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The taxonomy and phylogeny of *Austropleospora ochracea* sp. nov. (Didymosphaeriaceae) from Guizhou, China

LAKMALI S. DISSANAYAKE^{1,5}, NALIN N. WIJAYAWARDENE^{2,6}, MILAN C. SAMARAKOON^{3,7}, KEVIN D. HYDE^{3,4,8} & JI-CHUAN KANG^{1,3,9*}

¹ Engineering Research Center of the Utilization for Characteristic Bio-Pharmaceutical Resources in Southwest, Ministry of Education, Guizhou University, Guiyang, Guizhou Province 550025, People's Republic of China.

² Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing, Yunnan 655011, People's Republic of China.

³ Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

⁴ Innovative Institute of Plant Health, Zhongkai University of Agriculture and Engineering, Haizhu District, Guangzhou 510225, P.R. China.

⁵ ✉ dmsldlakmali.ld@gmail.com; <https://orcid.org/0000-0003-2933-3127>

⁶ ✉ nalinwijayawardene@yahoo.com; <https://orcid.org/0000-0003-0522-5498>

⁷ ✉ samare.ag.rjt@gmail.com; <https://orcid.org/0000-0002-4815-125X>

⁸ ✉ kdhyde3@gmail.com; <https://orcid.org/0000-0002-2191-0762>

⁹ ✉ jckang@gzu.edu.cn; <https://orcid.org/0000-0002-6294-5793>

*Corresponding author: ✉ jckang@gzu.edu.cn

Abstract

Austropleospora is a genus of Didymosphaeriaceae with only three extant species. A survey of saprobic ascomycetes in Guizhou Province, China, discovered a new *Austropleospora* species on dead twigs in a terrestrial habitat. The molecular phylogeny based on a combined SSU-LSU-*tef1*-ITS DNA sequence dataset confirmed the new species' taxonomic position in *Austropleospora*. *Austropleospora ochracea* sp. nov. is characterized by globose to subglobose and uni-loculate ascoma with a centric short papilla, brown to dark brown peridium with cells of *textura angularis* to *textura prismatica* and conical, comparatively smaller ascospores with narrowly rounded polar ends. The new taxon is compared with similar species in *Austropleospora*, and its taxonomic status is briefly discussed.

Keywords: Dothideomycetes, muriform, new species, taxonomy

Introduction

Didymosphaeriaceae was introduced by Munk (1958) to accommodate *Didymosphaeria* Fuckel as the type genus, which is currently one of the most species-rich families in Pleosporales (Hyde *et al.* 2013, Pem *et al.* 2019, Hongsanan *et al.* 2020a). Wijayawardene *et al.* (2020) accepted 31 genera in this family. The sexual morph of Didymosphaeriaceae is characterized by globose to subglobose, centrally ostiolate ascomata, a peridium with light to heavily pigmented cells of *textura angularis*, hyaline cellular or trabeculate pseudoparaphyses often in a gelatinous matrix, 2–4-spored or 8-spored, cylindrical or oblong, pedicellate asci with or without an ocular chamber and 1–2-seriate, overlapping, ellipsoid or oblong, pigmented ascospores that are 1–3-septate or muriform (Hongsanan *et al.* 2020a). The asexual morph can be fusicliadium-like and phoma-like (Hyde *et al.* 2013). Didymosphaeriaceae species have been recorded in terrestrial and aquatic habitats as endophytes, pathogens or saprobes on twigs, leaves, pods, herbaceous stems and occasionally human (Morin *et al.* 2010, Hyde *et al.* 2013, Ariyawansa *et al.* 2014a, Thambugala *et al.* 2017, Jayasiri *et al.* 2019, Phookamsak *et al.* 2019, Tennakoon *et al.* 2020, Hongsanan *et al.* 2020a).

