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A new host report of *Diaporthe manihotia* (Diaporthales, Ascomycota) from *Camellia* sp. in Yunnan province, China

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Abstract

A sexual morph of a *Diaporthe* species was collected from a dead aerial branch of *Camellia* sp., in Yunnan Province, China. Multi-locus phylogeny of internal transcribed spacer (ITS), beta-tubulin (BT), translation elongation factor 1-alpha (EF1-a), calmodulin (cal) and Histone (H3) genes showed that our strain clustered with *Diaporthe manihotia* (CBS 505.76) with high statistical support. A comprehensive description, photographs of micro-morphological characteristics and a phylogenetic tree to show the placement of the taxon are provided. This is the first host report of *D. manihotia* from *Camellia* sp.

Keywords – Diaporthaceae – Morphology – Phylogeny – Saprobes – Taxonomy – Tea

Introduction

Camellia belongs to Theaceae and is inclusive of evergreen shrubs or small plants used for human consumption as beverages and oils (Min 2000). *Camellia* species are also grown as ornamental plants in gardens, for their colourful flowers. China is considered as the main center for the origin of *Camellia* in the world. There are 97 *Camellia* species in China, of which 76 of them are endemic (Xin et al. 2015). Species of *Camellia* (especially *C. reticutala*) are mostly distributed throughout the Yunnan Province of China due to its adaptability to various geographical and climatic conditions (Zeng 2001, Chen 2005, Tong et al. 2015). These *Camellia* trees have been a part of Chinese culture for generations of many native ethnic groups that live in Yunnan (Kondo 1975, 1976, Long et al. 1999).

Diaporthaceae contains pathogenic, saprobic and endophytic species which inhabit terrestrial and rarely submerged hosts (Udayanga et al. 2011, Dissanayake et al. 2017, Senanayake et al.

2017). Castlebury et al. (2002) placed both *Diaporthe (Phomopsis)* and *Mazzantia* in Diaporthaceae based on phylogenetic analysis. Currently, Diaporthaceae comprises of *Allantoporthe*, *Apioporthella*, *Chaetoconis*, *Diaporthe*, *Leucodiaporthe*, *Mazzantia*, *Ophiodiaporthe*, *Phaeocytostroma*, *Phaeodiaporthe*, *Pustulomyces*, *Stenocarpella*, *Chiangraiomyces*, *Paradiaporthe* and *Hyaliappendispora* (Maharachchikumbura et al. 2015, Senanayake et al. 2017, Hyde et al. 2020).

Diaporthe was described by Nitschke (1870) with D. eres on Ulmus campestris in Germany as the type species. *Diaporthe* species have been recorded as endophytes or saprobes on a wide range of host plants in different geographical areas. Many economically significant crops are infected by pathogenic Diaporthe species leading to severe crop losses (Crous & Groenewald 2005, Rossman et al. 2007, Santos & Phillips 2009, Udayanga et al. 2011, 2012a, b, Gomes et al. 2013, Udayanga et al. 2014a, b, 2015, Guarnaccia & Crous 2018, Manawasinghe et al. 2019), with blights, fruit and root rots, cankers, diebacks, wilts and leaf spots (Mostert et al. 2001, Rensburg et al. 2006, Thompson et al. 2011). Initial studies on identifying Diaporthe species mainly based on morphology and host-specificity (Udayanga et al. 2011, Gomes et al. 2013). Studies have shown that many *Diaporthe* species are not host-specific, and can occur on multiple hosts (Brayford 1990, Rehner & Uecker 1994, Mostert et al. 2001, Farr et al. 2002, Crous & Groenewald 2005). Within one host, multiple species can live and colonize at the same time (Dissanayake et al. 2015). Morphological characteristics can overlap within the genus, making the morphological identification dubious. Therefore, multi-loci molecular data helps to reduce the complexity and gives a better understanding on the identification of *Diaporthe* species (Dissanayake et al. 2015, Huang et al. 2015, Udayanga et al. 2015, Santos et al. 2017, Manawasinghe et al. 2019).

Among 1111 names of *Diaporthe* and 986 names of *Phomopsis* in Index Fungorum (2020), many species lack molecular data (Gao et al. 2017). Many studies continuously research on the classification and delimitation of *Diaporthe* species. Recently most of the species identifications are based on combined morpho-molecular, phyto-pathological and cultural data (Gomes et al. 2013, Gao et al. 2017). Even though *Diaporthe* was thought to be monophyletic based on their *Phomopsis* asexual morph, a recent study by Gao et al. (2017) revealed its paraphyletic nature.

The United States Department of Agriculture (USDA) host- fungus database lists 1632 fungal records from *Camellia* worldwide, of which 520 are from China (Farr & Rossman 2020; from https://nt.ars-grin.gov/fungaldatabases/). Out of those 520 records, 72 are *Diaporthe*. In the present study, fruiting bodies of a diaporthe-like collection was found from a dead aerial branch of *Camellia* in Yunnan Province, China. A combined ITS, BT, EF1– α , cal and H3 phylogenetic analyses and morphological characteristics revealed that this fungus is the sexual morph of *Diaporthe manihotia*. Therefore, this study provides the new host and new geographical record of *D. manihotia* from a *Camellia* species.

Materials & Methods

Sample collections, examination

A dead aerial branch of a *Camellia* sp., with fungal fruiting bodies, was collected from the Kunming Institute of Botany Garden, Yunnan Province, China in February 2019. The specimen was taken into the laboratory and examined using an Olympus SZ61 stereomicroscope. Hand-cut sections of ascomata were mounted in sterile water for microscopic studies and photographed with a Canon EOS 600D digital camera fitted to a Nikon ECLIPSE Ni compound microscope. Measurements were performed using the Tarosoft (R) Image Frame Work program and images used for figures processed with Adobe Photoshop CS5 Extended version 10.0 software (Adobe Systems, USA). The herbarium specimen was deposited at the Mae Fah Luang University herbarium (MFLU). Faces of Fungi numbers were acquired by following Jayasiri et al. (2015).

