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Melansporellaceae: a novel family of Diaporthales (Ascomycota)

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Abstract

Melansporellaceae *fam. nov.* is introduced to accommodate a genus of diaporthalean fungi that is a phytopathogen causing walnut canker disease in China. The family is typified by *Melansporella gen. nov.* It can be distinguished from other diaporthalean families based on its irregularly uniseriate ascospores, and ovoid, brown conidia with a hyaline sheath and surface structures. Phylogenetic analysis shows that *Melansporella juglandium sp. nov.* forms a monophyletic group within Diaporthales (MP/ML/BI=100/96/1) and is a new diaporthalean clade, based on molecular data of ITS and LSU gene regions. Thus, a new family is proposed to accommodate this taxon.

Key words: diaporthalean fungi, fungal diversity, new taxon, Sordariomycetes, systematics, taxonomy

Introduction

The ascomycetous order Diaporthales (Sordariomycetes) are well-known fungal plant pathogens, endophytes and saprobes, with wide distributions and broad host ranges (Castlebury *et al.* 2002, Rossman *et al.* 2007, Maharachchikumbura *et al.* 2016). *Diaporthe* spp. cause various diseases, e.g., chestnut blight caused by *Cryphonectria parasitica* (Murrill) (Gryzenhout *et al.* 2006); stem-end rot of citrus fruits infected by *Diaporthe citri* Wolf (Huang *et al.* 2013), and willow and walnut canker or dieback disease caused by *Cytospora chrysosperma* (Pers.) Fr. (Fan *et al.* 2014, 2015). The order Diaporthales is characterized by brown to black perithecial ascomata immersed in stromata or the substrata, and a diaporthalean-type centrum development, i.e., lacking true paraphyses, and having unitunicate asci that commonly float free at maturity, often with a refractive ring at the apex (Barr 1978, Castlebury *et al.* 2002, Voglmayr *et al.* 2012, Maharachchikumbura *et al.* 2015).

The families of Diaporthales have been treated differently by various mycologists. Castlebury *et al.* (2002) postulated six major lineages within this order, recognized as the *Cryphonectria-Endothia* complex, Diaporthaceae *sensu stricto*, Gnomoniaceae *sensu stricto*, Melanconidaceae *sensu stricto*, Schizoparme complex and Valsaceae *sensu stricto*. When Rossman *et al.* (2007) reviewed the Diaporthales, nine families were recognized, i.e., Cryphonectriaceae, Diaporthaceae, Gnomoniaceae, Melanconidaceae, Pseudovalsaceae, Schizoparmeaceae, Sydowiellaceae, Togniniaceae and Valsaceae. Kirk *et al.* (2008) added Melogrammataceae and listed ten families in this order, whereas Jaklitsch & Voglmayr (2012) placed Melogrammataceae within Xylariales rather than Diaporthales based on ITS and LSU sequence data. Pseudoplagiostomaceae, Harknessiaceae and Tirisporellaceae were also added to the Diaporthales (Cheewangkoon *et al.* 2010, Crous *et al.* 2012, Suetrong *et al.* 2015). Voglmayr & Jaklitsch (2014) resurrected Stilbosporaceae, while Togniniaceae was reallocated to the Togniniales (Gramaje *et al.* 2015, Maharachchikumbura *et al.* 2015, 2016). Fan *et al.* (2016) revised the genus *Melanconis* from *Betula* and listed 12 known families of Diaporthales. Norphanphoun *et al.* (2016) indicated *Melanconis desmazieri* was synonymized under *Lamproconium desmazieri* with Lamproconiaceae *fam. nov.*, and listed 13 families in this order. In spite of these changes, the phylogenetic placement of many genera in the Diaporthales remains unknown, and many families still await to be elucidated.

In the present study, we examined taxonomy and phylogeny of new Melanconis-like specimens from China within the Diaporthales. A distinct family level clade from *Juglans regia* L. was found based on analysis of combined

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ITS and LSU gene regions. Species in this clade have different morphologies from the presently known families of Diaporthales. Thus, a new family is proposed to accommodate this taxon.

Materials and methods

Isolates

Fresh specimens of diaporthalean fungi were collected from infected branches or twigs during collecting trips in Gansu and Heilongjiang Provinces in China (Table 1). Five isolates were made by removing a mucoid spore mass from conidiomata or ascomata, 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 are deposited in the Museum of the Beijing Forestry University (BJFC). Axenic cultures are maintained in the China Forestry Culture Collection Center (CFCC). Facesoffungi and MycoBank number are provided (Jayasiri *et al.* 2015, MycoBank 2017).

