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RESEARCH ARTICLE

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Cytospora elaeagnicola sp. nov. Associated with Narrow-leaved Oleaster Canker Disease in China

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ABSTRACT

Cytospora is a genus including important phytopathogens causing severe dieback and canker diseases distributed worldwide with a wide host range. However, identification of *Cytospora* species is difficult since the currently available DNA sequence data are insufficient. Aside the limited availability of ex-type sequence data, most of the genetic work is only based on the ITS region DNA marker which lacks the resolution to delineate to the species level in *Cytospora*. In this study, three fresh strains were isolated from the symptomatic branches of *Elaeagnus angustifolia* in Xinjiang Uygur Autonomous Region, China. Morphological observation and multi-locus phylogenetic analyses (ITS, LSU, ACT and RPB2) support these specimens are best accommodated as a distinct novel species of *Cytospora*. *Cytospora elaeagnicola* sp. nov. is introduced, having discoid, nearly flat, pycnidial conidiomata with hyaline, allantoid conidia, and differs from its relatives genetically and by host association.

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1. Introduction

The genus *Cytospora* contains important phytopathogens causing dieback and stem canker disease on multiple woody plants [1,2]. It was introduced by Ehrenberg in 1818 [3] and belonged to the family Cytosporaceae in Diaporthales [4]. This disease has globally caused great losses on ecologically and commercially important woody plants. *Cytospora* is characterized by the diaporthalean-like perithecial ascoma, clavate to elongate obovoid asci with allantoid, hyaline, aseptate ascospores in sexual state; and the single or labyrinthine locules, filamentous conidiophores, phialidic conidiogenous cells with allantoid, hyaline, aseptate conidia in the asexual state [2,5]. The asexual name *Cytospora* (1818) is an older name than all of the sexual synonyms *Valsa* (1849), *Leucocytospora* (1917), *Leucostoma* (1917), *Valsella* (1870) and *Valseutypella* (1919), and thus has the priority in nomenclature [2,6–8]. More than 610 species named *Cytospora* are listed at present in Index Fungorum (2019). However, the amount of species in *Cytospora* was with 110 estimated species [9]. Species criteria of *Cytospora* were previously based on host affiliations and morphology in China, however these bases are unreliable due to the uninformative illustrations and descriptions, weak host specificity and overlapping morphological characteristics [10–12]. Recent studies have reported updated phylograms for the genus *Cytospora* on the basis of multigene phylogenetic analyses using ex-type or reference strains [6,7,13–15]. However, because availability of the ex-type sequence data is limited to few species, identification of a strain to species level is very difficult. Recently, only 14 new species were included to this genus [16].

Elaeagnus angustifolia is a drought-resistant tree that is grown as a major biomass energy source [17], and has high medicinal and ecological value as well [18]. Furthermore, during an investigation of phytopathogens in north of China, most *E. angustifolia* trees were observed to suffer from dieback and stem canker caused by *Cytospora* species. In the current study, three representative *Cytospora* strains were collected from *Elaeagnus angustifolia* in Xinjiang Uygur Autonomous Region, China. Multilocus phylogenetic analyses using combination of ITS, LSU, ACT and RPB2 sequences confirmed finding of a new species in *Cytospora*. In this paper, *C. elaeagnicola* sp. nov. is introduced, accompanied with descriptions, illustrations and comparison with other species in the genus.

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2. Materials and methods

2.1. Sample collection and isolation

Fresh samples of *Cytospora* were collected from infected branches and stems of *E. angustifolia* during investigations of phytopathogens in Xinjiang Uygur Autonomous Region, China. The samples placed in paper bags were brought to the laboratory for processing and experimental purpose using the same methodology as in Fan et al. [14,15]. Single conidia were isolated by taking fruiting bodies and suspend the mucoid spore mass removed from conidiomata or ascomata in a drop of sterile water. The spore suspension from each sample was then spread over the surface of 1.8% potato dextrose agar (PDA) medium in a petri-dish and incubated at 25 °C. After 24 h, a single germinating conidium was transferred to a fresh PDA plate. Samples and isolates of the new species were deposited in the Museum of Beijing Forestry University (BJFC) and single-spore cultures in the China Forestry Culture Collection Center (CFCC).

2.2. Morphology observation

Samples were observed on infected plant tissues including the structure and size of fruiting bodies. The photographs of the macro-morphological characteristics were recorded using a Leica stereomicroscope (M205 FA) while the micro-morphological observations were determined under a Leica compound microscope (DM 2500) with differential interference contrast (DIC). Over 20 fruiting bodies were sectioned, both vertically and horizontally, and 50 conidia were selected randomly to get the measurement of their length and width. Cultural characteristics, including the colony characters and the production of pigment of isolates on PDA incubated at 25 °C in the dark were recorded, after 3, 7, and 30-days growth [19].

2.3. DNA extraction, PCR amplification, and sequencing

Fungal mycelium grown on the cellophane of PDA was scraped for the extraction of genomic DNA following a modified CTAB approach [20]. The ITS region was amplified with the primers ITS1 and ITS4 [21]; the LSU region with LR0R and LR7 [22]; the partial ACT region with ACT512F and ACT783R [23] and the RPB2 region with RPB2-5F and fRPB2-7cR [24]. The PCR amplicons were estimated visually by electrophoresis in 2% agarose gels. Fragments were sequenced in both directions using the respective primers and the BigDye Terminater v.3.1 Cycle Sequencing Kit (Applied Biosystems; Foster City, CA). Sequences were joined and quality was examined with Seqman v.7.1.0 in the DNASTAR lasergene core suite software (DNASTAR Inc.; Madison, WI).

2.4. DNA sequence analysis

Sequences based on ITS region and the combined dataset (ITS, LSU, ACT and RPB2) were aligned using MAFFT v.6 [25] and edited manually using MEGA6 [26], and some characters were excluded from both ends of the alignments to approximate the size of our sequences to those included in the dataset.

MP analysis was carried out by using PAUP v.4.0b10 with a heuristic search option of 1000 random-addition sequences with a tree bisection and reconnection (TBR) as the branch swapping algorithm [27]. Zero length branches were collapsed, whereas all equally parsimonious trees were saved. Stability of the clade was assessed with a bootstrap analysis of 1000 replicates [28]. Other measures calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC) [27]. ML analysis was carried out by using RAxML v.7.2.8 with a GTR + G + I model of site substitution, including estimation of gamma-distributed rate heterogeneity and a proportion of invariant sites [29]. And the branch support from MP and ML analyses was evaluated with a bootstrapping method of 1000 replicates [28].

