



Identification and characterization of chestnut branch-inhabiting melanocratic fungi in China

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Abstract

Chinese chestnut (*Castanea mollissima*) is an important commercial tree species widely cultivated in most provinces in China. Chestnut branch-inhabiting fungi may be responsible for branch canker and dieback. In present study, dead corticated chestnut branches with superficial ascomata or conidiomata were collected from chestnut plantations in Hebei, Shaanxi and Shandong provinces. Fungi producing melanocratic ascospores or conidia were observed and identified based on both morphology and analyses of DNA sequence data. Specimens were identified to five species, viz. *Neopseudomelanconis castaneae* gen. et sp. nov., *Aplosporella javeedii* Jami, Gryzenh., Slippers and M.J. Wingf., *Coryneum umbonatum* Nees, *Lopadostoma americanum* Jaklitsch, J. Fourn., J.D. Rogers and Voglmayr and *Myrmaecium fulvopruinatum* Jaklitsch and Voglmayr.

Key words – 2 new taxa – canker – Diaporthales – Pseudomelanconidaceae – taxonomy

Introduction

Chestnuts are important fruit and timber trees worldwide. The four main species are commonly known as American, Chinese, European and Japanese chestnuts. Chinese sweet chestnut (*Castanea mollissima* Blume) is widely cultivated in China for its important economic value (Zhang et al. 2009, Lu & Guo 2017). Nevertheless, fungal diseases have greatly disturbed the healthy growth of chestnut (Tai 1979, Visentin et al. 2012, Gong et al. 2017, Jiang et al. 2018). Chestnut blight caused by *Cryphonectria parasitica* is the most notorious branch canker disease among various chestnut diseases worldwide (Jiang et al. 2018, Rigling & Prospero 2018).

Melanocratic fungi are an artificial taxonomic group which produces melanocratic conidia or ascospores (Wijayawardene et al. 2016). They are more recognizable than species which produce hyaline spores, hence we started our taxonomic work on branch-inhabiting fungi on *Castanea mollissima* with melanocratic taxa (Fig. 1). In this study, fresh specimens with melanocratic spores were collected from Hebei, Shaanxi and Shandong provinces in China, and were identified to five genera, viz. *Aplosporella*, *Coryneum*, *Lopadostoma*, *Myrmaecium* and *Neopseudomelanconis* gen. nov., with evidence from morphology and phylogenies.

Branch-inhabiting fungi on *Castanea mollissima* mainly contain canker pathogens and

saprobies. For example, *Cryphonectria parasitica* is an important canker pathogen and *C. radicalis* is considered as saprobe on chestnut trees (Gryzenhout et al. 2009). Determining whether fungi are parasitic or saprobic depends on their pathogenicity to their hosts, hence there is no absolute limit between pathogens and saprobies. In China, identification of branch-inhabiting pathogens and saprobies on chestnut is poor. Therefore, the present study was conducted (i) to identify branch-inhabiting melanocratic fungi on chestnut trees and (ii) to propose *Neopseudomelanconis* gen. nov. based on both morphological and phylogenetic evidences.



Figure 1 – Melanocratic spores observed from *Castanea mollissima*. A *Neopseudomelanconis castaneae*. B *Aplosporella javeedii*. C *Coryneum umbonatum*. D *Myrmaecium fulvopruinatum*. E *Lopadostoma americanum*. Scale bars: A–E = 10 μ m.

Materials & Methods

Isolates and morphology

Fresh specimens of dead corticated branches were collected from *Castanea mollissima* in Hebei, Shaanxi and Shandong provinces in China. Single conidial and ascospore isolates were established by removing a mucoid spore mass from conidiomata or ascomata, and spreading the suspension on the surface of PDA (potato dextrose agar) or MEA (malt extract agar). After inoculation, agar plates were incubated at 25 °C to induce germination of spores. Single germinating spores were then transferred to clean plates under a dissecting microscope with a sterile needle (Jaklitsch et al. 2014, 2015, Jami et al. 2014, Wijayawardene et al. 2016, Senanayake et al. 2017). Specimens and isolates were deposited in the Museum of Beijing Forestry University (BJFC). Axenic cultures are maintained in the China Forestry Culture Collection Center (CFCC).

Species identification was based on morphological features of the conidiomata or ascomata produced on infected chestnut branches. Cross-sections were prepared by hand using a double-edge blade under a Leica stereomicroscope (M205 FA). At least 20 conidiomata/ascomata, 10 asci and 50 conidia/ascospores were measured to calculate the mean size and standard deviation. Measurements were reported as maxima and minima in parentheses and the range representing the mean plus and minus the standard deviation of the number of measurements given in parentheses (Voglmayr et al. 2017). Microscopic photographs 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. Cultural characteristics of isolates incubated on PDA or MEA in the dark at 25°C were recorded.

Molecular characterization and multi-locus phylogenetic analysis

Genomic DNA was extracted from axenic living cultures with cellophane using a modified CTAB method (Doyle & Doyle 1990). To amplify the internal transcribed spacer (ITS), large subunit (LSU), translation elongation factor 1 α (TEF1- α) and the largest subunit of RNA polymerase II (RPB2), we used the primers LR0R and LR5 (Moncalvo et al. 1995, Vilgalys & Hester 1990), ITS1 and ITS4 (White et al. 1990), EF1-688F and EF1-986R (Alves et al. 2008,

Carbone & Kohn 1999), dRPB2-5f and dRPB2-7r (Voglmayr et al. 2016), respectively. The polymerase chain reaction assay was conducted as described by Fan et al. (2018). 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® Terminator Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Sequences from this study and reference sequences obtained from GenBank (Tables 1–4) 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), maximum likelihood (ML) analysis with PhyML v.3.1 (Guindon et al. 2010) and Bayesian inference (BI) with MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). Sequences data were deposited in GenBank (Tables 1–4). The multilocus file was deposited in TreeBASE (www.treebase.org) as accession S22783. Introduction of the new species based on molecular data follow the recommendations of Jeewon & Hyde (2016).

Table 1 Strains of Diaporthales species used in the molecular analyses in this study, the genes sequenced and GenBank accessions. The new strains from the current study are in red.

Species	Strain/Specimen	Host	GenBank numbers	
			ITS	LSU
<i>Apiosporopsis carpinea</i>	CBS 771.79	<i>Carpinus betulus</i>	NA	AF277130
<i>Apiosporopsis</i> sp.	Masuya 11Af2-1	<i>Alnus firma</i>	NA	AB669034
<i>Apharknessia insueta</i>	CBS 111377	<i>Eucalyptus pellita</i>	JQ706083	AY720814
<i>Asterosporium asterospermum</i>	MFLU 15-3555	<i>Fagus sylvatica</i>	NA	MF190062
<i>Auratiopycnidia tristaniopsidis</i>	CBS 132180	<i>Tristaniopsis laurina</i>	JQ685516	JQ685522
<i>Chiangraiomycetes bauhiniae</i>	MFLUCC 17-1669	<i>Bauhinia</i> sp.	MF190118	MF190064
<i>Coniella straminea</i>	CBS 149.22	<i>Fragaria</i> sp.	AY339348	AF362569
<i>Coniella wangiensis</i>	CBS 132530	<i>Eucalyptus</i> sp.	JX069873	JX069857
<i>Coryneum arausiacum</i>	MFLUCC 13-0658	<i>Quercus</i> sp.	MF190120	MF190066
<i>Coryneum arausiacum</i>	MFLUCC 15-1110	<i>Quercus</i> sp.	MF190121	MF190067
<i>Coryneum modonium</i>	AR 3558	<i>Castanea sativa</i>	NA	EU683073
<i>Coryneum depressum</i>	AR 3897	<i>Quercus cerris</i>	NA	EU683074
<i>Coryneum umbonatum</i>	AR 3541	<i>Quercus cerris</i>	NA	EU683072
<i>Cryphonectria macrospora</i>	CBS 122593	<i>Quercus mongolica</i>	EU199182	AF408340
<i>Cryphonectria parasitica</i>	ATCC 38755	<i>Castanea dentata</i>	AY141856	EU199123
<i>Cryptosporella hypodermia</i>	AR 3552	<i>Ulmus minor</i>	EU199181	AF408346
<i>Cytospora chrysosperma</i>	CFCC 89600	<i>Sophora japonica</i>	KR045623	KR045623
<i>Dendrostoma mali</i>	CFCC 52102	<i>Malus spectabilis</i>	MG682072	MG682012
<i>Diaporthe eres</i>	CBS 109767	<i>Acer campestre</i>	KC343075	AF408350
<i>Diaporthosporella cercidicola</i>	CFCC 51994	<i>Cercis chinensis</i>	KY852492	KY852515
<i>Diaporthostoma machili</i>	CFCC 52100	<i>Machilus leptophylla</i>	MG682080	MG682020
<i>Disculoides eucalypti</i>	CPC 17650	<i>Eucalyptus</i> sp.	JQ685517	JQ685523
<i>Ditopella ditopa</i>	CBS 109748	<i>Alnus glutinosa</i>	EU199187	EU199126
<i>Erythrogloeum hymenaeae</i>	CPC 18819	<i>Hymenaea courbaril</i>	JQ685519	JQ685525
<i>Gnomonia gnomon</i>	CBS 199.53	<i>Corylus avellana</i>	AY818956	AF408361

Table 1 Continued.

