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Morphology and phylogeny of *Colletotrichum dioscoreicola* sp. nov. related to anthracnose disease on *Dioscorea yunnanensis* (Yam) in China

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Abstract

Colletotrichum contains many important phytopathogenic species that pose serious crop threats. In this study, a novel species, *Colletotrichum dioscoreicola* associated with *Dioscorea yunnanensis* was obtained from Wenshan City, Yunnan Province, China. The multi-gene phylogenetic analysis of ITS, ACT, CHS-1, GAPDH, HIS3, and TUB2 sequence data revealed its close affinity with *C. sydowii*. Morphology of this species is similar to *C. sydowii* in having cylindrical conidia and presenting dark brown, septate, cylindrical to conical setae. However, the new species can be differentiated by having larger conidia and calabash-shaped appressoria. *Colletotrochum dioscoreicola* is the first *Colletotrichum* species reported from *D. yunnanensis*.

Keywords – 1 new species – *Colletotrichum* – multi-locus – plant disease

Introduction

Colletotrichum (Glomerellaceae, Glomerellales, Sordariomycetes) is an important genus of phytopathogenic fungi causing anthracnose that typically occurs on stems, leaves, flowers and fruits of the plant (Hyde et al. 2014, 2020b, Jayawardena et al. 2021, Talhinas & Baroncelli 2021). The infected region commonly presents the disease symptom of sunken spots, necrotic lesions, dieback and blight (Jayawardena et al. 2021a). *Colletotrichum* has been regarded as one of the world's ten most important plant pathogens and is treated as a regulated plant quarantine pest by many countries (Damm et al. 2019, Bhunjun et al. 2022). *Colletotrichum* species have been found in many economically important crops (Curry et al. 2002, Diao et al. 2017, Wu et al. 2020, Boufleur et al. 2021, Huang et al. 2021, Jayawardena et al. 2021, Talhinas & Baroncelli 2021). *Colletotrichum* species independently or concurrently cause disease in their hosts, leading to huge economic loss (Jayawardena et al. 2021). Some *Colletotrichum* species occur as endophytes without causing any

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disease symptoms until they switch lifestyles to pathogens that cause visible anthracnose symptoms, challenging the management of seedlings and post-harvest crops (Bhunjun et al. 2021a). In addition, some species are opportunistic pathogens to humans and animals, such as *C. dematium*, *C. gigasporum*, *C. gloeosporioides* and *C. truncatum*, which can cause ophthalmic and subcutaneous infections (Shivaprakash et al. 2011, Jayawardena et al. 2016c, Buchta et al. 2019, Hung et al. 2020).

Colletotrichum was established by Corda (1831) with *C. lineola* as the type species (Sharma and Shenoy 2016). Before the molecular era, *Colletotrichum* was identified based on morphological evidence and host plants. The characteristics of ascomata, asci and ascospores, conidiomata, conidiophores, conidia, appressoria, disease symptom, colony and setae have been used to demarcate *Colletotrichum* species (Damm et al. 2012, 2019, Talhinhas & Baroncelli 2021). However, these features are not highly informative for delineating *Colletotrichum* species. Thus, many of the names are likely synonyms (Soares et al. 2021). There are 1013 epithets listed in the Index Fungorum (accessed on 23 February 2023). However, only about 280 were confirmed with a combination of molecular and morphological analyses (Bhunjun et al. 2019, Hyde et al. 2020a, Liu et al. 2022, Zapata & Palfner 2022, Zheng et al. 2022). Liu et al. (2022) provided a modern taxonomic framework for *Colletotrichum* based on genome-scale and multi-locus phylogenetic analysis. The result showed that this genus mainly comprises 16 species complexes, namely acutatum, agaves, bambusicola, boninense, caudatum, dematium, destructivum, dracaenophilum, gigasporum, gloeosporioides, graminicola, magnum, orbiculare, orchidearum, spaethianum, and truncatum. However, some species that do not belong to the above complexes are considered as singleton species, including *C. hsienjenchang*, *C. metake*, *C. phaseolorum*, *C. rusci* and *C. sydowii* (Jayawardena et al. 2021a). Accurate identification of *Colletotrichum* species is essential for understanding biodiversity, host-parasite interaction, conservation and evolution, monitoring and controlling plant pathogens, and developing quarantine measures (Jayawardena et al. 2016b, 2021b, Bhunjun et al. 2021a). In this study, two isolates of *Colletotrichum* from *Dioscorea yunnanensis*, are identified as a new species, which is a sister taxon to the singleton species *C. sydowii*. We aim to provide the species with accurate taxonomic status and present its detailed illustration, colour photograph and phylogenetic analysis.

Materials & Methods

Sample collection, isolation, and morphological study

In October 2021, leaves of *Dioscorea yunnanensis* (Prain et Burkill), with typical anthracnose symptoms of leaf lesions, presenting irregular, dark brown dieback region with a yellow edge, were sampled. The host plants were found on farmland in Wenshan city, Yunnan Province, China. The lesional leaves were collected and processed for fungal isolation through the single spore isolation method (Zhang et al. 2013, Senanayake et al. 2020). Conidial masses on the leaf with the lesion were transferred to a small tube containing 100 µL sterile water for preparing spore suspension. The spore suspension was dropped on a potato dextrose agar (PDA) plate and incubated overnight in the biochemical incubator. The germinating conidia were observed using a microscope (Nikon Eclipse Ni M322E) and transferred to a fresh plate using a sterilized needle.

Morphological observations were performed using a dissecting microscope (Olympus SMZ745T) and compound microscope (Nikon Eclipse Ni M322E) equipped with a camera (Nikon DS-Ri2). The culture characteristics were documented from three standard media, potato dextrose agar, synthetic nutrient-poor agar medium (SNA) and oatmeal agar (OA). The cultures were incubated at 24 °C for 25 to 30 days. The growth rates were measured every three days. The specimens were deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN), and the living cultures were deposited at the Culture Collection of the Herbarium of Cryptogams Kunming Institute of Botany, Academia Sinica (KUNCC).

DNA Extraction, PCR and Sequencing

Fresh mycelia grown on PDA media were used to extract genomic DNA by using HP Fungal DNA Kit (OMEGA, USA) following the manufacturer's instructions. The primer pairs used for the

amplification of ACT, CHS-1, GAPDH, HIS3, ITS and TUB2 are listed in Table 1. Polymerase chain reaction (PCR) was performed in a final volume of 25 µL reaction mixture that contained 21 µL GoldenStar T6 Super PCR Mix (Tsingke), 1 µL (10 µM) of each primer and 2 µL DNA template using a T100 Thermal Cycler (T100™, Bio-Rad, USA). The procedures were composed of an initial denaturation of 3 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at the temperature listed in Table 1 for 15 s and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were purified and sequenced by Tsingke Biotechnology (Yunnan, China).

Table 1 The primers used in this study.

