ITS-based diversity of *Colletotrichum* from India

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Rapid and precise species identification of plant pathogens such as *Colletotrichum* is essential for their effective control. *Colletotrichum* species have been traditionally identified based on conidial morphology and host-specificity. Recently, extra emphasis has been placed on DNA sequence comparison for rapid and accurate identification. In this study, we have analyzed the suitability of using internal transcribed spacer (ITS)/ 5.8S rRNA gene, which is the universal fungal barcode marker towards accurate identification of *Colletotrichum* species. We have sequenced 207 isolates of *Colletotrichum* and compared their ITS/ 5.8S rRNA gene-sequence data with that of the 183 reference sequences available for the 143 accepted *Colletotrichum* type strains. The results of phylogenetic analysis based on ITS/ 5.8S rRNA gene-sequence dataset along with sequence-comparison hints at the nomenclatural ambiguity of many isolates, which were initially diagnosed based on morphological characters. A local-BLAST analysis of the 588 GenBank sequences from India shows that 79% of the sequences were erroneously named and 97% of the 159 isolates procured from Indian culture collection centres were found to be misidentified. Mycologists need to work on identification of a potential secondary barcode for this genus as ITS/ 5.8S rRNA is found to be ineffective in accurate identification of *Colletotrichum*. Researchers should also follow a polyphasic approach for species identification, which would definitely not be rapid; nevertheless would be more reliable and accurate.

Key words – Barcoding – NCBI-BLAST – GenBank – species identification

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Introduction

Identification is an important aspect of taxonomy. Accurate identification of an isolate facilitates the recognition of species, its morphological and biochemical features, with positive implications in ecological and biodiversity research (Hajibabaei et al. 2007, Seifert et al. 2007, Begerow et al. 2010). Identification is followed by taxonomic processes such as determination and classification (Shenoy et al. 2007a). Firstly, the essential attributes of a fungus such as: morphology, host specificity, DNA sequence-data and physiological features are characterized (identification), followed by determination of its relationships with the closely related extant fungi, leading to a hierarchy of biological classification system.

Developments in DNA barcoding have transformed the field of fungal taxonomy (Summerbell et al. 2005, Shenoy et al. 2007a, Seifert 2009). DNA barcoding (Hebert et al. 2003a) is a method to diagnose a species based on standard short DNA sequences such as mitochondrial cytochrome c oxidase1 (*cox1*) in animals (Folmer et al. 1994, Hebert et al. 2003a, b), large subunit of the ribulose-biphosphate carboxylase gene (*rbcL*) and maturase K gene of chloroplast (*matK*) in plants (Consortium for the Barcode of Life (CBOL) Plant Working Group 2009). The ITS/ 5.8S rRNA gene region has been widely used for species identification and phylogenetic analyses of many fungal genera (Soltis et al. 1999, Summerbell et al. 2005, Nilsson et al. 2009, Seifert 2009) and it has been projected as the best candidate for barcoding of fungi in the All Fungi Barcoding Meeting held in 2007 (Rossman 2007, Bellemain et al. 2010). This is attributed to the fact that this region is easy to sequence with the help of specific primers (White et al. 1990), provides good species-level resolution in many fungal groups of endophytes as well as phyto-pathogens and also has a high capability to evolve rapidly (Nilsson et al. 2008). Recently, Schoch et al. (2012) have declared nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*.

Colletotrichum is an important fungal genus whose members are known to cause various diseases in a variety of commercial, ornamental plants and perennial crops (Hyde et al. 2009a, b, Cannon et al. 2012). It is reported mainly from tropical regions (Freeman et al. 1998). This genus has been implicated in symptoms like fruit-lesions, fruit-rot, leaf-spot and leaf wilts from different parts of the world. *Colletotrichum* species can infect a growing crop directly (pre-harvest) as well as cause latent infections (fruit decay) as a post-harvest pathogen (Bailey et al. 1992, Waller 1992, Freeman et al. 1998, Farr and Rossman 2013). *Colletotrichum* has emerged as an important post-harvest pathogen owing to its ability to cause latent infection (Muirhead et al. 1981, Everett 1997, Fokulnang et al. 2000). *Colletotrichum* species have been implicated in serious damages to agricultural crops in India (Sharma et al. 2005, Shenoy et al. 2007b, Agarwal et al. 2012, Gautam et al. 2012, Hubballi et al. 2012, Kumar et al. 2012, Sawant et al. 2012, Sharma et al. 2012, Wilson and Naseema 2012, Sharma et al. 2013a, b).

Taxonomically, *Colletotrichum* is a complex genus. As reports on anamorph-teleomorph connection were limited (Hyde et al. 2009b, Cannon et al. 2012), *Colletotrichum* species have been traditionally identified based on conidial characters and host-specificity (Cai et al. 2009). Identification based on morphology, however, has been shown to be ineffective and unreliable in many cases such as *C. gloeosporioides* species-complex (Cannon et al. 2008, Weir et al. 2012) and *C. graminicola* species complex (Crouch et al. 2009).

Recently, taxonomic revisions have been made in the *Colletotrichum* genus by revisiting the phylogenies of members of *C. gloeosporioides*, *C. acutatum* and *C. boninense* species complexes based on multigene phylogeny and genealogical concordance phylogenetic species recognition (GCPSR) criterion (Damm et al. 2012 a, b, Weir et al. 2012). Cannon et al. (2012) have reviewed and enlisted 119 species under *Colletotrichum* genus and around 30 species are expected to possess a *Glomerella* sexual stage. At present there are 143 accepted species names under the genus *Colletotrichum* (Cannon et al. 2012, Noireung et al. 2012, Rakotoniriana et al. 2012, Barimani et al. 2013, Doyle et al. 2013, Huang et al. 2013, Liu et al. 2013b, Manamgoda et al. 2013, Peng et al. 2013, Tao et al. 2013, Udayanga et al. 2013).

DNA based species-identification, is expected to provide a potentially reliable framework for identification as DNA characters are not known to be directly affected by environmental factors (Cannon et al. 2000). Though ITS/ 5.8S rRNA gene region is a widely used gene marker in *Colletotrichum* taxonomy (Summerbell et al. 2005, Seifert 2007, Shenoy et al. 2007a), recent studies have shown that this gene region offers moderate species resolution in *Colletotrichum.* However, until other or better gene markers are described, it remains to be the best available sequence dataset till date (Cai et al. 2009, Hyde et al. 2009b). There is a need to revisit the taxonomy of this economically important

phytopathogenic fungal genus from India, as majority of cultures deposited in various culture collections have been identified based on morphological features. In addition, the NCBI-GenBank sequences deposited from India also needs amendments based on the latest ITS/ 5.8S rRNA sequence dataset (Cannon et al. 2012). This study reveals the nomenclatural ambiguity in many morphologically identified *Colletotrichum* species from India, based on the phylogenetic analysis and sequence comparison results.

Materials and Methods

Fungal isolates

Plant substrates from different hosts were collected from IMTECH campus and neighboring areas in Punjab. A total of 49 endophytic *Colletotrichum* were isolated from the collected tissues following the method described in Cai et al. (2009). The plant tissues were surface-sterilized in 70% ethanol for 1 minute, followed by 1% sodium hypochlorite for 1 minute, rinsed thrice with sterilized water and finally dried on sterilized tissue paper. The plant tissue were then placed on potato dextrose agar (PDA) medium (Difco, catalogue number 213400, Maryland, USA) containing 50 µg/ml chloromphenicol (BioBasic, catalogue number CB0118, Ontario, Canada). The fresh mycelia from tissues were sub-cultured on PDA medium and grown at 20 ºC for 7 days to isolate DNA for molecular study. A total of 158 *Colletotrichum* isolates were procured from different culture collection facilities across India and subcultured in a similar manner. All the 207 fungal isolates were preserved at -70 ºC and LN_2 in 10% glycerol for future use. *Colletotrichum* strains from this study are represented in Table 1 (list of endophytes) and Table 2 (list of isolates from different culture collection centre of India). List of *Colletotrichum* type species whose reference ITS/5.8S rRNA gene sequence has been used, are tabulated in Table 3.

DNA extraction, PCR amplification and sequencing of ITS / 5.8S rRNA gene region

Genomic DNA from fresh mycelia was isolated using DNA isolation kit (Zymo Research, catalogue number D6005, USA) following the manufacturer's protocol and stored at -20 ºC. Polymerase chain reaction (PCR) was done in a 50 µl reaction volume. Each reaction tube contained 3 µl of total genomic DNA, 10 µl of 5x GoTaq Green Buffer (Promega, USA), 1.5 µl each of 10 µM forward primer (ITS 5) and reverse (ITS 4) primer (White et al. 1990), 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 0.25 µl of 5U Go Taq Polymerase enzyme (Promega) and 29.75 µl of sterile water. The PCR reactions were carried out in Eppendorf Mastercycler (Eppendorf, Germany) with cycling parameters as described in White et al. (1990). The PCR products were run on a 1% Tris-Acetate-EDTA agarose gel stained with ethidium bromide (0.5 µg/ ml) at 100 V, 400 mA for 45 minutes to check the presence of desired band of 500-600 bp. The PCR products were further cleaned with QIAquick PCR purification kit (QIAGEN, catalogue number 28106) according to the manufacturer's protocol. The purified PCR products were quantified using Nanodrop Spectrophotometer ND-1000 (Thermo). The PCR products were sequenced using ITS 5 and ITS 4 primers (White et al. 1990) with ABI Big Dye v3.1 Terminator Ready Reaction cycle sequencing kit (Applied Biosystems) using manufacturer's protocol. The samples were purified to remove excess salt, denatured with HiDi-Formamide at 95 ºC for 3 minutes and analysed using 3730 DNA Analyzer (Applied Biosystems) at the Central DNA sequencing facility available at the CSIR-Institute of Microbial Technology, Chandigarh, India.

Sequence alignment and phylogenetic analysis

The forward and reverse sequences obtained for each strain were aligned using Sequencher version 4.10.1 (Gene Codes Corp., Ann Arbor, Michigan, USA) to generate a consensus sequence. The ends of the sequences were trimmed with the help of ITS 5 and ITS 4 primer sequences and aligned using MUSCLE algorithm (Edgar 2004) in MEGA version 5.2 (Tamura et al. 2011). Analysis of sequence divergence was done using Kimura-2 Parameter (K2P) distance (Kimura 1980) and the rate variation among sites was modeled with a gamma distribution (shape parameter = 5). The phylogram for ITS/ 5.8S rRNA gene

region was generated with Neighbor-Joining (NJ) method (Saitou and Nei 1987) with bootstrap analysis (Felsenstein 1985) of 100 replicates using MEGA. All positions containing alignment gaps and missing data were removed in pairwise sequence comparisons. The resulting NJ tree with branch support and bootstrap value is shown in Fig. 1. The sequences generated in this study have been deposited in NCBI-GenBank (Table 1 and 2) (Benson et al. 1997, 2006).

Retrieval of sequences from NCBI-GenBank and local BLAST analysis

A total of 588 sequences were retrieved from NCBI-GenBank on 29.03.2013 using the keywords "*Colletotrichum* ITS India" for local BLAST analysis. List of the *Colletotrichum* isolates from India whose sequences have been retrieved from NCBI-GenBank for local BLAST analysis, are tabulated in Supplementary Table 1. A local database including the type sequences of *Colletotrichum* species (Table 3) was created using BioEdit (Hall 1999). BLAST similarity search analysis (Altschul et al. 1990) of ITS/5.8S rRNA gene sequences retrieved from NCBI-GenBank were performed against this local database and the result is presented in Fig. 2 and the local BLAST score and % identity for each GenBank sequence from India is tabulated in detail in Supplementary Table 1. Similarly, local BLAST analysis was also done for the 207 *Colletotrichum* isolates sequenced in this study and the local BLAST score and % identity is tabulated in detail in Table 1 and 2.

Result

Phylogeny

The phylogenetic analysis included 391 sequences including the outgroup *Monilochaetes infuscans* (CBS 869.96) and a total of 719 characters. All ambiguous regions were excluded from the analysis. The phylogenetic tree generated based on Neighbor-Joining method with branch support in a bootstrap analysis of 100 replicates is shown in Fig. 1.

