



Morphological and phylogenetic studies of *Pleopunctum* gen. nov. (Phaeoseptaceae, Pleosporales) from China

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Abstract

A new hyphomycete genus, *Pleopunctum*, is introduced to accommodate two new species, *P. ellipsoideum* sp. nov. (type species) and *P. pseudoellipsoideum* sp. nov., collected from decaying wood in Guizhou Province, China. The genus is characterized by macronematous, mononematous conidiophores, monoblastic conidiogenous cells and muriform, oval to ellipsoidal conidia often with a hyaline, elliptical to globose basal cell. Phylogenetic analyses of combined LSU, SSU, ITS and *TEF1α* sequence data of 55 taxa were carried out to infer their phylogenetic relationships. The new taxa formed a well-supported subclade in the family Phaeoseptaceae and basal to *Lignosphaeria* and *Thyridaria macrostomoides*. Divergence time estimation based on LSU, SSU and *TEF1α* sequence data was performed to provide additional evidence for the establishment of Phaeoseptaceae, which diverged approximately 88 MYA.

Key words – 3 new taxa – Asexual morph – Divergence times – Dothideomycetes – Multi-genes – Taxonomy

Introduction

Hyphomycetes are the asexually reproducing part of the life cycle of ascomycetes and basidiomycetes (Seifert et al. 2011). They are a diverse group belonging to different classes, families and genera as shown by modern molecular phylogenetic analyses (Jeewon et al. 2003, Hyde et al. 2013, Maharachchikumbura et al. 2016, Dai et al. 2017, Doilom et al. 2017, Wijayawardene et al. 2017, Lu et al. 2018). Colonies of dematiaceous hyphomycetes on natural substrates are normally effuse, conspicuous, velvety and brown or black (Ellis 1971, 1976, Bhat 2010, Seifert et al. 2011, Luo et al. 2017). Punctiform colonies are common in dematiaceous hyphomycetes. Although punctiform colonies on natural substrates are similar when observed under the stereoscope, the morphology of conidia is quite diverse, and their phylogenetic affinities are rather intriguing. Historically, it has been uncommon to link hyphomycetes with their sexual morphs, since many asexual morphs have permanently lost the potential to produce a sexual morph (Seifert et al. 2011).

However, with the advent of DNA based methods, taxonomic placement of many hyphomycetes with or without sexual morphs has been possible (Tsui et al. 2006, Shenoy et al. 2007, Wang et al. 2007). This has shown that many morphologically similar hyphomycetous groups are polyphyletic (Shenoy et al. 2006, Pinnoi et al. 2007, Klaubauf et al. 2014, Lu et al. 2018).

The cheirosporous genus *Dictyosporium* Corda and their relatives are placed in Dictyosporiaceae (Boonmee et al. 2016, Yang et al. 2018). Both *Hermatomyces* Speg. and *Lentimurispora* N.G. Liu, Bhat & K.D. Hyde can produce cushion-like, lenticular conidia with dark brown central cells and subhyaline to pale brown peripheral cells. However, *Hermatomyces* belongs to Hermatomycetaceae (Hashimoto et al. 2017), while *Lentimurispora* is accommodated in Lentimurisporaceae (Liu et al. 2018). All of the families, Dictyosporiaceae, Hermatomycetaceae and Lentimurisporaceae belong to the Dothideomycetes. Yang et al. (2016) established the order Fuscosporellales in the subclass Hypocreomycetidae (Sordariomycetes) based on fungi isolated from freshwater. Two genera, *Fuscosporella* Jing Yang, Bhat & K.D. Hyde and *Parafuscosporella* Jing Yang, Bhat & K.D. Hyde form black, punctiform colonies with obpyriform conidia on submerged twigs. *Canalisporium* Nawawi & Kuthub. also resides in the subclass Hypocreomycetidae (Sordariomycetes). Unlike conidia of *Fuscosporella* and *Parafuscosporella* having a basal septum, *Canalisporium* produces muriform conidia (Goh et al. 1998, Sri-Indrasutthi et al. 2010). Besides two largest classes of Ascomycota (Dothideomycetes and Sordariomycetes), punctiform colonies have also been reported from other classes. *Aculeata* W. Dong, H. Zhang & K.D. Hyde was recently introduced by Dong et al. (2018) in Herpotrichiellaceae (Eurotiomycetes). Conidia of *Aculeata* are olive to brown, vesiculate, cruciately septate, bearing densely packed, subulate, obtuse, brown to black spines (Dong et al. 2018). There are also some hyphomycetous genera treated as *incertae sedis* due to lack of molecular data. For example, *Vanakripa* Bhat, W.B. Kendr. & Nag Raj was introduced by Bhat & Kendrick (1993) with the type species *V. gigaspora* Bhat, Kendrick & Nag Raj. Since then, eight species were described in *Vanakripa* but none of them have DNA sequence data in public repositories (Tsui et al. 2003, Hu et al. 2010, Leão-Ferreira et al. 2013). This genus is now assigned as ascomycetes *incertae sedis*. The conidial morphology in *Vanakripa* is similar to those of *Fuscosporella* and *Parafuscosporella*, however, their conidiogenous cells are distinguishable.

While investigating dematiaceous hyphomycetes in China and Thailand, two interesting hyphomycetes with punctiform colonies were collected from decaying wood in China. Phylogenetic analyses based on the combined LSU, SSU, ITS and *TEF1 α* sequence data indicated that these two taxa represent a new genus in Phaeoseptaceae (Pleosporales). Therefore, *Pleopunctum* gen. nov., is introduced to accommodate *P. ellipsoideum* and *P. pseudoellipsoideum* spp. nov. with detailed morphological studies and supported by multi-gene phylogenetic analyses. In addition, we also estimated the divergence time to further support the establishment of Phaeoseptaceae.

Materials & Methods

Collections and examination of specimens

Fresh samples of decaying wood were collected from Guizhou Province, China. The samples were processed and examined following the method described in Taylor & Hyde (2003). The samples were incubated in plastic boxes with sterile and moist tissue at 25–30 °C for 3 days, and then examined using a Motic SMZ 168 Series dissecting microscope. Fruiting bodies of the new taxa were mounted in a drop of water for microscopic studies and photomicrography. The species were examined with a Nikon ECLIPSE 80i compound microscope fitted with a Canon 600D digital camera. Measurements were performed using the Tarosoft (R) Image Frame Work software (Liu et al. 2010) and photo-plates were prepared using Adobe Photoshop CS3 software (Adobe Systems, USA).

Single conidium isolations were carried out following the method described in Chomnunti et al. (2014). Germinated conidia were individually transferred to potato dextrose agar (PDA) media plates and incubated at 25 °C. Dried specimens were deposited in the herbarium of Mae Fah Luang

University (MFLU), Chiang Rai, Thailand. Pure cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC). Facesoffungi (FoF) numbers were acquired as in Jayasiri et al. (2015) and Index Fungorum numbers as in Index Fungorum (2019).

DNA extraction, PCR amplification and sequencing

A sterile scalpel was used to scrape fresh mycelia from pure cultures growing on PDA medium for one month at 25 °C. Genomic DNA was extracted using DNA Extraction Kit (Sangon Biotech, Shanghai, P.R. China) following the manufacture's protocol. Four different gene regions, the nuclear large subunit rDNA (28S, LSU), the nuclear small subunit rDNA (18S, SSU), internal transcribed spacer (ITS) and the translation elongation factor 1-alpha gene (*TEF1 α*) were selected for study. Part of LSU locus was amplified with the primers LR0R and LR5 (Vilgalys & Hester 1990), part of SSU with primers NS1 and NS4 (White et al. 1990), part of ITS with primers ITS5 and ITS4 (White et al. 1990), and part of *TEF1 α* with primers 983F and 2218R (Rehner & Buckley 2005). Polymerase chain reaction (PCR) was carried out in 25 μ l reaction volume containing 12.5 μ l Taq PCR Master Mix (TIANGEN Co., P.R. China), 9.5 μ l ddH₂O, 1 μ l forward primer, 1 μ l reverse primer and 1 μ l DNA template. PCR conditions for LSU, SSU, ITS and *TEF1 α* were as follows: 3 min at 94 °C (initial denaturation), followed by 40 cycles of 45 s at 94 °C (denaturation), 50 s at 56 °C (annealing), 1 min at 72 °C (extension), with a final extension of 10 min at 72 °C. Purified PCR products were sequenced by Sangon Biotech (Shanghai, P.R. China).

