Phytotaxa 336 (2): 159–170
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.336.2.3

Phylogeny and morphology reveal two new species of *Diaporthe* from Traditional Chinese Medicine in Northeast China

QIN YANG [1], ZHUO DU [1] & CHENG-MING TIAN [1]*

[1] *The Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University, Beijing 100083, China*
** Correspondence author: chengmt@bjfu.edu.cn*

Abstract

Diaporthe sambucusii sp. nov and *D. schisandrae* sp. nov., collected from diseased branches of Traditional Chinese Medicine in Northeast China, *Sambucus williamsii* Hance and *Schisandra chinensis* (Turcz.) Baill, are herein described and illustrated. Recognition of the two new species are supported by both holomorphic morphology and phylogenetic analysis. Morphologically, *D. sambucusii* is distinguished by hyaline, aseptate, smooth, ovoid to subfusiform, biguttulate alpha conidia (7.0–9.5 × 2.0−2.5 µm) and beta conidia hyaline, aseptate, straight to hamate, 18.5–23.5 × 0.9–1.1 µm, similar to most species of *Diaporthe*. *Diaporthe schisandrae* is characterized by hyaline, aseptate, ellipsoidal to fusoid, somewhat tubercular at one end and obtuse at other, 1–3-guttulate alpha conidia (8.5–11.5 × 2.7−3.3 µm). Phylogenetic analysis using CAL, HIS, ITS, TEF1-α, and TUB molecular data shows that the isolates of the new species form two distinct clade within *Diaporthe* (MP/ML/BI=100/100/1).

Key words: Diaporthaceae, dieback, molecular phylogeny, taxonomy

Introduction

Members of *Diaporthe* are plant pathogens, endophytes or saprobes in a wide range of hosts and are responsible for several diseases, some of which are of economic importance (Uecker 1988, Crous & Groenewald 2005, Rossman *et al.* 2007, Santos & Phillips 2009, Udayanga *et al.* 2011, 2012a, b, 2014a, 2015, Gomes *et al.* 2013). For mycologists, studying on phytopathogenic *Diaporthe* species are therefore particularly important to work on a wide range of crops and economic trees (e.g. grapes, sunflowers, soybean and various diseases associated with ornamentals and forest trees) (Santos *et al.* 2011, Thompson *et al.* 2011, Baumgartner *et al.* 2013, Fan *et al.* 2015, Du *et al.* 2016, Yang *et al.* 2017a, b).

Sambucus williamsii (Caprifoliaceae) and *Schisandra chinensis* (Schisandraceae) have long been used as a Traditional Chinese Medicine (Han *et al.* 2008, Lu & Chen 2009). *Sambucus williamsii* is mainly used for the treatment of bone fractures and osteoporosis (Han *et al.* 2008). *Schisandra chinensis* is commonly applied to the treatment of night sweating, protracted diarrhea and diabetes (Lu & Chen 2009). *Sambucus* species are however, infected by a wide range of canker diseases, especially diaporthalean pathogens, which can cause serious reduction in growth. Deng (1963) reported *Diaporthe spiculosa* (Alb. & Schwein. : Fr.) Nitschke isolated from *Sambucus williamsii* in Jiangsu Province, China., causing annual bark canker disease. However, *Diaporthe spiculosa* is a synonym of *D. euonymi* Dearn., which has no available DNA data (Dearness 1916). *Diaporthe megalospora* Ellis & Everh. and *D. viticola* Nitschke (syn. *D. rudis*) have been recorded from *Sambucus* species (Gomes *et al.* 2013). The taxonomy and phylogeny of *Diaporthe* on *Schisandra* species in China have not been studied systematically.

In the last two decades, much progress has been made in the ability to define fungal species through the use of molecular data (Hibbett & Taylor 2013, Hyde *et al.* 2013). Multi-locus phylogenic analyses have become a conventional procedure to identify novel fungal species, especially in those genera that lack distinctive morphological characteristics, and to resolve species complex where traditional taxonomy has resulted in confusions (Lumbsch *et al.* 2005, Alves *et al.* 2006, Schoch *et al.* 2006, Cai *et al.* 2011a, b, Manamgoda *et al.* 2011, Udayanga *et al.* 2012b). As we all know, *Diaporthe* species in culture or under natural conditions do not produce all spore states of the asexual (alpha, beta and gamma conidia) or the sexual state (Gomes *et al.* 2013).

Accepted by Sajeewa Maharachchikumbura: 31 Dec. 2017; published: 30 Jan. 2018

During collecting trips in Heilongjiang Province, fresh specimens from symptomatic cankered branches of *Sambucus williamsii* and *Schisandra chinensis* were collected. Four fungal specimens were found with characters fitting the genus *Diaporthe.* Since the species of *Diaporthe* cannot easily be distinguished morphologically, a phylogenetic analysis was carried out based on ITS, CAL, HIS, TEF1-α, and TUB gene regions. This analysis determined that the two isolates are distinct from all other currently described and sequenced species in *Diaporthe*.

Materials and Methods

Isolation

Diseased samples were collected from infected branches or twigs during collecting trips in Heilongjiang Province, China (Table 1). Single conidia were obtained from fruiting bodies by removing a mucoid conidial mass from pycnidial ostioles, and spreading the suspension on the surface of 1.8 % potato dextrose agar (PDA), incubated at 25 °C for up to 24 h. Single germinating conidia were plated onto fresh potato dextrose agar (PDA) plates. Specimens and isolates of the new species are deposited in the Museum of Beijing Forestry University (BJFC). Axenic cultures are maintained in the China Forestry Culture Collection Center (CFCC).

Morphology

Species identification was based on the morphological and micromorphological features of the fruiting bodies produced on infected plant tissues, supplemented with cultural characteristics. Morphological characteristics of the fruiting bodies were recorded using a Leica stereomicroscope (M205 FA). Micromorphological observations determined under a Leica compound microscope (DM 2500). More than 20 fruiting bodies were sectioned, both vertically and horizontally, and 50 spores were selected randomly for measurement. Four strains were selected for the species, and three cultures were replicated for each strain. Cultural characteristics of isolates incubated on PDA in the dark at 25 °C were observed and recorded, including colony color, texture and the arrangement of the conidiomata.

DNA extraction, PCR amplification, and sequencing

Fungal mycelia from pure cultures of representative isolates was harvested from PDA plates with cellophane using a modified CTAB method (Doyle & Doyle 1990). Representative isolates of taxa were chosen based on their frequency and consistency of isolation from collected samples. DNA were estimated by electrophoresis in 1 % agarose gels, and the quality was measured by NanoDrop™ 2000 (Thermo, USA) according to the user's manual (Desjardins *et al.* 2009).

