UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM FITOPATOLOGIA

Dissertação de mestrado

Espécies de *Colletotrichum* associadas a antracnose do bastão do imperador (*Etlingera elatior*)

Ingrid Gomes Duarte

Recife – PE

2021

INGRID GOMES DUARTE

ESPÉCIES DE *Colletotrichum* ASSOCIADAS A ANTRACNOSE DO BASTÃO DO IMPERADOR (*Etlingera elatior*)

Dissertação apresentada ao Programa de Pós-Graduação em Fitopatologia da Universidade Federal Rural de Pernambuco, como parte dos requisitos para obtenção do título de Mestre em Fitopatologia.

COMITÊ DE ORIENTAÇÃO:

Orientador: Prof. Marcos Paz Saraiva Câmara, Ph.D. (DEPA/UFRPE)

Co-orientador: Dr. Willie Anderson dos Santos Vieira (DEPA/UFRPE)

Co-orientador: Dr. Humberson Rocha Silva (DEPA/UFRPE)

RECIFE- PE

JULHO – 2021

Dados Internacionais de Catalogação na Publicação
Universidade Federal Rural de Pernambuco
Sistema Integrado de Bibliotecas
Gerada automaticamente, mediante os dados fornecidos pelo(a) autor(a)

D812e Duarte, Ingrid Gomes

Espécies de Colletotrichum associadas a antracnose do bastão do imperador (Etlingera elatior) / Ingrid Gomes Duarte. - 2021.

53 f. : il.

Orientador: Marcos Paz Saraiva Camara.
Coorientador: Willie Anderson dos Santos Vieira.
Inclui referências.

Dissertação (Mestrado) - Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Fitopatologia, Recife, 2021.

1. Diversidade. 2. filogenia . 3. floricultura. 4. virulência. I. Camara, Marcos Paz Saraiva, orient. II. Vieira, Willie Anderson dos Santos, coorient. III. Título

CDD 632

ESPÉCIES DE *Colletotrichum* ASSOCIADAS A ANTRACNOSE DO BASTÃO DO IMPERADOR (*Etlingera elatior*)

INGRID GOMES DUARTE

Dissertação defendida e aprovada pela Banca Examinadora em: 29/07/2021

ORIENTADOR(A):

_____

Prof. Marcos Paz Saraiva Câmara, Ph.D. (UFRPE)

EXAMINADORES:

_____

Prof. Dr. Alexandre Reis Machado (UFPE)

_____

Prof. Dr. André Luiz Firmino (UFU)

RECIFE-PE
JULHO – 2021

AGRADECIMENTOS

À minha mãe Maria José por toda a dedicação na minha criação, amor e carinho indescritível.
Ao professor Marcos Câmara, pela orientação e confiança e pelos ensinamentos transmitidos.
Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa de estudo concedida e pelo suporte para a realização deste trabalho.
Aos integrantes do Laboratório de Micologia por compartilharem a rotina do dia-a-dia, em especial aos amigos Ana Gabriele e Athaise Ferreira pela paciência e apoio durante todo o curso; à Anthony Carlos, pela amizade tão espontânea e leve que sempre me traz ânimo; à Willie Anderson pela coorientação, pelo conhecimento transmitido e pela colaboração na realização desse trabalho.
A Dra. Josiene Veloso pela paciência, amizade, motivação e pelo conhecimento transmitido.
À Beatriz Torres e Beatriz Macêdo, pela amizade e compreensão desde a Graduação, nos momentos em que mais precisei de conforto.
À Otília Farias, pela amizade, ensinamentos, conselhos, paciência, carinho, muito carinho que sei que tens por mim.
À todos os professores do PPGF/UFRPE pelos ensinamentos transmitidos ao longo do curso.
À Romildo, secretário do PPGF, quem sempre auxilia os alunos no que for possível.
Aos amigos do ensino médio que perduraram, Beatriz Araújo, Micaelle Oliveira, Gabriella Nascimento pelos momentos de descontração e carinho, mesmo que a distância.
À Flaviano Fernandes e Nardiele Freitas com quem dividi maior parte da minha rotina recifense durante o curso. Agradeço o cuidado que tiveram comigo durante este tempo. E à Brener Gomes, que em pouco tempo já me afeiçoei e por quem tenho grande carinho.
À todos aqueles que de alguma forma contribuíram para a construção deste momento da minha vida.

SUMÁRIO

RESUMO GERAL

O bastão do imperador é uma das espécies de plantas ornamentais mais exploradas comercialmente no estado de Pernambuco, espécie muito apreciada pelas características das inflorescências. A ocorrência de doenças como a antracnose leva a perda de valor estético e comercial. A antracnose causada por espécies de *Colletotrichum* é uma das doenças mais importantes para o bastão do imperador. O presente estudo teve como objetivo caracterizar isolados de *Colletotrichum* spp. associados à antracnose do bastão do imperador em áreas produtoras dos estados de Ceará e Pernambuco. Um total de 48 isolados de *Colletotrichum* foram identificados a partir de dados de sequências de DNA (APN2, APN2/MAT-IGS, CAL, GAPDH, GAP2-IGS, GS e TUB2) sendo encontradas as seguintes espécies: *C. crysophilum*, *C. fructicola*, *C. siamense*, *C. theobromicola*, *C. tropicale*, e três novas espécies, descritas como *C. atlanticis*, *C. floscerae* e *C. zingibericola*. Todas as espécies representam o primeiro relato associadas ao bastão do imperador no Brasil. *C. atlanticis* foi a espécie mais prevalente. Todas as espécies foram patogênicas. Houve diferenças significativas na virulência entre as espécies de *Colletotrichum*. As espécies de *C. atlanticis*, *C. floscerae* e *C. zingibericola* foram as espécies mais virulentas.

Palavras-chave: Diversidade, filogenia, floricultura, virulência.

GENERAL ABSTRACT

Torch ginger is one of the most commercially exploited species in the state of Pernambuco. It is a highly appreciated species due to the characteristics of its inflorescences. The occurrence of diseases such as anthracnose leads to loss of aesthetic and commercial value. Anthracnose caused by *Colletotrichum* species is one of the most important diseases in torch ginger. The present study aimed to characterize *Colletotrichum* spp. associated with torch ginger anthracnose in producing areas in the states of Ceará and Pernambuco. A total of 48 *Colletotrichum* isolates were identified using DNA sequence data (APN2, APN2/MAT-IGS, CAL, GAPDH, GAP2-IGS, GS and TUB2) and the following species were found: *C. crysophilum*, *C. fructicola*, *C. siamense*, *C. theobromicola*, *C. tropicale*, and three novel species, described as *C. atlanticis*, *C. floscerae* and *C. zingibericola*. All *Colletotricum* species in this study represent the first report of torch ginger anthracnose in Brazil. C. atlanticis was the most prevalent species. All species were pathogenic in torch ginger. There were significant differences in virulence between species. The species of *C. atlanticis*, *C. floscerae* and *C. zingibericola* were the most virulent.

Keywords: diversity, phylogeny, floriculture, aggressiveness.

CAPÍTULO I

Introdução Geral

ESPÉCIES DE *Colletotrichum* ASSOCIADAS A ANTRACNOSE DO BASTÃO DO IMPERADOR (*Etlingera elatior*)

INTRODUÇÃO GERAL

As plantas ornamentais possuem atrativos aos consumidores por seu papel estético, por proporcionar melhor ambiência e também como atividade de lazer (jardinagem) (DE, 2017; SIVIERO et al., 2014). Características morfológicas de bom aspecto, principalmente externas como cor de flores, são alvos de melhoramento de plantas, devido a influência na escolha do consumidor (KULIGOWSKA et al., 2016; SU et al., 2019).

A floricultura comercial é um segmento do agronegócio presente em quase todos os países do mundo. Segundo dados do anuário de 2018 da United Nations (UN, 2019), no ano de 2017 obteve-se o valor de 20,22 bilhões de dólares em exportações e 16,32 bilhões de dólares em importações mundiais de produtos de floricultura. A Holanda contribui para estes valores como maior produtor e exportador mundial, enquanto a Índia possui a maior área plantada com cerca de 242.000 hectares (BRAINER, 2018).

O Brasil possui cerca de 15 mil hectares destinados à produção de plantas ornamentais, contando com mais de 4000 empresas concentradas no sudeste do país (AIPH, 2019). Nesta região, a produção de flores concentra-se principalmente no estado de São Paulo, com destaque para os municípios de Holambra e Atibaia (JUNQUEIRA; PEETZ, 2018). Entretanto, a floricultura brasileira não apresenta inserção significativa no mercado externo, sendo a produção de flores destinada majoritariamente para o mercado interno (AIPH, 2019).

A produção de flores é uma atividade explorada por pequenos produtores como fonte alternativa de renda (JUNQUEIRA; PEETZ, 2018). Este setor foi responsável pela geração de 215,8 mil empregos em toda a cadeia de produção (IBRAFLOR, 2015). Sua importância tem aumentado devido à contribuição ao agronegócio nacional, e também pelo objetivo de ganhar mercado externo (IBRAFLOR, 2015).

As espécies de plantas ornamentais de climas temperados e tropicais compõem maior parte da produção nacional, principalmente as espécies tropicais que requerem menos exigências de cultivo e possuem maior durabilidade (FRANÇA; MAIA, 2008; BUAINAIN; BATALHA, 2007). No Brasil, as condições de temperatura e umidade na região Nordeste favorecem o cultivo principalmente de espécies tropicais (LOGES et al., 2005). Apesar disso, a região Nordeste ocupa a terceira posição nacional em termos de Valor Bruto da Produção (VBP), concentrando apenas 9,01% de participação percentual do VBP da floricultura nacional

(SEBRAE, 2015).

Na região Nordeste a produção de flores é concentrada em sua maioria na Bahia, Pernambuco e Ceará (BRAINER, 2018). As principais espécies cultivadas nesta região são antúrio (*Anthurium* sp. Schott), helicônia (*Heliconia* sp. L.), alpínia (*Alpinia* sp. Roxburgh), strelitzia (*Strelitzia reginae* Banks ex Aiton), sorvetão (*Zingiber spectabile* Griff) e o bastão do imperador (*Etlingera elatior*) (SEBRAE, 2015). O estado de Pernambuco é o segundo polo produtivo da floricultura da região Nordeste (SEBRAE, 2015) e tem como regiões de maior relevância na produção de flores ornamentais a Região Metropolitana, Agreste, Zona da Mata, Vale do São Francisco e Sertão.

O gênero Etlingera é originário da região Indo-pacífico (UD-DAULA; BASHER 2019), e inclui aproximadamente 100 espécies aceitas (TPL, 2013). As plantas deste gênero possuem hábito herbáceo, perene, podendo alcançar oito metros de altura. Os rizomas são aromáticos, e podem ser subterrâneos ou aéreos. As folhas são grandes e em touceiras. As hastes florais surgem separadamente, com inflorescências terminais (CHAN; LIM; WONG, 2011; UD-DAULA; BASHER, 2019). As principais espécies descritas deste gênero são *Etlingera venusta* (Ridl.), *Etlingera corneri* Mood & Ibrahim, *Etlingera. junnanense* Mood & Ibrahim, *Etlingera. pyramidosphaera* (K.Schum.) e *Etlingera. elatior* Jack (LOGES et al., 2008). Muitas zingiberáceas são utilizadas na indústria farmacêutica por possuírem efeitos antiflamatório, antioxidantes, analgésico, antifúngico e antibacteriano (CHAN; LIM; TAN, 2011). As inflorescências são consumidas em saladas e os rizomas como condimento ou tempero (MOHAMAD; KALU, 2019). Alguns dos gêneros mais importantes são *Alpinia* Roxb., *Zingiber* Mill., *Curcuma* L. e *Etlingera* Giseke (WU; LARSEN, 2000).

O bastão do imperador (*Etilingera elatior*) pertence à família Zingiberaceae que compreende mais de 1600 espécies descritas, em cerca de 53 gêneros (WCSP, 2020) sendo a maior da ordem Zingiberales. Estas plantas são de hábito herbáceo e perene, rizomatosas e apresentam vistosas inflorescências terminais (SAENSOUK et al., 2016; KUMAR; SINGH, 2018). É uma espécie nativa da Malásia e Indonésia (Sudeste Asiático). Esta espécie pode crescer até 6 metros de altura, e suas inflorescências podem apresentar coloração vermelha, rosa, rosa-claro e branco (LINS; COELHO, 2004; LOGES et al., 2008). O bastão do imperador é muito utilizado de forma ornamental, na composição de cosméticos e fitoterápicos (apresenta metabólitos secundários e propriedades farmacológicas) e na culinária asiática (alto valor nutricional) (SUNGTHONG; SRICHAIKUL, 2018; JUWITA; PUSPITASARI; LEVITA, 2018).

No Brasil, está entre as principais espécies de flores tropicais produzidas juntamente ao

antúrio e helicônia (SEBRAE, 2015). A região Nordeste destaca-se em sua produção, principalmente nos estados de Pernambuco, Bahia e Sergipe (SEBRAE, 2015), sendo cultivadas as variedades vermelho (Red Torch), rosa (Pink Torch) e rosa-claro (Porcelana) (LOGES et al., 2008). O clima que favorece a produção de flores tropicais nessa região também é um fator que favorece a ocorrência de doenças fúngicas que interferem nas características do bastão do imperador (LINS; COELHO, 2004).

As principais doenças observadas em bastão do imperador são a antracnose, podridão do rizoma e das raízes (*Rhizoctonia solani* Kühn). Foram detectadas também fitonematoses por *Meloidogyne incognita* (Kofoid & White), Helicotylenchus Steiner, Xiphinema Cobb e Radopholus Thorne (GONÇALVES; CASTRO, 2014; LINS; COELHO, 2004). Dentre as doenças observadas, destaca-se a antracnose por apresentar incidência em toda a planta (LINS; COELHO, 2004).

A antracnose em bastão do imperador foi reportada pela primeira vez no Brasil, no estado de Pernambuco (LINS; COELHO, 2003). É uma das doenças pós-colheita mais importantes, e seus sintomas influenciam na taxa fotossintética e reduzem a produção de plantas ornamentais. A fase pós-colheita requer maiores cuidados, evitando principalmente ferimentos nas inflorescências por ser o local mais afetado pelos sintomas de antracnose (SILVA et al., 2018; LOGES et al., 2005; FERRARI, 2008). Os maiores danos são os causados nas brácteas florais, manchas escuras que desenvolvem para podridões encharcadas e até necrose (FERRARI, 2008; LINS; COELHO, 2004).

A espécie mais comum da antracnose em flores ornamentais tropicais é o *Colletotrichum gloeosporioides* (LINS; COELHO 2004). Quando em condições de alta umidade, surgem corpos de frutificação chamados de acérvulos, com massas de conídios (OO et al., 2018). Após serem formados, estes conídios são dispersos na chuva, na mesma planta ou nas mais próximas, por insetos e pelo ar (GASPAROTO et al., 2017; JOSHI et al., 2018). A ocorrência da antracnose é favorecida por clima quente e úmido, ocasionando danos em campo (FERRARI, 2008; GURGEL et al., 2016; LINS; COELHO, 2004).

