Phytotaxa 269 (2): 090–102
http://www.mapress.com/j/pt/

Article

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

http://dx.doi.org/10.11646/phytotaxa.269.2.2

Phylogeny and morphology reveal two new species of *Diaporthe* from *Betula* spp. in China

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Abstract

Diaporthe species are common pathogens, endophytes, or saprobes on a wide range of hosts. During our investigation of forest pathogens, we made collections of *Diaporthe* species associated with canker and dieback disease of *Betula platyphylla* and *B. albosinensis* in Sichuan and Shaanxi provinces in China. *Diaporthe betulae* sp. nov. and *D. betulicola* sp. nov. are introduced in this paper, with illustrations, descriptions and support from analysis of ribosomal DNA internal transcribed spacer (ITS), calmodulin (CAL), histone H3 (HIS), translation elongation factor 1-α (TEF1-α) and beta-tubulin (TUB2) sequence data. *Diaporthe betulae* is characterized by hyaline, ellipsoidal, aseptate, biguttulate, 8.5–11 × 3–4 µm alpha conidia. *Diaporthe betulicola* is characterized by pycnidial stromata with a single locule with one ostiole per disc. Alpha conidia are hyaline, oblong, aseptate, lack guttules and 9.9–14.7 × 1.3–2.5 µm, and beta conidia are hyaline, spindle-shaped, curved, aseptate and 17–24 × 0.7–1.2 µm.

Key words: birch, Diaporthales, pathogens, systematics, taxonomy

Introduction

Diaporthe species are associated with wide range of hosts and are commonly encountered as pathogens, endophytes, or saprobes of crops, ornamentals and forest trees (Brayford 1990, Mostert *et al.* 2001; Farr *et al.* 2002; Santos and Phillips 2009; Santos *et al.* 2011; Udayanga *et al.* 2011, 2012, 2014a, 2015; Gomes *et al.* 2013; Hyde *et al.* 2014; Fan *et al.* 2015). *Diaporthe* species can cause blights, cankers, decay and wilt, dieback, leaf spots, and root and fruit rots (Mostert *et al.* 2001; Anagnostakis 2007; Santos *et al.* 2011; Thompson *et al.* 2011; Fan *et al.* 2015; Lawrence *et al.* 2015). The names *Diaporthe* and *Phomopsis* are no longer used for different morphs of the same genus (Santos and Phillips 2009). Diogo *et al.* (2010) and Udayanga *et al.* (2011, 2012), Rossman *et al.* (2015) suggested that being the older name, *Diaporthe* should have priority over *Phomopsis*, and should be adopted as the generic name. The sexual morph of *Diaporthe* is characterized by immersed ascomata and an erumpent pseudostroma with more or less elongated perithecial necks. Asci are unitunicate, clavate to cylindrical and ascospores are hyaline, 1-septate, and sometimes with appendages (Udayanga *et al.* 2011). The asexual morph is characterized by ostiolate conidiomata, with cylindrical phialides producing up to three types of hyaline, aseptate conidia; alpha conidia however are most often produced (Udayanga *et al.* 2011).

Betula are common species that grow rapidly, and have a broad tolerance to various environmental conditions and less specific demand for factors such as temperature, soil, nutrients, water and solar radiation. Thus they have regarded as common trees and shrubs of boreal forests in the Northern hemisphere, with great ecological importance (Furlow 1990; Linder *et al.* 1997). *Betula* species are however, infected by a wide range of canker disease, especially diaporthalean pathogens, which can cause serious reduction in growth (Fan *et al.* 2016). Arnold (1967) reported *Diaporthe alleghaniensis* R.H. Arnold isolated from *Betula alleghaniensis* in Canada, causing annual bark canker and foliage disease. *Diaporthe alleghaniensis*, *D. eres* Nitschke and *D. melanocarpa* Dearn have been recorded from *Betula* species in Japan (Kobayashi 1970).

During our investigation of forest pathogens causing *Diaporthe* canker of *Betula* species in China, we made

 Accepted by Dhanushka Udayanga: 28 Jul. 2016; published: 3 Aug. 2016

four collections of two *Diaporthe* species from symptomatic trees in Shaanxi and Sichuan provinces. In the current paper we introduce these as two novel *Diaporthe* species and provide descriptions and illustrations and multi-gene phylogenetic evidence to delineate the taxa.

Materials and methods

Isolation

Fresh collections of *Diaporthe* were made from infected branches or twigs of *Betula* spp. during investigations of forest pathogens in China. Single conidial isolates were established following a modified method of Chomnunti *et al.* (2014). The modification involved making a suspension from the conidial masses exuding through pycnidial ostioles and spreading this on the surface of 1.8 % potato dextrose agar (PDA), and incubating at 25 °C for up to 24 h. Single germinating conidia were transferred to fresh PDA plates (Chomnunti *et al.* 2014). Four strains (two from each new species) were used in the phylogenetic analysis (Table 1). Specimens and axenic cultures are deposited in the Museum of Beijing Forestry University (BJFC) and China Forestry Culture Collection Center (CFCC).

TABLE 1. Isolates used in this study, the genes sequenced and GenBank accessions.