BI analysis employing a Markov Chain Monte Carlo (MCMC) algorithm was performed using in MrBayes v.3.1.2 with the inverse gamma rates (GTR + I + G) nucleotide substitution model, which was selected based on the AIC criterion, using MrModeltest v.2.3 [30,31]. Two MCMC chains were run from random trees for 1,000,000 generations, and trees were sampled every 100th generation, resulting in 10,000 total trees. The first 25% of trees were discarded as the burn-in phase of the analysis and the Bayesian posterior probabilities (BPP) were calculated using the remaining 7500 trees [32].

In all analyses, *C. elaeagnicola* was selected as a distinct and new grape. Phylograms were examined in Figtree v.1.3.1 [33]. Novel sequence data was deposited in GenBank (Table 1), the multilocus sequences alignment file was deposited in TreeBASE (www.treebase.org) accession S24181 and the taxonomic novelty was deposited in MycoBank.

3. Results

3.1. Phylogeny

The ITS sequences of the three isolates of *Cytospora* from *E. angustifolia* were aligned with

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

Species	Strain	Host	GenBank accession numbers			
			ITS	LSU	ACT	RPB2
C. abyssinica	CMW 10181[T]	*Eucalyptus globulus*	AY347353	–	–	–
C. abyssinica	CMW 10178	*Eucalyptus globulus*	AY347354	–	–	–
C. acaciae	CBS 468.69	*Ceratonia siliqua*	DQ243804	–	–	–
C. ampulliformis	MFLUCC 16-0583[T]	*Sorbus intermedia*	KY417726	KY417760	KY417692	KY417794
C. ampulliformis	MFLUCC 16-0629	*Acer platanoides*	KY417727	KY417761	KY417693	KY417795
C. atrocirrhata	CFCC 89615	*Juglans regia*	KR045618	KR045700	KF498673	KU710946
C. atrocirrhata	CFCC 89616	*Juglans regia*	KR045619	KR045701	KF498674	KU710947
C. austromontana	CMW 6735[T]	*Eucalyptus pauciflora*	AY347361	–	–	–
C. berberidis	CFCC 89927[T]	*Berberis dasystachya*	KR045620	KR045702	KU710990	KU710948
C. berberidis	CFCC 89933	*Berberis dasystachya*	KR045621	KR045703	KU710991	KU710949
C. berkeleyi	StanfordT3[T]	*Eucalyptus globulus*	AY347350	–	–	–
C. berkeleyi	UCBTwig3	*Eucalyptus globulus*	AY347349	–	–	–
C. brevispora	CBS 116829	*Eucalyptus grandis*	AF192321	–	–	–
C. brevispora	CBS 116811[T]	*Eucalyptus grandis* × *tereticornis*	AF192315	–	–	–
C. carbonacea	CFCC 89947	*Ulmus pumila*	KR045622	KP310812	KP310842	KU710950
C. carpobroti	CMW 48981[T]	*Carpobrotus edulis*	MH382812	MH411216	–	–
C. cedri	CBS 196.50	–	AF192311	–	–	–
C. centrivillosa	MFLUCC 16-1206[T]	*Sorbus domestica*	MF190122	MF190068	–	MF377600
C. centrivillosa	MFLUCC 17-1660	*Sorbus domestica*	MF190123	MF190069	–	MF377601
C. chrysosperma	CFCC 89629	*Salix psammophila*	KF765673	KF765689	KF765721	KF765705
C. chrysosperma	CFCC 89981	*Populus alba* subsp. *pyramidalis*	MH933625	MH933660	MH933533	MH933597
C. chrysosperma	CFCC 89982	*Ulmus pumila*	KP281261	KP310805	KP310835	KU710952
C. cinerostroma	CMW 5700[T]	*Eucalyptus globulus*	AY347377	–	–	–
C. cincta	ATCC 32673	–	DQ996041	–	–	–
C. cotini	MFLUCC 14-1050[T]	*Cotinus coggygria*	KX430142	KX430143	–	KX430144
C. curvata	MFLUCC 15-0865[T]	*Salix alba*	KY417728	KY417762	KY417694	KY417796
C. davidiana	CXY 1350[T]	*Populus davidiana*	KM034870	–	–	–
C. davidiana	CXY 1374	*Populus davidiana*	KM034869	–	–	–
C. diatrypelloidea	CMW 8549[T]	*Eucalyptus globulus*	AY347368	–	–	–
C. disciformis	CMW 6509[T]	*Eucalyptus grandis*	AY347374	–	–	–
C. disciformis	CMW 6750	*Eucalyptus globulus*	AY347359	–	–	–
C. donetzica	MFLUCC 16-0574[T]	*Rosa* sp.	KY417731	KY417764	KY417696	KY417798
C. donetzica	MFLUCC 15-0864	*Crataegus monogyna*	KY417729	KY417763	KY417695	KY417797
C. elaeagni	CFCC 89632	*Elaeagnus angustifolia*	KR045626	KR045706	KU710995	KU710955
C. elaeagni	CFCC 89633	*Elaeagnus angustifolia*	KF765677	KF765693	KU710996	KU710956
C. elaeagnicola	**CFCC 52882[T]**	***Elaeagnus angustifolia***	**MK732341**	**MK732338**	**MK732344**	**MK732347**
C. elaeagnicola	**CFCC 52883**	***Elaeagnus angustifolia***	**MK732342**	**MK732339**	**MK732345**	**MK732348**
C. elaeagnicola	**CFCC 52884**	***Elaeagnus angustifolia***	**MK732343**	**MK732340**	**MK732346**	**MK732349**
C. eriobotryae	IMI 136523[T]	*Eriobotrya japonica*	AY347327	–	–	–
C. erumpens	MFLUCC 16-0580[T]	*Salix* × *fragilis*	KY417733	KY417767	KY417699	KY417801
C. eucalypti	LSEQ	*Sequoia sempervirens*	AY347340	–	–	–
C. eucalypticola	ATCC 96150[T]	*Eucalyptus nitens*	AY347358	–	–	–
C. eucalypticola	CMW 5309	*Eucalyptus grandis*	AF260266	–	–	–
C. eucalyptina	CMW 5882	*Eucalyptus grandis*	AY347375	–	–	–
C. eugeniae	CMW 7029	*Tibouchina* sp.	AY347364	–	–	–
C. eugeniae	CMW 8648	*Eugenia* sp.	AY347344	–	–	–
C. fraxinigena	BBH 42442	*Fraxinus ornus*	MF190134	MF190079	–	–
C. fraxinigena	MFLUCC 14-0868[T]	*Fraxinus ornus*	MF190133	MF190078	–	–
C. friesii	CBS 194.42	*Abies alba*	AY347328	–	–	–
C. fugax	CXY1371	*Populus simonii*	KM034852	–	–	–
C. fugax	CXY1381	*Populus ussuriensis*	KM034853	–	–	–
C. germanica	CXY1322	*Elaeagnus oxycarpa*	JQ086563	JX524617	–	–
C. gigaspora	CFCC 89620[T]	*Juglans regia*	KR045628	KR045708	KU710997	KU710957
C. gigaspora	CFCC 89621	*Juglans regia*	KR045629	KR045709	KU710998	KU710958
C. gigaspora	CFCC 50014	*Juniperus procumbens*	KR045630	KR045710	KU710999.	KU710959
C. gigaspora	CFCC 89634[T]	*Salix psammophila*	KF765671	KF765687	KU711000	KU710960
C. hippophaës	CFCC 89639	*Hippophae rhamnoides*	KR045632	KR045712	KU711001	KU710961
C. hippophaës	CFCC 89640	*Hippophae rhamnoides*	KF765682	KF765698	KF765730	KU710962
C. japonica	CBS 375.29	*Prunus persicae*	AF191185	–	–	–
C. junipericola	BBH 42444	*Juniperus communis*	MF190126	MF190071	–	–
C. junipericola	MFLU 17-0882[T]	*Juniperus communis*	MF190125	MF190072	–	–
C. kantschavelii	CXY1383	*Populus maximowiczii*	KM034867	–	–	–
C. kantschavelii	CXY1386	*Populus maximowiczii*	KM034867	–	–	–
C. kunzei	CBS 118556	*Pinus radiata*	DQ243791	–	–	–
C. leucosperma	CFCC 89622	*Pyrus bretschneideri*	KR045616	KR045698	KU710988	KU710944
C. leucosperma	CFCC 89894	*Pyrus bretschneideri*	KR045617	KR045699	KU710989	KU710945
C. leucostoma	CFCC 50016	*Sorbus aucuparia*	MH820400	MH820393	MH820408	–
C. leucostoma	CFCC 50015	*Sorbus pohuashanensis*	KR045634	KR045714	KU711002	–
C. longiostiolata	MFLUCC 16-0628[T]	*Salix* × *fragilis*	KY417734	KY417768	KY417700	KY417802
C. mali	CFCC 50031	*Crataegus* sp.	KR045636	KR045716	KU711004	KU710965
C. mali	CFCC 50044	*Malus baccata*	KR045637	KR045717	KU711005	KU710966
C. melnikii	CFCC 89984	*Rhus typhina*	MH933644	MH933678	MH933551	MH933609
C. melnikii	MFLUCC 15-0851[T]	*Malus domestica*	KY417735	KY417769	KY417701	KY417803
C. melnikii	MFLUCC 16-0635	*Populus nigra*	KY417736	KY417770	KY417702	KY417804
C. mougeotii	ATCC 44994	*Picea abies*	AY347318	–	–	–
C. multicollis	CBS 105.89[T]	*Quercus ilex* subsp. *rotundifolia*	DQ243803	–	–	–