Gene	Primer	Primer sequence	Annealing temperatures	References
ACT	ACT-512F	5'-ATGTGCAAGGCCGGTTTCGC-3'	55	Carbone & Kohn (1999)
	ACT-783R	5'-TACGAGTCCTTCTGGCCCAT-3'		
CHS- 1	CHS- 79F	5'-TGGGGCAAGGATGCCTGGAAGAAG-3'	56	Carbone & Kohn (1999)
	CHS-354R	5'-TGGAAGAACCATCTGTGAGAGTTG-3'		
GAPDH	GDF1	5'-GCCGTCAACGACCCCTTCATTGA-3'	60	Guerber et al. (2003)
	GDR1	5'-GGGTGGAGTCGTACTTGAGCATGT-3'		
HIS3	CYLH3F	5'-AGGTCCACTGGTGGCAAG-3'	58	Crous et al. (2006)
	CYLH3R	5'-AGCTGGATGTCCTTGGACTG-3'		
ITS	ITS1	5'-TCCGTAGGTGAACCTGCGG-3'	56	White et al. (1990)
	ITS4	5'-TCCTCCGCTTATTGATATGC-3'		
TUB2	T1	5'-AACATGCGTGAGATTGTAAGT-3'	55	Glass & Donaldson (1995), O'Donnell (1997)
	Bt2b	5'-ACCCTCAGTGTAGTGACCCTTGGC-3'		

Phylogenetic analysis

The generated sequences were assembled using Chromas Pro (2.1.8). The BLAST tool in NCBI was used for the preliminary identification of the isolates. The sequences with high similarity to the isolates were downloaded from GenBank (Table 2). Each gene matrix was aligned with MAFFT v. 7 using default settings (Katoh & Standley 2013) and manually improved using BioEdit v. 7.0.5.2 (Hall 1999). The alignment sequence dataset was automatically trimmed using TrimAl v. v1.4.1 with the gappyout function (Capella-Gutiérrez et al. 2009). In addition, the introns were removed based on the amino acid sequence of previously published sequences, such as the ACT, CHS-1, GAPDH, HIS3 and TUB2. The alignments were concatenated with Sequence Matrix v.1.7.8 (Vaidya et al. 2011). Maximum likelihood (ML) analysis was performed using RAxML-HPC2 on XSEDE (Stamatakis 2014) on CIPRES (Miller et al. 2012). The ML phylogenetic tree was inferred from a dataset containing six matrixes, with the GTR+G+I model and 1,000 rapid bootstrap replicates. For the Bayesian inference (BI), the dataset was subdivided into 11 partitions: ITS position, ACT 1st, 2nd and 3rd codon positions, CHS-1 1st, 2nd and 3rd codon positions, GAPDH 1st, 2nd and 3rd codon positions, HIS3 1st, 2nd and 3rd codon positions, TUB2 1st, 2nd and 3rd codon positions. The best-fit partition and model were estimated using PartitionFinder 2 (Lanfear et al. 2016), with Akaike Information Criterion (AIC), using the 'greedy' search algorithm and 'linked' to estimate branch lengths (Costa-rezende et al. 2020). Bayesian inference analysis was performed in MrBayes on XSEDE 3.2.7a (Ronquist et al. 2012) with Markov chains running for 5,000,000 generations, and trees were sampled every 1000th generation. The first 25% of the resulting trees were discarded as burn-in, and posterior probabilities were calculated from the remaining sampled tree. ML bootstrap values and BI posterior probabilities greater than or equal to 70% and 0.90, respectively, were considered as significant support. A pairwise homoplasy index (Φw) test (PHI) was performed in Splits Tree (Huson 1998, Huson & Bryant 2006) to estimate the recombination level within

phylogenetically closely related species, using both LogDet transformation and splits decomposition. The pairwise homoplasy index below the 0.05 threshold (Φw < 0.05) indicates no significant recombination in the dataset. The relationship between closely related species was visualized by constructing a split graph.

Table 2 The taxa used in the phylogenetic analysis.

Species	Strain	ITS	GAPDH	CHS-1	HIS3	ACT	TUB2
Colletotrichum adenophorae	YMF1.04952 ^{T}	OK030859	OK513662	OK513558	–	–	–
C. agaves	LC0947	MZ595831	MZ664053	MZ799266	MZ673842	MZ664129	MZ673955
C. alatae	CBS 304.67 ^{T}	JX010190	JX009990	JX009837	–	JX009471	JX010383
C. bambusicola	CNUCC 307307 ^{T}	MT199632	MT192844	MT192871	–	MT188638	MT192817
C. bletillae	CGMCC 3.15117 ^{T}	JX625178	KC843506	MZ799322	MZ673854	KC843542	JX625207
C. boninense	CBS 123755 ^{T}	JQ005153	JQ005240	JQ005327	JQ005414	JQ005501	JQ005588
C. brevisporum	BCC 38876 ^{T}	JN050238	JN050227	MZ799287	MZ673841	JN050216	JN050244
C. cereale	CBS 129663	JQ005774	MZ664101	JQ005795	JQ005816	JQ005837	JQ005858
C. colombiense	CBS 129818 ^{T}	JQ005174	JQ005261	JQ005348	JQ005435	JQ005522	JQ005608
C. crousii	LC13858 ^{T}	MZ595876	MZ664059	MZ799281	MZ673896	MZ664174	MZ673995
C. curcumae	IMI 288937 ^{E}	GU227893	GU228285	GU228383	GU228089	GU227991	GU228187
C. dematium	CBS 125.25 ^{E}	GU227819	GU228211	GU228309	GU228015	GU227917	GU228113
C. dioscoreicola	**KUNCC-2210800** ^{T}	**OP454043**	**OP480024**	**OP480022**	**OP480027**	**OP480018**	**OP480028**
C. dioscoreicola	**KUNCC-2210801**	**OP454044**	**OP480025**	**OP480023**	**OP480026**	**OP480019**	**OP480029**
C. diversisporum	LC13890 ^{T}	MZ595911	MZ664122	MZ799302	MZ673931	MZ664209	MZ674029
C. dracaenophilum	CBS 118199 ^{T}	JX519222	JX546707	JX519230	JX546756	JX519238	JX519247
C. euphorbiae	CBS 134725 ^{T}	KF777146	KF777131	KF777128	KF777134	KF777125	KF777247
C. fioriniae	CBS 128517 ^{T}	JQ948292	JQ948622	JQ948953	JQ949283	JQ949613	JQ949943
C. fusiforme	MFLUCC 12-0437 ^{T}	KT290266	KT290255	KT290253	–	KT290251	KT290256
C. gloeosporioides	IMI 356878 ^{E}	JX010152	JX010056	JX009818	JQ005413	JX009531	JX010445
C. godetiae	CBS 133.44 ^{T}	JQ948402	JQ948733	JQ949063	JQ949393	JQ949723	JQ950053
C. graminicola	CBS 130836 ^{E}	JQ005767	–	JQ005788	–	JQ005830	JQ005851
C. guangxiense	CNUCC 310138 ^{T}	MT199633	MT192834	MT192861	–	MT188628	MT192805
C. guizhouensis	CGMCC 3.15112 ^{T}	JX625158	KC843507	MZ799321	MZ673850	KC843536	JX625185
C. hemerocallidis	CBS 130642 ^{T}	JQ400005	JQ400012	JQ399998	–	JQ399991	JQ400019
C. henanense	CGMCC 3.17354 ^{T}	KJ955109	KJ954810	MZ799256	MZ673835	KM023257	KJ955257
C. higginsianum	IMI 349061 ^{E}	KM105184	KM105535	KM105254	KM105324	KM105394	KM105464
C. hippeastri	CBS 125376 ^{T}	JQ005231	JQ005318	JQ005405	JQ005492	JQ005579	JQ005665
C. jiangxiense	CGMCC 3.17361 ^{T}	KJ955149	KJ954850	MZ799257	–	KJ954427	OK236389
C. karsti	CBS 106.91	JQ005220	JQ005307	JQ005394	JQ005481	JQ005568	JQ005654
C. lentis	CBS 127604 ^{T}	JQ005766	KM105597	JQ005787	JQ005808	JQ005829	JQ005850
C. lindemuthianum	CBS 144.31 ^{E}	JQ005779	JX546712	JQ005800	JQ005821	JQ005842	JQ005863
C. liriopes	CBS 119444 ^{T}	GU227804	GU228196	GU228294	GU228000	GU227902	GU228098
C. merremiae	CBS 124955 ^{T}	MG600765	MG600825	MG600872	MG600910	MG600969	MG601032
C. multiseptatum	NN055357 ^{T}	MZ595901	MZ664099	MZ799331	MZ673921	MZ664199	MZ674019
C. navitas	CBS 125086 ^{T}	JQ005769	–	JQ005790	JQ005811	JQ005832	JQ005853
C. neosansevieriae	CBS 139918 ^{T}	KR476747	KR476791	–	KR476792	KR476790	KR476797
C. nymphaeae	CBS 134233 ^{T}	KC293581	KC293741	KY856138	KY856309	KY855973	KC293661
C. orbiculare	CBS 570.97 ^{T}	KF178466	KF178490	KF178515	KF178539	KF178563	KF178587
C. orchidearum	CBS 135131 ^{T}	MG600738	MG600800	MG600855	MG600897	MG600944	MG601005
C. orchidophilum	CBS 632.80 ^{T}	JQ948151	JQ948481	JQ948812	JQ949142	JQ949472	JQ949802

