MYCOBIOLOGY
2021, VOL. 49, NO. 3, 267–274
https://doi.org/10.1080/12298093.2021.1912254

RESEARCH NOTE

OPEN ACCESS Check for updates

Diaporthe taoicola and *D. siamensis*, Two New Records on *Citrus sinensis* in China

Meng Jiao Cui[a], Xin Wei[a], Peng Liang Xia[b], Ji Ping Yi[c], Zhi He Yu[d], Jian Xin Deng[a] and Qi Li Li[e]

[a]Department of Plant Protection, College of Agriculture, Yangtze University, Jingzhou, China; [b]Enshi Tobacco Company of Hubei Province, Enshi, China; [c]Zigui Plant Protection Station, Yichang, China; [d]Department of Applied Microbiology, College of Life Sciences, Yangtze University, Jingzhou, China; [e]Institute of Plant Protection, Guangxi Academy of Agricultural Sciences and Guangxi Key Laboratory of Biology for Crop Diseases and Insect Pests, Nanning, China

ABSTRACT

Two *Diaporthe* species isolated from fruit of *Citrus sinensis* in China were characterized based on morphology and multilocus phylogeny of ITS, *tef1*, and *tub2* gene sequences. The phylogeny indicated that the two species match *Diaporthe taoicola* and *D. siamensis*. A critical examination of phenotypic characteristics confirmed the phylogenetic results. *Diaporthe taoicola* was morphologically characterized by producing Alpha conidia with tapering toward both ends. Meanwhile, *D. siamensis* produced cylindrical or ellipsoidal Alpha conidia with two oil drops. Pathogenicity tests revealed that both species were pathogenic to fruit of *C. sinensis*. To our knowledge, the two species were firstly reported on *Citrus sinensis* in China.

ARTICLE HISTORY
Received 3 December 2020
Revised 21 January 2021
Accepted 30 March 2021

KEYWORDS
Diaporthe; phylogeny; morphology; pathogenicity; *Citrus sinensis*

Citrus (Rutaceae) is worldwide cultivated because of its nutritional values and the medicinal benefits (e.g., anti-hypertensive) [1]. In 2018, citrus production in Zigui county, Hubei province, the larger *Citrus sinensis* production area in China, has reached 23.3 thousand ha. As citrus has diversified as a commercial crop, it became a host for various pathogens from nursery to the storage stage.

Diaporthe species are present as pathogens, endophytes, or saprophytes on a wide range of cultivated or wild trees and ornamentals [2–5]. Species in this genus have been reported as the pathogens of blight, canker, decay, dieback, wilt, leaf spot, fruit rot and root rot across a diverse range of plant species [6–9]. Members of *Diaporthe* are also frequently associated with citrus diseases worldwide [10,11]. *Diaporthe citri* (anamorph=*Phomopsis citri*) caused melanose and stem-end rot of fruit, which are important in most citrus-growing areas with high humidity. Besides, *D. foeniculina* has also been found from New Zealand, Spain and USA associated with stem end rot on fruit [11]. In China, *Diaporthe citri*, *D. eres* and *D. unshiuensis* have been reported on fruit of *Citrus* spp. [12].

Preliminarily, species in *Diaporthe* were identified mainly based on morphological characters and host associations. However, morphology has been conferred to be inconsistent for identification due to inter- and intra-species variability [13]. Molecular analyses inferred that *Diaporthe* species are not highly host-specific [14]. More than one species is often present on one host, or one species may occur on more than one host [15]. Studies converging on the diversity of *Diaporthe* have been progressed in recent years in China. Huang et al. (2015) studied *Diaporthe* on *Citrus* in China found eight known species and seven novel species based on morphological comparison and multi-gene analyses. Moreover, *Diaporthe* associated with peach trees [16], pear shoot canker [5], and dieback diseases involving 16 host genera [17] were reported in China. These references provided bounteous information for the study of *Diaporthe* in China.

During the investigation of fungal pathogens associated with *Citrus sinensis* in Zigui county, *Diaporthe* isolates were encountered based on morphology. Three *Diaporthe*-like isolates, YZU 181047, YZU 181403, and YZU 181223 were found pathogenic to fruit of *C. sinenesis*. The main objectives of this study were to identify them based on morphological observations and sequence analyses of multiple gene regions.

In 2018, diseased citrus fruit was collected from commercial orchards in Zigui county. Tissues from the margin of infected lesions were cut into segments, which contained both diseased and healthy parts. All segments were surface sterilized in 2% sodium hypochlorite for 2 min, followed by 75%

CONTACT Jian Xin Deng djxin555@yangtzeu.edu.cn; Qi Li Li 65615384@qq.com

Table 1. Isolates and GenBank accession numbers used in the phylogenetic analyses of *Diaporthe*.