Phylogenetic analyses

Fifty-five strains representing Amorosiaceae, Halotthiaceae, Lentimurisporaceae, Lophiostomaceae, Phaeoseptaceae, Sporormiaceae and Teichosporaceae, along with the outgroup *Lindgomyces ingoldianus* KH100 (Table 1) in the order Pleosporales used for this study were obtained from GenBank based on blast search results and other published studies (Mantle et al. 2006, Kruys & Wedin 2009, Mugambi & Huhndorf 2009, Zhang et al. 2013, Thambugala et al. 2015, Phukhamsakda et al. 2016a, Hyde et al. 2018, Liu et al. 2017, 2018). The multiple alignments were automatically performed by online MAFFT version 7 (Katoh & Standley 2013) and BioEdit (Hall 1999). Four genes were combined using BioEdit. Alignments were checked visually and optimized manually using AliView (Larsson 2014) where necessary. The final alignment was deposited in TreeBASE (submission ID: 24638). Sequences derived in this study were deposited in GenBank (Table 1).

Maximum likelihood analysis was performed using RAxML (Stamatakis 2006). The tree search included 1,000 non-parametric bootstrap replicates and the best scoring tree was selected from suboptimal trees under the GTRGAMMA substitution model. The resulting replicates were plotted on to the best scoring tree obtained previously.

Maximum parsimony analysis was performed with the heuristic search in PAUP v. 4.0b10 (Swofford 2002). Gaps in the alignment were treated as missing characters and all characters were unordered. Maxtrees were unlimited. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1,000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993).

Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist et al. 2012). The program MrModeltest 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model for each data partition. GTR+I+G substitution model with gamma rates and dirichlet base frequencies was decided for LSU, SSU, ITS and *TEF1 α* sequences. The Markov Chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP) (Rannala & Yang 1996). Bayesian analyses of four simultaneous Markov chains were run for 5,000,000 generations with trees sampled every 1,000th generations. The first 20% of trees, representing the burn-in phase of the analyses, were discarded and the remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree.

Fossil calibration, divergence time and evolutionary rate estimations

The fossil calibrations used in the analyses followed the methodology described in Phukhamsakda et al. (2016b). The related sequences in the class Dothideomycetes are listed in Table 2. Reliable fossils and one secondary calibration were selected for the divergence times estimations based on the phylogenetic analyses. The fossil *Metacapnodium succinum* (Metacapnodiaceae) was used to calibrate the minimum age of Capnariales (normal distribution, mean = 100, SD = 150, providing 95% credibility interval of 346 MYA) (Rikkinen et al. 2003, Hongsanan et al. 2016, Pérez-Ortega et al. 2016, Phukhamsakda et al. 2016b, Samarakoon et al. 2019). The fossil *Margaretbarromyces dictyosporus* was used to calibrate the crown age of *Aigialus* (Aigialaceae) (gamma distribution, offset = 35, shape = 1.0, scale = 25, providing 95% credibility interval of 110 MYA) (Mindell et al. 2007, Phukhamsakda et al. 2016b). The split between Arthoniomycetes and Dothideomycetes was calibrated using the results from Phukhamsakda et al. (2016b) as the secondary calibration (normal distribution, mean = 300, SD = 50, providing 95% credibility interval of 382 MYA).

Evolutionary estimation was performed by BEAST 1.8.0 (Drummond et al. 2012). Aligned sequence data were partitioned separately for each LSU, SSU, *TEF1 α* data set, and were loaded to prepare an XML file constructed with BEAUti v1.8.0. Clock and substitution models were set to be unlinked (independently estimated for each gene partition), while the tree prior parameters were set to be linked across partitions (concatenation). We applied a lognormal relaxed clock (uncorrelated). The tree prior was shared by all tree models; this consisted of a birth/death in complete sampling tree prior and was used to model the speciation of nodes in the topology with uniform prior on probability of splits and extinctions. The analysis was performed for 100 million generations in BEAST v1.8.0, and sampling parameters every 1,000 generations. Tracer v1.6 (Rambaut et al. 2014) was used to check the effective sample sizes (ESS) (ESS > 200). The first of 20% trees were discarded as a burn-in phase. The remaining trees were combined in LogCombiner v1.8.0. A maximum clade credibility tree was generated by Tree Annotator v1.8.0.

Trees were visualized with FigTree v1.4.0 (Rambaut 2006) and the layout was edited using Adobe Illustrator CS6 software (Adobe Systems, USA).

Table 1 Taxa used in in this study. The new taxa are indicated in bold.

Species	Strain numbers	LSU	SSU	ITS	<i>TEF1α</i>
<i>Alpestrisphaeria terricola</i>	SC-12	JX985750	JX985749	JN662930	
<i>Amorosia littoralis</i>	NN 6654	AM292055	AM292056	AM292047	
<i>Angustimassarina populi</i>	MFLUCC 13-0034	KP888642	KP899128	KP899137	KR075164
<i>Bahusandhika indica</i>	GUFCC 18001	KF460274		KF460273	
<i>Berkleasium micronesicum</i>	BCC 8141	DQ280272	DQ280268	DQ280262	
<i>Berkleasium nigroapicale</i>	BCC 8220	DQ280273	DQ280269	DQ280261	
<i>Biappendiculispora japonica</i>	MAFF 239452	AB619005	AB618686	LC001728	LC001744
<i>Brunneoclavispora bambusae</i>	MFLUCC 11-0177	KT426562		MG520957	
<i>Capulatispora sagittiformis</i>	JCM 15100	AB369267	AB618693	AB369268	LC001756
<i>Coelodictyosporium pseudodictyosporium</i>	MFLUCC 13-0451	KR025862		KR025858	
<i>Decaisnella formosa</i>	BCC 25617	GQ925847	GQ925834		GU479850
<i>Decaisnella formosa</i>	BCC 25616	GQ925846	GQ925833		GU479851
<i>Exosporium stylobatum</i>	CBS 160.30	JQ044447		JQ044428	
<i>Forliomyces uniseptata</i>	MFLUCC 15-0765	KU721762	KU721767	KU721772	

Table 1 Continued.