O gênero *Colletotrichum* foi descrito pela primeira vez por este nome através de revisão realizada por Corda (1831). Pertence ao filo Ascomycota, classe Sordariomycetes, ordem Glomerellales, família Glomerellaceae. O micélio é septado, conídios hialinos e unicelulares, reprodução assexuada (por meio de conídios produzidos em acérvulos). A colônia pode apresentar diferentes tonalidades, e pode ocorrer a presença de escleródios (SUTTON, 1992; WEIR; JOHNSTON; DAMM, 2012). O gênero *Colletotrichum* ocupa o oitavo lugar no ranking de fungos fitopatogênicos mais importantes do mundo (DEAN et al., 2012). Este gênero possui

grande importância por sua vasta gama de hospedeiros devido aos seus diferentes modos de colonização. Os principais modos de vida fitopatogênicos deste gênero são biotrófico, hemibiotrófico, endofítico e necrotrófico. Algumas espécies são específicas a um hospedeiro (MENEZES, 2006; DEAN et al., 2012).

Anterior a revisão de Corda (1831), o gênero era conhecido como *Vermicularia* (Tode, 1790), termo adotado para espécies de conídio curvado. Em 1837 o gênero *Colletotrichum* foi caracterizado por Corda pela morfologia dos conídios (hialinos, retos e fusiformes ou curvados). Algumas características taxonômicas como diferenças na estrutura e forma da conidiomata, presença ou ausência de seta marginal eram consideradas relevantes para separar espécies do gênero *Colletotrichum* de espécies do gênero *Vermicularia*. Como consequência muitas espécies passaram a ser consideradas pertencentes ao gênero *Colletotrichum* (DUKE, 1928). Até 1957 constavam 750 nomes de espécies no gênero *Colletotrichum*. No mesmo ano, Von Arx concluiu que as espécies de *Colletotrichum*, *Vermicularia* e *Gloeosporium* pertenciam ao gênero *Colletotrichum* considerando apenas características morfológicas, propondo então, a redução para 11 espécies deste gênero. Número este, que aumentou para 40 através do uso de um sistema de chave de identificação, baseada também por caracteres morfológicos e algumas considerações quanto à patogenicidade (SUTTON, 1980; 1992).

Os caracteres morfológicos foram por muito tempo utilizados como principal ou única forma de identificação de espécies. A diferenciação era realizada por meio de diagnóstico de características morfológicas, geográficas e ecológicas (MARIN-FELIX et al., 2017). A plasticidade apresentada por caracteres morfológicos utilizados na taxonomia convencional torna impossível o uso exclusivo destes para a identificação e delimitação de espécies de *Colletotrichum* (CAI et al., 2009; HYDE et al., 2009a; VIEIRA et al., 2017).

Considerações quanto a necessidade de outros critérios além dos morfológicos para a identificação e classificação das espécies do gênero *Colletotrichum* tornaram-se comuns. Em 1990, no primeiro International Workshop on *Colletotrichum*, realizado na Universidade de Bath, Reino Unido, a discussão quanto esta problemática já se mostrava presente entre pesquisadores da taxonomia e biologia molecular (CANNON et al., 2012). Novas ferramentas e técnicas de identificação através da comparação de ácidos nucleicos surgiram com o avanço nos estudos em biologia molecular, e foram considerados como úteis para elucidação de problemas de delimitação de espécies dentro deste gênero (BRUNS; WHITE; TAYLOR, 1991).

Os primeiros estudos filogenéticos dentro do gênero *Colletotrichum* (MILLS; SREENIVASAPRASAD; BROWN, 1992; SREENIVASAPRASAD; BROWN; MILLS, 1992) demonstraram eficiência na diferenciação de espécies utilizando a região ITS (Internal

Transcribed Spacer). Em 2002, foi publicada uma das primeiras análises multilocus, utilizando os genes TUB2 (β-tubulin) e HIS4 (Histone 4) (TALHINHAS et al., 2002), visto que o uso apenas da região ITS já demonstrava ser insuficiente. Posteriormente diversos estudos foram publicados com diferentes grupos de regiões gênicas, como tentativas de estabelecer metodologias (DAMM et al., 2009; PHOULIVONG et al., 2010). Em 2009, foi publicada uma revisão baseada no critério morfológico e na abordagem filogenética multilocus, resultando em 66 nomes de espécies de *Colletotrichum* e novamente foi enfatizada a necessidade do uso de métodos moleculares (HYDE et al., 2009a; 2009b). Em 2011, foram introduzidas mais 41 espécies ao gênero (CANNON et al., 2012).

De acordo com a última revisão feita para o gênero, existiam 188 espécies de *Colletotrichum* descritas (MARIN-FELIX et al., 2017) e uma parte destas estão distribuídas em onze complexos reconhecidos (8 em *C. caudatum* sensu lato, 14 em *C. graminicola* s. l., 9 em *C. spaethianum* s.l., 15 em *C.destructivum* s.l., 34 em *C. acutatum* s.l., 11 em *C. dematium* s.l., 6 em *C. gigasporum* s.l., 38 em *C. gloeosporioides* s.l., 19 em *C. boninense* s.l., 3 em *C. truncatum* s.l. e 8 em *C. orbiculare* s.l.). Em trabalhos posteriores, são relatadas novas espécies descritas e três novos clados propostos para representar novos complexos (*C. dracaenophilum* s.l., *C. magnum* s.l. e *C. orchidearum* s.l.) (CAO et al., 2019; DAMM et al., 2019).

Para o gênero *Colletotrichum*, complexos de espécies podem ser definidos como os principais grandes clados fortemente suportados na árvore do gênero *Colletotrichum*. Esses clados incluem espécies filogenéticas estreitamente relacionadas, as quais são indistinguíveis com base em caracteres fenotípicos (por exemplo, formato e tamanho de conídios e apressórios, taxa de crescimento, coloração das colônias), também denominadas espécies crípticas. Os complexos de espécies recebem o nome da espécie mais conhecida ou da que foi descrita primeiramente dentre as espécies do complexo. Em alguns casos, membros de um determinado complexo de espécies compartilham características peculiares referentes aos conídios: *C. acutatum* s. l. – conídios com extremidades afiladas; *C. boninense* s. l. – presença de uma cicatriz proeminente (hilo) na base do conídio; *C. caudatum* s. l. – conídios com apêndice filiforme no ápice; *C. gigasporum* s. l. – conídios com comprimento e largura extremamente grandes em comparação aos demais complexos de espécies (VIEIRA et al., 2020).

Atualmente, utiliza-se a filogenia multilocus para a diferenciação das diferentes espécies filogenéticas inseridas nos diversos complexos de espécies do gênero *Colletotrichum* (MARIN-FELIX et al., 2017). Vários trabalhos têm sido publicados nos últimos anos visando estabelecer um conjunto ideal de marcadores moleculares para identificação de espécies de *Colletotrichum*, em especial para o complexo *C. gloeosporioides* (CAI et al., 2009, SILVA et

al., 2012, VIEIRA et al., 2017). Mais recentemente, Vieira et al. (2020) publicaram um trabalho onde foram estabelecidos os melhores marcadores para a delimitação de espécies em 13 dos 14 complexos de espécies do gênero *Colletotrichum*. Entretanto, apesar de todos estes trabalhos publicados, pesquisadores de diferentes grupos de pesquisa parecem ignorar estes resultados. Em diversos trabalhos publicados recentemente, foram utilizados diferentes conjuntos de marcadores moleculares. Nestes trabalhos não são apresentadas justificativas para utilização dos referidos conjuntos de marcadores em substituição aos preestabelecidos na literatura.

Atualmente não existem muitos estudos disponíveis quanto a identificação de espécies de *Colletotrichum* associados ao bastão do imperador. Em 2009, foi publicado um trabalho com identificação de isolados de *C. gloeosporioides* em bastão do imperador (BARGUIL et al., 2009), no qual foi utilizada caracterização fenotípica dos isolados, em combinação com o sequenciamento do espaçador interno transcrito do DNA ribossomal (ITS). A região ITS também foi utilizada posteriormente para identificação de isolados de *Colletotrichum* associados a antracnose do bastão do imperador (GURGEL et al., 2016).

Os referidos estudos utilizando a região ITS para identificação de espécies de *Colletotrichum* associados ao bastão do imperador (BARGUIL et al., 2009; GURGEL et al., 2016) não foram utilizados marcadores informativos como os recomendados por Viera et al. (2020). Os resultados destes estudos são inconclusivos, uma vez que a identificação das espécies foi feita utilizando somente a região ITS, a qual não apresenta informatividade filogenética suficiente para discriminar espécies do gênero *Colletotrichum* (VIEIRA et al., 2020). Desta forma, identificação com marcadores moleculares para bastão do imperador faz-se necessário para que se possa ter mais informações quanto a diversidade nesse hospedeiro. Portanto, o objetivo do presente trabalho é caracterizar a diversidade e identificar as espécies filogenéticas do gênero *Colletotrichum* associadas à antracnose do bastão do imperador nos estados de Pernambuco e Ceará, por meio do uso de marcadores moleculares de maior precisão na identificação de espécies bem como caracterizá-las quanto a prevalência, e a patogenicidade e virulência.

REFERÊNCIAS BIBLIOGRÁFICAS

AIPH – INTERNATIONAL ASSOCIATION OF HORTICULTURAL PRODUCERS. Production and markets: the future of ornamentals. 2019. Disponível em: <http://aiph.org/international-statistics-flowers-and-plants-2019/>. Acesso em: 25 abr. 2020.

BARGUIL, B.M.; OLIVEIRA, S.M.A; COELHO, R.S.B.; BESERRA JUNIOR, J.E.A. Identificação e variabilidade genética de isolados de *Colletotrichum* causando antracnose em inflorescências de plantas ornamentais tropicais. **Ciência Rural**, Santa Maria, v. 39, n.6, p. 1639-1646, 2009.

BRAINER, M.S.C.P. **Quando nem tudo são flores a floricultura pode ser uma alternativa**. Fortaleza: Banco do Nordeste do Brasil, 2018. Disponível em:<https://www.bnb.gov.br/documents/80223/4049480/42_Flores_2018.pdf/022d87e8-c8db-1a98-b760-419661cf4e25>. Acesso em: 25 abr. 2020.

BRUNS, T.D.; WHITE, T.J.; TAYLOR, J.W. Fungal Molecular Systematics. **Annual Review of Ecology, Evolution, and Systematics**, v. 22, p. 525-564, 1991.

BUAINAIN, A.M.; BATALHA, M.O. **Cadeias produtivas de flores e mel. Brasília**: Instituto Interamericano de Cooperação para a Agricultura, 2007. 140 p.

CAI, L.; HYDE, K.D.; TAYLOR, P.W.J.; WEIR, B.S.; WALLER, J.M.; ABANG, M.M.; ZHANG, J.Z.; YANG, Y.L.; PHOULIVONG, S.; LIU, Z.Y.; PRIHASTUTI, H.; SHIVAS, R.G.; MCKENZIE, E.H.C.; JOHNSTON, P.R. A polyphasic approach for studying *Colletotrichum*. **Fungal Diversity**, Kunming, v. 39, p. 183-204, 2009.

CANNON, P.F.; DAMM, U.; JOHNSTON, P.R.; WEIR, B.S. *Colletotrichum* – current status and future directions. **Studies in Mycology**, Utrecht, v. 73, p. 181–213, 2012.

CAO, X.; XU, X.; CHE, H.; WEST, J.S.; LUO, D. Three *Colletotrichum* species, including a new species, are associated to leaf anthracnose of rubber tree in Hainan, China. **Plant Disease**, Saint Paul, v.103, n.1, p. 117–124, 2019.

CHAN, E.; LIM, Y.; TAN, S. Standardised herbal extract of chlorogenic acid from leaves of *Etlingera elatior* (Zingiberaceae). **Pharmacognosy Research**, v. 3, n. 3, p. 178, 2011.

CHAN, E.W.C.; LIM, Y.Y.; WONG, S.K. Phytochemistry and Pharmacological Properties of *Etlingera elatior*: A Review. **Pharmacognosy Journal**, v.3, n.22, p.6–10, 2011.

CORDA, A.C.I., 1831. Die Pilze Deutschlands. In: Deutschlands Flora in Abbildungen nach

der Natur mit Beschreibungen (Sturm, J, ed.). Sturm, Nürnberg. Vol. 3, Abt. 12: 33–64, tab. 21–32.

CORDA, A.C.I., 1837. Icones Fungorum hucusque cognitorum vol. 1. Calve, Prague.

DAMM, U.; SATO, T.; ALIZADEH, A.; GROENEWALD, J.Z.; CROUS, P.W. The *Colletotrichum dracaenophilum, C. magnum* and *C. orchidearum* species complexes. **Studies in Mycology**, Utrecht, v. 92, p. 1–46, 2019.

DAMM, U.; WOUDENBERG, J.H.C.; CANNON, P.F.; CROUS, P.W. *Colletotrichum* species with curved conidia from herbaceous hosts. **Fungal Diversity**, Kunming, v. 39, p. 45–87, 2009.

DE, L.C. Improvement of ornamental plants -a review. **International Journal of Horticulture**, v.7, n. 22, p.180–204, 2017.

DEAN, R.; VAN KAN, J.A.L.; PRETORIUS, Z.A.; HAMMOND-KOSACK, K.E.; DI PIETRO, A.; PIETRO, D.S.; RUDD, J.J.; DICKMAN, M.; KAHMANN, R.; ELLIS, J.; FOSTER, G.D. The Top 10 fungal pathogens in molecular plant pathology. **Molecular Plant Pathology**, Oxford, v. 13, n. 4, p. 414–430, 2012.

DUKE, M.M. The genera *Vermicularia* Fr. and *Colletotrichum* Cda. **Transactions of the British Mycological**, London, v.13, p. 156–184, 1928.

FERRARI, J.T. **Antracnose em Bastão do Imperador**. São Paulo: Instituto Biológico/APTA), 2008. p.1-6. (Documento Técnico 003).

FRANÇA, C.A.M; MAIA, M.B.R. Panorama do agronegócio de flores e plantas ornamentais no Brasil. In: CONGRESSO DA SOCIEDADE BRASILEIRA DE ECONOMIA, ADMINISTRAÇÃO E SOCIOLOGIA RURAL, 16. 2008. Rio Branco. **Anais Eletrônico…** Rio Branco: SOBER, 2008.

GASPAROTO, M.C.G.; LOURENÇO, S.A.; TANAKAA, F.A.O.; SPÓSITO, M.B.; MARCHINIC, L.C.; SILVA JUNIOR, G.J.; AMORIM, L. Honeybees can spread *Colletotrichum acutatum* and *C. gloeosporioides* among citrus plants. **Plant Pathology**, Hoboken: Wiley, v. 66, n. 5, p. 777-782, 2017.

GONÇALVES, C.; CASTRO, C.E.F. Bastão-do-Imperador. In: AGUIAR, A.T.E.; GONÇALVES, C.; PATERNIANI, M.E.G.Z.; TUCCI, M.L.; CASTRO, C.E.F. **Instruções**

agrícolas para as principais culturas econômicas – Boletim 200. 7ed. Campinas: IAC, 2014. v. 200, p. 313-316.

GURGEL, L.M.S.; COELHO, R.S.B.; OLIVEIRA, S.M.A.De.; SILVA, R.L.X.DA.; ROSA, R.C.T.Da.; ASSIS, T.C.De.; ANDRADE, D.E.G.T.de. Efeito de indutores de resistência no controle da antracnose do bastão do imperador (*Etlingera elatior*). **Anais da Academia Pernambucana de Ciência Agronômica**, Recife, v. 13, p. 223-233, 2016.

