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Colletotrichum species associated to anthracnose in passion fruit Brazil

Espécies de *Colletotrichum* associadas à antracnose em maracujazeiro no Brasil.

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ABSTRACT

Brazil is the world's leading producer of passion fruit. However, among the diseases that affect the culture of passion fruit, the anthracnose, caused by species of the *Colletotrichum* genus causes great socioeconomic losses in northeastern Brazil. The aims of this study were to identify species of the *Colletotrichum* genus that infect passion fruit leaves, using multi-locus phylogenetic analysis associated to morpho-cultural characteristics, as well as to evaluate the pathogenicity in different hosts. Isolates of the *Colletotrichum* genus were obtained from passion fruit leaves showing typical symptoms of the disease, collected in commercial plantations in the state of Alagoas, Brazil. The pathogenic isolates were identified based on the sequence of the *GAPDH*, *TUB*2, *CHS*-1 genes and the ITS-rDNA region. For the cultural characterization of the identified species, the mycelial growth rate and aspect of the colonies were evaluated. In the morphological characterization the length and width of 50 conidia and appressoria were measured. The multi-locus phylogenetic analysis associated to morpho-cultural characteristics of fiftee isolates revealed five species of *Colletotrichum*: *C. fructicola*, *C. theobromicola*, *C. plurivorum*, *C. brevisporum*, *Colletotrichum* sp. This is the first report of *C. fructicola* and *C. theobromicola* in the world and of *C. brevisporum* and *C. plurivorum*in Brazil. The *Colletotrichum* species were pathogenic to different inoculated hosts (mango, passion fruit, banana, papaya and guava), with the exception of the *C. brevisporum*, *Colletotrichum* sp. and *C. plurivorum* to guava fruit.

RESUMO

O Brasil é o maior produtor mundial de maracujá. Entretanto, dentre as doenças que acometem a cultura do maracujazeiro, a antracnose, causada por espécies do gênero *Colletotrichum* causa grandes prejuízos socioeconômicos no nordeste brasileiro. Os objetivos deste estudo foram identificar espécies do gênero *Colletotrichum* que infectam folhas de maracujazeiro, utilizando análise filogenética multilocus associada a características morfoculturais, bem como avaliar a patogenicidade em diferentes hospedeiros. Isolados do gênero *Colletotrichum* foram obtidos de folhas de maracujá com sintomas típicos da doença, coletados em plantios comerciais no estado de Alagoas, Brasil. Os isolados patogênicos foram identificados com base na sequência dos genes GAPDH, TUB2, CHS-1 e na região ITS-rDNA. Para a caracterização cultural das espécies identificadas, avaliou-se a taxa de crescimento micelial e o aspecto das colônias. Na caracterização morfológica foram medidos o comprimento e a largura de 50 conídios e apressórios. A análise filogenética multilocus associada às características morfoculturais de cinqüenta isolados revelou cinco espécies de *Colletotrichum*: *C. fructicola*, *C. theobromicola*, *C. plurivorum*, *C. brevisporum, Colletotrichum* sp. Este é o primeiro relato de *C. fructicola* e *C. theobromicola* no mundo e de *C. brevisporum* e *C. plurivorum* no Brasil. As espécies de *Colletotrichum* foram patogênicas para diferentes hospedeiros inoculados (manga, maracujá, banana, mamão e goiaba), com exceção *de C. brevisporum, Colletotrichum* sp. e *C. plurivorum* para goiaba.

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Introduction

The passion fruit (*Passiflora edulis* Sims *f. flavicarpa* Deg) is a plant that belongs to the Passifloraceae family, composed of 18 genera, *Passiflora* being the one with the largest number of species, estimated at more than 500 (Vanderplank, 1996; Crochemore et al., 2002), and many of them have edible fruits (Araújo, 2007). In addition to fresh consumption, the fruit is also used in the manufacture of pulp, jam and nectar. Its seeds, leaves and roots are used by the pharmaceutical industry as antispasmodics, anthelmintics and sedatives. Its flowers are used by the ornamentation market (Faleiro et al., 2005).

Brazil is the world's largest passion fruit producer with a total production of 690.364 thousand tons, which enables the generation of more than 200 thousand direct and indirect jobs (Ibge, 2020). However, different phytosanitary problems may occur and cause significant damage to the culture, causing losses in its production. Within these problems, anthracnose caused by species of the *Colletotrichum* genus is among the main and impacting diseases found in all producing regions, causing serious damage, especially in times of high temperature associated with high humidity (São José, 2015). Anthracnose symptoms in the leaves appear in the form of watery spots, which evolve in size, acquiring a brown color with dark brown edges. With the coalescence of lesions, large areas of necrotic tissue are formed, with cracks and intense leaf fall (Fischer & Rezende 2016).

The C*olletotrichum gloeosporioides* and *C. boninense* species were associated to the anthracnose in *Passiflora* spp. in Brazil, Colombia, Florida, Japan and Argentina. These studies were based in morpho-cultural and molecular characterization, using the ITS-rDNA region and RAPD (Wolcan & Larran 2000; Afanador-Kafuri et al., 2003; Almeida & Côelho 2007; Tozze Júnior et al., 2010; Gil et al., 2017). However, these taxonomic criteria have been considered insufficient for the reliable identification of the species from this genus (Cai et al., 2009; Hyde et al., 2009). Recently, the identification of the *Colletotrichum* genus has been based on the multi-locus phylogenetic analyzes combined with recognized phenotypic characters, such as morphology, pathogenicity and cultural characteristics (Vieira et al., 2017; Veloso et al., 2018; Costa et al., 2019; Damm et al., 2019). From this approach, new species have been reported in *Passiflora* spp.: *C. brasiliense*, *C. colombiense*, *C. karstii*, *C. plurivorum*, *C. brevisporum* and *C. torulosum* (Damm et al., 2012; Damm et al., 2019).

Thus, in order to contribute to a better knowledge of the *Colletotrichum* species that cause anthracnose in the passion fruit culture in the state of Alagoas, this study identified fungal isolates obtained from passion fruit leaves through the molecular and morpho-cultural characterization, as well as evaluated the pathogenicity of these isolates in different hosts.

Material and methods

The study was carried out at the Laboratório de Fitopatologia Molecular do Centro de Ciências Agrárias (CECA) of the Universidade Federal de Alagoas (UFAL), located at km 85 of BR 101 Norte (9°27'54.71" S – 35°49'39.27" O), in the city of Rio Largo, Alagoas, Brazil.

Obtaining the isolates

Passion fruit leaves exhibiting typical symptoms of anthracnose were collected from three commercial plantations in the state of Alagoas, one area of Alagoas' agreste mesoregion (Quebrangulo county) and two areas of eastern Alagoas mesoregion (Coruripe and Maragogi counties), Brazil. The plants were in their vegetative state. The leaves were washed in running water and dried in filter paper. Small fragments were obtained from the transition region of sick tissue. The foliar fragments were superficially disinfested with 70% ethanol for 30 s, NaClO 1% for 1 min, washed in sterile distilled water for 30 s and dried in sterilized filter paper. Then, these were transferred to Petri dishes containing potato-dextrose-agar (PDA) medium (Acumedia, Lansing, USA) and kept at 25 °C until growth (Alfenas et al., 2007). After the sporulation the isolates were morphologically identified as belonging to the *Colletotrichum* genus (Sutton, 1980).

Single-spore isolates were obtained from serial dilution of a spore suspension of up to 10^6 of the initial concentration. An aliquot of 20 µL was evenly distributed in Petri dishes containing WA (water agar) medium using a Drigalski handle. After 24 hours, the germinated spore of each isolate was transferred to new Petri dishes containing PDA medium. Single-spore isolates were preserved by the freeze storage method in strips of filter paper (Alfenas & Mafia 2007) and in Eppendorf tubes. Then, they were stored in the Phytopathogen Collection at the Universidade Federal de Alagoas (COUFAL). All studies were performed with single-spore cultures.

