



Additions to the genus *Cytospora* with sexual morph in Cytosporaceae

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Abstract

Cytospora species are important pathogens, which have a worldwide distribution, broad host range and are commonly associated with tree dieback and canker disease. Lack of ex-type cultures or inability to link multi-gene DNA sequence data in GenBank to phylogenetic analyses makes it difficult to classify *Cytospora* taxa to species level. In this study, ten specimens of *Cytospora* were collected from dead wood in China, Italy and Thailand. Based on their morphology and phylogenetic characterization, four new species (*C. diopuiensis*, *C. galegicola*, *C. pingbianensis* and *C. pubescentis*) and four known species (*C. cedri*, *C. cotini*, *C. predappioensis* and *C. prunicola*) are herein described, illustrated and compared with related taxa. Detailed morphological descriptions of the holomorph (*C. galegicola*, *C. prunicola*, *C. predappioensis* and *C. pubescentis*), the sexual morph (*C. cedri*, *C. cotini*, *C. diopuiensis* and *C. pingbianensis*) and a new record of *C. predappioensis* in China are provided. Phylogenetic analyses of a combined ITS, LSU, ACT and RPB2 DNA sequence dataset support their placement in the genus *Cytospora* and justify the new species and identification of known species.

Key words – 4 new taxa – Diaporthales – Multi-gene – phylogeny – taxonomy

Introduction

Cytospora (Cytosporaceae, Diaporthales, Sordariomycetes, Ascomycota) was established by Ehrenberg (1818) with *Cytospora chrysosperma* (Pers.) Fr. as the type species. *Cytospora* species are important phytopathogens, causing dieback and canker disease on a broad range of plants worldwide (Adams et al. 2005, 2006, Lawrence et al. 2018, Fan et al. 2020). It leads to commercial losses, such as reduction of economically important fruits and nut crops on Juglandaceae spp., Punicaceae spp., Rosaceae spp. and Rhamnaceae spp. (Wang et al. 2011, Du et al. 2013, Fan et al. 2015a, b, 2020, Palavouzis et al. 2015, Lawrence et al. 2018, Pan et al. 2018, Zhu et al. 2018). Species of *Cytospora* also result in ecological damage, for example, the destructive canker diseases of the anti-desertification plants on Elaeagnaceae spp. and Salicaceae spp. (Fan et al. 2015b). Members of *Cytospora* are also recognized as endophytes and saprobes, and commonly connected

with a wide range of hosts worldwide (Christensen 1940, Spielman 1983, Bills 1996). There are 647 epithets of *Cytospora* listed in Index Fungorum (November, 2019) and 110 estimated species in Kirk et al. (2008). The *Cytospora* species are characterized by the single or labyrinthine, loculate stromata, filamentous conidiophores or asci, and allantoid hyaline conidia or ascospores (Spielman 1983, 1985, Adams et al. 2005, Fan et al. 2015a, b, Norphanphoun et al. 2017). Recent studies have been carried out and dealt with the taxonomy of *Cytospora* species based on multi-gene analysis of combined ITS, LSU, ACT, RPB2, TEF and TUB2 sequence data (Fan et al. 2014a, b, 2015a, b, 2020, Yang et al. 2015, Lawrence et al. 2017, 2018, Norphanphoun et al. 2017, 2018, Pan et al. 2018, Zhu et al. 2018).

The sexual morphs of *Cytospora* species are reported as *Leucocytospora*, *Leucostoma*, *Valsa*, *Valsella*, and *Valseutypella* (Fries 1823, Saccardo 1884, Kobayashi 1970, Barr 1978, Gvritishvili 1982, Spielman 1983, 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, Hyde et al. 2016, Li et al. 2016, Norphanphoun et al. 2017, 2018, Zhu et al. 2018). Combining the morphological studies from several decades with the phylogenetic results of DNA sequence data, Adams et al. (2005) revised the genus *Cytospora* and synonymized all the sexual genera under *Valsa*, either as subgenera or species without additional infrageneric rank (von Höhnelt 1906, 1914, 1917, 1919, 1928, von Petrak 1919, 1969, Défago 1942, Urban 1957, Hubbes 1960, Barr 1978, 1990, Vasilyeva 1988, 1994, Adams et al. 2005). Following the International Code of Nomenclature for Algae, Fungi, and Plants (McNeill et al. 2012), which permits the single-name for pleomorphic taxa, *Cytospora* (1818) was treated as the recommended name instead of *Valsa* (1849) as it is older and more widely used (Adams et al. 2005, Rossman et al. 2015).

The sexual morph of *Cytospora* is rarely found in nature (Saccardo 1884, 1986, Sydow et al. 1916, Adams et al. 2005, Norphanphoun et al. 2017). In the asexual morph however, formal descriptions for some species of *Cytospora* (e.g., *C. coenobitica* and *C. rhodophila*) only provided details on conidial sizes (Saccardo 1884). Moreover, there is only ITS rDNA sequence data available for most *Cytospora* species in GenBank, and this makes it difficult to classify *Cytospora*, especially to species level. Adams et al. (2005) suggested that extensive fresh collections, especially with neotypes or epitypes, should be made and their molecular data obtained to clarify taxonomy of *Cytospora*.

The aim of this study is to provide more evidence to justify and stabilize the taxonomic identification of *Cytospora* species. Ten *Cytospora* specimens with their sexual morphs were collected from dead branches in China, Italy and Thailand. All specimens were characterized by multi-loculate, conspicuous stromata, perithecial ascomata, 8-spored, unitunicate asci and hyaline, allantoid, aseptate ascospores. Based on the morphological comparison and phylogenetic analyses, four new species are introduced, four known species are identified and detailed descriptions and illustrations are provided. The holomorphs of *C. galegicola*, *C. prunicola*, *C. predappioensis* and *C. pubescentis* are provided from fresh specimens and pure cultures. In addition, *Cytospora predappioensis* is reported as a new record in China. A phylogenetic tree based on a combined ITS, LSU, ACT and RPB2 sequence analysis is provided.

Materials and Methods

Isolation and morphology

Dead wood samples were collected from Yunnan Province, China, Forlì-Cesena Province, Italy and Chiang Mai Province, Thailand. The samples were taken back to the laboratory in plastic ziplock bags and maintained at room temperature. The specimens were examined following the methods described in Dai et al. (2017). Macro-morphological characters were examined using a Motic SMZ-140 dissecting microscope and photographed using a Carl Zeiss GmbH (AxioCam ERc 5s) stereo microscope. A Canon EOS 600D camera connected to a Nikon Eclipse 80i compound microscope was used to examine and capture the fungal micro-morphology. Fungal structures were measured using the Tarosoft (R) Image Frame Work software (Version 0.9.7) (Liu et al. 2010).

Pure cultures were obtained by single spore isolation as described in Chomnunti et al. (2014) and deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Culture Collection (KUMCC). Herbarium materials were deposited in the herbarium of Mae Fah Luang University (MFLU) and duplicated in the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS). Faces of Fungi and Index Fungorum numbers are registered following the outline of Jayasiri et al. (2015) and Index Fungorum (2019), respectively. New taxa are established based on recommendations as outlined by Jeewon & Hyde (2016).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh fungal mycelium scraped from the margin of a colony grown on potato dextrose agar (PDA) for 1–4 weeks (which incubated at 16°C for Italy specimens, 25°C for specimens from China and 30°C for specimens from Thailand) by using a DNA extraction kit (Biospin Fungus Genomic DNA Extraction Kit, BioFlux®, China) following the protocols in the manufacturer's instructions.

The DNA amplification was performed through polymerase chain reaction (PCR), following details as below. The internal transcribed spacers (ITS), large subunit rDNA (LSU), α -actin (ACT) and RNA polymerase II largest subunit (RPB2) gene regions were amplified using the primer pairs ITS4 with ITS5 (White et al. 1990), LR0R with LR5 (Vilgalys & Hester 1990), ACT512F with ACT783R (Carbone & Kohn 1999) and RPB2-5F with fRPB2-7cR (Liu et al. 1999), respectively. The PCR thermal cycle profile for the ITS, LSU and ACT regions were; initially at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, elongation at 72°C for 90 seconds and a final extension at 72°C for 10 minutes. PCR profile for the RPB2 locus was as follows: initially at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute, elongation at 72°C for 90 seconds, and a final extension at 72°C for 10 minutes. Purification and sequencing of the PCR products with the same PCR primers mentioned above were carried out at Shanghai Sangon Biological Engineering Technology and Services Co., Shanghai, P.R. China.

Phylogenetic analyses

The sequences generated from this study were analyzed with related *Cytospora* taxa and other representative genera in Diaporthales which were derived from GenBank and recent publications (Table 1) (Lawrence et al. 2018, Norphanphoun et al. 2018, Pan et al. 2018, Zhu et al. 2018, NCBI 2019, Fan et al. 2020). The consensus sequences were initially aligned by MAFFT v. 7.310 (Katoh & Standley 2013) and further improved where necessary by using Bioedit v. 7.0.9.1 (Hall 1999). Individual DNA sequence data from ITS, LSU, ACT and RPB2 were initially analyzed separately for comparing the tree topologies. In addition, a concatenated dataset of the ITS, LSU, ACT and RPB2 gene region was also assembled and analyzed under different optimality criteria. Phylogenetic trees were generated from maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses.

An evolutionary model for BI was estimated independently for each locus using MrModeltest v. 2.3 (Nylander 2008). The best-fit model with GTR+I+G was selected for each locus under the Akaike Information Criterion (AIC). Bayesian Inference analysis was performed via the web portal CIPRES Science Gateway v. 3.3 (Miller et al. 2010) using MrBayes v. 3.2.6 on XSEDE. Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo Sampling (MCMC). Six simultaneous Markov Chains were run from random trees for 20,000,000 generations and trees were sampled every 100th generation with a total of 200,000 trees. The first 25% trees representing the burn-in phase were discarded and the remaining trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree (the standard deviation of split frequencies were reached to 0.01).

A maximum likelihood analysis was performed by Randomized Axelerated Maximum Likelihood (RAxML) using program raxmlGUI v. 1.3 (Silvestro & Michalak 2012). A general time reversible model (GTR) was applied with a discrete gamma distribution which was complemented

for each substitution model (Silvestro & Michalak 2012). The optimal ML tree search was conducted with 1000 rapid bootstrapping. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR+GAMMAI substitution model.

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

Taxon	Source *	Host	Origin	GenBank accession				Reference
				ITS	LSU	ACT	RPB2	
<i>Cytospora abyssinica</i>	CMW 10181 ^T	<i>Eucalyptus globulus</i>	Wondo Genet, Ethiopia	AY347353	-	-	-	Adams et al. (2005)
<i>C. acaciae</i>	CBS 468.69	<i>Ceratonia siliqua</i>	Spain, Mallorca	DQ243804	-	-	-	Adams et al. (2006)
<i>C. ailanthicola</i>	CFCC 89970 ^T	<i>Ailanthus altissima</i>	Ningxia, China	MH933618	MH933653	MH933526	MH933592	Fan et al. (2020)
<i>C. ambiens</i>	CFCC 89894	<i>Pyrus bretschneideri</i>	Ningxia, China	KR045617	KR045699	KU710989	KU710945	Unpublished
<i>C. ampulliformis</i>	MFLUCC 16-0583 ^T	<i>Sorbus intermedia</i>	Russia	KY417726	KY417760	KY417692	KY417794	Norphanphoun et al. (2017)
<i>C. amygdali</i>	CBS 144233 ^T	<i>juglans regia</i>	California, USA	MG971853	-	MG972002	-	Lawrence et al. (2018)
<i>C. atrocirrhata</i>	CFCC 89615 ^T	<i>juglans regia</i>	Qinghai, China	KR045618	KR045700	KF498673	KU710946	Fan et al. (2015a), Zhu et al. (2018)
<i>C. austromontana</i>	CMW 6735 ^T	<i>Eucalyptus pauciflora</i>	Australia	AY347361	-	-	-	Adams et al. (2005)
<i>C. beilinensis</i>	CFCC 50493 ^T	<i>Pinus armandii</i>	Beijing, China	MH933619	MH933654	MH933527	-	Fan et al. (2020)
<i>C. berberidis</i>	CFCC 89927 ^T	<i>Berberis dasystachya</i>	China	KR045620	KR045702	KU710990	KU710948	Liu et al. (2015)
<i>C. berkeleyi</i>	StanfordT3 ^T	<i>Eucalyptus globulus</i>	California, USA	AY347350	-	-	-	Adams et al. (2005)
<i>C. brevispora</i>	CBS 116811 ^T	<i>Eucalyptus grandis</i> × <i>tereticornis</i>	Democratic Republic of the Congo	AF192315	-	-	-	Adams et al. (2005)
<i>C. bungeanae</i>	CFCC 50495 ^T	<i>Pinus bungeana</i>	Shanxi, China	MH933621	MH933656	MH933529	MH933593	Fan et al. (2020)
<i>C. californica</i>	CBS 144234 ^T	<i>Juglans regia</i>	California, USA	MG971935	-	MG972083	-	Lawrence et al. (2018)
<i>C. carbonacea</i>	CFCC 89947	<i>Ulmus pumila</i>	Qinghai, China	KR045622	KP310812	KP310842	KU710950	Yang et al. (2015)
<i>C. carbonacea</i>	CFCC 50055	<i>Ulmus pumila</i>	Shanxi, China	KP281262	KP310808	KP310838	-	Yang et al. (2015)
<i>C. carpobroti</i>	CMW 48981 ^T	<i>Carpobrotus edulis</i>	Cape Town, South Africa	MH382812	MH411216	-	-	Jami et al. (2018)
<i>C. cedri</i>	MFLUCC 18-1219a	<i>Ostrya carpinifolia</i>	Forli-Cesena, Italy	MK912132	MK571760	MN685814	MN685823	This study
<i>C. cedri</i>	MFLUCC 18-1219b	<i>Ostrya carpinifolia</i>	Forli-Cesena, Italy	MK912133	MK571761	MN685815	MN685824	This study
<i>C. cedri</i>	CBS 196.50	-	Italy	AF192311	-	-	-	Adams et al. (2002)
<i>C. celtidicola</i>	CFCC 50497 ^T	<i>Celtis sinensis</i>	Anhui, China	MH933623	MH933658	MH933531	MH933595	Fan et al. (2020)
<i>C. centravillosa</i>	MFLUCC 16-1206 ^T	<i>Sorbus domestica</i>	Italy	MF190122	MF190068	-	MF377600	Senanayake et al. (2017)
<i>C. ceratophora</i>	CBS 192.42	<i>Taxus baccata</i>	Italy	AY347333	-	-	-	Adams et al. (2005)
<i>C. ceratosperma</i>	MFLUCC 16-0625	<i>Acer platanoides</i>	Russia	KY563246	KY563248	KY563242	KY563244	Tibpromma et al. (2017)

Table 1 Continued.

Taxon	Source *	Host	Origin	GenBank accession				Reference
				ITS	LSU	ACT	RPB2	
<i>C. ceratospermopsis</i>	CFCC 89626 ^T	<i>Juglans regia</i>	Shaanxi, China	KR045647	KR045726	KU711011	KU710978	Fan et al. (2020)
<i>C. ceratospermopsis</i>	CFCC 89627	<i>Juglans regia</i>	Shaanxi, China	KR045648	KR045727	KU711012	KU710979	Fan et al. (2020)
<i>C. chrysosperma</i>	CFCC 89982 ^T	<i>Ulmus pumila</i>	Xizang, China	KP281261	KP310805	KP310835	-	Yang et al. (2015)
<i>C. cincta</i>	CFCC 89956	<i>Prunus cerasifera</i>	China	KR045624	KR045704	KU710993	KU710953	Zhu et al. (2018)
<i>C. cinerostroma</i>	CMW 5700 ^T	<i>Eucalyptus globulus</i>	Chile	AY347377	-	-	-	Adams et al. (2005)
<i>C. coenobitica</i>	CBS 283.74	<i>Betula verrucosa</i>	Netherlands	JX438610	-	-	-	Adams et al. (2002)
<i>C. cotini</i>	MFLUCC 14-1050 ^T	<i>Cotinus coggygria</i>	Russia	KX430142	KX430143	-	KX430144	Norphanphoun et al. (2017)
<i>C. cotini</i>	MFLUCC 18-1203	<i>Ostrya carpinifolia</i>	Forli-Cesena, Italy	MK912134	MK571762	MN685816	MN685825	This study
<i>C. curvata</i>	MFLUCC 15-0865 ^T	<i>Salix alba</i>	Russia	KY417728	-	KY417694	-	Norphanphoun et al. (2017)
<i>C. cypri</i>	CBS 201.42 ^T	<i>Syringa</i> sp.	Switzerland	DQ243801	-	-	-	Adams et al. (2006)
<i>C. davidiana</i>	CXY1350 ^T	<i>Populus davidiana</i>	China	KM034870	-	-	-	Wang et al. (2015)
<i>C. decorticans</i>	CBS 116.21	<i>Fagus sylvatica</i>	Netherlands	AY347335	-	-	-	Adams et al. (2005)
<i>C. diatrypelloidea</i>	CMW 8549 ^T	<i>Eucalyptus globulus</i>	Orbost, Australia	AY347368	-	-	-	Adams et al. (2005)
<i>C. diopuiensis</i>	MFLUCC 18-1419 ^T	Undefined wood	Chiang Mai, Thailand	MK912137	MK571765	MN685819	-	This study
<i>C. diopuiensis</i> = “ <i>Phomopsis theae</i> ”	GJJM16	-	-	JN638438	-	-	-	Jayanthi et al. (2018)
<i>C. disciformis</i>	CMW 6509 ^T	<i>Eucalyptus grandis</i>	Uruguay	AY347374	-	-	-	Adams et al. (2005)
<i>C. donetzica</i>	MFLUCC 16-0574 ^T	<i>Rosa</i> sp.	Russia	KY417731	KY417765	KY417697	KY417799	Norphanphoun et al. (2017)
<i>C. elaeagni</i>	CFCC 89632 ^T	<i>Elaeagnus angustifolia</i>	Ningxia, China	KR045626	KR045706	KU710995	KU710955	Fan et al. (2015b), Zhu et al. (2018)
<i>C. eriobotryae</i>	IMI136523 ^T	<i>Eriobotrya japonica</i>	India	AY347327	-	-	-	Adams et al. (2005)
<i>C. erumpens</i>	MFLUCC 16-0580 ^T	<i>Salix × fragilis</i>	Russia	KY417733	KY417767	KY417699	KY417801	Norphanphoun et al. (2017)
<i>C. eucalypti</i>	LSEQ	<i>Sequoia sempervirens</i>	California, USA	AY347340	-	-	-	Adams et al. (2005)
<i>C. eucalyptina</i>	CMW 5882	<i>Eucalyptus grandis</i>	Cali, Columbia	AY347375	-	-	-	Adams et al. (2005)
<i>C. eugeniae</i>	CMW 8648	<i>Eugenia</i> sp.	Indonesia	AY347344	-	-	-	Adams et al. (2005)
<i>C. euonymicola</i>	CFCC 50499 ^T	<i>Euonymus kiautschovicus</i>	Shaanxi, China	MH933628	MH933662	MH933535	MH933598	Fan et al. (2020)
<i>C. euonymina</i>	CFCC 89993 ^T	<i>Euonymus kiautschovicus</i>	Shaanxi, China	MH933630	MH933664	MH933537	MH933600	Fan et al. (2020)
<i>C. fabianae</i>	ATCC 96150 ^T	<i>Eucalyptus nitens</i>	Tasmania, Australia	AY347358	-	-	-	Adams et al. (2005)
<i>C. fraxinigena</i>	MFLUCC 14-0868 ^T	<i>Fraxinus ornus</i>	Italy	MF190133	MF190078	-	-	Senanayake et al. (2017)

Table 1 Continued.