HYDE, K.D.; CAI, L.; CANNON, P.F.; CROUCH, J.A.; CROUS, P.W.; DAMM, U.; GOODWIN, P.H.; CHEN, H.; CHEN, H.; JOHNSTON, P.R.; JONES, E.B.G.; LIU, Z.Y.; MCKENZIE, E.H.C.; MORIWAKI, J.; NOIREUNG, P.; PENNYCOOK, S.R.; PFENNING, L.H.; PRIHASTUTI, H.; SATO, T.; SHIVAS, R.G.; TAN, Y.P.; TAYLOR, P.W.J.; WEIR, B.S.; YANG, Y.L.; ZHANG, J.Z. *Colletotrichum*—names in current use. **Fungal Diversity**, Kunming, v. 39, n. 1, p. 147-182, 2009a.

HYDE, K.D.; CAI, L.; MCKENZIE, E.H.C.; YANG, Y.L.; ZHANG, J.Z.; PRIHASTUTI, H. *Colletotrichum*: a catalogue of confusion. **Fungal Diversity**, Kunming, v. 39, p. 1-17, 2009b.

IBRAFLOR - INSTITUTO BRASILEIRO DE FLORICULTURA. Mercado Interno 12.2014. Holambra, IBRAFLOR, 2015. Disponível em: <http://www.ibraflor.com/publicacoes/vw.php?cod=234>. Acesso em: 20 abr. 2020.

JOSHI, R. A Review on *Colletotrichum* spp. Virulence mechanism against host plant defensive factors. **Journal of Medicinal Plants Studies**, v. 6, n. 6, p. 64-67, 2018.

JUNQUEIRA, A.H.; PEETZ, M.S. Sustainability in Brazilian floriculture: introductory notes to a systemic approach. **Ornamental Horticulture**, São Paulo, v. 24, n. 2, p. 155-162, 2018.

JUWITA, T.; PUSPITASARI, I.M.; LEVITA, J. Torch ginger (*Etlingera elatior*): A review on its botanical aspects, phytoconstituents and pharmacological activities. **Pakistan Journal of Biological Sciences**, v. 21, n. 4, p. 151-165, 2018.

KULIGOWSKA, K.; LÜTKEN, H.; MÜLLER, R. Towards development of new ornamental plants: status and progress in wide hybridization. **Planta**, New York, v. 244, n. 1, p. 1-17, 2016.

KUMAR, P.; SINGH, P. Conservation assessment of two rare gingers (Zingiberaceae) from Dampa Tiger Reserve, Mizoram, India. **Journal of Threatened Taxa**, v. 10, n. 5, p. 11599-

11605, 2018.

LINS, S.R.O.; COELHO, R.S.B. Antracnose em inflorescências de bastão do imperador (*Etlingera elatior*): ocorrência e métodos de inoculação. **Summa Phytopathologica**, Botucatu, v.29, n.4, p.355-358, 2003.

LINS, S.R.O.; COELHO, R.S.B. Ocorrência de doenças em plantas ornamentais tropicais no Estado de Pernambuco. **Fitopatologia Brasileira**, Brasília, v. 29, p. 332-335, 2004.

LOGES, V.; CASTRO, A.C.R. de; MARTINS, L.S.S.; WILLADINO, L.G.; TEXEIRA, M.C.F.; LIRA JUNIOR, M.A.; PINHEIRO, P.G.L.; SOUZA, J.W.O.; MELO, M.G. Floriculture research in Pernambuco. **Acta Horticulturae**, Brussels, v. 683, p. 345-350, 2005.

LOGES, V.; COSTA, A.S.; GUIMARÃES, W.N.R.; TEIXEIRA, M.C.F. Potencial de mercado de Bastão-do-imperador. **Revista Brasileira de Horticultura Ornamental**, Campinas, v. 14, n. 1, p. 15-22, 2008.

MARIN-FELIX, Y.; GROENEWALD, J.Z.; CAI, L.; CHEN, Q.; MARINCOWITZ, S.; BARNES, I.; BENSCH, K.; BRAUN, U.; CAMPORESI, E.; DAMM, U.; DE BEER, Z.W.; DISSANAYAKE, A.; EDWARDS, J.; GIRALDO, A.; HERNÁNDEZ-RESTREPO, M.; HYDE, K.D.; JAYAWARDENA, R.S.; LOMBARD, L.; LUANGSA-ARD, J.; MCTAGGART, A.R.; ROSSMAN, A.Y.; SANDOVAL-DENIS, M.; SHEN, M.; SHIVAS, R..G; TAN, Y.P.; VAN DER LINDE, E.J.; WINGFIELD, M.J.; WOOD, A.R.; ZHANG, J.Q.; ZHANG, Y.; CROUS, P.W. Genera of phytopathogenic fungi: GOPHY 1. **Studies in. Mycology**, Utrecht, v. 86, p. 99-216, 2017.

MENEZES, M. Aspectos biológicos e taxonômicos de espécies do gênero *Colletotrichum*. **Anais da Academia Pernambucana de Ciência Agronômica**, Recife, v. 3, p.170-179, 2006.

MILLS, P.R.; SREENIVASAPRASAD, S.; BROWN, A.E. Detection and differentiation of *Colletotrichum gloeosporioides* isolates using PCR. **FEMS Microbiology Letters**, Amsterdam, v. 98, p. 137-143, 1992.

MOHAMAD, S.; KALU, M. Assessment of Zingiberaceae (Tribe *Alpinieae*) from North East Sarawak, Malaysia. In: INTERNATIONAL CONFERENCE ON BIODIVERSITY. **IOP Conf. Series: Earth and Environmental Science**, 1. 2019, Bristol. **Anais**… Bristol: Institute of Physics, 2019. v. 269.

OO, M.M.; YOON, H-Y.; JANG, H.A.; OH, S.K. Identification and Characterization of *Colletotrichum* Species Associated with Bitter Rot Disease of Apple in South Korea. **Plant Pathology Journal**, v. 34, n. 6, p. 480-489, 2018.

PHOULIVONG, S.; CAI, L.; CHEN, H., MCKENZIE, E.H.C.; ABDELSALAM, K.; CHUKEATIROTE, E.; HYDE, K.D. *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. **Fungal Diversity**, Kunming, v. 44, p. 33-43, 2010.

SAENSOUK, S.; SAENSOUK, P.; PASORN, P.; CHANTARANOTHAIC, P. Diversity and uses of Zingiberaceae in Nam Nao National Park, Chaiyaphum and Phetchabun provinces, Thailand, with a new record for Thailand. **Agriculture and Natural Resources**, Bangkok, v.50, p. 445-453, 2016.

SEBRAE–SERVIÇO BRASILEIRO DE APOIO ÀS MICRO E PEQUENAS EMPRESAS. **Flores e plantas ornamentais do Brasil**: volume 1 -o mercado brasileiro de flores e plantas ornamentais. Brasília, DF: SEBRAE, 2015. Disponível em: <http://www.bibliotecas.sebrae.com.br/chronus/ARQUIVOS_CHRONUS/bds/bds.nsf/7ed114f4eace9ea970dadf63bc8baa29/$File/5518.pdf>. Acesso em: 19 abr. 2020.

SILVA, D.N.; TALHINHAS, P.; VÁRZEA, V.; CAI, L.; PAULO, O.S.; BATISTA, D. Application of the Apn2/MAT locus to improve the systematics of *the Colletotrichum gloeosporioides* complex: an example from coffee (*Coffea* spp.) hosts. **Mycologia**, Bronx, v. 104, p. 396-409, 2012.

SILVA, S.S.L.; FERREIRA, I.V.S.; SILVA, F.C.C.; MUSSER, R.S.; MELO, M.G.P.; LOGES, V. Cut flowers and leaves farmer market in Recife-PE, Brazil. **Acta Horticulturae**, Brussels, v. 1, p. 233-236, 2018.

SIVIERO, A.; DELUNARDO, T.A.; HAVERROTH, M.; OLIVEIRA, L.C. DE; ROMAN, A.L.C.; MENDONÇA, Â.M.S. Plantas ornamentais em quintais urbanos de Rio Branco, **Boletim do Museu Paraense Emílio Goeldi**, Belém, v. 9, n. 3, p. 797-813, 2014.

SREENIVASAPRASAD, S.; BROWN, A.E.; MILLS, P.R. DNA sequence variation and inter- relationship among *Colletotrichum* species causing strawberry anthracnose. **Physiological and Molecular Plant Pathology**, London, v. 41, p. 265-281, 1992.

SU, J.; JIANG, J.; ZHANG, F.; LIU, Y.; DING, L.; CHEN, S.; CHEN, F. Current achievements and future prospects in the genetic breeding of chrysanthemum: a review.

Horticulture Research, v. 6, n. 109, 2019.

SUNGTHONG, B.; SRICHAIKUL, B. Antioxidant Activities, Acute Toxicity and Chemical Profiling of Torch Ginger (*Etlingera elatior* Jack.) Inflorescent Extract. **Pharmacognosy Journal**, v. 10, n. 5, p. 979-82, 2018.

SUTTON, B.C. **The Coelomycetes. Fungi imperfecti with pycnidia acervuli and stromata**. Kew: Commonwealth Mycological Institute, 1980. 696 p.

SUTTON, B. C. **The genus *Glomerella* and its anamorph *Colletotrichum***. In: BAILEY, J.A.; JEGER, M.J. ***Colletotrichum* – Biology, Pathology and Control**. Wallingford: CAB International, 1992. p. 1-26.

TALHINHAS, P.; SREENIVASAPRASAD, S.; NEVES-MARTINS, J.; OLIVEIRA, H. Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupins. **Phytopathology**, Saint Paul, v. 92, p. 986-996, 2002.

TODE, H.J. Fungi Mecklenburgenses Selecti, I. 1790.

TPL–THE PLANT LIST. Version 1.1. 2013. Disponível em: <http://www.theplantlist.org/>. Acesso em: 20 abr. 2020.

UD-DAULA, A.F.M.S.; BASHER, M.A. Genus *Etlingera* - A review on chemical composition and antimicrobial activity of essential oils. **Journal of Medicinal Plants Research**, v. 13, n.7, p.135-156, 2019.

UN– UNITED NATIONS. International Trade Statistics Yearbook 2018. New York; United Nations, 2019. Disponível em: <https://comtrade.un.org/pb/downloads/2018/VolII2018.pdf>. Acesso em 15 abr. 2020.

VIEIRA, W.A.S.; LIMA, W.G.; NASCIMENTO, E.S.; MICHEREFF, S.J.; CÂMARA, M.P.S.; DOYLE, V.P. The impact of phenotypic and molecular data on the inference of *Colletotrichum* diversity associated with *Musa*. **Mycologia**, Bronx, v. 109, p. 912-934, 2017.

VIEIRA, W.A.S.; BEZERRA, P.A.; SILVA, A.C.; VELOSO, J.S.; CÂMARA, M.P.S.; DOYLE, V.P. Optimal markers for the identification of *Colletotrichum* species. Molecular Phylogenetics and Evolution, v. 143, p.106694, 2020.

WCSP. 'World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew, 2020. Disponível em: <http://wcsp.science.kew.org>. Acesso em: 20 abr.

2020.

WEIR, B.S.; JOHNSTON, P.R.; DAMM, U. The *Colletotrichum* gloeosporioides species complex. Studies in Mycology, v. 73, p.115–180, 2012.

WU, D.; LARSEN, K. Zingiberaceae. Flora of China, v. 24, p.322–377, 2000.

CAPÍTULO II

Diversity of *Colletotrichum* species associated with torch ginger

Diversity of *Colletotrichum* species associated with torch ginger

I.G. Duarte[1], W.A.S. Vieira[1], J.S. Veloso[1], A.G.G. Amara[1], A.C. Silva[1], V.Q. Balbino[2], V.P. Doyle[3], M.P.S. Câmara[1]

[1] Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Recife, 52171-900, PE, Brazil; corresponding author e-mail: marcos.camara@ufrpe.br.
[2] Departamento de Genética, Universidade Federal de Pernambuco, Recife, 50670-901, PE, Brazil.
[3] Department of Plant Pathology and Crop Physiology, Louisiana State University AgCenter, Baton Rouge, Louisiana, United States of America.

ABSTRACT

Anthracnose caused by *Colletotrichum* species is one of the most important diseases of torch ginger. The disease leads to loss of aesthetic and commercial value of torch ginger stems. This study aimed to characterize *Colletotrichum* spp. associated with torch ginger anthracnose production areas of Pernambuco and Ceará. A total of 48 *Colletotrichum* isolates were identified using molecular techniques. Pathogenicity tests were performed on torch ginger with representative isolates. Phylogenetic analyses based on seven loci (APN2, APN2/MAT-IGS, CAL, GAPDH, GAP2-IGS, GS and TUB2) revealed that they belong to 5 known *Colletotrichum* species, including *C. crysophilum*, *C. fructicola*, *C. siamense*, *C. theobromicola*, *C. tropicale*, and three new species, described here as *C. atlanticis*, *C. floscerae* and *C. zingibericola*. Of these, *C. atlanticis* was the most dominant. Pathogenicity assays showed that all isolates were pathogenic to torch ginger bracts. All species are reported for the first time associated with torch ginger in Brazil. The present study contributes to the current understanding of the diversity of *Colletotrichum* species associated with anthracnose on torch ginger and demonstrate the importance of accurate species identification for effective disease management strategies.

Keywords: *Colletotrichum gloeosporioides*, *Etlingera*, aggressiveness.

INTRODUCTION

Cultivation of flowers has world economic importance since it is explored in several countries. The development of flower cultivation in Brazil was highly influenced by Japanese and Dutch immigration, especially in the state of São Paulo. Emergence of cooperatives in the

country initially served as a new source of income for small producers and to supply local markets. Since 70's, cultivated floriculture industry in Brazil has increased becoming an agricultural activity in expressive grown. In 2015, R$ 4.5 billion, and financial transactions of R$ 10.2 billion were generated from the production to the final sale of floriculture products (Neves & Pinto 2015). The growth of floriculture in Brazil is due to factors such as the variety of species, weather conditions and improvements in infrastructure and logistics (Neves & Pinto 2015; SEBRAE 2015).

Among the tropical species cultivated in the country, the torch ginger (*Etlingera elatior* (Jack) R.M.Smith) is favored by the characteristics of humidity and temperature of Northeast Brazil (Loges et al. 2005) and the production in Pernambuco stand out in the Northeast. However, the climate conditions which favor cultivation also increase the disease occurrence, especially in commercial parts of the plant (Lins & Coelho 2004).

Anthracnose caused by *Colletotrichum* is the most important disease of torch ginger, causing significant yield loss to the crop. Symptoms appear mainly in the floral stem, compromising the quality of the inflorescences. Initially, spots appear on the outer bracts and then progress to the inner bracts as water-soaked lesions. Lesions are dark brown, depressed and rounded, and bracts become dry when disease progress (Lins & Coelho 2003; Ferrari 2008).

