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Identification and characterization of *Colletotrichum* species associated with durian fruit in northern Thailand

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Abstract

Colletotrichum is one of the most important plant pathogenic genera affecting different plant species, particularly tropical and sub-tropical crops and fruits. Species of the genus can cause many diseases, including fruit rots, crown rots, stem end rots, and anthracnose. The objective of the present study was to identify the *Colletotrichum* species associated with durian fruit rots in northern Thailand. Based on morphological study and phylogenetic analyses of five loci (internal transcribed spacer (ITS), actin (*ACT*), chitin synthase 1 (*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and ß-tubulin (*TUB2*)), four species belonging to three complexes were identified and described. *Colletotrichum durionigenum* is introduced as a new species, and *C. gigasporum*, *C. pandanicola*, and *C. truncatum* are described and illustrated as new host records.

Keywords – 1 new species – Diversity – *Durio* spp. – Glomerellaceae – Phylogeny – Phytopathogen

Introduction

Durian (*Durio* sp., Malvaceae) is a tropical fruit native to Southeast Asia and one of the most popular fruits in Thailand (Teh et al. 2017). This fruit is known as the 'king of fruits' for its formidable spiny husk, unique overwhelming flavor and odor (Bampenrat et al. 2020, Li et al. 2012). The three leading durian-producing countries are Thailand, Malaysia, and Indonesia. Thailand is the main durian producer in the world. Thailand's commercial durian production was 1,111.93 thousand metric tons in 2020 (National Agricultural Big Data Center; OAE 2021).

Durian is one of the most economically important exported fruits in Thailand (Charoensumran et al. 2021). Thailand's most popular cultivars for consumption and export include 'Chanee', 'Gaan Yaow', 'Gradumtong', 'Nok Yib', 'Puang Mani' and 'Monthong' (Lim and Sangchote 2003). Sweeter cultivars with a mild odor are popular in Thailand (Monthong), whereas pungent and bitter varieties are popular in Malaysia and Singapore (Musang King) (Teh et al. 2017). The complexity of different cultivars and distinctive odors is due to differences in sulfur volatiles, esters, alcohol, and acid percentages (Siriphanich 2011). A draft genome assembly of *D. zibethinus* was provided by Teh et al. (2017).

Durian is also subjected to different kinds of fungal diseases: fruit rots, stem cankers and root rots, resulting in low yield and quality. Among these diseases, fruit rots are a serious problem in

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durian production for domestic consumption and export (Siriphanich 2011). *Colletotrichum* includes important plant pathogens, endophytes, saprobes, and human pathogens (Jayawardena et al. 2021a). As plant pathogens, *Colletotrichum* is a member of the family Glomerellaceae (Glomerellales, Sordariomycetes) (Hyde et al. 2020, Wijayawardene et al. 2022), and the species are known to cause anthracnose disease, fruit rots, crown rots, leaf spots, and stem end rots in pre- and postharvest (Cannon et al. 2012, Jayawardena et al. 2021a). Two hundred and eighty species with molecular data are accepted in this genus with 16 species complexes and 15 singleton species (Liu et al. 2022). The use of polyphasic approaches has enabled the correct identification of *Colletotrichum* species (Bhunjun et al. 2021, Damm et al. 2018, Hyde et al. 2014, Liu et al. 2022). However, knowledge of the overall species diversity and host distribution is largely incomplete (Jayawardena et al. 2021a, Bhunjun et al. 2022, Liu et al. 2022). Hence, to fill this gap, this study aims to identify *Colletotrichum* species associated with durian in Thailand based on morphology and phylogenetic analyses.

Materials & Methods

Sample collection, examination, and isolation

Fresh samples of durian fruit were collected from orchards in Chiang Rai province, Thailand, from 2021 to 2022. Samples were brought to the laboratory in Zip-lock plastic bags for examination. The fruiting bodies on natural substrates were observed and photographed using a stereo-microscope (OLYMPUS-SZX16). Morphological characters were observed using a LEICA-EZ4 stereo-microscope and photographed with an optical microscope equipped with a Nikon DS-Ri2 camera. The photo plates were made by Adobe Photoshop v. 21.1.2 software. Measurements were done using the Tarosoft (R) Image Frame Work software.

Single spore isolation was employed to obtain pure cultures, following the methods described by Senanayake et al. (2020). The pure cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand. Specimens were deposited in the herbarium of Mae Fah Luang University (MFLU). Faces of Fungi (FoF) and Index Fungorum numbers were acquired for the new species as described in Jayasiri et al. (2015). Moreover, the novel species was submitted to the GMS webpage (Chaiwan et al. 2021). Based on the recommendations provided by Chethana et al. (2021) and Jayawardena et al. (2021b), the new species has been confirmed.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fresh mycelia grown on potato dextrose agar (PDA) for 10 days using the DNA Extraction Kit (Omega Bio-Tek) according to the manufacturer's instructions. The internal transcribed spacer (ITS), actin (*ACT*), chitin synthase (*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and β-tubulin (*TUB2*) were amplified using primers given in Table 1. The polymerase chain reaction was performed in a total volume of 25 µL, containing 12.5 µL of 2 × Power Taq PCR Master Mix, 1 µL of each primer (20 µM), 1 µL genomic DNA, and 9.5 µL deionized water. The PCR procedure was performed using the following conditions: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation for 30 s at 95 °C; annealing at 53 °C for 60 s (ITS), 55 °C for 50 s (*ACT*), 58 °C for 30 s (*CHS-1*); 58 °C for 50 s (*GAPDH*), 58 °C for 90 s (*TUB2*); extension at 72 °C for 60 s; and the final extension at 72 °C for 10 min. PCR amplification was performed in an Eppendorf (Master cycler X50s) thermal cycler. PCR products were sequenced by the SolGent Co, Republic of Korea.

Phylogenetic analyses

The retrieved sequences were BLAST-searched, and comparable reference sequences were downloaded from GenBank (Table 2) in accordance with the results of those searches and recently published papers on *Colletotrichum* (Jayawardena et al. 2020, 2021a, Liu et al. 2022). Utilizing MAFFT v.7 under the web server (http://mafft.cbrc.jp/alignment/server), the individual loci were manually adjusted (Katoh et al. 2019). Where necessary, the alignment was further adjusted using BioEdit v. 7.0.9.0 (Hall 1999). Command-based TrimAl software and the gappyout approach were

used to trim aligned sequences automatically. Maximum likelihood (ML) analysis with the GTR + GAMMA model of nucleotide evolution was carried out using RAxML-HPC2 on XSEDE with bootstrapping of 1000 replicates. The Bayesian analysis was conducted using a Markov Chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities (BYPP) using MrBayes on XSEDE (Ronquist et al. 2012). On the CIPRES online platform, the best-fit evolutionary models for each dataset were assessed using jModeltest 2.1.10 through the Akaike Information Criterion (AIC). Four MCMC chains were run from random trees for 1,000,000 generations and sampled every 100th generation. The first 25% of the generated trees were discarded as burn-in, and the remaining trees were used for calculating posterior probabilities. Using PAUP XSEDE (Swofford 2002), a maximum parsimony (MP) analysis was performed. Gaps were deemed to be missing data, and ambiguously aligned areas were discarded. The analyses were performed on the CIPRES Science Gateway (https://www.phylo.org/portal2) (Miller et al. 2011). A multi-locus concatenated sequence dataset (ITS, *ACT*, *CHS-1*, *GAPDH*, and *TUB2*) of closely related species was used for a pairwise homoplasy index (PHI) test using Splits Tree 4 (version 4.14.2) to determine the recombination level (Fu et al. 2019). The phylograms were visualized in FigTree v. 1.4.0 (Rambaut 2014) and annotated in Adobe Illustrator CC 22.0.0 (Adobe Systems, San Jose, CA, USA).

Table 1 Primers used in the study.

Gene	Primer	Sequence (5'-3')	References
ITS	ITS 5	GGA AGT AAA AGT CGT AAC AAG G	White et al. (1990)
	ITS 4	TCC TCC GCT TAT TGA TAT GC	
ACT	ACT-512F	ATG TGC AAG GCC GGT TTC GC	Carbone & Kohn (1999)
	ACT-783R	TAC GAG TCC TTC TGG CCC AT	
CHS-1	CHS-79F	TGG GGC AAG GAT GCT TGG AAG AAG	Carbone & Kohn (1999)
	CHS-345R	TGG AAG AAC CAT CTG TGA GAG TTG	
GAPDH	GDF	GCC GTC AAC GAC CCC TTC ATT GA	Templeton et al. (1992)
	GDR	GGG TGG AGT CGT ACT TGA GCA TGT	
TUB2	BT-2Fd	GTB CAC CTY CAR ACC GGY CAR TG	Woudenberg et al. (2009)
	BT-4Rd	CCR GAY TGR CCR AAR ACR AAG TTG TC	

Table 2 Taxa with their respective GenBank accession numbers used in the phylogenetic analyses.

Taxa	Strains	GenBank accession numbers				
		ITS	***GAPDH***	***CHS-1***	***ACT***	***TUB***
Colletotrichum Acidae	MFLUCC 17-2659*	MG996505	MH003691	MH003694	MH003697	MH003700
C. acidae	MFLU 18-0233	MG996506	MH003692	MH003695	MH003698	MH003701
C. aenigma	ICMP 18608*	JX010244	JX010044	JX009774	JX009443	JX010389
C. aeschynomenes	ICMP 17673*, ATCC 201874	JX010176	JX009930	JX009799	JX009483	JX010392
C. alatae	CBS 304.67*, ICMP 17919	JX010190	JX009990	JX009837	JX009471	JX010383
C. alienum	ICMP 12071*	JX010251	JX010028	JX009882	JX009572	JX010411
C. aotearoa	ICMP 18537*	JX010205	JX010005	JX009853	–	JX010420
C. arecicola	CGMCC 3.19667*	MK914635	–	MK935541	MK935374	MK935498

Table 2 Continued.