DNA extraction and PCR amplification

Since we were unable to obtain a culture, DNA was extracted directly from the ascomata of

the fungus. First, the ascomata were freeze-dried in 1.5 ml micro-centrifuge tubes using liquid nitrogen and ground into a powder. Then, the OMEGA E.Z.N.A. (B) Forensic DNA Kit was used to extract DNA following the manufacturer's instructions. The genomic DNA was used to amplify gene regions ITS, TUB2, TEF1– α , CAL and HIS as described in Manawasinghe et al. (2019). The PCR products were visualized on 1% agarose electrophoresis gels after staining with ethidium bromide. The amplified PCR fragments were sequenced at Biomed Co. LTD, Beijing, China. The nucleotide sequences were deposited at the GenBank (Supplementary Table 1).

Sequence alignment and phylogenetic analyses

The consensus sequences of the fungus were subjected to a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest matches. Based on the results of blast searches and recent publications, reference sequences were retrieved from the GenBank (Marin-Felix et al. 2019) and automatically aligned using default settings in MAFFT v. 7, Katoh et al 2019, http://mafft.cbrc.jp/alignment/server/).

The combined dataset of the five gene regions (ITS, TUB2, TEF1– α , CAL and HIS) was prepared and manually adjusted using BioEdit where necessary. Maximum likelihood trees were generated in the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010). The GTRGAMMA was used as the model of evolution, and Bootstrap support values were obtained by running 1000 pseudo-replicates. Bayesian Inference (BI) analysis was conducted using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Six simultaneous Markov chains were run for 2,000,000 generations, and trees were sampled at every 1000th generation. The first 25% of generated trees representing the burn-in phase of the analyses were discarded, and the remaining 75% of trees were used to calculate posterior probabilities (BYPP) in the majority rule consensus tree. Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012), and reorganized in Microsoft Power Point (2016).

Results

The fungus observed and sequenced in this study was identified as *Diaporthe manihotia* using morphology and molecular phylogenetic data.

Phylogenetic analyses

The initial dataset (ITS, TUB2, TEF1 $-\alpha$, CAL and HIS) consisted of 214 taxa, including our fungal strain (Diaporthe manihotia: MFLU 19-2826). Phylogenetic trees are rooted with Diaporthella corylina (CBS 121124). The complete RAxML tree with 214 Diaporthe taxa and a final optimization likelihood value of - 79807.880799 is shown in Supplementary Figure 1. Placement of the Diaporthe manihotia (MFLU 19-2826) is confirmed, and trees re-constructed with 66 taxa. Maximum likelihood and Bayesian posterior probability analyses resulted in trees with similar topologies that did not differ significantly (data not shown). The RAxML tree with the final optimization likelihood value of - 28926.462839 is shown in Fig. 1. The matrix had 1589 distinct alignment patterns, with 25.13% of undetermined characters or gaps. Parameters for the GTRGAMMA model of the combined data set (ITS, TUB2, TEF1-α, CAL and HIS) were as follows: Estimated base frequencies; A = 0.207598, C = 0.334229, G = 0.235010, T = 0.223163; substitution rates AC = 1.105931, AG = 3.876866, AT = 3.876866, CG = 1.050143, CT = 4.981350, GT = 1.000000; proportion of invariable sites I = 0.347363; gamma distribution shape parameter $\alpha = 1.001672$. The sexual morph of *Diaporthe manihotia* (MFLU 19–2826) clustered with Diaporthe manihotia (CBS 505.76) in a well-supported clade (97% ML, 1.00%, BYPP, Fig. 1).

Diaporthe manihotia Punith., Kavaka 3: 29 (1976) [1975]

Index Fungorum number: 312932; Facesoffungi number: FoF07324

Saprobic on a dead aerial branch of *Camellia* spp., visible as raised black spots or black necks immerging through the host surface. Sexual morph: *Ascomata* 200–360 µm high, 180–300

Fig. 2

μm diam. ($\bar{x} = 262 \ \mu m \times 231 \ \mu m$, n = 5), immersed in the host epidermis, globose to sub–globose, solitary or occur in clusters, black, ostiolate. *Ostiole* 45–70 μm wide×150–200 μm long ($\bar{x} = 54 \ \mu m \times 262 \ \mu m$, n = 5). *Peridium* 10–15 μm wide, comprising 4–6 layers, outer layers heavily pigmented, thin-walled, comprising dark brown cells of *textura angularis*, inner layers composed of hyaline to brown thin-walled cells of *textura angularis*. *Paraphyses* 2.5–4 μm wide, septate, wide at base, tapering towards the apex, thin-walled. *Asci* 45–60 μm × 7–8 μm ($\bar{x} = 49.2 \ \mu m \times 7.3 \ \mu m$, n = 10), 8-spored, unitunicate, clavate to subclavate, straight to slightly curved, sessile, with a J– apical ring. *Ascospores* 8–13 μm × 2.6–3.7 μm ($\bar{x} = 10.2 \ \mu m \times 3.07 \ \mu m$, n = 20), overlapping uniseriate to biseriate, 1-septate, constricted at the septum, often tetra-guttulate, ellipsoidal, straight, hyaline, without appendages or a mucilaginous sheath. Asexual morph: See Swarup et al. (1966).

