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Host and geographic range extensions of *Melanconiella*, with a new species *M. cornuta* in China

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Abstract

Members of *Melanconiella* are opportunistic pathogens and endophytic fungi, and have been found to confined so far, on the collection of host family Betulaceae. Moreover, two fresh specimens associated with canker and dieback of *Cornus controversa* and *Juglans regia* collected in Shaanxi, China were found as distinct and new species of *Melanconiella,* based on morphological and multi-gene, combined, phylogenetic analyses (ITS, LSU, rpb2 and tef1-α). Results also revealed the host and geographic range extensions of this genus. *Melanconiella cornuta sp. nov.* is introduced with an illustrated account and differs from similar species in its host association and multigene phylogeny.

Key words: Diaporthales, Melanconidaceae, systematics, taxonomy

Introduction

Melanconiella was introduced by Saccardo (1882) to accommodate *Melanconis spodiaea* Tul. & C. Tul. and an asexual state placed in *Melanconium* Link. The type of *Melanconiella* is confirmed as *M. spodiaea*. *Melanconiella* is characterized by forming circularly arranged perithecia immersed in the substrate with oblique or lateral ostioles convergent and erumpent through an ectostromatic disc and dark coloured ascospores (Saccardo 1882). The genus subsequently entered a long period of confusion with a broad concept of the melanconidacous genera *Melanconium* and *Melanconis* Tul. & C. Tul. (Wehmeyer 1937, 1941; Barr 1987). Castlebury *et al.* (2002) excluded *Melanconiella* from *Melanconidaceae* based on LSU phylogenetic analysis, and maintained *Melanconis* as the only genus in this family followed by Rossman *et al.* (2007). Senanayake *et al.* (2017) revisited families of Diaporthales and introduced Melanconiellaceae to clarify the classification of *Melanconiella*.

Voglmayr *et al.* (2012) revised the generic circumscriptions of *Melanconiella* with 13 species, and confirmed that it is genetically distinct from the genus *Melanconis* based on morphology and multi-gene phylogeny (ITS, LSU, rpb2 and tef1-α). Previously, all species of *Melanconiella* were observed to be highly host-specific as they were found to be confined mostly to the host family Betulaceae (Voglmayr *et al.* 2012), and *Melanconiella* species are confined to the north temperate zone, i.e. Europe and North America (Voglmayr *et al.* 2012). But during the investigation of forest pathogens that cause canker or dieback disease in China, two *Melanconiella* specimens were collected from *Cornus controversa* and *Juglans regia* in Shaanxi, China. These specimens were characterized by slightly erumpent, conidiomatal stromata with solitary and with single neck erumpent through the host bark, and hyaline, cylindrical to ellipsoidal, aseptate conidia with distinct hyaline sheath, arising from narrowly cylindrical, conidiogenous cells. Interestingly, phylogenetic analysis of combined ITS, LSU, rpb2 and tef1-α sequence data suggested that these specimens represent a new species belonging to *Melanconiella*, supported by high bootstrap values. Because the species of *Melanconiella* associated with cankers in China have not been identified and the asexual morph was not impressed and emphasized in taxonomy, we initiated this project. Therefore, the objectives of this study were (i) to extent the hosts and geographic ranges of *Melanconiella*, and (ii) to introduce *Melanconiella cornuta sp. nov.* with descriptions and illustrations and compare it with other species in the genus.

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Materials and Methods

Isolates

Two specimens of diaporthalean fungi were collected from infected branches or twigs of *Cornus controversa* and *Juglans regia* during collecting trips in Shaanxi, China. Two strains of *Melanconiella* were obtained following the method of Fan *et al.* (2016), where a mucoid spore mass was removed from the conidiomata, and spreading the suspension onto the surface of 1.8 % potato dextrose agar (PDA) in a Petri-dish, and incubating at 25 °C for up to 24 h. Single germinating conidia were transferred onto fresh PDA plates. Specimens were deposited in the Museum of the Beijing Forestry University (BJFC) under collection numbers BJFC-S1375 and BJFC-S1345. Axenic cultures were deposited and now maintained at the China Forestry Culture Collection Center (CFCC) under collection numbers CFCC 51990 and CFCC 51991.

