



Fungi from Asian Karst formations III. Molecular and morphological characterization reveal new taxa in Phaeosphaeriaceae

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Abstract

An ongoing study on ascomycetes from Karst landforms in Guizhou Province, China yielded 6 new taxa that belong to the Phaeosphaeriaceae. These include 2 novel monotypic genera: *Hydeopsis* (with *H. verrucispora* sp. nov.) and *Pseudoophiosphaerella* (with *P. huishuiensis* sp. nov.), and two new species: *Hydeomyces pinicola* and *Leptospora hydei*. Multi-gene phylogenetic analyses of a concatenated LSU-ITS-SSU-TEF1- α sequence data were performed to infer their phylogenetic relationships. *Hydeopsis* is morphologically characterized by subglobose, papillate, dark brown to black ascomata, cellular pseudoparaphyses, clavate, bitunicate, fissitunicate asci and ellipsoidal to fusiform, hyaline to pale pigmented, 3-septate ascospores with a mucilaginous sheath. *Hydeopsis* is phylogenetically related to *Dactylidina* and *Phaeopectea*, but can be recognized as a distinct lineage and these genera can also be distinguished based on morphology. *Pseudoophiosphaerella* is morphologically similar to *Ophiosphaerella* in having cylindrical-clavate asci and scolecospores, but is phylogenetically distant from the latter and more closely related to *Dlhawksworthia*. Comprehensive illustrations and detailed descriptions of these taxa are provided, and relationships with allied genera or species are discussed based on comparative morphology and phylogenetic evidence.

Key words – 6 new taxa – Dothideomycetes – Multi-gene – Sexual morph – Phylogeny – Taxonomy

Introduction

Pleosporales is the largest order of the Dothideomycetes and comprise around 80 families with a quarter of all described dothideomycetous fungi (Kodsueb et al. 2006, Kirk 2008, Zhang et al. 2008, 2012, Hyde et al. 2013, Tanaka et al. 2015, Jaklitsch & Voglmayr 2016, Dai et al. 2017,

Phookamsak et al. 2017, Liu et al. 2017, Ariyawansa et al. 2018, Wijayawardene et al. 2018). Most members of this order have been reported as playing important roles, and they can also change their lifestyles depending on environmental conditions/nature of substrates, and degrade dead plant materials or cause serious diseases in crops (Wong & Hyde 2001, Promptutha et al. 2007, 2010, Ghimire & Hyde 2008, Szilagy-Zecchin et al. 2016, Yang et al. 2016). Following extensive collections, a number of novel taxa have been described from this order (Jayasiri et al. 2016, Wanasinghe et al. 2017, 2018). Phaeosphaeriaceae, one of the most species-rich families in Pleosporales, was initially introduced to accommodate 15 genera by Barr (1979) with *Phaeosphaeria* as type genus and *P. oryzae* as the type species. As of now, Phaeosphaeriaceae comprises 55 genera, with a number of novel species introduced recently (Huang et al. 2017, Senanayake et al. 2018, Yang et al. 2019).

Members of Phaeosphaeriaceae are distributed worldwide, and normally characterized by having small to medium-sized, depressed global or conical ascomata, bitunicate asci, hyaline or pigmented ascospores with or without septation, and coelomycetous asexual morphs (Zhang et al. 2012, Hyde et al. 2013, Phookamsak et al. 2014). Many species of this group are known as important plant pathogens, saprobes, endophytes and lichenicolous (Wang et al. 2007, Lawrey et al. 2012, Zhang et al. 2012, Hyde et al. 2013, Quaedvlieg et al. 2013). A recent study reported establishment of a new genus, *Tintelnotia* which can even cause cornea and nail infections in humans (Ahmed et al. 2017).

Fungi from karst landforms in the province of Guizhou in China have been documented in recent studies (Ariyawansa et al. 2016 a, b, c, Chen et al. 2017, Zhang et al. 2017a, b, 2018). As part of our ongoing studies on ascomycetous fungi from this region, four new isolates were obtained and identified as members of Phaeosphaeriaceae. Based on morphological characteristics and in-depth phylogenetic analyses of a combined LSU-ITS-SSU-TEF1- α sequence dataset, two novel monotypic genera *Hydeopsis* and *Pseudoophiosphaerella*, with 4 new species viz. *Hydeomyces pinicola*, *Hydeopsis verrucispora*, *Leptospora hydei* and *Pseudoophiosphaerella huishuiensis* are introduced in this study. The aim of this study is to describe these new taxa with detailed descriptions and illustrations, and also investigate their phylogenetic relationships within Phaeosphaeriaceae based on DNA sequence data derived from multi-genes.

Materials & Methods

Collection, examination and isolation of specimens

Dead woody or herbaceous plant materials were collected from Guizhou Province of China from 2016 to 2017. The samples were taken back to laboratory and examined using a stereomicroscope (Motic SMZ 168), and the macro-morphological characters were captured by a stereomicroscope (Nikon SMZ800N) with a Cannon EOS 70D digital camera. Hand sections of fruiting bodies were made by a razor blade and mounted in a water drop for microscope studies and photomicrography. Micro-morphological characters were observed by differential interference contrast (DIC) using a Nikon ECLIPSE 80i compound microscope and captured by the Cannon EOS 600D digital camera. Measurements were processed in a Tarosoft (R) Image Frame Work version 0.9.7 program, and photographic plates were edited in Adobe Photoshop CS6 (Adobe Systems Inc., USA).

The fungi were isolated using single spore isolation as described in Chomnunti et al. (2014). The single germinated ascospore was picked up and transferred to potato dextrose agar (PDA; 39 g/l distilled water, Difco potato dextrose) for recording growth rates and culture characteristics (Liu et al. 2010). The specimens are deposited at the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand, and Guizhou Academy of Agricultural Sciences (GZAAS), Guizhou, China. Living cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC) Chiang Rai, Thailand, and Guizhou Culture Collection (GZCC), Guizhou, China. Index Fungorum and Facesoffungi numbers are provided as stated in Jayasiri et al. (2015) and Index Fungorum (2019). New species are established as outlined by Jeewon & Hyde (2016).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh fungal mycelium with Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, Shanghai, P. R. China) following the manufacturer's protocol. DNA amplifications were performed by Polymerase Chain Reaction (PCR) in a 50 µl reaction volume, which contained 19 µl Distilled-Deionized-water, 25 µl of 2 × Power Taq PCR Master Mix (TIANGEN Co., China), 2 µl of DNA template and 2 µl of each forward and reverse primers. Four partial gene portions were used in this study. The primers of ITS4 and ITS5, NS1 and NS4 (White et al. 1990), LR0R and LR5 (Vilgalys & Hester 1990), were used for amplifying the internal transcribed spacers (ITS), small subunit of the nuclear ribosomal RNA genes (SSU) and large subunit of the nuclear ribosomal RNA genes (LSU) respectively. The translation elongation factor 1-alpha region (TEF1- α) was amplified by the primers of EF1-983F and EF1-2218R (Rehner 2001). The PCR thermal cycle programs for these four genes are as follows: 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 50 s, elongation at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Quality of PCR products was checked by 1.2% agarose gel electrophoresis stained with ethidium bromide and then sent to Sangon Biotechnology Co., Ltd (Shanghai, P. R. China) for sequencing.

Phylogenetic analyses

Newly generated sequence data were initially subjected to blast search in NCBI. Appropriate sequences used for phylogenetic analyses were selected and downloaded from GenBank based on blast results and recent literature (Phookamsak et al. 2014, 2017, Liu et al. 2015, Abd-Elsalam et al. 2016, Ahmed et al. 2017, Tennakoon et al. 2016, Tibpromma et al. 2016, Hyde et al. 2017, Bakhshi et al. 2018, Maharachchikumbura et al. 2019, Yang et al. 2019). Each gene region was aligned with the MAFFT v. 7 online interface using default settings (<http://mafft.cbrc.jp/alignment/server/>), then improved manually and combined in the program BioEdit v. 7.2 (Hall 1999).

Phylogenies of the aligned sequence dataset were inferred from maximum likelihood (ML) and Bayesian Inference (BI) analyses. The ML analyses including 1000 bootstrap replicates were performed in the program of RAxMLGUI v. 1.5b1 (Silvestro & Michalak 2012), and the best scoring tree was selected among suboptimal trees from each replicate by comparing likelihood scores under the GTR+GAMMA substitution model. Bayesian analyses were carried out by using MrBayes v. 3.2 (Ronquist et al. 2012), and the suitable models of evolution were estimated in MrModeltest 2.3 for each gene (Nylander 2008). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2. Six simultaneous Markov chains were run for 10000000 generations and trees were sampled every 100th generation. The temperature values were lowered to 0.15, burn-in was set to 0.25, and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01. Trees were visualized with TreeView (Page 1996), and the layouts were done in the program of Adobe Illustrator CS v. 5 (Adobe Systems Inc., USA).

