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Tese de Doutorado

Filogenia e epidemiologia de espécies de *Colletotrichum* associadas à antracnose do cajueiro

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

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A Deus!

OFEREÇO

Os meus melhores resultados às pessoas que eu mais amo

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Minha irmã Josilane

Meu noivo Paulo

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SUMÁRIO

RESUMO GERAL

Antracnose é a principal doença do cajueiro, ocorrendo tanto na fase vegetativa quanto reprodutiva de plantas cultivadas (*Anacardium occidentale*) e silvestres (*Anacardium humile* e *Anacardium othonianum*). Comumente atribuída a *Colletotrichum gloeosporioides*, a exata etiologia da antracnose do cajueiro permanecia até então desconhecida. Objetivou-se com o presente estudo identificar as espécies de *Colletotrichum* associadas à antracnose do cajueiro no Brasil, determinar suas relações filogenéticas, distribuição geográfica e fornecer algumas informações sobre os fatores bióticos e abióticos que podem influenciar a composição da comunidade. Investigou-se ainda a influência da temperatura nos parâmetros biológicos das espécies de *Colletotrichum*, sua agressividade em folhas de *A. occidentale* e frutos de seis hospedeiros alternativos, e sua sensibilidade a três fungicidas. Sete espécies de *Colletotrichum* (*C. chrysophilum*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides sensu stricto*, *C. queenslandicum*, *C. siamense* e *C. tropicale*) foram identificadas a partir de tecidos sintomáticos de *A. occidentale*, *A. humile* e *A. othonianum*. Ocorrendo na Caatinga, Cerrado, Floresta Amazônica e Mata Atlântica, *C. siamense* foi a espécie mais comum em *A. occidentale* e *A. othonianum*, enquanto *C. fructicola* foi a prevalente no Cerrado e em *A. humile*. Em relação ao hospedeiro, *A. occidentale* e folhas foram os estratos mais diversos, enquanto a Mata Atlântica e Pernambuco foram os estratos mais diversos relacionados à distribuição geográfica. As temperaturas ótimas para o crescimento micelial e germinação conidial variaram entre 25–30 °C e 27–37 °C, respectivamente. Todas as espécies de *Colletotrichum* foram patogênicas a folhas de cajueiro com ferimento, mas nenhuma causou lesão em superfície foliar intacta. Variando quanto à agressividade, todas as espécies, exceto *C. fragariae* e *C. fructicola* em abacate, foram patogênicas a frutos com ferimento de abacateiro, bananeira, goiabeira, mangueira e mamoeiro, enquanto algumas também induziram lesões em tecidos intactos, exceto em abacateiro. Nenhum sintoma de antracnose foi observado em frutos de maracujazeiro, independente do método de inoculação. Em maior ou menor nível, as espécies de *Colletotrichum* se mostraram sensíveis a azoxistrobina, difenoconazole e tiofanato-metíllico.

PALAVRAS-CHAVE: *Anacardium* spp., filogenia, distribuição geográfica, epidemiologia, fungicidas.

GENERAL ABSTRACT

Anthracnose is the most important disease on cashew plants, occurring on both vegetative and reproductive stages of cultivated (*Anacardium occidentale*) and wild cashew species (*Anacardium humile* and *Anacardium othonianum*). Usually ascribed to *Colletotrichum gloeosporioides*, the precise etiology of cashew anthracnose is just now revealed. The present study aimed to determine the *Colletotrichum* species associated with cashew anthracnose in Brazil, infer their phylogenetic relationships and geographical distribution, and provide information about biotic and abiotic factors that may affect community composition. The influence of temperature on the biological traits of *Colletotrichum* spp., their aggressiveness on leaves of *A. occidentale* and fruits of six alternative hosts, in addition to their sensibility to three fungicides were also investigated. Seven *Colletotrichum* species (*C. chrysophilum*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides sensu stricto*, *C. queenslandicum, C. siamense* and *C. tropicale*) were identified from symptomatic-tissues of *A. occidentale*, *A. humile* and *A. othonianum*. Found across the Caatinga, Cerrado, Amazon Rainforest and Atlantic Forest, *C. siamense* was the most common species associated with *A. occidentale* and *A. othonianum*, whereas *C. fructicola* prevailed in the Cerrado on *A. humile*. The cultivated cashew and leaves were the most diverse host-associated strata, whereas the Atlantic Forest and Pernambuco were the most diverse strata related to geographical distribution. The optimum temperatures for mycelial growth and conidial germination varied between 25–30 °C and 27–37 °C, respectively. All *Colletotrichum* species were pathogenic to wounded cashew leaves, but none of them induced lesions on intact leaf-surface. Varying in the aggressiveness, all *Colletotrichum* species, but *C. fragariae* and *C. fructicola* on avocado, were pathogenic to wounded fruits of avocado, banana, guava, mango and papaya, while some of them also caused lesions on non-wounded host-tissues, except on avocado. There were no symptoms of anthracnose on passion fruits, regardless of the inoculation method. In a greater or lesser extension, *Colletotrichum* species were sensitive to azoxystrobin, difenoconazole and thiophanate-methyl.

KEY WORDS: *Anacardium* spp., phylogeny, geographical distribution, epidemiology, fungicides.

CAPÍTULO I

Introdução Geral

Filogenia e epidemiologia de espécies de *Colletotrichum* associadas à antracnose do cajueiro

Introdução geral

Descrição e importância das espécies de cajueiro

A vasta extensão territorial e multiplicidade climática favorecem a fruticultura brasileira que tem como uma de suas características fundamentais a diversidade de espécies cultivadas tanto nativas quanto exóticas (SILVA JUNIOR; BEZERRA; LEDERMAN, 1999). No Brasil, além dos cultivos comerciais altamente tecnificados, algumas espécies de frutas nativas como umbu, pequi, mangaba, castanha-do-Pará e caju são exploradas de forma extrativista (APIZ, 2008; ASSUNÇÃO, 2012; MACÊDO, 2012). Em 2014, atrás apenas da China e Índia, o Brasil ocupou a terceira colocação no *ranking* mundial de produção de frutas, com destaque para a laranja, banana, melancia, abacaxi, mamão e caju (FAO, 2017).

No contexto botânico, o cajueiro pertence à família Anacardiaceae e ao gênero *Anacardium*, este compreendendo pelo menos 38 espécies de plantas (TPL, 2017). Os principais centros de diversidade do gênero *Anacardium* estão na região amazônica e nos cerrados brasileiro (PAIVA et al., 2002; AGOSTINI-COSTA et al., 2006). As espécies *Anacardium humile* St. Hilaire, *Anacardium nanum* St. Hilaire, *Anacardium corymbosum* Barb. Rodr. e *Anacardium othonianum* Rizzini são nativas nas regiões de cerrado, enquanto *A. occidentale* L., distribuído por quase todo o território brasileiro, adapta-se melhor ao litoral e ao semi-árido nordestinos (CRISÓSTOMOS et al., 2000; PAIVA; BARROS, 2004; AGOSTINI-COSTA et al., 2006). Além da melhor adaptação a determinadas regiões do país, essas espécies apresentam algumas características que as diferenciam umas das outras.

Conhecido popularmente como caju-do-campo, cajuí, cajuzinho-do-cerrado, caju-mirim, cajuzinho-do-mato e caju-anão, *A. humile* apresenta porte subarbustivo medindo de 30 a 150 cm de altura (AGOSTINI-COSTA et al., 2006). É encontrado em Santa Cruz na Bolívia, região oriental do Paraguai e, no Brasil, nos cerrados da Bahia, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, São Paulo e no Distrito Federal (LORENZI et al., 2006; GRANDO, 2009). Ocorre com frequência em campo sujo e no Cerrado *stricto senso* (VIEIRA et al., 2010). *Anacardium humile*

apresenta raízes profundas (freatófitas) e tronco subterrâneo (AGOSTINI-COSTA et al., 2006), características que conferem proteção contra a seca e as queimadas (LÓPEZ-NARANJO; PERNÍA 1990) frequentes no cerrado brasileiro durante a transição da estação seca para a chuvosa (RAMOS-NETO; PIVELLO, 2000; PIVELLO, 2011). A parte aérea é formada por folhas e flores que emergem na superfície do solo e possuem comportamento ortotrópico de crescimento apical (MITCHELL; MORI, 1987). As folhas coriáceas podem atingir 27,5 cm de comprimento e 9,5 cm de largura. Apresentam base geralmente atenuada e assimétrica, glabras nas duas superfícies e geralmente apresentam pecíolos de até 15 mm (AGOSTINI-COSTA et al., 2006). As inflorescências de *A. humile* são pubescentes, cuja floração inicia-se em julho e a frutificação geralmente ocorre entre setembro e outubro (DALPONTE; LIMA, 1999; BATALHA; MANTOVANI, 2000; LORENZI et al., 2006).

Já a espécie *A. nanum*, conhecida como cajuzinho e caju-rasteiro, também é um subarbusto de tronco subterrâneo com a mesma altura de *A. humile*, mas difere deste por suas folhas coriáceas de base geralmente auriculada e assimétrica irem de pubescentes a vilosas abaixialmente e de glabras a puberosas adaxialmente, além de apresentar inflorescências vilosas (AGOSTINI-COSTA et al., 2006). Esta espécie se distribui entre a região central de Goiás, Distrito Federal e regiões sul, central e oeste de Minas Gerais (MENDONÇA et al., 1998). Bastante semelhante às espécies anteriores, *A. corymbosum* é um subarbusto de 50 a 150 cm de altura cujas folhas, coriáceas e sem pecíolo, são vilosas abaixialmente e glabras a puberosas adaxialmente (MITCHELL; MORI, 1987). Essa espécie é endêmica do estado de Mato Grosso e suas flores apresentam antera globular, o que a diferencia de *A. nanum*, que apresenta antera normal (MITCHELL; MORI, 1987; AGOSTINI-COSTA et al., 2006).

Diferentemente das anteriores, *A. othonianum*, popularmente denominado caju-de-árvore-do-cerrado, cajuzinho ou cajuí, apresenta porte arbóreo variando entre 3 e 6 m de altura, folhas coriáceas glabras de até 17 cm de comprimento e base subcortada com pecíolo de até 8 mm (MITCHELL; MORI, 1987; AGOSTINI-COSTA et al., 2006). Sendo o principal cajueiro de importância econômica para a região central do Brasil, cuja frutificação ocorre de setembro a outubro (AGOSTINI-COSTA et al., 2006). Esta espécie é mais comum no cerrado, principalmente em neossolos litólicos e solos com concreções ou cascalho (AGOSTINI-COSTA et al., 2006). Pelas semelhanças, *A. othonianum* já foi classificado como ecótipo de *A. occidentale* (MITCHELL; MORI, 1987), mas atualmente, estas são consideradas espécies distintas (TPL, 2017).

A única espécie do gênero *Anacardium* cultivada comercialmente, *A. occidentale*, compreende árvores de médio e pequeno porte, sendo denominadas como cajueiro comum e

cajueiro anão-precoce, respectivamente (CRISÓSTOMO et al., 2004; AGOSTINI-COSTA et al., 2006). O cajueiro comum, de caule atarracado e tortuoso, pode atingir de 8 a 15 m de altura com copa de até 20 m de diâmetro, ao passo que o cajueiro anão-precoce atinge até 5 m de altura e sua copa, mais compacta e homogênea, chega a 8 m de diâmetro (BARROS et al., 2002). Período de florescimento varia de cinco a sete meses no tipo comum (de julho/agosto a dezembro/janeiro) e de seis a oito meses (de junho/julho a janeiro/fevereiro) no tipo anão precoce (SERRANO; OLIVEIRA, 2013).

Apesar de ser frequentemente confundida como fruto, a parte carnosa e suculenta do caju na verdade corresponde ao pedúnculo floral superdesenvolvido (pseudofruto), enquanto o fruto verdadeiro, um aquênio de casca coriácea que abriga internamente a parte comestível, é representado pela castanha (MAZZETTO; LOMONACO; MELE, 2009). O pseudofruto, junto à castanha, constitui o "duplo fruto" característico do gênero *Anacardium* (AGOSTINI-COSTA et al., 2006). Esses frutos podem ser consumidos *in natura* ou destinados para agroindústrias, servindo como matéria-prima na fabricação de sucos, polpas, aguardente, rapadura, licores e doces, ou simplesmente comercializados como castanha torrada (PAIVA et al., 2000; TORREGGIANI; BERTOLO, 2001; PETINARI; TARSITANO, 2002). Assim, a cadeia produtiva do caju, seja ele cultivado ou explorado de forma extrativista, gera milhares de empregos diretos e indiretos, representando um complemento à renda familiar de pequenos produtores rurais ou mesmo de trabalhadores autônomos circunvizinhos às áreas de produção (SILVA et al., 2001; BARROS et al., 2002; AGOSTINI-COSTA et al., 2006).

Em 2014, quatro países comercializaram o pseudofruto do caju, sendo o Brasil o maior produtor mundial com 1.889.934 toneladas, seguido por Madagascar, Mali e Guyana (FAO, 2017). A castanha, por sua vez, é explorada em 33 países e em 2014, sua produção atingiu 3.713.731 toneladas, destacando-se a Nigéria como o principal produtor seguido pela Índia, Costa do Marfim, Vietnã e o Brasil ocupando o décimo lugar (FAO, 2017). A produção brasileira de castanha de caju em 2014 atingiu 107.713 toneladas (FAO, 2017), impulsionada principalmente pelos estados do Ceará, Rio Grande do Norte, Piauí e Bahia que juntos contribuiu com 69,23% do total efetivamente exportado (CONAB, 2017; IBGE, 2017). Em 2016, o Brasil exportou 14.506 toneladas de castanha de caju, tendo como principais destinos os Estados Unidos, Canadá e os Países Baixos (CONAB, 2017).

Antracnose e seu impacto na cajucultura

Diversos fatores limitam a expansão da cajucultura no Brasil, incluindo o domínio do mercado por atravessadores, que geram assimetria na rentabilidade entre o produtor e o comerciante, baixo aproveitamento do peseudofruto e a baixa qualidade do que é produzido (CARMELIO, 2010). Na verdade, esses fatores correlacionam-se à medida que o produtor, ao não captar recursos suficientes com o que comercializa em uma safra, deixa de investir em práticas culturais para garantir boa produção na safra seguinte. Ao negligenciar tais práticas, as plantas podem tornar-se mais suscetíveis à incidência de pragas e doenças, destacando-se o mofo-preto (*Pilgeriella anacardii* Arx & Müller), oídio (*Oidium anacardii* Noack), mancha-angular (*Septoria anacardii* Freire), crestamento foliar (*Xanthomonas campestres* pv. *Mangiferaeindicae*, resinose (*Lasiodiplodia theobromae* (Pat.) Griffon et. Maubl, *Lasiodiplodia brasiliense* M. S. B. Netto, M. W. Marques & A. J. L. Phillips, *Lasiodiplodia euphorbicola* A. R. Machado & O. L. Pereira, *Lasiodiplodia gonubiensis* Pavlic, Slippers & M. J. Wingf, *Lasiodiplodia iraniensis* Abdollahzadeh, Zare & A. J. L. Phillips, *Lasiodiplodia jatrophicola* A. R. Machado & O. L. Pereira, *Lasiodiplodia gravistriata* M.S.B. Netto & M.P.S. Câmara, *Lasiodiplodia pseudotheobromae* A. J. L. Phillips, A. Alves & Crous, *Neofusicoccum batangarum* Begoude, Jol. Roux & Slippers e *Pseudofusicoccum stromaticum* Mohali, Slippers & M.J. Wingf (NETO et al., 2016)) e antracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. et Sacc (FREIRE et al., 2002; FREIRE; CARDOSO, 2003; MENEZES, 2005).

Antracnose é a doença fúngica mais severa do cajueiro no Brasil (CAVALCANT et al., 2000; FREIRE et al., 2002; FREIRE; CARDOSO, 2003; MENEZES, 2005; RIBEIRO; VAL; NETO, 2009). Ocorre em todas as regiões produtoras tanto na fase vegetativa quanto reprodutiva da cultura (FREIRE et al., 2002, FREIRE; CARDOSO, 2003; MENEZES, 2005). Essa doença incide tanto em pomares geneticamente diversificados, quanto em áreas de cultivo comercial, onde frequentemente são utilizados clones melhorados (FEIRE; CARDOSO, 2003), além de ser observada também em espécies de cajueiro não cultivadas.

Os sintomas da antracnose no cajueiro, caracterizados inicialmente por manchas irregulares de coloração parda que tornam-se avermelhadas com o envelhecimento, podem ser observadas nos ramos, inflorescências, pedúnculo, castanha e, principalmente, nas folhas (FEIRRE et al., 2002; FEIRE; CARDOSO, 2003; MENEZES, 2005). Os ramos infectados apresentam lesões necróticas, deprimidas e escuras (MENEZES, 2005). Pode haver exudação de goma nas lesões das inflorescências, enquanto no pedúnculo ocorrem rachaduras que levam ao seu apodrecimento, ao

passo que os frutos novos atacados tornam-se escuros, deformados e atrofiados (MENEZES, 2005). Em infecções severas, devido ao crescimento desigual do tecido sadio em relação ao tecido afetado, as folhas apresentam-se retorcidas e deformadas com aparência de queima (FREIRE et al., 2002; CARDOSO; FEIRE 2002; MENEZES, 2005; RIBEIRO; VAL; NETO, 2009). A antracnose é mais severa durante ou logo após o período chuvoso, quando novas brotações e/ou inflorescências são emitidas. Quando o período de elevada umidade prolonga-se até o início da frutificação, as perdas na produção são mais acentuadas (FEIRE; CARDOSO, 2003).

Mensurar as perdas de produção ocasionadas pela antracnose na cajucultura é uma tarefa complicada, principalmente ao considerar-se todos os fatores que influenciam sua epidemiologia, por exemplo, o genótipo do hospedeiro, estágio fenológico da cultura, agressividade do(s) patógeno(s) envolvido(s), práticas culturais adotadas nos pomares e as condições ambientais predominantes nas áreas de cultivo.

Gênero *Colletotrichum*

Considerado como um dos gêneros fúngicos mais importantes economicamente no mundo (CANNON et al., 2012; DEAN et al., 2012; HUANG et al., 2013; UDAYANGA et al., 2013), *Colletotrichum* abrange espécies endofíticas, epifíticas, saprofíticas e fitopatogênicas (PHOTITA et al., 2003; OSONO et al., 2009; LIMA et al., 2013; MANAMGADA et al., 2013; TAO et al., 2013). Este patógeno pode penetrar o tecido hospedeiro de forma direta ou indireta em qualquer fase de desenvolvimento. Por ser hemibiotrófico, a interação de *Colletotrichum* com sua planta hospedeira é caracterizada por uma curta fase biotrófica, quando os dois organismos mantêm contato direto na superfície celular, seguido de uma fase necrotrófica destrutiva. A infecção ocorre através da formação de um apressório que se desenvolve a partir da germinação do esporo na superfície da planta hospedeira, seguida pela penetração direta na cutícula. Após a penetração, o patógeno pode permanecer latente ou desenvolver-se intra e/ou intercelularmente, resultando em lesões oriundas do desenvolvimento das hifas secundárias e colonização dos tecidos adjacentes (BAILEY et al., 1992; PERFECT et al., 1999; AGRIOS, 2005; DAMM et al., 2009; DEAN et al., 2012). Por ser um gênero altamente diversificado, espécies de *Colletotrichum* pertencentes a um mesmo complexo ou até isolados de uma mesma espécie podem apresentar diferentes níveis de virulência dependendo da suscetibilidade do hospedeiro, presença de ferimentos e das condições ambientais, dentre outros fatores (FREEMAN et al., 1998; PHOULIVONG et al., 2012; KENNY et al., 2012; AGOSTEO et al., 2015).

As colônias de *Colletotrichum* apresentam coloração que varia do branco-gelo ao cinza-escuro, micélio aéreo de densidade variável, conídios hialinos asseptados, retos, fusiformes ou curvados, apressórios marrons com margens contínuas ou irregulares, acérvulo subcuticular, epidermal, subperidermal ou peridermal, além da presença de escleródios em algumas espécies (SUTTON, 1980, 1992). Os acérvulos, formados abaixo da cutícula do tecido hospedeiro, produzem conídios que são protegidos por uma matriz mucilaginosa de coloração alaranjada constituída de polissacarídeos e proteínas solúveis em água que protegem os esporos da dessecação, autogerminação e radiação ultravioleta (AGRIOS, 2005; MENEZES, 2005).

Gama de hospedeiros de *Colletotrichum*

Durante muito tempo assumiu-se que as espécies fitopatogênicas de *Colletotrichum* apresentavam especificidade/preferência a determinadas espécies de plantas, levando à descrição de táxons com base na identidade de seu hospedeiro (CONNON et al., 2012). No entanto, muitas espécies de *Colletotrichum* ocorrem endofiticamente, o que constitui uma complicação a mais na compreensão da especificidade/preferência hospedeira dos representantes desse gênero (ROJAS et al., 2010). Além disso, algumas espécies patogênicas permanecem em latência no interior dos tecidos do hospedeiro, ao passo que alguns saprófitas também podem se comportar como parasitas facultativos (PHOTITA et al., 2003). A estratégia de infecção destes fungos também influencia na sua patogenicidade, sendo os fungos biotróficos altamente específicos, enquanto os hemibiotróficos apresentam fase inicial biotrófica seguida de uma fase necrotrófica virulenta (SHEN et al., 2001).

A gama de hospedeiros de *Colletotrichum* spp. é bastante diversificada, sendo comum que uma única espécie infecte múltipos hospedeiros e/ou que diversas espécies ocorram em um único hospedeiro (FREEMAN et al., 1998; CAI et al., 2009; HYDE et al., 2009; PHOULIVONG et al., 2010; PHOULIVONG et al., 2012). *Colletotrichum fructicola* Prihastuti, L. Cai & K.D. Hyde, por exemplo, é relatado em plantas de *Citrus* sp., *Vitis* sp., *Ficus* sp., *Mangifera indica* L., *Coffea arabica* L., *Annona muricata* L. e *Capsicum annuum* L. (WEIR et al., 2012; PENG et al., 2013; ÁLVAREZ et al., 2014; LIU et al., 2016), enquanto *Colletotrichum karstii* Y.L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai tem sido comumente relatado em *Citrus* sp., *M. indica, A. muricata, C. sinensis* e plantas da família Orchidaceae (YANG et al., 2011; DAMM et al., 2012; PENG et al., 2012; LIMA et al., 2013; ÁLVAREZ et al., 2014; WANG et al., 2016). Por outro lado, *C. karstii*, *Colletotrichum citri* F. Huang, L. Cai, K.D. Hyde & H.Y. L, *Colletotrichum citricola* F. Huang, L. Cai, K.D. Hyde & H.Y. Li, *Colletotrichum gloeosporioides*, *Colletotrichum truncatun* (Schwein.)

Andrus & W.D. Moore e *C. fructicola* foram associados à antracnose em *Citrus* (HUAMG et al., 2013), ao passo que *Colletotrichum siamense* Prihastuti, L. Cai & K.D. Hyde, *Colletotrichum theobromicola* Delacr., Bulletin e *C. tropicale* E.I. Rojas, S.A. Rehner & Samuels foram coletados de tecidos sintomáticos de *Theobroma cacao* L., *C. arabica*, *C. annuum*, *M. indica*, *Citrus* sp., *Persea americana* Mill, *Olea europaea* L. e *Carica papaya* L. (DAMM et al., 2012b; WEIR et al., 2012; LIMA et al., 2013; SCHENA et al., 2013; UDAYANGA et al., 2013).

Apesar da maioria das espécies de *Colletotrichum* apresentar ampla gama de hospedeiros, algumas são restritas a determinados grupos de plantas. O gênero *Poaceae*, por exemplo, compreende várias espécies hospedeiro-específicas do grupo 'graminicola' (CROUCH et al., 2009). As espécies do complexo *C. orbiculare* parecem restritas às famílias Asteraceae, Cucurbitaceae, Fabaceae e Malvaceae, enquanto *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara infecta especificamente *Phaseolus vulgaris* L. e *Phaseolus coccineus* L. (DAMM et al., 2013). Mesmo no complexo *C. gloeosporioides*, cujas espécies ocorrem em plantas de mais de 400 gêneros (KIRK et al., 2001), existem algumas hospedeiro-específicas, como é o caso de *Colletotrichum horii* B. Weir & P.R. Johnst em caqui, *Diospryos* sp., e *Colletotrichum musae* (Berk. & M.A. Curtis) Arx, Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk em plantas do gênero *Musa* (WEIR et al., 2012). Portanto, as espécies de *Colletotrichum* apresentam substancial variação patogênica (CANNON et al., 2012), assim justificando estudos que acessem sua gama hospedeira e possíveis riscos de infecção cruzada.

Caracterização, sistemática e conceito de espécies para *Colletotrichum*

Proposto primeiramente como *Vermicularia* Tode (TODE, 1790), o gênero *Colletotrichum* tem sido reclassificado ao longo dos anos. *Colletotrichum* pertence à família Glomerellaceae, ordem Glomerellales e filo Ascomycota (CAI et al., 2009). A taxonomia de *Colletotrichum* tem sofrido profundas mudanças nos últimos anos e, consequentemente, o conceito de espécie também tem apresentado variações, envolvendo os conceitos morfológico, biológico, ecológico e filogenético (CAI et al., 2011). Todos esses critérios de reconhecimento de espécies tentam identificar linhagens evolutivas independentes (TAYLOR et al., 2000; GIRAUD et al. 2008a, CAI et al. 2011), sendo as espécies morfológicas definidas com base na divergência morfológica entre as espécies; as espécies biológicas relacionadas à compatibilidade sexual entre as espécies, que são reprodutivamente isoladas de outros grupos; as espécies ecológicas relacionadas à adaptação a um nicho geográfico

específico; e as espécies filogenéticas defendem a divergência molecular entre linhagens estreitamente relacionadas com base em dados de sequências de DNA.

Por muito tempo, os conceitos de espécie morfológica foram utilizados na identificação das espécies de *Colletotrichum* (PHOTITA et al., 2005; THAUNG, 2008). Entretanto, a plasticidade dos caracteres morfológicos e culturais em função dos métodos e condições experimentais levantou alguns conflitos em relação à sua classificação (CAI et al., 2009). Com base nos caracteres morfológicos, Von Arx (1957) concluiu que 600, das 750 espécies assumidas até aquele momento, tratavam-se na verdade de sinonímias de *C. gloeosporioides*, levando-o a reduzir para 11 o número de espécies. Posteriormente, avaliando as diferenças dos conídios, apressórios e características culturais das colônias, Sutton (1980) obteve 22 espécies de *Colletotrichum* e, ao considerar também as características patogênicas além das morfoculturais, esse número passou para 40 (SUTTON, 1992). Ao reunir as informações disponíveis, Kirk et al. (2008) registraram a existência de 60 espécies de *Colletotrichum*, enquanto Hyde et al. (2009) em uma abordagem morfológica e filogenética obtiveram 66 espécies, das quais 52 ainda não apresentam a fase sexual conhecida. Posteriormente mais 41 espécies foram incluídas nesse gênero, elevando assim o número de espécies para mais de 100 (CANNON et al., 2012), enquanto o *Index Fungorum* contabiliza 830 nomes (INDEX FUNGORUM, 2017).

Apesar das características culturais, conidiais e apressoriais permitirem a diferenciação de taxons entre complexos de espécies, essas, mesmo quando associadas às características patogênicas, não são suficientes para separar as espécies de *Colletotrichum* dentro do mesmo complexo (WHITELAW-WECKERT et al., 2007; PERES et al., 2008; THAN et al., 2008; CAI et al., 2009). Assim, a combinação dos métodos moleculares e morfológicos é considerada atualmente uma abordagem adequada no que diz respeito à identificação de espécies e de taxas sub-específicos desse gênero (CANNON et al., 2000; CAI et al., 2009; PRIHASTUTI et al., 2009). O estudo de caracteres moleculares como dados de sequências de DNA representa uma ferramenta útil para a taxonomia de *Colletotrichum*, uma vez que permite entender as relações filogenéticas entre suas espécies (CAI et al., 2009; CANNON et al., 2012).

