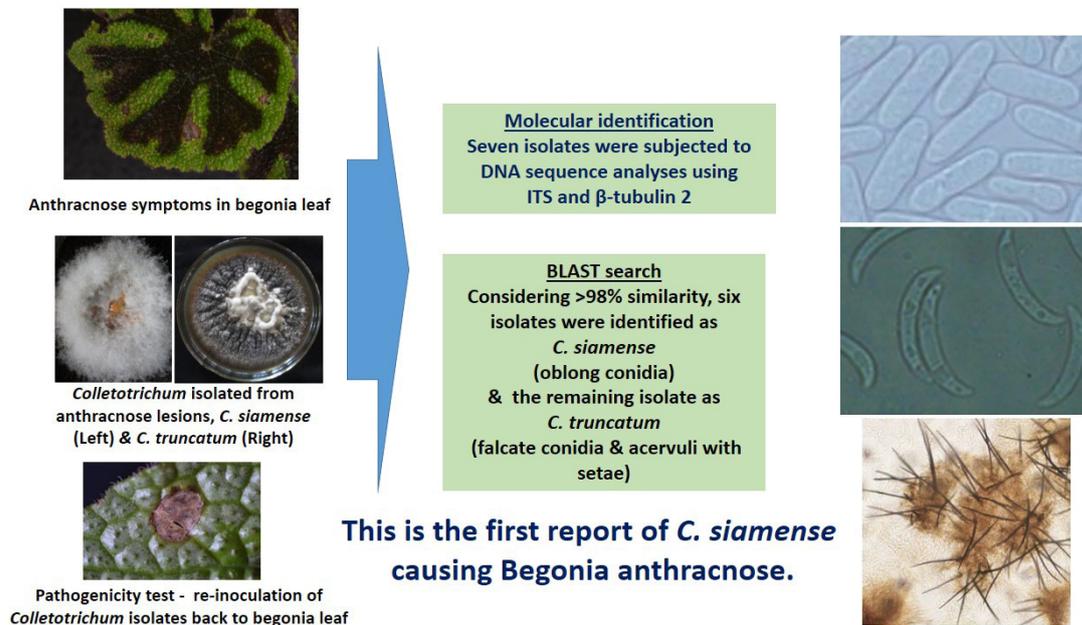


Molecular characterization of *Colletotrichum* species causing *Begonia* anthracnose in Sri Lanka

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Highlights

- Popularity of foliage plants is because of their attractive leaves which must be kept free of diseases or blemishes.
- *Begonia* plants are affected by several diseases of which anthracnose is most common.
- Using DNA sequence analyses, causal agents of the disease were identified as *Colletotrichum siamense* and *C. truncatum*.
- Use of disease-free plants, good sanitation and fungicide application, if necessary, may control the disease.
- This is the first report of *C. siamense* as a causal agent of begonia anthracnose.

RESEARCH ARTICLE

Molecular characterization of *Colletotrichum* species causing *Begonia* anthracnose in Sri Lanka

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Abstract: Anthracnose disease is known to affect many tropical and subtropical fruits, vegetables and also certain cut-flowers and foliage plants. The disease was known to be caused by *Colletotrichum gloeosporioides* or *C. acutatum* which are presently accepted as species complexes. The present study was conducted with the main objective of identifying *Colletotrichum* species causing begonia anthracnose using morphological and molecular data. *Begonia* is an ornamental foliage plant grown worldwide. Anthracnose symptoms appear in begonia leaves as brownish, irregular, necrotic lesions. *Colletotrichum* was isolated from three *Begonia* species showing anthracnose symptoms, collected from three Provinces of Sri Lanka. Thirty isolates were obtained in the study of which 29 formed oblong conidia and the remainder produced falcate conidia. Six randomly selected isolates forming oblong conidia and the isolate with falcate conidia were sequenced for Internal Transcribed Spacer (ITS) and β -tubulin 2 (TUB2) regions. Considering >98% similarity with NCBI GenBank database for both sequences, the isolates with oblong conidia were identified as *C. siamense* and the isolate with falcate conidia as *C. truncatum*. Newly generated sequences were subjected to phylogenetic analysis with the closely related ex-type and authenticated isolates sequences. Phylogenetic analysis confirmed the species as *C. siamense* and *C. truncatum*. Koch's postulates were performed to establish whether the fungi isolated from anthracnose lesions were actually causing anthracnose disease in *Begonia* leaves. This is the first report of *C. siamense* causing *Begonia* anthracnose.

Keywords: *Begonia*, anthracnose disease, *Colletotrichum*, DNA sequence analysis.

INTRODUCTION

Begonia, belonging to the Family Begoniaceae, is one of the largest angiosperm genera comprising over 1500 species (Hughes and Hollingsworth, 2008). They are native to moist tropical and subtropical climates and cultivated as indoor or outdoor houseplants and as edgings in gardens for their elegant, succulent, often varicolored leaves and waxy flowers.

