



# *Cytospora* (*Diaporthales*) in China

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## Key words

canker disease  
new taxa  
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*Valsa*

**Abstract** Members of the genus *Cytospora* are often reported as endophytes, saprobes or phytopathogens, primarily causing canker diseases of woody host plants. They occur on a wide range of hosts and have a worldwide distribution. Although several species have in the past been reported from China, the vast majority are not known from culture or DNA phylogeny. The primary aim of the present study was thus to clarify the taxonomy and phylogeny of a large collection of *Cytospora* species associated with diverse hosts in China. *Cytospora* spp. were collected in northeast, northwest, north and southwest China, indicating that the cold and dry environments favour these fungi. In this paper, we provide an assessment of 52 *Cytospora* spp. in China, focussing on 40 species represented by 88 isolates from 28 host genera. Based on a combination of morphology and a six-locus phylogeny (ITS, LSU, *act1*, *rpb2*, *tef1-α* and *tub2*), 13 new species and one new combination are introduced. The majority of the species investigated here appear to be host-specific, although further collections and pathogenicity studies will be required to confirm this conclusion.

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## INTRODUCTION

Members of *Cytospora* are cosmopolitan in distribution and have often been regarded as phytopathogens, endophytes or saprobes occurring on a broad host range. Several species have been reported as pathogens causing severe branch or trunk disease on monocotyledonous, dicotyledonous and gymnosperm hosts (e.g., *Anacardiaceae*, *Elaeagnaceae*, *Fabaceae*, *Juglandaceae*, *Myrtaceae*, *Rosaceae*, *Salicaceae*, *Ulmaceae*) (Adams et al. 2005, 2006, Mehrabi et al. 2011, Fan et al. 2014a, b, 2015a, b, Zhang et al. 2014, Jami et al. 2018).

As plant pathogens, *Cytospora* species are primarily associated with canker diseases, although other maladies have also been reported such as root rot of Chinese jujube and collar rot of pomegranate (Du et al. 2013, Palavouzis et al. 2015). *Cytospora* canker symptoms include elongate, slightly sunken and discoloured areas in the bark, which often splits along the canker margin. Symptoms vary with host species and stage of disease development (Fig. 1). Diseased inner-bark and the bark above the infected cambium may appear sunken and yellow, brown, reddish brown, grey or black, becoming watery and odorous as the tissues deteriorate. Wood below the cambium is stained brown. Histopathologically, the rapid colonisation of the cortex and phloem are via wide intercellular hyphae, while the cell contents are digested by narrower intracellular hyphae following the forming of chambers (Fig. 2). Later, these fungi quickly girdle and kill branches and twigs, forming several prominent black sporocarps (Fig. 1). Species of *Cytospora* have a single or labyrinthine of locules (and/or diaporthalean-like perithecia), filamentous conidiophores (and/or clavate to elongate obovoid asci) and allantoid, hyaline conidia (and/or ascospores) (Spielman 1983, 1985, Adams et al. 2005). Under

moist conditions, the conidia emerge from the fructifications in the form of yellow, orange to red gelatinous tendrils (Fig. 1).

## Classification and history

The ascomycete order *Diaporthales* (*Sordariomycetes*) is well-known to contain fungal phytopathogens, endophytes and saprobes, with wide distributions and broad host ranges (Castlebury et al. 2002, Rossman et al. 2007, Fan et al. 2018). Members of this order cause various serious diseases, e.g., chestnut blight disease caused by *Cryphonectria parasitica* (Gryzenhout et al. 2006); stem-end rot of citrus fruits infected by *Diaporthe citri* (Huang et al. 2013); poplar and willow canker disease caused by *Cytospora chrysosperma* (Fan et al. 2014b), birch canker and dieback caused by *Melanconis stilbostoma* (Fan et al. 2016), leading to severe ecological and economic losses worldwide. The *Diaporthales* is characterised by forming brown to black perithecial ascomata immersed in stromata or substrata, and a diaporthalean-type centrum development, i.e., lacking true paraphyses, and having unitunicate asci that commonly float free at maturity, often with a refractive ring at the apex (Barr 1978, Castlebury et al. 2002, Rossman et al. 2007, Voglmayr et al. 2012, Fan et al. 2018). Recent studies accepted 30 families in *Diaporthales*, of which *Cytosporaceae* contains more than 600 species epithets (Senanayake et al. 2017, 2018, Fan et al. 2018, Crous et al. 2019, Guterres et al. 2019, Xavier et al. 2019).

*Cytosporaceae* was introduced by Fries (1825) but later placed in synonymy under *Valsaceae* (1861), only to be resurrected again as family name for this complex (*Cytosporaceae*; Rossman et al. 2015). Nitschke (1867) published the first important study of the family including four genera, i.e., *Anthostoma*, *Diaporthe*, *Thyridium* and *Valsa* (= *Cytospora*). Von Höhnelt (1918) believed the subfamily *Valseen* comprised six allantoid-spored genera, i.e., *Leucostoma*, *Peroneutypa*, *Scoptria*, *Valsa*, *Valsella* and *Valseutypella*. Nannfeldt (1932) elevated *Valseen* to ordinal level as *Valsales*, but Gilman et al. (1957) considered these taxa as the subfamily *Valseae* within *Diaporthaceae*, comprising *Endothia*, *Fenestella*, *Valsa*, *Valsaria* and *Valsella*, a decision which was adopted by Dennis (1968) and Kobayashi

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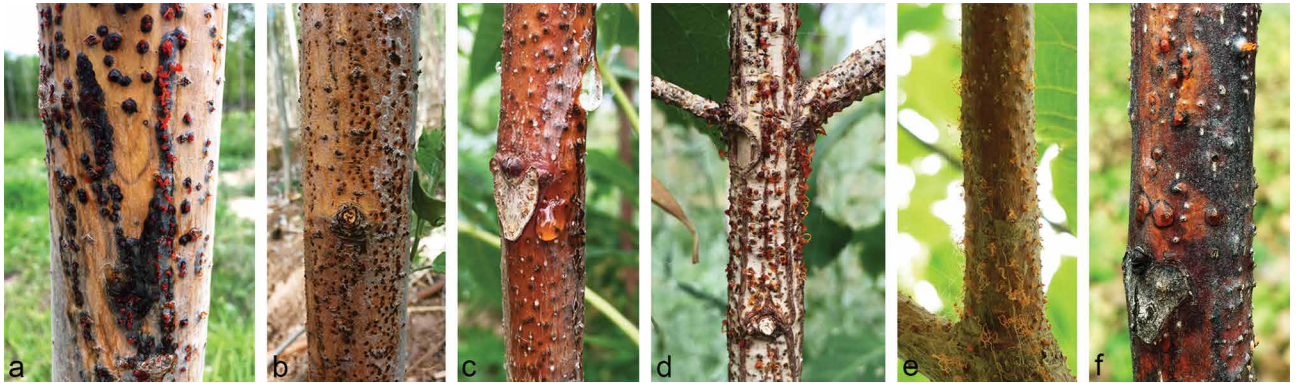


Fig. 1 Disease symptoms associated with *Cytospora* spp. a–b. *Populus alba* subsp. *pyramidalis*; c. *Ailanthus altissima*; d. *Euonymus maackii*; e–f. *Juglans regia*.

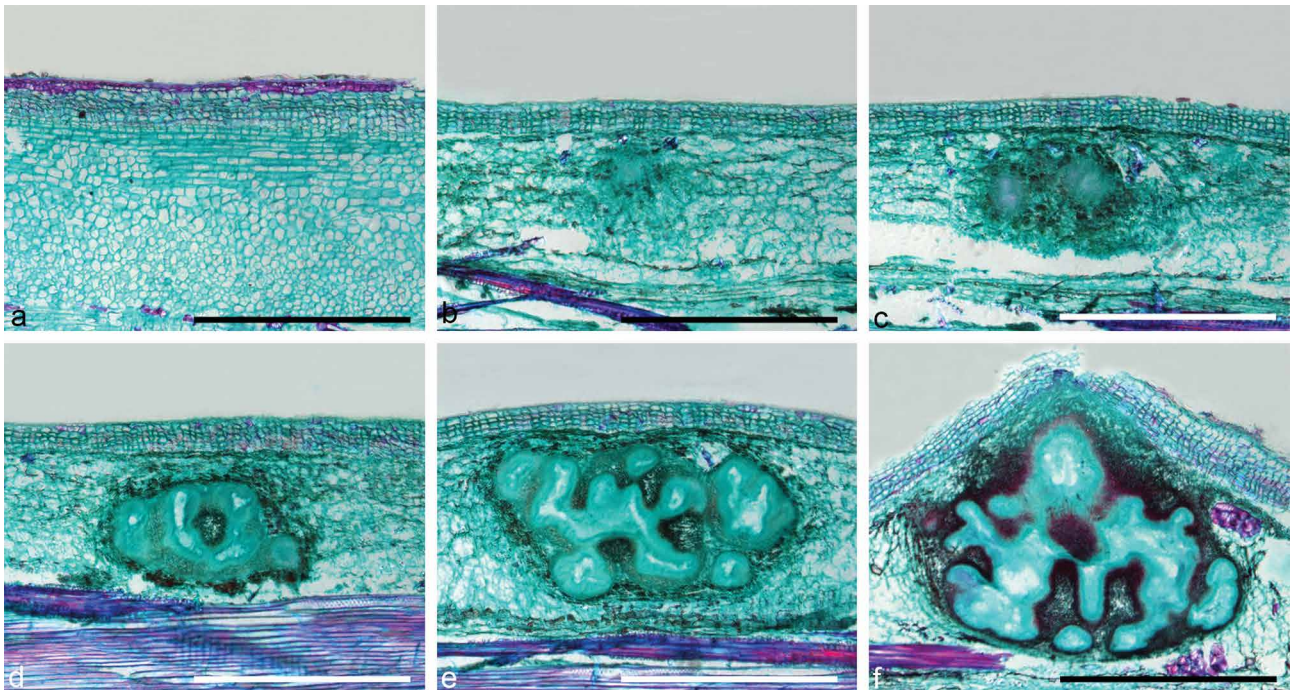


Fig. 2 Infection process of *Cytospora chrysosperma* on *Populus alba* subsp. *pyramidalis*. a. Uninfected tissues; b. initial stage of pycnidial development (aggregation of mycelial cells in the cortex tissues) (6 d); c. formation of locules in the centre of pycnidial primordium (10 d); d–e. combination of adjacent locules (12 d); f. mycelial mass above locules rupturing the cuticle and forming an ostiole through the apical rupture (15 d). — Scale bars = 0.5 mm.

(1970). Wehmeyer (1975) separated *Valsaceae* from *Diaporthaceae* with *Leucostoma*, *Glomerella*, *Scoptria*, *Valsa* and *Valsella*. Dennis (1978) merged almost all allantoid-spored genera (including *Leucostoma*, *Valsa* and *Valsella*) into the *Diatripaceae*. Barr (1978) recognised the connection of *Valsaceae* and *Diaporthaceae* based on the characters of ascomata, and treated *Diaporthaceae* as synonym of *Valsaceae*, including 15 genera (*Amphiporthe*, *Apioplagoistoma*, *Clypeoporthella*, *Cryphonectria*, *Cryptodiaporthe*, *Diaporthe*, *Hypospilina*, *Leucostoma*, *Linospora*, *Ophiovalsa*, *Plagiosphaera*, *Plagiostoma*, *Pleuroceras*, *Valsa* and *Valsella*). Most genera from Barr's system have since been separated in different families in *Diaporthales* (*Amphiporthe*, *Apioplagoistoma*, *Cryptodiaporthe* (now *Plagiostoma*), *Plagiostoma*, *Pleuroceras* in *Gnomoniaceae*; *Cryphonectria*, *Endothia* in *Cryphonectriaceae*; *Clypeoporthella* (now *Diaporthe*), *Diaporthe* in *Diaporthaceae*; *Linospora*, *Ophiovalsa* (now *Cryptosporella*) in *Gnomoniaceae*), and some genera are not classified in *Diaporthales* (*Anthostoma*, *Fenestella*, *Glomerella* (now *Colletotrichum*), *Peroneutypa*, *Scoptria* (now *Eutypella*), *Thyridium* and *Valsaria*) or remain unresolved (*Hypospilina*, *Plagiosphaera*) (Senanayake et al. 2017, 2018, Fan et al. 2018). Castlebury et al. (2002) suggested

three genera (*Leucostoma*, *Valsa* and *Valsella*) in *Valsaceae* based on an LSU phylogeny, while Adams et al. (2005) treated *Cytospora* and *Valsa* in this family, regarding the sexual genera (*Leucocytospora*, *Leucostoma*, *Valsella* and *Valseutypella*) as synonyms of *Valsa*. Based on the one fungus = one name initiative (Wingfield et al. 2012), Fan et al. (2015a, b) and Rossman et al. (2015) recommended to use *Cytospora*, the oldest name having priority over *Valsa*, and *Cytosporaceae* having priority over *Valsaceae*. Senanayake et al. (2017) regarded *Cytospora*, *Pachytrype*, *Paravalsa*, *Waydora* and *Xenotypa* as genera of *Cytosporaceae*.

*Cytospora* was introduced by Ehrenberg (1818) with four species (*C. betulina*, *C. epimyces*, *C. resinae* and *C. ribis*). Fries (1823) described 18 *Cytospora* species, but he considered it as *Cytispora* (orthographic variant). Saccardo (1884) revised the name to *Cytospora* and recorded 144 species. Gvritshvili (1982) and Spielman (1983, 1985) regarded the morphologically similar genera *Cytophoma*, *Cytospora*, *Cytosporopsis*, *Lamyella*, *Leucocytospora* and *Torsellia* as six sections based on the structure of their conidiomatal locules. The first molecular phylogeny of *Cytospora* was inferred from ITS sequences which suggested six groups, with a new species *C. parapersoonii*



(*Leucostoma parapersonii*) (Adams et al. 2002). Adams et al. (2005) formally treated these genera as synonyms of *Cytospora* with eight sections, and described 28 *Cytospora* species from *Eucalyptus* based on ITS phylogeny and morphology. Several recent papers have subsequently described new species of *Cytospora* using multi-locus DNA sequence data (Wang et al. 2013, Zhang et al. 2014, Fan et al. 2014a, b, 2015a, b, Yang et al. 2015, Norphanphoun et al. 2017, Senanayake et al. 2017, 2018, Jami et al. 2018, Zhu et al. 2018).

### Nomenclature of *Cytospora* and associated sexual morphs

*Cytospora* has priority over several sexual genera in the previous dual-nomenclature system, i.e., *Leucocytospora*, *Leucostoma*, *Valsa*, *Valsella* and *Valseutypella* (Adams et al. 2005, Fan et al. 2015a, b, Rossman et al. 2015). The type species of *Cytospora*, *C. chrysosperma* (sexual morph *Valsa sordida*), is the most common species causing *Cytospora* cankers on *Salicaceae*, especially in China (Fan et al. 2014b), having a remarkably wide host range. The genus *Valsa*, typified by *V. ambiens*, is commonly associated with its *Cytospora* asexual morph (Spielman 1985, Hayova & Minter 1998). The type species of *Leucostoma*, *L. massarianum*, clustered within the genus *Cytospora*, and was regarded as synonym (Adams et al. 2002, 2005). Adams et al. (2005) also listed *Leucocytospora* (based on *L. corni*) as a synonym of *Cytospora*. *Leucostoma* and *Leucocytospora* were previously distinguished from *Valsa* by an obvious dark conceptacle delimiting the stroma from host tissue (Adams et al. 2005). The genus *Valsella* (based on *V. salicis*) is characterised by a leucostoma-like conceptacle, and more than eight ascospores per ascus. This species was considered as a synonym of *Cytospora fertilis* and grouped with other *Cytospora* species (Castlebury et al. 2002). The type species of *Valseutypella* (based on *V. tristicha*), was recognised by the presence of a parenchymatous stroma surrounding its perithecia (Fries 1823, Saccardo 1884, Spielman 1985, Adams et al. 2005). Adams et al. (2005) regarded these genera as synonyms of *Valsa*, which are now classified under the oldest name, *Cytospora* (Fan et al. 2015a, b, Rossman et al. 2015).

### Identification and species numbers

In the past, species identification in *Cytospora* was largely based on host affiliation, with morphological descriptions. Several species of *Cytospora* associated with canker diseases have been systematically studied using a geography- or host-centred strategy. Adams et al. (2005) combined morphology and phylogeny using ITS sequence data to describe 28 species of *Cytospora* from *Eucalyptus*, of which 11 species were new to science. Adams et al. (2006) described 14 additional species from South Africa using the same methodology. Fotouhifar et al. (2010) identified 12 species from Iran using morphology and ITS phylogeny. Mehrabi et al. (2011) used morphology to identify six species of *Cytospora* from apple trees in Iran and also described their sexual morphs. Although more than 600 *Cytospora* species epithets have been listed in Index Fungorum (<http://www.indexfungorum.org/>; 2018), and 110 were recognised in Kirk et al. (2008), details pertaining to ex-type strains of *Cytospora* are available for only a few species. Donk (1964) selected *C. chrysosperma* as the lectotype species, but to date there are no living cultures linked to the type specimen.

In China, previous *Cytospora* records were based on old literature, generally lacking cultures (Teng 1963, Tai 1979, Wei 1979, Zhuang 2005). Recent identifications were performed mainly according to the ITS rDNA gene (Wang et al. 2013, Zhang et al. 2014, Fan et al. 2014a, b, 2015a, b, Yang et al. 2015, Zhu et al. 2018). To facilitate species recognition of *Cytospora* in China, a phylogenetic backbone is urgently required. The objectives of the present study were:

- i. to clarify species boundaries among *Cytospora* isolates from various host genera distributed over 12 provinces in China;
- ii. to provide a multi-gene phylogeny for the genus *Cytospora* based on a large set of freshly collected specimens in China;
- iii. to link Chinese *Cytospora* names to recent collections based on epitypification; and
- iv. to elucidate host specificity and the relationship between *Cytospora* species and their respective host plants.

## MATERIALS AND METHODS

### Isolates

Fresh specimens exhibiting *Cytospora* canker disease symptoms were collected from infected branches or twigs of 28 host genera (40 species) during collecting trips in 12 Provinces in China (Table 1). A total of 88 isolates were established by removing a mucoid spore mass from conidiomata and/or ascomata, 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. Specimens are deposited in the Museum of the Beijing Forestry University (BJFC) and the working Collection of X.L. Fan (CF) housed at the Beijing Forestry University. Axenic cultures are maintained in the China Forestry Culture Collection Centre (CFCC).

### DNA isolation, amplification and sequencing

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions, from fungal mycelium growing on PDA. The internal transcribed spacer (ITS) region was amplified with the primers ITS1 and ITS4 (White et al. 1990), the nuclear ribosomal large subunit (LSU) region with the primers LR0R and LR7 (Vilgalys & Hester 1990), the partial actin (*act1*) region was amplified using primers ACT512F and ACT783R (Carbone & Kohn 1999), the partial RNA polymerase II subunit (*rpb2*) region with primers RPB2-5F and rRPB2-7cR (Liu et al. 1999), the partial translation elongation factor 1-alpha (*tef1-α*) gene with the primers EF1-728F and EF1-986R (Carbone & Kohn 1999) and the partial beta-tubulin (*tub2*) gene was amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995). The PCR mixture for all genes consisted of 1 μL genomic DNA, 3 mM MgCl<sub>2</sub>, 20 μM of each dNTP, 0.2 μM of each primer and 0.25 U rTaq DNA polymerase (TAKARA). Conditions for PCR of ITS, LSU and *tef1-α* genes constituted an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 48 °C and 1 min at 72 °C, and a final denaturation step of 8 min at 72 °C. Conditions for *act1* and *tub2* genes constituted an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C, and a final denaturation step of 8 min at 72 °C. For the *rpb2* gene, the amplification consisted of five cycles of 45 s at 95 °C, 45 s at 56 °C and 2 min at 72 °C, then five cycles with 53 °C annealing temperature and 30 cycles with 50 °C annealing temperature. The PCR products were sequenced in both 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.

### Phylogenetic analyses

DNA sequencing electropherograms generated by each primer combination were assembled using Seqman v. 7.1.0 in the DNASTAR Lasergene core suite software (DNASTAR Inc.,

Table 1 Details of the species and isolates included in this study.

Species	Strain <sup>1</sup>	Host	Origin	GenBank accession numbers					tub2
				ITS	LSU	act1	rpb2	tef1- $\alpha$	
<i>Cytophora acaciae</i>	CBS 468.69	Ceratonia siliqua fruit	Spain	DQ243804	NA	NA	NA	NA	NA
<b><i>Cytophora ailanthicola</i></b>	<b>CFCC 89970<sup>T</sup></b>	<i>Ailanthus altissima</i>	Ningxia, China	MH933618	MH933653	MH933526	MH933592	MH933494	MH933565
<i>Cytophora abyssinica</i>	CMW 10181 <sup>T</sup>	<i>Eucalyptus globulus</i>	Ethiopia	AY347353	NA	NA	NA	NA	NA
	CMW 10178	<i>Eucalyptus globulus</i>	Ethiopia	AY347354	NA	NA	NA	NA	NA
	CMW 10179	<i>Eucalyptus globulus</i>	Ethiopia	AY347352	NA	NA	NA	NA	NA
<i>Cytophora ampulliformis</i>	MFLUCC 16-0583 <sup>T</sup>	<i>Sorbus intermedia</i>	Russia	KY417726	KY417760	KY417692	KY417794	NA	NA
	MFLUCC 16-0629	<i>Acer platanoides</i>	Russia	KY417727	KY417761	KY417693	KY417795	NA	NA
<i>Cytophora amygdali</i>	CBS 144233 <sup>T</sup>	<i>Prunus dulcis</i>	California, USA	MG971853	NA	MG972002	NA	MG971659	MG971718
<b><i>Cytophora atrocirrhatta</i></b>	<b>CFCC 89615</b>	<i>Juglans regia</i>	Qinghai, China	KR045618	KR045700	KF498673	KU710946	KP310858	KR045659
	<b>CFCC 89616</b>	<i>Juglans regia</i>	Qinghai, China	KR045619	KR045701	KF498674	KU710947	KP310859	KR045660
	22	<i>Salix excelsa</i>	Iran	EF447305	NA	NA	NA	NA	NA
<i>Cytophora austromontana</i>	CMW 6735 <sup>T</sup>	<i>Eucalyptus pauciflora</i>	Australia	AY347361	NA	NA	NA	NA	NA
<b><i>Cytophora beilimensis</i></b>	<b>CFCC 50493<sup>T</sup></b>	<i>Pinus armandii</i>	Beijing, China	MH933619	MH933654	MH933527	NA	MH933495	MH933561
	<b>CFCC 50494</b>	<i>Pinus armandii</i>	Beijing, China	MH933620	MH933655	MH933528	NA	MH933496	MH933562
<b><i>Cytophora berberidis</i></b>	<b>CFCC 89927<sup>T</sup></b>	<i>Berberis dasystachya</i>	Qinghai, China	KR045620	KR045702	KU710990	KU710948	KU710913	KR045661
	<b>CFCC 89933</b>	<i>Berberis dasystachya</i>	Qinghai, China	KR045621	KR045703	KU710991	KU710949	KU710914	KR045662
<i>Cytophora berkeleyi</i>	StanfordT3 <sup>T</sup>	<i>Eucalyptus globulus</i>	USA	AY347350	NA	NA	NA	NA	NA
	UCBTwig3	<i>Eucalyptus globulus</i>	USA	AY347349	NA	NA	NA	NA	NA
<i>Cytophora brevispora</i>	CBS 116829	<i>Eucalyptus grandis</i>	Venezuela	AF192321	NA	NA	NA	NA	NA
	CBS 116811 <sup>T</sup>	<i>Eucalyptus grandis</i> × <i>tereticornis</i>	Congo	AF192315	NA	NA	NA	NA	NA
<b><i>Cytophora bungeanae</i></b>	<b>CFCC 50495<sup>T</sup></b>	<i>Pinus bungeana</i>	Shanxi, China	MH933621	MH933656	MH933529	MH933593	MH933497	MH933563
	<b>CFCC 50496</b>	<i>Pinus bungeana</i>	Shanxi, China	MH933622	MH933657	MH933530	MH933594	MH933498	MH933564
<i>Cytophora californica</i>	CBS 144234 <sup>T</sup>	<i>Juglans regia</i>	California, USA	MG971935	NA	MG972083	NA	MG971645	NA
<b><i>Cytophora carbonacea</i></b>	<b>CFCC 89947</b>	<i>Ulmus pumila</i>	Qinghai, China	KR045622	KP310812	KP310842	KU710950	KP310855	KP310825
<i>Cytophora carobroti</i>	CMW 48981 <sup>T</sup>	<i>Carobrotus edulis</i>	South Africa	MH382812	MH411216	NA	NA	MH411212	MH411207
<i>Cytophora cedri</i>	CBS 196.50	NA	Italy	AF192311	NA	NA	NA	NA	NA
<b><i>Cytophora celtidicola</i></b>	<b>CFCC 50497<sup>T</sup></b>	<i>Celtis sinensis</i>	Anhui, China	MH933623	MH933658	MH933531	MH933595	MH933499	MH933566
	<b>CFCC 50498</b>	<i>Celtis sinensis</i>	Anhui, China	MH933624	MH933659	MH933532	MH933596	MH933500	MH933567
<i>Cytophora centrivillosa</i>	MFLUCC 16-1206 <sup>T</sup>	<i>Sorbus domestica</i>	Italy	MF190122	MF190068	NA	MF377600	NA	NA
	MFLUCC 17-1660	<i>Sorbus domestica</i>	Italy	MF190123	MF190069	NA	MF377601	NA	NA
<i>Cytophora ceratosperma</i>	CBS 116.21	<i>Fagus sylvatica</i>	Netherlands	AY347335	NA	NA	NA	NA	NA
	CBS 192.42	<i>Taxus baccata</i>	Switzerland	AY347333	NA	NA	NA	NA	NA
<b>CFCC 89624</b>	<b>CFCC 89624</b>	<i>Juglans regia</i>	Gansu, China	KR045645	KR045724	NA	KU710976	KP310860	KR045686
<b>CFCC 89625</b>	<b>CFCC 89625</b>	<i>Juglans regia</i>	Gansu, China	KR045646	KR045725	NA	KU710977	KP310866	KR045687
<b>CFCC 89626<sup>T</sup></b>	<b>CFCC 89626<sup>T</sup></b>	<i>Juglans regia</i>	Shaanxi, China	KR045647	KR045726	KU711011	KU710978	KU710934	KR045688
<b>CFCC 89627</b>	<b>CFCC 89627</b>	<i>Juglans regia</i>	Shaanxi, China	KR045648	KR045727	KU711012	KU710979	KU710935	KR045689
<b>CFCC 89629</b>	<b>CFCC 89629</b>	<i>Salix psammophila</i>	Shaanxi, China	KF765673	KF765689	NA	KF765705	NA	NA
<b>CFCC 89981</b>	<b>CFCC 89981</b>	<i>Populus alba</i> subsp. <i>pyramidalis</i>	Gansu, China	MH933625	MH933660	MH933533	MH933597	MH933501	MH933568
<b>CFCC 89982</b>	<b>CFCC 89982</b>	<i>Ulmus pumila</i>	Tibet, China	KP281261	KP310805	KP310835	NA	KP310848	KP310818
	CMW 5700 <sup>T</sup>	<i>Eucalyptus globulus</i>	Chile	AY347377	NA	NA	NA	NA	NA
<i>Cytophora cinerostroma</i>	MFLUCC 14-1050 <sup>T</sup>	<i>Cotinus coggygria</i>	Russia	KX430142	KX430143	NA	KX430144	NA	NA
<i>Cytophora cotini</i>	MFLUCC 15-0865 <sup>T</sup>	<i>Salix alba</i>	Russia	KY417728	KY417762	KY417694	KY417796	NA	NA
<i>Cytophora curvata</i>	CXY 1350 <sup>T</sup>	<i>Populus davidiana</i>	Inner Mongolia, China	KM034870	NA	NA	NA	NA	NA
<i>Cytophora davidiana</i>	CXY 1374	<i>Populus davidiana</i>	Heilongjiang, China	KM034869	NA	NA	NA	NA	NA
<i>Cytophora diatrypelloidea</i>	CMW 8549 <sup>T</sup>	<i>Eucalyptus globulus</i>	Australia	AY347374	NA	NA	NA	NA	NA
<i>Cytophora disciformis</i>	CMW 6509 <sup>T</sup>	<i>Eucalyptus grandis</i>	Uruguay	AY347374	NA	NA	NA	NA	NA
	CMW 6750	<i>Eucalyptus globulus</i>	Australia	AY347359	NA	NA	NA	NA	NA
<b><i>Cytophora elaeagni</i></b>	<b>CFCC 89632</b>	<i>Elaeagnus angustifolia</i>	Ningxia, China	KR045626	KR045706	KU710995	KU710955	KU710918	KR045667
	<b>CFCC 89633</b>	<i>Elaeagnus angustifolia</i>	Ningxia, China	KF765677	KF765693	KU710996	KU710956	KU710919	KR045668
<i>Cytophora eriobotryae</i>	IMI 136523 <sup>T</sup>	<i>Eriobotrya japonica</i>	India	AY347327	NA	NA	NA	NA	NA
<b><i>Cytophora erumpens</i></b>	<b>CFCC 50022</b>	<i>Prunus padus</i>	Shanxi, China	MH933627	MH933661	MH933534	NA	MH933502	MH933569
	MFLUCC 16-0580 <sup>T</sup>	<i>Salix × fragilis</i>	Russia	KY417733	KY417767	KY417699	KY417801	NA	NA





Table 1 (cont.)

