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Revisiting the genus *Cytospora* and allied species

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Abstract

Cytospora species are important plant pathogens causing dieback and canker diseases on a wide range of hosts, worldwide. However, species level identification is difficult due to poor phylogenetic understanding and lack of sequenced type species. ITS sequence data are only available for most Cytospora strains in GenBank. In this study, samples of Cytospora were collected from symptomatic twigs and branches in European Russia. A combination of morphological characters and multi-gene phylogenetic analysis (ITS, LSU, RPB2, ACT) were used to identify taxa. A total of 34 collections, representing 17 fungal species of Cytospora were studied. Of these, 14 new species are described and illustrated. Three species were known taxa and identified as C. nivea, C. parasitica, and C. salicicola. Newly introduced species are C. ampulliformis, C. curvata, C. donetzica, C. erumpens, C. longiostiolata, C. melnikii, C. parakantschavelii, C. paratranslucens, C. rusanovii, C. salicacearum, C. salicina, C. sorbi, C. sorbicola and C. ulmi. Descriptions, illustrations and notes are provided for all studied taxa in this study. The distribution patterns of Cytospora species on different hosts are discussed. The study represents a preliminary study of Cytospora species from a small region and provides an initial contribution to the understanding of the genus.

Key words – Diaporthales – Morphology – Phylogenetic analyses – Plant pathogenic fungi – Russia – *Valsaceae*

Introduction

Cytospora was introduced by Ehrenberg (1818) and is one of the most important forest pathogenic genera causing canker disease on branches of various tree species. The disease often leads to large areas of dieback on a wide range of plants (Adams et al. 2005, 2006). Cytospora is the asexual morph of Valsa, which is the type genus of Valsaceae Tul. & C. Tul. in Diaporthales Nannf. comprising the genera Amphicytostroma, Chadefaudiomyces, Cryptascoma, Cytospora,

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Ditopellina, Durispora, Harpostroma, Hypospilina, Kapooria, Leptosillia, Maculatipalma, Pachytrype and Paravalsa (Adams et al. 2005, Wang et al. 2011, Ariyawansa et al. 2015, Fan et al. 2015a, b, Liu et al. 2015, Maharachchikumbura et al. 2015, 2016, Hyde et al. 2016).

Cytospora has been also considered as the asexual morph of other genera such as, Leucocytospora, Leucostoma, Valsella and Valseutypella (Fries 1823, Saccardo 1884, Gvritishvili 1982, Spielman 1985, Adams et al. 2002, 2005, Castlebury et al. 2002, Bulgakov 2010, Maharachchikumbura et al. 2015, 2016, Rossman et al. 2015, Yang et al. 2015, Li et al. 2016, Hyde et al. 2016). Therefore, all of these sexual genera were synonymized under Valsa, either as a subgenus or species (Adams et al. 2006). The International Code of Nomenclature for Algae, Fungi, and Plants (ICN, McNeill et al. 2012) permits a single name for biological species and genera (Hyde et al. 2009, Wikee et al. 2011, Huang et al. 2013, Wei et al. 2013, Udayanga et al. 2014, Fan et al. 2015a, b). Therefore, Valsa (1849) was treated as synonym of Cytospora (1818), with the latter being the oldest and most widely used name (Adams et al. 2005, Fotouhifar et al. 2010, Fan et al. 2014, Rossman et al. 2015). Most of the names previously recognized in Valsa already have an older epithet in Cytospora in the SMML Fungal Database (http://nt.ars-grin.gov/fungaldatabases/), while some new names for the common species of Cytospora, previously placed in Valsa, were provided by Rossman et al. (2015).

Cytospora species usually produce asexual fruiting bodies and contain a single or labyrinthine of locules, filamentous conidiophores or asci, and allantoid hyaline conidia or allantoid hyaline ascospores (Spielman 1983, 1985, Adams et al. 2005, Fan et al. 2015a, b). In moist conditions, the conidia will emerge from the fruiting bodies forming yellow, orange to red gelatinous tendrils (Adams et al. 2005, 2006). Identification of Cytospora species has generally been established according to host association, while morphological descriptions generally lack significant differences. Therefore, a single species of Cytospora may occur on several unrelated host plants, or a single host plant may support more than one Cytospora species (Adams et al. 2005, Wang et al. 2011, Ariyawansa et al. 2015, Fan et al. 2015a, b, Liu et al. 2015, Hyde et al. 2016). Thus, there are 585 epithets for Cytospora in Index Fungorum (2017) with an estimated number of 110 species in Kirk et al. (2008). However, ex-type sequence data are available for only 23 species in GenBank (accessed 2017). Thus, it is difficult to identify species from a phylogenetic perspective alone (Ariyawansa et al. 2015, Liu et al. 2015, Hyde et al. 2016, Li et al. 2016) as many taxa are morphologically similar. The have been several recent papers dealing with Cytospora with a limited number of multi-gene sequence trees or larger trees based on ITS sequence data, however there has been no comprehensive multi-gene study for the genus (Adams et al. 2002, Fotouhifar et al. 2010, Hyde et al. 2010, 2014, Ariyawansa et al. 2015, Fan et al. 2015a, b, Liu et al. 2015, Yang et al. 2015, Hyde et al. 2016, Li et al. 2016).

The aim of the present study was to identify *Cytospora* species in a small region of Rusia based on morphological studies and phylogenetic analysis, and provide a multi-locus phylogeny using ITS, LSU, RPB2 and ACT sequence data for resolution of *Cytospora* species. The distribution of *Cytospora* species on hosts is also discussed.

Materials & Methods

Sample collection and specimen examination

The specimens were collected from Russia during 2015 by Timur Bulgakov. The specimens were returned to the laboratory in small paper bags for observation, examination and description, following the methods described in Norphanphoun et al. (2015, 2016). Micro-morphological characters were studied using a Motic SMZ 168 dissecting microscope. Hand sections of the fruiting structures were mounted in water and examined for morphological details. Specimens were examined under a Nikon Ni compound microscope and photographed using a Canon EOS 600D digital camera fitted to the microscope. Photo-plates were made by using Adobe Photoshop CS6 Extended version 13.0×64 (Adobe Systems Inc., The United States) and the Tarosoft (R) Image Frame Work program v. 0.9.7 was used for taking measurements. The contents inside the

conidiomata were picked with a sterile needle and soaked in sterile water in a glass container for examination.

Cultures were obtained by single spore isolation following the method described in Chomnunti et al. (2014). Spore germination was observed and photographed under a Nikon Ni compound microscope fitted with a Canon EOS 600D digital camera. Geminated spores were transferred aseptically to malt extract agar (MEA) and incubated at 18–25°C. Colony characters were recorded and measured after one week and also one month.

The herbarium specimens were deposited in the Mae Fah Luang University Herbarium, Chiang Rai, Thailand (MFLU) and duplicated in New Zealand Fungal Herbarium, New Zealand (PDD). Living cultures were deposited at Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Culture Collection (KUMCC). Facesoffungi and Index Fungorum numbers were registered (Jayasiri et al. 2015, Index Fungorum 2017).

DNA extraction, PCR amplification and sequencing

Thirty-four isolates were selected for the molecular analyses (Table 1). DNA was amplified from the complete the internal transcribed spacer regions (ITS1-5.8S-ITS2), large nuclear ribosomal RNA subunit region (LSU), RNA polymerase II subunit (RPB2), and α-actin (ACT) genes of each *Cytospora* isolate, which were performed using fungal mycelia growing on MEA at 18–25°C for one month. The genomic DNA was obtained using a E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA) following the manufacturer's protocols.

Polymerase chain reactions (PCR) were carried out using primer pairs of ITS1 and ITS4 to amplify the ITS region (White et al. 1990), the partial LSU region was amplified with primers NL1 and NL4 (O'Donnell 1993), the partial RPB2 region was amplified using primers bRPB-6F and bRPB-7.1R (Matheny 2005), and the partial ACT region was amplified using primers ACT512F and ACT783R (Carbone & Kohn 1999) (Table 1). The amplification reaction was performed in 50 μ l reaction volume containing, 2 μ l of DNA template, 2 μ l of each forward and reverse primers, 25 μ l of 2 × Bench TopTMTaq Master Mix (mixture of Taq DNA Polymerase (recombinant): 0.05 units/ μ L, MgCl₂: 4 mM, and dNTPs: 0.4 mM) and 19 μ l of double-distilled water (ddH₂O). The PCR conditions used in this study for each gene are shown in Table 1. The quality of PCR products were checked by using 1% agarose gel electrophoresis stained with ethidium bromide. Purification and sequencing of PCR product were carried out at Life Biotechnology Co., Shanghai, China.

Phylogenetic analysis

Blast searches were made to identify the closest matches in GenBank and reliable sequences were downloaded (viz. Fotouhifar et al. 2010, Fan et al. 2014, 2015a, b, Hyde et al. 2016). The phylogenetic analyses were initially performed using ITS sequence data and then determined using combined multi-gene phylogeny (ITS, LSU, RPB2, ACT) with 143 and 97 sequences respectively. Cytospora species were selected for combined multi-gene phylogeny based on fungal group relationships between closely related taxa of type strains with our strains from the ITS phylogenetic tree. Phomopsis vaccinii Shear (ATCC 18451) was selected as the outgroup taxon in individual and combined analyses. The individual datasets were combined as this has been previously found to increase phylogenetic accuracy (Cunningham 1997, Bull et al. 1993). The combined sequence alignment was obtained from MEGA7 version 7.0.14 (Kumar et al. 2015) and ambiguously aligned regions were excluded and gaps were treated as missing data. Alignments were checked visually and manually adjusted for errors. The partition homogeneity test (PHT) or the Incongruence Length Difference (ILD) test were used to testing the congruence and combinability of the individual datasets (Farris et al. 1995a, b) with 1,000 heuristic search replicates in PAUP v. 4.0b10 (Swofford 2003). In addition, the datasets were optimized manually where necessary. Phylogenetic trees were reconstructed using maximum parsimony, maximum likelihood and Bayesian inference analyses.

Maximum parsimony (MP) analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003). The trees were inferred using the heuristic search option with tree bisection—reconnection (TBR) as the branch swapping algorithm and 1000 random

sequence additions. Maxtrees were setup to 1000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for the maximum parsimonious tree (MPT). The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications, each with ten replicates of random stepwise addition of taxa (Felsenstein 1985). The Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed to determine whether the trees were significantly different.

Table 1 The PCR thermal cycle program in this study

Gene and the primers used	Step	Temp	Time	Cycle
	Initialization	95 °C	3 minutes	
ITC	Denaturation	95 °C	30 seconds	
ITS ITS1/ITS4	Annealing	55 °C	50 seconds	40 Cycles
1131/1134	Extension/Elongation	72 °C	1 minute	
	Final Elongation	72 °C	7 minutes	
	Initialization	94 °C	2 minutes	
1 011	Denaturation	94 °C	30 seconds	
LSU NL1/NL4	Annealing	52 °C	30 seconds	30 Cycles
INL1/INL4	Extension/Elongation	72 °C	1 minute	
	Final Elongation	72 °C	7 minutes	
	Initialization	95 °C	5 minutes	
RPB2	Denaturation	95 °C	1 minute	
bRPB-6F/bRPB-7.1R	Annealing	52 °C	2 minutes	40 Cycles
UKFD-017 UKFD-7.1K	Extension/Elongation	72 °C	90 seconds	
	Final Elongation	72 °C	10 minutes	
ACT ACT512F/ACT783R	Initialization	95 °C	5 minutes	
	Denaturation	95 °C	40 seconds	
	Annealing	58 °C	30 seconds	40 Cycles
ACIJIZF/ACI/OJK	Extension/Elongation	72 °C	1 minute	
	Final Elongation	72 °C	5 minutes	

The best-fit nucleotide substitution models for each dataset were separately determined using MrModeltest version 2.2 for both ML and Bayesian analyses (Nylander 2004). Maximum-likelihood (ML) analysis was performed in RAxML (Stamatakis 2006) implemented in raxmlGUI v.1.3 (Silvestro & Michalak 2012). The 1000 rapid bootstrap replicates were run with generalized time reversible GTRGAMMA model of nucleotide substitution and searches for model selected for ML were applied.

Bayesian inference (BI) analysis was performed using the Markov Chain Monte Carlo (MCMC) method with MrBayes v.3.2.2 (Ronquist et al. 2012). GTR+I+G was selected as the best-fitting model for individual ITS and combined ITS, LSU, RPB2 and ACT. The Markov ChainMonte Carlo sampling (MCMC) analyses, with four chains, were started from random tree topology and lasted 5,000,000 generations and sampled every 100 generations. The Tracer v. 1.5.0 program was used to check the effective sampling sizes (ESS) that should be above 200, the stable likelihood plateaus and burn-in value (Rambaut et al. 2013). The first 5000 generations were excluded as burn-in.

The resulting trees were viewed with FigTree v1.4.0 (Rambaut 2012) and edited in Adobe Illustrator CS6 and Adobe Photoshop CS6 Extended version 13.0×64 (Adobe Systems Inc., The United States). Sequences data from this study were deposited in GenBank (Table 2) and alignments were saved in TreeBASE (www.treebase.org) submission ID: 20491 (ITS sequence alignment) and 20492 (combined sequence alignment).

 Table 2 Cytospora species introduced in this study (ex-type strains are bold)