Begonias are susceptible to several diseases caused by fungi, bacteria, and viruses, as well as abiotic disorders. Among fungal diseases, the grey mould caused by *Botrytis cinerea*, powdery mildew incited by *Erysiphe cichoracearum* and *Rhizoctonia* crown rot are common

and important (Benson and Cartwright, 1996). A leaf rust caused by *Coleosporium solidaginis* has also been reported which produces orange-red pustules on the under surface of leaves resulting in shedding them prematurely. Leaf and stem rot, vein yellowing, and wilting of *Begonia elatior* hybrids (*Begonia* x *hiemalis*), caused by *Fusarium foetens*, were reported from Japan (Sekine *et al.*, 2008). One of the most common and wide-spread diseases in *Begonia* is bacterial leaf spot or blight with small blister-like spots on leaves caused by *Xanthomonas campestris* pv. *begonia* (Vauterin *et al.*, 1990). Affected leaves may shed prematurely.

Anthracnose is a common foliage disease in potted begonias and is more serious in nurseries, often resulting in outbreaks (Park *et al.*, 2006). The disease affects mainly the foliage, directly reducing the plant's horticultural value. Anthracnose in *Begonia* in South Korea was reported to be caused by *C. acutatum* using RAPD analysis and morphological characteristics (Park *et al.*, 2006). *Colletotrichum truncatum* was more recently identified as the cause of anthracnose in China using morphological and multigene sequence data (Zhai *et al.*, 2018). Here the anthracnose symptoms appeared as large, sunken and brown lesions, mostly at the leaf margin, with numerous black acervuli on *Begonia* stems.

In recent years, a considerable progress has been made in delineating the taxonomy of different *Colletotrichum* species that cause anthracnose. Molecular phylogeny and genomics data have enabled distinguish *Colletotrichum* species complexes and identify genetically distinct subgroups. Anthracnose disease in *Begonia* in Sri Lanka has not been subject to any detailed studies. This present study was conducted to characterize the *Colletotrichum* species causing *Begonia* anthracnose in Sri Lanka by DNA sequence analysis.

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MATERIALS AND METHODS

Collection of diseased material

Leaves showing symptoms of anthracnose disease were mostly collected from a nursery of the Royal Botanical Gardens, Peradeniya. Diseased leaves were also collected from Hantana (Kandy District), Kothmale (Nuwara Eliya District) in the Central Province, Kurunegala (Kurunegala District) in the North Western Province and Kegalle (Kegalle District) in the Sabaragamuwa Province of Sri Lanka during the period from July – December 2018. Four different *Begonia* varieties, *Begonia rex* var. *Rex*, *B. rex* var. *Glass begonia*, *B. peltata* (unknown variety) and *B. masoniana* var. *Iron cross* were encountered in the study. Diseased leaves from each variety were brought to the Plant Pathology laboratory at the Department of Botany, University of Peradeniya in punch-aerated polyethylene bags within a few hours after collection.

Isolation of *Colletotrichum*

Small segments (5x5 mm²), cut from the diseased areas of the leaf, were surface sterilized in sodium hypochlorite (Clorox[®], USA) for 3 min. After rinsing the segments in sterilized distilled water (SDW), the excess liquid was removed by placing them on sterilized filter papers before transferring aseptically on to Potato Dextrose Agar (PDA) plates (4 segments per plate), supplemented with Tetracycline to suppress bacterial growth. The plates were incubated at 28 -30 °C or 5-7 d to allow fungal growth. A small portion of the mycelium from each colony was mounted in lactophenol on a glass slide and examined under light microscope for the presence or absence of conidia typical to *Colletotrichum*. The isolates, tentatively recognized as *Colletotrichum*, were sub-cultured by transferring 6 mm diameter discs of mycelium on to fresh PDA plates and the plates were incubated at 28 -30 °C for 7-14 d.

Preparation of mono-conidial cultures

A suspension of conidia was prepared by scraping mycelia from two weeks old cultures and suspending in sterile distilled water (SDW) in 50 ml flasks. The suspension was filtered through sterile glass wool, the concentration of conidia was adjusted to 1 x 10⁶ conidia ml⁻¹. A loopful of the suspension from each isolate was streaked on tap water agar plates and the plates were incubated at 26-28 °C for 18 h. A single germinated conidium was located by moving the objective lens (x 25) of a light microscope (Olympus BX53 with Olympus DP72 digital camera) across the streak line of the inverted agar plate. A small piece of agar with the germinated conidium was cut and transferred on to fresh PDA plates. The plates were incubated for seven days at 28-30 °C and the isolates were sub-cultured on PDA to be used for morphological and molecular studies.

Morphological study

Two weeks old mono-conidial cultures were used in triplicate to study the colony morphology viz. colony color, texture, pigmentation underneath, the presence or absence of concentric rings, sectoring and reproductive

morphology, acervuli or the conidia masses. A drop (20 µl) of suspension of conidia was mounted on a clean slide and, after placing a coverslip, the conidial characteristics such as shape and color of conidia, presence of septa etc. were recorded (Olympus BX53 with Olympus DP72 digital camera and Olympus cellSens software). The dimensions were measured in µm on 50 randomly selected conidia with eyepiece graticule connected to a light microscope. The average length and width were calculated.