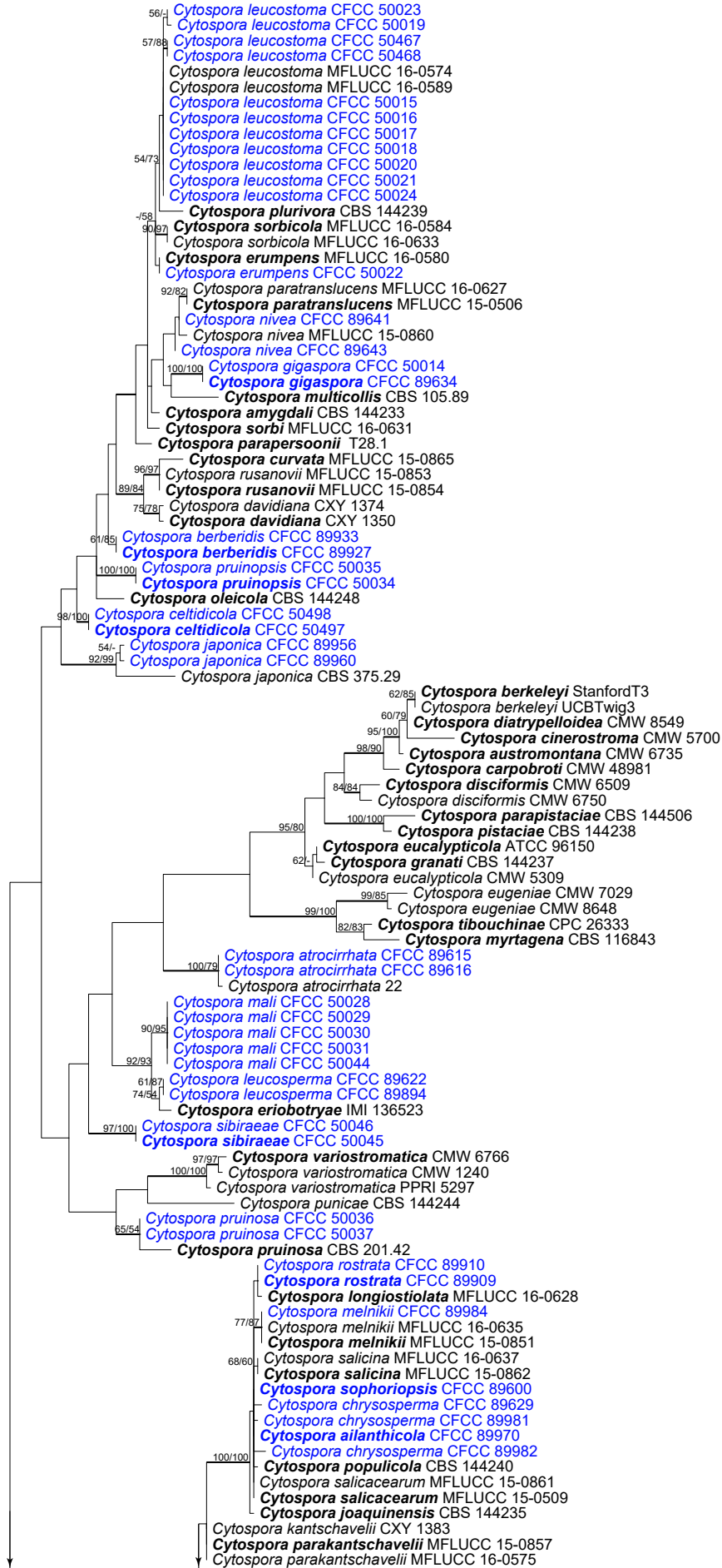
Species	Strain <sup>1</sup>	Host	Origin	ITS	LSU	act1	rpb2	terf1-α	tub2
<i>Cytospora longispora</i>	CBS 144236 <sup>T</sup>	<i>Prunus domestica</i>	California, USA	MG971905	NA	MG972054	NA	MG971615	MG971764
<i>Cytospora lummitzericola</i>	MFLUCC 17-0508 <sup>T</sup>	<i>Lummitzera racemosa</i>	Thailand	MG975778	MH253453	MH253457	MH253461	NA	NA
<b><i>Cytospora mali</i></b>	<b>CFCC 50028</b>	<i>Malus pumila</i>	Gansu, China	MH933641	MH933675	MH933548	MH933606	MH933513	MH933577
	<b>CFCC 50029</b>	<i>Malus pumila</i>	Ningxia, China	MH933642	MH933676	MH933549	MH933607	MH933514	MH933578
	<b>CFCC 50030</b>	<i>Malus pumila</i>	Shaanxi, China	MH933643	MH933677	MH933550	MH933608	MH933524	MH933579
	<b>CFCC 50031</b>	<i>Crataegus</i> sp.	Shanxi, China	KR045716	KR045717	KU711004	KU710965	KU710927	KR045677
	<b>CFCC 50044</b>	<i>Malus baccata</i>	Qinghai, China	KR045637	KR045717	KU711005	KU710966	KU710928	KR045678
<b><i>Cytospora melnikii</i></b>	<b>CFCC 89984</b>	<i>Rhus typhina</i>	Xinjiang, China	MH933644	MH933678	MH933551	MH933609	MH933515	MH933580
	MFLUCC 15-0851 <sup>T</sup>	<i>Malus domestica</i>	Russia	KY417735	KY417769	KY417701	KY417803	NA	NA
	MFLUCC 16-0635	<i>Populus nigra</i> var. <i>italica</i>	Russia	KY417736	KY417770	KY417702	KY417804	NA	NA
<i>Cytospora mougeotii</i>	ATCC 44994	<i>Picea abies</i>	Norway	AY347329	NA	NA	NA	NA	NA
<i>Cytospora multiflora</i>	CBS 10589 <sup>T</sup>	<i>Quercus ilex</i> subsp. <i>rotundifolia</i>	Spain	DQ243803	NA	NA	NA	NA	NA
<i>Cytospora myrtilloides</i>	CBS 116843 <sup>T</sup>	<i>Tibouchina urvilleana</i>	USA	AY347363	NA	NA	NA	NA	NA
<i>Cytospora nitschkii</i>	CMW 10180 <sup>T</sup>	<i>Eucalyptus globulus</i>	Ethiopia	AY347356	NA	NA	NA	NA	NA
	CMW 10184	<i>Eucalyptus globulus</i>	Ethiopia	AY347355	NA	NA	NA	NA	NA
<i>Cytospora nivea</i>	MFLUCC 15-0860	<i>Salix acutifolia</i>	Russia	KY417737	KY417771	KY417703	KY417805	NA	NA
	<b>CFCC 89641</b>	<i>Eleagnus angustifolia</i>	Ningxia, China	KF765683	KF765699	KU711006	KU710967	KU710929	KR045679
	<b>CFCC 89643</b>	<i>Salix psammophila</i>	Shaanxi, China	KF765685	KF765701	NA	KU710968	KP310863	KP310829
<i>Cytospora oleicola</i>	CBS 144248 <sup>T</sup>	<i>Olea europaea</i>	California, USA	MG971944	NA	MG972098	NA	MG971660	MG971752
<i>Cytospora palm</i>	CXY 1276	<i>Cotinus coggynia</i>	Beijing, China	JN402990	NA	NA	NA	KJ781296	NA
	MFLUCC 15-0857 <sup>T</sup>	<i>Cotinus coggynia</i>	Beijing, China	JN411939	NA	NA	NA	KJ781297	NA
<i>Cytospora parakantschavelii</i>	MFLUCC 16-0575	<i>Populus x sibirica</i>	Russia	KY417738	KY417772	KY417704	KY417806	NA	NA
	T28.1 <sup>T</sup>	<i>Pyrus pyraeaster</i>	Russia	KY417739	KY417773	KY417705	KY417807	NA	NA
<i>Cytospora paraperisoonii</i>	CBS 144506 <sup>T</sup>	<i>Prunus persica</i>	USA	AF191181	NA	NA	NA	NA	NA
<i>Cytospora parapistaciae</i>	MFLUCC 15-0507 <sup>T</sup>	<i>Pistacia vera</i>	California, USA	MG971804	NA	MG971954	NA	MG971519	MG971669
<i>Cytospora parasitica</i>	XJAU 2542-1	<i>Malus domestica</i>	Russia	KY417740	KY417774	KY417706	KY417808	NA	NA
	MFLUCC 15-0506 <sup>T</sup>	<i>Malus</i> sp.	Xinjiang, China	MH798884	MH798897	NA	NA	MH813452	NA
	MFLUCC 16-0627	<i>Populus alba</i> var. <i>bolleana</i>	Russia	KY417741	KY417775	KY417707	KY417809	NA	NA
<i>Cytospora pini</i>	CBS 197.42	<i>Populus alba</i>	Russia	KY417742	KY417776	KY417708	KY417810	NA	NA
	CBS 224.52 <sup>T</sup>	<i>Pinus sylvestris</i>	Switzerland	AY347332	NA	NA	NA	NA	NA
	CBS 144238 <sup>T</sup>	<i>Pinus strobus</i>	USA	AY347316	NA	NA	NA	NA	NA
<i>Cytospora platanicola</i>	MFLU 17-0327 <sup>T</sup>	<i>Pistacia vera</i>	California, USA	MG971802	NA	MG971952	NA	MG971517	MG971667
<b><i>Cytospora platycladi</i></b>	<b>CFCC 50504<sup>T</sup></b>	<i>Platanus hybrida</i>	Italy	MH253451	MH253452	MH253449	MH253450	NA	NA
	<b>CFCC 50505</b>	<i>Platycladus orientalis</i>	Yunnan, China	MH933645	MH933679	MH933552	MH933610	MH933516	MH933581
	<b>CFCC 50506</b>	<i>Platycladus orientalis</i>	Yunnan, China	MH933646	MH933680	MH933553	MH933611	MH933517	MH933582
	<b>CFCC 50038<sup>T</sup></b>	<i>Platycladus orientalis</i>	Yunnan, China	MH933647	MH933681	MH933554	MH933612	MH933518	MH933583
	<b>CFCC 50039</b>	<i>Platycladus orientalis</i>	Gansu, China	KT222840	MH933682	MH933555	MH933613	MH933519	MH933584
<i>Cytospora plumvara</i>	MFLUCC 17-2458 <sup>T</sup>	<i>Platycladus orientalis</i>	Gansu, China	KR045642	KR045721	KU711008	KU710973	KU710931	KR045683
<i>Cytospora populicola</i>	MFLU 17-0995 <sup>T</sup>	<i>Olea europaea</i>	California, USA	MG971861	NA	MG972010	NA	MG971572	MG971726
<b><i>Cytospora populina</i></b>	<b>CFCC 89644</b>	<i>Populus deltoides</i>	California, USA	MG971891	NA	MG972040	NA	MG971601	MG971757
<b><i>Cytospora populinoensis</i></b>	<b>CFCC 50032<sup>T</sup></b>	<i>Salix psammophila</i>	Shaanxi, China	KF765686	KF765702	KU711007	KU710969	KU710930	KR045681
	MFLUCC 17-2458 <sup>T</sup>	<i>Sorbus aucuparia</i>	Ningxia, China	MH933648	MH933683	MH933556	MH933614	MH933520	MH933585
<i>Cytospora predappioensis</i>	MFLU 17-0995 <sup>T</sup>	<i>Sorbus aucuparia</i>	Ningxia, China	MH933649	MH933684	MH933557	MH933615	MH933521	MH933586
<i>Cytospora prunicola</i>	<b>CFCC 50033</b>	<i>Platanus hybrida</i>	Italy	MG873484	MG873480	NA	NA	NA	NA
<b><i>Cytospora pruinopsis</i></b>	<b>CFCC 50034<sup>T</sup></b>	<i>Prunus</i> sp.	Italy	MG742350	MG742351	MG742353	MG742352	NA	NA
	<b>CFCC 50035</b>	<i>Ulmus pumila</i>	Shaanxi, China	KP281259	KP310806	KP310836	KU710970	KP310849	KP310819
	CBS 201.42	<i>Ulmus pumila</i>	Jilin, China	KP281260	KP310807	KP310837	KU710971	KP310850	KP310820
	<b>CFCC 50036</b>	<i>Syringa</i> sp.	Switzerland	DQ243801	NA	NA	NA	NA	NA
	<b>CFCC 50037</b>	<i>Syringa oblata</i>	Qinghai, China	KP310800	KP310802	KP310832	NA	KP310845	KP310815
<i>Cytospora punicea</i>	CBS 144244	<i>Syringa oblata</i>	Qinghai, China	MH933650	MH933685	MH933558	NA	MH933522	MH933589
<i>Cytospora quercicola</i>	MFLU 17-0881	<i>Punica granatum</i>	California, USA	MG971943	NA	MG972091	NA	MG971654	MG971798
	MFLUCC 14-0867 <sup>T</sup>	<i>Quercus</i> sp.	Italy	MF190128	MF190074	NA	NA	NA	NA
		<i>Quercus</i> sp.	Italy	MF190129	MF190073	NA	NA	NA	NA

Table 1 (cont.)

Species	Strain <sup>1</sup>	Host	Origin	GenBank accession numbers						
				ITS	LSU	act1	rbp2	tef1-α	tub2	
<i>Cytospora rhizophorae</i>	MUCC302		Australia	EU301057	NA	NA	NA	NA	NA	NA
<b><i>Cytospora ribis</i></b>	<b>CFCC 50026</b>	<i>Eucalyptus grandis</i>	Qinghai, China	KP281267	KP310813	KP310843	KU710972	NA	KP310856	NA
	<b>CFCC 50027</b>	<i>Ulmus pumila</i>	Qinghai, China	KP281268	KP310814	KP310844	NA	NA	KP310857	KP310826
	CBS 187.36	<i>Ribes rubrum</i>	Netherlands	DQ243810	NA	NA	NA	NA	NA	NA
<i>Cytospora roseae</i>	MFLUCC 14-0845 <sup>T</sup>	<i>Rosa canina</i>	Italy	MF190131	MF190075	NA	NA	NA	NA	NA
<b><i>Cytospora rostrata</i></b>	<b>CFCC 89909<sup>T</sup></b>	<i>Salix cupularis</i>	Gansu, China	KR045643	KR045722	KU711009	KU710974	KU710932	NA	KR045684
<i>Cytospora rusanovii</i>	MFLUCC 15-0853	<i>Salix cupularis</i>	Gansu, China	KR045644	KR045723	KU711010	KU710975	KU710933	NA	NA
	MFLUCC 15-0854 <sup>T</sup>	<i>Populus × sibirica</i>	Russia	KY417743	KY417777	KY417709	KY417811	NA	NA	NA
	MFLUCC 15-0861	<i>Salix babylonica</i>	Russia	KY417744	KY417778	KY417710	KY417812	NA	NA	NA
	MFLUCC 15-0509 <sup>T</sup>	<i>Salix × fragilis</i>	Russia	KY417745	KY417779	KY417711	KY417813	NA	NA	NA
	MFLUCC 16-0576	<i>Salix alba</i>	Russia	KY417746	KY417780	KY417712	KY417814	NA	NA	NA
	MFLUCC 16-0587	<i>Populus nigra</i> var. <i>italica</i>	Russia	KY417747	KY417775	KY417707	KY417809	NA	NA	NA
<i>Cytospora salicicola</i>	MFLUCC 15-0866	<i>Prunus cerasus</i>	Russia	KY417748	KY417776	KY417708	KY417810	NA	NA	NA
	MFLUCC 14-1052 <sup>T</sup>	<i>Salix alba</i>	Russia	KY417749	KY417783	KY417715	KY417817	NA	NA	NA
	MFLUCC 15-0862 <sup>T</sup>	<i>Salix alba</i>	Russia	KU982636	KU982635	KU982637	NA	NA	NA	NA
<i>Cytospora salicina</i>	MFLUCC 15-0862 <sup>T</sup>	<i>Salix alba</i>	Russia	KY417750	KY417784	KY417716	KY417818	NA	NA	NA
	MFLUCC 16-0637	<i>Salix × fragilis</i>	Russia	KY417751	KY417785	KY417717	KY417819	NA	NA	NA
<b><i>Cytospora schulzeri</i></b>	<b>CFCC 50040</b>	<i>Malus domestica</i>	Ningxia, China	KR045649	KR045728	KU711013	KU710980	KU710936	NA	KR045690
	<b>CFCC 50042</b>	<i>Malus asiatica</i>	Qinghai, China	KR045650	KR045729	KU711014	KU710981	KU710937	NA	KR045691
<b><i>Cytospora sibiricae</i></b>	<b>CFCC 50045<sup>T</sup></b>	<i>Sibiraea angustata</i>	Gansu, China	KR045651	KR045730	KU711015	KU710982	KU710938	NA	KR045692
	<b>CFCC 50046</b>	<i>Sibiraea angustata</i>	Gansu, China	KR045652	KR045731	KU711015	KU710983	KU710939	NA	KR045693
<b><i>Cytospora sophorae</i></b>	<b>CFCC 50047</b>	<i>Styphnolobium japonicum</i>	Shanxi, China	KR045653	KR045732	KU711017	KU710984	KU710940	NA	KR045694
	<b>CFCC 50048</b>	<i>Magnolia grandiflora</i>	Shanxi, China	MH820401	MH820394	MH820409	MH820397	MH820405	NA	MH820390
	<b>CFCC 89598</b>	<i>Styphnolobium japonicum</i>	Gansu, China	KR045654	KR045733	KU711018	KU710985	KU710941	NA	KR045695
<b><i>Cytospora sophoricola</i></b>	<b>CFCC 89596</b>	<i>Styphnolobium japonicum</i> var. <i>pendula</i>	Gansu, China	KR045656	KR045735	KU711020	KU710987	KU710942	NA	KR045697
	<b>CFCC 89595<sup>T</sup></b>	<i>Styphnolobium japonicum</i> var. <i>pendula</i>	Gansu, China	KR045655	KR045734	KU711019	KU710986	KU710942	NA	KR045696
<b><i>Cytospora sophoropsis</i></b>	<b>CFCC 89600<sup>T</sup></b>	<i>Styphnolobium japonicum</i>	Gansu, China	KR045623	KP310804	KU710992	KU710951	KU710915	NA	KP310817
<i>Cytospora sorbi</i>	MFLUCC 16-0631 <sup>T</sup>	<i>Sorbus aucuparia</i>	Russia	KY417752	KY417786	KY417718	KY417820	NA	NA	NA
<i>Cytospora sorbicola</i>	MFLUCC 16-0584 <sup>T</sup>	<i>Acer pseudoplatanus</i>	Russia	KY417755	KY417789	KY417721	KY417823	NA	NA	NA
	MFLUCC 16-0633	<i>Cotoneaster melanocarpus</i>	Russia	KY417758	KY417792	KY417724	KY417826	NA	NA	NA
<b><i>Cytospora spiraeae</i></b>	<b>CFCC 50049<sup>T</sup></b>	<i>Spiraea salicifolia</i>	Gansu, China	MG707859	MG707643	MG708199	MG708199	NA	NA	NA
	<b>CFCC 50050</b>	<i>Spiraea salicifolia</i>	Gansu, China	MG707860	MG707644	MG708196	MG708200	NA	NA	NA
<b><i>Cytospora tamaricicola</i></b>	<b>CFCC 50507</b>	<i>Rosa multiflora</i>	Yunnan, China	MH933651	MH933686	MH933559	MH933616	MH933525	NA	MH933587
	<b>CFCC 50508<sup>T</sup></b>	<i>Tamarix chinensis</i>	Yunnan, China	MH933652	MH933687	MH933560	MH933617	MH933523	NA	MH933688
<i>Cytospora tanaitica</i>	MFLUCC 14-1057 <sup>T</sup>	<i>Betula pubescens</i>	Russia	KT459411	KT459412	KT459413	NA	NA	NA	KM034895
<i>Cytospora thailandica</i>	MFLUCC 17-0262 <sup>T</sup>	<i>Xylocarpus moluccensis</i>	Thailand	MG975776	MH253455	MH253459	MH253463	NA	NA	NA
	MFLUCC 17-0263 <sup>T</sup>	<i>Xylocarpus moluccensis</i>	Thailand	MG975777	MH253456	MH253460	MH253464	NA	NA	NA
<i>Cytospora tibouchinae</i>	CPC 26333 <sup>T</sup>	<i>Tibouchina semidecandra</i>	France	KX228284	KX228335	NA	NA	NA	NA	NA
<i>Cytospora translucens</i>	CXY 1351	<i>Populus davidiana</i>	Inner Mongolia, China	KM034874	NA	NA	NA	NA	NA	NA
<i>Cytospora ulmi</i>	MFLUCC 15-0863 <sup>T</sup>	<i>Ulmus minor</i>	Russia	KY417759	NA	NA	NA	NA	NA	NA
<i>Cytospora valsoidea</i>	CMW 4310	<i>Eucalyptus grandis</i>	Indonesia	AF192312	NA	NA	NA	NA	NA	NA
	CMW 6766 <sup>T</sup>	<i>Eucalyptus grandis</i>	Indonesia	AF192312	NA	NA	NA	NA	NA	NA
<b><i>Cytospora variostromatica</i></b>	<b>CMW 1240</b>	<i>Eucalyptus globulus</i>	Australia	AY347366	NA	NA	NA	NA	NA	NA
	CBS 118086 = PPRI 5297	<i>Eucalyptus grandis</i>	South Africa	AF260263	NA	NA	NA	NA	NA	NA
<i>Cytospora vinacea</i>	CBS 141585 <sup>T</sup>	<i>Vitis interspecific hybrid 'Vidal'</i>	USA	AF260264	NA	NA	NA	NA	NA	NA
<i>Cytospora viticola</i>	CBS 141586 <sup>T</sup>	<i>Vitis vinifera</i> 'Cabernet Franc'	USA	KX256256	NA	NA	NA	NA	NA	KX256235
<i>Cytospora xylocarpi</i>	MFLUCC 17-0251 <sup>T</sup>	<i>Xylocarpus granatum</i>	Thailand	KX256239	NA	NA	NA	NA	NA	KX256218
<i>Diaporthe vaccini</i>	CBS 160.32	<i>Vaccinium macrocarpon</i>	USA	MG975775	MH253462	MH253458	MH253454	NA	NA	NA
				KC343228	NA	JQ807297	NA	KC343954	NA	KC344196

<sup>1</sup> Acronyms: ATCC: American Type Culture Collection, Virginia, USA; BBH: BIOTEC Bangkok Herbarium, National Science and Technology Development Agency, Thailand; CBS: Westerdijk Fungal Biodiversity Institute (CBS-KNAW Fungal Biodiversity Centre), Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Centre, Beijing, China; CPC: Culture collection of Pedro Crous, The Netherlands; ICMP: International Collection of Microorganisms from Plants, New Zealand; IMI: Culture collection of the International Mycological Institute, CAB International, Egham, Surrey, UK; MFLU: Mae Fah Luang University herbarium, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Thailand; MUCC: Murdoch University Culture Collection, Perth, Australia; PPRI: Culture collection of the Plant Protection Research Institute, Agriculture Research Center, Pretoria, South Africa; XJAU: Xinjiang Agricultural University, Xinjiang, China; NA: not applicable. All the new isolates used in this study are in bold and the type materials are marked with <sup>T</sup>.





**Fig. 3** Maximum parsimony phylogram of *Cytospora* obtained from the ITS rDNA gene matrix. MP bootstrap support values above 50 % and posterior probabilities above 0.95 from BI are given at the first and second position. — Scale bar = 20 nucleotide substitutions. Ex-type strains are in bold; strains obtained in the current study in blue.

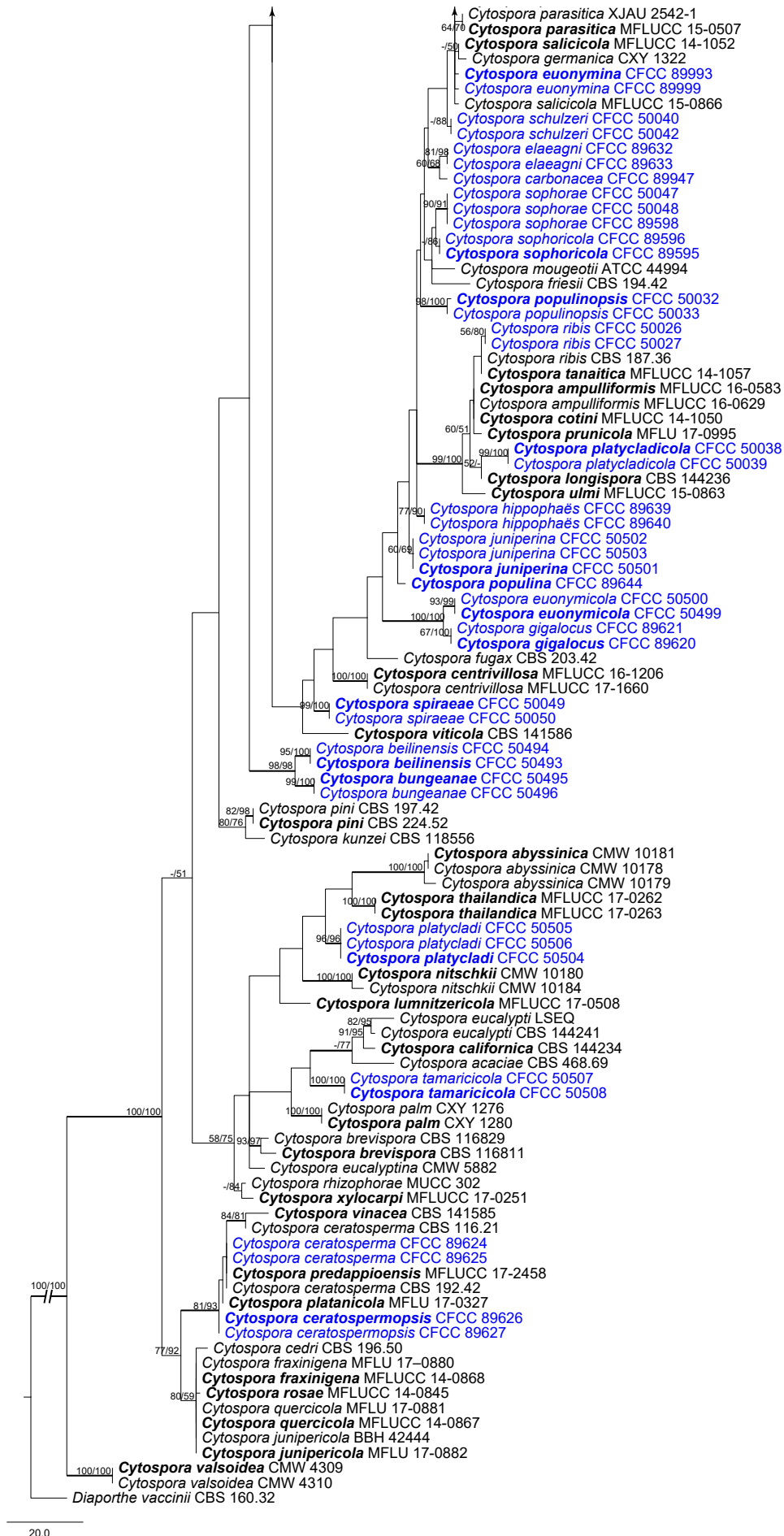
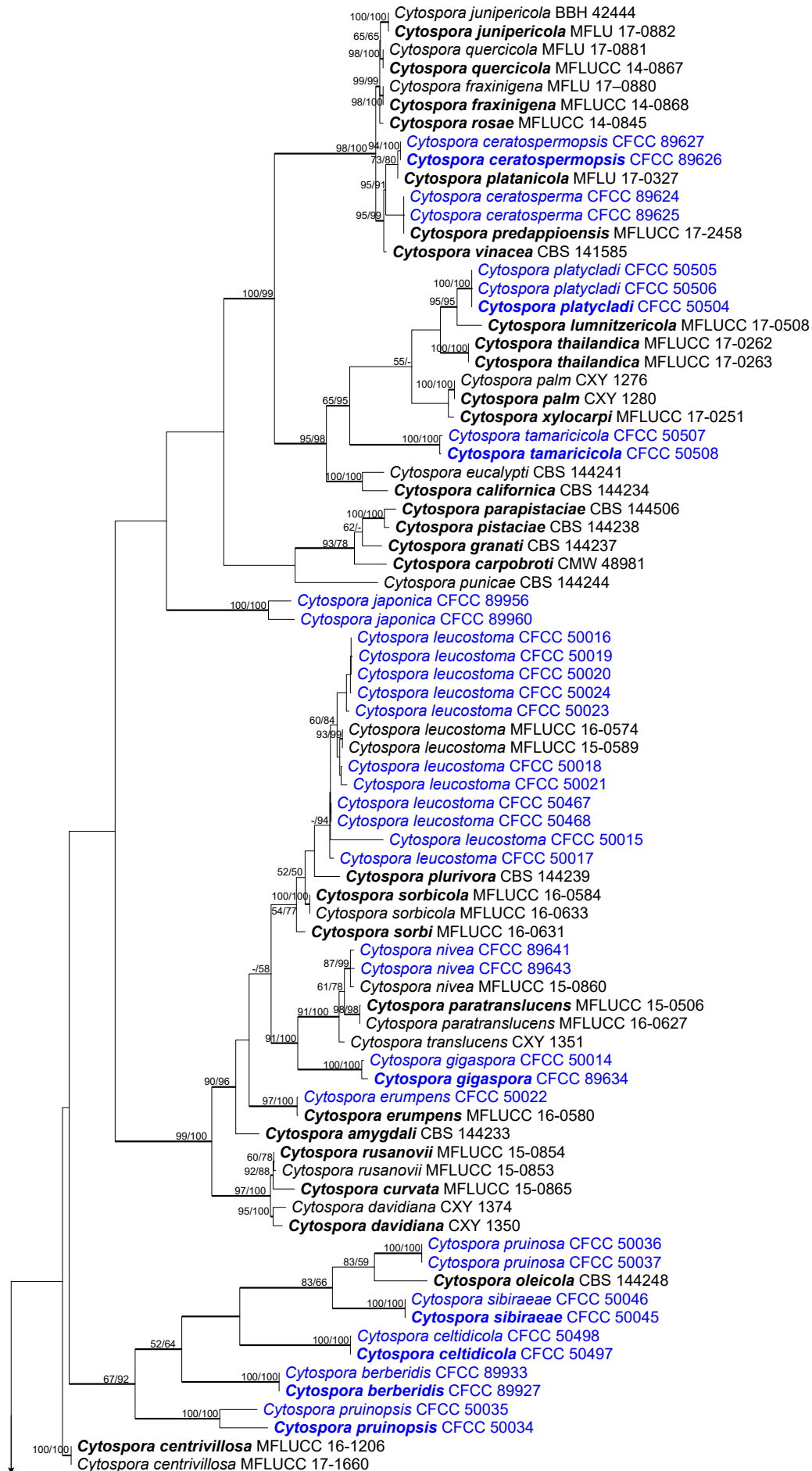


Fig. 3 (cont.)



**Fig. 4** Maximum parsimony phylogram of *Cytospora* obtained from the combined matrix of ITS, LSU, *act1*, *tpb2*, *tef1- $\alpha$*  and *tub2* genes. MP bootstrap support values above 50 % and posterior probabilities above 0.95 from BI are given at the first and second position. — Scale bar = 200 nucleotide substitutions. Ex-type strains are in **bold**; strains obtained in the current study in blue.



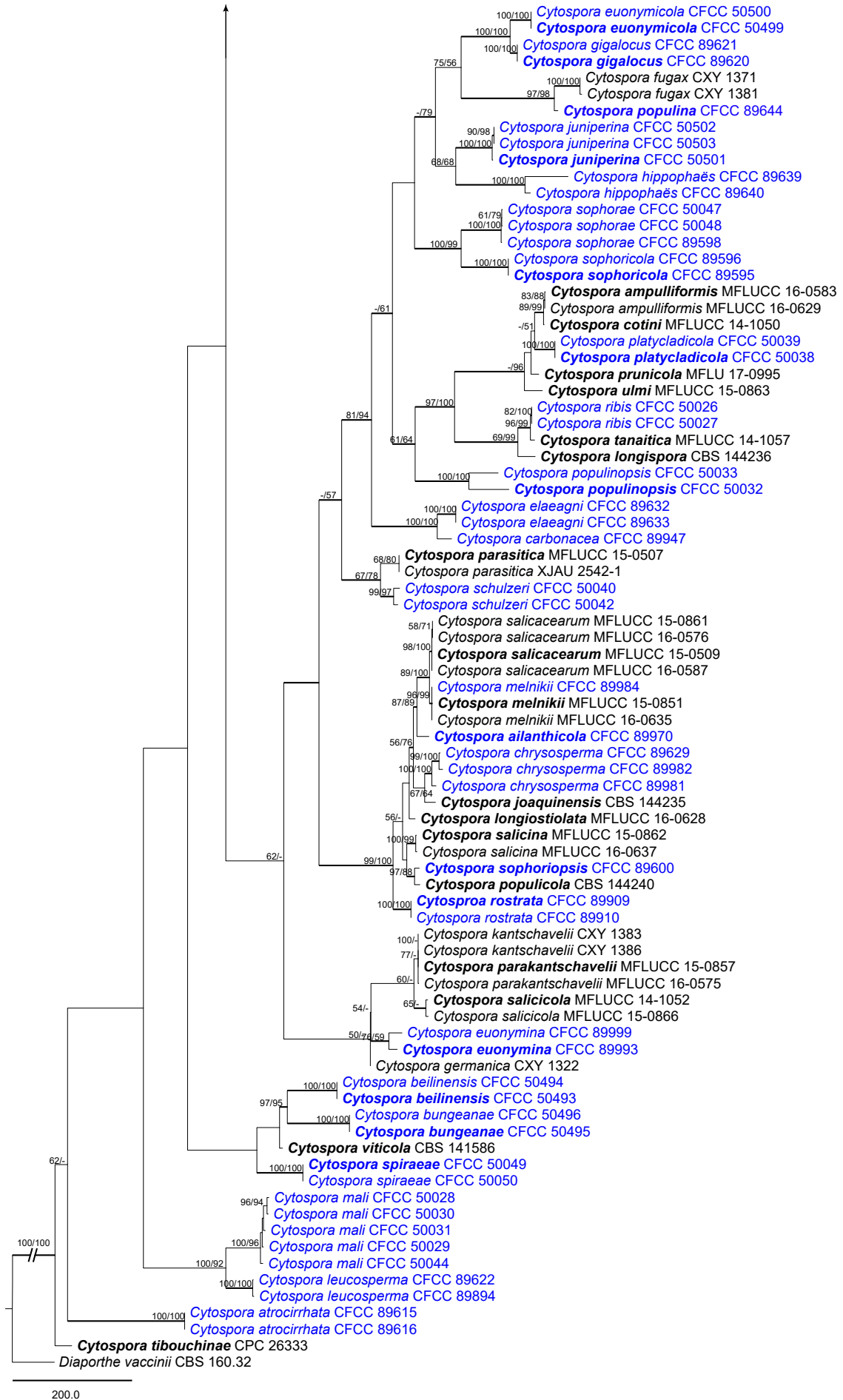


Fig. 4 (cont.)

Madison, WI, USA). To infer a preliminary phylogenetic relationship for the new sequences, an initial alignment of the ITS rDNA sequences was performed using MAFFT v. 6 (Katoch & Standley 2013) and edited manually using MEGA v. 6.0 (Tamura et al. 2013), and some characters were excluded from both ends of the alignments to approximate the size of our sequences to those included in the dataset. This alignment was used to infer a preliminary phylogenetic relationship for the new sequences based on Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses.

The second alignment based on a combined matrix was constructed using available ITS, LSU, *act1*, *rpb2*, *tef1- $\alpha$*  and *tub2* sequences. For individual datasets, sequences were aligned using MAFFT v. 6 and edited manually using MEGA v. 6.0, and some characters were excluded from both ends of the alignments. A partition homogeneity test (PHT) with heuristic search and 1000 homogeneity was performed using PAUP v. 4.0b10 to test the discrepancy among the ITS, LSU, *act1*, *rpb2*, *tef1- $\alpha$*  and *tub2* sequence dataset in reconstructing phylogenetic trees.

The parsimony optimality criterion was used to perform the MP analysis in PAUP v. 4.0b10. The MP analysis was conducted using a heuristic search (1000 bootstrap) (Hillis & Bull 1993), with random sequence addition as option to stepwise-addition (1000 replicates and one tree held at each addition step), and maxtrees limited to 200 by replicate. The tree bisection and reconnection (TBR) was selected as option to the branch swapping algorithm (Swofford 2003). The branches of zero length were collapsed using the command minbrlen and all equally most 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 2003). Maximum likelihood analysis was performed by PhyML v. 3.0 with a GTR site substitution model, including a gamma-distributed rate heterogeneity and a proportion of invariant sites (Guindon et al. 2010). Bayesian inference was performed using the best-fit evolutionary models for each partitioned locus estimated in MrModeltest v. 2.3 (Posada & Crandall 1998) following the Akaike Information Criterion (AIC), with a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Two MCMC chains were run from random trees for 10 M generations and stopped when average standard deviation of split frequencies fell below 0.01. Trees were saved each 1000 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 analyses were evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993). *Diaporthe vaccinii* (CBS 160.32) was selected as outgroup in all analyses. Phylograms were shown using Figtree v. 1.3.1 (Rambaut & Drummond 2010). Novel sequences generated in the current study were deposited in GenBank (Table 1) and the aligned matrices used for phylogenetic analyses and the resulting trees in TreeBASE (www.treebase.org; accession number: S23600).

### Morphology

Descriptions of the asexual and sexual morphs are based on host materials. The macro-morphological photographs were captured using a Leica dissecting microscope (M205 FA), including size and arrangement of stromata; presence or absence of special structures 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). Stromata were mounted in sterile water, more than 20 stromata were sectioned, and 50 measurements determined per structure, with extremes of conidial measurements given in parentheses. Colony diameters were measured and the colony colours described after 3 and 30 d according to the colour charts of Rayner (1970). Adobe Photoshop CS v. 5 was used for the manual editing. Nomenclatural novelties were deposited in MycoBank (Crous et al. 2004).

## RESULTS

### Phylogenetic analyses

The phylogenetic analysis of ITS sequence data contained 200 *Cytospora* ingroup strains with a total of 617 characters including gaps, of which 345 characters are constant, 69 variable characters are parsimony-uninformative and 203 characters are variable and parsimony-informative. MP analyses generated 200 parsimonious trees, one of which is presented in Fig. 3 (TL = 1088, CI = 0.379, RI = 0.853, RC = 0.323). ML and Bayesian analyses were similar to the MP tree.

The second phylogenetic analysis was performed based on a combined dataset of the available ITS, LSU, *act1*, *rpb2*, *tef1- $\alpha$*  and *tub2* sequences. The multi-gene analyses include 162 *Cytospora* ingroup strains with a total of 4066 characters including gaps (661 characters for ITS, 525 for LSU, 354 for *act1*, 731 for *rpb2*, 779 for *tef1- $\alpha$*  and 1016 for *tub2*), of which 2277 characters are constant, 207 variable characters are parsimony-uninformative and 1582 characters are variable and parsimony-informative. Each individual alignment has the following constant characters (ITS = 432, LSU = 426, *act1* = 199, *rpb2* = 464, *tef1- $\alpha$*  = 402 and *tub2* = 490), variable characters parsimony-uninformative (ITS = 61, LSU = 39, *act1* = 24, *rpb2* = 6, *tef1- $\alpha$*  = 34 and *tub2* = 49) and variable characters and parsimony-informative (ITS = 164, LSU = 56, *act1* = 164, *rpb2* = 257, *tef1- $\alpha$*  = 411 and *tub2* = 529). MP analysis generated six most parsimonious trees, one of which is presented in Fig. 4 (TL = 8888, CI = 0.348, RI = 0.789, RC = 0.274). For BI analysis, the general time reversible model with inverse gamma rates (GTR + I + G) was determined to be the best for ITS, LSU, *act1- $\alpha$*  and *tub2* loci by MrModeltest, while the most appropriate model of the *act1* and *rpb2* loci were Hasegawa-Kishino-Yano with inverse gamma rates model (HKY + I + G). The results of the Bayesian analyses were similar to the MP tree. The MP bootstrap support values (BS) equal to or above 50 % are shown above the branches in Fig. 3 and 4. The branches with significant Bayesian posterior probabilities (BPP) equal to or above 0.95 are shown in the phylogram.

### Taxonomy

Based on phylogenetic analyses and morphological examination of 88 isolates, we recognise 40 species in the present study, of which 13 are described as new species below, and we propose one new combination. The identification of many old taxa described below can currently only be preliminary and tentative, as many of them require typification before a stable species concept can be achieved.

**Cytosporaceae** Fr. (as '*Cytispori*'), Syst. Orb. Veg. (Lundae) 1: 118. 1825

*Synonym.* *Valsaceae* Tul. & C. Tul. (as '*Valsarum*'), Select. Fung. Carpol. (Paris) 1: 180. 1861.

*Type genus.* *Cytospora* Ehrenb., Sylv. Mycol. Berol. (Berlin): 28. 1818.