Austropleospora R.G. Shivas & L. Morin was introduced by Shivas & Morin (2010) with *A. osteospermi* R.G. Shivas & L. Morin as the type species. The sexual morph of *Austropleospora* has scattered and immersed, black, ostiolate ascomata, with a protruding neck, 6–8-spored, clavate to cylindrical asci and ellipsoidal, yellowish brown, smooth-walled ascospores (Morin *et al.* 2010). The asexual morph has coelomycetous, pycnidial, globose conidiomata, with a central ostiole, brown to reddish brown conidiomata walls and brown to yellowish brown, globose to obovate

conidia (Morin *et al.* 2010, Verkley *et al.* 2014, Jayasiri *et al.* 2019). *Austropleospora* comprises three species viz. *A. archidendri* (Verkley Göker & Stielow) Ariyaw. & K.D. Hyde, *A. keteleeriae* Jayasiri, E.B.G. Jones & K.D. Hyde and *A. osteospermi*. *Austropleospora osteospermi* was introduced with both sexual and asexual morphs as a pathogen on stems of *Chrysanthemoides monilifera* ssp. *rotundata* (Asteraceae), in Australia, while *A. archidendri* was introduced with only an asexual morph as a saprobe on *Archidendron bigeminum* (Fabaceae) leaves from Myanmar (CBS 168.77) and on a decaying pod of *Leucaena* spp. (Fabaceae) in Thailand (MFLU 18-2143) (Verkley *et al.* 2014, Jayasiri *et al.* 2019). *Austropleospora keteleeriae* was introduced from its asexual morph as a saprobe on a decaying cone of *Keteleeria fortunei* (Pinaceae) from China (Jayasiri *et al.* 2019).

During a survey of ascomycetous fungal diversity in Guizhou Province, China, a novel taxon that is morphologically similar to *Austropleospora* was collected. Morpho-molecular analysis results confirmed that the new collection is a new species that is thus introduced as *Austropleospora ochracea*.

Materials and methods

Morphological observations

Dead woody twigs were collected from Guizhou University premises, Guiyang, Guizhou Province, China, in October 2019. Samples were observed using a stereomicroscope (SZX16, Olympus). Hand sections of the ascomata were mounted in distilled water. The following characters were measured: diameter and height of ascomata, width of the peridium, diameter and height of ostioles, length and width of asci and ascospores. Images were captured with a Canon EOS70D digital camera fitted to a compound microscope. Measurements were made with the Tarosoft (R) Image Frame Work program, and images used for figures were processed with Adobe Photoshop CS6 software (Adobe Systems, USA).

Single spore isolation was done according to Senanayake *et al.* (2020), and germinating spores were transferred to potato dextrose agar (PDA). The pure cultures were incubated at 18–20 °C for 4 weeks. The type specimens were deposited in the Cryptogamic Herbarium, Kunming Institute of Botany, Academia Sinica (HKAS), Chinese Academy of Science, Kunming and Chinese Academy of Science Herbarium (HMAS), Beijing, China. The ex-type culture was deposited in the Kunming Institute of Botany Culture Collection (KUMCC). Facesoffungi and Index Fungorum numbers were provided as outlined in Jayasiri *et al.* (2015) and Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>) respectively.

DNA extraction, PCR amplification and sequencing

EZgne™ fungal gDNA kit (BIOMIGA, Hangzhou city, Zhejiang Province, China) was used to extract total genomic DNA from fresh mycelium grown on PDA for 14 days at 20 °C, by following the instructions of the manufacturer. DNA sequence data was obtained from the partial sequences of three ribosomal coding genes, including internal transcribed spacer region (ITS: ITS1-5.8S-ITS2), 28S large subunit rDNA (LSU), 18S small subunit rDNA (SSU) and one protein-coding gene including translation elongation factor 1-alpha gene (*tef1*) using ITS5/ITS4, LR0R/LR5, NS1/NS4 (Vilgalys & Hester 1990, White *et al.* 1990) and EF1-983F/EF1-2218R (Rehner & Buckley 2005) primers respectively. Polymerase chain reaction (PCR) was carried out in a volume of 25 µl, which contained 9.5 µL of ddH₂O, 12.5 µL of 2X PCR Master Mix (2x Bench Top™ Taq Master Mix, BIOMIGA, China), 1 µL of DNA template and 1 µL of forward and reverse primers (10 µM each) in each reaction. PCR thermal cycle programs for all gene amplifications were as follows: initialization at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 50 s, elongation at 72 °C for 90 s, and final extension at 72 °C for 10 min. Purification and sequencing of PCR products were done by Sangon Biotech, Shanghai, China. Both directions of the PCR products were sequenced using the same primer pairs as used in PCR amplification to ensure the integrity of the sequences.

