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Identification of *Colletotrichum* isolates from *Capsicum chinense* in Amazon

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ABSTRACT. Chili pepper (*Capsicum chinense*) is a great economic important culture on the State of Amazonas, and it represents, approximately, a production of 1.9 thousand tons per year. It is one of the hosts of *Colletotrichum* genus in the North region of Brazil. The aim of the study was to differentiate and to identify isolates of *Colletotrichum* collected from *C. chinense* in Amazon. Molecular characterization, using RFLP-PCR, ERIC-PCR and ISSR, was carried out initially for screening of morphologically similar isolates. Furthermore, phylogenetic analyses were performed using combined regions: Actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for the three isolates, INPA 2066, INPA 2286 and INPA 1858, plus superoxide dismutase (SOD2) for INPA 2066. We showed that the molecular markers were able to distinguish the isolates of *Colletotrichum* studied and these results were confirmed with the phylogenetic analyses, three different occurrences of *Colletotrichum* species (*C. siamense*, *C. scovillei* and *C. brevisporum*) causing anthracnose in *C. chinense* in the State of Amazonas. This study

represents the first report of the species *C. siamense* and *C. scovillei* in this host in Brazil.

Key words: *Colletotrichum*; Molecular markers; Chili pepper; Phylogenetic analysis

INTRODUCTION

Colletotrichum (teleomorph *Glomerella*), comprises a big range of cosmopolitan fungi species and are usually described as being the anthracnose disease agent. It is known ~600 species of the genus *Colletotrichum*, which are pathogens of over 3000 species of plants including cereals, legumes, vegetables, perenial crops, and tree fruits (O'Connell et al., 2012). There are some *Colletotrichum* species complexes already described in the literature, such as *C. gloeosporioides*, *C. acutatum*, *C. boninense* and *C. orbiculare* (Weir et al., 2012; Damm et al., 2012a,b, 2013). In Brazil, there are many reports of plant infection caused by *Colletotrichum* species, especially in economically important plants and fruits, such as coffee berries, sugarcane, strawberry, maize, sorghum, banana, avocado and many others. The tropical and subtropical climate favors the spread of *Colletotrichum* species on these plants.

It is notable the great economic interest of chili pepper (*Capsicum chinense* Jacq.) culture in many parts of the world. It happens because of its great potential of growth in almost every country, since it is a tropical regions native, and its use on food, pharmaceutical, cosmetic, and ornamental products (Dias et al., 2013). *C. chinense* is one of the most cultivated vegetables in Brazil, mainly in the North region. The anthracnose is a disease that more affects the culture and five species of *Colletotrichum* were described as the pathogen for this host, *C. capsici* and *C. gloeosporioides* in India, Indonesia, Korea, and Thailand, *C. acutatum* in Australia and Indonesia, *C. coccodes* in Korea and New Zealand (Ratanacherdchai et al., 2010) and *C. brevisporum* in Brazil (Almeida et al., 2017).

The identification of *Colletotrichum* based on morphological characters is problematic due to the few morphological traits that can be used to separate species in this genus (Than et al., 2008). Therefore, it is necessary a precise study and characterization based not only on morphological but also on molecular data utilized for species delimitation and defining of inter- and intraspecific relationships as it has been performed in the past decades. Several molecular techniques have been developed to characterize and to identify different *Colletotrichum* species. Multilocus phylogenetic analysis using partial sequences of gene such as actin (ACT), calmodulin (CAL), chitin synthase (CHS-1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glutamine synthetase (GS), and manganese superoxide dismutase (SOD2) have been utilized to identify different species of this genus (Weir et al., 2012; Damm et al., 2012a).

Additionally, molecular markers are generally recognized as a reliable method to evaluate genetic diversity and differentiation of *Colletotrichum* spp isolates, such as restriction fragments length polymorphism (RFLP-PCR), inter-simple sequence repeats or microsatellites (ISSR) and enterobacterial repetitive intergenic consensus (ERIC-PCR). In the present paper, the objective was to differentiate and identify isolates of *Colletotrichum* collected from *C. chinense* in Amazon, northern of Brazil, using molecular markers and phylogenetic analysis.

MATERIAL AND METHODS

Isolation of *Colletotrichum* from *C. chinense*

Fruits of chili pepper (*C. chinense* Jacq.) with typical anthracnose symptoms were observed in Manaus, Amazonas, Brazil.

The isolation was performed by collecting spores directly from the surface of the lesions in *C. chinense* fruit and then plated on PDA culture medium. A monosporic culture was performed to ensure that this work would be upon a single genetic uniformity.

