Phytotaxa 338 (1): 049–062
http://www.mapress.com/j/pt/
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Article

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

https://doi.org/10.11646/phytotaxa.338.1.4

Multigene phylogeny and morphology reveal *Cytospora spiraeae* sp. nov. (Diaporthales, Ascomycota) in China

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Abstract

Members of *Cytospora* encompass important plant-associated pathogens, endophytes and saprobes, commonly isolated from a wide range of hosts with a worldwide distribution. Two specimens were collected associated with symptomatic canker and dieback disease of *Spiraea salicifolia* in Gansu, China. These isolates are characterized by its hyaline, biseriate, aseptate, elongate-allantoid ascospores and allantoid conidia. *Cytospora spiraeae* sp. nov. is introduced based on its holomorphic morphology plus support from phylogenetic analysis (ITS, LSU, ACT and RPB2), and differs from similar species in its host association.

Key words: Cytosporaceae, plant pathogen, systematics, taxonomy, *Valsa*

Introduction

The genus *Cytospora* (Ascomycota: Diaporthales) was established by Ehrenberg (1818). It is commonly famous as the important phytopathogens that cause dieback and canker disease on a wide range of plants, causing severe commercial and ecological damage and significant losses worldwide (Adams *et al.* 2005, 2006). Previous *Cytospora* species and related sexual genera *Leucostoma*, *Valsa*, *Valsella*, and *Valseutypella* were listed by old fungal literatures without any living culture and sufficient evidence to identify (Fries 1823; Saccardo 1884; Kobayashi 1970; Barr 1978; Gvritishvili 1982; Spielman 1983, 1985). Adams *et al.* (2005) revised genus *Cytospora* from *Eucalyptus* with 28 species and accepted all sexual genera were combined under *Valsa*, either as subgenera or species without additional infrageneric rank. Following the single-name for pleomorphic taxa, *Cytospora* (1818) was proposed to be the recommended name to against *Valsa* (1849) according to the older name and more common in nature (Rossman *et al.* 2015). More than 600 species epithets named *Cytospora* have been recorded in Index Fungorum (2017) with an estimated 110 species in Kirk *et al.* (2008). Recent studies have subsequently emphasized on part of *Cytospora* species using multiphase approaches to solve the confused frame (Fan *et al.* 2014a, 2014b, 2015a, 2015b; Yang *et al.* 2015; Lawrence *et al.* 2017; Norphanphoun *et al.* 2017). However, only ITS gene are available for most known *Cytospora* species, and ex-type sequence data are available for only a very few species and many taxa need epitypifying (Adams *et al.* 2005). Thus many of *Cytospora* species still need re-clarification and the identification to species level is difficult.

To facilitate species recognition of *Cytospora*, an investigation of forest pathogens that cause canker or dieback disease in China was performed. Three *Cytospora* specimens were collected from *Spiraea salicifolia* in Gansu Province, China. This species is characterized by hyaline, biseriate, aseptate, elongate-allantoid ascospores and allantoid conidia. Phylogenetic analysis inferred from combined ITS, LSU, ACT and RPB2 sequence data provided strong support that this is a new species. We introduce *Cytospora spiraeae* sp. nov. in this paper with a description and illustrations and compare it with other species in the genus.

Materials and methods

Isolates

Three isolates were isolated from infected branches or twigs of *Spiraea salicifolia* during collecting trips in Gansu Provinces in China (Table 1). The suspension of conidia was established by removing a mucoid spore mass from

Accepted by Sajeewa Maharachchikumbura: 31 Dec. 2017; published: 9 Feb. 2018

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conidiomata or ascomata, and spreading the suspension on the surface of 1.8 % potato dextrose agar (PDA) in a petri-dish, and incubating at 25 °C for up to 24 h. Single germinating conidia were transferred onto fresh PDA plates. Living cultures are deposited and now maintained at the China Forestry Culture Collection Center (CFCC) and specimens are deposited in the Museum of the Beijing Forestry University (BJFC).

Morphology

Specimens were described based on the morphological characteristics of their fruiting bodies from infected host materials. The macro-morphological photographs were captured using a Leica stereomicroscope (M205 FA), including size and arrangement of stromata; presence or absence of special structure such as conceptacle and central column; number and diameter of ostioles per ectostromatic disc; shape and size of discs; number of locules. Micro-morphological observations include size and shape of conidiophores and conidia (asci and ascospores) determined under a Leica compound microscope (DM 2500) with differential interference contrast (DIC). Over 20 conidiomata were sectioned and 50 conidia were selected randomly to measure their lengths and widths. Colony diameters were measured and the colony colours described after 3 days and 30 days according to the colour charts of Rayner (1970). Adobe Bridge CS v. 6 and Adobe Photoshop CS v. 5 were used for the manual editing. Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al.* 2004).

DNA isolation, amplification and sequencing

Fungal mycelium grown on PDA was scraped from the agar and used for genomic DNA extraction using a CTAB method (Doyle & Doyle 1990). The ITS region was amplified using the primers ITS1 and ITS4 (White *et al.* 1990). The LSU region was amplified using the primers LR0R and LR7 (Vilgalys & Hester 1990). The partial ACT region was amplified using primers ACT512F and ACT783R (Carbone & Kohn 1999). The RPB2 region was amplified using primers RPB2-5F and fRPB2-7cR (Liu *et al.* 1999). The PCR products were sequenced in two directions using the PCR primers and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and performed with an ABI Prism 3730XL Sequencer (Applied Biosystems) according to the instructions of manufacturer. The multiple sequences generated by each primer combination were used to obtain consensus sequences using Seqman v. 7.1.0 in the DNASTAR lasergene core suite software (DNASTAR Inc., Madison, WI, USA).

Phylogenetic analyses

Sequences were aligned using MAFFT v. 6 (Katoh & Standley 2013) and edited manually using MEGA v. 6.0 (Tamura *et al.* 2013). Phylogenetic relationships were inferred by maximum parsimony (MP), and confirmed using maximum likelihood (ML) and Bayesian inference (BI). A MP analysis was performed using PAUP v. 4.0b10 with a heuristic search option of 1000 random-addition sequences with a tree bisection and reconnection (TBR) branch swapping algorithm (Swofford *et al.* 2003). The branches of zero length were collapsed and all equally parsimonious trees were saved. Other parsimony scores such as tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC) were calculated (Swofford *et al*. 2003). A ML tree analysis was performed with GTR+G+I model of site substitution including estimation of Gamma-distributed rate heterogeneity and a proportion of invariant sites using RAxMLv.7.2.8 (Stamatakis 2006).

MrModeltest v. 2.3 was performed to estimate the best nucleotide substitution model settings for each gene (Posada & Crandall 1998). Bayesian inference (BI) employing a Markov Chain Monte Carlo (MCMC) algorithm was performed using in MrBayes v. 3.1.2 based on the individual DNA dataset from the results of the MrModeltest (Ronquist & Huelsenbeck 2003). Two MCMC chains were run from random trees for 1000000 generations and trees were sampled each 100th generations. The first 25 % of trees were discarded as the burn-in phase of each analysis, and the posterior probabilities (BPP) were calculated to assess the remaining trees (Rannala & Yang 1996). The branch support from MP and ML analysis were evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993). *Diaporthe vaccinii* was selected as outgroup in all analyses. Phylograms are shown using Figtree v. 1.3.1 (Rambaut & Drummond 2010). Novel sequences data was deposited in GenBank (Table 1) and the multilocus sequences alignment file and ITS sequence-alignment file were maintained in TreeBASE (www.treebase.org; accession number: S22039).