Taxon	Source *	Host	Origin	GenBank accession				Reference
				ITS	LSU	ACT	RPB2	
<i>C. fraxinigena</i>	BBH42442	<i>Fraxinus ornus</i>	Italy	MF190134	MF190079	-	-	Senanayake et al. (2017)
<i>C. friesii</i>	CBS 194.42	<i>Abies alba</i>	Switzerland	AY347328	-	-	-	Adams et al. (2005)
<i>C. galegicola</i>	MFLUCC 18-1199 ^T	<i>Galega officinalis</i>	Forli-Cesena, Italy	MK912128	MK571756	MN685810	MN685820	This study
<i>C. gelida</i>	MFLUCC 16-0634 ^T	<i>Cotinus coggygria</i>	Russia	KY563245	KY563247	KY563241	KY563243	Tibpromma et al. (2017)
<i>C. germanica</i>	CXY1322	<i>Elaeagnus oxycarpa</i>	China	JQ086563	JX524617	-	-	Zhang et al. (2013)
<i>C. gicalocus</i>	CFCC 89620 ^T	<i>Juglans regia</i>	Qinghai, China	KR045628	KR045708	KU710997	KU710957	Fan et al. (2015a), Zhu et al. (2018)
<i>C. gigaspora</i>	CFCC 89634 ^T	<i>Salix psammophila</i>	Shaanxi, China	KF765671	KF765687	KU711000	KU710960	Fan et al. (2015b), Zhu et al. (2018)
<i>C. granati</i>	CBS 144237 ^T	<i>Punica granatum</i>	California, USA	MG971799	-	MG971949	-	Lawrence et al. (2018)
<i>C. gutnerae</i>	214	<i>Platanus orientalis</i>	Iran	EF447365	-	-	-	Fotouhifar et al. (2010)
<i>C. japonica</i>	CBS 375.29	<i>Prunus persica</i>	Japan	AF191185	-	-	-	Adams et al. (2002)
<i>C. joaquinensis</i>	CBS 144235 ^T	<i>Populus deltoides</i>	California, USA	MG971895	-	MG972044	-	Lawrence et al. (2018)
<i>C. junipericola</i>	BBH42444	<i>Juniperus communis</i> (Cupressaceae)	Italy	MF190125	MF190072	-	-	Senanayake et al. (2017)
<i>C. junipericola</i>	MFLU 17-0882 ^T	<i>Juniperus communis</i> (Cupressaceae)	Italy	MF190126	MF190071	-	-	Senanayake et al. (2017)
<i>C. juniperina</i>	CFCC 50501 ^T	<i>Juniperus przewalskii</i>	Sichuan, China	MH933632	MH933666	MH933539	MH933602	Fan et al. (2020)
<i>C. kantschavelii</i>	287-2	<i>Populus deltoides</i>	Iran	EF447367	-	-	-	Fotouhifar et al. (2010)
<i>C. kunzei</i>	CBS 118556	<i>Pinus radiata</i>	Eastern Cape, South Africa	DQ243791	-	-	-	Adams et al. (2006)
<i>C. leucostoma</i>	CFCC 50015	<i>Sorbus pohuashanensis</i>	China	KR045634	KR045714	KU711002	KU710963	Yang et al. (2015)
<i>C. longiostiolata</i>	MFLUCC 16-0628 ^T	<i>Salix × fragilis</i>	Russia	KY417734	KY417768	KY417700	KY417802	Norphanphoun et al. (2017)
<i>C. longispora</i>	CBS 144236 ^T	<i>Prunus domestica</i>	California, USA	MG971905	-	MG972054	-	Lawrence et al. (2018)
<i>C. lumnitzericola</i>	MFLUCC 17-0508 ^T	<i>Lumnitzera racernosa</i>	Phetchaburi, Thailand	MG975778	MH253453	MH253457	MH253461	Norphanphoun et al. (2018)
<i>C. mali</i>	CFCC 50031	<i>Crataegus</i> sp.	Shanxi, China	KR045636	KR045716	KU711004	KU710965	Zhu et al. (2018)
<i>C. malicola</i>	CBS 118570	<i>Malus domestica</i>	Michigan, USA	DQ243802	-	-	-	Adams et al. (2005)
<i>C. mali-sylvestris</i>	MFLUCC 16-0638	<i>Malus sylvestris</i> (Rosaceae)	Russia	KY885017	KY885018	KY885019	KY885020	Hyde et al. (2017)
<i>C. melnikii</i>	MFLUCC 15-0851 ^T	<i>Malus domestica</i>	Russia	KY417735	KY417769	KY417701	KY417803	Norphanphoun et al. (2017)
<i>C. mougeotii</i>	ATCC 44994	<i>Picea abies</i>	Norway	AY347329	-	-	-	Adams et al. (2005)

Table 1 Continued.

Taxon	Source *	Host	Origin	GenBank accession				Reference
				ITS	LSU	ACT	RPB2	
<i>C. multicollis</i>	CBS 105.89 ^T	<i>Quercus ilex</i> subsp. <i>rotundifolia</i>	Spain	DQ243803	-	-	-	Adams et al. (2006)
<i>C. myrtagena</i>	HiloTib1	<i>Tibouchina urvilleana</i>	Hawaii, USA	AY347363	-	-	-	Adams et al. (2005)
<i>C. nitschkii</i>	CMW10180 ^T	<i>Eucalyptus globulus</i>	Wondo Genet, Ethiopia	AY347356	-	-	-	Adams et al. (2005)
<i>C. nitschkii</i>	CMW10184	<i>Eucalyptus globulus</i>	Wondo Genet, Ethiopia	AY347355	-	-	-	Adams et al. (2005)
<i>C. nivea</i>	CFCC 89642 ^T	<i>Salix psammophila</i>	Yulin, Shanxi, China	KF765684	KF765700	KF765732	KF765716	Fan et al. (2015b), Zhu et al. (2018)
<i>C. oleicola</i>	CBS 144248 ^T	<i>Olea europaea</i>	California, USA	MG971944	-	MG972098	-	Lawrence et al. (2018)
<i>C. palm</i>	CXY1280 ^T	<i>Cotinus coggygria</i>	Beijing, China	JN411939	-	-	-	Zhang et al. (2014)
<i>C. parakantschavelii</i>	MFLUCC 15-0857 ^T	<i>Populus</i> × <i>sibirica</i>	Russia	KY417738	KY417772	KY417704	KY417806	Norphanphoun et al. (2017)
<i>C. parapersonii</i>	T28.1 ^T	<i>Prunus persicae</i>	Michigan, USA	AF191181	-	-	-	Adams et al. (2002)
<i>C. parapistaciae</i>	CBS 144506 ^T	<i>Pistacia vera</i>	California, USA	MG971804	-	MG971954	-	Lawrence et al. (2018)
<i>C. parasitica</i>	MFLUCC 15-0507 ^T	<i>Malus domestica</i>	Russia	KY417740	KY417774	KY417706	KY417808	Norphanphoun et al. (2017)
<i>C. paratranslucens</i>	MFLUCC 15-0506 ^T	<i>Populus alba</i> var. <i>bolleana</i>	Otto	KY417741	KY417775	KY417707	KY417809	Norphanphoun et al. (2017)
<i>C. piceae</i>	CFCC 52841 ^T	<i>Picea crassifolia</i>	Xinjiang, China	MH820398	MH820391	MH820406	MH820395	Pan et al. (2018)
<i>C. pingbianensis</i>	MFLUCC 18-1204 ^T	Undefined wood	Yunnan, China	MK912135	MK571763	MN685817	MN685826	This study
<i>C. pini</i>	CBS 224.52 ^T	<i>Pinus strobus</i>	New York	AY347316	-	-	-	Adams et al. (2005)
<i>C. pistaciae</i>	CBS 144238 ^T	<i>Pistacia vera</i>	California, USA	MG971802	-	MG971952	-	Lawrence et al. (2018)
<i>C. platycladi</i>	CFCC 50504 ^T	<i>Platycladus orientalis</i>	Yunnan, China	MH933645	MH933679	MH933552	MH933610	Fan et al. (2020)
<i>C. platycladi</i>	CFCC 50505	<i>Platycladus orientalis</i>	Yunnan, China	MH933646	MH933680	MH933553	MH933611	Fan et al. (2020)
<i>C. platycladicola</i>	CFCC 50038 ^T	<i>Platycladus orientalis</i>	Gansu, China	KT222840	MH933682	MH933555	MH933613	Fan et al. (2020)
<i>C. plurivora</i>	CBS 144239 ^T	<i>Olea europaea</i>	California, USA	MG971861	-	MG972010	-	Lawrence et al. (2018)
<i>C. populicola</i>	CBS 144240 ^T	<i>Populus deltoides</i>	California, USA	MG971891	-	MG972040	-	Lawrence et al. (2018)
<i>C. populina</i>	CFCC 89644 ^T	<i>Salix psammophila</i>	Shanxi, China	KF765686	KF765702	KU711007	KU710969	Fan et al. (2015b), Zhu et al. (2018)
<i>C. populinopsis</i>	CFCC 50032 ^T	<i>Sorbus aucuparia</i>	Ningxia, China	MH933648	MH933683	MH933556	MH933614	Fan et al. (2020)
<i>C. predappioensis</i>	MFLUCC 18-1202	<i>Ostrya carpinifolia</i>	Forlì-Cesena, Italy	MK912131	MK571759	MN685813	MN685822	This study
<i>C. predappioensis</i>	MFLUCC 17-2458 ^T	<i>Platanus hybrida</i>	Italy	MG873484	MG873480	-	-	Hyde et al. (2018)
<i>C. predappioensis</i>	MFLUCC 17-0327	<i>Platanus hybrida</i>	Italy	MH253451	MH253452	MH253449	MH253450	Hyde et al. (2018)
<i>C. predappioensis</i>	MFLUCC 18-1205	<i>Cupressus</i> sp.	Yunnan, China	MK912136	MK571764	MN685818	MN685827	This study
<i>C. pruinopsis</i>	CFCC 50034 ^T	<i>Ulmus pumila</i>	Shanxi, China	KP281259	KP310806	KP310836	KU710970	Yang et al. (2015)

Table 1 Continued.

Taxon	Source *	Host	Origin	GenBank accession				Reference
				ITS	LSU	ACT	RPB2	
<i>C. pruinosa</i>	CFCC 50036	<i>Syzygium aromaticum</i>	Qinghai, China	KP310800	KP310802	KP310832	-	Yang et al. (2015)
<i>C. prunicola</i>	MFLUCC 18-1200	<i>Quercus pubescens</i>	Forlì-Cesena, Italy	MK912129	MK571757	MN685811	-	This study
<i>C. prunicola</i>	MFLU 17-0995 ^T	<i>Prunus</i> sp.	Italy	MG742350	MG742351	MG742353	MG742352	Hyde et al. (2018)
<i>C. pubescentis</i>	MFLUCC 18-1201 ^T	<i>Quercus pubescens</i>	Forlì-Cesena, Italy	MK912130	MK571758	MN685812	MN685821	This study
<i>C. quercicola</i>	MFLUCC 14-0867 ^T	<i>Quercus</i> sp.	Italy	MF190129	MF190073	-	-	Senanayake et al. (2017)
<i>C. quercicola</i>	BBH42443	<i>Quercus</i> sp.	Italy	MF190128	MF190074	-	-	Senanayake et al. (2017)
<i>C. rhizophorae</i>	MUCC302	<i>Eucalyptus grandis</i>	Australia	EU301057	-	-	-	Unpublished
<i>C. rhodophila</i>	ATCC 38695	<i>Rosa</i> sp.	-	DQ243809	-	-	-	Yang et al. (2015)
<i>C. ribis</i>	CFCC 50026 ^T	<i>Ulmus pumila</i>	Qinghai, China	KP281267	KP310813	KP310843	KU710972	Yang et al. (2015)
<i>C. rosae</i>	MFLUCC 14-0845 ^T	<i>Rosa canina</i>	Italy	MF190131	MF190075	-	-	Senanayake et al. (2017)
<i>C. rosae</i>	MFLUCC 17-1664 (BBH 42447)	<i>Rosa canina</i>	Italy	MF190130	MF190076	-	-	Senanayake et al. (2017)
<i>C. rosarum</i>	218	<i>Rosa canina</i>	Iran	EF447387	-	-	-	Fotouhifar et al. (2010)
<i>C. rostrata</i>	CFCC 89909 ^T	<i>Salix cupularis</i>	Gansu, China	KR045643	KR045722	KU711009	KU710974	Fan et al. (2014b), Zhu et al. (2018)
<i>C. rusanovii</i>	MFLUCC 15-0854 ^T	<i>Salix babylonica</i>	Russia	KY417744	KY417778	KY417710	KY417812	Norphanphoun et al. (2017)
<i>C. sacculus</i>	HMBF281	<i>Juglans regia</i>	China	KF225615	KF225629	KF498678	-	Fan et al. (2015a), Zhu et al. (2018)
<i>C. sacculus</i>	HMBF282	<i>Juglans regia</i>	China	KF225616	KF225630	KF498679	-	Fan et al. (2015a), Zhu et al. (2018)
<i>C. sacculus</i>	CFCC 89624 ^T	<i>Juglans regia</i>	Shaanxi, China	KR045645	KR045724	-	KU710976	Fan et al. (2015a), Zhu et al. (2018)
<i>C. salicacearum</i>	MFLUCC 15-0509 ^T	<i>Salix alba</i>	Russia	KY417746	KY417780	KY417712	KY417814	Norphanphoun et al. (2017)
<i>C. salicicola</i>	MFLUCC 15-0866	<i>Salix alba</i>	Russia	KY417749	KY417783	KY417715	KY417817	Norphanphoun et al. (2017)
<i>C. salicina</i>	MFLUCC 15-0862 ^T	<i>Salix</i> sp.	Russia	KY417750	KY417784	KY417716	KY417818	Norphanphoun et al. (2017)
<i>C. schulzeri</i>	CFCC 50040	<i>Malus domestica</i>	Ningxia, China	KR045649	KR045728	KU711013	KU710980	Unpublished
<i>C. sibiraeae</i>	CFCC 50045 ^T	<i>Sibiraeae angustata</i>	Gansu, China	KR045651	KR045730	KU711015	KU710982	Liu et al. (2015)
<i>C. sophorae</i>	CFCC 89598	<i>Styphnolobium japonicum</i>	Gansu, China	KR045654	KR045733	KU711018	KU710985	Fan et al. 2014a, Zhu et al. (2018)
<i>C. sophoricola</i>	CFCC 89595 ^T	<i>Styphnolobium japonicum</i> var. <i>pendula</i>	Gansu, China	KR045655	KR045734	KU711019	KU710986	Fan et al. 2014a, Zhu et al. (2018)
<i>C. sophoriopsis</i>	CFCC 89600 ^T	<i>Styphnolobium japonicum</i>	Gansu, China	KR045623	KP310804	KU710992	KU710951	Fan et al. (2020)
<i>C. sorbi</i>	MFLUCC 16-0631 ^T	<i>Sorbus aucuparia</i>	Russia	KY417752	KY417786	KY417718	KY417820	Norphanphoun et al. (2017)

Table 1 Continued.

