Article

Cryptosporella platyphylla, a new species associated with *Betula platyphylla* in China

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Abstract

Members of *Cryptosporella* are well-known as common endophytes, and occasionally, as pathogens on a narrow range of hosts in Betulaceae, Tiliaceae and Ulmaceae. Two fresh specimens associated with canker and dieback of *Betula platyphylla* were made in Beijing, China in 2015. Morphological and multi-gene, combined, phylogenetic analyses (ITS, tef1-α and β-tub) support these speciemens as a distinct and new species of *Cryptosporella*, from a unique host, *Betula platyphylla*. *Cryptosporella platyphylla* sp. nov. is introduced with an illustrated account and differs from similar species in its host association and multigene phylogeny.

Key words: Diaporthales, *Disculina*, Gnomoniaceae, systematics, taxonomy

Introduction

Cryptosporella was introduced by Saccardo (1877) and was distinguished from *Cryptospora* based on the morphology of the ascospores. The genus subsequently entered a long period of confusion with the gnomoniaceous genera *Ophiovalsa* Petr. and *Winterella* (Sacc.) O. Kuntze. Mejía *et al.* (2008) regarded *Ophiovalsa* and *Winterella* as synonyms of *Cryptosporella,* which is based on type species *C. hypodermia* (Fr.) Sacc., and accepted nine species in this genus. Mejía *et al.* (2011) added a further ten species to the genus supported by DNA data from three genes (ITS, tef1-α and β-tub). Most *Cryptosporella* species are distributed in temperate regions, e.g., Europe, Japan, and North America as endophytes, and occasionally as saprobes and pathogens on hardwood trees, i.e., Betulaceae, Tiliaceae and Ulmaceae (Saccardo 1877, Kobayashi 1970, Barr 1978, Mejía *et al.* 2011). *Cryptosporella* is characterized by aggregated ascomata below the bark surface, with converging necks and ellipsoid to elongated, aseptate or rarely 1-septate ascospores. Additionally, their *Disculina* Höhn. asexual morph is characterized by hyaline, aseptate conidia, arising from proliferating conidiogenous cells (Rossman *et al.* 2007, Mejía *et al.* 2008, 2011). *Cryptosporella* is presently placed in Gnomoniaceae (Castlebury *et al.* 2002, Maharachchikumbura *et al.* 2015).

During an investigation of forest pathogens that cause canker or dieback disease in China, two *Cryptosporella* specimens were collected from *Betula platyphylla* Sukaczev in Beijing, China. These specimens are characterized by slightly erumpent, conidiomatal stromata with conical central column and dark brown ectostromatic disc, and hyaline, cylindrical to ellipsoidal, aseptate conidia (51 × 4 μm), arising from narrowly cylindrical, conidiogenous cells. Phylogenetic analysis of combined ITS, tef1-α and β-tub sequence data suggested that these specimens represent a new species, supported by high bootstrap values. Therefore, the objective of this study is to introduce *Cryptosporella platyphylla* sp. nov. with a description and illustrations and compare it with other species in the genus.

Materials and Methods

Isolates

Two specimens of gnomoniaceous fungi were collected from infected branches or twigs of *Betula platyphylla* during collecting trips in Beijing, China. Two strains of *Cryptosporella* were obtained following the method of Chomnunti *et al.* (2014), where a mucoid spore mass was removed from the conidiomata, and transferred to the surface of 1.8 % potato dextrose agar (PDA) in a Petri-dish, and incubated at 25 °C for up to 24 h. Single germinating conidia were transferred to fresh PDA plates. The representative strains were deposited and now maintained at the China Forestry Culture Collection Center (CFCC) and Beijing Forestry University (BJFU) under strain numbers CFCC 50465 and CFCC 50466. Specimens are deposited in the Museum of the Beijing Forestry University (BJFC) under collection numbers BJFC-S1313 and BJFC-S1314.