(*continued*)

Table 1. Continued.

Species	Strain	Host	GenBank accession numbers			
			ITS	LSU	ACT	RPB2
C. myrtagena	CBS 116843[T]	*Tibouchiina urvilleana*	AY347363	–	–	–
C. nivea	MFLUCC 15-0860	*Salix acutifolia*	KY417737	KY417771	KY417703	KY417805
C. nivea	CFCC 89641	*Elaeagnus angustifolia*	KF765683	KF765699	KU711006	KU710967
C. nivea	CFCC 89643	*Salix psammophila*	KF765685	–	–	KU710968
C. palm	CXY1276	*Cotinus coggygria*	JN402990	–	–	–
C. palm	CXY1280[T]	*Cotinus coggygria*	JN411939	–	–	–
C. parakantschavelii	MFLUCC 15-0857[T]	*Populus × sibirica*	KY417738	KY417772	KY417704	KY417806
C. parakantschavelii	MFLUCC 16-0575	*Pyrus pyraster*	KY417739	KY417773	KY417705	KY417807
C. parapersoonii	T28.1[T]	*Prunus persicae*	AF191181	–	–	–
C. parasitica	MFLUCC 15-0507[T]	*Malus domestica*	KY417740	KY417774	KY417706	KY417808
C. paratranslucens	MFLUCC 15-0506[T]	*Populus alba* var. *bolleana*	KY417741	KY417775	KY417707	KY417809
C. paratranslucens	MFLUCC 16-0627	*Populus alba*	KY417742	KY417776	KY417708	KY417810
C. pini	CBS 197.42	*Pinus Sylvestirs*	AY347332	–	–	–
C. pini	CBS 224.52[T]	*Pinus strobus*	AY347316	–	–	–
C. populina	CFCC 89644[T]	*Salix psammophila*	KF765686	KF765702	KU711007	KU710969
C. predappioensis	MFLUCC 17-2458[T]	*Platanus* sp.	MG873484	MG873480	–	–
C. pruinopsis	CFCC 50034[T]	*Ulmus pumila*	KP281259	KP310806	KP310836	KU710970
C. pruinosa	CFCC 50035	*Ulmus pumila*	KP281260	KP310807	KP310837	KU710971
C. pruinosa	CFCC 50036	*Syzygium aromaticum*	KP310800	KP310802	KP310832	–
C. pruinosa	CFCC 50037	*Syzygium aromaticum*	MH933650	MH933685	MH933558	–
C. prunicola	MFLU 17-0995[T]	*Prunus* sp.	MG742350	MG742351	MG742353	MG742352
C. quercicola	MFBBH 42443	*Quercus* sp.	MF190128	MF190074	–	–
C. quercicola	MFLUCC 14-0867[T]	*Quercus* sp.	MF190129	MF190073	–	–
C. rhizophorae	MUCC302	*Eucalyptus grandis*	EU301057	–	–	–
C. ribis	CFCC 50026	*Ulmus pumila*	KP281267	KP310813	KP310843	KU710972
C. ribis	CFCC 50027	*Ulmus pumila*	KP281268	KP310814	KP310844	–
C. rosae	MFLU 17-0885[T]	*Rosa canina*	MF190131	MF190075	–	–
C. rostrata	CFCC 89909[T]	*Salix cupularis*	KR045643	KR045722	KU711009	KU710974
C. rostrata	CFCC 89910	*Salix cupularis*	KR045644	KR045723	KU711010	KU710975
C. rusanovii	MFLUCC 15-0853	*Populus × sibirica*	KY417743	KY417777	KY417709	KY417811
C. rusanovii	MFLUCC 15-0854[T]	*Salix babylonica*	KY417744	KY417778	KY417710	KY417812
C. sacculus	CFCC 89624	*Juglans regia*	KR045645	KR045724	KM401888	KU710976
C. sacculus	CFCC 89625	*Juglans regia*	KF225616	KM401887	KM401889	–
C. salicacearum	MFLUCC 15-0509[T]	*Salix alba*	KY417746	KY417780	KY417712	KY417814
C. salicacearum	MFLUCC 15-0861	*Salix × fragilis*	KY417745	KY417779	KY417711	KY417813
C. salicacearum	MFLUCC 16-0587	*Prunus cerasus*	KY417748	KY417782	KY417714	KY417816
C. salicicola	MFLUCC 15-0866	*Salix alba*	KY417749	KY417783	KY417715	KY417817
C. salicicola	MFLUCC 14-1052[T]	*Salix alba*	KU982636	KU982635	KU982637	–
C. salicina	MFLUCC 15-0862[T]	*Salix alba*	KY417750	KY417784	KY417716	KY417818
C. salicina	MFLUCC 16-0637	*Salix × fragilis*	KY417751	KY417785	KY417717	KY417819
C. schulzeri	CFCC 50040	*Malus domestica*	KR045649	KR045728	KU711013	KU710980
C. schulzeri	CFCC 50042	*Malus asiatica*	KR045650	KR045729	KU711014	KU710981
C. sibiraeae	CFCC 50045[T]	*Sibiraea angustata*	KR045651	KR045730	KU711015	KU710982
C. sibiraeae	CFCC 50046	*Sibiraea angustata*	KR045652	KR045731	KU711015	KU710983
C. sophorae	CFCC 50047	*Styphnolobium japonicum*	KR045653	KR045732	KU711017	KU710984
C. sophorae	CFCC 89598	*Styphnolobium japonicum*	KR045654	KR045733	KU711018	KU710985
C. sophoricola	CFCC 89596	*Styphnolobium japonicum*	KR045656	KR045735	KU711020	KU710987
C. sophoricola	CFCC 89595[T]	*Styphnolobium japonicum* var.	KR045655	KR045734	KU711019	KU710986
C. sorbi	MFLUCC 16-0631[T]	*Sorbus aucuparia*	KY417752	KY417786	KY417718	KY417820
C. sorbicola	MFLUCC 16-0584[T]	*Acer pseudoplatanus*	KY417755	KY417789	KY417721	KY417823
C. sorbicola	MFLUCC 16-0633	*Cotoneaster melanocarpus*	KY417758	KY417792	KY417724	KY417826
C. spiraeae	CFCC 50049[T]	*Spiraea salicifolia*	MG707859	MG707643	MG708196	MG708199
C. spiraeae	CFCC 50050	*Spiraea salicifolia*	MG707860	MG707644	MG708197	MG708200
C. tanaitica	MFLUCC 14-1057[T]	*Betula pubescens*	KT459411	KT459412	KT459413	–
C. tibouchinae	CPC 26333[T]	*Tibouchina semidecandra*	KX228284	KX228335	–	–
C. translucens	CXY1351	*Populus davidiana*	KM034874	–	–	–
C. ulmi	MFLUCC 15-0863[T]	*Ulmus minor*	KY417759	–	–	–
C. valsoidea	CMW 4309[T]	*Eucalyptus grandis*	AF192312	–	–	–
C. valsoidea	CMW 4310	*Eucalyptus grandis*	AF192312	–	–	–
C. variostromatica	CMW 6766[T]	*Eucalyptus globulus*	AY347366	–	–	–
C. variostromatica	CMW 1240	*Eucalyptus grandis*	AF260263	–	–	–
C. vinacea	CBS 141585[T]	*Vitis interspecific*	KX256256	–	–	–
C. viticola	CBS 141586[T]	*Vitis vinifera*	KX256239	–	–	–
Diaporthe vaccinii	CBS 160.32	*Vaccinium macrocarpon*	KC343228	–	JQ807297	–

