International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2019): 7.583

Characterization of *Colletotrichum* Species Causing Mango Anthracnose in Senegal

Yaya Diallo*[1], P. M. Diedhiou[2], Elisabeth Bush[3], Mizuho Nita[4], Anton Baudoin[5]

[1, 2] UFR des Sciences Agronomiques d'Aquaculture et des Technologies Alimentaires (UFR S2ATA), Université Gaston Berger de Saint-Louis (UGB), Saint-Louis, Sénégal

[3, 4, 5]Virginia Polytechnic Institute and State University in Blacksburg, Virginia, USA (*Yaya Diallo)

Abstract: ***A number of species in the Colletotrichum gloeosporioides complex, as well as several additional Colletotrichum species, have been reported as causal agents of mango anthracnose. Although morphological characters are more commonly usedfor the identification of fungal species, these criteria are howeveroften overlapping and variable. In such cases, a more reliable species identification is achieved with molecular characteristics, a trend with a huge development in recent years. In Senegal, mango anthracnose is very common in wet production conditions, likewise during the rainy season. However, no accurate identification of the causal species was made. To fill this gap, thirty isolates of Colletotrichum were isolated from anthracnose lesions of mango from several regions in Senegal. The isolates were identified and grouped based on colony morphology, as well as size and shape of conidia and appressoria. Several color variations were noticed among these isolates, with small differences in spore shape, spore size, and presence of black pigmentation in the colony. Sequence analysis of the rDNA internal transcribed spacer region indicated that all 30 isolates belonged to the C. gloeosporioides complex. Based on sequence analysis of the ApMat intergenic region, all of them were further identified as most closely resembling C. siamense.***

Keywords: ApMat intergenic region, identification of*Colletotrichum gloeosporioides*complex, ITS region, mango anthracnose, Senegal

1. Introduction

Senegal ranks second for mango production among West African countries, and has the potential to competitively produce high mango tonnage over a long period of the year for the European Market. However, the fruit is subjected to attacks by several pests, among which the fungal disease known as anthracnose is the most important. Anthracnose causes both pre- and post- harvest fruit rot. *Colletotrichum gloeosporioides* Penz. and Sacc., the anamorph of *Glomerella cingulata*, has historically been named as the causal pathogen of anthracnose on mango and other fruits. It has more than 600 synonyms and many morphological and physiological variations [1]. In addition, seven formae speciales were described by [23], who showed that *C. gloeosporioides* is aheterogeneous group with much variation in morphology.

The morphological characters are useful for distinguishing some species within *C. gloeosporioides* group [11]. However, many of these morphological characters can change under different growing conditions (growth media, temperature, light, etc.), or with repeated subculturing. [5] used the shape and the size of the conidia and the shape of the appressoria as morphological criteria to separate groups within the *C. gloeosporioides* species complex. Conidial morphology, appressoria formation, color of colonies, and presence or absence of setae are traditional morphological characteristics used for differentiating *C. gloeosporioides*.

More recently, molecular methods have allowed further resolution of *Colletotrichum gloeosporioides* and it is now considered a species complex [6],[27]. At least nine different species within this complex have been reported as causal agents of mango anthracnose, but additional species of *Colletotrichum* in other groups can cause anthracnose on mango as well [12], [18]. Molecular methods have been used successfully to differentiate species of *Colletotrichum*, and the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA); glyceraldehyde–3–phosphate dehydrogenase (GPDH); histone 4 (his4); glutamine synthase (GS); b–tubulin 2 (TUB 2) were used for this purpose[19],[6], [12], [20], [26] , [17], [14]. The ITS locus has been proposed as a universal barcode, but, for Colletotrichum, it is highly evolutionarily conserved [3]. It is helpful in distinguishing Colletotrichum species from different complexes, but does not resolve the issue of differentiating species within *C.gloeosporioides* s.l. [27]. [27] discussed secondary barcode loci for the *C. gloeosporioides*within species complex, and indicated that the TUB2 or GS loci are best for distinguishing *C. aenigma* (syn: *C. populi*)from *C. alienum* and some *C. siamense* isolates. In addition, the Apn2/MAT locus has been used by [22] to improve phylogenetic resolution of the *Colletotrichum gloeosporioides* species complex. Moreover, [13] showed that the combination of the ApMat (intergenic region of Apn2/MAT locus) and GS loci in a phylogenetic analysis is able to resolve all currently accepted species in the *C. gloeosporioides* species complex.