Molecular phylogenetic analyses

Sequence alignment

The sequence data generated in this study were analyzed with closely related taxa retrieved from GenBank (Table 1)

based on BLAST searches (<https://www.ncbi.nlm.nih.gov>) and recently published data (Jayasiri *et al.* 2019, Hyde *et al.* 2020). Sequence homologies were searched by using the NCBI BLASTn (<https://www.ncbi.nlm.nih.gov>). Sequences (SSU, LSU, *tef1* and ITS) were aligned using MAFFT v. 6.864b (Katoh *et al.* 2017) and manually improved when necessary in BioEdit v. 7.0 (Hall 1999). The single-gene alignments (SSU, LSU, *tef1* and ITS) were separately used to perform model tests in MrModeltest 2.3 to estimate the best-fit evolutionary model under the Akaike Information Criterion (AIC) (Nylander 2004) and resulted in GTR+I+G substitution model for each. Phylogenetic analyses were constructed based on SSU, LSU, *tef1* and ITS sequence data. Ambiguously aligned areas of each gene region were excluded and gaps were treated as missing data. Missing characters were assessed to be unordered and equally weighted.

Phylogenetic analyses

A maximum likelihood analysis was performed using RAxML GUI v. 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 1,000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores with the GTR+I+G nucleotide substitution model. Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) to evaluate Bayesian posterior probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 200th generation. The distribution of log-likelihood scores was examined to determine the stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.5 (Rambaut & Drummond 2007). The first 10 % of generated trees were discarded and the remaining 90 % of trees were used to calculate posterior probabilities of the majority rule consensus tree. BYPP values greater than 0.95 are given above each node (Figure 1).

All trees were visualized in FigTree v1.4.0 (Rambaut 2012) and the final layout was done with Microsoft PowerPoint (2013). The final alignment and tree were registered in TreeBASE under the submission ID26787 (<http://www.treebase.org/>).

TABLE 2. Taxa used in the phylogenetic analyses and their corresponding GenBank accession numbers.

Species	Strain no.	GenBank Accession no.				Reference
		SSU	LSU	<i>tef1</i>	ITS	
<i>Alloconiothyrium aptrootii</i>	CBS 980.95*	NA	JX496234	NA	JX496121	Verkley <i>et al.</i> 2014
<i>A. aptrootii</i>	CBS 981.95*	NA	JX496235	NA	JX496122	Verkley <i>et al.</i> 2014
<i>Austropleospora archidendri</i>	MFLUCC 17-2429	MK347863	MK347974	MK360044	MK347757	Jayasiri <i>et al.</i> 2019
<i>A. archidendri</i>	CBS 168.77	NA	JX496162	NA	JX496049	Verkley <i>et al.</i> 2014
<i>A. keteleeriae</i>	MFLUCC 18-1551	MK347910	MK348021	MK360045	MK347802	Jayasiri <i>et al.</i> 2019
<i>A. ochracea</i>	KUMCC 20-0020	MT808321	MT799860	MT872714	MT799859	This study
<i>Bimuria novae-zelandiae</i>	CBS 107.79	AY016338	AY016356	NA	NA	Lumbsch & Lindemuth 2001
<i>Chromolaenicola thailandensis</i>	MFLUCC 17-1475*	MN325013	MN325007	NA	MN325019	Mapook <i>et al.</i> 2020
<i>C. lampangensis</i>	MFLUCC 17-1462*	MN325010	MN325004	NA	MN325016	Mapook <i>et al.</i> 2020
<i>C. nanensis</i>	MFLUCC 17-1473*	MN325009	MN325003	NA	MN325015	Mapook <i>et al.</i> 2020
<i>Cylindroaseptospora leucaenae</i>	MFLUCC 17-2424*	MK347856	NG_066310	MK360047	NR_163333	Jayasiri <i>et al.</i> 2019
<i>Deniquelata barringtoniae</i>	MFLUCC 11-0422*	JX254656	NG_042696	NA	NR_111779	Ariyawansa <i>et al.</i> 2013
<i>D. barringtoniae</i>	MFLUCC 16-0271	MH260333	MH260291	MH412766	MH275059	Tibpromma <i>et al.</i> 2018
<i>D. quercina</i>	ABRIICC 10068*	MH316155	MH316157	NA	MH316153	Alidadi <i>et al.</i> 2018
<i>Didymocrea sadasivanii</i>	CBS 438.65*	DQ384074	DQ384103	NA	MH858658	Kruys <i>et al.</i> 2006
<i>Didymosphaeria rubi ulmifolii</i>	MFLUCC 140023*	KJ436588	KJ436586	NA	NA	Ariyawansa <i>et al.</i> 2014a
<i>D. rubi-ulmifolii</i>	MFLUCC 14-0024	KJ436587	KJ436585	NA	NA	Ariyawansa <i>et al.</i> 2014a
<i>Kalmusia italica</i>	MFLUCC 14-0560*	KP325442	KP325441	NA	KP325440	Thambugala <i>et al.</i> 2015
<i>K. variisporum</i>	CBS 121517*	NA	JX496143	NA	NR_145165	Verkley <i>et al.</i> 2014
<i>K. ebuli</i>	CBS 123120*	JN851818	JN644073	NA	KF796674	zhang <i>et al.</i> 2014
<i>Kalmusibambusa triseptata</i>	MFLUCC 13-0232*	KY682696	KY682695	NA	KY682697	Thambugala <i>et al.</i> 2017

