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LAVRAS – MG
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RESUMO

Caryocar brasiliense Camb. é uma espécie arbórea típica do bioma Cerrado no Brasil, popularmente conhecida como pequizeiro. O fruto dispõe de elevada importância para alimentação e para a indústria de cosméticos. Durante o período de frutificação da espécie, inúmeras famílias se beneficiam da colheita direta dos frutos, sendo essa muitas vezes sua única fonte de renda no período. Durante a condução de pesquisas de campo em regiões de Cerrado no estado de Minas Gerais, foi verificada a presença de cancro nos troncos de árvores de *C. brasiliense*, as quais muitas vezes já estavam mortas. Constatou-se, também, a presença de estruturas com características semelhantes àquelas da família Cryphonectriaceae, a qual abriga fungos majoritariamente causadores de cancro. Objetivou-se com esse estudo isolar e identificar o fungo causador de cancro em *C. brasiliense* e avaliar sua patogenicidade. A identificação foi baseada na caracterização morfológica e em dados de sequenciamento de DNA da região ITS (Internal Transcribed Spacer), dos fragmentos BT1 e BT2 do gene beta - tubulina, do gene actina e da subunidade maior do rDNA. Os dados obtidos pelo sequenciamento foram comparados com sequências de outros gêneros da família Cryphonectriaceae. Para avaliação dos testes de patogenicidade, mudas de *C. brasiliense* foram inoculadas artificialmente. Por meio das análises filogenéticas, constatou-se que todos os isolados provenientes de *C. brasiliense* formaram um clado único, fortemente suportado e distinto dos demais gêneros em Cryphonectriaceae. O fungo apresenta marcadores morfológicos que o diferenciam dos outros gêneros de Cryphonectriaceae, especialmente quanto à presença de hifas ostiolares septadas agrupadas ou individuais. Os isolados estudados apresentam capacidade de causar lesões escurecidas no caule. Para completar os postulados de Koch, o fungo foi reisolado das plantas inoculadas de *C. brasiliense*. Esses resultados evidenciam que o fungo patogênico obtido de *C. brasiliense* se trata de um novo gênero e uma nova espécie em Cryphonectriaceae, então denominada *Cryptoporthe reticulata.*

Palavras-chave: Pequizeiro. Cerrado. Filogenia.

ABSTRACT

Caryocar brasiliense Camb. (*Ca. brasiliense*) is a typical tree of the Brazilian Cerrado commonly known as pequi. The pequi fruits have a high potential for use in cosmetic and food industries. Due to its economic importance, during the fruiting period, numerous families living in the Cerrado biome benefit from direct fruit harvesting, which is often their only income source. There are no commercial plantations and the only source of the pequi fruits is the natural Brazilian Cerrado. During a disease survey in the Brazilian Cerrado, an unknown fungus was observed on stem cankers of dying trees. The fungus has similar characteristics to the well-known family of canker pathogens, the Cryphonectriaceae. Thus, the aim of this study was to isolate and identify the fungus from those canker symptoms and assess its pathogenicity. Identification of the fungus was based on morphological characteristics as well as DNA sequenced data. DNA from the Internal Transcribed Spacer (ITS) regions, two fragments of the b-tubulin gene (BT1 and BT2), large subunit of rDNA (LSU) and actin (ACT), was sequenced and compared with published sequences for 20 genera in the Cryphonectriaceae family. Pathogenicity tests were conducted on *Ca. brasiliense* seedlings. Morphological characterizations revealed that the fungus isolated from *Ca. brasiliense* differed from those typically found in the Cryphonectriaceae, especially for the presence of ostiolar septate single or branched hyphae. Phylogenetic analyses showed that this novel fungus from *Ca. brasiliense* grouped separately from other genera in the Cryphonectriaceae. Pathogenicity tests on *Ca. brasiliense* showed that the fungus is able to cause stem cankers. Taking all findings together, we propose that the pathogenic fungus isolated from *Ca. brasiliense* is a novel genus and a novel species in the Cryphonectriaceae and, thus, we named it as *Cryptoporthe reticulata*.

Keywords: Pequi. Brazilian Cerrad. Phylogeny.

SUMÁRIO

PRIMEIRA PARTE- INTRODUÇÃO E REFERENCIAL TEÓRICO GERAL

1 INTRODUÇÃO

O pequizeiro, *Caryocar brasiliense* Camb., é uma espécie arbórea tipicamente brasileira que se distribui amplamente pelo bioma Cerrado em diversos estados (ALMEIDA et al., 1998).O fruto produzido pela espécie é popularmente conhecido como pequi e possui elevada importância alimentar e industrial, devido ao seu alto teor de óleo e nutrientes. Além disso, na época de colheita do fruto, inúmeras famílias se beneficiam de sua comercialização (ALMEIDA; SILVA, 1994; ANGELO et al., 2012; SANTOS et al., 2013).

Assim como outras espécies florestais nativas, o pequizeiro tem sido acometido por doenças causadas por fungos fitopatogênicos, como o mal-do-cipó, a mais grave dessas doenças (CARVALHO, 2008). Apesar disso, até o momento não existem registros de doenças provocadas por fungos pertencentes à família Cryphonectriaceae nessa espécie. Entretanto durante pesquisas de campo realizadas na região do Cerrado, observou-se em *C. brasiliense* a presença cancros e galhos secos e mortos com estruturas fúngicas semelhantes àquelas da família Cryphonectriaceae.

Cryphonectriaceae pertence à ordem Diaphortales e abriga ascomicetos que são, em sua maioria, causadores de cancro em troncos de espécies arbóreas (GRYZENHOUT; WINGFIELD; WINGFIELD, 2009). Essa família possui algumas diferenças das outras famílias da ordem, tais como: apresentar tecido estrómatico preto-fosco; formar um tecido estromático alaranjado em algum estágio do ciclo de vida do patógeno; produzir coloração púrpura em contato com KOH e amarelada ao ser colocado em contato com ácido lático. (GRYZENHOUT et al., 2006).

As variações morfológicas entre gêneros e espécies dentro da família se referem à características como a presença ou ausência de pescoço nos corpos de frutificação, coloração do tecido e da massa de esporos, tipo e textura do tecido, presença ou ausência de paráfases e tamanho e forma dos conídios e ascósporos (GRYZENHOUT; WINGFIELD; WINGFIELD, 2009).

Contudo, muitas vezes, apenas características morfológicas não são suficientes para distinguir gêneros e espécies dentro da família, pois as diferenças podem ser muito sutis. Além disso, muitas vezes não se encontram simultaneamente estruturas sexuadas e assexuadas do fungo, dificultando a sua correta identificação (GRYZENHOUT;

WINGFIELD; WINGFIELD, 2009). Em alguns casos, como em *Chrysoporthe* spp., só é possível se alcançar a identificação conclusiva de espécies fazendo-se uso de comparações de sequências de DNA (GRYZENHOUT, 2004; VAN DER MERWE et al., 2013).

O sequenciamento de DNA, especialmente dos genes beta-tubulina e actina e das regiões internal transcribed spacer (ITS) e subunidade maior do rDNA (LSU) (CHEN; WINGFIELD; ROUX, 2013; CHEN et al., 2016; GRYZENHOUT et al., 2005,2006), têm sido utilizado na identificação de espécies já existentes e também na classificação de novas espécies e gêneros da família Cryphonectriaceae.

No Brasil há relatos apenas das espécies *Chrysoporthe cubensis e C. doradensis* ocorrendo em plantas de *Tibouchina* spp. e *Eucalyptus* spp. (ALFENAS et al., 2009; HODGES; REIS; MAY, 1973; SANTOS, 2015). Entretanto devido à condição de clima tropical do País, acredita-se que outras espécies de Cryphonectriaceae, ainda não descritas, possam estar associadas a esses e a outros hospedeiros. O cancro encontrado em *C. brasiliense* associado a um fungo característico de Cryphonectriaceae reforça essa hipótese. Desta forma, buscando-se esclarecer a identidade desse fungo e comprovar a existência de um novo patossistema, o presente estudo teve como objetivos realizar a caracterização filogenética e morfológica dos isolados, bem como comprovar sua patogenicidade, além de descrever sua sintomatologia em campo.

2 REFERENCIAL TEÓRICO

2.1 *Caryocar brasiliense* Camb.

O pequizeiro, *C. brasiliense*, é também conhecido popularmente como pequi, piqui, piquiá-bravo, piquiá, suari, pequiá-verdadeiro, dependendo da região (ALMEIDA et al. 1998; LORENZI, 2008). É uma árvore típica do Cerrado brasileiro e que ocorre nos estados da Bahia, Distrito Federal, Goiás, Minas Gerais, Mato Grosso, Mato Grosso do Sul, São Paulo, Ceará, Maranhão, Pará, Piauí, Rio de Janeiro e Tocantins (ALMEIDA et al., 1998). A espécie é protegida por lei, de acordo com a Portaria Federal 54 de 1987 do IBAMA, sendo assim proibidos o corte e comercialização da madeira em todo território brasileiro (SANTOS et al., 2013).

As árvores possuem troncos tortuosos revestidos por uma casca com ritidoma suberoso. São semidecíduas, exigentes em luz, preferencialmente xerófitas e de solos ácidos

com baixa fertilidade (ALMEIDA; SILVA, 1994; LEITE et al., 2012; SANTOS et al., 2013). As folhas são compostas e trifolioladas, apresentando em sua face abaxial pilosidades que se concentram nas nervuras proeminentes. Os frutos são do tipo drupas subglobosas, a polpa possui coloração amarelada e as sementes são muricadas ou espinescentes (LORENZI, 2008). Também é considerada como árvore ornamental, graças à beleza de suas alvas flores e ao formato de sua copa (SANTOS et al., 2013).

O nome do gênero *Caryocar* possui origem grega, se referindo a *caryon* (núcleo ou noz) e *kara* (cabeça), fazendo alusão ao fruto globoso; já o epíteto específico *brasiliense* se atribui à sua origem brasileira. Seu nome popular pequi é originário do tupi, *py* (casca) e *qui* (espinho), se referindo aos espinhos do endocarpo (CARVALHO, 2008).

É uma espécie que apresenta elevada variação fenotípica, caracterizada por diferenças no porte da árvore e em características dos frutos como peso, tamanho, número de pirênios e sua adequabilidade ao mercado (MOURA; NAVES; CHAVES, 2013).

A madeira é moderadamente pesada, macia, resistente e de boa durabilidade natural, possuindo coloração amarelo-parda e alburno bege-escuro. No passado, foi utilizada para construção de esteios de curral, mourões e dormentes na zona rural e também para a prática de xilografia (ALMEIDA et al, 1998; LORENZI, 2008). A espécie é protegida por lei, de acordo com a Portaria Federal 54 de 1987 do IBAMA, sendo assim proibidos o corte e comercialização da madeira em todo território brasileiro (SANTOS et al., 2013).

