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Rildo Alexandre Fernandes da Silva

DIVERSIDADE E IDENTIFICAÇÃO DE FUNGOS FITOPATOGÊNICOS EM FRUTOS NÃO CONVENCIONAIS NO BRASIL

Brasília
Setembro de 2022

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Tese de doutorado apresentado ao Programa de Pós-Graduação em Fitopatologia no Departamento de Fitopatologia, como parte dos requisitos necessários à obtenção do título de Doutor em Fitopatologia.

Orientador:
Danilo Batista Pinho

Brasília
Setembro de 2022

"*Só podemos ver um pouco do futuro, mas o suficiente para nos darmos conta de que há muito o que fazer*."

Alan M. Turing

Dedico aos meus familiares e a toda uma sociedade que não acreditava, eu consegui

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DIVERSIDADE E IDENTIFICAÇÃO DE FUNGOS FITOPATOGÊNICOS EM FRUTOS NÃO CONVENCIONAIS NO BRASIL

RILDO ALEXANDRE FERNANDES DA SILVA

Tese _____ em 28 de julho de 2023 por:

_____

Prof. Robert Neil Gerard Miller

Examinador Interno

_____

Profa. Maria Alves Ferreira

Examinador Externo

_____

Profa. Flávia Rodrigues Barbosa

Examinador Externo

_____

Prof. Danilo Batista Pinho

Orientador (Presidente)

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Sumário

RESUMO

Frutos não convencionais à agricultara brasileira, como *Syzygium jambos* (Myrtaceae) apesar de possuir significante importância na medicina tradicional, possuem um número raro de estudos focados em doenças que podem afetar esta espécie. Gêneros de fungos das famílias Nectriaceae, Bionectriaceae e Botryosphaeriaceae são frequentemente encontrados como fitopatógenos de plantas, relatados como associados a doenças de importância agrícola e florestal mas raramente associados a plantas nativas ou ao sintomas de podridão de frutos. A identificação desses fungos é desafiadora, especialmente o grupo *Calonectria*-like (Nectriaceae) por englobar fungos relacionados filogeneticamente e morfologicamente ao gênero *Calonectria*. Com isso técnicas moleculares foram implementadas para o grupo a partir de 1995 com a submissão sequências depositadas no Genbank, que se tornou um valoroso recurso para os pesquisadores deste grupo. O crescente volume de dados na plataforma dificulta ou impossibilita estudos de diversidade de hospedeiros, localidade e dispersão geográfica, devido à falta de uniformidade dos dados depositados. O presente estudo teve como objetivo fazer uma meta-análise do banco de dados para gêneros de *Calonectria*-like, além de identificar por análises morfo-filogeneticamente os isolados de *Calonectria*-like, *Clonostachys* e *Neofusicoccum* obtidos a partir de frutos nos estados de Distrito Federal, Goiás, Minas Gerais, Paraná, Pernambuco, Santa Catarina e São Paulo. Com a meta-análise revelou disparidades na amostragem de regiões genéticas, com os genes tef (n=5.125) e tub (n=5.287) os mais frequentemente depositados. Observando o “Efeito Matryoshka” por haver uma super-representação de isolados, devido a transferência entre laboratórios. Os metadados revelaram uma perda de 21.7% de dados de localidade, 88% para substrato/hospedeiros. No entanto os metadados permitiram identificar que o isolamento geográfico dos gêneros *Xenocylindrocladium* (América do Sul) e *Curvicladiella* (Ásia), além de Brasil (n=64) e China (n=51) apresentarem maior diversidade de espécies destes gêneros. Foram obtidos 86 isolados, os quais foram submetidos a análises morfo-filogenéticas, segregando dentro dos gêneros *Calonectria* (n=15), *Clonostachys* (n=8), *Cylindrocladiella* (n=28), *Gliocladiopsis* (n=18), *Gliocephalotrichum* (n=2) e *Neofusicoccum* (n=15). Desse total, sete espécies conhecidas foram documentadas enquanto seis táxons serão propostos como novas espécies. As espécies *Cylindrocladiella* vitis, *Gliocladiopsis* hennebertii e *Neofusicoccum* occulatum e N. umdonicola são relatadas pela primeira vez no Brasil. Enquanto novas combinações de hospedeiros foram registradas para *Ch. Pseudocholeuca, Ch. rogersoniana, Cy. infestans, Cy. lageniformis, Cy. peruviana*, *Gliocephalotrichum* simplex, *Gliocladiopsis tenuis, N. batangarum*, e *N. parvum* em frutos de *Dypsis madagascariensis, Eugenia aggregata, Eugenia involucrata, Euterpe edulis, Spondia mombin, Syzygium jambos* e *Terminalia catappa*. Esse estudo demonstra a necessidade de levantamentos abrangentes sobre a diversidade de espécies fungos em plantas nativas e alerta sobre o risco de transmissão para as plantas de interesse econômico.

Palavras-chave: Hypocreaceae; Cerrado; Meta-análise; Filogenia; Taxonomia de Fungos.

ABSTRACT

Non-conventional fruits in Brazilian agriculture, such as *Syzygium jambos* (Myrtaceae), despite their significant importance in traditional medicine, have scarce studies focused on diseases that can affect this species. Genera of fungi from the families Nectriaceae, Bionectriaceae, and Botryosphaeriaceae are frequently found as plant pathogens, associated with diseases of agricultural and forestry importance but rarely associated with native plants or fruit rot symptoms. The identification of these fungi is challenging, especially the *Calonectria*-like group (Nectriaceae) that includes phylogenetically and morphologically related fungi to the genus *Calonectria*. Molecular techniques have been implemented for this group since 1995 with the submission of sequences deposited in the GenBank, which has become a valuable resource for researchers in this field. The increasing volume of data in the platform hinders or prevents studies on host diversity, location, and geographical dispersion due to the lack of data uniformity. This study aimed to perform a meta-analysis of the database for *Calonectria*-like genera, as well as to identify morpho-phylogenetically the isolates of *Calonectria*-like, *Clonostachys,* and *Neofusicoccum* obtained from fruits in the states of Federal District, Goiás, Minas Gerais, Paraná, Pernambuco, Santa Catarina, and São Paulo. The meta-analysis revealed disparities in the sampling of genetic regions, with the genes tef (n=5,125) and tub (n=5,287) being the most frequently deposited. The "Matryoshka Effect" was observed, indicating an over-representation of isolates due to transfers between laboratories. The metadata revealed a loss of 21.7% of location data and 88% of substrate/host data. However, the metadata allowed for the identification of the geographic isolation of the genera *Xenocylindrocladium* (South America) and *Curvicladiella* (Asia), with Brazil (n=64) and China (n=51) presenting a higher species diversity of these genera. A total of 86 isolates were obtained, which were subjected to morpho-phylogenetic analyses, segregating into the genera *Calonectria* (n=15), *Clonostachys* (n=8), *Cylindrocladiella* (n=28), *Gliocladiopsis* (n=18), *Gliocephalotrichum* (n=2), and *Neofusicoccum* (n=15). Among these, seven known species were documented, while six taxa are proposed as new species. The species *Cylindrocladiella* vitis, *Gliocladiopsis* hennebertii, *Neofusicoccum occulatum*, and *N. umdonicola* are reported for the first time in Brazil. Furthermore, new host combinations were recorded for *Ch. pseudocholeuca, Ch. rogersoniana, Cy. infestans, Cy. lageniformis, Cy. peruviana, Gliocephalotrichum simplex, Gliocladiopsis tenuis, N. batangarum,* and *N. parvum* in fruits of *Dypsis madagascariensis, Eugenia aggregata, Eugenia involucrata, Euterpe edulis, Spondia mombin, Syzygium jambos*, and *Terminalia catappa*. This study demonstrates the need for comprehensive surveys on the diversity of fungal species in native plants and highlights the risk of transmission to economically important plants.

Keywords: Hypocreaceae; Cerrado; Meta-analysis; Phylogeny; Fungal Taxonomy.

Capítulo 1: Distribuição global, diversidade de hospedeiros e diversidade genética dos gêneros de *Calonectria*-like

1.1 Introdução

O grupo denominado *Calonectria*-like, detém atualmente oito gêneros de fungos *Aquanectria, Calonectria, Curvicladiella, Cylindrocladiella, Gliocephalotrichum, Gliocladiopsis, Penicillifer* e *Xenocylindrocladium.* Durante a história estes gêneros foram assim nomeados por possuírem morfologias similares a *Calonectria* descrito há mais de 150 anos. Atualmente é constatado que além de compartilharem características morfológicas eles também compartilham um ancestral comum dentro da família *Nectriaceae* (Ordem *Hypocreales* - Lombard et al. 2015a) Ao longo do desenvolvimento dos estudos relacionados a estes fungos, o conceito morfológico de espécie foi sendo lapidado com a proposição de novos gêneros (*Cylindrocladiella* e *Gliocephalotrichum*) ou sinonimização de gêneros que posteriormente foram revalidados (*Gliocladiopsis*). Essas mudanças melhoraram o entendimento da real definição do conceito morfológico de espécie, fazendo com que os próximos gêneros similares a *Calonectria* fossem já descritos separadamente (Ellis and Hesseltine 1962; Boesewikel 1982; Lombard and Crous 2012a).

Calonectria-like inclui atualmente 227 espécies. Algumas das espécies são globalmente encontradas em vários países e em diferentes ambientes, outros recebem destaque devido a importância econômica por causarem doenças em inúmeras espécies vegetais. A exemplo do gênero *Calonectria* que é relatado associado a aproximadamente 335 espécies de plantas em quase 30 famílias (Crous 2002; Lombard et al. 2010b) ou *Gliocephalotrichum* que recentemente foi estudado como causador de podridão de frutos em diversas espécies e locais no Brasil, ou seu impacto na comercialização de rambutãn em diversos países do globo (Lombard et al. 2014; Silva et al. 2020a). Devido à importância destes gêneros nos últimos anos foram adotadas diversas novas ferramentas para a correta identificação de espécies, o principal destes é a aplicação do conceito filogenético de espécie, baseado em análises de sequencias de DNA. A aplicação desta ferramenta, fez

crescer exponencialmente o reconhecimento e descrição de espécies, assim consequentemente a deposição de sequencias em bancos de dados públicos. Em casos como *Calonectria* o gênero chegou a possuir 169 espécies em 2019, o que no ano seguinte foi revisado e viram que 54 destas espécies eram erros de interpretação do conceito devido a reconstruções filogenéticas usando números e tipos de marcadores variados, sem uniformidade (Liu et al. 2020). Tal estudo ressalta a necessidade de padronizações, mas também sugere padrões como o uso de seis marcadores moleculares para o uso em reconstruções filogenéticas.

Apesar de acelerar a identificação de espécies usando ferramentas moleculares, muitos laboratórios ainda não possuem tal ferramenta ou não possuem recursos econômicos suficientes para atender determinados padrões como os sugeridos por Liu et al. (2020) Com isso análises morfológicas muitas vezes é a metodologia adotada, seja para primeiro *screening* ou para uma diagnose rápida a nível de gênero. Porém como *Calonectria*-like possui grandes similaridades morfológica a análise e classificação deste grupo se torna um desafio para pesquisadores inexperientes. Outro desafio é quanto a dispersão de informação em muitos locais, o único trabalho que une muitas descrições de gêneros contendo imagens de espécies é o de Lombard et al. (2015a) no entanto para pesquisadores iniciantes a ausência de um glossário dificulta o entendimento. Além do significado de cada estrutura existe uma lacuna quanto a descrição de espécie, pois os padrões de medições é ausente em publicações, é uma informação metodológica que atualmente ficam restritas a uso interno de laboratórios que trabalham com os gêneros.

Calonectria-like é relacionado comumente causando doenças em espécies vegetais, no entanto eles são caracterizados como habitantes do solo, que podem infectar plantas com muitos destes gêneros já caracterizados como hemibiotróficos (Crous 2002). Gêneros como *Calonectria*, *Cylindrocladiella, Gliocladiopsis* e *Gliocephalotrichum* vêm reportadas em inúmeros patossistemas, como causadores de manchas foliares, podridão

de raiz e podridão de frutos além de outros. Apesar de alguns patossistemas o agente causal ter sido comprovado através do postulado de Koch, existem várias espécies dentro de *Cylindrocladiella* e *Gliocladiopsis* que apesar de associadas a sintomas em plantas não foram submetidas ao postulado (Pham et al. 2018). Estas ausências impedem uma melhor compreensão do impacto atual e futuro destas espécies em plantas de importância econômica e ambiental. Cerca de 5 espécies de *Calonectria* tiveram alguns dos seus patossistemas investigados a nível molecular, identificando quais fatores da relação estão envolvidos nos processos de infecção, penetração e colonização pelo fungo, bem como quais os fatores de resistência e defesa da planta (Malapi-Wight et al. 2019; Liu et al. 2021; Salgado-Salazar et al. 2022). Estes estudos ainda são iniciais e mais patossistemas precisam ser explorados, no entanto os estudos atualmente publicados compõe uma base de dados para o melhor entendimento desta relação.

Outro enfoque que tem se iniciado no grupo, mas que se mantem inicialmente dentro do gênero *Calonectria* são os estudos genômicos, estes podem ser aplicados com diversas finalidades e a partir de diversas hipóteses. Atualmente estão publicados genomas de 17 espécies de *Calonectria* dentro do NCBI, não tendo disponível genoma de nenhum outro gênero de *Calonectria*-like. O que deixa uma lacuna enorme para aplicações desde definição de espécies, identificações de padrões evolutivos compartilhados entre os gêneros, reconstruções de ancestralidade, análises de diferenciação de gêneros, identificação de promotores de variabilidade etc. É visto que, apesar da grande quantidade de estudos voltados para o grupo, ainda existem ausência de informação para vários dos gêneros de menor importância econômica e mesmo os que ainda possuem tal relevância ainda é identificado a carência de alguns estudos, indicando que necessita de uma melhor atenção a todos os indivíduos do grupo para uma melhor compreensão.

1.2 Frutos não convencionais à agricultura

O Brasil, atualmente está entre os 5 maiores produtores agrícolas do mundo, acompanhado de Estados Unidos, Índia, China e Rússia (IBGE 2023). No que se relaciona a produção de frutos o Brasil ocupou em 2020 a terceira posição no ranking mundial de maiores produtores, com 58 milhões de toneladas em “frutas” (Aragão and Contini 2020). Segundo censo de 2020 os frutos mais produzidos no País ficaram com a Laranja e Banana. Entre os frutos mais produzidos no Brasil consta apenas uma listagem de 20 frutos, entre estes somente duas tem origem na América do Sul (Cacau e Goiaba – Tabela 1).

Tabela 1. Ranking de frutos mais produzidos no Brasil em 2020 segundo o IBGE.

Principais produtos das lavouras temporárias e permanentes	Quantidade produzida (t)	Valor da produção (1 000 R$)
Laranja	16.980.379	8.299.850
Banana	6.625.211	8.123.040
Melancia	2.088.048	1.347.431
Coco-da-baía	1.756.264	1.127.157
Abacaxi	1.706.078	2.264.492
Mamão	1.296.940	1.388.767
Limão	1.234.691	1.273.656
Uva	1.113.345	2.391.852
Manga	1.094.358	944.859
Maçã	1.055.383	1.667.655
Tangerina	1.013.067	965.521
Maracujá	618.298	956.731
Melão	596.430	597.724
Goiaba	420.809	515.830
Cacau (em amêndoa)	213.871	2.007.189
Abacate	196.545	230.129
Pêssego	193.480	403.587
Caqui	162.184	287.613
Figo	26.910	78.618
Pera	14.915	33.770

É notável a dominância de frutos de origem de países asiáticos (Melancia, banana, mamão, manga etc.), e europeus (Laranja, pera, maçã, pêssego, uva etc.) estes foram e introduzidos no Brasil desde a sua colonização (Teixeira et al. 2019 – Tabela 1). Estes frutos podem ser considerados convencionais à agricultura, por serem amplamente conhecidos, cultivados e estarem nas prateleiras dos mercados. Em contrapartida, frutos como: abricó-da-praia (*Mimusopsis comersonii*), acerola (*Malpighia emarginata*), angá (*Inga laurina*), araçá-boi (*Eugenia stipitata*), buriti (*Mauritia flexuosa*), cajá (*Spondia mombin*), Cerejeira-da-praia (*Eugenia involucra*), dovialis (*Dovyalis abyssinica*), jambo amarelo (*Syzygium jambos*), jambolão (*Syzygium jambolanum*), jaracatiá (*Jaracatia spinosa*), pequí (*Caryocar brasiliensis*), pitanga (Eugenia uniflora), pitangatuba (Eugenia selloi), seriguela (*Spondias purpúrea)* e sete-copas (*Termninalia catappa*) e muitos outros, são considerados como não convencionais por estarem ligados a sazonalidade da produção, conhecimento tradicional, o desconhecimento da população, e a não existência de áreas dedicadas à produção destes frutos.

Apesar dos frutos não convencionais terem passado por muito tempo de desconhecimento, existem estudos focados em utilizar estes frutos para descoberta de novas fontes de alimentos para humanos, fármacos e cosméticos (Mohanty and Cock 2010; Infante et al. 2016; Ferreira et al. 2019; Ochieng et al. 2022). Todos estes possíveis novos usos para os frutos não convencionais são sugeridos pela observação do uso constante das espécies por povos tradicionais, conhecimento que é pobremente conhecido no Brasil (Teixeira et al. 2019). Como exemplo, o pequí é citado como rico em proteínas, vitaminas A e B2, ferro, cobre e fósforo, enquanto, a cagaita (*Eugenia dysenterica*), caju (*Anacardium occidentale*), graviola (*Annona muricata*) e outros possuem alto teor de antioxidantes (Luzia and Jorge 2014; Infante et al. 2016). Entre os frutos não convencionais está o jambo amarelo, que tem sido demonstrado alta aplicabilidade por seus compostos antioxidantes,

anti-inflamatórios, anti-diabetes, protetor hepático e antimicrobiano (Mohanty and Cock 2010).

A importância da pesquisa em relação ao aproveitamento e direcionamento da produção destes frutos, entra em conflito direto com o número de estudos relacionando estes frutos como as possíveis doenças que podem ocasionar perdas na comercialização destes frutos, ou na viabilidade destes para indústrias de fármacos e cosméticos. Doenças causadas por fungos podem ser consideradas uma importante ameaça a tais setores. A exemplo disto, em pequí foram relacionadas doenças causadas por *Phomopsis* spp, *Calonectria* clavatum, *Botryodiplodia theobromae, Capillaureum caryovora, Cerotelium* spp, *Colletotrichum acutatum, Ceratocystis fimbriata* e *Gliocephalotrichum* sp. (Hodges and May 1972; Ferreira et al. 2019; Silva et al. 2020). Em 2020, Silva et al. (2020) relataram espécies de *Gliocephalotrichum* causando doenças em frutos de cajazinho (*Spondias mombin*), jambo amarelo (*Syzygium jambos*), jambolão (Syzygium cumini), jerivá (*Syagrus romanzoffiana*), mangostin (*Garcinia mangostana*), palmeira laca (*Cyrtostachys renda*), palmeira locuba (*Dypsis madagascariensis*) e seriguela (*Spondias purpurea*). Trabalhos como os anteriormente citados, são apenas o início e ainda existe uma imensa lacuna a ser preenchida para o sucesso de muitas das aplicações pretendidas às diversas espécies de frutos não convencionais encontrados no Brasil.

1.3 Histórico do grupo *Calonectria*-Like

História de *Calonectria* e seus gêneros aliados ao longo dos mais de 150 anos. O gênero *Calonectria* que dá nome ao grupo atualmente é constituído por cerca de 138 espécies relatadas. Teve seu início em 1867 por De Notaris, que identificou o este sob folhas de *Magnolia grandiflora* em Daldini (Itália), descrevendo *Calonectria daldiniana* como a espécie tipo do gênero, posteriormente esta espécie foi sinonimizada por Rossman,

(1979) como *Calonectria pyrochroa* devido à similaridade com *Nectria pyrochroa*. Vinte e cinco anos após a descrição de *Calonectria* foi descrito o gênero *Cylindrocladium* baseado na morfologia de *Cylindrocladium scoparium* descrito por Morgan 1892 como saprófita em ramos de *Gledtsia triacanthos* nos EUA (Morgan 1892; Lombard et al. 2010c), entretanto este autor falhou em descrever a espécie deixando fora a extensão da estipe terminando em uma vesícula tão característica do gênero (Crous 2002). Nos anos seguintes ambos os gêneros formam relacionados como teleomorfo (*Calonectria*) e anamorfo (*Cylindrocladium*).

Rossman, (1979) ao sinonimizar o tipo de *Calonectria*, descreveu o anamorfo com uma superfície peritecial de amarelo, vermelho, marrom de coloração pálida em KOH+. com paredes em três camadas e distintas texturas, com células da parede de finas a espessas, pigmentadas, ascos caracterizados como unitunicados, evanescentes quando maduros, ápice indiferenciado, 8 ascósporos por ascos, estes são elípticos a fusiformes, hialinos, uni ou multiseptados (Figura 1). O anamorfo caracterizado frequentemente macroconidiósporos, consistindo em uma estipe com arranjo penicilado dos ramos férteis, uma extensão da estipe terminando em uma vesícula terminal, estipe hialina ou ligeiramente pigmentada na base, lisa ou finamente verrugosa, extensão da estipe septada, ereta ou flexionada (na mesma espécie pode ser observado extensão da estipe adicional formada em um angulo de 90º na estipe), terminando em uma vesícula de fina parede com formato característico. Aparato conidiogênico com um ou nenhum septo no primeiro ramo, podendo ter até 6 ramos adicionais, geralmente asseptados, no final de cada ramo é produzido 1-6 fiálides, estas comumente cilíndricas para alantoides, ligeiramente curvadas, ou doliformes para reniformes, hialinas, asseptadas, ápice com espessamento periclinal e coloração indistinta. Conídios cilíndricos arredondados nas pontas, eretos ou curvados, 1 ou multiseptados, cicatriz aparente ausente, conídios formados em aglomerados cilíndricos unidos por massa mucilaginosa incolor. Algumas espécies podem apresentar megaconidióforos e microconidióforos (Figura 1.2 - Crous, 2002).

Até 1950, somente sete espécies eram conhecidas, Boedjin & Reitsma, (1950) realocaram dentro do gênero *Cylindrocladium* espécies que estavam fora deste,

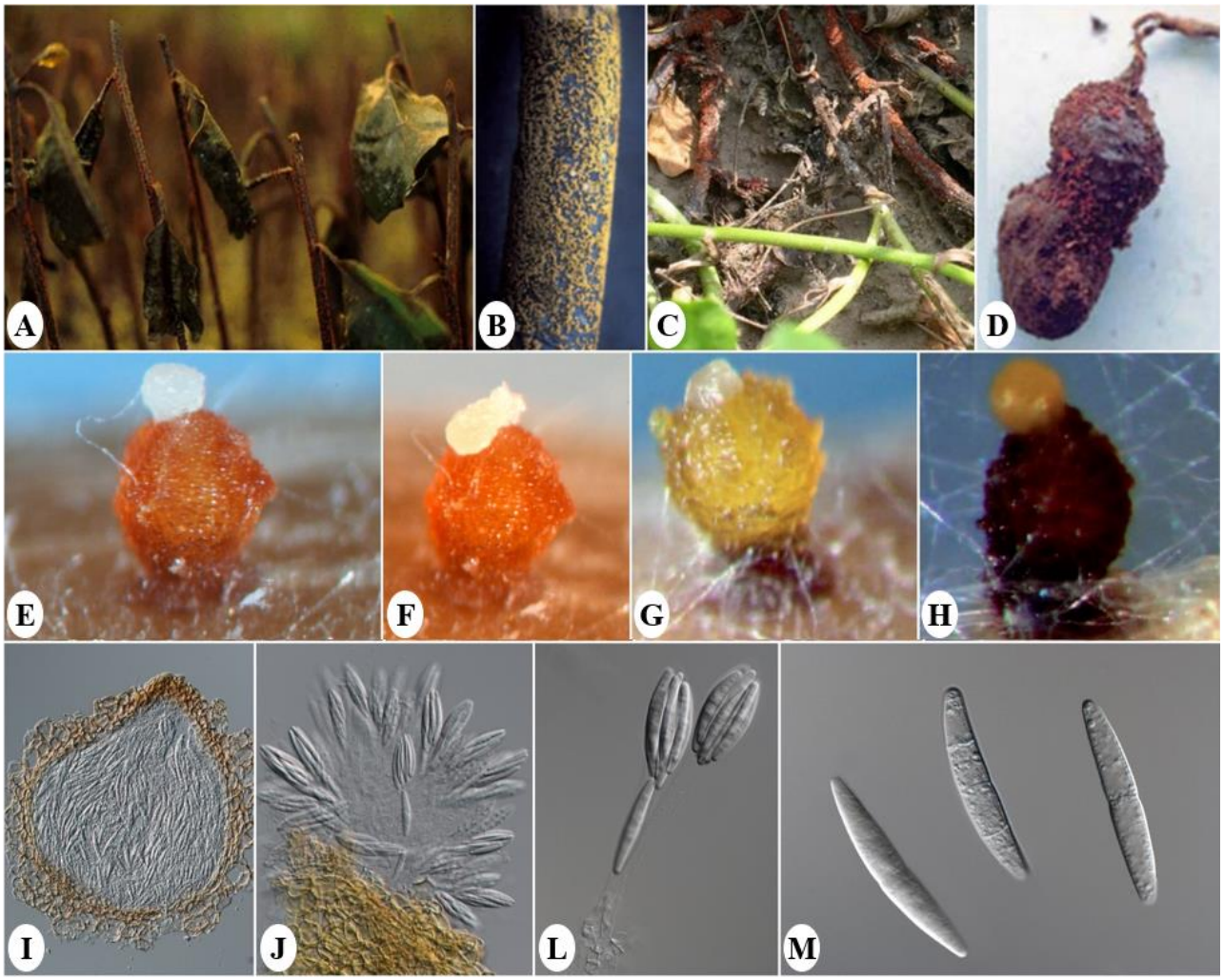

Figura 1: Estruturas típicas da fase sexuada de *Calonectria*. (A-D) Presença de peritécio em tecidos doentes, (E-H) peritécio brilhante de coloração viva, (I) peritécio contendo ascas clavadas, (J-K) ascas clavadas contendo ascósporos hialinos e fusiformes e (L) ascósporos típicos de Calonectria. (Alfenas et al., 2015).

constituindo assim a primeira monografia sobre o gênero no mundo, posteriormente muitas espécies de *Calonectria* foram descritas, estas novas descrições não necessariamente possuíam as duas fases do fungo. Quatro anos após a primeira revisão no gênero *Calonectria,* foi descrito um novo gênero, baseado na morfologia de *Gliocladiopsis sagariensis,* coletado a partir de amostras de solo, o gênero *Gliocladiopsis* caracterizado tendo conidióforos penicilados, hialinos, produzindo conídios hialinos e cilíndricos, no entanto o gênero não produzia extensão da estipe (Lombard and Crous 2012a). O gênero

Calonectria ao longo dos anos foi revisitado para refinar e caracterizar melhor quais são os padrões morfológicos do gênero, para que seja bem distinguido dos seus gêneros similares. Em 1962 a primeira lapidada ocorreu com a retirada de *Calonectria simplex* e *Ca. simplex* var*. microchlamidosporum* do gênero e alocado dentro de um novo gênero, *Gliocephalotrichum* devido os conidióforos multipenicilados e múltiplas extensões da estipe (Ellis and Hesseltine 1962).

Em 1968 o gênero *Gliocladiopsis* foi sinonimizado como espécie de *Calonectria* por este segundo poder perder a extensão da estipe de acordo com a idade do conidióforo (Lombard & Crous, 2012). No mesmo ano um outro gênero com características similares ao anamorfo de *Calonectria,* foi descrito. *Penicillifer* foi proposto a partir da espécie *Pe. pulcher* com conidióforo penicilado, hialino, conídios monoseptado cilíndricos, oblongo para elipsóide (Van Emden 1968).

Em 1982 o gênero *Cylindrocladium* agrupava 24 espécies, 20 destas com conídios grandes e outras quatro com conídios pequenos. Com base na diferença dos grupos de dentro de *Calonectria* Boesewikel, (1982) ergue o gênero *Cylindrocladiella* composto por *Cylla. parva* (=*Cy. parvum*), *Cylla.camelliae* (=*Cy. camelliae*), *Cylla. peruviana* (=*Cy. peruvianum*) e *Cylla. novae-zelandiae* (=*Cy. novae-zelandiae*) retirados de *Calonectria* por possuírem conídios menores que 20 µm, 0-1septos e extensão da estipe asseptada com paredes finas. Neste mesmo trabalho foi adicionado *Cylla. infestans* como uma nova espécie.

A reclassificação de *Gliocladiopsis* foi rejeitada em 1993 e novamente elevado à categoria de gênero devido a comprovação da estabilidade morfológica, e como padrão morfológico o gênero não produz nenhuma extensão da estipe (Crous and Wingfield 1993b; Lombard and Crous 2012a). Outros dois gêneros foram sendo descritos tomando como base a morfologia, *Xenocilindrocladium* sp. e *Curvicladiella* sp. ambos baseados somente

em características morfológicas (Decock et al. 1997; Decock and Crous 1998). *Aquanectria* é o gênero mais recente do grupo, proposto em 2015 a partir de caracterização polifásica, baseada em características morfológicas e moleculares (Lombard et al. 2015a).

A fase de anamorfo começou a ser citada como importante para a distinção de espécies de *Calonectria* (Peerally 1991) , que foi mais bem discutido três anos depois por Crous & Wingfield, (1993). Com esta nova abordagem os próximos trabalhos se basearam nesta característica para descreverem novas espécies de *Calonectria*. Crous, (2002) publicou um importante trabalho que compilou 54 espécies de *Calonectria* e seus gêneros aliados. Na revisão foi padronizado a morfologia, mostrando cada uma das descrições, distribuição, diversidade de hospedeiros e extensivos dados sobre etiologia e sintomatologia das doenças causadas por estes gêneros, servindo de base para o estudo destes gêneros no mundo (Crous 2002).

Outro importante trabalho que trouxe luz ao gênero foi publicado em 2010, este trabalho reviu e estabeleceu o conceito de espécie de *Calonectria*, discorrendo em torno dos parâmetros, morfológicos, biológicos e filogenéticos para nomenclatura (Lombard et al. 2010d). Neste mesmo ano foi publicado o divisor de águas dos estudo filogenéticos de *Calonectria*. Lombard et al., (2010a) estudou sete marcadores moleculares (Actina, Beta-tubulina, Histona, Transcritor Interno do Ribossomo 1 e 2 e o 5.8S do RNA ribossomal, 28S Subunidade maior do RNA e Fator de elongação 1-alfa), mostrando na época, quais marcadores proviam uma maior acurácia no reconhecimento de espécies, tais marcadores ainda têm sido amplamente utilizados. Já nos primeiros estudos morfológicos do gênero *Calonectria* Lombard et al. (2010c) propõe que o gênero apresenta dois grandes grupos um com vesículas do tipo prolata e outra do tipo esfaero-naviculata com base em observações morfológicas. Os dois grupos foram posteriormente confirmados por análises filogenéticas, e estabeleceu que a melhor estratégia para descrição de espécies é a polifásica devido a presença de espécies crípticas (Lombard et al. 2010c).

Mesmo os gêneros *Calonectria* e *Cylindrocladium* terem sido relacionados um ao outro desde 1892, ambos os gêneros foram tratados separadamente durante anos, no entanto o uso de dois nomes para um único fungo não fazia sentido e causava muitas confusões entre pesquisadores, especialmente para os que não são taxonomistas (Lombard et al. 2010d). Com intuito de evitar tais confusões, algumas mudanças no Código Internacional de Nomenclatura de Fungos, Algas e Plantas (ICBN, McNeill, et al. 2005) e sob acordo da comunidade científica, desde 2009 foi adotado *Calonectria* como o nome oficial para o gênero independente da fase observada, isso se deu pelo nome ter sido publicado primeiramente (Wingfield et al. 2012). Hoje, todos os artigos publicados por referência a esta normatização de 2009 usam o nome de *Calonectria*. Uma pesquisa feita no Index Fungorum (www.indexfungorum.org) por ambos os nomes, encontramos dados de 434 registros para *Calonectria* e 91 para *Cylindrocladium*. Apesar destas considerações em uma pesquisa no indexador do Google Acadêmico (www.scholar.google.com.br) é observado publicações feitas em 2022 com o título referenciando somente *Cylindrocladium* sem o atual nome devidamente referenciado (Ndifon 2022).

Em trabalho reunindo ampla amostragem de espécimes em solos e folhas de *Eucalyptus* spp. Alfenas et al. (2015) propõe o conceito de complexo de espécies, baseado nas características morfológicas compartilhadas entre espécies que compartilham um ancestral em comum. Tal conceituação permitiu agrupar 57 espécies em cinco complexos de espécies (*Ca. brassicae, Ca. candelabra, Ca. cylindrospora, Ca. pteridis* e *Ca. naviculata*). Em meados de 2019 o gênero *Calonectria* chegou a possuir 171 espécies aceitas baseadas em sequências de DNA e comparações morfológicas (Li et al. 2017; Marin-Felix et al. 2017; Pham et al. 2019; Liu et al. 2020) ao se deparar com o grande número de espécies e a desuniformidade de marcadores moleculares usados para descrever tais espécies, fez um extenso estudo revendo a maioria das espécies adicionando aos bancos de dados moleculares. Com isso pode analisar amplamente qual

dos marcadores poderia ser utilizado como *DNA barcode* e refinando melhor o conceito filogenético de espécie para o gênero. Liu et al. (2020) reduziu o número de espécies de 171 para 124, sinonimizando muitas delas devido a nova abordagem. Os autores também reafirmaram a segregação em dois grandes grupos e o conceito de complexo de espécies. A nova revisão definiu que o gênero *Calonectria* é composto por dois grupos maiores o prolato e esfaero-naviculado, o primeiro composto por 9 complexos de espécies (*Ca. brassicae, Ca. candelabrum, Ca. colhounii, Ca. cylindrospora, Ca. gracilipes, Ca. mexicana, Ca. pteridis, Ca. reteaudii* e *Ca. spathiphylli*) e dois complexos de espécies para o segundo grupo (*Ca. kyotensis* e *Ca. naviculata*), mantendo um total de 124 espécies (Liu et al. 2020). Atualmente foram adicionadas ao gênero seis novas espécies entre 2020 e 2023, (Pham et al. 2022; Sanchez-Gonzalez et al. 2022) totalizando 131 espécies aceitas no gênero (Tabela 1).

Recentemente um trabalho propôs sete novas espécies de *Calonectria*, no entanto as espécies deste trabalho não foram consideras na conta total, devido a abordagem utilizada para descrever as espécies. Lang et al. (2023) propõe as espécies baseado em análises polifásicas (morfologia e filogenia) no entanto nas análises filogenéticas os autores optaram por utilizar somente três marcadores (*tef, cmda* e *his3*). Enquanto, desde 2020 pelo indicado por Liu et al. (2020) devido à similaridade genética entre espécies do gênero espécies deveriam ser propostas baseadas em seis marcadores para ter melhore resolução e definição da espécie (*act, cmdA, his3, rpb2, tef1* e *tub2*).

Diferentemente do gênero *Calonectria* outros gêneros não tiveram sua história tão mudada ao longo dos anos, a exemplo disto *Cylindrocladiella* após ser proposto a única mudança grande foi em 2013, com a unificação do nome, deixando *Nectricladiella* como sinônimo (Taylor 2011; Wingfield et al. 2012). Dez anos após o surgimento do gênero *Cylindrocladiella* eram compostos por seis espécies, este número permaneceu estável por mais 10 anos somente o conhecimento de duas fases sexuais. O maior aumento de

espécies acontece em 2012 com o acréscimo de 18 novas espécies, neste mesmo trabalho foi introduzido o conceito de complexo de espécies trazido do seu "gênero irmão" (*Calonectria*), assim, 5 complexos de espécies (*Cylla. camelliae, Cylla. infestans, Cylla. elegans, Cylla. parva* e *Cylla. peruviana*) foram propostos (Lombard et al. 2012). Os próximos acréscimos foram nos anos de 2017 e 2018 com a adição de 11 novas espécies encontradas no Sudeste Asiático (Lombard et al. 2017; Pham et al. 2018), deixando assim o gênero com 43 espécies aceitas (Figura 3).

Outro gênero com número de espécies e relatos muito restritos é *Gliocephalotrichum* (Figura 4), este gênero proposto em 1962 derivado de espécies coletadas de amostras de solo e espécimes revisados trazidos do gênero *Calonectria*. Dez anos após a sua proposição foi acrescido duas espécies e feita a primeira inferência sobre a separação destes entre dois grupos, observando o ponto de inserção das extensões da estipe (Wiley and Simmons 197). Os estudos usando marcadores moleculares foi introduzido ao gênero em 2006, ajudando Decock et al. (2006) a identificar e caracterizar duas novas espécies, aumentando de cinco para sete espécies conhecidas. Em 2014 o gênero aumentou o número de marcadores moleculares e foram descritas mais cinco espécies, fortificando o conceito de que este gênero está comumente ligado em podridão de frutos em áreas tropicais (Lombard et al. 2014), posteriormente em 2020 foram adicionadas mais 3 espécies ao gênero, totalizando 16 espécies. este trabalho abriu a discussão quanto a presença ou não de extensão da estipe, identificando a primeira espécie do gênero sem as características extensões da estipe, bem como confirmando que análises filogenéticas não considera válida a segregação de dois grupos como de acordo com o ponto de inserção da extensão da estipe como Wiley and Simmons (1971) sugere (Silva et al. 2020).

Atualmente o gênero tem 16 espécies descritas (Tabela 1). *Gliocephalotrichum* foi caracterizado com base na espécie tipo *Gliocephalotrichum bulbilium* como: saprófita, de rápido crescimento, conidióforos com coloração, livres, septados, poliverticilados com formação de ramos primários, secundários e frequentemente terciários, e fiálides nas pontas dos ramos; conidióforos com extensões estéreis saindo abaixo das primeiras ramificações penicilado; conídios oblongos a elipsoidais, hialinos, formados sucessivamente e unindo-se por gotículas mucilaginosas (Figura 1.4 - Ellis and Hesseltine

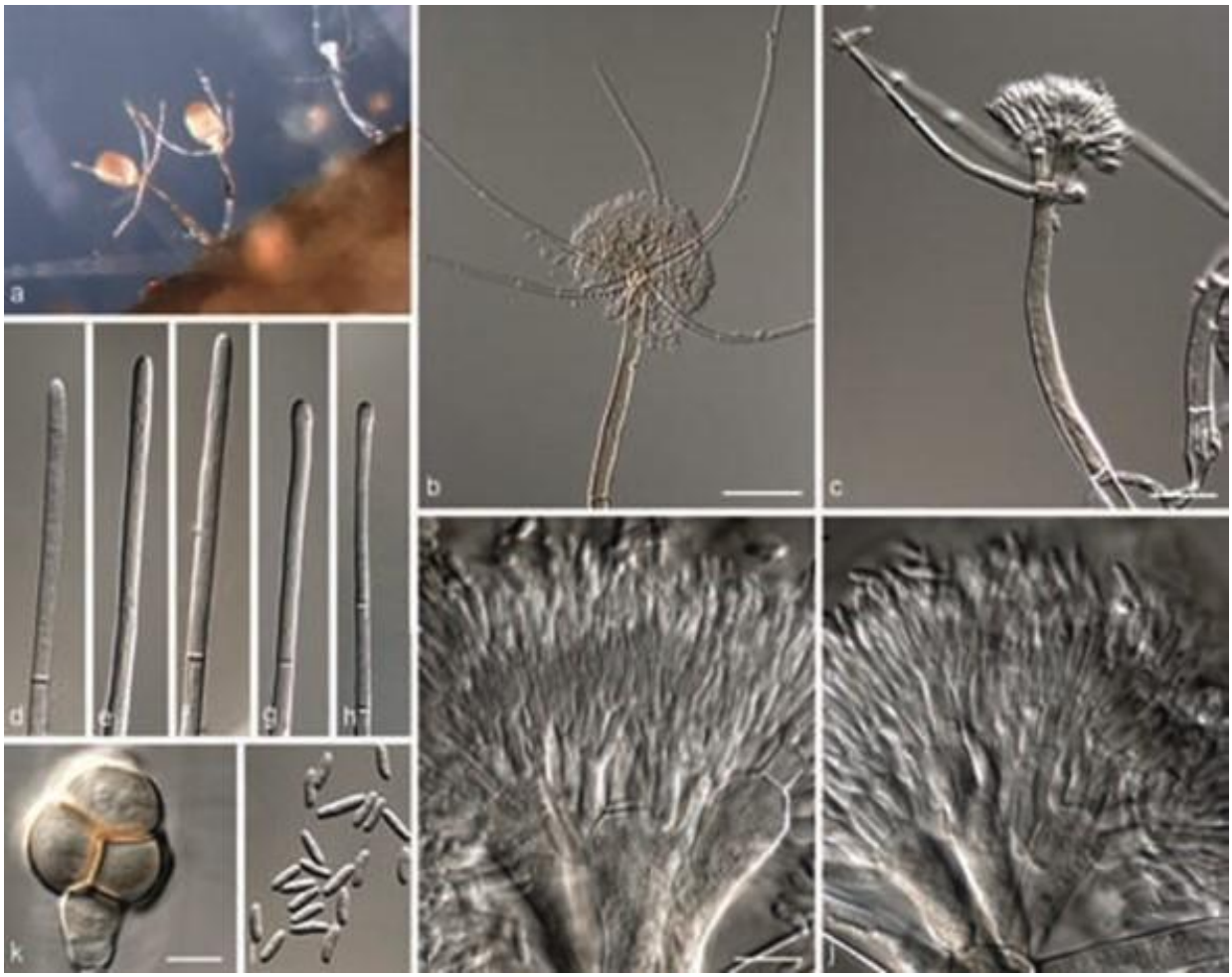

Figura 2: Gliocephalotrichum bulbilium, Conidióforos (A-C); ápice da extensão da estipe (D-H); ramificações do conidióforo (I-J); agregado bulbiloide de clamidósporos (K) e conídios (L). Barras: B = 50 µm; C = 20 µm; D-L= 10 µm (Fonte: Lombard et al. 2014).

1962).

Gliocladiopsis é um gênero ainda muito pouco estudado, considerando o número de reportes no Index Fungorum, 19 no total (Tabela 1). Os primeiros trabalhos com filogenia

molecular foram feitos por Schoch et al. (2000) que demonstrou a soberania deste como gênero, relacionado filogeneticamente com *Gliocephalotrichum, Cylindrocladiella, Cylindrocarpon* e *Calonectria.* Posteriormente o gênero foi relacionado a *Glionectria* este representando sua fase sexual (Lombard and Crous 2012a). Historicamente até 2012 o gênero comportava somente 9 espécies (Lombard and Crous 2012a), nos últimos anos ao gênero foram acrescidas onze novas espécies(Liu and Cai 2013; Parkinson et al. 2017; Gordillo and Decock 2019a; Zhai et al. 2019; Perera et al. 2023). Atualmente são aceitas 20 espécies, sob o gênero *Gliocladiopsis*.(Lombard and Crous 2012)

O teleomorfo de *Gliocladiopsis* foi caracterizado com peritécios superficiais em densos grupos, obovoides para fortemente piriformes, tornando-se vermelho amarronzado com a adição de KOH+ 3% com uma base estromática avermelhada, colapsando lateralmente quando seco, consistindo em duas camadas: regiões com parede fina de textura globulosa, com células do interior comprimidas de textura angulosa; perífises ostiolares, tubulares com pontas arredondas. Ascos unitunicados. 8 esporos, cilíndricos, sésseis, com ápice achatado. Ascósporos unisseriados, hialino, elipsoidais, lisos com um septo, tornando-se marrom e verrugoso com a idade (Lombard and Crous 2012a). A fase assexual (Figura 1.5) possui conidióforos penicilados e subverticilado, consistindo em um septo, estipe hialina e penicilada ou/e subvercicilado arranjo de ramos férteis, com ausência de uma extensão da estipe e uma vesícula terminal. Aparato conidiogênico com muitas séries de ramos asseptados ou com um septo, cada um terminando em 2-7 fiálides de formato doliforme a cimbiforme a cilíndrica, hialina, asseptada, com coloração óbvia. Conídio cilíndrico, ereto para curvado, 0-1 septos, produzido em uma massa mucilaginosa amarela nos conidióforos (Lombard and Crous 2012a).

Os quatro gêneros menos diversos em número de espécies *Aquanectria, Penicillife*, *Xenocylindrocladium* e *Curvicladiella* possuem no total 25 espécies, somando (Figura 5). O primeiro, *Aquanectria* foi recentemente descrito a partir de uma extensa revisão para definir

o conceito de gênero dentro de Nectriaceae (Lombard et al. 2015b) Atualmente o gênero tem sete espécies, a maioria delas descritas somente a partir de um único isolado (Lombard et al. 2015b; Pem et al. 2018; Gordillo and Decock 2019b).

Aquanectria é então caracterizado por produzir peritécio superficial, em agregados ou individualizados, colapsando lateralmente quando com a idade, de coloração marrom alaranjado para vermelho alaranjado e papilas ostiolares. Ascos cilíndricos para clavados, produzindo oito ascósporos. Ascósporos elipsoides para fusiformes, hialinos, monosseptados, com ligeira constrição no septo. Conidióforos em ambientes aquáticos são eretos, solitários, septados e hialinos, são ramificados de forma penicilada com 1-4 fiálides. Fiálides cilíndricas, ponta com espessamento periclinal e sem coloração aparente e achatamento (Figura 5). Conídios filiformes, curvados para ligeiramente sigmoides, monosseptados, hialinos e lisos. Clamidósporos formados de forma intercalar nas hifas, pálidos para marrom escuro, contendo uma grande gútula de óleo, podendo se agregar para formar escleródios (Lombard et al. 2015b).

Tabela 2. Relação de espécies dos oito gêneros que compõem o grupo, juntamente com os isolados de referência para cada espécie

Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Aquanectria devians	MUCL 48197	Folhas submersas	Singapura	A. Gordillo & C. Decock	N/A	N/A	KX671150	KX671144	N/A	N/A	KX671136	KX611506
A. filiformis	MUCL 54681	Raiz	Equador	A. Gordillo & C. Decock	N/A	N/A	KX671145	KX671137	N/A	N/A	KX671129	KX611499
A. jacinthicolor	KUMCC 18-0017	Madeira morta	China	Shi-Ke Huang	N/A	N/A	N/A	MH051231	MH051233	N/A	N/A	MH051235
	KUMCC 17-0146	Madeira morta	China	Shi-Ke Huang	N/A	N/A	N/A	MH051230	MH051232	N/A	N/A	MH051234
A. penicillioides	CBS 257.54	*Acer* sp.	USA	F.V. Ranzoni	KM231110	KM231275	N/A	KM231743	KM231613	KM232299	KM231865	KM232000
A. submersa	CBS 394.62	-	UK	H.J. Hudson	KM231109	N/A	KM231458	HQ897796	KM231612	HQ897728	N/A	KM231999
A. tenuispora	MUCL 48047	Folhas submersas	Singapura	C. Decock	N/A	N/A	KX671149	KX671143	N/A	N/A	KX671135	KX611505
	MUCL 48016	Folhas submersas	Singapura	C. Decock	N/A	N/A	KX671147	KX671141	N/A	N/A	KX671133	KX611503
A. tenuissima	MUCL 53250	-	Guiana Francesa	C. Decock	N/A	N/A	KX671148	KX671142	N/A	N/A	KX671134	KX611504
Calonectria acaciicola	CMW 47173^{T}	Solo (*Acacia auriculiformis* plantação)	Do Luong, Nghe An, Vietnam	N.Q. Pham & T.Q. Pham	MT334933	MT335160	MT335399	MT359620	MT359380	MT412474	MT412690	MT412930
	CMW 47174	Solo (*A.auriculiformis* plantação)	Do Luong, Nghe An, Vietnam	N.Q. Pham & T.Q. Pham	MT334934	MT335161	MT335400	MT359621	MT359381	MT412475	MT412691	MT412931
Ca. acicola	CMW 30996^{T}	*Phoenix canariensis*	Northland, Nova Zelândia	H. Pearson	MT334935	MT335162	MT335401	MT359622	MT359382	MT412476	MT412692	MT412932
	CBS 114812	*P. canariensis*	Northland, Nova Zelândia	H. Pearson	MT334936	MT335163	MT335402	MT359623	MT359383	MT412477	MT412693	MT412933
Ca. aciculata	CERC 5342^{T}	*Eucalyptus urophylla x E. grandis*	YunNan, China	S.F. Chen & J.Q. Li	MT334937	MT335164	MT335403	MT359624	MT359384	MT412478	MT412694	MT412934
Ca. aconidialis	CMW 35174^{T}	Solo (*Eucalyptus* plantação)	HaiNan, China	X. Mou & S.F. Chen	MT334938	MT335165	MT335404	MT359625	MT359385	MT412479	MT412695	N/A11
	CMW 35384	Solo (*Eucalyptus* plantação)	HaiNan, China	X. Mou & S.F. Chen	MT334939	MT335166	MT335405	MT359626	MT359386	N/A	MT412696	N/A
Ca. aeknauliensis	CMW 48253^{T}	Solo (*Eucalyptus* plantação)	Aek Nauli, North Sumatra, Indonésia	M.J. Wingfield	MT334953	MT335180	MT335419	MT359640	MT359400	MT412486	MT412710	N/A
	CMW 48254	Solo (*Eucalyptus* plantação)	Aek Nauli, North Sumatra, Indonésia	M.J. Wingfield	MT334954	MT335181	MT335420	MT359641	MT359401	MT412487	MT412711	N/A
Ca. amazonica	CBS116250^{T}	*E. tereticornis*	Amazônia, Brasil	P.W. Crous & A.C. Alfenas	MT334955	MT335182	MT335421	MT359642	MT359402	MT412488	MT412712	MT412935
	CBS 115486	*E. tereticornis*	Amazônia, Brasil	P.W. Crous & A.C. Alfenas	MT334956	MT335183	MT335422	MT359643	MT359403	MT412489	MT412713	MT412936

Continua...

Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Ca. angustata	CMW 30990^{T}	*Tillandsia capitata*	Sarasota nursery, Florida, USA	R.M. Leahy	MT334963	N/A	MT335429	MT359650	MT359410	MT412493	MT412720	MT412943
	CBS 112133	*Tillandsia capitata*	Sarasota nursery, Florida, USA	R.M. Leahy	GQ280427	GQ267362	DQ190695	GQ280549	GQ280670	KY653360	FJ918552	DQ190593
Ca. asiatica	CBS 114073^{T}	Folhas	Prathet Thai, Tailandia	N.L.Hywel-Jones	GQ280428	AY725741	AY725658	GQ280550	GQ280672	N/A10	AY725705	AY725616
Ca. auriculiformis	CMW 47178^{T}	Solo (*A. auriculiformis* plantação)	Hau Loc, Thanh Hoa, Vietnam	N.Q. Pham & T.Q. Pham	MT334964	MT335190	MT335430	MT359651	MT359411	MT412494	MT412721	MT412944
	CMW 47179	Solo (*A. auriculiformis* plantação)	Hau Loc, Thanh Hoa, Vietnam	N.Q. Pham & T.Q. Pham	N/A	MT335191	MT335431	MT359652	MT359412	MT412495	MT412722	MT412945
Ca. australiensis	CMW 23669^{T}	*Ficus pleurocarpa*	Queensland, Austrália	C. Pearce & B. Paulus	MT334965	MT335192	MT335432	MT359653	MT359413	MT412496	MT412723	MT412946
Ca. avesiculata	CBS 313.92^{T}	*Ilex vomitoria*	Cairo, Georgia, USA	S.A. Alfieri	GQ280431	GQ267364	DQ190620	GQ280553	GQ280675	N/A	GQ267294	AF333392
Ca. borneana	CMW50832=CBS144551	Solo	Malásia	M.R.B.A Rauf	OL635113	OL635065	OL635041	N/A	N/A	OL635089	OL635017	N/A
	CMW50833=CBS144552	Solo	Malásia	M.R.B.A Rauf	OL635114	OL635066	OL635042	N/A	N/A	OL635090	OL635018	N/A
Ca. brachiatica	CMW 25298^{T}	*Pinus maximinoi*	Buga, Colômbia	M.J. Wingfield	N/A	MT335195	MT335435	MT359656	MT359416	MT412499	MT412726	MT412948
	CMW 25302	*Pi. tecunumanii*	Buga, Colômbia	M.J. Wingfield	N/A	MT335196	MT335436	MT359657	MT359417	MT412500	MT412727	MT412949
Ca. brasiliana	CBS 111484^{T}	Solo	Brasil	A.C. Alfenas	MT334968	MT335198	MT335438	MT359659	MT359419	MT412502	MT412729	MT412951
	CBS 111485	Solo	Brasil	A.C. Alfenas	MT334969	MT335199	MT335439	MT359660	MT359420	MT412503	MT412730	MT412952
Ca. brasiliensis	CBS 230.51^{T}	*Eucalyptus* sp.	Ceará, Brasil	T.R. Ciferri	MT334970	MT335200	MT335440	MT359661	MT359421	MT412504	MT412731	MT412953
	CMW 32949	*Eucalyptus* sp.	Aracruz, Brasil	A.C. Alfenas	MT334971	MT335201	MT335441	MT359662	MT359422	MT412505	MT412732	MT412954
Ca. brassiana	CBS 134855^{T}	Solo (*Eucalyptus brassiana* plantação)	Teresina, Piauí, Brasil	R.F. Alfenas	N/A	KM396056	KM396139	N/A	N/A	N/A		
	CBS 134856	Solo (*E. brassiana* plantação)	Teresina, Piauí, Brasil	R.F. Alfenas	N/A	KM396057	KM396140	N/A	N/A	N/A		
Ca. brassicae	CBS 111869^{T}	*Argyreia splendens*	Indonésia	F. Bugnicourt	MT334972	MT335202	MT335442	MT359663	MT359423	MT412506	MT412733	MT412955
Ca. brassicicola	CBS 112841^{T}	Solo (*Brassica* sp.)	Indonésia	M.J. Wingfield	N/A	KX784561	N/A	N/A	N/A	N/A	KX784689	KX784619
Ca. brevistipitata	CBS 115671^{T}	Solo	México	P.W. Crous	MT334973	MT335203	MT335443	MT359664	MT359424	MT412507	MT412734	MT412956

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
	CBS 110928	Solo	México	P.W. Crous	MT334974	MT335204	MT335444	MT359665	MT359425	MT412508	MT412735	MT412957
Ca. bumicola	CMW 48257^{T}	Solo (*Eucalyptus* plantação)	Aek Nauli, North Sumatra, Indonésia	M.J. Wingfield	MT334975	MT335205	MT335445	MT359666	MT359426	MT412509	MT412736	N/A
Ca. canadiana	CMW 23673^{T}	*Picea* sp.	Canada	S. Greifenhagen	MT334976	MT335206	MT335446	MT359667	MT359427	MT412510	MT412737	MT412958
	CERC 8952	Solo	HeNan, China	S.F. Chen	MT335058	MT335290	MT335530	MT359751	MT359511	MT412587	MT412821	MT413035
Ca. candelabrum	CMW 31000	*Eucalyptus* sp.	Amazonas, Brasil	A.C. Alfenas	MT334977	MT335207	MT335447	MT359668	MT359428	MT412511	MT412738	MT412959
	CMW 31001	*Eucalyptus* sp.	Amazonas, Brasil	A.C. Alfenas	MT334978	MT335208	MT335448	MT359669	MT359429	MT412512	MT412739	MT412960
Ca. cerciana	CMW 25309^{T}	*E. urophylla* × *E. grandis* hybrid cutting	CERC nursery, GuangDong, China	M.J. Wingfield & X.D. Zhou	MT334981	MT335211	MT335451	MT359672	MT359432	MT412515	MT412742	MT412963
	CMW 25290	*E. urophylla* ×*E. grandis* hybrid cutting	CERC nursery, GuangDong, China	M.J. Wingfield & X.D. Zhou	MT334982	MT335212	MT335452	MT359673	MT359433	MT412516	MT412743	MT412964
Ca. chinensis	CMW 23674^{T}	Solo	Hong Kong, China	E.C.Y. Liew	MT334990	MT335220	MT335460	MT359681	MT359441	MT412524	MT412751	MT412972
	CMW 30986	Solo	Hong Kong, China	E.C.Y. Liew	MT334991	MT335221	MT335461	MT359682	MT359442	MT412525	MT412752	MT412973
Ca. citri	CMW 23675^{T}	*Citrus sinensis*	Florida, USA	H.S. Fawcett	MT334992	MT335222	MT335462	MT359683	MT359443	MT412526	MT412753	MT412974
Ca. clavata	CMW 23690^{T}	*Callistemon viminalis*	Lake Placid, Florida, USA	C.P. Seymour & E.L. Barnard	MT334993	MT335223	MT335463	MT359684	MT359444	MT412527	MT412754	MT412975
	CMW 30994	Root debris in peat	Lee County, Florida, USA	D. Ferrin	MT334994	MT335224	MT335464	MT359685	MT359445	MT412528	MT412755	MT412976
Ca. cochinchinensis	CMW 49915^{T}	Solo (*Hevea brasiliensis* plantação)	Duong Minh Chau, Tay Ninh, Vietnam	N.Q. Pham, Q.N. Dang & T.Q. Pham	MT334995	MT335225	MT335465	MT359686	MT359446	MT412529	MT412756	MT412977
	CMW 47186	Solo (*A. auriculiformis* plantação)	Song May, Dong Nai, Vietnam	N.Q. Pham & T.Q. Pham	MT334996	MT335226	MT335466	MT359687	MT359447	MT412530	MT412757	MT412978
Ca. colhounii	CBS 293.79T	*Camellia sinensis*	Mauritius	A. Peerally	GQ280443	GQ267373	DQ190639	GQ280565	GQ280687	KY653376	GQ267301	DQ190564
Ca. Colômbiana	CBS 115127^{T}	Solo	La Selva, Colômbia	M.J. Wingfield	GQ280538	GQ267455	FJ972442	GQ280660	GQ280782	N/A	FJ972492	FJ972423
	CBS 115638	Solo	La Selva, Colômbia	M.J. Wingfield	GQ280539	GQ267456	FJ972441	GQ280661	GQ280783	N/A	FJ972491	FJ972422
Ca. colombiensis	CMW 23676^{T}	Solo (*E. grandis* árvores)	La Selva, Colômbia	M.J. Wingfield	MT334998	MT335228	MT335468	MT359689	MT359449	MT412532	MT412759	MT412980
	CMW 30985	Solo (*E. grandis* árvores)	La Selva, Colômbia	M.J. Wingfield	MT334999	MT335229	MT335469	MT359690	MT359450	MT412533	MT412760	MT412981
Ca. crousiana	CMW 27249^{T}	*E. grandis*	FuJian, China	M.J. Wingfield	MT335000	MT335230	MT335470	MT359691	MT359451	MT412534	MT412761	MT412982

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
	CMW 27253	*E. grandis*	FuJian, China	M.J. Wingfield	MT335001	MT335231	MT335471	MT359692	MT359452	MT412535	MT412762	MT412983
Ca. curvispora	CMW 23693^{T}	Solo	Tamatave, Madagascar	P.W. Crous	MT335002	MT335232	MT335472	MT359693	MT359453	MT412536	MT412763	N/A
	CMW 48245	Solo (*Eucalyptus* plantação)	Aek Nauli, North Sumatra, Indonésia	M.J. Wingfield	MT335003	MT335233	MT335473	MT359694	MT359454	MT412537	MT412764	N/A
Ca. cylindrospora	CBS 136425	*Blephilia ciliata*	Ellerbe, North Carolina, USA	T. Sharp	MT335005	MT335235	MT335475	MT359696	MT359456	MT412539	MT412766	MT412984
	CBS 119670	*Pistacia lentiscus*	Italy	N/A	MT335006	MT335236	MT335476	MT359697	MT359457	MT412540	MT412767	MT412985
	CMW 30978	*Ilex vomitoria*	Florida, USA	N.E. El-Gholl	MT335007	MT335237	MT335477	MT359698	MT359458	MT412541	MT412768	MT412986
Ca. densa	CMW 31182^{T}	Solo	Las Golondrinas, Pichincha, Equador	M.J. Wingfield	MT335008	MT335238	MT335478	MT359699	MT359459	N/A	MT412769	MT412987
	CMW 31184	Solo	Las Golondrinas, Pichincha, Equador	M.J. Wingfield	MT335009	MT335239	MT335479	MT359700	MT359460	N/A	MT412770	MT412988
Ca. duoramosa	CBS 134656^{T}	Solo (Floresta tropical)	Monte Dourado, Pará, Brasil	R.F. Alfenas	N/A	KM396027	KM396110	N/A	N/A	N/A		
	LPF453	Solo (*Eucalyptus* plantação)	Monte Dourado, Pará, Brasil	R.F. Alfenas	N/A	KM396028	KM396111	N/A	N/A	N/A		
Ca. Equadorae	CMW 23677^{T}	Solo	Equador	M.J. Wingfield	MT335012	MT335242	MT335482	MT359703	MT359463	MT412544	MT412773	MT412991
	CBS 111706	Solo	Equador	M.J. Wingfield	MT335010	MT335240	MT335480	MT359701	MT359461	MT412542	MT412771	MT412989
	–	Solo	Equador	M.J. Wingfield	MT335011	MT335241	MT335481	MT359702	MT359462	MT412543	MT412772	MT412990
Ca. eucalypti	CMW 18444^{T}	*E. grandis*	Aek Nauli, Sumatra Utara, Indonésia	M.J. Wingfield	MT335013	MT335243	MT335483	MT359704	MT359464	MT412545	MT412774	MT412992
	CMW 18445	*E. grandis*	Aek Nauli, Sumatra Utara, Indonésia	M.J. Wingfield	MT335014	MT335244	MT335484	MT359705	MT359465	MT412546	MT412775	MT412993
Ca. eucalypticola	CBS 134847^{T}	*Eucalyptus* sp. (mudas)	Santa Barbara, Minas Gerais, Brasil	A.C. Alfenas	N/A	KM396051	KM396134	N/A	N/A	N/A		
	CBS 134846	*Eucalyptus* sp. (folhas)	Eunápolis, Bahia, Brasil	A.C. Alfenas	N/A	KM396050	KM396133	N/A	N/A	N/A		
Ca. fragariae	CBS 133607^{T}	*Fragaria* × *ananassa*	Santa Maria do Jetibá, Espírito Santo, Brasil	U.P. Lopes	N/A	KM998966	KM998964	N/A	N/A	N/A		
	LPF141.1	*Fragaria* × *ananassa*	Santa Maria do Jetibá, Espírito Santo, Brasil	U.P. Lopes	N/A	KX500191	KX500194	N/A	N/A	N/A		

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
	LPF141.2	*Fragaria* × *ananassa*	Santa Maria do Jetibá, Espírito Santo, Brasil	U.P. Lopes	N/A	KX500192	KX500193	N/A	N/A	N/A		
Ca. fujianensis	CMW 27257^{T}	*E. grandis*	FuJian, China	M.J. Wingfield	MT335019	MT335249	MT335489	MT359710	MT359470	MT412551	MT412780	MT412998
	CMW 27254	*E. grandis*	FuJian, China	M.J. Wingfield	MT335020	MT335250	MT335490	MT359711	MT359471	MT412552	MT412781	MT412999
	CBS 131802	*Nymphaea tetragona*	Guiyang, Guizhou, China	S.Y. Qin	MT335070	MT335302	MT335542	MT359763	MT359523	MT412599	MT412833	MT413047
Ca. glaebicola	CBS 134852^{T}	Solo (*Eucalyptus* plantação)	Martinho Campos, Minas Gerais, Brasil	A.C. Alfenas	N/A	KM396053	KM396136	N/A	N/A	N/A		
	CBS 134853	*Eucalyptus* sp. (folhas)	Tocantins, Bico do Papagaio, Brasil	R.F. Alfenas	N/A	KM396054	KM396137	N/A	N/A	N/A		
Ca. gordoniae	CMW 23694^{T}	*Gordonia lasianthus*	Florida, USA	D. Chiappini	MT335021	MT335251	MT335491	MT359712	MT359472	MT412553	MT412782	MT413000
Ca. gracilipes	CBS 115674^{T}	Solo	La Selva, Colômbia	M.J. Wingfield	MT335022	MT335252	MT335492	MT359713	MT359473	MT412554	MT412783	MT413001
	CBS 111141	Solo	La Selva, Colômbia	M.J. Wingfield	MT335023	MT335253	MT335493	MT359714	MT359474	MT412555	MT412784	MT413002
Ca. gracilis	CBS 111807^{T}	*Manilkara zapota*	Pará, Brasil	F. Carneiro de Albuquerque	GQ280488	GQ267407	DQ190646	GQ280610	GQ280732	KY653390	GQ267323	AF232858
	CBS 111284	Solo	Imbrapa, Brasil	P.W. Crous	GQ280489	GQ267408	DQ190647	GQ280611	GQ280733	KY653389	GQ267324	DQ190567
Ca. hawksworthii	CBS 111870^{T}	*Nelumbo nucifera*	Pamplemousses garden, Mauritius	A. Peerally	MT335024	MT335254	MT335494	MT359715	MT359475	MT412556	MT412785	MT413003
	CMW 14878	*Eucalyptus* sp.	Sulawesi, Indonésia	M.J. Wingfield	MT335141	MT335378	MT335618	MT359839	MT359599	MT412670	MT412909	MT413119
	–	*Eucalyptus* sp.	Sulawesi, Indonésia	M.J. Wingfield	MT335142	MT335379	MT335619	MT359840	MT359600	MT412671	MT412910	MT413120
Ca. henricotiae	CBS 138102^{T}	*Buxus sempervirens*	Lokeren, East Flanders, Belgium	B. Gehesquiere & K. Heungens	N/A	KF815157	KF815185	JX535322	N/A	N/A		
	CB041	*B. sempervirens*	Lokeren, East Flanders, Belgium	B. Gehesquiere & K. Heungens	N/A	KF815156	KF815184	N/A	N/A	N/A		
Ca. heveicola	CMW 49913^{T}	Solo (*Hevea brasiliensis* plantação)	Bau Bang, Binh Duong, Vietnam	N.Q. Pham, Q.N. Dang & T.Q. Pham	MT335025	MT335255	MT335495	MT359716	MT359476	N/A	MT412786	MT413004

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					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
	CMW 49928	Solo	Bu Gia Map National Park, Binh Phuoc, Vietnam	N.Q. Pham,Q.N. Dang &T.Q. Pham	MT335048	MT335280	MT335520	MT359741	MT359501	MT412577	MT412811	MT413025
	CMW 49935	Solo	Bu Gia Map National Park, Binh Phuoc, Vietnam	N.Q. Pham, Q.N. Dang & T.Q. Pham	MT335049	MT335281	MT335521	MT359742	MT359502	MT412578	MT412812	MT413026
Ca. honghensis	CERC 5572^{T}	Solo (*Eucalyptus* plantação)	HongHe, YunNan, China	S.F. Chen & J.Q. Li	MT335026	MT335256	MT335496	MT359717	MT359477	MT412557	MT412787	MT413005
	CERC 5571	Solo (*Eucalyptus* plantação)	HongHe, YunNan, China	S.F. Chen & J.Q. Li	MT335027	MT335257	MT335497	MT359718	MT359478	MT412558	MT412788	MT413006
Ca. hongkongensis	CBS 114828^{T}	Solo	Hong Kong, China	M.J. Wingfield	MT335028	MT335258	MT335498	MT359719	MT359479	MT412559	MT412789	MT413007
	–	Solo (*Eucalyptus* plantação)	GuangXi, China	N/A	MT335029	MT335259	MT335499	MT359720	MT359480	MT412560	MT412790	MT413008
Ca. humicola	CMW 31183^{T}	Solo	Las Golondrinas, Pichincha, Equador	M.J. Wingfield	MT335032	MT335262	MT335502	MT359723	MT359483	N/A	MT412793	MT413011
	CMW 31186	Solo	Las Golondrinas, Pichincha, Equador	L. Lombard	MT335033	MT335263	MT335503	MT359724	MT359484	N/A	MT412794	MT413012
	CMW 31187	Solo	Las Golondrinas, Pichincha, Equador	L. Lombard	MT335034	MT335264	MT335504	MT359725	MT359485	N/A	MT412795	MT413013
Ca. hurae	CBS 114182	*Rumohra adiantiformis*	Brasil	A.C. Alfenas	MT335035	MT335265	MT335505	MT359726	MT359486	MT412563	MT412796	MT413014
Ca. ilicicola	CMW 30998^{T}	*Solanum tuberosum*	Bogor, Java, Indonésia	K.B. Boedijn & J. Reitsma	MT335036	MT335266	MT335506	MT359727	MT359487	MT412564	MT412797	N/A
Ca. imperata	CCDCA 11649	*E. urophylla*	Maranhão, Brasil	M.A. Ferreira	ON009351	OM974330	OM974339	N/A	N/A	OM974348	OM974357	OM974366
	PFC7	*E. urophylla*	Maranhão, Brasil	M.A. Ferreira	ON009352	OM974331	OM974340	N/A	N/A	OM974349	OM974358	OM974367
Ca. Indonésiae	CMW 23683^{T}	*Syzygium aromaticum*	Warambunga, Indonésia	M.J. Wingfield	MT335037	MT335267	MT335507	MT359728	MT359488	MT412565	MT412798	MT413015
	CBS 112840	*S. aromaticum*	Warambunga, Indonésia	M.J. Wingfield	MT335038	MT335268	MT335508	MT359729	MT359489	MT412566	MT412799	MT413016
Ca. indusiata	CBS 144.36^{T}	*Camellia sinensis*	Sri Lanka	N/A	GQ280536	GQ267453	GQ267262	GQ280658	GQ280780	KY653396	GQ267332	GQ267239
	CBS 114684	*Rhododendron* sp.	Florida, USA	N.E. El-Gholl	GQ280537	GQ267454	DQ190653	GQ280659	GQ280781	N/A	GQ267333	AF232862
Ca. insularis	CMW 30991^{T}	Solo	Tamatave, Madagascar	P.W. Crous	N/A	MT335269	MT335509	MT359730	MT359490	MT412567	MT412800	MT413017
	CMW 30992	Solo	Conejos, Veracruz, México	M.J. Wingfield	N/A	MT335270	MT335510	MT359731	MT359491	MT412568	MT412801	MT413018