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TABLE 1 (Continued)

Species	Strain no.	GenBank Accession no.				Reference
		SSU	LSU	<i>tef1</i>	ITS	
<i>K. rhodostoma</i>	CBS 691.94	AB797241	AB807531	AB808506	LC014559	Tanaka <i>et al.</i> 2015
<i>Laburnicola muriformis</i>	MFLUCC 16-0290*	KU743199	KU743198	KU743213	KU743197	Wanasinghe <i>et al.</i> 2016
<i>L. muriformis</i>	MFLUCC 14-0921	KU743202	KU743201	NA	KU743200	Wanasinghe <i>et al.</i> 2016
<i>L. hawksworthii</i>	MFLUCC 13-0602*	KU743196	KU743195	NA	KU743194	Wanasinghe <i>et al.</i> 2016
<i>Letendreaa cordylinicola</i>	MFLUCC 11-0150	KM214002	KM213999	NA	KM213996	Ariyawansa <i>et al.</i> 2014b
<i>L. cordylinicola</i>	MFLUCC 11-0148*	KM214001	NG_059530	NA	NR_154118	Ariyawansa <i>et al.</i> 2014b
<i>Montagnula scabiosae</i>	MFLUCC 14-0954*	KT443905	KT443903	NA	KT443907	Hongsanan <i>et al.</i> 2015
<i>M. cirsi</i>	MFLUCC 13-0680*	KX274255	KX274249	KX284707	KX274242	Hyde <i>et al.</i> 2016
<i>M. bellevaliae</i>	MFLUCC 14-0924*	KT443904	KT443902	KX949743	KT443906	Hongsanan <i>et al.</i> 2015
<i>M. krabiensis</i>	MFLUCC 16-0250*	MH260343	MH260303	MH412776	MH275070	Tibpromma <i>et al.</i> 2018
<i>Neokalmusia arundinis</i>	MFLUCC 16-0405*	NG_063637	KY706132	KY706145	KY706142	Thambugala <i>et al.</i> 2017
<i>N. thailandica</i>	MFLU 16-2787*	NA	NG_059792	NA	NR_154255	Thambugala <i>et al.</i> 2017
<i>N. thailandica</i>	MFLUCC 16-0399*	KY706136	KY706131	NA	KY706141	Thambugala <i>et al.</i> 2017
<i>Neptunomyces aureus</i>	CMG12	NA	NA	MK948000	MK912121	Goncalves <i>et al.</i> 2019
<i>N. aureus</i>	CMG13	NA	NA	MK948001	MK912122	Goncalves <i>et al.</i> 2019
<i>N. aureus</i>	CMG14	NA	NA	MK948002	MK912123	Goncalves <i>et al.</i> 2019
<i>Paracamarosporium fagi</i>	CPC 24890	NA	KR611904	NA	KR611886	Crous <i>et al.</i> 2015a
<i>P. fagi</i>	CPC 24892*	NA	KR611905	NA	KR611887	Crous <i>et al.</i> 2015a
<i>P. hawaiiense</i>	CBS 120025*	EU295655	JX496140	NA	JX496027	Verkley <i>et al.</i> 2015
<i>Paraconiothyrium cyclothyrioides</i>	CBS 972.95*	AY642524	JX496232	NA	JX496119	Verkley <i>et al.</i> 2015
<i>P. estuarinum</i>	CBS 109850*	AY642522	JX496129	NA	JX496016	Verkley <i>et al.</i> 2015