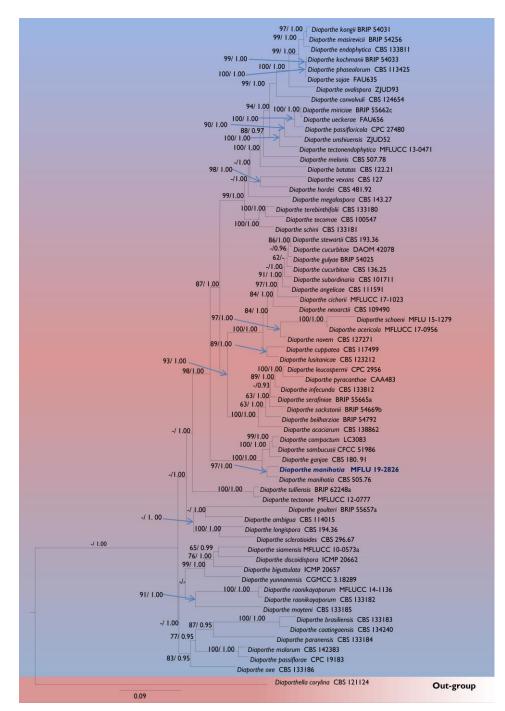


Fig. 1 – Phylogram generated from maximum likelihood analysis of combined ITS, TUB2, TEF1– α , CAL and HIS sequence data set. *Diaporthe manihotia* (MFLU 19–2826) is indicated in blue and the types and ex-types are in bold.

Material examined – China, Yunnan Province, Heilongtan, Kunming Institute of Botany, Botanical Gardens (25.137711°N, 102.745185°E), on a dead aerial branch of *Camellia* sp., 2 February 2019, D.N. Wanasinghe, CG003 (MFLU 19–2826).



Fig. 2 – *Diaporthe manihotia* (MFLU 19–2826) sexual morph on the host. a Appearance of ascomata on a dead aerial branch of *Camellia* spp. b Close up view of elongate ascoma neck. c Cross-section through the ascoma. d Cell layers of ascomatal neck area. e Peridium wall comprising layers of cells. f. Paraphyses. g Immature and mature asci. i, j Ascospores. Scale bars:

a, b = 200 μ m, c, f, g, h = 20 μ m, i, j = 10 μ m.

Discussion

Diaporthe manihotia collected from a *Camellia* sp. was identified using morphology and molecular phylogenetic data. Since we were unable to obtain a culture from our *D. manihotia* collection; hence we extracted the genomic DNA from the ascomata. Our strain was grouped with *D. manihotia* strain CBS 505.76, which was collected from leaves of *Manihot utilissima* (Euphorbiaceae) in Rwanda (Fig. 1) (Gomes et al. 2013). However, the holotype of *D. manihotia* (IMI 180613) was recovered from the stems of *Manihot esculenta* in Colombia (Punithalingam 1975).

It is reported that *D. manihotia* causes leaf spot disease on Cassava (referred to as Phomopsis blight of tapioca) (Rajan et al. 1974, Punithalingam 1975, Gomes et al. 2013). Symptoms of early disease infection on young leaves are visible as pale green, water-soaked, and small round spots, while petioles rapidly enlarge and turn brown. When the disease is severe, defoliation occurs. Affected plant parts become withered and pycnidia observed in the tissues (Rajan et al. 1974, Punithalingam 1975, Gomes et al. 2013). This disease is distributed worldwide, including Africa (Ethiopia, Nigeria, Rwanda), Asia (India), Central America, West Indies and South America (Colombia) (Gomes et al. 2013, Farr & Rossman 2020).

Our *D. manihotia* strain shares similar morphology such as immersed ascomata, erumpent pseudostroma with elongated perithecial necks, unitunicate clavate to cylindrical asci and hyaline ascospores as other *Diaporthe* sexual morphs (Udayanga et al. 2011). Our *D. manihotia* strain shares similar morphology with the holotype specimen of *D. manihotia* (IMI 180613) with minor dimensional differences. The ascomata of *D. manihotia* isolate MFLU 19–2826 are comparatively smaller than those of isolate IMI 180613 (180–300 µm diam. *vs* up to 500 µm wide) (Punithalingam 1975). Furthermore, IMI 180613 has relatively smaller asci (30–40 × 6–8 µm), while our collection has relatively longer asci (45–60 µm × 7–8 µm). Both have similar-sized ascospores with slight dimensional differences (8–12 × 3–4 µm *vs* 8–13 µm × 2.6–3.7 µm) (Punithalingam 1975). These dimensional differences are probably due to environmental variation or/and host associations.

In our multi-gene phylogenetic analyses, the strain *Diaporthe manihotia* (CBS 505.76) formed a monophyletic clade with our isolate (MFLU 19–2826) with 97% ML, and 1.00 BYPP statistical support (Fig. 1). Nucleotide comparisons for the ITS, BT, EF1– α and cal gene regions between our isolate MFLU 19–2826 and CBS 505.76 revealed 99%, 98%, 92% and 97% base pair similarities respectively. However, we could not obtain the H3 sequence for our isolate. According to both morphological and molecular data, our collection from *Camellia* was confirmed as the sexual morph of *D. manihotia*.

Recently identified *Diaporthe* species associated with *Camellia* spp. were either pathogenic or endophytic (Gao et al. 2016, 2017, Li et al. 2017, Guarnaccia & Crous 2018). Pathogenic species are responsible for leaf spots or dieback in tea (Gao et al. 2016, 2017, Guarnaccia & Crous 2018). As we were unable to obtain a culture, we could not do a pathogenicity test to check whether this species can cause dieback of *Camellia* shoots.