The NJ tree thus generated is very poorly resolved which shows that ITS/ 5.8S rRNA signal is poor in case of *Colletotrichum* genus. ITS/ 5.8S rRNA gene marker is able to cluster the *Colletotrichum* isolates only to their respective species complex level. A total of 163 *Colletotrichum* isolates from his study clustered within the *C. gloeosporioides* species complex clade comprising 31 type strain sequences (Clade gloeosporioides) (Weir et al. 2012). MTCC 2175 did not align with any of the existing *Colletotrichum* type strain, however; it remained basal to the type strains of recently described two new species: *C. thailandicum* (Noireung et al. 2012) and *C. gigasporum* (Rakotoniriana et al. 2012).

Six isolates clustered within the *C. boninense* species complex clade comprising 18 type strain sequences (Clade boninense) (Damm et al. 2012a). A total of 23 isolates clustered within the *C. truncatum* species complex clade comprising three type strain sequences (Clade truncatum). Four isolates clustered within the *C. graminicola* species complex clade comprising 18 type strain sequences (Clade graminicola). Three isolates (GS30, ITCC 6271, ITCC 6272) clustered with a recently described new species *C. brevisporum* (Noireung et al. 2012), while four isolates (ITCC 4765, ITCC 5213, ITCC 6032, GUFCC 15503) clustered with type strain of *C. cliviae*. ITCC 6079 clustered with type strain of *C. nigrum* (Liu et al. 2013a). There were no representative isolates for 14 *Colletotrichum* type strains in our study including: *C. agaves, C. chlorophyti, C. coccodes, C. dracaenophilum, C. lindemuthianum, C. orbiculare, C. orchidophilum, C. phaseolorum, C. pseudoacutatum, C. rusci, C. sansevieriae, C. trichellum, C. tropicicola* and *C. yunnanense*.

Recently, Cannon et al. (2012) reviewed the current status of *Colletotrichum* systematics and have described nine major clades within this genus. In our study, the ITS/ 5.8S rRNA gene marker was able to identify species belonging to different species complexes into their respective clades: for eg. Clade gloeosporioides, Clade boninense, Clade dematium, Clade spaethianum, Clade destructivum and Clade acutatum. However, species belonging to the Clade graminicola were not fully resolved based on ITS/ 5.8S rRNA gene marker. Similar finding has been reported earlier by Crouch et al. (2009).

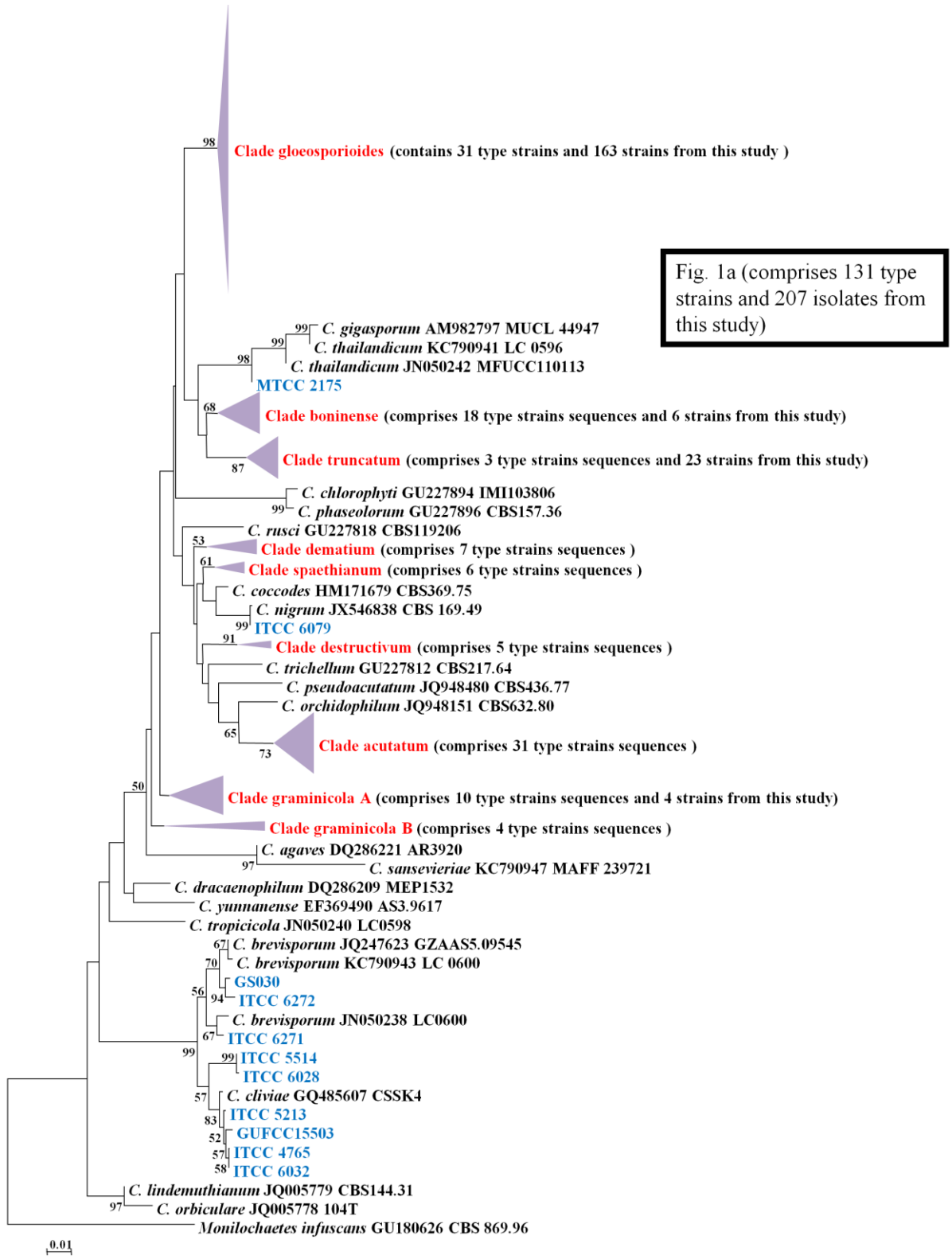


Fig. 1 – **a** NJ tree based on ITS/ 5.8S rRNA gene sequences of 207 *Colletotrichum* isolates from India and 131 type strain sequences (Sequences highlighted in blue are from this study; clade names highlighted in red contains isolates from India, details about each clade has been mentioned in the clade title)

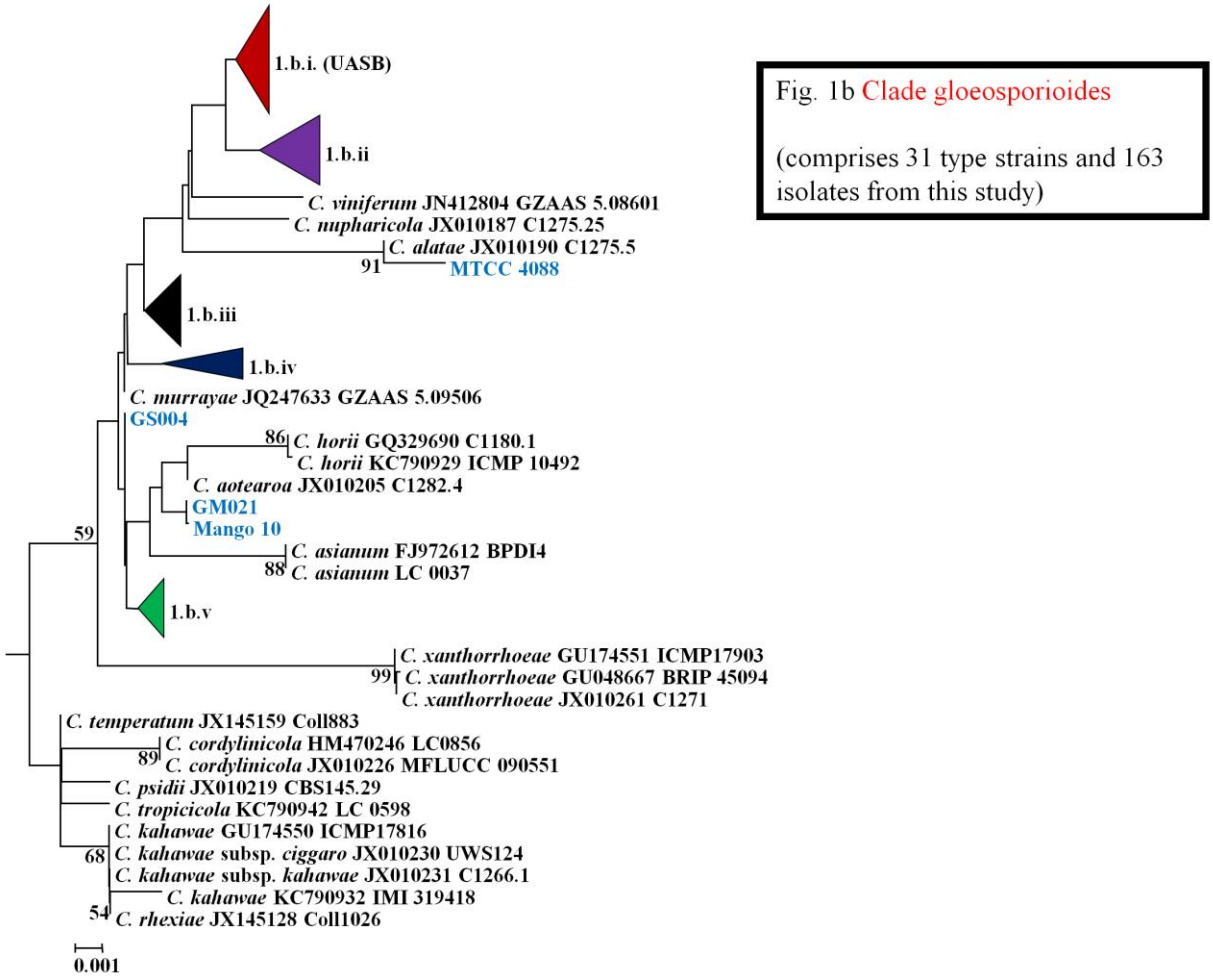


Fig. 1 – (contd.) b. Isolates in clade gloeosporioides (further split in parts i-v)

Local BLAST analysis

A total of 588 sequences were retrieved from NCBI-GenBank for the local BLAST analysis and to validate the authenticity of the sequences deposited from India. Identification was based on the sequence comparison with the DNA sequence data of ITS/ 5.8S rRNA gene region of 183 *Colletotrichum* type strain sequences using Local-BLAST application in BioEdit. The results of local BLAST have been summarized in Supplementary Table 1.

Approximately 79% (463/588) of the DNA sequence entries of Indian origin retrieved from NCBI-GenBank were found to be erroneously named (Fig. 2a). However, all the sequences named "*G. tucumanensis*" were correctly named and were found to show 98-99% identity with the type strain sequence of *C. falcatum*. One sequence each of *C. curcumae*, *C. chlorophyti* and *C. siamense* were correctly named and showed 100% identity with their respective type strain sequence (Fig. 3). Out of the 277 sequences deposited as *C. gloeosporioides* only two sequences showed identity with the type strain sequence of *C. gloeosporioides sensu stricto. Colletotrichum musae* (5/7) remained the most correctly named strain within the members of *C. gloeosporioides* species complex. Besides, approximately 97% (155/159) of the isolates procured from various culture collections of India (Fig. 2b) were found to be misidentified.