<i>Paramassariosphaeria anthostomoides</i>	CBS 615.86	GU205246	GU205223	NA	MH862005	Verkley <i>et al.</i> 2015
<i>P. clematidicola</i>	MFLU 16-0172*	KU743208	KU743207	NA	KU743206	Wanasinghe <i>et al.</i> 2016
<i>Paraphaeosphaeria rosae</i>	MFLUCC 17-2547*	MG829150	MG829044	MG829222	MG828935	Wanasinghe <i>et al.</i> 2018
<i>P. rosae</i>	MFLUCC 17-2549	MG829152	MG829046	MG829223	MG828937	Wanasinghe <i>et al.</i> 2018
<i>P. rosicola</i>	MFLUCC 15-0042*	MG829153	MG829047	NA	NR_157528	Wanasinghe <i>et al.</i> 2018
<i>Periconia pseudodigitata</i>	KT1395*	AB797274	AB807564	AB808540	LC014591	Tanaka <i>et al.</i> 2015
<i>P. pseudodigitata</i>	KT1195A	AB797273	AB807563	AB808539	LC014590	Tanaka <i>et al.</i> 2015
<i>Phaeodothis winteri</i>	CBS 182.58	GU296183	GU301857	NA	NA	Schoch <i>et al.</i> 2009
<i>Pseudocamarosporium ulmi-minoris</i>	MFLUCC 17-0671*	MG829167	MG829062	NA	NR_157537	Wanasinghe <i>et al.</i> 2018
<i>P. pteleae</i>	MFLUCC 17-0724*	MG829166	MG829061	MG829233	NR_157536	Wanasinghe <i>et al.</i> 2018
<i>P. propinquum</i>	MFLUCC 13 0544	KJ819949	KJ813280	NA	KJ747049	Wijayawardene <i>et al.</i> 2014
<i>Pseudopithomyces kunmingensis</i>	MFLUCC 17-0314*	MF173606	MF173605	NA	MF173607	Hyde <i>et al.</i> 2017
<i>P. entadae</i>	MFLUCC 17-0917*	MK347835	NG_066305	MK360083	NA	Jayasiri <i>et al.</i> 2019
<i>Pseudopithomyces rosae</i>	MFLUCC 15-0035*	MG829168	MG829064	NA	MG828953	Wanasinghe <i>et al.</i> 2018
<i>Spegazzinia deightonii</i>	yone 212	AB797292	AB807582	AB808558	NA	Tanaka <i>et al.</i> 2015
<i>S. tessartha</i>	SH 287	AB797294	AB807584	AB808560	JQ673429	Tanaka <i>et al.</i> 2015
<i>S. radermacherae</i>	MFLUCC 17-2285*	MK347848	MK347957	MK360088	MK347740	Jayasiri <i>et al.</i> 2019
<i>Trematea guiyangensis</i>	GZAAS01*	KX274253	KX274247	KX284705	KX274240	Hyde <i>et al.</i> 2016
<i>T. arundicola</i>	MFLU 16-1275*	KX274254	KX274248	KX284706	KX274241	Hyde <i>et al.</i> 2016
<i>Verrucoconiothyrium nitidae</i>	CBS 119209	NA	EU552112	NA	EU552112	Marincowitz <i>et al.</i> 2008
<i>Xenocamarosporium acaciae</i>	CPC 24755*	NA	NG_058163	NA	NR_137982	Crous <i>et al.</i> 2015b
<i>X. acacia</i>	MFLUCC 17-2432	MK347873	MK347983	MK360093	MK347766	Jayasiri <i>et al.</i> 2019