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					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Ca. kyotensis	CBS 114525^{T}	*Robinia pseudoacacia*	Japão	T. Terashita	MT335039	MT335271	MT335511	MT359732	MT359492	MT412569	MT412802	MT413019
	CBS 114550	Solo	China	M.J. Wingfield	MT335016	MT335246	MT335486	MT359707	MT359467	MT412548	MT412777	MT412995
Ca. ladang	CMW50776 T=CBS144550	Solo	Malásia	M.R.B.A Rauf	OL635122	OL635075	OL635051	N/A	N/A	OL635099	OL635027	N/A
	CMW50775=CBS144549	Solo	Malásia	M.R.B.A Rauf	OL635121	OL635074	OL635050	N/A	N/A	OL635098	OL635026	N/A
Ca. lageniformis	CBS 111324^{T}	*Eucalyptus* sp. (folhas)	Rivière Noire, Mauritius	H. Smith	N/A	KX784574	N/A					
Ca. lantauensis	CERC 3302^{T}	Solo	LiDao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MT335040	MT335272	MT335512	MT359733	MT359493	MT412570	MT412803	N/A
	CERC 3301	Solo	LiDao, Hong Kong, China	M.J. Wingfield & S.F. Chen	MT335041	MT335273	MT335513	MT359734	MT359494	N/A	MT412804	N/A
Ca. lateralis	CMW 31412^{T}	Solo (*Eucalyptus* plantação)	GuangXi, China	X. Zhou, G. Zhao & F. Han	MT335042	MT335274	MT335514	MT359735	MT359495	MT412571	MT412805	MT413020
Ca. lauri	CMW 23682^{T}	*Ilex aquifolium*	Vijlen, Vijlenerbos, South-East Limburg, Holanda	H.A. van der Aa	MT335043	MT335275	MT335515	MT359736	MT359496	MT412572	MT412806	MT413021
Ca. leguminum	CMW 23684^{T}	*Annona squamosa*	São Paulo, Brasil	M.B. Figueiredo	MT335044	MT335276	MT335516	MT359737	MT359497	MT412573	MT412807	MT413022
Ca. leucothoes	CMW 30977^{T}	*Leucothoe axillaris*	Florida, USA	N.E. El-Gholl	MT335045	MT335277	MT335517	MT359738	MT359498	MT412574	MT412808	N/A
Ca. lichi	CERC 8866^{T}	Solo	HeNan, China	S.F. Chen	MT335046	MT335278	MT335518	MT359739	MT359499	MT412575	MT412809	MT413023
	CERC 8850	Solo	HeNan, China	S.F. Chen	MT335047	MT335279	MT335519	MT359740	MT359500	MT412576	MT412810	MT413024
Ca. lombardiana	CMW 30602^{T}	*Xanthorrhoea australis*	Victoria, Austrália	T. Baigent	MT335156	MT335395	MT335635	MT359856	MT359616	MT412686	MT412926	MT413133
Ca. macroconidialis	CBS 114880^{T}	*E. grandis*	Sabie, Mpumalanga, África do Sul	P.W. Crous	MT335050	MT335282	MT335522	MT359743	MT359503	MT412579	MT412813	MT413027
	CBS 110798	*E. grandis* (raiz)	Sabie, Mpumalanga, África do Sul	P.W. Crous	MT335051	MT335283	MT335523	MT359744	MT359504	MT412580	MT412814	MT413028
Ca. madagascariensis	CMW 23686^{T}	Solo	Rona, Madagascar	J.E. Taylor	MT335052	MT335284	MT335524	MT359745	MT359505	MT412581	MT412815	MT413029
	CMW 30993	Solo	Rona, Madagascar	J.E. Taylor	MT335053	MT335285	MT335525	MT359746	MT359506	MT412582	MT412816	MT413030
Ca. malesiana	CMW 23687^{T}	Solo	Northern Sumatra, Indonésia	M.J. Wingfield	MT335054	MT335286	MT335526	MT359747	MT359507	MT412583	MT412817	MT413031
	CBS 112710	Folhas	Prathet, Tailandia	N.L. Hywel-Jones	MT335055	MT335287	MT335527	MT359748	MT359508	MT412584	MT412818	MT413032

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					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Ca. maranhensis	CBS 134811^{T}	*Eucalyptus* sp. (folhas)	Açailândia, Maranhão, Brasil	A.C. Alfenas	N/A	KM396035	KM396118	N/A	N/A	N/A		
	CBS 134812	*Eucalyptus* sp. (folhas)	Açailândia, Maranhão, Brasil	A.C. Alfenas	N/A	KM396036	KM396119	N/A	N/A	N/A		
Ca. matogrossensis	GFP006	E. urophylla	Mato Grosso, Brasil	R.F. Alfenas	N/A	MH837653	MH837648	N/A	N/A	N/A	MH837659	MH837664
	GFP018	E. urophylla	Mato Grosso, Brasil	R.F. Alfenas	N/A	MH837657	MH837652	N/A	N/A	N/A	MH837663	MH837668
Ca. metrosideri	CBS 133604	*Metrosideros polymorpha*	Viçosa, Minas Gerais, Brasil	R.F. Alfenas	MT335056	MT335288	MT335528	MT359749	MT359509	MT412585	MT412819	MT413033
	CBS 133603^{T}	*Metrosideros polymorpha*	Viçosa, Minas Gerais, Brasil	R.F. Alfenas	N/A	KC294304	KC294307	N/A	N/A	N/A		
Ca. mexicana	CBS 110918^{T}	Solo	Uxmal, Yucatan, México	M.J. Wingfield	GQ280474	GQ267396	FJ972460	GQ280596	GQ280718	KY653412	FJ972526	AF210863
Ca. minensis	CSF9941	Solo (*Eucalyptus* plantação)	Xinluo, Longyan,Fujian, China	S.F. Chen,Q.L. Liu	OK253121	OK253259	OK253403	N/A	N/A	OK253477	OK253814	OK253967
	CSF9974	Solo (Floresta natural)	Liancheng,Longyan ,Fujian, China	S.F. Chen,Q.L. Liu	OK253122	OK253260	OK253404	N/A	N/A	OK253478	OK253815	OK253968
Ca. monticola	CBS 140645^{T}	Solo	Chiang Mai, Tailandia	P.W. Crous	N/A	KT964771	N/A	KT964775	KT983443	N/A	KT964773	KT964769
	CPC 28836	Solo	Chiang Mai, Tailandia	P.W. Crous	N/A	KT964772	N/A	KT964776	KT983444	N/A	KT964774	KT964770
Ca. multilateralis	CBS 110932^{T}	Solo	Uxmal, México	P.W. Crous	MT335060	MT335292	MT335532	MT359753	MT359513	MT412589	MT412823	MT413037
	CBS 110926	Solo	Uxmal, México	P.W. Crous	MT335061	MT335293	MT335533	MT359754	MT359514	MT412590	MT412824	MT413038
Ca. multinaviculata	CBS 134858^{T}	Solo (*Eucalyptus* plantação)	Mucuri, Bahia, Brasil	E. Zauza	N/A	KM396072	KM396155	N/A	N/A	N/A		
	CBS 134859	Solo (*Eucalyptus* plantação)	Monte Dourado, Pará, Brasil	R.F. Alfenas	N/A	KM396073	KM396156	N/A	N/A	N/A		
Ca. multiphialidica	CMW 23688^{T}	Solo (raiz de *Musa* sp.)	Cameroon	Abadie	MT335066	MT335298	MT335538	MT359759	MT359519	MT412595	MT412829	MT413043
Ca. multiseptata	CMW 23692^{T}	*E. grandis*	North Sumatra, Indonésia	M.J. Wingfield	MT335067	MT335299	MT335539	MT359760	MT359520	MT412596	MT412830	MT413044
Ca. naviculata	CBS 101121	Folhas	Joao Pessoa, Brasil	R.F. Castaneda	GQ280478	GQ267399	GQ267252	GQ280600	GQ280722	KM232309	GQ267317	GQ267211
	CBS 116080	Solo	Manaus, Amazonas, Brasil	M.J. Wingfield	GQ280477	GQ267398	GQ267251	GQ280599	GQ280721	KY653417	GQ267316	AF333409
Ca. nemoricola	CBS 134837^{T}	Solo (Floresta tropicalt)	Araponga, Minas Gerais, Brasil	A.C. Alfenas & P.W. Crous	N/A	KM396066	KM396149	N/A	N/A	N/A		

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
	CBS 134838	Solo (Floresta tropicalt)	Araponga, Minas Gerais, Brasil	A.C. Alfenas & P.W. Crous	N/A	KM396067	KM396150	N/A	N/A	N/A		
Ca. octoramosa	CBS 111423^{T}	Solo	Equador	M.J. Wingfield	MT335071	MT335303	MT335543	MT359764	MT359524	MT412600	MT412834	MT413048
Ca. orientalis	CMW 20291^{T}	Solo	Langam, Indonésia	M.J. Wingfield	MT335072	MT335304	MT335544	MT359765	MT359525	MT412601	MT412835	MT413049
	CMW 20273	Solo	Teso East, Indonésia	M.J. Wingfield	MT335073	MT335305	MT335545	MT359766	MT359526	MT412602	MT412836	MT413050
Ca. ovata	CMW 16724^{T}	*E. urophylla*	Monte Dourado, Pará, Brasil	N.E. El-Gholl	MT335075	MT335307	MT335547	MT359768	MT359528	N/A	MT412838	MT413052
	CMW 30979	*E. tereticornis*	Tucuruí, Pará, Brasil	P.W. Crous	MT335076	MT335308	MT335548	MT359769	MT359529	N/A	MT412839	MT413053
Ca. pacifica	CMW 16726^{T}	*Araucaria heterophylla*	Hawaii, USA	M. Aragaki	MT335079	MT335311	MT335551	MT359772	MT359532	MT412604	MT412842	N/A
	CMW 30988	*Ipomoea aquatica*	Auckland, Nova Zelândia	C.F. Hill	MT335080	MT335312	MT335552	MT359773	MT359533	MT412605	MT412843	N/A
Ca. paracolhounii	CBS 114679^{T}	-	USA	A.Y. Rossman	N/A	KX784582	N/A					
	CBS 114705	*Annona reticulata* (fruit)	Austrália	D. Hutton	N/A	N/A	N/A					
Ca. paraensis	CBS 134669^{T}	Solo (*Eucalyptus* plantação)	Monte Dourado, Pará, Brasil	R.F. Alfenas	N/A	KM396011	KM396094	N/A	N/A	N/A		
	LPF429	Solo (Floresta tropical)	Monte Dourado, Pará, Brasil	R.F. Alfenas	N/A	KM396015	KM396098	N/A	N/A	N/A		
Ca. paragominensis	CCDCA 11648	E. grandis x E. brassiana	Pará, Brasil	M.A. Ferreira	ON009346	OM974325	OM974334	N/A	N/A	OM974343	OM974352	OM974361
	PF2	E. grandis x E. brassiana	Pará, Brasil	M.A. Ferreira	ON009347	OM974326	OM974335	N/A	N/A	OM974344	OM974353	OM974362
Ca. parvispora	CBS 111465^{T}	Solo	Brasil	A.C. Alfenas	MT335082	MT335314	MT335554	MT359775	MT359535	MT412607	MT412845	MT413057
	–	Solo	Brasil	A.C. Alfenas	MT335081	MT335313	MT335553	MT359774	MT359534	MT412606	MT412844	MT413056
Ca. pauciphialidica	CMW 30980^{T}	Solo	Equador	M.J. Wingfield	MT335083	MT335315	MT335555	MT359776	MT359536	MT412608	MT412846	MT413058
Ca. pauciramosa	CBS 138824^{T}	Solo	Knysna, África do Sul	P.W. Crous	MT335093	MT335325	MT335565	MT359786	MT359546	MT412618	MT412856	MT413068
	–	*E. nitens*	África do Sul	M.J. Wingfield	MT335094	MT335326	MT335566	MT359787	MT359547	MT412619	MT412857	MT413069
Ca. penicilloides	CMW 23696^{T}	*Prunus* sp.	Hatizyo Island, Japão	M. Ookubu	MT335106	MT335338	MT335578	MT359799	MT359559	MT412631	MT412869	MT413081

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Ca. piauiensis	CBS 134850^{T}	Solo (*Eucalyptus* plantação)	Teresina, Piauí, Brasil	R.F. Alfenas	N/A	KM396060	KM396143	N/A	N/A	N/A		
	CBS 134851	Solo (Floresta tropical)	Teresina, Piauí, Brasil	R.F. Alfenas	N/A	KM396061	KM396144	N/A	N/A	N/A		
Ca. pini	CMW 31209^{T}	*Pinus patula*	Buga, Valle del Cauca, Colômbia	C.A. Rodas	MT335107	MT335339	MT335579	MT359800	MT359560	MT412632	MT412870	MT413082
	CBS 125523	*Pinus patula*	Buga, Valle del Cauca, Colômbia	C.A. Rodas	GQ280518	GQ267437	GQ267274	GQ280640	GQ280762	N/A	GQ267345	GQ267225
Ca. plurilateralis	CBS 111401^{T}	Solo	Equador	M.J. Wingfield	N/A	MT335340	MT335580	MT359801	MT359561	MT412633	MT412871	MT413083
Ca. propaginicola	CBS 134815^{T}	*Eucalyptus* sp. (mudas)	Santana, Pará, Brasil	A.C. Alfenas	N/A	KM396040	KM396123	N/A	N/A	N/A	N/A	N/A
	CBS 134816	*Eucalyptus* sp. (mudas)	Santana, Pará, Brasil	A.C. Alfenas	N/A	KM396041	KM396124	N/A	N/A	N/A	N/A	N/A
Ca. pseudobrassicae	CBS 134662^{T}	Solo (*Eucalyptus* plantação)	Santana, Pará, Brasil	A.C. Alfenas	N/A	KM396023	KM396106	N/A	N/A	N/A	N/A	N/A
	CBS 134661	Solo (*Eucalyptus* plantação)	Santana, Pará, Brasil	A.C. Alfenas	N/A	KM396022	KM396105	N/A	N/A	N/A	N/A	N/A
Ca. pseudoEquadoriae	CBS 111402^{T}	Solo	Equador	M.J. Wingfield	N/A	KX784589	N/A	N/A	N/A	N/A	N/A	N/A
Ca. pseudomalesiana	CMW50821 T=CBS144563	Solo	Malásia	M.R.B.A Rauf	OL635123	OL635076	OL635052	N/A	N/A	OL635100	OL635028	OL635137
	CMW50779	Solo	Malásia	M.R.B.A Rauf	OL635124	OL635077	OL635053	N/A	N/A	OL635101	OL635029	OL635138
Ca. pseudometrosideri	CBS 134845^{T}	Solo (*Eucalyptus* plantação)	Maceió, Alagoas, Brasil	M.M. Coutinho	N/A	KM395995	KM396083	N/A	N/A	N/A		
	CBS 134843	*Metrosideros polymorpha*	Viçosa, Minas Gerais, Brasil	A.C. Alfenas	N/A	KM395993	KM396081	N/A	N/A	N/A		
Ca. pseudomexicana	CBS 130354^{T}	*Callistemon* sp.	Tunis, Carthage, Tunísia	G. Polizzi	MT335110	MT335343	MT335583	MT359804	MT359564	MT412636	MT412874	MT413086
	CBS 130355	*Callistemon* sp.	Tunis, Carthage, Tunísia	G. Polizzi	MT335111	MT335344	MT335584	MT359805	MT359565	MT412637	MT412875	MT413087
Ca. pseudonaviculata	CBS 116251^{T}	*Buxus sempervirens*	Kumeu, West Auckland, Nova Zelândia	N/A	N/A	MT335345	MT335585	MT359806	MT359566	MT412638	MT412876	MT413088
	CMW 23672	*B. sempervirens*	Nova Zelândia	C. Crepel	N/A	MT335346	MT335586	MT359807	MT359567	MT412639	MT412877	MT413089
Ca. pseudopteridis	CBS 163.28^{T}	*Washingtonia robusta*	USA	C.D. Sherbakoff	MT335112	MT335347	MT335587	MT359808	MT359568	MT412640	MT412878	N/A

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Ca. pseudoreteaudii	CMW 25310^{T}	*E. urophylla* × *E. grandis*	GuangDong, China	M.J. Wingfield & X.D. Zhou	MT335119	MT335354	MT335594	MT359815	MT359575	MT412647	MT412885	MT413096
	CMW 25292	*E. urophylla* × *E. grandis*	GuangDong, China	M.J. Wingfield & X.D. Zhou	MT335120	MT335355	MT335595	MT359816	MT359576	MT412648	MT412886	MT413097
Ca. pseudospathiphylli	CBS 109165^{T}	Solo	Equador	M.J. Wingfield	GQ280493	GQ267412	AF348241	GQ280615	GQ280737	KY653435	FJ918562	FJ918513
Ca. pseudospathulata	CBS 134841^{T}	Solo (Floresta tropical)	Araponga, Minas Gerais, Brasil	A.C. Alfenas & P.W. Crous	N/A	KM396070	KM396153	N/A	N/A	N/A		
	CBS 134840	Solo (Floresta tropical)	Araponga, Minas Gerais, Brasil	A.C. Alfenas & P.W. Crous	N/A	KM396069	KM396152	N/A	N/A	N/A		
Ca. pseudouxmalensis	CBS 110924^{T}	Solo	México	P.W. Crous	MT335123	MT335358	MT335598	MT359819	MT359579	MT412651	MT412889	MT413100
	CBS 110923	Solo	México	P.W. Crous	MT335124	MT335359	MT335599	MT359820	MT359580	MT412652	MT412890	MT413101
Ca. pseudovata	CBS 134674^{T}	Solo (*Eucalyptus* plantação)	Santana, Pará, Brasil	A.C. Alfenas	N/A	KM396032	KM396115	N/A	N/A	N/A		
	CBS 134675	Solo (*Eucalyptus* plantação)	Santana, Pará, Brasil	A.C. Alfenas	N/A	KM396033	KM396116	N/A	N/A	N/A		
Ca. pteridis	CBS 111793^{T}	*Arachniodes adiantiformis*	USA	F. Schickedanz	GQ280494	GQ267413	DQ190679	GQ280616	GQ280738	KY653438	FJ918563	DQ190578
Ca. putriramosa	CBS 111449^{T}	*Eucalyptus* sp.	Brasil	A.C. Alfenas	MT335129	MT335364	MT335604	MT359825	MT359585	MT412657	MT412895	MT413105
	CBS 111470	Solo	Brasil	A.C. Alfenas	MT335130	MT335365	MT335605	MT359826	MT359586	MT412658	MT412896	MT413106
Ca. queenslandica	CMW 30604^{T}	*E. urophylla*	Lannercost, Queensland, Austrália	B. Brown	MT335132	MT335367	MT335607	MT359828	MT359588	MT412660	MT412898	MT413108
	CMW 30603	*E. pellita*	Lannercost, Queensland, Austrália	P.Q Thu & K.M. Old	MT335133	MT335368	MT335608	MT359829	MT359589	MT412661	MT412899	MT413109
Ca. quinqueramosa	CBS 134654^{T}	Solo (*Eucalyptus* plantação)	Monte Dourado, Pará, Brasil	R.F. Alfenas	N/A	KM396029	KM396112	N/A	N/A	N/A		
	CBS 134655	Solo (*Eucalyptus* plantação)	Santana, Pará, Brasil	A.C. Alfenas	N/A	KM396030	KM396113	N/A	N/A	N/A		
Ca. reteaudii	CMW 30984^{T}	*E. camaldulensis*	Chon Thanh, Binh Phuoc, Vietnam	M.J. Dudzinski & P.Q. Thu	MT335135	MT335370	MT335610	MT359831	MT359591	MT412663	MT412901	MT413111
	CMW 16738	*Eucalyptus* sp. (folhas)	Binh Phuoc, Vietnam	M.J. Dudzinski & P.Q. Thu	MT335136	MT335371	MT335611	MT359832	MT359592	MT412664	MT412902	MT413112

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Ca. robigophila	CBS 134652^{T}	*Eucalyptus* sp. (folhas)	Açailândia, Maranhão, Brasil	R.F. Alfenas	N/A	KM396024	KM396107	N/A	N/A	N/A		
	CBS 134653	*Eucalyptus* sp. (folhas)	Açailândia, Maranhão, Brasil	R.F. Alfenas	N/A	KM396025	KM396108	N/A	N/A	N/A		
Ca. rumohrae	CMW 23697^{T}	*Rumohra adiantiformis*	Volkan, Panama	J.W. Miller & R.M. leahy	MT335137	MT335372	MT335612	MT359833	MT359593	N/A	MT412903	MT413113
	CMW 30989	*Adiantum* sp.	The Holanda	R. Pieters	MT335138	MT335373	MT335613	MT359834	MT359594	MT412665	MT412904	MT413114
Ca. silvicola	CBS 135237^{T}	Solo (Floresta tropical)	Mucuri, Bahia, Brasil	A.C. Alfenas & P.W. Crous	N/A	KM396065	KM396148	N/A	N/A	N/A		
	CBS 134836	Solo (Floresta tropical)	Araponga, Minas Gerais, Brasil	A.C. Alfenas & P.W. Crous	N/A	KM396062	KM396145	N/A	N/A	N/A		
Ca. spathiphylli	CMW 16742^{T}	*Spathiphyllum* sp.	Florida, USA	C.L. Schoulties	N/A	MT335374	MT335614	MT359835	MT359595	MT412666	MT412905	MT413115
	CMW 30997	*Spathiphyllum* sp.	Suíça	L. Petrini	N/A	MT335375	MT335615	MT359836	MT359596	MT412667	MT412906	MT413116
Ca. spathulata	CMW 16744^{T}	*E. viminalis*	Brasil	N.E. El-Gholl	MT335139	MT335376	MT335616	MT359837	MT359597	MT412668	MT412907	MT413117
	CBS 112513	*Eucalyptus* sp.	Colômbia	M.J. Wingfield	MT335140	MT335377	MT335617	MT359838	MT359598	MT412669	MT412908	MT413118
Ca. sumatrensis	CMW 23698^{T}	Solo	Northern Sumatra, Indonésia	M.J. Wingfield	MT335145	MT335382	MT335622	MT359843	MT359603	MT412674	MT412913	N/A
	CMW 30987	Solo	Northern Sumatra, Indonésia	M.J. Wingfield	MT335146	MT335383	MT335623	MT359844	MT359604	MT412675	MT412914	N/A
Ca. syzygiicola	CBS 112831^{T}	*Syzygium aromaticum*	Sumatra, Indonésia	M.J. Wingfield	N/A	N/A	N/A	N/A	N/A	N/A		
Ca. tanah	CMW50777	Solo	Malásia	M.R.B.A Rauf	OL635134	OL635088	OL635064	N/A	N/A	OL635112	OL635040	OL635146
	CMW50772	Solo	Malásia	M.R.B.A Rauf	OL635133	OL635087	OL635063	N/A	N/A	OL635111	OL635039	OL635145
Ca. terricola	CBS 116247^{T}	Solo (*Eucalyptus* plantação)	Brasil	P.W. Crous	N/A	N/A	N/A	N/A	N/A	N/A		
Ca. tonkinensis	CMW 47430^{T}	Solo (*Eucalyptus* plantação)	Bavi, Hanoi, Vietnam	N.Q. Pham & T.Q. Pham	MT335147	MT335384	MT335624	MT359845	MT359605	MT412676	MT412915	MT413122
Ca. uniseptata	CBS 413.67^{T}	*Paphiopedilum callosum*	Celle, Alemanha	W. Gerlach	GQ280451	GQ267379	GQ267248	GQ280573	GQ280695	N/A	GQ267307	GQ267208
Ca. uxmalensis	CBS 110925^{T}	Solo	Uxmal, México	P.W. Crous	MT335153	MT335390	MT335630	MT359851	MT359611	MT412681	MT412921	MT413128
	CBS 110919	Solo	Uxmal, México	P.W. Crous	MT335154	MT335391	MT335631	MT359852	MT359612	MT412682	MT412922	MT413129
Ca. variabilis	CMW 3187^{T}	*Schefflera morototoni*	Pará, Brasil	F.C. de Albuquerque	N/A	MT335392	MT335632	MT359853	MT359613	MT412683	MT412923	MT413130
	CMW 2914	*Theobroma grandiflorum*	Pará, Brasil	F. Carneiro	N/A	MT335393	MT335633	MT359854	MT359614	MT412684	MT412924	MT413131

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Ca. venezuelana	CBS 111052^{T}	Solo	Acarigua, Venezuela	M.J. Wingfield	MT335155	MT335394	MT335634	MT359855	MT359615	MT412685	MT412925	MT413132
Ca. yunnanensis	CERC 5339^{T}	Solo (*Eucalyptus* plantação)	YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MT335157	MT335396	MT335636	MT359857	MT359617	MT412687	MT412927	MT413134
	CERC 5337	Solo (*Eucalyptus* plantação)	YunNan, China	S.F. Chen, J.Q. Li & G.Q. Li	MT335158	MT335397	MT335637	MT359858	MT359618	MT412688	MT412928	MT413135
Curvicladiella Cignea	CBS 109167^{T}	Folhas em decomposição	Guiana Francesa	C. Decock	KM231122	KM231287	KM231461	AF220973	AY793431	KM232311	KM231867	KM232002
	CBS 109168	Sementes em decomposição	Guiana Francesa	C. Decock	KM231121	KM231286	KM231460	KM231745	JQ666074	KM232312	KM231868	KM232003
Cu. Paphiopedili	MFLUCC20-0110	Folhas doentes de *Paphiopedilum* sp	China, Gui Zhou Province	L.C. Song	N/A	MT294104	MT294105	MT279198	MT279199	N/A	MT294103	MT294102
Cylindrocladiella addiensis	CBS143794T	Solo	Etiópia	P.W. Crous	N/A	N/A	N/A	MH111383	N/A	N/A	MH111395	MH111388
	CBS143793	Solo	Etiópia	P.W. Crous	N/A	N/A	N/A	MH111385	N/A	N/A	MH111394	MH111390
Cylla. Arbusta	CMW 47295T	Solo	Vietnan	N.Q. Pham and T.Q. Pham	N/A	N/A	MH016996	MH017015	N/A	N/A	MH016978	MH016958
	CMW 47296	Solo	Vietnan	N.Q. Pham and T.Q. Pham	N/A	N/A	MH016997	MH017016	N/A	N/A	JN099060	MH016959
Cylla. australiensis	CBS129567T	Solo	Austrália	P.W. Crous	N/A	N/A	JN098932	JN100624	N/A	N/A	JN099059	JN098747
	CBS129568	Solo	Austrália	P.W. Crous	N/A	N/A	JN098931	JN100623	N/A	N/A	MF444940	JN098748
Cylla. brevistipitata	CBS 142786T	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	N/A	N/A	N/A	JN099090	MF444926
Cylla. camelliae	CPC234	*Eucalyptus grandis*	África do Sul	P.W. Crous	N/A	N/A	JN098839	JN100573	N/A	N/A	JN099086	JN098749
	CBS114891T	*Eucalyptus grandis*	África do Sul	P.W. Crous	N/A	N/A	AY793510	AF220953	N/A	N/A	JN098975	AY793472
Cylla. clavata	CBS129563	Solo	Austrália	P.W. Crous	N/A	N/A	JN098859	JN099096	N/A	N/A	JN098974	JN098751
	CBS129564T	Solo	Austrália	P.W. Crous	N/A	N/A	JN098858	JN099095	N/A	N/A	JN098988	JN098752
Cylla. cymbiformis	CBS129553T	Solo	Austrália	P.W. Crous	N/A	N/A	JN098866	JN099103	N/A	N/A	JN098989	JN098753
	CBS129554	Solo	Austrália	P.W. Crous	N/A	N/A	JN098867	JN099104	N/A	N/A	JN099039	JN098754
Cylla. elegans	CBS338.92T	Folhas	África do Sul	L. Rong	N/A	N/A	AY793512	AY793444	N/A	N/A	JN099044	AY793474
	CBS110801	Folhas	África do Sul	P.W. Crous	N/A	N/A	JN098916	JN100609	N/A	N/A	JN099073	JN098755
Cylla. ellipsoidea	CBS129572	Solo	Austrália	P.W. Crous	N/A	N/A	JN098943	JN100636	N/A	N/A	JN098973	JN098756
	CBS129573T	Solo	Austrália	P.W. Crous	N/A	N/A	JN098857	JN099094	N/A	N/A	JN687562	JN098757
Cylla. hahajimaensis	PD684	Solo	Japão	T. Watanabe	N/A	N/A	N/A	JN687561	N/A	N/A	JN098996	N/A
Cylla. hawaiiensis	CBS118704	Solo	Hawaii	Y. Degawa	N/A	N/A	JN098878	JN099115	N/A	N/A	JN099057	JN098760

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Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	*cmdA*	*his3*	*ITS*	*LSU*	*rpb2*	*tef*	*tub2*
	CBS129569T	Solo	Hawaii	Y. Degawa	N/A	N/A	JN098929	JN100621	N/A	N/A	MF444938	JN098761
Cylla. horticola	CBS 142784T	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444911	N/A	N/A	MF444939	MF444924
	CBS 142785	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444912	N/A	N/A	MF444931	MF444925
Cylla. humicola	CBS 142777	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444904	N/A	N/A	MF444933	MF444917
	CBS 142779	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444906	N/A	N/A	JN099037	MF444919
Cylla. infestans	CBS111795T	*Pinus pinea*	Nova Zelândia	H.J. Boesewinkel	N/A	N/A	AY793513	AF220955	N/A	N/A	JN099036	AF320190
	CBS191.50	*Arena pinnata*	Indonésia	K.B. Boedijin & J. Reisma	N/A	N/A	AY793514	AF220956	N/A	N/A	JN099001	AY793475
Cylla. kurandica	CBS129576	Solo	Austrália	P.W. Crous	N/A	N/A	JN098941	JN100634	N/A	N/A	JN099083	JN098764
	CBS129577T	Solo	Austrália	P.W. Crous	N/A	N/A	JN098953	JN100646	N/A	N/A	JN099003	JN098765
Cylla. lageniformis	CBS340.92T	*Eucalyptus* sp.	África do Sul	A.C. Alfenas	N/A	N/A	AY793520	AF220959	N/A	N/A	JN099046	AY793481
	CBS111060	*Eucalyptus* sp.	África do Sul	P.W. Crous	N/A	N/A	JN098918	JN100611	N/A	N/A	N/A	JN098770
Cylla. lanceolata	CBS129566T	Solo	Austrália	P.W. Crous	N/A	N/A	JN098862	JN099099	N/A	N/A	JN099019	JN098789
	CBS114950	*Eucalyptus* sp.		P.W. Crous	N/A	N/A	JN098898	JN100591	N/A	N/A	MF444941	JN098787
Cylla. lateralis	CBS 142787	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444913	N/A	N/A	MF444942	MF444927
	CBS 142788T	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444914	N/A	N/A	JN098966	MF444928
Cylla. longiphialidica	CBS129557T	Solo	Tailandia	P.W. Crous	N/A	N/A	JN098851	JN100585	N/A	N/A	JN098967	JN098790
	CBS129558	Solo	Tailandia	P.W. Crous	N/A	N/A	JN098852	JN100586	N/A	N/A	JN099025	JN098791
Cylla. longistipitata	CBS112953	*Opisthiolepsis heterophylla*	Austrália	C. Pearce & B. Paulus	N/A	N/A	JN098902	JN100595	N/A	N/A	JN098993	JN098792
	CBS116075T	Solo	China	M.J. Wingfield	N/A	N/A	AY793546	AF220958	N/A	N/A	MH016979	AY793506
Cylla. malesiana	CMW 48278T	Solo	Malásia	M.J. Wingfield,	N/A	N/A	MH016998	MH017017	N/A	N/A	MH016980	MH016960
	CMW 48277	Solo	Malásia	M.J. Wingfield,	N/A	N/A	MH016999	MH017018	N/A	N/A	MH016981	MH016961
	CMW 48276	Solo	Malásia	M.J. Wingfield,	N/A	N/A	MH017000	MH017019	N/A	N/A	JN099041	MH016962
Cylla. microcylindrica	CBS111794T	*Echeveria elegans*	Indonésia	C.F. Hill	N/A	N/A	AY793523	AY793452	N/A	N/A	N/A	AY793483
	STE-U 10452	*Agalonema commutatum*	USA	C.F. Hill	N/A	N/A	AY793524	AY793453	N/A	N/A	JN099043	AY793484
Cylla. natalensis	CBS110800	Solo	África do Sul	P.W. Crous	N/A	N/A	JN098915	JN100608	N/A	N/A	JN099016	JN098793

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					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
	CBS114943T	*Arachis hypogaea*	África do Sul	M.J. Wingfield	N/A	N/A	JN098895	JN100588	N/A	N/A	MH111397	JN098794
Cylla. nauliensis	CBS 143792T	Solo	Indonésia	M.J. Wingfield,	N/A	N/A	N/A	MH111387	N/A	N/A	MH111396	MH111392
	CBS 143791	Solo	Indonésia	M.J. Wingfield,	N/A	N/A	N/A	MH111386	N/A	N/A	JN099011	MH111391
Cylla. Nedelandica	CBS146.94	*Rhododendron* sp.	Holanda		N/A	N/A	JN098889	JN099127	N/A	N/A	JN099033	JN098799
	CBS152.91T	*Pelargorium* sp.	Holanda	J.W. Veenbass-Rijks	N/A	N/A	JN098910	JN100603	N/A	N/A	JN099050	JN098800
Cylla. Novaezelandica	CBS486.77T	*Rhododendron indicum*	Nova Zelândia	H.J. Boesewinkel	N/A	N/A	AY793525	AF220963	N/A	N/A	MH016984	AY793485
Cylla. obpyriformis	CMW 47194T	Solo	Vietnan	N.Q. Pham and T.Q. Pham	N/A	N/A	MH017003	MH017022	N/A	N/A	MH016985	MH016965
	CMW 49940;	Solo	Vietnan	N.Q. Pham and T.Q. Pham	N/A	N/A	MH017004	MH017023	N/A	N/A	JN099009	MH016966
Cylla. parva	CBS114524T	*Telopea speciossima*	Nova Zelândia	H.J. Boesewinkel	N/A	N/A	AY793526	AF220964	N/A	N/A	MH016986	AY793486
Cylla. parvispora	CMW 47193	Solo	Vietnan	N.Q. Pham and T.Q. Pham	N/A	N/A	MH017005	MH017024	N/A	N/A	MH016987	MH016967
	CMW 47197T	Solo	Vietnan	N.Q. Pham and T.Q. Pham	N/A	N/A	MH017006	MH017025	N/A	N/A	MH016988	MH016968
	CBS113022	*Eucalyptus* sp.	África do Sul	P.W. Crous	N/A	N/A	JN098906	JN100599	N/A	N/A	JN098968	JN098801
Cylla. postalofficium	CBS146060T	Folhas	África do Sul	L. Lombard	N/A	N/A	MN556796	MN562148	N/A	N/A	JN098958	MN556845
Cylla. pseudocamelliae	CBS129555T	Solo	Tailandia	P.W. Crous	N/A	N/A	JN098843	JN100577	N/A	N/A	JN098961	JN098814
	CBS129556	Solo	Tailandia	P.W. Crous	N/A	N/A	JN098846	JN100580	N/A	N/A	JN099012	JN098815
Cylla. pseudohawaiiensis	CBS210.94T	*Eucalyptus* sp.	Brasil	A.C. Alfenas	N/A	N/A	JN098890	JN099128	N/A	N/A	JN099024	JN098819
	CBS115610	-	Madagascar	J.E. Taylor	N/A	N/A	JN098901	JN100594	N/A	N/A	JN099010	JN098820
Cylla. pseudoinfestans	CBS114530	Solo	Madagascar	J.E. Taylor	N/A	N/A	JN098888	JN099126	N/A	N/A	JN099004	JN098821
	CBS114531T	Solo	Madagascar	J.E. Taylor	N/A	N/A	AY793548	AF220957	N/A	N/A	JN099002	AY793508
Cylla. pseudoparva	CBS113624	*Quercus* sp.	Suíça	L. Petrini	N/A	N/A	JN098883	JN099121	N/A	N/A	JN099030	JN098822
	CBS122594	*Vitis riparia*	Nova Zelândia	K. Paice	N/A	N/A	JN098907	JN100600	N/A	N/A	JN099056	JN098823
	CBS129560T	Solo	Holanda	P.W. Crous	N/A	N/A	JN098927	JN100620	N/A	N/A	JN098977	JN098824
Cylla. queenslandica	CBS129574T	Solo	Austrália	P.W. Crous	N/A	N/A	JN098861	JN099098	N/A	N/A	JN098976	JN098826

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					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
	CBS129575	Solo	Austrália	P.W. Crous	N/A	N/A	JN098860	JN099097	N/A	N/A	MF444935	JN098827
Cylla. reginae	CBS 142781	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444908	N/A	N/A	MF444936	MF444921
	CBS 142782 T	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444909	N/A	N/A	JN099048	MF444922
Cylla. stellenboschensis	CBS386.67	*Fragaria* sp.	Holanda	P.W. Crous	N/A	N/A	JN098920	JN100613	N/A	N/A	JN099051	JN098828
	CBS110668T	Solo	África do Sul	P.W. Crous	N/A	N/A	JN098922	JN100615	N/A	N/A	MF444943	JN098829
Cylla. terretris	CBS 142789	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444915	N/A	N/A	MF444944	MF444929
	CBS 142790	Solo	Tailandia	P.W. Crous	N/A	N/A	N/A	MF444916	N/A	N/A	JN098962	MF444930
Cylla. Tailandiaica	CBS129570	Solo	Tailandia	P.W. Crous	N/A	N/A	JN098847	JN100581	N/A	N/A	JN098963	JN098833
	CBS129571T	Solo	Tailandia	P.W. Crous	N/A	N/A	JN098848	JN100582	N/A	N/A	JN099000	JN098834
Cylla. variabilis	CBS375.93	*Mangifera indica*	Índia	P.N. Chow	N/A	N/A	JN098881	JN099119	N/A	N/A	JN099080	JN098836
	CBS129561T	Solo	Austrália	P.W. Crous	N/A	N/A	JN098950	JN100643	N/A	N/A	JN099064	JN098719
Cylla. viticola	CBS112897T	*Vitis vinifera*	África do Sul	G.J. Van Coller	N/A	N/A	AY793544	AY793468	N/A	N/A	JN099047	AY793504
	CBS114682	*Amorphophallus* sp.	Tailandia	R. Stevenson	N/A	N/A	JN098919	JN100612	N/A	N/A	KY979891	JN098723
Cylla. vitris	CBS142517T	*V. vinifera*	Nova Zelândia	D. Davis	N/A	N/A	N/A	KY979751	N/A	N/A	N/A	KY979918
Gliocladiopis aquaticus	MFLUCC17-2028	*Cassia fistula*	Tailandia	R.H. Perera	N/A	N/A	MG734183	MG543925	N/A	N/A	N/A	MG574422
	MFLUCC17-1811	Madeira em decomposição	Tailandia	R.H. Perera	N/A	N/A	MG734182	MG543924	N/A	N/A	N/A	MG574421
Glio. curvata	CBS 194.80	*Persea americana*	Equador	J.P. Laoh	N/A	N/A	JQ666010	JQ666044	N/A	N/A	JQ666086	JQ666120
	CBS 112365	*Archontophoenix purpurea*	Nova Zelândia	F. Klassen	N/A	N/A	JQ666016	JQ666050	N/A	N/A	JQ666092	JQ666126
Glio. Equadoriensis	MUCL 54740	*Polybotrya* sp.	Equador	A. Gordillo and C. Decock	N/A	N/A	KX671146	N/A	N/A	N/A	KX671131	KX611501
Glio. elghollii	CBS 206.94	*Chamaedorea elegans*	USA	N.E. El-Gholl	N/A	N/A	JQ666020	JQ666054	N/A	N/A	JQ666096	JQ666130
	CBS 116104	*Chamaedorea elegans*	USA	N.E. El-Gholl	N/A	N/A	JQ666021	JQ666055	N/A	N/A	JQ666097	JQ666131
Glio. forsbergii	BRIP 60984	*Grevillea* sp.	Austrália	K.G. Pegg	N/A	N/A	KX274053	KX274070	N/A	N/A	N/A	KX274036
	BRIP 61349a	*Persea americana*	Austrália	L.E. Parkinson	N/A	N/A	KX274054	KX274071	N/A	N/A	N/A	KX274037
Glio. guangdongensis	LC 1340	Madeira submersa	China	F. Liu and L. Cai	N/A	N/A	KC776120	KC776122	N/A	N/A	KC776119	KC776124
	LC 1349	Madeira submersa	China	F. Liu and L. Cai	N/A	N/A	KC776121	KC776123	N/A	N/A	KC776118	KC776125

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					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Glio. hennebertii	MUCL 54818	*Costus scaber*	Equador	A. Gordillo and C. Decock	N/A	N/A	N/A	KX671140	N/A	N/A	KX671132	KX611502
Glio. indonesiensis	CBS 116090	Solo	Indonésia	A.C. Alfenas	N/A	N/A	JQ666022	JQ666056	N/A	N/A	N/A	JQ666132
Glio. irregularis	CBS 755.97	Solo	Indonésia	A.C. Alfenas	N/A	N/A	JQ666023	AF220977	N/A	N/A	JQ666099	JQ666133
Glio. mexicana	CBS 110938	Solo	México	M.J. Wingfield	N/A	N/A	JQ666027	JQ666060	N/A	N/A	JQ666103	JQ666137
Glio. peggii	BRIP 53654	*Persea americana*	Austrália	E.K. Dann and A.W.	N/A	N/A	N/A	JN255246	N/A	N/A	N/A	JN255247
	BRIP 60983	*Persea americana*	Austrália	K.G. Pegg	N/A	N/A	KX274065	KX274083	N/A	N/A	N/A	KX274038
Glio. pseudotenuis	CBS 114763	*Vanilla* sp.	Indonésia	M.J. Wingfield	N/A	N/A	JQ666029	JQ666062	N/A	N/A	JQ666105	JQ666139
	CBS 116074	Solo	China	M.J. Wingfield	N/A	N/A	JQ666030	AF220981	N/A	N/A	JQ666106	JQ666140
Glio. siamensis	MFLUCC 18-0576	Ramos	Tailândia	R.H. Perera	N/A	N/A	ON364457	ON361571	ON352629	N/A	N/A	ON364481
Glio. singaporiensis	MUCL 48728	*Olivier laurence*	Singapura	C. Decock	N/A	N/A	N/A	KX671138	N/A	N/A	KX671130	KX611500
Glio. sumatrensis	CBS 754.97	Solo	Indonésia	M.J. Wingfield	N/A	N/A	JQ666032	JQ666064	N/A	N/A	JQ666108	JQ666142
	CBS 111213	Solo	Indonésia	M.J. Wingfield	N/A	N/A	JQ666034	JQ666066	N/A	N/A	JQ666110	JQ666144
Glio. tenuis	IMI 68205	*Indigofera* sp.	Indonésia	F. Bugnicourt	N/A	N/A	JQ666040	AF220979	N/A	N/A	JQ666116	JQ666150
	CBS 111964	*Coffea* sp.	Vietnam	P.W. Crous	N/A	N/A	JQ666037	JQ666068	N/A	N/A	JQ666113	JQ666147
Glio. sagariensis	CBS 199.55	Solo	Índia	S.B. Saksena	N/A	N/A	JQ666031	JQ666063	N/A	N/A	JQ666107	JQ666141
Glio. swieteniae	MFLU 18-2767	*Swietenia mahagoni*	Tailandia	R.H. Perera	N/A	N/A	MT212194	MT215501	N/A	N/A	N/A	MT212214
Glio. whileyi	BRIP 61430	*Persea americana*	Austrália	E.K. Dann	N/A	N/A	KX274069	KX274086	N/A	N/A	N/A	KX274052
Glio. wuhanensis	HEAC17307	Solo	China	Niping Zhai	N/A	N/A	MH255786	MH024520	N/A	N/A	N/A	MH169602
Gliocladiopsis sp. 1	CBS 111038	Solo	Colômbia	M.J. Wingfield	N/A	N/A	JQ666041-	JQ666071-	N/A	N/A	JQ666117	JQ666151-
Gliocladiopsis sp. 2	CBS 116086	Solo	Indonésia	A.C. Alfenas	N/A	N/A	JQ666042-	JQ666072-	N/A	N/A	JQ666118	JQ666152-
Gliocephalotrichum abrachium	CCUB10	*C. brasiliense*	Piauí, Brasil	A. Reis	N/A	N/A	MN508738	MN450200	N/A	N/A	MN508678	MN508721
	CCUB301	*C. brasiliense*	Minas Gerais, Brasil	A. Reis	N/A	N/A	MN508745	MN450207	N/A	N/A	MN508691	MN508728
G. bacillisporum	CBS 250.91	Raiz	Brasil	L. Pfenning	N/A	N/A	KF513323	KF513251	N/A	N/A	KF513405	KF513182
	CBS 126572	Folha	Guiana Francesa	C. Decock & V. Robert	N/A	N/A	KF513324	DQ374408	N/A	N/A	KF513406	DQ374413
G. bulbilium	*CBS 118.68*	*Ar*	*Central African Republic*	*J. Nicot*	*N/A*	*N/A*	*KF513327*	*KF513252*	*N/A*	*N/A*	*KF513409*	*KF513183*

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					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
	CBS 562.75	*Flacourtia* sp.	Indonésia	I. Gandjar	N/A	N/A	KF513328	KF513253	N/A	N/A	KF513410	KF513184
G. brasiliense	CCUB232	*D. madagascariensis*	Distrito Federal, Brasil	A. Reis	N/A	N/A	MN508747	MN450209	N/A	N/A	MN508698	MN508730
	CCUB355	*S. purpurea*	Distrito Federal, Brasil	A. Reis	N/A	N/A	MN508748	MN450210	N/A	N/A	MN508701	MN508731
G. caryocaris	CCUB229	*C. brasiliense*	Minas Gerais, Brasil	A. Reis	N/A	N/A	MN508744	MN450206	N/A	N/A	MN508689	MN508727
	CCUB231	*C. brasiliense*	Minas Gerais, Brasil	A. Reis	N/A	N/A	MN508746	MN450208	N/A	N/A	MN508692	MN508729
G. cylindrosporum	CBS 902.70	Solo	Tailandia	C. Klinsukont	N/A	N/A	KF513353	DQ366705	N/A	N/A	KF513435	DQ377841
	CBS 903.70	Solo	Tailandia	S. Chomchalow	N/A	N/A	KF513354	KF513277	N/A	N/A	KF513436	KF513208
G. grande	HMAS	Folhas	China	W.Y. Zhuang & Y. Nong	N/A	N/A	N/A	EF121859	N/A	N/A	HM054075	EU984072
G. humicola	CBS 135945	Solo	Taiwan	P.W. Crous	N/A	N/A	KF513356	KF513278	N/A	N/A	KF513438	KF513209
	CBS 135946	Solo	Taiwan	P.W. Crous	N/A	N/A	KF513357	KF513279	N/A	N/A	KF513439	KF513210
G. longibrachium	CBS 126571	Folhas	Guiana Francesa	C. Decock & V. Robert	N/A	N/A	KF513367	N/A	N/A	N/A	KF513449	DQ377835
	CBS 132043	Folhas	Guiana Francesa	C. Decock & V. Robert	N/A	N/A	KF513368	DQ278422	N/A	N/A	KF513450	DQ377836
G. mexicanum	CBS 135947	*N. lappaceum*	México	L.M. Serrato-Diaz	N/A	N/A	KF513369	KF513289	N/A	N/A	KF513451	KF513220
	CBS 135948	*N. lappaceum*	México	L.M. Serrato-Diaz	N/A	N/A	KF513370	KF513290	N/A	N/A	KF513452	KF513221
G. microchlamydosporum	CBS 345.64	Solo	Zaïre	J.A. Meyer	N/A	N/A	KF513371	DQ366699	N/A	N/A	KF513453	DQ374410
	CPC 21862	–	Zaïre	–	N/A	N/A	N/A	DQ366700	N/A	N/A	KF513454	DQ374411
G. nephelii	CBS 135949	*N. lappaceum*	Guatemala	L.M. Serrato-Diaz	N/A	N/A	KF513372	KF513291	N/A	N/A	KF513456	KF513222
	CBS 135950	*N. lappaceum*	Guatemala	L.M. Serrato-Diaz	N/A	N/A	KF513373	KF513292	N/A	N/A	KF513457	KF513223
G. ohiense	CBS 567.73	Solo	USA	L.H. Huang	N/A	N/A	N/A	DQ366707	N/A	N/A	KF513458	DQ374415
G. queenslandicum	CBS 112956	*E. angustifolius*	Austrália	I. Steer & B. Paulus	N/A	N/A	KF513374	KF513293	N/A	N/A	KF513459	KF513224
	CBS 114868	*E. angustifolius*	Austrália	I. Steer & B. Paulus	N/A	N/A	KF513375	KF513294	N/A	N/A	KF513460	KF513225
G. simmonsii	CBS 135951	*N. lappaceum*	Guatemala	L.M. Serrato-Diaz	N/A	N/A	KF513376	KF513295	N/A	N/A	KF513461	KF513226
	CBS 135953	*N. lappaceum*	Guatemala	L.M. Serrato-Diaz	N/A	N/A	KF513378	KF513297	N/A	N/A	KF513463	KF513228
G. simplex	CBS 267.65	Solo	África do Sul	H.J. Swart	N/A	N/A	KF513379	DQ366702	N/A	N/A	KF513464	DQ377838
	CBS 983.69	Solo	Brasil	C. Ram	N/A	N/A	KF513380	KF513298	N/A	N/A	KF513465	KF513229

Continua...

Espécie	Isolado	Hospedeiro/ Substrato	País	Coletor	Acessos do GenBank							
					act	***cmdA***	***his3***	***ITS***	***LSU***	***rpb2***	***tef***	***tub2***
Penicillifer bipapillatus	CBS 420.88^{T}	Casca	C.T. Rogerson	Venezuela	KM231105	KM231270	KM231454	KM231740	KM231608	KM232295	KM231860	KM231996
P. diparietisporus	CBS 376.59^{T}	Solo	A.A. Foster	USA	KM231106	KM231271	KM231455	KM231741	KM231609	KM232296	KM231861	KM231997
P. macrosporus	CBS 42388	-	G.J. Samuels	Guyana	KM231104	KM231269	KM231453	MH862133	KM231607	KM232294	KM231859	KM231995
P. martinii	BRIP 59225	*Cynodon dactylon*	P.T.W. Wong	Austrália: New South Wales	N/A	N/A	N/A	NR168155	NG068753	N/A	KJ869241	N/A
P. penicilliferi	CBS 423.88	-	G.J. Samuels	Guyana	KM231104	KM231269	KM231453	KM231739	KM231607	KM232294	KM231859	KM231995
P. pulcher	CBS 560.67^{T}	Solo	J.H. van Emden	The Holanda	KM231107	KM231272	KM231456	KM231742	KM231610	KM232297	KM231862	KM231998
P. sinicus	HMAS 247865	Raiz	Z.Q. Zeng & H.D. Zheng	CHINA, Guangxi Zhuang	N/A	N/A	N/A	OP223439	OP223435	OP272863	OP272864	OP586763
Xenocylindrocladium guianense	CBS 112179^{T}	Planta morta	C. Decock	Guiana Francesa	KM231124	KM231289	KM231463	AF317348	JQ666073	KM232314	KM231895	AF320197
X. serpens	CBS 128439	Casca	G.L. Hennebert	Equador	KM231125	KM231290	KM231464	N/A	MH876378	KM232315	KM231894	N/A
X. subverticillatum	CBS 113660	Planta morta	C. Decock	Singapura	KM231123	KM231288	KM231462	N/A	KM231687	KM232313	KM231893	N/A

Penicillifer também possui doze espécies atualmente descritas, assim como *Aquanectria* (Tabela 1). O gênero foi primeiramente descrito a partir da espécie *P. pulcher* em 1968 de amostras de solo que apresentavam longas e hialinas cadeias de esporos(Van Emden 1968). Apesar de no Index Fungorum ser possível encontrar 12 nomes relatados, é constatado algumas mudanças dentro do gênero. As espécies *P. simplex*, *P. superimpositus* e *P. variabilis* foram sinonimizadas nos gêneros *Cephalosporiopsis, Mariannaea* e *Septofusidium* em 1989 (Samuels 1989). Já as espécies *P. fragariae* (Watanabe 1990) e *P. furcatus* (Polishook et al. 1991) só foram relatas nestas únicas vezes, não possuindo dados moleculares disponíveis. A última espécie adicionada ao gênero aconteceu em 2022 com a descrição de *P. sinicus* (Zeng and Zhuang 2022a).

A definição de gênero para *Penicillifer* são espécime que possuem ascoma não estromático, superficial, solitário, globoso para piriforme, vermelho, laranja amarronzado ou marrom, não reagindo ou mudando para vermelho em KOH. Ascos clavados, com ápice simples, Ascósporos verdes, monosseptados e lisos. Conidióforos eretos, solitários, septados, hialinos não ramificados e monofiálidicos ou biverticilados. Fiálides cilíndricas, com espessamento periclinal, não achatadas. Conídios cilíndricos para levemente naviculados, monosseptados, lisos e com papila em uma ou nas duas pontas (Lombard et al. 2015a).

O segundo menor gênero do grupo possui apenas três espécies e com um limitado número de isolados. *Xenocylindrocladium* foi proposto em 1997 baseado na espécie *X. serpens,* o gênero foi proposto por se diferenciar de *Calonectria* quanto a sua estipe terminando em uma ponta espiral e por seu teleomorfo estar em *Nectria* (Decock et al. 1997). A partir disto o gênero só teve adição de mais espécies em 2001 com a descrição de *X. guianense* e *X. subverticillatum* ambas propostas baseadas em análises morfológicas e moleculares (Crous et al. 2001). Com isso o gênero é estabelecido por espécies que apresentam peritécio superficial, solitário ou agregado, globoso para subgloboso, amarelo

para vermelho com um vermelho escuro na base estromática. Perífises ostiolares hialinas, tubulares e arredondadas na ponta. Asco unitunicados, com oito ascósporos, cilíndrico com uma longa base, achatado no ápice e com um aparato apical refrativo. Ascósporos agregados acima do primeiro terço do asco, hialinos, fortemente para levemente elipsoidal, lisos, em média um septo. Conidióforos consistindo em uma estipe com arranjo penicilado de ramos férteis e uma extensão da estipe avesiculada. Estipe septada, hialina, lisa extensão da estipe septada, reta para flexuosa ou sinuosa. Aparato conidiogênico com primeiros ramos asseptados ou monosseptados, segundo, terceiro e quarto asseptados, cada um terminando na produção de 2-6 fiálides. Fiálides doliformes para reniformes, hialinas, asseptadas e com ápice apresentando espessamento periclinal e coloração inconspícua. Conídio cilíndrico, arredondado em ambas as pontas, ereto ou curvado, septado, ausência de cicatriz de abscisão evidente. Formados em paralelos clusters cilíndricos unidos por uma mucilagem (Lombard et al. 2015a).

O menor gênero do grupo, possui somente duas espécies uma relatada em 1998 quando o *Curvicladiella* foi proposto baseada na espécie *Cu. cigneum*, e outra em 2022 com a descrição de *Cu. paphilopedili* (Decock and Crous 1998; Song et al. 2022– Tabela 2). Ambas as espécies descritas no gênero possuem dados moleculares a primeira foi estudada em filogenias da família Nectriaceae e a segunda já foi proposta usando esta nova abordagem (Lombard et al. 2015a; Song et al. 2022). Ambas as espécies são definidas por possuírem teleomorfo desconhecido, mas com formação de esporodóqui ou sinêmio, consistindo em numeroso conidióforos penicilados formando um estroma amarronzado de clamidósporos de espessas paredes. Conidióforos consiste em uma parede grossa, lisa para finamente verrucosa, septada, beje amarronzado para marro na base da estipe, um aparato conidiogênico e muitas extensões da estipe estéreis que podem apresentar 1(-2) septos no ápice ou um na base, extensão da estipe avesiculada, parede apical grossa, verrucosa, beje amarronzado, geralmente curvada e afunilamento em direção ao ápice,

terminando de forma aguda. Aparato conidiogênico com muitos ramos, hialinos, lisos, subcilíndricos, eretos para ligeiramente curvados, e inconspícuos. Conídio cilíndricos septados, ausência de um visível cicatriz de abscisão, agrupados em cabeças de mucilagem incolor. Clamidósporos arranjados de forma intercalar nas hifas, ou em agregados formando microescleródios (Lombard et al. 2015a).

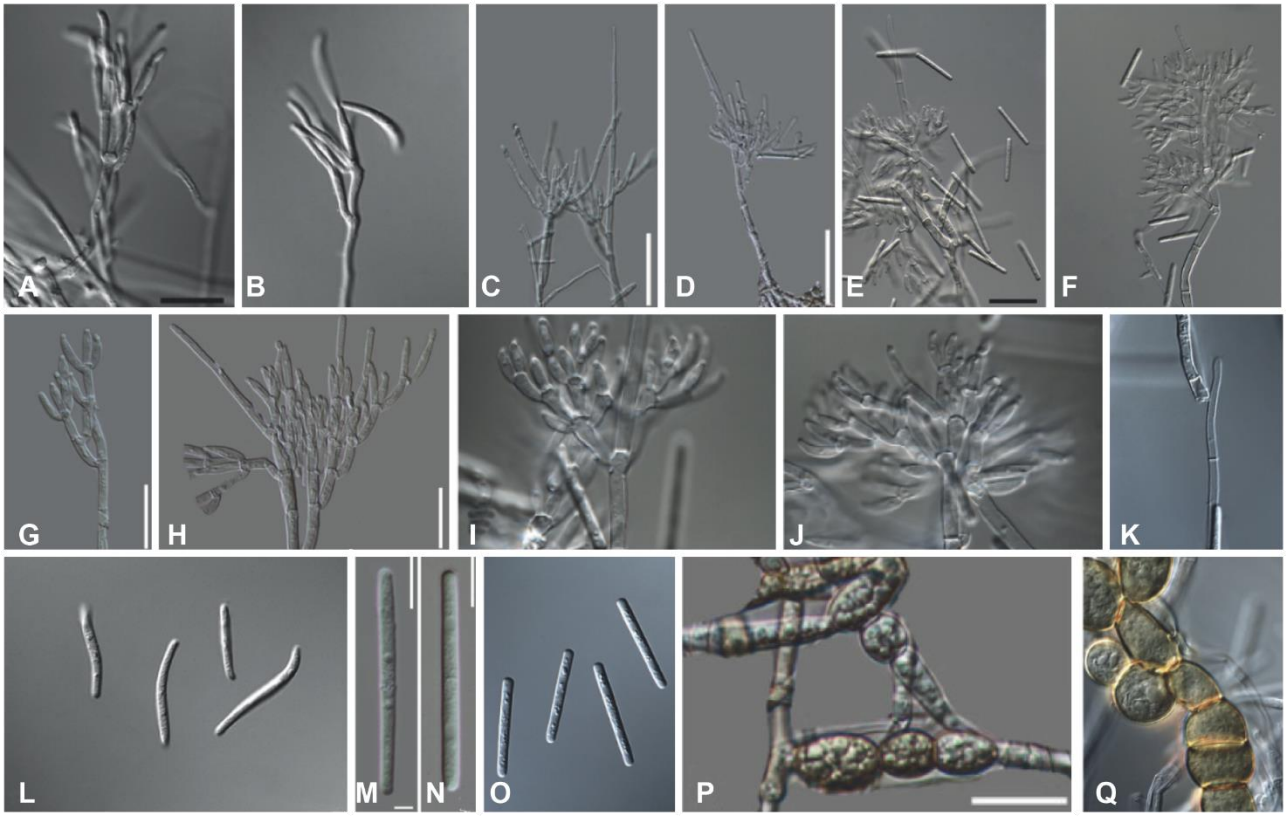

Figura 3. Morfologia dos gêneros Aquanectria, Curvicladiella e Xenocylindrocladium. A - F. conidióforos de Aquanectria (A e B), Curvicladiella (C e D) e Xenocylindrocladium (E e F). G – J. detalhe do aparelho conidiogênico de Curvicladiella (G e H) e Xenocylindrocladium (I e J). K. Detalhe da vesícula de Xenocylindrocladium. L – O. Conídios de Aquanectria (L), Curvicladiella (M e N) e Xenocylindrocladium (O). P e Q. Clamidósporos de Curvicladiella (P) e Xenocylindrocladium (Q). Fonte: imagens A-B, E-F, I-L, O e Q de Lombard et al. (2015), imagens C-D, H-H, M-N e P de Song et al. (2022).

1.4 Distribuição global dos *Calonectria*- like

Atualmente *Calonectria* é reconhecidamente como um gênero cosmopolita por ser encontrado em quase todos os continentes. Atualmente o gênero foi relatado em 74

diferentes países, sobretudo em países tropicais. Alfenas et al. (2015) relata que é percebido uma distinção biogeográfica entre os complexos de espécies de *Calonectria*. posteriormente Liu et al. (2020) em seu estudo reafirmaram o observado por Alfenas et al. (2015), o autor cita que espécies dentro dos complexos de espécies *Ca. candelabrum* e *C. navidulata* ocorrem em todos os continentes (com exceção da Antártida), e em contraste espécies dentro dos complexos *Ca. gracilipe, Ca. pteridis* e *Ca. spathiphylli* são mais comumente encontradas nas Américas.

Apesar das inferências quanto a distribuição geográfica do gênero feita pelos autores anteriormente citados, estes analisaram a informação contida em 1.017 isolados (Alfenas et al. 2015) e 316 isolados (Liu et al. 2020). Quando observamos a quantidade de informação dentro dos repositórios podemos constatar que o número é muito maior que o que ambos os autores avaliaram. Atualmente dentro do GBIF (*Global Biodiversity Information Facility*) existem 9.133 registros destes gêneros distribuídos no mundo. Esta é a soma do que são reportados por centenas de instituições ao redor do mundo, entre elas herbários, fungários, coleções de culturas, repositórios de sequencias etc. Em um panorama geral, é possível afirmar que estes gêneros estão presentes globalmente, com relatos em todos os continentes, incluindo a Antártica. Entretanto existe uma variação entre gêneros quanto a sua distribuição geográfica. *Aquanectria* possui 256 registros distribuídos em 33 países, com destaque para a América Central e norte da América do Sul. *Calonectria* e *Cylindrocladiella,* possuem perfis de distribuição similares, tendo na plataforma 2.309 e 2.195 registros, 101 e 73 países, respectivamente e com uma distribuição similar e intensa nas Américas, sudestes Asiático, Europa e Oceania. *Curvicladiella* é um gênero que ainda se encontra restrito em poucas localidades pelos dados do GBIF, é mostrado que está somente no norte da América do sul e Asia. *Gliocephalotrichum* e *Gliocladiopsis* é possível observar 2.049 e 1.530 relatos distribuídos em 53 e 59 países, respectivamente, e com destaque para a América central e sul, além de África, Índia Papua e Nova Guiné. *Penicillifer*

possui 470 relatos em 37 países com predominância de países abaixo do linha do Equador, como os que estão na América do Sul, África, Austrália e no sudeste Asiático. *Xenocylindrocladium* apesar de poucas espécies conhecidas, é relatado 301 vezes, com distribuição em 21 países, principalmente em países da África Colômbia e Tailândia (Figura 7).

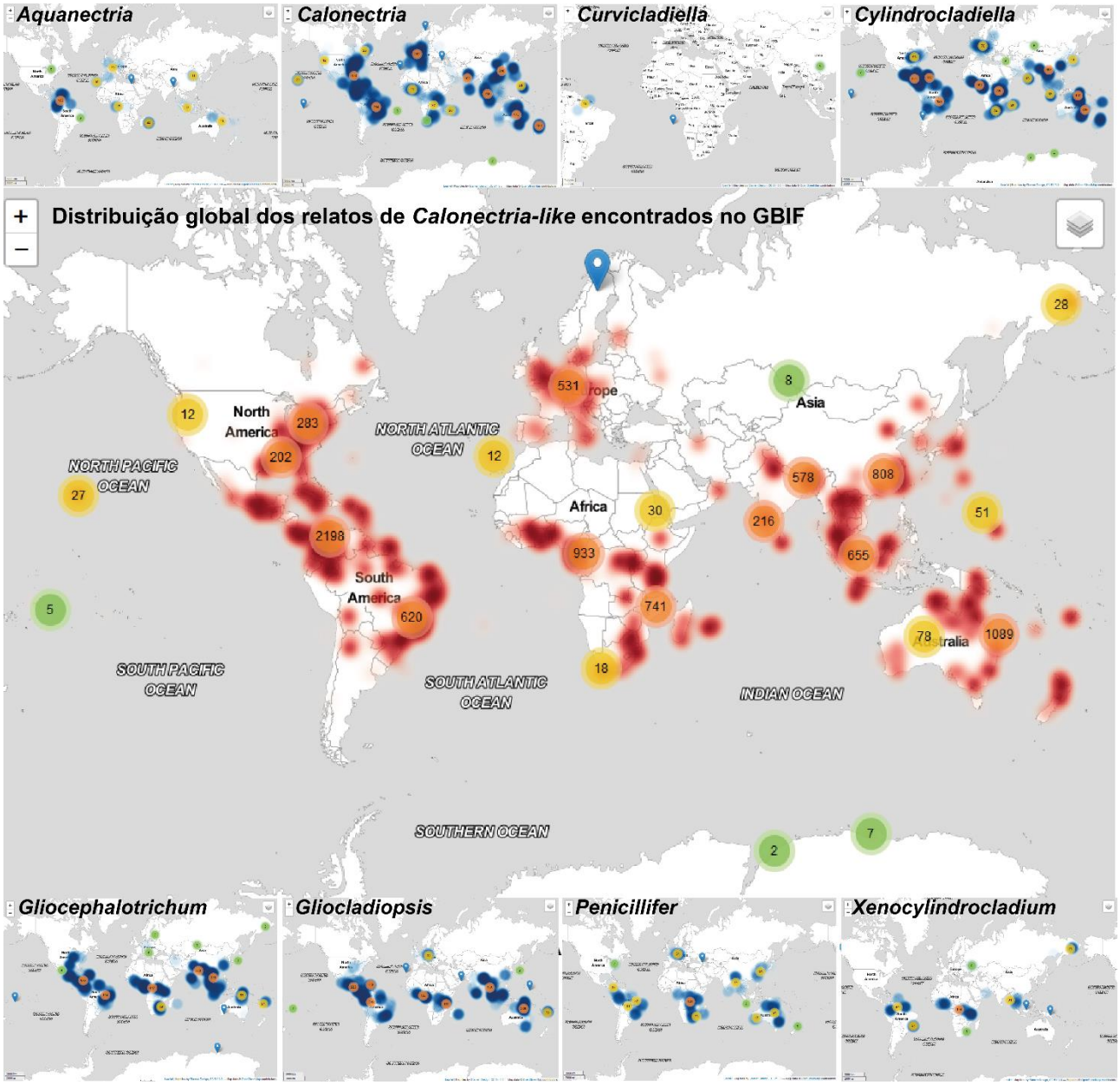

Figura 4. Distribuição global dos relatos encontrados no GBIF para todos os oito gêneros que compõem o grupo. Sombras em azul ou vermelho indicam a concentração de relatos, tornando mais escuro quando maior o número de relatos no mesmo loca e círculos com número indicam o número das quantidades encontrados naquela zonal. Mapa central com sombras em vermelho é a distribuição total de todos os oito gêneros. Mapas menores acima e abaixo com sombras azuis representam a distribuição individual de cada gênero.

Com base nos dados do GBIF podemos observar que o Brasil é o território com maior número de espécies, 8 gêneros tendo 58 espécies representados no território. Seguido da Austrália com 52 espécies e China com 48 espécies, no primeiro país ainda não é relatado espécies de *Curvicladiella* e nos dois ainda não tiveram relatos de espécies de *Xenocylindrocladium*. Observando pelo lado dos fungos, podemos citar que *Cylindrocladiella variabilis* é a mais cosmopolita, por estar presente em 49 países diferentes, seguida de *Gliocladiopsis elghollii* (36 países), *Gliocephalotrichum cylindrosporum* (35 países), *Gliocladiopsis tenuis* (32 países)*, Penicillifer diparietisporus* (29 países). O ranqueamento, mostra que nos dados do GBIF uma espécie não identificada de *Calonectria* é relatada em mais de 50 países, mas que espécies identificadas ao nível de epíteto específico não estão entre as 10 mais cosmopolitas. Considerando o número total de países por gênero temos uma frequência quase que ligada a quantidade de amostragens, com *Calonectria* em 101, *Cylindrocladiella* em 73, *Gliocladiopsis* com 59 e *Gliocephalotrichum* com 53 países (Figura 6).

Em *Calonectria,* podemos observar que o gênero é distribuído ao longo do globo, mas os complexos de espécies parecem ter distintas separações (Alfenas et al. 2015; Liu et al. 2020). Em questão de complexos cosmopolitas podemos ver que 54% deles estão presentes em cinco continentes, somente *Ca. gracilipes* é o menos cosmopolita entre os complexos, por estar presente nos continentes Americano e Europeu, com predominância de espécies relatadas no primeiro. Desta forma, *Ca. kyotensis* é o complexo de espécie encontrado em 35 países, com 23 espécies encontradas em 14 diferentes países Asiáticos (China e Indonésia) e 8 espécies em 10 países Americanos. O complexo *Ca. candelabrum* é encontrado predominantemente nas Américas em nove países, sobretudo o Brasil possui 17 diferentes espécies deste complexo relatadas em seu território. Em número de países que possuem relatos os complexos *Ca. naviculata* e *Ca. colhounii* se empatam, com 23

países, o primeiro com maior número de espécies encontradas nos países Europeus (12 países) e a segunda nos Asiáticos (12 países).

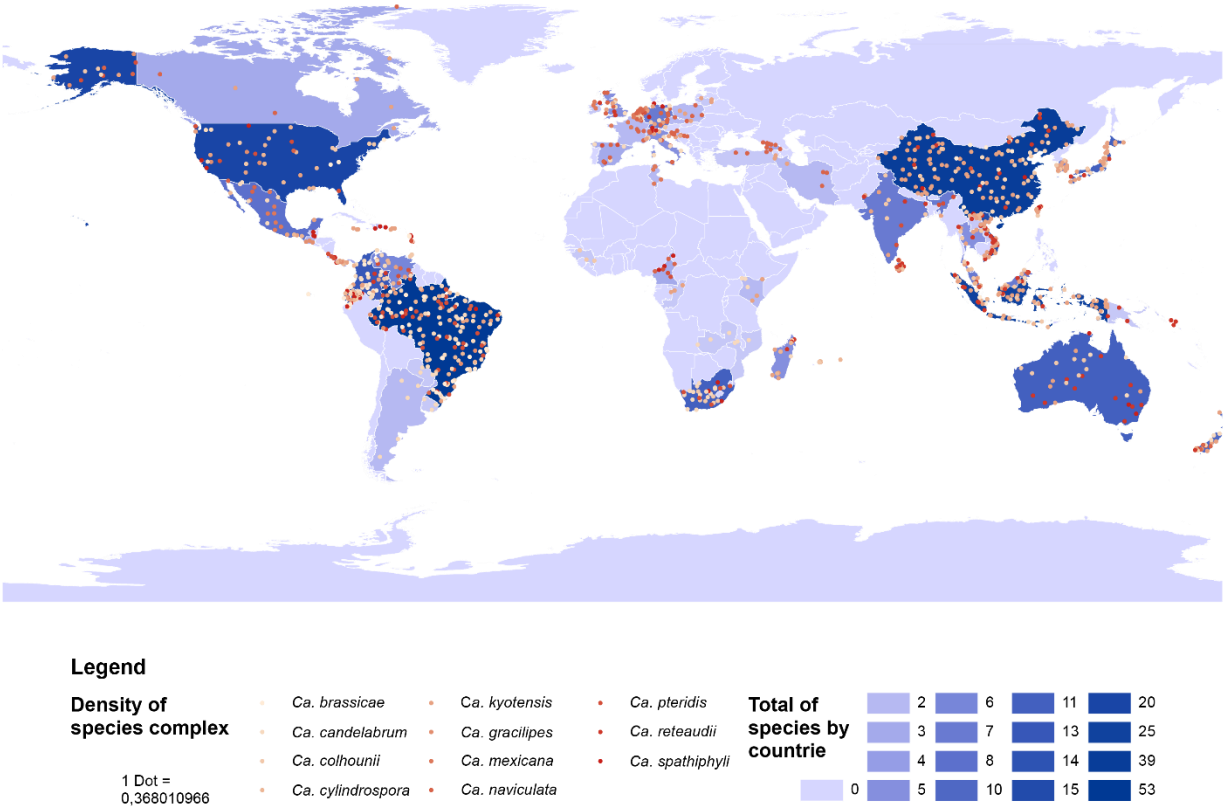

Figura 5: Distribuição geográfica das espécies de Calonectria por complexo de espécie.

Olhando a nível de espécies, vemos *Ca. kyotensis* e *Ca. pseudonaviculata* em 21 diferentes países, com a diferença que o último não foi relatado no continente Africano. Seguindo, vemos *Ca. ilicicola* relatado em 20 países, *Ca. reteaudii* em 18 países, *Ca. pteridis* e *Ca. spathiphylli* em 17 países todos os 5 continentes. *Calonectria* mostra grande adaptação nas diferentes latitudes e longitudes do planeta, um padrão cosmopolita, no entanto intra-gênero podemos ver o zoneamento de algumas espécies a determinadas regiões do mundo, para muitos fungos se especula-se sobre os efeitos do ambiente associados a diversidade das espécies e as estratégias evolucionárias do gênero, apesar de *Calonectria* ter uma longa história os estudos relacionados a estas relações ainda são

escassos. Os bancos de dados e extensas revisões bibliográficos oferecem suporte a trabalhos de zoneamento espacial de fungos, com esta base criada, aumenta a possibilidade da criação de medidas protetivas para evitar ou mitigar o translado de patógenos entre nações, diminuindo a possibilidade de introdução de novas espécies/genótipos em locais suscetíveis.