NO	TAYON	CITID A INI	TIOST.		GENBANK ACCESSION NUMBERS		
NO	TAXON	STRAIN	HOST	ITS	LSU	RPB2	ACT
1	Cytospora ampulliformis	MFLUCC 16-0583	Sorbus intermedia (Ehrh.) Pers.	KY417726	KY417760	KY417794	KY417692
2	Cytospora ampulliformis	MFLUCC 16-0629	Acer platanoides L.	KY417727	KY417761	KY417795	KY417693
3	Cytospora curvata	MFLUCC 15-0865	Salix alba L.	KY417728	KY417762	KY417796	KY417694
4	Cytospora donetzica	MFLUCC 15-0864	Crataegus monogyna Jacq.	KY417729	KY417763	KY417797	KY417695
5	Cytospora donetzica	MFLUCC 16-0641	Pyrus pyraster (L.) Burgsd	KY417730	KY417764	KY417798	KY417696
6	Cytospora donetzica	MFLUCC 16-0574	Rosa sp.	KY417731	KY417765	KY417799	KY417697
7	Cytospora donetzica	MFLUCC 16-0589	Salix alba L.	KY417732	KY417766	KY417800	KY417698
8	Cytospora erumpens	MFLUCC 16-0580	Salix imes fragilis L.	KY417733	KY417767	KY417801	KY417699
9	Cytospora longiostiolata	MFLUCC 16-0628	Salix imes fragilis L.	KY417734	KY417768	KY417802	KY417700
10	Cytospora melnikii	MFLUCC 15-0851	Malus domestica Borkh	KY417735	KY417769	KY417803	KY417701
11	Cytospora melnikii	MFLUCC 16-0635	Populus nigra L. var. italica Münchh	KY417736	KY417770	KY417804	KY417702
12	Cytospora nivea	MFLUCC 15-0860	Salix acutifolia Willd.	KY417737	KY417771	KY417805	KY417703
13	Cytospora parakantschavelii	MFLUCC 15-0857	Populus × sibirica G.V. Krylov & G.V. Grig. ex A.K. Skvortsov	KY417738	KY417772	KY417806	KY417704
14	Cytospora parakantschavelii	MFLUCC 16-0575	Pyrus pyraster (L.) Burgsd.	KY417739	KY417773	KY417807	KY417705
15	Cytospora parasitica	MFLUCC 16-0507	Malus domestica Borkh	KY417740	KY417774	KY417808	KY417706
16	Cytospora paratranslucens	MFLUCC 16-0506	Populus alba L. var. bolleana (Lauche) Otto	KY417741	KY417775	KY417809	KY417707
17	Cytospora paratranslucens	MFLUCC 16-0627	Populus alba L.	KY417742	KY417776	KY417810	KY417708
18	Cytospora rusanovii	MFLUCC 15-0853	Populus × sibirica G.V. Krylov & G.V. Grig. ex A.K. Skvortsov	KY417743	KY417777	KY417811	KY417709
19	Cytospora rusanovii	MFLUCC 15-0854	Salix babylonica L.	KY417744	KY417778	KY417812	KY417710
20	Cytospora salicacearum	MFLUCC 15-0861	Salix imes fragilis L.	KY417745	KY417779	KY417813	KY417711
21	Cytospora salicacearum	MFLUCC 16-0509	Salix alba L.	KY417746	KY417780	KY417814	KY417712
22	Cytospora salicacearum	MFLUCC 16-0576	Populus nigra L. var. italica Münchh.	KY417747	KY417781	KY417815	KY417713
23	Cytospora salicacearum	MFLUCC 16-0587	Prunus cerasus L.	KY417748	KY417782	KY417816	KY417714
24	Cytospora salicicola	MFLUCC 15-0866	Salix alba L.	KY417749	KY417783	KY417817	KY417715
25	Cytospora salicina	MFLUCC 15-0862	Salix alba L.	KY417750	KY417784	KY417818	KY417716
26	Cytospora salicina	MFLUCC 16-0637	Salix imes fragilis L.	KY417751	KY417785	KY417819	KY417717
27	Cytospora sorbi	MFLUCC 16-0631	Sorbus aucuparia L.	KY417752	KY417786	KY417820	KY417718
28	Cytospora sorbicola	MFLUCC 16-0581	Sorbus aucuparia L.	KY417753	KY417787	KY417821	KY417719
29	Cytospora sorbicola	MFLUCC 16-0582	Cotoneaster melanocarpus Fisch. ex Blytt,	KY417754	KY417788	KY417822	KY417720
30	Cytospora sorbicola	MFLUCC 16-0584	Acer pseudoplatanus L.	KY417755	KY417789	KY417823	KY417721
31	Cytospora sorbicola	MFLUCC 16-0585	Sorbaronia mitschurinii (A.K. Skvortsov & Maitul.) Sennikov	KY417756	KY417790	KY417824	KY417722
32	Cytospora sorbicola	MFLUCC 16-0586	Prunus cerasus L.	KY417757	KY417791	KY417825	KY417723
33	Cytospora sorbicola	MFLUCC 16-0633	Cotoneaster melanocarpus Fisch. ex Blytt	KY417758	KY417792	KY417826	KY417724
34	Cytospora ulmi	MFLUCC 15-0863	Ulmus minor Mill.	KY417759	KY417793	KY417827	KY417725

Results

Phylogenetic analyses

The individual ITS dataset contained 143 sequences. The total alignment length had 609 total characters including alignment gaps. Parsimony analyses indicate that 391 characters were constant, 72 variable characters were parsimony uninformative and 146 characters were parsimony informative. The parsimony analysis of the data matrix resulted in 1000 equally parsimonious trees and the first tree (TL = 773, CI = 0.4373, RI = 0.8446, RC = 0.3693, HI = 0.5627) is shown in Fig. 1. Based on MP, ML and BI analyses of individual ITS dataset can separate some species of Cytospora (Fig. 1). However, it cannot resolve some species. For example C. longiostiolata (MFLUCC 16-0628), C. melnikii (MFLUCC 15-0851, MFLUCC 16-0635), C. salicacearum (MFLUCC 16-0509, MFLUCC 16-0576, MFLUCC 16-0587, MFLUCC 15-0861), C. salicina (MFLUCC 15-0862, MFLUCC 16-0637), C. chrysosperma (334, CFCC 89629, CFCC 89630, HMBF CGHs10, HMBF 151, HMBF 158, HMBF 17) and Cytospora sp. (30 NC5, 37 NC14) (Fig. 1). Thus, a combined ITS, LSU, RPB2 and ACT tree was used to delimit the Cytospora species. The combined ITS, LSU, RPB2 and ACT analysis comprised 97 sequences. The total alignment length of 2337 characters including alignment gaps was used of which 609, 610-1163, 1164-2044 and 2045-2337 were derived from ITS, LSU, RPB2 and ACT sequence data respectively. The result from the partition homogeneity test (PHT) was not significant level (95%), indicating that the individual data sets were congruent and could be combined. Parsimony analysis indicate that 1545 characters were constant, 240 variable characters were parsimony uninformative and 552 characters were parsimony informative and yielded 1000 most parsimonious trees (TL = 2020, CI = 0.554, RI = 0.864, RC = 0.479, HI = 0.446) (Fig. 2). The BI and ML tree analyses of individual and combined phylogenies had similar topologies to the MP tree as shown in Figs. 1 and 2 respectively. Bootstrap support values of MP, ML $(\geq 70\%)$ and Bayesian posterior probabilities (≥ 0.90) are shown on the upper branches (Figs. 1 and 2). The molecular support obtained from phylogenetic topologies are discussed under notes of each species.

Taxonomy

In this study, we introduce 14 new species and re-describe three known species. We follow Jeewon and Hyde (2016) for establishing species boundaries and Dayarathne et al. (2016) for using old names.

Cytospora ampulliformis Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552601, Facesoffungi Number: FoF 02736 Fig. 3 Etymology: The specific epithet 'ampulliformis' refers to the flask-shaped conidiogenous cells.

Holotype: MFLU 15-2187

Associated with twigs and branches of *Sorbus intermedia* (Ehrh.) Pers. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 680–1200 \times 350–480 μ m diameter, semi-immersed in host tissue, solitary, erumpent, scattered, discoid, circular to ovoid, with 3–4 locules, and ostiolar neck. *Ostioles* 200–300 μ m long, at the same level as the disc surface. *Peridium* comprising few to several layers of cells of *textura angularis*, with inner most layer thin, hyaline to pale brown, outer layer brown to dark brown. *Conidiophores* unbranched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, flask-shaped phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* (5–)5.6–9 \times 1.3–1.6(–1.7) μ m (\overline{x} = 7.5 \times 1.6 μ m, n = 30), unicellular, subcylindrical, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 7.5 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Rostov-on-Don City, Botanical Garden of Southern Federal University, Systematic Arboretum, parkland, dead and dying branches (necrotrophic) of *Sorbus intermedia* (*Rosaceae*), 30 May 2015, T. Bulgakov, T-483 (MFLU 15-2187, holotype, KUN, isotype), ex-type living culture, MFLUCC 16-0583, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye Forest, artificial forest, dying twigs and branches (necrotrophic) on *Acer platanoides* L. (*Sapindaceae*), 27 October 2015, T. Bulgakov, T-1094 (MFLU 15-3756, KUN), living culture, MFLUCC 16-0629, KUMCC.

Notes – *Cytospora* species associated with *Sorbus* sp. were reported in previous studies such as *C. leucostoma* and *Valsa massariana* (Adams et al. 2002, 2005). In this study, *C. ampulliformis*, *C. sorbi* and *C. sorbicola* are also reported from *Sorbus* sp. (Table 3). Morphologically, *C. ampulliformis* is similar to *C. sorbi* in its conidiomata having 3–4 locules and in the length of ostiolar neck (*C. ampulliformis*: 200–300 μ m versus 250–300 μ m: *C. sorbi*). However, *C. ampulliformis* differs from *C. sorbi* in having larger conidia (*C. ampulliformis*: 7.5 × 1.6 μ m, versus 6.5 × 1.5 μ m: *C. sorbi*). Based on phylogenetic analyses, both species form separate lineages within the genus *Cytospora* (Figs. 1 and 2).

We obtained two isolates of *Cytospora ampulliformis* (MFLUCC 16-0583, MFLUCC 16-0629) and these formed a close relationship with *C. cotini* (MFLUCC 14-1050) isolated from *Cotinus coggygria*, *C. tanaitica* (MFLUCC 14-1057) isolated from *Betula pubescens*, *C. rosarum* (218) isolated from *Rosa canina* and *C. ulmi* (MFLUCC 15-0863) isolated from *Ulmus minor*. *Cytospora ampulliformis* (7.5 \times 1.6 μ m) differs from *C. cotini* in forming black-discoid conidiomata on the host and lobate circular colonies on MEA (Hyde et al. 2016), while it differs from *C. cotini* (5.9 \times 1.2 μ m), *C. tanatica* (3.4 \times 0.7 μ m), *C. rosarum* (5–6 \times 1.5 μ m) and *C. ulmi* (5.4 \times 1.4 μ m) by its larger conidia.

Cytospora curvata Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552602, Facesoffungi Number: FoF 02737 Fig. 4 Etymology: The specific epithet "curvata" refers to the conidia character (Latin 'curvata' means 'crooked', 'bent', 'curved').

Holotype: MFLU 15-2229

Associated with twigs and branches of *Salix alba* L. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 900–1100 \times 470–580 μ m diameter, semi-immersed in host tissue, scattered, erumpent, unilocular, with ostiolar neck. *Ostioles* 140–160 μ m, same level as the disc surface. *Peridium* comprising a few to several layers of cells of *textura angularis*, with inner most layer thin, brown, outer later dark brown to black. *Conidiophores* unbranched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* (5.4–)5.8–7.7 \times 1.2–1.3(–1.4) μ m (\overline{x} = 5.9 \times 1.3 μ m, n = 30), unicellular, elongate-allantoid, slightly curved, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 8 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, white, margin rough, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye Forest, shore of little pond, on dead and dying branches of *Salix alba* (*Salicaceae*), 28 June 2015, T. Bulgakov, T-525 (MFLU 15-2229, holotype, KUN, isotype), ex-type living culture, MFLUCC 15-0865, KUMCC.

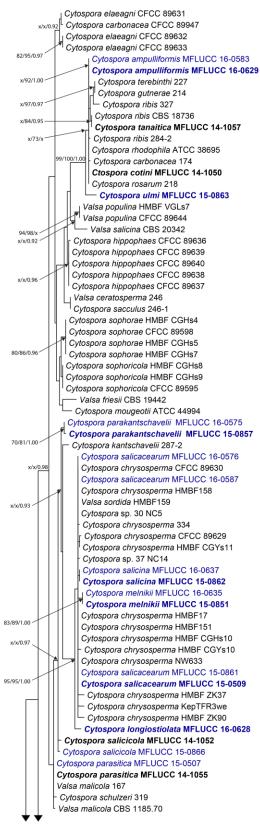


Fig. 1 – Phylogram generated from one of 1000 most parsimonious trees based on analysis of ITS sequence data of *Cytospora* isolates. The tree is rooted to *Phomopsis vaccinii* (ATCC 18451). Maximum parsimony and maximum likelihood bootstrap values ≥70%, Bayesian posterior probabilities ≥0.90 (MPBS/MLBS/PP) are given at the nodes. The species obtained in this study are in blue and ex-types from the study are in blue bold. Ex-type taxa from other studies are in black bold.

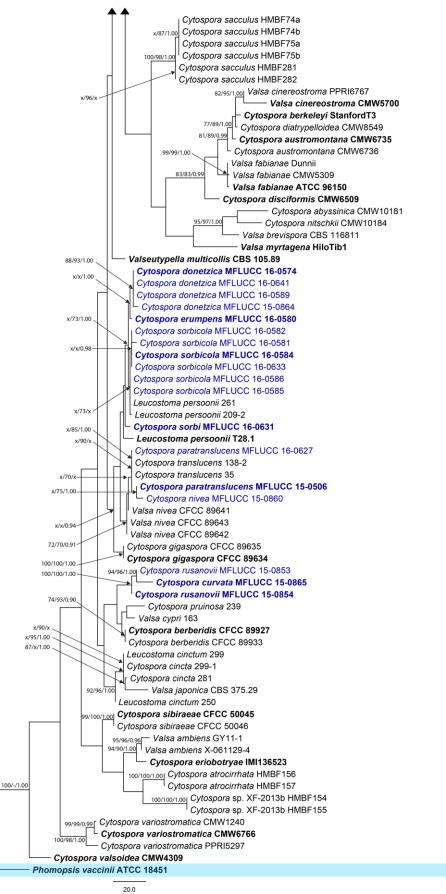


Fig. 1 – continued

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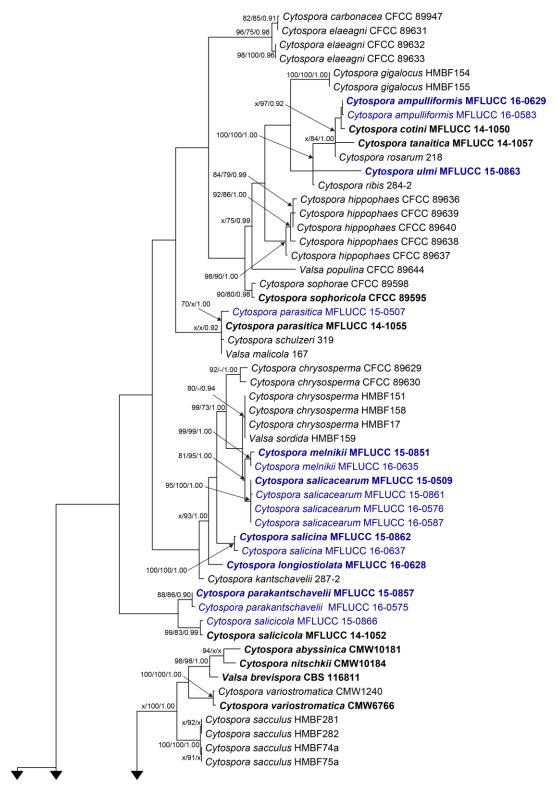


Fig. 2 – Phylogram generated from one of 1000 most parsimonious trees based on analysis of combined ITS, LSU, RPB2 and ACT sequence data of *Cytospora* isolates. The tree is rooted to *Phomopsis vaccinii* (ATCC 18451). Maximum parsimony and maximum likelihood bootstrap values \geq 70%, Bayesian posterior probabilities \geq 0.90 (MPBS/MLBS/PP) are given at the nodes. The species obtained in this study are in blue and ex-types from the study are in blue bold. Ex-type taxa from other studies are in black bold.

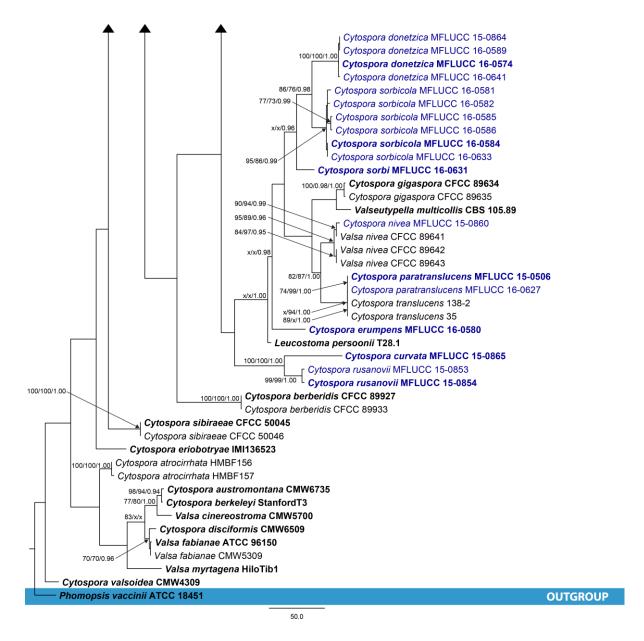


Fig. 2 continued

Notes – Many *Cytospora* species have been reported from *Salix* worldwide (Table 3; Adams et al. 2002, 2005, 2006, Défago 1942, Fotouhifar et al. 2010, Singh et al. 2007). However, they can be distinguished based on phylogenetic analyses (Fan et al. 2014, Wang et al. 2015). Ten novel species are introduced in this study, which were collected from *Salix* viz. *C. curvata*, *C. donetzica*, *C. erumpens*, *C. longiostiolata*, *C. rusanovii*, *C. salicacearum*, *C. salicina*, *C. salicina*, *C. salicina*, *C. salicina* (MFLUCC 16-0628), but the former species differs in having shorter ostiolar necks (*C. curvata*: 140–160 μm versus 400–500 μm: *C. longiostiolata*) and larger conidia (*C. curvata*: 5.9 × 1.3 μm versus 5.5 × 1.3 μm: *C. longiostiolata*).

