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Etiologia e epidemiologia da antracnose do inhame em área de cultivo no Brasil

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ETIOLOGIA E EPIDEMIOLOGIA DA ANTRACNOSE DO INHAME EM ÁREA DE CULTIVO NO BRASIL

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SUMÁRIO

RESUMO GERAL

A antracnose é uma das principais doenças foliares do inhame (*Dioscorea* spp.), gerando perdas de até 85% da produção. Essa doença era frequentemente atribuída a *Colletotrichum gloeosporioides* e a exata etiologia da antracnose do inhame no Brasil permanecia até então desconhecida. O presente estudo teve como objetivo identificar as espécies de *Colletotrichum* associadas à antracnose do inhame no Brasil, através do uso de marcadores moleculares de alta precisão, e determinar a distribuição geográfica dessas espécies. Também foram determinados componentes epidemiológicos, tais como agressividade, temperatura ótima de crescimento, taxa máxima de crescimento micelial e sensibilidade a fungicidas. Um total de 105 isolados de *Colletotrichum* foram identificados a partir de dados de sequências de DNA (ACT, APN2, APN2/MAT-IGS, CAL, GAPDH, GAP2-IGS, GS, HIS3, ITS e TUB2). Os isolados de *Colletotrichum* obtidos de inhame foram distribuídos entre oito espécies previamente descritas (*C. chrysophilum*, *C. karsti*, *C. queenslandicum*, *C. plurivorum*, *C. siamense*, *C. sojae*, *C. tropicale*, *C. truncatum*), e duas espécies novas introduzidas no presente trabalho como *C. inhamicola* e *C. vitoriense*. É o primeiro relato das espécies *C. chrysophilum*, *C. inhamicola*, *C. queenslandicum*, *C. plurivorum*, *C. sojae* e *C. vitoriense* asssociados a antracnose do inhame no mundo. *Colletotrichum plurivorum* foi a espécie prevalente nos estados de Alagoas, Pernambuco, Paraíba e também no Distrito Federal. Esta espécie também prevaleceu nas espécies *D. alata* e *D. cayennensis*. Todas as espécies de *Colletotrichum* encontradas foram patogênicas em folhas de inhame. *Colletotrichum siamense* foi a espécie mais agressiva e *C. queenslandicum* a menos agressiva. A temperatura ótima para crescimento micelial variou entre 24,3 e 28,8 ºC e a taxa máxima de crescimento micelial variou de 9,8 a 13,9 mm. Todas as espécies de *Colletotrichum* apresentaram o crescimento micelial reduzido na presença dos fungicidas azoxistrobina, difenoconazol e tiofanato-metílico. A resposta de sensibilidade variou de acordo com o fungicida e a espécie de *Colletotrichum*. Nossos resultados oferecem subsídios para o desenvolvimento de estratégias de manejo eficientes para a antracnose do inhame no Brasil.

Palavras-chave: Distribuição geográfica, epidemiologia, filogenia.

GENERAL ABSTRACT

Anthracnose is one of the main foliar diseases of yam (*Dioscorea* spp.), causing losses of up to 85% of production. This disease was often attributed to *Colletotrichum gloeosporioides* and the exact etiology of yam anthracnose in Brazil remained unknown until then. The present study aimed to identify the species of *Colletotrichum* associated with yam anthracnose in Brazil. Epidemiological components such as aggressiveness, optimum growth temperature, maximum mycelial growth rate, and sensitivity to fungicides were also determined. A total of 104 *Colletotrichum* isolates were identified from DNA sequence data (ACT, APN2, APN2/MAT-IGS, CAL, GAPDH, GAP2-IGS, GS, HIS3, ITS, and TUB2). The *Colletotrichum* isolates obtained from yams were distributed among eight previously described species (*C. chrysophilum*, *C. karsti*, *C. queenslandicum*, *C. plurivorum*, *C. siamense*, *C. sojae*, *C. tropicale*, and *C. truncatum*), and two new species introduced in the present work (*C. inhamicola* and *C. vitoriense*). It is the first report of the species *C. chrysophilum*, *C. inhamicola*, *C. queenslandicum*, *C. plurivorum*, *C. sojae*, and *C. vitoriense* associated with yam anthracnose in the world. *Colletotrichum plurivorum* was the prevalent species in the in the state of Alagoas, Paraiba, Pernambuco and also Distrito Federal. This species also prevailed in the species *D. alata* and *D. cayennensis*. All *Colletotrichum* species found were pathogenic in yam leaves. *Colletotrichum siamense* was the most aggressive species and *C. queenslandicum* was the least aggressive. The optimum temperature for mycelial growth ranged from 24.3 to 28.8 ºC and the maximum mycelial growth rate ranged from 9.8 to 13.9 mm. All *Colletotrichum* species showed reduced mycelial growth in the presence of azoxystrobin, difenoconazole, and thiophanate-methyl fungicides. The sensitivity response varied according to the fungicide and *Colletotrichum* species. Our results offer subsidies for the development of efficient management strategies for yam anthracnose in Brazil.

Keywords: Geographic distribution, epidemiology, phylogeny.

CAPÍTULO I

Introdução Geral

ETIOLOGIA E EPIDEMIOLOGIA DE ESPÉCIES DE *Colletotrichum* ASSOCIADAS À ANTRACNOSE DO INHAME NO BRASIL

1. INTRODUÇÃO GERAL

1.1 A cultura do inhame

O gênero *Dioscorea* L. pertencente à família *Dioscoreaceae* possui como centro de origem o oeste do Africano. Espécies desse gênero são popularmente conhecidas como inhame da costa ou cará-da-costa (LEBOT, 2009). Os primeiros relatos do cultivo de inhame no Brasil datam do período da colonização portuguesa, onde eram realizadas trocas de mercadorias entre a costa africana a caminho da Índia (MESQUITA, 2002).

O gênero *Dioscorea* é composto por mais de 600 espécies, dessas apenas 11 são comestíveis: *D. rotundata* Poir, *D. cayannensis* Lam, *D. dumerotum* (Kunth) Pax, *D. oppositifolia* L., *D. alata* L., *D. esculenta* (Lour.) Burk, *D. bulbifera* L., *D. japonica* (Thunb.), *D. trifida* L., *D. numularia* Lam. e *D. pentaphylla* L. (NTUI *et al*., 2021). No Brasil, as espécies predominantemente cultivadas são *D. cayannensis* e *D. alata* (NORONHA, 2014).

Plantas do gênero *Dioscorea* apresentam porte herbáceo, crescimento escandente, e produzem túberas alongadas de cor castanho-claro. Possui caule volumoso com folhas dispostas de forma oposta e com raras exceções de forma alternada. A propagação pode ser realizada por rizóforo-sementes ou rizóforos inteiros (NETO, 2000; SANTOS, 2002).

O inhame é uma cultura de ciclo anual que apresenta quatro estádios fenológicos: dormência fisiológica, vegetativo, reprodutivo e maturação fisiológica. O ciclo da cultura pode durar de oito a doze meses. A planta pode produzir dois tipos de túberas a depender da época de colheita: túbera-semente e túbera comercial. Para obtenção de túberas-sementes é realizada a técnica da capação. A capação consiste em separar a túbera da planta mãe para facilitar a formação da túbera semente. Já a túbera comercial é colhida no fim do ciclo quando as folhas ficam amarelas e os ramos secam, sendo então destinadas à comercialização (SANTOS, 1996; OLIVEIRA, 2006).

A túbera do inhame é rica em vitaminas do complexo A, C, D e principalmente B (tiamina, riboflavina, niacina, adermina) e baixa porcentagem de gordura. Além de ser rico em minerais como Ca, P e Fe, amido, aminoácidos essenciais e propriedades medicinais que garantem seu uso na farmacologia principalmente para síntese de cortisona e hormônios esteroides (MESQUITA, 2002; OLIVEIRA *et al*., 2006; OLIVEIRA *et al*., 2011). Todas essas características junto ao valor comercial do rizóforo, fazem com que o inhame tenha uma ampla aceitação e consumo, participando da dieta alimentar da população.

O inhame é uma cultura de grande importância econômica para países africanos, algumas regiões da Ásia, Índia, Japão e países do Caribe. Entretanto o continente africano domina o cenário da produção mundial, correspondendo a 80% da produção da cultura (FAO, 2021). O Brasil ocupa a 13º posição, com centro de cultivo principalmente na região Nordeste, com produção de aproximadamente 38,2 mil toneladas (SEPLANDE, 2012; MOURA, 2016).

O Nordeste do Brasil é detentor de 90% da produção nacional, sendo os estados da Paraíba, Pernambuco, Bahia, Alagoas e Maranhão os que possuem maior produção (SEPLANDE, 2012). A concentração da produção nessa região se deve ao cultivo ter grande importância como base da alimentação regional, devido ao valor agregado de suas rizofóros em relação a outras fontes de carboidrato, como batata-doce e mandioca, e pela alta necessidade de mão-de-obra durante seu ciclo, principalmente na colheita, gerando emprego e renda (OLIVEIRA *et al*., 2002). Grande parte dessa produção é encaminhada para o comércio interno, sendo as rizofóros de melhor qualidade destinadas à exportação. Os Estados Unidos, Reino Unido, Países Baixos, Canadá e França destacam-se como os principais importadores (MESQUITA, 2002).

Apesar da importância econômica e social que o inhame representa para a região Nordeste, sua produtividade ainda é considerada baixa (10.500 kg ha^{-1}). Esta baixa produtividade é decorrente de vários fatores, como baixa fertilidade do solo, baixo nível tecnológico dos produtores, manejo inadequado da cultura, uso de rizofóros-sementes de baixa qualidade e problemas fitossanitários (SANTOS et al., 2009). Dentre os problemas fitossanitários, a ocorrência de doenças de origem biótica, caracteriza um dos principais problemas para baixa produtividade.

1.2 Doenças na cultura do inhame

Durante o seu desenvolvimento, o inhame é acometido por diversas doenças que irão reduzir sua produção e produtividade. Os patógenos responsáveis por essas doenças infectam desde a parte aérea como folhas, hastes e pecíolos até a parte subterrânea, rizóforos ou túberas (NORONHA, 2014).

Entre as doenças causadas por nematóides têm-se: as meloidoginoses [*Meloidogyne incognita* Kofoid & White, Chitwood, *M. javanica* (Treub) Chitwood e *M. arenaria* Neal, Chitwood], e a casca preta ou podridão seca [*Scutellonema bradys* (Steiner & Lehew) Andrassy, *Pratylenchus coffeae* (Zimmermmann) Filipjev & Schuurmans Stekhovem e *P. brachyurys* (Godfrey) Filipjev & Schuurmans Stekhoven]. Dentre as doenças fúngicas

infectando os rizóforos/túberas e causando podridões têm-se a podridão verde [*Penicilium sclerotigenum* Yamam], podridão aquosa [*Rhizopus oryzae* Went & Prins. Geerl e *Sclerotium rolfsii* Sacc.], as de parte aérea, como a pinta preta [*Curvularia eraglostidis* (Henn, Meyer)] e a antracnose [*Colletotrichum* spp.], além do mosaico do inhame infectando as folhas, pelo vírus *Yam mosaic vírus* [YMC] (NORONHA, 2014). Dentre as doenças foliares, a antracnose é uma das responsáveis por ocasionar as perdas mais expressivas na cultura.

1.3 Antracnose do inhame

A antracnose do inhame causada pelo fungo do gênero *Colletotrichum* Corda é um dos principais fatores que contribuem para redução da produção da cultura (NWANTIKI; ENE, 1984; WINCH, *et al*., 1984). Embora muitas vezes negligenciada e não sendo vista como um grande problema fitossanitário, a antracnose já foi relatada em alguns países ocasionando grandes perdas na cultura. Em Rajasthan, na Índia, epidemias levaram a perdas de safra de até 80% (PRASAD; SINGH, 1966, 1967). Perdas semelhantes foram registradas no Caribe (FOURNET *et al*., 1974; GOODING; HOAD, 1967). Na Nigéria, onde concentra mais de 70% da produção mundial de inhame, a antracnose foi encontrada em todo o cinturão de produção do país (GREEN, 1998; FAO, 2021). No Brasil, não existem estudos quantificando as perdas de produção geradas por essa doença.

A antracnose se manifesta através de manchas acastanhadas arredondadas, encharcadas e afundadas ou padrões formados por anéis concêntricos devido à massa de conídios. Infecções severas resultam em desfolha da planta, morte das nervuras e podridão seca da túbera (FOKUNANG *et al*., 2000). A doença ainda causa diminuição da área de superfície fotossintética das plantas de inhame, e consequentemente reduz o acúmulo de substâncias nas túberas, que são o produto comercial (ABANG *et al*., 2003). Devido à redução da área fotossintética da planta, epidemias que começam antes ou durante a formação do tubérculo podem resultar em perdas de rendimento superiores a 85% (GREEN, 1994).

O ciclo da doença inicia a partir da germinação de esporos na superfície do tecido hospedeiro, seja na folha, caule ou túbera, formando estruturas chamadas de apressórios. Em seguida, ocorre a penetração no tecido hospedeiro, onde as hifas primárias de infecção infectam as células, sendo esse conhecido como o estágio biotrófico da infecção, onde não são observados nenhum sintoma (Figura 1) (SILVA *et al*., 2017). Após essa fase, segue-se para fase necrotrófica, onde serão formadas hifas secundárias, que surgem a partir das hifas primárias e iniciam a colonização das células vizinhas. Nessa fase, ocorre o desenvolvimento de lesões nos locais de infecção sendo observados o início dos sintomas. Essas lesões são

resultado da secreção de enzimas e de metabólitos secundários fitotóxicos que degradam a parede celular e matam as células hospedeiras (AMUSA *et al*., 1993). Por fim, os conídios são formados na superfície do tecido infectado e, em seguida, são dispersos pelo ar, chuvas ou insetos para iniciar outro ciclo de infecção (SILVA *et al*., 2017; SHARMA; KULSHRESTHA, 2015; NTUI *et al*., 2021).

Como forma de sobrevivência, *Colletotrichum* spp. persistem em restos culturais, túberas infectadas e hospedeiros alternativos. Essas formas poderão servir como fonte de inóculo para o ciclo seguinte na próxima estação (ABANG *et al*., 2003; GREEN, 1994). Dentre os hospedeiros alternativos, tem-se plantas daninhas como *Acalypha ciliata* Forssk, *Calapogonium mucunoides* Desv., *Chromolaena odorata* L., *Commelina* spp. L., *Euphobia heterophylla* L., *Ipomoea triloba* L. e *Spigelia anthelmia* L. (ALLEYNE, 2001).

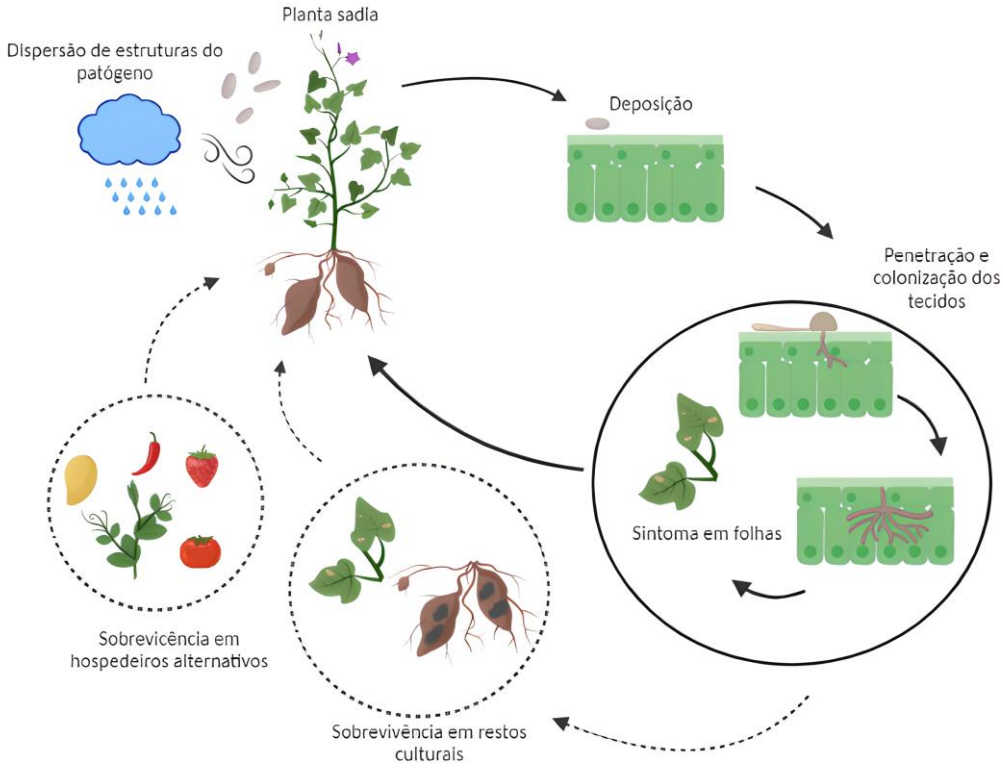

Figura 1 – Ciclo da antracnose do inhame ocasionada por *Colletotrichum* spp. (Ilustração produzida utilizando o Biorender, por Autor, 2023).

Algumas espécies de *Colletotrichum* já foram relatadas ocasionando antracnose em *Dioscorea* no mundo. Isolados provenientes de países africanos foram identificados como: *C. alatae*, *C. fructicola*, e *C. siamense*; (WEIR *et al*., 2012). Na China, *C. alatae* (LIN *et al*., 2018). Na Colômbia, *C. gloeosporioides* e em Porto Rico, *C. truncat*um (FUENTES, 2015). No Brasil, *C. siamense* (SOUSA JUNIOR; ASSUNÇÃO 2021) e *C. karsti* (AMARAL *et al*., 2023). Entretanto as espécies relatadas até então no Brasil, não são representativas, uma vez

que a amostragem realizada para essas identificações foi limitada e pontual. As espécies foram amostradas de pequenos campos de cultivo nos estados do Ceará e Paraíba. Diante disso, a diversidade de espécies de *Colletotrichum* causando antracnose no inhame no Brasil é desconhecida.

1.5 O gênero *Colletotrichum*

O gênero *Colletotrichum* caracteriza-se por possuir estilo de vida hemibiotrófico durante sua interação com o hospedeiro. O início da infecção é estabelecido por uma rápida fase biotrófica assintomática, seguida da fase necrotrófica destrutiva (JAYAWARDENA *et al*., 2016; VELOSO *et al*., 2022). Esse gênero inclui elevado número de espécies com expressiva importância econômica, que levam à redução da produção de muitas plantas em regiões tropicais e temperadas (ZHOU *et al*., 2023).

Colletotrichum ocupa o oitavo lugar em importância econômica dentre os fungos que acometem plantas em todo mundo. Essa importância está relacionada a praticamente todas as culturas cultivadas serem acometidas por uma ou mais espécies de *Colletotrichum* (DEAN *et al*., 2012).

O gênero foi descrito pela primeira vez por Corda em 1831, pertencendo ao filo Ascomycota, classe *Sordariomycetes*, ordem *Glomerellales* e família *Glomerellaceae*. Possui como características morfológicas micélio septado, conídios hialinos e unicelulares, com conídios produzidos em acérvulos e durante a fase sexuada produção de peritécios de coloração marrom a marrom escuro. A colônia pode apresentar diferentes tonalidades, e pode ocorrer a presença de escleródios. Em 1837, Corda caracterizou a morfologia dos conídios como hialinos, retos e fusiformes ou curvados (SUTTON, 1992; WEIR; JOHNSTON; DAMM, 2012).

Anterior a essa classificação, o gênero passou por várias outras classificações, sendo descrito como *Vermicularia* T. (TODE, 1790). Outra classificação foi como *Gloeosporium* M. (MONTAGNE, 1849) sendo adotado durante todo o final do século XIX e início do século XX. Mais outros 10 sinônimos genéricos para *Colletotrichum* foram listados por Sutton (1980) porém nenhum está de uso recente. Entretanto, em 1957 Von Arx concluiu que as espécies de *Colletotrichum*, *Vermicularia* e *Gloeosporium* deveriam ser sinonimizadas no gênero *Colletotrichum*.

Como consequência dessa confusão taxonômica, muitas espécies passaram a ser consideradas pertencentes ao gênero *Colletotrichum*, porém a monografia de Von Arx (1957) teve grande impacto para uma nova era da taxonomia do gênero. Sua abordagem com base em

caracteres morfológicos levou a uma redução de espécies aceitas de 750 para 11 (CANNON *et al*., 2012; DEAN *et al*., 2012). Essa redução foi possível devido a desassociação de espécies a hospedeiros específicos. Posteriormente, esse número aumentou para 40 com o uso de um sistema de chave de identificação, baseada em caracteres morfológicos, especialmente formato dos apressórios e conídios (SUTTON, 1980; 1992).