Species	Strain/Specimen	Host	GenBank numbers	
			ITS	LSU
<i>Harknessia eucalypti</i>	CBS 342.97	<i>Eucalyptus regnans</i>	AY720745	AF408363
<i>Harknessia molokaiensis</i>	CBS 109779	<i>Eucalyptus robusta</i>	NA	AF408390
<i>Hercospora tiliae</i>	CBS 109746	<i>Tilia tomentosa</i>	NA	AF408365
<i>Juglanconis appendiculata</i>	D96	<i>Juglans nigra</i>	KY427139	KY427139
<i>Juglanconis juglandina</i>	ME23	<i>Juglans nigra</i>	KY427150	KY427150
<i>Lamproconium desmazieri</i>	MFLUCC 15-0870	<i>Tilia tomentosa</i>	KX430134	KX430135
<i>Lasmenia</i> sp.	CBS 124123	<i>Nephelium lappaceum</i>	GU797406	JF838338
<i>Macrohilum eucalypti</i>	CPC 10945	<i>Eucalyptus</i> sp.	DQ195781	DQ195793
<i>Melanconiella ellisii</i>	BPI 878343	<i>Carpinus caroliniana</i>	JQ926271	JQ926271
<i>Melanconiella spodiaea</i>	MSH	<i>Carpinus betulus</i>	JQ926298	JQ926298
<i>Melanconis betulae</i>	CFCC 50471	<i>Betula albosinensis</i>	KT732952	KT732971
<i>Melanconis stilbostoma</i>	CFCC 50475	<i>Betula platyphylla</i>	KT732956	KT732975
<i>Nakataea oryzae</i>	CBS 243.76	NA	KM484861	DQ341498
<i>Neopseudomelanconis castaneae</i>	CFCC 52787	<i>Castanea mollissima</i>	MH469162	MH469164
<i>Neopseudomelanconis castaneae</i>	CFCC 52788	<i>Castanea mollissima</i>	MH469163	MH469165
<i>Pachytrype princeps</i>	Rogers S	NA	NA	FJ532382
<i>Paradiaporthe artemisiae</i>	MFLUCC 14-0850	<i>Artemisia</i> sp.	MF190155	MF190100
<i>Prosopidicola mexicana</i>	CBS 113530	<i>Prosopis glandulosa</i>	AY720710	NA
<i>Pseudomelanconis caryae</i>	CFCC 52110	<i>Carya cathayensis</i>	MG682082	MG682022
<i>Pseudoplagiostoma eucalypti</i>	CBS 124807	<i>Eucalyptus urophylla</i>	GU973512	GU973606
<i>Pseudoplagiostoma oldii</i>	CBS 115722	<i>Eucalyptus camaldulensis</i>	GU973535	GU973610
<i>Pyricularia grisea</i>	Ina168	NA	AB026819	AB026819
<i>Rossmania ukurunduensis</i>	AR 3484	<i>Acer ukurunduense</i>	NA	EU683075
<i>Stegonsporium pyriforme</i>	CBS 124487	<i>Acer heldreichii</i>	KF570160	KF570160
<i>Stilbospora macrosperma</i>	CBS 121883	<i>Carpinus betulus</i>	JX517290	JX517299
<i>Sydowiella fenestrans</i>	CBS 125530	<i>Chamerion angustifolium</i>	JF681956	EU683078
<i>Synnemasporella aculeans</i>	CFCC 52094	<i>Rhus chinensis</i>	MG682086	MG682026
<i>Synnemasporella toxicodendri</i>	CFCC 52097	<i>Toxicodendron sylvestri</i>	MG682089	MG682029

Table 2 Strains of *Aplosporella* species used in the molecular analyses in this study, the genes sequenced and GenBank accessions. The new strains from the current study are in red.

Species	Strain/Specimen	Host	GenBank numbers	
			ITS	TEF1- α
<i>Aplosporella africana</i>	CBS 121777	<i>Acacia mellifera</i>	EU101315	EU101360
<i>Aplosporella africana</i>	CBS 121779	<i>Acacia mellifera</i>	EU101317	EU101362
<i>Aplosporella artocarpi</i>	CPC 22791	<i>Artocarpus heterophyllus</i>	KM006450	KM006481
<i>Aplosporella hesperidica</i>	CBS 208.37	NA	JX681069	NA
<i>Aplosporella javeedii</i>	CFCC 50052	<i>Gleditsia sinensis</i>	KP208838	KP208844

Table 2 Continued.

Species	Strain/Specimen	Host	GenBank numbers	
			ITS	TEF1- α
<i>Aplosporella javeedii</i>	CFCC 50053	<i>Sophora japonica</i>	KP208839	KP208845
<i>Aplosporella javeedii</i>	CFCC 52777	<i>Castanea mollissima</i>	MH458901	MH482840
<i>Aplosporella ginkgonis</i>	CFCC 89660	<i>Morus alba</i>	KR045623	KP310847
<i>Aplosporella ginkgonis</i>	CFCC 89661	<i>Ginkgo biloba</i>	KM030583	KM030597
<i>Aplosporella macropycnidia</i>	CGMCC3.17725	<i>Cerasus yedoensis</i>	KT343648	KX011176
<i>Aplosporella macropycnidia</i>	CGMCC3.17726	<i>Cerasus yedoensis</i>	KT343649	KX011177
<i>Aplosporella papillata</i>	CBS 121780	<i>Acacia tortillas</i>	EU101328	EU101373
<i>Aplosporella papillata</i>	CBS 121781	<i>Acacia erioloba</i>	EU101329	EU101374
<i>Aplosporella prunicola</i>	CBS 121167	<i>Prunus persica</i> var. <i>nucipersica</i>	KF766147	NA
<i>Aplosporella prunicola</i>	STE-U 6326	<i>Prunus persica</i> var. <i>nucipersica</i>	EF564375	NA
<i>Aplosporella thailandica</i>	MFLU 16-0615	NA	KX423536	KX423537
<i>Aplosporella yalgorensis</i>	MUCC 511	<i>Eucalyptus gomphocephala</i>	EF591926	EF591977
<i>Aplosporella yalgorensis</i>	MUCC 512	<i>Mimetes cucullata</i>	EF591927	EF591978
<i>Saccharata proteae</i>	CBS 115206	NA	KC343004	KC343730

Table 3 Strains of *Lopadostoma* species used in the molecular analyses in this study, the genes sequenced and GenBank accessions. The new strains from the current study are in red.

Species	Strain/Specimen	Host	GenBank numbers		
			ITS	LSU	RPB2
<i>Lopadostoma americanum</i>	CBS 133211	<i>Quercus</i> sp.	KC774568	KC774568	KC774525
<i>Lopadostoma americanum</i>	CFCC 52313	<i>Castanea mollissima</i>	MH454484	MH454486	MH482841
<i>Lopadostoma americanum</i>	CFCC 52314	<i>Castanea mollissima</i>	MH454485	MH454487	MH482842
<i>Lopadostoma</i> cf. <i>amoenum</i>	MUCL 51842	<i>Fagus sylvatica</i>	KC774569	KC774569	NA
<i>Lopadostoma dryophilum</i>	CBS 133213	<i>Quercus petraea</i>	KC774570	KC774570	KC774526
<i>Lopadostoma dryophilum</i>	LG23	<i>Quercus petraea</i>	KC774571	KC774571	KC774527
<i>Lopadostoma dryophilum</i>	LG24	<i>Quercus petraea</i>	KC774572	KC774572	KC774528
<i>Lopadostoma fagi</i>	LF	NA	KC774574	KC774574	KC774530
<i>Lopadostoma fagi</i>	CBS 133206	<i>Fagus sylvatica</i>	KC774575	KC774575	KC774531
<i>Lopadostoma fagi</i>	LF2	<i>Fagus sylvatica</i>	KC774576	KC774576	KC774532
<i>Lopadostoma gastrinum</i>	LG1	<i>Carpinus betulus</i>	KC774579	KC774579	KC774535
<i>Lopadostoma gastrinum</i>	CBS 133210	<i>Ulmus glabra</i>	KC774581	KC774581	KC774536
<i>Lopadostoma gastrinum</i>	CBS 134632	<i>Ulmus minor</i>	KC774584	KC774584	KC774537
<i>Lopadostoma insulare</i>	LG32	<i>Quercus coccifera</i>	KC774588	KC774588	KC774541
<i>Lopadostoma insulare</i>	CBS 133214	<i>Quercus ilex</i>	KC774589	KC774589	KC774542
<i>Lopadostoma lechatii</i>	CBS 133694	<i>Carpinus betulus</i>	KC774590	KC774590	KC774543
<i>Lopadostoma linospermum</i>	CBS 133208	<i>Pistacia lentiscus</i>	KC774591	KC774591	KC774544

Table 3 Continued.

Species	Strain/Specimen	Host	GenBank numbers		
			ITS	LSU	RPB2
<i>Lopadostoma linospermum</i>	LPL1	<i>Pistacia lentiscus</i>	KC774592	KC774592	KC774545
<i>Lopadostoma meridionale</i>	CBS 133209	<i>Quercus ilex</i>	KC774593	KC774593	KC774546
<i>Lopadostoma meridionale</i>	LG29	<i>Quercus ilex</i>	KC774594	KC774594	KC774547
<i>Lopadostoma meridionale</i>	LG33	<i>Quercus coccifera</i>	KC774595	KC774595	KC774548
<i>Lopadostoma</i> cf. <i>polynesium</i>	LAG	<i>Amelanchier ovalis</i>	KC774600	KC774600	KC774553
<i>Lopadostoma pouzarii</i>	CBS 103.96	<i>Fraxinus excelsior</i>	KC774601	KC774601	KC774554
<i>Lopadostoma pouzarii</i>	MUCL 47149	<i>Fraxinus excelsior</i>	KC774602	KC774602	NA
<i>Lopadostoma quercicola</i>	CBS 133212	<i>Quercus cerris</i>	KC774603	KC774603	KC774555
<i>Lopadostoma quercicola</i>	LG16	<i>Quercus cerris</i>	KC774606	KC774606	KC774556
<i>Lopadostoma quercicola</i>	CBS 134633	<i>Quercus pubescens</i>	KC774610	KC774610	KC774558
<i>Lopadostoma turgidum</i>	LT	<i>Fagus sylvatica</i>	KC774616	KC774616	KC774561
<i>Lopadostoma turgidum</i>	LT1	<i>Fagus sylvatica</i>	KC774617	KC774617	KC774562
<i>Lopadostoma turgidum</i>	CBS 133207	<i>Fagus sylvatica</i>	KC774618	KC774618	KC774563

Results

Diaporthales based on analyses of LSU and ITS sequence data.

Diaporthales is a well-resolved ascomycetous order comprising phytopathogenic, saprobic, and endophytic fungi (Senanayake et al. 2017). Pseudomelanconidaceae was proposed in Diaporthales by Fan et al. (2018), and can be distinguished from the other diaporthalean taxa by conidiogenous cells with apical collarets and discreet annellations, and the inconspicuous hyaline conidial sheath when mature. *Pseudomelanconis caryae* on *Carya cathayensis* is the sole species in Pseudomelanconidaceae and causes branch canker of hickory trees in China (Fan et al. 2018).

The taxa in Diaporthales were selected based on Wijayawardene et al. (2017, 2018). The combined LSU and ITS data set consisted of 53 strains with *Nakataea oryzae* (CBS 243.76) and *Pyricularia grisea* (Ina168) as outgroup taxa. Alignment comprises 1537 characters after alignment. Of these, 920 characters were constant, 120 variable characters were parsimony-uninformative and 507 characters were parsimony informative. The MP analysis resulted in ten equally most parsimonious trees, with the first tree (TL = 2609, CI = 0.419, RI = 0.606, RC = 0.254) was shown in Fig. 2. The topology of phylogenetic tree obtained from ML was similar with the MP tree. The novel genus appeared in a distinct clade with high bootstrap support (Fig. 2).

Neopseudomelanconis C.M. Tian & N. Jiang, gen. nov.

MycoBank number: MB 825183; Facesoffungi number: FoF 04969

Etymology – Named after its morphological similarity to *Pseudomelanconis*.

Type species: *Neopseudomelanconis castaneae* C.M. Tian & N. Jiang.

Original description – Sexual morph: Undetermined. Asexual morph – *Conidiomata* in bark, acervular, immersed in host bark to erumpent. *Ectostromatic* disc inconspicuous, causing a more or less pustulate bark surface. *Central column* beneath the disc more or less conical. The marginal part of the central column comprises conidiophores and their basal cell layers. *Conidiophores* branched

or unbranched, aseptate, cylindrical, hyaline to pale brown, smooth-walled, sometimes reduced to conidiogenous cells. *Conidiogenous cells* annellidic, sometimes with apical collarette. *Conidia* hyaline when immature, becoming brown at maturity, ellipsoid to oblong, 2-celled, multiguttulate, with distinct hyaline sheath, becoming inconspicuous when mature. Conidial wall smooth on the outer surface, with inconspicuous to distinct, sometimes confluent irregular verrucae on the inner surface.

