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Morphological and molecular identification of two novel species of *Melanops* in China

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Abstract

Melanops is an uncommon genus in Botryosphaeriales that until now has been found only on *Fagaceae* hosts. It can be distinguished from the other Botryosphaeriales by having large, multiloculate ascomata and conidiomata with locules arranged at various levels within the stroma and a narrow, persistent mucous sheath surrounding the ascospores and conidia. In the present study, the morphology and phylogenetic relationships of *Melanops* specimens collected from *Castanea mollissima* and *Quercus* sp. in China were studied. As a result, *Melanops castaneicola* sp. nov. and *Melanops chinensis* sp. nov. are introduced, and a comparison with accepted *Melanops* species is presented.

Key words – Botryosphaeriales – *Castanea* – *Melanopsaceae* – *Quercus* – Taxonomy

Introduction

Melanops (*Melanopsaceae*, Botryosphaeriales) is a small genus comprising only *M. tulasnei* and an unnamed culture, CBS 118.39, possibly *M. quercuum*. *Melanops* is the sole genus representing *Melanopsaceae* (Crous et al. 2006, Phillips & Alves 2009, Slippers et al. 2013, Wijayawardene et al. 2017). Morphologically, *Melanopsaceae* differs from the other genera in Botryosphaeriales on account of the relatively large, multiloculate ascomata and condiomata with locules arranged at various levels within the stroma, and ascospores and conidia surrounded by a narrow, persistent mucous sheath (Phillips & Pennycook 2004, Phillips & Alves 2009, Slippers et al. 2013). *Melanopsaceae* groups most basal in the Botryosphaeriales, together with *Aplosporellaceae*, *Planistromellaceae* and *Saccharataceae* (Phillips et al. 2013, 2018, Slippers et al. 2013, Wyka & Broders 2016, Yang et al. 2017).

Botryosphaeriales are widespread, common and important fungal pathogens of woody plants, and many are also known to exist as endophytes in healthy plant tissues (Slippers et al. 2017). However, whether *Melanops tulasnei* is pathogenic or endophytic is not known, as it appears to infect woody tissue and sporulate on the dead tissue similar to other Botryosphaeriales.

In an ongoing study of Botryosphaeriales in China, specimens with typical characteristics of *Melanops* were collected from *Fagaceae* trees. The purpose of the work presented here was to identify the fungi in terms of morphology and phylogeny.

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Materials & Methods

Isolates and morphology

Two fresh specimens from dead and dying *Castanea mollissima* and two from *Quercus* sp. trees were collected from Hebei and Shaanxi Provinces in China. Single conidial isolates were established by spreading a mucoid spore mass taken from conidiomata onto the surface of PDA plates. After 48 h of incubation at 25 °C, plates were examined with a dissecting microscope and single germinating spores were transferred with a sterile needle to fresh plates of PDA. Herbarium specimens were deposited in the Museum of Beijing Forestry University (BJFC) and cultures maintained in the China Forestry Culture Collection Center (CFCC).

Species were identified based on morphological features of the conidiomata and conidia produced on the dead and dying plant tissues. Cross-sections of conidiomata were cut by hand with a razor blade. Specimens for microscopy were mounted in 100% lactic acid. At least 20 conidiomata and 50 conidia were measured to calculate their mean size and standard deviation. Dimensions are reported as maximum and minimum in parentheses and the range representing the mean, plus or minus the standard deviation with the number of measurements given in parentheses (Voglmayr et al. 2017). Microscope images were captured with a Nikon Eclipse 80i microscope equipped with a Nikon digital sight DS-Ri2 high definition colour camera, using differential interference contrast (DIC) illumination and the Nikon software NIS-Elements D Package v. 3.00. Culture characteristics of isolates were recorded after one month of incubation on PDA in the dark at 25°C.

Molecular characterization and multi-locus phylogenetic analysis

Genomic DNA was extracted from cultures grown on PDA overlaid with cellophane using a modified CTAB method (Doyle & Doyle 1990). The internal transcribed spacer (ITS), ribosomal large subunit (LSU), part of the translation elongation factor 1α (*tef1*) and part of the beta-tubulin (*tub2*) gene, were amplified with the primers ITS1 and ITS4 (White et al. 1990), LR0R and LR5 (Moncalvo et al. 1995, Vilgalys & Hester 1990), EF1-688F and EF1-986R (Alves et al. 2008, Carbone & Kohn 1999), and Bt2a and Bt2b (Glass & Donaldson 1995), respectively. PCR was done by the methods described by Phillips & Alves (2009). The PCR amplification products were visualised by electrophoresis in 2 % agarose gels and sequenced by Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Sequences obtained from this study and reference sequences obtained from GenBank (Table 1) were aligned and edited manually using MEGA6 (Tamura et al. 2013). The alignments were concatenated for phylogenetic analyses. Maximum parsimony (MP) analysis was conducted with PAUP v.4.0b10 (Swofford 2003), and maximum likelihood (ML) analysis with PhyML v.7.2.8 (Guindon et al. 2010). Sequences of novel species were deposited in GenBank (Table 1). The multilocus file was deposited in TreeBASE (www.treebase.org) as accession S23609. Introduction of the new species based on molecular data followed the recommendations of Jeewon & Hyde (2016).

