranched, 3-4-septate, hyaline, smooth. *Asexual morph*: not observed.

Culture characteristics: in preparation

Holotype: Brazil, Santa Catarina, Luizinho, Highway to São José dos Ausentes, roadside, on fronds of *Dicksonia sellowiana* (*Dicksoniaceae*), 16 Apr 2013, *E. Guatimosim*, (VIC 42478, culture ex-type CPC 24713).

Habitat/Distribution: Known from *D. sellowiana* in the southern of Brazil.

Additional specimens examined: Brazil, Santa Catarina, Luizinho, Highway to São José dos Ausentes, roadside, on fronds of *Dicksonia sellowiana* (*Dicksoniaceae*), 16 Apr 2013, *E. Guatimosim*, (VIC 42481) Santa Catarina, Urubici, roadside, on fronds of *Dicksonia sellowiana* (*Dicksoniaceae*), 15 Apr 2013, *E. Guatimosim*, (VIC 42507, culture CPC 24723).

Notes: Based on the ITS phylogenetic study (Fig. 2) *L. catarinense* has *L. varians* as sister clade, and differs from it by having long sub-cylindrical to fusoid ascospores, rather small and elliptical in the latter (Haines and Dumont 1984). Among other *Lachnum* species known from tropical ferns in Brazil, *L. brasiliense* is rather similar to *L. catarinense*, but differs from it by having cylindrical hairs with hemispherical tips and non-septate ascospores (hairs obclavate and 3-septate ascospores in the latter) (Haines and Dumont 1984). Additionally, *L. brasiliense* is phylogenetically different from the newly described species (Fig. 2).

Lachnum varians (Rehm) M.P. Sharma, Nova Hedwigia 43: 411. 1986. (Fig. 7).

MycoBank: MB 129277

Frond spots amphigenous, randomly affecting pinnulets, irregular, pale brown becoming necrotic, where ascomata are formed. *Apothecia* scattered, hypophyllous, stipitate, disc goblet-shaped, 180–1000 × 260–1500 µm, stipe 40–315 × 35–290 µm, cream to ochre. *Receptacle* concolorous with the disc, densely clothed with pale brown to ochre hairs. *Ectal excipulum* of *textura prismatica,* composed of cells 9–11 × 3–5 µm, thin-walled, oriented at low angle, more intricate towards the base, pale brown, smooth. *Hairs* sub-cylindrical, straight, 40–70 × 2.5–5 µm, 3–4-septate, tapering toward the apex, pale straw yellow, thin-walled, roughened with hyaline, rod-shaped granules,

more concentrated towards the apex, non-amyloid. *Asci* unitunicate, 8-spored, cylindrical straight, 52–62 × 6–8 µm, with a tapered base and hemispherical apex, not arising from croziers, hyaline, thin-walled, smooth, pore non-amyloid. *Ascospores* uniseriate, overlapping, ellipsoid and fusiform, 13–19 × 2.5–6 µm, 1-septate, tapering towards acute ends, guttulate, hyaline, smooth. *Ascospores germination* not seen. *Paraphyses* narrowly lanceolate or sub-cylindrical, 47–87 × 2–4.5 µm, tapered apex, exceeding the asci, straight, unbranched, 1-septate at the base, hyaline, smooth. *Asexual morph*: not observed.

Specimen examined: Brazil, Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, Serra das cabeças, atlantic rainforest, on fronds of *Dicksonia sellowiana* (*Dicksoniaceae*), 27 Apr 2013, *P.B. Scwatzburd, A.P. Fortuna*, (VIC 44526, culture CPC 24742, COAD 1429).

Notes: Lachnum varians is the most common and widespread discomycete inhabiting decaying remains of tropical ferns (Haines and Dumont 1984, Spooner 1987), ranging from northern and western South America (including Brazil), till the Caribbean, Hawaii , New Guinea and New Zealand (Haines 1980). Among tropical ferns, it was already recorded on members of *Cyatheaceae*, *Dicksoniaceae*, and *Gleicheniaceae* including *Cyathea delbata, Gleichenella pectinata*, *Dicksonia antartica* and *D. squarrosa* (Haines 1980, Spooner 1987). Despite most of records lacks identification about the host from where the fungus was found, this is the first record of *L. varians* on *Dicksonia sellowiana* from Brazil. At this point, it is not possible to state whether *L. varians* is pathogen or saprobe as conjectured, but the isolate from Brazil was obtained from apothecia found only on frond spots.

Psilachnum pteridimi Guatimosim, R.W. Barreto & Crous **sp. nov.** (Fig. 8).

MycoBank: MB 813048

Etymology: Refers to the generic name of the host species, *Pteridium arachnoideum*

Frond spots amphigenous randomly affecting individual pinnulets, irregular, pale brown becoming necrotic, where ascomata are formed. *Apothecia* scattered, hypophyllous, sessile, disc initially closed and cupulate, becoming opened and shallow concave when mature, 150–270 × 260–310 µm, centrally cream and white periphery when opened, margin elevated. *Receptacle* concolorous with the disc. *Medulary excipulum* of *textura angularis,* composed of cells 4–10 µm diam, thin-walled, oriented perpendicular to the host tissue, hyaline, smooth. *Ectal excipulum* of *textura epidermoidea,* composed of cells 1–2.5 µm diam, thin-walled, oriented at low angle, more intricated toward the base, hyaliane, smooth. *Hairs* filiform, 13–16 × 5–6.5 µm, non-septate, hyaline,

thin-walled, smooth, no crystals and resinous matters observed, non-amyloid. *Asci* unitunicate, 8-spored, sub-cylindrical, straight, 54–100 × 2–18 µm, pediculate, tapering towards the apex into a small cap, with a distinctive pore, not arising from croziers, slightly thick-walled, hyaline, smooth, pore amyloid. *Ascospores* uniseriate, overlapping, initially clavate becoming sub-cylindrical, straight, 44–57 × 1.5–3 µm, initially non-septate becoming 3-septate, tapering toward one end and the other rounded, guttulate, hyaline, smooth. *Ascospores germination* not seen. *Paraphyses* filiforms, 1 µm wide, as long as the asci, flexuous, unbranched, non-septate, apex rounded, hyaline, smooth. *Asexual morph*: not observed.

Culture characteristics: in preparation

Holotype: Brazil, Rio de Janeiro, Nova Friburgo, on fronds of *Pteridium arachnoideum* (*Dennstaedtiaceae*), 13 June 2011, *R.W. Barreto*, (VIC 42544, culture ex-type CPC 24666).

Habitat/Distribution: Known from *Pteridium aracnoideum* in the states of Pernambuco and Rio de Janeiro, Brazil.

Additional specimens examined: Brazil, Pernambuco, Taquaritinga do Norte, trilha do mirante, Serra da Taquara, *Pteridium arachnoideum*, 09 Jul 2014, *D.J. Soares*, (VIC 42921, culture CPC 25778, COAD 1796).

Notes: Based on both phylogenetic studies (Fig. 1 and Fig. 2), *P. pteridimi* has *Hyphodiscus*, as sister clade. These two genera however, are not related, given the size and shape of the ascospores (long, septate and sub-cylindrical on the former, rather small, non-septate and ellipsoid on all described species of the latter) (Zhuang 1988, Hosoya 2002). Additionally, the genus *Hyphodiscus* is known as having gelatinous ectal excipulum (Hosoya 2002, Untereiner et al. 2006) absent in *Psilachnum*.

The genus *Psilachnum* Höhn accommodates lachnoid species with smooth hairs, and like *Lachnum*, it is also known from tropical ferns (Galán and Raitviir 1999). *Psilachnum pteridimi* clearly differs from other species in the genus, due to its longer (>20 µm) ascospores: clavate and non-septate when immature, becoming sub-cylindrical and septate at maturity.