Especialmente em *Eucalyptus* sp. o gênero *Calonectria* é conhecido por causar manchas foliares, com consequência de intensa desfolha (doença conhecida como *Calonectria leaf blight* ou CLB) (Crous 2002). Tal doença tem impacto diretamente a capacidade fotossintética, metabolismo de ácidos nucleicos e proteínas, detoxificação de fitoalexinas e degradação de parede celular devido a ação de cutinases, pectinases e ligninases (Chen et al. 2015; Ye et al. 2018). Em uma recente revisão da distribuição global desta doença, foi identificado que ela pode ser encontrada relatada em 13 países dos 5 continentes, além de que esta doença pode ser causada por 24 espécies de *Calonectria* (Figura 7 - Bose et al., 2022).

É possível notar que CLB é causada por diferentes espécies de acordo com a localidade, a exemplo disso, comparando Brasil e China, que possuem relatadas 18 e 14 espécies associadas a *Eucalyptus*, respectivamente, somente *Ca. kyotensis* é relatada nos dois países, e somente *Ca. reteaudii* é reportada em 8 países (Figura 7). No território brasileiro é possível notar que existem 14 espécies associadas a *Eucalyptus* mas somente nove foram reportadas causando CLB (Bose et al. 2022). *Calonectria pteridis* somente é reportada causando CLB no Brasil, a doença foi relatada a primeira vez em 1990, posteriormente ela tem sido citada como a espécie mais comumente isolada de plantios comerciais (Alfenas et al. 2015; Freitas et al. 2019).

Também encontrado em muitas regiões do globo, *Cylindrocladiella* apesar do número reduzido de espécies quando comparada a *Calonectria*, pode ser encontrada

também nos cinco continentes. Atualmente a Oceania e a Ásia são os locais de maior diversidade em números de espécies (22 e 15 espécies), porém algumas espécies estão restritas a determinadas localidades como por exemplo *Cylla. pseudoinfestans* em Madagascar, *Cylla. camelliae* e *Cylla. elegans* na África do Sul, *Cylla. hawaiiensis* no Havaí, *Cylla. stellanboschensis* no Canadá, *Cylla. brevistipitata, Cylla. humicola, Cylla.horticola, Cylla. lateralis, Cylla. reginae, Cylla. terrestris, Cylla. Tailandiaica, Cylla. longiphialidica,* e *Cylla. pseudocamelliae* na Tailândia. *Gliocephalotrichum* é somente encontrado em quatro continentes, ainda não há relato deste gênero na Europa, em número de espécies por país podemos observar que o gênero tem seu maior número nas Américas, apesar de ter sido relatado em 17 países ao longo do mundo. Atualmente o Brasil após o relato de três novas espécies de *Gliocephalotrichum* no país, representa a nação que mais possui espécies relatadas, logo na frente da Guatemala, que possui 4 espécies.

1.5 Diversidade de hospedeiros e seus sintomas causadas pelo grupo *Calonectria*-like

O primeiro relato deste *Calonectria* foi relacionado sob em folhas de *Magnolia grandiflora* em Daldini (Itália), posteriormente vinte e cinco anos após foi descrito como saprófita em ramos de *Gledtsia triacanthos* nos EUA (Lombard et al. 2010b), a partir daí o gênero começou a ser encontrado e relacionado como causador de inúmeras doenças em plantas. Atualmente espécies de *Calonectria* são relatadas principalmente causando manchas foliares, Lombard et al., (2010b) cita sintomas como podridão de raízes, *"damping-off"*, manchas foliares e cancro na haste, em que estes sintomas costumam aparecer com mais frequência em viveiros de mudas, ressaltando que o patógeno também tem sido bastante relatado em plantios florestais comerciais mais velhos resultando em intensa desfolha com diminuição de vigor da planta. A desfolha causada por *Calonectria* spp. pode

em plantios de materiais suscetíveis chegar a 80% das plantas com sintomas desta doença (Alfenas et al., 2009). Guimaraes et al., (2010) em comparação com podas cita que o patógeno pode reduzir em até 45% o incremento volumétrico de madeira em materiais de alta suscetibilidade.

Até 2010 *Calonectria* era citada como causando doenças em aproximadamente 30 famílias de plantas, no mesmo ano, Lombard et al., (2010b) compilando os dados em sua revisão, relatou que este número era aproximadamente 100 famílias chagando a 335 espécies de plantas, atualmente este número não se diferenciou muito, podemos encontrar as mesmas 100 famílias distribuídas em 345 espécies vegetais. Com isso é indicado que o gênero possui uma gama de hospedeiros de importância agrícola, florestal e horticultura, segundo Lombard et al., (2010b) o impacto desta doença nestes setores ainda não foi descrito.

Ao observarmos o número de hospedeiros que *Cylindrocladiella* possui, podemos constatar que o gênero infecta 40 famílias de plantas em 85 espécies diferentes, neste gênero 30% dos isolados obtidos para estudo vem de amostras de solo. Ao longo dos anos *Cylindrocladiella* vem sendo relacionada a sintomas como: manchas foliares, podridão de frutos, podridão de raízes, morte descendente e mais frequentemente associadas a doenças no sistema radicular (Pham et al. 2018). Diferentemente de *Calonectria*, *Cylindrocladiella* possui espécies que não foram identificadas tendo nenhum hospedeiro, exclusivamente encontradas em solo até o momento, como é o caso de *Cylla. lanceolata, Cylla. longiphialidica, Cylla. clavata, Cylla. peruviana* e *Cylla. pseudocamelliae*.

Tabela 2: Famílias e número de espécies vegetais que são citadas como hospedeiras de Calonectria.

Família	Nº. sp.	Família	Nº. sp.	Família	Nº. sp.
Actinidiaceae	2	Dipterocarpaceae	1	Pinaceae	21
Altingiaceae	1	dryopteridaceae	2	Piperaceae	1
Anacardiaceae	3	Ebenaceae	1	Platanaceae	1

Annonaceae	4	Ericaceae	19	Phumbaginaceae	1
Aparagaceae	1	Euphorbiaceae	6	Poaceae	6
Apiaceae	1	Fabaceae	59	Polygalaceae	1
Apocynaceae	2	Fagaceae	4	Polygonaceae	3
Aquifoliaceae	4	Ginkgoaceae	1	Polypodiaceae	1
Araceae	5	Jiglandaceae	2	Proteaceae	7
Araliaceae	2	Lauraceae	6	Pteridaceae	1
Arecaceae	21	Laxmanniaceae	1	Rhamnaceae	1
Araucariaceae	2	lecythidaceae	1	Rhizophoraceae	1
Aspleniaceae	1	leeaceae	1	Rosaceae	11
Asteraceae	5	linaceae	1	Rubiaceae	2
Berberidadeae	2	lamariopsidaceae	1	Ruscaceae	1
Betulaceae	1	lythraceae	1	Rutaceae	3
Bixaceae	1	Magnoliaceae	2	Salicaceae	3
Bromeliaceae	3	Malpighiaceae	2	Sapindaceae	4
Buxaceae	1	Malvaceae	6	Sapotaceae	3
Caricaceae	2	Meliaceae	2	Sarraceniaceae	1
Caryophyllaceae	1	Moraceae	2	Saxifragaceae	1
Celastraceae	1	Musaceae	2	Solanaceae	4
Chenopodiaceae	1	Myristicaceae	1	Sterculiaceae	2
Combretaceae	3	Myrsinaceae	1	Strelilziaceae	2
Convolvulaceae	1	Myrtaceae	36	Theaceae	1
Cornaceae	1	Nelumbonaceae	1	Ulmaceae	1
Crassulaceae	1	Nepenthaceae	1	Verbenaceae	1
Cupressaceae	4	Nothofagaceae	1	Vitaceae	2
Cucurbitaceae	3	Nymphaeaceae	1	Vochysiaceae	1
Cycadaceae	1	Oleaceae	1	Xanthorrhoeaceae	1
Davalliaceae	1	Onagraceae	2	Zingberaceae	2
Dennstaedtiaceae	1	Orchidaceae	1		
Dilleniaceae	1	Phytolaccaceae	1		

Tanto *Calonectria* e *Cylindrocladiella* são relacionados majoritariamente a espécies das famílias Fabaceae, Myrtaceae, Pinaceae e Arecaceae, estas famílias totalizam 30% do total de espécies hospedeiras de *Cylindrocladiella* e 39% para *Calonectria*, indicando uma preferência destes gêneros de fitopatógenos por hospedeiros destas famílias. Para *Glyocephalotrichum* e *Gliocladiopsis* a variação de hospedeiros é muito menor, o primeiro pode ser encontrado como parasita em 10 diferentes famílias e o segundo em 8. Assim com

Calonectria e *Cylindrocladiella*, *Glyocephalotrichum* e *Gliocladiopsis* também apresentam gêneros hospedeiros dentro das famílias Myrtaceae e Arecaceae.

Como é possível observar os gêneros dentro de *Calonectria*-like em muitos casos podem ser considerados como polífagos, e com uma certa tendência a parasitarem plantas das famílias Fabaceae, Myrtaceae, Pinaceae e Arecaceae. Do lado do patógeno, exclusivamente do gênero *Calonectria* é observado muitas espécies encontradas parasitando plantas da família Myrtaceae, um total de 55%, bem como tendo espécies dos 11 complexos de espécies já relatados parasitando algumas espécies desta família de plantas, tornando Myrtaceae a única família de plantas que hospedeira dos 11 complexos de espécies.

Acompanhando pelo lado do patógeno, é possível inferir que *Ca. kyotensis* e *Ca. cylindrospora* são os complexos de espécies mais polífagos do gênero, conseguindo infectar 116 e 85 gêneros de plantas distribuídos em 53 e 45 famílias, respectivamente. Em uma classificação do mais para o menos polífago, podemos ter o seguinte posicionamento: em primeiro, *Ca. cylindrospora* (71 gêneros e 38 famílias), em segundo *Ca. ilicicola* e *Ca. kyotensis* (61 gêneros e 31 famílias cada) e em terceiro *Ca. reteaudii* (40 gêneros e 24 famílias). Cerca de 31% espécies de *Calonectria* até o momento foram encontradas somente parasitando plantas da Família Myrtaceae, outras famílias também têm esta relação específica com algumas espécies do gênero, no entanto o número de ocorrência é muito menor que na Família Myrtaceae. Tal indicação de preferência dos gêneros dentro de *Calonectria*-like por determinadas famílias ou gêneros de hospedeiros, abre hipóteses de relações cofilogenéticas ainda não exploradas.

Tabela 3: Famílias e número de espécies vegetais que são citadas como hospedeiras de Cylindrocladiella.

Família	Nº. sp.	Família	Nº. sp.	Família	Nº. sp.
Adoxaceae	1	Fabaceae	8	Primulaceae	1
Anacardiaceae	2	Fagaceae	2	Proteaceae	4
Annonaceae	2	Geraniaceae	1	Rhizophoraceae	1

Araceae	5	Juglandaceae	1	Rosaceae	4
Arecaceae	7	Lauraceae	2	Rubiaceae	1
Brassicaceae	1	Liliaceae	1	Rutaceae	1
Canellaceae	1	-	-	Sapindaceae	1
Celastraceae	1	Malvaceae	1	Sapotaceae	1
Clusiaceae	1	Meliaceae	1	Saxifragaceae	1
Crassulaceae	1	Moraceae	1	Solanaceae	1
Cupressaceae	1	Myristicaceae	1	Theaceae	3
Dilleniaceae	1	Myrtaceae	8	Vitaceae	3
Ericaceae	2	Pinaceae	5		
Euphorbiaceae	2	Polygonaceae	2		

Apesar dos dados levantados aqui serem substanciais, em comparação com a história destes gêneros, a informação sobre hospedeiros, localização e caracterização da doença, bem como a confirmação destes gêneros como causador da doença, acaba sendo escassa e pobremente documentada e em alguns casos erroneamente registrada. Tais ausências ou erros de registro de informação torna trabalhos de diversidade, cofilogenia e o posicionamento histórico geográfico de espécies mais complexo. Drenkhan et al., (2020) com o objetivo de determinar a disponibilidade de dados históricos globalmente elaborando modelos de predição de doença e uma lista de hospedeiros suscetíveis a *Fusarium circinatum* levantou extensos dados em diversos bancos de dados, no entanto ele relata que apesar de globalmente conhecido, os dados encontrados em muitos países são escassos, com anotações erradas ou pobremente documentadas, dificultando a criação de modelos e compreensão ampla dos hospedeiros.

Trabalhos de cofilogenia pode mostrar eventos históricos que aconteceram ao longo da história evolutiva de fungos e plantas, ajudando a explicar hábitos e preferencias de alguns fungos fitopatogênicos. Um exemplo disto é o estudo de Refrégier et al., (2008) em que ao analisa a cofilogenia de alguns basidiomicetos e seus hospedeiros da família Caryophyllaceae pode observar eventos de salto de hospedeiros como um importante

evento na especiação deste tipo de fungo, com este salto possivelmente sendo dependente de alguns fatores como a ecologia, geografia do hospedeiro e especializações genéticas.

1.6 Relação patógeno/hospedeiro

Devido a quantidade de hospedeiros associado as espécies de *Calonectria*-like, é surpreendente que as interações entre os patógenos e os hospedeiros ainda não tenham sido estudadas a fundo. No entanto existem alguns trabalhos para *Calonectria* que buscaram elucidar a relação patógeno/hospedeiro em dois patossistemas, *Ca. pseudoreteuadii – Eucalytpus* sp. e *Ca. henricotiae/ Ca. pseudonaviculata – Buxus sempervirens.* No primeiro patossistema os autores do estudo propuseram um modelo representando o modo de ação de *Ca. pseudoreteaudii* com base na observação dos transcritos obtidos 12, 24 e 48 horas após a inoculação em folhas de *Eucalyptus*.

A infecção por *Calonectria* começa com a deposição de um esporo viável em um hospedeiro suscetível, e condições de temperatura e umidade ideais para a germinação do esporo. O conídio sob a superfície folear pode germinar entre 6-8h, gerando de um a dois tubos germinativos, podendo germinar por ambos as pontas do conídio (Graça et al. 2007; Ye et al. 2017). Estudos mostram que a penetração ocorre por estômatos, e em maior frequência na face abaxial em folhas novas ou velhas, na face adaxial apesar de haver adesão do esporo não foi comprovada a infecção apesar de haver germinação (Graça et al. 2007). Em *Ca. pseudoreteaudii* os conídios germinado começam a se ramificar de 5 a 8 horas após a inoculação (hpi) e em torno de 12 hpi ao encontrar o estômato o fungo penetra e ocorre uma expansão da hifa ocupando toda a cavidade do estômato, em seguida é produzida uma hifa que invade o limbo foliar (Ye et al. 2017; Guo et al. 2020). Em estômatos fechado no momento da penetração, Graça et al., (2007) observou que o tubo germinativo estava intumescido, semelhante a um apressório.

A análise temporal de Ye et al., (2017) não apresenta diferença estatística entre os genes super expressos nos três tempos após infecção, demonstrando que os genes acessados durante as primeiras 48 hpi estão envolvidos na infecção/colonização do hospedeiro. A primeira barreira da linha de defesa da planta contra a entrada de patógenos é a estrutural (mecanismo de resistência pré-formada), entre estas estruturas estão a cutícula, estômatos, parece celular e lamela média (Amorim et al. 2018), tais estruturas precisam ser primeiramente ultrapassadas para o sucesso da colonização por *Calonectria.* Como estratégia de ataque, durante as horas iniciais da infecção (12 hpi) é possível observar uma super expressão do gene que codifica a enzima cutinase, molécula crucial na penetração do fungo devido a degradação da cutina. No genoma de *Ca. pseudoreteaudii* foram encontrados mais genes que codificam cutinase que em fungos como *Fusarium, Colletotrichum, Neurospora, Botritys, Sclerotinia* e *Ustilago*, este mesmo gene também já foi descrito nas espécies *Ca. henricotiae* e *Ca. pseudonaviculata* (Ye et al. 2017, 2018; Rogers et al. 2022). Do lado do hospedeiro, já foi demonstrado que cutículas mais espessas são encontradas em genótipos resistentes de *Eucalyptus* e considerado como um index importante para a espécie contra a infecção por *Calonectria* (Ye et al. 2017).

A partir das 24 hpi, Ye et al., (2017) identificou uma super expressão do gene que codifica endopolygalacturonase (endo-PG), este gene é responsável por hidrolisar pectina, molécula constituinte da lamela média de plantas. Também foi identificado pelos autores a expressão de genes envolvidos na degradação de celulose e hemicelulose (beta-galactosidase e beta-glocosidase) além de alta expressão de gene codificador de feruloil esterase que participam na redução de pectinas e xilanas presentes na lignina e celulose. Todas estas três classes de genes participam diretamente na degradação da parede celular, permitindo o sucesso da colonização. Tais genes são relatados tanto no genoma de *Ca. pseudoreteaudii, Ca. henricotiae* e *Ca. pseudonaviculata,* em comparação Rogers

et al., (2022) observaram que somente os fungos causadores de mancha foliar em *B. sempervirens* possuem uma contração dos genes que codificam genes responsáveis por codificar proteínas relacionadas a degradação de parede celular. Tal contração poderia indicar uma coevolução com seus hospedeiros, as duas espécies são relatadas somente como patógenos de plantas dentro da família *Buxaceae*. No entanto, o mesmo, não foi observado para *Ca. leucothoe* e *Ca. pseudoreteaudii* apesar de serem restritos a um número limitado de hospedeiros a contração de genes envolvidos na patogenicidade não foi observado (Rogers et al., 2022).

Na interação *Ca. pseudoreteaudii* x *Eucalyptus* ao analisar o secretoma extraído do isolado YA51, foi apontado a presença de 1.178 proteínas secretadas indicando possíveis efetores (Ye et al. 2018) Efetores são proteínas secretadas que desenvolvem essencial papel durante o processo de patogênese, seja por degradar parede celular e facilitar a infecção e penetração, ou também manipular o a célula do hospedeiro para promover a infecção(Lo Presti et al. 2015; Ye et al. 2018). Ye et al. (2018) ressalta que os possíveis efetores encontrados, são significativamente enriquecidos em atividade como: hidrolases, proteólises, dehidrogenase acetilmuramate, celulase, peroxidase e catabolismo de macromoléculas da parede celular (Ye et al. 2018). No genoma do hospedeiro é dito que existem grande número de genes que codificam terpenos, genes responsáveis pela regulação de fenilpropanoides como compostos participantes da resposta de defesa a infecção, o que olhando pelo lado de *Ca. pseudoretaudii* fez com que se desenvolvesse um efetivo sistema de detoxificação em resposta a estas moléculas (Ye et al. 2018). No patossistema *Calonectria* x *B. sempervirens,* ao analisar espécies de *Ca. henricotiae* e *Ca. pseudonaviculata* foi encontrado 630 proteínas preditas como efetores (Yang et al. 2021). Além dos putativos efetores identificados com função documentada em outros

patossistemas, outros 120 permanecem sem a identificação dele no desenvolvimento do patossistema (Yang et al. 2021).

Para ambas as espécies do patossistema *Calonectria* x *B. sempervirens,* efetores como elicitores de resposta a patogênese e relacionado a virulência estão relacionados a um único cluster gênico, um segundo cluster apresenta proteínas de parede celular *PhiA* e seus homólogos conhecidos por desempenhar papel na formação do conídio e de resposta ao estresse, também identificado dois clusters de cutinases, homólogos de corismato mutase, guanil ribonuclease, lipase entre outras (Yang et al. 2021). Apesar de ser encontrado tais homólogos de efetores, eles ainda precisam ser mais bem estudados no futuro para validações quanto a sua função na patogênese. Outros dois patossistemas foram estudados em 2022: *Ca. ilicicola* x *Arachis hypogeae* (Chen et al. 2022) e *Ca. hawksworthii* x *Persea americana* (Salgado-Salazar et al. 2022), no primeiro foi relatado 122 proteínas como putativos efetores e no segundo 80 proteínas das 677 preditas como proteínas secretadas foram identificadas como efetores (Chen et al. 2022; Salgado-Salazar et al. 2022). Entre os 80 efetores encontrados em *Ca. hawksworthii* Salgado-Salazar et al. (2022) identificou que o genoma apresenta duas vezes mais proteases que outras espécies em Nectriaceae e proteínas como Serina peptidases e metalopeptidases também são mais abundantes nesta espécie, correlacionando este fatoro com a associação destas moléculas na reposta de defesa do fungo contra os peptídeos produzidos pelo hospedeiro durante a infecção. Enzimas auxiliares identificadas no proteoma de *Ca. hawksworthii* com função lignocelulase, glucoolisacarideo oxidadese podem estar desenvolvendo papel no processo de infecção, invasão, formação de lesão e expansão do patógeno dentro da planta, além da detoxificação de compostos lignocelulosicos (Salgado-Salazar et al. 2022).

Uma vez reconhecido os fatores de virulência e qual o arsenal o fitopatógeno usa para causar doenças em seus hospedeiros é importante olhar pelo lado do hospedeiro, qual

é seu arsenal de defesa. De forma geral a resistência genética é a mais efetiva e mais barato método, no entanto este método necessita de conhecimento prévio de cultivares resistentes ou de fatores genéticos que só ou em combinação agem como fatores de resistência (Alfenas et al. 2009; Zarpelon et al. 2015).Entre os patossistemas em que *Calonectria*-like faz parte somente o *Calonectria* x *Eucalyptus* foi abordado até o momento. Dentro disso Zarpelon et al. (2015) investigou a arquitetura das QTL's envolvidas na resistência de *Eucalyptus* sp. à *Ca. pteridis.* O primeiro ponto relatado pelos autores é quanto a relação desfolha/reação observada, em que os indivíduos mais resistentes apresentaram em 3,2% e os mais suscetíveis 81,4% de desfolha (Clone resistente = *E. grandis*, clone suscetível = *E. grandis*×*E. urophylla*). Então foram encontrados cinco QTLs segregando com relação a intensidade de desfolha, três em parentais de *E. urophylla* EU11 (Rd1 e Rd2 no Grupo de Ligação 1 – LG1, Rd4 no LG6) explicando 22, 15 e 17% da variação fenotípica observada; e duas QTLs em parentais de *E. camaldulensis* EC06 (Rd3 no LG4 e Rd5 no LG8) explicando 16 e 49% da variação fenotípica (Zarpelon et al. 2015). Os autores ainda citam que apesar das poucas QTLs explicando uma parte substancial da resistência *Calonectria* em *Eucalyptus* pode estar envolvida somente a alguns locus de efeito relativamente importantes (Zarpelon et al. 2015). Os trabalhos levando em consideração os cinco patossistemas anteriormente citados, representam o pontapé inicial, mas que ainda existe um gap no conhecimento da patogêneses deste fungo, seja na interação com *Arachys*, *Buxus, Eucalyptus* ou *Persea.* Estudos referenciando possíveis efetores e fazendo a correta anotação provêm um repositório de potenciais novos efetores a serem analisados e validados no futuro

1.7 Identificação morfológica clássica e seus problemas

Por muitos anos, a identificação de fungos era feita por estritamente a partir de mensurações de caracteres visíveis, seja eles qualitativos (cor, forma, odor) ou quantitativos (comprimento, largura, número de septos etc.), ou seja, por caracteres fenológicos. Até o presente momento tal caracterização é feita como pré-requisito para a descrição de novas espécies ou identificação da mesma pelo ICBN (McNeill et al. 2005). Recentemente esta metodologia tem dado lugar a identificações observando o genótipo do fungo, a partir de fragmentos de DNA ou do sequenciamento genômico. Tais técnicas objetivam identificar espécies olhando para diferentes caracteres, a molecular analisando caracteres moleculares, com baixa taxa de variação e sem interação com o ambiente e a morfologia, que se trata de um caractere fenológico, resultante da interação da genética do organismo com o ambiente. Com isso características morfológicas são dependentes do meio de cultivo que estão sendo observados, podendo sofrer mudanças de acordo com a concentração de carbono/nitrogênio.

Os estudos usando caracteres fenológicos apesar de estarem limitados a descrições de novas espécies (Lombard et al., 2010), em centros educacionais e/ou centros de pesquisas com baixo recurso para investimento em técnicas moleculares, optam por esta via, devido aos seus custos menores.

Um dos problemas de estudos morfológicos é quanto ao conhecimento de qual característica mensurar e como deve ser a preparação para tal identificação, estes problemas podem levar a identificações equivocadas e dados pobremente documentados. Até o presente momento somente uma literatura especializada nos gêneros *Calonectria*-like possui a discrição detalhada de cada caractere morfológico a ser tratado ao observar o trabalho de Crous (2002) podemos identificar 12 principais caracteres morfológicos, são eles: estipe, extensão da estipe, vesícula terminal, aparato conidiogênico, ramos férteis,

fiálides e conídios para forma assexual, peritécio, células da parede do peritécio, asco, ascósporos para a fase sexual e clamidósporos. Para cada um destes é necessário fazer mensurações quanto ao comprimento e largura, a forma, presença ou ausência e em alguns casos a coloração.

Em análise da morfologia Crous (2002) cita 12 parâmetros morfológicos a serem quantificados e qualificados para descrever espécies do grupo *Calonectria*-Like, tais caracteres foram selecionados de acordo com a estabilidade/frequência dentro do gênero. Com isso Crous (2002) recomenda o uso do meio de cultura sólido de ágar e contendo folhas de cravo (CLA - *Canation-Leaf Agar*), cultivando o fungo durante 7 dias em temperatura a 25ºC sob baixa luz ultravioleta, só após o período eram iniciadas as preparações micológicas, com atenção que somente eram retiradas estruturas que haviam crescido sob as folhas de cravo. Em 2009 Lombard et al. (2009) inicia os estudos de morfologia utilizando uma cultura monospórica crescida em MEA e ágar com nutrientes sintéticos (SNA, Nirenburg, 1981), retirando da primeira metodologia a necessidade de folhas de cravo, neste mesmo trabalho o autor recomenda a quantificação do crescimento dos isolados em meio de cultura sob diferentes temperaturas (6-36ºC) e no escuro, além da coloração do verso da colônia em placa Petri contendo 2% de extrato de malte com ágar (MEA) também crescido no escuro durante 7 dias e em temperatura de 24ºC classificando a cor com base em uma carta de cor (Rayner, 1970). A metodologia descrita por Lombard et al. (2009) tem sido utilizada até hoje nas novas descrições de espécies.

1.7.1 Características mensuradas

A partir das colônias crescida de acordo com a metodologia descrita por Lombard et al. (2009), o próximo passo é a elaboração de lâminas utilizando liquido de montagem transparente (não se usa corantes para montagem de lâminas para caracterização

morfológica devido a alteração da cor das estruturas, mascarando algumas características) para exames em microscópio, como os gêneros dentro de *Calonectria*-like são hialinos, é recomendado o uso de microscopia de contraste de interferência diferencial. Em consenso a comunidade científica especializada nestes gêneros adotou a mensuração de 30 repetições para cada parâmetro obedecendo a magnificação de 1.000x, para cada isolado analisado. Em aspectos amplos, os caracteres a serem mensurados são:

- **Aparato conidiogênico:** Representa o conjunto de ramos e fiálides existentes no conidióforo, este parâmetro é mensurado a largura e comprimento, em que a largura se inicia no septo onde é gerado a primeira ramificação e se estende até a ponta da última fiálide do lado esquerdo, uma segunda medida de largura é feita do mesmo ponto do primeiro septo até a ponta da última fiálide do lado direito, para a largura é mensurada da ponta a última fiálide do lado direito até a última do lado esquerdo (Figura 8, A);

- **Ramos férteis:** É o conjunto de ramos (ramificações) no conidióforo que dá origem as fiálides, dando a característica penicilada para estes gêneros. Os ramos devem ter sua largura e comprimento mensurados, e contado o número de ramos até a fiálide, o número de ramos é representado pela quantidade mais frequente e sua variação (Figura 8, B);

- **Fiálides:** Estas são as estruturas que comportam a célula conidiogênica que dá origem ao conídio, na ponta das fiálides fica a uma fina cicatriz onde o conídio é prezo durante a sua formação, em fungos como *Calonectria*-like esta cicatriz é indistinta e sem coloração. A fiálide deve ser mensurada em comprimento e largura, e sua forma distinguida, esta pode ser: Doliforme, reniforme, ampuliforme etc. (Figura 8, B);

- **Conídios:** Os conídios dos gêneros de *Calonectria*-like em sua maioria são cilíndricos (Conídios de *Gliocephalotrichum*, podem ser elipsoides, obovoides ou cilíndricos), podem ser curvados ou eretos, com as pontas arredondadas. Conídios de *Calonectria* podem ter um ou mais septos, enquanto de *Cylindrocladiella* e *Gliocladiopsis* possuem somente um, *Gliocladiopsis* possui conídios com septos ou asseptados, geralmente nestes gêneros não há uma constrição no septo, (Figura 8, C);

- **Vesícula terminal:** Muito importante na caracterização de espécies, a vesícula terminal é um forte marcador morfológico para gênero, e em alguns casos pode dar suporte na identificação de complexo de espécies em *Calonectria*. *Cylindrocladiella* possui vesículas de paredes delgadas e muito delicada, *Calonectria* e *Gliocephalotrichum*, possui uma septação logo abaixo da vesícula e tem paredes espessas, *Gliocladiopsis* não possui vesícula terminal. Este parâmetro é medido a largura e comprimento e a forma, podendo ser acicular, clavada, espatulada, elipsoidal, piriforme, esfaeropendunculada, obpiriforme, naviculada e globosa, podendo ter variações nestes formatos (Figura 8, D);

- **Extensão da estipe e estipe:** Este é um importante marcador morfológico de gênero, por se diferenciar em número dentro destes. *Gliocladiopsis* não possui extensão da estipe e estipe, *Gliocephalotrichum* possui de 2-6, *Calonectria* e *Cylindrocladiella* possui uma e em algumas espécies é encontrado extensões da estipe adicionais. A extensão da estipe é mensurada a largura e comprimento se iniciando no septo da primeira ramificação até a ponta da vesícula, a estipe se inicia no septo da última ramificação até o septo da vesícula terminal (Figura 8, E);

- **Ascósporos:** Estes são formados em número de 8 dentro dos ascos, de forma geral são muito semelhantes entre os gêneros. possuem um septo com constrição nele,

podem ser eretos a curvados e com pontas arredondadas. Desta forma são mensurados a largura e comprimento, contado o número por asco, número de septo, formato do ascósporo se ereto ou curvado e se há coloração (Figura 8, F);

- **Ascos:** São geralmente hialinos, com um pescoço longo, formado na base do peritécio. É mensurado a largura e comprimento, assim como verificado se possui coloração, e quantas túnicas são, além do tipo de deiscência (Figura 8, G);

- **Peritécio:** A estrutura que comporta todo o resultado do cruzamento entre dois matings complementares, com isto algumas espécies não são conhecidas esta fase por isso é relatada a presença e ausência desta fase, posteriormente se presente é qualificado observando o formato, coloração ao natural e quando exposto a KOH+, textura da parede celular, e se é formado solitariamente ou em agrupamentos (Figura 8, H);

- **Células da parede peritecial:** Mais internamente ao peritécio é observado qual a coloração das células, contada quantas camadas de células presente, é mensurado o diâmetro destas, anotado a textura e o formato, se angular, globosa e elipsoidal, sempre analisando do ostíolo para a base (Figura 8, H);

- **Clamidósporos:** Compreendem estruturas de resistência destes fungos, para *Gliocephalotrichum* é um marcador morfológico, desenvolvidos a partir do espessamento da parede celular de uma ou mais hifas, podem ser encontrados em agregados ou solitários. É medido a largura e comprimento, bem como anotado se em agregados ou solitários, terminal ou intercalar na hifa e quanto ao formato da célula que os compõe.

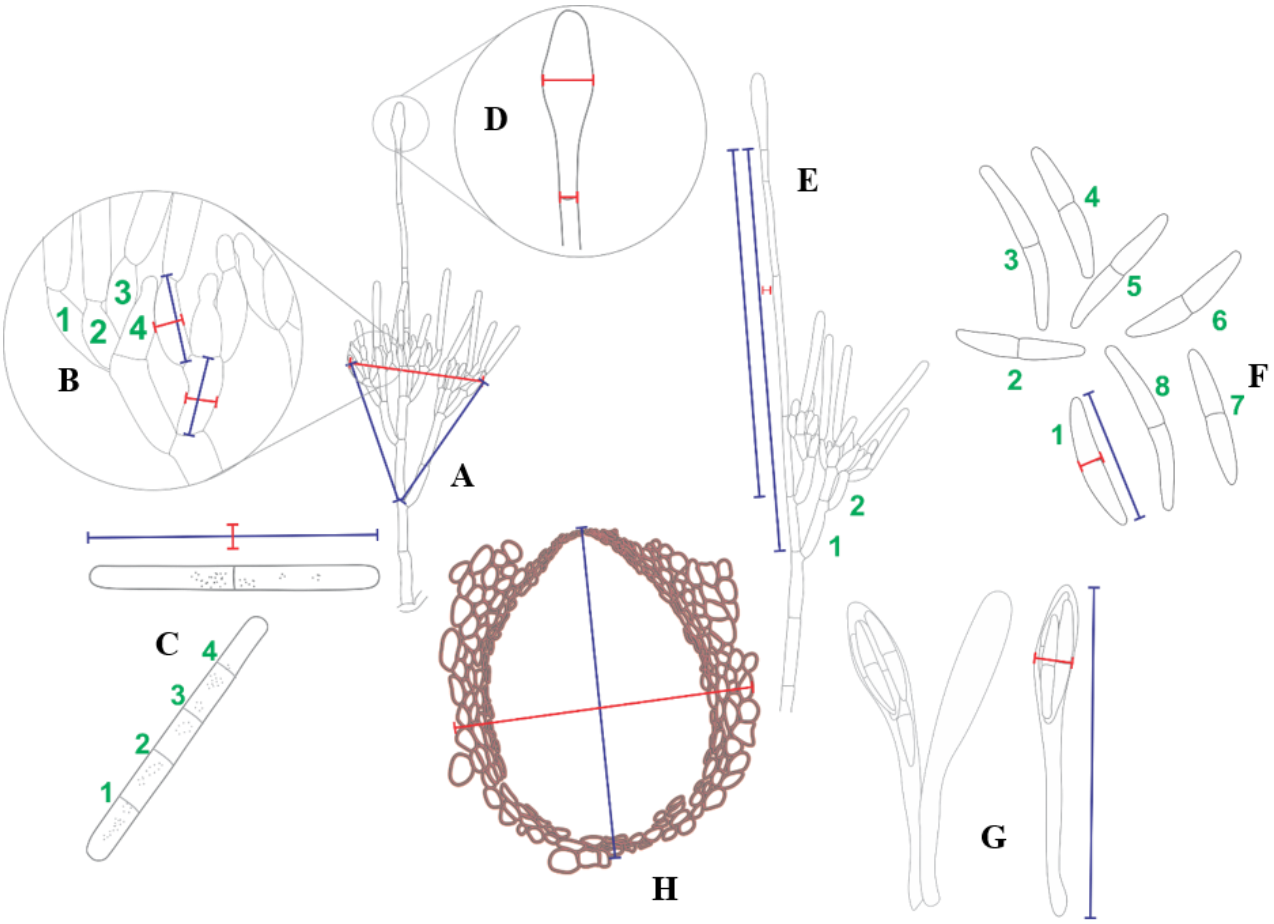

Figura 6: Esquema representando parâmetros morfológicos para caracterização morfológica de Calonectria, linhas azuis indicam largura, linhas vermelhas comprimento, número em verde representa a contagem dos parâmetros. (A) Aparato conidiogênico, (B) Fiálides e ramos, (C) Conídio, (D) Vesícula terminal, (E) Extensão da estipe e estipe, (F) ascósporos, (G) Ascos, (H) Peritécio

Alguns gêneros de *Calonectria*-like podem apresentar variações diferentes das principais anteriormente citadas, como a presença de macro ou microconidióforos em *Calonectria*, ou conidióforos subverticilados sem extensão da estipe em *Cylindrocladiella*, ou ainda vesículas terminais com paredes rugosas e curvada como em *Curvicladiella*, ou alongada e curva como em *Xenocilindrocladium*. Todas as medidas tomadas durante as análises são sempre relatadas observando um nível de confiança de 95% (Lombard et al. 2009), indicando os limites mínimos e máximos para cada parâmetro, as medidas que ficam fora do intervalo de confiança são tratadas como extremos, ficando indicados entre parênteses.

1.7.2 Dúvidas na interpretação de conceito morfológico de espécies

Embora a morfologia tenha desempenhado um papel importante na identificação de *Calonectria* e seus similares, muitas vezes essa identificação pode ser confusa, especialmente para fungos pleomórficos, que apresentam morfologias distintas em relação às conexões da fase sexual-assexual, e morfologicamente muito similares. Sobretudo estes problemas podem ocorrer se a metodologia usada para a preparação de lâminas microscópicas não está de acordo com o citado na metodologia. A exemplo disto foram as constantes realocações de espécies dentro de *Calonectria*-like.

Pesquisadores que não possuem experiência na identificação morfológica de *Calonectria*-like podem facilmente identificar erroneamente espécimes entre os gêneros, o principal motivo pode ser as similaridades morfológicas entre os gêneros. Um pesquisador que fizer uma lâmina de um isolado de *Calonectria, Cylindrocladiella* ou *Gliocephalotrichum* que esteja em meio de cultura a muito tempo, pode identificar aquelas estruturas que está vendo como sendo do gênero *Gliocladiopsis* devido a estes fungos perderem suas extensões da estipe em determinada idade. Outro exemplo são espécies do complexo *Ca. kyotensis* que podem apresentar mais que uma extensão da estipe nos seus conidióforos, confundindo o avaliador fazendo com que seja identificado com *Gliocephalotrichum* devido ao grande número de extensão da estipe que este possui.

Um exemplo clássico que pode ocorrer com observadores inexperientes é a identificação entre *Calonectria* e *Cylindrocladiella*, ambos possuem muitas características em comum, no entanto o segundo gênero pode ser identificado por seu tamanho diminuto, conídios com um septo, extensão da estipe asseptada, além de que a extensão da estipe

de *Calonectria* possui padrão de ramificação dicotômico enquanto *Cylindrocladiella* é verticilada (Pham et al. 2018).

A idade do fungo na placa, pode gerar também deformações a algumas estruturas dos gêneros, induzindo ao observador a cometer alguns equívocos, como por exemplo a germinação de esporos sem que estes estejam fora do conidióforo, ou a germinação da vesícula a qual pode ser confundida facilmente com as vesículas de *Cylindrocladiella* ou *Xenocylindrocladium*. As vesículas de algumas espécies do complexo *Ca. candelabrum* sob determinadas condições de cultivo e idade sofrem deformações facilmente, podendo formar dilatações na vesícula terminal (Figura 9).

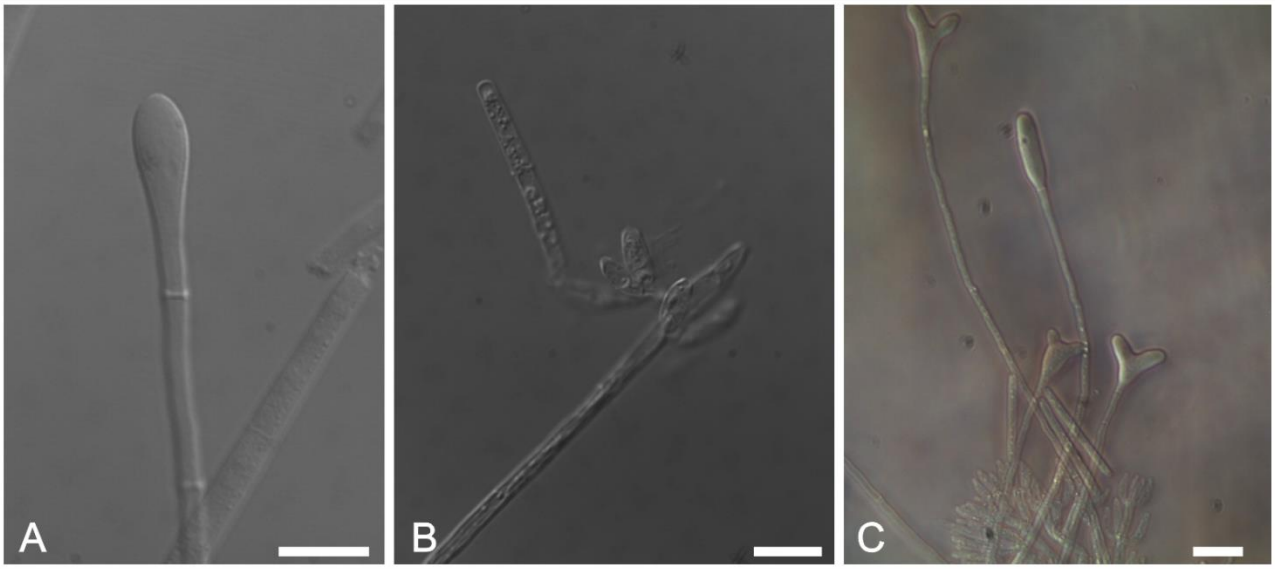

Figura 7: Deformações na vesícula terminal de Calonectria, devido a idade da cultura. (A) vesícula normal, (B) vesícula com germinada e com ramificações, (C) vesícula com dilatação. Escala 10µm.

Gêneros como *Curvicladiella* e *Xenocylindrocladium* apesar de mais raramente encontrados, podem ser confundidos com qualquer um dos gêneros anteriormente citados, devido às suas características similares. Principalmente quando se trata da observação da extensão da estipe e do final dela, onde comumente em *Calonectria* e *Cylindrocladiella* é encontrada uma vesícula, nestes não é encontrado, no entanto em *Calonectria* quando o conidióforo se encontra jovem a vesícula terminal pode estar totalmente formada, dando a impressão da sua ausência. Algumas espécies como as do complexo de espécie *Ca.*

brassicae, Ca. colhounii, Ca. gracilipes e *Ca. reteaudii* que possuem como vesícula terminal tipicamente clavada para levemente clavada (Liu et al. 2020)(Liu et al. 2020)(Liu et al. 2020)(Liu et al. 2020) também podem ser confundidas com espécimes dos gêneros *Curvicladiella* e *Xenocylindrocladium,* ou mesmo o contrário. Alguns pontos chaves podem ser usados para diferenciar e evitar confusões, o primeiro é quanto a formação de conidióforos, enquanto *Calonectria, Cylindrocladiella* e *Gliocephalotrichum* são formados individualmente, *Curvicladiella* e *Xenocylindrocladium* são encontrados em grupamentos. Outros dois chave e quanto a terminação de extensão da estipe, a de *Curvicladiella* termina torta para um dos lados em um ângulo agudo e na maioria dos espécimes possui rugosidade nesta região, já *Xenocylindrocladium* a terminação acaba de forma helicoidal e lisa (Lombard et al. 2015a)(Lombard et al. 2015a)(Lombard et al. 2015a)(Lombard et al. 2015a).

Aquanectria na sua forma anamórfica apesar de ser diferente da maioria dos gêneros, por ter seus conidióforos sem ramificações penicilados (Gordillo and Decock 2019b)(Gordillo and Decock 2019b)(Gordillo and Decock 2019b)(Gordillo and Decock 2019b), pode haver alguma confusão com o conidióforo secundário que é encontrado em algumas espécies de *Cylindrocladiella* (Lombard et al. 2012)(Lombard et al. 2012)(Lombard et al. 2012)(Lombard et al. 2012). Para diferenciar ambos os gêneros, basta olhar a forma dos conídios, conídios de *Cylindrocladiella* são todos cilíndricos e septados, enquanto os de *Aquanectria* podem ser curvados para ligeiramente sigmoides (Gordillo and Decock 2019b)(Gordillo and Decock 2019b)(Gordillo and Decock 2019b)(Gordillo and Decock 2019b).

1.8 Identificação de *Calonectria* baseados na biologia molecular

Os trabalhos em biologia molecular baseados em sequências, se iniciaram em 1997 com o emprego de sequencias do gene que codifica o 5.8S do RNA ribossomal e parte que espaçador interno do ribossomo (ITS) para distinguir isolados de *Cylindrocladium scoparium* e *Cylindrocladium floridanum* (Jeng et al. 1997). Posteriormente, foi usado parte do gene de *beta-tubulina* juntamente com ITS para distinguir espécies dentro de dois complexos de espécies (Crous et al. 1999). Nestes dois trabalhos foi observado que sequencias da região ITS continham pouca informação importante a análises, por ser uma

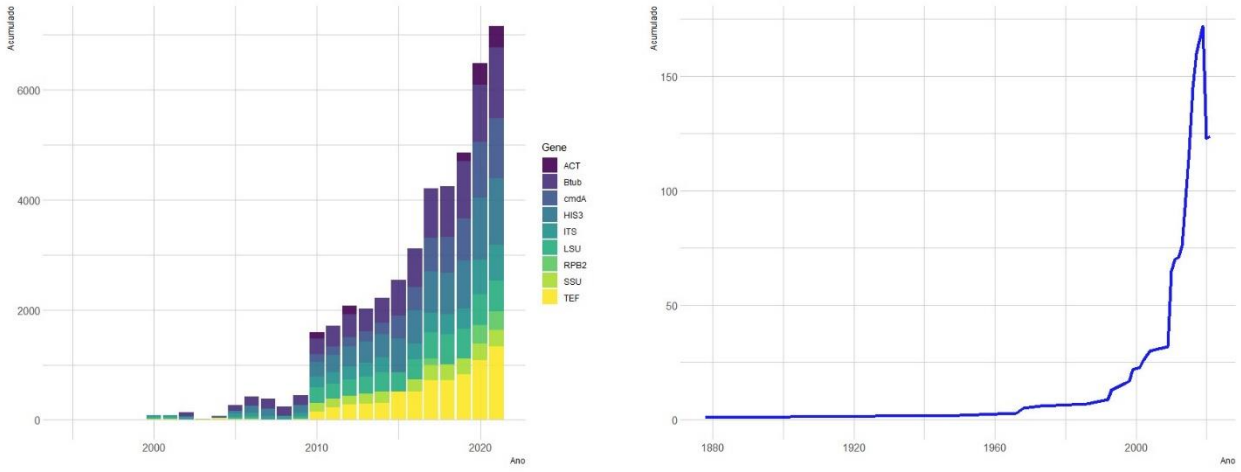

Figura 8: Número de sequencias nucleotídicas depositadas na plataforma do GenBank por ano (esquerda) e número de novas espécies de Calonectria relatadas por ano até 2021

região muito conservada, indicando que *beta-tubulina* proporcionava melhor resolução na separação de espécies de *Calonectria*.

Um ano após os estudos de Crous (1999) usando dois marcadores genéticos, Schoch et al. (2000) faz a separação dos gêneros dentro de *Calonectria-like* também baseado em filogenia, este foi o marco para gêneros como *Cylindrocladiella* que até o momento havia dúvida se era válido como gênero. A análise filogenética do gênero *Calonectria* só foi elaborada em 2001, considerado assim como o primeiro estudo do tipo a

unir à esta análise morfológica para definição de grupos (Schoch et al. 2001 e Crous, 2002). Com o objetivo de aumentar a resolução de análises filogenéticas em 2001 foi inserido mais um marcador molecular, a Histona H3 (Kang et al. 2001), posteriormente foi adicionado Fator de Elongação 1-*alpha* em 2004 (Lombard et al. 2004) e somente em 2010 foi introduzido nas análises filogenéticas do gênero *Calonectria* (Lombard et al., 2010).

Mas com o avanço das técnicas moleculares, a identificação com base em sequências de DNA tem revolucionado a taxonomia desse grupo de fungos, com a descrição de várias novas espécies morfologicamente semelhantes (Lombard et al., 2010; Lombard et al., 2012; Alfenas et al., 2015; Lopes et al., 2018). Além disso, com base na análise de sequências de DNA foi possível observar que as espécies de *Cylindrocladium* tem conexão com *Calonectria*, provando a conexão das diferentes fases não sendo necessário usar nomes distintos, pois trata-se de um mesmo organismo (Wingfield et al., 2012).

Lombard et al., (2010) cita que no ano de publicação da sua revisão haviam 734 sequencias disponíveis nos bancos de dados de *Calonectria* e *Clyindrocladium*, este número atualmente se encontra na casa dos 8 mil aumentando em mais de 1000% em apenas 11 anos, considerando assim sequencias de *Mating-type* entre outras, das regiões utilizadas para filogenia, iremos ter: 388 sequencias de Actina, 1.292 de *beta-tubulina Tub2*, 1.089 de Calmodulina *Cmda*, 1.216 de Histonas H3 *His3*, 649 para ITS, 342 para RNA polimerase II *rpb2*, 1.331 pra Fator de Elongação 1-*alpha tef1* (Figura 10).

Analisando a quantidade de sequencias hoje disponíveis no GenBank (NCBI) é possível destacar uma explosão de estudo utilizando sequencias de DNA em 2010, um outro aumento substancial após 2015 e um terceiro em 2020. Estes tempos de aumento grande do número de sequencias está relacionado aos principais trabalhos publicados neste tempo, em 2010 por exemplo temos, quatro dos mais importantes trabalhos

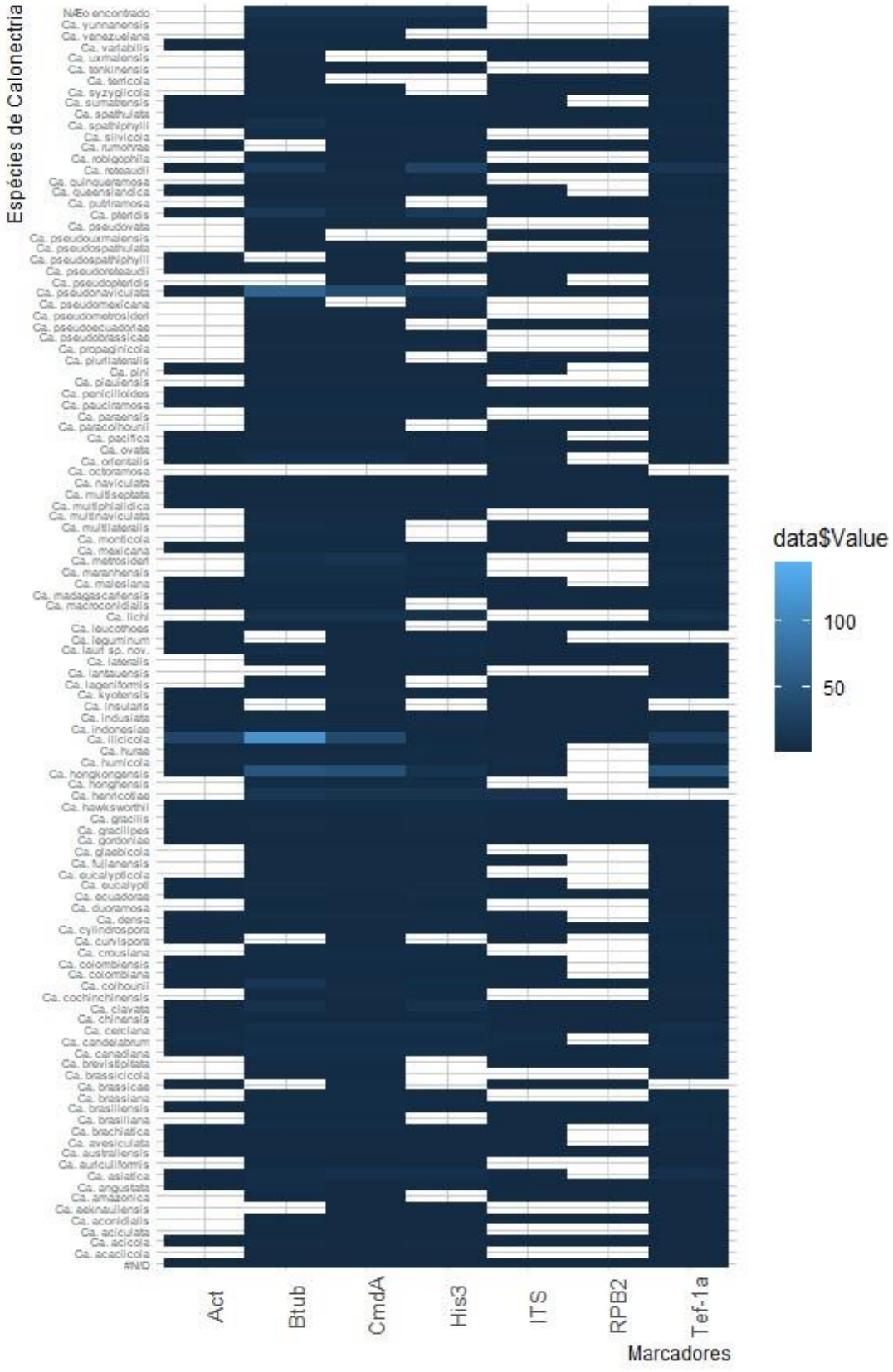

Figura 9: Heat map do número de sequencias depositadas no GenBank de cada marcador dentro das espécies de Calonectria até 2021.

publicados para o gênero *Calonectria* desde 2002, todos publicados em uma única edição da Studies in Mycology nº66. Em 2015 foi publicado o trabalho de Alfenas et al. (2015) em que sequencias para 1.017 isolados e mais recentemente em outubro de 2020 Liu et al. (2020) publicou seu trabalho analisando e gerando novas sequencias para os tipos de 128 espécies.

Observando o número de sequencias depositadas por estes trabalhos versus o número de espécies aceitas no gênero, é possível constatar que o impacto também é grande, podemos ver que nas duas primeiras datas anteriormente citadas o número de novas espécies saltou bruscamente, fazendo com que o número aumentasse. Apesar deste número de sequência estarem diretamente ligados ao número de espécies descritas, este aumento acabou sendo desuniforme entre as regiões genéticas analisadas, causando uma ausência de informação em alguns estudos, Liu et al. (2020) cita que esta ausência de informação está presente principalmente em genes como *act, His3* e *rpb2* (Figura 11).

A uniformidade na quantidade de genes analisados para descrever uma espécie de *Calonectria* foi questionada devido a desuniformidade nos números nos bancos de dados. Liu et al. (2020) cita que, em um patógeno com tamanha relevância a falta de padronização para descrição de espécies com base em filogenias abre perguntas quanto a real identificação impactando na diagnose de fitopatógenos e seu manejo correto. Com o intuito de verificar o posicionamento filogenético das espécies de *Calonectria* Liu et al. (2020) gerou as sequencias faltantes para 128 espécies do gênero e utilizando sequencias das outras 48 espécies que não teve acesso, o autor reduziu para 120 espécies o gênero.

Esta redução substancial de espécies aceitas segue o padrão contrário do observado nos anos de 2010 e 2015 quando relacionamos o número de sequencias depositadas ao número de espécies (Figura 10), em 2020 número de sequencias aumentou em 25% em relação ao ano anterior, no entanto o número de sequencias aceitas diminuiu 27%. Para a decisão de retirar o status de espécie de algumas, Liu et al. (2020) usou a

concordância de múltiplas genealogias de genes baseados em dois critérios: (*i*) isolados que se diferenciam em uma linhagem diferente de outros isolados a qual se repete em ao menos duas das oito regiões analisadas e não contraditas por outros locus e (*ii*) quando linhagens independentes são suportadas por altos valores de bootstraps em filogenias concatenadas com os oitos locus e apresentando Polimorfismos de único nucleotídeo (SNPs - *Single Nucleotide Polymorphisms*) o diferenciando das outras linhagens.

Apesar de Liu et al. (2020) ter diminuído o número de espécies para 120 em seu trabalho, nota-se que o autor deixou de analisar três espécies *Ca. hemileiae, Ca. matogrossensis* e *Ca. vietnamensis*, subsequente a este trabalho foi publicado mais uma espécie *Ca. Singapuransis (Crous et al. 2021)* baseado no estudo de seis diferentes marcadores e a comparação morfológica. Os seis marcadores utilizados para descrever a nova espécie de *Calonectria* foi delimitado obedecendo os resultados obtidos por Liu et al. (2020), o autor cita que *act, cmdA, his3, rpb2, tef1* e *tub2* podem servir como barcode para a delimitação de espécies de *Calonectria* devido a sua robusta informação filogenética. Liu et al. (2020) reporta que *tef1* e *tub2* são marcadores que fornecem informação suficiente para diferenciar a maioria das espécies indicando estes dois marcadores para identificações preliminares, o autor cita que *tef1* é um ótimo candidato a *barcode* para o gênero.

Observando os outros gêneros que compõe os *Calonectria-like*, notamos que a quantidade de sequencias depositadas no Genbank é muito menor, ficando *Cylindrocladiella* com 855, *Gliocephalotrichum* com 505 e *Gliocladiopsis* com 339 sequencias. Assim como em *Calonectria* notamos que existe uma falta de uniformidade nos números de sequência existente nos bancos de dados para estes gêneros (Figura 3.1). Lombard et al. (2014) relata que para *Gliophalotrichum tub2* é o marcador que fornece a melhor resolução para filogenia do gênero, seguido pelo *tef1* e *his3*, Silva et al. (2020) utilizou *tef1* como marcador inicial para segregar isolados deste gênero com posterior aplicação de estratégia de multigene o que demonstrou que *tef1* possuía o maior número

de sítios informativos a parcimônia seguida de *tub2* e *its*. As análises de Lombard et al. (2014) e Silva et al. (2020) foram feitas com 92% dos isolados tendo todos os quatro marcadores analisados, o que tornou a análise deste robusta devido a homogeneidade entre os marcadores.

Em *Gliocladiopsis* o uso de marcadores segue o mesmo caminho que os outros gêneros, ficando retidos dentro de quatro marcadores *his3, its, tef1* e *tub2*, Lombard e Crous (2012) relatam que analises individuais de sequencias de *its* e *tub2* só foram capazes de distinguir cinco linhagens das 11 analisadas, e que *lsu* não é um bom marcador para o gênero por agrupar todas as linhagens em um único grupo monofilético.

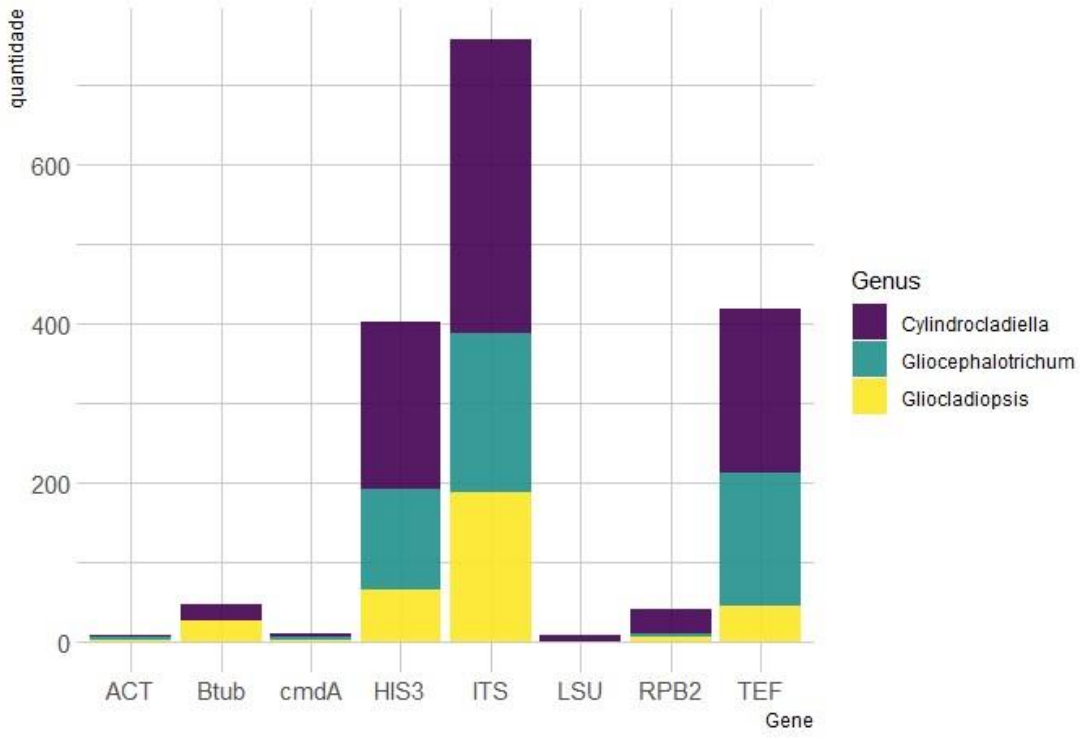

Figura 10: Quantidade de sequencias de nucleotídeos depositadas no GenBank para cada marcador dentro de Cylindrocladiella, Gliocephalotrichum e Gliocladiopsis

1.9 Variabilidade genética nos gêneros de *Calonectria*-like

Fungos em geral se reproduzem de duas principais formas em geral, de forma assexual (ciclo mitótico) e sexual (ciclo meiótico), nos gêneros de *Calonectria-like* ambos os ciclos podem ocorrer. O ciclo mitótico representa a reprodução dos fungos de forma clonal, onde não há troca de gametas podendo ser iniciado por impulsos ambientais ou fenológicos do fungo (Piperbring, 2015). Ciclo meiótico existe a necessidade de troca de gametas dentro de um único organismo quando este é homotálico ou distintos organismos quando em heterotalismo, tal reconhecimento se dá por complementariedade de *Mating-type* estes para desencadear o ciclo sexual precisam ser diferentes e complementares entre si (Wilken et al. 2017). Os complexos de espécies deste gênero possuem ao menos uma fase sexual descrita, para *Calonectria* existem 37 espécies com sua fase sexual relatada, cerca de 29%, entre os complexos de espécies deste gênero é possível reconhecer que *Ca. pteridis* e *Ca. naviculata* não possuem nenhuma de suas espécies com a fase sexual reconhecida (Liu et al., 2020). Para *Cylindrocladiella* a fase sexual é somente relatada para 1 espécies *Cylla. pseudinfestans*, para *Gliocephalotrichum* até o momento é conhecido somente as fases sexuais de *Glio. bulbilium* e *Glio. grande* e para *Gliocladiopsis* somente a espécie *G. pseudotenuis* é conhecida.

Em *Calonectria* é relatados os dois tipos de sistemas de *mating*, heterotálico e homotálico, mas raramente a fase sexual é vista na natureza ou em laboratório (Crous, 2002 e Lombard et al. 2010). Crous (2002) cita que o conceito biológico de espécie determina que isolados que conseguem cruzar e gerar abundante progênie fértil são componentes de uma mesma espécie, em sequência cita que estudos de cruzamento de interespecíficas em *Calonectria* podem gerar descendentes, mas com fertilidade baixa. No

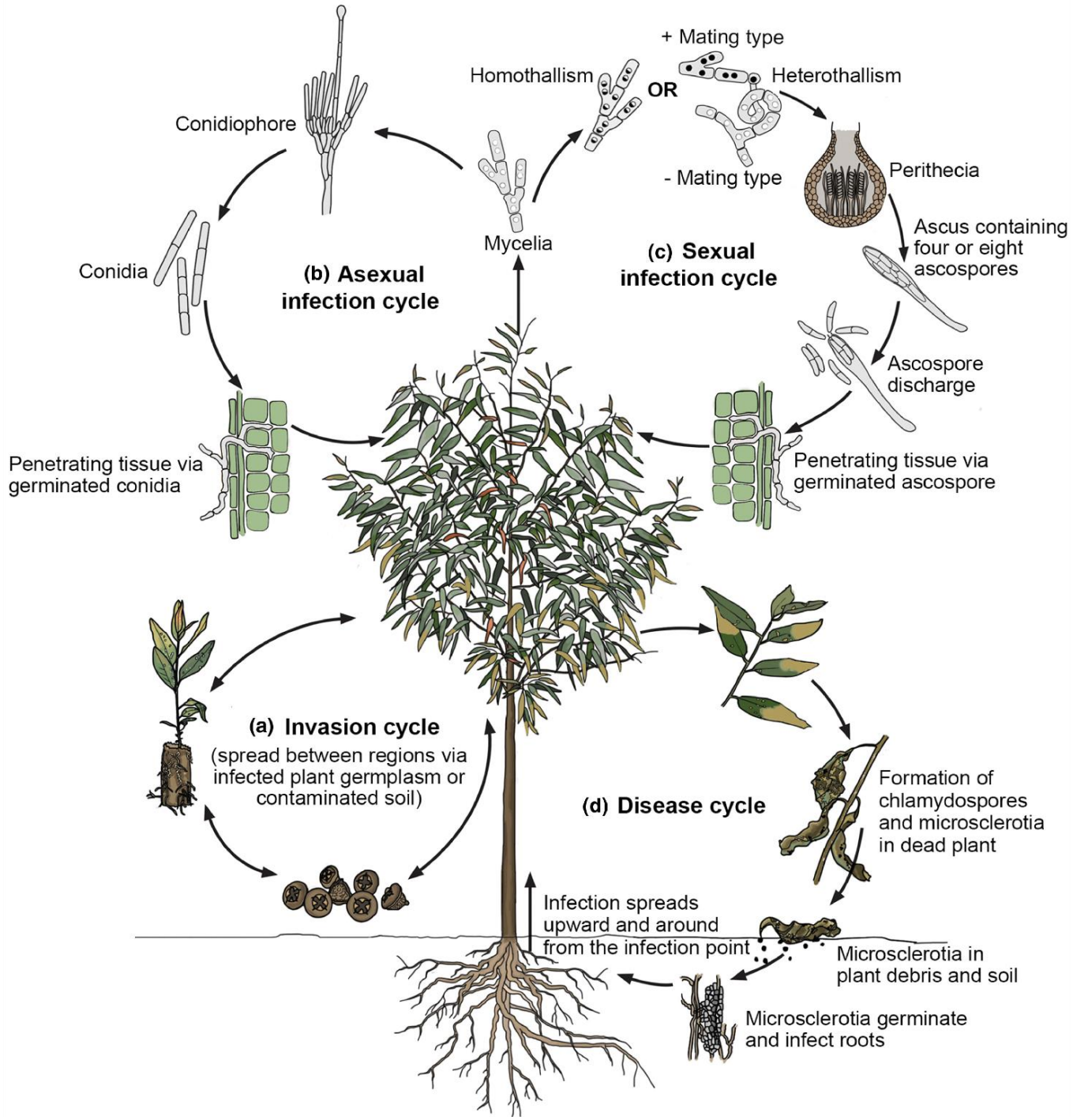

Figura 11: Provável ciclo de espécies de Calonectria. (a) Calonectria pode ser dispersa por plantas infectadas ou solo contaminado. (b) Após a infecção o patógeno pode germinar na forma de micélio e infectar plantas em condições ambientais favoráveis para o fungo. O micélio pode rapidamente iniciar a fase sexual formando um grande número de conidióforos em um curto período. (c) Sob condições desfavoráveis Calonectria pode entrar em seu ciclo sexual pela união de indivíduos com matings opostos e complementares (Heterotalismo) ou por autofertilização (homotalismo). Ascósporos haploides são formados e dispersados pelo vento ou carreados pelas gotas de chuva para penetrar novamente no tecido sadio de novos hospedeiros. (d) Infecções usualmente começam na base de uma árvore ou muda. Estruturas de resistência, microescleródios, podem ser encontrados sobrevivendo durante longos tempos em tecidos mortos e no solo (Crous, 2002; Phipps et al., 1976). Em condições ideais os microescleródios podem germinar infectando raízes e iniciando um novo ciclo. Li et al. 2022.

entanto a ausência de estruturas sexuais nestes gêneros não diz que eles não são capazes de se reproduzir sexualmente (Biliard et al. 2012).

Os estudos de compatibilidade sexual entre isolados eram recomendados em publicações até 2013, onde cada um dos isolados eram postos em lados opostos de uma placa de Petri contendo meio solido, a partido do crescimento e interação destes isolados, se compatíveis era possível observar a produção de peritécios, caso contrário as estruturas sexuais eram ausentes. Tal interação era anotada e os peritécios produzidos eram analisados morfologicamente. Com o crescimento das bases moleculares, descobriu-se que a compatibilidade sexual era regida por um conjunto de genes denominados *mating-types*.

Mating types em Pexixomicotina foi definido em 1990 a partir de estudo com *Neurospora crassa* e formalmente definido como um loci com uma única posição no genoma, responsável por controlar a reprodução sexual (Turgeon and Yoder 2000; Wilken et al. 2017). Por convenção *MAT*1 indica o lócus, porém não impede que exista mais de um loci identificado, também pode haver versões, chamadas de idiomorfos podendo haver mais de uma variação dentro da população. Em organismos heterotálicos estes idiomorfos estão em organismos diferentes, identificados como *MAT1-1* e *MAT1-2.* O lócus ainda pode ser flanqueado por pseudogenes o qual é anotado ao *mating type* adicionando um terceiro número no final, ficando *MAT1-2-3* (Turgeon and Yoder 2000; Billiard et al. 2012; Wilken et al. 2017; Malapi-Wight et al. 2019).

A reprodução sexual é um importante mecanismo de geração de diversidade, eliminação de mutações deletérias, assegurando a sobrevivência de espécies e fazendo a manutenção para que a população se mantenha saudável (Li et al. 2020). Em *Calonectria* este sistema que controla a reprodução sexual é bipolar possuindo um gene de *mating-type* (MAT) em um locus simples com duas formas não alélicas referidas como idiomorfo *MAT1-1* e *MAT1-2* (Wilken et al. 2017). Em organismos heterotálicos dois idiomorfos

complementares existem em diferentes isolados e em organismos homotálicos um único indivíduo possui os dois idiomorfos complementares, e pode completar o ciclo sexual sozinho (Li et al., 2020).

A partir da análise dos genomas das espécies: *Ca. henricotiae* CBS 138102*, Ca. leucothoes* CBS 109166*, Ca. naviculata* CBS 101121 e *Ca. pseudonaviculata* CBS 139394 Malapi-Wight et al., (2019) constatou que todos os quatro isolados analisados são heterotálicos, contendo somente o locus *MAT1* e dois idiomorfos. Para *Ca. naviculata* e *Ca. henricotiae* foram encontrados típicos arranjos do idiomorfo *MAT1-1* como: *MAT1-1-1, MAT1-1-2* e *MAT1-1-3* que possuem os domínios ligação α, PFF e HMG respectivamente,

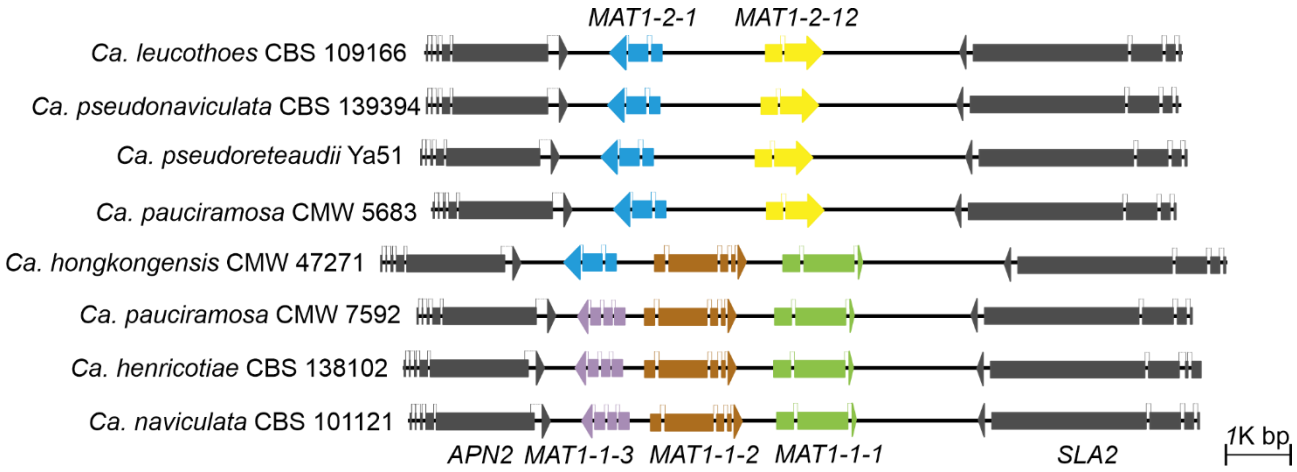

Figura 12. Mapa dos MAT's para Calonectria, indicando espécies homotálicas e heterotálicas

para *Ca. leucothoes* e *Ca. pseudonaviculata* foi observado o idiomorfo *MAT1-2* nas variações contendo o domínio de ligação HMG box (*MAT1-2-1*) e uma proteína de função não determinada (*MAT1-2-2*; Malapi-Wight et al., 2019). Este mesmo padrão foi encontrado ao analisar genomas de outras três espécies: *Ca. pseudoreteaudii* YA51*, Ca. pauciramosa* CMW 5683 e CMW 7592*, Ca. hongkongensis* CMW 47271, com a diferença que o *MAT1-2-2* foi renomeado para *MAT1-2-12* por passar a ser considerado um novo *MAT* somente encontrado em *Calonectria* (Liu et al. 2020 – Figura 12).