Pathogenicity in different hosts

All isolates were tested for pathogenicity in *Passiflora edulis*. The inoculation was performed on asymptomatic leaves obtained from passion fruit, washed and dried on paper towel. The inoculum consisted of a drop of 30 µL of the spore suspension at a concentration of 10^6 conidia/mL. Then, the inoculum together with a drop of Tween 20% was deposited on the adaxial surface of the leaves, injured with a sterile needle, with four repetitions. The control consisted only of sterilized distilled water (SDW). The leaves were placed in a Gerbox (11 × 11 × 3.5 cm) with sterile filter paper, moistened with SDW and incubated in a Biochemistry Oxygen Demand (BOD) incubator stove at 25 °C and photoperiod of 12 h. The

leaf lesions were evaluated seven days after inoculation. The pathogen was re-isolated to prove the pathogenicity, thus completing Koch's postulates.

The pathogenicity of the *Colletotrichum* species identified was assessed in the host range consisting of mango (cv. Tommy Atkins) at stage 4 of ripeness (Ássis, 2004), papaya (cv. Golden) at stage 3 (Oliveira et al., 2002), banana (cv. Pacovan), guava (cv. Paluma) and yellow passion fruit at stages 5 of ripeness (Azzolini et al., 2004; Pbmh & Pif 2006; Silva et al., 2008). The fruits were washed with detergent in running water, superficially disinfested in NaClO at 1% for 3 min and washed again with distilled water. After drying, the epidermis of each fruit was perforated in the middle region, at a depth of 3mm, with a sterile needle. The inoculum consisted of a 30 μL drop of spore suspension with a concentration of 10^6 conidia/mL, deposited on each point of the wounded epidermis. For the control, sterile distilled water (SDW) was used. The fruit were placed, separately, in plastic trays covered with a polyethylene bag containing a SDW-soaked cotton to keep the relative humidity high. The trays were kept in room temperature. After 48h, the polyethylene bags were removed and the fruits were kept in the same temperature. The fruits were inoculated separately, the experimental design was completely randomized, with four repetitions per treatment (species) and two repetitions per fruit. The pathogenicity of the isolates was assessed seven days after inoculation, by measuring the lesion diameter (cm) in two perpendicular directions and calculating the lesion diameter (DLE). The obtained data were submitted to analysis of variance (ANOVA), and the means compared by the Tukey tests at a level of 5% of probability using the Statistix Program V. 10 (Analytical Software, Tallahassee, USA).

Sequencing and phylogenetic analysis

The total genomic DNA of the isolates was obtained from 5-day-old single-spore cultures grown in L-asparagine medium (Zauza et al., 2007) using Doyle e Doyle (1987) protocol.

The DNA of all isolates were used as a template for PCR amplification of the partial sequence of the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) using the pair of primers GDF1 and GDR1 (Templeton et al., 1992). A subset of isolates of the *Colletotrichum* species identified with the *GAPDH* gene was selected to represent the range of diversity genetic and to determine definitive taxonomic positioning of the species. For this, the partial sequences of the β-tubulin gene (*TUB*2), chitin synthase (*CHS*-1), and the rDNA Internal transcribed spacer (ITS) region of these isolates were amplified by PCR (Table 1).

Table 1. Primers used to identify species of the genus *Colletotrichum*.

Gene	Primers	Sequence (5' a 3')	References
GAPDH	GDR1	GGGTGGAGTCGTACTTGAGCATGT	Guerberet al. 2003
	GDF1	GCCGTCAACGACCCCTTCATTGA	
	T1	AACATGCGTGAGATTGTAAGT	O'Donnell e Cigelnik (1997)
	T2	TAGTGACCCTTGGCCCAGTTG	
*TUB*2	BT2b	ACCCTCAGTGTAGTGACCCTTGGC	Glass e Donaldson (1995)
	BT2a	GGTAACCAAATCGGTGCTGCTTTC	
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC	
CHS-1	CHS-79F	TGGAAGAACCATCTGTGAGAGTTG	Carboneand Kohn, 1999
	CHS-345R	TGGGGCAAGGATGCTTGGAAGAAG	

The PCR cycling conditions for the *GAPDH* gene consisted of initial denaturation at 95 ºC for 4 min, followed by 35 cycles at 95 ºC for 30 s, 60 ºC for 30 s, 72 ºC for 45 s and a final cycle at 72 ºC for seven min. The annealing temperature differed for *CHS*-1 at 58 ºC and *TUB2* at 55 ºC. When the amplification resulted in multiple bands, the primer combination, annealing temperature and/or time were adjusted. For the ITS region the PCR cycling condition consisted of initial denaturation at 95 ºC for 2 min, followed by 38 cycles at 95 ºC for 1 s, 55 ºC for 30 s, 72 ºC for 45 s and a final cycle at 72 ºC for 10 min. Each mix of PCR reactions contained: 10X buffer (3 µL), 50 mM MgCl2 (0.9 µL), 10 mM DNTP's (2.4 µL), 10 µM of each oligonucleotide (2 µL), 1U Taq DNA polymerase (0.2 µL) and DNA (1µL, 25ng/µL). The final reaction volume was adjusted for 30 µL with sterile deionized water (Milli-Q). PCR products were submitted to electrophoresis on 1% agarose gel, stained in ethidium bromide and examined under ultraviolet light (UV). Positive PCR samples were sent for sequencing at Macrogen Inc., Seoul, South Korea.

Consensus sequences were assembled using the Staden Package software. These were initially compared with the GenBank sequence database using the BLASTn algorithm to determine the species with greater sequence similarity. Based on the results of the BLASTn analysis, sequences of ex-type isolates of *Colletotrichum* species available in GenBank for each genomic region were obtained and added for phylogenetic analysis (Supplementary Table 1). Multiple sequence alignments were prepared using the MUSCLE algorithm (Edgar, 2004), implemented in MEGA software v.7 (Molecular Evolutionary Genetics Analysis) (Tamura et al., 2011) for the dataset.

Bayesian Inference (BI) analysis were performed with all sequences for the *GAPDH* gene and with multi-locus dataset (*GAPDH, TUB2, CHS*-1 and ITS region*)* for the representative isolates of the identified species. The best nucleotides substitution models for each genomic region was determined using Mr. Modeltest 2.3 (Posada & Buckley 2004) according to the Akaike Information Criterion (AIC). The robustness of each individual branch of the tree was determined through 1000 bootstrap replications.

BI-based phylogenies were inferred in MrBayes v. 3.0 b4 (Ronquist & Huelsenbeck 2003) employed the Markov Chain Monte Carlo (MCMC) method at CIPRES web portal

(http://www.phylo.org). Four MCMC chains were simultaneously conducted, starting the trees randomly up to 10 million generations for each dataset. The trees were sampled every 1,000 generations resulting in 10.000 trees. The first 2.500 trees were discarded from the analysis, as a *burn-in* phase. The posterior probability values (Rannala & Yang 1996) were determined from a *majority-rule* consensus tree generated with the remaining 7.500 trees. The trees were visualized in the FigTree v. 1.4 program (ztree.bio.ed.ac.uk/software/figtree) and edited in the Inkscape 0.91 program (https://inkscape.org/pt-br/release/inkscape-0.91).

