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Resolving the *Colletotrichum siamense* species complex using *ApMat* marker

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Abstract *Colletotrichum gloeosporioides sensu lato* has been associated with anthracnose in diverse commercial crops. It is now established that *C. gloeosporioides sensu lato* comprises 33 phylogenetic species and *C. gloeosporioides sensu stricto* is not a common pathogen of tropical fruits. In this study, we investigated the phylogenetic relationships of 85 *Colletotrichum* isolates associated with select tropical fruits and flowering plants from India. In the *ApMat* marker analysis, the 85 isolates clustered with 7 known *Colletotrichum* species (*C. aotearoa*, *C. dianesei*, *C. endomangiferae*, *C. musae*, *C. siamense*, *C. theobromicola, Glomerella cingulata* f. sp. *camelliae*) and six novel lineages. One of the novel lineages is described and illustrated in this paper as *Colletotrichum communis sp. nov.*, while new-host pathogen associations for *C. aotearoa*, *C. endomangiferae*, *C. dianesei* and *C. theobromicola* are reported from India. Out of the 85 isolates analysed in this paper, 73 isolates clustered within the *C. siamense* species complex, indicating that *C. siamense* species complex, not *C. gloeosporioides sensu stricto*, is common on tropical fruits. In comparison with *act*, *cal*, *gapdh*, ITS and *tub2* gene markers, we recommend the use of the *ApMat* marker for accurate identification of cryptic species within the *C. siamense* species complex. We believe that the *ApMat* marker, in combination with one or two similar 'phylogenetically superior' gene markers, is a better candidate for species-level classification of fungi that were traditionally identified as '*Colletotrichum gloeosporioides*'.

Keywords Apn2/MAT IGS · Ascomycota · Pathogenicity testing · Phylogeny · Tropical crops

Introduction

India is known as the fruit and vegetable basket of the world (Yeledhalli et al. 2012). In the fiscal year 2012–13, India produced 81.29 million metric tons of fruits, 162.19 million metric tons of vegetables and 1.73 million metric tons of floricultural plants (Mistry et al. 2014). Indian fruits and vegetables are mainly exported to Middle East and South East Asian countries (DCGIS Annual Report 2014) and it is a major source of revenue for the economy (Kapila 2009). The pre- and post-harvest infections caused by *Colletotrichum* species result in severe losses in crop yield and quality, thus affect the export of fruits to other countries (Chadha 2009). It is essential that *Colletotrichum* species that cause anthracnose disease are accurately identified so as to develop effective disease-control strategies.

Colletotrichum gloeosporioides, in its traditional sense, was regarded as a major pre- and post-harvest pathogen causing anthracnose in economically important tropical crops. Recent taxonomic revisions reveal that *C. gloeosporioides sensu lato* is a species complex including 33 phylogenetic species and one subspecies (Lima et al. 2013; Liu et al. 2013; Manamgoda et al. 2013; Sharma et al. 2013; Udayanga et al. 2013; Vieira et al. 2014). It has been demonstrated that *C. gloeosporioides sensu stricto* is not a common pathogen of tropical fruits (Phoulivong et al. 2010; Sharma et al. 2013; Udayanga et al. 2013). Thus, it is important to revisit the old host-pathogen records and update it with molecular data as per recent nomenclatural revisions (Damm et al. 2012a, b; Weir et al. 2012). However, it is challenging to deal with the taxonomy of morphologically cryptic fungal species (Hibbett and Taylor 2013). This issue is very relevant and critical while

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dealing with identification of fungi that cause diseases in plants, animals and humans.

Colletotrichum siamense H. Prihastuti, L. Cai & K.D. Hyde, one of the challenging and controversial taxa, was described as a species associated with coffee berries by Prihastuti et al. (2009). In a major revision of the *C. gloeosporioides* species complex, Weir et al. (2012) later synonymised *C. jasmini-sambac* S. Wikee, K.D. Hyde, L. Cai and E. H. C. McKenzie (Wikee et al. 2011) and *C. hymenocallidis* Y.L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai (Yang et al. 2009) with *C. siamense*. Using the intergenic sequence of *apn2* and *Mat1-2* gene region (*ApMat* marker) and the translation elongation factor 1-α gene region (*5′ tef1*), Sharma et al. (2013), however, have demonstrated that *C. jasmini-sambac*, *C. hymenocallidis*, *C. melanocaulon* V.P. Doyle, P.V. Oudem. & S.A. Rehner (= *C. dianesei* N.B. Lima, M.P.S. Câmara & S. J. Michereff) (Vieira et al. 2014) and *C. siamense* are four distinct species within the *C. siamense* species complex. Some recent studies also support this hypothesis (Doyle et al. 2013; Udayanga et al. 2013; Vieira et al. 2014). *Colletotrichum melanocaulon* has recently been synonymised with *C. dianesei* and a new species *C. endomangiferae* W.A.S. Vieira, M.P.S. Câmara & S.J. Michereff has been described within the *C. siamense* species complex based on *ApMat* sequence data (Vieira et al. 2014). The species status of *C. murrayae* L.J. Peng & K.D. Hyde (Peng et al. 2012) within this complex is ambiguous, due to its illegitimate nomenclature (Liu et al. 2013).

Recent studies have demonstrated that *ApMat* marker is capable of efficient resolution of species within the *C. gloeosporioides sensu lato* (Rojas et al. 2010; Silva et al. 2012; Doyle et al. 2013) and *C. siamense* species complex (Sharma et al. 2013; Vieira et al. 2014). The occurrence of high level of fixed polymorphism among different species is believed to be responsible for the efficiency of the *ApMat* marker towards better phylogenetic species resolution (Silva et al. 2012). This study, therefore, aimed to unravel and describe novel *Colletotrichum* lineages/ taxa associated with anthracnose diseases of various host plants from India based on morphology, *ApMat*-marker phylogeny and pathogenicity data.

Materials and methods

Sample collection

Apparently healthy plant-tissue samples from selected ornamental and flowering plants (*Bauhinia*, *Cassia,* and *Ficus*) and guava (*Psidium*) fruits were collected from CSIR-Institute of Microbial Technology (CSIR-IMTECH) campus in Chandigarh. Symptomatic tea (*Camellia*) leaves were collected from tea gardens in Bir and Palampur regions of Kangra district in Himachal Pradesh. Banana (*Musa*) and orange (*Citrus*) fruits with lesions were procured from fruit-markets in Chandigarh. Banana fruits with lesions and neem (*Azadirachta*) leaves were purchased from a supermarket in Mysore, Karnataka. Infected coffee (*Coffea*) berries were collected and supplied by Mr. Deepak M. (CSIR-CFTRI, Mysore) from Virajpet Taluk in Karnataka. Fungal isolation from host-plant tissues was carried out as described by Cai et al. (2009).

Fungal isolates

Thirty-six isolates were recovered as endophytes or potential pathogens from the collected plant tissue samples as described above. One isolate was procured from Goa University Fungal Culture Collection (GUFCC), Goa. Two isolates were procured from National Fungal Culture Collection of India (NFCCI), Pune. Three isolates were accessed from the Microbial Type Culture Collection (MTCC), Chandigarh and 14 isolates from the Indian Type Culture Collection (ITCC), New Delhi. In addition, 29 isolates belonging to the *C. siamense* species complex associated with mango from our previous study (Sharma et al. 2013) were also included in this paper. Information on host and geographic location of sample collection of the isolates are detailed in Table 1. Fungal isolates were subcultured on potato dextrose agar (PDA, HiMedia, India) medium, grown at 20 °C for 7 days and preserved at −70 °C and liquid nitrogen in 10 % glycerol for future use.

DNA extraction, PCR amplification and sequencing of gene markers

Genomic DNA from fresh mycelia was isolated using the DNA isolation kit (catalogue number D6005, Zymo Research, USA) and stored at −20 °C. Fifty-six isolates from this study were subjected to polymerase chain reaction (PCR) amplification of the *ApMat* marker. Out of the 56 isolates, a subset of thirty-four *Colletotrichum* isolates was selected based on uniqueness of host and geographical location and subjected to PCR amplification of actin (*act*), calmodulin (*cal*), chitin synthase (*chs1*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), ITS and β-tubulin (*tub2*) gene regions. The reactions were carried out in an Eppendorf Mastercycler with the cycling parameters and primers as specified in previous papers (Damm et al. 2009–ITS, *act, chs1, gapdh, tub2*; Silva et al. 2012–*ApMat* and Weir et al. 2012–*cal*). The PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN, catalogue number 28106), quantified using Nanodrop Spectrophotometer ND-1000 (Thermo) and sequenced using respective forward and reverse primers with the ABI Big Dye v3.1 Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems). Post sequencing

Table 1 List of *Colletotrichum* isolates from this study with information on isolate designation, host, geographic location and GenBank accession numbers of the *act, Apmat, cal*, *chs1*, *gapdh,* ITS and *tub2* gene sequences ($^{\#}$ = Sequence not used in multigene phylogeny, *N.A.* information not available, *N. S.* not sequenced). All the isolates are of Indian origin