A partir da caracterização das variações dos locus responsáveis pela reprodução sexual em *Calonectria* usando os genomas, autores produziram primers específicos para identificar no genoma de outras espécies ou isolados qual estratégia de reprodução era usada. Em no trabalho de Malapi-Wight et al., (2019) foi desenhado dois pares de primers um para o idiomorfo *MAT1-1* e outro para *MAT1-2*, com base em 14 genomas das quatro espécies de *Calonectria* citadas anteriormente (Tabela 4). A partir destes primers o autor amplificou e sequenciou 268 isolados de *Ca. henricotiae* e *Ca. pseudonaviculata* em coletados em 15 países diferentes entre 1999 e 2014, o sequenciamento mostrou que os idiomorfos encontrados estão separados de acordo com a espécie, o idiomorfo *MAT1-1* só foi encontrado em *Ca. henricotiae* enquanto *MAT1-2* somente em *Ca. pseudonaviculata* (Malapi-Wight et al. 2019).

Tabela 4. Primers desenhados para os estudos de Mating type.

Gene	Primer	Sequência (5' - 3')	Fragmento (bp)	Referência
MAT1-1	Che_mat-1F	GCAAGGGATGAGGTTGGTAA	583	Malapi-Wight et al., 2019
	Che_mat-1R	ACTGTTCTCGGCCTCAGTGT		Malapi-Wight et al., 2019
MAT1-1-1	Cal_MAT111_F	ATGCTTCCTCAGTCTTTGCT	330	Li et al., 2020
	Cal_MAT111_R	CTTGAAYRGGGTTGGTGG		Li et al., 2020
MAT1-1-3	Cal_MAT113_F	CCTCCAGAAGTACCGACT	430	Li et al., 2020
	Cal_MAT113_R	GCTGTCGTTCTTCTTCCT		Li et al., 2020
MAT1-2	Cps_mat-2F	CCAATCCTTCTCCTGCTGAG	452	Malapi-Wight et al., 2019
	Cps_mat-2R	CGTCGTTGGAGTCATCATTG		Malapi-Wight et al., 2019
MAT1-2-1	Cal_MAT121_F	GCAAGGAYCGCCACCRAAT	240	Li et al., 2020
	Cal_MAT121_R	GACACCTCKGCGTTTCTTCTCAG		Li et al., 2020
MAT1-2-12	Cal_MAT1212_F	TCATCAGTTTCGCCCATT	670	Li et al., 2020
	Cal_MAT1212_R	CGTCGTACTTCTTCTTCCG		Li et al., 2020

O mapeamento dos idiomorfos para as duas espécies causadoras de Podridão do buxinho (Boxwood blight), mostrou que o *MAT1-1* é restrito somente a regiões da Europa e Reino Unido, e está em menor frequência na população de *Ca. henricotiae* e *Ca. pseudonaviculata* ficando restrito a 5 países (Malapi-Wight et al. 2019). A presença dos dois

idiomorfos em cinco países indica que os dois fungos podem estar coexistindo em sintonia por no mínimo uma década em países como Alemanha e Holanda, mas sem evidências de ambos infectando a mesma planta (Malapi-Wight et al. 2019). Os resultado de Malapi-Wight et al., (2019) corroboram com a hipótese levantada por LeBlanc et al., (2018) de que nos Estados Unidos não havia indícios de reprodução sexual naquela população e de que *Ca. pseudonaviculata* havia se dispersado de forma clonal nos mais de 20 estados Americanos, tudo isso devido a presença apenas de um *Mating* no país. Na Europa também não foram encontradas evidências de reprodução sexual para nenhuma das duas espécies indicando outro caminho para a manutenção de variabilidade genética (Malapi-Wight et al. 2019).

No trabalho de Li et al., (2020) foram desenhados quatro pares de primers para identificação de cada variação de idiomorfo (Tabela 4). A partir disto o autor amplificou as regiões para 123 isolados de 65 espécies de *Calonectria* encontrado que destes 21 espécies foram identificadas como homotálicas, 22 heterotálicas e outras 36 identificadas como heterotálicas por conterem somente um idiomorfo entre seus isolados (*MAT1-1-1* ou *MAT1-2-1*). Com base na comparação das sequências geradas no estudo, Li et al. (2020) relata que possivelmente o ancestral de *Calonectria* tenha sido heterotálico, tendo uma transição para homotalismo em dois pontos distintos na história evolutiva do gênero, uma na separação do complexo *Ca. colhounii* e outra na separação de *Ca. kyotensis*, neste último o heterotalismo permanece e em um pequeno grupo houve o retorno a este sistema mais recentemente.

A partir da reconstrução do ancestral comum, e da evidência do surgimento de dois pontos de transição para o homotalismo na história evolutiva de *Calonectria.* Li et al. (2020) levanta que esta transição pode ser explicada pelas hipóteses de que o homotalismo pode ter surgido por eventos de recombinação independentes (*Unequal Crossover*) ou que um ancestral homotálico que continha todos os *MAT* tenha mudado para heterotálico por dois eventos de deleção (Li et al. 2020). Em estudos de com o gênero *Neurospora* também foi

constatado que o ancestral comum do gênero era heterotálico, neste caso o autor relata que a transição de heterotálico para homotálico tem dois possíveis modelos um mediado por transposons e outro por recombinação (*Unequal Crossover*) assim como o levantado para *Calonectria*(Gioti et al. 2012; Li et al. 2020).

A hipótese de *unequal crossover* aponta quatro possíveis pontos de recombinação entre dois ancestrais heterotálicos, em três pontos levantados os recombinantes resultantes são heterotálicos o que pode justificar a maior presença deste padrão dentro do gênero (Figura 14 - a, b e c). Um possível ponto de recombinação é sugerido produzindo um recombinante contendo todos os genes *MAT* (Figura 14 – d). A hipótese alternativa ao *unequal crossover* é a de deleção de genes em um ancestral que possuía todos os genes no mesmo genoma, o que geraria somente dois recombinantes heterotálicos (Figura 14 – a', a'') e todo o resto homotálico (Figura 14 – b, c e d), mostrando que a primeira hipótese é mais plausível que a última (Li et al. 2020).

Curiosamente nos estudos de Malapi-Wight et al., (2019) foram encontrados *matings* opostos em duas espécies distintas e que coabitam o mesmo território em cinco países. Uma das hipóteses levantadas pelo autor para explicar a adaptabilidade e variabilidade genética de *Ca. henricotiae* e *Ca. pseudonaviculata* é que possa haver hibridização interespecífica entre eles. A hibridização interespecífica em *Calonectria* é citada como possível de acontecer entre espécies relacionadas filogeneticamente, porém a progênie possui baixos níveis de fertilidade (Crous 2002; Lombard et al. 2010d). Outro estudo aponta que tal fenômeno ocorre por estas espécies reterem a habilidade para recombinar dos ancestrais comuns, mas que uma espécie pode induzir outra a produzir peritécio mas não contribuir geneticamente com a progênie (Lombard et al. 2010d). Apesar da constatação de baixa fertilidade, outros estudos não quantificaram está o quão baixa é, se a progênie produzida possui genes de um ou das duas espécies participantes e ainda se as progênies viáveis possuem *fitiness* diferentes dos seus parentais.

A hibridização intraespecífica caso aconteça em *Calonectria* pode gerar grandes impactos em como os programas de melhoramento são conduzidos, devido ao potencial de mudanças rápidas nestas populações pelo surgimento de novas características como: aumento da virulência, alteração da adaptação a novos hospedeiros e locais, e redução a sensibilidade à princípios ativos usados no controle (Malapi-Wight et al. 2019).

O conhecimento dos diferentes *mating-types* de uma população de fungos em uma localidade, suporta as regulamentações de quarentena impedindo que um *mating* diferente seja introduzido (Li et al. 2020). A introdução de novos e opostos *matings* em uma população heterotálica pode desencadear o ciclo sexual que envolve o *crossover* fazendo com que a prole recombinante tenha diferente *fitness* por gerar novas combinações de alelos, aumentar a diversidade genética, evitar o acúmulo de mutações deletérias (Roach and Heitman 2014). Apesar do conhecimento já gerado e a afirmação de Li et al. (2020) de que o ancestral comum de *Calonectria* tenha sido heterotálico e a maioria das espécies não terem o *mating* oposto conhecido, como o gênero possui tanta diversidade entre e intraespécie. Roach & Heitman, (2014) discutem que populações com reprodução somente sexual eventualmente podem sofrer com mutações nocivas com possível impacto no *fitness* do fungo e que ao longo das gerações esta perda de *fitness* pode continuar e eventualmente levar a população a extinção. Para isso uma terceira via de manutenção de variabilidade é predita em populações de fungos, o ciclo unissexual, que não dispensa um *mating* oposto(Roach et al. 2014). Este não tradicional ciclo acontece quando o fungo trabalha com se fosse homotálico, produzindo estruturas sexuais, mas ao contrário do ciclo homotálico o organismo só possui um *mating* no seu genoma (Roach et al. 2014). Casos da presença do ciclo unissexual são relatadas em espécies de *Cryptococcus neoformans, Candida albicans* e *Neurospora* (Roach et al. 2014).

O ciclo unissexual é um mecanismo é citado como uma hipótese alternativa à hibridização intraespecífica em *Ca. henricotiae* e *Ca. pseudonaviculata*. Este mecanismo

pode explicar a manutenção de diversidade intraespecífica destas espécies e a capacidade de adaptação de novos nichos como ocorreu nos últimos nove anos nos Estados Unidos (Malapi-Wight et al. 2019). A longo tempo o mecanismo pode ser muito significante em populações clonais por permitir a recombinação e expurgar mutações deletérias da progênie, evitando a possível extinção da espécie (Roach et al. 2014; Malapi-Wight et al. 2019). As bases genéticas deste mecanismo ainda não são definidas e também são conhecidos poucos casos entre os fungos.

Uma outra via de geração de variabilidade é conhecida em alguns fungos e tem ganhado números de publicações. O ciclo parassexual é quando um fungo com diferentes genótipos em diferentes hifas sofrem anastomose, os dois núcleos podem se fundir gerando um organismos diploide, este por sua vez pode sofrer mitose e se manter neste estado gerando micélio e esporos diploides o que pode ou não sofrer aneuploidia voltando ao estado haploide, ou após a mitose os núcleos podem sofres recombinação mitótica e os seus recombinantes gerar descendentes diploides com posterior aneuploidia formando organismos haploides ou permanecer no estado diploide por vários ciclos (Debuchy e Turgeon, 2006). A recombinação sem fora dos ciclos sexuais foi primeiramente observada em espécies dos gêneros *Aspergillus, Candida, Fusarium, Macrophomina, Metarhizium, Paecilomyces, Penicillium e Verticilium* (Hastie 1964; Garber and Beraha 1965; Riba and Ravelojoana 1984; Forche et al. 2008; Nielsen et al. 2021; Sybuia et al. 2022).

O ciclo parassexual pode ser um mecanismo que aumenta a diversidade genética em fungos que se propagam predominantemente de forma assexual, com potencial para gerar proles que se adaptam melhor as contínuas mudanças do ambiente ou melhores características que seus parentais (Strom and Bushley 2016). Em *Metarhizium* muitos trabalhos têm demostrado estruturas assexuais como conídios na forma diploide, chegando a proporções de 25% dos coletados em infecções em insetos(Wang et al. 2011; Strom and

Bushley 2016). O ciclo parassexual apesar de conhecido desde a década de 50, ainda não foi explorado para muitos fungos, deixando uma lacuna quando a geração de variabilidade em populações de fungos que só é conhecida seu ciclo assexual.

1.10 Estudos genômicos relacionados aos gêneros *Calonectria*-like

Sequenciamentos genômicos tem recebido destaque nos últimos anos devido as enormes aplicações possíveis, uma delas é a resolução de divergências em reconhecimento de espécies, a filogenômica ajuda neste tipo de análise por aumentar o sinal filogenético e dar uma robusta resolução por analisar sequencias de diferentes genes (Li et al. 2022) Para este e demais estudos a disponibilização destes genomas em plataformas de bancos de dados como Genbank e Mycocosm (https://mycocosm.jgi.doe.gov/mycocosm/home) é parte crucial para a perpetuação de pesquisas de comparação genômica. Observando no banco de dados do Genbank é possível notar que para a família Nectriaceae existem cerca de 1.477 genomas disponibilizados. Do total de genomas nota-se que os estudos genômicos dentro desta família estão focados em dois gêneros principais, *Fusarium* e *Calonectria* com 94% e 4% dos genomas, respectivamente. Os 2% restantes são genomas dos gêneros *Aquanectria, Ilyonectria, Mariannaea, Microcera, Neonectria, Pseudonectria, Rugonectria, Stylonectria, Thelonectria* e *Xenoacremonio.* Na plataforma Mycocosm a predominância de dados de *Fusarium* é repetida, no entanto não existem dados para *Calonectria*. Em ambas as plataformas não há registro de genomas disponibilizados para os outros gêneros que compõe o grupo *Calonectria*-like além do gênero principal.

Em 2016 começaram os estudos genômicos relacionados ao gênero *Calonectria*, a partir da disponibilização do genoma de *Ca. pseudonaviculata* CBS 139395 na plataforma

do Genbank. No mesmo ano a primeira publicação é feita, com o objetivo de demonstrar como as técnicas de sequenciamento de nova geração (*NGS* do inglês *Next Generation sequencing*) podem ser usadas para a rápida caracterização de fitopatógenos e novas doenças (Malapi-Wight et al. 2016). Foram submetidos à plataforma do GenBank outros 69 genomas entre os anos de 2017 e 2022, entre estes, constam 17 espécies; são elas: *Ca. aciculata; Ca. crousiana; Ca. fujianensis; Ca. hawksworthii; Ca. henricotiae; Ca. honghensis; Ca. hongkongensis; Ca. ilicicola; Ca. leucothoes; Ca. montana; Ca. multiphialidica; Ca. naviculata; Ca. pauciramosa; Ca. pseudonaviculata; Ca. pseudoreteaudii; Ca. pseudoturangicola e Ca. pteridis.*

De forma geral, com os dados atuais é possível notar que o gênero *Calonectria* possui um genoma variando entre 30,8 e 75,4 Mb, e uma porcentagem de GC entre 46 e 51,5%. Entre as espécies amostradas se destacam *Ca. ilicicola* (40 genomas)*, Ca. pseudonaviculata* (9 genomas) e *Ca. henricotiae* (5 genomas). As duas últimas espécies se destacam devido à recente importância como patógenos em *Buxus sempervivérens* na Europa e nos Estados Unidos(Malapi-Wight et al. 2019) Genomas de *Ca. ilicicola* fazem parte de dois trabalhos principais o primeiro reporta o sequenciamento dos genomas(Liu et al. 2021) e o segundo estuda o a relação desta espécie com ouros fungos de outros grupos taxonômicos, ressaltando o uso de genoma mitocondrial para criar bases no entendimento da evolução mitocondrial destes fungos (Gai et al. 2020). Apesar de *Ca. ilicicola* ser a espécie que possui 40 genomas, não foi dado nenhuma outra abordagem a eles além dos estudos citados acima.

Um único estudo de filogenômica que buscou datar tempo de divergência entre espécies, utilizou genomas de cinco espécies, três do complexo de espécies *Ca. naviculata* (*Ca. henricotiae, Ca. naviculata, Ca. pseudonaviculata*) uma do complexo *Ca. mexicana* (*Ca. leuchothoes*) e outra do complexo *Ca. reteaudii* (*Ca. pseudoreteaudii*). Segundo os resultados, as espécies analisadas do complexo *Ca. naviculata* se separaram das demais

a cerca de 40,2 Milhões de anos atrás (Malapi-Wight et al. 2019), na era Cenozóica, período do Eoceno, este período é considerado como o mais quente e húmido do perído Cenozóico, e é levantado a hipótese que devida as condições ideais tenha sido um período de intensa migração e diversificação de inúmeras plantas e animais (Gradstein et al. 2004; Cai et al. 2019). A separação entre espécies citadas como causadoras de doenças em *Buxus sempervirens* ocorreu durante o final do Oligoceno a 26,5 Milhões de anos e a separação das espécies *Ca. henricotiae* e *Ca. pseudonaviculata* já acontece bem recentemente no final do Plioceno a 2,6 Milhões de anos, este foi um período em que foi marcado pela expansão de gramíneas C4 devido a uma expansão global de aridificação (Gradstein et al. 2004; Cai et al. 2019; Malapi-Wight et al. 2019).

Estudos filogenômicos que buscam datar por estimativa eventos de especiação dentro de um gênero correlacionados com eventos históricos e processos de especiação, extinção, migração e expansão de hospedeiros podem oferecer melhor entendimento sobre a história evolutiva destes patógenos. Os dados estimados por Malapi-Wight et al., (2019) indicando a separação do complexo de espécies *Ca.* naviculata no Eoceno coincidem com a estimativa de separação do gênero *Buxus* (estimado entre 40 e 50 milhões de anos) dos seus parentais (Krutzsch 1989) Outro estudo mostra que na Itália a 130 mil anos atras já existiam espécies de *Buxus sempervirens* devido a presença de pólen fossilizados (Carvalho et al. 2016). O complexo *Ca. naviculata* possui maior número de associações todas a espécies de *Buxus* entre todos os outros complexos de *Calonectria*. Estas evidências podem apontar para uma coevolução entre as espécies durante a história evolutiva dos gêneros.

Outro estudo genômico que aborda de forma pioneira as estratégias de patogenicidade e adaptação de *Ca. pseudoreteudii* foi publicado em 2018. Ao analisar o genoma da espécie os autores observaram, que tem um tamanho de 63,7 Mb, com taxa de CG em 48,3%, 14.355 genes, destes 1.178 são proteínas secretadas 87% das quais

suportadas por dados de RNA-seq(Ye et al. 2018). Do total de genes encontrados em *Ca. pseudoreteaudii* cerca de 46,1% também é encontrado em espécies filogeneticamente próximas como *Nectria ditíssima, Fusarium graminearum*, *F. oxysporum, F. solani* e *F. verticilliodes,* outros 12,3% são encontrados somente em *Ca. pseudoreteaudii* fazendo com

Espécie	Isolado	Genoma	Tamanho (Mb)	GC (%)	Scaffold/Nº de contigs	Cobertura	N50 (bp)	Hospedeiro	Projeto WGS	Método de sequenciamento	Instituição	Referência
Calonectria aciculata	CMW 47645	ASM1340699v1	61,6	49,5	334	40	365.6	*Eucalyptus urophylla*	VTGE01	Illumina HiSeq	FABI	Liu et al. (2019)
Ca. crousiana	CMW 27249	ASM1340698v1	58,1	50,0	417	52	332.9	*Eucalyptus grandis*	VTGD01	Illumina HiSeq	FABI	Liu et al. (2019)
Ca. fujianensis	CMW 27257	ASM1340696v1	61,5	49,5	263	31	505.8	*E. grandis*	VTGC01	Illumina HiSeq	FABI	Liu et al. (2019)
Ca. hawksworthii	S6964	ASM2097541v1	64,8	49,0	153	15	1.5Mb	*Persea americana*	JAHXZK01	Illumina MiSeq	USDA-ARS	
Ca. henricotiae	CB077	ASM438093v1	47,5	49,5	6145	839	16.9kb	*Buxus sempervirens*	PGSE01	Illumina MiSeq	USDA-ARS	Crouch et al. (2017)
	CBS 138102	ASM438088v1	53,5	48,5	9584	1020	15.3kb	*B. sempervirens*	PGWR01	Illumina MiSeq; PacBio	USDA-ARS	Crouch et al. (2017)
	NL009	ASM438096v1	49,1	49,0	10144	1108	12kb	*B. sempervirens*	PGSF01	Illumina MiSeq	USDA-ARS	Crouch et al. (2017)
	NL017	ASM438220v1	43,3	49,5	28985	7711	1.8	*B. sempervirens*	PHMY01	Illumina MiSeq	USDA-ARS	Crouch et al. (2017)
	JAC13-131	ASM2062369v1	53,0	46,5	1018	140	107.9kb	*Buxus* sp.	JAHVYB01	Illumina MiSeq	USDA-ARS	Roger et al. (2022)
Ca. honghensis	CMW 47669	ASM1340385v1	61,7	49,5	175	23	918.3kb	Solo	VTGB01	Illumina HiSeq	FABI	Liu et al. (2019)
Ca. hongkongensis	CMW 47271	ASM1714075v1	61,7	48,5	110	19	1.2Mb	Solo	JAACJA01	Illumina HiSeq	FABI	Li et al. (2020)
Ca. ilicicola	FJLY41	ASM1462202v1	70,2	46,0	303	36	641kb	*Arachis hypogaea*	JACVOE01	Illumina HiSeq	Zhejiang University	Liu et al. (2021)
	GDBL01	ASM1462209v1	68,6	46,5	328	43	478317	*Glycine max*	JACVOJ01	Illumina HiSeq	Zhejiang University	Liu et al. (2021)
	GDBL02	ASM1462210v1	69,6	46,5	351	35	555473	*A, hypogaea*	JACVOI01	Illumina HiSeq	Zhejiang University	Liu et al. (2021)
	GDBL60	ASM1462203v1	68,8	46,5	329	39	510771	*G. max*	JACVOH01	Illumina HiSeq	Zhejiang University	Liu et al. (2021)
	GDMZ12	ASM1462201v1	69,9	46,0	353	43	520.7kb	*A, hypogaea*	JACVOG01	Illumina HiSeq	Zhejiang University	Liu et al. (2021)
	JXLN31	ASM1462199v1	69,9	46,5	485	45	432kb	*A, hypogaea*	JACVOF01	Illumina HiSeq	Zhejiang University	Liu et al. (2021)
	ZJHZ01	ASM1462200v1	70,4	46,0	307	29	689kb	*G. max*	JACVOD01	Illumina HiSeq	Zhejiang University	Liu et al. (2021)

Continua...

Espécie	Isolado	Genoma	Tamanho (Mb)	GC (%)	Scaffold/Nº de contigs	Cobertura	N50 (bp)	Hospedeiro	Projeto WGS	Método de sequenciamento	Instituição	Referência
	AHDT12	ASM2101936v1	68,5	46,5	302	35	490165	*A, hypogaea*	JAIZDK01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	AHDT6	ASM2101933v1	68,5	46,5	312	34	525308	*A, hypogaea*	JAIZDJ01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	AHSZ11	ASM2101931v1	68,5	46,5	262	36	536426	*A, hypogaea*	JAJAHW01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	AHSZ7	ASM2101929v1	68,3	46,5	342	42	446200	*A, hypogaea*	JAIZDI01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDCH127	ASM2101926v1	69,8	46,0	287	38	598381	*A, hypogaea*	JAIZDH01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDGZ125	ASM2074036v1	73,7	48,0	5981	1073	17654	*A, hypogaea*	JAJALN01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDYF159	ASM2074035v1	69,6	46,5	337	50	447822	*A, hypogaea*	JAJALM01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDYJ169	ASM2080970v1	69,9	46,5	12	5	6.8Mb	*A, hypogaea*		Oxford Nanopore	Beijing Forestry University	Liu et al. (2021)
	GDYJ183	ASM2101925v1	69,7	46,5	374	54	506045	*A, hypogaea*	JAIZDG01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDZQ186	ASM1650864v1	69,7	46,0	350	49	438287	*A, hypogaea*	JADDLS01	Illumina HiSeq	Zhejiang University	Liu et al. (2021)
	HNYC11	ASM2101921v1	67,1	47,0	782	72	262077	*A, hypogaea*	JAJAHV01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	HNYC15	ASM2101919v1	68,6	46,5	279	37	527601	*A, hypogaea*	JAJAHU01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	HNYC23	ASM2101922v1	68,6	46,5	245	32	585074	*A, hypogaea*	JAJAHT01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)

Espécie	Isolado	Genoma	Tamanho (Mb)	GC (%)	Scaffold/Nº de contigs	Cobertura	N50 (bp)	Hospedeiro	Projeto WGS	Método de sequenciamento	Instituição	Referência
	HNYC24	*ASM2101917v1*	*68,6*	*46,5*	*256*	*35*	*553048*	*A, hypogaea*	*JAJAHS01*	*Illumina HiSeq*	*Beijing Forestry University*	*Liu et al. (2021)*
	HNYC36	ASM2101915v1	68,5	46,5	307	37	524568	*A, hypogaea*	JAIZDF01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	HNYC58	ASM2101910v1	68,6	46,5	196	28	727719	*A, hypogaea*	JAIZDE01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	HNYC70	ASM2101907v1	68,5	46,5	349	40	487517	*A, hypogaea*	JAJAHR01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	SCMS200	ASM2074034v1	58,6	51,5	923	69	220882	*A, hypogaea*	JAJALL01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	SDJN1	ASM2101909v1	68,5	46,5	311	35	531443	*A, hypogaea*	JAJAHQ01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	SDJN13	ASM2101908v1	67,7	46,5	244	35	553047	*A, hypogaea*	JAJAHP01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	SDJN15	ASM2101905v1	68,6	46,5	301	34	509966	*A, hypogaea*	JAIZDM01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	SDJN6	ASM2101901v1	68,4	46,5	369	38	481441	*A, hypogaea*	JAIZDL01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	F018	ASM2451573v1	69,0	46,5	16	5	6053737	*G. max*	JAKVRC01	Oxford Nanopore GridION	National Taiwan University	Liu et al. (2021)
	GDCH194	ASM2301553v1	69,6	46,5	424	54	434235	*A, hypogaea*	JAJIAB01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDHY120	ASM2301551v1	68,4	47,0	728	47	259980	*A, hypogaea*	JAJIAC01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDHY151	ASM2301548v1	70,5	46,5	394	44	477770	*A, hypogaea*	JAJIAD01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)

Espécie	Isolado	Genoma	Tamanho (Mb)	GC (%)	Scaffold/Nº de contigs	Cobertura	N50 (bp)	Hospedeiro	Projeto WGS	Método de sequenciamento	Instituição	Referência
	GDHY27	ASM2301546v1	70,9	46,5	379	40	549956	*A, hypogaea*	JAJIAE01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDMZ26	ASM2301544v1	75,4	47,0	456	45	481907	*A, hypogaea*	JAJIAF01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDSG115	ASM2301543v1	70,3	46,0	376	43	511546	*A, hypogaea*	JAJIAG01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDYJ176	ASM2301540v1	70,6	46,5	418	40	497366	*A, hypogaea*	JAJIAH01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDYJ177	ASM2301538v1	69,9	46,0	352	45	482386	*A, hypogaea*	JAJIAI01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	GDYJ182	ASM2301537v1	69,8	46,0	329	43	530120	*A, hypogaea*	JAJIAJ01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
	JXLN134	ASM2301534v1	70,6	46,0	294	33	685330	*A, hypogaea*	JAJIAK01	Illumina HiSeq	Beijing Forestry University	Liu et al. (2021)
Ca. leucothoes	CBS 109166	ASM217983v1	63,0	49,5	3806	89	204521	*Leucothoe axillaris*	NAJI01	Illumina MiSeq	USDA-ARS	Malapi-Wight et al. (2019)
Ca. montana	PCam007	ASM2260643v1	59,3	47,0	13	4	6356788	tree peony	JAIZZI01	Oxford Nanopore PromethION	Henan University of Science & Technology	Tian et al. (2022)
Ca. multiphialidica	CBS 112678	ASM2062366v1	62,9	50,0	1162	64	255455	Solo	JAHVYC01	Illumina MiSeq	USDA-ARS	Roger et al. (2022)
Ca. naviculata	CBS 101121	ASM303170v1	65,7	50,5	5805	299	55554	*Folhas*	NAGG01	Illumina MiSeq	USDA-ARS	Roger et al. (2022)
Ca. pauciramosa	CBS 138824	ASM1714078v1	62,5	49,0	154	13	1618990	*E. grandis*	JAACIZ01	Illumina HiSeq	FABI	Li et al. (2020)
	CMW 7592	ASM1714065v1	62,3	49,0	113	20	1000055	*E. grandis*	JAACIY01	Illumina HiSeq	FABI	Li et al. (2020)
Ca. pseudonaviculata	CBS 139394	ASM169650v1	51,4	47,5	2267	178	83301	*Sarcococca hookeriana*	JYJY01	Illumina MiSeq	USDA-ARS	Yang et al. (2022)
	CB002	ASM650590v1	49,9	48,5	7686	877	16997	*B. sempervirens*	QGQV01	Illumina GAIIx	USDA-ARS	Yang et al. (2022)

Espécie	Isolado	Genoma	Tamanho (Mb)	GC (%)	Scaffold/Nº de contigs	Cobertura	N50 (bp)	Hospedeiro	Projeto WGS	Método de sequenciamento	Instituição	Referência
	CBS 139395	ASM438091v1	55,0	46,0	3424	272	62248	*B. sempervirens*	PGGA01	Illumina MiSeq; PacBio	USDA-ARS	Yang et al. (2022)
	CBS 14417	ASM438100v1	47,3	50,0	5848	855	16943	*B. sempervirens*	PHMX01	Illumina MiSeq	USDA-ARS	Yang et al. (2022)
	CT13	ASM438098v1	47,8	49,5	5194	706	20785	*B. sempervirens*	PGWW01	Illumina MiSeq	USDA-ARS	Yang et al. (2022)
	ICMP 14368	ASM438224v1	30,8	49,5	26064	7135	1370	*B. sempervirens*	PHNA01	Illumina MiSeq	USDA-ARS	Yang et al. (2022)
	NC-BB1	ASM438103v1	39,8	49,5	21937	4560	2591	*B. sempervirens*	PHMZ01	Illumina MiSeq	USDA-ARS	Yang et al. (2022)
	ODA1	ASM438222v1	45,2	50,0	16367	2840	4586	*B. sempervirens*	PHNB01	Illumina MiSeq	USDA-ARS	Yang et al. (2022)
	JAC13-27	ASM2062367v1	54,0	46,5	2390	291	52534	*Buxus* sp.	JAHVYD01	Illumina MiSeq	USDA-ARS	Roger et al. (2022)
Ca. pseudoreteaudii	YA51	ASM187950v1	63,7	48,0	2740	236	79404	*Eucalyptus* sp.	MOCD01	Illumina HiSeq	Fujian Agriculture and Forestry University	Ye et al. (2018)
Ca. pseudoturangicola	CMW 47496	ASM1340382v1	62,1	48,5	209	26	682760	Solo	VTGA01	Illumina HiSeq	FABI	Liu et al. (2019)
Ca. pteridis	LPF059	ASM2283700v1	58,4	50,0	1207	55	276469	*Eucalyptus* sp.	JAKZGU01	Illumina NovaSeq	UFV	Queiroz et al. (2022)

que os autores levantem a hipótese de que possam genes formados para adaptação a um hospedeiro específico (Ye et al. 2018).

A análise do genoma de *Ca. pseudoreteaudii* mostrou que este organismos possui um arsenal genômico muito poderoso e distinto de vários outros Hypocreales fitopatógenos. A exemplo disto: foram identificados 57 genes relacionados ao metabolismo responsável pela invasão e colonização do hospedeiro; 679 genes transportadores de membrana, sobretudo ABC transportador (ATP-binding cassette transporter), fator que aumenta a resistência do patógeno a toxinas liberadas pelas células do hospedeiro, podendo ser fator determinante no sucesso na colonização; alta quantidade de genes relacionado a transcrição de cutinases, pectinase, e 1,4-benzoquinona que atua na degradação de lignina e proteção do fungo a compostos reativos de quinona, tais genes transcrevem enzimas importantes na colonização por degrada a primeira linha de defesa da planta (Ye et al. 2018).

O trabalho de Ye et al., (2018) demonstra que várias famílias de genes foram expandidas ou contraídas em *Ca. pseudoreteaudii*, principalmente famílias de genes relacionadas a processo de oxido-redução, compostos aromáticos, transporte transmembrana estão em expansão, o que pode ser relacionado a adaptação á diferentes nichos ecológicos. Atualmente a espécie é reportada somente causando doenças em *Eucalyptus* sp. na China, porém esta expansão pode ser compartilhada entre espécies que compõem o complexo de espécie *Ca. reteaudii*, uma vez que este complexo está distribuído em 5 continentes, 19 países sendo relatadas associadas a 27 famílias de plantas.

Posteriormente Rogers et al., (2022) analisou genomas de 24 espécies relacionadas a doenças em *B. sempervirens,* e espécies de *Calonectria* ainda não associadas a doenças, buscando entender como os genes destas espécies estão

evoluindo. Pela análise dos genomas os autores constataram que entre as seis espécies de *Calonectria* analisadas (patogênicas a *B. sempervirens* = *Ca. henricotiae* e *Ca. pseudonaviculata,* patogênicas a outras espécies = *Ca. leucothoes* e *Ca. pseudoreteaudii,* não associadas como patógenos = *Ca. multiphialidica* e *Ca. naviculata,*) somente as associadas a doenças em *B. sempervirens* apresentaram alta taxa de contração e expansão de famílias gênicas e nas espécies não associadas como patógenos exibiram também altas taxas de expansão de famílias gênicas. Rogers et al., (2022) discutem quem esta rápida contração em famílias de genes relacionados no processo de infecção das espécies patogênicas a *B. sempervirens* aponta para uma evolução no comportamento destes fungos, se tornado mais biotróficos que hemibiotrófico como é relatado para *Calonectria*, porém como os estudos relacionado ao comportamento destas duas espécies ainda não foi feito esta correlação permanece no campo da hipótese. Associando a rápida contração de genes relacionados a infecção das espécies *Ca. henricotiae* e *Ca. pseudonaviculata*, com os indícios de possível co-evolução com a família botânica hospedeira e a baixa diversidade de hospedeiros relatados para estas espécies (Malapi-Wight et al. 2013; Liu et al. 2020; Li et al. 2022; Rogers et al. 2022), indicam uma especialização entre estes patossistemas, em que os patógenos estão, geneticamente mudando para um hábito diferente dos seus ancestrais.

Espécies também patogênicas como *Ca. leucothoes* e *Ca. pseudoreteaudii* apesar de terem a sua diversidade de hospedeiros restrito somente a uma família botânica, não foram encontrados padrões de contração e expansão de famílias gênicas como as patogênicas a *B. sempervirens*(Rogers et al. 2022). As espécies não associadas como patógenos, *Ca. multiphialidica* e *Ca. naviculata,* por apresentarem famílias gênicas com função ainda desconhecida e outras responsáveis por

biossíntese do metabolismo secundário e metabolismo de carboidrato nas categorias COG. Expansão destas famílias de genes são comuns em fungos que possuem hábito saprofítico devido a estratégias de defesas contra outros fungos e degradação de nutrientes (Rogers et al. 2022). Interessantemente nas espécies não patogênicas foram encontradas famílias de genes também relacionados a patogenicidade e comuns entre seus parentes patogênicos. O que aponta para uma evolução em que estes genes advêm de um ancestral comum, ou que as espécies não patogênicas estão evoluindo de forma similar a patógenos de plantas (Rogers et al., 2022).

Uma abordagem genômica aplicada a *Calonectria* foi a mitogenomica, que consiste no sequenciamento e análise do genoma mitocondrial. A ferramenta oferece um potencial inexplorado como relógio molecular por ter alta taxa de mutação e baixa taxa de recombinação (Gai et al. 2020). Atualmente a técnica foi aplicada no sequenciamento de cinco mitogenomas de *Ca. ilicicola* que mostrou tamanho variando de 39,9Kb a 64,4Kb e é marcado pela permanência desta espécie próximo a gêneros filogeneticamente relacionados em análises de DNA nuclear como *Ilyonectria* e *Fusarium* (Gai et al. 2020).

Apesar do número dos estudos citados anteriormente, análises genômicas (nuclear e mitocondrial) para o gênero *Calonectria* são escassas, mantendo ainda muitas questões em aberto. Apesar disto, o número de genomas disponibilizado nos bancos de dados vem crescendo, o que serve de insumo para futuras análises. Bons exemplos de estudos utilizando genomas podem ser observados em outros grupos taxonômicos, como em *Zymoseptoria tritici* onde foi identificado e estimado as datas de introdução da espécie em novas áreas devido à domesticação e expansão global do cultivo de trigo e que a resistência a defensivos agrícolas foi mediados por

elementos transponíveis, isso a partir da análise de mil genomas (Feurtey et al. 2023). Este primeiro exemplo serve de base para iniciar os estudos em patossistemas como *Eucalyptus* sp. e *Calonectria,* levando em consideração que a interação produz uma das doenças mais devastadores no hospedeiro (Bose et al. 2022) é amplamente distribuído em regiões subtropicais e tropicais e a interação é gênero-gênero (múltiplas espécies de *Calonectria* podem causar doenças e múltiplas espécies de *Eucalyptus*) e não espécie-espécie.

Estudos com *Trichoderma* utilizando genoma, podem ser citadas como base para futuros estudos em *Calonectria:* (Kubicek et al. 2011) mostra em seus trabalhos como foi a evolução de espécies de *Trichoderma* de acordo com o tipo de habito das espécies analisadas foi possível inferir sobre quais os genes são importantes no processo de micoparasitismo, indicando que o ancestral comum do gênero possa ter sido micoparasita de basidiomicetos decompositores de madeira e que tenha evoluído para características saprofíticas a para sobreviver no substrato do seu hospedeiro basidiomiceto. Similar a esta abordagem podemos notar que muitas espécies de *Calonectria* até o momento não são associadas a doenças, enquanto outras são somente associadas aos seus hospedeiros. Não possuímos conhecimento de que durante a evolução do gênero qual o hábito do ancestral comum e como foi a evolução, se no início o gênero tinha hábito saprofítico e evoluiu para o hábito parasítico, ou o contrário.

Além das perguntas acima pode ser citado que os próximos estudos com genômica de *Calonectria* podem tomar as seguintes direções: *i.* expandir banco de dados de genoma para ter ao menos um isolado de cada espécie sequenciado; *ii.* Identificação do core genoma do gênero; *iii.* Elaborar backbone filogenômico a partir

do core genoma; *iv.* Identificar possíveis locus que ofereçam melhor delimitação de espécies do que os atuais marcadores; *v.* Identificar famílias de genes que estão relacionadas a fatores de patogenicidade; *vi.* Identificar pan genoma e entender o processo evolutivo de especiação das espécies; *vii.* Identificar como os elementos transponíveis possam ter agidos como direcionadores de evolução; *viii.* Identificar possíveis transferências horizontais de genes ao longo da história evolutiva; *ix.* Estimar processos migratórios e dispersão de espécies nos territórios e nas espécies hospedeiras; *x.* Analisar vias alternativas de geração de variabilidade genética.

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Capítulo 2: A meta-analysis of the representativity of Genbank with regard to *Calonectria* and closely related genera.

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A meta-analysis of the representativity of Genbank regarding *Calonectria* and closely related genera.

2.1 Abstract

Since 1995, when the first *Calonectria* sequence was submitted to Genbank, this databank turned a valuable resource to *Calonectria* -like researchers, acting as a cut between the traditional taxonomic based on phenotypic measurements to molecular approaches. Due to the exponential increase in the sequence of data in Genbank with metadata, this resource presents some discrepancies, making some studies difficult or impossible, mainly because of the uniformity in the number of sequences, metadata information, and lack of accuracy by the submitters. In this way, a search in Genbank using the keywords *Aquanectria, Calonectria, Curvicladiella, Cylindrocladiella, Gliocladiopsis, Gliocephalotrichum, Penicillifer,* and *Xenocylindrocladium* 20,236 accessions were downloaded to Genbank, totaling 430,504 databank lines. It was shown that *Calonectria* is the most sampled genus, followed by *Cylindrocladiella, Gliocephalotrichum,* and *Gliocladiopsis.* There are many gaps in marker sampling in all genera, and that *tef* and *tub* are the two most frequently markers submitted with 5,125 and 5,287 sequences each. 6,414 isolates were identified, but these numbers can be overrepresented due to the “Matryoshka doll effect” of providing the new number to isolate each time it is stored in a new laboratory. The data show the group is globally spread, but the analysis was only possible for 78.3% of access, as they were filled with the information. Genera such *Xenocylindrocladium* and *Curvicladiella* stay isolated in South America and Asia. Brazil and China (64 and 51 species respectively) are the countries with greatest diversity of reported species, and *Calonectria pauciramosa* is the most cosmopolitan species (12 countries). In the metadata

host/substrate information was retrieved in only 22% of all access, indicating that the authors ignored this information. In total, 146 genera, 82 families and 45 orders of plant were observed with the eight genera. Highlighted the order Myrtales, Ericales, Fabales, Pinales, and Sapindales, the top five orders with the greatest number of associated species. Despite the quantities of data presented in this study, metadata analysis shows that the health of the databank is in decline, due to poor metadata submission. Therefore, the databank is wasted and offside of growth by the implementations of new search tools and used as data source for host diversity, spatial analysis, and epidemiological approaches. The gaps found in this paper made us propose a standardization and the authors followed standards such as filling in a minimum of information about sequences, updating the currently name and using the first isolate number as reference independent of where it was currently stored, and living the new number to internal use and referred in the metadata note.

Keywords: Hypocreales, Botryosphaeraceae, Myrtaceae, Medicine, Drugs

2.2 Introduction

The historic of *Calonectria* and seven other genera (*Aquanectria, Curvicladiella, Cylindrocladiella, Gliocladiopsis, Gliocephalotrichum, Penicillifer* and *Xenocylindrocladium*), have been associated due to similarities shared by the anamorphous phase, such as the presence of white penicillate conidiophores, the presence of stipe extension with the presence of vesicle at the end (exception *Gliocladiopsis*), produce cylindrical conidia and are commonly isolate from soil samples (Boesewikel 1982; Crous 2002; Silva et al. 2020a). The group called *Calonectria*-like (Crous 2002) had its taxonomy intertwined for years. *Calonectria* was raised in 1867 by De Notoris, in next its anamorph state was described in 1892

(Morgan 1892; Crous 2002). Almost 60 years later, *Gliocladiopsis* was proposed as a genus (Barron and Peterson 1968; Lombard and Crous 2012a). In 1962, *Gliocephalotrichum* was removed from *Calonectria* on the basin of different spore morphology and number of stipe extensions (Ellis and Hesseltine 1962), in this same decade the genus *Penicillifer* was published, and specimens of *Gliocladiopsis* were demoted as species within genus *Calonectria* (Barron and Peterson 1968; Van Emden 1968; Lombard and Crous 2012a). *Cylindrocladiella* was proposed to group specimens of *Calonectria* that had small conidiophores (less than 20µ) and small conidia (Boesewinkel 1982). The genus *Gliocladiopsis* was only resurrected in 1993 based only in morphological characteristics (Lombard and Crous 2012b) The last two genera described based only in non-molecular approaches were *Xenocylindrocladium* and *Curvicladiella* (Decock et al. 1997; Decock and Crous 1998). *Aquanectria* is the most recent genus described in this group and join morphological and phylogenetics characteristics (Lombard et al. 2015a).

Throughout the revision increase of this group, we can separate into two moments, before and after the implementation of molecular data Before, all descriptions were made based in morphological, physiological and/or biological concepts (Lücking et al. 2020) causing some misunderstanding in the development of the process. After, the phylogenetic concept of species is introduced, later changed to Genealogical Concordance Phylogenetic Specie Recognition (GCPSR - Taylor et al. 2000). But this made it necessary to make sequences available on public dates, for features comparisons. With this main objective, began the International Sequence Database Collaboration (INSDC) join the National Center for Biotechnology Information (NCBI), the European Bioinformatics Institute (EMBL-EBI) and the DNA Data Bank of Japan (DDJB) and American nucleic acid sequence database Genbank.

The author mentions that this initiative contains more nucleotides than “the number of stars in the Milky Way”(Strasser 2008). Genbank currently represents a valuable resource for global science by detain nowadays more than 500 million of nucleotide sequences (https://www.ncbi.nlm.nih.gov/genbank/). The importance of this databank has been cited as innovative of biology due join molecular sequences and values of natural history (Strasser 2008).

The first *Calonectria* -like sequence submitted to Genbank was in April 1995, being a *Calonectria pteridis* clone cyl3 with 305 nucleotides. This was the first step until we reached about 20,000 sequences currently, totaling eight genera.. Over the years, with the increase and implementation of new molecular markers, the pattern of sequences submitted in Genbank changed, starting with ribossomal markers (*its, lsu* and *ssu*) to partial sequences that participated in other molecules (*act, cmda, his3, repb1, rpb2, tef, tub,* etc. – Liu et al. 2020). The change caused some uniformity in the sample quantities of these markers inner the databank, inducing researchers to some misunderstanding, in the same case providing phylogenetics analysis without uniformity. Such lack of uniformity triggered the over-inference about the number of *Calonectria* species needed summary review and establishment of markers number and refining the phylogenetical *Calonectria* specie concept (Liu et al. 2020). The review by Liu et al. (2020) reinforces the importance of utilization barcode markers for species, before proposed in 2017 for *Calonectria* and 2019 for *Cylindrocladiella* (Marin-Felix et al. 2017, 2019), while the others six genus remain undefined. Such a review needs some physical resources, such as the presence of an isolate available to be newly processed in DNA extraction, thus the phenomenon of migration of isolates between laboratories occurs.. The phenomena can be caused by applying this biological resource in a new study or just to store it in a reference culture collection. In this transfer

process, its common new publication uses the new isolate number gave by the new location, in some cases, referring to the old number in the paper table and in Genbank metadate.

As Genbank is a public databank provided by the authors, who submitted sequences accompanied by the metadata, the richness of the databank is in the hands of these authors. Some practices put the usability of this resource in risk: *i)* revisions in taxonomy, causing some species to be synonymized inner others without revision in databanks; *ii)* transfer of material between laboratories without the proper use of only the first published isolate number, disregarding where the isolate is now; *iii)* submit the sequence with wrong nomenclatures; *iv)* submit sequences without metadata, poorly filled or information in wrong qualifiers. Due to these problems, the database suffers from the impossibility of improving the filter, even using complex keywords nowadays it is impossible to consult sequences of a determinate group of specifications, for example, location on earth, substrate or associated host, height or same collector (Yilmaz et al. 2011). As an effort to care for the integrity of the Genbank database, the authors reinforce that minimal information about a marker gene sequence (MIMARKS) and about any sequences (MixS) suggesting that environment (any information about where the sample was collected), geographical location and historic (collector number, personal number of the isolate, collector) as minimums to be inputted in the moment of sequence submission (Yilmaz et al. 2011; Aime et al. 2021a).

As the objective of this study, we posed to answer the following questions: *i)* how healthy is the databank in the concern the eight genera that compose *Calonectria*-like? *ii)* How much is the metadata filled with richness? *iii)* what is the sampling of each major marker used in the phylogeny for this group since the first sequence was

submitted? *iv)* the number of sequences is related to the number of recognized species? *V)* how is the distribution of sequence inner species and genus, are they representative? *vi)* Using Genbank databank, how is the worldwide spatial distribution of isolates and species? *vii)* Is the currently cited number of host species for the genus possible to find representativeness in the Genbank database? *viii)* how is the distribution of data reported in association with host or substrate?

2.3 Material and Methods

Data extraction – Was searched by the genus that compose *Calonectria*-like group (*Aquanectria, Calonectria, Curvicladiella, Cylindrocladiella, Gliocladiopsis, Gliocephalotrichum, Penicillifer* and *Xenocylindrocladium*- Crous 2002 - Crous 2002- Crous 2002 - Crous 2002) in Genbank (https://www.ncbi.nlm.nih.gov/genbank/search) toolbar, using the parameter nucleotide as the primary filter. On the next page, with all dates uploaded, was applied the filter by molecule type, choosing only data of genomic DNA/RNA, after was required a create file complete record in a file under the format INSDSeq XML, sorted by default order and downloaded. This process was made for all genera separately. All XML files were saved in one directory.

Pre-process and tags filtering – One of the compiled files was opened on Excel® as resource of XML panel, after in resource panel, was chosen the tags. These tags refer to columns that contain all data used as resources. The tags chosen were: INSDSeq_locus, INSDSeq_organism, INSDSeq_create-date, INSDQualifier_name, INSDQualifier_value, INSDAuthor, INSDReference_title and INSDSquence (Table 1). In following the add all tags the data was uploaded and altered the sheet name to refer to the genus uploaded. In a new sheet, the process was repeated to the next genus.

Each genus data was uploaded in different sheets separately. After the file was saved as.xlsx in one directory.

Assemble, data extraction and improving metadata – A blank Excel® file, was opened to assemble all archive.xlsx created as a primary dataset. To this, using the data tools, in this new sheet, was get data from Excel® workbook and selected one of.xlsx file. In the next window the was selected the sheet named “Sheet1” and followed to data transformation. After the Power Query Editor opened, in panel of all steps made, was deleted the two last, remained only step sources. In the main panel was selected all genus data sheets, using a filter of the first column (name). In sequence, on column data, was used the expand function to merge all sheets in one databank. Followed by getting close and loading the data.

After all, data has loaded on the main sheet of Excel® has applied a filter in INSDSeq_locus to take off data following parameters: *i.* sequences generated to genomic studies; *ii.* sequences of another genus downloaded together; *iii.* without locus information. The column INSDQualifier_name was used to segregate data inner four categories: Country (country and note), Gene (gene, organelle, and product), Host/substrate (host, isolate_source and note) and Isolate (culture_collection, isolate, note, speciemen_voucher and strain - Table 1). Each category was worked in a separate sheet, and to link all data was used the tag INSDSeq_locus. Data containing nomenclatures such as genus, species, and host species, was confirmed using the currently accepted name searched in the literature. Names of partial sequences patterned by the

Table 1. Tags chosen as classifiers and what is expected to be found in each.

Tag	Note
INSDSeq_locus	Access number of sequence generated after the sequence was submitted and approved by Genbank.
INSDSeq_organism	Name of organism according the submitted by author in sequence submission moment, according to the Nomenclature Committee of Fungi (NCF)
INSDSeq_create-date	Date that the sequence was applied to the platform.
INSDQualifier_name *	Union of metadata that refer to specifies about the sequence, they tend to be unique to isolate and share to sequence that was sequenced by the isolate.
country	Country where the sample was collected/isolated. To precision recommends that Country needs be followed by state/province, city or place separated by ":" (*ex. Brazil: Brasília, Asa Norte, Parque olhos d'agua*)
culture_collection	Culture collection number after the stored in culture collection, in general this is compound by the initials of culture collection institute followed by the individual number (*ex. CCUB1030*).
gene	Name of whole or partial gene that sequence was identified (*ex*. *Its, tef, tub,* etc.)
host	Wherever organism that isolate are collected (animal, plant, fungal, etc.), been mandatory the use of binominal name or the abbreviation to specie when the specific epithet was indefinite (*ex. Syzygium jambos*).
isolate	Isolate number give the culture obtained, in general had been used to the internal labs.
isolation_source	To discriminated physical or environment source and substrate that isolate was obtained, like dead wood, leaf litter, soil, fresh water, etc.
lat_lon	Geographic coordinate of the place collected, recommended the use in decimal format.
note	Free tags be filled what the submitter wants, like specifications of DNA extraction, collection, or culture medium protocols.
organelle	Name of organelle that sequence refer.
product	Which products are synthetized or are made part by the sequence
specimen_voucher	Used to dried specimens, follow the same pattern than culture_collection.
strain	General this is recommend using when the pure culture or than not deposited in culture collection
INSDQualifier_value	The tag is the value of all INSDQualifier_name sub tags.
INSDAuthor	Name the authors in publications
INSDReference_title	Title of the publication, when the sequence is dispatched before the publication use to been cited as directed submission.
INSDSquence.	Nucleotide sequence submitted.

* To some INSD qualifiers are strongly the use of pattern nomenclature standardized by the team, and can be consulted in https://www.insdc.org/submitting-standards/insdc-controlled-vocabularies/

gene abbreviation. The data country when cited only the country name was searched and added the capital to geolocate, in complete records like having city, province, region, or state was segregated in different columns, all data obtained added the latitude and longitude when unavailable in the dataset. To access that had lat_lon filled and country missing, was used the geographic coordinates to filled country information.

Source final analysis were maintained using the Excel® tools and all graphics was built in RStudio (2020) submitted all data to the ggplot2 package (Villanueva and Chen 2019) and geographical analysis was submitted to the leaflet (Cheng et al. 2023) and leaflet.extras (Karambelkar et al. 2023) packages, to create the bipartition analysis was used networkD3 (Allaire et al. 2023).

2.4 Results

The unfiltered data present in the Genbank platform show a total of 430.504 lines of data, referring to the 20.236 sequences (Genbank access) available. Among these unfiltered sequences, the research captured 0.4% of other genera that are not part of the *Calonectria*-like group.. Inner the 99.6% of *Calonectria*-like resulting four genera represent 98,4% of this total (*Calonectria, Cylindrocladiella, Gliocephalotrichum* and *Gliocladiopsis*), and *Calonectria* alone had 86.4% of all sequences available sequence this group. The genus *Curvicladiella* and *Xenocylindrocladium* are the two with less sequence submitted in platform, both summed had 81 access or 0.4%.

The number of isolates submitted in Genbank maintain the rating, as *Calonectria* (n = 5.586) as first placed, followed by *Cylindrocladiella* (n = 331)*,* *Gliocephalotrichum* (n = 275), and *Gliocladiopsis* (n = 135) (Figure 1 – A). Was observed that 369 access was submitted without the number of isolates. When we see the dispersion of sequence by specie the number differ expressively, with *Calonectria* and *Gliocephalotrichum* presenting 126 and 43 sequences by specie, respectively, and the other genus inner the 13 – 27 range, that factor may be related to the number of isolates by specie, that follow the same pattern (Figure 1).

Concerning the number of new species proposed by the literature can observe that *Calonectria* had an oscillation since 2020, when 39 species were synonymized

inner other species. Although this genus was described as the first species in 1867, the age of most specie inclusion was the 2010's early until 2020, with the inclusion of 33 new species in 2010, 42, and 27 in 2015 and 2016. Like *Calonectria*, the genus *Cylindrocladiella* shows the same trend in the same time interval, which had 76.6% of the species described from 2010 onwards. Unlike these two genera, the genus *Xenocylindrocladium* did not have any new species in the last twenty-two years (Figure 1C).

Looking at the distribution of partial genes implemented to all eight *Calonectria*-like genera was possible to see the dynamic of applying different markers to recognize species. The first sequences submitted were of *Calonectria, Penicillifer,* and *Gliocephalotrichum* in 1995, all three genera had the ribosomal (*lsu* and *its*) sequence deposited. Ribosomal sequences stayed predominant in the databank until 2000 when beta-tubulin (*tub*) sequence was introduced to *Calonectria* and 2001 to another genus in *Calonectria*-like. During 2003 and 2004, the histone-3 (his3), calmodulin (cmda), translation elongation factor (tef), and the second major subunit of RNA polymerase II (rpb2) regions were introduced as molecular markers. Later in 2010, the actin regions (act) and major subunit of RNA polymerase II (rpb1) were introduced. Currently, *tub* is the most sampled region with 5.287 sequences, followed by *tef* with 5.125 sequences, his3, cmda and its regions with 2,477, 1,832 and 1,752 sequences, respectively.. Both the most sampled partial genes are unique present as most sampled in *Calonectria* and *Gliocephalotrichum,* excluding this genus, in the other six, genes like *its* and *lsu* are predominant (Figure 1 – D).The dynamics of submitting new sequences has been consistence in *Calonectria* studies, with approximately 764 sequences being verified on average per year. Furthermore, Furthermore, there is a pattern of implementing new molecular markers over the years, which become widely used when they provide

a better phylogenetic signal. In *Calonectria* the implementation of non-ribosomal marker in 2000 was followed by a decrease in ribosomal sequences analysis.

Except for *Calonectria,* the analysis shows that ribosomal markers are important in the studies of these genera, and partial genes like *act, cmda, his3* and *rpb2* have fewer sequences, almost always presented in 2015 and 2020 (Figure 1 – D). In general, the rapid increase in the number of sequences is linked to the description of new species or studies with large territorial sampling.

We observed that within the genus there are some variations in the quantity and presence/absence of sequences between the species (Figure 1 - B). It was observed that the number of sequences per isolatevaries greatly, ranging from a single sequence to over 11, with the majority of sampled genus having an average of 3-4 sequences per isolate. The genus Aquanectria was the only one that had an average of two sequences per isolate, and all species have tub sequences, except for the unidentified organism that only has its sequence sequenced (Figure 1 - B and Figure 2).

In *Calonectria*, 71% of the species have more than six available loci and 17% have four or fewer. No locus covers the entire genus *Calonectria*, but the three most sampled are *cmda, tef* and *tub,* these two last in some species are over sampled, like in *Calonectria aconidialis, Ca. hongkongensis, Ca. kyotensis* and *Ca. pseudoreteaudii* were observed in over a thousand sequences. *Curvicladiella* and *Xenocylindrocladium,* by having only a few species recognized both have 100% of species with five or more loci sequenced. Every species of *Cylindrocladiella* and *Gliocephalotrichum* is down the sample loci media, but we see that 65% and 70% of species have five or more loci

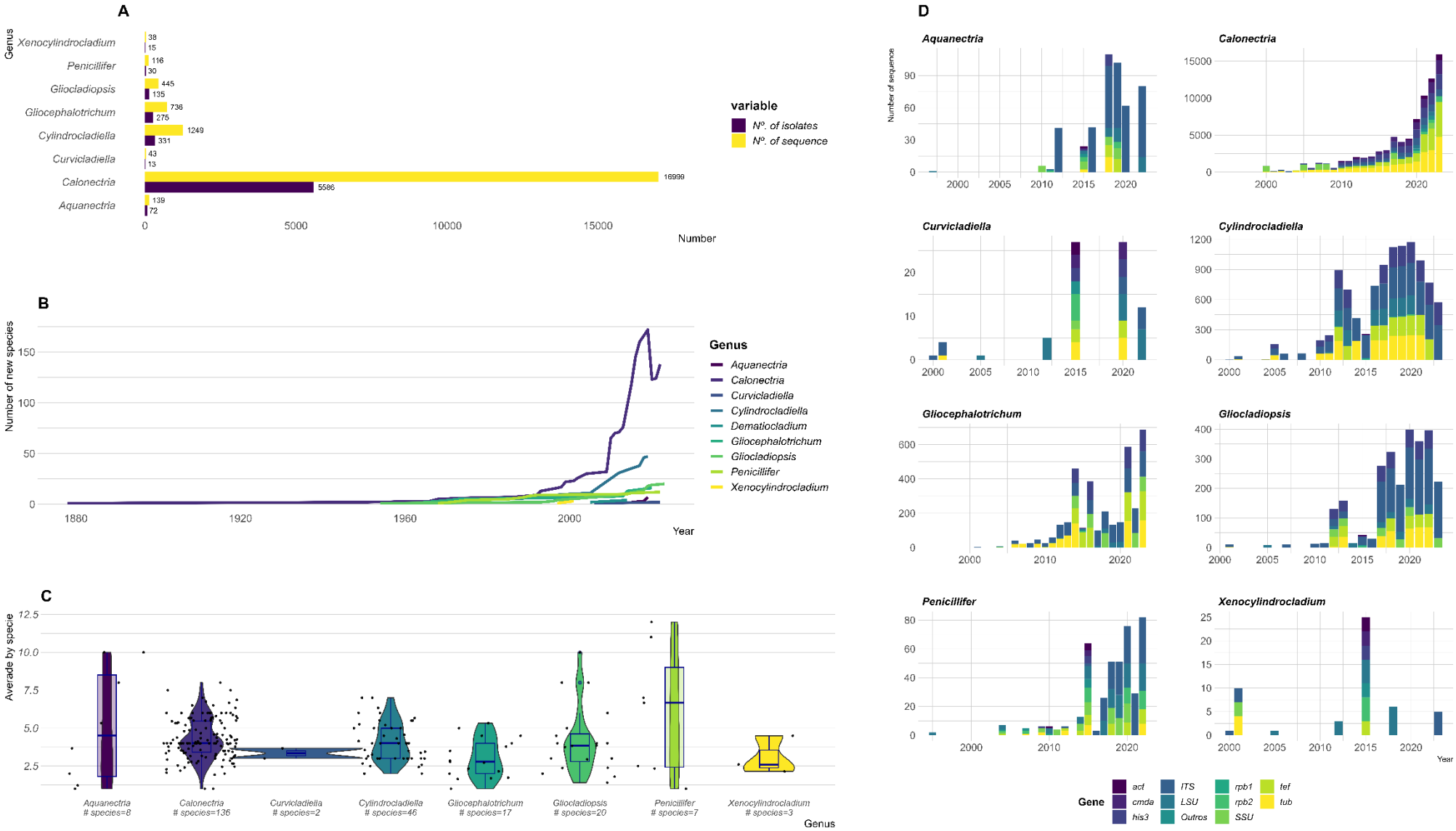

Figure 1. Distribution of data collected in Genbank. (A) Number of sequences and isolates by Calonectria-like genus. (B) Number of new species described all over the time. (C) Distribution of sequence by isolate for each genus inner the Calonectria-like, above of genus identification has number of specie founded on Genbank. (D) Number of sequences of each partial genus sequence by genus according to the year that was submitted to Genbank.


Specie
Xenocylindrocladium subverticillatum
Xenocylindrocladium serpens
Xenocylindrocladium guaiense
Penicillifer sp.
Penicillifer sinicus
Penicillifer pulcher
Penicillifer martinii
Penicillifer macrosporus
Penicillifer gloriosporus
Penicillifer bipapillatus
Gliocladiopsis wuhanensis
Gliocladiopsis whiteyi
Gliocladiopsis tenuis
Gliocladiopsis swieteniae
Gliocladiopsis pumilaensis
Gliocladiopsis sp.
Gliocladiopsis singaporiensis
Gliocladiopsis sagamiensis
Gliocladiopsis pseudotenuis
Gliocladiopsis peggii
Gliocladiopsis mexicana
Gliocladiopsis irregularis
Gliocladiopsis indonesiensis
Gliocladiopsis henneberti
Gliocladiopsis guangdongensis
Gliocladiopsis forsbergii
Gliocladiopsis elphidii
Gliocladiopsis ecuadoriensis
Gliocladiopsis curvata
Gliocladiopsis aquaticus
Gliocephalotrichum sp.
Gliocephalotrichum simplex
Gliocephalotrichum simmonsii
Gliocephalotrichum queenslandicum
Gliocephalotrichum oniense
Gliocephalotrichum nophcili
Gliocephalotrichum microchlamydosporum
Gliocephalotrichum frerianum
Gliocephalotrichum longibrachium
Gliocephalotrichum humicola
Gliocephalotrichum grande
Gliocephalotrichum cylindrosporum
Gliocephalotrichum cerycana
Gliocephalotrichum bubilium
Gliocephalotrichum brasiliense
Gliocephalotrichum bacillisporum
Gliocephalotrichum abrachium
Cylindrocladiella xishuangbannaensis
Cylindrocladiella vitis
Cylindrocladiella vitricola
Cylindrocladiella variabilis
Cylindrocladiella thailandica
Cylindrocladiella terrestris
Cylindrocladiella stellenboschensis
Cylindrocladiella sp.
Cylindrocladiella solicola
Cylindrocladiella reginae
Cylindrocladiella queenslandica
Cylindrocladiella pseudoparva
Cylindrocladiella pseudoinfestans
Cylindrocladiella pseudohawaiensis
Cylindrocladiella pseudocamelliae
Cylindrocladiella postalofficum
Cylindrocladiella peruviana
Cylindrocladiella parvispora
Cylindrocladiella parva
Cylindrocladiella obpyriformis
Cylindrocladiella novae-zelandiae
Cylindrocladiella nederlandica
Cylindrocladiella naulensis
Cylindrocladiella nalinensis
Cylindrocladiella microcylindrica
Cylindrocladiella malesiana
Cylindrocladiella longistipitata
Cylindrocladiella longiphialidica
Cylindrocladiella lateralis
Cylindrocladiella lanceolata
Cylindrocladiella lageniformis
Cylindrocladiella kuroshica
Cylindrocladiella infestans
Cylindrocladiella humicola
Cylindrocladiella hortiicola
Cylindrocladiella hawaiensis
Cylindrocladiella hahajimaensis
Cylindrocladiella ellipsoidea
Cylindrocladiella elegans
Cylindrocladiella cymbiformis
Cylindrocladiella clavata
Cylindrocladiella camelliae
Cylindrocladiella brevistipitata
Cylindrocladiella australiensis
Cylindrocladiella arbusta
Cylindrocladiella addisonii
Curvicladiella paphiopedili
Curvicladiella cignea
Calonectria yunnanensis
Calonectria xiamenensis
Calonectria venezuelana
Calonectria variabilis
Calonectria uxmalensis
Calonectria unisepiata
Calonectria tonkinensis
Calonectria terricola
Calonectria tanahensis
Calonectria syzygiicola
Calonectria sumatrensis
Calonectria stipitata
Calonectria spathulata
Calonectria spathiphylli
Calonectria sp.
Calonectria singaporensis
Calonectria silvicola
Calonectria rumohrae
Calonectria robigophila
Calonectria reteaudii
Calonectria quinqueseptata
Calonectria queenslandica
Calonectria pyrochroa
Calonectria pulchramosa
Calonectria pteridis
Calonectria pseudovata
Calonectria pseudoumalensis
Calonectria pseudospathulata
Calonectria pseudospathiphylli
Calonectria pseudoreteaudii
Calonectria pseudopteridis
Calonectria pseudopteviciata
Calonectria pseudonymphaeae
Calonectria pseudonectrioides
Calonectria pseudomalesiana
Calonectria pseudocuadriseptata
Calonectria pseudobrassicae
Calonectria propagenicola
Calonectria plumbicolor
Calonectria pini
Calonectria pinicola
Calonectria penicillioides
Calonectria pauciramosa
Calonectria pauciphialidica
Calonectria parvispora
Calonectria paragominensis
Calonectria parasitans
Calonectria paracolhounii
Calonectria pacifica
Calonectria ovata
Calonectria orientalis
Calonectria ochroramosa
Calonectria nymphicola
Calonectria naviculata
Calonectria multiseptata
Calonectria multiphialidica
Calonectria multinaviculata
Calonectria multilateralis
Calonectria monticola
Calonectria mexicana
Calonectria metrosideri
Calonectria madagascariensis
Calonectria malesiana
Calonectria madagascariensis
Calonectria macroconidialis
Calonectria lombardiana
Calonectria lichi
Calonectria leucothoes
Calonectria leguminum
Calonectria lauri
Calonectria lateralis
Calonectria lantauensis
Calonectria lageniformis
Calonectria ladung
Calonectria kyotensis
Calonectria inquinans
Calonectria indusiata
Calonectria inforesiae
Calonectria imperata
Calonectria ilicicola
Calonectria hunan
Calonectria humicola
Calonectria hongkongensis
Calonectria honghensis
Calonectria heveicola
Calonectria henricopiae
Calonectria hemileiae
Calonectria hawksworthii
Calonectria gracilis
Calonectria gracilipes
Calonectria glorioniae
Calonectria glabicola
Calonectria fujianensis
Calonectria fragariae
Calonectria eucalyptigena
Calonectria eucalypti
Calonectria ecuadoriae
Calonectria duoramosa
Calonectria densa
Calonectria cylindrospora
Calonectria convispora
Calonectria curvata
Calonectria crousiana
Calonectria colombiensis
Calonectria colombiana
Calonectria colhounii
Calonectria cochinchinensis
Calonectria clavata
Calonectria citri
Calonectria chinensis
Calonectria cerciana
Calonectria candelabrum
Calonectria canadiana
Calonectria californiensis
Calonectria bomiensis
Calonectria brevistipitata
Calonectria brasiliensis
Calonectria brassicae
Calonectria brassiasis
Calonectria brasiliensis
Calonectria breviana
Calonectria brachiatica
Calonectria borneana
Calonectria aviculata
Calonectria australiensis
Calonectria auriculiformis
Calonectria asiatica
Calonectria angustata
Calonectria amazoniensis
Calonectria amazonica
Calonectria aekrauiensis
Calonectria acicrotalis
Calonectria aciculata
Calonectria acicola
Calonectria acaricola
Aquanectria tenuissima
Aquanectria tenuispora
Aquanectria submersa
Aquanectria sp.
Aquanectria penicilloides
Aquanectria jacinthicolor
Aquanectria filiformis
Aquanectria deviana
act
cmda
his3
its
lsu
Outros
rpb1
rpb2
ssu
tef
tub
Gene
0
3
6
9
N
Genus
Aquanectria
Calonectria
Curvicladiella
Cylindrocladiella
Gliocephalotrichum
Gliocladiopsis
Penicillifer
Xenocylindrocladium
n
1000
500

Figure 2. Heatmap of sequence frequency by each gene available in databank inner species of genus Calonectria-like. The scale in blue represents the number of access available increasing while the blue tone turns in light blue. In right, the bars show the number of genes available by specie. The vertical line is the general media of all species (aprox. 6 sequences by specie).

sampled, respectively. When we examined the loci more frequently between these species, we observed that *its, tef,* and *tub* are present in 90% of species. Only *Cylindrocladiella brevistipitata* was two loci sampled until now. *Gliocladiopsis* saw a smaller number of species with more than five loci sampled (55%) and more number species in groups with less than 3 loci (30%). *Penicillifer* is the third genera with most species sampled in five or more loci. (Figure 2). In general, the frequency of loci over sequenced and submitted in the databank, refers to the species inner the genera *Calonectria* centered in five meanly loci (*act, cmda, his3, tef,* and *tub*) (Figure 2).

. Analyzing the records sent along with the sequence, the tags within the INSDQualifier_name provide essential information about host/substrate, country, isolate etc. It was possible to verify incomplete and inaccurately placed information by the data authors. Looking specifically at the categories "isolate," "country," and "host/substrate," there is a loss of approximately 19,647 data points. When classifying the categories by aggregated value, the “host/substrate” ranks first, accounting for 76.4% of all missing data, followed by “country” is the second with 21.7%, and “isolate” with 1.8% (Supplementary Figure 2 - A). The absence of these data is particularly pronounced in genres such as *Curvicladiella, Gliocladiopsis* and *Penicilifer* which showed a 27 to 41% lack of isolate identification, while *Calonectria* was observed in only 4% of all sampled sequences. Both “host/substrate” and “country” categories have more than 66 and 71% missing data, respectively, across all analyzed genera (Supplementary Figure 2 - B). To further analysis we eliminate accesses that have some missing data according to the category analyzed.

There are 6,414 isolates identified in the database, among which 5,567 (86.8%) belong to the genus *Calonectria* against 847 isolates from the other 7 genera (Supplementary Figure 3 - A).In summary, there are some species with a considerable number of isolates, an example is *Calonectria,* it has 4.4% of the species have 200 or more isolates, while 39.3% of all species have only one isolate reported in database. In total genera such *Curvicladiella, Penicillifer* and *Xenocylindrocladium* all have species with less than 16 isolates (such *Pe. Diparietisporus*). *Aquanectria, Cylindrocladiella,* and *Gliocladiopsis* have all their species with less than 80 than isolates reported. *Gliocephalotrichum* presented 58.8% of the species with less than 10 isolates, and only 5.9% between 40 and 80 isolates, but no species exceeded 100 isolates.

An alert data concerns the number of species with one isolate, within seven genus, five present 41- 55% of the species in this situation (Supplementary Figure 3 - B). While 17.4% of all species have a single strain, *Curvicladiella*, *Gliocephalotrichum,* and *Xenocylindrocladium* had two or more isolates per species. The most studied genus, *Calonectria* presents 2.9% of the species with a single isolate, on the other hand, the genus is the only one that has six species with more than 200 isolates, which are: *Ca. aconidialis* (1.378), *Ca. hongkongensis* (1.026), *Ca. pseudoreteaudii* (468), *Ca. kyotensis* (374), *Ca. ilicicola* (269) and *Ca. acaciicola* (204 - Supplementary Figure 3 - B).