Taxon	Source *	Host	Origin	GenBank accession				Reference
				ITS	LSU	ACT	RPB2	
<i>C. sorbicola</i>	MFLUCC 16-0584 ^T	<i>Acer pseudoplatanus</i>	Russia	KY417755	KY417789	KY417721	KY417823	Norphanphoun et al. (2017)
<i>C. sordida</i>	HMBF159	<i>Juglans regia</i>	China	KF225613	KF225627	KF498676	-	Fan et al. (2015a)
<i>C. spiraeae</i>	CFCC 50049 ^T	<i>Spiraea salicifolia</i>	Gansu, China	MG707859	MG707643	MG708196	MG708199	Zhu et al. (2018)
<i>C. subclypeata</i>	CBS 117.67	<i>Rhododendron ponticum</i>	Netherlands	AY347331	-	-	-	Adams et al. (2005)
<i>C. tamaricicola</i>	CFCC 50508 ^T	<i>Tamarix chinensis</i>	Yunnan, China	MH933652	MH933687	MH933560	MH933617	Fan et al. (2020)
<i>C. tanaitica</i>	MFLUCC 14-1057 ^T	<i>Betula pubescens</i>	Russia	KT459411	KT459412	KT459413	-	Ariyawansa et al. (2015)
<i>C. terebinthi</i>	227	<i>Pistacia khinjuk</i>	Iran	EF447402	-	-	-	Fotouhifar et al. (2010)
<i>C. thailandica</i>	MFLUCC 17-0262 ^T	<i>Xylocarpus moluccensis</i>	Ranong, Thailand	MG975776	MH253455	MH253459	MH253463	Norphanphoun et al. (2018)
<i>C. thailandica</i>	MFLUCC 17-0263	<i>Xylocarpus moluccensis</i>	Ranong, Thailand	MG975777	MH253456	MH253460	MH253464	Norphanphoun et al. (2018)
<i>C. tibouchinae</i>	CPC 26333 ^T	<i>Tibouchina semidecandra</i>	La Reunion, France	KX228284	KX228335	-	-	Crous et al. (2013)
<i>C. ulmi</i>	MFLUCC 15-0863 ^T	<i>Ulmus minor</i>	Russia	KY417759	KY417793	KY417725	KY417827	Norphanphoun et al. (2017)
<i>C. ulmicola</i>	MFLUCC 18-1227 ^T	<i>Ulmus pumila</i> (Ulmaceae)	Russia	MH940220	MH940218	MH940216	-	Phookamsak et al. (2019)
<i>C. valsoidea</i>	CMW 4309 ^T	<i>Eucalyptus grandis</i>	North Sumatra, Indonesia	AF192312	-	-	-	Adams et al. (2005)
<i>C. variostromatica</i>	CMW 6766 ^T	<i>Eucalyptus globulus</i>	Australia	AY347366	-	-	-	Adams et al. (2005)
<i>C. vinacea</i>	CBS 141585 ^T (Cyt5)	<i>Eucalyptus globulus</i>	New Hampshire, USA	KX256256	-	-	-	Lawrence et al. (2017)
<i>C. xylocarpi</i>	MFLUCC 17-0251 ^T	<i>Xylocarpus granatum</i>	Ranong, Thailand	MG975775	MH253454	MH253458	MH253462	Norphanphoun et al. (2018)
<i>Diaporthe eres</i>	CBS 145040	<i>Lactuca sativa</i>	Netherlands	MK442579	MK442521	MK442634	MK442663	Crous et al. (2019)

* **ABBREVIATIONS:** **ATCC:** American Type Culture Collection, Manassas, America; **BBH:** National Science and Technology Development Agency, Thailand; **CBS:** Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; **CFCC:** China Forestry Culture Collection Center; **CMW:** The culture collection of Mike Wingfield housed at TPCP, FABI, University of Pretoria. **IMI:** International Mycological Institute, CABI-Bioscience, Egham, BAKEHAM Lane, UK; **CPC:** Culture collection of Pedro Crous, housed at CBS; **MFLU:** Mae Fah Luang University Herbarium Collection; **MFLUCC:** Mae Fah Luang University Culture Collection, Chiang Rai, Thailand. ^T denotes the sources of holotype and epitype. The newly generated sequences are in blue.

Maximum parsimony analysis (MP) was performed by PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002) using the heuristic search option. Starting tree(s) were obtained via stepwise addition with 1,000 replicates of random sequence additions and the branch swapping was performed by using tree-bisection-reconnection (TBR) swapping algorithm. Maxtrees were set up at 1000 with all characters were treated as unordered and of equal weight. Gaps were treated as missing data and the branches of zero length were collapsed. All multiple equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993). Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for all parsimonious trees. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to compare tree topologies obtained under different optimality criteria.

Phylograms were viewed in FigTree v. 1.4.2 (Rambaut 2012). The resulting tree was edited and annotated in Microsoft Power Point (2016) and converted to jpeg file in Adobe Photoshop CS6 software (Adobe Systems, USA). Sequences derived from this study were deposited in GenBank (Table 1). The final alignment and tree have been deposited in TreeBASE under submission ID: 25123 (TreeBASE 2019).

Results

Phylogenetic analysis

Phylogenetic analysis of a combined ITS, LSU, ACT and RPB2 dataset was used to determine the taxonomic placement of our new taxa and other *Cytospora* species. Phylogenetic analyses obtained from ML, MP and BI analyses resulted in trees with similar topologies and were not significantly different (data not shown). Trees recovered from single gene analyses did not result in any topological conflicts with respect to phylogenies generated with the combined dataset (data not shown). The sequence data comprises 147 *Cytospora* taxa with *Diaporthe eres* (CBS 145040) as the outgroup taxon (Crous et al. 2019). The best scoring ML tree (shown in Fig. 1) with the final ML optimization likelihood value of -23739.011018 (ln) is selected to represent and discuss the phylogenetic relationships among taxa. The dataset for maximum parsimony comprised 2761 characters (including the gaps), of which 1868 are constant characters, 664 are parsimony-informative characters and 229 are variable parsimony-uninformative characters, yielded 10 equally most parsimonious trees and the first parsimonious tree was represented as the best tree (TL = 4069, CI = 0.342, RI = 0.728, RC = 0.249, HI = 0.658). The BI analysis for final split frequency critical value for the topological convergence diagnostic is 0.009995.

The phylogenetic result (Fig. 1) depicts the relationships of other *Cytospora* taxa within Diaporthales which is congruent to phylogeny recovered by Norphanphoun et al. (2018) and Pan et al. (2018). Most of the isolates clustered together with other *Cytospora* species and eight species can be reorganized in the tree. Six taxa grouped together with ex-type strains of known species and can be identified as *C. cedri*, *C. cotini*, *C. predappioensis* and *C. prunicola*; and four isolates represent phylogenetically distinct species and are introduced as the new species, *C. diopuensis*, *C. galegicola*, *C. pingbianensis* and *C. pubescentis*. The details of the relationships of the new taxa with others are discussed in the notes.

Taxonomy

Cytospora cedri Syd., P. Syd. & E.J. Butler, *Annls mycol.* 14(3/4): 193 (1916)

Fig. 2

Index Fungorum number: IF 184521; Facesoffungi number: FoF 05104

Saprobic on the bark. Sexual morph *Stromata* 1048–1422 µm wide, with the poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent the bark by the ostiolar canal, dark brown to black, glabrous, circular in shape, arranged with conspicuous, clustered, roundish to cylindrical prominent ostioles. *Ascomata* (excluding necks) 212–334 µm

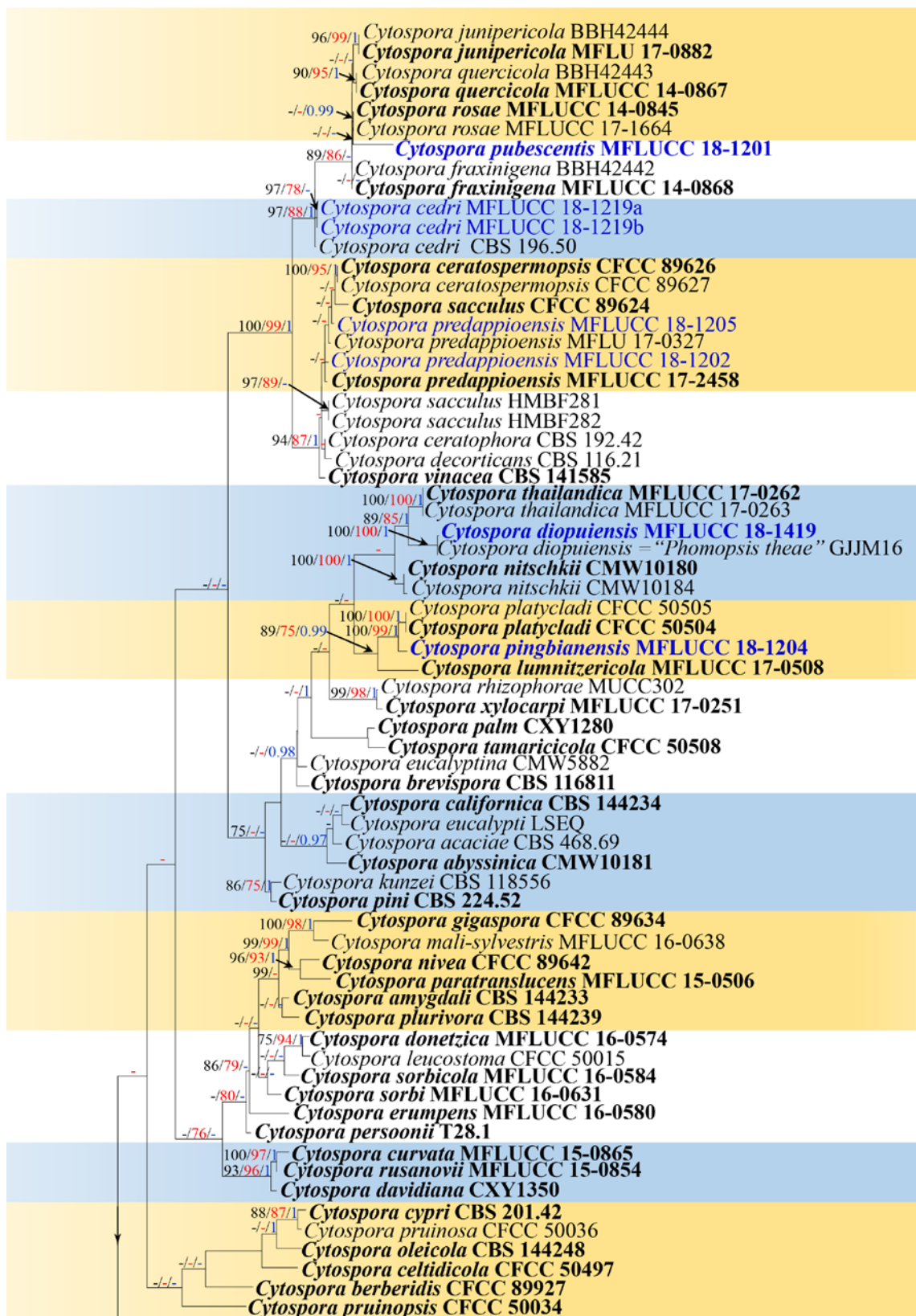


Figure 1 – Phylogenetic tree generated from MP analysis based on a combined dataset of ITS, LSU, ACT and RPB2 sequence data. Bootstrap support values for ML (back) and MP (red) equal to or greater than 75% are shown above the nodes. The BI values (blue) greater than 0.95 are also indicated above the nodes. The new isolates are in blue and ex-type strains are in bold. The tree is rooted to *Diaporthe eres* (CBS 145040).

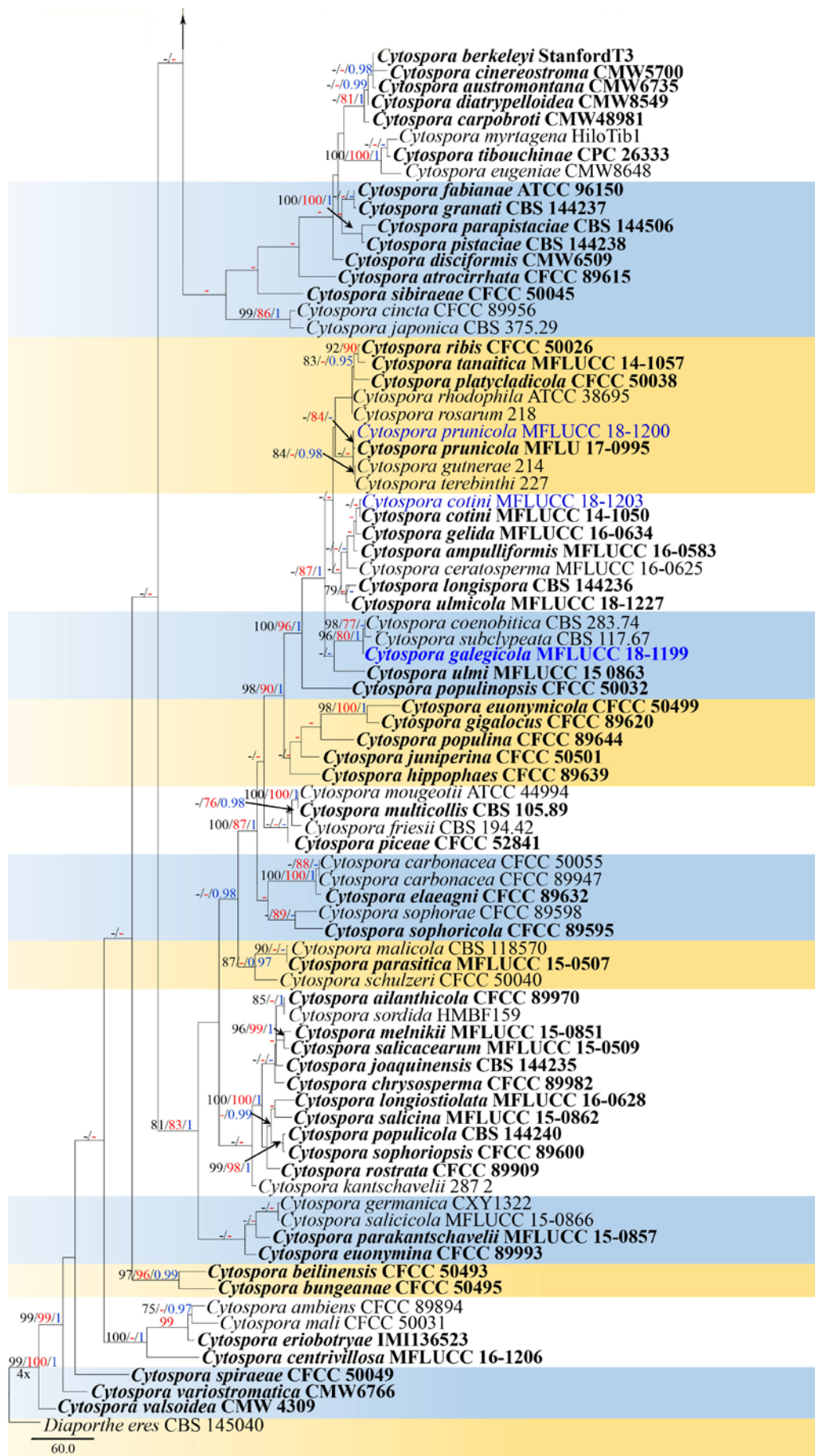


Figure 1 – Continued.

high, 222–373 μm diam. (\bar{x} = 273 \times 297 μm , n = 30), perithecial, immersed in a stroma, dark brown to brown, globose to subglobose, glabrous, individual ostiole with the neck. *Ostiolar canal* 287–493 μm high, 59–90 μm diam. (\bar{x} = 390 \times 75 μm , n = 18), cylindrical, sulcate, periphysate. *Peridium* 25–38 μm wide, composed of two section layers, outer section comprising 3–5 layers, of relatively small, brown to dark brown, thick-walled cells, arranged in *textura angularis*, the inner part comprising 2–3 layers of hyaline cells of *textura angularis*. *Hamathecium* comprising only asci. *Asci* (30–)32–39(–42) \times (4–)4.5–6(–6.5) μm (\bar{x} = 35 \times 5.2 μm , n = 35), 8-spored, unitunicate, clavate, sessile, apically rounded to truncate, with a J- apical ring. *Ascospores* (5.5–)7–8.5(–10) \times (1.5–)1.8–2.5(–3) μm (\bar{x} = 7.6 \times 2 μm , n = 110), overlapping 1–2-seriate, hyaline, allantoid, aseptate, smooth-walled. Asexual morph Undetermined.

Culture characteristics – Ascospores germinating on PDA within 24 hrs. Germ tubes produced from all sides. Colonies on PDA reaching 5–5.5 cm diam. after 15 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairly fluffy, dark brown from above, dark brown to black from below; not producing pigments in agar.

Material examined – ITALY, near Galeata (Province of Forlì-Cesena [FC]), on a dead land branch of *Ostrya carpinifolia*, 13 April 2017, E. Camporesi, IT 3289C (MFLU 18-1387, KUN-HKAS 100917), living culture, MFLUCC 18-1219.