Na reavaliação dos complexos de espécies, vários estudos têm sido conduzidos para tentar elucidar as relações filogenéticas do gênero *Colletrotrichum* (CAI et al., 2009; CANNON et al., 2012; DAMM et al., 2012a-b; WEIR et al., 2012). As 11 espécies consideradas inicialmente por Von Arx (1957), por exemplo, atualmente estão incluídas nos complexos *Colletotrichum orbiculare*, *Colletotrichum acutatum* e *C. gloeosporiodes* (CANNON et al., 2012). Por outro lado,

os complexos *C. acutatum*, *C. gloeosporioides* e *Colletotrichum boninense* passaram a compreender 30, 22 e 17 espécies, respectivamente (DAMM et al., 2012a-b; WEIR et al., 2012).

A sistemática atual do gênero *Colletotrichum* tem envolvido uma abordagem polifásica na identificação das espécies, destacando a filogenia multilocus combinada com caracteres fenotípicos (CAI et al., 2009; WEIR et al., 2012). A comparação de sequências da região ITS (Internal Transcribed Spacer) do rDNA entre espécies de *Colletotrichum* detectou variações que levaram ao desenvolvimento de *primers* baseados nesta região, como por exemplo o CgInt, um *primer* taxon-específico para *C. gloeosporioides sensu lato* (*s.l.*) (MILLS et al., 1992). Além da região ITS, os genes GAPDH (glicerol-3-fosfato desidrogenase), GS (glutamina sintase) CAL (calmodulina), ACT (actina), CHS (quitina sintase), TUB2 (β-tubulina) e Apn2 (DNA ligase) também têm sido recomendados para inferir as relações filogenéticas de *Colletotrichum* (CAI et al., 2009; DAMM et al., 2009; HYDE et al., 2009; ROZAS et al., 2010; CANNON et al., 2012). Segundo Sharma et al. (2013), a filogenia baseada no ApMat (Região intergênica dos genes Apn2 e MAT1-2-1) promoveu uma resolução mais precisa dentro do complexo 'gloeosporioides' mas, apesar de todos os esforços, ainda não se tem um consenso entre os micologistas sobre quais marcadores devem ser empregados para definir e delimitar uma espécie dentro de determinado complexo (DOYLE et al., 2013). Além disso, as regiões gênicas podem apresentar incongruências geneológicas e inconsistências topológicas, uma vez que os nós suportados em uma única árvore de gene podem estar em conflito com a árvore concatenada, assim como em outras árvores de genes individuais (Liu et al., 2016b). Os eventos de especiação podem ser erroneamente estimados se simplesmente reconhecer os clados bem suportados como espécies distintas sem implementar um exame cuidadoso do limite de espécies (LIU et al., 2016b). De acordo com os critérios de GCPSR (reconhecimento de espécies filogenéticas através da concordância genealógica), o conflito entre genealogias se dá pela recombinação entre indivíduos dentro de uma espécie, e a incongruência dos nos sãos identificados como o ponto de isolamento genético e limites de espécies (TAYLOR et al., 2000). O GCPSR é especialmente prático para delimitar espécies em fungos morfologicamente homogêneas ou quando não há conhecimento suficiente a respeito da existência de reprodução sexuada do microrganismo (LAURENCE et al., 2014; LIU et al., 2016b).

De modo geral, os estudos filogenéticos de *Colletotrichum* são direcionados a um pequeno grupo dentro do gênero e/ou associados a uma cultura em particular (DAMM et al., 2012 a-b; LIMA et al., 2013; TAO et al., 2013; UDAYANGA et al., 2013). Baseado em inferência filogenética e caracteres morfológicos e testes de patogenicidade, Huang et al. (2013) diferenciaram as espécies de *Colletotrichum* associadas a *citrus* na China em *C. gloeosporiodes, C. fruticola, C.*

karstii, *C. truncatum*, *C. citricola* e *C. citri* F. Huang, L. Cai, K.D. Hyde & H.Y. Li, sendo estas duas últimas relatadas como novas espécies e *C. truncatun* como o primeiro relato nesta cultura. Recentemente, as espécies *C. asianum* Prihastuti, L. Cai & K.D. Hyde, *C. fructicola*, *C. tropicale*, *C. karstii* e *C. dianesei* N. B. Lima, M. P. S. Câmara & S. J. Michereff foram identificadas como agentes etiológicos da antracnose em manga na principal região produtora desse fruto no Brasil (LIMA et al., 2013). Na China, as espécies *C. fructicola*, *C. karstii*, *C. truncatun*, *C. camelliae* Massee, *C. cliviae* Yan L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, *C. fioriniae* (Marcelino & Gouli) R.G. Shivas & Y.P. Tan, *C. siamense*, *C. aenigma* B.S. Weir & P.R. Johnston, *C. endophytica* Manamgoda, Udayanga, L. Cai & K.D. Hyde, *C. wuxiense* Y.C. Wang, X.C. Wang & Y.J. Yang e uma espécie indeterminada (*Colletotrichum* sp.) foram identificadas como responsáveis pela antracnose em *Camellia sinensis* L. (WANG et al., 2016). Na Austrália, *C. siamense*, *C. truncatum*, *C. simmondsii R.G. Shivas & Y.P. Tan*, *Colletotrichum queenslandicum* B.S. Weir & P.R. Johnston e *C. cairnsense* D. D. De Silva, R. Shivas & P. W. J. Taylor foram associados à antracnose em pimenta (DE SILVA et al., 2016), enquanto *C. gloeosporioides*, *C. siamense*, *C. fructicola*, *C. truncatum*, *C. scovillei* P.F. Cannon & Crous, *C. brevisporum* Noireung, Phoulivong, L. Cai & K.D. Hyde e *Colletotrichum sichuanensis* G.S. Gong & F.L. Liu foram identificados em *Capsicum annuum* na China (LIU et al., 2016). A antracnose em *A. occidentale* tem sido atribuída unicamente a *C. gloeosporioides* (LOPEZ; LUCAS, 2010; SERRA et al., 2011; UACIQUETE et al., 2013), enquanto os agentes etiológicos dessa doença nas espécies de caju nativas do cerrado brasileiro permanecem até o momento desconhecidas.

Embora a delimitação das espécies de *Colletotrichum* seja dificultada pela ausência de caracteres taxonômicos específicos, grande diversidade biológica e ampla distribuição pelo mundo influenciada pela atividade agrícola, os estudos filogenéticos têm apresentado grande utilidade para esse fim. Assim, considera-se que o correto reconhecimento das espécies de *Colletotrichum* seja fundamental para compreender suas interações com os fatores ambientais que influenciam na epidemiologia e agressividade da antracnose em diversos hospedeiros (CAI et al., 2011) e também para se adotar medidas de controle efetivas contra cada espécie ou grupo de espécies predominantes em cada cultura.

Efeito da temperatura sobre espécies de *Colletotrichum*

Os fatores climáticos exercem papel crucial na infecção e colonização dos tecidos hospedeiros por *Colletotrichum* spp. (AGRIOS, 2005). Os primeiros eventos desse processo

incluem o reconhecimento da superfície hospedeira, germinação dos conídios e formação de apressórios, os quais garantem a sobrevivência do patógeno sob condições adversas do ambiente antes de penetrarem e colonizarem o tecido hospedeiro (FERNANDO et al., 1994; KENNY et al., 2012; AGOSTEO et al., 2015).

A temperatura, em especial, influencia tanto o crescimento micelial quanto a esporulação e germinação de conídios de *Colletotrichum* spp. (DIAS et al., 2005; MAIA et al., 2011; FERNANDO et al. 2000; AMORIM et al., 2013; BARONCELLI et al., 2015). Por exemplo, 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 25-31 °C, enquanto temperaturas <9 °C e >35 °C foram desfavoráveis para o crescimento micelial desses patógenos (KENNY et al., 2012). Em comparação, temperaturas acima de 10 °C permitiram o desenvolvimento de lesões em frutos de manga inoculados com cinco espécies de *Colletotrichum*, sendo a maior virulência observada nas temperaturas de 25-30 °C (LIMA et al., 2015). Embora estudos prévios tenham demonstrado que a maior severidade da antracnose do cajueiro ocorre durante a estação chuvosa com temperaturas entre 22-28 °C (FREIRE et al., 2002), nenhum investigou a influência de diferentes temperaturas nos parâmetros biológicos de *Colletotrichum* spp. obtidos dessa cultura. No geral, patógenos de regiões tropicais e subtropicais se desenvolvem em uma ampla faixa de temperaturas (AMORIN et al., 2013), sendo o crescimento micelial favorecido entre 20 ºC e 25 °C (POLTRONIERI et al., 2013). Essa variação na amplitude térmica das espécies de *Colletotrichum* está relacionada com sua capacidade de adaptação a diferentes condições climáticas (DIAS et al., 2005), assim justificando sua ampla distribuição pelo mundo.

Controle da antracnose e Sensibilidade a fungicidas

A capacidade de *Colletotrichum* spp. causar infecções quiescentes ou latentes nos estádios pré e pós-colheita de diversas culturas (BAILE; JEGER, 1992; AGRIOS, 2005; DEAN et al., 2012; CANNON et al., 2012), somada à disponibilidade de hospedeiros suscetíveis e condições ambientais favoráveis (DAMM et al., 2010) asseguram rápido acúmulo de inóculo nas zonas tropicais. Como agravante, a seleção de linhagens resistentes pela utilização frequente de determinados fungicidas dificulta ainda mais o manejo da antracnose nas áreas de cultivo (BAILEY; JEGER, 1992).

Além das caracterizações já descritas, as espécies de *Colletotrichum* também têm sido discriminadas quanto à sensibilidade a fungicidas, sendo esta característica utilizada para estimar o

potencial de tais compostos no manejo da antracnose (FREEMAN et al., 1998). Os fungicidas podem atuar como marcadores fenotípicos permitindo diferenciar populações de *Colletotrichum* spp. (ADASKAVEG; FOSTER, 2000), enquanto a sensibilidade diferencial a fungicidas auxilia na caracterização da variabilidade entre isolados de uma mesma espécie. Por exemplo, *C. dianesei* foi mais sensível ao tiofanato metílico do que *C. fructicola*, embora ambos tenham sido isolados de frutos de mangueira e expostos à mesma concentração do referido fungicida (LIMA et al., 2015).

Conhecer como as espécies de *Colletotrichum* diferem na sua sensibilidade a determinados fungicidas é de crucial importância para o manejo desses patógenos, principalmente em agroecossistemas onde existem populações mistas como os complexos *C. acutatum* e *C. gloeosporioides* (FREEMAN et al., 1998). O controle da antracnose nos pomares de mangueira do nordeste brasileiro inclui o uso intensivo dos fungicidas tiofanato metílico, difenoconazole e azoxistrobina, os quais se mostraram efetivos na inibição do crescimento micelial de *C. asianum*, *C. dianesei*, *C. fructicola*, *C. karstii* e *C. tropicale* (LIMA et al., 2015). O monitoramento da sensibilidade a fungicidas em uma população de patógenos é importante para determinar a presença de isolados resistentes (GHINI; KIMATI, 2000). Avaliando a inibição do crescimento micelial de isolados de *C. gloeosporioides* associados à mancha da mela da macieira, Hamada et al. (2009) identificaram um grupo de isolados resistentes ao fungicida benomil que diferiram de todos os isolados dessa espécie até então relatados neste patossistema. Tal caracterização se mostra fundamental para o desenvolvimento de estratégias de manejo da resistência e consequentemente controle de doenças de plantas.

Como o patógeno pode sobreviver internamente em tecidos infectados e em restos culturais, uma das medidas de controle aplicáveis à antracnose consiste na poda de limpeza, remoção e destruição de restos culturais (CARDOSO; FREIRE, 2002; FREIRE et al., 2002; AGROFIT, 2017). Pulverizações com fungicidas, como oxicloreto de cobre, hidróxido de cobre e mancozeb, aplicados no inicio da emissão das folhas, bem como durante a floração, também são recomendadas (BARROS et al., 1993; FREIRE et al., 2002; MENEZES, 2005). Dentre estes, apenas oxicloreto de cobre apresenta registro no Ministério da Agricultura para utilização no manejo da antracnose do cajueiro no Brasil (AGROFIT, 2017). A extensa área plantada, a baixa produtividade e o custo do fungicida mostram-se como empecilhos ao controle da antracnose (FREIRE et al., 2002). Fontes de resistência a essa doença têm sido encontradas em cajueiro anão-precoce, como relatam Cavalcanti et al. (2000) para os clones CAP-14, CAP-17, CAP-05 e CAP-07, indicando que esta característica pode, futuramente, ser implementada no manejo da doença. Apesar das vantagens econômicas e ecológicas proporcionadas pelo uso da resistência varietal, esta técnica pode falhar caso não se

conheça os agentes etiológicos realmente envolvidos, o que demonstra a importância da correta identificação das espécies de *Colletotrichum* associadas à antracnose o cajueiro em diferentes espécies de *Anacardium* de várias regiões do Brasil.

Objetivou-se com o presente estudo identificar e inferir as relações filogenéticas entre espécies de *Colletotrichum* associadas à antracnose em cajueiros cultivados (*A. occidentale*) e nativos (*A. humile* e *A. othonianum*) distribuídos em quatro biomas brasileiros (Caatinga, Cerrado, Floresta Amazônica e Mata Atlântica). Avaliaram-se ainda seus parâmetros biológicos (crescimento micelial e germinação de conídios) em diferentes temperaturas, sua agressividade em folhas de *A. occidentale* e frutos de seis hospedeiros alternativos com e sem ferimentos, e sua sensibilidade (inibição do crescimento micelial, esporulação e germinação de conídios) a três fungicidas. Tais pontos são fundamentais para o desenvolvimento de estratégias eficientes de manejo e elucidação da epidemiologia das doenças de plantas cultivadas ou nativas.

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CAPÍTULO II

Why species delimitation matters for fungal ecology: *Colletotrichum* **diversity on wild and cultivated cashew in Brazil**

Why species delimitation matters for fungal ecology: *Colletotrichum* diversity on wild and cultivated cashew in Brazil

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Resumo

A antracnose, causada por espécies de *Colletotrichum*, é uma das doenças de plantas mais importantes a nível mundial, ocorrendo numa vasta gama de espécies hospedeiras cultivadas e selvagens. O presente estudo objetivou identificar as espécies de *Colletotrichum* associadas à antracnose do cajueiro no Brasil, determinar suas relações filogenéticas e distribuição geográfica e fornecer algumas informações sobre os fatores bióticos e abióticos que podem estar influenciando a composição da comunidade. Um total de 280 isolados de *Colletotrichum* foram coletados de folhas sintomáticas, caules, inflorescências e frutos de *Anacardium occidentale* e *Anacardium humile* e *Anacardium othonianum* em quatro biomas brasileiros (Floresta Amazônica, Mata Atlântica, Caatinga e Cerrado) representados por 10 locais de amostragem geográfica. Com base em na análise filogenética multilocus, foram identificadas sete espécies de *Colletotrichum*, denominada *C. chrysophilum*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides sensu stricto*, *C. queenslandicum*, *C. siamense* e *C. tropicale*. Embora a antracnose do caju tenha sido anteriormente atribuída a *C. gloeosporioides*, esta espécie era rara nas amostras, enquanto *C. siamense* foi a espécie dominante, ocorrendo nos quatro biomas amostrados, e presente nas folhas, caules, inflorescências e / ou fruto de ambos cultivados e selvagens. Em geral, a maior riqueza de espécies de *Colletotrichum* foi encontrada associada a *A. occidentale*; as folhas abrigaram mais espécies do que os outros órgãos; a Mata Atlântica abrangeu mais espécies do que os outros biomas; e Pernambuco foi o local mais rico em espécies. No entanto, considerando a abundância relativa de espécies de *Colletotrichum* e as diferenças no tamanho da amostra entre os estratos, a interpretação de qual comunidade é a mais diversa depende de como as espécies são delimitadas. O presente estudo fornece informações valiosas sobre o patosistema *Colletotrichum*/cajueiro, esclarece a identificação dos agentes causais que podem auxiliar no desenvolvimento de medidas de controle efetivas e destaca o impacto que a delimitação de espécies pode ter nos estudos ecológicos de fungos.

Palavras-chave: *Anacardium* spp., filogenia multigene, reconhecimento de espécies, índices ecológicos, distribuição de *Colletotrichum*.

Abstract

Anthracnose, caused by species of *Colletotrichum*, is one of the most important plant diseases globally, occurring on a wide range of cultivated and wild host species. The present study aimed to identify the *Colletotrichum* species associated with cashew anthracnose in Brazil, determine their phylogenetic relationships and geographical distribution, and provide some insight into the biotic and abiotic factors that may be influencing community composition. A total of 280 *Colletotrichum* isolates were collected from symptomatic leaves, stems, inflorescences and fruit of cultivated (*Anacardium occidentale*) and wild cashew (*Anacardium humile* and *Anacardium othonianum*) across four Brazilian biomes (Amazon Rainforest, Atlantic Forest, Caatinga and Cerrado) represented by 10 geographical sampling sites. Based on a multilocus phylogenetic analysis, seven *Colletotrichum* species were identified, namely *C. chrysophilum*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides sensu stricto*, *C. queenslandicum*, *C. siamense* and *C. tropicale*. Although cashew anthracnose was previously ascribed to *C. gloeosporioides*, this species was rare within our sample, while *C. siamense* was the most dominant species, occurring across the four sampled biomes, and present on leaves, stems, inflorescences and/or fruit of both cultivated and wild hosts. Overall, the greatest *Colletotrichum* species richness was found associated with *A. occidentale*; leaves harbored more species than the other organs; the Atlantic Forest encompassed more species than the other biomes; and Pernambuco was the most species-rich location. However, accounting for the relative abundance of *Colletotrichum* species and differences in sample size across strata, the interpretation of which community is most diverse depends on how species are delimited. The present study provides valuable information about the *Colletotrichum*/cashew pathosystem, sheds light on the identification of the causal agents which may assist in developing effective control measures, and highlights the impact that species delimitation can have on ecological studies of fungi.

Key words: *Anacardium* spp., multigene phylogeny, species recognition, ecological indices, *Colletotrichum* distribution.

INTRODUCTION

The genus *Anacardium* L. comprises about 38 species of wild and cultivated plants, each with socioeconomic and/or ecological relevance (Agostini-Costa et al. 2006, TPL. 2016). One such group of *Anacardium* species includes plants from the Amazon Forest and the Brazilian 'Cerrado' popularly known as cashew (Agostini-Costa et al. 2006; Barros et al. 2002; Cardoso and Freire 2002; Mitchell and Mori 1987), Among the cashew species, only *Anacardium occidentale* L. is cultivated for commercial purposes in different parts of the world. *Anacardium ottonianum* Rizzini is wild-harvested for subsistence in poor communities in the Central region of Brazil, while other wild species, such as *Anacardium humile* St. Hilaire, *Anacardium nanum* St. Hilaire and *Anacardium corymbosum* Barb. Rodr., serve as a source of food for native animals (Agostini-Costa et al. 2006).

Currently *A. occidentale* is cultivated in at least 33 countries around the world, among which Brazil is ranked among the top (FAO 2017). The juicy part of the cashew is a pseudo-fruit or accessory fruit, as it is not derived from the ovary but is a swollen pedicel. It can be directly consumed, or may be used in the production of juice, jam, alcoholic beverages, and soft drinks, whereas the fruit is usually eaten as roasted nuts (Agostini-Costa et al. 2006; Freire et al. 2002; Feirre and Cardoso 2003). Brazil is the leading producer of cashew pseudo-fruit and the tenth largest producer of cashew nuts (FAO 2017). Most of the cashew nuts produced in Brazil come from the Northeast region (Brazilian Institute of Geography and Statistics – IBGE 2017), where small farmers frequently organize themselves in cooperatives aiming to raise the quantity and quality of their products. Aside from stressful abiotic conditions, several diseases compromise yield and quality of cashew, among which anthracnose is reported as the most important due to yield loss that can surpass 40% (Freire and Cardoso 2003). This is usually associated with *Colletotrichum* Corda (1831) species and occurs during both vegetative growth and fruiting in commercial (Freire et al. 2002) and wild species of cashew. In general, symptoms of anthracnose start with water-soaked lesions, which become orange to slightly reddish during sporulation (Freire et al 2002, Freire and Cardoso 2003, Menezes 2005). Infection may result in premature abscission of leaves or depressed lesions on the fruit (Menezes 2005).

Colletotrichum is a cosmopolitan genus of fungi that includes pathogenic and non-pathogenic species (Cannon et al. 2012; Hyde et al. 2009; Prihastuti et al. 2009; Vieira et al. 2014). It has

been found associated with approximately 2200 plant species (Farr and Rossman 2015), and represents one of the most important etiological agents of plant diseases worldwide (Dean et al. 2012). However, reliable species recognition within *Colletotrichum* eluded fungal taxonomists prior to the advent of molecular systematics due to morphological plasticity and a paucity of taxonomically-informative morphological characters (Cai et al. 2009; Cannon et al. 2012; Freeman et al. 1998; Sutton 1992). Multilocus phylogenetic data have become the standard for the delimitation of species (Dettman et al. 2003; Doyle et al. 2013; Liu et al. 2015; Liu et al. 2016; Taylor et al. 2000; Rojas et al. 2010) and provide the opportunity to more reliably characterize diversity and understand the biology of important but morphologically cryptic lineages within *Colletotrichum* (Damm et al. 2012 a, b; De Silva et al. 2016; Haung et al. 2013; Manamgoda et al. 2013; Prihastuti et al. 2009; Tao et al. 2013; Sharma et al. 2015; Wang et al. 2016; Weir et al. 2012).

The identification of etiological agents is particularly important in agroecosystems because it is the foundation from where pest management strategies are developed. For example, it was thought that anthracnose in mango was caused only by *Colletotrichum gloeosporioides*, but multilocus phylogenetic data revealed eight distinct lineages associated with mango (Lima et al. 2013; Sharma et al. 2013; Sharma et al. 2015; Vieira et al. 2014,). *Colletotrichum gloeosporioides* is the only species thus far associated with anthracnose on *A. occidentale* (Freire et al. 2002; Lopez and Lucas 2010; Menezes 2005; Serra et al. 2011, Uaciquete et al. 2013). However, the studies published to date have used molecular phylogenetic data with relatively low information content, making it impossible to recognize the many cryptic species that have been described within the *C. gloeosporioides* species complex, and there is no information about the species that infect wild species of cashew. Moreover, cashew species are distributed across several biomes with remarkable biodiversity and diverse agricultural environments and the aforementioned studies tend to be geographically restricted. The purpose of this study was to investigate the diversity of *Colletotrichum* species associated with cultivated and wild species of cashew across a broad geographic distribution representing four Brazilian biomes. We employed multilocus phylogenetic data to infer evolutionary relationships among the species of *Colletotrichum*, as well as their prevalence by geography, biome, host species, and plant organ.

MATERIALS AND METHODS

Sampling and Fungal isolation

Cultivated and wild species of cashew were collected from nine Brazilian states, including Alagoas (AL), Ceará (CE), Minas Gerais (MG), Pará (PA), Pernambuco (PE), Paraíba (PB), Goiás (GO), Santa Catarina (SC), and Rio Grande do Norte (RN), plus Distrito Federal (DF). The collection sites are distributed across the following four Brazilian biomes that are unique with respect to climate, biodiversity, and human intervention: Atlantic Forest, Amazon Rainforest, Caatinga, and Cerrado. Cashew organs showing symptoms of anthracnose were collected and shipped in plastic bags to the Mycology Laboratory of the Universidade Federal Rural de Pernambuco (UFPRE) in Recife, Brazil. Fragments bordering healthy and necrotic zones in leaves, stems, inflorescences, and fruit were cut and surface disinfested by submersing in 70% ethanol for 30 s, 1.5% sodium hypochlorite for 1 min, rinsed three times with sterile water, and dried on sterilized filter paper. Five fragments of each plant organ were evenly spaced in glass Petri dishes containing potato-dextrose-agar (PDA: 200 g of potato, 20 g of dextrose and 20 g of glucose, in 1 L of distilled water) and amended with 0.5 g streptomycin sulfate (PDAS) to suppress bacterial growth. The Petri dishes were incubated at ambient temperature for 5 days and mycelium from the colony edges were transferred to new Petri dishes containing PDA.

Pure cultures were established from single spores for those isolates identified as *Colletotrichum* spp. based on morphological characteristics. Spores were suspended in 30 µL of sterile water and a 5 µL aliquot was uniformly distributed in glass Petri dishes containing PDA using a Drigalski spatula. A single conidium of each isolate was transferred to a new PDA plate for preservation and DNA extraction. Mycelium discs from these colonies were preserved by placing them in microtubes filled with 700 µL of sterile water. Representative isolates were deposited as vouchers at the "Coleção de Fungos Fitopatogênicos Professora Maria Menezes (CMM)" of the Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil.

Extraction of DNA, PCR amplification and sequencing

Single spore cultures of *Colletotrichum* isolates were grown on PDA incubated for five to seven days at ambient temperature. Mycelium was scraped from the colony surface using a sterile pipette tip and genomic DNA was extracted using the Sodium Dodecyl Sulfate (SDS) protocol with some modifications (Moller et al. 1992). Stock DNA was suspended in 1x TE buffer and stored at -20 ºC. The stock DNA concentration was measured with the NanoVue Plus spectrometer (GE Healthcare, USA) and diluted to 25ng/µL for PCR.

The intergenic spacer between the 3' end of the DNA-lyase and the mating type locus MAT1-2 (APN2/MAT-IGS) was sequenced for all isolates in order to select representative isolates for multilocus sequencing. Distinct haplotypes were identified using DnaSP 4.0 (Rozas et al. 2003) and the selection of representative isolates was based on host species, host organ, Brazilian biome, and geographical sampling site. PCR amplification and sequencing of DNA-lyase (APN2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glyceraldehyde-3-phosphate dehydrogenase-IGS (GAP2-IGS), β-tubulin (TUB2), glutamine synthetase (GS), and calmodulin (CAL) were done for all representative isolates. Information on primers used for PCR amplification and sequencing is shown in Supplementary Table S1.

The PCR cycling parameters for APN2/MAT-IGS and APN2 consisted of initial denaturation step at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 62 °C for 45 s, 72 °C for 1 min and a final cycle at 72 °C for 10 min. For GAPDH and GAP2-IGS they involved an initial denaturation step at 95 °C for 5 min, 35 cycles at 95 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min and a final cycle at 72 °C for 10 min. The optimal annealing temperature differed for each of the other genes: TUB2 - 53 °C; GS - 55 °C; and CAL - 57 °C. When amplification resulted in multiple bands, annealing temperatures and/or time were adjusted. PCR primers used during this study are listed in Table 1. Each 12.5 µL PCR reaction volume included 4.0 µL of PCR-grade water, 0.625 µL of each primer (10 µM), 1.0 µL of DNA template and 6.25 µL of PCR Master Mix (2X) (Promega GoTaq® Master Mix, Wisconsin, USA). The PCR amplification products were visualized on an 1.5% agarose gel stained with GelRed™ (Biotium). PCR purification and sequencing were carried out by Beckman Coulter Genomics (Danvers, Massachusetts, USA).

