University of São Paulo
“Luiz de Queiroz” College of Agriculture

Molecular characterization of *Colletotrichum* spp. associated with fruits in Brazil

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Thesis presented to obtain the degree of Doctor of Science. Area: Plant Pathology

Piracicaba
2013

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"we can allow satellites, planets, suns, universe, nay whole systems of universe[s,] to be governed by laws, but the smallest insect, we wish to be created at once by special act"

(Charles Darwin)

The man's strength is in the ability to realize the abstract

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RESUMO

Caracterização molecular de espécies de *Colletotrichum* associadas a frutos no Brasil

Fungos do gênero *Colletotrichum* são considerados um dos mais importantes economicamente na Fitopatologia. Espécies desse gênero são encontradas amplamente disseminadas e estão associadas a diversas espécies de plantas hospedeiras. Em regiões tropicais e subtropicais, espécies dos complexos *C. gloeosporioides* e *C. acutatum* são a principal causa das antracnoses em pré e pós-colheita de frutos e consequentemente causam significantivas perdas. Ainda há muitos aspectos a serem compreendidos sobre o gênero *Colletotrichum*, como a biologia e a sistemática. A acurada identificação das espécies associadas a antracnoses é de suma importância para o estabelecimento de estratégias de controle. No entanto, apesar dos grandes avanços na sistemática desse gênero, complexos de espécies como aquelas citadas acima são tratados de modo genérico no Brasil. Estes complexos de espécies foram recentemente estudados e considerados geneticamente e geograficamente variáveis. Neste sentido, o presente trabalho teve como objetivo caracterizar isolados de *Colletotrichum* spp. associados a diferentes frutos e regiões do Brasil por meio de análise filogenética. Para análise multilocus, foram utilizadas sequências parciais dos genes ITS, GAPDH, CHS-1, TUB2 and CAL ou HIS3. Sequências de espécies-tipos disponíveis no GenBank e de isolados de diferentes países foram adicionadas ao conjunto de dados. Com base nos resultados obtidos por meio de filogenia multilocus, seis isolados do complexo *C. gloeosporiodes* e cinco do complexo *C. acutatum* foram escolhidos para testes de patogenicidade cruzada. A espécie *C. siamense*, pertencente ao complexo *C. gloeosporioides*, foi a mais variável geneticamente e quanto ao hospedeiro de origem. Diferentemente, apenas isolados obtidos de manga se agruparam no clado *C. asianum.* Isolados agrupados neste clado não infectaram abacate e um dos isolados (CPC 20969) causou sintomas apenas em manga. No clado *C. fructicola*, isolados coletados no Brasil se agruparam em um subclado e parecem representar um grupo geneticamente distinto. A espécie *C. theobromicola* é relatada pela primeira vez em acerola. Foram identificadas três novas espécies, *C. polyphialidicum*, *C. paranaense* e *C. pruni*, pertencentes ao complexo *C. acutatum*. Isolados brasileiros agrupados no clado *C. nymphaeae* parecem representar um grupo geneticamente distinto, todos se agruparam em um subclado. Isolados do complexo *C. acutatum* utilizados no teste de patogenicidade provocaram sintomas nos hospedeiros testados, porém, em algumas inoculações, as lesões foram maiores no hospedeiro de origem.

Palavras-chave: Filogenia multilocus; Patogenicidade; Sistemática; Identificação

ABSTRACT

Molecular characterization of *Colletotrichum* spp. associated with fruits in Brazil

Colletotrichum species are considered one of the most economically important plant pathogens. They cause many losses in tropical, subtropical and temperate regions affecting a wide range of plant species. In tropical and subtropical regions *C. gloeosporioides* and *C. acutatum* are associated with significant losses on pre and post-harvest anthracnoses. There are still many features to understand about *Colletotrichum* biology and its systematics. The accurate identification of species involved with each anthracnose is of high relevance to establish management strategies to control the disease. Although the great advances on *Colletotrichum* systematics, species complex such as *C. gloeosporioides* and *C. acutatum* are used in a broad sense in Brazil. These complexes were recently investigated and showed to be highly genetic and geographic variable. In this study multigene analysis were carried out based on ITS, GAPDH, CHS-1, TUB2 and CAL or HIS3 partial sequences for strains of *C. gloesporioides* and *C. acutatum* complexes collected from fruit crops in Brazil. Strains from different countries and ex-epitypes and others sequences available on GenBank from the species accepted on both complexes were added on dataset. Six strains from *C. gloeosporiodes* complex and five for *C. acutatum* were selected based on multigene phylogeny to investigate the pathogenicity through inoculations on detached fruit. The multigene phylogenies showed the occurrence of species in Brazil related to those complexes with a high genetic variability among them. The phylogeny of Brazilian strains belonging to the *C. gloeosporioides* complex showed that *C. siamense* represents the most genetically and host-specific variable clade. In contrast, *C. asianum* clade grouped only strains isolated from mango. The strains from this clade used on pathogenic test were not able to infect avocado and one of the strains caused symptoms only on mango. All strains from Brazil grouped in one subclade within the *C. fructicola* clade and seem to represent a genetically distinct group. *C. theobromicola* is first reported causing anthracnose on acerola fruit. Three new species (*C. polyphialidicum*, *C. paranaense* and *C. pruni*) belonging to the *C. acutatum* complex were recognized and their morphologic descriptions were provided. The pathogenic test for the strains in the *C. acutatum* complex showed their cross infection ability, but in some cases the larger lesions were produced on the original host. Most brazilian strains from *C. acutatum* complex grouped in one subclade within the *C. nymphaeae* clade and seem to be genetically distinct.

Keywords: Multilocus phylogeny; Pathogenicity; Systematics; Identification

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1 INTRODUCTION

Colletotrichum species are considered one of the most economically important plant pathogens. They cause many losses in tropical, subtropical and temperate regions affecting a wide range of plant species (SUTTON, 1992). The most common symptoms associated with the genus are sunken necrotic lesions where orange conidial masses are produced, known as anthracnose (FREEMAN et al., 1998). Even though *Colletotrichum* has been considered rank highly as one of the most studied and important plant pathogen genera (DEAN et al., 2012; LATUNDE-DADA, 2001), there are many features to understand about *Colletotrichum* biology and their systematic (CANNON et al., 2012).

The accurate identification and comprehension of species involved with each anthracnose are of high relevance to establish strategies of management (CAI et al., 2009). Traditionally, taxonomy of *Colletotrichum* species was based in morphological and cultural characters such as size and shape of conidia and appressoria, presence or absence of setae, colony color and growth rate (SUTTON, 1992; THAUNG, 2008; HYDE et al, 2009). However, these characteristics are not always reliable to differentiate among the species due to variation in morphology and phenotype under environmental influences (HYDE et al., 2009). Another aspect that has made identification of *Colletotrichum* species difficult is the host range. Several studies have demonstrated the cross infection ability of *Colletotrichum* species (LAKSHMI et al.,2011; PHOULIVONG et al., 2012; de SOUZA et al., 2012; PENG et al., 2013; PERES et al., 2002). Those species, in many cases, are able to infect different hosts or different species can be associated with one host species (FREEMAN et al., 1998).

Despite that problem, great efforts have been made to achieve an accurate identification of species within *Colletotrichum* genus. Physiological, pathogenic and molecular studies have been used together to clarify specific groups of *Colletotrichum* (CAI et al; PRIHASTUTI et al; HYDE et al, 2009; SHIVAS and TAN, 2009). Regarding molecular analyses, phylogeny based on partial gene sequencing had successfully differentiated species within the genus (PRIHASTUTI et al, 2009; SHIVAS and TAN, 2009). In these analyses, many genes have been used, e.g. ITS, calmodulin, glutamine synthetase, glyceraldehyde-3-phosphate dehydrogenase, actin and β-tubulin (PRIHASTUTI et al., 2009). Although phylogenetic analyses have

solved taxonomic problems, there is some possibility to infer erroneously the identification, because some genes cannot distinguish within the group being more useful in population level rather than systematic studies (CAI et al, 2009; CROUCH et al, 2009). Therefore, the right choices of genes and multi-gene phylogenies are important aspects considered on *Colletotrichum* species studies (CANNON et al., 2012; CAI et al., 2009).

Despite the great advances on systematic of *Colletotrichum*, species complex such as *C. gloeosporioides* and *C. acutatum* are used in a broad sense in Brazil (DE SOUZA et al., 2012; SERRA et al., 2011). These complexes were recently investigated and shown to be highly genetic and geographic variable (WEIR et al.; DAMM et al., 2012). The acutatum clade is defined as a collective of *C. acutatum* and 29 closely related species (DAMM et al., 2012). In the *C. gloeosporioides* species complex, most of the 22 species morphologically similar to this species are genetically defined based on multilocus analysis (WEIR et al., 2012).

In this context, all previously designed *C. gloeosporiodes* and *C. acutatum* strains should be compared through multilocus phylogeny to epitypes of these species complexes to characterize the populations. This knowledge would guarantee an accurate identification of the species, their occurrence and effective control.

The aim of this work is to characterize *Colletotrichum* strains associated with different fruit crops and from different localities in Brazil by phylogenetic inferences.

2 DEVELOPMENT

2.1 Literature review

2.1.1 Economic Importance

Species of the genus *Colletotrichum* and its teleomorph *Glomerella* are considered plant pathogens of global importance. Only in South America, they are reported in 803 plant host species (FARR and ROSSMAN, 2013). They can cause significant losses in a wide variety of crop including cereals, vegetables, ornamentals and fruit trees. However, in tropical and subtropical regions those pathogens are the main cause of anthracnose in pre and postharvest fruit (FREEMAN et al., 1998).

Their economic importance and biological features have led to studies on different aspects such as infection process (PRUSKY et al., 2000), genetic diversity (GUERBER et al., 2003; WEEDS et al., 2003), plant-pathogen interactions (O'CONNELL et al., 2004), epidemiology (FÖSTER and ADASKAVEG, 1999) and systematic (CAI et al., 2009; THAN et al., 2008; PRIHASTUTI et al., 2009; DAMM et al., 2012 WEIR et al., 2012). Recently, the genus was considered one of the most studied and economically important groups of plant pathogen in the world (DEAN, et al., 2012).

2.1.2 Biology

Colletotrichum includes species with epiphytic, endophytic, saprobic and plant pathogenic lifestyles (Kumar et al., 2004; Zou et al., 2000, Sutton, 1992; PRIHASTUTI et al., 2009). Many studies have reported a pathogenic species living as endophytic (KUMAR et al., 2004; LIU et al., 2010; WANG et al., 2008). For example, *C. fructicola, C. siamense* and *C. asianum,* species widespread on several hosts, were encountered living as endophyte, epiphyte or pathogen in coffee berries in Thailand (PRIHASTUTI et al., 2009). Although it is known the ability of those species to survive on different ecological niches is well known, the mechanisms associated with the change of their life styles from non-pathogenic to pathogenic need to be studied (HYDE et al., 2009).

The typical symptoms of fruit rot associated with *Colletotrichum* species are known as anthracnose, characterized by round sunken necrotic tissue where orange conidial mass are produced (FREEMAN et al., 1998). However, those species are

able to affect different parts of plants causing symptoms such as defoliation, blossom blight, crown rot, leaf spot, fruit drop and root necrosis (WHARTON and DIÉGUEZ-URIBEONDO, 2004). Several species have been associated with quiescent infections and this symptomless period leads to postharvest damage in several fruits (Prusky and Plumbley, 1992).

Some interactions between *Colletotrichum* species and their host are features that need to be better understood. A few species have a narrow host range or are associated with specific host, but in many cases one species can be associated with several host plants. On the other hand, one host can be affected by different species (FREEMAN et al., 1998; HYDE et al., 2009). For example, *C. siamense*, *C. fructicola*, *C. nymphaeae* are species that have a wide host range affecting different crop fruits (PRIHASTUTI et al., 2009). Although some studies have indicated association between *Colletotrichum* species and specific hosts, the genus is considered non-specific. This is due to poor details on pathogenic interaction studies, nonrepresentative sampling of the population and studies of strains restricted to single host (CANNON et al., 2012).

Pathogenic assays have been reported the cross infection ability of several *Colletotrichum* species. This ability may be differentiated depending on the pathogen-host interaction (PERES et al., 2002; MACKENZIE et al., 2009; PENG et al., 2013). For example, Phoulivong et al. (2012) studying the cross infection of *Colletotrichum* in six host plants reported that *C. asianum* isolated from infected coffee berries was able to infect chili and rose apple, whereas the strain isolated from mango infected chili and mango. In Brazil, cross infection of *C. acutatum* in different fruits has been reported. Peres et al. (2002) showed that *C. acutatum* from strawberry could infect avocado, papaya, mango, guava and passion fruit except banana and the *C. musae* produced symptoms only in banana and avocado. Additionally, the strains were more aggressive to the original host except for mango and passion fruit. In the field, *C. acutatum* from strawberry has been reported surviving on different cultivated plant species such as pepper, eggplant, tomato, bean, as well as on weed species without causing symptoms. It means that these plants may serve as potential inoculum reservoir for strawberry (FREEMAN, 2008).

2.1.3 *Colletotrichum* Systematics

The systematics of *Colletotrichum* species has undergone drastic changes and has motivated worldwide discussions and studies about this genus (VON ARX, 1957; SUTTON, 1992; HYDE et al., 2009; CAI et al., 2009; DAMM et al., 2012; WEIR et al., 2012). The name *Colletotrichum* was introduced by Corda (1831) as *C. lineola*, species considered for a long time as synonym of *C. dematium*, but was recently investigated and re-established as an independent species based on multilocus analysis (DAMM et al., 2009).

The genus *Vermicularia* could be the precursor name for *Colletotrichum* (SUTTON, 1992). The taxonomic features used to separate *Colletotrichum* from this genus were conidiomatal structure, shape and setae presence and disposition. However, Duke (1928) demonstrated that these features are variable and not significant at generic level and, consequently all the species named as *Vermicularia* changed to *Colletotrichum* (SUTTON, 1992).

Before Von Arx's monograph around 750 *Colletotrichum* species were recognized based mainly on host-specificity (CANNON et al., 2012). The Von Arx's study made a remarkable change on *Colletotrichum* systematics (Von Arx, 1957), since the author considered only morphological features and little or no pathogenic characteristics, reducing the number of species to 11. However, *Colletotrichum* has few morphological characteristics that could distinguish the species and these features are variable in culture and overlappings among the species may happen (CANNON et al., 2000; HYDE et al., 2009). This is the main reason why species were recognized inaccurately and many taxa were considered as synonym of *C. gloeosporioides* and *C. graminicola* (CAI et al., 2009; CROUCH et al., 2009).

Other significant studies on *Colletotrichum* systematics were carried out after Von Arx's contribution. Simmonds (1965) separated *C. acutatum* from *C. gloeosporioides* based on shape of the conidia. He described this species as a wide host range pathogen and noted that conidia were variable in length showing fusiform shape. Species of this genus have been identified based on morphological features such as size and shape of conidia and appressoria, presence or absence of setae, sclerotia, acervuli, teleomorph state, colony color, growth rate and texture (CANNON, 2000; FREEMAN et al., 1998; SUTTON, 1992). Therefore, the changes on

systematics for this genus are still advancing and the numbers of species are increasing.

It is evident that the traditional methods might lead to an inaccurate identification and, consequently, compromise the understanding of the host-pathogen relationships; diagnosing and controlling the disease (HYDE et al., 2009). To overcome this problem, molecular techniques, such as those based on RAPD, AFLP markers, have started help on this task. These techniques are useful to detect DNA polymorphism in *Colletotrichum* species, evidencing genetic differences among the strains (FREEMAN, 2000; SREENIVASAPRASAD et al., 1992; WHITELAW-WECKERT et al., 2007; HEILMANN et al., 2006; SILVA-MANN et al., 2005).

Although studies have made improvements to understand the biology and to the detection of the genetic differences among species, the taxonomy still is confusing due to identification based on morphological and cultural criteria. Late 1990's the first International Workshop on *Colletotrichum* met experts on taxonomy, DNA analysis, host-plant interaction and pathology in order to attempt to elucidate these aspects (BARLEY and JEGER, 1992). This meeting revealed new ideas which revolutionized researches on this genus (CANNON et al., 2012).

The use of molecular data became essential to investigate *Colletotrichum* species. Millis et al. (1992) were able to differentiate strains of *Colletotrichum gloeosporioides* on avocado, papaya and mango based on digestion of DNA by restriction enzymes (RFLP technique) and amplification of ITS and IGS regions. The DNA sequencing represented the main breakthrough on *Colletotrichum* systematic. It revealed to be sensitive enough to distinguish species that morphologic features failed. Consequently, the number of using this method and incorporating new gene regions or combining different methods has increased (DAMM et al., 2009; ROJAS et al., 2010; PRIHASTUTI et al., 2009; SHIVAS et al., 2009; WEIR et al., 2012; DAMM et al., 2012). Photita et al. (2005) distinguished strains of *Colletotrichum* in five morpho-groups (*C. musae*, *C. gloeosporioides* representing group 1; *C. gloeosporioides* groups 2 and 3 and *C. truncatum*). Whitelaw-Weckert et al. (2007) proposed a new *C. acutatum* group, based on morphological features, RAPD markers and sequencing part of rDNA regions and β-tubulin gene. The numbers of genes revealed to be useful for systematics and the cost reduction of sequencing permitted to perform multilocus analysis on *Colletotrichum*. Talhinhas et al. (2002)

used ITS, TUB2 and HIS4 to include *Colletotrichum* strains from lupin on *C. acutatum* group.

Although the number of genes and multilocus analyses have been used to clarify the *Colletotrichum* systematics, some species are treated as species complex. Molecular data used to identify species has also been a problem. Crouch et al. (2009) revealed that more than 86% of the sequences from ITS investigated in their study and named as *C. gloeosporiodes* in the GenBank were not related to this species. Thus, the resolution of ITS sequences to differentiate *Colletotrichum* species was questioned.

Due to the difficulties on *Colletotrichum* systematics and the importance of the genus for plant pathology, one volume of the Fungal Diversity journal compiled important studies and reviews on systematics of this genus. These studies represent the new guides to identify those species. In that volume, Cai et al. (2009) proposed a polyphasic approach based on multi-gene phylogeny, morphology, pathogenicity and other techniques for the epitypification and description of *Colletotrichum* species. Hyde et al. (2009) provided a list of the recognized species and a brief summary of those, their hosts and associated diseases. This paper represents the first summary of the genus to incorporate molecular data and phylogenetic analysis.