Taxa	Strains	GenBank accession numbers				
		ITS	***GAPDH***	***CHS-1***	***ACT***	***TUB***
C. artocarpicola	MFLUCC 18-1167*	MN415991	MN435568	MN435569	MN435570	MN435567
C. arxii	CBS 132551*	KF687716	KF687843	KF687780	KF687802	KF687881
C. arxii	CBS 169.59, IMI 304050, IMI 309371	KF687717	KF687824	KF687781	KF687784	KF687868
C. asianum	ICMP 18580*, CBS 130418	JX010196	JX010053	JX009867	JX009584	JX010406
C. analogum	YMF1.06943*	OK030860	OK513663	OK513559	OK513599	OK513629
C. camelliae	CGMCC 3.14925, LC1364*	KJ955081	KJ954782	MZ799255	KJ954363	KJ955230
C. cangyuanensis	YMF1.05001*	OK030864	OK513667	OK513563	OK513603	OK513633
C. changpingense	CGMCC 3.17582*, SA0016, MFLUCC 15-0022	KP683152	KP852469	KP852449	KP683093	KP852490
C. chiangmaiense	MFLUCC 18-0945*	MW346499	MW548592	MW623653	MW655578	–
C. chrysophilum	URM 7368, CMM4268*	KX094252	KX094183	KX094083	KX093982	KX094285
C. cigarro	ICMP 18539*	JX010230	JX009966	JX009800	JX009523	JX010434
C. clidemiae	ICMP 18658*	JX010265	JX009989	JX009877	JX009537	JX010438
C. cobbittiense	BRIP 66219*	MH087016	MH094133	MH094135	MH094134	MH094137
C. conoides	CGMCC 3.17615, CAUG17, LC6226*	KP890168	KP890162	KP890156	KP890144	KP890174
C. cordylinicola	MFLUCC 090551*, ICMP 18579	JX010226	JX009975	JX009864	HM470234	JX010440
C. crousii	LC13858, MH0588*	MZ595876	MZ664059	MZ799281	MZ664174	MZ673995
C. crousii	LC13860, MH0592	MZ595878	MZ664061	MZ799282	MZ664176	MZ673997
C. curcumae	IMI 288937*	GU227893	GU228285	GU228383	GU227991	GU228187
C. dimorphum	YMF1.07309*	OK030867	OK513670	OK513566	OK513606	OK513636
C. dracaenigenum	MFLUCC 19-0430*	MN921250	MT215577	MT215575	MT313686	–
C. durionigenum	**MFLUCC 22-0111***	**OP740244**	**OP744505**	**OP744504**	**OP744503**	**OP744506**

Table 2 Continued.

Taxa	Strains	GenBank accession numbers				
		ITS	***GAPDH***	***CHS-1***	***ACT***	***TUB***
C. endophytica	MFLUCC 13-0418, LC0324*	KC633854	KC832854	MZ799261	KF306258	MZ673954
C. fici-septicae	MFLU 19-2770*	MW114367	MW183774	MW177701	MW151585	–
C. fructicola	ICMP 18581*, CBS 130416	JX010165	JX010033	JX009866	FJ907426	JX010405
C. fructivorum	Coll1414, BPI 884103, CBS 133125*	JX145145	MZ664047	MZ799259	MZ664126	JX145196
C. fusiforme	MFLU 13-0291*	KT290266	KT290255	KT290253	KT290251	KT290256
C. gigasporum	CBS 133266, MUCL 44947*	KF687715	KF687822	KF687761	–	KF687866
C. gigasporum	CBS 101881	KF687736	KF687841	KF687777	KF687797	KF687886
C. gigasporum	CBS 181.52	KF687734	KF687838	KF687775	KF687799	KF687885
C. gigasporum	CBS 109355	KF687729	KF687827	KF687774	KF687798	KF687870
C. gigasporum	CBS 125385	KF687732	KF687835	KF687764	KF687787	KF687872
C. gigasporum	CBS 125387	KF687733	KF687834	KF687765	KF687788	KF687873
C. gigasporum	CBS 125730	KF687735	KF687840	KF687770	KF687793	KF687878
C. gigasporum	CBS 125476	KF687728	KF687833	KF687767	KF687790	KF687875
C. gigasporum	CBS 124947	KF687731	KF687828	KF687763	KF687786	KF687871
C. gigasporum	CBS 125731	KF687727	KF687837	KF687771	KF687794	KF687879
C. gigasporum	CBS 132884	KF687730	KF687830	KF687773	KF687796	–
C. gigasporum	CBS 125475	KF687723	KF687836	KF687766	KF687789	KF687874
C. gigasporum	CBS 132881	KF687725	KF687829	KF687772	KF687795	KF687880
C. gigasporum	CBS 159.75	KF687726	KF687839	KF687776	KF687783	KF687884
C. gigasporum	**MFLUCC 22-0108**	**OP740245**	**OP744509**	**OP744508**	**OP744507**	**OP744510**
C. gloeosporioides	IMI 356878*, ICMP 17821, CBS 112999	JQ005152	JQ005239	JQ005326	JQ005500	JQ005587
C. gracile	YMF1.06939*	OK030868	OK513671	OK513567	OK513607	OK513637

Table 2 Continued.

Taxa	Strains	GenBank accession numbers				
		ITS	***GAPDH***	***CHS-1***	***ACT***	***TUB***
C. grevilleae	CBS 132879, CPC 15481*	KC297078	KC297010	KC296987	KC296941	KC297102
C. grossum	CGMCC3.17614, CAUG7, LC6227*	KP890165	KP890159	KP890153	KP890141	KP890171
C. hebeiense	MFLUCC 13-0726*	KF156863	KF377495	KF289008	KF377532	KF288975
C. hederiicola	MFLU 15-0689*	MN631384	–	MN635794	MN635795	–
C. helleniense	CBS 142418, CPC 26844*	KY856446	KY856270	KY856186	KY856019	KY856528
C. henanense	LC3030, CGMCC 3.17354, LF238*	KJ955109	KJ954810	MZ799256	KM023257	KJ955257
C. horii	NBRC 7478*, ICMP 10492, MTCC 10841	GQ329690	GQ329681	JX009752	JX009438	JX010450
C. hystricis	CBS 142411, CPC 28153*	KY856450	KY856274	KY856190	KY856023	KY856532
C. jiangxiense	CGMCC 3.17363*	KJ955201	KJ954902	–	KJ954471	KJ955348
C. jishouense	GMBC 0209*, GZU_HJ2_G3	MH482929	MH681658	–	MH708135	MH727473
C. jishouense	GZU_HJ2_G2	MH482931	MH681657	–	MH708134	MH727472
C. jishouense	GZU_HJ2_G4	MH482932	MH681659	–	MH708136	MH727474
C. jishouense	GZU_HJ3_J5	MH482930	MH492706	–	MH708137	–
C. kahawae	IMI 319418*, ICMP 17816	JX010231	JX010012	JX009813	JX009452	JX010444
C. magnisporum	CBS 398.84*	KF687718	KF687842	KF687782	KF687803	KF687882
C. makassarense	CBS 143664*	MH728812	MH728820	MH805850	MH781480	MH846563
C. mengyinense	SAUCC200702*	MW786742	–	MW883686	MW883695	MW888970
C. musae	CBS 116870*, ICMP	HQ596292	HQ596299	JX009896	HQ596284	HQ596280

Table 2 Continued.

Taxa	Strains	GenBank accession numbers				
		ITS	***GAPDH***	***CHS-1***	***ACT***	***TUB***
	19119, MTCC 11349					
C. nanhuaensis	YMF1.04993*	OK030870	OK513673	OK513569	OK513609	OK513639
C. nullisetosum	YMF1.06946*	OK030872	OK513675	OK513571	OK513611	OK513641
C. nupharicola	CBS 470.96*, ICMP 18187	JX010187	JX009972	JX009835	JX009437	JX010398
C. oblongisporum	YMF1.06938*	OK030874	OK513677	OK513573	–	OK513643
C. parvisporum	YMF1.06942*	OK030876	OK513679	OK513575	OK513613	OK513645
C. pandanicola	MFLUCC 17-0571*	MG646967	MG646934	MG646931	MG646938	MG646926
C. pandanicola	**MFLUCC 22-0109**	**OP740246**	**OP744513**	**OP744512**	**OP744511**	**OP744514**
C. perseae	CBS 141365*, GA100	KX620308	KX620242	MZ799260	KX620145	KX620341
C. proteae	CBS 132882*	KC297079	KC297009	KC296986	KC296940	KC297101
C. pseudomajus	CBS 571.88*	KF687722	KF687826	KF687779	KF687801	KF687883
C. pseudotheobromicola	MFLUCC 18-1602*	MH817395	MH853675	MH853678	MH853681	MH853684
C. psidii	CBS 145.29*, ICMP 19120	JX010219	JX009967	JX009901	JX009515	JX010443
C. queenslandicum	ICMP 1778*	JX010276	JX009934	JX009899	JX009447	JX010414
C. radicis	CBS 529.93*	KF687719	KF687825	KF687762	KF687785	KF687869
C. rhexiae	Coll1026, BPI 884112, CBS 133134*	JX145128	MZ664046	MZ799258	MZ664127	JX145179
C. salsolae	ICMP 19051*	JX010242	JX009916	JX009863	JX009562	JX010403
C. serranegrense	COAD 2100*	KY400111	–	KY407894	KY407892	KY407896
C. siamense	ICMP 18578*, CBS 130417	FJ972613	FJ972575	JX009865	FJ907423	FJ907438
C. siamense	HSI-3	OM654563	OM831360	OM831354	OM831342	OM831384
C. sp.	CBS 159.50	KF687724	KF687823	KF687778	KF687800	KF687867
C. subacidae	LC13857, LH01*	MZ595846	MZ664068	MZ799307	MZ664144	MZ673967
C. subacidae	NN054605	MZ595893	MZ664075	MZ799309	MZ664191	MZ674011

Table 2 Continued.