DNA amplification, sequencing and phylogeny

Genomic DNA was obtained from colonies grown on PDA using a CTAB method (Doyle and Doyle 1990). The ITS region was amplified using primers ITS1 and ITS4 (White *et al.* 1990). The partial translation elongation factor 1-alpha (tef1-α) gene region was amplified using primers EF1-728F and EF1-986R (Carbone and Kohn 1999). The partial β-tubulin (β-tub) was amplified using primers Bt2a and Bt2b (Glass and Donaldson 1995). DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China). DNA sequences were deposited in GenBank (Table 1) and a data matrix (expanded from the work of Mejía *et al.* 2011) were deposited in TreeBASE (www.treebase.org) as accession S18571. The current sequences and ex-type reference sequences selected from recent studies and GenBank were aligned in MAFFT v.7 (Katoh and Standley 2013) and checked manually.

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

Species	Strain	Host	GenBank Assession Number		
			ITS	**tef**	**β-tub**
C. alnicola	CBS121074	*Corylus cornuta*	EU199204	EU199160	EU219138
C. alni-rubrae	CBS126120	*Alnus rubra*	GU826092	GU826051	GU826010
C. alni-rubrae	LCM411	*Alnus rubra*	GU826090	GU826049	GU826008
C. alni-rubrae[T]	LCM499.01	*Alnus rubra*	GU826096	GU826055	GU826014
C. alni-sinuatae	AR4200	*Alnus viridis* ssp. *sinuata*	GU826086	GU826045	GU825989
C. alni-sinuatae[T]	CBS125662	*Alnus viridis* ssp. *sinuata*	GU826087	GU826046	GU826005
C. alni-tenuifoliae[T]	CBS125663	*Alnus incana* ssp. *tenuifol*	GU826097	GU826056	GU826015
C. amistadensis	LCM618.01	*Alnus acuminata*	GU826109	GU826073	GU826032
C. amistadensis[T]	CBS125664	*Alnus acuminata*	GU826108	GU826072	GU826031
C. betulae	CBS121078	*Betula pendula*	EU199213	GU826057	GU826016
C. betulae	LCM477.01	*Betula pendula*	GU826098	GU826059	GU826018
C. betulae	CBS121079	*Betula pendula*	EU199216	GU826058	GU826017
C. confusa	CBS121063	*Betula papyrifera*	EU199219	-	-
C. corylina	LCM391.04	*Corylus avellana*	GU826100	GU826063	GU826022
C. femoralis	LCM196.04	*Alnus incana* ssp. *rugosa*	GU826102	GU826067	GU826025
C. femoralis[T]	CBS121076	*Alnus incana* ssp. *rugosa*	EU199220	EU219139	EU221951
C. hypodermia	CBS109753	*Ulmus minor*	EU199224	GU826064	GU826023
C. hypodermia[T]	CBS122593	*Ulmus minor*	EU199181	GU826066	GU826024
C. jaklitschii	LCM112.01	*Alnus serrulata*	GU826089	GU826048	GU826007

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TABLE 1. (Continued)

Species	Strain	Host	GenBank Assession Number		
			ITS	**tef**	**β-tub**
C. jaklitschii[T]	CBS125665	*Alnus serrulata*	GU826088	GU826047	GU826006
C. marylandica	LCM386.05	*Alnus maritima*	GU826106	GU826070	GU826029
C. marylandica	LCM581.01	*Alnus maritima*	GU826107	GU826071	GU826030
C. marylandica[T]	CBS125666	*Alnus maritima*	GU826105	GU826069	GU826028
C. multicontinentalis	CBS126119	*Alnus incana* ssp. *rugosa*	GU826081	GU826040	GU825999
C. multicontinentalis	LCM93b.02	*Alnus incana* ssp. *rugosa*	GU826082	GU826041	GU826000
C. multicontinentalis	LCM427.01	*Alnus glutinosa*	GU826085	GU826044	GU826004
C. multicontinentalis[T]	LCM401.01	*Alnus glutinosa*	GU826083	GU826042	GU826001
C. pacifica	CBS122311	*Alnus viridis* ssp. *sinuata*	EU199208	GU826034	GU825991
C. pacifica	LCM453.01	*Alnus incana* ssp. *tenuifolia*	GU826077	GU826037	GU825995
C. pacifica[T]	LCM461.01	*Alnus incana* ssp. *tenuifolia*	GU826076	GU826036	GU825994
C. platyphylla	**CFCC50466**	**Betula platyphylla**	**KT732947**	**KT733015**	**KT733019**
C. platyphylla[T]	**CFCC50465**	**Betula platyphylla**	**KT732946**	**KT733014**	**KT733018**
C. suffusa	CBS121077	*Alnus incana*	EU199184	EU221891	EU219127
C. suffusa	LCM576.01	*Alnus* sp.	GU826079	GU826039	GU825997
C. suffusa[T]	CBS109750	*Alnus incana*	EU199207	EU221945	EU219106
C. tomentella	CBS121078	*Betula* sp.	EU199213	GU826057	GU826016
C. tomentella	CBS121079	*Betula* sp.	EU199216	GU826058	GU826017
C. tomentella	LCM184b.01	*Betula alleghaniensis*	GU826099	GU826062	GU826021
C. wehmeyeriana	CBS121085	*Tilia sp.*	EU199205	EU221959	EU219110
C. wehmeyeriana	LCM85.02	*Tilia americana*	GU826104	GU826068	GU826027
Ditopella ditopa	LCM94.02	*Alnus incana* ssp. *rugosa*	GU826075	GU826033	GU825990
Plagiostoma petiolophillum	LCM181.01	*Acer spicatum*	GU367078	GU367112	GU367023