Table 1 Isolates used in the phylogenetic analysis, new strains from the current study are in red and ex-type strains in bold face.

Species	Isolate no.[a]	ITS	LSU	*tef1*	*tub2*
Aplosporella javeedii	CMW 38166	KC769939	KC769980	KC769847	KC769908
Aplosporella prunicola	CBS 121167	KF766147	JX681071	N/A	N/A
Aplosporella yalgorensis	**MUCC 511**	EF591927	EF591944	EF591978	EF591961
Bagnisiella examinans	CBS 551.66	KF766148	KF766316	GU349056	KF766126
Barriopsis fusca	**CBS 174.26**	KF766149	DQ377857	KF766395	EU673109
Botryosphaeria dothidea	**CBS 115476**	KF766151	KF766319	AY236898	AY236927

Table 1 Continued.

Species	Isolate no.[a]	ITS	LSU	*tef1*	*tub2*
Cophinforma atrovirens	**MFLUCC 110425**	JX646800	JX646817	JX646865	JX646848
Diplodia rosulata	**CBS 116470**	EU430265	DQ377896	EU430267	EU673132
Dothiorella longicollis	**CBS 122068**	KF766162	KF766328	EU144069	KF766130
Dothiorella viticola	CBS 117009	AY905554	DQ377873	AY905559	EU673104
Endomelanconiopsis endophytica	**CBS 120397**	EU683656	EU683629	EU683637	KF766131
Guignardia bidwellii	CBS 111645	FJ824766	DQ377876	EU683653	FJ824777
Guignardia citricarpa	CBS 102374	FJ824767	DQ377877	FJ538376	FJ824778
Kellermania crassispora	CBS 131714	KF766175	KF766345	KF766406	KF766135
Kellermania dasylirionis	CBS 131715	KF766177	KF766347	KF766408	KF766137
Kellermania macrospora	CBS 131716	KF766178	KF766348	KF766409	KF766138
Kellermania nolinae	CBS 131717	KF766180	KF766350	KF766411	KF766140
Kellermania plurilocularis	CBS 131719	KF766181	KF766351	KF766412	KF766141
Kellermania yuccifoliorum	CBS 131726	KF766185	KF766355	KF766416	KF766144
Lasiodiplodia theobromae	**CBS 164.96**	AY640255	EU673253	AY640258	EU673110
Lecanosticta acicola	LNPV 252	JX901755	JX901844	JX901639	JX902213
Macrophomina phaseolina	**CBS 227.33**	KF531825	DQ377906	KF952000	KF531806
Marasasiomyces karoo	CBS 118718	KF531828	DQ377939	KF531807	KF531808
Melanops castaneicola	**CFCC 52980**	MK203065	MK203069	MK204619	MK204615
Melanops castaneicola	CFCC 52981	MK203066	MK203070	MK204620	MK204616
Melanops chinensis	**CFCC 52982**	MK203067	MK203071	MK204621	MK204617
Melanops chinensis	CFCC 52983	MK203068	MK203072	MK204622	MK204618
Melanops sp.	CBS 118.39	FJ824771	DQ377856	FJ824776	FJ824782
Melanops tulasnei	**CBS 116805**	FJ824769	FJ824764	FJ824774	FJ824780
Melanops tulasnei	CBS 116806	FJ824770	FJ824765	FJ824775	FJ824781
Neodeightonia phoenicum	CBS 169.34	EU673338	EU673259	EU673307	EU673138
Neofusicoccum umdonicola	**CMW 14058**	EU821904	KF766373	EU821874	EU821844
Neoscytalidium dimidiatum	CBS 499.66	AY819727	DQ377925	EU144063	FM211167
Phaeobotryon mamane	CPC 12264	EU673331	DQ377898	EU673297	EU673125
Phaeobotryosphaeria citrigena	**ICMP 16818**	EU673329	EU673247	EU673295	EU673141
Phyllosticta minima	CBS 111635	KF766215	EU754194	KF766433	N/A
Phyllosticta philoprina	CBS 616.72	KF766171	KF766341	KF766402	N/A
Phyllosticta podocarpi	CBS 111647	KF766217	KF766383	KF766434	N/A
Pseudofusicoccum stromaticum	CBS 117448	KF766223	DQ377931	N/A	EU673094
Saccharata capensis	CBS 122693	KF766224	KF766390	EU552095	N/A
Saccharata kirstenboschensis	CBS 123537	KF766225	FJ372409	N/A	N/A
Saccharata proteae	CBS 115206	KF766226	DQ377882	KF766438	KF531790
Septorioides pini-thunbergii	**CBS 473.91**	KF251243	KF251746	N/A	KF252727
Sphaeropsis visci	CMW 39386	KF575004	KF575065	KF575036	KF575100
Septorioides strobi	**CBS 141443**	KT884699	KT884685	KT884713	KT884721
Septorioides strobi	SW14-36	KT884692	KT884678	KT884706	KT884720