A frutificação de *C. brasiliense* ocorre durante o período chuvoso que se estende de novembro até fevereiro e os frutos são de ampla utilização culinária, sendo consumidos juntamente com arroz, farinha, carne, frango e sob a forma de licor. Possuem alto teor de proteínas e são ricos em vitaminas A e B2, ferro, cobre e fósforo. Apresentam propriedades medicinais contra resfriados e bronquites. O óleo extraído da polpa é apreciado pela indústria de cosméticos para produção de sabonetes e cremes. Os frutos são ainda fonte de alimentação para a fauna silvestre e são dispersos por aves e marsupiais (ALMEIDA; SILVA, 1994; CARVALHO, 2008).

Estudos mostram um elevado potencial para o uso do óleo de pequi como biodiesel, apresentando vantagens sobre culturas como soja e girassol quanto ao teor de óleo e meses de colheita por ano. A maior parte do óleo é de excelente qualidade, constituído por ácidos graxos insaturados (SANTOS et al., 2013).

As sementes possuem germinação relativamente baixa e lenta, devido à dormência causada tanto pelo endocarpo rígido, quanto pelo embrião (ALMEIDA; SILVA, 1994; PEREIRA et al., 2000).

A exploração do pequi apresenta características puramente extrativistas, sendo a coleta e o processamento realizados de forma artesanal. A comercialização se dá, na maioria das vezes, por ambulantes e em feiras livres. Mas mesmo com essas características extrativistas, inúmeras famílias se beneficiam da época de safra do pequi, tendo a cultura como fonte de renda e emprego (ANGELO et al., 2012; SANTOS et al., 2013).

O Cerrado é o bioma brasileiro com maiores ameaças à biodiversidade, estando entre elas: as queimadas não controladas, a introdução de espécies exóticas e a redução do habitat pela expansão urbana e agropecuária (ANGELO et al., 2012). Desse modo, ocorre uma intensa fragmentação de populações e alto isolamento genético, fazendo com que a destruição do pequizeiro não seja acompanhada pela sua regeneração natural em escala significativa e impondo um custo às populações que se beneficiam com a sua comercialização (ANGELO et al., 2012; MELO et al. 2012).

Estudos realizados no Norte de Minas evidenciaram que o pequi é considerado como um recurso comum entre as famílias sertanejas que o extraem de suas propriedades e de propriedades vizinhas. Em consequência de seu impacto econômico, cultural e simbólico para essas famílias o pequizeiro é considerado como patrimônio cultural sertanejo (SILVA; TUBALDINI, 2014).

2.2 Doenças fúngicas em *Caryocar brasiliense*

Até os dias atuais, as doenças fúngicas relatadas em *C. brasiliense* são o mal-do-cipó causado por *Phomopsis* spp, a podridão de raízes provocada por *Cylindrocladium clavatum*, a morte descendente dos ramos acarretada por *Botryodiplodia theobromae*, a ferrugem causada por *Cerotelium giacometii*, *a p*odridão dos frutos ocasionada por *B. theobromae* e por *Phomopsis* spp. (CARVALHO, 2008; SILVA et al.,2001;), a antracnose causada por *Colletotrichum acutatum* (ANJOS; CHARCHAN; AKIMOTO, 2002) e a recentemente relatada murcha de *Ceratocystis* produzida por *C. fimbriata* (SILVA et al., 2017).

O mal-do-cipó é a mais grave das doenças citadas. O patógeno ataca preferencialmente pequizeiros adultos da região Centro-Oeste, podendo também ocorrer na fase de viveiro. Nas

mudas provoca estiolamento ou alongamento, deformação nos ramos mais tenros e folhas novas, fazendo com que posteriormente as mudas sequem ou parem de crescer. Nas árvores adultas causa alongamento dos internódios e estiolamento dos ramos mais novos, tornando-os extremamente flexíveis e retorcidos, adquirindo aspecto de cipó (CARVALHO, 2008; SILVA et al., 2001).

A podridão de raízes causada por *C. clavatum*, ataca as raízes das mudas, fazendo com que haja apodrecimento das mesmas. Provoca a morte da árvore ou retarda, consideravelmente, seu crescimento. Os primeiros sintomas da doença são a presença de lesões escurecidas no coleto, baixo desenvolvimento das mudas, seguido de amarelecimento e queda das folhas. A morte descendente dos ramos desencadeada por *Botryodiplodia theobromae* ocorre em pequizeiros adultos e provoca secamento dos ramos mais novos e de suas folhas. Posteriormente, o patógeno atinge os galhos e causa a morte da árvore. Sob os galhos, ramos ou tronco afetados pode-se observar a presença de um tecido escuro e necrosado. A podridão dos frutos causada por *B. theobromae* e por *Phomopsis* spp. ocorre após a colheita, deixando-os com uma podridão mole e escurecida e com gosto amargo (CARVALHO, 2008; SILVA et al., 2001).

A antracnose causada por *C. acutatum* foi relatada pela primeira vez em 2000 no Distrito Federal causando grande quantidade de lesões irregulares e de coloração marrom-escura nos folíolos (ANJOS; CHARCHAR; AKIMOTO, 2002).

Recentemente foi relatada nas regiões de Montes Claros e Lontra- MG a ocorrência de murcha ocasionada por *Ceratocystis fimbriata*. Os sintomas iniciais da doença são amarelecimento e murcha das folhas dos ramos laterais. Posteriormente as árvores perdem a maior parte de suas folhas e morrem (SILVA et al., 2017).

2.3 Cryphonectriaceae

A família Cryphonectriaceae pertence à ordem Diaporthales e filo Ascomycota. Cryphonectriaceae abriga um grupo de fungos majoritariamente causadores de cancro em tronco de espécies arbóreas, com exceção dos gêneros *Filiocryphia* e *Aurantiosacculus* que estão associado à manchas foliares (CHEEWANGKOON et al., 2009; CROUS et al., 2012; GRYZENHOUT; WINGFIELD; WINGFIELD, 2009). Essa família se diferencia das outras pertencentes à mesma ordem quanto à sua característica de formar um tecido estromático

alaranjado em algum estágio do ciclo de vida do patógeno. Quando colocado em contato com KOH, o tecido produz uma reação que lhe confere coloração púrpura e ao ser colocado em contato com ácido lático adquire coloração amarelada. (GRYZENHOUT et al., 2006).

No Brasil, até os dias atuais, foi reportada a presença apenas do gênero *Chrysoporthe*, espécies *C. cubensis* e *C. doradensis* causando o cancro em *Tibouchina* spp e *Eucalyptus* spp.(ALFENAS et al., 2009; SANTOS, 2015; SEIXAS et al., 2004). O gênero abriga, também, espécies que são muito similares morfologicamente, sendo diferenciadas por um sutil tamanho dos esporos e temperatura ótima de crescimento que pode ser de 25 ou 30°C. A identificação conclusiva só é possível com a comparação das sequências de fragmentos de DNA, sendo os mais utilizados para essa diferenciação o gene beta tubulina e a região ITS do rDNA (GRYZENHOUT et al., 2004; VAN DER MERWE et al., 2013).

Chrysoporthe cubensis ocorre em vários países da América tropical e subtropical, África e Austrália (NAKABONGE et al., 2007; RODAS et al., 2005; VAN DER MERWE et al., 2010). O primeiro relato da espécie foi em 1917, em Cuba causando cancro em *Eucalyptus* spp, sendo denominada *Diaporthe cubensis* (BRUNER, 1917). Posteriormente foi recolocada dentro do gênero *Cryphonectria* devido ao fato de suas características culturais e morfológicas se assemelharem às do gênero. Porém, em 2004, estudos das sequências genéticas ITS e beta-tubulina demonstraram diferenças filogenéticas entre *C. cubensis* e o gênero *Cryphonectria*, sendo então denominado *Chrysoporthe cubensis* (GRYZENHOUT et al., 2004).

O patógeno existe em regiões tropicais e subtropicais onde a temperatura média da maioria dos meses do ano é superior a 23°C e a precipitação anual próxima ou superior a 1200mm (FERREIRA, 1989).

No Brasil, o primeiro relato de *C. cubensis* foi em 1973, causando cancro em plantios de *Eucalyptus* spp (HODGES; REIS; MAY, 1973). Na década de 1970, foi a enfermidade biótica mais importante da eucaliptocultura brasileira, sendo um dos fatores que mais pressionou o desenvolvimento da eucaliptocultura, patologia florestal e melhoramento genético na busca de resistência à doença (FERREIRA, 1989). Até o momento *C. cubensis* foi identificado no Brasil em *Eucalyptus* spp. e *Tibouchina* spp. (ALFENAS et al., 2009; SEIXAS et al., 2004).

Os sintomas do cancro são mortes esporádicas e lesões basais em desenvolvimento. Ocorrem em plantios jovens até os dois anos de idade, havendo lesões próximas ao coleto que podem até mesmo se transformar em anelamento devido ao diâmetro reduzido das plantas,

levando-as à morte. Podem surgir lesões pouco profundas, com formação de uma nova casca resistente abaixo da casca infectada. Ainda podem ocorrer sintomas de cancro típico, onde uma lesão margeada por calos resulta em uma lesão profunda na casca, matando assim o câmbio de uma porção da circunferência do tronco (ALFENAS, 2009; FERREIRA, 1989). Manifesta-se em várias alturas do tronco, sendo mais comum próximo ao solo devido aos ferimentos existentes na região entre raiz e coleto e à maior umidade. (FERREIRA, 1989).

Os sinais da doença são a presença de corpos de frutificação semelhantes a pelos escuros (picnídios e/ou peritécios) produzidos sobre a casca morta (ALFENAS et al., 2009).

Pode acarretar retardamento no crescimento da planta, prejudicar o valor da madeira para serraria, reduzir seu rendimento em termos de celulose e poder calorífico, além de afetar a rebrota das árvores após o corte devido ao local de incidência dos cancros (FERREIRA, 1989; GUIMARÃES et al. 2010; SOUZA, 2010).

O controle se dá por meio da resistência genética, selecionando-se clones ou espécies resistentes. Há ampla variabilidade intraespecífica quanto à resistência, permitindo a clonagem e seleção de genótipos resistentes, já o controle em jardins clonais se dá reduzindo o estresse nas cepas por meio de coletas seletivas e contínuas de brotações (ALFENAS et al., 2009).

Chrysoporthe doradensis foi relatado pela primeira vez causando cancro em *E. grandis* e *E. deglupta* no Equador (GRYZENHOUT et al., 2005). No Brasil foi relatado em 2015, atacando *Eucalyptus* spp. dos Estados do Maranhão e Minas Gerais e *Tibouchina* granulosa em Minas Gerais (SANTOS, 2015). Seus sintomas são semelhantes aos sintomas causados por *C. cubensis* (GRYZENHOUT et al., 2005).

Até o presente momento não há relatos da presença de patógenos da família Cryphonectriaceae em *C. brasiliense*.

