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Article

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Diaporthe collariana sp. nov., with prominent collarettes associated with *Magnolia champaca* fruits in Thailand

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Abstract

We are studying seed and fruit inhabiting fungi in Thailand and this paper introduces a new species, *Diaporthe collariana*, from *Magnolia champaca* fruits, collected in Chiang Rai Province. Molecular analysis of a combined ITS, TEF1, TUB and CAL sequence DNA and morphological data provide evidence to justify the new species. *Diaporthe collariana* is characterized by producing alpha and beta conidia, and conidiogenous cells with prominent, flared collarettes. The new species is compared with closely related species in the genus.

Key words – *Diaporthaceae* – morphology – new species – phylogeny – seed/fruit fungi

Introduction

Diaporthe species are plant pathogens, endophytes or saprobes, found on a wide range of hosts (Gomes et al. 2013, Gao et al. 2014, Dissanayake et al. 2017a, b, c). Previously species of this genus were considered as host-specific. However, as the same species can be found on more than one host, this is no longer valid (Rehner & Uecker 1994, Gomes et al. 2013, Dissanayake et al. 2017b). Currently, 171 species of *Diaporthe*, have been described from various plant hosts worldwide and species rank supported with molecular data (Gomes et al. 2013, Dissanayake et al. 2017a, b, c, Gao et al. 2017). However, most old epithets of *Diaporthe* lack molecular data and some morphological descriptions lack informative data (Dayarathne et al. 2016, Gao et al. 2017, Index Fungorum 2017). Taxonomy of the genus relies largely on molecular phylogenies (Udayanga et al. 2012, Gomes et al. 2013), as few morphological characters can be used in species delimitation

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(Sutton 1980, Rehner & Uecker 1994, Chi et al. 2007, Hyde et al. 2011, Dissanayake et al. 2017b, Gao et al. 2017). Currently, the pairwise dissimilarities of the internal transcribed spacer (ITS), translation elongation factor 1-α (TEF1), partial beta tubulin (TUB), histone H3 (HIS) and calmodulin (CAL) loci are useful when defining a new species (Udayanga et al. 2012, Gomes et al. 2013, Jeewon & Hyde 2016, Dissanayake et al. 2017b, Gao 2017, Santos et al. 2017).

The leaf spot causing pathogenic species of *Diaporthe* (as *Phomopsis micheliae* Sankaran et al.) was identified from leaves of *Magnolia champaca* (=*Michelia champaca*) in India (Sankaran et al. 1987). It is characterized by simple septate conidiophores (9–36 × 1–1.5 µm), fusiform to ellipsoid alpha conidia, and filiform, hamate beta conidia (16–34 × 1.5 µm) (Sankaran et al. 1987). A homonym, *P. micheliae* C.Q. Chang et al., which was collected from living branches of *Michelia alba* in China, was introduced by Chang et al. (2005). However, this was not considered as a validly published species, since the name was already published by Sankaran et al. (1987) (Hawksworth & David 1989 – Art. 53.1). Gao et al. (2017) treated *Phomopsis micheliae* as a synonymy of *Diaporthe michelina* (C.Q. Chang et al.) Y.H. Gao & L. Cai.

In the current study, an undescribed species of *Diaporthe* is recognized by DNA sequence analysis, together with morphological characterization of asexual morphic structures.

Materials & methods

Sample collection, morphological examination and isolation

Specimens were collected from Chiang Rai, Thailand during August 2017, and macroscopic and microscopic characters were observed in the laboratory. Fungal structures were observed using a Motic dissecting microscope (SMZ 168) and a Nikon ECLIPSE 80i compound microscope. Free hand sections of conidiomata were taken and mounted in water for microscopic study. Conidiophores, conidiogenous cells and conidia were mounted in Congo red for detailed observations. Photomicrography was carried out using a Canon 450D digital camera fitted to the microscope. Measurements were taken with the Tarosoft (R) Image Frame Work software. The images used for illustrating the fungi were processed with Adobe Photoshop CS5 v. 12.0 software (Adobe Systems, USA). Single conidial colonies were established as described in Chomnunti et al. (2014). Pure cultures were obtained on Potato Dextrose Agar (PDA) and incubated at room temperature of 28°C. To induce sporulation, cultures were incubated at 28 °C, in the dark. Conidiomata produced on PDA, were also illustrated following the above procedure.

Herbarium specimens were deposited in the Mae Fah Luang University (MFLU) herbarium, Chiang Rai, Thailand. Living cultures were deposited in the Culture Collection at Mae Fah Luang University (MFLUCC). Facesoffungi and Index Fungorum numbers were registered as explained in Jayasiri et al. (2015) and Index Fungorum (2017). Species are delineated based on DNA sequence data as in Jeewon & Hyde (2016).

Table 1 Information on loci and PCR protocols used in the study.