In Senegal, anthracnose of mango has been attributed to *C. gloeosporioides*[15], [8] and the identities and relative representation of the species according to modern criteria are unknown because no molecular characterization of *Colletotrichum* species associated with mango has been carried out. Therefore, the purpose of this study was to characterize isolates of *Colletotrichum* from orchards and markets in several production areas and cities and achieve an accurate identification in Senegal based on DNA sequence analysis.

2. Materials and Methods

Sampling and fungal isolation

Mangoes were collected from orchards and markets in several cities in Senegal in July and August of 2015 (Table 1), both in the North (Pout, Notto Gouye Diama), and in the South (Kolda, Sedhiou, and Ziguinchor). Samples were brought to the laboratory in plastic bags and mangoes were held in bags at room temperature for 8 days at which time lesions were cultured for recovery of Colletotrichum.

In order to isolate the pathogen, the mangoes were first soaked in a 10% household bleachsolution (0.8% sodium hypochlorite) for 5 minutes, and two crossed incisions in the form of a V were made at the edge of the lesion with a sterile scalpel. A piece of flesh was taken from under the peel and placed in a Petri dish containing potato dextrose agar (PDA), which was incubated at room temperature. Two days later, the mycelium growing out of the mango flesh was transferred into new Petri dishes to obtain pure culture of the fungi. The isolates were then incubated for 4 to 10 days to allow sporulation. The presence of Colletotrichum was confirmed based on morphological characteristics (i.e. mycelium color, conidial size and shape, and appressoria size and shape). Thirty isolates were used for further characterization.

Table 1: Description of Colletotrichum isolates from Senegal, collected in summer 2015

Locations	Code	Variety	Source
Notto Gouye Diama (North)	1A 1B	Keitt	Orchard
	1C 1D	Keitt and Kent	Orchard
	1E	Keitt	Market
Pout (North)	2A 2B 2C 2D 2E	Kent and Keitt	Market
Kolda (South)	3A 3B 3C	Keitt	Orchards and market
Sedhiou (South)	3D 3E	Keitt	Orchards and market
Ziguinchor (South)	4A 4B 4C 4D 4E	Keitt and Kent	Orchards and market
Badjo (Djibelor, South)	5A 5B 5C 5D 5E	Keitt	Orchard
Jean (Djibelor, South)	6A 6B 6C 6D 6E	Keitt	Orchard

Morphological characterization of isolates

A total of 30 *Colletotrichum* isolates were collected from mango in eight cities located in the principal mango production areas. They were characterized morphologically based on traits like colony color, conidial size and shape, and size and shape of appressoria. Colony pigment was determined after colonies were grown 7 to 8 d on PDA at 27^0C under constant black light illumination (F15T8-BLB lamp). In addition, spore sizewere measured by placingfifty conidia on a slide in a drop of lactic acid and observation under a stereoscopic microscope equipped with appropriate devices.

Appressoria were produced using a slide culture technique [11]. A small piece of PDA was placed on a microscope slide and covered with a cover slip. After 1 to 2 days, the cover slip was removed and placed in a drop of lactic acid on a microscope slide, and twenty appressoria were observed under 400X magnification in bright field.