Morpho-cultural Characterization

Representative isolates of the *Colletotrichum* species identified based on the phylogenetic multi-locus analysis were selected for the morpho-cultural characterization. The length and width of fifty conidia and appressoria were evaluated from the deposition of a drop (40 µL) of SDW together with the conidia in a sterile glass slide conditioned in a Petri dish lined with filter paper moistened with SDW, to keep the environment moist and allow the conidia to germinate. After 24 hours, images of the conidia and appressoria were captured under a microscope (Olympus CKX41SF, image capture software CellSens Standard – Olympus 2010).

The cultural characterization was obtained from the growth of a seven-day-old mycelium plug (5mm) plated on synthetic PDA medium and kept in a BOD incubator stove at 25 ºC ± 1ºC and photoperiod of 12 h. Five repetitions per isolate were used. The mycelial growth rate was estimated by measuring the colonies diameter (mm) daily for seven days. The appearance and color of the colonies were also recorded.

The data obtained were subjected to analyzes of variance (ANOVA) and the means compared by the Tukey Test at 5% probability, using the variance analysis system program for balanced data (SISVAR), developed by Ferreira (2000).

Results

Obtaining the isolates and pathogenicity

Fifteen isolates were obtained from passion fruit leaves with anthracnose symptoms in commercial plantation areas in the state of Alagoas. All isolates were pathogenic, inducing small brownish circular spots on the leaves' surface seven days after inoculation. No disease symptoms were observed on the control leaves (Supplementary Figure 1).

The species of *Colletotrichum* of this study induced depressed, soggy, dark brown necrotic lesions, with variable sizes in guava, papaya, mango, banana and passion fruit seven days after inoculation, with significant differences in the severity of the symptoms. No disease symptoms were observed on the control fruits. However, *C. brevisporum, C.*

plurivorum and *Colletotrichum* sp. did not induce lesions in guava fruits. The lesions caused by *C. theobromicola* were significantly bigger on tested fruits, except on guava, with an average between 3.50 and 2.20 cm (Table 2 and Figure 1).

Table 2. Aggressive of five species of *Colletotrichum* associated with anthracnose of passion fruit in papaya (cv. Golden), mango (cv. Tommy Atkins), banana (cv. Pacovan), guava (cv. Paluma) and yellow passion fruit.

	Lesion diameter (cm)				
	Papaya	Mango	Banana	Guava	Passion fruit
C. brevisporum	2.17	3.38	1.05	0.00	1.89
C. fructicola	3,37	2.45	1.00	2.55	1.75
Colletotrichum sp.	2.05	1.85	1.33	0.00	1.05
C. plurivorum	2.73	2.25	1.20	0.00	2.38
C. theobromicola	3.18	3.50	2.20	1.80	2.48

Phylogenetic Analysis

The initial analysis of the partial sequences of the *GAPDH* gene carried out with fifteen isolates from passion fruit leaves indicated the presence of four complexes (*C.*

Figure 1. Aggressive of five species of *Colletotrichum* associated with anthracnose of passion fruit in papaya (cv. Golden), mango (cv. Tommy Atkins), banana (cv. Pacovan), guava (cv. Paluma) and yellow passion fruit.

C. brevisporum COUFAL 0269 | *C. plurivorum* | *Colletotrichum* sp. COUFAL 0279 | *C. fructicola* COUFAL 0280 | *C. theobromicola* COUFAL 0281 | TESTEMUNHA

Magnum | **Orchidearum** | **Boninense** | **Gloeosporioides**

gloeosporioides, *C. boninense*, *C. magnum* and *C. orchidearum*) belonging to the *Colletotrichum* genus (Figure 1 and 2). To confirm the identified species in the *GAPDH* gene analysis, eleven isolates were selected and submitted to phylogenetic analyzes multi-locus for the *C. gloeosporioides* complex (*GAPDH*, *TUB*2 and ITS region) and for the other complexes (*GAPDH*, *TUB2*, *CHS*-1 and ITS region).

The alignment for the *C. gloeosporioides* complex showed 1589 characters, of which 220 are parsimony informational sites and 1173 conserved sites. The locus limits in the alignment were: *GAPDH*: 1-304, ITS: 305-858 and *TUB*2: 859-1589. For the *C. boninense* complex together with other complexes, presented 1861 characters, in which 459 are parsimony informational sites and 1198 conserved sites. The locus limits in the alignment were: *GAPDH*: 1-282, ITS: 283-858, *TUB*2: 859-1581 e *CHS*-1: 1582-1861. The replacement models selected based on the Akaike Information Criterion (AIC) of the genes and ITS region for the *C. gloeosporioides* complex was HKY + G and for the other complexes it was GTR + G.

The multi-locus phylogeny inferred by BI allowed the identification of four species and one unknown species in four complexes of the *Colletotrichum* genus, corroborating with the preliminary analysis based on the *GAPDH* gene. Two isolates were clustered in two clades well supported included in the *C. gloeosporioides* complex: the isolate COUFAL0279 grouped with *C. fructicola* species and isolate COUFAL0280 grouped in clade with *C. theobromicola* (Figure 2).

The phylogeny of the species from the *C. boninense*, *C. magnum* and *C. orchidearum* complexes included nine representative isolates, which were grouped in three clades with high support for Bayesian posterior probability. The isolate COUFAL0283, named *Colletotrichum* sp., from Maragogi county showed high similarity with the species *C. parsonsiae* and *C. brasiliense*, belonging to the *C. boninense* complex. However, it formed a separate clade (although close) of these species in the multi-locus phylogenetic analysis, making their identification inconclusive. Two isolates (COUFAL0274 and COUFAL0278) from Quebrangulo county and two isolates (COUFAL0276 and COUFAL0275) from Coruripe formed a clade with the species *C. plurivorum* (*C. orchidearum* complex). Whereas the isolates COUFAL0267, COUFAL0273, COUFAL0269 and COUFAL0271, from three different collection counties, were belonging to the *C. brevisporum* clade (*C. magnum* complex) (Figure 3). The alignments and trees were deposited at TreeBASE (http://www.treebase.org/) with the access number 27807 for the *C. gloeosporioides* complex and 27809 for the other complexes.

Figure 2. Bayesian phylogenetic tree of *Colletotrichum* spp. isolates from *Passiflora edulis*. The tree was built using concatenated sequences of the *GAPDH*, *TUB2* genes and ITS region for *gloeosporioides* complex. The sequences generated in this study are highlighted in bold.

Ex-type cultures are marked with an asterisk. The scale bar (0.02) represents nucleotide substitutions per site. This tree is rooted with *C. boninense* species (CBS 123755).