Table 1 Isolates used in the phylogenetic analyses. Newly generated sequences are in red.

Taxon	Strain/Specimen No.	GenBank accession numbers			
		LSU	ITS	SSU	TEF1- α
<i>Acericola italica</i>	MFLUCC 13-0609	MF167429	MF167428	MF167430	---
<i>Allophaeosphaeria muriformia</i>	MFLUCC 13-0349	KP765681	KP765680	KP765682	---
<i>Allophaeosphaeria subcylindrospora</i>	MFLUCC 13-0380	KT314183	KT314184	KT314185	---
<i>Amarenographium ammophilae</i>	MFLUCC 16-0296	KU848197	KU848196	KU848198	MG520894
<i>Amarenomyces dactylidis</i>	MFLUCC 14-0207	KY775575	KY775577	---	---
<i>Ampelomyces quisqualis</i>	CBS 129.79	EU754128	HQ108038	EU754029	---
<i>Bhatiellae rosae</i>	MFLUCC 17-0664	MG828989	MG828873	MG829101	---
<i>Camarosporioides phragmitis</i>	MFLUCC 13-0365	KX572345	KX572340	KX572350	KX572354
<i>Chaetosphaeronema achilleae</i>	MFLUCC 16-0476	KX765266	KX765265	---	---

Table 1 Continued.

Taxon	Strain/Specimen No.	GenBank accession numbers			
		LSU	ITS	SSU	TEF1- α
<i>Chaetosphaeronema hispidulum</i>	CBS 216.75	KF251652	KF251148	EU754045	---
<i>Dactylidina dactylidis</i>	MFLUCC 13-0618	KP744473	KP744432	KP753946	---
<i>Dactylidina dactylidis</i>	MFLUCC 14-0966	MG829002	MG828886	MG829113	MG829199
<i>Dematiopleospora donetzica</i>	MFLU 15-2199	MG829005	---	MG829116	---
<i>Dematiopleospora mariae</i>	MFLUCC 13-0612	KJ749653	KJ749654	KJ749652	KJ749655
<i>Diederichomyces ficuzzae</i>	CBS 128019	JQ238616	KP170647	---	---
<i>Diederichomyces xanthomendozae</i>	CBS 129666	JQ238634	KP170651	---	---
<i>Dlhawksworthia clematidicola</i>	MFLUCC 14-0910	MG829011	MG828901	MG829120	MG829202
<i>Dlhawksworthia loniceriae</i>	MFLUCC 14-0955	MG829012	MG828902	MG829121	MG829203
<i>Embarria clematidis</i>	MFLUCC 14-0652	KT306593	KT306949	KT306956	---
<i>Embarria clematidis</i>	MFLUCC 14-0976	MG828987	MG828871	MG829099	MG829194
<i>Equiseticola fusispora</i>	MFLUCC 14-0522	KU987669	KU987668	KU987670	MG520895
<i>Galliicola pseudophaeosphaeria</i>	MFLUCC 14-0524	KT326693	KT326692	---	MG520896
<i>Hydeomyces desertipleosporoides</i>	SQUCC 15259	MK290839	MK290841	MK290843	MK290848
<i>Hydeomyces desertipleosporoides</i>	SQUCC 15260	MK290840	MK290842	MK290844	MK290849
<i>Hydeomyces pinicola</i>	GZCC 19-0003	MK522496	MK522506	MK522502	MK523386
<i>Hydeopsis verrucispora</i>	MFLUCC 19-0163	MK522498	MK522508	MK522504	MK523388
<i>Italica achilleae</i>	MFLUCC 14-0959	MG829013	MG828903	MG829122	MG829204
<i>Juncaceicola italica</i>	MFLUCC 13-0750	KX500107	KX500110	KX500108	MG520897
<i>Juncaceicola luzulae</i>	MFLUCC 16-0780	KX449530	KX449529	KX449531	MG520898
<i>Juncaceicola typharum</i>	CBS 296.54	KF251695	KF251192	---	GU456287
<i>Leptosphaeria doliolum</i>	CBS 505.75	GU301827	JF740205	GU296159	GU349069
<i>Leptospora galii</i>	KUMCC 15-0521	KX599548	KX599547	KX599549	MG520899
<i>Leptospora rubella</i>	CPC 11006	DQ195792	DQ195780	DQ195803	---
<i>Leptospora rubella</i>	MFLU 16-0965	KX757834	KX757835	---	---
<i>Leptospora thailandica</i>	MFLUCC 16-0385	KX655549	KX655559	KX655554	KX655564
<i>Leptospora hydei</i>	GZCC 19-0004	MK522497	MK522507	MK522503	MK523387
<i>Loratospora luzulae</i>	MFLUCC 14-0826	KT328495	KT328497	KT328496	---
<i>Melnikia anthoxanthii</i>	MFLUCC 14-1010	KU848204	---	KU848205	---
<i>Muriphaeosphaeria galatellae</i>	MFLUCC 14-0614	KT438329	KT438333	KT438331	MG520900
<i>Muriphaeosphaeria galatellae</i>	MFLUCC 15-0769	KT438330	---	KT438332	---
<i>Neosetophoma garethjonesii</i>	MFLUCC 14-0528	KY496738	KY496758	KY501126	KY514402
<i>Neosetophoma rosigena</i>	MFLUCC 17-0768	MG829037	MG828928	MG829143	---
<i>Neostagonospora arrhenather</i>	MFLUCC 15-0464	KX910091	KX926417	KX950402	MG520901
<i>Neostagonospora caricis</i>	CBS 135092	KF251667	KF251163	---	---
<i>Neostagonospora phragmitis</i>	MFLUCC 16-0493	KX910090	KX926416	KX950401	MG520902
<i>Neostagonospora sichuanensis</i>	MFLU 18-1223	MH394690	MK296469	MH394687	MK313854
<i>Neostagonospora sichuanensis</i>	MFLUCC 18-1228	MH368073	MH368088	MH368079	MK313851
<i>Neosulcatispora agaves</i>	CPC 26407	KT950867	KT950853	---	---
<i>Neosulcatispora strelitziae</i>	CPC 25657	KX228305	KX228253	---	---
<i>Nodulosphaeria guttulatum</i>	MFLUCC 15-0069	KY496726	KY496746	KY501115	KY514394
<i>Nodulosphaeria multiseptata</i>	MFLUCC 15-0078	KY496728	KY496748	KY501116	KY514396
<i>Nodulosphaeria scabiosae</i>	MFLUCC 14-1111	KU708846	KU708850	KU708842	KU708854
<i>Ophiobolopsis italica</i>	MFLUCC 17-1791	MG520959	MG520939	MG520977	MG520903
<i>Ophiobolus artemisiae</i>	MFLUCC 14-1156	KT315509	KT315508	MG520979	MG520905
<i>Ophiobolus disseminans</i>	MFLUCC 17-1787	MG520961	MG520941	MG520980	MG520906
<i>Ophiobolus ponticus</i>	MFLUCC 17-2273	MG520963	MG520943	MG520982	MG520908
<i>Ophiosimulans tanacetii</i>	MFLUCC 14-0525	KU738891	KU738890	KU738892	MG520910
<i>Ophiosphaerella agrostidis</i>	MFLUCC 11-0152	KM434281	KM434271	KM434290	KM434299
<i>Ophiosphaerella aquaticus</i>	MFLUCC 14-0033	KX767089	KX767088	KX767090	MG520911
<i>Ophiosphaerella herpotricha</i>	KY423	KP690989	KP690989	KP690989	KP691011

Table 1 Continued.