2.1.4 Species complex and recognition of species

The concept of *Colletotrichum* species has changed in the last 10 years and, due to unreliable identification of those species, mostly based on traditional methods, the definition of the species actually is based on multilocus analysis (DAMM et al.; WEIR et al., 2012). Cannon et al. (2012) provided a review of the current status of the recognition of *Colletotrichum* species and the species clusters that need to be more comprehended. In that review, currently nine major clades represent the *Colletotrichum* species complex. Although ITS has been proposed as fungal barcode marker, on *Colletotrichum* this gene is considered evolutionarily conservative to distinguish most of the species in the *Colletotrichum* complex (CROUCH et al., 2009; CANNON et al.; DAMM et al., WEIR et al., 2012). DAMM et al. (2012) could split 29

subclades in *C. acutatum* complex using multilocus analysis, but the ITS sequences alone distinguished only 11 subclades. In this complex the sequences from TUB2 and GAPDH could split all subclades. WEIR et al. (2012), studying the *C. gloeosporiodes* complex came to the same conclusion. The multilocus analyses based on 8 genes could separate 22 species, but the ITS alone did not distinguish some of these species. Therefore, in the study of *Colletotrichum* species, the multilocus analyses are recommended.

2.2 Material and methods

2.2.1 Obtention and preservation of the Isolates

A total of 85 strains isolated from diverse fruits and different localities of Brazil were used in this study (Table 1 and 2). To represent the species in the *Colletotrichum gloeosporioides* complex around the world, 61 strains from CBS collection (Centraalbureau voor Schimmelcultures) were added to the dataset. To study the *Colletotrichum acutatum*, complex 17 strains were used.

Almost all the strains from Brazil had been stored as single spore cultures at -80 °C in a 5% glycerol/water solution and mineral oil at 10 °C. Those strains as well as the fungal herbarium from new species described here are located in the CPC collection (Collection Pedro Crous – CBS), Utrecht, The Netherlands.

2.2.2 Multilocus Phylogeny

2.2.2.1 PCR and sequencing

Total DNA used in this study was extracted in Brazil according to Murray and Thompson (1980) or in the Netherlands using the method described by Damm et al. (2008).

PCR reactions were performed in a 2720 Thermal Cycler (Applied Biosystems) in a total volume of 12.5 µL. The PCR mixture to amplify partly GAPDH, CHS-1, TUB2, ACT, CAL, ITS and HIS3 genes contained 1 µL 20x diluted genomic DNA, 0.2 µM of each primer, 1x PCR buffer (Bioline, Luckenwalde, Germany), 2 mM MgCl2, 20 µM of each dNTP, 0.7 µL of DMSO and 0.25 U Taq DNA polymerase

(Bioline). The conditions for PCR of ITS were the same as described by Woudenberg (2009), while for the other genes were carried out with an initial denaturation step at 94 °C for 5 min followed by 40 cycles of 30 s at 94 °C, 30 s at 52 °C and 30 s at 72 °C, and a final step at 72 °C for 7 min. The amplicons were visualized in agarose gel at 1% and stained with GelRed™ (Biotium).

The ribosomal DNA flanking two internal transcribed spacers (ITS; 540 pb), chitin synthase 1 (CHS-1; 282 pb), glyceraldehyde-3-phosphate dehydrogenese (GAPDH; 250 pb), actin (ACT; 248), beta-tubulin (TUB2; 490 pb or 700 pb) calmodulin (CAL; 730 pb) and histone 3 (HIS3; 490 pb) genes were amplified and sequenced using the primers ITS-1F (GARDES & BRUNS, 1993) and ITS-4 (WHITE et al., 1990), CHS-354R and CHS-79F (CARBONE & KOHN, 1999), GDF1 and GDR1 (GUERBER et al., 2003), ACT-512F and ACT-783R (CARBONE & KOHN, 1999), BT2Fd (WOUDENBERG et al., 2009) or T1 (O'DONNELL & CIGELNIK, 1997) and Bt-2b (GLASS & DONALDSON, 1995), CL1C and CL2C (WEIR et al., 2012), CYLH3F and CYLH3R (CROUS et al., 2004), respectively. The sequencing was performed using the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems, U.S.A.) and an ABI PRISM® 3100 DNA sequencer (Applied Biosystems) to obtain both directions of the sequences.

The forward and reverse sequences generated were assembled using the software SeqMan 9.0.4 (DNASTAR®) and the multiple sequence alignments from each gene were performed with MAFFT v.6.7 (KATOH & TOH, 2008). The aligned sequences were manually edited when necessary.

2.2.2.2 Phylogenetic analysis

Phylogenetic analyses based on Bayesian Inference were performed separately with the alignment of ITS, TUB2 and CHS-1 sequences to separate the strains by complex. The analysis split the strains in two groups (data not shown). BLAST searches of GenBank were carried out to check the similarity of those sequences.

Evolution models were estimated in Modeltest v. 3.7 (HUELSENBECK and RONQUIST, 2001; RONQUIST AND HUELSENBECK, 2003) using the Akaike information criterion (AIC) for each locus. A Bayesian inference was used to

reconstruct the phylogeny based on multilocus alignment (ITS, ACT, CHS-1, BTUB2 and HIS3 for *C. acutatum* dataset or CAL for the *C. gloeosporioides* dataset). To represent species boundaries and their variability, 52 ex-type sequences and some others related to these, available on GenBank, were added to the *C. gloeosporioides* dataset, while in *C. acutatum* dataset 51 were used.

The partitioned analyses were run twice in MrBayers v.3.2 (RONQUIST et al. 2012) using Markov Chain Monte Carlo (MCMC) algorithm to generate the phylogenetic trees with Bayesian posterior probabilities (BPP). Four MCMC chains were run simultaneously for random trees for 1 x 10^7 generations. Samples were taken every 1000 generations and the first 25% of generations were discarded as burn-in phase of each analysis and posterior probabilities were determined from the remaining trees.

2.2.3 Morphological analysis

To describe the new species of the *C. acutatum* complex based on phylogenetic analysis, those strains were cultivated on synthetic nutrient-poor agar medium – SNA (NIRENBERG 1976) with autoclaved filter paper and *Anthriscus sylvestris* stems placed on surface and oatmeal agar medium - OA (CROUS *et al*. 2009). The cultures were incubated at 20 °C under near UV light with 12 h photoperiod for 10 d. Analyses of taxonomic features and its measurements were made according to Damm *et al*. (2007). Microscopic preparations were made in clear lactic acid and 30 measurements per structure were made for each strain under Nikon Eclipse 80i microscope using differential interference contrast (DIC) illumination. Appressoria were observed on the reverse side of SNA medium.

Colony characteristics on SNA and OA medium were observed after the incubation period. To obtain the growth rates, the diameters of colonies were measured after 7 and 10 d. The colors of colonies were determinated according to Rayner (1970).

2.2.4 Cross infection test

The pathogenicity test was conducted by inoculations on physiologically mature peach (*Prunus persica* cv. chimarrita), apple (*Malus domestica* cv. gala)

guava (*Psidium guajava* cv. kumagai), melon (*Cucumis melo* cv. amarelo), for strains grouped in C. acutatum complex. Avocado (*Persia americana* cv. quintal), mango (*Mangifera indica* cv. palmer), guava (*Psidium guajava* cv. kumagai) were used for strains grouped in *C. gloeosporioides* complex. All fruits used in the inoculation tests were standardized according the maturation stage which was determined through peel color and pulp firmness by a Minolta colorimeter and a penetrometer, respectively. Before the inoculation, the fruits were immersed into 0.5% hypochlorite solution for 3 min., then rinsed in sterile distilled water twice and air dried.

The strains CPC 20938, CPC 20940, CPC 20923, CPC 20969 CPC 20904, CPC 20954 (Table 3), CPC 20894, CPC 20897, CPC 20912, CPC 20916, CPC 20928 and Col 20, (Table 4) were grown on PDA for 7 days at 26 °C ±1 under 12 h photoperiod, to induce the sporulation (CAI et al., 2009). After the incubation, the spores were harvested by placing 10 mL of sterile distilled water onto the cultures followed by scraping it with sterile brush. The spore suspensions were filtered through sterile cheese cloth and the spores concentration was adjusted to 1 x 10^5.mL^{-1} using a hemocytometer.

The fruits were placed in plastic box, with soaked cotton wool, in five replicates and inoculated by deposition of 40 µL of spore suspension on their surfaces followed by wounding the fruits with a sterile needle. Control fruits were inoculated with sterile distilled water. The plastic boxes were kept in moist chamber at 25 °C for 48h. After this period, moist chamber was removed and the boxes remained at 25 ^{o}C, 80% RH and 12h photoperiod for 5 days. Fruits were checked daily to determinate the beginning of the symptoms (incubation period). After the symptoms appeared, the lesions were measured daily to obtain lesions growth rate. At the 7th day the final lesion size was measured. The latency period was also determined through daily observations of the beginning of sporulation on the lesions with the help of a 20X magnification lens. Due to no development of symptoms in fruits wounded by needle, the melon fruits were inoculated again by deposition of 4mm diameter colonized PDA plugs, removed from the edge of the colonies, in a 5mm deep wound made with a 5mm diameter cork borer.

2.3 Results and Discussion

2.3.1 Phylogenetic analysis of species in the *C. gloeosporioides* complex

The multigene analysis of 198 strains of *C. gloeosporioides* including the outgroup (*C. boninense* MAFF 305972) was performed with 2911 characters in the alignment, in which 488 were parsimony-informative, 332 were single site and 2006 constant. The gene boundaries were: CAL: 1-764, TUB2: 765-1508, ITS: 1509-2047, ACT: 2048-2346, CHS-1: 2347-2629, GAPDH: 2630-2911. For the Bayesian Inference, the selected evolution models based on AIC criteria (GTR+G for CAL and BTUB, SYM+I for ITS, HKY+I for GAPDH and ACT, K80+I for CHS-1) were included in the partitioned analysis. Some gene trees could not distinguish some species in the *C. gloeosporioides complex* (data not shown). Bayesian analysis of the partitioned data set and posterior probability are showed in Fig. 1.

The multilocus Bayesian analysis split 23 species in the *C. gloeosporioides* complex, according to Weir et al., (2012), however strains from Brazil grouped only in 7 species. The most genetically distant clades were *C. horii, C. theobromicola*. Posterior probabilities of some clades were low.

In this study, the species of *C. gloeosporioides* complex found affecting fruits in Brazil were *C. horii*, *C. thebromicola*, *C. gloeosporioides*, *C. tropicale*, *C. siamense*, *C. fructicola* and *C. asianum.* Almost all isolates from avocado (*Persia americana*) and mango (*Mangifera indica*) collected in Brazil were clustered in the *C. siamense* and *C. asianum* clades, respectively. For the *C. siamense* clade, the intraspecific variability was the highest, showing a considerable number of well supported subclades. Some of these subclades were related to the host of origin of the strains. Four strains isolated from avocado in South Africa clustered in the *C. siamense* clade, as well. The only isolate from acerola fruit (*Malpighia* sp.) and one from peach (*Prunus persica*) were included in the *C. theobromicola* and *C. siamense* clade, respectively. Despite reported species (*C. psidii*) in guava, no isolate from this host grouped with this species.

Considering the Bayesian inference obtained all brazilian strains showed a considerable genetic variability. The *C. siamense* represents the most genetically variable and the less host-specific clade. In the *C. asianum* clade only strains from mango from different geographic origin were grouped. The *C. fructicola* clade was split in two subclades and isolates from Brazil were included in only one of them.

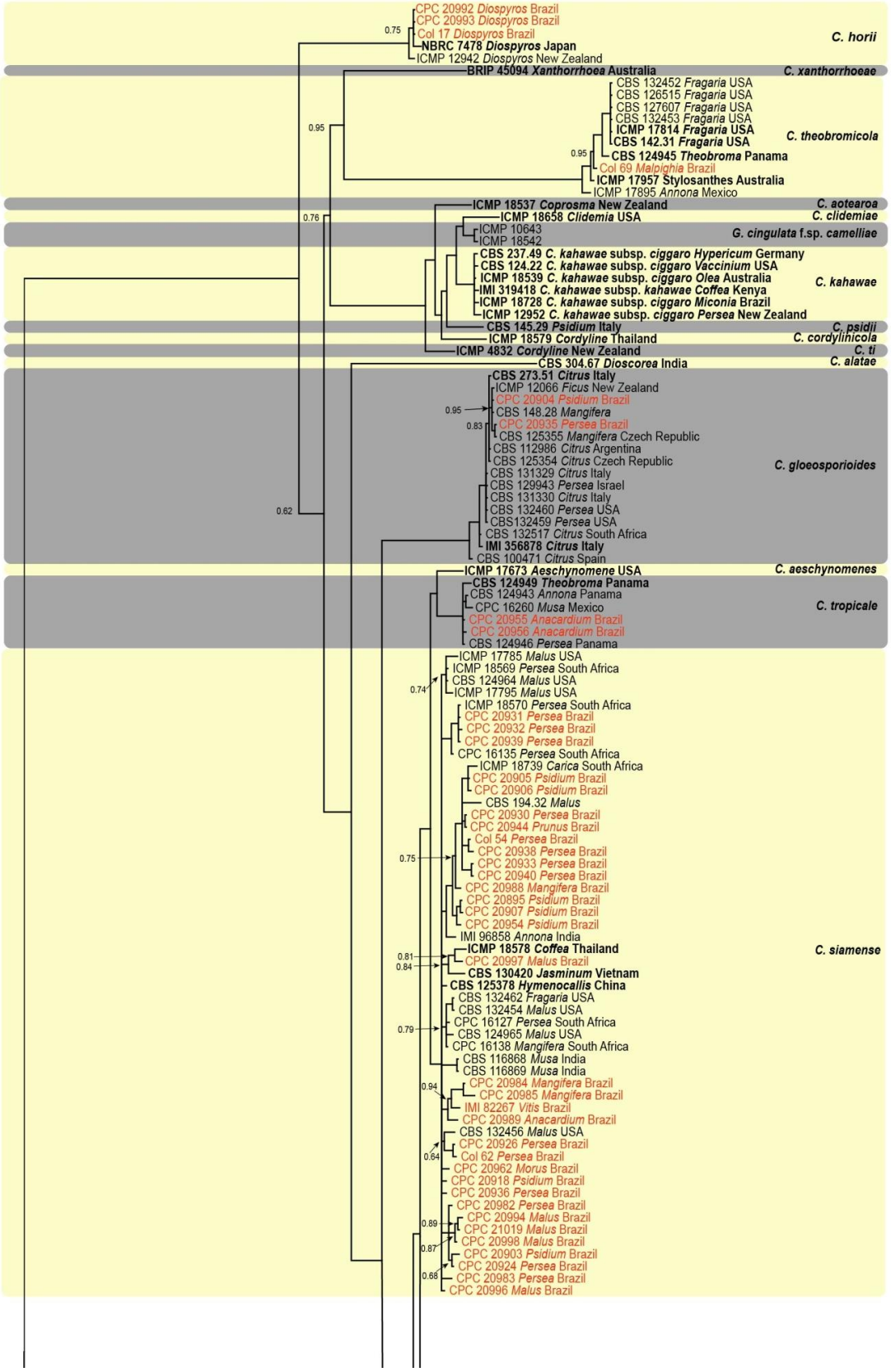

Figure 1 - Bayesian Inference phylogenetic tree of 198 isolates of the *C. gloeosporioides* complex species. The tree was reconstructed using partitioned data from sequences of the ITS, CAL, CHS-1, TUB, GAPDH and ACT. Bayesian posterior probability values ≤ 0.95 are shown in the node. Ex-type cultures are emphasized in bold font. Specie delimitations are indicated with the boxes. Isolates obtained from Brazil are emphasized in red font. The scale bar represents the number of expected changes per site (continue)

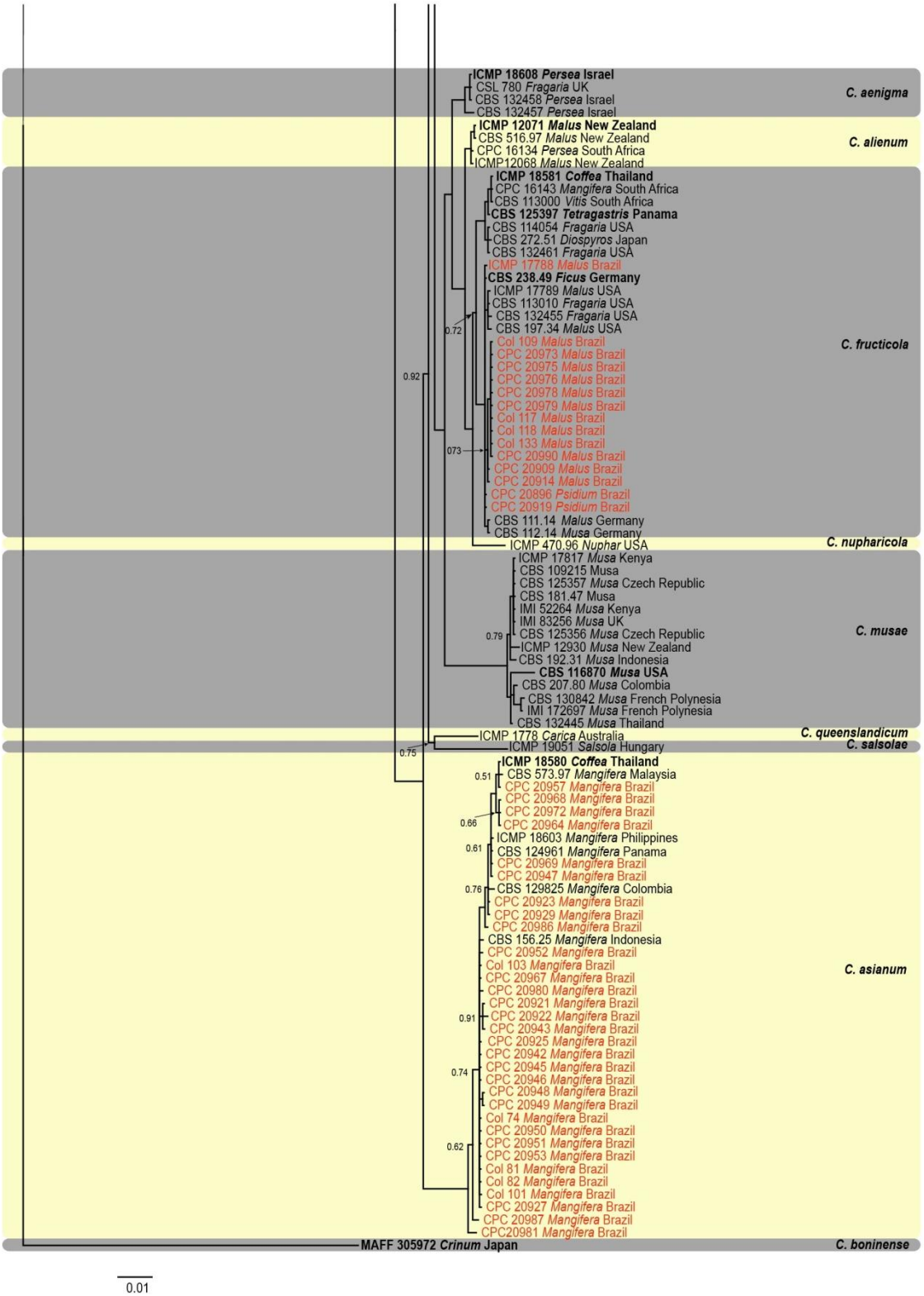

Figure 2 - Bayesian Inference phylogenetic tree of 198 isolates of the *C. gloeosporioides* complex species. The tree was reconstructed using partitioned data from sequences of the ITS, CAL, CHS-1, TUB, GAPDH and ACT. Bayesian posterior probability values ≤ 0.95 are shown in the node. Ex-type cultures are emphasized in bold font. Specie delimitations are indicated with the boxes. Isolates obtained from Brazil are emphasized in red font. The scale bar represents the number of expected changes per site (conclusion)