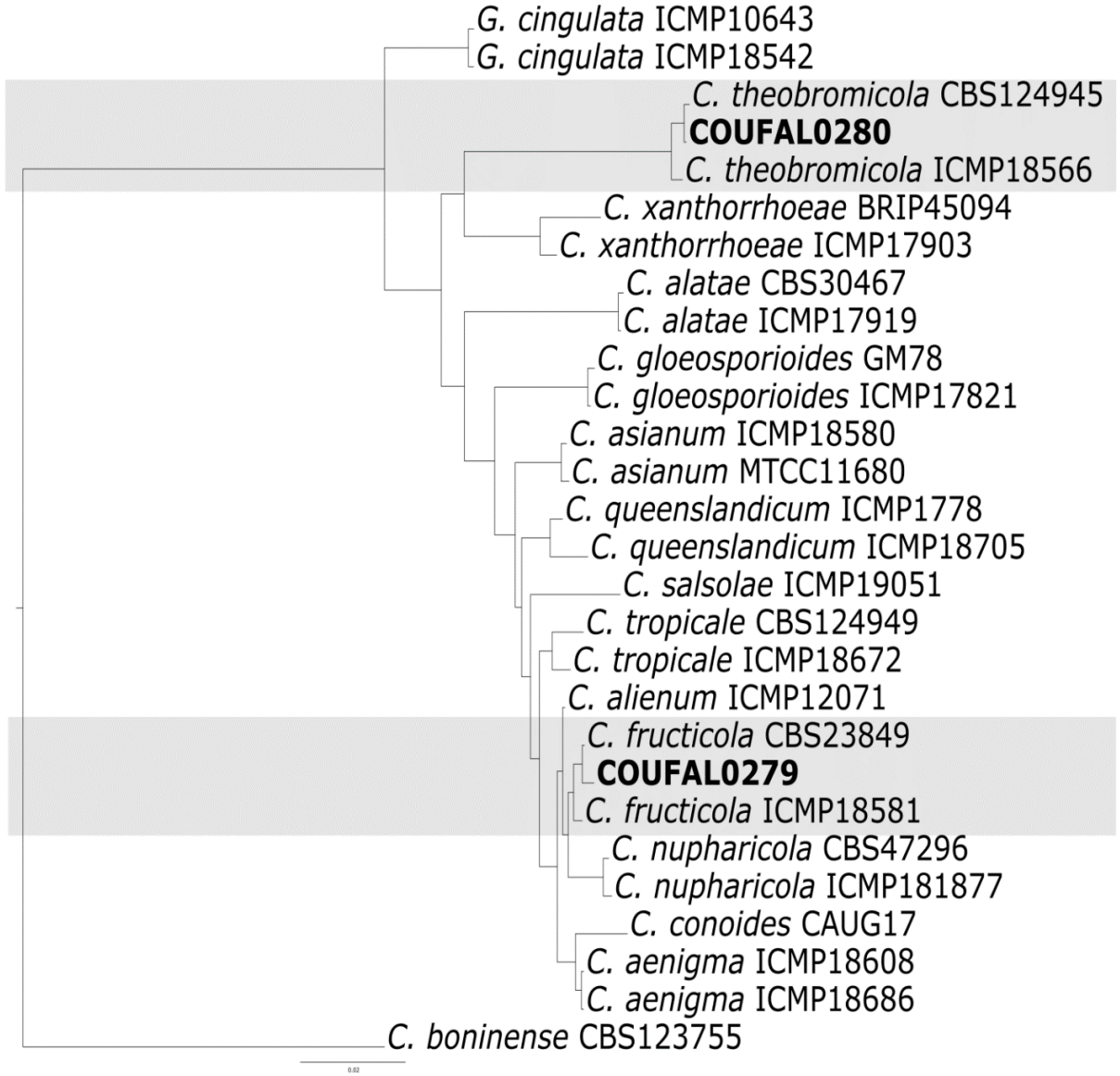
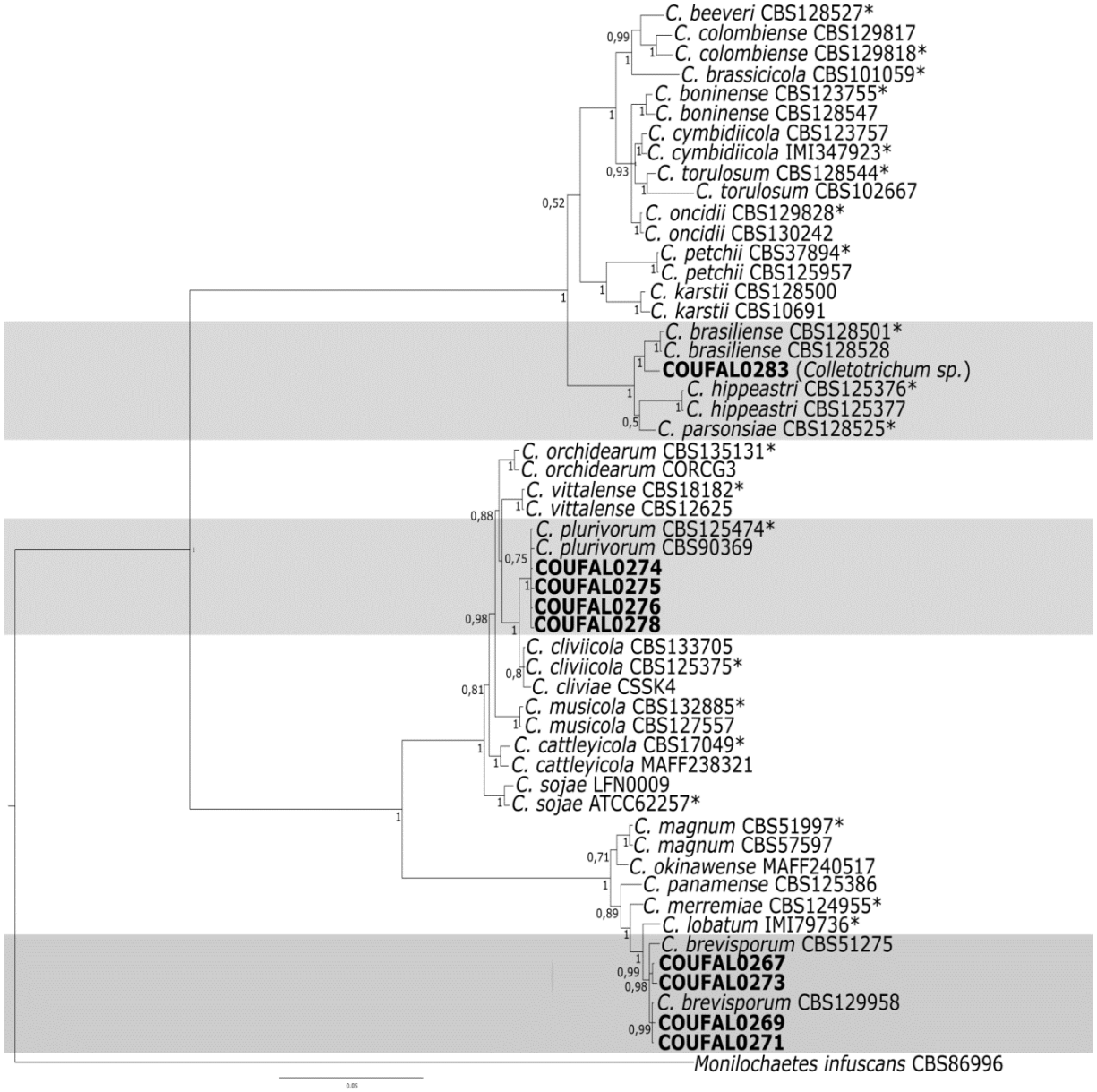


Figure 3. Bayesian phylogenetic tree of *Colletotrichum* spp. isolates from *Passiflora edulis*. The tree was built using concatenated sequences of the *GAPDH, TUB2*, *CHS*-1 genes and ITS region for the *C. boninense*, *C. magnum* and *C. orchidearum* complexes. The sequences generated in this study are highlighted in bold. Ex-type cultures are marked with an asterisk. The scale bar (0.05) represents nucleotide substitutions per site. This tree is rooted with *Monilochaetes infuscans* species (CBS 86996).