Species	Strain numbers	LSU	SSU	ITS	TEF1α
<i>Guttulispora crataegi</i>	MFLUCC 13–0442	KP888639	KP899125	KP899134	KR075161
<i>Halotthia posidoniae</i>	BBH 22481	GU479786	GU479752		
<i>Lentimurispota urniformis</i>	MFLUCC 18–0497	MH179144	MH179160		MH188055
<i>Lignosphaeria fusispora</i>	MFLUCC 11–0377	KP888646		KP899140	
<i>Lignosphaeria thailandica</i>	MFLUCC 11–0376	KP888645		KP899139	
<i>Lindgomyces ingoldianus</i>	KH 100	AB521737	AB521720		
<i>Lophiohelichrysum helichrysi</i>	MFLUCC 15–0701	KT333436	KT333437	KT333435	KT427535
<i>Lophiopoacea paramacrostoma</i>	MFLUCC 11–0463	KP888636	KP899122		
<i>Lophiostoma macrostomum</i>	JCM 13544	AB619010	AB618691	JN942961	LC001751
<i>Massarina corticola</i>	CBS 154.93	FJ795448	FJ795491		
<i>Mauritiana rhizophorae</i>	BCC 28866	GU371824	GU371832		GU371817
<i>Neolophiostoma pigmentatum</i>	MFLUCC 10–0129	KT324588	KT324589	KT324587	KT324590
<i>Neotrematosphaeria biappendiculata</i>	KTC 1124	GU205227	GU205256		
<i>Paucispota quadrispora</i>	KH 448	LC001722	LC001720	LC001733	LC001754
<i>Phaeoseptum aquaticum</i>	CBS 123113	JN644072			
<i>Phaeoseptum terricola</i>	MFLUCC 10–0102	MH105779	MH105780	MH105778	MH105781
<i>Platystomum compressum</i>	MFLUCC 13–0343	KP888643	KP899129		KR075165
<i>Pleopunctum ellipsoideum</i>	MFLUCC 19–0390	MK804517	MK804514	MK804512	MK828510
<i>Pleopunctum pseudoellipsoideum</i>	MFLUCC 19–0391	MK804518		MK804513	MK828511
<i>Preussia funiculata</i>	CBS 659.74	GU301864	GU296187		GU349032
<i>Pseudolophiostoma vitigenum</i>	JCM 13534	AB619015	AB618697	LC001735	LC001761
<i>Pseudoplatystomum scabridisporum</i>	BCC 22835	GQ925844	GQ925831		GU479857
<i>Sigarispota ravnica</i>	MFLUCC 14–0005	KP698414	KP698415	KP698413	
<i>Sparticola junci</i>	MFLUCC 15–0030	KU721765	KU721770	KU721775	KU727898
<i>Sporormia fimetaria</i>	UPS:Dissing Gr.81.194	GQ203729		GQ203769	
<i>Sporormiella minima</i>	CBS 52450	DQ468046		DQ468026	
<i>Sulcosporium thailandicum</i>	MFLUCC 12–0004	KT426563	KT426564	MG520958	
<i>Teichospora aurantiacinotata</i>	GKM 1238	GU385173			
<i>Teichospora austroafricana</i>	CBS 122674	EU552116		EU552116	
<i>Teichospora cruciformis</i>	SMH 5151	GU385211			
<i>Teichospora kenyensis</i>	GKML 100Na	GU385189			GU327766
<i>Teichospora parva</i>	GKM 169N	GU385165			GU327768
<i>Teichospora striata</i>	JK 5678I	GU301813	GU296149		GU479852
<i>Teichospora tennesseensis</i>	ANM 911	GU385207			GU327769
<i>Teichospora thailandica</i>	MFLUCC 13–0284	KP888647	KP899131	KP899141	KR075167

Table 1 Continued.

Species	Strain numbers	LSU	SSU	ITS	TEF1 α
<i>Teichospora uniseriata</i>	ANM 909	GU385206			
<i>Thyridaria macrostomoides</i>	GKM 1033	GU385190			GU327776
<i>Thyridaria macrostomoides</i>	GKM 1159	GU385185			GU327778
<i>Thyridaria macrostomoides</i>	GKM 224N	GU385191			GU327777
<i>Vaginatispora aquatica</i>	MFLUCC 11-0083	KJ591576	KJ591575	KJ591577	
<i>Westerdykella ornata</i>	CBS 379.55	GU301880	GU296208	NR103587	GU349021

Table 2 Taxa used for divergence time estimate in this study.

Species	Strain	LSU	SSU	TEF1 α
<i>Aigialus grandis</i>	BCC 18419	GU479774	GU479738	GU479838
<i>Aigialus mangrovei</i>	BCC 33563	GU479776	GU479741	GU479840
<i>Aigialus parvus</i>	BCC 18403	GU479778	GU479744	GU479842
<i>Aigialus rhizophorae</i>	BCC 33572	GU479780	GU479745	GU479844
<i>Aliquandostipite khaoyaiensis</i>	CBS 118232	GU301796		GU349048
<i>Amniculicola immersa</i>	CBS 123083	FJ795498	GU456295	GU456273
<i>Amorosia littoralis</i>	NN 6654	AM292055	AM292056	
<i>Angustimassarina populi</i>	MFLUCC 13-0034	KP888642	KP899128	KR075164
<i>Anteaglonium abbreviatum</i>	GKM 219N	GQ221881		GQ221916
<i>Anteaglonium parvulum</i>	GKM 1029	GQ221878		GQ221915
<i>Arthonia dispersa</i>	UPSC 2583	AY571381	AY571379	
<i>Ascochyta pisi</i>	AFTOL-ID 1583	DQ678070	DQ678018	DQ677913
<i>Ascocratera manglicola</i>	BCC 09270	GU479782	GU479747	GU479846
<i>Astrosphaeriella fusispora</i>	MFLUCC 10-0555	KT955462	KT955443	KT955425
<i>Bahusandhika indica</i>	GUFCC 18001	KF460274		
<i>Berkleasmiium micronesicum</i>	BCC 8141	DQ280272	DQ280268	
<i>Berkleasmiium nigroapicale</i>	BCC 8220	DQ280273	DQ280269	
<i>Bimuria novae-zelandiae</i>	CBS 107.79	NG_058623	NG_061017	
<i>Boeremia exigua</i>	CBS 431.74	JX681074	EU754084	KY484687
<i>Botryosphaeria dothidea</i>	CBS 115476	NG_027577	DQ677998	DQ767637
<i>Capnodium salicinum</i>	CBS 131.34	DQ678050	DQ677997	
<i>Caryospora minima</i>		EU196550	EU196551	
<i>Cladosporium cladosporioides</i>	CBS 170.54	AY213694	DQ678004	
<i>Coelodictyosporium pseudodictyosporium</i>	MFLUCC 13-0451	KR025862		
<i>Corynespora cassiicola</i>	CBS 100822	GU301808	GU296144	GU349052
<i>Corynespora smithii</i>	CABI 5649b	GU323201		GU349018
<i>Cyclothyriella rubronotata</i>	CBS 141486	KX650544	KX650507	KX650519
<i>Decaisnella formosa</i>	BCC 25617	GQ925847	GQ925834	GU479850
<i>Decaisnella formosa</i>	BCC 25616	GQ925846	GQ925833	GU479851
<i>Delitschia chaetomioides</i>	SMH 3253.2	GU390656		GU327753
<i>Delitschia winteri</i>	CBS 225.62	DQ678077	DQ678026	DQ677922
<i>Dendrographa leucophaea f. minor</i>		AF279382	AF279381	
<i>Dissoconium aciculare</i>	CBS 204.89	GU214419	GU214523	
<i>Exosporium stylobatum</i>	CBS 160.30	JQ044447		
<i>Gloniopsis calami</i>	MFLUCC 15-0739	KX646363	KX669034	KX671965

Table 2 Continued.