The isolates of *Colletotrichum* selected and used for screening analysis (Figures 1, 2 and 3) and phylogenetic study are deposited in the culture collection of the Phytopathology Laboratory of National Institute of Amazonian Research - INPA (INPA 2286, INPA 2066 and INPA 1858) (Table 1).

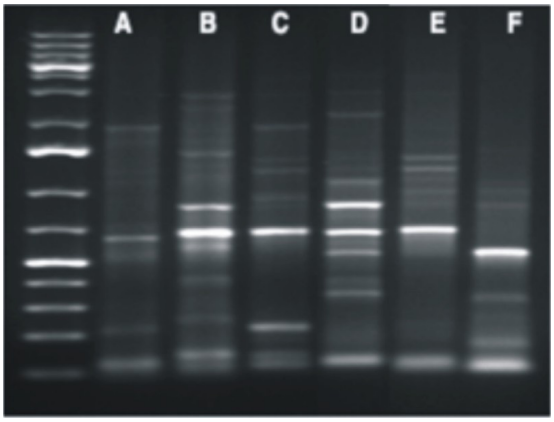

Figure 1. ERIC-PCR of the three isolates from *Capsicum chinense*. **A.** *Colletorihcum fructicola*. **B.** *C. gloeosporioides*. **C.** *C. fragariae*. **D.** Isolate 2286. **E.** Isolate 2066. **F.** Isolate 1858.

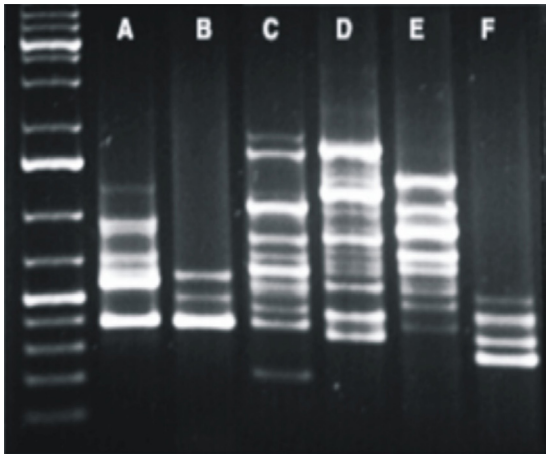

Figure 2. PCR amplification of ISSR of the three isolates from *Capsicum chinense*. **A.** Isolate 1858. **B.** Isolate 2066. **C.** Isolate 2286. **D.** *Colletorihcum fructicola*. **E.** *C. fragariae*. **F.** *C. gloeosporioides*.

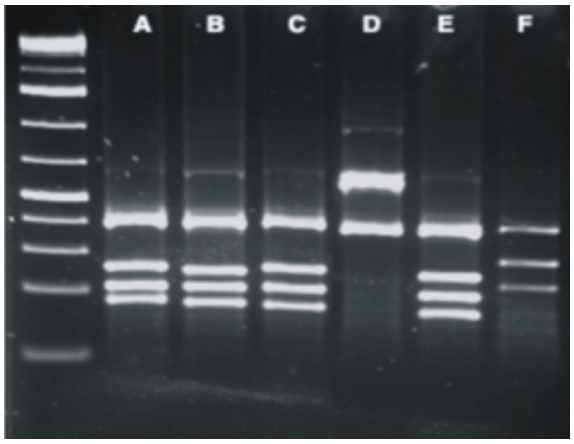

Figure 3. PCR amplification of the 1-kb GS intron based on *Pst*I enzyme digestion (RFLP-PCR), of the three isolates from *Capsicum chinense*. **A.** *Colletorihcum fructicola*. **B.** *C. gloeosporioides*. **C.** *C. fragariae*. **D.** Isolate 2286. **E.** Isolate 1858. **F.** Isolate 2286.

Table 1. List of primers used for molecular screening and phylogenetic analysis.

Region	Primers	Sequences (5'-3')
Enterobacterial Repetitive Intergenic Consensus	ERIC1	ATGTTAAGTCCCTGGGGATTCAC
Enterobacterial Repetitive Intergenic Consensus	ERIC2	AGTAAGTGACTGGGGTGAGCG
Glutamine synthetase	GSF1	ATGGCCGAGTACATCTGG
Glutamine synthetase	GSR1	AACCGTCGAAGTTCCAC
Inter Simple Sequence Repeats	UBC 885	BHBGAGAGAGAGAGAGA
Actin	ACT 512 F	ATGTGCAAGGCCGGTTTCGC
Actin	ACT 783 R	TACGAGTCCTTCTGGCCCAT
Superoxide dismutase	SODglo2-F	CAG ATC ATG GAG CTG CAC CA
Superoxide dismutase	SODglo2-R	TAG TAC GCG TGC TCG GAC AT
Glyceraldehyde 3-Phosphate dehydrogenase	GAPDH-F	GCCGTCAACGACCCCTTCATTGA
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH-R	GGGTGGAGTCGTACTTGAGCATGT