Morpho-cultural Characterization

The morpho-cultural characteristics of the *Colletotrichum* species identified in this study were similar to those belonging to the *C. gloeosporioides* complex (Weir et al., 2012), *C. boninense* complex (Damm et al., 2012), *C. magnum* complex and *C. orchidearum* complex (Damm et al., 2019). Information on the size and shape of conidia and appressoria,

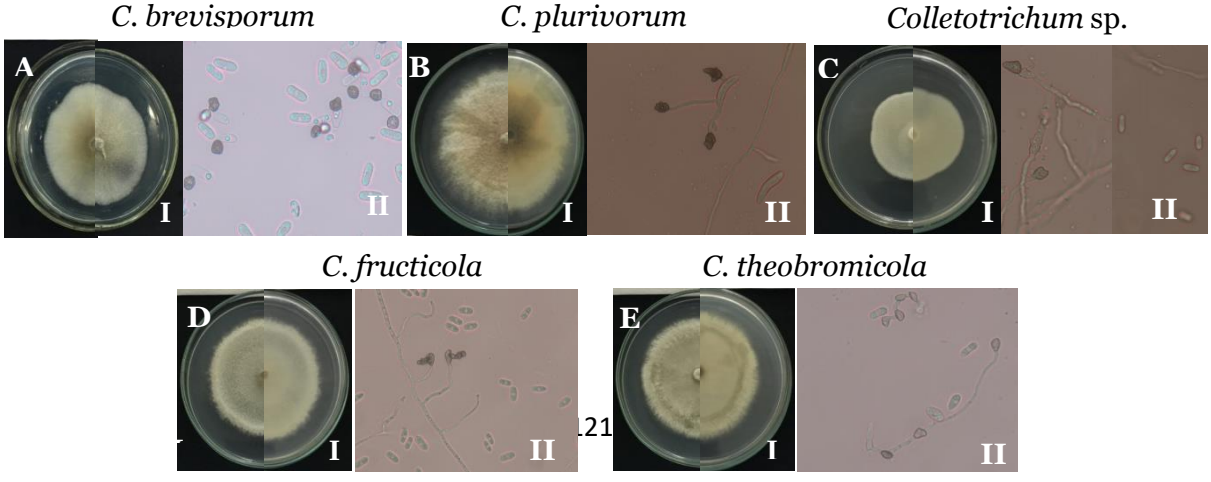
mycelial growth rate and cultural characteristics were described in Table 3 and Figure 4. Regarding colonies colors, these were heterogeneous, with predominance of white color, possessing alterations of tones in the center and/or at the edges.

Table 3. Summary of morpho-cultural data for *Colletotrichum* species

Species	Conidia (µm)			Appressoria (µm)		Growth rate (mm/day)	Colonies
	Length	Width	Shape	Length	Width		
C. brevisporum ([a]COUFAL0269)	**15.44** (12.42-18.65)	**6.70** (5.56-7.70)	Cylindrical	**9.38** (7.40-11.45)	**7.49** (6.11-9.12)	**6.64**	Dark green with white edges and dense mycelium
C. fructicola (COUFAL0279)	**14.12** (10.77-16.64)	**5.55** (4.20-7.05)	Cylindrical	**11.68** (6.79-18.00)	**6.91** (3.20-21.14)	**6.16**	White with pink center and dense mycelium
***Colletotrichum* sp.** (COUFAL0283)	**14.75** (12.20-18.10)	**5.77** (4.22-7.72)	Cylindrical	**10.75** (5.66-17.46)	**8.91** (5.26-13.06)	**4.08**	White and dense mycelium
C. plurivorum (COUFAL0275)	**27.75** (19.46-39.08)	**7.66** (5.38-11.88)	Cylindrical	**13.77** (7.61-18.20)	**9.41** (6.38-15.68)	**7.34**	Pink with brown center, dense mycelium and presence of sectors
C. theobromicola (COUFAL0280)	**20.55** (13.75-26.48)	**6.22** (4.00-7.89)	Cylindrical	**9.97** (8.06-12.52)	**7.54** (5.66-9.43)	**7.68**	Dark green with white edges and dense mycelium

[a]COUFAL (Phytopathogen collection of the Universidade Federal de Alagoas).

Figure 4. Morphocultural characteristics of *Colletotrichum* species. AI, BI, CI, DI and EI- Aspects of colonies. AII, BII, CII, DII and EII – Conidia and appressoria of *Colletotrichum* spp.



Discussion

In our study, the molecular approach using the *GAPDH, TUB*2, *CHS*-1 genes and ITS-rDNA region reliably differentiated the *Colletotrichum* species obtained from passion fruit leaves, showing a high genetic diversity. Four species and one unknown species belonging to four *Colletotrichum* complexes were revealed, including: *C. fructicola* e *C. theobromicola* (*C. gloeosporioides* complex), *C. brevisporum* (*C. magnum* complex), *C. plurivorum* (*C. orchidearum* complex) and *Colletotrichum* sp. (*C. boninense* complex).

The members from *C. gloeosporioides* complex identified in this study (*C. fructicola* and *C. theobromicola*) are biologically diverse and geographically disseminated pathogens, causing fruit rotting and leaf spots in numerous hosts around the world such as *Coffea arabica* L., *Theobromae cacao* L.*, Persea americana* Mill., *Dioscorea* spp., *Fragaria vesca* L., *Capsicum frutensens* L., *Annona muricata* L. (Rojas et al., 2010; Weir et al., 2012; Álvarez et al., 2014; James et al., 2014; Rodrigues et al., 2014; Diao et al., 2017; Veloso et al., 2018; Costa et al., 2019). Although *C. fructicola* and *C. theobromicola* have been associated with wide range of important hosts in tropical and subtropical regions, they have never been reported in the genus *Passiflora* before.

Four species from *C. boninense* complex occur in plants of the *Passiflora* genus: *Colletotrichum brasiliense* reported in fruits in Brazil (Tozze Júnior et al., 2010), *C. colombiense* in Colombia (in leaves), *C. torulosum* in New Zealand (in leaves) and *C. karstii* in Japan, Colombia (in leaves) and in Brazil (in fruits) (Damm et al., 2012). Nevertheless, neither of these species were identified in this study and only the isolate COUFAL0283, named *Colletotrichum* sp., grouped to the *C. boninense* complex, forming a clade close to *C. brasiliense*, *C. parsonsiae* and *C. hippeastri*. This possibly indicates the occurrence of speciation among *Colletotrichum* species, which can contribute for emergence of resistance to the fungicides used anthracnose control, causing serious problem for the management of the disease.

Colletotrichum brevisporum was considered one of the 23 *singleton* species, as it did not belong to any complex recognized in the *Colletotrichum* genus (Hyde et al., 2014; Jayawardena et al., 2016), however, it was recently inserted into the *C. magnum* complex by Damm et al. (2019). This species has been described as pathogen in plants of economic importance for agriculture including *Citrus medica* L., *Capsicum* spp. L. (China), *Neoregalia* sp., *Pandamus pygmaceus*, *Anthurium* (Thailand) and *Carica papaya* L. (Australia) and as endophyte of *Lycium chinense* in Korea (Noireung et al., 2012; Peng et al., 2012; Paul et al., 2014; Liu et al., 2016; Shivas et al., 2016; Damm et al., 2019). In Brazil, *C. brevisporum* is considered a relatively new pathogen, causing rot in fruits of *Carica papaya* (Vieira et al., 2013), *Capsicum* sp. (Almeida et al., 2017; Silva et al., 2017) and *Phaseolus lunatus* L. (Cavalcante et al., 2019). *Colletotrichum brevisporum* has been reported in passion fruit

using ITS sequences by Shivas et al. (2016), but the ITS-rDNA region alone is unreliable for identifying *Colletotrichum* species (Cannon et al., 2012; Weir et al., 2012; Sharma et al., 2013). However, different strains included in the study by Damm et al. (2019) confirmed the occurrence of this species in this host using multi-locus analysis. Du et al. (2017), also confirm the occurrence of this pathogen in stem, fruit, leaves and tendrils of *Passiflora edulis* in Sanming, Fujian province, China.