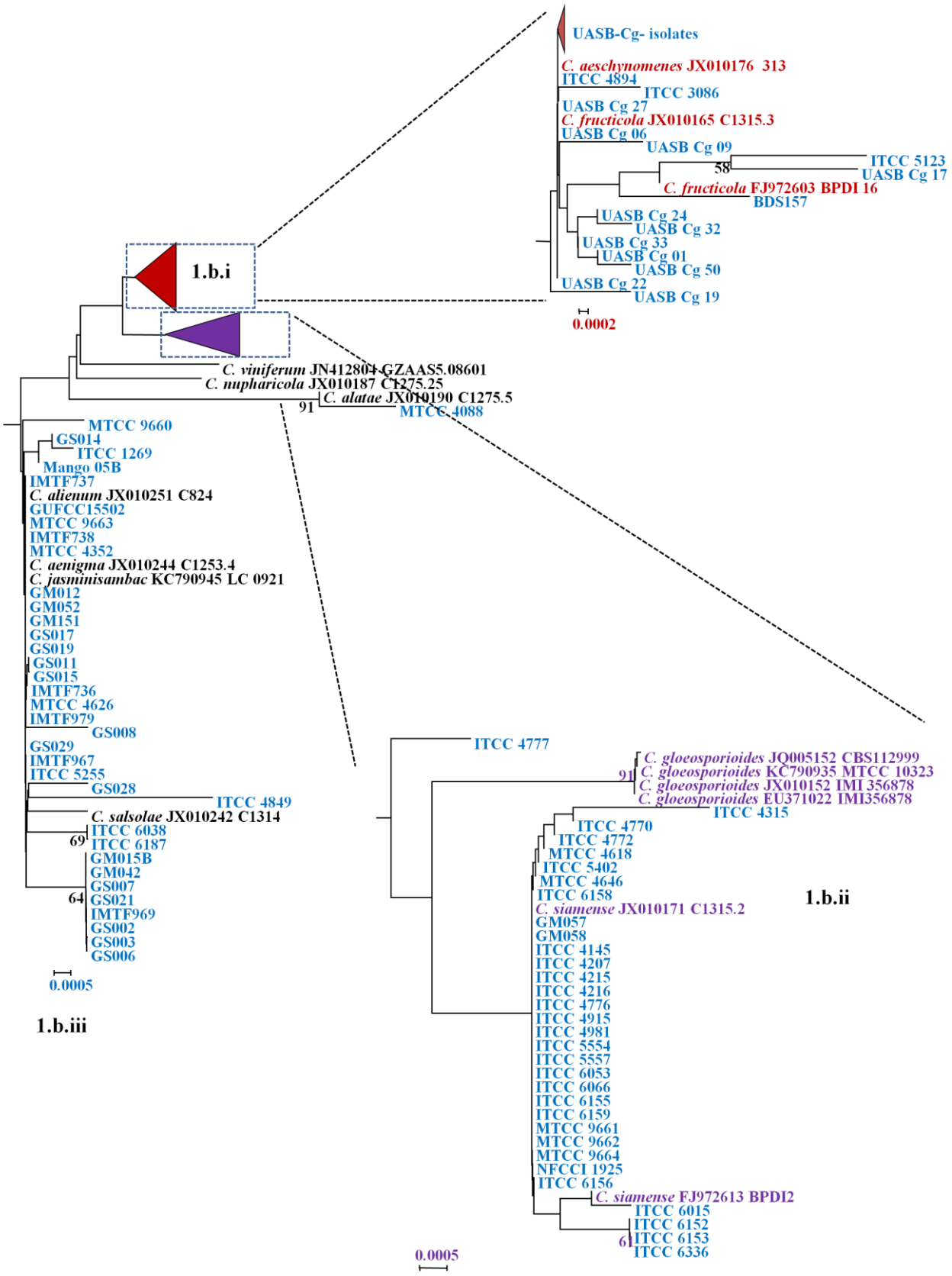
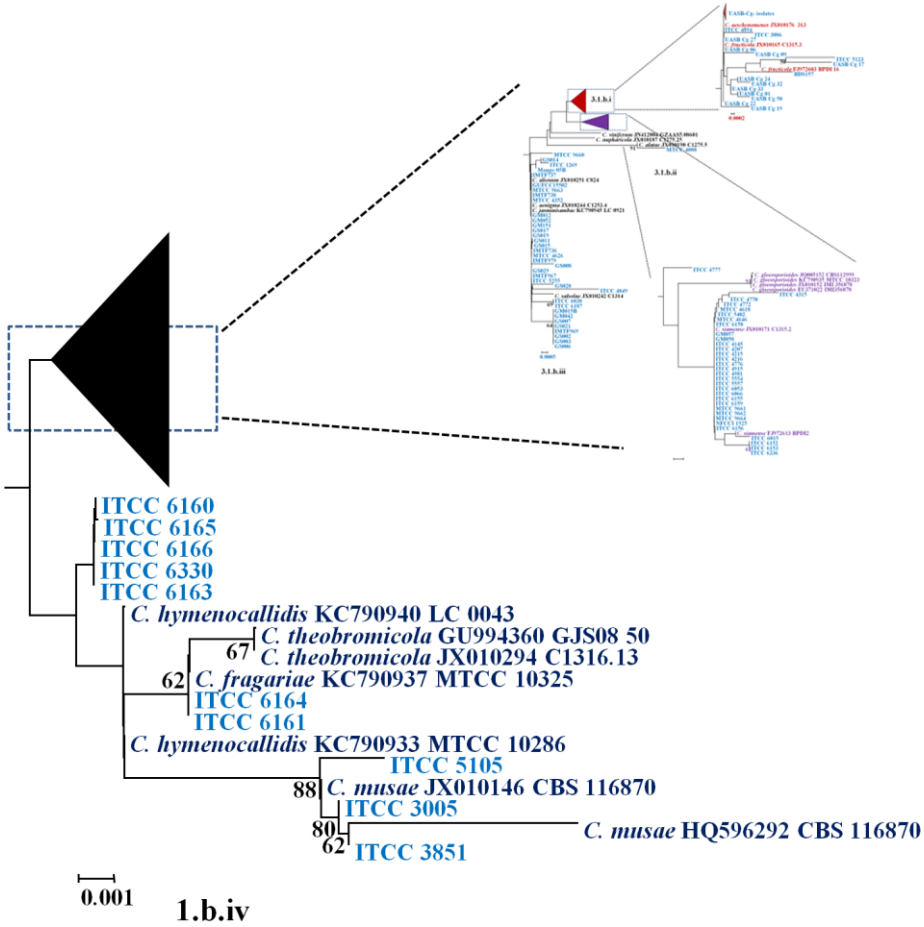


Fig. 1 – (contd.) b. Isolates in clade gloeosporioides (parts i-iii)



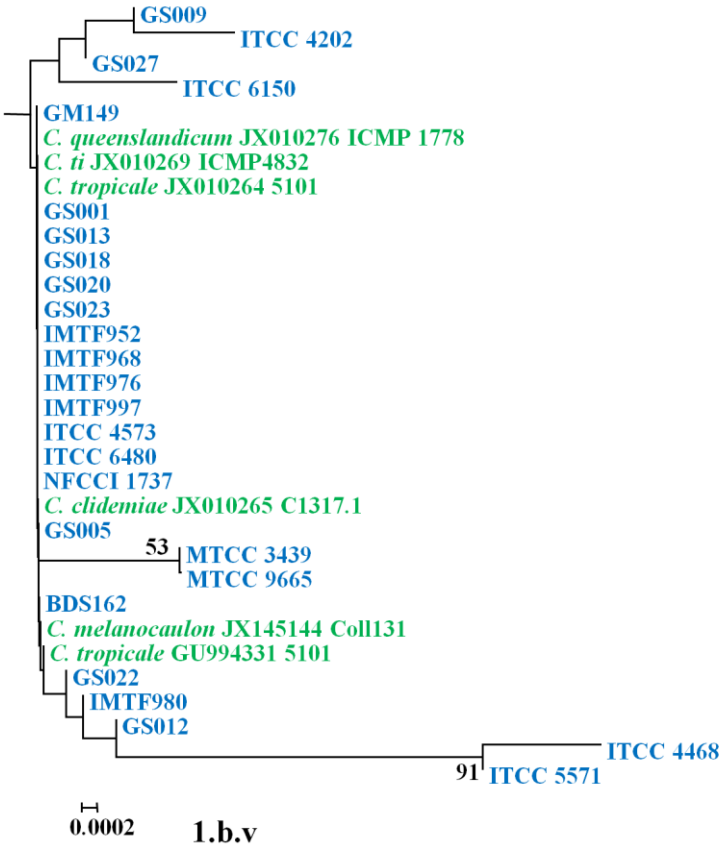


Fig. 1 – **(contd.) b**. Isolates in clade gloeosporioides (parts iv-v)

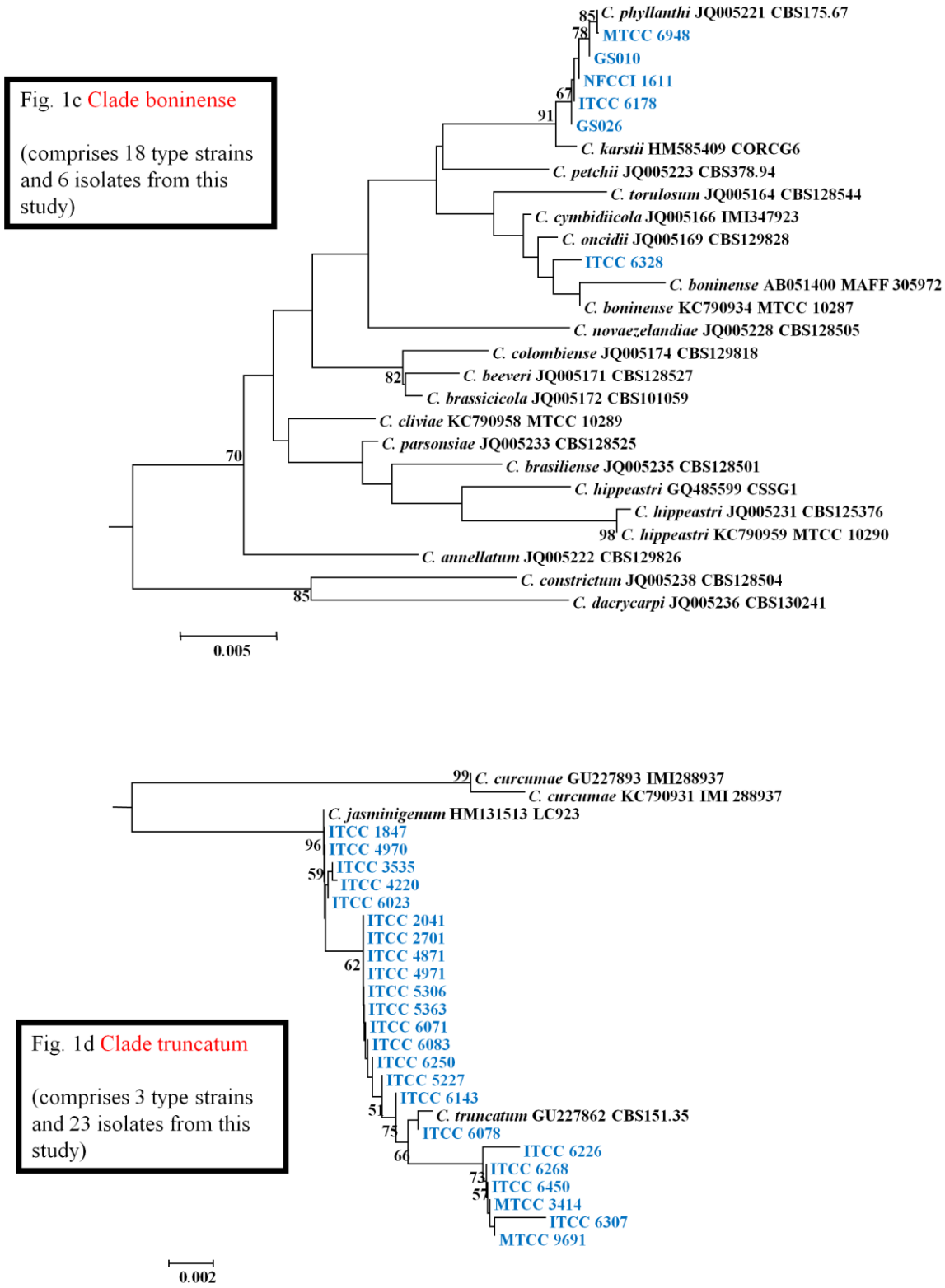


Fig. 1 – (contd.) c. Isolates in clade boninense **d**. Isolates in clade truncatum