Species	Strain	LSU	SSU	TEF1a
<i>Gloniopsis praelonga</i>	CBS 112415	FJ161173	FJ161134	FJ161090
<i>Guttulispora crataegi</i>	MFLUCC 13-0442	KP888639	KP899125	KR075161
<i>Halothia posidoniae</i>	BBH 22481	GU479786	GU479752	
<i>Herpotrichia diffusa</i>	CBS 250.62	DQ678071	DQ678019	DQ677915
<i>Hypsostroma caimitalense</i>	GKM 1165	GU385180		
<i>Hypsostroma saxicola</i>	SMH 5005	GU385181		
<i>Hysterium angustatum</i>	CBS 236.34	FJ161180	GU397359	FJ161096
<i>Jahnula seychellensis</i>	SS 2113	EF175665	EF175644	
<i>Katumotoa bambusicola</i>	KT 1517a	AB524595	AB524454	AB539108
<i>Lentimurispora urniformis</i>	MFLUCC 18-0497	Mh179144	MH179160	MH188055
<i>Leptosphaeria doliolum</i>	CBS 505.75	GQ387576	GQ387515	GU349069
<i>Leptoxyphium cacuminum</i>	MFLUCC 10-0049	JN832602	JN832587	
<i>Ligninsphaeria jonesii</i>	MFLUCC 15-0641	KU221037		
<i>Ligninsphaeria jonesii</i>	GZCC 15-0080	KU221038		
<i>Lignosphaeria fusispora</i>	MFLUCC 11-0377	KP888646		
<i>Lignosphaeria thailandica</i>	MFLUCC 11-0376	KP888645		
<i>Lindgomyces ingoldianus</i>	ATCC 200398	AB521736	AB521719	
<i>Lindgomyces rotundatus</i>	KT 1096	AB521740	AB521723	
<i>Lophiostoma macrostomum</i>	KT 508	AB619010	AB618691	LC001751
<i>Lophiotrema lignicola</i>	CBS 122364	GU301836	GU296166	GU349072
<i>Lophiotrema nucula</i>	CBS 627.86	GU301837	GU296167	GU349073
<i>Massaria anomia</i>	CBS 591.78	GU301839	GU296169	
<i>Massaria inquinans</i>	M 19	HQ599402	HQ599444	HQ599342
<i>Massarina corticola</i>	CBS 154.93	FJ795448	FJ795491	
<i>Massarina eburnea</i>	CBS 473.64	MH877786	GU296170	GU349040
<i>Massariosphaeria phaeospora</i>	CBS 611.86	GU301843	GU296173	
<i>Mauritiana rhizophorae</i>	BCC 28866	GU371824	GU371832	GU371817
<i>Melanomma pulvis-pyrius</i>	CBS 124080	GU456323	GU456302	GU456265
<i>Murilentithecium clematidis</i>	MFLUCC 14-0562	KM408759	NG_061185	KM454445
<i>Murispora rubicunda</i>	IFRD 2017	FJ795507	GU456308	GU456289
<i>Neoastrosphaeriella krabiensis</i>	MFLUCC 11-0025	JN846729	JN846739	
<i>Neoroussoella bambusae</i>	MFLUCC 11-0124	KJ474839		KJ474848
<i>Neotrematosphaeria biappendiculata</i>	KTC 1124	GU205227	GU205256	
<i>Nigrograna fuscidula</i>	CBS 141476	KX650547	KX650509	KX650522
<i>Nigrograna mackinnonii</i>	CBS 110022	GQ387614	GQ387553	KF407985
<i>Occultibambusa bambusae</i>	MFLUCC 13-0855	KU863112	KU872116	KU940193
<i>Occultibambusa chiangraiensis</i>	MFLUCC 16-0380	KX655546	KX655551	KX655561
<i>Ohleria modesta</i>	MGC	KX650562		KX650533
<i>Ohleria modesta</i>	OM	KX650563	KX650513	KX650534
<i>Paradictyoarthrinium diffractum</i>	MFLUCC 13-0466	KP744498	KP753960	
<i>Paradictyoarthrinium hydei</i>	MFLUCC 17-2512	MG747497	MH454349	
<i>Phaeoseptum aquaticum</i>	CBS 123113	JN644072		
<i>Phaeoseptum terricola</i>	MFLUCC 10-0102	MH105779	MH105780	MH105781
<i>Phyllosticta capitalensis</i>	CBS 226.77	KF206289	KF766300	
<i>Piedraia hortae</i>	CBS 480.64	GU214466	AY016349	
<i>Pleomassaria siparia</i>	CBS 279.74	DQ678078	DQ678027	DQ677923
<i>Pleopunctum ellipsoideum</i>	MFLUCC 19-0390	MK804517	MK804514	MK828510

Table 2 Continued.

Species	Strain	LSU	SSU	<i>TEF1α</i>
<i>Pleopunctum pseudoellipsoideum</i>	MFLUCC 19–0391	MK804518		MK828511
<i>Pleospora herbarum</i>	CBS 191.86	MH873624	GU238232	KC584731
<i>Preussia funiculata</i>	CBS 659.74	GU301864	GU296187	GU349032
<i>Prosthemium orientale</i>	MAFF 239509	AB553748	AB553641	
<i>Pseudoastrosphaeriella bambusae</i>	MFLUCC 11–0205	KT955475		KT955437
<i>Pseudoastrosphaeriella thailandensis</i>	MFLUCC 10–0553	KT955477	KT955456	KT955439
<i>Psilogonium araucanum</i>	CBS 112412	FJ161172	FJ161133	FJ161089
<i>Pteridiospora javanica</i>	MFLUCC 11–0159	KJ742940	KJ739607	KJ739605
<i>Racodium rupestre</i>	L 346	EU048583	EU048575	
<i>Racodium rupestre</i>	L 424	EU048582	EU048577	
<i>Rimora mangrovei</i>	JK 5246A	GU301868	GU296193	
<i>Roccella fuciformis</i>	Tehler 8171	FJ638979		
<i>Roussoella nitidula</i>	MFLUCC 11–0634	KJ474842		KJ474851
<i>Salsuginea ramicola</i>	KT 2597.1	GU479800	GU479767	GU479861
<i>Salsuginea ramicola</i>	KT 2597.2	GU479801	GU479768	GU479862
<i>Schismatomma decolorans</i>	Ertz 5003 (BR)	NG_027622	NG_013155	
<i>Scorias spongiosa</i>	CBS 325.33	KF901821		
<i>Sigarispora ravennica</i>	MFLUCC 14–0005	KP698414	KP698415	
<i>Sporormia fimetaria</i>	UPS:Dissing Gr.81.194	GQ203729		
<i>Teichospora parva</i>	GKM 169N	GU385165		GU327768
<i>Teichospora striata</i>	JK 5678I	GU301813	GU296149	GU479852
<i>Teichospora tennesseensis</i>	ANM 911	GU385207		GU327769
<i>Tetraplosphaeria sasicola</i>	MAFF 239677	AB524631	AB524490	
<i>Thyridaria acaciae</i>	CBS 138873	KP004497		
<i>Thyridaria broussonetiae</i>	CBS 141481	KX650568	KX650515	KX650539
<i>Thyridaria macrostomoides</i>	GKM 1033	GU385190		GU327776
<i>Thyridaria macrostomoides</i>	GKM 1159	GU385185		GU327778
<i>Thyridaria macrostomoides</i>	GKM 224N	GU385191		GU327777
<i>Torula herbarum</i>	CBS 111855	KF443386	KF443391	KF443403
<i>Torula hollandica</i>	CBS 220.69	KF443384	KF443389	KF443401
<i>Triplosphaeria maxima</i>	MAFF 239682	AB524637	AB524496	
<i>Tubeufia chiangmaiensis</i>	MFLUCC 11–0514	KF301538	KF301543	KF301557
<i>Tubeufia javanica</i>	MFLUCC 12–0545	KJ880036	KJ880035	KJ880037
<i>Verruculina enalia</i>	BCC 18401	GU479802	GU479770	GU479863
<i>Westerdykella ornata</i>	CBS 379.55	GU301880	GU296208	GU349021
<i>Wicklowia aquatica</i>	F 76-2	GU045445	GU266232	
<i>Zopfia rhizophila</i>	CBS 207.26	DQ384104		

Results

Phylogenetic analyses

The manually adjusted LSU, SSU, ITS and *TEF1 α* alignment comprised a total of 3,622 characters (1,037 for LSU, 1,010 for SSU, 630 for ITS and 945 for *TEF1 α*), including coded alignment gaps. Among them, 2,468 characters were constant, 353 variable characters were parsimony-uninformative, and number of parsimony-informative characters was 801. Four hundred fifty-seven equally most parsimonious trees (Tree length = 3741, CI = 0.458, RI = 0.605, RC =

0.277, HI = 0.542) were yielded from the heuristic search. Maximum parsimony, maximum likelihood and Bayesian analyses of the combined dataset inferred similar topologies, respectively.