Taxa	Strains	GenBank accession numbers				
		ITS	***GAPDH***	***CHS-1***	***ACT***	***TUB***
C. subvariabile	LC13876, NN040649*	MZ595883	MZ664054	MZ799343	MZ664181	MZ674001
C. syzygiicola	DNCL021, MFLUCC 10-0624*	KF242094	KF242156	–	KF157801	KF254880
C. tainanense	CBS 143666*	MH728818	MH728823	MH805845	MH781475	MH846558
C. temperatum	CBS 133122*, Coll883, BPI 884100	JX145159	MZ664045	MZ799254	MZ664125	JX145211
C. tengchongense	YMF 1.04950*	OL842169	OL981264	OL981290	OL981238	–
C. theobromicola	CBS 124945*, ICMP 18649	JX010294	JX010006	JX009869	JX009444	JX010447
C. ti	ICMP 4832*	JX010269	JX009952	JX009898	JX009520	JX010442
C. tropicale	CBS 124949*, ICMP 18653, MTCC 11371	JX010264	JX010007	JX009870	JX009489	JX010407
C. truncatum	CBS 151.35*	GU227862	GU228254	GU228352	GU227960	GU228156
C. truncatum	CBS 120709	GU227877	GU228269	GU228367	GU227975	GU228171
C. truncatum	CBS 141.79	GU227873	GU228265	GU228363	GU227971	GU228167
C. truncatum	IMI 135524	GU227874	GU228266	GU228364	GU227972	GU228168
C. truncatum	CBS 710.70	GU227864	GU228256	GU228354	GU227962	GU228158
C. truncatum	**MFLUCC 22-0110**	**OP740247**	**OP744517**	**OP744516**	**OP744515**	**OP744518**
C. variabile	LC13875, NN040656*	MZ595884	MZ664055	MZ799344	MZ664182	MZ674002
C. vietnamense	CBS 125478, LD16 (L2)*	KF687721	KF687832	KF687769	KF687792	KF687877
C. vietnamense	CBS 125477, BMT25 (L3)	KF687720	KF687831	KF687768	KF687791	KF687876
C. viniferum	GZAAS 5.08601*, yg1	JN412804	JN412798	–	JN412795	JN412813
C. vulgaris	YMF 1.04940*	OL842170	OL981265	OL981291	OL981239	–
C. wuxiense	CGMCC 3.17894*	KU251591	KU252045	KU251939	KU251672	KU252200
C. xanthorrhoeae	BRIP 45094*, ICMP 17903, CBS 127831	JX010261	JX009927	JX009823	JX009478	JX010448

Table 2 Continued.

Taxa	Strains	GenBank accession numbers				
		ITS	***GAPDH***	***CHS-1***	***ACT***	***TUB***
C. xishuangbannaense	MFLUCC 19-0107*	MW346469	MW537586	MW660832	MW652294	–
C. yulongense	CFCC 50818*	MH751507	MK108986	MH793605	MH777394	MK108987
C. yunajiangensis	YMF1.04996*	OK030885	OK513686	OK513583	OK513620	OK513649
C. zhaoqingense	LC13877, NN058985*	MZ595905	MZ664065	MZ799304	MZ664203	MZ674023
C. zhaoqingense	LC13878, NN071035	MZ595906	MZ664066	MZ799305	MZ664204	MZ674024

"*" indicates type strains and the newly generated sequences are in bold.

Results

Multi-locus Phylogeny

Phylogenetic analyses were performed based on a five-locus concatenated alignment of ITS, *ACT*, *CHS-1*, *GAPDH*, and *TUB2*. We constructed three phylograms separately for the *C. gigasporum*, *C. gloeosporioides*, and *C. truncatum* species complexes. The concatenated alignments for each complex were subjected to ML, MP, and BYPP analyses.

The dataset for the *C. gigasporum* species complex comprised 35 taxa and 1708 characters, including gaps, with 2 outgroup taxa (*C. crousii* LC13858 and *C. crousii* LC13860). Parsimony analysis revealed the presence of 1318 constant characters (proportion = 0.771663), 104 uninformative characters, and 286 parsimony-informative characters. A single most parsimonious tree (Tree Length (TL) = 690, Consistency Index (CI) = 0.696, Retention Index (RI) = 0.861, Rescaled Consistency (RC) = 0.599, Homoplasy Index (HI) = 0.304) was obtained by the parsimony analysis (Fig. 1).

The data matrix for the *C. gloeosporioides* species complex included 72 taxa and 1636 characters, including gaps, with 2 outgroup taxa (*C. acidae* MFLUCC 17-2659 and *C. truncatum* CBS:151.35). Parsimony analysis showed 987 constant characters (proportion = 0.603301), 267 variable characters, and 382 parsimony-informative characters. The most parsimonious tree (TL = 1296, CI = 0.676, RI = 0.812, RC = 0.549, HI = 0.324) was shown (Fig. 5).

The dataset for the *C. truncatum* species complex consisted of 13 taxa and 1637 characters, including gaps, with 1 outgroup (*C. arxii* CBS 132511). Parsimony analysis indicated 1240 constant characters (proportion = 0.757483), 214 variable characters, and 183 parsimony-informative characters. The most parsimonious tree (TL = 532, CI = 0.889, RI = 0.914, RC = 0.813, HI = 0.111) constructed by parsimony analysis was shown (Fig. 7).

The phylogenetic tree showed that two isolates belong to the *Colletotrichum gigasporum* species complex clade. Of these, one isolate clustered with *C. gigasporum*, representing 100% ML, 100% MP and 100% BYPP values (Fig. 1). Another obtained isolate made a separate branch from *C. magnisporum* with 73% ML, 74% MP, and 94% BYPP values and introduced as a new species, namely *Colletotrichum durionigenum* (Fig. 1). *Colletotrichum durionigenum* differs from *C. magnisporum* (CBS 398.84) by a 0.96% bp difference in ITS, 1.51% bp difference in *ACT*, 1.68% bp difference in *CHS-1*, 9.02% bp difference in *GAPDH*, and 4.602% bp difference in *TUB2* (Table 3). Since more strains of *C. magnisporum* were not available, we compared only the type strain with our species. A pairwise homoplasy index (PHI) test indicated no significant recombination ($\Phi w = 1.0$) between *C. durionigenum* and its closely related taxa (Fig. 3). *CHS-1* and *TUB2* sequences were not available for *C. jishouense* (GZU_HJ3_J5), hence it has not been included in the PHI test. Phylogenetic analyses showed that one obtained isolate fell into the *C. gloeosporioides* species complex and clustered with *C. pandanicola*, showing 99% BYPP. Finally, this isolate was identified as *C. pandanicola* based on the phylogenetic result and morphological comparison (Fig. 5).

One isolate belongs to the *Colletotrichum truncatum* species complex, clustering with *C. truncatum* strains with 100% ML, 100%, MP, and 100% BYPP. We identified this isolate as *C. truncatum* based on morpho-molecular results (Fig. 7).

Table 3 Base pair differences between *C. durionigenum* and *C. magnisporum* (CBS 398.84).

Taxon	Gene regions				
	ITS	***ACT***	***CHS-1***	***GAPDH***	***TUB2***
C. magnisporum (CBS 398.84)	5/519 bp	4/264 bp	5/298 bp	12/133 bp	22/478 bp