The genus *Colletotrichum* is present worldwide and includes both pathogenic and non-pathogenic species (Cannon et al. 2012; Hyde et al. 2009; Prihastuti et al. 2009; Vieira et al. 2014). This genus has more than 200 accepted species (Marin-Felix et al. 2017; Cao et al. 2019; Damm et al. 2019). The number of species increase through time due to the different criteria that were considered for the description of species. Initially, only morphological characteristics were used as diagnostic character for species (Duke 1928; Hyde et al. 2009; Cai et al. 2009). Later, host range and pathogenicity criteria were added (Sutton 1980; 1992). These criteria proved to be insufficient and new approaches were developed for the identification, characterization, and delimitation of species within the genus.

A polyphasic approach was proposed for the identification of species of the *Colletotrichum* genus (Cai et al. 2009). This approach uses different data (molecular, morphological, physiological, pathogenic, environmental and others), which allow a more reliable identification. Multilocus phylogeny has become the standard, and many studies emerged about the informativity of different markers.

Characterization and identification of the etiologic agent of a disease is important for the management of a disease. Torch ginger inflorescence anthracnose has been associated with *C. gloeosporioides* (Lins & Coelho, 2004; Barguil et al. 2009) in Brazil, using species-specific

oligonucleotides CgInt and CaInt2, along with partial sequencing of the ITS region (Internal Transcribed Spacer) (Barguil et al. 2009; Gurgel et al. 2016). The results of both studies showed only the species of *C. gloeosporioides* as the etiological agent of anthracnose of torch ginger in Brazil. However, it is known that these markers are not efficient to precisely identify *Colletotrichum* species, and the results of these studies are not conclusive.

Therefore, it is crucial to carry out studies using more accurate molecular markers to identify *Colletotrichum* species associated with torch ginger. Thus, the aim of the present study was: i) to identify *Colletotrichum* species associated with torch ginger anthracnose in Northeast Brazil; ii) to evaluate the *Colletotrichum* species prevalence; iii) and to compare the aggressiveness of *Colletotrichum* species in torch ginger bracts.

MATERIAL AND METHODS

Sampling and fungal isolation

Cultivated torch ginger plants were collected from two fields from Brazilian states between February and June 2017: Pernambuco (Cabo de Santo Agostinho, Gravatá and Paulista municipalities) and Ceará (Guaramiranga and Pacoti). Twelve floral stems presenting typical anthracnose symptoms were collected in each area with a minimum of 10 meters between sampled plant. Samples were returned to lab, washed on running water and air dried prior to isolation. *Colletotrichum* isolates were obtained via indirect isolation from symptoms in bracts. Fragments from the margin between necrotic and healthy tissues were surface disinfested in 70% ethanol for 30 s, 1.5% sodium hypochlorite for 2 min, rinsed three times with sterile distilled water (SDW) and air dried. Fragments were plated onto potato dextrose agar medium (PDA, Merck) amended with 0.5 g L-1 streptomycin sulfate. Plates were incubated at 25 ± 2 °C under 12 h light period and inspect daily. Colonies with phenotypical characteristics similar with *Colletotrichum* (Sutton 1980) were grown on pure culture. Isolates were preserved in cryogenic tubes containing SDW and deposited in the working collection of “Laboratório de Micologia” (LM) at Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil. Holotypes from novel species were deposited on “Herbário do Departamento de Biologia Vegetal” (VIC) herbarium, and ex-types on “Coleção Octávio de Almeida Drummond” (COAD) culture colection.

DNA extraction, PCR, sequencing

Colletotrichum isolates were grown on PDA at 25 ± 2 °C for 7 day and a 12 h light. Mycelia were scraped from cultures and the genomic DNA extracted using CTAB (cetyltrimethylammonium bromide) protocol described by Doyle & Doyle (1990) with minor changes.

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) partial region was amplified for all isolates to estimate haplotype diversity among the isolates with DnaSP v5 (Librado & Rozas 2009). Up to two representative isolates from each haplotype were randomly chosen for the multilocus analysis. GAPDH sequences were compared to GenBank sequences using BLAST to identify from which *Colletotrichum* species complex the isolates belong. Since the BLAST indicates that all isolates presented high similarity with species from *C. gloeosporioides* species complex, the following remaining locus were amplified for multilocus analyses: DNA lyase (APN2), intergenic spacer between DNA lyase and the mating-type locus MAT1-2-1 (APN2/MAT-IGS), calmodulin (CAL), intergenic spacer between GAPDH and a hypothetical protein (GAP2-IGS), glutamine synthetase (GS) and β-tubulin (TUB2). These genomic regions are reported as the most informative to precisely identify species within *C. gloeosporioides* species complex (Vieira et al. 2020). Primers used in the present study are listed above.

The APN2 region was amplified and sequenced using primers CgDL_R1 and ColDL_F3 (Rojas et al. 2010); APN2/MAT-IGS with CgDL_F6 (Rojas et al. 2010) and AMR (Silva et al. 2012); CAL with CL1C and CL2C (Weir et al. 2012); GAPDH with GAP-95 and GAP-1174, GAP2-IGS with GAP-1041 and GAP/IGS-2044, and GS with GS-64F and GS-967R (Vieira et al. 2017); and TUB2 with T1 and T22 (O'Donnell & Cigelnik 1997). PCR amplifications were performed in a 12.5 μL volume reaction containing 4 μL PCR-grade water, 1 μL DNA template, 0.625 μL of each primer (10 μM), and 6.25 μL of PCR master mix (2x) (Promega GoTaq Master Mix; Madison, Wisconsin, USA).

PCR reactions were carried out following the cycling parameters: APN2 and APN2/MAT-IGS – initial denaturing for 3 min at 95 C, followed by 35 cycles of 95 C for 30 s, 62 C for 45 s, and 72 C for 1 min, followed by a final extension at 95 C for 30 s; CAL – initial denaturing for 5 min at 95 C, followed by 35 cycles of 95 C for 30 s, 57 C for 30 s, and 72 C for 1 min, followed by a final extension at 72 C for 10 min; GS – initial denaturing for 3 min at 95 C, followed by 35 cycles of 95 C for 30 s, 57 C for 45 s, and 72 C for 1 min, followed by a final extension at 72 C for 10 min; GAPDH and GAP2-IGS – initial denaturing for 5 min at 95 C, followed by 35 cycles of 95 C for 30 s, 56 C for 1 min, and 72 C for 1 min 30 s, followed by

a final extension at 72 C for 10 min; and TUB2 – initial denaturing for 5 min at 95 C, followed by 35 cycles of 95 C for 30 s, 55 C for 1 min, and 72 C for 1 min and 30 s, followed by a final extension at 72 C for 10 min.

PCR products were purified and sequenced on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, California, USA) on DNA sequencing platform located at Laboratório de Bioinformática e Biologia Evolutiva – LABBE from Universidade Federal de Pernambuco (Pernambuco, Recife, Brazil). Sequence reads were assembled into contigs and edited using the Staden package (Staden et al. 1998). Sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

Sequences of *Colletotrichum* ex-type and reference isolates from previous studies were retrieved from GenBank and included in phylogenetic analyses. Multiple sequence alignments (MSA), uncertainty estimation and alignment filtering were performed on GUIDANCE2 server (http://guidance.tau.ac.il/ver2/) (Sela et al. 2015). Unaligned sequences (no gaps) were built on MEGA 7 (Kumar et al., 2016), uploaded on GUIDANCE2 server, and alignment confidence scores were calculated under the following parameters: MAFFT as the MSA algorithm; max-iterate=1000; pairwise alignment method=global pair; 100 bootstrap replicates. Unreliable alignment regions were filtered by masking residues with scores below the lowest cutoff as proposed by Vieira et al. (2017).

Phylogeny for each individual locus and concatenated alignments was inferred using Maximum Likelihood analyses (ML) and Bayesian Inference (BI). MSAs were converted, concatenated, and partitioned in multilocus matrices using SequenceMatrix 1.8 (Vaidya et al. 2011). ML and BI analyses were performed on RAXML-HCP2 v.7.0.4 (Stamatakis 2014) and MrBayes v 3.2.1 (Ronquist et al. 2012) respectively, both implemented on the CIPRES Science Gateway (https://www.phylo.org/portal2/home.action).

ML analyses were performed with 1000 bootstrap pseudoreplicates under the GTR-GAMMA model (-m GTRGAMMA -p 12345 -k -f a -N 1000 -x 12345). Bayesian inferences were performed using MrBayes 3.2.6 (Ronquist et al. 2012) also implemented on the CIPRES Science Gateway. Analyses were conducted using the best-fit models of nucleotide substitution selected according to AICc by MrModeltest 2.3 (Nylander 2004). Four runs were conducted with four Markov chain Monte-Carlo (MCMC) search chains per run for 107 generations, sampling every 1000 generations. Convergence of all parameters was checked in the “pstat” file (Estimated Sample Size – EES ≥ 100, and Potential Scale Reduction Factor – PSRF = 1),

and posterior probabilities were calculated after discarding the first 25% of generations as burn-in. Clades were considered well supported when ML bootstrap support ≥ 70 and BI posterior probability ≥ 0.95.

Species recognition

Genealogical Concordance Phylogenetic Species Recognition – GCPSR approach was applied to identify independent phylogenetic lineage (Dettman et al. 2003; Taylor et al. 2000). A clade is considered an independent phylogenetic lineage if at least one of the two GCPSR criteria is satisfied: genealogical concordance criterion – the clade is present in most of individual gene trees; genealogical non-discordance criterion – the clade is strongly supported in at least one of the individual gene trees in both ML and BI analysis, and not contradicted in any other individual gene tree at the same level of support.

Novel species were accepted if the clade: i) was recognized as an independent phylogenetic lineage as described above; ii) present significant support in both ML and BI multilocus analyses; iii) does not contain the type of any previously described species.

Morphological characterization

For the phenotypical characterization of novel species, isolates were firstly grown on corn meal agar (CMA – 30 g of corn meal and 20 g of agar per liter) during 7 days at 25 ± 2 ºC and 12 h light period. Five millimeters plugs were taken from the edge of colonies grown on CMA and transferred to the center of 100 × 15 mm polystyrene Petri dishes with PDA. Plates were incubated in the same conditions described above. Radial measurements were taken from the edge of the plug to the margin of the colony every 48 hours across 6 days and used to calculate the mycelial growth rate (mm/day). The cultures were maintained in the same incubation conditions and the colony features were recorded from 7-day-old colonies. Rayner's chart (1970) was followed for colonies colors.

Conidiogenous cells, conidiophores and setae from PDA, and conidia from PDA and CMA were mounted in 10% lactic for microscopic observation. Appressoria were induced using CMA slide culture technique (Johnston & Jones 1997) with minor changes: 25 µL of 104 conidia mL-1 conidial suspension was deposited onto 10 mm2 squares of CMA placed on a sterile microscope slide, covered with a sterile coverslip, and incubated at 25°C in the dark during 24h. Microscopic images were made with a DS-L3 digital camera attached to a Nikon Eclipse Ni-U transmitted light microscope.

Summary statistics were calculated in Statistix 10 (Statistix 2013). Measurements are

presented as follow: lower extreme – upper extreme (average ± standard deviation) for growth rates; (lower extreme-) 1st quartile – average – 3rd quartile (-upper extreme) (average ± standard deviation) for conidiophores, conidiogenous cells, setae, conidia and appressoria.

Prevalence of *Colletotrichum* species

Prevalence of *Colletotrichum* species in torch ginger were established using the following equation: P (%) = (Nx / Nt) × 100, where P = prevalence, Nx = number of isolates of the same species and Nt = total number of isolates.

Pathogenicity and aggressiveness assay

Pathogenicity tests were carried out on inflorescences of torch ginger. Inflorescences were washed in water and surface sterilized in 1.5% sodium hypochlorite for 3 min, rinsed two times in sterile distilled water, and air dried. Ten microliters of conidial suspension (10^6 conidia mL^{-1}) were deposited onto the wounds point on the surface of bracts. The negative control was represented by bracts inoculated with 10 μL of sterile distilled water. Inflorescences were kept in a humid chamber during 48 h under 25 °C and a light period of 12 h. The humid chamber was removed after 48 h, and the inflorescences were kept under the same conditions.

Pathogenicity and aggressiveness were accessed 5 days after inoculation. Pathogenicity was confirmed by the presence of typical torch ginger anthracnose lesions. Aggressiveness was accessed by measuring orthogonal diameter of. The experiment was conducted with four replicates, being each replicate represented by one inflorescence with eight bracts inoculated. The experiment was repeated once.

Differences in aggressiveness were analyzed by one-way analysis of variance – ANOVA, and means were compared with Tukey's range test (p=0.05) using the program Statistix 10 (Statistix 2013).

RESULTS

Sampling and fungal isolation

A total of 48 *Colletotrichum* isolates were obtained from typical anthracnose lesions on torch ginger: 7 isolates were obtained from Ceará (6 from Guaramiranga and 1 from Pacoti) and 41 from Pernambuco (1 from Cabo de Santo Agostinho, 18 from Gravatá and 22 from Paulista).

Phylogenetic analyses and species assignment

The GAPDH sequences revealed a total of 11 haplotypes among *Colletotrichum* isolates from torch ginger. Sixteen representative isolates were randomly chosen for further multilocus analysis. Isolates from torch ginger were distributed in eight main clades according to the multilocus analysis (Fig. 1). All species were recognized as independent phylogenetic lineages according to GCPSR approach, fulfilling both concordance and non-discordance criteria.

Three haplotypes were identified as *C. siamense*, two haplotypes as *C tropicale*, and one haplotype as *C. theobromicola*. The three species presented maximum support in both multilocus analyses. *Colletotrichum tropicale* was recovered as monophyletic in all individual gene trees. Most markers recovered *C. theobromicola* as monophyletic, in exception of TUB2 which grouped *C. theobromicola* and *C. grevilleae* in the same lineage. *Colletotrichum siamense* was not recovered as monophyletic only in GAPDH and GS trees.

Two haplotypes were separately placed in *C. fructicola* and *C. chrysophilum* clade. Both species were strongly supported in multilocus analyses. All seven loci separate *C. chrysophilum* from *C. fructicola*, however *C. chrysophilum* was recovered as paraphyletic in APN2/MAT-IGS tree.

Finally, the tree remaining haplotypes comprised three sister clades with maximum support in both ML and BI. The clades do not contain any species previously described, and each clade represent different independent phylogenetic lineages. Thus, we are describing the novel species *C. atlanticis sp. nov.*, *C. floscerae sp. nov.* and *C. zingibericola sp. nov.*. Details about species description and genetic identification are presented in the Taxonomy section.

Based on initial genetic diversity recovered by GAPDH sequences, it was the distribution of *Colletotrichum* isolates from torch ginger anthracnose: 16 isolates of *C. atlanticis sp. nov.*, 7 isolates of *C. chrysophilum*, 2 isolates of *C. floscerae sp. nov.*, 2 isolates of *C. fructicola*, 10 isolates of *C. siamense*, 4 isolates of *C. theobromicola*, 5 isolates of *C. tropicale* and 2 *C. zingibericola sp. nov.*.

TAXONOMY

Colletotrichum atlanticis I.G. Duarte, W.A.S. Vieira & M.P.S. Câmara, *sp. nov.* – Mycobank _____; Fig. 2.

Etymology. Name refers to Atlantic Forest, where it was collected.