Species	Isolate	host	location	GenBank accession numbers				
				ITS	cal	his	tef1-α	β-tub2
D. acaciigena[T]	CBS 129521	*Acacia retinodes*	Australia	KC343005	KC343247	KC343489	KC343731	KC343973
D. alleghaniensis[T]	CBS 495.72	*Betula alleghaniensis*,	Canada	KC343007	KC343249	KC343491	KC343733	KC343975
D. alnea[T]	CBS 146.46	Betulaceae	Netherlands	KC343008	KC343250	KC343492	KC343734	KC343976
D. ampelina[T]	CBS 114016	*Vitis vinifera*	France	AF230751	AY745026	-	AY745056	JX275452
D. amygdali[T]	CBS 126679	*Prunus dulcis*	Portugal	KC343022	KC343264	KC343506	KC343748	KC343990
D. apiculatum[T]	LC3418	*Camellia sinensis*	China	KP267896	-	-	KP267970	KP293476
D. arctii[T]	DP0482	*Arctium* sp.	-	KJ590736	KJ612133	KJ659218	KJ590776	KJ610891
D. australafricana[T]	CBS 111886	*Vitis vinifera*	Australia	KC343038	KC343280	KC343522	KC343764	KC344006
D. batatas[T]	CBS 122.21	*Ipomoea batatas*	USA	KC343040	KC343282	KC343524	KC343766	KC344008
***D. betulae*[T]**	**CFCC 50469**	***Betula platyphylla***	**China**	**KT732950**	**KT732997**	**KT732999**	**KT733016**	**KT733020**
D. betulae	**CFCC 50470**	***Betula platyphylla***	**China**	**KT732951**	**KT732998**	**KT733000**	**KT733017**	**KT733021**
***D. betulicola*[T]**	**CFCC 51128**	***Betula albosinensis***	**China**	**KX024653**	**KX024659**	**KX024661**	**KX024655**	**KX024657**
D. betulicola	**CFCC 51129**	***Betula albosinensis***	**China**	**KX024654**	**KX024660**	**KX024662**	**KX024656**	**KX024658**
D. biconispora[T]	ICMP20654	*Citrus grandis*	China	KJ490597	-	KJ490539	KJ490476	KJ490418
D. bicincta[T]	CBS 121004	*Juglans* sp.	USA	KC343134	KC343376	KC343618	KC343860	KC344102
D. biguttulata[T]	ICMP20657	*Citrus limon*	China	KJ490582	-	KJ490524	KJ490461	KJ490403
D. biguttusis[T]	CGMCC 3.17081	*Lithocarpus glabra*	China	KF576282	-	-	KF576257	KF576306
D. brasiliensis[T]	CBS 133183	*Aspidosperma tomentosum*	Brazil	KC343042	KC343284	KC343526	KC343768	KC344010
D. canthii[T]	CBS 132533	*Canthium inerme*	South Africa	JX069864	KC843174	-	KC843120	KC843230
D. carpini	CBS 114437	*Carpinus betulus*	Sweden	KC343044	KC343286	KC343528	KC343770	KC344012
D. caulivora[T]	CBS 127268	*Glycine max*	Croatia	KC343045	KC343287	KC343529	KC343771	KC344013
D. celastrina[T]	CBS 139.27	*Celastrus sp.*	USA	KC343047	KC343289	KC343531	KC343773	KC344015

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TABLE 1. (Continued)

Species	Isolate	host	location	GenBank accession numbers				
				ITS	cal	his	tef1-α	β-tub2
D. citri[T]	CBS 135422	*Citrus* sp.	USA	KC843311	KC843157	-	KC843071	KC843187
D. citrichinensis[T]	ZJUD34	*Citrus* sp.	China	JQ954648	KC357494	-	JQ954666	-
D. convolvuli[T]	FAU 649	*Convolvulus arvensis*	Canada	KJ590721	KJ612130	KJ659210	KJ590765	-
D. cotoneastri[T]	CBS 439.82	*Cotoneaster* sp.	UK	KC343090	KC343332	KC343574	KC343816	KC344058
D. crotalariae[T]	CBS 162.33	*Crotalaria spectabilis*	USA	KC343056	KC343298	KC343540	KC343782	KC344024
D. cuppatea[T]	CBS 117499	*Aspalathus linearis*	South Africa	KC343057	KC343299	KC343541	KC343783	KC344025
D. cynaroidis[T]	CBS 122676	*Protea cynaroides*	South Africa	KC343058	KC343300	KC343542	KC343784	KC344026
D. cytosporella[T]	FAU 461	*Citrus limon*	Spain	KC843307	KC843141	-	KC843116	KC843221
D. detrusa	CBS 109770	*Berberis vulgaris*	Austria	KC343061	KC343303	KC343545	KC343787	KC344029
D. discoidispora[T]	ICMP20662	*Citrus unshiu*	China	KJ490624	-	KJ490566	KJ490503	KJ490445
D. ellipicola[T]	CGMCC 3.17084	*Lithocarpus glabra*	China	KF576270	-	-	KF576245	KF576291
D. endophytica[T]	CBS 133811	*Schinus terebinthifolius*	Brazil	KC343065	KC343307	KC343549	KC343791	KC344033
D. eres[T]	AR5193	*Ulmus* sp.	Germany	KJ210529	KJ434999	KJ420850	KJ210550	KJ420799
D. fibrosa	CBS 109751	*Rhamnus cathartica*	Austria	KC343099	KC343341	KC343583	KC343825	KC344067
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. gardeniae	CBS 288.56	*Gardenia florida*	Italy	KC343113	KC343355	KC343597	KC343839	KC344081
D. helianthi[T]	CBS 592.81	*Helianthus annuus*	Serbia	KC343115	KC343357	KC343599	KC343841	KC344083
D. helicis[T]	AR5211	*Hedera helix*	France	KJ210538	KJ435043	KJ420875	KJ210559	KJ420828
D. hongkongensis[T]	CBS 115448	*Dichroa febrifuga*	China	KC343119	KC343361	KC343603	KC343845	KC344087
D. impulsa	CBS 114434	*Sorbus aucuparia*	Sweden	KC343121	KC343363	KC343605	KC343847	KC344089
D. kongii[T]	BRIP 54031	*Portulaca grandiflora*	Australia	JF431301	-	-	JN645797	KJ197272
D. litchicola[T]	BRIP 54900	*Litchi chinensis*	Australia	JX862533	-	-	JX862539	KF170925
D. longicicola[T]	CGMCC 3.17089	*Lithocarpus glabra*	China	KF576267	-	-	KF576242	KF576291
D. longicolla[T]	ATCC 60325	*Glycine max*	USA	KJ590728	KJ612124	KJ659188	KJ590767	KJ610883
D. lusitanicae[T]	CBS 123212	*Foeniculum vulgare*	Portugal	KC343136	KC343378	KC343620	KC343862	KC344104
D. maritima[T]	DAOMC 250563	*Picea rubens*	Canada	KU552025	-	-	KU552023	KU574615
D. multigutullata[T]	ICMP20656	*Citrus grandis*	China	KJ490633	-	KJ490575	KJ490512	KJ490454
D. neilliae[T]	CBS 144. 27	*Spiraea* sp.	USA	KC343144	KC343386	KC343628	KC343870	KC344112
D. neoarctii[T]	CBS 109490	*Ambrosia trifida*	USA	KC343145	KC343387	KC343629	KC343871	KC344113
D. nomurai	CBS 157.29	*Morus* sp.	Japan	KC343154	KC343396	KC343638	KC343880	KC344122
D. nothofagi[T]	BRIP 54801	*Nothofagus cunninghamii*	Australia	JX862530	-	-	JX862536	KF170922
D. novem[T]	CBS 127270	*Glycine max*	Croatia	KC343156	KC343398	KC343640	KC343882	KC344124