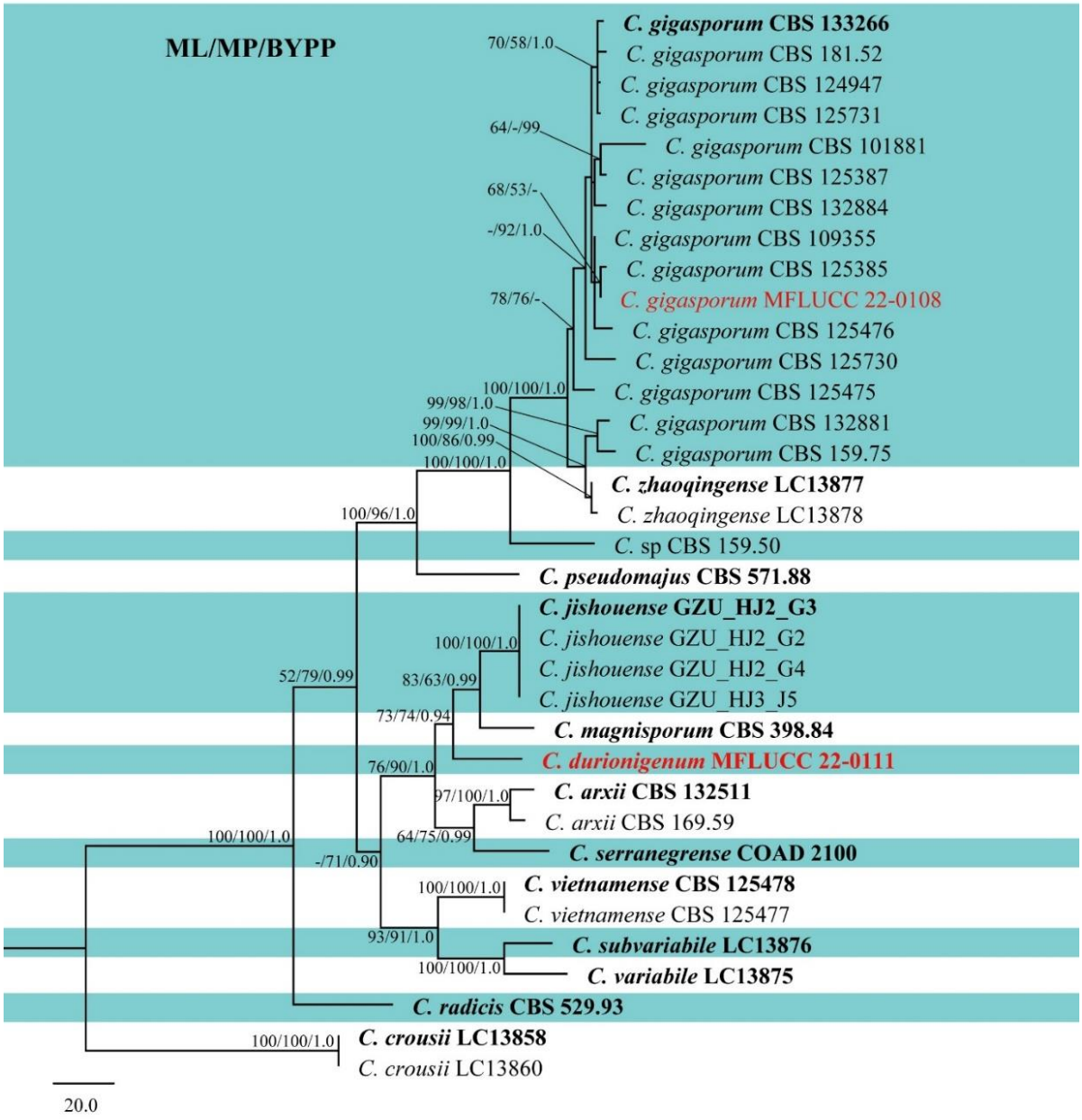

Figure 1 – Phylogenetic tree of the *Colletotrichum gigasporum* species complex generated by maximum parsimony analysis of combined ITS, *ACT*, *CHS-1*, *GAPDH* and *TUB2* sequence data. The tree was rooted with *Colletotrichum crousii* (LC13858) and *Colletotrichum crousii* (LC13860). Maximum likelihood and maximum parsimony bootstrap support values ≥50% (BT) as well as bayesian posterior probabilities ≥0.90 (PP) are shown respectively near the nodes. Type strains are in bold, and the newly generated isolates are in red.

Colletotrichum durionigenum A. Armand, Jayawar. & K.D. Hyde, sp. nov. Fig. 2

Index Fungorum number: IF559996; Facesoffungi number: FoF13362

Etymology – "*durio*" refers to the plant genus from which the fungus was isolated, and "*durionigenum*" is a Latin combination meaning "Durio-borne".

Holotype – MFLU 22-0194

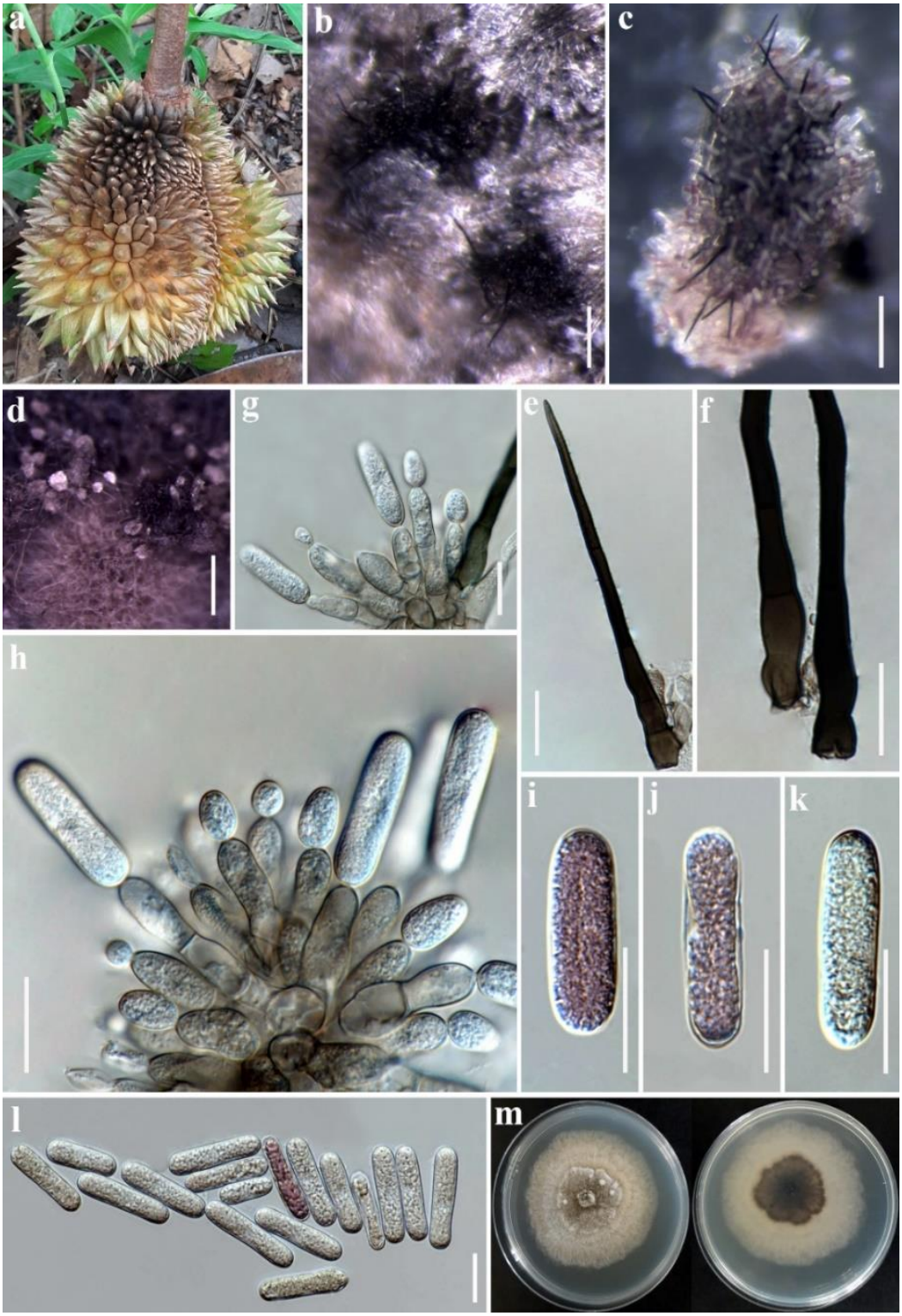

Figure 2 – *Colletotrichum durionigenum* (MFLU 22-0194, holotype). a Rotting durian fruit. b Acervuli on the host. c Acervulus and conidial mass in water drop. d Acervuli and conidial masses on the PDA. e, f Setae. g, h Conidiophores, conidiogenous cells, and conidial attachment. i–l Conidia. m Upper and reverse view of culture on PDA. Scale bars: b–d = 100 µm, e–l = 20 µm.

Associated with durian fruit rot. Sexual morph: Not observed. Asexual morph: *Vegetative hyphae* hyaline to brown, smooth-walled, septate, branched. *Conidiomata* acervular, dark brown, bearing conidial masses and setae. *Setae* brown to dark brown, smooth-walled, 2–5-septate, 107–176 μm ($\bar{x}$ = 135.5 μm, n = 10) long, base conic or inflated, 8.5–12.5 μm diam. ($\bar{x}$ = 10.5 μm, n = 10), tip acute or obtuse. *Conidiophores* medium brown to brown, septate, branched. *Conidiogenous cells* hyaline to pale brown, cylindrical or clavate, 18.5–39 × 6.5–10 μm ($\bar{x}$ = 28.5 × 8 μm, n = 30). *Conidia* hyaline or pale purple, aseptate, smooth-walled, cylindrical with rounded ends, guttulate, 29–40 × 8.5–11.5 μm ($\bar{x}$ = 35 × 10 μm, n = 30).

Culture characteristics – Colonies on PDA 56–60 mm in diam. after 7 days, velvety, medium sparse, flat with undulated margin, circular. Surface olivaceous-grey in the centre, becoming olivaceous black after 30 days; reverse olivaceous-grey to olivaceous-black. Colonies on OA 59–65 mm in diam. after 7 days, flat with entire margins; surface olivaceous-grey, reverse iron-grey. *Appressoria* not observed.

Material examined – Thailand, Chiang Rai Province, Wiang Chiang Rung District, Thung Ko Sub-district. On durian rotting fruit, 04 July 2022, Alireza Armand, P132-2 (MFLU 22-0194, holotype), ex-holotype culture, MFLUCC 22-0111.

Notes – Phylogenetically, *C. magnisporum* (CBS 398.84) is the closest taxon to *Colletotrichum durionigenum*. However, *C. durionigenum* (MFLU 22-0194) can be differentiated from *C. magnisporum* based on phylogenetic analyses and morphological features. The base pair differences between these two species were 48 bp in all five gene regions (48 bp/1676 bp). *Colletotrichum durionigenum* differs from *C. magnisporum* by producing non-verruculose setae that are considerably bigger than those of *C. magnisporum* (107–176 μm in *C. durionigenum* vs. 42.5–105 μm in *C. magnisporum*). Moreover, *C. durionigenum* is distinguishable by producing longer conidiogenous cells (18.5–39 × 6.5–10 μm in *C. durionigenum* vs. 18–33.5 × 5.5–10 μm in *C. magnisporum*). Although the two species produce conidia with a same shape, the conidia of *C. durionigenum* are slightly larger than those of *C. magnisporum* (29–40 × 8.5–11.5 μm in *C. durionigenum* vs. 28–39 × 8.5– 10.5 μm in *C. magnisporum*). Additionally, *C. durionigenum* (MFLU 22-0194) produces conidia with pale purple pigments which have not been described in *C. magnisporum* (Liu et al. 2014).