A identificação com base em caracteres morfológicos foi amplamente utilizada por longos anos, junto a isso características geográficas e ecológicas também eram levadas em consideração. Porém a identificação baseada nessas características se torna problemática devido à plasticidade e variação induzida por condições experimentais (MARIN-FELIX *et al*., 2017; VEIRA *et al*., 2017).

O primeiro passo para a mudança em relação as características e métodos que deveriam ser adotados para identificação de espécies do gênero *Colletotrichum* ocorreu em 1990 com o Workshop Internacional sobre *Colletotrichum*, onde reuniu vários especialistas de diversas áreas marcando o início da aplicação em larga escala de métodos moleculares nos estudos de *Colletotrichum* (BAILEY; JEGER, 1992).

Os primeiros estudos filogenéticos de *Colletotrichum* usando sequências de DNA foram publicados por Mills *et al*. (1992) e Sreenivasaprasad *et al*. (1992). Esses estudos identificaram variação de sequência na região ITS1 do mtDNA (Espaçador transcrito interno do DNA mitocondrial) entre seis espécies de *Colletotrichum*, bem como detectou polimorfismos na mesma região entre isolados de *C. gloeosporioides* de diferentes hospedeiros. Embora a região ITS seja a região amplamente sequenciada e escolhida como o locus do código de barras para os fungos, essa região se mostra ineficiente e limitada para estudos sistemáticos desse gênero (VIEIRA *et al*., 2017). Devido a esse fato, em 2002, foi publicada uma das primeiras análises multilocus, utilizando além do ITS, os genes TUB2 (beta-tubulina) e HIS4 (histona 4) (TALHINHAS *et al*., 2002). Guerber *et al*. (2003) usaram sequências de nucleotídeos gliceraldeído3-fosfato desidrogenase (GAPDH) e glutamina sintetase (GS) em um estudo posterior com *C. acutatum*, e a seção HMG-box dos genes de compatibilidade sexual MAT-1 foi considerada um valioso marcador evolutivo (DU *et al*., 2005).

Outro marco na sistemática desse gênero foi alcançado com a publicação de uma edição especial da revista Fungal Diversity no final de 2009, onde uma revisão baseada no critério morfológico e na abordagem filogenética multilocus, resultou em 66 nomes de espécies de *Colletotrichum* enfatizando a necessidade do uso de métodos moleculares (DAMM *et al*., 2009; HYDE *et al*., 2009). Ainda nessa edição da revista, Cai *et al*. 2009

propõem uma abordagem polifásica para o reconhecimento e identificação de espécies dentro de *Colletotrichum*, combinando distinção genética com caracteres morfológicos e biológicos informativos. Além de fornecer um conjunto de protocolos e metodologias como guia para futuros estudos, epitipificação e descrição de novas espécies. Posteriormente, foram introduzidas mais 41 espécies ao gênero (CANNON *et al*., 2012).

Alguns estudos foram publicados com diferentes grupos de regiões gênicas, como tentativas de estabelecer metodologias (DAMM et al., 2009; PHOULIVONG *et al*., 2010). Porém a última sinopse do gênero *Colletotrichum* contabilizou 280 espécies, das quais 265 estão distribuídas em 16 complexos (10 em *C. caudatum sensu lato*, 21 em *C. graminicola s. l.*, 5 em *C. bambusicola s. l.*, 11 em *C. spaethianum s.l.*, 20 *em C. destructivum s.l.*, 48 em *C. acutatum s.l.*, 21 em *C. dematium s.l.*, 12 em *C. gigasporum s.l.*, 56 em *C. gloeosporioides s.l.*, 29 em *C. boninense s.l.*, 5 em *C. agaves s.l.*, 5 em *C. truncatum s.l.*, 12 em *C. dracaenophilum s.l.*, 11 em *C. orchidearum s.l.*, 9 em *C. magnum s.l.*, 8 em *C. orbiculare s.l.*) e 15 não estão associadas a nenhum complexo (LIU *et al*., 2022).

Os complexos de espécies do gênero *Colletotrichum* compreendem as espécies crípticas, ou seja, espécies morfologicamente semelhantes, porém filogeneticamente distintas. Os complexos de espécies são os principais clados fortemente suportados na árvore desse gênero. O nome do complexo de espécies é dado pela espécie mais conhecida desse complexo ou que foi descrita pela primeira vez. Em alguns casos, os membros de um determinado complexo de espécies compartilham características peculiares dos conídios: *C. acutatum* - conídios com extremidades agudas; *C. boninense* - presença de cicatriz proeminente (hilo) na base do conídio; *C. caudatum* - conídios com apêndice filiforme no ápice; *C. gigasporum* - conídios mais longos e mais largos do gênero (VIEIRA *et al*., 2020).

Atualmente para diferenciação das espécies inseridas nos complexos do gênero *Colletotrichum*, utiliza-se a filogenia multilocus (MARIN-FELIX *et al*., 2017). Diversas tentativas foram feitas com o objetivo de determinar qual conjunto de marcador seria ideal para identificação de espécies de *Colletotrichum*, com ênfase para o complexo *C. gloeosporioides* (CAI *et al*., 2009; SILVA *et al*., 2012; VIEIRA *et al*., 2017). Porém, recentemente o estudo de Vieira *et al*. (2020) estabeleceu os melhores marcadores para delimitação de espécie em 13 dos 16 complexos existentes para o gênero *Colletotrichum*. Entretanto, não está sendo observado nas últimas publicações a utilização desses marcadores propostos por Vieira *et al*. (2020), pois os autores tendem a utilizar marcadores de escolha própria sem justificativa da escolha.

1.7 Epidemiologia comparativa de espécies

As doenças de plantas são resultadas da interação entre o hospedeiro suscetível, patógeno virulento e ambiente favorável. Qualquer modificação em algum desses fatores irá culminar em alterações na ocorrência, na intensidade ou desenvolvimento da doença. Uma vez estabelecido o contato entre o patógeno virulento e o hospedeiro suscetível, os fatores ambientais serão determinantes para que a doença ocorra ou não. Dentre esses fatores tem-se: temperatura, umidade, luz, nutrientes e o pH, porém com destaque para a temperatura e umidade (AGRIOS, 2005).

O desenvolvimento mais rápido de uma doença, ou seja, o menor tempo necessário para a conclusão do ciclo da doença, geralmente ocorre quando a temperatura é ótima para o desenvolvimento do patógeno. O progresso de uma doença é dificultado em temperaturas muito abaixo ou acima do valor ótimo que permite o desenvolvimento do patógeno. (AGRIOS, 2005).

Para a antracnose do inhame, os fatores climáticos exercem papel fundamental no estabelecimento de *Colletotrichum* sobre o tecido hospedeiro. A umidade afeta a sobrevivência do inóculo, a disseminação e o processo infeccioso do patógeno (AMORIM *et al*., 2018). Enquanto a temperatura influencia no crescimento micelial, esporulação e germinação dos conídios. Em geral, a infecção por espécies de *Colletotrichum* é favorecida em temperaturas entre 20 e 30 ° C (FITZELL *et al*., 1984; ARAUZ, 2000), porém esses valores variam de espécie para espécie. Por exemplo, Poltroinere *et al*. (2013) constataram que isolados de *C. gloeosporioides* obtidos de palmeira obtiveram temperatura de crescimento ótimo na faixa de 24,6 a 28ºC. Enquanto as temperaturas ótimas para a germinação dos conídios e a formação de apressório de *C. acutatum* e *C. gloeosporioides* em bagas de café variaram entre 25 a 31 °C (KENNY *et al*., 2012). No estudo de Maia *et al*. (2011), a temperatura ótima de crescimento e esporulação para *Colletotrichum* spp. oriundos de frutos sintomáticos de manga foram temperaturas entre 20 e 25ºC.

De maneira geral, doenças ocasionadas por espécies do gênero *Colletotrichum* ocorrem de forma mais severa sob condições chuvosas, temperaturas elevadas e alta umidade (LIMA, 2013). Não obstante, a antracnose do inhame é favorecida por longos períodos de chuva pois os conídios do fungo são espalhados por respingos de chuva. Um grande número de conídios é formado em massas mucilaginosas de coloração variada, sobre folhas e demais órgãos infectados. Surtos graves se desenvolvem em variedades suscetíveis após tempestades. Os esporos são formados em grande número nas manchas das folhas e são salpicados pela

chuva e/ou carregados pelo orvalho que goteja para as folhas e caules adjacentes e inferiores. (JACKSON; NEWHOOK; WINCH, 2002).

Sweetmore *et al*. (1994), Green (1994) e Ekefan (1996) investigaram os aspectos temporais e espaciais do desenvolvimento da antracnose no inhame. Epidemias de antracnose foram iniciadas a partir de focos distribuídos aleatoriamente e a disseminação secundária da doença a partir desses focos resultou em um padrão espacial agregado que finalmente se tornou uniforme. A taxa de progressão através desta sequência de padrões espaciais foi afetada pela suscetibilidade da cultivar, idade da folha, idade da planta, estágio da epidemia, pluviosidade e práticas agronômicas como a aplicação de fungicidas (SWEETMORE *et al*., 1994; WHARTON, 1994).

Os patógenos de plantas respondem a mudanças ambientais a quais estão inseridos, e isso está atrelado a numerosas populações que emergem em um curto intervalo de tempo. Não obstante espécies de *Colletotrichum* têm capacidade de adaptação a diferentes condições climáticas, justificando sua ampla distribuição no mundo e em relação à amplitude térmica (SUTHERST *et al*., 1996; DIAS *et al*., 2005).

Em patossistemas agrícolas, a ação do homem é culminante para que a doença ocorra. Essa interferência pode se dá por exemplo através da introdução de fungicidas. Os fungicidas podem atuar como marcadores fenotípicos permitindo diferenciar populações de *Colletotrichum* spp. enquanto a sensibilidade diferencial a fungicidas irá auxiliar na caracterização da variabilidade entre isolados de uma mesma espécie (ADASKAVEG; FOSTER, 2000). Diferenças em relação a sensibilidade principalmente em áreas onde ocorre populações de diferentes espécies de *Colletotrichum*, implicam diretamente em seu manejo (LIMA *et al*., 2015). Isolados de *C. gloeosporioides* oriundos de campos de pesquisa de inhame na Nigéria, apresentaram diferentes níveis de sensibilidade aos fungicidas benomil, tiabenzol e procloraz (ABANG, 2003). Não se tem estudos sobre a sensibilidade a fungicidas de espécies de *Colletotrichum* oriundos de plantios de inhame no Brasil.

O conhecimento dos componentes epidemiológicos de uma epidemia é de extrema importância, pois possibilita a melhor compreensão dos mecanismos que governam a doença em uma população possibilitando a definição de estratégias de manejo mais eficientes.

Com a descoberta de novas espécies de *Colletotrichum*, é importante estabelecer se essas espécies são específicas do hospedeiro, se infectam uma ampla gama de hospedeiros e se há variabilidade na virulência em função dos cultivares e das condições ambientais, bem como estabelecer suas diferentes sensibilidades aos fungicidas. Esses fatores têm implicações

direta para o manejo da doença com base no controle químico, na resistência genética do hospedeiro e nas estratégias de quarentena (PHOULIVONG, 2011).

Diante disso, os objetivos do presente estudo foram: 1) identificar espécies do gênero *Colletotrichum* associadas à antracnose do inhame no Brasil, com o uso de marcadores moleculares de maior precisão na identificação de espécies; 2) determinar a prevalência das espécies de *Colletotrichum* associadas a antracnose do inhame; e 3) Comparar as espécies de *Colletotrichum* associadas a antracnose do inhame de acordo com os componentes epidemiológicos agressividade, temperatura e sensibilidade a fungicidas.

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CAPÍTULO II

Diversity of *Colletotrichum* species associated with yam anthracnose

Submissão: **Fungal Biology**

Diversity of *Colletotrichum* species associated with yam anthracnose

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Abstract

Anthracnose caused by *Colletotrichum* species is one of the most important diseases of yam (*Dioscorea* spp.). The disease affects the photosynthetic area of the plant, directly interfering with the tuber development, the commercial product. This study aimed to characterize *Colletotrichum* spp. associated with yam anthracnose in Brazil and evaluate the pathogenicity and aggressiveness of the isolates obtained. The isolates obtained were also evaluated for their prevalence by sampled region and species of *Dioscorea*. A total of 105 *Colletotrichum* isolates from the states of Alagoas, Paraíba, Pernambuco and the Federal District were obtained. The isolates were identified using molecular techniques. Pathogenicity tests were performed on yam plants cv. "Da Costa" with representative isolates. Phylogenetic analyzes based on eleven loci (ACT, APN2, APN2/MAT-IGS, CAL, CHS-1, GAPDH, GAP2-IGS, HIS-3, nrITS, GS e TUB2) revealed that the isolates belong to 8 species belonging to the 5 species complexes: *C. boninense*, *C. gloeosporioides*, *C. orchidearum*, *C. magnum* and *C. truncatum*. The species were identified as: *C. chrysophilum*, *C. karsti*, *C. plurivorum*, *C. queenslandicum*, *C. siamense*, *C. sojae*, *C. tropicale* and *C. truncatum*. Two new species are described here as *C. inhamicola* sp. nov. and *C. vitoriense* sp. nov.. *Colletotrichum plurivorum* was prevalent in the sampled regions. Pathogenicity assays showed that all isolates were pathogenic in yam plants. This is the first report of the species *C. chrysophilum*, *C. plurivorum*, *C. queenslandicum*, *C. sojae*, and *C. tropicale* associated with yam anthracnose worldwide. Our results contribute to the understanding of the diversity of *Colletotrichum* species associated with yam anthracnose and provide input for the development of management strategies.

Keywords: Agressiveness, *Dioscorea* spp., multilocus, prevalence, phylogeny.

Introduction

Yam (*Dioscorea* spp.) is a tuberose that has great economic and social importance in the regions where it is cultivated, serving as the basis of the population's diet (Oliveira et al. 2011). The biggest yam producer in the world is Nigeria, which is responsible for more than 65% of world production. Brazil occupies the 13th position, with its cultivation center mainly in the Northeast region, with a production of approximately 252,878 thousand tons (FAO 2021). The genus *Dioscorea* is composed of more than 600 species, of which it has cultivated and non-cultivated species. Among the cultivated species, the predominant species in Brazil are *D. cayennensis*, and *D. alata*, which are widely used in food (Bressan, 2005; Lebot, 2009).

During its development, the crop can be affected by diseases that affect its production and productivity. Yam anthracnose, caused by *Colletotrichum* spp. can cause losses of more than 80% of production (Singh and Prasad 1966, 1967). The disease manifests itself through small blackened lesions on the leaves that tend to coalesce to form large necrotic areas. Due to these lesions, there is a decrease in the photosynthetic area of the plant that affects the development of the tuber (Jackson et al. 2002; Achar et al. 2013).

Yam anthracnose was first reported in 1908 and classified as the causal agent *Gloeosporium pestis* from yam leaves in Fiji (Massee 1908). Later the pathogen was classified as *C. gloeosporioides* (Abang et al. 2001). The *Colletotrichum* species already listed as associated with yam anthracnose in African countries are *C. alatae*, *C. siamense*, *C. fructicola* and *C. truncatum* (Weir et al. 2012; Fuentes, 2015). The species *C. siamense* and *C. karsti* were related to Brazil, occasioning anthracnose in yam in the states of Pernambuco and Paraíba, in the Northeast region of the country (Souza and Asssunção 2021; Amaral et. al. 2023)

Although these studies were important to elucidate species of *Colletotrichum* associated with yams in Brazil, the sampled area was small and therefore not representative.

Since the correct taxonomic identification is important to disease management (Arce et al. 2019). Thus, the aim of the present study was: i) to identify *Colletotrichum* species associated with yam anthracnose in Brazil; ii) to evaluate the *Colletotrichum* species prevalence; iii) and to compare the aggressiveness of *Colletotrichum* species in yam plants.

Material and Methods

Sampling and isolation

Yam leaves with symptoms of anthracnose were collected between January and December 2020. Samples were collected from fields locates in four Brazilian states [Alagoas (Arapiraca, Campestre, Feira Grande, Limoeiro and Novo Lino), Pernambuco (Bonito, Recife and Vitoria de Santo Antão), Paraíba (Alhandra, Conde and Marcação)], and from Distrito Federal (Gama). Fragments between necrotic and healthy tissues were taken from symptomatic leaves, surface disinfected in 70% ethanol for 30 s and 1.5% sodium hypochlorite for 1 min, then rinsed three times with sterile distilled water. Fragments were plated onto potato dextrose agar (PDA) medium (Merck Millipore) amended with tetracycline (100 μLmL-1). Plates were incubated at 25 ◦C for 5 days under continuous light. Colonies presenting morphology similarity with *Colletotrichum* (Suton, 1980) were grown on pure culture and stored in cryogenic tubes with sterile distilled water (Figueiredo, 1967). Isolates were preserved and deposited in the working collection of “Laboratório de Micologia” (LM) at Universidade Federal Rural de Pernambuco (UFRPE), Recife, Pernambuco, Brazil. Holotypes from novel species were deposited in “Herbário do Departamento de Biologia Vegetal” (VIC) herbarium, and ex-type cultures in “Coleção Octávio de Almeida Drummond” (COAD) culture collection.

DNA extraction, PCR and sequencing

Colletotrichum isolates were grown on PDA media at 25 ± 2 °C for 7 days and a 12 h light. Genomic DNA was extracted using the CTAB (cetyl trimethyl ammonium bromide) protocol described by Doyle and Doyle (1990).

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) partial region was amplified for all isolates to identify haplotype diversity through DnaSP v.6 (Librado and Rozas 2009). One isolate was randomly chosen from each haplotype to proceed to multilocus analysis. Sequences obtained were compared with those from GenBank using the BLAST tool.

The following loci were amplified for multilocus analyses: actin (ACT), DNA lyase (APN2), the intergenic spacer between DNA lyase and the mating-type locus MAT1-2-1 (APN2/MAT-IGS), calmodulin (CAL), chitin synthase (CHS-1), the intergenic spacer between GAPDH and a hypothetical protein (GAP2-IGS), glutamine synthetase (GS), histone (HIS3), internal transcribed spacer (ITS) and β-tubulin (TUB2). These genomic regions are reported to be the most informative for identifying species from several *Colletotrichum* species complexes (Vieira et al. 2020).

PCR amplifications were performed in a 12.5 μL volume reaction containing 4 μL PCR-grade water, 1 μL DNA template, 0.625 μL each primer (10 μM), and 6.25 μL 2× PCR master mix (Promega GoTaq Master Mix; Madison, Wisconsin, USA). PCR products were visualized in a 1.5% agarose/ TAE gel, by electrophoresis. The primers and cycles used in this study are listed in Table 1.

PCR products were purified by ethanol and ammonium acetate precipitation, and sequenced on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, California, USA) on a 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 2).

Phylogenetic analyses

Sequences from *Colletotrichum* ex-type and reference isolates from previous studies were retrieved from GenBank and included in phylogenetic analyses (Table S1). Multiple sequence alignments (MSA) were generated with the online version of MAFFT 7 with the Q-INS-i iterative refinement method (Katoh et al. 2002; Katoh and Toh 2013). For the multilocus analysis, the loci were concatenated using Sequence Matrix v. 1.8 (Vaidya et al. 2011).

A phylogeny for each locus and concatenated alignments were inferred using maximum likelihood (ML) and Bayesian inferences. ML analyses were performed using IQ-TREE v. 2.1.2 (Nguyen et al. 2015), keeping identical sequences in the alignment. The ML tree search was estimated using a specific substitution model for each locus. Model parameters were separately estimated for each partition using ModelFinder (Kalyaanamoorthy et al. 2017; Minh et al. 2020), allowing each partition to have its evolution rate (-m MFP -p). The best ML tree was found after 1000 iterations with a perturbation strength of 0.2. ML analyses were carried out 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 Markov chain Monte-Carlo (MCMC) chains were run for $3x10^6$ generations, sampling every 1000 generations. Convergence of all parameters was checked in the summary file (Estimated Sample Size – EES $\geq$ 100, and Potential Scale Reduction Factor – PSRF $\cong$ 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 ≥ 80 and BI posterior probability ≥ 0.95.