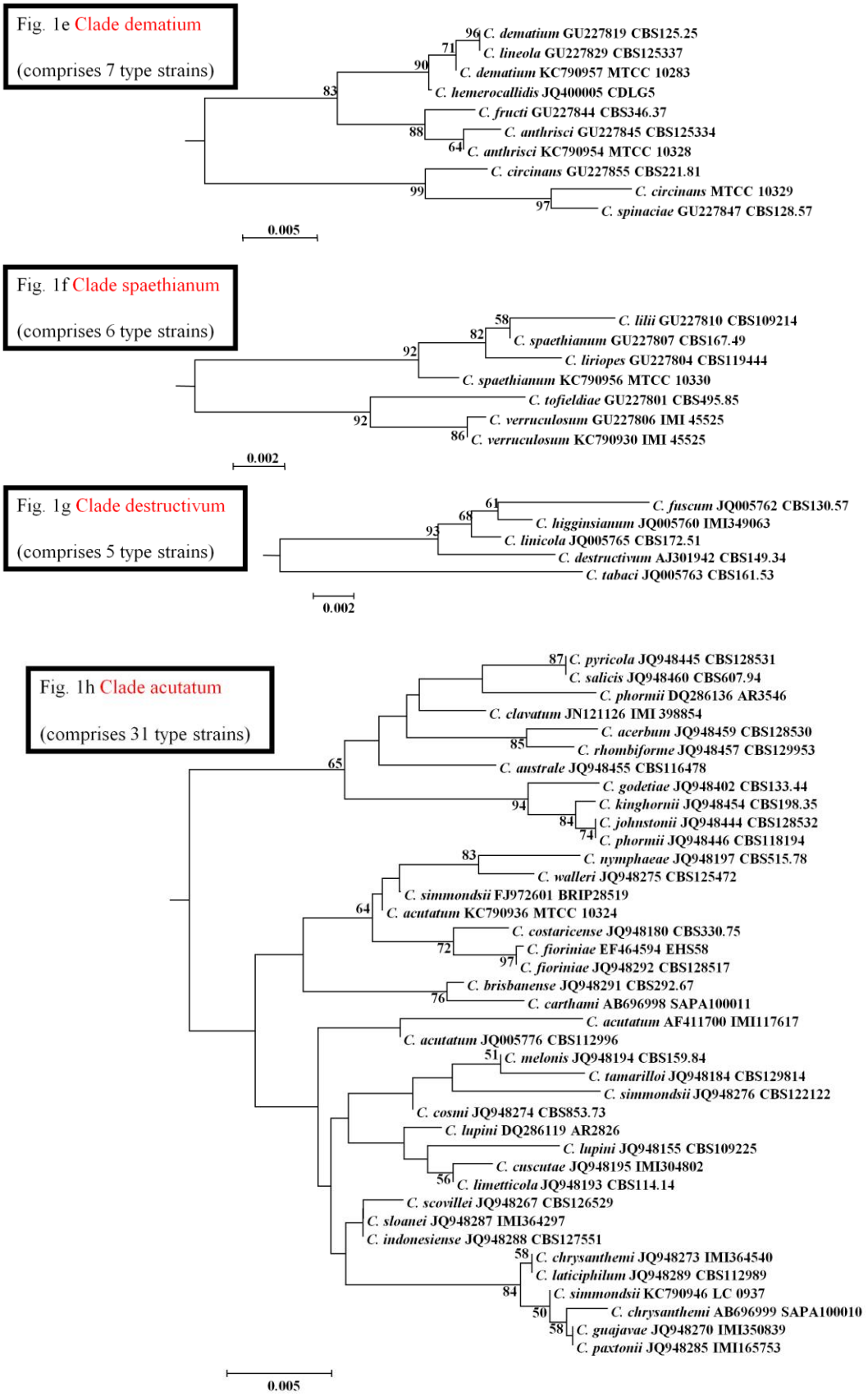


Fig. 1 – (contd.) e. Isolates in clade dematium **f**. Isolates in clade spaethianum **g**. Isolates in clade destructivum **h**. Isolates in clade acutatum

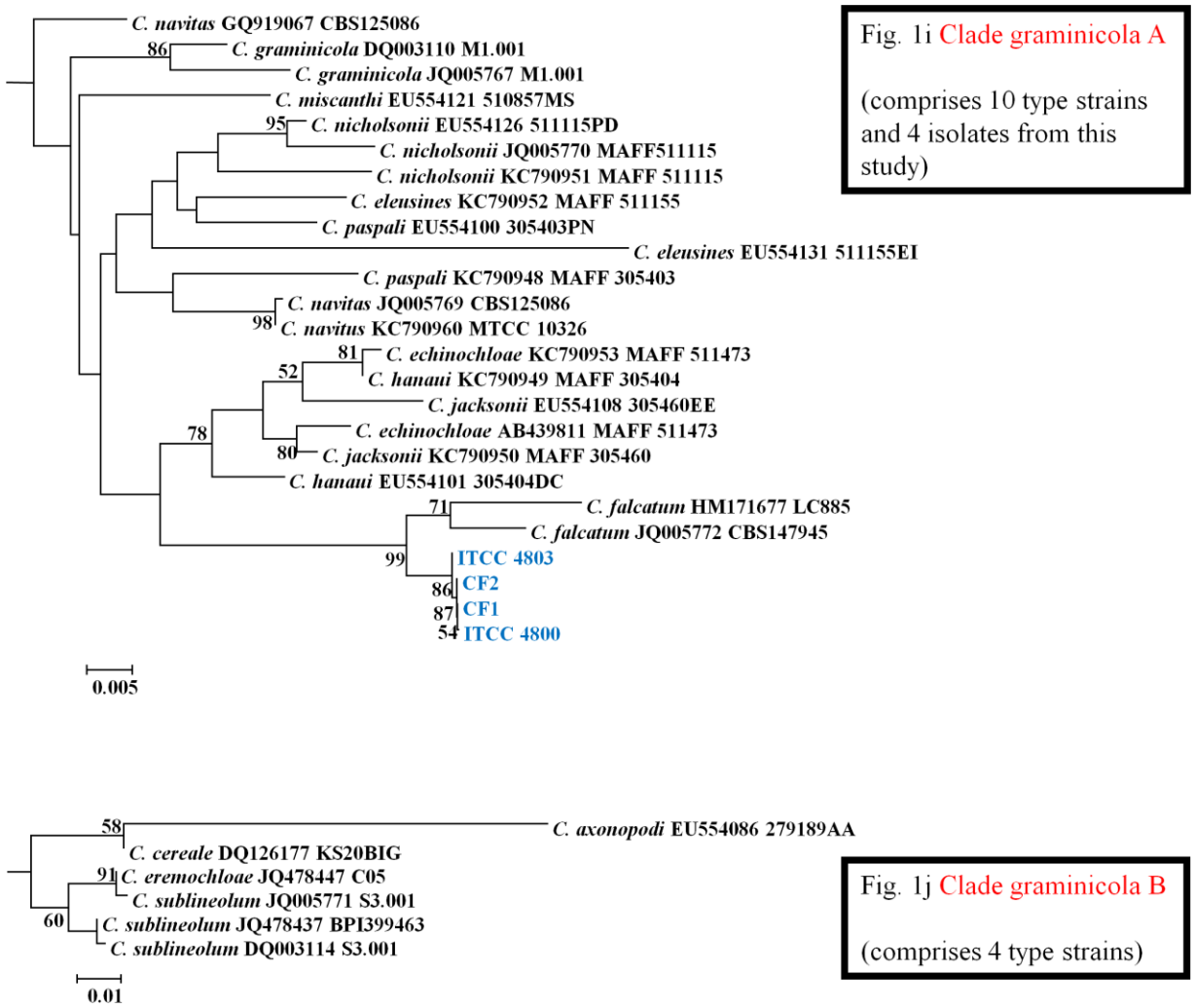


Fig. 1 – **(contd.) i**. Isolates in clade graminicola A **j.** Isolates in clade graminicola B

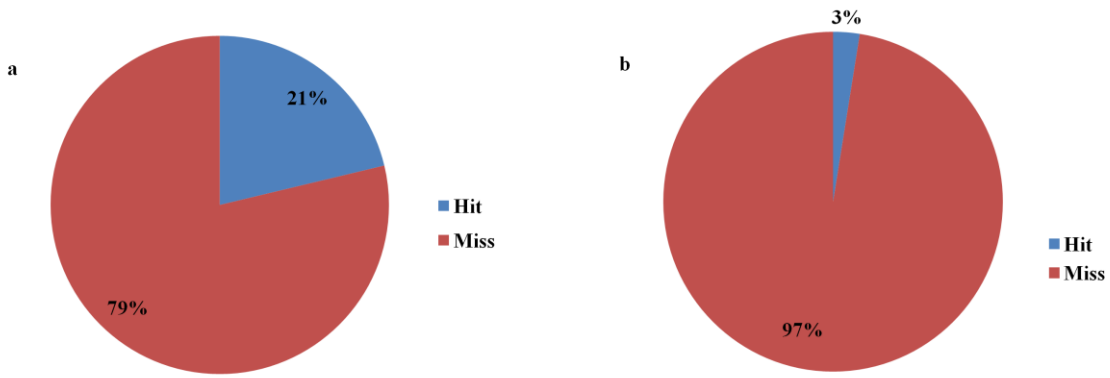


Fig. 2 – Pie chart showing the percentage of correct hits and mismatches in the local BLAST sequence comparison for **a**. the 558 GenBank sequences submitted from India **b**. the 159 isolates procured from Indian culture collection centres

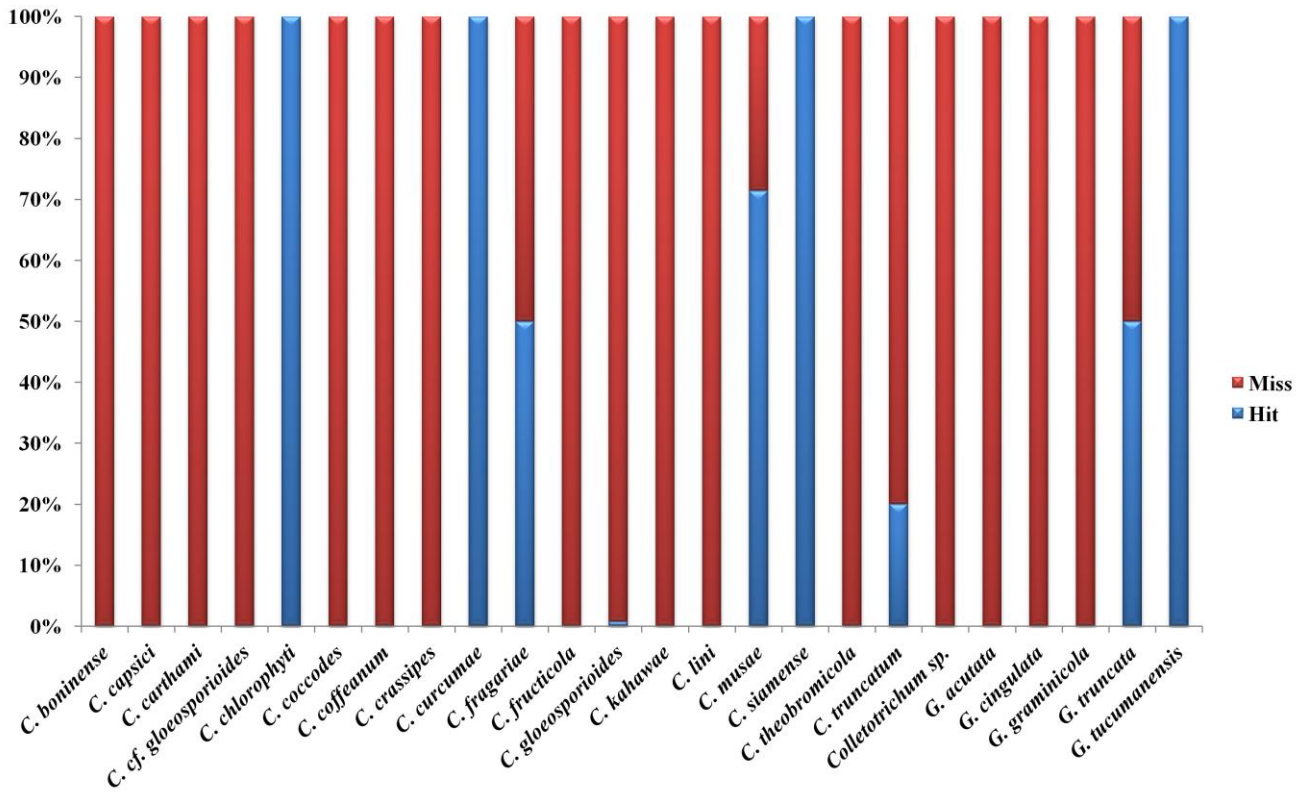


Fig. 3 – Bar diagram showing the percentage of correctly (Hit) or erroneously named (Miss) GenBank sequences submitted from India

Discussion

Accurate identification of economically important plant pathogenic fungi is a major concern at present, as it aids in early and accurate disease diagnosis. Anthracnose diseases caused by *Colletotrichum* species have had a considerable impact on world agricultural production through their capacity to cause economic losses on a number of important cereal, legume, fruit, vegetables and cash crops (Bailey et al. 1992, Waller 1992, Pakdeevaraporn et al. 2005). The fruits and vegetables, infected by anthracnose are not acceptable for export market (Waller 1992). The major bio-security concern regarding plant quarantine also involves accurate species identification. Thus, it becomes necessary to move ahead of traditional approach based on morphology and incorporate modern ways for species recognition.

