BRUNO WESLEY FERREIRA

TAXONOMIC PLACEMENT OF SELECTED PLANT PATHOGENIC FUNGI REVISITED: *Acroconidiella*, *Ceratobasidium lantanae-camarae*, *Duosporium*, *Korunomyces* AND Perisporiopsidaceae

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*.

Orientador: Robert Weingart Barreto

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Assentimento:

Bruno Wesley Ferreira
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Aos meus pais, Vera Lúcia e José Antônio

Ao meu irmão Elielson

Aos meus amigos e familiares.

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“Sabio é todo aquele que, através do relativo conhecimento que possui, identifica a ignorância de que ainda é portador”.

(Marco Prisco)

RESUMO

FERREIRA, Bruno Wesley, D.Sc., Universidade Federal de Viçosa, março de 2021. **Posicionamento taxonômico de selecionados fungos fitopatogênicos revisitados: *Acroconidiella*, *Ceratobasidium lantanae-camarae*, *Duosporium*, *Korunomyces* e Perisporiopsidaceae.** Orientador: Robert Weingart Barreto.

Desde os primórdios da micologia, a classificação dos fungos baseou-se principalmente em caracteres morfológicos, com substrato, distribuição geográfica, informações quimiotaxonômicas ou ecológicas, desempenhando um papel complementar para alguns grupos. Isso, às vezes, resultava em sistemas provisórios ou duvidosos de classificação e em afinidades incorretamente assumidas e na aplicação equivocada de nomes a táxons de fungos. Com o advento da biologia molecular e sua incorporação na abordagem polifásica da taxonomia de fungos, tornou-se progressivamente possível elucidar melhor as relações entre os membros do Reino Fungi e construir um sistema de classificação mais confiável e natural desses organismos. Uma reavaliação taxonômica de táxons selecionados de fungos fitopatogênicos, variando de espécies a famílias, foi conduzida com base em espécimes coletados no Brasil. A obtenção de culturas puras desses fungos permitiram a extração e o sequenciamento de regiões do DNA e resolver a questão-chave das afinidades filogenéticas e a aplicação correta de nomes para esses fungos. Entre os espécimes coletados durante este estudo, alguns foram selecionados e indicados como neótipos ou epitipos para táxons erigidos no passado. Foi realizado o reexame das características morfológicas, assim como a obtenção de informações moleculares, possibilitando, pela primeira vez, a aplicação da abordagem polifásica a esses táxons. Como resultado, os seguintes táxons foram "desmascarados": família Perisporiopsidaceae (considerada um sinônimo de Phaeosphaeriaceae); os gêneros *Acroconidiella* (considerado um sinônimo tardio de *Alternaria*), *Duosporium* (sin. de *Curvularia*) e *Korunomyces* (sin. de *Coniella*). *Ceratobasidium lantanae-camarae*, foi considerada distinta de *C. cornigerum* - para a qual foi erroneamente sinonimizada - e reintegrada. Novidades taxonômicas foram tratadas, conforme determinado pelas presentes regras nomenclaturais, para publicação.

Palavras-chave: Filogenia. Fungos. Taxonomia.

ABSTRACT

FERREIRA, Bruno Wesley, D.Sc., Universidade Federal de Viçosa, March, 2021. **Taxonomic placement of selected plant pathogenic fungi revisited: *Acroconidiella*, *Ceratobasidium lantanae-camarae*, *Duosporium*, *Korunomyces* and Perisporiopsidaceae**. Adviser: Robert Weingart Barreto.

Since the early days of mycology, the classification of fungi has been based mainly on morphological characters, with substrate, geographical distribution, chemotaxonomic or ecological information playing a complementary role for some groups. This, sometimes, resulted in provisional or dubious sytems of classification and to incorrectly assumed affinities and mistaken application of names to fungal taxa. With the advent of molecular biology and its incorporation in the polyphasic approach for the taxonomy of fungi, it became progressively possible to better elucidate the relationships between members of the Kingdom Fungi and to erect a more reliable and natural system of classification of these organisms. A taxonomic reassessment of selected taxa of plant pathogenic fungi, ranging from species to families, was conducted based on specimens recollected in Brazil. Obtaining pure cultures of these fungi allowed for extraction and sequencing of DNA regions and to address the key issue of the phylogenetic affinities and correct application of names for those fungi. Among the specimens recollected during this study some were selected and indicated as neotypes or epitypes for taxa which were erected in the past. The reexamination of morphological features was carried out, as well as the obtaining of molecular information, enabled, for the first time, the application of the polyphasic approach to these taxa. As a result, the following taxa were “debunked”: family Perisporiopsidaceae (considered a synonym of Phaeosphaeriaceae); the genera *Acroconidiella* (found to be a late synonym of *Alternaria*), *Duosporium* (syn. of *Curvularia*) and *Korunomyces* (syn. of *Coniella*). *Ceratobasidium lantanae-camarae*, was found to be distinct from *C. cornigerum* – to which it was mistakenly synonymized – and reinstated. Taxonomic novelties were treated, as determined by the present nomenclatural rules, for publication.

Keywords: Phylogeny. Fungi. Taxonomy.

SUMÁRIO

1. INTRODUÇÃO

Os fungos compreendem um amplo grupo de microrganismos com diversos estilos de vida, incluindo saprófitas, simbiontes, patógenos de plantas e animais (Carris et al., 2012; Kuo et al., 2014; Bunney et al., 2017; Muszewska et al., 2017). Eles são o segundo grupo de organismos mais rico em espécies depois dos insetos (Purvis e Hector 2000). Estimativas recentes indicam a existência de aproximadamente 12 milhões de espécies de fungos no mundo (Wu et al. 2019). Dessas, pouco mais de 120.000 espécies foram nomeadas (Hawksworth & Lücking 2017).

No passado, a classificação de fungos baseou-se principalmente em caracteres morfológicos e ecológicos (Guarro et al. 1999). Isso resultou em muitas classificações incertas e provisórias ou na aplicação incorreta de nomes. Até a década passada, por exemplo, dar nomes científicos diferentes a fases distintas (sexuada e assexuada) de uma mesma espécie de fungo, baseando-se nas diferenças entre os esporos, iniciada no século IX (Fuckel 1870, Saccardo 1987) era prática aceita pelas regras de nomenclatura vigentes. No caso de fungos fitopatogênicos, um peso excessivo dado a supostas especializações na relação patógeno-hospedeiro, causou muitos erros na aplicação de nomes, resultando numa profusão de sinônimos para fungos, por vezes simplesmente polífagos, por exemplo, *Cercospora* (Chupp 1953).

Nos últimos anos, o reconhecimento de espécies filogenéticas de concordância genealógica (genealogical concordance phylogenetic species recognition – GCPSR), que é uma adaptação do conceito de espécie filogenética, tornou-se um método comum empregado por micologistas e fitopatologistas para distinguir espécies (Taylor et al. 2000). O uso da abordagem polifásica para identificar espécies combinando dados morfológicos, ecológicos e filogenéticos forneceram a base para a moderna classificação e taxonomia de fungos, e têm sido amplamente utilizadas para descrever novas espécies, reexaminar taxas já conhecidos, ou para o estudo das relações evolutivas entre diferentes grupos de

fungos (Hyde et al 2014, Groenewald et al. 2013, Woudenberg et al. 2013, Lombard et al. 2019, Wijayawardene et al. 2020).

Os dados moleculares também nos permitiram relacionar formas assexuadas e sexuadas do mesmo fungo (Taylor 2011), bem como elucidar a identidade de espécies crípticas - morfoespécies que representam um conjunto de táxons indistinguíveis ou quase indistinguíveis que são claramente diferentes com base em inferência filogenética e que muitas vezes também diferem em ecologia (incluindo gama de hospedeiros e patogenicidade) ou distribuição. Muitas dessas espécies receberam o mesmo nome no passado, embora sejam espécies filogeneticamente diferentes (Groenewald et al. 2013, Damm et al. 2012 a, Damm et al. 2012 b) . A identificação e nomeação dessas espécies crípticas são complicadas, sobretudo, pela falta de material ex-tipo vivo para servir como ponto de referência básico para inferência filogenética. (Lombard et al. 2020).

O grande problema com a maioria dos estudos iniciais é que eles se concentraram em técnicas e utilizaram materiais ou isolados de fungos que na maioria dos casos não foram cuidadosamente referenciados, e em um número preocupante de casos, foram erroneamente nomeados. A maioria das espécies clássicas, particularmente de microfungos, não são representadas por material tipo adequado, que podem, inclusive, servir como fontes de DNA para estudos filogenéticos ou para desenvolver sistemas de identificação robustos. As classificações naturais de fungos, portanto, sofrem com a falta de isolados de referência nas árvores filogenéticas resultantes (Crous et al. 2015). Em muitos casos, apesar de haver um tipo designado, o material encontra-se em péssimo estado de conservação, foram perdidos, ou destruídos.

Em determinadas situações, a neotipificação ou a epitipificação podem resolver esse problema (Hyde & Zhang 2008, Ariyawansa 2014). Esse expediente está sendo cada vez mais usado para resolver a confusão taxonômica e estabilizar a aplicação de nomes para espécies, gêneros, famílias ou ordens de fungos (Groenewald et al. 2013, Woudenberg et al. 2013, Lombard et al. 2019). No entanto, a missão de recoletar no seu habitat natural um microfungo, descrito por um autor do passado distante, a partir de coletas feitas por naturalistas em um mundo muito

menos impactado pela atividade humana, pode ser dispendiosa e desafiadora, senão impossível. Muitas vezes recoleta, epitificação e aplicação do procedimento polifásico em materiais representativos de taxa fúngicos descritos no passado, depende de sorte e oportunidade.

O presente estudo representa o resultado de alguns “aproveitamentos de oportunidades” para o avanço da taxonomia de microfungos fitopatogênicos. Ele é composto de uma reavaliação taxonômica de algumas espécies, gêneros e de uma família, propostos antes do advento da filogenia molecular e sua aplicação na taxonomia de fungos. Assim, espécimes foram recoletados e neótipos e epitipos foram indicados a partir desses materiais para amparar os resultados da análise polifásica feita para a família Perisporiopsidaceae, os gêneros *Acroconidiella*, *Duosporium*, *Korunomyces* e a espécie *Ceratobasidium lantanae-camarae*.

2. ESTRUTURA DA TESE

A pesquisa apresentada nesta tese se relaciona a vários aspectos taxonômicos de microfungos agentes causais de doenças em plantas cultivadas ou não. A saber: *Acroconidiella tropaeoli* – patógeno de chagas (*Tropaeolum majus*); *Duosporium yamadanum* – patógeno da tiririca (*Cyperus rotundus*), *Korunomyces prostratus* – patógeno de *Miconia calvescens*, *Ceratobasidium lantanae-camarae* – patógeno do cambará (*Lantana camara*), *Perisporiopsis lantanae* – idem e *Perisporiopsis struthanthi* em erva-de-passarinho (*Struthanthus* sp.). Esses estudos foram desencadeados a partir da recoleta no Brasil de materiais representativos de cada espécie, gênero ou família tratados.

ARTIGOS DA TESE

ARTIGO 1: Debunking *Acroconidiella*

Acroconidiella foi proposta para acomodar *Acroconidiella tropaeoli*, uma espécie de fungo que causa manchas nas folhas do *Tropaeolum majus*. Embora o fungo tivesse semelhanças com *Alternaria*, não apresentava conídios muriformes formados em cadeias. Observações mais recentes de *A. tropaeoli* em cultura

formando cadeias conidiais acropetais e o reconhecimento de várias espécies não dictioconidiais como pertencentes a *Alternaria*, levaram a uma reavaliação do gênero, começando com o reexame da espécie-tipo. Amostras de *A. tropaeoli*, e também de *Acroconidiella trisepta*, foram coletadas no Brasil, e um estudo envolvendo a análise de sua morfologia, em microscopia de luz e MEV, e uma análise filogenética molecular foram realizados.

ARTIGO 2: Debunking *Duosporium*

Duosporium é um gênero monotípico que inclui apenas a espécie-tipo *Duosporium yamadanum*, que foi tratada na literatura como "Pezizomycotina anamórfico". No entanto, isso é apenas uma conjectura, uma vez que suas verdadeiras afinidades filogenéticas permanecem desconhecidas. Este fungo é conhecido por causar manchas nas folhas em vários membros de *Cyperus* spp. Foi intensamente investigado na década de 1990 como um candidato potencial para uso como agente de biocontrole inundativo contra tiririca - uma importante erva daninha tropical. Sua morfologia foi reconhecida, como algo próximo a de *Curvularia* e *Bipolaris*. No entanto, foi mantido em um gênero separado com base em duas características morfológicas distintas: produção de dois tipos de esporos e macroconídios retos. Nenhum estudo molecular foi feito para elucidar a localização do *Duosporium*. Aqui, uma análise filogenética multilocus foi construída para mostrar o posicionamento filogenético do gênero e detalhes morfológicos adicionais foram registrados e adicionados à descrição original do fungo.

ARTIGO 3: Debunking *Korunomyces*

Korunomyces é um pequeno gênero erigido para incluir fungos que produzem estruturas reprodutivas assexuadas multicelulares estipuladas, ramificadas (propágulos) em folhas e em cultura. Três espécies são aceitas no gênero: *Korunomyces terminaliae* - a espécie-tipo, *K. zapatensis* e *K. prostratus*. Nenhum estudo molecular foi feito para elucidar a colocação filogenética de *Korunomyces*. Nesse trabalho, um estudo filogenético foi realizado a partir de sequencias de DNA de *K. terminaliae* e *K. prostratus*. Características morfológicas adicionais foram relatadas e o posicionamento taxonômico do Gênero foi esclarecido.

ARTIGO 4: Reinstating *Ceratobasidium lantanae-camarae* the white thread blight fungus on the pantropical weed *Lantana câmara*

Ceratobasidium lantanae-camarae foi um nome proposto para um fungo associado à doença queima-do-fio em *Lantana camara*: uma planta nativa brasileira listada entre as dez piores plantas daninhas do mundo. Este fungo foi encontrado durante levantamentos no Brasil - e posteriormente, descrito como uma nova espécie - para avaliar o potencial de patógenos fúngicos co-evoluídos como agentes de biocontrole clássicos nos Paleotrópicos e na Australásia. Posteriormente, em uma monografia sobre fungos *Rhizoctonia*-like, *C. lantanae-camarae* foi sinonimizada com *C. cornigerum*: uma espécie polífaga registrada em todo o mundo, mas nunca anteriormente na América Latina. A falta de culturas puras de *C. lantanae-camarae* não permitiu a verificação do verdadeiro status do fungo da queima-do-fio em *Lantana câmara*. Neste estudo isolados de *C. lantanae-camarae* foram coletados no Rio de Janeiro e Amazonas. Culturas puras foram obtidas, DNA foi extraído e análises filogenéticas foram realizadas visando esclarecer a verdadeira identidade do fungo em *L. camara*.

ARTIGO 5: Debunking Perisporiopsidaceae

A família Perisporiopsidaceae inclui espécies com ascomas superficiais, com micélio circundantes, ascósporos hialinos, oblongos a elipsoidais, com um ou mais septos. A taxonomia de suas espécies foi baseada, principalmente, em caracteres morfológicos e hospedeiro. Há informações limitadas sobre sua posição taxonômica em Dothideomycetes. Aqui, uma avaliação da posição filogenética de Perisporiopsidaceae é fornecida com base em uma análise filogenética multilocus de dados de sequência obtidos de material fresco de espécies selecionadas coletadas no Brasil. Três espécies foram incluídas: *Perisporiopsis struthanthi*, que é a espécie-tipo para o género-tipo de Perisporiopsidaceae, *Perisporiopsis lantanae* e *Perisporiopsis lateritia*.

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4. ARTIGOS DA TESE

4.1. ARTIGO 1

Debunking *Acroconidiella*

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ORIGINAL ARTICLE

Debunking *Acroconidiella*

Bruno W. Ferreira [1] · Robert W. Barreto [1]

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Abstract

Acroconidiella was proposed to accommodate *Acroconidiella tropaeoli*, a fungal species causing leaf spots on *Tropaeolum majus*. At the time, it was recognized as deserving to be treated as a distinct genus because, although being somewhat similar to *Alternaria*, it did not present muriform conidia formed in chains. More recent observations of *A. tropaeoli* in culture forming acropetal conidial chains, and the recognition of several non-dictioconidial species as belonging to *Alternaria*, prompted a reappraisal of the genus, starting with the re-examination of the type species. Samples of *Acroconidiella tropaeoli*, and also of *Acroconidiella trisepta*, were recollected in Brazil, and a study involving an analysis of their morphology, under light microscopy and SEM, and a molecular phylogenenetic analysis was performed. A multi-gene phylogeny, including the large subunit of the nrDNA (nc LSU rDNA), internal transcribed spacer (ITS) region, translation elongation factor 1-α (*EF1*), and polymerase II second largest subunit (*RPB2*), placed *A. tropaeoli* within *Alternaria*, close to *A. sonchi* and *A. cinerariae*. The ITS and nc LSU rDNA phylogenetic study of *A. trisepta* placed it within *Dendryphiella*. The new combination *Dendryphiella trisepta* comb. nov is proposed to accommodate *A. trisepta.* Nevertheless, the new name *Alternaria obtusa* is proposed for *Acroconidiella tropaeoli* since it could not be recombined into *Alternaria tropaeoli* because this name is already in use for another valid (and distinct) species in this genus described from India. This study showed that *Acroconidiella* is an artificial genus which is now rejected, since its type species belongs to *Alternaria*—which has nomenclatural priority over *Acroconidiella*. Other species placed in *Acroconidiella*, given below, await reappraisal in order to determine their correct taxonomic affinity.

Keywords *Alternaria* · *Dendryphiella* · Multi-gene phylogeny · New taxa · Reappraisal · Taxonomy

Introduction

The genus *Acroconidiella* was proposed by Lindquist and Alippi (1964) to accommodate fungi with macronematous, mononematous, simple, or occasionally branched conidiophores with integrated, terminal, polytretic sympodial conidiogenous cells bearing solitary ellipsoidal, septate echinulate conidia (Ellis 1971). Baker (1947) made the first report of a disease in *Tropaeolum majus* (garden nasturtium—chagas, in Brazil) in California and considered that it was caused by a fungus belonging to *Heterosporium*, but without giving it a name. In the same year, Bond (1947) independently described the fungus on *Tropaeolum majus* in Ceylon (Sri Lanka) naming it *Heterosporium tropaeoli*. De Vries (1952), considered that *Heterosporium* should be regarded as analogous to *Cladosporium*. However, the fungus described as *Heterosporium tropaeoli* has morphological features which were recognized as clearly distinct from members of *Cladosporium*. The fungus on garden nasturtium has porospores instead of blastospores—typical of *Cladosporium*—and a scar morphology which is also distinct from those of *Cladosporium*. Lindquist mentioned a personal communication made to him by M. B. Ellis (Lindquist and Alippi 1964) "*H. tropaeoli* is closer to *Alternaria*, than to *Curvularia* and *Cladosporium*". However, this fungus did not present muriform conidia in chains, thought to be diagnostic for *Alternaria*, at the time, and the conidia did not have its central cells larger than the apical cells, accepted as key for *Curvularia* at the time [now much changed after the works of Berbee et al. 1999 and Manamgoda et al. 2012]. Based on Ellis' views, Lindquist and Alippi (1964) proposed a new genus *Acroconidiella* to accommodate this fungus and designated it *Acroconidiella tropaeoli*.

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Presently, there are five species in the genus *Acroconidiella*: *A. eschscholtziae*, *A. indicus*, *A. manoharacharii*, *A. tropaeoli*, and *A. trisepta* (Lindquist and Alippi 1964; Ellis 1971, 1976; Muchovej 1980; Prasher and Verma 2015).

The lack of molecular studies for any of the species in this genus and the observations made by Vieira and Barreto (2002), particularly the *in vitro* production of short acropetal conidial chains, prompted a reappraisal of *Acroconidiella*. This was based on freshly collected material of two members of *Acroconidiella*, namely *Acroconidiella tropaeoli* and *Acroconidiella trisepta*.

Materials and methods

Sample collection processing and observation of fungus morphology

Samples of diseased foliage of *Tropaeolum majus* and dead branches of *Glycine max* were collected, the former from the original host of *Acroconidiella tropaeoli*, but the latter both from the substrate plant species and exactly from the type locality (Viçosa, state of Minas Gerais, Brazil). These were screened under a stereomicroscope, and parts of the samples bearing sporulating colonies of the fungi were selected and dried in a plant press. Fungal structures were scraped from the sample surface with a scalpel and mounted in lactophenol and lactofuchsin. Observations were made with an Olympus BX53 adapted with differential contrast lighting and equipped with digital capture system (Olympus Q-Color 3™). Representative specimens were deposited in the local herbarium (Herbarium Universidade Federal de Viçosa, VIC).

Isolations were performed by aseptic transfer of conidia from the leaf surfaces onto potato dextrose-agar (PDA) plates with a sterile fine-pointed needle. Culture descriptions were based on the observation of 7-day-old (*A. tropaeoli*) and 14-day-old (*A. trisepta*) colonies formed in plates containing either potato dextrose-agar (PDA) or potato carrot-agar (PCA), maintained at 25 °C under a 12-h daily/light regime (light provided by two white and one near-UV lamps placed 35 cm above the plates). The color terminology followed Rayner (1970).

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 analyze and generate images from the samples.

DNA isolation

Total genomic DNA was extracted from 7-day-old cultures formed on PDA 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 large subunit of the nrDNA (nc LSU rDNA) and internal transcribed spacer (ITS) regions from each fungus included in the study were sequenced with the primers LSU1Fd (Crous et al. 2009) and LR5 (Vilgalys and Hester 1990) and IT5 + ITS4 (White et al. 1990), respectively. For *Acroconidiella tropaeoli*, two additional loci, polymerase II second largest subunit (*RPB2*) and translation elongation factor 1-α (*TEF1*), were amplified and sequenced with the primer pairs RPB2-5F2 (Sung et al. 2007) and fRPB2-7R (Liu et al. 1999) and EF1-728F + EF1-986R (Carbone and Kohn 1999). PCR amplifications were performed in a total volume of 12.5 μL 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, and 1.0 U BioTaq DNA polymerase (Bioline GmbH Luckenwalde, Germany). Conditions for PCR amplification consisted of an initial denaturation step of 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 48 °C, and 90 s at 72 °C for nc LSU rDNA, ITS and 40 cycles of 30 s at 94 °C, 30s at 52 °C/59 °C and 45 s at 72 °C for *TEF1*, and a final elongation step of 7 min at 72 °C. The partial *RPB2* gene was obtained by using a touchdown PCR protocol of 5 cycles of 45 s at 94 °C, 45 s at 60 °C, and 2 min at 72 °C, followed by 5 cycles with a 58 °C annealing temperature and 30 cycles with a 54 °C annealing temperature. Amplicons were analyzed on 0.8% agarose electrophoresis gels stained with GelRed (InstantAgarose) in a 1× TAE buffer and visualized under UV light to check for amplification size and purity. PCR products were purified and sequenced by Macrogen Inc. (http://www.macrogen.com).

Phylogenetic analysis

DNA consensus sequences were generated and imported into MEGA v. 6 (Tamura et al. 2013) for initial alignment and the construction of sequence datasets. Sequences obtained from the GenBank (www.ncbi.nlm. nih.gov) and the novel sequences generated on this study were aligned and edited using DNA Dragon program (http://www.dna-dragon.com\index.php) (Table 1).

Bayesian inference analyses were conducted, and the best-fit evolutionary model was determined by comparing different evolutionary models via the Akaike information criterion using PAUP (version 4.0b10, Sinauer Associates) and MrModeltest 2.2 (Nylander 2004). 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

Table 1 Taxa and collections used for multi-gene phylogenetic analyses in this study

Species name	Strain number	GenBank accession numbers			
		nc LSU rDNA	*RPB2*	ITS	*TEF1*
Alternaria alternata	CBS 916.96*	DQ678082	KC584375	AF347031	KC584634
Alternaria alternantherae	CBS 124392	KC584251	KC584374	KC584179	KC584633
Alternaria anigozanthi	CBS 121920*	KC584252	KC584376	KC584180	KC584635
Alternaria argyranthemi	CBS 116530*	KC584254	KC584378	KC584181	KC584637
Alternaria armoraciae	CBS 118702*	KC584255	KC584379	KC584182	KC584638
Alternaria avenicola	CBS 121459*	KC584256	KC584380	KC584183	KC584639
Alternaria brassicae	CBS 116528	KC584258	KC584382	KC584185	KC584641
Alternaria brassicicola	CBS 118699	KC584259	KC584383	JX499031	KC584642
Alternaria calycipyricola	CBS 121545*	KC584260	KC584384	KC584186	KC584643
Alternaria capsici-annui	CBS 504.74	KC584261	KC584385	KC584187	KC584644
Alternaria chlamydospora	CBS 491.72*	KC584264	KC584388	KC584189	KC584647
Alternaria cinerariae	CBS 116495	KC584265	KC584389	KC584190	KC584648
Alternaria conjuncta	CBS 196.86*	KC584266	KC584390	FJ266475	KC584649
Alternaria cumini	CBS 121329*	KC584267	KC584391	KC584191	KC584650
Alternaria dianthicola	CBS 116491	KC584270	KC584394	KC584194	KC584653
Alternaria ellipsoidea	CBS 119674*	KC584272	KC584396	KC584196	KC584655
Alternaria eryngii	CBS 121339	KC584273	KC584397	JQ693661	KC584656
Alternaria ethzedia	CBS 197.86*	KC584274	KC584398	AF392987	KC584657
Alternaria gaisen	CBS 632.93	KC584275	KC584399	KC584197	KC584658
Alternaria geniostomatis	CBS 118701*	KC584276	KC584400	KC584198	KC584659
Alternaria gypsophilae	CBS 107.41*	KC584277	KC584401	KC584199	KC584660
Alternaria helianthiinficiens	CBS 117370	KC584278	KC584402	KC584200	KC584661
	CBS 208.86*	KC584279	KC584403	JX101649	EU130548
Alternaria infectoria	CBS 210.86*	KC584280	KC584404	DQ323697	KC584662
Alternaria japonica	CBS 118390	KC584281	KC584405	KC584201	KC584663
Alternaria leucanthemi	CBS 421.65*	KC584347	KC584472	KC584240	KC584732
	CBS 422.65	KC584348	KC584473	KC584241	KC584733
Alternaria limaciformis	CBS 481.81*	KC584283	KC584407	KC584203	KC584665
Alternaria longipes	CBS 540.94	KC584285	KC584409	AY278835	KC584667
Alternaria mimicula	CBS 118696*	KC584287	KC584411	FJ266477	KC584669
Alternaria molesta	CBS 548.81*	KC584288	KC584412	KC584205	KC584670
Alternaria mouchaccae	CBS 119671*	KC584289	KC584413	KC584206	KC584671
Alternaria nepalensis	CBS 118700*	KC584290	KC584414	KC584207	KC584672
Alternaria oregonensis	CBS 542.94*	KC584292	KC584416	FJ266478	KC584674
Alternaria panax	CBS 482.81	KC584293	KC584417	KC584209	KC584675
Alternaria petroselini	CBS 112.41*	KC584295	KC584419	KC584211	KC584677
Alternaria photistica	CBS 212.86*	KC584296	KC584420	KC584212	KC584678
Alternaria porri	CBS 116698	KC584297	KC584421	DQ323700	KC584679
Alternaria radicina	CBS 245.67	KC584299	KC584423	KC584213	KC584681
Alternaria selini	CBS 109382*	KC584302	KC584426	AF229455	KC584684
Alternaria septorioides	CBS 106.41*	KC584303	KC584427	KC584216	KC584685
Alternaria solani	CBS 116651	KC584306	KC584430	KC584217	KC584688
Alternaria soliaridae	CBS 118387*	KC584307	KC584431	KC584218	KC584689
Alternaria solidaccana	CBS 118698*	KC584308	KC584432	KC584219	KC584690
Alternaria sonchi	CBS 119675	KC584309	KC584433	KC584220	KC584691
Alternaria tenuissima	CBS 918.96	KC584311	KC584435	AF347032	KC584693
Alternaria thalictrigena	CBS 121712*	KC584312	KC584436	EU040211	KC584694

Table 1 (continued)

Species name	Strain number	GenBank accession numbers			
		nc LSU rDNA	*RPB2*	ITS	*TEF1*
Alternaria triglochinicola	CBS 119676*	KC584313	KC584437	KC584222	KC584695
Alternaria obtusa	COAD 2389*	**MK277356**	**MK317924**	**MK278897**	**MK981886**
	COAD 2799	**MK968119**	**MK988439**	**MK968121**	**MK981887**
Alternaria vaccariae	CBS 116533	KC584314	KC584438	KC584223	KC584696
Aquaticheirospora lignicola	RK 2006a*	AY736378	–	AY864770	–
Dendryphiella eucalyptorum	CPC 22927*	KJ869196	–	KJ869139	–
Dendryphiella fasciculata	MFLUCC 17–1074*	MF399214	–	MF399213	–
Dendryphiella paravinosa	CPC 26176*	KX228309	–	KX228257	–
Dendryphiella trisepta	COAD 2388*	**MK277357**	–	**MK278898**	–
Dendryphiella vinosa	NBRC 32669		–	EU848590	–
Dictyocheirospora aquatica	KUMCC 15–0305*	KY320513	–	KY320508	–
Dictyocheirospora bannica	KH 332*	AB807513	–	LC014543	–
Dictyocheirospora garethjonesii	MFLUCC 16–0909*	KY320514	–	KY320509	–
Dictyocheirospora pseudomusae	KH 412	AB807516	–	LC014549	–
Dictyocheirospora pseudomusae	yone 234*	AB807520	–	LC014550	–
Dictyocheirospora rotunda	MFLUCC 14–0293*	KU179100	–	KU179099	–
Dictyosporium alatum	ATCC34953*	DQ018101	–	NR077171	–
Dictyosporium elegans	NBRC 32502*	DQ018100	–	DQ018087	–
Dictyosporium olivaceosporum	KH 375*	AB807514	–	LC014542	–
Dictyosporium sexualis	MFLUCC 10–0127*	KU179106	–	KU179105	–
Dictyosporium stellatum	CCFC 241241*	JF951177	–	JF951154	–
Dictyosporium thailandicum	MFLUCC 13–0773*	KP716707	–	KP716706	–
Digitodesmium bambusicola	CBS 110279*	DQ018103	–	DQ018091	–
Gregarithecium curvisporum	KT 922*	AB807547	–	AB809644	–
Jalapriya inflata	NTOU 3855	JQ267363	–	JQ267362	–
Jalapriya pulchra	MFLUCC 15–0348*	KU179109	–	KU179108	–
Jalapriya toruloides	CBS 209.65	DQ018104	–	DQ018093	–
Paradendryphiella arenariae	CBS 181.58*	KC793338	–	KF156010	–
Paradendryphiella salina	CBS 142.60	KC793339	–	DQ411540	–
Pseudocoleophoma calamagrostidis	KT 3284*	LC014609	–	LC014592	–
Pseudocoleophoma polygonicola	KT 731*	AB807546	–	AB809634	–
Pseudocoleophoma typhicola	MFLUCC 16–0123*	KX576656	–	KX576655	–
Pseudodictyosporium elegans	CBS 688.93*	DQ018106	–	DQ018099	–
Pseudodictyosporium wauense	NBRC 30078	DQ018105	–	DQ018098	–
Stemphylium herbarum	CBS 191.86	GU238160	KC584471	KC584239	KC584731
	BRIP 65181	–	KY009907	KY009903	KY009905
Stemphylium botryosum	CBS 714.68	–	AF107804	KC584238	KC584729

Sequences produced in the present study are in bold. The other sequences are from Woudenberg et al. (2013) and Liu et al. (2017) except those of *Stemphylium herbarum* and *Stemphylium botryosum* from Moslemi et al. (2017)

Ex-type strains are indicated with "*" after collection number. nc LSU rDNA = large subunit of the nrDNA, *RPB2* = polymerase II second largest subunit, ITS = internal transcribed spacer, *TEF1* = translation elongation factor 1-α

10,000 trees were obtained. The first 2000 trees representing the burn-in phase were discarded, whereas the remaining 8000 trees were used for calculating the posterior probabilities. Bayesian posterior probabilities are presented on the left of each node. The analysis was hosted by CIPRES Science Gateway portal at San Diego Supercomputer Center (Miller et al. 2010). Phylogenetic trees were visualized with the program FigTree v1.3.1 (Rambaut 2009).

For maximum parsimony (MP), analyses were conducted using PAUP v. 4.0b10 (Swofford 2003) with phylogenic

relationships estimated by heuristic searches with 1000 random stepwise addition sequences and tree bisection and reconstruction (TBR) branch swapping. Alignment gaps were treated as missing data, and all characters were weighted equally. Measures calculated for parsimony included tree length (TL), retention index (RI), consistency index (CI), rescaled consistency index (RC), and homoplasy index (HI). Statistical support for branch nodes in the most parsimonious trees was obtained by performing 1000 bootstrap replicates.

Maximum likelihood (ML) tree was generated with the nearest-neighbor-interchange (NNI) ML heuristic method and the Tamura-Nei substitution model as tree inference options in MEGA. The branch stabilities of the phylogenetic tree were assessed by using the bootstrap re-sampling strategy with 1000 bootstrap test replicates. The resulting tree topologies using the three methods (MP, ML, and BI) were then compared, and the phylogram was edited with CorelDRAW Graphics Suite 2017.

Sequences of *Stemphylium herbarum* (CBS 191.86 and BRIP 65181) and *Stemphylium botryosum* (CBS 714.68) were used as the outgroups in the *Alternaria* phylogeny, and sequences of *Paradendryphiella arenariae* (CBS 181.58) and *Paradendryphiella salina* (CBS 142.60) were used in the *Dendryphiella* phylogeny. Sequences derived in this study were lodged in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1). The alignment and tree were deposited in TreeBASE (http://www.treebase.org) (study numbers S24915 and S24916) and taxonomic novelties in MycoBank (www.MycoBank.org).

Results

Phylogeny

Phylogenetic analysis using the ITS, nc LSU rDNA, *RPB2*, and *TEF1* regions were based on 51 *Alternaria* strains, 2 isolates of *Acroconidiella tropaeoli*, and 3 outgroup sequences (Fig. 1). The combined alignment was comprised of 2731 characters with gaps (625 for ITS, 852 for nc LSU rDNA, 865 for *RPB2*, and 389 for *TEF1*). The phylogenetic analyses generated by maximum parsimony, maximum likelihood (ML), and Bayesian analysis indicate that *Acroconidiella tropaeoli* grouped within the genus *Alternaria* and formed a monotypic lineage near sect. *Sonchi* with a well-supported clade (99%/100%/1.00, MP/ML/BI supports, respectively).

Another phylogenetic analysis was performed using the ITS and nc LSU rDNA loci to clarify the phylogenetic position of *Acroconidiella trisepta* (Fig. 2). The alignment of combined ITS and nc LSU rDNA sequence data comprised the total of 2168 characters with gaps (772 for ITS, 1396 for nc LSU rDNA). The dataset comprised 30 strains including 1 newly sequenced taxon and 2 outgroup sequences, *Paradendryphiella arenariae* and *P. salina* (Pleosporaceae). The phylogenetic analyses generated by maximum parsimony (MP), maximum likelihood (ML), and Bayesian analysis indicate that *Acroconidiella trisepta* belongs within the genus *Dendryphiella* with high support (100%/98%/1.00, respectively). The isolate of *Acroconidiella trisepta* clustered together with 2 other *Dendryphiella* species. Additionally, *Acroconidiella trisepta* formed a distinct lineage and was a sister to a strain of *D. eucalyptorum*.

Taxonomy

Alternaria Nees, Syst. Pilze (Würzburg): 72 (1816)

≡ Syn. nov. *Acroconidiella* J.C. Lindq. & Alippi, Darwiniana 13: 612, 1964

Alternaria obtusa **B.W. Ferreira & R.W. Barreto**, nom. nov. (Fig. 3).