Taxon	Strain/Specimen No.	GenBank accession numbers			
		LSU	ITS	SSU	TEF1- α
<i>Ophiosphaerella korrae</i>	ATCC 56289	KC848509	KC848509	KC848509	KC848515
<i>Ophiosphaerella narmari</i>	ATCC 64688	KC848510	KC848510	KC848510	KC848516
<i>Paraleptosphaeria dryadis</i>	CBS 643.86	GU301828	JF740213	KC584632	GU349009
<i>Paraophiobolus arundinis</i>	MFLUCC 17-1789	MG520965	MG520945	MG520984	MG520912
<i>Paraophiobolus plantaginis</i>	MFLUCC 17-0245	KY815010	KY797641	KY815012	MG520913
<i>Paraphoma chrysanthemicola</i>	CBS 522.66	KF251670	FJ426985	GQ387521	---
<i>Paraphoma radicina</i>	CBS 111.79	KF251676	KF251172	EU754092	---
<i>Parastagonospora italica</i>	MFLUCC 13-0377	KU058724	KU058714	MG520985	MG520915
<i>Parastagonospora minima</i>	MFLUCC 13-0376	KU058723	KU058713	MG520986	MG520916
<i>Parastagonospora nodorum</i>	CBS 110109	KF251681	KF251177	---	---
<i>Parastagonospora fallopiae</i>	CBS 135981	MH460545	MH460543	---	---
<i>Parastagonospora fallopiae</i>	CCTU 1151.1	MH460546	MH460544	---	---
<i>Phaeopoea festucae</i>	MFLUCC 17-0056	KY824767	KY824766	KY824769	---
<i>Phaeosphaeria chiangraina</i>	MFLUCC 13-0231	KM434280	KM434270	KM434289	KM434298
<i>Phaeosphaeria oryzae</i>	CBS 110110	KF251689	KF251186	GQ387530	---
<i>Phaeosphaeriopsis glaucopunctata</i>	MFLUCC 13-0265	KJ522477	KJ522473	KJ522481	MG520918
<i>Phaeosphaeriopsis triseptata</i>	MFLUCC 13-0271	KJ522479	KJ522475	KJ522484	MG520919
<i>Phaeosphaeriopsis yuccae</i>	MFLUCC 16-0558	KY554481	KY554482	KY554480	MG520920
<i>Poaceicola arundinis</i>	MFLUCC 15-0702	KU058726	KU058716	MG520988	MG520921
<i>Poaceicola italica</i>	MFLUCC 13-0267	KX910094	KX926421	KX950409	MG520924
<i>Populocrescentia ammophilae</i>	MFLUCC 17-0665	MG829059	MG828949	MG829164	MG829231
<i>Populocrescentia forlicesenensis</i>	MFLUCC 14-0651	KT306952	KT306948	KT306955	MG520925
<i>Populocrescentia rosae</i>	MFLU 17-0128	MG829060	---	MG829165	MG829232
<i>Pseudoophiobolus mathieui</i>	MFLUCC 17-1784	MG520969	MG520949	MG520991	MG520928
<i>Pseudoophiobolus rosae</i>	MFLUCC 17-1786	MG520972	MG520952	MG520993	MG520930
<i>Pseudoophiobolus urticicola</i>	KUMCC 17-0168	MG520975	MG520955	MG520996	MG520933
<i>Pseudoophiosphaerella huishuiensis</i>	MLUCC 19-0164	MK522499	MK522509	MK522505	MK523389
<i>Pseudophaeosphaeria rubi</i>	MFLUCC 14-0259	KX765299	KX765298	KX765300	MG520934
<i>Sclerostagonospora ericae</i>	CPC 25927	KX228319	KX228268	---	---
<i>Sclerostagonospora opuntiae</i>	CBS 118224	DQ286772	DQ286768	---	---
<i>Scoliosporium minkeviciusii</i>	MFLUCC 12-0089	KF366382	---	KF366383	---
<i>Septoriella phragmitis</i>	CPC 24118	KR873279	KR873251	---	---
<i>Setomelanomma holmii</i>	CBS 110217	GQ387633	KT389542	GQ387572	GU349028
<i>Setophoma terrestris</i>	CBS 335.29	KF251749	KF251246	GQ387526	---
<i>Stagonospora neglecta</i>	CBS 343.86	EU754218	AJ496630	EU754119	---
<i>Tintelnotia destructans</i>	CBS 127737	KY090664	KY090652	KY090698	---
<i>Tintelnotia opuntiae</i>	CBS 376.91	GU238123	KY090651	GU238226	---
<i>Vagicola arundinis</i>	MFLUCC 15-0027	KY706129	KY706139	KY706134	MG520936
<i>Vrystaatia aloecicola</i>	CBS 135107	KF251781	KF251278	---	---
<i>Wojnowicia italica</i>	MFLUCC 13-0447	KX430001	KX342923	KX430002	KX430003
<i>Wojnowicia rosicola</i>	MFLUCC 15-0128	MG829091	MG828979	MG829191	---
<i>Wojnowiciella eucalypti</i>	CBS 139904	KR476774	KR476741	---	---
<i>Xenoseptoria neosaccardoi</i>	CBS 128665	KF251784	KF251281	---	---
<i>Xenoseptoria neosaccardoi</i>	CBS 120.43	KF251783	KF251280	---	---
<i>Yunnanensis phagmitis</i>	MFLUCC 17-0315	MF684863	MF684862	MF684867	MF683624
<i>Yunnanensis phagmitis</i>	MFLUCC 17-1361	MF684865	MF684869	MF684864	MF683625

Abbreviations: CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC Collection of Pedro Crous housed at CBS; CCTU Culture Collection of Tabriz University, Tabriz, Iran; GZCC Guizhou Culture Collection, Guizhou, China. MFLUCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

Results

Phylogenetic analyses

A combined LSU-ITS-SSU-TEF1- α matrix was used to infer the phylogenetic affinities of these new taxa among members of Phaeosphaeriaceae. The dataset comprised 106 taxa (including the new strains), with *Leptosphaeria doliolum* (CBS 505.75) and *Paraleptosphaeria dryadis* (CBS 643.86) as the outgroup taxa. The dataset comprised 3107 characters including gaps, of which 822 characters belonged to LSU (1–822), 456 characters to ITS (826–1281), 932 characters to SSU (1285–2216) and 888 characters to TEF1- α (2220–3107). The best ML tree revealed by the RAxML analysis is selected to represent the relationships among these taxa and shown in Fig. 1. The trees generated from Bayesian Inference analyses had similar topologies and not presented here.

Phylogeny indicates that all of the newly collected taxa belong to the Phaeosphaeriaceae and the new genus *Pseudoophiosphaerella* constitutes an independent lineage and closely related to *Dlhawksworthia* with strong statistical support (MLBP 94% and BIPP 1.0). *Hydeopsis verrucispora* sp. nov. is nested in between *Dactylidina* and *Phaeopoacea* as a distinct lineage with good support in ML (MLBP 82%) and BI (BIPP 1.0) analyses. In addition, the new species *Hydeomyces pinicola* clustered together with the type species of *Hydeomyces* (*H. desertipleosporoides*) with high support (MLBP 100%, and BIPP 1.0), while *Leptospora hydei* sp. nov. is assigned within the genus *Leptospora*, and related to *L. thailandica*.

Taxonomy

Pseudoophiosphaerella J.F. Zhang, J.K. Liu & Z.Y. Liu, gen. nov.

Index Fungorum number: IF 556244; Facesoffungi Number: FoF 05835

Etymology – Refers to the morphological resemblance to *Ophiosphaerella*.

Type species – *Pseudoophiosphaerella huishuiensis*.

Saprobic on dead culms of herbaceous plant. Sexual morph: *Ascomata* scattered, solitary to gregarious, globose to subglobose, dark brown to black, immersed to erumpent, ostiolate. *Hamathecium* comprising numerous, hyphae-like, trabeculate, branched pseudoparaphyses. *Asci* cylindrical, 8-spored, bitunicate, fissitunicate, short pedicellate, apically rounded. *Ascospores* filiform, fasciculate, hyaline and aseptate when young, become pale-yellowish and multi-septate at maturation, lying parallel or spirally coiled, obtuse in one end and towards acute in another end, smooth-walled, guttulate. Asexual morph: Undetermined.

Notes – Our phylogeny indicates that *Pseudoophiosphaerella* shares a close relationship to *Dlhawksworthia* with good support. However, it is morphologically different from the latter in having filiform ascospores, while the ascospores in *Dlhawksworthia* were observed as ellipsoidal or muriform. Although *Pseudoophiosphaerella* is morphologically similar to *Ophiosphaerella* (in having long cylindrical asci with septate, filiform ascospores), our multi-gene analyses indicate that they are phylogenetically distant. Hyde et al. (2013), Phookamsak et al. (2014) indicated that many sexual genera within Phaeosphaeriaceae share similar morphological characters, and it is difficult to distinguish these genera based only on morphological examination. *Pseudoophiosphaerella* is introduced herein as a new genus to accommodate the new species *Pseudoophiosphaerella huishuiensis*.

Pseudoophiosphaerella huishuiensis J.F. Zhang, J.K. Liu & Z.Y. Liu sp. nov.