For this class of data, we observe that 15.498 sequences have the country tag filled, among which only 94 sequences have coordinates information (Supplementary Figure 2 – A). It has been reported that at least one species of *Calonectria*-like is found in 66 countries across six continents (Figure 3 – C. In spatial analysis we observed that 79.2% of species are found between the tropics, and 46.3% are found down to

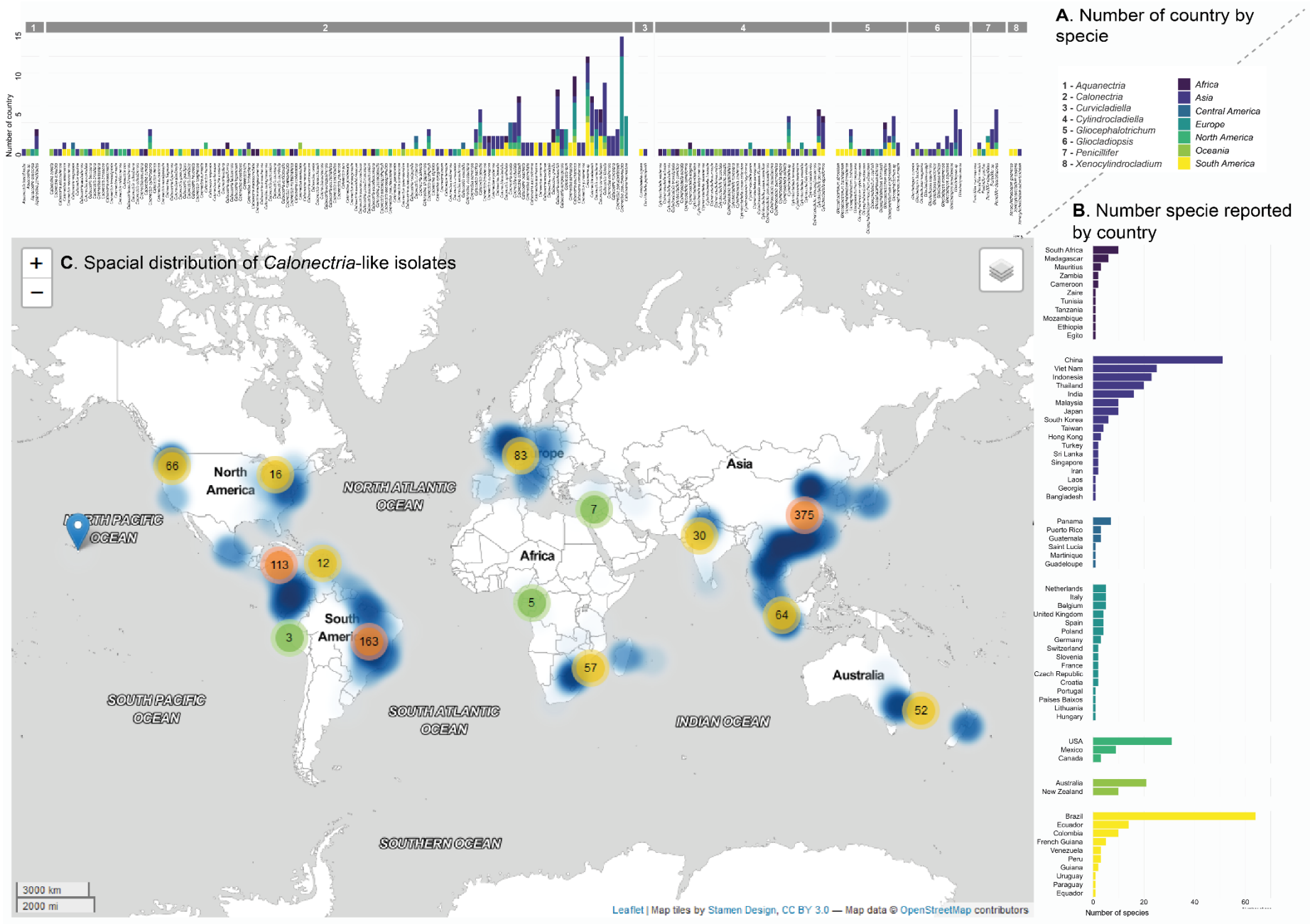
A. Number of country by specie
1 - Aquanectria
2 - Calonectria
3 - Curvicladiella
4 - Cylindrocladiella
5 - Gliocephalotrichum
6 - Gliocladiopsis
7 - Penicillifer
8 - Xenocylindrocladium
Africa
Asia
Central America
Europe
North America
Oceania
South America
Number of country
B. Number specie reported by country
South Africa
Madagascar
Mauritius
Zambia
Cameroon
Zaire
Tunisia
Tanzania
Mozambique
Ethiopia
Egito
China
Viet Nam
Indonesia
Thailand
India
Malaysia
Japan
South Korea
Taiwan
Hong Kong
Turkey
Sri Lanka
Singapore
Iran
Laos
Georgia
Bangladesh
Panama
Puerto Rico
Guatemala
Saint Lucia
Martinique
Guadeloupe
Netherlands
Italy
Belgium
United Kingdom
Spain
Poland
Germany
Switzerland
Slovenia
France
Czech Republic
Croatia
Portugal
Países Baixos
Lithuania
Hungary
USA
Mexico
Canada
Australia
New Zealand
Brazil
Ecuador
Colombia
French Guiana
Venezuela
Peru
Guiana
Uruguay
Paraguay
Equador
Number of species
C. Spacial distribution of Calonectria-like isolates
North America
NORTH ATLANTIC OCEAN
NORTH PACIFIC OCEAN
Africa
Asia
Australia
SOUTH PACIFIC OCEAN
SOUTH ATLANTIC OCEAN
INDIAN OCEAN
SOUTHERN OCEAN
66
16
83
7
375
30
113
12
5
64
3
163
57
52
3000 km
2000 mi
Leaflet | Map tiles by Stamen Design, CC BY 3.0 — Map data © OpenStreetMap contributors

Figure 3. Global distribution of all species inner Calonectria-like group. A. Number of country by specie, B. Number of specie by country. C. Global distribution of number of specie, different intensity of blue indicates the number of specie find in that place, blue shade represents the intensity of diversity species, how more intense the red more different species are present in that place.

Tropic of Capricorn and up to Tropic of Cancer. No species are found within the Arctic and Antarctic circles. On the other hand, the number of isolates followed the opposite pattern, with 77% outside tropics and 23% in tropical boundary. In summary we see that diversity decreases up to 2.300km to south and 4.500 km north of the Equator line. Due to the extension, Asia presented 103 species spread across 17 different countries, followed by South America with 87reported in six countries and North America with 40 species across three countries (Figure 3). In Asia, China is the country with bigger number of species (a total of 51), with 66% of these being *Calonectria* species. Similar proportions are seen in countries like Indonesia and Vietnam, where approximately 66 of all species found in Asia are endemic to this continent. Among them, 33 belong to the *Calonectria* genus, and 18 belong *Cylindrocladiella* genus (Figure 3 – A and B).

According to the data, South America is the continent with the highest number of species in eight genera and the most diverse in *Calonectria*-like and 53 species of *Calonectria* reported in its territory (Figure 3 – A and B).

Of all species found in South America 62% are endemic, this percentage is the majority of *Calonectria* species, out of a total of 45 endemic species. Looking at the global distribution of each genus, we can see that the two countries act as diversity hotspots.. Brazil can be considered a hotspot for *Calonectria* and *Gliocephalotrichum* due to the number of species (60 in total and 48 endemic), isolate (247), territorial distribution (27 places) and host/substrate register (30 different hosts). While China can be considered *Cylindrocladiella, Gliocladiopsis*, and *Penicillifer* hotspot.

Xenocylindrocladium based on reported data is present in two South America countries, French Guyana, and Ecuador.

Classifying the species according to the number of countries in which it occurs, we observed that *Calonectria pauciramosa* is the only species present on six continents, distributed in twelve different countries. *Calonectria pseudonaviculata* is present on four continents, but the number of countries and locations is greater than *Ca. pauciparous*, being present in 18 countries and 37 different locations. The same relationship happens with *Ca. ilicicola, Ca. hawksworthii, Ca. colhounii* and *Ca. pteridis* where all three can be found on five continents, but the number of countries is less than *Ca. ilicicola* that is only reported on four continents (Figure3 – C).

A different pattern to filled and blank data was seen in host/substrate tag. The total data filled in is 22% of all sequences analyzed. Compared to the total number of isolates submitted to the databank, there is a loss of 76.3% location information (Supplementary Figure 3 - A). Summarizing the losses, we observed that *Aquanectria* has few species, they present 62.5% of their species without host/substrate information, and the number of isolates without this information grow to 76.1%. *Calonectria* remain 78.6% in of its isolates and 28.6% of species without host/substrate information. Only *Curvicladiella* and *Xenocylindrocladium* have all species with at least one host/substrate referenced isolate. In data available on databank, it was possible to detect that 226 host species are classified, distributed in 146 genus, 82 families and 45 orders (Figure 4).

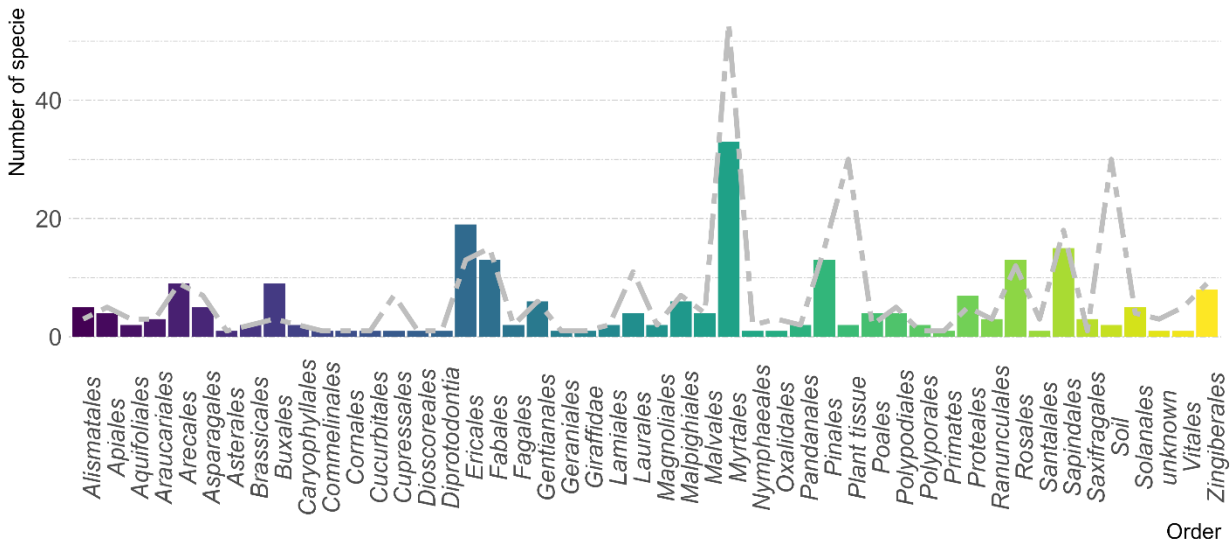

Figure 4. Bars represent the number host species by each order and the grey dash line represent the number of fungal specie reported in each host order.

In databank was registered as host/substrate species in plant, animal, and fungi kingdom, these two in less frequency (Figure 4). Was observed that Myrtales, Ericales, Sapindales, Pinales and Fabales, are the five orders with most species associated with *Calonectria*-like sequences. Seen the number of sequences by host order, the raking change, maintain Myrtales as the first, followed by Pinales, Buxales, Sapindales and Fabales. Myrtales beyond of been the order with a greater number of host species (33 species distributed in 9 genus) and *Calonectria*-like sequences associated (2.133 sequences) distributed in 53 species of this fungi group and 579 isolates associated with these hosts. Nevertheless, Myrtales order is associated with three fungi genus, *Calonectria, Cylindrocladiella* and *Gliocephalotrichum.* While Sapindales remains present in five genera, and Fabales, Ericales and Arecacea in four. Inner all 45 orders compiled on databank, around 58% are associated with only one genus, among these 84% are exclusively found in *Calonectria* records and 16% in *Cylindrocladiella.*

On the fungal side, it was observed that *Curvicladiella* has sequences obtained from isolates collected from plant tissue without plant species identification.

Other genera, such as like *Aquanectria* and *Xenocylindrocladiella,* remain associated with some plant orders such as Arecales, Pinales Alismatales and Sapindales. *Gliocephalotrichum* and *Penicillifer* are associated with eight and nine orders respectively, with the difference that the first is associated with 16 plant genera while the second with eight genera. *Gliocephalotrichum* has 58 isolates collected from species of *Nephelium*, 27 in *Caryocar* and 13 in *Spondias*, with no one-to-one relationship (a genus for a species fungi) in these reports. *Cylindrocladiella and Gliocladiopsis had few differences in relation to associated hosts, differing in only three orders.* The first, plant tissue source is the substrate most reported. Soil is the second most mentioned substrate with association of ten species (21 isolates). To *Cylindrocladiella* two species present the relation one to one (one fungi to one host), like *Cylla. xishuangbannaensis – Pandanaceae* and *Cylla. postalofficium*. *Acacia* species had four *Cylindrocladiella* exclusively reported. In *Gliocladiopsis* 78 % of species (11 species and 44 isolates) are reported as collected in plant tissue source. In association with some plant genera, we see twelve reports of species, and example is *Glio. indonesiensis* and *Glio. peggii* were collected exclusively in dead plants tissue and soil.

As *Calonectria* is the genus with the highest number of species in the group, it was analyzed separately. The majority of species were reported in association with Myrtales, accounting for 57% of the species and 568 isolates. Myrtales is present in ten out of the eleven species within the *Calonectria* complex, with the exception of the Ca. naviculata species complex, which is not related to this order.. Specie complexes such *Ca. reteaudii, Ca. kyotensis* and *Ca. candelabrum* are the most sampled within the Myrtales order, collectively representing 75% of the reports. Within this host order, the genus *Eucalyptus* is the most frequent, with 535 isolates (93%) identified from this

source, including in *Ca. pteridis, Ca. reteaudii* and *Ca. spathiphylli,* which are the only genera from this order present. *Ca. reteaudii* species complex alone accounts for 38.4% (142 isolates) of the registered isolates with *Eucalyptus* as the host, making *Ca. pseudoreteaudii* as the most frequently submitted fungus to Genbank from the *Eucalyptus* samples.

The second order with the highest number of related isolates is Pinales with 120 isolates, followed by Buxales with 114 isolates and Fabales with 82 isolates. Pinales is a short order in number of genera sampled, with Picea and Pinus the only two related to *Calonectria*, being *Pinus massniana* very common having 88 isolates

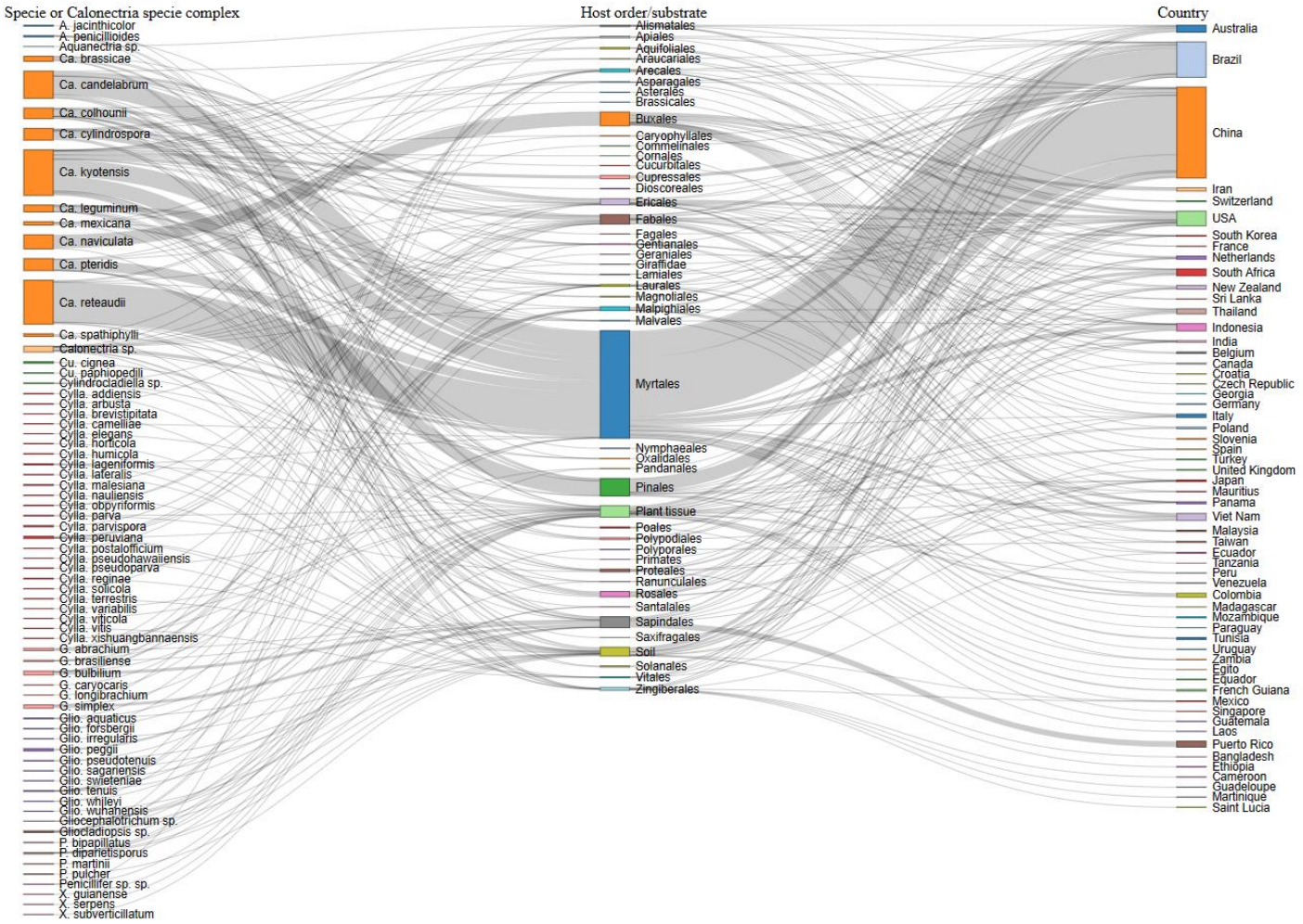

Figure 5. Sankey network between genus of Calonectria-like and host orders inputted together in database. The bar size indicates the number of reports in database.

collected in samples of this species. A similar is observed in Buxales, which includes

the genera *Buxus, Pachysandra,* and *Sarcococca*, but most isolates are collected from samples of *Buxus semperviverens* (52.9% or 64 isolates). This order is limited to the *Ca. naviculata* species complex and *Ca. pseudonaviculata* and *Ca. henricotiae* are the two species commonly isolated in the databank. The order Fabales, further down the list, encompasses seven 7 genera associated with the fungi, with *Arachis* and *Glycine* standing out in terms of the number of reported isolates, with 52 and 17 isolates, respectively.

Looking for the side of fungi, the databank shows that *Calonectria* has a more restrict species complex. For example, *Ca. naviculata* is associated with only nine species within two host orders, or *Ca. spathiphylli* reported in six species within three host orders. Meanwhile, *Ca. kyotensis* species complex can be considered as multi-host by the number of host order information, it is present in 22 orders, reported in 51 host species (Figure 5). Regarding the number of host orders, *Ca. ilicicola* was associated with 13 orders, while *Ca. pauciramosa* had eight orders, this last, has less diversity in terms of the number of family and genera number, but it has one more host species than the *Ca. ilicicola.* We observed that more species are restricted to the host order and species than multi-hosts. Species that have only one reported host genus represent 53.7%, and those with one host species represent 46.2%, while species present in five or more host orders are 16.2% and with 10 or more host species are 10%.

2.5 Discussion

All the metadata are provided by the author submitting the sequence, in a file named by the “source”. In this file the author can fill in information about 39 categories, an example is provided by the platform during the submission process. Despite this

information is required, many authors don't fill it in, causing a large gap in Genbank metadata. Healthily databases can provide support for studies of fungal communities, global distribution, hypothesis about temperature, altitude host preference and fungal habits (Větrovský et al. 2019). But without a necessary supply, databases with missing or incomplete data can generate bias in analysis and decision-making (Hunt et al. 2021). An example of application of this type of study is in quarantine barrier, as in the case of *Ca. henricotiae* and *Ca. pseudonaviculata* both species are related as pathogens of *Buxus sempervirens* but currently, different populations and matings of this species are globally restricted in European, Asian countries, the United States of America, and New Zealand (Malapi-Wight et al. 2019). Such species are absent in South America, which produces *B. sempervirens* as an ornamental plant, and are necessary a to monitor the risk of entrance in these countries. The observation that South America is free of fungi that cause boxwood blight disease is confirmed by the global distribution analyzed in this study.

We observed that most of the access available in the Genbank database is from *Calonectria*. This correlates significantly with which hosts are associated and what economical damage this interaction can cause. Evidence of this lies in the fact that a *Eucalyptus* sp. is the genus with 93% within Myrtaceae Order with several associate *Calonectria* accessions d. It is knowledge that *Calonectria* is a threat of global importance to the sustainability of *Eucalyptus* production because they cause disease that affect all stages and parts of the plant, with *Calonectria* leaf blight (CLB) being the most devasting (Crous 2002; Liu et al. 2020; Bose et al. 2022). A recent revision (Bose et al. 2022) identified 35 species infecting *Eucalyptus* in 13 countries. Coincidentally, the pattern of increasing access to the databank is similar to the increase of *Eucalyptus* plantation areas (Zhang and Wang 2021). Our data show that

the number of countries reporting this interaction is currently 18, the number of *Calonectria* species grew to 44, and affect 15 different *Eucalyptus* species. Our study shows that there are *Eucalyptus* reported with susceptibility to more than one *Calonectria* species, such *E. grandis, E. urophylla* and *E. tereticornis.* The information that *E. grandis* has 24 species of *Calonectria* associated, is due to the fact that this species is the most planted in the world (Zhang and Wang 2021), and it has already been demonstrated that specie is highly susceptible, with 80% of defoliation in commercial plantation in Brazil (Ferreira et al. 1995; Alfenas et al. 2015).

Applying efforts to one genus, such *Calonectria,* due to its economic importance, can provide patterns for studying another closely genus, like what happens with the other six allied genera. This pattern can be observed in the standardization of culture medium for morphological characterization -, sexual mating test, and better markers. But it can create some challenges for major genera and their allied. One of these challenges is related to the divergent number of sequences for markers. In the databank, we can see the predominance of *tub* and *tef* sequences (5.287 and 5.125 sequences, respectively) while the others all markers add up to 48% of submitted sequences. Liu et al. (2020)mention that some *Calonectria* species were recognized in different studies using different markers, making inconsistency a complete phylogenetic analysis and consequently a lack of interpretation of what define a phylogenetic specie. The standardization of markers to be used as genus and species barcodes had been proposed and discussed since 2012 (Schoch et al. 2012), and defined that it *its* and *lsu* are barcodes for genus, and for *Calonectria* species n uses *cmda, his3, tub, tef* and, *rpb2* (Marin-Felix et al. 2017) and to *Cylindrocladiella, his3, tef1* and, *tub* are used (Marin-Felix et al. 2019). Nevertheless *Aquanectria, Gliocephalotrichum, Gliocladiopsis, Penicillifer* and *Xenocylindrocladium* haven't been

defined. By analyzing the frequency of each marker in this non-standard DNA barcode, we suggest that future studies of *Aquanectria, Gliocephalotrichum* and *Gliocephalotrichum* proceed using *his3, tef* and *tub* as barcodes. In recent year all three genera had new species well supported using these markers (Lombard and Crous 2012a; Gordillo and Decock 2019b; Zhai et al. 2019; Silva et al. 2020a). *Penicillifer* and *Xenocylindrocladium,* both, have a small number of recognized species, and both have more than 66% of their species with six markers (*act, cmda, his3, rpb2, tef* and *tub*), excluding the ribossomal markers. For these two genera, we recommend that future studies follow the same pattern as for *Gliocephalotrichum* and *Cylindrocladiella*, and when the number of species increase, a deep analysis of resolution and of the best phylogenetical signals can be performed.

Despite the DNA barcode proposal for *Calonectria* in 2017, many gaps were made before and after this patterning. As a compromise to resolve and enforce the barcode standard in 2020, a review was published that sequenced the missing genes for 128 species to analyze which marker separately or in concatenate formation provided the best resolution. This publication studied phylogenetic construction of 8 markers (*act, cmda, his3, its, lsu, rpb2, tef* and *tub*) and suggest that to describe and identify all *Calonectria* species with high confidence is recommended to use of six region (*act, cmda, his3, rpb2, tef* and *tub*) as best approach (Liu et al. 2020). Similar studies have been published and recommend that for *Colletotrichum* species the use of *gapdh, tub* and *his3* provide high support to species in all species complex, and the set of markers may be different according to the analyzed complex (Vieira et al. 2020). Some studies, besides proposing a set of markers such as barcoding, review and refine knowledge about phylogenetic concept of species, and therefore, the more complete the databank, the more robust and unbiased is the conclusions. Liu et al.

(2020) reviewing the species in the paper, demonstrate that some species are the same phylogenetic species and, the morphological difference isn't sufficient to support the specie status. The author reduced 51 species of *Calonectria* to the synonym status for another based in six-markers analysis approach. In Maryani et al. (2019) the use of databank suggests the author segregate *Fusarium oxysporum* f. sp. *cubensis* and raised some clades to specie level using only three markers (*rpb1, rpb2* and *tef*). But these new proposed species are questionable in Bedoya et al. (2021) meanly in the databank formed by the first author, due de absence of some sequences to key isolates, resulting in a phylogenetic analysis with divergent number of markers among the proposal species, and lack of clade support in key clades.

The gap in genus with reduced number of species described is less than in genera with substantial number of species, like *Calonectria, Cylindrocladiella, Gliocephalotrichum* and *Gliocladiopsis.* But the number of isolates that are best locus number sampled is minimal, staying restrict to one isolate by species, or even, species are not raisin without barcode sequences, in the case using only *its* as DNA barcode (Schoch et al. 2012). In total we found 20 species submitted to the database with a single isolation, most of them in *Gliocladiopsis.* By convention, the taxonomic community recommends that to propose new species, evidence needs be based on multiple observations, to possible enable comparisons, encouraging a minimal of two isolates (Aime et al. 2021a). The same community, referring to the introduction of new species, isn't recommended using a single locus, spatially *its* sequences, only in exceptional cases. In phylogenetic analysis, the application of a minimal number of isolates sequenced help to support the clade and accuracy, due to the recognition of parsimony informative loci that are unable with a single sequence(Aime et al. 2021a; Chethana et al. 2021).

The submission of new species without a strong support means that the community needs revisions in a short time, this process leads in some cases to the synonym of a valid species, like what happens in the revision of 2020 to *Calonectria*. As a result, due to the responsibility for correcting and updating the actual name of the submitted author, this update often doesn't occur, causing less experienced researcher misunderstanding. An important improvement in fungal nomenclature is the abolition of dual nomenclature to distinguish the sexual morph, adopting only one name (Taylor 2011; Aime et al. 2021a). Despite this change proposed in 2011, currently Genbank accessions named after old name for the anamorph state, *Cylindrocladium*. Even in recent publications such as (Liu et al. 2020)where 51 specie were synonymized, they remain unaltered in Genbank, waiting author revisions.

Database analysis faces another challenge regarding the number of isolates/strains, with many divergences regarding this identification. The main divergence occurs due to the transfer of isolates between collections. In general, many laboratories start the studies using the number of personal or local isolates and submit with this number all the data generated in public databank, and even in some cases, these isolates are transferred to another collection receiving a new number of isolate, and may, in turn, participate in another publication with a new number of isolate. These multiple identifications are usually placed in the access table as different isolates. An example is *Calonectria angustata* CMW 30990 (type isolate), it was first published under isolate number P99-0454 (specimen voucher = STE-U 2347) and submitted for *tub* sequence only (AF207543- Crous et al. 2000), then implemented under CPC 2347 in other collection, followed by deposit in CBS collection, the number CBS114544 and submitted six new sequences to the Genbank (*act, cmda, his3, its, lsu* and *tef* - Crous et al. 2000; Lombard et al. 2010). In 2020, the same isolate received a fourth isolate

number, CMW 30990, and submitted four other sequences (*act, his3, Its* and *rpb2* - Liu et al. 2020) In the case of *Ca. angustata* old single isolate numbers are cited in tag notes, an inefficient way to refer to historical numbers. There are other examples like *Ca. pacifica, Ca. leucothoes, Ca. gracilis, Ca. gordoniae* and *Ca. clavata* that have five different culture collection numbers (Liu et al. 2020).

Such an event commonly happens, taking a publication and looking at the Genbank accessions that cited 240 isolates, only 13 of these had one isolate number. We call this as “Matryoshka doll effect” because the specimen remains the same, only the new isolate number is changed, and the data is sent. The proposal unifies the name of the fungi was intended to avoid misunderstandings and all data to be under the same name,, but the constant of isolate numbers can provoke some errors as what occurs before the standardization of a name for different forms (sexual and asexual). The constant change in the number of isolates can cause: *i)* Interpretation of different and not equal isolates; *ii)* Absence of a sequence set due to a different number; *iii)* Excessive interpretation of real number of isolates available in databanks; *iv)* Lengthy to construct the table of accession numbers; *v)* Difficulties in tracking the specimen's history due the multiples isolated numbers and data and publications linked to the specimen. To reduce such problems and make the databank clearer, trackable, and uniform for all levels of researcher expertise, we propose that a universal number be adopted for submission in the Genbank databank. This can be the first isolated number that specimen received when it was stored, independently of whether or not the collections that are stored change during the current publication. The others isolated numbers particularly from each collection, become internal works and cited in notes on Genbank, but staying out of publications.

There is a great paper summarizing good practices for publishing a new fungus species, it contains recommendations on what are valuable information should be uploaded with the sequences in a databank (Seifert and Rossman 2010; Aime et al. 2021a). In total, the author cites seven minimal data that are important to compose the metadata sequence, which are: place of collected the isolate, habitat, substrate and host, geographic coordinates, altitude, collection and/or isolation data, collector and its number, access number or barcode in fungarium/herbarium (Aime et al. 2021a). As we demonstrated in results, a lot of these minimal data, observing at location information, are 21.6% missing from the database. The presence of location in the metadata is an important source for future studies of global distribution, support for barrier politics, prevention of entrance of genetic variant foreign pathogens, population genetics and dynamics comprehensions, prevention of breeding programs, understand of spatial dispersion and diversity drivers and possibles host jumping of important pathogens.

Only a few studies have focused on large-scale fungal distribution, many are centered on country scales and only a few host-species interactions, such *Calonectria* in *Eucalyptus* and *Pinus* plantation soils in China (Alfenas et al. 2015; Liu et al. 2022; Lang et al. 2023; Liu and Chen 2023). The large registers of places data accumulated in public metadate repositories across numerous studies allowed us to analyze them in combination. Some examples of meta-analysis to be comprehend to clarify which factor is important for the distribution of fungi in the soil,, summarized 36 papers with data from more than three thousand samples (Větrovský et al. 2019).Another explored 843 papers util 2019 margin sequence of *its* and geographical coordinates available to create a public interface to research (available to consult in https://globalfungi.com - Větrovský et al. 2020). In Nectriaceae, a notable example is *Fusarium circinatum*, the

causal agent of Pine Pitch Canker, which had a global distribution of 6.297 samples collected in public databanks (Drenkhan et al. 2020).

The unique review in *Calonectria* is about the global distribution of this genus causing leaf blight in *Eucalyptus,* which observed that disease is reported in thirteen countries (Bose et al. 2022). In this study, database meta-analysis showed that *Calonectria*-like is spread across six continents and 66 countries, with Brazil and China being the two countries with the highest numbers of isolates and species. The global distribution of isolates and specie changes according to the genera we analyze, like *Calonectria, Cylindrocladiella,* and *Penicillifer* follow a pattern present in six continents, *Gliocephalotrichum* and *Gliocladiopsis* the first is more reported in Central and South America, while the second in Asia, *Aquanectria* and *Xenocylindrocladium* are restricted in South America and Asia. All use 5.982 reports with locations indicated in the Genbank database.

A platform that gathers data from global repository to facilitate direct access to all type of life on Earth, is the Global Biodiversity Information Facility (GBIF.org 2023). There are 3.706 register more records than we analyzed here, otherwise, some conflicts with some literature, such as the number of species for each genus, totaling 421 species, while the currently accepted species are around 217 species. All this is stored in 94 datasets such as Fungarium/Herbarium and shows in GBIF platform (GBIF.org 2023), so perhaps some reports are not passed by the review, which is a gap to characterize the studies. Initiatives to gather data and release it in one local to provide an important source for composite phylogeographic studies. Despite the amount of data available in databases such as Genbank, this approach wasn't applicable in nether of these genera. Such an approach provides a better understanding of the migrations process, population size, emergence patterns, spread

of pathogens, and new more infectious genotypes or follows gene flow over time (Rasmussen and Grunwald 2021). An example of a similar study is in relation to the point of divergence when *Ca. naviculata* specie complex separates with the divergence point of *Buxus sempervirens* dated in 40 – 50 million years ago, that can indicate a coevolution process between these organisms (Krutzsch 1989; Carvalho et al. 2016; Malapi-Wight et al. 2019). Another direction was presented to *Ca. henricotiae* and *Ca. pseudonaviculata* both are restricted in Asia, Europe, North America, and Oceania continent and all over the 20 years the last species continue to spread in the USA and this population is clonally disseminated due to the absence of a compatible mating type identified in the USA territory(Malapi-Wight et al. 2019).

Proportionally, we see some tendency for species diversity to be in the tropic region near the Equator line, decreasing diversity the farther the locations are from the Ecuador line. The high frequencies observed in the intervals from 30º to 40 º north are due to the presence of 49 species described in China without precise geographical coordinates, considering the capital of the country as the location of the species for these cases. Richness of fungal are cited as decreasing exponentially at the same time as to increase the latitude, in response to environment and vegetation conditions, suggesting that there are more endemic species endemic in the tropical than extratropical region (Tedersoo et al. 2014). Species diversity may be linked to spatial location, with higher diversity at low latitudes and characteristics of soil, climate, vegetation, and fungal community composition (Větrovský et al. 2019), but the endemism is more connected to the capacity of spread and adaption to a new condition of infecting and colonizing a new host.

Within the *Calonectria* – like genus, soil fungi are those capable of infecting parts of living plants, causing tissue necrosis due do their hemibiotrophic habit (Yang

et al. 2022). *Calonectria* is cited as associated with closer 100 plant families and approximately 335 species of hosts plants (Crous 2002; Lombard et al. 2010d) and remains unchanged since 2010. Nevertheless, when we compiled all metadata informed in Genbank, there is a reduction in number, 178 species distributed in 67 families and in 42 orders only for *Calonectria*. As the author compiled data from many publications, a lot of data not referenced in Genbank, causing this gap. As the cited by Lombard et al. (2010b), the five most important families for the genus are *Myrtaceae, Pinaceae, Fabaceae, Ericaceae* and *Buxaceae,* here in a different order from that observed by the author. For all seven genera, the number increases to 215 host plant species within 79 families and 46 orders and repeats the patterns of the most important number of associated plant species. In the concern number of isolating the rate change, add *Sapindales* as fourth followed by Fabales*.* As most species of the sexual genus are obtained from samples of soil or dead plant tissue they detain 146 isolates.

Brazil and China domain in the number of isolates reported in Myrtales *Myrtales,* within the genus *Eucalyptus,* both countries where the genus is exotic, and introduced for commercial purpose (Crous et al. 2019). Only in *Eucalyptus* are 46 species found in two genera of *Calonectria*-like in eighteen countries, a hypothesis arises that this is associated with the rapid growth of plantations gathering about 20 million hectares worldwide, causing plantations involved to selection pressure (Crous et al. 2019). As the impact of these pathogens is dangerous to success of *Eucalyptus* plantation, efforts are being rapidly increased to identify and control them. Community associated with *Eucalyptus* differs by country, as shown here and in Bose et al. (2022). *Eucalyptus* is native to Australia and was part of 74% of forested land, in the analyzed data we found only *Ca. reteaudii* and *Ca. queenslandica* infecting this genus or even in *Myrtales*.

In the total of the databank analyzed, there were presented around 81 restricted species associated with one plant genus, meanly in *Calonectria* (45 species) and *Cylindrocladiella* (19 species), the number of exclusive associations increases according to the increase in the taxonomic level of the plant, reaching up to 87 species of fungi at the level of order. Despite *Cylindrocladiella* is commonly associated with plants, there is no pathogenicity test probe for the host-parasite association. Meta-analysis using plant reports in Genbank databank acting as survey to systematics of register flora inner country boundaries, accompaniment of increase plant-fungal association range, tracking plant species in dangerous and cophylogeny studies. Using the Genbank databank, the Mexican authors summarized 14,615 species one representation of 43% of all Mexican flora, and 27% of these are endemic species (Maya-Lastra et al. 2022). The author cited that Genbank records are underrepresented, these authors cite this due to few publications reducing the number of taxonomic units in the analysis of phylogenetics tree (Maya-Lastra et al. 2022).

Another type of study that the databank can be used is in cophylogeny approaches, in which hypothesis of coespeciations, extinctions, duplications, and host-shift events contempt the parallel comparisons between host and parasite phylogenies (Refrégier et al. 2008b)(Refrégier et al. 2008)(Refrégier et al. 2008b)(Refrégier et al. 2008). In *Calonectria* a similar hypothesis was proposed in the analysis of the mating type population in *Ca. henricotiae* and *Ca. pseudonaviculata,* due they are reported causing disease only in *Buxaceae* species and are geographically restricted in few countries (Malapi-Wight et al. 2019). Another hypothesis to be investigated in the feature is the Nectriaceae – Myrtaceae relationship*,* based on the number of plant species affected by the fungus and the number of species restricting associated with this plant. Recent studies place the Nectriaceae and Myrtaceae in same period of birth.

Nectriaceae is estimated to have diverged by ~97.28 Mya, and Myrtales by ~116 Mya, while Myrtaceae was by ~92.78 Mya in the late Cretaceous west of Godwana, super land that gave origins to southern countries, such South America and Africa (Torsvik and Cocks 2013; Berger et al. 2016; Balbinott et al. 2022; Yp et al. 2023). The unique time convergence for *Calonectria* placed five species converging in ~40.22 Mya (Malapi-Wight et al. 2019)leaving this genus within the time range of the Myrtaceae diversification process. Following the same pattern, phylogeography in conjunction with plant association data can show the migration process due to the commercial of infected plant tissue.

The examples cited before suffer threat numbers to succeed in these approaches: As the case shown, the divergence between Genbank database and the presented by GBIF, happens due to some records being stored in herbarium/fungarium, being public database under-estimated of diversity collected. Failing to enter minimal data in the records, filing in wrong tags, or misidentifying place, plant or fungal can lead to distorted interpretations. Even when surveying some markers, global distribution can be a challenge. Kõljalg et al. (2013) increase in your methodology steps to process taxonomic annotations of Genbank access collect relevant metadata (voucher specimen/culture, country of origin and host/ substrate of collection) consulting publications manually or getting in touch the original authors, while this information can be part of submission process of sequence in Genbank database.

This lack of annotation is also cited in studies about genomic and metagenomic (Yilmaz et al. 2011). This absence of data integration makes even complex searches in data bank impossible to get datasets segregated by the environment's location of host/substrate. Yilmaz et al. (2011)cite that only way to solve this is stablish a group of

requirements to be fulfilled and deposited at the time of sequence submission. Taxonomic precision in annotations metadata represents state-of-art data due enables even no mycologists to study fungal ecological perspectives, turning easy-to-collect data into meta-studies on phylogeny, evolutionary ecology, and biogeography ways (Kõljalg et al. 2013). Porter and Hajibabaei (2018) analyzing 2.5 million *Cytochrome c oxidase subunit I* (*COI*) highlight that the studies are dependent on few steps and one of these is the adherence to the minimum information about marker sequence (MIMARKS) guidelines. MIMARKS is a checklist that was developed to guide authors to help construct a public databank healthily, this was based on Genomic Standards Consortium (CGS) extending what was proposed as a minimum annotation to genomic submissions to single gene submissions in Genbank (Yilmaz et al. 2011). Genbank was cited as a time division in natural history due the merged values of natural history with molecular science (Strasser 2008) and a reliable resource for biodiversity research (Leray et al. 2019).

In the big data era, the Genbank is a valuable and wasted resource to scientists, especially to *Calonectria*-like research due centered information in one platform and many have the vision of it only serving to store or release access number to fill tables in publications, the healthily of this resource is in risk if scientists don't start feeding this resource with quality and complete data information.

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Capítulo 3: Morphological and phylogenetic analysis reveals five new species in *Calonectria*-like (Nectriaceae) associated with tropical fruits.

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Morphological and phylogenetic analysis reveals five new species in *Calonectria*-like (Nectriaceae) associated with tropical fruits.

3.1 Abstract

Calonectria-like represent a group of genera closely related phylogenetically and morphologically, but the efforts applied in both are different, doing the genus that named the group largely studied, the genera that compose the group are commonly associated with disease in important economic plants. In Brazil, *Calonectria*-like genus are regularly reported in soils or in tissues of *Eucalyptus* sp. and rarely in native plants or associated with rot fruits. The aim of this study was identified the collection of *Calonectria*-like isolates from fruit rots on different species and localities in Brazil, using on phylogenetic and morphological approaches. The study revealed that six previously described species: *Cy. infestans, Cy. lageniformis, Cy. peruviana, Cy. vitis, G. hennebertii* and *G. tenuis*. As well five new species have been discovered and described here as *Calonectria arborius, Ca. cerradense, Cylla. albus, Cylla. catarinensis* and *Cylla. biestipitata.* Despite the genus *Calonectria* are broadly studied in Brazil the collection of this study show that remain species to be discovered, meanly in systems out off economic plantations. This species unknowledge are more evident in genus like *Cylindrocladiella* that most of species founded was inner this genus.

Keywords: Hypocreales, Brazilian cerrado, Diversity

3.2 Introduction

The phylogenetic and morphologic group named as *Calonectria*-like is composed by ten genera: *Aquanectria, Calonectria, Corallonectria, Curvicladiella, Cylindrocladiella,*

Dematiocladium, Gliocephalotrichum, Gliocladiopsis, Xenocylindrocladium, Penicillifer and Xenocylindrocladium. The *Calonectria* that gave the name to the group is the most knowledge and the first genus to be described in 1867 among these. Nowadays is recognized as an important pathogen of many forestry, agricultural and horticultural plants due they are being associated with than 335 plants species (Q. L. Liu et al., 2020). In number of species *Calonectria* is on the top of rating with 132 species, followed by *Cylindrocladiella* with 43 species and *Gliocladiopsis* 19 species comprehend the top tree genera with most species related.

The first report registered in Brazil for *Cylindrocladiella* was published by Hodges and May in 1972, these authors reported a new species named as *Cylla. clavata* (= *Cylindrocladium clavatum*) causing roots of dying in *Araucaria angustifoilia* on Paraná, and isolated of *Pinus* spp. and seedlings of *Eucalyptus saligna* on Minas Gerais, São Paulo e Espírito Santo. In 1962 was collected *Cylla. peruviana* by M.P. Herrera on Ant and published by Boeserwikel in 1982. Nine years before, in 1993 was reported two other species on Brazilian territory, an isolate of *Cylla. infestans* and a new specie *Cylla. lageniformis* both on *Eucalyptus* sp. An important compiled report several new isolates from Brazil in 2002 (Crous, 2002), but all isolates was collected in 1993, an apparently year that some researcher applied several efforts on create a culture collection of this genus. The *Cylla. pseudohawaiiensis* was the third new specie described in Brazil by Lombard et al. 2012, but there was described using an isolate collected ten years after. *Gliocladiopsis* are reported by one collection made by collected by C.S. Hodges on soil (Lombard & Crous, 2012).

Calonectria-like is related with disease like cutting rot, damping off, leaf spot, defoliation, shoot blight, stem canker, root, and tuber disease (Chen et al., 2022; Crous,

2002; H. H. Liu et al., 2021; Q. L. Liu et al., 2020), but the disease association of this group tends to leaf and root disease, by the type and focus of sampling and study already published. Most of studies are focused on identified *Calonectria* in *Eucalyptus* plantations, an example, in a critical study in Brazil was collected 1 017 isolates, 94% were from symptomatic leaves or soil samples of *Eucalyptus* plantations, only 6% were collected in native vegetation (Alfenas et al. 2015). On genus that compose *Calonectria*-like there are many studies show as an important agent of fruit rot is *Gliocephalotrichum*. This genus is reported causing fruit rot in twelve plant species in nine countries, only in Brazil there are report of these fungi causing disease on fruit of nine plant species (Silva et al., 2020).

In the rare studies related *Calonectria* with fruit diseases was founded *Ca. cylindrospora* (*syn.= Cylindrocladium scoparium*), *Ca. ilicicola* and *Ca. fragariae* causing rot fruit on *Durio graveolens* in Brunei Darussalam, *Malpighia glabra* and *Fragaria x ananassa* in Brazil (Ferreira et al., 1995; Lopes et al., 2018; Sivapalan et al., 1998). In *Cylindrocladiella* and *Gliocladiopsis* there is no literature showing these genera associated with fruit rot. The frequency of studies, host diversity and geographical distribution of this genus in Brazil is rare. This study will abroad the knowledge about this genus diversity and host associate on Brazilian territory. Then the aim of this study was describing a new species of *Calonectria* causing fruit rot in Brazil.

3.3 Material and Methods

Isolates collection - Was collected fruits with rot symptoms from: *Araucaria angustifolia* (Araucariaceae); *Citrus* x *limonia* (Rutaceae); *Dypsis madagascariensis*; *Euterpe edulis; Euterpe oleracea; Syagrus oleraceae* (Arecaceae); *Eugenia aggregata;*

Eugenia involucrata (Myrtaceae); *Inga laurina* (Fabaceae); *Persea americana* (Lauraceae); *Spondia mombin; Spondia purpurea* (Anacardiaceae); *Terminalia catappa* (Combretaceae). They were storage on paper bags to transport until the Laboratório de Micologia of Departamento de Fitopatologia at Universidade de Brasília. These symptomatic fruits were submitted to method of direct and indirect isolation (Alfenas & Mafia, 2016). All cultures were purified take one single hypha and placed on a new Petri dish with Malt Extract Agar (MEA 2%), this culture was grown on BOD at 25ºC. After 7 days all cultures were stored on Castallani, Glycerol 10% and Mineral Oil methods. These cultures were banked in Coleção de Culturas da Universidade de Brasília under a laboratory codification.

DNA extraction, amplification, and sequencing - The genomic DNA was extracted by monohyphae culture grew on Petri dish filled with MEA during 7 days on environment temperature. Was used the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), modified by Pinho et al. (2012). The PCR reaction (Polymerase Chain Reaction) were made with a final aliquot of 12.5*µ*L, compound by: 6.25*µ*L of MyTaq™ Polymerases and Mixes (Bioline, London, UK), 4,65 *µ*L of Milli-q sterilized water, 1*µ*L of PCR product, and 0,3 *µ*L of each pair of primers. Was amplified and sequenced portion primarily for (*i*) translation elongation factor 1- *α* (*TEF1*) used as barcode, to this locus was used primers EF-1F e EF-2R (Jacobs et al. 2004). Other three loci were amplified and sequenced to multigenic analysis, (*ii*) *β*-tubulin (*BTUB*), with primer T1 (O'Donnell et al., 2007) and Bt2b (Glass & Donaldson, 1995), (*iii*) histone H3 (*HIS3*) using CYLH3F e CYLH3R (Crous et al. 2004) and the nuclear rDNA internal transcribed spacer (*ITS*) using the primers LR5 (Vilgalys & Hester, 1990) e V9G (Hoog & Ende, 2009). PCR was made follow the conditions described for Silva et al. (2020). PCR products were purified using 2 *µ*L de ExoSAP-IT™ (TermoFisher,

Santa Clara, USA) follow the brand protocol, all products were sent to Macrogen (Seoul, Korea) to sequencing.

Data sets and phylogenetic analysis - The sequences generated were verified the quality and edited with DNA Dragon software (Hepperle D., 2011). All sequences were assessed and verified ambiguous position manually and the contigs generated was submitted to Mega Blast comparisons against GenBank database (www.ncbi.nlm.nih.gov). Reference sequences were downloaded from GenBank. The outgroup was chosen separately for each dataset in separate genus analysis. All new sequences generated were deposited in GenBank (TABLE 1). Alignments were made inner MAFFT 7.463 (Katoh & Standley, 2013) platform using the default parameters. The Bayesian Inference (BI) and Maximum likelihood (ML) were used to phylogenetic inferences. For BI analyses, the best substitution models for each partial gene were determined with MrModeltest (Nylander, 2004). and submitted to run in MrBayes 3.2.2 (Ronquist et al., 2012), the posterior probabilities (PP) were calculated considering only 75% of saved trees (the first 25%were discarded as the burn-in). The Markov chain Monte Carlo (MCMC) analysis was run with a total of 10 million generations and one tree sampled every 1000 generations, to set. The ML analysis was performed using RAxML 8.2.9 (Stamatakis, 2014), starting with a randomized stepwise-addition parsimony tree under a GTR+GAMMA model for and 1000 bootstrap (BS) replicates under the same model. Both analyses were performed using the web portal CIPRES (Miller et al., 2010). The convergence of each tree generated was confirmed using TRACER 1.7.1 (Rambaut & Drummond, 2018). All trees were edited in FigTree 1.4 (Rambaut, 2018) and values of PP and ML adds to the final tree using TreeGrath 2 (http://treegraph.bioinfweb.info/). The nucleotide matrices and phylogenetic trees from all five data sets (individual and four-gene combined).

Morphological characterization - All new phylogenetic lineage were specimens characterized. These news species were cultivated on SNA (Sintetic Nutrient Agar) with croton dry leaf (Lombard et al., 2010b; Nirenberg, 2011) on 25ºC under alternate photoperiod (12h bright/12h dark) for 7 days. Microscopical characteristics are examined by mounting fungal structures in clear lactoglycerol and plyvinyllactoglycerl (PVLG). Thirty measurements were mad for each trait using Leica DM2500 microscope (Leica Microsystem, Nassloch, Germany). The Colony coloration was determined using MEA and PDA cultures grew for 7 days, comparing with chat published by (Rayner, 1970). Measurements were tabulated as 95% confidence intervals and average, minimum, and maximum values.

Table 1. Collection details and GenBank accessions of isolates included in the phylogenetic analysis.

				GenBank Access**						
Species	**Isolator***	**Host/substrate**	**Locality**	**ITS**	**TUB2**	**HIS3**	**TEF1**	**RPB2**	**CMDA**	**Collector**
Calonectria auriculiformis	**CMW 47178^{T}**	Soil	Vietnam	MT359651	MT412944	MT335430	MT412721	MT412494	MT335190	N.Q. Pham and T.Q. Pham
	CMW 47179	Soil	Vietnam	MT359652	MT412945	MT335431	MT412722	MT412495	MT335191	N.Q. Pham and T.Q. Pham
Ca. brasiliensis	**CBS 230.51^{T}**	*Eucalyptus* sp.	Brazil	MT359661	MT412953	MT335440	MT412731	MT412504	MT335200	T.R. Ciferri
	CBS 114257	*Eucalyptus* sp.	Brazil	MT359662	MT412954	MT335441	MT412732	MT412505	MT335201	A.C. Alfenas
Ca. cerciana	**CMW 25309 ^{T}**	*Eucalyptus urograndis*	China	MT359672	MT412963	MT335451	MT412742	MT412515	MT335211	M.J. Wingfield & X.D. Zhou
	CMW 25290	*Eucalyptus urograndis*	China	MT359673	MT412964	MT335452	MT412743	MT412516	MT335212	M.J. Wingfield & X.D. Zhou
Ca. cerradense	CCUB1013	*S. purpurea*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis
	CCUB1039 ^{T}	*Spondia mombin*	Goiás, Brazil	-	-	-	-	-	-	A. Reis
	CCUB1054	*I. laurina*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis
Ca. colombiensis	**CMW 23676 ^{T}**	Soil	Colombia	MT359689	MT412980	MT335468	MT412759	MT412532	MT335228	M.J. Wingfield
	CMW 30985	Soil	Colombia	MT359690	MT412981	MT335469	MT412760	MT412533	MT335229	M.J. Wingfield
Ca. cylindrospora	**CBS 110666S ^{T}**	*Ilex vomitoria*	USA	MT359698	MT412986	MT335477	MT412768	MT412541	MT335237	N.E. El-Gholl
	CBS 119670	*Pistacia lentiscus*	Italy	MT359697	MT412985	MT335476	MT412767	MT412540	MT335236	N.A.
Ca. arborius	CCUB418	*Spondia purpurea*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis
	CCUB1008	*Terminalia catappa*	Goiás, Brazil	-	-	-	-	-	-	A. Reis
Ca. hawksworthii	**CMW 14878 ^{T}**	*Eucalyptus* sp.	Indonesia	MT359839	MT413119	MT335618	MT412909	MT412670	MT335378	A. Peerally

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Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
	CBS 111870 T	*Nelumbo nucifera*	Mauritius	MT359715	MT413003	MT335494	MT412785	MT412556	MT335254	M.J. Wingfield
Ca. insularis	**CMW 30991 T**	Soil	Madagascar	MT359730	MT413017	MT335509	MT412800	MT412567	MT335269	P.W. Crous
	CMW 30992	Soil	Mexico	MT359731	MT413018	MT335510	MT412801	MT412568	MT335270	M.J. Wingfield
Ca. maranhensis	**CBS 134811 T**	*Eucalyptus* sp.	Brazil	N.A.	KM395948	KM396118	KM395861	N.A.	KM396035	A.C. Alfenas
	CBS 134812	*Eucalyptus* sp.	Brazil	N.A.	KM395949	KM396119	KM395862	N.A.	KM396036	A.C. Alfenas
Ca. plurilateralis	**CBS 111401 T**	Soil	Ecuador	MT359801	MT413082	MT335580	MT412870	MT412632	MT335340	M.J. Wingfield
Ca. propaginicola	**CBS 134815 T**	*Eucalyptus* sp.	Brazil	N.A.	KM395953	KM396123	KM395866	N.A.	KM396040	A.C. Alfenas
	CBS 134816	*Eucalyptus* sp.	Brazil	N.A.	KM395954	KM396124	KM395867	N.A.	KM396041	A.C. Alfenas
Ca. tonkinensis	**CMW 47430 T**	Soil	Vietnam	MT359845	MT413122	MT335624	MT412915	MT412676	MT335384	N.Q. Pham and T.Q. Pham
Ca. variabilis	CMW 2914	*Theobroma grandiflorum*	Brazil	MT359854	MT413131	MT335633	MT412924	MT412684	MT335393	F.C. Albuquerque
	CMW 3187 T	*Schefflera morototoni*	Brazil	MT359853	MT413130	MT335632	MT412923	MT412683	MT335392	F. Carneiro
Calonectria sp.	CCUB1065	*Euterpe edulis*	Santa Catarina, Brazil	-	-	-	-	-	-	A. Reis
Calonectria sp.	CCUB1051	*S. mombin*	Pernambuco, Brazil	-	-	-	-	-	-	A. Reis
Calonectria sp.	CCUB1053	*Inga laurina*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis
Calonectria sp.	CCUB1040	*S. mombin*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis
Calonectria sp.	CCUB425	*S. purpurea*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis
Calonectria sp.	CCUB409	*S. purpurea*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis

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Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
Calonectria sp.	CCUB1056	*I. laurina*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis
Calonectria sp.	CCUB1057	*I. laurina*	Distrito Federal, Brazil	-	-	-	-	-	-	A. Reis
Cylla. addiensis	**CBS143794 T**	*Soil*	Ethiopia	MH111383	MH111388	N.A.	MH111393	N.A.	N.A.	P.W. Crous
	CBS143793	*Soil*	Ethiopia	MH111385	MH111390	N.A.	MH111395	N.A.	N.A.	P.W. Crous
	CBS143795	*Soil*	Ethiopia	MH111384	MH111389	N.A.	MH111394	N.A.	N.A.	P.W. Crous
Cylla. albus	CCUB410	*S. jambos*	Distrito Federal - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1137	*Dypsis madagascariensis*	São Paulo - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1010	*T. catappa*	Santa Catariana - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
Cylla. arbusta	**CMW 47295 T**	*Soil*	Vietnan	MH017015	MH016958	MH016996	MH016977	N.A.	N.A.	N.Q. Pham and T.Q. Pham
	CMW 47296	*Soil*	Vietnan	MH017016	MH016959	MH016997	MH016978	N.A.	N.A.	N.Q. Pham and T.Q. Pham
Cylla. australiensis	**CBS129567 T**	*Soil*	Australia	JN100624	JN098747	JN098932	JN099060	N.A.	N.A.	P.W. Crous
	CBS129568	*Soil*	Australia	JN100623	JN098748	JN098931	JN099059	N.A.	N.A.	P.W. Crous
Cylla. brevistipitata	**CBS 142786 T**	*Soil*	Thailand	N.A.	MF444926	N.A.	MF444940	N.A.	N.A.	P.W. Crous
Cylla. biestipitata	CCUB1012	*T. catappa*	Distrito Federal - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1035		Minas Gerais - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
Cylla. camelliae	CPC234	*Eucalyptus grandis*	South Africa	JN100573	JN098749	JN098839	JN099090	N.A.	N.A.	P.W. Crous
	CBS114891 T	*Eucalyptus grandis*	South Africa	AF220953	AY793472	AY793510	JN099086	N.A.	N.A.	P.W. Crous

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Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
Cylla. catarinensis	**CCUB1006**	*T. catappa*	Santa Catariana Brazil -	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1031	*T. catappa*	Santa Catariana Brazil -	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1015	*T. catappa*	Santa Catariana Brazil -	-	-	-	-	N.A.	N.A.	A. Reis
Cylla. clavata	CBS129563	*Soil*	Australia	JN099096	JN098751	JN098859	JN098975	N.A.	N.A.	P.W. Crous
	CBS129564 T	*Soil*	Australia	JN099095	JN098752	JN098858	JN098974	N.A.	N.A.	P.W. Crous
Cylla. cymbiformis	**CBS129553 T**	*Soil*	Australia	JN099103	JN098753	JN098866	JN098988	N.A.	N.A.	P.W. Crous
	CBS129554	*Soil*	Australia	JN099104	JN098754	JN098867	JN098989	N.A.	N.A.	P.W. Crous
Cylla. elegans	**CBS338.92 T**	Leaf litter	South Africa	AY793444	AY793474	AY793512	JN099039	N.A.	N.A.	L. Rong
	CBS110801	Leaf litter	South Africa	JN100609	JN098755	JN098916	JN099044	N.A.	N.A.	P.W. Crous
Cylla. ellipsoidea	CBS129572	*Soil*	Australia	JN100636	JN098756	JN098943	JN099073	N.A.	N.A.	P.W. Crous
	CBS129573 T	*Soil*	Australia	JN099094	JN098757	JN098857	JN098973	N.A.	N.A.	P.W. Crous
Cylla. hahajimaensis	PD684	*Soil*	Japan	JN687561	N.A.	N.A.	JN687562	N.A.	N.A.	T. Watanabe
Cylla. hawaiiensis	CBS118704	*Soil*	Hawaii	JN099115	JN098760	JN098878	JN098996	N.A.	N.A.	Y. Degawa
	CBS129569 T	*Soil*	Hawaii	JN100621	JN098761	JN098929	JN099057	N.A.	N.A.	Y. Degawa
Cylla. horticola	**CBS 142784 T**	*Soil*	Thailand	MF444911	MF444924	N.A.	MF444938	N.A.	N.A.	P.W. Crous
	CBS 142785	*Soil*	Thailand	MF444912	MF444925	N.A.	MF444939	N.A.	N.A.	P.W. Crous
Cylla. humicola	**CBS 142777 T**	*Soil*	Thailand	MF444904	MF444917	N.A.	MF444931	N.A.	N.A.	P.W. Crous
	CBS 142779	*Soil*	Thailand	MF444906	MF444919	N.A.	MF444933	N.A.	N.A.	P.W. Crous
Cylla. infestans	**CBS111795 T**	*Pinus pinea*	New Zealand	AF220955	AF320190	AY793513	JN099037	N.A.	N.A.	H.J. Boesewinkel
	CBS191.50	*Arena pinnata*	Indonésia	AF220956	AY793475	AY793514	JN099036	N.A.	N.A.	K.B. Boedijin & J. Reisma
	CBS 192.50	*Theobroma cacoa*	Indonésia	JN099120	JN098762	JN098882	JN099001	N.A.	N.A.	K.B. Boedijin & J. Reisma

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Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
	CCUB1037	*Eugenia aggregata*	Santa Catariana - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
Cylla. kurandica	CBS129576	Soil	Australia	JN100634	JN098764	JN098941	JN099071	N.A.	N.A.	P.W. Crous
	CBS129577 T	Soil	Australia	JN100646	JN098765	JN098953	JN099083	N.A.	N.A.	P.W. Crous
Cylla. lageniformis	**CBS340.92 T**	*Eucalyptus* sp.	South Africa	AF220959	AY793481	AY793520	JN099003	N.A.	N.A.	A.C. Alfenas
	CBS111060	*Eucalyptus* sp.	South Africa	JN100611	JN098770	JN098918	JN099046	N.A.	N.A.	P.W. Crous
	CCUB1052	*S. mombin*	Pernambuco - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
Cylla. lanceolata	**CBS129566 T**	Soil	Australia	JN099099	JN098789	JN098862	JN098978	N.A.	N.A.	P.W. Crous
	CBS114950	*Eucalyptus* sp.		JN100591	JN098787	JN098898	JN099019	N.A.	N.A.	P.W. Crous
Cylla. lateralis	CBS 142787	Soil	Thailand	MF444913	MF444927	N.A.	MF444941	N.A.	N.A.	P.W. Crous
	CBS 142788 T	Soil	Thailand	MF444914	MF444928	N.A.	MF444942	N.A.	N.A.	P.W. Crous
Cylla. longiphialidica	**CBS129557T**	Soil	Thailand	JN100585	JN098790	JN098851	JN098966	N.A.	N.A.	P.W. Crous
	CBS129558	Soil	Thailand	JN100586	JN098791	JN098852	JN098967	N.A.	N.A.	P.W. Crous
Cylla. longistipitata	CBS112953	*Opisthiolepsis heterophylla*	Australia	JN100595	JN098792	JN098902	JN099025	N.A.	N.A.	C. Pearce & B. Paulus
	CBS116075 T	Soil	China	AF220958	AY793506	AY793546	JN098993	N.A.	N.A.	M.J. Wingfield
Cylla. malesiana	**CMW 48278 T**	Soil	Malaysia	MH017017	MH016960	MH016998	MH016979	N.A.	N.A.	M.J. Wingfield,
	CMW 48277	Soil	Malaysia	MH017018	MH016961	MH016999	MH016980	N.A.	N.A.	M.J. Wingfield,
	CMW 48276	Soil	Malaysia	MH017019	MH016962	MH017000	MH016981	N.A.	N.A.	M.J. Wingfield,
Cylla. microcylindrica	**CBS111794 T**	*Echeveria elegans*	Indonésia	AY793452	AY793483	AY793523	JN099041	N.A.	N.A.	C.F. Hill
	STE-U 10452	*Agalonema commutatum*	USA	AY793453	AY793484	AY793524	N.A.	N.A.	N.A.	C.F. Hill
Cylla. natalensis	CBS110800	Soil	South Africa	JN100608	JN098793	JN098915	JN099043	N.A.	N.A.	P.W. Crous

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Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
	CBS114943 T	*Arachis hypogaea*	South Africa	JN100588	JN098794	JN098895	JN099016	N.A.	N.A.	M.J. Wingfield
Cylla. nauliensis	**CBS 143792 T**	Soil	Indonésia	MH111387	MH111392	N.A.	MH111397	N.A.	N.A.	M.J. Wingfield,
	CBS 143791	Soil	Indonésia	MH111386	MH111391	N.A.	MH111396	N.A.	N.A.	M.J. Wingfield,
Cylla. nedelandica	CBS146.94	*Rhododendron* sp.	Netherlands	JN099127	JN098799	JN098889	JN099011	N.A.	N.A.	
	CBS152.91 T	*Pelargorium* sp.	Netherlands	JN100603	JN098800	JN098910	JN099033	N.A.	N.A.	J.W. Veenbass-Rijks
Cylla. novaezelandica	**CBS486.77 T**	*Rhododendron indicum*	New Zealand	AF220963	AY793485	AY793525	JN099050	N.A.	N.A.	H.J. Boesewinkel
Cylla. obpyriformis	**CMW 47194 T**	Soil	Vietnan	MH017022	MH016965	MH017003	MH016984	N.A.	N.A.	N.Q. Pham and T.Q. Pham
	CMW 49940	Soil	Vietnan	MH017023	MH016966	MH017004	MH016985	N.A.	N.A.	N.Q. Pham and T.Q. Pham
Cylla. parva	**CBS114524 T**	*Telopea speciossima*	New Zealand	AF220964	AY793486	AY793526	JN099009	N.A.	N.A.	H.J. Boesewinkel
Cylla. parvispora	CMW 47193	Soil	Vietnan	MH017024	MH016967	MH017005	MH016986	N.A.	N.A.	N.Q. Pham and T.Q. Pham
	CMW 47197 T	Soil	Vietnan	MH017025	MH016968	MH017006	MH016987	N.A.	N.A.	N.Q. Pham and T.Q. Pham
	CMW 47207	Soil	Vietnan	MH017026	MH016969	MH017007	MH016988	N.A.	N.A.	N.Q. Pham and T.Q. Pham
	CBS113022	*Eucalyptus* sp.	South Africa	JN100599	JN098801	JN098906	JN099029	N.A.	N.A.	P.W. Crous
	CPC2404	*Ants*	Peru	AF220966	AY793500	AY793540	JN098968	N.A.	N.A.	M.P. Herre
	CCUB417	*Syzygium jambos*	Distrito Federal - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1016	*Spondia mombin*	Goiás - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1007	*Terminalia catappa*	Santa Catariana - Brazil	-	-	-	-	N.A.	N.A.	A. Reis

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Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
Cylla. postalofficium	**CBS146060 T**	leaf litter	South africa	MN562148	MN556845	MN556796	N.A.	N.A.	N.A.	L. Lombard
Cylla. pseudocamelliae	**CBS129555 T**	Soil	Thailand	JN100577	JN098814	JN098843	JN098958	N.A.	N.A.	P.W. Crous
	CBS129556	Soil	Thailand	JN100580	JN098815	JN098846	JN098961	N.A.	N.A.	P.W. Crous
Cylla. pseudohawaiiensis	**CBS210.94 T**	*Eucalyptus* sp.	Brasil	JN099128	JN098819	JN098890	JN099012	N.A.	N.A.	A.C. Alfenas
	CBS115610	-	Madagascar	JN100594	JN098820	JN098901	JN099024	N.A.	N.A.	J.E. Taylor
Cylla. pseudoinfestans	CBS114530	Soil	Madagascar	JN099126	JN098821	JN098888	JN099010	N.A.	N.A.	J.E. Taylor
	CBS114531 T	Soil	Madagascar	AF220957	AY793508	AY793548	JN099004	N.A.	N.A.	J.E. Taylor
Cylla. pseudoparva	CBS113624	*Quercus* sp.	Switzerland	JN099121	JN098822	JN098883	JN099002	N.A.	N.A.	L. Petrini
	CBS122594	*Vitis riparia*	New Zealand	JN100600	JN098823	JN098907	JN099030	N.A.	N.A.	K. Paice
	CBS129560 T	Soil	Netherlands	JN100620	JN098824	JN098927	JN099056	N.A.	N.A.	P.W. Crous
Cylla. queenslandica	**CBS129574 T**	Soil	Australia	JN099098	JN098826	JN098861	JN098977	N.A.	N.A.	P.W. Crous
	CBS129575	Soil	Australia	JN099097	JN098827	JN098860	JN098976	N.A.	N.A.	P.W. Crous
Cylla. reginae	CBS 142781	Soil	Thailand	MF444908	MF444921	N.A.	MF444935	N.A.	N.A.	P.W. Crous
	CBS 142782 T	Soil	Thailand	MF444909	MF444922	N.A.	MF444936	N.A.	N.A.	P.W. Crous
Cylla. stellenboschensis	CBS386.67	*Fragaria* sp.	Netherlands	JN100613	JN098828	JN098920	JN099048	N.A.	N.A.	P.W. Crous
	CBS110668 T	Soil	South Africa	JN100615	JN098829	JN098922	JN099051	N.A.	N.A.	P.W. Crous
Cylla. terretris	CBS 142789	Soil	Thailand	MF444915	MF444929	N.A.	MF444943	N.A.	N.A.	P.W. Crous
	CBS 142790	Soil	Thailand	MF444916	MF444930	N.A.	MF444944	N.A.	N.A.	P.W. Crous
Cylla. thailandica	CBS129570	Soil	Thailand	JN100581	JN098833	JN098847	JN098962	N.A.	N.A.	P.W. Crous
	CBS129571 T	Soil	Thailand	JN100582	JN098834	JN098848	JN098963	N.A.	N.A.	P.W. Crous
Cylla. variabilis	CBS375.93	*Mangifera indica*	India	JN099119	JN098836	JN098881	JN099000	N.A.	N.A.	P.N. Chow
	CBS129561 T	Soil	Australia	JN100643	JN098719	JN098950	JN099080	N.A.	N.A.	P.W. Crous
Cylla. viticola	**CBS112897 T**	*Vitis vinifera*	South Africa	AY793468	AY793504	AY793544	JN099064	N.A.	N.A.	G.J. Van Coller

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Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
	CBS114682	*Amorphophallus* sp.	Thailand	JN100612	JN098723	JN098919	JN099047	N.A.	N.A.	R. Stevenson
Cylla. vitis	**CBS142517 T**	*V. vinifera*	New Zealand	KY979751	KY979918	N.A.	KY979891	N.A.	N.A.	D. Davis
	CCUB1055	*Araucaria angustifolia*	Santa Catariana - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
Cylindrocladiella sp.	CCUB1003	*T. catappa*	Santa Catariana - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1014	*T. catappa*	Distrito Federal - Brazil	-	-	-	-	N.A.	N.A.	A. Reis
Gliocladiopsis aquaticus	MFLUCC17-2028	*Cassia fistula*	Thailand	MG543925	MG574422	MG734183	N.A.	N.A.	N.A.	R.H. Perera
	MFLUCC17-1811	Decaying wood	Thailand	MG543924	MG574421	MG734182	N.A.	N.A.	N.A.	R.H. Perera
	MFLUCC19-0317	*Cassia fistula*	Thailand	MT215502	N.A.	MT215552	N.A.	N.A.	N.A.	R.H. Perera
G. curvata	CBS 194.80	*Persea americana*	Ecuador	JQ666044	JQ666120	JQ666010	JQ666086	N.A.	N.A.	J.P. Laoh
	CBS 112365 T	*Archontophoenix purpurea*	New Zealand	JQ666050	JQ666126	JQ666016	JQ666092	N.A.	N.A.	F. Klassen
	CBS 112935	*Syzygium aromaticum*	Indonesia	JQ666051	JQ666127	JQ666017	JQ666093	N.A.	N.A.	M.J. Wingfield
G. ecuadoriensis	MUCL 54740	*Polybotrya* sp.	Ecuador	KX67113	KX611501	KX671146	KX671131	N.A.	N.A.	A. Gordillo and C. Decock
G. elghollii	CBS 206.94	*Chamaedorea elegans*	USA	JQ666054	JQ666130	JQ666020	JQ666096	N.A.	N.A.	N.E. El-Gholl
	CBS 116104 T	*Chamaedorea elegans*	USA	JQ666055	JQ666131	JQ666021	JQ666097	N.A.	N.A.	N.E. El-Gholl
G. forsbergii	BRIP 60984	*Grevillea* sp.	Australia	KX274070	KX274036	KX274053	N.A.	N.A.	N.A.	K.G. Pegg
	BRIP 61349a T	*Persea americana*	Australia	KX274071	KX274037	KX274054	N.A.	N.A.	N.A.	L.E. Parkinson

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Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
G. guangdongensis	**LC 1340 T**	Submerged wood	China	KC776122	KC776124	KC776120	KC776119	N.A.	N.A.	F. Liu and L. Cai
	LC 1349	Submerged wood	China	KC776123	KC776125	KC776121	KC776118	N.A.	N.A.	F. Liu and L. Cai
G. hennebertii	MUCL 54818	*Costus scaber*	Ecuador	KX671140	KX611502	N.A.	KX671132	N.A.	N.A.	A. Gordillo and C. Decock
	CCUB1030	*Eugenia involucrata*	Santa Catarina, Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB1069	*Euterpe edulis*	Santa Catarina, Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB419	*Euterpe edulis*	Santa Catarina, Brazil	-	-	-	-	N.A.	N.A.	A. Reis
G. indonesiensis	**CBS 116090 T**	Soil	Indonesia	JQ666056	JQ666132	JQ666022	N.A.	N.A.	N.A.	A.C. Alfenas
G. irregularis	**CBS 755.97 T**	Soil	Indonesia	AF220977	JQ666133	JQ666023	JQ666099	N.A.	N.A.	A.C. Alfenas
G. mexicana	**CBS 110938 T**	Soil	Mexico	JQ666060	JQ666137	JQ666027	JQ666103	N.A.	N.A.	M.J. Wingfield
G. peggii	BRIP 53654	*Persea americana*	Australia	JN255246	JN255247	N.A.	N.A.	N.A.	N.A.	E.K. Dann and A.W.
	BRIP 54019	*Persea americana*	Australia	JN243765	JN243766	JN243767	N.A.	N.A.	N.A.	E.K. Dann and A.W.
	BRIP 60983 T	*Persea americana*	Australia	KX274083	KX274038	KX274065	N.A.	N.A.	N.A.	K.G. Pegg
G. pseudotenuis	CBS 114763	*Vanilla* sp.	Indonesia	JQ666062	JQ666139	JQ666029	JQ666105	N.A.	N.A.	M.J. Wingfield
	CBS 116074 T	Soil	China	AF220981	JQ666140	JQ666030	JQ666106	N.A.	N.A.	M.J. Wingfield
G. singaporiensis	MUCL 48728	*Olivier laurence*	Singapore	KX671138	KX611500	N.A.	KX671130	N.A.	N.A.	C. Decock
G. sumatrensis	**CBS 754.97 T**	Soil	Indonesia	JQ666064	JQ666142	JQ666032	JQ666108	N.A.	N.A.	M.J. Wingfield
	CBS 111213	Soil	Indonesia	JQ666066	JQ666144	JQ666034	JQ666110	N.A.	N.A.	M.J. Wingfield
G. tenuis	**IMI 68205 T**	*Indigofera* sp.	Indonesia	AF220979	JQ666150	JQ666040	JQ666116	N.A.	N.A.	F. Bugnicourt
	CBS 111964	*Coffea* sp.	Vietnam	JQ666068	JQ666147	JQ666037	JQ666113	N.A.	N.A.	P.W. Crous

Continue...