MycoBank: MB832351

Etymology: having blunt/rounded-ended conidia

≡ *Heterosporium tropaeoli* Bond, Ceylon Journal of Science 12: 185, 1947

≡ *Acroconidiella tropaeoli* (Bond) Lindquist & Alippi, Darwiniana 13: 613, 1964

On fruits or, most abundantly, on leaves forming irregular to subcircular brownish or purple amphigenous spots, up to 1 cm diameter coalescing and leading to the blight of large areas of the leaf with yellow periphery. Colonies predominantly hypophyllous. Mycelium immersed, branched, septate, hyaline, smooth, hyphae 3–7 μm diameter. Conidiophores arising singly or in small groups through stomata or breaking through epidermis, erect, flexuous, simple, or occasionally branched, up to 180 μm long, 5–10 μm diameter, septate, often geniculate, sometimes slightly swollen at the apex, pale to mid olivaceous brown, smooth, conidial scars similar to those formed in *Drechslera* and *Curvularia*. Conidiogenous cells polytretic, integrated, terminal, subcylindrical to cylindrical, sometimes geniculate with sympodial proliferation, 6–22 × 4–5 μm, with one locus on a broadly obtuse apex, with a pigmented alternarioid scar, 2–4 μm wide. Conidia tretic, solitary or in short (2–3) unbranched chains, ellipsoidal, 30–50 (av. 41) μm long, 15–27 (av. 21) μm wide in the broadest part, lacking longitudinal septa, (1–) 2 (−3) transverse septa, strongly constricted at the septa, olivaceous brown, thin-walled, verruculose.

In culture: Fast growing (4–7 cm diam after 7 days), umbonate, cottonose, either fimbriate (in PCA) or entire edged (in PDA), smoke gray becoming white, gray olivaceous with white edges reverse. No sporulation was observed after 7 days of incubation.

Material examined: Brazil: Rio de Janeiro, Nova Friburgo, Ponte da Saudade, on *Tropaeolum majus*, 23 May 2017, R. W. Barreto (VIC 44410 – neotype designated here, neotype culture COAD 2389, MBT 388771); Minas Gerais, Antônio

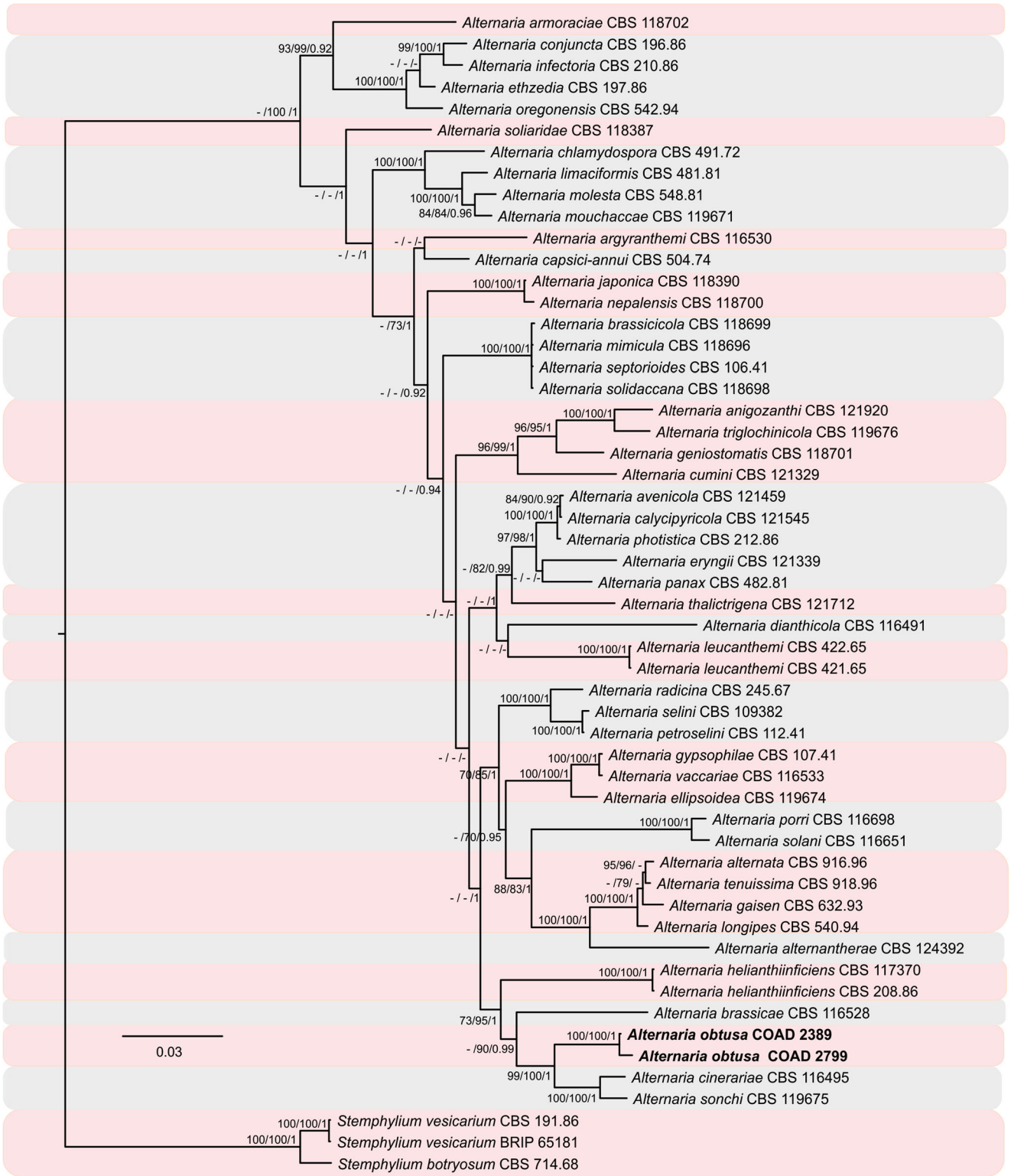

Fig. 1 Phylogenies based on combined nc LSU rDNA, ITS, *RPB2*, and *TEF1* showing the relationship of *Alternaria obtusa* with other closely related species within *Alternaria*. Bootstrap support values (MP and ML) or Bayesian posterior probabilities higher than 70% or 0.90 are indicated above or below the thickened branches ("–" indicates lack of support). Isolates from this study are indicated by bold text

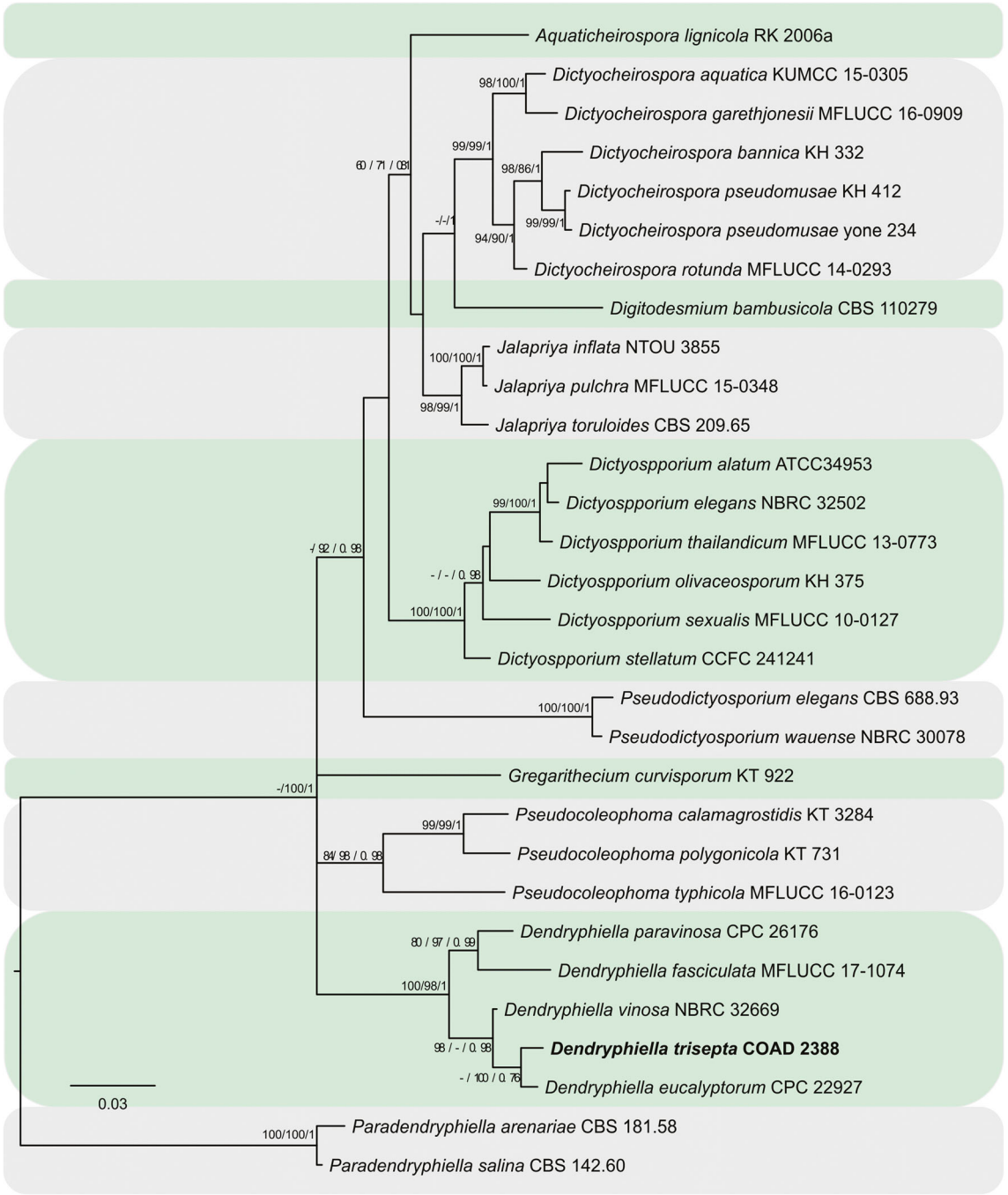

Fig. 2 Phylogenies based on combined nc LSU rDNA and ITS showing the relationship of *Dendryphiella trisepta* with other closely related genera from family Dictyosporiaceae. Bootstrap support values (MP and ML) or Bayesian posterior probabilities higher than 70% or 0.90 are indicated above or below the thickened branches ("–" indicates lack of support). Isolates from this study are indicated by bold text

Carlos, Sítio São Jorge, on *Tropaeolum majus*, 20 May 2019, B. W. Ferreira (VIC 47206, culture COAD 2799).

Notes: *Heterosporium tropaeoli* was originally described from a specimen collected from a non-designated site in Sri Lanka on living leaves of *T. majus*. No herbarium specimen is known for this specimen, and no ex-type culture was designated. It was hence considered necessary to designate a neotype and ex-neotype culture here. It might be argued that this should be obtained from Sri Lanka. Nevertheless, *A. tropaeoli* is a pathogen known only from *T. majus*, which is a plant native to South America. The fungus has been reported from many countries ranging from Australasia to Africa and the Americas, but not from Eurasia (Farr and Rossman 2019). In South America, it has been first reported

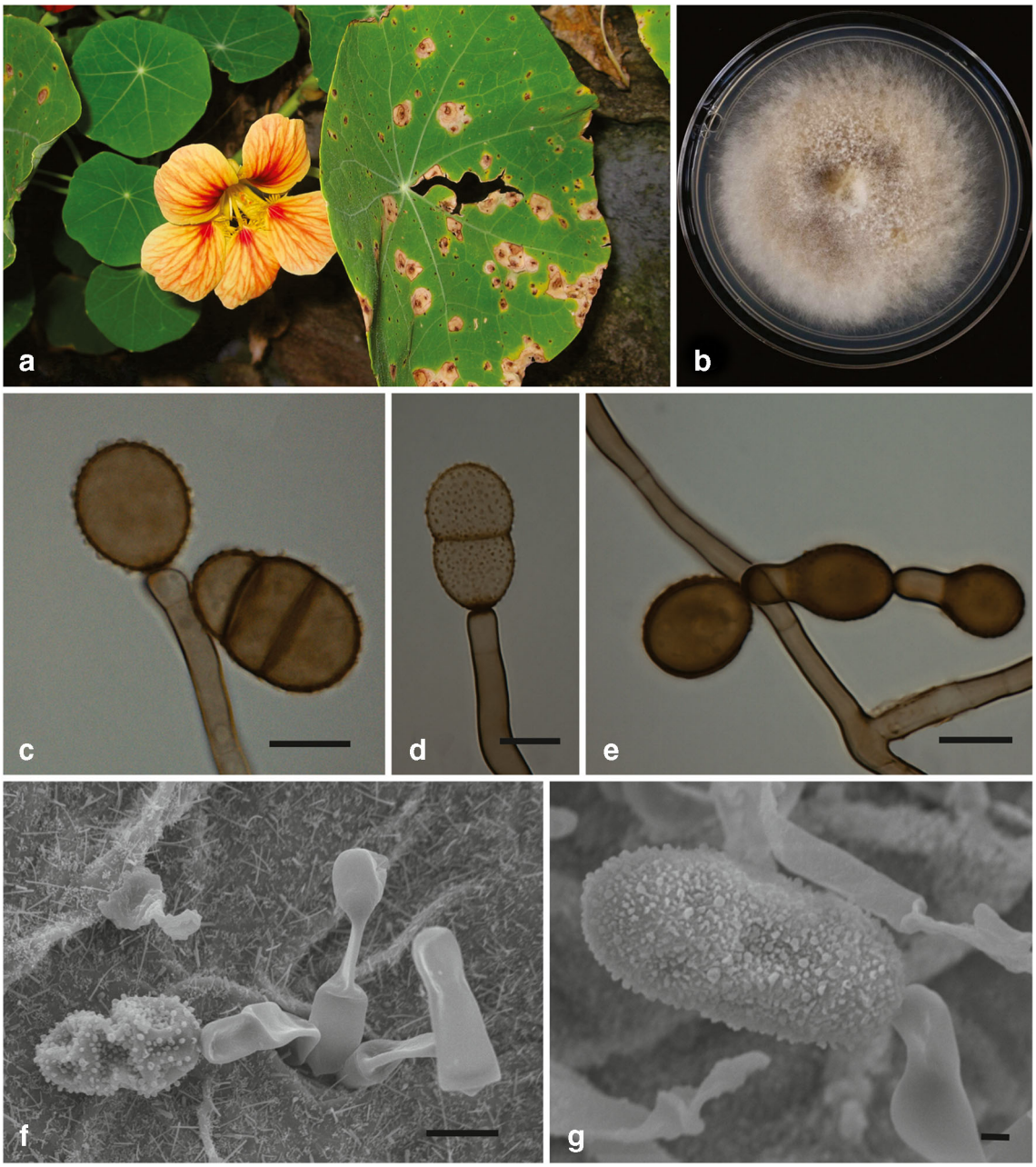

Fig. 3 *Alternaria obtusa* (VIC 44410). **a** Leaf spots on *Tropaeolum majus*. **b** Colony on PDA after 7 days (after incubation at 25 °C in 12 h light/dark cycle). **c** Detail of tretic conidiogenous cell bearing attached immature conidium and one mature conidium (note constrictions at septa of mature conidium) and verrucose surface on both immature and mature conidia. **d** *Ibid* one-septate immature conidium. **e** Acropetal conidial chain. **f** SEM image showing smooth conidiophores arising through the stoma and bearing one verrucose conidium (collapsed). **g** SEM image of one verrucose conidium. Bars = 10 μm except **g** = 2 μm

by Vieira and Barreto (2002), in Brazil. It is likely that its original occurrence and original description from Sri Lanka is fortuitous, and the fungus is actually native to South America which spread to other regions of the globe in planting material of its host but without being noticed in its center of origin until recently.

The new name *Alternaria obtusa* is proposed above for *Acroconidiella tropaeoli*. It could not be recombined into *Alternaria tropaeoli* because this name is already in use for another valid (and distinct) species in this genus described from India on *T. majus*.

***Dendryphiella trisepta* (Muchovej) B.W. Ferreira & R.W. Barreto, comb. nov.** (Fig. 4)

MycoBank: MB832343

Basionym. *Acroconidiella trisepta* J.J. Muchovej, Mycologia 72: 1045 (1980)

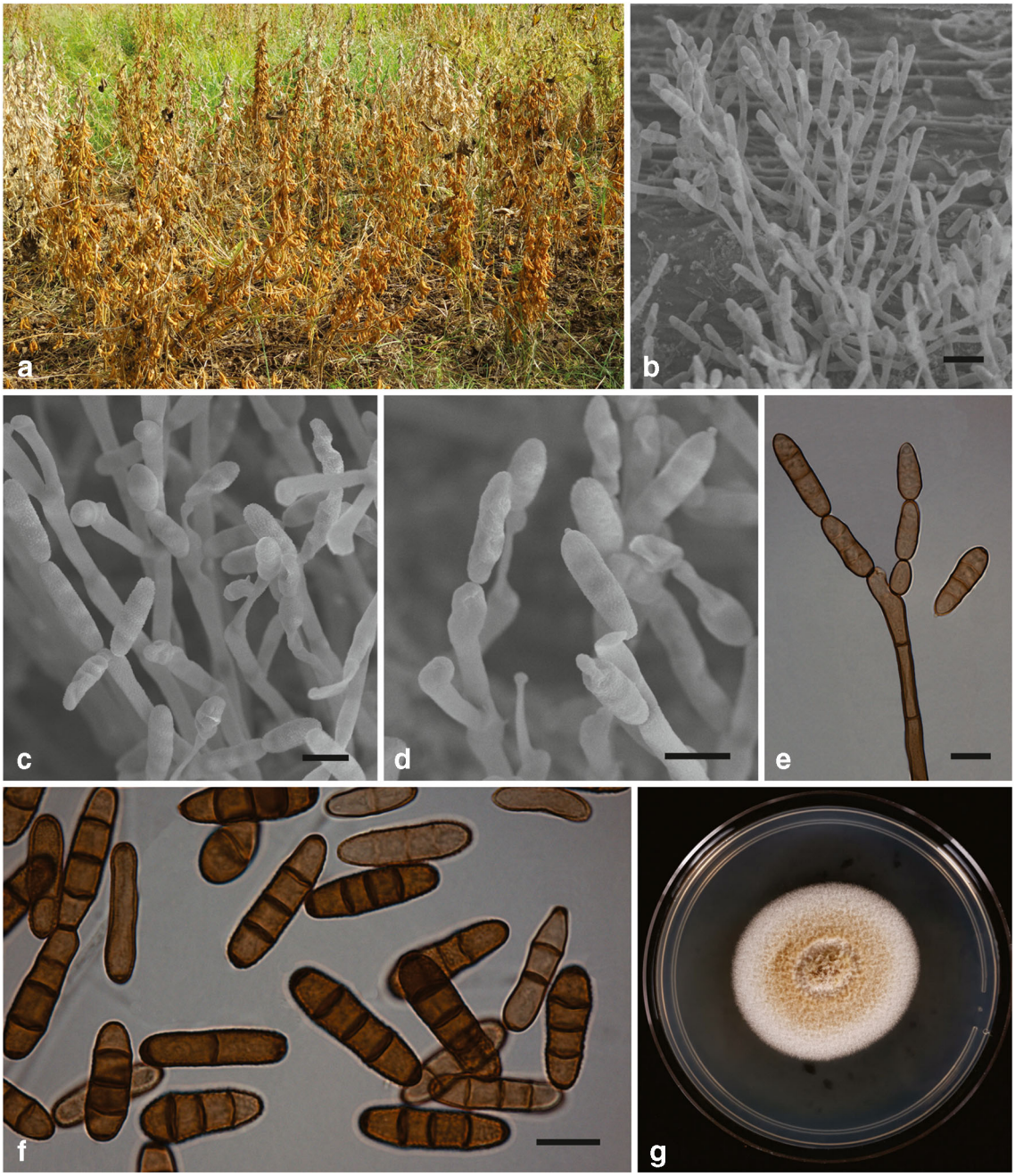

Fig. 4 *Dendryphiella trisepta* (VIC 44409). **a** *Glycine max* debris at the type locality (experimental area of the campus of the Universidade Federal de Viçosa, Viçosa, state of Minas Gerais, Brazil). **b** SEM images of *D. trisepta* colony on the surface of dead soybean stems. **c**, **d** SEM images of conidial chains. **e** Conidial chains under light microscopy. **f** Group of typical predominantly triseptate conidia (note: thickened and darkened conidial scars). **g** Colony on PDA after 15 days (incubation at 25 °C in 12-h light/dark cycle). Bars = 10 μm except **b** = 20 μm

Colonies bursting through the cuticle and forming dark spots amidst colonies of a range of other saprophytic fungi on rotting stems of *Glycine max*. Conidiophores erect, macronemateous, forming loose tufts, cylindrical, flexuous, geniculate, up to 140 μm, 5–6.5 μm diam, reddish-brown, smooth. Conidiogenous cells polytretic, cylindrical, terminal, proliferating sympodially, slightly swollen apically, 5–13 μm in width. Conidia tretic, cylindrical, straight, 22–27(–33) × 8–10(–12) μm, apex obtuse to rounded, base rounded, mostly 3-septate, in acropetal unbranched chains, pale to mid reddish-brown, echinulate, hila thickened and darkened, 1 μm diameter.

In culture: Slow-growing (4.5–4.8 cm diameter after 15 days), umbonate, felty, edges entire, either entirely white

or buff centrally with white edges, honey reverse with white edges. No sporulation was observed after 15 days of incubation.

Type material: Brazil: Minas Gerais: Viçosa, Universidade Federal de Viçosa, on dead branches *Glycine max*, 8 Jul 1979, J. J. Muchovej, Herbarium Universidade Federal de Viçosa (holotype lost); Minas Gerais: Viçosa, Universidade Federal de Viçosa, on dead branches *Glycine max*, 9 May 2017, R. W. Barreto (VIC 44409 – neotype designated here, ex-neotype culture COAD 2388, MBT 388772).

Notes: *Dendryphiella trisepta* was only known from soybean debris at the type locality. Colonies of the fungus were readily found in this substrate at the type locality in the first attempt of recollecting the fungus. As the original type material has been lost, and also because no ex-type culture was designated by Muchovej, a neotype and ex-neotype culture are designated here.

Species included in *Acroconidiella*

Acroconidiella eschscholziae (Harkn.) M.B. Ellis, nom. dub. More dematiaceous hyphomycetes. 407 (1976)

Basionym. Heterosporium escholtziae Harkn., Bull. Calif. Acad. Sci. 1: 38 (1884)

Colonies effuse, dark olivaceous. Mycelium immersed. Conidiophores solitary or in small groups developing between the epidermal cells, erect, flexuous, simple or occasionally branched, up to 85 μm long, 5–8 μm wide, often geniculate, pale olivaceous or golden-brown, smooth, with several dark-brown scars. Conidiogenous cells polytretic, sympodial. Conidia solitary or occasionally in short chains, mostly cylindrical, 28–90 × 9–19 μm, with 1–7 transverse and occasionally 1–2 longitudinal or oblique septa, often constricted at the septa, pale to mid-golden-brown, echinulate—as described in Ellis (1976).

Type material: USA: California, San Francisco, on leaves of *Eschscholtzia californica*, Jan 1884, Harkness (holotype destroyed)

Note: The presence of longitudinal septa in *A. eschscholziae* strongly suggests it to belong to *Alternaria.*

Acroconidiella indica I. B. Prasher & R. K. Verma, nom. dub. Journal on New Biological Reports 4: 111 (2015)

Colonies on natural substrate effuse, superficial on the substratum forming large stromatoid masses, made up of mycelium 3.2–6.4 μm wide, dark-brown, thick-walled, slightly roughened, extensively branched (branches close), short celled, bearing erect vertical conidiophores. Conidiophores 8–126 × 3.2–11.2 μm, brown, short or elongate, cylindrical, straight or slightly curved, septate, thick-walled, pigmented opaque, with a swollen basal cell, bearing conidiogenous cells. Conidiogenous cells rachiform, pale-brown to colorless, straight or flexuous, geniculated, geniculations thickened and minute, few (1–2), poroid. Conidia phaeo-, ceteri-, phragmo-, porosporous, acrogenous, 3–33.5 × 1.5–10.5 μm, brown to dark-brown, thick-walled, oval to elliptical or elongate, cylindrical, straight or slightly curved, dry, (1–6 celled), with transverse septa only, smooth, constricted at the septum: septa thick, distinct, apical cells rounded or sometimes pointed; basal cells more or less triangular and narrowed towards the hilum; hilum protruding, thickened. Germination of the conidia starts in situ or on the substrate by short germ tube which bears secondary conidia on them—as described in Prasher and Verma (2015).

Holotype: India: Himachal Pradesh: Solan, on dead twigs of unidentified tree, 10 Feb 2009, I. B. Prasher (PAN 30076)

Note: Prasher and Verma (2015) did not provide cultural or molecular data for *A. indica* and the morphology described for this fungus might place it in a range of similar genera such as: *Dendryphiella*, *Paradendryphiella*, or *Alternariaster.*

Acroconidiella manoharacharii I. B. Prasher & R. K. Verma, nom. dub. Journal on New Biological Reports 4: 113(2015)

Colonies on natural substratum black, minute, velvety, distributed throughout the substrate forming a scum. Mycelium immersed in the substrate, composed of branched, septate, brown, smooth-walled hyphae. Conidiophores 7–51 × 3–9.5 μm, branched, brown, short, cylindrical, straight or slightly curved, septate, with a swollen basal cell, pigmented opaque, thick-walled, bearing an apical conidiogenous cell. Conidiogenous cell rachiform, pale-brown, straight or flexuous, geniculated, geniculations thickened and minute, few (1–2) poroid. Conidia phragmo-, ceteri-, phaeo-, porosporous, borne singly, 8–20 × 3–8 μm, brown, thick-walled, oval to elliptic, straight or slightly curved, dry, (1–3 celled) with transverse septa, smooth, constricted at the septum; septa thick-walled, distinct; apical cell round or occasionally pointed; basal cell more or less triangular and narrowed towards the hilum; hilum protruding and thickened—as described in Prasher and Verma (2015).

Holotype: India: Himachal Pradesh: Shimla, Tara Devi, on angiospermous sticks, 23 Sep 2010, I. B. Prasher (PAN 30077)

Notes: There are no DNA sequences available in public databases for *A. manoharacharii* or any other fungi described in *Acroconidiella* except for those in this publication. It was not possible to examine specimens of *A. manoharacharii* deposited in PAN herbarium, and it appears that no culture of this fungus was obtained by the authors, since no description of cultural features is provided in Prasher and Verma (2015). As for *A. indica*, morphological features alone would allow its placement in a range of dematiaceous genera. It is hence better to leave the two species as *nomen dubium* until the fungus is recollected allowing for a proper re-evaluation including molecular typing.

Discussion

Alternaria was originally described by Nees von Esenbeck (1816), based on *A. tenuis*. The genus grew in number of species and included an assemblage of dematiaceous hyphomycetes producing dark-colored phaeodictyospores ended in a tapering beak and usually forming conidial chains (Ellis 1976). *Alternaria* was monographed by Simmons (2007) who, based on morphological and cultural features, recognized 273 *Alternaria* species and segregated several similar genera such as *Aternariaster*, *Chalastospora*, *Prathoda*, and *Teretispora*. A modern re-evaluation of *Alternaria* and related genera is presently under way (Lawrence et al. 2013; Woudenberg et al. 2013) and is producing major changes in the delimitation of this important group of fungi. Our results provide yet another contribution by modern-day mycologists and clarified that *Acroconidiella* is not a valid genus since its type species is a member of the genus *Alternaria.*

Lindquist and Alippi (1964) treated the genus *Acroconidiella* as distinct from *Alternaria* because it did not produce conidial chains or longitudinal or oblique septa. This combination of features suggested that it might be inadequately placed in *Alternaria*. This appeared a logical decision, based on morphology, at the time. Nevertheless, modern studies of *Alternaria* have indicated that the absence of longitudinal or oblique septae alone is not sufficient for excluding a species from *Alternaria.* Some examples of species of *Alternaria* having solely transverse septa are as follows: *A. leucanthemi*, *A. thalictrina*, *A. thalictricola*, *A. thalictrigena*, and members of sect. *Nimbya* (Schubert et al. 2007; Lawrence et al. 2012; Woudenberg et al. 2013). As for the presence or absence of conidial chains, a morphological feature which had taxonomic weight for erecting *Acroconidiella*, Vieira and Barreto (2002) had documented the presence of short conidial chains in *A. trapaeoli*—also observed and illustrated in the present study and also confirmed to be a dominant feature in *A. trisepta*, as already observed in the original description by Muchovej (1980). *Acroconidiella eschscholziae* described by Ellis (1976) presents conidia with longitudinal or oblique septa (as for the majority of the members of *Alternaria*) which are occasionally produced in short chains. It is very likely that *A. eschscholziae* is yet another species to be recombined into *Alternaria. Acroconidiella tropaeoli* was found here to be phylogenetically close to *Alternaria brassicae*, *Alternaria cinerariae*, and *Alternaria sonchi*. None of these taxa is morphologically similar to *A. obtusa.* Although sharing the feature of the absence of longitudinal/oblique septae with *A. obtusa* neither *A. leucanthemi* nor *A. thalictrigena* grouped with *A. obtusa.* There are no *A. thalictrina* or *A. thalictricola* sequences in GenBank available for comparison with the fungus on *T. majus*. However, these latter taxa have clear morphological distinctions separating them from *A. obtusa.* Both have longer, wider, rostrate conidia (Schubert et al. 2007). According to Lawrence et al. (2013), section *Sonchi* includes *Alternaria cinerariae* and *Alternaria sonchi* and is characterized morphologically by producing large subcylindrical, broadly ovoid, broadly ellipsoid or obclavate, formed singly or in short chains, with multiple transverse and few longitudinal septa, slightly constricted at the septa, with a blunt taper which can form secondary conidiophores. Lawrence et al. (2013) included *A. brassicae* as the basal lineage in sect. *Sonchi*, which was supported by a monotypic lineage in the analysis of Woudenberg et al. (2013). *Alternaria brassicae* has conidia which are straight or slightly curved, obclavate, rostrate, with transverse septa and longitudinal or oblique septa. Conversely, conidia of *A. obtusa* are ellipsoidal with rounded tips and lack longitudinal septa. Additionally, two species of *Alternaria*, *A. tropaeoli* (Deshpande and Rajderkar 1964) and *A. tropaeolicola* (Zhang 2000), have been described on *Tropaeolus majus* from India and China, respectively. Although there are no DNA sequences in public databases for these two species, they are clearly morphologically distinct from *Alternaria obtusa*. Both *A. tropaeoli* and *A. tropaeolicola* have rostrate, non-verrucous conidia which have transverse and longitudinal septae (Deshpande and Rajderkar 1964; Simmons 2007).

The fungus recollected from soybean debris was found here to be misplaced by Muchovej (1980). *Acroconidella trisepta* was found to clearly fit phylogenetically within the genus *Dendryphiella*. *Dendryphiella* was established by Ranojevic (1914) with the type species *D. interseminata* (Berk. & Ravenel) Bubák. Morphological characteristics used for delimitation of this genus included macronematous conidiophores with polytretic, integrated conidiogenous cells formed at the swollen tip and at intercalary swellings of conidiophores from which catenate or solitary conidia are formed (Ellis 1971; Matsushima 1971; Rai and Kamal 1986; Guo and Zhang 1999; Crous et al. 2014, 2016). Boonmee et al. (2016) performed phylogenetic analyses of *Dendryphiella* based on DNA sequence data for three loci (SSU, nc LSU rDNA, *TEF1*). The results showed that *Dendryphiella* represents a distinct genus within the Dictyosporiaceae. Different from other asexual morphs, genera belonging to this family—which form blastic conidiogenous cells—members of *Dendryphiella* have tretic conidiogenous cells.

Dendryphiella trisepta, as recombined in the present publication, has a distinct morphology from that of other species of *Dendryphiella.* Its conidiophores are shorter than those of *D. eucaliptorum* and *D. aspera* (up to 140 μm in length vs. up to 500 μm and 136–544 μm, respectively), and its conidia are larger [22–27 (–33) × 8–10 (–12) μm vs. (19–) 20–23 (–25) × 5 (–7) μm and 10–22 × 4–6 μm respectively]. In *D. indica*, *D. paravinosa*, *D. eucalypti*, *D. vinosa*, *D. uniseptata*, *D. infuscans*, and *D. dregeae*, conidiophores are mostly

solitary, whereas in *D. trisepta*, these are formed in tufts. *Dendryphiella broussonetiae* and *D. lycopersicifolia* have conidiophores which are longer than those of *D. trisepta* (129–303 μm and 100–500 μm vs. 140 μm). *Dendryphiella fasciculata* has longer conidiophores (170–250 μm) and narrower conidia (4.3–7.4 μm) than those of *D. trisepta.*

Nuclear DNA of *A. tropaeoli*, the type species of *Acroconidiella*, was extracted and supported a phylogenetic evaluation of *Acroconidiella* performed here, for the first time. It demonstrated that the morphological delimitation utilized by the scientists who dealt with this genus in the past does not mirror the true phylogenetic placement of this taxon. As it has happened with other genera in the past, *Acroconidiella* became a "dumping ground" for somewhat morphologically similar taxa of uncertain placement, a fact demonstrated here by *D. trisepta*. By transferring *A. tropaeoli* to *Alternaria*, the generic name *Acroconidiella* can no longer be applied to the other species placed in the genus. There are doubts as for the correct taxonomic placement of *A. eschscholziae*, *A. indicus*, and *A. manoharacharii*. For *D. trisepta*, samples needed for re-evaluating the taxon were within the type locality, in the campus of the Universidade Federal de Viçosa where the work was performed. Nevertheless, it was surprisingly easy to recollect (in the first attempt) the material of *A. trisepta* from old soybean stems in the field, nearly forty years after it was collected and described by Muchovej (1980), suggesting that a stable population of this saprophytic fungus exists at this locality (an experimental area belonging to the Departamento de Fitotecnia of the Univesidade Federal de Viçosa). Although no other record exists of this fungus outside this area, it is likely that this fungus is present in soybean debris in other fields in Brazil and perhaps other parts of the world. Although it appears that this is a saprophytic species, no studies on its relationship with soybean or other plant species have ever been conducted (Baird et al. 1997, 2003; Almeida et al. 2001).

Recollecting, isolating and re-evaluating the other species in the former genus *Acroconidiella* is a pending challenge, among so many others posed to mycologists worldwide of recollecting, neotypifying, or epitipifying the fungal taxa of the past (Hyde and Zhang 2008). Until then, these species are to be regarded as *Incertae sedis.*

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4.2. ARTIGO 2

Debunking *Duosporium*

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ORIGINAL ARTICLE

Debunking *Duosporium*

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Abstract
Duosporium is a monotypic genus including only the type species *Duosporium yamadanum* which has been treated in the literature as "anamorphic Pezizomycotina." Nevertheless, this is just a conjecture since its true phylogenetic affinities remain unknown. This fungus is known to cause leaf spots on several members of *Cyperus* spp. It has been intensively investigated in the 1990s as a potential candidate for use as an inundative biocontrol agent against purple nutsedge—a major tropical weed. Its morphology was recognized, as somewhat close to those of *Curvularia* and *Bipolaris*. Nevertheless, it was kept in a separate genus based on two distinctive morphological features: production of two kinds of spores (as indicated by its generic name) and straight versicolored macroconidia. No molecular studies have ever been made to elucidate the placement of *Duosporium*. In a relatively recent publication, *Curvularia americana* and *C. chlamydospora* were found to produce macro- and microconidia, as in *Duosporium.* Besides, there are several species of *Curvularia* known to have predominantly straight conidia. Here, a multilocus phylogenetic analysis including the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) and translation elongation factor 1-α (*TEF1*) sequences placed *D. yamadanum* within *Curvularia*, close to *C. tuberculata*, *C. oryzae*, and *C. reesi*. This led to the proposal of the new combination *C. yamadana* and the synonymization of *Duosporium* with *Curvularia.*

Keywords *Curvularia* · Multilocus phylogeny · Reappraisal · Taxonomy

Introduction

Duosporium is a genus treated by Kirk et al. (2008) as "anamorphic Pezizomycotina." Only its asexual dematiaceous hyphomycete stage is known. Its correct taxonomic placement is uncertain since no molecular study has ever been made to elucidate its true phylogenetic affinities. *Duosporium* was proposed by Thind and Rawla (1961) based on a fungus found infecting the leaves of *Cyperus iria* in India. Since its proposal, no additions were made to the genus which remains monotypic and only contains *Duosporium yamadanum*. Tsuda and Ueyama (1982) in their literature survey found that well before *D. cyperi* was proposed by Thind and Rawla (1961), Matsuura (1931) had described a highly morphologically similar fungus from Japan (Honshu). This was found attacking *Cy. iwasakii.* Matsuura named it *Brachysporium yamadanum*. Tsuda and Ueyama (1982) concluded upon this observation that the name *D. cyperi* was inadequate since *B. yamadanum* had priority over *D. cyperi.* The new combination *D. yamadanum* having *B. yamadaeanum* as basionym was then proposed by Tsuda and Ueyama (1982).