Colletotrichum plurivorum was previously described as *C. sichuanensis* in the culture of *Capsicum annuum* in China (Liu et al., 2016). Posteriorly, *C. sichuanensis* was considered synonymous with *C. cliviicola* (as *C. cliviae*) in study by Douanla-Meli et al. (2017). Nonetheless, it was recently reclassified as *C. plurivorum* and attributed to the *C. orchidearum* complex where eight closely related species are present, confirming that it is a distinct species of *C. cliviicola* (Damm et al., 2019). From this new classification, *C. plurivorum* was associated with different botanical families: Passifloraceae, Malvaceae, Anacardiaceae, Araceae, Caricaceae, Fabaceae, Solanaceae, Musaceae, Orchidaceae, Rubiaceae, Theaceae and Rosaceae (Fu et al., 2019; Damm et al., 2019; Silva et al., 2019). In Brazil, there are few reports of this pathogen, which can be found only in *Mangifera indica* and *Gossypium* sp. (Damm et al., 2019).

Among the identified species in this study, *C. brevisporum* and *C. plurivorum* appear more frequently among isolates, with 46.66% and 33.33%, respectively, raising hypothesis that these species are likely the most prevalent in *Passiflora edulis* in the state of Alagoas. In contrast, the low incidence found for the species of the *C. boninense* complex, only 6.66%, may be related to the type of tissue (leaves) chosen for our analysis e/or number of samples. Since *C. brasiliense*, reported only in *P. edulis f. flavicarpa* in Brazil, was isolated from fruit by Tozze Júnior et al. (2010). In turn, the species *C. fructicola* and *C. theobromicola* represented 13.33% of the isolates. The occurrence of species belonging to *C. gloeosporioides* complex was already expected in the present study, since these species have often been reported in several cultures in northeastern Brazil (Silva et al., 2017; Veloso et al., 2018; Vieira et al., 2018; Costa et al., 2019). Additionally, this is the first report of *C. fructicola* and *C. theobromicola* in *P. edulis* in the world and of *C. brevisporum* and *C. plurivorum* in Brasil.

The morphological characteristics were similar to those described in previous studies for species within the different *Colletotrichum* genus complexes (Weir et al., 2012; Damm et al., 2012; Damm et al., 2019). The cultural characteristics of the *Colletotrichum* species were quite variable, possibly due to factors such as culture medium, environmental aspects and storage conditions of the isolates (Weir et al., 2012).

All identified species in this study induced circular brownish spots consistent with typical symptoms of anthracnose found in passion fruit leaves described by Fischer e Rezende (2016). Pathogenicity in different hosts demonstrated the nonspecificity in the

parasitic relationship of the five *Colletotrichum* species in papaya, mango, banana, guava and yellow passion fruit. These results corroborate with other pathogenicity studies of this genus, where it is common for a single species to infect multiple hosts (Hyde et al., 2009; Phoulivong et al., 2010; Youlian et al., 2011; Lima et al., 2015). However, our results indicate a variation of the aggressiveness of these species, depending on the inoculated fruit. The preference and/or adaptation of the species to host could explain this response. For example, the larger lesions shown by *C. theobromicola* may have been influenced by the fact that this species is a common pathogen in many fruit trees (Weir et al., 2012; James et al., 2014). While *C. plurivorum* and *C. brevisporum* were not pathogenic to guava, and until now there are no reports of these species in this host. In addition, the variation of the aggressiveness presented by *Colletotrichum* species in this study may be related to pathogenic variability and/or factors as variety and conditions of the fruit (Lima et al., 2015). The pathogenic variability was demonstrated by Rogério et al. (2016), where different isolates of *C. truncatum* revealed a high degree of variation in aggressiveness when inoculated in soybean seed. In turn, isolates of *C. capsici* and *C. acutatum* from chilli showed variation in the pathogenic ability on ripe and unripe fruits, indicating that the stage of fruit development should be considered in the management strategies of the disease (Saxena et al., 2014). These information are important to improve our understanding of the biology and lifestyle of the genus.

The present study showed a considerable diversity of *Colletotrichum* species associated with anthracnose in passion fruit leaves in Alagoas. Four species and one unknown species were identified as etiological agents of the anthracnose in this culture, most of it included in the *C. gloeosporioides* complex, followed by the *C. orchidearum*, *C. magnum* and *C. boninense* complexes. The application of multi-locus phylogeny for *Colletotrichum* identification has contributed to increase the number of species associated with anthracnose in different hosts, as observed in other research conducted in northeastern Brazil (Lima et al., 2013; Vieira et al., 2017; Silva et al., 2017; Costa et al., 2019). Moreover, the sampled producing areas are considered agricultural centers, where the nearby cultivation of different agricultural crops is common, an approach that can favor the pathogens jump from one host to another.

The results of this study may contribute to the development of adequate techniques for anthracnose control in the culture of passion fruit in the state of Alagoas and in other parts of the world. In face of the diversity of pathogens present in the *P. edulis* crop, is extremely necessary a management plan that involves easily applied practices aimed at the socioeconomic conditions of farmers. In addition, this study confirms the need for future research on the *Colletotrichum* species in the passion fruit culture in other producing regions of Brazil to better understand the disease etiology.

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References

Afanador-Kafuri, L., Minz, D., Maymon, M., Freeman, S. (2003). Characterization of *Colletotrichum* isolates from tamarillo, passiflora, and mango in Colombia and identification of a unique species from the genus. *Phytopathology,* 93, 579-587.

Alfenas, A.C., Mafia, R.G. (2007). *Métodos em fitopatologia*. Editora UFV, Viçosa, Brasil. 382 p.

Alfenas, A. C., Ferreira, F. A., Mafia, R. G., Gonçalves, R. C. (2007). Isolamento de fungos fitopatogênicos. In: Alfenas, A. C., Mafia, R. G. (ed) *Métodos em fitopatologia*. (pp. 53-90). UFV

Almeida, L.B., Matos, K.S., Assis, L.A.G., Hanada, R.E., Silva, G.F. (2017). First Report of Anthracnose of *Capsicum chinense* in Brazil Caused by *Colletotrichum brevisporum*. *Plant Disease,* 101, 1035.

Almeida, L.C.C., Coêlho, R.S.B. (2007). Caracterização da agressividade de isolados de *Colletotrichum* de maracujá amarelo com marcadores bioquímico, fisiológico e molecular. *Fitopatologia Brasileira*, 32, 318-328.

Álvarez, E., Gañán, L., Rojas-Triviño, A., Mejía, J.F., Llano, G.A., González, A. (2014). Diversity and pathogenicity of *Colletotrichum* species isolated from soursop in Colombia. *European Journal of Plant Pathology,* 139, 325-338.

Araújo, F.P. (2007). *Caracterização da variabilidade morfoagronômica de maracujazeiro (Passiflora cincinnata Mast.) no semi-árido brasileiro*. Tese, Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, Botucatu, Brasil.

Ássis, J.S. (2004). Cultivo da mangueira: colheita e pós-colheita. http://www.cpatsa.embrapa.br:8080/sistema_producao/spmanga/colheita.htm <Acesso em 23 de nov. de 2019>

Azzolini, M., Jacomino, A.P., Bron, I.U. (2004). Índices para avaliar qualidade pós-colheita de goiabas em diferentes estádios de maturação. *Pesquisa agropecuária brasileira,* 39, 139-145.

Cai, L., Hyde, K.D., Taylor, P.W.J., Weir, B., Waller, J., Abang, M.M., Zhang, J.Z., Yang, Y.L., Phoulivong, S., Liu, Z.Y., Prihastuti, H., Shivas, R.G., McKenzie, E.H.C., Johnston, P.R. (2009). A polyphasic approach for studying *Colletotrichum*. *Fungal Diversit,* 39, 183-204.