TABLE 1. Strains used in this study and their GenBank accession numbers.

Species	Isolate	**Location**	**Host**	**Genbank accession numbers**	
				ITS	**Lsu**
Cainiella johansonii	Kruys 731	Sweden	*Dryas octopetala*	-	JF701920
Chapeckia nigrospora	AR 3809	USA	*Betula* sp.	-	EU683068
Coniella musaiensis	AR 3534 = CBS 109757	India	Soil	KX833589	AF408391
Cryphonectria macrospora	AR 3444 = CBS 109764	Russia	*Quercus mongolica*	DQ120760	AF408340
Cryphonectria nitschkei	AR 3433 = CBS 109776	Russia	*Quercus mongolica*	DQ120761	AF408341
Cryphonectria parasitica	ATCC 38755	USA	*Castanea dentata*	AY141856	EU199123
Cryptodiaporthe salicella	AR 3455 = CBS 109775	Austria	*Salix* sp.	DQ323529	DQ323529
Cryptosporella hypodermia	AR 3552	Austria	*Ulmus minor*	EU199181	AF408346
Cytospora cenisia	AR 3522 1583 = CBS 109752	Austria	*Juniperus communis*	-	AF408385
Cytospora chrysosperma	CFCC 89600	China	*Sophora japonica*	KR045623	KR045623
Cytospora elaeagni	CFCC 89633	China	*Elaeagnus angustifolia*	KF765677	KF765693
Cytospora leucostoma	CFCC 50468	China	*Betula platyphylla*	KT732949	KT732968
Cytospora nivea	AR 3512 = CBS 109743	Austria	*Salix purpurea*	-	AF408367
Cytospora sacculus	AR 3416= CBS 109756	Russia	*Quercus mongolica*	-	AF408386
Cytospora sacculus	AR 3426 = CBS 109777	Austria	*Quercus robur*	-	AF408387
Diaporthe decedens	AR 3459 = CBS 109772	Austria	*Corylus avellana*	KC343059	AF408348
Diaporthe detrusa	AR 3424 = CBS 109770	Austria	*Berberis vulgaris*	KC343061	AF408349
Diaporthe eres	AR 3538 = CBS 109767	Austria	*Acer campestre*	KC343075	AF408350
Ditopella ditopa	AR 3423 = CBS 109748	Austria	*Alnus glutinosa*	DQ323526	AF408360
Gnomonia gnomon	CBS 199.53	Italy	*Corylus avellana*	AY818956	AF408361
Harknessia eucalypti	CBS 342.97	Australia	*Eucalyptus regnans*	AY720745	AF408363
Hercospora tiliae	AR 3526	Austria	*Tilia tomentosa*	-	AF408365
Lamproconium desmazier	MFLUCC 14-1047	Russia	*Tilia cordata*	KX430132	KX430133
Lamproconium desmazier	MFLUCC 15-0870	Russia	*Tilia tomentosa*	KX430134	KX430135
Magnaporthe grisea	Ina168	-	-	AB026819	AB026819
Magnaporthe salvinii	CBS 243.76	-	-	KM484861	DQ341498
Melanconiella ellisii	BPI 878343	USA	*Carpinus caroliniana*	JQ926271	JQ926268
Melanconiella hyperopta	AR 3832 = CBS 131492	Austria	*Carpinus betulus*	JQ926278	JQ926278
Melanconiella spodiaea	MSH	Austria	*Carpinus betulus*	JQ926298	JQ926298
Melanconis alni	AR 3748	Austria	*Alnus viridis*	EU199195	EU199130
Melanconis betulae	CFCC 50471	China	*Betula albosinensis*	KT732952	KT732971
Melanconis itoana	CFCC 50474	China	*Betula albosinensis*	KT732955	KT732974
Melanconis marginalis	AR 3442 = CBS 109744	Canada	*Alnus rubra*	-	AF408373
Melanconis stilbostoma	CFCC 50475	China	*Betula platyphylla*	KT732956	KT732975
Melansporella juglandium	CFCC 51725	China	*Juglans regia*	KY363852	KY363857
Melansporella juglandium	CFCC 51726	China	*Juglans regia*	KY363853	KY363858
Melansporella juglandium	CFCC 51727	China	*Juglans regia*	KY363854	KY363859