Notes – *Cytospora cedri* was reported by Sydow et al. (1916) as a coelomycete which is characterized by having black stromata and allantoid conidia. There are no records of the sexual morph (Adams et al. 2002, 2005, 2006). The phylogenetic result (Fig. 1) shows that our isolates (MFLUCC 18-1219a and MFLUCC 18-1219b) cluster together with *C. cedri* (CBS 196.50) with support (57% ML, 58% MP and 0.56 PP). The single gene comparison of ITS (Table 4) showed that there is no sequence difference among these three isolates. Therefore, based on the guidelines of Jeewon & Hyde (2016), we identify our isolates (MFLUCC 18-1219a and MFLUCC 18-1219b) as *C. cedri* and provide the first sexual morph description for the species.

Cytospora cotini Norph., Bulgakov & K.D. Hyde, Fungal Diversity 80: 176 (2016) Fig. 3

Index Fungorum number: IF552231; Facesoffungi number: FoF02365

Saprobic on the bark. Sexual morph *Stromata* 1190–2098 μm wide, with the poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent by the ostiolar canal, dark brown to black, glabrous, circular in shape, arranged with conspicuous, clustered, roundish to cylindrical prominent ostioles. *Ascomata* (excluding necks) 183–386 μm high, 364–613 μm diam. (\bar{x} = 284 \times 489 μm , n = 15), perithecial, immersed in a stroma, brown to dark brown, globose to subglobose, glabrous, individual ostiole with the neck. *Ostiolar canal* 250–310 μm high, 120–155 μm diam. (\bar{x} = 276 \times 139 μm , n = 10), cylindrical, sulcate, periphysate. *Peridium* 49–84 μm wide, composed of two section layers, outer section comprising 5–10 layers, of relatively small, brown to dark brown, thick-walled cells, arranged in *textura angularis*, the inner part comprising 3–5 layers of hyaline cells of *textura angularis*. *Hamathecium* comprising only asci. *Asci* (56–)61–71(–85) \times (6.5–)7.5–9.5(–11) μm (\bar{x} = 66 \times 8.4 μm , n = 50), 8-spored, unitunicate, clavate, short stalks, apically rounded to truncate, with a J-, refractive apical ring. *Ascospores* (10.5–)12.5–15(–17) \times (2.5–)3–4(–4.5) μm (\bar{x} = 14 \times 3.5 μm , n = 250), overlapping 1–2-seriate, hyaline, allantoid, aseptate, smooth-walled. Asexual morph Undetermined.

Culture characteristics – Ascospores germinating on PDA within 24 hrs. Germ tubes produced from all sides. Colonies on PDA reaching 5–5.5 cm diam. after 7 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairly fluffy, white from above, white to yellow from below; not producing pigments in agar.

Material examined – ITALY, Corniolo, Santa Sofia (Province of Forlì-Cesena [FC]), on a dead land branch of *Ostrya carpinifolia*, 29 March 2017, E. Camporesi, IT 3294 (MFLU 17-0864, KUN-HKAS 100919), living culture, MFLUCC 18-1203.

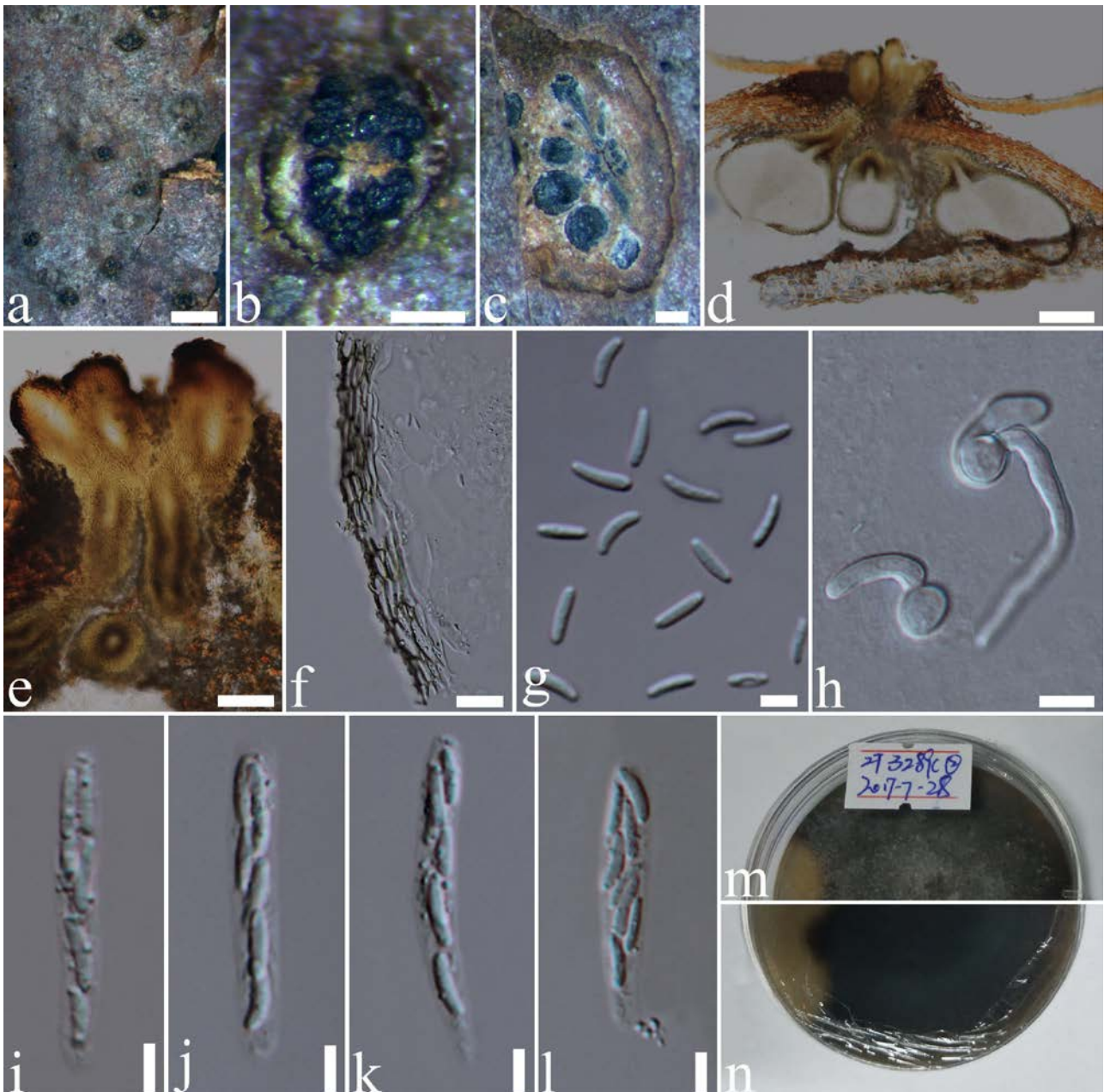


Figure 2 – *Cytospora cedri* (MFLU 18-1387). a Appearance of stromata on substrate. b Stroma. c Cross section through stroma. d Vertical section through stroma. e Ostiolar canal. f Peridium. g Ascospores. h Germinating ascospore. i–l Asci. m, n Culture characteristic on PDA after 10 days (m = colony from above, n = colony from below). Scale bars: a = 1 mm, b, c = 200 μ m, d = 100 μ m, e = 50 μ m, f = 20 μ m, g, i–l = 5 μ m, h = 10 μ m.

Notes – The phylogenetic result (Fig. 1) shows that our new strain (MFLUCC 18-1203) clusters together with a *Cytospora cotini* strain (MFLUCC 14-1050, holotype) and they share a sister relationship to *Cytospora ampulliformis* (MFLUCC 16-0583, holotype), *Cytospora ceratosperma* (MFLUCC 16-0625) and *Cytospora gelida* (MFLUCC 16-0634, holotype) with 46% ML, 60% MP and 0.80 PP support. Our specimen of *C. cotini* (MFLU 17-0864) differs from *C. ceratosperma* by its larger stromata, shorter ostiolar canal (Table 2) and its asci with J-, refractive apical ring (Tibpromma et al. 2017). The taxa of *C. ampulliformis*, *C. cotini* and *C. gelida* were only reported in their asexual morph, and it is impossible to compare with our samples which are sexual morphs (Norphanphoun et al. 2017, Tibpromma et al. 2017). Considering the unresolved phylogenetic result and the incomparable morphology, we compared the single gene regions of ITS, LSU and RPB2 (Table 4) for these taxa, which displayed the highest nucleotides sequence

similarity between strains MFLUCC 18-1203 and MFLUCC 14-1050. Based on the guidelines of Jeewon & Hyde (2016), we identify our specimen as *C. cotini* and provide the first sexual morph description of this species. However, our study indicates that taxonomic revision of *C. ampulliformis*, *C. ceratosperma*, *C. cotini* and *C. gelida* in *Cytospora* is needed based on type studies and further analyses from other informative genes and with more taxon sampling (Jeewon & Hyde 2016).

Cytospora diopuiensis Q.J. Shang, K.D. Hyde & J.K. Liu, sp. nov.

Fig. 4

Index Fungorum number: IF 555502; Facesoffungi number: FoF 05099

Etymology – Names after a famous mountain “Dio Pui” in Mueang Chiang Mai District of Thailand, of where the fungus was collected nearby.

Holotype – MFLU 18-1390

Saprobic on the bark. Sexual morph *Stromata* 0.8–1.2 mm wide, with the poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent by the ostiolar canal, dark brown to black, glabrous, circular in shape, arranged with conspicuous, clustered, roundish to cylindrical prominent ostioles. *Ascomata* (excluding necks) 117–192 μm high, 205–333 μm diam. (\bar{x} = 154 \times 269 μm , n = 15), perithecial, immersed in a stroma, brown to dark brown, globose to subglobose, glabrous, individual ostiole with the neck. *Ostiolar canal* 242–520 μm high, 95–121 μm diam. (\bar{x} = 381 \times 108 μm , n = 10), cylindrical, sulcate, periphysate. *Peridium* 15–25 μm wide, composed of two section layers, outer section comprising 3–5 layers, yellow to brown, thick-walled cells, arranged in *textura angularis*, the inner part comprising 3–4 layers of hyaline cells of *textura angularis*. *Hamathecium* composed of 2.2–2.7 μm wide, cylindrical, aseptate, hyaline, paraphyses. *Asci* (23–)25–31(–34) \times (4–)4.5–6(–6.5) μm (\bar{x} = 27.8 \times 5.2 μm , n = 50), 8-spored, unitunicate, cylindrical to clavate, sessile, apically rounded to truncate, with a J- apical ring. *Ascospores* (6.5–)7.5–8.5(–9) \times (1.2–)1.5–2.5(–3.6) μm (\bar{x} = 8 \times 1.9 μm , n = 75), overlapping 1–2-seriate, hyaline, oblong to elongate-allantoid, aseptate, smooth-walled. Asexual morph Undetermined.

Culture characteristics – Ascospores germinating on PDA within 12 hrs. Germ tubes produced from all sides. Colonies on PDA reaching 4–5 cm diam. after 7 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, felt-like, initially white, becoming yellow after 7 days. After 10–15 days, gray to black from above, black from below; not producing pigments in agar.

Material examined – THAILAND, Chiang Mai Province, Mueang Chiang Mai District, on dead wood, 27 July 2016, Qiuju Shang, DP03 (MFLU 18-1390, holotype; KUN-HKAS 99637, isotype), ex-type living culture, MFLUCC 18-1419.

Notes – *Cytospora diopuiensis* resembles *Cytospora thailandica* in having the conspicuous, clustered ostioles, sessile asci and elongate-allantoid ascospores (Norphanphoun et al. 2018). However, *Cytospora diopuiensis* is distinguished from *C. thailandica* by having larger stromata, asci and ascospores (Table 2). Moreover, *Cytospora diopuiensis* is distinct from *C. thailandica* in the asci having J-, apical ring, whereas the asci of *C. thailandica* having J-, refractive, apical ring (Norphanphoun et al. 2018). Phylogenetically, the isolate of *C. diopuiensis* (MFLUCC 18-1419) clusters with the strain *Phomopsis theae* (GJJM16), with high support (100% ML, 100% MP and 1 PP), and they are sister to *C. thailandica* with moderate support (89% ML, 85% MP and 1 PP) (Fig. 1). The strain of GJJM16, an endophyte was isolated and given the taxonomic placement as *P. theae* by Jayanthi et al. (2018) and *Phomopsis theae* (*Diaporthe theae*) was reported as an asexual morph taxon (Petch 1925, Rossman et al. 2015). However, Jayanthi et al. (2018) did not provide a detailed morphological description and phylogenetic analysis for this isolate to justify its taxonomic identification. Moreover, there is only ITS sequence provided for the strain of GJJM16 in GenBank. We carried out the ITS gene comparison between our isolate (MFLUCC 18-1419) and the strain (“*P. theae*” GJJM16), and there is no significant difference between them (Table 4), hence they could be identified as same species (Jeewon & Hyde 2016). Therefore, we introduce them as a new species *C. diopuiensis*.

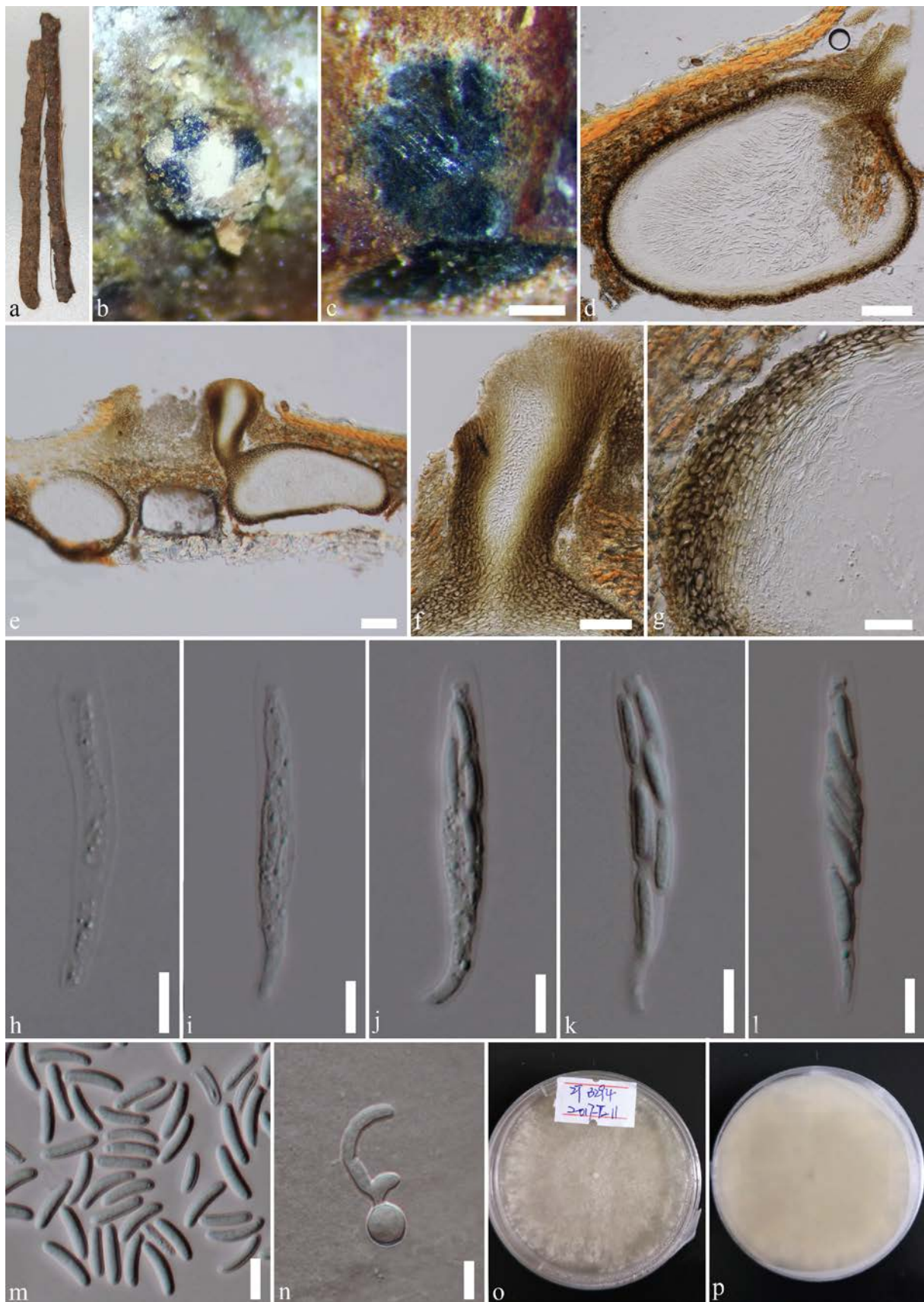


Figure 3 – *Cytospora cotini* (MFLU 17-0864). a Host substrate. b Stroma. c Cross section through stroma. d Ascoma. e Vertical section through stroma. f Ostiolar canal. g Peridium. h–l Asci. m Ascospores. n Germinating ascospore. o, p Culture characteristic on PDA after 10 days (o = colony from above, p = colony from below). Scale bars: c = 200 μ m, d, e = 100 μ m, f = 50 μ m, g = 20 μ m, h–n = 10 μ m.