Colletotrichum gigasporum E.F. Rakotoniriana & Munaut, Mycol. Progr. 12: 407. 2013

Fig. 4

Index Fungorum number: IF800175; Facesoffungi number: FoF10777

Associated with durian fruit rot. Sexual morph: Not observed. Asexual morph: *Vegetative hyphae* hyaline to brown, smooth-walled, septate, branched. *Conidiomata* acervular, dark brown, bearing conidial masses and setae. *Setae* brown to dark brown, smooth-walled to verruculose, 2–4-septate, 95–145 μm ($\bar{x}$ = 127.5 μm, n = 10) long, base cylindrical, 6–7.5 μm diam. ($\bar{x}$ = 6.5 μm, n = 10), tip obtuse to acute. *Conidiophores* pale brown to brown, septate, branched. *Conidiogenous cells* hyaline to pale brown, cylindrical or clavate, 15–27 × 5–7.5 μm ($\bar{x}$ = 19 × 6.5 μm, n = 30). *Conidia* hyaline, aseptate, smooth-walled, cylindrical with rounded ends, guttulate, 25–30.5 × 7–8.5 μm ($\bar{x}$ = 28 × 8 μm, n = 30).

Culture characteristics – Colonies on PDA 45–67 mm in diam. after 7 days, velvety, circular, entire in margins; surface olivaceous-grey, reverse olivaceous-grey to olivaceous-black. Colonies on OA 50–65 mm in diam. after 7 days. entire in margins, surface white to pale olivaceous-grey, reverse iron-grey. *Appressoria* produced on slide culture, pale brown to brown, undulated to strongly lobate, irregular in shape, 9.5–17.5 × 6.5–8 μm ($\bar{x}$ = 12.5 × 7.5 μm, n = 10), produced directly from mycelia.

Material examined – Thailand, Chiang Rai Province, Mueang Chiang Rai District, Huai Sak Sub-district. On rotting durian fruit, 27 June 2022, Alireza Armand, P103 (MFLU 22-0192), living culture, MFLUCC 22-0108.

Notes – Strains of *C. gigasporum* clustered together with our strain in a distinct clade with the highest bootstrap values (Fig. 1). The base pair differences between *C. gigasporum* (MFLUCC 22-0108) and *C. gigasporum* (CBS 133266, holotype) revealed no difference in ITS, 0.7% (2/298 bp) in

CHS-1, 2.2% (3/133 bp) in *GAPDH*, and 0.4% (2/478) in *TUB2*. Actin's sequences were not available for the type strain. Morphologically, *C. gigasporum* (MFLU 22-0192) is similar to *C. gigasporum* (CBS 133266, holotype). However, it produced slightly smaller conidia (25–30.5 × 7–8.5 μm in *C. gigasporum* (MFLU 22-0192) vs. (22)25–29(32) × (6)7–9 μm in *C. gigasporum* (CBS 133266, holotype)) and longer setae (95–145 μm in *C. gigasporum* (MFLU 22-0192) vs. 90–140 μm in *C. gigasporum* (CBS 133266, holotype)) (Rakotoniriana et al. 2013).

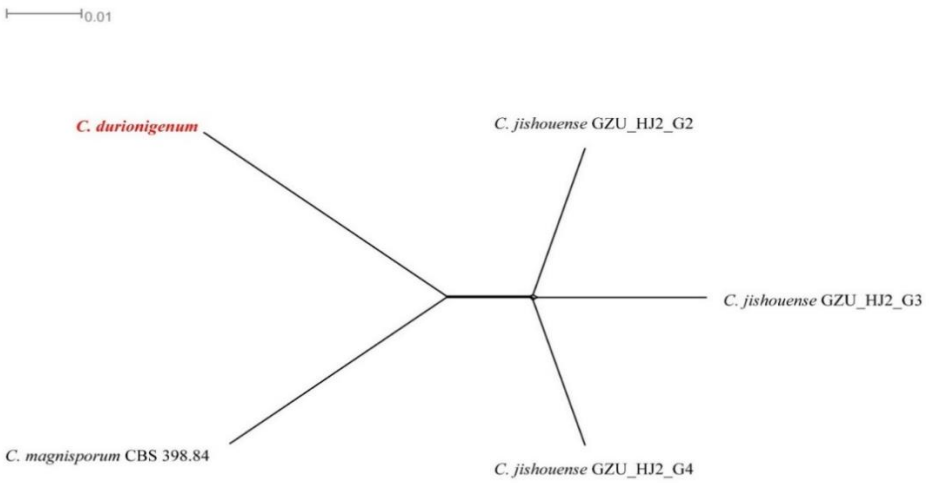

Figure 3 – Pairwise homoplasy index (PHI) test of *C. durionigenum* and closely related species using both LogDet transformation and splits decomposition. PHI test results (Φw) <0.05 indicate significant recombination among the species.

Colletotrichum pandanicola Tibpromma & K.D. Hyde, MycoKeys. 33: 25. 2018

Fig. 6

Index Fungorum number: IF823841; Facesoffungi number: FoF03906

Associated with durian fruit. Sexual morph: Not observed. Asexual morph: *Vegetative hyphae* hyaline to pale brown, smooth-walled, septate, branched. *Conidiomata* acervular, dark brown, bearing conidial masses. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, cylindrical to inflated. C*onidiogenous cells* hyaline, cylindrical, 13.5–19 × 2.7–3 μm ($\bar{x}$ = 16.5 × 3.3 μm, n = 25). *Conidia* hyaline, aseptate, smooth-walled, cylindrical with rounded ends tapering slightly towards the base, guttulate, 14–17.5 × 4.5–5.5 μm ($\bar{x}$ = 15.5 × 5 μm, n = 30).

Culture characteristics – Colonies on PDA 63–65 mm in diam. after 7 days, velvety, circular, entire in margins; surface white to olivaceous-grey, reverse same color. Colonies on OA 57–67 mm in diam. after 7 days. entire in margins, aerial mycelia abundant, surface white to whitish grey, reverse same color. *Appressoria* produced on slide culture, pale brown to dark brown, non-rounded, undulated to lobate, irregular in shape, 6–11 × 4.5–6 μm ($\bar{x}$ = 8.5 × 5 μm, n = 10), produced directly from mycelia.

Material examined – Thailand, Chiang Rai Province, Mueang Chiang Rai District, Huai Sak Sub-district. On rotting durian fruit, 27 June 2022, Alireza Armand, P103-2 (MFLU 22-0193), living culture, MFLUCC 22-0109.

Notes – Based on the phylogenetic tree, *C. pandanicola* (MFLUCC 22-0109) clustered with *C. pandanicola* (MFLUCC 17-0571, ex-holotype) with a high bayesian posterior probabilities value (0.99) (Fig. 5). The base pair differences between *C. pandanicola* (MFLUCC 22-0109) and the type strain revealed 0.4% (2/511 bp) differences in ITS, 0.8 (2/254 bp) differences in *ACT*, 1.4% (3/215 bp) differences in *CHS-1*, no difference in *GAPDH* and *TUB2*. Morphologically, *C. pandanicola* (MFLU 22-0193) is similar to *C. pandanicola* (MFLU 18-0003, holotype). However, the type strain produces slightly larger conidia (14–17.5 × 4.5–5.5 μm in *C. pandanicola* (MFLU 22-0193) vs. 9–18 × 4–8 μm in *C. pandanicola* (MFLU 18-0003, holotype)) (Tibpromma et al. 2018).