In the present study we have tried to analyze the suitability of using ITS/ 5.8S rRNA gene region as a marker for precise identification of *Colletotrichum* species from India. Although the ITS/ 5.8S rRNA based identification is a rapid way of preliminary identification of fungal species, but the reliability and accuracy of this method still remains a concern. ITS/ 5.8S rRNA region is rich in indels but its difficulty to align makes its usage in phylogenetic reconstruction difficult (Seifert 2009). In addition to it, ITS as a marker has its own limitation since it is not able to fully resolve many species of *C. gloeosporioides* species complex completely as is reflected in Fig. 1.

There are more than 6300 NCBI-GenBank (Benson et al. 1997, 2006) entries (6396 as on 7th June 2013) for ITS/ 5.8S rRNA gene, which is maximum (~ 29%) among all the gene sequences (21528 total nucleotide entries for the genus) available in NCBI (Ryberg et al. 2009), but majority of them are erroneous and not authentic (Bridge et al. 2003, Nilsson et al. 2006, Cai et al. 2009, Crouch et al. 2009). In a recent study, a high rate of misidentification (86%) was observed while using BLAST similarity search of ITS/ 5.8S rRNA sequences for *C. graminicola* species (Crouch et al. 2009). The reference type sequences have not been considered while analyzing in BLAST, which further leads to accumulation of wrong information in public databases in the longer run.

Table 1 List of the *Colletotrichum* endophytes included in this study with information on taxon, strain designation, host, geographic location, GenBank accession number for the ITS/ 5.8S rRNA sequences and results for the local BLAST sequence similarity search

Strain no.	Host	Geographic location	GenBank acc. no.	Seq. length	Local BLAST result	Score	E-value	Identity
GS01	*Bauhinia variegata*	IMTECH, Chandigarh	JN248668	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
GS02	*B. variegata*	IMTECH, Chandigarh	JN248669	541	KC790945 (*C. jasminisambac*)	1065	0	540/541 (99%)
GS03	*B. variegata*	IMTECH, Chandigarh	JN248670	538	KC790945 (*C. jasminisambac*)	1045	0	537/539 (99%)
GS04	*B. variegata*	IMTECH, Chandigarh	JN248671	556	JX010264 (*C. tropicale*)	1096	0	555/556 (99%)
GS05	*B. variegata*	IMTECH, Chandigarh	JN248672	555	JX010264 (*C. tropicale*)	1086	0	555/556 (99%)
GS06	*Saraca indica*	IMTECH, Chandigarh	JN248673	541	KC790945 (*C. jasminisambac*)	1065	0	540/541 (99%)
GS07	*Bauhinia variegata*	IMTECH, Chandigarh	JN248674	556	KC790945 (*C. jasminisambac*)	1094	0	555/556 (99%)
GS08	*B. variegata*	IMTECH, Chandigarh	JN248675	540	KC790945 (*C. jasminisambac*)	1063	0	539/540 (99%)
GS09	*B. variegata*	IMTECH, Chandigarh	JN248676	536	JX010264 (*C. tropicale*)	1063	0	536/536 (100%)
GS10	*B. variegata*	IMTECH, Chandigarh	JN248677	564	JQ005221 (*C. phyllanthi*)	1100	0	555/555 (100%)
GS11	*B. variegata*	IMTECH, Chandigarh	JN248678	533	KC790945 (*C. jasminisambac*)	1057	0	533/533 (100%)
GS12	*B. variegata*	IMTECH, Chandigarh	JN248679	527	KC790940 (*C. hymenocallidis*)	1037	0	526/527 (99%)
GS13	*Azadirachta indica*	IMTECH, Chandigarh	JN248680	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
GS14	*Ficus elastica*	IMTECH, Chandigarh	JN248681	497	KC790945 (*C. jasminisambac*)	985	0	497/497 (100%)
GS15	*Bauhinia variegata*	IMTECH, Chandigarh	JN248682	530	KC790945 (*C. jasminisambac*)	1051	0	530/530 (100%)
GS17	*Psidium guajava*	IMTECH, Chandigarh	JN248683	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
GS18	*P. guajava*	IMTECH, Chandigarh	JN248684	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
GS19	Unidentified plant	IMTECH, Chandigarh	JN248685	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
GS20	Unidentified plant	IMTECH, Chandigarh	JN248686	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
GS21	Unidentified plant	IMTECH, Chandigarh	JN248687	556	KC790945 (*C. jasminisambac*)	1094	0	555/556 (99%)
GS22	Unidentified plant	IMTECH, Chandigarh	JN248688	531	JX010264 (*C. tropicale*)	1053	0	531/531 (100%)
GS23	*Curcuma longa*	IMTECH, Chandigarh	JN248689	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
GS26	*Bougainvillea* sp.	IMTECH, Chandigarh	JN248690	576	JQ005221 (*C. phyllanthi*)	1100	0	555/555 (100%)
GS27	*Bougainvillea* sp.	IMTECH, Chandigarh	JN248691	537	JX010264 (*C. tropicale*)	1065	0	537/537 (100%)
GS28	Unidentified plant	IMTECH, Chandigarh	JN248692	557	KC790945 (*C. jasminisambac*)	1080	0	555/557 (99%)
GS29	Unidentified plant	IMTECH, Chandigarh	JN248693	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
GS30	Unidentified plant	IMTECH, Chandigarh	JN248694	544	KC790943 (*C. brevisporum*)	1047	0	537/540 (99%)
IMTF 736	*Cassia* sp.	IMTECH, Chandigarh	JN248695	539	KC790945 (*C. jasminisambac*)	1068	0	539/539 (100%)
IMTF 737	*Cassia* sp.	IMTECH, Chandigarh	JN248696	541	KC790945 (*C. jasminisambac*)	1072	0	541/541 (100%)

Strain no.	Host	Geographic location	GenBank acc. no.	Seq. length	Local BLAST result	Score	E-value	Identity
IMTF 738	*Cassia* sp.	IMTECH, Chandigarh	JN248697	548	KC790945 (*C. jasminisambac*)	1086	0	548/548 (100%)
IMTF 952	*Curcuma longa*	IMTECH, Chandigarh	JN248698	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
IMTF 967	Unidentified plant	IMTECH, Chandigarh	JN248699	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
IMTF 968	Unidentified plant	IMTECH, Chandigarh	JN248700	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
IMTF 969	Unidentified plant	IMTECH, Chandigarh	JN248701	556	KC790945 (*C. jasminisambac*)	1094	0	555/556 (99%)
IMTF 976	Unidentified plant	IMTECH, Chandigarh	JN248702	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
IMTF 979	*Psidium guajava*	IMTECH, Chandigarh	JN248703	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
IMTF 980	*P. guajava*	IMTECH, Chandigarh	JN248704	531	JX010264 (*C. tropicale*)	1053	0	531/531 (100%)
IMTF 997	Unidentified plant	IMTECH, Chandigarh	JN248705	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
Mango 5B	*Mangifera indica*	CSIO, Chandigarh	JN248706	534	KC790945 (*C. jasminisambac*)	1043	0	33/534 (99%)
Mango 10	*M. indica*	Sector 40 Market, Chandigarh	JN248707	531	JX010264 (*C. tropicale*)	1017	0	530/533 (99%)
GM 12	*M. indica*	CSIO, Chandigarh	JN248708	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
GM 15B	*M. indica*	CSIO, Chandigarh	JN248709	556	KC790945 (*C. jasminisambac*)	1094	0	555/556 (99%)
GM 21	*M. indica*	Sector 40 Market, Chandigarh	JN248710	549	JX010264 (*C. tropicale*)	1080	0	548/549 (99%)
GM 42	*M. indica*	Malihabad, Lucknow	JN248711	556	KC790945 (*C. jasminisambac*)	1094	0	555/556 (99%)
GM 52	*M. indica*	CISH, Lucknow	JN248712	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
GM 57	*M. indica*	Valsad, Nand Sagar, Gujrat	JN248713	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
GM 58	*M. indica*	Valsad, Nand Sagar, Gujrat	JN248714	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
GM 149	*M. indica*	Navsari, Gujrat	KF214633	557	KC790940 (*C. hymenocallidis*)	1096	0	556/557 (99%)
GM 151	*M. indica*	Navsari, Gujrat	KF214634	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)

In this study, ITS/ 5.8S rRNA region was sequenced for a few type strain sequences including *C. sansevieriae*, whose original sequence (AB212991) was too short and hence not included in the analysis. The newly generated type sequences have been highlighted in bold in Table 3. An updated file comprising the ITS/ 5.8S rRNA gene sequence for the 135 type strains of *Colletotrichum* (Post Cannon et al. 2012) was made and used to validate the identities of the *Colletotrichum* isolates deposited in various culture collection centres across India and the GenBank sequences deposited from India. Based on the NJ tree (Fig. 1) and the local-BLAST analysis done in BioEdit; almost 97 % (155/159) of the isolates procured from various culture collection centres of India were found to be misidentified. Also, about 79% of the GenBank sequences deposited from India were found to be erroneously named. The reason for this could be the fact that, in many Institutes and Universities of India, traditional morphology based approach for species-identification is still more popular. The sequence based identification is yet not popular owing to its high cost.

DNA sequence based species identification could be a recent trend, but based on our study, we are forced to think whether it is judicious to spend so much money on a process which ultimately leads us nowhere but towards a clutter of confusion. Thus, in current scenario ITS/ 5.8S rRNA based species

Table 2 List of the *Colletotrichum* isolates included in this study, procured from different culture collection centres of India; with information on taxon, strain designation, host, geographic location, GenBank accession number for the ITS/ 5.8S rRNA sequences and results for the local BLAST sequence similarity search (*=Taxon name as mentioned in GenBank)

Taxon name*	Strain no.	Host	Geographic location	GenBank acc. no.	Seq. length	Local BLAST result	Score	E-value	Identity
C. falcatum	CF1	*Saccharum* sp.	Coimbatore, Tamil Nadu	JN390936	535	JQ005772 (*C. falcatum*)	1029	0	531/535 (100%)
C. falcatum	CF2	*Saccharum* sp.	Coimbatore, Tamil Nadu	JN390937	535	JQ005772 (*C. falcatum*)	1029	0	531/535 (100%)
Colletotrichum sp.	GUFCC 15502	*Diffenbakia* sp.	unknown	JN390934	557	JX010251 (*C. alienum*)	1104	0	557/557 (100%)
Colletotrichum sp.	GUFCC 15503	*Calamus thwaitesii*	Netravali, Goa	JN390935	528	KC790943 (*C. brevisporum*)	975	0	520/528 (98%)
C. papayae	ITCC 1269	unknown	Delhi	JN390835	543	JX010251 (*C. alienum*)	1066	0	538/538 (100%)
C. truncatum	ITCC 1847	*Cajanus cajan*	unknown	JN390836	562	HM131513 (*C. jasminigenum*)	1114	0	562/562 (100%)
C. capsici	ITCC 2041	*Capsicum annum*	Nagpur, Maharashtra	JN390888	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. capsici	ITCC 2701	*Solanum melongena*	Ujjain, Madhya Pradesh	JN390889	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. gloeosporioides	ITCC 3005	*Cocoa sp.*	Ratnagiri, Maharashtra	JN390890	532	JX010146 (*C. musae*)	1055	0	532/532 (100%)
C. gloeosporioides	ITCC 3508	*Dalbergia*	Delhi	JN390892	557	JX010165 (*C. fructicola*)	1088	0	555/557 (99%)
C. dematium	ITCC 3535	*Gossypium* sp.	Dapoli, Maharashtra	JN390837	557	HM131513 (*C. jasminigenum*)	1104	0	557/557 (100%)
C. gloeosporioides	ITCC 3086	*Cyclamen sp.*	Solan, Himachal Pradesh	JN390891	557	JX010165 (*C. fructicola*)	1096	0	556/557 (99%)
C. gloeosporioides	ITCC 3851	*Pouteria sapota*	Navsari, Gujarat	JN390893	530	JX010146 (*C. musae*)	1051	0	530/530 (100%)
C. gloeosporioides	ITCC 4145	*Gossypium* sp.	Nagpur, Maharashtra	JN390894	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 4202	*Arachis hypogaea*	Udaipur, Rajasthan	JN390895	546	JX010264 (*C. tropicale*)	1078	0	544/544 (100%)
C. gloeosporioides	ITCC 4207	*Allium cepa*	Karnal, Haryana	JN390896	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 4215	*Fern*	Warangal, Andhra Pradesh	JN390897	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 4216	*Ixora*	Warangal, Andhra Pradesh	JN390898	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
Colletotrichum sp.	ITCC 4220	*Abelmoschus esculentus*	New Delhi	JN390838	550	HM131513 (*C. jasminigenum*)	1090	0	550/550 (100%)
C. gloeosporioides	ITCC 4315	-	Nasik, Maharashtra	JN390899	536	JX010171 (*C. siamense*)	1041	0	535/537 (99%)
C. gloeosporioides	ITCC 4468	*Cassia* sp.	Junagarh, Gujrat	JN390901	540	JX010264 (*C. tropicale*)	1013	0	517/519 (99%)
C. gloeosporioides	ITCC 4573	*Solanum melongena*	Siliguri, West Bengal	JN390902	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
C. lindemuthianum	ITCC 4765	*Phaseolus* sp.	unknown	JN390883	533	GQ485607 (*C. cliviae*)	983	0	506/508 (99%)
C. gloeosporioides	ITCC 4770	*Agalonema commutatum*	Kottayam, Kerala	JN390903	508	JX010171 (*C. siamense*)	1007	0	508/508 (100%)
C. gloeosporioides	ITCC 4772	*Begonia saachsen*	Kottayam, Kerala	JN390917	531	JX010171 (*C. siamense*)	1053	0	531/531 (100%)
C. gloeosporioides	ITCC 4776	*Lantana camara*	Kottayam, Kerala	JN390904	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)