***Cytospora* Ehrenb., Sylv. Mycol. Berol. (Berlin): 28. 1818**

*Synonyms.* *Valsa* Fr., *Summa Veg. Scand., Sectio Post.* (Stockholm): 410. 1849.

*Leucocytospora* (Pers.) Höhn., *Ber. Deutsch. Bot. Ges.* 35: 352. 1917.

*Leucostoma* (Nitschke) Höhn., *Ber. Deutsch. Bot. Ges.* 35: 637. 1917.

*Valsella* Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 23–24: 203. 1870.

*Valseutypella* Höhn., *Ann. Mycol.* 16: 224. 1919.

Additional synonyms in MycoBank.

Plant pathogenic, mostly causing canker diseases. Sexual morph: *Ascostromata* solitary, immersed in vascular plant tissues, slightly to strongly erumpent through the bark surface. *Stromatic tissues* prosenchymatous or pseudoparenchymatous, sometimes delimited by a black marginal line (conceptacle). *Perithecial ascomata* inclined to upright, in valsoid or diatrypelloid configurations, immersed, usually embedded in ectostromatic disc, with beaks converging at surface. *Ostioles* numerous per disc, periphysate; walls of perithecia bilayered, narrow, outer layer of *textura epdermoidea* to *textura angularis*. *Paraphyses* may be lacking at maturity but usually present, often collapsed and broad. *Asci* free, narrow, ellipsoid to clavate, apical ring refractive. *Ascospores* hyaline, allantoid, aseptate, thin-walled, smooth, biserial, 4–8 or polysporous per ascus. Asexual morph: *Pycnidial stromata* ostiolate, immersed in vascular plant tissues, slightly to strongly erumpent through the bark surface, sometimes delimited by a black marginal line (conceptacle). *Ectostromatic disc* prominent or lacking, one to few ostioles per disc. *Locules* single, undivided to multiple chambered with invaginations, globoid to flattened toroid, in ectostroma or embedded in entostroma, sometimes with a column; wall bilayered, outer layer prosenchymatous, ultimately sclerenchymatous. *Conidiophores* borne along the locules,

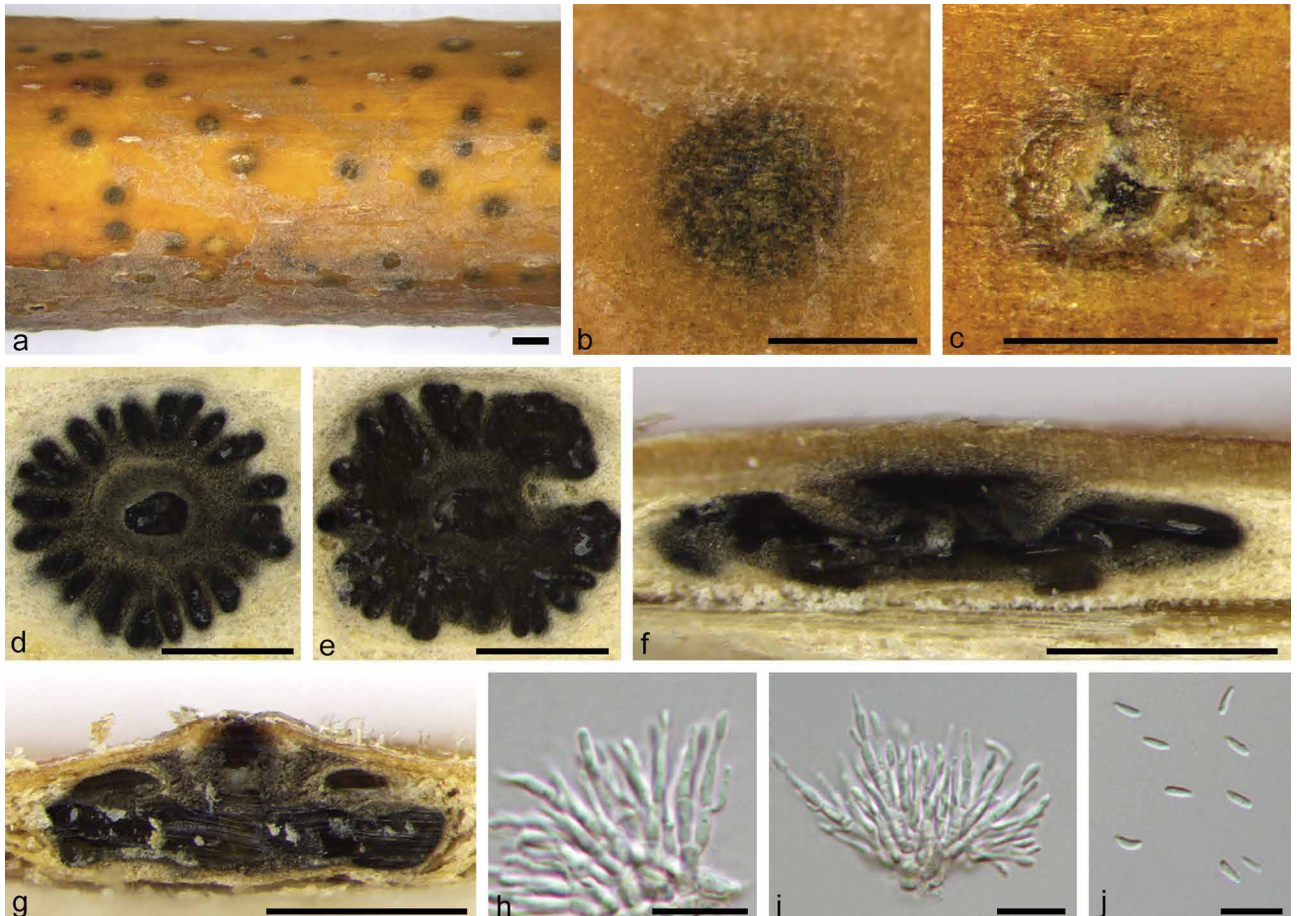
hyaline, branched or not, thin-walled, normally embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, tapering towards apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, relatively small and narrow (adapted from Adams et al. 2005).

***Cytospora ailanthicola* X.L. Fan & C.M. Tian, sp. nov.** — MycoBank MB830146; Fig. 5

*Etymology.* The name reflects the host genus from which it was collected, *Ailanthus*.

*Sexual morph* not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, producing black area on bark, circular to ovoid, with multiple locules, occasionally slightly erumpent through the surface. *Conceptacle* absent. *Ectostromatic disc* inconspicuous, producing one ostiole per disc when mature. *Ostiole* in the centre of the disc, black, conspicuous, (65–)70–100(–130) µm diam. *Locules* numerous, subdivided frequently by invaginations with common walls, (530–)600–1300(–1400) µm diam. *Conidiophores* borne along the locules hyaline, branched at base, middle, or unbranched, thin-walled, occasionally septate, (7–)8.5–17(–18) × 1–1.5 µm, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical, 4–7.5(–9) × 1–1.5 µm, tapering towards apices; arranged in rosettes. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (4–)4.5–5 × 1.5 µm.

*Culture characteristics* — On PDA, colonies fast growing, up to 9 cm diam after 3 d, with fluffy and whitish aerial mycelium, producing black pycnidia with cream to yellowish conidial drops exuding from the ostioles after 30 d. *Pycnidia* sparse and distributed irregularly on the medium surface.



**Fig. 5** *Cytospora ailanthicola* on *Ailanthus altissima* (BJFC-S550). a–c. Habit of conidiomata on branch; d–e. transverse section through conidiomata; f–g. longitudinal section through conidiomata; h–i. conidiogenous cells with attached conidia; j. conidia. — Scale bars: a = 1 mm; b–g = 0.5 mm; h–j = 10 µm.



**Material examined.** CHINA, Ningxia Province, Zhongwei City, Zhongning County, Qukou, N37°39'34.26" E105°50'45.59", from branches of *Ailanthus altissima*, 3 June 2012, X.L. Fan (holotype BJFC-S550, ex-type living culture CFCC 89970).

**Notes** — *Cytospora ailanthicola* is described being associated with canker disease of *Ailanthus altissima* in China. This species is characterised by having pycnidia immersed in the bark, and by producing a black area on the bark, with inconspicuous ectostromatic disc and multiple locules (600–1300 µm), which differs from *C. chrysosperma* by the grey to black, nearly flat, and circular to ovoid disc (Adams et al. 2005, Fan et al. 2014b). *Cytospora ailanthi* was recorded from *Ailanthus altissima*, but without any available morphological details, specimens or DNA data. Saccardo (1884) listed this name under the heading 'Species Imperfecte Descriptae' without supplementary information. Grove (1935) provided a description as having totally immersed small pycnidia (250–300 µm), and slender conidia (5–6 × 1 µm), which can be distinguished from *Cytospora ailanthicola*. Spielman (1985) treated *Cytospora ailanthi* as a synonym of *C. ceratosperma* (syn. *C. sacculus*). The current multi-gene phylogram indicated a distinction from all available *Cytospora* strains, thus *Cytospora ailanthicola* is considered to represent a new species from *Ailanthus* (Fig. 4).

***Cytospora atrocirrhatta*** Gvrit., Mikol. Fitopatol. 7: 547. 1973 — Fig. 6

**Description** — See Fan et al. (2015a).

**Materials examined.** CHINA, Qinghai Province, Xining City, Xishan Botanical Garden, E101°44'48.92" N36°37'25.89", from branches of *Juglans regia*, 19 Aug. 2012, X.L. Fan (BJFC-S649, living culture CFCC 89615); *ibid.*, BJFC-S650, living culture CFCC 89616.

**Notes** — *Cytospora atrocirrhatta* was previously recorded as pathogen associated with cankers on *Salix* and *Populus*

(Gvritishvili 1973, Fotouhifar et al. 2010), as well as associated with *Juglans regia* in China (Fan et al. 2015b). This species is identified by having an undivided single locule surrounded by a black conceptacle, and secreting a lightly red pigment in the PDA medium, representing a different morphological section from the known *Cytospora* sections of Adams et al. (2005).

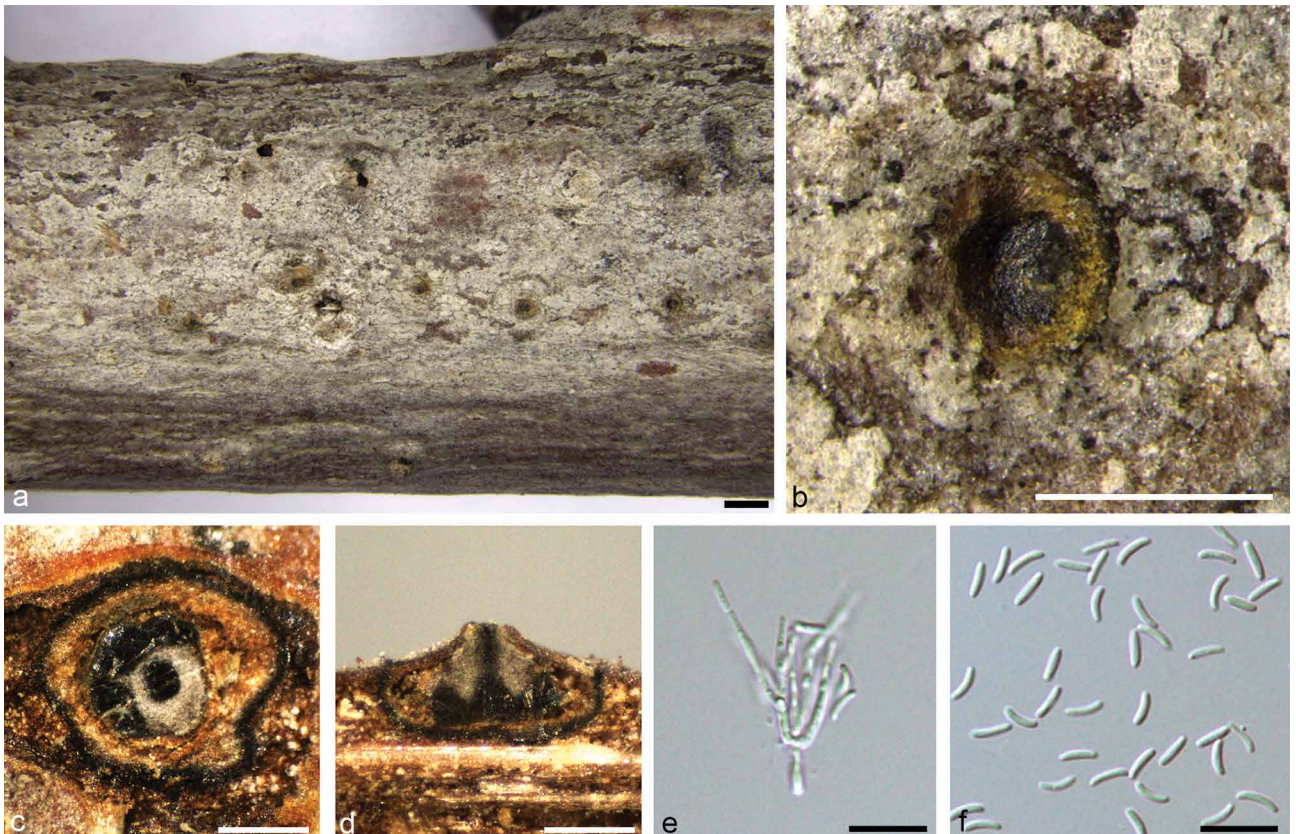
***Cytospora beilinensis*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830147; Fig. 7

**Etymology.** Named after the location from where it was collected, *Beilin*.

**Sexual morph** not observed. **Pycnidial stromata** ostiolate, immersed in bark, scattered, nearly flat, slightly erumpent through the bark surface, with multiple locules. **Conceptacle** absent. **Ectostromatic disc** amber to dark brick, conspicuous, triangular to circular, (200–)230–360(–400) µm diam, with one ostiole per disc. **Ostiole** black, conspicuous, (55–)60–90(–105) µm diam. **Locules** numerous, subdivided frequently by invaginations with common walls, (600–)650–800(–860) µm diam. **Conidiophores** borne along the locules, hyaline, branched at base or unbranched, thin-walled, (9–)9.5–18(–20) × 1.5–2 µm, embedded in a gelatinous layer. **Conidiogenous cells** enteroblastic, phialidic, sub-cylindrical to cylindrical, 6.5–9 × 1.5–2 µm, tapering towards the apices. **Conidia** hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (5–)6–6.5(–7) × 1–1.5 µm.

**Culture characteristics** — On PDA, colonies initially white, growing up to 6.5 cm diam after 3 d and entirely covering the 9 cm Petri dish after 5 d, lacking aerial mycelium, becoming dark mouse grey after 30 d. Colony margin regular. **Pycnidia** irregularly distributed on the medium surface.

**Materials examined.** CHINA, Beijing City, Haidian District, campus of Beijing Forestry University (Beilin), N40°00'64.31" E116°35'04.26", from stem and branches of *Pinus armandii*, 26 May 2015, X.L. Fan (holotype BJFC-S1108, ex-type living culture CFCC 50493); *ibid.*, living culture CFCC 50494.



**Fig. 6** *Cytospora atrocirrhatta* on *Juglans regia* (BJFC-S649). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10 µm.





**Fig. 7** *Cytospora beilinensis* on *Pinus armandii* (BJFC-S1108). a. Disease symptoms; b–c. habit of conidiomata on branch; d. transverse section through conidioma; e. longitudinal section through conidioma; f. conidiogenous cells with attached conidia; g. conidia. — Scale bars: a = 5 mm; c–e = 0.5 mm; f–g = 10  $\mu$ m.

**Notes** — *Cytospora beilinensis* is associated with canker disease of *Pinus armandii* at the Beijing Forestry University in Beijing. *Cytospora pini* was recorded from *Pinus sylvestris* (Saccardo 1884). *Cytospora beilinensis* differs from *C. pini* by the larger size of its conidia (6–6.5  $\times$  1–1.5 vs 4  $\times$  1  $\mu$ m; Saccardo 1884) and they cluster apart in the phylograms (Fig. 3, 4).

***Cytospora berberidis*** C.M. Tian et al., Fungal Diversity 72: 43. 2015 — Fig. 8

**Description** — See Liu et al. (2015).

**Materials examined.** CHINA, Qinghai Province, Haidong City, Huzhu County, E102°30'11.85" N36°56'12.62", from branches of *Berberis dasystachya*, 15 Aug. 2012, X.L. Fan (holotype BJFC-S681, ex-type living culture CFCC 89927); *ibid.*, CFCC 89933.

**Notes** — *Cytospora berberidis* is a member of a group with undivided single locules. *Berberis dasystachya* represents a new host record for this genus (Liu et al. 2015).

***Cytospora bungeanae*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830148; Fig. 9

**Etymology.** The name reflects the host species from which it was collected, *Pinus bungeana*.

**Sexual morph** not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, nearly flat, slightly erumpent through the bark surface in a large area, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* dark grey to black, inconspicuous, circular, (110–)130–205(–220)  $\mu$ m diam, with one ostiole per disc. *Ostiole* black, inconspicuous, (40–)50–70(–85)  $\mu$ m diam. *Locules* numerous, irregular, subdivided frequently by invaginations with common walls, (1150–)1220–1480(–1600)  $\mu$ m diam. *Conidiophores* borne along the locules, hyaline, branched at the base, in the middle, or unbranched, thin-walled, 15–27(–30)  $\times$  1.5–2  $\mu$ m, embedded in a gelatinous layer.

*Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 5.5–9(–10)  $\times$  1.5  $\mu$ m, tapering towards apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, 4–5(–5.5)  $\times$  1  $\mu$ m.

**Culture characteristics** — On PDA, colonies initially white to pale white, becoming cinnamon to brick in the centre and olivaceous to olivaceous black at the margin, secreting a sienna pigment. *Colonies* thin with a uniform texture, lacking aerial mycelium, growing up to 7.5 cm after 30 d, with an irregular edge. *Pycnidia* irregularly distributed on culture surface.

**Materials examined.** CHINA, Shanxi Province, Taiyuan City, Wanbailin Forest Park, N37°54'19.12" E112°31'46.75", on branches of *Pinus bungeana*, 16 Apr. 2014, X.L. Fan & B. Cao (holotype BJFC-S1106, ex-type living culture CFCC 50495); *ibid.*, living culture CFCC 50496.

**Notes** — *Cytospora bungeanae* is a unique *Cytospora* species isolated from *Pinus bungeana* in China. It has multiple locules without conceptacle which is commonly observed in *Cytospora* spp., but the molecular phylogenies show a position clearly distinct from all other strains included in this study. Therefore, we describe this species as new based on DNA sequence data and morphology.

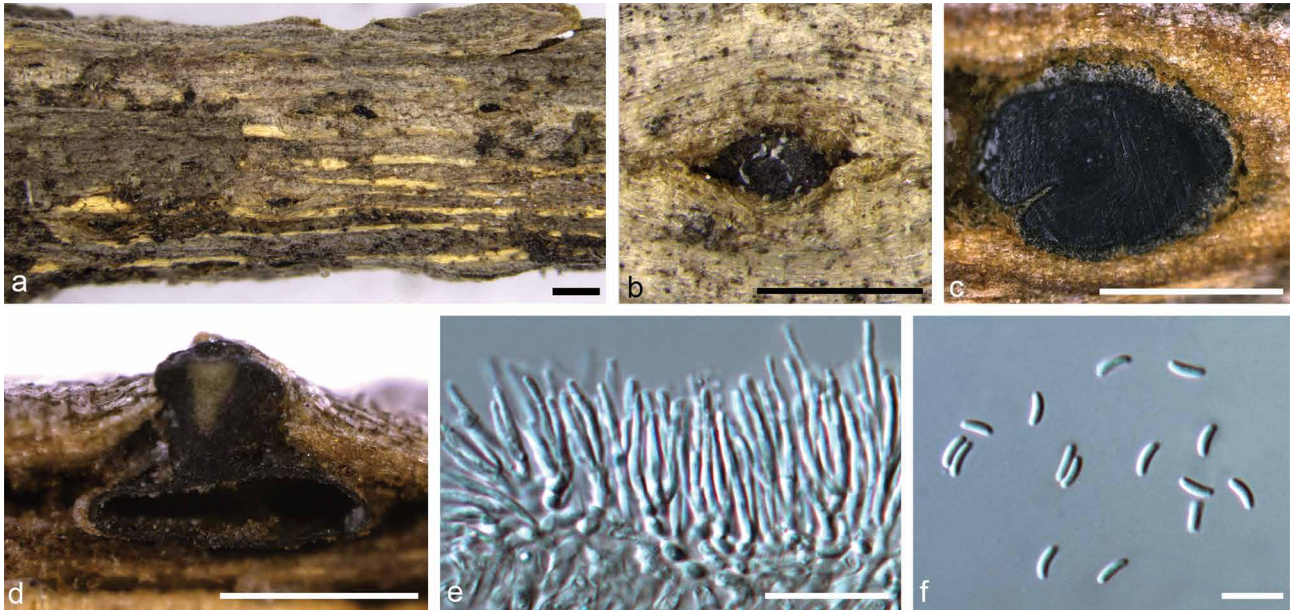
***Cytospora carbonacea*** Fr., Syst. Mycol. (Lundae) 2: 544. 1823 — Fig. 10

**Description** — See Yang et al. (2015).

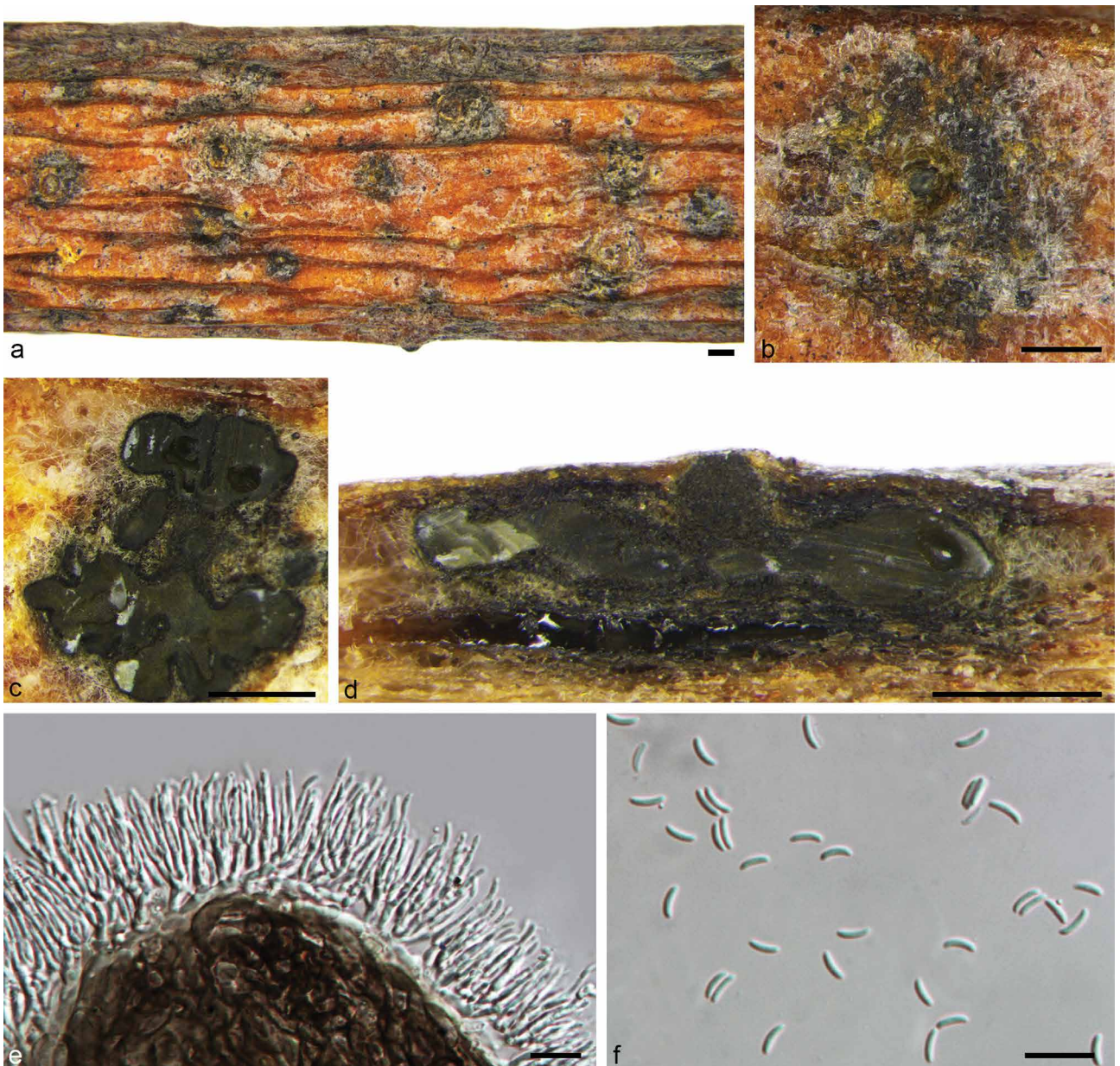
**Material examined.** CHINA, Qinghai Province, Haidong city, Pingan County, N36°28'50.48" E102°10'03.29", from twigs and branches of *Ulmus pumila*, 15 Aug. 2012, X.L. Fan (BJFC-S630, living culture CFCC 89947).

**Notes** — *Cytospora carbonacea* has been previously recorded from *Ulmus americana*, *U. campestris* and *U. minor* in Germany and Iran (Fotouhifar et al. 2010). The first report of this species in China was from *Syzygium aromaticum* (Zhang et al. 2014). Yang et al. (2015) described this species from *U. pumila* in China with a detailed description and wide distribution.



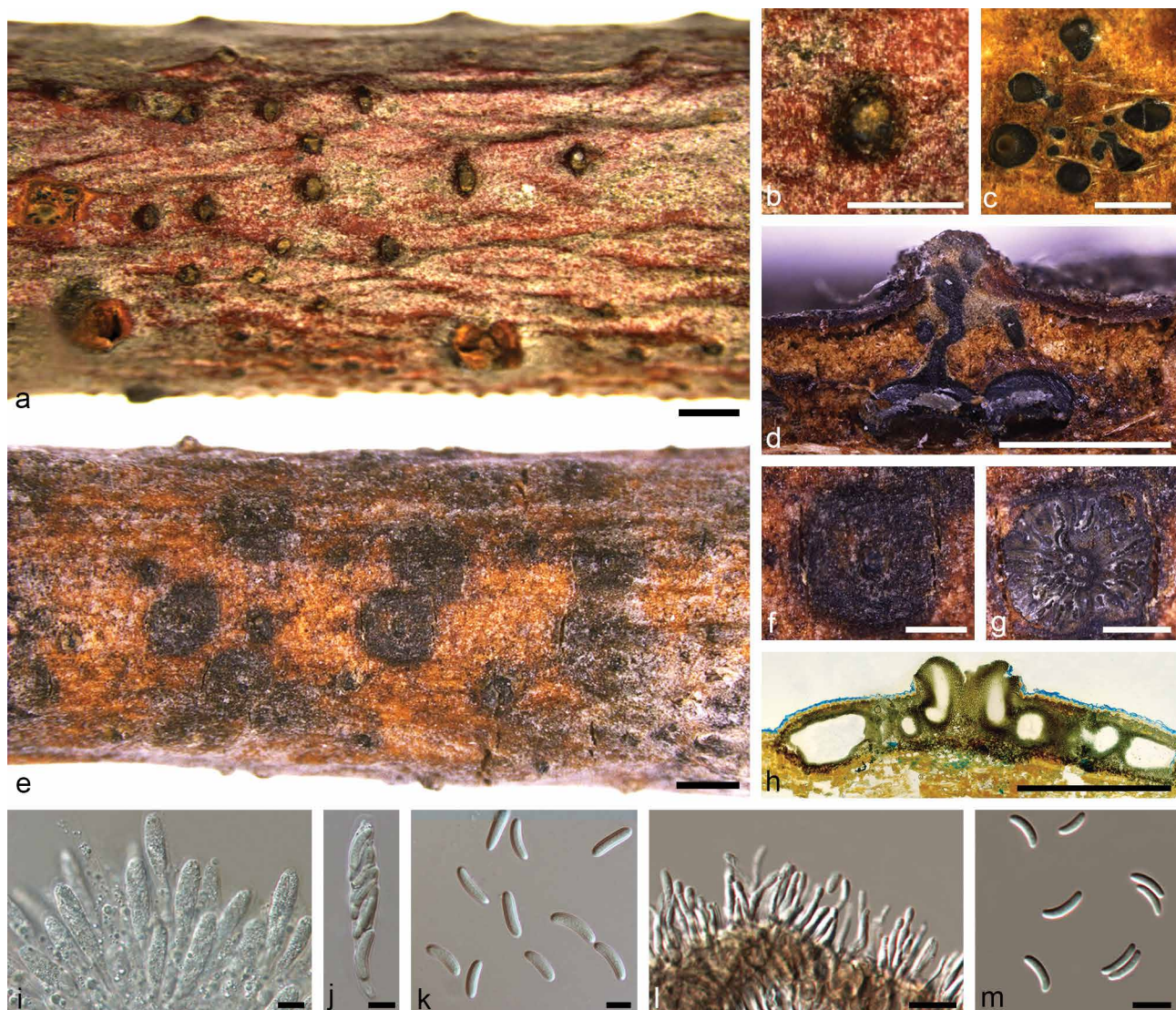


**Fig. 8** *Cytospora berberidis* on *Berberis dasystachya* (BJFC-S681). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.



**Fig. 9** *Cytospora bungeanae* on *Pinus bungeana* (BJFC-S1106). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.





**Fig. 10** *Cytospora carbonacea* on *Ulmus pumila* (BJFC-S630). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i–j. asci; k. ascospores; l. conidiogenous cells with attached conidia; m. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–m = 10  $\mu$ m.

***Cytospora celtidicola*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830149; Fig. 11

**Etymology.** The name reflects the host genus from which it was collected, *Celtis*.

**Sexual morph** not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, nearly flat, slightly erumpent through the bark surface in a large area, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* dark grey to black, inconspicuous, circular, (140–)150–220(–230)  $\mu$ m diam, with one ostiole per disc. *Ostiole* dark grey to black, inconspicuous, (40–)45–60(–70)  $\mu$ m diam. *Locules* undivided, circular to ovoid, occasionally wrinkled, (640–)700–1220(–1350)  $\mu$ m diam. *Conidiophores* borne along the locules, hyaline, branched at the base, in the middle or unbranched, thin-walled, 8–18(–21)  $\times$  1  $\mu$ m, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 3–7  $\times$  1.5–2  $\mu$ m, tapering towards the apices. *Conidia* hyaline, allantoid, egutulate, smooth, aseptate, thin-walled, 5–6  $\times$  1–1.5  $\mu$ m.

**Culture characteristics** — On PDA, colonies initially white, growing up 6 cm after 3 d, becoming pale vinaceous grey to vinaceous grey, entirely covering the 9 cm Petri dish after 10 d. *Colonies* flat, thin with a uniform texture, lacking aerial mycelium. *Pycnidia* irregularly distributed on medium surface.

**Materials examined.** CHINA, Anhui Province, Hefei City, West Mountain Forest Park, N31°85'85.13" E117°28'12.11", from branches of *Celtis sinensis*, 18 Aug. 2014, X.L. Fan & B. Cao (holotype BJFC-S1107, ex-type living culture CFCC 50497); *ibid.*, BJFC-S1109, living culture CFCC 50498.

**Notes** — *Cytospora celtidicola* is associated with canker disease of *Celtis sinensis* in China. It represents a unique species of *Cytospora* diagnosed by obvious symptoms which include orange-coloured branches. Additionally, the clear multi-gene phylogram placed it in a distinct clade with high support (MP/ML/BI = 100/100/1, Fig. 4).

***Cytospora ceratosperma*** (Tode) G.C. Adams & Rossman, IMA Fungus 6: 147. 2015 — Fig. 12

**Basionym.** *Sphaeria ceratosperma* Tode, Fung. Mecklenb. Sel. (Lüneburg) 2: 53. 1791.

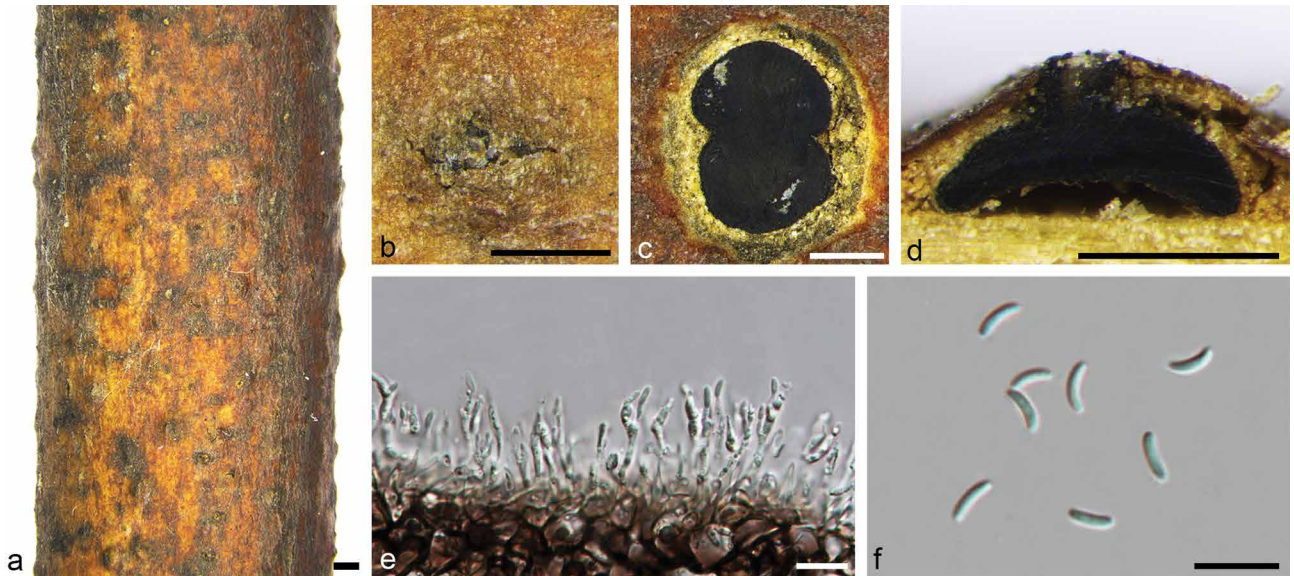
**Synonyms.** *Sphaeria sacculus* Schwein., Schr. Naturf. Ges. Leipzig 1: 26. 1822.

*Valsa ceratosperma* (Tode) Maire, Publ. Inst. Bot. 3(4): 20. 1937.