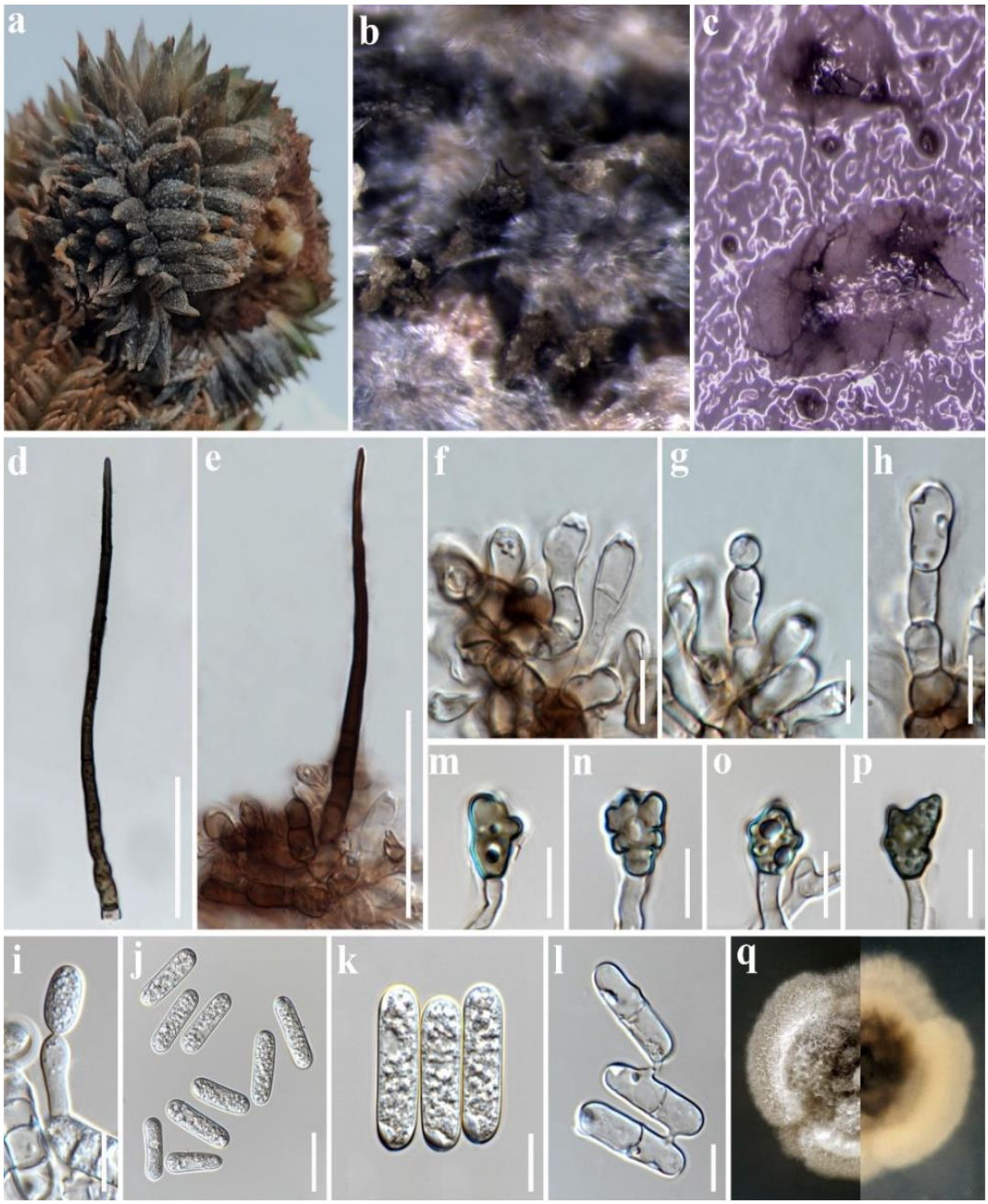

Figure 4 – *Colletotrichum gigasporum* (MFLUCC 22-0108). a Rotting durian fruit. b Acervuli on the host. c Acervuli on the PDA. d, e Setae. f–h Conidiophores and conidiogenous cells. i Conidial attachment. j, k Conidia. l Conidial anastomosis. m–p Appressoria. q Upper and reverse view of culture on PDA. Scale bars: d, e = 50 µm, f–i = 10 µm, j = 20 µm, k–p = 10 µm.

Colletotrichum truncatum (Schwein.) Andrus & W.D. Moore, Phytopathology 25: 122. 1935

Fig. 8

Index Fungorum number: IF280780; Facesoffungi number: FoF03827

Associated with durian fruit rot. Sexual morph: Not observed. Asexual morph: *Vegetative hyphae* hyaline, smooth-walled, septate, branched. *Conidiomata* acervular, dark brown, bearing conidial masses and setae. *Setae* brown to dark brown, smooth to verruculose, 2–4 septate, 125–175 µm long ($\bar{x}$ = 155.5 µm, n = 10), base cylindrical, 4–7 µm diam ($\bar{x}$ = 5 µm, n = 10), acute at the apex. *Conidiophores* hyaline, densely clustered. *Conidiogenous cells* hyaline, cylindrical, 18–30 × 2.5–4 µm ($\bar{x}$ = 23.5 × 3 µm, n = 30), collarette not visible. *Conidia* hyaline, smooth-walled, aseptate, curved with parallel walls at the middle part, round and truncate at the base, tapering towards the acute and curved apex, guttulate, 27.5–31 × 3.3–4.4 µm ($\bar{x}$ = 29 × 3.8 µm, n = 30).

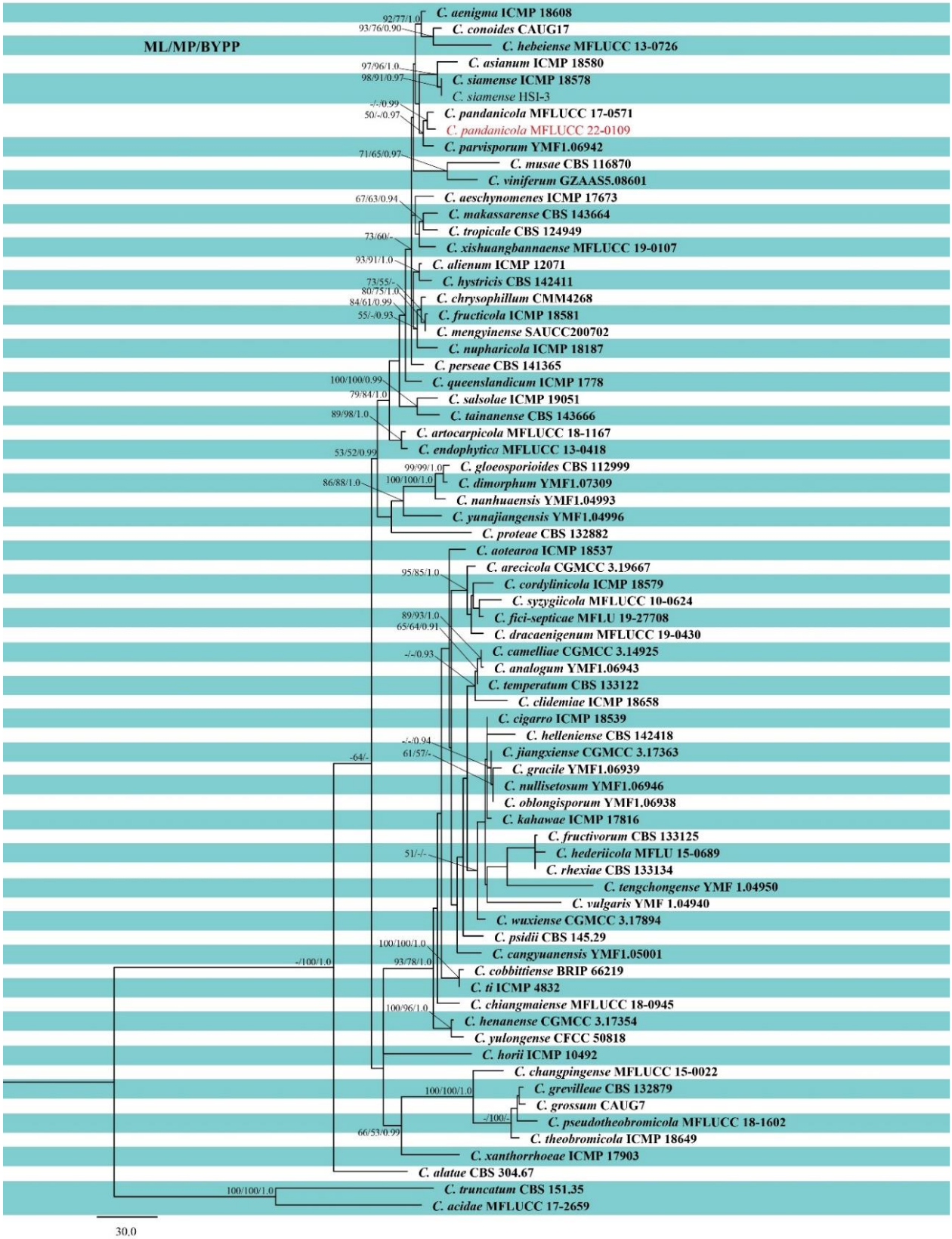

Figure 5 – Phylogenetic tree of the *Colletotrichum gloeosporioides* species complex generated by maximum parsimony analysis of combined ITS, *ACT*, *CHS-1*, *GAPDH* and *TUB2* sequence data. The tree was rooted with *Colletotrichum acidae* (MFLUCC 17-2659) and *Colletotrichum truncatum* (CBS:151.35). Maximum likelihood and maximum parsimony bootstrap support values ≥50% (BT) as well as bayesian posterior probabilities ≥0.90 (PP) are shown respectively near the nodes. Type strains are in bold, and the newly generated isolate is in red.