[a]Acronyms of culture collections: CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie pre-fecture, Japan; MFLUCC: Mae Fah Luang University Culture Collection,

Chiang Mai, Thailand; LNPV: Laboratoire National de la Protection des Vegetaux Mycologie, Malzeville, France; ICMP: International Collection of Microorganisms from Plants, Plant Diseases Division, DSIR, Auckland, New Zealand.

Results

Phylogenetic analyses

The combined LSU, ITS, *tef1* and *tub2* data set consisted of 46 strains with *Lecanosticta acicola* (CBS 164.96) as the out group taxon (Wyka et al. 2016). The alignment comprised 2459 characters of which 1245 characters were constant and 352 variable characters were parsimony-uninformative. MP analysis of the remaining 862 parsimony-informative characters resulted in 2 equally most parsimonious trees, and the first tree (TL = 4715, CI = 0.469, RI = 0.669, RC = 0.314) is shown in Fig. 1. The topology of the phylogenetic tree obtained from ML was similar to the MP tree. The two novel species appeared in two distinct clades with high bootstrap support (Fig. 1).

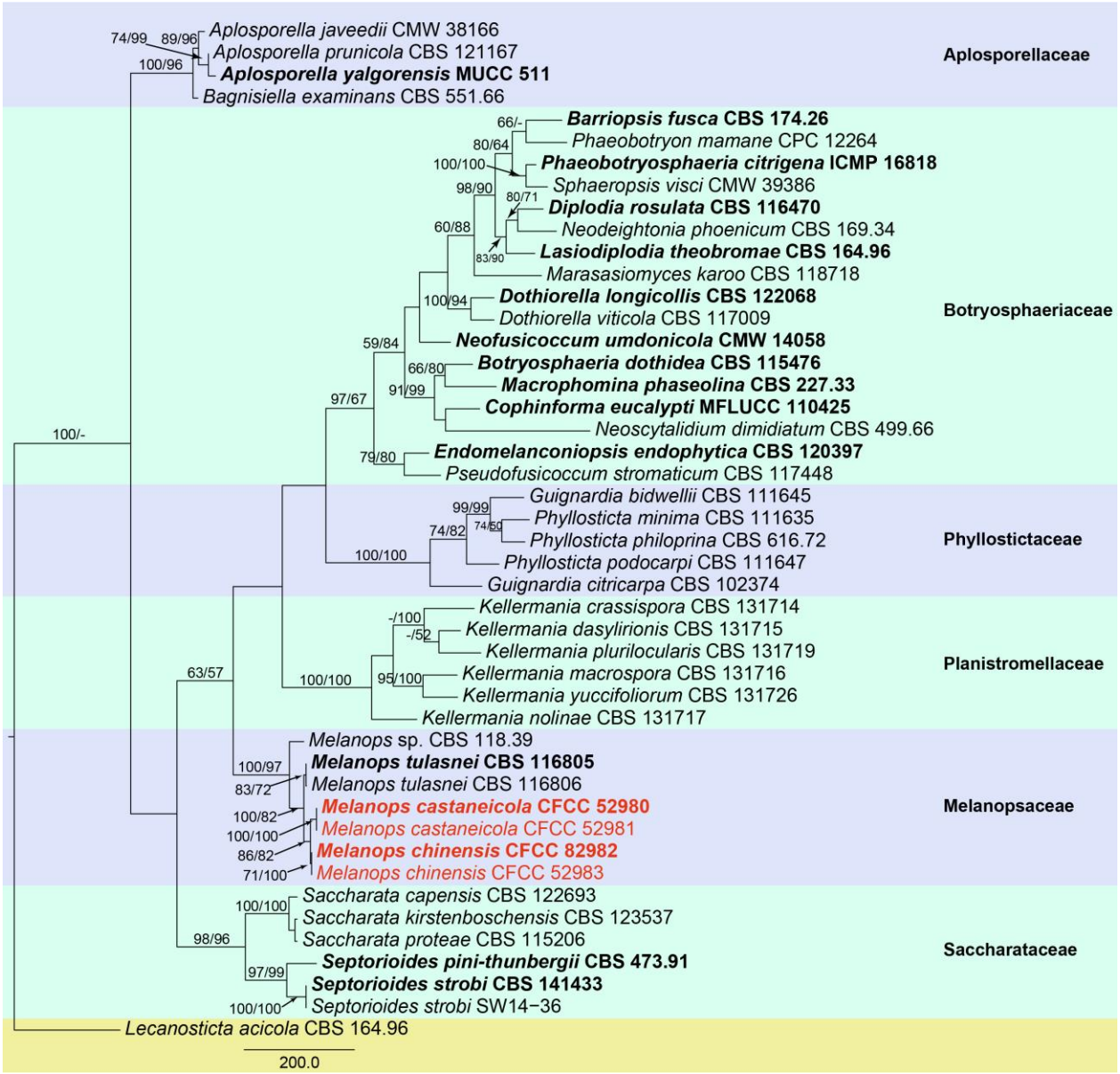

Figure 1 – Maximum parsimony tree of Botryosphaeriales based on combined LSU, ITS, *tef1* and *tub2* sequences. MP/ML bootstrap support values greater than 50 % are shown at the nodes. Scale bar = 200 nucleotide substitutions. The two new species are highlighted in red. The ex-strains are in bold face.

Melanops castaneicola C.M. Tian, A.J.L. Phillips & N. Jiang, sp. nov. Fig. 2

MycoBank number: MB 828676; Facesoffungi number: FoF05399

Etymology – Named after the host genus (*Castanea*) from which it was first isolated.

Typification – CHINA, Shaanxi Province, Ankang City, chestnut plantation, 33°39'27.29"N, 109°07'15.24"E, 2504 m asl, on dead and dying branches of *Castanea mollissima*, collected by N. Jiang, 8 July 2017 (BJFC-S1578, holotype), culture ex-holotype CFCC 52980.