As pointed out by Thind and Rawla (1961) and Tsuda and Ueyama (1982), *Duosporium* appeared to be related to either *Bipolaris* or *Curvularia*. However, in these earlier publications, it was recognized that conidiogenous cells leading to the formation of macroconidia of *Duosporium* were mostly monotretic whereas those where microconidia originated were often polytretic. Conversely, the conidiogenous cells on both *Bipolaris* and *Curvularia* are typically polytretic. Also, as emphasized in its generic name, *Duosporium* was found to typically produce two kinds of conidia: macro and microconidia. Such a feature was, at the time, unknown for members of *Bipolaris* and *Curvularia*. This justified the proposal of *Duosporium* to accommodate the fungus on *Cyperus* spp.

Duosporium yamadanum is only known in association with members of the genus *Cyperus*. Four species of

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Cyperus have been reported as hosts of this fungus: *Cyperus ferax* in Venezuela (Urtiaga 1986), *Cy. iria* in India and Venezuela (Ellis 1971), and *Cy. iwasakii* Matsuura (1931) in Japan and *Cy. rotundus* in Brazil (Barreto and Evans 1995). Additional records of *D. yamadanum* have been published but without a full identification of their *Cyperus* host. These records are from Cuba (Mercado Sierra 1984) and West Indies (Minter et al. 2001).

This fungus attracted great interest when it was first collected in association with severe leaf spots and blight of foliage of *Cy. rotundus* in Brazil during surveys for potential weed biocontrol agents (Barreto and Evans 1995). *Cyperus rotundus* is considered as one of the worst tropical weeds worldwide (Holm et al. 1991) and a challenging target for mechanical or chemical management (Edenfield et al. 2005). A series of intensive studies on the biology and management of *D. yamadanum* was initiated at the Universidade Federal de Viçosa (state of Minas Gerais, Brazil) by Pomella (1999) and later retaken by Macedo (2006). Results of the attempts at developing a *D. yamadanum*-based mycoherbicide produced inconsistent results and this project was interrupted. Observations made during these years of study led to speculation that the taxonomic treatment for *Duosporium* might be incorrect and that its affinity with the *Curvularia*/*Bipolaris* complex should be reevaluated. A study including the use of molecular tools was then conducted in order to better clarify the taxonomic status of *Duosporium* and results are presented herein.

Materials and method

Isolates and morphology

Samples deposited in the herbarium at the Universidade Federal de Viçosa (VIC) during the 1990s were re-examined under a dissecting microscope in the laboratory. Fungal structures growing externally on plant tissues were scraped from colonized plant surfaces and mounted in lactophenol or lactofuchsin. Observations of fungal structures were performed with an Olympus BX53 microscope adapted with differential interference contrast lighting and digital image capture system (Olympus Q-Color 3 ™). Biometric data was obtained from the measurement of at least 30 representative fungal structures.

Existing pure cultures from the studies performed in the 1990s and deposited in the culture collection of the Universidade Federal de Viçosa - Coleção Octávio de Almeida Drumond (COAD) in silica gel, as described in Dhingra and Sinclair (1995), were recovered by aseptically transferring fragments of filter paper carrying fungal structures onto Petri dishes containing potato carrot-agar (PCA) and maintained in a controlled temperature room at 25 °C under a 12-h daily light/12-h dark regime (light provided by two white and one near-UV lamps placed 35 cm above the plates).

Culture description

Colony descriptions were based on fungal growth on PDA and PCA, after 7 days. The fungus was grown either under a daily 12-h light regime as mentioned above, or in the dark (Petri dishes wrapped in aluminum foil). Color terminology followed Rayner (1970).

Molecular characterization and multilocus phylogenetic analysis

Genomic DNA was extracted from COAD 141, COAD 359, and COAD 375 grown in vegetable broth-agar (VBA)-medium described in Pereira et al. (2003) under a 12-h daily light regime for 2 weeks. Approximately 50 mg of mycelium was scraped from the surface of the colonized medium and placed inside sterile plastic tubes containing zirconium spheres and placed in a grinder (L-Beader-3, Loccus Biotecnologia). After 5-s grinding, the resulting suspension was drained into a sterile plastic tube and used for DNA extraction. This was performed with the Wizard Genomic DNA Purification Kit following the manufacturer's protocol. The PCR reaction was performed as described by Pinho et al. (2012). The primers ITS4 and ITS5 (White et al. 1990) were used to amplify the ITS region and the 5.8S rRNA gene. A partial region of *TEF1* was amplified using the primer pair EF1-983 and EF1-2218R (Schoch et al. 2009). PCR products were analyzed on GelRed ™ (Biotium Inc., Hayward, CA, E.U.A.) and visualized under UV light to verify the size and purity of amplification. The PCR products were sequenced by Macrogen Inc., South Korea (http://www.macrogen.com). The nucleotide sequences were edited with software SeqAssem ver. 07/2008 (Hepperle 2004). All sequences were manually verified and nucleotides with ambiguous positions were clarified using sequences from both directions.

The ITS and *TEF1* consensus sequences were compared with others deposited in the GenBank database using the MegaBLAST program. Sequences from GenBank and Tan et al. (2018) were aligned using MUSCLE (Edgar 2004) and built in MEGA v.6 (Tamura et al. 2011). All of the ambiguously aligned regions within the dataset were excluded from the analyses. Gaps (insertions/deletions) were treated as missing data.

Bayesian inference (BI) analyses employing a Markov Chain Monte Carlo method were performed with all sequences, first with each locus separately and then with the concatenated sequences. Before launching the BI, the best nucleotide substitution models were determined for each gene with MrMODELTEST 2.3 (Posada and Buckley 2004). Once

the likelihood scores were calculated, the models were selected according to the Akaike Information Criterion (AIC). The GTR + I + G model of evolution was used for ITS, whereas GTR + G was used for *TEF1*. One concatenated tree with ITS and *TEF1* was generated with Mesquite v. 3.1 (Maddison and Maddison 2011) and estimated on the CIPRES web portal using MrBayes on XSEDE 3.2.6 (Miller et al. 2010).

Additionally, a maximum likelihood (ML) tree was generated with the nearest-neighbor-interchange (NNI) ML heuristic method and the Tamura-Nei substitution model as tree inference options, using CIPRES web portal. The chain stabilities of the phylogenetic tree were assessed by using the bootstrap re-sampling strategy with 1000 bootstrap test replicates. The resulting tree topologies using the two methods (ML and BI) were then compared and the phylogram layout (BI tree) was edited with CorelDRAW Graphics Suite 2017.

Sequences derived from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1). The alignment and tree were deposited in TreeBASE (http://www.treebase.org) (study number S25966).

Results

Phylogenetic analyses

The alignment to construct phylogenetic trees included 52 strains (Table 1), representing many of the known *Curvularia* species, some isolates of *Bipolaris*, three isolates of *Duosporium yamadanum*, and the outgroup taxon (*Alternaria alternata*). The combined matrix consisted of 1911 characters including alignment gaps (ITS: 802 and *EF1*: 1109). The number of conserved sites was 1155 (ITS: 582 and *EF1*: 573). The number of variable and parsimony uninformative sites was 745 (ITS: 210 and *EF1*: 535) and 571 sites were variable and parsimony informative (ITS: 141 and *EF1*: 430). The trees obtained with ML and BI agreed on topology. The phylogenetic analyses inferred from the combined dataset (Fig. 1) indicated that the three strains of the fungus on *Cy. rotundus* (COAD 141, COAD 359, and COAD 375) clustered together with 100% (ML) and 1.0 (BI) support. These isolates of *D. yamadanum* clustered together with three *Curvularia* species: *C. oryzae*, *C. reesii*, and *C. tuberculata*. Additionally, *D. yamadanum* formed a distinct lineage within *Curvularia*. Based on the results of the molecular study, the status of *Duosporium* as a synonym of *Curvularia* became clear. The new combination *C. yamadana* was then proposed.

Taxonomy

Curvularia Boedijn, Bulletin du Jardin Botanique de Buitenzorg 13 (1): 123 (1933).

= *Duosporium* K.S. Thind & Rawla, American Journal of Botany 48 (10): 862 (1961). **Syn. nov.**

Curvularia yamadana (Matsuura) B.W. Ferreira and R.W. Barreto, **comb. nov.** (Fig. 2)

Basionym. Brachysporium yamadanum Matsuura, Byochu-gai Zasshi [J. Pl. Prot. Tokyo] 17: 419, 1931 (as "*yamadaeanum*"). = *Duosporium cyperi* Thind & Rawla, American Journal of Botany 48: 862, 1961.

≡ *Duosporium yamadanum* (Matsuura) Tsuda and Ueyama, Mycotaxon 14: 145, 1982.

Lesions on living leaves; starting as dark brown, linear necrosis at apex or in the middle of the midrib, expanding to cover large portions of leaves, causing the blight of leaves and, sometimes, to death of all aerial part of plants. Internal mycelium intracellular, 1–6-μm diam, branched, septate, constricted at septae, concentrated adaxially under the epidermis, hyaline. External mycelium absent. Stromata absent. Macroconidiophores arising through stomata, amphigenous, mostly in loose fascicles of few conidiophores, rarely solitary, cylindrical, 43–132 × 4–7 μm, straight to slightly curved, inflated at the base and apex, septate, unbranched, smoky brown, smooth. Conidiogenous cells terminal, integrated, monotretic or polytretic, proliferating sympodially, or with percurrent proliferation with enteroblastic regenerative growth, cylindrical, 10–45 × 7 μm, inflated apical portion up to 11 μm wide, smoky brown becoming paler towards the apices. Conidiogenous loci indistinct to darkened. Macroconidia dry, solitary, tretic, oblong, 29–43 × 15–25 μm, apex and base rounded, hilum mostly indistinct, but visible in some spores as a darkened basal area, 3–4 μm wide, 3-septate, eguttulate, central cells dark brown to brown, end cells hyaline to subhyaline, smooth. Microconidiophores only seen in culture, arising from macroconidia or vegetative hyphae, cylindrical, strongly geniculate, straight to flexuous, mononematous, macronematous, 11–86 × 4–5 μm, walls thicker than on vegetative hyphae, brown, paler towards apex. Conidiogenous cells integrated, terminal or intercalary, cylindrical, proliferating sympodially, 6–11 × 3–5 μm, pale brown to brown, smooth, mono- or polytretic. Microconidia globose to subglobose to ellipsoidal, 5–13 × 5–9 μm, aseptate, brown to dark brown, warted, warts prominent, 3 × 2.5 μm.

Culture characters—slow-growing (3-cm diam on PDA and 3.4-cm diam on PCA after 7 days), flat to raised centrally with depressed marginal ring, undulate, edges entire, felty, center pale mouse gray becoming olivaceous gray, margin white, reverse fuscous black and white margin (PDA); center pale mouse gray, scarlet near the edge, border orange, reverse bay with orange margin (PCA). Not sporulating except on PCA in the dark.

Known distribution—Brazil, Cuba, India, Japan, Venezuela, West Indies.

Material examined: Brazil: Bahia, Itabuna, CEPLAC Brasil, on *Cyperus rotundus*, 11 Apr 2000, A. W. V. Pomella

Table 1 DNA sequences used for the phylogenetic tree

Species	Isolate	Host	Genbank ITS	*TEF1*	Reference
Alternaria alternata	EGS 34.016	*Arachis hypogaea*	AF347031	KC584634	Woudenberg et al. (2013)
Bipolaris bamagaensis	BRIP 13577*	*Brachiaria subquadripara*	KX452445	KX452462	Tan et al. (2016)
	BRIP 10711	*Dactyloctenium aegyptium*	KX452444	KX452461	Tan et al. (2016)
	BRIP 14847	*Dactyloctenium aegyptium*	KX452446	KX452463	Tan et al. (2016)
B. cookei	MAFF 51191	*Sorghum bicolor*	KJ922392	KM093777	Tan et al. (2016)
B. drechsleri	CBS 136207*	*Microstegium vimineum*	KF500530	KM093760	Tan et al. (2016)
B. maydis	CBS 137271/C5	*Zea mays*	AF071325	KM093794	Manamgoda et al. (2014)
	CBS 136.29*	*Zea mays*	HF934926	KJ415463	Manamgoda et al. 2014
B. shoemakeri	BRIP 15806	*Ischaemum rugosum var. segetum*	KX452452	KX452469	Tan et al. (2016)
	BRIP 15929*		KX452453	KX452470	Tan et al. (2016)
B. sivanesaniana	BRIP 15847*	*Paspalidium distans*	KX452455	KX452472	Tan et al. (2016)
	BRIP 15822	*Setaria sphaecelata*	KX452456	KX452473	Tan et al. (2016)
Curvularia aeria	CBS 294.61*	Air	HF934910	–	Tan et al. (2016)
C. americana	UTHSC 08-3414*	*Homo sapiens*	HE861833	–	Tan et al. (2018)
C. beasleyi	BRIP 10972*	*Chloris gayana*	MH414892	MH433654	Tan et al. (2018)
	BRIP 15854	*Leersia hexandra*	MH414893	MH433655	Tan et al. (2018)
C. boeremae	IMI 164633*	*Portulaca oleracea*	MH414911	–	Tan et al. (2018)
C. buchloës	CBS 246.49*	*Buchloë dactyloides*	KJ909765	KM196588	Tan et al. (2018)
C. carica-papayae	CBS 135941*	*Carica papaya*	HG778984	–	Tan et al. (2018)
C. chlamydospora	UTHSC 07-2764*	*Homo sapiens*	HG779021	–	Tan et al. (2018)
C. clavata	BRIP 61680b	*Oryza rufipogon*	KU552205	KU552159	Tan et al. (2018)
C. colbranii	BRIP 13066*	*Crinum zeylanicum*	MH414898	MH433660	Tan et al. (2018)
C. dactyloctenii	BRIP 12846*	*Dactyloctenium radulans*	KJ415545	KJ415447	Tan et al. (2018)
C. eragrostidis	CBS 189.48	*Sorghum* sp.	HG778986	–	Tan et al. (2018)
C. harveyi	BRIP 57412*	*Triticum aestivum*	KJ415546	KJ415446	Raza et al. (2019)
C. hawaiiensis	BRIP 11987*	*Oryza sativa*	KJ415547	KJ415445	Tan et al. (2018)
C. homomorpha	CBS 156.60*	Air	JN192380	JN601014	Tan et al. (2018)
C. kusanoi	CBS 137.29	*Eragrostis major*	JN192381	JN601016	Tan et al. (2018)
C. lamingtonensis	BRIP 12259*	*Microlaena stipoides*	MH414901	MH433663	Tan et al. (2018)
C. mebaldsii	BRIP 12900*	*Cynodon transvaalensis*	MH414902	MH433664	Tan et al. (2018)
	BRIP 13983	*Cynodon dactylon x transvaalensis*	MH414903	MH433665	Tan et al. (2018)
C. muehlenbeckiae	CBS 144.63*	*Muehlenbeckia sp.*	HG779002	–	Raza et al. (2019)
	LC11988	*Saccharum officinarum*	MN215681	MN263975	Raza et al. (2019)
	LC11989	*Saccharum officinarum*	MN215682	MN263976	Raza et al. (2019)
C. neoindica	IMI 129790*	*Brassica nigra*	MH414910	MH433667	Tan et al. (2018)
C. nicotiae	BRIP 11983*	Soil	KJ415551	KJ415442	Tan et al. (2018)
C. nodulosa	CBS 160.58	*Eleusine indica*	JN601033	JN601019	Tan et al. (2018)
C. oryzae	CBS 169.53*	*Oryza sativa*	KP400650	KM196590	Tan et al. (2018)
C. pallescens	CBS 156.35*	Air	KJ922380	KM196570	Tan et al. (2018)
C. portulacae	BRIP 14541*	*Portulaca oleracea*	KJ415553	KJ415440	Tan et al. (2018)
C. prasadii	CBS 143.64*	*Jasminum sambac*	KJ922373	KM230408	Tan et al. (2018)
C. pseudolunata	UTHSC 09-2092*	*Homo sapiens*	HE861842	–	Tan et al. (2018)
C. reesii	BRIP 4358*	Air	MH414907	MH433670	Tan et al. (2018)
C. spicifera	CBS 274.52	Soil	JN192387	JN601023	Tan et al. (2018)

Table 1 (continued)

Species	Isolate	Host	Genbank ITS	*TEF1*	Reference
C. tsudae	ATCC 44764*	*Chloris gayana*	KC424596	KC503940	Tan et al. (2018)
C. tuberculata	CBS 146.63*	*Zea mays*	JX256433	JX266599	Tan et al. (2018)
C. variabilis	CPC 28815*	*Chloris barbata*	MF490822	MF490865	Tan et al. (2018)
C. verruculosa	CBS 150.63	*Punica granatum*	KP400652	KP735695	Tan et al. (2018)
C. warraberensis	BRIP 14817*	*Dactyloctenium aegyptium*	MH414909	MH433672	Tan et al. (2018)
C. yamadana	COAD 375	*Cyperus rotundus*	MN954704	MT008259	This study
	COAD 359	*Cyperus rotundus*	MN954705	MT008260	This study
	COAD 141	*Cyperus rotundus*	MN954706	MT008261	This study

Ex-type strains are indicated in asterisk after collection number

(VIC 27784F–culture COAD 141, MBT390712); Minas Gerais, Viçosa, Chácara Cristal, on *Cyperus rotundus*, 11 Apr 1998, R. W. Barreto, (culture COAD 375); Rio de Janeiro, Carmo, Fazenda São José, on *Cyperus rotundus*, 20 Jan 1998, R. W. Barreto, (culture COAD 359).

Notes: *Duosporium* was originaly described from a specimen collected in Punjab, India, on living leaves of *Cyperus iria*, but collected earlier in Honshu, Japan, on leaves of *Cyperus iwasakii* and mistakenly placed in *Brachysporium*, as *B. yamadanum*. No herbarium specimen or ex-type culture were designated by these authors.

The multilocus phylogenetic analyses indicated *C. yamadana* to be close to *C. tuberculata*, *C. oryzae*, and *C. reesii*. *Curvularia reesii* was recently described from colonies obtained from an air sample (Tan et al. 2018) and has not known to have *Cyperus* spp. as a substrate. Nevertheless, Farr and Rossman (2020) lists *C. tuberculata* and *C. oryzae* on *Cyperus* spp. *C. oryzae* was reported on *Cy. rotundus* from West Indies (Minter et al. 2001) and *C. tuberculata* was reported on *Cy. malaccensis* from Taiwan (Matsushima 1980). *Curvularia yamadana* has conidia similar in size to *C. tuberculata* (23–52 × 13–20 μm), *C. oryzae* (24–40 × 12–22 μm) and *C. reesii* (31–35 × 12–13 μm). However, conidia of *C. tuberculata* are sometimes curved, have 3–8-distoseptae and these are tuberculate at maturity. Conidia in *C. oryzae* and *C. reesii* are obclavate to ellipsoidal, whereas in *C. yamadana* are oblong having a rounded apex and base. Two other *Curvularia* species have been listed by Farr and Rossman (2020) on *Cyperus* spp. *Curvularia aeria* on *Cy. rotundus* from West Indies (Minter et al. 2001) and *Curvularia pallescens* on *Cy. antillanus* from Cuba (Mercado Sierra 1984). Both are morphologically rather different from *C. yamadana*. *Curvularia aeria* has conidia which are straight or curved, ellipsoidal, obovoid or clavate, 18–32 × 8–16. *Curvularia pallescens* has ellipsoidal to fusiform conidia, usually slightly curved, 17–32 × 7–12 μm. In the phylogenetic tree, both *C. aeria* and *C. pallescens* were phylogenetically distant from *C. yamadana*.

Discussion

The genus *Curvularia* includes species associated with plant diseases worldwide (Sivanesan 1987; Manamgoda et al. 2012a, b; da Cunha et al. 2013; Hyde et al. 2014; Manamgoda et al. 2015). It is characterized by the production of sympodial conidiophores with tretic, terminal, and intercalary conidiogenous cells and elongate, transversely septate conidia with a dark basal scar. Conidia are often curved because of it having asymmetrically swollen intermediate cells, (Sivanesan 1987; Manamgoda et al. 2015). For a long time, curvature of conidia was used as the key feature to differentiate fungi in the genus *Curvularia* from species belonging to related genera. However, it is now known that some *Curvularia* species such as *C. cymbopogonis*, *C. oryzae-sativae*, *C. protuberata*, and *C. ryleyi* have predominantly straight conidia (Manamgoda et al. 2015). Species delimitation in *Bipolaris* and *Curvularia* remained problematic for taxonomists for very long due to the overlapping morphological characters among many species (Manamgoda et al. 2014, 2015; Sivanesan 1987).

Such a subjectivity of the morphological distinction of taxa in *Bipolaris* and *Curvularia* was only resolved through the use of molecular data. In addition to ITS, other loci were found to be of high informative value in the phylogenetic analyses of sequence data from species belonging to these two genera. Particularly, the protein-coding loci of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), *TEF1*, and RNA polymerase II second largest subunit (*RPB2*) which became critical for such analyses (Hernández-Restrepo et al. 2018; Manamgoda et al. 2014; Marin-Felix et al. 2017).

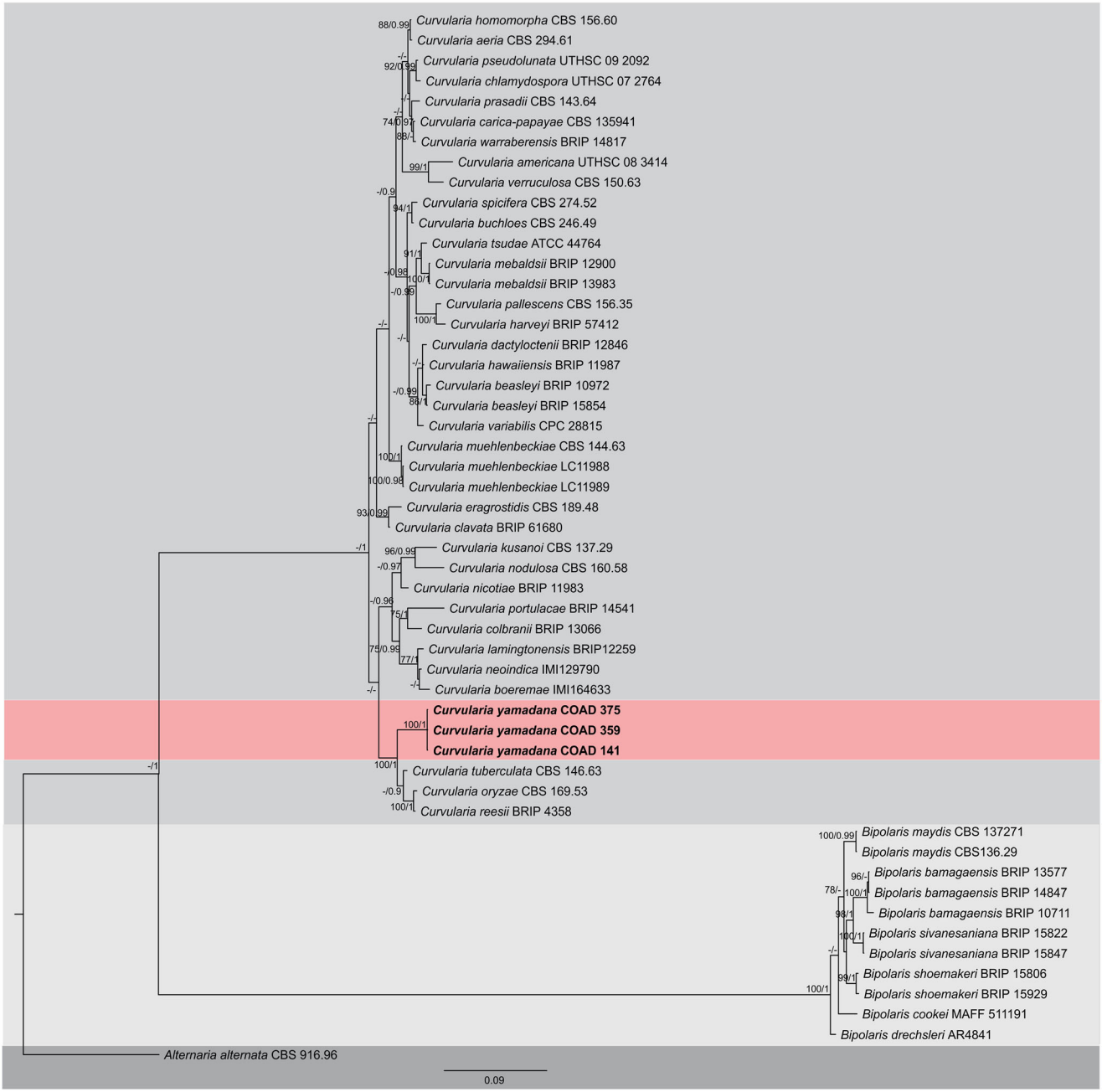

Fig. 1 Phylogeny based on Bayesian inference inferred of combined ITS and *TEF1* showing the relationship of *Curvularia yamadana* with other species within *Curvularia* and *Bipolaris*. Bootstrap support values (ML) or Bayesian posterior probabilities higher than 70% or 0.90 are indicated above or below thickened branches (– indicates lack of support). Isolates from this study are indicated by bold text

According to Thind and Rawla (1961) and Tsuda and Ueyama (1982), *Duosporium* was considered distinct from *Curvularia*, because conidiogenous cells generating macroconidia of *Duosporium* are mostly monotretic and those generating microconidia are polytretic whereas conidiogenous cells of *Curvularia* are, predominantly, polytretic. However, *C. muehlenbeckiae*, *C. beerburrumensis*, *C. boeremae*, *C. coatesiae*, *C. colbranii*, *C. kenpeggii*, *C. mebaldsii*, *C. petersonii*, *C. platzii*, *C. reesii*, and *C. warraberensis*, along with other species, were recognized to have either mono- or polytretic conidiogenous cells (Tan et al. 2018). *Duosporium* was also occasionally seen to produce polytretic macroconidiophores, as mentioned above and in the published description of Barreto and Evans (1995).

Another feature which led *Duosporium* to be treated as distinct from *Curvularia*, is the production of microconidia in *Duosporium*. This remains a partially valid difference, since it is now known that some species of *Curvularia* do in fact produce secondary conidia. Among them are *C. americana* and *C. chlamydospora* (Madrid et al. 2014). Nevertheless,

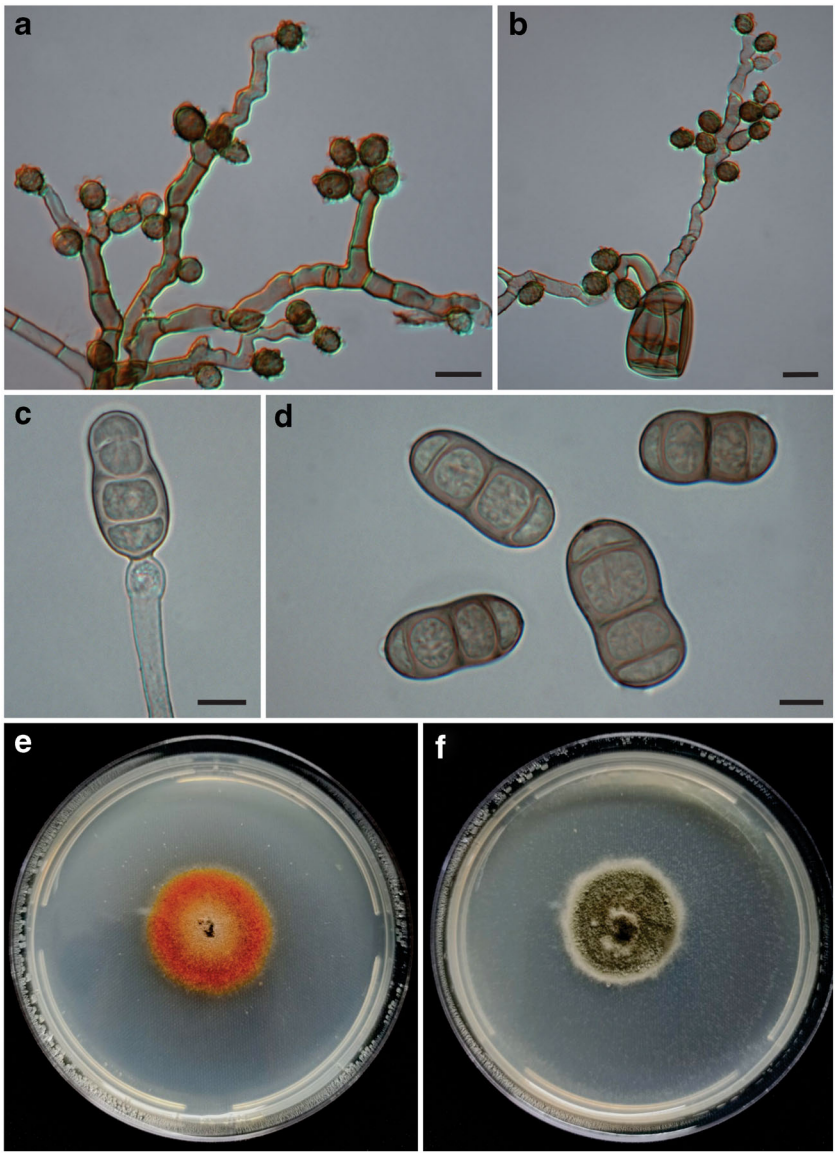

Fig. 2 *Curvularia yamadana* (COAD 141). **a** Microconidiophores producing warted microconidia. **b** Collapsed macroconidium germinating to produce microconidia. **c** Macroconidium attached to the conidiogenous cell. **d** Mature macroconidia. **e** Colony on PCA after 7 days (incubation at 25 °C in 12-h light/dark cycle). **f** Colony on PDA after 7 days (incubation at 25 °C in 12-h light/dark cycle). Bars = 10 μm

such secondary conidia in those two species are only formed directly from macroconidia and not from conidiophores as seen regularly in vitro for *C. yamadana.* In *C. americana*, microconidia are aseptate, pale brown, globose, and 5–6 μm wide. *C. chlamydospora* produce microconidia which are 1–2 celled, pale brown, globose to subglobose, and 4–6 μm diam. On the other hand, in *C. yamadana*, microconidia are aseptate, warted, brown to dark brown, globose to subglobose to ellipsoidal, and 5–13 × 5–9 μm. Microconidia remain a puzzle in the life cycle of *C. yamadana*. These are produced in large quantities in vitro in older cultures, but it was never seen in nature. Only a very small proportion of the microconidia were found to germinate, even after a series of treatments were attempted to break their dormancy (Pomella 1999). This led to the speculation that these structures would function as resting spores for *C. yamadana*, a hypothesis which remains to be better investigated.

The multilocus phylogenetic analyses clearly indicated that the fungus on *Cyperus* spp. belongs to *Curvularia*. *Curvularia yamadana* is close to *C. tuberculata*, *C. oryzae*, and *C. reesii*. Some species of *Curvularia* have some morphological resemblance to *C. yamadana* but the combination of oblong, straight versicolored 3-septate conidia and production of warted microconidia in culture is exclusive to the species formerly placed in *Duosporium.*

The need for epitipification of plant pathogenic fungi was emphasized by Cai et al. (2011) as the way forward towards clarification of the taxonomy and phylogeny of such taxa and towards the stability in the application of names. A recent case in point is that of the dematiaceous hyphomycete *Acroconidiella*, a genus which we recently "debunked" (Ferreira and Barreto 2019) showing its type species to be an "unusually shaped" *Alternaria.* Here, we provided a small contribution to this formidable task by showing that

Duosporium is an artificial genus which needs to be recognized as a late synonym of *Curvularia*. Nevertheless, the designation of an epitype, ideally a specimen from the type locality (Honshy, Japan) on *Cy. iwasakii*, would be of great value for a confirmation of our understanding on the taxonomy of *C. yamadana*.

The taxonomic placement of the fungus on *Cyperus* spp. has, hopefully, been finally resolved, but an important pending challenge remains. That of determining its true potential as a practical tool to be deployed against the "tropical scourge" *Cy. rotundus*, as placed by William (1976). Although the theme has been shelved long ago, improvements in tools for the mass production, formulation, and application of biological control agents have occurred along the years and may justify revisiting *C. yamadana* as an "ecologically benign" weed biocontrol product (mycoherbicide).

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4.3. ARTIGO 3

Debunking *Korunomyces*

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Debunking *Korunomyces*

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Abstract

Korunomyces is a small genus erected to include fungi that produce stipitate, profusely branched, multicellular asexual reproductive structures (propagules) on leaves and in culture. Three species are accepted in the genus: *Korunomyces terminaliae* – the type species, *K. prostratus* and *K. zapatensis*. No molecular studies have ever been made to elucidate the phylogenetic placement of *Korunomyces*. Recently, DNA sequences were obtained from pure cultures of *K. terminaliae* and *K. prostratus*, enabling an elucidtation of its taxonomic placement. Isolates of *K. prostratus* obtained from diseased tissues of *Miconia calvescens* were observed, for the first time, forming pycnidia in culture. A multi-gene phylogeny, including the large subunit of the nrDNA (nc LSU rDNA), internal transcribed spacer (ITS) region, polymerase II second largest subunit (*RPB2*) and translation elongation factor 1-α (*EF1*), placed *K. terminaliae* and *K. prostratus* within *Coniella*. As *Coniella* has nomenclatural priority over *Korunomyces*, this is reduced to a synonym for *Coniella* and new name and a new combination are proposed for both species, namely: *Coniella ferreirense* nom. nov. and *Coniella prostrata* comb. nov. An emended description of *Coniella* to include the occasional formation of distinct and elaborate asexual propagules is also provided.

Keywords *Coniella* • Multi-gene phylogeny • New taxa • Reappraisal • Taxonomy

Introduction

The genus *Korunomyces* was proposed by Hodges & Ferreira (1981) for a fungus found producing stipitate, profusely branched, multicellular asexual reproductive structures (propagules) on infected leaves and in culture. The type species of the genus was described as *Korunomyces terminaliae*. This was found causing leaf spot on *Terminaliae ivorensis* in Brazil (Hodges & Ferreira, 1981), *Korunomyces zapatensis* was later described from dead leaves of *Nectandra coriaceae* (Hol & Castaneda, 1986). The latest addition to the genus was

Korunomyces prostrates, found causing foliage blight on *Miconia calvescens* (Seixas et al., 2007).

Korunomyces terminaliae was originally described in Brazil associated with leaf and stem blast of seedlings of *Terminalia ivorensis* (Hodges & Ferreira, 1981). The fungus has also been reported to cause numerous leaf spots in members of the Combretaceae family, such as *T. ivorensis*, *T. catappa*, *T. myriocarpa* and *Bouchenavia* sp. (Hodges & Ferreira, 1981; Farr & Rossman, 2021). Hodges and Ferreira (1981) compared the fungus on *T. ivorensis* with *Cristulariella*, *Papulaspora viridis* (= *Trichoderma matsushimae*) and *Aegerita candida* (= *Bulbillomyces farinosus*), all of which are known to produce non-conidial asexual propagules with more or less elaborate morphology. The differences in the branching character, width and color of the hyphae, as well as the branching pattern of the propagules were regarded, at the time, by Hodges and Ferreira (1981), as sufficient to distinguish each of these agonomycetous fungi from *Korunomyces*. One additional distinctive feature of *Korunomyces* separating from *Aegerita candida* was the absence of clamp connections – present on the propagules and hyphae of *A. candida*. *Papulaspora*, besides being restricted to lignicolous (damp wood) habitat is a dematiaceous hyphomycete whereas *Korunomyces,* mycelium and propagules are hyaline. *Cristulariella depraedans*, similarly to *K. terminaliae* (and also *K protratus*) is a leaf parasite, but when growing in culture it produces phialoconidia and sclerotia (Redhead 1975). Based on such distinctions, Hodges and Ferreira (1981) decided to propose the new genus *Korunomyces* to accommodate the fungus on *T. ivorensis*.

Korunomyces zapatensis was the second species of *Korunomyces* included in the genus. Contrarily to the two other species, it was not described as a leaf spot fungus. It was originally found on dead leaves of *Nectandrae coriaceae* in Cuba. *Korunomyces zapatensis* produces asexual propagules which are morphologically similar to those of *K. terminaliae* but differences in the width of the propagule branches and sizes of the terminal cells and stalk were recognized as sufficient to propose the new species (Hol & Castaneda 1986).