DNA extraction

Six isolates, randomly selected from among the 29 isolates that produce oblong conidia, and the single isolate with falcate conidia, were taken for molecular studies. DNA was extracted using Promega Wizard Genomic DNA Purification Kit. Cell lysis solution (300 µl) and the nucleic acid lysis solution (300 µl) were added into each eppendorf tube. The mycelium, scraped from the colonies of each fungal isolate using sterilized micropipette tips, were placed in respective eppendorf tubes. They were vortexed for 2 min vigorously and incubated at 65 in a water bath for 1 h. After cooling down to room temperature, 20 µl of proteinase K solution was added to each tube. The eppendorf tubes were shaken vigorously and incubated at 65 for 1 h. 20 µl of protein precipitation solution was added to each eppendorf tube and vortexed vigorously and centrifuged at 14,000 rpm for 5 min. The supernatants were transferred into separate tubes respectively to avoid mycelia. Aliquot (600 µl) of cold isopropanol was added into each tube. They were kept in ice for 5 min and centrifuged at 14,000 rpm for 5 min. The supernatants were discarded and 600 µl of 70% ethanol was added to the pellet. The tubes were centrifuged at 14,000 rpm for 1 min and the supernatants were discarded. The tubes were kept inverted on a sterile filter paper to air-dry. Finally, 100 µl of DNA rehydration solution was added to each tube and the DNA samples were stored at -20 °C.

PCR amplification

Two gene regions, β-tubulin 2 (TUB2) (BT2a 5'-GGTAACCAAATCGGTGCTTTC-3') (BT2b 5'-ACCCTCAGTGTAGTGACCCCTTGC3') (Glass and Donaldson, 1995) and internal transcribed spacer (ITS) of the ribosomal DNA (ITS-1F 5'-CTTGGTCATTTAGAGGAAGTAA-3') (ITS-4 5'-TCCTCCGCTT ATTGATAATGC-3') (White *et al.*, 1990) were amplified. All PCR amplifications were carried out in a total 40 µl volume containing 1 × PCR reaction buffer, 2.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphate (dNTPs), 0.2 µM each forward and reverse primer, 1 U of Taq DNA polymerase (Promega) and 3 µl of unquantified DNA extract, using a Thermal Cycler (Applied Biosystems, Veriti). The PCR program consisted of an initial denaturation at 95 °C for 4 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C, 54 °C for TUB2, and ITS respectively for 30 s, extension at 72 °C for 45 s and a final extension at 72 °C for 7 min (Weir *et al.*, 2012).

PCR products were separated by electrophoresis in 1% agarose gel and visualized with a UV trans-illuminator. PCR products, confirmed for amplification, were