Species were recognized utilizing the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) criteria, as described by Taylor et al. (2000) and Dettman et al. (2003).

Morphological characterization

For phenotypic characterization of the new species, 5 mm diameter mycelial plugs were taken from the margin of 7-day-old colonies and transferred to the center of Petri dishes containing PDA. Plates were incubated at 25°C and continuous light. Orthogonal diameters were measured every 24 hours and used to calculate the mycelial growth rate (mm day-1). The culture was maintained in the same incubation conditions and the colony features were recorded from 7-day-old colonies. Rayner's chart (1970) was followed for colony colors.

The conidiogenous cells, conidiophores, conidia, and setae from PDA were prepared for examination under a microscope by mounting them in a solution of 10% lactic acid. A modified version of the slide culture technique developed by Johnston and Jones (1997) was used to induce the formation of appressoria. In this method, a small block of agar-agar medium (4%) was placed on a sterile microscope slide, and conidial masses were spread on the top edges of the block. The block was then covered with a sterile coverslip and left to incubate in the dark at a temperature of 25°C for 24 hours. Microscopic images of the samples were captured using a Nikon Eclipse Ni-U transmitted light microscope equipped with a DS-L3 digital camera. Summary statistics were calculated using Statistix 9.

Prevalence of *Colletotrichum* species

The prevalence of *Colletotrichum* spp. was determined by the formula: P (%) = (Cx/Ct) × 100, where P represents prevalence, Cx is the number of isolates of the same species, and Ct is the total number of isolates. Prevalence was also calculated by state and species of *Dioscorea*.

Pathogenicity and aggressiveness

Pathogenicity and aggressiveness tests were carried out on 45 days old yam plants, cultivar "da Costa", with isolated representatives of the species (Table 3). Four fully developed leaves were previously wound in two points with sterile need. Ten microliters of conidial suspension (10^6 conidia mL^{-1}) were deposited onto wounds. The negative control was represented by deposition of the 10 μL sterile distilled water on leaves. Plants were kept in a moist chamber for 48 hours and incubated at 25ºC. Pathogenicity was confirmed by the presence of typical yam anthracnose lesions. The aggressiveness was evaluated 4 days after inoculation and represented the mean of the orthogonal diameters of the lesions (mm). The experiment was conducted with four replicates, each replicate represented by one leaf with 2 inoculation points. 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 isolation

A total of 105 *Colletotrichum* isolates were obtained from yam leaves with typical symptoms of anthracnose. Of these isolates, 23 were obtained from Alagoas, 37 from Paraíba, 28 from Pernambuco, and 17 from Distrito Federal.

Phylogenetic analyses

A preliminary analysis among the 105 isolates using partial sequences of GAPDH identified the presence of 29 haplotypes among *Colletotrichum* yam isolates. The BLAST indicated that isolates presented high similarity with species from five of *Colletotrichum* species complex: 2 haplotypes in *C. boninense sensu lato*; 16 haplotypes in *C. gloeosporioides* s. l.; 1 haplotype *C. magnum* species s. l.;7 haplotype *in C. orchidearum s. l.*; and 3 haplotypes in *C. truncatum s. l.*

Colletotrichum isolates from yam were resolved into ten lineages in the multilocus tree and assigned to eight previously described species according to GCPSR criteria, while two strains were not aligned in a clade containing the former type of a previously described species.

One isolate was aligned within the *C. chrysophilum* clade, two within the *C. queenslandicum* clade, and eleven within the *C. siamense* clade in the multilocus analysis with strong support of support for both the ML and BI analyses. The *C. tropicale* clade was recovered in most single-gene trees with strong support in both analyses (Figure 2). Two isolates were aligned within the *C. karsti* clade in the multilocus analysis with strong support for both the ML and BI analyses (Figure 1). Five isolates were aligned within the *C. plurivorum* clade and one isolates within the *C. sojae* clade in the multilocus analysis with strong support (Figure 3). Three isolates were aligned within the strongly supported *C. truncatum* clade in both multilocus analyses (Figure 5). Two lineages did not group with the type of any previously described species and are described in the Taxonomy section. These new species are closely related to *C. musicola* and *C. cacao* respectively and were strongly supported in the ML and BI multilocus analyses (Figure 4). Thus, we are describing the novel species *C. inhamicola sp. nov*. and *C. vitoriense sp. nov*.

Based on the haplotypic diversity recovered by the GAPDH, the 105 *Colletotrichum* isolates from yam presented the following distribution within the species identified in the phylogenetic analysis: 11 isolates of *C. chrysophilum*, 1 *C. inhamicola* sp. nov., 3 *C. karsti*, 39 *C. plurivorum*, 3 *C. queenslandicum*, 27 in *C. siamense*, 1 *C. sojae*, 16 *C. tropicale*, 3 *C. truncatum* and 1 *C. vitoriense sp. nov*.

Taxonomy

Colletotrichum vitoriense A. G. G. Amaral, W.A.S. Vieira & M.P.S. Câmara, sp. nov. – Mycobank 848567; Fig. 6.

Systematic position: Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Glomerellales, Glomerellaceae

Etymology. —Named after the location where the fungus was isolated, Vitória de Santo Antão.

Type: —BRAZIL, PERNAMBUCO: Vitória de Santo Antão from foliar anthracnose lesions on *Dioscorea alata*, June 2021, A. G. G. Amaral. Holotype VIC 49407. Ex-type living culture LM808, COAD 3526.

Colonies on PDA greyish sepia and vinaceous buff in the edge aerial view, reverse hazel center become white in the edge aerial mycelium sparse, growth rate at 25°C 7.5–12 mm.dia-1 (average 9.0 ± 1.6 mm.dia-1). Conidiogenous cells hyaline, smooth-walled, cylindrical, monophialidic on 3.9–14.8 × 4.8–2.6 µm (av. 9.37 ± 2.9 × 3.6 ± 0.6 µm n = 15). Conidiophores reduced to conidiogenous cells. Conidia hyaline, aseptate, smooth-walled, cylindrical, both ends rounded 10.0–12.4 × 3.7–4.8 µm (av. 11.0 ± 0.4 × 4.3 ± 0.3 µm n = 30) length/width ratio 2.2–3.0 (av. 2.5 ± 0.2). Appressoria in slide cultures, single, medium to dark brown smooth-walled, clavate, sometimes with undulate margin, 6.5–10.1 × 4.0–8.6 µm (av. 8.1 ± 0.8 × 6.5 ± 1.0 µm n = 30) length/width ratio 0.9–1.9 (av. 1.2 ± 0.2). Setae dark brown, smooth-walled, base cylindrical, with two or more septa, tip ± rounded to slightly acute, 35.9–77.4 × 1.89–2.95 µm (av. 56.4 ± 11.7 × 3.1 ± 0.8 µm n = 30). Sexual morph not observed. Sclerotia and chlamydospores absent.

Notes — In the world, the species *C. alatae*, *C. siamense*, *C. fructicola*, *C. karsti* and *C. truncatum* have already been reported and phylogenetically identified causing anthracnose in yams (Weir et al. 2012; Amaral et al. 2023). All species previously reported for the genus *Dioscorea* belong to the *C. gloeosporioides* and *C. boninense* complex. Comparing the species *C. vitoriense* with the *Colletotrichum* species previously described, it can be seen that: the size of the conidium *C. alatae* (18.9 × 5.2 µm), *C. fructicola* (11.53 ± 1.03 × 3.55 ± 0.32

μm) and *C. karsti* (15.9 ± 1.4 ×6.8 ± 0.5 μm) are larger than those of the described new species. However, *C. siamense* presented smaller conidia (10.18 ± 1.74 × 3.46 ± 0.36 μm) than *C. vitoriense* (Weir et al. 2012; Prihastuti et al. 2009). These species are not easily phenotypically distinct from *C. vitoriense* which belongs to the *C. orchidearum* complex. Phylogenetically, though according to BLAST, *C. vitoriense* is closest to *C. musicola* and *C. sojae* but it clearly differs from both by analysis multi-locus (Figure 6). *Colletotrichum vitoriense* is recovered as monophyletic for most sequenced genes, except for CHS-1. Morphologically, *C. vitoriense* has smaller conidia when compared to *C. musicola* and *C. sojae* (14.5 ± 2.1 × 5.2 ± 0.5; 16.5 ± 1.5 × 4.9 ± 0.3 μm).

Colletotrichum inhamicola A. G. G. Amaral, W.A.S. Vieira & M.P.S. Câmara, sp. nov. – Mycobank 849394; Fig. 7.

Systematic position: Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Glomerellales, Glomerellaceae

Etymology. —The specific epithet refers to the common name of *Dioscorea* spp.

Type: —BRAZIL, DISTRITO FEDERAL: Gama from foliar anthracnose lesions on *Dioscorea trifida*, July 2021, A. Reis. Holotype VIC 49408. Ex-type living culture LM1118, COAD 3527.

Colonies on PDA greyish sepia and vinaceous buff in the edge aerial view, reverse hazel center become white in the edge aerial mycelium sparse, growth rate at 25°C 8.7–10.5 mm.dia-1 (average 9.4 ± 0.7 mm.dia-1). Conidiogenous cells smooth-walled, cylindrical to ampulliform, monophialidic on 21.9–5.58 × 4.2–2.7 μm (av. 15.1 ± 4.5 × 3.4 ± 0.4 μm n = 16). Conidiophores reduced to conidiogenous cells. Conidia one-celled, aseptate, smooth-walled, hyaline, cylindrical with rounded ends and sometimes oblong 12.6–8.8 × 5.2–3.6 μm (av. 11.0 ± 0.8 × 4.3 ± 0.3 μm n = 30) length/width ratio 2.9–2.0 (av. 2.6 ± 0.3). Appressoria in slide cultures, single, medium brown, smooth-walled, elliptical, subcircular or irregular in

outline, with an undulate to lobate margin, 9.2–6.2 × 6.7–3.8 µm (av. 7.7 ± 0.7 × 5.8 ± 0.7 µm n = 30) length/width ratio 2.0–1.0 (av. 1.3 ± 0.2). Setae medium brown, base somewhat inflated, with one or two septa, tip ± rounded to slightly acute, 78.0–42.4 × 25.1–16.4 µm (av. 61.3 ± 10.5 × 21.4 ± 2.4 µm n = 15). Sexual morph not observed. Sclerotia and chlamydospores absent.

Notes — Colletotrichum inhamicola was recovered as monophyletic in four of the five individual gene trees. This species is closely related to the sister species *Colletotrichum cacao*. The ACT, CHS-1 and HIS-3 sequences had highest similarity *C. cacao* CBS119297, identity = 98.7%, 100% and 99.2% respectively. The GAPDH sequences had highest similarity to *C. okinawense* LM140, identity = 99.1%. The nrITS sequences had highest similarity to *Colletotrichum* sp. CZPMP-22, identity = 91.9%. The TUB2 sequences had highest similarity to *C. okinawense* LM139, identity = 99.2%. Morphologically, *C. inhamicola* has smaller conidia when compared to C. cacao (17–13 × 5.5–5 µm) (Dam et al. 2019).

Prevalence

Of the *Colletotrichum* isolates obtained, 21.9% came from the state of Alagoas, 26.6% from Pernambuco, 35.2% from Paraíba, and 16.1% from the Distrito Federal. *Colletotrichum plurivorum* was a prevalence (37,5%) species associated with yam anthracnose in Brazil. The other species varied between 0.96 to 26%. *Colletotrichum plurivorum* was present in all the sampled states and was also prevalent in *D. cayennensis* and *D. alata* species (Figure 8 and 9). Most of the *Colletotrichum* isolates obtained originated from the *Dioscorea* species, *D. cayennensis* (71.4%) (Figure 9).

Pathogenicity and aggressiveness

All *Colletotrichum* species were pathogenic on yam leaves. Typical symptoms of anthracnose similar to those found in the field were observed. There was a significant difference ($P<0.05$)

regarding aggressiveness. The mean diameters of the lesions vary from 11.3 to 31 mm. *Colletotrichum siamense* was the most aggressive while *C. queenslandicum* was the least aggressive (Figure 10).

Discussion

This study identified ten species of *Colletotrichum* associated with yam anthracnose in Brazil, using a polyphasic approach. *Colletotrichum inhamicola* and *C. vitoriense* were introduced as new species. This work presents a great contribution to understanding *Colletotrichum* species associated with yam anthracnose in Brazil, as it represents a broad sampling of the main producing areas. Among the *Colletotrichum* species already described associated with yams, we found *C. siamense*, *C. karsti*, and *C. truncatum* (Weir et al. 2012; Souza and Assunção 2022; Amaral et al. 2023). This is the first report of *C. chrysophilum*, *C. queenslandicum*, *C. plurivorum*, *C. sojae*, and *C. tropicale*, causing anthracnose in yams worldwide. The abundance of species observed, indicating a significant degree of diversity, could potentially be attributed to the climatic conditions of tropical regions. This observation is consistent with previous investigations conducted on agricultural crops cultivated in various tropical areas of Brazil (Lima et al. 2013; Vieira et al. 2014).

Colletotrichum plurivorum was the overall prevalent species including two of the largest Brazilian-producing states of Alagoas and Pernambuco. The species *C. plurivorum* belongs to the *C. orchidearum* species complex and was initially identified as *C. sichuanensis* from *Capsicum annuum* in the Sichuan Province of China (Liu et al., 2016). This species derives its name from its broad host range, which encompasses various plant families including *Anacardiaceae*, *Araceae*, *Caricaceae*, *Fabaceae*, *Malvaceae*, *Musaceae*, *Orchidaceae*, and *Passifloraceae* (Dam et al. 2019). In Brazil, *C. plurivorum* has already been described in *Vitis* spp. (Santos et al. 2018), *Phaseolus lunatus* (Sousa et al. 2018, Cavalcante et al. 2018), *Passiflora edulis* (Silva et al. 2022), *Abelmoschus esculentus* (Batista et al. 2020),

and *Saccharum* spp. (Marins et al. 2022). Its occurrence has been reported across three continents: Africa, America, and Asia indicating its occurrence in a broad range of climatic zones. Similarly, these diverse climatic zones are found within the Brazilian territory, where many of the host plants are cultivated (Bragard et al. 2021). It could be concluded that *C. plurivorum* has the ability to develop in different climatic conditions of Brazil, thereby explaining its distribution across the country.

Colletotrichum siamense was the second most prevalent and the most aggressive species among the species found in this study. This species was also present in all sampled states and in the main producing regions. *Colletotrichum siamense* was originally reported causing coffee berry disease in Thailand (Prihastuti et al. 2009) and was recently reported causing yam anthracnose in Brazil (Souza and Assunção 2021). This species also has a broad host range and is the most common and geographically diverse species in the *C. gloeosporioides* complex.

There are no studies evaluating the aggressiveness of *Colletotrichum* species associated with this pathosystem. However, in epidemiological studies carried out for cashew and mango in Brazil, *C. siamense* also exhibited higher aggressiveness compared to other evaluated species (Lima et al. 2015; Veloso et al. 2021). These results demonstrate the aggressive potential of this species.

The new species *C. inhamicola* and *C. vitoriense* were found in the Distrito Federal and the state of Pernambuco respectively. *Colletotrichum inhamicola* was isolated from *D. trifida*, a species of yam with inexpressive commercial production in Brazil. This yam species is grown only in the central-west region of the country. While *C. vitoriense* was isolated from *D. alata*, one of the main cultivated species. These species were found in areas of the Atlantic Forest which are known for its high genetic diversity. It is believed that the speciation process

may have occurred, where these species may have left native species and specialized in yam culture.

Although the new species were not prevalent in the sampled regions, *C. vitoriense* was the second most aggressive species. This result may indicate that the species underwent a process of specialization or preference for this host. To confirm this hypothesis, a host range test should be performed.

Pathogenicity testing using isolates from the ten species of *Colletotrichum* showed that all species were pathogenic to yam plants. The species *C. siamense* and *C. vitoriense* are considered with high pathogenic potential. Management strategies must be taken for *C. siamense* due to its wide distribution and aggressiveness. However, *C. vitoriense* should also be considered since its high virulence in the most cultivated yam variety. At this moment this species was restricted to a small area in Pernambuco state but it has the potential to spread to other areas where yam is cultivated. This is particularly important because its host range is unknown.

Our work represents an important contribution to understanding the etiology and prevalence of *Colletotrichum* species associated with yam anthracnose in Brazil, using a significant sampling of the main producing states in Brazil. The identification of species has a direct impact on disease management, as different species may respond differently to various management strategies adopted. Due to wide distribution of *C. plurivorum* and *C. siamense* in the main areas, this species should be considered in any management strategy for this pathosystem. Epidemiological studies are necessary for a better understanding of the dynamics of this pathosystem as well as the establishment of management measures.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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506 **Table 1.** Primers and cycles were used in this study.

Gene	Primer	Reference	PCR amplification protocols				
			Initial denaturation (95 °C)	Denaturation (95 °C)	Annealing	Extension (72 °C)	Final extension (72 °C)
ACT	ACT-512F	Carbone and Kohn (1999)	3 min	30 sec	57 °C	1 min	10 min
	ACT-783R	Carbone and Kohn (1999)			45 sec		
APN2	CgDl_R1	Rojas et al. (2010)	3 min	30 sec	62° C	1 min	10 min
	ColDL_F3	Rojas et al. (2010)			45 sec		
APN2/MAT-IGS	AMR	Silva et al. (2012)	3 min	30 sec	50 °C	1 min	10 min
	AMF	Silva et al. (2012)			1 min		
CAL	CL1C	Weir et al. (2012)	3 min	30 sec	57 °C	1 min	10 min
	CL2C	Weir et al. (2012)			45 sec		
CHS-1	CHS-79F	Weir et al. (2012)	3 min	30 sec	55 °C	45 sec	10 min
	CHS-345R	Weir et al. (2012)			45 sec		
GAPDH	GAP-95	Vieira et al. (2017)	3 min	30 sec	60 °C	1 min 30 sec	10 min
	GAP-1174	Vieira et al. (2017)			45 sec		
GAP2-IGS	GAP-1041	Vieira et al. (2017)	3 min	30 sec	58 °C	1 min 30 sec	10 min
	GAP/IGS-2044	Vieira et al. (2017)			1 min		
GS	GS-64F	Vieira et al. (2017)	3 min	30 sec	55 °C	1 min	10 min
	GS-967R	Vieira et al. (2017)			45 sec		
HIS3	CYLH3F	Crous et al. (2004)	3 min	30 sec	53 °C	45 sec	10 min
	CYLH3R	Crous et al. (2004)			45 sec		
nrITS	ITS1	Gardes and Brun (1993)	3 min	30 sec	55 °C	45 sec	10 min
	ITS4	White et al. (1990)			45 sec		
TUB2	BT1	OminDonnell and Cigelnik (1997)	3 min	30 sec	53 °C	1 min 30 sec	10 min
	BT22	OminDonnell and Cigelnik (1997)			1 min		
	B2a	Glass and Donaldson (1995)	3 min	30 sec	55 °C	1 min	10 min
	B2b	Glass and Donaldson (1995)			45 sec		
	BT1	OminDonnell and Cigelnik (1997)	3 min	30 sec	55 °C	1 min	10 min
	B2b	Glass and Donaldson (1995)			45 sec		

507 **Table 2.** Collection details and GenBank accession numbers of isolates included in this study.