Table 1. Strains of *Cytospora* used in the molecular analyses in this study.

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	LSU	ACT	RPB2
Cytospora abyssinica	CMW 10181[T]	*Eucalyptus globulus*	Wondo Genet, Ethiopia	AY347353	-	-	-
Cytospora abyssinica	CMW 10178	*Eucalyptus globulus*	Wondo Genet, Ethiopia	AY347354	-	-	-
Cytospora abyssinica	CMW 10179	*Eucalyptus globulus*	Wondo Genet, Ethiopia	AY347352	-	-	-
Cytospora acaciae	CBS 468.69	*Ceratonia siliqua fruit*	Spain, Mallorca	DQ243804	-	-	-
Cytospora ambiens	CFCC 89622	*Pyrus bretschneideri*	Lanzhou, Gansu	KR045616	KR045698	KU710988	KU710944
Cytospora ambiens	CFCC 89894	*Pyrus bretschneideri*	Yichuan, Ningxia	KR045617	KR045699	KU710989	KU710945
Cytospora ampulliformis	MFLUCC 16-0583[T]	*Sorbus intermedia*	Russia	KY417726	KY417760	KY417692	KY417794
Cytospora ampulliformis	MFLUCC 16-0629	*Acer platanoides*	Russia	KY417727	KY417761	KY417693	KY417795
Cytospora atrocirrhata	CFCC 89615	*juglans regia*	Xining, Qinghai	KR045618	KR045700	KF498673	KU710946
Cytospora atrocirrhata	CFCC 89616	*juglans regia*	Xining, Qinghai	KR045619	KR045701	KF498674	KU710947
Cytospora atrocirrhata	22	*Salix excelsa*	Iran	EF447305	-	-	-
Cytospora austromontana	CMW 6735[T]	*Eucalyptus pauciflora*	Australia	AY347361	-	-	-
Cytospora berberidis	CFCC 89927[T]	*Berberis dasystachya*	China	KR045620	KR045702	KU710990	KU710948
Cytospora berberidis	CFCC 89933	*Berberis dasystachya*	China	KR045621	KR045703	KU710991	KU710949
Cytospora berkeleyi	StanfordT3[T]	*Eucalyptus globulus*	California, USA	AY347350	-	-	-
Cytospora berkeleyi	UCBTwig3	*Eucalyptus globulus*	California, USA	AY347349	-	-	-
Cytospora brevispora	CBS 116829	*Eucalyptus grandis*	Venezuela	AF192321	-	-	-
Cytospora brevispora	CBS 116811[T]	*Eucalyptus grandis*×*tereticornis*	Democratic Republic of the Congo	AF192315	-	-	-
Cytospora cabonacea	CFCC 50055	*Ulmus pumila*	Shaanxi, China	KP281262	KP310808	KP310838	-
Cytospora cabonacea	CFCC 50058	*Ulmus pumila*	Heilongjiang, China	KP281264	KP310810	KP310840	-
Cytospora carbonacea	CFCC 89947	*Ulmus pumila*	Qinghai, China	KR045622	KP310812	KP310842	KU710950
Cytospora cedri	CBS 196.50	-	Italy	AF192311	-	-	-
Cytospora chrysosperma	CFCC 89982	*Ulmus pumila*	Tibet, China	KP281261	KP310805	KP310835	-
Cytospora chrysosperma	CFCC 89630	*Salix psammophila*	Shaanxi, China	KF765674	KF765690	KF765722	-
Cytospora chrysosperma	CFCC 89600	*Sophora japonica*	Gansu, China	KR045623	KP310804	KU710992	KU710951
Cytospora cincta	CFCC 89956	*Prunus cerasifera*	China	KR045624	KR045704	KU710993	KU710953
Cytospora cincta	CFCC 89960	*Prunus cerasifera*	Ningxia, China	KR045625	KR045705	KU710994	KU710954
Cytospora cinerostroma	CMW 5700[T]	*Eucalyptus globulus*	Chile	AY347377	-	-	-
Cytospora cotini	MFLUCC 14-1050[T]	*Cotinus coggygria*	Russia	KX430142	KX430143	-	KX430144
Cytospora cotini	MFLUCC 15-0863[T]	*Ulmus minor*	Russia	KY417759	-	-	-
Cytospora curvata	MFLUCC 15-0865[T]	*Salix alba*	Russia	KY417728	KY417762	KY417694	KY417796
Cytospora davidiana	CXY1350[T]	*Populus davidiana*	China	KM034870	-	-	-
Cytospora davidiana	CXY1374	*Populus davidiana*	China	KM034869	-	-	-
Cytospora diatrypelloidea	CMW 8549[T]	*Eucalyptus globulus*	Orbost, Australia	AY347368	-	-	-
Cytospora disciformis	CMW 6509[T]	*Eucalyptus grandis*	Uruguay	AY347374	-	-	-

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Table 1. (Continued)