Discussion

The topology of both trees (Fig. 1 and Fig. 2) suggests that both, *C. lygodii* and *C. cyatheae* are related to *Chalara* but significantly distant from all the species included in this study, having *B. cyatheicola* as sister clade. However, this topology is corroborated by the fact that most part of the available DNA

information of *Chalara*, is related to species causing diseases on vascular plants, like the angiosperms and gymnosperms (Nag Raj and Kendrick 1975).

Only three species of *Chalara* are known from ferns, namely *C. crassipes* causing disease on *Pteridium aquilinum* from Germany, *C. parvispora* on *Cyathea medullaris* from New Zealand, and *C. pteridina* on *Pteridium aquilinum* from Austria, Australia, England, Germany, Poland, and the United Kingdom (Farr and Rossman 2015, Nag Raj and Kendrick 1975). However, only *C. crassipes* and *C. parvispora* have DNA information available (Cai et al. 2009), and besides it is only the LSU locus, they still are different from both *C. cyatheae* and *C. lygodii* (Fig. 1).

The genus *Bloxamia* is characterized as a tubercularіaceous fungus, presenting fructifications scattered or gregarious, black, disciform sporodochia, with pale brown superficial stromata composed of sub-hyaline to pale brown cells, arranged in dense palisades, from which arise the phialophores, where catenulate, hyaline conidia are produced (Nag Raj and Kendrick 1975). The genus is based on *B. truncata* occurring on dead decorticated wood of *Ulmus* sp. from England (Pirozynski and Morgan-Jones 1968). Currently, seven species are recognized within *Bloxamia*, as summarized in Table 2.

Berthet (1964) reported the development of *Bloxamia truncata* (type species of *Bloxamia*) from cultures of single ascospores isolations of *Bisporella sulphurina*. Johnston (1998) reproduced the same finding by recovering a *Bloxamia* asexual morph through the isolation of *Bisporella discedens* from New Zealand, however, the latter author did not propose a separate name for the asexual stage.

The genus *Bisporella* Sacc. is characterized by its small, bright yellow, sessile apothecia, which generally occur on woody substrata in temperate zones; in longitudinal section, the internal anatomy of the apothecium is characterized by a gelatinized or subgelatinized ectal excipulum, with little or no differentiation of a medullary excipulum; asci 8-spored, 1-septate (Carpenter and Dumont 1978, Saccarddo 1884). Over the years, this genus was treated as a repository of a huge variety of fungi, with significant differences in morphology (e.g. 3-septate ascospores like *B. triseptata* and non-septate ascospores like *B. calycellinoides, B. iodocyanescens* and *B. oritis*), achieving up to date 25 species (Kirk et al. 2008) and being probably a genus-complex. This idea is corroborated by *Bisporella resinicola* from which an asexual morph of *Eustibum* (completely different from *Bloxamia*) was described (Baranyay and Funk 1969, Seifert and Carpenter 1987). In addition, a recently published phylogeny has shown that some of the species recognized as members of *Bisporella* (namely *B. citrina, B. claroflava, B. drosodes, B. lactea*, and *B. scolochloae*) were in fact

members of *Calycina* Nees ex Gray, once they grouped with its type species *C. herbarum* (Baral et al. 2013).

For the clarification of the true evolutionary relationships within *Bisporella* it is necessary to recollect and epitipify its type species *B. monilifera*, with the assessment of the DNA. Despite the fact that species from Brazil shows both sexual and asexual morphs, we decided to describe it within *Bloxamia,* once this genus is as well circumscribed as older than *Bisporella*.

Except for *B. foliicola,* all species of *Bloxamia* were described from dead or decorticated wood, or from rotting plant material (Table 2), suggesting its habit as a saprobe. *Bloxamia cyatheicola* was found associated with frond spots on *Cyathea* spp. but also on living leaves associated with no symptoms, these findings suggests its habit as a pathogen or as a possible endophyte.

The genus *Lachnum* Retz. is widely distributed and characterized by small, discoid apothecia covered by numerous sub-cylindrical, septate and granulated hairs (Haines and Dumont 1984). The genus includes about 250 species (Kirk et al. 2008) and besides most part of them are not known from molecular data, it was already shown that the genus is polyphyletic (Han et al. 2014). The present phylogenetic survey (Fig.2), agrees with Zhao and Zuang (2011), whom demonstrated the locus ITS as a reliable source for verifying species boundaries within *Lachnum*. Further studies, based on the epitypification of *L. agaricinum* (type of *Lachnum*) and related species, are necessary to clarify the correct evolutionary placement of *Lachnum* and allied genera.

Regarding *Psilachnum*, there are two other sequences of the genus, available from GenBank: one determined only at the generic level, and the other one related to *Psilachnum staphyleae,* isolated from leaves of *Staphylea bumalda*, from Korea (Han et al. 2009). On both phylogenetic analyses (Fig. 1 and Fig. 2), the genus *Psilachnum* is clearly polyphyletic, since *P. pteridimi* and *P. staphyleae* cluster in non-related clades. The clarification of the evolutionary relationships within this genus, awaits a proper reassessment and epitypification of all species described within *Psilachnum,* including the type, *P. lateritioalbum*.

The present work contributes to a better understanding of lachnoid fungi, *Chalara* and allied genera within *Hyaloscyphaceae* sensu latu, by increasing the sampling, providing descriptions, images, and molecular data of these infrequently collected species.