					GenBank numbers										
Species	**Culture**	**Group**	**Host**	**Country**	**ACT**	**APN2**	**APN2/MAT-IGS**	**CAL**	**CHS-1**	**GAPDH**	**GAP2-IGS**	**GS**	**HIS3**	**ITS**	**TUB**
C. acidae	MFLUCC 17-2659	Truncatum	*Phyllanthus acidus*	Thailand	MH003697	-	-	-	MH003694	MH003691	-	-	-	MG996505	-
C. acidae	MFLU 18-0233	Truncatum	*Phyllanthus acidus*	Thailand	MH003698	-	-	-	MH003695	MH003692	-	-	-	MG996506	-
C. aenigma	ICMP 18608*	Gloeosporioides	*Persea americana*	Israel	-	-	KM360143	JX009683	-	JX010044	-	JX010078	-	-	JX010389
C. aeschynomenes	ICMP 17673*	Gloeosporioides	*Aeschynomene virginica*	USA	-	-	KM360145	JX009721	-	JX009930	-	JX010081	-	-	JX010392
C. alatae	CBS 304.67, ICMP 17919*	Gloeosporioides	*Dioscorea alata*	India	-	-	KC888932	JX009738	-	JX009990	-	JX010065	-	-	JX010383
C. alienum	ICMP 12071*	Gloeosporioides	*Malus domestica*	New Zealand	-	-	KM360144	JX009654	-	JX010028	-	JX010101	-	-	JX010411
C. annellatum	CBS 129826	Boninense	*Hevea brasiliensis*	Colombia	-	-	-	JQ005743	-	JQ005309	-	-	-	-	JQ005656
C. aotearoa	ICMP 18537*	Gloeosporioides	*Coprosma sp.*	New Zealand	-	-	-	JX009611	-	JX010005	-	JX010113	-	-	JX010420
C. arecicola	CGMCC 3.19667, HNBL5*	Gloeosporioides	*Areca catechu*	Wenchang	-	-	MK935413	-	-	MK935455	-	-	-	-	MK935498
C. artocarpicola	MFLUCC 18_1167*	Gloeosporioides	*Artocarpus heterophyllus*	Thailand	-	-	-	-	-	MN435568	-	-	-	-	MN435567
C. asianum	ICMP 18580, CBS 130418*	Gloeosporioides	*Coffea arabica*	Thailand	-	-	FR718814	FJ917506	-	JX010053	-	JX010096	-	-	JX010406
C. beeveri	CBS 128527*	Boninense	*Brachyglottis repanda*	New Zealand	-	-	-	JQ005692	-	JQ005258	-	-	-	-	JQ005605
C. boninense	CBS 123755*	Boninense	*Crinum asiaticum var. sinicum*	Japan	-	-	-	JQ005674	-	JQ005240	-	-	-	-	JQ005588
C. boninense	CBS 128547	Boninense	*Camellia sp*	New Zealand	-	-	-	JQ005680	-	JQ005246	-	-	-	-	JQ005593
C. brasiliense	CBS 128501*	Boninense	*Passiflora edulis*	Brazil	-	-	-	JQ005756	-	JQ005322	-	-	-	-	JQ005669
C. brasiliense	CBS 128528	Boninense	*Passiflora edulis*	Brazil	-	-	-	JQ005755	-	JQ005321	-	-	-	-	JQ005668
C. brassicicola	CBS 101059*	Boninense	*Brassica oleracea var. gemmifera*	New Zealand	-	-	-	JQ005693	-	JQ005259	-	-	-	-	JQ005606
C. brassicicola	CD015-1	Boninense	*Rubus glaucus Benth*	Colombia	-	-	-	KC860036	-	KC860010	-	-	-	-	-
C. brevisporum	BCC 38876*	Orchidearum	*Neoregalia sp*	Thailand	JN050216	-	-	-	MZ799287	JN050227	-	-	MZ673841	JN050238	JN050244
C. brevisporum	CBS 129957	Magnum	*Neoregalia sp.*	Thailand	MG600966	-	-	-	MG600869	MG600822	-	-	MG600908	MG600762	MG601029
C. brevisporum	CBS 129958	Magnum	*Anthurium sp*	Thailand	MG600967	-	-	-	MG600870	MG600823	-	-	MG600909	MG600763	MG601030
C. brevisporum	BCC 38876*	Magnum	*Neoregalia sp*	Thailand	JN050216	-	-	-	-	JN050227	-	-	-	JN050238	JN050244
C. brevisporum	CBS 512.75	Magnum	*Carica papaya*	Australia	MG600965	-	-	-	MG600868	MG600821	-	-	MG600907	MG600761	MG601028

508 **Table 2 (cont)**

					GenBank numbers										
Species	**Culture**	**Group**	**Host**	**Country**	**ACT**	**APN2**	**APN2/MAT-IGS**	**CAL**	**CHS-1**	**GAPDH**	**GAP2-IGS**	**GS**	**HIS3**	**ITS**	**TUB**
C. cacao	CBS 119297	Magnum	*Theobroma cacao*	Costa Rica	MG600976	-	-	-	MG600878	MG600832	-	-	MG600916	MG600772	MG601039
C. camelliae	CGMCC 3.14925, LC1364*	Gloeosporioides	*Camellia sinensis*	China	-	-	-	KJ954634	-	KJ954782	-	KJ954932	-	-	KJ955230
C. camelliae-japonicae	CGMCC3.18118*	Boninense	*Camellia japonica*	China	-	-	-	-	-	KX893584	-	-	-	-	KX893580
C. catinaense	CBS 142417	Boninense	*Citrus reticulata*	Italy	-	-	-	KY856053	-	KY856224	-	-	-	-	KY856482
C. cattleyicola	**CBS 170.49***	Orchidearum	*Cattleya sp*	Belgium	MG600963	-	-	-	MG600866	MG600819	-	-	MG600905	MG600758	MG601025
C. cattleyicola	MAFF 238321	Orchidearum	*Cattleya sp*	Japan	-	-	-	-	-	-	-	-	-	MG600759	MG601026
C. changpingense	MFLUCC 15-0022*	Gloeosporioides	*Rhizome of Fragaria × ananass*	China	-	-	-	-	-	KP852469	-	-	-	-	KP852490
C. chongqingense	CS0612	Boninense	*Camellia sinensis*	China	-	-	-	MT976097	-	MG602022	-	-	-	-	MG602044
C. chrysophilum	8395	Gloeosporioides	*Theobroma cacao*	Panama	-	GU994415	GU994444	KX094056	-	KX094176	KX094126	KX094209	-	-	GU994473
C. chrysophilum	CMM 4268*, URM 7362	Gloeosporioides	*Musa sp.*	Brazil	-	KX094018	KX094325	KX094063	-	KX094183	-	KX094204	-	-	KX094285
C. chrysophilum	Coll919	Gloeosporioides	*Terpsichore taxifolia*	Puerto Rico	-	JX145265	JX145317	KX094057	-	KX094177	KX094127	KX094207	-	-	KX094288
C. chrysophilum	E183	Gloeosporioides	*Genipa americana*	Panama	-	GU994414	GU994443	KX094058	-	KX094178	KX094128	KX094208	-	-	GU994472
C. chrysophilum	**LM1270**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818226**	**OQ791443**	**OQ831800**	-	**OQ745587**	**OQ818210**	**OQ791454**	-	-	**OQ818242**
C. citricola	CBS 134228*	Boninense	*Citrus unchiu*	China	-	-	-	KC293696	-	KC293736	-	-	-	-	KC293656
C. citricola	SXC161	Boninense	*Proteaceae*	China	-	-	-	KC293698	-	KC293738	-	-	-	-	KC293658
C. clidemiae	ICMP 18658*	Gloeosporioides	*Clidemia hirta*	USA	-	-	-	JX009645	-	JX009989	-	JX010129	-	-	JX010438
C. cliviicola	CBS 125375*	Orchidearum	*Clivia miniata*	China	MG600939	-	-	-	MG600850	MG600795	-	-	MG600892	MG600733	MG601000
C. colombiense	CBS 129818*	Boninense	*Passiflora edulis*	Colombia	-	-	-	JQ005695	-	JQ005261	-	-	-	-	JQ005608
C. colombiense	CBS 129817	Boninense	*Passiflora edulis*	Colombia	-	-	-	JQ005694	-	JQ005260	-	-	-	-	JQ005607
C. conoides	CGMCC 3.17615*	Gloeosporioides	*Capsicum annuum*	China	-	-	-	KP890150	-	KP890162	-	-	-	-	KP890174
C. constrictum	CBS 128504*	Boninense	*Citrus limon*	New Zealand	-	-	-	JQ005759	-	JQ005325	-	-	-	-	JQ005672
C. constrictum	CBS 128503	Boninense	*Solanum betaceum*	New Zealand	-	-	-	JQ005758	-	JQ005324	-	-	-	-	JQ005671

509 **Table 2 (cont)**

					GenBank numbers										
Species	**Culture**	**Group**	**Host**	**Country**	**ACT**	**APN2**	**APN2/MAT-IGS**	**CAL**	**CHS-1**	**GAPDH**	**GAP2-IGS**	**GS**	**HIS3**	**ITS**	**TUB**
C. cordylinicola	MFLUCC 090551*, ICMP 18579	Gloeosporioides	*Cordyline fruticosa*	Thailand	-	-	-	HM470238	-	JX009975	-	JX010122	-	-	JX010440
C. curcumae	IMI 288937	Truncatum	*Curcuma longa*	India	GU227991	-	-	-	GU228383	GU228285	-	-	-	GU227893	-
C. cymbidiicola	IMI 347923*	Boninense	*Cymbidium sp.*	Australia	-	-	-	JQ005687	-	JQ005253	-	-	-	-	JQ005600
C. cymbidiicola	CBS 123757	Boninense	*Cymbidium sp.*	Japan	-	-	-	JQ005689	-	JQ005255	-	-	-	-	JQ005602
C. dacrycarpi	CBS 130241*	Boninense	*Dacrycarpus dacrydioides*	New Zealand	-	-	-	JQ005757	-	JQ005323	-	-	-	-	JQ005670
C. diversisporum	LC13890	Singleton	*Dracaena angustifolia*	China	MZ664209	-	-		MZ799302	MZ664122	-	-	MZ673931	MZ595911	MZ674029
C. doitungense	MFLU14-0128	Boninense	*Dendrobium sp.*	Thailand	-	-	-	-	-	MH049480	-	-	-	-	MH351277
C. endophyticum	MFLUCC 130418, LC0324*	Gloeosporioides	*Pennisetum purpureum*	Thailand	-	-	-	KC810018	-	KC832854	-	-	-	-	-
C. feijoicola	CBS 144633	Boninense	*Acca sellowiana*	Portugal	-	-	-	-	-	MK876475	-	-	-	-	MK876507
C. fructicola	LC3427	Gloeosporioides	*Theobroma cacao*	Panama	-	GU994409	GU994438	KX094066	-	KX094174	KX094121	KX094198	-	-	KX094279
C. fructicola	CBS 125397*, ICMP 18646	Gloeosporioides	*Tetragastris panamensis*	Panama	-	GU994412	JQ807839	JX009674	-	JX010032	-	JX010099	-	-	JX010409
C. fructivorum	CBS 133125, Coll1414	Gloeosporioides	*Vaccinium macrocarpon*	Burlington	-		JX145300	-	-	-	-	-	-	-	JX145196
C. fusiforme	MFLUCC 12–0437	Truncatum	*Citrus*	China	KT290251	-	-	-	KT290253	KT290255	-	-	-	KT290266	-
C. gigasporum	CBS 133266, MUCL 44947*	Gigasporum	*Centella asiatica*	Madagascar	-	-	-	-	KF687761	KF687822	-	-	-	KF687715	-
C. gloeosporioides	IMI 356878*, ICMP 17821, CBS 112999	Gloeosporioides	*Citrus sinensis*	Italy	-	GU994416	JQ807843	JX009731	-	JX010056	-	JX010085	-	-	JX010445
C. grevilleae	CBS 132879*, CPC 15481	Gloeosporioides	*Grevillea sp.*	Italy	-	-	-	KC296963	-	KC297010	-	KC297033	-	-	KC297102
C. grossum	CGMCC3.17614, CAUG7, LC6227*	Gloeosporioides	*Chili pepper*	China	-	-	-	KP890147	-	KP890159	-	-	-	-	KP890171
C. hebeiense	JZB 330028*	Gloeosporioides	*Vitis vinifera*	China	-	-	-		-	KF377495	-	-	-	-	KF288975
C. helleniense	CBS 142419, CPC 27107*	Gloeosporioides	*Citrus reticulata*	Greece, Arta	-	-	-	KY856100	-	KY856271	-	-	-	-	KY856529
C. henanense	CGMCC 3.17354	Gloeosporioides	*Camellia sinensis*	China	-	-	KJ954524	KJ954662	-	KJ954810	-	KJ954960	-	-	KJ955257
C. hippeastri	CBS 125376*	Boninense	*Hippeastrum vittatum*	China	-	-	-	JQ005752	-	JQ005318	-	-	-	-	JQ005665
C. hippeastri	CBS 241.78	Boninense	*Hippeastrum sp.*	Netherlands	-	-	-	JQ005753	-	JQ005319	-	-	-	-	JQ005666
C. horii	ICMP 10492*	Gloeosporioides	*Diospyros kaki*	Japan	-	-	JQ807840	JX009604	-	GQ329681	-	JX010137	-	-	JX010450

510 **Table 2 (cont)**

Species	Culture	Group	Host	Country	GenBank numbers										
					ACT	APN2	APN2/MAT-IGS	CAL	CHS-1	GAPDH	GAP2-IGS	GS	HIS3	ITS	TUB
C. hystricis	CBS 142411 = CPC 28153*	Gloeosporioides	*Citrus hystrix*	Italy	-	-	-	KY856103	-	KY856274	-	-	-	-	KY856532
C. inhamicola sp. nov.	**LM1118***	Magnum	*Dioscorea trifida*	Brazil	**OQ789258**	-	-	-	**OQ789257**	**OQ745600**	-	-	**OQ818202**	**OQ831542**	**OR133745**
C. jiangxiense	CGMCC 3.1736*	Gloeosporioides	*Camellia sinensis*	China	-	-	KJ954607	KJ954752	-	KJ954902	-	KJ955051	-	-	KJ955348
C. kahawae	ICMP 17816*	Gloeosporioides	*Coffea arabica*	Kenya	-	-	JQ894579	JX009642	-	JX010012	-	JX010130	-	-	JX010444
C. kaifengense	CAASZK33	Magnum	*Citrullus lanatus*	China	OL449313	-	-	-	OL901183	OL456715	-	-	OM057724	MZ475245	OL456674
C. kaifengense	CAASZK32	Magnum	*Citrullus lanatus*	China	OL449312	-	-	-	OL901182	OL456714	-	-	OM057723	MZ475244	OL456673
C. karsti	CBS 132134*	Boninense	*Vanda sp.*	China	-	-	-	HM582013	-	HM585391	-	-	-	-	HM585428
C. karsti	CBS 128550	Boninense	*Annona cherimola*	Mexico	-	-	-	JQ005740	-	JQ005306	-	-	-	-	JQ005653
C. karsti	CBS 129927	Boninense	*Anthurium sp.*	Thailand	-	-	-	JQ005727	-	JQ005293	-	-	-	-	JQ005640
C. karsti	CBS 861.72	Boninense	*Bombax aquaticum*	Brazil	-	-	-	JQ005705	-	JQ005271	-	-	-	-	JQ005618
C. karsti	CBS 128545	Boninense	*Capsicum annuum*	New Zealand	-	-	-	JQ005728	-	JQ005294	-	-	-	-	JQ005641
C. karsti	CBS 106.91	Boninense	*Carica papaya*	Brazil	-	-	-	JQ005741	-	JQ005307	-	-	-	-	JQ005654
C. karsti	CBS 128524	Boninense	*Citrullus lanatus*	New Zealand	-	-	-	JQ005716	-	JQ005282	-	-	-	-	JQ005629
C. karsti	CBS 126532	Boninense	*Citrus sp.*	South Africa	-	-	-	JQ005730	-	JQ005296	-	-	-	-	JQ005643
C. karsti	CBS 128551	Boninense	*Citrus sp.*	New Zealand	-	-	-	JQ005729	-	JQ005295	-	-	-	-	JQ005642
C. karsti	ICMP 18597	Boninense	*Clivia miniata*	Japan	-	-	-	JQ005717	-	JQ005283	-	-	-	-	JQ005630
C. karsti	CBS 125468	Boninense	*Coffea sp.,*	Vietman	-	-	-	JQ005718	-	JQ005284	-	-	-	-	JQ005631
C. karsti	CBS 18599	Boninense	*Cucumis melo*	Japan	-	-	-	JQ005712	-	JQ005278	-	-	-	-	JQ005625
C. karsti	CBS 125468	Boninense	*Coffea sp.,*	Vietman	-	-	-	JQ005718	-	JQ005284	-	-	-	-	JQ005631
C. karsti	CBS 18599	Boninense	*Cucumis melo*	Japan	-	-	-	JQ005712	-	JQ005278	-	-	-	-	JQ005625
C. karsti	**LM544**	Boninense	*Dioscorea cayennensis*	Brazil	-	-	-	**OP093982**	-	**OP093980**	-	-	-	-	**OP093984**
C. karsti	**LM583**	Boninense	*Dioscorea cayennensis*	Brazil	-	-	-	**OP093981**	-	**OP093979**	-	-	-	-	**OP093983**
C. ledongense	LD1683, CGMCC 3.18888	Gloeosporioides	*Hevea brasiliensis*	China, Hainan	-	-	-	MG242013	-	MG242017	-	MG242021	-	-	MG242011
C. limonicola	CBS 142410	Boninense	*Citrus limon*	Malta	-	-	-	KY856125	-	KY856296	-	-	-	-	KY856554
C. lobatum	IMI 79736	Magnum	*Piper catalpaefolium*	Trinidad and Tobago	MG600972	-	-	-	MG600874	MG600828	-	-	MG600912	MG600768	MG601035

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					GenBank numbers										
Species	**Culture**	**Group**	**Host**	**Country**	**ACT**	**APN2**	**APN2/MAT-IGS**	**CAL**	**CHS-1**	**GAPDH**	**GAP2-IGS**	**GS**	**HIS3**	**ITS**	**TUB**
C. magnum	IMI 391662	Magnum	*Citrullus lanatus*	USA	MG600975	-	-	-	MG600877	MG600831	-	-	MG600915	MG600771	MG601038
C. magnum	CBS 575.97	Magnum	*Citrullus lanatus*	USA	MG600974	-	-	-	MG600876	MG600830	-	-	MG600914	MG600770	MG601037
C. magnum	CBS 519.97	Magnum	*Citrullus lanatus*	USA	MG600973	-	-	-	MG600875	MG600829	-	-	MG600913	MG600769	MG601036
C. makassarense	CBS 143664, CPC 28612*	Gloeosporioides	*Capsicum annuum*	Indonesia	-	-	MH728831	-	-	MH728820	-	MH748264	-	-	MH846563
C. merremiae	CBS 124955	Magnum	*Merremia umbellata*	Panama	MG600969	-	-	-	MG600872	MG600825	-	-	MG600910	MG600765	MG601032
C. monsterae	LC13871	Orchidearum	*Monstera deliciosa*	China	MZ664195	-	-	-	MZ799351	MZ664121	-	-	MZ673917	MZ595897	MZ674015
C. musae	CBS 116870*, ICMP 19119	Gloeosporioides	*Musa sp.*	USA	-	-	KC888926	JX009742	-	JX010050	-	JX010103	-	-	HQ596280
C. musicola	**CBS 132885***	Orchidearum	*Musa sp.*	Mexico	MG600942	-	-	-	MG600853	MG600798	-	-	MG600895	MG600736	MG601003
C. musicola	CBS 127557	Orchidearum	*Musa sp.*	Mexico	MG600943	-	-	-	MG600854	MG600799	-	-	MG600896	MG600737	MG601004
C. neosansevieriae	CBS 139918	Agaves	*Sansevieria trifasciata*	South Africa	-	-	-	-	-	KR476791	-	-	-	-	KR476797
C. novae-zelandiae	CBS 128505*	Boninense	*Capsicum annuum*	New Zealand	-	-	-	JQ005749	-	JQ005315	-	-	-	-	JQ005662
C. novae-zelandiae	CBS 130240	Boninense	*Citrus sp. (grapefruit)*	New Zealand	-	-	-	JQ005750	-	JQ005316	-	-	-	-	JQ005663
C. noveboracense	AFKH109*	Gloeosporioides	*Apple/Idared*	Columbia/NY	-	MN910262	MN640564	MN640566	-	MN640567	-	MN640568	-	-	MN640569
C. noveboracense	PMBrms-1	Gloeosporioides	*Apple/Un*[b]	Adams/PA	-	MN790765	MN741075	MN741056	-	MN741087	-	MN741100	-	-	MN741064
C. nupharicola	CBS 470.96*, ICMP 18187	Gloeosporioides	*Nuphar lutea subsp. polysepala*	USA	-	JX145275	JX145319	JX009661	-	JX009936	-	JX010088	-	-	JX010397
C. nupharicola	CBS 472.96, ICMP 17940	Gloeosporioides	*Nymphaea odorata*	USA	-	JX145276	JX145320	JX009662	-	JX010031	-	JX010089	-	-	JX010399
C. okinawense	MAFF 240517	Magnum	*Carica papaya*	Japan	MG600971	-	-	-	-	MG600827	-	-	-	MG600767	MG601034
C. oncidii	CBS 129828*	Boninense	*Oncidium sp.*	Germany	-	-	-	JQ005690	-	JQ005256	-	-	-	-	JQ005603
C. oncidii	CBS 130242	Boninense	*Oncidium sp.*	Germany	-	-	-	JQ005691	-	JQ005257	-	-	-	-	JQ005604
C. orchidearum	**CBS 135131***	Orchidearum	*Dendrobium nobile*	Netherlands	MG600944	-	-	-	MG600855	MG600800	-	-	MG600897	MG600738	MG601005
C. orchidearum	CBS 136877	Orchidearum	*Dendrobium nobile*	Netherlands	MG600945	-	-	-	MG600856	MG600801	-	-	MG600898	MG600739	MG601006
C. panamense	CBS 125386	Magnum	*Merremia umbellata*	Panama	-	-	-	-	-	MG600826	-	-	MG600911	-	MG601033