Species	Strain	Host/Locality	GenBank accession number		
			ITS	*tef1*	*tub2*
Diaporthe arecae	CBS 161.64	*Areca catechu*/India	KC343032	KC343758	KC344000
Diaporthe arengae	CBS 114979	*Arenga engleri*/China, HongKong	KC343034	KC343760	KC344002
Diaporthe batatas	CBS 122.21	*Ipomoea batatas*/USA	KC343040	KC343766	KC344008
Diaporthe citri	CFCC 53079	*Citrus* sp./China	MK573940	MK574615	MK574635
	CFCC 53080	*Citrus* sp./Brazil	MK573941	MK574616	MK574636
Diaporthe endophytica	CBS 133811	*Schinus terebinthifolius*	KC343065	KC343791	KC344033
Diaporthe eugeniae	CBS 444.82	*Eugenia aromatica*/Indonesia	KC343098	KC343824	KC344066
Diaporthe oxe	CBS 133186	*Maytenus ilicifolia*/Brazil	KC343164	KC343890	KC344132
Diaporthe perseae	CBS 151.73	*Persea americana*/Netherlands	KC343173	KC343899	KC344141
Diaporthe pseudomangiferae	CBS 101339	*Mangifera indica*/Dominican Republic	KC343181	KC343907	KC344149
Diaporthe pseudophoenicicola	CBS 462.69	*Phoenix dactylifera*/Spain	KC343184	KC343910	KC344152
Diaporthe siamensis	MFLUCC 10-0573a	*Dasymaschalon* sp./Thailand	JQ619879	JX275393	JX275429
	MFLUCC 10-0573 b	*Dasymaschalon* sp./Thailand	JQ619880	JX275395	JX275430
	MFLUCC 10-0573c	*Dasymaschalon* sp./Thailand	JQ619881	JX275396	JX275431
	MFLUCC 17-0591	Pandanaceae/Thailand	MT908796	MG646989	MG646925
	YZU 181403	***Citrus sinensis*/China**	**MW160357**	**MW160363**	**MW160360**
Diaporthe sojae	FAU 635	*Glycine max*/USA	KJ590719	KJ590762	KJ610875
Diaporthe taoicola	PSCG 292	*Pyrus pyrifolia*/China	MK626871	MK654800	MK691232
	PSCG 386	*Pyrus pyrifolia*/China	MK626868	MK654797	MK691222
	PSCG 413	*Pyrus pyrifolia*/China	MK626890	MK654814	MK691238
	PSCG 485	*Pyrus pyrifolia*/China	MK626869	MK654812	MK691238
	MFLUCC 16-0117	*Prunus persica*/China	KU557567	KU557635	KU557591
	MFLUCC 16-0118	*Prunus persica*/China	KU557568	KU557636	KU557592
	MFLUCC 16-0119	*Prunus persica*/China	KU557569	KU557637	KU557593
	MFLUCC 16-0120	*Prunus persica*/China	KU557570	KU557638	KU557594
	YZU 181047	***Citrus sinensis*/China**	**MW160355**	**MW160361**	**MW160358**
	YZU 181223	***Ctrus sinensis*/China**	**MW160356**	**MW160362**	**MW160359**
Diaporthe yunnanensis	LC 6168	*Coffea* sp.	KX986796	KX999188	-
	SAUCC 0254	Unknown/China	MT376663	MT376663	MT376634
Diaporthella corylina	CBS 121124	*Corylus* sp./China	KC343004	KC343730	KC343972

The present strains are shown in bold.

ethanol for 30 s and rinsed in sterile distilled water for three times. All samples were dried with sterile filter paper and plated onto potato dextrose agar (PDA, Difco, USA). Plates were incubated at 25 °C in darkness until mycelia grow. Then, mycelia from colony margin were taken and transferred on fresh PDA plates. Pure cultures were stored in the Fungi Herbarium of Yangtze University (YZU) in Jingzhou, China.

Genomic DNA was extracted from mycelium developed on PDA medium according to Cenis [18]. The primers ITS4 and ITS5 [19] was used to amplify the ITS region of the nuclear ribosomal RNA operon, including the 3′ end of the 18S rRNA, the first internal transcribed spacer region, the 5.8S rRNA gene; the second internal transcribed spacer region and the 5′ end of the 28S rRNA gene. The primers EF1-728F and EF1-986R [20] were used to amplify part of the translation elongation factor 1-α (*tef1*) gene, and the primers Bt2a and Bt2b [21] were used to amplify the partial beta-tublin (*tub2*) gene. The PCRs were performed in a 25 µL reaction mixture consisted of 12.5 µL of 2 × Taq PCR StarMix (Genstar, Beijing, China), 2 µL genomic DNA, 1.25 µL of each primer, and 8 µL distilled water (ddH2O). The thermal cycling program was completed on a thermal cycler using the following conditions: initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 60 s, annealing at (52 °C for ITS, and 56 °C for *tef1*, and 60 °C for *tub2*) for 30 s, extension at 72 °C for 60 s, with a final extension step at 72 °C for 5 min. Successful PCR amplification products were purified and sequenced at BGI (Beijing Genomics Institute).

All the obtained sequences were analyzed in the basic-local-alignment search tool (BLASTn) (http://blast.ncbi.nlm.nih.gov/) to retrieve the most similar taxa sequences. Relevant sequences were selected from the studies of Gomes et al. [3], Dissanayake et al. [16], Yang et al. [17], and Tibpromma et al. [22]. All sequences were aligned and combined in the MEGA 7.0 program [23]. Maximum Parsimony (MP) analysis was performed in PAUP version 4.0 b10 [24], generating a heuristic search option of 1000 random-addition replicates and a tree bisection-reconnection (TBR) as a branch-swapping algorithm. MaxTrees were set to 1000, branches of zero length collapsed, and all equally parsimonious trees were saved. Other scores in parsimony were calculated as tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency (RC). The maximum likelihood (ML) [25] phylogeny of the combined dataset was constructed with 1000 bootstrap replicates using GTRGAMMAI model. Additionally, Bayesian (BI) analysis was conducted in MrBayes v. 3.2.6 with 1,000,000 Markov chain Monte Carlo (MCMC) generations and a sampling frequency of every 100th generations. The best-fit evolutionary model was determined *via* MrModelTest v. 2.3 [26]. At the end of the analysis,