Species	Isolator*	Host/substrate	Locality	GenBank Access**						Collector
				ITS	TUB2	HIS3	TEF1	RPB2	CMDA	
	CBS 114148	Soil	Vietnam	JQ666070	JQ666149	JQ666039	JQ666115	N.A.	N.A.	P.W. Crous
	CCUB412	*Euterpe edulis*	Santa Catarina, Brazil	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB408	-	-	-	-	-	-	N.A.	N.A.	A. Reis
	CCUB407	*Dypsis madagascariensis*	Distrito Federal, Brazil	-	-	-	-	N.A.	N.A.	A. Reis
Gliocladiopsis tenuis	CCUB421	*Euterpe oleracea*	Pernambuco, Brazil	-	-	-	-	N.A.	N.A.	A. Reis
G. sagariensis	**CBS 199.55 T**	Soil	India	JQ666063	JQ666141	JQ666031	JQ666107	N.A.	N.A.	S.B. Saksena
G. swieteniae	**MFLU 18-2767 T**	*Swietenia mahagoni*	Thailand	MT215501	MT212214	MT212194	N.A.	N.A.	N.A.	R.H. Perera
G. whileyi	**BRIP 61430 T**	*Persea americana*	Australia	KX274086	KX274052	KX274069	N.A.	N.A.	N.A.	E.K. Dann
G. wuhanensis	HEAC17307	Soil	China	MH024520	MH169602	MH255786	N.A.	N.A.	N.A.	Niping Zhai
Gliocladiopsis sp. 1	CBS 111038	Soil	Colombia	JQ666071	JQ666151	JQ666041	JQ666117	N.A.	N.A.	M.J. Wingfield
Gliocladiopsis sp. 2	CBS 116086	Soil	Indonesia	JQ666072	JQ666152	JQ666042	JQ666118	N.A.	N.A.	A.C. Alfenas

T Ex-type cultures.

* BRIP: Biosecurity Queensland Plant Pathology Herbarium, Department of Agriculture and Fisheries, Dutton Park, Australia. CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. CPC: Working collection of Pedro Crous housed at CBS. IMI: International Mycological Institute, CABI-Bioscience, Egham, UK. ATCC: American Type Culture Collection, Manassas, USA. IMUR: Institute of Mycology, University of Recife, Recife, Brazil. LC: Herbarium of Microbiology, Academia Sinica, Taipei, Taiwan. CGMCC: China General Microbiological Culture Collection Center, Beijing, China. CCUB: Coleção de Culturas da Universidade de Brasília, Brasília, Brazil.

** Newly deposited sequences are showed in bold

3.4 Results

Isolates – Fifty-four *Calonectria*-like isolates are obtained from six Brazilian states: Distrito Federal (21), Goiás (6), Minas Gerais (1), Santa Catarina (18), São Paulo (1) and Pernambuco (3). There was isolated from fruit presenting circular pale to brown spot and/or presenting white hyphae or sporulation upper the skin, symptomatic fruits were found in fourteen species of plants: *Araucaria angustifolia* (Araucariaceae); *Citrus* x *limonia* (Rutaceae); *Dypsis madagascariensis*; *Euterpe edulis; Euterpe oleracea; Syagrus oleraceae* (Arecaceae); *Eugenia aggregata; Eugenia involucrata* (Myrtaceae); *Inga laurina* (Fabaceae); *Persea americana* (Lauraceae); *Spondia mombin; Spondia purpurea* (Anacardiaceae); *Terminalia catappa* (Combretaceae). Four isolates had the location and plant species annotation lost. In the field the symptoms and signals observed were pale to brown spot of oblong to circle shape above the epicarp, in fruits with rot more developed the intense white cottony hyphae was observed. Sporulation was rarely seen, in some fruits was detected slight sporulation bright white on fruit surface.

Phylogenetic analyses - The *Calonectria*-like alignment length was 575 bases ncluding gaps for the partial genus Elongation factor 1-alpha (TEF1). The matrix alignment presented 292 parsimonies informative, 361 variables and 187 conserved sites. The phylogeny analyses include 84 taxa count with three strains of *Thelonectria,* used as outgroup (Supplementary table 1) and the best evolutive model chosen for the data set was HKY+I+G. All genus was separated in clades with more than 0.89 Posterior probabilities (PP). The total of 54 new isolates are segregated into three genera: 44.4% identified as *Cylindrocladiella,* 31.5% as *Gliocladiopsis* and 24.1% as *Calonectria* species (Figure 1).

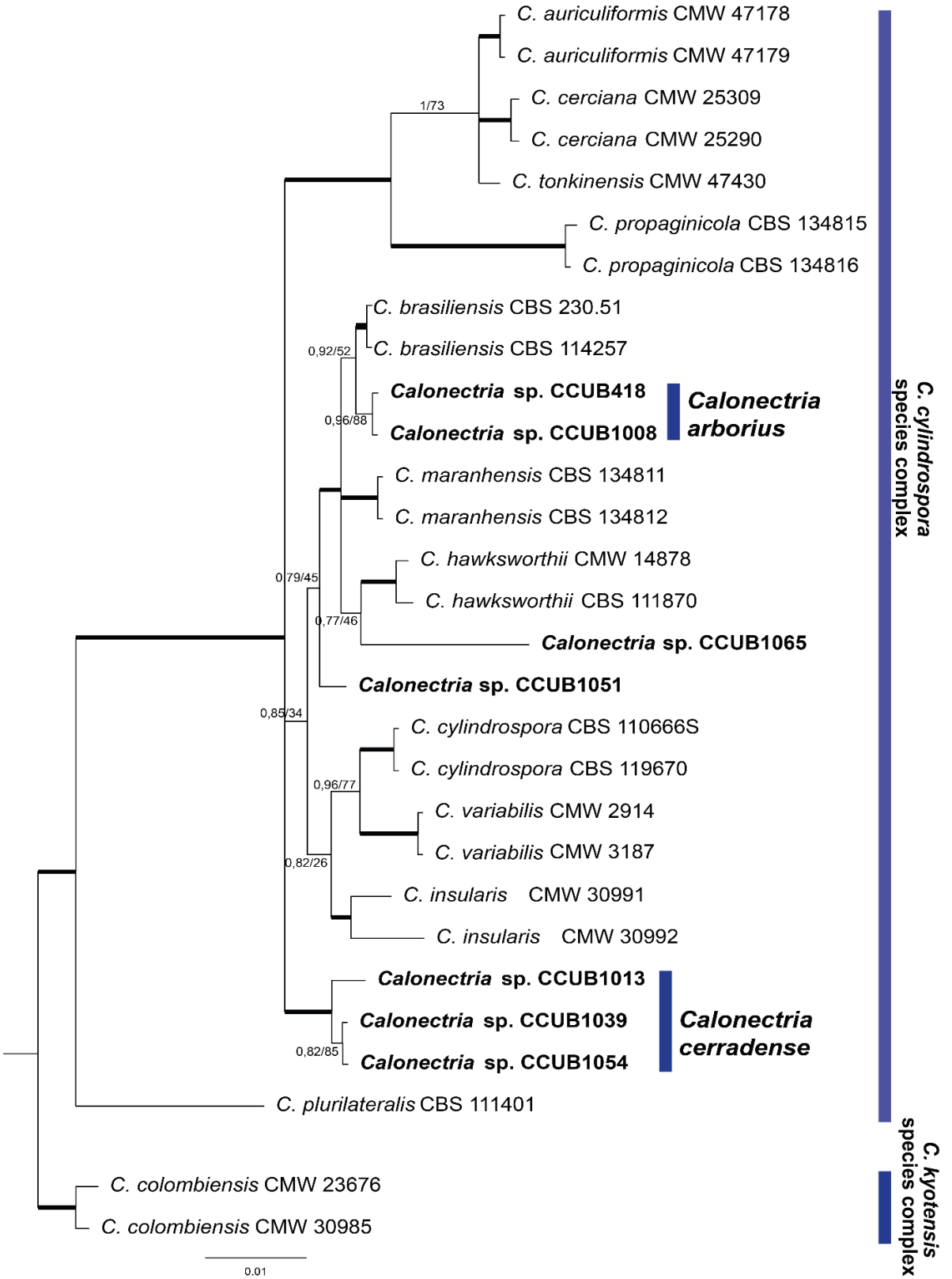


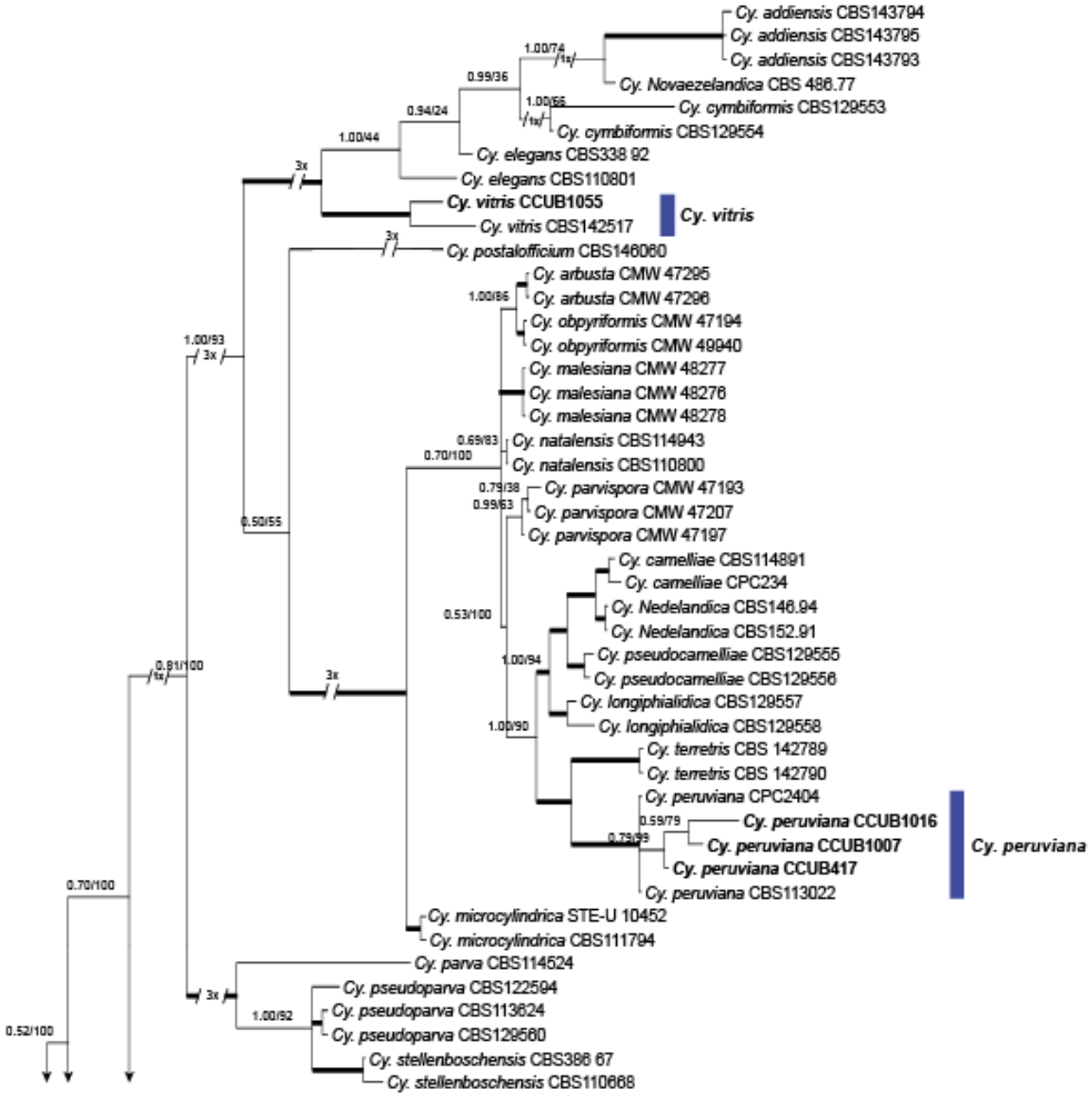
Figure 6. Phylogenetic tree of specie complex *Calonectria cylindrospora* based on Maximum Likelihood (ML) and Bayesian Inference (BI) of combined of five partial genes (*cmdA, his3, rpb2, tef1a, tub2*). *Ca. colombiensis* was used as outgroup. Values of bootstrap (ML) and posterior probability (PP) to BI are presented above the branches. Thicker branches mean values of ML ≥ 99% and PP ≥ 0.99. Taxonomic units in bold represent isolates obtained to this study.

obtained in this study grouping in *Calonectria cylindrospora* specie complex. Three isolates were forming a clade with *Ca. brasiliensis,* seven forming a new phylogenetic lineage and the last three wasn't possible segregate in screening with TEF1a. By the screening was selected seven representative isolates to multi-gene approach. The multi-gene alignment length 3021 bases including gaps for five gene regions (*cmdA, his3, rpb2, tef1a, tub2*). The alignment was compound by 645 variables, 576 parsimonies informative and 1917 conserved sites. The multi-gene phylogeny analysis included 27 *Ca. cylindrospora* complex specie ingroup plus two *Ca. colombiensis* taxa as outgroup. For Bayesian inference the best evolutive model chosen was K80 to *cmdA,* HKY+I to *his3*, SYM+G to *rpb2,* GTR+G to *tef1a* and HKY+G to *tub2.*The BI and ML tree topologies were not in conflict.

The multi-gene analysis confirmed that the isolates CCUB1013, CCUB1039 and CCUB1054 form a monophyletic new phylogenetic species close to *Ca. variabilis* and well supported (BI=1.00, ML=100). The isolates CCUB4018 and CCUB2008 grouped with *Ca. brasiliensis* with low support (BI=0.60, ML=71). Two isolates segregated separately, CCUB1051 and CCUB1065 with very low support (BI=0.54, ML=32) indicating that each can be a different lineage (Figure 1).

The screening using *tef1a* shows that the 24 new *Cylindrocladiella* isolates are segregated into eight clades. By the *tef1a* tree was selected 16 representative new isolates to the multi-gene approach. The multi-gene analysis for *Cylindrocladiella* used 103 taxa, composed for the selected isolates, 85 published isolates and two outgroups (*Ca. ilicicola* CBS 190.50 and *Ca. naviculata* CBS 101121). The concatenate matrix of five partial genes (*his3, its, lsu, tub2* and *tef1a*) has a 2359 bases, composed by 539 variable, 412 parsimony informative and 1776 conserved sites. Bayesian Inference analysis a GTR+I+G evolutive model as chosen for *his3* and *tef1a*, HKY+I+G to *lsu*

and *tub2,* and K80+I to *its.* Analysis of multi-gene phylogeny shows that six isolates are segregated with four species described by the literature, between these are *Cy.*



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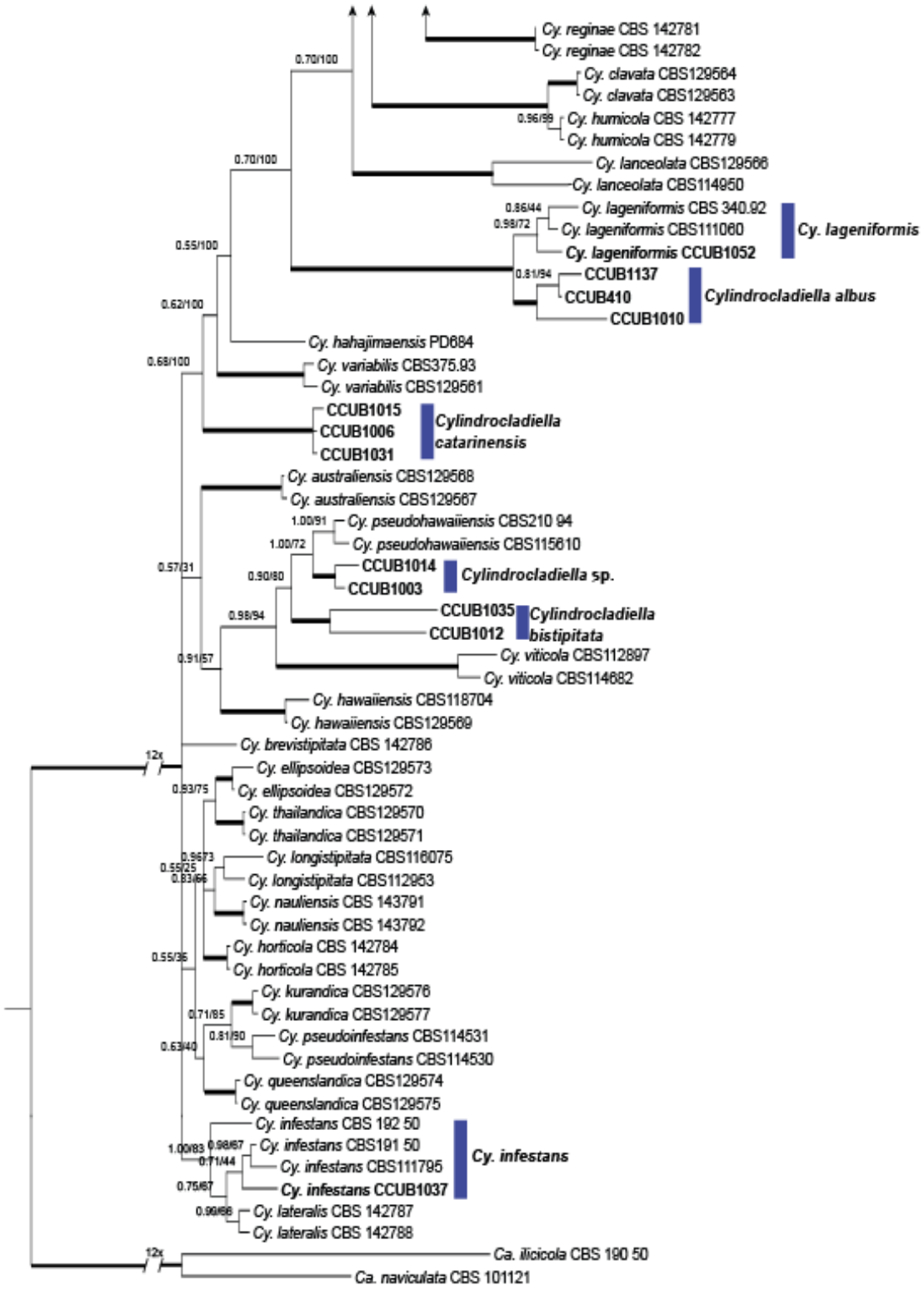

Figure 7. Most parsimonious tree of genera *Cylindrocladiella* obtained by the Bayesian Inference (BI) and Maximum Likelihood (ML) using the combination of five genes (*his3, its, lsu, tub2 and tef1a*). *Gliocladiopsis tenuis* was used as outgroup. Diagonal line followed by a number represent the number of times the branch was cut in relation of the scale. Values of bootstrap (ML) and posterior probability (PP) to BI are presented above the branches. Thicker branches mean values of ML ≥ 99% and PP ≥ 0.99. Taxonomic units in bold represent isolates obtained to this study.

infestans CCUB1037, *Cy. lageniformis* CCUB1052, *Cy. peruviana* CCUB417, CCUB1007 and CCUB1016, and *Cy. vitris* CCUB1055. The other ten isolates formed four new phylogenetic lineages.

The first new lineage compound by three isolates (CCUB410, CCUB1010 and CCUB1137) is a sister clade of *Cy. lageniformis* (BI=0.99, ML=100), the second has three isolate (CCUB1006, CCUB1015 and CCUB1031) and is close to *Cy variabilis,* the third and fourth are formed by two isolates each (CCUB1003 and CCUB1014 the third, CCUB 1012 and CCUB1035 the fourth) the clade that these two new phylogenetic lineages are located is possible a new specie complex, this new lineage is between *Cy. viticola* and *Cy pseudohawaiiensis* (Figura 2).

The first analysis used seventeen new isolates with sequence of *tef1a*, segregated these isolates in five clades. To multi-gene analysis was choose eight representative isolates. The matrix obtained to multi-gene analysis contains 1.328 bases including gaps, formed by concatenated of four partial gene (*his3, its, tef1a* and *tub2*) formed. for 44 taxa including the outgroup (*Cy. parva* ATCC 28272). The multi-gene matrix presented 459 variables, 148 parsimonies informative and 843 conserved sites. The evolutionary models used were GTR+G to *tef1a* and *tub2,* GTR+I+G to *his3* and SYM+I+G to *its.*

The phylogeny result shows that the seventeen new isolates segregated in three clades instead of five presented on initial screening. Four isolates were identified as *Glio. tenuis* (CCUB407, CCUB408, CCUB412 and CCUB421). The strain CCUB379 segregated separately close to *Glio. tenuis* and an unnamed specie *Gliocladiopsis* sp. CBS 111038. Another three isolates (CCUB419, CCUB1030 and CCUB1069) were identified as *G. hennerbetii* with a high support (IB=1.00 and ML=99 – Figure 3).

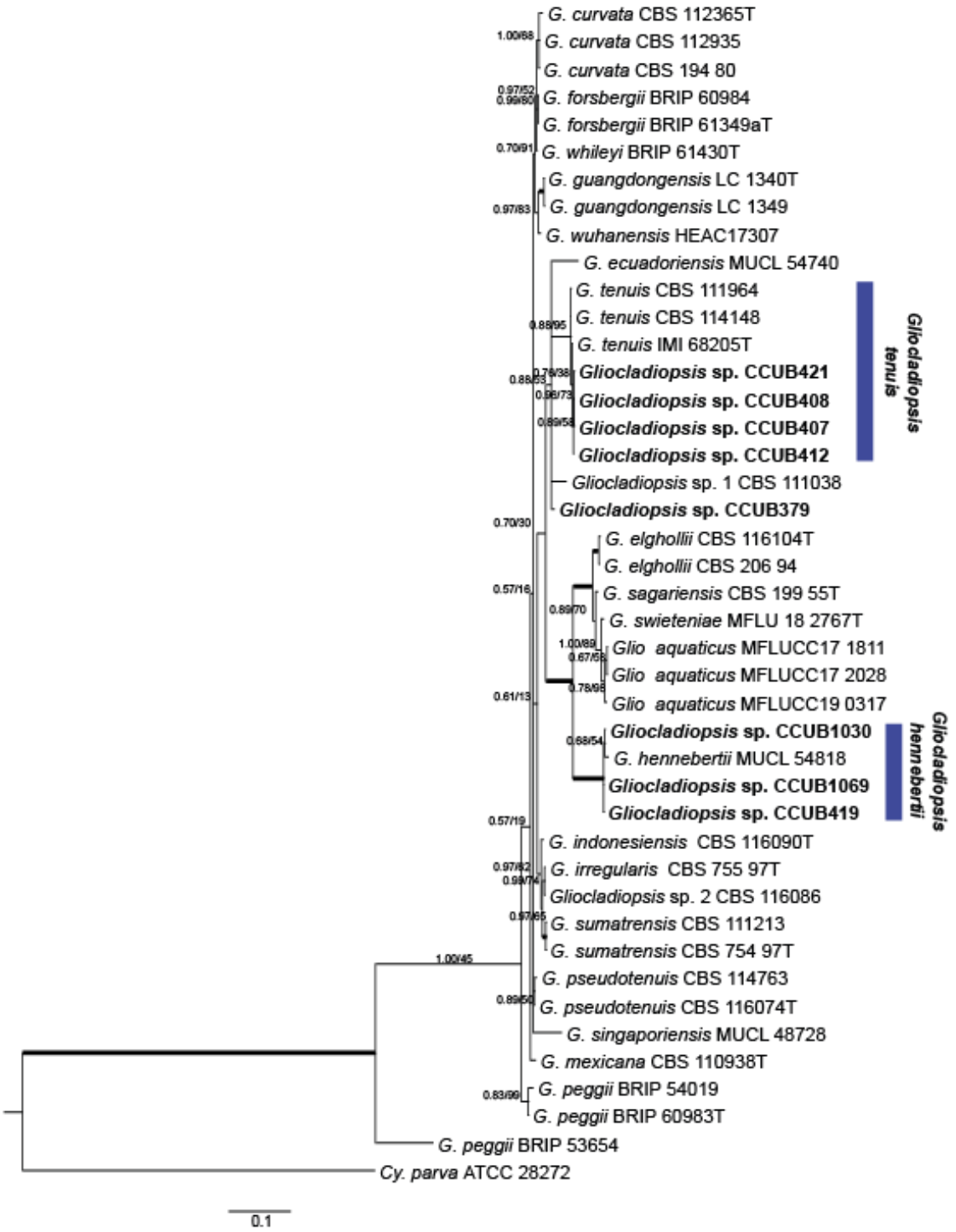

Figure 8. Most parsimonious tree of genera *Gliocladiopsis* from Bayesian Inference (BI) and Maximum Likelihood (ML) using the combination of five genes (*his3, its, lsu, tub2 and tef1a*). *Cylindrocladiella parva* ATCC 28272 was used as outgroup. Values of bootstrap (ML) and posterior probability (PP) to BI are presented above the branches. Thicker branches mean values of ML ≥ 99% and PP ≥ 0.99. Taxonomic units in bold represent isolates obtained to this study.

Taxonomy – Based on genealogical concordance phylogenetic species recognition

criterion (GCPSR) were detected that fifteen isolates formed seven strongly monophyletic groups, four in *Ca. cylindrospora* species complex *Cylindrocladiella* genera. Although two new lineages in *Calonectria* phylogeny that wasn't proposed as new species due the presence of one isolate each and in *Cylindrocladiella* the clade formed by the isolates CCUB1003 and CCUB1014 wasn't possible to characterize the morphology. The morphological characteristics of five species were characterized, and show distinguish differences of neighbor clades composed by other known species. Therefore, the fungi isolated in this study represent two and three new species of *Calonectria* and *Cylindrocladiella,* respectively.

Calonectria arborius **R.A. Fernandes, A. Reis & D. B. Pinho sp. nov. Figure 4**

Classification — Nectriaceae, Hypocreales, Sordariomycetes.

Etymology: *Arborius* (Latin), in reference to the presence of germinated vesicle in young colonies.

Sexual morph not observed. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and stipe extension terminating in a

vesicle; stipe septate hyaline, smooth, 179.5−275.5 × 3.5−5.5 µm; stipe extension septate, hyaline, straight to flexuous, 154.5−248 µm long, 3.5−4.5 µm wide at the apical septum, terminating in a vesicle ellipsoid to obpyriform, 6−9.5 µm diam, not observed lateral stipe extension. Conidiogenous apparatus 72−112 µm long and 75−112.5 µm wide; primary branches aseptate, 19.5−36(−49.4) × 4.5−6(−7) µm; secondary branches, aseptate, 17−24.5 × 4−6 µm; tertiary branches, aseptate, 11.5−18 × 3.5−5.5 µm, fourth branches, 10.5−15.5 × 3.5−5 µm aseptate, additionally branches (−5), aseptate 9.5−11.5 × 3−4.5 µm, each terminal branch producing 2−4 phialides, doliiform to reniform, hyaline, aseptate, 10−17 × 3−5 µm, apex with minute

periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, (32−)36.5−44 × (3−)3.5−4.5 µm (av. 39.5 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Mega- and microconidia not observed.

Culture characteristics — Colonies fast growing at 26 ºC on MEA (45−55 mm after 7 d), producing abundant white mycelium and sporulating on the medium surface under the cottony mycelium; culture with color blight brown to dark brown after 7 d; chlamydospores abundant throughout the medium, forming microsclerotia.

Typus. Brazil, Goiás, on *Terminalia catappa* fruits, 2018, A. Reis (holotype UB24559, *ITS, tef-1α, cmdA, his3 and tub2* sequences GenBank XXxxxxxx−XXxxxxxx, MycoBank Xxxxxxxx).

Notes — Calonectria arborius corresponds to the new member of the *Ca. cylindrospora* complex inner group (Alfenas et al. 2015). Sister group to the *Calonectria brasiliensis* and *Ca. maranhensis* (R. F. Alfenas et al., 2015). Morphologically and phylogenetically distinguish of closer species in *Ca. cylindrospora* complex. Phylogenetically *Ca. arborius* form a well-support clade (0.96 BI and 88% ML). Morphologically it differs from its nearest sister clade having conidiogenous apparatus bigger than *Ca. maranhensis* (45–65 × 45–71 µm) and *Ca. brasiliensis* (81–103 × 58–90 µm). Stipe extension smaller than *Ca. brasiliensis* (204–266 × 6–7 µm) and bigger than *Ca. maranhensis* (125–190 × 3–5 µm). *Ca. arborius* present four branches and 6−9.5 µm of vesicle while its nearest neighbor have only three branches and larger vesicles (7−11) µm. Another marker is the number of germinated vesicles in this proposal species, this had a frequency of 45% of all conidiophores analyzed.

Calonectria cerradense **R.A. Fernandes, A. Reis & D. B. Pinho sp. nov. Figure 5**

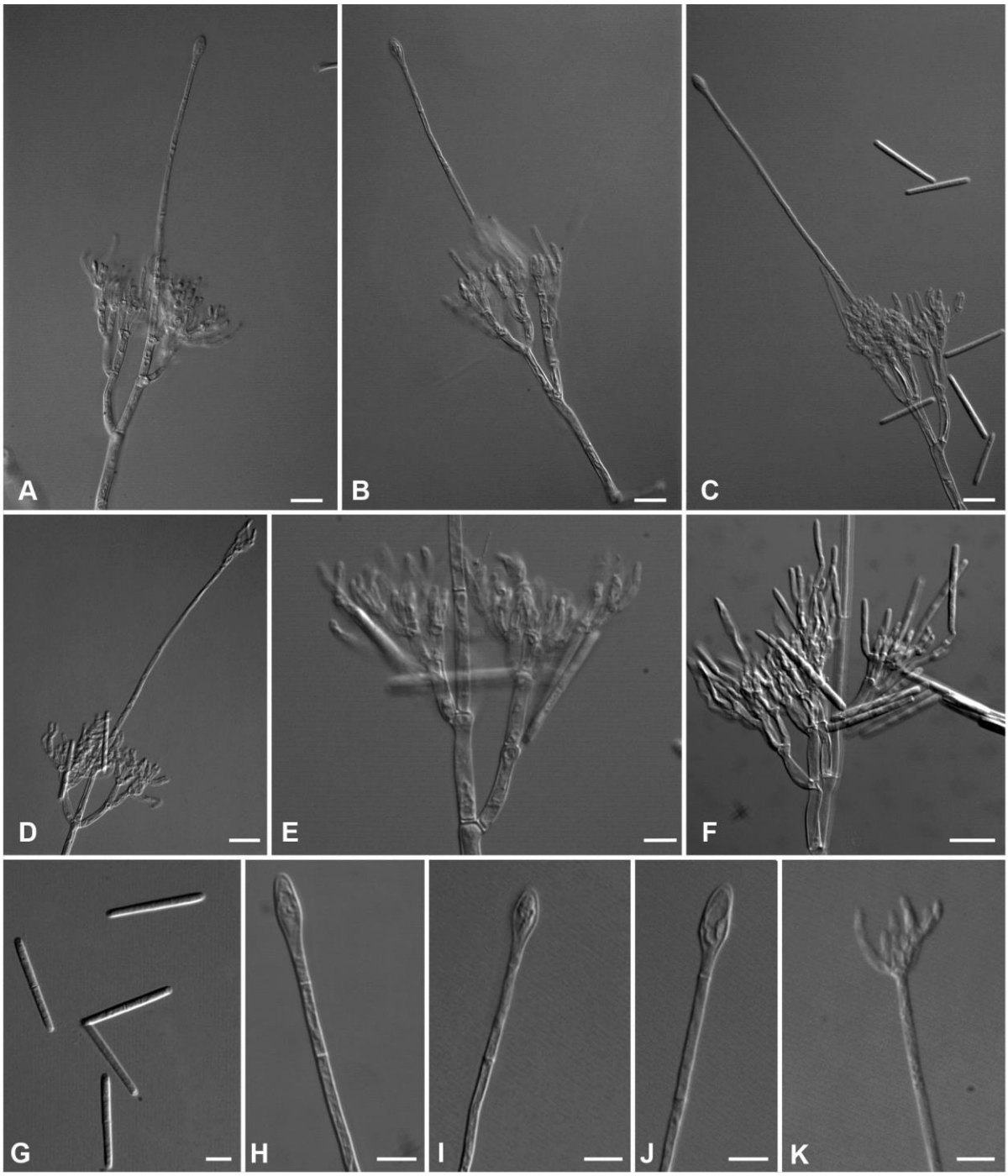

Figure 9. *Calonectria arborius sp. nov.* (CCUB1008) – A – C. Macroconidiophores in SNA after 7 dpi. D. Macroconidiophores with germinated vesicle. E-F. Conidiogenous apparatus with branches and detail of phialides. G. Macroconidia. H- K. Ellipsoidal to obpyriform vesicles and germinated vesicles forming phialide on the top. Scale bars: A = 20 µm (Apply to D-C); E = 10 µm (Apply to F-K).

Classification — Nectriaceae, Hypocreales, Sordariomycetes.

Etymology: *Cerradense* (Latin), in reference to the name of Brazilian savanna that the species origins.

Sexual morph not observed. *Macroconidiophores* consisting of a stipe bearing a penicillate arrangement of fertile branches, and stipe extension terminating in a vesicle; stipe septate hyaline, smooth, (186−)209.5–279 ×4.0–5,5(−7.0) ; stipe extension septate, hyaline, straight to flexuous, (163−)184−254 µm long, 3.5−4 µm

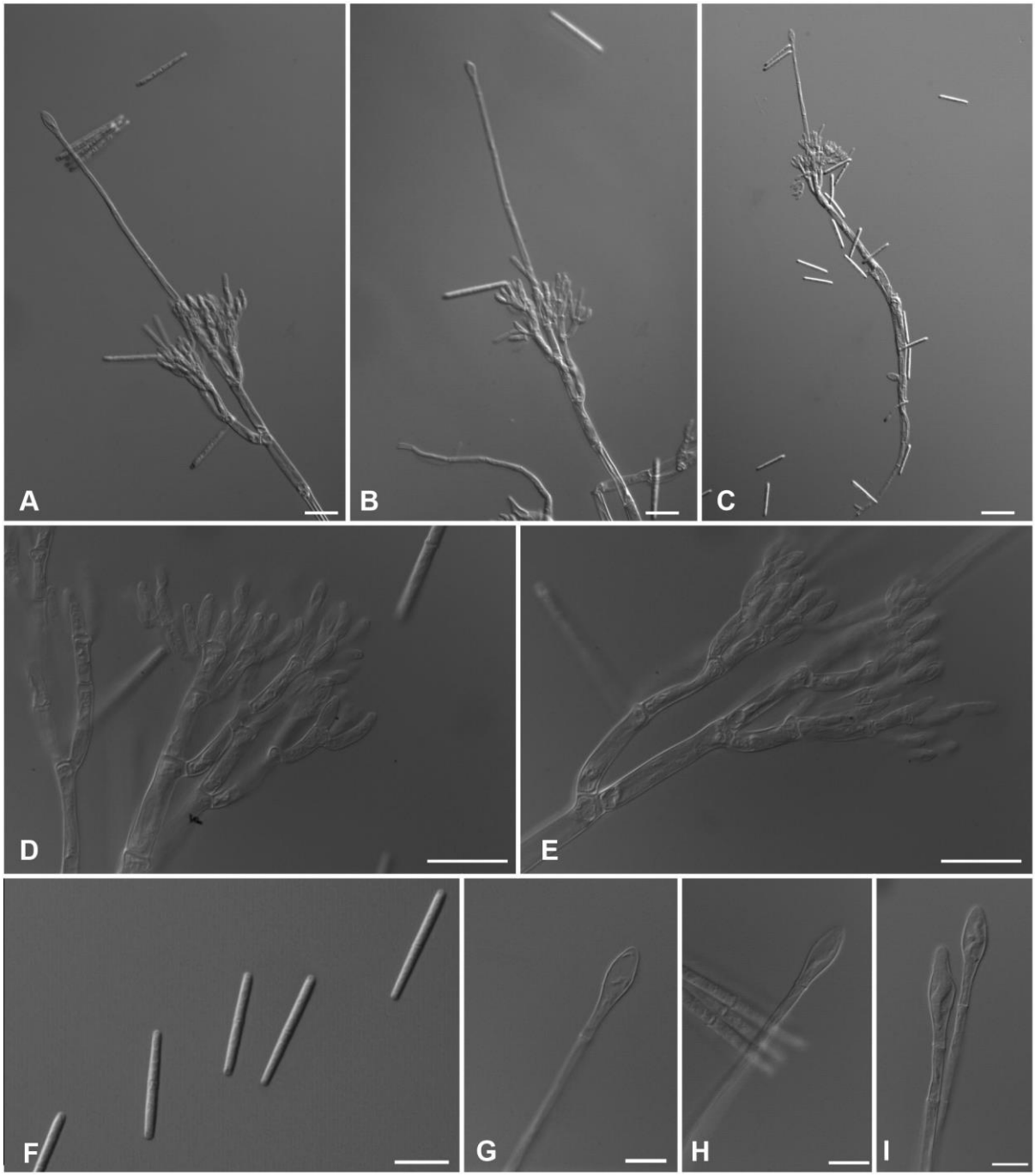

Figure 10. *Calonectria cerradense sp. nov.* (CCUB1039). A – C. Macroconidiophores. D-E. Conidiogenous apparatus with branches and phialides. F. Macroconidia. G-I. Ellipsoidal to obpyriform vesicles. Scale bars: A = 20 µm (Apply to B-E); F = 10 µm (Apply to G-I).

wide at the apical septum, terminating in a vesicle spathulate, 7−11.5 µm diam, not observed lateral stipe extension. Conidiogenous apparatus (38.5−)59,5–104 µm long and 68–107.5(–129) µm wide; primary branches aseptate, (18.5−)25−32 × (3.5−)5.5–7.5 µm; secondary branches, aseptate, 16.5−23(−28) × 4.5–6.5(−8.5) µm; tertiary branches, aseptate, 9.5−19(−28.5) × 4–5.5(−6.5) µm, additional branches (−4), aseptate, 11−14(−15.5) × 3.5–5 µm, ach terminal branch producing 2−4 phialides, doliiform to reniform, hyaline, aseptate, 8.5−13.5 × 3.5–4.5 µm, apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, (39.5−)40.5−44(−47) × (3.5−)4−4.5 µm (av. 43 × 4 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Mega- and microconidia not observed.

Culture characteristics — Colonies fast growing at 26ºC on MEA (55 mm after 7 d), producing abundant white mycelium, edges of colony with a dark brown halo and without sporulating on the medium surface, needed to be stressed; culture with color blight brown to dark brown after 7 d; chlamydospores abundant throughout the medium, forming microsclerotia.

Typus. Brazil, Goiás, Nova Veneza, on *Spondia mombin* fruits (Myrtaceae), 2017, A. Reis (holotype UB24025, *cmdA, his3, rpb2, tef1a,* and *tub2* sequences GenBank XXxxxxxx−XXxxxxxx, MycoBank XXxxxxxx).

Notes — Calonectria cerradense is a new member of the *Ca. cylindrospora* species complex (Alfenas et al. 2015). Phylogenetically it formed a new clade out of complex core, well-support clade (0.99 for Bayesian probability posterior and 99 % for maximum likelihood bootstrap support). Morphologically it differs from its nearest neighbors in having lateral stipe extensions. *Ca. cerradense* is close to *Ca. variabilis* and forms the outgroup of a subclade composed of *Ca. brasiliensis, Ca. maranhensis* and *Ca.*

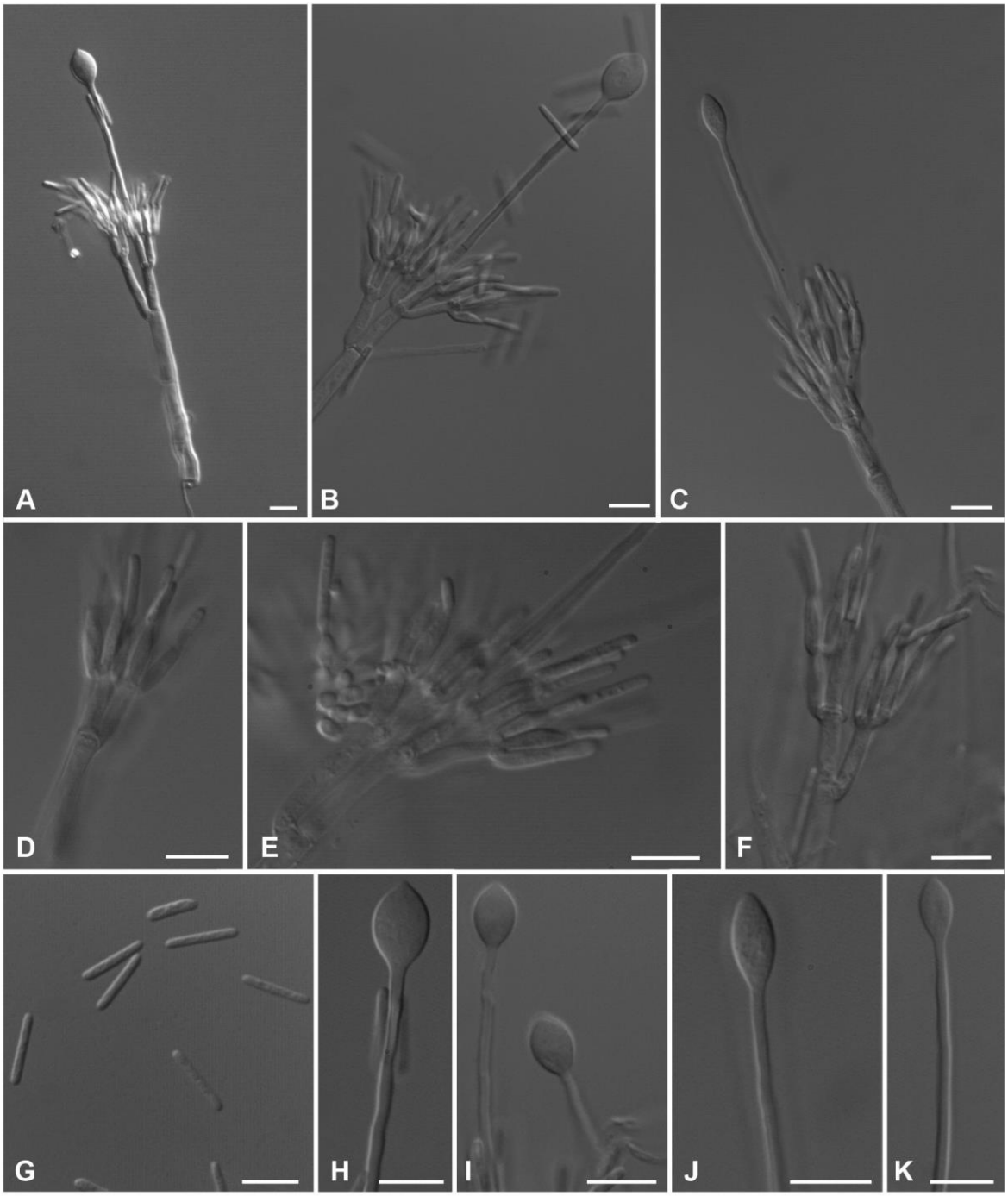

Figure 11. *Cylindrocladiella albus* sp. nov. (CCUB1137). A – C. Penicillate conidiophores. D-F. Conidiogenous apparatus with branches and phialides. G. Conidia. H-K. Ellipsoidal to obpyriform vesicles. Scale bars: A = 10 µm (Apply to B-K).

hawkswothii. Compared with *Ca. cerradense* with sister clade, this species has only macroconidia smaller and with one septum, and a stipe extension longer than *Ca. variabilis.* As to the subclade, the range of conidia size is smaller to *Ca. maranhesis* and *Ca. hawksworthii* and bigger than *Ca. brasiliensis.*

Cylindrocladiella albus **R.A. Fernandes, A. Reis & D. B. Pinho, sp. nov. MycoBank MBXXXXX. Figure 6.**

Classification — Nectriaceae, Hypocreales, Sordariomycetes.

Etymology: *Albus* (Latin). Name refers to the color of the colony in BDA medium.

Sexual morph not observed. *Conidiophores* monomorphic, penicillate, mononematous and hyaline; penicillate conidiophores comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth; stipe extension 82–116.5(–140) µm long, 2–3µm wide, aseptate, straight, thick-walled with one basal septum, terminating in thin-walled, broadly lageniform to ovoid vesicles. Conidiogenous apparatus (18.5−)29.5–45 µm long and 22.5–35.5(–43.5) µm wide, with primary branches aseptate 12–20 × 2.5–4.5 µm, secondary branches 9.5–12.5 × 2.5–4 µm, aseptate, each terminal branch producing 2–4 phialides; phialides 9–11.5 × 2–3 µm, elongate doliiform to reniform to cymbiform, hyaline, aseptate, apex with minute periclinal thickening and collarette. Conidia (11–)12.5–14.5(–16) × 1,5–2.5 µm (av. = 13 × 2 µm), cylindrical, rounded at both ends, straight, 1-septate, frequently slightly flattened at base, held in asymmetrical clusters by hyaline slime.

Culture characteristics: Colonies on MEA at 26 °C after 7 d. present fast growth, white cottony mycelium; chlamydospores absence; reaching 70–75 mm. Colonies on PDA at 26ºC after 7 d present fast growth reaching 55–60 mm, mycelia cottony, white to pale with smooth margins, reverse paleluteous to honey with sepia center.

Materials examined: Brazil, São Paulo, from decaying fruit of *Dypsis madagascariensis*, May 2018, collector A. Reis, isolated by R.A.F. Silva (holotype

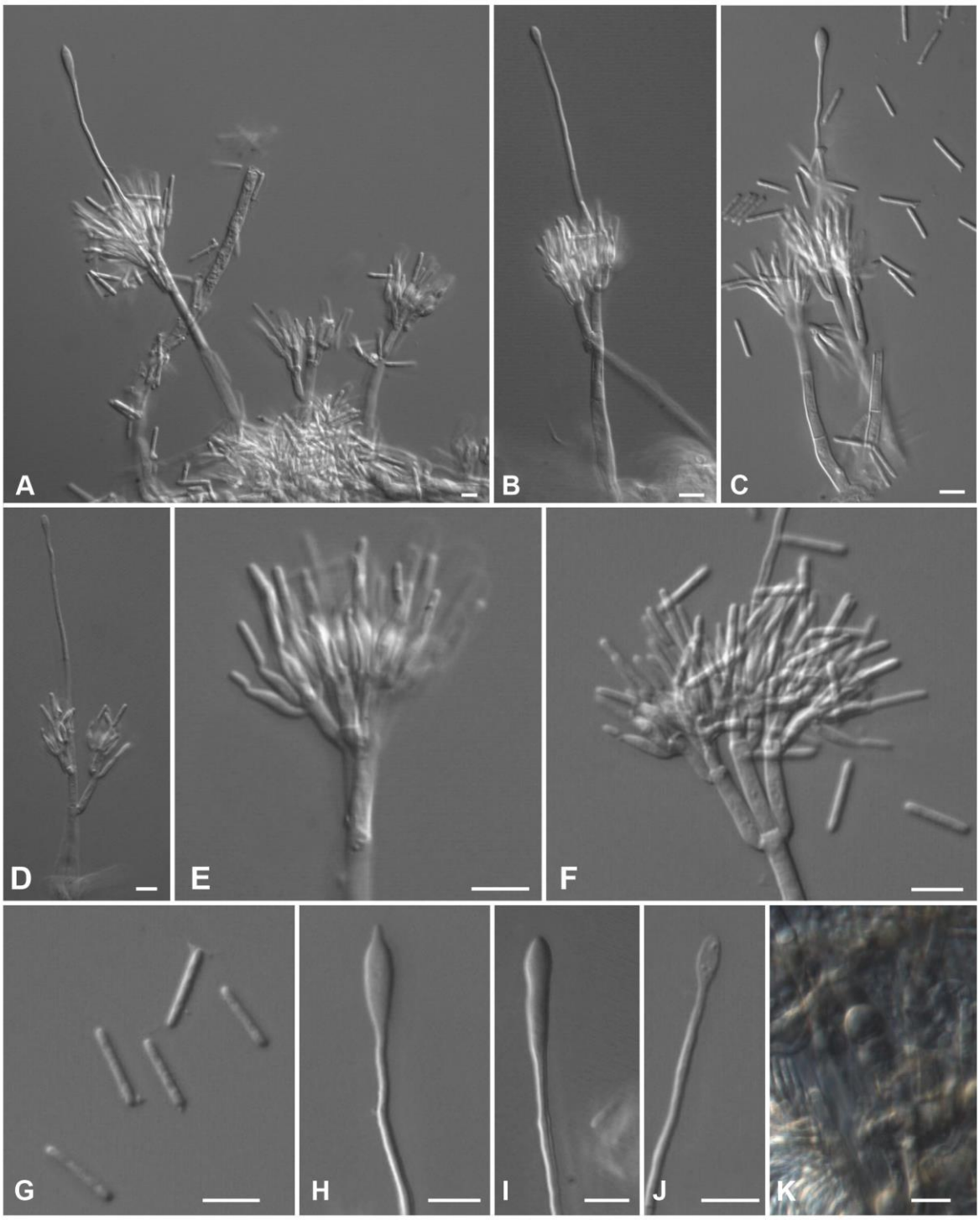

Figure 12. *Cylindrocladiella catarinensis* sp. nov. (CCUB1006). A – D. Penicillate conidiophores. E-F. Conidiogenous apparatus with branches and phialides. G. Conidia. H-J. Vesicles. K. Chlamydospores. Scale bars: A = 10 µm (Apply to B-K).

UB24553, culture ex-type CCUB1137), isotype culture CCUB410 (UB24562, metabolically inactive).

Notes: *Cylindrocladiella albus* closely related to *Cylla*. *lageniformis* (Crous & Wingfield, 1993a). Both species are distinguished by the absence of chlamydospores in the *Cylla. albus* and the presence in *Cylla. lageniformis* and the characteristics of vesicle restrict in lageniformis to ovoid in the first species, while in *Cylla. lageniformis* can present a third variation lageniformis to pyriform.

Cylindrocladiella catarinensis **R.A. Fernandes, A. Reis & D. B. Pinho, sp. nov. MycoBank MBXXXXX. Figure 7.**

Etymology: Name refers to the Santa Catarina, Brazilian state, from where this fungus was collected.

Sexual morph not observed. *Conidiophores* monomorphic, penicillate, mononematous and hyaline; penicillate conidiophores comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth; stipe extension 69–102(–134) µm long, 2–2.5µm wide, aseptate, straight, thick-walled with one basal septum, terminating in thin-walled, broadly ellipsoidal with a papillate apex, or clavate to ovoid vesicles. Conidiogenous apparatus (24,5−)30.5–42 µm long and 32.5–47.5(–50.5) µm wide, with primary branches aseptate 14–21.5 × 3.5–4.5 µm, secondary branches 9–12 × 2.5–3.5 µm, aseptate, each terminal branch producing 2–4 phialides; phialides 9–12 × 2.5–3.5 µm, elongate doliiform to reniform to cymbiform, hyaline, aseptate, apex with minute periclinal thickening and collarette. Conidia (13.5–)14–16(–17) × 2–3 µm (av. = 15 × 2.5 µm), cylindrical, rounded at both ends, straight, 1-septate, frequently slightly flattened at base, held in asymmetrical clusters by hyaline slime.

Culture characteristics: Colonies on MEA at 26 °C after 7 d. present fast growth, white cottony mycelium; chlamydospores absence; reaching 80 mm. Colonies on PDA at

26ºC after 7 d present fast growth reaching 60 mm, mycelia cottony, white reverse white in the edges and the center of colony tuning pale. Without presence of sporulation, and small hyaline to bright brown chlamydospores.

Materials examined: Brazil, Santa Catarina, from decaying fruit of *Terminalia catappa*, Abr. 2018, collector A. Reis, isolated by R.A. Fernandes (holotype UB24554, culture ex-type CCUB1006), isotype culture CCUB1015 (UB24566, metabolically inactive).

Notes: *Cylindrocladiella catarinensis* phylogenetically formed an outgroup of a subclade composed by *Cylla. variabilis, Cylla. albus, Cylla. lageniformis, Cylla. clavata, Cylla. humicola, Cylla. reginae* and *Cylla. lanceolata.* As *Cylla. variabilis* (Lombard et al., 2012), *Cylla catarinensis* present highly variable vesicles morphology, broadly ellipsoidal with a papillate apex, or clavate to ovoid vesicles, while the *Cylla. variabilis* was described clavate to fusoid to ovoid vesicles. The *Cylla. catarinensis* can be distinguished by the conidia, bigger than the closest species, as *Cylla. variabilis* ((9–)11–13(–14) × 2–3 µm).

Cylindrocladiella biestipitata **R.A. Fernandes, A. Reis & D. B. Pinho, sp. nov. MycoBank MBXXXXX. Figure 8.**

Etymology: Name refers to the species that can present two stipe extension in one conidiophore.

Sexual morph not observed. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline; penicillate conidiophores comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension, and a terminal vesicle; stipe septate, hyaline, smooth; stipe extension 107–140 µm long, 2–3.5µm wide, aseptate,

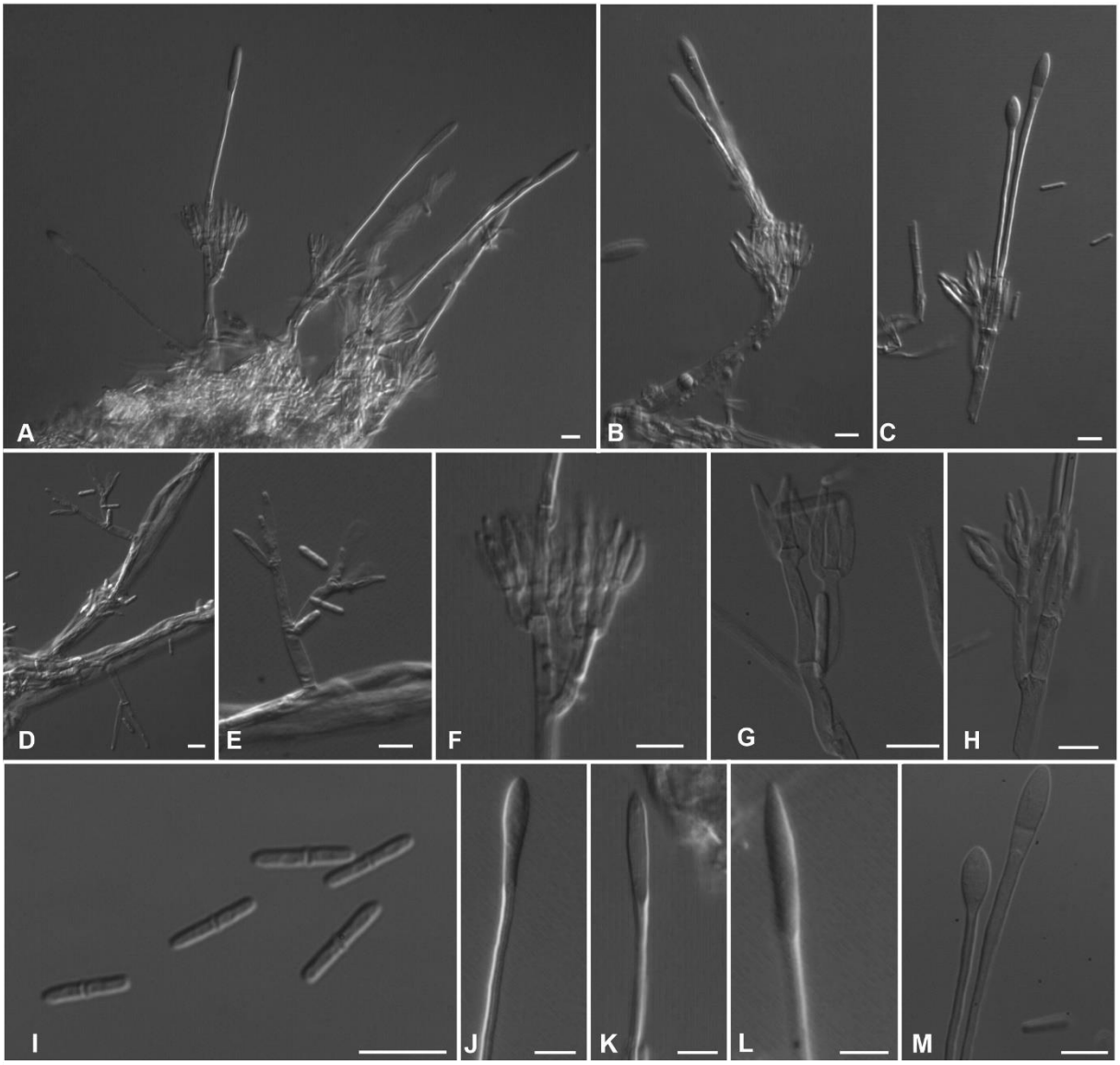

Figure 13. *Cylindrocladiella biestipitata* sp. nov. (CCUB1035). A – C. Penicillate conidiophores. D-E. Subverticillate conidiophores. F-H. Conidiogenous apparatus with branches and phialides. I. Conidia. J-M. Ellipsoidal to obpyriform vesicles. Scale bars: A = 10 µm (Apply to B-K).

straight, thick-walled with one basal septum, terminating in thin-walled, lageniformis to ellipsoidal vesicles. Conidiogenous apparatus (29.5−)33.5–44.5 µm long and 26–39.5(–45) µm wide, with primary branches aseptate 13.5–19.5 × 3–4.5 µm, secondary branches 9.5–11.5 × 2.5–3.5 µm, aseptate, each terminal branch producing 2–4 phialides; phialides 9.5–12.5 × 2.5–3.5 µm, elongate doliiform to reniform to cymbiform, hyaline, aseptate, apex with minute periclinal thickening and

collarette. *Subverticillate conidiophores* in less numbers, comprising of a septate stipe, and primary branches terminating in 2–3 phialides; primary branches straight, hyaline, aseptate, 16.5–21.5 × 3–4 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 12.5–24×3.5 µm, apex with minute periclinal thickening and collarette. Conidia 10.5–13(–15) × 2–3 µm (av. = 12.5 × 2.5 µm), cylindrical, rounded at both ends, straight, 1-septate, frequently slightly flattened at base, held in asymmetrical clusters by hyaline slime.

Culture characteristics: *Culture characteristics*: Colonies on MEA at 26 °C after 7 d. present fast growth, white cottony mycelium; chlamydospores absence; reaching 75 mm. Colonies on PDA at 26ºC after 7 d present fast growth reaching 55 mm, mycelia cottony, white reverse white in the edges and the center of colony tuning pale. Without presence of sporulation, and small hyaline chlamydospores.

Materials examined: Brazil, Minas Gerais, from decaying fruit of *Spondia purpurea*, Abr. 2018, collector A. Reis, isolated by R.A. Fernandes (holotype UB24561, culture ex-type CCUB1035), isotype culture CCUB1012 (UB24565, metabolically inactive).

Notes: *Cylindrocladiella biestipitata* phylogenetically locate inner of one subclade composed by *Cylla. pseudohawaiiensis, Cylla. australiensis* and *Cylla. hawaiiensis.* The species *Cylla. biestipitata* can be distinguished of its closely sister clade by the presence of two stipe extension in one conidiophore.

3.5 Discussion

Gliocladiopsis is a genus with a reduced number of species, especially in Brazilian territory, been only one *G. tenuis* reported by the samples of soil in 1994 (Crous, 2002). The species were founded in this study under *Euterpe edulis, Dypsis madagascariensis* and *Euterpe oleraceae* rot fruits in Distrito Federal and Santa

Catarina, representing the first association of this species with these plants. *G. hennebertii* was previously described in 2019 from samples of rhizoplane of *Cactus scaber* in Ecuador. Since the description the species are restricted to Ecuador (Gordillo & Decock, 2019). The identification of *G. hennebertii* in Brazil increase the territory knowledge about this species, and the association of this species with rot fruit of *Eugenia involucrate* and *E. edulis* on Santa Catarina, increase more one possible host to this species.

The *Ca. cylindrospora* species complex is distributed on the world, they are characterized by macroconidia with one septum, no had a distinct vesicle, varying form pyriform to obpyriform or ovoid to ellipsoidal, turning this species complex hard to differentiate from the other (R. F. Alfenas et al., 2015; Q. L. Liu et al., 2020). In this study the species complex knowledge extended by the increasing two new species, the first association they with fruit rot on Brazil. *Ca. arborium* is a new specie close to *Ca. brasiliensis* this study, composed by two isolates collected from symptomatic fruits of *S. purpurea* and *T. catappa* in Midwest Brazilian. The *Ca. brasiliensis* firstly identified on Brazil in 1948 as *Cylindrocladium pauciramosa* var. *brasiliensis* causing leaf spot on *Eucalyptus* sp. and *Corymbia citreodora* (Lombard et al., 2010a), before associated as *Anacarium* sp. (Lombard et al., 2016) even related as leaf disease. Nowadays *Ca. brasiliensis* had inclusion of two other species as it synonymous (*Ca. hodgesii* and *Ca. pseudohodgesii*) due the construction of back bone phylogeny using uniformity number of markers (Q. L. Liu et al., 2020). The vesicle germination is an event reported in this species complex associated with the observations of the old culture and conidiophores in small frequencies (Crous, 2002), in *Ca. arborium* we quantified the frequency of this event, calculated in 45% of all vesicles analyzed by the cultures with 7dpi. This event suggests that vesicle may develop a row in conidia production of this species.

Two new isolates segregated in individual clade inner the *Ca. cylindrospora* species complex and close to *Ca. hawkswothii,* both suggest that are a new phylogenetic species, but how they are single isolate. Forming a distinct clade out of the subclade with most species of *Ca. cylindrospora* species complex, three isolates presented in this work are proposed as new species, called *Ca. cerradense*. Nowadays, three species of *Calonectria* was reported causing fruit rot, *Ca. cylindropora*, *Ca. ilicicola* and *Ca. fragariae*, only the last was identified in Brazil (Sivapalan et al. 1998; Ferreira et al. 2001; Lopes et al. 2017). *Ca. fragariae* was the first species only reported causing fruit rot, but this species is located on *Ca. candelabrum* species complex. In the *Ca. cylindrospora* species complex the *Ca. arborium* and *Ca. cerradense* are the first species only reported causing fruit rot.

Inner the *Cylindrocladiella,* four species are known by the scientific community. This genus is small sampled in Brazil, and haven't a work exclusively dedicated to understanding the diversity and geographical distribution. Until this work only four species are known in Brazilian territory, *Cylla. infestans, Cylla. lageniformis, Cylla. peruviana* and *Cylla. pseudohawaiiensis* (Crous, 2002; Crous & Wingfield, 1993b; Lombard et al., 2012). In this work we found *Cylla. lageniformis* and *Cylla. peruviana* associated with the rot fruit on *Terminalia catappa* and *Spondia mombin,* there are the first time that both species are associated with these plants. In Brazil *Cylla. infestans* and *Cylla. lageniformis* is associated with *Eucalyptus* sp. and *Cylla. peruviana* with *Piptadenia* sp. and *Psidium guajava* (Crous, 2002; Lombard et al., 2012). *Cylla. vitis* was firstly reported in New Zealand on *Vitis vinifera* in 2017, this was the unique report of this species (Crous et al., 2017). This specie was collected under leaf of *Araucaria angustifolia* in Santa Catarina, Brazil, composing the first report of this specie in Brazilian territory and associated with this plant.

Three new species of *Cylindrocladiella* were proposed in this work. *Cylla. albus* composed by isolates CCUB1137, CCUB410 and CCUB1010 is sister clade of *Cylla. lageniformis.* The species was collected on fruits of *Dypsis madagascariensis, Syzygium jambos* and *Terminalia catappa* in three Brazilian states, Distrito Federal, São Paulo and Santa Catarina. The diversity in species and places can indicate that species have a great adaptability to different host and climate, due to the mean temperature and type of vegetation.

Distrito Federal is characterized by the Aw (koppen classification of climate patterns), with mean temperature of 21°C and are placed in Brazilian Cerrado (Brazilian Savanna), already the isolate collected in São Paulo is in Cwa climate, 20 ºC mean temp. and in transition between Brazilian Cerrado an Atlantic Forest, while the isolate collected in Santa Catarina, is in Cfa climate, in 19ºC mean temp. and in Atlantic Forest (IBGE, 2023). *Cylla. catarinensis* is one species restricted to Santa Catarina and *Terminalia catappa*, all isolates are collected under these conditions, this species phylogenetically form an outgroup of one subclade, like *Cylla. variabilis*. Observing the shape of terminal vesicle of *Cylla. catarinensis* we seen an intense variation going of broadly ellipsoidal with a papillate apex, or clavate to ovoid vesicles. In *Cylla. variabilis* the variation in shape of vesicle was observed too and referred in the name of specie (Lombard et al., 2012), apparently the variation in shape vesicle can be a morphological marker to these clades.

Cylla. biestiptata is one species that can present two stipe extension in one conidiophore, for the genus *Cylindrocladiella* represents one novelty, including in genera description as one new character. Genus closely related to *Cylindrocladiella,* as *Calonectria* and *Gliocephalotrichum* detain species with multiply stipe extension. In

Gliocephalotrichum the presence of more than one stipe extension is a morphological genus marker (Lombard et al., 2014; Silva et al., 2020), while in *Calonectria* this is a species complex marker (Crous, 2002), due in the first genus only *Gliocephalotrichum abrachium* is characterized without stipe extension all off species present this character. In *Calonectria* species inner the *Ca. kyotensis* species complex is commonly reported with one or more stipe extension forming 90º in relation the principal stipe extension (Crous, 2002; Q. L. Liu et al., 2020).

Calonectria species in Brazilian territory are reported since early 90's (Crous, 2002), especially 2015 when the genus gave the inclusion of 20 new species by the one monography entirely dedicate to the genus (R. F. Alfenas et al., 2015). Although the number of *Calonectria* founded in Brazil, most are associated with *Eucalyptus* sp. and a small percentage with native species, that open questions about the diversity of species founded in this country are native or was introduced? The present study shows that despite the intense studies, the diversity and native host remain unexplored. In contrast there aren't studies dedicate to *Cylindrocladiella* in Brazil, let the knowledge restrict to four species in few places. The identification of eight species of *Cylindrocladiella* by this work in different plants and places suggests that they can infect and cause disease on fruit. The presence of new species and new reports suggest that genus have a diversity undiscovered in Brazil. *Calonectria* and *Cylindrocladiella* clearly deserve studies that help understand the diversity and distribution on Brazil to help in development of resistant *Eucalyptus* clones to *Calonectria Leaf Blight* of prevent the new disease caused by these fungi.

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Supplemental Data

Supplementary table 1. GenBank accessions of isolates included in the *Calonectria*-like phylogenetic analysis using elongation factor 1-alpha.

