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Cytospora piceae sp. nov. associated with canker disease of *Picea crassifolia* in China

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Abstract

Cytospora species are common pathogens associated with stem canker diseases of woody plants, with a worldwide distribution and broad host range. The criteria of species level identification are difficult due to insufficient ex-type cultures with molecular data and phylogenetic understanding. Two fungal specimens were collected from *Picea crassifolia* associated with symptomatic canker and dieback disease in the Xinjiang Uygur Autonomous Region, China. They were identified as novel species based on morphology plus support from multilocus phylogenetic analyses of ITS, LSU, ACT, RPB2 and TEF1-α gene regions. *Cytospora piceae* is characterized by its ostiolated pycnidia with vesicularly arranged locules, and hyaline, eguttulate, aseptate, allantoid conidia, which differs from similar species in its host association and multilocus phylogeny.

Key words: canker disease, Cytosporaceae, Diaporthales, phylogeny, taxonomy

Introduction

Species of *Cytospora* cause cankers and dieback disease on more than 100 species of hardwoods and coniferous plants, which is one of the most important pathogenic genera causing severe commercial and ecological damage and significant losses worldwide (Sinclair *et al*. 1987, Adams *et al*. 2005, 2006, Fan *et al.* 2014a, 2014b, 2015a, 2015b, Zhu *et al.* 2018). The genus *Cytospora* (Ascomycota: Diaporthales) was established by Ehrenberg (1818). It is characterized by single or labyrinthine of pycnidial locules, filamentous conidiophores (enteroblastic and phialidic conidiogenous cells) producing hyaline, allantoid conidia in asexual morph; diaporthalean-like perithecia, clavate to elongate obovoid asci with 4- or 8- hyaline, allantoid ascospores in sexual morph (Spielman 1983, 1985, Adams *et al.* 2005). Under moist conditions, the conidia emerge from the pycnidia in the form of yellow, orange to red gelatinous tendrils. Over 615 species epithets of *Cytospora* are listed in Index Fungorum (2018) while Kirk *et al.* (2008) estimated 110 species. In the past it was difficult to name *Cytospora* species as morphology overlapped, and this caused confused species delimitation. The previous identification of *Cytospora* species is based mainly on their host affiliations, often with unclear morphological descriptions. Morphology and phylogeny using ITS sequence data was combined to describe 28 species of *Cytospora* from *Eucalyptus*, of which eleven species were new to science by Adams *et al*. (2005), and also described fourteen species from South Africa using the same methodology (Adams *et al*. 2006). However, only ITS gene is available for most known *Cytospora* species, and ex-type sequence data are available for only a few species and many taxa need epitypifying. Thus, 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, Zhu *et al.* 2018).

During an investigation of forest pathogens that cause canker or dieback disease in China, two *Cytospora* specimens were collected from *Picea crassifolia* with obvious symptoms. Both specimens were characterized by ostiolated pycnidia with vesicularly arranged locules, and hyaline, eguttulate, aseptate, allantoid conidia. Phylogenetic analyses inferred from combined ITS, LSU, ACT, RPB2 and TEF1-α gene regions provided strong support that this species is novel. Thus, we introduce *Cytospora piceae* as a new in this paper with a description and illustrations and compare it with other species in the genus.

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Materials and methods

Sampling and fungal isolates

Strains of *Cytospora* were isolated from diseased branches or twigs of *Picea crassifolia*, during collecting trips in the Xinjiang Uygur Autonomous Region of China (Table 1). Isolations were made directly from conidiomata, and spreading the suspension over the surface of a Petri dish with 1.8% of potato dextrose agar (PDA) at 25 ℃ for up to 24h. After incubation for up to 24h, single germinating conidia were transferred to fresh plates of PDA. Specimens have been deposited at the working Collection of X.L. Fan (CF) housed at the Beijing Forestry University (BJFC). Living cultures were deposited at China Forestry Culture Collection Centre (CFCC).

Morphology

Specimens were observed based on the morphological characteristics of their fruiting bodies from infected host materials. The macro-morphological photographs were captured using a Leica stereomicroscope (M205), including size of conidiomata; presence or absence of special structure such as conceptacle and central column; number and diameter of ostioles per ectostromatic disc; the color, shape and size of discs; number of locules. Micro-morphological observations such as size and shape of conidiophores and conidia were determined under a Nikon Eclipse 80i microscope equipped with a Nikon digital sight DS-Ri2 high definition colour camera with differential interference contrast (DIC). Over 30 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 14 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 extraction, PCR amplification, and sequencing

Mycelium for DNA extraction was grown on PDA with cellophane for 3 days and obtained from the surface of cellophane by scrapping. Genomic DNA was extracted using the modified CTAB method (Doyle & Doyle 1990). DNA concentrations were estimated visually by electrophoresis in 1% agarose gels by comparing band intensity with a DNA marker 1 kbp (Takara Biotech). PCR amplifications were performed in DNA Engine (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories, CA, USA). DNA were amplified from the ITS, LSU, TEF1-α, ACT, and RPB2. The ITS region was amplified using primers ITS1 and ITS4 (White *et al*. 1990). The LSU region was amplified using the primers LR0R and LR7 (Vilgalys & Hester 1990). The 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 TEF1-α region was amplified with the primer EF-688F and EF-1251R (Carbone & Kohn 1999). The PCR amplification products were estimated visually by electrophoresis in 2 % agarose gels. DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminater Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China). DNA 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

The first analysis using ITS sequence data was performed to compare *Cytospora* species from the current study with other strains in GenBank. Sequences were aligned using MAFFT v.6 (Katoh & Standley 2013) and edited manually using MEGA v.6.0 (Tamura *et al.* 2013). Ambiguously aligned sequences were excluded from analysis. Phylogenetic analysis was performed by PAUP v.4.0b10 for maximum parsimony (MP) method (Swofford 2003), MrBayes v.3.1.2 for Bayesian Inference (BI) method (Ronquist & Huelsenbeck 2003) and RAxML for maximum likelihood (ML) method (Stamatakis 2006). To clarify the phylogenetic position of our isolates, a second analysis based on the combined five concatenated sequences (ITS, LSU, ACT, RPB2 and TEF1-α) was performed. *Diaporthe vaccinii* was selected as the outgroup in all analyses.