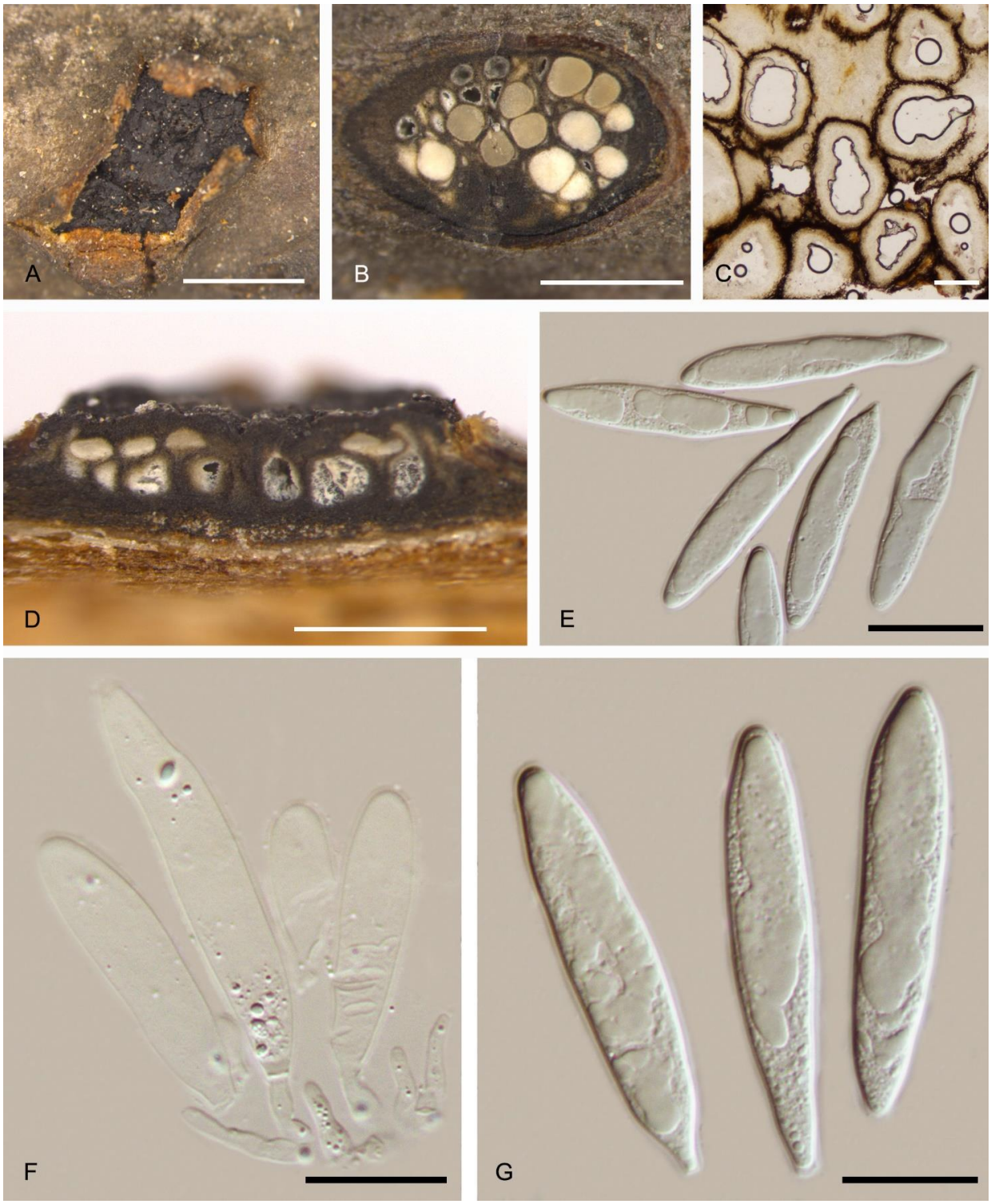

Figure 2 – *Melanops castaneicola* from *Castanea mollissima* (BJFC-S1578, holotype). A Habit of conidiomata erumpent through the bark. B, C Transverse sections through a conidioma. D Longitudinal section through a conidioma. E, G Conidia. F Conidiogenous cells. Scale bars: A, B, D = 0.5 mm; C = 50 μm; E = 30 μm; F, G = 20 μm.

Sexual morph – Not observed. Asexual morph – *Conidiomata* 1–2 mm wide, 0.2–0.5 mm high, pycnidial, multilocular, thick-walled, dark brown to black, semi-immersed in the host becoming erumpent when mature. Wall composed of dark-walled, thick-walled cells of *textura angularis* becoming progressively thinner-walled and paler towards the loculi, individual locules 50–200 μm diameter. *Ostioles* circular and central on each locule, non-papillate. *Paraphyses* 1.5–2 μm wide, up to 30 μm long, filiform, septate, unbranched, arising between the conidiogenous cells, tip rounded or slightly swollen. *Conidiogenous cells*, 5–15 × 2–4 μm, cylindrical, hyaline, unbranched, discrete, formed from the inner wall of the forming a single conidium at the tip and proliferating percurrently to form one or two annellations, rarely proliferating at the same level giving rise to periclinal thickenings. *Conidia* (50.2–)56.3–66.7(–72.1) × (12.4–)12.9–14.6 (–15.6) μm, l/w = (3.5–)4–5(–5.6) (n = 50), hyaline, aseptate, fusiform, widest in the middle, apex acute, base truncate with a minute marginal frill, surrounded by a narrow, persistent mucous sheath, contents granular.

Culture characters – On PDA at 25 °C, cultures initially white, becoming grey after 1 week. Colonies flat, with irregular margins; texture initially uniform, producing concentric circles within 1 month at 25 °C in the dark.

Habitat and host range – on dead and dying branches of *Castanea mollissima*.

Additional specimen examined – CHINA, Shaanxi Province, Ankang City, chestnut plantation, 32°13'43.51"N, 109°00'44.24"E, 1810 m asl, on dead and dying branches of *Castanea mollissima*, collected by N. Jiang, 3 July 2017 (BJFC-S1579, paratype), living culture CFCC 52981.

Notes – Two isolates of *Melanops castaneicola* cluster in a well-supported clade (MP/ML = 100/100) sister to *M. chinensis* (Fig. 1). *Melanops castaneicola* can be distinguished from *M. chinensis* by its shorter conidia (56.3–66.7 μm in *M. castaneicola* vs. 68.1–73.7 μm in *M. chinensis*). Additionally, these two species inhabit different host genera (*M. castaneicola* on *Castanea* vs. *M. chinensis* on *Quercus*) in *Fagaceae*. *Melanops castaneicola* is separated from *M. tulasnei* by 10 bp differences in ITS and 7 bp differences in *tef1*, while 5 bp differences in ITS and 4 bp differences in *tef1* separate it from *M. chinensis*.

Melanops chinensis C.M. Tian, A.J.L. Phillips & N. Jiang, sp. nov. Fig. 3

MycoBank number: MB828684; Facesoffungi number: FoF05398

Etymology – Named after the country where it was first found, China.

Typification – CHINA, Hebei Province, Qinhuangdao City, Zu Mountain, 40°14'13.22"N, 119°43'28.42"E, 1125 m asl, on dead and dying branches of *Quercus* sp., collected by N. Jiang, 5 April 2018 (BJFC-S1580, holotype), culture ex-holotype CFCC 52982.