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TABLE 1. (Continued)

Species	Isolate	host	location	GenBank accession numbers				
				ITS	cal	his	tef1-α	β-tub2
D. oraccinii[T]	LC3166	*Camellia sinensis*	China	KP267863	-	KP293517	KP267937	KP293443
D. ovalispora[T]	ICMP20659	*Citrus limon*	China	KJ490628	-	KJ490570	KJ490507	KJ490449
D. ovoicicola[T]	CGMCC 3.17092	*Lithocarpus glabra*	China	KF576264	KF576222	-	KF576239	KF576288
D. oxe[T]	CBS 133186	*Maytenus ilicifolia*	Brazil	KC343164	KC343406	KC343648	KC343890	KC344132
D. padi var. *padi*	CBS 114649	*Alnus glutinosa*	Sweden	KC343170	KC343412	KC343654	KC343896	KC344138
D. paranensis[T]	CBS 133184	*Maytenus ilicifolia*	Brazil	KC343171	KC343413	KC343655	KC343897	KC344139
D. pascoei[T]	BRIP 54847	*Persea americana*	Australia	JX862532	-	-	JX862538	KF170924
D. penetriteum[T]	LC3353	*Camellia sinensis*	China	KP714505	-	KP714493	KP714517	KP714529
D. perjuncta[T]	CBS 109745	*Ulmus glabra*	Austria	KC343172	KC343414	KC343656	KC343898	KC344140
D. phaseolorum[T]	AR4203	*Phaseolus vulgaris*	USA	KJ590738	KJ612135	KJ659220	KJ590739	KJ610893
D. pseudophoenicicola[T]	CBS 462.69	*Phoenix dactylifera*	Spain	KC343184	KC343426	KC343668	KC343910	KC344152
D. pterocarpi[T]	MFLUCC 100571	*Pterocarous indicus*	Thailand	JQ619899	JX197451	-	JX275416	JX275460
D. pterocarpicola[T]	MFLUCC 100580	*Pterocarpus indicus*	Thailand	JQ619887	JX197433	-	JX275403	JX275441
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. rostrate	CFCC 50063	*Juglans mandshurica*	China	KP208848	KP208850	KP208852	KP208854	KP208856
D. rudis[T]	AR3422	*Laburnum anagyroides*	Austria	KC843331	KC843146	-	KC843090	KC843177
D. schini[T]	CBS 133181	*Schinus terebinthifolius*	Brazil	KC343191	KC343433	KC343675	KC343917	KC344159
D. scobina	CBS 251.38	*Fraxinus excelsior*	UK	KC343195	KC343437	KC343679	KC343921	KC344163
D. sojae[T]	FAU 635	*Glycine max*	USA	KJ590719	KJ612116	KJ659208	KJ590762	KJ610875
D. stewartii[T]	CBS 193.36	*Cosmos bipinnatus*	-	FJ889448	JX197415	-	GQ250324	JX275421
D. subclavata[T]	ICMP20663	*Citrus unshiu*	China	KJ490587	-	KJ490529	KJ490466	KJ490408
D. terebinthifolii[T]	CBS 133180	*Schinus terebinthifolius*	Brazil	KC343216	KC343458	KC343700	KC343942	KC344184
D. thunbergii[T]	MFLUCC 100576	*Thunbergia laurifolia*	Thailand	JQ619893	JX197440	-	JX275409	JX275449
D. thunbergiicola[T]	MFLUCC 120033	*Thunbergia laurifolia*	Thailand	KP715097	-	-	KP715098	-
D. toxica[T]	CBS 534.93	*Lupinus angustifolius*	Australia	KC343220	KC343462	KC343704	KC343946	KC344188
D. ueckerae[T]	FAU 656	*Cucumis melo*	USA	KJ590726	KJ612122	KJ659215	KJ590747	KJ610881
D. unshiuensis[T]	CGMCC3.17569	*Citrus unshiu*	China	KJ490587	-	KJ490529	KJ490466	KJ490408
D. vaccinii[T]	CBS 160.32	*Oxycoccus macrocarpos*	USA	KC343228	KC343470	KC343712	KC343954	KC344196
D. virgiliae[T]	CMW 40755	*Virgilia oroboides*	South Africa.	KP247573	-	-	-	KP247582
D. woolworthii	CBS 148.27	*Ulmus americana*	-	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.