The third species to be included in the genus was *Korunomyces prostratus.* This was proposed by Seixas et al. (2007) based on a fungus found in Brazil associated to leaf spots which expand to cause leaf blight on *M. calvescens*. Despite the significant similarities with *K. terminaliae*, the propagules of *K. prostratus* – as indicated by the name – are always prostrate whereas in *K. terminaliae* these are always formed in an upright position. It was conjectured that the propagules in *K. prostratus* function as infection pads, where in *K.*

terminaliae whese might serve as "fungal analogues" of the parachute-like achenes of many Asteraceae.

No additions to the genus were made since 2007. The absence of a sexual stage or other morphological markers, such as clamp connections and the lack of molecular information for a phylogenetic study left *Korunomyces* taxonomic placement an unresolved issue until now.

Here we report the results of a study involving the recollection of *K. prostratus* combined with the study of the type culture of *K. terminaliae* aimed at resolving the taxonomy of this agonomicetous genus.

Materials and methods

Sample collection processing and observation of fungus morphology

Samples of diseased foliage of *Miconia calvescens* were collected from the type locality (Angra dos Reis, state of Rio de Janeiro, Brazil). These were screened under a stereo microscope and parts of the samples bearing sporulating colonies of the fungi were selected and dried in a plant press. Fungal structures were scraped from the sample surface with a scalpel and mounted in lactophenol and lactofuchsin. Observations were made with an Olympus BX53 adapted with differential contrast lighting and equipped with digital capture system (Olympus Q-Color 3 ™). A representative specimen was deposited in the local herbarium at the Universidade Federal de Viçosa – state of Minas Gerais, Brazil (Herbarium VIC).

Isolations were performed by aseptic transfer of hyphal tips from the leaf surfaces onto potato dextrose-agar (PDA) plates with a sterile scalpel. Culture descriptions were based on the observation of 14-day-old (*K. prostratus*) colonies formed in plates containing either potato dextrose-agar (PDA) or potato carrot-agar (PCA), maintained at 25 °C under a 12-h daily/light regime (light provided by two white and one near-UV lamps placed 35 cm above the plates). The colour terminology followed Rayner (1970).

DNA isolation

Total genomic DNA was extracted from 7-day-old cultures formed on PDA by using Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions and the steps described in Pinho et al. (2012).

PCR amplification

The large subunit of the nrDNA (nc LSU rDNA), internal transcribed spacer (ITS), polymerase II second largest subunit (*RPB2*) and translation elongation factor 1-α (*TEF1*) regions from each fungus included in the study were sequenced with the primers LSU1Fd (Crous et al. 2009) and LR5 (Vilgalys and Hester 1990) and IT5 + ITS4 (White et al. 1990), EF1Fd + EF2Fd (Groenewald et al. 2013) or EF1-728F + EF1- 986R (Carbone & Kohn 1999) or EF-2 (O'Donnell et al. 1998) and fRPB2-5F + fRPB2-7cR (Liu et al. 1999), respectively. PCR amplifications were performed in a total volume of 12.5 µL 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). Conditions for PCR amplification consisted of an initial denaturation step of 5 min at 94 ºC followed by 35 cycles of 30 s at 94 ºC, 30 s at 48 ºC and 90 s at 72 ºC for nc LSU rDNA, ITS and 40 cycles of 30 s at 94 ºC, 30s at 52 ºC / 59 ºC and 45 s at 72 ºC for *TEF1* and a final elongation step of 7 min at 72 ºC. The partial *RPB2* gene was obtained by using a touchdown PCR protocol: start step of 5 min at 94 °C, followed by 5 cycles of 45 s at 94 °C, 45 s at 60 °C annealing temperature, and 2 min at 72 °C; 5 cycles of 45 s at 94 °C, 45 s at 58 °C annealing temperature, and 2 min at 72 °C; 30 cycles of 45 s at 94 °C, 45 s at 54 °C annealing temperature, and 2 min at 72 °C followed by a final step of 8 min at 72 °C. Amplicons were analyzed on 0.8% agarose electrophoresis gels stained with GelRed (InstantAgarose) in a 1× TAE buffer and visualized under UV light to check for amplification size and purity. PCR products were purified and sequenced by Macrogen Inc. (http://www.macrogen.com).

Phylogenetic analysis

Contigs were generated by assembled from forward and reverse sequences using DNA Dragon program (http://www.dna-dragon.com\index.php).The resulting sequences were

aligned with other sequences retrieved from GenBank (www.ncbi.nlm. nih.gov), using MEGA v. 6 (Tamura et al. 2013).

Bayesian inference analyses were conducted and the best-fit evolutionary model was determined by comparing different evolutionary models via the Akaike information criterion using PAUP (version 4.0b10, Sinauer Associates) and MrModeltest 2.2 (Nylander 2004). 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, whereas the remaining 8 000 trees were used for calculating posterior probabilities. Bayesian posterior probabilities are presented on the left of each node. The analysis was hosted by CIPRES science gateway portal at San Diego supercomputer center (Miller et al. 2010). Phylogenetic trees were visualized with the program FigTree v1.3.1 (Rambaut 2009).

Maximum likelihood (ML) tree was generated with the Nearest-Neighbor-Interchange (NNI) ML heuristic method and the Tamura-Nei Substitution model as tree inference options in MEGA. The branch stabilities of the phylogenetic tree were assessed by using the bootstrap re-sampling strategy with 1000 bootstrap test replicates. The resulting tree topologies using the three methods (ML and BI) were then compared and the phylogram was edited with InkScape 0.91 (www.inkscape.org).

Sequences of *Melanconiella hyperopta* (CBS 131696) were used as the outgroups in the *Coniella* phylogeny.

Results

Phylogeny

Phylogenetic analysis using the ITS, nc LSU rDNA, *RPB2* and *TEF1* regions were based on 51 *Coniella* strains, one isolate of *K. terminaliae*, two isolates of *K. prostratus* and one outgroup sequences (Fig. 1). The combined alignment was comprised of 3576 characters with gaps (777 for ITS, 1314 for nc LSU rDNA, 768 for *RPB2* and 717 for *TEF1*). The phylogenetic analyses generated by Maximum likelihood (ML), and Bayesian inference (BI)

indicate that *K. terminaliae* and *K. prostratus* grouped within the genus *Coniella* and formed a monotypic well-supported clade (100%/1.00, ML/BI supports, respectively). Additionally, *K. prostratus* formed a distinct lineage and was a sister to a strain of *K. terminaliae*.

Taxonomy

Coniella Höhn., Ber. dt. bot. Ges. 36 (7): 316 (1918)

≡ Syn. nov. *Korunomyces* Hodges & F.A. Ferreira, Mycologia 73 (2): 335 (1981)

For more synonyms see Index Fungorum (2021)

Coniella ferreirense **B.W. Ferreira & R.W. Barreto, nom. nov.** (Fig. 3).

MycoBank:

Etymology: Named after the forest pathologist and mycologists Francisco Alves Ferreira (Chico Fungo) who first collected and described the fungus on *T. ivorensis*, and proposed the genus *Korunomyces*.

≡ *Korunomyces terminaliae* Hodges & F.A. Ferreira, Mycologia 73(2): 335 (1981)

Coniella prostrata **(Seixas & R. W. Barreto) B.W. Ferreira & R.W. Barreto, comb. nov. and emmend. descr.** (Fig. 3).

MycoBank:

≡ *Korunomyces prostratus* Seixas & R.W. Barreto, Mycologia 99 (1): 105, 2007

Lesions necrotic, initially circular, grayish-brown centrally with a brown periphery, becoming irregular with age with concentric dark-brown peripheral rings often resulting in a scale-like pattern, with a yellowish halo, coalescing and leading to an extensive leaf-blight; older parts of lesions tend to crack leaving irregular holes in the leaves. External mycelium amphigenous, branched, septate, initially hyaline becoming yellow or orange later. Internal mycelium indistinct. Propagulophores often difficult to distinguish from ordinary hyphae,

cylindrical, simple, length indeterminate, individual cells 11–27 × 3–4 µm, diam, below propagules 5–8 µm, hyaline, smooth, point of rupture indistinct or absent. Propagules subglobose to irregular when mature, formed on apex of usually prostrate hyphae or occasionally on erect propagulophores, multicellular, formed of primary branches with an initial dichotomous branching pattern, becoming dendritic later, 69–273 × 64–272 µm, branch elements 4–10 µm diam, terminal elements 4–5 × 7–13 µm, initally hyaline becoming orange when mature, smooth." (Seixas and Barreto, 2017).

In culture [on PDA]: Conidiomata pycnidial, globose to slightly depressed globose, 100–260 × 100–370 µm, wall composed of 1–3 cell-thick layers dark brown textura angularis, 7–12 µm, dark brown; dehiscence ostiolate, central. Conidiophores formed on a dense, basal, cushion-like aggregation of hyaline cells, mostly reduced to conidiogenous cells, subcylindrical, branched next to base, 7–13 × 3–4 µm, smooth, hyaline, 1–2-septate. Conidiogenous cells enteroblastic, phialidic with apical periclinal thickening, 7–12 × 2–3 µm, smooth, hyaline, with minute collarette. Conidia mostly broadly ellipsoidal, often somewhat flattened on one side, oblong, subreniform, ovoid to subovoid, 9–12 × 3–5 µm, apex rounded to subtruncate, hilum sometimes slightly protuberant, aseptate, hyaline when immature, becoming chestnut-brown at maturity, smooth, guttulate.

Culture description: In PDA and PCA, fast-growing (7–7.4 cm diam in 7 days), colonies of cottony-woolly aerial mycelium, orange centrally and becoming white at the margin, diurnal zonation distict between the center and margin; dark-orange to umber or ochreous to orange reverse in PDA; in PCA colonies of flattened aerial mycelium surrounded by isolated areas of sparse aerial mycelium and strongly irregular superficial growth, reverse like surface; sporulation abundant from pycnidia in both media (observed after 14 d).

Material examined: Holotype: Brazil: Rio de Janeiro: Angra dos Reis, Ilha Grande, 04 Jan 2000, VIC 22213. **Paratype**: Brazil. Rio de Janeiro: Angra dos Reis, Ilha Grande, road from Vila Abhraão to Dois Rios, 13 Jan 2002, VIC 22218. **Epitype:** Brazil: Rio de Janeiro: Angra dos Reis, Praia Brava, on *Miconia calvescens*, 28 Jul 2018, R. W. Barreto, Herbarium Universidade Federal de Viçosa (VIC 47147 – epitype designated here, ex-epitype culture COAD 2597). **Additional material**: Brazil: Rio de Janeiro, Estrada de Guapiaçu, on *Miconia calvescens*, 8 Jan 2021, R. W. Barreto VIC 47491, culture COAD 3306).

Note: The culture obtained from the type was no longer viable. Hence, a new isolate obtained from the same region from where the type material originated was collected to serve as

epitype, as indicatyed above. An ex-epitype culture was obtained, deposited in the culture collection and used, together with a supplementary specimen in the study.

Discussion

In the present study, multigenic phylogenetic analyzes showed that two among the three *Korunomyces* spp. (*K. terminaliae* and *K. prostratus*) formed a well-supported clade within the genus *Coniella*, making it clear that *Korunomyces* is an agonomycetous synanamorph of *Coniella,* and belongs into the Schizoparmaceae. Since *Coniella* has nomenclatural priority over *Korunomyces*, *Korunomyces* is reduced herein to a synonym for *Coniella* and a new name and combination are proposed here for *K. terminaliae* and *K. prostratus*: *Coniella ferreirense* and *Coniella prostrata*. The taxonomic affinities of *K. zapatensis* will remain unclear until this fungus is recollected and epitypified.

Coniella was introduced by von Höhnel (1918) and typified by *Coniella pulchella* (= *Coniella fragariae*). Many species of *Coniella* are known as plant pathogens, causing leaf, fruit, stem, and root diseases of a wide range of hosts, including economically important species, and have received considerable attention from in the phytopathological literature (van Niekerk et al. 2004; Alvarez et al. 2016; Chethana et al. 2017). Other species in this genus have a saprobic lifestyle, occurring in litter, decaying bark and in the soil whereas others occur as endophytes or as secondary invaders of plant tissues infected by other organisms or injured by other causes (Alvarez et al. 2016; Ferreira et al. 1997).

Seixas et al. (2007) when differentiating *C. prostrata* from *C. ferreirense*, conjectured that the propagules of *C. prostrata* would not be functional as dispersion units due to the prostrate condition of the structure and were likely to function, instead, as infection pads. The authors suggested that the dispersion in *C. prostrata* would probably depend on some spore stage that had not been observed until then. Several attempts to induce teleomorph formation of *Korunomyces* sp. were made by the authors, but without success. Here, we observed, for the first time, the formation of sporulating pycnidia in *C. prostrata* in culture and found it to produce a typical *Coniella* asexual morph. The sexual form of the fungus is yet to be observed, but it is likely that the pycnidial phase may be formed on infected leaves of *M. calvescens* at an advanced stage of the necrosis and will represent the dispersal stage of this fungus, as suggested earlier by Seixas et al. (2007).

In the phylogenetic tree *C. ferreirense* and the two isolates of *C. prostrata* formed distinct lineages within a clade separated from the other species of *Coniella*. A morphological comparison was made between *C. prostrata* and *C. ferreirense* and other *Coniella* spp., for which there are no DNA sequences available on GenBank (Table2).

Other species of *Coniella* have been described from *Terminalia* spp., namely: *C. crousii* on *T. chebula* from India (Alvarez et al. 2016), *C. macrospora* on *T. ferreirense* from Ivory Coast (Alvarez et al. 2016), *C. pseudogranati* on *T. stuhlmannii* from Zambia (Alvarez et al. 2016; Chethana et al. 2017), *C. fragariae* on *T. chebula* and *T. paniculata* from India (Rajeshkumar et al. 2011), *C. terminaliae* on *T. tomentosa* from India (Rajeshkumar et al. 2011; Alvarez et al. 2016) and *C. terminaliicola* on *T. superba* from Ecuador (Alvarez et al. 2016). *Coniella. crousii*, *C. macrospora*, *C. pseudogranati*, and *C. fragariae* are phylogenetically distant from *C. prostrata* and *C. ferreirense*. There are no sequences available on Genbank for *C. terminaliae* and *C. terminaliicola*. Pycnidia formation was not described for *C. terminaliicola* and *C. ferreirense*, making morphological comparison impossible. *C. terminaliae* has globose to subglobose spores, 2–8 × 2–3.5 µm, whereas *C. prostrata* has ellipsoidal conidia, 9–12 × 3–5 µm. Although the size, shape and color of the spores are overlapping characteristics in some species, the formation of propagules and propagulophores are unique for *C. ferreirense* and *C. prostrata*.

Seixas et al. (2007) and Hodges & Ferreira (1981) performed pathogenicity tests with *C. ferreirense* and *C. prostrata*. The results of these inoculations showed that *C. prostrata* was capable of causing necrosis in the leaves of *M. calvescens*, *T. ivorensis* and *E. grandis*, but not of *T. catappa*, whereas *C. ferreirense* was able to infect three species of *Terminalia*, but not *Eucalyptus grandis* (Hodges and Ferreira 1981). Despite the partial overlap of the host range, there are differences between the two species. Based on phylogeny and morphology presented here and host differences reported by Seixas et al. (2007) and Hodges & Ferreira (1981) the two species continue to be considered here as distinct.

Unfortunately, a phylogenetic study of *K. zapatensis* was not possible, because the species is only represented by herbarium material available only in Cuba and there are no ex-type cultures or DNA sequences available for this species (Hol & Castaneda, 1986). However, the morphological similarity with the other species previously belonging to *Korunomyces*, indicates that this is yet another agonomycetous sinanamorph of *Coniella*. For now, this particular species should be treated as *incertae sedis*.

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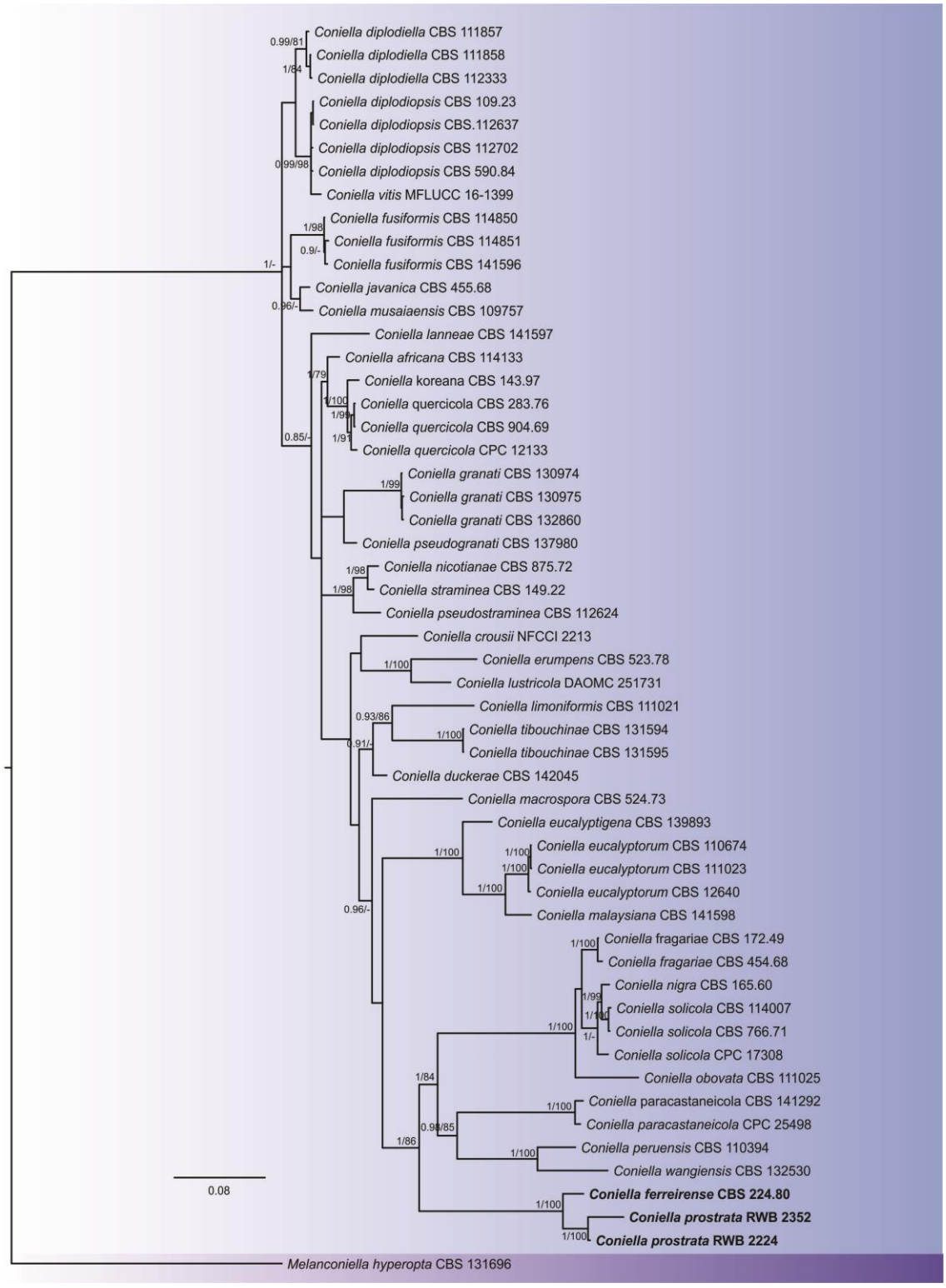

Fig. 1. Phylogeny based on Bayesian inference inferred of combined nc LSU rDNA, ITS, *RPB2* and *TEF1* showing the relationship of *C. ferreirense* and *C. prostrata* with other closely related species within *Coniella*. Bootstrap support values or Bayesian posterior probabilities higher than 70 % or 0.90 are indicated above or below thickened branches (– indicates lack of support). Isolates from this study are indicated by bold text.

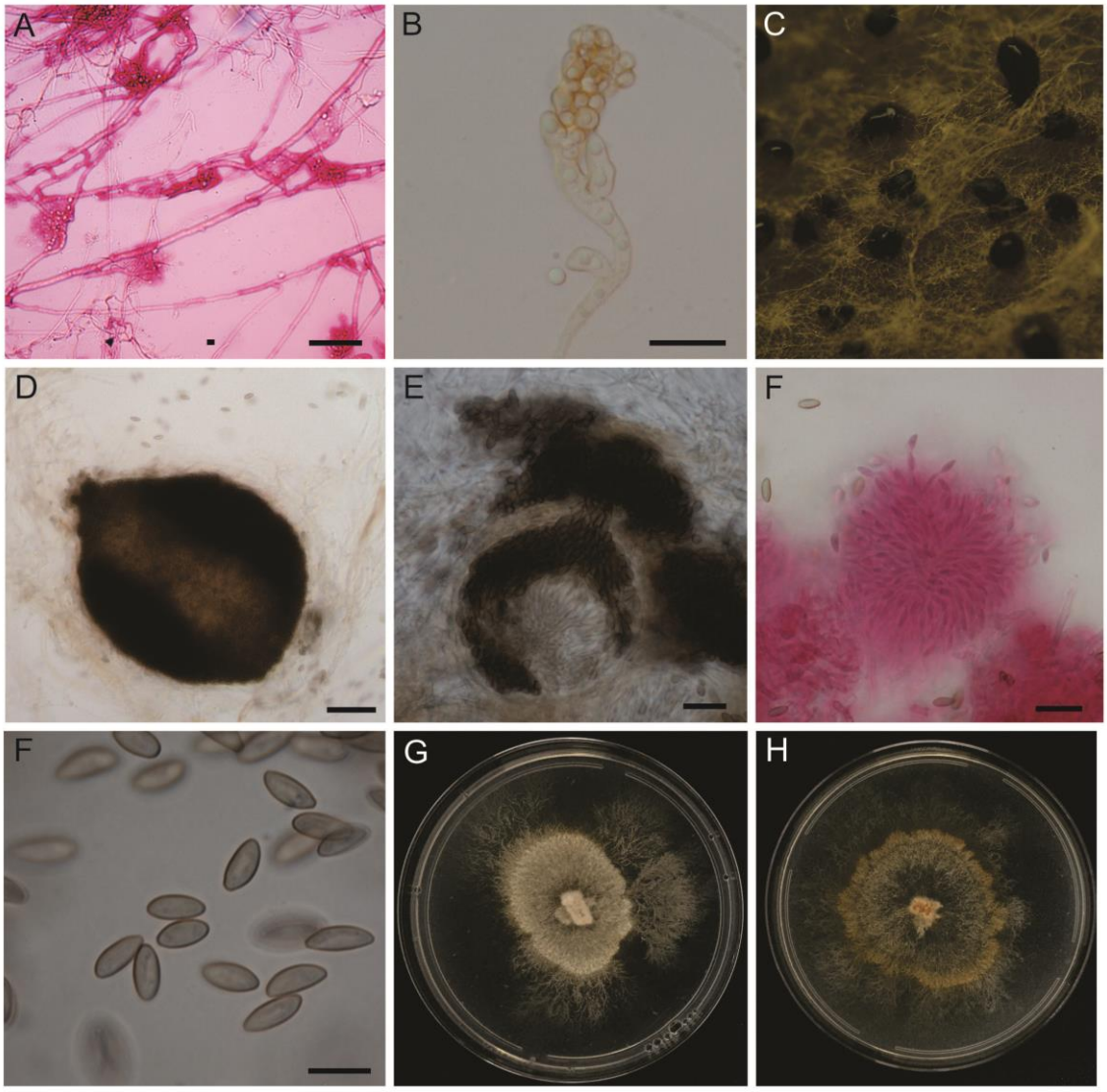

Fig. 2. *C. prostrata* (VIC 47147). **A–B** Propagules and propagulophores. **C** Mature conidiomata on PDA. **D–E** Mature conidiomata with ostiole. **F** Conidiogenous cells. **G** Conidia **H** Colony on PDA after 14 days (incubation at 25 °C in 12 h light/dark cycle). **I** Colony on PCA after 14 days (incubation at 25 °C in 12 h light/dark cycle). Scale bars: a = 50 µm; b = 20 µm; d = 50 µm; e = 20 µm; f = 20 µm; g = 10 µm.

Table 1. Taxa and collections used for multi-gene phylogenetic analyses in this study.

Species name	Strain accession no.	GenBank accession no.				References
		ITS	LSU	rpb2	tef1	
Coniella africana	CBS 114133T = CPC 405	AY339344	AY339293	KX833421	KX833600	Van Niekerk et al. (2004);Alvarez et al. (2016)
C. crousii		HQ264189	–	–	–	Rajeshkumar et al. (2011)
C. diplodiella	CBS 111858ET = CPC 3708	AY339323	KX833335	KX833423	KX833603	Van Niekerk et al. (2004);Alvarez et al. (2016)
	CBS 111857 = CPC 3735	AY339325	AY339285	KX833422	KX833602	Alvarez et al. (2016)
	CBS 112333 = CPC 3775	AY339329	KX833336	KX833424	KX833604	Alvarez et al. (2016)
C. diplodiopsis	CBS 590.84T = CPC 3940	AY339334	AY339288	–	–	Alvarez et al. (2016)
	CBS 10923 = CPC 3933	AY339332	AY339287	KX833440	KX833624	Van Niekerk et al. (2004);Alvarez et al. (2016)
	CBS 112637 = CPC 4228	KX833530	KX833355	KX833441	KX833625	Alvarez et al. (2016)
	CBS 112702 = CPC 3866	KX833531	KX833356	KX833442	KX833626	Alvarez et al. (2016)
C. duckerae	VPRI 13689 = CBS 142045T	KY924929	–	–	–	Marin-Felix et al. (2017)
C. erumpens	CBS 52378T	KX833535	KX833361	KX833446	KX833630	Alvarez et al. (2016)
C. eucalyptigena	CBS 139893T	KR476725	–	–	–	Crous et al. (2015a, b)
C. eucalyptorum	CBS 112640T = CPC3904	AY339338	AY339290	KX833452	KX833637	Van Niekerk et al. (2004);Alvarez et al. (2016)
	CBS 110674 = CPC 610	KX833536	KX833362	KX833447	KX833631	Alvarez et al. (2016)
	CBS 111023 = CPC 3843	KX833537	KX833363	KX833448	KX833632	Alvarez et al. (2016)
C. fragariae	CBS 17249NT = CPC 3930	AY339317	AY339282	KX833472	KX833663	Van Niekerk et al. (2004);Alvarez et al. (2016)
	CBS 45468	KX833571	KX833393	KX833477	KX833670	Alvarez et al. (2016)
C. fusiformis	CBS 141596T = CPC 19722	KX833576	KX833397	KX833481	KX833674	Alvarez et al. (2016)
	CBS 114850	KX833574	KX833395	KX833479	KX833672	Alvarez et al. (2016)
	CBS 114851	KX833575	KX833396	KX833480	KX833673	Alvarez et al. (2016)
C. granati	CBS 132860	KX833577	KX833400	KX833484	KX833677	Alvarez et al. (2016)
	CBS 130974 = CPC 19625	JN815312	KX833398	KX833482	KX833675	Alvarez et al. (2016)
	CBS 130975 = CPC 19626	JN815313	KX833399	KX833483	KX833676	Alvarez et al. (2016)
C. hibisci	CBS 109757ET	KX833589	–	–	KX833689	Marin-Felix et al. (2017)
C. javanica	CBS 45568T	KX833583	KX833403	KX833489	KX833683	Alvarez et al. (2016)

C. koreana	CBS 14397	KX833584	AF408378	KX833490	KX833684	Alvarez et al. (2016)
C. lanneae	CBS 141597T = CPC 22200	KX833585	KX833404	KX833491	KX833685	Alvarez et al. (2016)
C. limoniformis	CBS 111021T = PPRI 3870	KX833586	KX833405	KX833492	KX833686	Alvarez et al. (2016)
C. lustricola	DAOMC 251731T	MF631778	MF631799	MF651900	MF651899	Jayawardena et al. (2019)
C. macrospora	CBS 52473T = CPC 3935	KX833587	AY339292	KX833493	KX833687	Alvarez et al. (2016)
C. malaysiana	CBS 141598T = CPC 16659	KX833588	KX833406	KX833494	KX833688	Alvarez et al. (2016)
C. musaiaensis	CBS 109757 = AR 3534	KX833589	AF408337	–	KX833689	Alvarez et al. (2016)
C. nicotianae	CBS 87572T = PD 72/793	KX833590	KX833407	KX833495	KX833690	Alvarez et al. (2016)
C. nigra	CBS 16560T = IMI 181519	AY339319	KX833408	KX833496	KX833691	Van Niekerk et al. (2004);Alvarez et al. (2016)
C. obovata	CBS 111025 = CPC4196	AY339313	KX833409	KX833497	KX833692	Van Niekerk et al. (2004);Alvarez et al. (2016)
C. paracastaneicola	CBS 141292T = CPC 20146	KX833591	KX833410	KX833498	KX833693	Alvarez et al. (2016)
	CPC 25498	KX833592	KX833411	–	KX833694	Alvarez et al. (2016)
C. peruensis	CBS 110394T = RMF 7401	KJ710463	KJ710441	KX833499	KX833695	Crous et al. (2015a, b)
C. pseudogranati	CBS 137980T	KJ869132	–	–	–	Crous et al. (2014)
C. pseudostraminea	CBS 112624T = IMI 233050	KX833593	KX833412	KX833500	KX833696	Alvarez et al. (2016)
C. quercicola	CBS 90469NT	KX833595	KX833414	KX833502	KX833698	Alvarez et al. (2016)
	CBS 283.76	KX833594	KX833413	KX833501	KX833697	Alvarez et al. (2016)
	CPC 12133	KX833596	–	KX833503	KX833699	Alvarez et al. (2016)
C. solicola	CBS 76671T	KX833597	KX833416	KX833505	KX833701	Alvarez et al. (2016)
	CBS 114007 = IMI 253210	AY339320	KX833415	KX833504	KX833700	Alvarez et al. (2016)
	CPC 17308	KX833598	KX833417	–	KX833702	Alvarez et al. (2016)
C. straminea	CBS 14922 = CPC 3932	AY339348	AY339296	KX833506	KX833704	Van Niekerk et al. (2004);Alvarez et al. (2016)
C. tibouchinae	CBS 131595T = CPC 18512	JQ281774	KX833418	KX833507	JQ281778	Miranda et al. (2012); Alvarez et al. (2016)
	CBS 131594T = CPC 18511	JQ281774	KX833418	KX833507	JQ281778	Alvarez et al. (2016)
C. vitis	MFLUCC 16–1399*	KX890008	KX890083	–	KX890058	Jayawardena et al. (2019)
C. wangiensis	CBS 132530T = CPC 19397	JX069873	JX069857	KX833509	KX833705	Crous et al. (2012); Alvarez et al. (2016)
Melanconiella hyperopta	CBS 131696	JQ926281	JQ926281	KX833510	KX833706	Miranda et al. (2012); Alvarez et al. (2016)

Table 2. Morphology and host range differences among *Coniella* and its synonym *Pilidiella* species lacking molecular data.

Isolate	Conidia morphology	Host	Geography	Source
Coniella angustispora		*Psidium guajava*	Hawaii	Samuels et al. (1993)
C. australiensis	10–14 × 7–11 µm; ovoid, ellipsoid or subglobose conidia.	*Pelargonium australe*	Australia	Petrak (1955)
C. calamicola		*Daemonorops margaritae*	Hong Kong	Alvarez et al. (2016)
C. castaneicola	15–29 × 2.5–3.5 µm			Sutton (1980)
C. citri	8–19 × 3–4.5 µm	*Citrus medica*	India	Nag Raj (1993); Sharma and Agarwal (1977)
C. clypeata		decaying leaf	Japan	Alvarez et al. (2016)
C. costae	19–28 × 7–7.5 µm			Dianese et al. (1993)
C. delicata	7–9 × 2.5–3 µm, Ellipsoid	*Aerides crassifolia*	Thailand	Sutton (1980)
C. destruens	I= 12–13 µm, w = 4–5 µm, acutely rounded apices	*Eucalyptus grandis*	Hawaii	Van Niekerk et al. (2004)
C. eucalypticola	19–29 × 2.5–3.5 µm	*Eucalyptus sp.*	India	Nag Raj (1976)
C. genistae		*Genista tinctoria*	Germany	Alvarez et al. (2016)
C. minima	6.5–7.5 × 3.5–4.5 µm, Globoid to subgloboid	*Eucalyptus camaldulensis*	Burma (Myanmar)	Alvarez et al. (2016); Sutton (1980)
C. musaiaensis	15.5–22 × 4.5–5 µm	*Piliostigma thonningii, Acacia arabica, and from soil*	Sierra Leone, Pakistan, and India	Sutton (1980)
C. oryzae	ellipsoidal conidia	*Oryzae sativae*	Pakistan	Ahmad (1968)
C. petrakii	10–15.5 × 4.5–7 µm	*Vitis vinifera*	India	Sutton (1980)
C. petrakioidea	l:w = 1.9			Van Niekerk et al. (2004)

C. populina		*Populus tremula*	Russia	Alvarez et al. (2016)
C. stromatica	12.4–19.7 × 8–10 µm, longitudinal germ slit	tree bark	Brazil	Alvarez et al. (2016)
C. terminaliae	2–8 × 2–3.5 µm, Globose to subglobose	Terminalia tomentosa	India	Firdousi et al. (1994)
C. tibouchinae	10–13 × 6–8 µm, Broadly ellipsoid	*Tibouchina granulosa*		Miranda et al. (2012)
C. terminaliicola		*Terminalia superba*	Ecuador	Alvarez et al. (2016)
Pilidiella duvauicola		*Duvaue longifolia*	Argentina	Alvarez et al. (2016)
P. haraeana	l = 15–17.5 µm			Sydow and Sydow (1913)
P. jambolana	19–22 × 3.5–4 µm	*Eugenia jambolana*	Pakistan	Ahmad (1967)
P. tamaricina		*Tamarix articulata*	Pakistan	Ahmad (1967)
Coniella ferreirense		***Terminaliae ivorensis***	**Brazil**	**This study**
Coniella prostrata	**9–12 × 3–5 µm**	***Miconia calvescens***	**Brazil**	**This study**

4.4. ARTIGO 4

Reinstating *Ceratobasidium lantanae-camarae* the white thread blight fungus on the pantropical weed *Lantana camara*

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ORIGINAL PAPER

Reinstating *Ceratobasidium lantanae-camarae*: the white thread blight fungus on the pantropical weed *Lantana camara*

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Abstract
Ceratobasidium lantanae-camarae was a name proposed for a fungus associated with white thread blight disease of *Lantana camara* (common lantana): a Brazilian native plant listed amongst the world's ten worst weeds. This fungus was found during surveys in Brazil to assess the potential of coevolved fungal pathogens as classical biocontrol agents in the Paleotropics and Australasia. Later, in a monograph on *Rhizoctonia* forming-fungi, *C. lantanae-camarae* was synonymized with *C. cornigerum*: a polyphagous species recorded worldwide, but never previously from Latin America. The lack of pure cultures of *C. lantanae-camarae* did not allow a verification of the true status of the white thread blight fungus on lantana. Recently, the lantana disease was observed in localities in the Brazilian states of Rio de Janeiro and Amazonas. Pure cultures were obtained, DNA was extracted and phylogenetic analyses of the ITS-5.8S rDNA region were performed. Results showed that *C. lantanae-camarae* and *C. cornigerum* are phylogenetically distinct and that their synonymization was a taxonomic error. It was also found that, although *C. lantanae-camarae* is phylogenetically close to *C. papillatum*, morphological differences between these two species are evident. Thus, the name *C. lantanae-camarae* is re-established herein: an epitype and a reference ex-epitype sequence are indicated, resolving this taxonomic issue. Additionally, Koch's postulates were fulfilled for the first time. The significance of this taxonomic elucidation for weed biocontrol is discussed.

Keywords Biological control · Common lantana · Koch's postulates · Taxonomy

Introduction

Fungi belonging to the genus *Ceratobasidium* (*Ceratobasidiaceae*, *Cantharellales*) are characterized by producing effuse ceraceous fruitbodies, with globose to sphaeropedunculate basidia, produced directly from basal hyphae or in raceme-like groups, usually showing a division between hypo- and epibasidium and basidiospores with high rates of repetitive germination (Rogers 1935). *Ceratobasidium* spp. have, in common with members of *Thanatephorus*, a non-sporulating asexual *Rhizoctonia*-like stage. However, hyphal cells of *Ceratobasidium* spp. are binucleate whereas those of *Thanatephorus* spp. are multinucleate (González Garcia et al. 2006). Currently, there are several competing classifications based on the concept of anastomosis group (AG) for mono and binucleate *Rhizoctonia*-forming fungi. For *Ceratobasidium* (binucleate *Rhizoctonia*), the groups AG-A to AG-U and CAG-1 to CAG-7 are recognized (Carling 1996; González Garcia et al. 2006).