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Species	Culture	Group	Host	Country	GenBank numbers										
					ACT	APN2	APN2/MAT-IGS	CAL	CHS-1	GAPDH	GAP2-IGS	GS	HIS3	ITS	TUB
C. parsonsiae	CBS 128525*	Boninense	*Parsonsia capsularis*	New Zealand	-	-	-	JQ005754	-	JQ005320	-	-	-	-	JQ005667
C. parsonsiae	CGMCC 3.15126	Boninense	*Bletilla ochracea*	China	-	-	-	-	-	KC843500	-	-	-	-	JX625210
C. pereskiae	COAD 2994	Orchidearum	*Pereskia aculeata*	Brazil	MZ265332	-	-	-	MZ265334	MZ265336	-	-	MZ265338	MZ262420	MZ265340
C. pereskiae	COAD 2995*	Orchidearum	*Pereskia aculeata*	Brazil	MZ265333	-	-	-	MZ265335	MZ265337	-	-	MZ265339	MZ262421	MZ265341
C. perseae	GA100TCBS141365*	Gloeosporioides	*Persea americana*	Israel	-	-	KX620177	KX620206	-	KX620242	-	KX620275	-	-	KX620341
C. petchii	CBS 378.94*	Boninense	*Dracaena marginata*	Italy	-	-	-	JQ005744	-	JQ005310	-	-	-	-	JQ005657
C. petchii	CBS 118193	Boninense	*Dracaena sanderiana*	China	-	-	-	JQ005748	-	JQ005314	-	-	-	-	JQ005661
C. philodendricola	LZJZ1	Boninense	*Philodendron tatei cv. Congo*	China	-	-	-	MH105281	-	MH105261	-	-	-	-	MH105277
C. phyllanthi	CBS 175.67*	Boninense	*Phyllanthus acidus*	India	-	-	-	JQ005742	-	JQ005308	-	-	-	-	JQ005655
C. phyllanthi	GS10	Boninense	*Bauhinia variegata*	India	-	-	-	JX863678	-	JX576718	-	-	-	-	JX863674
C. piperis	CPC 21195*	Orchidearum	*Piper nigrum*	Malaysia	MG600964	-	-		MG600867	MG600820	-	-	MG600906	MG600760	MG601027
C. plurivorum	**CBS 125474***	Orchidearum	*Coffea sp*	Vietnam	MG600925	-	-		MG600841	MG600781	-	-	MG600887	MG600718	MG600985
C. plurivorum	CBS 132444	Orchidearum	*Gossypium sp.*	Brazil	MG600927	-	-		MG600843	MG600783	-	-	MG600889	MG600720	MG600987
C. plurivorum	**LM554**	Orchidearum	*Dioscorea cayennensis*	Brazil	-	-	-		**OQ789254**	**OQ745605**	-	-	**OQ818203**	-	**OR133740**
C. plurivorum	**LM811**	Orchidearum	*Dioscorea alata*	Brazil	-	-	-		**OQ789256**	**OQ745607**	-	-	**OQ818200**	-	**OR133739**
C. plurivorum	**LM1094**	Orchidearum	*Dioscorea cayennensis*	Brazil	-	-	-		**OQ789247**	**OQ745601**	-	-	**OQ818197**	-	**OR133744**
C. plurivorum	**LM1098**	Orchidearum	*Dioscorea cayennensis*	Brazil	-	-	-		**OQ789248**	**OQ745602**	-	-	**OQ818198**	-	**OR133743**
C. plurivorum	**LM1108**	Orchidearum	*Dioscorea cayennensis*	Brazil	-	-	-		**OQ789249**	**OQ745603**	-	-	**OQ818199**	-	**OR133742**
C. proteae	CBS 132882*, CPC 14859	Gloeosporioides	*Protea sp.*	South Africa	-	-	-	KC296960	-	KC297009	-	KC297032	-	-	KC297101
C. proteae	CBS 134301, CPC 14860	Gloeosporioides	*Protea sp.*	South Africa	-	-	-	KC842375	-	KC842379	-	KC842381	-	-	KC842387
C. pseudoboninense	LZJZ5	Boninense	*Philodendron tatei cv. Congo*	China	-	-	-	-	-	MK796573	-	-	-	-	MK796554
C. psidii	CBS 145.29*, ICMP 19120	Gloeosporioides	*Psidium sp.*	Italy	-	-	-	JX009743	-	JX009967	-	JX010133	-	-	JX010443
C. pyrifoliae	CGMCC318902	Singleton	*Pyrus pyrifolia cv. Jinshui*	China	-	-	-	-	-	MG747996	-	-	-	-	MG748158

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Species	Culture	Group	Host	Country	GenBank numbers										
					ACT	APN2	APN2/MAT-IGS	CAL	CHS-1	GAPDH	GAP2-IGS	GS	HIS3	ITS	TUB
C. qilinense	CAASZK13	Magnum	*Citrullus lanatus*	China	OL449292	-	-	-	OL901162	OL456694	-	-	OM057703	MZ475217	OL456653
C. qilinense	CAASZK12	Magnum	*Citrullus lanatus*	China	OL449291	-	-	-	OL901161	OL456693	-	-	OM057702	MZ475216	OL456652
C. queenslandicum	CMM3233	Gloeosporioides	*A. occidentale*	Brazil, Pernambuco state	-	MF110711	MF110639	MF110828	-	MF110849	MF110918	MF110996	-	-	MF111058
C. queenslandicum	CMM3241	Gloeosporioides	*A. occidentale*	Brazil, Pernambuco state	-	MF110714	MF110642	MF110832	-	MF110848	MF110915	MF111000	-	-	MF111059
C. queenslandicum	ICMP 1778*	Gloeosporioides	*Carica papaya*	Australia	-	-	KC888928	JX009691	-	JX009934	-	JX010104	-	-	JX010414
C. queenslandicum	**LM574**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818231**	**OQ791445**	**OQ831805**	-	**OQ745586**	**OQ818215**	**OQ791459**	-	-	**OQ818246**
C. queenslandicum	**LM579**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818233**	**OQ791446**	**OQ831807**	-	**OQ745585**	**OQ818217**	**OQ791461**	-	-	**OQ818248**
C. reniforme	LC8230*	Orchidearum	*Smilax cocculoides*	China	MZ664145	-	-	-	MZ799290	MZ664110	-	-	MZ673867	MZ595847	MZ673968
C. reniforme	LC8248	Orchidearum	*Paederia foetida*	China	MZ664148	-	-	-	MZ799295	MZ664111	-	-	MZ673870	MZ595850	MZ673971
C. rhexiae	CBS 133134, Coll1026*	Gloeosporioides	*Rhexia virginica*	Delaware, USA	-	-	JX145290	-	-	-	-	-	-	-	JX145179
C. salsolae	ICMP 19051*	Gloeosporioides	*Salsola tragus*	Hungary	-	-	KC888925	JX009696	-	JX009916	-	JX010093	-	-	JX010403
C. siamense	CMM 4083	Gloeosporioides	*M. indica*	Brazil	-	KX093998	KX094307	KX094052	-	KX094167	KX094147	KX094219	-	-	KX094271
C. siamense	CMM 4084	Gloeosporioides	*M. indica*	Brazil	-	KX093999	KX094310	KX094053	-	KX094166	KX094148	KX094220	-	-	KX094272
C. siamense	CMM 4248	Gloeosporioides	*Musa sp.*	Brazil	-	KX093992	KX094314	KX094037	-	KX094154	KX094136	KX094229	-	-	KX094300
C. siamense	ICMP 18578*, CBS 130417	Gloeosporioides	*Coffea arabica*	Thailand	-	-	JQ899289	FJ917505	-	JX009924	-	JX010094	-	-	JX010404
C. siamense	CMM 4244	Gloeosporioides	*Musa sp.*	Brazil	-	KX094014	KX094315	KX094055	-	KX094172	KX094135	KX094226	-	-	KX094299
C. siamense	CMM 4247	Gloeosporioides	*Musa sp.*	Brazil	-	KX094009	KX094301	KX094038	-	KX094155	KX094141	KX094196	-	-	KX094261
C. siamense	**LM553**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818227**	**OQ791444**	**OQ831801**	-	**OQ745595**	**OQ818211**	**OQ791455**	-	-	-
C. siamense	**LM557**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818229**	**OQ791438**	**OQ831803**	-	**OQ745590**	**OQ818213**	**OQ791457**	-	-	**OQ818244**
C. siamense	**LM559**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818230**	**OQ791439**	**OQ831804**	-	**OQ745589**	**OQ818214**	**OQ791458**	-	-	**OQ818245**
C. siamense	**LM576**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818232**	**OQ791440**	**OQ831806**	-	**OQ745584**	**OQ818216**	**OQ791460**	-	-	**OQ818247**
C. siamense	**LM591**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818235**	**OQ791447**	**OQ831809**	-	**OQ745596**	**OQ818219**	**OQ791463**	-	-	**OQ818250**
C. siamense	**LM1109**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818220**	-	**OQ831794**	-	**OQ745588**	**OQ818205**	**OQ791448**	-	-	**OQ818236**

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					GenBank numbers										
Species	**Culture**	**Group**	**Host**	**Country**	**ACT**	**APN2**	**APN2/MAT-IGS**	**CAL**	**CHS-1**	**GAPDH**	**GAP2-IGS**	**GS**	**HIS3**	**ITS**	**TUB**
C. siamense	**LM1120**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818221**	-	**OQ831795**	-	**OQ745592**	-	**OQ791449**	-	-	**OQ818237**
C. siamense	**LM1214**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818222**	-	**OQ831796**	-	**OQ745591**	**OQ818206**	**OQ791450**	-	-	**OQ818238**
C. siamense	**LM1220**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818223**	**OQ791435**	**OQ831797**	-	**OQ745594**	**OQ818207**	**OQ791451**	-	-	**OQ818239**
C. siamense	**LM1221**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818224**	**OQ791441**	**OQ831798**	-	**OQ745597**	**OQ818208**	**OQ791452**	-	-	**OQ818240**
C. siamense	**LM1224**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818225**	**OQ791442**	**OQ831799**	-	**OQ745593**	**OQ818209**	**OQ791453**	-	-	**OQ818241**
C. sojae	ATCC 62257*	Orchidearum	*Glycine max*	USA	KC110830	-	-	-	MG600860	MG600810	-	-	MG600899	MG600749	MG601016
C. sojae	CBS 134.87	Orchidearum	*Glycine max*	Italy	MG600957	-	-	-	MG600863	MG600813	-	-	MG600902	MG600752	MG601019
C. sojae	SAUCC 1407	Orchidearum	*Arctium lappa*	China	KT362189	-	-	-	KT362187	KT362188	-	-	-	KT362184	KT362185
C. sojae	**LM1111**	Orchidearum	*Dioscorea trifida*	Brazil	-	-	-	-	**OQ789251**	**OQ745604**	-	-	**OQ818201**	-	**OR133741**
C. subacidae	NN054605	Truncatum	*Asparagus officinalis*	China	MZ664191	-	-	-	MZ799309	MZ664075	-	-	-	MZ595893	-
*C. subacidae**	LC13857	Truncatum	*Tetrastigma obovatum*	China	MZ664144	-	-	-	MZ799307	MZ664068	-	-	-	MZ595846	-
C. syngoniicola	M745*	Orchidearum	*Syngonium*	China	MZ664161	-	-	-	MZ799296	MZ664117	-	-	MZ673883	MZ595863	MZ673982
C. syngoniicola	LC8895	Orchidearum	*Syngonium*	China	MZ664162	-	-	-	MZ799297	MZ664118	-	-	MZ673884	MZ595864	MZ673983
C. syngoniicola	LC8896	Orchidearum	*Syngonium*	China	MZ664163	-	-	-	MZ799298	MZ664119	-	-	MZ673885	MZ595865	MZ673984
C. syzygicola	MFLUCC 10–0624	Gloeosporioides	*Syzygium samarangense*	Thailand	-	-	-	KF254859	-	KF242156	-	KF242125	-	-	KF254880
C. tainanense	CBS 143666, Coll 1298*	Gloeosporioides	*Capsicum annuum*	Taiwan	-	-	MH728836	-	-	MH728823	-	MH748259	-	-	MH846558
C. temperatum	Coll883, BPI 884100, CBS 133122*	Gloeosporioides	*Vaccinium macrocarpon*	Bronx	-	-	JX145298	-	-	-	-	-	-	-	JX145211
C. theobromicola	CBS 124945*, ICMP 18649	Gloeosporioides	*Theobroma cacao*	Panama	-	GU994419	KC790726	JX009591	-	JX010006	-	JX010139	-	-	JX010447
C. ti	ICMP 4832*	Gloeosporioides	*Cordyline sp.*	New Zealand	-	-	-	JX009649	-	JX009952	-	-	-	-	JX010442
C. torulosum	CBS 128544*	Boninense	*Solanum melongena*	New Zealand	-	-	-	JQ005685	-	JQ005251	-	-	-	-	JQ005598
C. tropicale	8401	Gloeosporioides	*Theobroma cacao*	Panama	-	GU994400	GU994429	-	-	-	-	-	-	-	GU994458
C. tropicale	CBS 124949*, ICMP 18653	Gloeosporioides	*Theobroma cacao*	Panama	-	GU994396	GU994425	JX009719	-	JX010007	-	JX010097	-	-	GU994454
C. tropicale	CMM 4243	Gloeosporioides	*Musa sp.*	Brazil	-	KU213598	KU213597	KU213599	-	KU213601	KX094120	KU213602	-	-	KU213604

515 **Table 2 (cont)**

Species	Culture	Group	Host	Country	GenBank numbers										
					ACT	APN2	APN2/MAT-IGS	CAL	CHS-1	GAPDH	GAP2-IGS	GS	HIS3	ITS	TUB
C. tropicale	Coll918	Gloeosporioides	*Terpsichore taxifolia*	Puerto Rico	-	JX145264	JX145307	-	-	-	-	-	-	-	JX145214
C. tropicale	E1164	Gloeosporioides	*Trichilia tuberculata*	Panama	-	GU994397	GU994426	-	-	-	-	-	-	-	GU994455
C. tropicale	E406	Gloeosporioides	*Pentagonia macrophylla*	Panama	-	GU994395	GU994424	-	-	-	-	-	-	-	GU994453
C. tropicale	**LM556**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818228**	**OQ791436**	**OQ831802**	-	**OQ745599**	**OQ818212**	**OQ791456**	-	-	**OQ818243**
C. tropicale	**LM580**	Gloeosporioides	*Dioscorea cayennensis*	Brazil	-	**OQ818234**	**OQ791437**	**OQ831808**	-	**OQ745598**	**OQ818218**	**OQ791462**	-	-	**OQ818249**
C. truncatum	CBS 151.35	Truncatum	*Phaseolus lunatus*	USA	GU227960	-	-	-	GU228352	GU228254	-	-	-	MH855611	-
C. truncatum	CBS 120709	Truncatum	*Phaseolus lunatus*	USA	GU227975	-	-	-	GU228367	GU228269	-	-	-	GU227877	-
C. truncatum	CBS 50697	Truncatum	*Vigna unguiculata*	Burkina Faso	GU227969	-	-	-	GU228361	GU228263	-	-	-	GU227871	-
C. truncatum	CBS 141.79	Truncatum	*Stylosanthes hamata*	Australia	GU227971	-	-	-	GU228363	GU228265	-	-	-	GU227873	-
C. truncatum	CBS 260.85	Truncatum	*Crotalaria spectabilis*	USA	GU227973	-	-	-	GU228365	GU228267	-	-	-	GU227875	-
C. truncatum	CBS 146.32	Truncatum	*Opuntia sp*	USA	GU227982	-	-	-	GU228374	GU228276	-	-	-	GU227884	-
C. truncatum	CBS 125330	Truncatum	*Basella rubra*	Laos	GU227987	-	-	-	GU228379	GU228281	-	-	-	GU227889	-
C. truncatum	CBS 714.95	Truncatum	*Limonium sp*	Israel	GU227981	-	-	-	GU228373	GU228275	-	-	-	GU227883	-
C. truncatum	IMI 266002	Truncatum	*Homo sapiens*	Nepal	GU227988	-	-	-	GU228380	GU228282	-	-	-	GU227890	-
C. truncatum	CBS 335.75	Truncatum	*Capsicum annuum*	Indonesia	GU227977	-	-	-	GU228369	GU228271	-	-	-	GU227879	-
C. truncatum	**LM1110**	Truncatum	*Dioscorea alata*	Brazil	**OR133736**	-	-	-	**OQ789250**	**OQ745610**	-	-	-	**OQ831538**	-
C. truncatum	**LM1113**	Truncatum	*Dioscorea alata*	Brazil	**OR133738**	-	-	-	**OQ789252**	**OQ745609**	-	-	-	**OQ831540**	-
C. truncatum	**LM1115**	Truncatum	*Dioscorea trifida*	Brazil	**OR133737**	-	-	-	**OQ789253**	**OQ745608**	-	-	-	**OQ831539**	-
C. viniferum	GZAAS 5.08601*	Gloeosporioides	*Vitis vinifera*	China	-	-	-	JQ309639	-	JN412798	-	JN412787	-	-	JN412813
C. vitoriense sp. nov.	**LM808***	Orchidearum	*Dioscorea alata*	Brazil	**OQ789259**	-	-	-	**OQ789255**	**OQ745606**	-	-	-	**OQ831541**	**OQ884600**
C. vittalense	CBS 181.82*	Orchidearum	*Theobroma cacao*	India	MG600940	-	-	-	MG600851	MG600796	-	-	MG600893	MG600734	MG601001
C. watphraense	MFLU 14-0123	Boninense	*Dendrobium sp.*	Thailand	-	-	-	-	-	MH049479	-	-	-	-	MH351276

Table 2 (cont)

Species	Culture	Group	Host	Country	GenBank numbers										
					ACT	APN2	APN2/MAT-IGS	CAL	CHS-1	GAPDH	GAP2-IGS	GS	HIS3	ITS	TUB
C. wuxiense	CGMCC 3.17894	Gloeosporioides	*Camellia sinensis*	China	-	-	KU251722	KU251833	-	KU252045	-	KU252101	-	-	KU252200
C. xanthorrhoeae	BRIP 45094, ICMP 17903, CBS 127831*	Gloeosporioides	*Xanthorrhoea preissii*	Australia	-	-	KC790689	JX009653	-	JX009927	-	JX010138	-	-	JX010448

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.

ACT: actin; APN2: DNA lyase; APN2/MAT-IGS: intergenic spacer between DNA lyase and the mating-type locus MAT1-2; CAL: calmodulin; CHS-1: chitin synthase 1; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GAP2-IGS: intergenic spacer between GAPDH and a hypothetical protein; GS: glutamine synthetase; HIS3: histone; ITS: internal transcribed spacer; TUB2: β-tubulin.

528 **Table 1**. Isolates of ten species of *Colletotrichum* obtained from leaves of yam (*Dioscorea*
529 spp.) from cultivated areas in Brazil.