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Supplementary materials

Supplementary Table 1 Taxa names, culture collection/herbarium numbers and GenBank accession numbers of the taxa used in the phylogenetic analyses

Species	Culture collection/	GenBank accession numbers				
-	Herbarium number	ITS	TUB2	HIS	TEF1-α	CAL
	a,*					
Diaporthe acaciarum	CBS 138862*	KP004460	KP004509	KP004504	_	_
D. acaciigena	CBS 129521*	KC343005	KC343973	KC343489	KC343731	KC343247
D. acericola	MFLUCC 17-0956*	KY964224	KY964074	—	KY964180	KY964137
D. acerina	CBS 137.27	KC343006	KC343974	KC343490	KC343732	KC343248
D. acutispora	CGMCC 3.18285*	KX986764	KX999195	KX999235	KX999155	KX999274
D. alleghaniensis	CBS 495.72*	FJ889444	KC843228	KC343491	GQ250298	KC343249
D. alnea	CBS 146.46*	KC343008	KC343976	KC343492	KC343734	KC343250
D. ambigua	CBS 114015*	KC343010	KC343978	KC343494	KC343736	KC343252
D. ampelina	CBS 114016*	AF230751	JX275452	_	GQ250351	JX197443
D. amygdali	CBS 126679*	KC343022	KC343990	KC343506	KC343748	KC343264
D. anacardii	CBS 720.97*	KC343024	KC343992	KC343508	KC343750	KC343266
D. angelicae	CBS 111592*	KC343026	KC343994	KC343511	KC343752	KC343268
D. apiculatum	LC 3418*	KP267896	KP293476	KP293550	KP267970	_
D. aquatica	IFRDCC 3051*	JQ797437	_	_	_	_
D. arctii	CBS 136.25	KC343031	KC343999	KC343515	KC343757	KC343273
D. arecae	CBS 161.64*	KC343032	KC344000	KC343516	KC343758	KC343274
D. arengae	CBS 114979*	KC343034	KC344002	KC343518	KC343760	KC343276
D. aseana	MFLUCC 12-0299a*	KT459414	KT459432	_	KT459448	KT459464
D. asheicola	CBS 136967*	KJ160562	KJ160518	_	KJ160594	KJ160542
D. aspalathi	CBS 117169*	KC343036	KC344004	KC343520	KC343762	KC343278
D. australafricana	CBS 111886*	KC343038	KC344006	KC343522	KC343764	KC343280
D. baccae	CBS 136972*	KJ160565	MF418509	MF418264	KJ160597	_
D. batatas	CBS 122.21	KC343040	KC344008	KC343524	KC343766	KC343282
D. beckhausii	CBS 138.27	KC343041	KC344009	KC343525	KC343767	KC343283
D. beilharziae	BRIP 54792*	JX862529	KF170921	_	JX862535	_
D. benedicti	CFCC 50062*	KP208847	KP208855	KP208851	KP208853	KP208849
D. betulae	CFCC 50469*	KT732950	KT733020	KT732999	KT733016	KT732997