Sl. no.	Isolate designation	Taxon	Host	Geographic location	ITS	*gapdh*	*ApMat*	*act*	*tub2*	*cal*	*chs1*$^{\#}$
1	GBM02	*C. aotearoa*	*Musa* sp. (Banana)	Kuvempu Nagar, Mysore, Karnataka	KC790970	KC790731	KC790669	KC790617	KC790864	KF451949	KF451984
2	GO01 = MTCC 11696	*C. communis sp. nov.*	*Citrus* sp. (Orange)	Chandigarh	KC790977	KF452016	KC790720	KF451940	KF452029	KF451953	KF451988
3	GO02	*C. communis sp. nov.*	*Citrus* sp. (Orange)	Chandigarh	KC790978	N.S.	KC790721	N.S.	N.S.	N.S.	N.S.
4	GO03	*C. communis sp. nov.*	*Citrus* sp. (Orange)	Chandigarh	KC790979	N.S.	KC790722	N.S.	N.S.	N.S.	N.S.
5	GO04 = MTCC 11695	*C. communis sp. nov.*	*Citrus* sp. (Orange)	Chandigarh	KC790980	KF452017	KC790723	KF451941	KF452030	KF451954	KF451989
6	GO05	*C. communis sp. nov.*	*Citrus* sp. (Orange)	Chandigarh	KC790975	N.S.	KC790724	N.S.	N.S.	N.S.	N.S.
7	GO06	*C. communis sp. nov.*	*Citrus* sp. (Orange)	Chandigarh	KC790976	N.S.	KC790725	N.S.	N.S.	N.S.	N.S.
8	GS01 = MTCC 11697	*C. communis sp. nov.*	*Bauhinia variegata* (Orchid tree)	IMTECH, Chandigarh	JN248668	KC790736	KC790674	KC790622	KC790869	KF451955	KF451990
9	GS03	*C. communis sp. nov.*	*Bauhinia variegata* (Orchid tree)	IMTECH, Chandigarh	JN248670	KC790738	KC790676	KC790624	KC790871	N.S.	N.S.
10	GS06	*C. communis sp. nov.*	*Saraca indica* (Ashoka tree)	IMTECH, Chandigarh	JN248673	KC790739	KC790677	KC790625	KC790872	KF451956	KF451991
11	GS14 = MTCC 11699	*C. communis sp. nov.*	*Ficus elastica* (Rubber plant)	IMTECH, Chandigarh	JN248681	KC790741	KC790679	KC790643	KC790874	KF451957	KF451992
12	GS17 = MTCC 11700	*C. communis sp. nov.*	*Psidium guajava* (Guava)	IMTECH, Chandigarh	JN248683	KC790742	KC790680	KC790627	KC790875	KF451958	KF451993
13	GS18	*C. communis sp. nov.*	*Psidium guajava* (Guava)	IMTECH, Chandigarh	JN248684	KC790743	KC790681	KC790628	KC790876	N.S.	N.S.
14	GS19	*C. communis sp. nov.*	Unidentified plant	IMTECH, Chandigarh	JN248685	KC790744	KC790682	KC790644	KC790877	N.S.	N.S.
15	GS21	*C. communis sp. nov.*	Unidentified plant	IMTECH, Chandigarh	JN248687	KC790746	KC790684	KC790630	KC790879	N.S.	N.S.
16	GS22	*C. communis sp. nov.*	Unidentified plant	IMTECH, Chandigarh	JN248688	KC790747	KC790685	KC790631	KC790880	N.S.	N.S.
17	GS28	*C. communis sp. nov.*	Unidentified plant	IMTECH, Chandigarh	JN248692	KC790748	KC790686	KC790632	KC790881	KF451959	KF451994
18	GS29	*C. communis sp. nov.*	Unidentified plant	IMTECH, Chandigarh	JN248693	KC790749	KC790687	KC790633	KC790882	N.S.	N.S.
19	IMTF736	*C. communis sp. nov.*	*Cassia fistula* (Golden shower tree)	IMTECH, Chandigarh	JN248695	KC790751	KC790690	KC790636	KC790884	KF451962	KF451997
20	IMTF737	*C. communis sp. nov.*	*Cassia fistula* (Golden shower tree)	IMTECH, Chandigarh	JN248696	KC790752	KC790691	KC790637	KC790885	N.S.	N.S.
21	IMTF738	*C. communis sp. nov.*	*Cassia fistula* (Golden shower tree)	IMTECH, Chandigarh	JN248697	KC790756	KC790692	KC790638	KC790886	N.S.	N.S.
22	ITCC 5123	*C. communis sp. nov.*	*Psidium guajava* (Guava)	Allahabad, Uttar Pradesh	JN390843	KC790763	KC790700	KC790650	KC790896	KF451965	KF452000
23	ITCC 6152	*C. communis sp. nov.*	*Cassia* sp. (Cassias)	Rahuri, Maharashtra	JN390861	KC790766	KC790703	KC790653	KC790899	N.S.	N.S.
24	ITCC 6153	*C. communis sp. nov.*	*Cassia* sp. (Cassias)	Rahuri, Maharashtra	JN390862	KC790767	KC790704	KC790654	KC790900	KF451967	KF452002
25	ITCC 6155	*C. communis sp. nov.*	*Cassia* sp. (Cassias)	Rahuri, Maharashtra	JN390863	KC790768	KC790705	KC790655	KC790901	N.S.	N.S.
26	ITCC 6159	*C. communis sp. nov.*	*Psidium guajava* (Guava)	Rahuri, Maharashtra	JN390865	KC790769	KC790706	KC790656	KC790902	KF451968	KF452003
27	ITCC 6336	*C. communis sp. nov.*	*Cassia* sp. (Cassias)	Jobner, Rajasthan	JN390881	KC790775	KC790714	KC790664	KC790910	KF451973	KF452008
28	MTCC 4626	*C. communis sp. nov.*	*Psidium guajava* (Guava)	IMTECH, Chandigarh	JN390924	KC790777	KC790716	KC790666	KC790912	KF451974	KF452009
29	NFCCI 1925	*C. communis sp. nov.*	N. A.	N. A.	JN390942	KC790755	KC790696	KC790642	KC790890	KF451976	KF452011
30	GS02	*C. dianesei*	*Bauhinia variegata* (Orchid tree)	IMTECH, Chandigarh	JN248669	KC790737	KC790675	KC790623	KC790870	N.S.	N.S.

Table 1 (continued)

Sl. no.	Isolate designation	Taxon	Host	Geographic location	ITS	*gapdh*	*ApMat*	*act*	*tub2*	*cal*	*chs1*$^{\#}$
31	GS07	*C. dianesei*	*Bauhinia variegata* (Orchid tree)	IMTECH, Chandigarh	JN248674	KC790740	KC790678	KC790626	KC790873	N.S.	N.S.
32	GS20	*C. dianesei*	Unidentified plant	IMTECH, Chandigarh	JN248686	KC790745	KC790683	KC790629	KC790878	N.S.	N.S.
33	IMTF976	*C. dianesei*	Unidentified plant	IMTECH, Chandigarh	JN248702	KC790753	KC790693	KC790639	KC790887	KF451963	KF451998
34	IMTF997	*C. dianesei*	Unidentified plant	IMTECH, Chandigarh	JN248705	KC790754	KC790694	KC790640	KC790888	N.S.	N.S.
35	ITCC 4981	*C. dianesei*	*Cocos nucifera* (Coconut)	CPCRI, Kasaragod, Kerala	JN390909	KC790762	KC790699	KC790649	KC790895	KF451964	KF451999
36	MTCC 9663	*C. dianesei*	*Psidium guajava* (Guava)	Kurti-Ponda, Goa	JN390931	KC790760	KC790697	KC790647	KC790893	KF451946	KF451981
37	GUFCC 15502	*C. endomangiferae*	*Dieffenbachia* sp. (Dumb cane)	N. A.	JN390934	KC790758	KC790688	KC790645	KC790891	KF451960	KF451995
38	NFCCI 1737	*C. endomangiferae*	N. A.	N. A.	JN390941	KC790757	KC790695	KC790641	KC790889	KF451975	KF452010
39	GB07	*C. musae*	*Musa* sp. (Banana)	Chandigarh	KC790968	KC790729	KC790667	KC790615	KC790862	KF451948	KF451983
40	GB15	*C. musae*	*Musa* sp. (Banana)	Chandigarh, India	KC790969	KC790730	KC790668	KC790616	KC790863	N.S.	N.S.
41	GBM03	*C. musae*	*Musa* sp. (Banana)	Kuvempu Nagar, Mysore, Karnataka	KC790971	KC790732	KC790670	KC790618	KC790865	KF451950	KF451985
42	GN1	*C. siamense s. s.*	*Azadirachta indica* (Neem)	Mysore, Karnataka	KC790974	KC790735	KC790673	KC790621	KC790868	KF451952	KF451987
43	ITCC 6161	*C. theobromicola*	*Punica granatum* (Pomegranate)	Rahuri, Maharashtra	JN390867	KC790770	KC790708	KC790658	KC790904	KF451970	KF452005
44	ITCC 6164	*C. theobromicola*	*Punica granatum* (Pomegranate)	Rahuri, Maharashtra	JN390869	KF452019	KC790710	KC790660	KC790906	KF451971	KF452006
45	GC01	*Colletotrichum* sp. indet. 1	*Coffea robusta* (Coffee)	Virajpet Taluk, Karnataka	KC790972	KC790733	KC790671	KC790619	KC790866	KF451951	KF451986
46	GC02	*Colletotrichum* sp. indet. 1	*Coffea robusta* (Coffee)	Virajpet Taluk, Karnataka	KC790973	KC790734	KC790672	KC790620	KC790867	N.S.	N.S.
47	ITCC 6160	*Colletotrichum* sp. indet. 2	*Punica granatum* (Pomegranate)	Rahuri, Maharashtra	JN390866	KF452018	KC790707	KC790657	KC790903	KF451969	KF452004
48	ITCC 6163	*Colletotrichum* sp. indet. 2	*Punica granatum* (Pomegranate)	Rahuri, Maharashtra	JN390868	KC790771	KC790709	KC790659	KC790905	N.S.	N.S.
49	ITCC 6165	*Colletotrichum* sp. indet. 2	*Punica granatum* (Pomegranate)	Rahuri, Maharashtra	JN390870	KC790772	KC790711	KC790661	KC790907	KF451972	KF452007
50	ITCC 6166	*Colletotrichum* sp. indet. 2	*Punica granatum* (Pomegranate)	Rahuri, Maharashtra	JN390871	KC790773	KC790712	KC790662	KC790908	N.S.	N.S.
51	ITCC 6066	*Colletotrichum* sp. indet. 3	*Cocos nucifera* (Coconut)	Bhubaneshwar, Orissa	JN390914	KC790764	KC790701	KC790651	KC790897	KF451966	KF452001
52	MTCC 9664	*Colletotrichum* sp. indet. 4	*Carica papaya* (Papaya)	Panjim Market, Goa	JN390932	KC790761	KC790698	KC790648	KC790894	KF451947	KF451982
53	TB01 = MTCC 11728	*G.lomerella cingulata* f. sp. *camelliae*	*Camellia sinensis* (Tea)	Bir, Kangra, Himachal Pradesh	KF452025	KF452020	KF452024	KF451942	KF452031	KF451977	KF452012
54	TP02 = MTCC 11731	*G. cingulata* f. sp. *camelliae*	*Camellia sinensis* (Tea)	IHBT, Palampur, Himachal Pradesh	KF452027	KF452022	KC790718	KF451944	KF452033	KF451979	KF452015
55	TP05 = MTCC 11730	*G. cingulata* f. sp. *camelliae*	*Camellia sinensis* (Tea)	IHBT, Palampur, Himachal Pradesh	KF452028	KF452023	KC790719	KF451945	KF452034	KF451980	KF452014
56	TP01 = MTCC 11729	*Glomerella cingulata* f. sp. *camelliae*	*Camellia sinensis* (Tea)	IHBT, Palampur, Himachal Pradesh	KF452026	KF452021	KC790717	KF451943	KF452032	KF451978	KF452013

ITCC Indian Type Culture Collection, New Delhi, India; *GUFCC* Goa University Fungal Culture Collection, Goa, India; *MTCC* Microbial Type Culture Collection and Gene Bank, Chandigarh, India; *NFCCI* National Fungal Culture Collection of India, Pune, India reaction clean-up was performed to remove excess salt from samples, which were further denatured with HiDi-Formamide at 95 °C for 3 min and analysed using 3730 DNA Analyzer (Applied Biosystems) at the central DNA sequencing facility of CSIR-IMTECH, Chandigarh. The sequences generated in this study are deposited in NCBI-GenBank with accession numbers as listed in Table 1.