			Localization	
***Colletotrichum* species**	**Isolate**	**Dioscorea host**	**City**	**State**
C. chyrsophilum	LM 1270	*D. cayennensis*	Campestre	Alagoas
C. inhamicola	LM 1118	*D. trifida*	Gama	Distrito Federal
C. karsti	LM 544	*D. cayennensis*	Bonito	Pernambuco
	LM 583	*D. cayennensis*	Conde	Paraíba
C. plurivorum	LM 554	*D. cayennensis*	Marcação	Paraíba
	LM 1098	*D. alata*	Bonito	Pernambuco
C. queenslandicum	LM 574	*D. cayennensis*	Conde	Paraíba
C. siamense	LM 576	*D. cayennensis*	Conde	Paraíba
	LM 1109	*D. cayennensis*	Gama	Distrito Federal
C. sojae	LM 1111	*D. trifida*	Gama	Distrito Federal
C. tropicale	LM 556	*D. cayennensis*	Conde	Paraíba
	LM 579	*D. cayennensis*	Conde	Paraíba
C. truncatum	LM 1110	*D. alata*	Gama	Distrito Federal
	LM 1113	*D. alata*	Gama	Distrito Federal
C. vitoriense	LM 808	*D. alata*	Vitória de Santo Antão	Pernambuco

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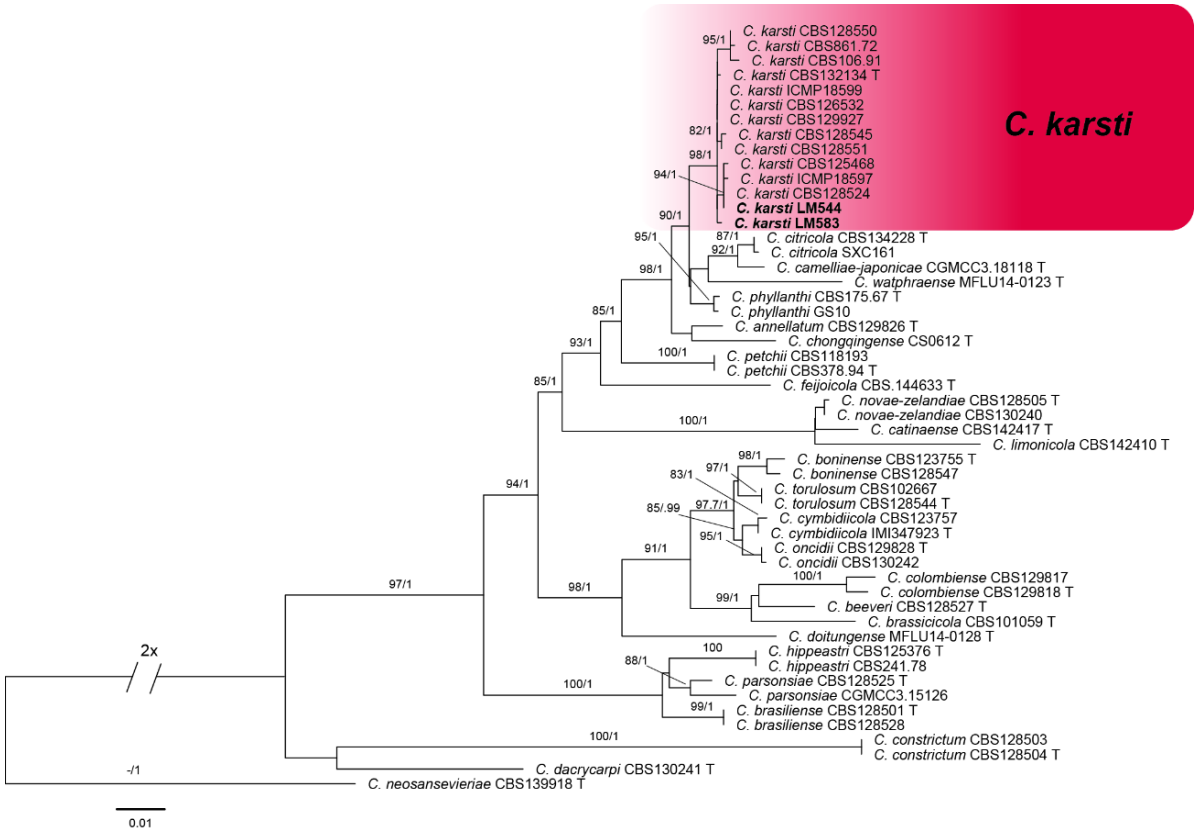

Fig. 1 - Maximum likelihood tree of the *Colletotrichum boninense* species complex inferred on IQ-TREE from a concatenated alignment of CAL, GAPDH and TUB2. Significant supports for ML (SH-alrt bootstrap ≥ 80) and Bayesian inference analysis (posterior probability ≥0.95) are shown above the nodes. The tree was rooted with *Colletotrichum neosansevieriae*. Ex-type isolates are indicated with "T" in the end of the taxa labels. Isolates from *Dioscorea* spp. are highlighted in bold. The scale bar indicates the average number of substitutions per site.

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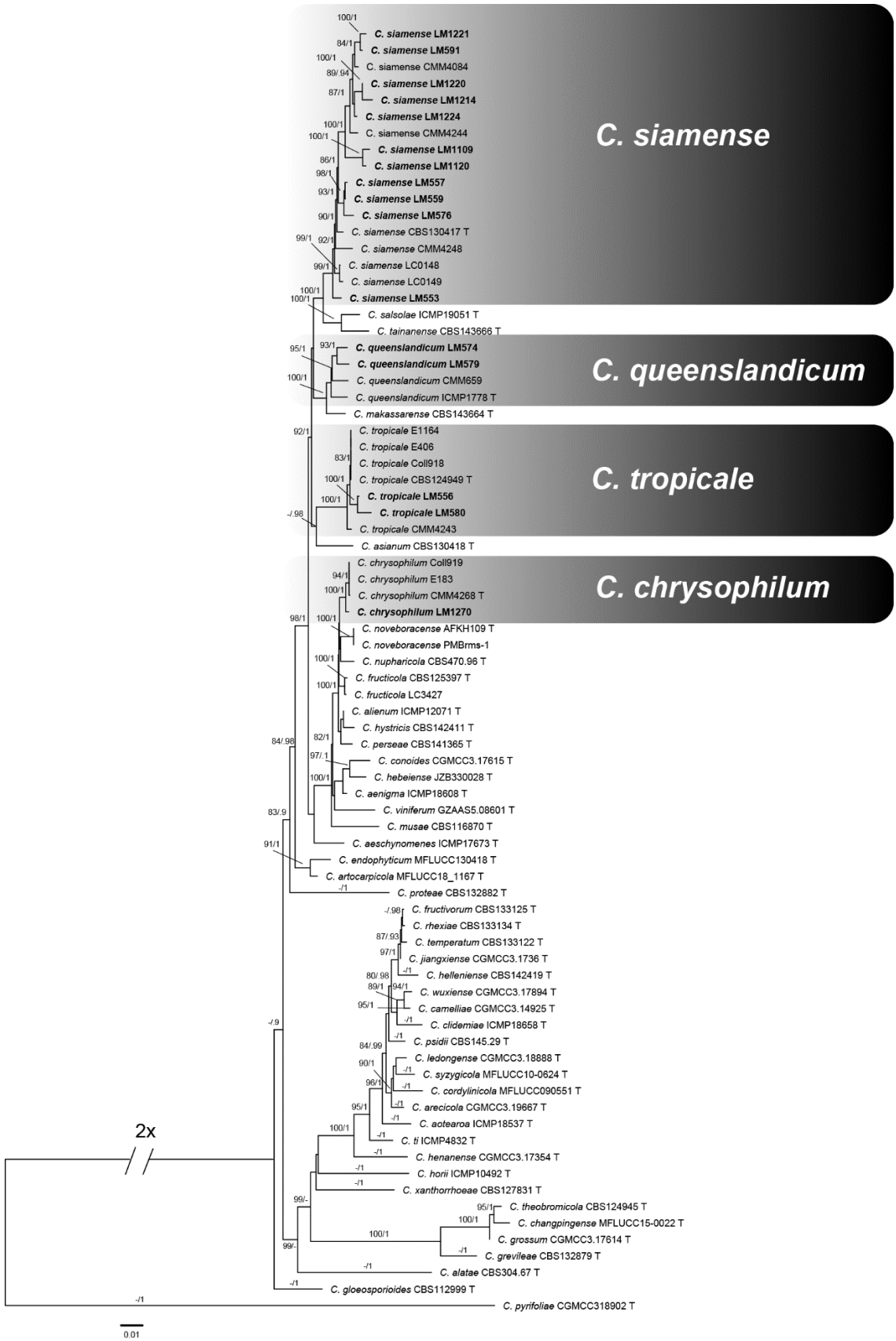

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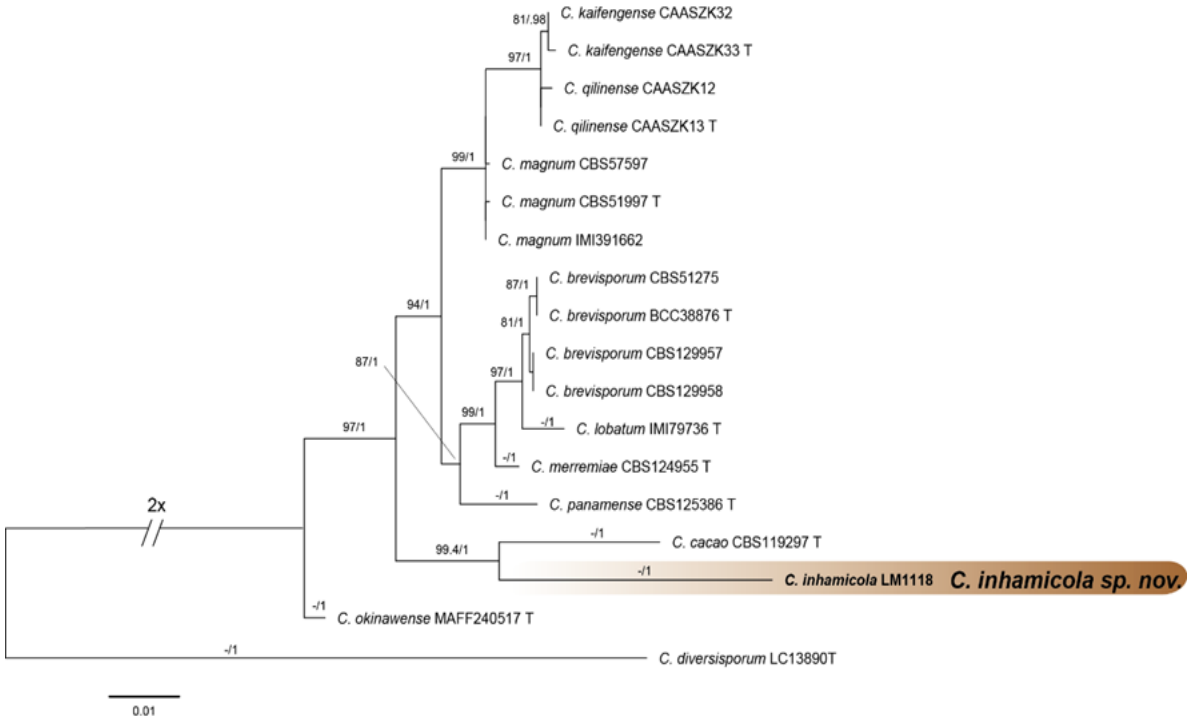
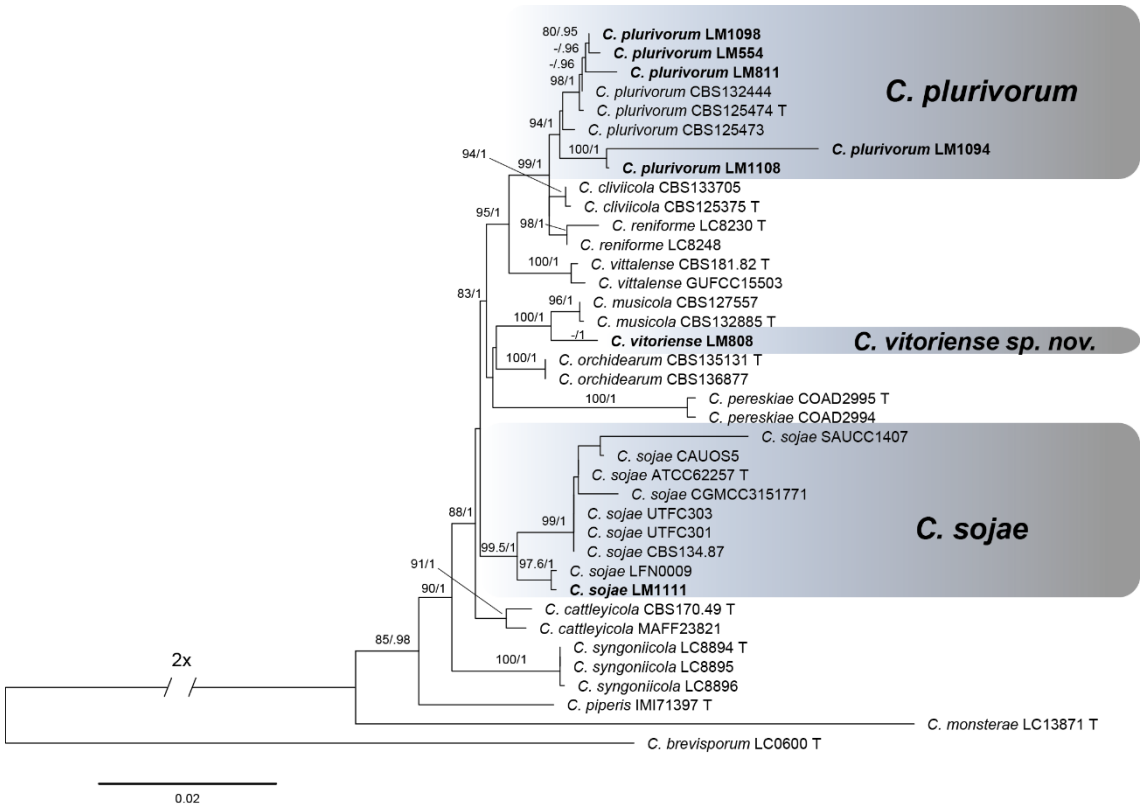
545
546

Fig. 2. - Maximum likelihood tree of the *Colletotrichum gloeosporioides* species complex inferred on IQ-TREE from a concatenated alignment of APN2, APN2/MAT-IGS, CAL, GAPDH, GAP2-IGS, GS and TUB2. Significant supports for ML (SH-alrt bootstrap ≥ 80) and Bayesian inference analysis (posterior probability ≥0.95) are shown above the nodes. The tree was rooted with *Colletotrichum pyrifoliae*. Ex-type isolates are indicated with "T" in the end of the taxa labels. Isolates from *Dioscorea* spp. are highlighted in bold. The scale bar indicates the average number of substitutions per site.



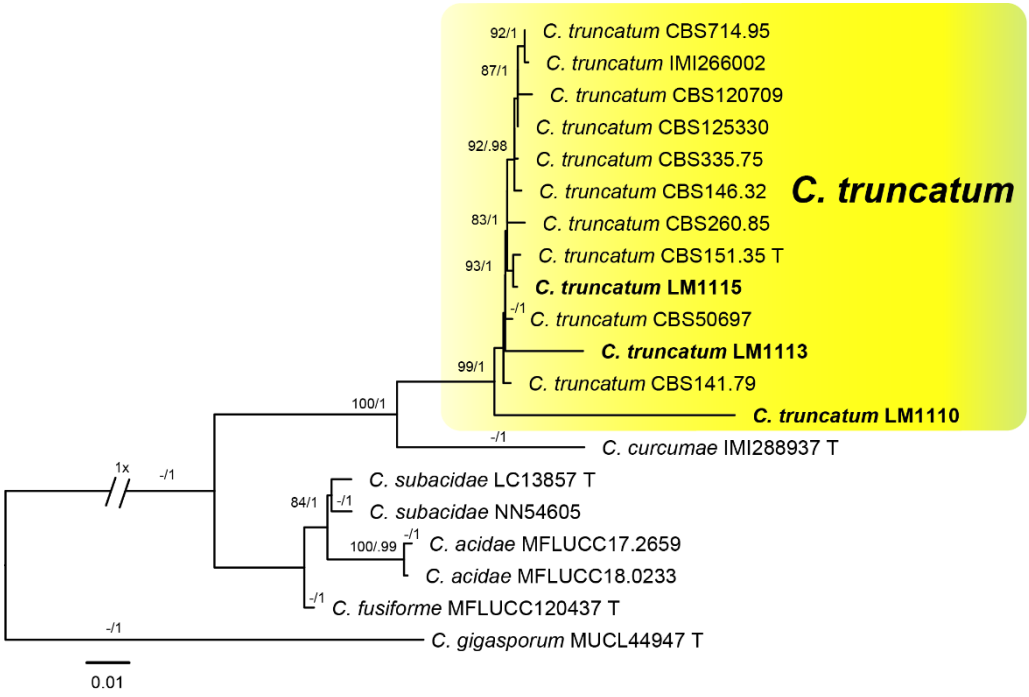
554

555 **Fig. 3**. - Maximum likelihood tree of the *Colletotrichum magnum* species complex inferred on
556 IQ-TREE from a concatenated alignment of ACT, CHS-1, GAPDH, HIS3, ITS and TUB2.
557 Significant supports for ML (SH-alrt bootstrap ≥ 80) and Bayesian inference analysis
558 (posterior probability ≥0.95) are shown above the nodes. The tree was rooted with
559 *Colletotrichum diversisporum*. Ex-type isolates are indicated with "T" in the end of the taxa
560 labels. Isolates from *Dioscorea* spp. are highlighted in bold. The scale bar indicates the
561 averagenumber of substitutions per site.



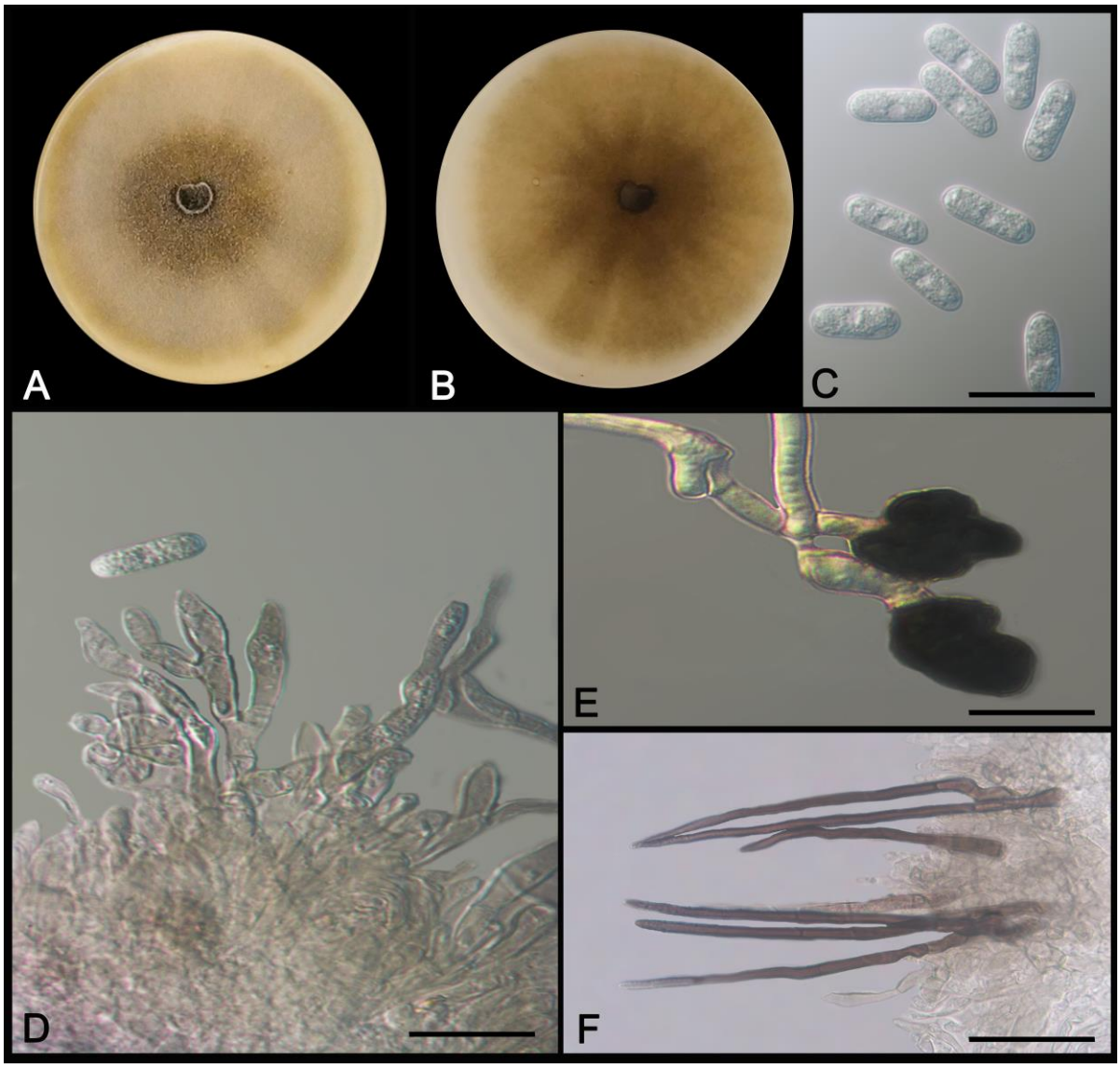
562

563 **Fig. 4**.- Maximum likelihood tree of the *Colletotrichum orchidearum* species complex
564 inferred on IQ-TREE from a concatenated alignment of ACT, CHS-1, GAPDH, HIS3, ITS
565 and TUB2. Significant supports for ML (SH-alrt bootstrap ≥ 80) and Bayesian inference
566 analysis (posterior probability ≥0.95) are shown above the nodes. The tree was rooted with
567 *Colletotrichum brevisporum*. Ex-type isolates are indicated with "T" in the end of the taxa
568 labels. Isolates from *Dioscorea* spp. are highlighted in bold. The scale bar indicates the
569 average number of substitutions per site.