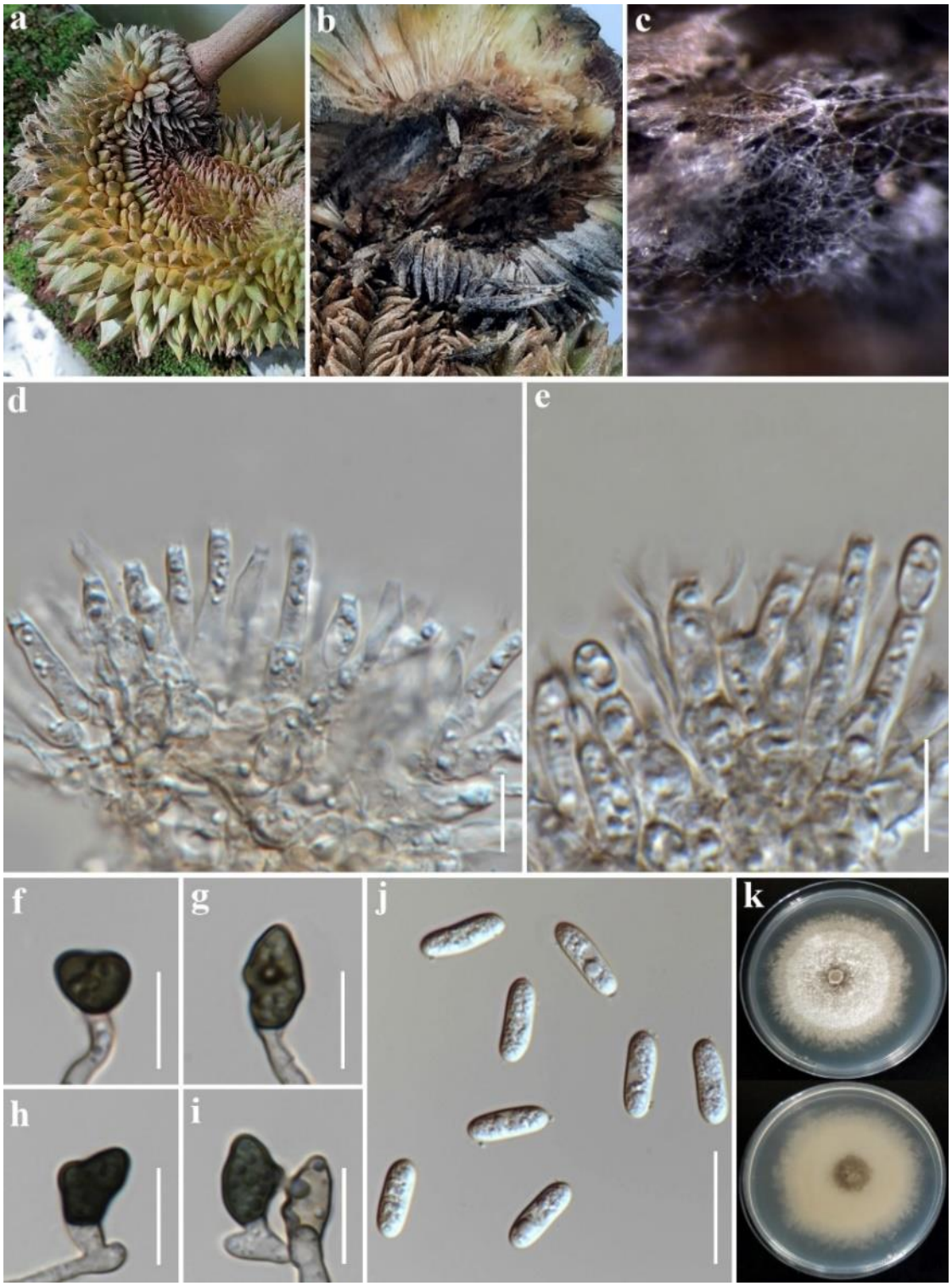

Figure 6 – *Colletotrichum pandanicola* (MFLU 22-0193). a, b Rotting durian fruit. c Acervuli on the host. d, e Conidiophores, conidiogenous cells and conidial attachment. i Conidial attachment. f–i Appressoria. j Conidia. k Upper and reverse view of culture on PDA. Scale bars: d–i = 10 µm, j = 20 µm.

Culture characteristics – Colonies on PDA 30 mm in diam. after 7 days, with pigment diffusion into PDA, flat, undulated; surface buff; reverse pale luteous. Colonies on OA 25 mm in diam. after 7 days. flat with entire in margins; surface buff, reverse buff to pale olivaceous-grey. *Appressoria* produced on slide culture, pale brown to brown, circular or undulated, non-lobate, 4.5–9 µm in diam. ($\bar{x}$ = 6.5 µm, n = 20), produced from both mycelia and conidia.

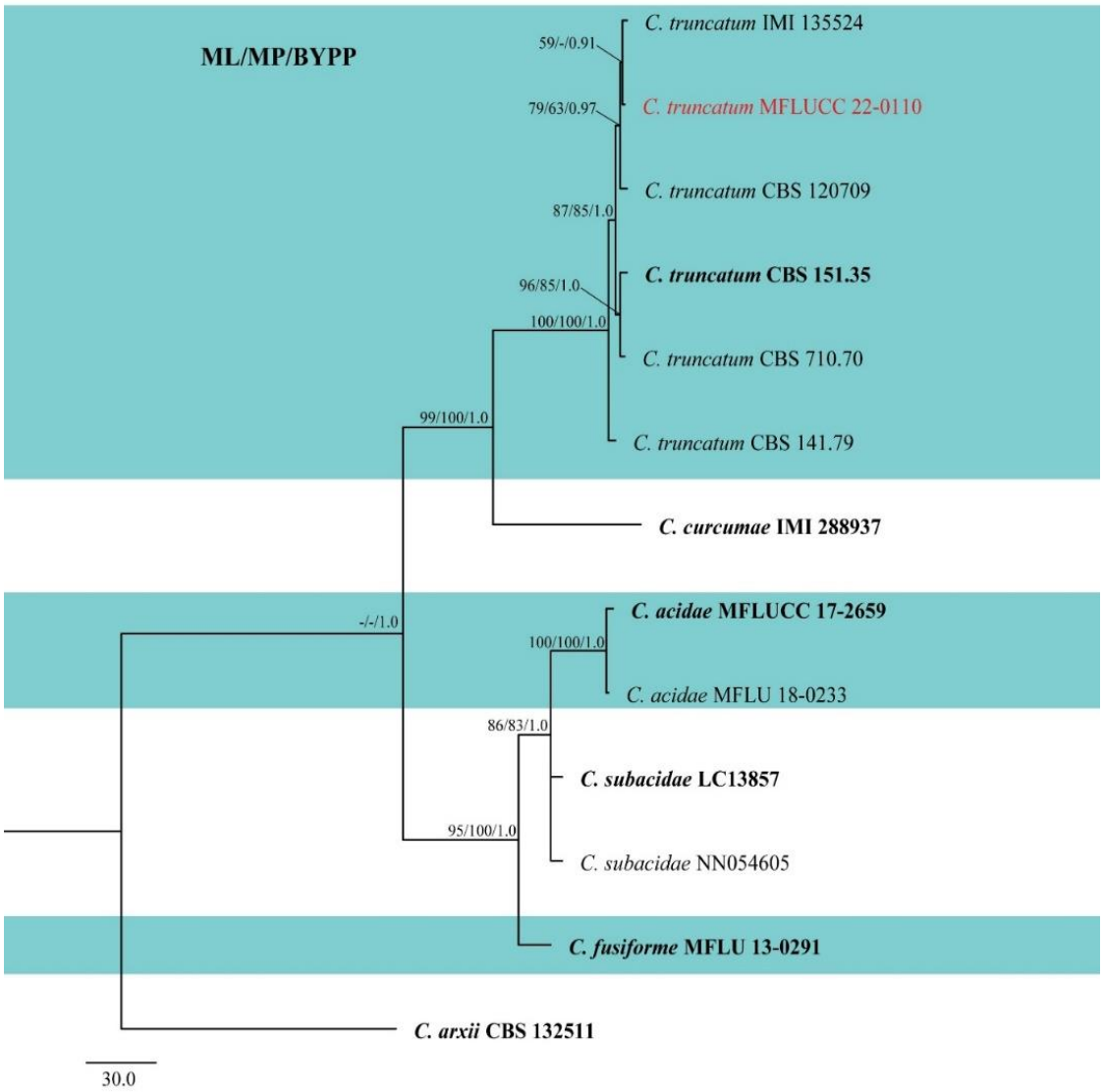

Figure 7 – Phylogenetic tree of the *Colletotrichum truncatum* species complex generated by maximum parsimony analysis of combined ITS, *ACT*, *CHS-1*, *GAPDH* and *TUB2* sequence data. The tree was rooted with *Colletotrichum arxii* (CBS 132511). Maximum likelihood and maximum parsimony bootstrap support values ≥50% (BT) as well as bayesian posterior probabilities ≥0.90 (PP) are shown respectively near the nodes. Type strains are in bold, and the newly generated isolate is in red.

Material examined – Thailand, Chiang Rai Province, Wiang Chiang Rung District, Thung Ko Sub-district. On durian fruit, 04 July 2022, Alireza Armand, P130 (MFLU 22-0191), living culture, MFLUCC 22-0110.

Notes – Strains of *C. truncatum* clustered together with our strain in a distinct clade with the highest bootstrap value (Fig. 7). The base pair differences between *C. truncatum* (MFLUCC 22-0110) and *C. truncatum* (CBS:151.35, ex-epitype) revealed no difference in ITS and *TUB2*, 0.9% (2/224 bp) in *ACT*, 1.8% (4/225 bp) in *CHS-1*, and 1.3% (3/231 bp) in *GAPDH*. Morphologically, *C. truncatum* (MFLU 22-0191) is similar to *C. truncatum* (CBS:151.35). However, it produced considerably longer setae (125–175 µm in *C. truncatum* (MFLU 22-0191) vs 80–150 µm in *C. truncatum* (CBS:151.35)), longer conidiogenous cells (18–30 in *C. truncatum* (MFLU 22-0191) vs 6–20 in *C. truncatum* (CBS:151.35)) and longer conidia (27.5–31 µm in *C. truncatum* (MFLU 22-0191) vs 20–23.5 (–26) µm in *C. truncatum* (CBS:151.35)) (Damm et al. 2009).