Morphology

Species identification was based on morphological features of the fruiting bodies produced on infected plant tissues and micromorphology, as well as cultural characteristics. Morphological characteristics of the fruiting bodies were examined using a Leica stereomicroscope (M205 FA), including size and shape of stromata, shape and size of ectostromatic disc and ostiole. Micro-morphological observations included colour, size and shape of conidiophores or conidiogeous cells; conidia and presence or absence of sheath. More than 20 fruiting bodies were sectioned, and 50 spores were selected randomly for measurement. Cultural characteristics of isolates incubated on PDA in the dark at 25 °C were recorded, including the colony colour and conidiomata distribution. Microscopic photographs were captured using a Nikon Eclipse 80i microscope, using differential interference contrast (DIC) illumination and the Nikon software NIS-Elements D Package v. 3.00. Adobe Bridge CS v. 6 and Adobe Photoshop CS v. 5 were used for the manual editing. Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al.* 2004).

DNA amplification, sequencing and phylogeny

Genomic DNA was extracted from colonies grown on PDA using a CTAB method (Doyle & Doyle 1990). DNA was estimated by electrophoresis in 1 % agarose gels. PCR amplifications were performed in a DNA Engine (PTC-200) Peltier Thermal Cycler (Bio-Rad Laboratories, CA, USA). The ITS region was amplified using primers ITS1 and ITS4 (White *et al.* 1990), and the partial large nuclear ribosomal RNA subunit (LSU) region was amplified using primers LR0R and LR5 (Vilgalys & Hester 1990). The partial translation elongation factor 1-alpha (tef1-α) gene region was amplified using primers EF1728F (Chaverri & Samuels 2003) and TEF1LLErev (Jaklitsch *et al.* 2006). The RNA polymerase II subunit B (rpb2) was amplified using primers fRPB2-5f and fRPB2-7cr (Liu *et al.* 1999). 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 Voglmayr *et al.* 2012) were deposited in TreeBASE (www.treebase.org) as accession S21285. The datasets were initially aligned with ClustalW as implemented in MEGA 6 and improved in MAFFT v.7 (Katoh & Standley 2013; Tamura *et al.* 2013).

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

Species	Isolate[1]	Location	Host	Genbank accession numbers			
				ITS	LSU	rpb2	tef1
Melanconiella carpinicola	MNM	Austria	*Carpinus betulus*	JQ926232	JQ926232	JQ926304	JQ926370
Melanconiella carpinicola	MNUK	UK	*Carpinus betulus*	JQ926234	JQ926234	JQ926306	JQ926372
Melanconiella carpinicola[T]	MSMI	Austria	*Carpinus betulus*	JQ926235	JQ926235	JQ926307	JQ926373
Melanconiella cornuta[T]	CFCC 51990	China	*Cornus controversa*	MF360006	MF360008	MF360002	MF360004

...Continued on next page

TABLE 1. (Continued)