Types strains are indicated with (*) and newly generated sequences are shown in bold. Unavailable sequences are indicated by "NA".

ABRIICC—Agriculture Biotechnology Research Institute of Iran Culture Collections; **CBS**—Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CMG**—M. Gonçalves, living cultures; **CPC**—Culture Collection of Pedro Crous, housed at CBS; **KT**—K. Tanaka; **yone**—H. Yonezawa; **MFLU**—Mae Fah Luang University Herbarium, Chiang Rai, Thailand; **MFLUCC**—Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

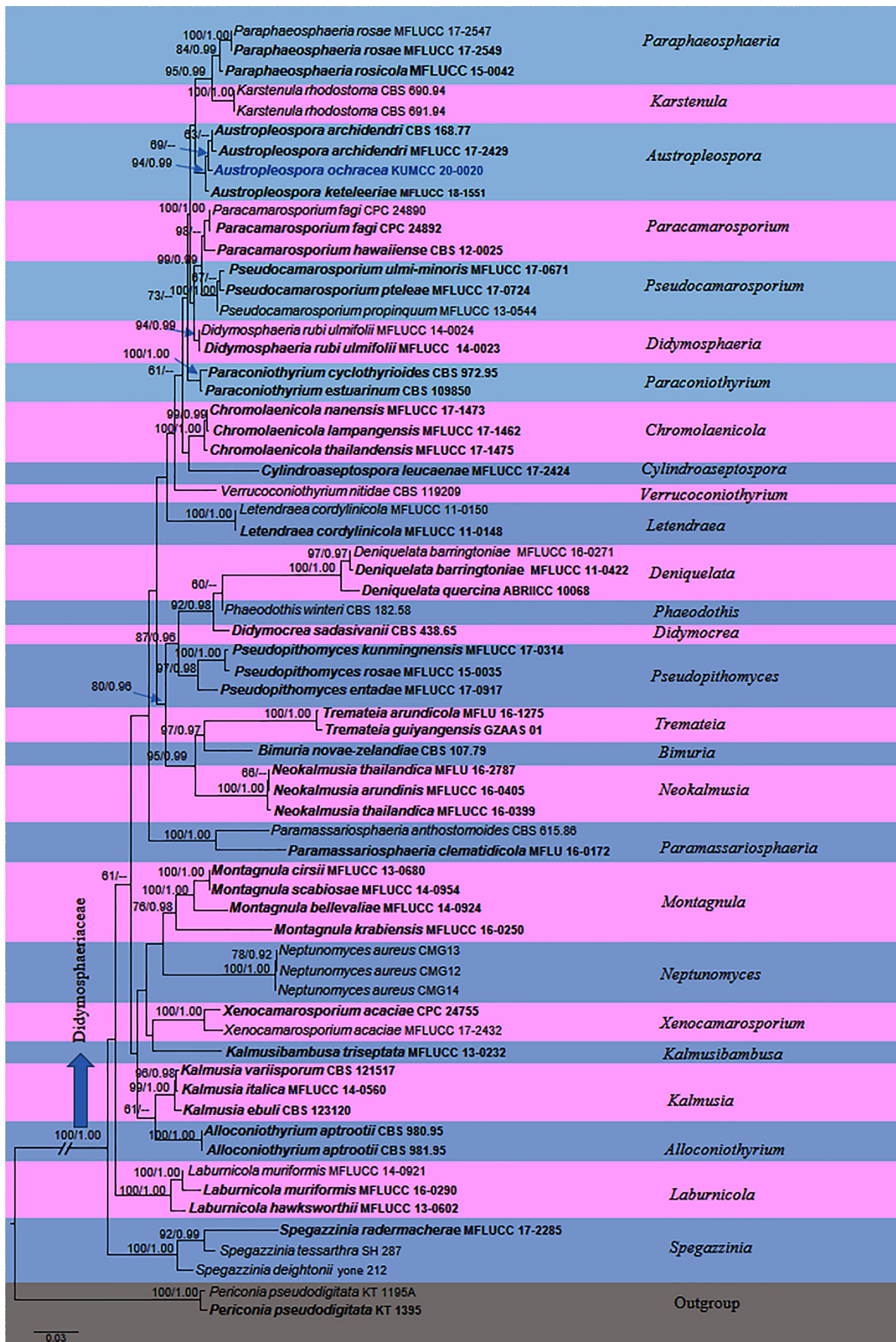


FIGURE 1. RAxML tree based on a combined dataset of partial SSU, LSU, *tef1* and ITS sequence analyses. Bootstrap support values for ML equal to or greater than 60 %, Bayesian posterior probabilities (BYPP) equal to or greater than 0.95 are shown as ML/BYPP above the nodes. The new isolate is in blue. The scale bar represents the expected number of nucleotide substitutions per site.



FIGURE 2. *Austropleospora ochracea* (HMAS 248367, holotype). a, b. Ascomata on host substrate, c, d. Vertical sections of ascomata, e. Peridium, f. Pseudoparaphyses, g–j. Asci, k–n. Ascospores, o. Germinating ascospore, p. Culture on PDA from above. Scale bars: c, d = 100 μ m, e = 10 μ m, f = 4 μ m, g–j = 20 μ m, k–o = 5 μ m.