A partition homogeneity test (PHT) with heuristic search and 1000 replicates was performed using PAUP v.4.0b10 to test the discrepancy among the ITS, LSU, ACT, RPB2 and TEF1-α sequence dataset in reconstructing phylogenetic trees. MP analysis was performed using a heuristic search option of 1,000 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. Clade stability was assessed with a bootstrap analysis of 1,000 replicates (Hillis and Bull 1993). Other parsimony scores such as tree length (TL), consistency index (CI),

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

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	LSU	ACT	RPB2	TEF1-α
C. abyssinica	CMW 10181[T]	*Eucalyptus globulus*	Ethiopia	AY347353	-	-	-	-
C. abyssinica	CMW 10178	*Eucalyptus globulus*	Ethiopia	AY347354	-	-	-	-
C. abyssinica	CMW 10179	*Eucalyptus globulus*	Ethiopia	AY347352	-	-	-	-
C. acaciae	CBS 468.69	*Ceratonia siliqua*	Spain	DQ243804	-	-	-	-
C. ambiens	CFCC 89622	*Pyrus bretschneideri*	China	KR045616	KR045698	KU710988	KU710944	KU710911
C. ambiens	CFCC 89894	*Pyrus bretschneideri*	China	KR045617	KR045699	KU710989	KU710945	KU710912
C. ampulliformis	MFLUCC 16-0583[T]	*Sorbus intermedia*	Russia	KY417726	KY417760	KY417692	KY417794	-
C. ampulliformis	MFLUCC 16-0629	*Acer platanoides*	Russia	KY417727	KY417761	KY417693	KY417795	-
C. atrocirrhata	CFCC 89615	*Juglans regia*	China	KR045618	KR045700	KF498673	KU710946	KP310858
C. atrocirrhata	CFCC 89616	*Juglans regia*	China	KR045619	KR045701	KF498674	KU710947	KP310859
C. austromontana	CMW 6735[T]	*Eucalyptus pauciflora*	Australia	AY347361	-	-	-	-
C. berberidis	CFCC 89927[T]	*Berberis dasystachya*	China	KR045620	KR045702	KU710990	KU710948	KU710913
C. berberidis	CFCC 89933	*Berberis dasystachya*	China	KR045621	KR045703	KU710991	KU710949	KU710914
C. berkeleyi	StanfordT3[T]	*Eucalyptus globulus*	California, USA	AY347350	-	-	-	-
C. berkeleyi	UCBTwig3	*Eucalyptus globulus*	California, USA	AY347349	-	-	-	-
C. brevispora	CBS 116829	*Eucalyptus grandis*	Venezuela	AF192321	-	-	-	-
C. brevispora	CBS 116811[T]	*Eucalyptus grandis* × *tereticornis*	Democratic Republic of the Congo	AF192315	-	-	-	-
C. cabonacea	CFCC 50055	*Ulmus pumila*	Shanxi, China	KP281262	KP310808	KP310838	-	-
C. cabonacea	CFCC 50058	*Ulmus pumila*	Heilongjiang, China	KP281264	KP310810	KP310840	-	-
C. carbonacea	CFCC 89947	*Ulmus pumila*	Qinghai, China	KR045622	KP310812	KP310842	KU710950	KP310855
C. carpobroti	CMW 48981[T]	*Carpobrotus edulis*	Cape Town, South Africa	MH382812	MH411216	-	-	MH411212
C. cedri	CBS 196.50	-	Italy	AF192311	-	-	-	-
C. centrivillosa	MFLUCC 16-1206[T]	*Sorbus domestica*	Italy	MF190122	MF190068	-	MF377600	-
C. centrivillosa	MFLUCC 17-1660	*Sorbus domestica*	Italy	MF190123	MF190069	-	MF377601	-
C. chrysosperma	CFCC 89629	*Salix psammophila*	Shanxi, China	KF765673	KF765689	KF765721	KF765705	-
C. chrysosperma	CFCC 89600	*Sophora japonica*	Gansu, China	KR045623	KP310804	KU710992	KU710951	-
C. cincta	CFCC 89956	*Prunus cerasifera*	China	KR045624	KR045704	KU710993	KU710953	KU710916
C. cinerostroma	CMW 5700[T]	*Eucalyptus globulus*	Chile	AY347377	-	-	-	-
C. cotini	MFLUCC 14-1050[T]	*Cotinus coggygria*	Russia	KX430142	KX430143	-	KX430144	-
C. curvata	MFLUCC 15-0865[T]	*Salix alba*	Russia	KY417728	KY417762	KY417694	KY417796	-
C. davidiana	CXY 1350[T]	*Populus davidiana*	China	KM034870	-	-	-	-

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TABLE 1. (Continued)

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	LSU	ACT	RPB2	TEF1-α
C. davidiana	CXY 1374	*Populus davidiana*	China	KM034869	-	-	-	-
C. diatrypelloidea	CMW 8549[T]	*Eucalyptus globulus*	Orbost, Australia	AY347368	-	-	-	-
C. disciformis	CMW 6509[T]	*Eucalyptus grandis*	Uruguay	AY347374	-	-	-	-
C. disciformis	CMW 6750	*Eucalyptus globulus*	Australia	AY347359	-	-	-	-
C. donetzica	MFLUCC 16-0574[T]	*Rosa* sp.	Russia	KY417731	KY417764	KY417696	KY417798	-
C. donetzica	MFLUCC 15-0864	*Crataegus monogyna*	Ukraine	KY417729	KY417763	KY417695	KY417797	-
C. donetzica	MFLUCC 16-0589	*Salix alba*	Russia	KY417732	KY417766	KY417698	KY417800	-
C. elaeagni	CFCC 89632	*Elaeagnus angustifolia*	Ningxia, China	KR045626	KR045706	KU710995	KU710955	KU710918
C. elaeagni	CFCC 89633	*Elaeagnus angustifolia*	Ningxia, China	KF765677	KF765693	KU710996	KU710956	KU710919
C. eriobotryae	IMI 136523[T]	*Eriobotrya japonica*	India	AY347327	-	-	-	-
C. erumpens	MFLUCC 16-0580[T]	*Salix* × *fragilis*	Russia	KY417733	KY417767	KY417699	KY417801	-
C. eucalypti	LSEQ	*Sequoia sempervirens*	California, USA	AY347340	-	-	-	-
C. eucalypticola	ATCC 96150[T]	*Eucalyptus nitens*	Tasmania, Australia	AY347358	-	-	-	-
C. eucalypticola	CMW 5309	*Eucalyptus grandis*	Entebbe, Uganda	AF260266	-	-	-	-
C. eucalyptina	CMW 5882	*Eucalyptus grandis*	Cali, Columbia	AY347375	-	-	-	-
C. eugeniae	CMW 7029	*Tibouchina* sp.	Brisbane, Australia	AY347364	-	-	-	-
C. eugeniae	CMW 8648	*Eugenia* sp.	Indonesia	AY347344	-	-	-	-
C. fraxinigena	BBH 42442	*Fraxinus ornus*	Italy	MF190134	MF190079	-	-	-
C. fraxinigena	MFLUCC 14-0868	*Fraxinus ornus*	Italy	MF190133	MF190078	-	-	-
C. friesii	CBS 113.81	*Abies alba*	Germany	AY347318	-	-	-	JX438592
C. friesii	CBS 194.42	*Abies alba*	Switzerland	AY347328	-	-	-	-
C. fugax	CXY1371	*Populus simonii*	Heilongjiang, China	KM034852	-	-	-	-
C. fugax	CXY1381	*Populus ussuriensis*	Heilongjiang, China	KM034853	-	-	-	-
C. germanica	CXY1322	*Elaeagnus oxycarpa*	China	JQ086563	JX524617	-	-	-
C. gigaspora	CFCC 89620[T]	*Juglans regia*	Xining, Qinghai	KR045628	KR045708	KU710997	KU710957	-
C. gigaspora	CFCC 89621	*Juglans regia*	Xining, Qinghai	KR045629	KR045709	KU710998	KU710958	-
C. gigaspora	CFCC 50014	*Juniperus procumbens*	Shanxi, China:	KR045630	KR045710	KU710999.	KU710959	KR045671
C. gigaspora	CFCC 89634[T]	*Salix psammophila*	China	KF765671	KF765687	KU711000	KU710960	KR045672
C. hippophaës	CFCC 89639	*Hippophae rhamnoides*	Gansu, China	KR045632	KR045712	KU711001	KU710961	KR045673
C. hippophaës	CFCC 89640	*Hippophae rhamnoides*	Gansu, China	KF765682	KF765698	KF765730	KU710962	KR045674
C. japonica	CBS 375.29	*Prunus persicae*	Japan	AF191185	-	-	-	-
C. junipericola	BBH 42444	*Juniperus communis*	Italy	MF190126	MF190071	-	-	MF377579
C. junipericola	MFLU 17-0882	*Juniperus communis*	Italy	MF190125	MF190072	-	-	MF377580
C. kantschavelii	CXY1383	*Populus maximowiczii*	China	KM034867	-	-	-	-
C. kantschavelii	CXY1386	*Populus maximowiczii*	Chongqing, China	KM034867	-	-	-	-
C. kunzei	CBS 118556	*Pinus radiata*	South Africa	DQ243791	-	-	-	-
C. leucostoma	CFCC 50016	*Sorbus aucuparia*	China	MH820400	MH820393	MH820408	-	MH820404