The new strains from the current study are in bold. Ex-type taxa are marked with a T.

Phylogenetic analyses were performed by maximum parsimony (MP), and confirmed using MrBayes v.3.1.2 for Bayesian inference (BI) and PhyML v.7.2.8 for maximum likelihood (ML) (Ronquist and Huelsenbeck 2003, Guindon *et al.* 2010). MP analysis was performed by a heuristic search algorithm (1,000 random-addition sequences) with a tree bisection and reconnection (TBR) branch swapping. Maxtrees were set to 5,000, branches of zero length were collapsed and all equally parsimonious trees were saved. Descriptive tree statistics (Tree Length [TL], Consistency Index [CI], Retention Index [RI] and Rescaled Consistency [RC] were calculated. ML analysis was also performed with a GTR site substitution model following previous studies (Guindon *et al.* 2010, Fan *et al.* 2015). A bootstrapping (BS) analysis (1,000 replicates) was calculated to assess the branch support (Hillis and Bull 1993). Bayesian inference (BI) analysis was done by a Markov Chain Monte Carlo (MCMC) algorithm (Rannala and Yang 1996). A nucleotide substitution model was estimated by MrModeltest v.2.3 (Posada and Crandall 1998). For the analysis, two MCMC chains were run from random trees for 1,000,000 generations, and trees were sampled every 100th generation, resulting in 10,000 total trees. The first 25 % of trees were discarded as the burn-in phase of each analysis. Branches with significant Bayesian posterior probabilities (BPP) were estimated in the remaining 7,500 trees. Trees are shown using FigTree v.1.4.0 (http://tree.bio.ed.ac.uk software/figtree) and the layout was performed by Adobe Illustrator CS v.6.
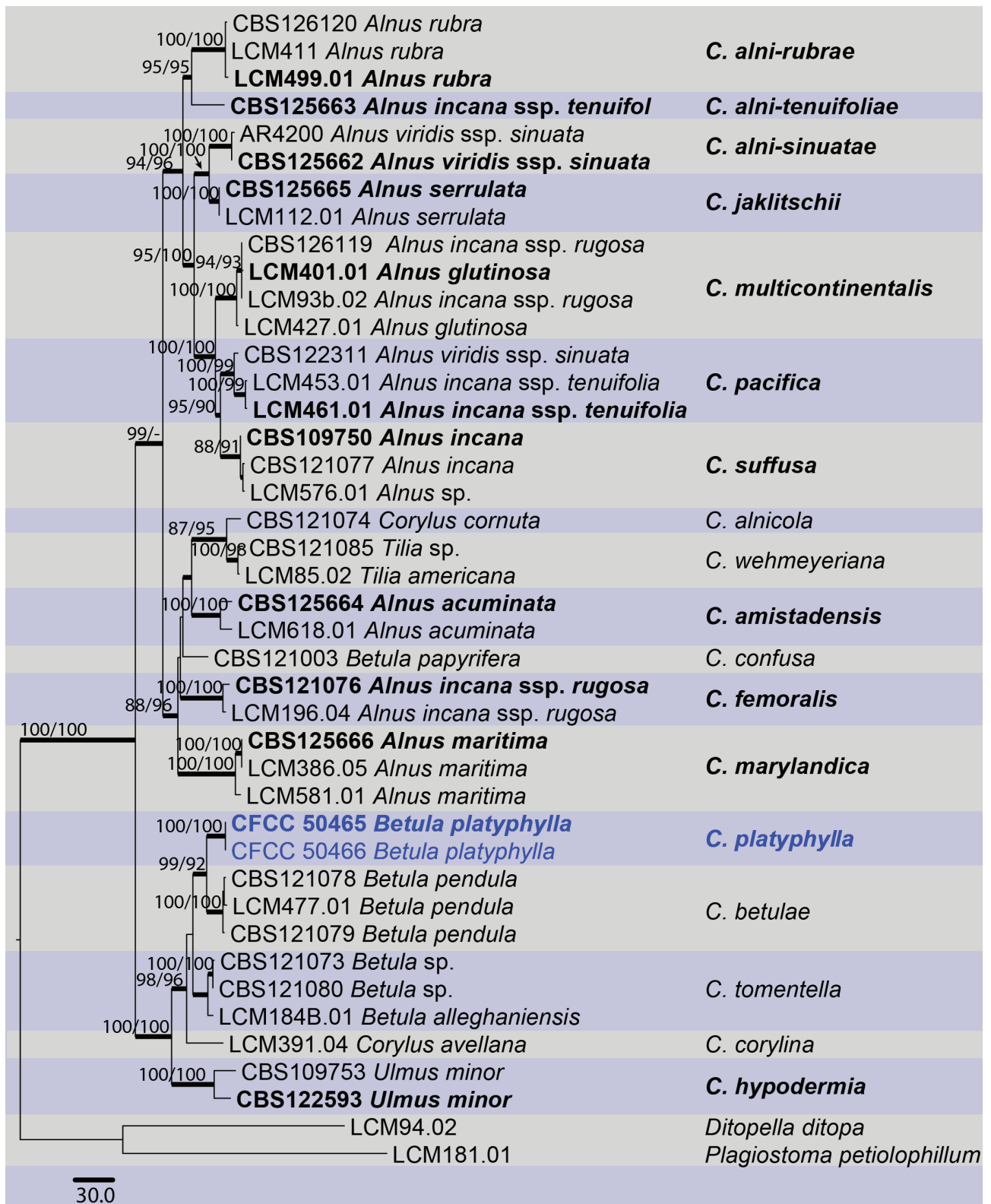