Screening *Colletotrichum* isolates by molecular markers

To confirm if *C. chinense*, in the State of Amazonas, is host of different species of *Colletotrichum*, a initial screening on the obtained isolates was carried out only using different molecular profile isolates for phylogenetic analysis. For this screening, we used ERIC-PCR, ISSR and PCR-RFLP of GS (Glutamine sintase) intron techniques. For comparison, we used isolates of reference of *C. fructicola*, *C. gloeosporioides* and *C. fragariae*.

DNA extraction was carried out at the Molecular Biology Laboratory - Embrapa Western Amazon, according to Doyle and Doyle (1987). The primers used for amplification are listed in Table 2.

To ERIC-PCR marker, ERIC1 and ERIC2 primers were used at a concentration of 0.2 µM; 1X Buffer [100 mM Tris-HCl pH 8.8; 500 mM KCl, 0.8% (v/v)], 25 mM $MgCl_2$; 0.5 mM dNTPs; 50 ng DNA; 1 U Taq polymerase (DNA Express®), and reaction was set up to a final volume of 25 µL. The programming on thermal cycler (Applied Biosystems Veriti™96-Well Thermal Cycler) initiated with 94°C for 1 min followed by 35 cycles of denaturation (94°C for 1 min), annealing (48°C for 1 min) and elongation (65°C for 5 min) and further extension of 65°C for 6 min.

Table 2. Strains of *Colletotrichum* spp used for phylogenetic analysis, with collection details and GenBank accessions.