ApMat marker-based phylogenetic analysis

Fifty-six *Colletotrichum* isolates belonging to the *C. gloeosporioides* species complex were selected for this analysis. Twenty-nine sequences belonging to the *C. siamense* species complex and associated with mango tissues were retrieved from Sharma et al. (2013). Information on GenBank accession numbers is detailed in Tables 1 and 2. A maximum parsimony (MP) analysis of the *ApMat* dataset was performed using PAUP version 4.0b10 (Swofford 2003). The ambiguously aligned regions were not included in the analysis. The gaps in the alignment were treated as missing data. All the characters in the analysis were unordered and had equal weight. Trees were inferred using the heuristic search option with 20 random sequence additions and tree bisection and reconstruction (TBR) as the branch swapping algorithm. Maxtrees were set to 10.000; the branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics such as Tree Length (TL), Consistency Index (CI), Retention Index (RI), Related Consistency Index (RCI) and Homoplasy Index (HI) were calculated for the generated trees. The clade stability was assessed by 100 bootstrap replicates (Felsenstein 1985) and addition of 10 random sequences. Kishino-Hasewaga tests (Kishino and Hasewaga 1989) were performed to determine whether trees were significantly different. Trees were viewed in TreeView version 1.6.6 (Page 1996) and edited in MEGA version 5.2 (Tamura et al. 2011) and Microsoft PowerPoint version 2007 (Microsoft Corp. USA). The alignment file and tree are deposited in TreeBase (www.treebase.org; Study ID: 14671).

5-gene based phylogenetic analysis of the *C. siamense* species complex

A multigene dataset including: newly generated *act*, *cal, gapdh*, ITS and *tub2* gene sequences of 23 isolates of the *C. siamense* species complex and the homologous sequences retrieved from GenBank of seven reference taxa was prepared using SequenceMatrix version 1.7.8 (Vaidya et al. 2011). The sequences from the ex-type isolates of *C. dianesei*, *C. endomangiferae*, *C. hymenocallidis*, *C. jasmini-sambac*, *C. murrayae* and *C. siamense* were included in the analysis. All the details related to gene sequences are presented in Tables 1 and 3. The *chs1* gene region was not included in the multigene dataset as it was missing for the following taxa: *C. dianesei* and *C. murrayae.* The MP analysis of the 5-gene dataset was performed using PAUP as described in *ApMat* marker based phylogenetic analysis. The alignment files and trees are deposited in TreeBase (www.treebase.org; Study ID: 14671).

Table 2 List of GenBank accession numbers of the *ApMat* sequences from Sharma et al. (2013) used in this study with information on taxa and isolate designation. All the isolates are from mango samples collected from India

Taxon	Isolate designation	*ApMat*
C. communis sp. nov.	GM010	JQ894555
	GM018 = MTCC 11672	JQ894556
	GM043A	JQ894558
	GM043B	JQ894559
	GM147	JQ894560
	GM150	JQ894561
	GM192	JQ894563
	GM301	JQ894565
	GM314	JQ894566
	GM397	JQ894571
	ITCC 6158	JQ894580
	NK22	JQ894586
	NK23	JQ894587
	NK24* = MTCC 11599*	JQ894582
	NK25	JQ894583
	NK28 = MTCC 115993	JQ894588
	NK29	JQ894585
C. dianesei	GM057 = MTCC 11590	JQ894551
	GM063	JQ894552
	GM172 = MTCC 11591	JQ894562
	GM291	JQ894564
	GM388	JQ894569
	GM409	JQ894572
	GM514	JQ894574
C. endomangiferae	GM473 = MTCC 11589	JQ894553
	GM529 = MTCC 11592	JQ894575
	MTCC 9660	JQ894548
Colletotrichum sp. indet. 3	GM385	JQ894568
Colletotrichum sp. indet. 5	GM390 = MTCC 11677	JQ894570

[* = ex-type isolate; Abbreviation: *ITCC* Indian Type Culture Collection, New Delhi, India; *MTCC* Microbial Type Culture Collection and Gene Bank, Chandigarh, India]

Morphological characterisation

For selected isolates (Table 4), morphological characterisation was carried out based on the 7-day old cultures grown at 20 °C on PDA. Micro-morphological characters such as colour, shape and size of conidia and conidiogenous cells were observed and photographed using a trinocular differential interference contrast (DIC) microscope (Olympus U-CMAD3)

Table 3 List of GenBank accession numbers for the ITS, *gapdh*, *cal*, *act*, *chs1*, *tub2* and *ApMat* gene sequences of the ex-type isolates belonging to the *Colletotrichum gloeosporioides* species complex with information on taxa, host and geographic location

Taxon	Isolate designation	Host	Geographic location	ITS	*gapdh*	*cal*	*act*	*chs1*	*tub2*	*ApMat*
C. aenigma	ICMP 18608*	*Persea americana*	Israel	JX010244	JX010044	JX009683	JX009443	JX009774	JX010389	N. S.
C. aeschynomenes	ICMP 17673*	*Aeschynomene virginica*	USA	JX010176	JX009930	JX009721	JX009483	JX009799	JX010392	N. S.
C. alatae	ICMP 17919*	*Dioscorea alata*	India	JX010190	JX009990	JX009738	JX009471	JX009837	JX010383	KC888932
C. alienum	ICMP 12071*	*Malus domestica*	New Zealand	JX010251	JX010028	JX009654	JX009572	JX009882	JX010411	KC888927
C. aotearoa	ICMP 18537*	*Coprosma* sp.	New Zealand	JX010205	JX010005	JX009611	JX009564	JX009853	JX010420	KC888930
C. asianum	ICMP 18580* = MTCC 10987*	*Coffea arabica*	Thailand	FJ972612	JX010053	FJ917506	JX009584	JX009867	JX010406	FR718814
C. clidemiae	ICMP 18658*	*Clidemia hirta*	USA, Hawaii	JX010265	JX009989	JX009645	JX009537	JX009877	JX010438	KC888929
C. cordylinicola	ICMP 18579* = MTCC 10995*	*Cordyline fruticosa*	Thailand	JX010226	JX009975	HM470238	HM470233	JX009864	JX010440	JQ899274
C. dianesei	MFLUCC 1300058*	*Mangifera indica*	Brazil	KC329779	KC517194	KC517209	KC517298	N. S.	KC517254	N. S.
C. endophytica	MFLUCC 13–0418*	*Pennisetum purpureum*	Thailand	KC633854	KC832854	KC810018	KF306258	N. S.	N. S.	N. S.
C. endomangiferae	CMM3814*	*Mangifera indica*	Brazil	KC702994	KC702955	KC992372	KC702922	KC598113	KM404170	KJ155453
C. fructicola	ICMP 18581*	*Coffea arabica*	Thailand	JX010165	JX010033	FJ917508	FJ907426	JX009866	JX010405	JQ807838
C. fructicola (syn. *C. ignotum*)	ICMP 18646* = MTCC 10906*	*Tetragastris panamensis*	Panama	JX010173	JX010032	JX009674	JX009581	JX009874	JX010409	JQ807839
C. fructivorum	Coll1414 = CBS 133125*	*Vaccinium macrocarpon*	USA	JX145145	N. S.	N. S.	N. S.	N. S.	JX145196	JX145300
C. gloeosporioides	ICMP 17821* = MTCC 10323*	*Citrus sinensis*	Italy	JX010152	JX010056	JX009731	JX009531	JX009818	JX010445	JQ807843
C. grevilleae	CBS 132879*	*Grevillea* sp.	Italy	KC297078	KC297010	KC296963	KC296941	KC296987	KC297102	N. S.
G. cingulata "f. sp. *camelliae*"	ICMP 18542	*Camellia sasanqua*	USA	JX010223	JX009994	JX009628	JX009488	JX009857	JX010429	N. S.
C. horii	ICMP 10492* = MTCC 10841*	*Diospyros kaki*	Japan	GQ329690	GQ329681	JX009604	JX009438	JX009752	JX010450	JQ807840
C. kahawae subsp. *ciggaro*	ICMP 18539*	*Olea europaea*	Australia	JX010230	JX009966	JX009635	JX009523	JX009800	JX010434	N. S.
C. kahawae subsp. *kahawae*	ICMP 17816* = MTCC 11049*	*Coffea arabica*	Kenya	JX010231	JX010012	JX009642	JX009452	JX009813	JX010444	JQ899282
C. dianesei (syn. *C. melanocaulon*)	Coll131 = CBS 133251*	*Vaccinium macrocarpon*	USA	JX145144	N. S.	N. S.	N. S.	N. S.	JX145195	JX145313
C. murrayae	GZAAS 5.09506*	*Murraya* sp.	China	JQ247633	JQ247609	JQ247596	JQ247657	N. S	JQ247644	N. S
C. musae	ICMP 19119* = MTCC 11349*	*Musa* sp.	USA	JX010146	JX010050	JX009742	JX009433	JX009896	HQ596280	KC888926
C. nupharicola	ICMP 18187*	*Nuphar lutea* subsp. *polysepala*	USA	JX010187	JX009972	JX009663	JX009437	JX009835	JX010398	JX145319
C. proteae	CBS 132882	*Protea* sp.	South Africa	KC297079	KC297009	KC296960	KC296940	KC296986	KC297101	N. S.
C. psidii	ICMP 19120*	*Psidium* sp.	Italy	JX010219	JX009967	JX009743	JX009515	JX009901	JX010443	KC888931
C. queenslandicum	ICMP 1778*	*Carica papaya*	Australia	JX010276	JX009934	JX009691	JX009447	JX009899	JX010414	KC888928
C. rhexiae	Coll1026 = CBS 133134*	*Rhexia virginica*	USA	JX145128	N. S.	N. S.	N. S.	N. S.	JX145179	JX145290
C. salsolae	ICMP 19051*	*Salsola tragus*	Hungary	JX010242	JX009916	JX009696	JX009562	JX009863	JX010403	KC888925
C. siamense	ICMP 18578* = MTCC 10173*	*Coffea arabica*	Thailand	JX010171	JX009924	FJ917505	FJ907423	JX009865	JX010404	JQ899289
C. hymenocallidis	ICMP 18642* = MTCC 10992*	*Hymenocallis americana*	China	JX010278	JX010019	JX009709	GQ856775	GQ856730	JX010410	JQ807842