Sequence alignment and phylogenetic analyses

Sequence reads were edited and consensus sequences automatically assembled using Geneious 8.1 (Kearse et al. 2012). All consensus sequences were used as queries against the National Center for Biotechnology Information (NCBI) nucleotide database using the *blastn* algorithm (Johnson et al. 2008). Sequences representing ex-types and related published sequences were retrieved from GenBank (Supplementary Table S2). Multiple sequence alignments (MSA) of each locus were estimated with the online version of MAFFT version 7 (Katoh et al. 2002; Katoh and Toh 2013) with the G-INS-i iterative refinement method and the 200PAM / κ=2 nucleotide scoring matrix. Each MSA was manually edited in MEGA5 (Tamura et al. 2011). All sequences generated for the present study were deposited in GenBank (Supplementary Table S2). The alignment length, number of parsimony informative characters, percentage of parsimony informative characters and substitution model of each locus are given in Supplementary Table S3.

Evolutionary relationships were inferred using both Bayesian (BI) and maximum likelihood (ML) approaches for each individual locus and the concatenated matrix. BI analysis was carried out using MrBayes v. 3.2.6 program (Ronquist et al. 2012) implemented in the CIPRES cluster (https://www.phylo.org/portal2/home.action) using the best-fit model of nucleotide evolution estimated by MrModeltest 2.3 (Nylander 2004) following the Akaike Information Criterion (AIC) (Table 3). Each analysis was run for 5×10^7 generations, sampling every 1000, with four Markov Chain Monte Carlo (MCMC) chains (3 heated, 1 cold). The first 25% of the samples were discarded as burnin and convergence of the parameter estimates and likelihoods was visually confirmed in the program Tracer v. 1.6 (Rambaut and Drummond 2007). Each analysis was also considered to have converged when effective sample sizes (ESS) were greater than 200. Maximum likelihood topologies were inferred in GARLI v. 2.01 (Zwickl 2006) using the High Performance Computational Resources at Louisiana State University applying the best-fit models of nucleotide substitution selected using Akaike's Information Criterion corrected for small sample sizes (AICc) in jModelTest2 v. 2.1.6 (Darriba et al. 2012; Guindon and Gascuel 2003). Each maximum likelihood tree represents the best tree from 20 replicate searches when the ML tree was found more than once or 100 replicate searches when the best tree was not found at least twice. Independent searches were terminated after 10,000 generations without an improvement in the likelihood score greater than 0.01 log-likelihood units. Node support was

estimated in a bootstrap analysis with 1008 pseudoreplicates, with the tree with the highest likelihood after 10 replicate searches to represent each bootstrap pseudoreplicate dataset. Support values were mapped to the ML tree using the program SumTrees 3.3.1, which utilizes the DendroPy Phylogenetic Computing Library version 3.12.0 (Sukumaran and Holder 2010). All sequence alignments and phylogenetic trees are archived in TreeBase (Study ID: S20942; treebase.org).

Species recognition

Genealogical Concordance Phylogenetic Species Recognition (GCPSR) (Dettman et al. 2003; Doyle et al. 2013; Taylor et al. 2000) was applied to recognize *Colletotrichum* species associated with cashew. Based on GCPSR a lineage is considered as independently evolved if it is strongly supported as monophyletic in the concatenated analysis and fits at least one of the following criteria: a clade is concordant when it is present in the majority of the individual gene trees (i.e., at least in 4 of the 7); a clade is non-discordant when the clade is well supported by both BI (posterior probability $\geq$ 0.95) and ML (bootstrap $\geq$ 70%) analysis in a single gene tree and is not in conflict with any other single genealogy at the same level of support.

Given recent discussions in the literature about the species-level status of several lineages within what is currently recognized as *Colletotrichum siamense*, strongly supported monophyletic lineages within *C. siamense* were treated under two alternate delimitation regimes. The first represents the inclusive view of Liu et al. (2016) that *C. siamense* represents a diverse and broadly distributed species composed of many monophyletic subclades. The alternate approach treats *C. siamense* as a species complex and recognizes previously described species (recently synonymized by Liu et al. 2016), strongly supported monophyletic subclades within *C. siamense*, and divergent singletons as independent lineages. The latter view is one that has been taken in previous publications (e.g. Sharma et al. 2015) and represents the other end of the spectrum when it comes to species diversity within *Colletotrichum gloeosporioides s.l.* These alternate approaches were devised to explore the impact that different approaches to species recognition can have on diversity analyses.

Prevalence of *Colletotrichum* species

The prevalence of *Colletotrichum* species collected from different cashew species, host organ, Brazilian biome and geographical sampling site was determined by calculating the Isolation Rate (IR) as follows: IR(%) = (Cx / Ct) x 100, where Cx is the number of isolates belonging to one species and Ct is the number of isolates per host, plant organ, biome and geographical site, respectively.

Rarefaction and extrapolation curves with Hill numbers

The diversity of *Colletotrichum* species associated with cultivated and wild cashew, host organ, Brazilian biome and geographical sampling site was determined by estimating rarefaction (interpolation) and prediction (extrapolation) curves based on Hill numbers. These are known as the effective number of species, and represent a mathematically unified group of diversity indices that differ from each other only by an exponent q (Hill 1973). As proposed by Chao et al. (2014), we applied a unified approach for individual-based data to estimate rarefaction and extrapolation curves for the first three Hill numbers: species richness ($q = 0$), Shannon diversity ($q = 1$, the exponential of Shannon entropy), and Simpson diversity ($q = 2$, the inverse of Simpson concentration). The exponent q determines the sensitivity of the measure to the relative abundance, where $q = 0$ counts species equally, regardless of their relative abundances; $q = 1$ counts individuals equally, thus considering species proportionally to their abundances; and $q = 2$ discounts all but the dominant species, and can be interpreted as the effective number of dominant species in the community (Chao et al. 2014; Hsieh et al. 2016). Species diversity was estimated as the mean of 200 bootstrap replications with 95% confidence intervals. All analyses were performed using the iNEXT library (Hsieh et al. 2016) in R v. 3.1.3 (R Core Team, 2013).

Results

Isolation of *Colletotrichum* strains

A total of 280 *Colletotrichum* strains were collected from both commercial and wild cashew species presenting typical symptomatology of anthracnose, and all of them produced conidia similar to *C. gloeosporioides* on PDA. Most isolates were collected from *A. occidentale* (215),

intermediate numbers were obtained from *A. othonianum* (39), and the smallest number of isolates were isolated from *A. humile* (26). Regardless of the host species, the largest number of isolates were found on leaves (219), while intermediate numbers were collected from stems (41) and inflorescences (19), and a single isolate was obtained from fruit. With respect to biome, most *Colletotrichum* isolates were taken from the Atlantic Forest (126), followed by Cerrado (73), Caatinga (71) and the Amazon Rainforest (10). The majority of them were obtained in Pernambuco (116), followed by Rio Grande do Norte (36), Distrito Federal (36), Minas Gerais (35), Paraíba (28), Pará (10), Alagoas (9), Ceará (7), Goiás (2), and Santa Catarina (1).

Phylogenetic analysis and species recognition

There were 30 Apn2/MAT-IGS haplotypes among the 280 isolates, all of which we assigned to the *C. gloeosporioides* complex based on similarity to representative sequences in the NCBI database. The seven loci described by Vieira et al. (in review) as the most informative for species recognition were sequenced for seventy-five isolates representing the breadth of collection localities across Brazil, host species, host organ, and genetic diversity at APN2/MAT-IGS. These data were included in a multilocus phylogenetic analysis to assign representative haplotypes to species.

The *Colletotrichum* isolates associated with cultivated and non-cultivated cashew were assigned to seven species previously described within the *Colletotrichum gloeosporioides* complex. Most of them were strongly supported by both ML and BI analysis in the concatenated tree, and satisfied the GCPSR criteria across the individual gene trees (Fig 1). Two isolates were assigned to *C. gloeosporioides sensu stricto* with strong support in all individual gene trees. Seven isolates were identified as *Colletotrichum chrysophilum*, recently described by Vieira et al. (in review) from Brazil, which formed a clade with strong support in the majority of single gene trees, except CAL (high support only in the BI) and GAPDH (not supported by ML or BI). Six isolates were assigned to *Colletotrichum fructicola*, a clade resolved with strong support in the majority of single gene trees from either ML or BI, with the exception of CAL. Six isolates were assigned to *Colletotrichum queenslandicum* with significant support in the the multilocus analysis and the single gene trees of Apn2/MAT-IGS, GAPDH and TUB2. Five isolates were recognized to be conspecific with *Colletotrichum fragariae* with strong support in both the Apn2/MAT-IGS and GAP2-IGS gene trees and not

contradicted in any other single gene tree at the same level of support. Four isolates were nested with *Colletotrichum tropicale*, which was supported by the majority of the independent gene trees and the concatenated analysis. Forty five isolates were assigned to *Colletotrichum siamense* in a strongly supported clade in the multilocus analysis and the majority of individual gene trees (Fig 1).

Communities of *Colletotrichum* species across sampling strata

The composition of *Colletotrichum* species differed among host species, host organ, biome, and geographical sampling site (Table 1, Fig 2). *Colletotrichum siamense* and *C. fructicola* were common to all three sampled *Anacardium* species (*A. occidentale*, *A. othonianum* and *A. humile*), while *C. gloeosporioides* and *C. chrysophilum* were isolated from *A. occidentale* and *A. humile*; and *C. fragariae*, *C. queenslandicum* and *C. tropicale* were restricted to *A. occidentale*. With respect to the host organ, *C. siamense* was isolated from leaves, stems, inflorescences and fruit, while *C. chrysophilum* was found on leaves, stems and inflorescences; *C. fragariae*, *C. tropicale* and *C. fructicola* on leaves and stems; and *C. queenslandicum* and *C. gloeosporioides* on leaves and inflorescences (Table 1, Fig 2).

With respect to geographical distribution, most of the *Colletotrichum* species occurred in two or three of the sampled Brazilian biomes (Atlantic Forest, Amazon Rainforest, Caatinga, and/or Cerrado), with *C. siamense* common to all of them and *C. queenslandicum* restricted to the Atlantic Forest (Table 1, Fig 2). In contrast, *C. tropicale* was found in the Atlantic Forest, the Amazon Rainforest, and Caatinga; *C. chrysophilum* occurred in the Atlantic Forest, Caatinga, and Cerrado; *C. fragariae* was found in the Atlantic Forest and Caatinga; *C. gloeosporioides* was restricted to the Caatinga and Cerrado; and C. *fructicola* was collected in the Atlantic Forest and Cerrado (Table 1, Fig 2).

While *C. siamense* was collected from all sampled federative units of Brazil (AL, CE, DF, GO, MG, PA, PB, PE, RN and SC), *C. chrysophilum* was common to five locations; *C. tropicale* was distributed across four sites; *C. fragariae*, *C. fructicola* and *C. gloeosporioide*s occurred in three; and *C. queenslandicum* was restricted to Pernambuco (PE) (Table 1, Fig 2).

Prevalence of *Colletotrichum* species across sampling strata

Overall, *C. siamense* was the most prevalent species among 280 *Colletotrichum* isolates collected from cultivated and wild cashew plants across Brazil. It represented approximately 62% of the isolates, with other species representing 1.4–14.3%. Nearly 66.5% and 69.2% of the isolates collected from *A. occidentale* and *A. othonianum*, respectively (Fig 2A), were assigned to *C. siamense*, while *C. fructicola* was the most frequent (73.1%) in *A. humile*. Regardless of the host, *C. siamense* was the most common species on leaves, stems, inflorescences and fruit (Fig 2B).

With respect to geographical distribution, *C. siamense* was the most common species across the four sampled biomes and all geographical sampling sites, except in Minas Gerais, where *C. fructicola* was the most abundant species (Fig 2C and Fig 2D).

Comparison of *Colletotrichum* species diversity based on abundance data

To compare the diversity of *Colletotrichum* species associated with anthracnose on different organs of cultivated and wild cashew in Brazil, individual-based rarefaction and extrapolation curves were computed for the first three Hill numbers: species richness ($q = 0$), Shannon diversity ($q = 1$), and Simpson diversity ($q = 2$). Given recent discussion about recognizing *C. siamense* as a single species or a complex (Liu et al. 2016; Sharma et al. 2015), we computed rarefaction and extrapolation curves considering both situations (Fig 3 and Fig 4, respectively). In the first case, maximum species richness comprised seven *Colletotrichum* species, namely *C. chrysophilum*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides s.s.*, *C. queenslandicum*, *C. siamense* and *C. tropicale*. On the other hand, the second scenario includes 21 independent lineages (the former seven species but *C. siamense s.s.*, plus *C. communis*, *C. endomangiferae*, *C. dianesei* and 12 lineages not currently recognized as species – see Supplementary Figure 1 for species assignments). While *C. dianesei* was supported by neither ML nor BI, other lineages within *C. siamense* s.l. formed sub clades or singletons identified as independent in the concatenated analysis and concordant across APN2, APN2/MAT-IGS, and GS individual gene trees. However, conflicts were observed when these trees were compared to GAPDH and GAP2-IGS trees. Overall, the number of significant differences in *Colletotrichum* diversity among sampling strata decreased as the exponent q was increased, i.e. the richness index detected more significant differences among

sources of *Colletotrichum* isolates than did Shannon, which depicted more differences than Simpson diversity (Figs 3 and 4, Tables 2 and 3).

Considering *C. siamense* as a single species, there was virtually no expected increase in species diversity measures due to extrapolation (double the reference sample size) curves (Fig 3A, 3B, 3C, 3D) across sampling strata and diversity metrics with the exception of *A. humile* ($q = 0$; Fig 3A), inflorescence ($q = 0$; Fig 3B), Cerrado ($q = 0$, Fig 3C), Rio Grande do Norte and Minas Gerais ($q = 0$; Fig 3D) and Ceará ($q = 2$; Fig 3D). *Anacardium occidentale* exhibited greater diversity than *A. humile* and *A. othonianum* on the basis of species richness, but was not significantly greater on the basis of Shannon or Simpson diversity measures. Similarly, on the basis of species richness, cashew leaves harbored more *Colletotrichum* species than stems, inflorescences and fruit (Table 2), but not when accounting for relative abundance and species dominance ($q = 1, 2$). Species richness for the Atlantic Forest and Caatinga did not differ and were significantly greater than that observed in the Amazon Rainforest, with the Cerrado intermediate (Table 2). The Atlantic Forest also exhibited the greatest diversity based on Shannon index, and was significantly different from Cerrado and the Amazon Rainforest, with Caatinga intermediate. In contrast, no significant difference was detected among biomes based on Simpson diversity. With respect to geographical sampling site, Pernambuco was the most diverse location (Table 2), but overlapping with Rio Grande do Norte in species richness. It was also the most diverse location based on Shannon diversity, but was overlapping with Paraíba and Ceará. While Ceará and Pernambuco were the most diverse on the basis of Simpson diversity, they were only significantly greater than Alagoas, Goiás, and Rio Grande do Norte.

When *C. siamense* was treated as a species complex, conclusions about the most diverse sampling strata (Table 3, Fig 4) differed from treating *C. siamense* as a single species. In this case, extrapolation (double the reference sample size) based on $q = 0$ predicted an increase in diversity by host species (Fig 4A), host organ (Fig 4B), biome, and geographical sampling site, while $q = 1$ and $q = 2$ predicted an increase only for the Amazon Rainforest and Pará (Fig 4C and Fig 4D). The trends in community diversity with respect to host were similar to that previously described when treating *C. siamense* as a single species, with *A. occidentale* hosting the most diverse assemblage of species. However, when treating putatively independent lineages within *C. siamense* as distinct, *A. occidentale* is also more diverse even when accounting for relative abundance ($q = 1$) and dominance ($q = 2$). Similarly, leaves were

the most diverse host organ, but were so on the basis of both species richness and accounting for relative abundance ($q = 1$). While the Atlantic Forest was still the most diverse on the basis of species richness, in contrast to treating *C. siamense* as a single lineage, the second most diverse biome (overlapping with the Atlantic Forest) was the Cerrado (Fig 4C, Table 3). The Atlantic Forest and the Amazon Rainforest were the most diverse with respect to both Shannon and Simpson diversity ($q = 1, 2$; Fig 4C, Table 3). Pernambuco was the most diverse location on the basis of species richness (Table 3) followed by Minas Gerais. However, Pará was the most diverse alongside Pernambuco based on Shannon diversity and was the most diverse location when focusing on dominant species ($q=2$).

DISCUSSION

In a broad phylogenetic analysis involving seven genes (Apn2/MAT-IGS, TUB2, CAL, GAP2-IGS, GAPDH, GS and APN2), the present study revealed a highly diverse group of *Colletotrichum* species associated with anthracnose on cultivated and wild species of cashew in Brazil. These included *C. chrysophilum*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides s.s.*, *C. queenslandicum*, *C. siamense*, and *C. tropicale*. However, when *C. siamense* was treated as a species complex rather than a single species, the diversity pattern among and within some strata changed depending upon the order of Hill numbers ($q = 0, 1$ or 2). According to Chao et al. (2014), researchers using Hill numbers should report at least the diversity of all species ($q = 0$), of 'typical' species ($q = 1$), and of dominant species ($q = 2$), keeping in mind that inferences for diversities of $q \geq 1$ are more reliable. Based on $q = 1$, and considering *C. siamense* as a single species, the diversity of the assemblage harbored by *A. occidentale* was similar to that of *A. humile* and more diverse than *A. othonianum*, while no significant differences were observed based on $q = 2$. This result is supported by the prevalence analysis (Fig 2), showing that the relative abundance ($q = 1$) of *C. fructicola* in *A. humile* is similar to that of *C. siamense* in *A. occidentale*, and that these *Colletotrichum* species were also dominant ($q = 2$) in *A. othonianum*. In contrast, when *C. siamense* was treated as a species complex with multiple independent lineages, *A. occidentale* was the host harboring the most diverse assemblage of *Colletotrichum* species regardless of q order.

Considering *C. siamense* as a single species or as a species complex leads to the conclusion that the Atlantic Forest is the most diverse biome, however the rank order of diversity among the remaining biomes changes depending on approaches to species delimitation. While the

Amazon Rainforest is the least diverse when treating *C. siamense* as a single species, it is comparable in diversity to the Atlantic Forest ($q = 1$) or the most diverse biome ($q = 2$) when treating it as a species complex. Variation in the diversity rank among strata is also observed with geographical sampling sites and the divergence in diversity among host organs is similarly impacted by choices with respect to species delimitation. The seeming instability of results across strata reflects the fact that *C. siamense* treated as a single species is represented by individuals of 15 lineages (*C. communis*, *C. endomangiferae*, *C. dianesei* and 12 undetermined lineages), whereas its consideration as a species complex treats these lineages as independent species with lower relative abundance. These results suggest taking an inclusive view and delimiting species more broadly may mask important differences in community composition among sampling strata. When two communities are viewed as comparable in diversity, future studies may ignore continued sampling in one or the other expecting inferences drawn from one to be transferable to another. However, our results indicate that one may want to consider alternate delimitation scenarios before drawing conclusions and planning future sampling efforts.

Gazis et al. (2011) highlighted the inadequacy of the internal transcribed spacer (ITS) for delimiting species and making biogeographical and ecological inferences for endophytic fungi, including *Colletotrichum*. Our results support their conclusions, but also highlight the impact that different choices about how to delimit species even in the presence of strong phylogenetic data can have on downstream inferences. Collectively, our results indicate that different approaches to species recognition may affect our understanding of how *Colletotrichum* communities are structured with respect to host, plant organ and geographical distribution. Given the global distribution of *Colletotrichum* and its prevalence as a plant pathogen, this has important implications for improving our understanding of the biology and life history of the genus.

The observed diversity of *Colletotrichum* species associated with cashew anthracnose in Brazil was not a surprise for two reasons. First, Brazil is a primary center of diversity for *Anacardium* spp. (Agostini-Costa et al. 2006; Barros et al. 2002) reflecting a long evolutionary history in this part of the world. This history allows for the potential coevolution of *Anacardium* with both beneficial and pathogenic organisms. Second, prior investigations related to cashew anthracnose reported lower levels of diversity presumably because their estimates were based on morphological characterization and/or phylogenetic analyses using

few genes with limited phylogenetic signal (Lopez and Lucas 2010; Serra et al. 2011; Uaciquete et al. 2013). These reports had ascribed cashew anthracnose exclusively to *C. gloeosporioides*, but this species represented only 1.4% of the 280 isolates evaluated in the present study, while *C. siamense* and *C. fructicola* were the most common species associated with anthracnose on cashew plants in Brazil. Our findings are consistent with other studies showing that *C. gloeosporioides s.s.* is not a common pathogen of tropical fruits (Phoulivong et al. 2010). Similarly, *C. fructicola* and *C. tropicale* were collected from symptomatic (Lima et al. 2013) and asymptomatic (Vieira et al. 2014) mango organs in northeastern Brazil, while *C. gloeosporioides s.s.* was neither isolated as a pathogen nor as an endophyte.

Most of the *Colletotrichum* species identified in the present study represent the first report on cashew, and it seems like their occurrence throughout multiple Brazilian biomes may be associated with their host distribution and environmental conditions. All species were found on *A. occidentale*, a host from which samples were taken across all sampled biomes. In contrast, six *Colletotrichum* species were associated with *A. humile* and *A. othonianum*, both of which are restricted to the Cerrado. While structural and chemical variation among *Anacardium* spp. may contribute to differences in diversity among host species, given that anthracnose incidence on cashew orchards correlates with rainfall (Freire et al. 2002; Uaciquete et al. 2013) we expect that environmental conditions also influences the distribution of *Colletotrichum* spp. across the sampled biomes. While precipitation is regular throughout the year ($mm \cdot yr^{-1}$) in the Amazon Rainforest (generally > 2000 $mm \cdot yr^{-1}$ [Ronchail et al. 2002]) and the Atlantic Forest (ca. 1300–1900 $mm \cdot yr^{-1}$ [Forti et al. 2003]), distinct dry and wet seasons characterize the climatic conditions of the Caatinga and Cerrado (usually $<$ 750 $mm \cdot yr^{-1}$ within three months [Leal et al. 2005; Prado et al. 2003], and 800–2000 $mm \cdot year^{-1}$ within six to seven months [Pivello 2011; Ratter et al. 1997], respectively). Our isolates were collected during the rainy season, which is coincident with peak growth and the highest incidence/severity of cashew anthracnose. *Colletotrichum* spp. are also influenced by temperature (Baroncelli et al. 2015; Fernando et al. 2000; Zhang et al. 2012), another environmental factor that may influence their geographical distribution. A recent epidemiological study revealed that the *Colletotrichum* species associated with cashew anthracnose identified in the present study displayed optimum mycelial growth and conidial germination at temperatures ranging between 25–30 °C and 27–37 °C, respectively (Veloso et al. in review). These temperatures are typical for tropical zones, which may explain the widespread occurrence of cashew anthracnose throughout Brazil.

The greatest diversity of *Colletorichum* spp. on *A. occidentale* may have been influenced by the fact that the largest proportion of sampling effort was concentrated on this host (as suggest by both interpolation and extrapolation curves in Fig 3, but see Fig 4), but it may also reflect its domestication and the cropping system in which most cashew orchards are cultivated in Brazil. The transition from natural habitats to agricultural environments may have reduced the bioactivity of some compounds originally used for chemical defense against plant pathogens. In addition, cashew orchards are typically cultivated with few or without managed cropping practices (Cardoso et al. 1999; Freire et al. 2002), meaning that *A. occidentale* plants are usually grown without artificial limiting forces such as chemical spraying. Also, Brazilian smallholder farmers commonly exploit multiple fruit species in the same orchard, an approach that may enhance cross-infections among *Colletotrichum* spp. on a variety of host plants. This may particularly be the case for *C. fructicola* and *C. tropicale* which are also reported among those responsible for anthracnose on mango fruits in northeastern Brazil (Lima et al. 2013). Likewise, a recent study revealed *C. fragariae*, *C. siamense* and *C. tropicale* among those associated with anthracnose on banana (Vieira et al. in review), another common fruit cultivated on small farms and urban backyards in Brazil. These results suggest that anthropogenic activities may represent a major driving force impacting the diversity of *Colletotrichum* species in both wild and agricultural habitats.

Other phylogenetic studies have investigated the relationships among *Colletotrichum* species associated with cultivated and wild plants. Based on genealogical concordance phylogenetic species recognition (GCPSR) and a multilocus analysis involving four genes, Doyle et al. (2013) identified seven different species within *C. gloeosporioides s.l.* associated with five host species from wild and commercial cranberry bogs (*Vaccinium macrocarpon* Aiton) in North America. Four of them, namely *C. temperatum*, *C. melanocaulon*, *C. rhexiae* and *C. fructivorum*, were found on *V. macrocarpon*, the latter of which is the principle fruit-rot pathogen of cultivated cranberry but is also capable of infecting alternative hosts such as *Vaccinium oxycoccos* and *Rhexia virginica* in commercial cranberry bogs. A multilocus analysis was also applied by Udayanga et al. (2013) to identify which *Colletotrichum* species were associated with anthracnose on commercially available cultivated and wild fruits in northern Thailand. They demonstrated that *C. gloeosporioides s.s.* was associated with *Citrus aurantifolia* and *Syzygium samarangense*, while *C. fructicola* was isolated from *Hylocereus undatus* and *Ziziphus* sp., and *C. endophytica* from an unknown wild fruit. However, the most predominant species in wild fruit was *C. siamense*, which was also identified as a pre- and/or

postharvest pathogen in *Coffea arabica*, *Annona reticulata*, *Ficus racemosa*, *Azadirachta indica*, *Carica papaya* and *Musa* sp. Study performed with five *Colletotrichum* species collected from symptomatic mango fruits in northeastern Brazil revealed them as pathogenic to banana, guava, mango and papaya fruit, although varying in aggressiveness (Lima et al. 2014). These data are consistent with previous reports that a single *Colletotrichum* species may infect a broad range of hosts while a single host species may harbor several *Colletotrichum* species (Bragança et al. 2016; Freeman et al. 1998; Lima et al. 2015; Phoulivong et al. 2012), making accurate identification important for developing precise and effective control measures against anthracnose.

The research presented here shows high levels of *Colletotrichum* diversity associated with cashew anthracnose in Brazil, including species previously described as endophytes and others responsible for pre- and/or postharvest diseases on other cultivated and wild plant species. Most of the *Colletotrichum* isolates associated with cashew anthracnose nested within *C. siamense*, which was revealed as the most common species. *Colletotrichum siamense* was collected across all four sampled Brazilian biomes, across all sampled host species, and across all sampled plant organs, which reflects its capacity to be a generalist pathogen and endophyte. Phylogenetic studies involving *Colletotrichum* species from different hosts and geographical areas are crucial to better understand the host and geographical distribution in order to gain insight into the biotic and abiotic factors that have shaped their evolutionary history and diversification. Since the correct identification of the causal agent is essential to define effective and adequate disease control measures, the present study will contribute to improving the management of cashew anthracnose.

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Table 1 – Strains of the *Colletotrichum* species studied in this paper with information about culture collection number, *Anacardium* host, plant organ, Brazilian biomes and geographical sampling sites.