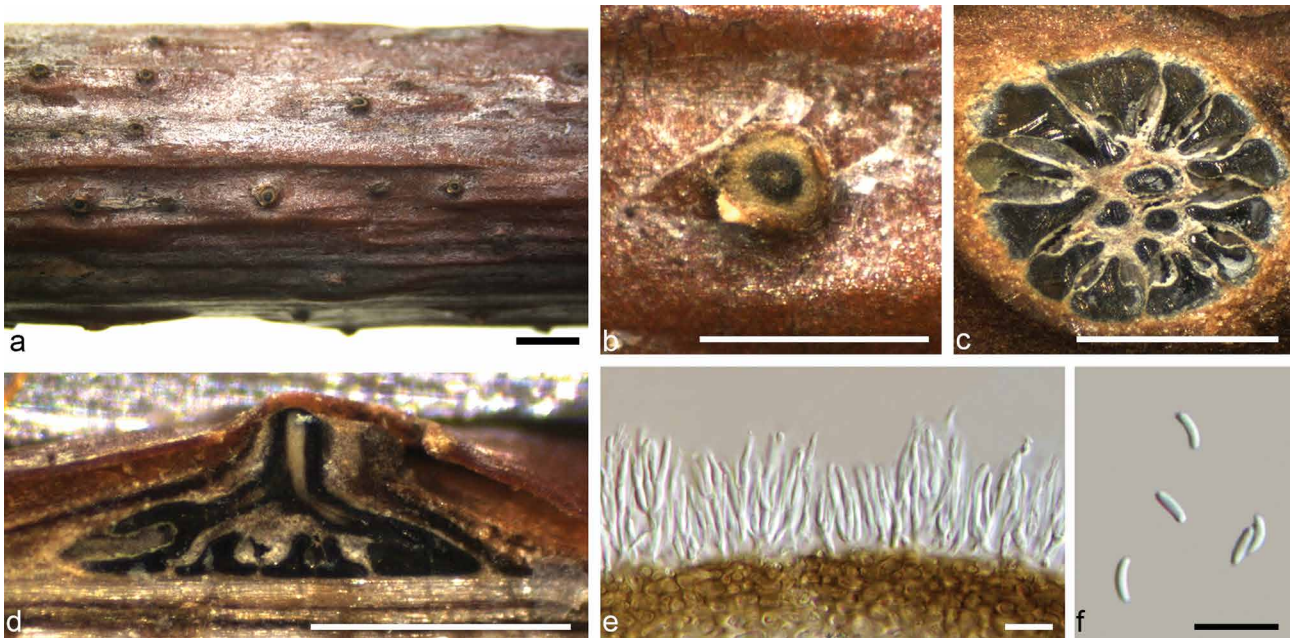
*Cytospora sacculus* (Schwein.) Gvrit., Mikol. Fitopatol. 3: 207. 1969.

**Sexual morph** not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the bark surface, discoid to conical, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* light brown to grey, circular to ovoid, (160–)170–240(–255)  $\mu$ m diam, with one ostiole per disc. *Ostiole*





**Fig. 11** *Cytospora celtidicola* on *Celtis sinensis* (BJFC-S1107). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.



**Fig. 12** *Cytospora ceratosperma* on *Juglans regia* (BJFC-S774). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.

in the centre of the disc, dark grey to black, conspicuous, (70–)80–100(–115)  $\mu$ m diam. *Locules* numerous, arranged circularly or elliptically with independent walls, (480–)510–960(–1050)  $\mu$ m diam. *Conidiophores* borne along the locules, hyaline, branched at base, middle, or unbranched, thin-walled, 12–23(–25.5)  $\times$  1.5–2  $\mu$ m, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 7.5–12(–14)  $\times$  1.5–2  $\mu$ m, tapering towards apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, 3.5–5(–5.5)  $\times$  1(–1.5)  $\mu$ m.

**Culture characteristics** — On PDA, colonies white with slight pale colour at the centre after 3 d, growing up to 3.5 cm diam, becoming greyish sepia after 7 d and finally olivaceous grey after 30 d, reaching 9 cm diam. *Colonies* flat, thin with a uniform texture, lacking aerial mycelium. *Pycnidia* are sparse and distributed irregularly on culture medium surface.

**Materials examined.** CHINA, Gansu Province, Gannan City, Diebu County, Luoda Town, Mogou, N33°56'46.77" E103°52'18.16", from stems of *Juglans regia*, 11 Aug. 2012, X.L. Fan (BJFC-S774, living culture CFCC 89624); *ibid.*, BJFC-S775, living culture CFCC 89625.

**Notes** — *Cytospora ceratosperma* has been recorded from a range of host plants, e.g., *Juglans*, *Malus*, *Pyrus*, *Rosa* and *Ziziphus* in China and Iran (Teng 1963, Tai 1979, Wang et al. 2007, Fotouhifar et al. 2010, Du et al. 2013, Fan et al. 2015a). *Cytospora ceratosperma* has many taxonomic synonyms and confusion exists regarding its morphology and DNA phylogeny. Adams et al. (2005) narrowed the morphological species concept of *Valsa ceratosperma* (syn. *C. sacculus*) and considered it as a distinct species with multi-ostioles per disc, independent locule walls and greyish to yellow-brown and brownish grey colonies, and provided the first ITS sequence data. Rossman et al. (2015) recommended to use *C. ceratosperma*, having priority over *V. ceratosperma*.



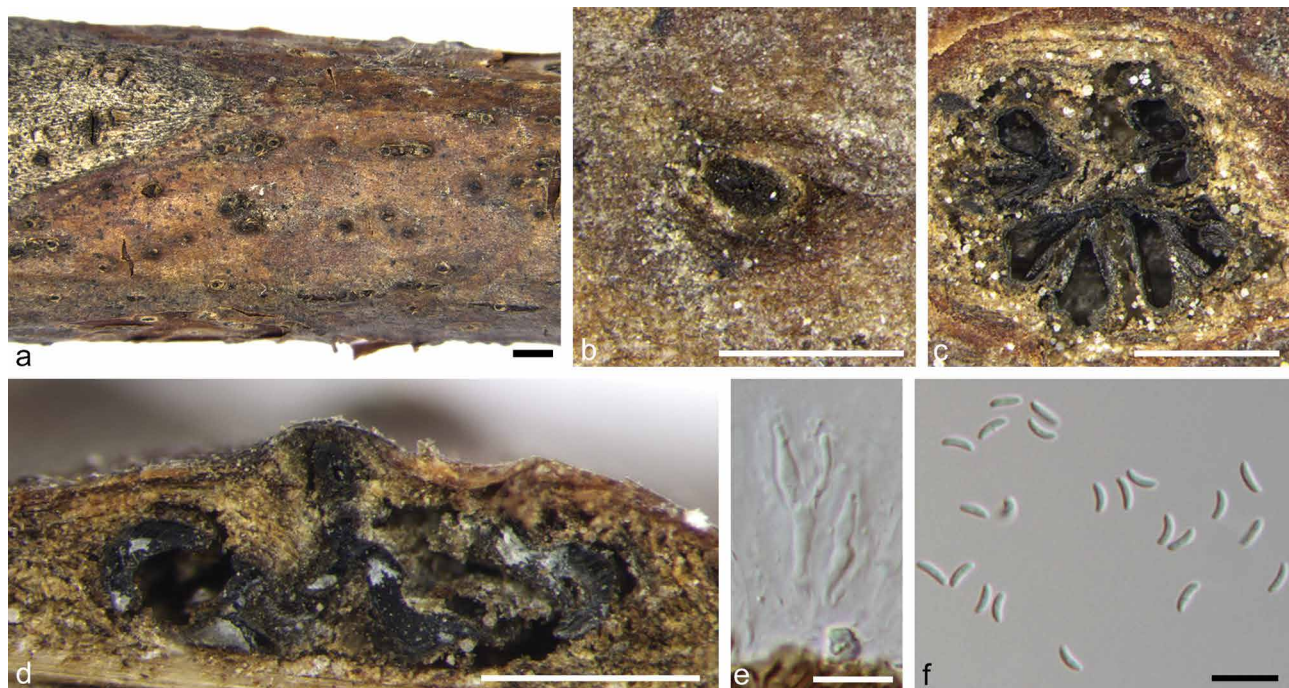


Fig. 13 *Cytospora ceratospermopsis* on *Juglans regia* (BJFC-S567). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.

***Cytospora ceratospermopsis*** C.M. Tian & X.L. Fan, sp. nov.  
— MycoBank MB830150; Fig. 13

*Etymology.* Named after its morphological similarity to *C. ceratosperma*.

*Sexual morph* not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the bark surface, discoid to conical, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* pale brown to grey, circular to ovoid, (170–)200–300(–340)  $\mu$ m diam, with one ostiole per disc. *Ostiole* in the centre of the disc, dark grey to black, conspicuous, (55–)60–90(–100)  $\mu$ m diam. *Locules* numerous, arranged circularly or elliptically with independent walls, (800–)850–1300(–1400)  $\mu$ m diam. *Conidiophores* borne along the locules, hyaline, branched at the middle or unbranched, thin-walled, 13–21(–22)  $\times$  2–2.5  $\mu$ m, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 5–8  $\times$  2–3  $\mu$ m, tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (4.5–)5–5.5(–6)  $\times$  1–1.5  $\mu$ m.

*Culture characteristics* — On PDA, colonies white with buff in the centre after 3 d, growing up to 6.5 cm diam, becoming greyish with isabelline in the centre, reaching 8.5 cm diam after 30 d; sterile.

*Materials examined.* CHINA, Shaanxi Province, Xianyang City, Yangling County, N34°14'06.08" E108°00'48.30", on stems of *Juglans regia*, 30 May 2012, X.L. Fan (holotype BJFC-S567, ex-type living culture CFCC 89626); *ibid.*, BJFC-S568, living culture CFCC 89627.

*Notes* — *Cytospora ceratospermopsis* is associated with canker disease of *Juglans regia* in China. Fan et al. (2015a) temporarily identified CFCC 89626 and CFCC 89627 as *Cytospora ceratosperma* (syn. *C. sacculus*) due to their similar morphology and phylogeny. Morphologically, it differs from *Cytospora ceratosperma* by larger multiple locules (850–1300 vs 510–960  $\mu$ m) with independent walls and conidial size (4.5–6  $\times$  1–1.5 vs 3.5–5  $\times$  1  $\mu$ m in *C. ceratosperma*). The phylogenetic inferences resolved *C. ceratospermopsis* as a distinct lineage from *C. ceratosperma*.

***Cytospora chrysosperma*** (Pers.) Fr., Sylv. Mycol. Berol. (Berlin): 28. 1818 — Fig. 14

*Basionym.* *Sphaeria chrysosperma* Pers., Neues Mag. Bot. 1: 82. 1794.

*Synonyms.* *Naemaspora chrysosperma* (Pers.) Pers., Observ. Mycol. (Lipsiae) 1: 80. 1796.

*Valsa sordida* Nitschke, Pyrenomyc. Germ. 2: 203. 1870.

*Description* — See Fan et al. (2014b).

*Materials examined.* CHINA, Gansu Province, Gannan City, Lintan County, Ligang Village, N34°39'07.89" E103°27'14.78", on twigs and branches of *Populus alba* subsp. *pyramidalis*, 8 Aug. 2012, X.L. Fan (BJFC-S750, living culture CFCC 89981); Tibet Province, Shigatse City, N29°27'34.53" E89°90'23.08", on twigs and branches of *Ulmus pumila*, 2 Feb. 2012, X.L. Fan (BJFC-S788, living culture CFCC 89982); Shaanxi Province, Yulin City, Airport East Road, N38°19'21.16" E109°39'54.73", from branches of *Salix psammophila*, 1 Aug. 2013, X.L. Fan (BJFC-S975, living culture CFCC 89629).

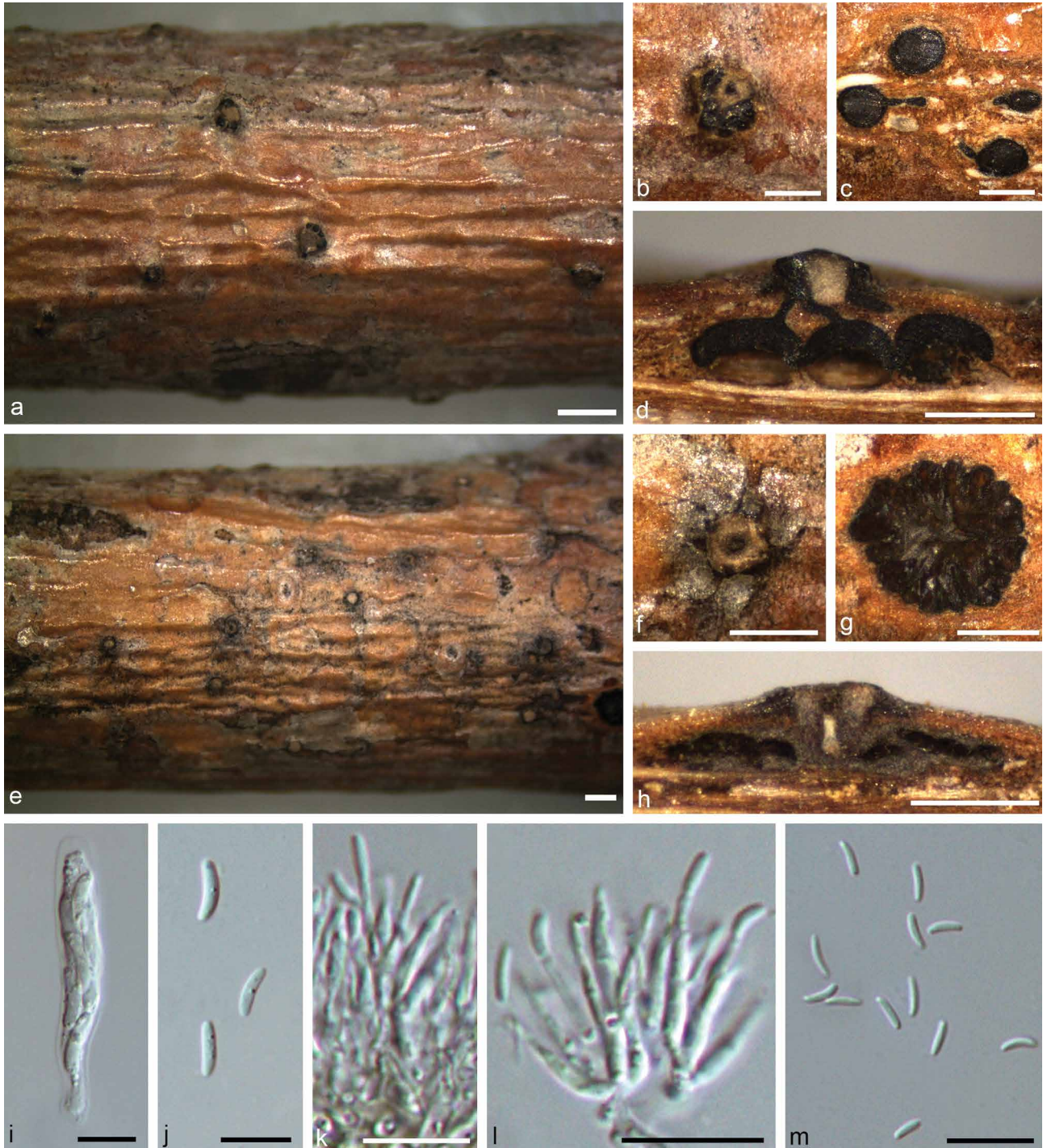
*Notes* — *Cytospora chrysosperma* is the type species of the genus *Cytospora*, and is also the most common species causing canker disease with a wide host range (Teng 1963, Tai 1979, Zhuang 2005, Adams et al. 2006, Fan et al. 2014b). According to earlier records, several plant genera are hosts to this species in China, e.g., *Castanea*, *Morus*, *Populus*, *Salix* and *Ulmus* (Teng 1963, Tai 1979, Zhuang 2005). Based on ITS and the multi-gene dataset the current investigation revisited the related fungi and delineated the *C. chrysosperma* complex, including *C. ailanthicola*, *C. chrysosperma*, *C. joaquinensis*, *C. longiostiolata*, *C. melnikii*, *C. populicola*, *C. salicacearum*, *C. salicina*, *C. sophoriopsis* and *C. rostrata* (Fig. 3, 4). These results suggest that this species is a common, severe pathogen in China, causing canker disease of *Salicaceae* hosts.

***Cytospora elaeagni*** Allesch., Hedwigia Beibl. 36: 162. 1897 — Fig. 15

*Description* — See Fan et al. (2015b).

*Materials examined.* CHINA, Ningxia Province, Guyuan City, Changchengliang, N36°03'01.77" E106°16'18.63", from twigs and branches of *Elaeagnus angustifolia*, 24 July 2013, X.L. Fan (BJFC-S965, living culture CFCC 89632); *ibid.*, living culture CFCC 89633.





**Fig. 14** *Cytospora chrysosperma* on *Populus alba* subsp. *pyramidalis* (BJFC-S750). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i. ascus; j. ascospores; k–l. conidiogenous cells with attached conidia; m. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–m = 10  $\mu$ m.

**Notes** — *Cytospora elaeagni* has been recorded from *Elaeagnus angustifolia* in China, Germany and North America, but these records lacked any detailed descriptions, illustrations and molecular data (Sydow 1897, Zhuang 2005). Morphologically, *C. elaeagni* is similar to *C. carbonacea* from *Ulmus* spp., but can be distinguished by the smaller size and fewer number of locules, cultures secreting a pale brown pigment, and by the plant hosts (Fan et al. 2015b, Yang et al. 2015).

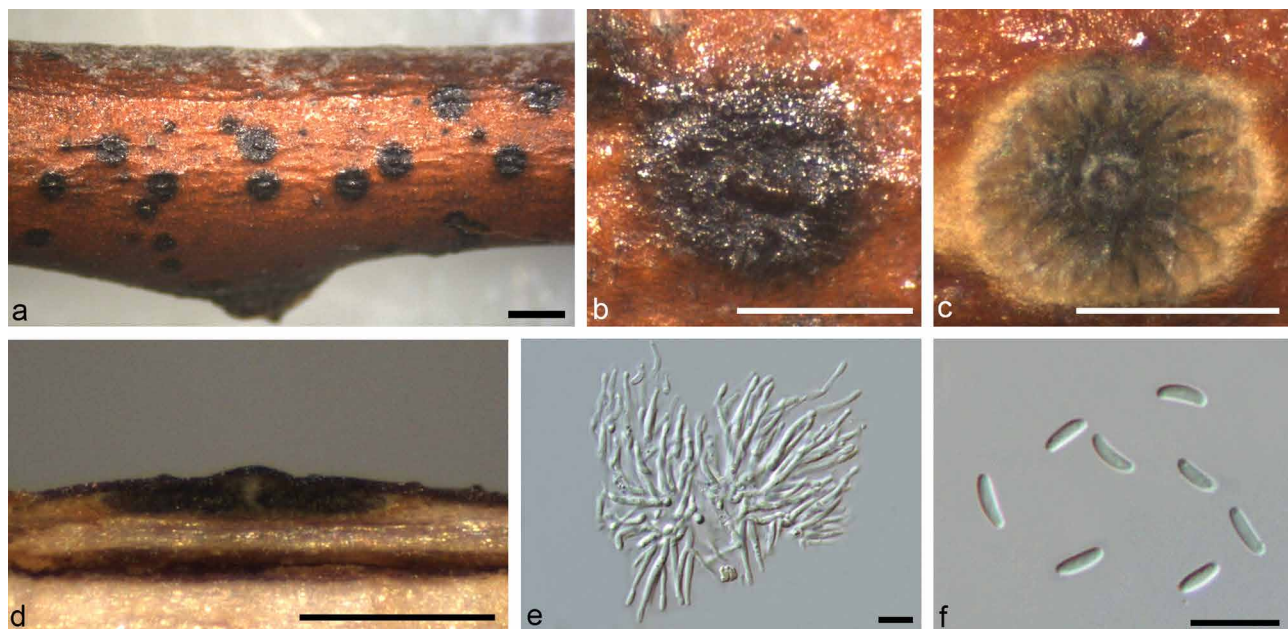
***Cytospora erumpens*** Norph. et al., *Mycosphere* 8: 64. 2017  
— Fig. 16

**Description** — See Norphanphoun et al. (2017).

**Material examined.** CHINA, Shanxi Province, Jincheng City, N35°25'43.27" E111°59'06.66", from branches of *Prunus padus*, 21 Apr. 2014 X.L. Fan & B. Cao (BJFC-S1064, living culture CFCC 50022).

**Notes** — *Cytospora erumpens* was described from dead and dying branches of *Salix* in Russia (Norphanphoun et al. 2017). This species can be identified as having typical leucostoma-like conidiomata. The current study extended its distribution to China, and its host range to include *Rosaceae*.





**Fig. 15** *Cytospora elaeagni* on *Elaeagnus angustifolia* (BJFC-S965). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.

***Cytospora euonymicola*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830151; Fig. 17

*Etymology.* The name reflects the host genus from which it was described, *Euonymus*.

*Sexual morph* not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the surface, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* isabelline to dark brick, conspicuous, circular to ovoid, (220–)240–350(–400)  $\mu$ m diam, with one ostiole per disc. *Ostiole* in the centre of the disc, black, conspicuous, (50–)60–120(–125)  $\mu$ m diam. *Locules* numerous, subdivided frequently by invaginations with common walls, (950–)1150–1400(–1550)  $\mu$ m diam. *Conidiophores* hyaline, branched at the base or unbranched, thin-walled, 13–21.5(–23)  $\times$  1.5–2  $\mu$ m, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 8–11(–13.5)  $\times$  1–2  $\mu$ m, tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (4–)4.5–5  $\times$  1  $\mu$ m.

*Culture characteristics* — On PDA, colonies initially white to greyish, growing up to 4 cm after 3 d, secreting a pale brown pigment, reaching up to 9 cm after 7–10 d, and becoming greyish sepia, secreting a fuscous black pigment after 30 d. *Colonies* felt-like with a uniform texture. *Pycnidia* irregularly distributed over the medium surface.

*Materials examined.* CHINA, Shaanxi Province, Yulin City, Economic Development Zone, N38°13'37.46" E109°44'60.93", from branches of *Euonymus kiautschovicus*, 31 July 2013, X.L. Fan (holotype BJFC-S1105, ex-type living culture CFCC 50499); *ibid.*, living culture CFCC 50500.

*Notes* — *Cytospora euonymicola* is associated with canker disease of *Euonymus kiautschovicus*. Morphologically, *C. euonymicola* has large, multiple locules (1150–1400  $\mu$ m) with large conidia (4.5–5  $\times$  1  $\mu$ m), compared to the smaller conidia of *C. euonymella* (2.5  $\times$  0.5  $\mu$ m) and the larger conidia of *Cytospora euonymi* (8  $\times$  2  $\mu$ m) and *C. euonymina* (6.5–7.5  $\times$  1.5–2  $\mu$ m) (Cooke 1885, Saccardo 1892). The multigene phylogenetic analysis supported this species as new with high support values (ML/MP/BI = 100/100/1, Fig. 4).

***Cytospora euonymina*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830152; Fig. 18

*Etymology.* The name reflects the host genus from which it was collected, *Euonymus*.

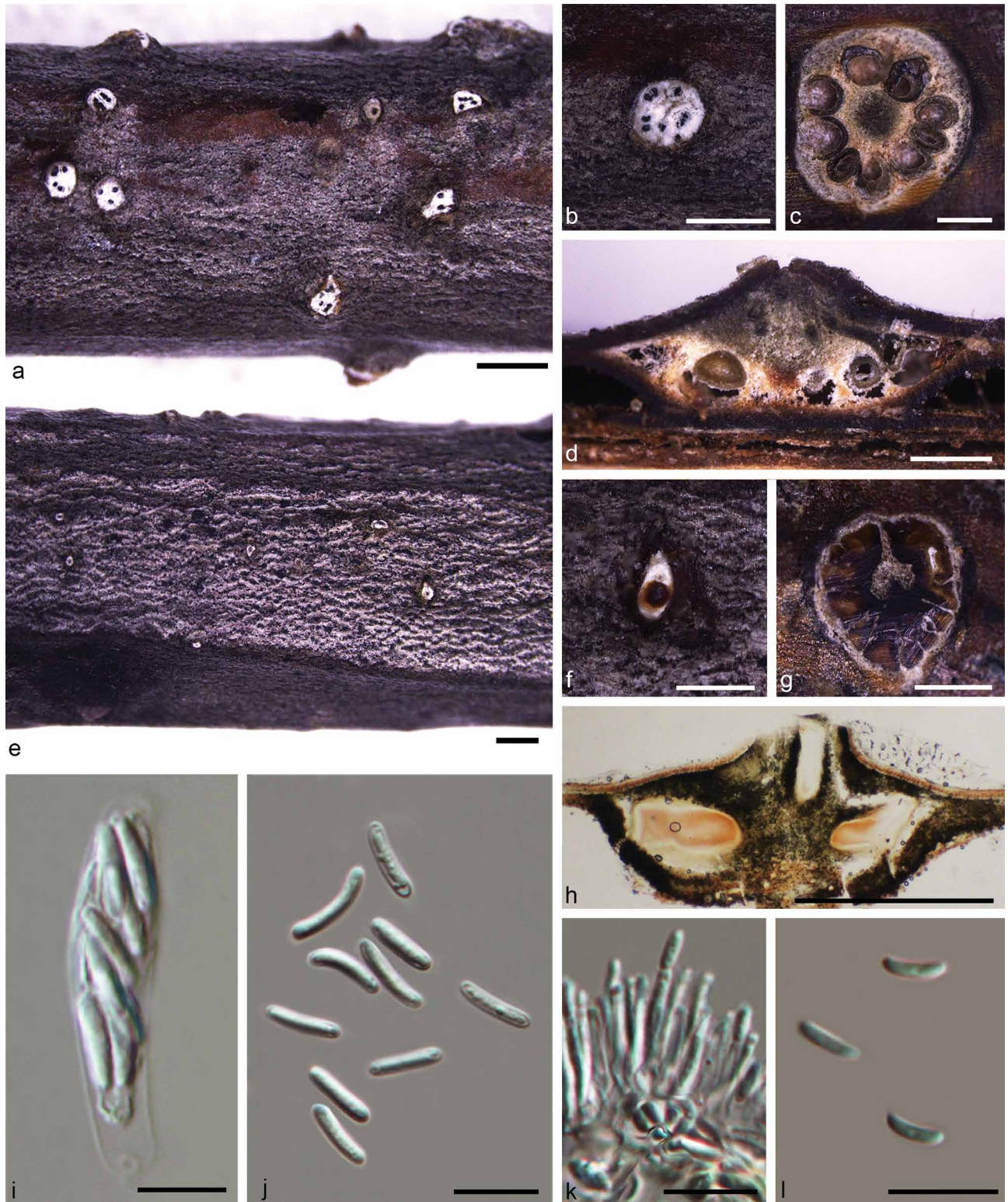
*Sexual morph* not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, producing black area on bark, erumpent through the surface, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* honey to dark mouse grey, conspicuous, circular to ovoid, (190–)200–230(–250)  $\mu$ m diam, with one ostiole per disc. *Ostiole* in the centre of the disc, black, conspicuous, (60–)70–115(–120)  $\mu$ m diam. *Locules* numerous, subdivided frequently by invaginations with common walls, (650–)700–900(–1150)  $\mu$ m diam. *Conidiophores* borne along the locules, hyaline, unbranched or occasionally branched at the base or in the middle, thin-walled, 12–16.5  $\times$  1.5–2  $\mu$ m, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 8.5–12  $\times$  1.5–2  $\mu$ m, tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, 6.5–7.5  $\times$  1.5–2  $\mu$ m.

*Culture characteristics* — On PDA, colonies initially white, irregular, lacking aerial mycelium, fast growing, reaching up to 9 cm diam after 3 d. *Colonies* pale white to light salmon after 30 d, lacking aerial mycelium. *Pycnidia* distributed sparsely over the medium surface, exuding a salmon conidia mass from the ostioles.

*Materials examined* (all on twigs and branches of *Euonymus kiautschovicus*). CHINA, Shanxi Province, Datong City, Wenying Ecological Garden, N40°04'32.40" E113°21'57.85", 15 Apr. 2014, X.L. Fan (holotype BJFC-S1025, ex-type living culture CFCC 89993); Datong City, Wenying Ecological Garden, N40°04'19.70" E113°22'20.29", 15 Apr. 2014, X.L. Fan (BJFC-S1026, living culture CFCC 89999).

*Notes* — *Cytospora euonymina* is associated with canker disease of *Euonymus kiautschovicus*. Morphologically, *C. euonymina* has larger conidia (6.5–7.5  $\times$  1.5–2  $\mu$ m) as compared with conidia of *C. euonymella* (2.5  $\times$  0.5  $\mu$ m; Saccardo 1892). *Cytospora euonymi* was similarly characterised by having pycnidia covered by the darkened cuticle compared to *C. euonymina*, whereas the author described it with 'rather small' pycnidia and larger conidia (8  $\times$  2  $\mu$ m) (Cooke 1885, Saccardo 1892). In the current study we treat this species as new, which is also reflected by its phylogeny (ML/MP/BI = 100/100/1).





**Fig. 16** *Cytospora erumpens* on *Prunus padus* (BJFC-S1064). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i. ascus; j. ascospores; k. conidiogenous cells with attached conidia; l. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–l = 10  $\mu$ m.

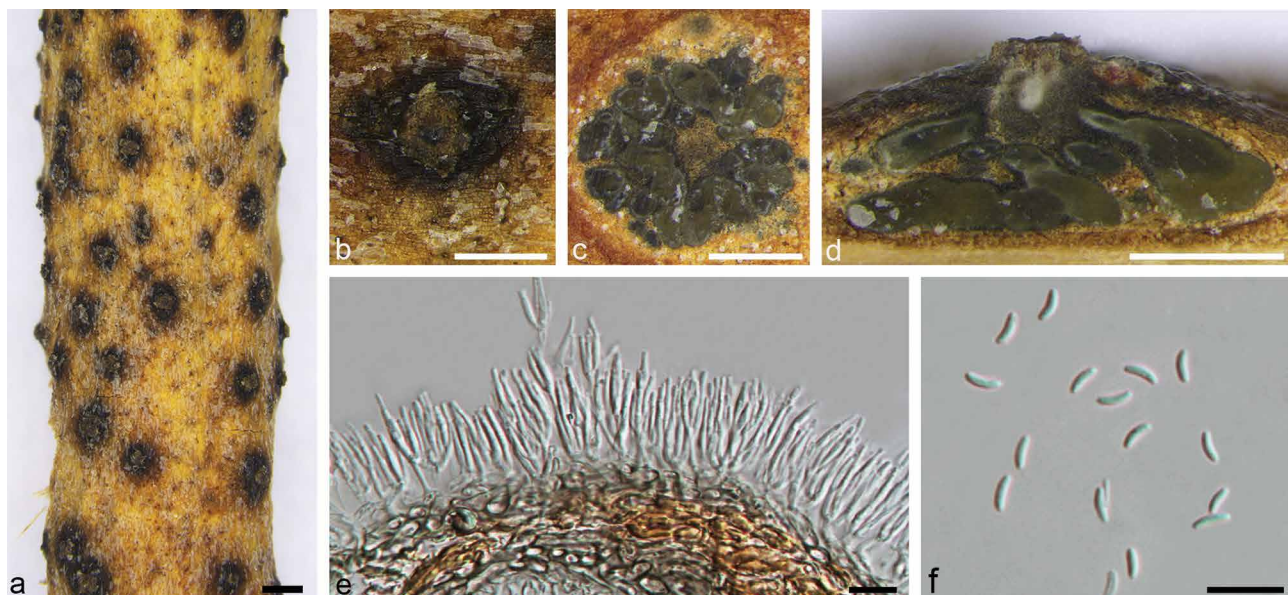
***Cytospora fortunea*** G.C. Zhao et al., J. Northeast For. Univ. 2: 105. 1991 — Fig. 19

*Synonym.* *Valsa fortunea* G.C. Zhao et al., J. Northeast For. Univ. 2: 105. 1991.

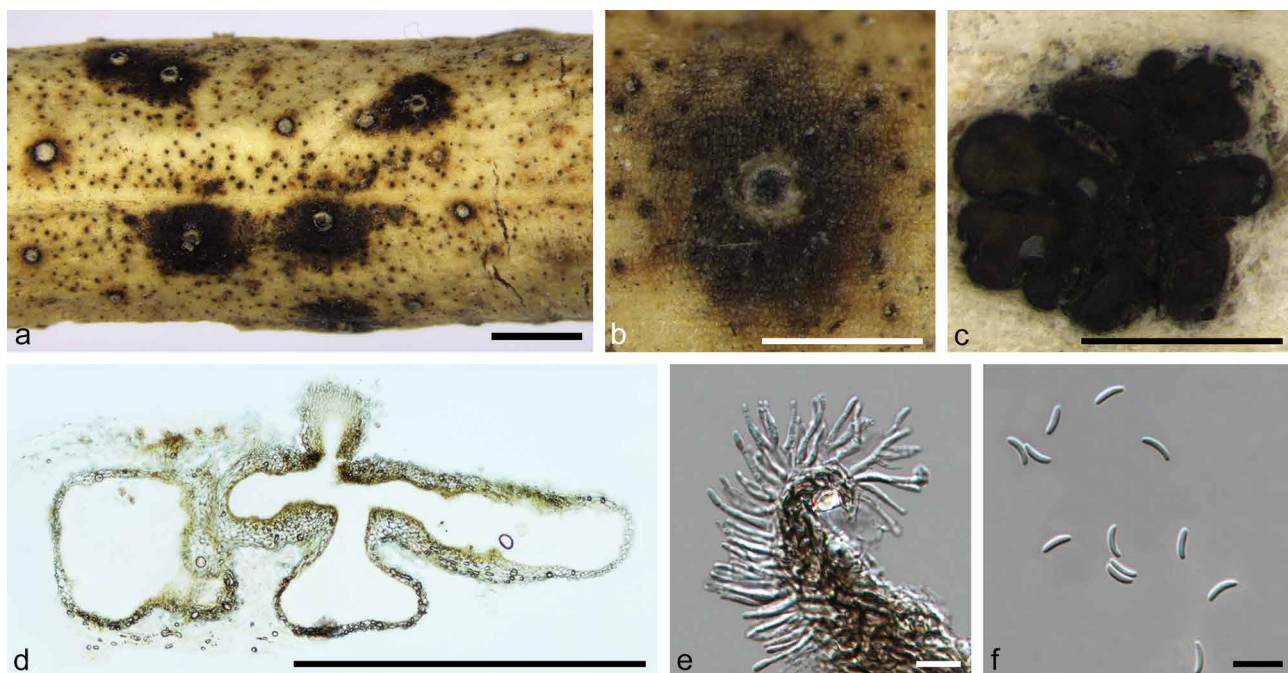
*Ascstromata* immersed in the bark, erumpent through the bark surface, scattered, (700–)750–1200(–1300)  $\mu$ m diam, with 5–18 perithecia arranged circularly or irregularly. *Conceptacle* absent. *Ectostromatic disc* inconspicuous, usually surrounded by tightly aggregated ostiolar necks, (120–)150–320(–350)  $\mu$ m

diam, with 5–18 ostioles per disc. *Ostioles* numerous, violaceous black to black, concentrated, arranged irregularly in a disc, (40–)45.5–55(–75)  $\mu$ m diam. *Perithecia* dark grey to black, flask-shaped to spherical, arranged circularly or irregularly, (145–)180–350(–400)  $\mu$ m diam. *Asci* free, clavate to elongate obovoid, (26–)28.5–35(–40)  $\times$  (3.5–)4–5.5(–6)  $\mu$ m, 8-spored. *Ascospores* biserial, elongate-allantoid, thin-walled, hyaline, aseptate, (7.5–)8–9(–9.5)  $\times$  1.5–2  $\mu$ m. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the surface, with multiple locules. *Conceptacle* absent. *Ectostro-*





**Fig. 17** *Cytospora euonymicola* on *Euonymus kiautschovicus* (BJFC-S1105). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.



**Fig. 18** *Cytospora euonymina* on *Euonymus kiautschovicus* (BJFC-S1025). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.