Locus	Primers (Reference)	PCR Conditions
ITS	ITS5/ITS4 (White et al. 1990)	[a]94 °C: 30 s, 48 °C: 1 min., 72 °C: 1 min. (37 cycles)[b]
TEF1	EF1-728F/ EF1-986R (Carbone & Kohn 1999)	[a]94 °C: 30 s, 55 °C: 30 s, 72 °C: 1.30 min. (35 cycles)[b]
TUB	Bt2a/Bt2b (Glass & Donaldson 1995)	[a]94 °C: 30 s, 55 °C: 50 s, 72 °C: 1 min. (35 cycles)[b]
CAL	CAL-228F/CAL-737R (Carbone & Kohn 1999)	[a]94 °C: 30 s, 58 °C: 1 min., 72 °C: 1 min. (35 cycles)[b]

[a]Initiation step of 94 °C: 3 min

[b]Final elongation step of 72 °C: 7 min. and final hold at 4 °C applied to all PCR thermal cycles

Table 2 GenBank accession numbers of the isolates used in this study

Species	Culture Collection No.	ITS	TEF1	TUB	CAL
Diaporthe alleghaniensis	CBS 495.72	KC343007	KC343733	KC843228	KC343249
D. alnea	CBS 146.46	KC343008	KC343734	KC343976	KC343977
D. alnea	CBS 159.47	KC343009	KC343735	KC343977	KC343251
D. apiculatum	LC3418	KP267896	KP267970	KP293476	-
D. apiculatum	LC3187	KP267866	KP267940	KP293446	-
D. betulae	CFCC 50469	KT732950	KT733016	KT733020	KT732997
D. betulae	CFCC 50470	KT732951	KT733017	KT733021	KT732998
D. bicincta	CBS 121004	KC343134	KC343860	KC344102	KC343376
D. biguttusis	CGMCC3.17081	KF576282	KF576257	KF576306	-
D.biguttusis	CGMCC3.17082	KF576283	KF576258	KF576307	-
D. celastrina	CBS 139.27	KC343047	KC343773	KC344015	KC343289
D. charlesworthii	BRIP54884m	KJ197288	KJ197250	KJ197268	-
D. citri	CBS 135422	KC843311	KC843071	KC843187	KC843157
D. citri	AR4469	KC843321	KC843081	KC843197	KC843167
D. citrichinensis	ZJUD34	JQ954648	JQ954666	KJ490396	KC357494
D. citrichinensis	ZJUD85	KJ490620	KJ490499	KJ490441	-
D. collariana	MFLUCC 17-2636	MG806115	MG783040	MG783041	MG783042
D. cotoneastri	CBS 439.82	FJ889450	GQ250341	JX275437	JX197429
D. discoidispora	ZJUD89	KJ490622	KJ490501	KJ490443	-
D. ellipicola	CGMCC3.17084	KF576270	KF576245	KF576291	-
D. ellipicola	CGMCC3.17085	KF576271	KF576246	KF576295	-
D. eres	AR5193	KJ210529	KJ210550	KJ420799	KJ434999
D. eres	LCM11401a = CBS 138598	KJ210521	KJ210545	KJ420787	KJ435027
D. eres	DLR12a	KJ210518	KJ210542	KJ420783	KJ434996
D. fukushii	MAFF 625029	JQ807469	JQ807418	-	-
D. gardeniae	CBS 288.56	KJ197289	KJ197252	KJ197270	-
D. helicis	AR5211	KJ210538	KJ210559	KJ420828	KJ435043
D. longicicola	CGMCC 3.17089	KF576267	KF576242	KF576291	-
D. longicicola	CGMCC 3.17090	KF576268	KF576243	KF576292	-
D. lonicerae	MFLUCC 17-0963	KY964198	KY964154	KY964082	KY964122
D. mahothocarpus	CGMCC3 15181	KC153096	KC153087	-	-
D. mahothocarpus	CGMCC3 15182	KC153097	KC153088	-	-
D. maritima	DAOMC 250563	KU552027	KU552022	KU574616	-
D. momicola	MFLUCC 16-0113	KU557563	KU557631	KU557587	KU557611
D. neilliae	CBS 144.27	KC343144	KC343870	KC344112	KC343386
D. nobilis	CBS 587.79	KC343153	KC343879	KC344121	KC343395
D. oraccinii	LC3166	KP267863	KP267937	KP293443	-

Table 2 Continued.

Species	Culture Collection No.	ITS	TEF1	TUB	CAL
D. penetriteum	LC3215	KP267879	KP267953	KP293459	-
D. penetriteum	LC3353	KP714505	KP714517	KP714529	-
D. penetriteum	LC3394	KP267893	KP267967	KP293473	-
D. phragmitis	CBS 138897	KP004445	-	KP004507	-
D. pulla	CBS 338.89	KC343152	KC343878	KC344120	KC343394
D. sennicola	CFCC 51634	KY203722	KY228883	KY228889	KY228873
D. sennicola	CFCC 51635	KY203723	KY228884	KY228890	KY228874
D. subclavata	ZJUD83	KJ490618	KJ490497	KJ490439	-
D. subclavata	ZJUD95	KJ490630	KJ490509	KJ490451	-
D. vaccinii	CBS 122116	KC343227	KC343953	KC344195	KC343469
D. vaccinii	CBS 160.32	AF317578	GQ250326	JX270436	KC343470
D. virgiliae	CMW 40748	KP247566	-	KP247575	-
Diaporthella corylina	CBS 121124	KC343004	KC343730	KC343972	KC343246

DNA isolation, amplification and analysis

Genomic DNA was extracted from fungal colonies growing on PDA, using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China), following the manufacturer's protocol. Gene regions of ITS, TEF1, TUB and CAL were amplified using the primer pairs and PCR protocols listed in Table 1. Amplifications were performed in 25 μl of PCR mixtures containing 9.5 μl ddH2O, 12.5 μl 2 × PCR Master Mix, 1 μl of DNA template and 1 μl of each primer (10 μM). The PCR products were visualized by staining with ethidium bromide after 1% agarose gel electrophoresis. Purification and sequencing of PCR products were done by Shanghai Sangon Biological Engineering Technology & Services Co., China. To ensure the integrity of the sequences, both directions of the PCR products were sequenced using the same primer pairs as used in PCR amplification. A consensus sequence for each gene region was assembled in ContigExpress (Vector NTI Suite 6.0).