PCR amplifications were performed in DNA Engine (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories, CA, USA). The primer pair ITS1/ITS4 (White *et al.* 1990) was used to amplify the ITS region. The primer pair EF1-728F/EF1-986R (Carbone & Kohn 1999) was used to amplify a partial fragment of the TEF1-alpha gene. The primer pair Bt2a/Bt2b (Glass & Donaldson 1995) was used to amplify the beta tubulin (TUB). The primer pair CAL228F/CAL737R (Carbone & Kohn 1999) was used to amplify the calmodulin gene (CAL). The HIS region was amplified using primers CYLH4F (Crous *et al.* 2004a) and H3-1b (Glass & Donaldson 1995). PCR amplification products were checked visually via electrophoresis in 2 % agarose gels. DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with a BigDye Terminater Kit v.3.1 (Invitrogen, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Phylogenetic analysis

Sequences generated in this study were compared to published sequences in GenBank and to those in the relevant published literature (Gomes *et al.* 2013, Gao *et al.* 2014, 2015, 2016, 2017, Huang *et al.* 2015, Udayanga *et al.* 2014b, Du *et al.* 2016, Tanney *et al.* 2016, Dissanayake *et al.* 2017a, b, c, Santos *et al.* 2017, Yang *et al.* 2017a, b), and are shown in Table 1. All sequences were aligned using MAFFT v.6 (Katoh & Toh 2010) and edited manually using MEGA6 (Tamura *et al.* 2013). Various methods of phylogenetic reconstruction were performed: maximum parsimony in PAUP v.4.0b10 (Swofford 2003); maximum likelihood (ML) in PhyML v.7.2.8 (Guindon *et al.* 2010); Bayesian Inference (BI) in MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). All analyses were performed on the combined multi-gene dataset (CAL, HIS, ITS, TEF1-α, TUB) to compare *Diaporthe* species from other ex-type reference in recent studies (Table 1). *Diaporthella corylina* (CBS 121124) was selected as outgroup in this analysis (Gomes *et al.* 2013). Trees are shown using FigTree v.1.3.1 (Rambaut & Drummond 2010).

TABLE 1. Isolates and GenBank accession numbers used in this study.

Species	Isolate	Host	Location	GenBank accession numbers				
				ITS	CAL	HIS	TEF1-α	TUB
D. acutispora[T]	CGMCC 3.18285	*Coffea* sp.	China	KX986764	KX999274	–	KX999155	KX999195
D. alnea[T]	CBS 146.46	*Alnus* sp.	Netherlands	KC343008	KC343250	KC343492	KC343734	KC343976
D. ampelina[T]	STEU2660	*Vitis vinifera*	France	AF230751	AY745026	–	AY745056	JX275452
D. anacardii[T]	CBS 720.97	*Anacardium occidentale*	East Africa	KC343024	KC343266	KC343508	KC343750	KC343992
D. angelicae[T]	CBS 111592	*Heracleum sphondylium*	Austria	KC343027	KC343269	KC343511	KC343753	KC343995
D. apiculata[T]	LC3418	*Camellia sinensis*	China	KP267896	–	–	KP267970	KP293476
D. arecae[T]	CBS 161.64	*Areca catechu*	India	KC343032	KC343274	KC343516	KC343758	KC344000
D. arengae[T]	CBS 114979	*Arenga enngleri*	Hong Kong	KC343034	KC343276	KC343518	KC343760	KC344002
D. aseana[T]	MFLUCC 12-0299a	Unknown dead leaf	Thailand	KT459414	KT459464	–	KT459448	KT459432
D. betulae[T]	CFCC 50469	*Betula platyphylla*	China	KT732950	KT732997	KT732999	KT733016	KT733020
D. betulicola[T]	CFCC 51128	*Betula albosinensis*	China	KX024653	KX024659	KX024661	KX024655	KX024657
D. bicincta[T]	CBS 121004	*Juglans* sp.	USA	KC343134	KC343376	KC343618	KC343860	KC344102
D. celastrina[T]	CBS 139.27	*Celastrus* sp.	USA	KC343047	KC343289	KC343531	KC343773	KC344015
D.chamaeropis	CBS 454.81	*Chamaerops humilis*	Greece	KC343048	KC343290	KC343532	KC343774	KC344016
D. cichorii[T]	MFLUCC 17-1023	*Cichorium intybus*	Italy	KY964220	KY964133	–	KY964176	KY964104
D. cinerascens	CBS 719.96	*Ficus carica*	Bulgaria	KC343050	KC343292	KC343534	KC343776	KC344018
D. cissampeli[T]	CBS 141331	*Cissampelos capensis*	South Africa	KX228273	–	KX228366	–	KX228384
D. citri[T]	AR 3405	*Citrus* sp.	USA	KC843311	KC843157	–	KC843071	KC843187
D. citrichinensis[T]	ZJUD 34	*Citrus* sp.	China	JQ954648	KC357494	–	JQ954666	–
D. compacta[T]	CGMCC 3.17536	*Camellia sinensis*	China	KP267854	–	KP293508	KP267928	KP293434
D. cucurbitae	CBS 136.25	*Arctium* sp.	Unknown	KC343031	KC343273	KC343515	KC343757	KC343999
D. cuppatea[T]	CBS 117499	*Aspalathus linearis*	South Africa	KC343057	KC343299	KC343541	KC343783	KC344025
D. detrusa	CBS 109770	*Berberis vulgaris*	Austria	KC343061	KC343303	KC343545	KC343787	KC344029
D. dorycnii[T]	MFLUCC 17-1015	*Dorycnium hirsutum*	Italy	KY964215	–	–	KY964171	KY964099
D. elaeagni	CBS 504.72	*Elaeagnus* sp.	Netherlands	KC343064	KC343306	KC343548	KC343790	KC344032
D. elaeagni-glabrae[T]	CGMCC 3.18287	*Elaeagnus glabra*	China	KX986779	KX999281	KX999251	KX999171	KX999212
D. eres[T]	AR5193	*Ulmus* sp.	Germany	KJ210529	KJ434999	KJ420850	KJ210550	KJ420799
D. eugeniae[T]	CBS 444.82	*Eugenia aromatica*	West Sumatra	KC343098	KC343340	KC343582	KC343824	KC344066
D. foeniculacea[T]	CBS 123208	*Foeniculum vulgare*	Portugal	KC343104	KC343346	KC343588	KC343830	KC344072
D. fraxini-angustifoliae[T]	BRIP 54781	*Fraxinus angustifolia*	Australia	JX862528	–	–	JX862534	KF170920
D. ganjae[T]	CBS 180.91	*Cannabis sativa*	USA	KC343112	KC343354	KC343596	KC343838	KC344080
D. gardeniae	CBS 288.56	*Gardenia florida*	Italy	KC343113	KC343355	KC343597	KC343839	KC344081
D. gulyae[T]	BRIP 54025	*Helianthus annuus*	Australia	JF431299	–	–	KJ197271	JN645803
D. helicis[T]	AR5211	*Hedera helix*	France	KJ210538	KJ435043	KJ420875	KJ210559	KJ420828
D. hickoriae[T]	CBS 145.26	*Carya glabra*	USA	KC343118	KC343360	KC343602	KC343844	KC344086
D. inconspicua[T]	CBS 133813	*Maytenus ilicifolia*	Brazil	KC343123	KC343365	KC343607	KC343849	KC344091
D. infecunda[T]	CBS 133812	*Schinus terebinthifolius*	Brazil	KC343126	KC343368	KC343610	KC343852	KC344094
D. juglandicola[T]	CFCC 51134	*Juglans mandshurica*	China	KU985101	KX024616	KX024622	KX024628	KX024634
D. kongii[T]	BRIP 54031	*Portulaca grandiflora*	Australia	JF431301	–	–	JN645797	KJ197272
D. longicicola[T]	CGMCC 3.17089	*Lithocarpus glabra*	China	KF576267	–	–	KF576242	KF576291