*matic disc* honey to primrose, conspicuous, circular to ovoid, (135–)155–280(–310)  $\mu$ m diam, with 1–3 ostioles per disc. *Ostiole* in the centre or arranged regularly in the disc, violaceous black to black, conspicuous, (50–)60–140(–150)  $\mu$ m diam. *Locules* numerous, subdivided frequently by invaginations with independent walls, (800–)880–1100(–1250)  $\mu$ m diam. *Conidiophores* and *conidiogenous cells* inconspicuous. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, 5–6(–6.5)  $\times$  1–1.5  $\mu$ m.

*Material examined.* CHINA, Yunan Province, Dali City, Binchuan County, Jizu Mountain, from branches of *Cryptomeria japonica* (as *Cryptomeria fortunei*), Nov. 1989, G.C. Zhao, S.F. Chen & N. Li (holotype HSFC 15206, no living culture).

*Notes* — *Cytospora fortunei* is associated with canker disease of *Cryptomeria japonica* (as *Cryptomeria fortunei*) in China (Zhao et al. 1991). This species can be characterised by the

multiple locules with independent walls from this host (Zhao et al. 1991). This species needs to be re-collected from *Cryptomeria japonica* in China, as presently no living culture is available.

***Cytospora gigalocus*** C.M. Tian et al., Fungal Biol. 119: 313. 2015 — Fig. 20

*Description* — See Fan et al. (2015a).

*Materials examined.* CHINA, Qinghai Province, Xining City, Xishan Botanical Garden, N36°37'25.89" E101°44'48.92", from twigs and branches of *Juglans regia*, 19 Aug. 2012, X.L. Fan (holotype BJFC-S647, ex-type living culture CFCC 89620); *ibid.*, BJFC-S648, living culture CFCC 89621.

*Notes* — *Cytospora gigalocus* has multi-loculate conidiomata with one to five ostioles and shares common walls, and is morphologically similar to *C. schulzeri* from *Malus* spp. and *C. carbonacea* on *Ulmus* spp. (Mehrabi et al. 2011). *Cytospora*





**Fig. 19** *Cytospora fortunea* on *Cryptomeria japonica* (HSFC 15206). a, Herbarium material; b, d, habit of ascostromata on branch; c, g, habit of conidiomata on branch; e, transverse section through ascostroma; f, longitudinal section through ascostroma; h, transverse section through conidioma; i, longitudinal section through conidioma; j–k, asci; l, ascospores; m–n, conidia. — Scale bars: b–c = 1 mm; d–i = 0.5 mm; j–n = 10  $\mu$ m.

*gigalocus* has larger locules with a central column as compared with *C. schulzeri* (1600–2200 vs 1400–1500  $\mu$ m) diam, and it has smaller conidia than *C. carbonacea* ( $4.8 \times 1.1$  vs  $11.7 \times 1.7$   $\mu$ m) (Adams et al. 2006, Zhang et al. 2014, Fan et al. 2015a).

***Cytospora gigaspora*** C.M. Tian et al., Phytotaxa 197: 232. 2015 — Fig. 21

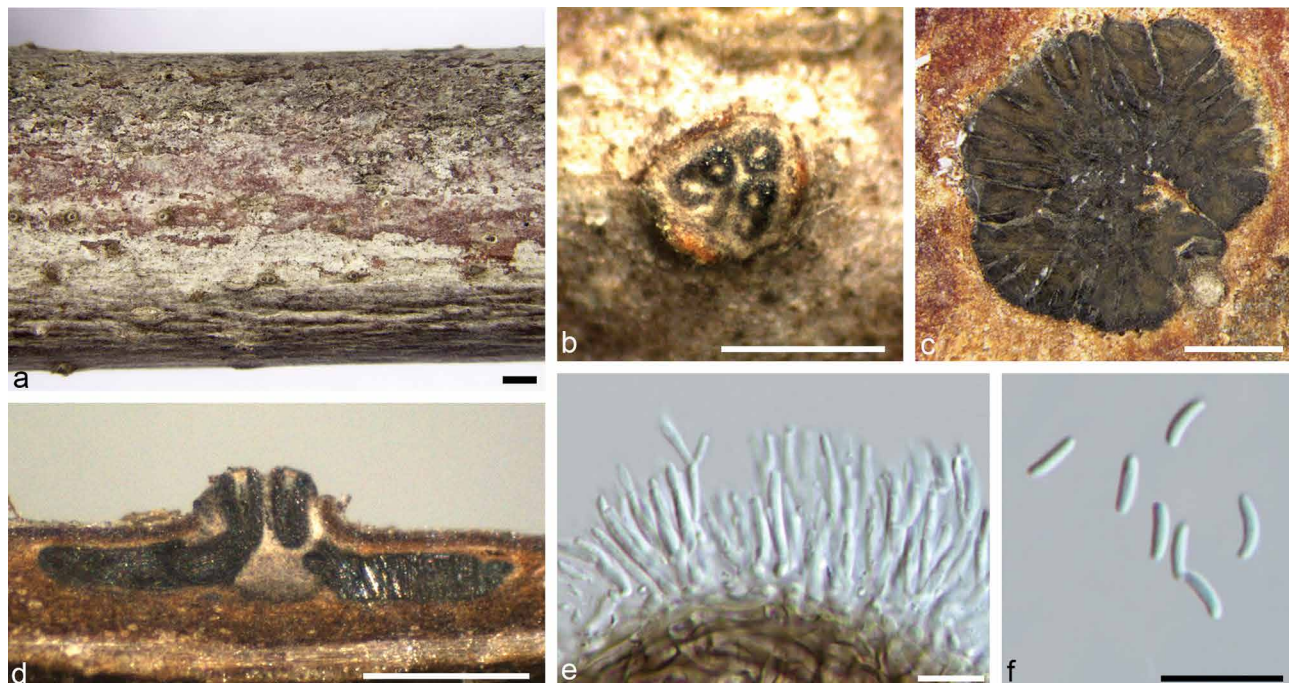
Description — See Fan et al. (2015b).

**Materials examined.** CHINA, Shaanxi Province, Yulin City, Airport East Road, N38°19'21.16" E109°39'54.73", on twigs and branches of *Salix psammophila*, 1 Aug. 2013, X.L. Fan (holotype BJFC-S975, ex-type living culture

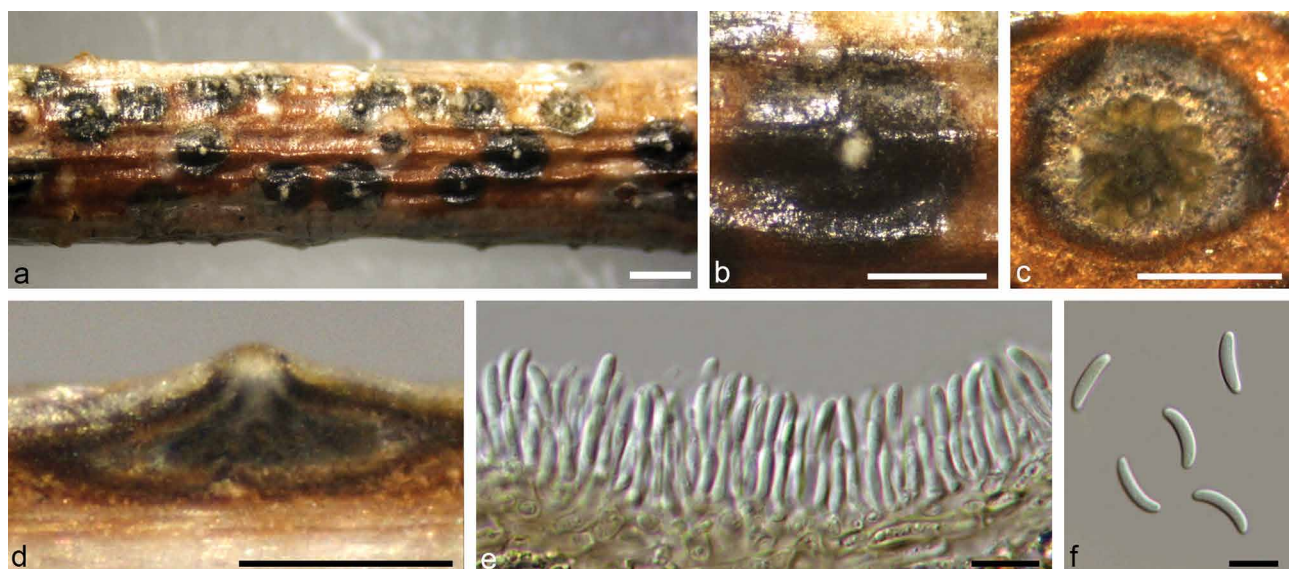
CFCC 89634); Shanxi Province, Datong, City, Wenyong Ecological Garden, N40°04'32.15" E113°21'55.18", on twigs and branches of *Juniperus procumbens*, 17 Apr. 2014, X.L. Fan (BJFC-S1045, living culture CFCC 50014).

**Notes** — *Cytospora gigaspora* has multi-loculate conidiomata with a single ostiole and black conceptacle, which is similar to *C. nivea* on *Populus* and *Salix*. However, *C. gigaspora* has unusually long conidia ( $9\text{--}12 \times 2\text{--}3$   $\mu$ m) and flat locules compared to those of other *Cytospora* species. *Cytospora chrysosperma*, *C. nivea* and *C. populina* have previously been reported from *Populus* and *Salix* spp. as the causative agents of poplar and willow canker disease (Saccardo 1884, Teng 1963, Tai 1979, Wei 1979, Zhuang 2005, Fan et al. 2014b).





**Fig. 20** *Cytospora gigalocus* on *Juglans regia* (BJFC-S647). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.



**Fig. 21** *Cytospora gigaspora* on *Salix psammophila* (BJFC-S975). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.

***Cytospora hippophaës*** Thüm., Fungi Austria. Exsicc.: 282.  
1872 — Fig. 22

Description — See Fan et al. (2015b).

**Materials examined.** CHINA, Gansu Province, Gannan City, Diebu County, N34°02'06.70" E103°12'06.25", from twigs and branches of *Hippophaë rhamnoides*, 7 Aug. 2012, X.L. Fan (BJFC-S779, living culture CFCC 89639); *ibid.*, living culture CFCC 89640.

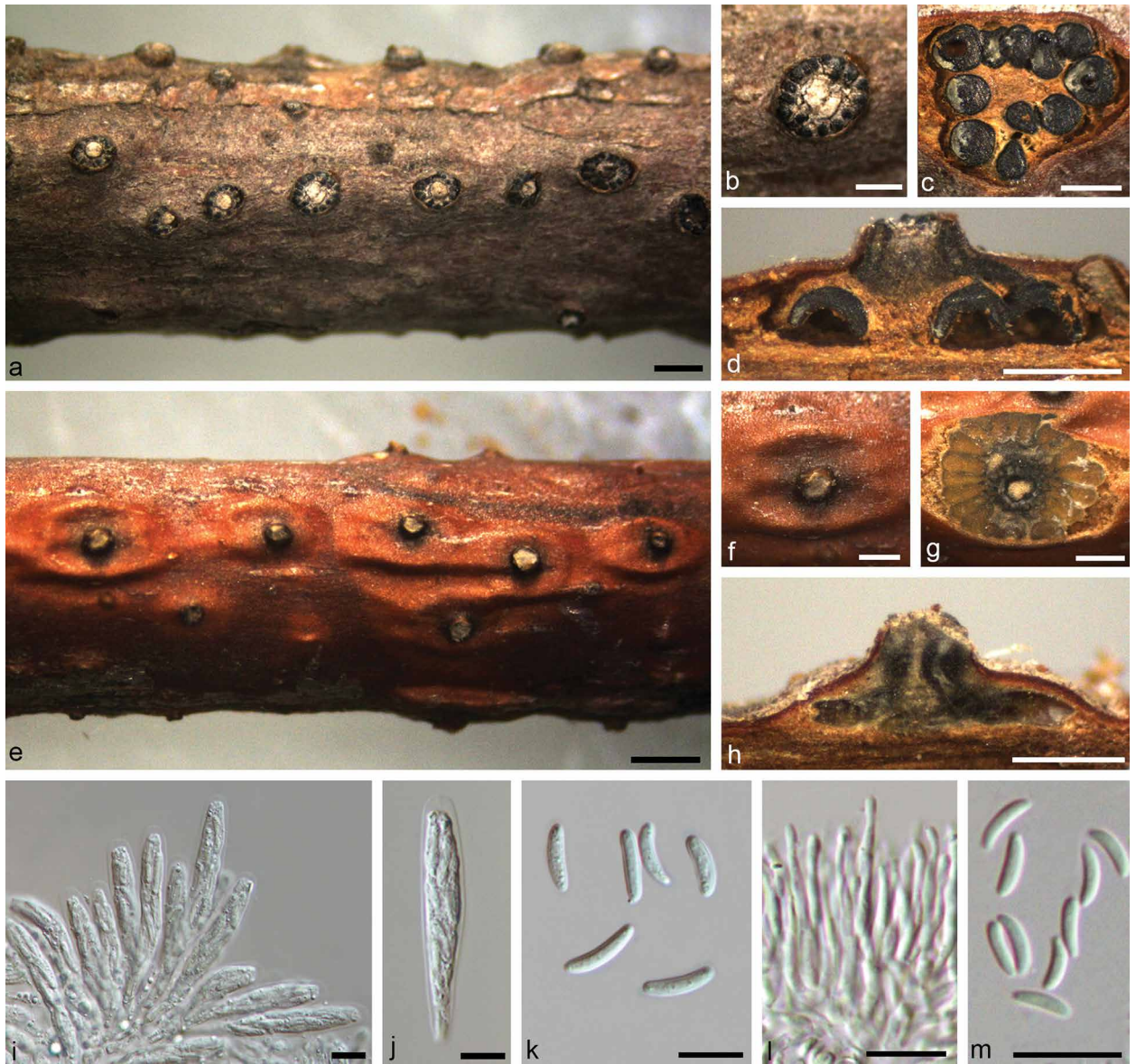
**Notes** — *Cytospora hippophaës* is a common pathogen on *Hippophaë rhamnoides* in China (Zhuang 2005, Fan et al. 2015b). This species represents one of the members of *Cytospora* with host plant specificity, and it is mainly characterised by ascostromata extending to a large circular area with distinct large ectostromatic disc, and flat to discoid pycnidia with multiple ostioles on each ectostromatic disc.

***Cytospora japonica*** (Miyabe & Hemmi) X.L. Fan, *comb. nov.*  
— MycoBank MB830153; Fig. 23

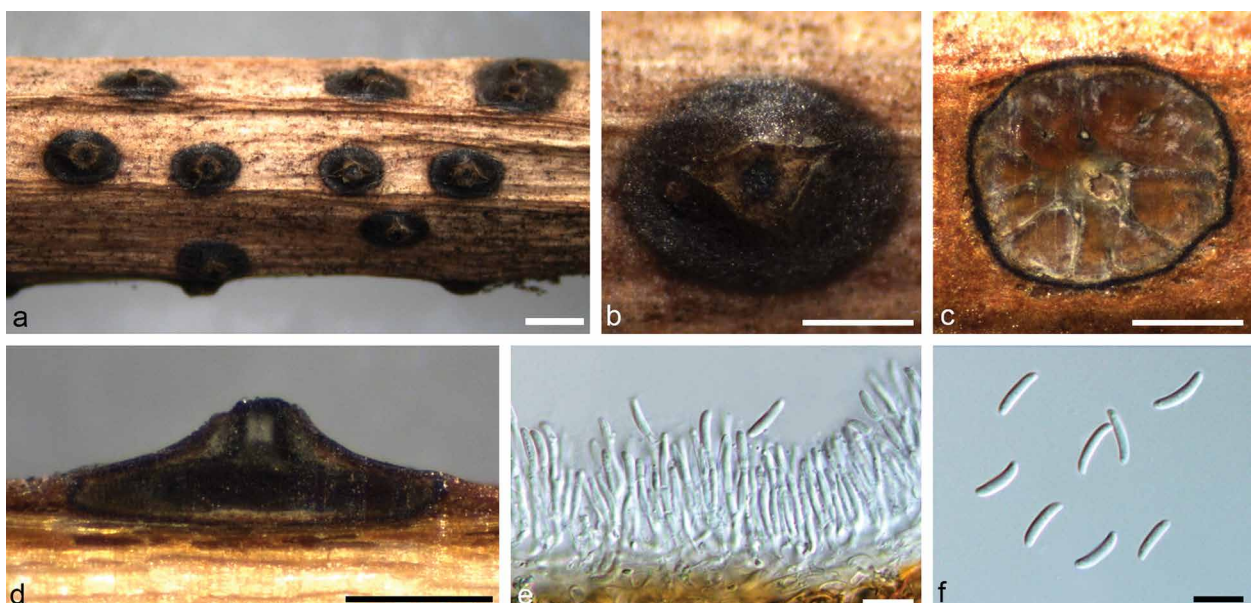
**Basionym.** *Valsa japonica* Miyabe & Hemmi, J. Coll. Agric., Imp. Univ. Sapporo 7(4): 296. 1917.

**Sexual morph** not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the bark surface in a large circular area, discoid to conoid, with large multiple locules. *Conceptacle* black. *Ectostromatic disc* dark grey to black, conspicuous, circular to ovoid, (145–)160–440(–500)  $\mu$ m diam, with one ostiole per disc. *Ostiole* in the centre of the disc, inconspicuous, dark grey to black, (55–)60–125(–130)  $\mu$ m diam. *Locules* numerous, arranged circularly or irregularly with common walls, (750–)810–1150(–1200)  $\mu$ m diam. *Conidiophores* borne along the locules, hyaline, branched at the



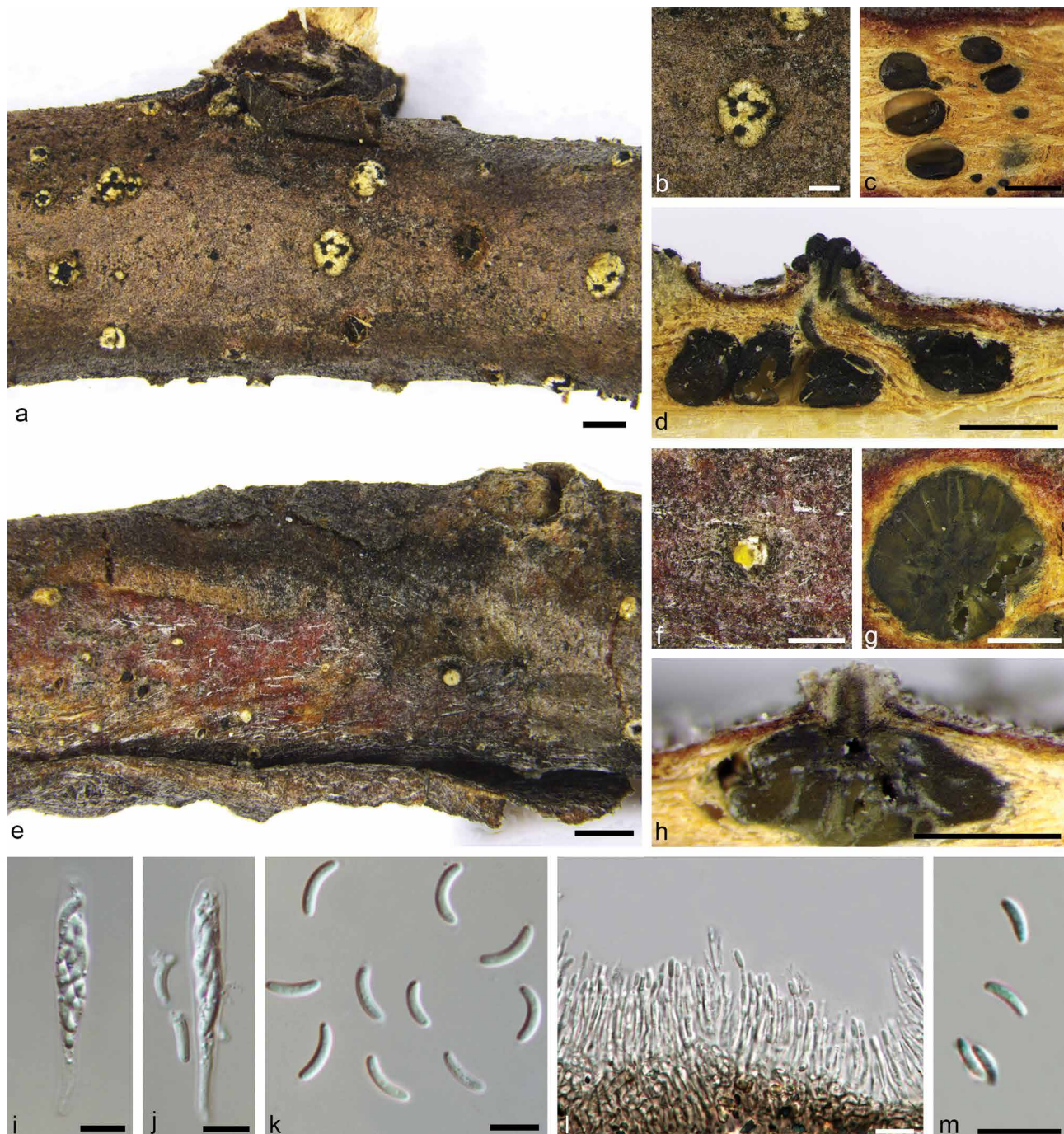


**Fig. 22** *Cytospora hippophaës* on *Hippophaë rhamnoides* (BJFC-S779). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i–j. asci; k. ascospores; l. conidiogenous cells with attached conidia; m. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–m = 10  $\mu$ m.



**Fig. 23** *Cytospora japonica* on *Prunus cerasifera* f. *atropurpurea* (BJFC-S832). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.





**Fig. 24** *Cytospora juniperina* on *Juniperus przewalskii* (CF-2015410). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i–j. asci; k. ascospores; l. conidiogenous cells with attached conidia; m. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–m = 10  $\mu$ m.

base, in the middle, or unbranched, thin-walled, occasionally septate,  $14.5\text{--}21 \times 1\text{--}1.5 \mu\text{m}$ , embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical,  $9\text{--}12.5 \times 1.5\text{--}2 \mu\text{m}$ , tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled,  $(5.5\text{--})6.5\text{--}8.5\text{--}(9) \times 1.5\text{--}2 \mu\text{m}$ .

**Culture characteristics** — On PDA, colonies fast growing, up to 8 cm diam, white greyish, flat, spreading after 3 d, reaching 9 cm diam after 4 d, becoming white greyish to pale greyish after 30 d. *Pycnidia* distributed concentrically on culture surface, extruding a hyaline to pale whitish conidial mass.

**Materials examined.** CHINA, Ningxia Province, Guyuan City, Jingyuan County, N35°28'25.73" E106°22'10.06", on twigs and branches of *Prunus cerasifera*, 22 July 2013, X.L. Fan (BJFC-S832, living culture CFCC 89956); *ibid.*, living culture CFCC 89960.

**Notes** — *Cytospora japonica* is a common species infecting *Rosaceae* hosts in China (Tai 1979). It can be distinguished from *C. leucostoma*, another common pathogen of *Prunoideae*, by the larger size of its conidia ( $6.5\text{--}8.5 \times 1.5\text{--}2$  vs  $4.5\text{--}6 \times 1\text{--}1.5 \mu\text{m}$ ) and colonies which tend to be white in culture.

***Cytospora juniperina*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830154; Fig. 24

**Etymology.** The name reflects the host genus from which it was collected, *Juniperus*.

*Ascostromata* immersed in the bark, erumpent through the bark surface, scattered,  $(1080\text{--})1250\text{--}1600\text{--}(1740) \mu\text{m}$  diam, with 5–12 perithecia arranged circularly or irregularly. *Conceptacle* absent. *Ectostromatic disc* sulphur yellow to greyish sepia, usually surrounded by tightly ostiolar necks, circular,  $(200\text{--})$



220–350(–380)  $\mu\text{m}$  diam. *Ostioles* numerous, dark brown to black, concentrated, arranged circularly in the disc, (45–)50.5–87(–95)  $\mu\text{m}$  diam. *Perithecia* dark grey to black, flask-shaped to spherical, arranged circularly, (250–)320–480(–550)  $\mu\text{m}$  diam. *Asci* free, clavate to elongate obovoid, (33–)35–45(–50)  $\times$  (5–)5.5–6(–6.5)  $\mu\text{m}$ , 8-spored. *Ascospores* biseriate, elongate-allantoid, thin-walled, hyaline, aseptate, (9–)10–13.5(–14)  $\times$  3–3.5(–4)  $\mu\text{m}$ . *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the surface, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* white to smoke grey, conspicuous, circular to ovoid, (210–)220–270(–305)  $\mu\text{m}$  diam, with one ostiole per disc. *Ostiole* in the centre of the disc, violaceous black to black, conspicuous, (42–)50–78(–80)  $\mu\text{m}$  diam. *Locules* numerous, subdivided frequently by invaginations with common walls, (980–)1120–1200(–1320)  $\mu\text{m}$  diam. *Conidiophores* borne along the locules, hyaline, branched at the base, in the middle, or unbranched, thin-walled, 14.5–20(–23)  $\times$  1–2  $\mu\text{m}$ , embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 5.5–11.5  $\times$  1–1.5  $\mu\text{m}$ , tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (5.5–)6–6.5  $\times$  1–1.5  $\mu\text{m}$ .

**Culture characteristics** — On PDA, colonies white, growing up to 3 cm diam after 3 d, becoming buff with cinnamon on the margin, reaching 7 cm diam after 10 d and covering the entire 9 cm Petri dish after 14 d. *Colonies* felt-like with a uniform texture. *Pycnidia* sparsely distributed over the medium surface.

**Materials examined** (all on twigs and branches of *Juniperus przewalskii*). CHINA, Sichuan Province, Aba City, Songtao County, Shuijing Town, N32°54'32.60" E103°40'59.19", 22 Apr. 2015, X.L. Fan & B. Cao (holotype CF-2015410, ex-type living culture CFCC 50501); *ibid.*, CF-2015411, living culture CFCC 50502; Aba City, Songtao County, Shuijing Town, N32°54'28.33" E103°40'58.59", 23 Apr. 2015, X.L. Fan & B. Cao (CF-2015412, living culture CFCC 50503).

**Notes** — *Cytospora juniperina* is associated with canker disease of *Juniperus przewalskii* in China, representing the first report of a *Cytospora* from this host. It is not common and easily diagnosed. It has a prominent white ectostromatic disc in symptomatic branches. It is also distinct based on sequence data and morphology.

***Cytospora leucosperma* (Pers.) Fr., Syst. Mycol. 2: 543. 1823**  
— Fig. 25

*Basionym*. *Naemaspora leucosperma* Pers., Observ. Mycol. 1: 81. 1796.

*Synonyms*. *Sphaeria ambiens* Pers., Syn. Meth. Fung. 1: 44. 1801.

*Valsa ambiens* (Pers.) Fr., Summa Veg. Scand., Sectio Post. (Stockholm): 412. 1849.

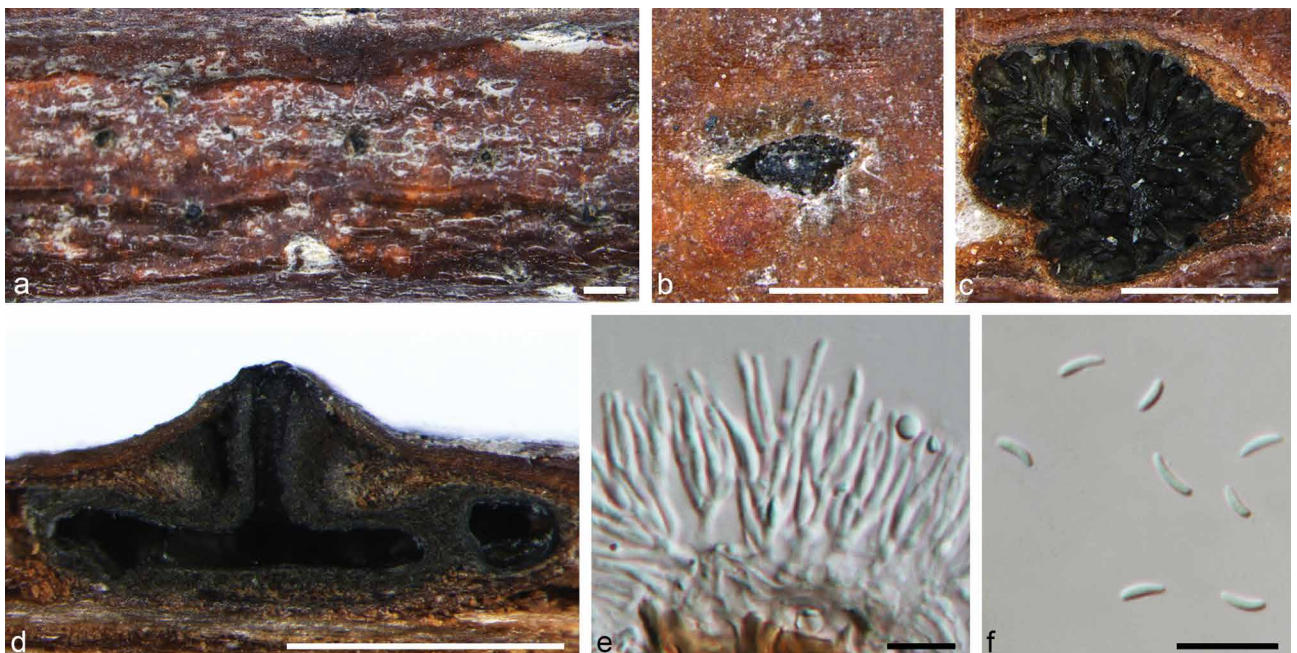
*Cytospora ambiens* Sacc., Michelia 1(5): 519. 1879.

**Sexual morph** not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the bark surface, discoid, nearly flat, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* prominent, flat, dark grey to black, circular to ovoid, (220–)270–370(–400)  $\mu\text{m}$  diam, with one ostiole per disc. *Ostiole* in the centre of the disc, conspicuous, dark grey to black, area below disc a lighter entostroma, (45–)55–100(–120)  $\mu\text{m}$  diam. *Locules* numerous, arranged circularly with common walls, (640–)720–980(–1030)  $\mu\text{m}$  diam. *Conidiophores* borne along the locules, hyaline, branched at the base, in the middle, or occasionally unbranched, thin-walled, 17–25  $\times$  2–2.5  $\mu\text{m}$ , embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 8–14.5  $\times$  2–2.5  $\mu\text{m}$ , tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (3.5–)4–5(–5.5)  $\times$  1–1.5  $\mu\text{m}$ .

**Culture characteristics** — On PDA, colonies fast growing, up to 8 cm diam after 3 d and entire 9 cm Petri dish after 5 d, white to light grey with a uniform texture, with an irregular margin, lacking aerial mycelium, becoming greyish and yellowish coloured after 30 d. *Pycnidia* distributed irregularly on the medium surface.

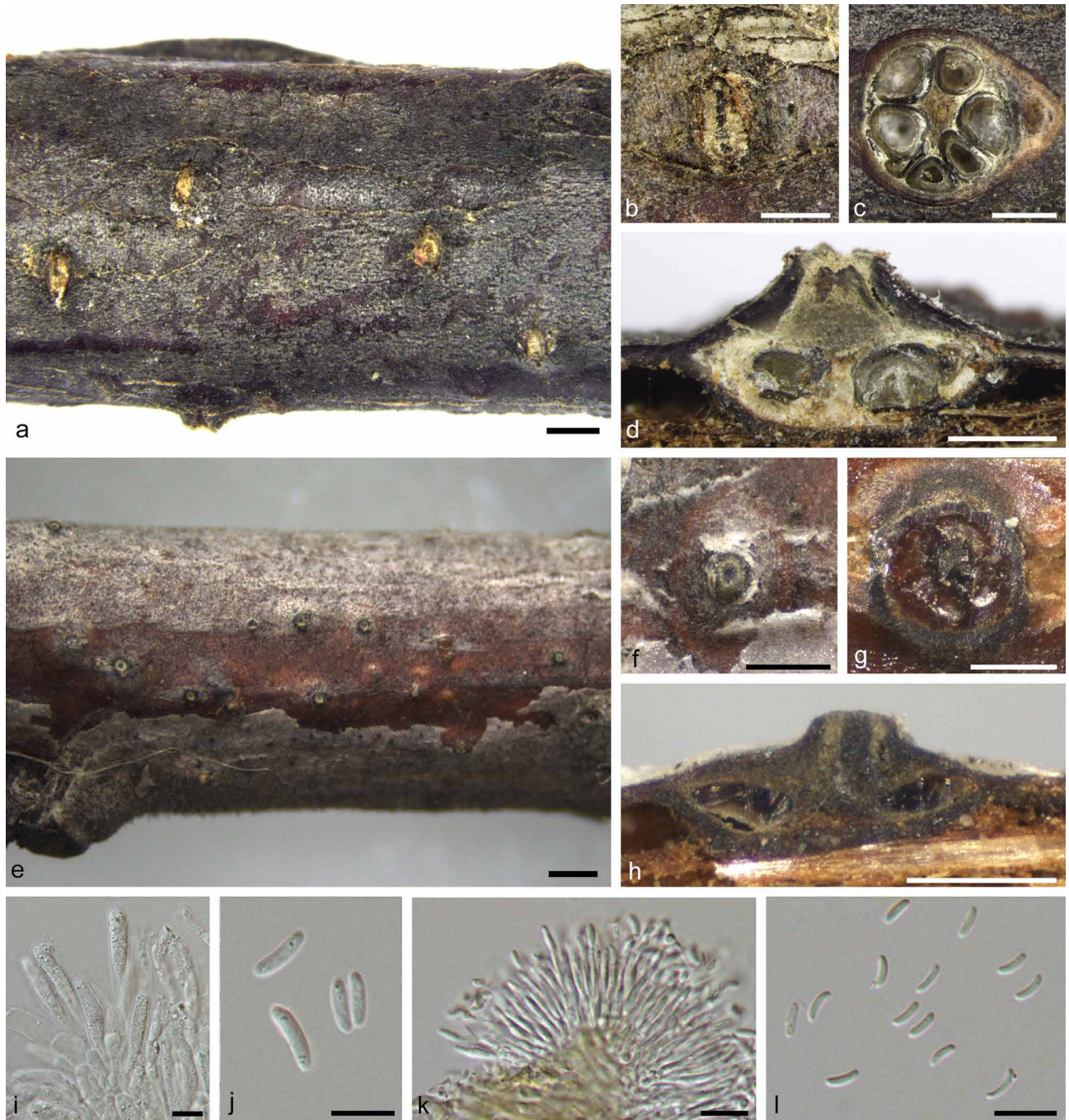
**Materials examined** (all on twigs and branches of *Pyrus bretschneideri*). CHINA, Qinghai Province, Haidong City, Pingan County, E102°09'18.38" N36°27'30.91", 15 Aug. 2012, X.L. Fan (BJFC-S618, living culture CFCC 89894); Gansu Province, Lanzhou City, Anning County, E103°43'18.29" N33°06'52.51", 13 Aug. 2012, X.L. Fan (BJFC-S772, living culture CFCC 89622).

**Notes** — *Cytospora leucosperma* (syn. *Valsa ambiens*) is the type species of the sexual genus *Valsa*, which was re-described by Adams et al. (2005). The asexual morph of *V. ambiens* has commonly been referred to as *C. ambiens*, but Spielman (1985) indicated that *C. leucosperma* is the earliest and correct name for this taxon. *Cytospora leucosperma* has a similar morphology and ITS phylogeny to that of *Cytospora mali* from *Malus* spp., leading to confusion in identifications (Wang et al. 2011).