Sexual morph – Not observed. Asexual morph – *Conidiomata* 2–4 mm wide, 1–2.5 mm high, pycnidial, multilocular, thick-walled, dark brown to black, immersed in the host. Wall composed of dark-walled, thick-walled cells of *textura angularis* becoming progressively thinner-walled and paler towards the loculi, individual locules 150–450 μm diameter. *Ostioles* circular and central on each locule, non-papillate. *Paraphyses* 1.5–2.5 μm wide, up to 25 μm long, filiform, septate, unbranched, arising between the conidiogenous cells, tip rounded or slightly swollen. *Conidiogenous cells* 5–20 × 1.5–3.5 μm, cylindrical, hyaline, unbranched, discrete, formed from the inner wall of the conidioma, forming a single conidium at the tip and proliferating percurrently to form one or two annellations, rarely proliferating at the same level giving rise to periclinal thickenings. *Conidia* (64.4–)68.1–73.7(–75.1) × (11.7–)12.4–14.5 (–15.6) μm, l/w = (4.4–)4.7–5.9(–6.4) (n = 50), hyaline, aseptate, fusiform, widest in the middle, apex acute, base truncate with a minute marginal frill, surrounded by a narrow, persistent mucous sheath, contents granular.

Culture characters – On PDA at 25 °C, cultures initially white, becoming grey to black after 1 week. Colonies are flat, with irregular margins; texture initially uniform, producing concentric circles within 1 month at 25 °C in the dark (Fig. 4).

Habitat and host range – on dead and dying branches of *Quercus* sp.

Additional specimen examined – CHINA, Qinhuangdao City, Zu Mountain, 40°14'13.15"N,

119°43'28.34"E, 1125 m asl, on dead and dying branches of *Quercus* sp., collected by N. Jiang, 5 April 2018 (BJFC-S1581, paratype), living culture CFCC 52983.