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SEGUNDA PARTE- ARTIGO

ARTIGO 1- *Cryptoporthe reticulata* gen. sp. nov. (Cryphonectriaceae) pathogenic to pequi (*Caryocar brasiliense*) in Brazil

Artigo redigido conforme as normas da revista Tropical Plant Pathology
(versão preliminar)

Cryptoporthe reticulata GEN. SP. NOV. (CRYPHONECTRIACEAE) PATHOGENIC TO PEQUI (*Caryocar brasiliense*) IN BRAZIL

ABSTRACT

Caryocar brasiliense Camb. (*Ca. brasiliense*) is a typical tree of the Brazilian Cerrado commonly known as pequi. The pequi fruits have a high potential for use in cosmetic and food industries. Due to its economic importance, during the fruiting period, numerous families living in the Cerrado biome benefit from direct fruit harvesting, which is often their only income source. There are no commercial plantations and the only source of the pequi fruits is the natural Brazilian Cerrado. During a disease survey in the Brazilian Cerrado, an unknown fungus was observed on stem cankers of dying trees. The fungus has similar characteristics to the well-known family of canker pathogens, the Cryphonectriaceae. Thus, the aim of this study was to isolate and identify the fungus from those canker symptoms and assess its pathogenicity. Identification of the fungus was based on morphological characteristics as well as DNA sequenced data. DNA from the Internal Transcribed Spacer (ITS) regions, two fragments of the b-tubulin gene (BT1 and BT2), large subunit of rDNA (LSU) and actin (ACT), was sequenced and compared with published sequences for 20 genera in the Cryphonectriaceae family. Pathogenicity tests were conducted on *Ca. brasiliense* seedlings. Morphological characterizations revealed that the fungus isolated from *Ca. brasiliense* differed from those typically found in the Cryphonectriaceae, especially for the presence of ostiolar septate single or branched hyphae. Phylogenetic analyses showed that this novel fungus from *Ca. brasiliense* grouped separately from other genera in the Cryphonectriaceae. Pathogenicity tests on *Ca. brasiliense* showed that the fungus is able to cause stem cankers. Taking all findings together, we propose that the pathogenic fungus isolated from *Ca. brasiliense* is a novel genus and a novel species in the Cryphonectriaceae and, thus, we named it as *Cryptoporthe reticulata*.

Key-words: canker disease, pequiá, Cerrado, Diaporthales.

Introduction

Caryocar brasiliense Camb. (*Ca. brasiliense*) is a typical Brazilian tree species of the Cerrado biome and is commonly known as pequi, piqui, piquiá-bravo among other names depending on the region. Fruits of the *Caryocar* genus are collectively called 'souari-nut'. This species is widely spread and occurs in 13 of the 26 Brazilian States (in Bahia, Distrito Federal, Goiás, Minas Gerais, Mato Grosso, Mato Grosso do Sul, São Paulo, Ceará, Maranhão, Pará, Piauí, Rio de Janeiro, and Tocantins) (Almeida and Silva 1994; Lorenzi 2008). The fruits are rich in proteins, vitamins A and B2, iron, copper, and phosphorus and have a high potential for use in cosmetic and food industries (Almeida and Silva 1994; Almeida et al. 1998). Due to these characteristics, during the fruiting period of the pequi tree, which occurs from November to February (Lorenzi, 2008), many families living in the Cerrado biome benefit from their exploitation, which is often the only income source for them (Ângelo et al. 2012, Santos et al. 2013). There is no commercial plantation of pequi and the extraction of fruits (removal of natural resources) is performed in naturally occurring trees. Brazilian law protects *Ca. brasiliense* and its cutting and commercialization of timber is prohibited (Santos et al. 2013).

The Brazilian Cerrado biodiversity is under several threats, for instance uncontrolled fires, introduction of exotic species, and habitat reduction by urban and agricultural expansion (Angelo et al., 2012). Thus, the pequi populations are very fragmented and in a high genetic isolation. The natural regeneration is not in pace with the extractivism and the major consequences negatively affect the communities that benefits from the commercialization of fruits (Ângelo et al. 2012; Melo et al. 2012). Another serious threat is the occurrence of fungal diseases on fruits and trees. A few pathogens were already reported occurring in this species, such as *Phomopsis* spp., *Cylindrocladium clavatum* Hodges & L.C. May, *Botryodiplodia theobromae* Pat., *Cerotelium* (Silva et al. 2001; Carvalho, 2008), *Colletotrichum acutatum* (Penz.) Sacc. (Anjos et al. 2002) and *Ceratocystis fimbriata* (Silva et al., 2017).

Currently, there is no report of any disease caused by fungi of the Cryphonectriaceae family in *Ca. brasiliense*. In Brazil, several species of the *Chrysoporthe* genus, which belongs to the Cryphonectriaceae family, cause canker in the *Myrtaceae* and *Melastomataceae* families of plants. *Chrysoporthe cubensis* is the most prominent species in Cryphonectriaceae family due to its high diversity in tropical and subtropical countries, including Brazil (Seixas et al. 2004). This fungus causes damage to eucalyptus plantations, being the causal agent of canker in several species of *Eucalyptus* as well as in other plant species such as *Tibouchina*

granulosa (Desr.) Cogn., *T. urvilleana* (DC.) Cogn., *Miconia thaezans* Cogn, *M. rubiginosa* (Bonpl.) DC. among others (Ferreira 1989; Rodas et al., 2005; Barreto et al. 2006; Alfenas et al. 2009).

During a disease survey in the field, symptoms and signs typical of pathogens causing canker, belonging to the Cryphonectriaceae family, were observed in *Ca. brasiliense* trees. Considering the high cultural and economic value of the local populations of *Ca. brasiliense*, the main goal of this study was to perform the morphological, phylogenetic, and pathogenic characterization of the fungus collected from *Ca. brasiliense*, as well as to describe the symptoms of the disease and evaluated the pathogenicity of the isolates by fulfilling the Koch's postulate.

Materials and methods

Survey, symptoms and fungal isolations

A survey was conducted in nine areas from seven locations (Lavras, Itumirum, Ingaí, Piumhi, São Roque de Minas, and Curvelo in Minas Gerais State; Três Lagoas in Mato Grosso do Sul State). Samples of trunk and dead stems were collected from pequi with symptoms and signs typical of the Cryphonectriaceae family. The collected samples were stored in paper bags and sent to the Laboratory of Forest Pathology, Federal University of Lavras. The fungal isolation from the tree samples consisted of single conidial masses from the top of pycnidia forming on the bark, dead trunk or stem and were transferred to plates containing YEMA medium (0.2% yeast extract, 2% malt extract, 2% agar) containing rifamycin (antibiotic) at100 μg L^{-1}. After germination, the single conidia were transferred to Petri plates and incubated at 25ºC for 10 days. Then, for long term storage, the cultures were stored in tubes containing sterilized water (Castellani 1938) and kept at 25ºC in the dark.

Molecular characterization

Ten pure cultures (CBL02, CBL04, CBL06, CBL11, CBI01, CBI03, CBING01, CBP03, CBSC01, and CBSC04) were grown on PDA medium at 25ºC for 10 days to obtain sufficient fungal biomass for DNA extraction. Mycelium was scraped from the surfaces of actively growing cultures and placed in frozen mortar and pestle containing liquid nitrogen, and was ground to a fine powder. DNA extractions were performed using the Wizard®Genomic DNA Purification Kit (Promega, Madson, WI, USA), according to the

manufacturer's instructions. DNA quality and quantity were checked using NanoDrop®. DNA samples were diluted to 50 ng/µl and 1µl was used in each PCR reaction.

The internal transcribed spacer (ITS1-5.8S-ITS2) region of rDNA was amplified using primers ITS1 and ITS4 (White et al. 1990). The β-tubulin gene (BT1 and BT2) was amplified using the primer pairs BT1a/BT1b and BT2a/BT2b, respectively (Glass and Donaldson 1995). The large subunit (LSU) of the ribosomal DNA was amplified using the primers LR0R and LR5 (White et al. 1990). The region of the actin (ACT) gene was amplified using the primers ACT512/ACT783 (Carbone and Kohn 1999). All PCR reactions were prepared in a final volume of 25 µl and were performed in a thermocycler (My Cycler^{TM} -BIO-RAD) and conditions were adjusted for each gene as previously described by Glass and Donaldson (1995), Carbone and Kohn (1999) and White et al. (1990).

Purification of the PCR products and sequencing was performed by Macrogen Company (Korea). The generated electropherograms were edited using SeqAssem Software (Hepperle 2004). Sequences from previous studies and publically available in GenBank (Table 1), for species in the Chryphonectriaceae family, were used for the phylogenetic analysis. The sequences generated in this study were deposited in the NCBI/GenBank. The sequences of CBL02 isolate were deposited with the accession numbers: MG192093 (ITS), MG192103 (LSU), MG192140 (ACT), MG192125 (BT1), and MG192133 (BT2).

Multiple alignments of nucleotide sequences were constructed using the CLUSTALW Program (Thompson et al. 1994) and MEGA 6.0 software (Tamura et al. 2013). Sequences were manually edited when needed. Phylogenetic trees were constructed using PAUP* software version 4.0 (Swofford 2002) using Maximum parsimony (MP) and MRBAYES v. 3.2.1 (Ronquist et al. 2012). A bootstrap analysis (1000 replicates) was also performed on the dataset to determine the confidence levels of the branches. Bayesian inference was used to generate posterior probabilities (PP) for consensus nodes using MRBAYES (Huelsenbeck 2001). The Monte Carlo Markov Chain (MCMC) (Larget & Simon 1999) was run with 1,000,000 generations using the appropriate substitution evolution model determined by jModelTest. Trees were viewed and edited in Fig.Tree 1.3.1. (http://tree.bio.ac.uk/software). The combination of the two portions of β-tubulin gene was determined with a partition homogeneity test (PHT) (Farris et al. 1995).

Morphological characterization

Fungal structures and fruiting bodies from bark or stem canker samples were selected based on the phylogenetic analysis and used for a morphological characterization. For this purpose, histological sections of fungal structures and fruiting bodies were examined. Conidiomata were then hand sectioned and mounted in KOH 3M and glycerol 50% for microscopic observation under a light microscope. Fifty structures were measured using light microscope and the pictures were taken using Nikon Eclipse E200, Infinity Analyze, Infinity 1 software image capture system. The structures measured were: pycnidial base, neck, ostiolar hyphae, locules, conidia, conidiophores and conidiogenous cells. The ostiolar hyphae were cut from pycnidia and placed in Calcofluor White 0.01% solution and samples were imaged in an epifluorescence microscope. Microscopy slides were prepared with Poly-L-lysine for adhesion of the conidia, and subsequently fixed using a modified Karnovsky solution (2.5% glutaraldehyde, 2.5% paraformaldehyde in 50 mM sodium cacodylate buffer pH 7.2), for 24 h at 4 °C. The slides were transferred to a 1% aqueous solution of osmium tetroxide for 1 h at room temperature, and subsequently dehydrated in a graded series of acetone solutions (25, 50, 75, 90 and 100%) for 10 min each. The slides were dried in a critical point dryer (model CPD 030, Balzers); then they were mounted on aluminum stubs with double-stick carbon tape on aluminum foil, with the sectioned side up in liquid nitrogen, sputter-coated with gold in a SCD 050 sputter (Balzers) and observed with a EVO 40 XVP scanning electron microscope (SEM) (Leo Electron Microscopy). All the imaging experiments were performed in the Microscopy Facility at the Federal University of Lavras.