All the new isolates used in this study are indicated in bold type and the strains from type materials are marked by an superscript (T).

available ITS sequences from related *Cytospora* species of published articles, resulting in an alignment containing 138 *Cytospora* ingroup strains and a total of 609 characters including gaps. In the alignment, 369 characters were constant, 72 variable characters were parsimony-uninformative and 168 characters were variable and parsimony-informative. MP analyses generated 145 parsimonious trees, one of which is presented in Figure 1 (TL = 927, CI = 0.409, RI = 0.830, RC = 0.339). ML and BI analyses resolved results similar to the MP tree. *C. elaeagnicola* represented a monophyletic clade with overall high bootstrap support values (MP/ML/BI = 99/100/1; marked in blue in

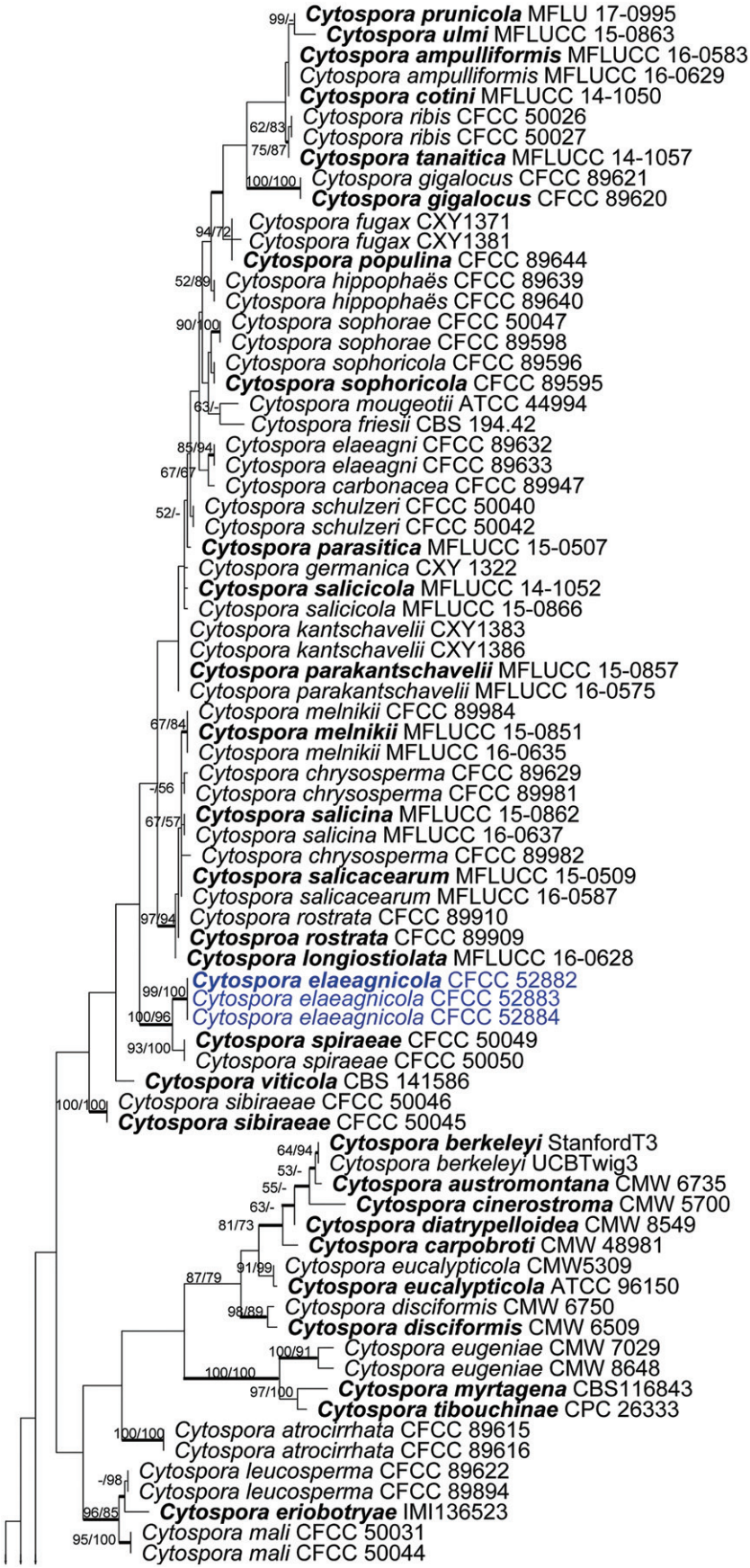

Figure 1. Phylogram of *Cytospora* based on ITS gene. MP and ML bootstrap support values above 50% are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type strains are in bold. Strains in current study are in blue.

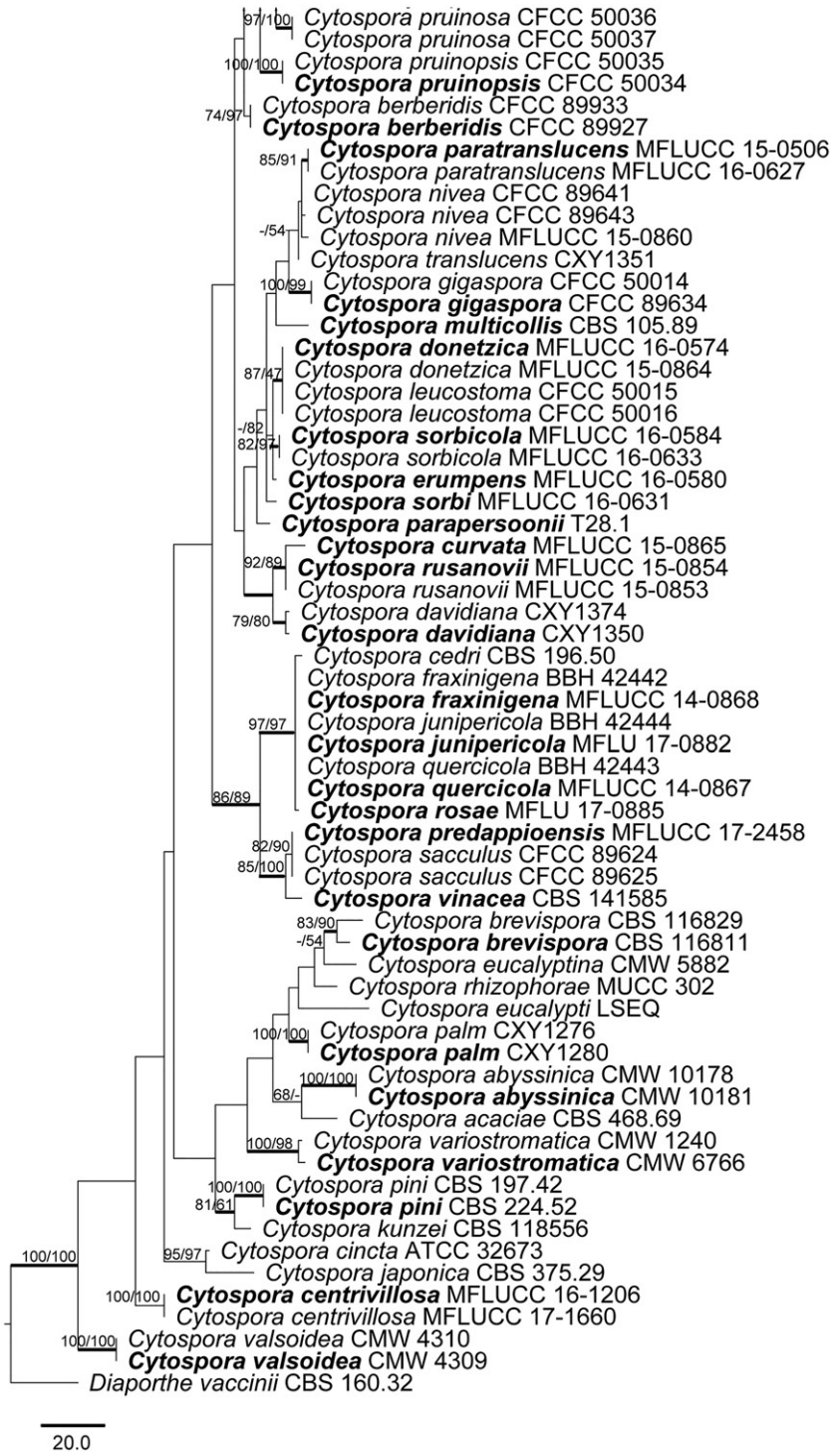

Figure 1. Continued

Figure 1). Subsequently, phylogenetic analyses were performed based on a concatenated alignment of ITS, LSU, ACT and RPB2 from published articles, comprised of 102 *Cytospora* ingroup strains with a total of 2207 characters including gaps. In the alignment, 1538 characters were constant, 104 variable characters were parsimony-uninformative and 565 characters were variable and parsimony-informative. MP analysis generated 105 parsimonious trees, one of which is presented in Figure 1 (TL = 2,350, CI = 0.412, RI = 0.827, RC = 0.341). ML and BI analyses were similar to the MP tree. *Cytospora elaeagnicola* represented a monophyletic clade with full support values (MP/ML/BI = 100/100/1) (marked in blue in Figure 2).

3.2. Taxonomy

Cytospora elaeagnicola X.L. Fan sp. nov. Figure 3

Mycobank: MB830292.