.....Continued on next page

TABLE 1. (Continued)

Species	Isolate	Location	Host	Genbank accession numbers	
				ITS	Lsu
Melansporella juglandium	CFCC 51728	China	*Juglans regia*	KY363855	KY363860
Melansporella juglandium	CFCC 51729	China	*Juglans regia*	KY363856	KY363861
Ophiovalsa betulae	AR 3524 = CBS 109763	Austria	*Betula pendula*	-	AF408375
Ophiovalsa suffusa	AR 3496 = CBS 109750	Austria	*Alnus incana*	-	AF408376
Phragmoporthe conformis	AR 3632 = CBS 109783	Canada	*Alnus rubra*	DQ323527	AF408377
Pilidiella castaneicola	CBS 143.97	Korea	-	-	AF408378
Pilidiella diplodiella	STE-U 3708	France	*Vitis vinifera*	AY339323	AY339284
Pilidiella wangiensis	CPC 19397	Australia	*Eucalyptus* sp.	JX069873	JX069857
Plagiostoma euphorbiae	CBS 340.78	Netherlands	*Euphorbia palustris*	EU199198	AF408382
Pseudoplagiostoma eucalypti	CBS 124807	Venezuela	*Eucalyptus urophylla*	GU973512	GU973606
Pseudoplagiostoma eucalypti	CBS 116382	Thailand	*Eucalyptus camaldulensis*	GU973514	GU973608
Pseudoplagiostoma oldii	CBS 115722	Australia	*Eucalyptus camaldulensis*	GU973535	GU973610
Pseudoplagiostoma variabile	CBS 113067	Uruguay	*Eucalyptus globulus*	GU973536	GU973611
Pseudovalsa longipes	AR 3541	Austria	*Quercus cerris*	-	EU683072
Pseudovalsa modonia	AR 3558	Austria	*Castanea sativa*	-	EU683073
Pseudovalsa umbonata	AR 3897	Austria	*Quercus cerris*	-	EU683074
Rossmania ukurunduensis	AR 3484	Russia	*Acer ukurunduense*	-	EU683075
Schizoparme straminea	CBS 149.22	USA	*Rosa rugosa*	-	AF362569
Sillia ferruginea	AR 3440	Austria	*Corylus avellana*	-	EU683076
Stilbospora macrosperma	CBS 121883	Austria	*Carpinus betulus*	JX517290	JX517299
Stilbospora macrosperma	CBS 121695	Netherlands	*Carpinus betulus*	JX517288	JX517297
Sydowiella depressula	CBS 813.79	Switzerland	*Rubus* sp.	-	EU683077
Sydowiella fenestrans	AR 3777	Russia	*Chamerion angustifolium*	-	EU683078
Thailandiomyces bisetulosus	BCC00018	Thailand	*Licuala longicalycata*	-	EF622230
Tirisporella beccariana	BCC36737	Thailand	*Nypa fruticans*	-	JQ655450
Wuestneia molokaiensis	AR 3578 = CBS 109779	USA	*Eucalyptus robusta*	-	AF408390

Note: Type species strains are in black bold

DNA amplification and sequencing

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madiso, WI, USA) following the manufacturer's instructions, from fungal mycelium growing on PDA. 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 with the primers ITS1 and ITS4 (White *et al.* 1990), and the partial large nuclear ribosomal RNA subunit (LSU) region was amplified using primers NL1 and NL4 (O'Donnell 1993). The PCR amplification products were estimated visually by electrophoresis in 2 % agarose gels. DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminater Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company (Beijing, China).

Phylogenetic analysis

DNA sequences generated by each forward and reverse primers were used to obtain consensus sequences using Seqman v.7.1.0 in the DNASTAR lasergene core suite software (DNASTAR, Madison, WI, USA). Reference sequences were selected based on type species in each family of Diaporthales available in GenBank. The single gene sequences were initially aligned with Clustal W as implemented in MEGA 6 and improved in MAFFT v.7 (Katoh & Standley 2013, Tamura *et al.* 2013). Sequences for the genes and genetic markers of each genus were selected based on the loci recently used for these genera in published literature (Table 1).