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	LSU	ACT	RPB2
Cytospora disciformis	CMW 6750	*Eucalyptus globulus*	Australia	AY347359	-	-	-
Cytospora donetzica	MFLUCC 16-0574[T]	*Rosa* sp.	Russia	KY417731	KY417764	KY417696	KY417798
Cytospora donetzica	MFLUCC 16-0589	*Salix alba*	Russia	KY417732	KY417766	KY417698	KY417800
Cytospora elaeagni	CFCC 89632	*Elaeagnus angustifolia*	Ningxia, China	KR045626	KR045706	KU710995	KU710955
Cytospora elaeagni	CFCC 89633	*Elaeagnus angustifolia*	Ningxia, China	KF765677	KF765693	KU710996	KU710956
Cytospora eriobotryae	IMI136523[T]	*Eriobotrya japonica*	India	AY347327	-	-	-
Cytospora erumpens	MFLUCC 16-0580[T]	*Salix* × *fragilis*	Russia	KY417733	KY417767	KY417699	KY417801
Cytospora eucalypti	LSEQ	*Sequoia sempervirens*	California, USA	AY347340	-	-	-
Cytospora eucalypticola	ATCC 96150[T]	*Eucalyptus nitens*	Tasmania, Australia	AY347358	-	-	-
Cytospora eucalypticola	CMW 5309	*Eucalyptus grandis*	Entebbe, Uganda	AF260266	-	-	-
Cytospora eucalyptina	CMW 5882	*Eucalyptus grandis*	Cali, Columbia	AY347375	-	-	-
Cytospora eugeniae	CMW 7029	*Tibouchina* sp.	Brisbane, Australia	AY347364	-	-	-
Cytospora eugeniae	CMW 8648	*Eugenia sp.*	Indonesia	AY347344	-	-	-
Cytospora fugax	CBS 203.42	*Salix* sp.	Switzerland	AY347323	-	-	-
Cytospora germanica	CXY1322	*Elaeagnus oxycarpa*	China	JQ086563	JX524617	-	-
Cytospora gigaspora	CFCC 89620[T]	*Juglans regia*	Xining, Qinghai	KR045628	KR045708	KU710997	KU710957
Cytospora gigaspora	CFCC 89621	*Juglans regia*	Xining, Qinghai	KR045629	KR045709	KU710998	KU710958
Cytospora gigaspora	CFCC 50014	*Juniperus procumbens*	Shanxi, China:	KR045630	KR045710	KU710999.	KU710959
Cytospora gigaspora	CFCC 89634[T]	*Salix psammophila*	China	KF765671	KF765687	KU711000	KU710960
Cytospora hippophaës	CFCC 89639	*Hippophae rhamnoides*	Gansu, China	KR045632	KR045712	KU711001	KU710961
Cytospora hippophaës	CFCC 89640	*Hippophae rhamnoides*	Gansu, China	KF765682	KF765698	KF765730	KU710962
Cytospora japonica	CBS375.29	*Prunus persicae*	Japan	AF191185	-	-	-
Cytospora kantschavelii	287-2	*Populus deltoides*	Iran	EF447367	-	-	-
Cytospora kantschavelii	CXY1383	*Populus maximowiczii*	China	KM034867	-	-	-
Cytospora kunzei	CBS 118556	*Pinus radiata*	Eastern Cape, SA	DQ243791	-	-	-
Cytospora leucostoma	CFCC 50015	*Sorbus pohuashanensis*	China	KR045634	KR045714	KU711002	-
Cytospora leucostoma	CFCC 50023	*Cornus alba*	China	KR045635	KR045715	KU711003	-
Cytospora longiostiolata	MFLUCC 16-0628[T]	*Salix* × *fragilis*	Russia	KY417734	KY417768	KY417700	KY417802
Cytospora mali	CFCC 50031	*Crataegus* sp.	Taiyuan, Shanxi	KR045636	KR045716	KU711004	KU710965
Cytospora mali	CFCC 50044	*Malus baccata*	Haidong, Qinghai	KR045637	KR045717	KU711005	KU710966
Cytospora melnikii	MFLUCC 15-0851[T]	*Malus domestica*	Russia	KY417735	KY417769	KY417701	KY417803
Cytospora melnikii	MFLUCC 16-0635	*Populus nigra* var. *italica*	Russia	KY417736	KY417770	KY417702	KY417804
Cytospora mougeotii	ATCC 44994	*Picea abies*	Norway	AY347318	-	-	-
Cytospora multicollis	CBS 105.89[T]	*Quercus ilex subsp. rotundifolia*	Spain	DQ243803	-	-	-
Cytospora myrtagena	CBS 116843[T]	*Tibouchiina urvilleana*	Hilo, Hawaii	AY347363	-	-	-
Cytospora nitschkii	CMW10180[T]	*Eucalyptus globulus*	Wondo Genet, Ethiopia	AY347356	-	-	-
Cytospora nitschkii	CMW10184	*Eucalyptus globulus*	Wondo Genet, Ethiopia	AY347355	-	-	-

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Table 1. (Continued)

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	LSU	ACT	RPB2
Cytospora nivea	MFLUCC 15-0860	*Salix acutifolia Willd.*	Russia	KY417737	KY417771	KY417703	KY417805
Cytospora nivea	CMW 5274	*Populus canescens*	South Africa: Belfast, Mpumalanga	DQ243794	-	-	-
Cytospora nivea	CFCC 89641	*Elaeagnus angustifolia*	China	KF765683	KF765699	KU711006	KU710967
Cytospora nivea	CFCC 89643	*Salix psammophila*	China	KF765685	-	-	KU710968
Cytospora palm	CXY1276	*Cotinus coggygria*	Beijing, China	JN402990	-	-	-
Cytospora palm	CXY1280[T]	*Cotinus coggygria*	Beijing, China	JN411939	-	-	-
Cytospora parakantschavelii	MFLUCC 15-0857[T]	*Populus* × *sibirica*	Russia	KY417738	KY417772	KY417704	KY417806
Cytospora parakantschavelii	MFLUCC 16-0575	*Pyrus pyraster*	Russia	KY417739	KY417773	KY417705	KY417807
Cytospora parapersoonii	T28.1[T]	*Prunus persicae*	Michigan, USA	AF191181	-	-	-
Cytospora parasitica	MFLUCC 15-0507[T]	*Malus domestica*	Russia	KY417740	KY417774	KY417706	KY417808
Cytospora paratranslucens	MFLUCC 15-0506[T]	*Populus alba var. bolleana (Lauche) Otto*		KY417741	KY417775	KY417707	KY417809
Cytospora paratranslucens	MFLUCC 16-0627	*Populus alba*	Russia	KY417742	KY417776	KY417708	KY417810
Cytospora pini	CBS 197.42	*Pinus Sylvestirs*	Switzerland	AY347332	-	-	-
Cytospora pini	CBS224.52[T]	*Pinus strobus*	New York	AY347316	-	-	-
Cytospora populina	CFCC 89644	*Salix psammophila*	Shaanxi, China	KF765686	KF765702	KU711007	KU710969
Cytospora pruinopsis	CFCC 50034[T]	*Ulmus pumila*	Shaanxi, China	KP281259	KP310806	KP310836	KU710970
Cytospora pruinopsis	CFCC 50035	*Ulmus pumila*	Heilongjiang, China	KP281260	KP310807	KP310837	KU710971
Cytospora pruinosa	CFCC 50036	*Syzygium aromaticum*	Qinghai, China	KP310800	KP310802	KP310832	-
Cytospora pruinosa	CBS 201.42[T]	*Syringa* sp.	Switzerland	DQ243801	-	-	-
Cytospora rhizophorae	MUCC302	*Eucalyptus grandis*	Australia	EU301057	-	-	-
Cytospora ribis	CFCC 50026	*Ulmus pumila*	Qinghai, China	KP281267	KP310813	KP310843	KU710972
Cytospora ribis	CFCC 50027	*Ulmus pumila*	Qinghai, China	KP281268	KP310814	KP310844	-
Cytospora ribis	CFCC 50038	*Platycladus orientalis*	Gansu, China	KT222840	-	-	-
Cytospora ribis	CFCC 50039	*Platycladus orientalis*	Gansu, China	KR045642	KR045721	KU711008	KU710973
Cytospora ribis	CBS 187.36	*Ribes rubrum*	Netherlands	DQ243810	-	-	-
Cytospora rostrata	CFCC 89909[T]	*Salix cupularis*	Gansu, China	KR045643	KR045722	KU711009	KU710974
Cytospora rostrata	CFCC 89910	*Salix cupularis*	Gansu, China	KR045644	KR045723	KU711010	KU710975
Cytospora rusanovii	MFLUCC 15-0853	*Populus* × *sibirica*	Russia	KY417743	KY417777	KY417709	KY417811
Cytospora rusanovii	MFLUCC 15-0854[T]	*Salix babylonica*	Russia	KY417744	KY417778	KY417710	KY417812
Cytospora sacculus	CBS 116.21	*Fagus sylvatica*	Netherlands	AY347335	-	-	-
Cytospora sacculus	CFCC 89624	*Juglans regia*	China	KR045645	KR045724	-	KU710976
Cytospora sacculus	CFCC 89626	*Juglans regia*	Shaanxi Province, China	KR045647	KR045726	KU711011	KU710978
Cytospora sacculus	CBS 192.42	*Taxus bacata*	Switzerland	AY347333	-	-	-
Cytospora salicacearum	MFLUCC 15-0861	*Salix* × *fragilis*	Russia	KY417745	KY417779	KY417711	KY417813
Cytospora salicacearum	MFLUCC 15-0509[T]	*Salix alba*	Russia	KY417746	KY417780	KY417712	KY417814
Cytospora salicicola	MFLUCC 15-0866	*Salix alba*	Russia	KY417749	KY417783	KY417715	KY417817