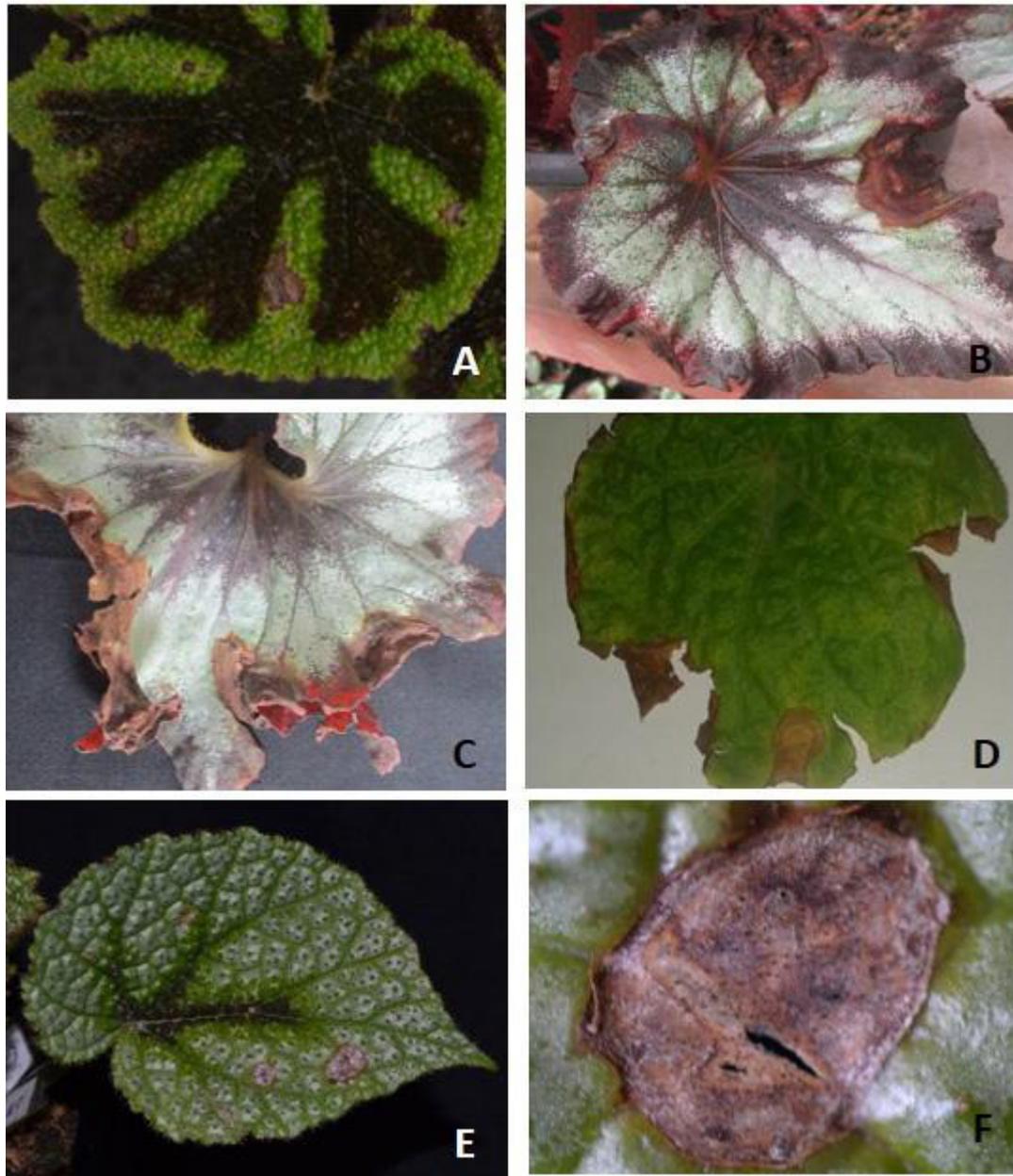


Figure 1: Anthracnose symptoms expressed in different begonia varieties, (A) *Begonia masoniana* var. Iron Cross, (B) *B. rex* var. Rex, (C) *B. rex* var. Glass begonia, and (D) *B. peltata* unknown variety, (E) Anthracnose lesions developed on *Begonia masoniana* var. Iron Cross artificially inoculated with by *C. truncatum* and, (F) Black tiny dots representing acervuli developed by *C. truncatum*.

sequenced (Applied Biosystems, 3500 genetic analyzer) at the Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Sri Lanka.

Identification of isolates

The species affiliations and identities were determined through similarity-based searches of the NCBI GenBank Database (<http://www.ncbi.gov>). Based on the identifications that resulted from the BLAST search, a combined phylogenetic analysis for ITS and TUB2 was conducted using the authenticated sequences (members of the *C. gloeosporioides* complex and *C. truncatum* complex) obtained from the GenBank. Chromatograms of all the sequences were edited and aligned using Bio Edit v7.2.5 software (Hall, 1999) and ClustalX 2.1 (Larkin *et*

al., 2007). Bayesian Inference analysis was performed for the combined matrix. The best fitting substitution model was determined with jModelTest v.2 (Darriba *et al.*, 2012) using the Akaike information criterion (AIC). The nucleotide substitution model Hasegawa-Kishino-Yano (HKY) was selected. Bayesian inference was conducted to obtain posterior probabilities using MrBayes ver. 3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with 10,000,000 generations Markov chain Monte Carlo (MCMC) chains with a sampling frequency of every 1,000 generations. The initial 25% samples from each run were discarded as burning. A majority rule consensus tree was calculated using the remaining trees to obtain the posterior probabilities for each node. The resulting tree was visualized and edited in FigTree ver. 1.4.3 (<http://tree.bio.ed.ac.uk/software/>

figtree/, last accessed 2018-12-04). *Colletotrichum hippeastri* was used as the out group. All newly generated sequences were deposited in GenBank and the accession numbers are given in the Table 1.

Koch's postulates

Begonia leaves showing anthracnose symptoms were examined and the symptoms were recorded. Scrapings obtained from anthracnose lesions were observed under light microscope to see the presence of the identifiable pathogen structures in all diseased leaves. The two pathogens, isolated from diseased tissues and grown in pure culture as described previously, were used for inoculation. For artificial inoculation, uniform-sized, healthy plants of *Begonia masoniana* var. Iron cross, devoid of any blemishes or visible symptoms of disease, and grown in medium-sized plastic pots without any fungicide treatment were used. A suspension of conidia of each isolate was prepared as described previously by scraping mycelium from 2-3 weeks old cultures of the *C. siamense* and *C. truncatum* and the concentration was adjusted to 1×10^6 conidia ml^{-1} . Drops (20 μl) of conidia suspension were applied on to wounded or unwounded sites of leaves (6 drops per leaf) of *Begonia*. Six replicate leaves in two plants were used for inoculation per isolate and another three leaves were treated with drops of sterile distilled water as controls. Fifty percent of the sites to be inoculated in the leaves were superficially wounded using a fine needle and the rest were left unwounded. Inoculated plants, covered with perforated polythene bags, were incubated at 28–30°C and after 24 h the polythene bags were taken off and the pots were transferred to a plant house for further incubation. Inoculated leaves were examined when symptoms appeared and compared with those of the plants used for original isolations. The pathogens were re-isolated from the symptomatic leaves on PDA. The colonies of the isolate were compared with the original isolate used for inoculation for colony and asexual reproductive morphology.

RESULTS

Symptoms

Anthracnose disease was observed in the four *Begonia* varieties belonging to three species, *Begonia rex* var. Rex, *B. rex* var. Glass begonia, *B. peltata* (unknown variety) and *B. masoniana* var. Iron cross, encountered in the study. Anthracnose symptoms in foliage varied slightly with the variety (Figure 1). In the var. Iron cross (*B. masoniana*), small, dark brown and circular lesions appeared initially, scattered over the mature leaves. The lesions gradually enlarged into irregular and large lesions up to about 1-4 cm diameter. The center of the lesions was light brown and the margin of the lesions was dark brown. In *B. rex* var. Rex, oval shaped or irregular, dark brown and 3 - 4 cm diameter lesions with concentric markings appeared towards the periphery of the leaf blade. In some plants, light brown, about 2 cm wide and up to 4 - 8 cm long lesions with wavy inner margin were also observed on the margin of the leaf. The outer margin of the lesion was often shredded. In heavily infected leaves, numerous pleomorphic lesions of

varied size could be seen. Anthracnose lesions were not observed in younger leaves or any of the petioles.