Colletotrichum species	Strain	*Anacardium* species	Host organ	Brazilian biomes	Geographical sampling sites
C. chysophilum	CMM3007	A. occidentale	Stem	Caatinga	CE
	CMM3204	A. humile	Leaf	Cerrado	MG
	CMM3217	A. occidentale	Leaf	Caatinga	PB
	CMM3218	A. occidentale	Leaf	Caatinga	PB
	CMM3231	A. occidentale	Stem	Atlantic Forest	PE
	CMM3239	A. occidentale	Leaf	Atlantic Forest	PE
	CMM3268	A. occidentale	Stem	Atlantic Forest	RN
C. fragariae	CMM3214	A. occidentale	Leaf	Caatinga	PB
	CMM3220	A. occidentale	Leaf	Caatinga	PB
	CMM3221	A. occidentale	Leaf	Atlantic Forest	PE
	CMM3224	A. occidentale	Stem	Atlantic Forest	PE
	CMM3245	A. occidentale	Leaf	Caatinga	RN
C. fructicola	CMM3013	A.humille	Leaf	Cerrado	DF
	CMM3102	A.othonianum	Leaf	Cerrado	DF
	CMM3207	A.othonianum	Leaf	Cerrado	MG
	CMM3208	A.humille	Leaf	Cerrado	MG
	CMM3209	A. occidentale	Leaf	Cerrado	MG
	CMM3238	A. occidentale	Leaf	Atlantic Forest	PE
C. gloeosporioides	CMM3272	A. occidentale	Inflorescence	Atlantic Forest	PE
	CMM3279	A. occidentale	Inflorescence	Atlantic Forest	PE
C. queenslandicum	CMM3233	A. occidentale	Leaf	Atlantic Forest	PE
	CMM3236	A. occidentale	Leaf	Atlantic Forest	PE
	CMM3237	A. occidentale	Leaf	Atlantic Forest	PE
	CMM3240	A. occidentale	Inflorescence	Atlantic Forest	PE
	CMM3241	A. occidentale	Inflorescence	Atlantic Forest	PE
	CMM3242	A. occidentale	Inflorescence	Atlantic Forest	PE
C. siamense	CMM2990	A. occidentale	Leaf	Atlantic Forest	AL
	CMM2994	A. occidentale	Leaf	Atlantic Forest	AL
	CMM2998	A. occidentale	Stem	Atlantic Forest	AL
	CMM2974	A. occidentale	Stem	Atlantic Forest	AL
	CMM3001	A. occidentale	Leaf	Caatinga	CE
	CMM3011	A. occidentale	Leaf	Cerrado	DF
	CMM3014	A. humile	Leaf	Cerrado	DF
	CMM3015	A.othonianum	Leaf	Cerrado	DF
	CMM3020	A.othonianum	Leaf	Cerrado	DF
	CMM3101	A.othonianum	Leaf	Cerrado	DF
	CMM3103	A.othonianum	Leaf	Cerrado	DF

Table 1 – (continued)

Colletotrichum species	Strain	*Anacardium* species	Host organ	Brazilian biomes	Geographical sampling sites
C. siamense	CMM3202	*A.othonianum*	Leaf	Cerrado	DF
	CMM3203	*A.othonianum*	Leaf	Cerrado	MG
	CMM3205	*A.othonianum*	Leaf	Cerrado	MG
	CMM3206	*A.othonianum*	Leaf	Cerrado	MG
	CMM3210	*A. occidentale*	Leaf	Cerrado	MG
	CMM3211	*A. occidentale*	Leaf	Cerrado	MG
	CMM3212	*A. occidentale*	Stem	Cerrado	MG
	CMM3215	*A. occidentale*	Leaf	Caatinga	PB
	CMM3216	*A. occidentale*	Leaf	Caatinga	PB
	CMM3219	*A. occidentale*	Leaf	Caatinga	PB
	CMM3222	*A. occidentale*	Leaf	Caatinga	PB
	CMM3223	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3225	*A. occidentale*	Stem	Atlantic Forest	PE
	CMM3226	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3227	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3229	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3232	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3234	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3235	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3243	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3251	*A. occidentale*	Leaf	Caatinga	RN
	CMM3253	*A. occidentale*	Leaf	Caatinga	RN
	CMM3260	*A. occidentale*	Inflorescence	Caatinga	RN
	CMM3261	*A. occidentale*	Stem	Caatinga	RN
	CMM3264	*A. occidentale*	Stem	Caatinga	RN
	CMM3284	*A. occidentale*	Leaf	Atlantic Forest	SC
	CMM3286	*A. humile*	Leaf	Cerrado	GO
	CMM3298	*A. occidentale*	Leaf	Amazon Rainforest	PA
	CMM3301	*A. occidentale*	Leaf	Amazon Rainforest	PA
	CMM3304	*A. occidentale*	Leaf	Amazon Rainforest	PA
	CMM3308	*A. occidentale*	Inflorescence	Amazon Rainforest	PA
	CMM3313	*A. occidentale*	Inflorescence	Amazon Rainforest	PA
	CMM3320	*A. occidentale*	Leaf	Amazon Rainforest	PA
	CMM3322	*A. occidentale*	Leaf	Amazon Rainforest	PA
C. tropicale	CMM2999	*A. occidentale*	Leaf	Caatinga	CE
	CMM3213	*A. occidentale*	Leaf	Caatinga	PB
	CMM3228	*A. occidentale*	Leaf	Atlantic Forest	PE
	CMM3303	*A. occidentale*	Leaf	Amazon Rainforest	PA

CMM: Culture Collection of Phythopathogenic Fungi "Prof. Maria Menezes", Recife, Brazil; AL = Alagoas, CE = Ceará, DF = Distrito Federal, GO = Goiás, MG = Minas Gerais, PA = Pará, PB = Paraíba, PE = Pernambuco, RN =Rio Grande do Norte, and SC = Santa Catarina.

Table 2 – Diversity measures represented by Hill numbers based on abundance data of *Colletotrichum* species (considering *C. siamense* as a single species) associated with different organs of cultivated and wild cashew in four Brazilian biomes and various geographical sampling sites.

Source of the *Colletotrichum* isolates	Reference sample size[a]	Hill numbers (95% confidence interval)		
		Richness ($q = 0$)[b]	Shannon ($q = 1$)[c]	Simpson ($q = 2$)[d]
Anacardium species				
A. humile	26	4.0 (2.46–5.54) b	2.3 (1.44–3.19) ab	1.8 (1.14–2.40) a
A. occidentale	215	7.0 (6.27–7.73) a	3.2 (2.75–3.73) a	2.1 (1.79–2.48) a
A. othonianum	29	2.0 (2.00–2.00) c	1.8 (1.62–2.09) b	1.7 (1.42–2.06) a
Host organ				
Fruit	1	1.0 (1.00–1.00) c	1.0 (1.00–1.00) b	NaN[e]
Inflorescences	19	4.0 (2.33–5.66) b	2.9 (2.03–3.74) a	2.4 (1.40–3.38) a
Leaves	219	7.0 (6.27–7.73) a	3.4 (2.89–4.00) a	2.4 (2.04–2.76) a
Stems	41	5.0 (4.02–5.98) b	2.8 (1.98–3.60) a	2.0 (1.35–2.67) a
Brazilian biome				
Amazon Rainforest	10	2.0 (1.72–2.28) b	1.6 (1.06–2.24) b	1.5 (0.89–2.05) a
Atlantic Forest	126	6.0 (5.27–6.73) a	3.5 (2.95–3.98) a	2.5 (2.05–2.89) a
Caatinga	71	5.0 (4.36–5.64) a	2.5 (1.85–3.19) ab	1.8 (1.37–2.21) a
Cerrado	73	4.0 (2.55–5.45) ab	2.4 (1.96–2.76) b	2.2 (1.97–2.34) a
Geographical sampling sites				
Alagoas	9	1.0 (1.00–1.00) c	1.0 (1.00–1.00) c	1.0 (1.00–1.00) b
Ceará	7	3.0 (2.15–3.85) b	2.9 (2.28–3.61) ab	2.9 (2.00–3.76) a
Distrito Federal	36	3.0 (2.02–3.98) b	2.0 (1.60–2.46) b	1.8 (1.36–2.21) ab
Goiás	2	1.0 (1.00–1.00) c	1.0 (1.00–1.00) c	1.0 (1.00–1.00) b
Minas Gerais	35	4.0 (3.13–4.87) b	2.4 (1.94–2.78) b	2.0 (1.51–2.53) ab
Pará	10	2.0 (1.27–2.73) b	1.6 (1.08–2.22) b	1.5 (0.94–2.00) ab
Paraíba	28	4.0 (3.21–4.79) b	2.8 (1.99–3.59) ab	2.3 (1.53–2.99) ab
Pernambuco	116	6.0 (5.21–6.79) a	3.7 (3.07–4.30) a	2.7 (2.10–3.27) a
Rio Grande do Norte	36	4.0 (2.22–5.78) ab	1.6 (0.89–2.29) bc	1.3 (0.95–1.57) b
Santa Catarina	1	1.0 (1.00–1.00) c	1.0 (1.00–1.00) c	NaN[e]

[a] Number of *Colletotrichum* isolates; [b] species richness; [c] exponential of the Shannon entropy; [d] inverse Simpson concentration; [e] not a number. Hill numbers for the same *Colletotrichum* source within the same exponent q (0, 1 or 2) followed by different letters were significantly different based on their 95% confidence intervals.

Table 3 – Diversity measures represented by Hill numbers based on abundance data of *Colletotrichum* species (considering *C. siamense* as a species complex) associated with different organs of cultivated and wild cashew in four Brazilian biomes and various geographical sampling sites.

Source of the *Colletotrichum* isolates	Reference sample size[a]	Hill numbers (95% confidence interval)		
		Richness ($q = 0$)[b]	Shannon ($q = 1$)[c]	Simpson ($q = 2$)[d]
Anacardium species				
A. humile	26	5.0 (3.26–6.74) b	2.6 (1.46–3.69) b	1.8 (1.13–2.48) b
A. occidentale	215	21.0 (16.76–25.23) a	7.8 (6.24–9.39) a	4.6 (3.51–5.63) a
A. othonianum	29	5.0 (3.75–6.25) b	2.9 (2.24–3.60) b	2.4 (1.82–2.94) b
Host organ				
Fruit	1	1.0 (1.00–1.00) c	1.0 (1.00–1.00) c	NaN[e]
Inflorescences	19	6.0 (3.97–8.03) b	4.9 (3.52–6.39) b	4.4 (3.13–5.78) a
Leaves	219	19.0 (14.96–23.04) a	9.1 (7.81–10.46) a	6.3 (4.97–7.73) a
Stems	41	10.0 (7.28–12.72) b	5.9 (4.07–7.65) b	3.9 (2.18–5.55) a
Brazilian biome				
Amazon Rainforest	10	7.0 (5.32–8.68) b	6.6 (4.64–8.55) ab	6.2 (4.08–8.42) a
Atlantic Forest	126	12.0 (10.26–13.74) a	6.7 (5.46–7.87) a	4.5 (3.25–5.76) ab
Caatinga	71	7.0 (5.18–8.82) b	4.2 (3.29–5.15) b	3.2 (2.28–4.06) b
Cerrado	73	10.0 (6.81–13.19) ab	4.2 (3.30–5.18) b	3.1 (2.45–3.77) b
Geographical sampling sites				
Alagoas	9	3.0 (1.71–4.29) c	2.0 (1.00–2.97) cd	1.6 (0.88–2.30) c
Ceará	7	3.0 (2.13–3.87) c	2.9 (2.04–3.84) bc	2.9 (1.99–3.78) bc
Distrito Federal	36	5.0 (3.84–6.16) bc	3.2 (2.54–3.88) bc	2.7 (1.96–3.35) bc
Goiás	2	1.0 (1.00–1.00) d	1.0 (1.00–1.00) d	1.0 (1.00–1.00) c
Minas Gerais	35	8.0 (4.71–11.29) ab	3.5 (2.03–5.06) bc	2.3 (1.13–3.50) bc
Pará	10	7.0 (4.99–9.01) b	6.6 (4.45–8.75) ab	6.2 (4.33–8.17) a
Paraíba	28	5.0 (3.97–6.03) b	4.1 (3.39–4.89) b	3.7 (2.56–4.77) ab
Pernambuco	116	10.0 (9.11–10.89) a	6.6 (5.47–7.57) a	4.7 (3.83–5.65) a
Rio Grande do Norte	36	6.0 (3.83–8.17) b	3.1 (1.98–4.20) bc	2.4 (1.71–3.02) bc
Santa Catarina	1	1.0 (1.00–1.00) d	1.0 (1.00–1.00) d	NaN[e]

[a] Number of *Colletotrichum* isolates; [b] species richness; [c] exponential of the Shannon entropy; [d] inverse Simpson concentration; [e] not a number. Hill numbers for the same *Colletotrichum* source within the same exponent *q* (0, 1 or 2) followed by different letters were significantly different based on their 95% confidence intervals.

Table S1 – Primers used in this study.

Gene	Primer	Sequence (5′–3′)	Reference
APN2/MAT-GS	CgDL_F6	AGTGGAGGTGCGGGACGTT	Rojas et al. 2010
	CgMAT1_F2	TGATGTATCCCGACTACCG	
APN2	CgDL_R1	GCCCGACGAGCAGAGGACGTAGTC	Rojas et al. 2010
	ColDL_F3	GGGAGAAGCGAACATACCA	
CAL	CL1C	GAA TTC AAG GAG GCC TTC TC	Weir et al. 2012
	CL2C	CTT CTG CAT CAT GAG CTG GAC	
GAPDH	GAP-95	CCGTCAACGACCCCTTCATT	Vieira et al. review
	GAP-1174	AACCCCACTCGTTGTCGTAC	
GAP2-IGS	GAP-1041	CTACACCGAGGACGATGTCG	Vieira et al. review
	GAP/IGS-2044	TTCTACGGGAAAACCAGGGC	
GS	GS-64F	CCGGAGAATYCTTTWCACGA	Vieira et al. review
	GS-967R	CTTCAGGTAGACGTCAGAGTTG	
TUB2	BT1	AACATGCGTGAGATTGTAAGT	O'Donnell and Cigelnik 1997
	BT22	TCTGGATGTTGTTGGGAATCC	
	Coll_Bt_F1int	TCACCTCCAGACCGGCCA	Rojas et al. 2010
	Coll_Bt_R1int	TGGACGTTGCGCATCTGG	

Table S2 – Strains of the *Colletotrichum* studied in this paper with information about culture collection number, host and location, and GenBank accessions of the sequences generated.

Colletotrichum species	Culture[1]	Host	Location	GenBank accessions						
				APN2	APN2/MAT-IGS	CAL	GAP2-IGS	GPDH	GS	TUB2
C. aenigma	ICMP18608*	*Persea americana*	Israel	-	KM360143	JX009683	-	JX010044	JX010078	JX010389
	ICMP18686	*Pyrus pyrifolia*	Japan	-	-	JX009684	-	JX009913	JX010079	JX010390
C. aeschynomenes	ICMP 17673*	*Aeschynomene virginica*	USA	-	KM360145	JX009721	-	JX009930	JX010081	JX010392
C. alienum	ICMP 12071*	*Malus domestica*	New Zealand	-	KC888927	JX009654	-	JX010028	JX010101	JX010411
	ICMP 18621	*Persea americana*	New Zealand	-	-	JX009657	-	JX009959	JX010075	JX010386
C. asianum	ICMP 18580*, CBS 130418	*Coffea arabica*	Thailand	-	FR718814	FJ917506	-	JX010053	JX010096	JX010406
	IMI 313839, ICMP 18696	*Mangifera indica*	Australia	-	-	JX009723	-	JX009915	JX010073	JX010384
C. chrysophilum	CMM 4268*, URM 7362	*Musa sp.*	Brazil	KX094285	KX094325	KX094063	KX094125	KX094183	KX094204	KX094018
C. chrysophilum (syn. C. ignotum)	8395	*Theobroma cacao*	Panama	GU994415	GU994444	KX094056	KX094126	KX094176	KX094209	GU994473
C. chrysophilum (syn. C. ignotum)	Coll919	*Terpsichore taxifolia*	Puerto Rico	JX145265	JX145317	KX094057	KX094127	KX094177	KX094207	KX094288
C. chrysophilum (syn. C. ignotum)	E183	*Genipa americana*	Panama	GU994414	GU994443	KX094058	KX094128	KX094178	KX094208	GU994472
C. chrysophilum	CMM3217	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3239	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3218	*A.ocidentale*	Brazil, Paraíba state	-	-	-	-	-	-	-

Table S2 – (Continued)

Colletotrichum species	Culture[1]	Host	Location	APN2	APN2/MAT-IGS	CAL	GAP2-IGS	GPDH	GS	TUB2
						GenBank accessions				
C. chrysophilum	CMM3231	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3268	*A.ocidentale*	Brazil, Rio Grande Do Norte state	-	-	-	-	-	-	-
	CMM3204	*A.humile*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3007	*A.ocidentale*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
C. endophytica	MFLUCC 130417, LC1216	*Pennisetum purpureum*	Thailand	-	-	KC810017	-	KC832853	-	-
C. endophytica	MFLUCC 130418, LC0324*	*Pennisetum purpureum*	Thailand	-	-	KC810018	-	KC832854	-	-
C. endophytica	MFLUCC 130419, LC0327	*Pennisetum purpureum*	Thailand	-	-	KC810016	-	KC832846	-	-
C. fragariae	Bra5	*Coffea sp.*	Brazil	-	FR718801	-	-	-	FR719907	FR719885
	Bra8	*Coffea sp.*	Brazil	-	FR718802	-	-	-	FR719908	FR719886
	CBS 142.31*, ICMP 17927*	*Fragaria × ananassa*	USA	-	JQ807844	JX009592		JX010024	JX010064	JX010373
	CMM3214	*A.ocidentale*	Brazil, Paraíba state	-	-	-	-	-	-	-
	CMM3220	*A.ocidentale*	Brazil, Paraíba state	-	-	-	-	-	-	-
	CMM3221	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3224	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3245	*A.ocidentale*	Brazil, Rio Grande Do Norte state	-	-	-	-	-	-	-

Table S2 – (continued)

Colletotrichum species	Culture[1]	Host	Location	GenBank accessions						
				APN2	APN2/MAT-IGS	CAL	GAP2-IGS	GPDH	GS	TUB2
C. fructicola	1087	*Theobroma cacao*	Panama	GU994409	GU994438	KX094066	KX094121	KX094174	KX094198	KX094279
	3589	*Theobroma cacao*	Panama	GU994411	GU994440	KX094067	KX094122	KX094175	KX094199	KX094280
	3679	*T. cacao*	Panama	GU994410	GU994439	-	-	-	-	GU994468
	7574	*T. cacao*	Panama	GU994413	GU994442	-	-	-	-	GU994471
	Coll1126	*Vaccinium macrocarpon*	New Jersey	JX145239	JX145315	-	-	-	-	JX145187
	Coll996	*Rhexia virginica*	New Jersey	JX145269	JX145324	-	-	-	-	JX145219
	CollP1	*V. corymbosum*	North Carolina	JX145273	JX145316	-	-	-	-	JX145223
	GM567= MTCC 11679	*Mangifera indica*	India	-	JQ894576	KC790787	-	JQ894630	-	JQ894600
	ICMP 18581*, CBS 130416	*Coffea arabica*	Thailand	-	JQ807838	FJ917508	-	JX010033	JX010095	JX010405
C. fructicola (syn. C. ignotum)	CBS 125397*, ICMP 18646	*Tetragastris panamensis*	Panama	GU994412	GU994441	JX009674	-	JX010032	JX010099	GU994470
	CMM3013	*A.humile*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3102	*A.othonianum*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3207	*A.othonianum*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3208	*A.humile*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3209	*A.ocidentale*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3238	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
C. gloeosporioides	IMI 356878*, CBS 112999	*Citrus sinensis*	Italy	GU994416	JQ807843	JX009731	-	JX010056	JX010085	JX010445

Table S2 – (continued)

Colletotrichum species	**Culture[1]**	**Host**	**Location**	**GenBank accessions**						
				APN2	**APN2/MAT-IGS**	**CAL**	**GAP2-IGS**	**GPDH**	**GS**	**TUB2**
C. gloeosporioides	LC3110, LF318	*Camellia sinensis*	China	-	KJ954541	KJ954680	-	KJ954828	KJ954978	KJ955275
C. gloeosporioides	C3312, LF534	*Camellia sinensis*	China	-	KJ954569	KJ954710	-	KJ954859	KJ955009	KJ955305
	CMM3279	*A.ocidentale*	Brazil, Rio Grande Do Norte state	-	-	-	-	-	-	-
	CMM3272	*A.ocidentale*	Brazil, Rio Grande Do Norte state	-	-	-	-	-	-	-
C. grevilleae	CBS 132879b, CPC 15481	*Grevillea sp.*	Italy	-		KC296963	-	KC297010	KC297033	KC297102
C. hebeiense	JZB330028*	*V.vinifera cv. Cabernet Sauvignon*	China	-	-		-	KF377495	-	KF288975
C. hebeiense	JZB330024	*V.vinifera cv. Cabernet Sauvignon*	China	-	-		-	KF377505	-	-
C. horii	ICMP 10492*	*Diospyros kaki*	Japan	-	JQ807840	JX009604	-	GQ329681	JX010137	JX010450
C. hymenocallidis	ICMP 18642*, CBS 125378, LC0043	*Hymenocallis americana*	China	-	JQ807842	JX009709	-	JX010019	JX010100	JX010410
C. jasmini-sambac	ICMP 19118*	*Jasminum sambac*	Vietnam	-	JQ807841	-	-	HM131497	-	JX010415
C. murrayae	GZAAS5.09506*	*Murraya sp.*	China			JQ247596		JQ247609	JQ247621	JQ247644
C. murrayae	GZAAS5.09538	*Murraya sp.*	China			JQ247597		JQ247608	JQ247620	JQ247645
C. musae	CBS 116870*, ICMP 19119			-	KC888926	JX009742	-	JX010050	JX010084	HQ596280
	IMI 52264, ICMP 17817			-	-	JX009689	-	JX010015	-	JX010395
	CMM4423	*Musa sp.*	Brazil	KX094010	KX094328	KX094028	KX094119	KX094195	KX094231	KX094294

Table S2 – (continued)

Colletotrichum species	**Culture[1]**	**Host**	**Location**	**GenBank accessions**						
				APN2	**APN2/MAT-IGS**	**CAL**	**GAP2-IGS**	**GPDH**	**GS**	**TUB2**
C. nupharicola	CBS 469.96, ICMP 17938	*Nuphar lutea subsp. polysepala*	USA	-	-	JX009661	-	JX009936	JX010087	JX010397
C. nupharicola	CBS 470.96*, ICMP 18187	*Nuphar lutea subsp. polysepala*	USA	JX145275	JX145319	JX009663	-	JX009936	JX010088	JX010397
C. nupharicola	CBS 472.96, ICMP 17940	*Nymphaea ordorata*	USA	JX145276	JX145320	JX009662	-	JX010031	JX010089	JX010399
C. queenslandicum	ICMP 1778*	*Carica papaya*	Australia		KC888928	JX009691	-	JX009934	JX010104	JX010414
C. queenslandicum	ICMP 18705	*Coffea sp.*	Fiji	-	-	JX009694	-	JX010036	JX010102	JX010412
C. queenslandicum	ICMP 1780	*Australia*	Australia	-	-	JX009693	-	JX010010	-	-
C. queenslandicum	ICMP 12564	*Persea americana*	Australia	-		JX009692	-	JX009919	-	-
	CMM3233	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3241	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3236	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3240	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3237	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3242	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
C. salsolae	ICMP 19051*	*Salsola tragus*	Hungary	-	KC888925	JX009696	-	JX009916	JX010093	JX010403
C. siamense	GN1			-	KC790673	KF451952	-	KC790735		KC790868

Table S2 – (continued)

Colletotrichum species	**Culture[1]**	**Host**	**Location**	**GenBank accessions**						
				APN2	**APN2/MAT-IGS**	**CAL**	**GAP2-IGS**	**GPDH**	**GS**	**TUB2**
C. siamense	ICMP 18578*, CBS 130417	*Coffea arabica*	Thailand	-	JQ899289	FJ917505	-	JX009924	JX010094	JX010404
	Thai4			-	HE655654					HE655600
	CMM3230	*A.ocidentale*	Brazil, Alagoas state	-	-	-	-	-	-	-
	CMM3231	*A.othonianum*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3232	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3221	*A.ocidentale*	Brazil, Rio Grande Do Norte state	-	-	-	-	-	-	-
	CMM3222	*A.ocidentale*	Brazil, Pará state	-	-	-	-	-	-	-
	CMM3223	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3224	*A.ocidentale*	Brazil, Rio Grande Do Norte state	-	-	-	-	-	-	-
	CMM3225	*A.ocidentale*	Brazil, Pará state	-	-	-	-	-	-	-
	CMM2990	*A.ocidentale*	Brazil, Alagoas state	-	-	-	-	-	-	-
	CMM3216	*A.ocidentale*	Brazil, Paraíba state	-	-	-	-	-	-	-
	CMM3243	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3264	*A.ocidentale*	Brazil, Rio Grande Do Norte state	-	-	-	-	-	-	-
	CMM2974	*A.ocidentale*	Brazil, Alagoas state	-	-	-	-	-	-	-

Table S2 – (continued)

Colletotrichum species	Culture[1]	Host	Location	GenBank accessions						
				APN2	APN2/MAT-IGS	CAL	GAP2-IGS	GPDH	GS	TUB2
C. siamense	CMM3001	*A.ocidentale*	Brazil, Ceará state	-	-	-	-	-	-	-
	CMM3206	*A.othonianum*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3219	*A.ocidentale*	Brazil, Paraíba state	-	-	-	-	-	-	-
	CMM3220	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3228	*Anacardium occidentale*	Brazil, Rio Grande Do Norte state	-	-	-	-	-	-	-
	CMM3227	*Anacardium occidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3226	*Anacardium occidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
C. siamense (syn Colletotrichum sp).	7767	*T. cacao*	Panama	GU994403	GU994432	-	-	-	-	GU994461
C. siamense (syn Colletotrichum sp).	GJS0852	*T. cacao*	Panama	GU994404	GU994433	-	-	-	-	GU994462
C. siamense	CMM 4244	*Musa sp.*	Brazil	KX094014	KX094315	KX094055	KX094135	KX094172	KX094226	KX094299
	CMM 4247	*Musa sp.*	Brazil	KX094009	KX094301	KX094038	KX094141	KX094155	KX094196	KX094261
	CMM3202	*A.othonianum*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3020	*A.othonianum*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3020	*A.othonianum*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3203	*A.othonianum*	Brazil, Distrito Federal state	-	-	-	-	-	-	-

Table S2 – (continued)

Colletotrichum species	**Culture[1]**	**Host**	**Location**	**GenBank accessions**						
				APN2	**APN2/MAT-IGS**	**CAL**	**GAP2-IGS**	**GPDH**	**GS**	**TUB2**
C. siamense	CMM3014	*A.humile*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3101	*A.othonianum*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3205	*A.othonianum*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3227	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3229	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3235	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3223	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3234	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM2994	*A.ocidentale*	Brazil, Alagoas state	-	-	-	-	-	-	-
	CMM3011	*A.ocidentale*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3015	*A.othonianum*	Brazil, Distrito Federal state	-	-	-	-	-	-	-
	CMM3260	*A.ocidentale*	Brazil, Rio Grande do norte state	-	-	-	-	-	-	-
	CMM3286	*A.othonianum*	Brazil, Goias state	-	-	-	-	-	-	-
	CMM3298	*A.ocidentale*	Brazil, Pará state	-	-	-	-	-	-	-

Table S2 – (continued)