The sequences generated in this study were supplemented with additional sequences obtained from GenBank (Table 2), selected based on recent publications (Gomes et al. 2013, Huang et al. 2015, Gao et al. 2016, 2017, Dissanayake et al. 2017a, b, c). The sequence data were aligned online with the MAFFT v. 7 server (http://mafft.cbrc.jp/ alignment/server/) and manually adjusted using MEGA6 v. 6.0 where necessary (Tamura et al. 2011). Phylogenetic analyses were based on Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) methods.

MP analysis was carried out using PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 (Swofford 2002). The trees were inferred using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. Ambiguously aligned regions were excluded from all analyses and gaps were treated as missing data. Maxtrees were setup to 5000, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Stability of the clade were assessed using a bootstrap (BT) analysis with 1000 replicates, each with 100 replicates of random stepwise addition of taxa (Hillis & Bull 1993). Tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], homoplasy index [HI], and log likelihood [-ln L] (HKY model) values were calculated. The robustness of the equally most parsimonious trees was evaluated by 1000 bootstrap replications (Felsenstein 1985) resulting from a maximum parsimony analysis, each with 10 replicates of random stepwise addition of taxa. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were significantly different. ML analysis was performed using RAxML GUI v. 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 1 000 separate runs, using the

default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores with the GTRGAMMA nucleotide substitution model. MrBayes v. v. 3.2.0 was used to generate the phylogenetic trees under optimal criteria per data partition (Ronquist & Huelsenbeck 2003). Bayesian analysis was performed using MrBayes v. 3.2.0. The best-fit evolutionary models for phylogenetic analyses were selected separately for ITS, TEF1, TUB and CAL gene regions using MrModeltest v. 2.2 (Nylander 2004). The GTR+I+G model was selected for ITS and TUB, while GTR+G was selected for TEF1 and CAL, separately, and incorporated into the analysis. Two parallel analyses of each consisting of six Markov Chain Monte Carlo (MCMC) chains, run from random trees for 6 000 000 generations were sampled every 100 generations resulting in 20 000 total trees. The first 10 000 trees, representing the burn in phase of the analyses were discarded from each run. The remaining trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree. Trees were viewed by FigTree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited using Microsoft PowerPoint 2010.

Results

Phylogenetic analyses

Single gene analyses of ITS, TEF1 and TUB were carried out for all the available sequences of *Diaporthe* species to compare the topology of the trees and clade stability (data not shown). Based on those analyses and blast results, 48 isolates were selected (including the outgroup taxon) for the combined gene analysis of ITS, TEF1, TUB and CAL (Table 2). The aligned dataset comprised 1766 characters including gaps (ITS: 1–468, TEF1: 469–922, TUB: 923–1283 and CAL: 1284–1766), of which 970 were constant, 393 parsimony-informative and 403 parsimony-uninformative. The parsimony analysis resulted in 8 equally most parsimonious trees (TL = 1628 steps, CI = 0.641, RI = 0.731, RC = 0.469, HI = 0.359). Bayesian inference and maximum parsimony analyses of the combined data set yielded trees with similar topologies to maximum likelihood tree. The best scoring RAxML tree with a final likelihood value of -10272.773331 is presented (Fig. 1). The matrix comprised 732 distinct alignment patterns, with 19.13% of undetermined characters or gaps. Estimated base frequencies were as follows; A = 0.220005, C = 0.316011, G = 0.233364, T = 0.230620; substitution rates AC = 1.337142, AG = 3.640863, AT = 1.113669, CG = 1.137669, CT = 5.044583, GT = 1.000000; gamma distribution shape parameter α = 0.501532.

In the phylogenetic analysis, our new isolate *Diaporthe collariana* clustered with *D. subclavata* isolate ZJUD83, with a high statistical support (100% MLBT, 100% MPBT, 1.00 PP). The ex-type strain of *D. subclavata* also shows a close relationship to *D. collariana*.

Taxonomy

Diaporthe collariana R. H. Perera & K. D. Hyde sp. nov. Figs 2, 3

Indexfungorum: IF554061; Facesoffungi: FoF03909

Etymology – Named after its prominently flared collarettes.