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Table 1. (Continued)

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	LSU	ACT	RPB2
Cytospora salicicola	MFLUCC 14-1052	-	-	KU982636	KU982635	KU982637	-
Cytospora salicina	MFLUCC 15-0862[T]	*Salix alba*	Russia	KY417750	KY417784	KY417716	KY417818
Cytospora salicina	MFLUCC 16-0637	*Salix × fragilis*	Russia	KY417751	KY417785	KY417717	KY417819
Cytospora schulzeri	CFCC 50040	*Malus domestica*	Ningxia, China	KR045649	KR045728	KU711013	KU710980
Cytospora schulzeri	CFCC 50042	*Malus asiatica*	Qinghai, China	KR045650	KR045729	KU711014	KU710981
Cytospora sibiraeae	CFCC 50045[T]	*Sibiraea angustata*	Gansu, China	KR045651	KR045730	KU711015	KU710982
Cytospora sibiraeae	CFCC 50046	*Sibiraea angustata*	Gansu, China	KR045652	KR045731	KU711015	KU710983
Cytospora sophorae	CFCC 50047	*Styphnolobium japonicum*	Shanxi, China	KR045653	KR045732	KU711017	KU710984
Cytospora sophorae	CFCC 89598	*Styphnolobium japonicum*	Gansu, China	KR045654	KR045733	KU711018	KU710985
Cytospora sophorae	CFCC 89597 = HMBF CGHs7	*Styphnolobium japonicum var. pendula*	Gansu, China	KC880151	-	-	-
Cytospora sophoricola	CFCC 89596	*Styphnolobium japonicum var. pendula*	Gansu, China	KR045656	KR045735	KU711020	KU710987
Cytospora sophoricola	CFCC 89595[T]	*Styphnolobium japonicum var. pendula*	Gansu, China	KR045655	KR045734	KU711019	KU710986
Cytospora sorbi	MFLUCC 16-0631[T]	*Sorbus aucuparia*	Russia	KY417752	KY417786	KY417718	KY417820
Cytospora sorbicola	MFLUCC 16-0584[T]	*Acer pseudoplatanus*	Russia	KY417755	KY417789	KY417721	KY417823
Cytospora sorbicola	MFLUCC 16-0633	*Cotoneaster melanocarpus*	Russia	KY417758	KY417792	KY417724	KY417826
Cytospora spiraeae	**CFCC 50049[T]**	***Spiraea salicifolia***	**Gansu, China**	MG707859	MG707643	MG708196	MG708199
Cytospora spiraeae	**CFCC 50050**	***Spiraea salicifolia***	**Gansu, China**	MG707860	MG707644	MG708197	MG708200
Cytospora spiraeae	**CFCC 50051**	***Spiraea salicifolia***	**Gansu, China**	MG707861	MG707645	MG708198	MG708201
Cytospora tanaitica	MFLUCC141057[T]	*Betula pubescens*	Russia	KT459411	KT459412	KT459413	-
Cytospora tibouchinae	CPC 26333[T]	*Tibouchina semidecandra*	La Reunion, France	KX228284	KX228335	-	-
Cytospora valsoidea	CMW 4309[T]	*Eucalyptus grandis*	Sibisa, North Sumatra	AF192312	-	-	-
Cytospora valsoidea	CMW 4310	*Eucalyptus grandis*	Sibisa, North Sumatra	AF192312	-	-	-
Cytospora variostromatica	CMW 6766[T]	*Eucalyptus globulus*	Australia	AY347366	-	-	-
Cytospora variostromatica	CMW 1240	*Eucalyptus grandis*	KwaMbonambi, SA	AF260263	-	-	-
Cytospora variostromatica	PPRI5297	*Eucalyptus grandis*	Pretoria, SA	AF260264	-	-	-
Cytospora vinacea	CBS 141585[T]	*Vitis interspecific*	New Hampshire, USA	KX256256	-	-	-
Cytospora viticola	CBS 141586[T]	*Vitis vinifera*	Connecticut, USA	KX256239	-	-	-
Diaporthe vaccinii	CBS 160.32	*Vaccinium macrocarpon*	Massachusetts, USA	KC343228	-	JQ807297	-
Valsa friesii	CBS 194.42	*Abies alba*	Switzerland	AY347328	-	-	-

The new strains from the current study are in bold. Ex-type taxa are marked with a T.

Results

The phylogenetic analysis of ITS sequence data contained 134 *Cytospora* ingroup strains with a total of 597 characters including gaps, of which 359 characters are constant, 63 variable characters are parsimony-uninformative and 175 characters are variable and parsimony-informative. MP analyses generated 200 parsimonious trees, one of which is presented in Fig. 1 (TL = 1009, CI = 0.378, RI = 0.815, RC = 0.308). ML and Bayesian analyses were similar to the MP tree. *Cytospora spiraeae* representde a monophyletic clade with high support value (MP/ML/BI = 99/95/0.99) (marked in blue in Fig. 1).