Maximum parsimony (MP) analysis was performed using PAUP v. 4.0b10 with a heuristic search option of 1000 random-addition sequences with a tree bisection and reconnection (TBR) branch swapping algorithm (Swofford 2003). The branches of zero length were collapsed and all equally parsimonious trees were saved. Other parsimony scores such as tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC) were

calculated (Swofford 2003). RAxMLv.7.2.8 was used to construct a maximum likelihood (ML) tree with GTR+G+I model of site substitution including estimation of Gamma-distributed rate heterogeneity and a proportion of invariant sites (Stamatakis 2006). The branch support was evaluated with bootstrapping method of 1000 replicates (Hillis & Bull 1993).

MrModeltest v. 2.3 was used to estimate the best nucleotide substitution model settings for each gene (Posada & Crandall 1998). Bayesian inference (BI) 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 10000000 generations and stopped when average standard deviation of split frequencies fell below 0.01. Trees were saved each 1000 generations. The first 25 % of trees were discarded as the burn-in phase of each analysis, and the posterior probabilities (BPP) were calculated to assess the remaining trees (Rannala & Yang 1996). The branch support from MP and ML analysis were evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993). *Magnaporthe grisea* (T.T. Hebert) M.E. Barr (Ina168) and *M. salvinii* (Catt.) R.A. Krause & R.K. Webster (CBS 243.76) were selected as outgroup in all analyses. Phylograms are shown using Figtree v. 1.3.1 (Rambaut & Drummond 2010). Novel sequences generated in the current study were deposited in GenBank (Table 1) and the aligned matrices used for phylogenetic analyses were maintained in TreeBASE (www.treebase.org; accession number: S20683).

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), and features recorded including size and shape of stromata and presence or absence of central column. Micro-morphological observations included colour, size and shape of conidiophores and conidia (asci and ascospores); presence or absence of sheath and surface structures in conidia determined using a Leica compound microscope (DM 2500). 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 pycnidium structure.

Results

The sequence dataset of Diaporthales including five strains from this study and 58 reference strains from recent studies were analyzed based on ITS and LSU (Castlebury *et al.* 2002, Gryzenhout *et al.* 2006, Mejía *et al.* 2008, Udayanga *et al.* 2011, Voglmayr *et al.* 2012, Gomes *et al.* 2013, Norphanphoun *et al.* 2016). The alignment consisted of 1281 characters including gaps, of which 737 characters are constant, 102 variable characters are parsimony-uninformative, and 442 are parsimony informative. A heuristic search generated 24 parsimonious trees, and the best tree (TL = 1689, CI = 0.513, RI = 0.803, RC = 0.412) is shown (Fig. 1). The 63 isolates clustered in 15 clades corresponding to 14 families in Diaporthales, and one clade referring to *Melanconiella*. The isolates of the current study clustered into a novel phylogenetic taxon at family level, which formed a strongly support clade (MP/ML/BI = 100/96/1). Tree topologies of all genera computed from MP, ML and BI analyses were similar for the individual gene region and in the combined dataset.

Taxonomy

Melansporellaceae C.M. Tian & Z. Du, *fam. nov.* (Fig. 2) MycoBank 820305

Type genus:—*Melansporella.*

Description:—Pathogen causing canker on branches or twigs of walnut and producing distinct pustules. *Ascomata* globose to subglobose, dark brown to black, with long black necks, penetrating through the ectostroma, convergent to disc. *Asci* inoperculate, unitunicate, oblong or fusoid, thin-walled, 8-spored. *Ascospores* irregularly uniseriate, fusoid, hyaline, 1-septate, constricted at the septum. *Pycnidial conidiomata* with waxy-gelatinous substance and a single