Ceratobasidium was initially proposed by Rogers (1935) to accommodate four taxa: *C. calosporum* (the type species), *C. cornigerum*, *C. sterigmaticum* and *C. obscurum*. Since this seminal work, the group has gone through numerous reviews with additions and changes in species names (González Garcia et al. 2006). The genus includes saprophytic species, symbiotic (orchid mycorrhizal) and plant parasitic taxa. *Ceratobasidium* includes pathogens of members of the Annonaceae, Rosaceae, Rubiaceae, Rutaceae and Theaceae, typically in tropical agroecosystems in Africa, Asia and South America (Farr and Rossman 2020). White thread blights have been reported in crops such as yerba mate (Lima et al. 2019), coffee (Belachew et al. 2015) and a series of unrelated plants in the Amazon, namely: African mahogany (*Khaya ivorensis*), 'araçá-boi' (*Eugenia stipitata*), bananas (*Musa* × *paradisiaca*, cv. yangambi), cinnamon

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(*Cinnamomum zeylanicum*), coconuts (*Cocos nucifera*), ixora (*Ixora coccinea*), neem (*Azadirachta indica*), heliconia (*Heliconia psitacorum*, cv. Golden Torch) and 'ipê-amarelo' (*Tabebuia serratifolia*) (Bechimol et al. 2001; Costa et al. 2013).

Ceratobasidium lantanae-camarae was described by Barreto et al. (1995) as one, among other fungi, of the Brazilian mycobiota of the weed *Lantana camara* (*Verbenaceae*). Common lantana was ranked among the worst weeds in the world by Holm et al. (1991). It is a neotropical prickly shrub which may be toxic to cattle and was distributed throughout the tropics as an ornamental, where it escaped to invade pasturelands and natural habitats. For complete and updated information see CABI (2020). Barreto et al. (1995) distinguished *C. lantanae-camarae* from other species of *Ceratobasidium* by host, growth habit and morphological characteristics, mainly basidiospore shape and size (Barreto et al. 1995). In a monograph on *Rhizoctonia* forming-fungi (Roberts 1999), the holotype, deposited in Herb IMI, was re-analysed but reported to be very scarce, few sporulating structures were found in the herborized material. Nevertheless, based on the type description (Barreto et al. 1995), especially, basidiospore size and morphology, *C. lantanae-camarae* was considered to be indistinguishable from *C. cornigerum* and, subsequently, reduced to synonymy (Roberts 1999; see Index Fungorum and MycoBank).

Historically, the taxonomy of *Ceratobasidium*, has been based mainly on morphological characteristics and host identity. This has led, over time, to the proposition of a series of uncertain names and opinion-based synonymizations (Rogers 1935; Roberts 1999; Oberwinkler et al. 2013). Few taxonomic studies of *Ceratobasidium* spp. have included molecular information. The exceptions are the phylogenetic studies based on the molecular marker rDNA ITS-5.8S of Ceresini et al. (2012) and De Melo et al. (2018). None of these, however, involved an elucidation of the status of *C. lantanae-camarae*.

Recent observations were made of white thread blight attacking *L. camara* in two localities in Brazil, offering the opportunity for a more detailed examination of the fungus and a reappraisal of its taxonomic status. Results of this study are presented here.

Material and methods

Sample collection and processing

Serendipitous findings of white thread blight of common lantana were made in Brazil in two distant and climatically different localities in the vicinities of Iracema Falls at Presidente Fiqueiredo, state of Amazonas – AM (01° 097′ 98" S, 60° 04′ 98" O) in 28 Jun 2019, and Riograndina, Nova Friburgo, state of Rio de Janeiro – RJ (22º 16′73" S, 42º 53′65" W) in 28 Apr 2018: the former in a lowland equatorial situation (*ca.* 120 m); and the latter in a subtropical highland habitat (*ca.* 1100 m). Samples were examined, while still fresh, under a dissecting microscope (Olympus SZX7). Selected specimens were dried in a plant press and representative specimens were deposited in the herbarium of the Universidade Federal de Viçosa (VIC).

Pure cultures were obtained by transfer of basidiospores, from the colonies formed on stems and leaves, onto potato dextrose-agar (PDA) plates, with a sterile fine-tipped needle. A representative isolate from each region was deposited in the culture collection of the Universidade Federal de Viçosa (Coleção Octávio de Almeida Drummond, COAD).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from single spore colonies grown in PDA at 25 ºC, under 12-h daily/light regime (light provided by two white and one near-UV lamps placed 35 cm above the plates), for seven days. *Ca.* 40 mg of mycelium were scraped from the surface of the PDA plates and placed inside sterile plastic tubes containing zirconium spheres and placed in a grinder (LBeader-3, Loccus Biotecnologia). After 5 s of crushing, the resulting suspension was drained into a sterile plastic tube and used for DNA extraction. The Wizard Genomic DNA Purification Kit was utilized for DNA extraction and the manufacturer's protocol was followed.

Primers ITS5 and ITS4 (White et al. 1990) were used to amplify the transcribed internal spacer (ITS) region. The thermal cycle consisted of 95 ºC for 5 min, followed by 35 cycles of 94 ºC for 1 min (denaturation), 52 ºC for 1 min (annealing), 72 ºC for 2 min (elongation) and 72 ºC for 10 min (final extension). The polymerase chain reaction (PCR) products were analyzed with 2% agarose electrophoresis gels stained with GelRed ™ (Biotium Inc., Hayward, CA, USA) and viewed under UV light to check the size and purity of the amplification.

The PCR products were purified and sequenced by Macrogen (South Korea). The nucleotide sequences were combined with SeqAssem (Hepperle 2004). All sequences were checked and adjusted manually, and ambiguous nucleotide positions were clarified using sequences from both DNA strands. The sequences were subjected to a BLASTn search for similar sequences in GenBank. The sequences were aligned with the most similar sequences found in GenBank, together with those provided in De Melo et al. (2018), using MUSCLE (Edgar 2004) and manually corrected with MEGAv. 6 (Tamura et al. 2013). The sequences obtained

in this study were deposited in GenBank (Accession Nos. MW361942 and MW361943).

Morphological characterization

Culture descriptions were based on observations of 4-day-old colonies on PDA and potato-carrot-agar (PCA), grown at 25 °C under a daily regime of alternating 12 h / light (light provided by two white lamps and a near UV lamp, placed 35 cm above the plates) and 12 h dark. Colour terminology followed Rayner (1970).

Morphology was described based on the observation of slides mounted by scraping fungal structures from the colonies on lantana with a scalpel and placing these on drops of either lactofuchsin or lactoglycerol. The slides were examined under a light microscope (Olympus, BX51) equipped with a digital capture system (Olympus Q-Color 3™). Biometric data was obtained through the measurement of at least 30 fungal structures.

Phylogenetic analysis

Three distinct phylogenetic analyses, based on the ITS-5.8S rRNA region, were performed with DNA sequences from the common lantana isolates and reference sequences from selected sequences from GenBank were included (Tables 1 and 2). The first study aimed to demonstrate the phylogenetic placement of *Ceratobasidium* from *L. camara* in the genus *Ceratobasidium*. The second study served to elucidate the relationship of *Ceratobasidium* from *L. camara* with members of the binucleate *Rhizoctonia* anastomosis groups. The third study aimed at clarifying the relationship of *Ceratobasidium* from *L. camara* with multinucleate *Rhizoctonia* (*Thanatephorus cucumeris*). Two analyzes were carried out: Bayesian Inference (BI) and Maximum Likelihood (ML).

BI phylogenetic analyses were performed on CIPRES portal (Miller et al. 2010) using MrBayes v. 3.2.6 (Ronquist et al. 2012), based on Markov Monte Carlo Chain (MCMC) with 10,000,000 generations using the nucleotide substitution model informed by the Akaike Information Criterion (AIC) on software MrModeltest v. 2.3 (Posada and Crandall 1998). Trees were sampled every 1000 generations, burning 25% of all trees obtained. Phylogenetic trees were visualized using the FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and exported to graphics programs.

ML trees were generated with the nearest-neighbor-interchange (NNI) ML heuristic method and the Tamura-Nei substitution model as tree inference options in MEGA. The branch stabilities of the phylogenetic tree were assessed by using the bootstrap re-sampling strategy with 1000 bootstrap test replicates. The resulting tree topologies using the two methods (ML and BI) were then compared, and the phylogram was edited with InkScape 0.91 (www.inkscape.org).

Pathogenicity tests

Young healthy 25 cm tall common lantana plants were selected for the pathogenicity test. Four plants were inoculated by placing 1 cm diam culture plugs (taken from the margin of two week-old colonies on PDA) on the surface of selected leaves (6 plugs per plant). After inoculation, the plants were kept in a dew chamber: two for a period of 48 h, and then taken to a greenhouse bench, and two were kept in the dew chamber until the onset of symptoms. Another group of four plants was inoculated with an asexual propagule suspension, at a concentration of 1.0×10^6 propagules/mL. The suspension was obtained by flooding seven days-old colonies on PDA plates with 5 mL of sterile water. The surface of the medium was then scraped with a rubber spatula to release the propagules. The contents of the plates were then sieved through a layer of cheesecloth and the concentration of the suspension was calibrated using a haemocytometer. The suspension was then sprayed on 4–6 leaves of each plant until runoff. Two plants sprayed only with sterile distilled water and two plants on which non-colonized PDA plugs placed over leaves served as controls. After the onset of symptoms, necrotic leaves were removed and observed under a stereomicroscope. Isolation of the fungus was performed by transfer of propagules, from the colonies formed on leaves, onto potato dextrose-agar (PDA) plates, with a sterile fine-tipped needle. Propagule-forming pure cultures of the fungus were obtained and compared to the original culture, in order to fulfill Koch's postulates.

Results

Although the disease has been observed at four instances, at localities at the Brazilian states of Amazonas, Pará and Rio de Janeiro, the fungus is difficult to isolate as the mycelial threads are exposed externally and contamination is very difficult to avoid. Additionally, there seems to be a quick loss of viability in herbarium samples. Only two isolates were obtained, after several attempts: COAD 2919 from Presidente Figueiredo, AM, Brazil and COAD 2918, from Nova Friburgo, RJ, Brazil.

Phylogeny

ITS amplicons were obtained,-generating sequences of approximately 574 and 617 bp for COAD2919 and COAD 2918, respectively. The alignment for the construction of

Table 1 Strains and GenBank accession numbers of *Ceratobasidium* spp. (binucleate *Rhizoctonia*) used in phylogenetic analyses in this study

Haplotype	Anastomosis groups	Species name	Culture collection number[a]	Isolate	Host/Substrate	Origin[b]	Accession (ITS)[c]
H1	AGA	–	–	C-662	–	Japan	AF354092
–	AGBo	–	–	SIR-2	*Ipomoea batatas*	Japan	AF354091
H2	AG-L	–	–	FK02–1	Soil	Japan	AF354093
H4	CAG7	–	–	BN22	*Pittosporum*	EUA	AF354084
H5	AGR	–	–	–	*Cucumis* sp.	EUA	AJ427407
H7	CAG6	–	–	BN74	*Erigeron*	EUA	AF354083
H8	AG-E	–	–	–	–	Netherlands	DQ279013
H9	AG-C	–	–	–	–	Netherlands	DQ279046
H11	CAG4	–	–	BN38	*Glycine max*	EUA	AF354081
H14	AGD	–	–	C-610	–	Japan	AF354090
H16	AG-Q	–	–	–	–	Netherlands	DQ279058
H17	CAG1	–	–	BN1	Grass	EUA	AF354086
H18	AG-D	–	–	–	–	Netherlands	DQ279060
H20	AG-U	–	–	MWR-20	*Rosa hybrida*	Japan	AB196664
H22	AG-P	–	–	–	–	Netherlands	DQ279015
H23	CAG3	–	–	BN31	*Arachis hypogaea*	EUA	AF354080
H25	CAG5	–	–	BN37	*Cucumis sativus*	EUA	AF354082
H26	AG-F	–	–	–	–	Netherlands	DQ279014
H27	AG-I	–	–	IMI 375130	–	United Kingdom	DQ279064
H29	AG-Bb	–	–	–	–	Netherlands	DQ279058
H30	AG-Ba	–	–	–	–	Netherlands	DQ279059
H31	AG-S	–	–	–	*Pittosporum* sp	EUA	AJ427400
H33	AG-G	–	–	–	–	Netherlands	DQ279049
H34	AGBa	–	–	C-460	*Oryza sativa*	Japan	AF354088
H35	AG-K	–	–	–	–	Netherlands	DQ279056
H36	AGQ	–	–	C-620	Soil	Japan	AF354095
H37	AGO	–	–	FK06–2	Soil	Japan	AF354094
H38	AG-O	–	–	–	–	Netherlands	DQ279045
H39	AGBo	–	–	–	–	Netherlands	DQ279057
H40	AGH	–	–	STC-9 s	Soil	Japan	AF354089
H41	AGH	–	–	–	–	Netherlands	DQ279065
H42	AGT	–	–	4Oit-800	*Reseda odorata*	Japan	AB196661
–	AGA	–	–	–	–	–	DQ279052
–	–	*C. chavesanum*	CML 3468	DK2c2a	*Diospyros kaki*	SP, Brazil	EU810049
–	–	*C. chavesanum*	CML 3469	DK12A2	*D. kaki*	SP, Brazil	EU810048
–	–	*C. chavesanum*	CML 3470	DK10c2a	*D. kaki*	SP, Brazil	EU810045
–	–	*C. chavesanum*	CML 3471	DK11c2a	*D. kaki*	SP, Brazil	EU810047
–	–	*C. chavesanum*	CML 3472	DK11b1a	*D. kaki*	SP, Brazil	EU810046
–	–	*C. chavesanum*	CML 3473	10BRCA21	*Coffea arabica*	ES, Brazil	KX870112
–	–	*C. chavesanum*	CML 3474 T	10BRCA25	*C. arabica*	ES, Brazil	KX870113
–	–	*C. chavesanum*	CML 3475	10BRCA15	*C. arabica*	ES, Brazil	KX870114
–	–	*C. chavesanum*	CML 3476	10BRCA22	*C. arabica*	ES, Brazil	KX870115
–	–	*C. chavesanum*	CML 3481	10BRCA13	*C. arabica*	ES, Brazil	KX870116
–	–	*C. niltonsouzanum*	CML 3477	CS161	*Camellia sinensis*	SP, Brazil	EU810032
–	–	*C. niltonsouzanum*	CML 3478	CS94a	*C. sinensis*	SP, Brazil	EU810043
–	–	*C. niltonsouzanum*	CML 3479	CS721	*C. sinensis*	SP, Brazil	EU810037
–	–	*C. niltonsouzanum*	CML 3480	CS512	*C. sinensis*	SP, Brazil	EU810031
–	–	*C. niltonsouzanum*	CML 3482	CS1021	*C. sinensis*	SP, Brazil	EU810028

Table 1 (continued)

Haplotype	Anastomosis groups	Species name	Culture collection number[a]	Isolate	Host/Substrate	Origin[b]	Accession (ITS)[c]
–	–	*C. niltonsouzanum*	CML 3483	CS1032	*C. sinensis*	SP, Brazil	EU810029
–	–	*C. niltonsouzanum*	CML 3596	MPM 201	*Azadirachta indica*	PI, Brazil	KX870111
–	–	*C. niltonsouzanum*	CML 3597	MPM 110	*Eugenia uniflora*	PI, Brazil	KU175889
–	–	*C. niltonsouzanum*	CML 3598 T	MPM 109	*E. uniflora*	PI, Brazil	KU175888
–	–	*C. albasitensis*	CBS 152.32	–	*Pteridium aquilinum*	UK	AJ427402
–	–	*C. bicorne*	–	1231	*Polytrichastrum formosum*	Filand	AF200514
–	–	*C. bulbillifaciens*	CBS 132236	–	*Bark of Fraxinus*	Germany	KC336072
–	–	*C. angustiporum*	CBS 568.83	–	*Pterostylis mutica*	South Australia	AJ427403
–	–	*C. cereale*	–	Sequence 17[d]	–	Switzerland	AX195385
–	–	*C. cereale*	–	Sequence 19[d]	–	Switzerland	AX195387
–	–	*C. cereale*	–	Sequence 22[d]	–	Switzerland	AX195390
–	–	*C. cereale*	–	Sequence 23[d]	–	Switzerland	AX195391
–	–	*C. cereale*	–	Sequence 24[d]	–	Switzerland	AX195392
–	–	*C. cereale*	CBS 558.77	–	*Secale cereale*	Germany	AJ302008
–	–	*C. cereale*	CBS 559.77	–	*Triticum aestivum*	Germany	AJ302009
–	–	*C. cereale*	–	–	*Poa annua*	USA	AF063019
–	–	*C. cereale*	–	99,125	*Agrostis palustris*	Canada	AF222793
–	–	*C. cornigerum*	CBS 133.82	–	*Pittosporum* sp.	USA	AJ301899
–	–	*C. cornigerum*	CBS 135.82	–	*Juniperus* sp.	USA	AJ301900
–	–	*C. cornigerum*	CBS 136.82	–	*Taxus* sp.	USA	AJ301901
–	–	*C. cornigerum*	CBS 137.82	–	*Erigeron canadenses*	USA	AJ301902
–	–	*C. cornigerum*	CBS 132.82	–	*Festuca* sp.	USA	AJ301903
–	–	*C. cornigerum*	CBS 139.82	–	*Pittosporum* sp.	USA	AJ302006
–	–	*C. lantanae-camarae*	COAD 2919	–	*Lantana camara*	AM, Brazil	MW361942
–	–	*C. lantanae-camarae*	COAD 2918 T	–	*Lantana camara*	RJ, Brazil	MW361943
–	–	*C. cornigerum*	–	Eab-aB	*Medicago sativa*	Spain	AJ302010
–	–	*C. noxium*	CBS 154.35	–	*Coffea arabica*	India	EU810056
–	–	*C. papillatum*	CBS 570.83	–	*Sarcochilus dilatatus*	Australia	AJ427401
–	–	*C. ramicola*	CBS 758.79	–	*Pittosporum* sp.	USA	AJ427404

[a]Culture collections *CBS* Centraalbureau voor Schimmelcultures-Fungal Biodiversity Center Utrecht The Netherlands *CML* Coleção Micológica de Lavras Universidade Federal de Lavras Lavras Brazil *COAD* Coleção Octávio de Almeida Drummond Universidade Federal de Viçosa Viçosa Brazil T Specimen type

[b]States of Brazil *AC* Acre AM Amazonas *ES* Espírito Santo, *PI* Piauí *SP* São Paulo *RJ* Rio de Janeiro

[c]The ITS-5.8S rDNA sequences used in the phylogenetic analyses

[d]Sequences from the patent WO0151653

the phylogenetic tree with the sequences available for *Ceratobasidium* spp. included 52 strains. The matrix consisted of 795 characters including alignment gaps. For the BI analysis, a GTR + G model was selected. The BI and ML tree topologies were not in conflict. Phylogeny indicates that the isolates formed a clade distant from *C. cornigerum* (to which *C. lantanae-camarae* was, mistakenly, synonymized) and close to *C. papillatum* (Fig. 1). A similar data set comparing the new sequences for *C. lantanae-camarae* with the reference isolates for Anastomosis Groups (AG) from binucleate *Rhizoctonia*, included 52 strains. The combined matrix consisted of 1767 characters including alignment gaps. The number of conserved sites was 1108. The number of variable sites were 525. For the BI analysis, a GTR + I + G model was selected. The trees obtained with ML and BI agreed on

Table 2 Information about *Thanatephorus cucumeris* (multinucleate *Rhizoctonia solani* Anastomosis Groups) reference isolates and their GenBank accession numbers

Haplotype	Anastomosis groups	Isolate	Host/Substrate	Origin[a]	Accession[b] (ITS)
H3	AG-12	CH1	–	Australia	AF153803
H4	AG-1 IA	SJ067	*Glycine max*	GO, Brazil	AY270011
H5	AG-1 IC	3Rs	*Pinus* spp.	Canada	AF354058
H7	AG-7	91ST8057-2A RSA	Soil	EUA	AF354100
H16	AG-2-2 IV	IV BC-10	–	Japan	AB000014
H18	AG-2-2 IIIB	15Rs	*Juncus* sp.	Japan	AF354116
H19	AG-2-2 LP	48R	–	Japan	AB054866
H20	AG-5	19Rs	*Glycine max*	Japan	AF354113
H22	AG-11	ROTH25	–	Japan	AB019027
H23	AG-4 HGII	HG-II UHBC	–	Japan	AB000045
H26	AG-2-3	237,258	–	Japan	AB019025
H27	AG-4 HGIII	44Rs	*Beta vulgaris*	EUA	AF354075
H29	AG-6 HGI	70Rs	Soil	Japan	AF354102
H32	AG-3 TB	1600NC	*Nicotiana tabacum*	EUA	AF153774
H33	AG-2-1	R144	–	Japan	AB054852
H35	AG-9 TP	TP V12 M	–	Japan	AB000046
H36	AG-9	65Rs	*Solanum tuberosum*	EUA	AF354109
H37	AG-9 TX	TX S4R1	–	Japan	AB000037
H38	AG-10	76,107	–	Japan	AB019026
H40	AG-4 HGI	HG-I GM-3	–	Japan	AB000018
H44	AG-1 ID	RCP13	*Coffea* sp.	Japan	AB122130
H48	AG-1 IB	1B SHIBA-1	–	Japan	AB000039
H49	AG-4 HGI	HG-I 78-23R-3	–	Japan	AB000007
H51	AG-6 GV	75Rs	Soil	Japan	AF354104
H53	AGBI	TE2–4	–	Japan	AB054873
H54	AG-8	(ZG1–4)88351	*Hordeum vulgare*	Australia	AF354068
H57	AG-3 PT	42Rs	*Solanum tuberosum*	EUA	AF354106

[a]State of Brazil *GO* Goiás

[b]The ITS-5.8S rDNA sequences used in the phylogenetic analyse

topology. The isolates formed a clade separate from the other anastomosis groups (AG), close to AG–S and AGQ (Fig. 2). The alignment with multinucleate *R. solani* AGs included 52 strains. The combined matrix consisted of 841 characters including alignment gaps. The number of conserved sites was 412. 337 sites were variable. For the BI analysis, a GTR + I + G model was selected. The BI and ML tree topologies were not in conflict. The isolates formed a clade separate from the other *R. solani* AGs (Fig. 3)

Morphology

Ceratobasidium lantanae-camarae H. C. Evans, R. W. Barreto & C. A. Ellison emend. descr. Figure 4

Original description: see Barreto et al. (1995).

Lesions initially absent from areas of advancing colonies, later appearing in direct connection with the expanding mycelial threads and fans on both sides of leaves as irregular to angular, necrotic, occasionally water-soaked areas, 1 – 20 mm wide, pale brown to tan with a distinct dark brown border; coalescing to cover entire leaf surface, leading to disintegration and death of the leaf; dead leaves remaining attached to branches by mycelial threads. Mycelium composed of loosely parallel-growing, sterile, branched, septate, 4 – 6 µm diam hyphae, aggregating to form strands (threads) or fans over the stems and leaves; forming clumps at the axil of branches or the base of petioles, associated with leaf blight or branch death. Fertile portions of colonies on leaves, hypophyllous, resupinate, forming a whitish web-like subiculum on young leaves over which sporulation is concentrated. Basal hyphae filamentous, 5 – 7 µm diam, septate, without clamp-connections, branched, hyaline, smooth. Basidia subglobose to obovate, 13 – 17 × 8 – 9 µm, with 4

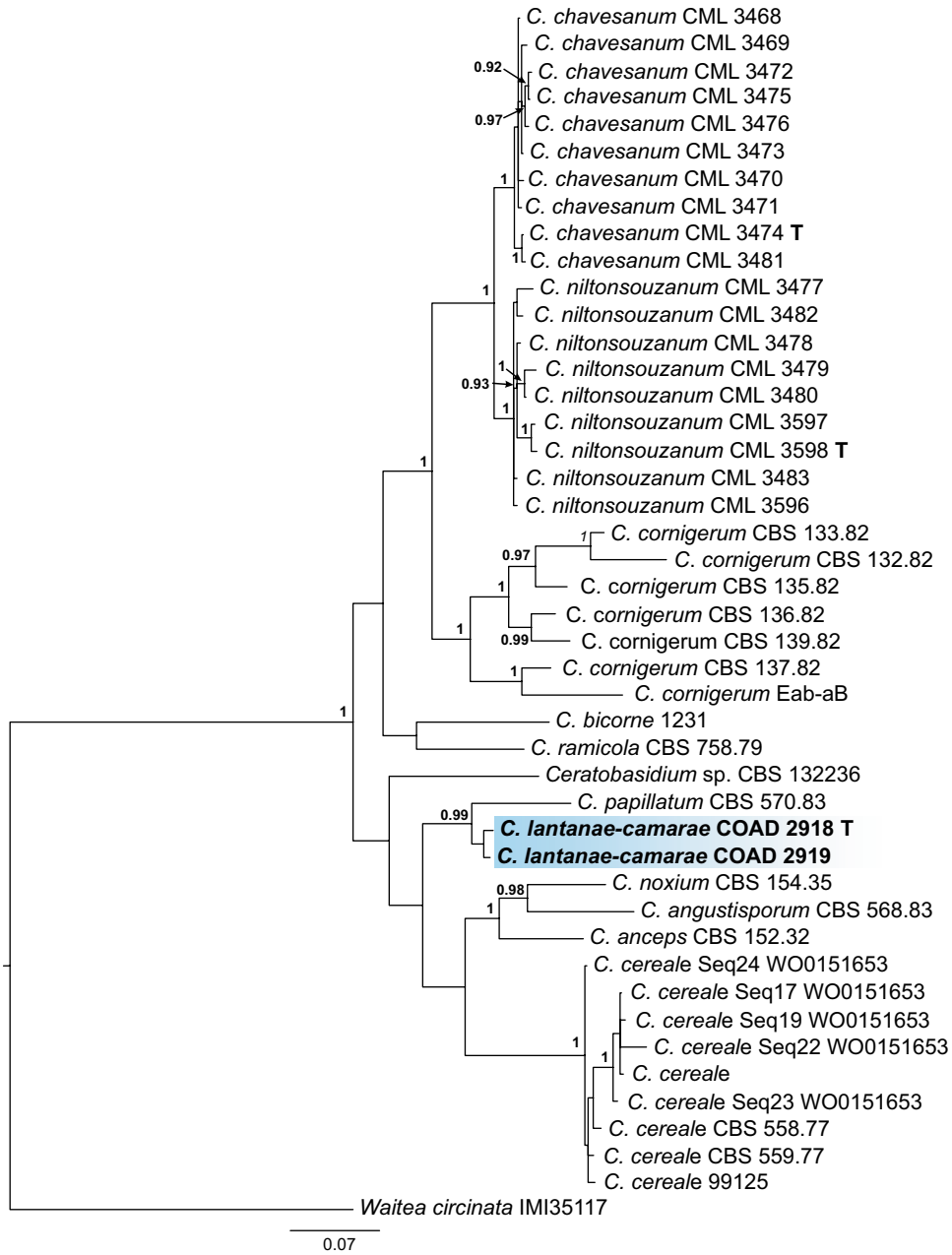

Fig. 1 Bayesian Inference phylogram based on ITS-5.8S rRNA showing the relationship of *Ceratobasidium lantanae-camarae* with other species within *Ceratobasidium*. Sequences from ex-type strains, indicated with a **t**. Probability values > 0.9 are presented above the nodes. Isolates from this study are indicated by bold text

digitate sterigmata, 6 – 12 × 2 – 3 µm, ending in a blunt apex. Basidiospores asymmetrically subglobose to broadly ellipsoid, 6.5 – 8 × 4 – 7 µm, aseptate, strongly apiculate, apiculus 1.0 – 1.5, hyaline, smooth. Asexual propagules, cylindrical, rounded at both ends, straight, 23 – 107 × 5 – 12 µm, 0 – 3 septate.

In culture: Colonies on PDA, fast-growing (7 cm diam in 4 days), raised, entire or undulate edges, aerial mycelium woolly white; on PCA fast-growing (6.5 cm diam in 4 days), flat, undulate edges, aerial mycelium felty to sparse, white. Abundant formation of asexual propagules and monilioid hyphae in the aerial mycelium on the entire surface of the colony after 7 days on PDA and PCA.

Material examined: Brazil: Amazonas, Presidente Fiqueiredo, Iracema Falls, on *Lantana camara*, 28 Jun 2019, R. W. Barreto (VIC 47,338– epitype designated here; epitype culture – COAD 2919, MBT395235); Rio de Janeiro, Nova Friburgo, Riograndina, Fazenda Barreto, on *Lantana camara*, 28 Apr 2018, R. W. Barreto, (VIC 47,204, culture COAD 2918).

Notes: The original description of *C. lantanae-camarae* was based on a relatively poorly preserved herbarium specimen and no cultures of the fungus were available (Barreto

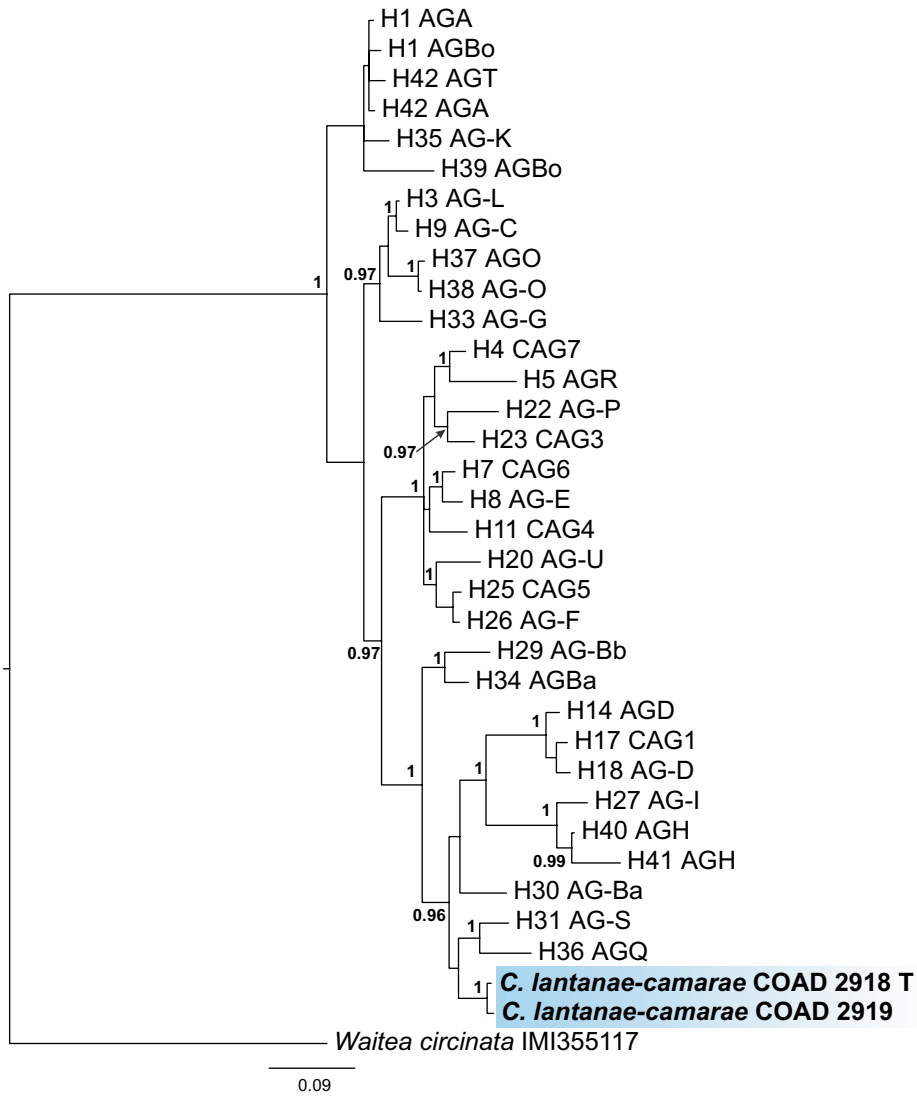

Fig. 2 Bayesian Inference phylogram based on ITS-5.8S rRNA showing the relationship of *Ceratobasidium lantanae-camarae* with *Ceratobasidium* species (binucleate *Rhizoctonia*). Anastomosis Groups (AG-A—AG-U and CAG-1—CAG-7), haplotype **h**, and sequences from ex-type **t** strains are indicated. Posterior probability values > 0.9 are presented above the nodes. Isolates from this study are indicated by bold text

et al. 1995). Here, an emended description is provided including a culture description and observations on asexual propagules which are readily produced in vitro but were not present on field samples. The morphological description of the recently collected isolates differs little from the type description (Barreto et al. 1995). The most striking difference is in relation to the length of the sterigmata, which are longer than those reported by Barreto et al. (1995), 6 – 12 µm here and up to 7 µm in the original description.

Pathogenicity tests

Leaves of plants inoculated with mycelial discs and asexual propagule suspension, and kept for 48 h in the dew chamber, did not show any symptoms. However, plants kept continuously in the dew chamber, showed symptoms after 14 days – when inoculated with mycelial discs – or 30 days – when inoculated with propagule suspension. The symptoms began with small, watersoaked necrotic patches, which grew in size and led to leaf blight. The infection progressed to the subjacent twig which also became necrotic. The death of the entire stem and leaves followed. Over time, a white fungal mycelial cord (typical of thread blights) appeared over the plant branches (Fig. 5c-f). Controls remained healthy under all conditions (Fig. 5a, b). *Ceratobasidium lantanae-camarae* was re-isolated from the diseased tissues.

Discussion

According to Oberwinkler et al. (2013), the shape of basidiospores is the most significant character for identifying species of *Ceratobasidium* and Roberts (1999) based his synonymization of *C. lantanae-camarae* with

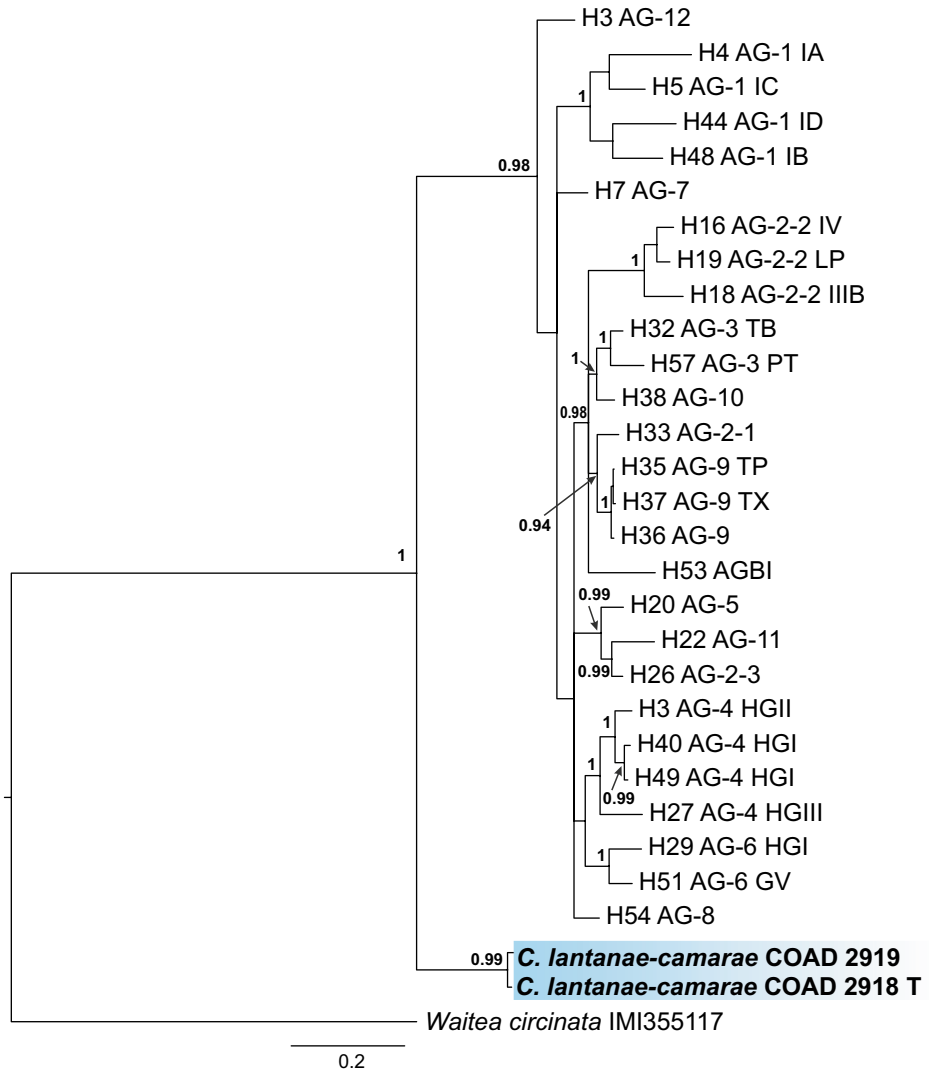

Fig. 3 Bayesian Inference phylogram based based on ITS-5.8S rRNA showing the relationship of *Ceratobasidium lantanae-camarae* with *Thanatephorus cucumeris* (multinucleate *R. solani* AGs). Anastomosis Groups (AG) and subgroups, haplotype **h**, and sequences from ex-type **t** strains are indicated. Posterior probability values > 0.9 are presented above the nodes. Isolates from this study are indicated by bold text

C. cornigerum on this character. Until the recent collections, it was not possible to contest this decision because of the lack of fresh specimens and cultures. The results of the phylogenetic study presented herein, now including DNA from pure cultures of *C. lantanae-camarae*, clearly demonstrates that *C. lantanae-camarae* is in a clade which is distant from that containing *C. cornigerum*. Therefore, the synonymization of the former with the latter was a taxonomic error.