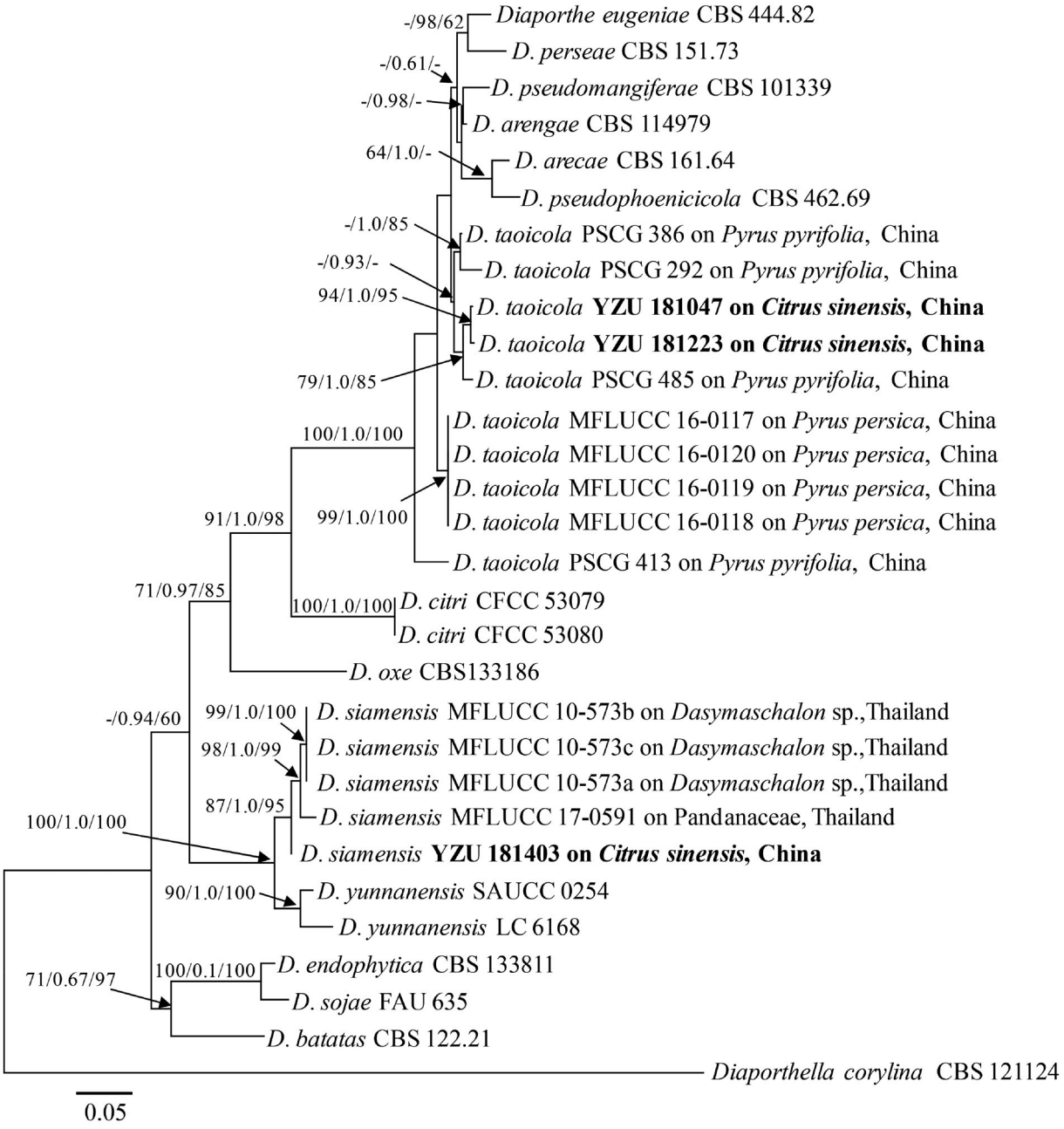

Figure 1. Phylogram of *Diaporthe* strains based on combined gene sequences of ITS, *tef1* and *tub2*. Values at the branch nodes indicated maximum parsimony bootstrap (MP BP $\geq$ 60%), Bayesian posterior (BI PP $\geq$ 0.6) and maximum likelihood bootstrap (ML BP $\geq$ 60%), respectively. The tree is rooted with *Diaporthella corylina*. Strains in the current study are in bold.

the first 25% of the samples were excluded as burn-in, and consensus trees were generated using the 50% majority-rule consensus tree criteria. The tree was viewed in Figtree v.1.3.1 [27]. Posterior probability (PP) values of BI analysis and bootstrap (BP) values of ML and MP analyses were shown at the nodes of branches. The out-group of the phylogeny was *Diaporthella corylina* CBS 121124.

Three isolates were characterized for their colonial and conidial morphology. Agar plugs (6 mm diam.) of each isolate were taken from the edge of actively growing cultures and transferred onto the center of petri dishes (9 cm diam), containing potato dextrose agar (PDA) and oatmeal agar (OA) for cultural feature. Plates containing 2% water agar (WA) with autoclaved *Citrus sinensis* leave tissues were incubated at 25 °C under a 12-h near-ultraviolet light/12-h dark cycle to induce sporulation. The culture was checked periodically for the development of ascomata and conidiomata. Morphology was recorded including colony color, texture, microconidia, and sporocarps formation. Conidia were mounted in sterile water for microscopic observation using a light microscope (Nikon DS-Ri2, Tokyo, Japan), equipped with a Nikon DS-Ri2 digital camera. Conidia ($n = 50$) were measured for each species.