Phylogenetic analysis of combined ITS, LSU, RPB2 and ACT sequence data indicate that *Cytospora curvata* (MFLUCC 15-0865) forms a separate branch as a sister taxon to *C. rusanovii* (MFLUCC 15-0853, MFLUCC 15-0854) (Figs. 1 and 2). *Cytospora rusanovii* is reported in this study, also from *Salix*. *Cytospora rusanovii* differs from *C. curvata* in having conidiomata with 4–6 locules, long ostioles (410–450 μ m), branched conidiophores and conidia that are shorter and wider (5.4 × 1.4 μ m) than *C. curvata*.

In ITS, *Cytospora rusanovii* differs from *C. curvata* at six polymorphisms, in ACT, they are different at six polymorphisms. Thus, based on phylogenetic analysis, polymorphic nucleotide comparisons and morphological differences, *C. melnikii* it is considered as a novel species.

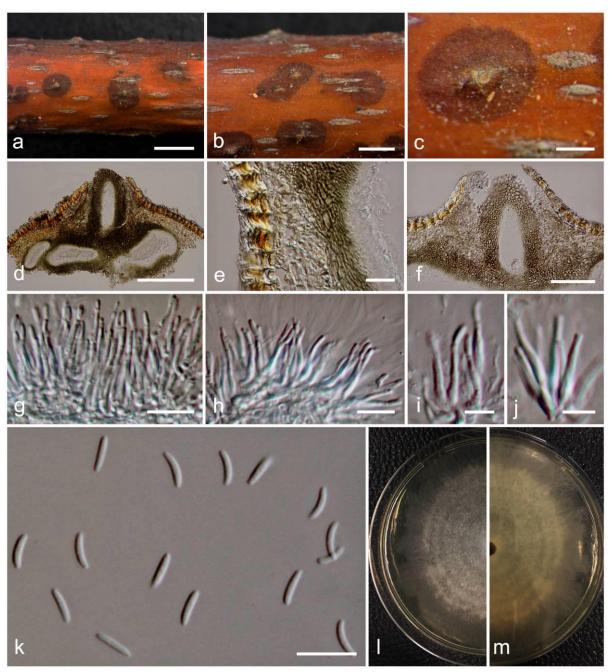


Fig. 3 – Cytospora ampulliformis on Sorbus intermedia (MFLU 15-2187, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–j Conidiogenous cells with attached conidia. k Mature conidia. l, m Colonies on MEA (l-from above, m-from below). Scale bars: a = 2000 μm , b = 1000 μm , c= 500 μm , d, f = 200 μm , e = 50 μm , f = 200 μm , g, h, k = 10 μm , i, j = 5 μm .

Cytospora donetzica Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, *sp. nov.* Index Fungorum number: IF552603, Facesoffungi Number: FoF 02738

Etymology: The specific epithet "donetzica" refers to biogeographical region of Donets ridge (Donets highland) and Seversky Donets river basin, where the type specimens were collected.

Holotype: MFLU 15-2093

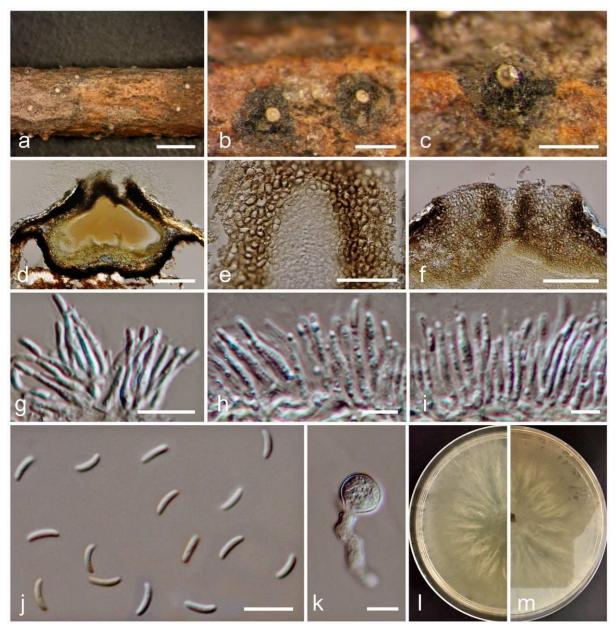


Fig. 4 – *Cytospora curvata* on *Salix alba* (MFLU 15-2229, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostioles. g—i Conidiogenous cells with attached conidia. j Mature conidia. k Germinating spore. l, m Colonies on MEA (l-from above, m-from below). Scale bars: $a=2000~\mu m$, $b, c=500~\mu m$, $d=200~\mu m$, $e=50~\mu m$, $f=100~\mu m$, g, j, $k=10~\mu m$, h, $i=5~\mu m$.

Associated with twigs and branches of *Crataegus monogyna* Jacq., *Pyrus pyraster* (L.) Burgsd., *Rosa* sp. and *Salix alba*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* $800-1200 \times 480-560 \, \mu m$ diameter, semi-immersed in host tissue, scattered, with 3–4 locules, with ostiolate. *Ostioles* $150-250 \, \mu m$ diameter, at the same level as the disc surface. *Peridium* comprising a few to several layers of cells of *textura angularis*, with inner

most layer thin, pale brown, outer later brown. *Conidiophores* branched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* (4.6–)5.4–6.4 ×1.2–1.4(–1.6) μ m ($\overline{x} = 5.5 \times 1.4 \mu$ m, n = 30), unicellular, elongate-allantoid, slightly curved, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 7 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, with aerial mycelium.

Material examined – RUSSIA, Rostov Region, Shakhty City, Grushevka steppe slopes near Grushevsky pond, ravine shrubbery, on dead and dying branches of *Rosa* sp. (*Rosaceae*), 14 May 2015, T. Bulgakov, T-389 (MFLU 15-2093, holotype, KUN, isotype), ex-type living culture, MFLUCC 16-0574, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, stony steppe, on dead and dying branches of *Crataegus monogyna* (*Rosaceae*), 28 June 2015, T. Bulgakov, T-523 (MFLU 15-2227, KUN), living culture, MFLUCC 15-0864, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, riparian forestry, on dead and dying branches of *Salix alba* (*Salicaceae*), 18 June 2015, T. Bulgakov, T-343 (MFLU 15-2047, KUN), living culture, MFLUCC 16-0589, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, ravine forest, on dying twigs and branches of *Pyrus pyraster* (*Rosaceae*), 27 October 2015, T. Bulgakov, T-1102 (MFLU 15-3764, KUN), living culture, MFLUCC 16-0641, KUMCC.

Notes – We observed four isolates of *Cytospora donetzica* (MFLUCC 16-0574, MFLUCC 15-0864, MFLUCC 16-0589, MFLUCC 16-0641) which clustered on a relative independent branch with high bootstrap support (100% MP/ 100% ML/ 1.00 PP, Fig. 2). The new species is introduced with the type from *Rosa* sp. *Cytospora donetzica* is most similar to *C. ceratosperma* (Tode) G.C. Adams & Rossman in its conidia size (5.5 × 1.4 μm versus 5–6 × 1.4 μm) (Saccardo 1884). However, based on combined gene phylogenetic analysis, *C. donetzica* is clearly separated from *C. ceratosperma* and sister to *C. sorbicola* with high bootstrap support (86% MP/76% ML/0.98 PP) (Fig. 2). In the polymorphic nucleotides of ITS, RPB2 and ACT sequence data, *C. donetzica* differs from *C. sorbicola* with five ITS polymorphisms, 23 RPB2 polymorphisms and seven ACT polymorphisms. Thus, *C. donetzica* is considered as a novel species.

Cytospora erumpens Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552604, Facesoffungi Number: FoF 02739 Fig. 6 Etymology: The specific epithet '*erumpens*' refers to the conidiomata characteristic as erumpent.

Holotype: MFLU 15-2165

Associated with twigs and branches of $Salix \times fragilis$ L. [S. alba L. \times S. euxina I.V. Belyaeva]. Sexual morph: Undetermined. Asexual morph: Conidiomata 720–1000 \times 470–550 μ m diameter, semi-immersed in host tissue, solitary, erumpent, with 1–3 locules, with ostiolar neck. Ostioles 280–350 μ m, at the same level as the disc surface. Peridium comprising a few to several layers of cells of textura angularis, with inner layer thin, pale brown, outer later brown to dark brown. Conidiophores unbranched or occasionally branched at the base, reduced to conidiogenous cells. Conidiogenous cells blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. Conidia (5.6–)6.4–6.7 \times 1.3–1.4(–1.7) μ m (\overline{x} = 6.4 \times 1.5 μ m, n = 30), unicellular, elongate-allantoid, hyaline, thin-walled, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 8.5 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Shakhty City, Grushevka steppe slopes, near Grushevsky pond, osier-bed near pond, on dead and dying branches of *Salix* × *fragilis* L. [*S. alba* L. × *S. euxina* I.V. Belyaeva]. (*Salicaceae*), 14 May 2015, T. Bulgakov, T-461 (MFLU 15-2165, holotype, PDD isotype), ex-type living culture, MFLUCC 16-0580, KUMCC.

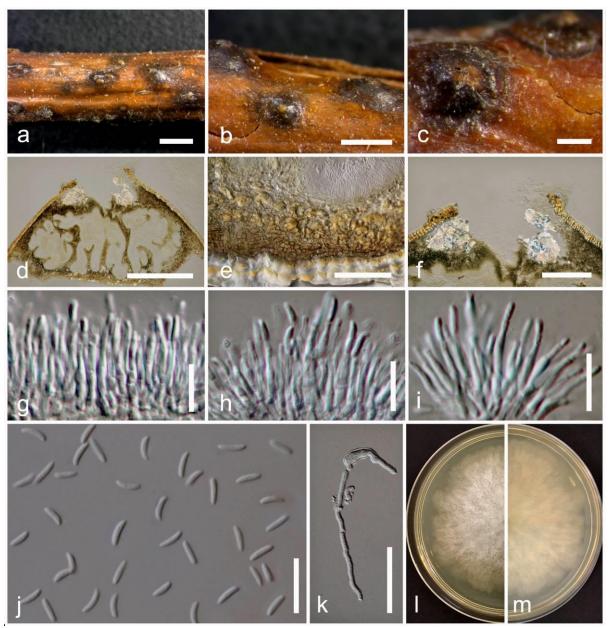


Fig. 5 – *Cytospora donetzica* on *Rosa* sp. (MFLU 15-2093, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostioles. g–i Conidiogenous cells with attached conidia. j Mature conidia. k Germinating spore. l, m Colonies on MEA (l-from above, m-from below). Scale bars: a = 2000 μm , b = 1000 μm , c, d = 500 μm , f = 200 μm , e, k = 50 μm , g–j = 10 μm .

Notes – Morphologically, *Cytospora erumpens* is most similar to *C. sorbi* (MFLUCC 16-0631) with conidia $6.4 \times 1.5 \, \mu m$ versus $6.5 \times 1.5 \, \mu m$. However, in the combined gene phylogenetic analysis, *C. erumpens* is clearly separated from *C. sorbi* (Fig. 2). *Cytospora*

erumpens and *C. rusanovii* are similar in morphology and also associated with the same host, *Salix* spp. However, *C. erumpens* differs from *C. rusanovii* in forming black-discoid conidiomata on the host and longer ostiolar necks (*C. erumpens*: 280–350 μm versus 155–170 μm: *C. rusanovii*).

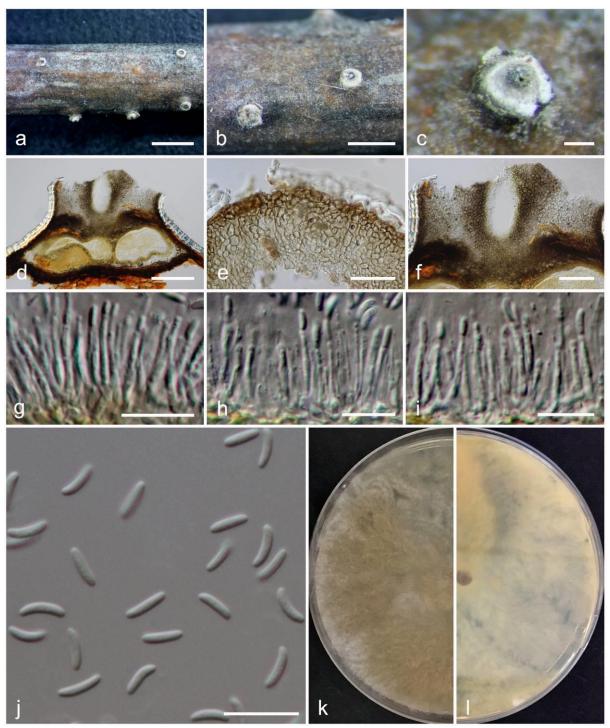


Fig. 6 – *Cytospora erumpens* on *Salix* × *fragilis* L. (MFLU 15-2165, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–i Conidiogenous cells with attached conidia. j Mature conidia. k, 1 Colonies on MEA (k-from above, 1-from below). Scale bars: $a = 2000 \,\mu m$, $b = 1000 \,\mu m$, c, $d = 200 \,\mu m$, $e = 50 \,\mu m$, $f = 100 \,\mu m$, $g-j = 10 \,\mu m$.

In phylogenetic study based on the ITS gene show that *Cytospora erumpens* is close to *C. donetzica*, but in the combined gene phylogenetic analysis, *C. erumpens* is separated from *C. donetzica* and some species in the genus (*C. donetzica*, *C. sorbicola*, *C. sorbi*, *C. gigaspora*, *C. nivea*, *C. paratranslucens*, *C. translucens*) with Bayesian posterior probabilities of 0.98 (Fig. 2). The ITS, RPB2 and ACT polymorphic nucleotides of sequence data, *C. erumpens* differs from *C. sorbi* with four ITS polymorphisms, 43 RPB2 polymorphisms and 13 ACT polymorphisms. Thus, it is considered that *C. erumpens* is a novel species.

Cytospora longiostiolata Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552605, Facesoffungi Number: FoF 02740 Fig. 7 Etymology: The specific epithet 'longiostiolata' refers to the long ostiolate of conidiomata.