Ceratobasidium lantanae-camarae is close to *C. papillatum*, a species proposed by Warcup and Talbot (1980) associated with *Sarcochilus dilatatus*, *S. olivaceus* and *Thrixspermum congestum*, which are all Australian Orchidaceae. *Ceratobasidium lantanae-camarae* and *C. papillatum* have a restricted disjunct geographic distribution and occupy rather distinct niches – pathogenic vs. endomycorrhizal. Additionally, their morphology is distinct. Basidiospores of *C. papillatum* are obovate to ellipsoid, 9 – 11.5 µm long, whereas in *C. lantanae-camarae* these are asymmetrically subglobose to broadly ellipsoid, 6 – 7 µm long. *Ceratobasidium papillatum* has basidia with sterigmata borne on a lateral swelling with the diagnostic papillate apical prolongation of the basidia. This pattern is not found in *C. lantanae-camarae*. A novel characteristic, not included in the original description of *C. lantanae-camarae*, because of the lack of pure cultures of the fungus at the time, is the production of propagules and monilioid cells in culture. The formation of such asexual propagules is common in *Rhizoctonia*-forming fungi (Shan et al. 2002; Hossain 2019) but has never been reported for *C. papillatum*.

Besides *C. lantanae-camarae*, the following species of *Ceratobasidium* have been reported in Brazil by De Melo et al. (2018), Farr and Rossman (2020) and Macedo et al. (2016): *C. anceps* on *Hevea brasiliensis* and *Hevea* sp.; *C. noxium* on *Piper nigrum*; *C. ochroleucum* on *Cinnamomum zeylanicum*, *Eugenia stipitata*, *Heliconia psittacorum* and

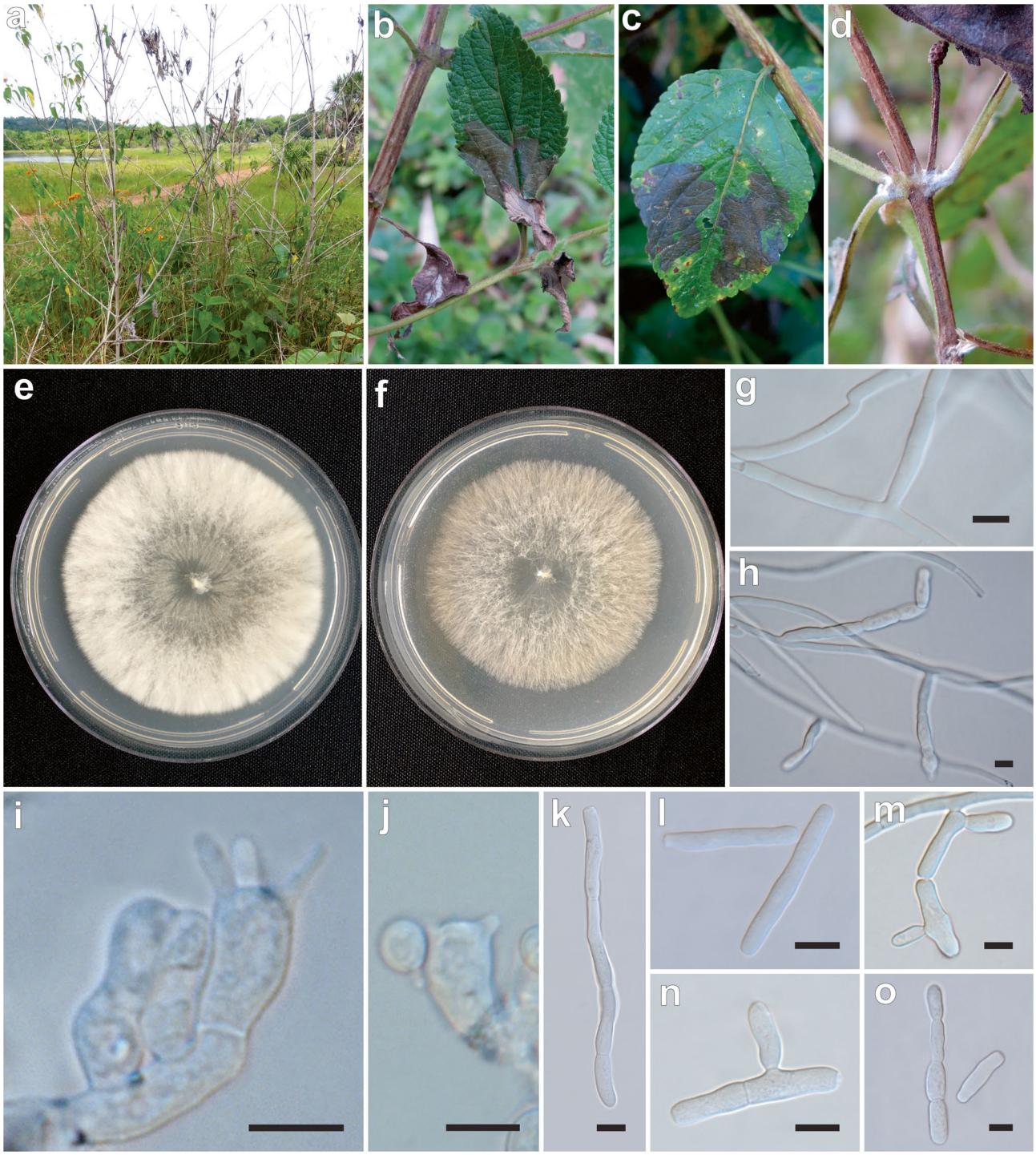

Fig. 4 **a** *Lantana camara* plants severely attacked by *Ceratobasidium lantanae-camarae* at Presidente Figueiredo (state of Amazonas, Brazil); **b–c** Leaf blight symptoms; **d** Mycelial cord, typical of white thread-blight on *Lantana camara* branch; **e** *Ceratobasidium lantanae-camarae* colony on PDA; **f** Colony on PCA; **g** Hypha showing typical *Rhizoctonia* 90° branching; **h** Monilioid hyphae; **i** Two immature basidia (left) and one mature basidium (right) – note well developed sterigmata on mature basidium; **j** Basidium bearing one subspherical basidiospore; **k–o** Asexual propagules of *Ceratobasidium lantanae-camarae* on PDA and PCA

Tabebuia serratifolia; *C. tradescantiae* on *Tradescantia fluminensis*; *C. chavesanum* on *Coffea arabica* and *Diospyros kaki*; and *C. niltonsouzanum* on *Azadirachta indica*, *Camellia sinensis* and *Eugenia uniflora*. *Ceratobasidium anceps*, *C. noxium*, *C. chavesanum* and *C. niltonsouzanum* belong to distant clades in the *Ceratobasidium* phylogenetic

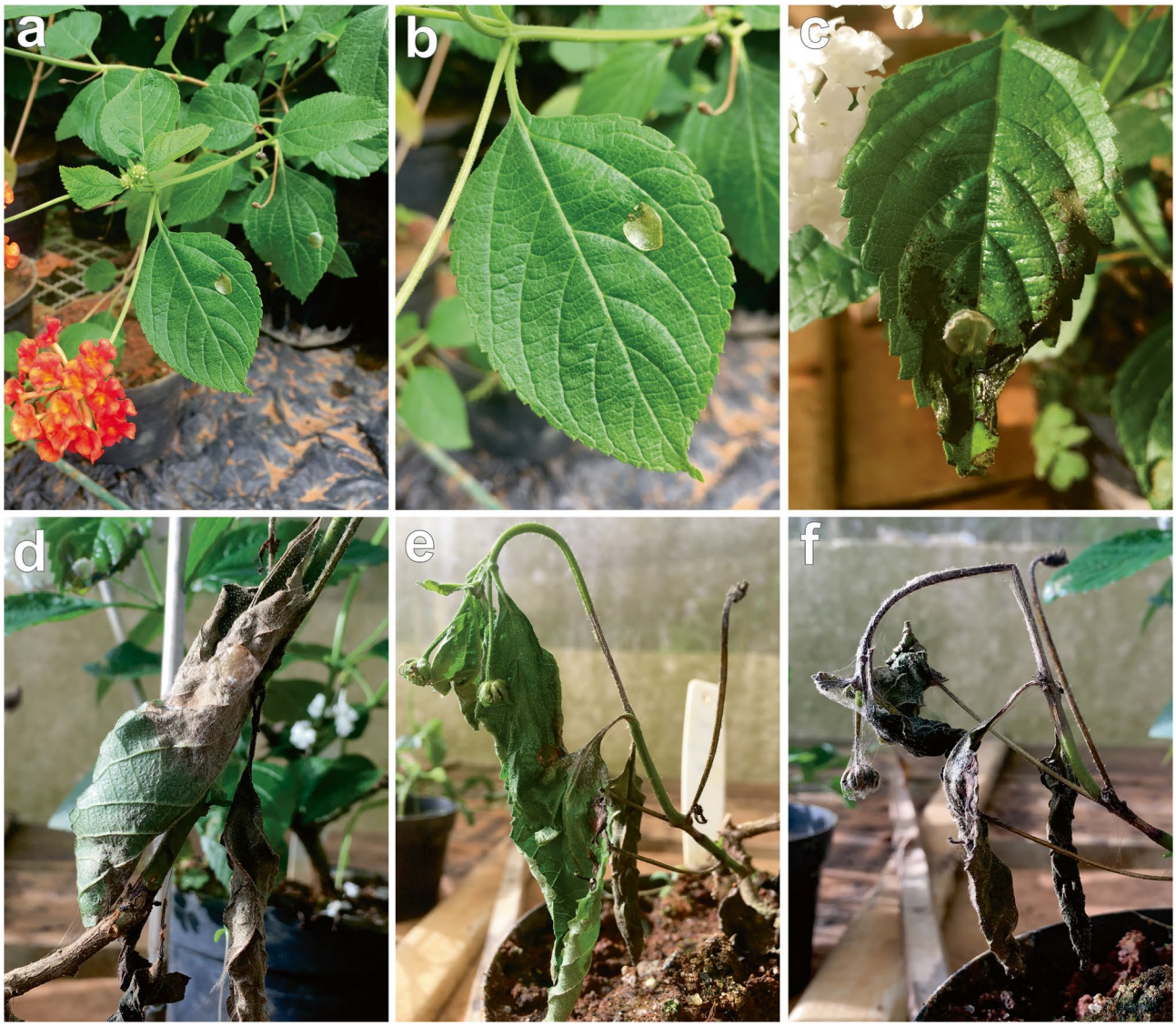

Fig. 5 Inoculation experiment of *Ceratobasidium lantanae-camarae* on *Lantana camara.* **a**–**b** Control plants receiving non-colonized disks of culture medium*;* **c**–**f** *L. camara* plants inoculated with mycelium disks of *C. lantanae-camarae;* **c** Symptoms starting as irregular to angular, pale brown necrotic spots on inoculated leaves; **d**–**e** Necrotic lesions coalescing and leading to blight of entire leaves and stems*;* **f** Branch dieback resulting from disease progress (note white mycelial cords typical of thread blight 30 days after inoculation)

tree. *Ceratobasidium ochroleucum* and *C. tradescantiae* are morphologically different from *C. lantanae-camarae*. *Ceratobasidium ochroleucum* (originally described as *Hypochnus ochroleucus* by Noack from *Pyrus malus* in Brazil) has longer basidiospores 8.5 – 10 µm *vs* 6.5 – 8 µm in *C. lantanae-camarae* and Noack (see Saccardo, 1902) made no mention of his fungus having apiculate basidiospores (as is the case of *C. lantanae-camarae*). In contrast to *C. lantanae-camarae*, the basidia in *C. tradescantiae* are shorter (8–14 µm *vs* 13 – 17 µm in *C. lantanae-camarae*) and bear shorter sterigmata (3–8 µm *vs* 6 – 12 µm in *C. lantanae-camarae*). Sterigmata of *C. tradescantiae* are also differently shaped: horn-like with acute base *vs* digitate with blunt base in *C. lantanae-camarae*.

Our conclusion is, therefore, that *C. lantanae-camarae* is a valid name for a distinct taxon of *Ceratobasidium* which was incorrectly synonymized with *C. cornigerum* and herein is reinstated. This conclusion is of practical relevance for the purpose of biological control of common lantana.

Common lantana has remained a challenging invasive weed, even after a hundred years of effort towards its biocontrol involving 200 releases of 39 biocontrol agents in 29 countries (Thomas and Ellison 2000; CABI 2020). Several potential biocontrol agents were collected in Brazil during the surveys of the mycobiota of *Lantana camara* (Barreto et al. 1995; Pereira and Barreto 2001). Most biocontrol agents which have been selected and released against this target-weed were insects, but a rust fungus, *Prospodium*

tuberculatum, from Brazil was released in Australia (Tomley and Riding 2002). Thomas and Ellison (2000) included *C. lantanae-camarae* among the fungi having potential for use in classical biocontrol of common lantana that had not been exploited. This potential had been flagged previously, particularly when it was noted that other *Lantana* species in close proximity to heavily-infected *L. camara* showed no disease symptoms; indicating a high level of host specificity (Barreto et al. 1995). However, confirmed evidence of *C. lantanae-camarae* being a pathogen was lacking at the time of publication, but is demonstrated in this publication. Our observation on the habitats where the fungus is found suggests that altitude and temperature are not limiting for the disease but there seems to be a need for continuously high humidity levels; particularly as the localities reported here are associated with humid forest ecosystems, not just in Brazil but also in Ecuador and Costa Rica (Barreto et al. 1995). This may be the case in some regions of the world, especially in forest situations, where *L. camara* is a weed problem.

Success of mitigating invasions by common lantana has been only partial in many countries (CABI 2020). It is clear that a combination of biocontrol agents will be required to achieve an adequate level of control of large-scale invasions in exotic situations in the tropics. Elucidating the life-cycle of possible candidates for introduction is among the top requirements for further consideration on their potential and safety-evaluation prior to their release. Likewise, the accurate taxonomic identification of a potential weed biocontrol agent is a basic requirement, before any classical introduction is further considered by quarantine authorities of a country (Barreto et al. 2012).

The results presented here demonstrate that *C. lantanae-camarae* is not conspecific with the polyphagous species *C. cornigerum* and provides justification for a closer look at this species as a potential candidate for use in classical biological control of *L. camara*.

Acknowledgments This work was conducted with funds provided by the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We would like to dedicate this paper to the late Dr. Carol A. Ellison who pioneered the research on *Ceratobasidium lantanae-camarae* and the use of fungal pathogens for the biological control of *Lantana camara*. Dr. H. C. Evans is acknowledged for reading and polishing this manuscript.

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4.5. ARTIGO 5

Debunking Perisporiopsidaceae

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Debunking Perisporiopsidaceae

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Abstract

Perisporiopsidaceae includes species with superficial ascomata seated on a subiculum, and ellipsoidal oblong, 1 or more septate, hyaline ascospores. Taxonomy of species in this family has been based, mainly, on morphological characters and host/substrate associations. There is limited information about the taxonomic placement of Perisporiopsidaceae within the Dothideomycetes. Here, an assessment of the phylogenetic position of the Perisporiopsidaceae was attempted based on a multilocus phylogenetic analysis of sequence data obtained from fresh material of two species of *Perisporiopsis* collected in Brazil. *Perisporiopsis struthanthi* – the type species of the type genus of Perisporiopsidaceae was recollected and an epitype was indicated. *Perisporiopsis lantanae* was also recollected and subjected to a molecular analysis. Additionally the status of *Perisporiopsis lateritia* was elucidated. *Perisporiopsis struthanthi* and *P. lantanae* were found to belong to the Phaeosphaeriaceae. *Perisporiopsis struthanthi* grouped with *Setophoma* spp. and *P. lantanae* isolates formed a separate clade within Phaeosphaeriaceae. *Perisporiopsis lateritia* grouped in the family Pyrenochaetopsidaceae, a family having nomeclatural priority over Perisporiopsidaceae. Thus, Perisporiopsidaceae is synonymized with Phaeosphaeriaceae. New combinations are proposed for *Setophoma* spp., since *Perisporiopsis* has nomenclatural priority over *Setophoma*. The new genus and combination are proposed for *P. lantanae*, namely *Sputnikia* and *Sputnikia lantanae* whereas *P. lateritia* is recombined as *Pyrenochaetopsis lateritia*. Other species and genera in Perisporiopsidaceae should be regarded as *Incertae sedis*, until DNA sequences are available and a molecular phylogenetic study clarifies their taxonomic position.

Keywords: Multi-gene phylogeny, New taxa, *Phaeosphaeriaceae*, *Pyrenochaetopsidaceae*, Reappraisal, Taxonomy.

Introdução

The family Parodiopsidaceae was invalidly introduced by Arnaud (1920) to accommodate the genera *Parodiopsis*, *Perisporiopsis* and *Perisporina*, based mainly on their habit in common of occurrence on leaf surfaces forming superficial ascomata over a subiculum. Arnaud (1915) considered the type of Parodiopsidaceae – *Parodiopsis* – a late synonym of *Perisporiopsis*. Therefore, *Perisporiopsis* became the type genus of the family (Hennings 1904). The name Perisporiopsidaceae was invalidly introduced by Müller and von Arx (1962) to combine the families of Parodiellinaceae and Parodiopsidaceae. Subsequently Kirschner et al. (2010) provided a diagnosis in Latin, validating Perisporiopsidaceae with Parodiopsidaceae as its synonym. Although Parodiopsidaceae was an earlier name, Perisporiopsidaceae was prioritized, since the type genus has the name *Perisporiopsis*.

Perisporiopsidaceae includes species forming colonies on living leaves, globose-pigmented ascomata forming ellipsoidal oblong, 1 or more septate, hyaline ascospores are formed over a subiculum (Hyde et al. 2013). Many genera were included in this family, including appressorial (hyphal hyphopodia)-forming fungi such as members of the genera *Alina*, *Balladyna* or *Balladynopsis* (Barr 1997; Sivanesan 1981, 1984; Lumbsch and Huhndorf 2010; Hyde 2013). However, later publications only accept five genera as belonging to Perisporiopsidaceae, namely: *Asteronia, Byssocallis, Chevalieropsis, Parodiellina* and *Perisporiopsis* (Boonmee et al. 2017, Wijayawardene et al. 2018, Pem et al. 2019, Hongsanan et al. 2020).

The asexual states of most genera in Perisporiopsidaceae are hyphomycetes producing simple conidiophores, with monoblastic, annellidic or sympodial conidiogenic cells, producing pigmented and septate conidia (Sivanesan 1984; Kirschner et al. 2010). However, an asexual state producing pycnidia was reported by Barreto et al. (1995) for *Perisporiopsis lantanae*.

Since the early introduction of the family under the name Parodiopsidaceae by Arnaud (1920), it has been compared with several families and orders (Arnaud 1918, 1921; Hansford 1946; Luttrell 1951, 1955; von Arx e Müller 1975; Barr 1976, 1979, 1987; Sivanesan 1984; Chaverri & Gazis 2011; Hyde et al. 2013; Boonmee et al. 2017). Hyde et al. (2013)

conjectured that Perisporiopsidaceae would be polyphyletic, based on the morphology of their genera. Sivanesan (1984) considered that *Perisporiopsis* might be taxonomically related to the Leptosphaeriaceae. Unfortunately, no complete phylogenetic study has been performed to elucidate the taxonomic position of the Perisporiopsidaceae. Molecular data is available only for putative strains of an endophytic *Perisporiopsis* (Chaverii and Gazis 2011). In that publication it is suggested that the genus is a possible member of the Pleosporales, with a close relationship with Leptosphaeriaceae or Phaeosphaeriaceae. However, sequences from the type species of *Perisporiopsis*, which is the type genus for the whole family are not available. There is a general lack of such data for most other taxa in Perisporiopsidaceae.

Here, results of a study aimed at elucidating the phylogenetic position of the Perisporiopsidaceae are provided based on the recollection of selected species in Brazil.

Materials and method

Sample collection processing and observation of fungus morphology

Samples of leaves of *Struthanthus* sp. – Loranthaceae (local name in Brazil "erva-de-passarinho") and *Lantana camara* – Verbenaceae (local name in Brazil 'cambará'), bearing dark fungal colonies, were collected in the state of Rio de Janeiro – Brazil. These were screened under a stereo microscope and parts of the samples bearing sporulating colonies of the fungi were selected and dried in a plant press. Fungal structures were scraped from the sample surface with a scalpel and mounted in lactophenol and lactofuchsin. Observations were made with an Olympus BX53 adapted with differential contrast lighting and equipped with digital capture system (Olympus Q-Color 3 ™). Representative specimens were deposited in the local herbarium (Herbarium Universidade Federal de Viçosa, VIC).

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 analyze and generate images from the samples.

Leaf fragments bearing ascomata were glued to the underside of Petri plate lids, over a layer of tap water-agar. The plates were kept at 25 ° C, with the ascomata facing the culture

medium. 12 hours later, single ascospores ejected onto the culture medium, were transferred with a sterile fine-pointed needle to other petri dishes containing either potato dextrose-agar (PDA) or potato carrot-agar (PCA), and maintained at 25 ° C under a 12-h daily / light regime (light provided by two white and one near-UV lamps placed 35 cm above the plates). Cultures of the fungus from *L. camara* were described based on the observation of 30-day-old. The color terminology followed Rayner (1970).

DNA isolation

DNA extraction from *P. struthanthi* was performed according to Guatimosim et. al. (2015). To obtain a representative fungal DNA, ca. 50 fertile perithecia were examined under a stereomicroscope to check for possible contamination by other fungi. They were removed with the aid of a fine needle and placed into a microcentrifuge tube (1.5 ml) containing 5 µl of double distilled water and stored at –20°C for later use. For *P. lantanae*, the fungus was cultivated grown in PDA under a 12-h daily light regime for 2 weeks. Approximately 50 mg of mycelium was scraped from the surface of the colonized medium and placed inside sterile plastic tubes containing zirconium spheres and placed in a grinder (L-Beader-3, Loccus Biotecnologia). After 5-s grinding, the resulting suspension was drained into a sterile plastic tube and used for DNA extraction. Genomic DNA was extracted for both fungi by using Wizard® Genomic DNAPurification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions and the steps described by Pinho et al. (2012).

PCR amplification

The large subunit of the nrDNA (LSU) and internal transcribed spacer (ITS) regions from each fungus included in the study were sequenced with the primers LSU1Fd (Crous et al. 2009) and LR5 (Vilgalys and Hester 1990) and ITS1 (Gardes and Bruns 1993) and ITS4 (White et al. 1990), respectively. PCR amplifications were performed in a total volume of 12.5 µL 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). Conditions for PCR amplification consisted of an initial denaturation step of 5 min at 94 ºC followed by 35 cycles of 30 s at 94 ºC, 30 s at 48 ºC and 90 s at 72 ºC for LSU, ITS and 40 cycles of 30 s at 94 ºC, 30s at 52 ºC / 59 ºC and 45 s at 72 ºC. Amplicons were analyzed on 0.8% agarose electrophoresis gels stained with GelRed

(InstantAgarose) in a 1× TAE buffer and visualized under UV light to check for amplification size and purity. PCR products were purified and sequenced by Macrogen Inc. (http://www.macrogen.com).

Phylogenetic analysis

Consensus sequences were assembled from forward and reverse sequences using DNA Dragon program (http://www.dna-dragon.com\index.php). Sequences obtained from GenBank (www.ncbi.nlm. nih.gov) and the novel sequences generated on this study were aligned in the MEGA v. 6 (Tamura et al. 2013) (Table 1).

Bayesian inference analyses were conducted and the best-fit evolutionary model was determined by comparing different evolutionary models via the Akaike information criterion using PAUP (version 4.0b10, Sinauer Associates) and MrModeltest 2.2 (Nylander 2004). 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, whereas the remaining 8 000 trees were used for calculating posterior probabilities. Bayesian posterior probabilities are presented on the left of each node. The analysis was hosted by CIPRES science gateway portal at San Diego supercomputer center (Miller et al. 2010). Phylogenetic trees were visualized with the program FigTree v1.3.1 (Rambaut 2009).

Maximum likelihood (ML) tree was generated with the Nearest-Neighbor-Interchange (NNI) ML heuristic method and the Tamura-Nei Substitution model as tree inference options in MEGA. The branch stabilities of the phylogenetic tree were assessed by using the bootstrap re-sampling strategy with 1000 bootstrap test replicates. The resulting tree topologies using the three methods (ML and BI) were then compared and the phylogram was edited with InkScape 0.91 (www.inkscape.org).

Results

Phylogeny

ITS and LSU amplicons were obtained for 2 isolates of *P. struthanthi* and 2 isolates of *P. lantanae*, generating sequences of approximately 500 and 800 bp, for ITS and LSU, respectively. Three phylogenetic trees were generated in this study. The first tree shows the relationship of *P. struthanthi* and *P. lantanae* with other species and genera in Phaeosphaeriaceae (Fig. 1). The alignment for the construction of this tree comprised 96 taxa as ingroup and 1 outgroup. The combined matrix consisted of 2531 characters including alignment gaps (ITS: 1075 and LSU: 1456). The number of conserved sites was 1155 (ITS: 314 and LSU: 1131). The number of variable and parsimony uninformative sites was 967 (ITS: 711 and LSU: 256) and 486 sites were variable and parsimony informative (ITS: 364 and LSU: 122). The phylogenetic tree containing several genera in Phaeosphaeriaceae confirmed that Perisporiopsidaceae does not support itself as a distinct family, since, *P. struthanthi*, type of *Perisporiopsis*, which in turn is type of Perisporiopsidaceae, grouped within the genus *Setophoma* with high support (96%). *P. lantanae* formed a monophyletic clade within Phaeosphaeriaceae, distant phylogenetically from *P. struthanthi* isolates (ML support = 77).

A second tree was built to study the taxonomic position of *P. struthanthi*, within the genus *Setophoma* (Fig.2). The alignment to construct phylogenetic trees included 35 strains (Table 1), representing many of the known *Setophoma* species, and one outgroup taxon (*Didymella pinodella*). Four gene regions were used for the study (ITS, LSU, TEF and TUB), although only ITS and LSU sequences were available for *P. struthanthi*. The combined matrix consisted of 2992 characters including alignment gaps (ITS: 612, LSU: 927, EF1: 899, TUB: 554). The number of conserved sites was 1923 (ITS: 401, LSU: 848, EF1: 320, TUB: 354). The number of variable and parsimony uninformative sites was 821 (ITS: 201, LSU: 58, EF1: 370, TUB: 192) and 593 sites were variable and parsimony informative (ITS: 158, LSU: 28, EF1: 264, TUB: 143). The tree containing isolates of various species in *Setophoma*, showed that the two isolates of *Perisporiopsis struthanthi* grouped in clades well supported in both BI (1.00) and ML (95%) within the genus. The topology of the tree indicates that the two isolates are a species phylogenetically distinct from the other species described in *Setophoma*.

A blast performed with ITS sequences deposited for the type of *P. lateritia*, resulted in high similarity with species of the Pyrenochaetopsidaceae family. Thus, a third tree was built to investigate the phylogenetic positioning of *P. laterítia* (Fig.3). Although there is only one ITS sequence listed in the GenBank for the type, the multigenic phylogenetic analysis was built with ITS, LSU, TUB and RPB2. The study included isolates in Pyrenochaetopsidaceae,

Neopyrenochaetaceae, Pseudopyrenochaetaceae, Cucurbitariaceae and two outgroups in Neocamarosporiaceae. The combined matrix consisted of 3405 characters including alignment gaps (ITS: 650, LSU: 1367, TUB: 398, RPB2: 990). The number of conserved sites was 2365 (ITS: 396, LSU: 1245, TUB: 198, RPB2: 526). The number of variable and parsimony uninformative sites was 1003 (ITS: 244, LSU: 107, TUB: 191, RPB2: 461) and 806 sites were variable and parsimony informative (ITS: 161, LSU: 81, TUB: 153, RPB2: 411). In phylogeny, we found that the isolated type of *P. lateritia* was grouped within the Pyrenochaetopsidaceae family, with species of the genus *Pyrenochaetopsis*. *P. lateritia* was in an unresolved clade with *P. microspora*.

Taxonomy

Phaeosphaeriaceae M.E. Barr, Mycologia 71: 948 (1979)

≡ Syn. nov. Perisporiopsidaceae E. Müll. & Arx ex R. Kirschner & T.A. Hofm.: 238 (2012)

Perisporiopsis Henn., Hedwigia 43: 83 (1904)

≡ Syn. nov. *Setophoma* Gruyter, Aveskamp & Verkley, Mycologia 102 (5): 1077 (2009)

Parasitic on living leaves. Sexual morph: Ascomata superficial, solitary to gregarious, seated on a subiculum, globose to subglobose to obovoid, brown, with an apical ostiole. Peridium relatively thick-walled, comprising of dark brown cells of textura angularis. Sterile filaments branched, septate, anastomosed, hyaline, pseudoparaphyses. Asci 8-spored, bitunicate, fissitunicate, broadly ellipsoid, with short pedicel, apically thickened, with an ocular chamber. Ascospores 2–3-seriate, ellipsoidal-oblong or fusiform, slightly curved and tapering toward the ends, 1-septate, hyaline, sometimes pale-yellow brown, with granulate cells, smooth-walled. Asexual morph: *Septoidium* sp. (Kirk et al. 2008, Seifert et al. 2011, Boonmee et al. 2017).

Type species – *Perisporiopsis struthanthi* Henn.

Notes –*Perisporiopsis* is characterized by superficial dark ascomata, lacking hyphopodia, indistinct pseudoparaphyses, broadly clavate asci and ellipsoidal oblong, septate, hyaline ascospores. Currently, the Index Fungorum (2021) lists 20 epithets under *Perisporiopsis*. The asexual morph was referred to as the *Septoidium* genus (Eriksson 1981, Kirk et al. 2008,

Seifert et al. 2011), but until then, it had never been found for *P. struthanthi*. In this work, the asexual morph of *P. struthanthi* was found, for the first time, in the materials collected during our survey, occurring sparsely amidst the ascomata.

The phylogenetic placement of *Perisporiopsis struthanthi* (represented by two specimens obtained in this work) was found to be in a clade with *Setophoma* spp. in the family Phaeosphaeriaceae. The genus *Setophoma* was proposed by de Gruytere et al. (2010) to accommodate two species previously placed in *Pyrenochaeta* – *Pyrenochaeta sacchari* and *Pyrenochaeta terrestris*. *Setophoma* is characterised by pycnidial conidiomata covered with setae, phialidic conidiogenous cells, and hyaline, ellipsoidal to subcylindrical, aseptate, guttulate conidia (de Gruyter et al., 2010, Quaedvlieg et al., 2013). Given that *Perisporiopsis* is an older name, *Setophoma* should be reduced to a synonym for *Perisporiopsis*. Thus, new combinations are proposed for the species in this genus (Table 2).

Perisporiopsis struthanthi Henn., Hedwigia 43: 83 (1904) (Fig. 4).

= *Parodiopsis struthanthi* (Henn.) G. Arnaud, Annls Épiphyt. 7: 54 (1921)

= *Perisporina struthanthi* (Henn.) Hansf., Proc. Linn. Soc. London 157: 144 (1946)

Lesions not produced, the fungus forms a sooty coating on the leaves of its host. Internal mycelium absent. External mycelium 5–10 µm diam., branched, septate, brown, smooth-walled with appressoria globose to tubglobose, 10–25 × 10–22 µm, brown. Sexual morph: Ascomata pseudothecioid, superficial, solitary to gregarious, seated on a subiculum, globose to subglobose, obovoid, brown, with apical ostiole, 150–250 × 185–275 µm. Wall of dark brown *textura angularis*, 40–45 µm thick; pseudoparaphyses branched, septate, anastomosed, 4–5 µm diam., hyaline. Asci bitunicate, fasciculate, fissitunicate, broadly ellipsoid, with short pedicel, apically thickened, with an ocular chamber, 102–163 × 25–45 µm, 8-spored. Ascospores 2-seriate, ellipsoidal-oblong or fusiform, slightly curved and tapering toward the ends, 62.5–80 × 12.5–15 µm, 0–1-septate, hyaline, sometimes pale-yellowish brown, with granulate cells, smooth.

Asexual morph: Sporodochial – sporodochium 200–250 × 75–100 µm, dark brown. Conidiophores arranged in the sporodochia in a palisade manner, erect, mononematous, macronematous, thick-walled, unbranched, 212–250 × 8.75–10 µm, 8–9 septate, hyaline, pale brown to dark brown, smooth-walled. Conidiogenous cells annellidic (holoblastic with percurrent proliferations), with conspicuous annellations, 27.5–30 × 5 µm, pale to moderately

brown. Macroconidia ovoid, fusiform to cymbiform, truncate at base, 70–92.5 × 17.5–20 µm, 3-septate, smooth, pale brown, sometimes with tinges of pale grayish rose.

Material examined: Brazil: Rio de Janeiro, Nova Friburgo, Debossan, on *Struthanthus* sp. parasitizing hibiscus garden fence, 30 Jul 2019, R. W. Barreto (VIC 47339 – epitype designated); Nova Friburgo, Janela das Andorinhas, on *Struthanthus* sp. parasitizing an avocado tree, 11 Apr 2019, R. W. Barreto (VIC 47340).

Notes: Attempts to obtain pure culture for *P. struthanthi* were unsuccessful. The species is probably biotrophic. Both specimens (VIC 47339 and VIC 47340) had a morphology which was very similar to the original description of Hennings (1904) and that of Boonmee et al. (2017) for Perisporiopsidaceae. The ascomata of our specimens were smaller than those described by Hennings (1904) and Boonmee et al. (2017) – 150–250 µm vs. 250–300 µm and 263.5–272 µm, respectively. Ascospores in the newly collected specimens were longer than those described in Hennings (1904) and Boonmee et al. (2017), 62.5–80 µm vs. 60–65 µm and 52.5–62 µm, respectively. Our specimens had ascospores which were 0-1 septate whereas Hennings (1904) reported the occurrence of ascospores with up to 7 septa. In Boonmee et al. (2017) ascospores were described as 1-septate. The phylogenetic placement of VIC 47339 and VIC 47340 was in the same clade of *Setophoma*. This is a genus described as having setose pycnidia. However, the only asexual morph observed for *P. struthanthi* in our study is an sporodochial anellidic hyphomycete. When *Setophoma* was introduced, the sexual morph had not been observed. Later, Phookamsak et al. (2014) described the sexual form of *Setophoma sacchari* as having: Ascomata immersed or semi-immersed, asci bitunicate, fissitunicate and ascospores cylindrical to cylindric-clavate, hyaline, overlapping or irregularly biseriate, 3-septate, usually widest at the second cell from apex. The differences found between *P. struthanti* and *S. sacchari* refer to (i) the location of ascomata on the leaf: immersed or semi-immersed in *S. sacchari* and superficial in *P. struthanthi*; (ii) wall thickness: 8.5–15 µm in *S. sacchari* and 40–46 µm in *P. struthanthi*; (iii) size of the ascospores: 20–23 (–25) × 5–6 µm in *S. sacchari* and 62.5–80 × 12.5–15 in *P. struthanthi*.