Saprobic on *Magnolia champaca.* Asexual morph from the natural substrate – *Conidiomata* 190–325 µm wide, 310–550 µm high, pycnidial, eustromatic, subepidermal, semi immersed, scattered, globose to ampullifom or irregular, black, outer surface smooth, convoluted to unilocular, singly ostiolate, with prominent necks 150–290 µm long. *Peridium* 18–25 µm thick, 5–9 cells thick, consisting brown to hyaline cells of *textura angularis*. *Conidial mass* globose or sometimes exuding in cirrhi, white to pale-yellow. *Alpha conidiophores* 12.1–20.6 × 2.4–3.2 µm ($\bar{x}$ = 16.6 × 2.8 µm), densely aggregated, ampulliform to subcylindrical, rarely septate and branched, hyaline. *Alpha conidiogenous cells* 10–17 × 1.3–2.4 µm ($\bar{x}$ = 13.7 × 1.8 µm) subcylindrical, tapering towards the apex, hyaline, with visible periclinal thickening, collarette prominent, up to 6 µm long, 5.7 µm wide. *Alpha conidia* 4.2–6.2 × 1.5–2 µm ($\bar{x}$ = 5.2 × 1.7 µm), less common than beta

conidia, oblong to ellipsoidal, apex bluntly rounded, base obtuse to subtruncate, aseptate, straight, guttulate, hyaline, smooth-walled. *Beta conidiophores* 10.3–19 × 1.4–3.5 µm ($\bar{x}$ = 14.6 × 2.6 µm), densely aggregated, subcylindrical, filiform or obconical, branched and septate, hyaline. *Beta conidiogenous cells* 3.8–14 × 1.4–2.2 µm ($\bar{x}$ = 7.9 × 1.8 µm) subcylindrical, tapering towards the apex, hyaline, with visible periclinal thickening, collarette prominent, up to 6.6 µm long, 5.7 µm wide. *Beta conidia* 22–31.3 × 0.8–1.6 µm ($\bar{x}$ = 27.7–1.2 µm), commonly found, straight, curved or hamate, hyaline, smooth-walled. *Gamma conidia* not observed. Asexual morph on PDA – *Conidiomata* 600–636 µm wide, 1045–1170 µm high, pycnidial, aggregated in small groups, globose to ampullifom, unilocular, black, with a prominent neck. *Peridium* consisting brown cells of *textura angularis*. *Conidial mass* globose or sometimes exuding in cirrhi, white to pale-yellow. *Alpha conidiophores* 12–20 × 2.4–3.2 µm ($\bar{x}$ = 17.2 × 2.8 µm), densely aggregated, ampulliform to subcylindrical, rarely septate and branched, hyaline. *Alpha conidiogenous cells* 11.1–17 × 1.3–2.4 µm ($\bar{x}$ = 14.4 × 1.8 µm) subcylindrical, tapering towards the apex, hyaline, with visible periclinal thickening, collarette prominent, up to 3.5 µm long, 3.2 µm wide. *Alpha conidia* 4.7–5.6 × 1.7–2.2 µm ($\bar{x}$ = 5.2 × 1.9 µm), less common than beta conidia, oblong to ellipsoidal, apex bluntly rounded, base obtuse to subtruncate, aseptate, straight, bi-guttulate, hyaline, smooth-walled. *Beta conidiophores* 13.2–20.8 × 1.3–4.1 µm ($\bar{x}$ = 17.4 × 3.6 µm), densely aggregated, subcylindrical, filiform or obconical, branched and septate, hyaline. *Beta conidiogenous cells* 8.8–13.4 × 1.7–2.3 µm ($\bar{x}$ = 10.8 × 2.1 µm) subcylindrical, tapering towards the apex, hyaline, with visible periclinal thickening, collarette prominent, up to 3.5 µm long, 3.2 µm wide. *Beta conidia* 22–31.7 × 1.1–1.6 µm ($\bar{x}$ = 28.8–1.3 µm), commonly found, straight, curved or hamate, hyaline, smooth-walled. *Gamma conidia* not observed. Sexual morph – Undetermined.

Culture characters – Conidia germinating on WA (Water Agar) within 12 h and germ tubes produced from one end. Colonies growing on PDA, reaching 6 cm in 7 days at 25°C, flat, initially white, aerial mycelium forming concentric rings with cottony texture, white to olivaceous, reverse zonate with white and ash-brown rings. Sporulate on PDA after 2 months incubation period in dark, at 25°C.

Material examined – THAILAND, Chiang Rai, Mae Fah Luang University premises, on dried fruits and pedicels of *Magnolia champaca* (L.) Baill. ex Pierre (Magnoliaceae), 17 August 2017, S. Boonmee, Fruit 3 (MFLU 17-2770, holotype), MFLU 17-2845 dried culture on PDA, ex-type living culture, MFLUCC 17-2636. (GenBank: LSU: MG806114)