Colletotrichum species	Culture[1]	Host	Location	GenBank accessions						
				APN2	APN2/MAT-IGS	CAL	GAP2-IGS	GPDH	GS	TUB2
C. siamense	CMM3320	*A.ocidentale*	Brazil, Pará state	-	-	-	-	-	-	-
	CMM3313	*A.ocidentale*	Brazil, Pará state	-	-	-	-	-	-	-
	CMM3284	*A.ocidentale*	Brazil, Santa Catarina state	-	-	-	-	-	-	-
	CMM3284	*A.ocidentale*	Brazil, Santa Catarina state	-	-	-	-	-	-	-
	CMM3210	*A.ocidentale*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3211	*A.ocidentale*	Brazil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3212	*A.ocidentale*	Braziil, Minas Gerais state	-	-	-	-	-	-	-
	CMM3301	*A.ocidentale*	Brazil, Pará state	-	-	-	-	-	-	-
	CMM 4248	*Musa sp.*	Brazil	KX093992	KX094314	KX094037	KX094136	KX094154	KX094229	KX094300
C. siamense (syn.C. communis)	GO01, MTCC11696	*Citrus sp.*	India	-	KC790720	KF451953	-	KF452016	-	KF452029
C. siamense (syn.C. communis)	NK24, MTCC11599*	*Mangifera indica*	India	-	JQ894582	KC790791	-	JQ894632	-	JQ894602
C. siamense (syn. C. dianesei)	CMM 3777	*Mangifera indica*	Brazil	KX093993	KX094302	KX094045	KX094142	KX094162	KX094214	KX094266
C. siamense (syn. C. dianesei)	CMM 3779	*Mangifera indica*	Brazil	KX093994	KX094303	KX094043	KX094143	KX094163	KX094215	KX094267
C. siamense (syn. C. dianesei)	CMM 4083	*Mangifera indica*	Brazil	KX093998	KX094307	KX094052	KX094147	KX094167	KX094219	KX094271
C. siamense (syn. C. dianesei)	CMM 4084	*Mangifera indica*	Brazil	KX093999	KX094310	KX094053	KX094148	KX094166	KX094220	KX094272
C. siamense (syn. C. dianesei)	CMM 4085*	*Mangifera indica*	Brazil	KX093995	KX094304	KX094044	KX094144	KX094156	KX094216	KX094268
C. siamense (syn. C. dianesei)	CMM4079	*Mangifera indica*	Brazil	KX093996	KX094305	KX094050	KX094145	KX094157	KX094217	KX094269

(continued on next page)

Table S2 – (continued)

Colletotrichum species	Culture[1]	Host	Location	GenBank accessions						
				APN2	APN2/MAT-IGS	CAL	GAP2-IGS	GPDH	GS	TUB2
C. siamense (syn. C. dianesei)	CMM4081	*Mangifera indica*	Brazil	KX093997	KX094306	KX094051	KX094146	KX094158	KX094218	KX094270
C. siamense (syn. C. dianesei)	CMM4089	*Mangifera indica*	Brazil	KX094000	KX094311	KX094054	KX094149	KX094164	KX094221	KX094273
C. siamense (syn. C. dianesei)	CMM4091	*Mangifera indica*	Brazil	KX094001	KX094313	KX094046	KX094150	KX094165	KX094222	KX094274
C. siamense (syn. C. dianesei)	CMM4093	*Mangifera indica*	Brazil	KX094002	KX094312	KX094047	KX094151	KX094159	KX094223	KX094275
C. siamense (syn. C. dianesei)	CMM4096	*Mangifera indica*	Brazil	KX094003	KX094308	KX094048	KX094152	KX094160	KX094224	KX094276
C. siamense (syn. C. dianesei)	CMM4099	*Mangifera indica*	Brazil	KX094004	KX094309	KX094049	KX094153	KX094161	KX094225	KX094277
C. siamense (syn. C. endomangiferae)	CMM 3740	*Mangifera indica*	Brazil	-	KJ155452	KC992371	-	KC702954	-	KM404169
C. siamense (syn. C. endomangiferae)	CMM 3814*	*Mangifera indica*	Brazil	-	KJ155453	KC992372	-	KC702955	-	KM404170
C. siamense (syn. C. melanocaulon)	Coll126, CBS133123	*V. macrocarpon*	New Jersey, USA	JX145245	JX145309	KX094035	KX094133	KX094186	KX094227	KX094289
C. siamense (syn. C. melanocaulon)	Coll131, CBS133251	*V. macrocarpon*	New Jersey	JX145247	JX145313	KX094036	KX094134	KX094187	KX094228	KX094290
C. theobromicola	CBS 124945*, ICMP 18649	*Theobroma cacao*	Panama	GU994419	KC790726	JX009591	-	JX010006	JX010139	JX010447
C. theobromicola	GJS0843	*Theobroma cacao*	Panama	GU994418	GU994447	-	-	-	-	GU994476
C. theobromicola	GJS0848	*Theobroma cacao*	Panama	GU994417	GU994446	-	-	-	-	GU994475
C. tropicale	CBS 124949*, ICMP 18653	*Theobroma cacao*	Panama	GU994396	GU994425	JX009719	-	JX010007	JX010097	GU994454
C. tropicale	CMM 3767	*Mangifera indica*	Brazil	-	KJ155464	KC992378	-	KC702960	-	KC992345
	CMM 3780	*Mangifera indica*	Brazil	-	KJ155467	KC992374	-	KC702961	-	KC992343
	Coll918	*Terpsichore taxifolia*	Puerto Rico	JX145264	JX145307	-	-	-	-	JX145214

Table S2 – (continued)

Colletotrichum species	Culture[1]	Host	Location	GenBank accessions						
				APN2	APN2/MAT-IGS	CAL	GAP2-IGS	GPDH	GS	TUB2
C. tropicale	CMM3213	*A.ocidentale*	Brazil, Paraíba state	-	-	-	-	-	-	-
	CMM2999	*A.ocidentale*	Brazil, Ceará state	-	-	-	-	-	-	-
	CMM3228	*A.ocidentale*	Brazil, Pernambuco state	-	-	-	-	-	-	-
	CMM3303	*A.ocidentale*	Brazil, Pará state	-	-	-	-	-	-	-
C.viniferum	GZAAS5.08601*	*Vitis vinifera cv. Shuijing*	China	-	-	JQ309639	-	JN412798	JN412787	JN412813
C.viniferum	GZAAS5.08608	*Vitis vinifera cv. Shuijing*	China	-	-	JN412782	-	JN412800	JN412784	JN412811

[1]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMM: Culture Collection of Phythopathogenic Fung "Prof. Maria Menezes", Recife, Brazil; CPC: Working collection of Pedro W. Crous, housed at CBS, The Netherlands; GZAAS: Guizhou Academy of Agricultural Sciences Herbarium, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; LC: Working collection of Lei Cai, housed at CAS, China; LF: Working collection of Fang Liu, housed at CAS, China; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand; MTCC: Microbial type culture collection and gene bank, India.* = ex-type culture. Strains/sequences studied in this paper are in **bold** font

Table S3 – Summary of locus and Nucleotide substitution models used in the phylogenetic analyses.

Gene	Length[a]	PI[b]	VC[c]	%PIC[d]	Substitution model	
					ML[e]	BI[f]
APN2/MAT-IGS	995	424	514	42.6	HKY+G	HKY+G
APN2	832	138	154	16.6	TIM1+I+G	GTR+I+G
CAL	755	134	157	17.7	TIM1ef+G	GTR+G
GAPDH	1033	120	153	11.6	TrN+I	GTR+I+G
GAP2-IGS	892	171	220	19.1	K80+G	K80+G
GS	951	192	257	20.2	TPM2uf+G	GTR+G
TUB2	1523	175	263	11.5	TrN+I	GTR+I+G

[a] Number of characters in the alignment; [b] Number of parsimony informative characters; [c] Number of variable characters; [d] Percentage of parsimony informative characters in the alignment; [e] Maximum likelihood; [f] Bayesian inference.

CMM4084 *Colletotrichum dianesei*
CMM4085 C. dianesei
1/100 CMM4083 *C.dianesei*
1/85 CMM3219 *C.siamense*
CMM3243 *C. siamense*
.99/- CMM2974 *C. siamense*
1/88 CMM3206 *C.siamense*
CMM2990 *C. siamense*
1/- CMM4079 *C.dianesei*
CMM4081 *C.dianesei*
-/- CMM3264 *C.siamense*
CMM3308 *C. siamense*
.96/- CMM3222 *C. siamense*
CMM3216 *C. siamense*
.99/- CMM3777 *C.dianesei*
.97/- CMM3779 *C.dianesei*
CMM3779 *C.dianesei*
1/88 CMM4099 *C.dianesei*
CMM4093 *C.dianesei*
-/- CMM3232 *C. siamense*
CMM3234 *C. siamense*
CMM3253 *C. siamense*
1/- CMM3251 *C. siamense*
CMM3001 *C. siamense*
CMM4089 *C.dianesei*
CMM4091 *C.dianesei*
CMM3304 *C. siamense*
-/- 1/98 **GZAAS 5.09506 C. murrayae**
GZAAS 5.09538 C. murrayae
CMM4244 *C. siamense.*
-/- CBS133123 *C. melanocaulon*
CBS13321 C. melanocaulon
1/85 **ICMP18642 C. hymenocallidis**
-/- CMM3301 *C. siamense*
CMM3261*C. siamense*
-/- CMM3226 *C. siamense*
-/- CMM3215 *C. siamense*
-/- 1/92 MTCC11696 *C. communis*
MTCC11599 C. communis
CMM3322 *C. siamense*
CMM3284 *C. siamense*
CMM3313 *C. siamense*
CMM3260 *C. siamense*
1/- CMM3011 *C. siamense*
1/82 CMM3286 *C. siamense*
.99/- CMM3015 *C. siamense*
-/- CMM3210 *C. siamense*
CMM2994 *C. siamense*
.99/78 CMM4248 *C. siamense*
1/85 CMM3212 *C. siamense*
-/- Thai 4 *C. siamense*
1/80 CMM3211 *C. siamense*
.98/69 1/- CMM3235 *C. siamense*
1/70 1/97 CMM3223 *C. siamense*
CBS130417 C. siamense
GN01 *C. siamense*
ICMP19118 C. jasmini-sambac
.99/70 CMM3020 *C. siamense*
1/71 CMM3202 *C. siamense*
.96/88 CMM3101 *C. siamense*
GJS0852 *Colletotrichum* sp.
7767 *Colletotrichum* sp.
.99/78 CMM3205 *C. siamense*
CMM4247 *C. siamense*
96/73 CMM3014 *C. siamense*
1/85 CMM3203 *C. siamense*
CMM3227 *C. siamense*
1/100 CMM3229 *C. siamense*
CMM3103 *C. siamense*
CMM3225 *C. siamense*
1/73 CMM3740 *C. endomangiferae*
1/100 **CMM3814 C. endomangiferae**
CMM2998 *C. siamense*
1/99 CMM3298 *C. siamense*
1/72 CMM3320 *C. siamense*
ICMP19051 C. salsolae
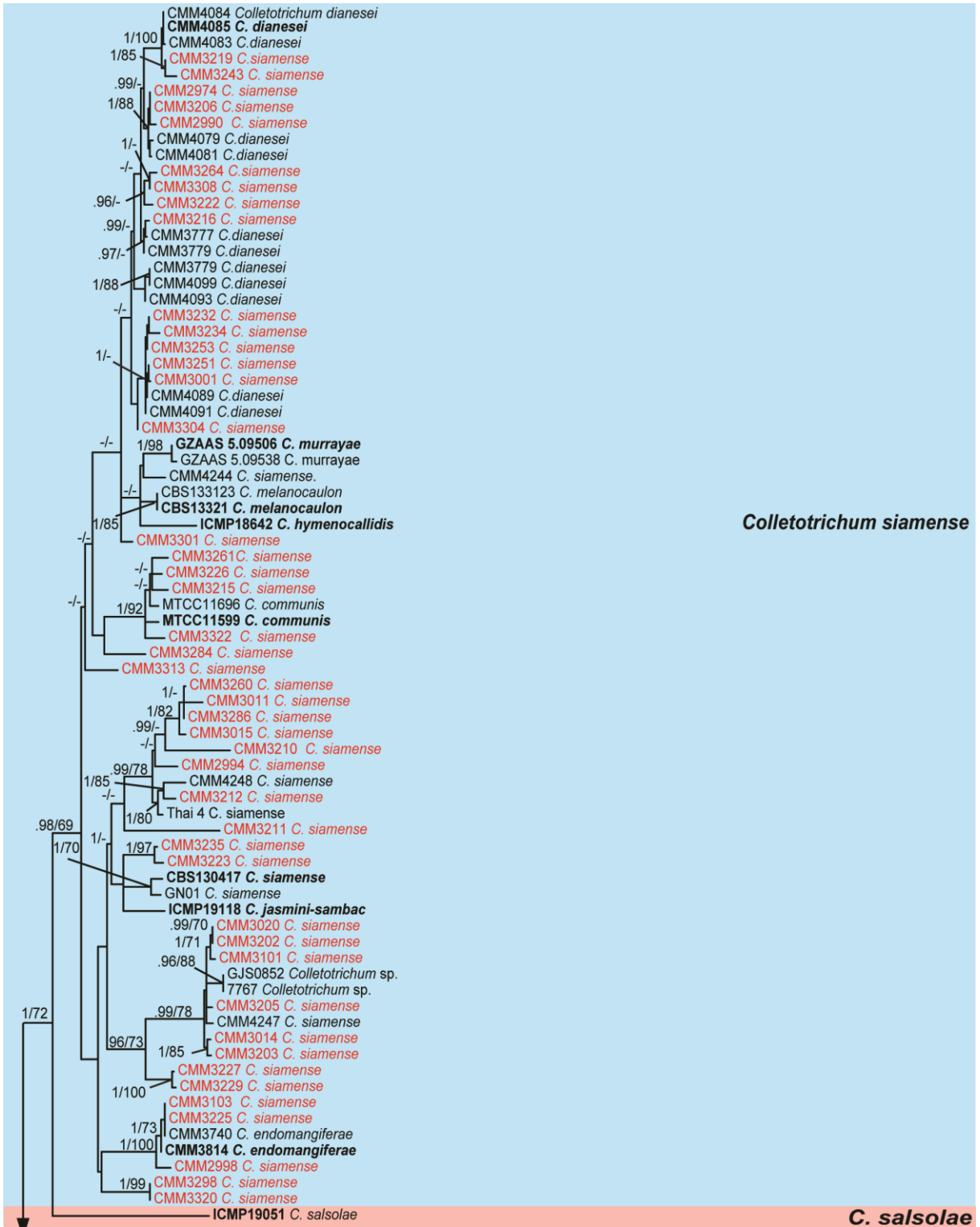
Colletotrichum siamense
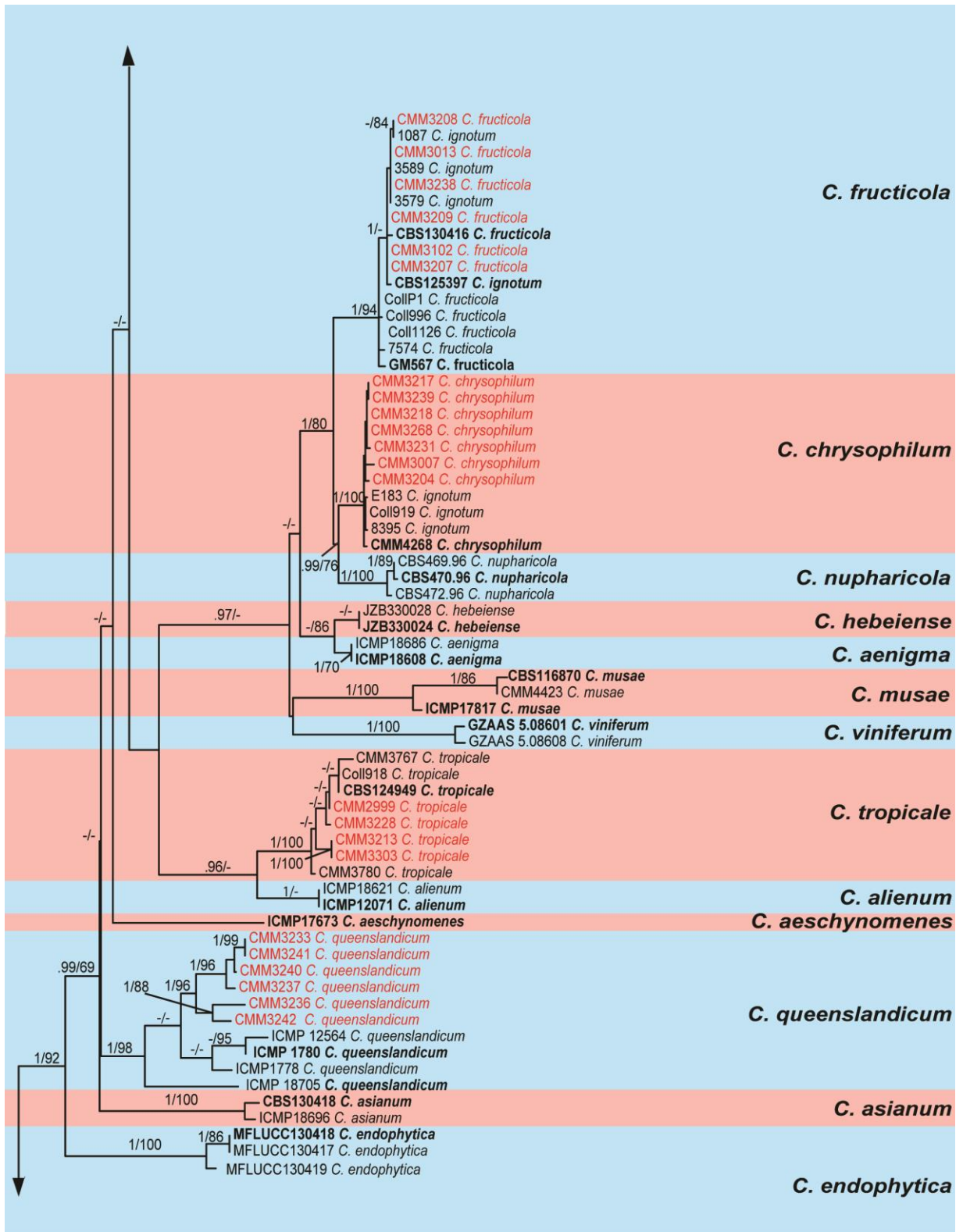
C. salsolae

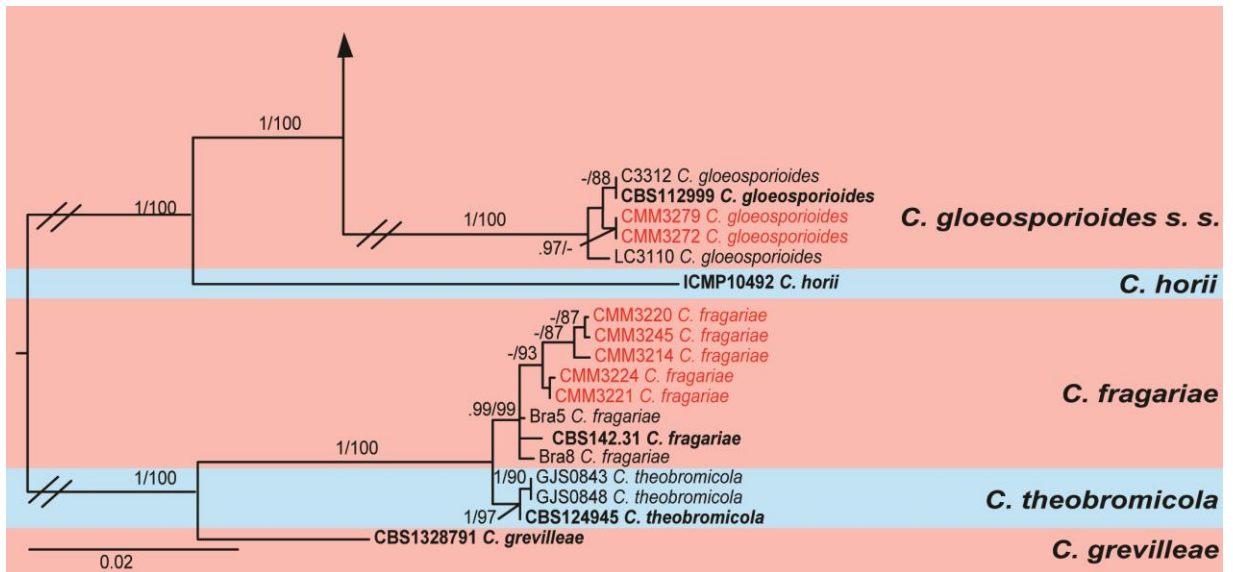**Fig 1 - (continued)**

Fig 1 (continued)