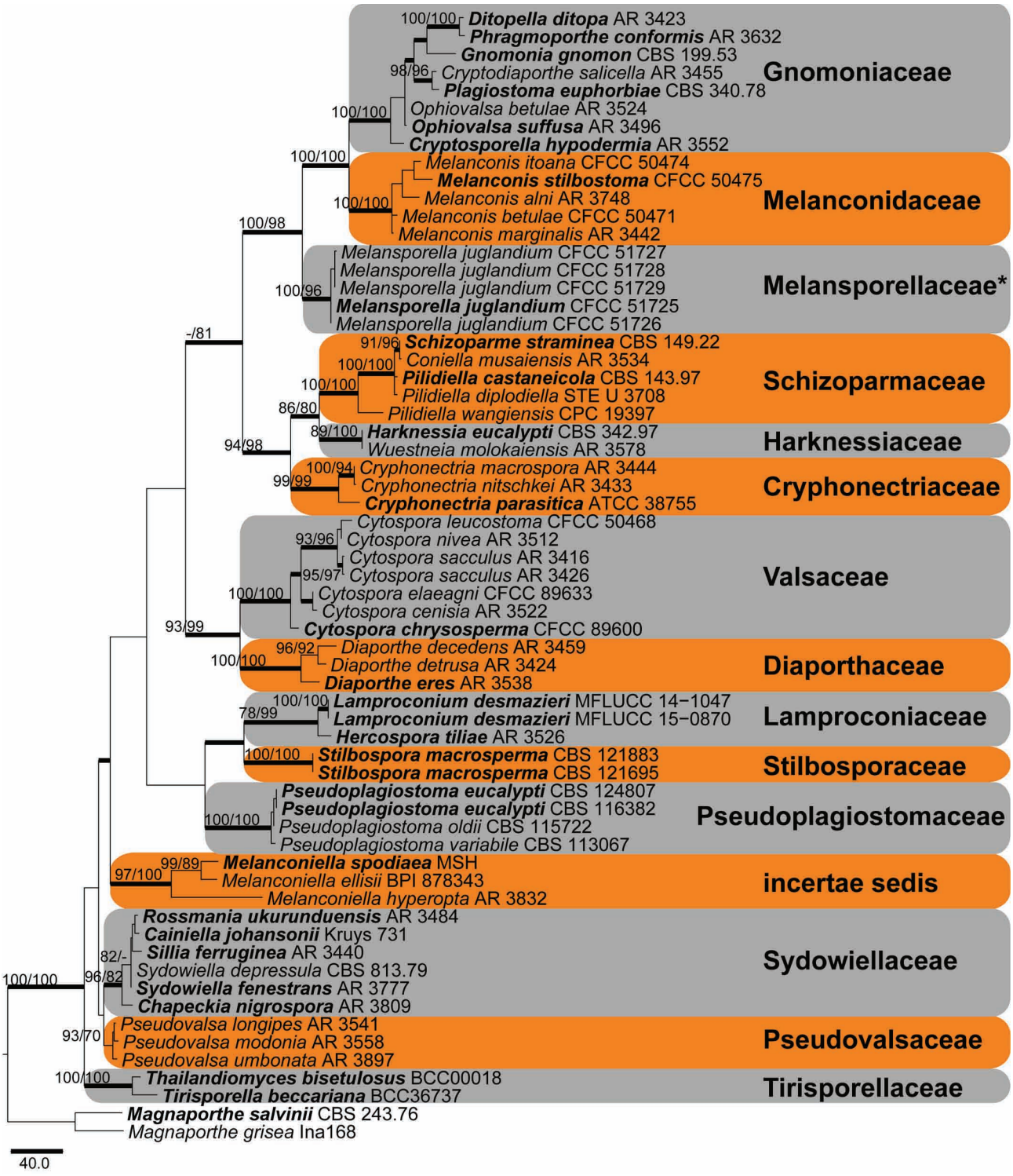

FIGURE 1. Maximum parsimony (MP) majority rule consensus tree of combined ITS and LSU sequence data based on MP, ML and Bayesian analyses. Values above the branches indicate MP and ML bootstrap (MPBS/MLBS) ≥ 70%. Thickened branches represent BI posterior probabilities (BIPP) ≥ 0.95. Scale bar = 40 nucleotide substitutions. Strain numbers are given following the taxon names. The new family resulting from this study is marked with an asterisk. Type species strains are in black bold.

locule. Ectostroma breaking through the bark, with saffron to black disc. Central column beneath the disc more or less conical and becoming light brown or olive coloured at maturity. The marginal part of ectostroma comprises conidiophores and their basal cell layers. *Conidiophores* unbranched. *Conidiogenous cells* cylindrical, hyaline to light brown, smooth-walled, forming a single conidium at the conidiophore apex. *Conidia* hyaline initially, becoming dark brown when mature, ovoid, with distinct hyaline sheath, unicellular, with verrucae structures on surface.