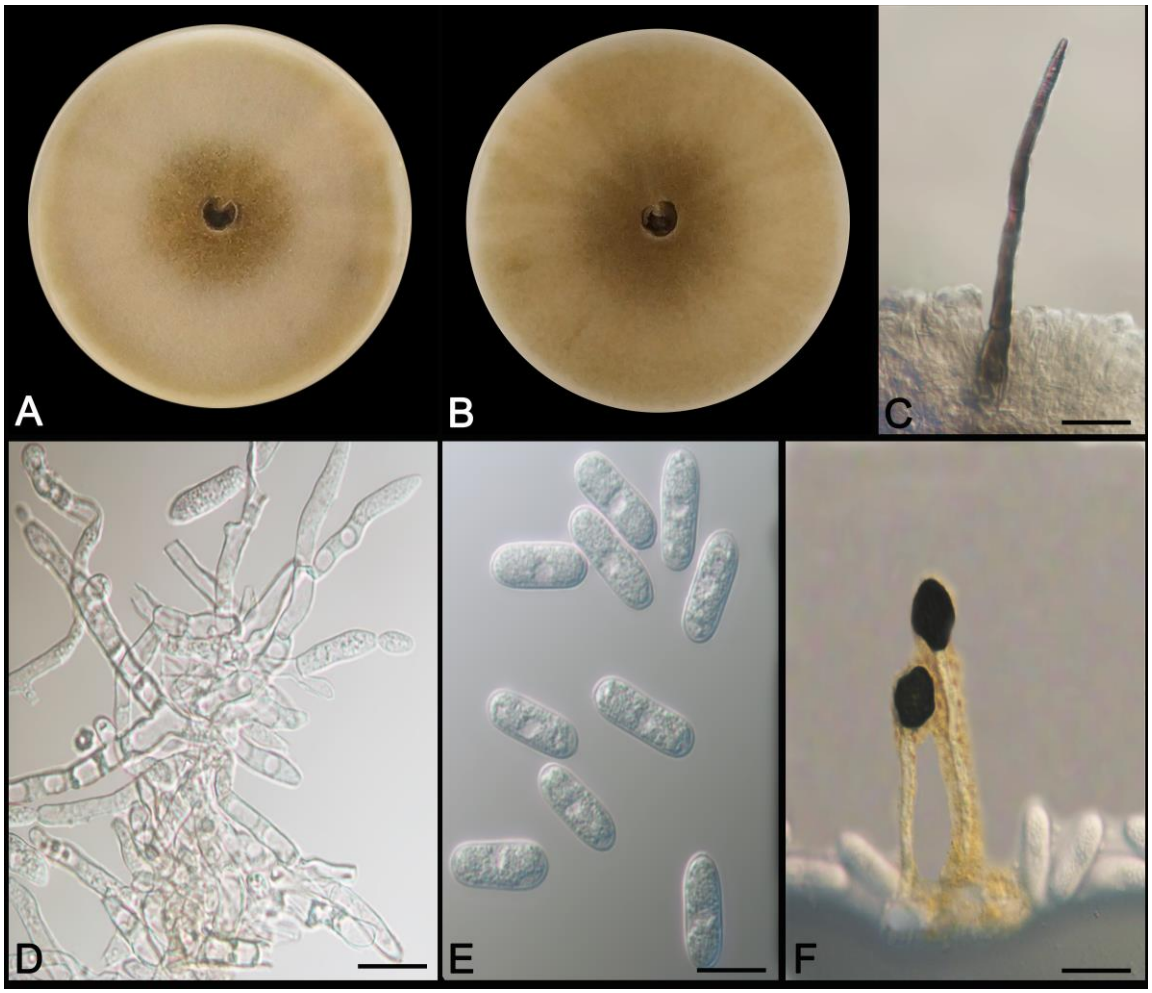
570

571 **Fig. 5**.- Maximum likelihood tree of the *Colletotrichum truncatum* species complex inferred
572 on IQ-TREE from a concatenated alignment of ACT, CHS-1, GAPDH and ITS. Significant
573 supports for ML (SH-alrt bootstrap ≥ 80) and Bayesian inference analysis (posterior
574 probability ≥0.95) are shown above the nodes. The tree was rooted with *Colletotrichum*
575 *gigasporum*. Ex-type isolates are indicated with "T" in the end of the taxa labels. Isolates
576 from *Dioscorea* spp. are highlighted in bold. The scale bar indicates the average number of
577 substitutions per site.



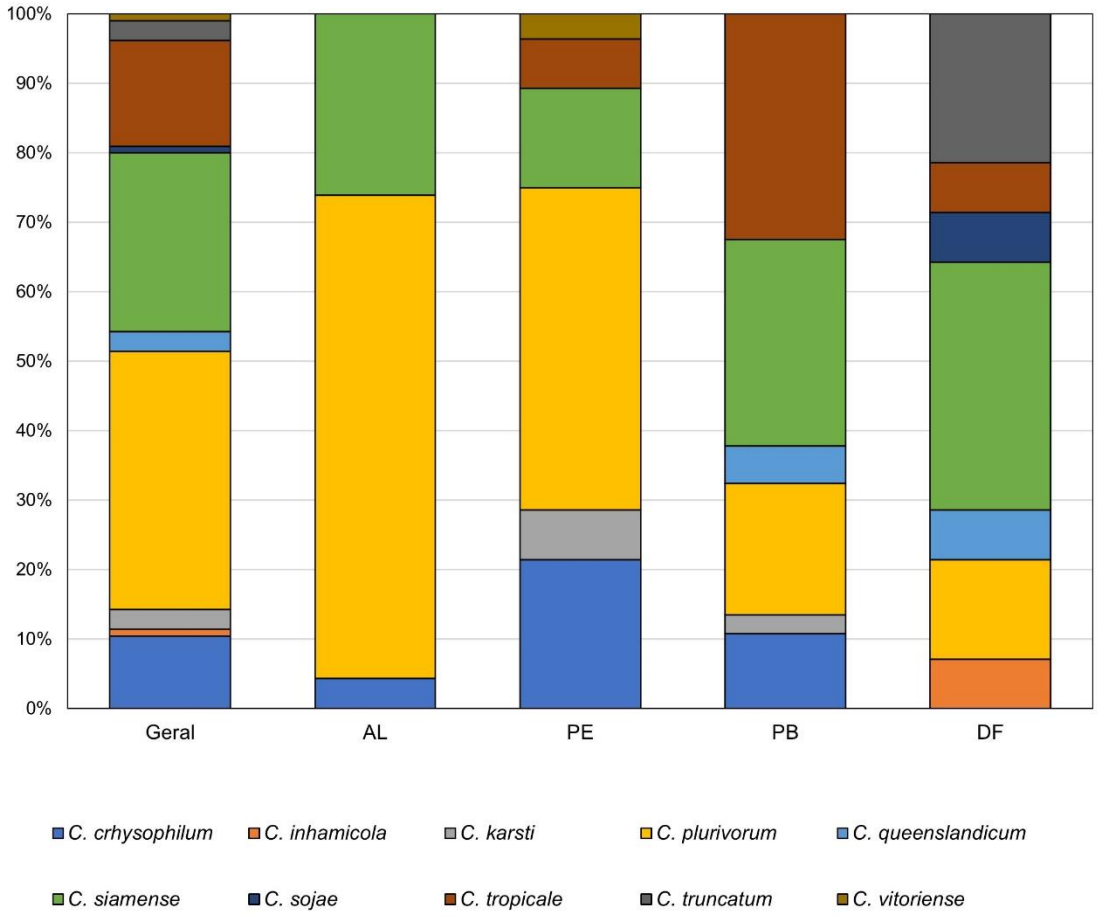
578

579 **Fig. 6.** *Colletotrichum vitoriense* (LM 808). A–B. Colonies on PDA above and below; C.
580 conidia. — Scale bars = 10 μm; D. conidiogenous cells; E. appressoria (Scale bars = 5 μm); F.
581 setae.



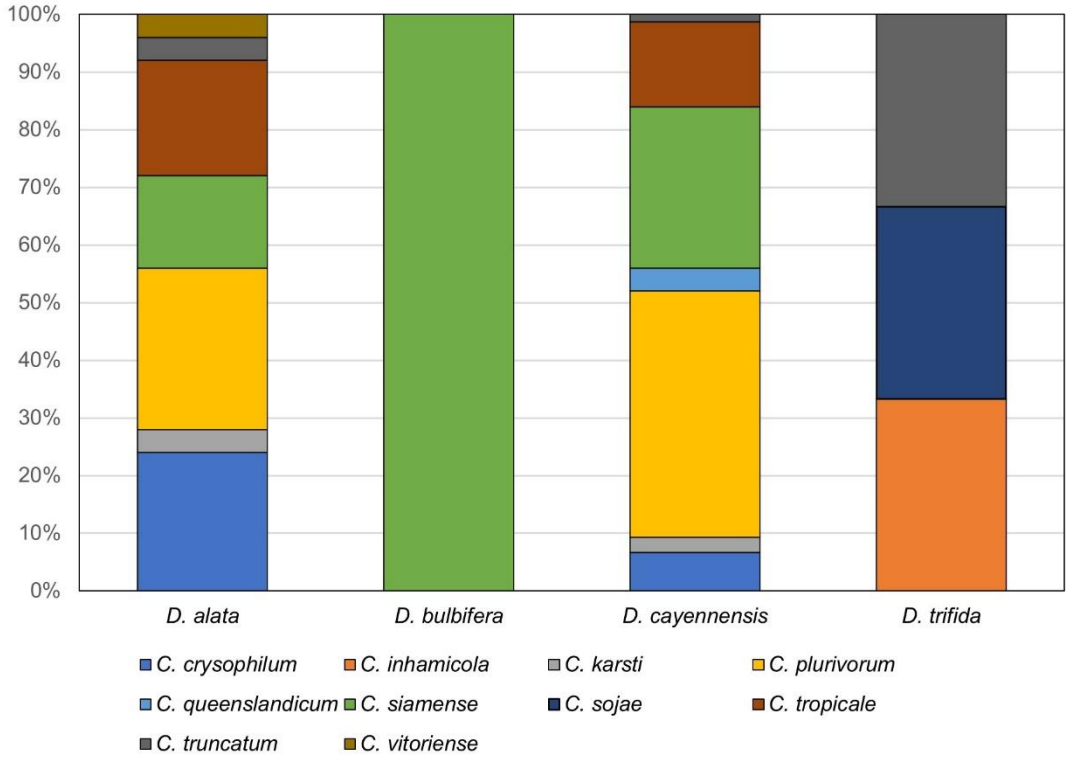
582

583 **Fig. 7**.- *Colletotrichum inhamicola* (LM 1118). A–B. Colonies on PDA above and below; C.
584 setae; D. conidiogenous cells Scale bars = 10 µm; E. conidia. — Scale bars = 10 µm; F.
585 appressoria (Scale bars = 5 µm).



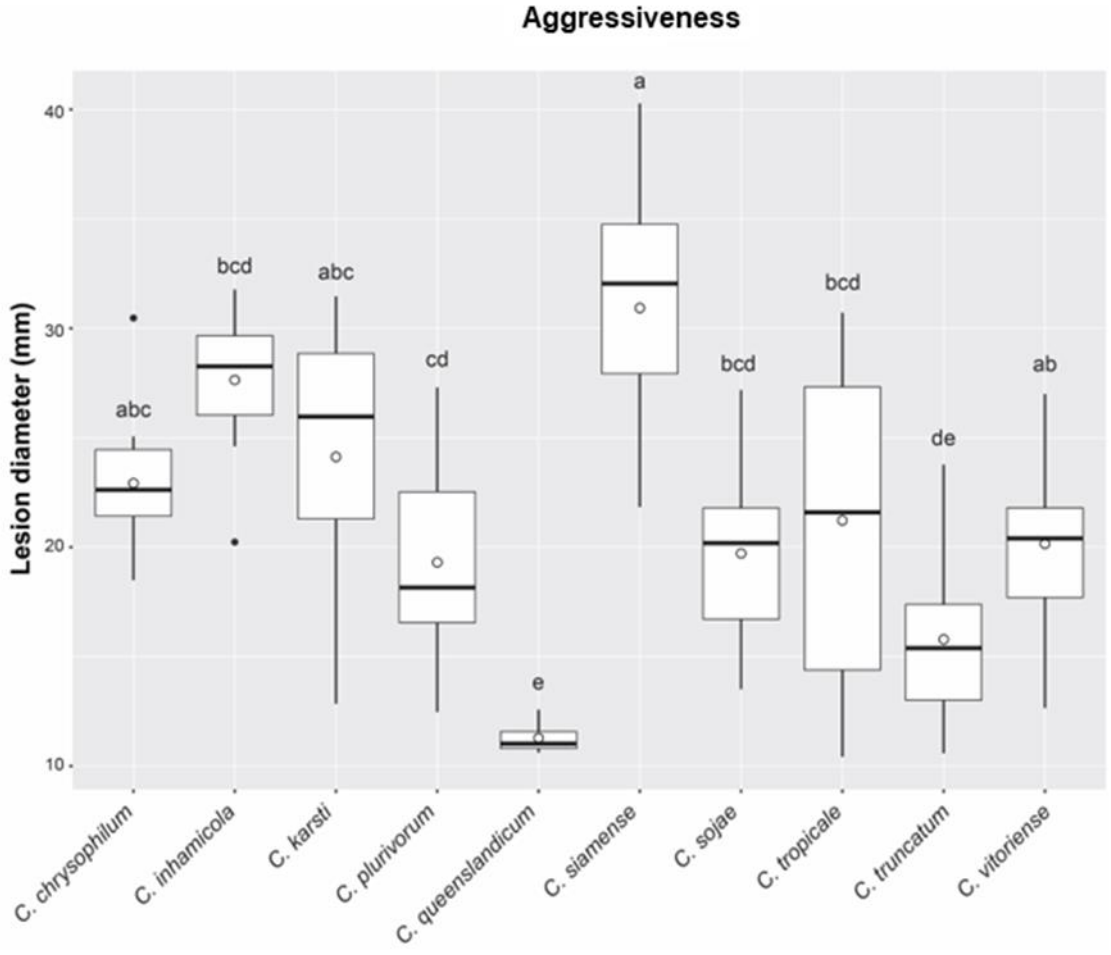
586

587 **Fig. 8**.- Prevalence of *Colletotrichum* species associated with yam anthracnose in Brazil.



588

589 **Fig. 9**.- Prevalence of *Colletotrichum* species associated with *Dioscorea* species in Brazil.



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591 **Fig. 10**.-. Aggressiveness (diameter of lesion) of the ten *Colletotrichum* species associated
592 with anthracnose in yam (cv. "Da Costa") in Brazil.

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594

CAPÍTULO III

Comparative epidemiology of *Colletotrichum* species associated with yam anthracnose in Brazil

Submissão: **Tropical Plant Pathology**

Comparative epidemiology of *Colletotrichum* species associated with yam anthracnose in Brazil

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Abstract

Anthracnose caused by *Colletotrichum* species is one of the main yam diseases in Brazil, causing production losses. The objectives of this study were to evaluate the optimal temperature and maximum growth rate, as well as sensitivity to fungicides *in vitro* and *in vivo* of ten species of *Colletotrichum* (*C. chrysophilum*, *C. inhamicola*, *C. karsti*, *C. plurivorum*, *C. queenslandicum*, *C. siamense*, *C. sojae*, *C. tropicale*, *C. truncatum*, and *C. vitoriense*) present in the main yam producing areas of Brazil. For all *Colletotrichum* species, the optimum growth temperature ranged from 24.3 to 28.8°C and the maximum growth rate from 9.82 to 13.86 mm/day. For *in vitro* fungicide tests, all *Colletotrichum* species showed mycelial growth reduction in the presence of azoxystrobin, difenoconazole, and thiophanate-methyl. For in vivo fungicide tests, *Colletotrichum* species were totally inhibited by fungicides at label rate. Our results provide important data for the development of forecasting models based on the weather forecast in the yam growing zones in Brazil, as well as the development of disease management strategies for this pathosystem.

Keywords: *Dioscorea*, chemical control, environmental conditions.

Introduction

Yam is a monocotyledonous plant, belonging to the *Dioscoreaceae* family, genus *Dioscorea*, with the species *D. cayennensis* Lam. and *D. alata* L., the most commercially cultivated in Brazil, with a predominance of the first species (Santos 1996). *Dioscorea cayennensis* has a single variety of commercial value in Brazil, commonly known as cará-da-costa, inhame-da-costa, or simply yam, and is characterized by having herbaceous stems, climbing, with the formation of tubers in their rhizomatic root system (Mesquita 2002; Moura 2006). The Brazilian Northeast region concentrates on the main yam-producing states, but national production is still considered low when compared to the main world producer, Nigeria (FAO 2021). Several factors are linked to the low productivity of yam in Brazil, such as infertile soils, low technology adopted in the planting fields, low-quality propagation material, and mainly fungal diseases, including anthracnose (Santos et al. 2009).

Yam anthracnose manifests itself through rounded brownish spots, waterlogged and sunken spots, or patterns formed by concentric rings due to the mass of conidia on the leaves. Severe infections result in plant defoliation, vein death, and tuber dry rot (Fokunang et al. 2000). The severity of anthracnose is determined by a combination of external physical and chemical factors, as well as the genetic composition of both the host and the *Colletotrichum* species (Miles et al. 2013; Zhang et al. 2014).

The disease occurs in the field when several factors coincide, among these are the susceptibility of the plant and the increase in the population of the pathogen, relative humidity, rainfall, strong winds; high intensity of light, and temperatures (Campo 2011).

With the discovery of new species of *Colletotrichum* associated with yam anthracnose in Brazil (Amaral et al. 2023 unpublished), it is important to establish whether there is any type of interference with epidemiological components, such as aggressiveness, optimum growth temperature, or sensitivity to fungicides, for example. These factors have direct

implications for disease management based on chemical control and host genetic resistance (Phoulivong 2011).

There are no studies on the comparative epidemiology of *Colletotrichu*m species in yam culture. Studies developed by Lima et al. (2015) and Veloso et al. (2021), would evaluate epidemiological components of *Colletotrichum* species associated with anthracnose in mango and cashew respectively. In these studies, it was possible to determine the influence of temperature, sporulation, conidia germination, and the severity of the disease induced by these *Colletotrichum* species and the effect of fungicides on the mycelial growth of the fungi, this information being extremely important for the adoption of management measures.

This work aimed to evaluate the aggressiveness of *Colletotrichum* species present in yam fields in Brazil, the optimal growth temperature of these species, as well as the sensitivity to azoxystrobin, difenoconazole, and thiophanate-methyl *in vitro* e *in vivo*.

Material and methods

Colletotrichum isolates

The experiments were carried out using representative isolates (Table 1) of *C. chrysophilum*, *C. inhamicola*, *C. karsti*, *C. plurivorum*, *C. queenslandicum*, *C. siamense*, *C. sojae*, *C. tropicale*, *C. truncatum*, and *C. vitoriense* from yam plantations from Brazil. The isolates were obtained from yam plants with typical symptoms of anthracnose and identified based on multilocus phylogenetics (Amaral et al. 2023 unpublished). All *Colletotrichum* isolates used in the assays were grown of 7-day-old colonies grown on PDA.

Influence of temperature on the mycelial growth of *Colletotrichum* spp.

A mycelial plug (5 mm in diameter) of each *Colletotrichum* isolate was transferred to the center of plastic Petri dishes containing PDA. The isolates were incubated in BOD at temperatures ranging from 15 °C to 35 °C in 5 °C intervals in the dark for five days. Each isolate's colony diameter was measured in two perpendicular directions to calculate the

growth rates (mm/day). The experiment was established in a completely randomized design with a factorial arrangement comprising three replicates (Petri dishes) per combination of *Colletotrichum* isolate/temperature.