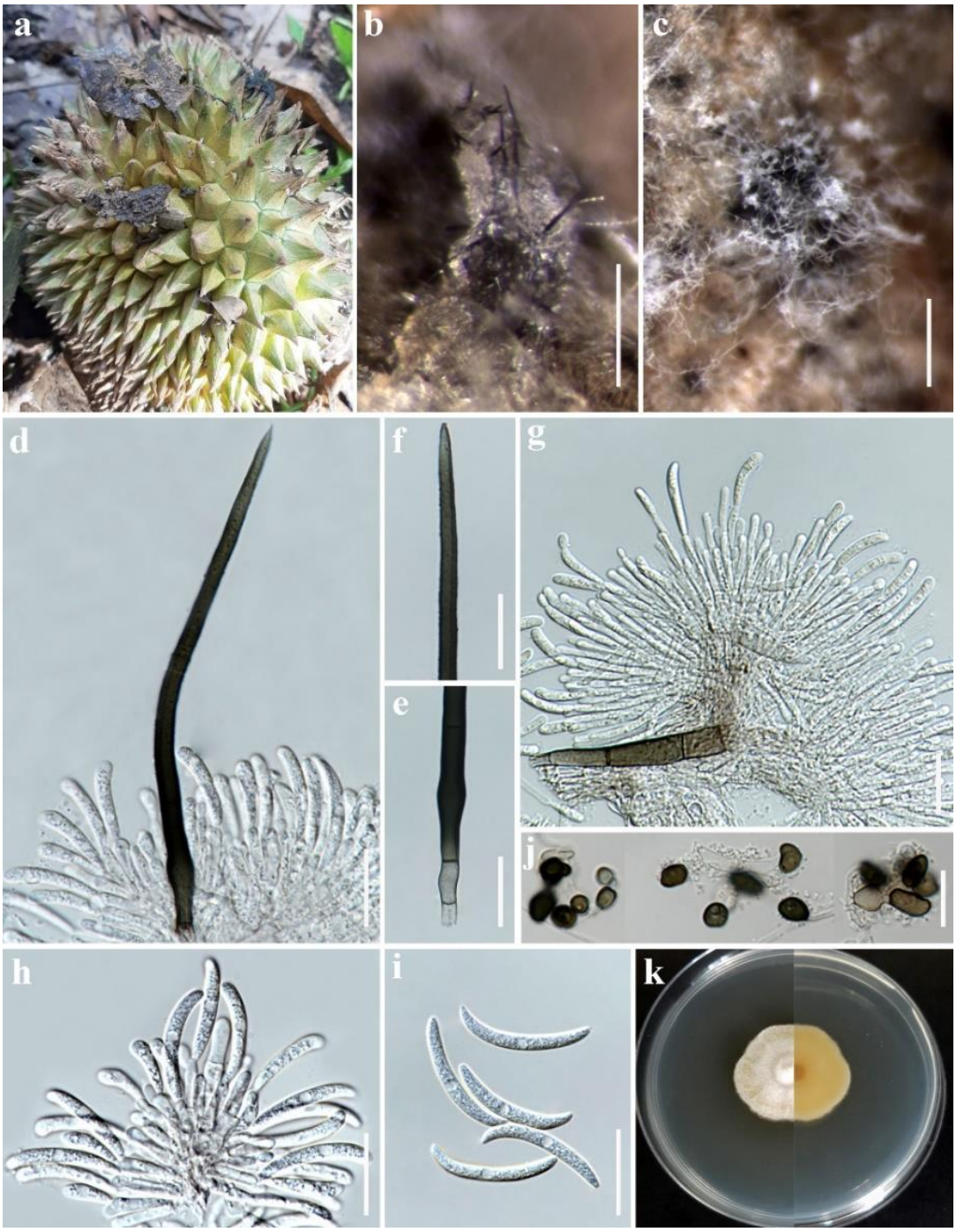

Figure 8 – *Colletotrichum truncatum* (MFLU 22-0191). a Rotting durian fruit. b, c Acervuli on the host. d–f Setae. g, h Conidiogenous cells and conidial attachment. i Conidia. j Appressoria. k Upper and reverse view of culture on PDA. – Scale bars: b = 200 µm, c = 250 µm, d–i = 20 µm, j = 10 µm.

Discussion

Fresh durian fruits with rot symptoms were collected from northern Thailand and examined. A combined morpho-molecular approach was adopted to identify *Colletotrichum* species associated with durian. To understand the species phylogenetic relationships in *C. gigasporum*, *C. gloeosporioides* and *C. truncatum* species complexes, we constructed three separate phylograms with ML, MP, and BYPP analyses based on five loci, including ITS, *ACT*, *CHS-1*, *GAPDH*, and *TUB2*.

According to the MP distance tree (Fig. 1), *Colletotrichum durionigenum* is a new species with a distinct lineage with 73/74/0.94 ML, MP, and BYPP bootstrap values, respectively, and is basal for *C. magnisporum* and *C. jishouense*. Moreover, morphological comparison confirmed the

phylogenetic results. *Colletotrichum durionigenum* differs from *C. magnisporum* in having larger setae, conidia and producing longer conidiogenous cells (Liu et al. 2014). Although conidial pigmentation is not a strong morphological feature, producing pale purple pigments in conidia of *C. durionigenum* (MFLU 22-0194, holotype) is worthy of documentation. *Colletotrichum durionigenum* was directly isolated from fresh durian fruit with rotting symptoms, as it was well developed and produced fruiting bodies on the fruit. However, *Colletotrichum magnisporum* was introduced from an unknown host (Liu et al. 2014), collected before 1984, and it was the only report of *C. magnisporum* in nature. Talhinhas & Baroncelli. (2021) speculated that this species might be extinct and suggested further studies for the assurance of its occurrence in nature.

The phylogenetic tree showed that *Colletotrichum gigasporum* strains clustered together, indicating high genetic variations within the species (Fig. 1). Morphological comparison revealed some minor differences between our strain (MFLUCC 22-0108) and *C. gigasporum* (CBS 133266, holotype) in the size of conidia and setae which are not surprising due to their high genetic variations. *Colletotrichum gigasporum* was introduced in 2013 from Stylo (*Stylosanthes guianensis*) and Kodavan (*Centella asiatica*) (Rakotoniriana et al. 2013). It has also been described from Thailand on Taro (*Alocasia* sp.) and Chinese Hibiscus (*Hibiscus rosa-sinensis*) (Liu et al. 2014). This study illustrated *C. gigasporum* as a new host record, associated with durian fruit rot.

Most species within the *C. gloeosporioides* species complex such as *C. asianum*, *C. fructicola*, *C. gloeosporioides*, *C. musae*, *C. siamense*, *C. tropicale*, and *C. viniferum* were originally isolated from tropical and sub-tropical fruits. *Colletotrichum pandanicola* was described on *Pandanus* sp. (Pandanaceae) from Thailand as an endophyte (Tibpromma et al. 2018). In this study, we described and illustrated *C. pandanicola* as a new record association with durian fruit. Phylogenetic analyses (Fig. 5) and morphological comparison both confirmed the identification accuracy.

Based on the phylogenetic tree (Fig. 7), *C. truncatum* strains clustered together with type strain and showed high genetic variations within the species. The morphological comparison confirmed the phylogenetic results. However, we found differences in the size of setae, conidiogenous cells, and conidia between *C. truncatum* (MFLU 22-0191) and *C. truncatum* (CBS 151.35) which can authenticate inter-specific variations. *Colletotrichum truncatum* has been reported on different plant hosts, including *Glycine max* (Giatgong 1980), *Glycine ussuriensis* (Lenne 1990), *Solanum melongena* (Richardson 1990), *Capsicum* sp. (Shenoy et al. 2007) and *Manihot esculenta* (Sangpueak et al. 2018) from Thailand. This is the first report of *C. truncatum* being associated with durian fruit in Thailand. Among *Colletotrichum* species recorded in Thailand, only *C. gloeosporioides* has been reported on durian as a fruit rot causal agent based on morphology alone (Sangchote et al. 2012). However, it is probably not a correct identification as it lacked molecular data.

Earlier, *Colletotrichum* species were identified based on morphology, cultural features, and pathogenicity studies (Cannon et al. 2000, Johnson et al. 1997, Sutton 1980). However, *Colletotrichum* species cannot be reliably identified due to changes in morphology and conidial shape and size with changes in substrate, host, and repetition of subculture. Besides, species identification based on host specificity is not reliable because of the possibility of more than one species occurring on the same host, leading to misidentification of the species. Damm et al. (2012) also indicated that more than one *Colletotrichum* species can colonize a single host based on a polyphasic approach and morphology. The present study supports this result, as four *Colletotrichum* species belonging to three complexes were isolated from durian fruits. Later, Weir and colleagues showed that the species earlier identified as *C. gloeosporioides* belonged to different distinct lineages (some remained as *C. gloeosporioides* sensu stricto) using molecular markers (Weir et al. 2012). Additionally, Udayanga et al. (2013) observed that, despite *C. gloeosporioides* sensu stricto's rather narrow host range, numerous species in the *C. gloeosporioides* complex comprise the predominant anthracnose pathogens in tropical Asia, emphasizing the use of molecular approaches for *Colletotrichum* species identification. However, two species within the *C. gloeosporioides* species complex, namely *C. siamense* and *C. gloeosporioides*, are the species associated with the largest number of host species worldwide (Talhinhas & Baroncelli 2021). *Colletotrichum* is a speciose genus with 247 accepted species (Bhunjun et al. 2021, Jayawardena et al. 2020). During 2022, 49 new species of

Colletotrichum have been introduced from different host plants (MycoBank 2023), and one new species from durian fruit is introduced in this study. However, much more species remained undiscovered in such speciose genera, according to Bhunjun et al. (2022). Therefore, it is clear that we are a long way from discovering all *Colletotrichum* species and having a deep understanding of species diversity, biology, host range, and distribution. Current research on *Colletotrichum* in tropical Asia has revealed a surprising species diversity present on a broad variety of hosts, producing significant fungal infections on fruits, vegetables, ornamentals, and other crops. Consequently, precise detection of pre- and postharvest diseases supported by molecular data has a significant influence on farming, biosecurity, and quarantine (Phoulivong et al. 2010, Hyde et al. 2013, Sharma et al. 2013, Udayanga et al. 2013).

Conclusion

This study proposed a new species (*C. durionigenum*) and recorded durian fruit as a new host to three known species (*C. gigasporum*, *C. pandanicola*, and *C. truncatum*), belonging to three complexes. The discovery of new species and new host records can provide a better understanding of fungal biodiversity, phylogenetic relationships, biology, and lifecycle, leading to enhancement of potential usages and functions. However, we did not conduct pathogenicity studies to confirm their pathogenicity on the durian fruits. Hence, future studies are recommended in this aspect.

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