Table 1 - Strains of *Colletotrichum* spp. In the *C. gloeosporioides* complex studied, accession numbers, host, country and GenBank numbers

(continue)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	ACT	CHS-1	TUB2	CAL
C. aenigma	CSL 780	Fragaria x ananassa	UK	KC566725	KC566579	KC566871	KC566293	KC566149	KC566438
	CBS 132457	*Persea americana*	Israel	KC566726	KC566580	KC566872	KC566294	KC566150	KC566439
	CBS 132458	*Persea americana*	Israel	KC566727	KC566581	KC566873	KC566295	KC566151	KC566440
	ICMP 18608	*Persea americana*	Israel	JX010244	JX010044	JX009443	JX009774	JX010389	JX009683
C. aeschynomenes	**ICMP 17673**	*Aeschynomene virginica*	USA	JX010176	JX009930	JX009483	JX009799	JX010392	JX009721
C. alatae	**CBS 304.67**	*Dioscorea alata*	India	JX010190	JX009990	JX009471	JX009837	JX010383	JX009738
C. alienum	CBS 516.97	*Malus domestica*	New Zealand	KC566728	KC566582	KC566874	KC566296	KC566152	KC566441
	CPC 16134	*Persea americana*	South Africa	KC566729	KC566583	KC566875	KC566297	KC566153	-
	ICMP 12071	*Malus domestica*	New Zealand	JX010251	JX010028	JX009572	JX009882	JX010411	JX009654
	ICMP 12068	*Malus domestica*	New Zealand	JX010255	JX009925	JX009492	JX009889	-	JX009660
C. aotearoa	**ICMP 18537**	*Coprosma* sp.	New Zealand	JX010205	JX010005	JX009564	JX009853	JX010420	JX009611
C. boninense	**MAFF 305972**	*Crinum asiaticum* var. *sinicum*	Japan	JX010292	JX009905	JX009583	JX009827	-	-
C. clidemiae	**ICMP 18658**	*Clidemia hirta*	USA, Hawaii	JX010265	JX009989	JX009537	JX009877	JX010438	JX009645
C. musae	CBS 109215	*Musa* sp.	unknown	KC566791	KC566645	KC566937	KC566358	KC566213	KC566500
	CBS 125357	*Musa sapientum*	Czech Republic, imported from Guinea	KC566795	KC566649	KC566941	KC566362	KC566217	KC566504
	CBS 181.47	*Musa sapientum*	unknown	KC566796	KC566650	KC566942	KC566363	KC566218	KC566505
	IMI 52264	*Musa sapientum*	Kenya	KC566797	KC566651	KC566943	KC566364	KC566219	KC566506
	IMI 83256	*Musa* sp.	UK	KC566799	KC566653	KC566945	KC566366	KC566221	KC566508
	CBS 125356	*Musa sapientum* , fruit	Czech Republic, imported from Ecuador	KC566800	KC566654	KC566946	KC566367	KC566222	KC566509
	CBS 192.31	*Musa* sp.	Indonesia	KC566798	KC566652	KC566944	KC566365	KC566220	KC566507
	CBS 207.80	*Musa* sp., dried leaf	Colombia	KC566790	KC566644	KC566936	KC566357	KC566212	KC566499
	CBS 130842	*Musa* sp.	Windward Islands (French Polynesia)	KC566792	KC566646	KC566938	KC566359	KC566214	KC566501
	IMI 172697	*Musa* sp.	Windward Islands (French Polynesia)	KC566793	KC566647	KC566939	KC566360	KC566215	KC566502
	CBS 132445	*Musa* sp.	Thailand	KC566794	KC566648	KC566940	KC566361	KC566216	KC566503
	CBS 116870	*Musa* sp.	USA	JX010146	JX010050	JX009433	JX009896	HQ596280	JX009742
	ICMP 17817	*Musa sapientum*	Kenya	JX010142	JX010015	JX009432	JX009815	JX010395	JX009689
	ICMP 12930	*Musa* sp.	New Zealand	JX010141	JX009986	JX009566	JX009881	-	JX009685
C. nupharicola	**CBS 470.96**	*Nuphar lutea* subsp. *Polysepala*	USA	JX010187	JX009972	JX009437	JX009835	JX010398	JX009663
C. psidii	**CBS 145.29**	*Psidium* sp.	Italy	JX010219	JX009967	JX009515	JX009901	JX010443	JX009743
C. queenslandicum	**ICMP 1778**	*Carica papaya*	Australia	JX010276	JX009934	JX009447	JX009899	JX010414	JX009691
C. salsolae	**ICMP 19051**	*Salsola tragus*	Hungary	JX010242	JX009916	JX009562	JX009863	JX010403	JX009696

Table 1 - Strains of *Colletotrichum* spp. In the *C. gloeosporioides* complex studied, accession numbers, host, country and GenBank numbers (continuation)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	ACT	CHS-1	TUB2	CAL
C. ti	**ICMP 4832**	*Cordyline* sp.	New Zealand	JX010269	JX009952	JX009520	JX009898	JX010442	JX009649
C. xanthorrhoeae	**BRIP 45094**	Xanthorrhoea preisii	Australia	JX010261	JX009927	JX009478	JX009823	JX010448	JX009653
C. cordylinicola	**MFLUCC 090551**	*Cordyline fruticosa*	Thailand	JX010226	JX009975	HM470235	JX009864	JX010440	HM470238
C. fructicola	**ICMP 18581**	*Coffea arabica*	Thailand	JX010165	JX010033	FJ907426	JX009866	JX010405	FJ917508
	ICMP 17789	*Malus domestica*	USA	JX010178	JX009914	JX009451	JX009809	-	JX009665
	ICMP 17788	*Malus domestica*	Brazil	JX010177	JX009949	JX009458	JX009808	-	JX009672
C. fructicola (syn *C. ignotum*)	**CBS 125397**	*Tetragastris panamensis*	Panama	JX010173	JX010032	JX009581	JX009874	JX010409	JX009674
C. fructicola G. cingulata var. *minor*	**ICMP 17921**	*Ficus edulis*	Germany	JX010181	JX009923	JX009495	JX009839	JX010400	JX009671
	CPC 16143	*Mangifera indica*	South Africa	KC566780	KC566634	KC566926	KC566347	KC566202	KC566489
	CBS 113000	*Vitis vinifera*	South Africa	KC566781	KC566635	KC566927	KC566348	KC566203	KC566490
	CBS 114054	*Fragaria vulgaris*	USA	KC566782	KC566636	KC566928	KC566349	KC566204	KC566491
	CBS 272.51	*Diospyros kaki*	Japan	KC566783	KC566637	KC566929	KC566350	KC566205	KC566492
	CBS 132461	*Fragaria* sp.	USA	KC566784	KC566638	KC566930	KC566351	KC566206	KC566493
	CBS 113010	*Fragaria* sp.	USA	KC566787	KC566641	KC566933	KC566354	KC566209	KC566496
	CBS 132455	*Fragaria* sp.	USA	KC566788	KC566642	KC566934	KC566355	KC566210	KC566497
	CBS 197.34	*Malus sylvestris*	USA	KC566789	KC566643	KC566935	KC566356	KC566211	KC566498
	Col 109	*Malus domestica*	Brazil	KC566766	KC566620	KC566912	-	KC566188	KC566475
	Col 117	*Malus domestica*	Brazil	KC566772	KC566626	KC566918	KC566339	KC566194	KC566481
	Col 118	*Malus domestica*	Brazil	KC566773	KC566627	KC566919	KC566340	KC566195	KC566482
	Col 133	*Malus domestica*	Brazil	KC566774	KC566628	KC566920	KC566341	KC566196	KC566483
	CPC 20973	*Malus domestica*	Brazil	KC566767	KC566621	KC566913	KC566334	KC566189	KC566476
	CPC 20975	*Malus domestica*	Brazil	KC566768	KC566622	KC566914	KC566335	KC566190	KC566477
	CPC 20976	*Malus domestica*	Brazil	KC566769	KC566623	KC566915	KC566336	KC566191	KC566478
	CPC 20978	*Malus domestica*	Brazil	KC566770	KC566624	KC566916	KC566337	KC566192	KC566479
	CPC 20979	*Malus domestica*	Brazil	KC566771	KC566625	KC566917	KC566338	KC566193	KC566480
	CPC 20990	*Malus domestica*	Brazil	KC566775	KC566629	KC566921	KC566342	KC566197	KC566484
	CPC 20909	*Malus domestica*	Brazil	KC566777	KC566631	KC566923	KC566344	KC566199	KC566486
	CPC 20914	*Malus domestica*	Brazil	KC566778	KC566632	KC566924	KC566345	KC566200	KC566487
	CPC 20896	*Psidium guajava*	Brazil	KC566776	KC566630	KC566922	KC566343	KC566198	KC566485
	CPC 20919	*Psidium guajava*	Brazil	KC566779	KC566633	KC566925	KC566346	KC566201	KC566488
	CBS 111.14	*Malus sylvestris*	Germany	KC566785	KC566639	KC566931	KC566352	KC566207	KC566494

Table 1 - Strains of *Colletotrichum* spp. In the *C. gloeosporioides* complex studied, accession numbers, host, country and GenBank numbers

(continuation)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	ACT	CHS-1	TUB2	CAL
C. fructicola	CBS 112.14	*Musa* sp.	Germany	KC566786	KC566640	KC566932	KC566353	KC566208	KC566495
C. siamense	CBS 124964	*Malus domestica* . Fruit rot	USA	KC566812	KC566666	KC566958	KC566379	KC566234	KC566521
	CPC 20931	*Persia americana*	Brazil	KC566809	KC566663	KC566955	KC566376	KC566231	KC566518
	CPC 20932	*Persia americana*	Brazil	KC566810	KC566664	KC566956	KC566377	KC566232	KC566519
	CPC 20939	*Persia americana*	Brazil	KC566811	KC566665	KC566957	KC566378	KC566233	KC566520
	CPC 16135	*Persea americana*	South Africa	KC566808	KC566662	KC566954	KC566375	KC566230	KC566517
	CPC 20905	*Psidium guajava*	Brazil	KC566844	KC566698	KC566990	KC566411	KC566266	KC566552
	CPC 20906	*Psidium guajava*	Brazil	KC566845	KC566699	KC566991	KC566412	KC566267	KC566553
	CPC 20930	*Persea americana*	Brazil	KC566846	KC566700	KC566992	KC566413	KC566268	KC566554
	CPC 20944	*Prunus persica*	Brazil	KC566847	KC566701	KC566993	KC566414	KC566269	KC566555
	CBS 194.32	*Malus sylvestris* , fruit	unknown	KC566824	KC566678	KC566970	KC566391	KC566246	KC566532
	Col 54	*Persea americana*	Brazil	KC566848	KC566702	KC566994	KC566415	KC566270	KC566556
	CPC 20938	*Persea americana*	Brazil	KC566849	KC566703	KC566995	KC566416	KC566271	KC566557
	CPC 20933	*Persea americana*	Brazil	KC566850	KC566704	KC566996	KC566417	KC566272	KC566558
	CPC 20940	*Persea americana*	Brazil	KC566851	KC566705	KC566997	KC566418	KC566273	KC566559
	CPC 20988	*Mangifera indica*	Brazil	KC566827	KC566681	KC566973	KC566394	KC566249	KC566535
	CPC 20895	*Psidium guajava*	Brazil	KC566826	KC566680	KC566972	KC566393	KC566248	KC566534
	CPC 20907	*Psidium guajava*	Brazil	KC566852	KC566706	KC566998	KC566419	KC566274	-
	CPC 20954	*Psidium guajava*	Brazil	KC566831	KC566685	KC566977	KC566398	KC566253	KC566539
	IMI 96858	*Annona squamosa*	India	KC566825	KC566679	KC566971	KC566392	KC566247	KC566533
	CPC 20997	*Malus domestica*	Brazil	KC566843	KC566697	KC566989	KC566410	KC566265	KC566551
	CBS 132462	*Fragaria* sp.	USA	KC566813	KC566667	KC566959	KC566380	KC566235	KC566522
	CBS 132454	*Malus* sp.	USA	KC566814	KC566668	KC566960	KC566381	KC566236	KC566523
	CPC 16127	*Persea americana*	South Africa	KC566820	KC566674	KC566966	KC566387	KC566242	KC566528
	CBS 124965	*Malus domestica,* Fruit rot	USA	KC566821	KC566675	KC566967	KC566388	KC566243	KC566529
	CPC 16138	*Mangifera indica*	South Africa	KC566822	KC566676	KC566968	KC566389	KC566244	KC566530
	CBS 116868	*Musa* sp.	India	KC566815	KC566669	KC566961	KC566382	KC566237	KC566524
	CBS 116869	*Musa* sp.	India	KC566816	KC566670	KC566962	KC566383	KC566238	KC566525
	CPC 20984	*Mangifera indica*	Brazil	KC566817	KC566671	KC566963	KC566384	KC566239	KC566526
	CPC 20985	*Mangifera indica*	Brazil	KC566818	KC566672	KC566964	KC566385	KC566240	KC566560
	IMI 82267	*Vitis* sp.	Brazil	KC566823	KC566677	KC566969	KC566390	KC566245	KC566531

Table 1 - Strains of *Colletotrichum* spp. In the *C. gloeosporioides* complex studied, accession numbers, host, country and GenBank numbers (continuation)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	ACT	CHS-1	TUB2	CAL
C. siamense	CPC 20989	*Anacardium occidentale*	Brazil	KC566841	KC566695	KC566987	KC566408	KC566263	KC566549
	CBS 132456	*Malus* sp.	USA	KC566819	KC566673	KC566965	KC566386	KC566241	KC566527
	CPC 20926	*Persea americana*	Brazil	KC566839	KC566693	KC566985	KC566406	KC566261	KC566547
	Col 62	*Persea americana*	Brazil	KC566840	KC566694	KC566986	KC566407	KC566262	KC566548
	CPC 20962	*Morus sp.*	Brazil	KC566828	KC566682	KC566974	KC566395	KC566250	KC566536
	CPC 20918	*Psidium guajava*	Brazil	KC566829	KC566683	KC566975	KC566396	KC566251	KC566537
	CPC 20936	*Persea americana*	Brazil	KC566830	KC566684	KC566976	KC566397	KC566252	KC566538
	CPC 20982	*Persea americana*	Brazil	KC566832	KC566686	KC566978	KC566399	KC566254	KC566540
	CPC 20994	*Malus domestica*	Brazil	KC566833	KC566687	KC566979	KC566400	KC566255	KC566541
	CPC 21019	*Malus domestica*	Brazil	KC566834	KC566688	KC566980	KC566401	KC566256	KC566542
	CPC 20998	*Malus domestica*	Brazil	KC566835	KC566689	KC566981	KC566402	KC566257	KC566543
	CPC 20903	*Psidium guajava*	Brazil	KC566836	KC566690	KC566982	KC566403	KC566258	KC566544
	CPC 20924	*Persea americana*	Brazil	KC566837	KC566691	KC566983	KC566404	KC566259	KC566545
	CPC 20983	*Persea americana*	Brazil	KC566838	KC566692	KC566984	KC566405	KC566260	KC566546
	CPC 20996	*Malus domestica*	Brazil	KC566842	KC566696	KC566988	KC566409	KC566264	KC566550
	ICMP 18578	*Coffea arabica*	Thailand	JX010171	JX009924	FJ907423	JX009865	JX010404	FJ917505
	ICMP 18739	*Carica papaya*	South Africa	JX010161	JX009921	JX009484	JX009794	-	JX009716
	ICMP 17795	*Malus domestica*	USA	JX010162	JX010051	JX009506	JX009805	JX010393	JX009703
	ICMP 18570	*Persea americana*	South Africa	JX010248	JX009969	JX009510	JX009793	-	JX009699
	ICMP 17785	*Malus domestica*	USA	JX010272	JX010051	JX009446	JX009804	-	JX009706
	ICMP 18569	*Persea americana*	South Africa	JX010262	JX009963	JX009459	JX009795	-	JX009711
C. siamense (syn. *C. hymenocallidis*)	**CBS 125378**	*Hymenocallis americana*	China	JX010278	JX010019	GQ856775	GQ856730	JX010410	JX009709
C. siamense (syn. *C. jasmini-sambac*)	**CBS 130420**	*Jasminum sambac*	Vietnam	HM131511	HM131497	HM131507	JX009895	JX010415	JX009713
C. tropicale	CPC 16260	*Musa* sp.	Mexico	KC566807	KC566661	KC566953	KC566374	KC566229	KC566516
	CPC 20955	*Anacardium occidentale*	Brazil	KC566804	KC566658	KC566950	KC566371	KC566226	KC566513
	CPC 20956	*Anacardium occidentale*	Brazil	KC566805	KC566659	KC566951	KC566372	KC566227	KC566514
	CBS 124946	*Persea americana*, leaf lession	Panama	KC566806	KC566660	KC566952	KC566373	KC566228	KC566515
	ICMP 18651	*Annona muricata*	Panama	JX010277	JX010014	JX009570	JX009868	-	JX009720
	CBS 124949	*Theobroma cacao*	Panama	JX010264	JX010007	JX009489	JX009870	JX010407	JX009719
C. asianum	CBS 573.97	*Mangifera indica*	Malaysia	KC566732	KC566586	KC566878	KC566300	KC566155	KC566444
	CPC 20957	*Mangifera indica*	Brazil	KC566736	KC566590	KC566882	KC566304	KC566158	KC566448

Table 1 - Strains of *Colletotrichum* spp. In the *C. gloeosporioides* complex studied, accession numbers, host, country and GenBank numbers