FIGURE 1. Phylogram of *Cryptosporella* based on combined ITS, tef1-α and β-tub genes. MP and ML bootstrap support values above 75 % are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Scale bar = 30 nucleotide substitutions. Ex-type strains are in bold. Strains in current study are in blue.

Morphology

Fruiting bodies produced on infected plant tissues and micromorphology and cultural characteristics were observed. Morphological characteristics of the fruiting bodies were recorded using a Leica stereomicroscope (M205 FA), including size and arrangement of stromata; presence or absence of central column in stromata; shape and size of ectostromatic

disc and ostiole. Micromorphological observations included the size and shape of conidiophores and conidia using a Leica compound microscope (DM 2500). More than 20 conidiomata were sectioned and 50 conidia were selected randomly for measurement. Cultural characteristics of strains incubated on PDA in the dark at 25 °C were recorded, including the colony colour and conidiomata distribution. Taxonomic novelties were deposited in MycoBank (Crous *et al.* 2004) with numbers MB 815811 and Faces of fungi numbers (FoF 01898) obtained as in Jayasiri *et al.* (2015).

Results

The combined ITS, tef1-α and β-tub dataset from 38 ingroup strains (sequences of two strains from this study and sequences of 36 strains available in GenBank mostly from Mejía *et al.* 2011) clustered in 18 clades representing species of *Cryptosporella* (Table 1). The alignment including gaps comprised 2864 characters of which 2062 characters were constant, 369 variable characters were parsimony-uninformative, and 433 were parsimony informative. The heuristic search generated one parsimonious tree (TL= 1238, CI = 0.823, RI = 0.883, RC = 0.726) as shown in Fig. 1. Strains CFCC 50465 and CFCC 50466, sequenced in this study, formed a well-supported clade (MP/ML/BI = 100/100/1) representing a new phylogenetic species.