Species names	Culture[a]	Host/Substrate	Origin	GenBank accession No.[b]		
				GAPDH	ACT	SOD2
C. aenigma	ICMP 18608*	*Persea americana*	Israel	JX010044	JX009443	JX010311
	ICMP 18686	*Pyrus pyrifolia*	Japan	JX009913	JX009519	JX010312
C. aeschynomenes	ICMP 17673*, ATCC 201874	*Aeschynomene virginica*	USA	JX009930	JX009483	JX010314
C. alienum	ICMP 12071*	*Malus domestica*	New Zeland	JX010028	JX009572	JX010333
	ICMP 18621	*Persea americana*	New Zeland	JX009959	JX009952	JX010308
C. communis (*C. cf. siamense*)	GS01	*Bauhinia variegata*	India	KC790736	KC790622	-
	GS06	*Saraca indica*	India	KC790739	KC790625	-
C. dianesei (syn. *C. melanocaulon*)	CMM 4083	*Mangifera indica*	Brazil	KC517194	KC517298	-
	Coll131 = CBS 133251*	*Vaccinium macrocarpon*	USA	KP703275	-	-
C. endomangiferae	CMM 3740	*Mangifera indica*	Brazil	KC517298	KC517298	-
	CMM 3814*	*Mangifera indica*	Brazil	KC517298	KC517298	-
C. fruticola	ICMP 18581*, CBS 130416	*Coffea arabica*	Thailand	JX010033	FJ907426	JX010327
	ICMP 17921, CBS 238.49*	*Ficus edulis*	Germany	JX009923	JX009495	JX010322
C. hebeiense	SD452	*Vitis vinifera*	China	KF377505	KF377542	-
	K3	*Vitis vinifera*	China	KF377495	KF377532	-
C. hymenocallidis (*C. cf. siamense*)	CSSN2*	*Hymenocallis americana*	China	-	GQ856775	-
	CSSN3	*Hymenocallis americana*	China	-	GQ856776	-
C. murrayae (*C. cf. siamense*)	GZAAS5.09538	*Murraya* sp	China	JQ247608	JQ247656	-
	GZAAS5.09506	*Murraya* sp	China	JQ247609	JQ247657	-
C. musae	ICMP 17817, IMI 52264	*Musa sapientum*	Kenya	JX010015	JX009432	JX010317
	ICMP 19119, CBS 116870*	*Musa* sp	USA	JX010050	JX009433	JX010335
C. nupharicola	CBS 469.96, IMCP 17938	*Nuphar lutea* subsp. *polysepala*	USA	JX009936	JX009486	JX010319
	CBS 470.96*, ICMP 18187	*Nuphar lutea* subsp. *polysepala*	USA	JX009972	JX009437	JX010320
C. queensalandicum	ICMP 1778*	*Carica papaya*	Australia	JX009934	JX009447	JX010336
	ICMP 18705	*Coffea* sp	Fiji	JXX10036	JX009490	JX010334
C. salsolae	ICMP 19051*	*Salsola tragus*	Hungary	JX009916	JX009562	JX010325
C. siamense	ICMP 18578*, CBS 130417	*Coffea arabica*	Thailand	JX009924	FJ907423	JX010326
	INPA 2066	***Capsicum chinense***	**Amazonas, Brazil**	KY435611	KY435609	KY435613
C. siamense (syn. *C. hymenocallidis*)	ICMP 18642, CBS 125378*	*Hymenocallidis americana*	China	JX010019	GQ856775	JX010332
C. tropicale	ICMP 18672, MAFF 239933	*Litchi chinensis*	Japan	JX010020	JX009480	JX010318
	ICMP 18653, CBS 124949*	*Theobroma cacao*	Panama	JX010007	JX009489	JX010329
C. acerbum	CBS 128530, ICMP 12921, PRJ, 1199.3*	*Malus domestica*	New Zeland	JQ948790	JQ949780	-
C. acutatum	CBS 112760, STE-U 4468	*Hakea sericea*	South Africa	JQ948723	JQ949713	-
	CBS 112761, STE-U 4461	*Hakea sericea*	South Africa	JQ948724	JQ949714	-
	CBS 113599, STE-U 3038	*Grevillea* sp	Australia	JQ948678	JQ949668	-
C. australe	CBS 116478, HKUCC 2616*	*Trachycarpus fortune*	South Africa	JQ948786	JQ949711	-
	CBS 131325, CPC 19820	*Hakea* sp.	Australia	JQ948787	JQ949777	-
C. brisbanense	CBS 292.67, DPI 11711*	*Capsicum annuum*	Australia	JQ948621	JQ949612	-
C. chrysanthemi	IMI 364540, CPC 18930	*Chrysanthemum coronarium*	China	JQ948603	JQ949594	-
	CBS 126518, PD 84/520	*Carthamus* sp	Netherlands	JQ948601	JQ949592	-
C. cosmi	CBS 853.73, PD 73/856*	*Cosmos* sp	Netherlands	JQ948604	JQ949595	-
C. costaricense	CBS 330.75*	*Coffea arabica*	Costa Rica	JQ948510	JQ949501	-
	CBS 211.78, IMI	*Coffea* sp	Costa Rica	JQ948511	JQ949502	-
C. cuscutae	IMI 304802, CPC 18873*	*Cuscuta* sp	Dominica	JQ948525	JQ949516	-
C. fioriniae	CBS 129946, PT170, RB021	*Olea europaea*	Portugal	JQ948672	JQ949663	-
	CSL 318	*Magnolia* sp	UK	JQ948676	JQ949667	-