(continuation)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	ACT	CHS-1	TUB2	CAL
C. asianum	CPC 20968	*Mangifera indica*	Brazil	KC566740	KC566594	KC566886	KC566308	KC566162	KC566452
	CPC 20972	*Mangifera indica*	Brazil	KC566741	KC566595	KC566887	KC566309	KC566163	KC566453
	CPC 20964	*Mangifera indica*	Brazil	KC566742	KC566596	KC566888	KC566310	KC566164	KC566454
	CPC 20969	*Mangifera indica*	Brazil	KC566737	KC566591	KC566883	KC566305	KC566159	KC566449
	CPC 20947	*Mangifera indica*	Brazil	KC566738	KC566592	KC566884	KC566306	KC566160	KC566450
	CPC 20923	*Mangifera indica*	Brazil	KC566743	KC566597	KC566889	KC566311	KC566165	KC566455
	CPC 20929	*Mangifera indica*	Brazil	KC566744	KC566598	KC566890	KC566312	KC566166	KC566456
	CPC 20986	*Mangifera indica*	Brazil	KC566731	KC566585	KC566877	KC566299	KC566154	KC566443
	CPC 20952	*Mangifera indica*	Brazil	KC566739	KC566593	KC566885	KC566307	KC566161	KC566451
	CPC 20967	*Mangifera indica*	Brazil	KC566746	KC566600	KC566892	KC566314	KC566168	KC566458
	CPC 20980	*Mangifera indica*	Brazil	KC566747	KC566601	KC566893	KC566315	KC566169	KC566459
	CBS 124961	*Mangifera indica*, fruit rot	Panama	KC566735	KC566589	KC566881	KC566303	KC566157	KC566447
	CBS 129825	*Mangifera indica*	Colombia	KC566734	KC566588	KC566880	KC566302	-	KC566446
	CBS 156.25	*Mangifera indica*, seedling	Indonesia	KC566730	KC566584	KC566876	KC566298	-	KC566442
	Col 103	*Mangifera indica*	Brazil	KC566745	KC566599	KC566891	KC566313	KC566167	KC566457
	CPC 20921	*Mangifera indica*	Brazil	KC566748	KC566602	KC566894	KC566316	KC566170	KC566460
	CPC 20922	*Mangifera indica*	Brazil	KC566749	KC566603	KC566895	KC566317	KC566171	KC566461
	CPC 20943	*Mangifera indica*	Brazil	KC566765	KC566619	KC566911	KC566333	KC566187	-
	CPC 20925	*Mangifera indica*	Brazil	KC566750	KC566604	KC566896	KC566318	KC566172	KC566462
	CPC 20942	*Mangifera indica*	Brazil	KC566751	KC566605	KC566897	KC566319	KC566173	KC566463
	CPC 20945	*Mangifera indica*	Brazil	KC566752	KC566606	KC566898	KC566320	KC566174	KC566464
	CPC 20946	*Mangifera indica*	Brazil	KC566753	KC566607	KC566899	KC566321	KC566175	KC566465
	CPC 20948	*Mangifera indica*	Brazil	KC566754	KC566608	KC566900	KC566322	KC566176	KC566466
	CPC 20949	*Mangifera indica*	Brazil	KC566755	KC566609	KC566901	KC566323	KC566177	KC566467
	Col 74	*Mangifera indica*	Brazil	KC566756	KC566610	KC566902	KC566324	KC566178	KC566468
	CPC 20950	*Mangifera indica*	Brazil	KC566757	KC566611	KC566903	KC566325	KC566179	KC566469
	CPC 20951	*Mangifera indica*	Brazil	KC566758	KC566612	KC566904	KC566326	KC566180	KC566470
	CPC 20953	*Mangifera indica*	Brazil	KC566759	KC566613	KC566905	KC566327	KC566181	KC566471
	Col 81	*Mangifera indica*	Brazil	KC566760	KC566614	KC566906	KC566328	KC566182	KC566472
	Col 82	*Mangifera indica*	Brazil	KC566761	KC566615	KC566907	KC566329	KC566183	KC566473
	CPC 20966	*Mangifera indica*	Brazil	KC566763	KC566617	KC566909	KC566331	KC566185	-

Table 1 - Strains of *Colletotrichum* spp. In the *C. gloeosporioides* complex studied, accession numbers, host, country and GenBank numbers (continuation)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	ACT	CHS-1	TUB2	CAL
C. asianum	CPC 20927	*Mangifera indica*	Brazil	KC566764	KC566618	KC566910	KC566332	KC566186	-
	CPC 20987	*Mangifera indica*	Brazil	KC566762	KC566616	KC566908	KC566330	KC566184	KC566474
	CPC 20981	*Mangifera indica*	Brazil	KC566733	KC566587	KC566879	KC566301	KC566156	KC566445
	ICMP 18603	*Mangifera indica*	Philippines	JX010195	JX009938	JX009579	JX009825	-	JX009725
	ICMP 18580	*Coffea arabica*	Thailand	FJ972612	JX010053	JX009584	JX009867	JX010406	FJ917506
C. gloeosporioides	CPC 20904	*Psidium guajava*	Brazil	KC566707	KC566561	KC566853	KC566275	KC566131	KC566420
	CBS 148.28	*Mangifera indica*, bud	India	KC566708	KC566562	KC566854	KC566276	KC566132	KC566421
	CPC 20935	*Persea americana*	Brazil	KC566709	KC566563	KC566855	KC566277	KC566133	KC566422
	CBS 125355	*Mangifera indica*	Czech Republic imported from Cuba	KC566714	KC566568	KC566860	KC566282	KC566138	KC566427
	CBS 112986	*Citrus* sp.	Argentina	KC566711	KC566565	KC566857	KC566279	KC566135	KC566424
	CBS 125354	*Citrus sinensis*, fruit	Czech Republic, imported from Spain	KC566712	KC566566	KC566858	KC566280	KC566136	KC566425
	CBS 131329	*Citrus sinensis*	Italy	KC566710	KC566564	KC566856	KC566278	KC566134	KC566423
	CBS 129943	*Persea americana*	Israel	KC566713	KC566567	KC566859	KC566281	KC566137	KC566426
	CBS 131330	*Citrus sinensis*	Italy	KC566715	KC566569	KC566861	KC566283	KC566139	KC566428
	CBS 132460	*Persea americana*	USA	KC566716	KC566570	KC566862	KC566284	KC566140	KC566429
	CBS 132459	*Persea americana*	USA	KC566717	KC566571	KC566863	KC566285	KC566141	KC566430
	CBS 132517	*Citrus* sp. (Soft citrus), leaves	South Africa	KC566718	KC566572	KC566864	KC566286	KC566142	KC566431
	CBS 100471	*Citrus aurantium*, leaf spot	Spain	KC566719	KC566573	KC566865	KC566287	KC566143	KC566432
	IMI 356878	*Citrus sinensis*	Italy	JX010152	JX010056	JX009531	JX009818	JX010445	JX009731
	ICMP 12066	*Ficus* sp.	New Zealand	JX010158	JX009955	JX009550	JX009888	-	JX009734
C. gloeosporioides	**ICMP 19121**	*Citrus limon*	Italy	JX010148	JX010054	JX009558	JX009903	-	JX009745
(syn. Gloeosporium pedemontanum)									
C. theobromicola	CBS 132452	*Fragaria* sp.	USA	KC566720	KC566574	KC566866	KC566288	KC566144	KC566433
	CBS 126515	Fragaria x ananassa	USA	KC566721	KC566575	KC566867	KC566289	KC566145	KC566434
	CBS 127607	Fragaria x ananassa	USA	KC566722	KC566576	KC566868	KC566290	KC566146	KC566435
	CBS 132453	*Fragaria sp.*	USA	KC566723	KC566577	KC566869	KC566291	KC566147	KC566436
	Col 69	*Malpighia emarginata*	Brazil	KC566724	KC566578	KC566870	KC566292	KC566148	KC566437
	CBS 124945	*Theobroma cacao*	Panama	JX010294	JX010006	JX009444	JX009869	JX010447	JX009591
	ICMP 17814	*Fragaria vesca*	USA	JX010288	JX010003	JX009448	JX009819	JX010379	JX009589
	ICMP 17895	*Annona diversifolia*	Mexico	JX010284	JX010057	JX009568	JX009828	JX010382	JX009600
C. theobromicola (syn. *C. fragariae*)	**CBS 142.31**	Fragaria × ananassa	USA	JX010286	JX010024	JX009516	JX009830	JX010373	JX009592

Table 1 - Strains of *Colletotrichum* spp. In the *C. gloeosporioides* complex studied, accession numbers, host, country and GenBank numbers

(conclusion)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	ACT	CHS-1	TUB2	CAL
C. theobromicola	**MUCL 42294**	*Stylosanthes viscosa*	Australia	JX010289	JX009962	JX009575	JX009821	JX010380	JX009597
(syn. C. gloeosporioides f. stylosanthis)									
C. horii	Col 17	*Diospyros kaki*	Brazil	KC566803	KC566657	KC566949	KC566370	KC566225	KC566512
	CPC 20992	*Diospyros kaki*	Brazil	KC566801	KC566655	KC566947	KC566368	KC566223	KC566510
	CPC 20993	*Diospyros kaki*	Brazil	KC566802	KC566656	KC566948	KC566369	KC566224	KC566511
	NBRC 7478	*Diospyros kaki*	Japan	GQ329690	GQ329681	JX009438	JX009752	JX010450	JX009604
	ICMP 12942	*Diospyros kaki*	New Zealand	GQ329687	GQ329685	JX009533	JX009748	JX010375	JX009603
C. kahawae subsp. *kahawae*	**IMI 319418**	*Coffea arabica*	Kenya	JX010231	JX010012	JX009452	JX009813	JX010444	JX009642
C. kahawae subsp. *ciggaro*	**ICMP 18539**	*Olea europaea*	Australia	JX010230	JX009966	JX009523	JX009800	JX010434	JX009635
	ICMP 12952	*Persea americana*	New Zealand	JX010214	JX009971	JX009431	JX009757	JX010426	JX009648
	ICMP 18728	*Miconia* sp.	Brazil	JX010239	JX010048	JX009525	JX009850	-	JX009643
C. kahawae subsp. *ciggaro*	**CBS 237.49**	*Hypericum perforatum*	Germany	JX010238	JX010042	JX009450	JX009840	JX010432	JX009636
(syn. Glomerella cingulata var. migrans)									
C. kahawae subsp. *ciggaro*	**CBS 124.22**	*Vaccinium* sp.	USA	JX010228	JX009950	JX009536	JX009902	JX010433	JX009744
(syn. Glomerella rufomaculans var. vaccinii)									
Glomerella cingulata "f.sp. *camelliae*"	**ICMP 10643**	Camellia × williamsii	UK	JX010224	JX009908	JX009540	JX009891	JX010436	JX009630
Glomerella cingulata "f.sp. *camelliae*"	**ICMP 18542**	*Camellia sasanqua*	USA	JX010223	JX009994	JX009488	JX009857	JX010429	JX009628

[1]Accession numbers in bold represent ex-type sequences;
[2]GenBank numbers started with KC were obtained in this study

Anthracnose diseases, caused by *Colletotrichum* species, can affect many high value crop plants, especially fruits (PERES et al., 2002; DAMM et al., 2012; WEIR et al, 2012). In tropical and subtropical regions, the species *C. gloeosporioides* and *C. acutatum* were considered the most common in a broad sense. But in recent years many species related to these have been recognized as part of a complex of species (cannon et al., 2012). Despite great advances in systematic of this genus, *C. gloeosporioides* and *C. acuatum* still have been used in a broad sense as causal agents of anthracnose on fruits in Brazil.

The results obtained in this study will clarify the status of *Colletotrichum* diseases on fruits in Brazil and will contribute to its systematics. Some species reported here, were not described previously and some others are first reported in Brazil. Furthermore, there are genetic differences between strains from Brazil and other countries in both species complexes studied. In the *C. gloeosporioides* complex, *C. asianum*, *C. siamense* and *C. fructicola*, were more frequent on mango, avocado and apple, respectively. These three species were first described by Prihastuti (2009) affecting coffee berries in Thailand and have been considered as biologically and geographically diverse, except for *C. asianum*, which is associated with mango (PHOULIVONG et al., 2010; WEIR et a., 2012).

The genetic variability of *Colletotrichum* species on different hosts have been studied in Brazil. Serra et al. (2011a), using morphological features, reported the occurrence of high variability in strains from mango. The same authors differentiated isolates from mango and cashew by pathogenicity tests, isozyme and RAPD analysis and considered *C. gloeosporioides* as the causal agent of anthracnose on those crops (SERRA et al., 2011b). The same species is associated with avocado in Brazil. Tozze Júnior (2012) studying isolates from peach, passion fruit (*Passiflora edulis*), mango and avocado noted high variability based on morphological features and association between host and isolate based on ITS and TUB2 phylogenetic analysis. The Bayesian analysis (Fig. 1) showed a similar result. Although different species were associated with the same host, the frequency of the species was different among the hosts studied and *C. asianum* and *C. horii* affected only mango and kaki.

Bitter rot is considered one of the most important diseases of apple and the species associated with this disease are known as *C. gloeosporioides*, *Glomerella cingulata* and *C. acutatum* (SUTTON, 1990). Populations of these pathogens are

noted as genetically variable. González et al., (2006) studied different populations from United States and Brazil and identified different morphological features and VCGs (vegetative compatibility groups) among strains associated with bitter rot. Based on mtDNA RFLP and phylogeny GAPDH intron, strains of *G. cingulata* causing Glomerela leaf spot were included in distinct haplotypes representing the populations of Brazil and United States. According to the Bayesian inference (Fig. 1), strains from Brazil seem to be a genetic distinct group in *C. fructicola* clade. The isolates from apple and those from United States grouped in a small clade.

The strain Col 69 grouped with a well supported PP in the *C. theobromicola* clade (Fig. 1). It was the first report of this species associated with acerola fruit. In Brazil anthracnose disease on this host is associated with *C. gloeosporioides* (ALMEIDA et al., 2003). *C. theobromicola* was described by Rojas et al. (2010) affecting leaves and fruits of cacao, but is considered a species widely distributed on different hosts in tropical and subtropical regions (WEIR et a., 2012). The same authors considered that this species contains several specialized pathogens. According to the Bayesian inference (Fig. 1), the strain Col 69 split in another branch and seems to be genetically distinct within *C. theobromicola* clade. However, to support this idea, more strains should be investigated.

2.3.2 Phylogenetic analysis of species in the *C. acutatum* complex

The Multigene analysis of 68 isolates of *C. acutatum* including outgroup (*C. orchidophilum* CBS 632.80) was performed with 2228 characters, in which 286 were parsimony-informative, 218 were single site and 1706 constant. The gene boundaries were: ITS: 1-544, GAPDH: 545-819, CHS-1: 820-1101, HS3: 1102-1488, ACT: 1489-1736, TUB2: 1737-2228. For the Bayesian Inference, selected evolution models based on AIC criteria were: GTR+G for ACT and GAPDH, HKY+G model for BTUB, K80+I+G for CHS-1, a GTR+I+G for HS3 and GTR+I for ITS. These models were considered in a partitioned analysis. The phylogram and posterior probabilities were shown in Fig. 2. According to the multilocus analysis, three new species (*C. polyphialidicum*, *C. paranaense* and *C. pruni*) were recognized and one strain was described as a possible new species (strain CPC 20912). The same result was obtained with maximum parsimony analysis, and because of the same topology, only

Bayesian tree was showed. A heuristic search retained 255 parsimony trees (length = 892 steps, CI =0.667, RI = 0.874, RC = 0.583, HI = 0.333).

The Bayesian analysis split the strains in 5 branches, but only two of them grouped more than 2 isolates. The *C. nymphaeae* clade was well supported with a Bayesian posterior probability value of 1.0. In this clade, almost all strains from apple, including one from Brazil (IMI 370491 – Table 2) and available on GenBank, grouped in a subclade with the same support value. It seems that the strains from Brazil from *C. nymphaeae* clade are genetically distinct. Although the strain CPC 20912 grouped separated from *C. melonis* with a BPP value of 1.0, in the TUB2, HIS3 and ACT gene trees (data not shown) this strain grouped with the *C. melonis* ex-type and so it was not recognized as a new species. To certificate that the CPC20912 branch represents a distinct species in the *C. acutatum* complex, more strains should be studied.

Two of the new species formed a long, well supported branch, and the third one grouped (BPP of 1.0) with a strain, also from Brazil and not described as a new species (DAMM et al., 2012) (Fig. 2). The strain CPC 20894 from guava, recognized in this study as a new species, is genetically distant from *C. guajavae* branch.

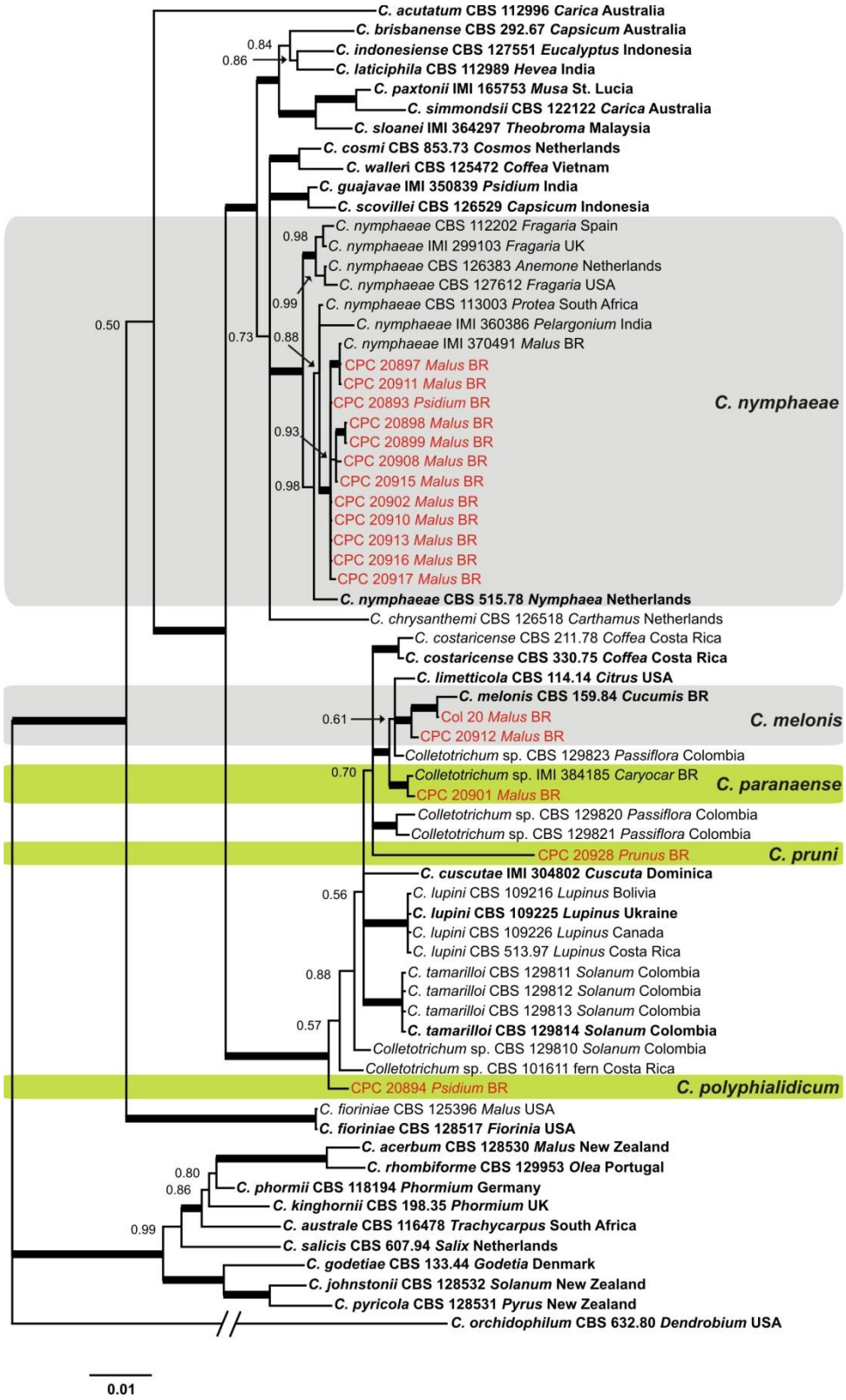

Figure 3 - Bayesian Inference phylogenetic tree of 68 isolates of the *C. acutatum* complex species. The tree was reconstructed using partitioned data from sequences of the ITS, HS3, CHS-1, TUB, GAPDH and ACT. Bayesian posterior probability (PP) values are shown in the node. The thick node represents 1 PP. Ex-type cultures are emphasized in bold font. New species are indicated with green boxes. Isolates obtained in this study are emphasized in red font. The scale bar represents the number of expected changes per site

Almost all strains from *Malus* were placed in a well supported subclade in the *C. nymphaeae* clade (Fig. 2) and seem to be a distinct population. The strain IMI 370471, also from Brazil and included in that subclade, represents a distinct branch in the *C. nymphaeae* (DAMM et al., 2012). This species is widely spread and genetically variable (DAMM et al., 2012).The authors showed that this species seems to be more important in strawberry than *C. fioriniae* in United Estates, so far considered the most important species on this host. The host specificity of *Colletotrichum* species is not clear due to poor knowledge about pathogenic effects and host-parasite interactions (CANNON et al., 2012). However, studies indicated that some species are naturally associated with a particular host, for example, *C. asianum* and *C. horii* were associated with mango and kaki respectively (WEIR et al., 2012). Mackenzie et al. (2009) found pathological differences between genetically distinct strains of *C. acutatum* species affecting strawberry, blueberry, citrus and fern.