.....continued on the next page

TABLE 1. (Continued)

Species	Isolate	Host	Location	GenBank accession numbers				
				ITS	CAL	HIS	TEF1-α	TUB
D. manihotia[T]	CBS 505.76	*Manihot utilissima*	Rwanda	KC343138	KC343380	KC343622	KC343864	KC344106
D. megalospora	CBS 143.27	*Sambucus canadensis*	Unknown	KC343140	KC343382	KC343624	KC343866	KC344108
D. melonis[T]	CBS 507.78	*Cucumis melo*	USA	KC343142	KC343384	KC343626	KC343868	KC344110
D. momicola[T]	MFLUCC 16-0113	*Prunus persica*	China	KU557563	KU557611	–	KU557631	KU55758
D. musigena[T]	CBS 129519	*Musa* sp.	Australia	KC343143	KC343385	KC343627	KC343869	KC344111
D. neilliae[T]	CBS 144. 27	*Spiraea* sp.	USA	KC343144	KC343386	KC343628	KC343870	KC344112
D. nobilis	CBS 113470	*Castanea sativa*	Korea	KC343146	KC343388	KC343630	KC343872	KC344114
D. nomurai	CBS 157.29	*Morus* sp.	Japan	KC343154	KC343396	KC343638	KC343880	KC344122
D. oncostoma	CBS 100454	*Robinia pseudoacacia*	Germany	KC343160	KC343402	KC343644	KC343886	KC344128
D. oraccinii[T]	LC3166	*Camellia sinensis*	China	KP267863	–	KP293517	KP267937	KP293443
D. ovalispora[T]	ICMP20659	*Citrus limon*	China	KJ490628	–	KJ490570	KJ490507	KJ490449
D. pascoei[T]	BRIP 54847	*Persea americana*	Australia	JX862532	–	–	JX862538	KF170924
D. passifloricola[T]	CBS 141329	*Passiflora foetida*	Malaysia	KX228292	–	KX228367	–	KX228387
D. penetriteum[T]	LC3353	*Camellia sinensis*	China	KP714505	–	KP714493	KP714517	KP714529
D. pescicola[T]	MFLUCC 16-0105	*Prunus persica*	China	KU557555	KU557603	–	KU557623	KU557579
D. podocarpi-macrophylli[T]	CGMCC 3.18281	*Podocarpus macrophyllus*	China	KX986774	KX999278	KX999246	KX999167	KX999207
D. pseudomangiferae[T]	CBS 101339	*Mangifera indica*	Dominican Republic	KC343181	KC343423	KC343665	KC343907	KC344149
D. pseudophoenicicola[T]	CBS 462.69	*Phoenix dactylifera*	Spain	KC343184	KC343426	KC343668	KC343910	KC344152
D. pyracanthae[T]	CAA483	*Pyracantha coccinea*	Portugal	KY435635	KY435656	KY435645	KY435625	KY435666
D. pulla[T]	CBS 338.89	*Hedera helix*	Yugoslavia	KC343152	KC343394	KC343636	KC343878	KC344120
D. rostrata[T]	CFCC 50062	*Juglans mandshurica*	China	KP208847	KP208849	KP208851	KP208853	KP208855
***D. sambucusii*[T]**	**CFCC 51986**	***Sambucus williamsii***	**China**	**KY852495**	**KY852499**	**KY852503**	**KY852507**	**KY852511**
	CFCC 51987	***Sambucus williamsii***	**China**	**KY852496**	**KY852500**	**KY852504**	**KY852508**	**KY852512**
D. schini[T]	CBS 133181	*Schinus terebinthifolius*	Brazil	KC343191	KC343433	KC343675	KC343917	KC344159
***D. schisandrae*[T]**	**CFCC 51988**	***Schisandra chinensis***	**China**	**KY852497**	**KY852501**	**KY852505**	**KY852509**	**KY852513**
	CFCC 51989	***Schisandra chinensis***	**China**	**KY852498**	**KY852502**	**KY852506**	**KY852510**	**KY852514**
D. sennae[T]	CFCC 51636	*Senna bicapsularis*	China	KY203724	KY228875	–	KY228885	KY228891
D. sennicola[T]	CFCC 51634	*Senna bicapsularis*	China	KY203722	KY228873	KY228879	KY228883	KY228889
D. sojae[T]	FAU635	*Glycine max*	USA	KJ590719	KJ612116	KJ659208	KJ590762	KJ610875
D. subclavata[T]	ICMP20663	*Citrus unshiu*	China	KJ490587	–	KJ490529	KJ490466	KJ490408
D. tecomae	CBS 100547	*Tabebuia* sp.	Brazil	KC343215	KC343457	KC343699	KC343941	KC344183
D. tectonae[T]	MFLUCC 12-0777	*Tectona grandis*	China	KU712430	KU749345	–	KU749359	KU743977
D. tectonendophytica[T]	MFLUCC 13-0471	*Tectona grandis*	China	KU712439	KU749354	–	KU749367	KU749354
D. tectonigena[T]	MFLUCC 12-0767	*Tectona grandis*	China	KU712429	KU749358	–	KU749371	KU743976
D. unshiuensis[T]	CGMCC 3.17569	*Citrus unshiu*	China	KJ490587	–	KJ490529	KJ490408	KJ490466
D. vaccinii[T]	CBS 160.32	*Oxycoccus macrocarpos*	USA	KC343228	KC343470	KC343712	KC343954	KC344196
D. woolworthii	CBS 148.27	*Ulmus americana*	Unknown	KC343245	KC343487	KC343729	KC343971	KC344213
Diaporthella corylina	CBS 121124	*Corylus* sp.	China	KC343004	KC343246	KC343488	KC343730	KC343972

New species are bold. Ex-type/ex-epitype isolates are marked by T.

MP analysis was performed by a heuristic search option of 1000 random-addition sequences with a tree bisection and reconnection (TBR) algorithm. Maxtrees was set to 5000, branches of zero length were collapsed and all of the most parsimonious trees were saved. Other calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC). ML analysis was performed with a GTR site substitution model (Guindon *et al.* 2010). Branch support was evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993).

MrModeltest v. 2.3 was used to estimate the best-fit model of nucleotide substitution model settings for each gene (Posada & Crandall 1998). The best fit model (GTR + I + G) was selected for CAL, HIS, ITS, TEF1-α and TUB sequence datasets. For the BI analyses, a Markov Chain Monte Carlo (MCMC) algorithm was performed (Rannala & Yang 1996). Sequences were submitted to GenBank (Table 1). The sequence alignment file was submitted to TreeBASE (www.treebase.org; accession number S21299). Our novel taxonomic descriptions were deposited in MycoBank (Crous *et al.* 2004b).