Species	Isolate nr.1	Substrate	Locality	Collector	GenBank Access
					tef1
Aquanectria devians	MUCL 48197	Leaf litter	Ecuador	A. Gordillo & C. Decock	KX671136
A. penicillioides	CBS 257.54	*Acer* sp.	USA	F.V. Ranzoni	KM231865
Calonectria brassicae	CBS 111869	*Argyreia* sp.	Brazil	AC. Alfenas	FJ918567
Ca. cylindrospora	CBS 110666	*Ilex vomitoria*	USA	N.E. El-Gholl	MT412768
Ca. ilicicola	CBS 190.50	*Solanum tuberosum*	Java	K.B. Boedjin & J. Reitsma	AY725726
Ca. naviculata	CBS 101121	Leaf litter	Brazil	R.F. Castañeda	GQ267317
Calonectria-like	CCUB408	-	-	A. Reis	*CNUB781*
Calonectria-like	CCUB413	-	-	A. Reis	*CNUB747*
Calonectria-like	CNUB1892	-	-	A. Reis	*CNUB1892*
Calonectria-like	CNUB1898	-	-	A. Reis	*CNUB1898*
Calonectria-like	CCUB1055	*Araucaria angustifolia*	Santa Catariana - Brazil	A. Reis	*CNUB1859*
Calonectria-like	CCUB1005	*Citrus x limonia*	Santa Catarina, Brazil	A. Reis	*CNUB1812*
Calonectria-like	CCUB1137	*Dypsis madagascariensis*	São Paulo - Brazil	A. Reis	*CNUB1890*
Calonectria-like	CCUB366	*D. madagascariensis*	Distrito Federal, Brazil	A. Reis	*CNUB694*
Calonectria-like	CCUB377	*D. madagascariensis*	Taguatinga	A. Reis	*CNUB701*
Calonectria-like	CCUB378	*D. madagascariensis*	Taguatinga	A. Reis	*CNUB702*
Calonectria-like	CCUB407	*D. madagascariensis*	Distrito Federal, Brazil	A. Reis	*CNUB745*
Calonectria-like	CCUB416	*D. madagascariensis*	Distrito Federal, Brazil	A. Reis	*CNUB750*
Calonectria-like	CCUB422	*D. madagascariensis*	Distrito Federal, Brazil	A. Reis	*CNUB754*
Calonectria-like	CCUB423	*D. madagascariensis*	Distrito Federal, Brazil	A. Reis	*CNUB756*
Calonectria-like	CCUB1037	*Eugenia aggregata*	Santa Catariana - Brazil	A. Reis	*CNUB1839*
Calonectria-like	CCUB1030	*Eugenia involucrata*	Santa Catarina, Brazil	A. Reis	*CNUB1832*
Calonectria-like	CCUB1065	*Euterpe edulis*	Santa Catarina, Brazil	A. Reis	*CNUB1883*
Calonectria-like	CCUB1069	*E. edulis*	Santa Catarina, Brazil	A. Reis	*CNUB1887*
Calonectria-like	CCUB373	*E. edulis*	Canelinha, SC	A. Reis	*CNUB703*
Calonectria-like	CCUB379	*E. edulis*	Santa Catarina, Brazil	A. Reis	*CNUB704*
Calonectria-like	CCUB412	*E. edulis*	Santa Catarina, Brazil	A. Reis	*CNUB751*
Calonectria-like	CCUB419	*E. edulis*	Santa Catarina, Brazil	A. Reis	*CNUB749*

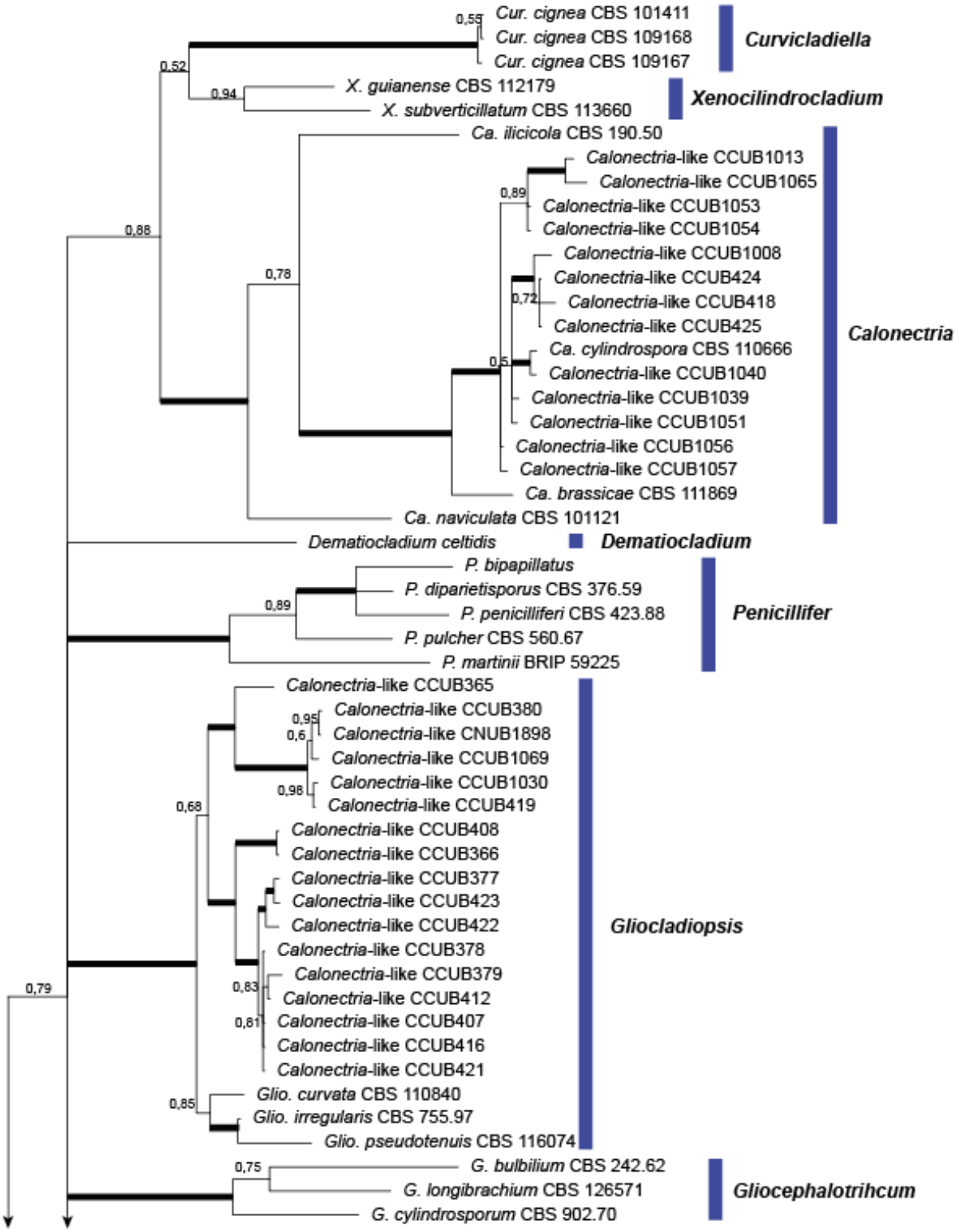
Calonectria-like	CCUB421	*Euterpe oleracea*	Pernambuco, Brazil	A. Reis	*CNUB755*
Calonectria-like	CCUB1054	*I. laurina*	Distrito Federal, Brazil	A. Reis	*CNUB1864*
Calonectria-like	CCUB1057	*I. laurina*	Distrito Federal, Brazil	A. Reis	*CNUB1881*
Calonectria-like	CCUB1053	*Inga laurina*	Distrito Federal, Brazil	A. Reis	*CNUB1863*
Calonectria-like	CCUB1056	*I. laurina*	Distrito Federal, Brazil	A. Reis	*CNUB1880*
Calonectria-like	CCUB406	*Palmeira locuba*	Distrito Federal, Brazil	A. Reis	*CNUB746*
Calonectria-like	CCUB414	*Persea americana*	Goiás	A. Reis	*CNUB758*
Calonectria-like	CCUB1051	*S. mombin*	Pernambuco, Brazil	A. Reis	*CNUB1860*
Calonectria-like	CCUB1052	*S. mombin*	Pernambuco - Brazil	A. Reis	*CNUB1861*
Calonectria-like	CCUB1013	*S. purpurea*	Distrito Federal, Brazil	A. Reis	*CNUB1821*
Calonectria-like	CCUB1039	*Spondia mombin*	Goiás, Brazil	A. Reis	*CNUB1841*
Calonectria-like	CCUB1016	*S. mombin*	Goiás - Brazil	A. Reis	*CNUB1824*
Calonectria-like	CCUB1040	*S. mombin*	Goiás, Brazil	A. Reis	*CNUB1842*
Calonectria-like	CCUB1017	*S. purpurea*	Distrito Federal, Brazil	A. Reis	*CNUB1809*
Calonectria-like	CCUB1035	*S. purpurea*	Minas Gerais - Brazil	A. Reis	*CNUB1837*
Calonectria-like	CCUB418	*S. purpurea*	Distrito Federal, Brazil	A. Reis	*CNUB759*
Calonectria-like	CCUB424	*Spondia purpurea*	Distrito Federal, Brazil	A. Reis	*CNUB1163*
Calonectria-like	CCUB425	*S. purpurea*	Distrito Federal, Brazil	A. Reis	*CNUB785*
Calonectria-like	CCUB365	*Syagrus oleraceae*	Distrito Federal, Brazil	A. Reis	*CNUB693*
Calonectria-like	CCUB380	*S. oleraceae*	Goiás	A. Reis	*CNUB740*
Calonectria-like	CCUB1003	*T. catappa*	Santa Catariana - Brazil	A. Reis	*CNUB1802*
Calonectria-like	CCUB1006	*T. catappa*	Santa Catariana - Brazil	A. Reis	*CNUB1813*
Calonectria-like	CCUB1010	*T. catappa*	Santa Catariana - Brazil	A. Reis	*CNUB1818*
Calonectria-like	CCUB1012	*T. catappa*	Distrito Federal - Brazil	A. Reis	*CNUB1820*
Calonectria-like	CCUB1014	*T. catappa*	Distrito Federal - Brazil	A. Reis	*CNUB1822*
Calonectria-like	CCUB1015	*T. catappa*	Santa Catariana - Brazil	A. Reis	*CNUB1823*
Calonectria-like	CCUB1031	*T. catappa*	Santa Catariana - Brazil	A. Reis	*CNUB1833*
Calonectria-like	CCUB370	*T. catappa*	Tijucas, SC	A. Reis	*CNUB698*
Calonectria-like	CCUB1008	*T. catappa*	Goiás, Brazil	A. Reis	*CNUB1816*
Calonectria-like	CCUB1007	*T. catappa*	Santa Catariana - Brazil	A. Reis	*CNUB1815*
Calonectria-like	CCUB369	*Terminalia catappa*	Tijucas, SC	A. Reis	*CNUB697*
Calonectria-like	CCUB415	*T. catappa*	DF	A. Reis	*CNUB757*
Coralonectria jatrophae	CBS 913.96	Unknown tree	Puerto Rico	G.J. Samuels	KM231863

Curvicladiella cignea	CBS 101411	Decaying seed	French Guiana	C. Decock	KM231866
Cu. cignea	CBS 109168	Decaying seed	French Guiana	C. Decock	KM231868
Cu. cignea	CBS 109167	Leaf litter	French Guiana	C. Decock	KM231867
Cylindrocladiella camelliae	CPC 234	*Eucalyptus grandis*	South Africa	P.W. Crous	JN099087
Cy. lageniformis	CBS 340.92	*Eucalyptus* sp.	Brazil	A.C. Alfenas	JN099003
Cy. parva	CBS 114524	*Telopea speciosissima*	New Zealand	H.J. Boesewinkel	JN099009
Dematiocladium celtidis		*Celtis tala*	Argentina	N. Allegrucci	KM231864
Gliocephalotrichum bulbilium	CBS 242.62	Soil	USA	L.J. Wickerham	KM231892
G. cylindrosporum	CBS 902.70	Soil	Thailand	C. Klinsukont	KF513435
G. longibrachium	CBS 126571	Leaf litter	French Guiana	C. Decock & V. Robert	KF513449
Gliocladiopsis Curvata	CBS 118040	Greenhouse	Belgium	C.Decock	JQ666087
Glio. irregularis	CBS:755.97	Soil	Indonesia	A.C. Alfenas	JQ666099
Glio. pseudotenuis	CBS:116074	Soil	China	M.J. Wingfield	JQ666106
Penicillifer martinii	BRIP 59225	*C. dactylon*	Australia	PT.W. Wong	KJ869241
Pe. bipapillatus		Bark	Venezuela	C.T. Rogerson	KM231860
Pe. diparietisporus	CBS 376.59	Soil	USA	A.A. Foster	KM231861
Pe. penicilliferi	CBS 423.88	Unknown	Guyana	G.J. Samuels	KM231859
Pe. pulcher	CBS 560.67	Soil	The Netherlands	J.H. van Emden	KM231862
Thelonectria discophora		*Pinus radiata*	New Zealand	A.Y. Rossman	KM231897
T. olida		*Asparagus* sp.	Germany	W. Gerlach	HM364345
T. trachosa	CBS 112467	Bark	Scotland	D. Bradford & G.J. Samuels	KM231896
Xenocylindrocladium guianense	CBS 112179	Plant litter	French Guiana	C. Decock	KM231895
X. subverticillatum	CBS 113660	Plant litter	Singapore	C. Decock & O. Laurence	KM231893

Supplementary table 2. The phylogenetic parameter of each dataset builds to phylogenetic analysis of *Calonectria*-like, *Ca. cylindrospora* specie complex, *Cylindrocladiella* sp. and *Gliocladiopsis* sp.

Parameter	Phylogeny 1 *Calonectria*-like	Phylogeny III *Calonectria* complex					Phylogeny IV *Cylindocladiella* genus					Phylogeny V- *Gliocladiopsis* genus			
	Partition														
	TEF1	**CMDA**	**HIS3**	**RPB2**	**TEF1**	**TUB2**	**HIS3**	**ITS**	**LSU**	**TUB2**	**TEF1**	**TUB2**	**HIS3**	**ITS**	**TEF1**
Nº taxa	84	29	29	23	28	29	82	99	74	103	101	41	38	39	30
Evlutive model	HKY+I+G	K80	HKY+I	SYM+G	GTR+G	HKY+G	GTR+I+G	K80+I	HKY+I+G	HKY+I+G	GTR+I+G	GTR+G	GTR+I+G	SYM+I+G	GTR+G
Likelihood	7039.1343	1141.2589	869.3916	1778.3975	1162.7135	1225.3448	3979.5525	1139.9004	1519.9801	2532.7917	2475.3052		1425.0981	890.4502	1264.0912
Matrix length	575	677	433	826	516	569	517	498	831	512	518	480	363	482	483
Variables sites	361	142	69	178	129	127	247	84	49	196	210	178	286	60	113
Parsimony informative sites	292	134	63	157	121	101	169	64	31	159	158	85	76	14	58
Conserved Sites	187	535	363	647	372	440	253	413	766	305	292	295	71	416	356
Base frequencies															
Freq. A	0.2320	Equal	0.2211	Equal	0.2375	0.2214	0.2403	Equal	0.2571	0.2223	0.2232	0.2292	0.2258	Equal	0.2196
Freq. C	0.3078		0.3838		0.3236	0.3194	0.3466		0.2099	0.2891	0.3211	0.3031	0.3965		0.3330
Freq. G	0.1718		0.2010		0.1934	0.2274	0.1829		0.2969	0.2130	0.1845	0.2391	0.2037		0.2008
Freq. T	0.2884		0.1941		0.2455	0.2317	0.2302		0.2361	0.2756	0.2712	0.2286	0.1740		0.2466
Transition rates															
[A-C]	1.3422	4.6146	3.0166	1.3912	3.5939	1.8376	1.1611	1.3932	4.1391	2.6080	1.2646	1.8249	0.5827	0.6160	9.6816
[A-G]				3.1497	8.5687		3.1855				3.9217	4.3324	2.7000	0.5168	22.2380
[A-T]				0.6983	7.2246		1.0819				2.1431	2.1982	1.1782	0.0000	14.6972
[C-G]				0.9065	2.6651		0.4690				0.7046	1.4212	0.1379	0.2800	3.7433

[C-T]				7.8908	9.4406		3.4067				3.8326	8.5104	5.8236	2.4131	38.9276
[G-T]				1.0000	1.0000		1.0000				1.0000	1.0000	1.0000	1.0000	1.0000
Proportion of invariable sites	0.2516	-	0.8328	-	-	-	0.2873	0.8444	0.8957	0.5424	0.5014	-	0.4397	0.8703	-
Gamma	1.0726	-		0.4865	0.2035	0.1937	0.7133	-	0.9058	10.822	0.7581	0.4581	0.3018	0.7208	0.0954


Cur. cignea CBS 101411
Cur. cignea CBS 109168
Cur. cignea CBS 109167
Curvicladiella
X. guianense CBS 112179
X. subverticillatum CBS 113660
Xenocilindrocladium
Ca. ilicicola CBS 190.50
Calonectria-like CCUB1013
Calonectria-like CCUB1065
Calonectria-like CCUB1053
Calonectria-like CCUB1054
Calonectria-like CCUB1008
Calonectria-like CCUB424
Calonectria-like CCUB418
Calonectria-like CCUB425
Ca. cylindrospora CBS 110666
Calonectria-like CCUB1040
Calonectria-like CCUB1039
Calonectria-like CCUB1051
Calonectria-like CCUB1056
Calonectria-like CCUB1057
Ca. brassicae CBS 111869
Ca. naviculata CBS 101121
Calonectria
Dematiocladium celtidis
Dematiocladium
P. bipapillatus
P. diparietisporus CBS 376.59
P. penicilliferi CBS 423.88
P. pulcher CBS 560.67
P. martinii BRIP 59225
Penicillifer
Calonectria-like CCUB365
Calonectria-like CCUB380
Calonectria-like CNUB1898
Calonectria-like CCUB1069
Calonectria-like CCUB1030
Calonectria-like CCUB419
Calonectria-like CCUB408
Calonectria-like CCUB366
Calonectria-like CCUB377
Calonectria-like CCUB423
Calonectria-like CCUB422
Calonectria-like CCUB378
Calonectria-like CCUB379
Calonectria-like CCUB412
Calonectria-like CCUB407
Calonectria-like CCUB416
Calonectria-like CCUB421
Glio. curvata CBS 110840
Glio. irregularis CBS 755.97
Glio. pseudotenuis CBS 116074
Gliocladiopsis
G. bulbilium CBS 242.62
G. longibrachium CBS 126571
G. cylindrosporum CBS 902.70
Gliocephalotrihcum
0,55
0,52
0,94
0,88
0,78
0,89
0,72
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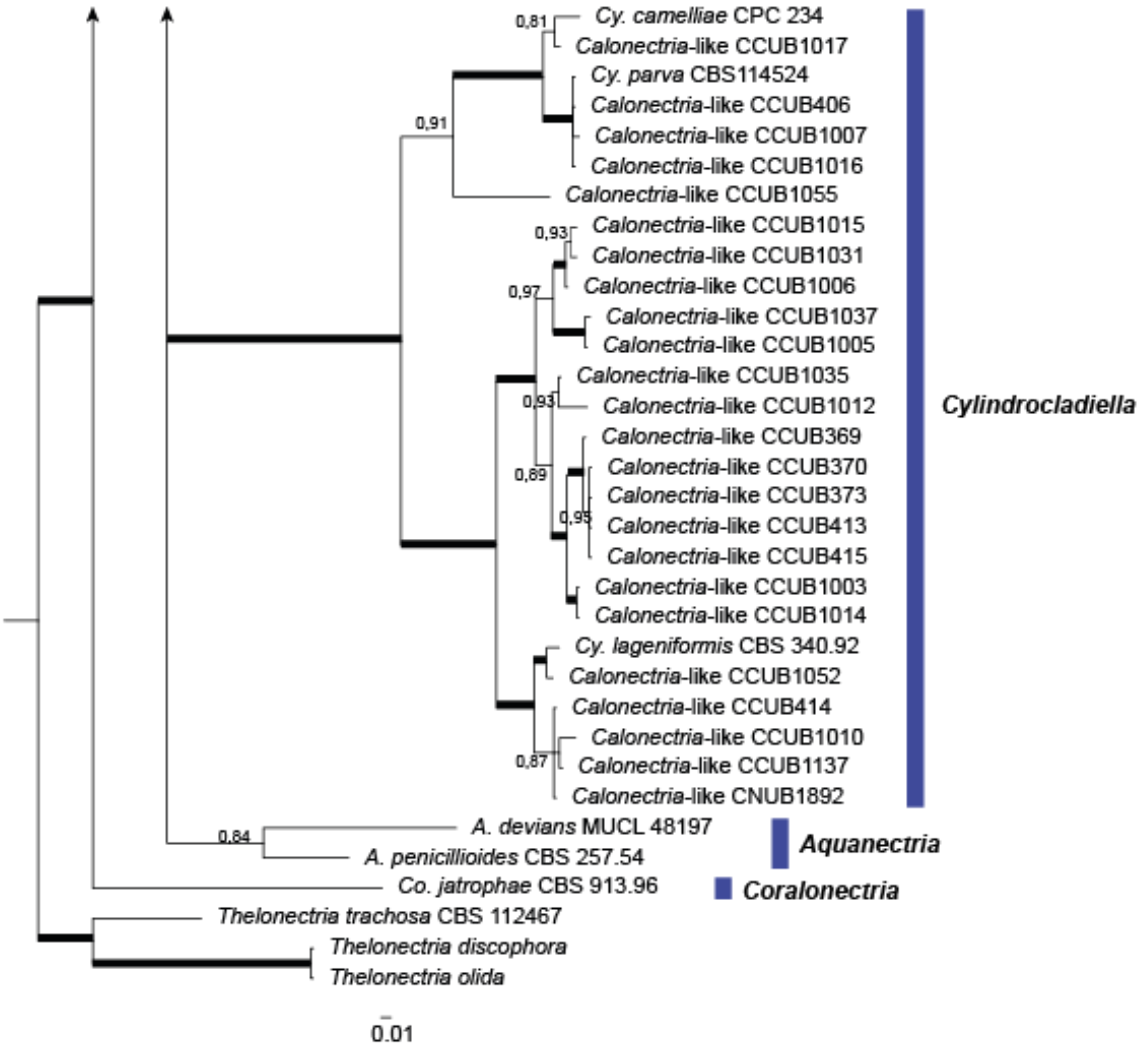
0,83
0,81
0,85
0,75



Supplementary figure 14. Phylogenetic tree of specie complex *Calonectria*-like based on Maximum Likelihood (ML) and Bayesian Inference (BI) of combined of five partial genes (*cmdA, his3, tef1a, tub2*). *Thelonectria disophora* and *Te. olinda* was used as outgroup. Values of bootstrap (ML) and posterior probability (PP) to BI are presented above the branches. Thicker branches mean values of ML ≥ 99% and PP ≥ 0.99. Taxonomic units in bold represent isolates obtained to this study.

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Capítulo 4: New disease on *Syzygium jambos* (L.) Alston causing fruit rot in Brazil.

Published in:

New report of Nectriaceous and Botriosphaeraceous species causing fruit rot on *Syzygium jambos* (L.) Alston in Brazil.

4.1 Abstract

The ornamental tree *Syzygium jambos* (Myrtaceae) was introduced in Brazil and distributed in almost all the territory. Its edible fruits have a unique aroma and flavor. In addition, this species has a significant importance in traditional medicine and a potential source to new drugs. However, the number of studies focused on the diseases associated to this species is limited. Our study expands the knowledge on fruit rot pathogens on *S. jambos* in three Brazilian states by applying phylogenetic approaches and pathogenicity tests. The results revealed twenty-four fungal isolates using DNA sequences of ITS nrDNA*, tef1a* and *tub2*. Multigene phylogenetic analysis confirms the presence of *Gliocephalotrichum bulbilium,* the first report of *Neofusicoccum occulatum* and *N. umdonicola* in Brazil*,* and the first record of *Cylindrocladiella lageniforms, C. peruviana, Gliocladiopsis tenuis, N. batangarum,* and *N. parvum* on *S. jambos*. In the pathogenicity tests, eleven isolates produced symptoms of fruit rot and confirmed Koch's postulates; only isolates of *C. peruviana, G. tenuis* and *N. parvum* did not cause disease. Although some of the species identified in this study were previously reported as important plant pathogens, the present study confirmed that they can cause fruit rot in *S. jambos.* Our study also supports previous studies suggesting that some of these fungi may remain in an endophytic asymptomatic stage in *S. jambos*. Symptoms start only after the fruit has been harvested, or infecting cracks of fallen and possibly damaged fruits. These fungal pathogens can difficult the use of *S. jambos* fruits in further pharmacological applications.

Keywords: Hypocreales, Botryosphaeraceae, Myrtaceae, Medicine, Drugs

4.2 Introduction

Syzygium jambos L. Alston. was introduced in South America to use as an ornamental and fruit tree in urban places (Tessmann et al. 2001). In Brazil, this plant is known as *jambo-amarelo, jambo-cheiroso,* or *jambo-da-índia*, among other names (Lorenzi 2003). This plant species originated in Southeast Asia and naturalized in India. It is an evergreen tree of the Myrtaceae family with edible fruits, smooth skin, abundant hard pale white pulp, attractive and unique rose-like aroma, and sweet taste with acid notes (Guedes et al. 2004).

In India *S. jambos* has a significant importance in traditional medicine, used as tonic for the brain and liver, as diuretic, to reduce fever, and treat diarrhea, dysentery, and catarrh (Mohanty and Cock 2010). In the last 20 years, numerous studies have focused on understanding more about the plant's chemistry and pharmacological applications (Ochieng et al. 2022). Many of these studies confirm its traditional uses in medicine, demonstrating that extracts of this plant have anti-inflammatory, antidiabetic, hepatoprotective, antioxidant, and antimicrobial activities, among others (Mohanty and Cock 2010). The identification of the chemical constituents and pharmacological properties of the extracts and compounds from *S. jambos* suggests a new economic potential.

Although the economic and medicinal potential of *S. jambos* has been increasing, little is known about the diseases affecting this plant. In USDA disease databank, approximately 225 records distributed on the world are reported, most of them in Cuba (Farr and Rossman 2020). Camino-Vilaró et al. (2019) identified 69 fungal species associated with *S. jambos*, but without inferences on their pathogenicity; all reported

species were isolated from stems and leaves. Some examples of fungal diseases reported in Brazil are the rust *Austropuccinia psidii,* which causes significant damage to young leaves, fruits, stems of juvenile flush growth, and flowers (Tessmann et al. 2001); *Colletotrichum pseudoacutatum*, causing anthracnose on fruits (Soares et al. 2017); or *Gliocephalotrichum simplex* causing fruit rot (Silva et al. 2020a). These three diseases are the only known reports on *S. jambos* in the Brazilian territory.

The diversity of fungal species that attack *S. jambos* is poorly known around the world, especially in Brazil. Thus far, the objectives of the present study were to identify several fungal isolates associated with fruit rot symptoms in different Brazilian states and determine their pathogenicity. This study constitutes the first research focused on broadening the knowledge about diversity of fungi causing fruit rot in *S. jambos* in Brazil.

4.3 Material and Methods

Isolates - Surveys were conducted in eight distinct locations: four in Distrito Federal (Brasília, Ceilândia, Gama, and Recanto das Emas), two in Goiás (Corumbá and Cidade de Goiás) and two in Santa Catarina (Canelinha and Tijucas). Samples of fruits presenting symptoms of rot or signs of the pathogen (e.g., conidiomata or conidia) were collected and transported to Laboratório de Micologia (Departamento de Fitopatologia, Universidade de Brasília). Fungal isolations were conducted by the method of direct and indirect isolation (Alfenas and Mafia 2016). All obtained cultures were classified according to the morphological characteristics. Then, fungi in taxonomic groups with pathogenic potential were selected for further study. The selected cultures were then purified by transferring hyphal tips onto a new Petri dish with Malt Extract Agar (MEA 2%) and incubated in growth chamber at 25ºC. After 7

days, all cultures were stored in Castallani, Glycerol 10% and Mineral Oil methods (Alfenas and Mafia 2016) in 2 mL tubes with five mycelial plugs each. These cultures are stored at the Coleção de Culturas da Universidade de Brasília (codes available in supplementary table 1).

Molecular identification and phylogenetic analyses - Twenty-three pure cultures were grown on MEA at room temperature for 7 days to produce a mycelial mat for DNA extraction. DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), with modifications (Pinho et al. 2012). Partial sequences of translation elongation factor 1-α (*tef1a*), with primers EF-1F and EF-2R (Jacobs et al. 2004), were used to obtain a preliminary identification. After the preliminary identification and depending on the taxonomic affinity, additional loci were amplified and sequenced to obtain a multilocus analysis. The additional markers used were *β*-tubulin (*tub2*) (primers T1 (O'Donnell et al. 2007) and Bt2b (Glass and Donaldson 1995)), and nuclear ribosomal DNA internal transcribed spacers (*ITS*) (primers LR5 (Vilgalys and Hester 1990) and V9G (Hoog and Ende 2009)). PCR conditions were the same described in Silva et al. (2020b). PCR products were purified using 2 *μ*L de ExoSAP-IT™ (TermoFisher, Santa Clara, USA) following the manufacturer's instructions and bidirectionally Sanger-sequenced. Quality of chromatograms were confirmed and edited in DNA Dragon software version 1.9.2 (Hepperle 2011). Forward and reverse strands were assembled in DNA Dragon. Consensus sequences were then submitted to a MegaBLAST search in GenBank. After the BLAST search, representative sequences of *Calonectria, Cylindrocladiella, Gliocephalotrichum,* and *Neofusicoccum* were downloaded from GenBank to place the query sequences in a phylogenetic context. All sequences used in this study are listed in TABLE 1. Alignments for each locus and genus were carried out in MAFFT 7 (Katoh

and Standley 2013) using the MUSCLE algorithm and the default parameters. Phylogenetic analyses were performed using Bayesian Inference (BI) and Maximum Likelihood (ML). For BI analyses, the best substitution models for each locus were determined with MrModeltest (Nylander 2004). The CIPRES portal (Miller et al. 2010) was used to run MrBayes 3.2.2 (Ronquist et al. 2012). The Markov chain Monte Carlo (MCMC) analysis was run with a total of 10 million generations and one tree sampled every 1000 generations. The convergence of log likelihoods was confirmed using TRACER 1.7.1 (Rambaut and Drummond 2018). The first 25% of saved trees were discarded as the burn-in and posterior probabilities (PPs) calculated for the remaining trees.

The ML analysis was performed using RAxML 8.2.9 (Stamatakis 2014) through the CIPRES portal, starting with a randomized stepwise-addition parsimony tree under a GTR+GAMMA model for each gene region and the four-gene combined data set and 1000 bootstrap (BS) replicates under the same model. All trees were edited in FigTree 1.4 (Rambaut 2018). The nucleotide matrices and phylogenetic trees from all data sets (individual and combined locus) are publicly available on GitHub (https://github.com/rildoalexandre/syzygium_jambos.git).

Morphological characterization - To compare the morphological characteristics of isolates obtained in this study with the related in literature, the new isolates were cultivated on SNA (Syntetic Nutrient Agar) (Lombard et al. 2010; Nirenberg 2011) for Hypocreales genera. For Botryosphaeriaceae, all isolates were placed in a Petri dish with PDA (Potato Dextrose Agar 2%) with autoclaved *Pinus* sp. needles (Crous et al. 2019). All cultures were incubated at 25ºC for 7 days with an alternating photoperiod (12h light/12h dark). Microscopical characteristics were examined by mounting fungal structures in clear lactoglycerol and polyvinyllactoglycerol (PVLG). At least fifteen

measurements were made for each character using a Leica DM2500 microscope (Leica Microsystem, Nassloch, Germany). Colony characteristics were described using MEA and PDA cultures grown for 7 days. Rayner (1970) was used as color standards. Measurements were tabulated as 95% confidence intervals and average, minimum, and maximum values.

Pathogenicity tests - Pathogenicity assays were performed on mature fruits of *Syzygium jambos*. One isolate of each species was selected. The inoculum was grown in Petri dishes containing 2% MEA and ten sterile toothpicks for 7 d at room temperature. Colonized toothpicks were used to poke the fruit's surface until they reached the mesocarp. Five fruits per isolate were inoculated. The control treatment was toothpicks without the fungus. Inoculated fruits were maintained in wet chambers with 90% relative humidity at room temperature. After 5-7 d post inoculation, the presence or absence of symptoms was evaluated, and the fungi re-isolation was carried for the symptomatic fruits to fulfill Koch's postulates.

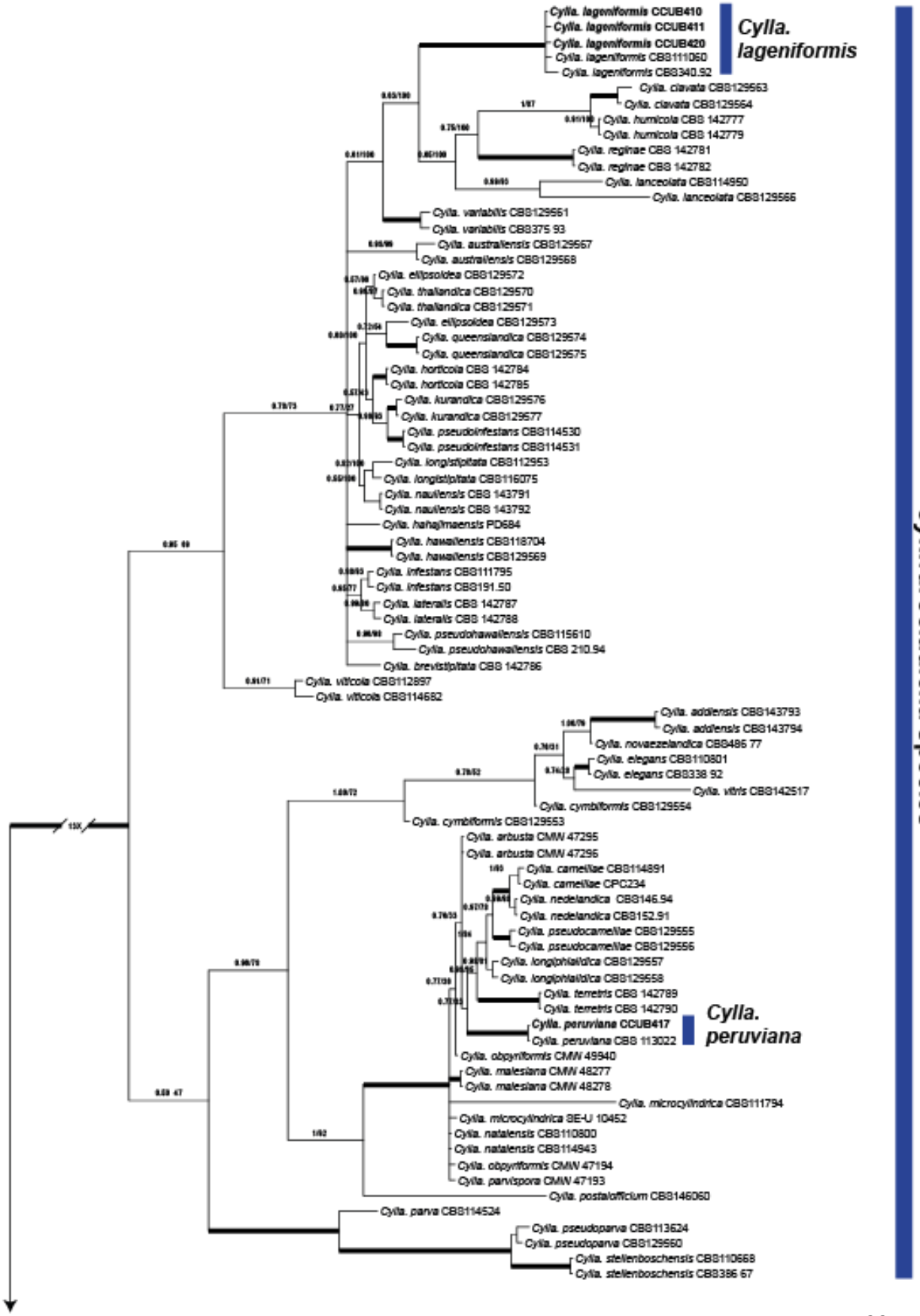
4.4 Results

Isolates – A total of twenty-four isolates were obtained from rotten fruits. Symptomatic fruits presented white hyphae under the surface. Epicarp and mesocarp were pale to dark brown, and in rare cases it was possible to observe fungal sporulation. The 24 isolates were collected in three Brazilian states: Distrito Federal (15 isolates), Goiás (5 isolates), and Santa Catarina (4 isolates). A preliminary screening using the morphology and growth rate of the colonies, the isolates were classified in two families, i.e., Botryosphaeriaceae (15 isolates) and Nectriaceae (9 isolates). Botryosphaeriaceae isolates were fast-growing with abundant aerial mycelia, initially white and turning black after 10-15 days. Nectriaceae isolates had fewer aerial mycelia

and moderate growth, and the colonies were initially white and, after four days, turning pale-brown to brown due the production of microsclerotia in the medium; some colonies presented white sporulation typical of this family.

Isolate identification and phylogenetic analyses – Sequences of 227 isolates were downloaded from GenBank for the phylogenetic analyses. These sequences were segregated into two matrices. The first consisted of 138 sequences of *Cylindrocladiella* spp*., Gliocephalotrichum* spp*.,* and *Gliocladiopsis* spp., and the second included 89 sequences of *Neofusicoccum* spp., including one sequence each of *Calonectria candelabrum* CPC1675 and *Botryosphaeria dothidea* CBS 100564 used as outgroup for Hypocreales and *Neofusicoccum* phylogenies, respectively. If available, at least two sequences per species were used (Supplementary Table S1). The Hypocreales concatenated alignment (ITS, *tef1a* and *tub2*) consisted of 1641 bp, 900 of these were conserved, 715 variables, and 623 parsimony-informative. The GTR+I+G for *tef1a* (589 bp), SYM+I+G for ITS (589 bp) and HKI+I+G for *tub2* (552 bp) were selected as best model to each loci. The *Neofusicoccum* multiloci matrix contained a total of 1232 bp (*tef1a* = 290 bp; ITS = 550 bp; *tub2* = 392 bp), 829 bp were conserved, 363 bp variable, and 218 bp parsimony-informative. Best model of nucleotide substitution was GTR+I+G for ITS, HKY+G to *tef1a* and GTR+G to *tub2*. For the

BI analysis, the best models of nucleotide substitutions were: GTR+G for *tub2* and ITS



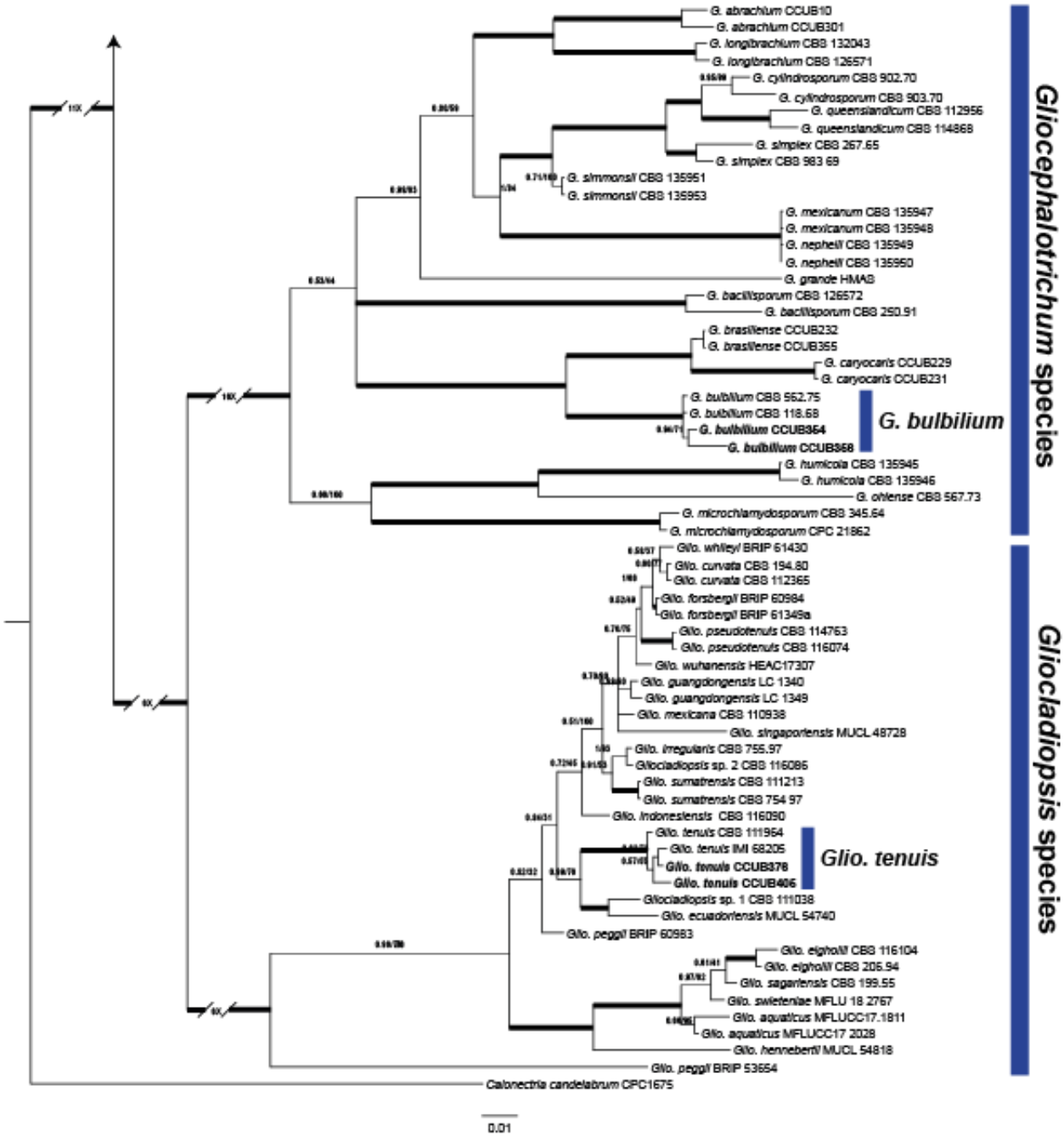


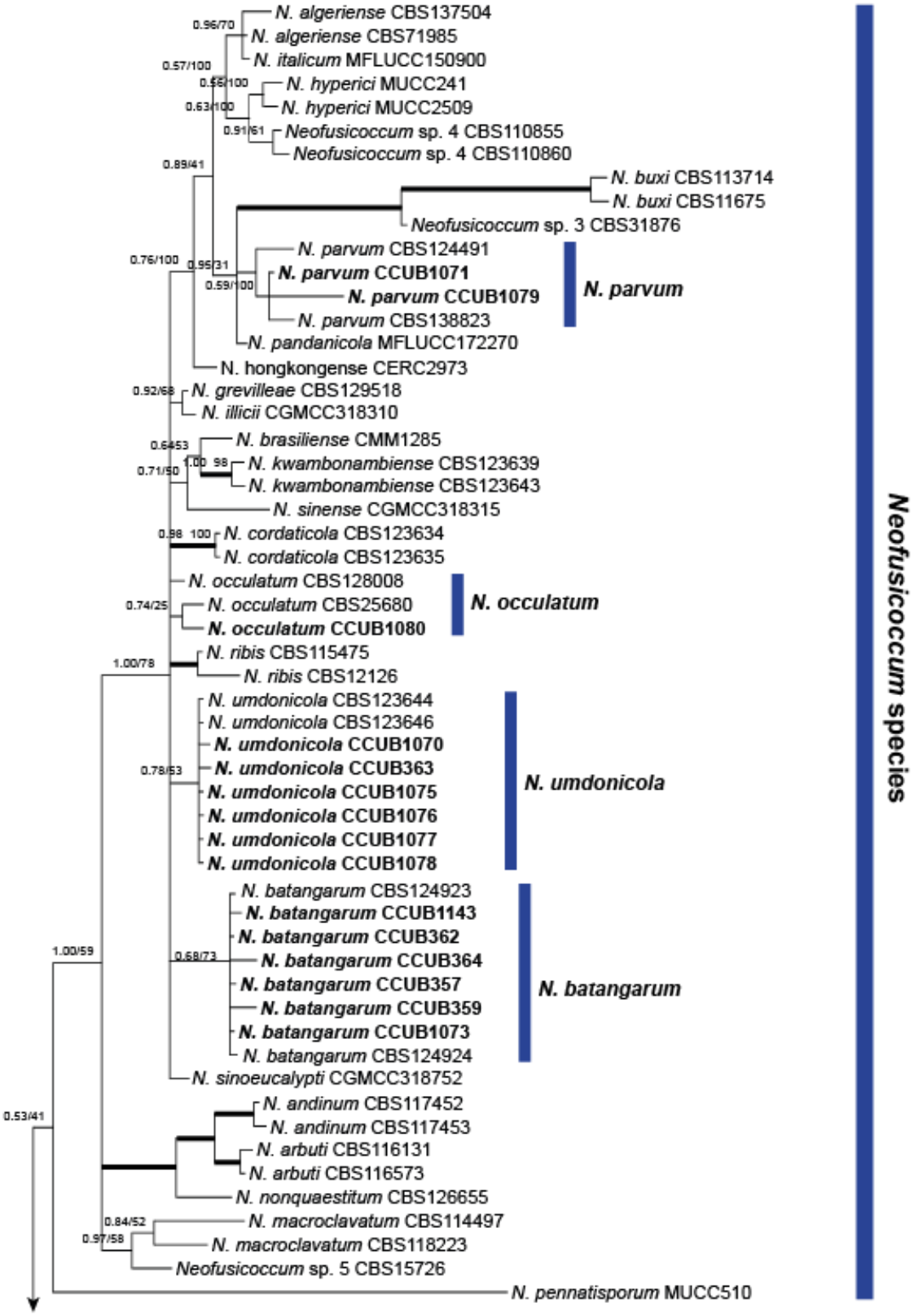
Figure 15. Figure 1. Multilocus (ITS, tef1a and tub2) phylogenetic tree of three Hypocreales genera based on Bayesian Inference analysis. Taxa in bold represents new isolates/species reported in this study. Posterior probability (PP) and Maximum likelihood (ML) bootstrap values are indicated at nodes. Thicker nodes represent PP/ML values above 0.99/99%. Long branches are trimmed according to the scale and the number is indicated. The scale bar represents the number of expected changes per site.

(plus proportion of invariable sites), and HKY+G for *tef1a* (Supplementary Table S2).

The multilocus phylogenetic analysis shows that the nine Hypocreales isolates were distributed among three genera and four species: *Cylindrocladiella lageniformis* (CCUB410, CCUB411 and CCUB420); *Cylla. peruviana* (CCUB417); *Gliocephalotrichum bulbilium* (CCUB354 and CCUB358); and *Gliocladiopsis tenuis* (CCUB378 and CCUB406). All clades representing the above species are supported by high PP (≥0.99) and ML bootstrap values (≥99%) (FIGURE 1). Four *Neofusicoccum* species were identified; six in *N. batangarum* (PP=0.58; ML=73%) and *N. umdonicola* (BI=0.78, ML=53%), one in *N. occulatum* (BI=0.74, ML=25%), and two in *N. parvum* (BI=0.95, ML=31% (FIGURE 2).

Pathogenicity tests – Five days post inoculation, 82% of the inoculated fruits presented symptoms. All fruits used as negative control remained healthy without any symptoms (FIGURE 3 – A and B). Fruits inoculated with isolates CCUB354 and CCUB356 of *G. bulbilium* produced lesions with soaked edges, transitioning from a pale color near the edges of the lesion to brownish in centers, and 2 d.p.i. affecting 50% of the fruit surface. Under the lesion, intense sporulation is observed, giving a velvety characteristic to the fruit. Under the dissecting scope it was possible to observe chlamydospores in the mesocarp. In a transversal section of the affected tissue, the soaked symptom was present in all the fruit, including the seed (FIGURE 3 – C-F). Fruits inoculated with *Glio. tenuis* CCUB376 produced smaller lesions than those observed in *G. bulbilium,* with slightly paler necrotic tissues not extending until the endocarp (FIGURE 3 – G and H). *Glio. tenuis* isolate CCUB405 did not produce symptoms; its growth extended just on

the surface under fruit's exocarp (FIGURE 3 – I and J). Sporulation was not observed in any of the fruits inoculated with *Glio. tenuis* isolates.



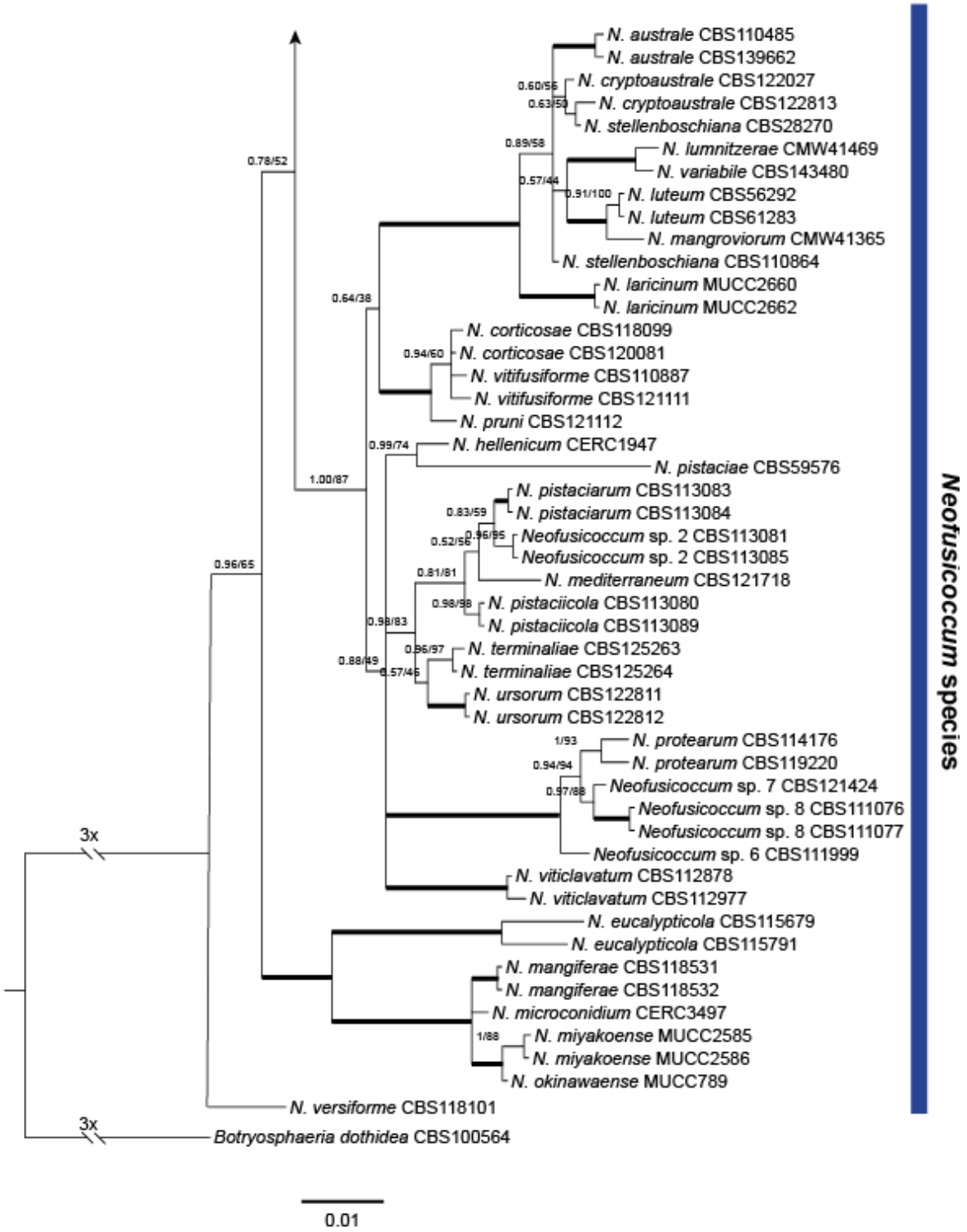


Figure 2. Neofussicoccum multilocus (tef1a, ITS and tub2) phylogenetic tree based on Bayesian Inference analysis. Taxa in bold represent new isolates/reports added from this study. Posterior probability (PP) and Maximum Likelihood (ML) bootstrap values are indicated at nodes. Thicker nodes represent PP/ML values above 0.99/99%. Long

branches are trimmed according to the scale and the number is indicated at branch. The scale bar represents the number of expected changes per site.

Syzygium jambos fruits inoculated with *Cylindrocladiella* isolates presented different levels of symptoms. The isolate of *Cylla. peruviana* CCUB417 did not induce fruit rot symptoms or grew on the fruit's exocarp (FIGURE 3 – Q and R). All three isolates of *Cylla. lageniformis* (CCUB410, CCUB411 and CCUB420) produced symptoms. For *Cylla. lageniformis* the characteristic symptoms were pale circular lesions on the edges and brownish in the center. The lesions extended all throughout the inside of the fruit. In the exocarp it was possible to see the signs of the pathogen, i.e., white mycelia; the mycelium was not obvious in the inner tissues of the fruit or seed (FIGURE 3 – K-P).

Fruits inoculated with isolates of *Neofusicoccum* showed different types of symptoms; only isolate *N. parvum* CCUB1079 did not produce symptoms or growth on the fruits (FIGURE 3 – W and X). Fruits inoculated with *N. umdonicola* CCUB1077 presented circular lesions, pale in edges and darker in the center, with some white mycelia on the fruit's surface. In a transversal section of the affected tissue, the lesion was characterized by a pale color that originated at the inoculation point (FIGURE 3 – Y and Z). Isolates of *N. batangarum* CCUB357, CCUB364 and *N. occulatum* CCUB1080 produced the same symptoms and signs as above. These isolates caused a brownish rot that extended throughout the entire fruit, with white to gray mycelia covering all fruit surface; the mycelium was also found in the inner tissues and seed (FIGURE 3 – S-V and Aa-Ab). No conidiomata or conidia were produced in the inoculated fruits. Koch's postulates were confirmed by pathogenicity assays for

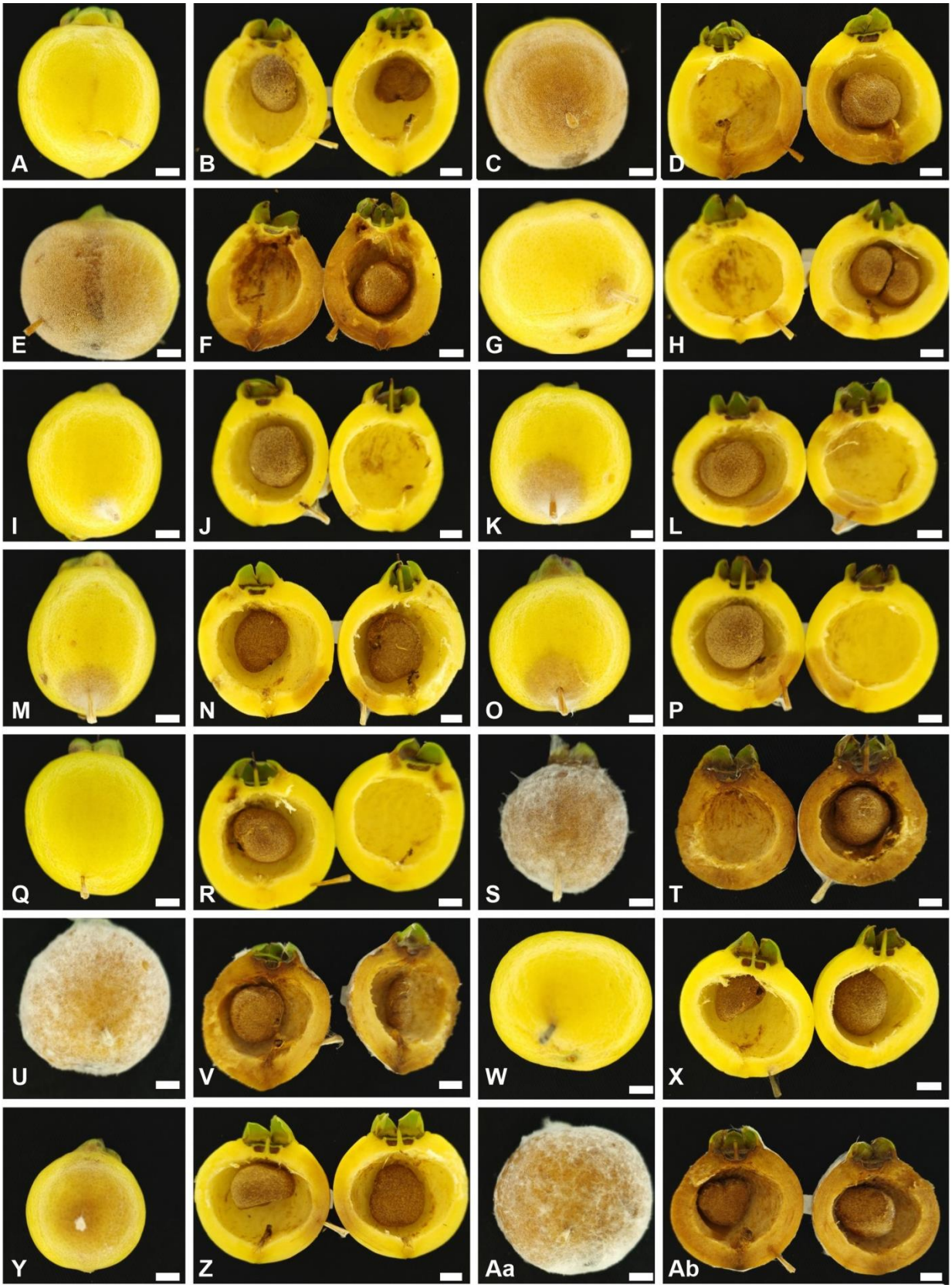


Figure 3. Pathogenicity assays on Syzygium jambos. (A-B) non inoculated, Gliocephalotrichum bulbilium CCUB354 (C-D) and CCUB356 (E-F); Gliocladiopsis tenuis CCUB376 (G-H) and CCUB405 (I-J); Cylindrocladiella lageniformis CCUB420

(K-L), CCUB410 (M-N) and CCUB411 (O-P); Cylla. peruviana CCUB417 (Q-R); Neofusicoccum bataguarum CCUB357 (S-T) and CCUB364 (U-V); N. parvum CCUB1079 (Y-Z); N. umdonicola CCUB1077 (Aa-Ab) and N. occulatum CCUB1080 (Ac-Ad). scale = 5mm.

six species analyzed by the reproduction of field symptoms in laboratory and post reisolation of causal agent from the inoculated fruits, only the species *Cylla. peruviana* and *N. parvum* were not confirmed in the Koch's postulates due to the absence of symptoms.

4.5 Discussion

In total, this study identified six species, inner *Gliocephalotrichum*, *Gliocladiopsis*, *Cylindrocladiella* and *Neofusicoccum*. Six species represent new reports causing fruit rot on *Syzygium jambos* (*Gliocephalotrichum bulbilium, Gliocladiopsis tenuis, Cylindrocladiella lageniformis, Neofusicoccum bataguarum, N. umdonicola* and *N. occulatum*) and two associated with *Syzygium jambos* (*Cylla*. *peruviana* and *N*. *parvum*). The species *Cylla*. *lageniformis*, *Cylla*. *peruviana*, *G. bulbilium, Glio. tenuis, N. batangarum* and *N. parvum* were previously found in Brazil (Crous 2002; de Oliveira Costa et al. 2010; Dissanayake et al. 2016; Silva et al. 2020b). However, the species *N. occulatum and N. umdonicola* are being reported for the first time in Brazil.

The pathogenicity assays showed that *Cylindrocladiella lageniformis* is one of the causal agents of fruit rot in *S. jambos* and that the isolate of *Cylla. peruviana* did not induce symptoms. Studies from different parts of the world have associated *Cylindrocladiella* with many symptoms; however, few of those have regarded those fungi as important plant pathogens (Lombard et al. 2012). This is probably because the efforts have been placed mostly on taxonomy and not on their potential as

phytopathogen (e.g., pathogenicity tests) (Pham et al. 2018). The authors highlighted that among the thirteen known *Cylindrocladiella* species, only four were associated with plant tissues but none had the pathogenicity tested. In the present study, *Cylla. lageniformis* and *Cylla. peruviana* were recovered from fruits of *S. jambos* in Distrito Federal, Brazil. Both species had already been reported in Brazil, but in *Eucalyptus* spp. plantations and associated with leaf spots and cutting rot (Crous et al. 1991; Crous and Wingfield 1993; Crous 2002). These species were also reported as the causal agents of black-foot disease on grape (van Coller et al. 2005) and, in stem inoculation, the authors observed differences in pathogenicity in these two species. Van Coller et al. (2005) showed that *Cylla. lageniformis* caused significant damage, while the lesions caused by *Cylla. peruviana* were not different from the negative control.

Gliocladiopsis bulbilium causing disease on *S. jambos* was first reported by Silva et al. (2020b). However, the authors tested the pathogenicity of only the three new species discovered in that study. In the present study, we confirmed that the symptoms observed by Silva et al. (2020b) are the same. However, the signs of the pathogen were slightly different, displayed as the brown velvety appearence in *G. bulbilium* due to the intense sporulation. In the species analyzed by Silva et al. (2020b), the sporulation was white and sparse. *Gliocladiopsis* species are commonly isolated from plant material, soil samples and even in freshwater (Crous 2002; Liu and Cai 2013). Although they have been associated with plants, this genus was never considered an important plant pathogen (Lombard and Crous 2012). The *Glio. tenuis* isolates recovered in this study had a different pattern of pathogenicity. Isolate CCUB376 produces discrete and small lesions, while CCUB405 only grow on the *S. jambos* fruit surface.

Two species of *Neofusicoccum* found in this study had already been reported in Brazil: *Neofusicoccum parvum* infecting mango (de Oliveira Costa et al. 2010) and *N batangarum* causing brown spot in cactus prickly pear (Conforto, et al. 2016). Although these species have been known in Brazil since 2010, information on their distribution, host diversity, and genetic variability are limited. For example, *N. parvum* has been reported on 205 plant species worldwide (Farr and Rossman 2020). In Brazil, these fungi species were reported in six plant species (e.g., *Carya illinoinensis, Fragaria ×ananassa, Mangifera indica, Psidium guajava, Vitis* sp*.,* and *Vitis vinifera*), but only in southeast and northeast regions of Brazil. Regarding *N. batangarum,* knowledge on distribution and host diversity is less than for *N. parvum.* This species has been reported in seven countries infecting ten plant species (Farr and Rossman 2020). In Brazil, they are associated with *Anacardium othonianum, Nopalea cochenillifera,* and *Vitis labrusca* (Brewer et al., 2021; de Oliveira Costa et al., 2010; Farr and Rossman, 2022; Lopes et al., 2014.; Rêgo et al., 2020).

Neofusicoccum occulatum was first described on *Eucalyptus* sp. and *Wollemia nobilis* in Australia (Sakalidis et al. 2011). There were later reports on *Eucalyptus* sp. in Hawaii, Uruguay, and Uganda (Sakalidis et al. 2011). In Uruguay and Uganda, they were also found infecting other Myrtaceae, such as *Grevillea* spp. and *Blepharocalyx salicifolius* (Sakalidis et al. 2013). We isolated *N. occulatum* from symptomatic fruits of *S. jambos,* also a Myrtaceae native of Southeast Asia (Ochieng et al. 2022) and introduced into Brazil. Sakalidis et al. (2013) hypothesized that *N. occulatum* and *N. parvum* were introduced to those countries from *Eucalyptus* germplasm brought from Australia. Their hypothesis was based on the report of this species in introduced *Eucalyptus* and native Myrtaceae in Uruguay. In Brazil, this is the first detection of *N.*

occulatum in a non-native plant, possibly supporting the Sakalidis et al. (2013) hypothesis; however, further studies are necessary to confirm this assumption.

Previous to the present study, *N. umdonicola* had been reported only with one native Myrtaceae (*Syzygium cordatum*) in four places in South Africa, and *Schizolobium parahyba* in Ecuador (Pavlic et al. 2009; Mehl et al. 2014, 2017; Osorio et al. 2016; Jami et al. 2017; Farr and Rossman 2020). The present study expands the geographic distribution of *N. umdonicola* to Brazil and on a new host *S. jambos.* Slippers and Wingfield (2007) reported that some Botryosphaeriaceae colonize the plant host without development of external symptoms, remaining as endophytes or latent pathogens, possibly becoming pathogenic as the plant's physiology (e.g., stress) changes. These fungi can then produce multiple disease symptoms such as fruit rots, leaf spots, seedling damping-off, and collar rot, among others (Mehl et al. 2017). Similarly, Pavlic et al. (2009) obtained several isolates from asymptomatic twigs, leaves, and fruits of *S. cordatum.* After testing their pathogenicity, they observed that the isolates produced lesions on stems.

Currently, there are reports of 172 species of fungi associated with *S. jambos.* Most of these were done meanly on Cuba and Hawaii (Camino-Vilaró et al. 2019; Farr and Rossman 2020). In Brazil, only four species of fungi are reported on *S. jambos*, i.e., *Austropuccinia psidii, Colletotrichum pseudoacutatum, Capinodium* sp., and *G. bulbilium* (Soares et al. 2017; Silva et al. 2020a). Most of these studies only described fungal disease, without any details about the disease cycle, which difficult the development and implementation of control methods. Based on our present study, we suggest that fruit rot in *S. jambos* may be caused by at least six fungal species. This

new information can now be used to investigate appropriate methods to control the disease.

Based on the results from our study, we hypothesize that this fruit rot can have two paths to infection: one caused by soil inoculum and other by aerial inoculum. The first may occur when the fruit drops from the tree. The drop causes the fruit to crack, serving as the entry point of infection. All the Hypocreales found in this work have been cited in literature as soil borne (Liu and Cai 2013). Hypocrealean fungi, such as *Cylindrocladiella* and *Gliocephalotrichum,* produce chlamydospores that can live in soil during years (Crous 2002). Likewise, *Neofusicoccum* and *Gliocladiopsis* can survive in leaf litter and dead material on the ground (Crous 2002; Slippers and Wingfield 2007; Lombard and Crous 2012; Liu and Cai 2013). With the cracked fruit on the ground and possibly in contact with the pathogen inoculum, penetration can occur through the wound or natural openings and start the infection. The infection expands until the seed is colonized.

The literature also suggests that fungi such as *Gliocladiopsis* and *Neofusicoccum* can remain as endophytes during the growth of seedling (Slippers and Wingfield 2007; Liu and Cai 2013; Osorio et al. 2016; Jami et al. 2017). There is no information on *Cylindrocladiella* or *Gliocephalotrichum* as endophytes, but due the known ability of these genera to cause root rot (Crous 2002), the most acceptable hypothesis is that during the initial plant development, *Cylindrocladiella* remains under the pulp and in soil, and when the root emerges, the fungi restarts the infection and kills the seedling. Many studies also suggest that *Neofusicoccum* has an endophytic phase (Slippers and Wingfield 2007). This phase can be associated with the aerial infection of the fruits (i.e., horizontal transmission). For example, Pavlic et al. (2009) obtained pathogenic

Neofusicoccum isolates from twigs, leaves, and healthy fruits. They suggested that the fungi could infect during the fruit development, then falling after already being infected, and only starting to show symptoms after the maturation process or some other external input. The aerial infection can occur by horizontal transmission from adjacent alternative hosts, possibly spores dispersed by wind or rain.

There is no information regarding other symptoms caused by these fungi in leaves, stems, or seedlings of *S. jambos*. The present study expands the knowledge about a disease that occurs in *S. jambos*, in three Brazilian states. However, further studies are necessary to understand their ecology, epidemiology, geographic distribution, and methods of control.

Acknowledgments

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Supplementary Data

Supplementary Table S1. List of *Cylindrocladiella, Gliocladiopsis* and *Gliocephalotrichum* (Hypocreales) and *Neofusicoccum* (Botryosphaeriaceae) species and GenBank accession numbers used in the phylogenetic analyses. The accession numbers in bold were newly generated as part of this study.