Table 2 - Strains of *Colletotrichum* spp. in the *C. acutatum* complex studied, accession numbers, host, country and GenBank numbers

(continue)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	CHS-1	HIS3	ACT	TUB2
C. acutatum	**CBS 112996, ATCC 56816, STE-U 5292**	*Carica papaya*	Australia	JQ005776	JQ948677	JQ005797	JQ005818	JQ005839	JQ005860
C. brisbanense	**CBS 292.67, DPI 11711**	*Capsicum annuum*	Australia	JQ948291	JQ948621	JQ948952	JQ949282	JQ949612	JQ949942
C. indonesiense	**CBS 127551, CPC 14986**	*Eucalyptus* sp.	Indonesia	JQ948288	JQ948618	JQ948949	JQ949279	JQ949609	JQ949939
C. laticiphilum	**CBS 112989, IMI 383015, STE-U 5303**	*Hevea brasiliensis*	India	JQ948289	JQ948619	JQ948950	JQ949280	JQ949610	JQ949940
C. paxtonii	**IMI 165753, CPC 18868**	*Musa* sp.	Saint Lucia	JQ948285	JQ948615	JQ948946	JQ949276	JQ949606	JQ949936
C. simmondsii	**CBS 122122, BRIP 28519**	*Carica papaya*	Australia	JQ948276	JQ948606	JQ948937	JQ949267	JQ949597	JQ949927
C. sloanei	**IMI 364297, CPC 18929**	*Theobroma cacao*	Malaysia	JQ948287	JQ948617	JQ948948	JQ949278	JQ949608	JQ949938
C. cosmi	**CBS 853.73, PD 73/856**	*Cosmos* sp.	Netherlands	JQ948274	JQ948604	JQ948935	JQ949265	JQ949595	JQ949925
C. walleri	**CBS 125472, BMT(HL)19**	*Coffea* sp., leaf tissue	Vietnam	JQ948275	JQ948605	JQ948936	JQ949266	JQ949596	JQ949926
C. guajavae	**IMI 350839, CPC 18893**	*Psidium guajava* , fruit	India	JQ948270	JQ948600	JQ948931	JQ949261	JQ949591	JQ949921
C. scovillei	**CBS 126529, PD 94/921-3, BBA 70349**	*Capsicum* sp.	Indonesia	JQ948267	JQ948597	JQ948928	JQ949258	JQ949588	JQ949918
C. nymphaeae	CBS 112202	*Fragaria* sp., fruit lesions	Spain	JQ948234	JQ948564	JQ948895	JQ949225	JQ949555	JQ949885
	IMI 299103, CPC 18871	*Fragaria vesca*	UK	JQ948231	JQ948561	JQ948892	JQ949222	JQ949552	JQ949882
	CBS 126383, PD 84/121	*Anemone coronaria* , cv. de Caen group	Netherlands	JQ948221	JQ948551	JQ948882	JQ949212	JQ949542	JQ949872
	CBS 127612, DAOM 213709, H-1984	*Fragaria × ananassa*	USA	JQ948230	JQ948560	JQ948891	JQ949221	JQ949551	JQ949881
	CBS 113003, STE-U 4457	*Protea* sp.	South Africa	JQ948209	JQ948539	JQ948870	JQ949200	JQ949530	JQ949860
	IMI 360386, CPC 18925	*Pelargonium graveolens* , petiole, leaf and twig	India	JQ948206	JQ948536	JQ948867	JQ949197	JQ949527	JQ949857
	IMI 370491, CPC 18932	*Malus pumila* , fruit	Brazil	JQ948204	JQ948534	JQ948865	JQ949195	JQ949525	JQ949855
	CPC 20897	*Malus domestica*	Brazil	KC204989	KC205023	KC205040	KC205008	KC205074	KC205057
	CPC 20911	*Malus domestica*	Brazil	KC204996	KC205023	KC205047	KC205014	KC205081	KC205064
	CPC 20893	*Psidium guajava*	Brazil	KC204987	KC205021	KC205038	KC205005	KC205072	KC205055
	CPC 20898	*Malus domestica*	Brazil	KC204990	KC205024	KC205041	KC205009	KC205075	KC205058
	CPC 20899	*Malus domestica*	Brazil	KC204991	KC205025	KC205042	KC205010	KC205076	KC205059
	CPC 20908	*Malus domestica*	Brazil	KC204994	KC205028	KC205045	KC205012	KC205079	KC205062
	CPC 20915	*Malus domestica*	Brazil	KC204999	KC205033	KC205050	KC205016	KC205084	KC205067
	CPC 20902	*Malus domestica*	Brazil	KC204993	KC205027	KC205044	KC205011	KC205078	KC205061
	CPC 20913	*Malus domestica*	Brazil	KC204998	KC205032	KC205049	KC205015	KC205083	KC205066
	CPC 20910	*Malus domestica*	Brazil	KC204995	KC205029	KC205046	KC205013	KC205080	KC205063
	CPC 20916	*Malus domestica*	Brazil	KC205000	KC205034	KC205051	KC205017	KC205085	KC205068

Table - 2 Strains of *Colletotrichum* spp. in the *C. acutatum* complex studied, accession numbers, host, country and GenBank numbers

(continuation)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	CHS-1	HIS3	ACT	TUB2
C. nymphaeae	CPC 20917	*Malus domestica*	Brazil	KC205001	KC205035	KC205052	KC205018	KC205086	KC205069
	CBS 515.78	*Nymphaea alba* , leaf spot	Netherlands	JQ948197	JQ948527	JQ948858	JQ949188	JQ949518	JQ949848
C. chrysanthemi	**CBS 126518, PD 84/520**	*Carthamus* sp., twisted stem	Netherlands	JQ948271	JQ948601	JQ948932	JQ949262	JQ949592	JQ949922
C. costaricense	CBS 211.78, IMI 309622	*Coffea* sp., twig	Costa Rica	JQ948181	JQ948511	JQ948842	JQ949172	JQ949502	JQ949832
	CBS 330.75	*Coffea arabica* , cv. Typica, berry	Costa Rica	JQ948180	JQ948510	JQ948841	JQ949171	JQ949501	JQ949831
C. limetticola	**CBS 114.14**	*Citrus aurantifolia* , young twig	USA, Florida	JQ948193	JQ948523	JQ948854	JQ949184	JQ949514	JQ949844
C. melonis	**CBS 159.84**	*Cucumis melo* , peel of fruit	Brazil	JQ948194	JQ948524	JQ948855	JQ949185	JQ949515	JQ949845
	Col 20	*Malus domestica*	Brazil	KC204986	KC205020	KC205037	KC205006	KC205071	KC205054
Colletotrichum sp.	CPC 20912	*Malus domestica*	Brazil	KC204997	KC205031	KC205048	KC205007	KC205082	KC205065
	CBS 129823, G8	*Passiflora edulis* , leaf, anthracnose	Colombia	JQ948192	JQ948522	JQ948853	JQ949183	JQ949513	JQ949843
	IMI 384185, CPC 18937	*Caryocar brasiliense*	Brazil	JQ948191	JQ948521	JQ948852	JQ949182	JQ949512	JQ949842
	CPC 20901	*Malus domestica*	Brazil	KC204992	KC205026	KC205043	KC205004	KC205077	KC205060
	CBS 129820, G5	*Passiflora edulis* , fruit, scab	Colombia	JQ948183	JQ948513	JQ948844	JQ949174	JQ949504	JQ949834
	CBS 129821, G6	*Passiflora edulis* , fruit, scab	Colombia	JQ948182	JQ948512	JQ948843	JQ949173	JQ949503	JQ949833
	CPC 20928	*Prunus persica*	Brazil	KC205002	KC205036	KC205053	KC205019	KC205087	KC205070
C. cuscutae	**IMI 304802, CPC 18873**	*Cuscuta* sp.	Dominica	JQ948195	JQ948525	JQ948856	JQ949186	JQ949516	JQ949846
C. lupini	CBS 109216, BBA 63879	*Lupinus mutabilis*	Bolivia	JQ948156	JQ948486	JQ948817	JQ949147	JQ949477	JQ949807
	CBS 109225, BBA 70884	*Lupinus albus*	Ukraine	JQ948155	JQ948485	JQ948816	JQ949146	JQ949476	JQ949806
	CBS 109226, BBA 71249	*Lupinus albus*	Canada	JQ948158	JQ948488	JQ948819	JQ949149	JQ949479	JQ949809
	CBS 513.97, LARS 401	*Lupinus polyphyllus*	Costa Rica	JQ948157	JQ948487	JQ948818	JQ949148	JQ949478	JQ949808
C. tamarilloi	CBS 129811, T.A.3	*Solanum betaceum* , fruit, anthracnose	Colombia	JQ948185	JQ948515	JQ948846	JQ949176	JQ949506	JQ949836
	CBS 129812, T.A.4	*Solanum betaceum* , fruit, anthracnose	Colombia	JQ948186	JQ948516	JQ948847	JQ949177	JQ949507	JQ949837
	CBS 129813, T.A.5	*Solanum betaceum* , fruit, anthracnose	Colombia	JQ948187	JQ948517	JQ948848	JQ949178	JQ949508	JQ949838
	CBS 129814, T.A.6	*Solanum betaceum* , fruit, anthracnose	Colombia	JQ948184	JQ948514	JQ948845	JQ949175	JQ949505	JQ949835
Colletotrichum sp.	CBS 129810, T.A.2	*Solanum betaceum* , fruit, anthracnose	Colombia	JQ948179	JQ948509	JQ948840	JQ949170	JQ949500	JQ949830
	CBS 101611	Fern	Costa Rica	JQ948196	JQ948526	JQ948857	JQ949187	JQ949517	JQ949847
	CPC 20894	*Psidium guajava* , fruit	Brazil	KC204988	KC205022	KC205039	KC205003	KC205073	KC205056
C. fioriniae	CBS 125396, GJS 08-140A	*Malus domestica* , fruit lesion	USA	JQ948299	JQ948629	JQ948960	JQ949290	JQ949620	JQ949950
	CBS 128517, ARSEF 10222, ERL 1257, EHS 58	*Fiorinia externa* (elongate hemlock scale, insect)	USA	JQ948292	JQ948622	JQ948953	JQ949283	JQ949613	JQ949943

Table 2. Strains of *Colletotrichum* spp. in the *C. acutatum* complex studied, accession numbers, host, country and GenBank numbers

(conclusion)

Species	Accession N.[1]	Host/Substrate	Country	GenBank N.[2]					
				ITS	GAPDH	CHS-1	HIS3	ACT	TUB2
C. acerbum	**CBS 128530, ICMP 12921, PRJ 1199.3**	*Malus domestica* , bitter rot of fruit	New Zealand	JQ948459	JQ948790	JQ949120	JQ949450	JQ949780	JQ950110
C. rhombiforme	**CBS 129953, PT250, RB011**	*Olea europaea*	Portugal	JQ948457	JQ948788	JQ949118	JQ949448	JQ949778	JQ950108
C. phormii	**CBS 118194, AR 3546**	*Phormium* sp.	Germany	JQ948446	JQ948777	JQ949107	JQ949437	JQ949767	JQ950097
C. kinghornii	**CBS 198.35**	*Phormium* sp.	UK	JQ948454	JQ948785	JQ949115	JQ949445	JQ949775	JQ950105
C. australe	**CBS 116478, HKUCC 2616**	*Trachycarpus fortunei*	South Africa	JQ948455	JQ948786	JQ949116	JQ949446	JQ949776	JQ950106
C. salicis	**CBS 607.94**	*Salix* sp., leaf, spot	Netherlands	JQ948460	JQ948791	JQ949121	JQ949451	JQ949781	JQ950111
C. godetiae	**CBS 133.44**	*Clarkia hybrida* , cv. Kelvon Glory, seed	Denmark	JQ948402	JQ948733	JQ949063	JQ949393	JQ949723	JQ950053
C. johnstonii	**CBS 128532, ICMP 12926, PRJ 1139.3**	*Solanum lycopersicum* , fruit rot	New Zealand	JQ948444	JQ948775	JQ949105	JQ949435	JQ949765	JQ950095
C. pyricola	**CBS 128531, ICMP 12924, PRJ 977.1**	*Pyrus communis* , fruit rot	New Zealand	JQ948445	JQ948776	JQ949106	JQ949436	JQ949766	JQ950096
C. orchidophilum	**CBS 632.80**	*Dendrobium* sp.	USA	JQ948151	JQ948481	JQ948812	JQ949142	JQ949472	JQ949802

[1] Species studied;
[2]GenBank numbers started with KC were generated in this study.

2.3.3 Morphology of *C. acutatum* strains

2.3.3.1 *C. pruni* (strain CPC 20928)

Sexual state not observed. Asexual state on SNA. Hyphae 2.5–5 µm diam, hyaline, sometimes pale brown, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* not developed, conidiophores formed directly on hyphae. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate, branched, to 31 µm long. *Conidiogenous cells* hyaline, smooth-walled, elongate-ampulliform to ampulliform, polyphialides sometimes observed, 7–13 × 2.5–3.5 µm, opening 1 µm diam, collarette 1–1.5 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, both ends acute, sometimes with one end round, (7–)10.5–15(–18) × (3.5–)3.5–4.5(–5) µm, mean ± SD = 12.8 ± 2.3 × 4.0 ± 0.3 µm, L/W ratio = 3.2. *Appressoria* single, dark brown, obovoidal, reniform or clavate, the edge entire, (4–)5–11(–16.5) × (3.5–)4–6(–6.5) µm, mean ± SD = 7.9 ± 2.9 × 5 ± 0.8 µm, L/W ratio = 1.6.

Asexual state on *Anthriscus* stem. *Conidiomata* acervular, conidiophores formed on pale brown angular basal cells 5.5–6.5 µm diam. *Setae* not observed. *Conidiophores*, hyaline, smooth-walled, septate, branched, to 30 µm long. *Conidiogenous cell*s hyaline, smooth-walled, elongate-ampulliform, 12–20 × 2.5–3 µm, opening 1–2 µm diam, collarette pale brown, 1–2 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth–walled, aseptate, cylindrical, both ends acute, sometimes with one end round, (11–)12.5–15.5(–17) × 4–4.5(–5) µm, mean ± SD = 14.1 ± 1.5 × 4.2 ± 0.3 µm, L/W ratio = 3.3.

Culture characteristics: Colonies on SNA flat with entire edge, hyaline, filter paper and center pale grey, medium, filter paper and *Anthriscus* stem partly covered by floccose white aerial mycelium, reverse same color, *Anthriscus* stem partly orange due to sporulation, growth rate 23.3–23.8 mm in 7 d and 34.3–34.5 mm in 10 d. Colonies on OA flat with entire edge, vinaceous buff to pale olivaceous grey, medium entirely covered by floccose-felty aerial mycelium with small black dots, reverse rosy buff, olivaceous grey to iron grey, growth rate 21.5–22.3 mm in 7 d and 32.3 mm in 10 d. *Conidia in mass* orange.