Morphology

Morphological observations were based on features of the fruiting bodies produced on infected plant tissues and micromorphology, supplemented by cultural characteristics. Morphological characteristics of the fruiting bodies were recorded using a Leica stereomicroscope (M205 FA), including size and shape of conidiomata and pycnidia; shape and diameter of ostioles per ectostromatic disc and locules. Micromorphological observations include size and shape of conidiophores and conidia were 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 measurements. A 5 mm diameter PDA section was cut from the edge of actively growing 3-day old cultures, and each was transferred to fresh PDA plates. Two strains were selected for each species, and three disks were replicated for each strain. All cultures incubated on PDA in the dark at 25 °C were observed and recorded. This included colony colour, texture and arrangement of the conidiomata in culture, at 3, 7, and 30-days.

DNA extraction and PCR

Genomic DNA was extracted from 7 days old colonies grown on cellophane-covered PDA using a modified CTAB method (Doyle and Doyle 1990). PCR amplifications were performed in a DNA Engine Peltier Thermal Cycler (PTC-200; Bio-Rad Laboratories, Hercules, CA, USA). Five genes were selected to clarify the phylogenetic relationships of *Diaporthe* species following Gomes *et al.* (2013). The internal transcribed spacer (ITS) region was amplified using ITS1 and ITS4 primer sets (White *et al.* 1990). The partial calmodulin (CAL) region was amplified using CAL-228F and CAL-737R primer sets (Carbone and Kohn 1999). The partial histone H3 (HIS) region was amplified using CYLH4F and H3-1b primer sets (Glass and Donaldson 1995; Crous *et al.* 2004). The partial translation elongation factor 1-alpha (TEF1-α) region was amplified using EF1-728F and EF1-986R primer sets (Carbone and Kohn 1999) and beta-tubulin (TUB2) region was amplified using Bt2a and Bt2b primer sets (Glass and Donaldson 1995). The PCR amplification products were estimated visually by electrophoresis in 1.8 % agarose gel. DNA sequencing was performed using an ABI PRISM®3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) with BigDye® Terminator Kit v.3.1 (Invitrogen, Carlsbad, CA, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China). Sequences used in the current study were aligned using MAFFT v.6 (Katoh and Toh 2010) and edited manually using MEGA6 (Tamura *et al.* 2013).

Phylogenetic analysis

A maximum parsimony (MP) analysis run in PAUP v.4.0b10 (Swofford 2003) was generated to show phylogenetic relationships among *Diaporthe* species including ex-type sequences available in GenBank as selected from recent studies (Hyde et al 2014; Dissanayake *et al.* 2015; Liu *et al.* 2015); and are shown in Table 1. Bayesian inference (BI) and maximum likelihood (ML) were performed using MrBayes v.3.1.2 and PhyML v.7.2.8 (Ronquist and Huelsenbeck 2003; Guindon *et al.* 2010). All analyses were performed based on the combined multi-locus dataset (ITS, CAL, HIS, TEF1-α, TUB2) to compare *Diaporthe* species with other ex-type and reference specimens in recent studies (Udayanga *et al.* 2011, 2012a, 2014a, b, 2015; Gomes *et al.* 2013; Gao *et al.* 2014, 2015, 2016; Hyde *et al.* 2014; Fan *et al.* 2015; Tanney *et al*. 2016). *Diaporthella corylina* (CBS 121124) was selected as outgroup taxon in the current analysis (Gomes *et al.* 2013). Trees are shown using FigTree v.1.3.1 (Rambaut and Drummond 2010) and the layout was edited in Adobe Illustrator CS v.6.

MP analysis was inferred using a heuristic search algorithm (1000 random sequence additions) with a tree bisection and reconnection (TBR) branch swapping. Maxtrees were set to 5000, branches of zero length were collapsed and all equally parsimonious trees were saved. Other calculated parsimony scores (Tree Length [TL], Consistency Index [CI], Retention Index [RI] and Rescaled Consistency [RC]) were calculated. ML analysis was performed with a GTR + I + G substitution model selected by MrModeltest v.2.3 (Posada and Crandall 1998). The branch supports of MP and ML analyses were evaluated using a bootstrapping (BS) method of 1000 replicates (Hillis and Bull 1993). Bayesian inference (BI) analysis was performed using a Markov Chain Monte Carlo (MCMC) algorithm to construct the topology of the tree (Rannala and Yang 1996). A nucleotide substitution model was also calculated in MrModeltest v.2.3 (Posada and Crandall 1998). Sequences data isolated in the current study are deposited in GenBank (Table 1). The multilocus file is deposited in TreeBASE (www.treebase.org) as accession S19539. The taxonomic novelties are deposited in Fungal Names (http://www.fungalinfo.net/) and Facesoffungi numbers were obtained as described in in Jayasiri *et al.* (2015).