**Fig. 25** *Cytospora leucosperma* on *Pyrus* sp. (BJFC-S618). a, b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu\text{m}$ .





**Fig. 26** *Cytospora leucostoma* on *Prunus persica* (BJFC-S918). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i. asci; j. ascospores; k. conidiogenous cells with attached conidia; l. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–l = 10 µm.

*Cytospora leucosperma* was chiefly isolated and recorded from infected *Pyrus* spp. in China (Teng 1963, Tai 1979, Zhuang 2005). Urban (1957) and Spielman (1985) designated PR 163781 from *Tilia* as neotype, but without any available living culture or DNA sequence data. Thus, the current identification is provisional and must await further studies of European material from the type host.

***Cytospora leucostoma* (Pers.) Sacc., Michelia 2: 264. 1881 — Fig. 26**

*Synonyms.* *Sphaeria leucostoma* Pers., Ann. Bot. 11: 23. 1794.

*Valsa leucostoma* (Pers.) Fr., Summa Veg. Scand., Section Post. (Stockholm): 411. 1849.

*Valsa persoonii* Nitschke, Pyrenomyc. Germ. 2: 222. 1870.

*Leucostoma persoonii* (Nitschke) Höhn., Mitt. Bot. Inst. Tech. Hochsch. Wien 5: 78. 1928.

*Cytospora donetzica* Norph. et al., Mycosphere 8: 62. 2017.

*Ascstromata* immersed in the bark, erumpent through the bark surface, scattered, (850–)1000–1500(–1600) µm diam, with 2–10 perithecia arranged circularly or irregularly. *Conceptacle* black. *Ectostromatic disc* conspicuous, circular, (200–)250–300(–350) µm diam, occasionally fusiform, with ostiolar necks, up to (600–)650–800(–900) µm diam, with 2–10 ostioles in disc. *Ostioles* numerous, violaceous black to black, concentrated, arranged straight or circular in the disc, (60–)70–100(–115) µm diam. *Perithecia* dark grey to fuscous black, flask-shaped to spherical, arranged circularly or irregularly, (300–)350–550(–600) µm diam. *Asci* free, clavate to elongate obovoid, (35–)40–50(–55) × (6.5–)7–8(–8.5) µm, 8-spored. *Ascospores* biseriolate, elongate-allantoid, thin-walled, hyaline, aseptate, (8.5–)9–11(–11.5) × 2.5–3 µm. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the surface, with multiple locules. *Conceptacle* black. *Ectostromatic disc* straw to honey, conspicuous, circular to ovoid, (100–)120–170(–200) µm diam,



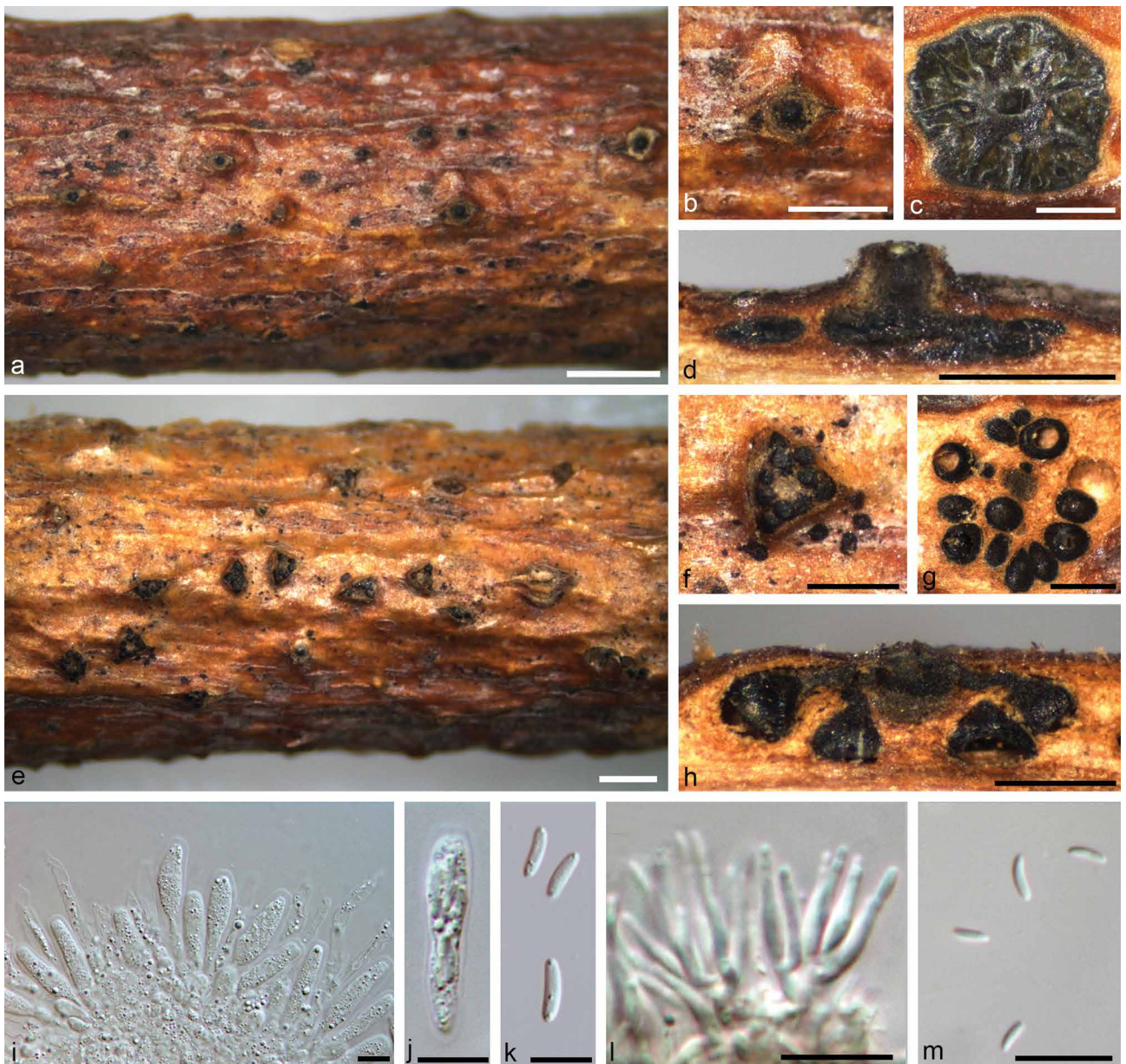
with one ostiole per disc. *Ostiole* in the centre, violaceous black to black, conspicuous, (40–)50–100(–115)  $\mu\text{m}$  diam. *Locules* numerous, subdivided frequently by invaginations with independent walls, (600–)650–1100(–1300)  $\mu\text{m}$  diam. *Conidiophores* borne along the locules, hyaline, branched at the base, in the middle, or occasionally unbranched, thin-walled, 11–21  $\times$  1–1.5  $\mu\text{m}$ , embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 10.5–16  $\times$  1–1.5  $\mu\text{m}$ , tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, 4.5–5.5  $\times$  1–1.5  $\mu\text{m}$ .

**Culture characteristics** — On PDA, colonies initially white to grey with a dark grey colour in the centre, growing up to 8 cm diam after 3 d, secreting a dark green to black pigment in culture medium after 7–10 d. *Colonies* dark grey to black, flat, felt-like after 30 d. *Pycnidia* irregularly distributed on the medium surface.

**Materials examined.** CHINA, Shanxi Province, Changzhi City, Taihang Park, E13°06'30.25" N36°12'07.48", on branches of *Cornus alba*, 20 Apr. 2014, X.L. Fan & B. Cao (BJFC-S1030, living culture CFCC 50023); Gansu Province, Tianshui City, Xiaolong Mountain, E106°11'23.59" N34°41'39.25",

on branches of *Rosa helena*, 16 July 2013, X.L. Fan (BJFC-S875, living culture CFCC 50019); Tianshui City, Manji Town, E105°57'49.94" N34°25'28.67", on branches of *Prunus serrulata*, 18 July 2013, X.L. Fan (BJFC-S847, living culture CFCC 50018); Qingyang City, Heshui County, E107°59'37.03" N35°47'04.66", on branches of *Prunus persica*, 13 July 2013, X.L. Fan (BJFC-S918, living culture CFCC 50020); Qingyang City, Heshui County, E107°59'38.71" N35°47'04.43", on branches of *Prunus salicina*, 13 July 2013, X.L. Fan (BJFC-S933, living culture CFCC 50021); Beijing City, Tongzhou District, Song Village, E116°39'32" N39°59'49", on branches of *Betula platyphylla*, 20 Mar. 2015, X.L. Fan (BJFC-S1315, living culture CFCC 50467); *ibid.*, BJFC-S1316, living culture CFCC 50468; Qinghai Province, Haidong City, Ledu District, Zhanbo Town, E102°27'00.56" N36°27'51.13", on branches of *Prunus pseudocerasus*, 14 Aug. 2012, X.L. Fan (BJFC-S605, living culture CFCC 50024); Ningxia Province, Guyuan City, The Great Wall Beam, E106°14'21.13" N36°02'13.51", on branches of *Prunus cerasifera*, 24 July 2013, X.L. Fan (BJFC-S823, living culture CFCC 50017); Guyuan City, Jingyuan County, Liupan Mountain, E106°23'12.05" N35°23'58.30", on branches of *Sorbus aucuparia*, 23 July 2013, X.L. Fan (BJFC-S805, living culture CFCC 50015); Guyuan City, Jingyuan County, Liupan Mountain, Nanzhuang Town, E106°23'32.20" N35°23'48.77", on branches of *Sorbus aucuparia*, 22 July 2013, X.L. Fan (BJFC-S819, living culture CFCC 50016).

**Notes** — *Cytospora leucostoma* is a common species causing canker disease of *Rosaceae* hosts, especially *Prunoideae*



**Fig. 27** *Cytospora mali* on *Malus pumila* (BJFC-S503). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i–j. asci; k. ascospores; l. conidiogenous cells with attached conidia; m. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–m = 10  $\mu\text{m}$ .



in China (Teng 1963, Tai 1979, Wei 1979, Zhuang 2005). This species is similar to *C. japonica* and *C. nivea*, of which the former can be distinguished by its smaller conidia ( $4.5\text{--}6 \times 1\text{--}1.5$  vs  $6.5\text{--}8.5 \times 1.5\text{--}2$   $\mu\text{m}$ ) and white colonies in culture. *Cytospora nivea* differs from *C. leucostoma* based on its distinct host associations (*Populus* and *Salix*), as well as DNA sequence data (Fan et al. 2014b). Based on currently available DNA data (Fig. 3, 4) and descriptions, *Cytospora donetzica* should be treated as synonym of *C. leucostoma*.

***Cytospora mali*** Grove, British Stem- and Leaf-Fungi (Coelomycetes) (Cambridge) 1: 279. 1935 — Fig. 27

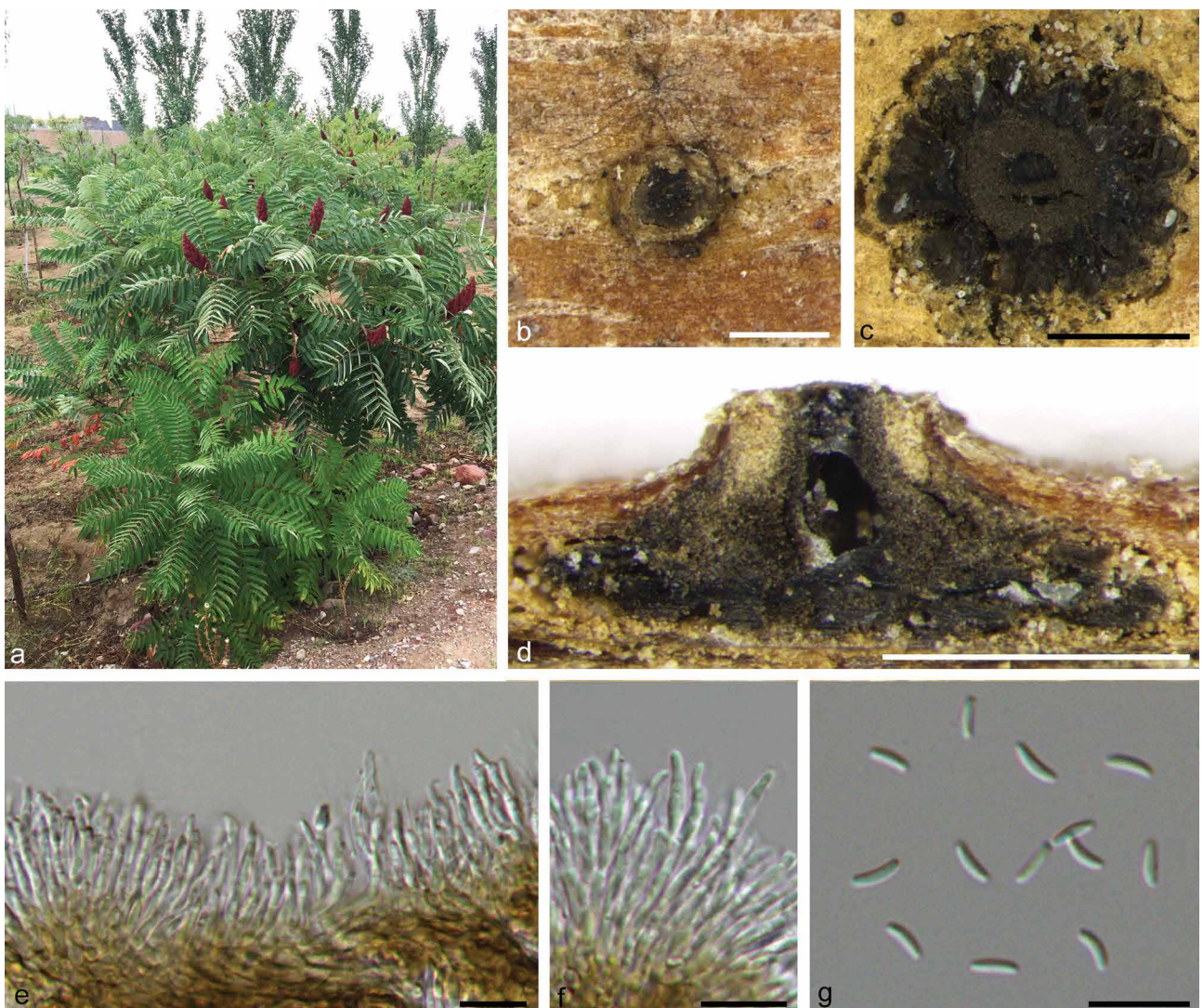
*Synonym.* *Valsa mali* Miyabe & G. Yamada, M. Miura Agr. Exp. Stn Bull. 4: 17. 1915.

*Ascstromata* immersed in the bark, erumpent through the bark surface, scattered, (1080–)1260–1660(–1780)  $\mu\text{m}$  diam, with 8–16 perithecia arranged circularly. *Conceptacle* absent. *Ectostromatic disc* light brown to grey, usually surrounded by tightly ostiolar necks, flat, circular to ovoid, (450–)510–660(–710)  $\mu\text{m}$  diam, with 8–16 ostioles arranged circularly or triangularly per disc. *Ostioles* grey to black, concentrated, dark brown to black, arranged circularly in a disc, (85–)96.5–154(–172)  $\mu\text{m}$  diam. *Perithecia* dark brown, flask-shaped to spherical, arranged irregularly, (260–)280–380(–450)  $\mu\text{m}$  diam. *Asci* free, clavate to elongate obovoid, (22.5–)25.5–33.5(–40)  $\times$  (4–)4.5–6  $\mu\text{m}$ ,

8-spored. *Ascospores* biseriolate, elongate-allantoid, thin-walled, hyaline, aseptate, (6–)6.5–10(–11)  $\times$  1–1.5  $\mu\text{m}$ . *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the bark surface, discoid, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* dark grey to black, circular to ovoid, (280–)300–480(–510)  $\mu\text{m}$  diam, with one ostiole per disc. *Ostiole* in the centre of the disc, conspicuous, black, (40–)54–102(–125)  $\mu\text{m}$  diam. *Locules* numerous, arranged irregularly with common walls, (600–)630–890(–950)  $\mu\text{m}$  diam. *Conidiophores* hyaline, branched at the base, in the middle, or occasionally unbranched, thin-walled, 7.5–15(–16.5)  $\times$  1.5  $\mu\text{m}$ , embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 8–11.5  $\times$  1.5–2  $\mu\text{m}$ , tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, 4–5(–5.5)  $\times$  1(–1.5)  $\mu\text{m}$ .

**Culture characteristics** — On PDA, colonies grey to pale greenish grey, with a dark grey colour in the centre, fast growing, reaching up to 7 cm diam after 3 d and entirely covering the 9 cm Petri dish after 5 d, becoming grey to dark grey, flat, thick, felt-like after 30 d. *Pycnidia* sparse, irregularly distributed on the culture medium surface.

**Materials examined.** CHINA, Gansu Province, Gannan City, Diebu County, E103°44'41.72" N33°56'57.01", on branches of *Malus pumila*, 13 Aug. 2012, X.L. Fan (BJFC-S767, living culture CFCC 50028); Ningxia Province, Yinchuan City, E106°03'07.12" N38°28'26.70", on branches of *Malus pumila*,



**Fig. 28** *Cytospora melnikii* on *Rhus typhina* (BJFC-XJHJS). a. Host; b. habit of conidioma on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e–f. conidiogenous cells with attached conidia; g. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–g = 10  $\mu\text{m}$ .



7 Feb. 2012, X.L. Fan (BJFC-S503, living culture CFCC 50029); Shaanxi Province, Yangling District, E108°00'49.89" N34°14'01.93", on branches of *Malus pumila*, 30 Mar. 2012, X.L. Fan (BJFC-S740, living culture CFCC 50030); Shanxi Province, Taiyuan City, E112°31'51.07" N37°54'28.11", on branches of *Crataegus pinnatifida*, 16 Apr. 2014, X.L. Fan & B. Cao (BJFC-S1066, living culture CFCC 50031); Qinghai Province, Xining City, Xishan botanical garden, E102°09'16.90" N36°27'32.44", on branches of *Malus baccata*, 19 Aug. 2012, X.L. Fan (BJFC-S691, living culture CFCC 50044).

**Notes** — *Cytospora mali* is a common species causing canker disease of *Maloideae* hosts, especially apple (Teng 1963, Tai 1979, Wei 1979, Zhuang 2005, Wang et al. 2011). This species is similar to *C. leucosperma* from *Pyrus* spp. in morphology, which is regarded as a sister clade in phylogeny. However, *C. mali* is mostly recorded from *Malus* spp.

***Cytospora melnikii*** Norph. et al., Mycosphere 8: 68. 2017 — Fig. 28

**Description** — Norphanphoun et al. (2017).

**Material examined.** CHINA, Xinjiang Province, Urumchi City, Anningqu nursery, N87°52'19.24" E43°58'59.50", on branches of *Rhus typhina*, X.L. Fan (BJFC-XJHJS, living culture CFCC 89984).

**Notes** — *Cytospora melnikii* is associated with canker disease of *Malus domestica* and *Populus nigra* in Russia (Norphanphoun et al. 2017). This species is similar to the type

species *C. chrysosperma*, but they are distinguished as separate taxa in the multigene analyses (Fig. 4). The current study represents the first report of this species from China.

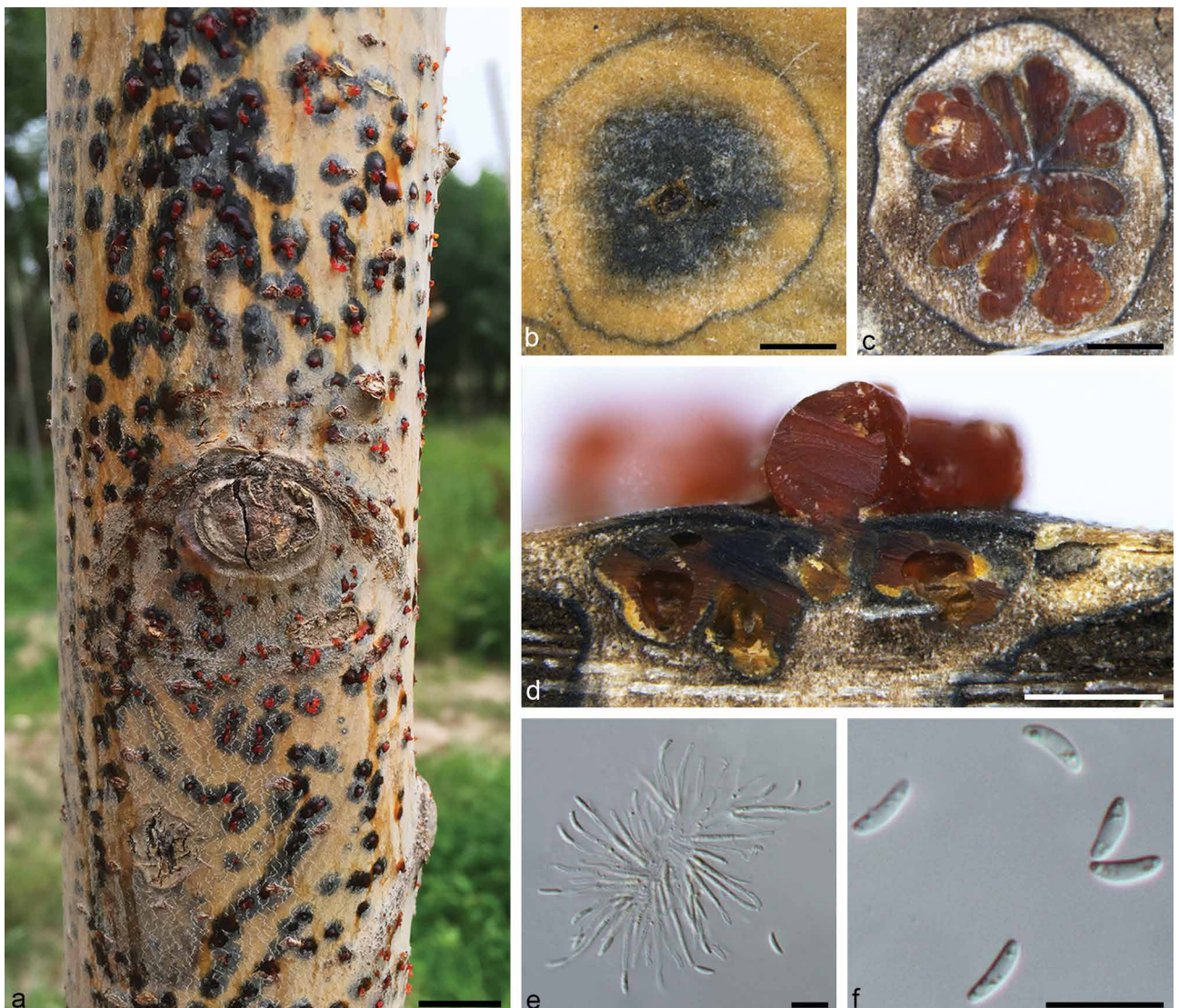
***Cytospora nivea*** (Hoffm.) Sacc., Michelia 2: 264. 1881 — Fig. 29

**Basionym.** *Sphaeria nivea* Hoffm., Veg. Crypt. 1: 28. 1787.

**Synonyms.** *Leucostoma niveum* (Hoffm.) Höhn., Mitt. Bot. Inst. Tech. Hochsch. Wien 5: 58. 1928.

*Valsa nivea* (Hoffm.) Fr., Summa Veg. Scand., Section Post. (Stockholm): 411. 1849.

**Sexual morph** not observed. *Pycnidial stromata* immersed in bark, erumpent through the bark surface, releasing the red gelatinous conidial tendrils when moist, discoid to conicoid, with multiple locules. *Conceptacle* black. *Ectostromatic disc* brown to dark grey, nearly flat, ovoid to ellipsoid, (110–)140–210(–270) µm diam, with one ostiole per disc. *Ostiole* dark grey to black, conspicuous, 52.5–93 µm diam. *Locules* numerous, arranged irregularly with common walls, (410–)470–650(–770) µm diam. *Conidiophores* borne along the locules, hyaline, unbranched, or occasionally branched at the base, 17–25.5(–27) × 1.5–2 µm, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 7–22 × 1.5–2 µm, tapering towards the apices. *Conidia* hyaline, allantoid, guttulate, smooth, aseptate, thin-walled, 6–9(–10) × 1.5–2.5(–3) µm.



**Fig. 29** *Cytospora nivea* on *Populus alba* subsp. *pyramidalis* (BJFC-S1307). a. Disease symptoms; b. habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 5 mm; b–d = 0.5 mm; e–f = 10 µm.



Culture characteristics — On PDA, colonies initially white to greyish, secreting a greenish olivaceous to olivaceous pigment, fast growing, entirely covering the 9 cm Petri dish after 3 d, becoming olivaceous grey to olivaceous black, flat, felt-like after 30 d. *Pycnidia* sparsely distributed on the culture medium.

**Materials examined.** CHINA, Ningxia Province, Guyuan City, Changchengliang, N36°03'01.78" E106°16'18.09", on twigs and branches of *Elaeagnus angustifolia*, 24 July 2013, X.L. Fan (BJFC-S964, living culture CFCC 89641); Shaanxi Province, Yulin City, Hongshi Gorge, N38°19'32.43" E109°42'00.69", on twigs and branches of *Salix psammophila*, 29 July 2013, X.L. Fan (BJFC-S979, living culture CFCC 89643).

**Notes** — *Cytospora nivea* is a commonly recorded species associated with *Salicaceae* hosts and commonly co-occurring with *C. chrysosperma*, which was regarded as the most common causative agent of poplar and willow canker disease in China (Saccardo 1881, 1884, Zhuang 2005, Fan et al. 2014b). *Cytospora nivea* can be distinguished from *C. chrysosperma* based on its black conceptacle surrounding the sexual and asexual stroma, usually presenting a huge black ectostromatic disc on the bark surface (Adams et al. 2005, Fan et al. 2014b).

***Cytospora platycladi*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830155; Fig. 30

**Etymology.** The name reflects the host genus from which it was collected, *Platycladus*.

**Sexual morph** not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the surface, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* dark grey to black, conspicuous, circular to ovoid, (210–)230–300 (–330)  $\mu\text{m}$  diam, with one ostiole per disc. *Ostiole* in the centre of the disc, black, conspicuous, (40–)45–75 (–85)  $\mu\text{m}$  diam. *Locules* numerous, arranged circularly or elliptically with independent walls, (750–)820–1370 (–1520)  $\mu\text{m}$  diam. *Conidiophores* borne along the locules, hyaline, branched at the base, in the middle, or occasionally unbranched, thin-walled, 11–18  $\times$  1–1.5  $\mu\text{m}$ , embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 5–12  $\times$  1–1.5  $\mu\text{m}$ , tapering towards the apices. *Conidia* hyaline, allantoid, guttulate, smooth, aseptate, thin-walled, (4–)4.5–5 (–5.5)  $\times$  1–1.5  $\mu\text{m}$ .

Culture characteristics — On PDA, colonies initially white, growing fast, entirely covering the 9 cm Petri dish after 4 d,

becoming leaden grey after 7–10 d, becoming flat, felt-like, and thin with a uniform texture and aerial mycelium after 30 d. *Pycnidia* sparse, concentrically and irregularly distributed on the medium surface.

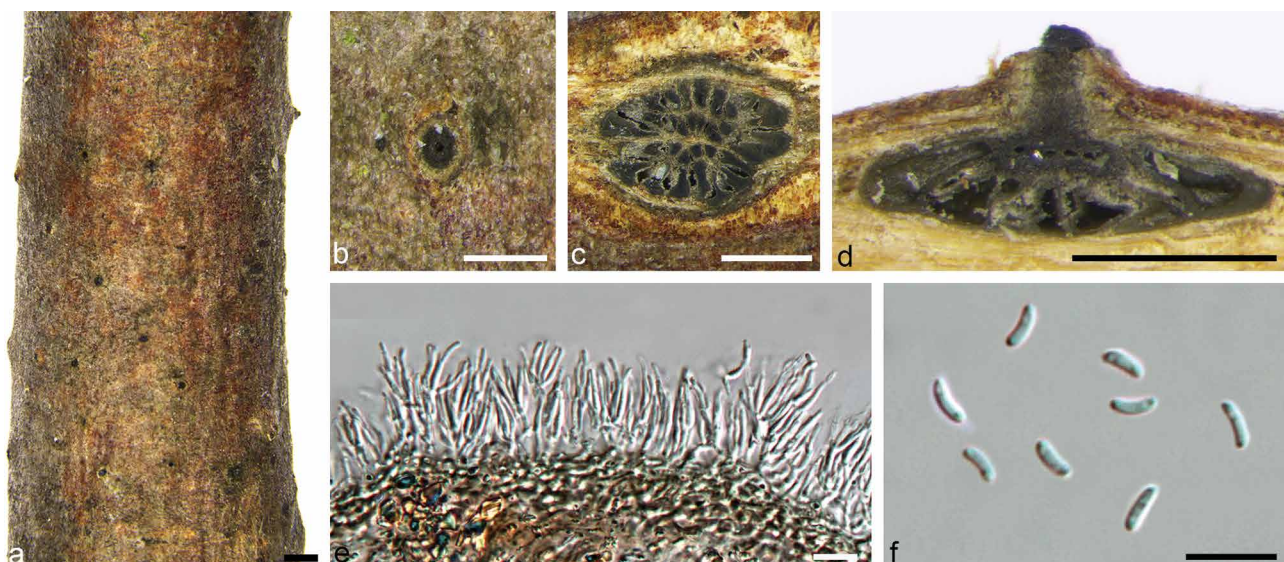
**Materials examined.** CHINA, Yunnan Province, Chuxiong City, Elu Park, N25°01'38.43" E101°32'12.44", on branches of *Platycladus orientalis*, 19 Mar. 2015, X.L. Fan, B. Cao, Q. Yang & Z. Du (holotype BJFC-S1111, ex-type living culture CFCC 50504); *ibid.*, living culture CFCC 50505; Chuxiong City, Elu Park, N25°01'37.55" E101°32'12.12", on branches of *Platycladus orientalis*, 20 Mar. 2015, B. Cao, Q. Yang & Z. Du (CF-2015515, living culture CFCC 50506).

**Notes** — *Cytospora platycladi* is associated with canker disease of *Platycladus orientalis* in China. *Cytospora platycladi* can be distinguished from *C. platycladicola* by the independent walls of its locules. Phylogenetically, *Cytospora platycladi* formed a close group with *C. lumnitzericola*, but differs in its disease symptoms, with erumpent pycnidia surrounding the cracked, buff coloured bark, and the size of its conidia (4.5–5 vs 5.5–6  $\mu\text{m}$ ) (Norphanphoun et al. 2018).

***Cytospora platycladicola*** X.L. Fan, *sp. nov.* — MycoBank MB830156; Fig. 31

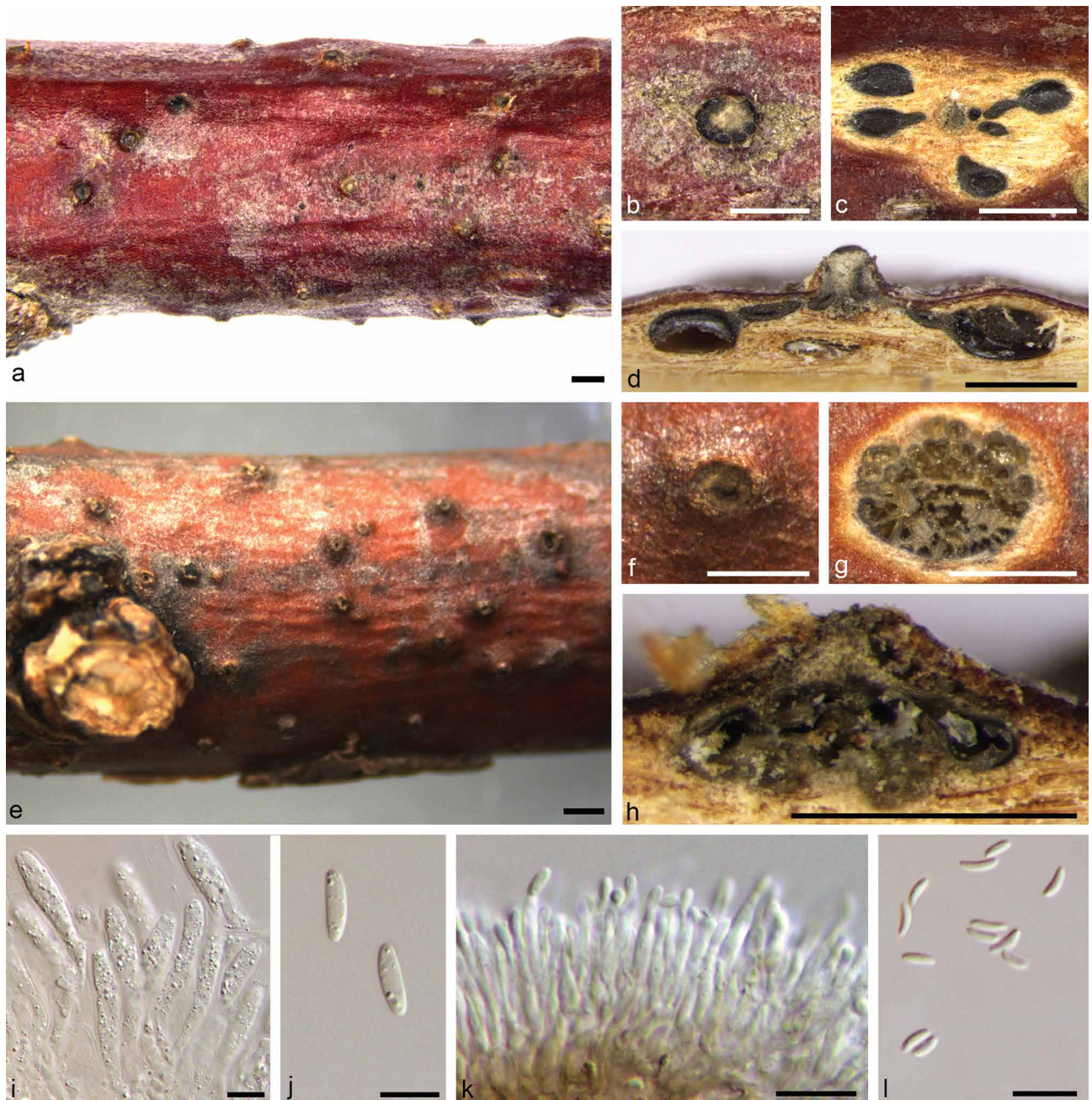
**Etymology.** The name reflects the host genus from which it was collected, *Platycladus*.