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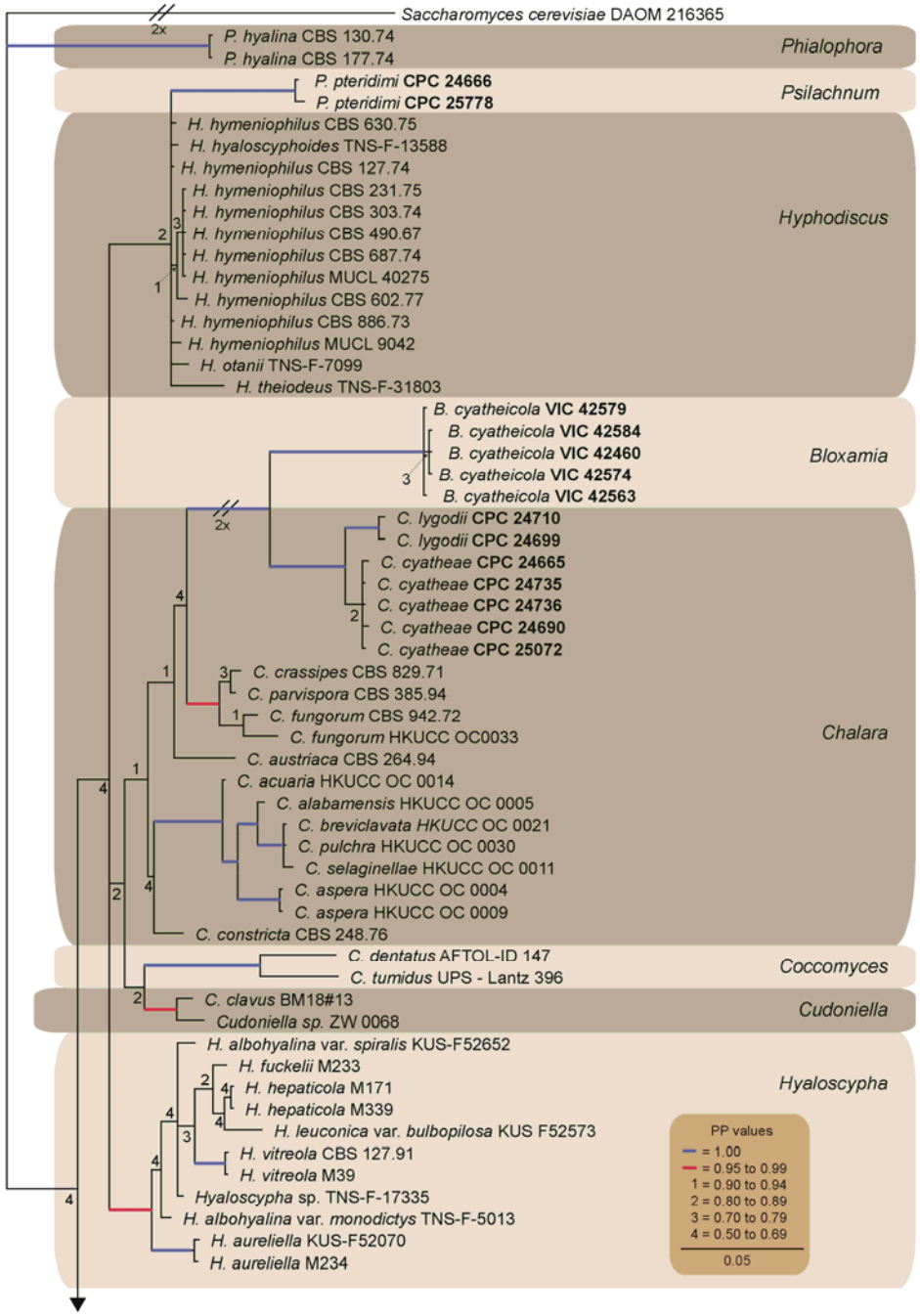
Figures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P. hyalina CBS 130.74
P. hyalina CBS 177.74
Phialophora
P. pteridimi CPC 24666
P. pteridimi CPC 25778
Psilachnum
H. hymeniophilus CBS 630.75
H. hyaloscyphoides TNS-F-13588
H. hymeniophilus CBS 127.74
H. hymeniophilus CBS 231.75
H. hymeniophilus CBS 303.74
H. hymeniophilus CBS 490.67
H. hymeniophilus CBS 687.74
H. hymeniophilus MUCL 40275
H. hymeniophilus CBS 602.77
H. hymeniophilus CBS 886.73
H. hymeniophilus MUCL 9042
H. otanii TNS-F-7099
H. theiodeus TNS-F-31803
Hyphodiscus
B. cyatheicola VIC 42579
B. cyatheicola VIC 42584
B. cyatheicola VIC 42460
B. cyatheicola VIC 42574
B. cyatheicola VIC 42563
Bloxamia
C. lygodii CPC 24710
C. lygodii CPC 24699
C. cyatheae CPC 24665
C. cyatheae CPC 24735
C. cyatheae CPC 24736
C. cyatheae CPC 24690
C. cyatheae CPC 25072
C. crassipes CBS 829.71
C. parvispora CBS 385.94
C. fungorum CBS 942.72
C. fungorum HKUCC OC0033
C. austriaca CBS 264.94
C. acuaria HKUCC OC 0014
C. alabamensis HKUCC OC 0005
C. breviclavata HKUCC OC 0021
C. pulchra HKUCC OC 0030
C. selaginellae HKUCC OC 0011
C. aspera HKUCC OC 0004
C. aspera HKUCC OC 0009
C. constricta CBS 248.76
Chalara
C. dentatus AFTOL-ID 147
C. tumidus UPS - Lantz 396
Coccomyces
C. clavus BM18#13
Cudoniella sp. ZW 0068
Cudoniella
H. albohyalina var. spiralis KUS-F52652
H. fuckelii M233
H. hepaticola M171
H. hepaticola M339
H. leuconica var. bulbopilosa KUS F52573
H. vitreola CBS 127.91
H. vitreola M39
Hyaloscypha sp. TNS-F-17335
H. albohyalina var. monodictys TNS-F-5013
H. aureliella KUS-F52070
H. aureliella M234
Hyaloscypha
PP values
= 1.00
= 0.95 to 0.99
1 = 0.90 to 0.94
2 = 0.80 to 0.89
3 = 0.70 to 0.79
4 = 0.50 to 0.69
0.05

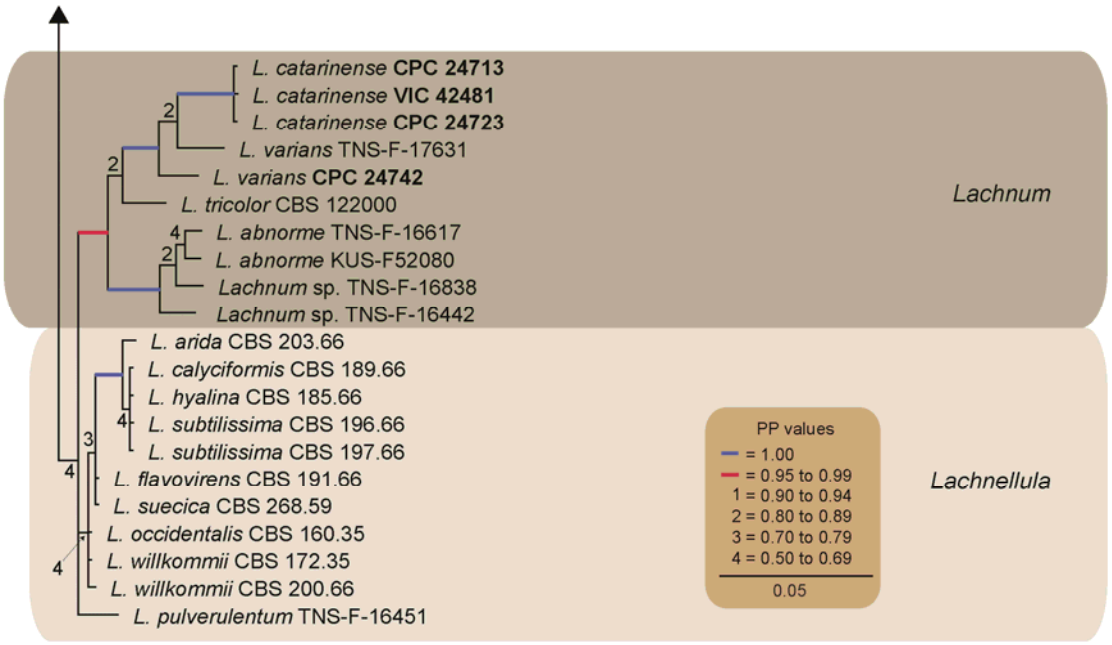
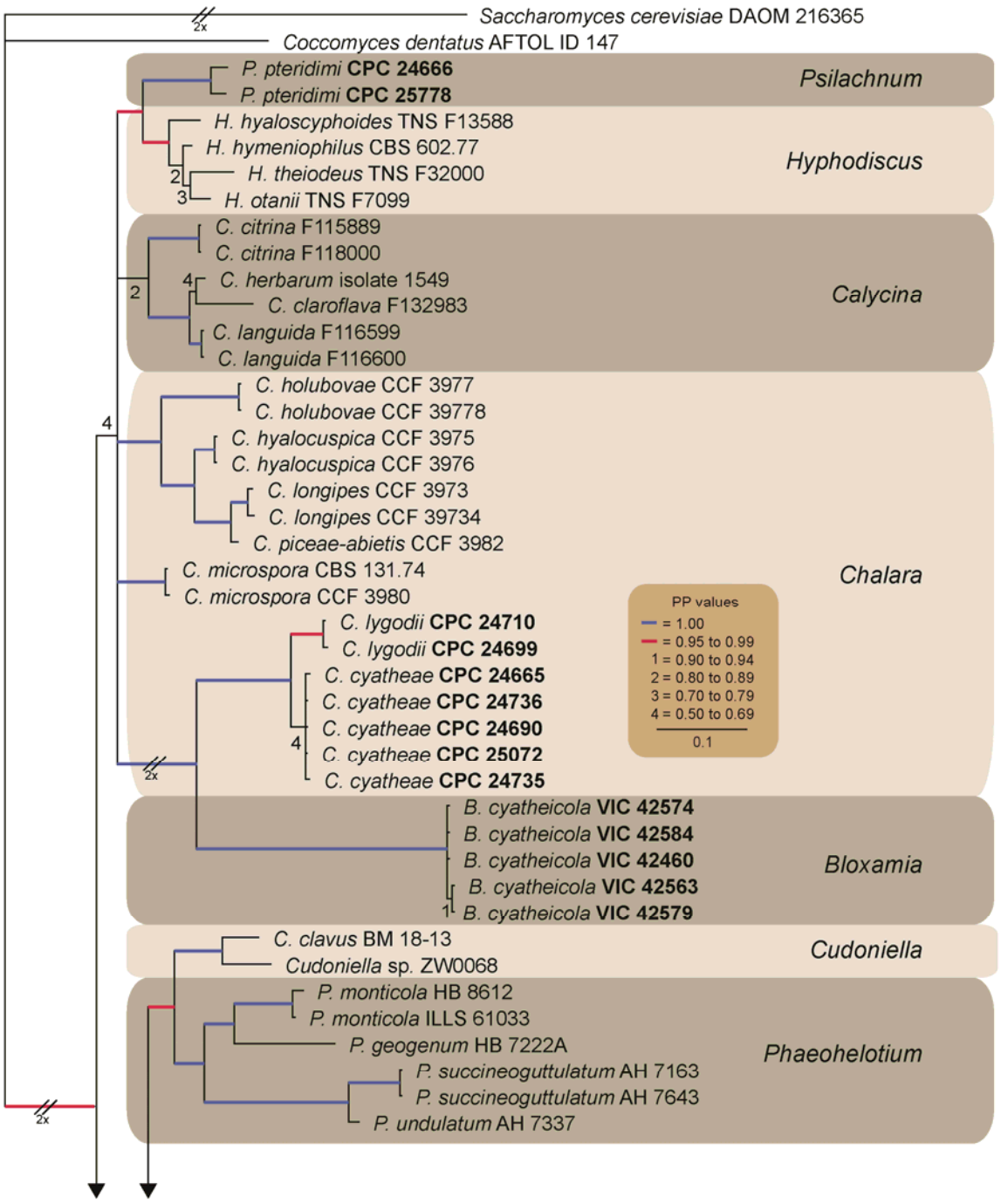