Fig 1 – Maximum likelihood tree of the *C. gloeosporioides* species complex inferred from a concatenated alignment of APN2, APN2/MAT-IGS,CAL, GAPDH, GAP2-IGS, GS and TUB2. Bootstrap support values (ML ≥70) and Bayesian posterior probability values (PP ≥0.95) are shown at the nodes. "-" indicates no-significant support or absence of the node. Ex-types are emphasized in bold and include the taxonomic name as originally described. Isolates from cashew are highlighted in red. *Colletotrichum fragariae, C. theobromicola* and *C. grevilleae* were used as outgroup. The scale bar indicates the number of expected changes per site.
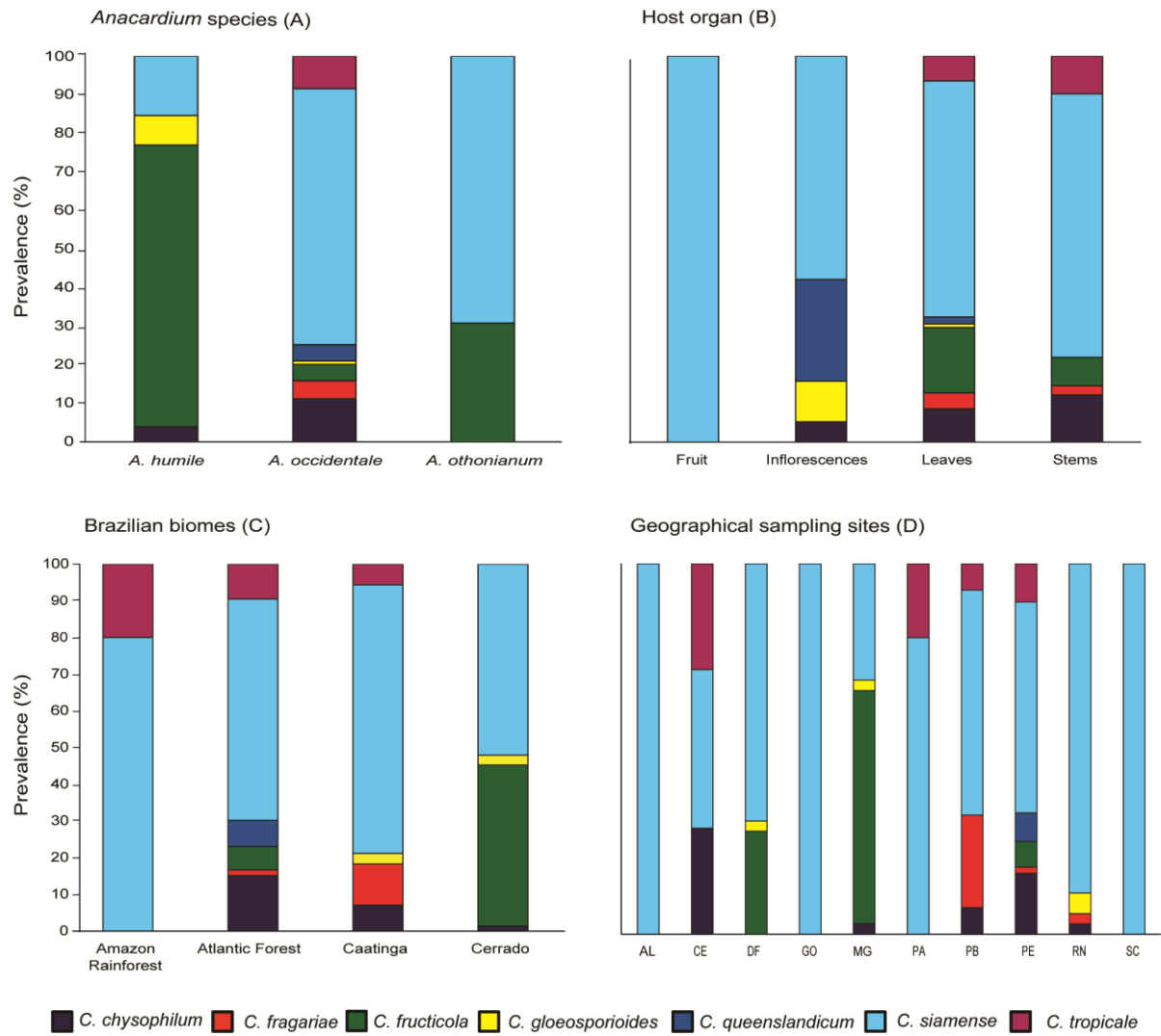
Fig 2 – Prevalence of *Colletotrichum* species associated with different *Anacardium* hosts (A), plant organs (B), Brazilian biomes (C) and 10 geographical sampling sites (D). AL = Alagoas, CE = Ceará, DF = Distrito Federal, GO = Goiás, MG = Minas Gerais, PA = Pará, PB = Paraíba, PE = Pernambuco, RN = Rio Grande do Norte, and SC = Santa Catarina.
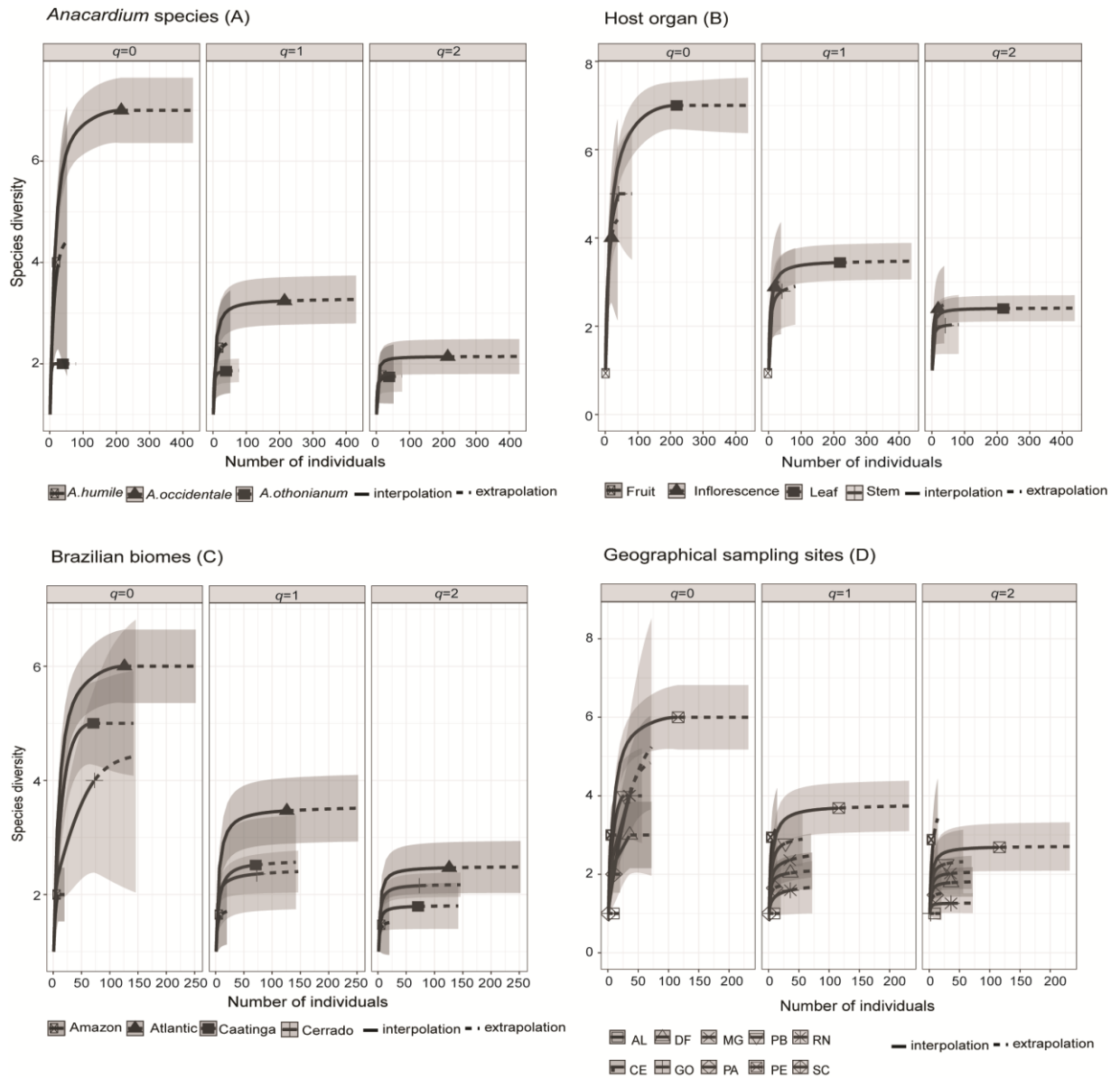
Fig 3 – Rarefaction (solid lines) and extrapolation (dashed lines) curves of *Colletotrichum* species diversity (considering *C. siamense* as a single species) based on the Hill numbers ($q = 0, 1, 2$) for the *Anacardium* species, host organ, Brazilian biomes and various geographical sampling sites. The 95% confidence intervals (gray-shaded regions) were obtained by a bootstrap method based on 200 replications. Reference samples are denoted by different symbols. AL = Alagoas, CE = Ceará, DF = Distrito Federal, GO = Goiás, MG = Minas Gerais, PA = Pará, PB = Paraíba, PE = Pernambuco, RN = Rio Grande do Norte, and SC = Santa Catarina.
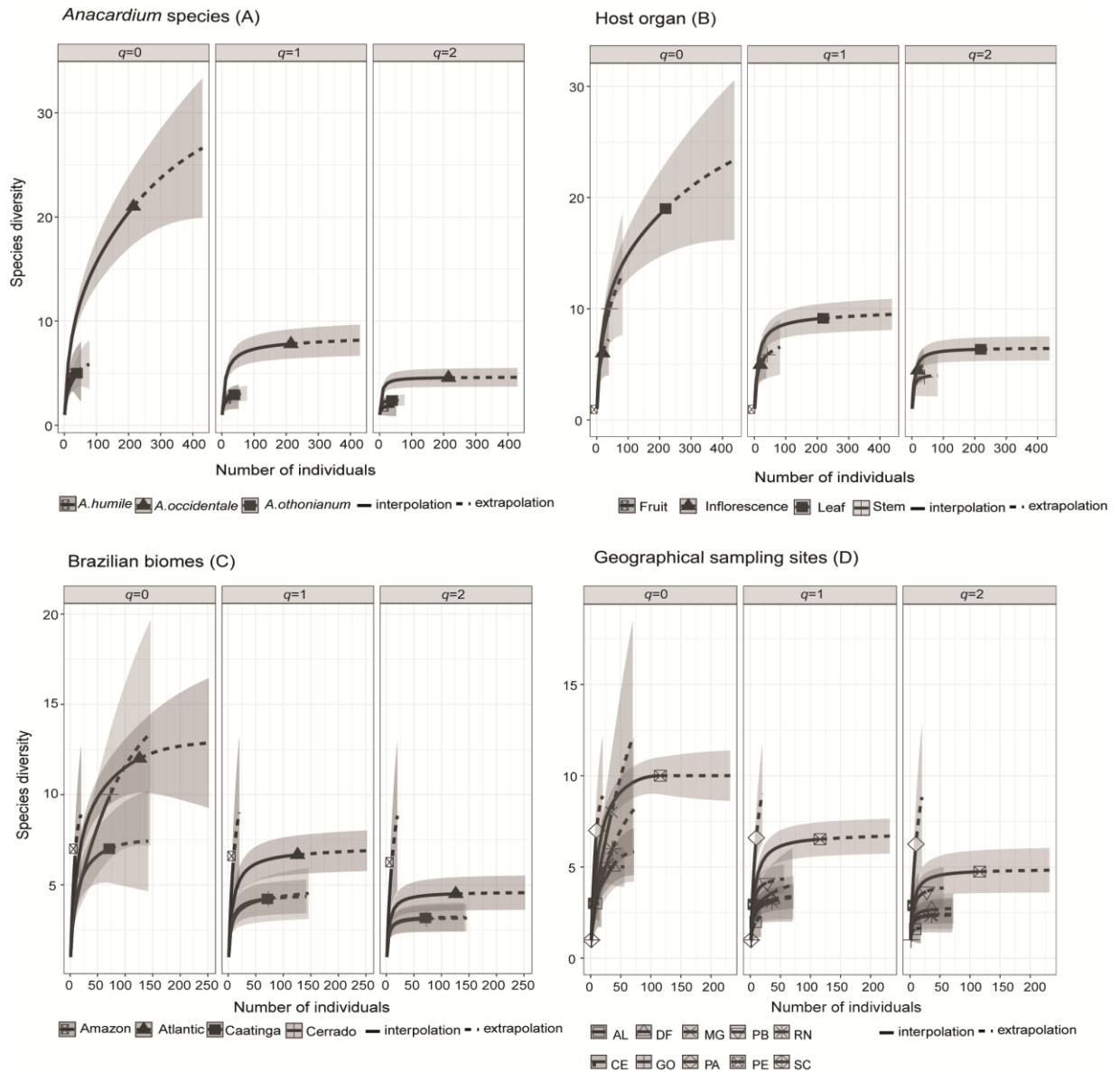
Fig 4 – Rarefaction (solid lines) and extrapolation (dashed lines) curves of *Colletotrichum* species diversity (considering *C. siamense* as a complex species) based on the Hill numbers (*q* = 0, 1, 2) for the *Anacardium* species, host organ, Brazilian biomes and various geographical sampling sites. The 95% confidence intervals (gray-shaded regions) were obtained by a bootstrap method based on 200 replications. Reference samples are denoted by different symbols. AL = Alagoas, CE = Ceará, DF = Distrito Federal, GO = Goiás, MG = Minas Gerais, PA = Pará, PB = Paraíba, PE = Pernambuco, RN = Rio Grande do Norte, and SC = Santa Catarina.
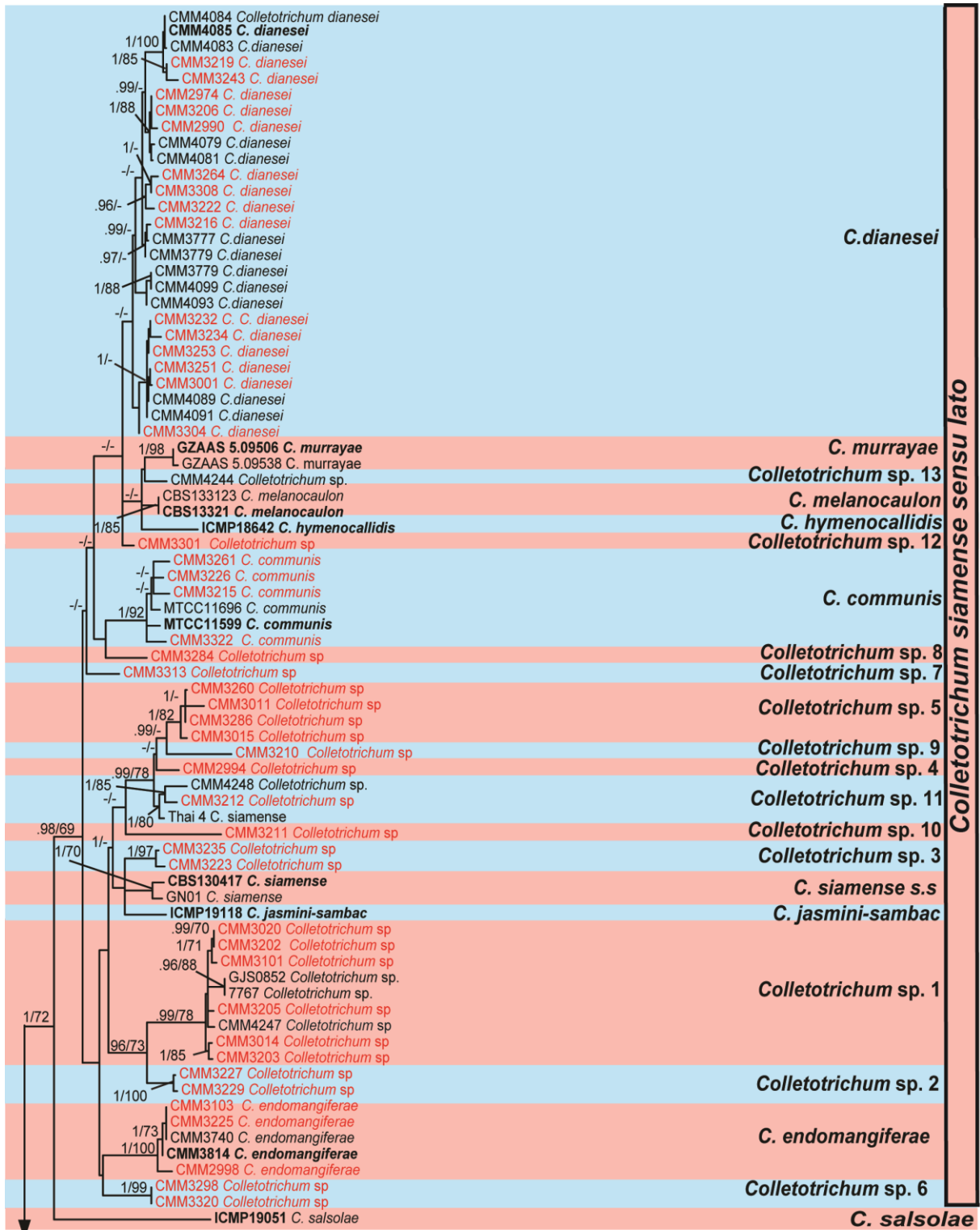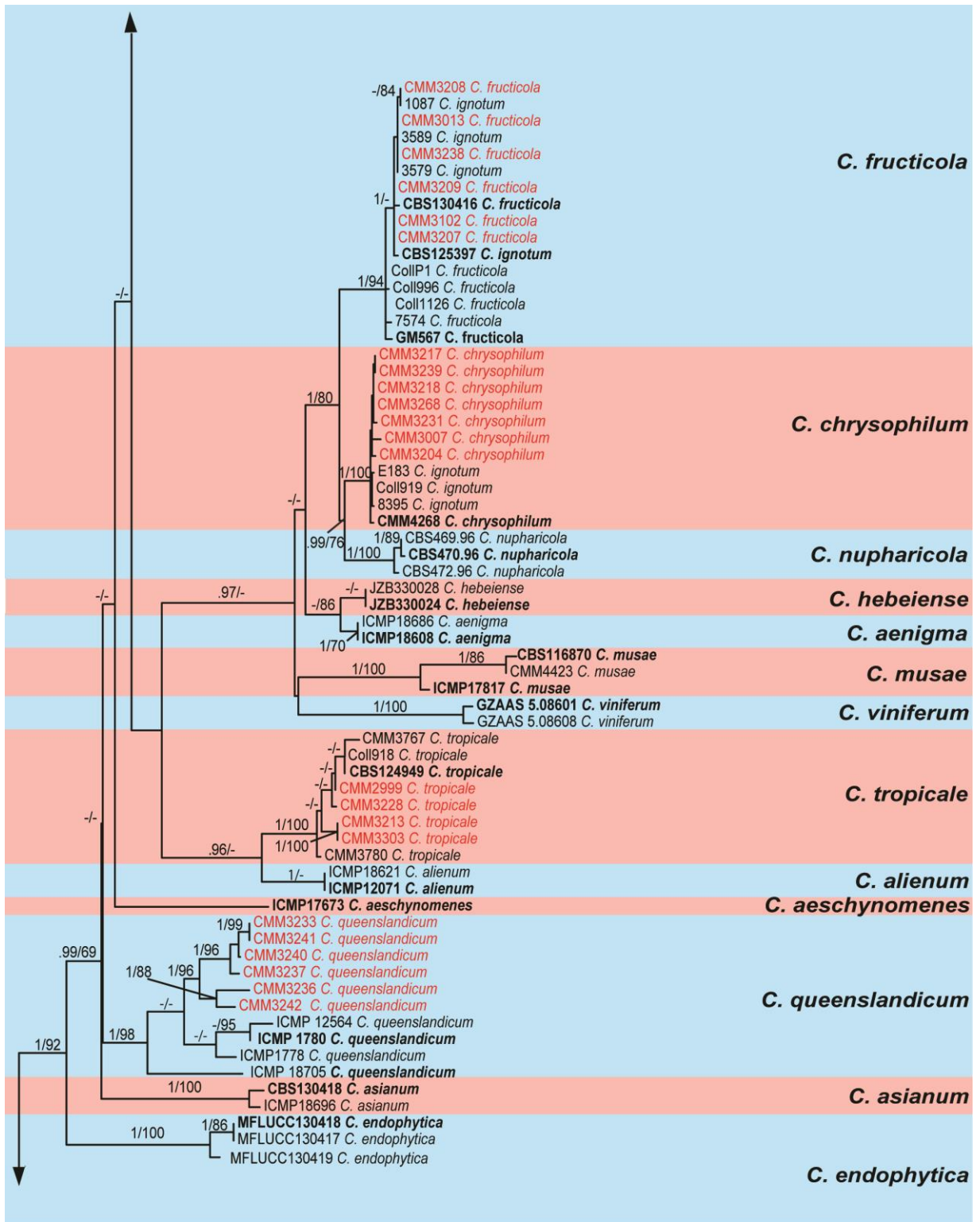
Fig S1 - (continued)

-/84 CMM3208 *C. fructicola*
1087 *C. ignotum*
CMM3013 *C. fructicola*
3589 *C. ignotum*
CMM3238 *C. fructicola*
3579 *C. ignotum*
CMM3209 *C. fructicola*
1/- **CBS130416 C. fructicola**
CMM3102 *C. fructicola*
CMM3207 *C. fructicola*
CBS125397 C. ignotum
1/94 CollP1 *C. fructicola*
Coll996 *C. fructicola*
Coll1126 *C. fructicola*
7574 *C. fructicola*
GM567 C. fructicola

C. fructicola

1/80 CMM3217 *C. chrysophilum*
CMM3239 *C. chrysophilum*
CMM3218 *C. chrysophilum*
CMM3268 *C. chrysophilum*
CMM3231 *C. chrysophilum*
CMM3007 *C. chrysophilum*
CMM3204 *C. chrysophilum*
1/100 E183 *C. ignotum*
Coll919 *C. ignotum*
8395 *C. ignotum*
CMM4268 C. chrysophilum

C. chrysophilum

-/-
.99/76
1/89 CBS469.96 *C. nupharicola*
1/100 **CBS470.96 C. nupharicola**
CBS472.96 *C. nupharicola*

C. nupharicola

.97/- -/- JZB330028 *C. hebeiense*
-/86 **JZB330024 C. hebeiense**

C. hebeiense

ICMP18686 *C. aenigma*
1/70 **ICMP18608 C. aenigma**

C. aenigma

1/100 1/86 **CBS116870 C. musae**
CMM4423 *C. musae*
ICMP17817 C. musae

C. musae

1/100 **GZAAS 5.08601 C. viniferum**
GZAAS 5.08608 *C. viniferum*

C. viniferum

CMM3767 *C. tropicale*
-/- Coll918 *C. tropicale*
-/- **CBS124949 C. tropicale**
-/- CMM2999 *C. tropicale*
-/- CMM3228 *C. tropicale*
1/100 CMM3213 *C. tropicale*
1/100 CMM3303 *C. tropicale*
.96/- CMM3780 *C. tropicale*

C. tropicale

1/- ICMP18621 *C. alienum*
ICMP12071 C. alienum

C. alienum

ICMP17673 C. aeschynomenes

C. aeschynomenes

.99/69
1/99 CMM3233 *C. queenslandicum*
CMM3241 *C. queenslandicum*
1/96 CMM3240 *C. queenslandicum*
1/88 1/96 CMM3237 *C. queenslandicum*
CMM3236 *C. queenslandicum*
-/- CMM3242 *C. queenslandicum*
1/98 -/95 ICMP 12564 *C. queenslandicum*
-/- **ICMP 1780 C. queenslandicum**
ICMP1778 *C. queenslandicum*
ICMP 18705 **C. queenslandicum**

C. queenslandicum

1/92 1/100 **CBS130418 C. asianum**
ICMP18696 *C. asianum*

C. asianum

1/100 1/86 **MFLUCC130418 C. endophytica**
MFLUCC130417 *C. endophytica*
MFLUCC130419 *C. endophytica*

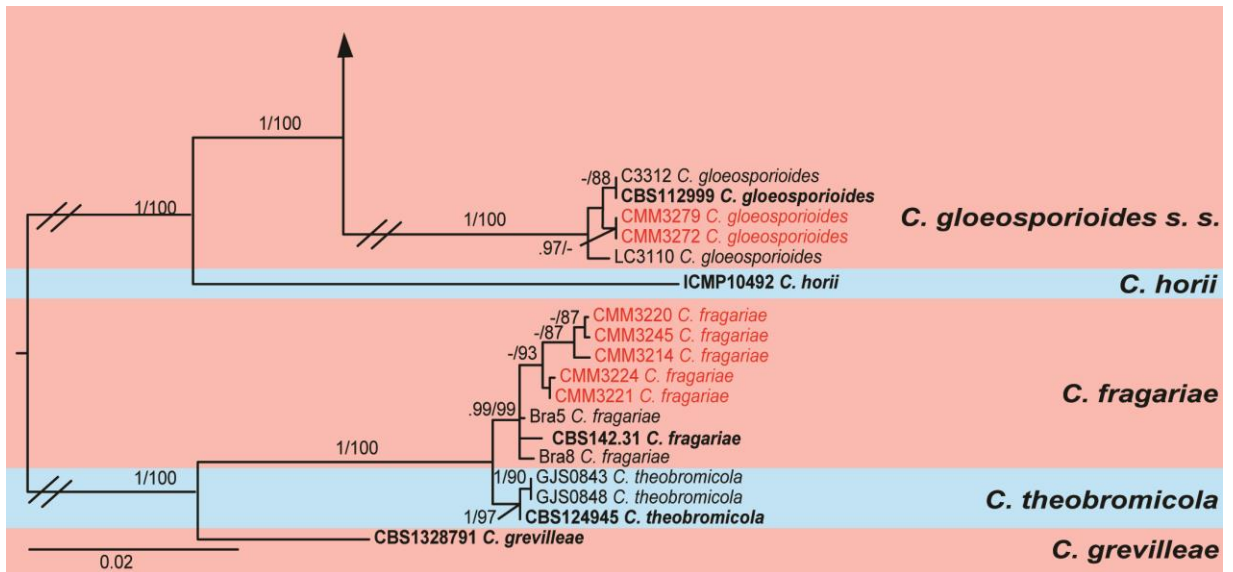***C. endophytica***

Fig S1 (continued)

Fig S1 – Maximum likelihood tree of the *C. gloeosporioides* species complex inferred from a concatenated alignment of APN2, APN2/MAT-IGS,CAL, GAPDH, GAP2-IGS, GS and TUB2. Bootstrap support values (ML ≥70) and Bayesian posterior probability values (PP ≥0.95) are shown at the nodes. "-" indicates no-significant support or absence of the node. Ex-types are emphasized in bold and include the taxonomic name as originally described. Isolates from cashew are highlighted in red. *Colletotrichum fragariae, C. theobromicola* and *C. grevilleae* were used as outgroup. The scale bar indicates the number of expected changes per site.

CAPÍTULO III

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

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

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Resumo

Antracnose é a principal doença fúngica nos pomares brasileiros de caju, infectando tanto órgãos vegetativos quanto reprodutivos de espécies cultivadas e silvestres. Compreender como os fatores físicos e químicos do ambiente influenciam nos parâmetros biológicos de *Colletotrichum* spp., e determinar sua gama hospedeira são essenciais para o controle adequado da antracnose. Objetivou-se com este trabalho estimar as temperaturas ótimas para o crescimento micelial e germinação conidial de sete espécies de *Colletotrichum* obtidas de cajueiros no Brasil (*C. chrysophilum*, *C. fragareae*, *C. fructicola*, *C. gloeosporioides*, *C. queenslandicum*, *C. siamense* and *C. tropicale*). Sua agressividade em folhas de caju e frutos de seis hospedeiros alternativos, bem como sua sensibilidade a fungicidas também foram avaliadas. As temperaturas ótimas para o crescimento micelial e germinação conidial variaram entre 25–30 °C e 27–37 °C, respectivamente. Todas as espécies de *Colletotrichum* induziram sintomas de antracnose em folhas de caju com ferimento, mas nenhuma delas causou lesão em superfície foliar intacta. Variando quanto à agressividade, todas elas foram patogênicas a frutos com ferimento de abacate (exceto *C. fragariae* e *C. fructicola*), banana, goiaba, manga e mamão, enquanto algumas também induziram lesões em tecidos intactos (exceto em abacate). Independente do método de inoculação, nenhum sintoma foi observado em frutos de maracujá. Em maior ou menor grau, o crescimento micelial, esporulação, germinação conidial e formação de apressório das sete espécies de *Colletotrichum* foram inibidos pelo azoxystrobina, difenoconazole e tiofanato-metíllico.

Palavras-chave: *Anacardium* spp., condições ambientais, contaminação cruzada, controle químico.

Abstract

Anthracnose is the main fungal disease on cashew orchards in Brazil, occurring on both vegetative and reproductive organs of cultivated and non-cultivated host plants. Understanding the influence of physical and chemical exogenous factors on the biological traits of *Colletotrichum* spp., and determining their host range is essential to developing appropriate anthracnose control measures. The present study aimed to estimate the optimum temperatures for mycelial growth and conidial germination of nine *Colletotrichum* species (*C. chrysophilum*, *C. fragareae*, *C. fructicola*, *C. gloeosporioides*, *C. queenslandicum C. siamense* and *C. tropicale*) associated with cashew anthracnose in Brazil. Their aggressiveness on cashew leaves and six alternative host fruits, in addition to their fungicide sensitivity were also investigated. The optimum temperatures for mycelial growth and conidial germination ranged between 25–30 °C and 27–37 °C, respectively. All *Colletotrichum* species induced anthracnose symptoms on wounded cashew leaves, but none of them produced lesions on intact leaf-surface. Although varying in the aggressiveness, all *Colletotrichum* species, but *C. fragariae* and *C. fructicola*, were pathogenic to wounded fruits of avocado, banana, guava, mango and papaya, while some induced lesions on non-wounded host-tissues (except on avocado). No symptoms were observed on passion fruits, regardless of the inoculation method. In a greater or lesser extension, the mycelial growth, sporulation, conidial germination and/or of the nine *Colletotrichum* species were inhibited by azoxystrobin, difenoconazole and thiophanate-methyl.

Keywords: *Anacardium* spp., environmental conditions, cross-infection, chemical control.

Introduction

Native to South America, cashew (*Anacardium occidentale* L.) is widely distributed throughout the Brazilian territory, growing as either a crop or spontaneous plants in natural habitats along with non-cultivated *Anacardium* species, such as *A. corymbosum* Barb. Rodr., *A. humile* St. Hilaire, *A. nanum* St. Hilaire, and *A. othonianum* Rizzini (Barros et al. 2002; Agostini-Costa et al. 2006). The fully ripened cashew fruit consists of a soft yellow and/or reddish edible hypocarp (5 to 11 cm in length) known as 'cashew apple', and a kidney-shaped drupe containing a single nut (Dendena and Corsi 2014), the main cashew product. In 2014, approximately 3.7 million tons of raw nuts was produced worldwide, most in Africa and Asia (ca. 60% and 36%, respectively), while ca. 92% over 1.9 million tons of cashew apples was produced in Brazil (FAO 2017), mainly by smallholder farmers located in the northeastern region (Conab 2017).

Apart from adverse environmental conditions and inappropriate management, fungi diseases are among the most important constraints to cashew production. These include anthracnose (Freire et al. 2002; Lopez and Lucas 2010; Uaciquete et al. 2013), a very common pre- and/or postharvest disease on tropical fruits caused by a range of *Colletotrichum* species (Phoulivong et al. 2010; Udayanga et al. 2013; Lima et al. 2013). Cashew anthracnose occur on both vegetative and reproductive organs, with symptoms on leaves manifested as water-soaked lesions that become orange-reddish prior defoliation; necrotic lesions on branches; abortion and drought of inflorescences; and dark depressed lesions on fruits, which may fall prematurely (Freire et al. 2002; Menezes 2005). Anthracnose severity is highly influenced by physical and chemical exogenous factors, in addition to the genetic makeup of both hosts and *Colletotrichum* species involved (Miles et al. 2013; Zhang et al. 2014).

Events leading to host-tissues infection by *Colletotrichum* species include host surface recognition followed by conidium germination, appressoria formation, hypha penetration, host cells colonization and finally, sporulation (Kenny et al. 2012; Agosteo et al. 2015). Each step is affected in a greater or lesser extension by temperatures, a major factor on mycelial growth, conidial germination, and spore production (Fernando et al. 2000; Baroncelli et al. 2015). Although previous studies coupled temperatures between 22–28 °C (Freire et al. 2002) during the rainy season (Cardoso et al. 2000; Uaciquete et al. 2013) with the highest severity of anthracnose on cashew orchards, none have investigated the influence of different temperatures on the biological traits of *Colletotrichum* spp. obtained from this crop. In addition, these researches had assumed *C. gloeosporioides* as the only etiological agent of

cashew anthracnose, whereas a phylogenetic analysis recently performed with isolates collected from symptomatic-tissues of cultivated and non-cultivated *Anacardium* plants in Brazil revealed its association with seven *Colletotrichum* species that lack epidemiological information, namely *C. chrysophilum*, *C. fragareae*, *C. fructicola*, *C. gloeosporioides*, *C. queenslandicum C. siamense* and *C. tropicale* (Veloso et al. rewiew).

Other phylogenetic studies have also shown that *C. gloeosporioides* is not so common on tropical fruits (Phoulivong et al. 2010; Udayanga et al. 2013; Lima et al. 2013), that one plant may often host a range of *Colletotrichum* species, and that a single *Colletotrichum* species may infect different hosts (e.g., Freeman et al. 1998; Hyde et al. 2009; Cannon et al. 2012; Phoulivong et al. 2012; Bragança et al. 2016). This information is particularly relevant to Brazilian smallholder farmers, who usually cultivate multiple fruit species in the same orchard, including cashew (*A. occidentale*), mango (*Mangifera indica* L.), avocado (*Persea americana* Mill.), banana (*Musa* spp.), citrus (*Citrus sinensis* L.), sugar-apples (*Anona squamosa* L.), and acerola (*Malpighia emarginata* Sessé & Moc ex DC.). Although enhancing the biodiversity within the orchards, this approach may favor cross-infections among *Colletotrichum* spp. on different plant groups (Zhang et al. 2014), especially if appropriate and effective agronomic practices are not applied.

Traditional cashew orchards consisting of heterozygous tall trees have been replaced with dwarf cashew cultivars that facilitate pruning, manual harvesting and spraying (Freire et al. 2002). For this moment, cooper oxychloride is the only fungicide registered against *C. gloeosporioides* on cashew trees in Brazil, compared to 40 and 13 commercial formulations registered against the same pathogen on mango and avocado, respectively (AGROFIT 2017), two other important crops that belong to the Anacardiaceae family. Given that azoxystrobin, difenoconazole and thiophanate-methyl significantly reduced the mycelial growth of five *Colletotrichum* species associated with mango anthracnose in northeastern Brazil (Lima et al. 2015), they might be a starting point in the searching for other active ingredients (a.i.) potentially useful to manage anthracnose on cashew orchards.