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TABLE 1. (Continued)

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	LSU	ACT	RPB2	TEF1-α
C. leucostoma	CFCC 50015	*Sorbus pohuashanensis*	China	KR045634	KR045714	KU711002	-	KU710925
C. longiostiolata	MFLUCC 16-0628^{T}	*Salix* × *fragilis*	Russia	KY417734	KY417768	KY417700	KY417802	-
C. mali	CFCC 50031	*Crataegus* sp.	China	KR045636	KR045716	KU711004	KU710965	KU710927
C. mali	CFCC 50044	*Malus baccata*	China	KR045637	KR045717	KU711005	KU710966	KU710928
C. melnikii	MFLUCC 15-0851^{T}	*Malus domestica*	Russia	KY417735	KY417769	KY417701	KY4178034309	-
C. mougeotii	ATCC 44994	*Picea abies*	Norway	AY347318	-	-	-	-
C. multicollis	CBS 105.89^{T}	*Quercus ilex subsp. rotundifolia*	Spain	DQ243803	-	-	-	-
C. myrtagena	CBS 116843^{T}	*Tibouchiina urvilleana*	Hawaii, USA	AY347363	-	-	-	-
C. nivea	MFLUCC 15-0860	*Salix acutifolia Willd.*	Russia	KY417737	KY417771	KY417703	KY417805	-
C. nivea	CFCC 89641	*Elaeagnus angustifolia*	China	KF765683	KF765699	KU711006	KU710967	KU710929
C. nivea	CFCC 89643	*Salix psammophila*	China	KF765685	-	-	KU710968	KP310863
C. palm	CXY1276	*Cotinus coggygria*	Beijing, China	JN402990	-	-	-	KJ781296
C. palm	CXY1280^{T}	*Cotinus coggygria*	Beijing, China	JN411939	-	-	-	KJ781297
C. parakantschavelii	MFLUCC 15-0857^{T}	*Populus* × *sibirica*	Russia	KY417738	KY417772	KY417704	KY417806	-
C. parakantschavelii	MFLUCC 16-0575	*Pyrus pyraster*	Russia	KY417739	KY417773	KY417705	KY417807	-
C. parapersoonii	T28.1^{T}	*Prunus persicae*	Michigan, USA	AF191181	-	-	-	-
C. parasitica	MFLUCC 15-0507^{T}	*Malus domestica*	Russia	KY417740	KY417774	KY417706	KY417808	-
C. paratranslucens	MFLUCC 15-0506^{T}	*Populus alba* var. *Bolleana (Lauche) Otto*	Russia	KY417741	KY417775	KY417707	KY417809	-
C. paratranslucens	MFLUCC 16-0627	*Populus alba*	Russia	KY417742	KY417776	KY417708	KY417810	-
C. piceae	**CFCC 52841^{T}**	*Picea crassifolia*	Xinjiang, China	MH820398	MH820391	MH820406	MH820395	MH820402
C. piceae	**CFCC 52842**	*Picea crassifolia*	Xinjiang, China	MH820399	MH820392	MH820407	MH820396	MH820403
C. pini	CBS 197.42	*Pinus Sylvestirs*	Switzerland	AY347332	-	-	-	-
C. pini	CBS 224.52^{T}	*Pinus strobus*	New York	AY347316	-	-	-	-
C. populina	CFCC 89644	*Salix psammophila*	Shanxi, China	KF765686	KF765702	KU711007	KU710969	KU710930
C. predappioensis	MFLU 17-0323	*Platanus hybrida*	Italy	MG873484	MG873480	-	-	-
C. pruinopsis	CFCC 50034^{T}	*Ulmus pumila*	Shanxi, China	KP281259	KP310806	KP310836	KU710970	KP310849
C. pruinosa	CFCC 50035	*Ulmus pumila*	Jilin, China	KP281260	KP310807	KP310837	KU710971	KP310850
C. pruinosa	CBS 201.42^{T}	*Syringa* sp.	Switzerland	DQ243801	-	-	-	-
C. prunicola	MFLU 17-0995^{T}	*Prunus* sp.	Italy	MG742350	MG742351	MG742353	MG742352	MG742354

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TABLE 1. (Continued)