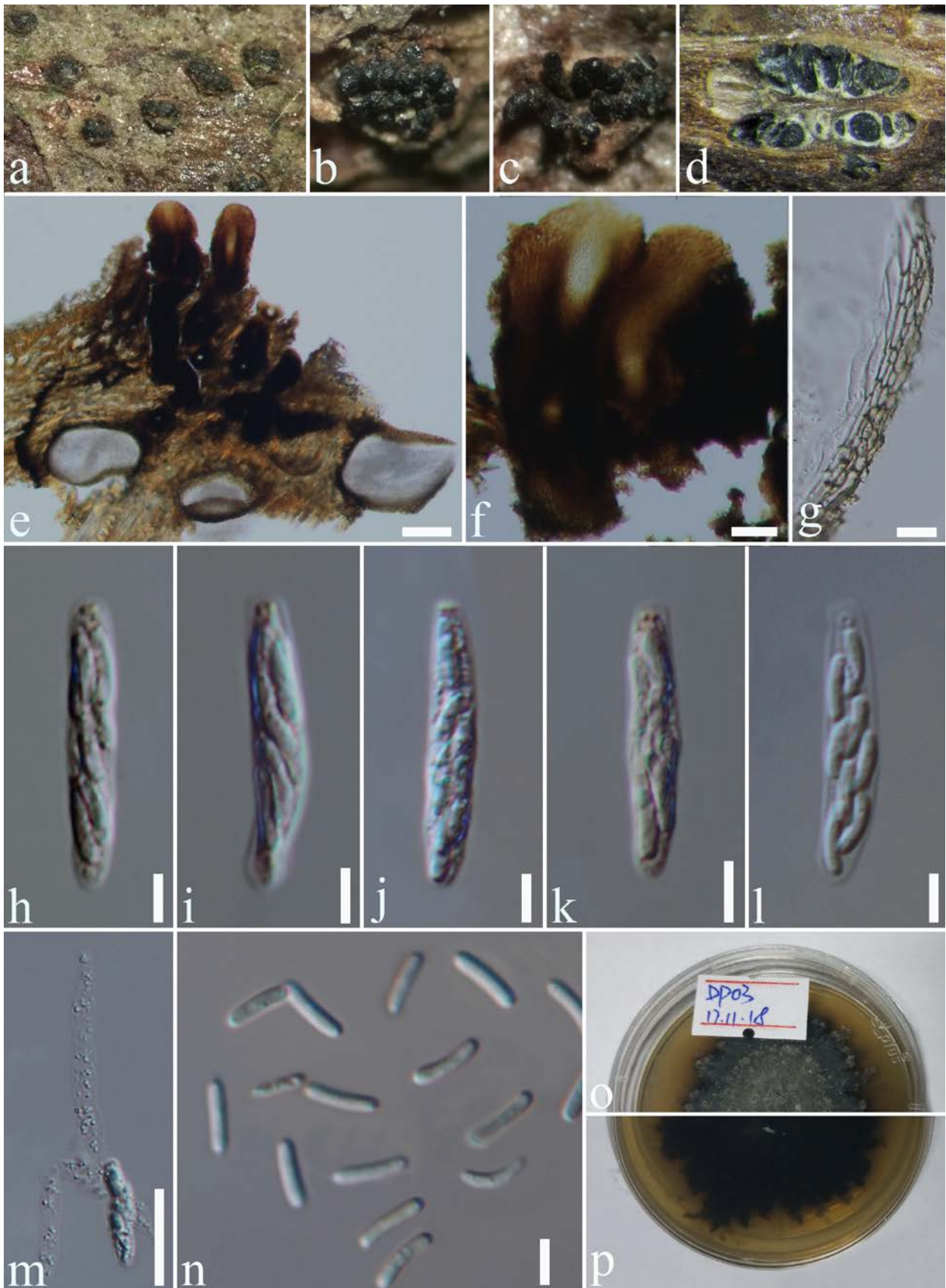


Figure 4 – *Cytospora diopuiensis* (MFLU 18-1390, holotype). a–c Appearance of stromata on the substrate. d Cross section through stroma. e Vertical section through stroma. f Ostiolar canal. g Peridium. h–l Asci (h–k = stained with Congo red). m Paraphyses. n. Ascospores. o, p Culture characteristic on PDA after 10 days (o = colony from above, p = colony from below). Scale bars: e = 100 μ m, f, m, n = 20 μ m, g = 10 μ m, h–l = 5 μ m.

Cytospora galegicola Q.J. Shang, E. Camporesi & K.D. Hyde, sp. nov.

Fig. 5

Index Fungorum number: IF 555503; Facesoffungi number: FoF 05100

Etymology – Names reflect the host, of which the fungus was collected from “*Galega officinalis*”.

Holotype – MFLU 16-2280

Saprobic on the bark. Sexual morph *Stromata* 0.6–1.3 mm wide, with the poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent by the ostiolar canal, dark brown to black, glabrous, irregular in shape, arranged with conspicuous, scattered, roundish to cylindrical prominent ostioles. *Ascomata* (excluding necks) 170–275 µm high, 210–390 µm diam. (\bar{x} = 223 × 300 µm, n = 20), perithecial, immersed in a stroma, yellow to brown, globose to subglobose, glabrous, individual ostiole with the neck. *Ostiolar canal* 170–210 µm high, 40–135 µm diam. (\bar{x} = 189 × 44 µm, n = 18), cylindrical, sulcate, concentrated, periphysate. *Peridium* 23–34 µm wide, composed of two section layers, outer section comprising 3–6 layers, of relatively small, yellow, thick-walled cells, arranged in *textura angularis*, inner part comprising 3–4 layers of hyaline cells of *textura angularis*. *Hamathecium* composed of 5–6 µm wide, dense, cylindrical, septate, hyaline, paraphyses. *Asci* (33–)38–45(–49) × (4.5–)5.5–6.5(–7.5) µm (\bar{x} = 41 × 6 µm, n = 45), 8-spored, unitunicate, clavate, with short stalks, apically rounded to truncate, with a J- apical ring. *Ascospores* (5.8–)6.8–10(–12.5) × (1.5–)2–3(–4) µm (\bar{x} = 8.4 × 2.5 µm, n = 75), overlapping 1–2-seriate, hyaline, oblong to allantoid, aseptate, smooth-walled. Asexual morph Coelomycetous. *Conidiomata* 430–589 µm, pycnidial, with multi-loculate, appearing as brown to black, watery, rounded, conidial masses, superficial, solitary or aggregated, subglobose, shiny, with white to brown mycelium covering the surface. *Pycnidial walls* 13–21 µm wide, comprising several layers of hyaline to brown, compressed hyphae, arranged in a *textura intricata*. *Conidiogenous cells* (7.2–)9.5–19.5(–23) × (1.5–)1.8–3.2(–4) (\bar{x} = 14.4 × 2.5 µm, n = 15), cylindrical to clavate, holoblastic, straight or curved. *Conidia* (4.8–)5–6.8(–10.7) × (0.8–)1–1.5(–2.0) \bar{x} = 7.6 × 1.3 µm, n = 150), hyaline, allantoid, some with strongly curved ends, unicellular, smooth-walled.

Culture characteristics – Ascospores germinating on PDA within 24 hrs. Germ tubes produced from all sides. Colonies on PDA reaching 5–5.5 cm diam. after 15 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairly fluffy, white from above, light yellow from below; not producing pigments in agar.

Material examined – Italy, Fiumicello, Premilcuore (Province of Forlì-Cesena [FC]), on dead aerial stem of *Galega officinalis*, 27 July 2016, E. Camporesi, IT 3045 (MFLU 16-2280, holotype; KUN-HKAS 100884, isotype), ex-type living culture, MFLUCC 18-1199.

Notes – The phylogenetic inference obtained in this study (Fig. 1) shows that the new taxon *Cytospora galegicola* (MFLUCC 18-1199) forms a distinct lineage close to *Cytospora coenobitica* (CBS 283.74) and *Cytospora subclypeata* (CBS 117.67) with high support (ITS, 96% ML, 80% MP and 1.00 PP). Morphologically, *Cytospora galegicola* has larger conidia than *C. coenobitica* and *C. subclypeata* (Saccardo 1884, 1986, Table 3). Therefore, *Cytospora galegicola* is described as a new species and the description and illustration of the holomorph are provided herein.

Cytospora pingbianensis Q.J. Shang, K.D. Hyde & J.K. Liu, sp. nov.

Fig. 6

Index Fungorum number: IF 555514; Facesoffungi number: FoF 05107

Etymology – The species epithet “*pingbianensis*” refers to the town *Ping bian* in China where the fungus was collected.

Holotype – KUN-HKAS 102161

Saprobic on the bark. Sexual morph *Stromata* 880–1524 µm wide, with the poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent by the ostiolar canal, dark brown to black, glabrous, circular in shape, arranged with conspicuous, clustered, roundish to cylindrical prominent ostioles. *Ascomata* (excluding necks) 142–248 µm high, 113–245 µm diam. (\bar{x} = 195 × 180 µm, n = 50), perithecial, immersed in a stroma, brown to dark brown, globose to subglobose, glabrous, individual ostiole with the neck. *Ostiolar canal* 185–722 µm high, 27–66 µm

diam. (\bar{x} = 453 × 47 μm, n = 10), cylindrical, sulcate, periphysate. *Peridium* 14–24 μm wide, composed of two section layers, outer section comprising 3–5 layers, of relatively small, brown to dark brown, thick-walled cells, arranged in *textura angularis*, the inner part comprising 3–5 layers of hyaline cells of *textura angularis*. *Hamathecium* comprising only asci. *Asci* (25–)27–30(–33) × (3.5–)4–5(–6) μm (\bar{x} = 28 × 4.7 μm, n = 70), 8-spored, unitunicate, clavate, sessile, apically rounded to truncate, with a J- apical ring. *Ascospores* (4.6–)5.8–6.7(–7.5) × (1–)1.5–2(–2.5) μm (\bar{x} = 6.2 × 1.7 μm, n = 210), overlapping 1–2-seriate, hyaline, allantoid, aseptate, smooth-walled. Asexual morph Undetermined.

Culture characteristics – Ascospores germinating on PDA within 12 hrs. Germ tubes produced from all sides. Colonies on PDA reaching 2.5–5.5 cm diam. after 5 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairly fluffy, initially white to yellow from above, yellow from below; After 10 days, yellow to brown from above, brown to dark brown from below; not producing pigments in agar.

Material examined – CHINA, Yunnan Province, Pingbian, on a dead branch of undetermined wood, 26 September 2017, Qiuju Shang, PB45 (KUN-HKAS 102161, holotype; MFLU 18-1389, isotype), ex-type living culture, MFLUCC 18-1204.

Notes – The phylogenetic result (Fig. 1) shows that our strain of *Cytospora pingbianensis* (MFLUCC 18-1204) forms a distinct lineage and is close to *Cytospora platycladi* (CFCC 50504, CFCC 50505) with the high support (100% ML, 99% MP and 1 PP). These taxa form a sister clade to *Cytospora lumnitzericola* with moderate support (89% ML, 75% MP and 0.99 PP). *Cytospora platycladi* and *C. lumnitzericola* were reported as asexual morph taxa associated with canker disease (Norphanphoun et al. 2018, Fan et al. 2020), while our taxon is only reported as a sexual morph. In this study, *C. pingbianensis* can be recognized as a phylogenetically distinct species (Fig. 1), and it is introduced as new species with detailed description and illustration of the sexual morph.

Cytospora predappioensis Q.J. Shang, Norphanph., Camporesi & K.D. Hyde, *Mycosphere* 9(2): 376 (2018) Figs 7–9

Index Fungorum number: IF554083; Facesoffungi number: FoF03936

Saprobic on the bark. Sexual morph *Stromata* 647–2225 μm wide, with the poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent the bark by the ostiolar canal, dark brown to black, glabrous, circular to irregular in shape, arranged with conspicuous, clustered, roundish to cylindrical prominent ostioles. *Ascomata* (excluding necks) 209–413 μm high, 194–360 μm diam. (\bar{x} = 300 × 277 μm, n = 10), perithecial, immersed in a stroma, dark brown to brown, globose to subglobose, glabrous, individual ostiole with the neck. *Ostiolar canal* 434–1185 μm high, 104–145 μm diam. (\bar{x} = 725 × 117 μm, n = 15), cylindrical, sulcate, periphysate. *Peridium* 23–40 μm wide, composed of two section layers, outer section comprising 5–8 layers, of relatively small, brown to dark brown, thick-walled cells, arranged in *textura angularis*, the inner part comprising 2–3 layers of hyaline cells of *textura angularis*. *Hamathecium* comprising only asci. *Asci* (23–)25–38(–50) × (3.5–)4–6.5(–8.5) μm (\bar{x} = 32 × 5.4 μm, n = 60), 8-spored, unitunicate, clavate, sessile, apically rounded to truncate, with a J- apical ring. *Ascospores* (6.5–)7–10 (–12) × (1.5–)1.7–3(–3.5) μm (\bar{x} = 8.4 × 2.2 μm, n = 120), overlapping 1–3-seriate, hyaline, allantoid, aseptate, smooth-walled. Asexual morph Coelomycetous. *Conidiomata* 540–665 μm, pycnidial, with 2–4-loculate, appearing as beige-white to brown, watery, rounded, conidial masses, superficial, solitary or aggregated, globose, shiny, with white to brown mycelium covering the surface. *Pycnidial walls* 13–21 μm wide, comprising several layers of brown, compressed hyphae, arranged in a *textura intricata*. *Conidiogenous cells* 8.5–10.5(–11) × 1.5–2.7(–3) (\bar{x} = 9.7 × 2 μm, n = 5), cylindrical to clavate, holoblastic, straight. *Conidia* (4.5–)5–6.5(–8) × (0.8–)1–1.5(–1.8) (\bar{x} = 6 × 1.4 μm, n = 75), hyaline, allantoid, unicellular, smooth-walled.

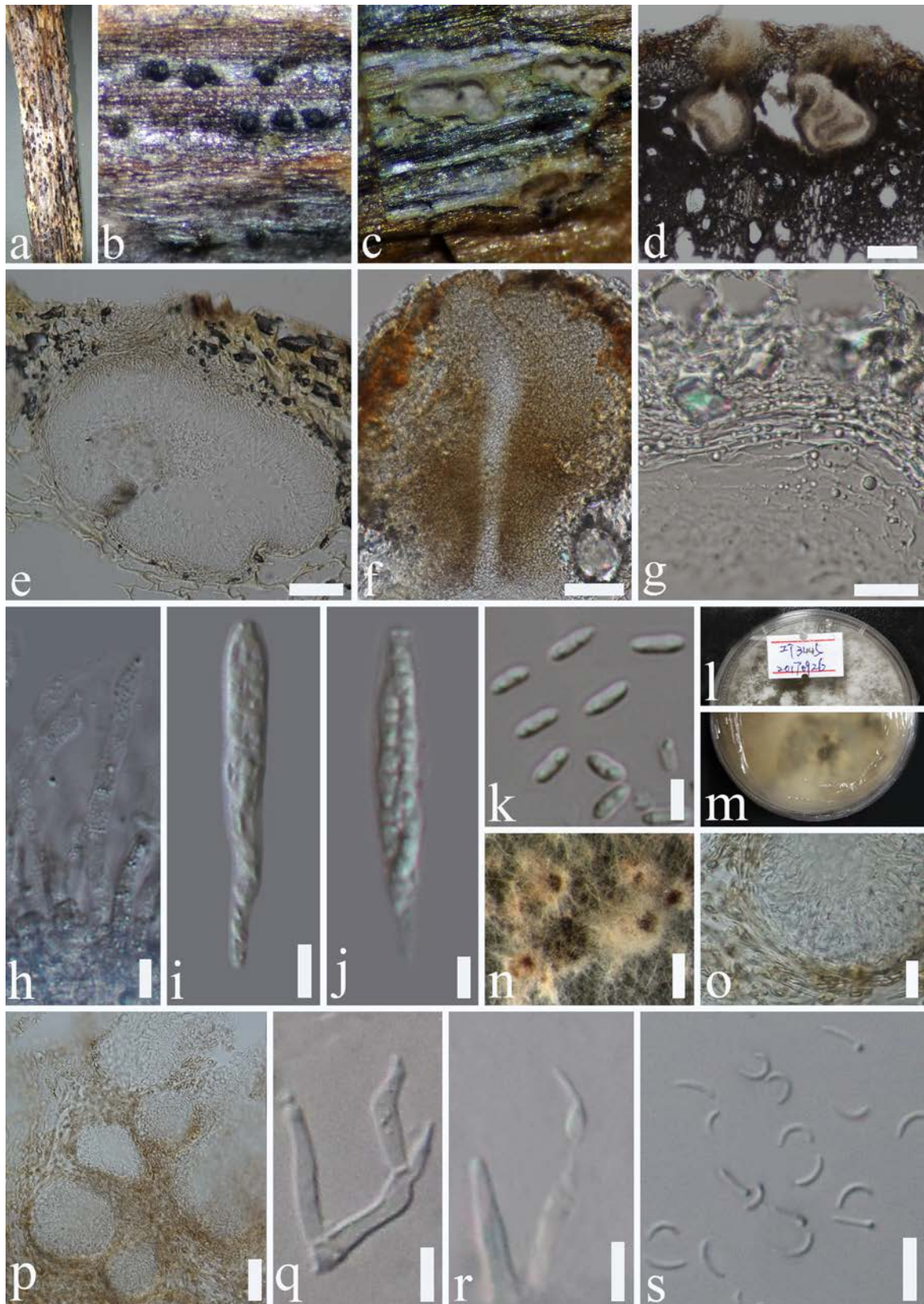


Figure 5 – *Cytospora galegaicola* (MFLU 16-2280, holotype). a Host substrate. b Appearance of stromata on the substrate. c Cross section through stroma. d Vertical section through stroma. e Ascoma. f Ostiolar canal. g Peridium. h Paraphyses. i, j Asci. k Ascospores. l, m Culture characteristic on PDA after 10 days (l = colony from above, m = colony from below). n Conidiomata on PDA. o. Peridium. p. Section of conidioma. q, r. Conidia attached to conidiogenous cells. s. Conidia. Scale bars: d = 200 μm, e, f = 100 μm, g = 50 μm, h, o, p = 20 μm, i–k, s = 10 μm, q, r = 5 μm.