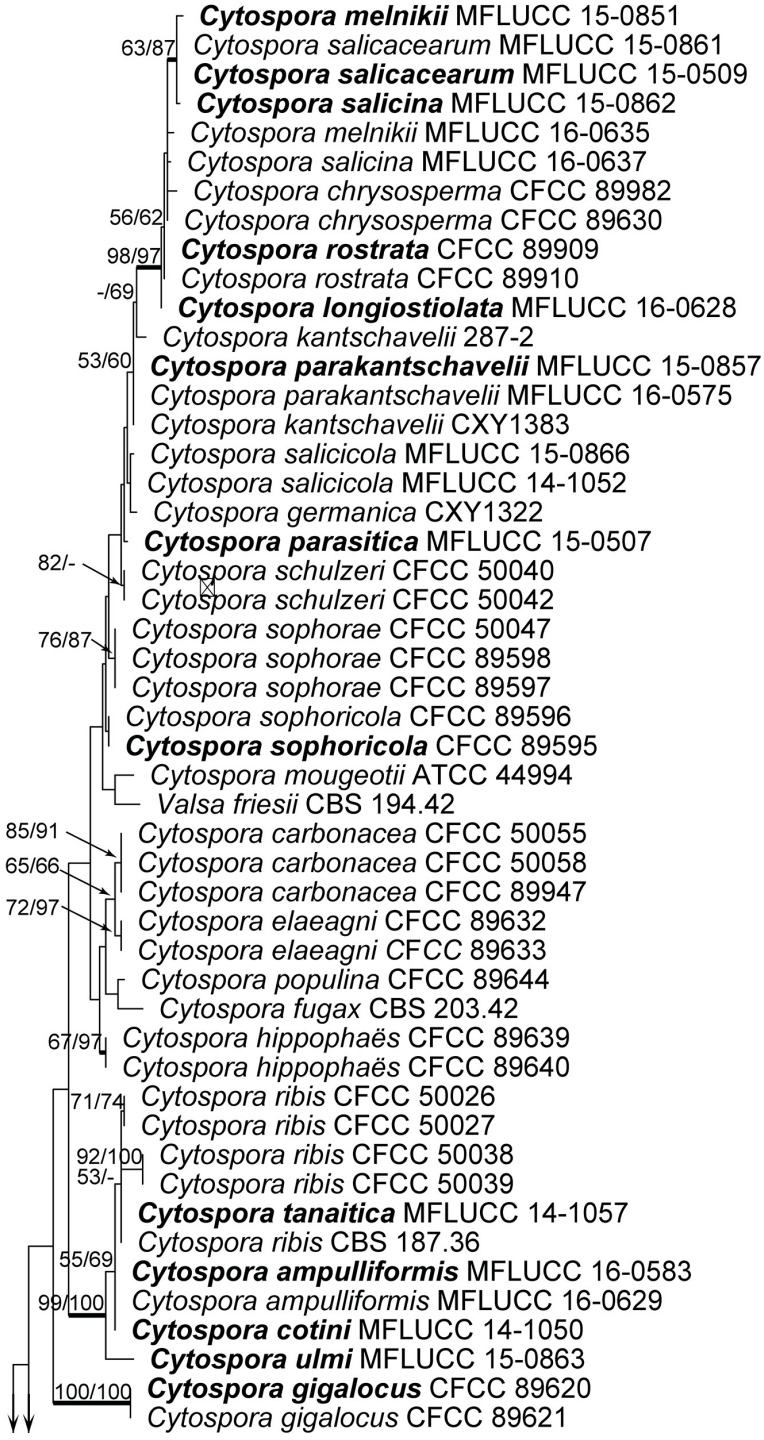


FIGURE 1. Phylogram of *Cytospora* based on ITS gene. MP and ML bootstrap support values above 50 % are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type strains are in bold. Strains in current study are in blue.

The second phylogenetic analysis are performed based on available ITS, LSU, ACT and RPB2 sequence dataset. The multi-locus analysis includes 75 *Cytospora* ingroup strains with a total of 2141 characters including gaps (566 characters for ITS, 522 for LSU, 326 for ACT and 727 for RPB2), of which 1497 characters are constant, 124 variable characters are parsimony-uninformative and 520 characters are variable and parsimony-informative. MP analyses generated six parsimonious trees, one of which is presented in Fig. 1 (TL = 2338, CI = 0.412, RI = 0.798, RC = 0.329). ML and Bayesian analyses were similar to the MP tree. *Cytospora spiraeae* also formed a monophyletic clade (MP/ML/BI = 100/100/1) (marked in blue in Fig 2). The MP bootstrap supports (BS) equal to or above 50 % were shown in branches in Fig. 1. The branches with significant Bayesian posterior probabilities (BPP) equal to or above 0.95 are shown in the phylogram (Table 1).

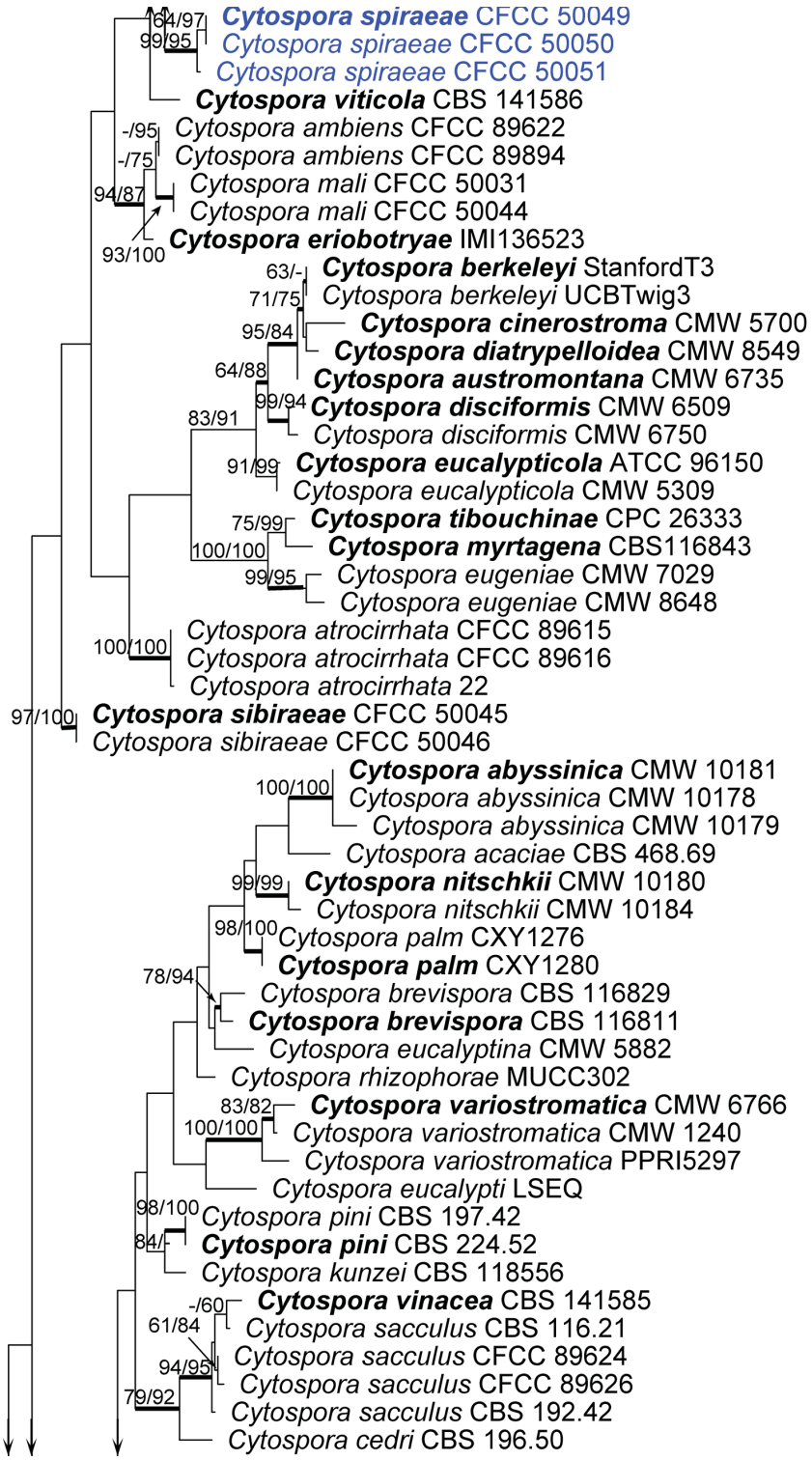


FIGURE 1. (continued)