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	LSU	ACT	RPB2	TEF1-α
C. quercicola	MFBBH 42443	*Quercus* sp.	Italy	MF190128	MF190074	-	-	-
C. quercicola	MFLUCC 14-0867^{T}	*Quercus* sp.	Italy	MF190129	MF190073	-	-	-
C. rhizophorae	MUCC302	*Eucalyptus grandis*	Australia	EU301057	-	-	-	-
C. ribis	CFCC 50026	*Ulmus pumila*	Qinghai, China	KP281267	KP310813	KP310843	KU710972	KP310856
C. ribis	CFCC 50027	*Ulmus pumila*	Qinghai, China	KP281268	KP310814	KP310844	-	KP310857
C. rusanovii	MFLUCC 15-0854^{T}	*Salix babylonica*	Italy	MF190131	MF190075	-	-	-
C. rostrata	CFCC 89909^{T}	*Salix cupularis*	Gansu, China	KR045643	KR045722	KU711009	KU710974	KU710932
C. rostrata	CFCC 89910	*Salix cupularis*	Gansu, China	KR045644	KR045723	KU711010	KU710975	KU710933
C. rusanovii	MFLUCC 15-0853	*Populus* × *sibirica*	Russia	KY417743	KY417777	KY417709	KY417811	-
C. rusanovii	MFLUCC 15-0854^{T}	*Salix babylonica*	Russia	KY417744	KY417778	KY417710	KY417812	-
C. sacculus	CFCC 89624	*Juglans regia*	China	KR045645	KR045724	KM401888	KU710976	KP310860
C. sacculus	CFCC 89625	*Juglans regia*	China	KF225616	KM401887	KM401889	-	-
C. salicacearum	MFLUCC 15-0509^{T}	*Salix alba*	Russia	KY417746	KY417780	KY417712	KY417814	-
C. salicacearum	MFLUCC 15-0861	*Salix* × *fragilis*	Russia	KY417745	KY417779	KY417711	KY417813	-
C. salicacearum	MFLUCC 16-0576	*Populus nigra* var. *italica*	Russia	KY417747	KY417781	KY417713	KY417815	-
C. salicacearum	MFLUCC 16-0587	*Prunus cerasus*	Russia	KY417748	KY417782	KY417714	KY417816	-
C. salicicola	MFLUCC 15-0866	*Salix alba*	Russia	KY417749	KY417783	KY417715	KY417817	-
C. salicicola	MFLUCC 14-1052	-	-	KU982636	KU982635	KU982637	-	-
C. salicina	MFLUCC 15-0862^{T}	*Salix alba*	Russia	KY417750	KY417784	KY417716	KY417818	-
C. salicina	MFLUCC 16-0637	*Salix* × *fragilis*	Russia	KY417751	KY417785	KY417717	KY417819	-
C. schulzeri	CFCC 50040	*Malus domestica*	Ningxia, China	KR045649	KR045728	KU711013	KU710980	KU710936
C. schulzeri	CFCC 50042	*Malus asiatica*	Qinghai, China	KR045650	KR045729	KU711014	KU710981	KU710937
C. sibiraeae	CFCC 50045^{T}	*Sibiraea angustata*	Gansu, China	KR045651	KR045730	KU711015	KU710982	KU710938
C. sibiraeae	CFCC 50046	*Sibiraea angustata*	Gansu, China	KR045652	KR045731	KU711015	KU710983	KU710939
C. sophorae	CFCC 50047	*Styphnolobium japonicum*	Shanxi, China	KR045653	KR045732	KU711017	KU710984	KU710940
C. sophorae	CFCC 50048	*Magnolia grandiflora*	China	MH820401	MH820394	MH820409	MH820397	MH820405

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TABLE 1. (Continued)

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	LSU	ACT	RPB2	TEF1-α
C. sophorae	CFCC 89598	*Styphnolobium japonicum*	Gansu, China	KR045654	KR045733	KU711018	KU710985	KU710941
C. sophoricola	CFCC 89596	*Styphnolobium japonicum*	Gansu, China	KR045656	KR045735	KU711020	KU710987	KU710943
C. sophoricola	CFCC 89595^{T}	*Styphnolobium japonicum* var.	Gansu, China	KR045655	KR045734	KU711019	KU710986	KU710942
C. sorbi	MFLUCC 16-0631^{T}	*Sorbus aucuparia*	Russia	KY417752	KY417786	KY417718	KY417820	-
C. sorbicola	MFLUCC 16-0584^{T}	*Acer pseudoplatanus*	Russia	KY417755	KY417789	KY417721	KY417823	-
C. sorbicola	MFLUCC 16-0633	*Cotoneaster melanocarpus*	Russia	KY417758	KY417792	KY417724	KY417826	-
C. spiraeae	CFCC 50049^{T}	*Spiraea salicifolia*	Gansu, China	MG707859	MG707643	MG708196	MG708199	-
C. spiraeae	CFCC 50050	*Spiraea salicifolia*	Gansu, China	MG707860	MG707644	MG708197	MG708200	-
C. spiraeae	CFCC 50051	*Spiraea salicifolia*	Gansu, China	MG707861	MG707645	MG708198	MG708201	-
C. tanaitica	MFLUCC 14-1057^{T}	*Betula pubescens*	Russia	KT459411	KT459412	KT459413	-	-
C. tibouchinae	CPC 26333^{T}	*Tibouchina semidecandra*	France	KX228284	KX228335	-	-	-
C. translucens	CXY1351	*Populus davidiana*	Inner Mongolia, China	KM034874	-	-	-	-
C. ulmi	MFLUCC 15-0863^{T}	*Ulmus minor*	Russia	KY417759	-	-	-	-
C. valsoidea	CMW 4309^{T}	*Eucalyptus grandis*	Sumatra, Indonisia	AF192312	-	-	-	-
C. valsoidea	CMW 4310	*Eucalyptus grandis*	Sumatra, Indonisia	AF192312	-	-	-	-
C. variostromatica	CMW 6766^{T}	*Eucalyptus globulus*	Australia	AY347366	-	-	-	-
C. variostromatica	CMW 1240	*Eucalyptus grandis*	South Africa	AF260263	-	-	-	-
C. variostromatica	PPRI5297	*Eucalyptus grandis*	Pretoria, South Africa	AF260264	-	-	-	-
C. vinacea	CBS 141585^{T}	*Vitis interspecific*	New Hampshire, USA	KX256256	-	-	-	KX256277
C. viticola	CBS 141586^{T}	*Vitis vinifera*	Connecticut, USA	KX256239	-	-	-	KX256260
Diaporthe vaccinii	CBS 160.32	*Vaccinium macrocarpon*	Massachusetts, USA	KC343228	-	JQ807297	-	KC343954

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retention index (RI) and rescaled consistency (RC) were calculated (Swofford *et al.* 2003). ML analysis was performed with GTR+G+I model of site substitution following recent studies (Zhu *et al.* 2018), including estimation of gamma-distributed rate heterogeneity and a proportion of invariant sites using RaxMl v.7.2.8 (Stamatakis 2006). The branch support was evaluated with a bootstrapping method of 1000 replicates (Hillis and Bull 1993). BI analysis was performed using a Markov Chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities (Rannala & Yang 1996). A nucleotide substitution model was estimated by MrModeltest v.2.3 (Posada and Crandall 1998), and a weighted Bayesian analysis was considered. Two MCMC chains were run from random trees for 1,000,000 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 1,000 replicates (Hillis & Bull 1993). Phylograms are shown using Figtree v.1.3.1 (Rambaut & Drummond 2010). Sequence data were deposited in GenBank. The ITS and multilocus sequence alignment file were deposited in TreeBASE (www. treebase. org) as the accession number S23479.