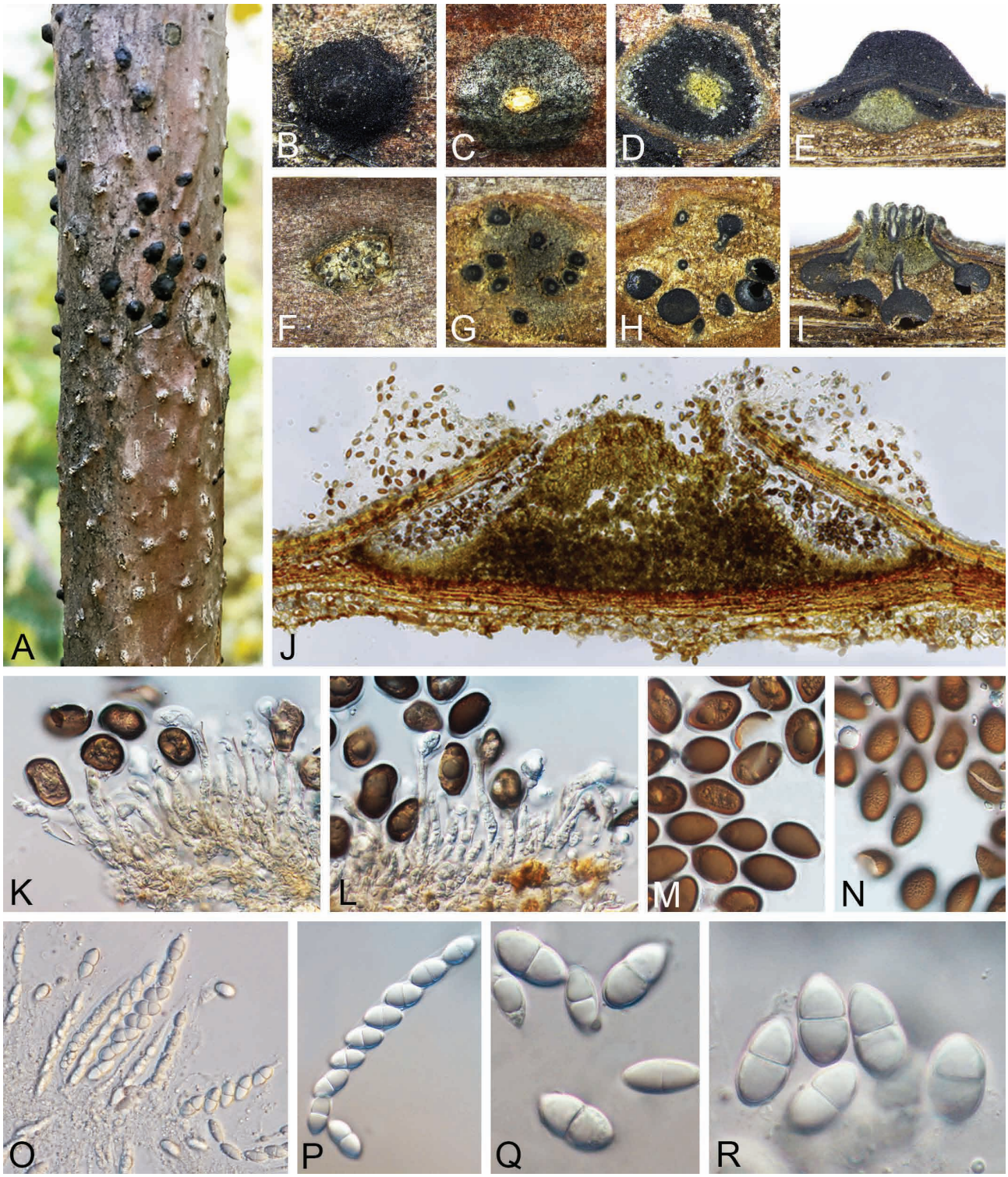

FIGURE 2. A. Habit of ascostroma and conidiomata on twig. B, C. Habit of conidiomata on twig. D. Transverse section through a conidioma. E, I, J. Longitudinal section through conidiomata. F–H. Habit of ascomata on twig. K, L. Conidiophores and conidia. M, N. Conidia. O, P. Asci and ascospores. Q, R. Ascospores. Scale bars: A = 5 mm, B–G, J = 500 µm, H, I = 1 mm, K–R = 20 µm.