Results

Phylogenetic analyses

The combined SSU, LSU, *tef1* and ITS matrix comprised 65 taxa, including the new taxon. *Periconia pseudodigitata* (KT1195A, KT1395) was selected as the outgroup taxon. The concatenated alignment comprised 3,391 total characters (SSU: 1–969 bp, LSU: 970–1860 bp, *tef1*: 1861–2771 bp, ITS: 2772–3391 bp), including gaps. The best scoring RAxML tree was selected to represent taxa relationships with a final likelihood value of -17819.961779 (Figure 1). The matrix had distinct alignment patterns with 27.41 % of undetermined characters or gaps. Estimated base frequencies were as A = 0.238644, C = 0.248783, G = 0.272124, T = 0.240449; and substitution rates as AC = 1.21954, AG = 1.923359, AT = 1.291790, CG = 0.868318, CT = 5.432812, GT = 1.00. The proportion of invariable sites I = 0.520772, the gamma distribution shape parameter alpha = 0.427153 and Tree-Length = 1.574462. Phylogenetic trees obtained from ML and BI analyses yielded trees with similar overall topology as previous work (Jayasiri *et al.* 2019).

Didymosphaeriaceae comprised 27 genera out of 31 genera with molecular data (Figure 1). All genera appear to be genetically closely related and monophyletic. Our strain *Austropleospora ochracea* clustered with two (CBS 168.77, MFLUCC 17-2429) strains of *Austropleospora archidendri* as a monophyletic clade but with low statistical support (ML = 69 %, BYPP = 0.92).

Taxonomy

Austropleospora ochracea L.S. Dissan., J.C. Kang & K.D. Hyde, *sp. nov.* (FIGURE 2)

Index Fungorum number: IF557841, *Facesoffungi number*: FoF 07933

Etymology:—The specific epithet refers to the color (yellowish brown) of the ascospores

Holotypus:—HMAS 248367

Saprobic on dead twigs. **Sexual morph**: *Ascomata* 150–230 µm high, 180–250 µm diam. (\bar{x} = 190 × 200 µm, n = 10), black, scattered, solitary to gregarious, immersed under host epidermis, slightly raised, globose to subglobose, uni-loculate, short ostiole in the center with a pore-like opening. *Peridium* 15–20 µm wide (\bar{x} = 18 µm, n = 15), of equal thickness, composed of several layers (3–5 layers), of flattened to broad, hyaline to brown cells, arranged in *textura angularis* to *textura prismatica*. *Hamathecium* composed of 1–2.5 µm wide (n = 30), numerous, filamentous, cellular, hyaline pseudoparaphyses. *Asci* 80–110 µm × 10–15 µm (\bar{x} = 97 × 12 µm, n = 25), 8-spored, bitunicate, fissitunicate, cylindrical to cylindric-clavate, short pedicellate. *Ascospores* 10–20 µm × 5–7 µm (\bar{x} = 15 × 6 µm, n = 30), overlapping uniseriate, muriform, mostly ellipsoidal, with 3 transverse septa, 0–1-longitudinal septa, initially hyaline, becoming yellowish-brown at maturity, asymmetrical, conical and narrowly rounded at the ends. **Asexual morph**: Undetermined.

Culture characteristics:—*Colonies on PDA*, reaching 2 cm diam., after 2 weeks at 20 °C, medium dense, circular to slightly irregular with uneven margin, slightly raised and cottony surface, colony from above: white at the margin, whitish-grey at the center, yellow concentric lines near to center; from below: yellowish white at the margin, yellow to brown at the center; mycelium greenish-grey.

Material examined:—CHINA, Guizhou Province, Guiyang, Guizhou University, on dead twigs of an undetermined host, 5 October 2019, L.S. Dissanayake (HMAS 248367, holotype), ex-type living culture, KUMCC 20-0020

Known distribution:—Guizhou Province, China

Discussion

In this study, we introduce *Austropleospora ochracea* from the dead twigs of an unidentified tree. This is the first record of this genus from China. The new taxon shares similar morphology to *Austropleospora* in having scattered, immersed, black, ostiolate ascomata and asci with 8 spored, clavate to cylindrical and yellowish brown ascospores. However, *A. ochracea* differs from other taxa in *Austropleospora* in having a globose to subglobose, uni-loculate ascomata with short papilla and a central ostiole, a comparatively thick peridium (15–18 µm), comprising brown to