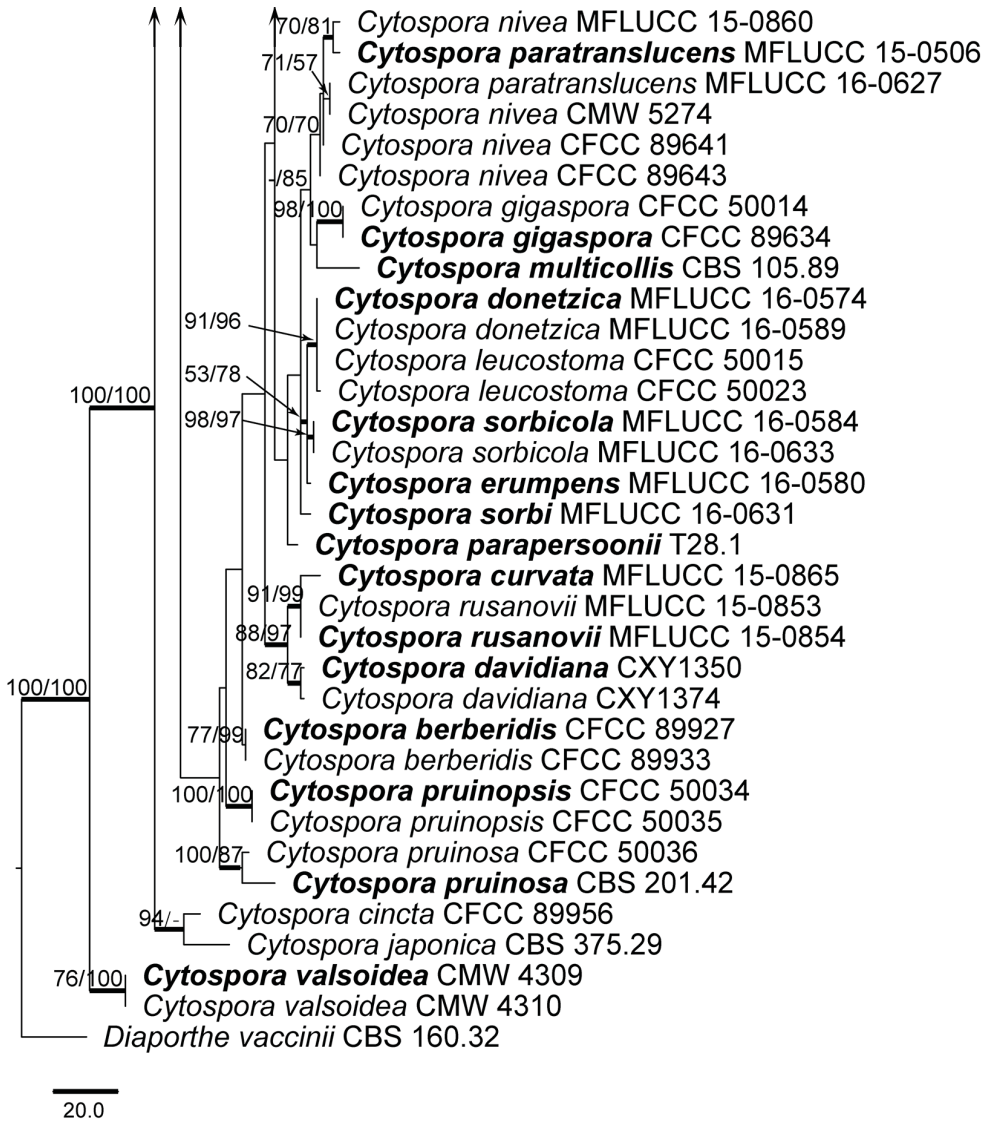


FIGURE 1. (continued)

Taxonomy

Cytospora spiraeae Fan ***sp. nov.*** Fig. 3
MycoBank MB 823824

Holotype:—**China**, **Gansu Province**, Gannan City, 34°17'22.16"N, 102°58'44.23"E, from branches of *Spiraea salicifolia*, Aug. 2012, X.L. Fan, deposited by X.L. Fan, holotype BJFC-S784, ex-type living culture CFCC 50049.

Etymology:—Named after the host genus on which it was collected, *Spiraea.*

Descriptions:—*Ascostromata* immersed in the bark, erumpent through the surface of bark in a large area, scattered, (920–)950–1100(–1240) µm in diam., with 5–8 perithecia arranged circularly or irregularly. *Conceptacle* absent. *Ectostromatic disc* dark grey to black, usually surrounded by tightly ostiolar necks, triangular to circular, (240–)280–340(–390) µm in diam., with 5–8 ostioles arranged circularly per disc. *Ostioles* numerous, dark brown to black, at the same or above level as the disc, concentrated, arranged triangularly to circularly in a disc, (50–)52.5–80(–98) µm in diam. *Perithecia* dark grey to black, flask-shaped to spherical, arranged circularly, (250–)270–400(–440) µm in diam. *Asci* free, clavate to elongate obovoid, (25.5–)27–35(–37) × (5–)6.5–8(–9.5) µm, 8-spored. *Ascospores* biseriate, elongate-allantoid, thin-walled, hyaline, aseptate, (6–)7–8(–8.5) × (1.5–)2–2.5 µm. *Pycnidial stromata* ostiolated, immersed in bark, scattered, erumpent slightly through the surface of bark, with multiple locules. *Conceptacle* absent.

Ectostromatic disc dark brown, unconspicuous, circular, (200–)225–290(–325) µm in diam., with 1–4 ostioles per disc. *Ostioles* black, unconspicuous, at the same level as the disc surface, arranged circularly in a disc, (40–)45–70(–85) µm in diam. *Locule* numerous, subdivided frequently by invaginations with common walls, (980–)1050–1250(–1320) µm in diam. *Conidiophores* hyaline, branched at base or not branched, thin walled, filamentous. *Conidiogenous cells* enteroblastic polyphialidic. *Conidia* hyaline, allantoid, smooth, aseptate, thin-wall, (5–)5.5–6.5(–7) × (1–)1.5 µm.

Culture characteristics: Culture on PDA is initially white, becoming fawn after 7–10 days. The colony is flat, felt-like, thin with a uniform texture, lacking aerial mycelium. Pycnidia distributed irregularly on medium surface.