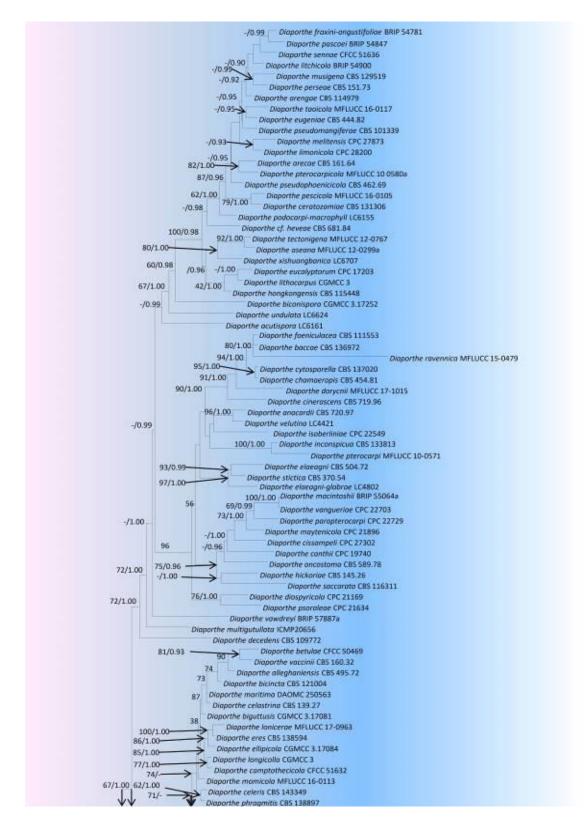
Species	Culture collection/	GenBank accession numbers					
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D. betulicola	CFCC 51128*	KX024653	KX024657	KX024661	KX024655	KX024659	
D. bicincta	CBS 121004*	KC343134	KC344102	KC343618	KC343860	KC343376	
D. biconispora	CGMCC 3.17252*	KJ490597	KJ490418	KJ490539	KJ490476	_	
D. biguttulata	ICMP20657*	KJ490582	KJ490403	KJ490524	KJ490461	_	
D. biguttusis	CGMCC 3.17081*	KF576282	KF576306	-	KF576257	_	
D. bohemiae	CBS 143347*	MG281015	MG281188	MG281361	MG281536	MG28171	
D. brasiliensis	CBS 133183*	KC343042	KC344010	KC343526	KC343768	KC343284	
D. caatingaensis	CBS 141542*	KY085927	KY115600	KY115605	KY115603	KY11559	
D. camptothecicola	CFCC 51632*	KY203726	KY228893	KY228881	KY228887	KY22887	
D. canthii	CBS 132533*	JX069864	KC843230	_	KC843120	KC843174	
D. carpini	CBS 112535 CBS 114437	KC343044	KC344012	 KC343528	KC343770	KC34328	
D. cassines	CBS 136440*	KF777155	KC344012	KC545520	KF777244	KCJ45200	
D. caulivora	CBS 127268*	KC343045	 KC344013	_ KC343529	KC343771	– KC34328'	
D. celastrina	CBS 139.27*	KC343045 KC343047	KC344015 KC344015	KC343531	KC343773	KC34328	
D. celeris	CBS 139.27 CBS 143349*	MG281017	MG281190	MG281363	MG281538	MG28171	
			MG201190	WIG281505	MG201330	MG28171	
D. ceratozamiae	CBS 131306* CBS 852.97	JQ044420	– KC344084	_ KC343600	– KC343842	– KC34335	
D. cf. heveae 1		KC343116					
D. cf. <i>heveae</i> 2	CBS 681.84	KC343117	KC344085	KC343601	KC343843	KC34335	
D. chamaeropis	CBS 454.81	KC343048	KC344016	KC343532	KC343774	KC34329	
D. charlesworthii	BRIP 54884m*	KJ197288	KJ197268	_	KJ197250	-	
D. cichorii	MFLUCC 17-1023*	KY964220	KY964104	- KG242524	KY964176	KY96413	
D. cinerascens	CBS 719.96	KC343050	KC344018	KC343534	KC343776	KC343292	
D. cissampeli	CBS 141331*	KX228273	KX228384	KX228366	-	-	
D. citri	CBS 135422*	KC843311	KC843187	MF418281	KC843071	KC84315	
D. citriasiana	CBS 134240*	JQ954645	KC357459	MF418282	JQ954663	KC35749	
D. citrichinensis	CBS 134242*	JQ954648	MF418524	KJ420880	JQ954666	KC357494	
D. compacta	LC3083*	KP267854	KP293434	KP293508	KP267928	_	
D. convolvuli	CBS 124654	KC343054	KC344022	KC343538	KC343780	KC34329	
D. crataegi	CBS 114435	KC343055	KC344023	KC343539	KC343781	KC34329	
D. crotalariae	CBS 162.33*	KC343056	KC344024	KC343540	KC343782	KC34329	
D. cucurbitae	DAOM 42078*	KM453210	KP118848	KM453212	KM453211	_	
D. cuppatea	CBS 117499*	AY339322	JX275420	KC343541	AY339354	JX197414	
D. cynaroidis	CBS 122676	KC343058	KC344026	KC343542	KC343784	KC34330	
D. cytosporella	CBS 137020*	KC843307	KC843221	MF418283	KC843116	KC84314	
D. decedens	CBS 109772	KC343059	KC344027	KC343543	KC343785	KC34330	
D. detrusa	CBS 109770	KC343061	KC344029	KC343545	KC343787	KC34330	
D. diospyricola	CBS 136552*	KF777156	_	_	_	_	
D. discoidispora	ICMP20662*	KJ490624	KJ490445	KJ490566	KJ490503	_	
D. dorycnii	MFLUCC 17-1015*	KY964215	KY964099	_	KY964171	_	
D. elaeagni-glabrae	CGMCC 3.18287*	KX986779	KX999212	KX999251	KX999171	KX99928	
D. eleagni	CBS 504.72	KC343064	KC344032	KC343548	KC343790	KC34330	
D. ellipicola	CGMCC 3.17084*	KF576270	KF576291	_	KF576245	_	
D. endophytica	CBS 133811*	KC343065	KC344033	KC343549	KC343791	KC34330	
D. eres	CBS 138594*	KJ210529	KJ420799	KJ420850	KJ210550	KJ434999	
D. eucalyptorum	CBS 132525*	JX069862	_	_	_	_	
D. eugeniae	CBS 444.82	KC343098	KC344066	KC343582	KC343824	KC34334(
D. fibrosa	CBS 109751	KC343099	KC344067	KC343583	KC343825	KC34334	
D. foeniculina	CBS 111553*	KC343101	KC344069	KC343585	KC343827	KC34334.	
D. fraxini-	BRIP 54781*	JX862528	KF170920	_	JX852534	_	
angustifoliae							
D. fusicola	CGMCC 3.17087*	KF576281	KF576305	_	KF576256	KF576233	
D. ganjae	CBS 180.91*	KC343112	KC344080	KC343596	KC343838	KC343354	
D. gardeniae	CBS 288.56	KC343113	KC344081	KC343597	KC343839	KC34335	
D. garethjonesii	MFLUCC 12-0542a*	KT459423	KT459441	-	KT459457	KT45947(