dark brown cells arranged in *textura angularis* to *textura prismatica* and smaller conical ascospores ($10\text{--}20 \times 5\text{--}7 \mu\text{m}$) narrowly rounded at the ends (Table 2). Out of three species in this genus, only one species (*A. osteospermi*) can be morphologically compared with our new taxon because the other two species were introduced only with asexual morphs. *Austropleospora osteospermi* was introduced with morphology (sexual and asexual) and molecular (ITS) data. We omitted *A. osteospermi* in our phylogenetic analysis because of the lack of molecular data. There is a close phylogenetic relationship between *A. ochracea* and *A. archidendri*; however, this is poorly supported in concatenated sequence data analyses (Figure 1). The sequence comparison shows 0.56 % (890 bp, including gaps) with the LSU and 1.45 % (619 bp, including gaps) with the ITS bp differences between *A. ochracea* and *A. archidendri* (MFLUCC 17–2429). According to the phylogenetic analysis, *Austropleospora* clade closely aligned with *Karstenula* (Figure 1). *Austropleospora ochracea* is similar in morphology to *Karstenula rhodostoma* in having globose to subglobose, immersed ascomata, 8-spored cylindrical asci and cylindrical to ellipsoid ascospores with 3 transverse and 0–1 longitudinal septa. However, *A. ochracea* has slightly raised ascomata, short asci ($80\text{--}110 \mu\text{m}$) and smaller ascospores ($10\text{--}20 \times 5\text{--}7 \mu\text{m}$), while *K. rhodostoma* has long asci ($145\text{--}175 \mu\text{m}$) and large ascospores ($22\text{--}26 \times 8\text{--}10 \mu\text{m}$) (Tanaka *et al.* 2015).

The pseudoparaphyses in the new species are cellular and anastomose above the asci and are embedded in a gelatinous matrix which may give the appearance of trabeculae. These, however, are not trabeculae *sensu stricto* which should anastomose between the asci (Liew *et al.* 2000, Hongsanan *et al.* 2020a). Trabeculae are only found in a small percentage of Dothideomycetes (Hongsanan *et al.* 2020a, b), but are important at the genus level and thus their interpretation must be considered carefully. *Austropleospora* is recorded as having cellular pseudoparaphyses in Hongsanan *et al.* (2020a). Morin *et al.* (2010) did not clearly illustrate or described the hamathecium characteristics of *A. osteospermi*. However, the pseudoparaphyses illustrated in Fig. 7 of Morin *et al.* (2010) appear to be cellular. We, therefore, conclude that *Austropleospora* species have cellular pseudoparaphyses.

TABLE 2. Synopsis of *Austropleospora* species with morphological features discussed in this study.

Species	<i>Austropleospora osteospermi</i> (Morin <i>et al.</i> 2010)	<i>Austropleospora ochracea</i> (This study)
Host	On stems of <i>Chrysanthemoides monilifera</i> ssp. <i>Rotundata</i>	Dead twigs of an undetermined deciduous tree
Country	Australia	China
Ascomata	Subglobose or slightly flattened, solitary or in groups, scattered, immersed immediately below the stem epidermis $125\text{--}250 \times 100 \mu\text{m}$	Globose to subglobose, black, scattered, solitary to gregarious, immersed under host epidermis, slightly raised, uni-loculate $150\text{--}230 \times 180\text{--}250 \mu\text{m}$
Ostiole	$25 \mu\text{m}$ diameter, with a protruding neck of $100\text{--}250 \times 75 \mu\text{m}$	Short ostiole in the center with a pore-like opening
Peridium	Dark reddish brown, composed of polyangular to irregular cells with rounded edges, $5\text{--}12 \mu\text{m}$ diam.	Brown to dark brown, composed of several layers, arranged in <i>textura angularis</i> to <i>textura prismatica</i> , $15\text{--}20 \mu\text{m}$ diam.
Asci	6–8 spored, cylindric-clavate, $75\text{--}120 \mu\text{m}$ long, $14\text{--}17 \mu\text{m}$ width	8 spored, cylindrical, $80\text{--}110 \mu\text{m}$ long, $10\text{--}15 \mu\text{m}$ width
Ascospores	Ellipsoidal $16\text{--}23 \times 7\text{--}10 \mu\text{m}$ 3 transverse septa 0–2 longitudinal septa Apex rounded to slightly tapered, yellowish brown	Ellipsoidal $10\text{--}20 \times 5\text{--}7 \mu\text{m}$ 3 transverse septa 0–1 longitudinal septum Conical and narrowly rounded at the ends, initially hyaline, becoming yellowish-brown at maturity

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