Molecular analysis

DNA extraction

The thirty *Colletotrichum* isolates were also used in the phylogenetic analysis. They were first grown on PDA and incubated for 7 days at 27°C. Fifty milligrams (50 mg) of mycelium were scraped from the surface of agar and placed into a tube containing three ceramic beads. The mycelium was homogenized using a FastPrep-24 homogenizer (MP, Santa Ana, CA) for 30 sec, or up to 60 sec, at a speed of 4 m/s. The Biosprint 15 DNA plant kit (Qiagen, Valencia, CA) was used for genomic DNA extraction, according to the manufacturer's protocol.

PCR amplification and DNA sequencing

The ITS region was amplified by PCR using the universal primers ITS5 forward (5' GGA AGT AAA AGT CGT AAC AAG G 3') and ITS4 reverse (5' TCC TCC GCT TAT TGA TAT GC 3') [19]. The PCR reactions were carried out in a Mastercycler® pro (Eppendorf, Hamburg, Germany) with the following cycling parameters: 10 min denaturation step at 95°C; 35 cycles of 95°C for 30 s, 60°C for 35 s and 72°C for 50 s; and a final extension step of 10 min at 72°C. The PCR reaction mixture consisted of: 8.9 µl nuclease free water, 12.5 µl ImmoMix (2X) (Bioline, Taunton, MA), 1.3 µl each primer (10 µM) and 1 µl genomic DNA. The PCR products were thereafter separated by electrophoresis on 1.5% agarose gel. The GelRed™ (Biotium, Hayward, CA) was added to the gel to stain the nucleic acid and the bands were visualized under UV light. Band sizes were estimated by comparison to a 100 bp molecular ladder.

The PCR products were then cleaned in preparation for sequencing with shrimp alkaline phosphatase/exonuclease I (SAP/Exo I) (Affymetrix, Santa Clara, CA) and the reaction mixture was: 5.0 µl nuclease free water, 1.0 µl shrimp alkaline phosphatase (1 unit/ µl), and 0.1 µl exonuclease I (10 units/µl).These reactions were placed in the mastercycler programmed as follows: 30 minutes at 37ºC, 20 minutes at 65ºC. The obtained PCR products were sequenced in both directions. Sequencing was performed by Eurofins Genomics (Huntsville, AL).

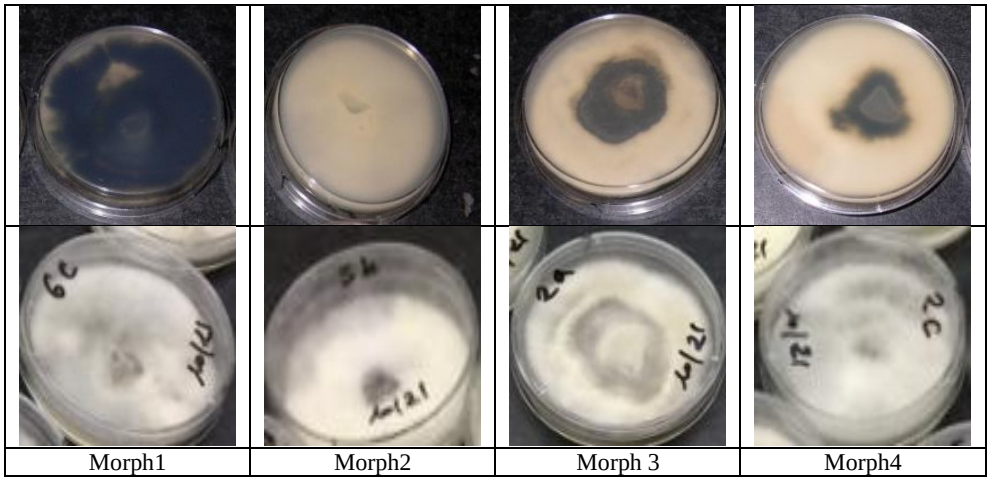
Two loci on the Apn2/MAT genes were also sequenced to further resolve identity of the isolates. The ApMat intergenic region (713 bp) was amplified by PCR using primer pair AM [AM-F (5'- TCATTCTACGTATGTGCCCG-3')/AM-R