Cannon, P.F., Damm, U., Johnston, P.R., Weir, B.S. (2012). *Colletotrichum* current status and future directions. *Studies in Mycology,* 73, 181–213.

Cavalcante, G.R.S., Barguil, M.B., Vieira, W.A.S., Lima, W.G., Michereff, S.J., Doyle, V.P.,

Câmara, M.P.S.(2019). Diversity, prevalence, and virulence of *Colletotrichum* species associated with lima bean in Brazil. *Plant Disease,* 103, 1961-1966.

Costa, J.F.O., Kamei, S.H., Silva, J.R.A., Miranda, A.R.D.S., Netto, M.B., Silva, S.J.C., Correia, K.C., Lima, G.S.A., Assunção, I.P. (2019). Species diversity of *Colletotrichum* infecting *annona*s pp in Brazil. *European Journal of Plant Pathology*, 153, 1119-1130.

Crochemore, M.L., Molinari, H.B., Stenzel, N.M.C. (2003). Caracterização agromorfológica do maracujazeiro (*Passiflora* spp.). *Revista Brasileira de Fruticultura,* 25, 5-10.

Damm, U., Cannon, P.F., Woudenberg, J.H.C., Johnston, P.R., Weir, B.S., Tan, Y.P., Shivas, R.G., Crous, P.W. (2012). The *Colletotrichum boninense* species complex. *Studies in Mycology,* 73, 1–36.

Damm, U., Cannon, P.F., Woudenberg, J.H.C., Crous, P.W. (2012). The *Colletotrichum acutatum* species complex. *Studies in Mycology,* 73, 37–113.

Damm, U., Sato, T., Alizadeh, A., Groenewald, J.Z., Crous, P.W. (2019). The *Colletotrichum dracaenophilum, C. magnum* and *C. orchidearum* species complexes. *Studies in Mycology,* 92, 1-46.

Diao, Y.Z., Zhang, C., Liu, F., Wang, W.Z., Liu, L., Cai, L., Liu, X.L. (2017). *Colletotrichum* species causing anthracnose disease of chilli in China. *Persoonia,* 38, 20-37.

Douanla-Meli, C., Unger, J.G., Langer, E. (2017). Multi-approach analysis of the diversity in *Colletotrichum cliviae* sensu lato. *Antonie van Leeuwenhoek,* 111, 423-435.

Doyle, J.J., Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin,* 19, 11-15.

Du, Y.X., Shi, N.N., Chen, W.L., Ruan, H.C., Yang, X.J., Gan, L., Dai, Y.L., Chen, F.R. (2017). Identification of *Colletotrichum brevisporum* causing anthracnose on passion fruit. *Canadian Journal of Plant Pathology,* 39, 527-532.

Edgar, R.C. (2004). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics,* 5, 1-19.

Faleiro, F.G., Junqueira, N.T.V., Braga, M.F. 2005. *Maracujá germoplasma e melhoramento genético*. Planaltina, DF: Embrapa Cerrados, 315-338 p.

Ferreira, D.F. 2000. Análises estatísticas por meio do Sisvar para Windows versão 5. 0. In: *Reunião Anual da Região Brasileira da Sociedade Internacional de Biometria*. (pp. 255-258).

Fischer, I.H., Rezende, J.A.M. (2016). Doenças do maracujazeiro (*Passiflora* spp.) In: Amorim, L., Rezende, J.A.M., Bergamim Filho, A., Camargo, L.E.A. (ed*) Manual de fitopatologia: doenças das plantas cultivadas*. (pp. 535-538). Agronômica Ceres.

Fu, M., Crous, P.W., Bai, Q., Zhang, P.F., Xiang, J., Guo, Y.S., Zhao, F.F., Yang, M.M., Hong, N., Xu, W.X., Wang, G.P. (2019). *Colletotrichum* species associated with anthracnose of Pyrus spp. in China. *Persoonia,* 42, 1-35.

Gil, J.G.R., Tamayo, P.J., Morales, J.G. (2017). Identification and pathogenicity of microorganisms affecting purple *passion fruit* in Colombia. *Revista Ceres,* 64, 250-257.

Hyde, K.D., Cai, L., McKenzie, E.H.C., Yang, Y.L., Zhang, J.Z., Prihastuti, H. (2009). *Colletotrichum*: a catalogue of confusion. *Fungal Diversity*, 39, 1–17.

Hyde, K.D., Nilsson, R.H., Alias, S.A., Ariyawansa, H.A., Blair, J.E., Cai, L., Cock, A.W.A.M., Dissanayake, A.J., Glockling, S.L., Goonasekara, I.D., Gorczak, M., Hahn, M., Jayawardena, R.S., Kan, J.A.L.V., Laurence, M.H., Lévesque, C.A., Li, X., Liu, J.K., Maharachchikumbura, S.S.N., Manamgoda, D.S., Martin, F.N., McKenzie. E.H.C., McTaggart, A.R., Mortimer, P.E., Nair, P.V.R., Pawłowska, J., Rintoul, T.L,, Shivas, R.G., Spies, C.F.J., Summerell, B.A., Taylor, P.W.J., Terhem, R.B., Udayanga, D., Vaghefi, N., Walther, G., Wilk, M., Wrzosek, M., Xu, J.C., Yan, J., Zhou, N. (2014). One stop shop: backbones trees for important phytopathogenic genera. *Fungal Diversity,* 67, 21-125.

IBGE - Instituto Brasileiro de Geografia e Estatística. (2020). Maracujá: Produção Agrícola Municipal. https://www.ibge.gov.br/explica/producao-agropecuaria/maracuja/brhttps://sidra.ibge.gov.br/tabela/1613 - resultado. <Acessado: 19 de abril de 2022>

James, R.S., Ray, J., Tan, Y.P., Shivas, R.G. (2014). *Colletotrichum siamense*, *C. theobromicola* and *C. queenslandicum* from several plant species and the identification of *C. asianum* in the Northern Territory. *Australasian Plant Disease*, 9, 138.

Jayawardena, R.S., Hyde, K.D., Damm, U., Cai, L., Liu, M., Li, X.H., Zhang, W., Zhao, W.S., Yan, J.Y. (2016). Notes on currently accepted species of *Colletotrichum*. *Mycosphere,* 7, 1192–1260.

Lima, N.B., Batista, M.V.A., Morais Jr, M.A., Barbosa, M.A.G., Michereff, S.J., Hyde, K.D., Câmara, M.P.S. (2013). Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil. *Fungal Diversity,* 61, 75–88.

Lima, N.B., Lima, W.G., Tovar-Pedraza, J.M., Michereff, S.J., Câmara, M.P.S. (2015). Comparative epidemiology of *Colletotrichum* species from mango in northeastern Brazil. *European Journal of Plant Pathology,* 141, 679-688.

Liu, F., Tang, G., Zheng, X., Li, Y., Sun, X., Qi, X., Zhou, Y., Xu, J., Chen, H., Chang, X., Zhang, S., Gong, G. (2016). Molecular and phenotypic characterization of *Colletotrichum* species associated with anthracnose disease in peppers from Sichuan Province, China. *Scientific Reports,* 6, 32761.

Noireung, P., Phoulivong, S., Liu, F., Cai, L., Mckenzie, E.H.C., Chukeatirote, E., Jones, E.B.G., Bahkali, A.H., Hyde, K.D. (2012). Novel species of *Colletotrichum* revealed by morphology and molecular analysis. *Cryptogam Mycol,* 33, 347–362.

Oliveira, M.A.B., Vianni, R., Souza, G., Araújo, T.M.R. (2002). Caracterização do estádio de maturação da papaia Golden em função da cor. *Revista Brasileira de Fruticultura,* 24, 559-561.