Taxonomy

Cryptosporella platyphylla C.M. Tian, X.L. Fan & K.D. Hyde, ***sp. nov.***, **Fig. 2**
MycoBank MB 815811; Facesoffungi number: FoF 01898

Holotype:—BJFC-S1313. CHINA, Beijing: Tongzhou District, Jingdong Forest Park, 39°59'44.37"N, 116°39'29.16"E, 26 m asl, on twigs and branches of *Betula platyphylla*, coll. X.L. Fan, 16 May 2015 (BJFC-S1313, holotype), ex-type culture, CFCC 50465.

Etymology:—*platyphylla*, referring to *Betula platyphylla*, the only host known for this species.

Host/Distribution:—from *Betula platyphylla* in China.

Original description:—Sexual morph: Undetermined. Asexual morph: *Conidiomatal stromata* immersed, slightly erumpent from surface of host branches, separate, discoid to conical, (880–)950–1330(–1460) μm (av. = 1130 μm, n = 20) diam. *Stromatic zones* lacking. *Central column* beneath the disc more or less conical, grey to black. *Ectostromatic disc* dark brown, circular to ovoid (400–)480–610(–790) μm (av. = 560 μm, n = 20) diam. *Ostiole* one per disc, emerging in central position in the ectostromatic disc. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* narrowly cylindrical, smooth, hyaline, producing a conidium at apex. *Conidia* hyaline, aseptate, cylindrical to clavate, gently curved, (45–)46.5–53(–59) × (3–)3.5–4.5 μm (av. = 51 × 4 μm, n = 50).

Cultures:—Colony growth on PDA initially white, becoming yellowish brown to brownish after 7–10 days. Colony flat, felt-like, with a uniform texture, extending to *ca*. 66 mm diam at room temperature. Conidiomata absent.

Material examined:—CHINA, Beijing: Tongzhou District, Song Village, 39°59'40"N, 116°39'29.31"E, 10 m asl, on twigs and branches of *Betula platyphylla*, coll. X.L. Fan, 20 March 2015 (BJFC-S1314, paratype), ex-paratype culture, CFCC 50466.

Discussion

In this study two strains of *Cryptosporella* associated with *Betula platyphylla* dieback or canker disease in China were identified. Species identification was supported by morphology and multigene DNA data (ITS, tef1-α and β-tub), which indicated that *Cryptosporella platyphylla* represents a distinct species (Fig. 1). Although *Cryptosporella platyphylla* represents a closely related sister group to *Cryptosporella betulae*, the two taxa are distinguished based on their unique host and multigene phylogenetic data (Fig. 2).

Cryptosporella is a well-defined genus which seems to have taxa with narrow host ranges, frequently limited to a single host species, especially in the host family Betulaceae (Castlebury *et al.* 2002, Mejía *et al.* 2008, 2011, Sogonov *et al.* 2008). Except for *C. wehmeyeriana* on *Tilia* spp. and type species *C. hypodermia* on *Ulmus* spp. all these species