Table 3 (continued)

Taxon	Isolate designation	Host	Geographic location	ITS	*gapdh*	*cal*	*act*	*chs1*	*tub2*	*ApMat*
C. jasmini-sambac	ICMP 19118* = MTCC 10990*	*Jasminum sambac*	Vietnam	HM131511	HM131497	JX009713	HM131507	JX009895	JX010415	JQ807841
C. syzygicola	DNCL021 = MFLUCC 10–0624*	*Syzygium samarangense*	Thailand	KF242094	KF242156	KF254859	KF157801	N. S.	KF254880	N. S.
C. temperatum	Coll883 = CBS 133122*	*Vaccinium macrocarpon*	USA	JX145159	N. S.	N. S.	N. S.	N. S.	JX145211	JX145298
C. theobromicola	ICMP 18649* = MTCC 11350*	*Theobroma cacao*	Panama	JX010294	JX010006	JX009591	JX009444	JX009869	JX010447	KC790726
C. theobromicola (syn. *C. fragariae*)	ICMP 17927* = MTCC 10325*	*Fragaria ananassa*	USA	JX010286	JX010024	JX009592	JX009516	JX009830	JX010373	JQ807844
C. ti	ICMP 4832*	*Cordyline* sp.	New Zealand	JX010269	JX009952	JX009649	JX009520	JX009898	JX010442	N. S.
C. tropicale	ICMP 18653* = MTCC 11371*	*Theobroma cacao*	Panama	JX010264	JX010007	JX009719	JX009489	JX009870	JX010407	KC790728
C. viniferum	GZAAS 5.08601*	*Vitis vinifera*	China	JN412804	JN412798	JQ309639	JN412795	N. S.	JN412813	N. S.
C. xanthorrhoeae	ICMP 17903* = MTCC 11050*	*Xanthorrhoea preissii*	Australia	JX010261	JX009927	JX009653	KC790635	JX009823	KC790913	KC790689

[*Abbreviation: *CBS* Culture Collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; *GZAAS* Guizhou Academy of Agricultural Sciences herbarium, China; *ICMP* International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand; *MFLUCC* Mae Fah Luang University Culture Collection, Thailand; *MTCC* Microbial Type Culture Collection and Gene Bank, Chandigarh, India; *N.S.* not sequenced]

equipped with an Olympus microscope camera. For each isolate, length and width of at least 30 randomly chosen conidia were measured using the CellB image analysis software (Olympus). The colony diameter was measured after 7 days to determine the growth rate (mm/ day). Selected morphological features of the isolates are listed in Table 4.

Pathogenicity testing

Pathogenicity testing was performed as outlined in Sharma et al. (2013). Following representative isolates were selected for pathogenicity tests: *Camellia*–*G. cingulata* f. sp. *camelliae* (TB01 = MTCC 11728); *Citrus*–*C. communis sp. nov.* (GO01 = MTCC 11696), *Psidium*–*C. communis sp. nov.* (MTCC 4626); *Mangifera*–*C. communis sp. nov.* (NK24 = MTCC 11599); *Mangifera*–*C. endomangiferae* (GM529 = MTCC 11592, GM473 = MTCC 11589); *Musa*–*C. aotearoa* (GBM2 = MTCC 11769), *C. musae* (GBM3 = MTCC 11768). Fresh, unripe fruits and leaves were surface-sterilized using 1 % sodium hypochlorite solution and wounded using a sterile needle. Four fruits/ leaves were inoculated with 6 μl of conidial suspension (1×10^6 spore/ ml) for each isolate and one was inoculated with sterile water and used as control. The fruits/ leaves were kept in a moist chamber. The appearance of disease symptoms was observed from 4 to 7 days of incubation at 20 °C (Figs. 3 and 4). Severity of disease was calculated by measuring the lesion size and scored on a 0–9 point scale based on the percentage of the infected area (Montri et al. 2009). Percent disease incidence (PDI) and percent disease severity (PDS) were calculated using the formula given below (Awa et al. 2012) and the resulting values for PDI and PDS are shown in Table 5.

$$\mathbf{PDI}(\%) = \mathbf{x}/\mathbf{N} \times \mathbf{100}$$

$$\mathbf{PDS}(\%) = [\Sigma\,(\mathbf{a}+\mathbf{b})/\mathbf{N} \times \mathbf{Z}] \times \mathbf{100}$$

Where; Σ (a+b) = Sum of score scales of all inoculated host tissue samples

N Total number of inoculated host tissue samples
Z Highest score scale
X Number of infected host tissue samples

Results

ApMat marker-based phylogenetic analysis

The *ApMat* dataset included 114 sequences and a total of 955 characters including gaps. Fifty-one characters from the ambiguously-aligned regions were excluded from the

Table 4 Comparison of morphological characters of selected *Colletotrichum* isolates with the ex-type isolates (*)

Taxon	Isolate	Colony morphology	Conidia length	Conidia width	Conidia shape	Growth rate
C. aotearoa	ICMP 18537*	Dense cottony, grey to dark grey	16–17.5 µm Mean =16.9 µm	5.0–5.5 µm Mean =5.2 µm	Cylindrical	8.5 mm/day
C. aotearoa	GBM2 = MTCC 11769	Dense cottony, grey to dark grey	11.8–18.3 µm Mean =14.4±0.1 µm (n =141)	4.0–5.7 µm Mean =4.7±0.1 µm (n =141)	Cylindrical	9.9 mm/ day
C. musae	ICMP 19119*	White to grey floccose mycelium	11.5–19.5 µm Mean =14.7±2.1 µm	4.0–5.0 µm Mean =4.6±0.41 µm	Cylindrical	17.6 mm/day
C. musae	GBM3 = MTCC 11768	White mycelium with orange conidial mass	10.9–18.2 µm Mean =15.0±0.2 µm (n =50)	4.5–6.3 µm Mean =5.3±0.1 µm (n =50)	Cylindrical	12.8 mm/ day
C. siamense	ICMP 18578*	Cottony, pale yellowish to pinkish mycelium	7.0–18.3 µm Mean =10.2±1.49 µm	3.0–6.0 µm Mean =3.6±0.5 µm	Fusiform to cylindrical	9.1 mm/day
C. hymenocallidis	ICMP 18642*	White to greyish mycelium	7.0–11.0 µm Mean =8.5±0.9 µm	5.0–7.5 µm Mean =6.6±0.6 µm	Fusiform	9.9 mm/ day
C. jasmini-sambac	ICMP 19118*	Cottony, white, aerial mycelium	13.0–15.0 µm Mean =14.0±0.7 µm	3.5–4.0 µm Mean =3.8±0.2 µm	Cylindrical	10.6 mm/ day
C. communis sp. nov.	GO01 = MTCC 11696	White to greyish mycelium	11.1–18.0 µm Mean =15.1±0.1 µm (n =100)	4.2–5.9 µm Mean =5.1±0.5 µm (n =100)	Cylindrical	10.8 mm/day
C. communis sp. nov.	MTCC 4626	White to greyish mycelium with orange conidial mass	12.3–18.2 µm Mean =14.9±0.2 µm (n =78)	3.7–5.9 µm Mean =4.8±0.1 µm (n =78)	Cylindrical	10.6 mm/ day
C. communis sp. nov.	NK24 = MTCC 11599*	White to greyish mycelium	13.6–17.1 µm Mean =14.7±0.1 µm (n =100)	4.4–7.3 µm Mean =5.7±0.1 µm (n =100)	Cylindrical	13.0 mm/ day
C. endomangiferae	CMM3814*	White to greyish mycelium	14.0–16.4 µm Mean =15.2±1.7 µm	4.5–5.2 µm Mean =4.8±0.5 µm	Cylindrical	15.5 mm/day
C. endomangiferae	GM529 = MTCC 11592	Cottony, white, aerial mycelium with orange conidial mass	10.2–16.6 µm Mean =13.8±0.1 µm (n =121)	4.2–6.6 µm Mean =5.5±0.1 µm (n =121)	Cylindrical with slightly obtuse ends	12.7 mm/ day
C. endomangiferae	GM473 = MTCC 11589	Cottony, white to greyish mycelium with orange conidial mass	11.1–18.5 µm Mean =15.1±0.3 µm (n =32)	4.0–6.7 µm Mean =5.5±0.1 µm (n =32)	Cylindrical with slightly obtuse ends	14.0 mm/ day
G. cingulata f. sp. *camelliae*	TB01 = MTCC 11728	Dense cottony, grey to dark grey	13.8–20.9 µm Mean =16.7±0.1 µm (n =84)	4.3–6.5 µm Mean =5.4±0.5 µm (n =84)	Cylindrical	8.1 mm/ day

Table 5 Disease score (DS) on a 0–9 scale for each fruit/ leaf and value of percent disease incidence (PDI) and percent disease severity (PDS) (* = ex-type isolate)

Isolate	Host tissue	Disease Score (DS) on a 0–9 scale					PDI (%) = x/N×100	Σ(a+b)	PDS (%) = [Σ(a+b)/N×Z]×100
		Control	Test 1	Test 2	Test 3	Test 4			
GBM02 = MTCC 11769 (*C. aotearoa*)	Banana fruit	0	1	1	1	1	100 %	4	11.1 %
GBM03 = MTCC 11768 (*C. musae*)	Banana fruit	0	5	5	5	5	100 %	20	55.5 %
MTCC 4626 (*C. communis sp. nov.*)	Guava fruit	0	9	9	3	9	100 %	30	83.3 %
GO01 = MTCC 11696 (*C. communis sp. nov.*)	Citrus fruit	0	5	5	7	9	100 %	26	72.2 %
NK24 = MTCC 11599* (*C. communis sp. nov.*)	Mango fruit	0	7	7	7	7	100 %	28	77.7 %
GM529 = MTCC 11592 (*C. endomangiferae*)	Mango fruit	0	5	7	7	7	100 %	26	72.2 %
GM473 = MTCC 11592 (*C. endomangiferae*)	Mango fruit	0	5	5	7	5	100 %	22	61.1 %
TB01 = MTCC 11728 (*G. cingulata* f. sp. *camelliae*)	Tea leaves	0	5	5	3	1	100 %	14	38.8 %

Where, Σ(a + b) =Sum of score scales of all inoculated host tissue samples

N = Total number of inoculated host tissue samples =4

Z = Highest score scale =9

X = Number of infected host tissue samples =4

analysis. Out of the remaining 904 characters, 409 characters were constant, 331 characters were parsimony-informative and 164 characters were parsimony-uninformative. The MP analysis resulted in 33 trees and based on the KH test, these trees were not significantly different (details not shown). One of the 33 trees (TL=815, CI=0.758, RI=0.939, RC=0.712, HI=0.242) generated in the MP analysis is shown in Fig. 1. The tree is rooted with *C. xanthorrhoeae* ICMP 17903. The bootstrap support values more than 50 % for the observed branching pattern are shown next to the branches. In the MP tree shown in Fig. 1, bootstrap support for majority of the clades is higher than 70 %. The *ApMat* analysis resolved most of the isolates to their species level. Seven known species (*C. aotearoa*, *C. dianesei*, *C. endomangiferae*, *C. musae*, *C. siamense*, *C. theobromicola* and *G. cingulata* f. sp. *camelliae*) and six novel lineages (designated as *C. communis sp. nov.* and *Colletotrichum* sp. indet. 1–5) were recovered in this analysis.