Table 2 Continued.

Species	Strain	ITS	GAPDH	CHS-1	HIS3	ACT	TUB2
C. panamense	CBS 125386 [T]	MG600766	MG600826	MG600873	MG600911	MG600970	MG601033
C. parabambusicola	NN058956 [T]	MZ595904	MZ664098	MZ799338	MZ673924	MZ664202	MZ674022
C. parsonsiae	CBS 128525 [T]	JQ005233	JQ005320	JQ005407	JQ005494	JQ005581	JQ005667
C. piperis	CPC 21195 [E]	MG600760	MG600820	MG600867	MG600906	MG600964	MG601027
C. plurivorum	CBS 125474 [T]	MG600718	MG600781	MG600841	MG600887	MG600925	MG600985
C. pseudoacutatum	CBS 436.77 [T]	JQ948480	JQ948811	JQ949141	JQ949471	JQ949801	JQ950131
C. pseudomajus	CBS 571.88 [T]	KF687722	KF687826	KF687779	KF687864	KF687801	KF687883
C. sansevieriae	MAFF 239721 [T]	AB212991	LC180130	LC180129	LC180126	LC180127	LC180128
C. siamense	CBS 130417 [T]	JX010171	JX009924	JX009865	–	FJ907423	JX010404
C. sidae	CBS 504.97 [T]	KF178472	KF178497	KF178521	KF178545	KF178569	KF178593
C. sojae	ATCC 62257 [T]	MG600749	MG600810	MG600860	MG600899	MG600954	MG601016
C. spinosum	CBS 515.97 [T]	KF178474	KF178498	KF178523	KF178547	KF178571	KF178595
C. subacidae	LH01 [T]	MZ595846	MZ664068	MZ799307	MZ673866	MZ664144	MZ673967
C. subvariabile	NN040649 [T]	MZ595883	MZ664054	MZ799343	MZ673903	MZ664181	MZ674001
C. sydowii	CBS 135819 [T]	KY263783	KY263785	KY263787	KY263789	KY263791	KY263793
C. sydowii	CBS 132889	KY263784	KY263786	KY263788	KY263790	KY263792	KY263794
C. tropicale	CBS 124949 [T]	JX010264	JX010007	JX009870	MZ673832	JX009489	JX010407
C. tropicicola	BCC 38877 [T]	JN050240	JN050229	MZ799279	MZ673840	JN050218	JN050246
C. truncatum	CBS 151.35	GU227862	GU228254	GU228352	GU228058	GU227960	GU228156
C. vietnamense	CBS 125478 [T]	KF687721	KF687832	KF687769	KF687855	KF687792	KF687877
C. vittalense	CBS 181.82 [T]	MG600734	MG600796	MG600851	MG600893	MG600940	MG601001
C. yunnanense	CBS 132135 [T]	JX546804	JX546706	JX519231	JX546755	JX519239	JX519248
C. zhaoqingense	NN058985 [T]	MZ595905	MZ664065	MZ799304	MZ673925	MZ664203	MZ674023
C. zhejiangense	NN076215 [T]	MZ595912	MZ664124	MZ799342	MZ673932	MZ664210	MZ674030
Monilochaetes infuscans	CBS 869.96	JQ005780	JX546612	JQ005801	JQ005822	JQ005843	JQ005864

The holotype strain and epitype are indicated with symbols "[T]" and "[E]", respectively. The symbol "-" means that the sequence is unavailable. Strains of this study are in **bold**.

Results

Phylogenetic analysis

In this study, 12 sequences including ITS, ACT, CHS-1, GAPDH, HIS3 and TUB2 were generated. All sequences were submitted to GenBank. The phylogenetic analysis was inferred from combined six genes matrix that is consisted of 66 taxa, of which 59 were from the type specimens. The Fig. 1 shows the comparison of maximum likelihood analysis of genus *Colletotrichum* based on the sequence dataset with and without the introns. Both phylogenetic trees shared similar tree topologies. A total of 16 clades and three singleton species were observed, of which 15 clades were species complexes, namely acutatum, agaves, bambusicola, boninense, caudatum, dematium, destructivum, dracaenophilum, gigasporum, gloeosporioides, graminicola, orbiculare, orchidearum, spaethianum, truncatum, and three clades present as singletons. Bootstrap support values increased at the main branch after moving the introns. On the contrary, bootstrap support values have a lesser decrease at the node. The combined dataset contained 1650 characters after removing the introns from the encoding gene, of which 1164 characters were constant, 475 characters were variable and 362 characters were parsimony-informative. The ML and BI analyses of *Colletotrichum* yielded similar tree topologies. The topology of ML tree was shown herein (Fig. 2). The new species, *C. dioscoreicola* formed a distinct clade, sister to *C. sydowii* and *C. adenophorae*, with strong support (BS = 100%, PP = 1.00). The clade of *C. dioscoreicola*, *C. sydowii* and *C. adenophorae* sister to gloeosporioides complex was proved. Additionally, only ITS, CHS-1 and GAPDH sequence data are available for *C. adenophorae* in the GenBank. In the phylogenetic tree, *C. adenophorae* and *C. sydowii* clustered into a clade without genetic distance, indicating the concatenated of these mentioned three genes has low resolution in distinguishing the two species. The PHI test inferred

from combined six genes, and the result showed that *C. dioscoreicola* has no significant recombination with *C. sydowii and C. adenophorae* (Fig. 3).

Colletotrichum dioscoreicola H. D. Yang & R. S. Jayawardena, sp. nov. Fig. 4

Index Fungorum number: IF559988; Facesoffungi number: FoF 12854

Etymology – referring to the host *Dioscorea yunnanensis* (Prain et Burkill).

Associated with leaf lesions of *D. yunnanensis*. Sexual morph: Not observed. Asexual morph: conidial mass developed on the leaf surface, solitary or scattered, pale salmon. *Setae* 79–160 × 4–7 ($\overline{x}$ = 114 × 5.5, n = 10) µm, medium brown, cylindrical, gradually dwindle and verruculose toward the upper, 1–8-septate, sometimes constricts at the last septum, basal intumescent, tip acute. *Conidiophores* hyaline, smooth-walled, septate. *Conidiogenous cells* 14–25 × 3.5–6 ($\overline{x}$ = 18 × 5, n = 5) µm, monophialidic, cylindrical, collarette. *Conidia* 20–23 × 6.5–7.7 ($\overline{x}$ = 21 × 6.5, n = 40) µm, L/W ratio = 3.2, hyaline, smooth-walled, straight, oblong with round ends, aseptate. Appressoria not observed.