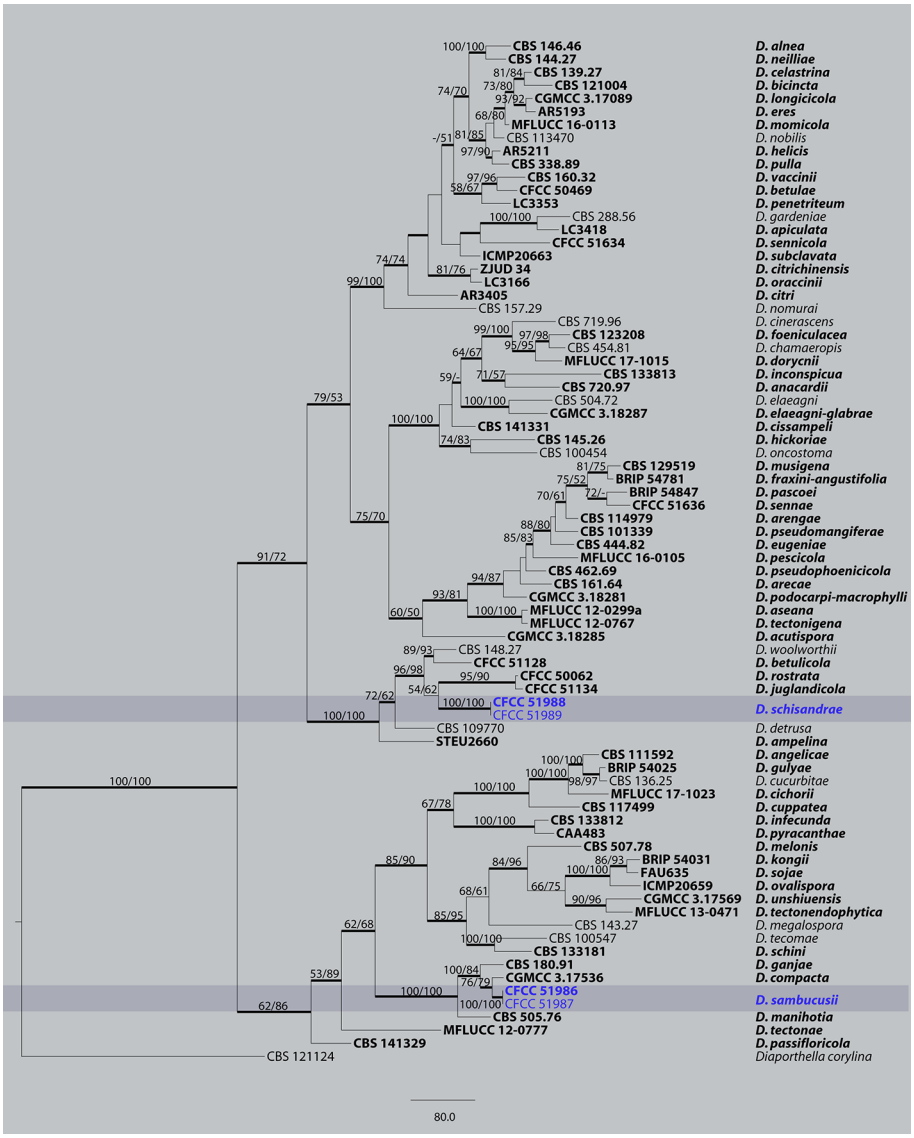

FIGURE 1. Phylogram of ITS regions based on MP, ML and Bayesian analysis. Values above the branches indicate maximum parsimony bootstrap (MP BP ≥ 50 %) and maximum likelihood bootstrap (ML BP ≥ 50 %). Values below branches represent posterior probabilities (BI PP ≥ 0.90) from Bayesian inference. Scale bar = 80 nucleotide substitutions. The new sequences resulting from the current study are in blue. Ex-type strains are in bold.

Results

Phylogeny

The aligned five-marker (CAL, HIS, ITS, TEF1-α and TUB) data set included 78 taxa (including one outgroup), comprising 2954 characters after alignment. Of these, 1455 characters were constant, 413 variable characters were parsimony-uninformative and 1086 characters were parsimony informative. The MP analysis resulted in 14 most parsimonious trees, with the first tree (TL = 6079, CI = 0.423, RI = 0.725, RC = 0.307) was shown in Fig. 1. The phylogenetic tree obtained from ML and Bayesian analyses with the MCMC algorithm was consistent with the previous MP tree. Based on the multi-locus phylogeny and morphology, 4 strains were identified to two novel species, which also supported by morphological traits. MP and ML bootstrap support values above 50 % are shown at the first and second position. The branches with significant Bayesian posterior probability (≥ 0.90) in Bayesian analyses were thickened in the phylogenetic tree. The sequences were determined to represent two new species as described in this paper.

Taxonomy

Diaporthe sambucusii C.M. Tian & Q. Yang, sp. nov. **FIGURE 2.**

MycoBank no: MB823869

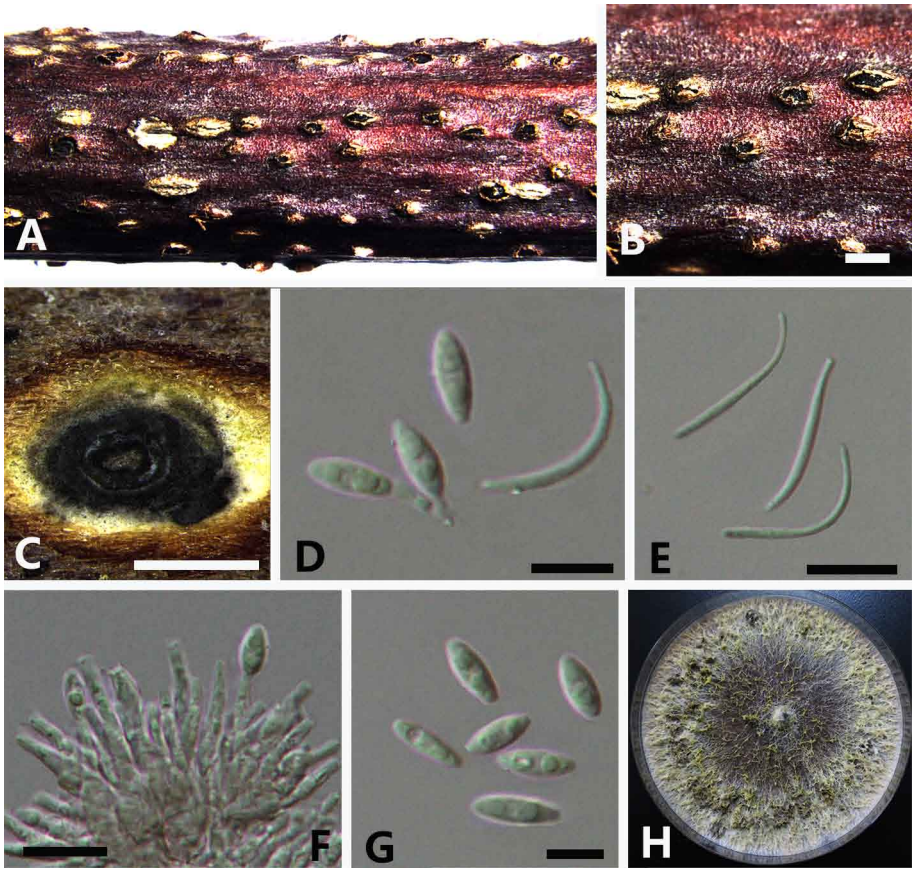

FIGURE 2. Morphology of *Diaporthe Sambucusii* from *Sambucus williamsii* (BJFC-S1368). A, B: Habit of conidiomata on branches. C: Transverse section of conidioma. D, E, G: Conidia. F: Conidiophores. H: Colonies on PDA at 30 days. Scale bars: B–C = 500 μm; D, G = 5 μm; E–F = 10 μm.