Fig. 1 Consensus phylogram (50 % majority rule) from a Bayesian analysis of the LSU sequence alignment. Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend) and the scale bar indicates 0.05 expected changes per site. Isolates from Brazil are indicated in bold. The tree was rooted to *Saccharomyces cerevisiae* (isolate DAOM 216365).


Saccharomyces cerevisiae DAOM 216365
Coccomyces dentatus AFTOL ID 147
P. pteridimi CPC 24666
P. pteridimi CPC 25778
Psilachnum
H. hyaloscyphoides TNS F13588
H. hymeniophilus CBS 602.77
H. theiodeus TNS F32000
H. otanii TNS F7099
Hyphodiscus
C. citrina F115889
C. citrina F118000
C. herbarum isolate 1549
C. claroflava F132983
C. languida F116599
C. languida F116600
Calycina
C. holubovae CCF 3977
C. holubovae CCF 39778
C. hyalocuspica CCF 3975
C. hyalocuspica CCF 3976
C. longipes CCF 3973
C. longipes CCF 39734
C. piceae-abietis CCF 3982
C. microspora CBS 131.74
C. microspora CCF 3980
C. lygodii CPC 24710
C. lygodii CPC 24699
C. cyatheae CPC 24665
C. cyatheae CPC 24736
C. cyatheae CPC 24690
C. cyatheae CPC 25072
C. cyatheae CPC 24735
Chalara
PP values
= 1.00
= 0.95 to 0.99
1 = 0.90 to 0.94
2 = 0.80 to 0.89
3 = 0.70 to 0.79
4 = 0.50 to 0.69
0.1
B. cyatheicola VIC 42574
B. cyatheicola VIC 42584
B. cyatheicola VIC 42460
B. cyatheicola VIC 42563
B. cyatheicola VIC 42579
Bloxamia
C. clavus BM 18-13
Cudoniella sp. ZW0068
Cudoniella
P. monticola HB 8612
P. monticola ILLS 61033
P. geogenum HB 7222A
P. succineoguttulatum AH 7163
P. succineoguttulatum AH 7643
P. undulatum AH 7337
Phaeohelotium
2x
2x
2x

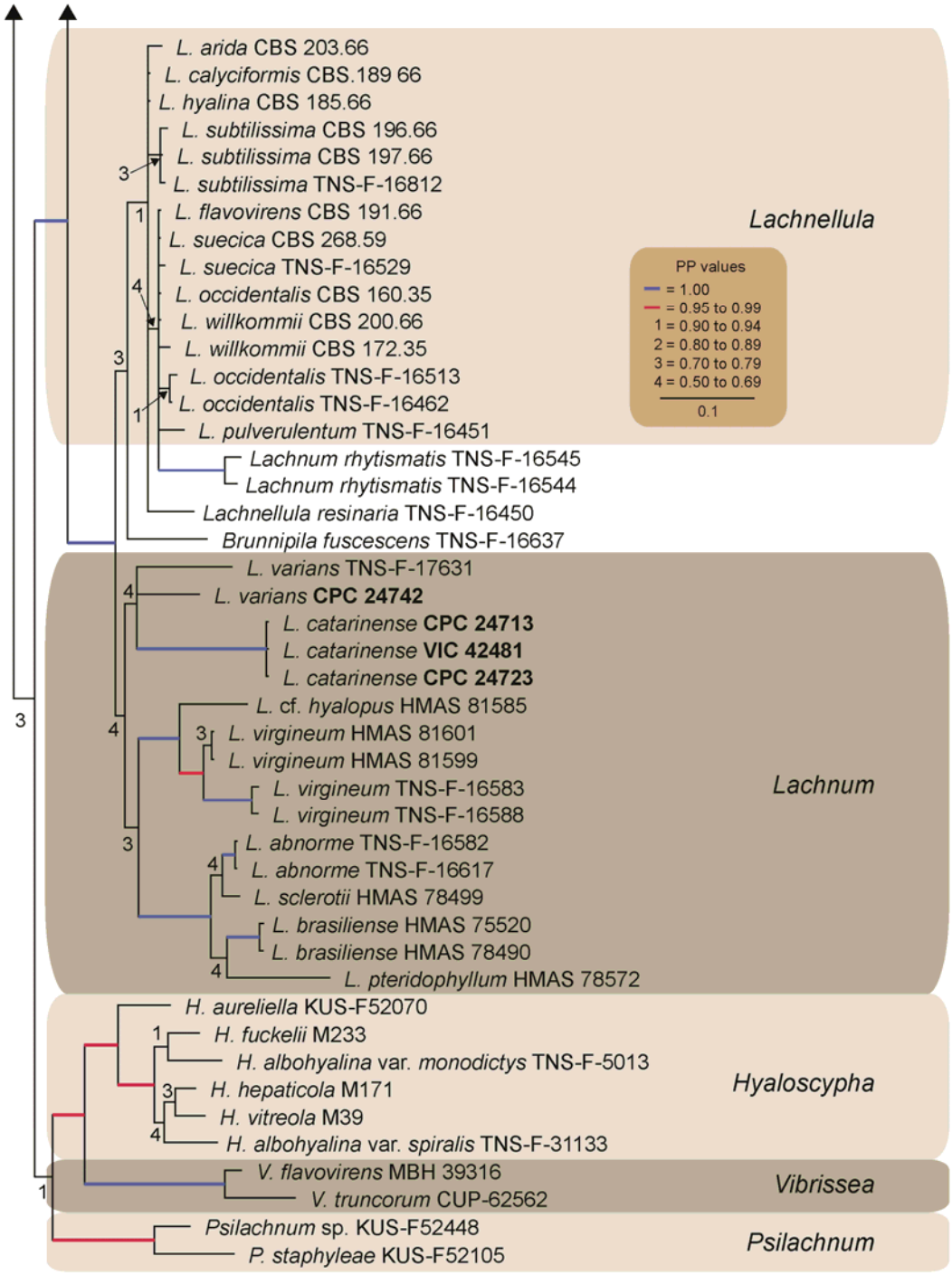


Fig. 2 Consensus phylogram (50 % majority rule) from a Bayesian analysis of the ITS sequence alignment. Bayesian posterior probabilities are indicated with colour-coded branches and numbers (see legend) and the scale bar indicates 0.01 expected changes per site. Isolates from Brazil are indicated in bold. The tree was rooted to *Saccharomyces cerevisiae* (isolate DAOM 216365).