On PDA, conidial masses developed on the surface of the medium, initially cream-white, becoming pale salmon with age. *Setae* not observed. *Conidiophores* 34–78 × 3.5–6.5 ($\overline{x}$ = 52 × 5.4, n = 15) µm, light brown to brown at base, becoming hyaline toward the apex, septate, branched at the basal part. *Conidiogenous cells* 5.5–11.5 × 2.5–7.5 ($\overline{x}$ = 8.2 × 6.2, n = 20) µm, hyaline, smooth-walled, ampulliform. *Conidia* 16–21.5 × 6–7.5 ($\overline{x}$ = 18.3 × 6.7, n = 50) µm, L/W ratio = 2.7, hyaline, straight, cylindrical with round ends, 0–1-septate. *Appressoria* 5.5–16.5 × 3.8–8.8 µm ($\overline{x}$ = 9.4 × 6.4, n = 20) µm, solitary, medium pale brown to brown, subglobose to oblong, or calabash-shaped, the edge entire or undulate.

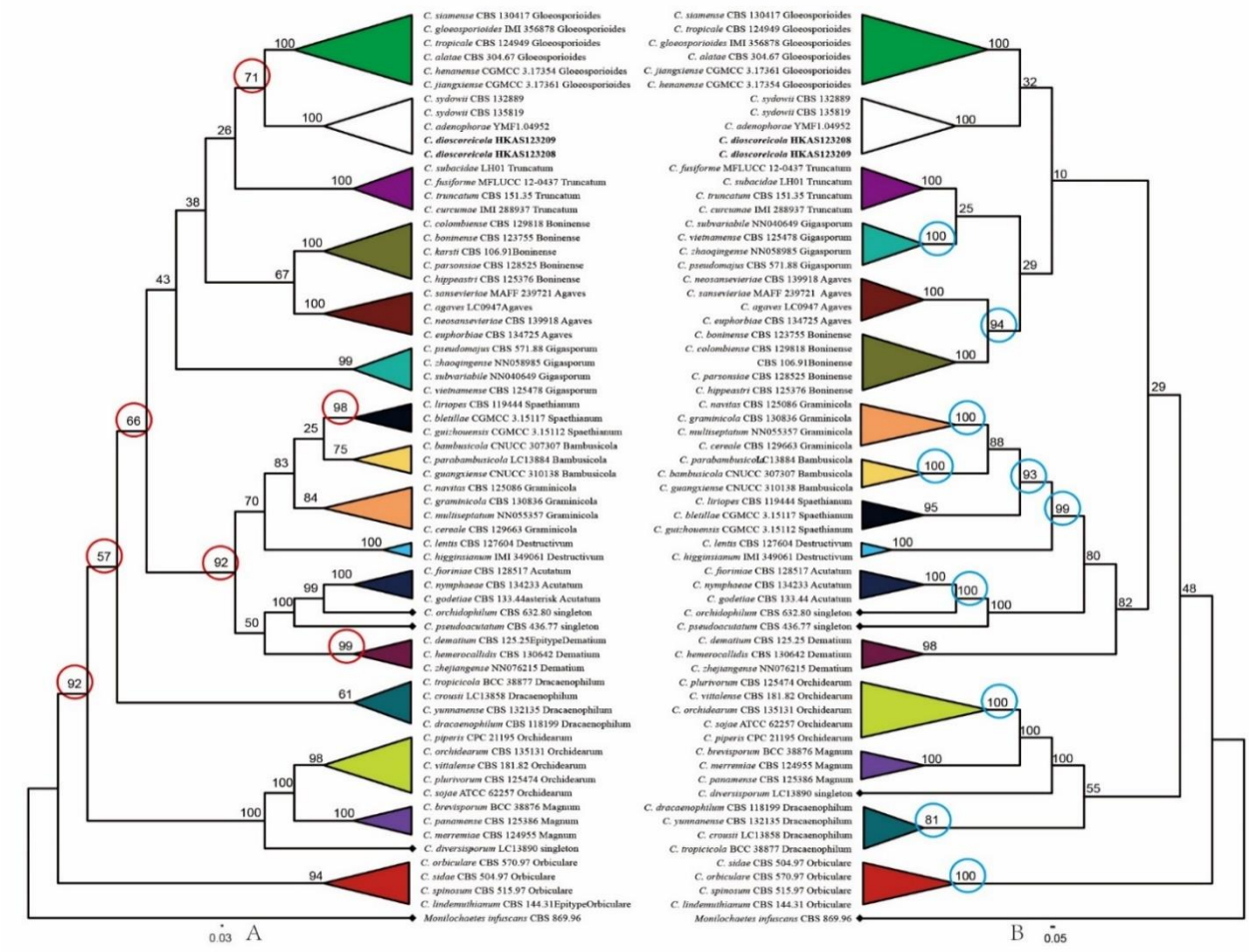

Fig. 1 – Maximum likelihood analysis was based on a combined ITS, ACT, CHS-1, GAPDH, HIS3 and TUB2 sequence dataset. A Sequence dataset without introns. B Sequence dataset contained

introns. The same species complex is labelled with the same color. Bootstrap support values increased and greater than 50 after removing the introns are circled in red. On the contrary, decreased values are circled in blue. The new species is in bold.