Pathogenicity assays

The pathogenicity assays were performed on one-year-old pequi seedlings and six isolates were evaluated (CBL02, CBSC04, CBI03, CBING01, CBP03, and CBSC01). For the artificial inoculation, wounds were made on stems of seedlings with, approximately, 7 mm of diameter and 70cm height (wound was made of 5 cm of soil line), with the aid of a central cylindrical spout of 5 mm diameter for the removal of discs from the bark and wood exposure. Then, 5 mm mycelial discs, from PDA medium, were placed in contact with the stem wounded tissues and covered with plastic film Parafilm®, which was used for disc fixation and protection against wound desiccation. The plastic film was kept on the wound for a period of 30 days after inoculation (dai), and the evaluation was performed at 60 dai by measuring

the length of the lesion on the stem. Discs containing PDA medium only were used as controls (Ferreira 1989; Alfenas et al. 2016).

The experiment was conducted in a greenhouse in the Federal University of Lavras during the summer, when temperatures ranged from 25°C to 35°C. A completely randomized experimental design with 10 replicates (plants) was used. An analysis of variance was performed with two treatments (inoculated and control), and the Scott-Knott ´s test ($P < 0.05$) was used for a multiple comparison test (for lesion length).

Results

Survey, Symptoms, and Isolations

The survey resulted in 34 samples collected in the nine areas from the seven locations (see Material and Methods for details). Ten individual isolations were analyzed for each sample. No symptomatic plants were found at Três Lagoas, Mato Grosso do Sul. Healthy (Fig. 1A-C) and diseased trees (Fig. 1D) were observed in a scattered distribution throughout the surveyed areas. Symptomatic and dying trees were commonly observed in the surveyed areas in Lavras (Fig. 1D). The observed symptoms were plant defoliation, dry branches and presence of fungal structures on broom-like shoots above and below the branches with lesions and fungal signs (Fig. 1E-H). The xylem discoloration in the symptomatic plants suggests that infections generally started in the stem ramification (Fig. 1I), moved upwards, ultimately reaching the upper branches of the entire plant and then leading to plant death.

In stem cross sections, discoloration of the host tissue was observed and often included the entire woody xylem. Dark-brown lesions in the cambium/inner bark region were observed (Fig. 1J). Thirty samples were successfully isolated and six to ten samples were analyzed (see Material and Methods for details).

Phylogenetic analysis

Sequences from 94 isolates representing the sequence diversity of the family Cryphonectriaceae were selected. Three regions (ITS, BT1 and BT2) were used for the phylogenetic analyses. The evolutionary history was inferred using the Maximum Parsimony method, and the most parsimonious tree with length of 820 is shown (Fig. 2). The consistency index is 0.63, the retention index is 0.86, and the composite index is 0.55 for all sites and parsimony-informative sites. Trees were similar in topology, and thus one tree was selected for illustration (Fig. 2). For the combined data set of ITS and BT regions, an alignment of

1750 characters of the 94 isolates resulted in 775 constant characters and 168 parsimony non-informative and 807 parsimony informative characters. The 10 isolates from *Ca. brasiliense* formed a group, which is separated from the other genera with high bootstrap support (100%) and high values of posterior probability (1.0). *Diaporthe ambigua* Nitschke fungal species was used as *outgroup* (Table 1).

For de LSU region, sequences from 63 isolates representing the sequence diversity of the family Cryphonectriaceae were analyzed. The most parsimonious tree with length of 132 is shown (Fig. 3). The consistency index is 0.57, the retention index is 0.86, and the composite index is 0.49 for all sites and parsimony-informative sites. The trees were similar in topology, and thus one tree was selected for illustration (Fig. 3). For the LSU region, an alignment of 1032 characters of the 63 isolates resulted in 940 constant characters and 25 parsimony non-informative and 67 parsimony informative characters. The 10 isolates from *Ca. brasiliense* formed a group, which is separated from the other genera with high bootstrap support (100%) and high values of posterior probability (1.0). Two sequences of *Harknessia* and two sequences of *Diaporthe* genera were used as outgroup.

For ACT gene, the analysis involved 28 sequences, and six sequences originated in this study. The most parsimonious tree with length of 123 is shown (Fig. 4). The consistency index is 0.88, the retention index is 0.96, and the composite index is 0.85 for all sites and parsimony-informative sites. For the ACT region, an alignment of 357 characters of the 28 isolates resulted in 218 constant characters and 16 parsimony non-informative and 123 parsimony informative characters. The six isolates from *Ca. brasiliense* formed a group, which is separated from the other genera with high bootstrap support (100%) and high values of posterior probability (1.0). *Amphilogia gyrosa* (Berk. & Broome) fungal species was used as outgroup (Fig. 4).

Morphology and taxonomy

Cryptoporthe, M.E.S. Oliveira G. A. Silva & M.A. Ferreira sp. nov.

CML (Coleção Micológica de Lavras): CML3847=CBL02

Etymology: *Greek*, *Crypto*, hidden, referring to the stromatic tissue of the base, and *porthe*, disease, describing that the fungus occurs on dead and dying bark and on stems/branches of living trees, and occurs on cankers.

Type species: *Cryptoporthe reticulata* M.E.S. Oliveira, G. A. Silva & M.A. Ferreira

Diagnosis: differs from other genera by internal cavities similar to convoluted locules and presence of ostiolar hyphae. *Cryptoporthe* is similar to species of *Aurapex*, *Celoporthe*, *Crysoporthe*, *Corticimorbus*, and *Luteocirrhus* considering the color of conidiomatal base fuscous black and shape of conidiomata. Conidiomata of the four genera are orange, not pulvinate, and without necks. The conidia are variable having cylindrical, fusoid, oval, and allantoid shapes. *Cryptoporthe* shares the presence of necks with mature *Aurantioporthe*, *Celoporthe*, *Chrysoporthe*, *Latruncellus*, *Rostraureum* and *Ursicullum* and distinguished for absence of paraphyses of the *Aurifilum*, *Celoporthe*, *Holocryphia*, *Immersioporthe*, *Luteocirrhus*, and *Microtia*. *Cryptoporthe* is the only genera with the presence of ostiolar hyphae (Table 2). Based on the phylogenetic analyses and combined analysis of 5.8S rRNA and BT genes, and LSU and ACT regions, this genus is separated from other genera in the Cryphonectriaceae. It is also different from any previously described genus and species in this family. Thus, a novel genus and species is consequently described to accommodate this fungus.

Description: The characteristics of the asexual structures on *Ca. brasiliense* are typical of fungi in the Cryphonectriaceae. These included fuscous-black stromatic tissue. Stromatic tissue stained purple in 3% KOH and orange in lactic acid staining. The fungus from *Ca. brasiliense* had superficial neck and immersed conidiomatal base, stromatic tissues of *textura globulosa* in the margin and *intricate* in the center, fuscous-black to orange in the center, conidiomata pyriform to pulvinate with internal cavities, similar multilocules and convoluted, with one neck, ostiolar septate single or branched hyphae. *Conidiophores* are aseptate, flask-shaped, hyaline. *Conidiogenous cells* are flask-shaped with attenuated apices. *Paraphyses* are absent. *Conidia* are hyaline, cylindrical, fusoid to ellipsoid, aseptate.

Cryptoporthe reticulata M.E.S. Oliveira & G. A. Silva, M.A. Ferreira sp. nov. (Fig. 5)
CML: CML3847=CBL02
Etymology: refers to the conidiomatal base forming a network (reticulata=latin) in the longitudinal section

Type: **Brazil**: Lavras, Minas Gerais State: Reserva Ecológica Quedas do Rio Bonito, isolated from pycnidia in branches or wood of *Caryocar brasiliense*, 14 Oct. 2016.

Description: *Conidiomata* on bark, fuscous-black to orange in the center, pyriform to pulvinate, immersed base and superficial neck (Fig. 5A-B), multilocular convoluted, ostiolate; stromatic tissue *textura globulosa* at the margin and *intricate* at the center; conidiomatal bases (Fig. 5C-E) 136.6- (295.7) -465.0 µm long always below bark surface, 243.9- (429.6) - 826.3 µm diam; *Hyphae ostiolars* 18.1 – (64.5) – 117.1 µm long (Fig. 4F), absent or shorter when young and present when mature with orange drops containing conidia, septate, and branched (Fig. 4G); *Locules* 33.9– (168.81) - 1030.0 x 16.4-(51,6)- 129.7 µm diam (Fig. 4H). Conidiomatal neck always above the bark surface, 214.4 – (563.97) – 931.2. *Conidiophores* non-septate, flask-shaped, 5.6 – (6.8) – 8.4 µm long. *Conidiogenous cells* 3.7 – (4.7) – 5.8 µm long flask-shaped with attenuated apices (Fig. 4I). Paraphyses absent. *Conidia* hyaline, cylindrical, fusoid to allantoid, aseptate, 2.3 – (4.3) – 6.5 x 0.8 – (1.42) – 2.2 µm (Fig.4J) exuded as orange droplets (Fig. 1A-B).

Culture characteristics: On PDA forms a fluffy mycelium with even margins, colony color varied, white and beige when young, turning yellow, orange or purple after 15 days. Optimal growth temperature varying from 25 to 30°C, no growth at 20°C. Sexual morph no found.

Substrate and distribution: On bark and wood of *Caryocar brasiliense* in Lavras, Minas Gerais State, Brazil.

Pathogenicity assays

Two months after inoculation, all six *C. reticulata* isolates produced lesions on *Ca. brasiliense* stems (Fig. 6). Four months after inoculation, the presence of typical pycnidia was observed on the bark and xylem of the inoculated plants. In the comparison tests the average lesion length caused by the six isolates were all significantly different ($P < 0.05$) from control (Fig. 7). Three groups were observed according to the size of lesion. Isolates CBP03 and CBSC01 grouped with 3.31 and 2.95 cm lesions. The isolates CBSC04, CBL03, CBING01, and CBL02 were grouped with lesions varying from 1.50 to 2.03 cm. After four months, lesions with 10 cm in length and pycnidia formation were observed. The Koch's postulates were confirmed by re-isolation of the fungus at the end of the pathogenicity assays. The fungi were recovered from all inoculated plants.

Discussion

In this study, we isolated and characterized a novel fungus from *Ca. brasiliense*, an important tree species that occurs in the Brazilian Cerrado biome. After the analyses, it was observed that the novel fungus belongs to the Cryphonectriaceae family. The fungus was present in different locations in the Minas Gerais State, in Brazil. The genus description of this fungus was based on sequencing data and on phylogenetic analyses from multiple genic regions as well as on morphological characteristics. The novel genus was named *Cryptoporthe* and the species *C. reticulata*.