Typus. Brazil, Pernambuco, Paulista City, from anthracnose lesions on *Etlingera elatior* bracts,

17 April 2017, J. S. Veloso (holotype VIC47502,
culture ex-type COAD3338 = LM938).

Sexual morph not observed. *Sclerotia* absent. *Acervuli* abundant, covered with orange mucilage. *Setae* brown, smooth-walled, septate, base truncate, tip ± acute, (35.3–) 36.3– 40.3–44 (45.9) ´ (2.4) 2.8– 3.6–4 (4.2) μm (40.2 ± 4.2)´ 3.4 ± 0.7) μm, L/W ratio = 11.9, (n = 5). *Conidiophores* hyaline, smooth-walled, septate, single, sometimes branched. *Conidiogenous* cells hyaline, smooth-walled, cylindrical to ampulliform, monophialidic. *Conidia* on PDA one-celled, smooth-walled, hyaline, cylindrical with rounded ends, sometimes oblong, contents appearing granular, (9.7) 10.6– 11.3–12 (–13.1) ´ (2.7) 2.9– 3–3.3 (–3.9) μm, (11.3 ± 0.8 ´ 3.1 ± 0.3 μm), L/W ratio = 3.7, (n = 30). *Appressoria* in slide cultures, single, medium to dark brown smooth-walled, clavate, with undulate margin, (5.5) 6.5– 7.2–8.3 (–12.3) ´ (3.8) 5.1 – 5.7 –6.9 (–8.8) μm, (7.6 ± 1.7 ´ 6 ± 1.3 μm), L/W ratio=1.4, (n = 30).

Culture characteristics — Colonies on PDA with surface mycelium flattened with entire margin, pale vinaceous margin from aerial view. Reverse apricot in the center, become fawn, buff towards edge. Growth rate at 25ºC 3.8–5.1 mm.dia-1 (4.6 ± 0.4 mm.dia-1).

Genetic identification — *Colletotrichum atlanticis* is recovered as monophyletic in all individual gene trees.

Notes — *Colletotrichum atlanticis* is closely related to the sister species *C. floscerae* and *C. zingibericola*. However, there is no conflict among the individual gene trees and the species can be easily identified as an independent phylogenetic lineage by all markers. *C. atlanticis* presented lower growth rate than *C. floscerae* (3.8–5.1 vs. 4.4–5.3 mm.dia^{-1}), and higher than *C. zingibericola* (3.8–5.1 vs. 1.3–3.1 mm.dia^{-1}). *C. atlanticis* presenter larger conidia on PDA (9.7–13.1 μm) than *C. floscerae* (8.9–11.5 μm) and *C. zingibericola* (8.6–13.1 μm).

Colletotrichum floscerae I.G. Duarte, W.A.S. Vieira & M.P.S. Câmara, *sp. nov.* – Mycobank _____; Fig. 3.

Etymology. The specific epithet refers to one of common names of the host *Etlingera elatior*, Philippine wax flower (from the Latin flos-, meaning flower, and –cera, meaning wax).

Typus. Brazil, Pernambuco, Paulista City, from anthracnose lesions on *Etlingera elatior* bracts, 16 February 2017, D. L. Neves (holotype VIC47501,
culture ex-type COAD3337= LM916).

Sexual morph not observed. *Sclerotia* absent. *Acervuli* abundant, covered with orange mucilage. *Setae* short, brown, smooth-walled, aseptate, base truncate, tip ± acute, (7.9–) 9.6-10.2-10.9 (–12) ´ (2.1–)2.7–2.8–3(–3.3) µm (10.2 ± 1 ´ 2.8 ± 0.2) µm, L/W ratio = 3.6, (n = 5). *Conidiophores* hyaline, smooth-walled, septate, unbranched. *Conidiogenous cells* hyaline, smooth-walled, cylindrical to ampulliform, monophialidic. *Conidia* on PDA one-celled, smooth-walled, hyaline, cylindrical with rounded ends, sometimes oblong, contents appearing granular, (8.4–)9.5–9.9–10.8(–11.4) ´ (2.7–)3.1–3.4–3.7(–3.9) µm, (10.1 ± 0.8 ´ 3.3 ± 0.3 µm), L/W ratio = 3.5, (n = 30). *Appressoria* in slide cultures, single, medium to dark brown smooth-walled, clavate, sometimes with undulate margin, (7.2–)8.5–9.4–10.4(–16.8) ´ (3.1–) 3.9–4.2–4.6(–5.4) µm, (9.8 ± 1.9 ´ 4.2 ± 0.5 µm), L/W ratio = 2.3, (n = 30).

Culture characteristics — Colonies on PDA with surface mycelium flattened, apricot in the center, became pale mouse grey, with colorless margin from aerial view. Reverse cinamon in the center, become mouse grey, buff towards edge. Growth rate at 25ºC 4.4–5.3 mm.dia^{-1} (4.9 ± 0.4 mm.dia^{-1}).

Genetic identification — *Colletotrichum floscerae* is recovered as monophyletic in APN2, APN2/MAT-IGS, GAP2-IGS and TUB2 individual gene trees.

Notes — see *C. zingibericola*.

Colletotrichum zingibericola I.G. Duarte, W.A.S. Vieira & M.P.S. Câmara, *sp. nov.* – Mycobank _____; Fig. 4.

Etymology. Name refers to Zingiberaceae, the botanical family of torch ginger, from which it was collected.

Typus. Brazil, Pernambuco, Paulista City, from anthracnose lesions on *Etlingera elatior* bracts, 17 April 2017, J. S. Veloso (holotype VIC47503,

culture ex-type COAD3339= LM942).

Sexual morph not observed. *Sclerotia* absent. *Acervuli* abundant, covered with orange mucilage. *Setae* short, brown, smooth-walled, with two or more septa, base truncate, tip ± acute, (58.8–) 65.5-79.6-115.6 (–128) ´ (2.3–)2.8–2.9–3.3(–3.9) µm (87.9 ± 24.4 ´ 3 ± 0.4 µm). *Conidiophores* hyaline, smooth-walled, septate, unbranched. *Conidiogenous cells* hyaline, smooth-walled, cylindrical, monophialidic. *Conidia* on PDA one-celled, smooth-walled, hyaline, cylindrical with rounded ends, sometimes oblong, contents appearing granular, (8.6–)10.5–11.1–11.8(–13.1) ´ (2.6–)2.8–3–3.3(–4.4) µm, (11.1 ± 1 ´ 3.1 ± 0.4 µm), L/W ratio = 29.7, (n = 14). *Atypical conidia* on PDA one-celled, smooth-walled, hyaline, subelliptic to obovoid with rounded ends, sometimes oblong, contents appearing granular, (7.9–)9.1 – 10 –11.5(–17.7) ´ (4.7) 5.5–6–6.6 (–8.5) µm, (10.6 ± 2.3 ´ 6.1 ± 1 µm), L/W ratio=1.8, (n = 30). *Appressoria* in slide cultures, single, medium to dark brown smooth-walled, clavate, sometimes with undulate margin, (5.4–)6.5–7.6–8.9(–10.7) ´ (3.8–)4.7–5.3–6.3(–8.1) µm (7.7 ± 1.4 ´ 5.5 ± 1.1 µm), L/W ratio=1.4, (n = 30).

Culture characteristics — Colonies on PDA with surface mycelium flattened with entire margin, apricot, growth rate at 25ºC 1.3–3.1 mm.dia^{-1} (2.4 ± 0.6 mm.dia^{-1}), Reverse cinnamon.

Genetic identification — *Colletotrichum zingibericola* is recovered as monophyletic in APN2, APN2/MAT-IGS, GAPDH and TUB2 individual gene trees.

Notes — *Colletotrichum floscerae* and *C. zingibericola* are sister species strongly supported in multilocus tree. Both species can be easily identified in APN2, APN2/MAT-IGS and GAP2-IGS trees. Although GAPDH clearly separate both species, only *C. zingibericola* is recovered as monophyletic, whereas *C. floscerae* isolates are grouped in a polythomous clade. The CAL and GS group all isolates of *C. floscerae* and *C. zingibericola* in a single clade, which represent a lack of resolution of these markers in separating those species, and not a discordance with other individual gene trees. *C. floscerae* and *C. zingibericola* are not easily separate by conidial size (8.9–11.5 ´ 2.6–3.4 vs. 8.6–13.1 ´ 2.6–4.4 µm). In contrast, *C. zingibericola* is clearly identified by the lower growth rate when compared with *C. floscerae* (1.3–3.1 vs. 4.4–5.3 mm.day^{-1}), and by the production of atypical subelliptic to obovoid conidia.

Prevalence

Colletotrichum species from ginger torch presented different prevalence (Fig. 5). *Colletotrichum. atlanticis* was the most prevalent species (33.3%), followed by *C. siamense* (20.8%), *C. chrysophilum* (14.6%), *C. tropicale* (10.4%), *C. theobromicola* (8.3%), *C. floscerae, C. fructicola* and *C. zingibericola* (4.2% each). *C. tropicale* was the prevalent species in Ceará (42.9%), whereas *C. siamense* was prevalent in Pernambuco (39%). *Colletotrichum. atlanticis*, *C. floscerae* and *C. zingibericola* were restricted to Pernambuco state.

Pathogenicity and aggressiveness

All *Colletotrichum* species were pathogenic on torch ginger bracts. All tested isolates produced typical anthracnose lesions remembering that occurring in torch ginger bracts in the field. Aggressiveness significantly differs among the species (P = 0.0019) (Table 2). *Colletotrichum atlanticis, C. floscerae* and *C. zingibericola* were the most aggressive species, showing the largest lesions. In contrast, *C. theobromicola* was the least aggressive. No symptoms were observed on the negative control.

DISCUSSION

This study reports eight *Colletotrichum species* in association with torch ginger anthracnose. Among these species, three were introduced as novel (*C. atlanticis*, *C. floscerae* and *C. zingibericola*). Based on the number of isolates sampled and the small size of the producing areas, we can consider high the *Colletotrichum* species diversity in torch ginger fields. This species diversity may be related to the climate on the tropical region, as observed in other several studies in crops cultivated in Brazilian tropical regions (Lima et al. 2013; Vieira et al. 2014, 2017). This also may be related to the high fungal diversity in natural ecosystems when compared with cultivated systems (O'Dell et al. 1996), since torch ginger is commonly cultivated near or inside forests areas.

Previous studies reported *C. gloeosporioides* as the causal agent of torch ginger. The first study (Lins & Coelho 2003) used morphological features for species identification. Further, the same species was identified by using nrITS sequences and species-specific primers (Barguil et al. 2009; Gurgel et al. 2016). Curiously, all species found in our study belong to *C. gloeosporioides* species complex, but the species *C. gloeosporioides* strictu sensu was not found. It is known that the taxonomy tools used in the previous studies are not useful to discriminate *Colletotrichum* species. Although nrITS region was proposed as a fungal barcode

marker, this region is phylogenetically informative only at species complex level, being necessary the use of more informative markers for a precise species identification (Cai et al. 2009, Crouch et al. 2009; Silva et al. 2012). Nowadays, several studies provide guidelines for *Colletotrichum* species identification using the polyphasic approach as well as the sets of best markers to include in phylogenetic studies (Cai et al. 2009; Vieira et al. 2017, 2020).

Colletotrichum atlanticis was the prevalent species in general and in Pernambuco state. This species, and *C. floscerae* and *C. zingibericola* were not found in Ceará state. Since the sampling in Pernambuco was significantly larger than in Ceará, we cannot confidently say that these species are restricted to Pernambuco. However, this hypothesis cannot be discarded, because it is suggested that in preserved forest areas it is possible to find endemic species. In contrast, the other species here reported in torch ginger (*C. chrysophilum*, *C. fructicola*, *C. siamense*, *C. tropicale* and *C. theobromicola*) are widely distributed in natural and cultivated ecosystems worldwide (Rojas et al. 2010; Weir et al. 2012; Doyle et al. 2013; Lima et al. 2013; Veloso et al. 2018; Vieira et al. 2014, 2017). Thus, the inclusion of more isolates from Ceará and from other producing regions may provide a better perspective of the distribution of *Colletotrichum* species in torch ginger, especially *C. atlanticis*, *C. floscerae* and *C. zingibericola*.

Colletotrichum atlanticis and *C. siamense* presented the highest prevalence. Additionally, *C. atlanticis* is also one of the most aggressive species, and together with *C. siamense* may be considered the species with highest pathogenic potential. Although a single small lesion is enough to depreciate ornamental plants, the combination of high distribution and high aggressiveness of *C. atlanticis* and *C. siamense* should be taken in consideration when choosing disease management strategies.

Our study clarify the *Colletotrichum* species diversity associated with torch ginger anthracnose in Brazil by using robust phylogenetic analysis. However, a larger sampling remains necessary to understand better whether *C. atlanticis*, *C. floscerae* and *C. zingibericola* is endemic in Pernambuco state. Epidemiologic aspects of *Colletotrichum* species found in torch ginger should be explored in the future to understand better this pathosystem, and so, determine the best way to manage the disease.

ACKNOWLEDGMENTS

Ingrid Gomes Duarte acknowledges “Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq” for the Masters scholarship. Marcos P. S. Câmara acknowledges CNPq

(Universal number 408724/2018-8) for the research productivity fellowship. Willie A. S. Vieira acknowledge the “Coordenação de Aperfeiçoamento Pessoal de Ensino Superior – CAPES” and the “Programa Nacional de Pós-Doutorado/CAPES – PNPD/CAPES” for the posdoctoral fellowships.

Table 1 Collection details and GenBank accession numbers of isolates included in this study.