*Ascstromata* immersed in the bark, erumpent through the bark surface, (900–)1000–1600 (–1750)  $\mu\text{m}$  diam, with 8–12 perithecia arranged irregularly. *Conceptacle* absent. *Ectostromatic disc* light grey to black, nearly flat, circular to ovoid, (300–)330–420 (–440)  $\mu\text{m}$  diam, with 6–10 ostioles arranged circularly per disc. *Ostioles* dark brown to black, occasionally the area below the disc with a paler entostroma, concentrated, arranged circularly in a disc, (44–)50–75 (–82)  $\mu\text{m}$  diam. *Perithecia* dark brown, flask-shaped to spherical, arranged circularly, (220–)240–330 (–370)  $\mu\text{m}$  diam. *Asci* free, clavate to elongate-obovoid, (45–)48–60 (–67)  $\times$  (8–)8.5–10 (–11)  $\mu\text{m}$ , 8-spored. *Ascospores* biserial, elongate-allantoid, thin-walled, hyaline, aseptate, 12–12.5 (–13)  $\times$  3–4  $\mu\text{m}$ . *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the bark surface, discoid, with favaginous, multiple locules. *Conceptacle* absent. *Ectostromatic disc* white to light brown, circular to ovoid, (250–)260–320 (–350)  $\mu\text{m}$  diam, with single ostiole per disc. *Ostiole* in the centre of the disc, conspicuous, dark grey to black, (80–)90–110 (–120)  $\mu\text{m}$  diam.



**Fig. 30** *Cytospora platycladi* on *Platycladus orientalis* (BJFC-S1111). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. Longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu\text{m}$ .





**Fig. 31** *Cytospora platycladicola* on *Platycladus orientalis* (BJFC-S758). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i. asci; j. ascospores; k. conidiogenous cells with attached conidia; l. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–l = 10  $\mu$ m.

*Locules* numerous, arranged irregularly with common walls, (500–)520–700(–750)  $\mu$ m diam. *Conidiophores* borne along the locules, hyaline, branched at the base or unbranched, thin-walled, 11–20  $\times$  1.5–2  $\mu$ m, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 8–15  $\times$  1.5–2  $\mu$ m, tapering towards the apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (4–)4.5–5(–5.5)  $\times$  1.5–2  $\mu$ m.

*Culture characteristics* — On PDA, colonies white to greyish, fluffy, growing up to 5 cm diam after 3 d, entirely covering the 9 cm Petri dish after 7 d, becoming yellowish to pale yellow after 30 d. *Pycnidia* extruding a pale white to yellowish conidial mass, sparse and distributed irregularly on the medium surface.

*Materials examined*. CHINA, Gansu Province, Longnan City, Wen County, Tianchi Lake, N33°14'33.21" E104°44'18.91", on twigs and branches of *Platycladus orientalis*, 11 Aug. 2012, X.L. Fan (holotype BJFC-S758, ex-type living culture CFCC 50038); *ibid.*, BJFC-S755, living culture CFCC 50039.

*Notes* — *Cytospora platycladicola* is associated with canker disease of *Platycladus orientalis* in China. In the combined analyses, the most closely related species are *C. ampulliformis* and *C. cotini* (Fig. 4). *Cytospora platycladicola* can be distinguished from *C. ampulliformis* and *C. cotini* by pycnidia having a central column with a single ostiole in the disc, as well as the smaller conidia (4.5–5  $\times$  1.5–2  $\mu$ m) (Hyde et al. 2016, Norphanphoun et al. 2017).

***Cytospora populina*** (Pers.) Rabenh., *Deutschl. Krypt.-Fl.* (Leipzig) 1: 148. 1844 — Fig. 32

*Basionym*. *Sphaeria populina* Pers., *Observ. Mycol.* (Lipsiae) 2: 67. 1800.  
*Synonyms*. *Valsa populina* (Pers.) Fuckel, *Jahrb. Nassauischen Vereins Naturk.* 25: 314. 1871.

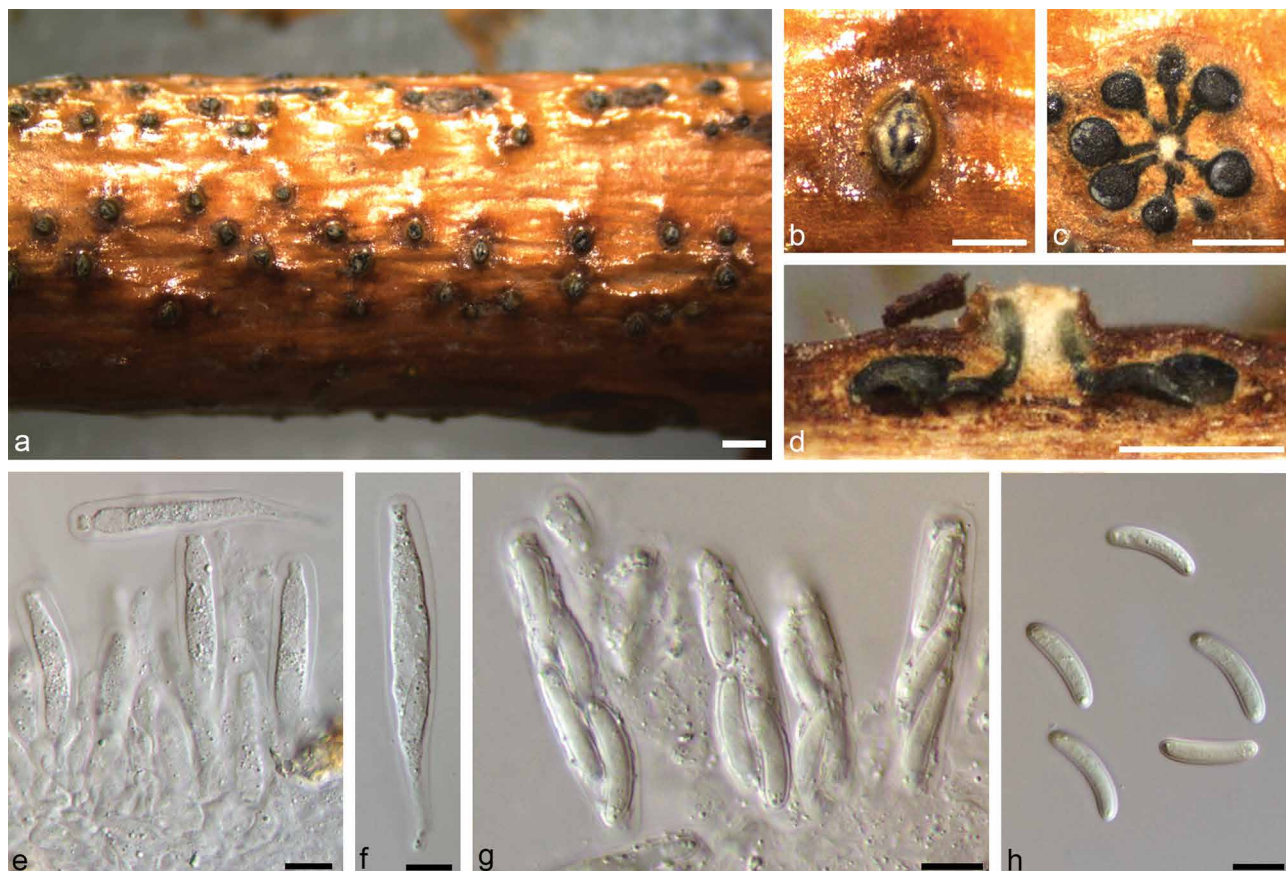
*Cryptosporella populina* (Pers.) Sacc., *Michelia* 1: 30. 1877.

*Diaporthe populina* (Pers.) Höhn., *Ann. Mycol.* 16: 106. 1918.

*Cryptodiaporthe populina* (Pers.) Petr., *Ann. Mycol.* 19: 119. 1921.

*Cytospora populina* (Fuckel) C.M. Tian et al., *Phytotaxa* 197: 234. 2015.





**Fig. 32** *Cytospora populina* on *Salix psammophila* (BJFC-S978). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–g. asci (e, f immature); h. ascospores. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–h = 10  $\mu$ m.

**Description** — See Fan et al. (2015b).

**Material examined.** CHINA, Shaanxi Province, Yulin City, Hongshi Gorge, N38°19'40.83" E109°42'37.98", from twigs and branches of *Salix psammophila*, 29 July 2013, X.L. Fan (BJFC-S978, living culture CFCC 89644).

**Notes** — *Cytospora populina* (syn. *Valsa populina*) is regarded as the pathogen responsible for poplar canker. Fan et al. (2015b) re-described this species from materials in China and observed that it has asci with four ascospores, which was also observed in the present study.

***Cytospora populinopsis*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830157; Fig. 33

**Etymology.** Named after its morphological similarity to *C. populina*.

**Ascstromata** immersed in the bark, erumpent through the bark surface, (1180–)1320–1750(–1910)  $\mu$ m diam, with 6–12 perithecia arranged irregularly. **Conceptacle** absent. **Ectostromatic disc** pale grey to black, nearly flat, circular to ovoid, (240–)280–470(–530)  $\mu$ m diam, with 6–12 ostioles arranged circularly per disc. **Ostioles** dark brown to black, the area below disc occasionally with a lighter entostroma, arranged circularly and tightly in a disc, (54–)61.5–96(–115)  $\mu$ m diam. **Perithecia** dark brown, flask-shaped to spherical, arranged circularly, (260–)310–460(–500)  $\mu$ m diam. **Asci** free, clavate to elongate obovoid, (42.5–)45–53.5(–58)  $\times$  (7–)8–10(–11)  $\mu$ m, 4-spored. **Ascospores** hyaline, biserial, elongate-allantoid, aseptate, guttulate, thin-walled, (13–)14–20(–21)  $\times$  (2.5–)3–4.5(–5)  $\mu$ m. **Asexual morph** not observed.

**Culture characteristics** — On PDA, colonies white to pale mouse grey, fluffy, growing up to 6 cm diam after 3 d and entirely covering the 9 cm Petri dish after 7 d. **Pycnidia** black, distributed circularly at the colony margin on the medium surface.

**Materials examined.** CHINA, Ningxia Province, Guyuan City, Jingyuan County, Liupan Mountain, N35°23'57.91" E106°23'11.94", on twigs and branches of *Sorbus aucuparia*, 23 July 2013, X.L. Fan (holotype BJFC-S802, ex-type living culture CFCC 50032); Guyuan City, Jingyuan County, Nanzhuang Town, N35°23'29.84" E106°23'29.84", on twigs and branches of *Prunus salicina*, 22 July 2013, X.L. Fan (BJFC-S818, living culture CFCC 50033).

**Notes** — *Cytospora populinopsis* is associated with canker disease of *Prunus salicina* and *Sorbus aucuparia* in China. It differs from other similar *Cytospora* species in having 4-spored asci, except from *C. populina*, which also has the same characteristic. *Cytospora populinopsis* can be distinguished from *C. populina* by its larger ascospores (13–21  $\times$  2.5–5  $\mu$ m vs 12–13  $\times$  3–4  $\mu$ m in *C. populina*).

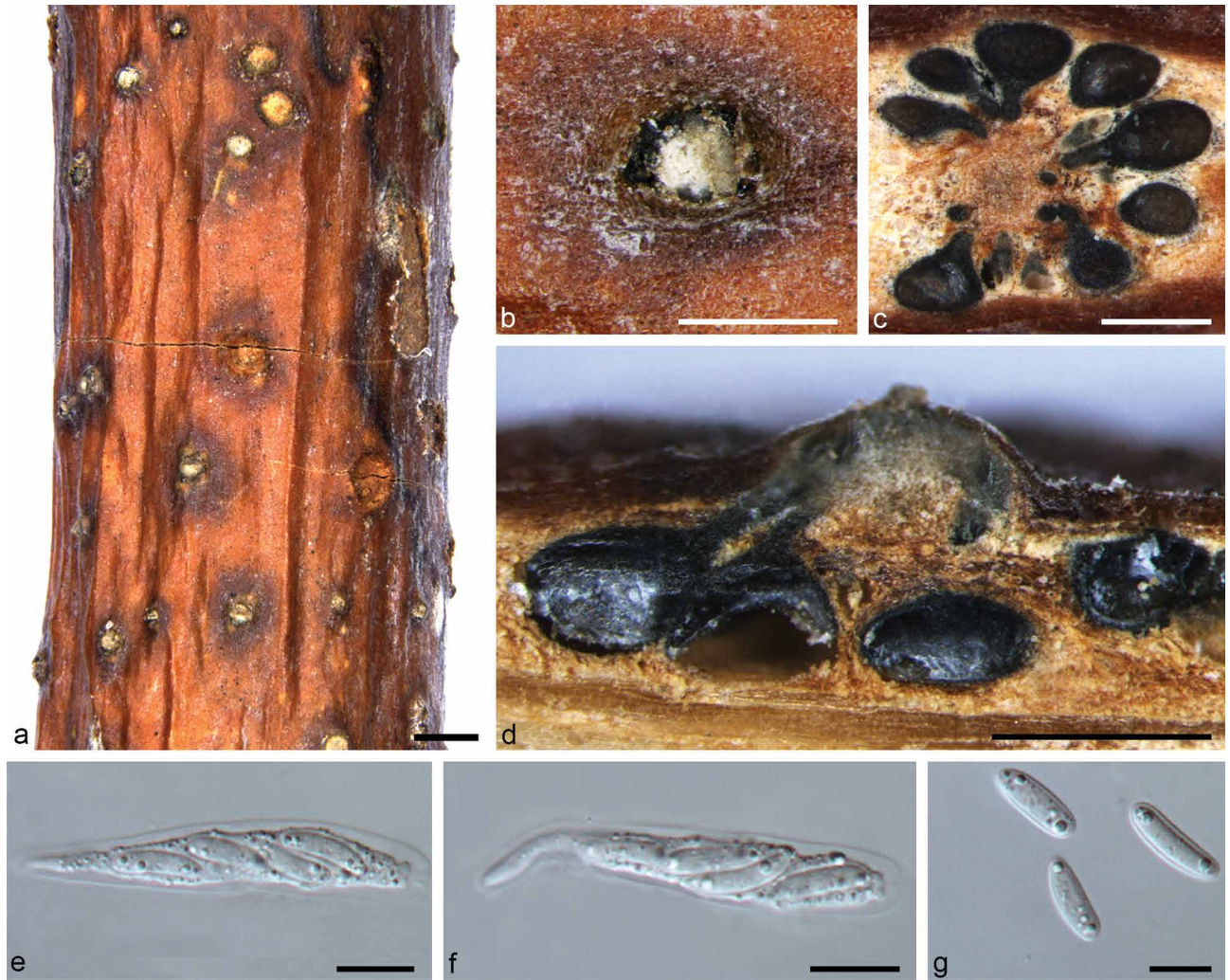
***Cytospora pruinopsis*** C.M. Tian & X.L. Fan, Mycol. Progr. 14: 74. 2015 — Fig. 34

**Description** — See Yang et al. (2015).

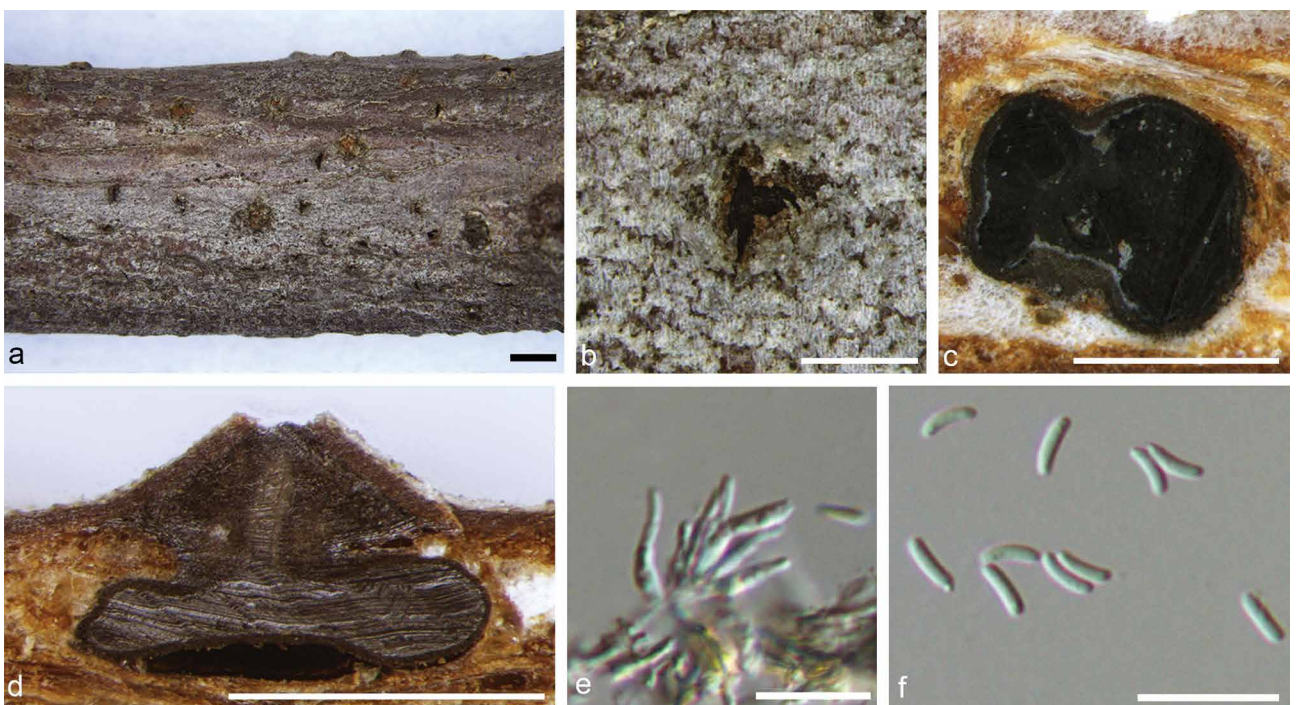
**Materials examined.** CHINA, Shaanxi Province, Yulin City, Yuyang District, Red Stone Gorge, N38°19'32.43" E109°42'00.63", on twigs and branches of *Ulmus pumila*, 29 July 2013, X.L. Fan (holotype BJFC-S1073, ex-type living culture CFCC 50034); Jilin Province, Tonghua City, N41°73'56.12" E125°96'85.84", on twigs and branches of *Ulmus pumila*, 3 June 2012, Y.B. Zhang (BJFC-S334, living culture CFCC 50035).

**Notes** — *Cytospora pruinopsis* was described to be associated with a canker disease of *Ulmus pumila* in China (Yang et al. 2015). This *Cytospora* species is characterised by a single locule, distinguishing it from *C. carbonacea*, *C. chrysosperma*, *C. leucosperma*, *C. leucostoma*, *C. pulchella* and *C. sacculus*, which are common species from *Ulmus* spp. with multiple locules (Yang et al. 2015). *Cytospora pruinopsis* is similar to *C. pruinosa*, but differs in the absence of a wing-like ectostroma



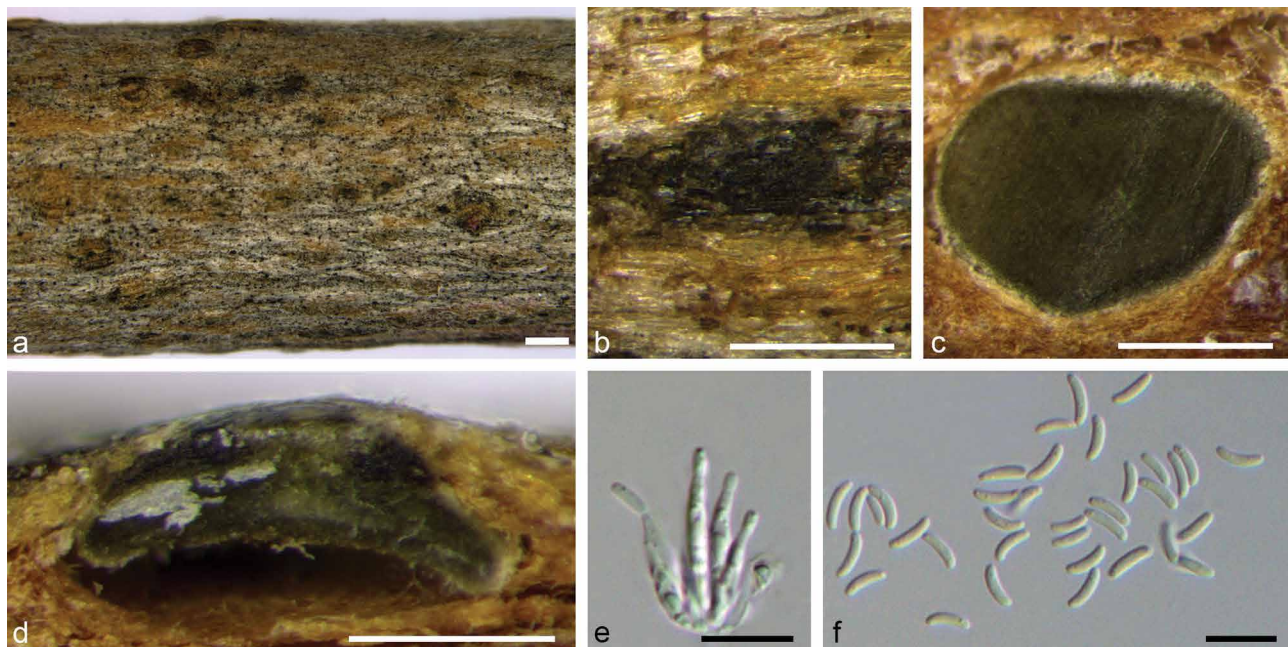


**Fig. 33** *Cytospora populinopsis* on *Sorbus aucuparia* (BJFC-S802). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. asci; g. ascospores. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–g = 10  $\mu$ m.



**Fig. 34** *Cytospora pruinopsis* on *Ulmus pumila* (BJFC-S1073). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 2 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.





**Fig. 35** *Cytospora pruinosa* on *Syringa oblata* (BJFC-S636). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.25 mm; e–f = 10  $\mu$ m.

around the ostiole, smaller conidial size ( $3.0 \times 0.8 \mu\text{m}$  vs  $5\text{--}7.5 \times 1\text{--}1.5$  in *C. pruinosa*), and host affiliations (Yang et al. 2015).

***Cytospora pruinosa*** (Fr.) Sacc., *Michelia* 1(5): 519. 1879 — Fig. 35

*Basionym.* *Sphaeria pruinosa* Fr., *Kongl. Svenska Vetensk.-Akad. Handl.* (Ser. 3) 39: 104. 1818.

*Synonym.* *Sphaeria cypri* Tul., *Compt. Rend. Hebd. Séances Acad. Sci.*, Paris 42: 706. 1856.

*Valsa cypri* (Tul.) Tul. & C. Tul., *Select. Fung. Carpol.* (Paris) 2: 194. 1863.

*Sexual morph* not observed. *Pycnidial stromata* immersed in bark, erumpent through the bark surface, discoid, with a solitary undivided locule. *Conceptacle* absent. *Ectostromatic disc* dark grey to black, inconspicuous, with one ostiole per disc. *Ostiole* dark grey to black, conspicuous, (61–)65–74(–78)  $\mu\text{m}$  diam. *Locules* undivided, circular to ovoid, with wing-like ectostroma around the ostiole, (400–)430–550(–590)  $\mu\text{m}$  diam. *Conidiophores* borne along the locules, hyaline, unbranched or occasionally branched at base,  $10\text{--}19(–20.5) \times 2\text{--}2.5 \mu\text{m}$ , embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical,  $10\text{--}17 \times 2\text{--}2.5 \mu\text{m}$ , tapering towards apices. *Conidia* hyaline, allantoid, occasionally guttulate, smooth, aseptate, thin-walled, (5–)5.5–7(–7.5)  $\times$  (1–)1.5  $\mu\text{m}$ .

*Culture characteristics* — On PDA, colonies white to slightly pale mouse grey at the centre, fluffy, growing up to 6 cm diam after 3 d, becoming flat, lacking aerial mycelium after 30 d, secreting a cinnamon pigment in the medium. *Pycnidia* are black, distributed irregularly on the medium surface.

*Materials examined.* CHINA, Qinghai Province, Haidong City, Pinan County, Pinan Town, N36°29'25.92" E102°08'34.11", from twigs and branches of *Syringa oblata*, 15 Aug. 2012, X.L. Fan (BJFC-S636, living culture CFCC 50036); *ibid.*, BJFC-S640, living culture CFCC 50037.

*Notes* — *Cytospora pruinosa* is a common species observed worldwide on *Fraxinus*, *Olea*, *Syringa* and *Viburnum lantana* (Saccardo 1879, Adams et al. 2006). Adams et al. (2006) re-described this species from *Olea europaea* in South Africa and provided ITS sequences.

***Cytospora ribis*** Ehrenb., *Sylv. Mycol. Berol.* (Berlin): 28. 1818

*Description* — See Yang et al. (2015).

*Materials examined.* CHINA, Qinghai Province, Xining City, N36°38'32.51" E101°44'42.89", from stems of *Ulmus pumila*, 19 Aug. 2012, X.L. Fan (BJFC S671, living culture CFCC 50026); *ibid.*, living culture CFCC 50027.

*Notes* — *Cytospora ribis* has been reported from China, Iran, the Netherlands and Poland (Mulencko et al. 2008, Fotouhifar et al. 2010, Yang et al. 2015). Yang et al. (2015) described it from *Ulmus* having typical *Cytospora* pycnidia with 1–4 ostioles and small elongate-allantoid conidia ( $3.5\text{--}4.5 \times 1\text{--}1.5 \mu\text{m}$ ) from *Ulmus pumila* in China.

***Cytospora rostrata*** C.M. Tian & X.L. Fan, *Mycotaxon* 129: 307. 2014

*Description* — See Fan et al. (2014b).

*Materials examined.* CHINA, Gansu Province, Ganan City, Diebu County, N34°04'48.85" E103°23'34.20", from stems of *Salix cupularis*, 9 Aug. 2012, Y.M. Liang & X.L. Fan (holotype BJFC S726, ex-type living culture CFCC 89909 = BJFC-CGLs251); Gansu Province, Ganan City, Diebu County, N34°04'48.35" E103°23'36.60", from stems of *Salix cupularis*, 9 Aug. 2012, Y.M. Liang & X.L. Fan (BJFC S727, living culture CFCC 89910 = BJFC-CGLs252).

*Notes* — *Cytospora rostrata* has small, single loculed pycnidia ( $455\text{--}851 \mu\text{m}$  diam), with thorn-like necks erumpent through the host bark, which differs from *C. chrysosperma* by its multiple locules ( $640\text{--}1260 \mu\text{m}$  diam) with nearly flat, circular to ovoid disc (Fan et al. 2014b). *Cytospora rostrata* was only recorded from stems of *Salix cupularis* in China (Fan et al. 2014b).

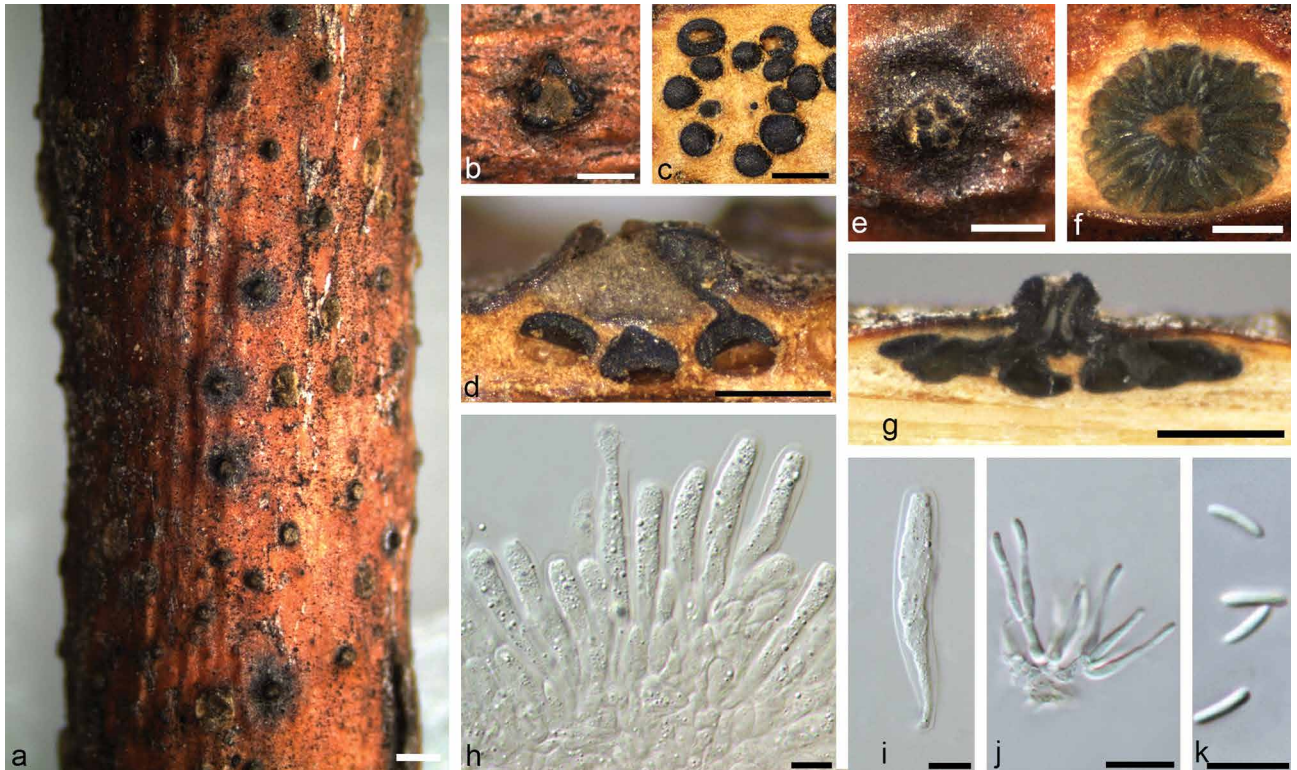
***Cytospora schulzeri*** Sacc. & P. Syd., *Syll. Fung.* (Abellini) 14(2): 918. 1899 — Fig. 36

*Synonyms.* *Cytospora capitata* Schulzer & Sacc., *Hedwigia* 23: 109. 1884, non *Cytospora capitata* Fuckel, *Reisen nach dem Nordpolarmeer* 2: 34 (1874).

*Valsa malicola* Z. Urb., *Česká Mykol.* 10: 209. 1956.

*Ascostromata* immersed in the bark, erumpent through the bark surface, scattered, (980–)1060–1430(–1600)  $\mu\text{m}$  diam, with 5–14 perithecia arranged circularly or irregularly. *Conceptacle*





**Fig. 36** *Cytospora schulzeri* on *Malus pumila* (BJFC-S538). a. Habit of conidiomata and ascostromata on branch; b. habit of ascostroma on branch; c. transverse section through ascostroma conidioma; d. longitudinal section through ascostroma; e. habit of conidioma on branch; f. transverse section through conidioma; g. longitudinal section through conidioma; h–i. asci; j. conidiogenous cells with attached conidia; k. conidia. — Scale bars: a = 1 mm; b–g = 0.5 mm; h–k = 10  $\mu$ m.

absent. *Ectostromatic disc* grey to black, usually surrounded by tightly ostiolar necks, circular to ovoid, (285–)310–450(–465)  $\mu$ m diam, with 5–14 ostioles arranged circularly per disc. *Ostioles* black, concentrated, dark brown to black, arranged circularly in a disc, (56–)63–103(–122)  $\mu$ m diam. *Perithecia* dark brown, flask-shaped to spherical, arranged circularly or irregularly, (235–)250–310(–350)  $\mu$ m diam. *Asci* free, clavate to elongate obovoid, (22.5–)37–45.5(–49)  $\times$  (4–)5.5–9(–10)  $\mu$ m. *Ascospores* not observed. *Pycnidial stromata* ostiolate, immersed in bark, scattered, erumpent through the bark surface, flat, discoid, with multiple locules. *Conceptacle* absent. *Ectostromatic disc* light brown, circular, (145–)160–390(–420)  $\mu$ m diam, with 1–7 ostioles per disc. *Ostiole* numerous, conspicuous, dark grey to black, arranged circularly, (25–)29–50(–71)  $\mu$ m diam. *Locules* numerous, arranged circularly with common walls, (830–)870–1320(–1410)  $\mu$ m diam. *Conidiophores* borne along the locules, hyaline, branched at base or unbranched, thin-walled, 12–19  $\times$  1.5–2  $\mu$ m, embedded in a gelatinous layer. *Conidiogenous cells* enteroblastic, phialidic, sub-cylindrical to cylindrical, 6.5–12(–13.5)  $\times$  2  $\mu$ m, tapering towards apices. *Conidia* hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (4.0–)4.5–6.5(–7)  $\times$  1–1.5  $\mu$ m.

**Culture characteristics** — On PDA, colonies fast growing, reaching up to 8 cm diam after 3 d and entirely covering the 9 cm Petri dish after 4 d, centrally white and olivaceous grey at the margin, becoming olivaceous black at the centre and iron-grey on the margin after 30 d. *Colonies* are slightly fluffy, thin with a uniform texture; sterile.

**Materials examined.** CHINA, Ningxia Province, Yinchuan City, N38°27'40" E106°01'00", on branches of *Malus pumila*, 2 June 2012, X.L. Fan (BJFC-S538, living culture CFCC 50040); Gansu Province, Gannan City, Diebu County, Zhouqu Town, N33°46'56.55" E104°20'12.65", from branches of *Malus pumila*, 10 Aug. 2012, X.L. Fan (BJFC-S773, living culture CFCC 50042).

**Notes** — *Cytospora schulzeri* is a common species causing apple canker disease (Teng 1963, Tai 1979, Wei 1979, Zhuang 2005, Wang et al. 2011). This species can be distinguished from *C. mali* by numerous ostioles and erumpent pycnidia.

***Cytospora sibiraeae*** C.M. Tian et al., Fungal Diversity 72: 44. 2015 — Fig. 37

**Description** — See Liu et al. (2015).

**Materials examined.** CHINA, Gansu Province, Gannan City, N34°31'50.10" E103°08'32.47", on branches of *Sibiraea angustata*, 8 Aug. 2012, X.L. Fan & Y.M. Liang (holotype BJFC-S783, ex-type living culture CFCC 50045); *ibid.*, living culture CFCC 50046.

**Notes** — *Cytospora sibiraeae* has been reported in China from twigs and branches of *Sibiraea angustata*, and this species is mainly characterised by ascostromata with black conceptacles (Liu et al. 2015). Few pathogenic fungi were reported in *S. angustata* from China, and *C. sibiraeae* represents the first *Cytospora* species reported from this host plant (Liu et al. 2015).