Species	Isolate[1]	Location	Host	Genbank accession numbers			
				ITS	LSU	rpb2	tef1
Melanconiella cornuta	CFCC 51991	China	*Juglans regia*	MF360007	MF360009	MF360003	MF360005
Melanconiella chrysodiscosporina[T]	MCH	Austria	*Carpinus betulus*	JQ926238	JQ926238	JQ926310	JQ926376
Melanconiella chrysodiscosporina	MEE	Austria	*Carpinus betulus*	JQ926240	JQ926240	JQ926312	JQ926378
Melanconiella chrysodiscosporina	MGG	Austria	*Carpinus betulus*	JQ926242	JQ926242	JQ926314	JQ926380
Melanconiella chrysomelanconium[T]	MCM	Austria	*Carpinus betulus*	JQ926247	JQ926247	JQ926319	JQ926385
Melanconiella chrysomelanconium	MEUK	UK	*Carpinus betulus*	JQ926249	JQ926249	JQ926321	JQ926387
Melanconiella chrysomelanconium	MGUK	UK	*Carpinus betulus*	JQ926255	JQ926255	JQ926327	JQ926393
Melanconiella chrysorientalis[T]	MGB	Croatia	*Carpinus orientalis*	JQ926256	JQ926256	JQ926328	JQ926394
Melanconiella chrysorientalis	MGP	Croatia	*Carpinus orientalis*	JQ926257	JQ926257	JQ926329	JQ926395
Melanconiella chrysorientalis	MVH	Croatia	*Carpinus orientalis*	JQ926259	JQ926259	JQ926331	JQ926397
Melanconiella decorahensis	CBS 159.26	USA	*Betula sp.*	JQ926260	JQ926260	JQ926332	JQ926398
Melanconiella decorahensis	MD	France	*Betula pendula*	JQ926261	JQ926261	JQ926333	JQ926399
Melanconiella decorahensis	MED	France	*Betula pendula*	JQ926262	JQ926262	JQ926334	JQ926400
Melanconiella echinata[T]	DAOM 121196	USA	*Carpinus caroliniana*	JQ926263	JQ926263	N/A	N/A
Melanconiella elegans	AR 3830	USA	*Carpinus caroliniana*	JQ926264	JQ926264	JQ926335	JQ926401
Melanconiella elegans[T]	BPI 843574	USA	*Carpinus caroliniana*	JQ926266	JQ926266	JQ926337	JQ926403
Melanconiella elegans	BPI 872067	USA	*Carpinus caroliniana*	JQ926267	JQ926267	JQ926338	JQ926404
Melanconiella ellisii	BPI 843491	USA	*Carpinus caroliniana*	JQ926268	JQ926268	N/A	JQ926405
Melanconiella ellisii	BPI 883227	USA	*Carpinus caroliniana*	JQ926269	JQ926269	N/A	N/A
Melanconiella ellisii	BPI 878343	USA	*Carpinus caroliniana*	JQ926271	JQ926271	JQ926339	JQ926406

...Continued on next page

TABLE 1. (Continued)

Species	Isolate[1]	Location	Host	Genbank accession numbers			
				ITS	LSU	rpb2	tef1
Melanconiella flavovirens	MFV1	Austria	*Corylus avellana*	JQ926274	JQ926274	JQ926342	JQ926409
Melanconiella flavovirens	MFV2	Austria	*Corylus avellana*	JQ926275	JQ926275	JQ926343	JQ926410
Melanconiella flavovirens	MFV3	Italy	*Corylus avellana*	JQ926276	JQ926276	JQ926344	JQ926411
Melanconiella hyperopta	MCHBV	Austria	*Carpinus betulus*	JQ926280	JQ926280	JQ926346	JQ926413
Melanconiella hyperopta	MCR	Austria	*Carpinus betulus*	JQ926283	JQ926283	JQ926349	JQ926416
Melanconiella hyperopta^{T}	MHG	Switzerland	*Carpinus betulus*	JQ926285	JQ926285	JQ926351	JQ926418
Melanconiella hyperopta var. orientalis	MHP	Croatia	*Carpinus orientalis*	JQ926288	JQ926288	JQ926352	JQ926420
Melanconiella hyperopta var. orientalis	MHVA	Croatia	*Carpinus orientalis*	JQ926287	JQ926287	JQ926353	JQ926419
Melanconiella hyperopta var. orientalis^{T}	MSK	Croatia	*Carpinus orientalis*	JQ926286	JQ926286	JQ926354	JQ926421
Melanconiella meridionalis	MOA	Austria	*Ostrya carpinifolia*	JQ926289	JQ926289	JQ926355	JQ926422
Melanconiella meridionalis	MOK	Croatia	*Ostrya carpinifolia*	JQ926290	JQ926290	JQ926356	JQ926423
Melanconiella meridionalis^{T}	MOM	Austria	*Ostrya carpinifolia*	JQ926291	JQ926291	JQ926357	JQ926424
Melanconiella ostryae	CBS 208.38*	USA	*Ostrya virginiana*	JQ926297	JQ926297	JQ926363	JQ926430
Melanconiella spodiaea	MSH	Austria	*Carpinus betulus*	JQ926298	JQ926298	JQ926364	JQ926431
Melanconiella spodiaea	MVS	Croatia	*Carpinus orientalis*	JQ926299	JQ926299	JQ926365	JQ926432
Melanconiella spodiaea	SPOD	Croatia	*Carpinus betulus*	JQ926300	JQ926300	JQ926366	JQ926433
Melanconis stilbostoma	MS = CBS 121894	Europe	*Betula pendula*	JQ926229	JQ926229	JQ926302	JQ926368