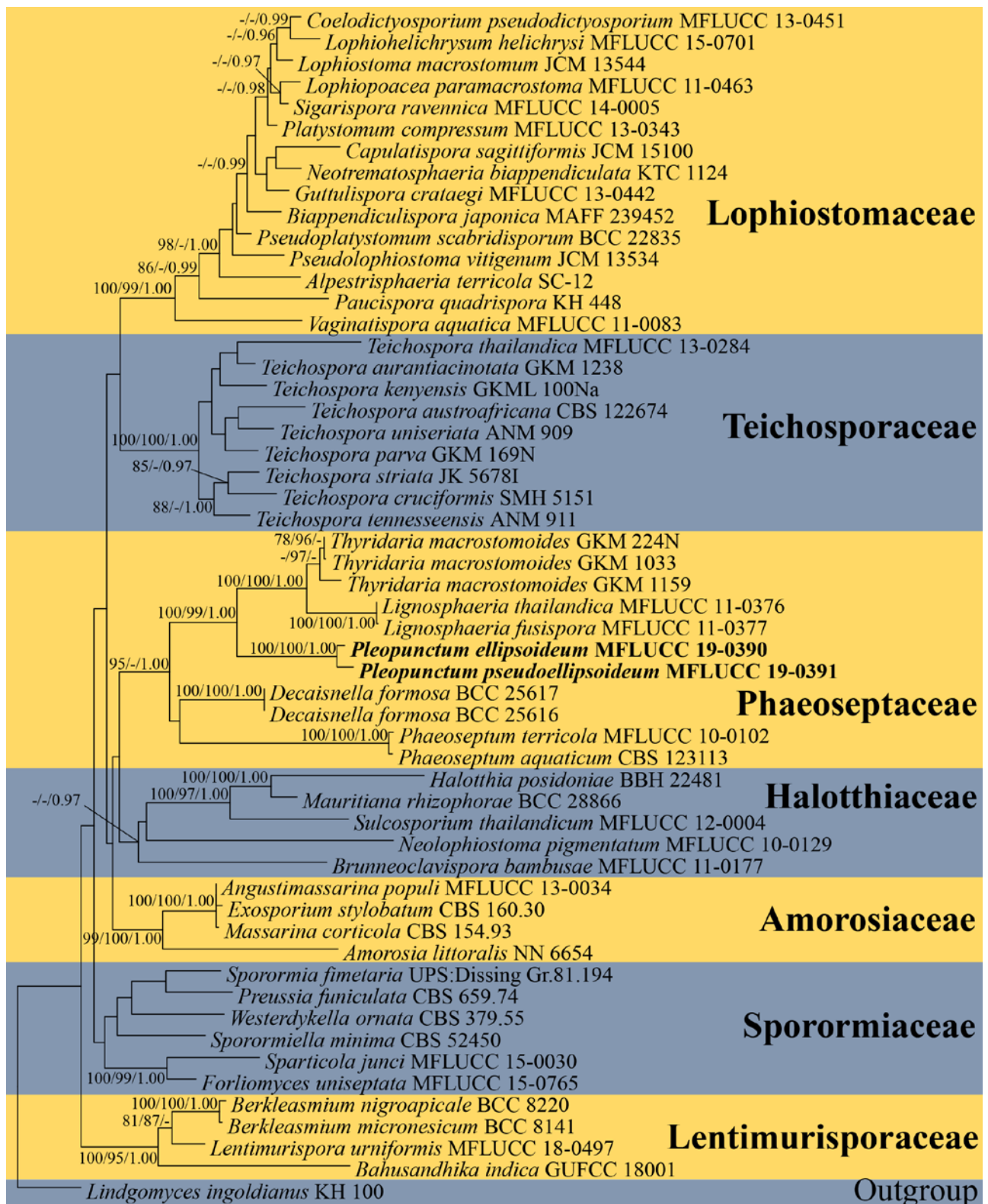


Figure 1 – Maximum likelihood (RAxML) tree based on analysis of a combined dataset of LSU, SSU, ITS and *TEF1α* sequence data. Bootstrap support values for ML and MP greater than 75% and Bayesian posterior probabilities above 0.95 are given near nodes, respectively. The tree is rooted with *Lindgomyces ingoldianus* (KH 100). The new taxa are indicated in bold.

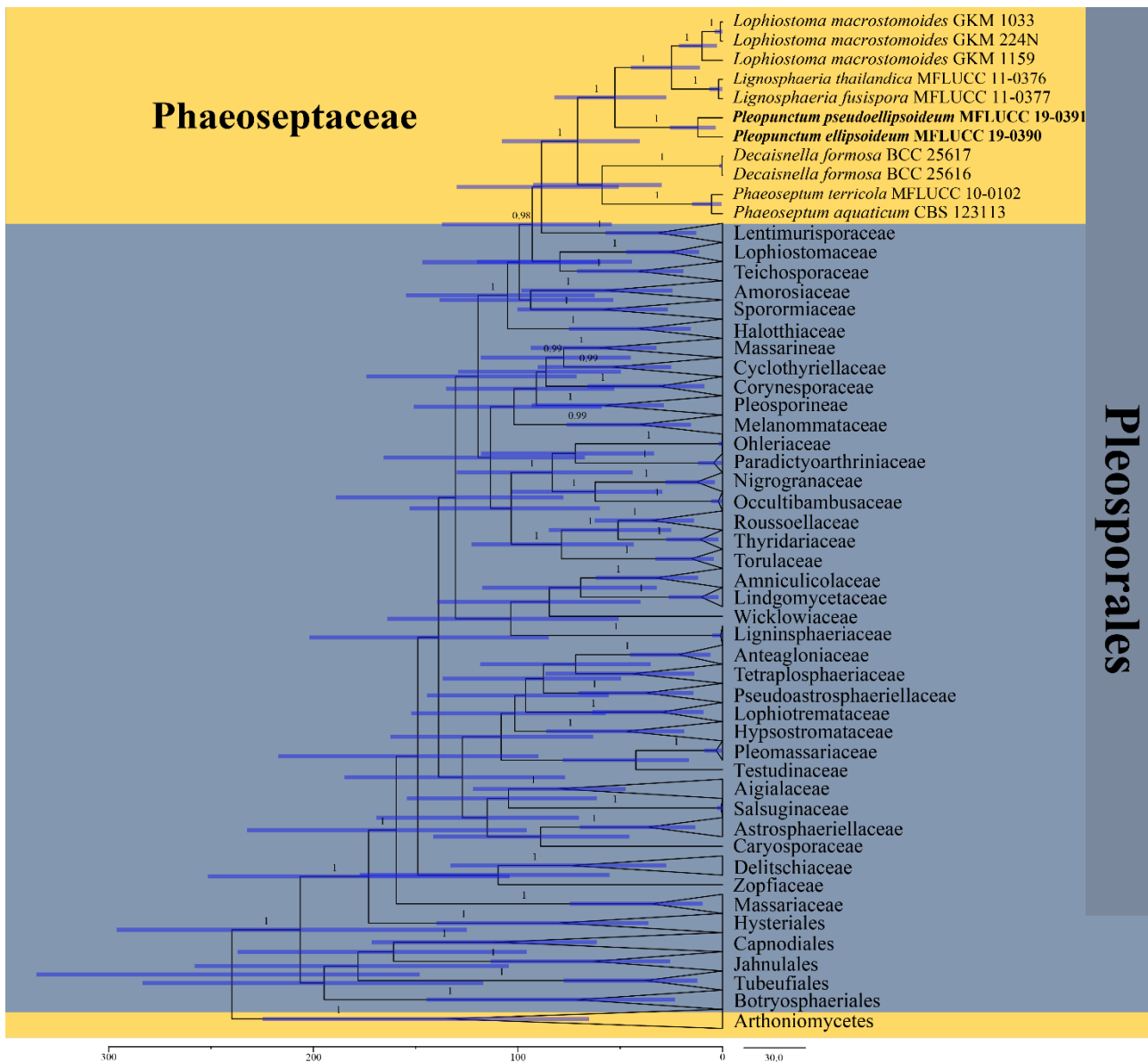


Figure 2 – Maximum clade credibility (MCC) tree with divergence times estimates obtained from BEAST. Posterior probabilities for branch support greater than 0.95 are given. Bars correspond to the 95% highest posterior density intervals. The new taxa are indicated in bold.

The most likely tree ($-\ln = 22570.586787$) is presented (Fig. 1). The matrix had 1,462 distinct alignment patterns with 38.91% undetermined characters or gaps. The two *Pleopunctum* taxa clustered together with maximal support (ML-bs = 100%, MP-bs = 100%, PP = 1.00) in Phaeoseptaceae. *Pleopunctum* subclade is close to *Lignosphaeria* and *Thyridaria macrostomoides* with maximal support (ML-bs = 100%, MP-bs = 100%, PP = 1.00) in Phaeoseptaceae.