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

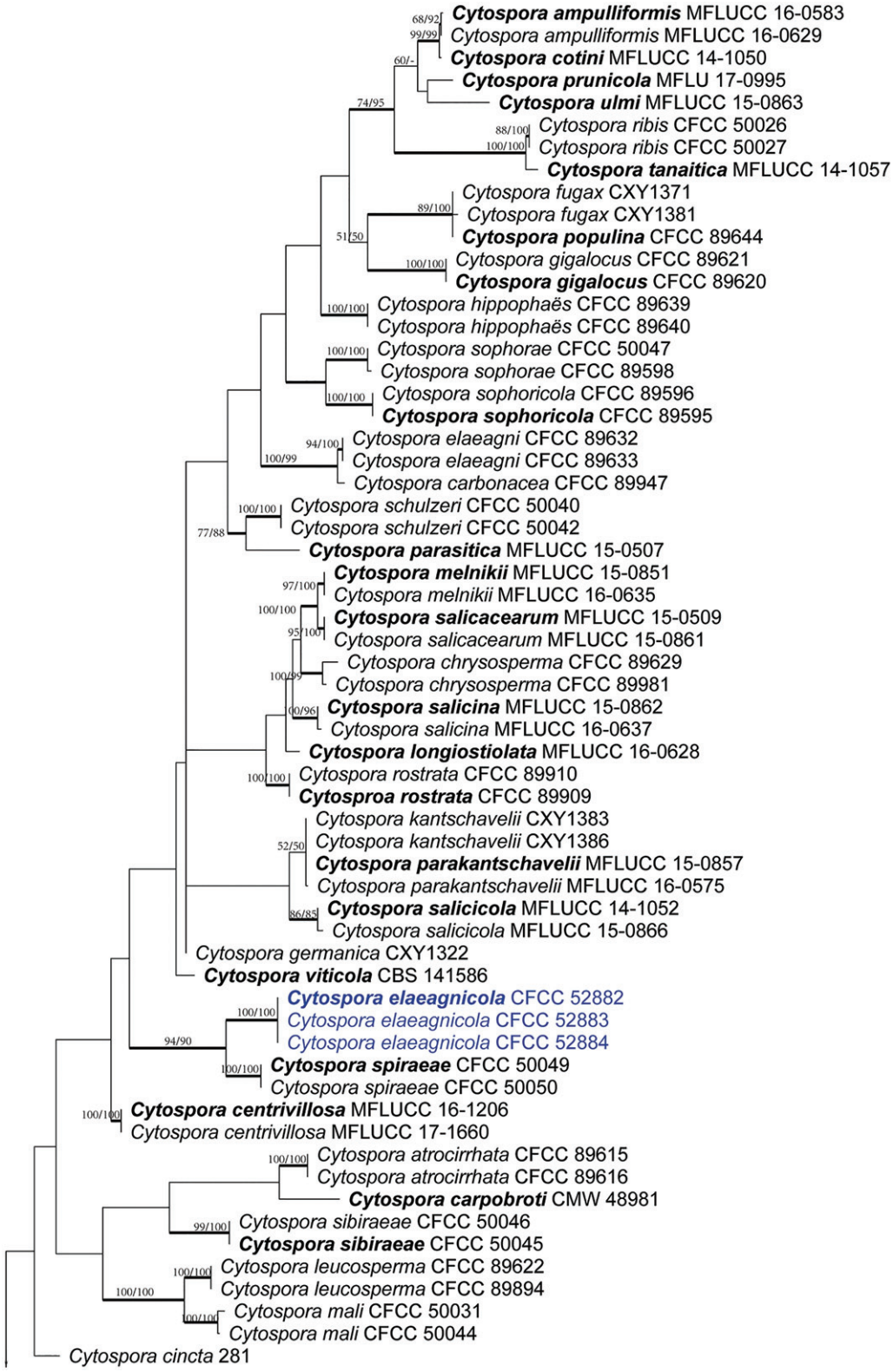

Figure 2. Phylogram of *Cytospora* based on combined ITS, LSU, ACT and RPB2 genes. MP and ML bootstrap support values above 50% are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type strains are in bold. Strains in current study are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

Holotype: CF 20175831.

Host/Distribution: from branches of *Elaeagnus angustifolia* in China.

Descriptions: Asexual state: Conidiomata pycnidial, ostiolate, discoid, nearly flat, immersed in bark, scattered, producing black area on bark, erumpent through the surface of bark when mature. Locules multiple, circular to ovoid, arranged irregularly with common walls, (890–)905–1160(–1240) µm ($\bar{x}$ = 1060 ± 120 µm, n = 30) in diameter. Conceptacle absent. Ectostromatic disc iron grey to violaceous black, circular, disc dark, (160–)170–310(–350) µm ($\bar{x}$ = 240 ± 60 µm, n = 30) in diameter, with one ostiole in the centre of disc. Ostiole conspicuous, circular to ovoid, iron grey to violaceous