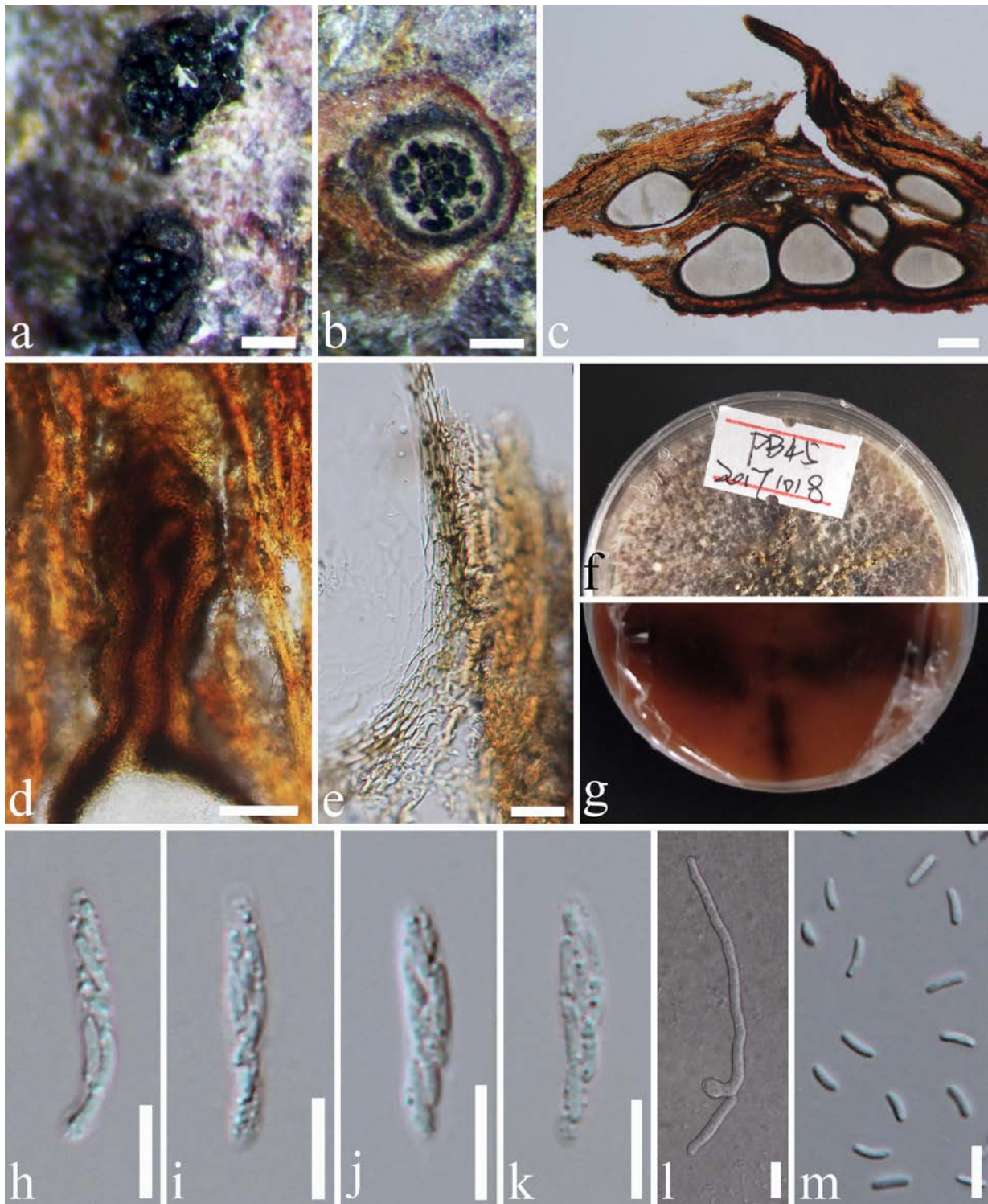


Figure 6 – *Cytospora pingbianensis* (KUN-HKAS 102161, holotype). a Stromata. b Cross section through stroma. c Vertical section through stroma. d Ostiolar canal. e Peridium. f, g Culture characteristic on PDA (f = colony from above, g = colony from below). h–k Asci. l Germinating ascospore. m Ascospores. Scale bars: a, b = 200 μm , c = 100 μm , d = 50 μm , e, l = 20 μm , h–k, m = 10 μm .

Culture characteristics – Ascospores germinating on PDA within 24 hrs. Germ tubes produced from all sides. Colonies on PDA reaching and 5–5.5 cm diam. after 15 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairly fluffy, white from above, white to yellow from below after 5 days, dark brown from above, brown to dark brown from below after 15 days; not producing pigments in agar.

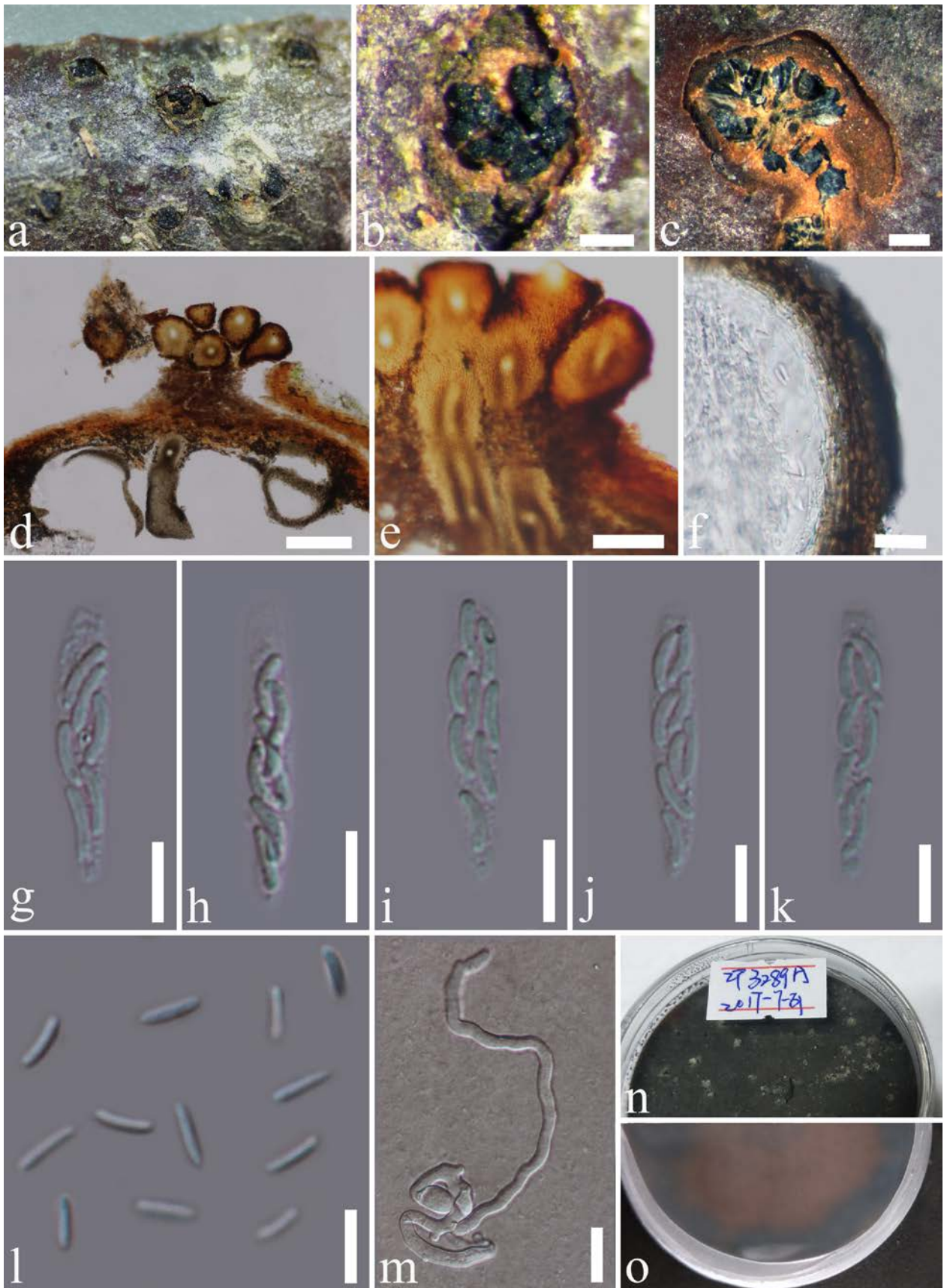


Figure 7 – *Cytospora predappioensis* (MFLU 17-0846, MFLU 17-0836). a Appearance of stromata on the substrate. b Stroma. c Cross section through stroma. d Vertical section through stroma. e Ostiolar canal. f Peridium. g–k Asci. l Ascospores. m Germinating ascospore. n, o Culture characteristic on PDA after 10 days (n = colony from above, o = colony from below). Scale bars: c–d = 200 μ m, e = 100 μ m, g–l = 10 μ m, m = 20 μ m.

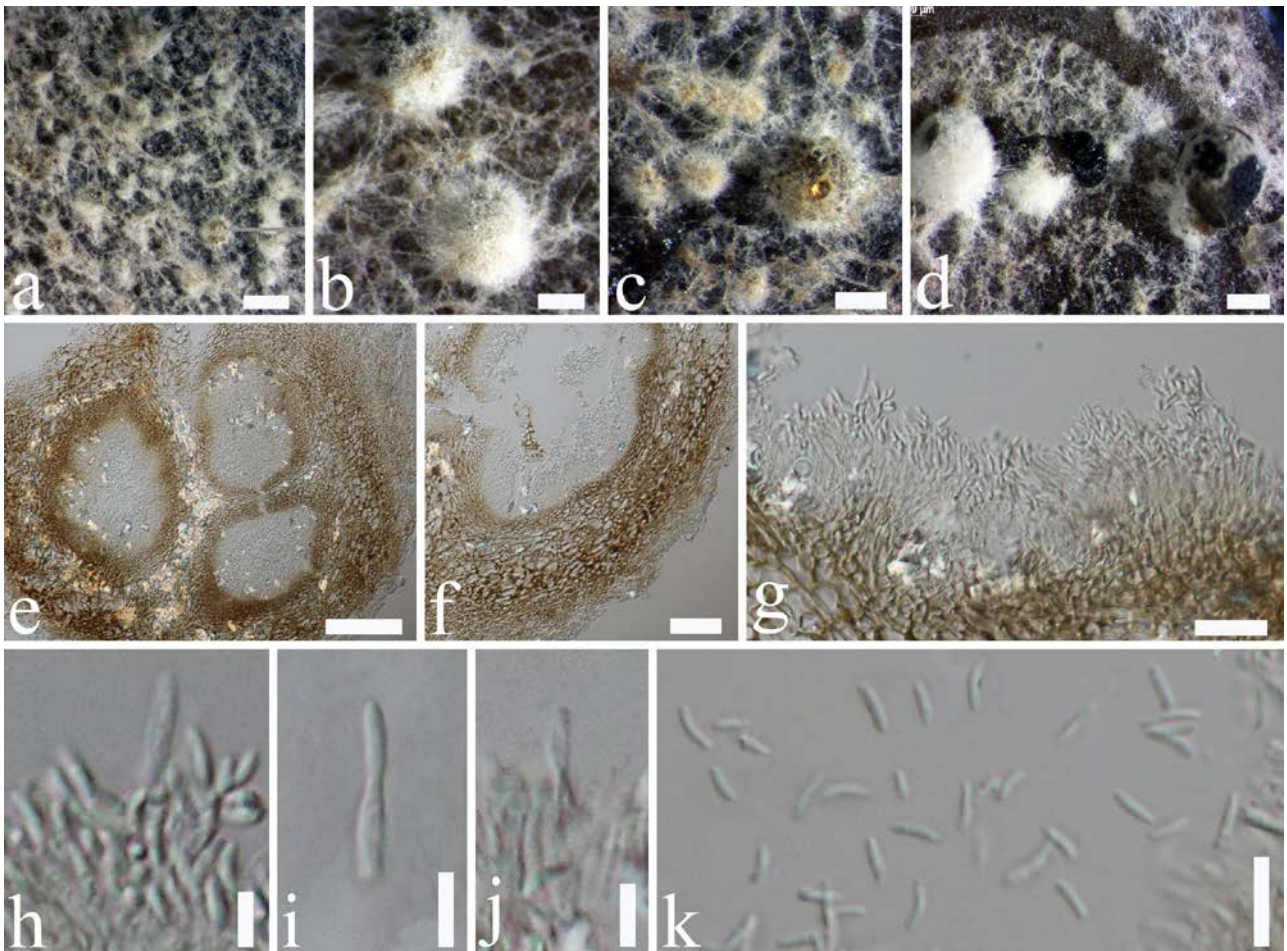


Figure 8 – *Cytospora predappioensis* (MFLUCC 18-1202). a Culture surface on PDA. b–d Conidiomata on PDA. e Section of the conidioma. f Peridium. g–j Conidia attached to conidiogenous cells. k Conidia. Scale bars: a = 1 mm, b–c = 500 μm , e = 100 μm , f = 50 μm , g = 20 μm , h, j = 5 μm , i, k = 10 μm .

Materials examined – ITALY, near Predappio (Province of Forlì-Cesena [FC]), on a dead land branch of *Ostrya carpinifolia*, 20 March 2017, E. Camporesi, IT 3289A (MFLU 17-0846, KUN-HKAS 100915), living culture, MFLUCC 18-1202. ITALY, Camposonardo, Santa Sofia (Province of Forlì-Cesena [FC]) Province, on a dead land branch of *O. carpinifolia*, 17 March 2017, E. Camporesi, IT 3289 (MFLU 17-0836, KUN-HKAS 102162). CHINA, Yunnan Province, Kunming, on a dead land branch of *Cupressus* sp., 29 March 2017, Qiuju Shang, SSHJ01 (MFLU 18-1388, KUN-HKAS 100951), living culture, MFLUCC 18-1205.

Notes – The phylogenetic result (Fig. 1) shows that the newly obtained isolates (MFLUCC 18-1202 and MFLUCC 18-1205) clustered together with taxa of *Cytospora ceratospermopsis* (CFCC 89626 and CFCC 89627), *Cytospora predappioensis* (MFLUCC 17-2458, MFLUCC 17-0327) and *Cytospora sacculus* (CFCC 89624) and can be identified as *C. predappioensis*. To confirm this identification, the single gene comparison of ITS, LSU and ACT gene regions was carried out between MFLUCC 18-1202, MFLUCC 18-1205 and MFLUCC 17-2458 (the ex-type of *C. predappioensis*), the result (Table 4) showed that there is no significant difference in nucleotides of ITS, LSU, and ACT gene. In addition, our new collections are morphologically identical to *C. predappioensis* except their longer ostiolar canals (Table 2, Hyde et al. 2018). Therefore, we conservatively identify our specimen as *C. predappioensis* and speculate that the phylogeny of this taxon is complex, and provide the holomorph description from a different host (MFLU 17-0836, MFLU 17-0846, *Ostrya carpinifolia* vs. MFLU 17-0323, *Platanus hybrida*, holotype; Figs 7, 8) and a new record (MFLU 18-1388, *Cupressus* sp., Fig. 9) from China (Hyde et al. 2018).