Table 4 Strains of *Myrmaecium* species used in the molecular analyses in this study, the genes sequenced and GenBank accessions. The new strains from the current study are in red.

Species	Strain/Specimen	Host	Country	GenBank numbers			
				ITS	LSU	TEF	RPB2
<i>Bambusaria bambusae</i>	MFLUCC 12-0851	<i>Thyrsostachys siamensis</i>	Thailand	KP687812	KP687812	KP687982	KP687890
<i>Bambusaria bambusae</i>	CBS 139763	<i>Thyrsostachys siamensis</i>	Thailand	KP687813	KP687813	KP687983	KP687891
<i>Myrmaecium fulvopruinatum</i>	CBS 139057	<i>Fagus sylvatica</i>	Austria	KP687858	KP687858	KP688027	KP687933
<i>Myrmaecium fulvopruinatum</i>	CBS 139058	<i>Fagus grandifolia</i>	USA	KP687861	KP687861	KP688030	KP687936
<i>Myrmaecium fulvopruinatum</i>	CBS 139059	<i>Quercus cerris</i>	Austria	KP687863	KP687863	KP688032	KP687938
<i>Myrmaecium fulvopruinatum</i>	VFJ1	Unidentified corticated twigs	China	KP687862	KP687862	KP688031	KP687937
<i>Myrmaecium fulvopruinatum</i>	CFCC 52769	<i>Castanea mollissima</i>	China	MH458908	MH458902	MH482828	MH482835
<i>Myrmaecium fulvopruinatum</i>	CFCC 52770	<i>Castanea mollissima</i>	China	MH458909	MH458903	MH482829	MH482836
<i>Myrmaecium fulvopruinatum</i>	CFCC 52771	<i>Castanea mollissima</i>	China	MH458910	MH458904	MH482830	MH482837
<i>Myrmaecium fulvopruinatum</i>	CFCC 52772	<i>Castanea mollissima</i>	China	MH458911	MH458905	MH482831	MH482838
<i>Myrmaecium fulvopruinatum</i>	CFCC 52773	<i>Castanea mollissima</i>	China	MH458912	MH458906	MH482832	MH482839
<i>Myrmaecium fulvopruinatum</i>	CFCC 52774	<i>Castanea mollissima</i>	China	MH458913	MH458907	MH482833	MH482834
<i>Myrmaecium rubricosum</i>	CBS 139067	Unidentified bark	France	KP687881	KP687881	KP688049	KP687955
<i>Myrmaecium rubricosum</i>	CBS 139068	<i>Quercus pubescens</i>	Croatia	KP687885	KP687885	KP688053	KP687958
<i>Myrmaecium rubrum</i>	CBS 109505	<i>Quercus sp.</i>	Italy	GU456324	GU456324	GU456260	GU456344
<i>Valsaria insitiva</i>	CBS 139056	<i>Acer monspessulanum</i>	Croatia	KP687847	KP687847	KP688016	KP687922
<i>Valsaria insitiva</i>	CBS 139061	unidentified corticated twigs	China	KP687866	KP687866	KP688035	KP687941

Table 4 Continued.

Species	Strain/Specimen	Host	Country	GenBank numbers			
				ITS	LSU	TEF	RPB2
<i>Valsaria lopadostomoides</i>	CBS 139062	<i>Quercus ilex</i>	Greece	KP687868	KP687868	KP688037	KP687943
<i>Valsaria neotropica</i>	CBS 139064	Unidentified corticated twig	France	KP687874	KP687874	KP688042	KP687948
<i>Valsaria robiniae</i>	CBS 121890	<i>Hippocrepis emerus</i>	Slovenia	KP687851	KP687851	KP688020	KP687926
<i>Valsaria robiniae</i>	CBS 128015	<i>Caragana arborescens</i>	Austria	KP687853	KP687853	KP688022	KP687928
<i>Valsaria rudis</i>	CBS 139065	<i>Quercus macrolepis</i>	Greece	KP687878	KP687878	KP688046	KP687952
<i>Valsaria rudis</i>	CBS 139066	<i>Quercus pubescens</i>	Austria	KP687879	KP687879	KP688047	KP687953
<i>Valsaria spartii</i>	CBS 121714	<i>Ceratonia siliqua</i>	Greece	KP687848	KP687848	KP688017	KP687923
<i>Valsaria spartii</i>	CBS 125584	<i>Cytisus scoparius</i>	Italy	EU040213	EU040213	KP688023	KP687929

Neopseudomelanconis castaneae C.M. Tian & N. Jiang, sp. nov.

Fig. 3

MycoBank number: MB 825198; Facesoffungi number: FoF 04970

Etymology – Named after the host genus from which it was isolated, *Castanea*.

Saprobic on *Castanea mollissima* Blume in terrestrial habitats. Sexual morph – Undetermined. Asexual morph – *Conidiomata*, 0.5–1.5 mm wide (\bar{x} = 0.8, SD \pm 0.2 mm, n = 20), 0.2–0.5 mm high (\bar{x} = 0.3, SD \pm 0.15 mm, n = 20), acervular, immersed in host bark to erumpent, covered by blackish conidial masses at maturity. *Central column* beneath the disc more or less conical. *Conidiophores* unbranched or branched at base, aseptate, cylindrical, hyaline to pale brown, smooth-walled, 10–35 \times 3.5–6 μ m (\bar{x} = 25, SD \pm 5.5 μ m \times \bar{x} = 4.5, SD \pm 1.5 μ m, n = 20). *Conidiogenous cells* enteroblastic, annellidic, with distinct annellations and collarettes. *Conidia* hyaline when immature, becoming greyish sepia to olivaceous, ellipsoid to oblong, multiguttulate, straight to slightly curved, 2-celled, 18–21.5 \times 4.8–7 μ m (\bar{x} = 19.5, SD \pm 1.2 μ m \times \bar{x} = 5.9, SD \pm 0.6 μ m, n = 50), l/w = (2.7–)2.9–3.7(–4.2), with distinct hyaline sheath, 0.5–1 μ m diam., becoming inconspicuous when mature. Conidial wall smooth on the outer surface.

Culture characteristics – On PDA at 25 °C, cultures are initially white, becoming grey olivaceous. The colonies are flat, with irregular margins; texture initially uniform, becoming compact in centre after 1 month.

Known distribution – Dead corticated branches of *Castanea mollissima*.

Material examined – CHINA, Shaanxi Province, Ankang City, chestnut plantation, 33°39'27.34"N, 109°07'15.23"E, 2504 m asl, on branches of *Castanea mollissima* Blume (Fagaceae), N. Jiang, 8 July 2017 (BJFC-S1444, holotype), ex-holotype CFCC 52787. Shaanxi Province, Ankang City, chestnut plantation, 32°13'43.51"N, 109°00'44.24"E, 1810 m asl, on branches of *Castanea mollissima*, N. Jiang, 3 July 2017 (BJFC-S1445, paratype), living culture CFCC 52788.

Notes – Two isolates of *Neopseudomelanconis castaneae* cluster in a well-supported clade (MP/ML = 86/100) sister to *Pseudomelanconis caryae* (Fig. 2). *Neopseudomelanconis castaneae* and *Pseudomelanconis caryae* are united in the family Pseudomelanconidaceae by conidiogenous cells with apical collarets and discreet annellations, and the hyaline conidial sheath. However, *Neopseudomelanconis* is different from *Pseudomelanconis* in having branched conidiophores and 2-celled conidia. Additionally, *Neopseudomelanconis castaneae* and *Pseudomelanconis caryae* are the only two species in Pseudomelanconidaceae, they can be distinguished in conidial dimension (18–21.5 × 4.8–7 μm in *Neopseudomelanconis castaneae* vs. 13–15 × 4–5 μm in *Pseudomelanconis caryae*), and ITS and LSU loci (35/512 in ITS and 11/557 in LSU).

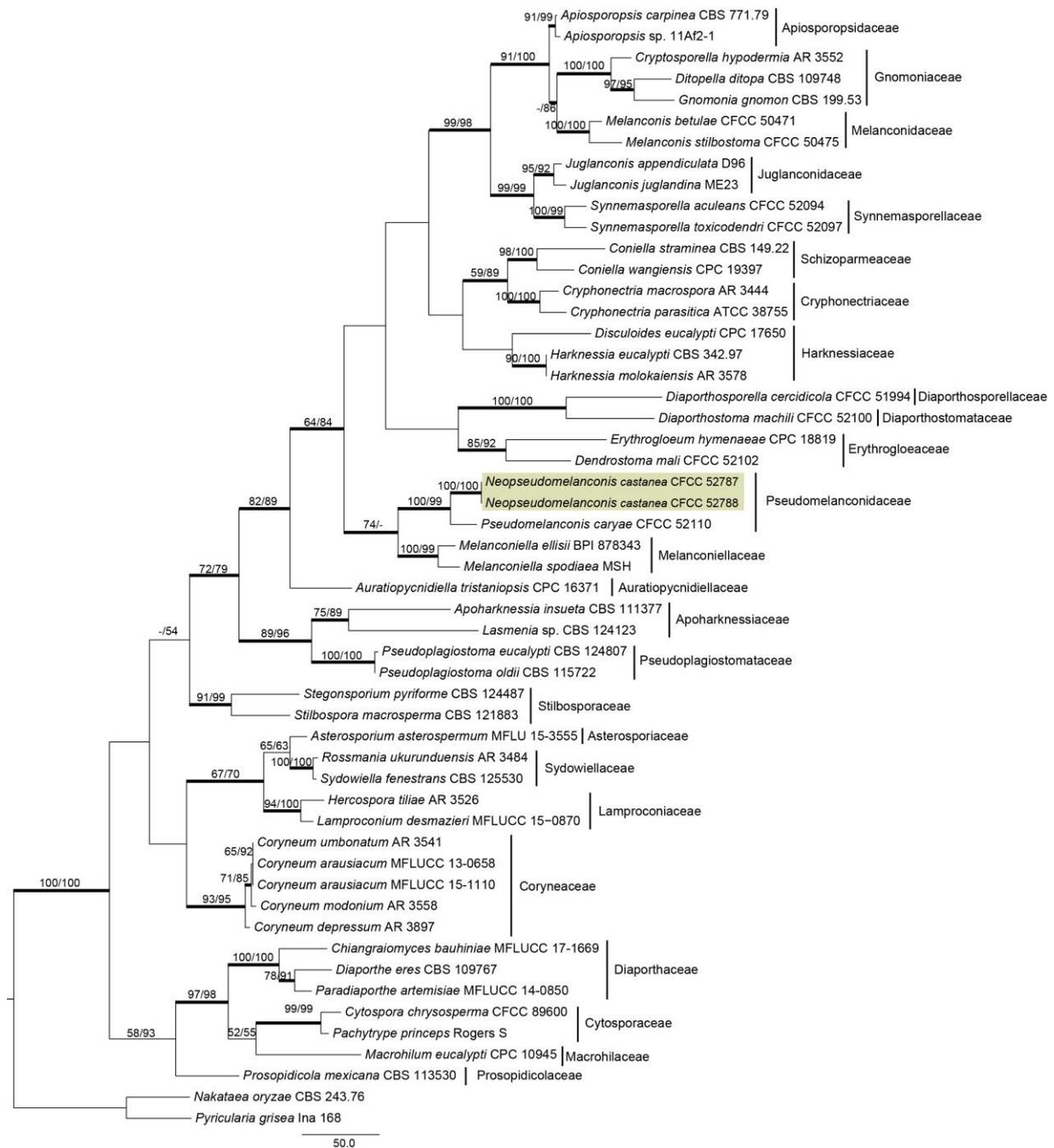


Figure 2 – Phylogram of Diaporthales based on combined LSU and ITS genes. MP and ML bootstrap support values above 50 % are shown at the first and second position. Thickened branches represent posterior probabilities above 0.90 from Bayesian inference. Scale bar = 50 nucleotide substitutions. The new sequences resulting from the current study are highlighted in yellow.