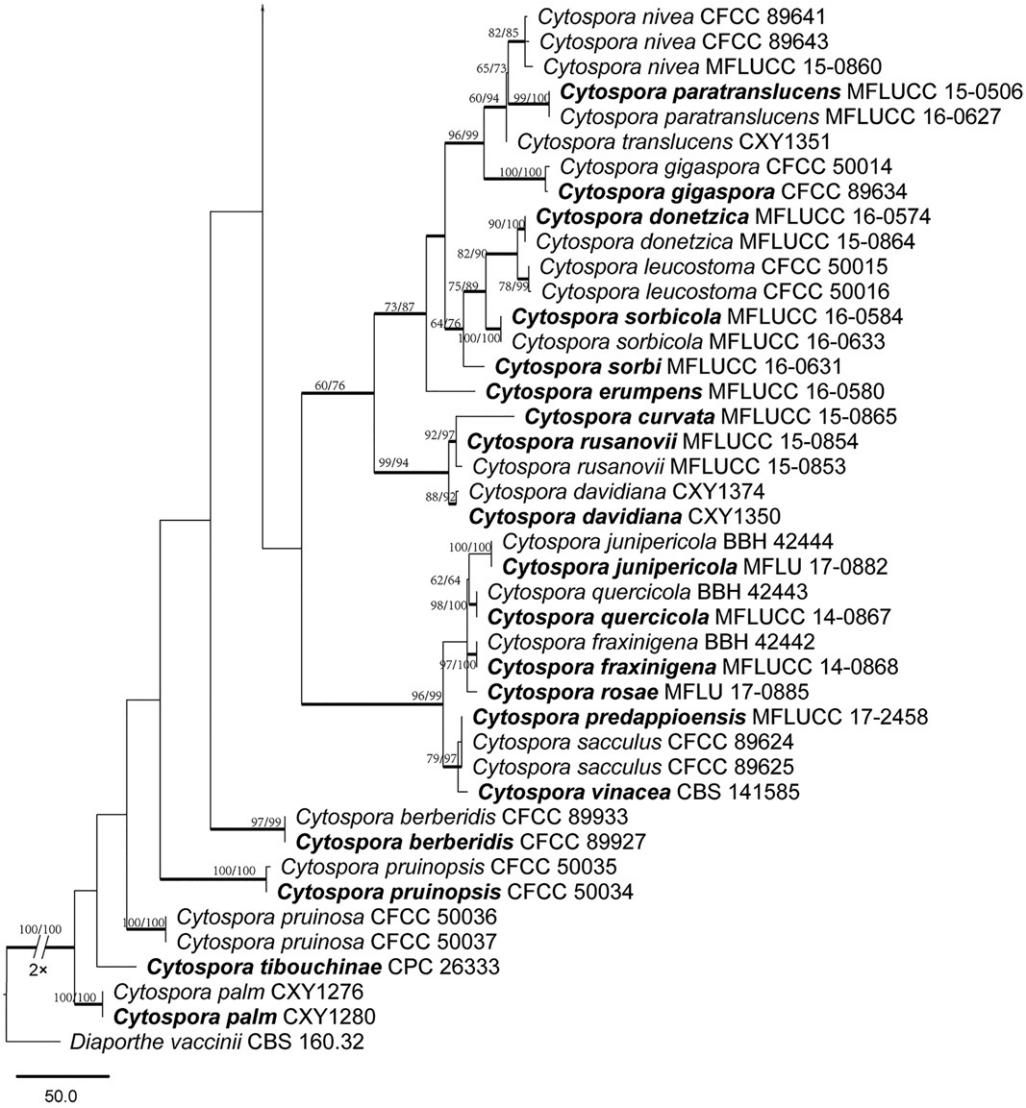

Figure 2. Continued

black at the same level as the disc, (48–)51–71(–78) µm ($\bar{x}$ = 60 ± 11 µm, n = 30) in diameter. 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, phialidic. Conidia hyaline, allantoid, eguttulate, smooth, aseptate, thin-wall, 5.5–6.5(–7) × (1–)1.5–2 µm ($\bar{x}$ = 6.1 ± 0.4 × 1.6 ± 0.1 µm, n = 50). Sexual morph: not observed.

Culture characteristics: On PDA, cultures are white. The colony is flat, felt-like with a thick texture at the center with thin surrounding texture. Pycnidia are sparse, distributed irregularly on medium surface.

Materials examined: China, Xinjiang Uygur Autonomous Region, Bole Mongol Autonomous Prefecture, Provincial Road 202, 45°06'29.50"N, 82°33'32.82"E, from branches of *Elaeagnus angustifolia*, July 2017, C.M. Tian & X.L. Fan, deposited by X.L. Fan, holotype CF 20175831, ex-type living culture CFCC 52882; *ibid.* CF 20175832, living culture CFCC 52883; CF 20175833, living culture CFCC 52884.

Notes: *Cytospora elaeagnicola* is associated with canker disease of *Elaeagnus angustifolia*. The phylogenetic inferences resolved this species as an individual clade both in ITS and combined multigene phylograms (Figures 1 and 2), which was closed to *C. spiraeae* from *Spiraea salicifolia*. Morphologically, *Cytospora elaeagnicola* has obvious symptoms with black area on bark, and smaller conidia (5.5–6.5 × 1.5–2 vs. 7–8 × 2–2.5 µm) as compared with *C. spiraeae*; the cultures of *C. elaeagnicola* are white, differing from the cultures of *C. spiraeae* which becomes fawn after 7–10 days [34]. Considering the clearly distinction between these two species based on molecular phylogenetic position and on the host affiliation, *Cytospora elaeagnicola* is thus described as a novel species.

4. Discussion

In the current study, *C. elaeagnicola* sp. nov. was described from infected branches and twigs of *E.*