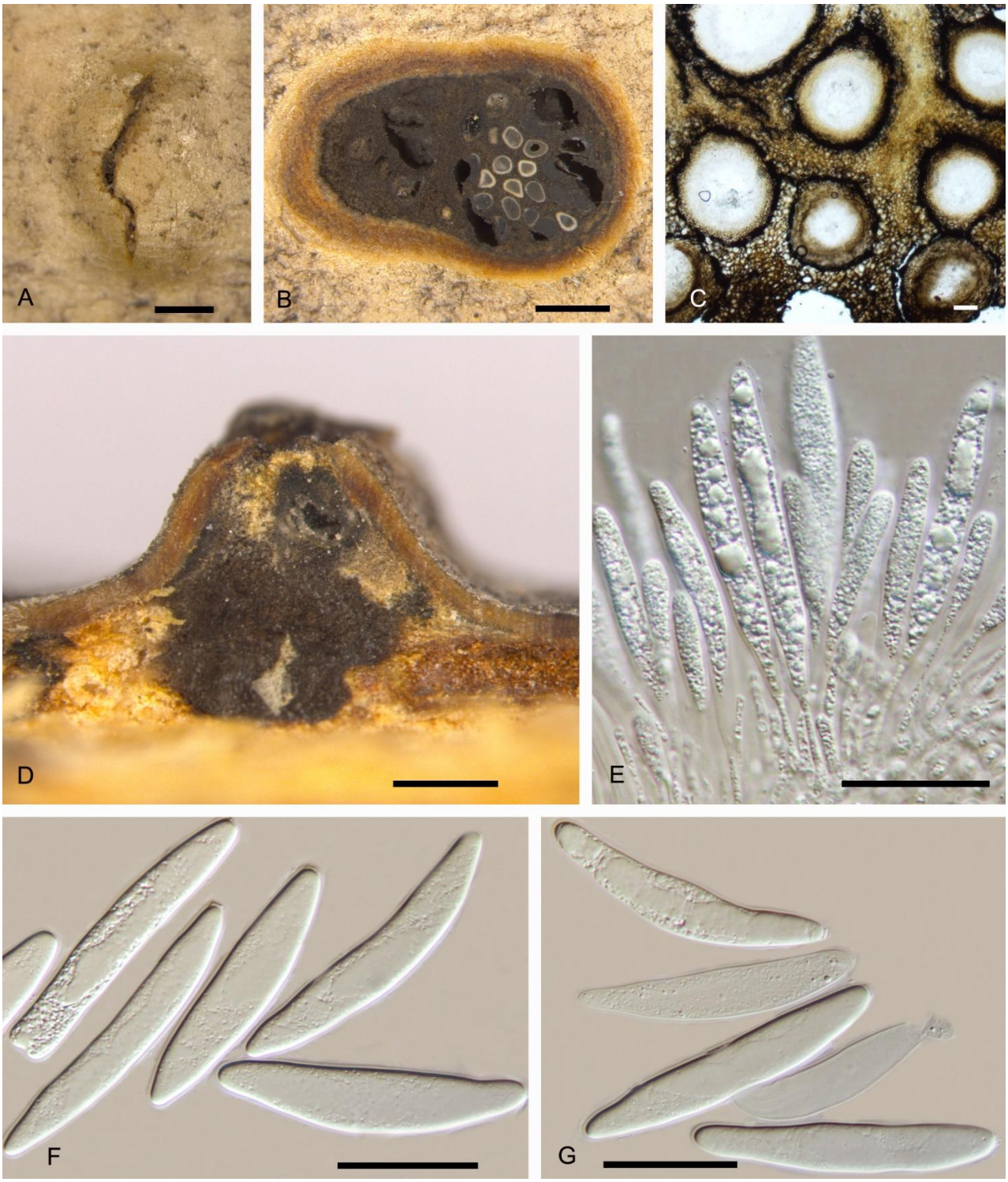

Figure 3 – *Melanops chinensis* from *Quercus* sp. (BJFC-S1580, holotype). A Habit of conidiomata immersed in the bark. B, C Transverse sections through conidiomata. D Longitudinal section through a conidioma. E Conidiogenous cells Conidia. F, G Conidia. Scale bars: A, B, D = 0.5 mm, C = 50 μm, E, G = 30 μm.

Notes – The two isolates of *Melanops chinensis* cluster in a well-supported clade (MP/ML = 71/100) (Fig. 1). Although *Melanops chinensis* was found on the same tree genus as *M. tulasnei* (*Quercus*), *M. chinensis* has larger conidia than *M. tulasnei* (68.1–73.7 × 12.4–14.5 μm in *M.*

chinensis vs. 45–46.8 × 9.1–9.7 μm in *M. tulasnei*) (Phillips & Alves 2009). Furthermore, the two species are separated by 5 bp differences in ITS and 3 bp differences in *tef1*.