Holotype: MFLU 15-3784

Associated with twigs and branches of $Salix \times fragilis$ L. [S. alba L. \times S. euxina I.V. Belyaeva]. Sexual morph: Undetermined. Asexual morph: Conidiomata 880–1000 \times 480–600 μ m diameter, semi-immersed in host tissue, solitary, erumpent, discoid, circular to ovoid, unilocular, with long ostiolar neck with ratio of conidiomata (3:4). Ostioles 400–500 μ m diameter, at the same level as the disc surface. Peridium comprising several layers of cells of textura angularis, with inner most layer thin, pale brown, outer layer brown. Conidiophores unbranched or occasionally branched at the base, reduced to conidiogenous cells. Conidiogenous cells blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. Conidia (3.9–)5.4–6.6 \times 1.0–1.2(–1.5) μ m ($\overline{x} = 5.5 \times$ 1.3 μ m, n = 30), unicellular, allantoid to subcylindrical, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 9 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, with aerial mycelium.

Material examined – RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, spinney ravine, on dying twigs and branches of $Salix \times fragilis$ L. [S. alba L. \times S. euxina I.V. Belyaeva] (Salicaceae), 27 October 2015, T. Bulgakov, T-1122 (MFLU 15-3784, holotype, KUN, isotype), ex-type living culture, MFLUCC 16-0628, KUMCC.

Notes – *Cytospora longiostiolata* is introduced as new species base on morphological characters and phylogenetic analyses. It has unilocular conidiomata with long ostioles. Phylogenetic analysis using ITS sequence data showed that *C. longiostiolata* is closely related to *C. salicina* (MFLUCC 15-0862, MFLUCC 16-0637) and *C. chrysosperma* species (Fig. 1). However, *C. salicina* (4.8 × 1.1 μ m) and *C. chrysosperma* (4.6 × 1.2 μ m) differ from *C. longiostiolata* (5.5 × 1.3 μ m) in having multiloculate conidiomata with smaller conidia. In the phylogenetic analyses based on combined ITS, LSU, RPB2 and ACT sequence data, *C. longiostiolata* forms a single lineage, separate from these taxa with high bootstrap support (93% ML/ 1.00 PP) (Fig. 2).

Cytospora longiostiolata was collected from Salix and is most similar to C. curvata and the differences are discussed under the species C. curvata. The species also resembles C. donetzica in conidia size (C. longiostiolata: 5.5×1.3 versus 5.5×1.4 µm: C. donetzica). However, C. donetzica differs C. longiostiolata in having 3–4 locules conidiomata and shorter ostiolar necks (150–250 µm).

In ITS, *Cytospora longiostiolata* differs from *C. chrysosperma* with two polymorphisms, with *C. salicina* in five polymorphisms. In RPB2 it differs with 21 polymorphisms from *C. chrysosperma*, 19 polymorphisms from *C. salicina*. In ACT it differed in 11 polymorphisms from *C. chrysosperma* and 11 polymorphisms from *C. salicina*.

Cytospora melnikii Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552606, Facesoffungi Number: FoF 02741

Etymology: The species named after the famous Russian mycologist Vadim Alexandrovich Melnik, researcher of anamorphic fungi.

Holotype: MFLU 15-1910

Associated with twigs and branches of *Malus domestica* Borkh. and *Populus nigra* L. var. *italica* Münchh. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 470–520 \times 370–420 μ m diameter, semi-immersed in host tissue, solitary, scattered, erumpent, discoid, circular to ovoid, unilocular, with long ostiolar neck. *Ostioles* 200–230 μ m long at the same

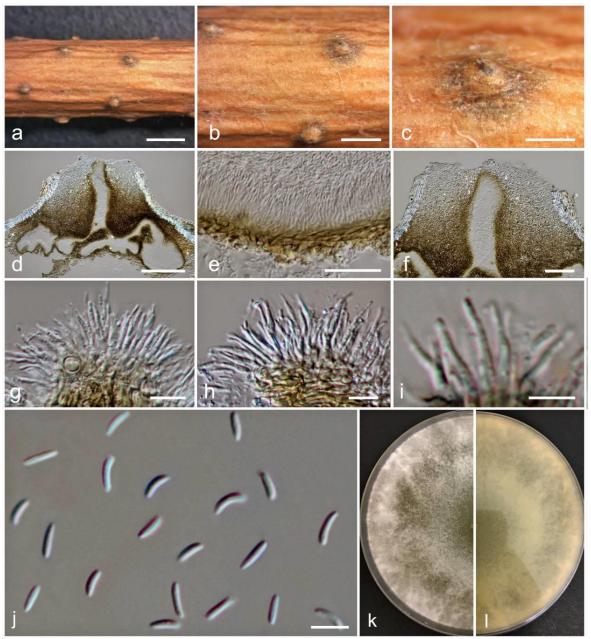


Fig. 7 – Cytospora longiostiolata on Salix × fragilis (MFLU 15-3784, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–i Conidiogenous cells with attached conidia. j Mature conidia. k, 1 Colonies on MEA (k-from above, 1-from below). Scale bars: $a = 2000 \, \mu m$, $b = 1000 \, \mu m$, $c = 500 \, \mu m$, $d = 200 \, \mu m$, $e = 20 \, \mu m$, $f = 100 \, \mu m$, g, h = $10 \, \mu m$, i, j = $5 \, \mu m$.

Fig. 8

level as the disc surface. *Peridium* comprising several layers of cells of *textura angularis*, with inner most layer thick, dark brown, outer layer dark brown to black. *Conidiophores* unbranched or occasionally branched at the base, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* $(3.1–)4.5–5 \times 1–1.2(-1.3)$ µm ($\overline{x} = 4.6 \times 1.2$ µm, n = 30), unicellular, allantoid to subcylindrical, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching the edge of the Petri-dish after 6-7 days at 25 °C, producing dense mycelium, circular, margin rough, white, with aerial mycelium.

Material examined – RUSSIA, Rostov Region, Oktyabrsky District, south edge of Persianovsky settlement, Khoruli gully, field-protecting shelterbelt, on dying branches of *Malus domestica* (*Rosaceae*), 28 April 2015, T. Bulgakov, T-206 (MFLU 15-1910, holotype, KUN, isotype), ex-type living culture, MFLUCC 15-0851, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, lining-out nursery, trees and shrubs, on dying twigs and branches of *Populus nigra* L. var. *italica* (*Salicaceae*), 27 October 2015, T. Bulgakov, T-1104 (MFLU 15-3766, KUN), living culture, MFLUCC 16-0635, KUMCC.

Notes – Cytospora melnikii was found on Malus domestica and Populus nigra, and has semi-immersed, unilocular conidiomata, with long ostioles and shares common walls with the host tissue. Cytospora melnikii can be distinguished based on the characteristics of fruiting bodies, conidia size, cultural characteristics and phylogenetic analyses. Cytospora melnikii is most similar to C. chrysosperma, which was found Malus. However, C. chrysosperma differs in having a multiloculate conidiomata (Mehrabi et al. 2011).

In phylogenetic analyses, using ITS sequence data (Fig. 1), *C. melnikii* groups with *C. chrysosperma* (HMBF151, HMBF158, HMBF17) and *C. salicacearum* (MFLUCC 16-0509, MFLUCC 16-0587, MFLUCC 15-0861, MFLUCC 16-0576). The tree using ITS, LSU, RPB2 and ACT sequence data demonstrate that *C. melnikii* is clearly separated from these taxa, with moderate bootstrap support (Fig. 2). Thus, based on phylogenetic analysis and morphological differences, it is considered that *C. melnikii* is a novel species.

Cytospora nivea (Hoffm.) Sacc., Michelia 2: 264 (1881)

For other possible synonyms see Index Fungorum

Facesoffungi Number: FoF 02742

Fig. 9

Associated with twigs and branches of *Salix acutifolia* Willd. Sexual morph: Undetermined. Asexual morph: *Conidiomata* $1000-1300 \times 340-380 \, \mu m$ diameter, semi-immersed in host tissue, scattered, erumpent, discoid, circular, with 3–4 locules, ostiolate. *Ostioles* $100-150 \, \mu m$ diameter, at the same level, with flattened top. *Peridium* comprising a few to several layers of cells of *textura angularis*, inner layer thick, brown, outer later dark brown. *Conidiophores* unbranched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* $(7.1-)7.4-8.8 \times 1.5-1.6(-1.9) \, \mu m$ ($\overline{x} = 7.5 \times 1.8 \, \mu m$, n = 30), unicellular, allantoid, slightly curved ends, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 2 cm diameter, after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Ust'-Donestsky District, near Nizhnekundryuchenskaya Village, arenal sandy forest, on dead and dying branches of *Salix acutifolia* (*Salicaceae*), 17 August 2015, T. Bulgakov, T-506 (MFLU 15-2210, PDD), living culture, MFLUCC 15-0860, KUMCC.

Notes – Fan et al. (2015b) have reported and illustrated *Cytospora nivea* (CFCC 89642). The morphology of our fresh collection resembles *C. nivea* (epitype) in Fan et al. (2015b) in having multiloculate conidiomata, allantoid, slightly curved, hyaline and 7.6×1.9

μm conidia and in the combined multi-gene phylogeny (Figs. 1 and 2). This is the first report of *C. nivea* from *Salix acutifolia* in Russia therefore, details are provided for this species to facilitate identification.

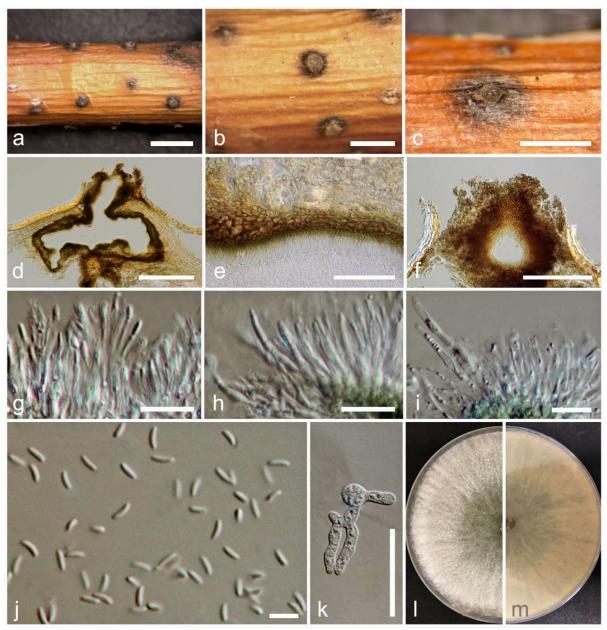


Fig. 8 – Cytospora melnikii on Malus domestica (MFLU 15-1910, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–i Conidiogenous cells with attached conidia. j Conidia. k Germinating spore. l, m Colonies on MEA with 7 days (l-from above, m-from below). Scale bars: a =2000 μ m, b = 500 μ m, c = 500 μ m, d = 200 μ m, e = 30 μ m, f = 100 μ m, g, h, i, j = 10 μ m.

Cytospora parakantschavelii Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov. Index Fungorum number: IF552607, Facesoffungi Number: FoF 02743 Fig. 10 Etymology: The specific epithet is composed from Greek prefix 'para-' meaning 'near' or 'beside' and 'kantschavelii' is from the species name, in reference to the occurrence on Cytospora species.

Holotype: MFLU 15-2094

Associated with twigs and branches of *Populus* × *sibirica* G.V. Krylov & G.V. Grig. ex A.K. Skvortsov [*P. balsamifera* L. × *P. nigra* L.] and *Pyrus pyraster* (L.) Burgsd.

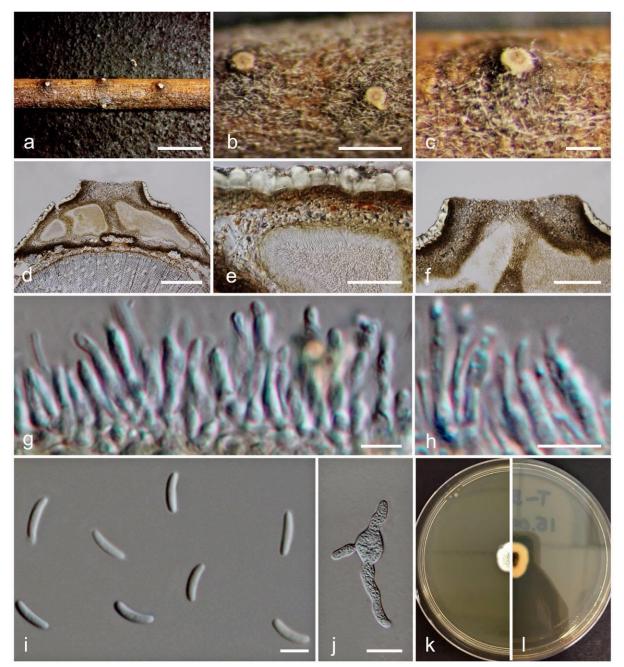


Fig. 9 – *Cytospora nivea* on *Salix acutifolia* (MFLU 15-2210). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing perithecia. e Peridium. f Ostioles. g–h Conidiogenous cells with intact conidia. i Conidia. j Germinating spore. k, 1 Colonies on MEA (k-from above, 1-from below). Scale bars: $a = 2000 \, \mu m$, $b = 500 \, \mu m$, c, $d = 200 \, \mu m$, e, $f = 50 \, \mu m$, $f = 20 \, \mu m$, $f = 50 \, \mu m$.

Sexual morph: Undetermined. Asexual morph: Conidiomata $750-900 \times 750-850 \, \mu m$ diameter, semi-immersed in host tissue, solitary, scattered, erumpent, with 3–6 locules, with ostiolar neck. Ostioles $540-680 \, \mu m$ diameter, at the same level as the disc surface. Peridium comprising several layers of cells of textura angularis, with inner most layer thin, hyaline to brown, outer layer brown to dark brown. Conidiophores branched, reduced to conidiogenous

cells. Conidiogenous cells blastic, enteroblastic, phialidic, hyaline, smooth-walled. Conidia (4.5–)5.3–5.7 \times 1.1–1.3(–1.6) μ m (\overline{x} = 5.3 \times 1.4 μ m, n = 30), unicellular, elongate-allantoid, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 8 cm diameter after 7 days at 25 °C, producing dense mycelium, margin rough, white, lacking aerial mycelium.

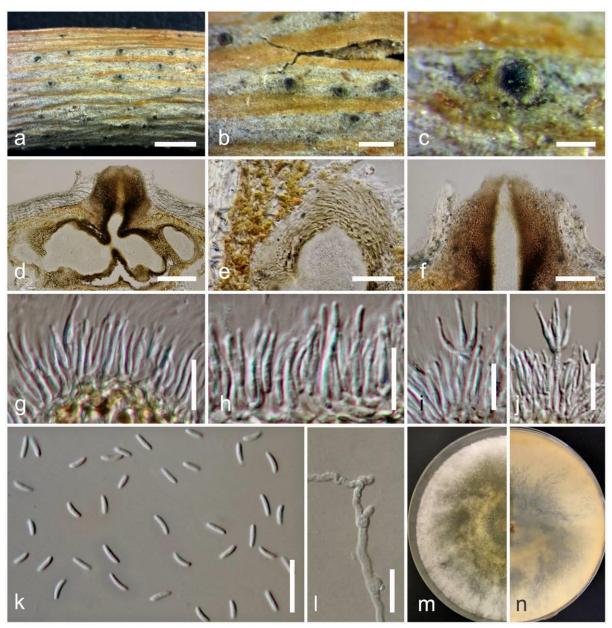


Fig. 10 – Cytospora parakantschavelii on Pyrus pyraster (L.) Burgsd. (MFLU 15-2094, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–j Conidiogenous cells with attached conidia. k Mature conidia. l Germinating spore. m, n Colonies on MEA (m-from above, n-from below). Scale bars: $a=2000~\mu m$, $b=500~\mu m$, c, d = $200~\mu m$, $f=100~\mu m$, $e=50~\mu m$, $l=20~\mu m$, $g-k=10~\mu m$.