Notes – Our new fungus *Diaporthe collariana* nested in between two *D. subclavata* strains and was more related to strain ZJUD83, which was collected from a fruit of *Citrus maxima* cv. Shatianyou in China, with very good support (Fig. 1). Nucleotide comparison reveals 5 (1.3%) differences in the ITS region, 10 (2.1%) in the TEF1 region, 11 (1.4%) in the TUB region. The ex-type strain of *D. subclavata* (ZJUD95) is the next phylogenetically closest isolate to *Diaporthe collariana* (Fig. 1). Nucleotide comparison reveals 15 (3.8%) were distinct in the ITS region, 46 (9.7%) in the TEF1 region, 10 (1.2%) in the TUB region. CAL region is not available for *D. subclavata* strains in GenBank (Huang et al. 2015). *Diaporthe collariana* differs from *D. subclavata* in the presence of beta conidia. Furthermore, *D. collariana* produces prominent collarettes while collarets are absent in *D. subclavata*. On PDA, *D. collariana* produces smaller alpha conidia, which are oblong to ellipsoidal (4.7–5.6 × 1.7–2.2 µm), while *D. subclavata* produces fusiform to clavate conidia (5.5–7.2 × 2.2–2.9 µm) (Huang et al. 2015). The placement of *D. collariana* in between two isolates of *D. subclavata* is rather intriguing. However, by comparing available gene sequences of *D. subclavata* strains, we confirm that ZJUD83 is different from its ex-type ZJUD95. This is further discussed below.

Diaporthe micheliae, is another species which lacks molecular data in the GenBank, and was also isolated from the same host as *D. collariana* (Sankaran et al. 1987). However, *D. collariana* can be distinguished from *D. micheliae* by having prominent collarettes which are absent in *D. micheliae*, branched conidiophores (vs. simple conidiophores), and smaller alpha conidia (4.7–5.6 × 1.7–2.2 vs. 4.6–8.2(-11.5) × 2–2.8 µm) (Sankaran et al. 1987).

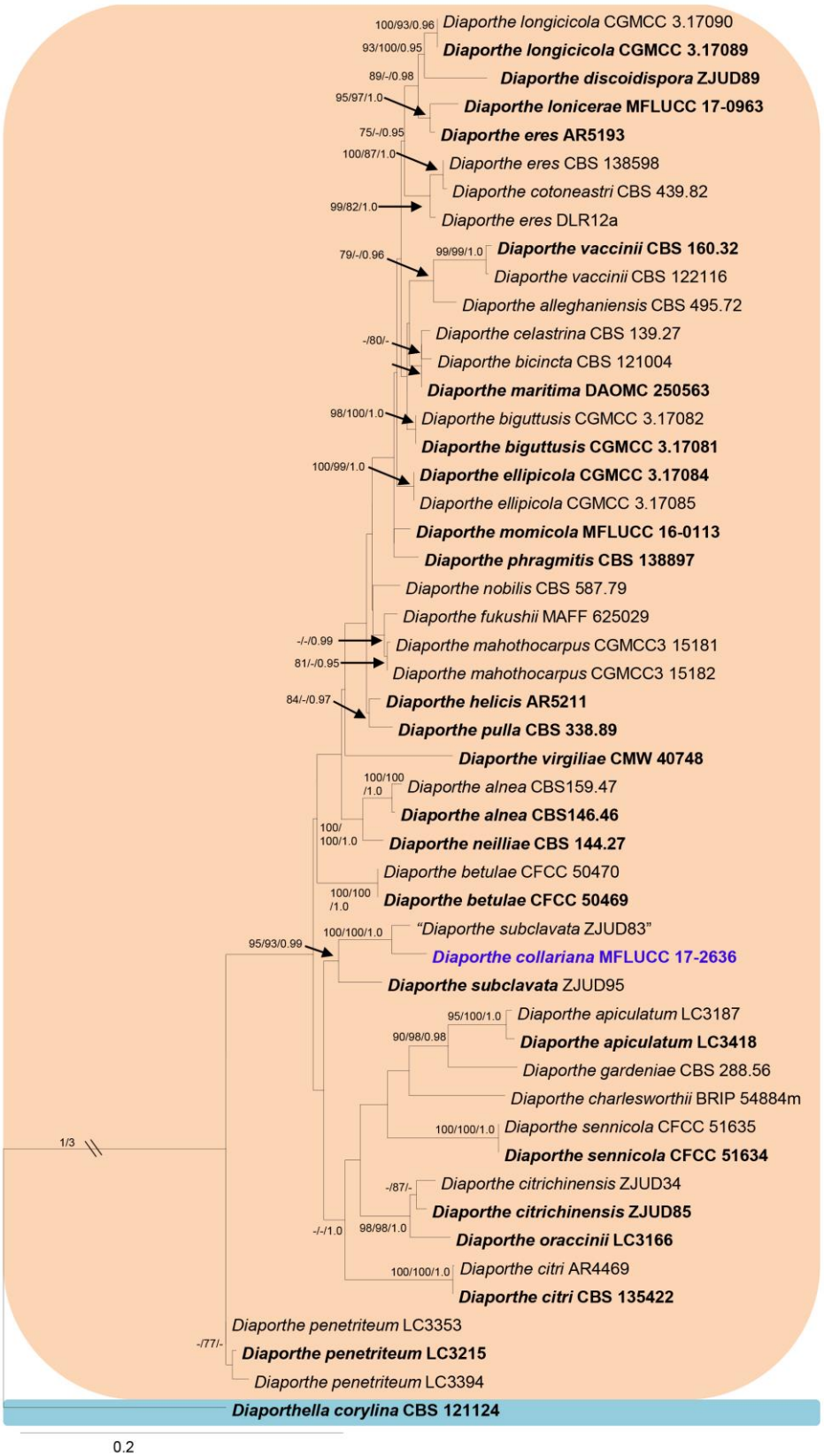

Fig. 1 – Phylogram generated from maximum likelihood analysis based on combined ITS, TEF1, TUB, and CAL sequence data of selected *Diaporthe* species. Values near the nodes indicate maximum likelihood and maximum parsimony bootstrap ≥75%, (MP/ML). Values at the third positions, respectively, represent posterior probabilities (PP ≥0.95) from Bayesian inference analysis. The ex-type strains are in bold and new isolates in blue. The scale bar indicates 0.2 nucleotide changes per site. The tree is rooted to *Diaporthella corylina* (CBS 121124).