Morphological characteristics

Thirty *Colletotrichum* isolates were obtained from the symptomatic *Begonia* leaves collected in the study. The colony morphology among the 6 isolates forming oblong conidia used for the study varied slightly from each other. Colony color ranged from white to light grey. Most isolates formed cottony, raised colonies with concentric markings and yellowish pink conidial masses. The lower surface of the colony was cream in color (Figure 2). Conidia were aseptate, hyaline, oblong, and the mean length ranged 12.7-18.1 μm .

The seventh isolate formed whitish, fluffy, raised colonies with yellowish pink center and dark grey to black color, the mycelium in the periphery was flat, consists of 1.5–2 cm wide radial sectors with dense conidia masses of 2-3 mm diameter. The lower surface of the colony was black color. Conidia were aseptate, hyaline and falcate with a prominent area in the center with granulate cytoplasm, the mean length varied from 18.2 – 20.1 μm . Setae were straight, dark brown and septate (Figure 2). From the morphological characteristics of acervuli and conidia (Zhai *et al.*, 2018), the solitary isolate was tentatively identified as *C. truncatum*.

Identification based on molecular data

The lengths of the sequences that resulted from the PCR reactions for the ITS region were between 580-600 bp, while the TUB2 region was between 500-520 bp. Based on the BLAST search results with authenticated sequences (GenBank, <http://www.ncbi.gov>) that gave > 98% similarity, six isolates (UPBTBCS01, UPBTBCS02, UPBTBCS03, UPBTBCS04, UPBTBCS05, and UPBTBCS06) were identified as *Colletotrichum siamense* and the isolate with falcate conidia was confirmed as *C. truncatum* (UPBTBCT01) (Figure 2), based on the sequences of the two gene regions.

Phylogenetic analysis

The phylogenetic tree that resulted from the Bayesian analysis is given in the Figure 3. The six isolates, identified as *C. siamense* during the present study, together with the authenticated ex-type culture of *Colletotrichum siamense*, have formed a monophyletic group with a posterior probability of 0.99 and is resolved within the CG complex. Similarly, the other isolate, identified as *C. truncatum*, has also formed a monophyletic group with the authenticated type isolate of *C. truncatum* with a posterior probability value of 1 as a separate clade (Figure 3).

Koch's postulates

The pathogen with oblong conidia, subsequently identified as *C. siamense*, was isolated from 29 diseased leaves of begonia indicating its consistent presence in lesions and grown on fresh PDA as pure cultures. *C. truncatum* with falcate conidia was isolated from only one diseased leaf. *Begonia* leaves artificially inoculated with *C. siamense*

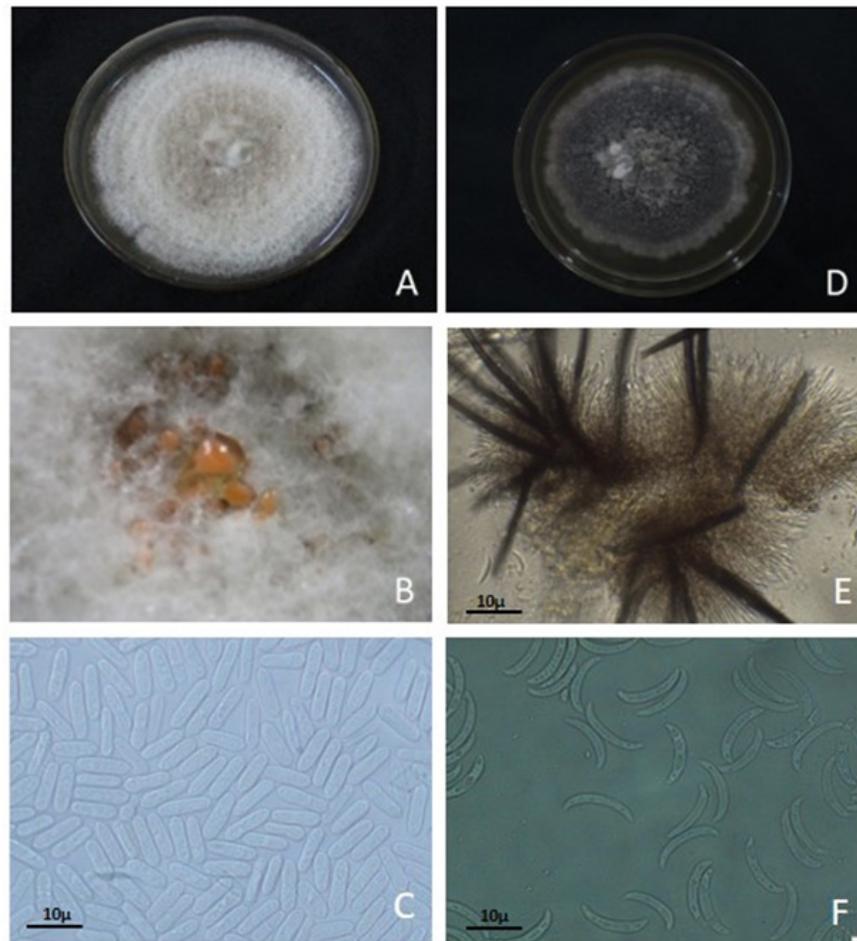


Figure 2: A to C - *Colletotrichum siamense* isolated from *Begonia*, A - 7-day old mono-conidial isolate on PDA, B – Conidia masses produced on culture, C – Conidia. D to F - *C. truncatum* isolated from *Begonia*, D – 7-day old culture of mono-conidial isolate on PDA, E – Acervuli with black, straight setae projecting out, and F – Conidia.