C. godetiae	IMI 381927, CPC 18935	*Rubus idaeus*	Turkey	JQ948769	JQ949759	-
	CBS 862.70	*Sambucus nigra*	Netherlands	JQ948768	JQ949758	-
C. guajavae	IMI 350839, CPC 18893*	*Psidium guajava*	India	JQ948600	JQ949591	-
C. indonesiense	CBS 127551, CPC 14986*	*Eucalyptus* sp	Indonesia	JQ948618	JQ949609	-
C. johnstonii	CBS 128532, ICMP 12926, PRJ 1139.3*	*Solanum lycopersicum*	New Zeland	JQ948775	JQ949765	-
C. kinghornii	CBS 198.35*	*Phormium* sp	UK	JQ948785	JQ949775	-
C. laticiphilum	CBS 112989, IMI 383015, STE-U 5303*	*Hevea brasiliensis*	India	JQ948619	JQ949610	-
	CBS 129827, CH2	*Hevea brasiliensis*	Colombia	JQ948620	JQ949611	-
C. limeticola	CBS 114.14*	*Citrus aurantifolia*	USA, Florida	JQ948523	JQ949514	-
C. melonis	CBS119142, CMW 9931	*Lupinus albus*	South Africa	JQ948505	JQ949496	-
	CBS 159.84*	*Cucumis melo*	Brazil	JQ948524	JQ949515	-
C. nymphaeae	CBS 129933, Gol99	*Fragaria x ananassa*	USA	JQ948592	JQ949583	-
	IMI 348177, CPC 18890	*Fragaria x ananassa*	USA	JQ948593	JQ949584	-
C. paxtonii	CBS 502.97, LARS 58	*Musa nana*	"West Indies"	JQ948616	JQ949607	-
	IMI 165753, CPC 18868*	*Musa* sp	Saint Lucia	JQ948615	JQ949606	-
C. phormii	CBS 118197, AR 3389	*Phormium* sp	New Zeland	JQ948781	JQ949771	-
	CBS 483.82	*Phormium tenax*	New Zeland	JQ948782	JQ949772	-
	CBS 118194, AR 3546*	*Phormium* sp	Germany	JQ948777	JQ949767	-
C. pyricola	CBS 128531, ICMP 12924, PRJ 977.1*	*Pyrus communis*	New Zeland	JQ948776	JQ949766	-
C. rhombiforme	CBS 129953, PT250, RB011*	*Olea europaea*	Portugal	JQ948788	JQ948778	-
	CBS 131322, DAOM 233253, C10, MS1L34	*Vaccinium macrocarpum*	USA	JQ948789	JQ949779	-
C. salicis	IMI 345585, CPC 19376	*Fragaria x ananassa*	New Zeland	JQ948807	JQ949797	-
	CBS 239.49	Unknown	Unknown	JQ948800	JQ949790	-
C. scovillei	CBS 126529, PD 94/924-3, BBA70349*	*Capsicum* sp	Indonesia	JQ948597	JQ949588	-
	CBS 126530	*Capsicum* sp	Indonesia	JQ948598	JQ949589	-
	CBS 120708	*Capsicum annuum*	Thailand	JQ948599	JQ949590	-
C. scovillei	**INPA 2286**	***Capsicum chinense***	**Amazonas, Brazil**	KY435612	KY435610	-
C. simmondsii	CBS 114494, STE-U 2964, STE-U 2964, STE-U 2088	*Protea cynaroides*	USA	JQ948613	JQ949604	-
	CBS 111531, STE-U 2090	*Protea cynaroides*	USA	JQ948612	JQ949603	-
C. solanei	IMI 364297, CPC 18929*	*Theobroma cacao*	Malaysia	JQ948617	JQ949608	-
C. tamarilloi	CBS 12814, T.A.6*	*Solanum betaceum*	Colombia	JQ948514	JQ949505	-
	CBS 129811, T.A.3	*Solanum betaceum*	Colombia	JQ948515	JQ949506	-
C. walerii	CBS 125472, BMT (HL)19*	*Coffea* sp	Vietnam	JQ948605	JQ949596	-
C. orbiculare	CBS 133198, KTU-K6	*Cucumis melo*	Japan	KF178482	KF178555	
C. brevisporum	L57, LC0600, BCC 38876*	*Neoregalia* sp	Thailand	JN050227	JN050216	-
	BTL23, LC0870, MFLUCC 100182	*Pandanus pygmaeus*	Thailand	JN050228	JN050217	-
	INPA 1858	***Capsicum chinense***	**Amazonas, Brazil**	KX878887	KX878886	-
C. boninense	CBS123755, MAFF 305972*	*Crinum asiaticum* var. *sinicum*	Japan	JQ005240	JQ005501	-
	ICMP 17904					
C. cliviae	CSSS1	*Clivia miniata*	China	GU085867	GU085861	-
	CSSS2	*Clivia miniata*	China	GU085868	GU085862	-
C. kahawae subsp. *ciggaro* (syn. *Glomerella rufomaculans* var. *vaccinii*)	CBS 124.22*, ICMP 19122	*Vaccinium* sp	USA	JX009950	JX009536	-
C. karstii	CBS 129833	*Musa* sp	Mexico	JQ005262	JQ005523	-
C. thailandicum	HR01MFU, LC0596, BCC 38879*	*Hibiscus rosa-sinensis*	Thailand	JN050231	JN050220	-
	CMSP34, LC0958, MFUCC 100192	*Alocasia* sp	Thailand	JN050232	JN050221	-
C. trifolii	CBS 128554, ICMP 12934,	*Medicago sativa*	USA	KF178500	KF178573	
C. tropicicola	L58, LC0598BCC, 38877*	*Citrus maxima*	Thailand	JN050229	JN050218	-
	BTL07, LC0957, MFLUCC 100167	*Paphiopedilum bellatolum*	Thailand	JN050230	JN050219	-