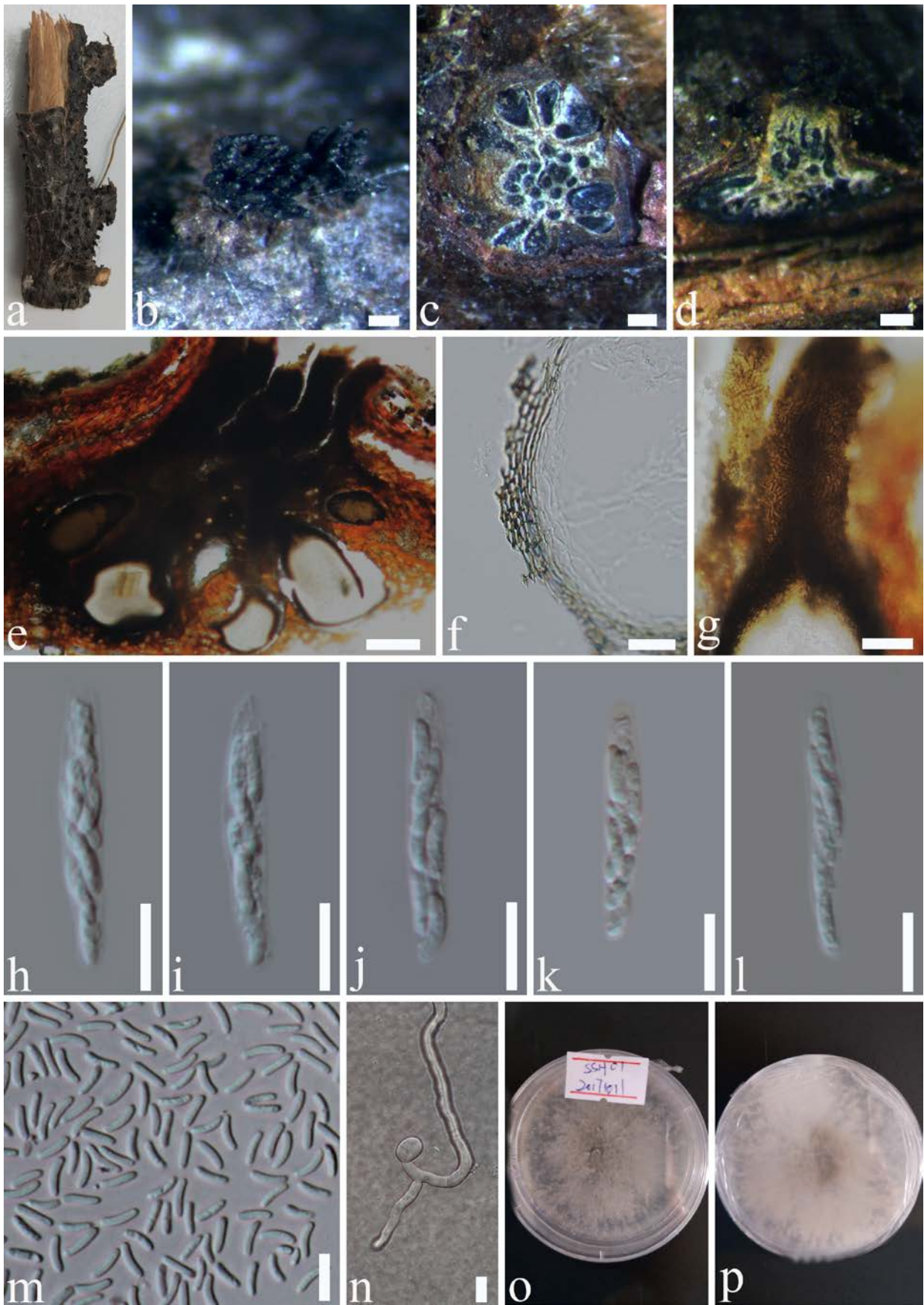


Figure 9 – *Cytospora predappioensis* (MFLU 18-1388). a Host substrate. b Stroma. c Cross section through stroma. d, e Vertical section through stroma. f Peridium. g Ostiolar canal. h–l Asci. m. Ascospores. n Germinating ascospore. o, p Culture characteristic on PDA (o = colony from above, p = colony from below). Scale bars: b–e = 200 μ m, f, n = 20 μ m, g–m = 10 μ m.

Index Fungorum number: IF 554078; Facesoffungi number: FoF04097

Saprobic on the bark. Sexual morph *Stromata* 622–908 µm wide, with the poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent the bark by the ostiolar canal, dark brown to black, glabrous, circular in shape, arranged with conspicuous, roundish to cylindrical prominent ostioles. *Ascomata* (excluding necks) 187–270 µm high, 159–289 µm diam. (\bar{x} = 229 × 224 µm, n = 15), perithecial, immersed in a stroma, dark brown to brown, globose to subglobose, glabrous, individual ostiole with the neck. *Ostiolar canal* 215–250 µm high, 57–122 µm diam., cylindrical, sulcate, periphysate. *Peridium* 29–48 µm wide, composed of two section layers, outer section comprising 2–5 layers, of relatively small, brown to dark brown, thick-walled cells, arranged in *textura angularis*, the inner part comprising 2–3 layers of hyaline cells of *textura angularis*. *Hamathecium* composed of 6–9 µm wide, dense, cylindrical, septate, hyaline, paraphyses. *Asci* (45–)55–68(–78) × (7.5–)8.5–10.5(–12) µm (\bar{x} = 62 × 9.5 µm, n = 50), 8-spored, unitunicate, clavate, with short stalks, apically rounded to truncate, with a J- apical ring. *Ascospores* (9.5–)10.5–13.5(–15.5) × (3–)3–4.5(–5) µm (\bar{x} = 12 × 3.7 µm, n = 80), overlapping 1–2-seriate, hyaline, allantoid, aseptate, smooth-walled. Asexual morph Coelomycetous. *Conidiomata* 259–535 µm, pycnidial, appearing as brown to black, watery, rounded, conidial masses, superficial, solitary or aggregated, subglobose, shiny, with white to brown mycelium covering the surface. *Pycnidial walls* 23–34 µm wide, comprising several layers of brown to dark brown, compressed hyphae, arranged in a *textura angularis*. *Conidiogenous cells* (6–)6.5–11(–13) × (1–)1.5–2.5(–3) (\bar{x} = 8.7 × 2 µm, n = 15), cylindrical to clavate, holoblastic, straight or curved. *Conidia* (4–)5–6.8(–8.2) × (0.8–)1–1.5(–2) (\bar{x} = 6 × 1.2 µm, n = 130), hyaline, allantoid, slightly curved ends, unicellular, smooth-walled.

Culture characteristics – Ascospores germinating on PDA within 24 hrs. Germ tubes produced from all sides. Colonies on PDA reaching 5–5.5 cm diam. after 5 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, initially fluffy to fairly fluffy, white from above, light yellow to brown from below; After 10 days, white to brown from above, brown to dark brown from below; not producing pigments in agar.

Material examined – Italy, Predappio Alta, Predappio (Province of Forlì-Cesena [FC]), on a dead land branch of *Ostrya carpinifolia*, 22 November 2016, E. Camporesi, IT 3162 (MFLU 16-2900, KUN-HKAS S100888), living culture, MFLUCC 18-1200.

Notes – The phylogenetic result (Fig. 1) shows that our strain MFLUCC 18-1200 clusters together with *Cytospora prunicola* (MFLU 17-0995) with 54% ML, 84% MP and 0.82 PP support and they share the sister relationship to *Cytospora gutnerae* (214) and *Cytospora terebinthi* (227). The conidia of our strain (MFLUCC 18-1200), on PDA, are similar in size to *C. prunicola* and smaller in size than *C. gutnerae* and *C. terebinthi* (Table 3). Therefore, the collection in the present study is identified as *C. prunicola* and the first sexual morph description of this species is provided here.

Cytospora pubescentis Q.J. Shang, E. Camporesi & K.D. Hyde, sp. nov.

Figs 12–13

Index Fungorum number: IF 555505; Facesoffungi number: FoF 05102

Etymology – Names reflect the host, of which the fungus was isolated from “*Quercus pubescens*”.

Holotype – MFLU 17-0727

Saprobic on the bark. Sexual morph *Stromata* 943–1461 µm wide, with the poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent the bark by the ostiolar canal, dark brown to black, glabrous, circular to irregular in shape, arranged with conspicuous, clustered, roundish to cylindrical prominent ostioles. *Ascomata* (excluding necks) 183–333 µm high, 158–259 µm diam. (\bar{x} = 258 × 209 µm, n = 18), perithecial, immersed in a stroma, brown to

dark brown, globose to subglobose, glabrous, individual ostiole with the neck. *Ostiolar canal* 300–460 μm high, 65–103 μm diam. (\bar{x} = 382 \times 84 μm , n = 15), cylindrical, sulcate, periphysate.



Figure 10 – *Cytospora prunicola* (MFLU 16-2900). a, b Appearance of stromata on the substrate. c Cross section through stroma. d Vertical section through stroma. e Peridium. f Ostiolar canal. g–k Asci. l Paraphyses. m Ascospores. n Germinating ascospores. o, p Culture characteristic on PDA (o = colony from above, p = colony from below). Scale bars: d = 100 μm , e, f = 50 μm , g–n = 10 μm .

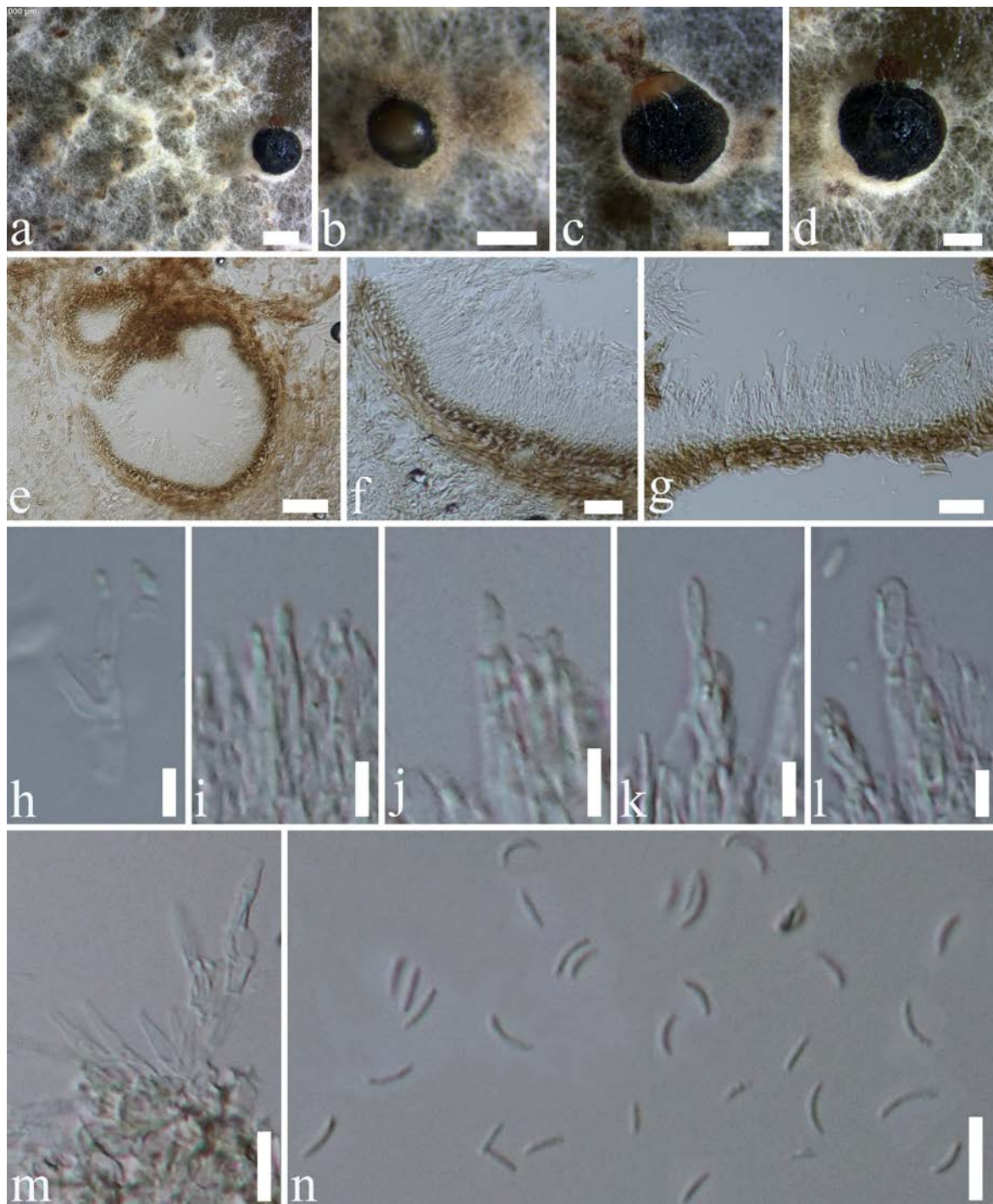


Figure 11 – *Cytospora prunicola* (MFLUCC 18-1200). a Culture surface on PDA. b–d Conidiomata on PDA. e Section of the conidioma. f Peridium. g–l Conidia attached to conidiogenous cells. m Conidiogenous cells. n Conidia. Scale bars: a = 1 mm, b–c = 500 μ m, e = 50 μ m, f, g = 20 μ m, h–l = 5 μ m, m, n = 10 μ m.

Peridium 22–38 μ m wide, composed of two section layers, outer section comprising 2–3 layers, brown to dark brown, thick-walled cells, arranged in *textura angularis*, inner part comprising 3–4 layers of hyaline cells of *textura angularis*. *Hamathecium* comprising only asci. *Asci* (28–)32–37(–40) \times (4–)5–6(–6.8) μ m (\bar{x} = 34 \times 5.5 μ m, n = 80), 8-spored, unitunicate, cylindrical to clavate, sessile, apically rounded to truncate, with a J- apical ring. *Ascospores* (5.7–)6.8–8.7(–10.3) \times (1.5–)1.9–2.7(–3.4) μ m (\bar{x} = 7.8 \times 2.3 μ m, n = 110), overlapping 1–2-seriate, hyaline, allantoid, aseptate, smooth-walled. Asexual morph Coelomycetous. *Conidiomata* pycnidial, with 2–4-loculate, appearing as brown to black, watery, rounded, conidial masses,

superficial, solitary or aggregated, globose, with beige-white to brown mycelium covering the surface. *Pycnidial walls* 60–83 μm wide, comprising several layers of brown, compressed hyphae, arranged in a *textura intricata*. *Conidiogenous cells* (7.5–) 9–22 (–28.5) \times (2.0–)2.2–4.5(–6) (\bar{x} = 15.5 \times 3.3 μm , n = 10), clavate to ampulliform, holoblastic, straight. *Conidia* (2.7–)5.8–7.5(–8.5) \times (1–)1.3–1.6(–1.8) (\bar{x} = 7.4 \times 1.5 μm , n = 85), hyaline, allantoid, unicellular, smooth-walled.

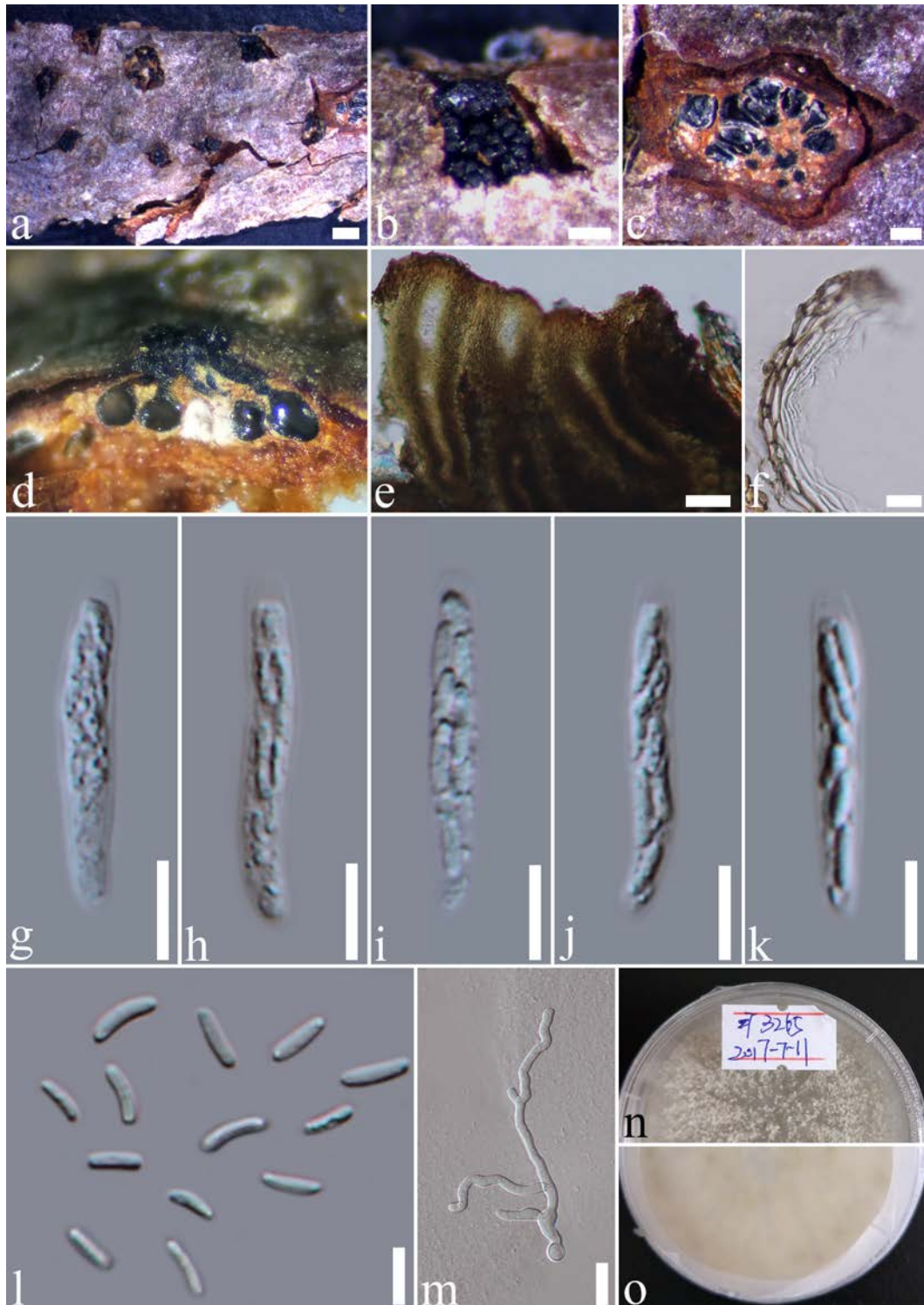


Figure 12 – *Cytospora pubescentis* (MFLU 17-0727, holotype). a Appearance of stromata on the substrate. b Stroma. c Cross section through stroma. d Vertical section through stroma. e Ostiolar canal. f Peridium. g–k Asci. l Ascospores. m Germinating ascospore. n, o Culture characteristic on PDA after 10 days (n = colony from above, o = colony from below). Scale bars: a = 500 μm , b, c = 200 μm , e = 50 μm , f–k = 10 μm , m = 20 μm .