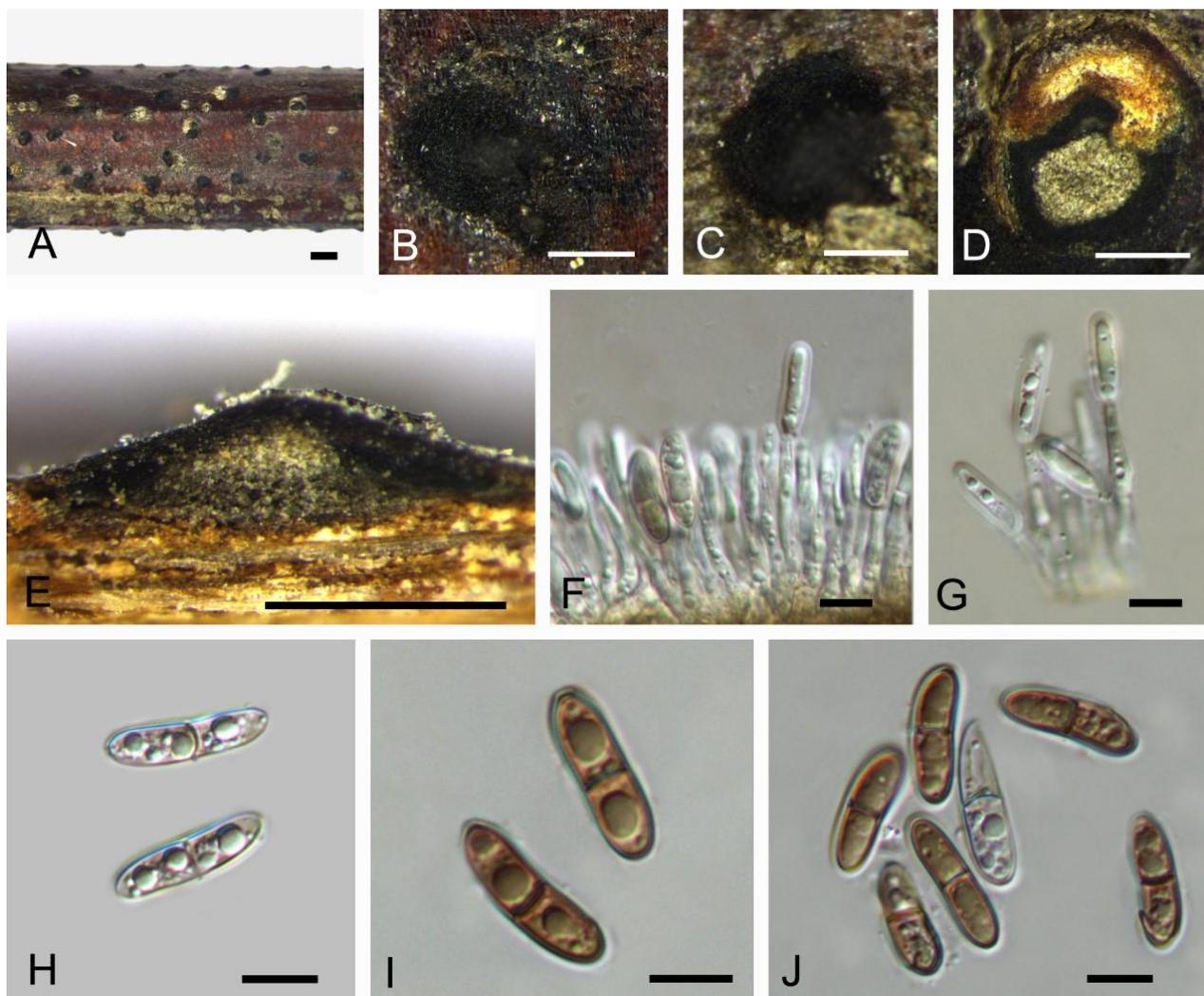


Figure 3 – Morphology of *Neopseudomelanconis castaneae* from *Castanea mollissima* (BJFC-S1444, holotype). A–C Habit of conidiomata on a stem. D Transverse sections through conidiomata. E Longitudinal sections through conidiomata. F, G Developing conidia attached to conidiogenous cells. H Immature conidia. I, J Mature conidia. Scale bars: A = 1 mm, B–E = 0.5 mm, F–J = 10 μ m.

***Aplosporella* based on analyses of ITS and TEF1- α sequence data.**

Aplosporella (Aplosporellaceae, Botryosphaerales) can be recognized by multiloculate conidiomata with a single ostiole, and brown, aseptate conidia (Du et al. 2017). The combined ITS and TEF1- α data set consists of 19 strains with *Saccharata proteae* (CBS 115206) as outgroup taxon. Alignment comprises 739 characters. Of these, 429 characters were constant, 229 variable characters were parsimony-uninformative and 81 characters were parsimony informative. The MP analysis resulted in 4 equally most parsimonious trees, with the first tree (TL = 383, CI = 0.903, RI = 0.805, RC = 0.727) was shown in Fig. 4. The topology of phylogenetic tree obtained from ML was similar with the MP tree.

Aplosporella javeedii Jami, Gryzenh., Slippers & M.J. Wingf., Fungal Biology 118: 174 (2014).

Fig. 5

Saprobic on dead and corticated branches of various hosts in terrestrial habitats. Sexual morph – Undetermined. Asexual morph – *Conidiomata* immersed in bark, erumpent from bark surface, separate, discoid, dark-brown to black, multi-locular, 0.5–1.5 mm diam. (\bar{x} = 0.8, SD \pm 0.25 mm, n = 20), wall consisting of 4–10 layers, thick outer layers composed of dark-brown textura angularis, becoming hyaline and thin-walled towards the inner region. *Disc* brown to black,

circular to ovoid, 0.42–0.76 mm (\bar{x} = 0.55, SD \pm 0.03 mm, n = 20), with 1–10 ostioles per disc. *Ostioles* multiple, conspicuous, level with the disc surface, sometimes covered below disc by lighter entostroma, 25.1–65.8 μ m in diam. (\bar{x} = 50.5, SD \pm 4.5 μ m, n = 20). *Locules* multiple, irregularly arranged, subdivided frequently by invaginations with common walls, 0.80–1.52 mm in diam. (\bar{x} = 1.2, SD \pm 0.15 mm, n = 20), *Paraphyses* hyaline, smooth-walled, 25.6–74.2 μ m long (\bar{x} = 45.5, SD \pm 15 μ m, n = 20), 3.2–5.5 μ m wide (\bar{x} = 4.1, SD \pm 0.5 μ m, n = 20). *Conidiogenous cells* formed from the cells lining the inner walls of the pycnidia, holoblastic, determinate, simple, ellipsoidal, and slightly tapered towards the apex, hyaline. *Conidia* aseptate, ellipsoid to oblong, smooth, ends rounded, initial hyaline, becoming brown when mature, 18.1–24.8 \times 7.5–11.7 μ m (\bar{x} = 21.3, SD \pm 2.2 μ m \times \bar{x} = 10.4, SD \pm 0.8 μ m, n = 50), l/w = (2–)2.1–2.2(–2.2).

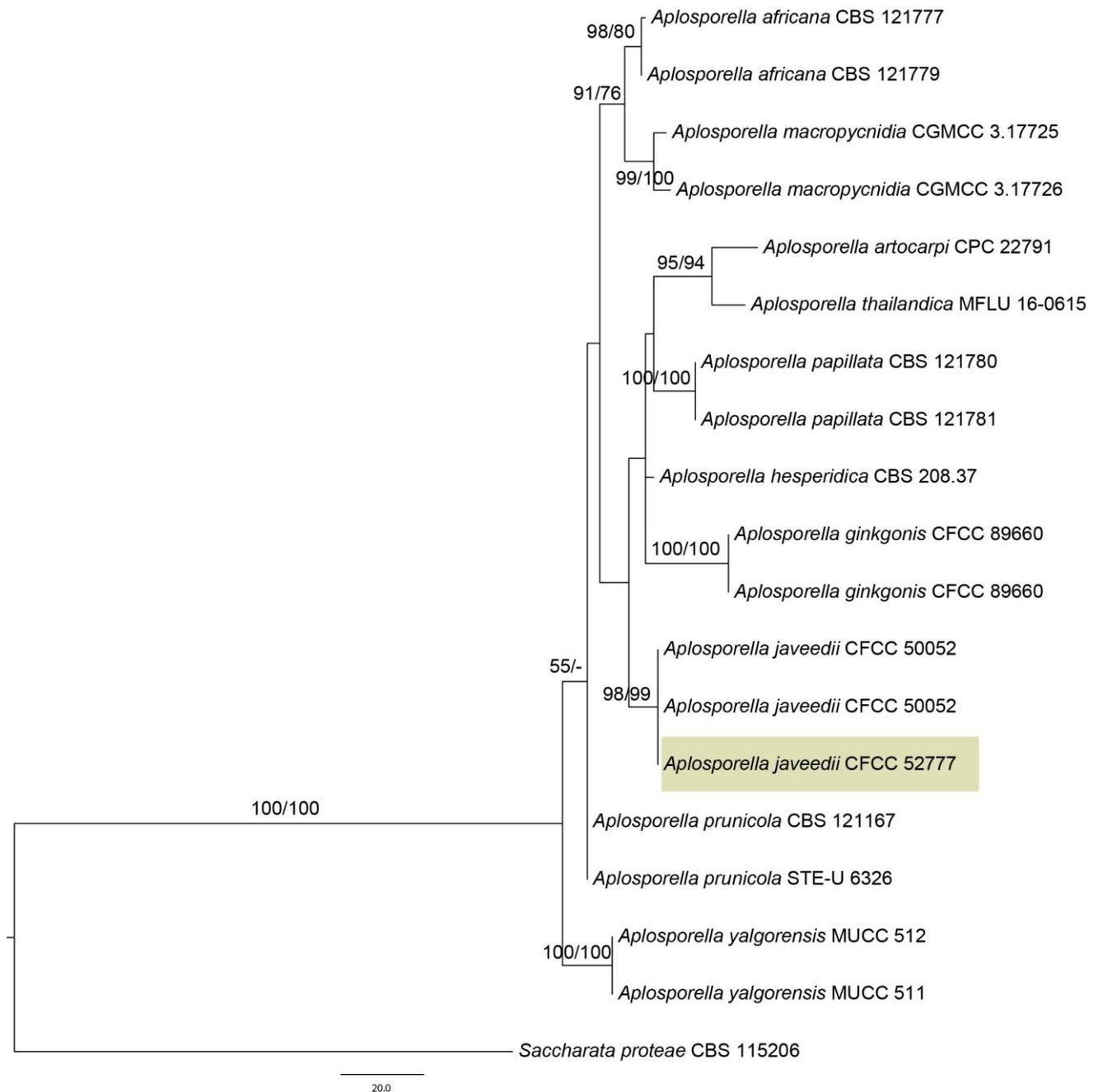


Figure 4 – Phylogram of *Aplosporella* based on combined ITS and TEF1- α genes. MP and ML bootstrap support values above 50 % are shown at the first and second position. Scale bar = 20 nucleotide substitutions. The new sequence resulting from the current study is highlighted in yellow.