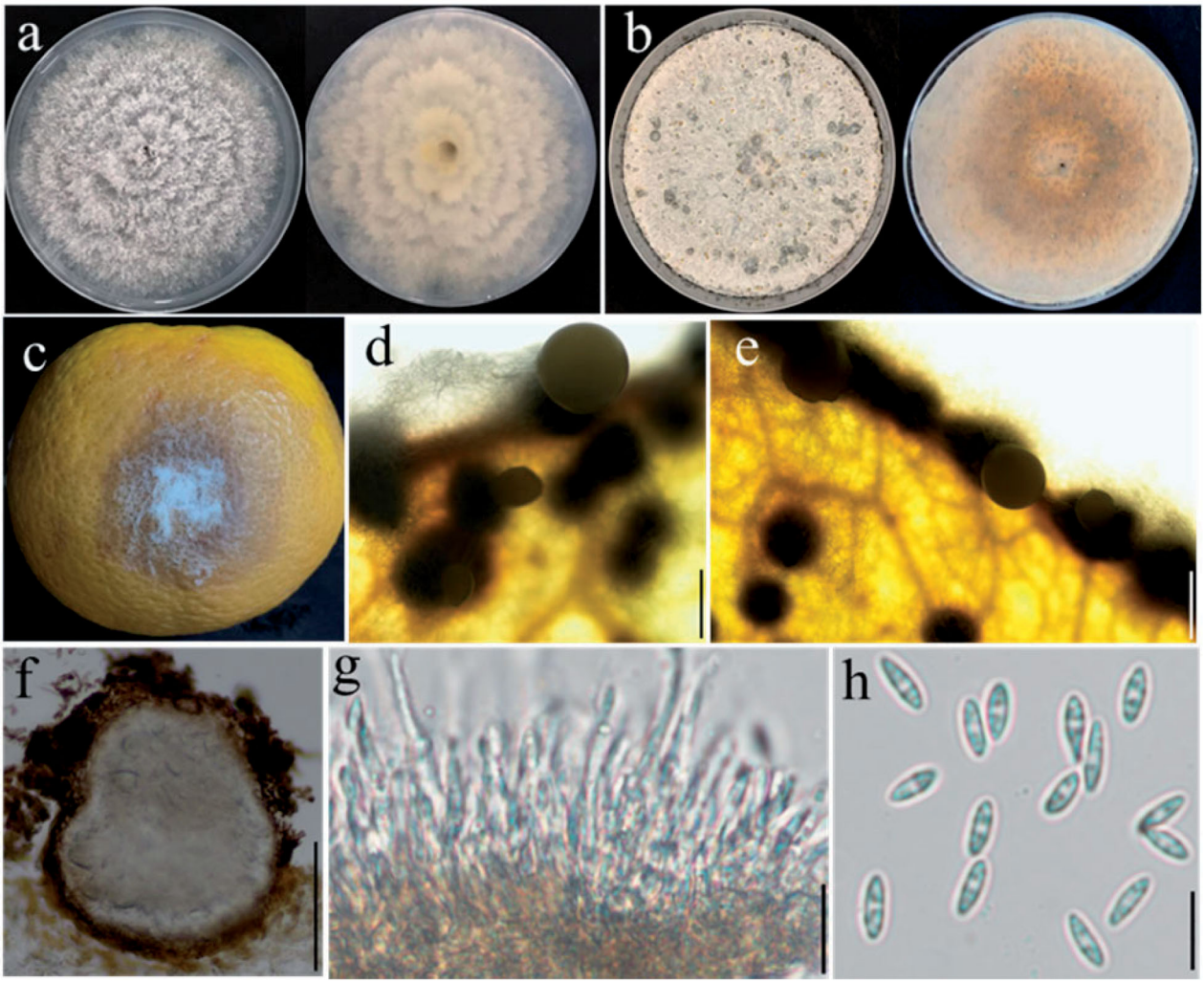

Figure 2. *Diaporthe taoicola* (YZU 181047). (a, b). Front and back view, respectively of colonies on PDA (a) and OA (b); (c). Pathogenicity test on *Citrus sinensis* fruit for 7 d; (d, e). Conidiomata; (f): Section view of conidiomata; (g). Conidiophores; (h): Alpha conidia. Scale bars: d, e, *f* = 100μm; g, h: 10 μm.

Pathogenicity tests were performed on fruit of *Citrus sinensis*. Mature and healthy fruit were surface-sterilized in 2% sodium hypochlorite for 2 min and washed three times with sterile distilled water. Fruit was wounded with a sterile scalpel around 6 mm × 6 mm in size. The mycelial plugs from 3 days old cultures grown on PDA were transferred onto the wounds. Controls were treated with sterile PDA. The inoculated fruits were maintained at 25 °C and 80 to 100% relative humidity (RH). The development of disease symptoms was checked daily for one week. The pathogen was re-isolated from the inoculated fruit and identified based on morphology to satisfy Koch's postulates. The pathogenicity tests were conducted with three replicates for each isolate and repeated three times.

A total of nine new sequences were generated and deposited in GenBank (Table 1). The combined multi-gene phylogeny (ITS, *tef1*, and *tub2*) contained 30 strains, of which 27 were obtained from NCBI (https://www.ncbi.nlm.nih.gov/) (Table 1, Figure 1). A total of 1276 characters (ITS 494, *tef1* 377, *tub2* 405) were included after alignment. Among them, 804 were constant, 190 were variable, and 282 were parsimony uninformative. The heuristic search generated 5 parsimonious trees (TL = 908, CI = 0.664, HI = 0.336, RI = 0.819, RC = 0.544). For the BI analysis, the HKY + I model was recommended by MrModeltest. The topology of ML phylogeny was identical to the results of BI and MP analyses, and it was used as a basal tree.

The phylogenetic tree showed that isolates YZU 181047 and YZU 181223 fell into a clade containing reference strains of *Diaporthe taoicola* supported with PP values of 0.93 and clustered together with *D. taoicola* PSCG 485 [5] with the BP or PP values of 94/1.0/95 (MP/BI/ML). The result indicated that both isolates were *D. taoicola*. However, the isolate YZU 181403 clustered with reference strains of *D. siamensis* with high BP or PP values of 87/1.0/95 (MP/BI/ML). The result showed that it was *D. siamensis*.

Morphological examination confirmed the phylogenetic results, the isolates YZU 181047 and YZU