Fig. 2

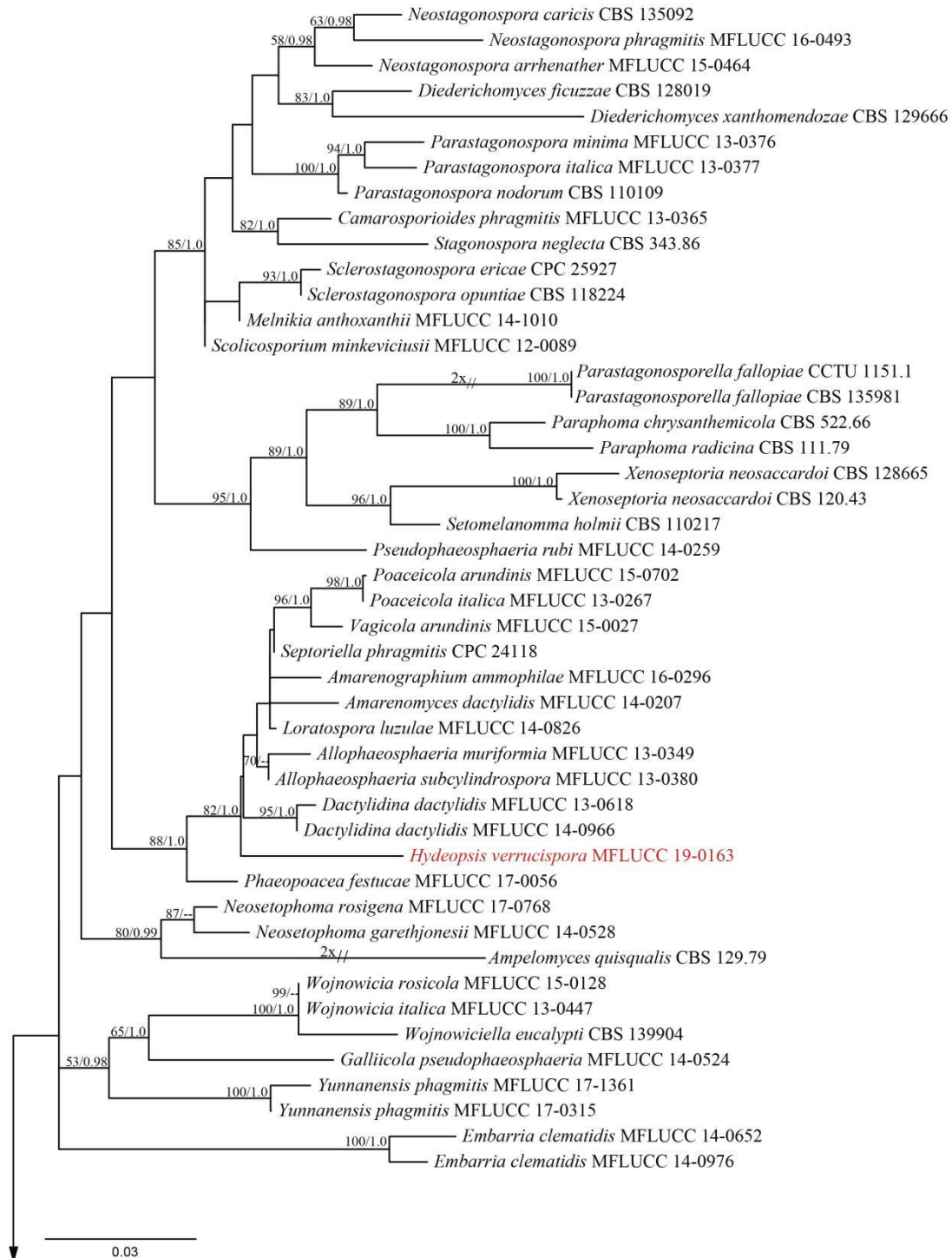
Index Fungorum number: IF 556246; Facesoffungi Number: FoF 05836

Etymology – Refers to the place Huishui county, where the fungus was collected.

Holotype – MFLU 18-2266

Saprobic on dead culms of unidentified herbaceous plant. Sexual morph: *Ascomata* scattered, solitary to gregarious, globose to subglobose, dark brown to black, immersed to erumpent, ostiolate. *Ostiole* central, papillate, conical, with a pore-like opening. *Peridium* 25–34 μm thick,

coriaceous, composed of several layers of thick-walled, brown to dark brown, pseudoparenchymatous cells of *textura angularis*, become pale brown to hyaline towards inner layers. *Hamathecium* comprising numerous, 1.5–3 µm wide, hyphae-like, pseudoparaphyses, trabeculate at the base and branched at the apex, anastomosing between and above asci, embedded in a gelatinous matrix. *Asci* 143–151.5 × 8–9.5 µm (\bar{x} = 148 × 8.7 µm, n=25), 8-spored, bitunicate, fissitunicate, long cylindrical, short pedicellate, apically rounded, with a minute ocular chamber. *Ascospores* 130.5–151.5 × 2.5–3.5 µm (\bar{x} = 142.5 × 3 µm, n=30), filiform, fasciculate, hyaline and aseptate when young, become pale-yellowish and multi-septate at maturation, multiseriate, lying parallel or spirally coiled, obtuse in one end and acute in another end, smooth-walled, guttulate. Asexual morph: Undetermined.



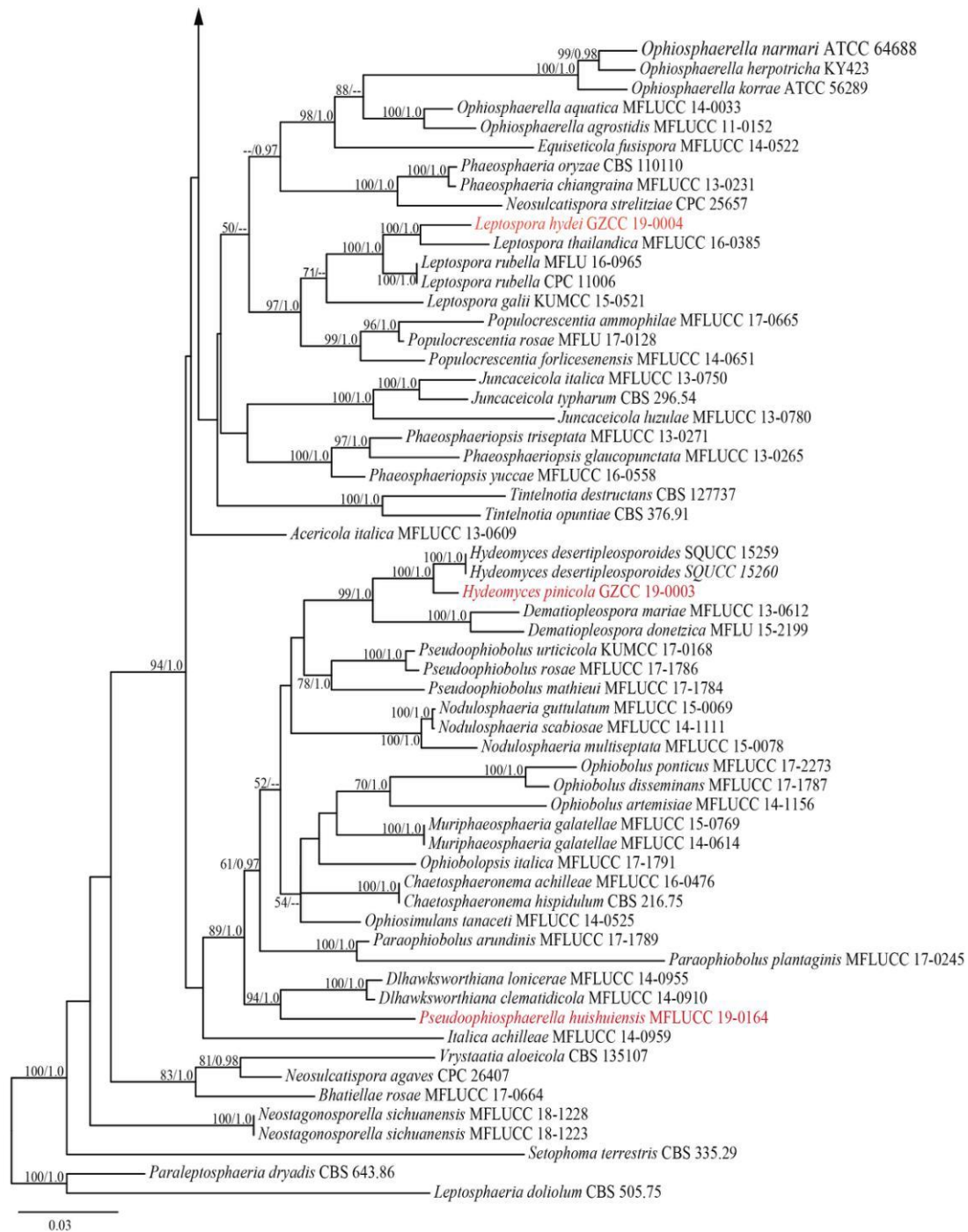


Fig. 1 – Phylogram of the best Maximum Likelihood tree revealed by RAxML analysis of the combined (LSU-ITS-SSU-TEF1- α) dataset with other members of the Phaeosphaeriaceae. RAxML bootstrap support values (MLBP) ($\geq 50\%$) and Bayesian posterior probabilities (BIPP) greater than 0.95 are shown near the nodes. The original isolate numbers are noted after the species name. The tree is rooted to *Leptosphaeria doliolum* (CBS 505.75) and *Paraleptosphaeria dryadis* (CBS 643.86), the scale bar shows 0.03 changes.