Fig. 3 *Bloxamia cyatheicola* (VIC 42579, holotype). **a** frond spots on *Cyathea delgadii*. **b–c** sporodochial conidiomata. **d–e** apothecia. **f** vertical section of conidioma. **g–h** phialophores. **i** phialoconidia. **j** vertical section of apothecia. **k** asci. **l** ascospores (**f–g, k–l** in lactofuchsin; **h–j** in lactic acid). *Scale bars* **f** 100 µm, **g–l** 10 µm

Fig. 4 *Chalara cyatheae* (VIC 42543, holotype). **a–b** frond spots on *Cyathea delgadii*. **c, e–f** phialophores. **d** phialoconidia. **e** colony on PDA (**d** in lactofuchsin; **c, f** in lactic acid). *Scale bars* **c–f** 10 µm

Fig. 5 *Chalara lygodii* (VIC 42470, holotype). **a–b** frond spots on *Lygodium volubile*. **c** phialophores. **d** phialoconidia. **e** colony on PDA (**d** in lactofuchsin; **c** in lactic acid). *Scale bars* **c–d** 10 µm

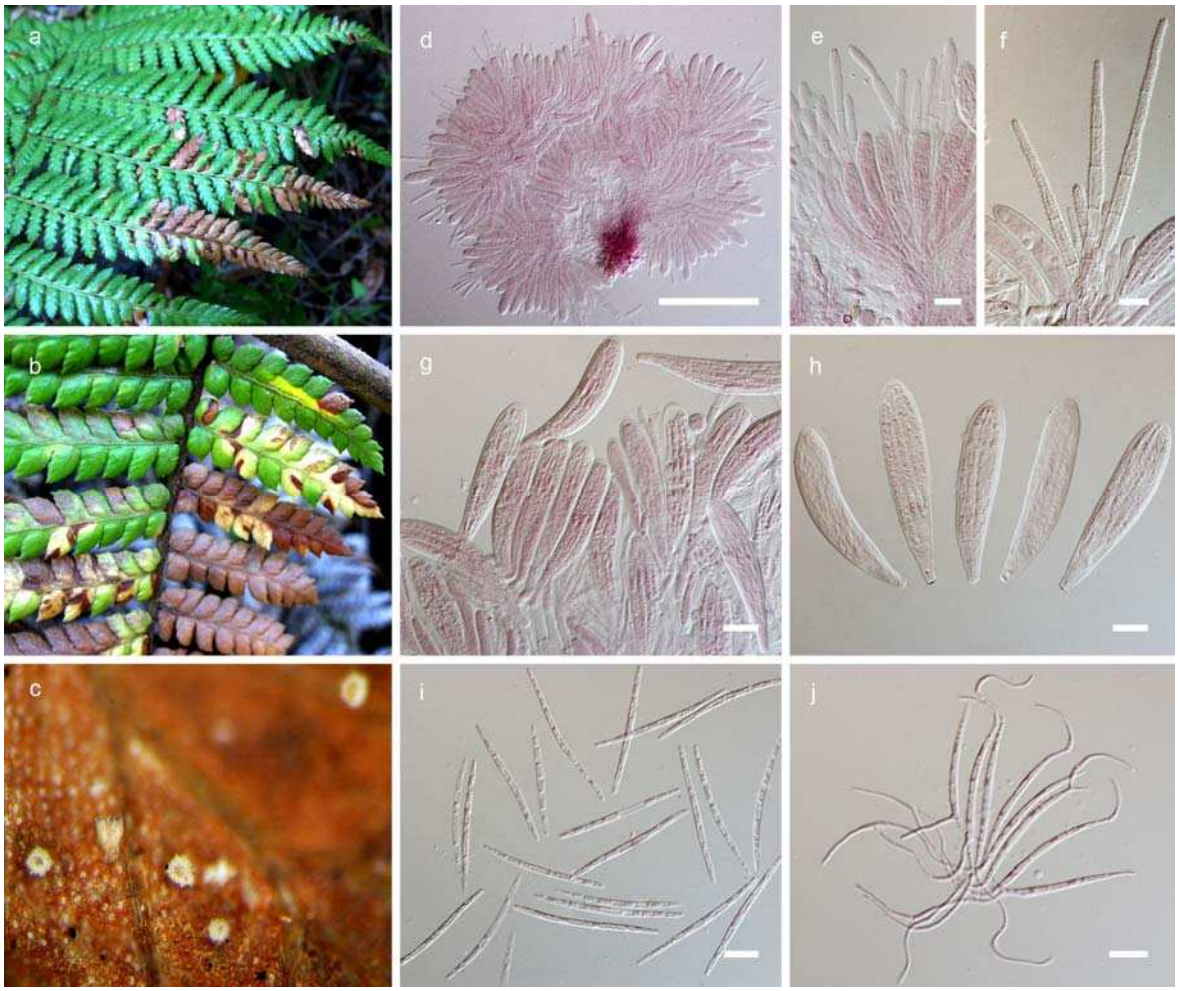


Fig. 6 *Lachnum catarinensis* (VIC 42478, holotype). **a–b** frond spots on *Dicksonia sellowiana*. **c** apothecia. **d** vertical section of apothecia. **e** detail of paraphyses with hemispherical apex, exceeding the asci. **f** roughened hairs, with hyaline rod-shaped granules. **g–h** asci. **i** ascospores. **j** ascospores germinating from both ends (**d–k** in lactofuchsin). *Scale bars* **d** 100µm, **e–j** 10 µm