Species	Culture collection/	GenBank accession numbers					
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D. goulteri	BRIP 55657a*	KJ197290	KJ197270	_	KJ197252	_	
D. gulyae	BRIP 54025*	JF431299	KJ197271	—	JN645803	_	
D. helianthi	CBS 592.81*	KC343115	KC344083	KC343599	KC343841	JX197454	
D. helicis	CBS 138596*	KJ210538	KJ420828	KJ420875	KJ210559	KJ435043	
D. heterophyllae	CBS 143769*	MG600222	MG600226	MG600220	MG600224	MG600218	
D. hickoriae	CBS 145.26*	KC343118	KC344086	KC343602	KC343844	KC343360	
D. hispaniae	CBS 143351*	MG281123	MG281296	MG281471	MG281644	MG281820	
D. hongkongensis	CBS 115448*	KC343119	KC344087	KC343603	KC343845	KC343361	
D. hordei	CBS 481.92	KC343120	KC344088	KC343604	KC343846	KC343362	
D. hungariae	CBS 143353*	MG281126	MG281299	MG281474	MG281647	MG281823	
D. impulsa	CBS 114434	KC343121	KC344089	KC343605	KC343847	KC343363	
D. incompleta	CGMCC 3.18288*	KX986794	KX999226	KX999265	KX999186	KX999289	
D. inconspicua	CBS 133813*	KC343123	KC344091	KC343607	KC343849	KC343365	
D. infecunda	CBS 133812*	KC343126	KC344094	KC343610	KC343852	KC343368	
D. infertilis	CBS 230.52*	KC343052	KC344020	KC343536	KC343778	KC343294	
D. isoberliniae	CBS 137981*	KJ869133	KJ869245	_	_	_	
D. juglandicola	CFCC 51134*	KU985101	KX024634	_	KX024628	KX024616	
D. kochmanii	BRIP 54033*	JF431295	_	_	JN645809	_	
D. kongii	BRIP 54031*	JF431301	KJ197272	_	JN645797	_	
D. leucospermi	CBS 111980*	JN712460	KY435673	KY435653	KY435632	KY435663	
D. limonicola	CBS 142549*	MF418422	MF418582	MF418342	MF418501	MF418256	
D. litchicola	BRIP 54900*	JX862533	KF170925	_	JX862539	_	
D. lithocarpus	CGMCC 3.15175*	KC153104	KF576311	_	KC153095	-	
D. litoricola	MFLUCC 16-1195*	MF190139	-	_	-	-	
D. longicicola	CGMCC 3.17089*	KF576267	KF576291	_	KF576242	_	
D. longicolla	FAU 599*	KJ590728	KJ610883	KJ659188	KJ590767	KJ612124	
D. longispora	CBS 194.36*	KC343135	KC344103	KC343619	KC343861	KC343377	
D. lonicerae	MFLUCC 17-0963*	KY964190	KY964073	-	KY964146	KY964116	
D. lusitanicae	CBS 123212*	KC343136	KC344104	KC343620	KC343862	KC343378	
D. macintoshii	BRIP 55064a*	KJ197289	KJ197269	_	KJ197251	-	
D. mahothocarpus	CGMCC 3.15181	KC153096	_	_	KC153087	_	
D. malorum	CBS142383*	KY435638	KY435668	KY435648	KY435627	KY435658	
D. manihotia	CBS 505.76	KC343138	KC344106	KC343622	KC343864	KC343380	
D. manihotia	MFLU 19-2826	MT012296	MW018927	-	MW014359	MW014358	
D. maritima	DAOMC 250563*	KU552025	KU574615	_	KU552023	_	
D. masirevicii	BRIP 57892a*	KJ197277	KJ197257	- KC242622	KJ197239	- KO242201	
D. mayteni	CBS 133185*	KC343139	KC344107	KC343623	KC343865	KC343381	
D. maytenicola	CBS 136441*	KF777157	KF777250	– KC343624	- VC242966	- KC242200	
D. megalospora D. melitensis	CBS 143.27	KC343140	KC344108 MF418584		KC343866	KC343382	
D. mellensis D. melonis	CBS 142551* CBS 507.78*	MF418424 KC343142	KC344110	MF418344 KC343626	MF418503 KC343868	MF418258 KC343384	
D. middletonii	BRIP 54884e*	KU343142 KJ197286	KU344110 KJ197266	KC343020	KU343808 KJ197248	KC343304	
D. miticiae	BRIP 54736j*	KJ197280 KJ197283	KJ197263	_	KJ197248 KJ197245	_	
D. monicola	MFLUCC 16-0113 [*]	KU557563	KU557587	—	KU557631	– KU557611	
D. multigutullata	ICMP20656*	KJ490633	KJ490454		KJ490512	K0357011	
D. musigena	CBS 129519*	KC343143	KJ4904J4 KC344111	KC343627	KC343869	 KC343385	
D. neilliae	CBS 129519 CBS 144.27*	KC343143 KC343144	KC344111 KC344112	KC343628	KC343809	KC343386	
D. neoarctii	CBS 109490	KC343144 KC343145	KC344112 KC344113	KC343629	KC343870	KC343387	
D. neourciii D.	MFLUCC 14-1136 [*]	KU712449	KU743988	_	KU749369	KU749356	
neoraonikayaporum	MI LOCC 17-1150	110/1277)	110/70/00		110/7/30/	110/7/000	
D. nomurai	CBS 157.29	KC343154	KC344122	KC343638	KC343880	KC343396	
D. nothofagi	BRIP 54801*	JX862530	KF170922	_	JX862536	_	
D. novem	CBS 127271*	KC343157	KC344125	KC343641	KC343883	KC343399	
D. novem	CDS 12/2/1	NC343137	NCJ4412J	NCJ4J041	NCJ4J00J	NCJ4JJ77	