(5'-CCAGAAATACACCGAACTTGC-3')] and the 5' end of the Apn2 gene (Apn25L, 883 bp) was amplified by PCR using primer pair A5L [A5L-F (5'-CAAGCGACGAAGTATACGAG-3')/A5L-R5'-GCATCACGGGAATAACTAGG-3')] primers [22]. The ApMat locus was amplified for all 30 isolates. Conditions were the same as those used for the ITS genomic region except for the annealing temperature, which was 60°C for both the AM and A5L primer pairs. Reactions were cleaned as described previously for the ITS region and sequenced in both directions by Eurofins Genomics.

3. Results

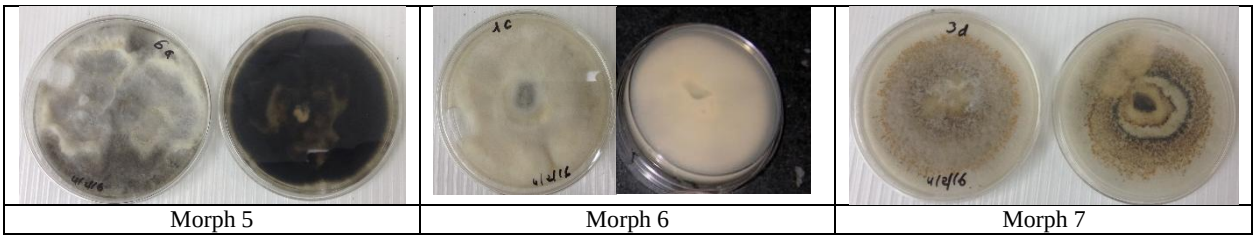
Morphological features of strains

Colletotrichum species complexes are characterized based on the size and shape of the conidia and appressoria. In order to determine whether the molecular species characterization matched the morphological characteristics, the variation among the 30 isolates was studied. Initially, four color variations were distinguished. Morphology 1 was distinguished by the production of a black pigment in most of the reverse side of PDA cultures.Morphology 2 produced white mycelial colonies without black pigment. Morphology 3 had a central round area of black pigment , and morphology 4 produced a central black area, of irregular shape (Figure 1). After several additional transfers, morphologies 1 and 2 were still present, although sometimes in different isolates (i.e., some isolates shifted from one morphology to another), butmorphologies 3 and 4 changed completely to another color, namely, white mycelium with many orange conidial masses all over the PDA plates (Figure 2).



Morph1 | Morph2 | Morph 3 | Morph4

Figure 1: Morphology (1 to 4) found among isolates of *Colletotrichum* spp. isolated from symptomatic mango fruit collected in several orchards and markets in Senegal. Upper side (top) and reverse side (bottom) of plate



Morph 5 | Morph 6 | Morph 7

Figure 2: New morphology (5 to 7) found among the same isolates of *Colletotrichum* spp after several additional transfers. isolated from symptomatic mango fruit collected in several orchards and markets in Senegal. Upper side (left) and reverse side (right) of plate.

The *Colletotrichum* isolates in this study showed only slight variation in conidial size and shape (Table 2). Spore shapes of most of the isolates were cylindrical with rounded ends (Table2), typical of *C. siamense* conidia, but some isolates also produced some conidia that were cylindrical with broadly rounded ends (Table2). Conidial length was considerably more variable than width (Table 2). The length/width (L/W) ratio of the conidia of the isolates ranged from 2.4 to 2.8.