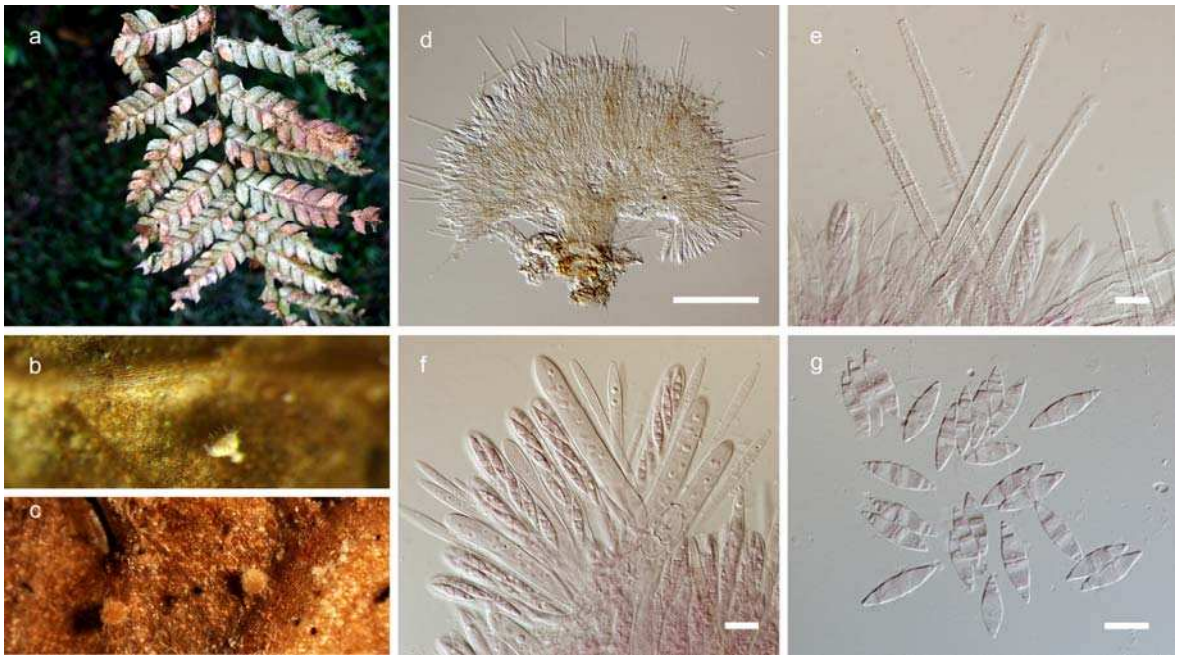


Fig. 7 *Lachnum varians* (VIC 44526). **a** frond spots on *Dicksonia sellowiana*. **b** initial stage of apothecia, bearing white color. **c** later stages of apothecia, bearing pale brown color. **d** vertical section of apothecia, note the pale brown to ochre hairs. **e** roughened hairs, with hyaline rod-shaped granules, more concentrated towards the apex. **f** asci intermixed with narrowly lanceolate or sub-cylindrical paraphyses. **g** ascospores (**e–g** in lactofuchsin; d in lactic acid). *Scale bars* **d** 100µm, **e–g** 10 µm

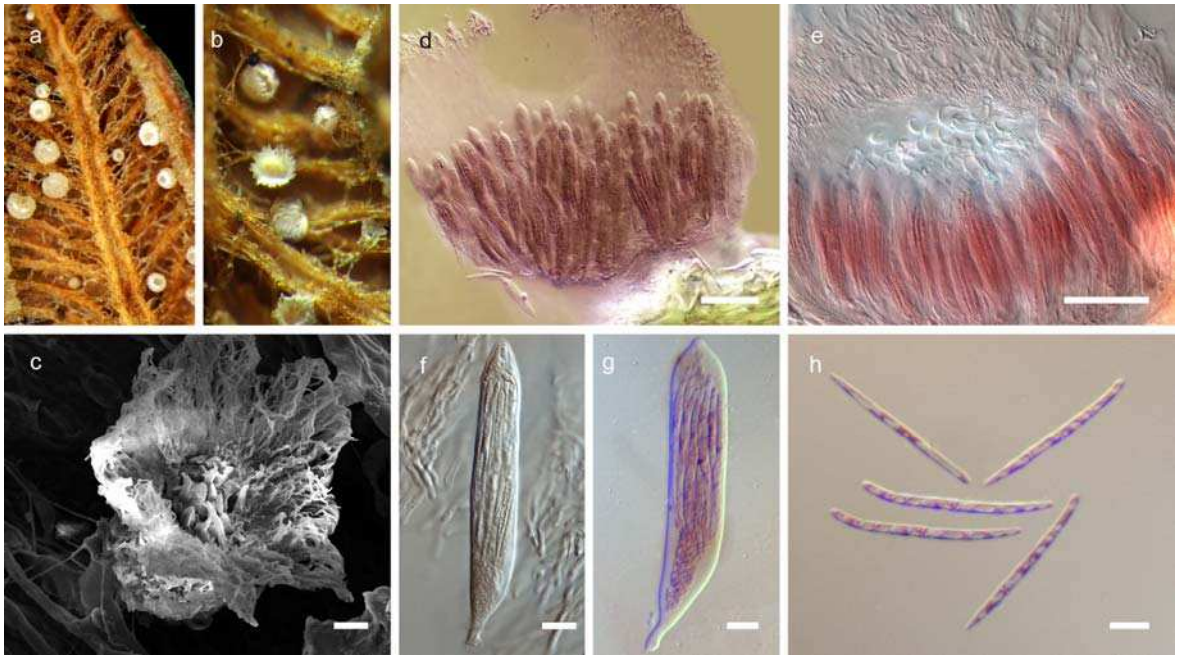


Fig. 8 *Psilachnum pteridimi* (VIC 42544, holotype). **a–b** hypophyllous apothecia on *Pteridium arachnoideum*. **c** SEM image of apothecia, note the smoothed hairs, typical of the genus. **d–e** vertical section of apothecia. **f–g** asci. **h** ascospores. (**d–e**, **g**, **h** in lactofuchsin; **f** in lactic acid). *Scale bars* **c** 20µm, **d–e** 50 µm, **f–h** 10 µm

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

Species	Culture / specimen accession numbers[1]	Host/isolation source	Country	Collector	GenBank accession numbers[2]	
					ITS	LSU
Phialophora hyalina	CBS 130.74	Soil of wheat field	Germany	W. Gams	–	GU727562
	CBS 177.74	Soil of wheat field	Germany	W. Gams	–	GU727563
Bloxamia cyatheicola	**VIC 42563**	*Cyathea delgadii*	Brazil	R.W. Barreto		
	VIC 42579	*Cyathea delgadii*	Brazil	R.W. Barreto		
	VIC 42574	*Cyathea atrovirens*	Brazil	R.W. Barreto		
	VIC 42584	*Cyathea delgadii*	Brazil	R.W. Barreto		
	VIC 42460	*Cyathea delgadii*	Brazil	E. Guatimosim		
Brunnipila fuscescens	TNS-F-16637	Lindera obtusiloba	Japan	R. Sasagawa	AB481254	–
Calycina citrina	F115889	*Fagus sylvatica*	Spain	–	KC412004	–
	F118000	*Quercus robur*	Spain	–	KC412005	–
Ca. claroflava	F132983	*Quercus ilex*	Spain	–	KC412006	–
Ca. herbarum	isolate 1549	–	–	–	AY348594	–
Ca. languida	F116599	*Fagus sylvatica*	Spain	–	KC412002	–
	F116600	*Fagus sylvatica*	Spain	–	KC412003	–
Chalara acuaria	HKUCC OC0014	–	–	–	–	FJ176248
Ch. alabamensis	HKUCC OC0005	–	–	–	–	FJ176247
Ch. aspera	HKUCC OC0004	–	–	–	–	FJ176244
	HKUCC OC0009	–	–	–	–	FJ176245
Ch. austriaca	CBS 264.94	*Hordeum vulgare*	Finland	T. Tuomi	–	FJ176255
Ch. breviclavata	HKUCC OC0021	–	–	–	–	FJ176243
Ch. constricta	CBS 248.76	decaying wood	Belgium	W. Gams	–	FJ176256
Ch. crassipes	CBS 829.71	*Pteridium aquilinum*	Germany	W. Gams	–	FJ176254
Ch. cyatheae	**CPC 24665 = COAD 1092**	*Cyathea delgadii*	Brazil	R.W. Barreto		
	CPC 24690	*Cyathea delgadii*	Brazil	R.W. Barreto		
	CPC 24735	*Cyathea delgadii*	Brazil	E. Guatimosim		
	CPC 24736	*Cyathea delgadii*	Brazil	E. Guatimosim		
	CPC 25072	*Cyathea delgadii*	Brazil	R.W. Barreto		
Ch. fungorum	CBS 942.72	*Picea abies*	Sweden	L. Beyer	–	FJ176252
	HKUCC OC0033	–	–	–	–	FJ176251
Ch. holubovae	CCF 3977	–	–	–	FR667221	FR667868
	CCF 3978	–	–	–	FR667222	FR667869