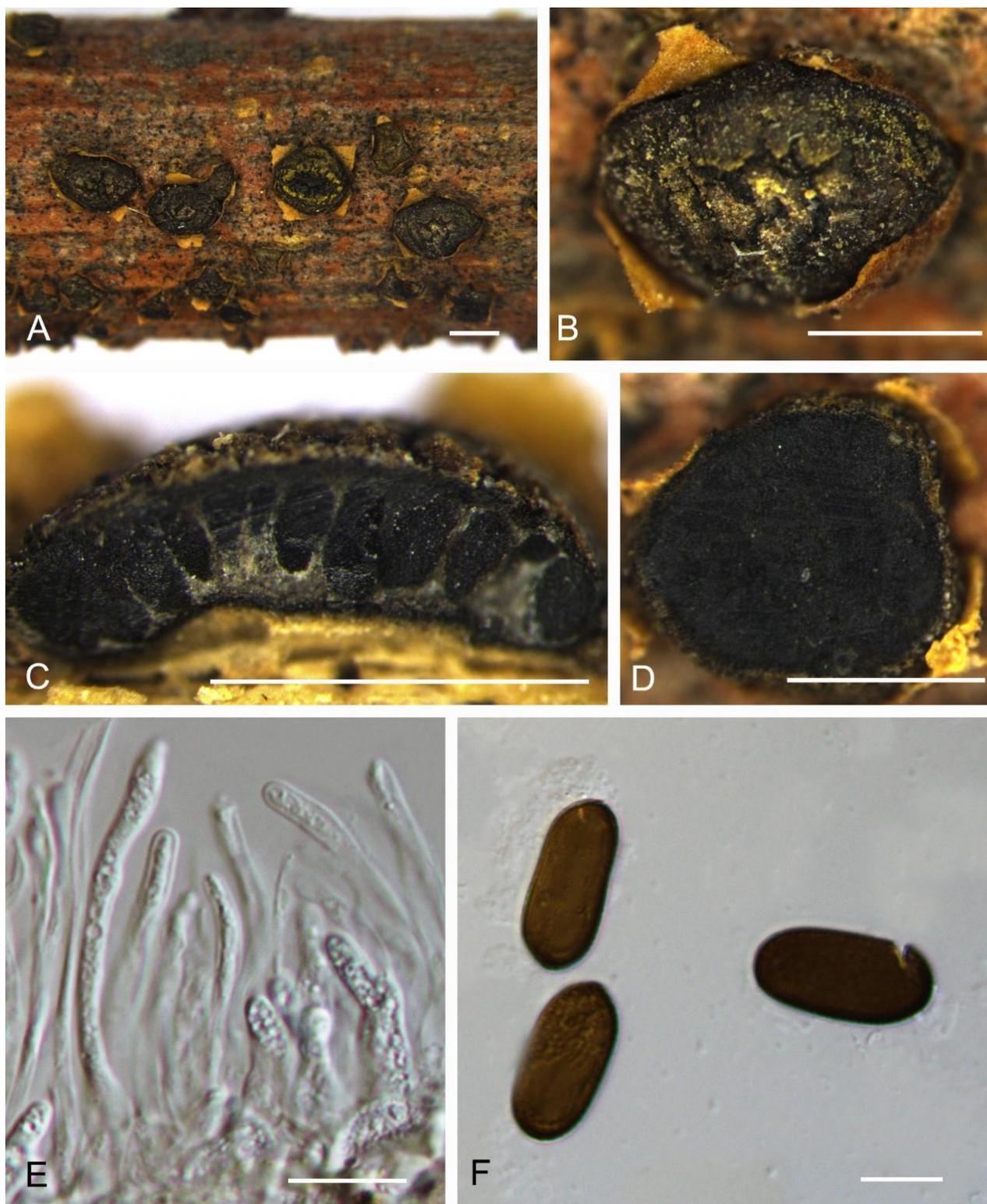


Figure 5 –*Aplosporella javeedii* from *Castanea mollissima* (BJFC-S1446). A, B Habit of conidiomata on a stem. C Longitudinal sections through conidiomata. D Transverse sections through conidiomata. E Conidiogenous cells and paraphyses. F conidia. Scale bars: A–D = 1 mm, E, F = 10 μ m.

Culture characteristics – On MEA at 25 °C, white at first, then grey-olivaceous, similar to reverse side after 8 days. Colony uniform with appressed aerial mycelium. After 3 weeks, conidiomata solitary, globose, unilocular, immersed to semi-immersed on MEA.

Known distribution – Dead corticated branches of *Albizia julibrissin* Durazz. (Leguminosae)

(BJFC-S867), *Broussonetia papyrifera* L'Hér. ex Vent. (Moraceae) (BJFC-S884), *Castanea mollissima* Blume (Fagaceae) (BJFC-S1446), *Gleditsia sinensis* Lam. (Leguminosae) (BJFC-S1022), *Juniperus chinensis* L. (Cupressaceae) (BJFC-S1044), and *Styphnolobium japonicum* L. (Leguminosae) (BJFC-S1042) in China and from *Celtis africana* (Ulmaceae) (CMW 38166) and *Searsia lancea* (Anacardiaceae) (CMW 38168) in South Africa.

Specimen examined – CHINA, Hebei Province, Chengde City, 40°37'17.15"N, 118°28'21.54"E, 325 m asl, asexual morphs on corticated branches of *Castanea mollissima*, N. Jiang, 13 October 2017 (BJFC-S1446), living culture CFCC 52777.

Notes – The specimen of *Aplosporella* collected from chestnut branches is identified as *Aplosporella javeedii* based on morphological observations and multi-gene phylogenetic analyses. Morphologically, it is characterized by solitary globose unilocular conidiomata on MEA and erumpent discoid conidiomata, multiple ostioles in branches with ellipsoid to oblong aseptate conidia averaging $21.3 \times 10.4 \mu\text{m}$, which matches the type (CBS 133954) perfectly (Jami et al. 2014).

Coryneaceae is characterized by having black perithecia, often immersed in wood, asci that deliquesce at maturity, and an asexual morph with transversely distoseptate brown conidia (Senanayake et al. 2017). *Coryneum* is the sole genus in the family Coryneaceae of Diaporthales, and asexual morph is very common in nature and sometimes the sexual morph is closely associated with it (Senanayake et al. 2017). *Coryneum* species are generally considered highly host specific, especially occurring on hardwood trees such as those in the Betulaceae and Fagaceae (Sutton 1980).

Coryneum umbonatum Nees, Syst. Pilze (Würzburg) 34 (1816).

Fig. 6

Pathogenic on *Castanea mollissima* branches. Sexual morph – Undetermined. Asexual morph – *Conidiomata* acervular, 0.2–0.8 mm wide ($\bar{x} = 0.5$, $\text{SD} \pm 0.25$ mm, $n = 20$), 0.2–0.7 mm high ($\bar{x} = 0.4$, $\text{SD} \pm 0.2$ mm, $n = 20$), solitary, erumpent through outer periderm layers of host, scattered, surface tissues above slightly domed. *Conidiophores* 15–30 μm long ($\bar{x} = 24$, $\text{SD} \pm 4.5$ μm , $n = 20$), 3–7 μm wide ($\bar{x} = 4.5$, $\text{SD} \pm 1.5$ μm , $n = 20$), branched at base, cylindrical, septate, hyaline at apex, pale brown at base. *Conidiogenous cells* holoblastic, annellidic, integrated, indeterminate, cylindrical, expanding towards apices, pale brown, smooth, with 0–1 percurrent proliferations. *Conidia* $42.2\text{--}82.5 \times 9.6\text{--}15.7 \mu\text{m}$ ($\bar{x} = 62.5$, $\text{SD} \pm 6.5 \mu\text{m} \times \bar{x} = 13.6$, $\text{SD} \pm 1.1 \mu\text{m}$, $n = 50$), $l/w = (4.2\text{--})4.4\text{--}4.7(-5.1)$, variable in shape, curved, broadly fusiform to fusiform cylindrical or clavate, dark brown, smooth-walled, 5–7-distoseptate, apical cell with a hyaline tip, truncate and black at base.

Known distribution – on stems of *Betula* sp. (BPI 871006A) in the USA, on twigs of *Quercus petraea* Libelb (Fagaceae) (IMI 362542) in the UK and on branches of *Castanea mollissima* in China.

Specimen examined – CHINA, Hebei Province, Qinhuangdao City, 40°22'51.22"N, 119°11'51.15"E, 235 m asl, asexual morphs on branches of *Castanea mollissima*, collected by N. Jiang, 16 October 2017 (BJFC-S1449).

Notes – *Coryneum umbonatum* could cause cankers, dieback in shoots and twigs (Sutton 1980). In the present study, a *Coryneum* specimen collected from dead chestnut branches is identified as *Coryneum umbonatum* exactly matched conidial dimensions (Sutton 1980). *Coryneum modonium* was reported on *Castanea* species, but conidial size can differ the two species ($42.2\text{--}82.5 \times 9.6\text{--}15.7 \mu\text{m}$ in *Coryneum umbonatum* vs. $50\text{--}71 \times 14\text{--}19 \mu\text{m}$ in *C. modonium*) (Sutton 1975, Teng 1996).