Culture characteristics – Ascospores germinated within 24 h in WA. Colonies on PDA reaching 40 mm diam. after 3 weeks at 25°C, medium dense, regular in shape, flattened, surface smooth, with edge fimbriate, colony from above cream, from below pale yellowish at the margin, orangish-brown in the centre.

Material examined – CHINA, Guizhou Province, Huishui county, on dead twigs of unidentified host, 21 July 2017, J.F. Zhang, HS-13 (MFLU 18-2266; GZAAS 18-0008); ex-type living culture (MFLUCC 19-0164, GZCC 19-0002).

Notes – The new taxon *Pseudoophiosphaerella huishuiensis* is phylogenetically related to *Dlhawksworthia* species with good support (MLBP 94% and BIPP 1.00). However, they can be distinguished on the basis of their morphological characteristics as the new taxon is characterized by long cylindrical asci with filiform, fasciculate, multiseriate ascospores, while the members of *Dlhawksworthia* are characterized by cylindric-clavate asci with ellipsoidal, fusiform, overlapping uniseriate ascospores (Wanasinghe et al. 2018).

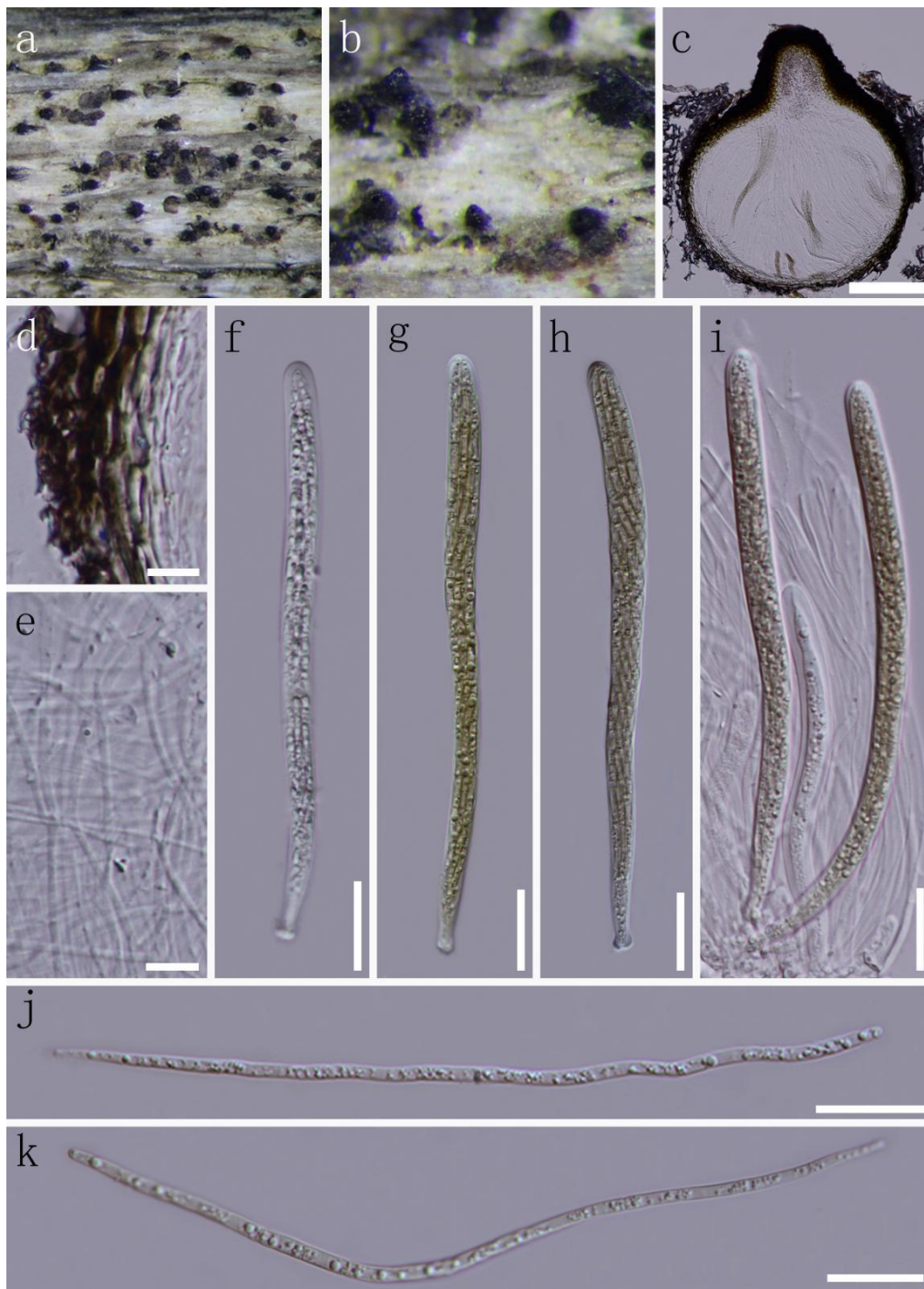


Fig. 2 – *Pseudoophiosphaerella huishuiensis* (MFLU 18-2266, holotype) a, b Appearance of ascostromata on host surface. c Vertical sections through ascostroma. d Section of peridium. e Pseudoparaphyses. f-i Asci. j, k Ascospores. Scale bars: c = 100 μ m, d, e = 10 μ m, f-k = 20 μ m

Hydeopsis J.F. Zhang, J.K. Liu & Z.Y. Liu, gen. nov.

Index Fungorum number: IF 556247; Facesoffungi Number: FoF 05837

Etymology – Named in honour of Prof. Kevin D. Hyde for his immense contribution to mycology.

Type species – *Hydeopsis verrucispora*.

Saprobic on dead culms of herbaceous plant. Sexual morph: *Ascomata* immersed, scattered, solitary, subglobose, dark brown to black, coriaceous, ostiolate. *Hamathecium* comprising numerous, hypha-like, cellular pseudoparaphyses, anastomosing between and among the asci. *Asci* clavate, 8-spored, bitunicate, fissitunicate, pedicellate, with a furcate pedicel at the base, rounded to truncate at the apex, with a developed ocular chamber. *Ascospores* ellipsoidal to fusiform, hyaline to pale pigmented, aseptate when young, and become septate at maturation, constricted at the median septum, surrounded by a mucilaginous sheath. Asexual morph: Undetermined.

Notes – Phylogeny indicates that *Hydeopsis* can be recognized as member of Phaeosphaeriaceae (Fig. 1), where it is phylogenetically close to *Dactylidina* and *Phaeopoacea*. However, it can be differentiated from these genera as it is characterized by ellipsoidal to fusiform, hyaline to pale pigmented, phragmosporous ascospores, while the members of *Dactylidina* and *Phaeopoacea* are described as having muriform, dark pigmented ascospores (Liu et al. 2015, Hyde et al. 2017, Wanasinghe et al. 2018b). Although *Hydeopsis* is morphologically similar to the genera *Acericola*, *Brickea*, *Juncaceicola*, *Loratospora*, *Phaeosphaeria*, *Pseudophaeosphaeria*, *Setomelanomma* and *Setophoma* in having 3-septate, fusiform ascospores (Phookamsak et al. 2014, Ariyawansa et al. 2015, Tennakoon et al. 2016, Hyde et al. 2016, 2017), our multigene phylogeny presented herein indicates that they are phylogenetically distinct genera.

Hydeopsis verrucispora J.F. Zhang, J.K. Liu & Z.Y. Liu, sp. nov.

Fig. 3

Index Fungorum number: IF 556248; Facesoffungi Number: FoF 05838

Etymology – In reference to the fungus having verrucose ascospores.