[a]ATCC: American Type Culture Collection, Virginia, USA; BBA: Culture collection of the Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin, Germany; BRIP: Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; CMM: Coleção de Culturas de Fungos Fitopatogênicos "Prof.Maria Menezes", Federal University of Pernambuco, Recife, Brazil; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; LARS: Culture collection of Long Ashton Research Station, Bristol, UK (no longer existing); MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; INPA: National Institute of Amazonian Research Manaus, Amazonas, Brazil; HKUCC: The University of Hong Kong Culture Collection, Hong Kong, China; PD: Plantenziektenkundige Dienst Wageningen, Nederland. [b]Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Actin (ACT) and Manganese superoxide dismutase (SOD2) were sequences used in the phylogenetic analyses. The *Colletotrichum* strains used in this study are indicated in bold. *Ex-holotype or ex-epitype cultures.

To ISSR marker analysis, the PCR was performed at a 25-µL final reaction volume, with 0.2 µM of the UBS primer 885; 1X Buffer [100 mM Tris-HCl pH 8.8; 500 mM KCl, 0.8% (v/v)]; 25 mM $MgCl_2$; 0.5 mM dNTPs; 50 ng DNA; 1.5 U *Taq* Polymerase (DNA Express®). The pre-cycle was on 94°C for 5 min, followed by 40 cycles of denaturation (94°C for 1 min), annealing (45°C for 1 min) and elongation (72°C for 1 min), followed by a final extension at 72°C for 7 min. Amplification of intron GS, PCR-RFLP was carried out according to Liu et al. (2012). All PCR and PCR-RFLP products ran at a 1.5% w/v agarose gel electrophoresis.

Phylogenetic analysis

Three loci were amplified by PCR and we used for phylogenetic analysis: ACT, GAPDH and SOD2. For these reactions, 1X Buffer [(100 mM Tris-HCl pH 8.8; 500 mM KCl, 0.8% (v/v)], 25 mM $MgCl_2$; 10 mM dNTPs; 5 µM for each primer; 50 ng/mL DNA; 5 U *Taq* Polymerase (DNA Express®). The first cycle initiated with 94°C for 4 min, followed by 35 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s) and elongation (72°C for 1 min), followed by a final extension at 72°C for 7 min. The primer sequences used for each gene are described in the Table 2. PCR products were purified and sequenced by the Applied Biosystems® 3500 Genetic Analyzers. Sequences from forward and reverse primers were aligned to obtain a consensus sequence (Table 1). The fungal DNA sequences were aligned using MEGA 6 (Tamura et al., 2013) with reference sequences of the *Colletotrichum* obtained from GenBank. The Bayesian inference analyses employing a Markov Chain Monte Carlo method were performed with all individual and combined sequences. The MrModeltest 2.3 (Posada and Buckley, 2004) was used to determine the best model of nucleotide evolution (HKY+I to ACT; HKY+G to GAPDH; GTR+I+G to SOD2). The phylogenetic analysis was performed on CIPRES web portal (Miller et al., 2010) using MrBayes version 3.2 (Ronquist et al., 2012). Markov chain Monte Carlo method was run for 10,000,000 generations, sampling every 1000 generations and discarding 2500 samples as burn-in. The resulting trees were rooted using outgroup taxon. Trees were visualized in FigTree 1.4.0 (Rambaut, 2012) and exported to graphic programs. The sequences obtained in this study were deposited in GenBank (Table 2).

RESULTS AND DISCUSSION

The results obtained reveal that simple molecular settings such as ERIC-PCR, PCR-RFLP and ISSR can be used with efficiency for screening of *Colletotrichum* isolates aiming to identify different species capable of causing anthracnose in the same host. ERIC-PCR and ISSR unique band profiles can be identified (Figures 1 and 2). GS RFLP-PCR also evidenced different profile bands for the isolates (Figure 3).

Phylogenetic results revealed that these different profiles correspond to different species of *Colletotrichum* associated with *C. chinense* (Figures 4, 5 and 6). This information could be confirmed by Bayesian inference methods from multiple gene sequences.

Sequences from fragments of ACT, GAPDH and SOD2 from isolate INPA 2066, ACT and GAPDH from isolates INPA 2286, and INPA 1858 from *C. chinense* were compared with sequences from strains of other *Colletotrichum* species and it showed approximately 99% of similarity with *Colletotrichum siamense*, 99% of similarity with *C. scovillei* Damm, P.F. Cannon & Crous, and 96% of identity with *C. brevisporum* Phoulivong, P. Noireung, L. Cai & K.D. Hyde, respectively.