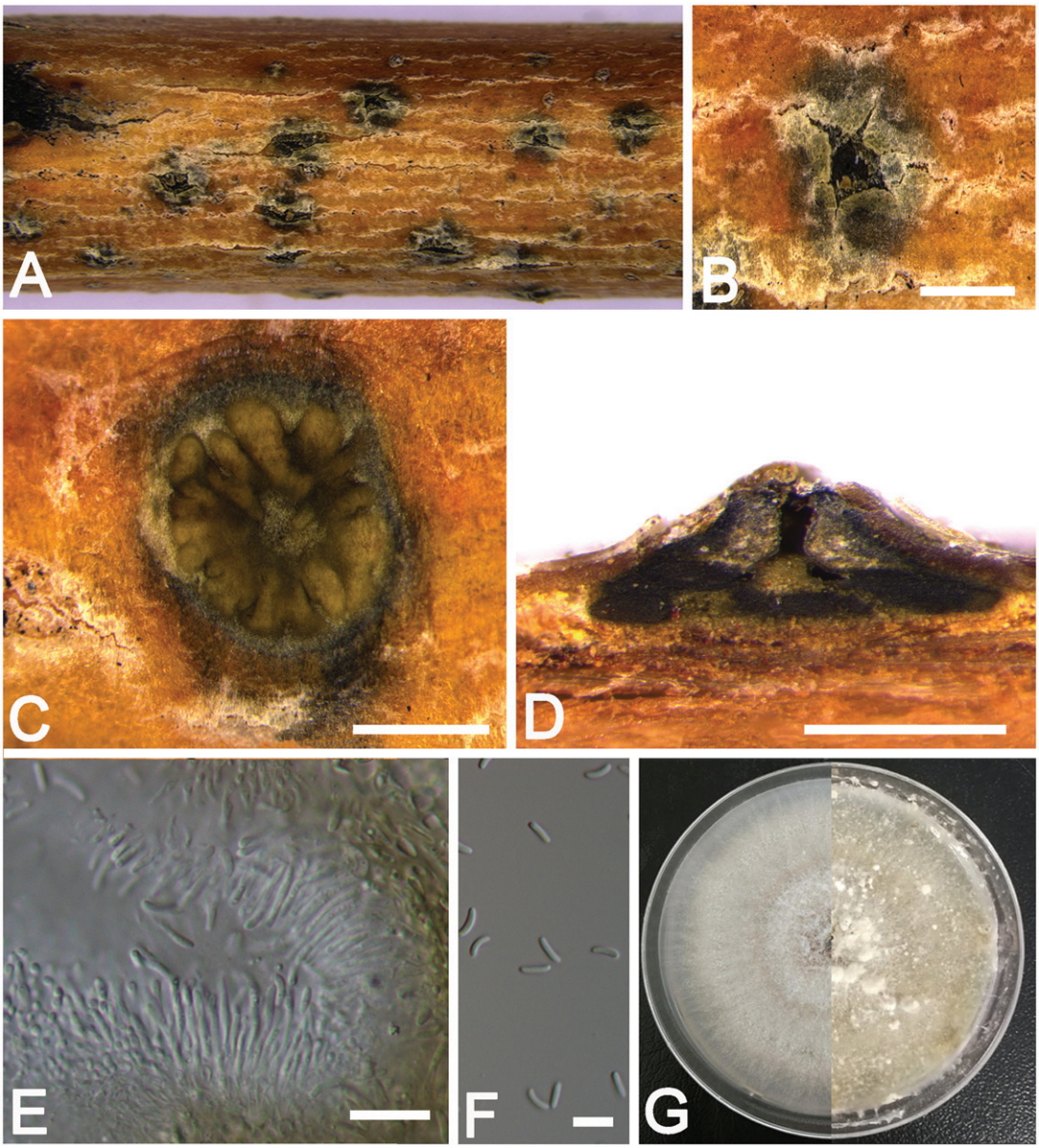

Figure 3. Morphology of *Cytospora elaeagnicola* from *Elaeagnus angustifolia* (CF 20175831). (A), (B) Habit of conidiomata on twig; (C) Transverse section of conidioma; (D) Longitudinal section through conidioma; (E) Conidiophores and conidiogenous cells; (F) Conidia; (G) Colonies on PDA after 3 d and 14 d (scale bars: B–C = 250 µm, D = 200 µm, E = 10 µm, F = 5 µm).

angustifolia in northwest region of China, an area that has undergone desertification at an alarming rate. Previously, Fan et al. [7] described *C. elaeagni* and *C. nivea* from *E. angustifolia* during the investigation of canker disease of three anti-desertification plants. Compared to *C. elaeagnicola*, *C. elaeagni* has smaller locules (630–920 µm) with larger conidia (6.3–9.3 × 2–2.9 µm) and dense cultures producing light brown pigment; *C. nivea* has obvious dark black conceptacle surrounding the conidiomata with larger conidia (6.2–9.2 × 1.7–2.4 µm), and cultures producing dark green to black pigment [7]. These morphological deviations are in line with the combined phylogenetic analyses which resolved *C. elaeagnicola* as a separate, highly supported clade, both in the single ITS analyses and the concatenated analyses.

Cytospora species were previously identified by host association and morphological characteristics. However, the uninformative illustrations and descriptions, overlapping morphological characteristics and low host-specificity have caused confusion in the identification of strains. Current study indicated more than one species of *Cytospora* are present on one host plant. In the future study, the taxonomy requires fresh collections from wide geographical ranges with comprehensive pathogenicity tests. Further studies are also needed in the clarification of the species diversity and in the understanding of their roles in plant diseases, especially for anti-desertification plants such as *E. angustifolia* in Northwestern China.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Spielman LJ. Taxonomy and biology of *Valsa* species on hardwoods in North America, with special reference to species on maples. New York, US: Cornell University; 1983.
[2] Adams GC, Wingfield MJ, Common R, et al. Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (Ascomycota, Diaporthales, Valsaceae) from Eucalyptus. Stud Mycol. 2005;52:1–144.
[3] Ehrenberg CG. Sylvae Mycologicae Berolinenses. Berlin: Formis Theophili Bruschcke;1818.
[4] Wijayawardene NN, Hyde KD, Lumbsch HT, et al. Outline of Ascomycota: 2017. Fungal Divers. 2018; 88:167–263.
[5] Spielman LJ. A monograph of *Valsa* on hardwoods in North America. Can J Bot. 1985;63: 1355–1378.
[6] Fan XL, Hyde KD, Liu M, et al. *Cytospora* species associated with walnut canker disease in China, with description of a new species C. *gigalocus*. Fungal Biol. 2015;119:310–319.
[7] Fan XL, Hyde KD, Yang Q, et al. *Cytospora* species associated with canker disease of three anti-desertification plants in northwestern China. Phytotaxa. 2015;197:227–244.