The present study evaluated the influence of temperature and three fungicides on the biological traits of nine *Colletotrichum* species associated with anthracnose on cultivated and non-cultivated cashew plants throughout four Brazilian biomes (i.e., Amazon Rainforest, Atlantic Forest, Caatinga and Cerrado). In addition, the aggressiveness of each *Colletotrichum* species was determined on attached leaves of *A. occidentale*, and detached fruits of six alternative hosts, both wounded and non-wounded.

Materials and Methods

Colletotrichum isolates

A series of experiments was performed using twenty-one representative isolates of seven *Colletotrichum* species (*C. chrysophilum*, *C. fragareae*, *C. fructicola*, *C. gloeosporioides*, *C. queenslandicum*, *C. siamense* and *C. tropicale*) associated with anthracnose on cultivated and non-cultivated cashew plants in Brazil (Table 1). They were obtained from symptomatic organs (i.e., leaves, stems and inflorescences), and identified through a phylogenetic analysis using sequences of the intergenic spacer between the 3' end of the DNA-lyase and the mating type locus MAT1-2 (APN2/MAT-IGS), DNA-lyase (APN2), calmodulin (CAL), glutamine synthetase (GS), β-tubulin (TUB2), glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), and glyceraldehyde-3-phosphate dehydrogenase-IGS (GAP2-IGS), as described by Veloso et al. (in review). The isolates were deposited in the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) at the Universidade Federal Rural de Pernambuco (Recife, Pernambuco, Brazil). The stock cultures were maintained on potato dextrose agar (PDA) media slants (Acumedia, Lansing, MI, USA) at 5 °C in the dark.

Biological characterization at different temperatures

These experiments evaluated the influence of temperature on mycelial growth conidial germination of the seven *Colletotrichum* species. A mycelial plug (5 mm in diameter) of each isolate was taken from the expanding margin of 7-day-old colonies grown on PDA, and transferred to the center of plastic Petri dishes (90 mm in diameter) containing 20 mL of PDA. They were incubated at temperatures ranging from 10 °C to 40 °C in 5 °C intervals in the dark for five days, when the colony diameter of each isolate was measured in two perpendicular directions to calculate their growth rates (mm/day).

A separate experiment was carried out to assess the effects of temperature on conidial germination. A volume of 30 μL spore suspension (10^6 spores·mL-1) of each isolate in sterile distilled water was placed into plastic Petri dishes (90 mm in diameter) randomly distributed in plastic trays lined with moistened paper towels. To prevent suspensions evaporation and guarantee a high relative humidity, the trays were placed into plastic bags. After incubation at temperatures ranging from 10°C to 40°C in 5°C intervals in the dark for 24 h, a 25 μL aliquot of lactoglycerol was added to each spore suspension to interrupt the germination process. At

this moment, 100 random spores were examined under an optical microscope to estimate the conidial germination percentage, and the optimum temperature for conidial germination. A conidium was considered germinated if having a primary germ tube equal in length to at least half of its width.

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

Aggressiveness of *Colletotrichum* species on cashew leaves

The aggressiveness of *Colletotrichum* spp. was evaluated on both wounded and non-wounded young cashew leaves using 2-month old *A. occidentale* seedlings (cv. CCP-76) maintained in a greenhouse. Given the age differences among the original leaves, cashew seedlings were completely defoliated, with the emergence of new ones stimulated through nitrogen fertilization. Each plant received one application of 10 g of a commercial fertilizer mixture 4-14-8 (N:P:K), followed by 20 mL of a nitrogen dilution (20 g of urea·L^{-1} water) applied every week around the plant base.

Two equidistant wounds (5 mm in diameter) were scratched on the adaxial epidermis of asymptomatic young leaves using a sterilized pin. A volume of 20 μL spore suspension (10^6 spores·mL^{-1}) of each isolate grown on PDA at 25 ± 2 °C was inoculated on the wounded and their respective non-wounded sites, thus resulting in four inoculation sites per leaf. Sterile distilled water was applied as the control. The seedlings soil was soaked with tap water and the whole plant was covered with a black plastic bag to maintain a high relative humidity. After 48 h, the plastic bags were removed and the plants remained in the greenhouse for 13 other days. At this moment (i.e., 15 days since the inoculation), the aggressiveness was evaluated by measuring the diameter of the lesions (mm) in two perpendicular directions and calculating the mean lesion diameter. This experiment was established using a completely randomized design with factorial arrangement of 22 treatments (21 *Colletotrichum* isolates and the control) with six replicates per treatment, each replicate represented by a cashew leaf bearing two wounded and two non-wounded inoculation sites.

Aggressiveness of *Colletotrichum* species on different hosts

The aggressiveness of *Colletotrichum* spp. was evaluated on six hosts, including papaya (cv. Golden), mango (cv. Tommy Atkins), banana (cv. Pacovan) and guava (cv. Paluma), at stages 3, 4, 5 and 5 of maturation, respectively, and passion fruit (cv. BRS Gigante Amarelo) and avocado (cv. Breda) at the time of harvesting. Asymptomatic fruits were surface disinfested by washing them with neutral detergent and tap water, dipping in a 1.5% sodium hypochlorite solution for 2 min, and double rinsing with sterile distilled water. After air-drying for one hour on paper towels, the epidermis of each fruit was perforated to a depth of 3 mm by five sterile pin, equidistantly attached to a cork (ca. 5 mm in diameter). Prior inoculation, the fruits were placed into glass Petri dishes (90 mm in diameter) lined with folded paper towel.

A volume of 20 µL spore suspension (10^6 spores·mL^{-1}) of each isolate grown on PDA at 25 ± 2 °C was inoculated on the wounded and its respective non-wounded sites, thus resulting in two inoculation sites per fruit. Sterile distilled water was applied as the control. Dishes were randomly distributed into plastic trays lined with moistened paper towels, covered with plastic bags, and incubated in the dark in a climate-controlled room set to 25 ± 2 ºC. After 48 h, the plastic bags were removed, and the fruits were maintained into the trays at the same physical conditions until the seventh day since the inoculation. At this moment, the aggressiveness of each *Colletotrichum* species was evaluated by measuring the diameter of the lesions (mm) in two perpendicular directions and calculating the mean lesion diameter. This experiment followed a completely randomized design with factorial arrangement of 22 treatments (21 *Colletotrichum* isolates and the control) comprising three replicates, each one represented by a fruit inoculated on both wounded and non-wounded sites.

Fungicide sensitivity assessments

This experiment aimed to investigate the sensitivity of the seven *Colletotrichum* species to three fungicides: azoxystrobin (Amistar 500 WG, 50.0% i.a., Syngenta, São Paulo, SP, Brazil), difenoconazole (Score EC, 25.0% i.a., Syngenta, São Paulo, SP, Brazil), and thiophanate-methyl (Cercobin 700 WP, 70.0% a.i., Iharabras, São Paulo, SP, Brazil).

Previous tests were performed to determine the required concentration (RC) of each fungicide dissolved in molten (~ 45 °C) PDA media (azoxystrobin = 1.0 µg i.a. ml^{-1}; difenoconazole = 0.5 µg de i.a. ml^{-1}; and thiophanate-methyl = 5.0 µg i.a. ml^{-1}). A mycelial plug (5 mm in diameter) of each isolate was taken from the expanding margin of 7-day-old colonies grown on PDA and placed in the center of plastic Petri dishes (150 mm in diameter) containing 20 mL of PDA supplemented with the fungicides. Mycelial plugs were plated onto non-

contaminated PDA as the controls. They were incubated in the dark at 25 2 °C for seven days, when the mycelial growth was determined in the same manner as described for the temperature assays. To determine the number of spores produced in contact with the fungicides, each Petri dish was flooded with 10 mL of sterile distilled water and the colony surface was mechanically disturbed with a scalpel to suspend conidia. The resulting suspensions were filtered through muslin cloth prior addition of 500-µl lactoglycerol to prevent conidia germination, and the spore concentrations (n^o spores·mL^{-1}) were determined using a Neubauer chamber under an optical microscope.

The effect of fungicides on conidial germination and appressoria formation were evaluated in a different assay. A 15-µL aliquot of spore suspension (10^6 spores·mL^{-1}) was added to the same volume of each fungicide solution (2x RC), thus yielding 30-µL spore suspensions (10^3 spores·mL^{-1}) supplemented with azoxystrobin, difenoconazole or thiophanate-methyl at 1.0 µg i.a. ml^{-1}, 0.5 µg de i.a. ml^{-1} and 5.0 µg i.a. ml^{-1}, respectively. Each isolate in sterile distilled water was placed into plastic Petri dishes (90 mm in diameter) randomly distributed in plastic trays lined with moistened paper towels. To prevent suspensions evaporation and guarantee a high relative humidity, the trays were placed into plastic bags. After incubation at temperature ranging from 25°C intervals in the dark for 24 h, a 25-µL aliquot of lactoglycerol was added to each spore suspension to interrupt the germination process. The germinated conidia and numbers of appressoria counted among 100 random spores were examined under an optical microscope to estimate the conidial germination and appresoria formation percentages. A conidium was tallied as germinated if presenting appressorium, or a primary germ tube equal in length to at least half of its width.

The inhibition percentage of mycelial growth (MG), sporulation (S), conidial germination (CG), and appressoria formation (AF) were calculated as follow: IMG = $[(C_m - F_m)/C_m]$ x 100, where IMG = % inhibition of MG, C_m = MG in control (without fungicide), and F_m = MG on PDA supplemented with the fungicides; IS = $[(C_s - F_s)/C_c]$ x 100, where IS = % inhibition of S, C_s = S in control (without fungicide), and F_c = S on PDA supplemented with the fungicides; ICG = $[(C_c - F_c)/C_c]$ x 100, where ICG = % inhibition of CG, C_c = CG in control (spore suspensions without fungicide), and F_c = CG on spore suspensions prepared with fungicide solutions; and IAF = $[(C_a - F_a)/C_a]$ x 100, where IAF = % inhibition of AF, C_a = AF in control (spore suspensions without fungicide), and F_a = AF on spore suspensions amended with fungicides.

All experiments were established in a completely randomized design with factorial arrangement comprising three replicates (Petri dishes) per combination of *Colletotrichum* isolate/fungicide.

Data analysis

In the experiments of temperatures, the mycelial growth rates were plotted against temperature and a curve was fitted by a cubic polynomial regression ($y=a+bx+cx^2+dx^3$), and conidial germination percentages were plotted against temperature and a curve was fitted by the Lorentzian model with three parameters ($y =a/(1+((x–b)/c)^2)$). The optimal temperatures, defined as the temperatures producing the highest mycelial growth rates and conidial germination percentages, were estimated using the regression models and the numerical summaries with the aid of the TableCurve™ 2D 5.01 software (Systat Software Inc., Chicago, IL, USA). The data obtained from each experiment were subjected to analyses of variance (ANOVA) and in all experiments the *Colletotrichum* isolates of the same species did not differ significantly (P>0.05) in relation to the evaluated variables. Therefore, values of each *Colletotrichum* species in experiments of optimal temperature, aggressiveness on cashew leaves and host range, and fungicide sensitivity were subjected to ANOVA, and the means were compared using the Fisher's least significant difference (LSD) test (P=0.05). The ANOVA and comparisons of means were performed with Statistix 9.0 software (Analytical Software, Tallahassee, FL, USA).

Results

Biological characterization at different temperatures

The *Colletotrichum* species significantly differed from each other with respect to the optimum growth temperature (P≤0.05). Although they grew within a temperature range between 10–35 °C, none of them did so at 40 °C. *Colletotrichum queenslandicum* exhibited the lowest optimum growth temperature (25.4 °C), and the highest one was estimated for *C. tropicale* (29.7 °C), while *C. chrysophilum*, *C. gloeosporioides*, *C. siamense*, *C. fructicola* and *C. fragariae* showed intermediate values ranging from 26.5 to 27.8 °C (Table 2). The maximum mycelial growth rate varied from 10.1 mm (*C. queensladicum*) to 14.7 mm (*C. gloeosporioides*) (Table 2), and significantly differed from each other. The first species did

not differ from *C. fructicola* (11.5 mm), while *C. gloeoporioides* was similar to *C. tropicale* (13.0 mm), *C. siamense* and *C. fragariae* (13.5 mm both) (Table 2).

Showing significant differences among treatments (P≤0.05), the optimum temperature and maximum conidial germination of *Colletotrichum* species ranged from 27.5-36.6 °C and 90.3-100%, respectively (Table 3). The highest temperatures were estimated for *C. chrysophilum* (32.6 °C) and *C. fructicola* (32.4 °C), which exhibited conidial germination of 97.2 and 100%, respectively. The lowest values were estimated for *C. gloeosporioides* (27.5 °C, conidial germination = 90.3%), while *C. queenslandicum*, *C. siamense*, *C. tropicale* and *C. fragariae* were intermediate, ranging from 28.8-32.1 °C with maximum conidial germination between 93.6-100%

Aggressiveness of *Colletotrichum* isolates on cashew leaves

None of the *Colletotrichum* species induced anthracnose symptoms on non-wounded tissues of *A. occidentale* leaves, but they showed significant differences in aggressiveness on wounded leaf-tissues (P≤0.05). *Colletotrichum siamense* caused the largest lesions (16.1 mm), which were significantly different from those inflicted by the other species, except *C. tropicale* (14.4 mm) and *C. gloeosporioides* (13.2 mm). Lesions caused by the latter two species did not differ significantly from each other (Fig. 1), and were also similar to *C. chrysophilum* (13.2 mm) and *C. fragariae* (11.5 mm), but significantly greater than those induced by C. *queenslandicum* (10.1 mm) and *C. fructicola* (8.3 mm), which were similar (Fig. 1).

Aggressiveness of *Colletotrichum* species on different hosts

The aggressiveness of *Colletotrichum* spp. on avocado, banana, guava, mango and papaya fruits varied significantly depending on both the alternative host and the inoculation method (i.e., on wounded or non-wounded tissues) (Table 4). All wounded fruits exhibited typical anthracnose symptoms, while no lesions were observed on intact avocado-tissues. Some *Colletotrichum* species were pathogenic to non-wounded tissues of the other alternative hosts, but no symptoms were observed on passion fruits, regardless of the inoculation method.

The lesion extensions induced by different *Colletotrichum* species on wounded avocado sites varied significantly (P≤0.05) (Table 4). While *C. fragariae* and *C. fructicola* produced no

symptoms, the largest lesions were caused by *C. queenslandicum* (ca. 18 mm) and *C. siamense* (16.3 mm), and the smallest ones were inflicted by *C. gloeosporioides* (5.9 mm).

The anthracnose symptoms on banana wounded and non-wounded sites differed significantly (P≤0.05) among the *Colletotrichum* species (Table 3). The largest and smallest lesions on wounded tissues were inflicted by *C. queenslandicum* (ca. 20.0 mm) and *C. chrysophilum* (7.2 mm), respectively. Only *C. tropicale*, *C. queenslandicum*, *C. gloeosporioides* and *C. siamense* induced symptoms on banana intact surface, with the former species significantly more aggressive (7.5 mm) than the latter two (ca. 3.0 mm), and *C. queenslandicum* intermediate.

Regardless of the inoculation method, all *Colletotrichum* species were pathogenic to guava, and significantly different (P≤0.05) with respect to the aggressiveness on wounded and non-wounded tissues (Table 3). The largest lesions on wounded sites were caused by *C. siamense* (19.1 mm), with *C. fragariae, C. fructicola* and *C. tropicale* revealed as the least aggressive (11.7, 11.0 and 12.9 mm respectively). In contrast, the largest lesions on non-wounded tissues were caused by *C. siamense* (12.3 mm) and *C. fragariae* (10.1 mm), while the smallest ones were inflicted by *C. tropicale* (2.1 mm).

On mango fruits, the aggressiveness of *Colletotrichum* spp. significantly (P≤0.05) differed from each other on wounded and non-wounded tissue (Table 3). In the first case, the largest lesions were induced by *C. fructicola* (26.0 mm) and the smallest ones by *C. gloeosporioides* (13.1 mm). However, *C. queenslandicum* was the most aggressive species on non-wounded mango-surface (8.0 mm), while *C. chrysophilum* caused the smallest lesions (2.1 mm).

Differing significantly (P≤0.05) in aggressiveness, all *Colletotrichum* species induced anthracnose symptoms on papaya wounded tissues, and four of them were pathogenic on non-wounded papaya-surface (Table 3). *Colletotrichum siamense* caused the largest lesions on wounded sites (18.8 mm), whereas *C. gloeosporioides* was the least aggressive (11.8 mm). On intact tissues, *C. siamense* induced the largest lesions (17.0 mm) and *C. chrysophilum* the smallest ones (2.0 mm), while *C. fragariae*, *C. fructicola* and *C. queenslandicum* induced no symptoms when inoculated on papaya non-wounded sites.

Fungicide sensitivity assessments

The mycelial growth of four *Colletotrichum* species inoculated on PDA supplemented with azoxystrobin was significantly inhibited. *Colletotrichum queenslandicum* was the most sensitive species with a mycelial growth inhibition of 19.9%, while *C. fragariae*, *C. fructicola* and *C. tropicale* were not affected (Fig. 2A). The sporulation of *C. fragariae* was not

inhibited, and the highest reduction level of spore production mediated by azoxystrobin was demonstrated by *C. tropicale* (65.7%) (Fig. 2B). This fungicide mediated significant reduction on conidial germination of all *Colletotrichum* species, with the highest values observed in *C. queenslandicum* (80.0%) and the lowest in *C. tropicale* (61.5%) (Fig. 2C). Azoxystrobin inhibited 100% appressoria formation of all *Colletotrichum* species, but *C. chrysophilum* (77.4%) (Fig. 2D).

All *Colletotrichum* species showed significant reduction on mycelial growth when inoculated on PDA amended with difenoconazole. The greatest mycelial growth inhibition was observed in *C. queenslandicum* (59.2%), while *C. tropicale* was the least sensitive (5.7%) (Fig. 3A). Regarding the inhibition of sporulation mediated by difenoconazole, *C. tropicale, C. siamense* and *C. fragariae* showed to be the most sensitive species (87.9, 86.76 and 83.33% respectively), and the lowest inhibition level was displayed by *C. chrysophilum* (24.9%) (Fig. 3B). Difenoconazole also inhibited the conidial germination of *Colletotrichum* spp., with *C. fragariae, C. chrysophilum* and *C. gloeosporioides* exhibiting the highest inhibition level (74.5, 70.1 and 66.2% respectively) and *C. siamense* the lowest (16.9%) (Fig. 3C). The appresoria formation of *C. fragariae*, *C. siamense*, *C. gloeosporioides* and *C. tropicale* was totally inhibited (100%), and the lowest inhibition percentage mediated by difenoconazole was demonstrated by *C. fructicola* (52.7%) (Fig. 3D).

Thiophanate-methyl significantly reduced the mycelial growth, sporulation and conidial germination of *Colletotrichum* spp. Regardless of the biological trait, *C. gloeosporioides* was the most sensitive species to this fungicide (100%), while *C. fructicola* exhibited the lowest inhibition of mycelial growth (78.6%) (Fig. 4A). *C. queenslandicum* the least sensitive with respect to sporulation (46.9%) (Fig. 4B) and *C. fructicola* the least affected on conidial germination (15.5%) (Fig. 4C). Thiophanate-methyl also inhibited appessoria formation of *Colletotrichum* spp., with *C. chrysophilum* the lowest percent (30%) (Fig. 4D).

Discussion

This is the first epidemiological study involving *Colletotrichum* species associated with anthracnose on cultivated and non-cultivated cashew plants in Brazil. The optimum temperatures for mycelial growth and maximum mycelial growth rate of *C. chrysophilum*, *C. fragareae*, *C. fructicola*, *C. gloeosporioides*, *C. queenslandicum*, *C. siamense* and *C. tropicale* ranged between 25.4–29.7 °C, whereas for maximum conidial germination varied from 27.5 to 36.6 °C. Although estimated *in vitro*, these temperatures match those typically found on

tropical zones, and neared those pointed out as the most favorable (22–28 °C) for cashew anthracnose development in northeastern Brazil (Freire et al. 2002). Likewise, our findings are consistent with the optimum temperatures for mycelial growth of *C. gloeosporioides* associated with anthracnose on coffee berries (25–31 °C) in Papua New Guinea (Kenny et al. 2012), and *Trichosanthes kirilowii* Maxim. (25–30 °C) in central China (Zhang et al. 2014). Since environmental conditions play major role on key steps of anthracnose epidemiology, i.e., mycelial growth and conidial germination of *Colletotrichum* spp. (Wang et al. 2015), our results may contribute to develop forecasting models based on prevailing weather of cashew cropping zones.

None of the 21 *Colletotrichum* isolates collected from cashew plants grew at 40 °C, which is consistent with previous reports of *Colletotrichum* spp. obtained from safflowers (*Carthamus tinctorius* L.) in central Italy (Baroncelli et al. 2015). However, *Colletotrichum* species from cashew formed regular colonies within three days after being transferred from 40 to 25 °C (*unpublished data*), but those from safflowers did not. Our isolates were collected from perennial, drought-resistant plants evolved in environments with high light intensity and optimal temperature between 24–30 °C (Agostini-Costa et al. 2006; Dendena and Corsi 2014; Royo et al. 2015), while theirs came from plants cultivated under average temperature of 17.3 °C (Baroncelli et al. 2015). These results suggest that temperature tolerance of *Colletotrichum* isolates is likely indicative of local adaptation rather than a general species characteristic. Other publications have shown behavioral differences within and among populations of *Colletotrichum* spp. in response to the environmental conditions (Estrada et al. 2000; Leandro et al. 2003; Moral et al. 2012; Miles et al. 2013; Wang et al. 2015), thus highlighting their phenotypic variation.

In the present study, all *Colletotrichum* species induced anthracnose symptoms on wounded sites of *A. occidentale* leaves (cv. CCP-76), which makes this cultivar a good host to compare the aggressiveness of *Colletotrichum* spp. A field survey performed in Mozambique revealed that cashew leaf anthracnose was caused exclusively by *C. gloeosporioides* (Uaciquete et al. 2013), but this species represented only 1.4% overall 280 isolates collected from cashew plants in Brazil (Veloso et al. in review). This species was revealed as aggressive as *C. chrysophilum*, *C. fragariae,* and *C. tropicale* on wounded cashew leaves, while *C. siamense* and *C. fructicola* induced the largest and the smallest lesions, respectively. In agreement, *C. endomangiferae,* recently synonymized as *C. siamense* (Liu et al. 2016) was significantly more aggressive than *C. fructicola* on wounded mango fruits, with *C. tropicale* causing intermediate lesions (Vieira et al. 2014). These authors first isolated the former species as

endophytes from leaves, stems and inflorescences of mango trees in northeastern Brazil, thus leading us to speculate that infected cashew leaves may serve as inoculum sources for other cashew organs.

None of our isolates inflicted lesions on intact cashew leaf surface. This result conforms to previous reports of *C. acutatum*, *C. boninense* and *C. capsici* associated with coffee berry anthracnose in Vietnam, which were revealed as pathogenic on wounded green berries and unable to induce symptoms on non-wounded coffee fruit (Nguyen et al. 2010). The same authors suggested that the host cuticular wax layer would inhibit the pathogen infection processes, an event that tend to be simpler through wounded tissues. In addition to the wax layer, Agosteo et al. (2015) claimed that the early events in host-pathogen interactions are influenced by competition with indigenous microorganisms, and the quality and quantity of solutes on plant surfaces, including nutrients (e.g. sugars, amino acids) and toxic molecules (e.g. phenols). Leaf histochemical analysis of Anacardiaceous plants indicated that the secretions inside glandular cells and outside the trichomes is a mixture containing mucilage, fatty acids, and phenolic compounds, which may serve for protection against desiccation and phytopathogens (Lacchia et al. 2016). Since this experiment was performed using attached *A. occidentale* leaves without previous surface disinfestation, their waxy epidermis, along with eventual indigenous competitors and phytochemical defenses may have inhibited the infection processes of the *Colletotrichum* species inoculated on non-wounded leaf sites. This assumption was supported by a screening test carried out with detached, surface-disinfested (i.e., the waxy layer and competitors were at least diminished) leaves of the same cashew cultivar, which revealed that all *Colletotrichum* species induced varying degrees of anthracnose symptoms on both wounded and non-wounded leaf-tissues (*unpublished data*).

Both host surface properties and wounding may have driven the aggressiveness of the *Colletotrichum* species inoculated on ripe fruits of avocado, banana, guava, mango and papaya. The largest lesions on wounded sites of avocado and banana were caused by *C. queenslandicum*, whereas *C. siamense* was the most aggressive species on wounded tissues of guava and papaya, with other *Colletotrichum* species inducing intermediate lesions on the other alternative hosts. This is consistent with previous work showing that different *Colletotrichum* species obtained from mango fruits in northeastern Brazil were also pathogenic to banana, guava, mango and papaya, although varying in the aggressiveness on wounded tissues (Lima et al. 2015). Regardless of the *Colletotrichum* species inoculated in the present study, the lesions on wounded host-tissues were greater than those developed on intact host-surface, which corroborates Phoulivong et al. (2012) who claimed that wounding

allows the pathogenic isolate right access to the internal fruit, thus enhancing infection. Given their relevance as etiological agents of anthracnose, several studies have tried to assess the host range of *Colletotrichum* species around the world, some of them suggesting potential cross-infections among different *Colletotrichum* species on a variety of host plants (Freeman et al. 1998; Than et al. 2008; Hyde et al. 2009; Cannon et al. 2012; Phoulivong et al. 2012; Zang et al. 2014). Since the ripe passionfruit exhibited no symptoms regardless of the inoculation method, one may argue that the *Colletotrichum* species obtained from cashew plants are not pathogenic to this host. However, a broader investigation comprising other cultivars, various maturation stages and different environmental conditions are essential to better answer this question. Our results demonstrated that some fruits usually exploited by smallholder farmers in Brazil could be infected by *Colletotrichum* species associated with cashew anthracnose, thus leading us to speculate that *Colletotrichum* cross-infections may occur into miscellaneous orchards, which may require adjustments of cultural practices.

One goal of this study was to determine if *Colletotrichum* species associated with cashew anthracnose in Brazil were sensitive to fungicides commonly used to manage this disease on other crops. Most *in vitro* fungicide sensitivity assessments involving *Colletotrichum* spp. focus only on mycelial growth inhibition (Chapin et al. 2006; Chung et al. 2006; Rampersad et al. 2012; Gang et al. 2015; Torres-Calzada et al. 2015), but we brought evidences that other biological traits can be differently affected depending upon the active ingredient. Overall, azoxystrobin mediated the greatest inhibition of conidial germination and appressoria formation of the seven *Colletotrichum* species evaluated in the present study. In contrast, their mycelial growth was moderately reduced by difenoconazole, but this fungicide mediated significant inhibition of sporulation (~ 25–96%) and conidial germination (~ 17–74%). On the other hand, the mycelial growth and sporulation of eight *Colletotrichum* species were highly inhibited ($> 80\%$) by thiophanate-methyl, while *C. gloeosporioides* and *C. fragariae* also exhibited significant reduction of conidial germination when exposed to this fungicide. Our results conform to other studies showing lower impact of azoxystrobin on mycelial growth of *Colletotrichum* spp. compared to that mediated by difenoconazole and/or thiophanate-methyl (Hu et al. 2015; Lima et al. 2015). Assuming that conidial germination is one of the earliest pre-infection processes of *Colletotrichum*, and that mycelial growth and sporulation represent post-infection events, we suggest that azoxystrobin and thiophanate-methyl would be good protective and curative chemicals to manage anthracnose on cashew orchards, respectively, while difenoconazole would serve for both purposes.