Sputnikia B.W. Ferreira & R.W. Barreto, gen. nov.

Type: ***Sputnikia lantanae***

Etymology: Having sphaerical pseudothecia bearing hyphal appendages which reminisce the shape of the pioneer Russian artificial satellite and also celebrating the first anti-covid 19 vaccine.

Diagnosis: Similar to *Perisporiopsis* but having external hyphae without appressoria and asexual morph with conidiomata pycnidial.

Sputnikia lantanae (F. Stevens) B.W. Ferreira & R.W. Barreto, comb. nov. (Fig. 5)

≡*Perisporiopsis lantanae* (F. Stevens) R.W. Barreto, Mycological Research 99 (7): 774 (1995)

=*Perisporina lantanae* F. Stevens, Transactions of the Illinois Academy of Science 10: 170 (1917).

Colonies a sooty coating on the underside of leaves of *Lantana camara*. Internal mycelium absent. External mycelium 3-4 µm diam., branched, septate, brown, smooth-walled. Sexual morph: Ascomata pseudothecioid, predominantly hypophyllous, superficial, abundant, subspherical, 132.5–250 × 120–250 µm; wall 1–3 cells thick, 3–10 µm, composed of dark brown *textura angularis*, ornamented with abundant brown septate setae (8–10 µm wide and up to 286 µm long), often with a group of shorter setae arranged as a crown surrounding the ostiole. Dehiscence ostiolate, one ostiole in the centre of each pseudothecium, circular, 31–52 µm diam., papillate. Interthecial filaments pseudoparaphyses, thin, branched, hyaline. Asci bitunicate, fasciculate, subclavate, 72.5–77.5 × 17.5–22.5 µm, 8-spored. Ascospores fusiform, rounded in one extremity subacute in the other, 25–37.5 × 7.5–10 µm, 3 septate, guttulate, greyish-brown, smooth-walled.

Asexual morph described by Barreto et al. (1995) – Conidiomata pycnidial, amphigenous, superficial, abundant, intermixed with ascomata, indistinguishable from ascomata in external appearance, wall thickness, composition and colour, 136–170 × 109–150 µm. Dehiscence as for teleomorph. Conidiophores absent. Conidiogenous cells arising from lower half of pycnidial wall, subcylindrical, 4–10 × 4–6 µm, hyaline. Conidia holoblastic, cylindrical, 60–80 × 6-8 µm, straight to slightly curved, apex round, base truncate, 3 µm wide, 4–6 septate, guttulate, greyish, smooth-walled.

In culture: Slow-growing (2.1–2.5 cm diameter after 35 days), umbonate, felty, edges entire, white centrally with olivaceous edges, reverse fully olivaceous in PCA, and smoke gray centrally with gray olicaveous edges, reverse olivaceous in PDA. Not sporulating.

Material examined: Brazil: Rio de Janeiro, Rio de Janeiro, Cachoeiras de Macacu, on *Lantana camara*, 22 Dec 2019, R. W. Barreto (RWB2296– epitype designated here); Rio de Janeiro, Nova Friburgo, Janela das Andorinhas, on *Lantana camara*, 13 Dec 2019, R. W. Barreto (RWB2298).

Notes: Stevens (1917) erected the species *Perisporina lantanae* based on material from Puerto Rico. Muller & Arx (1962) when they synonymized *Perisporina* under *Perisporiopsis*, did not propose a new combination for *Perisporina lantanae*. This was done later, by Barreto et al. (1995), who also provided illustrations and a more detailed description for *Perisporiopsis lantanae* based on materials collected in Rio de Janeiro-Brazil. The epitype designated here for *P. lantana* differs very little from the descriptions by Stevens (1917) and Barreto et al. (1995). The pycnidial stage was not found in the newly collected materials, but described in Barreto et al. (1995). The phylogenetic analysis performed here, showed that *P. lantana* is distantly related to *P. struthanthi*, the type of *Perisporiopsis*. The RWB 2296 and RWB 2298 isolates clustered close to the genera *Paraphoma* and *Parastagonosporella*. *Paraphoma* is characterized by setose pycnidia containing conidiogenous cells which are monophialidic, and enteroblastic, ellipsoid, aseptate, hyaline conidia. *Parastagonosporella* have conidiomata which are pycnidial, subepidermal, immersed, releasing conidia in cirrhi; wall of 4–8 layers; conidiogenous cells occasionally phialidic, with prominent periclinal thickening or annellidic, proliferating percurrently; conidia hyaline, scolecosporous, subcylindrical, granular to multi-guttulate, with obtuse apex and truncate to subtruncate base. The sexual morph of *Paraphoma* and *Parastagonosporella* are not known.

***Pyrenochaetopsis lateritia* (P. Chaverri & Gazis) B.W. Ferreira & R.W. Barreto, comb. nov.**

≡ ***Perisporiopsis lateritia*** P. Chaverri & Gazis, Mycotaxon 113: 164 (2010)

Chaverri & Gazis (2010) provided the following description for this taxon – Sexual morph: Mycelium superficial, hypophyllous, extensive, appearing black, anastomosing to form a close network, almost subiculum-like, with simple, knob-shaped stomatopodia. Ascomata superficial on mycelium, aggregated, associated with a hyphomycetous dematiaceous

anamorph (i.e. *Septoidium*). Ascomata dark brown to black, almost completely covered with a sienna to brick tomentum, except near the apex where they appear black, subglobose to obovoid, 300–310 × 420–450 µm (n = 5), non-ostiolate, irregularly dehiscent at apex; ascomatal wall composed of one region of 2–3 layers of thick-walled cells, textura angularis. Asci few, generally less than 5, 200–220 × 80–90 µm (n = 10), obovoid, sessile to short stalked, somewhat thickened at apex, eight-spored. Ascospores 1-septate, strongly constricted at septum, initially hyaline, later pale brown or fawn, smooth to slightly spinulose, broadly fusiform to ovoid, somewhat inequilateral, with apical cell slightly larger than basal cell, (65.0–) 66.0–75.5(–78.0) × 18.0–21.5(–23.0) µm (average = 70.5 × 20 µm, n = 30). Anamorph – Both macro- and microconidia of the hyphomycetous anamorph observed on natural substrata. For the macroconidial anamorph (i.e. *Septoidium*) no conidiogenous cells observed. Macroconidia ovoid, fusiform to cymbiform, truncate at base, smooth, pale brown, sometimes with tinges of pale grayish rose, 2-septate, (59.0–)61.5–69.0(–80.0) × (15.7–) 16.5–18.0(–19.3) µm (average = 65.2 × 17.2 µm, n = 10), length/width ratio 3.7–3.8(–4.2) (average = 3.8, n = 30). Microconidial anamorph with erect conidiophores, brown near base, pale brown almost hyaline near tip, simple, not branching, septate, with scattered denticles on upper part; conidiogenous cells polyblastic, sympodial, with small denticles; microconidia borne on denticles, globose to subglobose, unicellular, almost hyaline, sometimes apiculate at base, 4.5–5.5 × 4.8–5.5 µm (average = 5 × 5.2 um), length/width ratio 1.0–1.1 (average = 1.0, n = 8).

Habitat – On the underside of decaying *Hevea* spp. leaves in old growth forests. Known only from Peru.

Type: 17 June 2007, coll. R. Gazis, H.C. Evans, P. Chaverri; (Holotype BPI 880185, P.C.811) on underside of decaying leaves of *Hevea brasiliensis*, Picaflor Research Station, near Tambopata River, Prov. Tambopata: Dept. Madre de Dios, Peru. GenBank accession number FJ884129.

Notes: Sequences of the type of *P. lateritia* grouped with species of *Pyrenochaetopsis*, in the family Pyrenochaetopsidaceae. Pyrenochaetopsidaceae was spun off from Cucurbitareaceae by Valenzuela-Lopez et al. (2018) and comprises fungi with conidiomata pycnidial, pale brown to brown, solitary or confluent; pycnidial wall of textura angularis, glabrous or setose, subglobose to ovoid, with a non-papillate or papillate ostiolar neck. Conidiogenous cells phialidic, hyaline, discrete or integrated in septate, acropleurogenous conidiophores. Conidia

aseptate, hyaline, smooth- and thin-walled, ovoid, cylindrical to allantoid, guttulate. An asexual morph pycnidial phase was not described by Chaverri & Gazis (2010) for *P. lateritia*. The authors only deposited sequences of the ITS region for the type. *Pyrenochaetopsis* spp. have been commonly reported as saprobes (de Gruyter et al. 2010). Some species have been reported from samples of water, plants, soil, manure, air and as endophytes in grasses (de Gruyter et al. 2010, 2013, Crous et al. 2014, Farr and Rossman 2021). Here, we chose to adopt a new combination for *P. lateritia*, differentiating it from the other species in the genus, considering that only *P. lateritia* has its sexual morph described and has an asexual morph producing macro and microconidia.

Other species in *Perisporiopsis*

Currently, Index Fungorum (2020) lists 20 epithets under *Perisporiopsis*.

Perisporiopsis brachystegiae (Henn.) Arx 1962

Perisporiopsis brasiliensis (Bat. & Nascim.) Arx 1962

Perisporiopsis cecropiae (R.E.D. Baker) Arx 1962

Perisporiopsis cecropiae var. *cecropiae* (R.E.D. Baker) Arx 1962

Perisporiopsis clusiae (R.E.D. Baker) Arx 1962

Perisporiopsis clusiicola (Hansf.) Sivan. 1984

Perisporiopsis escharoides (Syd.) Arx 1962

Perisporiopsis fusispora (Pat.) Arx 1962

Perisporiopsis hurae (G. Arnaud) Arx 1962

Perisporiopsis kwangensis (Henn.) Arx 1962

Parodiopsis lophirae Deighton, in Petrak & Deighton, Sydowia 6(5-6): 315 (1952)

Perisporiopsis megalospora (Sacc. & Berl.) Arx 1962

Perisporiopsis megalospora var. *megalospora* (Sacc. & Berl.) Arx 1962

Perisporiopsis melioloides (Berk. & M.A. Curtis) Arx 1962

Perisporiopsis portoricensis (F. Stevens) Arx 1962

Perisporiopsis sydowii (Petr.) Arx 1962

Perisporiopsis torrendii (Bat. & H. Maia) Arx 1962

Other genera in Perisporiopsidaceae to be regarded as *incertae sedis*

Asteronia (Sacc.) Henn., Hedwigia 34: 104 (1895).

Index Fungorum number: IF425, Facesoffungi number: FoF 06215; – 2 morphological species (Species Fungorum 2020), molecular data unavailable.

Type species: *Asteronia sweetiae* Henn., Hedwigia 34:104 (1895).

= *Parodiopsis sweetiae* (Henn.) G. Arnaud, Annls Épiphyt. 7: 53 (1921).

Notes: *Asteronia* is a genus characterized by ascoma subglobose and gregarious, and 8-spored asci that are bitunicate with fusoid-ellipsoidal ascospores. The genus *Asteronia* was previously placed in Microthyriaceae by Lumbsch & Hundorf (2010). Wu et al. (2010) revisited Asteronia and provided a description and illustration of the *A. sweetiae* holotype and suggested placing this genus in the Asterinaceae or Meliolaceae families. Subsequently, Wu et al. (2011b) transferred *Asteronia* to Venturiales *incertae cedis*. Pem et al. (2019c) described and illustrated the genus based on the isotype of *A. sweetiae* and chose to transfer it to Perisporiopsidaceae, due to its morphological and subtract characteristics.

Byssocallis Syd., Annls mycol. 25(1/2): 14 (1927).

Index Fungorum number: IF 700, Facesoffungi number: FoF 06224; – 2 morphological species (Species Fungorum 2020), molecular data unavailable.

Type species: *Byssocallis phoebes* Syd., Annls mycol. 25(1/2): 14 (1927).

Notes: *Byssocallis* is characterized by reticulate, branched, and septate hyphae with colorless mycelium covering the host. Perithecia superficial, ostiolate, paraphyses are numerous, narrow, branched with mucous membrane. Asci cylindrical, bitunicate, octosporous. Ascospores are clavate, narrowly fusiform, transverse, hyaline, and multiseptate with the ends

often constricted. *Byssocallis* was previously accepted as a genus in Tubeufiaceae (Lumbsch & Huhndorf 2010) and then transferred to Dothideomycetes, genera *incertae sedis* (Hyde et al. 2013, Boonmee et al. 2014, Kirk et al. 2013, Wijayawardene et al. 2014). Pem et al. (2019) re-examined the syntype specimen of *Byssocallis phoebes* (E00455471) and found that it shares common characters with genera in Perisporiopsidaceae. Thus, they transferred *Byssocallis* to Perisporiopsidaceae.

Chevalieropsis G. Arnaud, Annls Épiphyt. 9: 2 (1923).

Index Fungorum number: IF 992, Facesoffungi number:

FoF 07894; – 1 morphological species (Species Fungorum 2020), molecular data unavailable.

Type species: *Chevalieropsis ctenotricha* (Pat. & Har.) G. Arnaud, Annls Épiphyt. 9: 2 (1923).

≡ *Dimerosporium ctenotrichum* Pat. & Har., J. Bot., Paris 14: 242 (1900).

Notes: *Chevalieropsis* is characterized by superficial ascostromata, with thick-walled asci and 1–4-septate, hyaline to pale brown ascospores. The genus was introduced by Arnaud (1923), with *C. ctenotricha* as the type species. Moreau & Moreau (1955) described two types of asexual conidia associated with *C. ctenotricha* on leaves of *Daniella oliveri* (Detarioideae), which were similar to species in the *Septoidium* genus. Eriksson (1999) included *Chevalieropsis* in Perisporiopsidaceae (= Parodiopsidaceae) based on its parasitic habit on leaves, ascomata features, thick-walled bitunicate asci and lightly pigmented ascospores.

Parodiellina Henn. ex G. Arnaud, Annals d'École National d'Agric. de Montpellier, Série 2 16(1–4): 21 (1918) [1917].

Index Fungorum number: IF 3750, Facesoffungi number: FoF 07895; – 1 morphological species (Species Fungorum 2020), molecular data unavailable.

Type species: *Parodiellina manaosensis* (Henn.) G.

Arnaud, Annals d'École National d'Agric. de Montpellier, Série 2 16(1–4): 21 (1918) [1917].

≡ *Parodiella manaosensis* Henn., Hedwigia 43(6): 358 (1904).

Notes: *Parodiellina* is characterized by oval to ellipsoid, obovoid-clavate, aseptate ascospores and a conspicuous sporodochial asexual morph. Aseptate ascospores and conidial features

differ from all genera in Perisporiopsidaceae (= Parodiopsidaceae). The genus was introduced by Arnaud (1918) to accommodate *Parodiellina manaosensis* (= *Parodiella manaosensis* Henn.) which was described by Hennings (1904). *Parodiellina* has been placed in various families and orders based on characters of ascomata, ascospores and the asexual morph (Gäumann & Dodge 1928, Hansford 1946, Müller & von Arx 1950). Luttrell (1955) re-evaluated the type species of *Parodiellina* and placed the genus in the family Perisporiopsidaceae, based on its parasitic habit on leaves and some morphological characteristics.

Discussion

Clearly, the morphological features, used for distinguishing genera and species of Perisporiopsidaceae, appear to be of little value as well as host/substrate associations. In the phylogeny performed here, we show that the isolates of *P. struthanthi*, the type species of *Perisporiopsis*, which is a type of Perisporiopsidaceae, groups with isolates of *Setophoma*, in the family Phaeosphaeriaceae. Thus, we proposed the synonymization of both families. Since the name Phaeosphaeriaceae is older than Perisporiopsidaceae, Phaeosphaeriaceae had priority and was retained.

Phaeosphaeriaceae is a family of the order Pleosporales, which includes species with different lifestyles: endophytic, epiphytic, lichenicolous, phytopathogenic, saprobic and even as human pathogens (Phookamsak et al. 2014). The family was introduced by Barr (1979) and typified by *Phaeosphaeria* with *Phaeosphaeria oryzae* (Miyake 1909) as a type species. Phaeosphaeriaceae that comprised quite heterogeneous genera has undergone several changes in recent years. New genera were introduced, while some others were transferred to other families (Zhang et al. 2012, Hyde et al. 2013, Quaedvlieg et al. 2013, Phookamsak et al. 2014, Trakunyingcharoen et al. 2014, Crous et al. 2015, Ertz et al. 2015, Phukhamsakda et al. 2015, Senanayake et al. 2015, Tennakoon et al. 2016, Tibpromma et al. 2016, Ahmed et al. 2017, Wanasinghe et al. 2018). Currently, more than 50 sexual and asexual genera are accepted in the family (Bakhshi et al. 2019).

The genus *Setophoma* was included in the family, to accommodate *Phoma terrestris* and *Pyrenochaeta sacchari* (de Gruyter et al. 2010). Species of *Setophoma* are characterized as

having setose pycnidia, phialidic conidiogenous cells and hyaline, ellipsoidal to subcylindrical, aseptate conidia (de Gruyter et al. 2010, Quaedvlieg et al. 2013). According to Index Fungorum and MycoBank, the genus currently has 14 species. All, except *S. terrestris*, are reported to occur in single host plants (Table 2). Since *P. struthanthi*, type of *Perisporiopsis* grouped in *Setophoma*, the two genera must be treated as synonyms, with *Perisporiopsis* having nomenclatural priority over *Setophoma*. Thus, we proposed new combinations for *Setophoma* spp. (Table 2).

All species of *Perisporiopsis*, have been described as having *Septoidium* macro and microconidial anamorphs (Chaverri & Gazis 2011). Only *P. lantanae* has a pycnidial anamorph. Based on this and on the morphological characteristics of the ascospores, Chaverri & Gazis (2011) conjectured that *P. lantanae* could have relations with Leptosphaeria and that it would not belong to *Perisporiopsis*. In fact, our phylogenetic analyzes showed that *P. lantanae* is distant from *P. struthanthi* (type of the genus), but within the family Phaeosphaeriaceae. *Perisporiopsis lantanae* (now *S. lantanae*) isolates formed a well-supported clade, separated from other genera in Phaeosphaeriaceae. Thus a new genus was proposed to accommodate the fungus on *Lantana camara*.

Phylogenetic analysis showed that *P. lateritia* belongs to the Pyrenochaetopsidaceae family. The isolates grouped with species of *Pyrenochaetopsis*. The phylogenetic distinction between *Pyrenochaetopsis microspora* and *P. lateritia* was not possible, using only ITS sequences. The morphological comparison was also not possible between *P. lateritia* and species of *Pyrenochaetopsis*, since only the pycnidial asexual morph is known for the members of *Pyrenochaetopsis* spp., and the asexual morph described for *P. lateritia*, belongs to *Septoidium*.

The list of sequences for fungi in the former Perisporiopsidaceae in public databases is still very limited. Although the epitification of *P. struthanthi* contributes towards a better understanding of this assemblage, much needs to be done. Many of the original materials designated as types are old, scarce or have been lost, or difficult to obtain. Hopefully mycologists will be able to recollect and epitify some of these old taxa, an increasingly challenging task in a world where the natural habitats of fungi and other unique organisms are being lost at a fast pace. Only the epitipification of such taxa, followed by molecular phylogenetic analysis, will resolve their taxonomic position and produce a more natural classification for the fungi formerly placed in the Perisporiopsidaceae.

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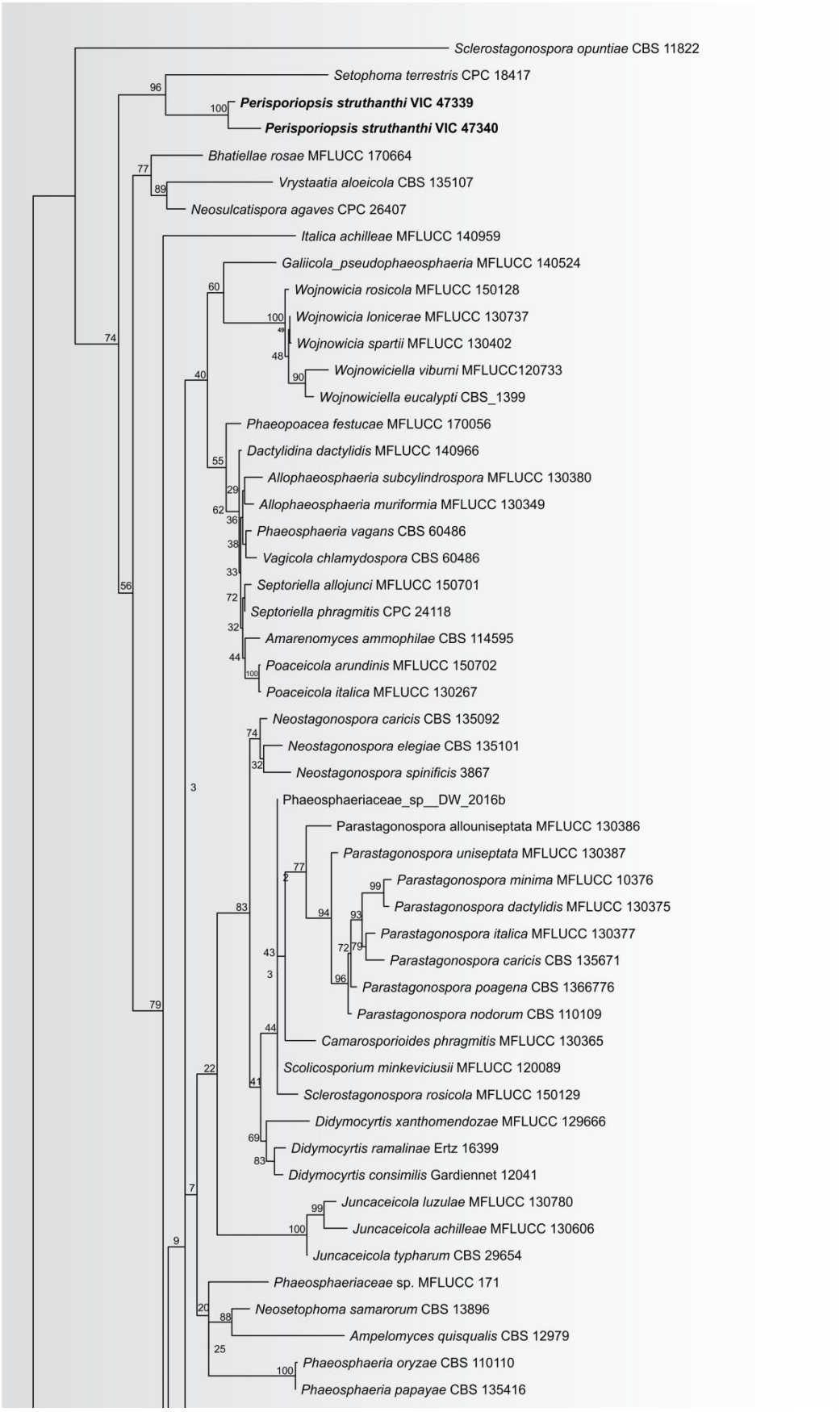
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Bhatiellae rosae MFLUCC 170664
Vrystaatia aloeicola CBS 135107
Neosulcatispora agaves CPC 26407
Italica achilleae MFLUCC 140959
Galiicola_pseudophaeosphaeria MFLUCC 140524
Wojnowicia rosicola MFLUCC 150128
Wojnowicia lonicerae MFLUCC 130737
Wojnowicia spartii MFLUCC 130402
Wojnowiciella viburni MFLUCC120733
Wojnowiciella eucalypti CBS_1399
Phaeopoacea festucae MFLUCC 170056
Dactylidina dactylidis MFLUCC 140966
Allophaeosphaeria subcylindrospora MFLUCC 130380
Allophaeosphaeria muriformia MFLUCC 130349
Phaeosphaeria vagans CBS 60486
Vagicola chlamydospora CBS 60486
Septoriella allojunci MFLUCC 150701
Septoriella phragmitis CPC 24118
Amarenomyces ammophilae CBS 114595
Poaceicola arundinis MFLUCC 150702
Poaceicola italica MFLUCC 130267
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Neostagonospora elegiae CBS 135101
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Parastagonospora nodorum CBS 110109
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Scolicosporium minkeviciusii MFLUCC 120089
Sclerostagonospora rosicola MFLUCC 150129
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Juncaceicola achilleae MFLUCC 130606
Juncaceicola typharum CBS 29654
Phaeosphaeriaceae sp. MFLUCC 171
Neosetophoma samarorum CBS 13896
Ampelomyces quisqualis CBS 12979
Phaeosphaeria oryzae CBS 110110
Phaeosphaeria papayae CBS 135416

Fig. 1. RAxML tree inferred from combined dataset of nc LSU rDNA and ITS of 96 strains of Phaeosphaeriaceae. The phylogenetic tree shows the relationship of *Perisporiopsis struthanthi* and *Perisporiopsis lantanae* with other genera in the family. Bootstrap support values ≥70% are given above or below each branch respectively. The tree was rooted to *Coniothyrium carteri* (CBS 105.91). Isolates from this study are indicated by bold text.

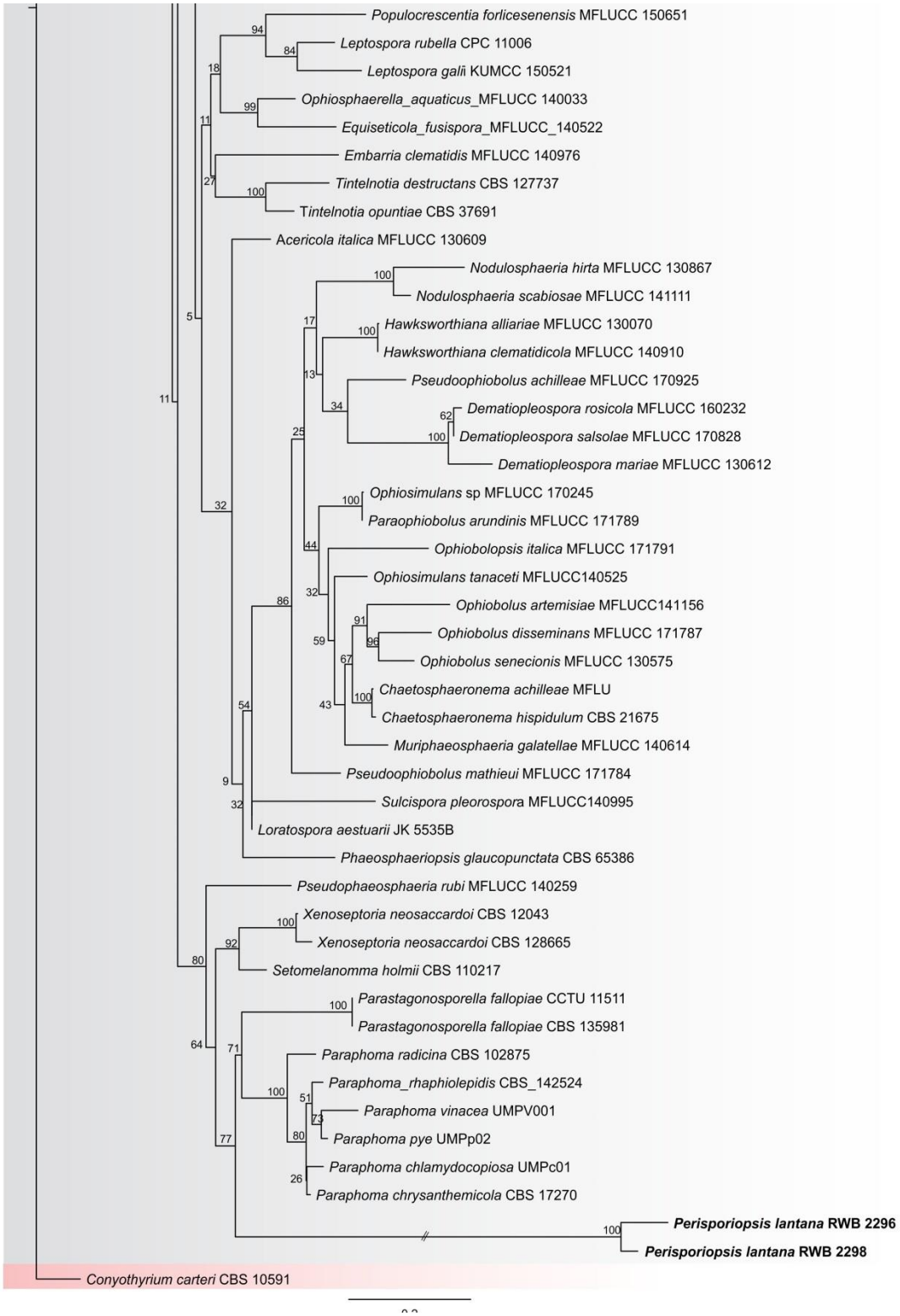

Fig. 1. (continuity) RAxML tree inferred from combined dataset of nc LSU rDNA and ITS of 96 strains of Phaeosphaeriaceae. The phylogenetic tree shows the relationship of *Perisporiopsis struthanthi* and *Perisporiopsis lantanae* with other genera in the family. Bootstrap support values ≥70% are given above or below each branch respectively. The tree

was rooted to *Coniothyrium carteri* (CBS 105.91). Isolates from this study are indicated by bold text.

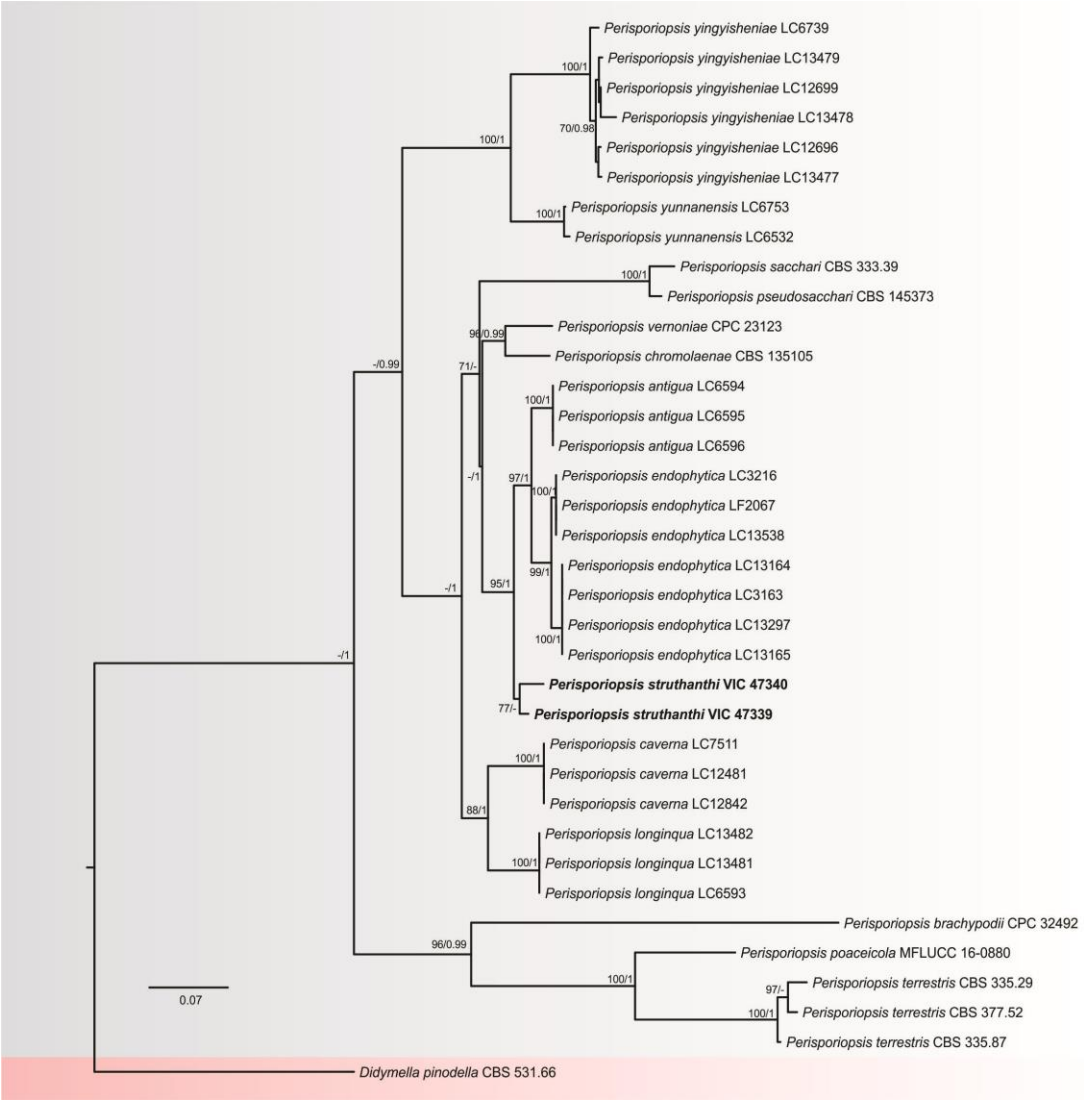

Fig. 2. RAxML tree inferred from combined dataset of nc LSU rDNA, ITS, TEF and TUB showing the relationship of *Perisporiopsis struthanthi* with other closely related *Setophoma* spp., now transferred to *Perisporiopsis*. Bootstrap support values (MP and ML) or Bayesian posterior probabilities higher than 70 % or 0.90 are indicated above or below thickened branches (– indicates lack of support). Isolates from this study are indicated by bold text.

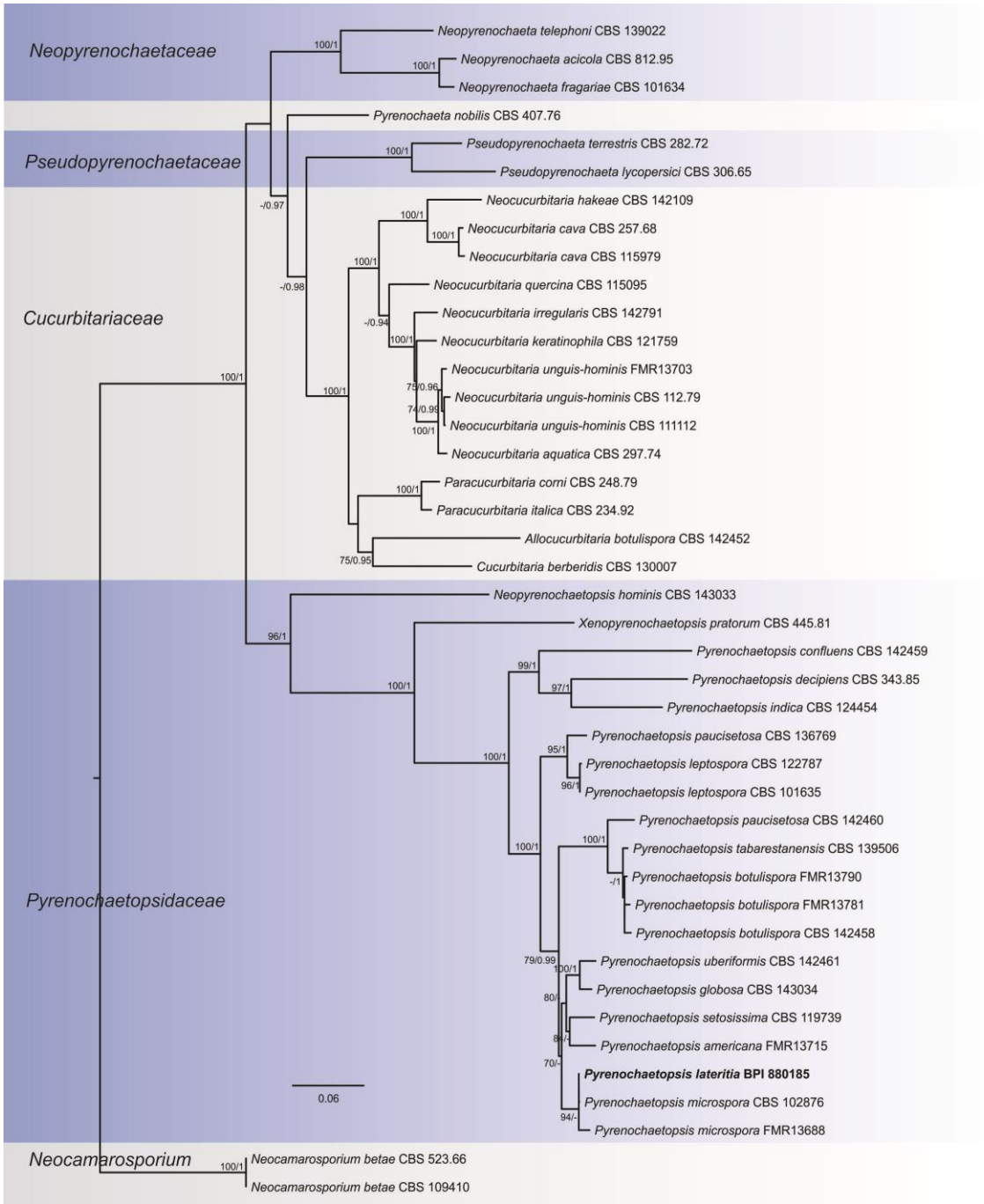

Fig. 3. RAxML tree based on combined nc LSU rDNA, ITS, *RPB2* and *TUB* showing the relationship of *Perisporiopsis lateritia* with other closely related family in Dothideomycetes. Bootstrap support values (MP and ML) or Bayesian posterior probabilities higher than 70 % or 0.90 are indicated above or below thickened branches (– indicates lack of support).