Table 2: Summary of morphological characteristics of Colletotrichum isolates grown on potato dextrose agar (PDA)

Morphology	Color	Length (µm)	Width (µm)	Isolates	Shape	Conidia
1	Reverse side of colony entirely black on PDA	14-15	5-6.25	6A; 6B; 6C; 6D; 6E; 5C; 5D; 4A; 4B; 2E; 4C; 4D; 5E	Cylindrical with rounded end	

2	White mycelial colonies	13-16	5-6.25	5A; 4E; 3A; 3D; 3E; 1D; 5B	Cylindrical with broadly rounded end	
3	Black color with round shape on the center of plate	14-18	5-6.25	3C; 2A; 1A; 1B; 1C; 2E	Cylindrical with rounded end	
4	Black irregular shape on the center of plate	13-16	5-6.25	3B; 2B; 2C; 1E	Cylindrical with rounded end	

Appressoria were produced within 24 hoursfor all isolates, and were light to dark brown (Figure 3). Appressoria varied in size with 5-7.5 µm to 3-5 µm for all *Colletotrichum* isolates.

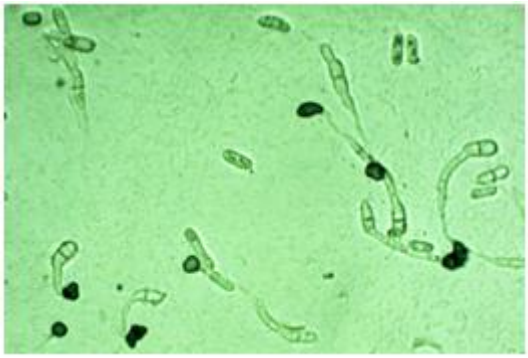


Figure 3: Appressoria light to dark brown under microscope

DNA analysis

Amplification of the ITS region of the 30 Colletotrichum isolates generated a fragment of approximately 600 bp for each isolate. Chromatograms and sequences were aligned, viewed and manually edited using Lasergene(DNASTAR, 13) and consensus sequences were created. The consensus sequences of all the isolates were identical. The consensus sequence was compared with nucleotide sequences in the NCBI database using BLAST. The query sequence had 100% similarity to several sequences from isolates belonging to the *C. gloeosporioides* species complex, i.e. *C. siamense* (accession numbers HM131511; JX010245) or *C. populi* (JX010243; JX010244). PCR products obtained for the ApMat intergenic locus of the 30 isolates were approximately 1,000 bp. Chromatograms and sequences were aligned, viewed and manually edited using Lasergene(DNASTAR, 13) and consensus sequences were created. The consensus sequences of all the isolates were identical. The consensus sequence was compared with nucleotide sequences in the NCBI database using BLAST. The query sequence had 99% similarity and 0.0 for the E-valuesto several sequences from isolates belonging to *C. siamense* (accession codes: KC790678; KC790694; KC790694.1; KJ954494; KJ954494.1; KJ954503.1; KJ954504.1; KJ954508; KJ954508.1; FR718813; HE657304; JQ899288).

4. Discussion

This study provides the first formal and accurate basis for the identification of the fungal species causing mango anthracnose in Senegal. The introduction of molecular taxonomy and its application to the genus *Colletotrichum* has demonstrated that what were formerly considered species are actually species complexes with members that are very difficult or impossible to differentiate and identify based on morphological characteristics [3].

Based on the conidial shape and the size, 50 conidia for each isolates were measured and they were not significantly different for both in length and width of conidia. The shape of these conidia was two types, rounded ends and broadly rounded end. Similar results have been reported by[19] saying that conidia of *C. siamense* from coffee berries in northern Thailand are fusiform with slightly rounded ends. In addition, [21] wrote that *C. siamense* shape is fusiform to cylindrical. The length/width ratios of conidia were in agreement with the observations of [19], in which the isolates of *C. siamense* from coffee berries were 2.3-4.2.
Appressorial size and shape did not show consistent differences among the four morphologies. Similar results about the length and width of appressoria of *C. siamense* from coffee berries were obtained by [19]. [24] showed that *Colletotrichum* species from chilli cannot be distinguished by the morphology of appressoria.

These morphological characters may not be reliable for identification within *Colletotrichum*, but a combination of molecular methods with morphological characters might be a more reliable approach for studying *Colletotrichum* species complexes [19].