Taxon name*	Strain no.	Host	Geographic location	GenBank acc. no.	Seq. length	Local BLAST result	Score	E-value	Identity
C. gloeosporioides	ITCC 4777	*Mussaenda frondasa*	Kottayam, Kerala	JN390905	509	KC790940 (*C. hymenocallidis*)	977	0	506/509 (99%)
C. falcatum	ITCC 4800	Saccharum sp.	Daurala, Uttar Pradesh	JN390906	533	JQ005772 (*C. falcatum*)	1025	0	529/533 (99%)
C. falcatum	ITCC 4803	*Saccharum* sp.	Daurala, Uttar Pradesh	JN390839	543	JQ005772 (*C. falcatum*)	1037	0	535/539 (99%)
C. gloeosporioides	ITCC 4849	*Nerium* sp.	New Delhi	JN390907	557	KC790945 (*C. jasminisambac*)	1080	0	555/557 (99%)
C. capsici	ITCC 4871	*Gomphrena* sp.	Sikkim	JN390840	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. gloeosporioides	ITCC 4894	*Morus alba*	Kalimpong, West Bengal	JN390900	557	JX010165 (*C. fructicola*)	1104	0	557/557 (100%)
C. gloeosporioides	ITCC 4915	*Dracena fragrans*	Lucknow, Uttar Pradesh	JN390908	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. dematium	ITCC 4970	*Passiflora foetida*	Kolkata, West Bengal	JN390841	562	HM131513 (*C. jasminigenum*)	1114	0	562/562 (100%)
C. dematium	ITCC 4971	*Gerbera* sp.	Chlutta, West Bengal	JN390842	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. gloeosporioides	ITCC 4981	*Cocos nucifera*	CPCRI, Kerala	JN390909	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 5105	Soil	Hyderabad, Andhra Pradesh	JN390910	557	JX010146 (*C. musae*)	1096	0	556/557 (99%)
C. gloeosporioides	ITCC 5123	*Psidium guajava*	Allahabad, Uttar Pradesh	JN390843	529	JX010264 (*C. tropicale*)	1021	0	529/531 (99%)
C. gloeosporioides	ITCC 5213	*Cattleya* sp.	Sikkim	JN390844	543	KC790943 (*C. brevisporum*)	989	0	533/543 (98%)
C. capsici	ITCC 5227	*Citrus* sp.	IARI, New Delhi	JN390845	558	HM131513 (*C. jasminigenum*)	1098	0	557/558 (99%)
C. gloeosporioides	ITCC 5255	*Anacardium occidentale*	Dapoli, Maharashtra	JN390846	556	KC790945 (*C. jasminisambac*)	1102	0	556/556 (100%)
C. dematium	ITCC 5306	*Withania somnifera*	Lucknow, Uttar Pradesh	JN390911	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. dematium	ITCC 5363	*Fragaria* sp.	NBPGR, New Delhi	JN390847	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. gloeosporioides	ITCC 5402	*M. indica*	IARI, New Delhi	JN390848	539	JX010171 (*C. siamense*)	1068	0	539/539 (100%)
C. gloeosporioides	ITCC 5514	*Amomum* sp.	Kalimpong, West Bengal	JN390849	549	KC790943 (*C. brevisporum*)	993	0	538/549 (97%)
C. gloeosporioides	ITCC 5554	*Rosa indica*	Kalyani, West Bengal	JN390850	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 5557	*Vanilla* sp.	unknown	JN390851	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 5571	*Elaeis* sp.	Padavegi, Andra Pradesh	JN390852	515	JX010264 (*C. tropicale*)	1005	0	513/515 (99%)
C. gloeosporioides	ITCC 6015	*Zamia neurophyllidia*	Chennai, Tamil Nadu	JN390853	551	JX010171 (*C. siamense*)	1076	0	550/551 (99%)
C. capsici	ITCC 6023	*Polianthes tuberose*	IARI, New Delhi	JN390854	562	HM131513 (*C. jasminigenum*)	1114	0	562/562 (100%)
G. tucumanensis	ITCC 6032	*Saccharum* sp.	Navasari, Gujarat	JN390913	535	GQ485607 (*C. cliviae*)	983	0	506/508 (99%)
C. gloeosporioides	ITCC 6038	*Rosa* sp.	Navsari, Gujarat	JN390884	550	KC790945 (*C. jasminisambac*)	1082	0	549/550 (99%)
C. gloeosporioides	ITCC 6053	*Elaeis* sp.	Padavegi, Andra Pradesh	JN390855	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 6066	*Cocos nucifera*	Bhuvaneshwar, Orissa	JN390914	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)

Taxon name*	Strain no.	Host	Geographic location	GenBank acc. no.	Seq. length	Local BLAST result	Score	E-value	Identity
C. capsici	ITCC 6071	*Capsicum* sp.	Jammu, Jammu & Kashmir	JN390856	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. capsici	ITCC 6078	*Capsicum* sp.	Jammu, Jammu & Kashmir	JN390885	535	HM131513 (*C. jasminigenum*)	1053	0	534/535 (99%)
C. gloeosporioides	ITCC 6079	*Capsicum* sp.	Solan, Himachal Pradesh	JN390857	558	HM171679 (*C. coccodes*)	1106	0	558/558 (100%)
C. capsici	ITCC 6083	*Capsicum* sp.	Jammu, Jammu & Kashmir	JN390858	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. capsici	ITCC 6143	*Raphanus sativus*	IARI, New Delhi	JN390859	546	HM131513 (*C. jasminigenum*)	1074	0	545/546 (99%)
C. gloeosporioides	ITCC 6150	*Areca catechu*	Rahuri, Maharashtra	JN390860	543	JX010264 (*C. tropicale*)	1068	0	542/543 (99%)
C. gloeosporioides	ITCC 6152	*Citrus* sp.	Rahuri, Maharashtra	JN390861	556	JX010171 (*C. siamense*)	1094	0	555/556 (99%)
C. gloeosporioides	ITCC 6153	*Citrus* sp.	Rahuri, Maharashtra	JN390862	555	JX010171 (*C. siamense*)	1086	0	555/556 (99%)
C. gloeosporioides	ITCC 6155	*Citrus* sp.	Rahuri, Maharashtra	JN390863	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 6156	*Mangifera indica*	Rahuri, Maharashtra	JN390864	557	JX010171 (*C. siamense*)	1088	0	556/557 (99%)
C. gloeosporioides	ITCC 6158	*Mangifera indica*	Rahuri, Maharashtra	JN390915	548	JX010171 (*C. siamense*)	1086	0	548/548 (100%)
C. gloeosporioides	ITCC 6159	*Psidium guajava*	Rahuri, Maharashtra	JN390865	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	ITCC 6160	*Punica granatum*	Rahuri, Maharashtra	JN390866	558	KC790945 (*C. jasminisambac*)	1066	0	555/558 (99%)
C. gloeosporioides	ITCC 6161	*P. granatum*	Rahuri, Maharashtra	JN390867	559	KC790937 (*C. fragariae*)	1104	0	557/557 (100%)
C. gloeosporioides	ITCC 6163	*Punica granatum*	Rahuri, Maharashtra	JN390868	558	KC790945 (*C. jasminisambac*)	1066	0	555/558 (99%)
C. gloeosporioides	ITCC 6164	*Punica granatum*	Rahuri, Maharashtra	JN390869	558	KC790937 (*C. fragariae*)	1088	0	556/557 (99%)
C. gloeosporioides	ITCC 6165	*P. granatum*	Rahuri, Maharashtra	JN390870	529	KC790945 (*C. jasminisambac*)	1025	0	527/529 (99%)
C. gloeosporioides	ITCC 6166	*Punica granatum*	Rahuri, Maharashtra	JN390871	557	KC790945 (*C. jasminisambac*)	1080	0	555/557 (99%)
C. gloeosporioides	ITCC 6178	*Passiflora edulis*	Mizoram	JN390872	576	JQ005221 (*C. phyllanthi*)	1100	0	555/555 (100%)
C. gloeosporioides	ITCC 6187	*Ricinus communis*	Navsari, Gujarat	JN390873	556	KC790945 (*C. jasminisambac*)	1094	0	555/556 (99%)
G. cingulata	ITCC 6028	Castor	Berhampur, Orissa	JN390912	535	GQ485607 (*C. cliviae*)	975	0	505/508 (99%)
C. dematium	ITCC 6226	unknown	unknown	JN390886	562	HM131513 (*C. jasminigenum*)	1098	0	560/562 (99%)
C. capsici	ITCC 6250	*Capsicum* sp.	Jaipur, Rajasthan	JN390874	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. capsici	ITCC 6268	*Curcuma longa*	Anand, Gujarat	JN390875	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. gloeosporioides	ITCC 6270	*Capsicum* sp.	Port Blair, Andaman Island	JN390876	534	JX010165 (*C. fructicola*)	1059	0	534/534 (100%)