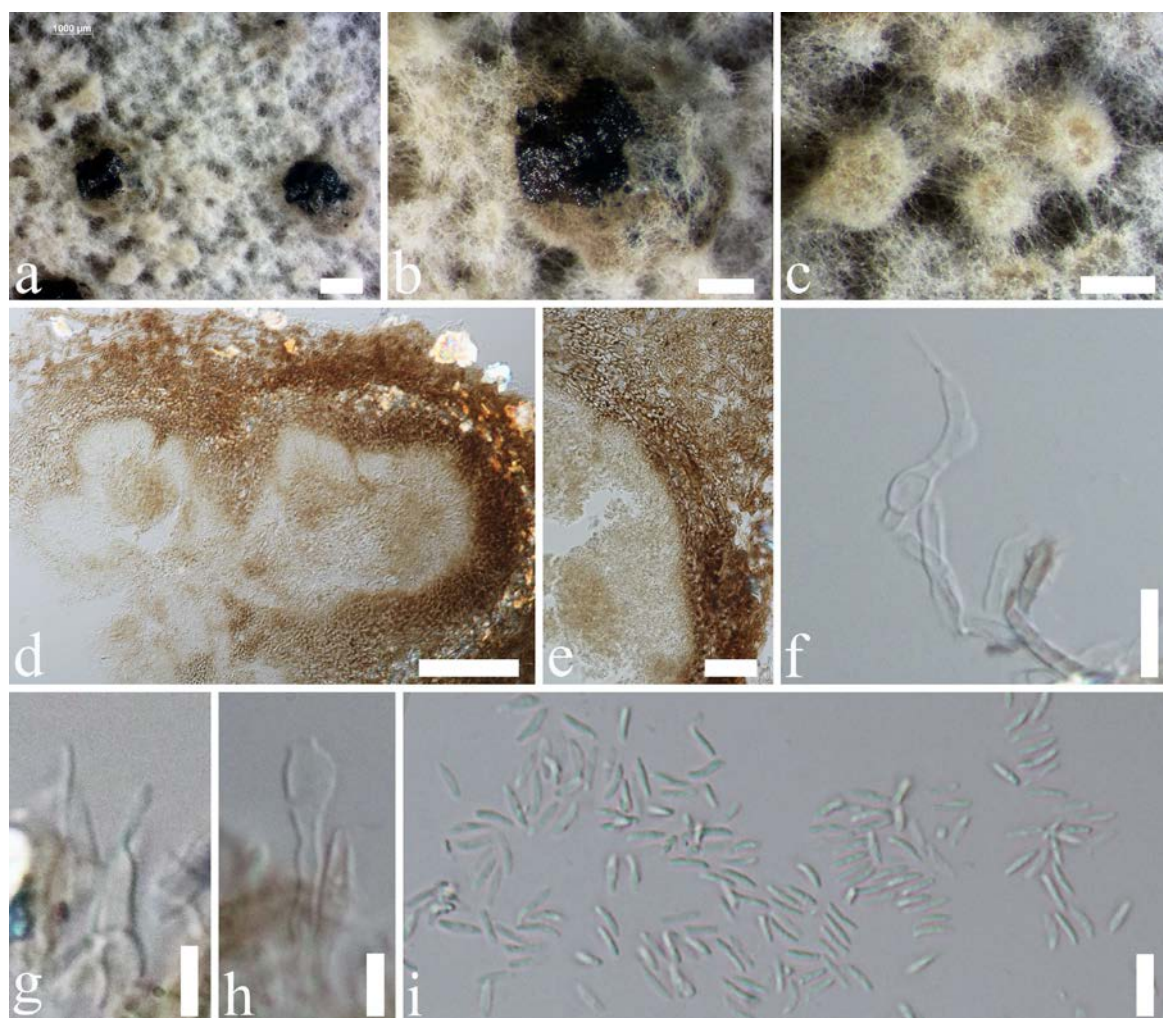


Figure 13 – *Cytospora pubescentis* (MFLUCC 18-1201, holotype). a Culture surface on PDA. b, c Conidiomata on PDA. d Section of the conidioma. e Peridium. f, g Conidia attached to conidiogenous cells. h Conidiogenous. i Conidia. Scale bars: a = 1 mm, b–c = 500 µm, d = 100 µm, e = 50 µm, f, i = 10 µm, g, h = 5 µm.

Culture characteristics – Ascospores germinating on PDA within 24 hrs. Germ tubes produced from all sides. Colonies on PDA reaching 5–5.5 cm diam. after 15 days at room temperature, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairly fluffy, white to gray from above, light yellow to brown from below; After 20 days, gray to brown from above, dark brown from below; not producing pigments in agar.

Material examined – Italy, Monte Mirabello, Predappio (Province of Forlì-Cesena [FC]), on a dead land branch of *Quercus pubescens*, 28 February 2017, E. Camporesi, IT 3265 (MFLU 17-0727, holotype; KUN-HKAS 100910, isotype), ex-type living culture, MFLUCC 18-1201.

Notes – *Cytospora pubescentis* can be distinguished from *Cytospora quercicola* by its smaller asci and ascospores. It differs from *Cytospora junipericola* by its shorter ascomata, more slender asci and larger ascospores. (Table 2, Senanayake et al. 2017). *Cytospora pubescentis* differs from *Cytospora fraxinigena* and *Cytospora rosae* by having larger asci, ascospores and ostioles (Senanayake et al. 2017). Moreover, the phylogenetic result (Fig. 1) shows that the strain *C. pubescentis* (MFLUCC 18-1201) forms a distinct lineage and shares a sister relationship to *C. fraxinigena* (MFLUCC 14-0868, BBH42442), *C. junipericola* (MFLUCC 17-0882, BBH42444), *C. quercicola* (MFLUCC 14-0867, BBH42443) and *C. rosae* (MFLUCC 14-0845, MFLUCC 17-1664) with moderate support (89% ML and 86% MP). Therefore, we identify our specimen as the new taxon *C. pubescentis* and provide the holomorph description for the species.

Table 2 Synopsis of sexual morph of *Cytospora* species and related species discussed in this study.

Species name	Stroma length/width (µm)	Ascoma length/width (µm)	Ascus length/width (µm)	Ascospore length/width (µm)	Ostiolar neck (µm)	Reference
<i>Cytospora ceratosperma</i>	300–450 × 500–550 (\bar{x} = 300 × 500)	–	(58–)60–65 × 10–11(–12) (\bar{x} = 63 × 11)	(11–)12.2–15 9 3.1–4(–4.2) (\bar{x} = 14.5 × 3.7)	450–550	Tibpromma et al. (2017)
<i>C. cotini</i> (MFLUCC 18-1203)	1190–2098	(excluding necks) 183–386 × 364–613 (\bar{x} = 284 × 489)	(56–)61–71(–85) × (6.5–)7.5–9.5(–11) (\bar{x} = 66 × 8.4)	(10.5–)12.5–15(–17) × (2.5–)3–4(–4.5) (\bar{x} = 14 × 3.5)	250–310 × 120–155 (\bar{x} = 276 × 139)	This study
<i>C. diopuiensis</i> (holotype)	800–1200	(excluding necks) 117–192 × 205–333 (\bar{x} = 154 × 269)	(23–)25–31(–34) × (4–)4.5–6(–6.5) (\bar{x} = 27.8 × 5.2)	(6.5–)7.5–8.5(–9) × (1.2–)1.5–2.5 (–3.6) (\bar{x} = 8 × 1.9)	242–520 × 95–121 (\bar{x} = 381 × 108)	This study
<i>C. fraxinigena</i> (holotype)	–	350–500 × 150–230 (\bar{x} = 429 × 189)	26–33 × 6.2–7.5 (\bar{x} = 30 × 6.7)	5.5–7.5 × 1.5–2 (\bar{x} = 6.4 × 1.7)	185–200 × 60–95 (\bar{x} = 193 × 79)	Senanayake et al. (2017)
<i>C. junipericola</i> (holotype)	–	630–700 × 150–250	30–35 × 5.5–7 (\bar{x} = 32 × 6)	5–10 × 1–2 (\bar{x} = 7 × 1.5)	300–500 × 45–65 (\bar{x} = 440 × 58)	Senanayake et al. (2017)
<i>C. pingbianensis</i> (holotype)	880–1524	(excluding necks) 142–248 × 113–245 (\bar{x} = 195 × 180)	(25–)27–30(–33) × (3.5–)4–5(–6) (\bar{x} = 28 × 4.7)	(4.6–)5.8–6.7(–7.5) × (1–)1.5–1.9(–2.5) (\bar{x} = 6.2 × 1.7)	185–722 × 27–66 (\bar{x} = 453 × 47)	This study
<i>C. predappioensis</i> (holotype)	875–2685	(excluding necks) 240–480 × 450–680 (\bar{x} = 365 × 567)	(25–)32–42(–54) × (4.5–)5.5–8(–9.8) (\bar{x} = 37 × 7.7)	(6.5–)8–10(–11) × (1–)1.5–3(–3.5) (\bar{x} = 9 × 2)	70–520 × 100–150 (\bar{x} = 444 × 124)	Hyde et al. (2018)
<i>C. predappioensis</i> (MFLUCC 18-1202, MFLUCC 18-1205)	647–2225	(excluding necks) 209–413 × 194–360 (\bar{x} = 300 × 277)	(23–)25–38(–50) × (3.5–)4–6.5(–8.5) (\bar{x} = 32 × 5.4)	(6.5–)7–10 (–12) × (1.5–)1.7–3(–3.5) (\bar{x} = 8.4 × 2.2)	434–1185 × 104–145 (\bar{x} = 725 × 117)	This study
<i>C. prunicola</i> (MFLUCC 18-1200)	622–908	(excluding necks) 187–270 × 159–289 (\bar{x} = 229 × 224)	(45–)55–68(–78) × (7.5–)8.5–10.5(–12) (\bar{x} = 62 × 9.5)	(9.5–)10.5–13.5(–15.5) × (3–)3–4.5(–5) (\bar{x} = 12 × 3.7)	215–250 × 57–122	This study
<i>C. pubescentis</i> (MFLUCC 18-1201)	943–1461	(excluding necks) 183–333 × 158–259 (\bar{x} = 258 × 209)	(28–)32–37(–40) × (4–)5–6(–6.8) (\bar{x} = 34 × 5.5)	(5.7–)6.8–8.7(–10.3) × (1.5–)1.9–2.7(–3.4) (\bar{x} = 7.8 × 2.3)	300–460 × 65–103 (\bar{x} = 382 × 84)	This study
<i>C. quercicola</i> (holotype)	–	550–725 × 160–215 (\bar{x} = 611 × 190)	75–85 × 15–19 (\bar{x} = 79 × 18)	16–20 × 4–6 (\bar{x} = 17 × 5)	285–430 × 90–130 (\bar{x} = 340 × 101)	Senanayake et al. (2017)
<i>C. rosae</i> (holotype)	–	235–255 × 130–150 (\bar{x} = 240 × 140)	20–23 × 3.2–3.7 (\bar{x} = 21 × 3.7)	4.2–6.3 × 1–1.5 (\bar{x} = 5.5 × 1.3)	27–140 × 70–90 (\bar{x} = 135 × 87)	Senanayake et al. 2017
<i>C. sordida</i> (holotype)	–	–	48–60 × 8	12 × 1.5–2	–	Nitschke (1870)
<i>C. thailandica</i> (holotype)	400–1000 × 70–250	–	(21–)23–25 × 4.1–4.7(–5) (\bar{x} = 22 × 4.3)	(5.6–)6–6.8 × 1.3–1.5(–2) (\bar{x} = 6.6 × 1.5)	70–150	Norphanhoun et al. (2018)

Table 3 Synopsis of asexual morph of *Cytospora* species and related species discussed in this study.

Species name	Conidioma length/width (µm)	Conidiogenous cell length/width (µm)	Conidium length/width (µm)	Reference
<i>Cytospora ampulliformis</i> (holotype)	680–1200 × 350–480	–	(5–)5.6–9 × 1.3–1.6(–1.7) (\bar{x} = 7.5 × 1.6)	Norphanphoun et al. (2017)
<i>C. coenobitica</i> (holotype)	–	–	5–6 × 1	Saccardo (1884)
<i>C. cotini</i> (holotype)	800–1000	–	(4.9–)5.6–6.5 × 0.8–1.4(–1.7) (\bar{x} = 5.9 × 1.2)	Hyde et al. (2016)
<i>C. galegicola</i> (holotype)	430–589	(7.2–)9.5–19.5(–23) × (1.5–)1.8–3.2(–4) (\bar{x} = 14.4 × 2.5)	(4.8–)5–6.8(–10.7) × (0.8–)1–1.5(–2.0) (\bar{x} = 7.6 × 1.3)	This study
<i>C. gelida</i> (holotype)	650–1000 × 350–450	–	(5.3–)5.7–8 × 1.4–1.8(–2) (\bar{x} = 6.9 × 1.8)	Tibpromma et al. (2017)
<i>C. gutnerae</i> (holotype)	800–1500 × 500–1000	–	8–12.5 × 3	Gvritschvili (1973)
<i>C. lumnitzericola</i> (holotype)	–	(8–)8.5–14 × 0.6–1.4(–1.6) (\bar{x} = 8.4 × 1.4)	(3.7–)4–4.5 × 1–1.3(–1.5) (\bar{x} = 4 × 1.2)	Norphanphoun et al. (2018)
<i>C. platyclade</i> (holotype)	(210–)230–300(–330) (Ectostromatic disc)	5–12 × 1–1.5	(4–)4.5–5(–5.5) × 1–1.5	Fan et al. (2020)
<i>C. predappioensis</i> (MFLUCC 18-1202)	540–665	8.5–10.5(–11) × (1.4–)1.5–2.7(–3) (\bar{x} = 9.7 × 2)	(4.5–)5–6.5(–8) × (0.8–)1–1.6(–1.8) (\bar{x} = 6 × 1.4)	This study
<i>C. prunicola</i> (holotype)	500–1000 × 450–500	–	(4–)5.2–6.6 × 1.1–1.3(–1.6) (\bar{x} = 5.5 × 1.3)	Hyde et al. (2018)
<i>C. prunicola</i> (MFLUCC 18-1200)	300–588 × 259–535	(6–)6.5–11(–13) × (1–)1.5–2.5(–3) (\bar{x} = 8.7 × 2)	(4–)5–6.8(–8.2) × (0.8–)1–1.5(–2) (\bar{x} = 6 × 1.2)	This study
<i>C. rhodophila</i> (holotype)	–	–	5–7 × 1	Saccardo (1884)
<i>C. rosae</i>	100–200	10–15 × 1–1.5 (\bar{x} = 12 × 1.2)	3–5 × 0.5–1 (\bar{x} = 2 × 1)	Senanayake et al. 2017
<i>C. subclypeata</i>	500–750	25 × 1	4–5 × 1	Saccardo (1896)
<i>C. terebinthi</i> (holotype)	500–667	–	6–7 × 1–1.5	Bresadola (1892)

Table 4 Nucleotide differences of *Cytospora* species and related species discussed in this study.

New Taxa	Strains	Taxa compared with	Fragment length, Gap, Identities (%), Query cover (%), Difference (%)				References
			ITS	LSU	ACT	RPB2	
<i>Cytospora cedri</i>	MFLUCC 18-1219a	<i>Cytospora cedri</i> (CBS 196.50)	542, 0, 99.81, 97, 0.19	–	–	–	Adams et al. (2002)
	MFLUCC 18-1219b	<i>C. cedri</i> (CBS 196.50)	551, 0, 100, 94, 0	–	–	–	Adams et al. (2003)
<i>C. cotini</i>	MFLUCC 18-1203	<i>C. cotini</i> (MFLUCC 14-1050 ^T)	557, 0, 99.61, 92, 0.39	811, 0, 100, 100, 0	–	784, 0, 100, 100, 0	Norphanphoun et al. (2017)
		<i>C. ampulliformis</i> (MFLUCC 16-0583 ^T)	557, 0, 99.60, 90, 0.4	811, 2, 99.3, 100, 0.7	235, 0, 99.55, 94, 0.45	784, 0, 99.73, 94, 0.27	Norphanphoun et al. (2017)
		<i>C. ceratosperma</i> (MFLUCC 16-0625)	577, 1, 99.80, 89, 0.2	811, 2, 98.8, 100, 1.2	235, 3, 97.7, 86.9, 2.3	784, 0, 99.06, 94, 0.94	Tibpromma et al. (2017)
		<i>C. gelida</i> (MFLUCC 16-0634 ^T)	557, 0, 98.62, 91, 1.38	811, 2, 98.6, 99.4, 1.4	235, 1, 98.6, 86.6, 1.4	784, 0, 100, 94, 0	Tibpromma et al. (2017)
<i>C. diopuiensis</i>	MFLUCC 18-1419	<i>C. diopuiensis</i> (GJJM16)	530, 0, 99.22, 96, 0.78	–	–	–	Jayanthi et al. (2018)
<i>C. predappioensis</i>	MFLUCC 18-1202	<i>C. predappioensis</i> (MFLUCC 17-2458 ^T)	533, 2, 99.81, 95, 0.19	650, 0, 99.7, 100, 0.19	174, 0, 98, 78.4, 2	–	Hyde et al. (2018)
	MFLUCC 18-1205	<i>C. predappioensis</i> (MFLUCC 17-2458 ^T)	586, 0, 99.3, 95, 0.7	650, 0, 100, 100, 0	173, 0, 96, 78.6, 4	–	Hyde et al. (2018)

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