Species	Culture collection/	GenBank accession numbers				
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D. obtusifoliae	CBS 143449*	MG386072	_	MG386137	_	_
D. ocoteae	CBS 141330*	KX228293	KX228388	_	_	_
D. oncostoma	CBS 589.78	KC343162	KC344130	KC343646	KC343888	KC343404
D. oraccinii	LC 3166 [*]	KP267863	KP293443	KP293517	KP267937	_
D. ovalispora	ICMP20659*	KJ490628	KJ490449	KJ490570	KJ490507	_
D. ovoicicola	CGMCC 3.17092*	KF576264	KF576288	_	KF576239	KF576222
D. ovoicicoid D. oxe	CBS 133186*	KC343164	KC344132	KC343648	KC343890	KC34340
D. oxe D. padi var. padi	CBS 114200	KC343169	KC344137	KC343653	KC343895	KC34341
D. paranensis	CBS 133184	KC343171	KC344139	KC343655	KC343897	KC34341
D. parapterocarpi	CBS 137986*	KJ869138	KJ869248		_	_
D. pascoei	BRIP 54847*	JX862532	KF170924	_	JX862538	_
D. passiflorae	CBS 132527*	JX069860	KY435674	KY435654	KY435633	KY43566
D. passifloricola	CBS 141329*	KX228292	KX228387	KX228367	-	_
D. penetriteum	LC 3353	KP714505	KP714529	KP714493	KP714517	_
D. perjuncta	CBS 109745*	KC343172	KC344140	KC343656	KC343898	– KC34341
D. perniciosa	CBS 124030	KC343172 KC343149	KC344140 KC344117	KC343633	KC343898 KC343875	KC34341 KC34339
D. perseae	CBS 124030 CBS 151.73	KC343149 KC343173	KC344117 KC344141	KC343033 KC343657	KC343875 KC343899	KC34339 KC34341
1	MFLUCC 16-0105*	KU557555	KU557579		KU557623	KU55760
D. pescicola				- KC242659		
D. phaseolorum	CBS 113425	KC343174	KC344142	KC343658	KC343900	KC34341
D. phragmitis	CBS 138897*	KP004445	KP004507	KP004503	- KV000167	- KX000077
D. podocarpi-	CGMCC3.18281*	KX986774	KX999207	KX999246	KX999167	KX99927
macrophylli	CDC 101000*	1/02/01/01	WGQ 44140	TROPAGES	1400 40007	12 (22 42 42
D. pseudomangiferae	CBS 101339*	KC343181	KC344149	KC343665	KC343907	KC34342
D. pseudophoenicicola	CBS 462.69*	KC343184	KC344152	KC343668	KC343910	KC34342
D. pseudotsugae	MFLU 15-3228	KY964225	KY964108	—	KY964181	KY96413
D. psoraleae	CBS 136412*	KF777158	KF777251	—	KF777245	-
D. psoraleae-pinnatae	CBS 136413*	KF777159	KF777252	—	_	_
D. pterocarpi	MFLUCC 10-0571	JQ619899	JX275460	—	JX275416	JX197451
D. pterocarpicola	MFLUCC 10-0580a	JQ619887	JX275441	-	JX275403	JX197433
D. pulla	CBS 338.89*	KC343152	KC344120	KC343636	KC343878	KC34339
D. pustulata	CBS 109742	KC343185	KC344153	KC343669	KC343911	KC34342
D. pyracanthae	CBS142384*	KY435635	KY435666	KY435645	KY435625	KY43565
D. racemosae	CBS 143770*	MG600223	MG600227	MG600221	MG600225	MG60021
D. raonikayaporum	CBS 133182*	KC343188	KC344156	KC343672	KC343914	KC34343
D. ravennica	MFLUCC 15-0479*	KU900335	KX432254	_	KX365197	_
D. rhoina	CBS 146.27	KC343189	KC344157	KC343673	KC343915	KC34343
D. rostrata	CFCC 50062*	KP208847	KP208855	KP208851	KP208853	KP20884
D. rudis	CBS 113201	KC343234	KC344202	KC343718	KC343960	KC34347
D. saccarata	CBS 116311*	KC343190	KC344158	KC343674	KC343916	KC34343
D. sackstonii	BRIP 54669b*	KJ197287	KJ197267	_	KJ197249	_
D. salicicola	BRIP 54825*	JX862531	KF170923	_	JX862537	_
D. sambucusii	CFCC 51986*	KY852495	KY852511	KY852503	KY852507	KY85249
D. schini	CBS 133181*	KC343191	KC344159	KC343675	KC343917	KC34343
D. schisandrae	CFCC 51988*	KY852497	KY852513	KY852505	KY852509	KY85250
D. schoeni	MFLU 15-1279*	KY964226	KY964109	_	KY964182	KY96413
D. sclerotioides	CBS 296.67*	KC343193	KC344161	KC343677	KC343919	KC34343
D. scobina	CBS 251.38	KC343195	KC344163	KC343679	KC343921	KC34343
D. sennae	CFCC 51636*	KY203724	KY228891	_	KY228885	KY22887
D. sennicola	CFCC 51634*	KY203724	KY228889	_	KY228883	KY22887
D. serafiniae	BRIP 55665a*	KJ197274	KJ197254	_	KJ197236	_
D. siamensis	MFLUCC 10-0573a	JQ619879	JX275429	_	JX275393	_
D. sojae	CBS 139282 [*]	KJ590719	KJ610875	 KJ659208	KJ590762	 KJ612116
D. sojae D. spartinicola	CBS 139282 CBS 140003*	KJ 390719 KR 611879	KR857695	KJ059208 KR857696		
D. sterilis	CBS 136969*	KJ160579	KK857095 KJ160528	MF418350	– KJ160611	– KJ160548

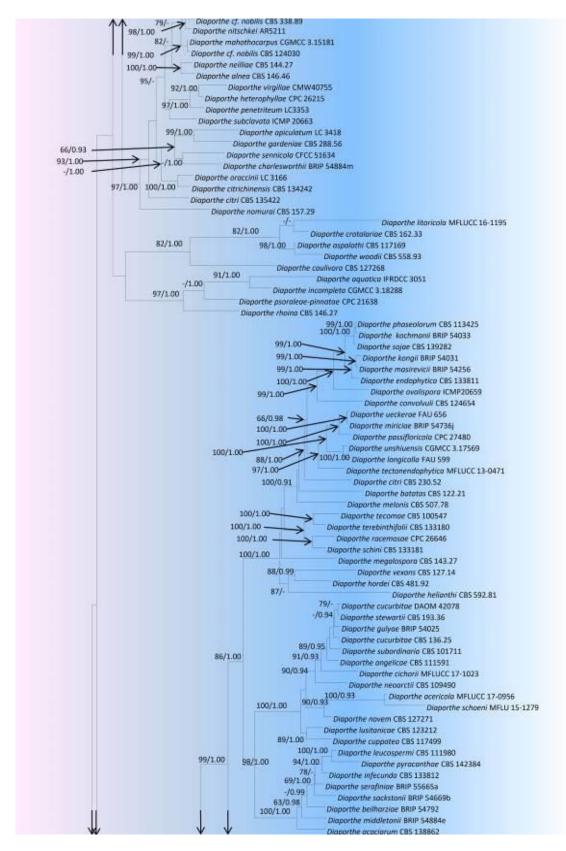
Species	Culture collection/	GenBank accession numbers				
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D. stewartii	CBS 193.36	FJ889448	_	_	GQ250324	_
D. stictica	CBS 370.54	KC343212	KC344180	KC343696	KC343938	KC343454
D. subclavata	ICMP20663*	KJ490630	KJ490451	KJ490572	KJ490509	_
D. subordinaria	CBS 101711	KC343213	KC344181	KC343697	KC343939	KC343455
D. taoicola	MFLUCC 16-0117*	KU557567	KU557591	_	KU557635	_
D. tecomae	CBS 100547	KC343215	KC344183	KC343699	KC343941	KC343457
D. tectonae	MFLUCC 12-0777*	KU712430	KU743977	_	KU749359	KU749345
D. tectonendophytica	MFLUCC 13-0471*	KU712439	KU743986	—	KU749367	KU749354
D. tectonigena	MFLUCC 12-0767*	KU712429	KU743976	_	KU749371	KU749358
D. terebinthifolii	CBS 133180*	KC343216	KC344184	KC343700	KC343942	KC343458
D. ternstroemia	CGMCC 3.15183*	KC153098	-	—	KC153089	_
D. thunbergii	MFLUCC 10-0756a	JQ619893	JX275449	—	JX275409	JX197440
D. torilicola	MFLUCC 17-1051*	KY964212	KY964096	—	KY964168	KY964127
D. toxica	CBS 534.93*	KC343220	KC344188	KC343704	KC343946	KC343462
D. toxicodendri	FFPRI420987	LC275192	LC275224	LC275216	LC275216	LC275200
D. tulliensis	BRIP 62248a	KR936130	KR936132	—	KR936133	_
D. ueckerae	FAU 656	KJ590726	KJ610881	KJ659215	KJ590747	KJ612122
D. undulata	CGMCC 3.18293*	KX986798	KX999230	KX999269	KX999190	_
D. unshiuensis	CGMCC3.17569*	KJ490587	KJ490408	KJ490529	KJ490466	_
D. vaccinii	CBS 160.32*	AF317578	KC344196	KC343712	GQ250326	KC343470
D. vangueriae	CBS 137985*	KJ869137	KJ869247	—	_	_
D. vawdreyi	BRIP 57887a	KR936126	KR936128		KR936129	_
D. velutina	CGMCC 3.18286*	KX986790	KX999223	KX999261	KX999182	_
D. vexans	CBS 127.14	KC343229	KC344197	KC343713	KC343955	KC343471
D. virgiliae	CBS 138788*	KP247573	KP247582	—	_	_
D. woodii	CBS 558.93	KC343244	KC344212	KC343728	KC343970	KC343486
D. woolworthii	CBS 148.27	KC343245	KC344213	KC343729	KC343971	KC343487
D. xishuangbanica	CGMCC 3.18282*	KX986783	KX999216	KX999255	KX999175	_
D. yunnanensis	CGMCC 3.18289*	KX986796	KX999228	KX999267	KX999188	KX999290