Results

Two isolates of *Cytospora* from *Picea crassifolia* were sequenced for ITS locus, which contained 143 *Cytospora* ingroup strains with a total of 604 characters including gaps. In the alignment, 178 characters were constant, 147 variable characters were parsimony- uninformative and 920 characters were variable and parsimony-informative. There were 178 variable sites of which 68 were parsimony informative. MP analyses generated 200 parsimonious trees, one of which was presented in Fig. 1 (TL = 949, CI = 0.413, RI = 0.844, RC = 0.349). ML and Bayesian analyses were similar to the MP tree. *Cytospora piceae* clustered with *C. friesii* and *C. mougeotii*, presenting an unstable clade with low support value.

To clarify the phylogenetic position of *Cytospora piceae*, the second phylogenetic analyses were performed based on available ITS, LSU, ACT, RPB2 and TEF1-α sequence dataset. The alignment included 98 *Cytospora* ingroup strains with a total of 2936 characters including gaps. In the alignment 1869 characters were constant, 147 variable characters were parsimony-uninformative and 920 characters were variable and parsimony-informative. MP analyses generated 12 parsimonious trees, one of which was presented in Fig. 1 (TL = 4386, CI = 0.397, RI = 0.766, RC = 0.304). ML and Bayesian analyses were similar to the MP tree. *Cytospora piceae* represented a monophyletic clade with high support value (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 were shown in the phylogram.

Taxonomy

Cytospora piceae Fan Fig. 3

MycoBank 828432

Holotype:—**China**, **Xinjiang Uygur Autonomous Region**, Bole Mongol Autonomous Prefecture, 44°46’13.44”N, 81°13’58.72”E, from branches of *Picea crassifolia*, July 2017, C.M. Tian & X.L. Fan, holotype CF 20176561, ex-type living culture CFCC 52841.

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

Descriptions:—Asexual state: *Conidiomata* pycnidial, ostiolated, immersed in bark, scattered, erumpent through the surface of bark when mature. *Locules* multiple, discoid, circular to ovoid, arranged vesicularly with common walls, (680–)720–1190(–1200) µm ($\bar{x}$ = 945 ± 130 µm, n = 30) in diam. *Conceptacle* absent. *Ectostromatic disc* white to light brown, circular, disc dark, (160–)230–290(–310) µm ($\bar{x}$ = 255 ± 36 µm, n = 30) in diam., with one ostiole in the centre of disc. *Ostiole* conspicuous, circular to ovoid, dark brown to black at the same level as the disc, (65–)70–115(–130) µm ($\bar{x}$ = 93 ± 17 µm, n = 30) in diam. *Conidiophores* hyaline, branched at base or not branched, thin walled, filamentous, (12–)13.5–19.5(–20) µm ($\bar{x}$ = 16.5 ± 3 µm, n = 30). *Conidiogenous* cells enteroblastic, polyphialidic. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-wall, (4.5–)5–5.5(–6) × 1–1.5 µm ($\bar{x}$ = 5.2 ± 0.3 × 1.3 ± 0.1 µm, n = 50). Sexual morph: not observed.