Paul, N.C., Lee, H.B., Lee, J.H., Shin, K.S., Ryu, T.H., Kwon, H.R., Kim, Y.K., Youn, Y.N., Yu, S.H. (2014). Endopytic fungi from *Lycium chinense* Mill and characterization of two new Korean records of *Colletotrichum*. *International Journal of Molecular Sciences,* 15, 15272-15286.

PBMH e PIF- Programa brasileiro para a modernização da horticultura e produção integrada de frutas, normas de classificação de banana, São Paulo: CEAGESP, 2006.

Peng, L., Yang, Y., Hyde, K.D., Bahkali, A.H., Liu, Z. (2012). *Colletotrichum* species on Citrus leaves in Guizhou and Yunnan provinces, China. *Cryptogam Mycol,* 33, 267–283.

Phoulivong, S., Cai, L., Chen, H., Mckenzie, E.H.C., Abd-Elsalam, K., Chukeatirote, E., Hyde, K.D. (2010). *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. *Fungal Diversity,* 44, 33-43.

Posada, D., Buckley, T.R. (2004). Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology,* 53, 793-808.

Rannala, B., Yang, Z. (1996). Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution,* 43, 304-311.

Rodrigues, A.L., Pinho, D.B., Lisboa, D.O., Nascimento, R.J., Pereira, O.L., Alfenas, A.C., Furtado, G.Q. (2014). *Colletotrichum theobromicola* causes defoliation, stem girdling and death of mini-cuttings of eucalyptus in Brazil. *Tropical Plant Pathology,* 39, 326-330.

Rogério, F., Ciampi-Guillardi, M., Barbieri, M.C.G., Bragança, C.A.D., Seixas, C.D.S., Almeida, A.M.R., Massola Jr, N.S. (2016). Phylogeny and variability of *Colletotrichum truncatum* associated with soybean anthracnose in Brazil. *Journal of Applied Microbiology,* 122, 402-415.

Rojas, E.I., Rehner, S.A., Samuels, G.J., Van Bael, S.A., Herre, E.A., Cannon, P., Chen, R., Pang, J., Wang, R., Zhang, Y., Peng, Y.Q., Sha, T. (2010). *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panamá: Multilocus phylogenies distinguish hostassociated pathogens from asymptomatic endophytes. *Mycologi,* 102, 1318–1338.

Ronquist, F., *Huelsenbeck,* J.P. *(2003).* MrBayes 3: Bayesian Phylogenetic Inference under Mixed Models. *Bioinformatics,* 19, 1572-1574.

São José, A.R. (2015). Controle fitossanitário do maracujá. Revista campo &negócios. http://www.revistacampoenegocios.com.br/controle-fitossanitario-do-maracuja<Acesso em: 26 de Setembro de 2019>

Saxena, A., Raghuwanshi, R., Singh, B. (2014). Molecular, phenotypic and pathogenic variability in *Colletotrichum* isolates of subtropical region in north-eastern India, causing fruit roto f chillies. *Journal of Applied Microbiology,* 117, 1422-1434.

Silva, T.V., Resende, E.D., Viana, A.P., Pereira, S.M.F., Carlos, L.A., Vitorazi, L. (2008). Determinação da escala de coloração da casca e do rendimento em suco do maracujá-amarelo em diferentes épocas de colheita. *Revista Brasileira de Fruticultura,* 30, 880-884.

Silva, J.R.A., Chaves, T.P., Silva, A.R.G., Barbosa, L.F., Costa, J.F.O., Ramos-Sobrinho, R., Teixeira, R.R.O., Silva, S.J.C., Lima, G.S.A., Assunção, I.P. (2017). Molecular andmorpho-cultural characterization of *Colletotrichum* spp. associated with anthracnose on *Capsicum* spp. in northeastern Brazil. *Tropical Plant Pathology,* 42, 315-319.

Silva, D.D., Groenewald, J.Z., Crous, P.W., Ades, P.K., Nasruddin, A., Mongkolporn, O., Taylor, P.W.J. (2019). Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia. *IMA Fungus,* 10, 8.

Sharma, G., Pinnaka, A.K., Shenoy, B.D. (2013). ITS-based diversity of *Colletotrichum* from India. *Current Research in Environmental & Applied Mycology,* 3, 194–220.

Shivas, R.G., Tan, Y.P., Edwards, J., Dinh, Q., Maxwell, A., Andjic, V., Liberato, J.R., Anderson, C., Beasley, D.R., Bransgrove, K., Coates, L.M., Cowan, K., Daniel, R., Dean, J.R., Lomavatu, M.F., Mercado-Escueta, D., Mitchell, R.W., Thangavel, R., Tran-Nguyen, L.T.T., Weir, B.S. (2016). *Colletotrichum* species in Australia. Australasian *Plant Pathology,* 45, 447-464.

Sutton, B.C. (1980). *The Coelomycetes, Fungi Imperfecti with acervuli, pycnidia and stromata*. Common wealth Mycological Institute, Califórnia, USA, 696 p.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011). MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731 – 2739.

Templeton, M.D., Rikkerink, E.H., Solon, S.L., Crowhurst, R.N. (1992). Cloning and molecular characterization of the glyceraldehyde-3-phosphate dehydrogenase-encoding gene and cDNA from the plant pathogenic fungus *Glomerella cingulata*. *Gene,* 122, 225–230.

Tozze Júnior, H.J., Fischer, I.H., Câmara, M.P.S., Massola Júnior, N.S. (2010). First report of *Colletotrichum boninense* infecting yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) in Brazil. *Plant Disease,* 5, 70–72.

Vanderplank, J. (1996). *Passion flowers*. MIT Press, Massachusetts, USA, 224 p.

Veloso, J.S., Câmara, M.P.S., Lima, W.G., Michereff, S.J., Doyle, V.P. (2018). Why species delimitation matters for fungal ecology: *Colletotrichum* diversity on wild and cultivated cashew in Brazil. *Fungal Biology,* 122, 677-691.

Vieira, W.A.S., Nascimento, R.J., Michereff, S.J., Hyde, K.D., Câmara, M.P.S. (2013)0. First report of papaya fruit anthracnose caused by *Colletotrichum brevisporum* in Brazil. *Plant Disease,* 97, 1659.

Vieira, W.A.S., Lima, W.G., Nascimento, E.S., Michereff, S.J., Câmara, M.P.S., Doyle, V.P. (2017). The impact of phenotypic and molecular data on the inference of *Colletotrichum* diversity associated with Musa. *Mycologia,* 109, 912-934.

Vieira, W.A.S., Lima, W. G., Nascimento, E.S., Michereff, S.J., Câmara, M.P.S., Doyle, V.P. (2018). The impact of phenotypic and molecular data on the inference of *Colletotrichum* diversity associated with Musa. *Mycologia,* 109, 912-934.

Weir, B.S., Johnston, P.R., Damm, U. (2012). The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology*, 73, 115-180.

Wolcan, S., Larran, S. (2000). First report of anthracnose caused by *Glomerella cingulata* on passion fruit in Argentina. *Plant Disease,* 84, 706.

Youlian, Y., Cai, L., Yu, Z., Liu, Z., Hyde, K.D. (2011). *Colletotrichum* species on Orchidaceae in southwest China. *Cryptogamie Mycologie,* 32, 229–253.

Zauza, E.A.V., Alfenas, A.C., Mafia, R.G. (2007). Esterilização, preparo, de meios de cultura e fatores associados ao cultivo de fitopatógenos. In: Alfenas, C.A., Mafia, R.G. (ed) *Métodos em fitopatologia*. (pp. 42). UFV