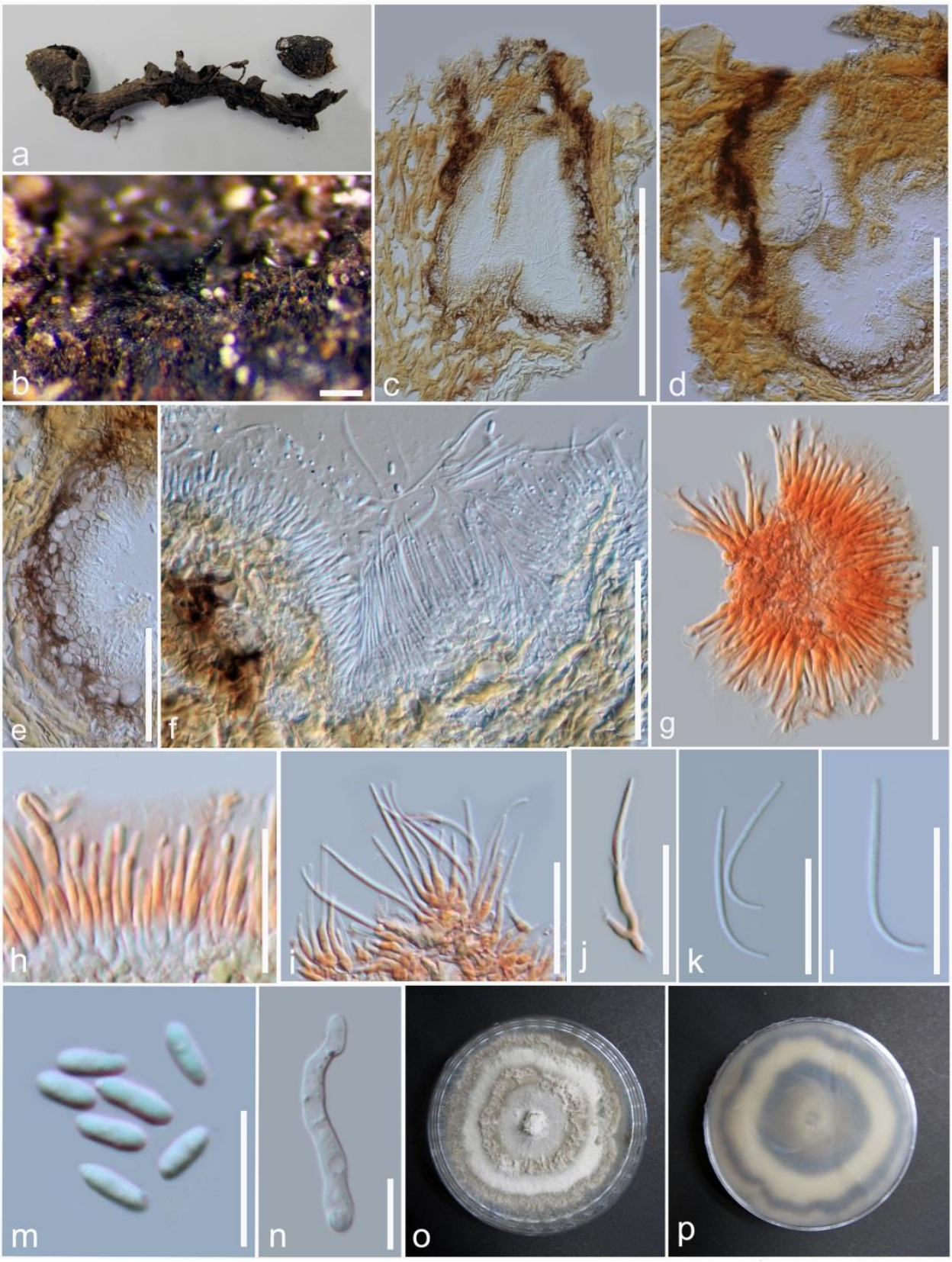

Fig. 2 – *Diaporthe collariana* (MFLU 17-2770, holotype). a Herbarium material. b Conidioma on host substrate. c, d Section through conidiomata. e Peridium. f Alpha and beta conidia inside the same conidiomata. g, h Conidiophores with alpha conidia (in Congo red). i, j Conidiophores with beta conidia (in Congo red). k, l Beta conidia. m Alpha conidia. n Germinating conidium. o, p Colony on PDA. Scale bars: b, c = 200 µm, d = 50 µm, e–g = 50 µm, h–l = 20 µm, m, n = 10 µm.