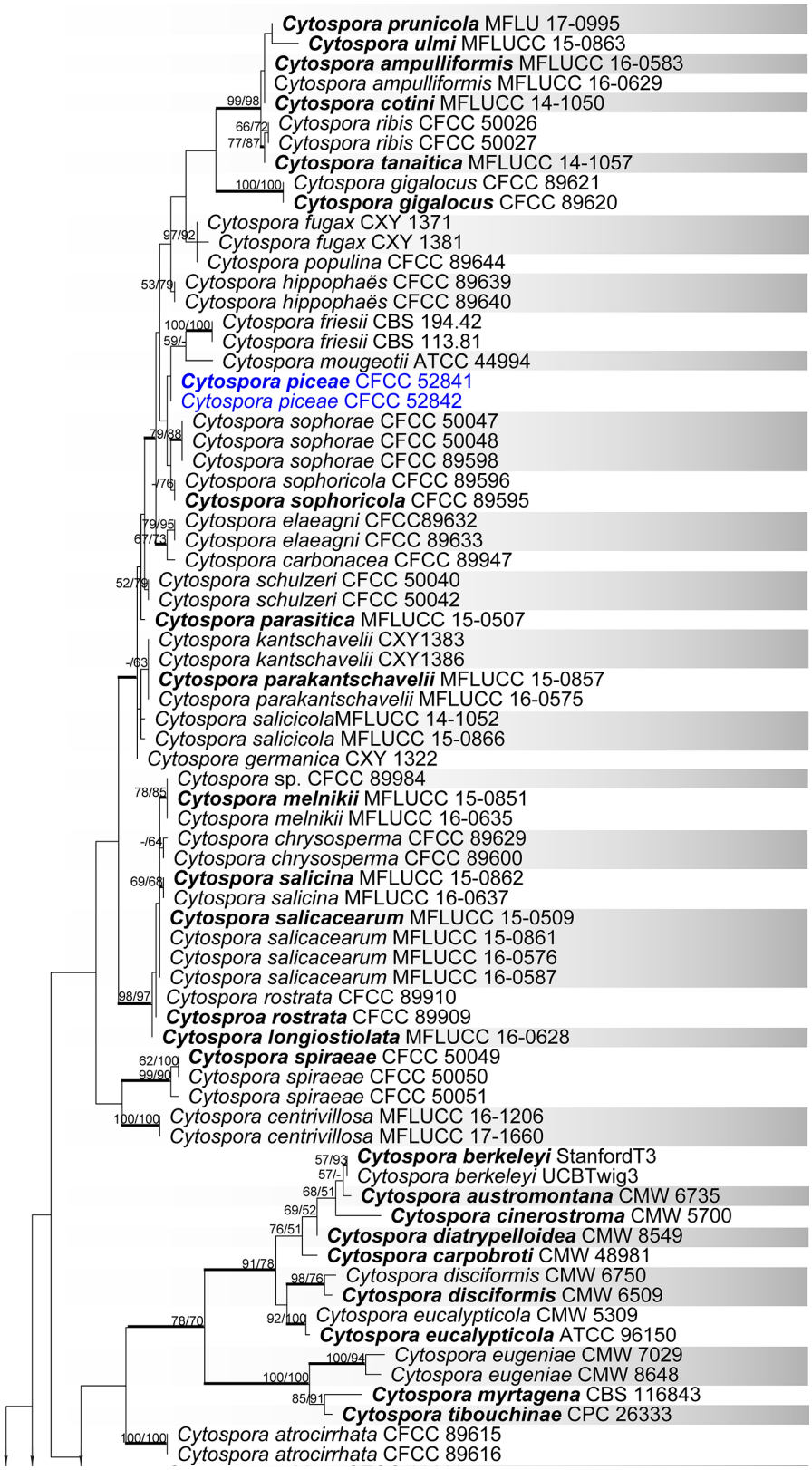

FIGURE 1. Phylogram of *Cytospora* based on ITS gene. MP and ML bootstrap support values above 50 % are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type strains are in bold. Strains in current study are in blue.

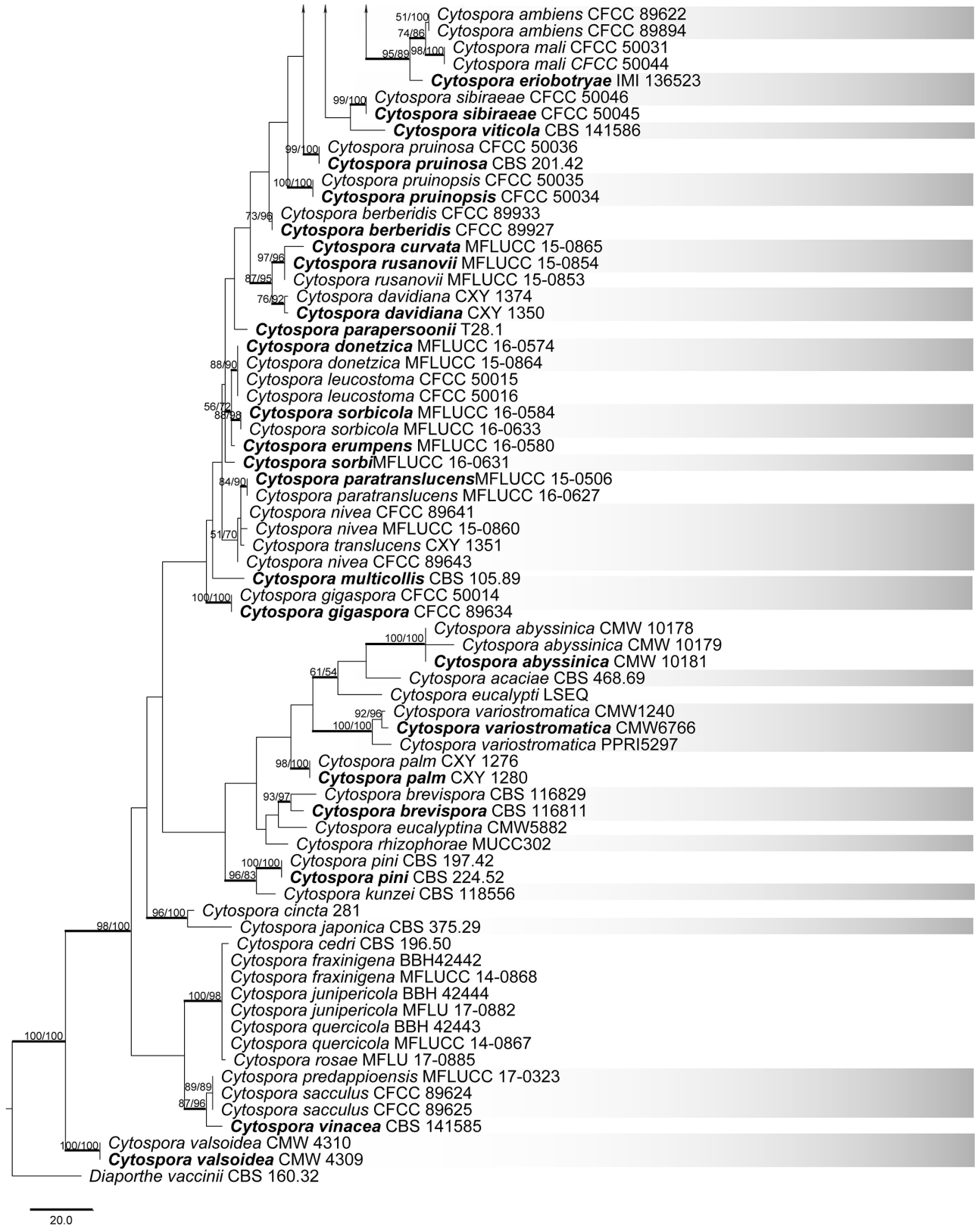

FIGURE 1 (Cont.)

Culture characteristics: Cultures on PDA are initially white, becoming saffron after one week. The colonies are tight, thin with a uniform texture, lacking aerial mycelium, up to 1.8 cm after four weeks. Sterile.

Materials examined:—**China, Xinjiang Uygur Autonomous Region**, Bole Mongol Autonomous Prefecture, 44°46’15.32”N, 81°13’57.54”E, from branches of *Picea crassifolia*, July 2017, C.M. Tian & X.L. Fan, deposited by X.L. Fan, CF 20176562, living culture CFCC 52842.

Notes:—*Cytospora piceae* is associated with canker disease of *Picea crassifolia.* The phylogenetic inferences resolved this species as a confused clade in ITS phylogram (Fig. 1), which was closed to *Cytospora friesii* and *C. mougeotii*. To clarify this clade, the second analysis indicated this species represented an individual clade with high support value (MP/ML/BI = 100/100/1) based on combined multilocus gene phylogenetic analysis, which was distinguish from other available species (Fig. 2). Morphologically, *Cytospora piceae* has larger conidia than those of *C. friesii* (5–5.5 × 1–1.5 vs. 4–5 × 1 µm), and wider than *C. mougeotii* (5–5.5 × 1–1.5 vs. 5–7 × 0.7–1 µm) (Saccardo 1884). *Cytospora piceae* is thus here considered as a novel species.