The first report of a species of Cryphonectriaceae in Brazil occurred in 1976 (Hodges et al., 1976) reported as *Diaporthe cubensis*, renamed as *Cryphonectria cubensis* and, currently, named as *C. cubensis* (Gryzenhout et al. 2009). No other genus of the family was reported on any tree species in Brazil so far. In general, among the species of Cryphonectriaceae that have been widely reported from Myrtaceae trees. In Brazil, *C. cubensis* is the most important disease of commercial *Eucalyptus* plantations in Brazil (Ferreira and Milani 2004). Resistant clones are selected through controlled crosses between species of *Eucalyptus* (Assis and Mafia 2007; Alfenas et al. 2009). This pathogen also causes canker in several other species in the *Myrtaceae* and *Melastomataceae* families. Previously, *C. cubensis* was reported in Brazil only from *T. granulosa* and *Marlierela edulis* Nied. (Boerboom and Maas 1970; Hodges et al.1976, 1979; Sharma et al. 1985; Myburg et al. 2003; Rodas et al. 2005; Seixas et al. 2004; Barreto et al. 2006; Gryzenhout et al. 2006). Recently, Santos (2015) described *Chrysoporthe doradensis* Gryzenh. & M. J. Wingf. in *Eucalyptus* spp., *T. granulosa* and *T. heteromalla* (D.Don) Cogn. in Brazil. The present study suggests that the Cryphonectriaceae have wider host and geographical ranges in Brazil.

The genus described in this study, C*ryptoporthe*, has similarities with other genera based on structure, shape and color, presence or absence of neck, type of stromatic tissue, presence or absence of paraphyses. In this study the morphological characteristics were compared with other 20 genera belonging to the Cryphonectriaceae family and *Cryptoporthe* was more similar to *Aurapex*, *Celoporthe*, *Crysoporthe* (Gryzenhout et al. 2004, 2006, 2009), *Corticimorbus* (Chen et al., 2016), and *Luteocirrhus* (Crane and Burgess, 2013) according to the color of conidiomatal base with fuscous black and shape of conidiomata. Conidia of *Cryptoporthe* were variable, having cylindrical, fusoid, oval, and allantoid shapes. *Cryptoporthe* shares the presence of neck with mature *Aurantioporthe* (Beier et al., 2015), *Celoporthe*, *Chrysoporthe* (Gryzenhout et al. 2009), *Latruncellus* (Vermeulen et al. 2011),

Rostraureum, and *Ursicullum* (Gryzenhout et al. 2009). The absence of paraphyses distinguishes *Cryptoporthe* from the *Aurifilum* (Begoud et al. 2010), *Celoporthe*, *Holocryphia* (Gryzenhout et al. 2009, Chen et al. 2013b), *Immersioporthe* (Chen et al. 2013a), *Luteocirrhus* (Crane and Burgess, 2013), and *Microtia* (Gryzenhout et al. 2009) species. There is a high variation in the description of stromatic tissue in the Chryphonectriaceae genera. However, the majority of genera were described as pseudoparenchymatous and prosenchymatous tissues, which were also found in for *Cryptoporthe* in this study. Some studies specify the different *textura* of stromatic tissues. For example, the base conidiomatal can have different tissue type in the neck, as *Chrysoporthe* species has (Gryzenhout et al., 2009). The genera used for the comparison to *Cryptoporthe*, usually, cause canker on trunk or stem. However, two genera compared, *Aurantiosacculus* and *Filiocryphia*, were found causing leaf spot as reported by Crous et al. 2012 and Cheewangkoon et al. 2009, respectively. Presence of ostiolar hyphae is observed only in the *Cryptoporthe* genera. This is the most important characteristic for its identification and can be used as a morphological marker for this genus. There is no other genus in Cryphonectriaceae with such marker.

The isolates collected from *Ca. brasiliense* were isolated from diseased or dead trees showing cankered stems and branches, and the results of the pathogenicity tests confirmed that the fungus produces lesions on stems of seedlings of *Ca. brasiliense*, and thus, meeting the Koch's postulates and confirming that *C. reticulata* is the causal agent of the cankers in the sampled plants. Some seedlings in the pathogenicity tests showed the presence of new shoots below the lesions confirming the symptoms of sprouting emission, which is also observed in the symptomatic plants under field conditions.

Caryocar brasiliense, family Caryocaraceae is a native tree of the Brazilian Cerrado. The absence of teleomorph stage in *C. reticulata* suggests that its origin can be from other places or hosts. For example, Carne and Brugess (2013) suggested that the center of origin of *L. shearii* C. Crne & T. I. Burgess is from elsewhere based on the absence of teleomorph and the same speculation was discussed for *Holocryphia. eucalypti* Gryzenh. & M.J. Wingf. (Nakabonge et at. 2008). In Brazil, there was no report of the teleomorph of *C. cubensis* on *Tibouchina* species, but in recent studies focusing in *Chrysoporthe* isolates (Oliveira, 2018), the teleomorph was observed in *T. heteromalla* at two sites in Minas Gerais. More studies on native trees are needed to confirm the presence or absence of teleomorph of *C. reticulata* in Brazil. Nevertheless, the importance of the pathogen for causing damages in *Ca. brasiliense* was identified in this study as well as high aggressiveness to the host was observed mainly in

rocky areas of the Brazilian Cerrado where the plant development is not ideal and thus perhaps facilitating fungal infection under some stress conditions.

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Table 1. Isolates of *Cryphonectriaceae* family and outgroup included in this study.

Species	Culture accession N°.	Host/ Substrate	Location	GenBank Accession Number				
				LSU	ACT	ITS	BT1	BT2
Amphilogia gyrosa	CMW10469	*Elaeocarpus dentatus*	New Zealand	AY194107	N/A	AF452111	AF525707	AF525714
	CMW10470	*E. dentatus*	New Zealand	AY194108	N/A	AF452112	AF525708	AF525715
Amphilogia gyrosa	YMJ91123101	*	Taiwan	N/A	EF025600	N/A	N/A	N/A
Aurantioporthe corni	ATCC66834	*Cornus alternifolia*	USA	AF277133	N/A	N/A	N/A	N/A
	CMW10526	*C. alternifolia*	USA	N/A	N/A	DQ120762	AH015163	AH015163
	CBS 24590	*C. alternifolia*	USA	AF408343	N/A	N/A	N/A	N/A
Aurantiosacculus eucalyptorum	CPC 13229	*Eucalytpus globulus*	Australia	N/A	N/A	N/A	N/A	N/A
Aurapex penicillata	CMW10030	*Miconia theaezans*	Colombia	N/A	N/A	AY214311	AY211439	AY214275
	CMW11295	*M. theaezans*	Colombia	AY194089	N/A	N/A	N/A	N/A
	CMW10035	*M. theaezans*	Colombia	N/A	N/A	AY214313	AY214241	AY214277
Aurifilum marmelostoma	CMW28285	*Terminalia mantaly*	Cameroon	HQ171215	N/A	FJ882855	FJ900585	FJ900590
	CMW28288	*T. ivorensis*	Cameroon	HQ171216	N/A	FJ882856	FJ900586	FJ900591
Celoporthe dispersa	CMW9976	*Syzygium cordatum*	South Africa	HQ730853	N/A	DQ267130	DQ267136	DQ267142
	CMW9978	*S.cordatum*	South Africa	HQ730854	N/A	AY214316	DQ267135	DQ267141
Celoporthe eucalypti	CMW26900	*Eucalyptus* sp.	China	HQ730862	JQ862992	HQ730836	HQ730816	HQ730826
	CMW26908	*Eucalyptus* sp.	China	HQ730863	JQ862993	HQ730837	HQ730817	HQ730827
Celoporthe fontana	CMW29375	*S. guineense*	Zambia	N/A	N/A	GU726940	GU726952	GU726952
	CMW29376	*S. guineense*	Zambia	N/A	N/A	GU726941	GU726953	GU726953
Celoporthe indonesiensis	CMW10781	*S.aromatium*	Indonesia	N/A	N/A	N/A	N/A	N/A
Celoporthe syzygii	CMW34023	*S. cumini*	China	N/A	N/A	HQ730831	HQ730811	HQ730821

	CMW24912	*S. cumini*	China	N/A	N/A	N/A	N/A	N/A
Celoporthe woodiana	CMW13936	*Tibouchina granulosa*	South Africa	N/A	N/A	DQ267131	DQ267137	DQ267143
Chysocrypta corymbiaie	CBS132528	*Corymbia* sp.	Australia	JX069851	N/A	N/A	N/A	N/A
Crysofolia colombiana	CPC19279	*Eucalyptus urophylla X E. grandis*	Colombia	KR476771	N/A	N/A	N/A	N/A
Crysoporthe austroafricana	CMW62	*Eucalyptus* sp.	South Africa	AY194097	N/A	N/A	N/A	N/A
	CMW 10192	*S. cordatum*	South Africa	N/A	GQ290163	AY142299	GQ290176	GQ290187
	CMW 9327	*T. granulosa*	South Africa	N/A	GQ290173	GQ290158	GQ290185	GQ290194
Crysoporthe cubensis	CMW 10028	*M. rubiginosa*	Colombia	N/A	QG290161	GQ290153	GQ290175	GQ290186
	CMW 10669	*Eucalyptus* sp.	Republicof Congo	N/A	GQ290171	GQ2900154	GQ290177	GQ290188
	CMW 12734	*Rhyncanthera mexicana*	Mexico	N/A	GQ290159	DQ368769	DQ368791	GQ290191
	CBS101281	E.urophylla	Cameroon	AF408338	N/A	N/A	N/A	N/A
	CMW10453	*E. saligna*	Democratic Republic of Congo	AF408339	N/A	N/A	N/A	N/A
	CE3	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639121	KX639087	KX639104
	CE12	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639122	KX639088	KX639105
	CE22	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639123	KX639089	KX639106
	CE26	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639124	KX639090	KX639107
	CC3	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639125	KX639091	KX639108
	CC4	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639126	KX639092	KX639109
	CT2	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639127	KX639093	KX639110
	CT24	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639128	KX639094	KX639111

	CT25	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639129	KX639095	KX639112
	CT30	*Eucalytptus* sp.	Brazil	N/A	N/A	KX639130	KX639096	KX639113
Chrysoporthe deuterocubenis	CMW8758	*E. grandis*	Venezuela	AY194098	N/A	N/A	N/A	N/A
C. deuterocubensis	CMW 8650	*S. aromaticum*	Indonesia	N/A	GQ290172	AY084001	AY084024	GQ290193
	CMW 2631	*E. marginata*	Australia	N/A	GQ290174	GQ290157	GQ290184	AF543825
	CMW 12745	*T. urvilleana*	Singapura	N/A	GQ290160	DQ368764	GQ290183	DQ368781
	CMW 17178	*T. urvilleana*	Tailandia	N/A	GQ290164	DQ368766	DQ368785	GQ290192
C. doradensis	CMW 11287	*E. grandis*	Ecuador	N/A	GQ290167	GQ290156	GQ290179	GQ290190
	CMW9126	*E. deglupta*	Ecuador	N/A	N/A	DQ224037	DQ224044	DQ224045
	CMW9125	*E. deglupta*	Ecuador	N/A	N/A	DQ224036	DQ224042	DQ224043
	CMW9123	*E. deglupta*	Ecuador	N/A	N/A	DQ224034	DQ224038	DQ224039
	CMW9124	*E. deglupta*	Ecuador	N/A	N/A	DQ224035	DQ224040	DQ224041
	CML3416	*Eucalyptussp.*	Brazil	N/A	N/A	KX639131	KX639097	KX639114
	CE34	*Eucalyptussp.*	Brazil	N/A	N/A	KX639132	KX639098	KX639115
	CE37	*Eucalyptussp.*	Brazil	N/A	N/A	KX639133	KX639099	KX639116
	CML3417	*Eucalyptussp.*	Brazil	N/A	N/A	KX639134	KX639100	KX639117
	CE45	*Eucalyptussp.*	Brazil	N/A	N/A	KX639135	KX639101	KX639118
	CT5	*T. granulosa*	Brazil	N/A	N/A	KX639136	KX639102	KX639119
	CT6	*T. granulosa*	Brazil	N/A	N/A	KX639137	KX639103	KX639120
Chrysoporthe hodesiana	CMW 9995	*T. semidecandra*	Colombia	N/A	GQ290162	AY956969	AY956978	AY956977
Chrysoporthe	CMW 10625	*T. theaezans*	Colombia	N/A	GQ290170	AY262399	AY262391	AY956979