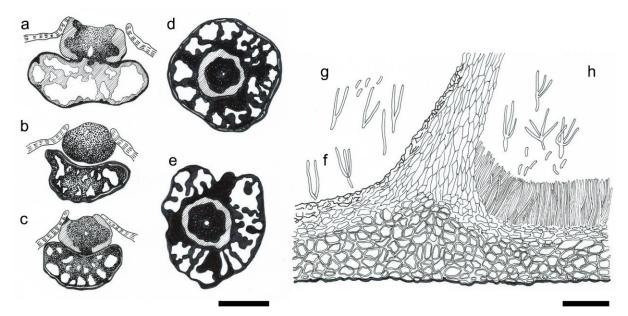


Fig. 11 – *Cytospora kantschavelii* on *Populus nigra* L. (Redrawn from Gvritishvili 1973), URSS (LE) holotype). a–c Stromata showing conidiomata. d, e Transverse sections through conidiomata. f, g Conidiophores and Conidiogenous cells with attached conidia. h Stroma showing peridium and conidiophores. Scale bars: a, b, c, d, e = $500 \, \mu m$, g, h = $20 \, \mu m$.

Material examined – RUSSIA, Rostov Region, Shakhty City, steppe slopes near Grushevsky pond, ravine shrubbery, on dead and dying branches of *Pyrus pyraster* (L.) Burgsd. [= *P. communis* auct.] (*Rosaceae*), 14 May 2015, T. Bulgakov (MFLU 15-2094, holotype, PDD isotype), ex-type living culture, MFLUCC 16-0575, KUMCC; RUSSIA, Rostov Region, Shakhty City, block landpark, on dying branches of *Populus* × *sibirica* G.V. Krylov & G.V. Grig. ex A.K. Skvortsov [*P. balsamifera L.* × *P. nigra* L.] (*Salicaceae*), 12 May 2015, T. Bulgakov (MFLU 15-1953, PDD), living culture, MFLUCC 15-0857, KUMCC.

Notes – The new species, *Cytospora parakantschavelii* (MFLUCC 16-0575, MFLUCC 15-0857) is similar to *C. kantschavelii* (287-2) in having multiloculate conidiomata. However, conidial dimensions of *C. parakantschavelii* (5.3 \times 1.4 μ m) are longer and wider than those of *C. kantschavelii* (4–5 \times 1.2 μ m) (Fig. 11) (Gvritishvili 1973).

Phylogenetic analysis of four combined gene loci place *Cytospora parakantschavelii* (MFLUCC 16-0575, MFLUCC 15-0857) on a separate branch from *C. kantschavelii*, forming as a sister taxon to *C. salicicola* Norphanphoun, Bulgakov & Hyde (MFLUCC 14-1052, MFLUCC 15-0866) (Fig. 2). However, *C. salicicola* differs from *C. parakantschavelii* in having shorter ostioles (170–200 μ m), unbranched conidiophores and larger conidia (6.8 \times 1.6 μ m).

Cytospora parasitica Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, in Ariyawansa et al., Fungal Diversity: 75(1): 146 (2015)

Facesoffungi Number: FoF 02744

Fig. 12

Associated with twigs and branches of *Malus domestica* Borkh. Sexual morph: Undetermined. Asexual morph: *Conidiomata* $1700-2000 \times 900-1200$ µm diameter, semi-immersed in host tissue, scattered, erumpent, multi-loculate, with ostiolar neck. *Ostioles* 320-400 µm diameter, at same level as the disc surface. *Peridium* comprising a few to several layers of cells of *textura angularis*, with most layer thin, brown to dark brown. *Conidiophores* unbranched or occasionally branched at the base, reduced to conidiogenous

cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* (5.5–)6.9–7.3 \times 1.5–1.7(–2) μ m (\overline{x} = 6.8 \times 1.8 μ m, n = 30), unicellular, elongate-allantoid, hyaline, smooth-walled.

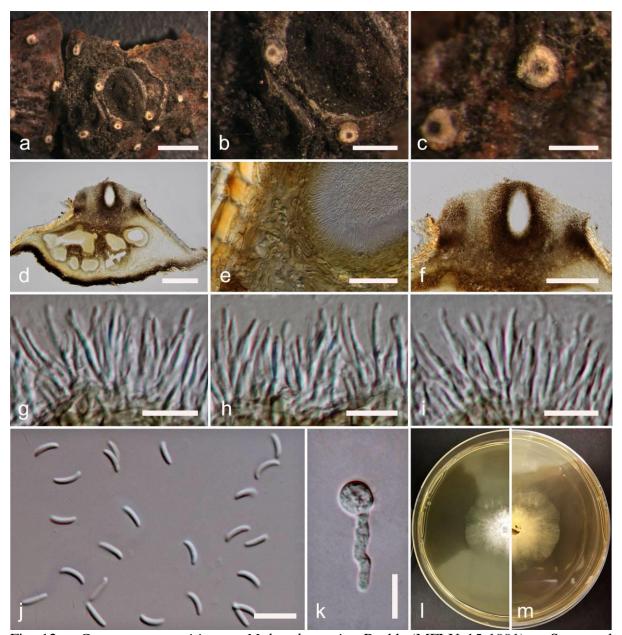


Fig. 12 – Cytospora parasitica on Malus domestica Borkh (MFLU 15-1991). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–i Conidiogenous cells with attached conidia. j Mature conidia. k Germinating spore. l, m Colonies on MEA (l-from above, m-from below). Scale bars: a = 2000 μ m, b = 1000 μ m, c= 500 μ m, d = 300 μ m, e = 30 μ m, f = 200 μ m, g, h, i, j, k = 10 μ m.

Culture characteristics – Colonies on MEA, reaching 4 cm diameter, after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Shakhty City, Grushevka steppe slopes near Grushevsky pond, ravine shrubbery, on dying branches (necrotrophic) of *Malus*

domestica (Rosaceae), 14 May 2015, T. Bulgakov (MFLU 15-1991, PDD), living culture, MFLUCC 16-0507, KUMCC.

Notes – *Cytospora parasitica* Norphanphoun, Bulgakov & K.D. Hyde was introduced by Ariyawansa et al. (2015) from *Malus domestica* in Russia (Ariyawansa et al. 2015). The morphology of this collection (MFLUCC 16-0507) is similar to *C. parasitica* (MFLUCC 14-1055) in having multi-loculate conidiomata and allantoid to slightly curved, unicellular, hyaline, $7 \times 1.75 \mu m$ conidia (Ariyawansa et al. 2015). In phylogenetic analyses, *C. parasitica* (MFLUCC 16-0507) groups with the ex-type strain (MFLUCC 14-1055) of *C. parasitica* (Fig. 2).

Cytospora paratranslucens Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552608, Facesoffungi Number: FoF 02745 Fig. 13

Etymology: The specific epithet is composed from Greek prefix 'para-' meaning 'near' or 'beside' and Latin 'translucens' is from the species name, in reference to the occurrence on Cytospora.

Holotype: MFLU 15-1986

Associated with twigs and branches of *Populus alba* L. Sexual morph: Undetermined. Asexual morph: *Conidiomata* $450-550 \times 270-350 \, \mu m$ diameter, semi-immersed in host tissue, scattered, erumpent, breaking through the outer branch, multiloculate, with ostiolar neck. *Ostioles* 70–150 μm diameter, at same level as the disc surface. *Peridium* comprising a few to several layers of cells of *textura angularis*, with inner most layer thin, red-brown, outer later dark brown to black. *Conidiophores* unbranched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* $(5.5-)6.5-7.3 \times 1.3-1.5(-1.8) \, \mu m$ ($\overline{x} = 6.8 \times 1.6 \, \mu m$, n = 30), unicellular, allantoid, slightly curved, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 8.5 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, with aerial mycelium.

Material examined – RUSSIA, Rostov Region, Shakhty City, Grushevka steppe slopes near Grushevsky pond, riparian grove, on dead and dying branches of *Populus alba*, 14 May 2015, T. Bulgakov, T-282 (MFLU 15-1986, holotype, PDD, isotype), ex-type living culture, MFLUCC 16-0506, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, lining-out nursery, trees and shrubs, dying twigs and branches on *Populus alba* L. var. *bolleana* (Lauche) Otto, 27 October 2015, T. Bulgakov, T-1016 (MFLU 15-3678, PDD), living culture, MFLUCC 16-0627, KUMCC.

Notes – *Cytospora* species associated with *Populus* have been reported worldwide (Table 3; Adams et al. 2002, 2005, 2006, Défago 1942, Fotouhifar et al. 2010, Singh et al. 2007). In this study, five novel species are introduced, which were collected from *Populus* viz. *C. melnikii*, *C. parakantschavelii*, *C. paratranslucens*, *C. rusanovii* and *C. salicacearum*. *Cytospora paratranslucens* is most similar to *C. germanica* and *C. nivea* (Adams et al. 2006). However, they can be distinguished by conidia size $(6.8 \times 1.6 \text{ versus } 5 \times 1, 6-7.5 \times 1.2 \text{ } \mu\text{m}$ respectively) (Adams et al. 2006). Furthermore, *C. paratranslucens* has multiloculate conidiomata sharing a single ostiole, while *C. germanica* and *C. nivea* have multiloculate conidiomata with individual or compressed ostioles in the conidiostromata.

In the ITS phylogenetic analyses, *Cytospora paratranslucens* groups with *C. translucens* Sacc. (138-2 and 35) with high bootstrap support (94% ML/ 1.00 PP). ITS sequence data is only available for *C. translucens* in GenBank. Therefore, we compare morphology of *C. paratranslucens* and *C. translucens*, from the original description in Saccardo (1884). *Cytospora paratranslucens* can be distinguished from *C. translucens* in having multiloculate conidiomata, while in *C. translucens* they are uniloculate. The conidia

of *C. paratranslucens* are larger than those of *C. translucens* ($6.8 \times 1.6 \mu m$ versus $4.6 \times 1.2 \mu m$).

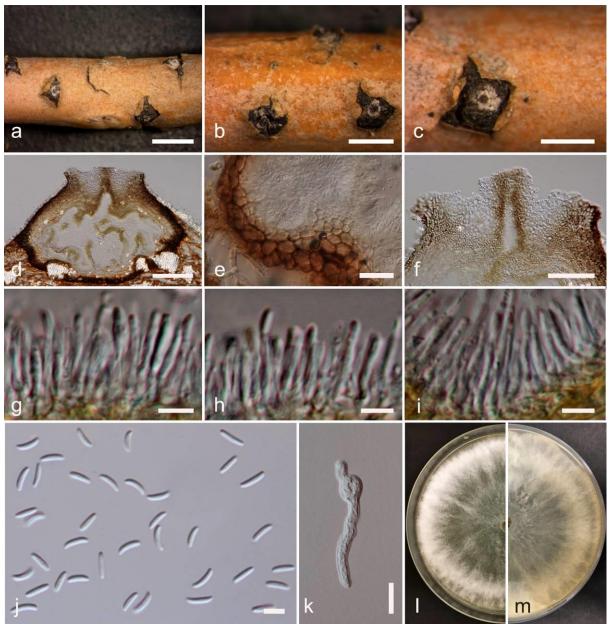


Fig. 13 – Cytospora paratranslucens on Populus alba (MFLU 15-1986, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–i Conidiogenous cells with attached conidia. j Mature conidia. k Germinating spore. l, m Colonies on MEA (I-from above, m-from below). Scale bars: $a = 2000 \mu m$, $b = 1000 \mu m$, $c = 1000 \mu m$, $d = 200 \mu m$, $e = 20 \mu m$, $e = 20 \mu m$, $e = 20 \mu m$, $e = 100 \mu m$.

Cytospora rusanovii Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552609, Facesoffungi Number: FoF 02746 Fig. 14 Etymology: The specific named after the Russian mycologist Victor Andreyevich Rusanov, researcher of plant pathogenic fungi in Rostov region of Russia.

Holotype: MFLU 15-1931

Associated with twigs and branches of *Salix babylonica* L. and *Populus* \times *sibirica* G.V. Krylov & G.V. Grig. ex A.K. Skvortsov [*P. balsamifera* L. \times *P. nigra* L.]. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 530–800 \times 280–350 μ m diameter, semi-immersed in host tissue, solitary, scattered, erumpent, discoid, circular to ovoid, with 1–3 locules, and long ostiolar neck. *Ostioles* 155–170 μ m diameter, at the same level as the disc surface. *Peridium* comprising several layers of cells of *textura angularis*, with inner most layer thick, dark brown, outer layer dark brown to black. *Conidiophores* unbranched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* (6–)6.5–7.1 \times 1.4–1.5(–1.6) μ m ($\overline{x} = 6.7 \times 1.5 \mu$ m, n = 30), unicellular, elongate-allantoid, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 8 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Shakhty City, Central Park, parkland, on dying branches of *Salix babylonica* (*Salicaceae*), 8 July 2015, T. Bulgakov (MFLU 15-1931, holotype, KUN, isotype), ex-type living culture, MFLUCC 15-0854, KUMCC; RUSSIA, Rostov Region, Shakhty City, Central Park, parkland, on dying branches of *Populus* × *sibirica* G.V. Krylov & G.V. Grig. ex A.K. Skvortsov [*P. balsamifera* L. × *P. nigra* L.] (*Salicaceae*), 9 July 2015, T. Bulgakov (MFLU 15-1929, KUN), living culture, MFLUCC 15-0853, KUMCC.

Notes – The new species has semi-immersed, 1–3-loculate conidiomata, with long ostioles and $6.7 \times 1.5 \, \mu m$, unicellular conidia. *Cytospora rusanovii* is most similar to *C. microspora* Rabenh. in conidia size $(6-7 \times 1-1.3)$ (Saccardo 1884). However, *C. microspora* differs from *C. rusanovii* in having multi-loculate conidiomata (>10 locules) (Corda 1839).

Phylogenetic analyses of ITS and multi-gene sequence data (Figs. 1 and 2) indicate that *Cytospora rusanovii* can be distinguished from *C. curvata* (MFLUCC 15-0865) and other species in *Cytospora*, with 98% bootstrap support and 1.00 Bayesian posterior probability in MP, ML and BI analyses (Fig. 2), while the polymorphic nucleotides differences are discussed under *C. curvata*.

Cytospora salicicola Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, in Li et al., Fungal Diversity 78: 10.1007/s13225-016-0366-9, [78] (2016)

Facesoffungi Number: FoF 02747

Fig. 15

Associated with twigs and branches of *Salix alba* L. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 750–1200 \times 300–450 μ m diameter, semi-immersed in host tissue, solitary, scattered, visible as raised areas, with 3–4 locules and long ostiolar neck. *Ostioles* 170–200 μ m diameter, at the base, same level as the host surface, black to brown, surrounded by a light coloured ostiolar disk. *Peridium* comprising several layers of cells of *textura angularis*, with inner most layer thin, hyaline to brown, outer layer brown to dark brown. *Conidiophores* unbranched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* (6.2–)6.9–7.6 \times 1.4–1.5(–1.7) μ m (\bar{x} = 6.8 \times 1.6 μ m, n = 30), unicellular, allantoid to subcylindrical, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 9 cm diameter, after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, Gremuchaya Balka (Thunderous gully), shore of pond, on dead and dying branches of *Salix alba* (*Salicaceae*), 28 June 2015, T. Bulgakov, T-527 (MFLU 15-2231, KUN), living culture, MFLUCC 15-0866, KUMCC.

Notes – *Cytospora salicicola* was introduced by Li et al. (2016) from *Salix alba* in Russia (Hyde et al. 2016, Li et al. 2016). In the phylogenetic analyses, our strain MFLUCC

15-0866 groups with the type material of *C. salicicola* (MFLUCC 14-1052) with high bootstrap support from all analyses (99% MP/ 83% ML/ 0.99 PP) (Fig. 2).