Holotype – MFLU 18-2269

Saprobic on dead culms of unidentified herbaceous plant. Sexual morph: *Ascomata* 191–235 μm high, 186–215 μm wide, immersed, scattered, solitary, subglobose, dark brown to black, coriaceous, ostiolate. *Ostirole* central, with a flat, pore-like opening. *Peridium* up to 19–37 μm thick, comprising several layers of dark brown to black, thick-walled cells of *textura angularis*, outwardly dark pigmented, and become pale brown to hyaline towards inwardly. *Hamathecium* comprising numerous, 2–3.5 μm wide, hypha-like, cellular pseudoparaphyses, anastomosing between and among asci. *Asci* (69–) 84.5–93.5 \times 11–16.5 μm (\bar{x} = 85 \times 14.5 μm , n = 20), 8-spored, bitunicate, fissitunicate, clavate, pedicellate, with a furcate stalk at the base, rounded and truncate at the apex, with a developed ocular chamber. *Ascospores* 18.5–20.5(–23.5) \times 6–7.5 μm (\bar{x} = 20 \times 6.5 μm , n = 25), ellipsoidal to fusiform, hyaline to pale brown, verrucose, aseptate when young, and become 3-septate upon maturation, constricted at the median septum, surrounded by a 4.5–7 μm wide, mucilaginous sheath. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinated within 12 h in WA. Colonies on PDA reaching 66 mm diam. after 10 days at 25°C, medium sparse, irregular in shape, flattened, with edge fimbriate, velvety, colony from above semiluculent to pale brown, from below pale grayish-brown.

Material examined – CHINA, Guizhou Province, Guiyang City, Huaxi district, dead culms of herbaceous plant, 03 April 2016, J.F. Zhang, SD-2016-5 (MFLU 18-2269; GZAAS 18-0007); ex-type living culture (MFLUCC 19-0163, GZCC 19-0001).

Notes – The type species of *Dactylidina* (*D. dactylidis*) and *Phaeopoacea* (*P. festucae*) share close phylogenetic affinities with *Hydeopsis verrucispora* in both ML (MLBP 82%) and BI (BIPP 1.0) analyses. *Hydeopsis verrucispora* also shares some similar morphological characteristics to those species in *Dactylidina* and *Phaeopoacea* by having globose or subglobose, glabrous ascomata, cylindrical to clavate asci. However, it can be easily distinguished from members of these two genera as the new taxon is observed to have 3-septate, fusiform ascospores, while the

members of *Dactylidina* are characterized by muriform ascospores with 5–6-transverse septate and 1-longitudinal septum (Wanasinghe et al. 2018), and *Phaeopoacea* species are described with narrowly fusiform ascospores with 5-transverse septate and 1-longitudinal septum in the mid cells (Thambugala et al. 2017).

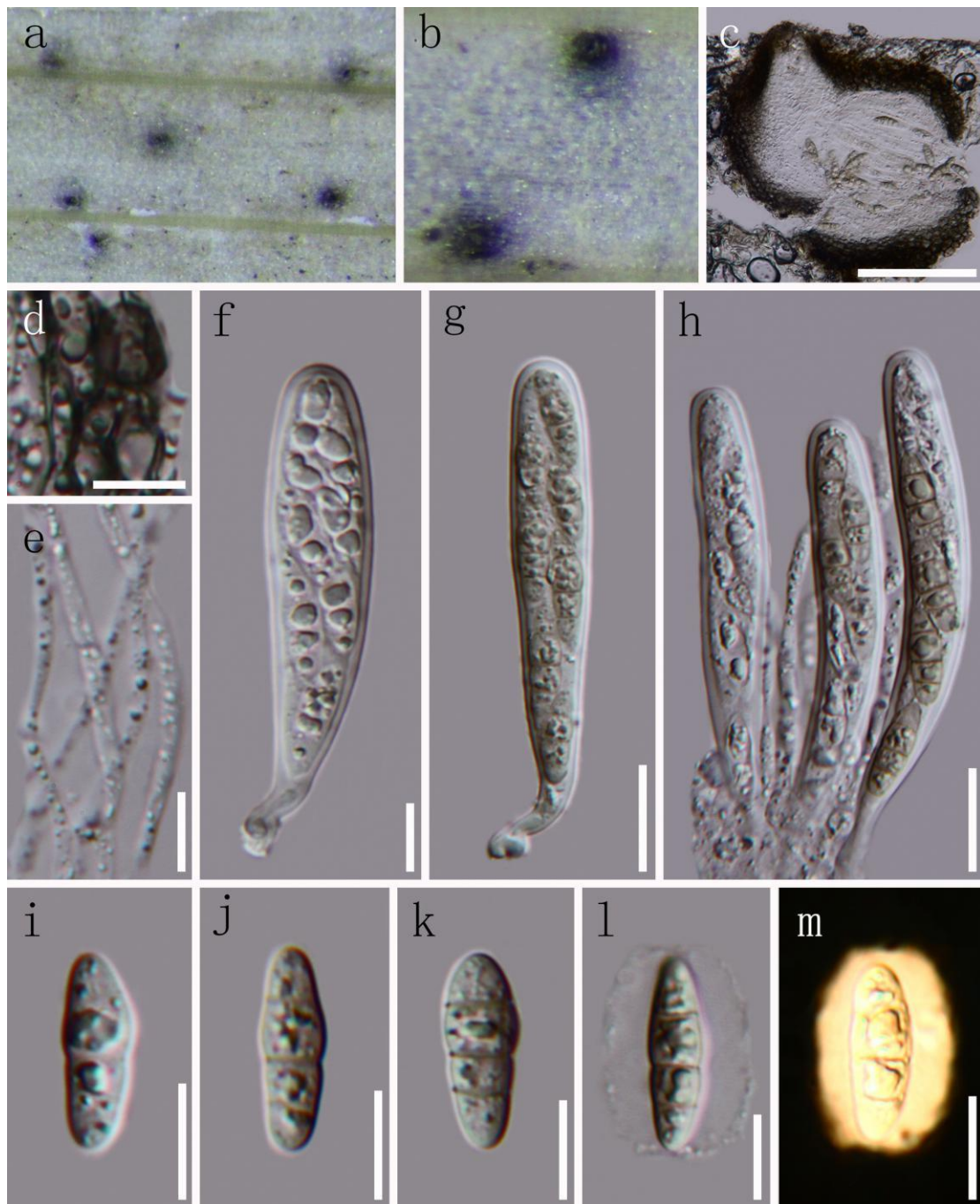


Fig. 3 – *Hydeopsis verrucispora* (MFLU 18-2269, holotype) a Appearance of ascostromata on host surface. b Ascomata with a central, pore-like opening ostiole. c Vertical sections through ascostroma. d Section of peridium. e Cellular pseudoparaphyses. f-h Asci. i-m Ascospores. Note: the m is stained with India ink to present the mucilaginous sheath. Scale bars: c = 100 µm, d-f, i-m = 10 µm, g, h = 20 µm

Hydeomyces Maharachch., H.A. Ariyaw., Wanas. and Al-Sadi., *Phytotaxa* 391(1): 33 (2019)

Hydeomyces was established by Maharachchikumbura et al. (2019) to accommodate the type species *Hydeomyces desertipleosporoides*. This genus is morphologically characterized by having

globose-depressed, brown to black ascomata, cylindrical, fissitunicate asci, ellipsoidal to subfusiform, muriform ascospores, and phoma-like asexual morphs. Until now, only the type species which was isolated from dead barks of *Juniperus excels* in Oman is known.

Hydeomyces pinicola J.F. Zhang, J.K. Liu & Z.Y. Liu, sp. nov.

Fig. 4

Index Fungorum number: IF 556249; Facesoffungi Number: FoF 05839

Etymology – Refers to the fungus being isolated from the dead wood of *Pinus* sp.

Holotype – MFLU 18-2289

Saprobic on dead wood of *Pinus* sp. Sexual morph: *Ascomata* 185–417 μm high, 210–341 μm wide, scattered, solitary to gregarious, immersed, semi-immersed to erumpent throughout the host tissue, subglobose, dark brown to black, coriaceous, ostiolate. *Ostirole* central, with a minute, broadly, flattened papilla, and a pore-like opening. *Peridium* up to 16–24 μm at the base, 42–59 μm at the sides, especially thick near the ostiole, comprising two strata, the outer stratum composed of black, thick-walled cells of *textura angularis*, fusing with the host tissues, inner stratum composed of several layers of brown to slightly pigmented, thin-walled cells of *textura angularis*. *Hamathecium* comprising numerous, 2–3.5 μm wide, hypha-like, septate pseudoparaphyses, branched at the apex, anastomosing between and among asci. *Asci* 103–111(–119) \times 9–11.5 μm (\bar{x} = 110 \times 10 μm , n = 20), 8-spored, bitunicate, fissitunicate, cylindrical, short pedicellate with a furcate pedicel, rounded at the apex, with a minute ocular chamber. *Ascospores* 15.5–19.5 \times 7–10 μm (\bar{x} = 17.5 \times 8 μm , n = 35), pyriform to subfusiform, muriform, asymmetrical, broader apically and narrower basally, round to truncate at the upper cell, uniseriate, partially overlapping, 3–5-transversely septate, with 0–3 vertical septa, initially hyaline, become greenish-brown at maturation, deeply constricted at the central septum, rounded at the ends, smooth-walled. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinated within 24 h in WA. Colonies on PDA reaching 39 mm diam. after 15 days at 25°C, medium sparse, regular in shape, flattened, colony from above yellowish green towards center and colourless at the margin, from below similar with above.