Both molecular and morphological characteristics were used to characterize the 30 Colletotrichum isolates from anthracnose-diseased mangoes in Senegal. The morphological characters, while matching the characteristics of C. *gloeosporioides* s. l. and of *C. siamense*, were not useful in resolving to species.

Many authors have stated in the past that *C. gloeosporioides* is the common pathogen that infects more than 1000 plant species [18]. In order to be able to obtain molecular data, [2]

obtained a culture from a necrotic spot of a living leaf of *Citrus sinensis* in Italy, the same host and location where the originally described type isolate was obtained, and designated it as ex-epitype cultureof *C. gloeosporioides*. When 25 isolates from eight tropical fruits were compared with the epitype, none of them was *C. gloeosporioides* [18]. Several studies since then have shown that the newly epitypified *C.gloeosporioides sensu stricto* is not a common pathogen on tropical fruits [18], [20].

The ITS sequence confirmed that the isolates belong to the C. *gloeosporioides* complex. The ITS sequence was the first barcode used [3] and supports a close relationship of the 30 isolates, confirming that they all belong to the genus *Colletotrichum*, and placed them all in the *C. gloeosporioides* complex. ITS sequencing analyses have been suggested by many authors to distinguish one *Colletotrichum* species complex from another [19], [27], [7], [12], [20], [26], [13], [17]. Based on the ITS sequence, the common causal agent of mango anthracnose in the main growing areas of Senegal belongs to the *C. gloeosporioides* species complex.

The results from analysis of sequences of the ApMat locus indicated that all 30 isolates were closest to *C. siamense*.[22] showed that the ApMat marker is helpful to separate species within the *C. gloeosporioides*complex. [20] used 6 genes (*act*, *cal*, *chs1*, *gapdh*, ITS and *tub2*), followed by ApMat sequence-analysis and statedthat 39 *Colletotrichum* isolates from India were separated into nine lineages based on ApMat, namely *C. fragariae sensu stricto*, *C. fructicola*,*C. jasmini-sambac*, *C. melanocaulon* and five undesignated groups. Going further, [21] concluded that *C. siamense* is a species complex based on ApMat marker analysis, and distinguished seven species within this complex (*C. hymenocallidis*, *C. jasmini-sambac*, *C. dianesei*, *C. endomangiferae*, *C. murrayae*, *C. siamense sensu stricto*, and *C. communis sp.*). They advised use of the ApMat marker as an efficient marker, to not only save time, but also the cost, compared with sequencing 5-8 gene loci. However, most recently, [13] disagreed and argued that *C. siamenses. l.* is single species rather than a species complex. These authors compared sequence results for eight loci (CAL, GAPDH, GS, ITS, TUB2, ApMat, Apn25L, MAT1-2-1) and also conducted morphological analysis and mating experiments; they concluded that the individual phylogenetic trees obtained for the different characters and loci did not agree, and did not support recognition of separate species according to the Genealogical Concordance Phylogenetic Species Recognition concept. *C. siamense sensu stricto*, *C. jasmini-sambac*, and *C. hymenocallidis* have high similarity and they could not be distinguished in phylogenetic analysis by [28] and [27].

Based on the ApMat marker analysis, *C. siamense sensu lato* is the probable causal agent of anthracnose on mango in Senegal. Additional evidence could be obtained by sequencing the GS locus [13] or other loci. Many researchers have shown the presence of *C. siamense sensu lato* in many plants hosts [27], [12], [20], [25], [26], [10], [4], [30]. [27] found that *C. siamense* is biologically and geographically diverse and this species can be in many hosts across many tropical and subtropical regions. [20] showed that *C. siamense sensu stricto* is one of the causal agents of anthracnose on mango in India. In addition, [25] showed that *C. siamensesensu lato* was the most common *Colletotrichum* species in tropical wild fruits in northern Thailand.