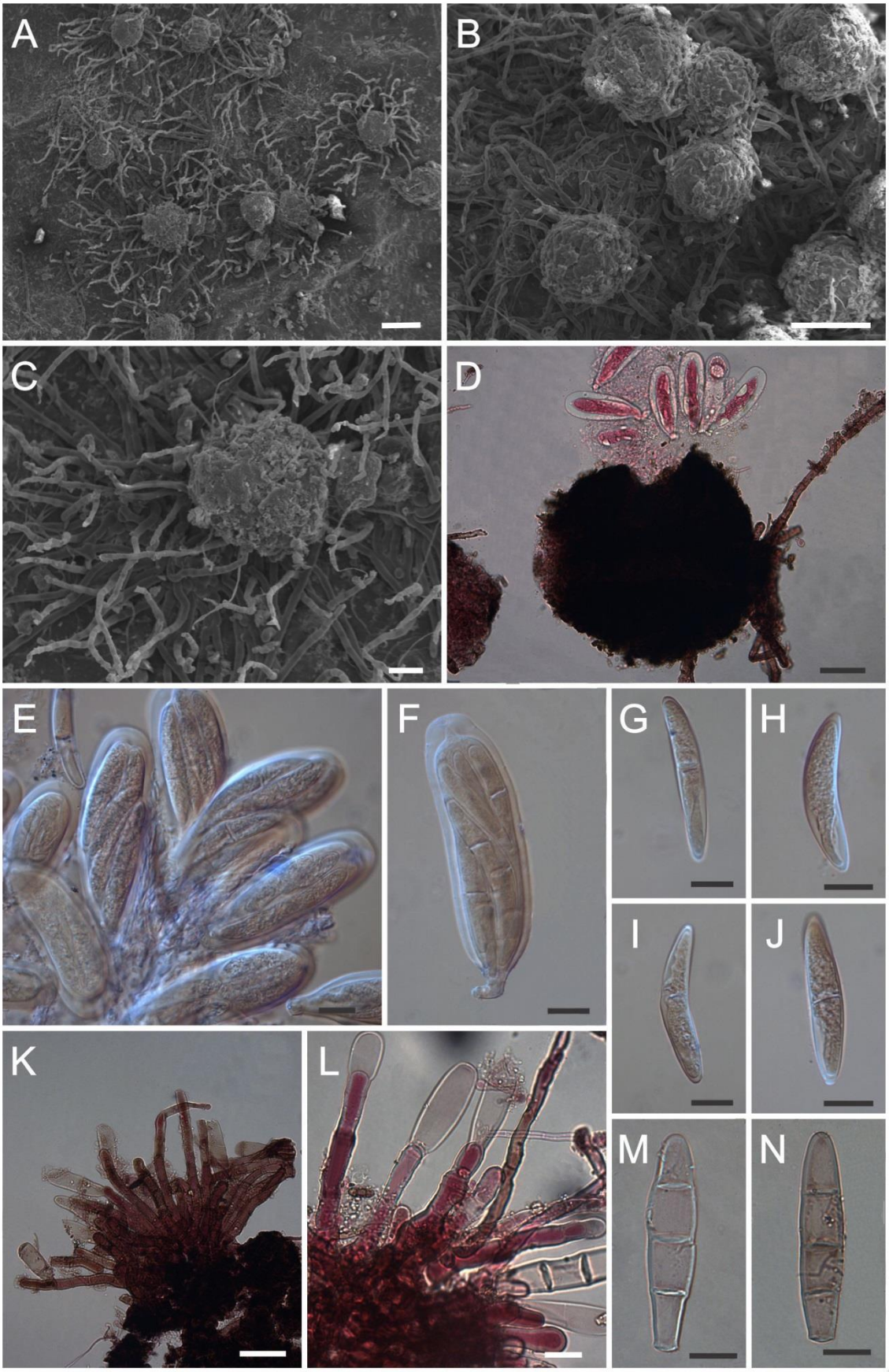
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Fig. 4. *Perisporiopsis struthanthi* (VIC 47339). **A-C** SEM image showing ascomata on leaf surface of *Struthanthus* sp. **D** Collapsed ascomata releasing Asci. **E** Mature and imature asci. **F** Detail of bitunicate asci. **G-J** Ascospores. **K** Conidiophores. **L** Close up of conidiophores and conidia. **M-N** Conidia. Scale bars: A-B = 100 µm, C = 30 µm, D and K = 50 µm, E-J and L-N = 20 µm.

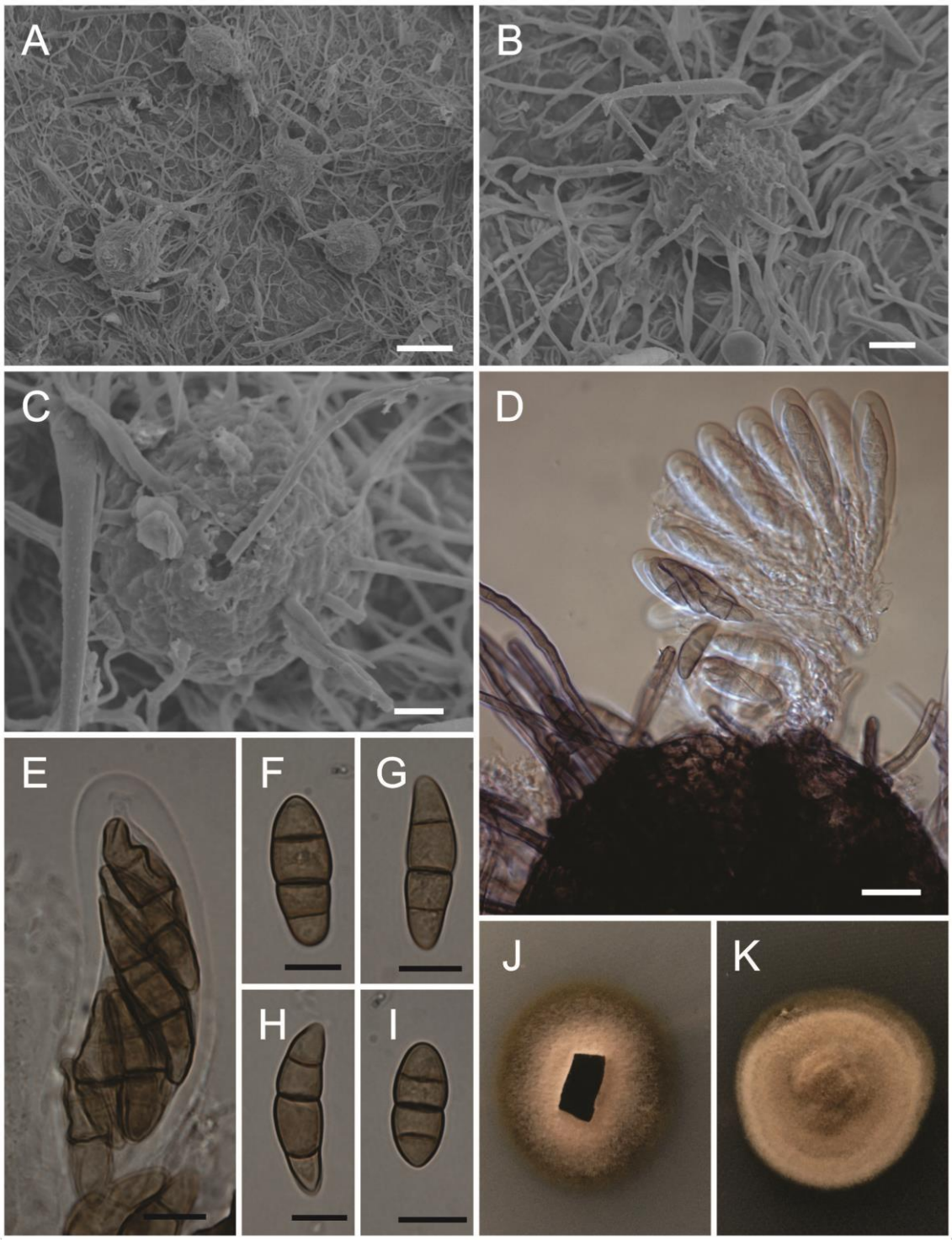

Fig. 5. *Sputnikia lantana* (RWB2296). **A-C** SEM image of ascomata on leaf surface of *Lantana camara*. **D** Ascomata releasing Asci. **E** Detail of bitunicate asci. **F-I** Ascospores. **J-K** Colony on PCA and PDA after 15 days (incubation at 25 °C in 12 h light/dark cycle). Scale bars: A = 100 μm, B = 30 μm, C-D = 20 μm, E-I = 10 μm.

Table 1 Taxa used in the phylogenetic analysis and GenBank accession numbers.

Taxon	Culture accession number	GenBank accession numbers					References
		LSU	ITS	tub2	rpb2	tef-1α	
Acericola italica	MFLUCC 13-0609*	MF167429	MF167428	–	–	–	Phookamsak et al. (2017)
Allocucurbitaria botulispora	CBS 142452*	LN907416	LT592932	LT593001	LT593070	–	Valenzuela-Lopez et al. (2018)
Allophaeosphaeria muriformia	MFLUCC 13-0349*	KP765681	KP765680	–	–	–	Liu et al. (2015)
Allophaeosphaeria subcylindrospora	MFLUCC 13-0380	KT314183	KT314184	–	–	–	Liu et al. (2015)
Amarenomyces ammophilae	CBS 114595*	GU301859	KF766146	–	–	–	Slippers et al. (2013)
Ampelomyces quisqualis	CBS 129.79*	EU754128	HQ108038	–	–	–	De Gruyter et al. (2009)
Bhatiellae rosae	MFLUCC 17-0664*	MG828989	MG828873	–	–	–	Wanasinghe et al. (2018)
Camarosporioides phragmitis	MFLUCC 13-0365*	KX572345	KX572340	–	–	–	Phookamsak et al. (2017)
Chaetosphaeronema achilleae	MFLUCC 16-0476	KX765266	KX765265	–	–	–	Hyde et al. (2016)
Chaetosphaeronema hispidulum	CBS 216.75*	KF251652	KF251148	–	–	–	Quaedvlieg et al. (2013)
Coniothyrium carteri	CBS 105.91	KF251712	KF251209	–	–	–	Quaedvlieg et al. (2013)
Cucurbitaria berberidis	MFLUCC 11-0387	KC506796	–	–	–	–	Valenzuela-Lopez et al. (2018)
	CBS 130007*	KC506793	LT717673	LT717676	LT854936	–	Valenzuela-Lopez et al. (2018)
Dactylidina dactylidis	MFLUCC 14-0966*	MG829002	MG828886	–	–	–	Wanasinghe et al. (2018)
Dematiopleospora	MFLUCC 13-0612*	KJ749653	KJ749654	–	–	–	Wanasinghe et al. (2014)

mariae							
Dematiopleospora rosicola	MFLU 16-0232	MG829006	MG828888	–	–	–	Wanasinghe et al. (2018)
Dematiopleospora salsolae	MFLUCC 17-0828	MG829007	MG828889	–	–	–	Wanasinghe et al. (2018)
Didymocyrtis consimilis	Gardiennet 12041*	KT383796	KT383813	–	–	–	Ertz et al. (2015)
Didymocyrtis ramalinae	Ertz 16399	KT383802	KT383838	–	–	–	Ertz et al. (2015)
Didymocyrtis xanthomendozae	CBS 129666	JQ238634	KP170651	–	–	–	Lawrey et al. (2012);Trakunyingcharoen et al. (2014)
Embarria clematidis	MFLUCC 14-0976*	MG828987	MG828871	–	–	–	Wanasinghe et al. (2018)
Equiseticola fusispora	MFLUCC 14-0522*	KU987669	KU987668	–	–	–	Abd-Elsalam et al. (2016)
Galiicola pseudophaeosphaeria	MFLUCC 14-0524*	KT326693	KT326692	–	–	–	Ariyawansa et al. (2015)
Hawksworthiana alliariae	MFLUCC 13-0070*	KX494877	KX494876	–	–	–	Wanasinghe et al. (2018)
Hawksworthiana clematidicola	MFLUCC 14-0910	MG829011	MG828901	–	–	–	Wanasinghe et al. (2018)
Italica achilleae	MFLUCC 14-0959	MG829013	MG828903	–	–	–	Wanasinghe et al. (2018)
Juncaceicola achilleae	MFLUCC 13-0606	KX449526	KX449525	–	–	–	Tennakoon et al. (2016)
Juncaceicola luzulae	MFLUCC 16-0780*	KX449530	KX449529	–	–	–	Tennakoon et al. (2016)
Juncaceicola typharum	CBS 296.54	KF251695	KF251192	–	–	–	Quaedvlieg et al. (2013)
Leptospora galii	KUMCC 15-0521	KX599548	KX599547	–	–	–	Phookamsak et al. (2017)
Leptospora rubella	CPC 11006*	DQ195792	DQ195780	–	–	–	Crous et al. (2006)
Loratospora aestuarii	JK 5535B*	GU301838	–	–	–	–	Schoch et al. (2009)
Melnikia anthoxanthii	MFLUCC 14-1010*	KU848204	–	–	–	–	Wijayawardene et al. (2016)
Muriphaeosphaeria galatellae	MFLUCC 14-0614*	KT438329	KT438333	–	–	–	Phukhamsakda et al. (2015)

Neocamarosporium betae	CBS 109410	EU754178	KY940790	–	GU371774	–	Valenzuela-Lopez et al. (2018)
	CBS 523.66	EU754179	FJ426981	KT389842	KT389670	–	Valenzuela-Lopez et al. (2018)
Neocamarosporium calvescens	CBS 246.79	EU754131	KY940774	–	KC584500	–	Valenzuela-Lopez et al. (2018)
Neocamarosporium goegapense	CBS 138008*	KJ869220	KJ869163	–	–	–	Valenzuela-Lopez et al. (2018)
Neocucurbitaria aquatica	CBS 297.74*	EU754177	LT623221	LT623238	LT623278	–	Valenzuela-Lopez et al. (2018)
Neocucurbitaria cava	CBS 115979	EU754198	AY853248	LT623234	LT623273	–	Valenzuela-Lopez et al. (2018)
	CBS 257.68*	EU754199	JF740260	KT389844	LT717681	–	Valenzuela-Lopez et al. (2018)
Neocucurbitaria hakeae	CBS 142109*	KY173526	KY173436	KY173613	KY173593	–	Valenzuela-Lopez et al. (2018)
Neocucurbitaria irregularis	CBS 142791*	LN907372	LT592916	LT592985	LT593054	–	Valenzuela-Lopez et al. (2018)
Neocucurbitaria keratinophila	CBS 121759*	LT623215	EU885415	LT623236	LT623275	–	Valenzuela-Lopez et al. (2018)
Neocucurbitaria quercina	CBS 115095*	GQ387619	LT623220	LT623237	LT623277	–	Valenzuela-Lopez et al. (2018)
Neocucurbitaria unguis-hominis	UTHSC:DI16-213; FMR 13703	LN907356	LT592910	LT592979	LT593048	–	Valenzuela-Lopez et al. (2018)
	CBS 111112	GQ387623	LT623222	LT623239	LT623279	–	Valenzuela-Lopez et al. (2018)
	CBS 112.79	GQ387622	LT717672	LT717675	LT717682	–	Valenzuela-Lopez et al. (2018)
Neopyrenochaeta acicola	CBS 812.95*	GQ387602	LT623218	LT623232	LT623271	–	Valenzuela-Lopez et al. (2018)
N. fragariae	CBS 101634*	GQ387603	LT623217	LT623231	LT623270	–	Valenzuela-Lopez et al. (2018)

N. inflorescentiae	CBS 119222*	EU552153	EU552153	LT623233	LT623272	–	Valenzuela-Lopez et al. (2018)
N. telephoni	CBS 139022*	KM516290	KM516291	LT717678	LT717685	–	Valenzuela-Lopez et al. (2018)
Neopyrenochaetopsis hominis	CBS 143033*	LN907381	LT592923	LT592992	LT593061	–	Valenzuela-Lopez et al. (2018)
Neosetophoma samarorum	CBS 138.96*	KF251664	KF251160	–	–	–	Quaedvlieg et al. (2013)
Neostagonospora caricis	CBS 135092*	KF251667	KF251163	–	–	–	Quaedvlieg et al. (2013)
Neostagonospora elegiae	CBS 135101	KF251668	KF251164	–	–	–	Quaedvlieg et al. (2013)
Neostagonospora spinificis	FU30120	KP676046	KP676045	–	–	–	Yang et al. (2016)
Neosulcatispora agaves	CPC 26407*	KT950867	KT950853	–	–	–	Crous et al. (2015)
Nodulosphaeria hirta	MFLUCC 13-0867*	KU708845	KU708849	–	–	–	Mapook et al. (2016)
Nodulosphaeria scabiosae	MFLUCC 14-1111	KU708846	KU708850	–	–	–	Mapook et al. (2016)
Ophiobolopsis italica	MFLUCC 17-1791*	MG520959	MG520939	–	–	–	Phookamsak et al. (2017)
Ophiobolus artemisiae	MFLUCC 14-1156	KT315509	KT315508	–	–	–	Ariyawansa et al. (2015)
Ophiobolus disseminans	MFLUCC 17-1787*	MG520961	MG520941	–	–	–	Phookamsak et al. (2017)
Ophiobolus senecionis	MFLUCC 13-0575	KT728366	KT728365	–	–	–	Tibpromma et al. (2015)
Ophiosimulans tanaceti	MFLUCC 14-0525*	KU738891	KU738890	–	–	–	Tibpromma et al. (2016)
Ophiosphaerella aquaticus	MFLUCC 14-0033	KX767089	KX767088	–	–	–	Ariyawansa et al. (2015)
Paracucurbitaria italica	CBS 234.92*	EU754176	LT623219	LT623235	LT623274	–	Valenzuela-Lopez et al. (2018)
Paracucurbitaria corni	CBS 248.79	GQ387608	LT903672	LT900365	LT903673	–	Valenzuela-Lopez et al. (2018)

Paraophiobolus arundinis	MFLUCC 17-1789*	MG520965	MG520945	–	–	–	Phookamsak et al. (2017)
Paraophiobolus plantaginis	MFLUCC 17-0245	KY815010	KY797641	–	–	–	Phookamsak et al. (2017)
Paraphoma chlamydocopiosa	UMPc01	–	KU999072	–	–	–	Moslemi et al. (2018)
Paraphoma chrysanthemicola	CBS 172.70	KF251669	KF251165	–	–	–	Quaedvlieg et al. (2013)
Paraphoma pye	UMPp02	–	KU999073	–	–	–	Moslemi et al. (2018)
Paraphoma radicina	CBS 102875*	KF251677	KF251173	–	–	–	Quaedvlieg et al. (2013)
Paraphoma rhaphiolepidis	CBS 142524	KY979813	KY979758	–	–	–	Crous et al. (2017)
Paraphoma vinacea	UMPV001	KU176888	KU176884	–	–	–	Moslemi et al. (2018)
Parastagonospora allouniseptata	MFLUCC 13-0386	KU058721	KU058711	–	–	–	Li et al. (2015)
Parastagonospora caricis	CBS 135671	KF251680	KF251176	–	–	–	Quaedvlieg et al. (2013)
Parastagonospora dactylidis	MFLUCC 13-0375	KU058722	KU058712	–	–	–	Li et al. (2015)
Parastagonospora italica	MFLUCC 13-0377	KU058724	KU058714	–	–	–	Li et al. (2015)
Parastagonospora minima	MFLUCC 13-0376	KU058723	KU058713	–	–	–	Li et al. (2015)
Parastagonospora nodorum	CBS 110109*	KF251681	KF251177	–	–	–	Quaedvlieg et al. (2013)
Parastagonospora poagena	CBS 136776	KJ869174	KJ869116	–	–	–	Crous et al. (2014)
Parastagonospora uniseptata	MFLUCC 13-0387	KU058725	KU058715	–	–	–	Li et al. (2015)
Parastagonosporella fallopiae	CBS 135981*	MH460545	MH460543	–	–	–	Thambugala et al. (2017)

Parastagonosporella fallopiae	CCTU 1151.1	MH460546	MH460544	–	–	–	Quaedvlieg et al. (2013)
Phaeopoacea festucae	MFLUCC 17-0056*	KY824767	KY824766	–	–	–	Quaedvlieg et al. (2013)
Phaeosphaeria oryzae	CBS 110110*	KF251689	KF251186	–	–	–	Quaedvlieg et al. (2013)
Phaeosphaeria papayae	CBS 135416	KF251690	KF251187	–	–	–	Li et al. (2015)
Phaeosphaeriopsis glaucopunctata	CBS 653.86*	KF251702	KF251199	–	–	–	Thambugala et al. (2017)
Poaceicola arundinis	MFLUCC 15-0702*	KU058726	KU058716	–	–	–	Ariyawansa et al. (2015)
Poaceicola italica	MFLUCC 13-0267	KX910094	KX926421	–	–	–	Phookamsak et al. (2017)
Populocrescentia forlicesenensis	MFLUCC 15-0651*	KT306952	KT306948	–	–	–	Phookamsak et al. (2017)
Pseudoophiobolus achilleae	MFLU 17-0925	MG520966	MG520946	–	–	–	Hyde et al. (2016)
Pseudoophiobolus mathieui	MFLUCC 17-1784*	MG520969	MG520949	–	–	–	Crous et al. (2012)
Pseudophaeosphaeria rubi	MFLUCC 14-0259*	KX765299	KX765298	–	–	–	Wanasinghe et al. (2018)
Pseudopyrenochaeta lycopersici	CBS 306.65*	EU754205	NR_103581	LT717674	LT717680	–	Valenzuela-Lopez et al. (2018)
Pseudopyrenochaeta terretris	CBS 282.72*	LT623216	LT623228	LT623246	LT623287	–	Valenzuela-Lopez et al. (2018)
Pyrenochaeta nobilis	CBS 407.76*	EU754206	EU930011	KT389845	LT623276	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis americana	UTHSC:DI16-225; FMR 13715	LN907368	LT592912	LT592981	LT593050	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis botulispora	UTHSC:DI16-289; FMR 13781	LN907432	LT592941	LT593010	LT593080	–	Valenzuela-Lopez et al. (2018)
	UTHSC:DI16-297; FMR 13790	LN907440	LT592945	LT593014	LT593084	–	Valenzuela-Lopez et al. (2018)
	CBS 142458*	LN907441	LT592946	LT593015	LT593085	–	Valenzuela-Lopez et al.

							(2018)
Pyrenochaetopsis confluens	CBS 142459*	LN907446	LT592950	LT593019	LT593089	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis decipiens	CBS 343.85*	GQ387624	LT623223	LT623240	LT623280	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis globosa	CBS 143034*	LN907418	LT592934	LT593003	LT593072	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis indica	CBS 124454*	GQ387626	LT623224	LT623241	LT623281	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis leptospora	CBS 101635*	GQ387627	JF740262	LT623242	LT623282	–	Valenzuela-Lopez et al. (2018)
	CBS 122787	EU754151	LT623225	LT623243	LT623283	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis microspora	UTHSC:DI16-198; FMR 13688	LN907341	LT592899	LT592968	LT593037	–	Valenzuela-Lopez et al. (2018)
	CBS 102876*	GQ387631	LT623226	LT623244	LT623284	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis paucisetosa	CBS 142460*	LN907336	LT592897	LT592966	LT593035	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis poae	CBS 136769*	KJ869175	KJ869117	KJ869243	LT623286	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis setosissima	CBS 119739*	GQ387632	LT623227	LT623245	LT623285	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis tabarestanensis	CBS 139506*	KF803343	KF730241	KX789523	–	–	Valenzuela-Lopez et al. (2018)
Pyrenochaetopsis uberiformis	CBS 142461*	LN907420	LT592935	LT593004	LT593074	–	Valenzuela-Lopez et al. (2018)
Sclerostagonospora opuntiae	CBS 118224	JX517293	JX517284	–	–	–	Wijayawardene et al. (2013)
Sclerostagonospora rosicola	MFLUCC 15-0129	MG829068	MG828957	–	–	–	Li et al. (2015)
Scolicosporium	MFLUCC 12-0089	KF366382	–	–	–	–	Crous et al. (2015)

minkeviciusii							
Septoriella allojunci	MFLUCC 15-0701	KU058728	KU058718	–	–	–	
Septoriella phragmitis	CPC 24118*	KR873279	KR873251	–	–	–	
Setomelanomma holmii	CBS 110217*	GU301871	KT389542	–	–	–	Schoch et al. (2009); Chen et al. (2015)
Didymella pinodella	CBS 531.66	GU238017	FJ427052	FJ427162	–	MK525067	Liu et al. (2019)
Setophoma antiqua	LC6594	MK511947	MK511909	MK524999	–	MK525070	Liu et al. (2019)
	LC6595	MK511948	MK511910	MK525000	–	MK525071	Liu et al. (2019)
	CGMCC 3.19525 = LC6596*	–	MK511911	MK525001	–	MK525072	Liu et al. (2019)
Setophoma brachypodii	CBS 145418*	–	MK539968	–	–	MK540161	Marin-Felix et al. (2019)
Setophoma caverna	LC12481	–	MK511927	MK525016	–	MK525088	Liu et al. (2019)
	LC12842	–	MK511928	MK525017	–	MK525089	Liu et al. (2019)
	CGMCC 3.19526 = LC7511	MK511965	MK511944	MK525032	–	MK525105	Liu et al. (2019)
Setophoma chromolaenae	CBS 135105*	KF251747	KF251244	KF252728	–	KF253195	Quaedvlieg et al. (2013)
Setophoma endophytica	LC13538	–	MK511923	MK525012	–	MK525084	Liu et al. (2019)
	LF2067	–	MK511924	MK525013	–	MK525085	Liu et al. (2019)
	CGMCC 3.19528 = LC3163*	MK511956	MK511931	MK525020	–	MK525092	Liu et al. (2019)
	LC3164	MK511957	MK511932	MK525021	–	MK525093	Liu et al. (2019)
	LC3165	–	MK511933	MK525022	–	MK525094	Liu et al. (2019)
	LC3216	MK511959	MK511938	MK525026	–	MK525099	Liu et al. (2019)
	LC3297	MK511962	MK511941	MK525029	–	MK525102	Liu et al. (2019)
Setophoma longinqua	CGMCC 3.19524 = LC6593*	MK511946	MK511908	MK524998	–	MK525069	Liu et al. (2019)
	LC13481	–	MK511925	MK525014	–	MK525086	Liu et al. (2019)
	LC13482	–	MK511926	MK525015	–	MK525087	Liu et al. (2019)

Setophoma pseudosacchari	CBS 145373*	–	MK539969	MK540176	–	–	Marin-Felix et al. (2019)
Setophoma sacchari	CBS 333.39*	–	KF251245	–	–	–	Quaedvlieg et al. (2013)
	LC12842	–	MK511928	MK525017	–	MK525089	Liu et al. (2019)
Setophoma terrestris	CBS 135470*	KF251739	KF251236		–		Quaedvlieg et al. (2013)
	CBS 335.29 = MUCL 9892 = LC6449*	KF251749	KF251246	KF252729	–	KF253196	Quaedvlieg et al. (2013)
	CBS 335.87	KF251750	KF251247	KF252730	–	KF253197	Liu et al. (2019)
	CBS 377.52	KF251751	KF251248	KF252731	–	KF253198	Liu et al. (2019)
Setophoma vervoniae	CBS 137988*	–	KJ869141	MK540177	–	MK540162	Crous et al. (2014)
Setophoma yingyisheniae	LC6739	–	MK511912	MK525002	–	MK525073	Liu et al. (2019)
	LC12696	MK511950	MK511914	–	–	MK525075	Liu et al. (2019)
	LC12699	MK511951	MK511915	MK525004	–	MK525076	Liu et al. (2019)
	LC13477	MK511952	MK511916	MK525005	–	MK525077	Liu et al. (2019)
	LC13478	–	MK511917	MK525006	–	MK525078	Liu et al. (2019)
	CGMCC 3.19527 = LC13479*	–	MK511918	MK525007	–	MK525079	Liu et al. (2019)
Setophoma yunnanensis	LC6532	MK511945	MK511907	MK524997	–	MK525068	Liu et al. (2019)
	CGMCC 3.19529 = LC6753*	MK511949	MK511913	MK525003	–	MK525074	Liu et al. (2019)
Sulcispora pleurospora	MFLUCC 14-0995*	KP271444	KP271443	–	–	–	Tibpromma et al. (2015)
Tintelnotia destructans	CBS 127737	KY090664	KY090652	–	–	–	Ahmed et al. (2017)
Tintelnotia opuntiae	CBS 376.91*	GU238123	KY090651	–	–	–	Ahmed et al. (2017)
Vagicola chlamydospora	MFLUCC 15-0177	KU163654	KU163658	–	–	–	Jayasiri et al. (2015)
Vagicola vagans	CBS 604.86*	KF251696	KF251193	–	–	–	Quaedvlieg et al. (2013)
Vrystaatia aloeicola	CBS 135107*	KF251781	KF251278	–	–	–	Quaedvlieg et al. (2013)
Wojnowicia lonicerae	MFLUCC 13-0737	KP684151	KP744471	–	–	–	Liu et al. (2015)

Wojnowicia rosicola	MFLUCC 15-0128	MG829091	MG828979	–	–	–	Wanasinghe et al. (2018)
Wojnowicia spartii	MFLUCC 13-0402	KU058729	KU058719	–	–	–	Li et al. (2015)
Wojnowiciella eucalypti	CPC 25024*	KR476774	KR476741	–	–	–	Crous et al. (2015)
Wojnowiciella viburni	MFLUCC 12-0733	KC594287	KC594286	–	–	–	Wijayawardene et al. (2013)
Xenoseptoria neosaccardoi	CBS 120.43	KF251783	KF251280	–	–	–	Quaedvlieg et al. (2013)
Xenoseptoria neosaccardoi	CBS 128665*	KF251784	KF251281	–	–	–	Quaedvlieg et al. (2013)
Xenopyrenochaetopsis pratorum	CBS 445.81*	GU238136	JF740263	KT389846	KT389671	–	Valenzuela-Lopez et al. (2018)
Yunnanensis phragmitis	MFLUCC 17-1361*	MF684865	MF684869	–	–	–	Karunarathna et al. (2017)

Ex-type strains are indicated with "*" after collection number. ITS = internal transcribed spacer, nc LSU rDNA = large subunit of the nrDNA, RPB2 = polymerase II second largest subunit, TUB2 = β-tubulin, TEF1 = translation elongation factor 1-α

Table 2 New name, substrate and distribution of *Setophoma* spp.

New name	Original name	Substrate	Distribution	References
Perisporiopsis antigua	*Setophoma antiqua*	*Camellia sinensis*	China	Liu et al. (2019)
P. brachypodii	*S. brachypodii*	*Brachypodium sylvaticum*	Belgium	Marin-Felix et al. (2019)
P. caverna	*S. caverna*	Carbonatite in cave	China	
P. chromolaenae	*S. chromolaenae*	*Chromolaena odorata*	Brazil	Quaedvlieg et al. (2013)
P. endophytica	*S. endophytica*	*Camellia sinensis*	China	Liu et al. (2019)
P. longinqua	*S. longinqua*	*Camellia sinensis*	China	Liu et al. (2019)
P. poaceicola	*S. poaceicola*	*Grass*	Thailand	Thambugala et al. (2017)
P. pseudosacchari	*S. pseudosacchari*	*Saccharum officinarum*	France	Marin-Felix et al. (2019)
P. sacchari	*S. sacchari*	*Saccharum officinarum*	Brazil	de Gruyter et al. (2010)

P. terrestris	*S. terrestris*	*Allium cepa*	North America, Senegal	de Gruyter et al. (2010)
		Allium sativum	United States	de Gruyter et al. (2010)
		Brassica sp.	Canada, Alberta	Yang et al. (2017)
		Cucurbita maxima	USA, Oregon	Rivedal et al. (2018)
		Cucurbita moschata	Japan	Ikeda et al. (2012)
		Solanum lycopersicum	Canada, Ontario	Johnston-Monje et al. (2017)
P. vernoniae	*S. vernoniae*	*Vernonia polyanthes*	Brazil	Crous et al. (2014)
P. yingyisheniae	*S. yingyisheniae*	*Camellia sinensis*	China	Liu et al. (2019)
P. yunnanensis	*S. yunnanensis*	*Camellia sinensis*	China	Liu et al. (2019)

5. CONCLUSÕES

Acroconidiella tropaeoli, espécie tipo do gênero *Acroconidiella* pertence a *Alternaria*. Um novo nome, *Alternaria obtusa* é proposto para *Acroconidiella tropaeoli*

Acroconidiella trisepta está filogeneticamente dentro de *Dendryphiella*. A nova combinação *Dendryphiella trisepta* comb. nov. é proposto para acomodar *A. trisepta*.

Acroconidiella é um gênero artificial que agora é rejeitado, uma vez que sua espécie-tipo pertence a *Alternaria* - que tem prioridade nomenclatural sobre *Acroconidiella*.

Outras espécies colocadas em *Acroconidiella*, aguardam reavaliação para determinar sua correta afinidade taxonômica.

Duosporium yamadanum, espécie tipo de *Duosporium*, está dentro de *Curvularia*. *Duosporium* torna-se sinônimo de *Curvularia*. Uma nova combinação é proposta para *D. yamadanum*: *Curvularia yamadana*.

Korunomyces terminaliae (espécie tipo de *Korunomyces*) e *Korunomyces prostratus* pertencem ao gênero *Coniella*. Como *Coniella* tem prioridade nomenclatural sobre *Korunomyces*, este, é reduzido a um sinônimo de *Coniella*. Um novo nome e uma nova combinação são propostas para ambas as espécies, a saber: *Coniella ferreirense* nom. nov. para *K. terminaliae* e *Coniella prostrata* comb. nov. para *K. prostratus.*

Uma descrição emendada de *Coniella* para incluir a formação ocasional de propágulos assexuados distintos e elaborados foi fornecida.

Ceratobasidium lantanae-camarae e *Ceratobasidium cornigerum* são filogeneticamente distintos, portanto, sua sinonimização foi um erro taxonômico.

O nome *C. lantanae-camarae* é restabelecido: um epitipo e uma sequência de ex-epitipo de referência são indicados, resolvendo sua taxonômia. Além disso, os postulados de Koch foram cumpridos pela primeira vez.

Perisporiopsis struthanthi, tipo do gênero *Perisporiopsis*, que por sua vez é o gênero tipo de Perisporiopsidaceae e *Perisporiopsis lantanae* pertencem à família Phaeosphaeriaceae. Uma vez que Phaeosphaeriaceae é um nome mais antigo, tem prioridade nomenclatural sobre Perisporiopsidaceae, que se torna um sinônimo do primeiro.

Perisporiopsis struthanthi, tipo do gênero *Perisporiopsis,* agrupou dentro do gênero *Setophoma*. Novas combinações são propostas para *Setophoma* spp. uma vez que *Perisporiopsis* tem prioridade nomenclatural sobre *Setophoma*.

O novo gênero e combinações são propostas para *P. lantanae*, nomeadamente *Sputnikia* e *Sputnikia lantanae*.

Perisporiopsis lateritia é recombinado como *Pyrenochaetopsis lateritia*.

Outras espécies e gêneros em Perisporiopsidaceae devem ser considerados como *Incertae sedis* até que sequências de DNA estejam disponíveis e um estudo filogenético molecular esclareça sua posição taxonômica.