Holotype:—BJFC-S1368.

Etymology:—*sambucusii*: named after the host genus, *Sambucus*.

Host/Distribution:—from *Sambucus williamsii* in northeast China.

Original description:—Sexual state: Undetermined. Asexual morph: *Conidiomata* pycnidial, conical to globose, embedded in bark, erumpent through the bark surface at maturity, dense, with a single locule. *Ectostromatic disc*

(400–)425–575(−600) µm (av. = 500 µm, n = 20), brown to black, one ostiole per disc. *Locule* undivided, (480–)550–700(−800) µm (av. = 650 µm, n = 20) in diam. *Wall* parenchymatous, consisting 3–4 layers of medium brown textura angularis. *Conidiophores* hyaline, unbranched, phialides, cylindrical, ampulliform, (10–)11–15.5(−17.5) × (1.2–)1.4−1.8(−2.0) µm (av. = 13.5 × 1.6 µm, n = 50), straight or slightly curved. *Conidiogenous cells* hyaline, phialides, cylindrical, terminal, slightly tapering towards the apex, 0.5–1 µm diam. *Paraphyses* absent. *Alpha conidia* abundant in twigs, (6.0–)7.0–9.5(−10.5) × (1.8–)2.0−2.5(−2.6) µm (av. = 8.5 × 2.2 µm, n = 50), hyaline, aseptate, oval to fusiform, conspicuously biguttulate. *Beta conidia* (16.5–)18.5–23.5(−25.5) × 0.9–1.1 µm (av. = 21 × 1.0 µm, n = 50), hyaline, aseptate, smooth, filiform, straight or curved, eguttulate.

Culture characters:—Cultures incubated on PDA at 25 °C in darkness, colony originally flat with white felty aerial mycelium, becoming yellowish-brown aerial mycelium at the centre and beige mycelium at the marginal area, hyphae dense with irregular margin, conidiomata sparse, irregularly distributed over agar surface.

Material examined:—CHINA, Heilogjiang Province, Yichun city, 46°41’56.95”N, 129°01’27.49”E, 373 m asl, on twigs and branches of *Sambucus williamsii*, Q. Yang and Z. Du, 27 July 2016 (BJFC-S1368, ***holotype***; living ex-type culture, CFCC 51986). Heilongjiang Province, Yichun city, 46°41’56.85”N, 129°01’27.30”E, 370 m asl, on twigs and branches of *Sambucus williamsii*, Q. Yang and Z. Du, 27 July 2016 (BJFC-S1369, ***paratype***; living culture, CFCC 51987).

Notes:—This new species is introduced as molecular data showed it to be distinct, and this is also supported by morphological traits. The phylogram clustered in 78 clades with 63 ex-type *Diaporthe* strains distinguished the new species with high support (MP/ML/BI=100/100/1) (Fig. 1). Morphologically, it is characterized by oval to subfusiform, aseptate, biguttulate alpha coidia and filiform, straight or curved, eguttulate beta conidia, which is similar with *D. ganjae* from *Cannabis sativa* and *D. compacta* from *Camellia sinensis*. However, *Diaporthe sambucusii* can be distinguished with *D. ganjae* in its smaller alpha conidia (7.0–9.5 × 2.0−2.5 µm in *D. sambucusii* vs. 5.0–11.5 × 2.0−4.0 µm in *D. ganjae*) (McPartland 1983); with *D. compacta* in its bigger alpha conidia (7.0–9.5 × 2.0−2.5 µm *D. sambucusii* vs. 6.0–7.5 × 2−3 µm in *D. compacta*) (Gao *et al.* 2017).

Diaporthe schisandrae C.M. Tian & Q. Yang, sp. nov. **FIGURE 3**
MycoBank no: MB823870

Holotype:—BJFC-S1370.

Etymology:—*schisandrae*: named after the host genus, *Schisandra*.

Host/Distribution:—from *Schisandra chinensis* in northeast China.

Original description:—Sexual state: Undetermined. Asexual morph: *Conidiomata* pycnidial, globose to ovoid, embedded in bark, erumpent through the bark surface at maturity, sparse, with a single locule. *Ectostromatic disc* (160)250–400(−500) µm (av. = 325 µm, n = 20), black, one ostiole per disc. *Locule* undivided, (320)400–700(−750) µm (av. = 575 µm, n = 20) in diam. *Wall* parenchymatous, consisting 3–4 layers of medium brown textura angularis. *Conidiophores* hyaline, unbranched, phialides, cylindrical, tapering towards the apex, (10.5–)13.5–20(−22) × (1.4–)1.7−2(−2.3) µm (av. = 16.5 × 1.8 µm, n = 50), straight or slightly curved. *Conidiogenous cells* hyaline, phialides, cylindrical, terminal, slightly tapering towards the apex, 0.5–1 µm diam. *Paraphyses* absent. *Alpha conidia* abundant in twigs, (7.5–)8.5–11.5(−12) × (2.5–)2.7−3.3(−3.5) µm (av. = 10 × 3 µm, n = 50), hyaline, aseptate, ellipsoidal to fusoid, somewhat tubercular at one end and obtuse at other, 1–3-guttulate. *Beta conidia* not seen.

Culture characters:—Cultures incubated on PDA at 25 °C in darkness, colony with white felty aerial mycelium, becoming yellow compact aerial mycelium at the centre, following the white aerial mycelium, and dark brown at the marginal area. Conidiomata dense, distributed in circularity over agar surface.

Material examined:—CHINA, Heilongjiang Province, Yichun city, Dailing District, 47°10’57.63”N, 128°53’35.15”E, 428 m asl, on twigs and branches of *Schisandra chinensis*, Q. Yang and Z. Du, 27 July 2016 (BJFC-S1370, ***holotype***; living ex-type culture, CFCC 51988). Heilongjiang Province, Yichun city, Dailing District, 47°10’57.70”N, 128°53’35.20”E, 430 m asl, on twigs and branches of *Schisandra chinensis*, Q. Yang and Z. Du, 29 July 2016 (BJFC-S1371, ***paratype***; living culture, CFCC 51989).

Notes:—Two isolates of *D. schisandrae* cluster in a well-supported clade and appeared closely related to *D. rostrata* and *D. juglandicola*. *Diaporthe schisandrae* differs from *D. rostrata* in smaller locules (400–700 µm in *D. schisandrae* vs. 620–1100 µm in *D. rostrata*) and narrower alpha conidia (2.7–3.3 µm in *D. schisandrae* vs. 4–5 µm in *D. rostrata*) (Fan *et al.* 2015); from *D. juglandicola* in bigger alpha conidia (8.5–11.5 µm in *D. schisandrae* vs. 8–9 µm in *D. juglandicola*) (Yang *et al.* 2017b).