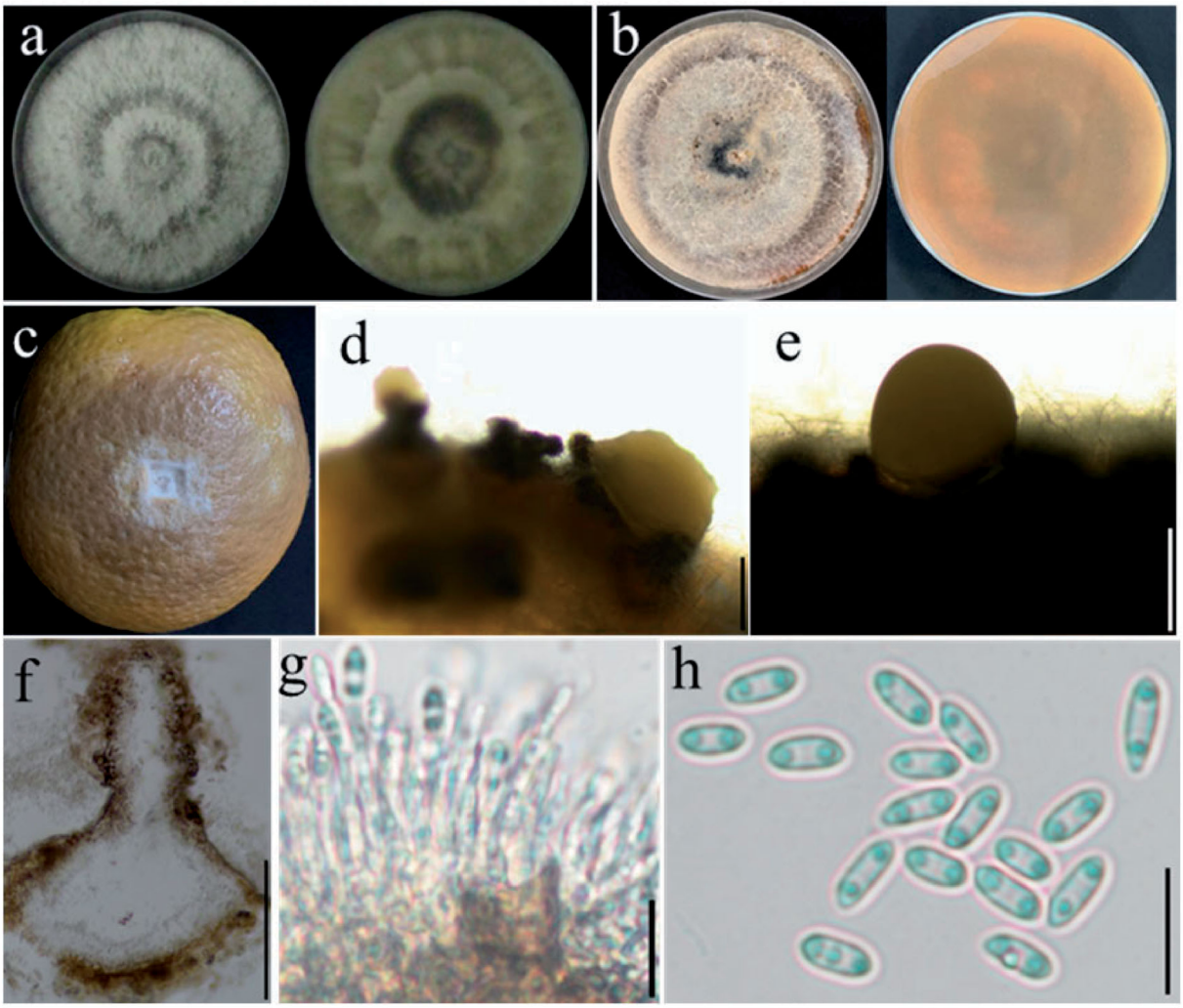

Figure 3. *Diaporthe siamensis* (YZU 181403). (a, b). Front and back view, respectively of colonies on PDA (a) and OA (b); (c). Pathogenicity test on *Citrus sinensis* fruit for 7 d; (d, e). Conidiomata; (f): Section view of conidiomata; (g). Conidiophores; (h): Alpha conidia. Scale bars: d, e, *f* = 100μm; g, h: 10 μm.

181223 were identified as *Diaporthe taoicola* [5,16], and the isolate YZU 181403 was *D. siamensis* [22,28] based on the colony and conidia characteristics.

Diaporthe taoicola **Dissanayake, X.H. Li & K.D. Hyde., Mycosphere 8: 543. (2017) (Figure 2)**

Sexual morph: Not observed. *Asexual morph*: *Conidiomata* 160–230 μm in size, pycnidial, subcuticular, scattered to confluent, dark brown to black, uniloculate, broadly spherical to flattened, cream conidial droplets exuding from central ostioles (Figure 2(d–f)). *Conidiophores* 16–28 × 2–3 μm, hyaline, smooth, densely aggregated, cylindrical, straight, or slightly curved, tapering toward the apex (Figure 2(g)). *Alpha conidia* 6–9 × 2–3 μm (av. 8 × 2.7 μm) hyaline, smooth, fusiform to ellipsoid, tapering toward both ends, straight (Figure 2(h)).

Colony morphology: Colonies on PDA covering the entire Petri dishes after 7 days, ropey with abundant tufted white aerial mycelium, reverse buff with zonate and irregular lines (Figure 2(a)), 79–81 mm in diam., with aerial mycelium dense in the center and sparse at the marginal area. Colonies on OA flat with white felty aerial mycelium, turning white to dark brown aerial mycelium, conidiomata irregularly distributed on the medium surface after 15-day incubation (Figure 2(b)).

Materials examined. China, Hubei province, Zigui county, on fruit of *Citrus sinensis*, August, 2018, M. J. Cui (cultures YZU 181047 and YZU 181223).

Notes: The size and shape of Alpha conidia from the present isolates were identical to that firstly reported by Dissanayake et al. (7–9 × 2–3 μm) [16]. However, Beta conidia were not observed during the present study, which was same as the strains found on pear shoot canker by Guo et al. [5].

Diaporthe siamensis **Udayanga, X.Z. Liu & K.D. Hyde., Cryptogamie Mycologie, 33(3): 295-309 (2012) (Figure 3)**

Sexual morph: Not observed. *Asexual morph*: *Conidiomata* 130–240 μm wide, 94–200 μm high,

Table 2. *Diaporthe* species isolated from various hosts in China.