***Lopadostoma* based on analyses of LSU, ITS and RPB2 sequence data.**

Lopadostoma (Lopadostomataceae, Xylariales) was reported as a saprobiotic genus with pustular pseudostroma development in bark of angiospermous trees (Jaklitsch et al. 2014). Species of *Lopadostoma* are generally not particularly common, but show a marked seasonal occurrence. *L. americanum*, *L. dryophilum*, *L. insulare*, *L. meridionale* and *L. quercicola*, were only collected

from *Quercus* trees, and these five species were close in the phylogram (Jaklitsch et al. 2014). However, none was recorded from China.

The combined LSU, ITS and RPB2 data set consists of 30 strains with *Lopadostoma* cf. *polynesium* (LAG) as outgroup taxon. Alignment comprises 2750 characters after alignment. Of these, 1917 characters were constant, 255 variable characters were parsimony-uninformative and 578 characters were parsimony informative. The MP analysis resulted in one parsimonious tree (TL = 1464, CI = 0.750, RI = 0.861, RC = 0.646), which was shown in Fig. 7. The topology of phylogenetic tree obtained from ML was similar with the MP tree.

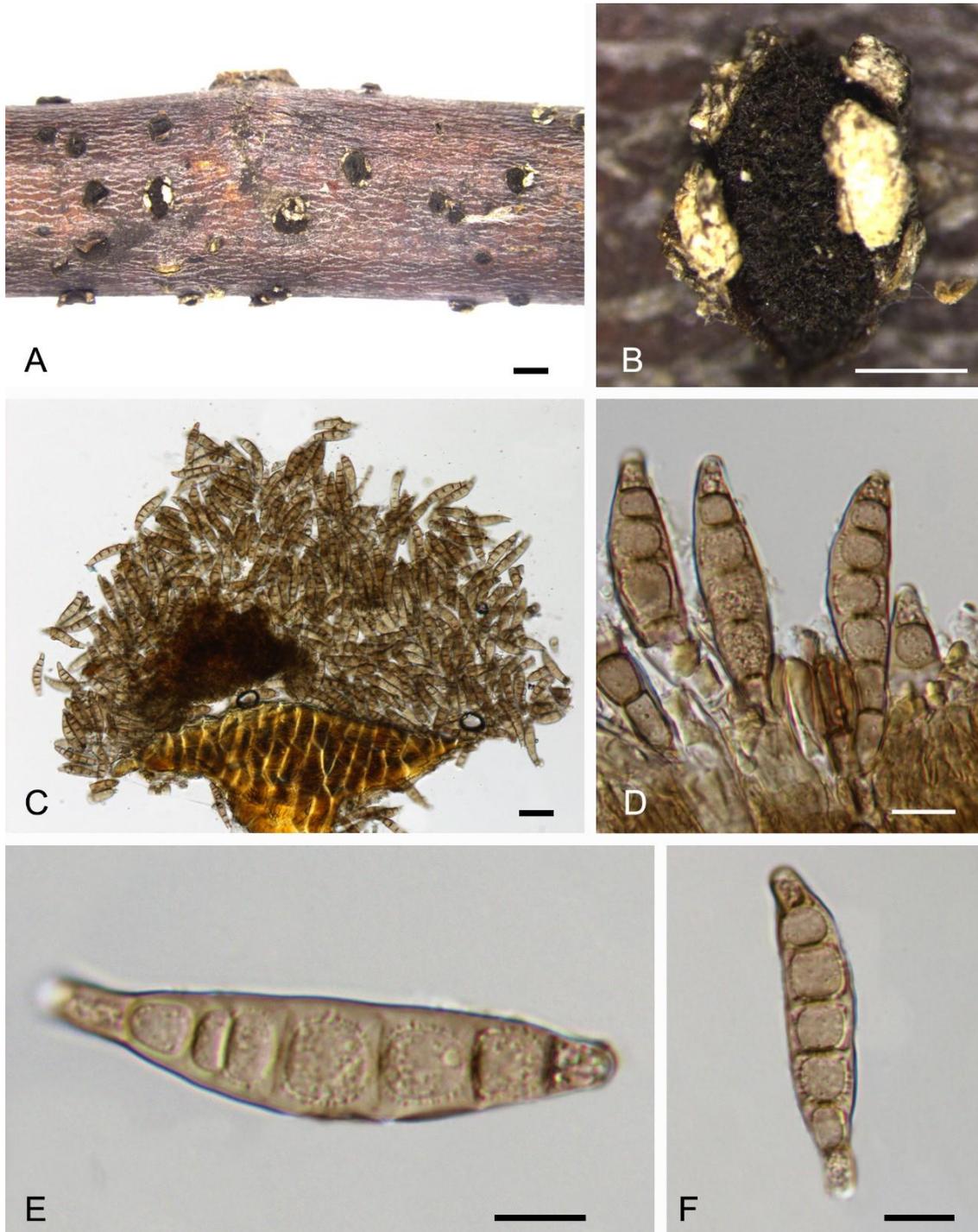


Figure 6 – Morphology of *Coryneum umbonatum* from *Castanea mollissima* (BJFC-S1449). A–B Habit of conidiomata on a stem. D Conidiogenous cells. C, E–F conidia. Scale bars: A = 1 mm, B = 0.5 mm, C = 30 μ m, E–F = 10 μ m.

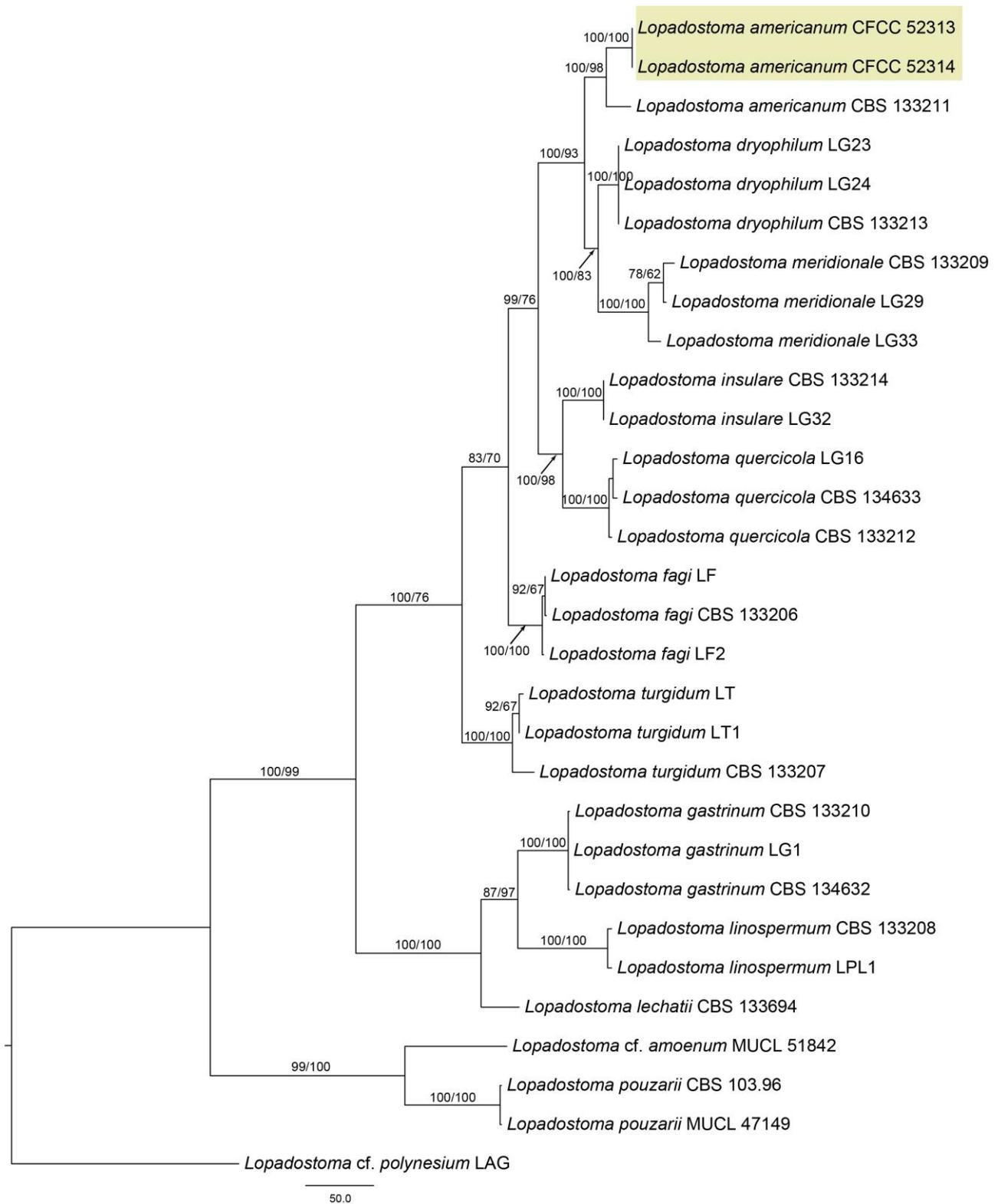


Figure 7 – Phylogram of *Lopadostoma* based on combined LSU, ITS and RPB2 genes. MP and ML bootstrap support values above 50 % are shown at the first and second position. Scale bar = 20 nucleotide substitutions. The new sequences resulting from the current study are highlighted in yellow.

Lopadostoma americanum Jaklitsch, J. Fourn., J.D. Rogers & Voglmayr, Persoonia 32: 58 (2014).

Fig. 8

Saprobic on *Castanea mollissima* branches. Sexual morph – *Ectostromatic* discs convex, circular or ellipsoid in outline, 0.8–1 mm in their greatest dimension, projecting to 0.5 mm above

the host surface, roughened, carbonaceous, shiny in places; ostioles opening separately in the disc, inconspicuous, umbilicate. *Stromata* for the most part immersed in bark, pustulate-erumpent, separate, scattered, subglobose, 1.5–2.5 mm diam. ($\bar{x} = 2$, $SD \pm 0.5$ mm, $n = 20$), delimited from surrounding bark by a black carbonized line; the latter 80–120 μm thick, irregular in outline, reaching the wood surface and spreading over it between adjacent stromata. Tissue between ostiolar necks dark yellow-brown, grey to black, woody; tissue around and beneath the perithecia composed of brownish, scarcely altered bark tissue, in places mixed with whitish mycelium. *Perithecia* 6–10 per stroma, arranged in valsoid configuration, monostichous in smaller stromata, polystichous in larger ones, subglobose to ovoid, often laterally compressed, 0.2–0.7 mm diam. ($\bar{x} = 0.5$, $SD \pm 0.25$ mm, $n = 20$), with long ostiolar necks converging toward the ectostromatic disc, 0.8–1.2 mm. *Asci* cylindrical, with 8 uniseriate ascospores, $52\text{--}88 \times 5.1\text{--}8.4$ μm , ($\bar{x} = 70$, $SD \pm 9.5$ $\mu\text{m} \times \bar{x} = 7.1$, $SD \pm 0.5$ μm , $n = 50$), sometimes fusiform due to partly overlapping biseriate or oblique ascospores, flat amyloid apical ring, stipe up to nearly as long as the spore part. *Ascospores* oblong to narrowly ellipsoid, $8\text{--}12 \times 3\text{--}4.2$ μm , ($\bar{x} = 10$, $SD \pm 0.5$ $\mu\text{m} \times \bar{x} = 3.6$, $SD \pm 0.3$ μm , $n = 50$), l/w = (2.1–)2.5–2.9(–3.3), dark to blackish brown, smooth, with 2 guttules when young, with commonly visible, straight, circumferential germ slit. Asexual morph – Undetermined.