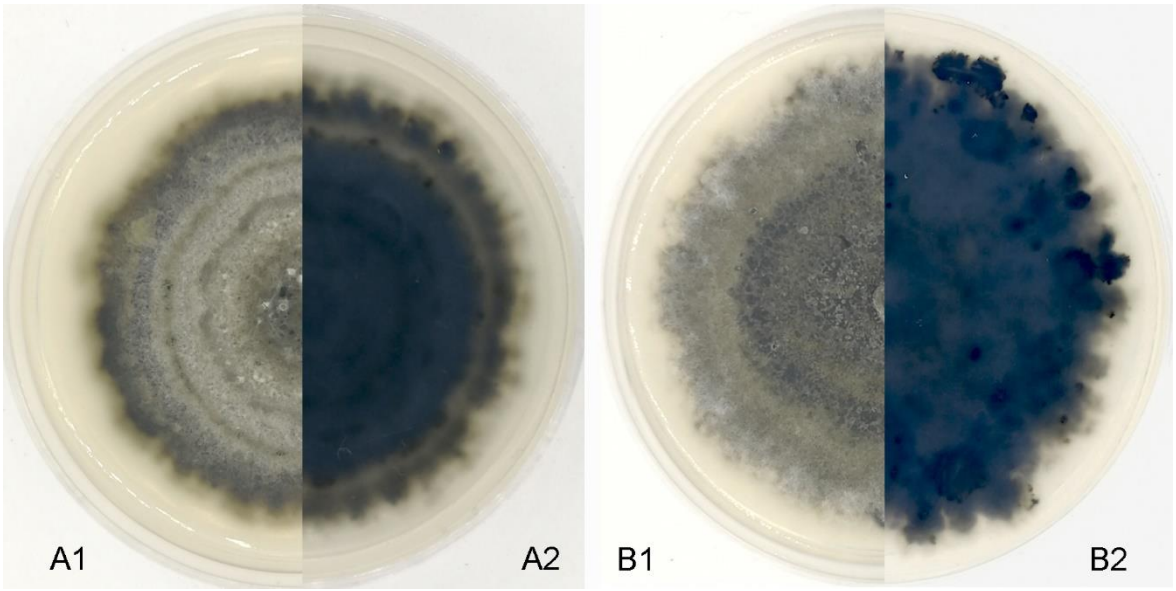

Figure 4 – Cultures on PDA after 1 month at 25 °C. A1–A2 *M. castaneicola*. B1–B2 *M. chinensis*. A1 and B1 surface view, A2 and B2 reverse side of colonies.

Discussion

In this study, *Melanops castaneicola* and *M. chinensis* are introduced based on morphological and phylogenetic evidence. Hence, at least three *Melanops* species are now known from cultures and can be distinguished by differences in asexual fruiting bodies and conidial dimension (Table 2). Interestingly, all three species inhabit *Fagaceae* hosts in the northern hemisphere.

Table 2. Morphological comparison of three *Melanops* species.