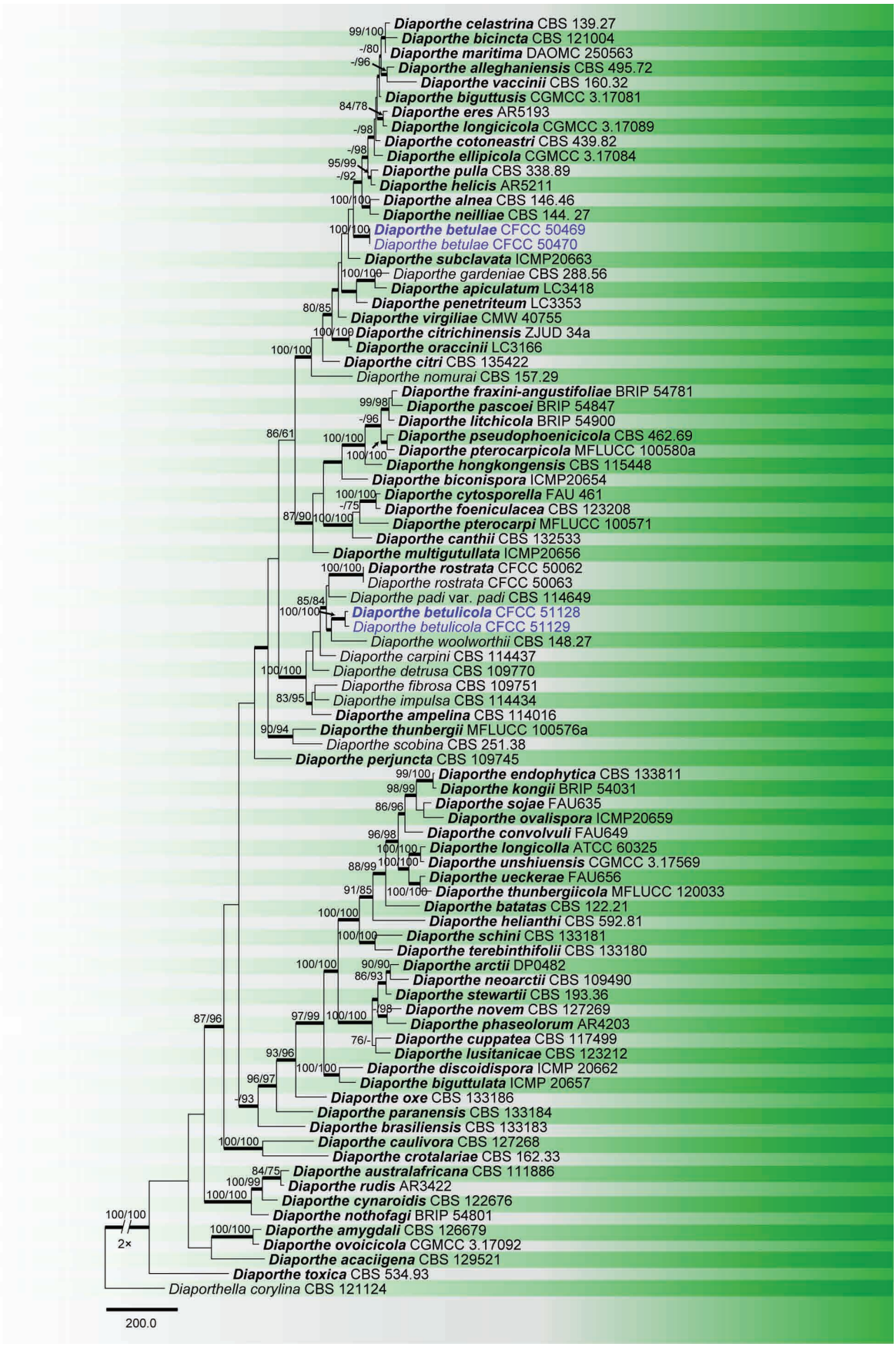

FIGURE 1. Phylogram of *Diaporthe* based on combined ITS, CAL, HIS, TEF1-α and TUB2. MP and ML bootstrap support values above 75 % are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Scale bar = 200 nucleotide substitutions. Ex-type strains are in bold. Strains in current study are in blue.

Result

The combined multi-locus dataset (ITS, CAL, HIS, TEF1-α and TUB2) contained 86 in-group isolates (sequences of four strains from this study and sequences of 82 strains available in GenBank). The alignment comprises 2999 characters including alignment gaps, of these 1460 characters are constant and 455 variable characters are parsimony uninformative. MP analysis of the remaining 1084 parsimony informative characters generated four equally parsimonious trees, and the first tree (TL = 7519, CI = 0.368, RI = 0.641, RC = 0.236) is shown in Fig. 1. Both ML analysis with a discrete gamma distribution with six rate categories (GTR+I+G) and BI analysis with a 0.01 average standard deviation of split frequencies resulted in the same topology as the presented MP phylogram. Isolates of *Diaporthe* clustered in 86 clades, corresponding to 83 known species with 74 ex-type isolates. Isolates in the current study clustered in two distinct clades with high support (MP/ML/BI = 100/100/1) in Fig. 1. They are recognized as two novel species, which are also supported by morphological traits.

Taxonomy

Diaporthe betulae C.M. Tian & X.L. Fan, sp. nov. **FIGURE 2**.
Fungal Names FN570261; Facesoffungi FoF02174

Holotype:—BJFC-S1317.

Etymology:—*betulae*, referring to *Betula platyphylla*, the host known for this species.

Host/Distribution:—from *Betula platyphylla* in China.

Original description:—Sexual morph: undetermined. Asexual morph: *Conidiomatal stromata* immersed, erumpent slightly from surface of host branches, separate, conical, with a single locule. *Ectostromatic disc* grey to black, with one ostiole per disc. *Ostiole* medium grey to black, up to the level of disc, (160–)170–220(–280) µm (av. = 250 µm, n = 20) diam. *Locule* undivided, conoid, (590–)600–1250(–1460) µm (av. = 1050 µm, n = 20) diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, phialides, straight or slightly curved, with periclinal thickening present. *Alpha conidia* hyaline, ellipsoidal, aseptate, smooth, conspicuously biguttulate, 8.5–11(–11.5) × 3–4(–4.5) µm (av. = 10 × 3.5 µm, n = 50). *Beta conidia* absent.