Colletotrichum isolates ITCC 6161 and ITCC 6164 clustered with the ex-type isolate of *C. theobromicola* (ICMP 18649). *Colletotrichum* isolate GBM2 clustered with the ex-type isolate of *C. aotearoa* (ICMP 18537). *Colletotrichum* isolates GB07, GB15 and GBM3 clustered with the ex-type isolate of *C. musae* (ICMP 19119). *Colletotrichum* isolates MTCC 11728, MTCC 11729, MTCC 11730 and MTCC 11731 associated with tea leaves clustered as a distinct clade. In the 5-gene analysis these four isolates clustered with the representative strain of *G. cingulata* f. sp. *camelliae* (ICMP 18542) (data not shown). However, in the *ApMat* analysis the representative sequence could not be included due to unavailability of ex-type isolate. Epitypification of *Glomerella cingulata* f. sp. *camelliae* is pending and expected to be completed soon (Lei Cai, personal communication).

In the *C. siamense* species complex, *Colletotrichum* isolate GN01 clustered with the ex-type isolate of *C. siamense sensu stricto* (ICMP 18578). The isolates GS02, GS07, GS20, IMTF976, IMTF997, ITCC 4981, MTCC 9663 from this study and the isolates GM057, GM063, GM172, GM291, GM388, GM409, GM514 from Sharma et al. (2013) clustered with the ex-type isolate of *C. dianesei* (MFLU 1300058). The isolates GUFCC 15502, NFCCI 1737 from this study and GM473, GM529, MTCC 9660 from Sharma et al. (2013) clustered with the ex-type isolate of recently described *C. endomangiferae* (CMM3814).

A strongly supported clade including isolates GO01–06, GS01, GS03, GS06, GS14, GS17–19, GS21, GS22, GS28, GS29, IMTF 736–738, ITCC 5123, ITCC 6152, ITCC 6153, ITCC 6155, ITCC 6159, ITCC 6336, MTCC 4626 and NFCCI 1925 from this study and isolates GM010, GM018, GM043A, GM043B, GM147, GM150, GM192, GM301, GM314, GM397, ITCC 6158, NK22–25 and NK28–29 from Sharma et al. (2013) was recovered as a novel lineage. This novel lineage is described in this paper as *C. communis sp. nov.* In addition, five novel lineages designated as *Colletotrichum* sp. indet 1–5 (Fig. 1) were recovered in this analysis. These novel lineages could not be described as new taxa due to inability of the isolates to sporulate or availability of only one representative isolate.

5-gene based phylogenetic analysis of the *C. siamense* species complex

The multigene dataset included 2253 characters including gaps. The gene boundaries in the dataset included: ITS: 1–576, *act*: 577–834, *tub2*: 835–1258, *cal*: 1259–1998 and *gapdh*: 1999–2253. The analysis involved 30 isolates. Twenty-one characters from the ambiguously-aligned regions were excluded from the analysis. Out of the remaining 2232 characters, 2066 characters were constant, 36 characters were parsimony-informative and 130 characters were parsimony-uninformative. The MP analysis resulted in 90 trees and based on the KH test, these trees were not significantly different (details not shown). One of the 90 trees (TL=206, CI=0.830, RI=0.696, RC=0.577, HI=0.170) generated in the MP analysis is shown in Fig. 2. The tree is rooted with *C. gloeosporioides* ICMP 17821. The bootstrap support values more than 50 % for the observed branching pattern are shown next to the branches. The MP tree shown in Fig. 2 is poorly supported.

Morphological comparison

The comparison of morphological characters (colony morphology on PDA, conidial measurements and shape) and growth rate is presented in Table 4. There were no apparent differences in the morphotaxonomic characters.

Taxonomy

Colletotrichum communis Sharma G., Pinnaka, A. K. & Shenoy, B. D., *sp. nov.* (Fig. 4)

MycoBank No.: MB808066

Etymology: named after the prevalent (common) distribution of this species over different tropical host plants

Description: *Colonies* on PDA attaining 74–91 mm diam. after 7 days at 20 °C, growth rate 10.6–13.0 mm per day (n=10), whitish to greyish black, reverse light orange to dark grey at the centre, aerial mycelium, dense, with orange conidial mass. *Sexual stage* not observed on PDA plate. *Asexual stage* widely observed. *Conidiomata* acervular. *Conidiophores* hyaline, septate, branched at base, smooth. *Conidiogenous cells* 14–18 µm in length (n=20) and 2–3 µm in width, enteroblastic, phialidic, hyaline, cylindrical or clavate shaped, the base is slightly wider than the apex, hyaline, arranged in clusters, unbranched. *Conidia* 11.1–18.2 µm in length and 3.7–7.3 µm in width (n=100), hyaline, smooth, cylindrical with rounded ends. *Appressoria* rarely formed on PDA, brown to dark brown, shape variable, 5–10 µm in length and 4–7 µm in width (n=10). *Setae* formed, dark brown to black in color, smooth, tapered towards apices, 70–90 µm in length and 2–5 µm in width (n=10).

Fig. 1 One of the 33 most parsimonious trees showing phylogenetic ▶ affinities of 85 *Colletotrichum* isolates from India (highlighted in blue), obtained from heuristic search of the *ApMat* dataset. *Colletotrichum xanthorrhoeae* ICMP 17903 is the designated outgroup. Bootstrap support values of more than 50 % are shown at the nodes. Ex-type isolates are marked with *

Geographic distribution and host range: *Bauhinia variegata* (orchid tree), *Cassia fistula* (golden shower tree), *Cassia* sp., *Citrus* sp. (orange), *Ficus elastica* (rubber plant), *Mangifera indica* (mango), *Psidium guajava* (guava) and *Saraca indica* (ashoka tree) in different locations of India as mentioned in Table 1.

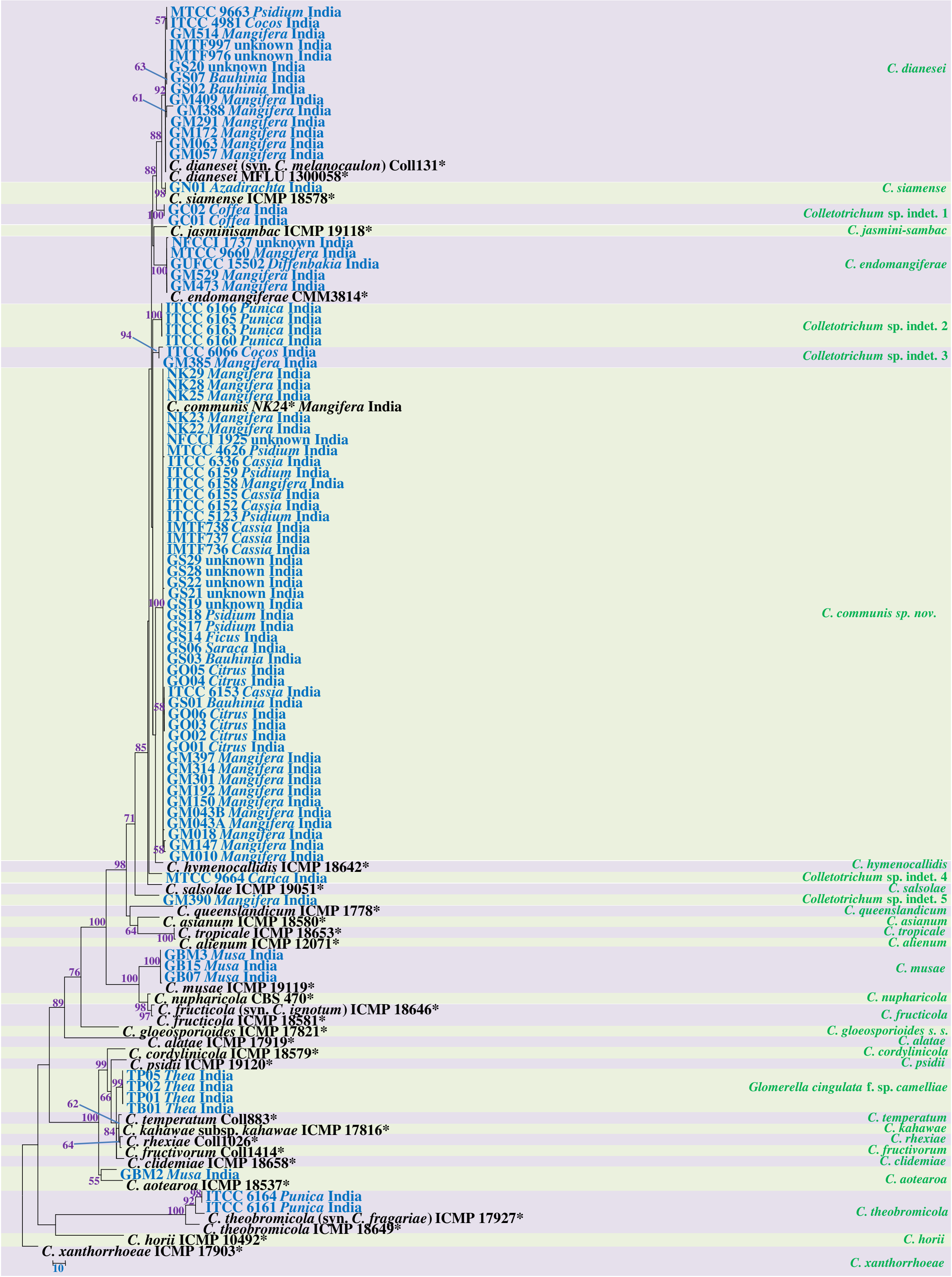
Materials examined: INDIA, Udupi district (13° 20′ N 74° 44′ E) in Karnataka state, on fruit lesion symptoms of *Mangifera indica* (mango) of *Neelam* variety, June 2011, Gunjan Sharma & Belle Damodara Shenoy (NK24* = MTCC 11599*, ex-type culture).