or *C. truncatum* developed brown color, irregular, necrotic lesions typical to begonia anthracnose observed in originally used begonia leaves for isolation, 7–14 days after inoculation. The leaves that were inoculated without wounding, with either of the two fungi, initiated lesions 2 - 3 days later and took a few more days for expansion than those in wound-inoculated leaves. *C. truncatum* developed necrotic lesions consisting numerous black color acervuli. The lesion development by *C. truncatum* was much quicker compared to *C. siamense* showing that *C. truncatum* was more aggressive on *Begonia* (Figure 1). There were no symptoms observed in the control plants. The culture plates prepared by re-isolation were morphologically similar to the cultures used for inoculation.

DISCUSSION

Begonias are primarily grown for their decorative foliage that offer different colors and textures and excellent alternatives to some of the flowering potted plants. They are affected by several diseases of those the anthracnose is one of the commonest and most destructive diseases adversely affecting the foliage quality. The main objective of the present study was to determine the *Colletotrichum* species causing *Begonia* anthracnose in Sri Lanka. Both morphological characteristics and multigene phylogenetic

analyses were utilized for species level identification.

Of a total of 30 isolates collected in the study, 29 produced oblong conidia. Six out of 29 isolates that were subjected to multigene DNA sequence analyses were identified as *Colletotrichum siamense* belonging to the *C. gloeosporioides* species complex. *C. siamense*, was first described as a causal agent of the anthracnose of coffee berries from the Northern Thailand (Prihastuti *et al.*, 2009) and subsequently from anthracnose disease in numerous plant species and fruits. The solitary isolate that produced falcate conidia from anthracnose in *Begonia* leaves was tentatively identified using conidial and acervuli morphology (Zhai *et al.*, 2018) as *C. truncatum*. DNA sequence data and phylogenetic analysis confirmed that the isolate was indeed *C. truncatum*. In a previous study in China, three *Colletotrichum* isolates from anthracnose in *Begonia* leaves were identified as *C. truncatum* based on cultural and morphological characteristics (Zhai *et al.*, 2018) and molecular data using ITS, TUB2 and GAPDH regions (Zhai *et al.*, 2018). *C. truncatum* belongs to the *C. truncatum* species complex which includes *C. truncatum* and three other closely related species. The complex can be distinguished by their curved conidia with truncated base and acute, more strongly curved apex (Damm *et al.*, 2009). *C. truncatum* was also frequently referred to as *C. capsici*

Table 1: Accession numbers of authenticated sequences of species of the *C. gloeosporioides* complex and *C. truncatum*, obtained from the GenBank and the sequences generated from the present study to be used for phylogenetic analysis.