Species	Authority	Host	Locality (Province)	Reference
Diaporthe acerigena	C.M. Tian & Q. Yang,	*Acer tataricum*	Shaanxi	Yang et al. [17]
D. acuta	Y.S. Guo & G.P. Wang	*Pyrus pyrifolia*	Hubei	Guo et al. [5]
D. acutispora	Y.H. Gao & L. Cai	*Coffea* sp.	Yunnan	Gao et al. [4]
D. alangii	C.M. Tian & Q. Yang	*Alangium kurzii*	Zhejiang	Yang et al. [17]
D. amygdali	Udayanga, Crous & K.D. Hyde	*Pyrus pyrifolia*	Jiangxi, Yunnan	Bai et al. [30]
		Camellia sp.	Sichuan	Gao et al. [4]
D. apiculata	Y.H. Gao & L. Cai	*Camellia* sp.	Jiangxi, Guangxi	Gao et al. [4]
D. aquatica	D.M. Hu, L. Cai & K.D. Hyde	*aquatic habitats*	Guizhou	Hu et al. [31]
D. betulae	C.M. Tian & X.L. Fan,	*Betula platyphylla*	Sichuan	Du et al. [8]
D. betulicola	C.M. Tian & Z. Du,	*Betula albo-sinensis*	Shaanxi	Du et al. [8]
D. betulina	C.M. Tian & Q. Yang	*Betula* sp.	Heilongjiang	Yang et al. [17]
D. biconispora	F. Huang, K.D. Hyde & H.Y. Li	*Citrus sinensis*	Jiangxi, Guangxi, Fujian	Huang et al. [12]
D. biguttulata	F. Huang, K.D. Hyde & H.Y. Li	*Citrus limon*	Yunnan	Huang et al. [12]
		Juglans regia	Zhejiang	Yang et al. [17]
D. biguttusis	Y.H. Gao & L. Cai	*Lithocarpus glabra*	Zhejiang	Gao et al. [4]
D. camptothecicola	C.M. Tian & Qin Yang	*Camptotheca acuminata*	Jiangsu	Yang et al. [32]
D. caryae	C.M. Tian & Q. Yang	*Carya illinoensis*	Jiangsu	Yang et al. [32]
		Pyrus pyrifolia	Jiangsu	Guo et al. [5]
D. cercidis	C.M. Tian & Q. Yang	*Carya illinoensis*	Jiangsu	Yang et al. [17]
		Pyrus pyrifolia	Shandong, Zhejiang, *et al.*	Guo et al. [5]
D. chensiensis	C.M. Tian & Q. Yang	*Abies chensiensis*	Shaanxi	Yang et al. [17]
D. chongqingensis	Y.S. Guo & G.P. Wang	*Pyrus pyrifolia*	Chongqing	Guo et al. [5]
D. cinnamomi	C.M. Tian & Q. Yang	*Cinnamomum sp.*	Zhejiang	Yang et al. [17]
D. citri	F.A. Wolf	*Citrus* sp.	Zhejiang, Huangyan, Jiangxi	Huang et al. [15]
D. citriasiana	F. Huang, K.D. Hyde & H.Y. Li	*Citrus* sp.	Shaanxi, Jiangxi, Zhejiang	Huang et al. [15]
D. citrichinensis	F. Huang, K.D. Hyde & H.Y. Li	*Citrus* sp.	Shaanxi, Guangxi, Fujian	Huang et al. et al. [15]
D. compacta	Y.H. Gao & L. Cai	*Camellia* sp.	Jiangxi	Gao et al. [4]
D. conica	C.M. Tian & Q. Yang	*Alangium chinense*	Zhejiang	Yang et al. [17]
D. discoidispora	F. Huang, K.D. Hyde & H.Y. Li	*Citrus* sp.	Jiangxi	Huang et al. [12]
D. elaeagni-glabrae	Y.H. Gao & L. Cai	*Elaeagnus glabra*	Jiangxi	Gao et al. [29]
D. ellipicola	Y. H. Gao & L. Cai	*Lithocarpus glabra*	Zhejiang	Gao et al. [4]
D. endophytica	R.R. Gomes, C. Glienke & Crous	*Citrus* sp.	Fujian	Huang et al. [12]
D. eres	Nitschke	*Aralia elata*	northeastern China	Bai et al. [30]
		Citrus sp.	Guangxi, Jiangxi, Zhejiang	Huang et al. [12]
		Vitis vinifera	Beijing, Zhejiang	Dissanayake et al. [16]
		Juglans regia	Zhejiang	Yang et al. [17]
		Camellia sp.	Sichuan	Gao et al. [29]
D. fraxinicola	C.M. Tian & Q. Yang,	*Fraxinus chinensis*	Shaanxi	Yang et al. [17]
D. fulvicolor	Y.S. Guo & G.P. Wang	*Pyrus pyrifolia*	Hubei	Guo et al. [5]
D. fusicola	Y.H. Gao & L. Cai	*Pyrus pyrifolia*	Jiangxi, Fujian, Zhejiang	Guo et al. [5]
D. ganjae	R.R. Gomes	*Pyrus pyrifolia*	Guizhou	Guo et al. [5]
D. hongkongensis	R.R. Gomes, C. Glienke & Crous	*Citrus* sp.	Zhejiang, Guangxi	Huang et al. [12]
		Vitis vinifera	Beijing	Dissanayake et al. [16]
		Camellia sp.	Guangxi	Gao et al. [4]
D. incompleta	Y.H. Gao & L. Cai	*Camellia sinensis*	Yunnan	Gao et al. [4]
D. juglandicola	C.M. Tian & Q. Yang	*Juglans mandshurica*	Beijing	Yang et al. [32]
D. kadsurae	C.M. Tian & Q. Yang	*Kadsura longipedunculata*	Jiangxi	Yang et al. [17]
D. lithocarpus	Y.H. Gao, W. Sun & L. Cai	*Lithocarpus* sp.	Zhejiang	Gao et al. [4]
D. longicolla	(Hobbs) J.M. Santos, Vrandečić & A.J.L. Phillips	*Pyrus pyrifolia*	Jiangxi, Fujian, Hubei	Bai et al. [30]
D. mahothocarpus	Y.H. Gao, W. Sun & L. Cai	*Lithocarpus* sp.	Zhejiang	Gao et al. [4]
D. multigutullata	F. Huang, K.D. Hyde & H.Y. Li	*Citrus* sp.	Fujian	Huang et al. [12]
D. neotheicola	A.J.L. Phillips & J.M. Santos	*Pyrus bretschneideri*	Yunnan, Jiangxi, Fujian	Bai et al. [30]
D. oraccinii	Y.H. Gao & L. Cai	*Camellia* sp.	Jiangxi	Gao et al. [29]
D. ovalispora	F. Huang, K.D. Hyde & H.Y. Li	*Citrus* sp.	Yunnan	Huang et al. [12]
D. ovoicicola	Y. H. Gao & L. Cai	*Citrus* sp.	Zhejiang	Gao et al. [4]
D. padina	C.M. Tian & Q. Yang	*Padus racemosa*	Jiangxi	Yang et al. [17]
D. parvae	Y.S. Guo & G.P. Wang	*Pyrus pyrifolia*	Yunnan	Guo et al. [5]
D. pentriteum	Y.H. Gao & L. Cai	*Camellia* sp.	Jiangxi	Gao et al. [29]
D. pescicola	Dissanayake *et al.*	*Pyrus bretschneideri*	Yunnan	Guo et al. [5]
D. phaseolorum	(Cooke & Ellis) Sacc.	*Vitis vinifera*	Beijing	Huang et al. [12]
D. phragmitis	Crous	*Phragmitis australis*	Beijing	Crous et al. [33]
D. podocarpi-macrophylli	Y.H. Gao & L. Cai	*Podocarpus macrophyllus*	Zhejiang	Gao et al. [29]
D. rostrata	C.M. Tian, X.L. Fan & K.D. Hyde	*Juglans mandshurica*	Gansu	Fan et al. [34]
D. sambucusii	C.M. Tian & Q. Yang	*Sambucus williamsii*	Heilongjiang	Yang et al. [35]
D. schisandrae	C.M. Tian & Q. Yang	*Schisandra chinensis*	Heilongjiang	Yang et al. [35]
D. sojae	Lehman	*Vitis vinifera*	Beijing	Huang et al. [12]
		Citrus sp.	Shaanxi	Huang et al. [12]
		Pyrus pyrifolia	Guizhou, Jiangsu	Guo et al. [5]
D. spinosa	Y.S. Guo & G.P. Wang	*Pyrus pyrifolia*	Zhejiang, Jiangsu, Guizhou	Guo et al. [5]
D. subclavata	F. Huang, K.D. Hyde & H.Y. Li	*Citrus* sp.	Fujian, Guangdong	Huang et al. [12]
D. taoicola	Dissanayake, X.H. Li & K. D	*Prunus persica*	Hubei	Dissanayake et al. [16]
		Pyrus pyrifolia	Zhejiang, Jiangsu, Guizhou	Guo et al. [5]
D. ternstroemia	Y.H. Gao, W. Sun & L. Cai	*Ternstroemia sp.*	Zhejiang	Gao et al. [4]
D. unshiuensis	F. Huang, K.D. Hyde & H.Y. Li	*Citrus* sp.	Guangxi	Huang et al. [12]
		Carya illinoensis	Jiangsu	Yang et al. [17]
D. velutina	Y.H. Gao & L. Cai	*Pyrus pyrifolia*	Fujian, Guizhou	Guo et al. [5]
D. xishuangbanica	Y.H. Gao & L. Cai	*Camellia sinensis*	Yunnan	Gao et al. [29]
D. yunnanensis	Y.H. Gao & L. Cai	*Coffea* sp.	Yunnan	Gao et al. [29]
D. zaobaisu	Y.S. Guo & G.P. Wang	*Pyrus bretschneideri*	Yunnan	Guo et al. [5]