		GenBank accession number				
Species	Isolates	*tub2*	ITS	*tef1a*	Substrate	Country
Cylla. addiensis	CBS143794	MH111388	MH111383	MH111393	Soil	Ethiopia
	CBS143793	MH111390	MH111385	MH111395	Soil	Ethiopia
Cylla. arbusta	CMW 47295	MH016958	MH017015	MH016977	soil	Vietnam
	CMW 47296	MH016959	MH017016	MH016978	soil	Vietnam
Cylla. australiensis	CBS129567	JN098747	JN100624	JN099060	Soil	Australia
	CBS129568	JN098748	JN100623	JN099059	Soil	Australia
Cylla. brevistipitata	CBS 142786	MF444926-	-	MF444940	Soil	Thailand
Cylla. camelliae	CPC234	JN098749	JN100573	JN099090	*Eucalyptus grandis*	South Africa
	CBS114891	AY793472	AF220953	JN099086	*Eucalyptus grandis*	South Africa
Cylla. clavata	CBS129563	JN098751	JN099096	JN098975	Soil	Australia
	CBS129564	JN098752	JN099095	JN098974	Soil	Australia
Cylla. cymbiformis	CBS129553	JN098753	JN099103	JN098988	Soil	Australia
	CBS129554	JN098754	JN099104	JN098989	Soil	Australia
Cylla. elegans	CBS338.92	AY793474	AY793444	JN099039	Leaf litter	South Africa
	CBS110801	JN098755	JN100609	JN099044	Leaf litter	South Africa
Cylla. ellipsoidea	CBS129572	JN098756	JN100636	JN099073	Soil	Australia
	CBS129573	JN098757	JN099094	JN098973	Soil	Australia
Cylla. hahajimaensis	PD684	-	JN687561	JN687562	Soil	Japan
Cylla. hawaiiensis	CBS118704	JN098760	JN099115	JN098996	Soil	Hawaii
	CBS129569	JN098761	JN100621	JN099057	Soil	Hawaii
Cylla. horticola	CBS 142784	MF444924	MF444911	MF444938	Soil	Thailand
	CBS 142785	MF444925	MF444912	MF444939	Soil	Thailand
Cylla. humicola	CBS 142777	MF444917	MF444904	MF444931	Soil	Thailand
	CBS 142779	MF444919	MF444906	MF444933	Soil	Thailand
Cylla. infestans	CBS111795	AF320190	AF220955	JN099037	*Pinus pinea*	New Zealand
	CBS191.50	AY793475	AF220956	JN099036	*Arena pinnata*	Indonesia
Cylla. kurandica	CBS129576	JN098764	JN100634	JN099071	Soil	Australia
	CBS129577	JN098765	JN100646	JN099083	Soil	Australia
Cylla. lageniformis	CBS340.92	AY793481	AF220959	JN099003	*Eucalyptus sp.*	South Africa
	CBS111060	JN098770	JN100611	JN099046	*Eucalyptus sp.*	South Africa

Species	Isolates	GenBank accession number			Substrate	Country
		tub2	ITS	*tef1a*		
Cylla. lanceolata	CBS129566	JN098789	JN099099	JN098978	Soil	Australia
	CBS114950	JN098787	JN100591	JN099019	*Eucalyptus sp.*	
Cylla. lateralis	CBS 142787	MF444927	MF444913	MF444941	Soil	Thailand
	CBS 142788	MF444928	MF444914	MF444942	Soil	Thailand
Cylla. longiphialidica	CBS129557	JN098790	JN100585	JN098966	Soil	Thailand
	CBS129558	JN098791	JN100586	JN098967	Soil	Thailand
Cylla. longistipitata	CBS112953	JN098792	JN100595	JN099025	*Opisthiolepsis heterophylla*	Australia
	CBS116075	AY793506	AF220958	JN098993	Soil	China
Cylla. malesiana	CMW 48278	MH016960	MH017017	MH016979	Soil	Malaysia
	CMW 48277	MH016961	MH017018	MH016980	Soil	Malaysia
Cylla. microcylindrica	CBS111794	AY793483	AY793452	JN099041	*Echeveria elegans*	Indonésia
	SE-U 10452	AY793484	AY793453	-	*Agalonema commutatum*	USA
Cylla. natalensis	CBS110800	JN098793	JN100608	JN099043	Soil	South Africa
	CBS114943	JN098794	JN100588	JN099016	*Arachis hypogaea*	South Africa
Cylla. nauliensis	CBS 143792	MH111392	MH111387	MH111397	Soil	Indonesia
	CBS 143791	MH111391	MH111386	MH111396	Soil	Indonesia
Cylla. nedelandica	CBS146.94	JN098799	JN099127	JN099011	*Rhododendron sp.*	The Netherlands
	CBS152.91	JN098800	JN100603	JN099033	*Pelargorium sp.*	The Netherlands
Cylla. novaezelandica	CBS486.77	AY793485	AF220963	JN099050	*Rhododendron indicum*	New Zealand
Cylla. obpyriformis	CMW 47194	MH016965	MH017022	MH016984	Soil	Vietnam
	CMW 49940;	MH016966	MH017023	MH016985	Soil	Vietnam
Cylla. parva	CBS114524	AY793486	AF220964	JN099009	*Telopea speciossima*	New Zealand
Cylla. parvispora	CMW 47193	MH016967	MH017024	MH016986	Soil	Vietnam
	CMW 47197	MH016968	MH017025	MH016987	Soil	Vietnam
Cylla. postalofficium	CBS146060	MN556845	MN562148	-	leaf litter	South Africa
Cylla. pseudocamelliae	CBS129555	JN098814	JN100577	JN098958	Soil	Thailand
	CBS129556	JN098815	JN100580	JN098961	Soil	Thailand
Cylla. pseudohawaiiensis	CBS210.94	JN098819	JN099128	JN099012	*Eucalyptus sp.*	Brazil
	CBS115610	JN098820	JN100594	JN099024	-	Madagascar
Cylla. pseudoinfestans	CBS114530	JN098821	JN099126	JN099010	Soil	Madagascar
	CBS114531	AY793508	AF220957	JN099004	Soil	Madagascar
Cylla. pseudoparva	CBS113624	JN098822	JN099121	JN099002	*Quercus sp.*	Switzerland
	CBS129560	JN098824	JN100620	JN099056	Soil	The Netherlands
Cylla. queenslandica	CBS129574	JN098826	JN099098	JN098977	Soil	Australia
	CBS129575	JN098827	JN099097	JN098976	Soil	Australia
Cylla. reginae	CBS 142781	MF444921	MF444908	MF444935	Soil	Thailand

	CBS 142782	MF444922-	MF444909	MF444936	Soil	Thailand
Cylla. stellenboschensis	CBS386.67	JN098828	JN100613	JN099048	*Fragaria sp.*	The
	CBS110668	JN098829	JN100615	JN099051	Soil	South Africa

		GenBank accession numbers				
Species	Isolates	*tub2*	ITS	*tef1a*	Substrate	Country
Cylla. terretris	CBS 142789	MF444929	MF444915	MF444943	Soil	Thailand
	CBS 142790	MF444930	MF444916	MF444944	Soil	Thailand
Cylla. thailandica	CBS129570	JN098833	JN100581	JN098962	Soil	Thailand
	CBS129571	JN098834	JN100582	JN098963	Soil	Thailand
Cylla. variabilis	CBS375.93	JN098836	JN099119	JN099000	*Mangifera indica*	India
	CBS129561	JN098719	JN100643	JN099080	Soil	Australia
Cylla. viticola	CBS112897	AY793504	AY793468	JN099064	*Vitis vinifera*	South Africa
	CBS114682	JN098723	JN100612	JN099047	*Amorphophallus sp.*	Thailand
Cylla. vitris	CBS142517	KY979918	KY979751	KY979891	*V. vinifera*	New Zealand
Gliocladiopis aquaticus	MFLUCC17-2028	MG574422	MG543925	-	*Cassia fistula*	Thailand
	MFLUCC17-1811	MG574421	MG543924	-	*Decaying wood*	Thailand
Glio. curvata	CBS 194.80	JQ666120	JQ666044	JQ666086	*Persea americana*	Ecuador
	CBS 112365	JQ666126	JQ666050	JQ666092	*Archontophoenix purpurea*	New Zealand
Glio. ecuadoriensis	MUCL 54740	KX611501	-	KX671131	*Polybotrya sp.*	Ecuador
Glio. elghollii	CBS 206.94	JQ666130	JQ666054	JQ666096	*Chamaedorea elegans*	USA
	CBS 116104	JQ666131	JQ666055	JQ666097	*Chamaedorea elegans*	USA
Glio. forsbergii	BRIP 60984	KX274036	KX274070	-	*Grevillea sp.*	Australia
	BRIP 61349a	KX274037	KX274071	-	*Persea americana*	Australia
Glio. guangdongensis	LC 1340	KC776124	KC776122	KC776119	Submerged wood	China
	LC 1349	KC776125	KC776123	KC776118	Submerged wood	China
Glio. hennebertii	MUCL 54818	KX611502	KX671140	KX671132	Costus scaber	Ecuador
Glio. indonesiensis	CBS 116090	JQ666132	JQ666056	-	Soil	Indonesia
Glio. irregularis	CBS 755.97	JQ666133	AF220977	JQ666099	Soil	Indonesia
Glio. mexicana	CBS 110938	JQ666137	JQ666060	JQ666103	Soil	Mexico
Glio. peggii	BRIP 53654	JN255247	JN255246	-	*Persea americana*	Australia
	BRIP 60983	KX274038	KX274083	-	*Persea americana*	Australia
Glio. pseudotenuis	CBS 114763	JQ666139	JQ666062	JQ666105	*Vanilla sp.*	Indonesia
	CBS 116074	JQ666140	AF220981	JQ666106	Soil	China
Glio. singaporiensis	MUCL 48728	KX611500	KX671138	KX671130	Olivier laurence	Singapore

Glio. sumatrensis	CBS 754.97	JQ666142	JQ666064	JQ666108	Soil	Indonesia
	CBS 111213	JQ666144	JQ666066	JQ666110	Soil	Indonesia
Glio. tenuis	IMI 68205	JQ666150	AF220979	JQ666116	*Indigofera sp.*	Indonesia
	CBS 111964	JQ666147	JQ666068	JQ666113	*Coffea sp.*	Vietnam

		GenBank accession numbers				
Species	Isolates	*tub2*	ITS	*tef1a*	Substrate	Country
Glio. sagariensis	CBS 199.55	JQ666141	JQ666063	JQ666107	Soil	India
Glio. swieteniae	MFLU 18-2767	MT212214	MT215501	-	Swietenia mahagoni	Thailand
Glio. whileyi	BRIP 61430	KX274052	KX274086	-	*Persea americana*	Australia
Glio. wuhanensis	HEAC17307	MH169602	MH024520	-	Soil	China
Gliocladiopsis sp. 1	CBS 111038	JQ666151-	JQ666071-	JQ666117	Soil	Colombia
Gliocladiopsis sp. 2	CBS 116086	JQ666152-	JQ666072-	JQ666118	Soil	Indonesia
G. abrachium	CCUB10	MN508721	MN450200	MN508678	*C. brasiliense*	Piauí, Brazil
	CCUB301	MN508728	MN450207	MN508691	*C. brasiliense*	Minas Gerais, Brazil
G. bacillisporum	CBS 250.91	KF513182	KF513251	KF513405	plant root	Brazil
	CBS 126572	DQ374413	DQ374408	KF513406	leaf litter	French Guiana
G. bulbilium	CBS 118.68	KF513183	KF513252	KF513409	air	Central African Republic
	CBS 562.75	KF513184	KF513253	KF513410	*Flacourtia sp.*	Indonesia
G. brasiliense	CCUB232	MN508730	MN450209	MN508698	*D. madagascariensis*	Distrito Federal, Brazil
	CCUB355	MN508731	MN450210	MN508701	*S. purpurea*	Distrito Federal, Brazil
G. caryocaris	CCUB229	MN508727	MN450206	MN508689	*C. brasiliense*	Minas Gerais, Brazil
	CCUB231	MN508729	MN450208	MN508692	*C. brasiliense*	Minas Gerais, Brazil
G. cylindrosporum	CBS 902.70	DQ377841	DQ366705	KF513435	Soil	Thailand
	CBS 903.70	KF513208	KF513277	KF513436	Soil	Thailand
G. grande	HMAS	EU984072	EF121859	HM054075	leaf litter	China
G. humicola	CBS 135945	KF513209	KF513278	KF513438	Soil	Taiwan
	CBS 135946	KF513210	KF513279	KF513439	Soil	Taiwan
G. longibrachium	CBS 126571	DQ377835	-	KF513449	leaf litter	French Guiana
	CBS 132043	DQ377836	DQ278422	KF513450	leaf litter	French Guiana
G. mexicanum	CBS 135947	KF513220	KF513289	KF513451	*N. lappaceum*	Mexico
	CBS 135948	KF513221	KF513290	KF513452	*N. lappaceum*	Mexico

G. microchlamydosporum	CBS 345.64	DQ374410	DQ366699	KF513453	Soil	Zaïre
	CPC 21862	DQ374411	DQ366700	KF513454	–	Zaïre
G. nephelii	CBS 135949	KF513222	KF513291	KF513456	*N. lappaceum*	Guatemala
	CBS 135950	KF513223	KF513292	KF513457	*N. lappaceum*	Guatemala
G. ohiense	CBS 567.73	DQ374415	DQ366707	KF513458	Soil	USA

		GenBank accession numbers				
Species	Isolates	*tub2*	ITS	*tef1a*	Substrate	Country
G. queenslandicum	CBS 112956	KF513224	KF513293	KF513459	E. angustifolius	Australia
	CBS 114868	KF513225	KF513294	KF513460	*E. angustifolius*	Australia
G. simmonsii	CBS 135951	KF513226	KF513295	KF513461	*N. lappaceum*	Guatemala
	CBS 135953	KF513228	KF513297	KF513463	*N. lappaceum*	Guatemala
G. simplex	CBS 267.65	DQ377838	DQ366702	KF513464	Soil	South Africa
	CBS 983.69	KF513229	KF513298	KF513465	Soil	Brazil
Gliocephalotrichum	**CCUB354**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Gliocephalotrichum	**CCUB356**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Gliocladiopsis	**CCUB376**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Cylindrocladiella peruviana	**CCUB417**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Cylindrocladiella lageniformis	**CCUB411**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Cylindrocladiella lageniformis	**CCUB410**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Gliocladiopsis	**CCUB405**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Cylindrocladiella lageniformis	**CCUB420**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Calonectria candelabrum	CPC1675	FJ972426	GQ280557	FJ972525	*Eucalyptus* sp.	Amazonas, Brazil
Botryosphaeria dothidea	CBS100564	KX464781	KX464085	KX464555	*Paeonia sp.*	The Netherlands
N. algeriense	CBS137504	-	KJ657702	KJ657721	*Vitis vinifera*	Algeria
N. algeriense	CBS719.85	KX464921	KX464151	KX464646	*Malus x domestica*	New Zealand
N. andinum	CBS117452	KX464922	DQ306263	DQ306264	*Eucalyptus sp.*	Venezuela
N. andinum	CBS117453	KX464923	AY693976	AY693977	*Eucalyptus sp.*	Venezuela
N. arbuti	CBS116131	KF531793	AY819720	KF531792	*Arbutus menziesii*	USA
N. arbuti	CBS116573	KX464925	KX464153	KX464648	*Arbutus menziesii*	USA
N. australe	CBS139662	AY339254	AY339262	AY339270	*Acacia sp.*	Australia
N. australe	CBS110485	KX464929	AY343411	KX464653	*Vitis vinifera*	South Africa
N. batangarum	CBS124924	FJ900634	FJ900607	FJ900653	*Terminalia catappa*	Cameroon

N. batangarum	CBS124923	FJ900635	FJ900608	FJ900654	*Terminalia catappa*	Cameroon
N. brasiliense	CMM1285	KC794030	JX513628	JX513608	*Mangifera indica*	Brazil
N. buxi	CBS116.75	-	KX464165	KX464678	*Buxus sempervirens*	France
N. buxi	CBS113714	KX464954	KX464164	KX464677	*Buxus sempervirens*	Sweden

		GenBank accession numbers				
Species	Isolates	*tub2*	ITS	*tef1a*	Substrate	Country
N. cordaticola	CBS123634	EU821838	EU821898	EU821868	*Syzygium cordatum*	South Africa
N. cordaticola	CBS123635	EU821843	EU821903	EU821873	*Syzygium cordatum*	South Africa
N. corticosae	CBS118099	KX464957	KX464168	KX464681	*Eucalyptus camaldulensis*	Australia
N. corticosae	CBS120081	KX464958	MN161920	KX464682	*Eucalyptus corticosa*	Australia
N. cryptoaustrale	CBS122027	EU375520	EU375516	EU375518	*Pistacia vera*	Spain
N. cryptoaustrale	CBS122813	FJ752756	FJ752742	FJ752713	*Eucalyptus sp.*	South Africa
N. eucalypticola	CBS115679	AY615125	AY615141	AY615133	–	–
N. eucalyptorum	CBS115791	AY236920	AF283686	AY236891	*E. grandis*	South Africa
N. grevilleae	CBS129518	-	JF951137	-	*Grevillea aurea*	Australia
N. hellenicum	CERC1947	KP217069	KP217053	KP217061	*Pistacia vera*	Greece
N. hongkongense	CERC2973	KX278261	KX278052	KX278157	*Araucaria cunninghamii*	China
N. hyperici	MUCC241	LC589147	LC589125	LC589137	*Hypericum patulum*	Japan
N. hyperici	MUCC2509	LC589148	LC589126	LC589138	*H. galioides*	Japan
N. illicii	CGMCC3.18310	KY350155	KY350149	-	*Illicium verum*	China
N. italicum	MFLUCC15-0900	-	KY856755	KY856754	*V. vinifera*	Italy
N. kwambonambiense	CBS123639	EU821840	EU821900	EU821870	*Syzygium cordatum*	South Africa
N. kwambonambiense	CBS123643	EU821864	EU821924	EU821894	*Syzygium cordatum*	South Africa
N. laricinum	MUCC2662	LC589151	LC589129	LC589140	*L. decidua*	Japan
N. laricinum	MUCC2660	LC589153	LC589131	LC589142	*Larix kaempferi*	Japan
N. lumnitzerae	CMW41469	KP860801	KP860881	KP860724	*Lumnitzera racemosa*	South Africa
N. luteum	CBS562.92	KX464968	KX464170	KX464690	*Actinidia deliciosa*	New Zealand
N. luteum	CBS612.83	KX464969	KX464171	KX464691	*Persea americana*	USA
N. macroclavatum	CBS118223	DQ093206	DQ093196	DQ093217	*E. globulus*	Australia
N. macroclavatum	CBS114497	KX464972	AF452534	KX464695	*Grevillea woosinii*	USA: Hawaii

N. mangiferae	CBS118531	AY615172	AY615185	DQ093221	*M. indica*	Australia
N. mangiferae	CBS118532	AY615173	AY615186	DQ093220	*Mangifera indica*	Australia
N. mangroviorum	CMW41365	KP860779	KP860859	KP860702	*Avicennia marina*	South Africa
N. mediterraneum	CBS121718	-	MH863145	-	*Eucalyptus sp.*	Greece
N. microconidium	CERC3497	KX278262	KX278053	KX278158	*E. urophylla* × *E. grandis*	China
N. miyakoense	MUCC2586	LC589155	LC589133	LC589144	*Mangifera indica*	Japan

		GenBank accession numbers				
Species	Isolates	*tub2*	ITS	*tef1a*	Substrate	Country
N. miyakoense	MUCC2585	LC589157	–	LC589146	*Coffea sp.*	Japan
N. nonquaestitum	CBS126655	GU251823	GU251163	GU251295	*Umbellularia californica*	USA
N. occulatum	CBS128008	EU339472	EU301030	EU339509	*Eucalyptus grandis*	Australia
N. occulatum	CBS256.80	KX464973	KX464175	KX464696	*Platanus acerifolia*	Italy
N. okinawaense	MUCC789	LC589156	LC589134	LC589145	*Lagerstroemia speciosa*	Japan
N. pandanicola	MFLUCC17-2270	-	MH275072	MH412778	*Pandanus sp.*	China
N. parvum	CBS138823	AY236917	AY236943	AY236888	*Populus nigra*	New Zealand
N. parvum	CBS124491	KX464961	FJ654999	KX464683	*Syzygium guineense*	Zambia
N. pennatisporum	MUCC510	EF591959	EF591925	EF591976	*Allocasuarina fraseriana*	Australia
N. pistaciae	CBS595.76	KX464953	KX464163	KX464676	*Pistacia vera*	Greece
N. pistaciarum	CBS113083	KX464998	KX464186	KX464712	*P. vera*	USA
N. pistaciarum	CBS113084	KX464999	KX464187	KX464713	*Redwood*	USA
N. pistaciicola	CBS113080	KX465013	KX464198	KX464726	–	–
N. pistaciicola	CBS113089	KX465014	KX464199	KX464727	*P. vera*	USA
N. protearum	CBS114176	KX465006	AF452539	KX464720	*Leucadendron salignum x*	South Africa
N. protearum	CBS119220	KX465007	EU552144	KX464721	*Leucadendron xanthoconus*	South Africa
N. pruni	CBS121112	KX465016	EF445349	EF445391	*Prunus salicina*	South Africa
N. ribis	CBS115475	AY236906	AY236935	AY236877	*Ribes sp.*	USA
N. ribis	CBS121.26	AY236908	AF241177	AY236879	*Ribes rubrum*	–
N. sinense	CGMCC3.18315	KY350154	KY350148	KY817755	*Unknown woody plant*	China
N. sinoeucalypti	CGMCC3.18752	KX278270	KX278061	KX278166	*E. urophylla* × *E. grandis*	China
N. sp. 2	CBS113081	KX465017	KX464201	KX464729	*Pistacia vera*	USA
N. sp. 2	CBS113085	KX465018	KX464202	KX464730	*Pistacia vera*	USA
N. sp. 3	CBS318.76	KX465028	KX464212	KX464740	*Vitis vinifera*	Italy
N. sp. 4	CBS110855	KX465029	AY343465	KX464741	*Vitis vinifera*	South Africa

N. sp. 4	CBS110860	KX465030	AY343470	AY343362	*Vitis vinifera*	South Africa
N. sp. 5	CBS157.26	KX465034	KX464214	KX464744	*Camellia sinensis*	Sri Lanka
N. sp. 6	CBS111999	KX465035	KX464215	KX464745	*Protea magnifica*	Australia
N. sp. 7	CBS121424	KX465036	KX464216	KX464746	*Protea obtusifolia*	South Africa
N. sp. 8	CBS111076	KX465037	KX464217	KX464747	*Protea sp.*	–
N. sp. 8	CBS111077	KX465038	KX464218	KX464748	*Protea sp.*	–
N. stellenboschiana	CBS110864	KX465047	AY343407	AY343348	*V. vinifera*	South Africa

		GenBank accession numbers				
Species	Isolates	*tub2*	ITS	*tef1a*	Substrate	Country
N. stellenboschiana	CBS282.70	KX465051	KX464225	KX464758	*Arum italicum*	Spain
N. terminaliae	CBS125263	KX465052	GQ471802	GQ471780	*Terminalia sericea*	South Africa
N. terminaliae	CBS125264	KX465053	GQ471804	GQ471782	*Terminalia sericea*	South Africa
N. umdonicola	CBS123646	EU821845	EU821905	EU821875	*Syzygium cordatum*	South Africa
N. umdonicola	CBS123644	KX465055	KX464226	KX464759	*Syzygium cordatum*	South Africa
N. ursorum	CBS122811	KX465056	FJ752746	FJ752709	*Eucalyptus sp.*	South Africa
N. ursorum	CBS122812	KX465057	KX464227	KX464760	*Eucalyptus sp.*	South Africa
N. variabile	CBS143480	MH569153	MH558608	-	*Mimusops caffra*	South Africa
N. versiforme	CBS118101	KF766128	KF766154	KX464757	*E. camaldulensis*	Australia
N. viticlavatum	CBS112878	KX465058	AY343381	AY343342	*V. vinifera*	South Africa
N. viticlavatum	CBS112977	KX465059	AY343380	AY343341	–	–
N. vitifusiforme	CBS110887	KX465061	AY343383	AY343343	*Vitis vinifera*	South Africa
N. vitifusiforme	CBS121111	KX465062	EF445347	EF445389	*Prunus salicina*	South Africa
Neofusicoccum sp.	**CCUB357 (713)**	**-**	**-**	**-**	***S. jambos Fruits***	**Brazil**
Neofusicoccum batangarum	**CCUB364 (715)**	**-**	**-**	**-**	***S. jambos Fruits***	**Brazil**
Neofusicoccum sp.	**CCUB359 (716)**	**-**	**-**	**-**	***S. jambos Fruits***	**Brazil**
Neofusicoccum batangarum	**CCUB362 (717)**	**-**	**-**	**-**	***S. jambos Fruits***	**Brazil**
Neofusicoccum batangarum	**CCUB363 (718)**	**-**	**-**	**-**	***S. jambos Fruits***	**Brazil**
Neofusicoccum batangarum	**CCUB1070 (1865)**	**-**	**-**	**-**	***S. jambos Fruits***	**Brazil**
Neofusicoccum umdonicola	**CCUB1075 (1867)**	**-**	**-**	**-**	***S. jambos Fruits***	**Brazil**

Neofusicoccum umdonicola	**CCUB1080 (1868)**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Neofusicoccum umdonicola	**CCUB1076 (1871)**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Neofusicoccum umdonicola	**CCUB1077 (1872)**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Neofusicoccum umdonicola	**CCUB1078 (1873)**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Neofusicoccum parvum	**CCUB1079 (1874)**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Neofusicoccum umdonicola	**CCUB1073 (1875)**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Neofusicoccum batangarum	**CCUB1143 (1897)**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**
Neofusicoccum parvum	**CCUB1071 (1903)**	**-**	**-**	**-**	***S. jambos* Fruits**	**Brazil**

T: Ex-type cultures.

* BRIP: Biosecurity Queensland Plant Pathology Herbarium, Department of Agriculture and Fisheries, Dutton Park, Australia. CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. CPC: Working collection of Pedro Crous housed at CBS. IMI: International Mycological Institute, CABI-Bioscience, Egham, UK. ATCC: American Type Culture Collection, Manassas, USA. IMUR: Institute of Mycology, University of Recife, Recife, Brazil. LC: Herbarium of Microbiology, Academia Sinica, Taipei, Taiwan. CGMCC: China General Microbiological Culture Collection Center, Beijing, China. CCUB: Coleção de Culturas da Universidade de Brasília, Brasília, Brazil.

** Newly deposited sequences are shown in bold.

Supplementary Table S2. Models of nucleotide substitution used in the phylogenetic analyses.

Parameter	**Hypocreales**			***Neofusicoccum***		
	ITS	**TEF1a**	**TUB2**	**TEF1a**	**ITS**	**TUB2**
Nº taxa	141	134	145	99	104	96
Model	SYM+I+G	GTR+I+G	HKY+I+G	HKY+G	GTR+I+G	GTR+G
Likelihood	-2114.0552	-6292.7305	-5482.9063	-1566.7107	-1885.2233	-1454.6644
Matrix length	500	589	552	290	550	392
Variables sites	125	328	262	139	123	101

Parsimony informative sites	102	289	232	95	58	65
Conserved Sites	368	255	277	139	413	277
Base frequencies						
Freq. A	Equal	0.2344	0.2404	0.1628	0.2040	0.2004
Freq. C		0.3080	0.3085	0.2921	0.3007	0.3552
Freq. G		0.1772	0.1911	0.3000	0.2606	0.2405
Freq. T		0.2804	0.2600	0.2451	0.2347	0.2039
Transition rates						
[A-C]	0.9910	1.7513	2.1730	3.0952	0.8901	1.2044
[A-G]	1.1289	4.5100			8.7035	4.7320
[A-T]	1.0403	2.9786			2.2271	0.3662
[C-G]	0.4495	1.4494			1.8003	2.0785
[C-T]	2.4119	6.4331			17.0396	9.6491
[G-T]	1.0000	1.0000			1.0000	1.0000
Proportion of invariable sites	0.6087	0.3037	0.4848	0	0.5651	0
Gamma	0.9453	0.8826	1.3298	0.7145	0.6757	0.2524

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Capítulo 5: *Clonostachys platoniensis* novel specie associated with Brazilian fruits

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Clonostachys platoniensis a novel specie associated with Brazilian fruits

5.1 Abstract

The *Clonostachys* genus despite very studied, in Brazil the information about it is limited to six species collected specially in litter and dead plants. This genus currently follows patterns of taxonomy using multiple approaches, molecular analysis, and morphological characterization, to determine new species. Using these approaches, this work identified *Clonostachys rogersoniana* and *Clonostachys pseudocroleuca* growing on dropped fruits in three Brazilian states. An isolate unnamed that indicates a new phylogenetic species was found. However, it was not proposed to be a new species due the absence of another isolate to compare, as suggested by the literature. In this work was identified *Clonostachys plationiensis* as a new specie by the phylogenetic analysis of four loci (*its, lsu, tef1a* and *tub2*) and morphological differences to a close species. This new species presented a level of inhibition when paired with other species of *Clonostachys,* indicating it is a candidate to future studies of biocontrol. The discovery of new species and the increase of knowledge about *Clonostachys* in Brazilian territory make possible the understanding of distribution and biology as well the possible application of these new genetic resources in bioproducts.

Keywords: Bionectriaceae, Biological control, Databank, Fungal barcode

5.2 Introduction

The genus *Clonostachys* was raised in 1839 by Corda, using *C. rosea* (syn. *C. araucaria*). This genus represents an asexual morph-type, characterized by penicillate conidiophores, commonly formed on sporodochial structures with conidia mostly asymmetric due the displaced hilum in one side, curved, and imbricately arranged that adhere in columns (Schroers et al. 1999). Subsequently, *Bionectria* was proposed as

genus with sexual morph-type based on type specie *B. tonduzi* Speg.1919 (Rossman et al. 2013). *B. tanduzi* was characterized by having solitary to gregarious, subglobose, or globose to ovoid perithecia, going from white to brown and KOH-, containing narrowly clavate to clavate asci producing 8-ascospores (Schroers et al. 1999; Zeng and Zhuang 2022).

Since the report of these two genera, both were treated separately but considered congeneric, until 2013 when it was proposed that each fungus would have only one name (one fungus one name concept). The scientists decided to adopt *Clonostachys* as the genus name (Rossman et al. 2013). One of the reasons for the asexual morph-type been maintained as the genus name, was the large use of *C. rosea* in biocontrol studies (Schroers et al. 1999; Rossman et al. 2013).

Species of *Clonostachys* were commonly found in soil samples, as endophytes, epiphytes, or saprotrophs and have been reported as mycoparasite and parasites of invertebrates (Torcato et al. 2020). The parasitic lifestyle of this genus provides valuable source to biocontrol of some microorganisms, insects, and nematodes. Nowadays, *C. rosea* is the species most studied as to such applications. This species is reported in studies of Biocontrol, biodegradation, biotransformation, fermentation, and biological energy source (Sun et al. 2020). The mycoparasite and saprophytic habit of *C. rosea* is assigned to secretion of biological control molecules as: chitinases, glucanases and proteases acting on enzymes to cell wall degradation; peptaibols, gliotoxin, viridin acting as antibiotics (Mukherjee et al. 2013; Karlsson et al. 2015). Despite the number of *Clonostachys* species described in literature is 103 (Index Fungorum, accessed on 26 December 2022) and around 65 accepted by literature

(Zeng and Zhuang 2022) only *C. rosea* is well studied, maintaining open the potential to the use of other species.

In Brazil, the studies with diversity and use of this genus are limited. An important work focused on to describe the diversity of *Clonostachys* by molecular approach identified four knowledge species (*C. byssicola, C. pseudochroleuca, C. rhizophaga* and *C. rogersoniana*), two special form of *C. rosea* and one new specie (*C. chroroleuca*) in six Brazilian states (Moreira et al. 2016). The Approach used by these authors is commonly accepted and reproduced by other authors for identifying new species. However, such approach has some divergence in number and how molecular markers are used to characterize the species. Some examples are *C. chroroleuca* described using four partial genes (*act*, *tub2*, *rpb1* and *tef1a*); *C. viticola* using three loci (*its, tef1a* and tub2); C*. chongqingensis, C. leptoderma* and *C. oligospora* was described using two partial gene (*its* and *benA*); and *C. moreaui, C. pilosella* and *C. pnagiana* with only *lsu* as molecular marker (Moreira et al. 2016; Lechat and Fournier 2020; Lechat et al. 2020; Torcato et al. 2020; Zeng and Zhuang 2022). This lack of uniformity of sequences used in phylogenetic recognition of species makes the genus confuse and impossible to have a robust analysis with all species. In this work, we pretend expand the knowledge of *Clonostachys* asexual morph-type occurring in Brazil, using four partial sequences of *its, lsu, tef1a* and *tub2* to determine species phylogenetically.

5.3 Material and Methods

Isolates collection – Strains were isolated from dropped fruits presenting white mycelia and sent to the Laboratório de Micologia of Departamento de Fitopatologia at Universidade de Brasília. The fungal isolation was conducted by the methods of direct

and indirect isolation (Alfenas and Mafia 2016). All cultures were selected then they were purified by taking one single hypha and growing it on a Petri dish with Malt Extract Agar (MEA 2%), in a BOD at 25ºC. After seven days of growth all cultures were stored on three methods: Castallani, Glycerol 10% and Mineral Oil methods. After being stored, they were added to the Coleção de Culturas da Universidade de Brasília.

Molecular characterization - Cultures were grown on MEA medium at B.O.D. at 26°C (±2°C) for 7 days to collect biomass for DNA extraction. The DNA extraction was conducted using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), modified by Pinho et al. (2012). Partial sequences of three primer pair was obtained by amplifying and sequencing: EF-1F/EF-2R (Jacobs et al. 2004) to translation elongation factor 1-alpha (*tef1a*), LR5/V9G (Vilgalys and Hester 1990; Hoog and Ende 2009) Internal transcribed spacer (*its*) and 28S large subunit RNA (*lsu);* and T1/Bt2b (Glass and Donaldson 1995; O'Donnell et al. 2007) to *β*-tubulin (*tub2*). PCR was made following the conditions described by Silva et al. (2020a). PCR products were purified using 2 *μ*L de ExoSAP-IT™ (TermoFisher, Santa Clara, USA) following the brand protocol. Purified PCR products were sent to sequencing procedures at Macrogen (Seoul, Korea). The quality of electropherograms were confirmed and edited. Both sense of sequences was used to assemble the contigs using DNA Dragon software (Hepperle D. 2011). Sequences of four partial genes (*its, lsu, tef1a* and *tub2*) of at list one strain by species were downloaded of Genbank to perform the phylogenetic comparisons. All sequences used in this study are listed in TABLE 1. Alignments for each locus and genus were conducted separately. Alignments were conducted in MAFFT 7 (Katoh and Standley 2013) using the algorithm MUSCLE and the default parameters. Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML) criteria. For BI analyses, the best

substitution models for each partial gene were determined with MrModeltest (Nylander 2004). The Web portal CIPRES (Miller et al. 2010) was used to run MrBayes 3.2.2 (Ronquist et al. 2012). The Markov chain Monte Carlo (MCMC) analysis was run with a total of ten million generations and one tree sampled every 1000 generations. The convergence of the log likelihoods was confirmed using TRACER 1.7.1 (Rambaut and Drummond 2018). The first 25% of saved trees were discarded as the burn-in, with posterior probabilities (PPs) calculated for the remaining trees.

The ML analysis was performed using RAxML 8.2.9 (Stamatakis 2014) through the CIPRES portal, starting with a randomized stepwise-addition par-simony tree under a GTR+GAMMA model for each gene region and the four-gene combined data set and 1000 bootstrap (BS) replicates under the same model. All trees were edited in FigTree 1.4 (Rambaut 2018). The nucleotide matrices and phylogenetic trees from all data sets (individual and combined locus) are available at TreeBASE (XXXXX).

Sexual compatibility test – Using a Petri dish fulfillment with BDA (2%) was laid a sterilized toothpick in the middle, on opposite sides were placed a stub contain one strain. Cultures were crossing in all combinations in pairs. All combinations were growth on environmental conditions under 4 weeks. Visually was identified the presence or absence of sexual morph-type.

Morphological characterization – Representative isolates of each phylogenetic species obtained were selected to morphological characteristics. The strains were cultivated on …, and all cultures were incubated on 25ºC under alternate photoperiod (12h bright/12h dark) for 7 days. Microscopical characteristics are examined by mounting fungal structures in clear lactoglycerol and plyvinyllactoglycerol (PVLG). At least thirteen measurements were made for each trait using Leica DM2500 microscope

(Leica Microsystem, Nassloch, Germany). For the colony characterization was analyzed the strains growth in AW (Agar Water), CA (Carrot Agar), MEA (Malt Extract Agar), SNA (Synthetic Nutrient Agar) and PDA (Potato Dextrose Agar) medium (Lombard et al. 2010; Nirenberg 2011; Alfenas and Mafia 2016). Isolates were incubated at 25ºC under alternate photoperiod (12h bright/12h dark) for 7 days. The Colony coloration was determined using the MEA and PDA cultures, comparing with the chat published by Rayner (1970). Measurements were tabulated as 95% confidence intervals and average, minimum, and maximum values.

Table 1. List of *Clonostachys* species used to phylogenetic comparisons in this study.

Specie	Collection n.*	Substrate	Place	GenBank access n.**			
				ITS	LSU	tef1	tub2
Clonostachys sp.	CCUB1029	Sete copas	Santa Catarina-BR	P16/P55	P16/P55	!/P30	P6
Clonostachys sp.	CCUB1030	Palmito jussara (fruto)	Santa Catarina-BR	P16	P16	?/P30	P6
Clonostachys sp.	CCUB1031	Butiá	Santa Catarina-BR	P16	P16	?/P30	P6
Clonostachys sp.	CCUB1032	Cereja do rio grande	Santa Catarina-BR	P16	P16	?/P30	P6
Clonostachys sp.	CCUB1033	Ingá mirim	Candangolândia - DF	P16/P55	P16/P55	P29/P30	P6
Clonostachys sp.	CCUB1034	Bacuri	Nova Veneza-GO	P16/P55	P16/P55	P29/P30	P6
Clonostachys sp.	CCUB1035	Bacuri	Nova Veneza-GO	P16	P16	?/P30	P6
Clonostachys sp.	CCUB1036	Bacabá	Santa Isabel - PA	P16/P55	P16/P55	P29/P30	P6
C. agrawalii	CBS 533.81	Deccomposing buffalo horn	India	AF358241	-	-	AF358187
C. ambigua	PAD S00003	-	Indonesia	MT554898	-	-	-
C. apocyni	CBS 130.87	Dead stem of *Apocynum cannabinum*	USA.	AF210688	-	-	AF358168
C. aranearum	WC-2015	Spider	China	KT895417	-	-	KU212400
C. aureofulvella	CBS 195.93	root of tree	New zealand	AF358226	-	-	AF358181
C. buxi	CBS 696.93	*Buxus sempervirens*	França	KM231840	KM231721	KM231977	KM232111
C. byssicolae	2510, 364.78T	Bark	Venezuela	-	-	KX184967	AF358153
C. byssicolae	2511, 365.78	Wood	Venezuela	-	-	KX184972	AF358154
C. byssicolae	2533	Bryophyte	Itumirim, MG	-	-	KX184973	KX185031
C. candelabrum	CML2512	Soil	Netherlands	-	-	KX185029	KF871189
C. capitata	CBS 218.93	Bark	Japan	MH862394	MH874054	-	AF358188
C. chlorina	CBS 287.90	Soil	Brazil	MH862212	MH873895	-	-
C. chloroleuca	1927, 141591	Soil under soybean field	Montividiu, GO	-	-	KX184987	KF871171
C. chloroleuca	1941, 141588 Td	Native soil from Cerrado	Montividiu, GO	-	-	KX184988	KF871172
C. chloroleuca	2537, 141592	Bryophyte	Itumirim, MG	-	-	KX184989	KX185038
C. coccicola	HD-2016	*Unaspis citri*	Australia	KU720552	KU720550	-	-
C. compactiuscula	CBS 377.65	-	Germany	MH858621	MH870261	-	-
C. compactiuscula	CBS 592.93	Bark of *Fagus* sp.	France	AF358247	-	-	AF358192
C. divergens	CBS 967.73	Soil	Germany	AF210677	-	-	AF358191
C. epichloe	CBS 101037	*Sasa* sp.	Japan	AF210675	-	-	AF358209

Continue…

Specie	Collection n.*	Substrate	Place	GenBank access n.**			
				ITS	LSU	tef1	tub2
C. eriocamporesiana	MFLUCC 17–2620	Hyperparasite on ascomata of botryosphaeriaceous fungus on dead stems of *Chromolaena odorata*	Thailand	NR_168235	-	MN699964	MN699965
C. eriocamporesii	MFLU 18-2718	dead stems of *Pennisetum polystachion*	Thailand	MN699133	MN699128	-	-
C. grammicospora	CBS 209.93	Standing dead tree	Franch Guiana	MH862392	-	-	AF358206
C. grammicosporopsis	CBS 114.87	Bark of *Metrosideros* sp.	New Zealand	MH862055	MH873743	-	-
C. grammicosporopsis	CBS 115.87	Bark of *Metrosideros* sp.	New Zealand	AF210679	-	-	AF358204
C. impariphialis	10503	-	China	KX096609	KX096606	-	-
C. indicus	RKV-2015	*Ficus virens*	India	KT291441	-	-	-
C. intermedia	CBS 508.82	Soil	Netherlands	AF210682	-	-	AF358205
C. kowhai	CBS 461.95	-	China	AF358250	-	-	AF358170
C. krabiensis	MFLUCC	Pandanaceae	Thailand	MH388335	MH376707	-	-
C. levigata	CBS 948.97	Branch of dead *Buxus sempervirens*	France	AF210680	-	-	AF358196
C. lucifer	CBS 100008	Bark of recently dead *Casearia arborea*	USA.	AF210683	-	-	AF358208
C. macrospora	CBS 130.87	-	-	AF210688	-	-	AF358168
C. miodochialis	CBS 997.69	Soil	Netherlands	AF210674	MH871287	-	AF358210
C. moreaui	CLL19024	dead corticated branches of *Laurus novocanariensis*	Portugal	-	MT160524	-	-
C. oblongispora	CBS 100285	-	-	AF358248	-	-	AF358169
C. pallens	PAD S00004	Herbarium	Indonesia	MT554899	-	-	-
C. phyllophila	CBS 921.97	Leaves of *Viscum album*	France	AF210664	-	-	-
C. pityrodes	CBS 126394	-	Sri Lanka	MH864280	MH875729	-	-
C. pilosella	BrFM 3113	Bark	French Guiana	-	Mt248415	-	-
C. pnagiana	BrFM 3057.	Bark	French Guiana	-	Mt248416	-	-
C. pseudochroleuca	18	*Solanum tuberosum*	S. Rita de Caldas, MG	KC806258	-	KX185004	KF871159
C. pseudochroleuca	CBS 187.94T	Base of decaying palm frond	French Guiana	MH874107	-	KX185003	KF871188
C. pseudochroleuca	CML 2562, CBS 192.94	Bark	French Guiana	AF358238	-	KX185016	AF358171

Continue…

Specie	Collection n.*	Substrate	Place	GenBank access n.**			
				ITS	LSU	tef1	tub2
C. pseudochroleuca	CML 2670	Unidentified	Lavras, MG	-	-	KX185013	KX185046
C. pseudostriata	CBS 120.87	Bark	Indonesia	MH862056	MH873744	-	AF358184
C. pseudostriata	CBS 119.87	Bark	Indonesia	AF358251	-	-	AF358183
C. pseudotriatopsis	MAFF239827	Bark of fallen twigs	Japan	-	-	-	AB237465
C. ralfsii	CBS 129.87	Bark	New Zealand	AF210676	-	-	AF358195
C. ralfsii	CBS 267.46		New Zealand	MH855796	MH867305	-	-
C. ralfsii	CBS 102845	Bark	Australia	AF358253	-	-	AF358219
C. rhizophaga	CML 1210	Soil under soybean field	Montividiu, GO	KC806272	-	KX184990	KF871156
C. rhizophaga	CML 1984	Native soil from Cerrado	Montividiu, GO	KC806274	-	KX184991	KF871155
C. rhizophaga	CML 2514, CBS 361.77	Culture contaminant	Switzerland	AF358228	-	KX184993	AF358158
C. rogersoniana	CML 2557, CBS 920.97T	Soil under *Araucaria* sp.	Batatais, SP	-	-	KX185022	KX185047
C. rogersoniana	CML 2558, CBS 582.89	Soil from Amazon Forest	Capitão Poço, PA	-	-	KX185023	AF358189
C. rogersoniana	CML 2646	Litter from dry place	Barroso, MG	-	-	KX185026	KX185050
C. rosea f. catenulata	CML 2516, CBS 154.27T	Soil	United States	MH854911	-	KX184995	AF358160
C. rosea f. catenulata	CML 2517, CBS 443.65	Soil	United States	MH858662	-	KX184996	AF358166
C. rosea f. rosea	CML 2518, CBS 710.86T	Soil, on sclerotia of *Sclerotinia minor*	Netherlands	-	-	KX184999	AF358161
C. rosea f. rosea	CML 2519, CBS 194.57	Decaying bulb of *Lilium auratum*	United States	-	-	KX185000	AF358165
C. rossmaniae	CBS 210.93	-	Brazil	AF358227	-	-	AF358213
C. rossmaniae	CML 2316	Soil	Brazil	KC806299	-	-	KF871190
C. samuelsii	CBS 699.97	Bark	Venezuela	AF358236	-	-	AF358190
C. sesquicillii	CBS 180.88	Twigs and lichen	Guyana	AF210666	MH873818	-	AF358214
C. setosa	CBS 834.91	*Trophis racemosa*	Cuba	AF210670	-	-	AF358211
C. solani	CBS 102418	-	Netherlands	MH862790	MH874384	-	-
C. solani	CBS 697.88	Bark	Germany	MH862150	MH873842	-	AF358216
C. solani	CBS 223.72	Soil	Germany	MH860460	MH872186	-	AF358223

Continue…

Specie	Collection n.*	Substrate	Place	GenBank access n.**			
				ITS	LSU	tef1	tub2
C. sporodochialis	CBS 101921	-	Venezuela	AF210685	-	-	AF358149
C. subquaternata	CBS 107.87	Wood	Venezuela	-	-	-	AF358207
C. swieteniae	MFLU 18-2769	-		MT215573	MT396164	MT212204	-
C. wenpingii	CBS 124067	-	China	MH863343	MH874867	-	-
C. wenpingii	W2792	-	China	-	-	HM054097	HM054127
C. viticola	CAA944	Root of *Vitis vinifera*	Peru	MK156282	-	MK156286	MK156290
C. viticola	CAA945	Root of *Vitis vinifera*	Peru	MK156283	-	MK156287	MK156291
C. viticola	CAA946	Root of *Vitis vinifera*	Peru	MK156284	-	MK156288	MK156292
C. zelandiae-novae	CBS 232.80	Bark of *Coprosma* sp.	New zealand	AF210684	-	-	AF358185

T Ex-type cultures.

* BRIP: Biosecurity Queensland Plant Pathology Herbarium, Department of Agriculture and Fisheries, Dutton Park, Australia. CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. CPC: Working collection of Pedro Crous housed at CBS. IMI: International Mycological Institute, CABI-Bioscience, Egham, UK. ATCC: American Type Culture Collection, Manassas, USA. IMUR: Institute of Mycology, University of Recife, Recife, Brazil. LC: Herbarium of Microbiology, Academia Sinica, Taipei, Taiwan. CGMCC: China General Microbiological Culture Collection Center, Beijing, China. CCUB: Coleção de Culturas da Universidade de Brasília, Brasília, Brazil.

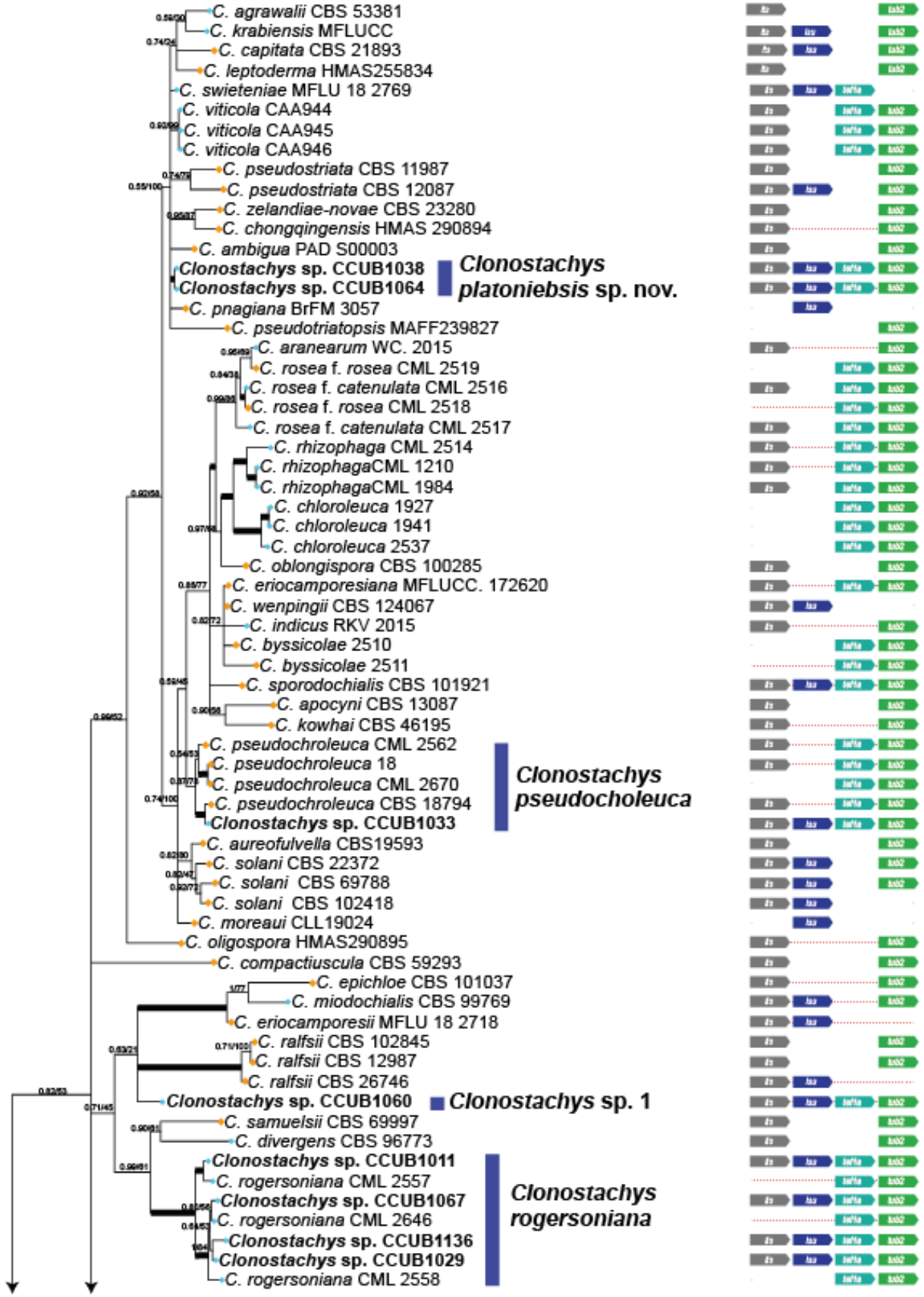
** Newly deposited sequences are shown in bold.

5.4 Results

Isolates - Eight isolates were obtained from dropped fruits of seven species and in four Brazilian states: Distrito Federal – *Inga laurina;* Goiás – *Platonia insignis;* Pará – *Oenocarpus bacaba;* Santa Catarina – *Butia archeri, Eugenia involucrate, Euterpe edulis* and *Terminalia catappa.*

Phylogenetic analysis – Amplicons of all isolates obtained were generated sequences of approximately 700bp to *tef1a* and *tub2,* and 900bp to *its* + *lsu*. The final matrix included 92 taxa, including isolates of *Calonectria brassicae* CBS 111869 and *Ca. ilicicola* CBS 19050 used as outgroup. The concatenated matrix had 2.460 sites including gaps, this also was compound by 1.446 conserved, 943 variables and 720 parsimony informative sites. The *tub2* and *tef1a* were the dataset that most contributed to parsimony informative sites, 277 and 213 sites, respectively. The Bayesian inference were chosen by individual dataset next best model of nucleotide substitution: SYM+I+G to *Its* using 523 sites*;* HKY+I+G to *lsu* using 807 sites*;* GTR+I to *tef* and *tub2* (+G) using 519 and 611 sites, respectively. Each data set was run individually, overall topologies were similar, although the relative position of some *Clonostachys s*pecies was slightly different between the IB trees.

The phylogeny result in segregation of the eight new isolates in four clades, two with species knowledge by the literature, and two new phylogenetic clades. The first strain identified was CCUB1033, they grouped with the type species of *C. pseudocholeuca* CBS 187.94 with high values (IB=1; ML = 100). Four new strains, CCUB1011, CCUB1029, CCUB1067 and CCUB1136 grouped with *C. rogersoniana,* although these isolates formed one clade only CCUB1011 was linked with the type of specie *C. rogersoniana* CML 2557. The strain CCUB1060 formed a new clade.


C. agrawalii CBS 53381
C. krabiensis MFLUCC
C. capitata CBS 21893
C. leptoderma HMAS255834
C. swieteniae MFLU 18 2769
C. viticola CAA944
C. viticola CAA945
C. viticola CAA946
C. pseudostriata CBS 11987
C. pseudostriata CBS 12087
C. zelandiae-novae CBS 23280
C. chongqingensis HMAS 290894
C. ambigua PAD S00003
Clonostachys sp. CCUB1038
Clonostachys sp. CCUB1064
Clonostachys platoniebsis sp. nov.
C. pnagiana BrFM 3057
C. pseudotriatopsis MAFF239827
C. aranearum WC. 2015
C. rosea f. rosea CML 2519
C. rosea f. catenulata CML 2516
C. rosea f. rosea CML 2518
C. rosea f. catenulata CML 2517
C. rhizophaga CML 2514
C. rhizophagaCML 1210
C. rhizophagaCML 1984
C. chloroleuca 1927
C. chloroleuca 1941
C. chloroleuca 2537
C. oblongispora CBS 100285
C. eriocamporesiana MFLUCC. 172620
C. wenpingii CBS 124067
C. indicus RKV 2015
C. byssicolae 2510
C. byssicolae 2511
C. sporodochialis CBS 101921
C. apocyni CBS 13087
C. kowhai CBS 46195
C. pseudochroleuca CML 2562
C. pseudochroleuca 18
C. pseudochroleuca CML 2670
C. pseudochroleuca CBS 18794
Clonostachys sp. CCUB1033
Clonostachys pseudocholeuca
C. aureofulvella CBS19593
C. solani CBS 22372
C. solani CBS 69788
C. solani CBS 102418
C. moreaui CLL19024
C. oligospora HMAS290895
C. compactiuscula CBS 59293
C. epichloe CBS 101037
C. miodochialis CBS 99769
C. eriocamporesii MFLU 18 2718
C. ralfsii CBS 102845
C. ralfsii CBS 12987
C. ralfsii CBS 26746
Clonostachys sp. CCUB1060
Clonostachys sp. 1
C. samuelsii CBS 69997
C. divergens CBS 96773
Clonostachys sp. CCUB1011
C. rogersoniana CML 2557
Clonostachys sp. CCUB1067
C. rogersoniana CML 2646
Clonostachys sp. CCUB1136
Clonostachys sp. CCUB1029
C. rogersoniana CML 2558
Clonostachys rogersoniana

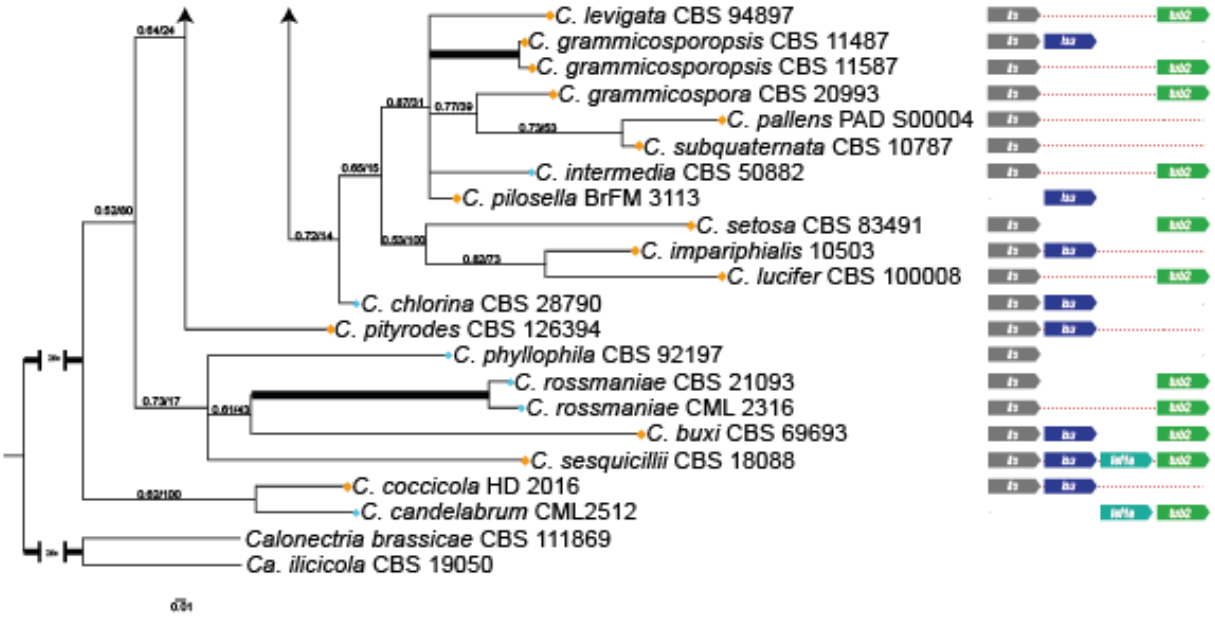
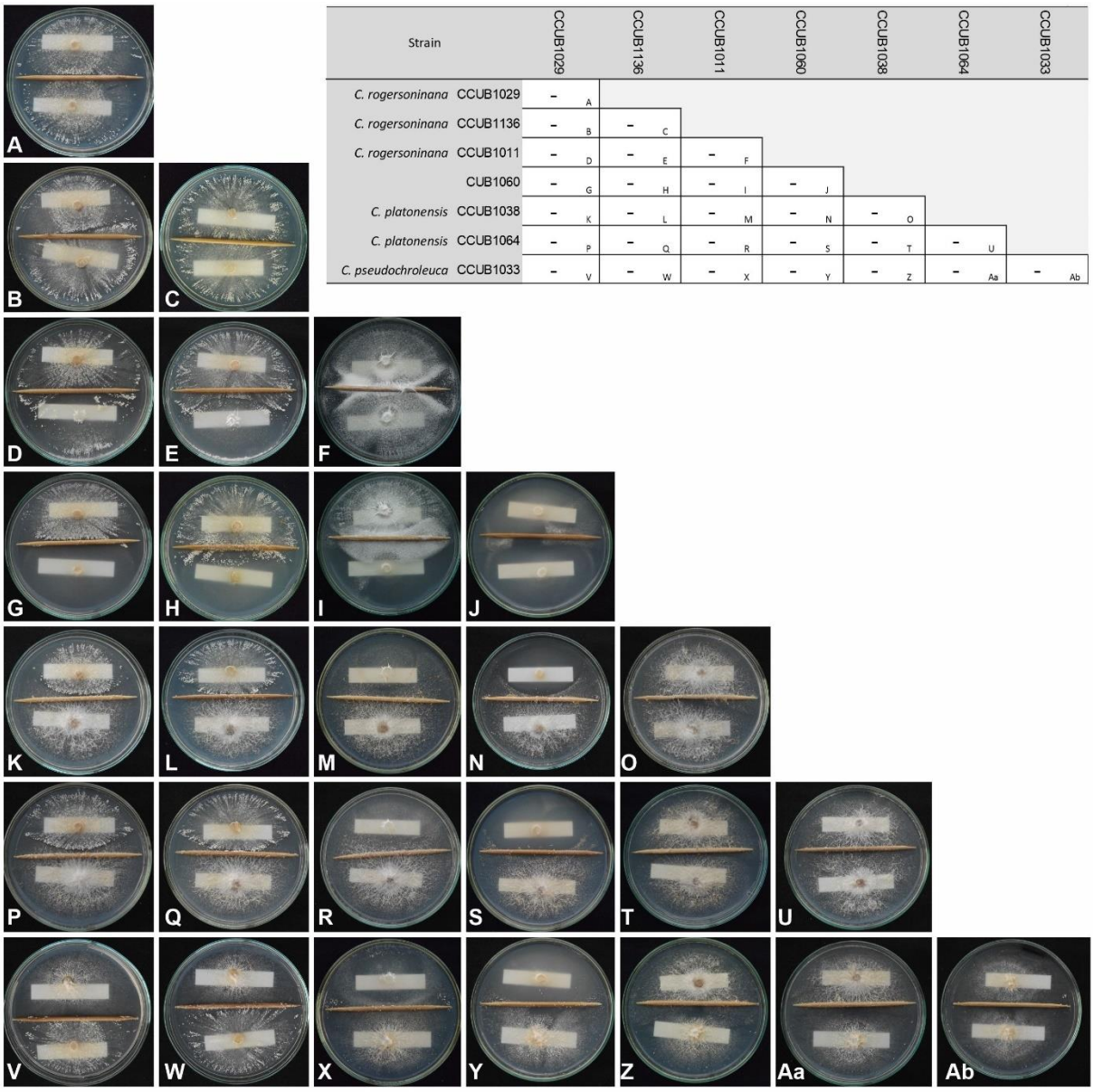


Figure 1. Phylogenetic tree of three of Clonostachys genus based on Bayesian inference analysis by combination of four partial loci represented in right side of tree. Arrows in gray represent its, dark blue tef1a, light blue lsu and green tub2, absence of some arrows represent that partial sequence is unavailable on Genbank database. Access in bold represents new isolates add by this study. Posterior probability (PP) and Maximum likelihood (ML) bootstrap value are indicated at nodes. Thicker nodes represent PP/ML values above 0.99/99. Dots on terminal branch represent the morph type founded until now, blue dot only founded asexual form and orange dot represent the sexual form. Long branches are cut according to the scale and the number was referred to in branch. The scale bar represents the number of expected changes per site.

separately of two other clades high supported one with three strains of *C. ralfsii* (IB≥0.99; ML ≥ 99), and other with *C. eriocamporesii, C. miodochialis* and *C. epichloe* (IB=1; ML ≥ 99). The last clade formed was compound by two new strains CCUB1038 and CCUB1064, these formed a new phylogenetic species close to *C. viticola* and *C. swieteniae* (Figure 1).

Sexual compatibility – After four weeks, no combination produced sexual structures indicating that they may have lost the ability to be self-fertile and the strains analyzed have only one mating-type (Figure 2). Although, during the process it was observed that the combinations involving *C. rogersoniana* CCUB1029 and CCUB1136, presented some inhibition under *C. rogersoniana* CCUB1011 and *Clonostachys* sp. 1 CCUB1060. Also was possible to see this pattern in combinations involving *C. platonensis* CCUB1038 and CCUB1064 under *C. rogersoniana* CCUB1029, CCUB1136 and *Clonostachys* sp. 1 CCUB1060.



Strain	CCUB1029	CCUB1136	CCUB1011	CCUB1060	CCUB1038	CCUB1064	CCUB1033
C. rogersoninana CCUB1029	– A						
C. rogersoninana CCUB1136	– B	– C					
C. rogersoninana CCUB1011	– D	– E	– F				
CUB1060	– G	– H	– I	– J			
C. platonensis CCUB1038	– K	– L	– M	– N	– O		
C. platonensis CCUB1064	– P	– Q	– R	– S	– T	– U	
C. pseudochroleuca CCUB1033	– V	– W	– X	– Y	– Z	– Aa	– Ab

Figure 2. Pairing of Clonostachys strains after four weeks. Table on top indicates the result of sexual compatibility, letters in box represent the pair of isolates used. From top to bottom and left to right the strains are in this sequence CCUB1029, CCUB1136, CCUB1011, CCUB1060, CCUB1038, CCUB1064, CCUB1033. The strain down to toothpick even are the isolate in line and up to toothpick is the column isolate.

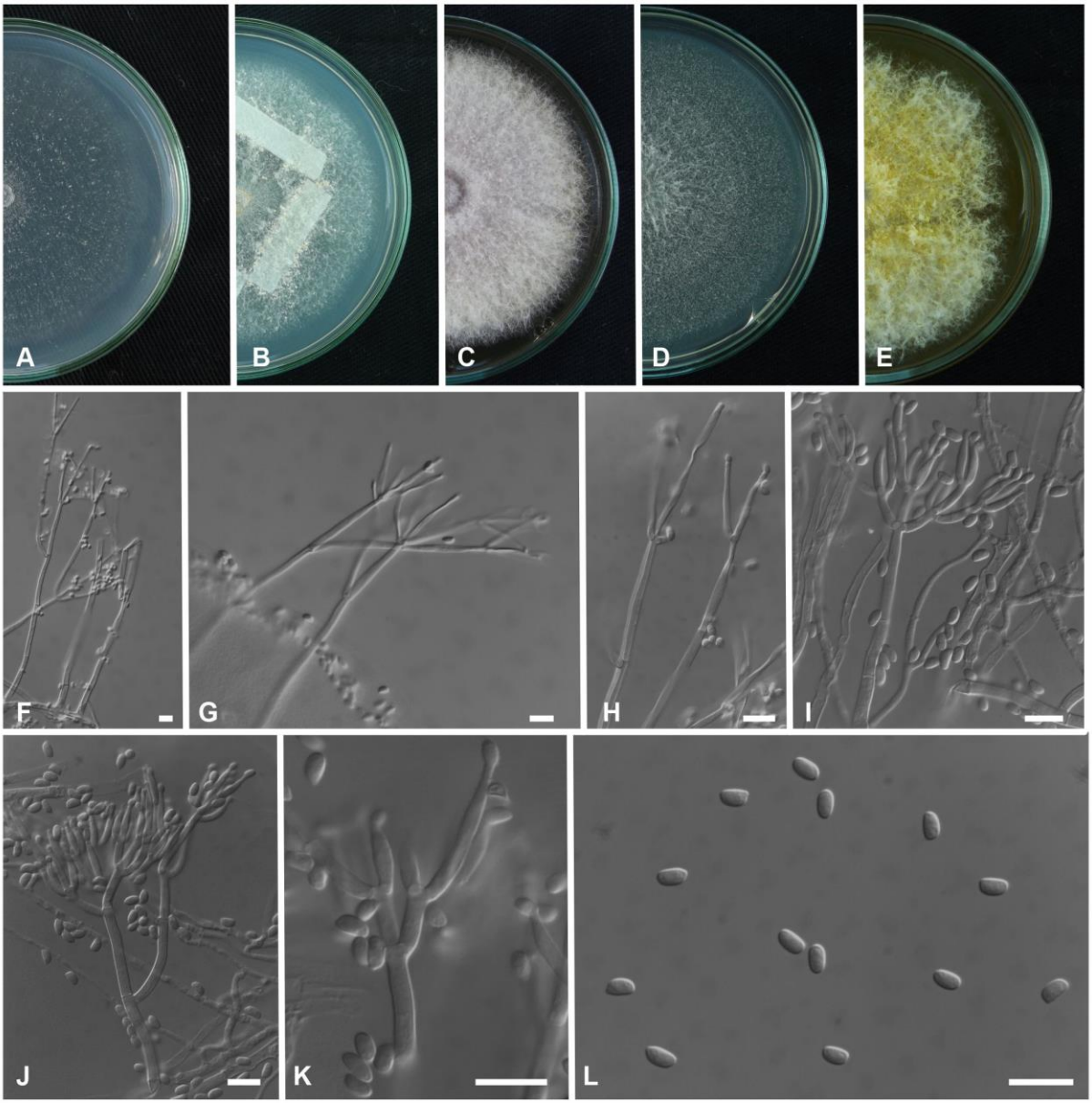
Taxonomy – Based on genealogical concordance phylogenetic species recognition criterion (GCPSR) was identified one new strongly monophyletic species. Morphological characteristics of this species were characterized and show distinct differences of neighboring clades composed by other known species. Using the GCPSR and morphological characteristics the strains CCUB1038 and CCUB1064 represent a new species of *Clonostachys* and are described as follow:

***Clonostachy platoniensis* sp. nov R.A. Fernandes, A. Reis & D. B. Pinho. Fig. 3**

Etymology – Named after the host genus Platonia *where this species was collected.*

Typus - Brazil, Nova Veneza, Goiás, isolated from the dropped fruit of *Platonia insignis*, 2018 (holotype CCUB1038, a dried culture sporulating on PDA). The GenBank accession numbers for the DNA sequences of its*, tef1a, lsu* and *tub2*.

Sexual morph not observed. Asexual morph with conidiophores dimorphic. Primary conidiophores verticillium-like, smooth-walled, 78–160 µm long, 3.5–5µm wide at the base, with branches at acute angles, ending to primary phialides 20.5–31(–40) × 2–3.5 µm (average= 27.5 × 2.5 µm) straight, cylindrical, slightly tapering towards tip. Secondary conidiophores broadly penicillate, 53–84 µm long and 43–75 µm wide, biverticillate to terverticillate, branches, phialides and metulae divergent. Each branch



ending in addressed phialides (2–6). Secondary phialides 9.5–13(–16) *Figure 3. Clonostachys platoniensis CCUB1038 – colonies after 7 days of growth on the (A)* agar Water, (B) synthetic nutrient agar, (C) carrot agar, (D) potato dextrose agar, (E) malt extract agar. (F-H) Primely conidiophores, (I-J) secondly conidiophores, (K) detail of phialide of secondly conidiophore and (L) conidia. Scale bar: F= 20µm, G-L = 10 µm.

× 2–3 µm (average= 12 × 2.5 µm). Conidia 4.5–5.5 × 2–3 µm (average= 5 × 2.5 µm) hyaline, ellipsoidal/oval, slightly curved, with a laterally displaced hilum.

Colony characteristics: On AW, reverse obverse without visible pigmentation, colony presenting concentric circles, scarce white mycelia on top, small granular points due to sporulation. On SNA with strip paper obverse white, little cottony aerial mycelium, and pale to rosea granular points around to strip paper, due the sporulation. On CA intense white and cottony aerial mycelia was observed, reverse present pale purple become more intense with the age of colony. On PDA as no AW the aerial mycelia white and scarce, no pigment was observed on reverse. On MEA strongly cottony mycelia observed, began pale yellow and turn yellow intense of border to center. Colonies reaching 59-64 mm diameter on AW, 54-62 mm on SNA, 70 mm on CA, 56 mm on PDA and 66-68 mm on MEA in 14 days at 25 °C.

Material examined – Nova Veneza, Goiás, isolated from the dropped fruit of *Platonia insignis*, 2018, CCUB1064. The GenBank accession numbers for the DNA sequences of its*, tef1a, lsu* and *tub2*, ex-type is MK156282, MK156286, MK156286 and MK156290.

Notes – The new strain phylogenetic specie identified in this study with *Clonostachys platoniensis* (CCUB1038) with high statistical support (IB = 1; ML= 100%, Figure 1) in phylogenetic analysis, and they are morphologically different of *Clonostachys viticola* and *C. switeniae* closers clades. In comparison with *C. viticola*, the new species had verticillate conidiophores, primary and secondary phialides bigger than this species, both species produced conidia in same shape and similar range of dimensions. Another difference between *C. viticola* and *C. platoniensis* is the colony growth in PDA, our specie does not present color in reverse, and the aerial mycelia is white and less cottoning than *C. viticola,* the comparison with other culture in different medium was not possible due the absence of colony characterization on *C. viticola.* In concern the

difference of *C. swieteniae* and *C. platoniensis*, is highlighted by the absence of verticilioide conidiophores in the first species, and the conidia dimensions of new species is bigger than the knowledge species.

5.5 Discussion

Clonostachys platoniensis is described as a new species in this study based on monophyletic clade observed on phylogenetic analysis with four loci (Fig 1) and has a distinct morphology characteristic when compared to other related species. Was found too *C. pseudocholeuca* and *C. rogersoniana*, both species was reported in Brazilian territory, in Amazonas, Goiás, Minas Gerais, Pará and São Paulo (Moreira et al. 2016). With this study the knowledge of *Clonostachys* distribution was increased for more two other Brazilian states, Distrito Federal and Santa Catarina. The isolate of *Clonostachys* sp. 1 CCUB1060 was not proposed as a new species although it formed an independent clade on phylogeny, due the presence of only one isolate, difficulty the understanding of morphologic and genetic variation, as recommended by Aime et al. (2021).

No sexual morph was observed during the sexual compatibility test indicating that all isolates obtained in this study are heterothallic. Nowadays almost 60 species are accepted by science, among these 41 had the sexual morph observed in natural environment of artificial medium. For approximately 17 species the sexual morph is unknow, the rate of sexual/asexual morph knowledge indicates that the homothallism is more common of been seen than others genus.

The identification of *Clonostachys* species based on molecular approaches started on 1994 with the use of the large subunit ribosomal DNA (lsu - Rehner and Samuels 1994). Since this time only two markers are commonly used in phylogenetic studies,

introduction of Internal transcribed spacer (*its*) (Park et al. 1999) and beta-tubulin (Paavanen-Huhtala et al. 2000) in Genbank they are the most representative in number of sequences, despite this databank presents sequences of seven other marker introduced during the last thirty years. In time the less representative marker introduced was: small subunit of ribosomal (*ssu)* in 1998; translation elongation factor 1-alpha (*tef1a*) in 2003; RNA polymerase II subunit larger (*rpb1*) in 2004; RNA polymerase II second largest subunit (*rpb2*) in 2007; ATC citrate lyase (*acl*) and calmodulin (*cmdA*) in 2015 (Rehner and Samuels 1994; Schroers 2001; Castlebury et al. 2004; Slippers et al. 2004; Spatafora et al. 2007; Lombard et al. 2015).

The introduction of many markers in various times without the revisiting old isolates already sequenced in previously study, construct unevenness databank, difficulty the phylogenies comparisons, increasing divergences, inconsistency and decrease consistency of species boundaries(Liu et al. 2020). In this study four partial loci were used to phylogenetic analysis, two of these the most representative in Genbank database (*its* and *tub2*). Despite some clades were highly supported many had low value of support. This lower support had two probable causes, the lack of sequences in databank and the absence of more than one isolate sampled in the specific description. As example of the lack of sequences impact, on clade formed by *C. ericamporesiana, C. wenpingii, C. indicus* and *C. byssicolae,* the analysis was not capable to distinguish these species forming a polytomy, due the absence of some sequences for these species. By the absence of more than one species to describe a new species in many clades we can see the low values of Posterior Probabilities or/and Maximum Likelihood, as in clades like *C. pallens* and *C. subquaternata.* Liu et al. (2020) highlighted in your study that the lack of consistency in multi-gene phylogenetic

analyses combined with a morphological characterization of a robust collection of isolates result in confusion and the report of many synonyms as new species.

More recently, the taxonomist community had applied efforts to clarify the backbone based on markers that can be used as barcode alone or combined. Important genus like *Calonectria* (Liu et al. 2020)*, Colletotrichum* (Vieira et al. 2020) and *Fusarium* (Lombard et al. 2019) already due the construction of what marker produce the more reliable backbone. On the same direction two publication made in 2017 and 2019 worked with many genera to determine the patterns of barcode according to the sampling on databank and marker that offer better resolutions (Marin-Felix et al. 2017, 2019). Until now, only Moreira et al. (2016) indicated *acl1* as useful molecular barcode, but they worked with 65 isolates distributed on seven species. Today *acl1* had 84 sequences distributed in 12 species, to work as barcode this marker needs to be evaluated in more species and isolates. The patterns of markers and indications of barcode markers represent a futures study in open that need attention due the crescent importance of *Clonostachys.???*

Clonostachys had gain attention by the constant report in biological control?? (Moreira et al. 2016). In our study, appear the compatibility test did not produce sexual morph, both isolates of *C. platoniensis* presented a level of inhibition when pairing with isolates of other species as *C. rogersoniana* and *C. pseudochroleuca.* Such patterns indicate that this species has potential to be applied in biocontrol studies of fungi. Besides the genus *Clonostachys* has almost 60 species a few species are used for such purpose, in highlight *C. rosea* domain as the most used species with this purpose (Karlsson et al. 2015). In analysis of *C. rosea* genome was observed that the mycoparasitism habit was developed by the selection in a high number of polyketide syntheses and ATP-

binding cassette transporter involved in drug resistance (Karlsson et al. 2015). The identification here of a new species with such potential to application on biocontrol show the importance of studies in fungal diversity in Brazilian territory and correct identification using bidirectional approach well standardized.

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Supplementary Data

Supplementary table 1. List of Hypocreaceos (*Cylindrocladiella, Gliocladiopsis* and *Gliocephalotrichum*) used for phylogeny.

Parameter	Partition			
	ITS	**TEF1**	**LSU**	**TUB**
Evolutive model	SYM+I+G	GTR+I	HKY+I+G	GTR+I+G
Likelihood	-3573.8057	-2805.1772	-1886.9279	-7151.5986
Matrix length	523	523	806	601
Variables sites	218	252	93	338
Parsimony informative sites	154	181	71	281
Conserved Sites	287	248	706	242
Base frequencies				
Freq. A	Equal	0.2292	0.2560	0.2071
Freq. C		0.2955	0.2170	0.2687
Freq. G		0.1892	0.3021	0.2343
Freq. T		0.2861	0.2249	0.2899
Transition rates				
[A-C]	2.8115		2.0832	0.8242
[A-G]	4.6265		11.6035	3.2891
[A-T]	2.5928	1.7427	3.7299	0.6735
[C-G]	0.9762		2.8135	0.6076
[C-T]	8.0770		28.2471	3.0437

[G-T]	1.0000		1.0000	1.0000
Proportion of invariable sites	0.4122	0.3531	0.8852	0.3880
Gamma	0.6274	1.4930		2.1536

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