[8] Rossman AY, Adams GC, Cannon PF, et al. Recommendations of generic names in Diaporthales competing for protection or use. IMA Fungus. 2015;6:145–154.
[9] Kirk PM, Cannon PF, Minter DW, et al. Ainsworth & Bisby's Dictionary of the Fungi, 10th edn. Wallingford, UK: CABI; 2008.
[10] Deng SQ. Fungi of China. Beijing, China;1963.
[11] Tai FL. Sylloge Fungorum Sinicorum. Beijing, China;1979.
[12] Wei JC. Identification of Fungus Handbook. Shanghai, China; 1979.
[13] Zhang YB, You CJ, Fan XL, et al. Taxonomy and phylogeny of *Cytospora* in Northeast China. Mycosystema. 2014;33:806–818.
[14] Fan XL, Liang YM, Ma R, et al. Morphological and phylogenetic studies of *Cytospora* (Valsaceae, Diaporthales) isolates from Chinese scholar tree, with description of a new species. Mycoscience. 2014;55:252–259.
[15] Fan XL, Tian CM, Yang Q, et al. *Cytospora* from *Salix* in northern China. Mycotaxon. 2014;129:303–315.
[16] Norphanphoun C, Doilom M, Daranagama DA, et al. Revisiting the genus *Cytospora* and allied species. Mycosphere. 2017;8:51–97.
[17] Chang W, Sui X, Fan XX, et al. Arbuscular mycorrhizal symbiosis modulates antioxidant response and ion distribution in salt-stressed *Elaeagnus angustifolia* seedlings. Front Microbiol. 2018;9:625.
[18] Mahboubi M. *Elaeagnus angustifolia* and its therapeutic applications in osteoarthritis. Ind Crops Prod. 2018;121:36–45.
[19] Rayner RW. A mycological colour chart. Kew, UK: Commonwealth Mycological Institute; 1970.
[20] Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13–15.
[21] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds), PCR Protocols: a Guide to Methods and Applications. London: Academic Press; 1990. pp. 315–322.
[22] Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol. 1990;172:423.
[23] Carbone I, Kohn L. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia. 1999;91:553–556.
[24] Liu YL, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molec Biol Evol. 1999;16: 1799–1808.
[25] Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molec Biol Evol. 2013; 30:772–780.
[26] Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol and Evol. 2013;30:2725–2729.
[27] Swofford DL. PAUP*: Phylogenetic analysis using parsimony, * and other methods. Version 4.0b10. Sinauer Associates, Sunderland; 2003.
[28] Hillis DM, Bull JJ. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol. 1993;42:182–192.
[29] Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006; 22:2688–2690.
[30] Posada D, Crandall KA. Modeltest: testing the model of DNA substitution. Bioinformatics. 1998; 14:817–818.
[31] Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003;19:1572–1574.
[32] Rannala B, Yang Z. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J Mol Evol. 1996;43:304–311.
[33] Rambaut A, Drummond A. FigTree v.1.3.1. Institute of evolutionary biology. Edinburgh, UK: University of Edinburgh; 2010.
[34] Zhu HY, Fan XL, Tian CM. Multigene phylogeny and morphology reveal *Cytospora spiraeae*. Phytotaxa. 2018;338:49–62.