solitary, single conical neck erumpent through leave tissues, 80–160 × 54–85 µm in size (Figure 3(d–f)). *Conidiophores* 11–23 × 1–2.5 µm, cylindrical, hyaline, straight, or curved, tapering toward the apex (Figure 3(g)). *Alpha conidia* 6–8 × 3–3.5 µm (av. 7 × 3.2 µm) hyaline, aseptate, ellipsoidal to oval, biguttulate, rounded at both ends (Figure 3(h)).

Colony morphology: Colonies on PDA mycelia growing full of Petri-dishes after 7 d with zones of the dirty white and umber, reverse umber patches (Figure 3(a)). Colonies on OA flat with white felty aerial mycelium, turning white to reddish-brown, with irregular black zones (Figure 3(b)).

Materials examined. China, Hubei province, Zigui county, on fruit of *Citrus sinensis*, August, 2018, M. J. Cui (culture YZU 181403).

Note: Alpha conidia of the present isolate were identical to that firstly reported by Udayanga et al. with (3.5–)4–5(–6) × (2–)2.5(–3) µm in size, collected from diseased leaves of *Dasymaschalon* sp. (Annonaceae) [28]. Besides, its cultural characteristics on PDA were identical to *D. siamensis* reported by Tibpromma *et al.*, as an endophytic fungus from a Pandanaceae host (*Pandanus* sp.) [22]. Unfortunately, Beta conidia and Gamma conidia were not observed in the present study.

In the pathogenicity tests, all isolates caused brown fruit rot (Figures 2(c), 3(c)) on *Citrus sinensis*, exposing mycelia on surface and severe rotting inside. The initial symptoms appeared as tiny, watery lesions, which gradually expanded eventually led to fruit rot on 7th day. However, the diameters of the lesions varied among different species; *D. siamensis* caused larger lesions (33–37 mm, av. 34 mm) than *D. taoicola* (26–30 mm, av. 28 mm) during the tests. In parallel, no lesions developed on the fruit that were inoculated with PDA disks as control. These results showed that all the present isolates were responsible agents for fruit rot of *Citrus sinensis*.

Presently, the identification of *Diaporthe* is mainly based on morphological characters and phylogenetic analysis [3,28]. In recent reported studies, nearly 65 *Diaporthe* species were associated with Chinese hosts, from which 15 were founded on *Citrus* spp. (Table. 2). According to Huang et al. [15] and Li et al. [36], phylogeny inferred from combined gene loci of ITS, *tef1*, and *tub2* could be used for further identification of *Diaporthe* species.

Diaporthe taoicola was firstly isolated from diseased shoots of *Prunus persica* in Hubei province, China, 2017, proved being able to cause necrotic lesions on detached peach shoots [16]. From then on, it had been only reported on *Pyrus pyrifolia* causing shoot canker symptoms in China with high phylogenetic diversity [5]. Guo et al. [5] inoculated *D. taoicola* from pear shoots on wounded twigs of different fruit crops to evaluate its host range, which could induce symptoms on citrus, apple, peach, and kiwifruit. It is worth noting that *D. taoicola* might pose threats to fruit trees in China. The present study firstly confirmed that it also pathogenic to *Citrus sinensis* fruit.

Diaporthe siamensis had been reported on diseased leaves of *Dasymaschalon* sp. in the family of Annonaceae in Thailand [28], but without pathogenicity test on the host plant. Then, it was also found as an endophytic fungus from *Pandanus* sp. (Pandanaceae) in Thailand [22], also probably as endophyte on *Garcinia parvifolia* from Malaysia [28]. Regretfully, the pathogenicity evaluation of the species remained a lack in previous studies. Except a detailed description of *D. siamensis* given in this study, the pathogenicity tests revealed that it could induce fruit rot on *Citrus sinensis*, stronger than *D. taoicola*. To the best of our knowledge, this is the first report of *Diaporthe taoicola* and *D. siamensis* from *Citrus sinensis* in China, which could induce fruit rot on the host.

Compliance with ethical standards

The research does not contain any studies with Human Participants and/or Animals, and the authors declare that they have no conflict of interest.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The research was supported by Guangxi Key Laboratory of Biology for Crop Disease and Insect Pests [2019-KF-01].