Sensitivity of *Colletotrichum* species to different fungicides *in vitro*

The sensitivity in vitro of *Colletotrichum* species to fungicides was determined in a liquid culture medium supplemented with a fungicide to determine the discriminatory dose. Suspensions of spores from representatives of different species were prepared at a concentration of 10^6 conidia ml^{-1}. The action of fungicides was determined by the resazurin reduction-based colorimetric antibiogram. The potato dextrose (BD) culture medium was supplemented with streptomycin sulfate to inhibit possible bacterial growth. Commercial formulations of thiophanate-methyl fungicides (Cercobin 700 WP, 700 g kg-1 active ingredient (a. i.), Iharabras, São Paulo, SP, Brazil), difenoconazole (Score EC, 250 g l-1 a.i., Syngenta, São Paulo, Brazil) and azoxystrobin (Amistar 500 WG, 500 g kg-1 a.i.., Syngenta, São Paulo, SP, Brazil). The fungicides were dissolved in water and added to 12 mL the culture media at the following final concentrations: thiophanate-methyl– 700 (positive control); 10; 5; 2.5; 1.25; 0.625; 0.312; 0.15; 0.07 and 0.03 µg/L; difenoconazole – 250 (positive control); 40; 20; 10; 5; 2.5; 1.25; 0.625; 0.31 and 0.15 µg/L; azoxystrobin – 500 (positive control); 50; 25; 12.5; 6.25; 3.125; 1.5; 0.75; 0.39 and 0.19 µg/L in culture medium supplemented with salicylhydroxamic acid (SHAM). 20 ul of conidia suspension was added to each well and the plates were incubated in the dark for 48 hours. The negative control consisted of two wells where the first contained only 120 mL of BD and the second BD plus the conidia suspension. After the incubation period, 25uL of resazurin 0,01%n was added to sodium resazurin in each of the wells, and the plates were incubated in the dark for 3 hours. After this period, discriminating concentration values were determined by visual reading after resazurin development, which is an oxidation-reduction indicator used to assess the viability

of microbial cells. The presence of fungal growth was detected by the pink color and the absence of growth by the blue color.

Fungicides were solubilized in sterile distilled water, except azoxystrobin was solubilidized in dimethylsulfoxide (DMSO) and added to a PDA medium at different concentrations: 6.25 µg/L for thiophanate-methyl; 20 µg/L for difeconazole; and 0.15 µg/L for azoxystrobin. Five-mm-diameter mycelial plugs were obtained from the edge of a seven-day-old colony of each isolate and transferred to a PDA medium with the fungicides at different concentrations. The PDA medium for azoxystrobin was supplemented with SHAM. Fungicide-free PDA medium was used as a control. Three replicates were used to evaluate each combination of isolate fungicide concentration. The cultures were incubated at 25 °C in the dark. The colony diameter was measured in two perpendicular directions after 3 days of incubation. After 3 days of incubation, the diameter of each colony was measured in two perpendicular directions. The percentage of mycelial growth inhibition (PIC) was calculated for all fungicide concentrations, using the formula PIC= [(C – F)/C]x100, where C corresponds to the diameter of the control colony and F is the diameter of the colony for the treatment with the fungicide.

Sensitivity of *Colletotrichum* species to different fungicides *in vivo*

The sensitivity in vivo of *Colletotrichum* species to fungicides was determined using yam plants cv. “Da Costa” with 45 days of age. The plants were previously injured with a sterile needle. The plants were sprayed with the at label rate of thiophanate-methyl, difenoconazole, and azoxystrobin, 4 hours before inoculation with the pathogen. It was prepared a suspension of concentration of 10^6 conidia ml^{-1} and 10 mL were sprayed in the plants. Yam plants untreated (control) were sprayed with sterile distilled water before inoculation.

The efficiency of in vivo control was evaluated by comparing the diameter of lesions on treated and untreated plants (10 leaves evaluated per plant). The lesion diameter (mm) was

measured in two perpendicular directions. The experiment was established in a completely randomized design with each yam plant comprising a replicate. Each *Colletotrichum* isolate/fungicide assay was carried out with three replicates.

Data analysis

The values estimated for each *Colletotrichum* species in the experiments related to aggressiveness on yam leaves and fungicide sensitivity *in vivo* 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 9. 0 (Analytical Software).

In the test involving varying temperatures, the relationship between lesion diameters and the temperature was analyzed using the cubic polynomial model ($y = a + bx^2 + cx^4 + dx^6$). The optimal temperature, which corresponds to the temperature resulting in the largest lesions, was determined by fitting the regression curves to the data and utilizing the TableCurve™ 2D 5.01 software for numerical summary and estimation.

The PMGI values were converted to heatmaps in the Heatmapper web server (http://www.heatmapper.ca) to aid in visualization (Babicki et al. 2016) of the percentage of PIC for the different fungicides.

Results

Influence of temperature on the mycelial growth of *Colletotrichum* spp.

Differences in optimal temperature and mycelial growth were observed between *Colletotrichum* species. All *Colletotrichum* species showed optimal growth temperature from 25 – 40ºC. The optimum temperature ranged from 24.3 a 28.8ºC. The species *C. sojae* and *C. karsti* were the ones that presented the highest and lowest optimum temperature, respectively. The optimum mycelial growth rate was recorded for the species *C. vitoriense* at 13.86 mm/day and the lowest for the species *C. chrysophilum* at 9.82 mm/day (Figure 1).

Sensitivity of *Colletotrichum* species to different fungicides *in vitro*

According to a resazurin reduction-based colorimetric antibiogram, the discriminatory concentrations were determined: 6.25 µg/L for thiophanate-methyl; 20 µg/L for difeconazole, and; 0.15 µg/L for azoxystrobin.

All *Colletotrichum* species showed lower sensitivity to difeconazole, with percentage inhibition of mycelial growth ranging from 7.2 to 28.1% corresponding to *C. vitoriense* and *C. inhamicola* species, respectively.

For azoxystrobin, *C. inhamicola* was the most sensitive among the species studied, with an inhibition of mycelial growth of 97.7% while *C. sojae* was the least sensitive 34,2%. The other species of *Colletotrichum* also showed high sensitivity to azoxystrobin: *C. chrysophilum*, *C. tropicale*, *C. siamense*, *C. karsti*, *C. queenslandicum*, *C. truncatum*, *C. plurivorum* and *C. vitoriense* with PIC 41.8, 50.1, 52, 54, 56.6, 58.7 70.8, and 71% respectively (Figure 2).

The PIC of six species of *Colletotricum* was 100% inhibited in a PDA culture medium supplemented with thiophanate-methyl at a discriminatory dose. *Colletotrichum chyrsophilum* was the least sensitive species to this chemical compound with PIC at 10.1%, followed by *C. sojae* at 43.3 %.

Sensitivity of *Colletotrichum* species to different fungicides *in vivo*

There was a difference between yam plants treated and not treated with fungicides at their respective label rates. Typical symptoms of anthracnose were observed in yam plants not treated with fungicides. In contrast, there was 100% control of the disease in plants treated with all tested fungicides.

Discussion

This is the first study on the comparative epidemiology of *Colletotrichum* species associated with yam anthracnose in Brazil. Optimum temperatures for growth and maximum mycelial

growth rate of *C. chrysophilum*, *C. inhamicola*, *C. karsti*, *C. plurivorum*, *C. queenslandicum*, *C. siamense*, *C. sojae*, *C. tropicale*, *C. truncatum*, and *C. vitoriense* ranged between 24.3–28.8 °C, while for maximum mycelial growth ranged from 9.8–13.9 mm. It is known that the optimum temperature for *Colletotrichum* infection is around 25 to 30 °C (Arauz 2000). In this study, most *Colletotrichum* species showed optimal growth temperatures close to 25 °C, differing from the results found by Wharton (1994), where the optimum growth temperature was in the range of 26 – 32 °C to *Colletotrichum* sp. Although estimated in vitro, these temperatures correspond to those normally found in tropical zones including the Brazilian areas where yam is grown. The most rapid development of a disease, i.e. the shortest time required for completion of the disease cycle, usually occurs when the temperature is optimal for pathogen development (Agrios 2005). There are no worldwide studies on the influence of temperature on the intensity of anthracnose in yams by the prevalent species of *Colletotrichum* in Brazil. Our results can contribute to the development of prediction models based on the weather forecast in the Yam growing zones.

According to the previous study (Amaral et. al. 2023b), the species prevalent in northeastern Brazil, the main producing region, were *C. plurivorum* and *C. siamense*. These species showed optimum growth temperatures between 26.3–25.8 °C respectively. This temperature range is commonly observed in the states from which these species were found. Although these species are prevalent in the main producing regions, the other species should not be excluded from any disease management programs since factors other than temperature, can interfere with the development of the disease, and these less prevalent species can eventually become dominant in the population.

Fungicides are widely used worldwide to control yam anthracnose. In our work, we aimed to determine whether *Colletotrichum* species associated with yam anthracnose in Brazil were sensitive to fungicides commonly used to control anthracnose in other crops. For *in vitro*

196 sensitivity tests, thiophanate-methyl would be efficient if the predominant species in that
197 cultivation region were: *C. karsti*, *C. plurivorum*, *C. queenslandicum*, *C. siamense*, *C.*
198 *tropicale*, *C. truncatum*, and *C. vitoriense*, but would have low efficiency if the predominant
199 species were: *C. crhysophilum*, *C. inhamicola*, and *C. sojae*. While azoxystrobin would be
200 efficient if the predominant species of *Colletotrichum* was: *C. inhamicola*, and little efficient
201 if the predominant species were *C. sojae*. In contrast, the use of difeconazole would have low
202 efficiency for any of the species evaluated in this study.

203 For in vivo tests, using "cv. Da Costa", fungicides at label rate proved to be efficient
204 for controlling yam anthracnose, completely inhibiting the development all 10 species.
205 However, these fungicides lack registration to be used in this crop in Brazil (AGROFIT
206 2023).

207 The identification of prevalent species in a growing area is crucial to improve the
208 efficiency in the use of fungicides since, in general, *Colletotrichu*m inoculum present in crop
209 fields in Brazil does not consist of a single species, but of a set of species.

210 In this comparative epidemiology study of *Colletotrichum* species, we offer subsidies
211 for the development of disease management strategies for yam anthracnose in Brazil. We
212 demonstrated that *Colletotrichum* species from yam plants in Brazil showed excellent
213 mycelial growth in a temperature range characteristic of tropical zones, which may explain
214 the wide occurrence of yam anthracnose in different Brazilian regions. How some fungicides
215 can be used and have a differential effect on *Colletotrichum* species in yam anthracnose. Our
216 results may contribute to the development of effective and appropriate management schemes
217 for yam anthracnose.

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Declarations

Conflict of interest

The author declares no competing interests.

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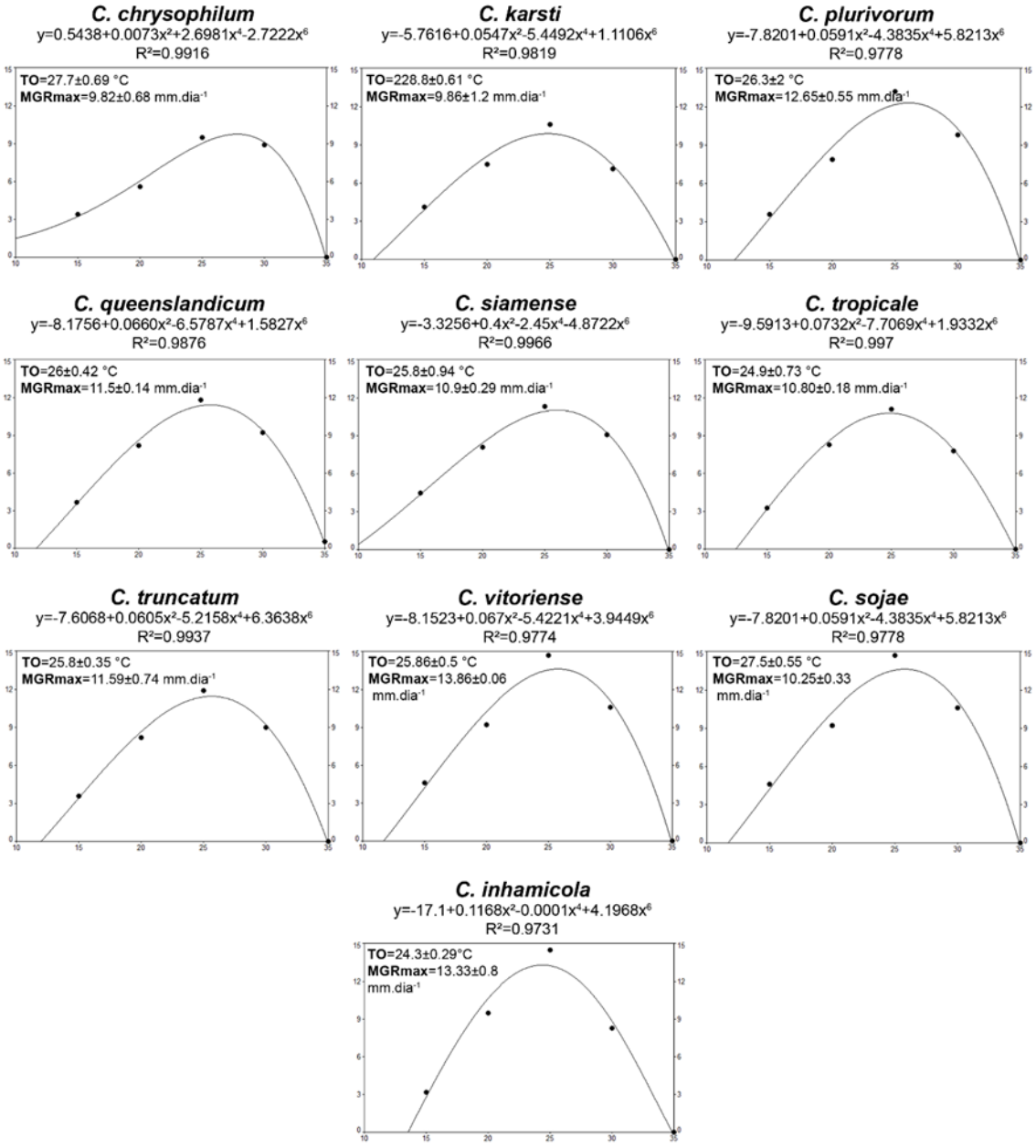
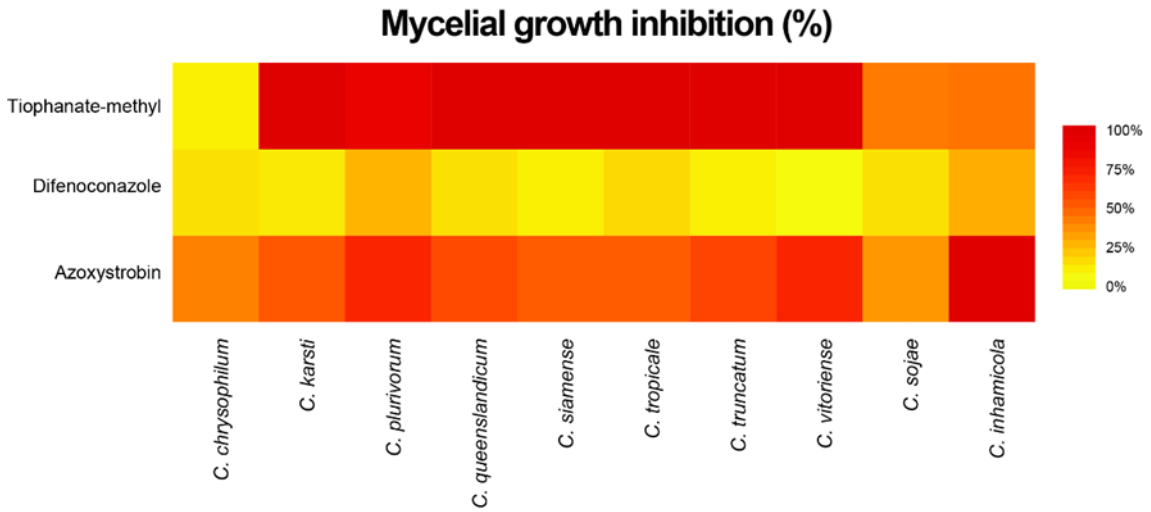


276

277 **Fig. 1** Optimum temperature for mycelial growth rate of ten *Colletotrichum* species associated

278 with yam anthracnose in Brazil. TO: Optimum temperature; MGR: Mycelial growth rate.



279

280 **Fig. 2** Heatmap showing the percentage inhibition of growth (PIC) of each *Colletotrichum*
281 species for thiophanate-methyl, difenoconazole and azoxystrobin *in vitro*. The Y-axis
282 corresponds to the fungicides used and the X-axis the PIC for each *Colletotrichum* species.

283

284 **Table 1**. Isolates of ten species of *Colletotrichum* obtained from leaves of yam (*Dioscorea*
285 spp.) from cultivated areas in Brazil.

			Localization	
***Colletotrichum* species**	**Isolate**	**Dioscorea host**	**City**	**State**
C. chyrsophilum	LM 1270	*D. cayennensis*	Campestre	Alagoas
C. inhamicola	LM 1118	*D. trifida*	Gama	Distrito Federal
C. karsti	LM 544	*D. cayennensis*	Bonito	Pernambuco
	LM 583	*D. cayennensis*	Conde	Paraíba
C. plurivorum	LM 554	*D. cayennensis*	Marcação	Paraíba
	LM 1098	*D. alata*	Bonito	Pernambuco
C. queenslandicum	LM 574	*D. cayennensis*	Conde	Paraíba
C. siamense	LM 576	*D. cayennensis*	Conde	Paraíba
	LM 1109	*D. cayennensis*	Gama	Distrito Federal
C. sojae	LM 1111	*D. trifida*	Gama	Distrito Federal
C. tropicale	LM 556	*D. cayennensis*	Conde	Paraíba
	LM 579	*D. cayennensis*	Conde	Paraíba
C. truncatum	LM 1110	*D. alata*	Gama	Distrito Federal
	LM 1113	*D. alata*	Gama	Distrito Federal
C. vitoriense	LM 808	*D. alata*	Vitória de Santo Antão	Pernambuco

Table S1. Percentage values of inhibition of mycelial growth (PIC) of *Colletotrichum* species to the fungicides thiophanate-methyl, difenoconazole and azoxystrobin used to obtain the heatmap.

	Colletotrichum species (**PIC**)									
Fungicide	***C. chrysophilum***	***C. inhamicola***	***C. karsti***	***C. plurivorum***	***C. queenslandicum***	***C. siamense***	***C. sojae***	***C. tropicale***	***C. truncatum***	***C. vitoriense***
Thiophanate-methyl	10.1	44.9	100	88.1	100	100	43.3	100	100	100
Difenoconazole	15.8	28.1	13.5	27.8	14.9	11	16.0	16.8	10.1	7.2
Azoxystrobin	41.8	97.7	54	70.8	56.6	52	34.9	50.1	58.7	71

CAPÍTULO IV

Conclusões gerais

CONCLUSÕES GERAIS

1. Dez espécies de *Colletotrichum* (*C. chrysophilum*, *C. inhamicola*, *C. karsti*, *C. plurivorum*, *C. queenslandicum*, *C. siamense*, *C. sojae*, *C. tropicale*, *C. truncatum* e *C. vitoriense*) são responsáveis pela antracnose do inhame no Brasil;
2. Duas espécies novas de *Colletotrichum* foram identificadas nesse estudo: *C. inhamicola* e *C. vitoriense*;
3. *Colletotrichum plurivorum* foi a espécie prevalente na maioria dos estados amostrados.
4. *Colletotrichum plurivorum* é a espécie prevalente nas espécies *Dioscorea alata* e *D. cayennensis*, *C. siamense* em *D. bulbifera* e *C. inhamicola*, *C. sojae* e *C. truncatum* em *D. trifida*;
5. A temperatura ótima para crescimento micelial das espécies de *Colletotrichum* identificadas, é similar as temperaturas das áreas de cultivo no Brasil;
6. As espécies de *Colletotrichum* se mostraram sensíveis a azoxistrobina, difenoconazole e tiofanato-metíllico em maior ou menor nível.
7. A identificação correta de espécies de *Colletotrichum* é essencial para o desenvolvimento de estratégias de manejo eficientes.