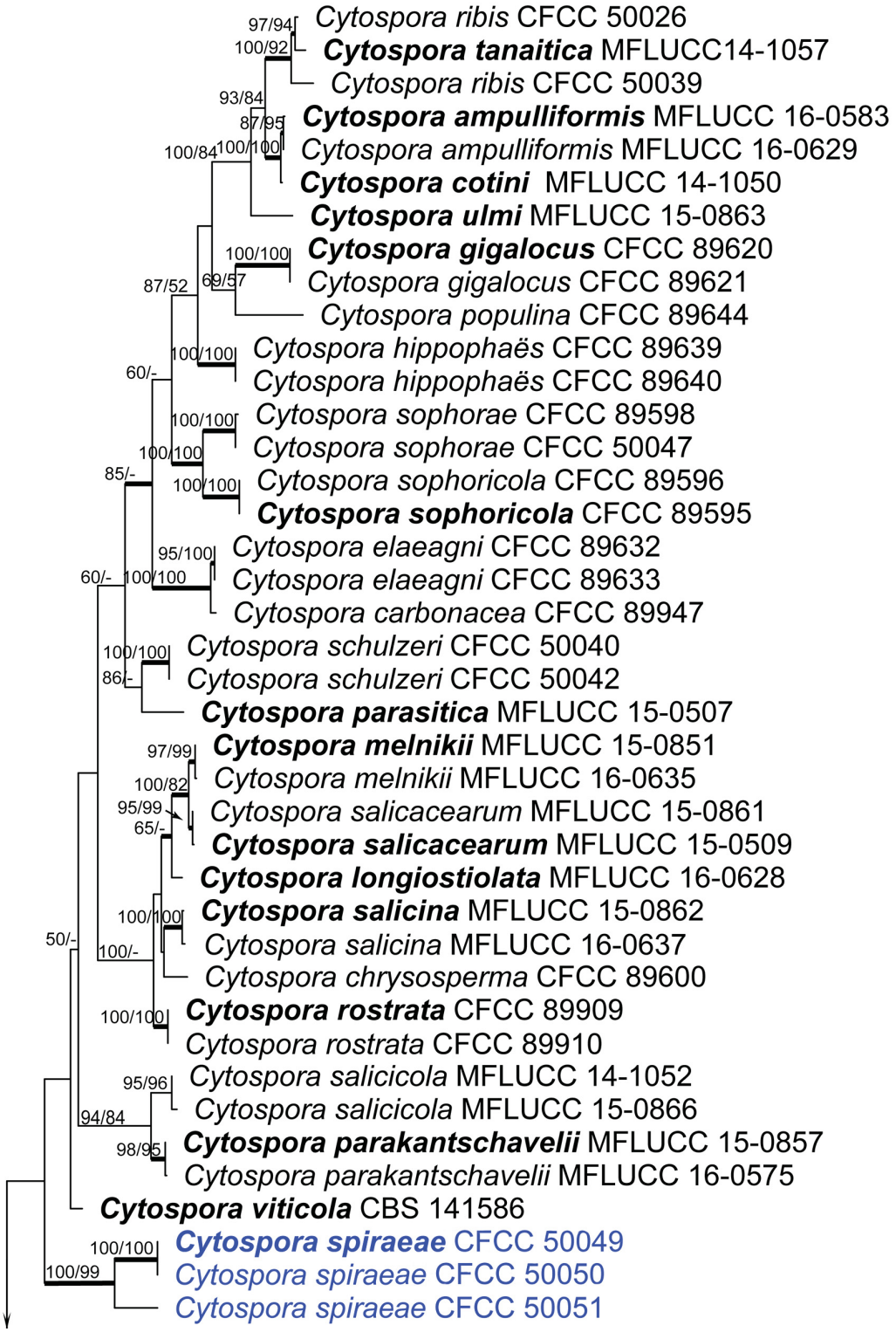


FIGURE 2. Phylogram of *Cytospora* based on combined ITS, LSU, ACT and RPB2 genes. MP and ML bootstrap support values above 50 % are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type strains are in bold. Strains in current study are in blue.

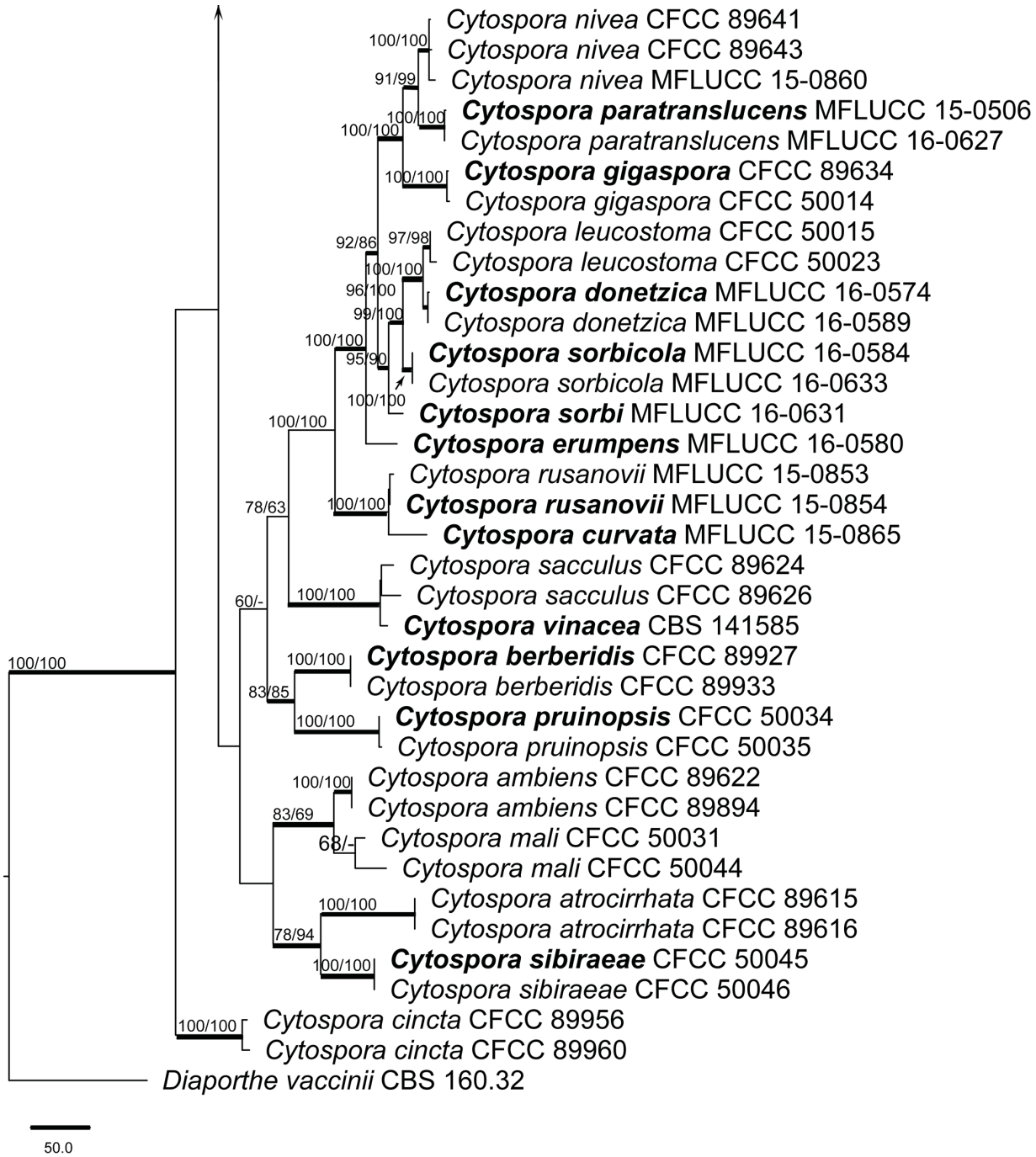


FIGURE 2. (continued)

Materials examined:—**China, Gansu Province**, Gannan City, 34°17'22.16"N, 102°58'44.23"E, from branches of *Spiraea salicifolia*, Aug. 2012, X.L. Fan, deposited by X.L. Fan, BJFC-S785, living culture CFCC 50050; **Shanxi Province**, Datong City, Wenying Lake Park, 40°04'32.02"N, 113°22'13.17"E, from branches of *Spiraea salicifolia*, Apr. 2014, X.L. Fan, deposited by X.L. Fan, BJFC-S1058, living culture CFCC 50051.