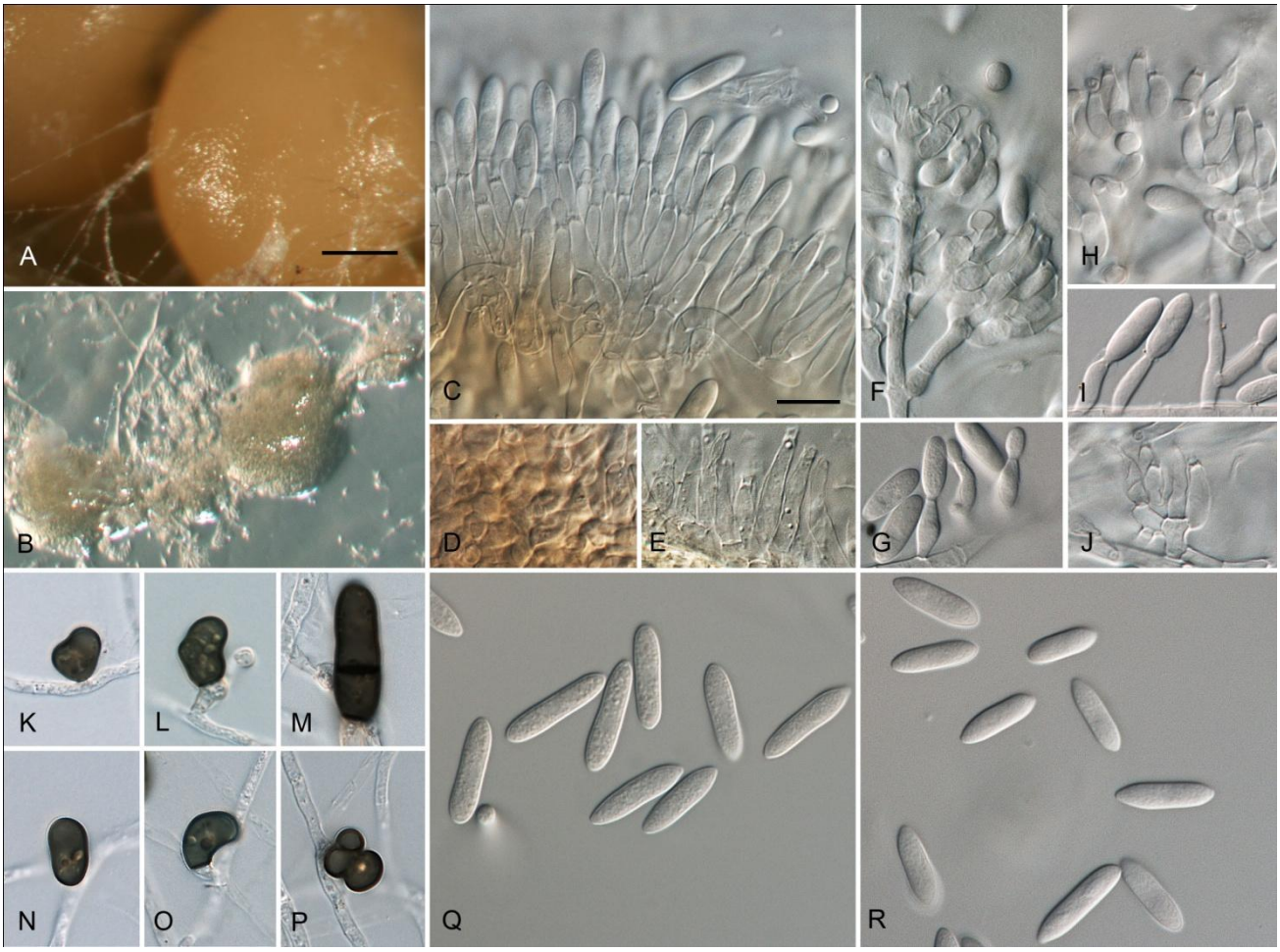

Figure 4 - *Colletotrichum* sp. Isolate CPC 20928. A-B. conidiomata. C-J. conidiophores. K-P. Apressoria. Q-R. conidia. A, C-E. from *Anthriscus* stem. B, F-R. from SNA. A-B. Dissecting microscope. C-R. Differential interference contrast. Scale bars: A = 100 µm, C = 10 µm

2.3.3.2 *C. polyphialidicum* (strain CPC 20894)

Sexual state not observed. Asexual state on SNA. *Vegetative hyphae* 2–3.5 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* not developed, conidiophores formed directly on hyphae. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate, sometimes branched,10–29 µm long. *Conidiogenous cells* hyaline, smooth-walled, elongate-ampulliform, 5–17.5 × 2–3 µm, sometimes integrated (not separated from ferile hyphae by a septum), sometimes polyphialides, opening 1–1.5 µm diam, collarette 1–1.5 µm long, periclinal thickening visible. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, both ends round, sometimes with one end acute, (10.5–)12–14.5(–16) × (3–)3.5–4(–

4.5) µm, mean ± SD = 13.2 ± 1.5 × 3.7 ± 0.4 µm, L/W ratio = 3.6. *Appressoria* single, pale to medium brown, obovoidal, ellipsoidal or clavate, the edge undulate to lobate and sometimes entire, (6–)7–12.5(–21) × (4.5–)5–6.5(–7.5) µm, mean ± SD = 9.8 ± 2.9 × 5.9 ± 0.7 µm, L/W ratio = 1.7.

Asexual state on *Anthriscus* stem. *Conidiomata*, acervular, conidiophores formed on hyaline to pale brown, angular, basal cells 5.5–6.5 µm diam. *Setae* not observed. *Conidiophores* hyaline to pale brown, smooth-walled, septate, branched, to 35 µm long. *Conidiogenous cell*s hyaline to pale brown, smooth-walled elongate-ampulliform, sometimes attenuated at the base, 12–15 × 2.4–3.3 µm, opening 1 µm diam, collarette pale brown, 1 µm long, periclinal thickening visible. *Conidia* hyaline, smooth–walled, aseptate, cylindrical, with one end acute, sometimes both ends acute, (8.5–)12–16(–17) × (3–)4–4.5(–5) µm, mean ± SD = 14 ± 1.9 × 4.3 ± 0.5 µm, L/W ratio = 3.3.

Culture characteristics: Colonies on SNA flat with entire edge, buff, filter paper partly covered by olivaceous felty aerial mycelium and *Anthriscus* stem partly covered by felty aerial mycelium and partly orange due to conidia mass, reverse same color, growth rate 21–22.3 mm in 7 d and 31–32.5 mm in 10 d. Colonies on OA flat to umbonate with entire edge, olivaceous to pale olivaceous, partly covered by white olivaceous grey floccose-felty aerial mycelium, reverse pale olivaceous grey to olivaceous grey, growth rate 16.8–17.5 mm in 7 d and 27.8–28.3 mm in 10 d. Conidia in mass saffron.

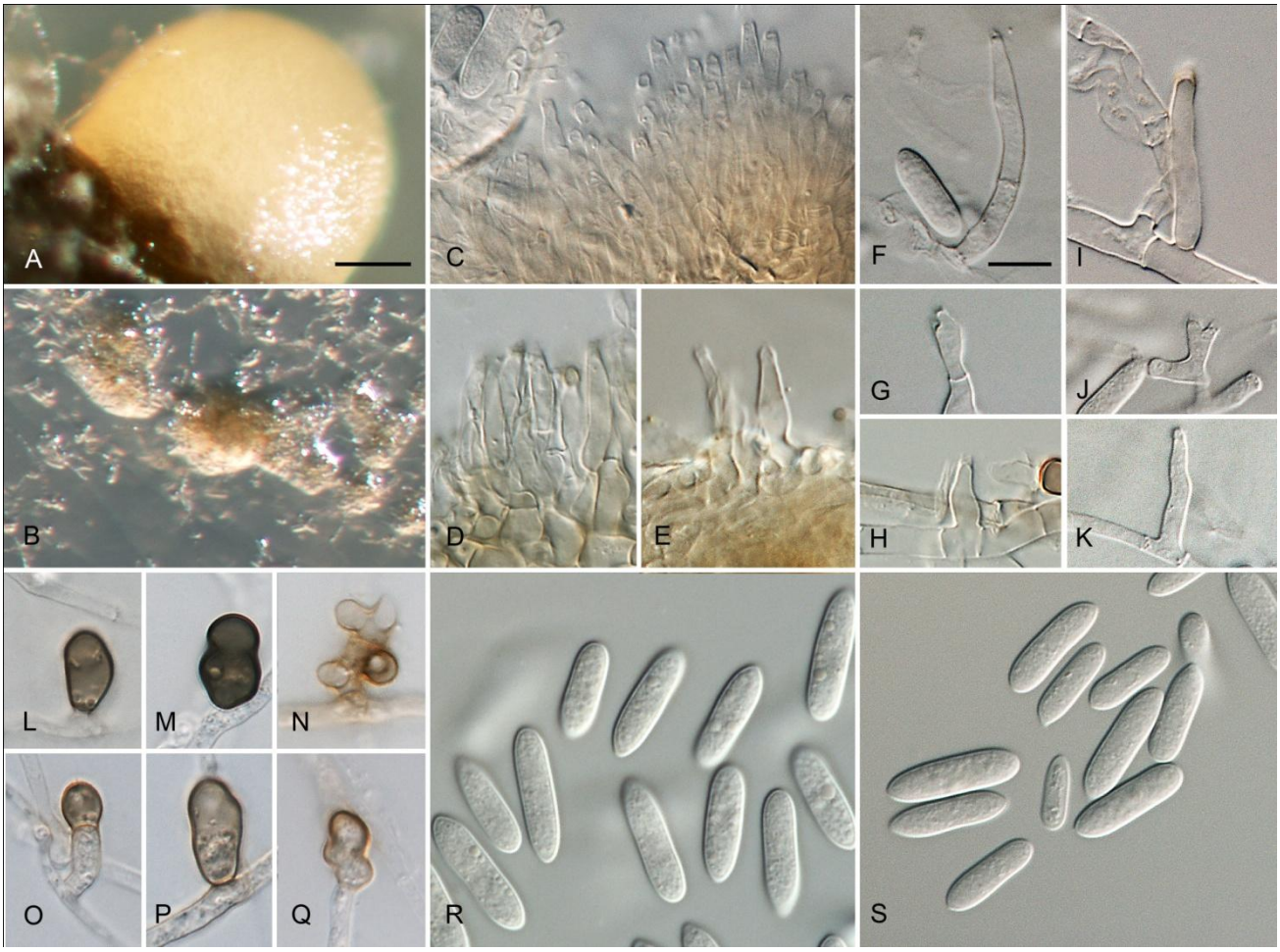

Figure 5 - *Colletotrichum* sp. Isolate CPC 20894. A-B. conidiomata. C-K. conidiophores. L-Q. Apressoria. R-S. conidia. A, C-E. from *Anthriscus* stem. B, F-S. from SNA. A-B. Dissecting microscope. C-S. Differential interference contrast. Scale bars: A = 100 µm, C = 10 µm

2.3.3.3 *C. paranaense* (strain CPC 20901)

Sexual state not observed. Asexual state on SNA. *Vegetative hyphae* 1–2.5 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* not developed, conidiophores formed directly on hyphae. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate, unbranched, 5–21 µm long. *Conidiogenous cells* hyaline, smooth–walled, elongate-ampulliform to subcylindrical, 4.5–21.5 × 1.5–2 µm, opening 1 µm diam, collarette sometimes not visible, 1–1.5 µm long, periclinal thickening sometimes visible. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, sometimes slightly constricted in the middle, both ends slightly acute or one end round, (4–)8–15(–22.5) × (2–)3–4(–5) µm, mean ± SD = 11.4 ± 3.6 x 3.4 ± 0.6 µm, L/W ratio = 3.4. *Appressoria* single, medium to pale

brown, ellipsoidal to obovoidal, the edge entire or sometimes lobate, (4.5–)5.5–10.5(–15.5) × (3.5–)4.5–7(–10.5) µm, mean ± SD = 7.9 ± 2.6 × 5.8 ± 1.4 µm, L/W ratio = 1.4.

Asexual state on *Anthriscus* stem. *Conidiomata*, acervular, conidiophores formed on pale brown, angular basal cells, 3–5.5 µm. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate, branched, to 36 µm long. *Conidiogenous cell*s hyaline to pale brown, smooth-walled, elongate-ampulliform to cylindrical, 13.5–20 × 3–3.5 µm, opening 1.5–2 µm diam, collarette 1–1.5 µm long, periclinal thickening visible, sometimes distinct. *Conidia* hyaline, smooth-walled, aseptate, straight, cylindrical, both ends slightly acute, sometimes one end round, sometimes slightly constricted in the middle, (8.5–)11–17.1(–19.5) × (3–)3.5–4.5(–4.5) µm, mean ± SD = 14.1 ± 3 × 4.1 ± 0.4 µm, L/W ratio = 3.5.

Culture characteristics: Colonies on SNA flat with entire edge, pale honey, filter paper partly covered by pale olivaceous grey, floccose felty aerial mycelium, *Anthriscus* stem partly covered by white to smoke grey aerial mycelium, reverse part pale isabelline to hazel, growth rate 22.8–23 mm in 7 d and 32.5–33 mm in 10 d. Colonies on OA flat with entire edge, covered by pale olivaceous grey to white floccose-felty aerial mycelium and few orange acervuli along the edge, reverse buff to olivaceous grey, honey in the center, growth rate 21.5–21.8 mm in 7 d and 29.8–32 mm in 10 d. Conidia in mass saffron.

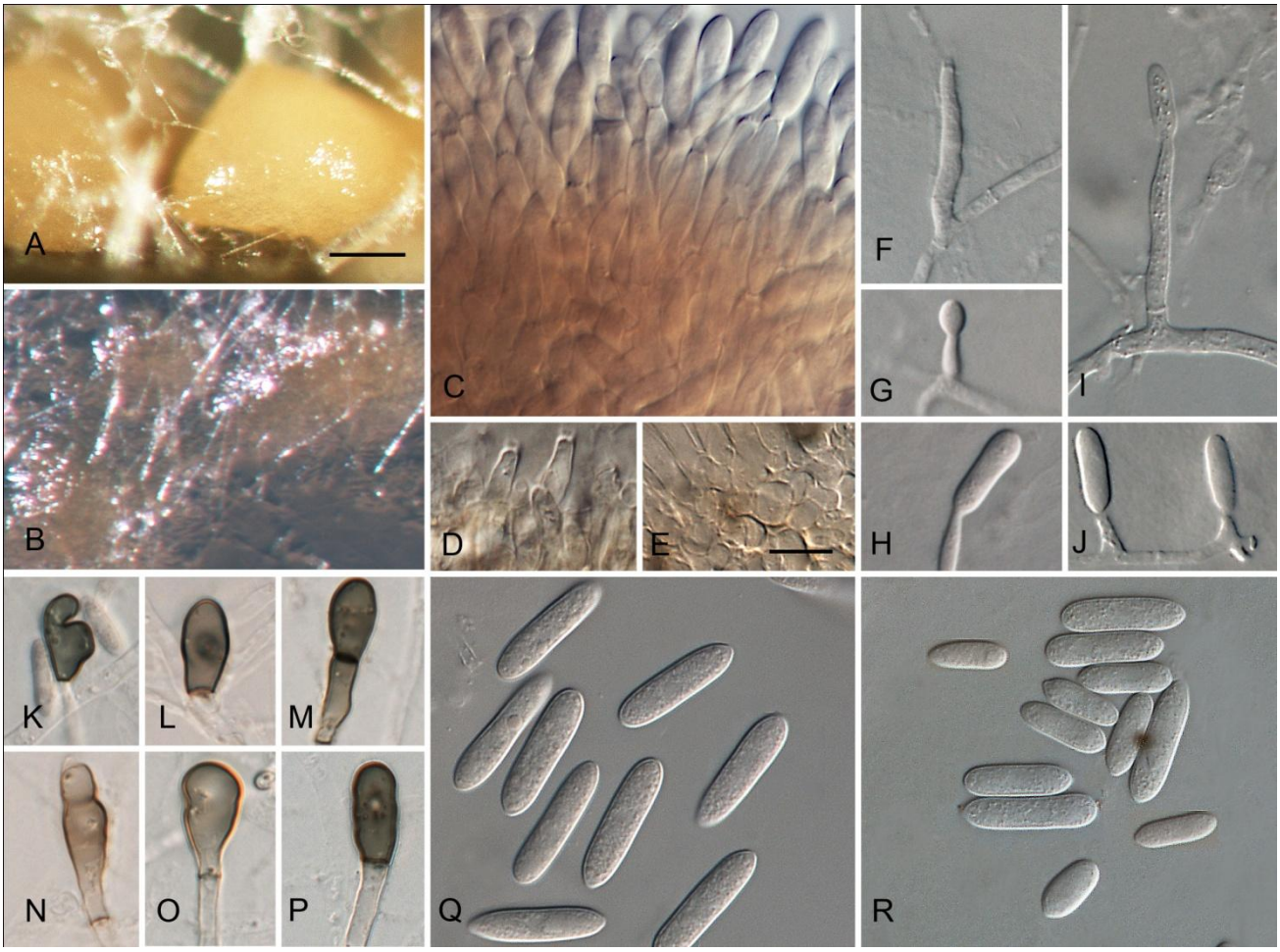

Figure 6 - *Colletotrichum* sp. Isolate CPC 20901. A-B. conidiomata. C-J. conidiophores. K-P. Apressoria. Q-R. conidia. A, C-E. from *Anthriscus* stem. B, F-R. from SNA. A-B. Dissecting microscope. C-S. Differential interference contrast. Scale bars: A = 100 µm, C = 10 µm

2.3.3.4 *C. melonis* (strain CPC 20912)

Sexual state not observed. Asexual state on SNA, *vegetative hyphae* 1.5–4 µm diam, pale brown, sometimes hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* not developed, conidiophores formed directly on hyphae. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate, unbranched, to 9 µm long. *Conidiogenous cells* hyaline, smooth-walled, ampulliform and constricted at the base, 3.5–4 × 5.5–8 µm, opening 1–1.5 µm diam, collarette 1–1.5 µm long, periclinal thickening visible. *Conidia* hyaline, smooth-walled, aseptate, cylindrical to clavate, both ends acute, sometimes one end round, (7–)9–13(–16) × (3–)3.5–4.5(–5.5) µm, mean ± SD = 11.1 ± 2.2 × 3.9 ± 0.5 µm, L/W ratio = 2.8. *Appressoria* single, medium to pale brown, bulled-shaped to clavate and

sometimes globose to obovoidal, the edge entire or sometimes lobate, (4.5–)6–14.5(–20.5) × (4–)4.5–6(–7) µm, mean ± SD = 10.4 ± 4.1 × 5.4 ± 0.7 µm, L/W ratio = 1.9.

Asexual state on *Anthriscus* stem. *Conidiomata*, acervular, not developed, conidiophores formed directly on hyphae. *Setae* not observed. *Conidiophores* hyaline to pale brown, septate, branched, to 35 µm long, *Conidiogenous cell*s hyaline to pale brown, smooth–walled, cylindrical to obclavate, 8.5–15.5 × 3–3.5 µm, opening 1–1.5 µm diam, collarette pale brown, 1–1.5 µm long, periclinal thickening visible. *Conidia* hyaline, smooth–walled, aseptate, cylindrical, both ends acute, sometimes one end round, (8–)11.5–15(–17.5) × (2.5–)4–5(–5) µm, mean ± SD = 13.3 ± 1.6 × 4.4 ± 0.4 µm, L/W ratio = 3.0.

Culture characteristics: Colonies on SNA flat with entire edge, hyaline to buff, *Anthriscus* stem partly covered by white floccose aerial mycelium, reverse same color. *Anthriscus* stem partly covered by orange conidia mass, growth rate 22.5–23 mm in 7 d and 343–34.5 mm in 10 d. Colonies on OA slightly umbonate with entire edge, saffron to olivaceous grey, almost entirely covered by orange conidia mass, partly covered by floccose pale olivaceous grey aerial mycelium, reverse salmon, growth rate 20.8 mm in 7 d and 32–32.3 mm in 10 d. Conidia in mass orange.