occur on hosts in Betulaceae (*Alnus*, *Betula* and *Corylus*) (Fig. 2). Seventy-four names for *Cryptosporella* are recorded in Index Fungorum (http://www.indexfungorum.org) and have caused confusion with related genera over a long time. Mejía *et al.* (2008, 2011) considered the accepted species with taxonomic evidence from living cultures and accepted 17 species in *Cryptosporella* chiefly from North America and Europe and one species, *C. amistadensis*, from Argentina and Panama. Although *Cryptosporella* species have in the past been recorded from Japan (Kobayashi 1970), specimens were not available for study. The description of *Cryptosporella platyphylla* in the present study, however, suggests that Asia could harbor many more species.
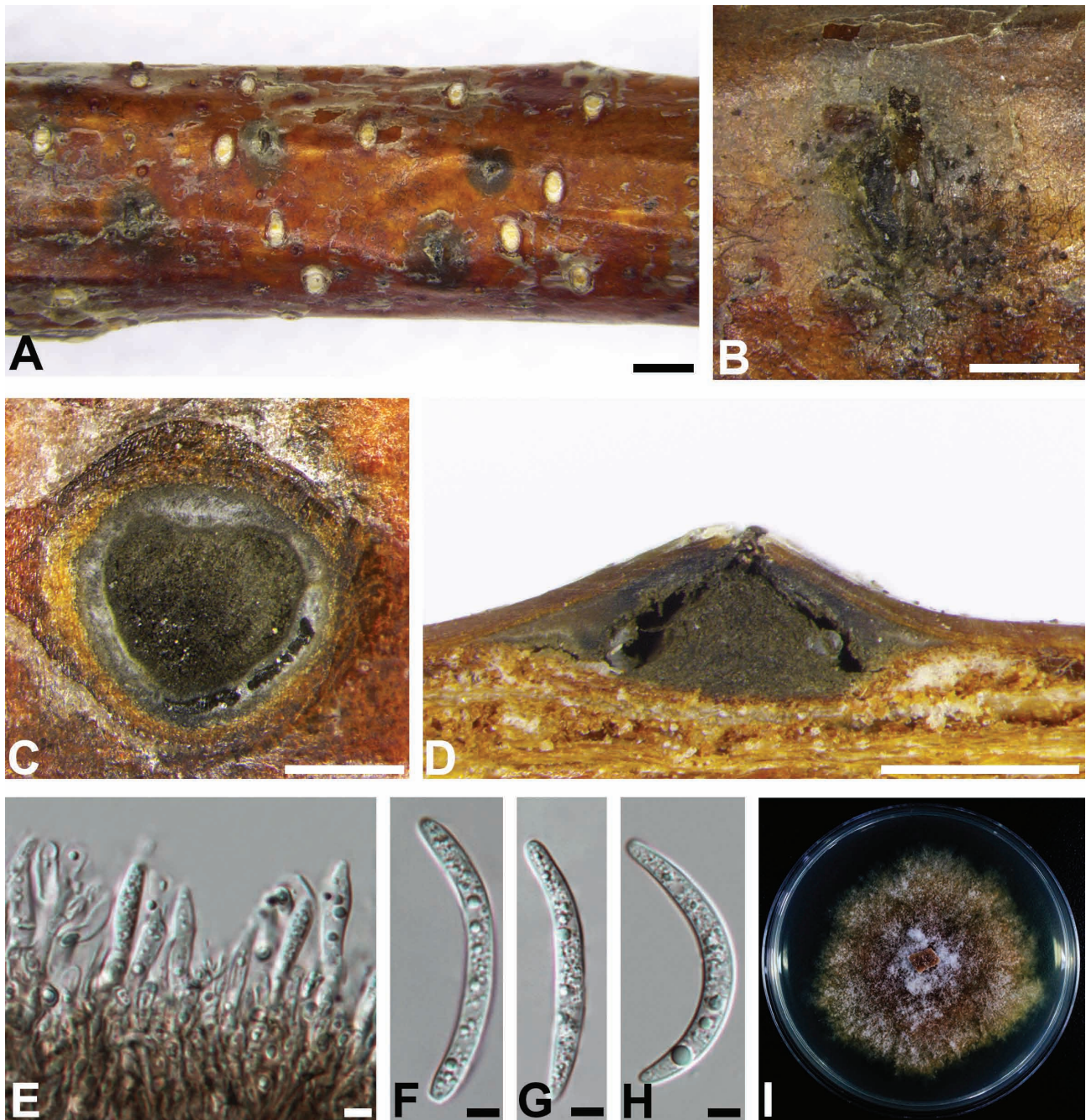
FIGURE 2. Morphology of *Cryptosporella platyphylla* from *Betula platyphylla* (BJFC-S1313). A, B: Habit of conidiomata on twig. C: Transverse section of conidioma. D: Longitudinal section through conidioma. E: Conidiophores. F–H: Conidia. I: Colonies on PDA at 30 days. Scale bars: B–D = 500 μm; E–H = 5 μm.

Acknowledgements

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