Additional specimens examined: INDIA, Chandigarh (U.T.) (30° 45′ N 76° 47′ E), on fruit spots of *Citrus*, December 2012, Gunjan Sharma (GO01 = MTCC 11696); INDIA, Chandigarh (U.T.) (30° 45′ N 76° 47′ E), from fruit lesions of Guava, Sandeep Jain (MTCC 4626).

Notes: *Colletotrichum communis sp. nov.* is morphologically similar to *C. siamense* but the former has a higher growth rate and slightly longer conidia. *Colletotrichum communis sp. nov.* is described as a pathogenic species associated with a broad host range in India based on the *ApMat* sequences.

Pathogenicity testing

The fruits/ leaves inoculated with conidial suspension of selected *Colletotrichum* isolates developed typical dark brown lesions of anthracnose disease around the wound (Figs. 3 and 4). The pathogens were re-isolated from the infected host tissues on to PDA medium to confirm the Koch's postulates. However, the control did not develop any symptoms after 7 days of inoculation. The results of pathogenicity testing are provided in Table 5. Based on the percent disease severity (PDS) calculations, *C. communis sp. nov.* proved to be highly pathogenic to the tropical fruits tested in this study. It produced anthracnose lesions on *Citrus* (Orange), *Mangifera* (Mango) and *Psidium* (Guava) fruits with 72.2, 77.7 and 83.3 % severity. *Colletotrichum endomangiferae* produced anthracnose lesions on mango fruits with 61.1–72.2 % severity. *Colletotrichum aotearoa* isolate was slightly pathogenic to *Musa* (banana) with 11.1 % disease severity while banana anthracnose pathogen *C. musae* was moderately pathogenic with 55.5 % disease severity. Tea anthracnose pathogen *G. cingulata* f. sp. *camelliae* isolate from this study was found to be moderately pathogenic with


MTCC 9663 Psidium India
ITCC 4981 Cocos India
GM514 Mangifera India
IMTF997 unknown India
IMTF976 unknown India
GS20 unknown India
GS07 Bauhinia India
GS02 Bauhinia India
GM409 Mangifera India
GM388 Mangifera India
GM291 Mangifera India
GM172 Mangifera India
GM063 Mangifera India
GM057 Mangifera India
C. dianesei (syn. C. melanocaulon) Coll131*
C. dianesei MFLU 1300058*
GN01 Azadirachta India
C. siamense ICMP 18578*
GC02 Coffea India
GC01 Coffea India
C. jasminisambac ICMP 19118*
NFCCI 1737 unknown India
MTCC 9660 Mangifera India
GUFCC 15502 Diffenbakia India
GM529 Mangifera India
GM473 Mangifera India
C. endomangiferae CMM3814*
ITCC 6166 Punica India
ITCC 6165 Punica India
ITCC 6163 Punica India
ITCC 6160 Punica India
ITCC 6066 Cocos India
GM385 Mangifera India
NK29 Mangifera India
NK28 Mangifera India
NK25 Mangifera India
C. communis NK24* Mangifera India
NK23 Mangifera India
NK22 Mangifera India
NFCCI 1925 unknown India
MTCC 4626 Psidium India
ITCC 6336 Cassia India
ITCC 6159 Psidium India
ITCC 6158 Mangifera India
ITCC 6155 Cassia India
ITCC 6152 Cassia India
ITCC 5123 Psidium India
IMTF738 Cassia India
IMTF737 Cassia India
IMTF736 Cassia India
GS29 unknown India
GS28 unknown India
GS22 unknown India
GS21 unknown India
GS19 unknown India
GS18 Psidium India
GS17 Psidium India
GS14 Ficus India
GS06 Saraca India
GS03 Bauhinia India
GO05 Citrus India
GO04 Citrus India
ITCC 6153 Cassia India
GS01 Bauhinia India
GO06 Citrus India
GO03 Citrus India
GO02 Citrus India
GO01 Citrus India
GM397 Mangifera India
GM314 Mangifera India
GM301 Mangifera India
GM192 Mangifera India
GM150 Mangifera India
GM043B Mangifera India
GM043A Mangifera India
GM018 Mangifera India
GM147 Mangifera India
GM010 Mangifera India
C. hymenocallidis ICMP 18642*
MTCC 9664 Carica India
C. salsolae ICMP 19051*
GM390 Mangifera India
C. queenslandicum ICMP 1778*
C. asianum ICMP 18580*
C. tropicale ICMP 18653*
C. aleinum ICMP 12071*
GBM3 Musa India
GB15 Musa India
GB07 Musa India
C. musae ICMP 19119*
C. nupharicola CBS 470*
C. fructicola (syn. C. ignotum) ICMP 18646*
C. fructicola ICMP 18581*
C. gloeosporioides ICMP 17821*
C. alatae ICMP 17919*
C. cordylinicola ICMP 18579*
C. psidii ICMP 19120*
TP05 Thea India
TP02 Thea India
TP01 Thea India
TB01 Thea India
C. temperatum Coll883*
C. kahawae subsp. kahawae ICMP 17816*
C. rhexiae Coll1026*
C. fructivorum Coll1414*
C. clidemiae ICMP 18658*
GBM2 Musa India
C. aotearoa ICMP 18537*
ITCC 6164 Punica India
ITCC 6161 Punica India
C. theobromicola (syn. C. fragariae) ICMP 17927*
C. theobromicola ICMP 18649*
C. horii ICMP 10492*
C. xanthorrhoeae ICMP 17903*
57
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92
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94
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85
71
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98
100
64
100
100
76
100
89
98
97
99
99
66
62
100
84
64
55
98
92
100
10
C. dianesei
C. siamense
Colletotrichum sp. indet. 1
C. jasmini-sambac
C. endomangiferae
Colletotrichum sp. indet. 2
Colletotrichum sp. indet. 3
C. communis sp. nov.
C. hymenocallidis
Colletotrichum sp. indet. 4
C. salsolae
Colletotrichum sp. indet. 5
C. queenslandicum
C. asianum
C. tropicale
C. alienum
C. musae
C. nupharicola
C. fructicola
C. gloeosporioides s. s.
C. alatae
C. cordylinicola
C. psidii
Glomerella cingulata f. sp. camelliae
C. temperatum
C. kahawae
C. rhexiae
C. fructivorum
C. clidemiae
C. aotearoa
C. theobromicola
C. horii
C. xanthorrhoeae

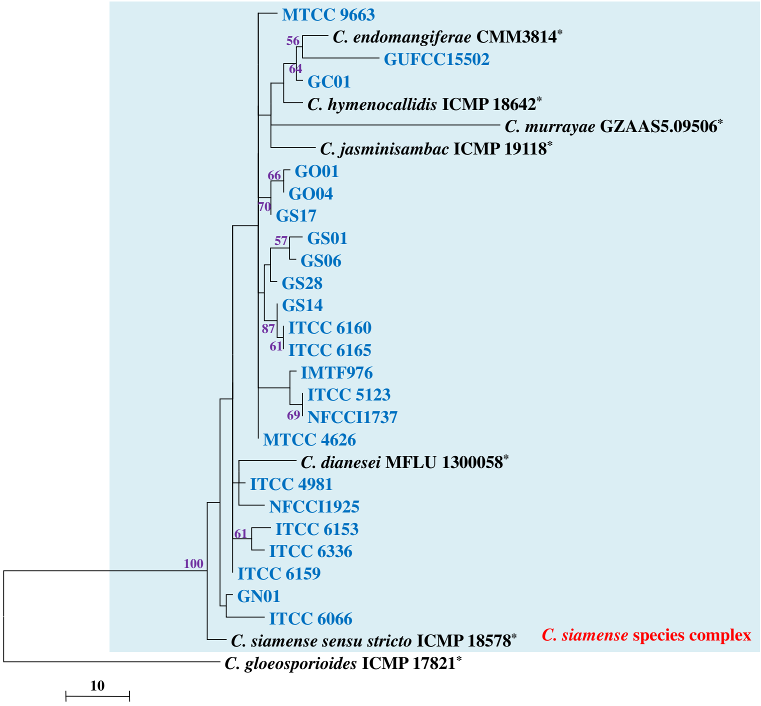


Fig. 2 One of the 90 most parsimonious trees showing phylogenetic affinities of 23 isolates of the *C. siamense* species complex from India (highlighted in blue), obtained from heuristic search of the 5-gene dataset (*act*, *cal*, *gapdh*, ITS and *tub2*). *Colletotrichum gloeosporioides* ICMP 17821 is the designated outgroup. Bootstrap support values of more than 50 %, are shown at the nodes. Ex-type isolates are marked with *

38.8 % disease severity, in the capacity to cause anthracnose lesion on *Camellia* (tea) leaves.