References

[1] ArxVonJ.A., 1957. Die Arten der Gattung *Colletotrichum* Cda. *PhytopathologischeZeitschrift* 29: 413–468
[2] Cannon, P.F., Buddie, A.G, and Bridge, P.D., 2008. The typification of *Colletotrichum gloeosporioides*. *Mycotaxon* 104, 189–204
[3] Cannon, P.F., Damm, U., Johnston, P.R., Weir, B.S., 2012. *Colletotrichum*– current status and future directions. Mycology 73, 181–213
[4] Chowdappa, P., Chethana, C.S., and Pavan, K.V. 2015. *Colletotrichum siamense* and *Colletotrichum truncatum* are responsible for severe outbreaks of anthracnose on onion in Southwest India. *Journal of Plant Pathology* 97, 77-86
[5] Cox, M.L., and Irwin, J.A.G. 1988. Conidium and appressorium variation in Australian isolates of the *Colletotrichum gloeosporioides* group and closely related species. *Australian Systematic Botany* 1, 139–149
[6] Damm, U., Baroncelli, R., Cai, L., Kubo, Y., O'Connell, R., Weir, B., Yoshino, K., and Cannon, P.F. 2010. *Colletotrichum*: species, ecology and interactions. *IMA Fungus* 1, 161–165
[7] Damm U., Cannon P.F., Woudenberg J.H.C., Johnston P.R., Weir B.S., Tan Y.P., Shivas R.G., and Crous P.W. 2012. The *Colletotrichum boninense* species complex. *Studies in Mycology* 73, 1–36
[8] Diedhiou, P.M., Diallo, Y., Faye, R., Mbengue, A.A, and Sene, A., 2014a**.** Efficacy of different fungicides against mango anthracnose in Senegalese Soudanian agroclimate. *American Journal of Plant Sciences*5, 2224-2229
[9] Ismail, A.M., Cirvilleri, G., Yaseen, T., Epifani, F., Perrone, G., and Polizzi G. 2015. Characterization of *Colletotrichum* species causing anthracnose disease of mango in Italy. *Plant Pathology* 97, 167-171
[10] James, R.S., Ray. J., Tan, Y.P., and Shivas, R.G. 2014. *Colletotrichum siamense*, *C. theobromicola* and *C. queenslandicum* from several plant species and the identification of *C. asianum* in the Northern Territory, Australia. *Australasian Plant Diseases Notes* 9, 138
[11] Johnson, D.A., Carris, L.M., and Rogers, J.D. 1997. Morphological and molecular characterization of *Colletotrichum nymphaeae* and *C. nupharicola* sp. nov. on water-lilies (*Nymphaea* and *Nuphar*). *Mycological Research* 101, 641–649
[12] Lima, N.B., Batista, M.V., Morais Jr, M.A., Barbosa, M.A.G.,Michereff, S.G., Hyde, K.D, and Câmara, M.P.S. 2013. Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil. *Fungal Diversity* 61, 75–88
[13] Liu, F., Weir, B.S., Damm, U., Crous, P.W., Wang, Y., Liu, B., Wang, M., Zhang, M., and Cai, L. 2015. Unravelling *Colletotrichum* species associated with *Camellia*: employing ApMat and GS loci to resolve

species in the *C. gloeosporioides* complex. *Persoonia* 35, 63–86

[14] Liu, F., Wang, M., Damm, U., Crous, P.W., and Cai, L. 2016. Species boundaries in plant pathogenic fungi: a Colletotrichum case study. *BMC Evolutionary Biology* 16, 81

[15] Mbaye N. (2006).Inventaire et caractérisation des champignons phytopathogénes responsables des maladies post-récoltes chez deux variétés de mangues (*Mangifera indica* L), Kent et Keitt, destinées à l'exportation dans la zone des Niayes du Sénégal. Dissertation: Département biologie végétale : Université Cheikh Anta Diop, 105 p.