Species	Isolate/Culture collection	Host	Gen Bank Accession number		Reference
			ITS	TUB2	
<i>C. aenigma</i>	ICMP:18608	<i>Persea americana</i>	JX010244	JX010389	Weir <i>et al.</i> (2012)
<i>C. aeschynomenes</i>	ICMP:18609	<i>Persea americana</i>	JX010245	JX010390	Weir <i>et al.</i> (2012)
<i>C. alatae</i>	ICMP:17919	<i>Dioscorea alata</i>	JX010190	JX010383	Weir <i>et al.</i> (2012)
<i>C. alienum</i>	ICMP:12071	<i>Malus domestica</i>	JX010251	JX010411	Weir <i>et al.</i> (2012)
<i>C. aotearoa</i>	ICMP:18537	<i>Coprosma</i> sp.	JX010205	JX010420	Weir <i>et al.</i> (2012)
<i>C. asianum</i>	BPDI4	<i>Coffea arabica</i>	FJ972612	JX010406	Weir <i>et al.</i> (2012)
<i>C. clidemiae</i>	ICMP:18658	<i>Clidemia hirta</i>	JX010265	JX010438	Weir <i>et al.</i> (2012)
<i>C. cordylinicola</i>	ICMP:18579	<i>Cordyline</i>	JX010226	JX010440	Weir <i>et al.</i> (2012)
<i>C. fructicola</i>	ICMP:18581	<i>Coffea arabica</i>	JX010165	JX010405	Weir <i>et al.</i> (2012)
<i>C. gloeosporioides</i>	CBS:112999	<i>Citrus sinensis</i>	JQ005152	JQ005587	Weir <i>et al.</i> (2012)
<i>C. horii</i>	ICMP:10492	<i>Diospyros kaki</i>	GQ329690	JX010450	Weir <i>et al.</i> , (2012)
<i>C. kahawae</i> subsp. <i>kahawae</i>	ICMP:17816	<i>Coffea arabica</i>	JX010231	JX010444	Weir <i>et al.</i> (2012)
<i>C. kahawae</i> subsp. <i>cigaro</i>	ICMP:19122	<i>Vaccinium</i> sp.	JX010231	JX010444	Weir <i>et al.</i> (2012)
<i>C. musae</i>	CBS:116870	<i>Musa</i> sp.	JX010146	HQ596280	Weir <i>et al.</i> (2012)
<i>C. nupharicola</i>	ICMP:18187	<i>Nuphar polysepala</i>	JX010187	JX010398	Weir <i>et al.</i> (2012)
<i>C. psidii</i>	ICMP:19120	<i>Psidium</i> sp.	JX010219	JX010443	Weir <i>et al.</i> (2012)
<i>C. queenslandicum</i>	PDD:28797	<i>Carica papaya</i>	JX010276	JX010414	Weir <i>et al.</i> (2012)
<i>C. salsolae</i>	ICMP:19051	<i>Salsola tragus</i>	JX010242	JX010403	Weir <i>et al.</i> (2012)
<i>C. siamense</i>	ICMP:18578	<i>Coffea arabica</i>	JX010171	JX010404	Weir <i>et al.</i> (2012)
<i>C. siamense</i>	UPBTBCS02	<i>Begonia</i> sp.	MK880372	--	Present study
<i>C. siamense</i>	UPBTBCS03	<i>Begonia</i> sp.	MK880373	MN701655	Present study
<i>C. siamense</i>	UPBTBCS04	<i>Begonia</i> sp.	MK880374	MN701656	Present study
<i>C. siamense</i>	UPBTBCS05	<i>Begonia</i> sp.	MK880375	MN701657	Present study
<i>C. siamense</i>	UPBTBCS06	<i>Begonia</i> sp.	MK880376	MN701658	Present study
<i>C. siamense</i>	UPBTBCS01	<i>Begonia</i> sp.	MK880377	MN701654	Present study
<i>C. theobromicola</i>	CBS:124945	<i>Theobroma cacao</i>	JX010294	JX010447	Weir <i>et al.</i> (2012)
<i>C. ti</i>	ICMP:4832	<i>Cordyline</i> sp.	JX010269	JX010442	Weir <i>et al.</i> (2012)
<i>C. tropicale</i>	CBS:124949	<i>T. cacao</i>	JX010264	JX010407	Weir <i>et al.</i> (2012)
<i>C. xanthorrhoeae</i>	BRIP:45094	<i>Xanthorrhoea</i> sp.	JX010261	JX010448	Weir <i>et al.</i> (2012)
<i>C. camelliae</i>	LC1364-strain	<i>Camellia</i>	KJ955081	KJ955230	Lie <i>et al.</i> (2015)
<i>C. changpingense</i>	SA0016	Strawberry	KP683152	KP852490	Jayawardena <i>et al.</i> (2016)
<i>C. conoides</i>	CAUG17	chili pepper	KP890168	KP890174	Liu <i>et al.</i> (2015)
<i>C. endophytica</i>	LC0324	<i>Pennisetum purpureum</i>	KC633854	KF254857	Udayanga <i>et al.</i> (2013)
<i>C. fructivorum</i>	Coll1414	<i>Vaccinium macrocarpon</i>	JX145145	JX145196	Doyle <i>et al.</i> (2013)
<i>C. grevilleae</i>	CBS:132879	<i>Grevillea</i> sp.	KC297078	KC297102	Liu <i>et al.</i> (2013)
<i>C. grossum</i>	CAUG7	chili pepper	KP890165	KP890171	Diao <i>et al.</i> (2017)
<i>C. hebeiense</i>	K3	<i>Vitis vinifera</i>	KF156863	KF288975	Yan <i>et al.</i> (2015)
<i>C. henanense</i>	LF238	<i>Camellia</i>	KJ955109	KJ955257	Liu <i>et al.</i> (2015)
<i>C. jiangxiense</i>	LF687	<i>Camellia</i>	KJ955201	KJ955348	Liu <i>et al.</i> (2015)
<i>C. temperatum</i>	Coll883	<i>Vaccinium macrocarpon</i>	JX145159	JX145211	Doyle <i>et al.</i> (2013)
<i>C. wuxiense</i>	JS1A32	<i>Camellia sinensis</i>	KU251591	KU252200	Wang <i>et al.</i> (2016)
<i>C. rhexiae</i>	Coll1026	<i>Rhexia virginica</i>	JX145128	JX145179	(Doyle <i>et al.</i> 2013)
<i>C. hippeastri</i>	CBS 125376	<i>Hippeastrum vittatum</i>	JQ005231	JQ005665	Weir <i>et al.</i> , (2012)
<i>C. truncatum</i>	CBS:151.35	<i>Phaseolus lunatus</i>	GU227862	GU228156	Damm <i>et al.</i> (2009)
<i>C. truncatum</i>	UPBTBCT01	<i>Begonia</i> sp.	MK880371	MN701659	Present study
<i>C. perseae</i>	GA100	<i>Persea americana</i>	KX620308	KX620341	Sharma <i>et al.</i> (2017)