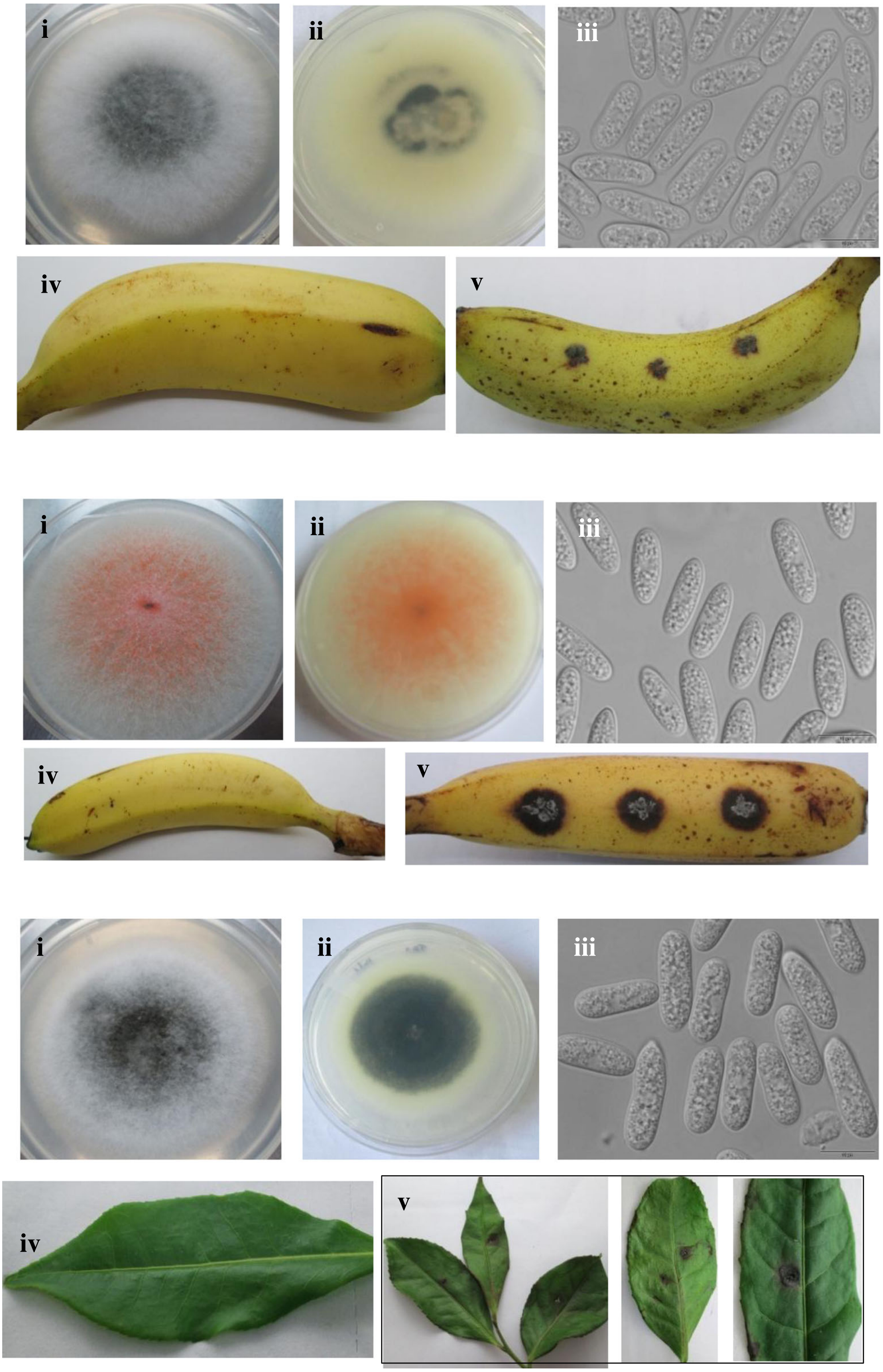
Discussion

Based on *ApMat* marker analysis, this study has established that the *C. siamense* species complex includes six previously known species (*C. dianesei*, *C. endomangiferae*, *C. hymenocallidis*, *C. jasmini-sambac*, *C. murrayae* and *C. siamense*) and one novel species *C. communis sp. nov.* (Table 6). We accept *C. hymenocallidis* Y.L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, *C. jasmini-sambac* S. Wikee, K.D. Hyde, L. Cai and E. H. C. McKenzie and *C. siamense* H. Prihastuti, L. Cai & K.D. Hyde as distinct species within the *C. siamense* species complex. Five novel lineages (potential new species), designated in this paper as *Colletotrichum* sp. indet 1–5, recovered within the *C. siamense* species complex (Table 6) are subject to further investigation with more isolates from diverse hosts.

The members of *C. siamense* species complex have been reported from several plants hosts (Yang et al. 2009; Wikee et al. 2011; Yang et al. 2011; Weir et al. 2012; Li et al. 2012; Cheng et al. 2013; Lima et al. 2013; Doyle et al. 2013; Sharma et al. 2013; Udayanga et al. 2013; James et al. 2014; Vieira et al. 2014). This study further reports the association of the members of *C. siamense* species complex with ashoka tree (*Saraca*), coconut (*Cocos*), coffee (*Coffea*), dumb cane (*Dieffenbachia*), golden shower tree (*Cassia*), guava (*Psidium*), mango (*Mangifera*), neem (*Azadirachta*), orange (*Citrus*), orchid tree (*Bauhinia*), papaya (*Carica*), pomegranate (*Punica*) and rubber plant (*Ficus*) in India. Additionally, this study reports *C. aotearoa* and *C. musae* from banana (*Musa*), *C. theobromicola* from pomegranate and *G. cingulata* f. sp. *camelliae* from tea (*Camellia*).

Though *Colletotrichum aotearoa* has been reported from New Zealand on a wide variety of native host plants (Weir et al. 2012), this is the first report on its association with banana fruits (*Musa* sp.). *Colletotrichum musae* is widely known as the pathogen of banana anthracnose (Abd-Elsalam et al. 2010; Su et al. 2011), but lesser known species such as *C. karstii* (Damm et al. 2012a) and *C. paxtonii* (Damm et al. 2012b) have also been associated with banana fruit. The pathogenic potential of *C. karstii* and *C. paxtonii* is poorly understood. In this study, we performed pathogenicity testing of *C. aotearoa* isolate (GBM2 = MTCC 11769) on banana fruits and the isolate exhibited a low pathogenic potential with 11.1 % disease severity, as compared to the *C. musae* isolate GBM3 with 55.5 % disease severity.

This study reports *G. cingulata* f. sp. *camelliae* from symptomatic tea (*Camellia sinensis*) leaves from tea gardens of Kangra district in Himachal Pradesh, India. As discussed in Weir et al. (2012), it was observed in our analysis that *G. cingulata* f. sp. *camelliae* isolates form a well-supported lineage within the Kahawae clade, both in the 5-gene (data not shown) and the *ApMat* marker based phylogenetic analyses (Fig. 1). The name *Glomerella cingulata* f. sp. *camelliae* was



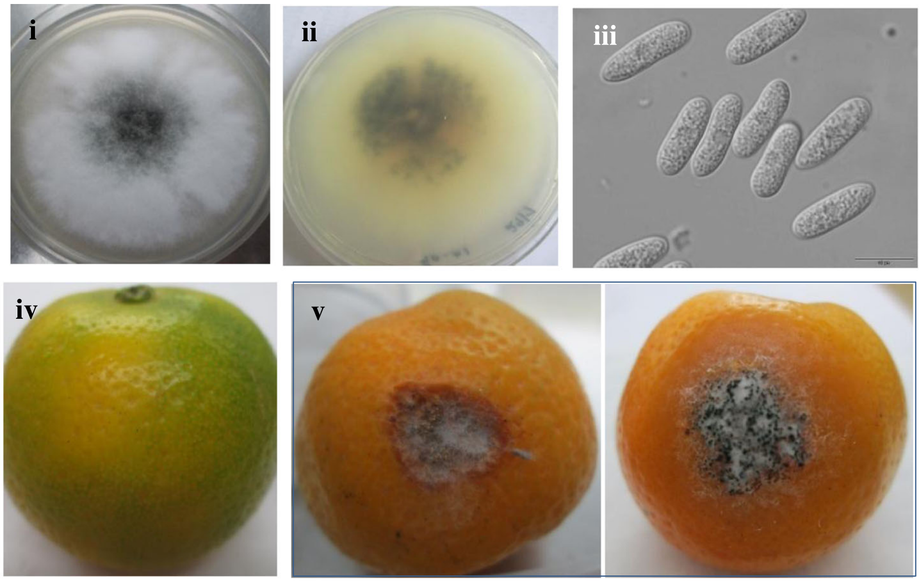
a. GBM2 = MTCC 11769 (*C. aotearoa*)

b. GBM3 = MTCC 11768 (*C. musae*)

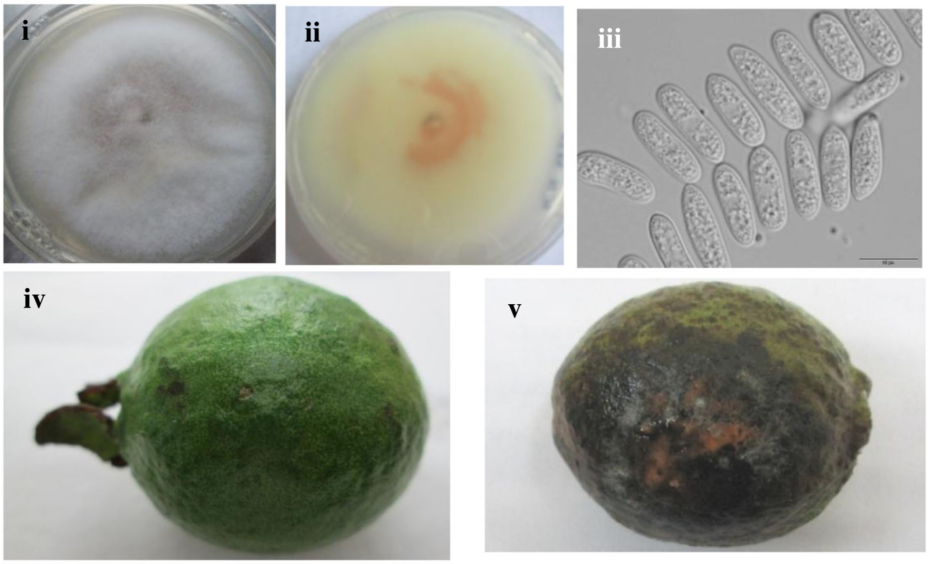
c. TB01 = MTCC 11728 (*G. cingulata* f. sp. *camelliae*)

Fig. 3 Morphology (after 7 days on PDA) and results of pathogenicity testing of selected isolates (**a**–**f**) on selected hosts i. Colony morphology (front), ii. Colony morphology (reverse), iii. Conidia (scale bar =10 μm), iv. Control fruit/ leaf, v. Fruit/ leaf seven days after inoculation