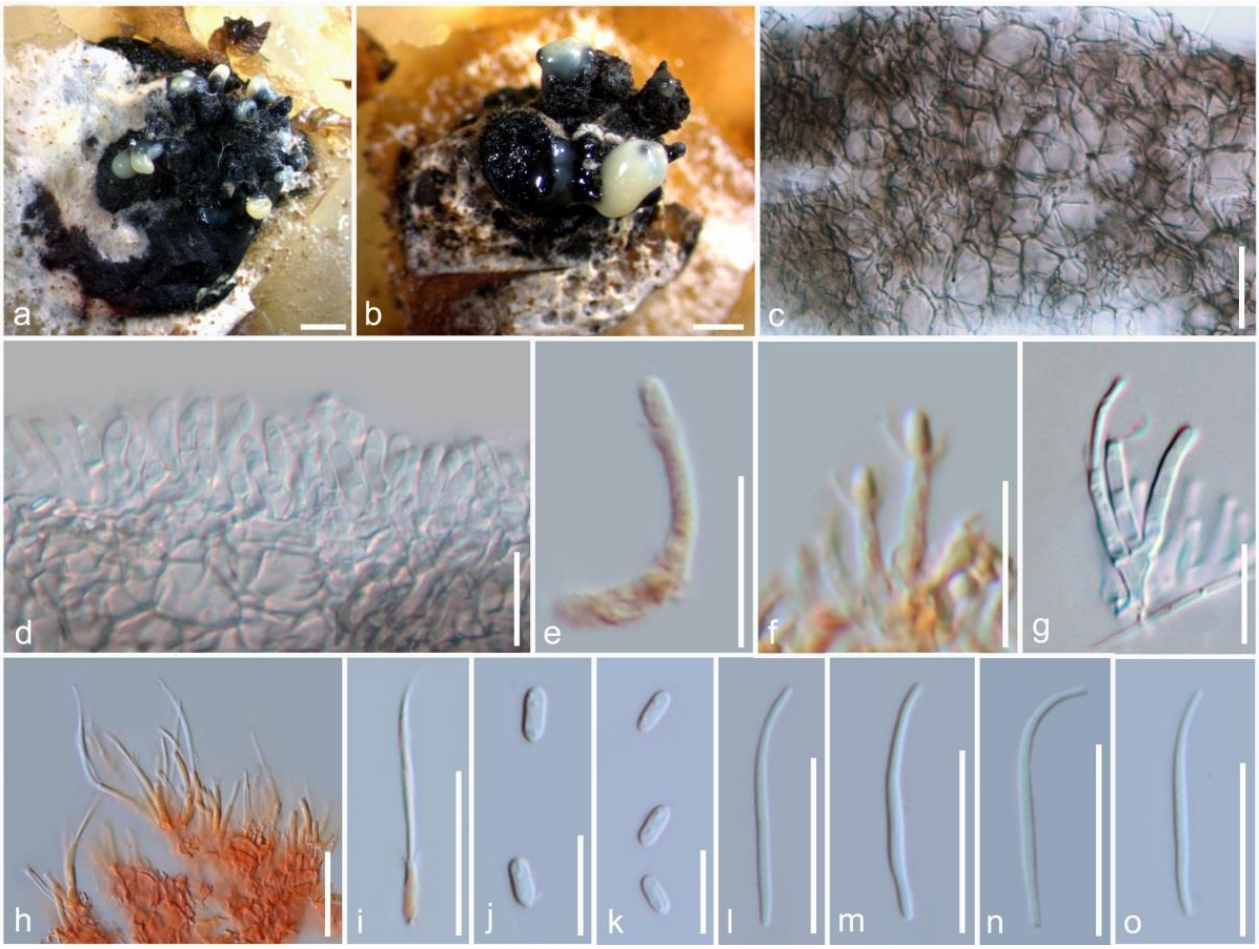

Fig. 3 – *Diaporthe collariana* (MFLU 17-2845) a, b Conidiomata on PDA. c Peridium in surface view. d Developing conidiophores with conidiogenous cells. e, f Conidiogenous cells with alpha conidia (in Congo red). g–i Conidiophores with beta conidia (h, i. in Congo red). j, k Alpha conidia. l–o Beta conidia. Scale bars: a, b = 1 mm, c = 20 µm, d–f = 10 µm, g = 10 µm, h, i = 20 µm, j, k = 10 µm, l–o = 20 µm.

Discussion

Magnolia champaca is an evergreen or semi-deciduous plant native to India, and exotic to many other Asian countries including Thailand (Orwa et al. 2009). Dried fruits of *M. champaca* were collected in rainy season, under trees in Mae Fah Luang University garden and examined for fungi. The garden floor is free of pesticides and herbicides. Here we illustrate the morphology of the fungus on natural substrates and *in vitro* on PDA. Conidiomata on PDA are larger and produce longer necks than the ones on the natural host (Figs 2, 3). However, other characters such as, conidiophores, conidiogenous cells and conidia are similar in both culture and natural substrates.