[1] **AR**: Isolates in culture collection of Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland, USA; **CBS**: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherland; **CFCC**: China Forestry Culture Collection Center, China.
The new strains from the current study are in bold. Ex-type taxa are marked with a T.

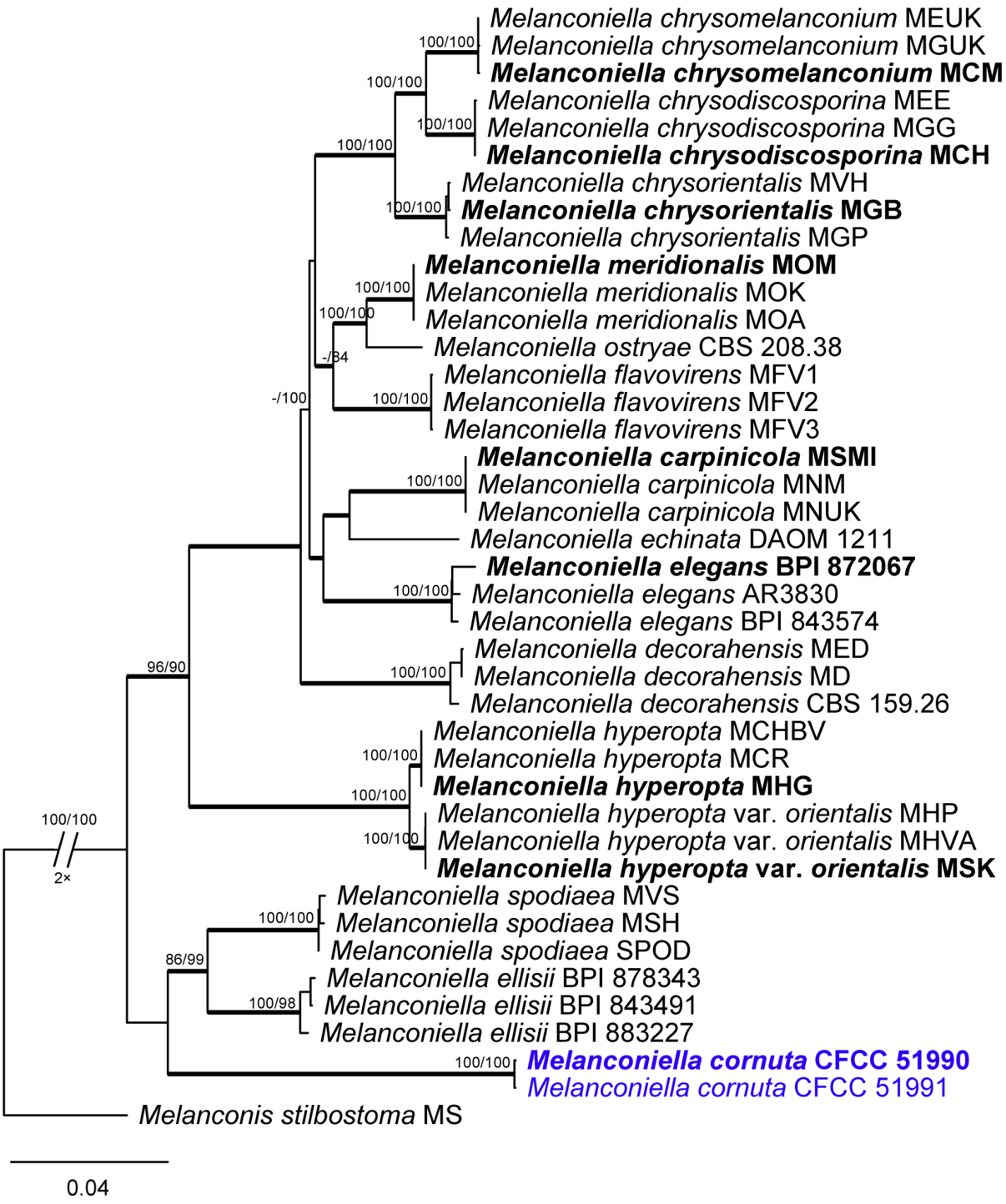

FIGURE 1. Phylogram of *Melanconiella* based on combined ITS, LSU, rpb2 and tef1-α genes. MP and ML bootstrap support values above 75 % are shown at the first and second position. Thickened branches represent posterior probabilities above 0.95 from BI. Scale bar = 30 nucleotide substitutions. Ex-type strains are in bold. Strains in current study are in blue.

Maximum parsimony (MP) analysis was performed using PAUP v. 4.0b10 with a heuristic search algorithm (1,000 random-addition sequences) with a tree bisection and reconnection (TBR) branch swapping (Swofford 2003). 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. MrModeltest v. 2.3 was used to estimate the best nucleotide substitution model settings for each gene (Posada & Crandall 1998). Maximum Likelihood (ML) analysis was also performed with a GTR+G+I model of site substitution including estimation of Gamma-distributed rate heterogeneity and a proportion of invariant sites (Stamatakis 2006). A bootstrapping (BS) analysis (1,000 replicates) was calculated to assess the branch supports

of MP and ML results (Hillis & Bull 1993). Bayesian inference (BI) analysis was performed based on the individual DNA dataset from the results of the MrModeltest, using a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Two MCMC chains were run from random trees for 1,000,000 generations, and stopped when average standard deviation of split frequencies fell below 0.01. Trees were sampled