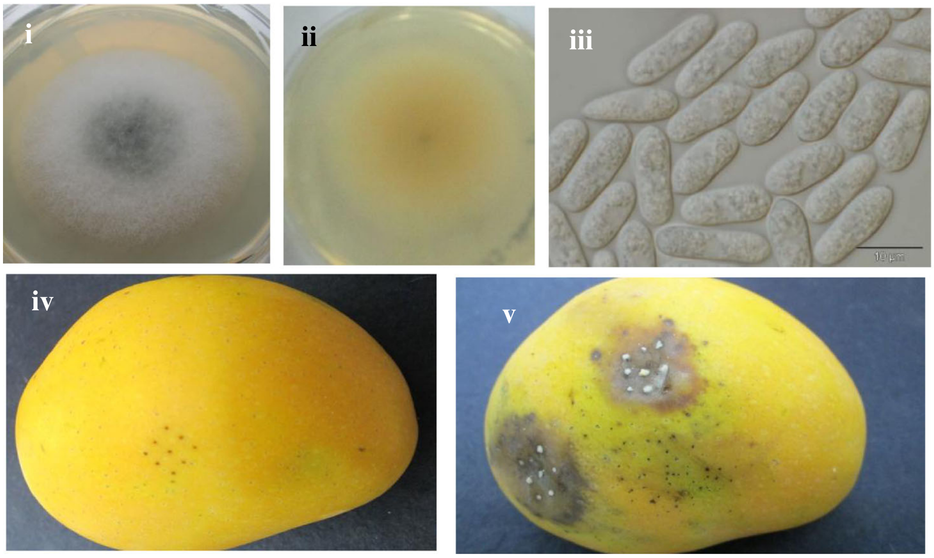
used by Dickens and Cook (1989) for the *C. gloeosporioides sensu lato* isolates associated with *Camellia* twig blight. This species has been reported from different *Camellia* hosts such as *Ca. japonica*, *Ca. oleifera*, *Ca. reticulata*, *Ca. saluenensis*, *Ca. sasanqua*, *Ca. sinensis* and *Ca.* × *williamsii* from Australia, China, France, Italy, Japan, Kenya, Korea, Malaysia,



d. GO01 = MTCC 11696
(*C. communis sp. nov.*)



e. MTCC 4626
(*C. communis sp. nov.*)



f. GM473 = MTCC 11589
(*C. endomangiferae*)

Fig. 3 (continued)

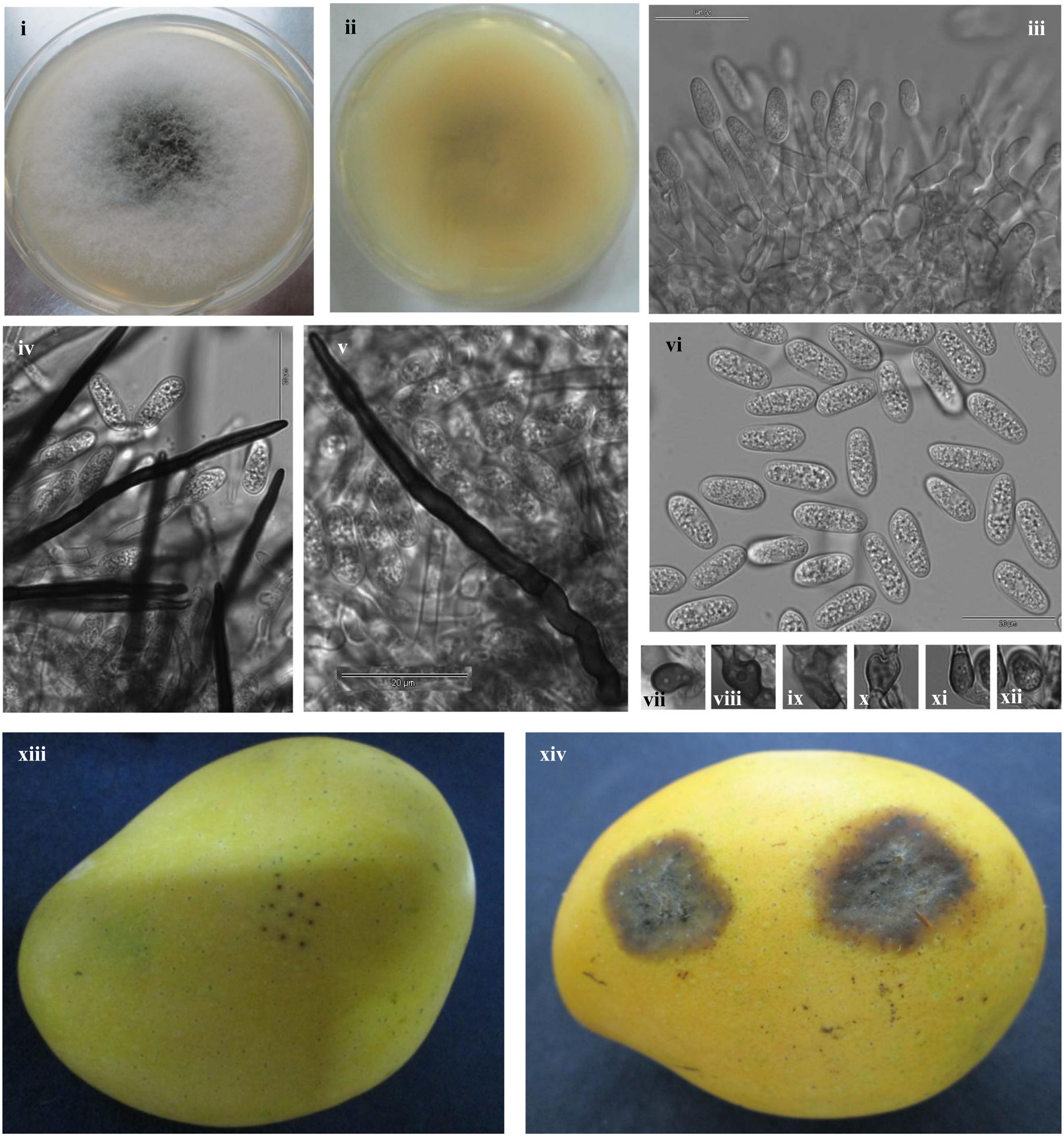


Fig. 4 Morphological features of *C. communis sp. nov.* (NK24* = MTCC 11599*) and results of pathogenicity testing **i-xii** Morphological features, **i** Colony morphology on PDA (front), **ii** Colony morphology on PDA (reverse), **iii** Conidiogenous cells, **iv-v** Setae, **vi** Conidia, **vii-xii** Appresoria (Scale bar of iii-vi =20 µm, Scale bar of vi applies to vii-xii), **xiii-xiv** Results of pathogenicity testing, **xiii** Control mango fruit, **xiv** symptoms 7 days after infection

Taiwan, Tanzania, UK, USA and Zimbabwe (Weir et al. 2012; Farr and Rossman 2014). However, the epitypification of this species is pending (Lei Cai, personal communication) and thus the identification of the tea isolates from this study is not definite in the absence of a valid type material.

This study has focussed on identification and description of cryptic species within the *C. siamense* species complex from India. Due to observed low level of genetic divergence among species complexes, we recommend the use of "powerful gene markers" such as *ApMat* marker. This will not only be time-saving, but also cost-effective in comparison with sequencing

Table 6 List of species in the *Colletotrichum siamense* species complex with information on host and geographic distribution

Sl. no.	Taxon	Authority	Host	Geographic distribution	Reference
1	*C. communis sp. nov.*	G. Sharma, A. K. Pinnaka, & B. D. Shenoy	*Bauhinia variegata*, *Cassia* sp., *Citrus* sp., *Ficus* sp., *Mangifera indica*, *Psidium* sp.,*Saraca indica*	India	This study
2	*C. dianesei* (syn. *C. melanocaulon*)	N. B. Lima, M. P. S. Câmara & S. J. Michereff	*Bauhinia variegata*, *Cocos* sp., *Mangifera indica*, *Psidium* sp., *Vaccinium macrocarpon*	Brazil, India	Doyle et al. 2013; Lima et al. 2013, This study
3	*C. endomangiferae*	W.A.S. Vieira, M.P.S. Câmara & S.J. Michereff	*Mangifera indica*, *Diffenbakia* sp.	Brazil, India	Vieira et al. 2014, This study
4	*C. hymenocallidis*	Y.L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai	*Hymenocallis americana*, *Hymenocallis* sp.	China	Cai et al. 2009; Yang et al. 2009; Yang et al. 2011; Li et al. 2012; Weir et al. 2012
5	*C. jasmini-sambac*	S. Wikee, K.D. Hyde, L. Cai and E. H. C. McKenzie	*Jasminum sambac*	China, Vietnam	Wikee et al. 2011; Li et al. 2012; Weir et al. 2012
6	*C. murrayae**	L.J. Peng & K.D. Hyde	*Murraya* sp.	China	Peng et al. 2012
7	*C. siamense*	H. Prihastuti, L. Cai & K.D. Hyde	*Arundina graminifolia*, *Azadirachta* sp., *Capsicum annuum*, *Carica papaya*, *Citrus reticulata*, *Coffea arabica*, *Coffea* sp., *Commelina* sp., *Dioscorea rotundata*, *Fragaria ananassa*, *Malus domestica*, *Mangifera indica*, *Persea americana*, *Pistacia vera*, *Protea cynaroides*, *Vitis vinifera*	Australia, Brazil, China, Colombia, India, Kenya, Malawi, Nigeria, South Africa, Thailand, USA, Zimbabwe	Cai et al. 2009; Prihastuti et al. 2009; Yang et al. 2009, 2011; Li et al. 2012; Silva et al. 2012; Weir et al. 2012; Cheng et al. 2013; Liu et al. 2013, This study
8	*Colletotrichum* sp. indet. 1	–	*Coffea robusta*	India	This study
9	*Colletotrichum* sp. indet. 2	–	*Punica granatum*	India	This study
10	*Colletotrichum* sp. indet. 3	–	*Cocos* sp., *Mangifera indica*	India	This study
11	*Colletotrichum* sp. indet. 4	–	*Carica papaya*	India	This study
12	*Colletotrichum* sp. indet. 5	–	*Mangifera indica*	India	This study

[* - not a legitimate name]

and analysing 5–8 genes. We promote the *ApMat* marker as an efficient marker for finer phylogenetic resolution within the *C. siamense* species complex and *C. gloeosporioides sensu lato*. There is a need to strengthen the *ApMat* dataset by sequencing missing type strains, so that a comprehensive phylogenetic analysis could be done by the researchers in future. A consensus among the researchers on gene markers to be used while describing a new *Colletotrichum* species is desirable.

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