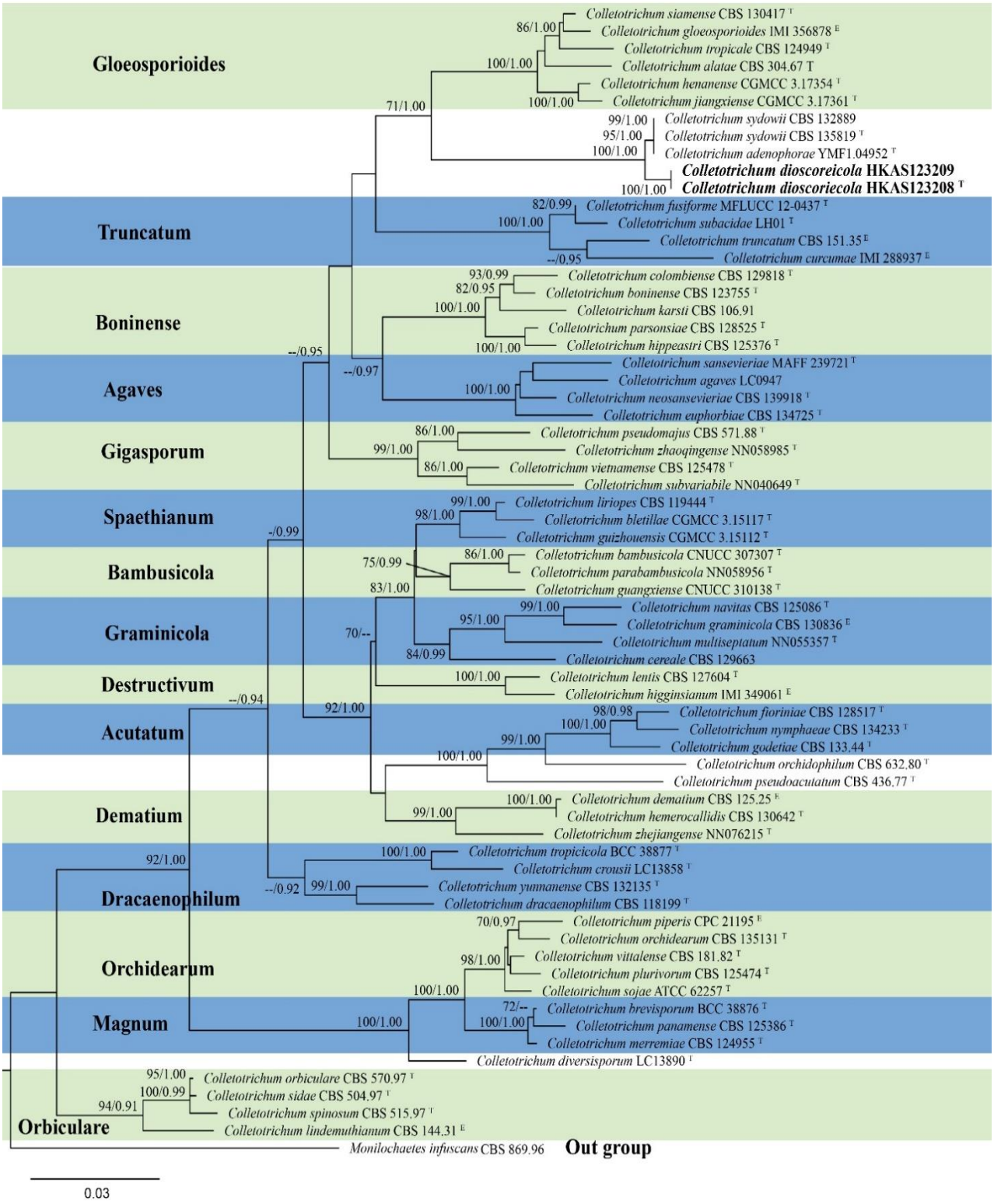

Fig. 2 – Phylogenetic tree of *Colletotrichum* inferred from a maximum likelihood analysis of the combined ITS, ACT, CHS-1, GAPDH, HIS3 and TUB2 sequence. Bootstrap values (left) equal to or greater than 70% and Bayesian posterior probability (right) equal to or greater than 0.90 are given at the nodes. The new species was in bold. The holotype strains were labelled with "T", and the epitype strains were denoted with "E". *Monilochaetes infuscans* was selected as the outgroup.

Culture characteristics – On SNA, vegetative hyphae are sparse, hyaline, reached 30–33 mm diam. in ten days at room temperature, no sporulation. On OA, the colony was white, circular, raised near the margin, edge entire, reverse white with a light brown ring, reached 40–46 mm diam in ten days at room temperature, no pigments, no sporulation. On PDA, the colony was white, cottony, circular, flat, or raised near the margin, edge entire, reverse white, reached 38–42 mm diam. in ten days at room temperature, sometimes reddish-orange pigment is present when cultivated under natural day/night lighting.

Material examined – China, Yunnan province, Wenshan city (23°46′39.01″N, 104°9′14.98″E), elevation 1536 m, on diseased leaves of *Dioscorea yunnanensis*, 7 October 2021, Hongde Yang, Holotype HKAS 123208, ex-type living culture KUNCC 22-10800. Paratype HKAS 123209, ex-paratype KUNCC 22-10801.

Notes – The phylogenetic analysis showed that *Colletotrichum dioscoreicola* clustered with *C. sydowii* and *C. adenophorae*. The pairwise dissimilarities of DNA sequences between *C. dioscoreicola* and *C. sydowii* were 3 bp (244/247 bp), 4 bp (224/228 bp), 5 bp (202/207 bp) and 4 bp (700/704 bp) in ACT, CHS-1, GAPDH and TUB2, respectively. The pairwise dissimilarities of DNA sequences between *C. dioscoreicola* and *C. adenophorae* were 4 bp (222/226 bp) and 4 bp (177/181) bp in CHS-1 and GAPDH, respectively. The PHI analysis detected that no significant recombination was observed within *C. dioscoreicola*, *C. sydowii* and *C. adenophorae*. Morphologically, *C. dioscoreicola* resembles *C. sydowii* in conidia and setae, but the *C. dioscoreicola* has larger conidia (20–23 × 6.5–7.7 μm *vs*. 14–20.5 × 5–6 μm), and light brown to brown, subglobose to oblong, or calabash-shaped appressoria, whereas *C. sydowii* has brown, subglobose, elliptical or irregular appressoria with lobate margin (Marin-Felix et al. 2017). *Colletotrichum dioscoreicola* differs from *C. adenophorae* by having ampulliform conidiogenous cells, while *C. adenophorae* has collarette inconspicuous conidiogenous cells (Yu et al. 2022). Based on molecular and morphological evidence following Chethana et al. (2021) and Jayawardena et al. (2021b) this species was identified as a new species in the genus.

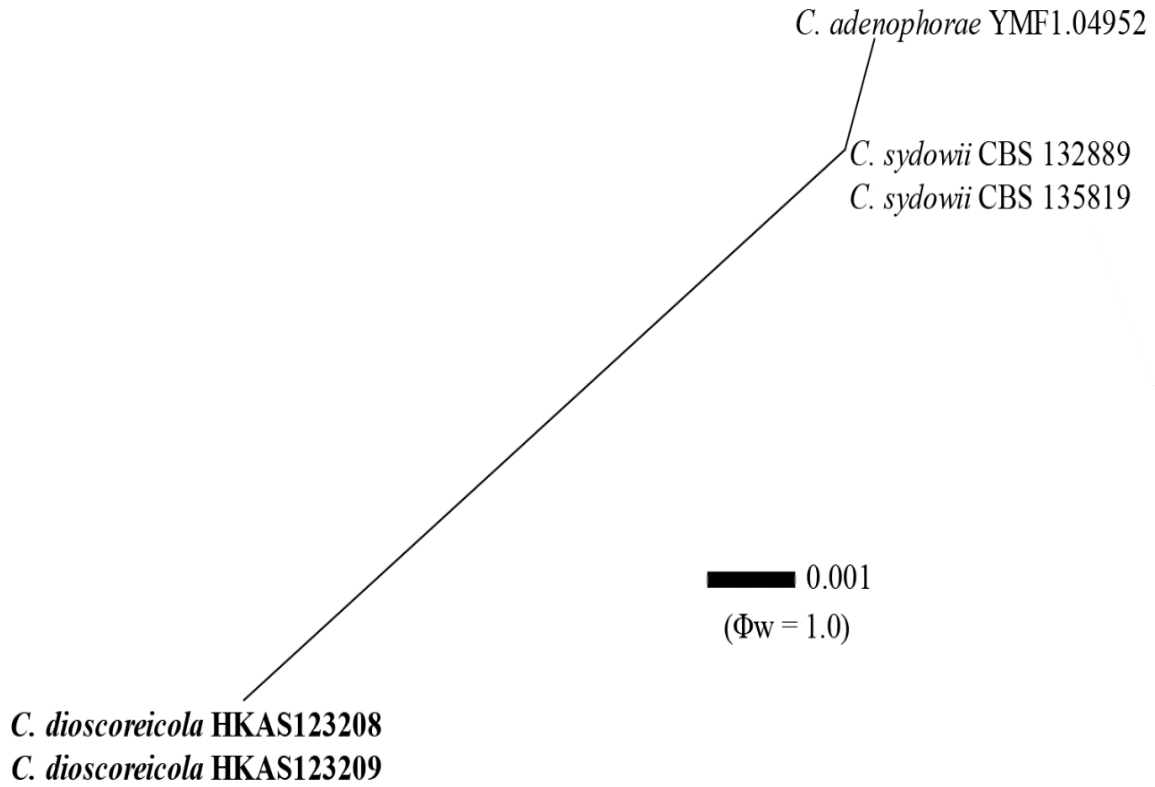

Fig. 3 – The results of the pairwise homoplasy index (PHI) test of closely related species using both LogDet transformation and splits decomposition. The new species is in bold.