Notes:—*Cytospora spiraeae* is associated with canker disease of *Spiraea salicifolia*. Morphologically, this species possesses obviously multiple ostioles and locules with common walls, which is similar with *Cytospora carbonacea* and *Cytospora schulzeri*, species but the host. *Cytospora spiraeae* has smaller locules with a centre column as compared with *C. schulzeri* (950–1100 vs. 1400–1500 μm) in diam., and it has smaller conidia than *C. carbonacea* (5.5–6.5 × 1.5 vs. 9–13 × 2–3 μm) (Fotouhifar *et al.* 2007; Zhang *et al.* 2014; Yang *et al.* 2015). The clear phylogenetic distinction with all other available strains included in this study, resulted in this species represented an individual clade. *Cytospora spiraeae* represented the unique *Cytospora* isolated from *Spiraea salicifolia* in China. Thus the current study indicated is as a new species and provides the clear phylogenetic distinction with all other available strains and holomorphic descriptions.

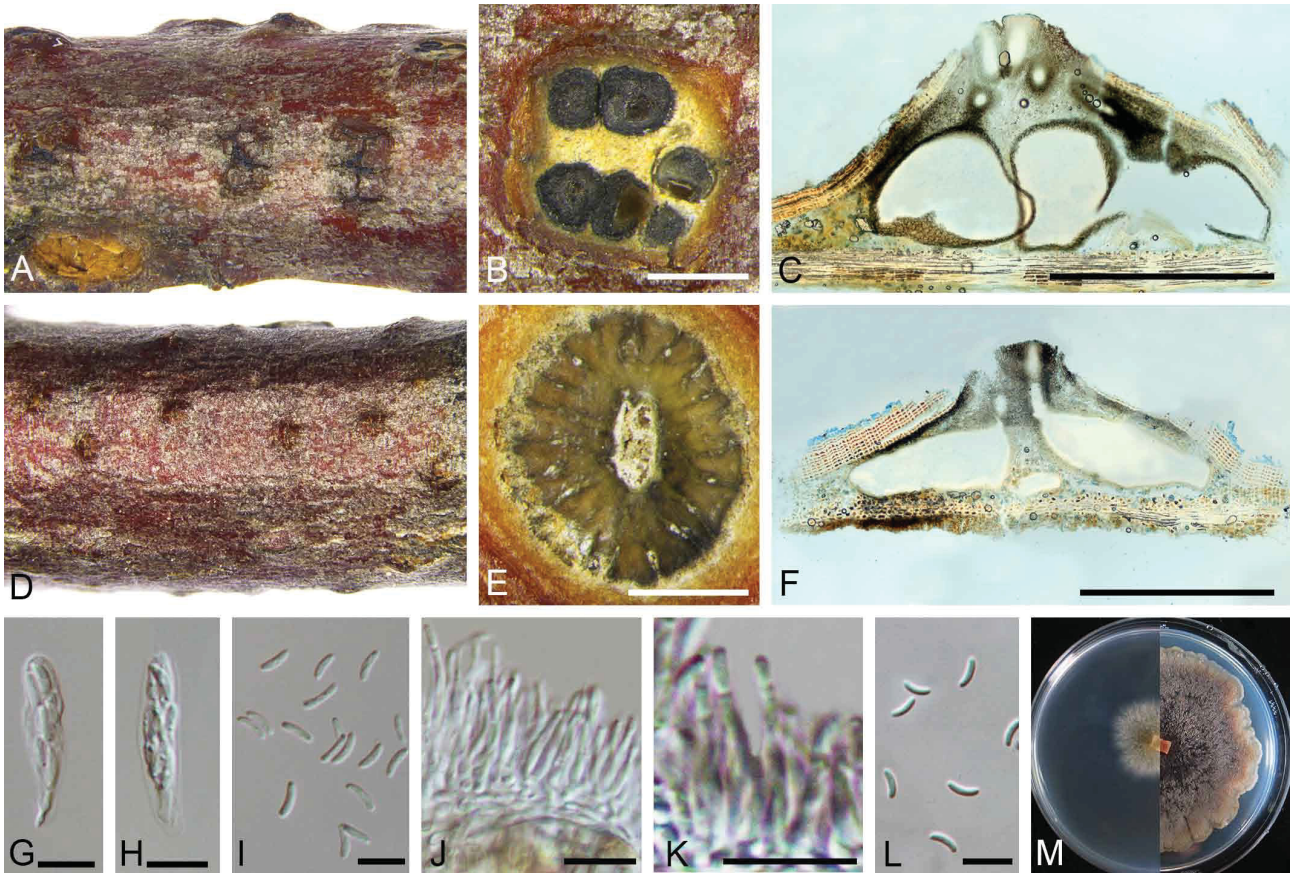


FIGURE 3. Morphology of *Cytospora spiraeae* from *Spiraea salicifolia* (BJFC-S784). A: Habit of ascomata on twig. B: Transverse section of ascomata. C: Longitudinal section through ascomata. D: Habit of conidiomata on twig. E: Transverse section of conidiomata. F: Longitudinal section through conidiomata. G–H: Asci. I: Ascospores. J–K: Conidiophores and Conidiogenous cells. L: Conidia. M: Colonies on PDA at 3 days (left) and 30 days (right). Scale bars: B–C, E–F = 500 μm; G–L = 10 μm.

Discussion

The taxa investigated in the current study revealed *Cytospora spiraeae* sp. nov. infecting branches and twigs of *Sibiraea angustata* in China. Phylogenetic studies published on the genus *Cytospora* in recent years have been substantially influenced by Adams *et al.* (2005). However, only ITS gene are available for most known *Cytospora* species, which caused confused species delimitation. In the current phylogram, *C. chrysosperma* complex, *C. leucostoma* complex, *C. nivea* complex and *C. ribis* complex were proposed to accommodate relative species due to the unclear separation among them (Fig. 1). In the past five years alone, the polyphasic approach (morphological identification and phylogenetic species recognition concept) has led to the descriptions of additional new species of *Cytospora* in China (Wang *et al.* 2013; Zhang *et al.* 2014; Fan *et al.* 2014a, 2014b, 2015a, 2015b; Yang *et al.* 2015; Lawrence *et al.* 2017; Norphanphoun *et al.* 2017), which were included here. The current study indicated that the *Cytospora spiraeae* represents a novel species with high support value (MP/ML/BI = 100/100/1) (Fig. 2).

In future studies of *Cytospora*, extensive fresh collections should be collected to help clarify the species concepts of taxa presently still lacking types linked to multigene DNA data, especially in northwestern China, the phytopathogen hotspot with many species of *Cytospora* from host families associated with canker disease, seems an attractive region for the discovery of new taxa in this genus.

Acknowledgements

This study is financed by Fundamental Research Funds for the Central Universities (Project No.: BLX201613) and National Natural Science Foundation of China (Project No.: 31670647).

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