Hodesiana								
C. inopina	CMW 12727	*T. lepidota*	Colombia	N/A	GQ290169	DQ368777	GQ290180	DQ368806
	CMW 12731	*T. lepidota*	Colombia	N/A	GQ290168	DQ368779	GQ290182	DQ368811
	CMW 12729	*T. lepidota*	Colombia	N/A	N/A	DQ368778	DQ368808	DQ368809
C. syzygiicola	CMW29942	*S. guineense*	Zambia	N/A	N/A	FJ655007	FJ805232	FJ805238
	CMW29940	*S. guineense*	Zambia	N/A	N/A	JN942335	FJ805230	FJ805236
C. zambiensis	CMW29930	*E. grandis*	Zambia	N/A	N/A	FJ655004	FJ858711	FJ805235
	CMW29928	*E. grandis*	Zambia	N/A	N/A	FJ655002	FJ858709	FJ805233
Corticimorbus sinomyrti	CERC629	*Rhodomyrtus tomentosa*	China	KT167179	N/A	KT167169	KT167189	KT167189
	CERC3631		China	KT167180	N/A	KT167170	KT167190	KT167190
Cryphonectria decipiens	CMW10436	*Quercus suber*	Portugal	JQ862750	N/A	AF452117	AF525703	AF525710
Cryphonectria macrospora	CMW10914	*Castanea cuspidata*	Japan	JQ862749	N/A	N/A	N/A	N/A
Cryphonectria parasitica	CMW7048	*Q. virginiana*	USA	AY194100	N/A	AF368330	AF273076	AF273470
	11552		USA	GU989031	N/A	N/A	N/A	N/A
Cryphonetria radicalis	CMW10477		Italy	AY194102	N/A	AF368328	AF368347	AF368347
Cryptometrion astuescens	CMW18790	*Eucalyptus grandis*	Indonesia	HQ171211	N/A	GQ369458	GQ369455	GQ369455
	CMW18793	*Eucalyptus grandis*	Indonesia	HQ171212	N/A	N/A	N/A	N/A
Diversimorbus metrosiderotis	CMW37321	*Meterosideros angustifolia*	South Africa	JQ862827	N/A	JQ862870	JQ862911	JQ862952
	CMW37322	*Met. angustifolia*	South Africa	JQ862828	N/A	JQ862871	JQ862912	JQ862953
Endothia gyrosa	AR3396	*Quercus* sp.	USA	AF362555	N/A	N/A	N/A	N/A
	CMW2091	*Q. palustres*	USA	AY194114	N/A	AF368325	AH011601	AH011601
	CMW10442	*Q. palustres*	USA	AY1941115	N/A	AF368326	AH011602	AH011602
Holocryphia	CMW37887	*Met angustifolia*	South Africa	JQ862811	N/A	JQ862854	JQ862895	JQ862936

capensis								
	CMW14545	*E. grandis*	South Africa	JQ862963	N/A	N/A	N/A	N/A
Holocryphia eucalypti	CMW7036	*E. grandis*	South Africa	JQ862962	N/A	N/A	N/A	N/A
	CMW7033	*E. grandis*	South Africa	JQ862794	N/A	JQ862837	JQ862878	JQ862919
	CMW7035	*E. grandis*	South Africa	JQ862795	JQ862961	JQ862838	JQ862879	JQ862920
Holocryphia gleniana	CMW37334	Met. angustifolia	South Africa	N/A	N/A	JQ862834	JQ862875	JQ862916
Holocryphia mzansi	CMW37337	Met. angustifolia	South Africa	JQ862798	N/A	JQ862841	JQ862882	JQ862923
Immersioporthe knoxdaviesiana	CMW37314	*Rapanea melanophloeos*	South Africa	JQ867555	N/A	JQ862765	JQ862785	JQ862775
	CMW37315	*R. melanophloeos*	South Africa	JQ862756	N/A	JQ862766	JQ862786	JQ862776
Latruncela aurorae	CMW28274	*Galpinia transvaalica*	Swaziland	HQ171213	N/A	GU726946	GU726958	GU726958
	CMW28276	*G. transvaalica*	Swaziland	HQ730872	N/A	GU726947	GU726959	GU726959
Luteocirrhus shearii	CMW130775	*Banksia baxteri*	Australia	KC197018	N/A	KC197024	KC197015	KC197009
	CMW130776	*B. baxteri*	Australia	KC197019	N/A	KC197021	KC197012	KC197006
Microthia havanensis	CMW11299	*Myricia faya*	Madeira	AY194087	N/A	N/A	N/A	N/A
	CMW11300	*M. faya*	Madeira	AY194088	N/A	N/A	N/A	N/A
	CMW11301	*M. faya*	Azores	N/A	N/A	AY214323	AY214251	AY214287
	CMW14550	*E. saligna*	Mexico	N/A	N/A	DQ368735	AH015792	AH015792
Rostraurerum tropicale	CMW9972	*T. ivorensis*	Ecuador	AY194092	N/A	AY167436	AY167426	AY167436
	CMW10796	*T. ivorensis*	Ecuador	N/A	N/A	AY167438	AY167428	AY167433
Ursicollum fallax	CMW18119	*Coccoloba uvifera*	USA	EF392860	N/A	DQ368755	DQ368758	DQ368759
	CMW18115	*C.uvifera*	USA	N/A	N/A	DQ368756	DQ368760	DQ368761
Diaporthe ambigua	CMW5587			N/A	N/A	AJ458388	AF543822	AF543820
Diaporthe eres	AR3538	*Acer campestre*	Austria	AF408350	N/A	N/A	N/A	N/A
Diaporthe fibrosa	AR3425	*Rhamnus*	Austria	AF408351	N/A	N/A	N/A	N/A

		catharticus						
				N/A	N/A	N/A	N/A	N/A
Harknessia eucalypti	CBS342	*E. regnans*	Australia	AF408363	N/A	N/A	N/A	N/A
	CBS 111122	*N/A*		AY720823	N/A	N/A	N/A	N/A

Abbreviations: Large subunit of rDNA (LSU), Internal transcribed spacer (ITS), Actin (ACT), and β-tubulin (BT) region 1 (BT1) and 2 (BT2).

Designation of isolates and culture collections: CERC= China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), China; CMW= Tree Protection Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. CML= Coleção Micológica de Lavras, Federal University of Lavras; CT= canker *Tibouchina*; CE= Canker eucalypti; CC= canker *Chrysoporthe*. N/A= not available.

Table 2. Morphological characteristics of *Cryptoporthe* compared with other genera of *Cryphonectriaceae*.

Genera	Structure of conidiomata			Conidial locules	Conidiogenous cells	Conidial shape	Paraphyses	Conidiomatal neck	References
	Color	Position in the bark	Shape						
Amphilogia	Orange	Superficial	Conical to pyriform to fluted	Unilocular	Cylindrical to flask-shaped	Oblong to slightly curved	Absent	Absent	Gryzenhout et al., 2009
Aurantioporthe	Orange	Immersed to semi-immersed	Irregularly subsferical to flattened	Unilocular	Obclavate	Fusiform to ellipsoidal	Absent	Present, single ostiole	Beier et al., 2015
Aurantiosaccullus	Bright orange	Subepidermal	Subglobose to flattened	N/D	Lageniform to subcylindrical	Sigmoid	N/D	N/D, ostiole central	Crous et al., 2012
Aurapex	Fuscous black	Superficial to slightly immersed	Globose to pyriform		Cylindrical to flask-shaped	Obtuse	Absent	Orange neck	Gryzenhout et al., 2009
Aurifilum	Orange	Semi-immersed, broadly convex	Pulvinate to pyriform	Uni to multilocular, even to convulated	Cylindrical	Cylindrical to allantoid	Present	Absent, ostiolar opening darkened	Begoud et al., 2010
Celoporthe	Fuscous black when mature	Superficial to slightly immersed	Pulvinte to conical	Unilocular	Cylindrical	Cylindrical to ovoid, occasionally allantoid	Present	With or without short attenuated necks	Gryzenhout et al., 2009
Cryphonectria	Orange	Semi-immersed	Pulvinate	Uninocular or multilocular	Cylindrical to flask-shaped	Cylindrical	Absent	Absent, non-ostiolate	Gryzenhout et al., 2009
Chrysomorbus	Orange when mature	Superficial to semi-immersed	Convex to globose	Uni to multilocular, often convulated	Cylindrical to flask-shaped	Fusoid to oval	Absent	Absent, non-ostiolate	Chen et al., 2017
Chrysoporthe	Fuscous-black	Superficial to slightly immersed	Pyriform to clavate, sometimes pulvinate	Convoluted occasionally multilocular	Cylindrical to flask-shaped	Oblong	Absent	One or several necks	Gryzenhout et al., 2009
Cryptometrion	Orange	Semi-immersed	Globose	N/D	Cylindrical to flask-shaped	Cylindrical	Absent	Absent	Gryzenhout et al., 2010
Cryptoporthe	Fuscous black to orange	Superficial neck and immersed base	Pulvinate to pyriform	Multiloculate and convoluted	Flask-shaped	Fusoid, oval, and ellipsoid	Absent	Present, one neck and presence of ostiolar hyphae	This study
Corticimorbus	Orange to black when mature	Superficial	Conical to globose	Uni to multilocular, often convulated	Cylindrical to flask-shaped	Fusoid to oval	Absent	Absent	Chen et al., 2016
Endothia	Orange	Superficial	Pulvinate	Multiloculate labyrinthine	Cylindrical to flask-shaped	Cylindrical	Absent	Absent	Gryzenhout et al., 2010
Filiocryphia	Pale to medium brown	Subsuperficial	Pulvinate to subglobose	Uni to multilocular, with convulated inner surface	Cylindrical	Elliposid, straight to irregulary curved	N/D	With or without ostiole	Cheewangkoon et al., 2009
Holocryphia	Orange	Semi-immersed	Pulvinate	Uni to multilocular, and convulated	Cylindrical	Cylindrical	Present	Absent	Gryzenhout et al.,2005, 2009
Immersiporthe	Orange	Immersed to semi-immersed	Puvinate	Uni to multilocular, and convulated	Cylindrical to flask-shaped	Cylindrical to fusoid, occasionally allantoid	Present	Absent	Chen et al., 2013
Latruncellus	Orange	Semi-immersed	Conical	Uni to multilocular, and convulated	Subulate to flask-shaped	Cylindrical	N/D	Present	Vermeulen et al., 2011