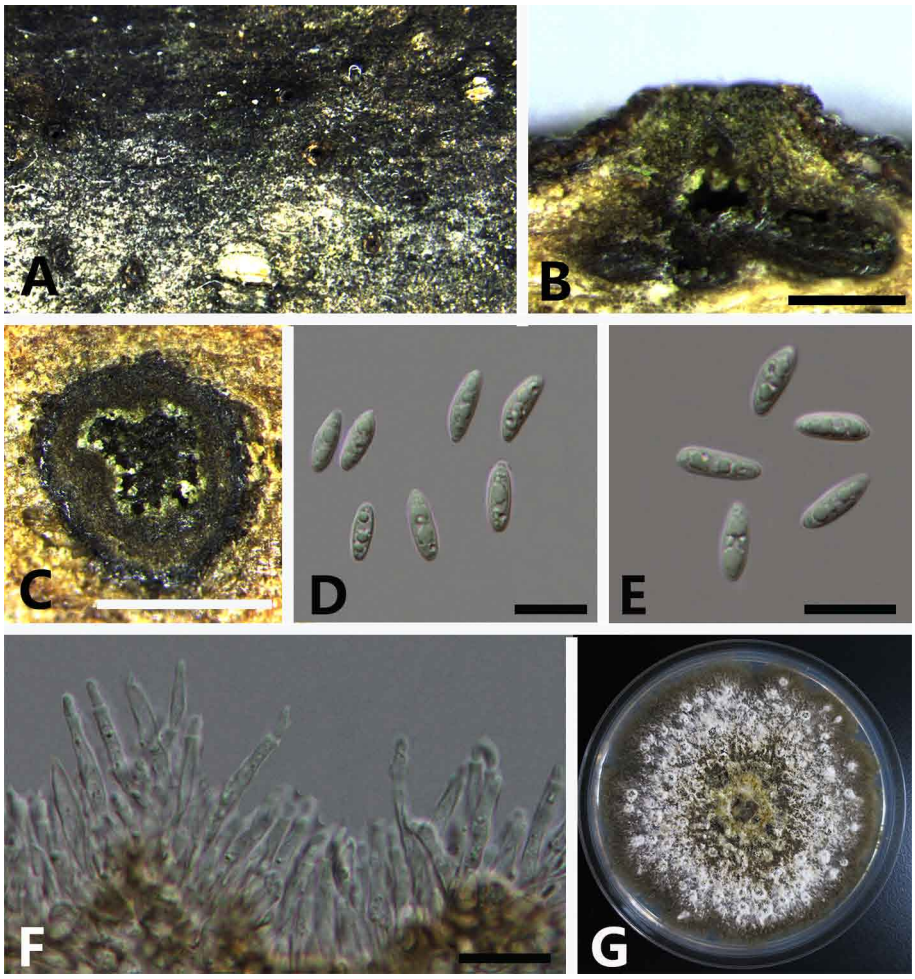

FIGURE 3. Morphology of *Diaporthe schisandrae* from *Schisandra chinensis* (BJFC-S1370). A: Habit of conidiomata on branches. B: Longitudinal section of conidioma. C: Transverse section of conidioma. D, E: Conidia. F: Conidiophores. G: Colonies on PDA at 30 days. Scale bars: B–C = 200 μm; D–F = 10 μm.

Discussion

Several *Diaporthe* species have been reported in China (Huang *et al.* 2013, 2015, Tan *et al.* 2013, Gao *et al.* 2014, 2015, 2016, Dissanayake *et al.* 2015, Fan *et al.* 2015, Du *et al.* 2016, Yang *et al.* 2017a, b). However, pathogens of trees associated with *Sambucus williamsii* and *Schisandra chinensis*, which are significant Traditional Chinese Medicine, have been poorly studied In this study, the two novel species (*D. sambucusii* and *D. schisandrae*) are introduced based on evidence from morphology and combined ITS, CAL, HIS, TEF1-α and TUB phylogenetic analyses.

Three *Diaporthe* species have been reported from *Sambucus*, i.e., *Diaporthe euonymi*, *D. megalospora* and *D. rudis* (Deng 1963, Gomes *et al.* 2013). However, *D. sambucusii* can be distinguished from *D. euonymi* in shorter condiophores (11–15.5 × 1.4–1.8 μm vs. 20–40 × 2 μm), and there is no available DNA data for this species (Deng 1963). *Diaporthe megalospora* is known to *Sambucus canadensis* from North America (Wehmeyer 1933, Farr & Rossman 2012), but there is no detailed morphological descriptions in Gomes *et al.* (2013) and it is required to designate an epitype, however, supported by the analysis of sequences data (Fig. 1). *Diaporthe viticola* is known from seveal hosts, especially from grapevines and is a synonym of *D. rudis* (Udayanga *et al.* 2014a). *Diaporthe sambucusii* can be distinguished from *D. rudis* in shorter condiophores (11–15.5 × 1.4–1.8 μm vs. 20–45 × 2–2.4 μm) and smaller beta conidia (18.5–23.5 × 0.9–1.1 μm vs. 27–31 × 3.4–3.8 μm) (Udayanga *et al.* 2014a). It is the first time to report *Diaporthe* species from *Schisandra chinensis.*

Previously, *Diaporthe* (syn. *Phomopsis*) have been primarily based on morphology, which has been shown to pay a minor role in species delimitation due to the simple and plastic morphological characters (Huang *et al.* 2013,

2015, Gao *et al.* 2016, Du *et al.* 2016, Yang *et al.* 2017a, b). Thus, analyses of rDNA ITS coupled with morphology, pathogenicity or multi-locus sequences data have been used in successful taxonomic revisions in contemporary molecular phylogenetic studies (Farr *et al.* 2002a, Santos & Phillips 2009, Diogo *et al.* 2010, Santos *et al.* 2011, Thompson *et al.* 2015, Udayanga *et al.* 2011, 2012a, 2012b, 2014a, 2014c, 2015, Gomes *et al.* 2013, Huang *et al.* 2015, Gao *et al.* 2015, 2016, Fan *et al.* 2015, Du *et al.* 2016). But confusion occurs when large number of species from a wide range of host species are analyzed. For example, Gao *et al.* (2016) reported that many isolates from *Camellia sinensis* belonging to the *D. eres* species complex, however, presented intermediated morphology and the phylogenetic tree also revealed the vague clades with short branch and moderate supports. *Diaporthe* represents a highly complex genus containing numerous cryptic species, it will be necessary to supplement the ITS, CAL, HIS, TEF1-α and TUB data by additional suitable single-copy markers like Apn2 and FG1093.

Acknowledgements

This study is financed by National Natural Science Foundation of China (Project No.: 31670647). We are grateful to Chungen Piao, Minwei Guo (China Forestry Culture Collection Center (CFCC), Chinese Academy of Forestry, Beijing.

References

Alves, A., Correia, A. & Phillips, A.J.L. (2006) Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. *Fungal Diversity* 23: 1–15.