References

[1] Rauf A, Uddin G, Ali J. Phytochemical analysis and radical scavenging profile of juices of *Citrus sinensis*, *Citrus anrantifolia*, and *Citrus limonum*. Org Med Chem Lett. 2014;4(1):5.

[2] Santos J, Phillips A. Resolving the complex of *Diaporthe* (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. Fungal Divers. 2009;34:111–125.

[3] Gomes RR, Glienke C, Videira SIR, et al. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia. 2013;31:1–41.

[4] Gao YHui, Su Y, Sun W, et al. *Diaporthe* species occurring on *Lithocarpus glabra* in China, with descriptions of five new species. Fungal Biol. 2015; 119(5):295–6146.

[5] Guo YS, Crous PW, Bai Q, et al. High diversity of *Diaporthe* species associated with pear shoot canker in China. Persoonia. 2020;45(1):132–162.
[6] Mostert L, Kang JC, Crous PW. *Phomopsis saccharata* sp. nov., causing a canker and die-back disease of Protea repens in South Africa. Sydowia. 2011;53:227–235.
[7] Udayanga D, Liu X, McKenzie EHC, et al. The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Diversity. 2011;50(1):189–225.
[8] Du ZHUO, Fan X-L, Hyde KD, et al. Phylogeny and morphology reveal two new species of *Diaporthe* from *Betula* spp. in China. Phytotaxa. 2016;269(2):90–102.
[9] Díaz GA, Latorre BA, Lolas M, et al. Identification and characterization of *Diaporthe ambigua*, *D. australafricana*, *D. novem*, and *D. rudis* causing a postharvest fruit rot in kiwifruit. Plant Dis. 2017; 101(8):1402–1410.
[10] Timmer LW, Garney SM, Graham J. Compendium of citrus diseases. 2nd ed. St. Paul, Minnesota, USA: APS Press; 2000. 19–21.
[11] Udayanga D, Castlebury LA, Rossman AY, et al. Species limits in *Diaporthe*: molecular re-assessment of *D. citri*, *D. cytosporella*, *D. foeniculina* and *D. rudis*. Persoonia. 2014;32:83–101.
[12] Huang F, Udayanga D, Wang XH, et al. Endophytic *Diaporthe* associated with citrus: a phylogenetic reassessment with seven new species from china. Fungal Biol. 2015;119(5):331–347.
[13] Santos JM, Correia VG, Phillips AJL. Primers for mating-type diagnosis in *Diaporthe* and *Phomopsis*: their use in teleomorph induction in vitro and biological species definition. Fungal Biol. 2010;114(2-3):255–270.
[14] Rehner SA, Uecker FA. Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. Can J Bot. 1994; 72(11):1666–1674.
[15] Huang F, Hou X, Dewdney M, et al. *Diaporthe* species occurring on citrus in China. Fungal Diversity. 2013;61(1):237–250.
[16] Dissanayake AJ, Zhang W, Liu M, et al. *Diaporthe* species associated with peach tree dieback in Hubei. China. Mycosphere. 2017;8(5):533–549.
[17] Yang Q, Fan XL, Vladimiro G, et al. High diversity of *Diaporthe* species associated with dieback diseases in China, with twelve new species described. MC. 2018;39:97–149.
[18] Cenis JL. Rapid extraction of fungal DNA for PCR amplification. Nucleic Acids Res. 1992;20(9):2380.
[19] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al., editors. PCR protocols: a guide to methods and applica-tions., San Diego, California: Academic Press; 1990. 315–322.
[20] Carbone I, Kohn LM. Method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 1999;91(3):553–556.
[21] Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Am Soc Microbiol. 1995;61:1323–1330.
[22] Tibpromma S, Hyde KD, Bhat JD, et al. Identification of endophytic fungi from leaves of Pandanaceae based on their morphotypes and DNA sequence data from southern. MycoKeys. 2018;33:25–67.
[23] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Evolutionary Gene-tics. Analysis. 2016;33:1870–1874.
[24] Swofford DL. Phylogenetic analysis using parsimony. Version 4b10. Sunderland, MA: Sinauer Associates; 2002.
[25] Larkin MA, Blackshields G, Brown NP, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23(21):2947–2948.
[26] ThailNylander JAA. MrModelTest v. 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. 2004.
[27] Rambaut A, Drummond A. FigTree v.1.3.1. Institute of Evolutionary Biology. Edinburgh, UK: University of Edinburgh; 2010.
[28] Udayanga D, Liua X, Mckenzie EHC, et al. Multilocus phylogeny reveals three new species of *Diaporthe* from Thailand. Cryptogamie Mycologie. 2012; 33(3):295–309.
[29] Gao YH, Liu F, Duan WJ, et al. *Diaporthe* is paraphyletic. Ima Fungus. 2017;8(1):153–187.
[30] Bai Q, Zhai LF, Chen XR, et al. Biological and molecular characterization of five *Phompsis* species associated with pear shoot canker in China. Plant Disease. 2015;99(12):1704–1712.
[31] Hu DM, Cai L, Hyde KD. Three new ascomycetes from freshwater in China. Mycologia. 2012;104(6): 1478–1489.
[32] Yang Q, Fan XL, Du Z. *Diaporthe juglandicola* sp. nov. (Diaporthales, Ascomycetes), evidenced by morphological characters and phylogenetic analysis. Mycosphere. 2017;8(5):817–826.
[33] Crous PW, Shivas RG, Quaedvlieg W, et al. Fungal planet description sheets: 214. Persoonia. 2014; 32(1):184–280.
[34] Fan XL, Hyde KD, Udayanga D, et al. *Diaporthe rostrata*, a novel ascomycete from *Juglans mandshurica* associated with walnut dieback. Mycol Progress. 2015;14(10):82.
[35] Yang Q, Du Z, Tian CM. Phylogeny and morphology reveal two new species of *Diaporthe* from Traditional Chinese Medicine in Northeast China. Phytotaxa. 2018;336(2):159–170.
[36] Li Y, Tan P, Zhao DG. *Diaporthe nobilis*, a new record on *Camellia sinensis* in Guizhou Province. China. Mycosphere. 2017;8(1):1–8.