Species	***Melanops tulasnei***	***Melanops castaneicola***	***Melanops chinensis***
Conidial length (μm)	(37.2–)45–46.8(–53)	(50.2–)56.3–66.7(–72.1)	(64.4–)68.1–73.7(–75.1)
Conidial width (μm)	(7.2–)9.1–9.7(–12.1)	(12.4–)12.9–14.6(–15.6)	(11.7–)12.4–14.5(–15.6)
Length/width ratio	N/A	(3.5–)4–5(–5.6)	(4.4–)4.7–5.9(–6.4)
Distribution	USA, Canada, Germany, Italy	China	China
Host	*Quercus* sp.	*Castanea mollissima*	*Quercus* sp.
Conidiomata	Semi-immersed	Semi-immersed	Immersed

Melanops chinensis forms asexual fruiting bodies fully immersed in the oak bark, different from *M. castaneicola* and *M. tulasnei*. It is reasonable that the formation of semi-immersed or fully immersed conidiomata is not related to the host, as *Melanops tulasnei* has semi-immersed conidiomata in oak barks (Phillips & Alves 2009). The most obvious feature to distinct the three species is the conidial dimension (Table 2).

Currently 103 *Melanops* species names have been recorded in Index Fungorum (http://www.indexfungorum.org, 2018) and 109 records are in MycoBank (http://www.mycobank.org, 2018). However, some have been transferred to other families (mostly in Botryosphaeriales), the rest remain unsolved. As in the previous research, Phillips & Alves could not establish any cultures of them (2009). Hence, more intensive sampling should be conducted to determine their correct taxonomic and phylogenetic position, which will reveal more unknown taxa.

Until now, whether *Melanops* species are pathogenic to their hosts or not is not known because they are not commonly found and seldom collected. Many Botryosphaeriales taxa have

been proven to be phytopathogens on different plant parts (Phillips & Alves 2009, Slippers et al. 2013, 2017), and *Melanops* species were collected from cankers on dead and dying branches in this study. Hence, *Melanops* species might contribute to canker diseases when hosts are stressed. Further collections and inoculation experiments are required, however, to confirm pathogenicity.

Acknowledgements

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References

Alves A, Crous PW, Correia A, Phillips AJL. 2008 – Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity 28, 1–13.

Carbone I, Kohn LM. 1999 – A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3), 553–556.

Doyle JJ, Doyle JL. 1990 – Isolation of plant DNA from fresh tissue. Focus 12, 13–15.

Crous PW, Slippers B, Wingfield MJ, Rheeder J et al. 2006 – Phylogenetic lineages in the *Botryosphaeriaceae*. Studies in Mycology 55, 235–253.

Glass NL, Donaldson GC. 1995 – Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61(4), 1323–1330.

Guindon S, Dufayard JF, Lefort V, Anisimova M et al. 2010 – New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic and biology 59(3), 307–321.

Jeewon R, Hyde KD. 2016 – Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. Mycosphere 7(11), 1669–1677.

Moncalvo JM, Wang HH, Hseu RS. 1995 – Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia 87, 223–238.

Phillips AJL, Alves A. 2009 – Taxonomy, phylogeny, and epitypification of *Melanops tulasnei*, the type species of *Melanops*. Fungal Diversity 38, 155–166.

Phillips AJL, Alves A, Abdollahzadeh J, Slippers B et al. 2013 – The *Botryosphaeriaceae*: genera and species known from culture. Studies in Mycology 76, 51–167.

Phillips AJL, Hyde KD, Alves A, Liu JK. 2018 – Families in Botryosphaeriales: a phylogenetic, morphological and evolutionary perspective. Fungal Diversity (In Press).

Phillips AJL, Pennycook SR. 2004 – Taxonomy of *Botryosphaeria melanops* and its anamorph, *Fusicoccum advenum*. Sydowia 56(2), 288–295.

Slippers B, Boissin E, Phillips AJL, Groenewald JZ et al. 2013 – Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. Studies in Mycology 76, 31–49.

Slippers B, Crous PW, Jami F, Groenewald JZ, Wingfield MJ. 2017 – Diversity in the Botryosphaeriales: Looking back, looking forward. Fungal Biology 121(4), 307–321.

Swofford DL. 2003 – PAUP*: Phylogenetic Analysis Using Parsimony, * and Other Methods, Version 4.0b10, Sinauer Associates, Sunderland.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 – MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 30(12), 2725–2729.

Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8), 4238–4246.

Voglmayr H, Castlebury LA, Jaklitsch WM. 2017 – *Juglanconis* gen. nov. on *Juglandaceae*, and the new family *Juglanconidaceae* (Diaporthales). Persoonia 38, 136–155.
White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18, 315–322.
Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL et al. 2017 – Notes for genera: Ascomycota. Fungal Diversity 86(1), 1–594.
Wyka SA, Broders KD. 2016 – The new family *Septorioideaceae*, within the Botryosphaeriales and *Septorioides strobi* as a new species associated with needle defoliation of *Pinus strobus* in the United States. Fungal Biology 120(8), 1030–1040.
Yang T, Groenewald JZ, Cheewangkoon R, Jami F et al. 2017 – Families, genera, and species of Botryosphaeriales. Fungal Biology 121(4), 322–346.