Culture characteristics – On PDA at 25 °C, colonies growing slowly, with uneven white mat, colourless, white to yellowish margin, and yellowish reverse, reaching 70 mm within 50 days, forming large thick yellowish pustules without conidial drops.

Known distribution – On *Quercus* spp. (CBS 133211) in North America and dead corticated branches of *Castanea mollissima* in China.

Specimen examined – CHINA, Shandong Province, Rizhao City, 119°46'23.18"N, 35°42'28.12"E, 452 m asl, on branches of *Castanea mollissima*, collected by N. Jiang, 23 April 2017 (BJFC-S1384, living culture CFCC 52313); Rizhao City, 119°46'23.18"N, 35°42'28.12"E, 452 m asl, on branches of *Castanea mollissima*, collected by N. Jiang, 23 April 2017 (BJFC-S1385), living culture CFCC 52314.

Notes – Two isolates of *Lopadostoma americanum* (CFCC 52313 and CFCC52314) cluster in a well-supported clade (MP/ML=100/100) sister to type strain of *Lopadostoma americanum* (CBS 133211). In addition, we compared morphological characteristics of *Lopadostoma americanum* between specimens collected from *Castanea mollissima* in China and type material recorded by Jaklitsch et al. (2014). Sexual morphs including pyrenocarp and ascospore characteristics and dimensions matched exactly. Hence we do not propose CFCC 52313 and CFCC52314 as a novel species.

***Myrmaecium* based on analyses of LSU, ITS, TEF1- α and RPB2 sequence data.**

Myrmaecium (Valsariaceae, Valsariales) is a genus traditionally treated as a synonym of *Valsaria*, but resolved by Jaklitsch et al. (2015) to comprise *M. fulvopruinatum*, *M. rubricosum* and *M. rubrum*. The combined LSU, ITS, TEF1- α , and RPB2 data set consists of 25 strains with *Bambusaria bambusae* (MFLUCC 12-0851) and *Bambusaria bambusae* (CBS 139763) as outgroup taxa. Alignment comprises 3898 characters after alignment. Of these, 2714 characters were constant, 110 variable characters were parsimony-uninformative and 1074 characters were parsimony informative. The MP analysis resulted in one parsimonious tree (TL = 2133, CI = 0.755, RI = 0.920, RC = 0.245), which was shown in Fig. 9. The topology of phylogenetic tree obtained from ML was similar with the MP tree.

***Myrmaecium fulvopruinatum* (Berk.) Jaklitsch & Voglmayr, Fungal Diversity 73: 190 (2015).**

Fig. 10

Pathogenic or *saprobic* on *Castanea mollissima* branches. Sexual morph – *Stromata* eustromatic, immersed to erumpent, causing small bumps in bark, subpulvinate, labiate or ring-like, often longish, separate, $1\text{--}3 \times 0.5\text{--}2$ mm ($\bar{x} = 2$, $SD \pm 0.6$ mm $\times \bar{x} = 1.2$, $SD \pm 0.3$ mm, $n = 20$), aggregated in linear rows. *Ascomata* 0.1–0.4 mm diam. ($\bar{x} = 0.25$, $SD \pm 0.1$ mm, $n = 20$), 0.1–0.4 mm high ($\bar{x} = 0.25$, $SD \pm 0.15$ mm, $n = 20$), arranged monostichously in valsoid groups of 5–10 at

near basal position or in the stroma middle, less commonly in diatrypoid configuration just below the stroma surface, flask-shaped to subglobose; peridium 15–25 μm thick, composed of a thin outer layer of flattened dark brown cells and an inner layer of flattened hyaline cells.

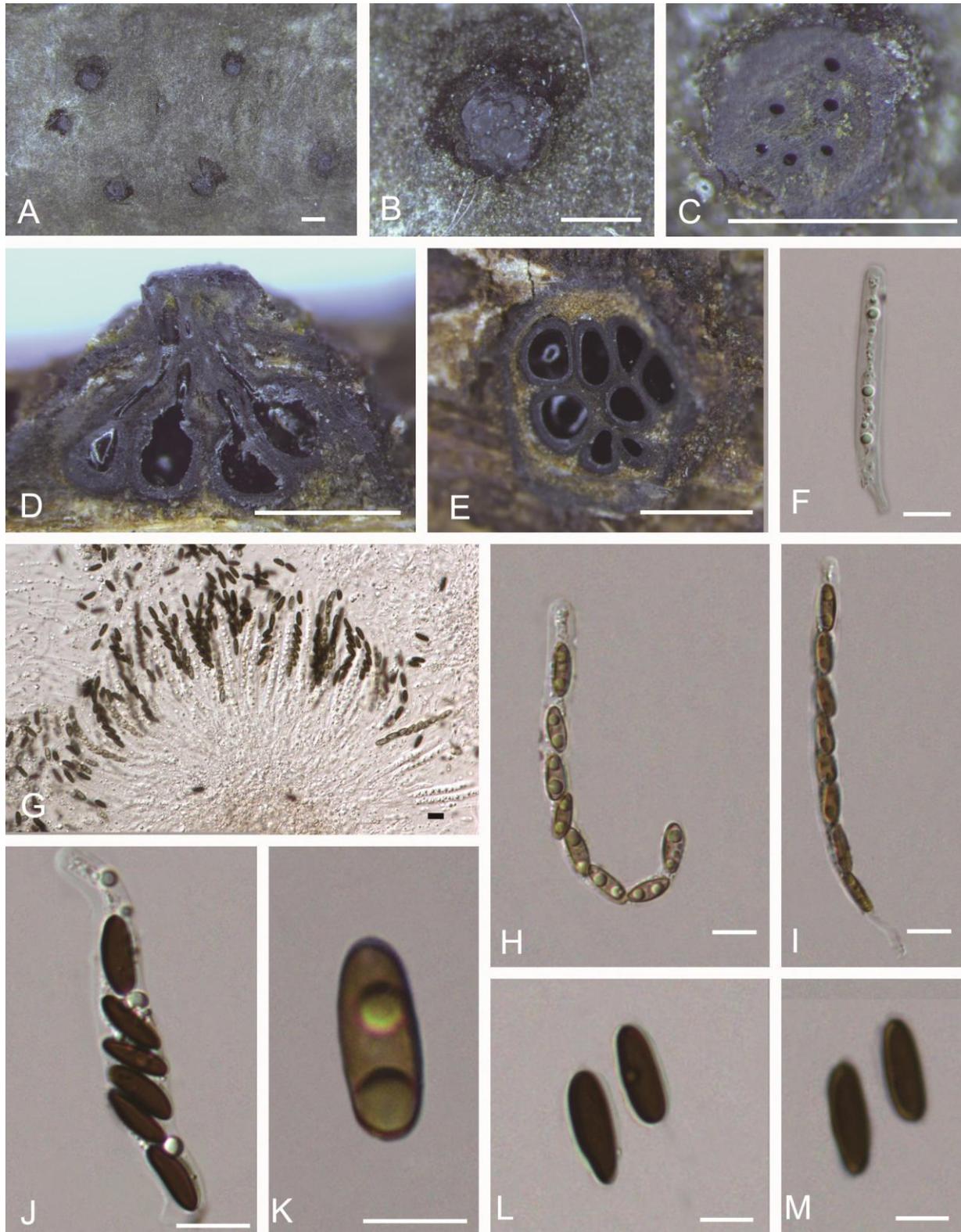


Figure 8 – Morphology of *Lopadostoma americanum* from *Castanea mollissima* (BJFC-S1384). A–B Habit of ascomata on a stem. C, E Transverse sections through ascomata. D Longitudinal sections through ascomata. F–J Asci. K: Immature ascospore. L–M Mature ascospores. Scale bars: A–E = 1 mm, F–M = 10 μm .

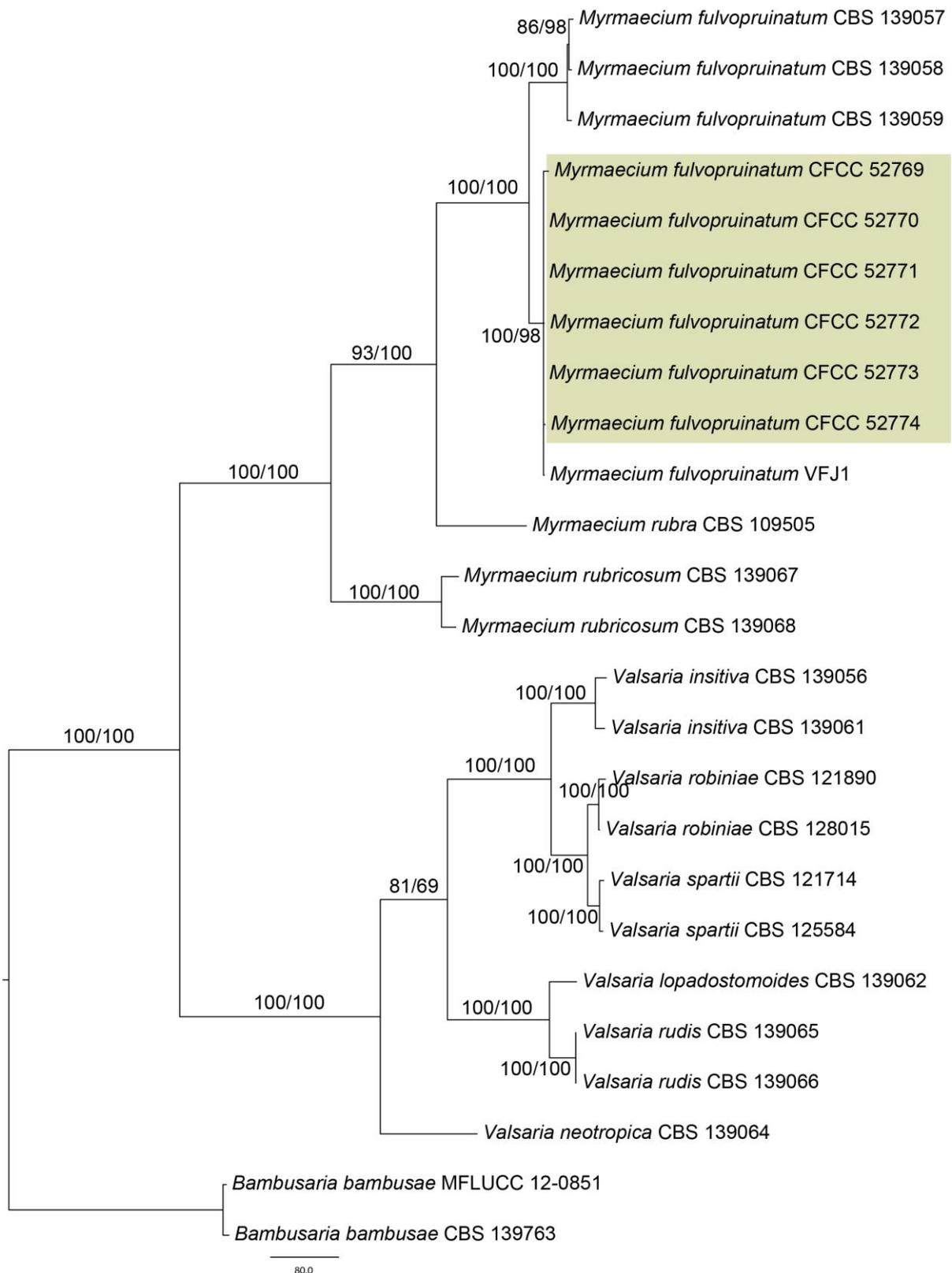


Figure 9 – Phylogram of *Myrmaecium* based on combined LSU, ITS, TEF1- α and RPB2 genes. MP and ML bootstrap support values above 50 % are shown at the first and second position. Scale bar = 80 nucleotide substitutions. The new sequences resulting from the current study are highlighted in yellow.