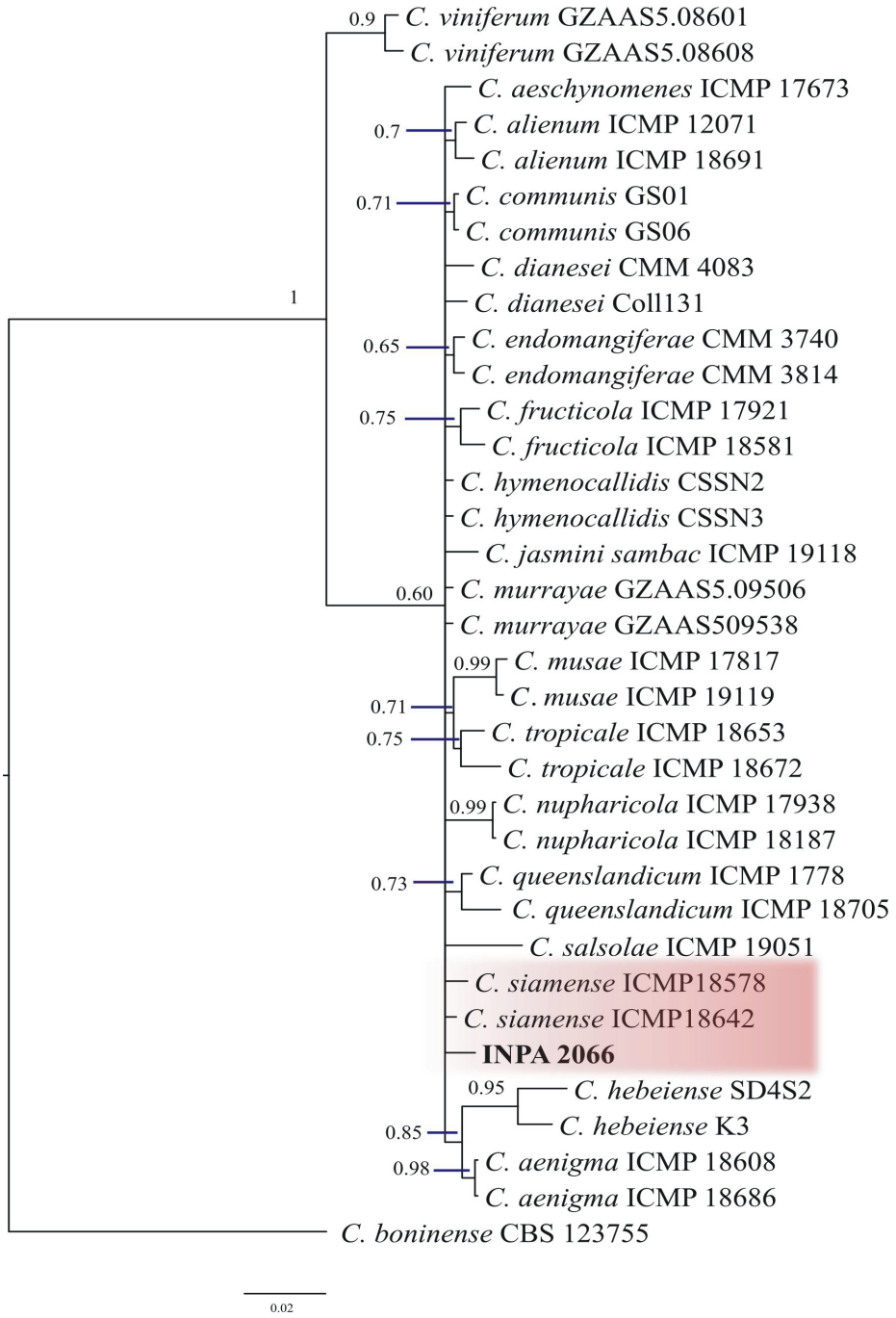

Figure 4. Phylogenetic tree generated by Bayesian inference upon a combinated ACT, GAPDH and SOD2 alignment sequences of *Colletotrichum gloeosporioides* species complex and the INPA 2066 isolate highlighted. This tree is rooted with *C. boninense*. Relevant bootstrap values are shown ate the nodes.