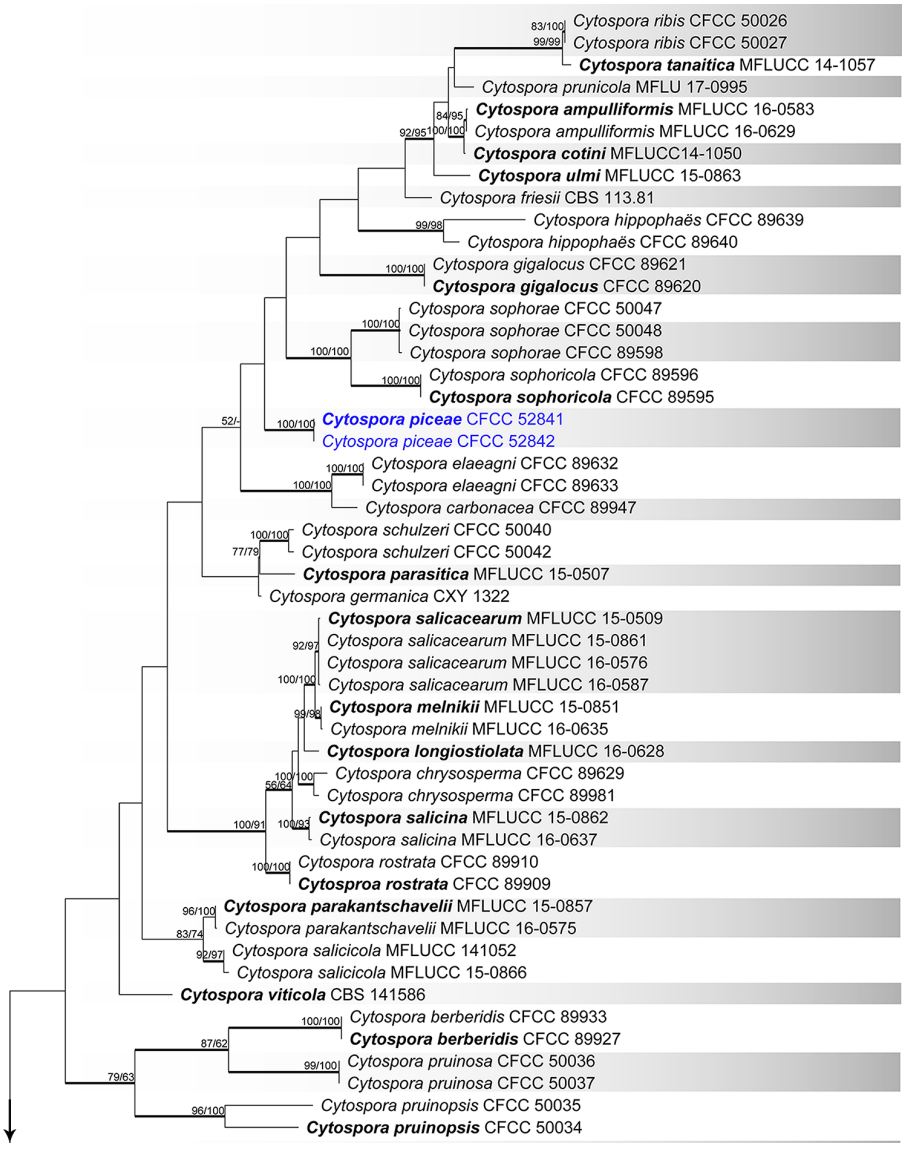

FIGURE 2. Phylogram of *Cytospora* based on combined ITS, LSU, ACT, RPB2 and TEF1-α genes. MP and ML bootstrap support values above 50 % are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type strains are in bold. Strains in current study are in blue.

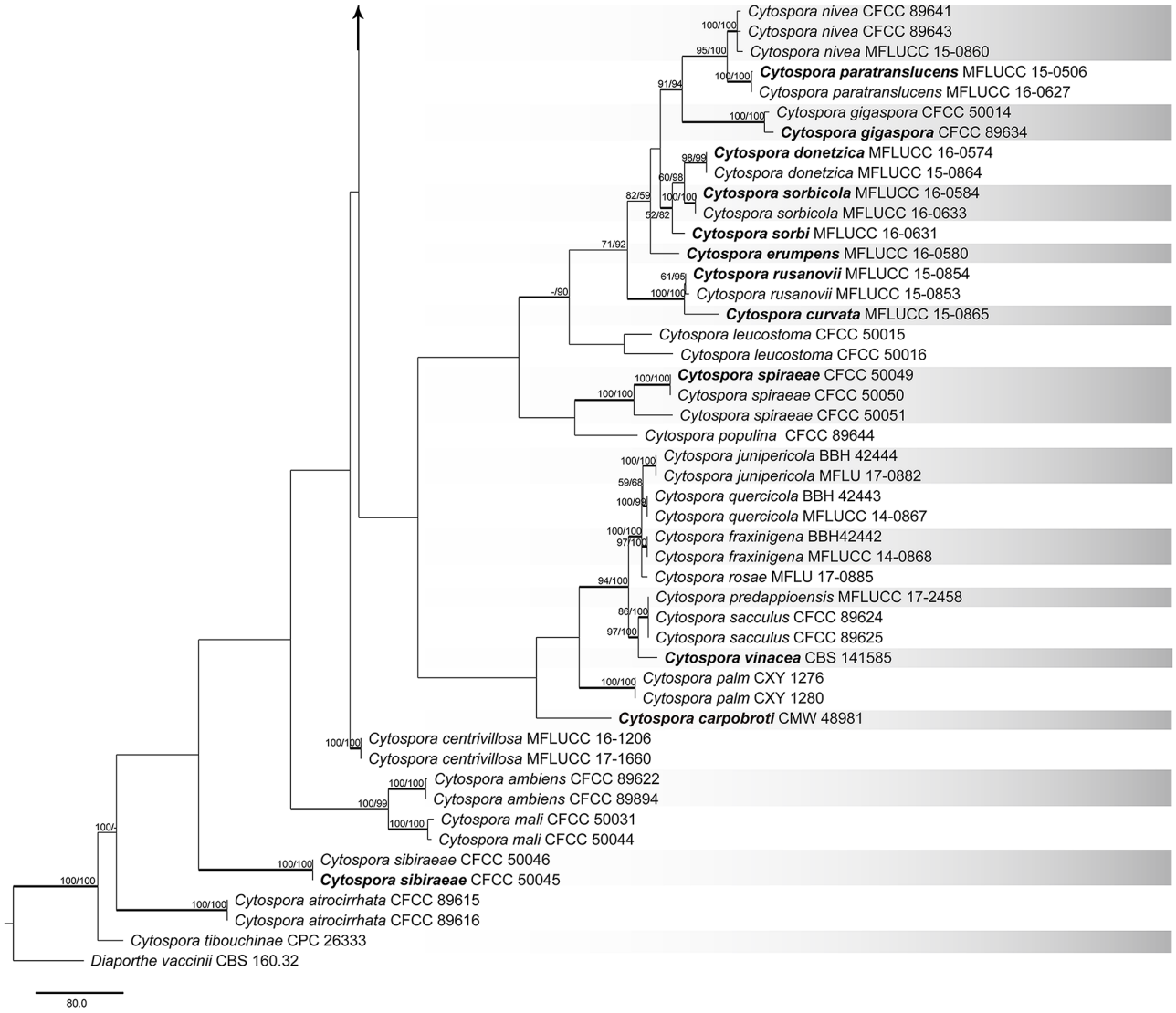

FIGURE 2 (Cont.)