every 1,000th generations. The first 25 % of trees were discarded as the burn-in phase of each analysis. Branches with significant Bayesian posterior probabilities (BPP) were estimated to assess the remaining 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. Novel sequences generated in the current study were deposited in GenBank (Table 1) and the aligned matrices used for phylogenetic analyses were maintained in TreeBASE (www.treebase.org; accession number: S21285).

Results

The combined ITS, LSU, rpb2 and tef1-α dataset from 40 ingroup strains clustered in 14 groups representing species of *Melanconiella* (Table 1). Sequences include two strains from this study and sequences of 38 strains available in GenBank mostly from Voglmayr *et al.* (2012). The alignment including gaps is composed of 4079 characters, of which 2897 characters were constant, 282 variable characters were parsimony-uninformative, and 900 were parsimony informative. The heuristic search generated one parsimonious tree (TL= 2258, CI = 0.705, RI = 0.881, RC = 0.621) as shown in Fig. 1. Strains CFCC 51990 and CFCC 51991, sequenced in this study, formed an individual clade which distinguished from other *Melanconiella* species with high supported value (MP/ML/BI = 100/100/1), representing a new phylogenetic species.

Taxonomy

Melanconiella cornuta C.M. Tian & Z. Du, ***sp. nov.***, **Fig. 2**
MycoBank MB 823037

Holotype:—BJFC-S1375. CHINA, Shaanxi: Ankang City, Ningshan County, Huoditang, 33°26’04.46”N, 108°26’59.91”E, 1610 m asl, on twigs and branches of *Cornus controversa*, coll. X.L. Fan, 3 July 2016 (BJFC-S1375, holotype), ex-type culture, CFCC 51990.

Etymology:—*cornuta*, referring to *Cornus controversa*, the new host known for this species.

Host/Distribution:—from *Cornus controversa* and *Juglans regia* in China.