***Colletotrichum* species**	**Culture**	**Host**	**Country**	GenBank Accession numbers						
				APN2	**APN2/MAT-IGS**	**CAL**	**GAP2-IIGS**	**GAPDH**	**GS**	**TUB2**
C. aenigma	GA512	*Persea americana*	Israel		KX620196	KX620230		KX620265	KX620296	KX620364
*C.aenigma**	ICMP18608	*Persea americana*	Israel		KM360143	JX009683		JX010044	JX010078	JX010389
C. aeschynomenes	ICMP17673	*Aeschynomene virginica*	USA		KM360145	JX009721		JX009930	JX010081	JX010392
*C. alatae**	CBS304.67	*Dioscorea alata*	India		KC888932	JX009738		JX009990	JX010065	JX010383
C. alatae	ICMP18122	*Dioscorea alata*	Nigeria			JX009739		JX010011	JX010136	JX010449
*C. alienum**	ICMP12071	*Malus domestica*	New Zealand		KM360144	JX009654		JX010028	JX010101	JX010411
C. alienum	LC3114	*Camellia sinensis*	China		KJ954545	KJ954684		KJ954832	KJ954982	KJ955279
*C. aotearoa**	ICMP18537	*Coprosma sp.*	New Zealand			JX009611		JX010005	JX010113	JX010420
C. arecicola	CGMCC3.19667	*Areca catechu*	China		MK935413			MK935455		MK935498
C. artocarpicola	MFLUCC18_1167	*Artocarpus heterophyllus*	Thailand					MN435568		MN435567
C. asianum	CBS124960	*Mangifera indica*	Panama			JX009724		JX010017		
C. asianum	Coll38	*Mangifera indica*	USA	JX145253	JX145308					JX145201
C. asianum	GJS08147	*Mangifera indica*	Panama	GU994408	GU994437					GU994466
*C. asianum**	CBS130418	*Coffea arabica*	Thailand		FR718814	FJ917506		JX010053	JX010096	JX010406
C. atlanticis sp. nov.	**LM898**	***Etlingera elatior***	**Brazil**	**MZ264109**	**MZ264125**	**MZ264141**	**MZ229415**	**MZ264093**	**MZ264077**	**MZ270516**
C. atlanticis sp. nov.	**LM938**	***Etlingera elatior***	**Brazil**	**MZ264123**	**MZ264139**	**MZ264155**	**MZ229429**	**MZ264107**	**MZ264091**	**MZ270523**
C. camelliae	CGMCC3.14925	*Camellia sinensis*				KJ954634		KJ954782	KJ954932	KJ955230
C. changpingense	MFLUCC15-0022	*Fragaria x Ananassa*	China					KP852469		KP852490
C. chrysophilum	8395	*Theobroma cacao*	Panama	GU994415	GU994444	KX094056	KX094126	KX094176	KX094209	GU994473
*C. chrysophilum**	CMM4268	*Musa sp.*	Brazil	KX094018	KX094325	KX094063	KX094125	KX094183	KX094204	KX094285
C. chrysophilum	Coll919	*Terpsichore taxifolia*	Puerto Rico	JX145265	JX145317	KX094057	KX094127	KX094177	KX094207	KX094288
C. chrysophilum	E183	*Genipa americana*	Panama	GU994414	GU994443	KX094058	KX094128	KX094178	KX094208	GU994472
C. chrysophilum	**LM909**	***Etlingera elatior***	**Brazil**	**MZ264114**	**MZ264130**	**MZ264146**	**MZ229420**	**MZ264098**	**MZ264082**	**MZ270517**
C. clidemiae	ICMP18658	*Clidemia hirta*	USA			JX009645		JX009989	JX010129	JX010438
C. conoides	CGMCC3.17615	*Capsicum spp*	China			KP890150		KP890162		KP890174
C. cordylinicola	MFLUCC090551	*Cordyline fruticosa*	Thailand			HM470238		JX009975	JX010122	JX010440
C. endophyticum	MFLUCC 130417	*Pennisetum purpureum*	Thailand			KC810017		KC832853		
*C. endophyticum**	MFLUCC 130418	*Pennisetum purpureum*	Thailand			KC810018		KC832854		
C. floscerae sp. nov.	**LM891**	***Etlingera elatior***	**Brazil**	**MZ264108**	**MZ264124**	**MZ264140**	**MZ229414**	**MZ264092**	**MZ264076**	**MZ270515**
C. floscerae sp. nov.	**LM916**	***Etlingera elatior***	**Brazil**	**MZ264115**	**MZ264131**	**MZ264147**	**MZ229421**	**MZ264099**	**MZ264083**	**MZ270518**
C. fructicola	1087	*Theobroma cacao*	Panama	GU994409	GU994438	KX094066	KX094121	KX094174	KX094198	KX094279
C. fructicola	3589	*Theobroma cacao*	Panama	GU994411	GU994440	KX094067	KX094122	KX094175	KX094199	KX094280

Table 1 (cont.)

***Colletotrichum* species**	**Culture**	**Host**	**Country**	GenBank Accession numbers						
				APN2	**APN2/MAT-IGS**	**CAL**	**GAP2-IGS**	**GAPDH**	**GS**	**TUB2**
*C. fructicola**	CBS125397	*Tetragastris panamensis*	Panama	GU994412	JQ807839	JX009674		JX010032	JX010099	JX010409
C. fructicola	**LM925**	***Etlingera elatior***	**Brazil**	**MZ264118**	**MZ264134**	**MZ264150**	**MZ229424**	**MZ264102**	**MZ264086**	**MZ270519**
C. fructivorum	CBS133125	*Vaccinium macrocarpon*	USA		JX145300					JX145196
C. gloeosporioides	CMM3272	*Anacardium occidentale*	Brazil, Rio Grande Do Norte state	MF110708		MF110826		MF110863	MF110994	MF111057
C. gloeosporioides	CMM3279	*Anacardium occidentale*	Brazil, Rio Grande Do Norte state	MF110709		MF110827	MF110975	MF110864	MF110995	MF111056
*C. gloeosporioides**	CBS112999	*Citrus sinensis*	Italy	GU994416	JQ807843	JX009731		JX010056	JX010085	JX010445
C. grevilleae	CBS132879	*Grevillea sp.*	Italy			KC296963		KC297010	KC297033	KC297102
C. grossum	CGMCC3.17614	*Capsicum spp.*	China			KP890147		KP890159		KP890171
C. hebeiense	JZB330028	*Vitis vinifera*	China					KF377495		KF288975
C. helleniense	CBS142419	*Citrus reticulata*	Greece, Arta			KY856100		KY856271		KY856529
C. henanense	CGMCC3.17354	*Camellia sinensis*			KJ954524	KJ954662		KJ954810	KJ954960	KJ955257
C. horii	ICMP10492	*Diospyros kaki*	Japan		JQ807840	JX009604		GQ329681	JX010137	JX010450
*C. hystricis**	CBS142411	*Citrus hystrix*	Italy			KY856103		KY856274		KY856532
C. hystricis	CBS142412	*Citrus hystrix*	Italy			KY856104		KY856275		KY856533
C. jiangxiense	CGMCC3.1736	*Camellia sinensis*			KJ954607	KJ954752		KJ954902	KJ955051	KJ955348
C. kahawae subsp. kahawae	ICMP 17816	*Coffea arabica*	Kenya		JQ894579	JX009642		JX010012	JX010130	JX010444
C. ledongense	CGMCC3.18888	*Hevea brasiliensis*	China, Hainan			MG242013		MG242017	MG242021	MG242011
*C. makassarense**	CBS143664	*Capsicum spp.*	Indonesia		MH728831			MH728820	MH748264	MH846563
C. makassarense	CPC28555	*Capsicum annuum*	Indonesia		MH728834			MH728822	MH748261	MH846560
C. makassarense	CPC28556	*Capsicum annuum*	Indonesia		MH728833			MH728821	MH748262	MH846561
*C. musae**	CBS116870	*Musa sp.*	USA		KC888926	JX009742		JX010050	JX010103	HQ596280
C. musae	ICMP17817	*Musa sp.*	Kenya			JX009689		JX010015	JX010084	JX010395
C. noveboracense	AFK423	*Malus domestica*	Ulster/NY	MN701186	MN701184	MN701191		MN741085	MN741099	MN701194
*C. noveboracense**	AFKH109	*Malus domestica*	Columbia/NY	MN910262	MN640564	MN640566		MN640567	MN640568	MN640569
C. noveboracense	PMBrms_1	*Malus domestica*	Adams/PA	MN790765	MN741075	MN741056		MN741087	MN741100	MN741064
C. nupharicola	CBS470.96	*Nuphar polysepala*	USA	JX145275	JX145319	JX009661		JX009936	JX010088	JX010397
*C. nupharicola**	CBS472.96	*Nymphaea odorata*	USA	JX145276	JX145320	JX009662		JX010031	JX010089	JX010399
C. perseae	GA039	*Persea americana*	Israel		KX620172	KX620200		KX620236	KX620269	KX620335
*C. perseae**	CBS141365	*Persea americana*	Israel		KX620177	KX620206		KX620242	KX620275	KX620341
*C. proteae**	CBS132882	*Protea sp.*	South Africa			KC296960		KC297009	KC297032	KC297101
C. proteae	CBS134301	*Protea sp.*	South Africa			KC842375		KC842379	KC842381	KC842387

Table 1 (cont.)

***Colletotrichum* species**	**Culture**	**Host**		**Country**	GenBank Accession numbers						
					APN2	**APN2/MAT-IGS**	**CAL**	**GAP2-IGS**	**GAPDH**	**GS**	**TUB2**
C. psidii	CBS145.29	*Psidium sp.*	Italy				JX009743		JX009967	JX010133	JX010443
C. queenslandicum	CMM3233	*Anacardium occidentale*	Brazil, Pernambuco state	MF110711	MF110639		MF110828	MF110918	MF110849	MF110996	MF111058
C. queenslandicum	CMM3241	*Anacardium occidentale*	Brazil, Pernambuco state	MF110714	MF110642		MF110832	MF110915	MF110848	MF111000	MF111059
*C. queenslandicum**	ICMP1778	*Carica papaya*	Australia				JX009691		JX009934	JX010104	JX010414
C. rhexiae	CBS133134	*Rhexia virginica*	Delaware, USA		JX145290						JX145179
C. salsolae	CBS119296	*Glycine max*	Hungary				JX009695		JX009917		
C. salsolae	ICMP19051	*Salsola tragus*	Hungary		KC888925		JX009696		JX009916	JX010093	JX010403
C. siamense	CMM4083	*Mangifera indica*	Brazil	KX093998	KX094307		KX094052	KX094147	KX094167	KX094219	KX094271
C. siamense	CMM4084	*Mangifera indica*	Brazil	KX093999	KX094310		KX094053	KX094148	KX094166	KX094220	KX094272
C. siamense	CMM4248	*Mangifera indica*	Brazil	KX093992	KX094314		KX094037	KX094136	KX094154	KX094229	KX094300
C. siamense	CBS130417	*Coffea arabica*	Thailand		JQ899289		FJ917505		JX009924	JX010094	JX010404
C. siamense	CMM4244	*Musa sp.*	Brazil	KX094014	KX094315		KX094055	KX094135	KX094172	KX094226	KX094299
C. siamense	CMM4247	*Musa sp.*	Brazil	KX094009	KX094301		KX094038	KX094141	KX094155	KX094196	KX094261
C. siamense	**LM902**	***Etlingera elatior***	**Brazil**	**MZ264110**	**MZ264126**		**MZ264142**	**MZ229416**	**MZ264094**	**MZ264078**	
C. siamense	**LM904**	***Etlingera elatior***	**Brazil**	**MZ264111**	**MZ264127**		**MZ264143**	**MZ229417**	**MZ264095**	**MZ264079**	
C. siamense	**LM905**	***Etlingera elatior***	**Brazil**	**MZ264112**	**MZ264128**		**MZ264144**	**MZ229418**	**MZ264096**	**MZ264080**	
C. siamense	**LM917**	***Etlingera elatior***	**Brazil**	**MZ264116**	**MZ264132**		**MZ264148**	**MZ229422**	**MZ264100**	**MZ264084**	
C. syzygicola	MFLUCC10_0624	*Syzygium samarangense*	Thailand				KF254859		KF242156	KF242125	KF254880
C. tainanense	CBS143666	*Capsicum annuum*	Taiwan		MH728836				MH728823	MH748259	MH846558
C. tainanense	UOM1119	*Capsicum annuum*	Taiwan		MH728824				MH728819	MH748271	MH846570
C. temperatum	CBS133122	*Vaccinium macrocarpon*	USA		JX145298						JX145211
C. theobromicola	CBS124945	*Theobroma cacao*	Panama	GU994419	KC790726		JX009591		JX010006	JX010139	JX010447
C. theobromicola	GJS0843	*Theobroma cacao*	Panama	GU994418	GU994447						GU994476
C. theobromicola	**LM936**	***Etlingera elatior***	**Brazil**	**MZ264121**	**MZ264137**		**MZ264153**	**MZ229427**	**MZ264105**	**MZ264089**	**MZ270521**
C. ti	ICMP4832	*Cordyline sp.*	New Zealand				JX009649		JX009952		JX010442
C. ti	ICMP5285	*Cordyline australis*	New Zealand				JX009650		JX009910	JX010124	JX010441
C. tropicale	8401	*Theobroma cacao*	Panama	GU994400	GU994429						GU994458
C. tropicale	CBS124949	*Theobroma cacao*	Panama	GU994396	GU994425		JX009719		JX010007	JX010097	GU994454
C. tropicale	CMM4243	*Musa sp.*	Brazil	KU213598	KU213597		KU213599		KU213601	KU213602	KU213604
C. tropicale	Coll918	*Mycopteris taxifolia*	Puerto Rico	JX145264	JX145307						JX145214

Table 1 (cont.)

***Colletotrichum* species**	**Culture**	**Host**	**Country**		GenBank Accession numbers						
					APN2	**APN2/MAT-IGS**	**CAL**	**GAP2-IGS**	**GAPDH**	**GS**	**TUB2**
C. tropicale	E1164	*Trichilia tuberculata*	Panama	GU994397	GU994426						GU994455
C. tropicale	E406	*Pentagonia macrophylla*	Panama	GU994395	GU994424						GU994453
C. tropicale	**LM907**	***Etlingera elatior***	**Brazil**	**MZ264113**	**MZ264129**		**MZ264145**	**MZ229419**	**MZ264097**	**MZ264081**	
C. tropicale	**LM922**	***Etlingera elatior***	**Brazil**	**MZ264117**	**MZ264133**		**MZ264149**	**MZ229423**	**MZ264101**	**MZ264085**	
C. tropicale	**LM927**	***Etlingera elatior***	**Brazil**	**MZ264119**	**MZ264135**		**MZ264151**	**MZ229425**	**MZ264103**	**MZ264087**	
C. viniferum	GZAAS5.08601	*Vitis vinifera cv. Shuijing*	China				JQ309639		JN412798	JN412787	JN412813
C. viniferum	GZAAS5.08608	*Vitis vinifera cv. Hongti*	China				JN412782		JN412800	JN412784	JN412811
C. wuxiense	CGMCC3.17894	*Camellia sinensis*	China		KU251722		KU251833		KU252045	KU252101	KU252200
C. xanthorrhoeae	CBS127831	*Xanthorrhoea sp.*	Australia		KC790689		JX009653		JX009927	JX010138	JX010448
C. zingibericola sp. nov.	**LM937**	***Etlingera elatior***	**Brazil**	**MZ264122**	**MZ264138**		**MZ264154**	**MZ229428**	**MZ264106**	**MZ264090**	**MZ270522**
C. zingibericola sp. nov.	**LM942**	***Etlingera elatior***	**Brazil**	**MZ264120**	**MZ264136**		**MZ264152**	**MZ229426**	**MZ264104**	**MZ264088**	**MZ270520**

CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMM: Culture Collection of Phythopathogenic Fung "Prof. Maria Menezes", Recife, Brazil; GZAAS: Guizhou Academy of Agricultural Sciences Herbarium, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; LC: Working collection of Lei Cai, housed at CAS, China; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand; LM: Working collection of Laboratório de Micologia, housed at UFRPE, Brazil; CGMCC: China General Microbiological Culture Collection Center, China; GZAAS:herbarium of Guizhou Academy of Agricultural Sciences, China; JZB: Culture collections of Beijing Academy of Agricultural and Forestry Sciences, China.

* = ex-type culture. Strains collected and sequences generated for this study are in **bold** font.

APN2: DNA lyase; APN2/MAT-IGS: intergenic spacer between DNA lyase and the mating-type locus MAT1-2; CAL: calmodulin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GAP2-IGS: intergenic spacer between GAPDH and a hypothetical protein; GS: glutamine synthetase; TUB2: β-tubulin.

Table 2 Aggressiveness of sixteen *Colletotrichum* species associated with torch ginger anthracnose in Brazil when inoculated on wounded bract tissues of torch ginger.