Discussion

The current study introduced *Cytospora piceae sp. nov.* associated with stem cankers on *Picea crassifolia.* Although *C. piceae* indicated an ambiguous related group to *Cytospora friesii* and *C. mougeotii* in only ITS phylogram (Fig. 1), it is distinguished based on the vesicularly arranged locules, the size of conidia, and multilocus phylogenetic data as well as its unique host (Fig. 2). The results showed that *Cytospora* species still needs multilocus DNA data to define the criteria in species level, and we recommended the combination of five genes (ITS, LSU, ACT, RPB2 and TEF1-α) in genus *Cytospora*.

The *Cytospora* species is a weak parasitic or saprophytic fungus with strong ecological adaptability and a wide range of hosts. *Picea crassifolia* is an important ornamental tree species to afforestation in China, whereas few relative taxonomic studies of Cytospora canker or dieback disease from coniferous plants was performed, such as *Cytospora ambiens, C. friesii* (as *Valsa friesii*)*, C. kunzei*, *C. leucostoma* and *C. pini* (Adams *et al*. 2005). In China, *Cytospora* species from deciduous tree have attracted the attention by studies. *Cytospora* species from cankered apple and pear bark were examined and compared with morphology and rDNA-ITS sequences (Wang *et al.* 2007, 2011). Fan *et al.*

(2014) clarified and illustrated *C. chrysosperma*, *C. sophorae* and *C. sophoricola* from the *Sophora japonica* using only ITS region. Subsequently, the multilocus phylogeny has led to the illustrations and 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, Zhu *et al.* 2018). The current study implies that many additional undiscovered species of *Cytospora* from coniferous tree exist in China, and further studies are needed to discover the species of *Cytospora* associated with branch and twig dieback or canker disease in China and other countries.

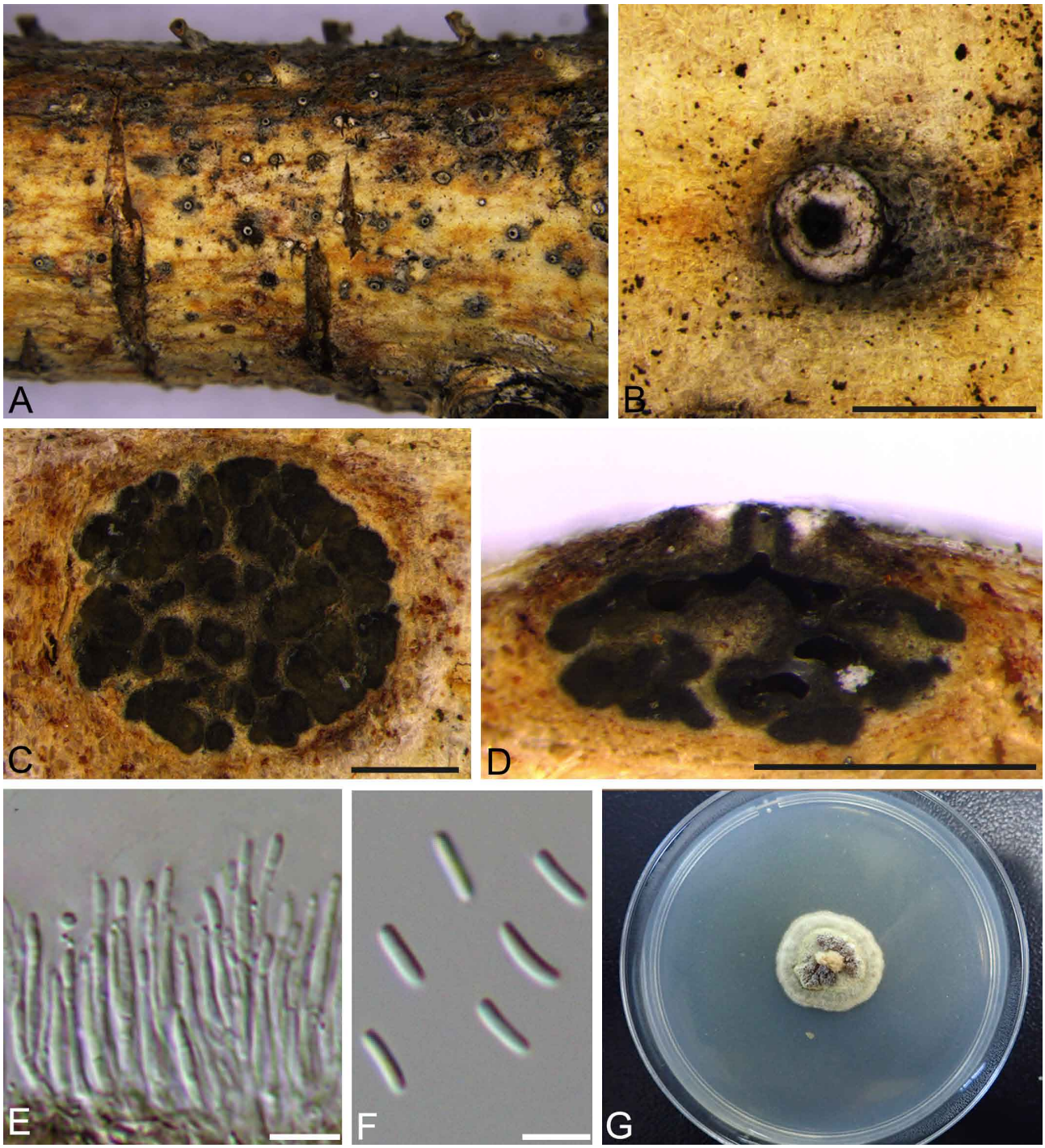

FIGURE 3. Morphology of *Cytospora piceae* from *Picea crassifolia* (CF 20176561). A, B: Habit of conidiomata on twig. C: Transverse section of conidioma. D: Longitudinal section through conidioma. E: Conidiophores and conidiogenous cells. F: Conidia. G: Colonies on PDA after two weeks. Scale bars: B–D = 500 µm; E–F = 5 µm.

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