Cultures:—Colony originally compact and flat with white aerial mycelium, then developing dark green to brown aerial mycelium at the centre and dark green mycelium at the marginal area, zonate with 4–5 well defined zones 0.5 cm wide with an irregular edge; conidiomata sparse, irregularly distributed over agar surface.

Material examined:—CHINA, Sichuan Province: Guangyuan City, Tianzhao Mountain, 32°29’22.79”N, 105°43’32.78”E, 1422 m asl, on twigs and branches of *Betula platyphylla*, coll. X.L. Fan, 28 April 2015 (BJFC-S1317, **holotype**), ex-type culture, CFCC 50469. Sichuan Province: Guangyuan City, Tianzhao Mountain, 32°29’21.49”N, 105°43’32.60”E, 1422 m asl, on twigs and branches of *Betula platyphylla*, coll. X.L. Fan, 28 April 2015 (BJFC-S1318, **paratype**), living culture, CFCC 50470.

Notes:—This new species is introduced as molecular data show it to be distinct clade with high support (MP/ML/BI=100/100/1). Morphologically, it is characterized by ellipsoidal, aseptate, smooth alpha conidia, which are conspicuously biguttulate, which is similar with *Diaporthe vaccinii* from *Vaccinium macrocarpon* Aiton and *V. oxycoccos* L., with a geographic range in the USA (Shear *et al.* 1931). However, *Diaporthe betulae* can be distinguished by its larger alpha conidia (8.5–11 × 3–4 µm, av. 10 × 3.5 µm vs. 6–11 × 2–4 µm, av. 8 × 3 µm) and dark green to brown colonies on PDA, as compared to the white to yellowish colonies in *D. vaccinii* (Chao and Glawe 1985; Farr *et al.* 2002).

Diaporthe betulicola C.M. Tian & Z. Du, sp. nov. **FIGURE** 3
Fungal Names FN570262; Facesoffungi FoF02173

Holotype:—BJFC-S1333.

Etymology:—*betulicola*, referring to *Betula albosinensis*, the known host for this species.

Host/Distribution:—Pathogen on twigs and branches of *Betula albosinensis* in China.

Original description:—Sexual morph: undetermined. Asexual morph: *Conidiomatal* pycnidial, conical, immersed, scattered, with a single locule. *Ectostromatic disc* brown to black, one ostiole per disc. *Ostiole* medium black, up to the