Table 3 Anthracnose disease caused by *Colletotrichum* species on *Dioscorea*.

Species	Complex	Host	Location	References
C. aeschynomenes	Gloeosporioides	*D. alata*	India	Weir et al. (2012)
C. alatae	Gloeosporioides	*D. alata*,	Nigeria,	Lin et al. (2018)
		D. rotundata	China: Hainan	Okon et al. (2022)
C. capsica	Truncatum	*D. alata*	India	Jehani et al. (2019)
C. cliviae	Orchidearum	*D. alata*	Puerto Rico	Douanla-Meli et al. (2018)
C. dioscoreae	Gloeosporioides	*D.* sp.	Brazil	Averna-Saccá (1917)
C. fructicola	Gloeosporioides	*D. alata*	Nigeria	Weir et al. (2012)
C. gloeosporioides	Gloeosporioides	*D. alata*	French	Frézal et al. (2012)
		D. opposita	China	Chong et al. (2018)
		D. batatas	China	Yang et al. (2021)
		D. bulbifera	America	Xiao et al. (2004)
C. jiangxiense	Gloeosporioides	*D. zingiberensis*	China: Jiangxi	Liu et al. (2022)
C. jinshuiense	Dematium	*D. zingiberensis*	China: Fuzhou	Liu et al. (2022)
C. siamense	Gloeosporioides	*D. rotundata*	Nigeria	Weir et al. (2012)
		D. rotunda	Nigeria	Jayawardena et al. (2016a)
		D. cayennensis	Brazil	de Souza Junior & Assunção. (2021)
C. truncatum	Truncatum	*D. alata*	France	Dentika et al. (2021)

Discussion

Colletotrichum has been extensively studied in evolution and host specialization, and hence, this genus was regarded as a model for studying plant pathogens (Talhinhas & Baroncelli 2021). In the last decades, studies were wildly carried out on populations affecting crop and ornamental plants, whereas fewer reports from non-cultivational crops and native woody plants in natural ecosystems (Zapata & Palfner 2022).

In this study, *Colletotrichum* species was isolated from *Dioscorea yunnanensis*, which is a less studied plant host. The multi-loci sequences ACT, CHS-1, GAPDH, HIS3, ITS and TUB2 were applied to delimit this species. Those genes were also commonly adopted to identify the species complex and study *Colletotrichum* phylogenetic relationship (Cannon et al. 2012, Jayawardena et al. 2021a, Bhunjun et al. 2021b). However, the protein-coding genes such as ACT, CHS-1, GAPDH, HIS3 and TUB2 have a high genetic variability within *Colletotrichum*, especially in the introns (Silva et al. 2020). It is difficult to align these highly various genes when sequence matrices from different complexes were included. The unaligned data matrix results in lower support values and unstable tree topologies (Höhl & Ragan 2007). Though we used with gappyout function to automatically align sequence dataset, the phylogenetic analysis did not provide a compelling tree topology. Hence, we removed the introns from the codon genes to obtain more stable tree topologies. As a consequence, bootstrap support values increased at the main branch indicating the tree topology was enhanced. Phylogenetic analyses based on exon obtained a more stable topology, which agrees with Talhinhas and Baroncelli (2021) in species complexes. The inclusive of 15 species complexes acutatum, agaves, bambusicola, boninense, caudatum, dematium, destructivum, dracaenophilum, gigasporum, gloeosporioides, graminicola, orbiculare, orchidearum, spaethianum, truncatum in our phylogenetic tree were well presented and the tree topology was more consistent with the genome tree (Liu et al. 2022). Our new species *C. dioscoreicola*, clustered with *C. sydowii* and *C. adenophorae* forming a distinct clade that is distant from other complexes and singletons. The PHI test result also shows clear evidence that our strain is a new species. *Colletotrichum sydowii* was regarded as a singleton species in previous study (Liu et al. 2022). However, together with the newly introduced *C. adenophorae* by Yu et al. (2022) and our new species *C. dioscoreicola*, this clade may form a new complex.

Yams (*Dioscorea* spp.) are one of the top ten most important edible tuber and root plants (Cao et al. 2021). *Dioscorea* has over 600 species and 11 are important cultivational crops worldwide (Ntui et al. 2021). Among the *Dioscorea* disease, anthracnose is the primary threat to yam production, accounting for 50% ~ 100% yield losses (Frézal et al. 2012, Okon et al. 2022). *Colletotrichum*

gloeosporioides is the most destructive species and has a wide range host of *Dioscorea*, such as *D. alata*, *D. opposite*, *D. batatas* and *D. bulbifera* (Frézal et al. 2012). Statistics on those ten-year publications associated with yam anthracnose disease were carried out (Table 3). The result shows that yam anthracnose mainly belongs to gloeosporioides, truncatum, orchidearum and dematium complex. Additionally, to our knowledge, this study provides the first report of *Colletotrichum* species from *D. yunnanensis*.