Colletotrichum species	Diameter of lesions (mm)
C. atlanticis	17.85 ± 6.04 a
C. chrysophilum	13.25 ± 3.46 ab
C. floscerae	15.12 ± 4.91 a
C. fructicola	15.56 ± 11.29 ab
C. siamense	12.46 ± 3.98 ab
C. theobromicola	6.71 ± 4.05 b
C. tropicale	12.28 ± 1.77 ab
C. zingibericola	16.40 ± 2.74 a
p	0.0019

Means ± SE followed by the same letter within columns do not differ significantly by Tukey's test ($p \leq 0.05$). For anlysis, meas were transformed using $\sqrt{x+0.5}$.

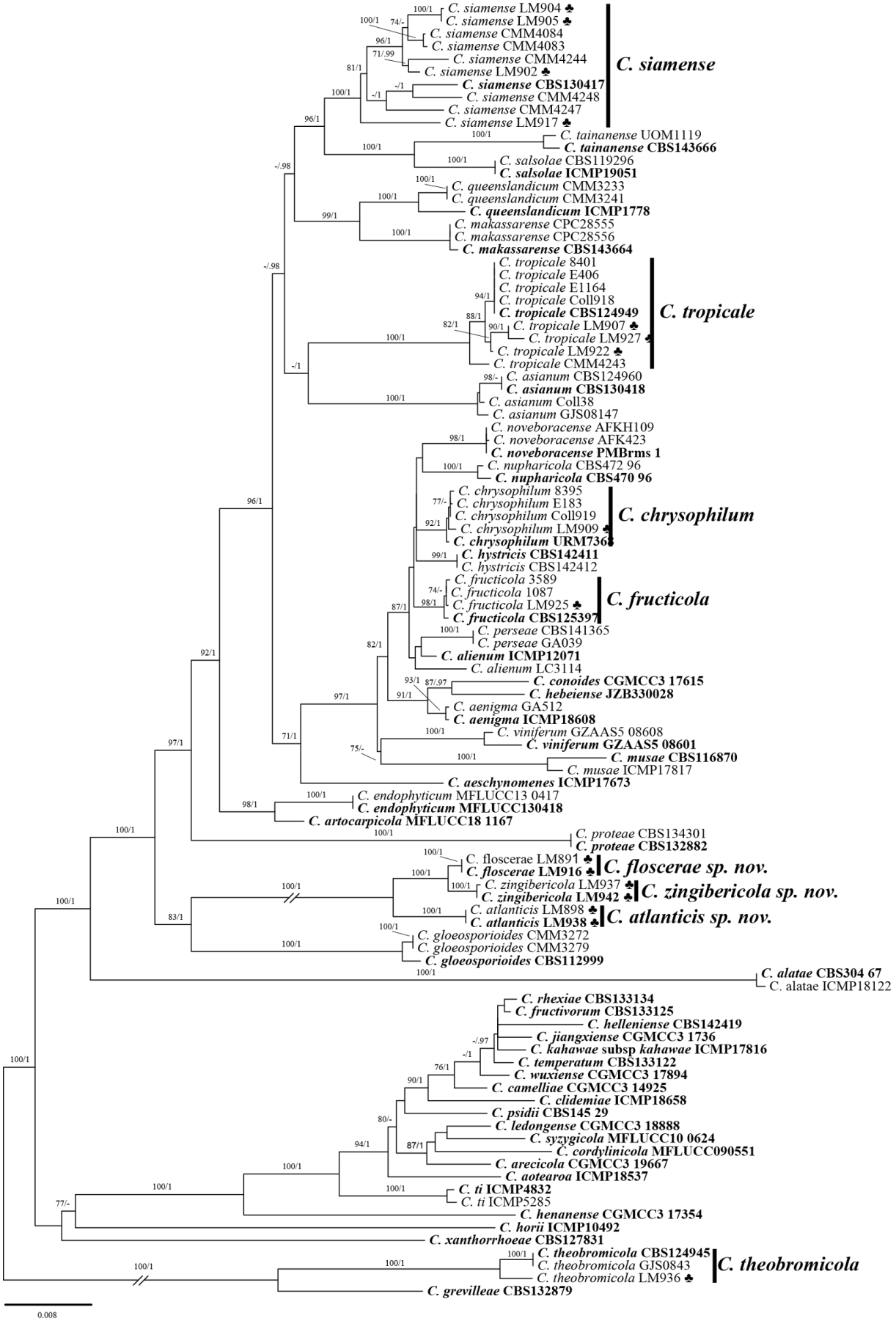
C. siamense LM904 ♣
C. siamense LM905 ♣
C. siamense CMM4084
C. siamense CMM4083
C. siamense CMM4244
C. siamense LM902 ♣
C. siamense CBS130417
C. siamense CMM4248
C. siamense CMM4247
C. siamense LM917 ♣
C. siamense
C. tainanense UOM1119
C. tainanense CBS143666
C. salsolae CBS119296
C. salsolae ICMP19051
C. queenslandicum CMM3233
C. queenslandicum CMM3241
C. queenslandicum ICMP1778
C. makassarense CPC28555
C. makassarense CPC28556
C. makassarense CBS143664
C. tropicale 8401
C. tropicale E406
C. tropicale E1164
C. tropicale Coll918
C. tropicale CBS124949
C. tropicale LM907 ♣
C. tropicale LM927 ♣
C. tropicale LM922 ♣
C. tropicale CMM4243
C. tropicale
C. asianum CBS124960
C. asianum CBS130418
C. asianum Coll38
C. asianum GJS08147
C. noveboracense AFKH109
C. noveboracense AFK423
C. noveboracense PMBrms 1
C. nupharicola CBS472 96
C. nupharicola CBS470 96
C. chrysophilum 8395
C. chrysophilum E183
C. chrysophilum Coll919
C. chrysophilum LM909 ♣
C. chrysophilum URM7368
C. chrysophilum
C. hystricis CBS142411
C. hystricis CBS142412
C. fructicola 3589
C. fructicola 1087
C. fructicola LM925 ♣
C. fructicola CBS125397
C. fructicola
C. perseae CBS141365
C. perseae GA039
C. alienum ICMP12071
C. alienum LC3114
C. conoides CGMCC3 17615
C. hebeiense JZB330028
C. aenigma GA512
C. aenigma ICMP18608
C. viniferum GZAAS5 08608
C. viniferum GZAAS5 08601
C. musae CBS116870
C. musae ICMP17817
C. aeschynomenes ICMP17673
C. endophyticum MFLUCC13 0417
C. endophyticum MFLUCC130418
C. artocarpicola MFLUCC18 1167
C. proteae CBS134301
C. proteae CBS132882
C. floscerae LM891 ♣
C. floscerae LM916 ♣
C. floscerae sp. nov.
C. zingibericola LM937 ♣
C. zingibericola LM942 ♣
C. zingibericola sp. nov.
C. atlanticis LM898 ♣
C. atlanticis LM938 ♣
C. atlanticis sp. nov.
C. gloeosporioides CMM3272
C. gloeosporioides CMM3279
C. gloeosporioides CBS112999
C. alatae CBS304 67
C. alatae ICMP18122
C. rhexiae CBS133134
C. fructivorum CBS133125
C. helleniense CBS142419
C. jiangxiense CGMCC3 1736
C. kahawae subsp kahawae ICMP17816
C. temperatum CBS133122
C. wuxiense CGMCC3 17894
C. camelliae CGMCC3 14925
C. clidemiae ICMP18658
C. psidii CBS145 29
C. ledongense CGMCC3 18888
C. syzygicola MFLUCC10 0624
C. cordylinicola MFLUCC090551
C. arecicola CGMCC3 19667
C. aotearoa ICMP18537
C. ti ICMP4832
C. ti ICMP5285
C. henanense CGMCC3 17354
C. horii ICMP10492
C. xanthorrhoeae CBS127831
C. theobromicola CBS124945
C. theobromicola GJS0843
C. theobromicola LM936 ♣
C. theobromicola
C. grevilleae CBS132879
0.008

Fig.1. Maximum likelihood tree of the *C. gloeosporioides* species complex inferred from a concatenated alignment of APN2, APN2/MAT-IGS, CAL, GAPDH, GAP2-IGS, GS and TUB2. Bootstrap support values (ML ≥ 70) and Bayesian posterior probability values (PP ≥ 0.95) are shown above the branches. "-" indicates no-significant support or absence of the branch. Ex-types are emphasized in bold and include the taxonomic name as originally described. "♣" indicates isolates from torch ginger. The scale bar indicates the estimated number of substitutions per site. The tree is rooted at the midpoint.

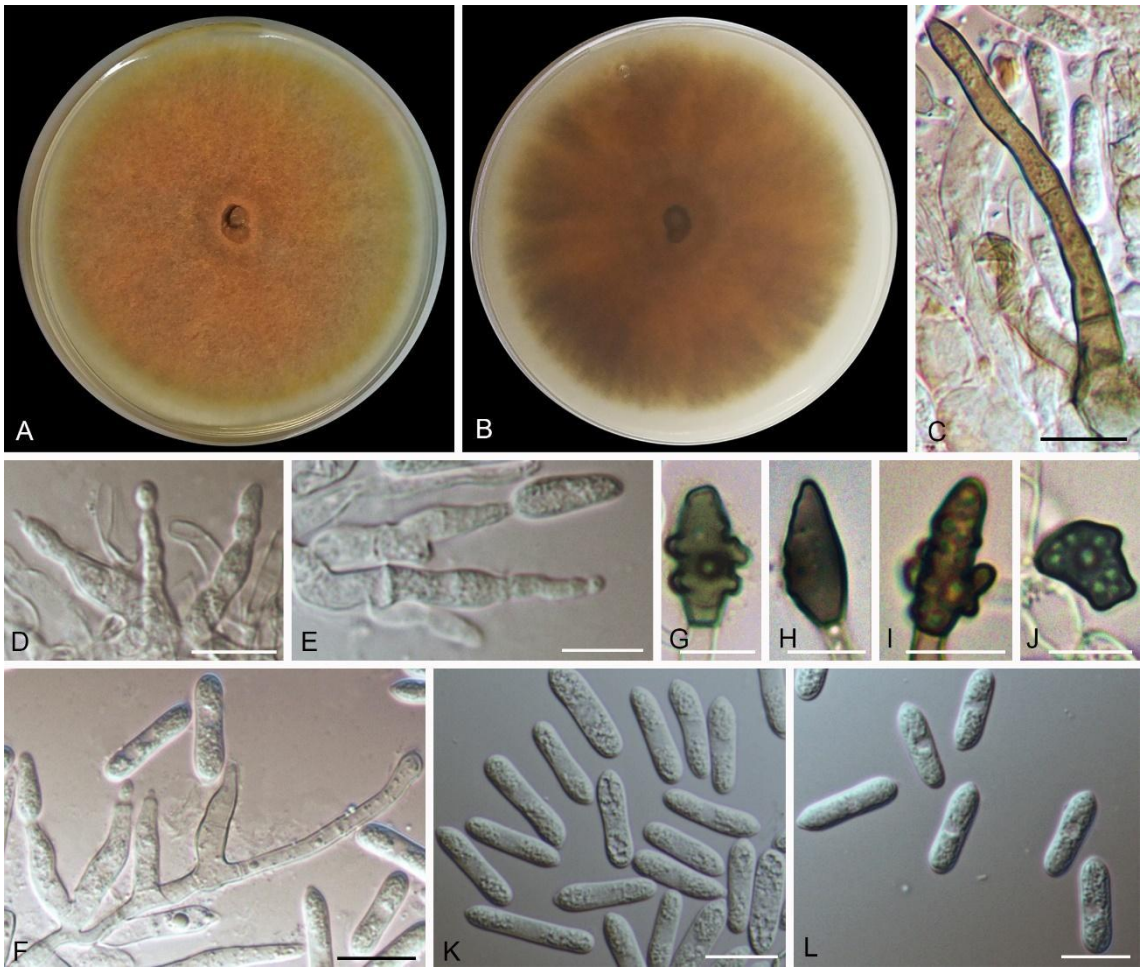

Fig. 2. *Colletotrichum atlanticis* (LM 938). A–B. Colonies on PDA above and below; C. setae; D–E. conidiogenous cells; G–J. appressoria (Scale bars = 5 μm); F. conidiophore; K–L. conidia. — Scale bars = 10 μm.

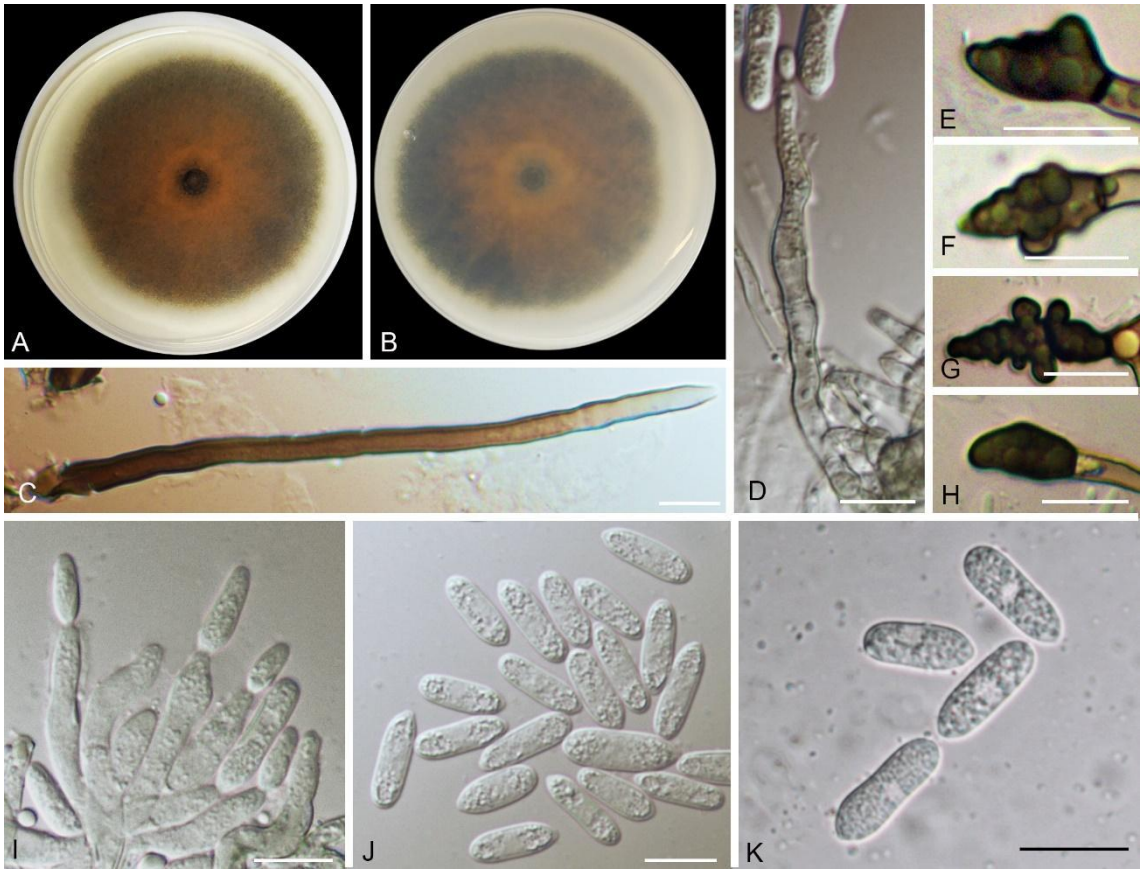

Fig. 3. *Colletotrichum floscerae* (LM 916). A–B. Colonies on PDA above and below; C. setae; D. conidiogenous cells; E–H. appressoria (Scale bars = 5 μm); I. conidiophore; J–K. conidia. —Scale bars = 10 μm.