Neolophiostoma pigmentatum Boonmee & K.D. Hyde (strain MFLUCC 10-0129) was included in Phaeoseptaceae and formed a basal subclade in the phylogenetic analyses of combined LSU, SSU and *TEF1 α* sequence data carried out by Hyde et al. (2018). However, in our analysis, *Neolophiostoma* belongs to Halotthiaceae. The monophyly of Phaeoseptaceae is well-supported by ML and Bayesian analyses, while Halotthiaceae is only supported by Bayesian analysis. Moreover, Halotthiaceae and Phaeoseptaceae clades had a sister relationship, but the monophyly of these two clades is not supported by ML, MP and Bayesian analyses. Among the seven families, the monophyly of Amorosiaceae, Lentimurisporeaceae, Lophiostomaceae, Phaeoseptaceae and Teichosporaceae are well-supported.

Divergence time estimations

According to the divergence times estimates (Fig. 2), the crown age of Dothideomycetes (which comprised taxa in Botryosphaerales, Capnodiales, Hysteriales, Jahnulales, Pleosporales and Tubeufiales) is around 206 MYA. The orders Hysteriales and Pleosporales diverged approximately 172 MYA. The crown age of Pleosporales is around 159 MYA. Among the Pleosporales families, Phaeoseptaceae diverged with Lentimurisporaceae approximately 88 MYA and the crown age of Phaeoseptaceae is around 71 MYA. Thus, the establishment of Phaeoseptaceae is supported and corresponds to previous studies (Liu et al. 2017, Zhang et al. 2019).

Taxonomy

Pleopunctum N.G. Liu, K.D. Hyde & J.K. Liu, gen. nov.

Index Fungorum: IF556522; Facesoffungi number: FoF 06113

Etymology – “Pleo-” an abbreviation of Pleosporales, the order in which this fungus is classified; “punctum” in reference to the punctiform colonies on natural substrate.

Saprobic on decaying wood in terrestrial habitats. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Colonies* on natural substrate sporodochial, superficial, brown, scattered, gregarious, punctiform. *Mycelium* immersed in the substratum, composed of septate, branched, subhyaline to greyish brown hyphae. *Conidiophores* macronematous, mononematous, cylindrical, branched, septate, medium brown, smooth-walled, thick-walled. *Conidiogenous cells* monoblastic, cylindrical, brown. *Conidia* acrogenous, solitary, muriform, constricted at septa, broadly oval to ellipsoidal, smooth-walled, pale brown when immature, broadly obtuse at apex and dark brown, truncate at base and paler brown when mature, often with a hyaline, elliptical to globose basal cell.

Type species – *Pleopunctum ellipticum*

Notes – *Pleopunctum* is the first hyphomycetous genus in Phaeoseptaceae. It contains two species, namely *P. ellipticum* and *P. pseudoellipticum*, and they formed a distinct subclade in Phaeoseptaceae in the phylogenetic tree. The sexual morph of *Pleopunctum* is unknown, and the asexual morph has a unique morphology compared to those phylogenetically related species. *Pleopunctum* is characterized by its macronematous, mononematous conidiophores, monoblastic conidiogenous cells and muriform, oval to ellipsoidal conidia often with a hyaline, elliptical to globose basal cell. We hereby introduce the new genus based on the distinctiveness of morphology and multi-gene phylogeny.

Pleopunctum ellipsoideum N.G. Liu, K.D. Hyde & J.K. Liu, sp. nov. Fig. 3

Index Fungorum: IF556523; Facesoffungi number: FoF 06114

Etymology – in reference to the ellipsoidal conidia

Holotype – MFLU 19-0685

Saprobic on decaying wood. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Colonies* on natural substrate sporodochial, superficial, brown, scattered, gregarious, punctiform. *Mycelium* immersed in the substratum, composed of septate, branched, subhyaline to greyish brown hyphae. *Conidiophores* 1.5–3.5 μm wide (\bar{x} = 2.5 μm , n = 15), macronematous, mononematous, cylindrical, branched, septate, medium brown, smooth-walled, thick-walled. *Conidiogenous cells* monoblastic, terminal, integrated, medium brown. *Conidia* 39–51 \times 17–24 μm (\bar{x} = 45 \times 20 μm , n = 30), acrogenous, solitary, muriform, constricted at septa, oval to ellipsoidal, smooth-walled, pale brown when immature, broadly obtuse at apex and dark brown, truncate at base and paler brown when mature, often with a hyaline, elliptical to globose basal cell, 8–20 \times 8.5–18.5 μm (\bar{x} = 13 \times 13 μm , n = 30).

Culture characteristics – Conidium germinated on water agar within 24 hours. Germ tubes produced from basal cell. Mycelia superficial, irregular circular, grey in the central cycle and pale grey in the outer circle from above. Dark brown in the central cycle and yellowish brown in the outer circle from below.

Material examined – CHINA, Guizhou Province, Guiyang, Guizhou Academy of Agricultural

Sciences, on decaying wood, 7 June 2018, N.G. Liu, NKY027 (MFLU 19–0685); ex-type living culture, MFLUCC 19–0390.

Pleopunctum pseudoellipsoideum N.G. Liu, K.D. Hyde & J.K. Liu, sp. nov.

Fig. 4

Index Fungorum: IF556524; Facesoffungi number: FoF 06115

Etymology – in reference to its similar morphology with *P. ellipsoideum*.

Holotype – MFLU 19–0686

Saprobic on decaying wood. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. Colonies on natural substrate sporodochial, superficial, black, scattered, gregarious, punctiform. Mycelium immersed in the substratum, composed of septate, branched hyphae. Conidiophores 1.5–4.5 µm wide (\bar{x} = 3 µm, n = 15), macronematous, mononematous, cylindrical, wider at the tip, septate, medium brown, smooth-walled, thick-walled. Conidiogenous cells monoblastic, terminal, integrated, medium brown. Conidia 39–59 × 19–28 µm (\bar{x} = 50 × 24 µm, n = 30), acrogenous, solitary, muriform, constricted at septa, oval to ellipsoidal, smooth-walled, broadly obtuse at apex and dark brown, truncate at base and paler brown, often with a hyaline, elliptical to subglobose basal cell, 6.5–13.5 × 11–15.5 µm (\bar{x} = 11–13 µm, n = 15).

Culture characteristics – Conidium germinated on water agar within 24 hours. Germ tubes produced from basal cell. Mycelia superficial, irregular circular, grey in the center and greyish white near the edge from above. Dark brown in the center, becoming paler towards the edge from below.

Material examined – China, Guizhou Province, Zunyi, Wangcao, on decaying wood, 16 September 2018, N.G. Liu, KKS020 (MFLU 19–0686); ex-type living culture, MFLUCC 19–0391.

Notes – *Pleopunctum ellipsoideum* and *P. pseudoellipticum* are morphologically similar. They both have sporodochial conidiomata, mononematous, cylindrical conidiophores, monoblastic, terminal conidiogenous cells and muriform, oval to ellipsoidal conidia often with a hyaline, elliptical to globose basal cell. The conidia size is also similar (39–51 × 17–24 µm vs. 39–59 × 19–28 µm). However, ITS comparison between *P. ellipsoideum* and *P. pseudoellipsoideum* showed that there are 12 bp differences (including the gaps) in a total of 524 bp, and 30 bp differences in a total of 985 bp for *TEF1α* (Table 3). Therefore, we identify *P. ellipsoideum* and *P. pseudoellipsoideum* as distinct species following the guidelines for species delineation in Jeewon & Hyde (2016).