Species	Culture / specimen accession numbers[1]	Host/isolation source	Country	Collector	GenBank accession numbers[2]	
					ITS	LSU
Ch. hyalocuspica	CCF 3975	–	–	–	FR667220	FR667867
	CCF 3976	–	–	–	FR667221	FR667868
Ch. longipes	CCF 3973	–	–	–	FR667213	FR667862
	CCF 3974	–	–	–	FR667214	FR667863
Ch. lygodii	**CPC 24710**	*Lygodium volubile*	Brazil	E. Guatimosim		
	CPC 24699	*Lygodium volubile*	Brazil	R.W. Barreto		
Ch. microspora	CBS 131.74	*Pinus sylvestris*	Netherlands	W. Gams	FR667228	FR667875
	CCF 3980	–	–	–	FR667226	FR667873
Ch. parvispora	CBS 385.94	–	Czech Republic	V. Holubová-Jechová	–	FJ176253
Ch. piceae-abietis	CCF 3982	–	–	–	FR667230	FR667877
Ch. pulchra	HKUCC OC0030	–	–	–	–	FJ176242
Ch. selaginellae	HKUCC OC0011	–	–	–	–	FJ176241
Coccomyces dentatus	AFTOL-ID 147	*Berberis nervosa*	USA	K. Hosaka	DQ491499	AY544657
Co. tumidus	UPS - Lantz 396	*Quercus robur*	Sweden	H. Lantz		HM140510
Cudoniella clavus	BM 18#13	–	–	–	AY789374	AY789373
Cudoniella sp.	ZW 0068	–	–	–	AY789342	AY789341
Hyaloscypha albohyalina var. *monodictys*	TNS-F-5013	unidentified wood	Japan	T. Hosoya	JN033456	JN086756
	KUS-F52652	unidentified wood	Korea	–	JN033426	JN086729
Hya. albohyalina var. spiralis	TNS-F-31133	unidentified wood	Japan	T. Hosoya	AB546941	–
Hya. aureliella	KUS-F52070	unidentified wood	Korea	–	JN033394	JN086697
	M234	–	UK	S. Huhtinen	EU940228	EU940152
Hya. fuckelii	M233	–	UK	Leonard	EU940230	EU940154
Hya. hepaticola	M171	–	Finland	Nieminen	EU940194	EU940118
	M339	–	Finland	Kukkonen	EU940226	EU940150
Hya. leuconica var. bulbopilosa	KUS-F52573	unidentified wood	Korea	–	JN033423	JN086726
Hyaloscypha sp.	TNS-F-17335	unidentified wood	Japan	T. Hosoya	JN033432	JN086735
Hya. vitreola	CBS 127.91	*Sorbus aucuparia*	Finland	S. Huhtinen	JN033378	JN086681
	M39	–	Finland	Söderholm	EU940231	EU940155
Hyphodiscus hymeniophilus	CBS 630.75	decaying wood	Belgium	W. Gams	GU727559	GU727559
	TNS-F-13588	*Betula ermanii*	Japan	T. Hosoya	–	AB546945
Hyp. hymeniophilus	CBS 127.74	*Piptoporus betulinus*	Germany	W. Gams	–	GU727551

Species	Culture / specimen accession numbers[1]	Host/isolation source	Country	Collector	GenBank accession numbers[2]	
					ITS	LSU
	CBS 231.75	decaying bark	Czech Republic	W. Gams	–	DQ227260
	CBS 303.74	stained bark	Netherlands	W. Gams	–	GU727550
	CBS 490.67	*Piptoporus betulinus*	Germany	W. Gams	–	DQ227261
	CBS 687.74	*Quercus pubescens*	France	W. Gams	–	DQ227262
	MUCL 40275	*Prunus spinosa*	Luxemburg	–	–	DQ227258
	CBS 602.77	*Alnus viridis*	Switzerland	P. Raschle	DQ227264	DQ227264
	CBS 886.73	*Piptoporus betulinus*	Netherlands	W. Gams	–	DQ227263
	MUCL 9042 = CBS 335.53	*Betula* sp.	France	F. Mangenot	–	DQ227259
Hyp. otanii	TNS-F-7099	unidentified wood	Japan	T. Hosoya	AB546949	AB546947
Hyp. theiodeus	TNS-F-31803	decaying wood	Japan	–	AB546953	AB546952
Lachnellula arida	CBS 203.66	*Pinus cembra*	Switzerland	E. Müller	KC464635	KC492972
La. calyciformis	CBS 189.66	*Pinus montana*	Italy	E. Müller	KC464636	KC492973
La. flavovirens	CBS 191.66	*Pinus sylvestris*	Switzerland	E. Müller	KC464637	KC492975
La. hyalina	CBS 185.66	*Pinus montana*	Switzerland	C.G. Dharne	KC464638	KC492976
La. occidentalis	CBS 160.35	*Larix decidua*	USA	G.G. Hahn	KC492977	KC464639
	TNS-F-16513	twig	Japan	R. Sasagawa	AB481245	–
	TNS-F-16462	twig	Japan	R. Sasagawa	AB481244	–
La. resinaria	TNS-F-16450	unidentified wood	Japan	R. Sasagawa	AB481246	–
La. subtilissima	CBS 196.66	*Abies alba*	Switzerland	E. Müller	KC464640	KC492978
	CBS 197.66	*Picea abies*	Switzerland	E. Müller	KC464641	KC492979
	TNS-F-16812	twig	Japan	R. Sasagawa	AB481247	–
La. suecica	CBS 268.59	*Larix decidua*	France	E. Müller	KC464642	KC492980
	TNS-F-16529	*Larix kaempferi*	Japan	R. Sasagawa	AB481248	–
La. willkommii	CBS 172.35	–	–	G.G. Hahn	KC464644	KC492982
	CBS 200.66	–	–	E. Müller	KC464645	KC492983
Lachnum abnorme	TNS-F-16617	twig	Japan	R. Sasagawa	AB481250	AB481309
	TNS-F-16582	unidentified wood	Japan	R. Sasagawa	AB481249	–
	KUS-F52080	unidentified wood	–	–	JN033395	JN086698
Lac. brasiliense	HMAS 75520	–	China	–	JF937579	–
	HMAS 78490	–	China	–	JF937580	–
Lac. catarinensis	**CPC 24713**	*Dicksonia sellowiana*	Brazil	E. Guatimosim		
	VIC 42481	*Dicksonia sellowiana*	Brazil	E. Guatimosim		
Lac. catarinense	**CPC 24723**	*Dicksonia sellowiana*	Brazil	E. Guatimosim		

Species	Culture / specimen accession numbers[1]	Host/isolation source	Country	Collector	GenBank accession numbers[2]	
					ITS	LSU
Lac. cf. hyalopus	HMAS 81586	–	China	–	JF937581	–
Lac. pteridophyllum	HMAS 78572	–	China	–	JF937583	–
Lac. pulverulentum	TNS-F-16451	*Pinus densiflora*	Japan	R. Sasagawa	AB481260	AB481295
Lac. rhytismatis	TNS-F-16545	*Symplocos coreana*	Japan	R. Sasagawa	AB481263	–
	TNS-F-16544	*Symplocos coreana*	Japan	R. Sasagawa	AB481264	–
Lac. sclerotii	HMAS 78499	–	China	–	JF937584	–
Lachnum sp.	TNS-F-16838	Leaf of evergreen wood	Japan	R. Sasagawa	AB481280	AB481327
Lachnum sp.	TNS-F-16442	unidentified wood	Japan	R. Sasagawa	AB481270	AB481305
Lac. tricolor	CBS 122000	*Quercus robur*	Germany	H.-O. Baral	KC464643.1	KC492981.1
Lac. varians	**CPC 24742 = COAD 1429**	*Dicksonia sellowiana*	Brazil	P.B. Scwatzburd, A.P. Fortuna		
	TNS-F-17631	*Pteris wallichiana*	Japan	T. Hosoya	AB481267	AB481293
Lac. virgineum	TNS-F-16583	unidentified wood	Japan	R. Sasagawa	AB481268	–
	TNS-F-16588	unidentified wood	Japan	R. Sasagawa	AB481269	–
	HMAS 81601	–	China	–	JF937586	–
	HMAS 81599	–	China	–	AF505518	–
Phaeohelotium geogenum	HB 7222A	*Fagus sylvatica*	Germany	–	KC411992	–