Material examined – CHINA, Guizhou Province, Guiyang City, Huaxi district, Guizhou academy of agricultural sciences, on the dead wood of *Pinus* sp., 25 September 2016, J.F. Zhang, GZ-06 (MFLU 18-2289, GZAAS 18-0009); ex-type living culture (GZCC 19-0003).

Notes – Phylogenetic analyses place the new species *H. pinicola* in the genus *Hydeomyces*, where it is close to the type species *H. desertipleosporoides* with high support (MLBP 100% and BIPP 1.00). Although *H. pinicola* is morphologically similar to *H. desertipleosporoides* in having globose, brown to black ascomata, cylindrical, fissitunicate asci, and subfusiform, muriform ascospores, it can be distinguished from the latter by its larger asci (103–119 μm vs. 70–85 μm) and ascospores (15.5–19.5 \times 7–10 μm vs. 10–15 \times 5–6.5 μm) (Maharachchikumbura et al. 2019). They are phylogenetically distinct species.

Leptospora Rabenh., *Hedwigia* 1: 116 (1857)

Leptospora is typified by *L. rubella* and characterized by large, flask-shaped ascomata with long cylindrical asci and thin, filiform, multi-septate ascospores, and members of this genus had also been reported to produce a reddish-purple pigmentation to stain the host tissue around the ostiole (Walker 1980, Hyde et al. 2016). So far, there are 16 species included in Index Fungorum (February, 2019), but sequence data are only available in GenBank for a few species.

Leptospora hydei J.F. Zhang, J.K. Liu & Z.Y. Liu, sp. nov.

Fig. 5

Index Fungorum number: IF 556264; Facesoffungi Number: FoF 05840

Etymology – In honour of Prof. Kevin D. Hyde for his contribution to ascomycete taxonomy.

Holotype: MFLU 19-0564

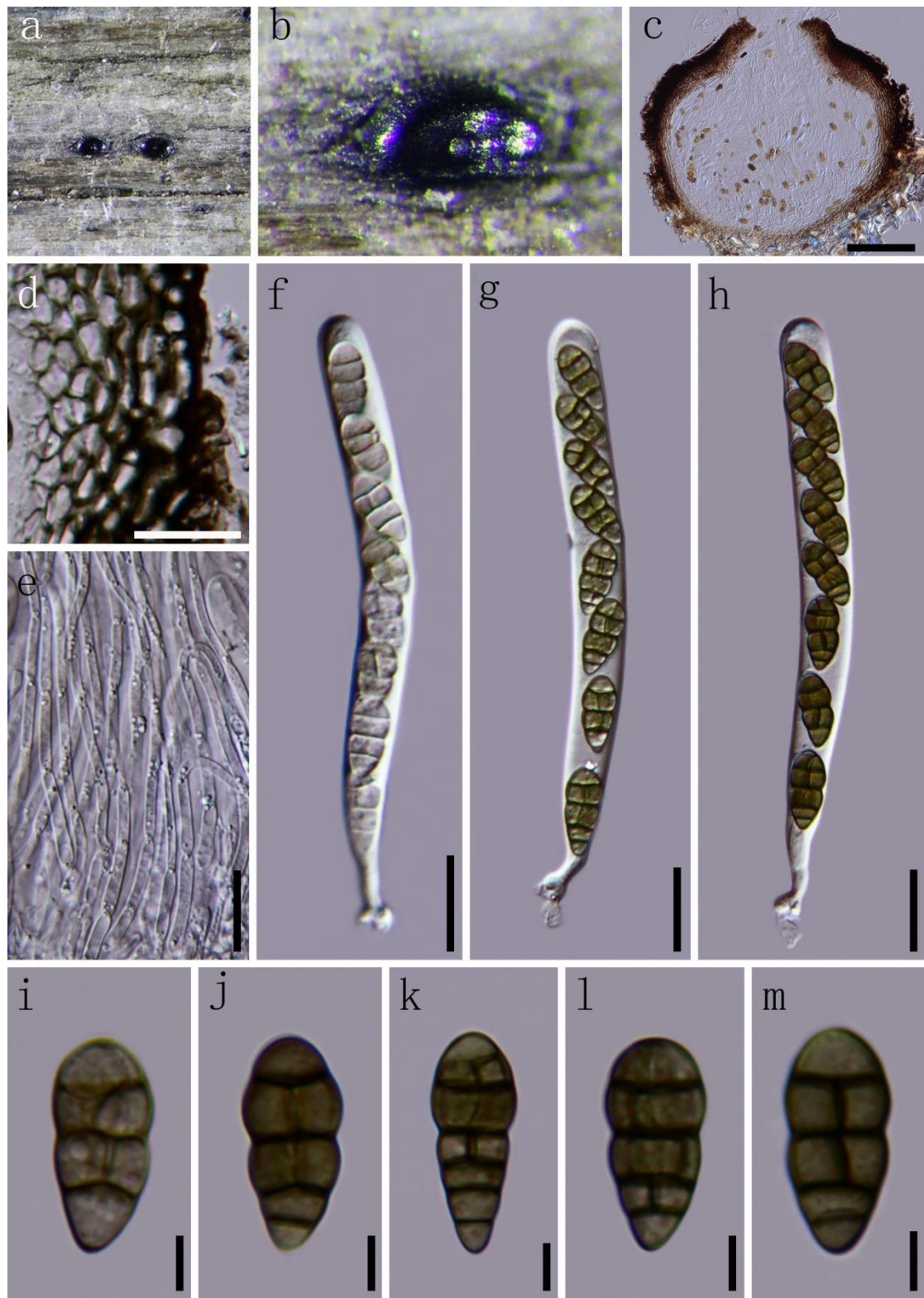


Fig. 4 – *Hydeomyces pinicola* (MFLU 18-2289, holotype) a Appearance of ascomata on host surface. b Ascomata with a central, pore-like opening ostiole. c Vertical sections through ascoma. d Section of peridium. e Pseudoparaphyses. f-h Asci. i-m Ascospores. Scale bars: c = 100 μ m, d-h = 20 μ m, i-m = 5 μ m

Saprobic on decaying culms of unidentified herbaceous plant. Sexual morph: *Ascomata* 196–267 μ m high, 168–187 μ m diam, immersed to erumpent through host tissue with papilla, solitary, scattered, depressed globose to subglobose, brown to dark brown, ostiolate. *Ostiole* central, papillate, cylindrical, truncate at the apex, dark brown to light brown, ostiole canal filled with

periphyses, and produces a reddish-purple discoloration in host tissue around ostiole. *Peridium* up to 11–24 μm wide, coriaceous, composed of several layers of thick-walled, brown to dark brown, pseudoparenchymatous cells, arrange in a *textura angularis* to *prismatica*, thin at the base, becoming thick and dark-pigmented towards ostiole. *Hamathecium* comprising numerous, 1.5–3 μm wide, filamentous, branched, trabeculate pseudoparaphyses anastomosing above and between the asci. *Asci* 147–165(–180) \times 13–17(–19.5) μm (\bar{x} = 161 \times 15.2 μm , n = 25), 8-spored, bitunicate, cylindrical to cylindrical-clavate, with very short swollen pedicel, apically rounded to truncate with a minute ocular chamber. *Ascospores* (104–)124–138(–146) \times 4–4.8 μm (\bar{x} = 133.5 \times 4.4 μm , n = 30), fasciculate, tri- to multiseriate, scolecosporous, filiform, tapering towards the ends, minute guttules in each cell, hyaline when young, and become pale brown at maturation, normally 11-septate, not constricted at the septa. Asexual morph Undetermined.