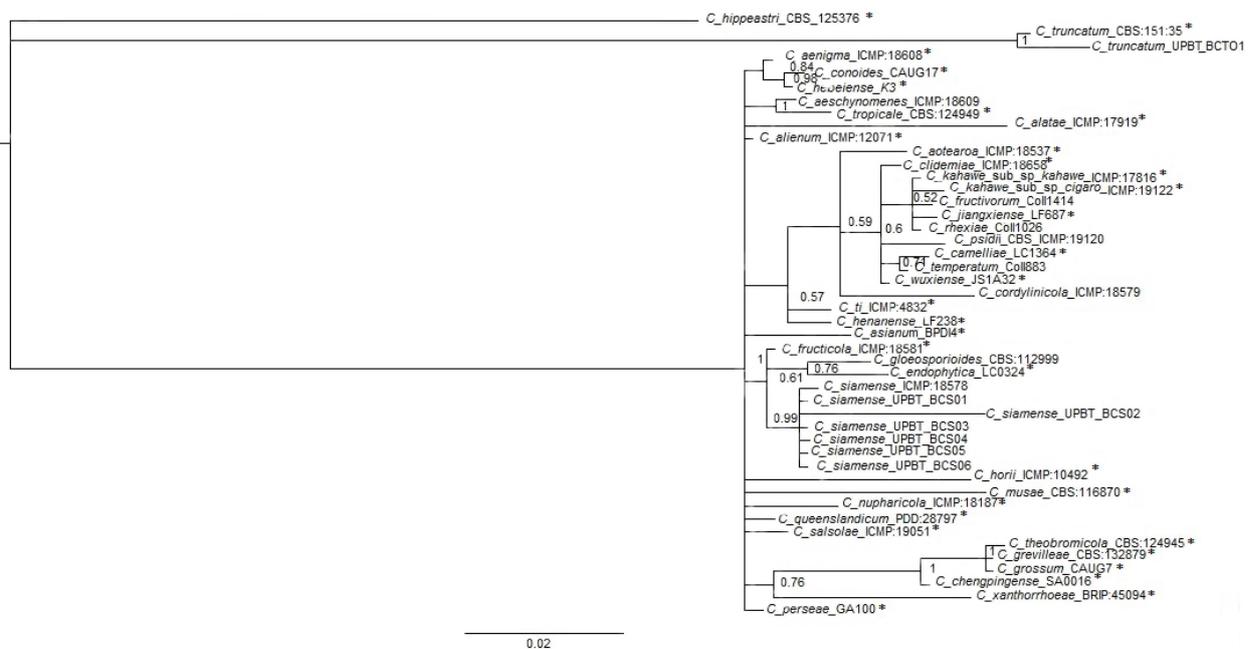


Figure 3: Bayesian inference phylogenetic tree for ITS and TUB2 sequences of *Colletotrichum* isolates of the present study together with ex-type and authentic culture sequences of *C. gloeosporioides* and *C. truncatum* species complexes; Bayesian posterior probability values ≥ 0.5 are shown at the nodes. The ex-type cultures are denoted by an *.

(Sawant *et al.* 2012) which is reported as an economically destructive pathogen of many tropical crops including legumes and solanaceous plants (Damm *et al.*, 2009).

The symptoms induced following *C. siamense* and *C. truncatum* infections in *Begonia* leaves showed a clear difference. *C. truncatum* infection resulted in numerous acervuli in the dotted center of well-developed necrotic lesions. In the present study, *C. truncatum* was isolated from the anthracnose in *Begonia* leaves at a very low frequency compared to *C. siamense*. However, *C. truncatum* was observed to be more aggressive on *Begonia* leaves on artificial inoculations than *C. siamense*. Koch's postulates, performed using *C. siamense* and *C. truncatum*, firmly established that both fungi were the pathogens involved in causing anthracnose disease in *Begonia*. *C. truncatum* has been previously reported as a pathogen causing *Begonia* anthracnose (Zhai *et al.*, 2018).

Colletotrichum species are well known pathogens worldwide in a wide variety of crop, ornamental and plantation plants and harvested fresh produce, fruits, vegetables and cut-flowers. They are also ubiquitous asymptomatic foliar endophytes in a vast range of hosts (Osono, 2008). *Colletotrichum* species appear to live as endophytes quite commonly in the leaves of *Begonia* species. Population of endophytic fungi has been studied in the leaves of three *Begonia* species in the Brazilian Atlantic Rainforest, *Begonia fischeri*, *B. olsoniae*, and *B. venosa*. A total of 426 fungal endophytes in 19 genera were isolated in pure culture. Among the isolates, *Colletotrichum* was the most abundant making 51.6% of the total isolates, followed by *Diaporthe* (22.5%) (Correia *et al.*, 2018). It has been suggested that endophytic fungi may be latent pathogens, saprophytes and/or mutualists (Saikkonen *et al.*, 2010). There is a possibility that endophytic *Colletotrichum*

species in *Begonia* may become pathogenic causing anthracnose disease in the leaves. However, the interaction between most plants and endophytes are still undetermined (Rojas *et al.*, 2010).

In summary, the *Colletotrichum* species causing anthracnose disease in *Begonia*s in Sri Lanka were identified as *C. siamense* and *C. truncatum*. None of the *Colletotrichum* isolates isolated in this study were either *C. acutatum* as reported previously (Park *et al.*, 2006; Jayawardena *et al.*, 2016) or *C. gloeosporioides sensu stricto*. There were no previous reports on the association of *C. siamense* with the *Begonia* either in Sri Lanka or elsewhere in the world. The present work has shed light on taxonomic aspects of *Begonia* anthracnose in Sri Lanka, which may lay the foundation for future studies related to management of the disease under nursery or marketing conditions.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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