Discussion

In our studies, *Pleopunctum ellipsoideum* and *P. pseudoellipsoideum* were collected from the same province but different cities in China. They are both saprobic on unidentified decaying woods. Based on phylogenetic analyses of combined LSU, SSU, ITS and *TEF1α* sequence data, *P. ellipsoideum* and *P. pseudoellipsoideum* formed a well-supported subclade and did not belong to any existing genera in Phaeoseptaceae. Moreover, although *P. ellipsoideum* and *P. pseudoellipsoideum* share similar morphology, they formed independent and distinct lineages with different branch lengths. Sufficient nucleotide differences are noted between *P. ellipsoideum* and *P. pseudoellipsoideum* in the ITS, LSU and *TEF1α* regions (Table 3). Therefore, we propose *Pleopunctum* as a new genus in Phaeoseptaceae and identify *P. ellipsoideum* and *P. pseudoellipsoideum* as different species.

Hyde et al. (2018) established the family Phaeoseptaceae to accommodate the genera *Lignosphaeria* Boonmee et al., *Neolophiostoma* Boonmee & K.D. Hyde and *Phaeoseptum* Ying Zhang, J. Fourn. & K.D. Hyde, along with putatively named species *Decaisnella formosa* Abdel-Wahab & E.B.G. Jones and *Thyridaria macrostomoides* (De Not.) M.E. Barr, based on LSU, SSU and *TEF1α* sequence data. Phaeoseptaceae is characterized by subglobose to globose ascomata with short papilla, bitunicate, long pedicellate, 8-spored asci with a small ocular chamber, and light brown, multi-septate ascospores (Hyde et al. 2018). No hyphomycetous asexual morphs were reported in this family. Thus, morphological comparison between *Pleopunctum* and other genera in Phaeoseptaceae is not available. The monotypic genus *Neolophiostoma* was introduced by

Ariyawansa et al. (2015) with the type species *N. pigmentatum* in the family Halotthiaceae. However, *Neolophiostoma* was transferred to Phaeoseptaceae by Hyde et al. (2018). In our analysis, *Neolophiostoma* remained within Halotthiaceae, although without good support, but agrees with Ariyawansa et al. (2015) based on LSU, SSU, *RPB2* sequence data; Hyde et al. (2016) based on LSU and SSU sequence data and Phukhamsakda et al. (2016a) based on LSU, SSU, ITS, *TEF1α* and *RPB2* sequence data.

Neolophiostoma resembles *Halotthia*, the type genus of Halotthiaceae, in having ostiolate ascomata, bitunicate, 8-spored and fissitunicate asci with an ocular chamber, but they differ in that *Neolophiostoma* has hyaline, 3–5-septate ascospores with a thin gelatinous sheath while *Halotthia* has brown, 1-septate ascospores. Therefore, *Neolophiostoma* probably belongs to Halotthiaceae or has its own family, but more collections and analyses are warranted to verify any taxonomic assumption. Our divergence time estimation shows that Phaeoseptaceae diverged with Lentimurisporaceae approximately 88 MYA, the establishment of the Phaeoseptaceae agrees well with ages (50–150 MYA) proposed by Hyde et al. (2017), with the genus *Neolophiostoma* excluded. Our work generated similar results with Liu et al. (2017) and Zhang et al. (2019). *Decaisnella* was introduced by Fabre (1879) based on *D. spectabilis* Fabre, and there are fourteen epithets listed in Index Fungorum (May 2019). However, sequence data are only available for *D. formosa*. Because of the lack of molecular sequence data of *D. spectabilis*, the phylogenetic placement of the genus *Decaisnella* requires further confirmation. *Thyridaria macrostomoides* was described by Barr (1990), until twenty year later, Mugambi & Huhndorf (2009) provided the DNA sequence data of *T. macrostomoides* from three newly obtained collections (GKM 1033, GKM 1159, GKM 224N) and placed them in Lophiostomaceae. Thambugala et al. (2015) restudied Lophiostomaceae and they excluded these three *T. macrostomoides* strains from Lophiostomaceae and assigned them as Dothideomycetes genera *incertae sedis*. However, *Thyridaria* Sacc. was accommodated to Thyridariaceae based on the type species *T. broussonetiae* (Sacc.) Traverso (Hyde et al. 2013, Jaklitsch & Voglmayr 2016). Therefore, these three *T. macrostomoides* collections may have been wrongly identified because they are distant from *T. broussonetiae*, and they probably can be recognized as a new genus, but we are not willing to introduce it in this study until we examine the type materials of *T. macrostomoides*.

Table 3 Nucleotide differences in the ITS, LSU and *TEF1α* regions for *P. ellipsoideum* and *P. pseudoellipsoideum*. Numbers are in reference to the nucleotide position of DNA sequences (*P. ellipsoideum*) submitted in GenBank.

Species	ITS												LSU			<i>TEF1α</i>							
	12	15	45	91	143	144	145	338	339	359	462	525	85	101	160	8	12	33	42	150	153	171	186
<i>P. ellipsoideum</i>	C	G	T	G	A	A	A	T	C	C	A	–	C	G	C	T	T	T	T	T	T	G	T
<i>P. pseudoellipsoideum</i>	T	C	–	A	–	–	–	C	T	T	–	T	T	A	T	C	C	C	C	C	C	T	C
Species	<i>TEF1α</i>																						
	252	258	343	366	397	571	572	591	684	705	876	909	915	924	936	951	965	969	975	979	984	986	
<i>P. ellipsoideum</i>	C	C	T	C	T	A	C	T	T	C	C	C	C	G	T	C	G	T	C	G	A	A	
<i>P. pseudoellipsoideum</i>	T	T	A	T	A	G	T	C	C	T	T	T	T	T	C	T	T	C	T	C	T	C	