Taxon name*	Strain no.	Host	Geographic location	GenBank acc. no.	Seq. length	Local BLAST result	Score	E-value	Identity
C. gloeosporioides	ITCC 6271	Bean	Port Blair, Andaman Island	JN390916	559	KC790943 (*C. brevisporum*)	1076	0	555/559 (99%)
C. gloeosporioides	ITCC 6272	*Lagenaria* sp.	Port Blair, Andaman Island	JN390877	545	KC790943 (*C. brevisporum*)	1037	0	532/535 (99%)
C. capsici	ITCC 6307	*Dioscorea alata*	Navsari, Gujarat	JN390878	543	HM131513 (*C. jasminigenum*)	1068	0	542/543 (99%)
C. gloeosporioides	ITCC 6328	*Cymbidium* sp.	Sikkim	JN390879	561	JQ005166 (*C. cymbidiicola*)	1098	0	554/554 (100%)
C. gloeosporioides	ITCC 6330	*Mangifera indica*	unknown	JN390880	558	KC790945 (*C. jasminisambac*)	1066	0	555/558 (99%)
C. gloeosporioides	ITCC 6336	*Citrus* sp.	Jobner, Rajasthan	JN390881	550	JX010171 (*C. siamense*)	1061	0	549/551 (99%)
C. capsici	ITCC 6450	*Vigna mungo*	Navsari, Gujarat	JN390882	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. gloeosporioides	ITCC 6480	unknown	unknown	JN390887	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
C. crassipes	MTCC 2175	*Citrus jambher*	Haryana	JN390918	500	JN050242 *(C. thailandicum)*	765	0	403/406 (99%)
C. capsici	MTCC 3414	*Capsicum annum*	Pondicherry	JN390919	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. gloeosporioides	MTCC 3439	*Capsicum annum*	Pondicherry	JN390920	550	JX010264 (*C. tropicale*)	1082	0	549/550 (99%)
C. gloeosporioides	MTCC 4088	*Dioscorea alata*	Udaipur, Rajasthan	JN390921	513	JX010190 (*C. alatae*)	1003	0	513/514 (99%)
C. musae	MTCC 4352	*Avicennia marina*	Pichavaram, Tamil Nadu	JN390922	548	KC790945 (*C. jasminisambac*)	1086	0	548/548 (100%)
C. gloeosporioides	MTCC 4618	*Mangifera indica*	IMTECH , Chandigarh	JN390923	539	JX010171 (*C. siamense*)	1068	0	539/539 (100%)
C. gloeosporioides	MTCC 4626	*Psidium guajava*	IMTECH , Chandigarh	JN390924	539	KC790945 (*C. jasminisambac*)	1068	0	539/539 (100%)
C. gloeosporioides	MTCC 4646	Soil	IMTECH , Chandigarh	JN390925	541	JX010171 (*C. siamense*)	1072	0	541/541 (100%)
C. gloeosporioides	MTCC 6948	*Carica papaya*	Rehman Khera, Uttar Pradesh	JN390926	555	JQ005221 (*C. phyllanthi*)	1078	0	544/544 (100%)
C. gloeosporioides	MTCC 9660	*Mangifera indica*	Vasco Market, Goa	JN390928	556	JX010171 (*C. alienum*)	1088	0	556/557 (99%)
C. gloeosporioides	MTCC 9661	*M. indica*	Merces-Bardez, Goa	JN390929	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	MTCC 9662	*M. indica*	Vasco Market, Goa	JN390930	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	MTCC 9663	*Psidium guajava*	Kurti-Pondo, Goa	JN390931	557	JX010171 (*C. alienum*)	1104	0	557/557 (100%)
C. gloeosporioides	MTCC 9664	*Carica papaya*	Panjim Market, Goa	JN390932	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	MTCC 9665	*Murraya* sp.	Mysore, Karnataka	JN390933	556	JX010264 (*C. tropicale*)	1094	0	555/556 (99%)
C. capsici	MTCC 9691	*Capsicum annum*	Kharar, Punjab	JN390927	562	HM131513 (*C. jasminigenum*)	1106	0	561/562 (99%)
C. gloeosporioides	NFCCI 1611	unknown	unknown	JN390940	576	JQ005221 (*C. phyllanthi*)	1100	0	555/555 (100%)
Colletotrichum sp.	NFCCI 1737	unknown	unknown	JN390941	556	JX010264 (*C. tropicale*)	1102	0	556/556 (100%)
Colletotrichum sp.	NFCCI 1925	unknown	unknown	JN390942	556	JX010171 (*C. siamense*)	1102	0	556/556 (100%)
C. gloeosporioides	UASB-Cg-01	*Capsicum* sp.	Kolar, Karnataka	JN248618	496	JX010165 (*C. fructicola*)	983	0	496/496 (100%)

Taxon name*	Strain no.	Host	Geographic location	GenBank acc. no.	Seq. length	Local BLAST result	Score	E-value	Identity
C. gloeosporioides	UASB-Cg-02	*Capsicum* sp.	Tumkur, Karnataka	JN248619	492	JX010165 (*C. fructicola*)	975	0	492/492 (100%)
C. gloeosporioides	UASB-Cg-03	*Capsicum* sp.	Raichur, Karnataka	JN248620	525	JX010165 (*C. fructicola*)	1035	0	524/525 (99%)
C. gloeosporioides	UASB-Cg-04	*Capsicum* sp.	Raichur, Karnataka	JN248621	557	JX010165 (*C. fructicola*)	1096	0	556/557 (99%)
C. gloeosporioides	UASB-Cg-05	*Capsicum* sp.	Raichur, Karnataka	JN248622	531	JX010165 (*C. fructicola*)	1037	0	530/531 (99%)
C. gloeosporioides	UASB-Cg-06	*Capsicum* sp.	Gulbarga, Karnataka	JN248623	557	JX010165 (*C. fructicola*)	1104	0	557/557 (100%)
C. gloeosporioides	UASB-Cg-07	*Capsicum* sp.	Gulbarga, Karnataka	JN248624	530	JX010165 (*C. fructicola*)	1051	0	530/530 (100%)
C. gloeosporioides	UASB-Cg-08	*Capsicum* sp.	Gulbarga, Karnataka	JN248625	530	JX010165 (*C. fructicola*)	1051	0	530/530 (100%)
C. gloeosporioides	UASB-Cg-09	*Capsicum* sp.	Bellary, Karnataka	JN248626	526	JX010165 (*C. fructicola*)	1029	0	526/527 (99%)
C. gloeosporioides	UASB-Cg-10	*Capsicum* sp.	Nellore, Andhra Pradesh	JN248627	529	JX010165 (*C. fructicola*)	1049	0	529/529 (100%)
C. gloeosporioides	UASB-Cg-11	*Capsicum* sp.	Nellore, Andhra Pradesh	JN248628	549	JX010165 (*C. fructicola*)	1088	0	549/549 (100%)
C. gloeosporioides	UASB-Cg-12	*Capsicum* sp.	Nellore, Andhra Pradesh	JN248629	527	JX010165 (*C. fructicola*)	1031	0	527/528 (99%)
C. gloeosporioides	UASB-Cg-13	*Capsicum* sp.	Nellore, Andhra Pradesh	JN248630	528	JX010165 (*C. fructicola*)	1047	0	528/528 (100%)
C. gloeosporioides	UASB-Cg-14	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248631	540	JX010165 (*C. fructicola*)	1070	0	540/540 (100%)
C. gloeosporioides	UASB-Cg-15	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248632	532	JX010165 (*C. fructicola*)	1023	0	530/532 (99%)
C. gloeosporioides	UASB-Cg-16	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248633	532	JX010165 (*C. fructicola*)	1055	0	532/532 (100%)
C. gloeosporioides	UASB-Cg-17	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248634	524	JX010165 (*C. fructicola*)	1015	0	519/520 (99%)
C. gloeosporioides	UASB-Cg-18	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248635	557	JX010165 (*C. fructicola*)	1096	0	556/557 (99%)
C. gloeosporioides	UASB-Cg-19	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248636	521	JX010165 (*C. fructicola*)	1011	0	520/522 (99%)
C. gloeosporioides	UASB-Cg-20	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248637	557	JX010165 (*C. fructicola*)	1096	0	556/557 (99%)
C. gloeosporioides	UASB-Cg-21	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248638	530	JX010165 (*C. fructicola*)	1051	0	530/530 (100%)
C. gloeosporioides	UASB-Cg-22	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248639	557	JX010165 (*C. fructicola*)	1104	0	557/557 (100%)
C. gloeosporioides	UASB-Cg-23	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248640	546	JX010165 (*C. fructicola*)	1082	0	546/546 (100%)
C. gloeosporioides	UASB-Cg-24	*Capsicum* sp.	Guntur, Andhra Pradesh	JN248641	507	JX010165 (*C. fructicola*)	1005	0	507/507 (100%)
C. gloeosporioides	UASB-Cg-25	*Capsicum* sp.	Prakasham, Andhra Pradesh	JN248642	554	JX010165 (*C. fructicola*)	1098	0	554/554 (100%)

Taxon name*	Strain no.	Host	Geographic location	GenBank acc. no.	Seq. length	Local BLAST result	Score	E-value	Identity
C. gloeosporioides	UASB-Cg-26	*Capsicum* sp.	Prakasham, Andhra Pradesh	JN248643	555	JX010165 (*C. fructicola*)	1100	0	555/555 (100%)
C. gloeosporioides	UASB-Cg-27	*Capsicum* sp.	Kammam, Andhra Pradesh	JN248644	557	JX010165 (*C. fructicola*)	1104	0	557/557 (100%)
C. gloeosporioides	UASB-Cg-28	*Capsicum* sp.	Kammam, Andhra Pradesh	JN248645	555	JX010165 (*C. fructicola*)	1100	0	555/555 (100%)
C. gloeosporioides	UASB-Cg-29	*Capsicum* sp.	Kammam, Andhra Pradesh	JN248646	555	JX010165 (*C. fructicola*)	1100	0	555/555 (100%)
C. gloeosporioides	UASB-Cg-30	*Capsicum* sp.	Kammam, Andhra Pradesh	JN248647	555	JX010165 (*C. fructicola*)	1100	0	555/555 (100%)
C. gloeosporioides	UASB-Cg-31	*Capsicum* sp.	Kammam, Andhra Pradesh	JN248648	555	JX010165 (*C. fructicola*)	1100	0	555/555 (100%)
C. gloeosporioides	UASB-Cg-32	*Capsicum* sp.	Kammam, Andhra Pradesh	JN248649	552	JX010165 (*C. fructicola*)	1074	0	542/542 (100%)
C. gloeosporioides	UASB-Cg-33	*Capsicum* sp.	Kammam, Andhra Pradesh	JN248650	527	JX010165 (*C. fructicola*)	1025	0	526/528 (99%)
C. gloeosporioides	UASB-Cg-34	*Capsicum* sp.	Gadag, Karnataka	JN248651	555	JX010165 (*C. fructicola*)	1100	0	555/555 (100%)
C. gloeosporioides	UASB-Cg-35	*Capsicum* sp.	Dharwad, Karnataka	JN248652	557	JX010165 (*C. fructicola*)	1096	0	556/557 (99%)
C. gloeosporioides	UASB-Cg-36	*Capsicum* sp.	Dharwad, Karnataka	JN248653	549	JX010165 (*C. fructicola*)	1068	0	546/547 (99%)
C. gloeosporioides	UASB-Cg-37	*Capsicum* sp.	Dharwad, Karnataka	JN248654	555	JX010165 (*C. fructicola*)	1100	0	555/555 (100%)
C. gloeosporioides	UASB-Cg-38	*Capsicum* sp.	Dharwad, Karnataka	JN248655	549	JX010165 (*C. fructicola*)	1088	0	549/549 (100%)
C. gloeosporioides	UASB-Cg-39	*Capsicum* sp.	Dharwad, Karnataka	JN248656	551	JX010165 (*C. fructicola*)	1092	0	551/551 (100%)
C. gloeosporioides	UASB-Cg-40	*Capsicum* sp.	Dharwad, Karnataka	JN248657	530	JX010165 (*C. fructicola*)	1051	0	530/530 (100%)
C. gloeosporioides	UASB-Cg-41	*Capsicum* sp.	Dharwad, Karnataka	JN248658	540	JX010165 (*C. fructicola*)	1043	0	537/540 (99%)
C. gloeosporioides	UASB-Cg-42	*Capsicum* sp.	Kolar, Karnataka	JN248659	540	JX010165 (*C. fructicola*)	1070	0	540/540 (100%)
C. gloeosporioides	UASB-Cg-43	*Capsicum* sp.	Belgaum, Karnataka	JN248660	539	JX010165 (*C. fructicola*)	1068	0	539/539 (100%)
C. gloeosporioides	UASB-Cg-44	*Capsicum* sp.	Belgaum, Karnataka	JN248661	539	JX010165 (*C. fructicola*)	1068	0	539/539 (100%)
C. gloeosporioides	UASB-Cg-45	*Capsicum* sp.	Belgaum, Karnataka	JN248662	545	JX010165 (*C. fructicola*)	1080	0	545/545 (100%)
C. gloeosporioides	UASB-Cg-46	*Capsicum* sp.	Chikkamaglur, Karnataka	JN248663	539	JX010165 (*C. fructicola*)	1068	0	539/539 (100%)
C. gloeosporioides	UASB-Cg-47	*Capsicum* sp.	Chikkamaglur, Karnataka	JN248664	537	JX010165 (*C. fructicola*)	1065	0	537/537 (100%)
C. gloeosporioides	UASB-Cg-48	*Capsicum* sp.	Chikkamaglur, Karnataka	JN248665	538	JX010165 (*C. fructicola*)	1066	0	538/538 (100%)
C. gloeosporioides	UASB-Cg-49	*Capsicum* sp.	Bengaluru, Karnataka	JN248666	552	JX010165 (*C. fructicola*)	1094	0	552/552 (100%)
C. gloeosporioides	UASB-Cg-50	*Capsicum* sp.	Hassan, Karnataka	JN248667	553	JX010165 (*C. fructicola*)	1082	0	548/549 (99%)
C. gloeosporioides	BDS 157			JN390938	556	JX010165 (*C. fructicola*)	1066	0	553/557 (99%)
C. kahawae	BDS 162			JN390939	553	JX010264 (*C. tropicale*)	1096	0	553/553 (100%)

Note: **GUFCC** = Goa University, Fungal Culture Collection, Goa; **ITCC** = Indian Type Culture Collection, IARI, New Delhi; **MTCC** = Microbial Type Culture Collection and Gene Bank, CSIR-IMTECH, Chandigarh; **UASB** = University of Agricultural Sciences, Bengaluru.