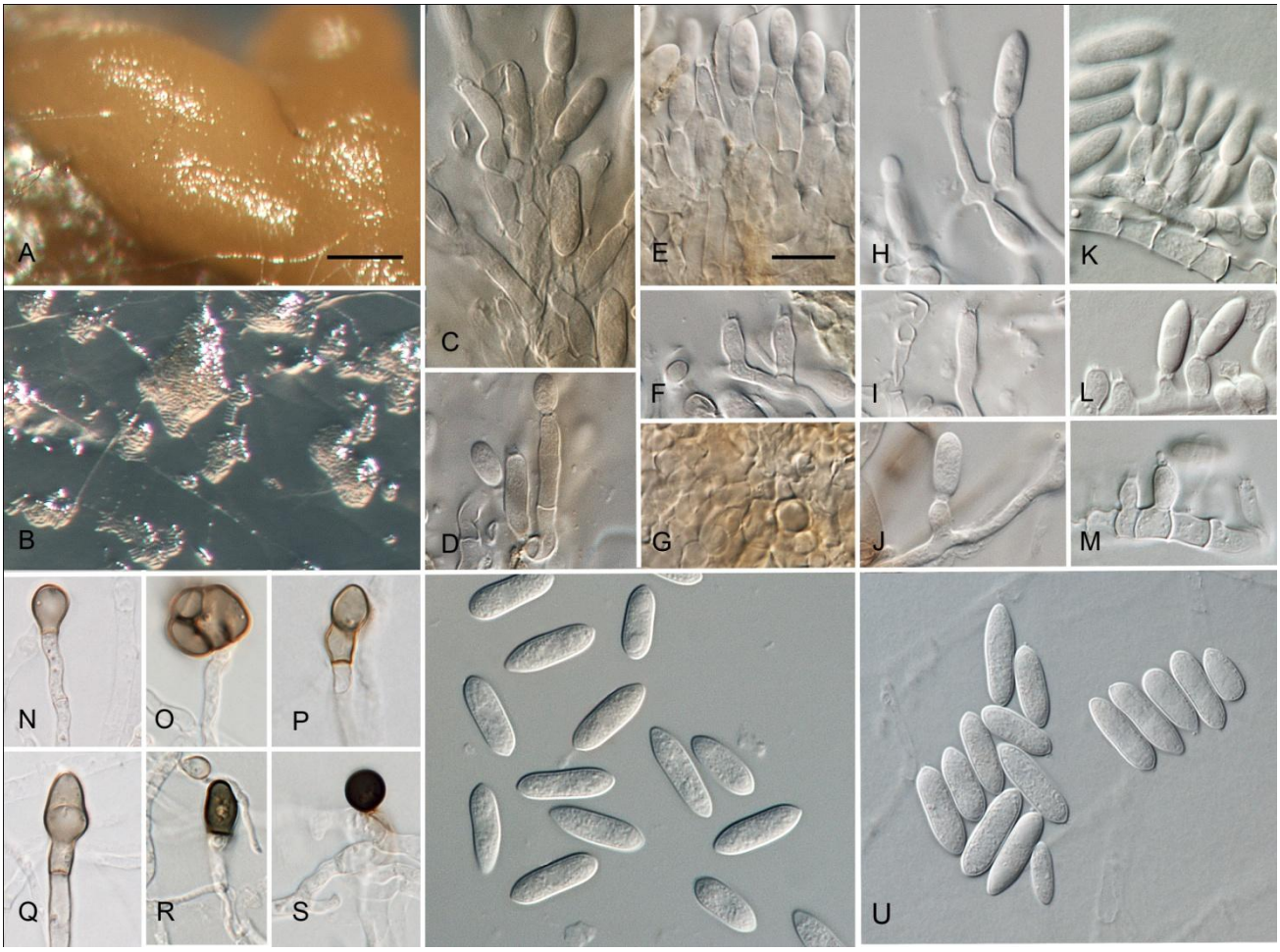

Figure 7 - *Colletotrichum* sp. Isolate CPC 20912. A-B. conidiomata. C-M. conidiophores. N-S. Apressoria. T-U. conidia. A, C-G. from *Anthriscus* stem. B, H-U. from SNA. A-B. Dissecting microscope. C-U. Differential interference contrast. Scale bars: A = 100 µm, C = 10 µm

The acute ends of the conidia were one of the most important morphological features to distinguish *C. acutatum* (SIMMONDS, 1965). However, the shape of the conidia can show significant variation within the species and even among the strains (DAMM et al., 2012). Several papers have demonstrated that v-shape is not a rule in *C. acutatum* species. For example, Talhinhas et al (2002), studying *Colletotrichum* isolates from *Lupinus* spp., found different proportions of conidia with round ends or one round and one acute end. In a recent study, many species that were identified as *C. gloeosporioides,* based on morphology, were included in *C. acutatum* complex (DAMM et al., 2012). Additionally, species with acute-ended conidia might not belong to the *C. acutatum* complex. For example, *C. pseudoacutatum* forms conidia with acute ends but it is phylogenetically distinct from *C. acutatum* complex (CANNON et al., 2012).

2.3.4 Cross infection tests

2.3.4.1 *C. gloeosporioides* complex species

The strains CPC 20938 and CPC 20940 from avocado and CPC 20954 from guava were able to infect all host tested. However, the strains CPC 20923 from mango caused lesion in mango and guava and the strain CPC 20969, also from mango, caused lesion only in mango. All The tested stains caused larger lesions in guava, except the strain CPC 20969 (Table 3 and Fig. 7). Only mango was infected by all tested strains,however only *C. siamense* was able to infect avocado.

The tested strains showed higher growth rates in guava, exept to the CPC 20969. The strains CPC 20938 and CPC 20940 showed higher growth rates in avocado. Sporulation was more frequent on guava (Table 3).

Table 3 - Pathogenicity tests and cross infection of *Colletotrichum* species on avocado, guava and mango

Species	Strain	Original host	Lesion size (mm^2)[a]			Growth rate ($mm.dia^{-1}$)			Frequence of infection and sporulation		
			Avocado	Guava	Mango	Avocado	Guava	Mango	Avocado	Guava	Mango
C. siamense	CPC 20938	avocado	62.2[b]	757.5	177.5	2.3	3.3	1.4	2/1/5[c]	5/5/5	5/0/5
C. siamense	CPC 20940	avocado	165.6	434.5	99.9	2.1	4.4	2	5/4/5	5/5/5	5/2/5
C. asianum	CPC 20923	mango	0	101.6	76.3	0	2	1.4	0/0/5	2/1/5	5/0/5
C. asianum	CPC 20969	mango	0	0	230.5	0	0	2.2	0/0/5	0/0/5	5/0/5
C. gloeosporioides	CPC 20904	guava	0	472.3	32.9	0	3.5	0.7	0/0/5	4/2/5	5/0/5
C. siamense	CPC 20954	guava	21.9	436.2	192.7	1	3.3	2.1	4/0/5	5/5/5	5/1/5

[a]Incubation periods were 4 days, exept for CPC 20938 in avocado and CPC 20923 in guava which were 5 days; latency periods were 7 days, exept for CPC 20904, CPC 20954, CPC 20938 and CPC 20940 in guava which were 6 days;
bMean of infected fruits;
[c]number of infected fruits/number of fruits with sporulation/number of fruits inoculated.

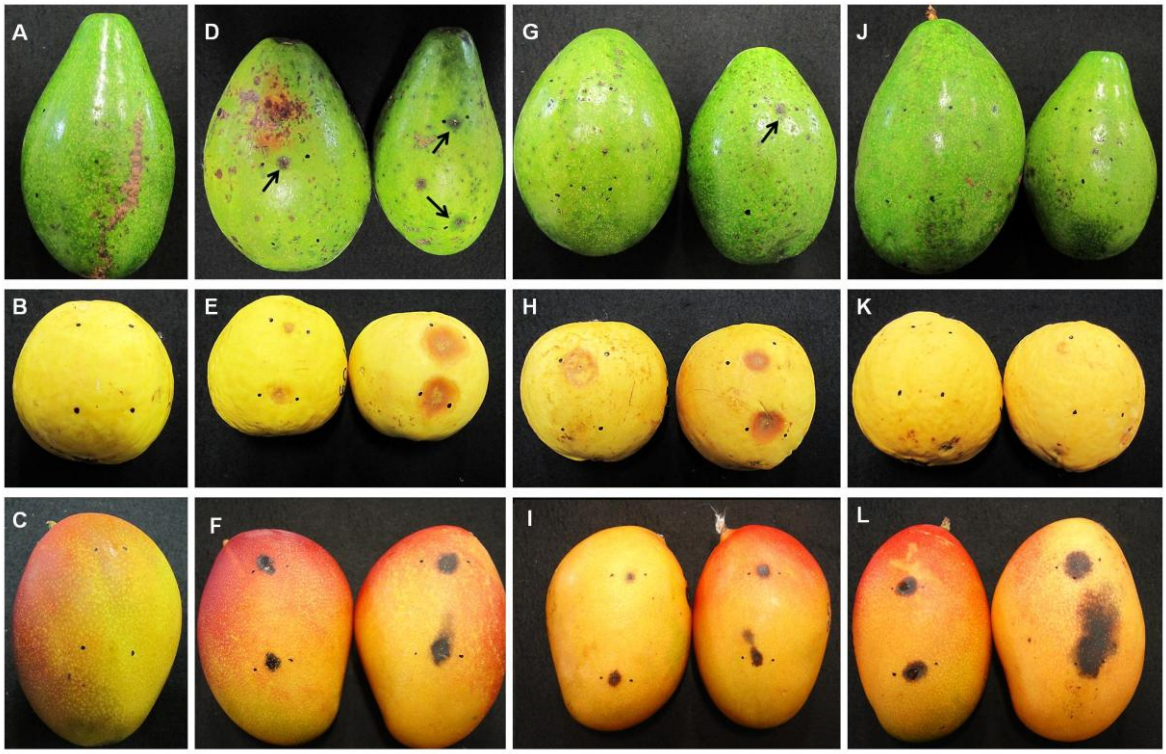

Figure 8 - *Colletotrichum* symptons on avocado, guava and mango 7 days after inoculation. A-C Control fruits. D-F *C. siamense* (CPC 20938 on the left and CPC 20940 on the right) from avocado; G-I on the left side – *C. gloeosporioides*, on the right side - *C. siamense* from guava; J-L *C. asianum* (CPC 20923 on the left and CPC 20969 on the right) from mango

2.3.4.2 *C. acutatum* complex species

All the tested strains were able to infect the original and different hosts, but in some cases, there were differences among them (Table 4 and Fig. 8). The strains CPC 20894 and CPC 20928 from guava and peach, for example, caused larger lesions and higher growth rate in the original host than in the others. The strains CPC 20897 and Col 20 from apple caused larger lesions in peach than in apple. However, the strain CPC 20916, also from apple, caused the opposite. The strain CPC 20916 from apple showed higher growth rate in the tested fruits. All inoculated fruits were infected by the strains. Only one apple fruit inoculated with the strain CPC 20897 showed sporulation on the lesion (Table 4). All the strains inoculated in melon caused simptoms (Table 5 and Fig. 9). The strain CPC 20912 caused higher lesion , but only one fruit was infected. Only one fruit showed sporulation.

Table 4 - Pathogenicity testing and cross infection of *Colletotrichum* species on peach, guava and apple

Species	Strain	Original host	Lesion size (mm^2)[a]			Growth rate ($mm.dia^{-1}$)			Frequence of infection and sporulation		
			Peach	Guava	Apple	Peach	Guava	Apple	Peach	Guava	Apple
C. nymphaeae	CPC 20897	apple	237.9[b]	32.4	76.3	2.5	0.9	1.3	5/3/5[c]	2/2/5	5/1/5
C. nymphaeae	CPC 20916	apple	294.2	436.2	360.3	2.8	3.3	3.3	5/2/5	5/5/5	5/5/5
C. melonis	Col 20	apple	316.2	174.9	234.4	2.8	2.1	2.4	5/5/5	3/3/5	5/5/5
C. polyphialidicum	CPC 20894	guava	174.1	271.2	103.3	2.1	2.5	1.6	5/5/5	4/4/5	5/5/5
C. pruni	CPC 20928	peach	363.1	329.5	222.5	3.1	2.7	2.2	5/5/5	5/5/5	4/4/5

[a]Incubation periods were 4 days, except for CPC 20938 in avocado and CPC 20923 in guava which were 5 days; latency period in peach, guava and apple were 6,7,8 for CPC 20894; 7,8,7 for Col 20; 6,7,7 for CPC 20916; 5,7,8 for CPC 20928 and 6,8 for CPC 20897 in peach and apple.
[b]Mean of infected fruits
[c]number of infected fruits/number of fruits with sporulation /number of fruits inoculated

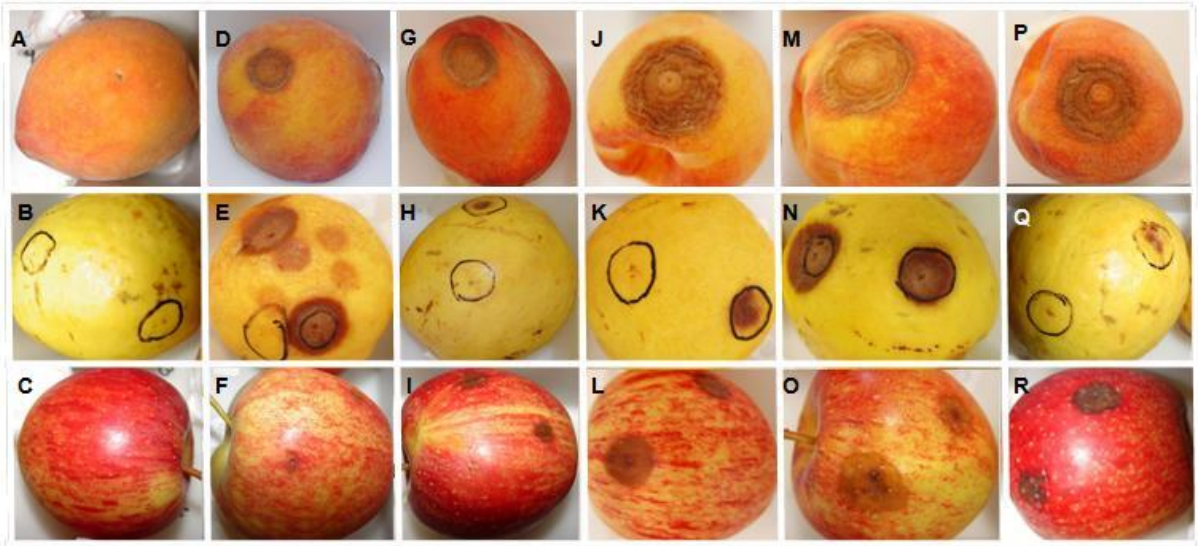

Figure 9 - *Colletotrichum* symptons on peach, guava and apple 7 days after inoculation. A-C control fruits D-F *C. polyphialidicum* (CPC 20894) from guava; G-I *C. nymphaeae* (CPC 20897) from apple; J-L *C. melonis* (Col 20) from apple; M-O *C. nymphaeae* (CPC 20916) from apple; P-R (*C. pruni*) CPC 20928 from peach

Table 5 - Pathogenicity testing of *Colletotrichum* species on melon

Species	Strain	Lesion size (mm^2)[a]	Growth rate ($mm.dia^{-1}$)	Frequence of infection and sporulation
C. melonis	Col 20	251[b]	0.4	4/1/4[c]
C. melonis	CPC 20912	986.4	5.3	1/1/4
C. nymphaeae	CPC 20910	448.2	4.2	4/1/4

[a]Incubation periods were 7 days for all strains; latency period were 14 days for all strais.
[b]Mean of infected fruits
[c]number of infected fruits/number of fruits with sporulation /number of fruits inoculated

Figure 10 - *Colletotrichum* symptoms on melon 7 days after inoculation. 31 = CPC 20912 (*C. melonis*); 20 = Col 20 (*C. melonis*); 29 = CPC 20902 (*C. nymphaeae*) from apple; C = control

The strains tested were able to infect different hosts, however they produced different reactions. Several studies have demonstrated the lack of host specificity of *Colletotrichum* species (LAKSHMI et al.,2011; PHOULIVONG et al., 2012; de SOUZA et al., 2012; PENG et al., 2013; MACKENZIE et al., 2009, PERES et al., 2002), in which the same species can be associated with different hosts or one host can be affected by different species (FREEMAN, 1998). However, the same species isolated from different hosts might show different cross infection ability and it should be considered when studying new species (PHOULIVONG et al., 2012). For example, Peng et al. (2013) reported a new species on grape, *C. viniferum*, and

based on pathogenicity test this species behaved differently on the tested hosts. In another study, *C. acutatum* strains were able to infect different host, but the highest incidence and the biggest lesions were observed on original the host. Additionally, the strains were genetically distinct based on Maximum parsimony analysis of three different genes fragments (MACKENZIE et al., 2009). Another study reported the ability of *C. asianum*, *C. fructicola*, *C. siamense* and *C. simmondsii* to infect chili, guava, mango, papaya and rose apple (PHOULIVONG et al., 2010). The knowledge of cross infection ability of the species is important to establish the host range and, consequently, to support quarantine control (PHOULIVONG et al., 2012).

The Bayesian trees obtained in this study showed high genetic variability among the species in Brazil and which ones are more frequently associated with a specific host. This knowledge may be used to create control strategies. For example, a population of a plant pathogen with high genetic variation can evolve rapidly, and this information can contribute to predict how long a control measure is likely to be effective (MCDERMOTT and MCDONALD, 1993). Furthermore, the accurate identification of the species can lead to better understanding the epidemiology. For effective control, it allows knowing the occurrence and distribution of a species in a specific host, supporting the breeding for resistance (FREEMAN et al., 1998). Additionally, the identification of species may guarantee the control based on fungicides. Some species may be more sensitive to specific groups of chemical compounds than others species (FREEMAN et al., 1998; WONG and MIDLAND, 2007; SANDERS et al., 2000). For example, *C. gloeosporioides*, in general, is considered highly sensitive to benomyl, whereas *C. acutatum* is relatively insensitive (FREEMAN et al., 1998).

The results of this work clarified the species and its occurrence on the studied hosts in Brazil as well as their genetic variability. However, some species, such *C theobromicola* and *C. tropicale*, need to be further investigated. The number of strains of these species used in this work was probably not enough to represent the hosts affected by them. Due to high genetic variation, studies on population genetics should be the next step to determine the genetic structure of these populations.

3 CONCLUSIONS

- There are different species of *Colletotrichum* associated with fruits in Brazil
- The population studied is highly variable from the genetic point of view.
- *C. siamense* is more frequently associated with avocado in Brazil and represents the most genetically variable and less host-specific species in the population studied.
- *C. asianum* is associated with mango in natural infections.
- *C. asianum* is not able to infect avocado and has low ability to infect guava.
- Three new, *C. polyphialidicum*, *C. paranaense* and *C. pruni*, species were recognized in the *C. acutatum* complex in Brazil.

REFERENCES

ARX, J.A.V. **Revision der zu Gloeosporium gestellten pilze**. Verhandligen des koninklijke Nederlandse Akadademie van Wetenschappen, Afd. Natuurkunde, 2, v. 51. 153p.