Original description:—Sexual morph: Undetermined. Asexual morph: Pycnidial storma yellow, immersed, conical, with a single locule, necks erumpent through the host bark, tissue around the necks is rostrate, (250–)270–330(–410) µm (av. = 300 µm, n = 20) diam. *Stromatic zones* lacking. *Conidiophores* reduced to conidiogenous cells, 17.5–23.5(–25) × 2.5–4(–4.5) µm (av. = 22 × 3.5 µm, n = 50). *Conidiogenous cells* cylindrical, smooth, hyaline, conidia are produced at apex. *Conidia* hyaline, aseptate, cylindrical to ellipsoidal, (18–)19–22.5(–23.5) × (8–)8.5–10(–11) µm (av. = 21.5 × 10 µm, n = 50), with distinct hyaline sheath (1.5–)2–2.5 µm (av. = 2 µm, n = 20).

Cultures:—Colony growth on PDA originally white, becoming pale yellowish after 7–10 days. Colony flat, felt-like, with a uniform texture, and yellowish to dark brown conidiomata irregular on the medium surface.

Material examined:—CHINA, Shaanxi: Ankang City, Ningshan County, Huoditang, 36°26’13.30”N, 108°26’48.32”E, 1432 m asl, on twigs and branches of *Juglans regia*, coll. Q. Yang, 3 Auguest 2015 (BJFC-S1345, paratype), ex-paratype culture, CFCC 51991.

Discussion

In this study, two strains of *Melanconiella* associated with *Cornus controversa* and *Juglans regia* dieback or canker disease in China were identified. Species identification was supported by morphology and multigene DNA data (ITS, LSU, rpb2 and tef1-α), which indicated that *Melanconiella cornuta* represents a distinct species (Fig. 1). Although *Melanconiella cornuta* represents a closely related group to *M. ellisii* and *M. spodiaea*, these two taxa are distinguished based on their unique host and multigene phylogenetic data (Fig. 2). Morphologically, *Melanconiella cornuta* has hyaline discosporina-like conidia which is different from type species *M. spodiaea* with dark brown melanconium-like

conidia, but similar to the *M. ellisii*. However, it can be distinguished by the absence of central or eccentric stromatic column and shape and size of conidia. Hence, we regard this taxon as a novel species of *Melanconiella*.