Baumgartner, K., Fujiyoshi, P.T., Travadon, R., Castlebury, L.A., Wilcox, W.F. & Rolshausen, P.E. (2013) Characterization of species of *Diaporth*e from wood cankers of grape in eastern North American vineyards. *Plant Disease* 97: 912–920. https://doi.org/10.1094/PDIS-04-12-0357-RE

Cai, L., Giraud, T., Zhang, N., Begerow, D., Cai, G. & Shivas, R.G. (2011a) The evolution of species concepts and species recognition criteria in plant pathogenic fungi. *Fungal Diversity* 50 (1): 121–133. https://doi.org/10.1007/s13225-011-0127-8

Cai, L., Udayanga, D., Manamgoda, D.S., Maharachchikumbura, S.S.N., Liu, X.Z. & Hyde, K.D. (2011b) The need to carry out re-inventory of tropical plant pathogens. *Tropical Plant Pathology* 36: 205–213. https://doi.org/10.1590/S1982-56762011000400001

Carbone, I. & Kohn, L.M. (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 3: 553–556. https://doi.org/10.2307/3761358

Crous, P.W., Gams, W., Stalpers, J.A., Robert, V. & Stegehuis, G. (2004a) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* 50 (1): 19–22.

Crous, P.W. & Groenewald, J.Z. (2005) Hosts, species and genotypes: opinions versus data. *Australasian plant pathology* 34 (4): 463–470. https://doi.org/10.1071/AP05082

Crous, P.W., Groenewald, J.Z., Risède, J.M., Simoneau, P. & Hywel-Jones, N.L. (2004b) *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Studies in Mycology* 50 (2): 415–430.

Dearness, J. (1916) New or noteworthy species of fungi. *Mycologia* 8 (2): 98–107. https://doi.org/10.2307/3753433

Deng, S.Q. (1963) *Fungi of China.* Beijing, China. [in Chinese]

Desjardins, P., Hansen, J.B. & Allen, M. (2009) Microvolume protein concentration determination using the NanoDrop 2000c spectrophotometer. *Journal of visualized experiments: JoVE* 33: 1–3. https://doi.org/10.3791/1610

Diogo, E., Santos, J.M. & Phillips, A.J. (2010) Phylogeny, morphology and pathogenicity of *Diaporthe* and *Phomopsis* species on almond in Portugal. *Fungal Diversity* 44 (1): 107–115. https://doi.org/10.1007/s13225-010-0057-x

Dissanayake, A.J., Camporesi, E., Hyde, K.D., Zhang, W., Yan, J.Y. & Li, X.H. (2017b) Molecular phylogenetic analysis reveals seven new *Diaporthe* species from Italy. *Mycosphere* 8: 853–877.

https://doi.org/10.5943/mycosphere/8/5/4

Dissanayake, A.J., Liu, M., Zhang, W., Chen, Z., Udayanga, D., Chukeatirote, E., Li, X.H., Yan, J.Y. & Hyde, K.D. (2015) Morphological and molecular characterisation of *Diaporthe* species associated with grapevine trunk disease in China. *Fungal Biology* 119 (5): 283–294.
https://doi.org/10.1016/j.funbio.2014.11.003

Dissanayake, A.J., Phillips, A.J.L., Hyde, K.D., Yan, J.Y. & Li, X.H. (2017c) The current status of species in *Diaporthe*. *Mycosphere* 8: 1106–1156.

Dissanayake, A.J., Zhang, W., Liu, M., Hyde, K.D., Zhao, W.S., Li, X.H. & Yan, J.Y. (2017a) *Diaporthe* species associated with peach tree dieback in Hubei, China. *Mycosphere* 8: 533–549.
https://doi.org/10.5943/mycosphere/8/5/3

Doyle, J.J. & Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.

Du, Z., Fan, X.L., Hyde, K.D., Yang, Q., Liang, Y.M. & Tian, C.M. (2016) Phylogeny and morphology reveal two new species of *Diaporthe* from *Betul*a spp. in China. *Phytotaxa* 269 (2): 90–102.
https://doi.org/10.11646/phytotaxa.269.2.2

Fan, X.L., Hyde, K.D., Udayanga, D., Wu, X.Y. & Tian, C.M. (2015) *Diaporthe rostrata*, a novel ascomycete from *Juglans mandshurica* associated with walnut dieback. *Mycological Progress* 14 (10): 1–8.
https://doi.org/10.1007/s11557-015-1104-5

Farr, D.F., Castlebury, L.A. & Rossman, A.Y. (2002a) Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of *Phomopsis* from blueberry and cranberry in the eastern United States. *Mycologia* 94: 494–504.
https://doi.org/10.2307/3761783

Farr, D.F. & Rossman, A.Y. (2012) *Fungal databases, systematic mycology and microbiology laboratory, ARS, USDA*. Retrieved from: http://nt.ars-grin.gov/fungaldatabases/ (accessed December 2012)

Gao, Y.H., Liu, F. & Cai, L. (2016) Unravelling *Diaporthe* species associated with *Camellia*. *Systematics and Biodiversity* 14: 102–117.
https://doi.org/10.1080/14772000.2015.1101027

Gao, Y.H., Liu, F., Duan, W., Crous, P.W. & Cai, L. (2017) *Diaporthe* is paraphyletic. *IMA fungus* 8: 153–187.
https://doi.org/10.5598/imafungus.2017.08.01.11

Gao, Y.H., Su, Y.Y., Sun, W. & Cai, L. (2015) *Diaporthe* species occurring on *Lithocarpus glabra* in China, with descriptions of five new species. *Fungal Biology* 115: 295–309.
https://doi.org/10.1016/j.funbio.2014.06.006

Gao, Y.H., Sun, W., Su, Y.Y. & Cai, L. (2014) Three new species of *Phomopsis* in Gutianshan Nature Reserve in China. *Mycological Progress* 13 (1): 111–121.
https://doi.org/10.1007/s11557-013-0898-2

Glass, N.L. & Donaldson, G.C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61 (4): 1323–1330.

Gomes, R.R., Glienke, C., Videira, S.I.R., Lombard, L., Groenewald, J.Z. & Crous, P.W. (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia*: *Molecular Phylogeny and Evolution of Fungi* 31: 1.
https://doi.org/10.3767/003158513X666844

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59 (3): 307–321.
https://doi.org/10.1093/sysbio/syq010

Han, H., Yan, X.Y., Kuang, H.X. & Dong, P.L. (2008) The research progress of *Sambucus williamsii. Information on Traditional Chinese Medicine* 25(6): 14–16. [in Chinese]

Hibbett, D.S. & Taylor, J.W. (2013) Fungal systematics: is a new age of enlightenment at hand? *Nature Reviews Microbiology* 11: 129–133.
https://doi.org/10.1038/nrmicro2963

Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42 (2): 182–192.
https://doi.org/10.1093/sysbio/42.2.182

Huang, F., Hou, X., Dewdney, M.M., Fu, Y., Chen, G., Hyde, K.D. & Li, H. (2013) *Diaporthe* species occurring on *citrus* in China. *Fungal Diversity* 61 (1): 237–250.
https://doi.org/10.1007/s13225-013-0245-6

Huang, F., Udayanga, D., Wang, X., Hou, X., Mei, X., Fu, Y., Hyde, K.D. & Li, H. (2015) Endophytic *Diaporthe* associated with *Citrus*: A phylogenetic reassessment with seven new species from China. *Fungal Biology* 119 (5): 331–347.
https://doi.org/10.1016/j.funbio.2015.02.006

Hyde, K.D., Udayanga, D., Manamgoda, D.S., Tedersoo, L., Larsson, E., Abarenkov, K., Bertrand, Y.J.K., Oxelman, B., Hartmann, M., Kauserud, H., Ryberg, M., Kristiansson, E. & Nilsson, R.H. (2013) Incorporating molecular data in fungal systematics: a guide for aspiring researchers. *Current Research in Environmental and Applied Mycology* 3: 1–32.