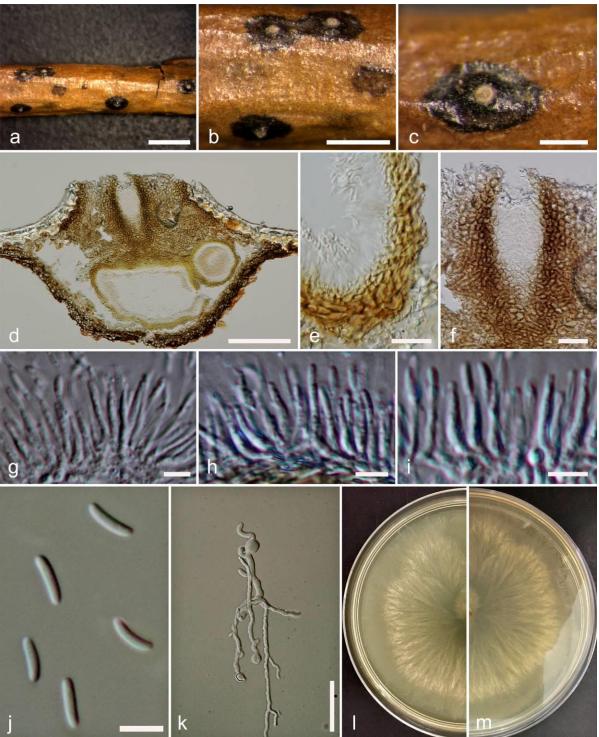


Fig. 14 – *Cytospora rusanovii* on *Salix babylonica* (MFLU 15-1931, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostioles. g–i Conidiogenous cells with attached conidia. j Mature conidia. k Germinating spore. l, m Colonies on MEA (l-from above, m-from below). Scale bars: $a = 2000 \, \mu m$, $b = 1000 \, \mu m$, $c = 500 \, \mu m$, $d = 100 \, \mu m$, $e = 10 \, \mu m$

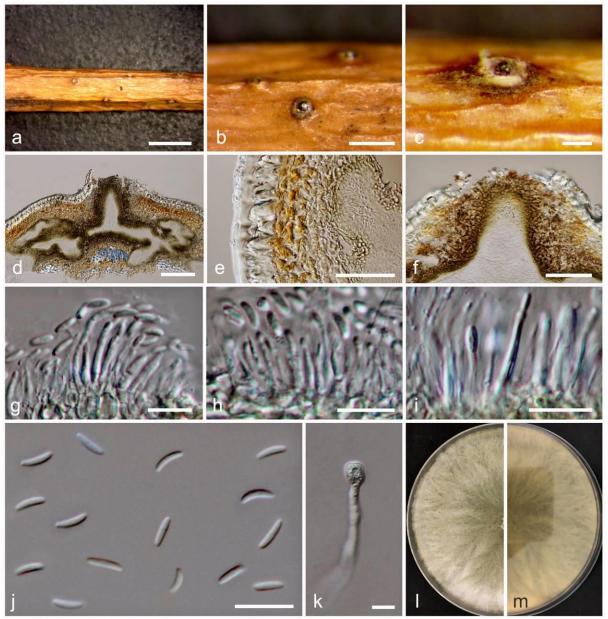


Fig. 15 – Cytospora salicicola on Salix alba (MFLU 15-2231). a Stromatal habit in wood. b Fruiting bodies on host surface. c Surface of fruiting bodies showing the black Ostioles. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–i Conidiogenous cells with intact conidia. j Mature conidia. k Germinating conidia. l, m Colonies on MEA (l-from above, m-from below). Scale bars: $a = 2000 \mu m$, $b = 500 \mu m$, $c = 200 \mu m$, $d = 100 \mu m$, $e = 50 \mu m$, $f = 100 \mu m$, $g-k = 10 \mu m$.

Cytospora salicacearum Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552610, Facesoffungi Number: FoF 02748 Fig. 16 Etymology: The specific epithet *salicacearum* refers to host plant family *Salicaceae*, on which the fungus was first collected.

Holotype: MFLU 15-2061

Associated with twigs and branches of *Salix alba* L., *Salix* \times *fragilis* L. [*S. alba* L. \times *S. euxina* I.V. Belyaeva], *Populus nigra* L. var. *italica* Münchh. and *Prunus cerasus* L. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 570–750 \times 320–380 μ m diameter, semi-immersed in host tissue, solitary, scattered, erumpent, discoid, circular to ovoid, with 1–2 locules, ostiolate. *Ostioles* 160–200 μ m diameter, at the same level as the

disc surface. *Peridium* comprising a few to several layers of cells of *textura angularis*, with inner most layer thin, dark brown, outer layer dark brown to black. *Conidiophores* unbranched or occasionally branched at the base, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* $(3.6-)4.9-6.4\times0.9-1(-1.1)$ µm ($\overline{x}=5\times1$ µm, n=30), unicellular, allantoid to subcylindrical, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 1.5 cm diameter after 7 days at 25 °C, producing rhizoid mycelium, undulate, margin rough, white, lacking aerial mycelium.

Material examined — RUSSIA, Rostov Region, Shakhty City, steppe slopes near Grushevsky pond, osier-bed near pond, on dead and dying branches of *Salix alba* (*Salicaceae*), 14 May 2015, T. Bulgakov, T-357 (MFLU 15-2061, holotype, KUN, isotype), ex-type living culture, MFLUCC 16-0509, KUMCC; RUSSIA, Rostov Region, Shakhty City, steppe slopes near Grushevsky pond, riparian grove, dead and dying branches on *Populus nigra* L. var. *italica* Münchh (*Salicaceae*), 14 May 2015, T. Bulgakov, T-393 (MFLU 15-2097, KUN), living culture, MFLUCC 16-0576, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, old orchard, dead and dying branches on *Prunus cerasus* L., 18 June 2015, T. Bulgakov, T-503 (MFLU 15-2207, KUN), living culture, MFLUCC 16-0587, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, riparian forest, on dead and dying branches of *Salix* × *rubens* Schrank [*S. alba* L. × *S. fragilis* L.] (*Salicaceae*), 18 June 2015, T. Bulgakov, T-509 (MFLU 15-2213, KUN), living culture, MFLUCC 15-0861, KUMCC.

Notes – *Cytospora salicacearum* has 1–2 loculate conidiomata with a single ostioles and shares common walls. In the phylogenetic analyses based on combined ITS, LSU, RPB2 and ACT sequence data, *C. salicacearum* is a close relative to *C. melnikii* (MFLUCC 15-0851, MFLUCC 16-0635) and *C. chrysosperma* (HMBF17, HMBF151, HMBF158, HMBF159) (Fig. 2). However, they are different in morphological characteristics, *C. melnikii* and *C. chrysosperma* differ from *C. salicacearum* in having shorter and wider conidia (4.6 × $1.2 \mu m$, $4.6 \times 1.2 \mu m$, $5 \times 1 \mu m$, respectively).

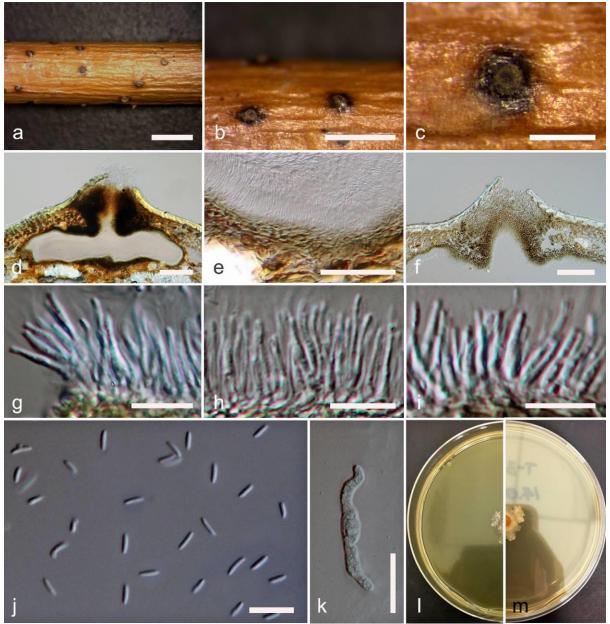
Cytospora salicacearum differs from C. melnikii and C. chrysosperma in the polymorphic nucleotides of the RPB2 and ACT sequence data. In RPB2 it differed with 35 polymorphisms from C. chrysosperma, four polymorphisms from C. melnikii. In ACT it differed with eight polymorphisms from C. chrysosperma and differs from C. melnikii with four polymorphisms.

Cytospora salicina Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552611, Facesoffungi Number: FoF 02749 Fig. 17 Etymology: The specific epithet 'salicina' refers to the host plant genus Salix, on which the type specimens were collected.

Holotype: MFLU 15-2212

Associated with twigs and branches of *Salix* alba L. and *Salix* × *rubens* Schrank [*S. alba* L. × *S. fragilis* L.]. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 1200–1500 × 550–600 μ m diameter, semi-immersed in host tissue, solitary, scattered, erumpent, discoid, circular to ovoid, with 2–5 locules, and ostiolar neck. *Ostioles* 300–350 μ m. at the same level as the disc surface. *Peridium* comprising several layers of cells of *textura angularis*, with inner most layer thick, dark brown, outer layer dark brown to black. *Conidiophores* branched or occasionally branched at the base, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, hyaline, smooth-walled. *Conidia* (3.6–)4.2–4.7 × 1–1.1(–1.3) μ m (\bar{x} = 4.3 × 1 μ m, n = 30), unicellular, allantoid to subcylindrical, hyaline, smooth-walled.



Culture characteristics – Colonies on MEA, reaching 9 cm diameter, after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined — RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, riparian forest, on dead and dying branches of *Salix alba* L. (*Salicaceae*), 18 June 2015, T. Bulgakov, T-508 (MFLU 15-2212, holotype, KUN, isotype), ex-type living culture, MFLUCC 15-0862, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, ravine spinney, on dying twigs and branches of *Salix* × *rubens* Schrank [*S. alba* L. × *S. fragilis* L.] (*Salicaceae*), 27 October 2015, T. Bulgakov, T-1017 (MFLU 15-3679, KUN), living culture, MFLUCC 16-0637, KUMCC.

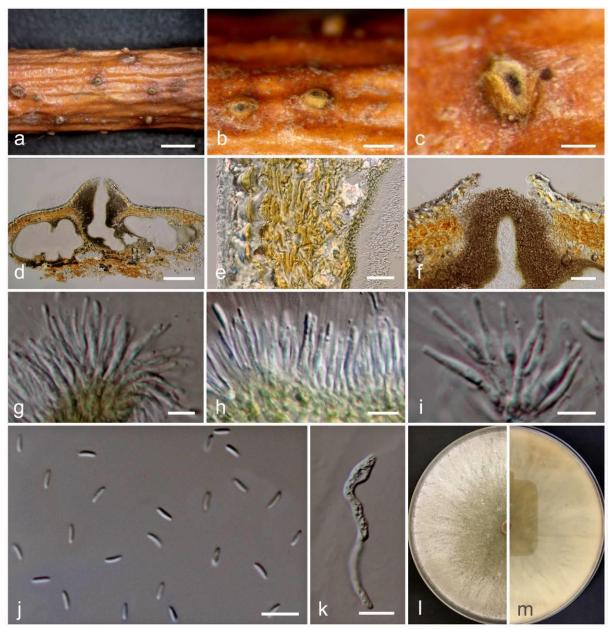


Fig. 17 – Cytospora salicina on Salix alba (MFLU 15-2212, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostioles. g–i Conidiogenous cells with attached conidia. j Mature conidia. k Germinating spore. l, m Colonies on MEA (l-from above, m-from below). Scale bars: a = 2000 μm , b = 500 μm , c, d = 200 μm , e = 20 μm , f = 50 μm , j, k = 10 μm , g, h, i = 5 μm .

Notes – *Cytospora salicina* (MFLUCC 15-0862, MFLUCC 16-0637) was found on *Salix* spp. causing canker disease. *Cytospora salicina* is most similar to *C. chrysosperma* in having multi-locules, but differs in having smaller conidia (4.6 × 1.2 μm) and a peridium comprising several layers. Phylogenetic analysis based of combined ITS, LSU, RPB2 and ACT sequence data indicates that *C. salicina* can be distinguished from *C. chrysosperma* (HMBF17, HMBF151, HMBF158, HMBF159), *C. melnikii* (MFLUCC 15-0851, MFLUCC 16-0635) and *C. salicacearum* (MFLUCC 16-0509, MFLUCC 16-0587, MFLUCC 15-0861, MFLUCC 16-0576) (Fig. 2).

In ITS, *Cytospora salicina* differs from *C. chrysosperma* with 28 polymorphisms, three polymorphisms from *C. melnikii* and a single polymorphism from *C. salicacearum*. In RPB2 it differs in 32 polymorphisms from *C. chrysosperma*, 30 polymorphisms from *C. melnikii* and 29 polymorphisms from *C. salicacearum*. In ACT it differs with ten polymorphism from *C. melnikii* and ten polymorphisms from *C. salicacearum*.

Cytospora sorbi Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552612, Facesoffungi Number: FoF 02750 Fig. 18 Etymology: The specific epithet "sorbi" refers to the host plant genus Sorbus, on which the type specimen was first collected.

Holotype: MFLU 15-3781

Associated with twigs and branches of *Sorbus aucuparia* L. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 900–1200 \times 420–550 μm diameter, semi-immersed in host tissue, scattered, erumpent, with 3–4 locules, and ostiolar neck. *Ostioles* 250–300 μm diameter, at the same level as the disc surface. *Peridium* comprising a few to several layers of cells of *textura angularis*, with inner most layer thick, brown, outer dark brown. *Conidiophores* unbranched or occasionally branched at the base, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* (6–)6.4–7.6 \times 1.4–1.5(–1.8) μm (\overline{x} = 6.5 \times 1.5 μm , n = 30), unicellular, elongate-allantoid, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 5 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, dying twigs and branches on *Sorbus aucuparia (Rosaceae)*, 27 October 2015, T. Bulgakov, T-1119 (MFLU 15-3781, holotype, KUN, isotype), ex-type living culture, MFLUCC 16-0631, KUMCC.

Notes – *Cytospora sorbi* was collected from *Sorbus aucuparia*. In phylogenetic analysis (Figs. 1 and 2) *C. sorbi* forms a separate branch as a sister taxon to *C. sorbicola* and *C. donetzica* (Fig. 2). *Cytospora sorbicola* and *C. donetzica* differs from *C. sorbi* in having smaller conidia $(5.6 \times 1.5 \, \mu m, 5.5 \times 1.4 \, \mu m, 6.5 \times 1.5 \, \mu m, respectively).$

In ITS, *Cytospora sorbi* differs from *C. sorbicola* in eleven polymorphisms and eight polymorphisms from *C. donetzica*. In RPB2 it differs from *C. sorbicola* with 20 polymorphisms and 28 polymorphisms from *C. donetzica*. In ACT, it differs from *C. sorbicola* with nine polymorphisms and eight polymorphisms from *C. donetzica*.

Cytospora sorbicola Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, *sp. nov.* Fig. 19 Index Fungorum number: IF552613, Facesoffungi Number: FoF 02751

Etymology: The specific epithet "sorbicola" refers to the host plant genus Sorbus, on which the type specimens were collected.