Notes:—Order Diaporthales includes 13 families (Norphanphoun *et al.* 2016). The new family Melansporellaceae forms a monophyletic lineage using combined ITS and LSU sequence data (Fig. 2). Although Melansporellaceae shares some morphological similarities to the family Melanconidaceae, several characters separate them. Species of Melanconidaceae, are characterized by ovoid, olive-brown, aseptate alpha conidia (Kobayashi 1970, Castlebury *et al.* 2002, Voglmayr *et al.* 2012, Fan *et al.* 2016). Melansporellaceae can be distinguished by its sheathed conidia, which are larger (24 × 14 µm vs. 12 × 5.5 µm) and brown with surface structures. In addition, ascospores are usually biseriate

in Melanconidaceae species, but uniseriate in Melansporellaceae (Kobayashi 1970). The former always occur on members of the Betulaceae (Castlebury *et al.* 2002, Voglmayr *et al.* 2012), whereas Melansporellaceae were collected only from Juglandaceae in the current study.

Melansporella C.M. Tian & Z. Du, *gen. nov.* (Fig. 2) MycoBank MB 820306
Type species:—*Melansporella juglandium.*

Etymology:—referring to the dark conidia.

Description:—*Perithecia* immersed in the substrate, arranged irregularly, ostioles convergent and erumpent through the disc. *Asci* oblong or fusoid, irregularly uniseriate, 8-spored. *Ascospores* fusoid, 1-septate, hyaline, lacking appendages. *Pycnidial conidiomata* with a single locule. *Conidiophores* hyaline to light brown, simple. *Conidiogenous cells* cylindrical. *Conidia* ovoid, brown, sheathed, with surface structures.

Melansporella juglandium C.M. Tian & Z. Du, *sp. nov.* (Fig. 2) MycoBank MB820307

Etymology:—*juglandium*, referring to *Juglans regia*, the only host known for this species.

Holotype:—BJFC-S1374.

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

Description:—Pustules on bark of cankered or dead stems and twigs. *Ascostroma* immersed, slightly erumpent from surface of host branches, conical, (1.5–)2–2.5(–3) mm (av. = 2.5 mm, n = 20) in diam. *Perithecia* globular to subglobular (430–)550–700(–800) µm (av. = 580 µm, n = 20) in diam., long black necks (500–)600–850(–950) µm (av. = 825 µm, n = 20), clustered beneath the disc at the top. *Asci* (120–)122–135 × (12.5–)13–16.5 (–17) µm (av. = 126.5 × 15 µm, n = 20), oblong or fusoid, 8-spored. *Ascospores* irregularly uniseriate, fusoid, 2-celled, hyaline, (17–)17.5–22(–23.5) × (7.5–)8–10.5(–11) µm (av. = 19.5 × 9.5 µm, n = 50). *Pycnidial stroma* black, conspicuous, with a single locule, 1.5–2(–2.5) mm (av. = 2 mm, n = 20) diam. Ectostromatic disc saffron to black. *Conidiophores* cylindrical, hyaline to light brown, simple, formed at the conidiomatal wall, (24–)28–52(–60) µm (av. = 43 µm, n=20) long. *Conidiogenous cells* subcylindrical to cylindrical, smooth-walled, produce conidia acrogenously. *Conidia* ovoid, with distinct hyaline sheath, brown to dark brown, unicellular, surface structures, (21–)21.5–26(–26.5) × (12–)13–15.5(–16) µm (av. = 24 × 14 µm, n = 50).

Culture characterisctics:—Culture growth on PDA is initially white, becoming yellowish after 3–5 days. The colony is flat, with an irregular edge, texture uniform, producing dark green to black pigment after 7–10 days. Conidiomata sparse, irregularly distributed over agar surface, black mucous conidia were produced on the colony.

Material examined:—CHINA. Heilongjiang Province: Harbin City: Heilongjiang Botanical Garden, 45°42’21.10”N, 126°38’42.87”E, 128 m asl, on twigs and branches of *Juglans regia*, coll. Z. Du & Q. Yang, 2 Aug 2016 (BJFC-S1374, holotype**),** ex-holotype living culture, CFCC 51725).