Luteocirrhus	Fuscous black	Semi-immersed	Pulvinate	Uni, multilocular or convoluted	N/D	Cylindrical or slightly allantoid	Present	Ostiolate	Crane et al., 2013
Microthia	Orange	Semi-immersed to superficial	Pulvinate	Uni, multilocular or convoluted	Cylindrical	Cylindrical	Present	Absent	Gryzenhout et al.,2005, 2009
Rostraureum	Yellow to orange	Superficial to slightly immersed	Clavate or rostrate	Unilocular	Cylindrical	Cylindrical	Absent	One to three necks	Gryzenhout et al.,2005, 2009
Ursicollum	Orange	Superficial to slightly immersed	Pyriform to rostrate	Unilocular strongly convoluted	Cylindrical	Cylindrical	Absent	One to three necks	Gryzenhout et al.,2005, 2009

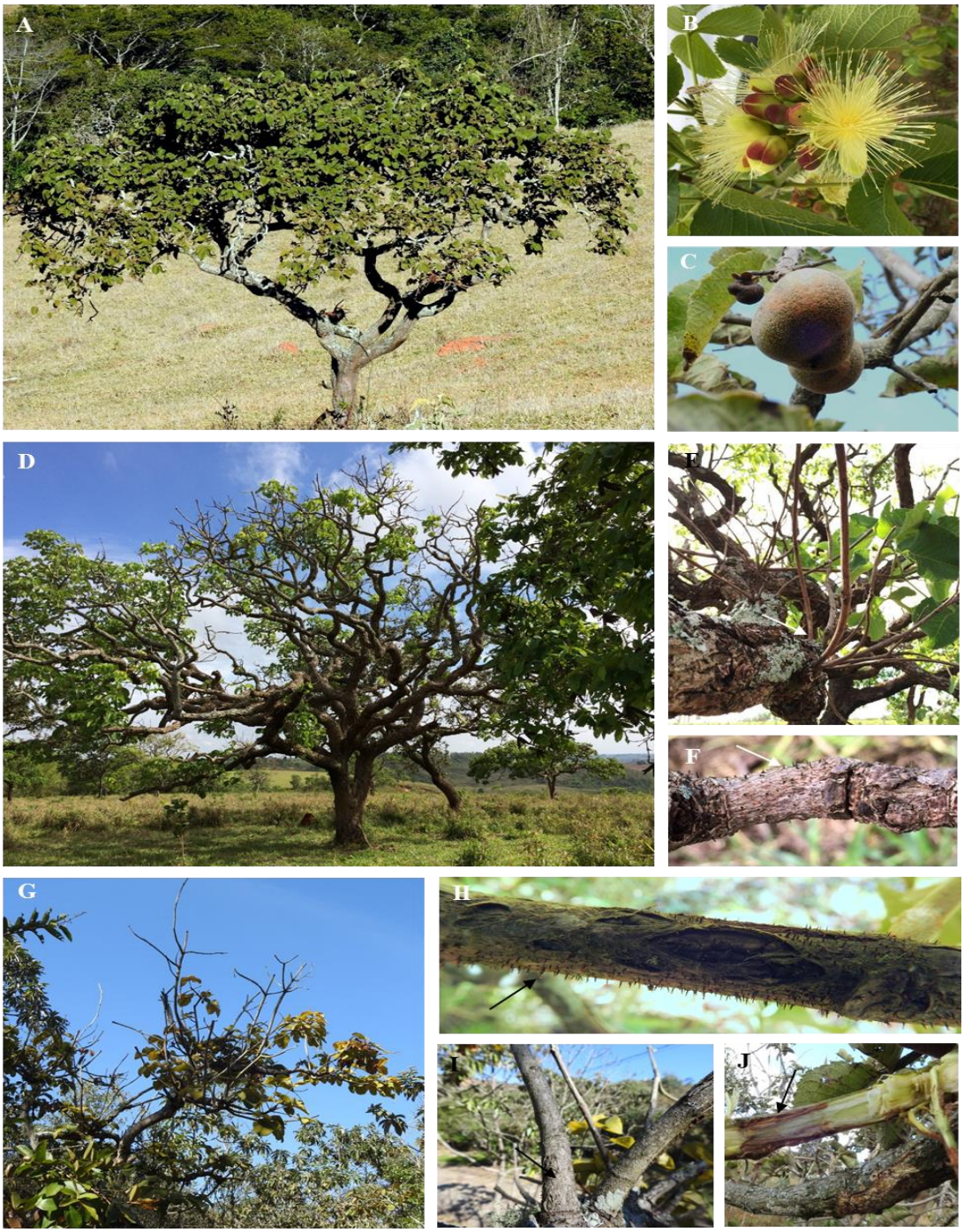

Fig 1. Symptomatology of *Cryptoporthe reticulata* on *Caryocar brasiliense*. A. Healthy tree of *Ca. brasiliense* in Lavras, Minas Gerais. B. Flower of *Ca. brasiliense*. C. Fruit in development on *Ca. brasiliense*. D. Diseased tree in the Piumhi, Minas Gerais. E. Presence of broom-like shoots above and below the branches with lesions. F. Presence of signs on branches. G. Diseased tree in Lavras, Minas Gerais with small development. H. Branches showing pycnidia exuding orange masses. I. Shoots showing signs. J. Discoloration of shoots caused by *Cryptoporthe reticulata*

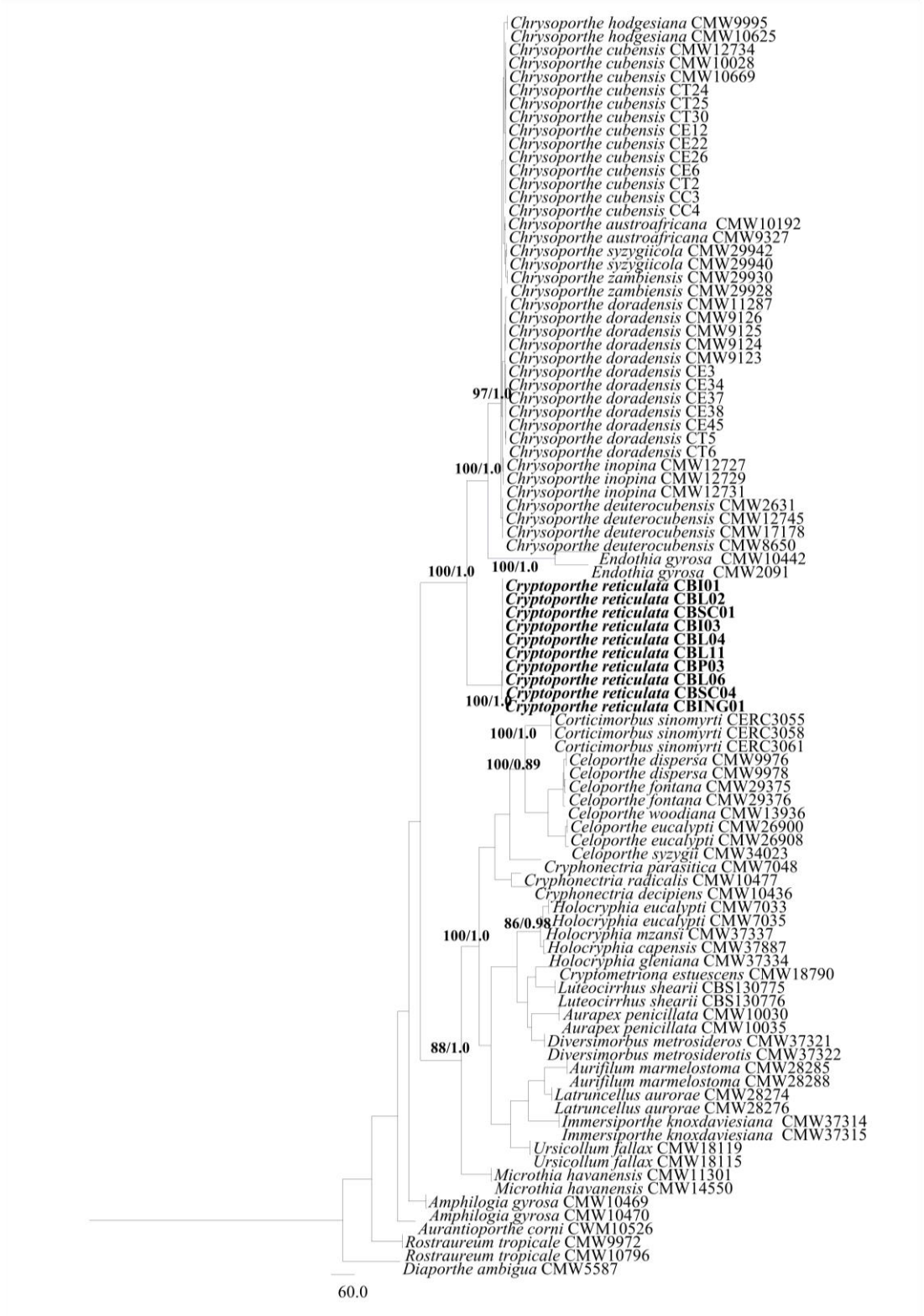

Fig 2. Cladogram based on maximum parsimony (MP) analysis of a combined DNA sequence data set of regions of the Internal Transcribed Spacer of rRNA region (ITS), and Beta-tubulin (BT) gene. Bootstrap value >70% for MP (maximum parcimony) analyses are presented at the branches (MP/posterior probability). Samples isolated from *Ca. brasiliense* are highlighted.

Fig 3. Cladogram based on maximum parsimony (MP) analysis of LSU DNA sequences for various genera in the *Diaporthales*. Bootstrap value >70% for MP (maximum parcimony) analyses are presented at branches (MP/posterior probability). Samples isolated from *Ca. brasiliense* are highlighted.