Holotype: MFLU 15-2203

Associated with twigs and branches of *Acer pseudoplatanus* L. and *Cotoneaster melanocarpus* (Bunge) Loudon, *Prunus cerasus* L., *Sorbus aucuparia* L. and × *Sorbaronia mitschurinii* (Skvortsov & Maitul.) Sennikov. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 850–1200 × 480–600 µm diameter, semi-immersed in bark, solitary, erumpent, with 1–2 locules, ostiolate. *Ostioles* 250–350 µm diameter, at the same level as the disc surface. *Peridium* comprising several layers of cells of *textura angularis*, with inner most layer thick, pale brown, outer later dark brown. *Conidiophores* unbranched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, formed from the

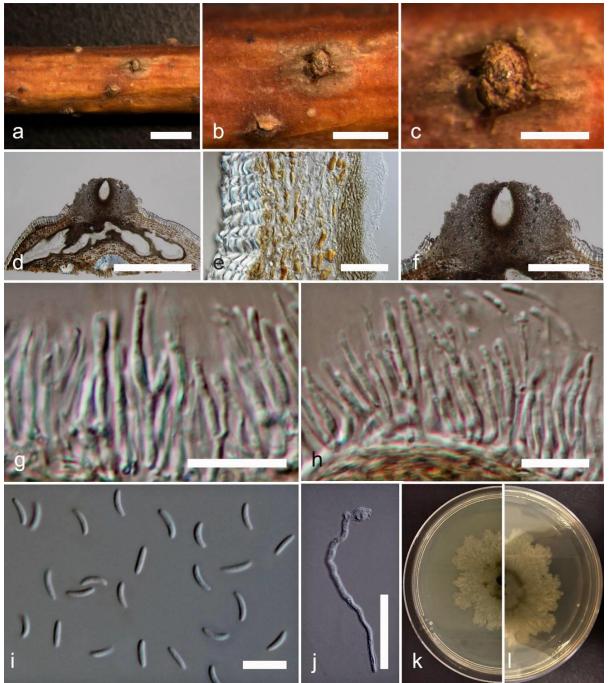


Fig. 18 – *Cytospora sorbi* on *Sorbus aucuparia* L. (MFLU 15-3781, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–h Conidiogenous cells with attached conidia. i Mature conidia. j Germinating spore. k, 1 Colonies on MEA (k-from above, 1-from below). Scale bars: $a = 2000 \, \mu m$, $b = 1000 \, \mu m$, $c = 500 \, \mu m$, $d = 500 \, \mu m$, $e = 500 \, \mu m$, $e = 1000 \, \mu m$

inner most layer of pycnidial wall, hyaline, smooth-walled. *Conidia* (4.4–)5.6–6.1 \times 1.3–1.5(–1.6) μ m ($\overline{x}=5.6\times1.5$ μ m, n = 30), unicellular, elongate-allantoid, slightly curved, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 8 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined - RUSSIA, Rostov Region, Krasnosulinsky District, Donskove forestry, artificial forest, dead and dying branches on Acer pseudoplatanus (Sapindaceae), 18 June 2015, T. Bulgakov, T-499 (MFLU 15-2203, holotype, KUN, isotype), ex-type living culture, MFLUCC 16-0584, KUMCC; RUSSIA, Rostov Region, Rostov-on-Don City, Botanical Garden of Southern Federal University, parkland, on dead and dying branches on Sorbus aucuparia L. (Rosaceae), 30 May 2015, T. Bulgakov, T-472 (MFLU 15-2176, KUN), living culture, MFLUCC 16-0581, KUMCC; RUSSIA, Rostov Region, Rostov-on-Don City, Botanical Garden of Southern Federal University, Systematic Arboretum, on dead and dying branches on Cotoneaster melanocarpus (Bunge) Loudon (Rosaceae), 30 May 2015, T. Bulgakov, T-476 (MFLU 15-2180, KUN), living culture, MFLUCC 16-0582, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, old orchard, on dead and dying branches on Sorbaronia mitschurinii (Skvortsov & Maitul.) Sennikov (Rosaceae), 18 June 2015, T. Bulgakov, T-501 (MFLU 15-2205, KUN), living culture, MFLUCC 16-0585, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, artificial forest platation, forest nursery, dead and dying branches on Prunus cerasus L. (Rosaceae), 18 June 2015, T. Bulgakov, T-502 (MFLU 15-2206, KUN), living culture, MFLUCC 16-0586, KUMCC; RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, Kabanya Balka (Boar gully), steppe on slope of gully, dying twigs and branches (necrotrophic) on Cotoneaster melanocarpus Lodd., G. Lodd. & W. Lodd., 27 October 2015, T. Bulgakov (MFLU 15-3768, KUN), living culture, MFLUCC 16-0633, KUMCC.

Notes – Six collections of *Cytospora sorbicola* (MFLUCC 16-0581, MFLUCC 16-0582, MFLUCC 16-0584, MFLUCC 16-0585, MFLUCC 16-0586, MFLUCC 16-0633) were made. *Cytospora sorbicola* is similar to *C. donetzica* and *C. ceratosperma* (Tode) G.C. Adams & Rossman in conidia size (5.6 × 1.5 μm, 5.5 × 1.4 μm, 5–6 × 1.4 μm, respectively) (Saccardo 1884), but *C. donetzica* differs in having multiloculate conidiomata and shorter ostiolar necks (150–250 μm). Phylogenetic analysis (Figs. 1 and 2) place *C. sorbicola* in a separate clade from *C. ceratosperma* which is sister to *C. donetzica* (MFLUCC 16-0574, MFLUCC 15-0864, MFLUCC 16-0589, MFLUCC 16-0641) with bootstrap support (86% MP/ 76% ML/ 0.98 PP). *Cytospora sorbicola* differs from *C. donetzica* with five ITS polymorphisms, 23 RPB2 polymorphisms and seven ACT polymorphisms.

Cytospora ulmi Norphanphoun, Bulgakov, T.C. Wen & K.D. Hyde, sp. nov.

Index Fungorum number: IF552614, Facesoffungi Number: FoF 02752 Fig. 20

Etymology: Name refers to the host genus *Ulmus*

Holotype: MFLU 15-1910

Associated with twigs and branches of *Ulmus minor*. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 980–1200 \times 500–600 μ m diameter, semi-immersed in host tissue, solitary, erumpent, scattered, discoid, circular to ovoid, with 4–6 locules, with long ostiolar neck. Ostioles 410–450 μ m, at the same level as the disc surface. *Peridium* comprising a few to several layers of cells of *textura angularis*, with inner most layer thin, pale brown. *Conidiophores* branched, reduced to conidiogenous cells. *Conidiogenous cells* blastic, enteroblastic, phialidic, hyaline, smooth-walled. *Conidia* (4.8–)5.4–6.4 \times 1.3–1.5(–1.6) μ m ($\overline{x} = 5.4 \times 1.4 \mu$ m, n = 30), unicellular, elongate-allantoid, hyaline, smooth-walled.

Culture characteristics – Colonies on MEA, reaching 6 cm diameter after 7 days at 25 °C, producing dense mycelium, circular, margin rough, white, lacking aerial mycelium.

Material examined – RUSSIA, Rostov Region, Krasnosulinsky District, Donskoye forestry, ravine forest, on dead and dying branches (necrotrophic/saprobic) of *Ulmus minor* Mill., 28 June 2015, T. Bulgakov, T-521 (MFLU 15-2225, holotype, KUN, isotype), ex-type living culture, MFLUCC 15-0863, KUMCC.

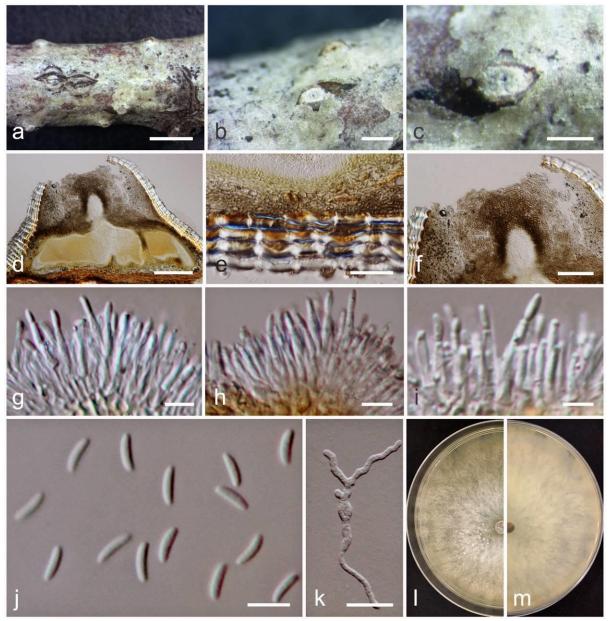


Fig. 19 – Cytospora sorbicola on Acer pseudoplatanus (MFLU 15-2203, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of stroma. d Vertical section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–i Conidiogenous cells with attached conidia. j Mature conidia. k Germinating spore. l, m Colonies on MEA (l-from above, m-from below). Scale bars: $a=2000~\mu m$, $b, c=500~\mu m$, $d=200~\mu m$, $e=50~\mu m$, $f=100~\mu m$, $g, h, i, j=5~\mu m$, $k=10~\mu m$.

Notes – Phylogenetic analysis of four combined gene loci indicate that *Cytospora ulmi* (MFLUCC 15-0863), forms a distinct branch as a sister taxon to *C. ribis* Ehrenb. (284-2) isolated from *Thuja orientalis* L., *C. rosarum* Grev. (218) isolated from *Rosa canina* L. and *C. tanaitica* Norphanphoun, Bulgakov & Hyde (MFLUCC 14-1057) isolated from *Betula pubescens* Ehrh. (Fig. 2) (Adams et al. 2002, Ariyawansa et al. 2015, Fotouhifar et al. 2010).

Cytospora ulmi is most similar to C. rosarum in having long conidia ((4.8–)5.4–6.4 μ m versus 4.5–6.7 μ m), but has narrower conidia (1.3–1.5(–1.6) μ m versus 0.9–1.1 μ m). Cytospora ribis and C. tanaitica are similar to C. ulmi in having multi-loculate conidiomata. However, C. ulmi (5.4 \times 1.4 μ m) has larger conidia than C. ribis (4 \times 1 μ m) and C. tanaitica (3.4 \times 0.7 μ m) (Adams et al. 2002, Fotouhifar et al. 2010, Ariyawansa et al. 2015).

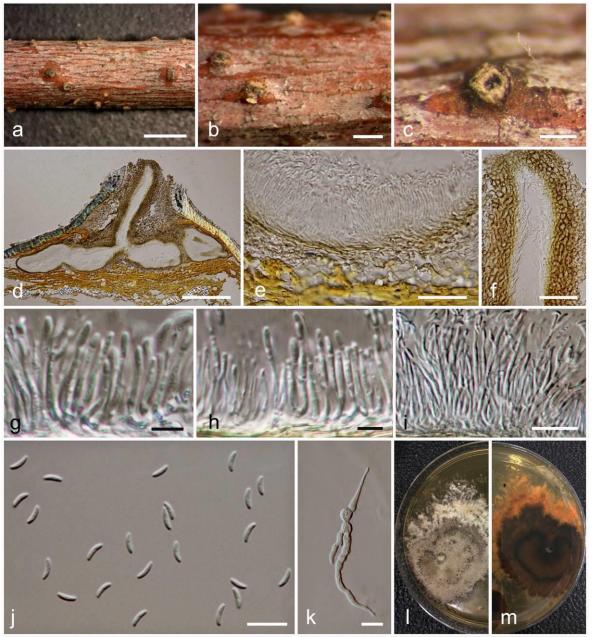


Fig. 20 – *Cytospora ulmi* (MFLU 15-2225, holotype). a Stromatal habit in wood. b Fruiting bodies on substrate. c Surface of fruiting bodies. d Cross section of the stroma showing conidiomata. e Peridium. f Ostiolar neck. g–i Conidiogenous cells with attached conidia. j Mature conidia. k Germinating spore. l, m Colonies on MEA (l-from above, m-from below). Scale bars: a = 2000 μ m, b= 500 μ m, c, d = 200 μ m, e, f = 50 μ m, g, h = 5 μ m, i, j = 10 μ m, k = 20 μ m.

Discussion

Cytospora species are important plant pathogens causing dieback and canker disease with a worldwide distribution and host range including Armeniaca, Eucalyptus, Fraxinus, Ligustrum, Malus, Mangifera, Platanus, Populus, Punus, Robinia, Salix and Ulmus to name a few (Adam et al. 2006, Fotouhifar et al. 2010, Ariyawansa et al. 2015, Fan et al. 2015a, 2015b, Liu et al. 2015, Li et al. 2016, Maharachchikumbura et al. 2015, 2016, Hyde et al. 2016). In this study, all samples were collected from European Russia from dying twigs and symptomatic branches.

A phylogenetic analysis was constructed using maximum parsimony, maximum likelihood and Bayesian inference for an individual ITS dataset and was able to resolve some species of *Cytospora* (Fig. 1). However, the combined analysis of ITS, LSU, RPB2 and ACT sequence was more appropriate for resolving *Cytospora* species (Fig. 2). It has repeatedly been shown that multi-gene dataset will resolve species in pathogenic (e.g. *Colletotrichum*, Cai et al. 2009, *Diaporthe*, Udayanga et al. 2011, 2015, *Pestalotiopsis*, Maharachchikumbura et al. 2011, 2012) and other genera (e.g. *Stachybotrys*, Lombard et al. 2016, *Nodulosphaeria*, Mapook et al. 2016, *Lasiodiplodia*, Doilom et al. 2017), better than single genes.

Species of *Cytospora* may be relatively host-specific (Zhou et al. 2001), such as *C. australiae* Speg., *C. eucalyptina* Speg., *C. eucalypticola* Van der Westh., and *C. agarwalii* Soni, Dadwal & Jamaluddin which have only been reported from *Eucalyptus* sp. (Adams et al. 2005). Previous reports have however, indicated that many *Cytospora* species have broad host ranges. However, those reports lacked identifications based on molecular data (Fries 1823, Deng 1963, Saccardo 1884, Tai 1979, Wei 1979). Some widespread species have been identified and verified by molecular data to occur in many host species, such as *C. chrysosperma* and *C. nivea* (Adams et al. 2005, Fan et al. 2014, Ariyawansa et al. 2015, Fan et al. 2015a, 2015b, Liu et al. 2015, Maharachchikumbura et al. 2015, 2016, Hyde et al. 2016, Li et al. 2016,). In Table 3 we summarize the species (identified with sequence data) and their host distribution.

Our study provides data on the distribution of 17 *Cytospora* species with a multi-gene phylogeny. Several hosts appear to support several *Cytospora* species and several *Cytospora* species are known from several hosts (Table 3). Some taxa on the other hand, are only known from a single host. Thus we regard the data we provide on *Cytospora* are preliminary and future studies are likely establish numerous new species and further resolve their distribution.