[16] Moriwaki, J., Tsukiboshi, T., and Sato, T. 2002. Grouping of *Colletotrichum* species in Japan based on rDNA sequences. *Journal of General Plant Pathology*68, 307–320

[17] Pardo-De la Hoz, C.J., Calderon, C., Rincon, A.M., Cardenas, M., Danies, G.,Lopez-Kleine, L., Restrepo, S., and Jimenez, P. 2016. Species from the *Colletotrichum acutatum*, *Colletotrichum boninense* and *Colletotrichum gloeosporioides* species complexes associated with tree tomato and mango crops in Colombia. *Plant Pathology* 65, 227–237

[18] Phoulivong, S., Cai, L., Chen, H., McKenzie, E.H.C., Abdelsalam, K., Chukeatirote, E., and Hyde, K.D. 2010.*Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. *Fungal Diversity* 44, 33–43

[19] Prihastuti, H., Cai, L., Chen, H., McKenzie, E.H.C. and Hyde, K.D. 2009. Characterization of Colletotrichum species associated with coffee berries in northern Thailand. *Fungal Diversity* 39, 89-109

[20] Sharma, G., Kuma, N., Weir, B.S., Hyde, and Shenoy, B.D. 2013. The ApMat marker can resolve *Colletotrichum* species: a case study with *Mangifera indica*. *Fungal Diversity* 61, 117-138

[21] Sharma, G., Pinnaka, A.K., and Shenoy, B.D. 2015. Resolving the *Colletotrichum siamense* species complex using ApMat marker. *Fungal Diversity* 71, 247–264

[22] Silva, D.N., Talhinhas, P., Várzea, V., Cai, L., Salgueiro O.P., and Batista, L.D. 2012b. Application of the Apn2/MAT locus to improve the systematics of the *Colletotrichum gloeosporioides* complex: an example from coffee (Coffea spp.) hosts. *Mycologia* 104, 396–409

[23] Sutton, B.C. 1992. The genus *Glomerella* and its anamorph *Colletotrichum*, p. 1-26. *In* J. A. Bailey and M. J. Jeger (ed.), *Colletotrichum* biology, pathology and control. CAB International, Wallingford, United Kingdom.

[24] Than, P.P., Jeewon, R., Hyde, K.D., Pongsupasamit, S., Mongkolporn, O. and Taylor, P.W.J. 2008a. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology* 57, 562-572

[25] Udayanga D., Manamgoda D.S., Xingzhong L., Chukeatirote E., and Hyde K.D. 2013. What are the common anthracnose pathogens of tropical fruits? *Fungal Diversity* 61, 165–179

[26] Vieira, W.A.S., Michereff, S.J., de Morais Jr., M.A., Hyde, K.D., and Câmara, M.P. S. 2014. Endophytic species of *Colletotrichum* associated with mango in northeastern Brazil. *Fungal Diversity* 67, 181-202

[27] Weir B.S., Johnston P.R., and Damm U. 2012. The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology* 73, 115–180

[28] Wikee, S., Cai, L., Pairin, N., McKenzie, E.H.C., Su, Y.Y., Chukeatirote, E., Thi, H.N., Bahkali, A.H., Moslem, M.A., Abdelsalam, K., and Hyde, K.D., 2011 — *Colletotrichum* species from Jasmine (*Jasminum sambac*). *Fungal Diversity* 46, 171–182

[29] Yang, Y.L., Liu, Z.Y., Cai, L., Hyde, K.D., Yu, Z.N. and McKenzie, E.H.C. 2009. Colletotrichum anthracnose of Amaryllidaceae. *Fungal Diversity* 39, 123-146

[30] Ye, Y., Qin, L., Jiang, S., Wei, J., Cui, Y., Powell, C.A., and Zhang, M. 2016. First report of anthracnose caused by *Colletotrichum siamense* on *Sarcandra glabra* in China. *Plant Disease*100, 862