Table 3 List of the *Colletotrichum* type strains included in this study with information on taxon, strain designation, and GenBank accession number for the ITS/ 5.8S rRNA sequences

Taxon	GenBank acc. no.	Strain no.
C. acerbum	JQ948459	CBS 128530
C. acutatum	JQ005776, AF411700, **KC790936**	CBS 112996, IMI 117617, MTCC 10324
C. aenigma	JX010244	ICMP 18608
C. aeschynomenes	JX010176	ICMP 17673
C. agaves	DQ286221	CBS 118190
C. alatae	JX010190	ICMP 17919
C. alienum	JX010251	ICMP 12071
C. annellatum	JQ005222	CBS 129826
C. anthrisci	GU227845, **KC790954**	CBS 125334, MTCC 10328
C. aotearoa	JX010205	ICMP 18537
C. asianum	FJ972612, **KC790939**	CBS 130418, LC0037
C. australe	JQ948455	CBS 116478
C. axonopodi	EU554086	IMI 279189
C. beeveri	JQ005171	CBS 128527
C. bletillum	JX625178*	CGMCC 3.15117
C. boninense	AB051400, **KC790934**	MAFF 305972, MTCC 10287
C. brasiliense	JQ005235	CBS 128501
C. brassicicola	JQ005172	CBS 101059
C. brevisporum	**KC790943**, JN050238, JQ247623	LC0600, BCC 38876, GZAAS 5.09545
C. brisbanense	JQ948291	CBS 292.67
C. carthami	AB696998	SAPA100011
C. caudasporum	JX625162*	CGMCC 3.15106
C. cereale	DQ126177	KS-20BIG
C. chlorophyti	GU227894	IMI 103806
C. chrysanthemi	JQ948273, AB696999	IMI 364540, SAPA100010
C. circinans	GU227855, **KC790955**	CBS 221.81, MTCC 10329
C. citri	KC293581*	CGMCC3.15228
C. citricola	KC293576*	CBS 134228
C. clavatum = C. godetiae	JN121126	IMI 398854
C. clidemiae	JX010265	ICMP 18658
C. cliviae	GQ485607,**KC790958**	CBS 125375, MTCC 10289
C. coccodes	HM171679	CBS 369.75
C. colombiense	JQ005174	CBS 129818
C. constrictum	JQ005238	CBS 128504
C. cordylinicola	JX010226, HM470246	ICMP 18579,MFLUCC090551
C. cosmi	JQ948274	CBS 853.73
C. costaricense	JQ948180	CBS 330.75
C. curcumae	GU227893, **KC790931**	IMI 288937
C. cuscutae	JQ948195	IMI 304802
C. cymbidiicola	JQ005166	IMI 347923
C. dacrycarpi	JQ005236	CBS 130241
C. dematium	GU227819, **KC790957**	CBS 125.25, MTCC 10283
C. destructivum	AJ301942	CBS 149.34
C. dianesei (= *C. siamense*)	KC329805*	MFLU 1300062
C. dracaenophilum	DQ286209	CBS 118199
C. duyunensis	JX625160*	CGMCC 3.15105
C. echinochloae	AB439811, **KC790953**	MAFF 511473
C. eleusines	EU554131, **KC790952**	MAFF 511155
C. endophytica	KC633854*	LC0324
C. endophytum	JX625177*	CGMCC 3.15108
C. eremochloae	JQ478447	CBS 129661
C. excelsum-altitudum	HM751815	CGMCC 3.15130
C. falcatum	JQ005772, HM171677	CBS 147945, CGMCC 3.14187
C. fioriniae	JQ948292, EF464594	CBS 128517, EHS58
C. fragariae s. s. (= *C. theobromicola*)	**KC790937**	MTCC 10325
C. fructi	GU227844	CBS 346.37
C. fructicola	FJ972603, JX010165	BPDI16, CBS 130416
C. fructivorum	JX145145	CBS 133125
C. fuscum	JQ005762	CBS 130.57
C. gigasporum	AM982797	MUCL 44947
C. gloeosporioides	JQ005152, JX010152, EU371022, **KC790935**	CBS 112999, IMI 356878, MTCC 10323
C. godetiae	JQ948402	CBS 133.44
C. graminicola	JQ005767, DQ003110	CBS 130836, M1.001

Taxon	GenBank acc. no.	Strain no.
C. grevilleae	KC297078*	CBS 132879
C. guajavae	JQ948270	IMI 350839
C. guizhouensis	JX625158*	CGMCC 3.15112
C. hanaui	EU554101, **KC790949**	MAFF350404
C. hemerocallidis	JQ400005	CDLG5
C. higginsianum	JQ005760	IMI 349063
C. hippeastri	JQ005231, GQ485599, **KC790959**	CBS 125376, CSSG1, MTCC 10290
C. horii	GQ329690, **KC790929**	ICMP 10492
C. hymenocallidis s. s. (= *C. siamense*)	**KC790940, KC790933**	LC0043, MTCC 10286
C. indonesiense	JQ948288	CBS 127551
C. jacksonii	EU554108, **KC790950**	MAFF 305460
C. jasminigenum	HM131513	CGMCC LLTX-01
C. jasmini-sambac s. s. (= *C. siamense*)	**KC790945**	LC0921
C. johnstonii	JQ948444	CBS 128532
C. kahawae subsp. *kahawae*	GU174550, JX010231, **KC790932**	ICMP 17816, IMI 319418
C. karstii	HM585409	CBS 132134
C. kinghornii	JQ948454	CBS 198.35
C. laticiphilum	JQ948289	CBS 112989
C. lilii	GU227810	CBS 109214
C. limetticola	JQ948193	CBS 114.14
C. lindemuthianum	JQ005779	CBS 144.31
C. lineola	GU227829	CBS 125337
C. linicola	JQ005765	CBS 172.51
C. liriopes	GU227804	CBS 119444
C. lupini	DQ286119, JQ948155	BBA 70884, CBS 109225
C. malvarum	not available*	LW1
C. melanocaulon	JX145144	CBS 133251
C. melonis	JQ948194	CBS 159.84
C. miscanthi	EU554121	MAFF 510857
C. murrayae (= *C. siamense*)	JQ247633	GZAAS5.09506
C. musae	HQ596292, JX010146	CBS 116870
C. navitas	GQ919067, JQ005769, **KC790960**	CBS 125086, MTCC 10326
C. nicholsonii	EU554126, JQ005770, **KC790951**	MAFF511115
C. nigrum	JX546838	CBS 169.49
C. novae-zelandiae	JQ005228	CBS 128505
C. nupharicola	JX010187	ICMP 18187
C. nymphaeae	JQ948197	CBS 515.78
C. ochracea	JX625168	CGMCC 3.15104
C. oncidii	JQ005169	CBS 129828
C. orbiculare	JQ005778	CBS 514.97
C. orchidophilum	JQ948151	CBS 632.80
C. parsonsiae	JQ005233	CBS 128525
C. paspali	EU554100, **KC790948**	MAFF 305403
C. paxtonii	JQ948285	IMI 165753
C. petchii	JQ005223	CBS 378.94
C. phaseolorum	GU227896	CBS 157.36
C. phormii	DQ286136, JQ948446	CBS 118194
C. phyllanthi	JQ005221	CBS 175.67
C. proteae	KC297079*	CBS 132882
C. pseudoacutatum	JQ948480	CBS 436.77
C. psidii	JX010219	CBS 145.29
C. pyricola	JQ948445	CBS 128531
C. queenslandicum	JX010276	ICMP 1778
C. rhexiae	JX145128	CBS 133134
C. rhombiforme	JQ948457	CBS 129953
C. rusci	GU227818	CBS 119206
C. salicis	JQ948460	CBS 607.94
C. salsolae	JX010242	ICMP 19051
C. sansevieriae	AB212991[#], **KC790947**	MAFF239721
C. scovillei	JQ948267	CBS 126529
C. siamense	FJ972613, JX010171	ICMP 18578
C. simmondsii	FJ972601, JQ948276, **KC790946**	BRIP28519, CBS 122122, LC0937
C. sloanei	JQ948287	IMI 364297
C. spaethianum	GU227807, **KC790956**	CBS 167.49, MTCC 10330
C. spinaciae	GU227847	CBS 128.57
C. sublineolum	DQ003114, JQ478437, JQ005771	S3.001, BPI399463, CBS 131301
C. syzygicola	KF242094*	MFLUCC 10-0624

Taxon	GenBank acc. no.	Strain no.
C. tabacum	JQ005763	CBS 161.53
C. tamarilloi	JQ948184	CBS 129814
C. tanaceti	JX218228*	CBS 132693/BRIP 57314
C. temperatum	JX145159	CBS 133122
C. thailandicum	JN050242, **KC790941**	BCC38879, LC0596
C. theobromicola	JX010294, GU994360	CBS 124945, ICMP 18649
C. ti	JX010269	ICMP 4832
C. tofieldiae	GU227801	CBS 495.85
C. torulosum	JQ005164	CBS 128544
C. trichellum	GU227812	CBS 217.64
C. tropicale	GU994331, JX010264	CBS 124949, ICMP 18653
C. tropicicola	JN050240, **KC790942**	BCC38877, LC0598
C. truncatum	GU227862	CBS 151.35
C. verruculosum	GU227806, **KC790930**	IMI 45525
C. viniferum	JN412804	GZAAS5.08601
C. walleri	JQ948275	CBS 125472
C. xanthorrhoeae	GU048667, JX010261, GU174551	BRIP 45094, CBS 127831, ICMP 17903
C. yunnanense	EF369490	CGMCC AS3.9617

[Abbreviations: **BBA** = Biologische Bundesanstalt für Land- und Forstwirtschaft, Germany; **BCC** = BIOTEC Culture Collection, Thailand; **BRIP** = Queensland Plant Pathology Herbarium, Australia; **CBS** = Culture Collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity centre, Utrecht, The Netherlands; **CGMCC** = China General Microbiological Culture Collection Center, China; **GZAAS** = Guizhou Academy of Agricultural Sciences herbarium, China; **ICMP** = International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand; **IMI** = Culture Collection of CABI Europe, UK centre, Egham, UK ; **LC** = Cultures shared by Dr. Lei Cai, China; **MAFF** = Microorganisms Section of the NIAS (National Institute of Agrobiological Sciences) Genebank, Japan; **MFLUCC** = Mae Fah Luang University Culture Collection, Thailand; **MTCC** = Microbial Type Culture Collection and Gene Bank, Chandigarh; **MUCL** = Belgian Co-ordinated Collections of Micro-organisms, (agro)industrial fungi & yeasts, Belgium. Note: * = sequence not used in the analyses due to unavailability; # = sequence not used in the analyses due to short length; sequences in bold have been generated in this study]

identification does not suffice the objective of accurate identification of fungus. So, we need better methodology for identification of *Colletotrichum* species-complexes. We could employ polyphasic approach of identification as suggested by Cai et al. (2009), along with multi-gene phylogeny using different gene markers as suggested by Damm et al. (2009). As recently recommended by Pires and Marinoni (2010) Integrative Taxonomy, an integrated approach employing both traditional taxonomy and DNA barcoding, could also serve the purpose of accurate identification of fungal species. It is also apparent from the study that we need to search for new gene-markers that can help us to resolve the species-complex which can not be resolved effectively with the help of ITS/ 5.8S rRNA gene. One such marker is the intergenic region between the *Apn2* and *Mat* 1-2-1 gene (*Apmat*) which has been recently shown as a candidate secondary barcode marker for resolving the members of *C. gloeosporioides* species complex members (Sharma et al. 2013a). Researchers should also follow a polyphasic approach for species identification, which would definitely not be rapid; nevertheless would be more reliable and accurate.

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