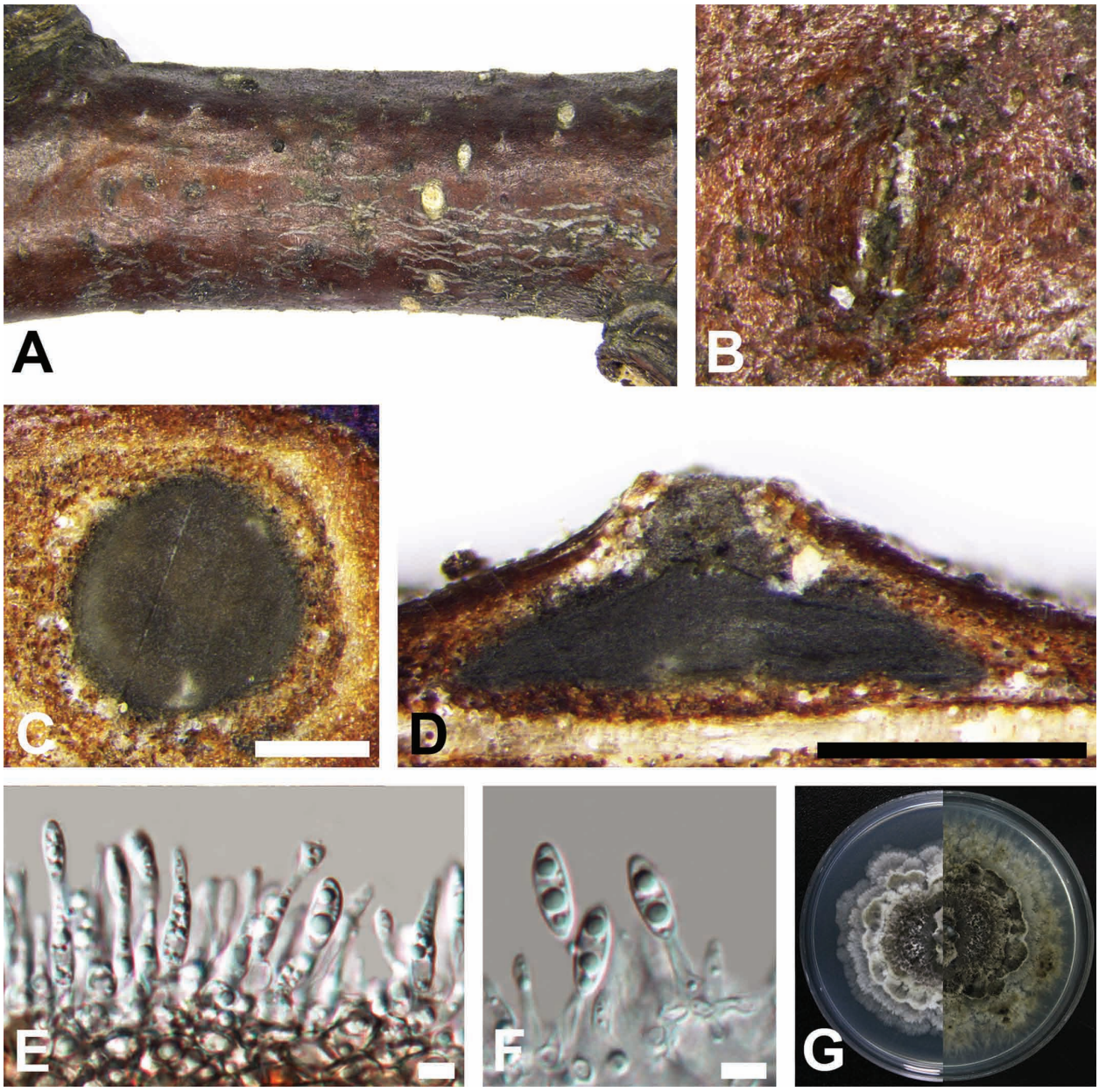

FIGURE 2. Morphology of *Diaporthe betulae* from *Betula platyphylla* (BJFC-S1317). A, B: Habit of conidiomata on twig. C: Transverse sections through conidiomata. D: Longitudinal sections through conidiomata. E: Conidiophores. F: Alpha conidia. G: Colonies on PDA at 3 days (left) and 30 days (right). Scale bars: B–D = 500 µm; E–F = 5 µm.

level of disc, (110–)130–220(–240) µm (av. = 180 µm, n = 20) diam. *Locule* undivided, (680–)700–1300(–1350) µm (av. = 960 µm, n = 20) diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, cylindrical, filiform, straight to curved. *Alpha conidia* hyaline, oblong, and acute at two sides, aseptate, smooth, not biguttulate, 10–14.5(–15) × 1.5–2.5 µm (av. = 12 × 2 µm, n = 50). *Beta conidia* hyaline, filiform, straight or curved, eguttulate, aseptate, apex acutely rounded, tapering from lower fourth towards base, 17–24 × 0.5–1(–1.5) µm (av. = 20 × 1 µm, n = 50).

Cultures:—Colony originally compact and flat with white felty aerial mycelium, then developing white to light brown aerial mycelium, zonate with 3–5 well defined zones 0.5–1 cm wide with a regular smooth edge; conidiomata distributed in circularity over agar surface.

Material examined:—CHINA, Shaanxi Province: Ankang City, Ningshan County, Huoditang, 33°26’24.15”N, 108°26’46.30”E, 1625 m asl, on twigs and branches of *Betula albosinensis*, coll. Qin Yang, 3 April 2015 (BJFC-S1333, **holotype**), ex-type culture, CFCC 51128. Shaanxi Province: Baoji City, Feng County, Tangzang Town, Tongtian River Forest Park, 34°16’26.21”N, 106°31’39.58”E, 2127 m asl, on twigs and branches of *Betula albosinensis*, coll. Qin Yang, 31 July 2015 (BJFC-S1334, **paratype**) living culture, CFCC 51129.