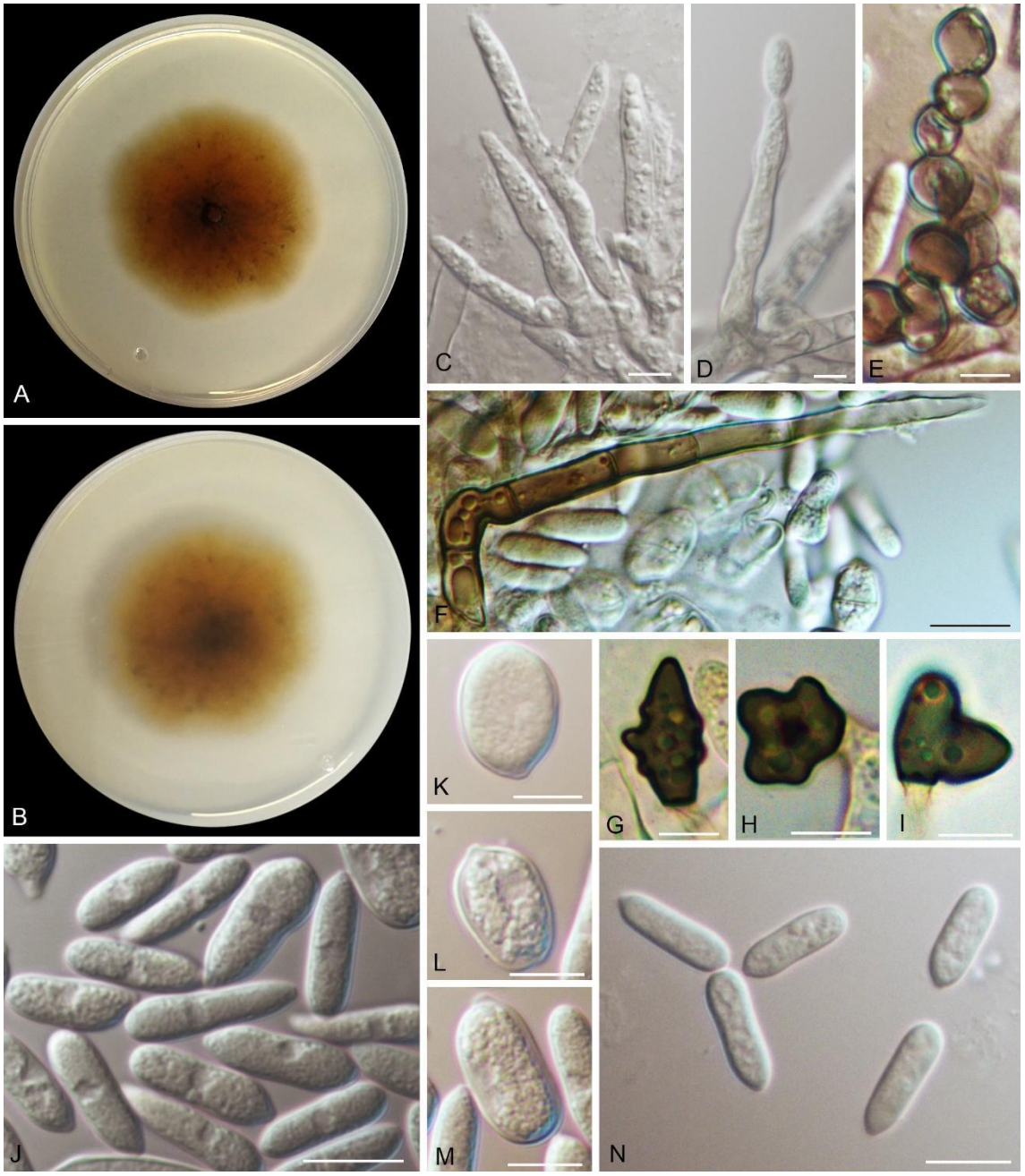

Fig. 4. *Colletotrichum zingibericola* (LM 942). A–B. Colonies on PDA above and below; C. conidiophore; D. conidiogenous cells; E. appressoria chain.; F. setae; G–I. appressoria (Scale bars = 5 μm); J and N. conidia; K–M. atypical conidia. —Scale bars = 10 μm.

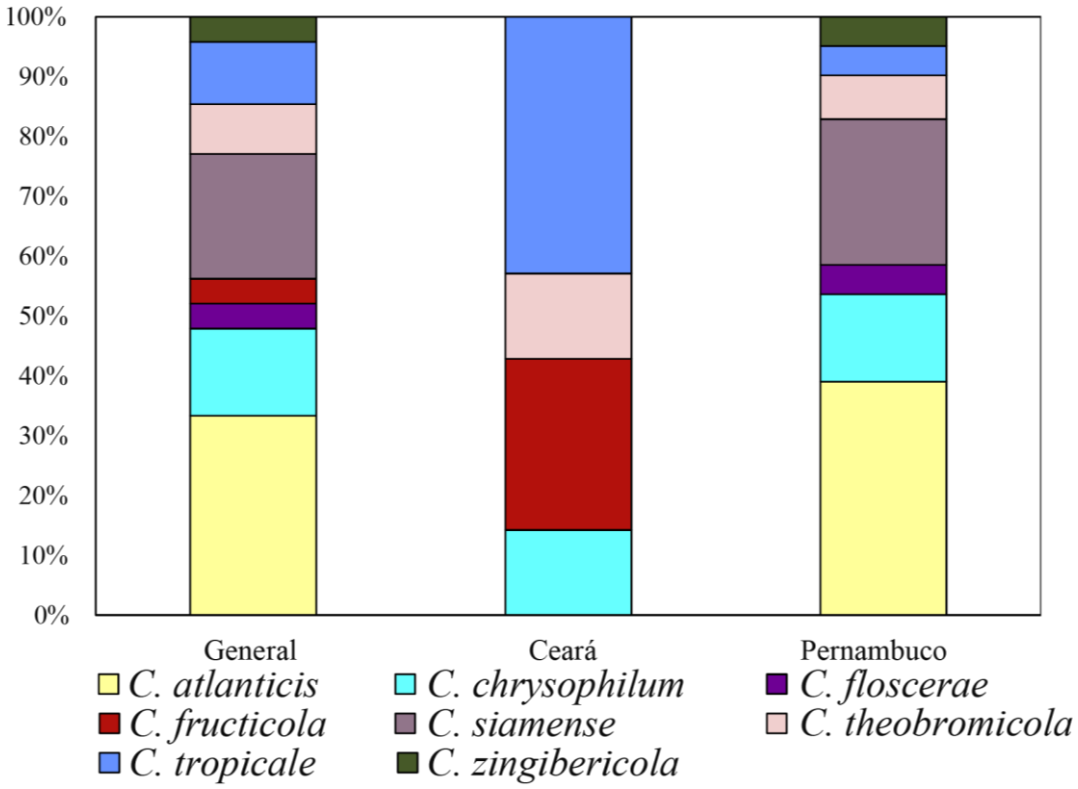

Fig.5. Prevalence of *Colletotrichum* species associated with torch ginger.

REFERENCES

Barguil BM, Oliveira SMA, et al. 2009. Identificação e variabilidade genética de isolados de *Colletotrichum* causando antracnose em inflorescências de plantas ornamentais tropicais. Ciência Rural 6:1639-1646.

Cai L, Hyde KD, et al. 2009. A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39:183-204.

Cannon PF, Damm U, et al. 2012. *Colletotrichum* – current status and future directions. Studies in Mycology 73:181-213.

Cao X, Xu X, et al. 2019. Three *Colletotrichum* species, including a new species, are associated to leaf anthracnose of rubber tree in Hainan, China. Plant Disease 103(1):117-124.

Crouch JA, Clarke BB, et al. 2009. What is the value of ITS sequence data in *Colletotrichum* systematics and species diagnosis? A case study using the falcate-spored graminicolous *Colletotrichum* group. Mycologia 101(5):648-656.

Damm U, Sato T,et al. 2019. The *Colletotrichum dracaenophilum, C. magnum and C. orchidearum* species complexes. Studies in Mycology 92:1-46.

Dettman JR, Jacobson DJ, et al. 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. Evolution 57:2703-2720.

Doyle JJ, Doyle JL, 1990. Isolation of plant DNA from freshtissue. Focus 12:13-15.

Doyle VP, Oudemans PV, et al. 2013. Habitat and host indicate lineage identity in *Colletotrichum gloeosporioides* s.l. from wild and agricultural landscapes in North America. Plos One 8: e 62394.

Duke MM, 1928. The genera Vermicularia Fr. and *Colletotrichum* Cda. Transactions of the British Mycological 13:156-184.

Ferrari, J.T. Antracnose em Bastão do Imperador. São Paulo: Instituto Biológico/APTA), 2008. p.1-6. (Documento Técnico 003).

Gurgel LMS, Coelho RSB, et al.2016. Efeito de indutores de resistência no controle da antracnose do bastão do imperador (*Etlingera elatior*). Anais da Academia Pernambucana de

Ciência Agronômica 13:223-233.

Hyde KD, Cai L, et al. 2009. *Colletotrichum*: a catalogue of confusion. Fungal Diversity 39:1-17.

Johnston PR, Jones D. 1997. Relationship among *Colletotrichum* isolates from fruit rots assessed using rDNA sequences. Mycologia 89:420-430.

Kumar, S, Stecher, G, Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution, 33(7): 1870-1874.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451-1452.

Lima NB, Batista, MVA, et al. 2013. Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil. Fungal Diversity 61:75-88.

Lins SRO, Coelho RSB. 2003. Antracnose em inflorescências de bastão do imperador (*Etlingera elatior*): ocorrência e métodos de inoculação. Summa Phytopathologica 29:355-358.

Lins SRO, Coelho RSB. 2004. Ocorrência de doenças em plantas ornamentais tropicais no Estado de Pernambuco. Fitopatologia Brasileira 29:332-335.

Loges V, Castro ACR. et al. 2005. Floriculture research in Pernambuco. Acta Horticulturae 638:345-350.

Marin-Felix Y, Groenewald JZ, et al. 2017. Genera of phytopathogenic fungi: GOPHY 1. Studies in Mycology 86:99-216.

Neves MF, Pinto MJA, 2015. Mapeamento e quantificação da cadeia de flores e plantas ornamentais do Brasil. São Paulo: Organização das Cooperativas do Estado de São Paulo.

Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

O'Dell T, Castellano MA, et al. 1996. Diversity and conservation of forest fungi. USDA Forest Service – General Technical Report.

O'Donnell K, Cigelnik E, 1997. Two divergent intragenomic rDNA ITS2 types within a

monophyletic lineage of the fungus fusarium are non orthologous. Molecular phylogenetics and evolution 7(1): 103-116.

Prihastuti H, Cai L, et al.2009. Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. Fungal Diversity 39:89-109.

Rayner RW, 1970. A mycological colour chart. Commonwealth Mycological Institute and British Mycological. Society, Kew.

Rojas EI, Rehner SA, et al. 2010. *Colletotrichum gloeosporioides* sl associated with *Theobroma cacao* and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102(6):1318-1338.

Ronquist F, Teslenko M, et al. 2012. MrBayes v. 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539-542.

SEBRAE–SERVIÇO BRASILEIRO DE APOIO ÀS MICRO E PEQUENAS EMPRESAS. Flores e plantas ornamentais do Brasil: volume 1 -o mercado brasileiro de flores e plantas ornamentais. Brasília, DF: SEBRAE, 2015. Disponível em: <http://www.bibliotecas.sebrae.com.br/chronus/ARQUIVOS_CHRONUS/bds/bds.nsf/7ed114f4eace9ea970dadf63bc8baa29/$File/5518.pdf>. Acesso em: 19 abr. 2020.

Sela I, Ashkenazy H, Katoh K, Pupko T. 2015. GUIDANCE2: accurate detection of unreliable alignment regions accounting for the uncertainty of multiple parameters. Nucleic Acids Research 43: W7-W14.

Silva DN, Talhinhas P, et al. 2012. Application of the apn2/mat locus to improve the systematics of the *Colletotrichum gloeosporioides* complex: an example from coffee (*Coffea* spp.) hosts. Mycologia 104:396-409.

Staden R, Beal KF, et al. 1998. The Staden package. In:Misener S, Krawetz SA, (eds) Bioinformatics methods and protocols:115-130 Humana, New York, p. 115-130.

Stamatakis A, 2014. RAxML version 8: A tool for Phylogenetic Analysis and Post Analysis of Large Phylogenies. Bioinformatics 30:1312-1313.

Statistix. 2013. Statistix 10, Version 10. Analytical Software, Tallahassee, FL, USA.

Sutton BC, 1980. The Coelomycetes. Fungi imperfecti with pycnidia acervuli and stromata.

Kew: Commonwealth Mycological Institute, p. 696.

Sutton BC, 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. In: Bailey JA, JEGER MJ,(eds) *Colletotrichum* – Biology, Pathology and Control. Wallingford: CAB International, p. 1-26.

Taylor JW, Jacobson DJ,et al.2000. Phylogenetic species recognition and species concepts in fungi. Fungal genetics and biology 31(1): 21-32.

Vaidya, G, Lohman, DJ, Meier, R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27(2): 171-180.

Veloso JS, Câmara MP, et al. 2018. Why species delimitation matters for fungal ecology: *Colletotrichum* diversity on wild and cultivated cashew in Brazil. Fungal biology 122(7):677-691.

Vieira WAS, Bezerra PA, et al. 2020. Optimal markers for the identification of *Colletotrichum* species. Molecular Phylogenetics and Evolution 143.

Vieira WAS, Lima WG, et al. 2017. The impact of phenotypic and molecular data on the inference of *Colletotrichum* diversity associated with *Musa*. Mycologia 109:912-934.

Vieira WAS, Micheref SJ, et al. 2014. Endophytic species of *Colletotrichum* associated with mango in northeastern Brazil. Fungal Diversity 67: 181–202.

Weir BS, Johnston PR, et al. 2012. The *Colletotrichum gloeosporioides* species complex. Studies in mycology 73: 115-180.

CAPÍTULO III

Conclusões Gerais

CONCLUSÕES GERAIS

- A diversidade inicial de espécies de *Colletotrichum* spp.em bastão do imperador foi de onze haplótipos utilizando o gene GAPDH;
- A combinação dos genes APN2, APN2/MAT-IGS, CAL, GAPDH, GAP2-IGS, GS e TUB2 foi eficiente para a identificação de oito espécies de *Colletotrichum* associadas a antracnose do bastão do imperador no Brasil;
- Três espécies novas foram identificadas e descritas como *C. atlanticis*, *C. floscerae* e *C. zingibericola*;
- Cinco espécies pertencentes ao complexo de espécies *C. gloeosporioides* foram identificadas: *C. crysophylum*, *C. fructicola*, *C. siamense*, *C. theobromicola* e *C. tropicale*;
- A espécie *C. atlanticis* é a mais prevalente e a mais virulenta.