Additional materials examined:—CHINA. Gansu Province: Qingyang City, Shenshe Town, 35°38’17.08”N, 107°47’48.68”E, 1253m asl, on twigs and branches of *Juglans regia*, coll. X.L. Fan, 14 Jul 2013 (BJFC-S908, living culture, CFCC 51727). CHINA. Gansu Province: Qingyang City, Zhongwan Forest Centre, 35°26’26.33”N, 108°34’09.38”E, 1553m asl, on twigs and branches of *J. regia*, coll. X.L. Fan, 11 Jul 2013 (BJFC-S947, living culture, CFCC 51728). CHINA. Gansu Province: Qingyang City, Zhongwan Forest Centre, 35°26’25.52”N, 108°34’09.03”E, 1580m asl, on twigs and branches of *J. regia*, coll. X.L. Fan, 11 Jul 2013 (BJFC-S955, living culture, CFCC 51729).

Notes:—*Melansporella juglandium* is the type species of *Melansporella*, and only occurs on *Juglans regia*. Isolates were identified as *Melansporella juglandium* based on holomorphic morphology characters, host, and high support in the phylogeny (MP/ML/BI=100/96/1). Hence, we treat this taxon as a novel species of *Melansporella.*

Discussion

The present study revealed Melansporellaceae *fam. nov.* infecting walnut trees in China, resided in Diaporthales (Fig. 1). Characteristics of the new family agree with Diaporthales, i.e., perithecia immersed with long necks and a diaporthalean-type centrum development, lack of true paraphyses, and septate ascospores (Barr 1978, Samuels & Blackwell 2001, Castlebury *et al.* 2002, Kruys & Castlebury 2012, Voglmayr *et al.* 2012). Melansporellaceae is distinct from other families in Diaporthales in having oblong or fusoid 8-spored, irregularly uniseriate asci and ovoid, brown

conidia, with a distinct hyaline sheath and surface structures. Phylogenetic analyses showed Melansporellaceae to be a distinct group based on combined ITS and LSU sequence data from 63 taxa.

The family Melansporellaceae based on type genus *Melansporella* is recognized as a separate group in Diaporthales, however the relationship between several known genera remains unresolved. The type species *Melansporella juglandium* is morphologically similar to *Melanconium oblongum* Berk. (Graves 1919), which is the asexual morph of *Melanconis juglandis* (Ellis & Everh.) A.H. Graves (Graves 1919, Kobayashi 1970), residing in Melanconidaceae (Wijayawardene *et al.* 2016). *Melansporella juglandium* clusters in a separate clade from Melanconidaceae with high support (MP/ML/BI=100/96/1). *Melanconis* is the only genus in Melanconidaceae, and it has five species, i.e., *Melanconis alni* Tul. & C. Tul., *M. betulae* C.M. Tian & X.L. Fan., *M. marginalis* (Peck) Wehm., *M. itoana* Tak. Kobay., *M. stilbostoma* (Fr.) Tul. & C. Tul. (Rossman *et al.* 2007, Voglmayr *et al.* 2012, Fan *et al.* 2016). *Melanconis juglandis* does not belong in Melanconidaceae.

Melanconiella is similar to *Melanconis* but *Melanconis juglandis* has been separated into *Melanconiella* (Voglmayr *et al.* 2012). *Melanconis juglandis* has no molecular data linked to type materials, so that the relationships between *Melanconis*, *Melanconiella* and *Melanconium*-like dark-spored taxa need resolving by epitypification of their types (*sensu* Ariyawansa *et al.* 2014). Several reports have shown that the morphological characteristics traditionally used for circumscribing families may not be as useful as previously indicated (Barr 1978, 1990, Castlebury *et al.* 2002, Gryzenhout *et al.* 2006, Moročko & Fatehi 2007).

Currently, Melansporellaceae is not as species rich as other families in Diaporthales, although the current study extends our knowledge of the order Diaporthales. Strains of Melansporellaceae were found only from *Juglans regia* as a phytopathogen causing canker and dieback disease. The status of the new family is supported by phylogenetic analysis of ITS and LSU sequence data. Establishing taxa at family and ordinal ranks are, however, often fraught with disagreement, as such ranking is usually subjective (Liu *et al.* 2016, Samarakoon *et al.* 2016a). It would be beneficial to establish the divergence times across the families of Diaporthales to establish if they fall within the range of other fungal families (see Phukhamsakda *et al.* 2016, Samarakoon *et al.* 2016b). This would provide further evidence for the introduction of Melansporellaceae. Further collections and molecular studies are therefore necessary to understand and support the introduction of Melansporellaceae.

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