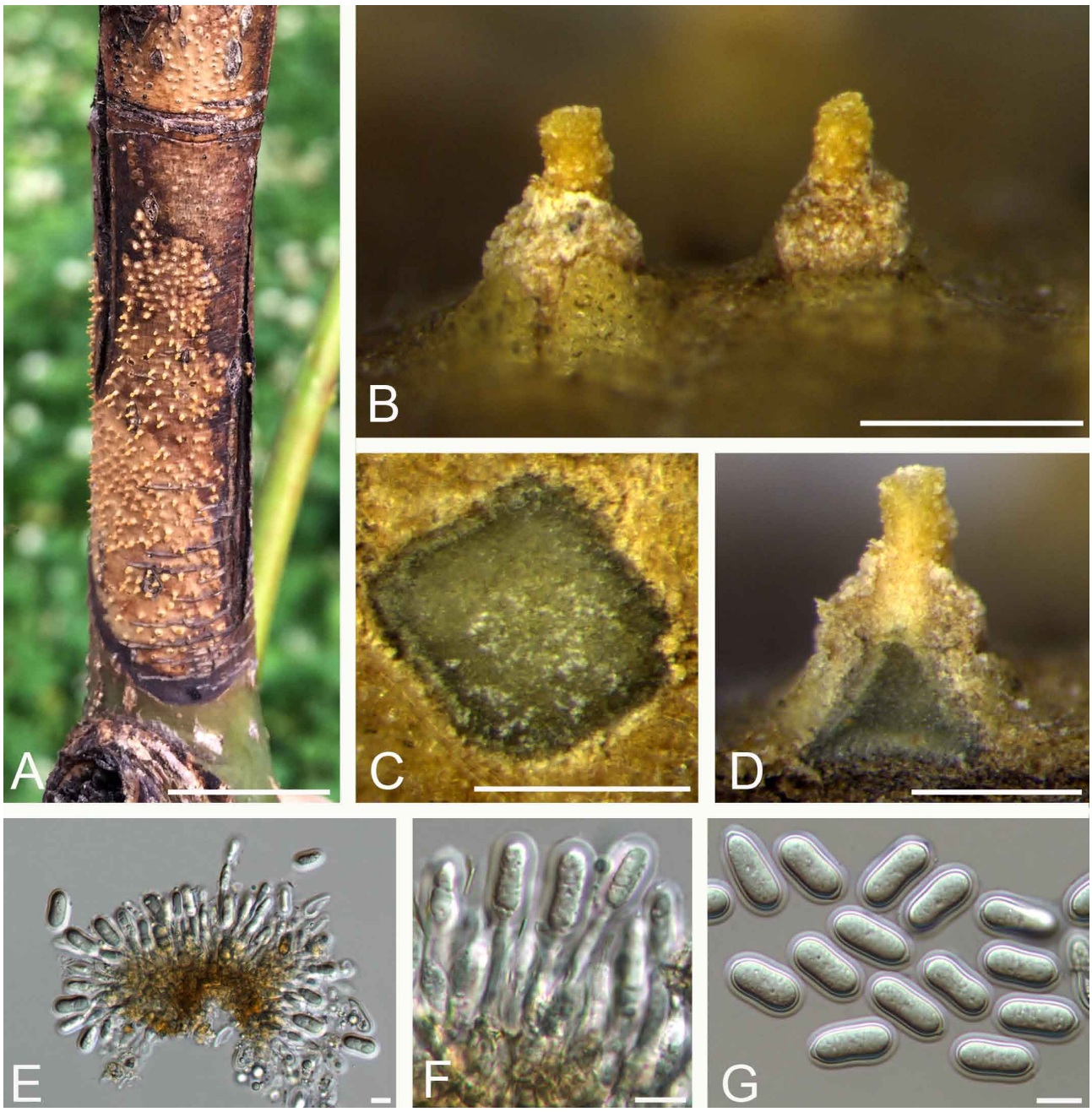

FIGURE 2. Morphology of *Melanconiella cornuta* from *Cornus controversa* (BJFC-S1375). A, B: Habit of conidiomata on twig. C: Transverse section of conidioma. D: Longitudinal section through conidioma. E: Conidiogenous cells. F, G: Conidia. Scale bars: A=1 cm; B–D = 300 µm; E–G = 10 µm.

Thirty-six names for *Melanconiella* were listed in the Index Fungorum (www.indexfungorum.org) and have caused confusion with related genera over a long period time. Anamorphs of *Melanconiella* and related genus *Melanconis* are usually referred to as *Melanconium*, which was treated as synonym of *Melanconis* by Maharachchikumbura *et al.* (2016), whereas the recent studies suggested species of *Melanconium* could refer to a huge number of cryptic fungi, such as *Juglanconis, Melanconiella* and *Melanconis* (Kobayashi 1970; Rossman *et al.* 2007; Voglmayr *et al.* 2012; Fan *et al.* 2016; Voglmayr *et al.* 2017). Voglmayr *et al.* (2012) performed the accepted 13 species of *Melanconiella* with taxonomic and DNA evidences from living cultures. Their results indicated that all species were observed to be highly host-specific, as they were found to be confined mostly to the host family Betulaceae from Europe and North America (Voglmayr *et al.* 2012). The description of *Melanconiella cornuta* in the present study extends the host range to the Cornaceae and Juglandaceae, suggesting that Asia could harbor many more species awaiting collection and description in various hosts.

Although the current study further extended our knowledge of the *Melanconiella*, which have been revised by Voglmayr *et al.* (2012), the ex-type sequence data are, however, not available for many species of *Melanconiella*, including the type species *Melanconiella spodiaea*. Senanayake *et al.* (2017) introduced Melanconiellaceae to accommodate *Dicarpella*, *Greeneria*, *Melanconiella*, *Microascospora* and *Tubakia*, whereas the original material of *M. spodiaea* is often unavailable for study, thus it cannot be confidently connected to the currently accepted *Melanconiella* species. Thus, a thorough revision of the genus *Melanconiella* based on robust sampling, cultures and DNA data is urgently needed.

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