ALMEIDA, F.A.; ARAÚJO, E.; GONÇALVES JUNIOR, H.; BARRETO, A.F.; CARVALHO, R.A.G. Diagnóstico e quantificação de doenças da acerola no Estado da Paraíba. **Fitopatologia Brasileira, Brasília**, v. 28, p. 176-179, Mar./Apr. 2003.

CAI, L.; HYDE, K.D.; TAYLOR, P.W.J.; WEIR, B.S.; 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. A polyphasic approach for studying *Colletotrichum*. **Fungal Diversity**, Yunnan, v. 39, p. 183-204, Dec. 2009.

BAILEY, J.A.; O'CONNELL, R.J.; PRING, R.J.; NASH, C. Infection strategies of *Colletotrichum* species. In: BAILEY, J.A.; JEGER, M.J. (Ed.).***Colletotrichum***: biology, pathology and control. Oxon: CAB International, 1992, p. 88–120.

CANNON, P.F.; BRIDGE, P.D.; MONTE, E. Linking the past, present and future of *Colletotrichum* systematics. In: PRUSKY, D.; FREEMAN, S.; DICKMAN, M.B. (Ed.).***Colletotrichum***: host specificity, pathology, and host-pathogen interaction. St. Paul: APS Press, 2000 chap. 1, p. 10-13.

CANNON, P.F.; DAMM, U.; JOHNSTON, P.R.; WEIR, B.S. *Colletotrichum* status and future directions. **Studies in Mycology**, Utrecht, v. 73, p.181-213, Sept. 2012.

CARBONE, I.; KOHN, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. **Mycologia**, New York, v. 91, p. 553–556, May/Jun. 1999.

CROUCH, J.A.; CLARKE, B.B.; HILLMAN, B.I. What is the value of ITS sequence data in *Colletotrichum* systematics and species diagnosis? A case study using the falcate-spored graminicolous *Colletotrichum* group. **Mycologia**, New York, v. 101, p. 648–656, Sept./Oct. 2009.

CROUS, P.W.; GROENEWALD, J.Z.; RISEDE, J.M.; HYWEL-JONES, N.L. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. **Studies in Mycology**, Utrecht, v. 50, p. 415–430, Sept. 2004.

CROUS, P.W.; VERKLEIJ, G.J.M.; GROENEWALD JZ, SAMSON RA (Ed.). **Fungal Biodiversity***.* Centraalbureau voor Schimmelcultures, Utrecht, 2009.(CBS Laboratory Manual Series, 1.)

DAMM, U.; CROUS, P.W.; FOURIE, P.H. *Botryosphaeriaceae* as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* spp. nov. **Mycologia**, New York, v. 99, p. 664–680, Sep./Oct. 2007.

DAMM, U.; MOSTERT, L.; CROUS, P.W.; FOURIE, P.H. Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. **Persoonia**, Utrecht, v. 20, p. 87–102, Jun. 2008.

DAMM, U.; WOUDENBERG, J.H.C.; CANNON, P.F.; CROUS, P.W. *Colletotrichum* species with curved conidia from herbaceous hosts. **Fungal Diversity**, Yunnan, v. 39, p. 45–87, Dec. 2009.

DAMM, U.; CANNON, P.F.; WOUDENBERG, J.H.C.; CROUS, P.W. The *Colletotrichum acutatum* species complex. **Studies in Mycology**, Utrecht, v. 73, p. 37-113, Set. 2012.

de SOUZA, A., DELPHINO CARBONI, R. C., WICKERT, E., DE MACEDO LEMOS, E. G. and de Goes, A. Lack of host specificity of *Colletotrichum* spp. isolates associated with anthracnose symptoms on mango in Brazil. **Plant Pathology**, Oxford. < http://onlinelibrary.wiley.com/doi/10.1111/ppa.12021/full>. 2012.

DEAN, R.; VAN KAN, J.A.L.; PRETORIUS, Z.A.; HAMMOND-KOSACK, K.E.; DI PIETRO, A.; SPANU, P.D.; RUDD, J.J.; DICKMAN, M.; KAHMANN, R.; ELLIS, J.; FOSTER, G.D. The Top 10 fungal pathogens in molecular plant pathology. **Molecular Plant Pathology**, Malden, v.13, p. 414-430, May. 2012.

DUKE, M.M. The genera *Vermicularia* Fr. and *Colletotrichum* Cda. **Transsactions of the British Mycological**, Ardwick, v. 97, p. 641-658, 1928.

FARR, D.F.; ROSSMAN, A.Y. **Fungal Databases, Systematic mycology and Microbiology** Laboratory, ARS, USDA. Retrieved 14 Fev. 2013, from http://nt.ars-grin.gov/fungaldatabases/

FÖRSTER, H.; ADASKAVEG, J.E. Identification of subpopulations of *Colletotrichum acutatum* and epidemiology of almond anthracnose in California. **Phytopathology**, St. Paul, v. 89, p. 1056-65, Nov. 1999.

FREEMAN, S. Management, survival strategies, and host range of *Colletotrichum acutatum* on strawberry. **Hortscience**, Alexandria, v. 43, p. 66-68, Feb. 2008.

FREEMAN, S.; KATAN, T.; SHABI, E. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. **Plant Disease**, St. Paul, v. 82, p. 596-605, Jun. 1998.

GARDES, M.; BRUNS, T.D. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. **Molecular Ecology**, Malden, v. 2, 113–118, Apr. 1993.

GLASS, N.L.; DONALDSON, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Applied and **Environmental Microbiology**, Washington v. 61, p. 1323-1330, Apr. 1995.

GONZÁLEZ, E.; SUTTON, T.B.; CORRELL, J.C. Clarification of the etiology of Glomerella leaf spot and bitter rot of apple caused by *Colletotrichum* spp. based on morphology and genetic, molecular, and pathogenicity tests. **Phytopathology**, St. Paul, v. 96, p. 982-992, Sept. 2006.

GUERBER, J.C.; LIU, B.; CORRELL, J.C. Characterization of diversity in Colletotrichum acutatum sensu lato by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. **Mycologia**, New York, v. 95, p. 872-895, Sept./Oct. 2003.

HEILMANN, L.J.; NITZAN, N.; JOHNSON, D.A.; PASCHE, J.S.; DOETKOTT, C.; GUDMESTAD, N.C. Genetic variability in the potato pathogen *Colletotrichum coccodes* as determined by amplified fragment length polymorphism and vegetative compatibility group analyses. **Phytopathology**, St. Paul, v. 10, p. 1097-107, Oct. 2006.

HUELSENBECK, J.P.; RONQUIST, F. MRBAYES: Bayesian inference of phylogeny. **Bioinformatics**, Oxford, v. 17, p. 754-755. Aug. 2001.

HYDE, K.D.; CAI, L.; CANNON, P.F.; CROUCH, J.A.; CROUS, P.W.; DAMM, U.; GOODWIN, P.H.; CHEN, H.; JOHNSTON, P.R.; JONES, E.B.G.; LIU, Z.Y.; MCKENZIE, E.H.C.; MORIWAKI, J.; NOIREUNG, P.; PENNYCOOK, S.R.; PFENNING, L.H.; PRIHASTUTI,H.; SATO, T.; SHIVAS, R.G.; TAN, Y.P.; TAYLOR, P.W.J.; WEIR, B.S.; YANG, Y.L.; ZHANG, J.Z. *Colletotrichum* – names in current use. **Fungal Diversity**, Yunnan, v. 39, p. 147-182, Dec. 2009.

KATOH, K.; TOH, H. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. **BMC Bioinformatics**, London, v. 9, p. 212, Apr. 2008.

KUMAR, D.S.S.; HYDE, K.D. Biodiversity and tissue-recurrence of endophytic fungi from *Tripterygium wilfordii*. **Fungal Diversity**, Yunnan, v. 17, p. 69-90, Jun. 2004.

LAKSHMI, B.K.M.; REDDY, P.N.; PRASAD, R.D. Cross-infection potential of *Colletotrichum gloeosporioides* Penz. isolates causing anthracnose in subtropical fruit crops. **Tropical Agricultural Research**, Peradeniya, v. 22, p.183–193, 2011.

LATUNDE-DADA, A.O. *Colletotrichum*: tales of forcible entry, stealth, transient confinement and breakout. **Molecular Plant Pathology**, Malden, v. 2, p. 187-198, Jun. 2001.

LIU, C.; LIU, T.; YUAN, F.; GU, Y. Isolating endophytic fungi from evergreen plants and determining their antifungal activities. **African Journal of Microbiology Research**, Lagos, v. 4, p. 2243-2248, Nov. 2010.

MACKENZIE, S.J.; PERES, N.A.; BARQUERO, M.P.; ARAUZ, L.F.; TIMMER, L.W. Host range and genetic relatedness of *Colletotrichum acutatum* isolates from fruit crops and leatherleaf fern in Florida. **Phytopathology**, St. Paul, v. 99, p. 620-631, May. 2009.

MCDERMOTT, J.M.; MCDONALD, B.A. Gene flow in plant pathosystems. **Annual Reviews of Phytopathology**, Palo Alto, v. 31, p. 353-373, 1993.

MILLS, P.R.; HODSON, A.; BROWN, A.E. Molecular differentiation of *Colletotrichum gloeosporioides* isolates infecting tropical fruits. In: BAILEY, J.A.; JEGER, M.J. (Ed.). ***Colletotrichum***: biology, pathology and control. Oxon: CAB International, p. 269–288. 1992.

MURRAY, M.G.; THOMPSON, W.S. Rapid isolation of high molecular weight DNA. **Nucleic Acids Research**, Oxford, v. 8, p. 4321-4325, Oct. 1980.

Nirenberg, H.I. Untersuchungen über die morphologische und biologische differenzierung in der Fusarium-Sektion Liseola. **Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft**, Dahlem, v. 169, p. 1–117, 1976.

O'CONNELL, R.; HERBERT, C.; SREENIVASAPRASAD, S.; KHATIB, M.; ESQUERRÉ-TUGAYÉ, M.T.; DUMAS, B. A novel *Arabidopsis–Colletotrichum* pathosystem for the molecular dissection of plant–fungal interactions. **Molecular Plant-Microbe Interactions**, St. Paul, v. 3, p. 272-282, Mar. 2004.

PENG, L.J.; SUN, T.; YANG, Y.L.; CAI, L.; HYDE, K.D.; BAHKALI, A.H.; LIU, Z.Y. *Colletotrichum* species on grape in Guizhou and Yunnan provinces, China. **Mycoscience**, Tokyo, v. 54, p. 29–41, Jan. 2013.

PERES, N.A.R.; KURAMAE, E.E.; DIAS; M.S.C.; DE SOUZA, N.L. Identification and characterization of *Colletotrichum* spp. affecting fruit after harvest in Brazil. **Journal of Phytopathology**, Malden, v. 150, p. 128–134. Mar. 2002.

PHOTITA, W.; TAYLOR, P.W.J.; FORD, R.; HYDE, K.D.; LUMYONG, S. Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. **Fungal Diversity**, Yunnan, v. 18, p. 117–133, Feb. 2005.

PHOULIVONG, S.; CAI, L.; CHEN, H.; MCKENZIE, E.H. C.; ABDELSALAM, K.; CHUKEATIROTE, E.; HYDE, K.D. *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. **Fungal Diversity**, Yunnan, v. 44, p. 33-43, Oct. 2010.

PHOULIVONG, S.; MCKENZIE, E.H.C.; HYDE, K.D. Cross infection of *Colletotrichum* species; a case study with tropical fruits. **Current Research in Environmental & Applied Mycology**, Chiang Rai, v. 2, p. 99–111, 2012. Doi 10.5943/cream/2/2/2.

POSADA, D.; CRANDALL, K.A. Selecting the best-fit model of nucleotide substitution. **Systematics Biology**, Oxford, v. 50, p. 580-601, Aug. 2001.

PRIHASTUTI, H.; CAI, L.; CHEN, H.; MCKENZIE, E.H.C.; HYDE, K.D. Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. **Fungal Diversity**, Yunnan, v. 39, p. 89-109, Dec. 2009.

PRUSKY, D.; PLUMBLEY, R.A. Quiescent infections of *Colletotrichum* in tropical and subtropical fruit. In: BAILEY, J.A.; JEGER, M.J. (Ed.). ***Colletotrichum***: biology, pathology and control. Oxon: CAB International, 1992. p. 337-357.

PRUSKY, D.; FREEMAN, S.; DICKMAN, M. *Colletotrichum*: Host specificity, pathology, and host-pathogen interaction. St. Paul, USA. :APS Press, 2000. 393p.

RAYNER, R.W. **A mycological colour chart***.* Common wealth Mycological Institute, Kew, UK. 1970. p. 34.

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. *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panama´: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. **Mycologia**, New York, v. 102, p. 1318–1338, Nov./Dec. 2010.

RONQUIST, F.; HUELSENBECK, J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. **Bioinformatics**, Oxford, v. 19, p. 1572-1574, Aug. 2003.

SANDERS, G.M.; KORSTEN, L.; WEHNER, F.C. Survey of fungicide sensitivity in *Colletotrichum gloeosporioides* from different avocado and mango production areas in South Africa. **European Journal of Plant Pathology**, Dordrecht, v. 106, p. 745-752, Oct. 2000.

SERRA, I.M.R.S.; COELHO, R.S.B.; FERRAZ, G.M.G.; MONTARROYOS, A.V.V.; SILVA, D.S. Diversidade fenotípica e patogênica de *Colletotrichum*, agente causal da antracnose em mangueira, e identificação de espécie. **Summa Phytopathologica**, Botucatu, v. 37, p. 42-51, Jan./Mar. 2011.

SERRA, I.M.R.S.; Menezes, M.; COELHO, R.S.B.; FERRAZ, G.M.G.; MONTARROYOS, MARTINS, L.S.M. Molecular analysis in the differentiation of *Colletotrichum gloeosporioides* isolates from the cashew and mango trees. **Brazilian Archives of Biology and Technology**, Curitiba, v. 54, p. 1099-1108, Nov./Dec. 2011.

SHIVAS, R.G.; TAN, Y.P. A taxonomic re-assessment of *Colletotrichum acutatum*, introducing *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov. **Fungal Diversity**, Yunnan, v. 39, p. 111-122, Dec. 2009.

SILVA-MANN, R.; VIEIRA, M.G.G.C; MACHADO, J.C.; BERNANDINO FILHO J.R.; SALGADO, K.C.C.; STEVENS, M.R. AFLP markers differentiate isolates of *Colletotrichum gossypii* from *C. gossypii* var. *cephalosporioides*. **Fitopatologia Brasileira**, Brasília, v. 30, p.169–172, Mar./Apr. 2005.

SIMMONDS, J.H. A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. **Queensland Journal of Agricultural and Animal Science**, Brisbane, v. 22, p. 437-459, 1965.

SREENIVASAPRASAD, S.; BROWN, A.E.; MILLS, P.R. DNA sequence variation and interrelationships among *Colletotrichum* species causing strawberry anthracnose. **Physiological and Molecular Plant Pathology**, London, v. 41, p. 265-281, Oct. 1992.

SUTTON, B.C. (1992) The genus *Glomerella* and its anamorph *Colletotrichum*. In: BAILEY, J.A. and JEGER, M.J. (Ed.). ***Colletotrichum***: biology, pathology and control. Oxon: CAB International, 1992. p. 1-26.

SUTTON, T.B. Bitter rot. In: JONES, A.L.; ALDWINCKLE, H.S. (Ed.). **Compendium of Apple and Pear Diseases**. St. Paul: American Phytopathological Society, 1990. p.15-16.

TALHINHAS, P.; SREENIVASAPRASAD, S.; NEVES-MARTINS, J.; OLIVEIRA, H. Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of Lupins. **Phytopathology**, St. Paul, v. 92, p. 986-996, Sept. 2002.

TOZZE JUNIOR, H.J. **Antracnose do abacateiro**: danos pós-colheita, **caracterização do agente causal, quantificação de parâmetros da pré-penetração e monocíclicos e controle químico**. 2012. 124 p. Tese (Doutorado em Fitopatologia) – Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba, 2011.

THAUNG, M.M. Coelomycete systematic with special reference to *Colletotrichum*. **Mycoscience**, Tokyo, v. 49, p. 345-350, Dec. 2008.

WANG, Y.T.; LO, H.S.; WANG, P.H. Endophytic fungi from *Taxus mairei* in Taiwan: first report of *Colletotrichum gloeosporioides* as an endophyte of *Taxus mairei*. **Botanical Studies**, Taipei, v. 49, p. 39-43. Jan. 2008.

WHARTON, P.S.; DIÉGUEZ-URIBEONDO, J. The biology of *Colletotrichum acutatum*. **Anales del Jardín Botánico de Madrid**, Madrid, v. 61, p. 3-22, Jan. 2004.

WHITE, T.J.; BRUNS. T.; LEE, S.; TAYLOR, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS M.A.; GELFAND, D.H.; SNINSKY, J.J.; WHITE, T.J. (Ed.). **PCR Protocols**: a guide to methods and applications. San Diego: Academic Press, 1990. p. 315–322.

WHITELAW-WECKERT, M.A.; CURTIN, S.J.; HUANG, R.; STEEL, C.C.; BLANCHARD, C.L.; ROFFEY, P.E. Phylogenetic relationships and pathogenicity of *Colletotrichum acutatum* isolates from grape in subtropical Australia**. Plant Pathology**, London, v. 56, p. 448- 463, Jun. 2007.

WEEDS, P.L.; CHAKRABORTY, S.; FERNANDES, C.D.; D'A CHARCHAR, M.J.; RAMESH, C.R.; KEXIAN, Y.; KELEMU, S. Genetic diversity in *Colletotrichum gloeosporioides* from *Stylosanthes* spp. at centers of origin and utilization. **Phytopathology**, St. Paul, v. 93, p.176-185, Feb. 2003.

WEIR, B.S.; JOHNSTON, P.R.; DAMM, U. The *Colletotrichum gloeosporioides* species complex. **Studies in Mycology**, Utrecht, v. 73, p. 115–180, Sep. 2012.

WONG, F.P.; MIDLAND, S.L. Sensitivity distributions of California populations of *Colletotrichum cereale* to the DMI fungicides propiconazole, myclobutanil, tebuconazole, and triadimefon. **Plant Disease**, St. Paul, v. 91, p. 1547-1555. Dec. 2007.

WOUDENBERG, J.H.C.; AVESKAMP, M.M.; GRUYTER, J.; SPIERS, A.G.; CROUS, P.W. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype**. Persoonia**, Utrecht, v. 22, p. 56–62, Jun. 2009.

ZOU, W.X.; MENG, J.C.; LU, H.; CHEN, G.X.; SHI, G.X.; ZHANG, T.Y.; TAN, R.X. Metabolites of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*. **Journal of Natural Products**, Columbus, v. 63, p. 1529–1530, Nov. 2000.