There are two strains of *D. subclavata* available in the GenBank including the ex-type. However, ex-type ZJUD95 and the putative strain of *D. subclavata* (ZJUD83) do not cluster together in our analysis (Fig. 1). Isolate ZJUD83 was collected from fruit of *C. maxima* cv. Shatianyou in Guangdong province, China, while the ex-type stain of *D. subclavata* (ZJUD95) was collected from *C. unshiu* in Fujian Province, China (Huang et al. 2015). Nucleotide comparison between *D. subclavata* isolates ZJUD95 and ZJUD83 reveals 14 (3.5%) were distinct in the ITS region, 52 (10.9%) in the TEF1 region, 9 (1.1%) in the TUB region and 31 (6.6%) in the HIS region, which means ZJUD83 isolate is probably a different species. However, no morphological descriptions are available for ZJUD83 for any comparison with holotype of *D. subclavata* (Huang et al. 2015). Given that *Diaporthe* species are of economic importance, it would be wise to relook into the herbarium material of ZJUD83, recollect the sample and perform phylogenetic analyses on same to clarify its taxonomy.

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References

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

Chang CQ, Cheng YH, Xiang MM, Jiang ZD, Chi PK. 2005 – New species of *Phomopsis* on woody plants in Fujian province. Mycosystema 24, 6–11.

Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q et al. 2014 – The sooty moulds. Fungal Diversity 66, 1–36.

Chi P, Jiang Z, Xiang M. 2007 – Flora Fungorum Sinicorum 34: *Phomopsis*. Science, Beijing.

Dayarathne MC, Boonmee S, Braun U, Crous PW et al. 2016 – Taxonomic utility of old names in current fungal classification and nomenclature: Conflicts, confusion and clarifications. Mycosphere 7, 1622–1648.

Dissanayake AJ, Camporesi E, Hyde KD, Zhang Wei, Yan JY, Li XH. 2017a – Molecular phylogenetic analysis reveals seven new *Diaporthe* species from Italy. Mycosphere 8, 853–877.

Dissanayake AJ, Phillips AJL, Hyde KD, Yan JY, Li XH. 2017b – The current status of species in *Diaporthe*. Mycosphere 8, 1106–1156.

Dissanayake AJ, Zhang W, Liu M, Hyde KD et al. 2017c – *Diaporthe* species associated with peach tree dieback in Hubei, China. Mycosphere 8, 533–549.

Felsenstein J. 1985 – Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Gao Y, Liu F, Cai L. 2016 – Unravelling *Diaporthe* species associated with *Camellia*. Systematics and Biodiversity 14, 102–117.

Gao Y, Liu F, Duan W, Crous PW, Cai L. 2017 – *Diaporthe* is paraphyletic. IMA Fungus 8, 153–187.

Gao YH, Su YY, Sun W, Cai L. 2014 – *Diaporthe* species occurring on *Lithocarpus glabra* in China, with descriptions of five new species. Fungal Biology 119, 295–309.

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.

Gomes RR, Glienke C, Videira SI, Lombard L, Groenewald JZ, Crous PW. 2013 – *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia: Molecular Phylogeny and Evolution of Fungi. 31, 1–41.

Hawksworth DL, David JC. 1989 – Family names. Index of Fungi Supplement – C.A.B. International. Wallingford, U.K.

Hillis DM, Bull JJ. 1993 – An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42, 182–192.

Huang F, Udayanga D, Wang X, Hou X et al. 2015 – Endophytic *Diaporthe* associated with *Citrus*: A phylogenetic reassessment with seven new species from China. Fungal Biology 119, 331–347.

Ronquist F, Huelsenbeck JP. 2003 – MrBayes 3: Bayesian phylogenetic inference under mixed models Bioinformatics 19, 1572–1574.

Hyde KD, McKenzie EHC, KoKo TW. 2011 – Towards incorporating anamorphic fungi in a natural classification–checklist and notes for 2010. Mycosphere 2, 1–88.

Index Fungorum. 2017 – www.indexfungorum.org.
Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J et al. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.
Jeewon R, Hyde KD. 2016 – Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. Mycosphere 7, 1669–1677.
Kishino H, Hasegawa M. 1989 – Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data. Journal of Molecular Evolution 29, 170–179.
Nylander JAA. 2004 – MrModeltest v2. Program distributed by the author Evolutionary Biology Centre, Uppsala University, Sweden.
Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. 2009 – Agroforestree Database:a tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/treedb/AFTPDFS/Michelia_champaca.PDF)
Rehner SA, Uecker FA. 1994 – Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. Canadian Journal of Botany 72, 1666–1674.
Sankaran KV, Florence EJM, Sharma JK. 1987 – Two new species of *Phomopsis* from India. Transactions of the British Mycological Society 89, 404–407.
Santos L, Alves A, Alves R. 2017 – Evaluating multi-locus phylogenies for species boundaries determination in the genus *Diaporthe*. PeerJ 5, e3120.
Silvestro D, Michalak I. 2011 – raxmlGUI: a graphical front-end for RAxML. Organisms. Diversity and Evolution 12, 335–337.
Swofford DL. 2002 – PAUP*: phylogenetic analysis using parsimony, version 4.0 b10. Sinauer Associates, Sunderland.
Sutton BC. 1980 – The coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute, Kew, Surrey, England.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011 – MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28, 2731–2739.
Udayanga D, Liu XZ, Crous PW, McKenzie EH, Chukeatirote E, Hyde KD. 2012 – A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). Fungal Diversity 56, 157–171.
White TJ, Bruns T, Lee S, Taylor J. 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 Press, New York, pp. 315–322.