Species	Culture / specimen accession numbers[1]	Host/isolation source	Country	Collector	GenBank accession numbers[2]	
					ITS	LSU
Ph. monticola	HB 8612	*Fagus sylvatica*	Germany	–	KC411991	–
	ILLS 61033	unidentified wood	USA	–	JQ256414	–
Ph. succineoguttulatum	AH 7163	humus under *Eucalyptus*	Spain	–	KC411990	–
	AH 7143	humus under *Eucalyptus*	Spain	–	KC411989	–
Pha. undulatum	AH 7337	humus under *Eucalyptus*	Spain	–	KC411988	–
Psilachnum pteridimi	CPC 24666	*Pteridium arachnoideun*	Brazil	R.W. Barreto	–	–
Psilachnum sp.	KUS-F52448	*Philadelphus schrenckii*	Korea	–	JN033415	–
Ps. staphyleae	KUS-F52105	*Staphylea bumalda*	Korea	–	JN033396	–
Vibrissea flavovirens	MBH 39316	–	–	–	AY789427	–
V. truncorum	CUP-62562	–	USA	–	AY789403	–

[1] AH: Herbarium of the Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CCF: Culture Collection of Fungi, Charles University in Prague, Faculty of Science, Prague, Czech Republic; COAD: Coleção Octávio de Almeida Drumond, Viçosa, Minas Gerais, Brazil; CPC: Culture collection of Pedro Crous, housed at CBS; F : Fundación Medina's Fungal Culture collection; HB: private herbaria of Hans-Otto Baral, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain; HKUCC: The University of Hong Kong culture collection, Hong Kong, Japan; HMAS: Herbarium of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China; KUS: Korea University Herbarium, Seoul, Korea; MUCL: Mycothèque del'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; TNS: National Museum of Nature and Science, Tsukuba, Japan; UPS : Botanical Museum, Uppsala University, Sweden; VIC: Herbário da Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

[2] ITS: internal transcribed spacers and intervening 5.8S nrDNA, LSU: 28S nrRNA gene.

Table 2 Comparison of described *Bloxamia* species

Species	Substrate	Host	Country	Conidiomata		Conidigenous cells		Phialoconidia			Reference
				type	color	feature	size	shape	proliferation	size	
B. bohemica	rotting needles	*Pinus sylvestris*	Czechoslovakia	sporodochial	amber	simple, lageniform, pale brown	8–11 × 1.5–2 µm	cylindrical	catenate	3–5.5 ×1 µm	Minter and Holubová-Jechová (1981)
B. cremea	rotting stems	unknown	Argentina	sporodochial	white to cream	branched, cylindrical, dark brown	24–26 × 2.5–3 µm	cylindrical	long and slimy chains	3–4 ×1–1.5 µm	Arambarri et al (1992)
B. foliicola	living leaves	*Oxyspora paniculata*	China	synnematal	brown	branched, cylindrical, brown	64–95 × 10–11 µm	cubic, with truncate ends	dry chains	6–9 ×5–8 µm	Liu and Zhang (1998)
B. hesterae	submerged litter	*Schoenoplectus tabernaemontani*	Netherlands	sporodochial	opaque to black	simple, lageniform, black	14–24 × 2–3 µm	oblong to clavate	single or in slimy chains	5–6 × 2–3 µm	Spooren (2014)
B. nilagirica	dead twigs	unknown	India	synnematal	brown			rectangular	long and slimy chains	4–5 × 3–3.5 µm	Nag Raj and Kendrick (1975)
B. sanctae-insulae	dead wood	unknown	The United Kingdom	sporodochial	brown to black	simple, lageniform, pale brown	10–14 × 1.5–2.5 µm	globose with tiny hilum	catenate	ca. 2 µm	Coppins and Minter (1980)
B. truncata	decorticated wood	*Ulmus* sp.	England	sporodochial	black	simple, cylindrical to sub–cylindrical, pale brown	15–32 × 2–3 µm	short cylindrical to oblong	single or in easily dispersable chains	2–4 × 1.5–2.5 µm	Pirozynski and Morgan-Jones (1968)
B. cyatheicola	living fronds	*Cyathea* spp.	Brazil	sporodochial	amber to black	branched, sub–culindrical, light brown	17–41 × 1.5–3.5 µm	cylindrical, truncate at both ends	single or in easily dispersable chains	2.5–8 × 1–3 µm	This study

CONCLUSÕES GERAIS

O estudo sistemático de fungos associados à pteridófitas, inédito para o Brasil, resultou em diversas novidades em diversos níveis taxonômicos. Até o presente momento, podem ser listadas 23 novas espécies, a saber: *Bloxamia cyatheicola, Cercospora samambaiae, Chalara cyatheae, Chalara lygodii, Inocyclus angularis, Lachnum catarinense, Lembosia abaxialis, Paramycosphaerella blechni, Paramycosphaerella cyatheae, Paramycosphaerella dicranopteridis-flexuosae, Paramycosphaerella sticheri, Phaeophleospora pteridivora, Psilachnum pteridimi, Pseudocercospora brackenicola, Pseudocercospora paranaensis, Pseudocercospora trichogena, Pseudocercospora serpocaulonicola, Clypeosphaerella sticheri, Rhagadolobiopsis thelypteridis, Xenomycosphaerella alsophilae, Xenomycosphaerella cyatheae, Xenomycosphaerella diplazii* e *Zasmidium cyatheae,* bem como dois novos gêneros: *Clypeosphaerella* e *Rhagadolobiopsis.* Adicionalmente, durante o estudo que visou elucidar o posicionamento evolutivo da ordem *Asterinales*, uma nova família – *Asterotexiaceae* (não relacionada à fungos oriundos de samambaias) – foi proposta, bem como o posicionamento filogenético dos gêrenos *Batistinulla* e *Prillieuxina* foi elucidado.

Cem novas sequências das regiões genômicas ITS e LSU, 57 novas sequências da região genômica ACT, 77 novas sequências da região genômica TEF, 14 novas sequências da região genômica CAL bem como 11 novas sequências da região genômica β-Tub, foram geradas e depositadas no GenBank.

Até o presente momento, cerca de 48 fungos eram conhecidos como associados a pteridófitas no Brasil (Farr & Rossman 2015, Mendes & Urben 2015). O trabalho aqui

realizado, acrescentou 23 espécies a este total, aumentando de forma significativa este número e fornecendo novas informações moleculares que podem ser úteis para uma melhor compreensão da evolução dos grupos de fungos apresentados.

A presente pesquisa indica claramente o valor científico de estudos de microfungos focados em determinado grupo de plantas hospedeiras, como fonte de novidades micológicas. Ela também confirma que micologistas e fitopatologistas nos trópicos ainda têm dado pouca atenção aos fungos em hospedeiros vegetais que têm limitada relevância econômica, como é o caso das samambaias.

Fungos de pteridófitas no Brasil e em outras regiões tropicais, parecem representar uma parte importante de uma micobiota altamente diversificada, a qual ainda aguarda ser descoberta.