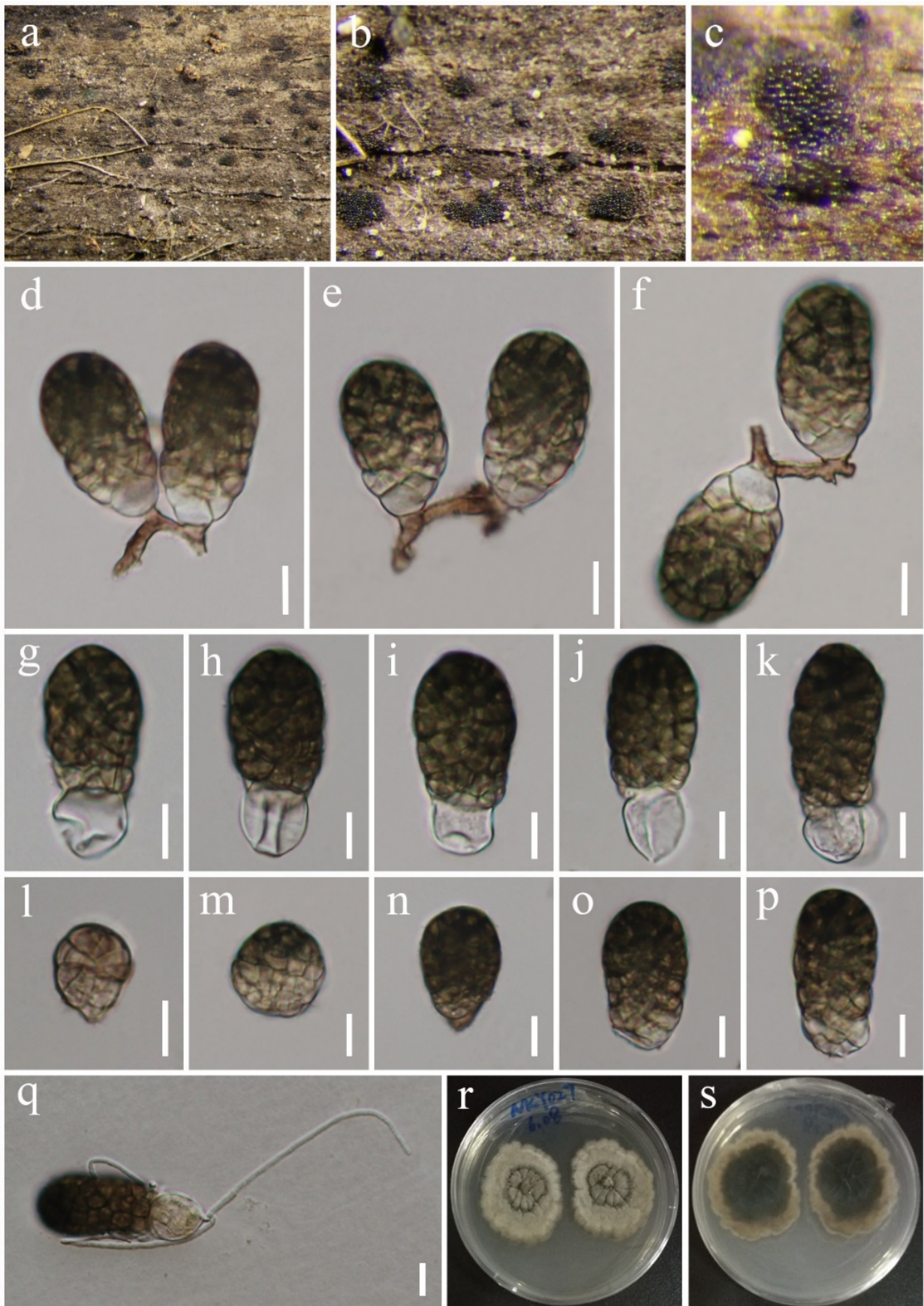


Figure 3 – *Pleopunctum ellipsoideum* (MFLU 19–0685, holotype). a–c Colonies on natural substrates. d–f Conidiophores and conidia. g–k Conidia with basal hyaline cells. l–p Conidia without basal hyaline cells. q Germinated conidium. r, s Colonies on PDA media. Scale bars: d–q = 10μm

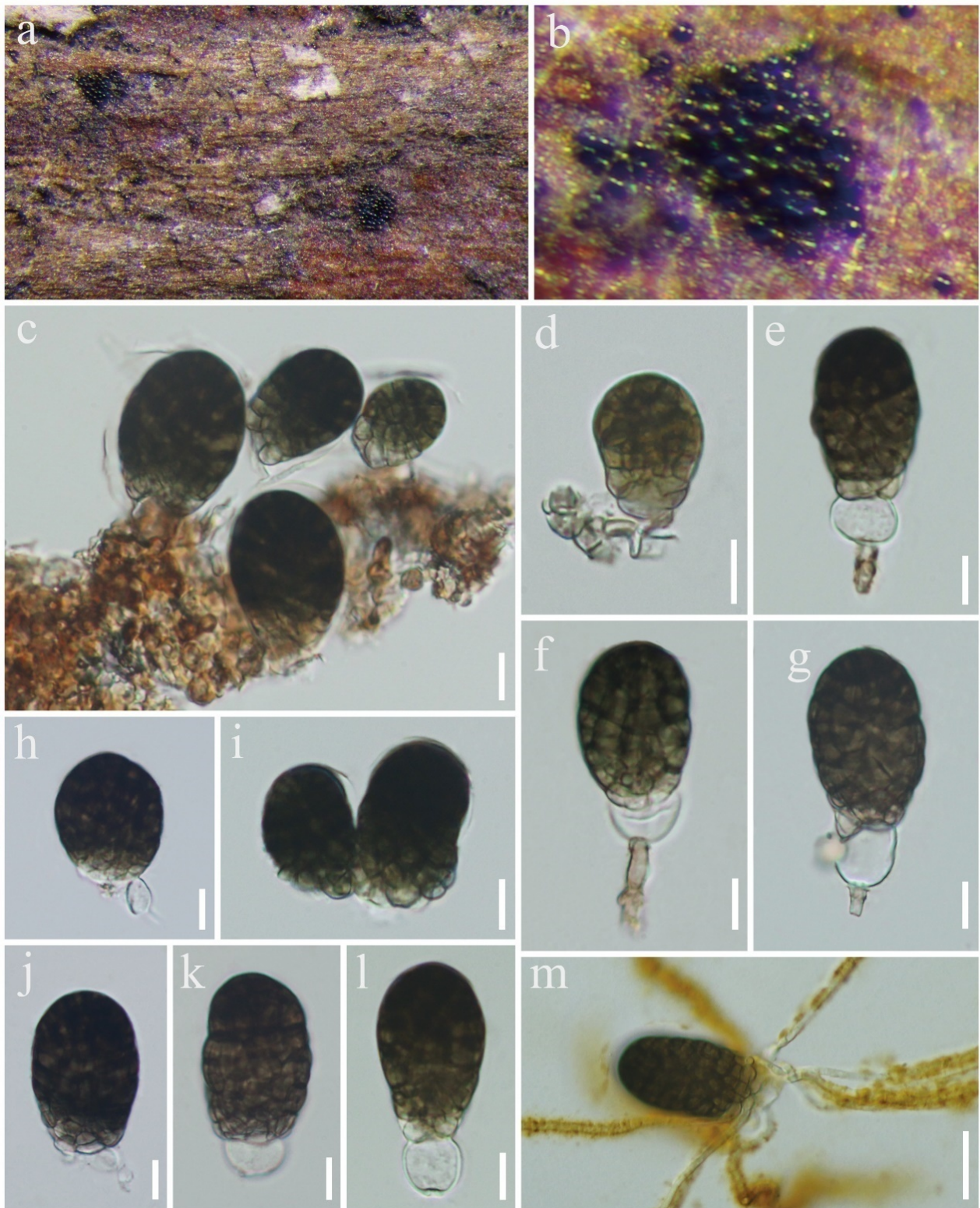


Figure 4 – *Pleopunctum pseudoellipsoideum* (MFLU 19–0686, holotype). a–b Colonies on natural substrates. c Conidia on substrate. d–g Conidiophores and conidia. h–l Conidia with or without basal hyaline cells. m Germinated conidium. Scale bars: c–l = 10 μm, m = 20 μm

Among the six phylogenetically close families in this study, only coelomycetous asexual morphs have been reported in Halotthiaceae, Lophiostomaceae and Teichosporaceae (Hyde et al. 2013, Thambugala et al. 2015). Amorosiaceae includes two genera, *Amorosia* Mantle & D. Hawksw. and *Angustimassarina* Thambugala, Kaz. Tanaka & K.D. Hyde, along two putative strains, *Exosporium stylobatum* CBS 160.30 and *Massarina corticola* CBS 154.93 (Thambugala et

al. 2015). *Amorosia* and *Angustimassarina* can produce chlamydospores in cultures, which are characterized by micronematous to semi-macronematous conidiophores, elongate-clavate conidia. However, *Amorosia* has 3–4-septate conidia with a distinct central pore in each septum, while *Angustimassarina* has 1–3-septate conidia (Mantle et al. 2006, Thambugala et al. 2015). *Exosporium* is a hyphomycetous genus with loosely aggregated conidiophores, terminal or lateral conidiogenous cells with prominent loci, and 5-distoseptate conidia (Crous et al. 2011). Lentimurisporeaceae is a dematiaceous hyphomycetous family without known sexual morphs. The type genus *Lentimurispora* produces muriform, lenticular conidia with dark brown central cells and subhyaline to pale brown peripheral cells (Liu et al. 2018). *Bahusandhika* Subram. is a torula-like genus with fusiform, cylindrical or rhomboidal conidia formed simply or in branched chains. *Berkleasmiium* Zobel has been shown to be not monophyletic (Pinnoi et al. 2007). Conidial morphology of *Berkleasmiium* is oval to ellipsoidal, often with a protruding hilum instead of a hyaline, elliptical to globose basal cell (Ellis 1971, Bussaban et al. 2001). The genus *Sparticola* Phukhams., Ariyaw., Camporesi & K.D. Hyde in Sporormiaceae can form hyphomycetous asexual morph in culture which is characterized by semi-macronematous to macronematous, branched conidiophores, annellidic, doliform conidiogenous cells, and dictyosporous conidia with granules. Other genera in Sporormiaceae, such as *Forliomyces* Phukhams., Camporesi & K.D. Hyde, produce coelomycetous asexual morphs. Therefore, *Pleopunctum* has a unique asexual morph morphology in the family Phaeoseptaceae, as well as compared to its phylogenetically close families in Pleosporales.

Acknowledgements

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