Without data from *in vivo* assays under field conditions, the efficacy of these fungicides at controlling cashew anthracnose in the present study is only tentative. Azoxystrobin would effectively protect cashew plants of the *Colletotrichum* species, perhaps preventing new infections, but failing at healing tissues/organs already infected. Cashew orchards would be protected against new infections of *C. chrysophilum*, *C. fragariae*, *C. gloeosporioides*, *C. dianesei* and *C. tropicale* using difenoconazole, and this fungicide would also effectively control sporulation of *C. communis*, *C. endomangiferae*, *C. fragariae* and *C. tropicale*, but would be less effective at controlling lesions expansion caused by *C. chrysophilum*, *C. dianesei*, *C. endomangiferae* and *C. tropicale*. If the main goals were to inhibit the mycelial growth and sporulation, thiophanate-methyl would be the best choice against the *Colletotrichum* species (but *C. queenslandicum* sporulation), and would effectively control spore germination of *C. gloeosporioides*. In our point of view, these shifting responses may indicate a genotypic variation within and among *Colletotrichum* species obtained from cashew plants, a crop for which azoxystrobin, difenoconazole and thiophanate-methyl still lack registration in Brazil (AGROFIT 2017).

In summary, we demonstrated that *Colletotrichum* species collected from, and proved pathogenic to cashew plants in Brazil displayed optimum mycelial growth and conidial germination in a range of temperatures characteristic from tropical zones, which may explain the broad occurrence of cashew anthracnose in different Brazilian biomes. The *Colletotrichum* species were pathogenic to some alternative hosts usually exploited along with or nearby cashew orchards, thus raising concerns related to potential cross-infections among *Colletotrichum* species on different plant groups. Our results may contribute to develop effective and adequate management schemes of cashew anthracnose.

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Table 1. Isolates of the seven *Colletotrichum* species obtained from leaves, stems and inflorescences of cultivated (*Anacardium occidentale*) and non-cultivated (*Anacardium humile* and *Anacardium othonianum*) cashew plants throughout four Brazilian biomes (Amazon Rainforest, Atlantic Forest, Caatinga and Cerrado).

Colletotrichum species	Isolate	*Anacardium* host	Host organ	Biome (FU)[a]
C. chrysophilum	CMM3204	*A. humile*	Leaf	Cerrado (MG)
	CMM3218	*A. occidentale*	Leaf	Caatinga (PB)
	CMM3231	*A. occidentale*	Stem	Atlantic Forest (PE)
C. fragariae	CMM3220	*A. occidentale*	Leaf	Caatinga (PB)
	CMM3224	*A. occidentale*	Stem	Atlantic Forest (PE)
	CMM3245	*A. occidentale*	Leaf	Caatinga (RN)
C. fructicola	CMM3207	*A. othonianum*	Leaf	Cerrado (MG)
	CMM3208	*A. humile*	Leaf	Cerrado (MG)
	CMM3238	*A. occidentale*	Leaf	Atlantic Forest (PE)
C. gloeosporioides	CMM3331	*A. humile*	Leaf	Cerrado (DF)
	CMM3272	*A. occidentale*	Inflorescence	Caatinga (RN)
	CMM3279	*A. occidentale*	Inflorescence	Caatinga (RN)
C. queenslandicum	CMM3236	*A. occidentale*	Leaf	Atlantic Forest (PE)
	CMM3240	*A. occidentale*	Inflorescence	Atlantic Forest (PE)
	CMM3242	*A. occidentale*	Inflorescence	Atlantic Forest (PE)
C. siamense	CMM3227	*A. occidentale*	Leaf	Atlantic Forest (PE)
	CMM3224	*A. occidentale*	Leaf	Caatinga (RN)
	CMM3222	*A. occidentale*	Inflorescence	Amazon Rainforest (PA)
C. tropicale	CMM3213	*A. occidentale*	Leaf	Caatinga (RN)
	CMM3228	*A. occidentale*	Inflorescence	Atlantic Forest (PE)
	CMM3303	*A. occidentale*	Leaf	Amazon Rainforest (PA)

[a] Federative Units of Brazil: *AL* Alagoas, *DF* Distrito Federal, *MG* Minas Gerais, *PA* Pará, *PB* Paraíba, *PE* Pernambuco, and *RN* Rio Grande do Norte.

Table 2. Temperature-mycelial growth rate relationship for seven *Colletotrichum* species associated with cashew anthracnose in Brazil.

Colletotrichum species	Adjusted model[w]					T_{opt} (°C)[x]	MGR_{max} (mm/day)[y]
	a	*b*	*c*	*d*	R^2		
C. chrysophylum	-0.5991	-0.2437	0.0695	-0.0016	0.95	27.4 (0.68) bc[z]	12.3 (0.21) bc
C. fragariae	7.9395	-1.5535	0.1304	-0.0024	0.97	28.4 (0.77) ab	13.5 (0.16) ab
C. fructicola	-5.3236	0.5241	0.0324	-0.0011	0.96	26.5(0.34) cd	11.5 (0.28) cd
C. gloeosporioides	2.6235	-0.6425	0.0948	-0.0020	0.98	27.5 (0.55) bc	14.7 (0.19) a
C. queenslandicum	-9.7649	1.2647	-0.0072	-0.0005	0.96	25.4 (0.61) d	10.1 (0.10) d
C. siamense	2.5995	-0.7134	0.0936	-0.0019	0.98	27.8 (0.89) bc	13.5 (0.27) ab
C. tropicale	16.4178	-3.0816	0.1962	-0.0032	0.96	29.7 (0.74) a	13.0 (0.23) ab

[w] Mycelial growth on potato dextrose agar at 10 to 40°C was adjusted to a thirty-degree polynomial model: $Y = a+bT+cT2+dT3$, in which Y = mycelial growth (mm/day); *a*, *b*, *c*, and *d* are the regression coefficients; and R^2 = coefficient of determination.

[x] Optimal temperature estimated by the adjusted model.

[y] Maximum mycelial growth rate estimated by the adjusted model.

[z] Values in parentheses represent the standard errors. For each column, means with the same letter are not significantly different according to the Fisher's LSD test ($P = 0.05$).

Table 3. Temperature-spore germination relationship for seven *Colletotrichum* species associated with cashew anthracnose in Brazil.

Colletotrichum species	Adjusted model[w]		T_{opt} (°C)[x]	G_{max} (%)[y]
	a	R^2		
C. chrysophylum	7.4957	0.96	32.6 (0.63) a[z]	97.2 (8.56) ab
C. fragariae	12.8872	0.94	32.1 (1.00) ab	99.5 (6.68) a
C. fructicola	19.3733	0.94	32.4 (1.37) a	100.0 (4.61) a
C. gloeosporioides	6.9071	0.95	27.5 (0.56) c	90.3 (8.00) c
C. queenslandicum	4.6588	0.99	28.8 (0.18) bc	93.6 (3.58) bc
C. siamense	5.1004	0.95	29.7 (0.56) bc	97.6 (8.27) ab
C. tropicale	17.3327	0.94	32.0 (1.18) ab	100.0 (5.04) a

[w] Spore germination at 10 to 40°C was adjusted to Lorentzian model: $G = G_{max}/(1+((T-T_{opt})/a)^2)$, in which G = spore germination (%); G_{max} = maximum spore germination; T = temperature, T_{opt} = optimum temperature; and a is the model parameter; and R^2 = coefficient of determination.

[x] Optimal temperature estimated by the adjusted model.

[y] Maximum spore germination estimated by the adjusted model.

[z] Values in parentheses represent the standard errors. For each column, means with the same letter are not significantly different ($P = 0.05$) according to the Fisher's LSD test.

Table 4. Aggressiveness (diameter of lesion) of the seven *Colletotrichum* species associated with cashew anthracnose in Brazil when inoculated on wounded (W) and non-wounded (N-w) tissues of avocado (cv. Breda), banana (cv. Pacovan), guava (cv. Paluma), mango (cv. Tommy Atkins) and papaya (cv. Golden).

Colletotrichum Species	Diameter of lesion (mm)									
	Avocado		Banana		Guava		Mango		Papaya	
	W	N-w	W	N-w	W	N-w	W	N-w	W	N-w
C. chrysophilum	6.5 (4.3) bc[w]	0.0 (0.0) a	10.9 (2.4) c	0.0 (0.0) c	13.4 (1.9) bc	7.5 (0.8) ab	17.8 (3.2) abc	2.1 (1.1) b	12.0 (2.5) b	2.0 (1.3) bc
C. fragariae	0.0 (0.0) c	0.0 (0.0) a	16.3 (1.8) ab	0.0 (0.0) c	11.7 (2.6) c	10.1 (1.6) a	14.7 (1.4) bc	3.7 (1.1) ab	15.5 (2.8) ab	0.0 (0.0) c
C. fructicola	0.0 (0.0) c	0.0 (0.0) a	16.3 (2.6) ab	0.0 (0.0) c	11.0 (2.9) c	4.3 (2.2) bc	20.6 (2.6) ab	3.0 (1.2) b	14.5 (3.5) ab	0.0 (0.0) c
C. gloeosporioides	5.9 (3.9) bc	0.0 (0.0) a	18.1 (2.2) a	3.1 (2.1) bc	14.2 (1.7) bc	7.9 (2.5) ab	13.1 (2.3) c	5.7 (2.2) ab	11.8 (3.9) b	3.0 (1.5) b
C. queenslandicum	17.9 (3.6) a	0.0 (0.0) a	19.9 (2.1) a	4.3 (2.2) ab	18.1 (1.4) ab	8.4 (1.8) ab	20.0 (3.1) ab	8.0 (2.7) a	16.6 (1.1) ab	0.0 (0.0) c
C. siamense	16.3 (4.2) a	0.0 (0.0) a	13.3 (2.5) bc	1.9 (0.7) bc	19.9 (2.1) a	12.3 (1.9) a	20.1 (2.9) ab	6.2 (1.6) ab	18.8 (2.7) a	7.2 (2.7) a
C. tropicale	7.8 (4.9) b	0.0 (0.0) a	16.6 (2.4) ab	7.5 (2.4) a	12.9 (2.2) c	2.1 (1.5) c	19.6 (3.7) ab	4.6 (1.6) ab	15.1 (2.3) ab	3.2 (2.2) b

[w] Values in parentheses represent the standard errors. For each column, means with the same letter are not significantly different according to the Fisher's LSD test (α ≤0.05).
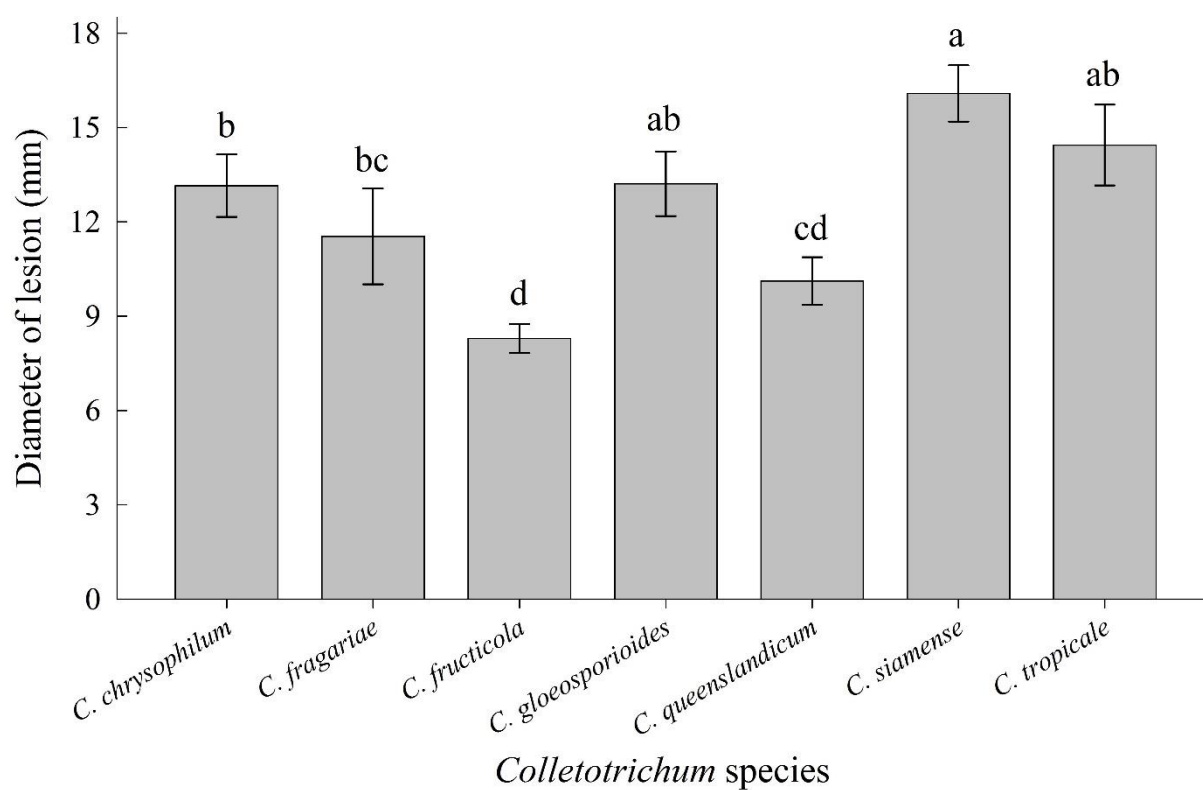
Fig. 1 Aggressiveness (mean ± standard error of the lesion diameter) of seven *Colletotrichum* species associated with anthracnose on wounded leaves of *Anacardium occidentale* following 15 days of inoculation in a greenhouse. Columns bearing different letters were significantly different (Fisher's LSD, α ≤0.05).
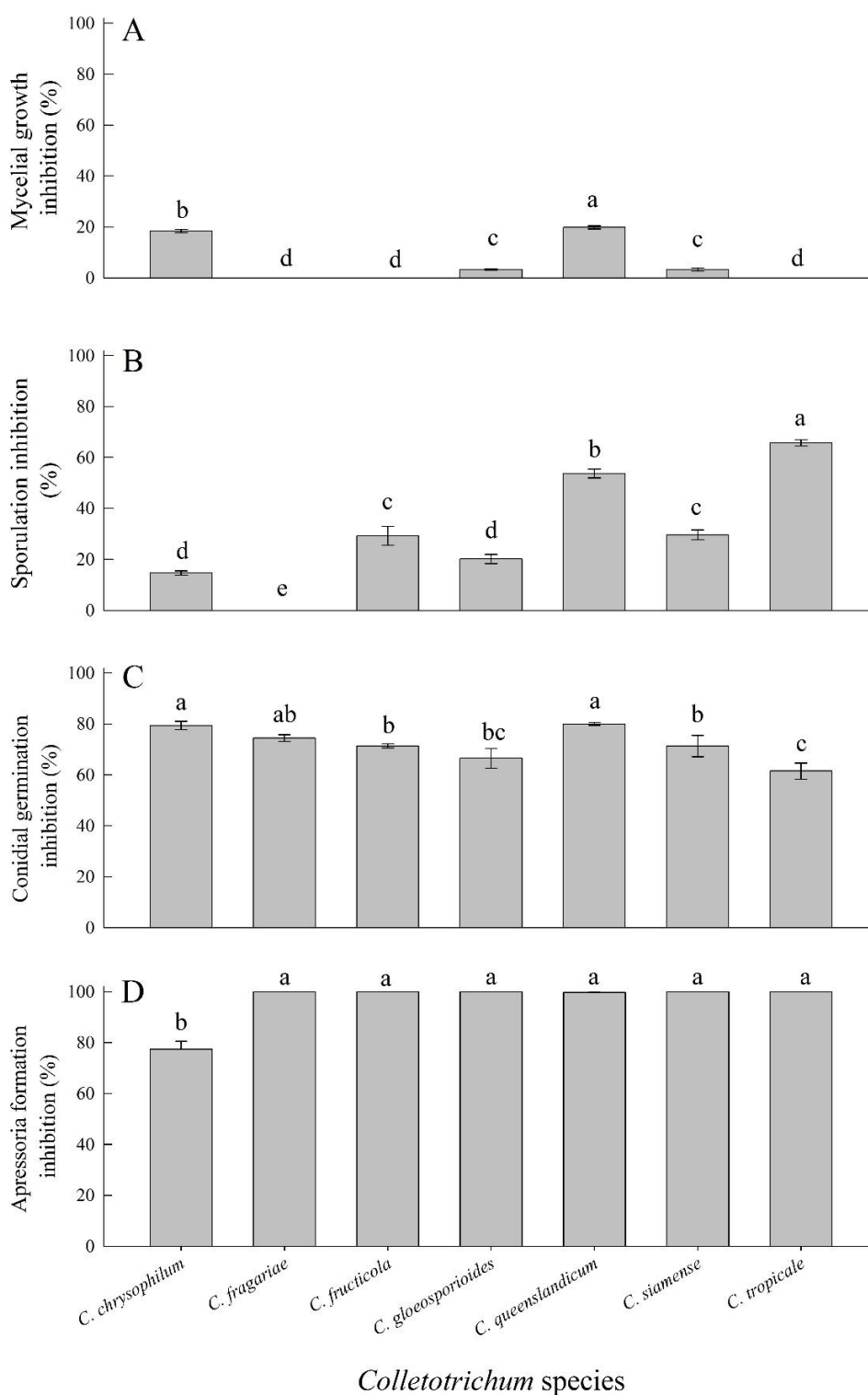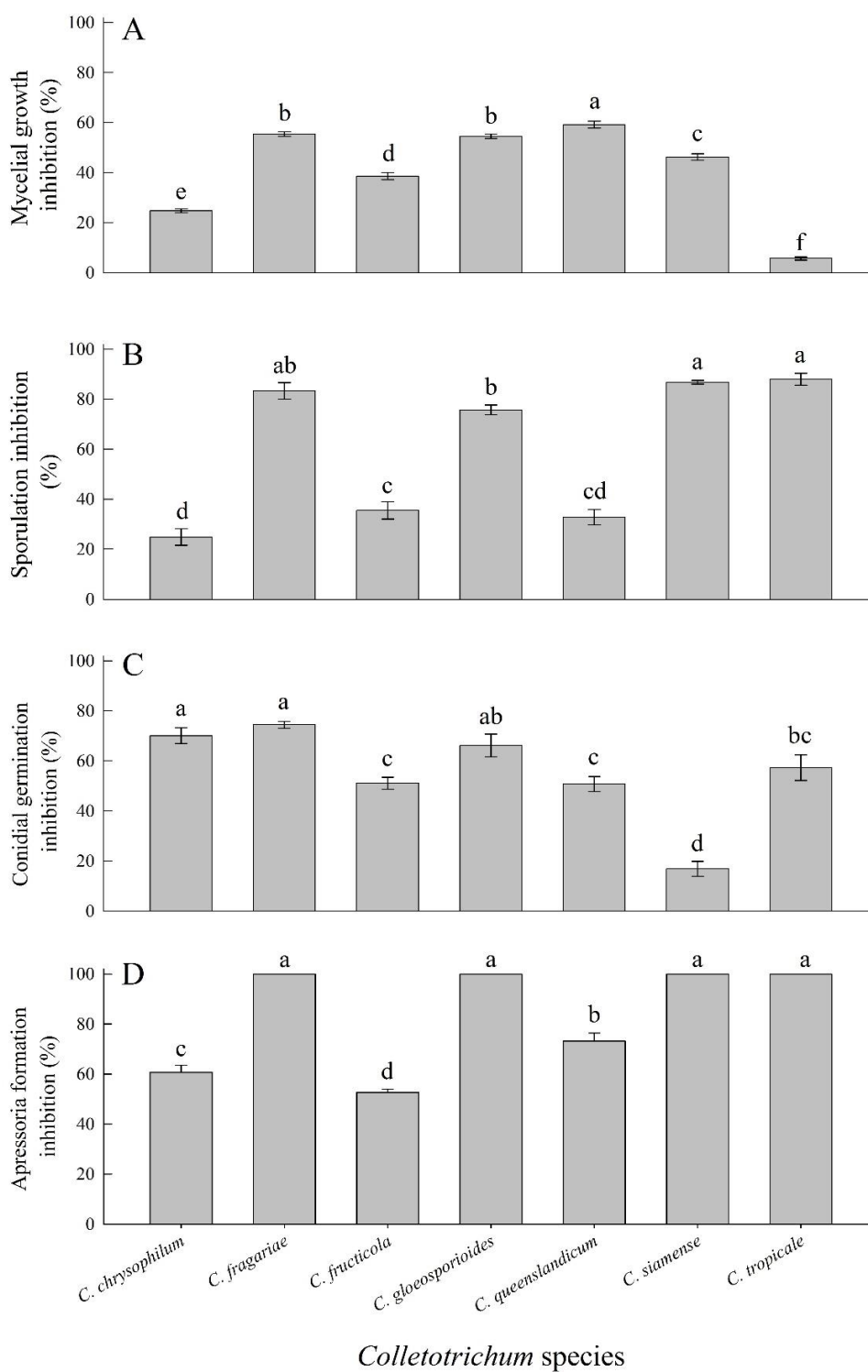
Fig. 2 Inhibition (%) of the mycelial growth (A), sporulation (B), conidial germination (C), and apressoria formation (D) of seven *Colletotrichum* species associated with cashew anthracnose to the fungicide azoxystrobina (1.0 μg i.a. ml^{-1}). Columns of the same inhibition trait bearing different letters were significantly different (Fisher's LSD, α = 0.05).

Fig. 3 Inhibition (%) of the mycelial growth (A), sporulation (B), conidial germination (C), and apressoria formation (D) of seven *Colletotrichum* species associated with cashew anthracnose to the fungicide difenoconazole (0.5 μg de i.a. ml^{-1}). Columns of the same inhibition trait bearing different letters were significantly different (Fisher's LSD, α = 0.05).
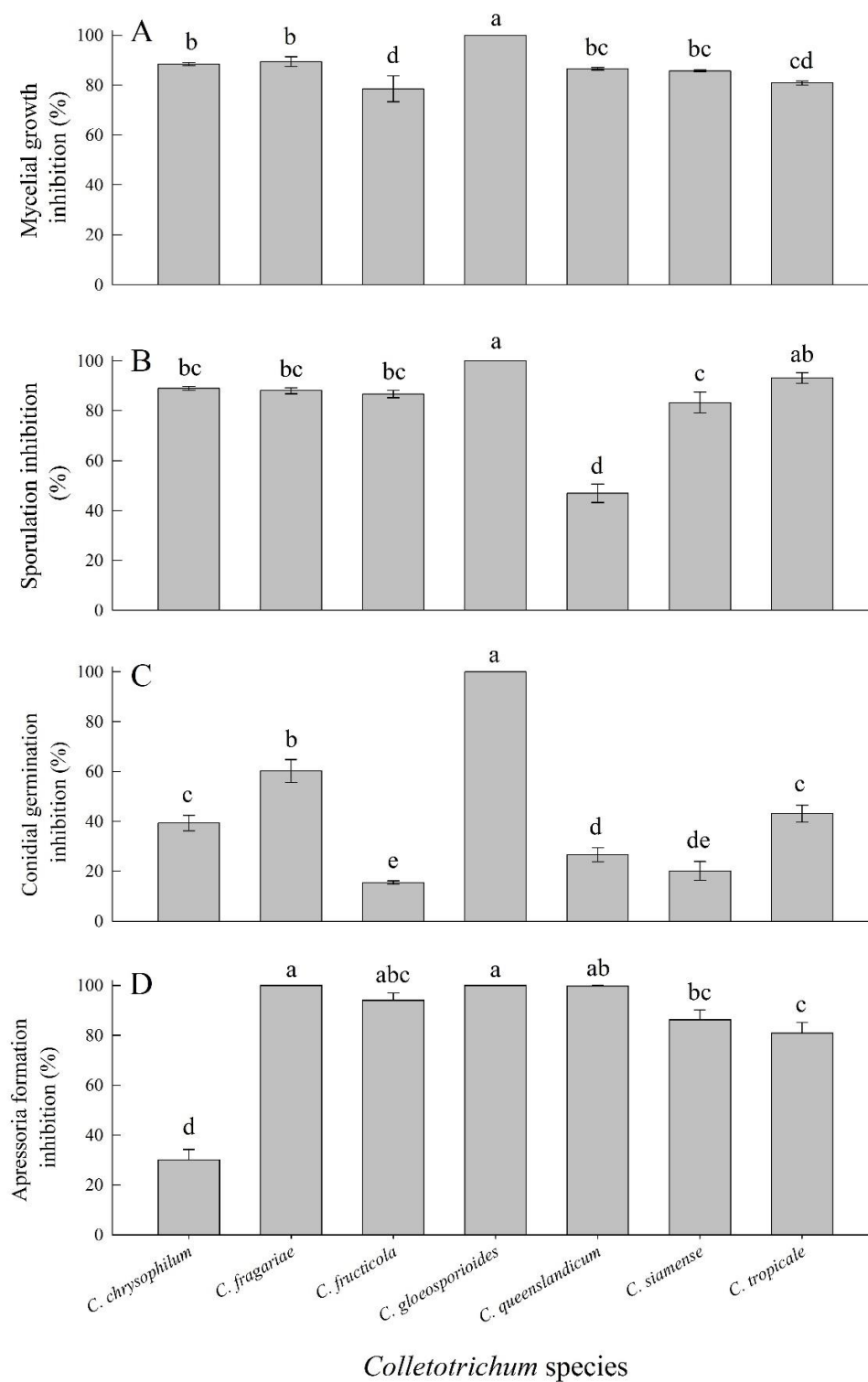
Fig. 4 Inhibition (%) of the mycelial growth (A), sporulation (B), conidial germination (C), and apressoria formation (D) of seven *Colletotrichum* species associated with cashew anthracnose to the fungicide tiophanate-methyl (5.0 μg i.a. ml^{-1}). Columns of the same inhibition trait bearing different letters were significantly different (Fisher's LSD, α = 0.05).

CAPÍTULO IV

Conclusões Gerais

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✓ Sete espécies de *Colletotrichum* (*C. chrysophilum*, *C. fragariae*, *C. fructicola*, *C. gloeosporioides sensu stricto*, *C. queenslandicum*, *C. siamense* e *C. tropicale)* são responsáveis pela antracnose do cajueiro cultivado e silvestre ao tratar linhagens dentro de *C. siamense* como uma única espécie;

✓ A interpretação dos fatores que influenciam a composição da comunidade de *Colletotrichum* depende da forma como as espécies crípticas são delimitadas;

✓ *C. gloeosporioides sensu stricto* foi rara, enquanto *C. siamense* foi a espécies dominate;

✓ Ocorrendo em todos os biomas amostrados, *C. siamense* foi a espécie mais comum em *A. occidentale* e *A. othonianum*, enquanto *C. fructicola* foi a prevalente no Cerrado e em *A. humile*;

✓ O cajueiro cultivado (*Anacardium occidentale*) e as folhas foram os estratos mais diversos de espécies de *Colletotrichum* associados ao hospedeiro

✓ A Mata Atlântica e o estado de Pernambuco foram os estratos mais diversos de espécies de *Colletotrichum* relacionados à localização

✓ As temperaturas ótimas para o crescimento micelial e germinação conidial variaram entre 25–30 °C e 27–37 °C, respectivamente;

✓ As espécies de *Colletotrichum* foram patogênicas a folhas de caju com ferimento, mas nenhuma causou lesão em superfície foliar intacta;

✓ A ampla gama de hospedeiros das espécies de *Colletotrichum* associadas ao cajueiro constitui um sério problema para o manejo da antracnose;

✓ As espécies de *Colletotrichum* se mostraram sensíveis a azoxistrobina, difenoconazole e tiofanato-metíllico em maior ou menor nível;

✓ A identificação correta das espécies de *Colletotrichum* é fundamental para compreender suas interações com os fatores ambientais que influenciam na epidemiologia e agressividade da doença em diversos hospedeiros, bem como também para se adotar estratégias efetivas de controle.