Katoh, K. & Toh, H. (2010) Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* 26 (15): 1899–1900. https://doi.org/10.1093/bioinformatics/btq224

Lu, Y. & Chen, D.F. (2009) Analysis of *Schisandra chinensis* and *Schisandra sphenanthera*. *Journal of Chromatography A* 1216 (11): 1980–1990. https://doi.org/10.1016/j.chroma.2008.09.070

Lumbsch, H.T., Schmitt, I., Lindemuth, R., Miller, A., Mangold, A., Fernandez, F. & Huhndorf, S. (2005) Performance of four ribosomal DNA regions to infer higher-level phylogenetic relationships of inoperculate euascomycetes (Leotiomyceta). *Molecular phylogenetics and evolution* 34: 512–524. https://doi.org/10.1016/j.ympev.2004.11.007

Manamgoda, D.S., Cai, L., Bahkali, A.H., Chukeatirote, E. & Hyde, K.D. (2011) *Cochliobolus*: an overview and current status of species. *Fungal Diversity* 51: 3–42. https://doi.org/10.1007/s13225-011-0139-4

McPartland, J.M. (1983) *Phomopsis ganjae* sp. nov. on *Cannabis sativa* [Fungi, new taxa, parasitic on hemp]. *Mycotaxon* (USA).

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14 (9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Rambaut, A. & Drummond, A. (2010) *FigTree v.1.3.1.* Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK.

Rannala, B. & Yang, Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of molecular evolution* 43 (3): 304–311. https://doi.org/10.1007/BF02338839

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rossman, A.Y., Farr, D.F. & Castlebury, L.A. (2007) A review of the phylogeny and biology of the Diaporthales. *Mycoscience* 48 (3): 135–144. https://doi.org/10.1007/S10267-007-0347-7

Santos, J.M. & Phillips, A.J.L. (2009) Resolving the complex of *Diaporthe* (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. *Fungal Diversity* 34 (11): 111–125.

Santos, L., Phillips, A.J.L., Crous, P.W. & Alves, A. (2017) *Diaporthe* species on Rosaceae with descriptions of *D. pyracanthae* sp. nov. and *D. malorum* sp. nov. *Mycosphere* 8: 485–511. https://doi.org/10.5943/mycosphere/8/5/2

Santos, J.M., Vrandečić, K., Ćosić, J., Duvnjak, T. & Phillips, A.J.L. (2011) Resolving the *Diaporthe* species occurring on soybean in Croatia. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 27: 9–19. https://doi.org/10.3767/003158511X603719

Schoch, C.L., Shoemaker, R.A., Seifert, K.A., Hambleton, S., Spatafora, J.W. & Crous, P.W. (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98: 1041–1052. https://doi.org/10.1080/15572536.2006.11832632

Swofford, D.L. (2003) *PAUP*: Phylogenetic analysis using parsimony, * and Other Methods, Version 4.0b10.* Sinauer Associates, Suderland.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution* 30 (12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Tanney, J.B., Mcmullin, D.R., Green, B.D., Miller, J.D. & Seifert, K.A. (2016) Production of antifungal and antiinsectan metabolites by the *Picea* endophyte *Diaporthe maritima* sp. nov. *Fungal Biology* 120 (11): 1448–1457. https://doi.org/10.1016/j.funbio.2016.05.007

Tan, Y.P., Edwards, J., Grice, K.R.E. & Shivas, R.G. (2013) Molecular phylogenetic analysis reveals six new species of *Diaporthe* from Australia. *Fungal Diversity* 61 (1): 251–260. https://doi.org/10.1007/s13225-013-0242-9

Thompson, S.M., Tan, Y.P., Shivas, R.G., Neate, S.M., Morin, L., Bissett, A. & Aitken, E.A.B. (2015) Green and brown bridges between weeds and crops reveal novel *Diaporthe* species in Australia. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 35: 39. https://doi.org/10.3767/003158515X687506

Thompson, S.M., Tan, Y.P., Young, A.J., Neate, S.M., Aitken, E.A.B. & Shivas, R.G. (2011) Stem cankers on sunflower (*Helianthus*

annuus) in Australia reveal a complex of pathogenic *Diaporthe* (*Phomopsis*) species. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 27: 80.
https://doi.org/10.3767/003158511X617110

Udayanga, D., Castlebury, L.A., Rossman, A.Y., Chukeatirote, E. & Hyde, K.D. (2014b) Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. *Fungal Diversity* 67 (1): 203–229.
https://doi.org/10.1007/s13225-014-0297-2

Udayanga, D., Castlebury, L.A., Rossman, A.Y., Chukeatirote, E. & Hyde, K.D. (2015) The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. *Fungal Biology* 119 (5): 383–407.
https://doi.org/10.1016/j.funbio.2014.10.009

Udayanga, D., Castlebury, L.A., Rossman, A.Y. & Hyde, K.D. (2014a) Species limits in *Diaporthe*: molecular re-assessment of *D. citri*, *D. cytosporella*, *D. foeniculina* and *D. rudis*. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 32: 83–101.
https://doi.org/10.3767/003158514X679984

Udayanga, D., Liu, X., Crous, P.W., McKenzie, E.H., Chukeatirote, E. & Hyde, K.D. (2012a) A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). *Fungal Diversity* 56 (1): 157–171.
https://doi.org/10.1007/s13225-012-0190-9

Udayanga, D., Liu, X., McKenzie, E.H., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011) The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. *Fungal Diversity* 50 (1): 189–225.
https://doi.org/10.1007/s13225-011-0126-9

Udayanga, D., Liu, X., Mckenzie, E.H., Chukeatirote, E. & Hyde, K.D. (2012b) Multi-locus phylogeny reveals three new species of *Diaporthe* from Thailand. *Cryptogamie, Mycologie* 33 (3): 295–309.
https://doi.org/10.7872/crym.v33.iss3.2012.295

Uecker, F.A. (1988) A world list of *Phomopsis* names with notes on nomenclature, morphology and biology. *Mycological Memoirs* 13: 1–231.

Wehmeyer, L.E. (1933) *The Genus Diaporthe Nitschke And Its Segretes*. Michigan, USA.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* 18: 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yang, Q., Fan, X.L., Du, Z. & Tian, C.M. (2017a) *Diaporthe* species occurring on *Senna bicapsularis* in southern China, with descriptions of two new species. *Phytotaxa* 302 (2): 145–155.
https://doi.org/10.11646/phytotaxa.302.2.4

Yang, Q., Fan, X.L., Du, Z. & Tian, C.M. (2017b) *Diaporthe juglandicola* sp. nov. (Diaporthales, Ascomycetes), evidenced by morphological characters and phylogenetic analysis. *Mycosphere* 8 (5): 817–826.