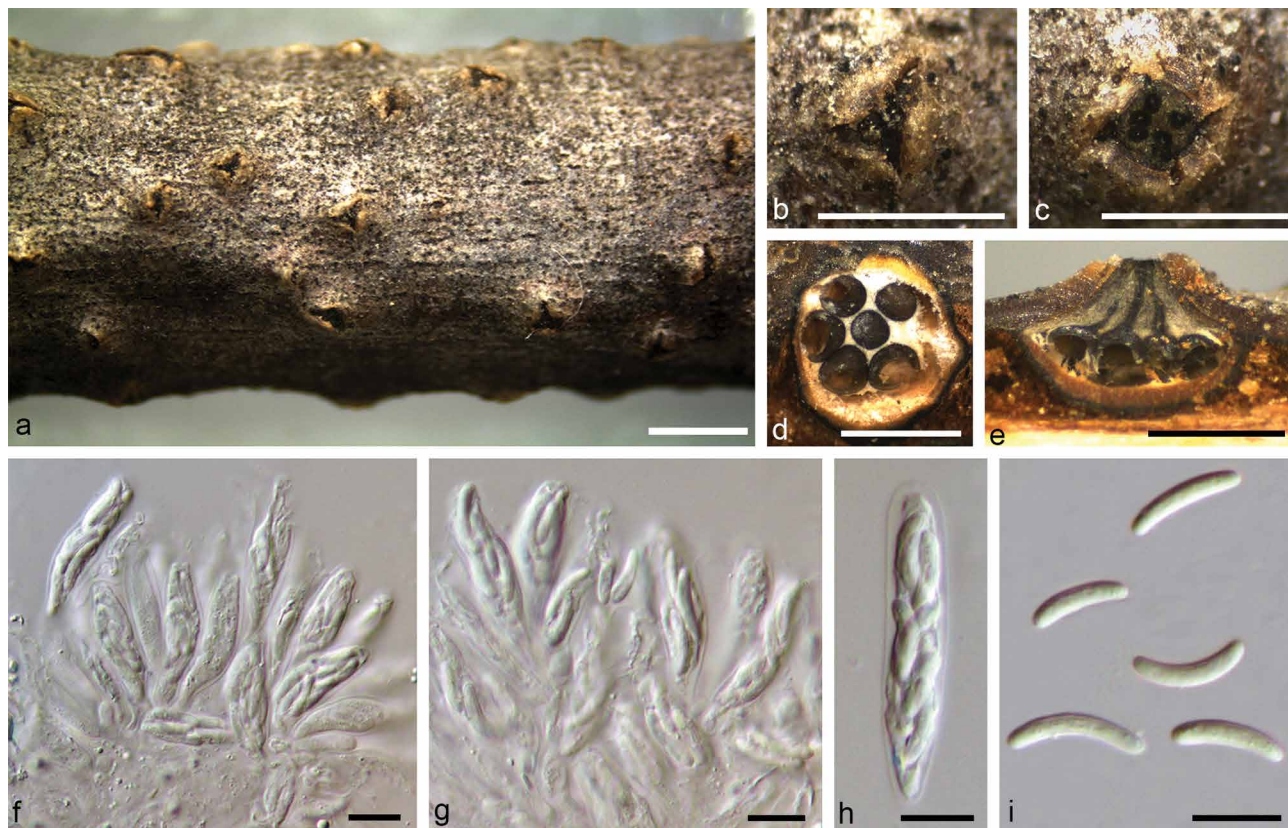
***Cytospora sophorae*** Bres., Fungi Trident. 2: 44. 1892 — Fig. 38

**Description** — See Fan et al. (2014a).

**Materials examined.** CHINA, Gansu Province, Lanzhou City, N36°03'45.37" E103°47'61.72", on branches of *Styphnolobium japonicum*, 13 Aug. 2012, X.L. Fan (BJFC-S698, living culture CFCC 89598); Shanxi Province, Datong City, Children's Park, N40°04'45.36" E113°16'39.56", on branches of *Styphnolobium japonicum*, 14 Apr. 2014, X.L. Fan (BJFC-S1037, living culture CFCC 50047); Changzhi City, Guyi Nursery Garden, N36°21'13.36" E113°12'14.56", on branches of *Magnolia grandiflora*, 19 Apr. 2014, X.L. Fan (BJFC-S1069, living culture CFCC 50048).

**Notes** — *Cytospora sophorae* is a common plant pathogenic fungus causing *Cytospora* canker on *Styphnolobium japonicum* (syn. *Sophora japonica*). The current identification follows previ-





**Fig. 37** *Cytospora sibiraeae* on *Sibiraea angustata* (BJFC-S783). a–b. Habit of ascostromata on branch; c. ectostromatic disc with ostioles; d. transverse section through ascostroma; e. longitudinal section through ascostroma; f–h. asci; i. ascospores. — Scale bars: a = 1 mm; b–e = 0.5 mm; f–i = 10  $\mu$ m.

ous descriptions and records in China, whereas a typification for a stable species concept is required (Tai 1979, Teng 1996, Fan et al. 2014a). *Cytospora sophorae* can be identified by cultural characters (cultures grow up to 7 cm after 30 d with an irregular edge, having a dark brown pigment), which differs from *C. sophoricola* (beak-shaped pycnidia produced in white cultures without a dark pigment) and *C. sophoriopsis* (cultures are white in the centre and straw at the margins). This is the first report of this fungus from *Magnolia grandiflora*.

***Cytospora sophoricola*** C.M. Tian & X.L. Fan, Mycoscience 55: 254. 2014 — Fig. 39

Description — See Fan et al. (2014a).

**Materials examined.** CHINA, Gansu Province, Gannan City, Lintan County, N34°57'56.17" E103°40'00.09", on branches of *Styphnolobium japonicum* var. *pendula*, 5 Aug. 2012, X.L. Fan (holotype BJFC-S694, ex-type living culture CFCC 89595); *ibid.*, BJFC-S695, living culture CFCC 89596.

**Notes** — *Cytospora sophoricola* is similar to *C. schulzeri* (recorded from *Malus*) and to *C. carbonacea* (reported from *Ulmus*) by the presence of multiple locules with a single ostiole, but can be distinguished from those based on the diameters of the disc and locules, number of ostioles, as well as the beak-shaped pycnidia produced in culture, size of the conidia, and cultural features. This species is only known from *Styphnolobium japonicum* var. *pendula* in China (Fan et al. 2014a).

***Cytospora sophoriopsis*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830158; Fig. 40

**Etymology.** Named after its morphological similarity to *C. sophorae*.

**Sexual morph** not observed. **Pycnidial stromata** ostiolate, immersed in bark, scattered, erumpent through the surface, with multiple locules. **Conceptacle** absent. **Ectostromatic disc** honey

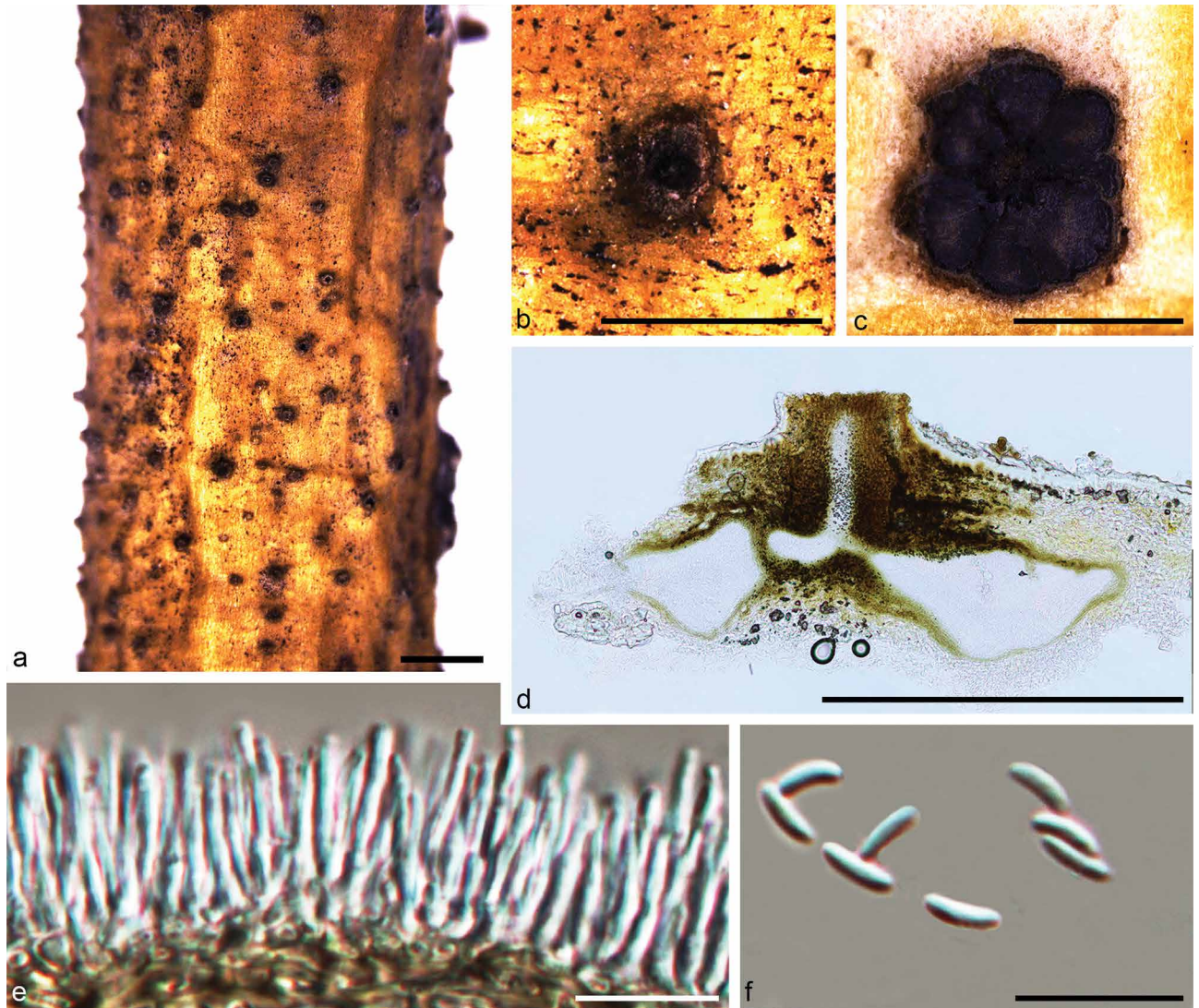
to isabelline, conspicuous, circular to ovoid, (120–)150–230 (–250)  $\mu$ m diam, with one ostiole per disc. **Ostiole** in the centre of the disc, black, conspicuous, (20–)25–35(–40)  $\mu$ m diam. **Locules** numerous, subdivided frequently by invaginations with common walls, (570–)600–700(–810)  $\mu$ m diam. **Conidiophores** borne along the locules, hyaline, branched at the base, in the middle, or unbranched, thin-walled, occasionally septate, 8–15  $\times$  1–1.5  $\mu$ m, embedded in a gelatinous layer. **Conidiogenous cells** enteroblastic, phialidic, sub-cylindrical to cylindrical, 5.5–9  $\times$  1–1.5  $\mu$ m, tapering towards the apices. **Conidia** hyaline, allantoid, eguttulate, smooth, aseptate, thin-walled, (3.5–)4–4.5(–5)  $\times$  1–1.5  $\mu$ m.

**Culture characteristics** — On PDA, colonies white, slightly fluffy, thin with a uniform texture, fast growing, reaching up to 7.5 cm diam after 3 d entirely covering the 9 cm Petri dish after 5 d, becoming thin in the centre and gradually straw at the margins after 30 d. **Pycnidia** black, distributed circularly at the colony margin on medium surface.

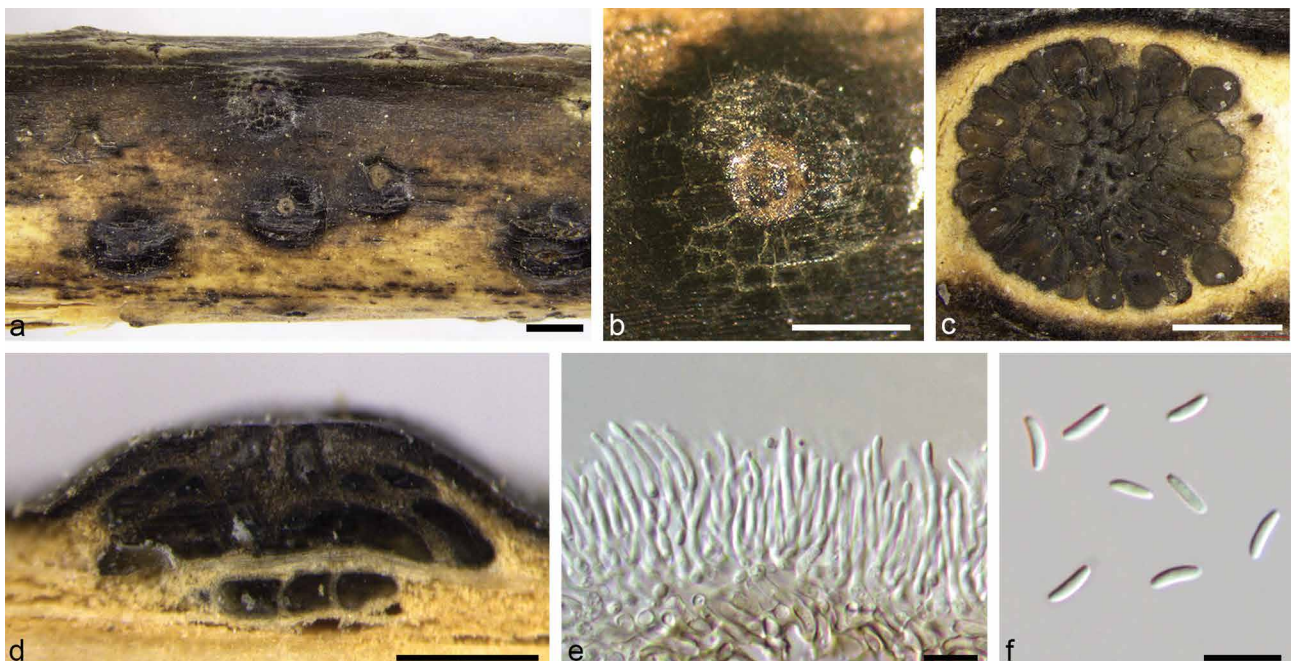
**Material examined.** CHINA, Gansu Province, Ganan City, Diebu County, N34°04'05.76" E103°11'33.63", on branches of *Styphnolobium japonicum*, 10 Aug. 2012, X.L. Fan (holotype BJFC-S713 = BJFC-CGHs10, ex-type living culture CFCC 89600).

**Notes** — *Cytospora sophoriopsis* is associated with canker disease of *Styphnolobium japonicum* (syn. *Sophora japonica*) in China. The strain CFCC 89600 was regarded as representative of *C. chrysosperma* due to its similar morphology, and the ITS phylogram based on a restricted dataset (Fan et al. 2014a). The current study revisited this fungus and introduced *C. sophoriopsis* as a new species based on its smaller, multiple locules (600–700 vs 640–1260  $\mu$ m) with honey to isabelline ectostromatic disc, and the host affiliation. The phylogenetic inferences of the combined matrix resolved *C. sophoriopsis* as distinct from the *C. chrysosperma* complex.



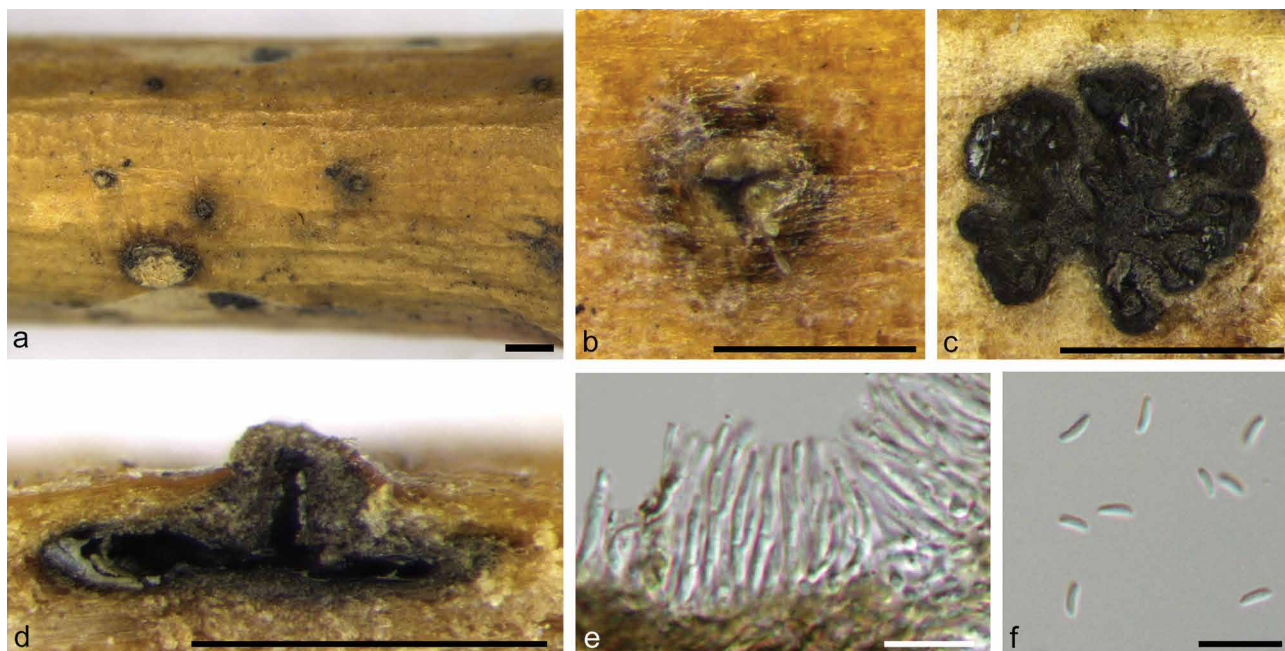


**Fig. 38** *Cytospora sophorae* on *Styphnolobium japonicum* (BJFC-S698). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.



**Fig. 39** *Cytospora sophoricola* on *Styphnolobium japonicum* (BJFC-S694). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.





**Fig. 40** *Cytospora sophoriopsis* on *Styphnolobium japonicum* (BJFC-S713). a–b. Habit of conidiomata on branch; c. transverse section through conidioma; d. longitudinal section through conidioma; e. conidiogenous cells with attached conidia; f. conidia. — Scale bars: a = 1 mm; b–d = 0.5 mm; e–f = 10  $\mu$ m.

***Cytospora spiraeae*** X.L. Fan, Phytotaxa 338: 57. 2018

Description — See Zhu et al. (2018).

**Materials examined** (all on twigs and branches of *Spiraea salicifolia*). CHINA, Gansu Province, Gannan City, N34°17'22.16" E102°58'44.23", 8 Aug. 2012, X.L. Fan (holotype BJFC-S784, ex-type living culture CFCC 50049); Gannan City, N34°17'22.16" E102°58'44.23", 8 Aug. 2012, X.L. Fan (BJFC-S785, living culture CFCC 50050); Shanxi Province, Datong City, Wenying lake Park, N40°04'32.02" E113°22'13.17", 15 Apr. 2014, X.L. Fan (BJFC-S1058, living culture CFCC 50051).

**Notes** — *Cytospora spiraeae* was described by Zhu et al. (2018) associated with symptomatic canker and dieback disease of *Spiraea salicifolia* in China. It is characterised by flask-shaped to spherical perithecia with hyaline, biseriate, aseptate, elongate-allantoid ascospores (6–8.5  $\times$  1.5–2.5  $\mu$ m), and pycnidia with numerous locules as well as allantoid conidia (5–7  $\times$  1–1.5  $\mu$ m) (Zhu et al. 2018). In the current phylogenetic trees (Fig. 3, 4), it clusters in a separate, well-supported clade.

***Cytospora tamaricicola*** X.L. Fan & C.M. Tian, *sp. nov.* — MycoBank MB830159; Fig. 41

**Etymology.** The name reflects the host genus from which it was collected, *Tamarix*.

**Ascstromata** immersed in the bark, erumpent through the bark surface, scattered, (1000–)1100–1400(–1550)  $\mu$ m diam, with 6–16 perithecia arranged circularly or irregularly. **Conceptacle** absent. **Ectostromatic disc** umber to sepia, usually surrounded by tightly aggregated ostiolar necks, (290–)310–400(–415)  $\mu$ m diam, with 6–16 ostioles arranged circularly per disc. **Ostioles** numerous, violaceous black to black, arranged irregularly and tightly in a disc, (45–)55.5–95(–115)  $\mu$ m diam. **Perithecia** dark grey to black, flask-shaped to spherical, arranged circularly or irregularly, (225–)250–380(–405)  $\mu$ m diam. **Asci** free, clavate to elongate obovoid, (31–)33.5–39(–42)  $\times$  (4–)4.5–5.5(–6)  $\mu$ m, 8-spored. **Ascospores** biseriate, elongate-allantoid, thin-walled, hyaline, aseptate, (8–)9–11.5(–12)  $\times$  2–2.5  $\mu$ m. **Pycnidial stromata** ostiolate, immersed in bark, scattered, erumpent through the surface, with multiple locules. **Conceptacle** absent. **Ectostromatic disc** greyish sepia to smoke grey, conspicuous, circular to ovoid, (175–)200–280(–305)  $\mu$ m diam, with one

ostiole per disc. **Ostiole** in the centre of the disc, violaceous black to black, conspicuous, (50–)55–85(–100)  $\mu$ m diam. **Locules** numerous, subdivided frequently by invaginations with independent walls, (950–)1080–1350(–1470)  $\mu$ m diam. **Conidiophores** borne along the locules, hyaline, branched at the base, in the middle, or unbranched, thin-walled, 10–19(–22)  $\times$  1–1.5  $\mu$ m, embedded in a gelatinous layer. **Conidiogenous cells** enteroblastic, phialidic, sub-cylindrical to cylindrical, 8–14.5  $\times$  1–1.5  $\mu$ m, tapering towards the apices. **Conidia** hyaline, allantoid, occasionally guttulate, smooth, aseptate, thin-walled, (5–)5.5–6(–6.5)  $\times$  1–1.5  $\mu$ m.

**Culture characteristics** — On PDA, colonies initially white and growing up to 5 cm diam after 3 d, becoming slightly buff at the margin with aerial mycelium, felt-like to slightly floccose, entirely covering the 9 cm Petri dish after 10 d, thick with a uniform texture after 30 d; sterile.

**Materials examined.** CHINA, Yunan Province, Kunming City, Kunming World Expo, N25°04'48.15" E102°45'30.60", on branches of *Tamarix chinensis*, 18 Mar. 2015, B. Cao, Q. Yang & Z. Du (holotype CF-2015510, ex-type living culture CFCC 50508); Dali City, Binchuan County, Jizu Mountain, N25°57'37.04" E100°23'05.77", on branches of *Rosa multiflora*, 23 Mar. 2015, B. Cao, Q. Yang & Z. Du (CF-2015512, living culture CFCC 50507).

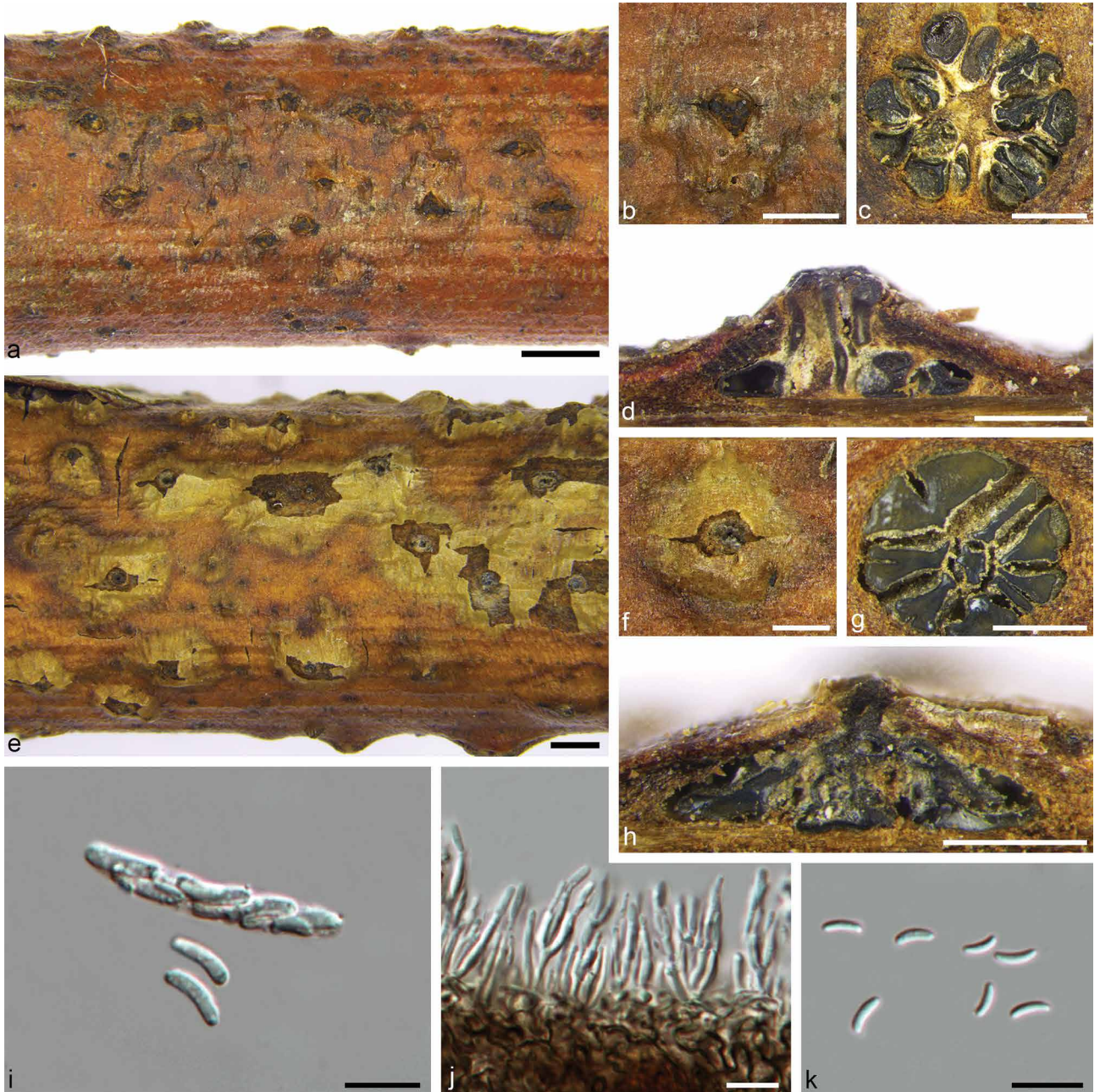
**Notes** — *Cytospora tamaricicola* is associated with canker disease of *Rosa multiflora* and *Tamarix chinensis* in China. *Cytospora tamaricella* was recorded from *Tamarix*, but without any available materials and DNA data. *Cytospora tamaricicola* differs from *C. tamaricella* by having smaller conidia (5.5–6  $\times$  1–1.5 vs 7–9  $\times$  1–1.5  $\mu$ m in *C. tamaricella*) (Sydow & Sydow 1904). Morphologically, this species has pycnidial locules with independent walls, which is similar to *C. ceratosperma* which has torsellioid chambers, but can be distinguished based on its phylogenetic position (Fig. 3, 4), and the unique host *Tamarix chinensis*.

**OTHER SPECIES REPORTED FROM CHINA**

***Cytospora davidiana*** Y.L. Wang & X.Y. Zhang, Fungal Biol. 119: 427. 2015

**Notes** — *Cytospora davidiana* was described and illustrated from stems of *Populus davidiana* in Inner Mongolia and Heilongjiang Provinces, China (Wang et al. 2015).





**Fig. 41** *Cytospora tamaricicola* on *Tamarix chinensis* (CF-2015510). a–b. Habit of ascostromata on branch; c. transverse section through ascostroma; d. longitudinal section through ascostroma; e–f. habit of conidiomata on branch; g. transverse section through conidioma; h. longitudinal section through conidioma; i. ascus and ascospores; j. conidiogenous cells with attached conidia; k. conidia. — Scale bars: a, e = 1 mm; b–d, f–h = 0.5 mm; i–k = 10  $\mu$ m.

***Cytospora fugax*** (Bull.) Fr., Syst. Mycol. (Lundae) 2: 544. 1823

Notes — *Cytospora fugax* was recorded from stems of *Populus* sp. in Inner Mongolia, Jilin and Heilongjiang Provinces, China (Wang et al. 2015). It needs further study to supplement the description.

***Cytospora kantschavelii*** Gvrit., Mikol. Fitopatol. 7: 547. 1973

Notes — *Cytospora kantschavelii* was reported from stems of *Populus* sp. in Inner Mongolia and Sichuan Provinces, China (Wang et al. 2015). It needs further study to supplement the illustration and description.

***Cytospora palm*** Q.T. Zhang & X.Y. Zhang, Cryptog. Mycol. 35: 216. 2014

Notes — *Cytospora palm* was described and illustrated from twigs of *Cotinus coggygria* in Beijing, China (Zhang et al. 2014).

***Cytospora parasitica*** Norph., Bulgakov & K.D. Hyde, Fung. Diversity 75: 146. 2015

Notes — *Cytospora parasitica* was described from dead branches of *Malus sylvestris* in Russia (Ariyawansa et al. 2015). Ma et al. (2018) reported that it caused a stem canker disease of apple in the Xinjiang Uygur Autonomous Region, China.

***Cytospora translucens*** Sacc., Syll. Fung. (Abellini) 3: 261. 1884

Notes — *Cytospora translucens* was recorded based on DNA sequence data from stems of *Populus* sp. and *Salix* sp. in Inner Mongolia and Heilongjiang Provinces, China (Wang et al. 2015).

***Cytospora tritici*** Punith., Nova Hedwigia 32: 586. 1980

Notes — *Cytospora tritici* was recorded as a new pathogen causing canker disease of *Populus* spp. in Sichuan Province, China (Zhang et al. 2012).

## UNCONFIRMED RECORDS FROM CHINA

***Cytospora curreyi*** Sacc., Syll. Fung. (Abellini) 3: 269. 1884

Notes — *Cytospora curreyi* was recorded from *Abies* sp. in the Sichuan Province of China (Teng 1963).

***Cytospora microspora*** Rabenh., Deutschl. Krypt.-Fl. (Leipzig) 1: 147. 1844

Notes — *Cytospora microspora* was recorded from *Quercus* sp. in the Hebei Province of China (Teng 1963).

***Cytospora personata*** (Fr.) Sacc., Syll. Fung. (Abellini) 1: 138. 1882

*Basionym.* *Sphaeria personata* Fr., Kongl. Svenska Vetensk.-Akad. Handl. (Ser. 3) 40: 105. 1819.

*Synonym.* *Valsa subclypeata* Cooke & Peck, Ann. Rep. N.Y. State Mus. Nat. Hist. 27: 109. 1875.

Notes — *Cytospora personata* was recorded from *Betula* spp. and *Rhododendron* sp. in the Hebei Province and North-west China (Teng 1963, Zhuang 2005).

***Cytospora rhodophila*** Sacc., Syll. Fung. (Abellini) 3: 253. 1884

Notes — *Cytospora rhodophila* was recorded from *Rosa* sp. in China (Teng 1963, Zhuang 2005).

***Cytospora sacchari*** E.J. Butler, Mem. Dept. Agric. India, Bot. Ser. 1: 31. 1906

Notes — *Cytospora sacchari* was recorded from *Saccharum* sp. in the Guangdong, Guangxi and Yunan Provinces of China (Teng 1963).

## DISCUSSION

The taxa investigated in the present study represent the largest number of *Cytospora* strains and species in China ever subjected to DNA sequence analyses. Phylogenetic studies published on the genus *Cytospora* based on ITS rDNA gene data have substantially influenced its taxonomy (Adams et al. 2005). In the past five years, the polyphasic approach (morphological identification and phylogenetic species recognition concept) has led to the description of several additional new species of *Cytospora* (Wang et al. 2013, Zhang et al. 2014, Fan et al. 2014a, b, 2015a, b, Yang et al. 2015, Lawrence et al. 2017, 2018, Norphanphoun et al. 2017). In this paper, we summarised 52 *Cytospora* species reported from China (including seven species in recent studies and five uncertain species). The current study recognised 40 *Cytospora* species from 88 isolates, including 13 new species and one new combination, which were sampled from 28 host genera distributed over 12 provinces in China (Table 1).

As is the case with many phytopathogenic genera of ascomycetous fungi, the most common phylogenetic problem related to *Cytospora* taxonomy is that the identification of the most important species (e.g., *C. chrysosperma*, *C. leucostoma* and *C. mali*) are only based on the ITS gene without type materials. In the first taxonomic phylogenetic study of *Cytospora*, Adams et al. (2002) used ITS sequence data to evaluate the phylogenetic significance of three species from fruit trees. In the Dictionary of Fungi, Kirk et al. (2008) recognised about 110 species in *Cytospora*, although more than 600 species have been described to date (see Index Fungorum (<http://www.indexfungorum.org/>) and MycoBank (<http://www.mycobank.org/>)). Previous taxonomists listed six *Cytospora* species in their list of fungi known from China, although this is based on old nomenclature and local reports (Teng 1963, Tai 1979, Zhuang

2005). We therefore regard these taxa as dubious, pending their re-examination. Few lists of *Cytospora* based on living cultures and authentic specimens are available for China. A significant result of the present study was thus to significantly increase the knowledge about *Cytospora* spp. in China based on DNA analyses of numerous additional isolates, as well as to extend the criteria of identification and number of genes used in *Cytospora* phylogenetic studies.

Most of the isolates obtained from branches and twigs of *Populus* and *Salix* stems displaying symptoms of canker disease were identified as *Cytospora chrysosperma* in this study. *Cytospora chrysosperma* is regarded as the most important causal agent of canker disease from *Salicaceae* with quarantine significance throughout China. This species was first described in Europe by Fries (1823), causing canker disease of *Populus* sp. Thereafter, this fungal pathogen has been reported on various plant hosts with worldwide distribution, including *Fraxinus*, *Prunus*, *Triticum*, *Ulmus* and, remarkably, even *Homo sapiens* (Kalkanci et al. 2006). Past taxonomic studies concluded that many species of *Cytospora* shared the morphology of *C. chrysosperma*, and thus it should be regarded as a species complex unless their DNA sequences could be obtained to resolve their identification (Adams et al. 2005). Tai (1979) separated different species from *Salicaceae* in China with various symptoms, but also assumed that they could possibly represent the same species. The current results restricted *C. chrysosperma* to *Populus* and *Salix* (*Salicaceae*). With regard to host associations, species of *Cytospora* seem to have wide host ranges, whereas they proved to be mostly limited to a single host species in the current study. Phylogenetic studies of *Cytospora* based on type materials are hampered by a lack of authentic cultures of previously described species, and thus typification studies from original type materials and/or fresh collections are required to ensure a stable and workable taxonomy. A monographic revision based on the type specimens along with phylogenetic analyses to reassess all species in the genus is urgently required.

The ecology, host specificity and pathogenicity of many *Cytospora* species are poorly known. Only a few important pathogens on apple and poplar are well studied with respect to their biology, infection and populations. Species of *Cytospora* are known as opportunistic pathogens mainly on woody hosts and some of the species occur on a wide host range (Adams et al. 2005). For example, the important and highly virulent plant pathogen, *C. chrysosperma*, has been reported on many other hosts in Australia, Asia, Africa, Europe and America (Spielman 1983, 1985, Adams et al. 2005). Most of the earlier *Cytospora* species identifications was based on morphology, and their host ranges must therefore be re-evaluated with the application of recent molecular data. Although some *Cytospora* species, such as *C. chrysosperma* and *C. leucostoma*, have been confirmed from a broad host range, extensive sampling of most other *Cytospora* species and accumulation of molecular data are required to improve our understanding of their host range and distribution.

In the present study, *Cytospora* spp. were found to occur in the northeast, northwest, north and southwest China, indicating that the cold and dry environments are always favourable for these taxa in China.

In future studies, extensive fresh specimens should be collected to help clarify the species concepts of taxa still lacking multigene DNA sequence data, especially in west China. The difficulty to observe typical sexual structures in many taxa and the overlapping morphological characteristics are common impediments. We hope that the descriptions and molecular data of *Cytospora* spp. provided in this study will be a resource for plant pathologists, plant quarantine officials and taxonomists



to aid in the identification of *Cytospora* species in China and other countries, as well as supplement our knowledge of their biology, ecology and geographic distribution.

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