^a BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; CGMCC: Chinese General Microbiological Culture Collection Center, Beijing, China; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; DAOMC: Canadian Collection of Fungal Cultures, Ottawa, Canada; FAU: Isolates in culture collection of Systematic Mycology and Microbiology Laboratory; FFPRI: Forestry and Forest Products Research Institute, Japan; ICMP: International Collection of Microorganisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IFRDCC: International Fungal Research and Development Culture Collection; MFLU: Mae Fah Luang University herbarium, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; LC: Working collection of Lei Cai, housed at Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. * Ex-types.

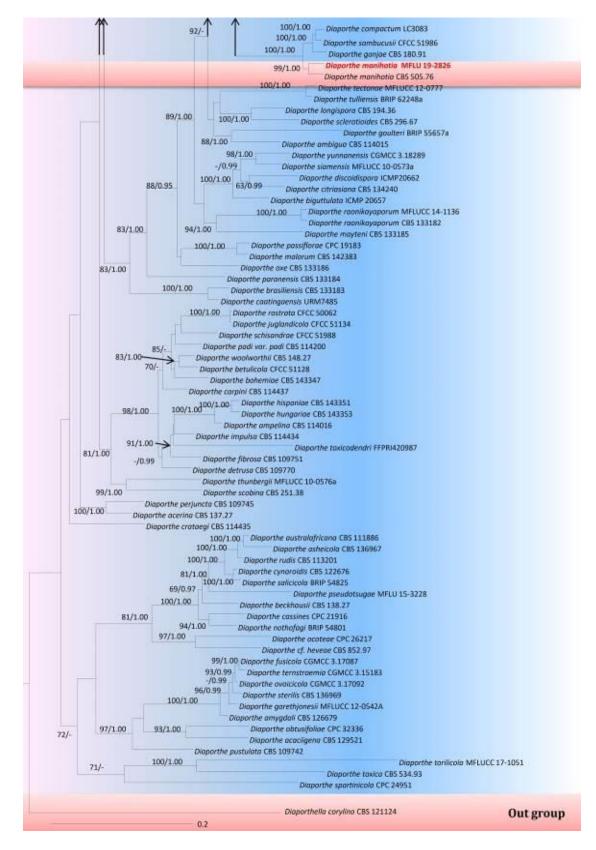


Supplementary Fig. 1 – Phylogram generated from maximum likelihood analysis based on combined ITS, TUB2, TEF1- α , CAL and HIS sequences. Bootstrap support values for maximum likelihood (ML) equal to or higher than 60% (left) and bayesian posterior probabilities (PP) equal to or greater than 0.95 (right) are provided on the nodes. The combined ITS, TUB2, TEF1- α , CAL and HIS dataset consisted of 214 taxa including our collection (*Diaporthe manihotia* MFLU 19-2826 is indicated in red bold). Phylogenetic trees were rooted with *Diaporthella corylina* (CBS 121124). The final RAxML tree with the final ML optimization likelihood value of -79807.880799. The matrix had 2531 distinct alignment patterns, with 39.19% of undetermined characters or gaps. Parameters for the GTRGAMMA model of the combined data set (ITS, TUB2, TEF1- α , CAL and

HIS) were as follows: Estimated base frequencies; A = 0.249825, C = 0.260011, G = 0.255881, T = 0.234283; substitution rates AC = 1.090057, AG = 3.447351, AT = 1.253285, CG = 0.913844, CT = 4.652094, GT = 1.000000; proportion of invariable sites I = 0.236988; gamma distribution shape parameter α = 0.826901.



Supplementary Fig. 1 – Continued.



Supplementary Fig. 1 – Continued.