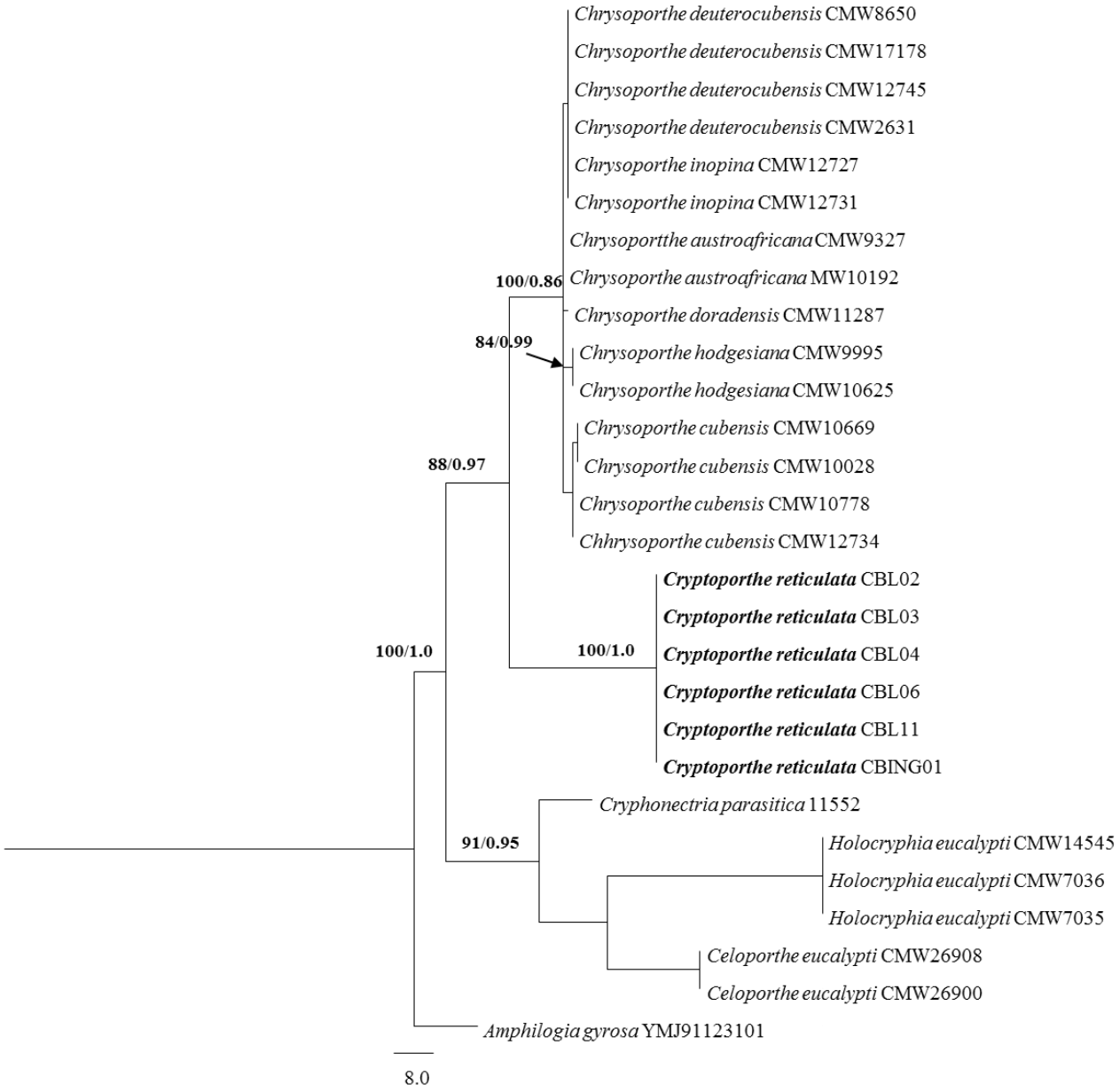

Fig 4. Cladogram based on maximum parsimony (MP) analysis and basyane of ACT DNA sequences for various genera in the *Diaporthales*. Bootstrap value >70% for MP (maximum parcimony) analyses are presented at the branches (MP/posterior probability). Samples isolated from *Ca. brasiliense* are highlighted.

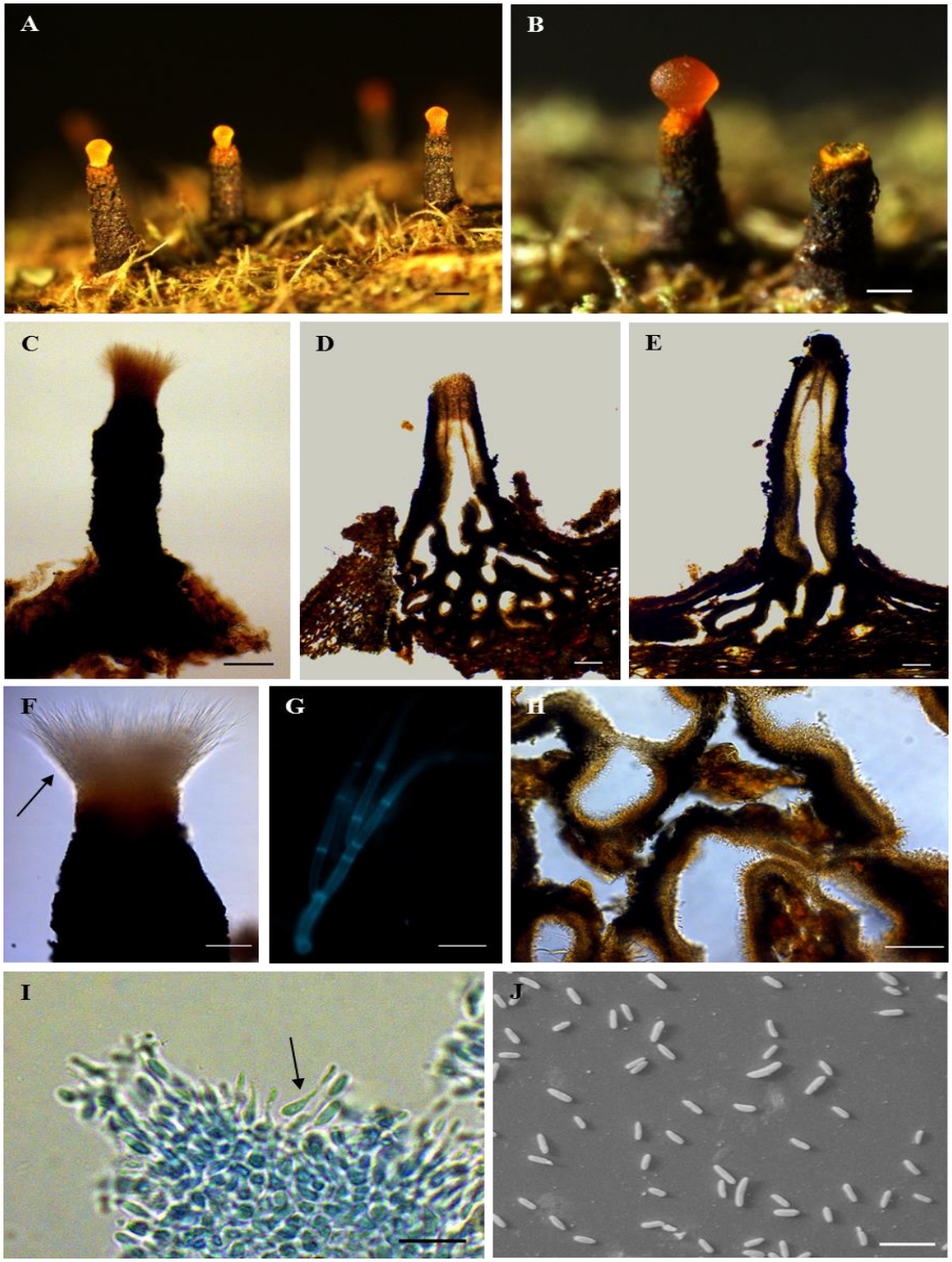

Fig 5. *Cryptoporthe reticulata* (CBI03). A. Coniodamata with orange masses of conidia. B. Conidiomata (young) without masses of conidia. C. Conidiomata showing ostiolar hyphae with orange masses of conidia. D-E. Horizontal cross sections of conidioma. F. Ostiolar hyphae at top of neck. G. Ostiolar hyphae containing septate branches. H. Conidiogenious cells flask-shaped (arrow). I. Conidia. Bars: A and B=200µm; C, D, and E=100µm; F and H=50µm; and G=10µm.

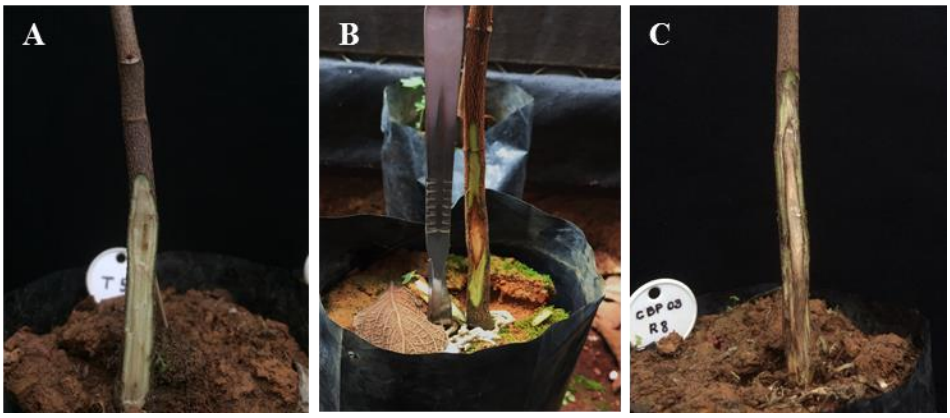

Fig 6. Pathogenicity assays on *Ca. brasiliense* (pequi). A. Control. B. Seedlings showing lesion 60 days after inoculation. C. Seedlings showing lesion 120 days after inoculation.

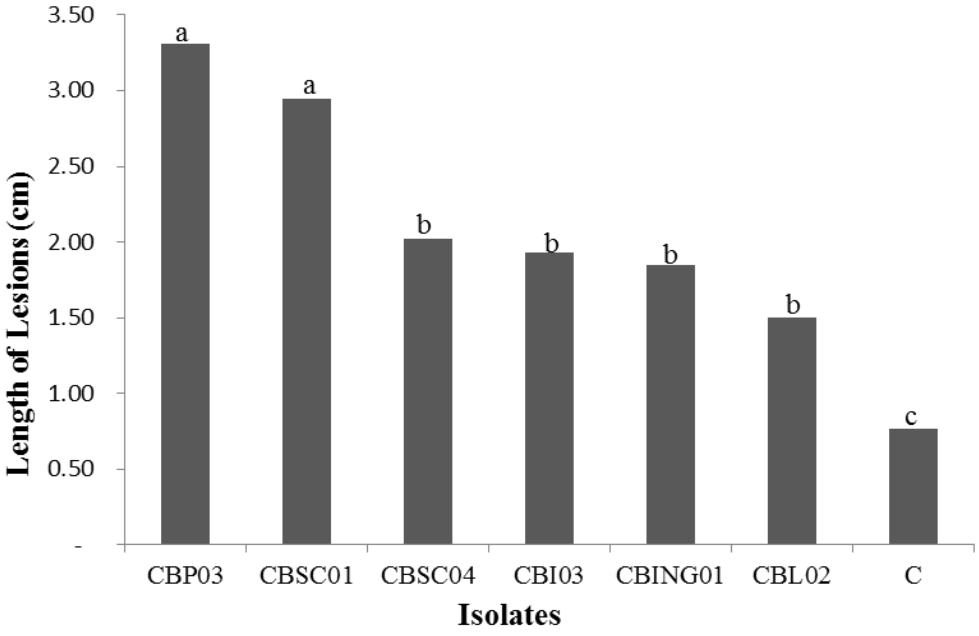

Fig 7. Lesion length (cm) of *Cryptoporthe reticulata* 60 days after the inoculation. Mean followed by the same letter were not grouped by the Scott-Knott test. C= Control.