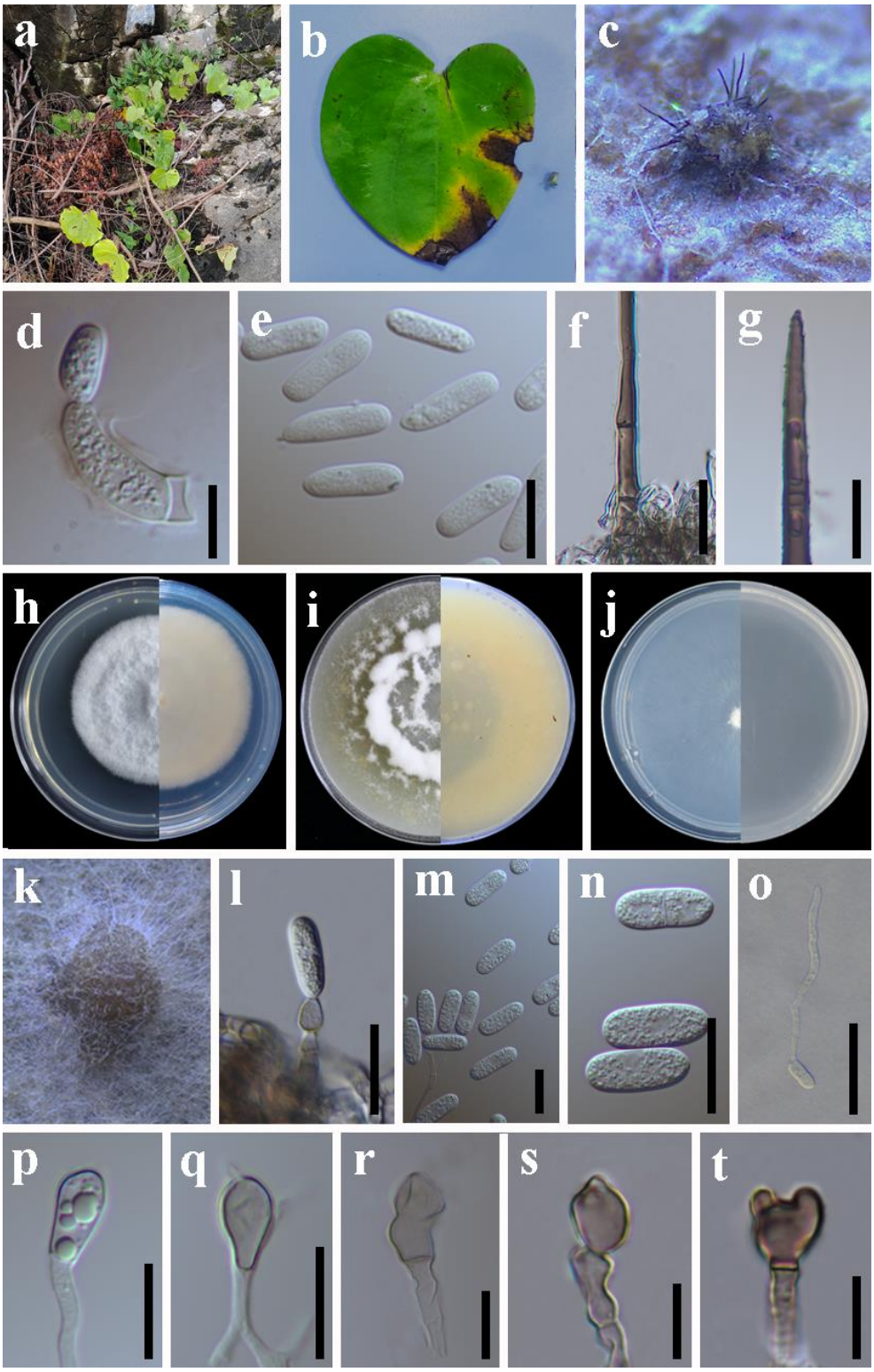

Fig. 4 – *Colletotrichum dioscoreicola* (a–g from holotype: HKAS 123208, k–t from ex-type: KUNCC 22-10800). a–b Lesions on leaves of *Dioscorea yunnanensis*. c, k Conidial mass.

d, l Conidiogenous cell. e, m–n Conidia. f–g. Setae. h. Colony on PDA. i. Colony on OA. j. Colony on SNA. o. Germinated conidium p–t. Appressoria. Scale bars: d, g, p, q, r, s, t = 10 μm, e, f = 20 μm, l, m, n = 20 μm, o = 50 μm.

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References

Averna-Saccá R. 1917 – As moléstiascryptogamicas das plantashorticolas. Boletim de Agricultura São Paulo 18, 634–654.

Bhunjun CS, Jayawardena RS, Wei DP, Huanraluek N et al. 2019 – Multigene phylogenetic characterisation of *Colletotrichum artocarpicola* sp. nov. from *Artocarpus heterophyllus* in northern Thailand. Phytotaxa 418, 273–286.

Bhunjun CS, Phillips AJL, Jayawardena RS, Promputtha I et al. 2021a – Importance of molecular data to identify fungal plant pathogens and guidelines for pathogenicity testing based on Koch's Postulates. Pathogens 10, 1096.

Bhunjun CS, Phukhamsakda C, Jayawardena RS, Jeewon R et al. 2021b – Investigating species boundaries in *Colletotrichum*. Fungal Diversity 107, 107–127.

Bhunjun CS, Niskanen T, Suwannarach N, Wannathes N et al. 2022 – The numbers of fungi: are the most speciose genera truly diverse?. Fungal Diversity 27, 1–76.

Boufleur TR, Ciampi-Guillardi M, Tikami Í, Rogério F et al. 2021 – Soybean anthracnose caused by *Colletotrichum* species: Current status and future prospects. Molecular Plant Pathology 22, 393–409.

Buchta V, Nekolová J, Jirásková N, Bolehovska R et al. 2019 – Fungal keratitis caused by *Colletotrichum dematium*: Case Study and Review. Mycopathologia 184, 441–453.

Cannon PF, Damm U, Johnston PR, Weir BS. 2012 – *Colletotrichum* – current status and future directions. Studies in Mycology 73, 181–213.

Cao TX, Sun JY, Shan N, Chen X et al. 2021 – Uncovering the genetic diversity of yams (*Dioscorea* spp.) in China by combining phenotypic trait and molecular marker analyses. Ecology and Evolution 11, 9970–9986.

Carbone I, Kohn LM. 1999 – A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553–556.

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009 – TrimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25, 1972–1973.

Chethana KT, Manawasinghe IS, Hurdeal VG, Bhunjun CS et al. 2021 – What are fungal species and how to delineate them?. Fungal Diversity 109, 1–25.

Chong ZW, Zhong BF, Huang DY, Xie J et al. 2018 – Pathogenicity differentiation of *Dioscorea opposita* Thunb. anthracnose pathogen in southern China. Journal of Southern Agriculture 49, 501–507.

Corda ACI. 1831 – Die Pilze Deutschlands. In: Deutschlands Flora in Abbildungennach der Natur mit Beschreibungen (Sturm, J). Sturm, Nürnberg vol. 3, Abt. 12: 33–64, tab. 21–32.

Costa-rezende DH, Robledo GL, Drechsler-santos ER, Glen M et al. 2020 – Taxonomy and phylogeny of polypores with ganodermatoid basidiospores (Ganodermataceae). Mycological Progress 19, 725–741.

Crous PW, Groenewald JZ, Risède JM, Simoneau P et al. 2006 – *Calonectria* species and their *Cylindrocladium* anamorphs: Species with clavate vesicles. Studies in Mycology 55, 213–226.
Curry KJ, Abril M, Avant JB, Smith BJ et al. 2002 – Strawberry anthracnose: Histopathology of *Colletotrichum acutatum* and *C. fragariae*. Mycology 92, 1055–1063.
Damm U, Cannon PF, Woudenberg JHC, Crous PW et al. 2012 – The *Colletotrichum acutatum* species complex. Studies in Mycology 73, 37–113.
Damm U, Sato T, Alizadeh A, Groenewald JZ et al. 2019 – The *Colletotrichum dracaenophilum*, *C. magnum* and *C. orchidearum* species complexes. Studies in Mycology 92, 1–46.
Dentika P, Ozier-Lafontaine H, Penet L. 2021 – Weeds as pathogen hosts and disease risk for crops in the wake of a reduced use of herbicides: Evidence from yam (*Dioscorea alata*) fields and *Colletotrichum* pathogens in the tropics. Journal of Fungi 7, 283.
Diao YZ, Zhang C, Liu F, Wang WZ et al. 2017 – *Colletotrichum* species causing anthracnose disease of chili in China. Persoonia 38, 20–37.
Douanla-Meli C, Unger JG, Langer E. 2018 – Multi-approach analysis of the diversity in *Colletotrichum cliviae* sensu lato. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology 111, 423–435.
Frézal L, Desquilbet L, Jacqua G, Neema C et al. 2012 – Quantification of the aggressiveness of a foliar pathogen, *Colletotrichum gloeosporioides*, responsible for water yam (*Dioscorea* alata) anthracnose. European Journal of Plant Pathology 134, 267–279.
Glass NL, Donaldson GC. 1995 – Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61, 1323–1330.
Guerber JC, Liu B, Correll JC, Johnston PR. 2003 – Characterization of diversity in *Colletotrichum acutatum* sensu lato by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. Mycologia 95, 872–895.
Hall TA. 1999 – BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuclc Acids Symposium Series 41, 95–98.
Huang R, Sun WX, Wang LR, Li QL et al. 2021 – Identification and characterization of *Colletotrichum* species associated with anthracnose disease of banana. Plant Pathology 70, 1827–1837.
Hung N, Hsiao CH, Yang CS, Lin HC et al. 2020 – *Colletotrichum keratitis*: a rare yet important fungal infection of human eyes. Mycoses 63, 407–415.
Huson DH. 1998 – SplitsTree: analyzing and visualizing evolutionary data. Bioinformatics 14, 68–73.
Huson DH, Bryant D. 2006 – Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23, 254–267.
Höhl M, Ragan MA. 2007 – Is multiple-sequence alignment required for accurate inference of phylogeny?. Systematic biology 56, 206–21.
Hyde KD, Nilsson RH, Alias SA, Ariyawansa HA et al. 2014 – One stop shop: backbones trees for important phytopathogenic genera: I. Fungal Diversity 67, 21–125.
Hyde KD, Jeewon R, Chen YJ, Bhunjun CS et al. 2020a – The numbers of fungi: is the descriptive curve flattening?. Fungal Diversity, 103, 219–271.
Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bhat DJ et al. 2020b – Refined families of Sordariomycetes. Mycosphere 11, 305–1059.
Jayawardena RS, Bhunjun CS, Hyde KD, Gentekaki E et al. 2021a – *Colletotrichum*: lifestyles, biology, morpho-species, species complexes and accepted species. Mycosphere 12, 519–669.
Jayawardena RS, Hyde KD, Damm U, Cai L et al. 2016a – Notes on currently accepted species of *Colletotrichum*. Mycosphere 7, 1192–1260.
Jayawardena RS, Hyde KD, Jeewon R, Li XH et al. 2016b – Mycosphere essay 6: Why it is important to correctly name *Colletotrichum* species?. Mycosphere 7, 1076–1092.
Jayawardena RS, Hyde KD, de Farias ARG, Bhunjun CS et al. 2021b – What is a species in fungal plant pathogens?. Fungal Diversity 109, 239–266.