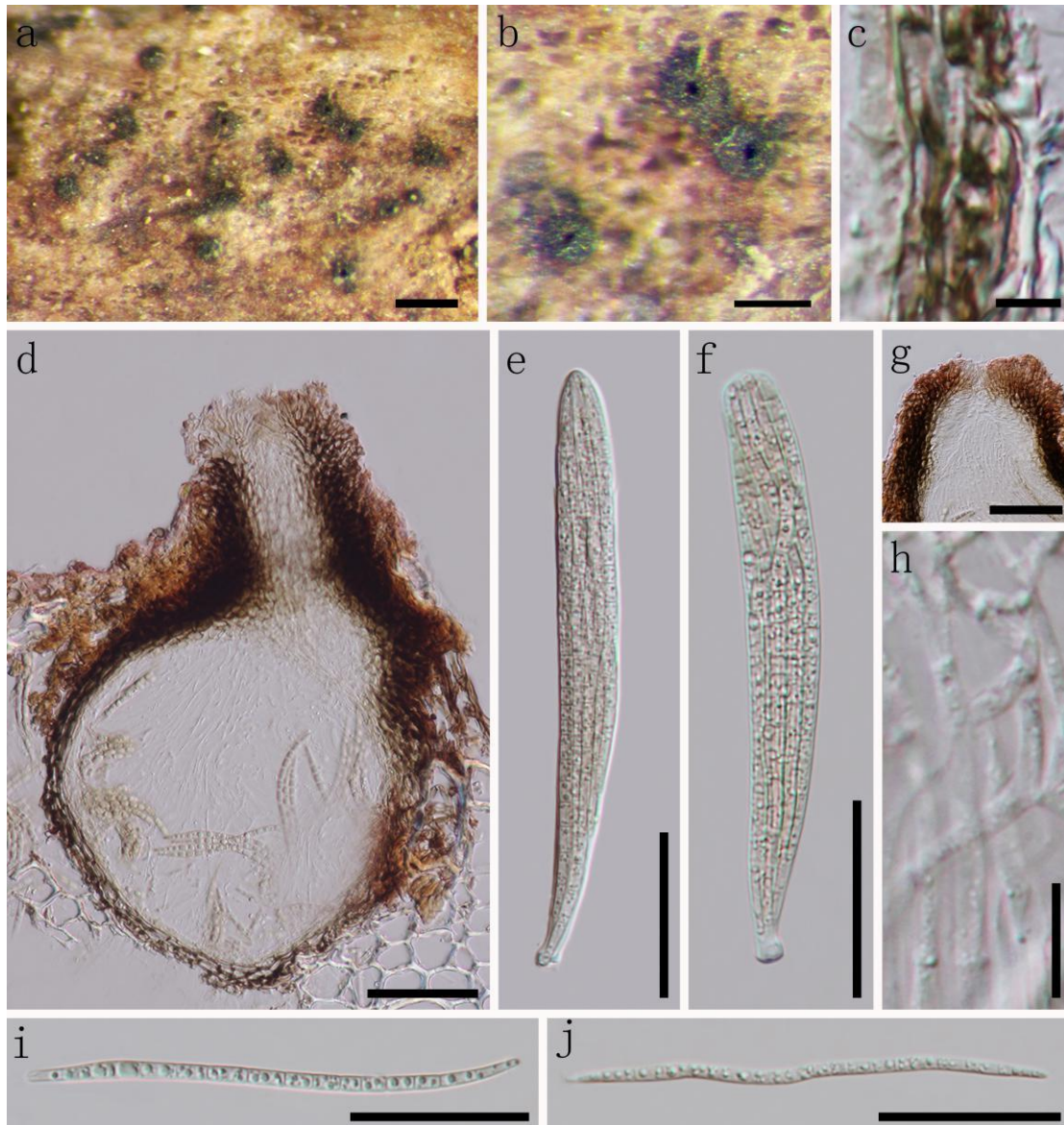


Fig. 5 – *Leptospora hydei* (MFLU 19-0564, holotype) a Appearance of ascostromata on host surface. b The ostiole on face view. c Peridium. d Vertical section through ascostromata. e, f Asci. g Ostiolar canal filled with numerous periphyses. h Pseudoparaphyses. i, j Ascospores. Scale bars: a = 200 μm , b = 100 μm , c = 5 μm , d-g, i-j = 50 μm , h = 10 μm

Culture characteristics – Ascospores germinated within 24h in WA. Colonies on PDA reaching 35 mm diam. after 4 weeks at 25°C, medium dense, irregular in shape, raised at center,

with edge fimbriate, colony from above greyish-green, from below olive in center, and become pale yellowish towards margin.

Material examined – CHINA, Guizhou Province, Guiyang City, Huaxi district, on decaying branch, 20 September 2016, J.F. Zhang, SD-02 (MFLU 19-0564, GZAAS 18-0010); ex-type living culture (GZCC 19-0004).

Notes – *Leptospora hydei* shares key morphological characters with existing *Leptospora* species in staining the host tissue with reddish-purple pigmentation around ostiole canal, and filiform, multi-septate ascospores. The phylogenetic result also assigns *L. hydei* to the genus *Leptospora*, as it clusters together with *L. thailandica* with strong statistical support (MLBP 100%, BIPP 1.00). However, it differs from *L. thailandica* in having larger asci (147–180 × 13–19.5 μm vs. 68–114 × 7–13 μm) and ascospores (104–146 × 4–4.8 μm vs. 63–89 × 1.8–3.8 μm), and with less number of septum in ascospores (11-septate vs. 14–22-septate) (Hyde et al. 2016).

Discussion

Phaeosphaeriaceae taxa are known to be polyphyletic with large overlap in morphological characters among taxa (Phookamsak et al. 2015). There is still confusion between taxa with similar morphological characteristics, and it is difficult to differentiate them based only on observation of phenotypic traits, and therefore DNA sequence data has become more important in their taxonomy (Phookamsak et al. 2017). In the present study, the new genus *Pseudoophiosphaerella* is quite similar to *Ophiosphaerella* in having cylindrical asci and filiform ascospores, but it is phylogenetically distant from *Ophiosphaerella* in both ML and BI analyses, and can be recognized as a distinct genus. Recent molecular studies have indicated that multi-gene analyses are essential to clarify the phylogenetic affinities among the taxa within Phaeosphaeriaceae (de Gruyter et al. 2010, Hyde et al. 2013, Phookamsak et al. 2014, 2017), and we also carried out multi-loci analyses based on LSU, ITS, SSU and TEF1- α sequence data in this study and recovered phylogenies similar to previous studies.

The taxonomic placement of most genera in Phaeosphaeriaceae has been rather well resolved in recent studies (Phookamsak et al. 2014, 2017, Ahmed et al. 2017, Tennakoon et al. 2016, Karunarathna et al. 2017, Maharachchikumbura et al. 2019). There are, however, still, several genera viz. *Amarenographium*, *Amarenomyces*, *Chaetosphaeronema*, *Melnikia*, *Scolicosporium*, *Septoriella* and *Lorotospora* that are still unresolved lineages and this further complicates intergeneric relationships. There is also insufficient taxa having DNA sequence data for all commonly used genes in phylogeny and this could result in wrong phylogenetic inferences associated with limited taxon sampling (Quaedvlieg et al. 2013, Ariyawansa et al. 2015, Abd-Elsalam et al. 2016, Hyde et al. 2016, 2017, Wijayawardene et al. 2016, Phookamsak et al. 2017). Therefore, more taxon sampling of these above mentioned genera and the new genera (*Hydeopsis* and *Pseudoophiosphaerella*) are needed to gain better understanding of phylogenetic affinities within Phaeosphaeriaceae. In the present study, it was found that, both *Hydeopsis* and *Pseudoophiosphaerella* were phylogenetically distinct from other existing genera by high support. Besides, generic delineation between the genera *Wonjnowicia* and *Wonjnowiciella* is still unclear despite having phylogenetic data (Wanasinghe et al. 2018). Future taxonomic studies are needed to clarify their taxonomy.

In this study, *Hydeomyces pinicola* was introduced based on morphological and molecular data. It was isolated from dead wood of *Pinus* sp. in Guizhou Province of China (subtropical region), while the type species of this genus *H. desertipleosporoides* was reported from dead barks of *Juniperus excels* in Oman (tropical region) (Maharachchikumbura et al. 2019). It can be inferred herein that *Hydeomyces* is neither host specific nor geographically restricted. However, there are only two accepted species until now and more samples of this genus should be collected and analyzed to confirm the above statement. In addition, *Leptospora hydei* is also established in this study. So far, 16 species have been included in this genus (Index Fungorum, February, 2019), and these species inhabit a wide range of hosts and distributed around the world. However, there are no

records available from China, and this is the first report, and also establishes a new *Leptospora* species from China.

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