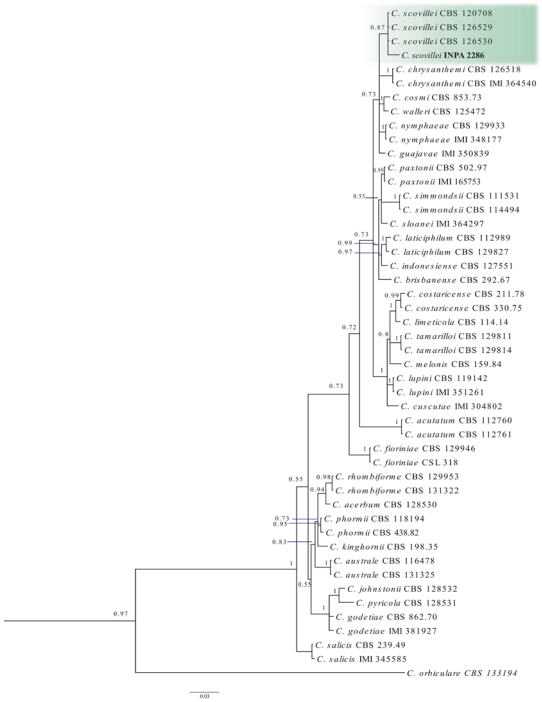

Figure 5. Phylogenetic tree generated by Bayesian inference upon a combinated ACT and GAPDH alignment sequences of *Colletotrichum acutatum* species complex among with the INPA 2286 isolate, highlighted. This tree is rooted with *C. orbiculare*. Relevant bootstrap values are shown ate the nodes.

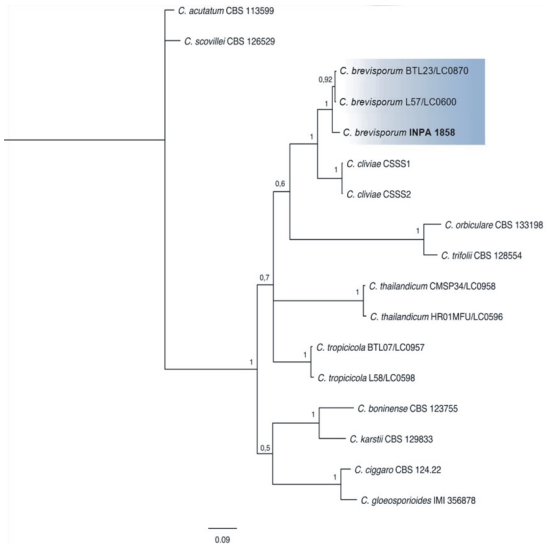

Figure 6. Phylogenetic tree generated by Bayesian inference upon a combinated ACT and GAPDH alignment sequences of *Colletotrichum* species including *C. brevisporum* among with the INPA 1858 isolate, highlighted. This tree is rooted with *C. acutatum* and *C. scovillei*. Relevant bootstrap values are shown ate the nodes.

The combined dataset generated from Bayesian analysis shows similar topology with individual trees. In the phylogenetic trees based on combined dataset of ACT, GAPDH and SOD2 comprised 873 characters including alignment gaps, which showed that the isolate INPA 2066 is closely related to *C. siamense* H. Prihastuti, L. Cai & K.D. Hyde. This tree was rooted to *C. boninense* (Figure 4). *C. siamense* is biologically and geographically diverse, found in many hosts across several tropical and subtropical regions, including *Capsicum annuum* in Thailand (Weir et al., 2012). Recently, Sharma et al. (2015) using multilocus analysis demonstrated that *C. siamense* are four distinct species forming the *C. siamense* species complex.

The analysis of the combined dataset of ACT and GAPDH showed that the isolate INPA 2286 formed a monophyletic clade supported (Bayesian posterior probability = 0.87) with three strains of *C. scovillei* (Figure 5). And isolate INPA 1858 formed a monophyletic clade with high support (Bayesian posterior probability = 1) with two isolates of *C. brevisporum* (Figure 6). The trees were rooted to *C. orbiculare*, and two species from each complex (*C. acutatum* and *C. scovillei*), respectively (Figures 5 and 6). *Colletotrichum scovillei* belongs to *C. acutatum* species complex and was associated with chilli in Indonesia and Thailand (Than et al. 2008; Weir et al., 2012).

In Brazil, the first report of anthracnose on pepper fruit (*C. annuum* L.) caused by *C. scovillei* was by Caires et al. (2014). *Colletotrichum brevisporum*, which is still not inserted in any *Colletotrichum* species complex has been reported in *Neoregalia* sp and *Pandanus pigmaeus* in Thaliand (Noireung et al., 2012). In Brazil, it has been notified the presence of this pathogen in papaya fruit (Vieira et al., 2013), chaoyte fruits (Bezerra et al., 2016) and chili pepper (Almeida et al., 2017).

In the present study, we showed that the molecular markers were able to distinguish the isolates of *Colletotrichum* studied through the different band profiles and was possible to differentiate isolates of the *C. gloeosporioides* and *C. acutatum* species complex. The phylogenetic analysis results confirmed the occurrence of *C. siamense*, *C. scovillei* and *C. brevisporum* causing anthracnose in *C. chinense* in the State of Amazonas.

This study represents the first report of the species *C. siamense* and *C. scovillei* in this host.

Conflicts of interest

The authors declare no conflict of interest.

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