Jayawardena RS, Li XH, Liu M, Zhang W et al. 2016c – Mycosphere essay 16: *Colletotrichum*: biological control, biocatalyst, secondary metabolites and toxins. Mycosphere 7, 1164–1176.
Jehani MD, Patel PR, Chaudhary AK. 2019 – Evaluation of bioagents against *Colletotrichum capsici* caused anthracnose disease of yam (*Dioscorea alata* L.). Journal of Pharmacognosy and Phytochemistry 8, 4788–4790.
Katoh K, Standley DM. 2013 – MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30, 772–780.
Lanfear R, Frandsen PB, Wright AM, Senfeld T et al. 2016 – Partitionfinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34, 772–773.
Lin CH, Wu WQ, Liao XM, Liu WB et al. 2018 – First report of leaf anthracnose caused by *Colletotrichum alatae* on water yam (*Dioscorea alata*) in China. Plant Disease 102, 248.
Liu F, Ma ZY, Hou LW, Diao YZ et al. 2022 – Updating species diversity of *Colletotrichum*, with a phylogenomic overview. Studies in Mycology 101, 1–56.
Marin-Felix Y, Groenewald JZ, Cai L, Chen Q et al. 2017 – Genera of phytopathogenic fungi: GOPHY 1. Studies in Mycology 86, 99–216.
Miller MA, Pfeiffer W, Schwartz T. 2012 – The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. Proceedings of the 1st conference of the Extreme Science and Engineering Discovery Environment. Bridging from the extreme to the campus and beyond. Association for Computing Machinery 39, 1–8.
Ntui VO, Uyoh EA, Ita EE, Markson AA et al. 2021 – Strategies to combat the problem of yam anthracnose disease: Status and prospects. Molecular Plant Pathology 22, 1302–1314.
O'Donnell K, Cigelnik E. 1997 – Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7, 103–116.
Okon NI, Markson AA, Okon EI, Ita E et al. 2022 – Characterization of some fungal pathogens causing anthracnose disease on yam in Cross River State, Nigeria. PLoS ONE 17, e0270601.
Ronquist F, Teslenko M, Van Der Mark P, Ayres DL et al. 2012 – MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61, 539–542.
Senanayake IC, Rathnayaka AR, Marasinghe DS, Calabon MS et al. 2020 – Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. Mycosphere 11, 2678–2754.
Sharma G, Shenoy BD. 2016 – *Colletotrichum* systematics: Past, present and prospects. Mycosphere 7, 1093–1102.
Shivaprakash MR, Appannanavar SB, Dhaliwal M, Gupta A et al. 2011 – *Colletotrichum truncatum*: an Unusual Pathogen Causing Mycotic Keratitis and Endophthalmitis. Journal of Clinical Microbiology 49, 2894–2898.
Silva LL, Moreno HL, Correia HL, Santana MF et al. 2020 – *Colletotrichum*: species complexes, lifestyle, and peculiarities of some sources of genetic variability. Applied microbiology and biotechnology 104, 1891–904.
Soares MG, Alves E, Silveira AL, Pereira FD et al. 2021 – *Colletotrichum siamense* is the main aetiological agent of anthracnose of avocado in south-eastern Brazil. Plant Pathology 70, 154–166.
de Souza Junior FJC, Assunção MC. 2021 – First report of *Colletotrichum siamense* causing anthracnose in *Dioscorea cayennensis* in Brazil. Journal of Plant Pathology 103, 1065–1065.
Stamatakis A. 2014 – RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Talhinhas P, Baroncelli R. 2021 – *Colletotrichum* species and complexes: geographic distribution, host range and conservation status. Fungal Diversity 111, 109–198.

Vaidya G, Lohman DJ, Meier R. 2011 – SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27, 171–180.
Weir BS, Johnston PR, Damm U. 2012 – The *Colletotrichum gloeosporioides* species complex. Studies in Mycology 73, 115–180.
White TJ, Bruns T, Lee S, Taylor J et al. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols, a guide to methods and applications. Academic, San Diego, 315–322.
Wu CJ, Chen HK, Ni HN. 2020 – Identification and characterization of *Colletotrichum* species associated with mango anthracnose in Taiwan. European Journal of Plant Pathology 157, 1–15.
Xiao CL, Mackenzie SJ, Legard DE. 2004 – Genetic and pathogenic analyses of *Colletotrichum gloeosporioides* isolates from Strawberry and Noncultivated hosts. Population Biology 94, 446–453.
Yang XL, Zhang YH, Wang ZQ, Chang XQ et al. 2021 – Pathogens infecting Chinese yams planted on ridges in northern Hubei. Fujian Journal of Agricultural Sciences 36, 59–64.
Yu ZF, Jiang XW, Zheng H, Zhang H et al. 2022 – Fourteen New Species of Foliar *Colletotrichum* Associated with the Invasive Plant *Ageratina adenophora* and Surrounding Crops. Journal of Fungi 8, 185.
Zapata M, Palfner G. 2022 – *Colletotrichum* (Ascomycota, Glomerellaceae) associated with native woody plants in natural ecosystems: What do we know?. Forest Pathology 52, e12748.
Zhang K, Su YY, Cai L. 2013 – An optimized protocol of single spore isolation for fungi. Cryptogamie Mycologie 34, 349–356.
Zheng H, Yu ZF, Jiang XW, Fang L et al. 2022 – Endophytic *Colletotrichum* Species from Aquatic Plants in Southwest China. Journal of Fungi 8, 87.