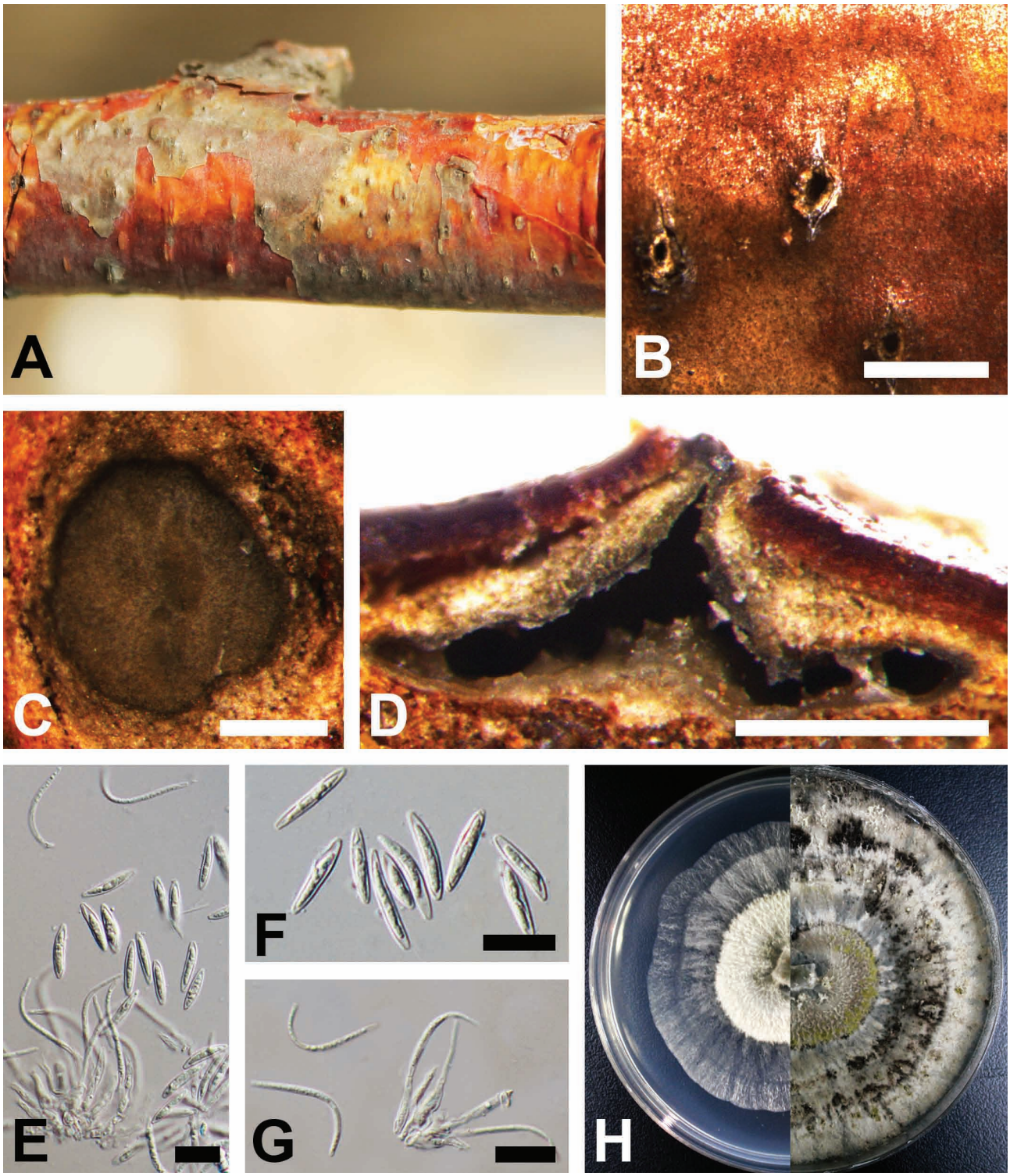

FIGURE 3. Morphology of *Diaporthe betulicola* from *Betula albosinensis* (BJFC-S1333). A, B: Habit of conidiomata on twig. C: Transverse sections through conidiomata. D: Longitudinal sections through conidiomata. E: Conidiophores and conidia. F: Alpha conidia. G: Beta conidia. H: Colonies on PDA at 3 days (left) and 30 days (right). Scale bars: B–D = 500 μm; E–G = 10 μm.

Notes:—This new species is distinguished from other *Diaporthe* species by its distinctive hyaline, oblong alpha conidia which are acute at both ends and lack guttules. It also has larger (10–14.5 × 1.5–2.5 μm) conidia, as compared to many other *Diaporthe* species. The most closely related species in the phylogram are *D. woolworthii* (Peck) Sacc. from *Ulmus americana* and *Diaporthe rostrata* C.M. Tian, X.L. Fan & K.D. Hyde from *Juglans mandshurica*. *Diaporthe woolworthii* was introduced from *Quercus* from America (Saccardo 1882), but there are no illustrations, detailed descriptions or loanable specimens, and thus the species needs to be epitypified or provided with a reference specimen from the same country and host (*sensu* Ariyawansa *et al.* 2014). Gomes *et al.* (2013) provided DNA data for

this species using a putatively named strain, CBS 148.27, from *Ulmus* (a different host) in America. Two strains of *D. betulina* clustered in distinct clade in combined sequence analysis with high support (MP/ML/BI = 100/100/1, Fig. 1), and differs from the strain of *D. woolworthii* (Gomes *et al.* 2013). The new taxon also can be distinguished from *D. rostrata*, which has central perithecial necks, with a black conceptacle and shorter ellipsoidal alpha conidia (8.5–11.5 × 4–5 µm) (Fan *et al.* 2015).

Discussion

The current study identified two novel species (*Diaporthe betulae* and *D. betulicola*) from *Betula* species in China. The species are introduced based on evidence from morphology and combined ITS, CAL, HIS, TEF1-α and TUB2 phylogenetic analyses. Although several plant pathogenic *Diaporthe* species have been described from China (Huang *et al.* 2013; Gao *et al.* 2014, 2015, 2016), *Diaporthe* species associated with *Betula* spp., which have significant economic and ecological value, have been poorly studied. *Diaporthe betulae* and *D. betulicola* were only found on infected branches or twigs and appeared to be the cause of birch dieback with typical dieback symptoms (Fig. 2A–B, Fig. 3A–B).

The two novel species cluster in separate clades compared to known species with high support values (MP/ML/BI = 100/100/1). Three *Diaporthe* species have been reported from *Betula*, i.e., *Diaporthe alleghaniensis*, *D. eres* and *D. melanocarpa* (Kobayashi 1970; Gomes *et al.* 2013). *Diaporthe betulae* (8.5–11 × 3–4 µm) and *D. betulicola* (10–14.5 × 1.5–2.5 µm) can be distinguished from *D. alleghaniensis* (5–8 × 1.5–2 µm) and *D. eres* (6.5–8.5 × 3–4 µm) in having larger alpha conidia, and support from analysis of sequence data (Fig. 1) (Arnold 1967; Anagnostakis 2007; Gomes *et al.* 2013). *Diaporthe melanocarpa* was described from *Pyrus melanocarpa* in London, and then recorded from *Amelanchier*, *Betula* and *Cornus*, but there is no available DNA data for this species (Dearness 1926; Wehmeyer 1933; Kobayashi 1970).

Species identification criteria in Diaporthe were previously based on host association and proliferation of names resulted from species being described from each host from which they were isolated (Saccardo 1882; Deng 1963; Tai 1979; Wei 1979; Uecker 1988; Mostert *et al.* 2001; Udayanga *et al.* 2012). Recent studies have shown that many species colonize a diverse range of hosts, as opportunists, and that several species could even co-occur on the same host or lesion (Udayanga *et al.* 2014a, 2014b, 2015; Fan *et al.* 2015; Gao *et al.* 2016). It is now recognized that host-specificity generally has limited value; therefore phylogenetic relationships are needed to accurately distinguish *Diaporthe* species (Udayanga *et al.* 2011, 2012, 2014a, 2015; Gomes *et al.* 2013; Gao *et al.* 2014, 2015, 2016; Fan *et al.* 2015). Udayanga *et al*. (2012) established a starting point for resolving Diaporthe species with taxonomic evidence and living cultures, rather than using the older taxon names, which lacked detailed descriptions and molecular data, unless they had been epitypified. Udayanga *et al.* (2014a, 2015) further clarified the Diaporthe group by resolving some species complexes such as D. eres and D. sojae. However, the taxonomy of Diaporthe species still requires extensive sampling from a wide distribution and host range, as numerous undescribed species associated with important hosts can be undiscovered worldwide.

Acknowledgements

This study is financed by National Natural Science Foundation of China (Project No.: 31170603) and the China Scholarship Council (CSC). We are grateful to China Forestry Culture Collection Center (CFCC) for support of strain preservation during this study.

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