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Table 3 Occurrence of Cytospora species on hosts (species identifications confirmed with molecular data)

Host	Species record	Genes used	References	
Abies	Valsa abietis	ITS	Adams et al. (2005)	
	Valsa friesii	ITS	Défago (1942)	
Acacia	Cytospora sp. "Uncertain lineage"	ITS	Adams et al. (2006)	
Acer	Cytospora ampulliformis	ITS, LSU, RPB2, ACT	In this study	
	Cytospora rosarum	ITS, LSU, RPB2, ACT	Tibpromma et al. (personal comm)	
	Cytospora sorbicola	ITS, LSU, RPB2, ACT	In this study	
	Valsa ambiens subsp. ambiens	ITS	Spielman (1985)	
	Valsa ambiens subsp. leucostomoides	ITS	Spielman (1985)	
Amygdalus	Cytospora cincta	ITS	Fotouhifar et al. (2010)	
7.0	Cytospora leucosperma	ITS	Fotouhifar et al. (2010)	
Armeniaca	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)	
	Cytospora cincta	ITS	Fotouhifar et al. (2010)	
	Cytospora cinctum	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al.	
	· · · · · · · · · · · · · · · · · · ·		(2010), Singh et al. (2007)	
	Cytospora leucostoma	ITS	Fotouhifar et al. (2010)	
Berberis	Cytospora berberidis	ITS, LSU, RPB2, ACT	Liu et al. (2015)	
Betula	Cytospora tanaitica	ITS, LSU, ACT	Ariyawansa et al. (2015)	
Celtis	Cytospora translucens	ITS	Fotouhifar et al. (2010)	
Cerasus	Cytospora cincta	ITS	Fotouhifar et al. (2010)	
	Cytospora schulzeri	ITS	Fotouhifar et al. (2010)	
Ceratonia	Cytospora acacia African-Mediterranean lineage	ITS	Adams et al. (2006)	
Cercis	Valsa viridistroma comb. nov.	ITS	Wehmeyer (1936)	
Chamaecyparis	Valsa abietis sp. complex 1 (syn. Valsa weiriana)	ITS	Adams et al. (2002)	
Colutea	Cytospora schulzeri	ITS	Fotouhifar et al. (2010)	
Cotinus	Cytospora cotini	ITS, LSU, RPB2, ACT	Hyde et al. (2016)	
	Cytospora gelida	ITS, LSU, RPB2, ACT	Tibpromma et al. (personal comm)	
Cotoneaster	Cytospora sorbicola	ITS, LSU, RPB2, ACT	In this study	
Crataegus	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)	
	Cytospora cincta	ITS	Fotouhifar et al. (2010)	
	Cytospora donetzica	ITS, LSU, RPB2, ACT	In this study	
	Cytospora malicola	ITS	Fotouhifar et al. (2010)	
	Cytospora schulzeri	ITS	Fotouhifar et al. (2010)	
Cydonia	Cytospora cincta	ITS	Fotouhifar et al. (2010)	
C , ac ma	Cytospora cinctum	ITS	Fotouhifar et al. (2010)	
Elaeagnus	Cytospora elaeagni	ITS, LSU, RPB2, ACT	Fan et al. (2015b)	
2.0.00.51000	Cytospora viacagni Cytospora nivra	ITS, LSU, RPB2, ACT	Fan et al. (2015b)	
	Cytospora ribis	ITS	Fotouhifar et al. (2010)	
Eriobotrya	Cytospora riobotryae	ITS	Adams et al. (2005, 2006)	

Host	Species record	Genes used	References
Eucaliptus	Cytospora abyssinica	ITS	Adams et al. (2005)
	Cytospora aff. austromontana	ITS	Adams et al. (2005)
	Cytospora agarwalii	ITS	Adams et al. (2005)
	Cytospora australiae	ITS	Adams et al. (2005)
	Cytospora austromontana	ITS	Adams et al. (2005)
	Cytospora berkeleyi	ITS	Adams et al. (2005)
	Cytospora chrysosperma	ITS	Adams et al. (2005)
	Cytospora diatrypelloideae	ITS	Adams et al. (2005)
	Cytospora disciformis	ITS	Adams et al. (2005)
	Cytospora eucalypticola	ITS	Adams et al. (2005, 2006)
	Cytospora-like species	ITS	Adams et al. (2005)
	Cytospora nitschkii	ITS	Adams et al. (2005)
	Cytospora putative	ITS	Smith et al. (1996)
	Cytospora putative species 1	ITS	Adams et al. (2005)
	Cytospora valsoidea	ITS	Adams et al. (2005)
	Cytospora variostromatica	ITS	Adams et al. (2005)
	Valsa aff. cinereostroma (Cytospora austealia)	ITS	Crous et al. (1990)
	Valsa brevispora	ITS	Adams et al. (2005)
	Valsa cinereostroma	ITS	Adams et al. (2005)
	Valsa eucalypti	ITS	Adams et al. (2005)
	Valsa eugeniae	ITS	Adams et al. (2005)
	Valsa fabianae	ITS	Adams et al. (2005)
	Valsa luecostoma	ITS	Adams et al. (2002)
	Vala myrtagena	ITS	Adams et al. (2005)
	Valsa salicina	ITS	Adams et al. (2006)
Eugenia	Valsa eugeniae	ITS	Adams et al. (2005)
O	Valsa eugeniae	ITS	Sivanesan and Holliday (19701)
Fagus	Cytospora decorticans	ITS	Adams et al. (2005)
Ficus	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
Fraxinus	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
	Cytospora minuta	ITS	Adams et al. (2006)
	Cytospora pruinosa	ITS	Fotouhifar et al. (2010)
	Valsa cypri	ITS	Défago (1942)
Hippophae	Cytospora hippophaes	ITS, LSU, RPB2, ACT	Fan et al. (2015b)
Jacaranda	Valsa ceratosperma	ITS	Adams et al. (2006)
	Cytospora atrocirrhata	ITS, LSU, TUB2, ACT	Fan et al. (2015a)
Juglans	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
0	Cytospora chrysosperma	ITS, LSU, TUB2, ACT	Fan et al. (2015a)
	Cytospora cincta	ITS	Fotouhifar et al. (2010)
	Cytospora gigaspora	ITS, LSU, TUB2, ACT	Fan et al. (2015a)
	Cytospora leucostoma	ITS	Fotouhifar et al. (2010)

Host	Species record	Genes used	References
	Cytospora sacculus	ITS, LSU, TUB2, ACT	Fan et al. (2015a)
	Cytospora sordida	ITS	Fotouhifar et al. (2010)
Lepidium	Cytospora ribis	ITS	Fotouhifar et al. (2010)
Ligustrum	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
Malus	Cytospora ceratosperma	ITS	Adams et al. (2005)
	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010), Mehrabi et al. (2011)
	Cytospora cincta	ITS	Fotouhifar et al. (2010), Mehrabi et al. (2011)
	Cytospora cinctum	ITS	Fotouhifar et al. (2010)
	Cytospora lucostoma	ITS	Mehrabi et al. (2011)
	Cytospora malicola	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al. (2010), Singh et al. (2007)
	Cytospora melnikii	ITS, LSU, RPB2, ACT	In this study
	Cytospora parasitica	ITS, LSU, RPB2, ACT	In this study, Ariyawansa et al. (2015)
	Cytospora schulzeri	ITS	Fotouhifar et al. (2010), Mehrabi et al. (2011)
	Valsa cincta	ITS	Adams et al. (2002)
	Valsa mali	ITS	Adams et al. (2002)
	Valsa malicola	ITS	Adams et al. (2002)
	Valsa melostoma	ITS	Adams et al. (2002)
	Valsa nivea	ITS	Adams et al. (2006)
Mangifera	Valsa cinereostroma (Cytospora)	ITS	Adams et al. (2005)
Morus	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
	Cytospora cypsi	ITS	Fotouhifar et al. (2010)
	Cytospora pruinosa	ITS	Fotouhifar et al. (2010)
Olea	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
	Valsa cypri	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al. (2010), Singh et al. (2007)
Persica	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
	Cytospora leucostoma	ITS	Fotouhifar et al. (2010)
Picea	Cytospora mougeotii	ITS	Adams et al. (2005)
	Valsa kunzei	ITS	Adams et al. (2005)
	Valsa kunzei var. piceae	ITS	Proffer and Hart (1988)
Pistacia	Cytospora terebinthi	ITS	Fotouhifar et al. (2010)
Pinus	Valsa friesii	ITS	Adams et al. (2005)
	Valsa kunzei	ITS	Adams et al. (2006)
	Valsa pini	ITS	Adams et al. (2005), Défago (1942)
Platanus	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
	Cytospora gutnerae	ITS	Fotouhifar et al. (2010)
	Cytospora nivea	ITS	Fotouhifar et al. (2010)
	Cytospora ribis	ITS	Fotouhifar et al. (2010)
Populus	Cytospora atrocirrhata	ITS	Fotouhifar et al. (2010)
1	Cytospora chrysosperma	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al.

Host	Species record	Genes used	References
			(2010), Singh et al. (2007)
	Cytospora fugax	ITS	Fotouhifar et al. (2010)
	Cytospora germanica	ITS	Adams et al. (2006)
	Cytospora hariotii	ITS	Adams et al. (2006)
	Cytospora kantschavelii	ITS	Fotouhifar et al. (2010)
	Cytospora melnikii	ITS, LSU, RPB2, ACT	In this study
	Cytospora nivea	ITS	Adams et al. (2006)
	Cytospora parakantschavelii	ITS, LSU, RPB2, ACT	In this study
	Cytospora paratranslucens	ITS, LSU, RPB2, ACT	In this study
	Cytospora rusanovii	ITS, LSU, RPB2, ACT	In this study
	Cytospora salicacearum	ITS, LSU, RPB2, ACT	In this study
	Cytospora sordida	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al.
			(2010), Singh et al. (2007)
	Cytospora translucens	ITS	Fotouhifar et al. (2010)
	Cytospora variostromatica	ITS	Adams et al. (2006)
	Valsa nivea	ITS	Défago (1942), Fotouhifar et al. (2010)
Prunus (Sakura)	Cytospora salicacearum	ITS, LSU, RPB2, ACT	In this study
	Cytospora sorbicola	ITS, LSU, RPB2, ACT	In this study
	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
	Cytospora cincta	ITS	Fotouhifar et al. (2010)
	Cytospora cinctum	ITS	Fotouhifar et al. (2010)
	Cytospora persoonii	ITS	Fotouhifar et al. (2010)
	Cytospora leucostoma	ITS	Adams et al. (2002)
	Valsa cincta	ITS	Adams et al. (2002)
	Valsa leucostoma f. sp. insititiae	ITS	Défago (1942)
	Cytospora eutypelloides	ITS	Adams et al. (2006)
	Valsa parapersoonii comb. Nov.	ITS	Adams et al. (2006)
Pseudotsuga	Valsa abietis sp. complex 1 (syn. Valsa weiriana)	ITS	Adams et al. (2002)
Pyrus	Cytospora donetzica	ITS, LSU, RPB2, ACT	In this study
<i>y</i>	Cytospora parakantschavelii	ITS, LSU, RPB2, ACT	In this study
	Cytospora sacculus	ITS	Fotouhifar et al. (2010)
	Valsa ceratosperma	ITS	Fotouhifar et al. (2010)
Ouercus	Cytospora intermedia	ITS	Fotouhifar et al. (2010)
2	Cytospora sacculus	ITS	Adams et al. (2005)
	Valseutypella multicollis	ITS	Adams et al. (2006)
Rhododendron	Valsa subclypeata	ITS	Adams et al. (2005)
Rhus	Valsa ceratosperma s.lat.	ITS	Adams et al. (2005)
Ribes	Cytospora ribis	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al.
	y <u>1</u>	**	(2010), Singh et al. (2007)
Rosa	Cytospora cincta	ITS	Fotouhifar et al. (2010)
	Cytospora donetzica	ITS, LSU, RPB2, ACT	In this study

Host	Species record	Genes used	References
	Cytospora leucostoma	ITS	Fotouhifar et al. (2010)
	Cytospora rhodophila	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al.
			(2010), Singh et al. (2007)
	Cytospora rosarum	ITS	Fotouhifar et al. (2010)
Robinia	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
	Cytospora leucostoma	ITS	Fotouhifar et al. (2010)
Saccharum	Cytospora sacchari	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al
	, 1		(2010), Singh et al. (2007)
Salix	Cytospora atrocirrhata	ITS	Fotouhifar et al. (2010)
	Cytospora aurora	ITS	Fotouhifar et al. (2010)
	Cytospora curvata	ITS, LSU, RPB2, ACT	In this study
	Cytospora chrysosperma	ITS, LSU, RPB2, ACT	Fan et al. (2015b)
	Cytospora donetzica	ITS, LSU, RPB2, ACT	In this study
	Cytospora erumpens	ITS, LSU, RPB2, ACT	In this study
	Cytospora fugax	ITS	Fotouhifar et al. (2010)
	Cytospora gigaspora	ITS, LSU, RPB2, ACT	Fan et al. (2015b)
	Cytospora longiostiolata	ITS, LSU, RPB2, ACT	In this study
	Cytospora nivea	ITS, LSU, RPB2, ACT	In this study, Fan et al. (2015b)
	Cytospora populina	ITS, LSU, RPB2, ACT	Fan et al. (2015b)
	Cytospora rusanovii	ITS, LSU, RPB2, ACT	In this study
	Cytospora salicacearum	ITS, LSU, RPB2, ACT	In this study
	Cytospora salicina	ITS, LSU, RPB2, ACT	In this study
	Cytospora salicacearum	ITS, LSU, RPB2, ACT	In this study
	Cytospora salicicola	ITS, LSU, RPB2, ACT	In this study, Li et al. (2016)
	Cytospora salicina	ITS, LSU, RPB2, ACT	In this study
	Leucostoma translucens	ITS 1157, 1161	Fotouhifar et al. (2010)
	Valsa salicina	ITS	Defago (1942), Fotouhifar et al. (2010)
	Valsa sordida	ITS	Adams et al. (2006), Fotouhifar et al. (2010)
	Valsa translucens	ITS	Defago (1942)
Seguoia	Cytospora eucalypti	ITS	Adams et al. (2005)
Sibiraea	Cytospora sibiraeae	ITS, LSU, RPB2, ACT	Liu et al. (2015)
Sophora	Cytospora chrysosperma	ITS	Dar &Rai (2014)
sop.io. u	Cytospora sophorae	ITS	Dar &Rai (2014)
	Cytospora sophoricola	ITS	Dar &Rai (2014)
Sorbaronia	Cytospora sorbicola	ITS, LSU, RPB2, ACT	In this study
Sorbus	Cytospora ampulliformis	ITS, LSU, RPB2, ACT	In this study
~	Cytospora leucostoma	ITS	Adams et al. (2005)
	Cytospora sorbicola	ITS, LSU, RPB2, ACT	In this study
	Cytospora sorbi	ITS, LSU, RPB2, ACT	In this study
	Valsa massariana	ITS, ESO, RI B2, ACT	Adams et al. (2002)
Syringa	Valsa cypri	ITS	Défago (1942)

Host	Species record	Genes used	References
Tamarix	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
Taxus	Valsa ambiens	ITS	Adams et al. (2005), Défago (1942)
	Valsa ceratophora	ITS	Adams et al. (2005), Défago (1942)
Thuja	Cytospora chrysosperma	ITS	Fotouhifar et al. (2010)
	Cytospora ribis	ITS	Fotouhifar et al. (2010)
	Cytospora schulzeri	ITS	Fotouhifar et al. (2010)
Tibouchina	Valsa eugeniae (Cytospora state)	ITS	Adams et al. (2005)
	Valsa myrtagena	ITS	Fotouhifar et al. (2010)
Triticum	Cytospora tritici	ITS	Adams et al. (2006)
Ulmus	Cytospora carbonacea	ITS	Adams et al. (2002, 2005, 2006), Fotouhifar et al.
			(2010), Singh et al. (2007)
	Cytospora eutypelloides	ITS	Défago (1942)
	Cytospora subclypeata	ITS	Adams et al. (2005)
	Cytospora ulmi	ITS, LSU, RPB2, ACT	In this study
Vitis	Cytospora cincta	ITS	Fotouhifar et al. (2010)
	Cytospora leucostoma	ITS	Fotouhifar et al. (2010)

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