Ostiolar necks converging into a common neck, less commonly separate, 200–1100 μm long, (\bar{x} = 500, SD \pm 150 μm , n = 20), 90–300 μm diam. (\bar{x} = 200, SD \pm 50 μm , n = 20), black, smooth; apices

at the surface usually with distinct circular outline, flush with the stroma surface or projecting to 120 μm ; periphysate. Paraphyses filiform, unbranched, apically free, up to 150 μm long, 1–5 μm wide, attenuated upwards, slightly enlarged at the tip. *Asci* 88–142 \times 10.8–19.5 μm (\bar{x} = 118, SD \pm 6 μm \times \bar{x} = 14.5, SD \pm 2.1 μm , n = 20), numerous, cylindrical to subclavate, containing 8 uniseriate ascospores. *Ascospores* 14.3–19 \times 7.1–8.6 μm (\bar{x} = 16.5, SD \pm 1.8 μm \times \bar{x} = 7.8, SD \pm 0.5 μm , n = 50), l/w = (1.9–)2–2.4(–2.5), ellipsoid, 2-celled, dark brown to black, plump or attenuated towards apices with 1 large drop per cell and densely reticulate surface ornamentation. Asexual morph – Immature stromata often with a dark and gelatinous flat central surface, containing *conidiomata* as irregular, labyrinthine locules at ostiolar levels above immature ascomata; interior of locules hyaline to orange or brown, walls lined by palisades of densely clustered, lageniform to cylindrical, often basally curved phialides, 6.3–13.2 \times 1.9–3.3 μm (\bar{x} = 9.5, SD \pm 1.5 μm \times \bar{x} = 2.7, SD \pm 0.3 μm , n = 50), l/w = (2.8–)3.1–4.7(–6.5), with broad collarete, originating from large brown incrustated cells 8–15 μm diam. and hyaline cylindrical cells. *Conidia* 2.1–3.4 \times 1.4–2.2 μm (\bar{x} = 2.7, SD \pm 0.4 μm \times \bar{x} = 1.7, SD \pm 0.2 μm , n = 50), l/w (1.3–)1.4–1.9(–2.1), oblong to bullet-shaped or subglobose, 1-celled, hyaline, orange-brown in mass, with rounded upper and truncate lower end.

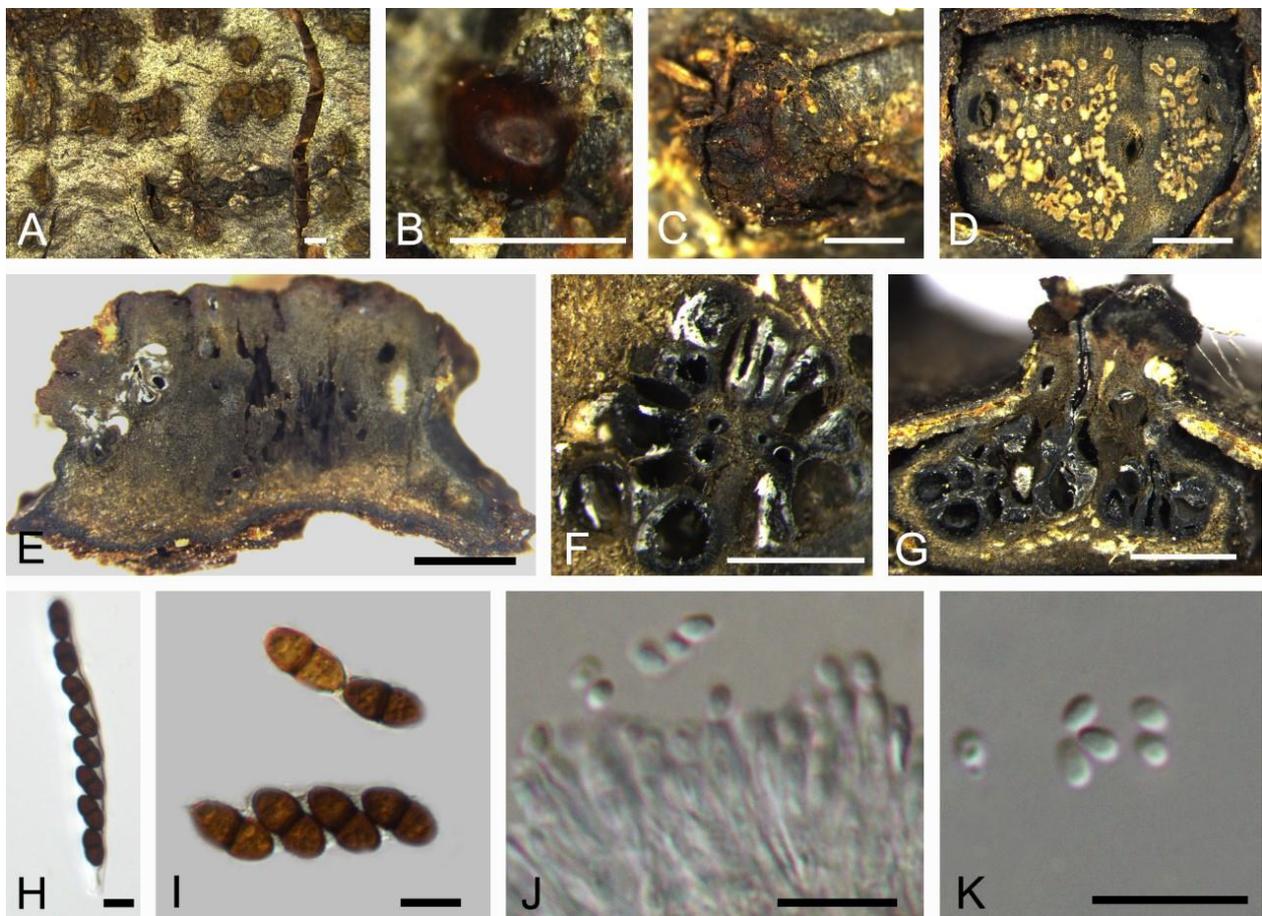


Figure 10 – Morphology of *Myrmaecium fulvopruinatum* from *Castanea mollissima* (BJFC-S1450). A Habit of Stromata on the bark. B Conidiomata. C Ascomata. D Transverse sections through conidiomata. E Longitudinal sections through conidiomata. F Transverse sections through ascomata. G Longitudinal sections through ascomata. H–I Asci and ascospores. J–K Conidiogenous cells and conidia. Scale bars: A–E, G = 1 mm, F = 0.5 mm, H–K = 10 μm .

Culture characteristics – On MEA at 25 $^{\circ}\text{C}$, colony radius 14–19 mm after 3 days, 26–33 mm after 5 days; centrally inoculated 90 mm plates entirely or nearly entirely covered by mycelium within 1 week; growth sometimes ceasing earlier. Colony zonate, surface and aerial hyphae with

distinct macroscopically visible radial arrangement; aerial hyphae forming white radial streaks; first white, soon turning yellowish to pale orange or rosy to yellow-brown. Odour pungent, cresol-like.

Known distribution – on sun-exposed, corticated logs and branches of coniferous and broadleaf trees, worldwide, but uncommon.

Specimen examined – CHINA, Shaanxi Province, Ankang City, Xiangxidong Garden, 32°40'32.36"N, 109°18'57.21"E, 1055 m asl, asexual morph on branches of *Castanea mollissima*, collected by N. Jiang, 1 July 2017 (BJFC-S1450, living culture CFCC 52769); Beijing City, Mentougou District, 39°17'24.56"N, 115°45'23.33"E, 452 m asl, sexual morph on branches of *Castanea mollissima*, collected by N. Jiang, 20 August 2017 (BJFC-S1451, living culture CFCC 52770); Hebei Province, Chengde City, 40°24'32.24"N, 117°28'55.24"E, 262 m asl, asexual morph on branches of *Castanea mollissima*, collected by N. Jiang, 11 October 2017 (BJFC-S1452, living culture CFCC 52771); Hebei Province, Chengde City, 40°21'44.18"N, 117°51'29.07"E, 256 m asl, sexual and asexual morph on branches of *Castanea mollissima*, collected by N. Jiang, 12 October 2017 (BJFC-S1453, living culture CFCC 52772 and CFCC 52773); Hebei Province, Chengde City, 40°37'39.14"N, 118°27'22.45"E, 350 m asl, sexual and asexual morph on branches of *Castanea mollissima*, collected by N. Jiang, 14 October 2017 (BJFC-S1454, culture CFCC 52774).

Notes – Six isolates of *Myrmaecium fulvopruinatum* (CFCC 52769, CFCC 52770, CFCC 52771, CFCC 52772, CFCC 52773, CFCC 52774 and VFJ1) from China cluster in a well-supported clade (MP/ML = 100/98) sister to three isolates of *M. fulvopruinatum* (CBS 139057, CBS 139058 and CBS 139059) from Austria and America. Although strains of *Myrmaecium fulvopruinatum* are divided into two clades in Fig. 9, there is not obvious morphological differences among different strains in both sexual and asexual morphs, thus we identified *Myrmaecium* specimens from China as *M. fulvopruinatum*.

Discussion

Chinese sweet chestnut (*Castanea mollissima*) represents an important fruit and timber tree species, branch cankers are common fungal diseases occurring in almost all chestnut plantations in China. However, fungal species that caused canker symptoms are complex and various, for example, *Cryphonectria parasitica* and *C. japonica* share similar orange fruiting bodies on the diseased chestnut branches (Gryzenhout et al. 2009). In the present study, we carried the taxonomy work of fungi diversity on chestnut trees in China started with fungi producing melanocratic spores which are more discernible. In addition, more fungi producing hyaline spores should be solved in the future.

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