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The evolution of Massarineae with Longipedicellataceae *fam. nov.*

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Abstract

Massarineae is a suborder of Pleosporales, the latter being the largest order in Dothideomycetes. Massarineae comprises 14 families and six taxa of uncertain placement. In this study, we introduce an additional new family, Longipedicellataceae in Massarineae, which accommodates the genera *Longipedicellata* and *Pseudoxylomyces*. The family inhabits submerged culms of plants in freshwater habitats. The family can be distinguished by its very long pedicellate asci and chlamydospore-like structures, which are produced in culture. A LSU, SSU, and RPB2 dataset from representative strains used in our phylogenetic analyses shows the separation of Longipedicellataceae from the other families of Massarineae. In addition, divergence times of families in Massarineae were estimated using a molecular clock methodology. We used an Eocene fossil of *Margaretbarromyces dictyosporus* to estimate dates in Pleosporales with emphasis on Massarineae. In this study, the crown of Pleosporales is dated to the late Triassic (211 Mya), while the suborder Massarineae is dated to the Cretaceous (130 Mya) and family Longipedicellataceae is dated to Eocene (56 Mya).

Key words – BEAST – Chlamydospores – Fossil fungi – freshwater fungi – *Margaretbarromyces dictyosporus* – Pleosporales

Introduction

Pleosporales is the largest order in the class Dothideomycetes and has thus been the focus of recent research (Zhang et al. 2012, Hyde et al. 2013, Wijayawardene et al. 2014, Ariyawansa et al. 2015). Taxa from this order occur in terrestrial, marine and freshwater habitats and have a worldwide distribution (Campbell et al. 2006, Kruys & Wedin 2009, Zhang et al. 2009, Tanaka et al. 2015, Jones et al. 2015, Ertz et al. 2015). The order comprises two main suborders, Massarineae and Pleosporineae, which is supported in morphological and phylogenetic studies (Zhang et al. 2012, Ariyawansa et al. 2015, Hyde et al. 2016). The suborder Massarineae was established by Barr

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(1979) to accommodate Arthopyreniaceae Watson, Massarinaceae Munk (family typified), and Pleomassariaceae Barr. Massarineae was characterized by depressed ascomata, with ostioles and thick peridia at the sides (Barr 1979). However, modern classifications using phylogenetic analysis together with morphological circumscription have demonstrated that Arthopyreniaceae and Pleomassariaceae are distinct from Massarinaceae. Thus, they were excluded from the suborder Massarineae (Schoch et al. 2009, Zhang et al. 2012, Hyde et al. 2013, Liu et al. 2014).

The suborder Massarineae currently includes 12 families: Bambusicolaceae, Dictyosporiaceae, Didymosphaeriaceae, Lentitheciaceae, Latoruaceae, Macrodiplodiopsidaceae, Massarinaceae, Morosphaeriaceae, Parabambusicolaceae, Periconiaceae, Sulcatisporaceae, and Trematosphaeriaceae (Zhang et al. 2012, Hyde et al. 2016). Mapook et al. (2016) placed *Phaeodimeriella* (Pseudoperisporiaceae) in the suborder Massarineae based on their phylogenetic analysis. The placements of *Asteromassaria pulchra* (Harkness) Shoemaker & P.M. LeClair, *Inflatispora pseudostromatica* Zhang, J. Fournier & K.D. Hyde *Monodictys capensis* Sinclair, S. Boshoff & A. Eicker, *Bactrodesmium cubense* (Castañeda & Arnold) Zucconi & D. Lunghini, *Pseudoxylomyces elegans* Tanaka & K. Hirayama and *Fuscostagonospora sasae* Tanaka & K. Hirayama are uncertain, thus they are included as species and genera *incertae sedis* within Massarineae (Boonmee et al. 2016). Zhang et al. (2016) introduced *Longipedicellata aptrootii* (Hyde & Wong) Zhang, K.D. Hyde & J.K. Liu to accommodate *Didymella aptrootii*, a species collected from submerged bamboo in freshwater (Hyde & Wong 1999) and assigned it to Bambusicolaceae. However, our phylogenetic analysis indicates that the species is closely related to *Pseudoxylomyces elegans* (Goh, W.H. Ho, K.D. Hyde & K.M. Tsui) Tanaka & K. Hirayama, where they form a distinct clade.

Divergence time estimates using molecular clock methodologies have been developed for the fungi (Berbee & Taylor 1993, Li et al. 2005, Taylor & Berbee 2006, Vijaykrishna et al. 2006). Various pleosporalean fossils have been discovered and can be compared to extant genera, introducing new fossil for dating in molecular clocks (Mindell 2007, Taylor et al. 2009, 2015). In the present study, we (1) estimate divergent times for families of the suborder Massarineae (Pleosporales) using a molecular clock methodology based on LSU, SSU, and RPB2 sequence data and (2) introduce Longipedicellataceae *fam. nov*. based on phylogenetic and evolutionary evidence, with includes the genera *Longipedicellata* and *Pseudoxylomyces*.

Material & Methods

Sample collection, morphological study and isolation

Fresh materials were collected from submerged stems of Bambusodeae in Chiang Rai, Thailand during 2015 and recollected in 2016. Fresh specimens were kept in plastic Ziploc bags with a small amount of water and returned to the laboratory. Pure cultures were established from single ascospores on 2% potato dextrose agar (PDA; 39 g/L Difco potato dextrose in distilled water) and malt extract agar (MEA; 62 g/L Criterion in distilled water) as described in Chomnunti et al. (2014). Cultures were incubated at 25°C for up to 8 weeks. Type specimens were deposited in Mae Fah Luang University (MFLU) herbarium. Ex-type living cultures were deposited at the Mae Fah Luang Culture Collection (MFLUCC). Faces of fungi numbers and Index Fungorum numbers are provided (Jayasiri et al. 2015, Index Fungorum 2016). Samples were examined under a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 600D digital camera fitted to the microscope. Measurements were made using Tarosoft (R) Image Frame Work program and photo-plate were made by using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, United States).

DNA extraction, amplification and sequencing

DNA was extracted from mycelium with Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) (Hangzhou, P. R. China); following the manufacturer's protocol. PCR amplification was carried out using primers LROR/LR5 for the nuclear ribosomal large subunit 28S rRNA gene (LSU), NS1/NS4 for the nuclear ribosomal small subunit 18S rRNA gene (SSU), and RPB2-

5f2/RPB2-7cr for RNA polymerase subunit II gene region (RPB2) (Vilgalys & Hester 1990, White et al. 1990, Carbone & Kohn 1999). Primer sequences are available at the WASABI database at the AFTOL website (aftol.org). Amplification reactions for LSU and SSU were performed according to Phukhamsakda et al. (2015). The PCR thermal cycle program for RPB2 was set for denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 2 min and extension at 72 °C for 2 min, with a final extension step at 72 °C for 10 min. DNA extracted and PCR proliferation products were checked on 1% Agarose gels with added 6 µl in 100 ml of 4S green dyes. Purified PCR products and the sequencing were performed by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China).

Sequence alignment and phylogenetic analysis

SeqMan v. 7.0.0 (DNASTAR, Madison, WI) was used to assemble consensus sequences. Sequences of closely related strains were retrieved using BLAST searches against GenBank (http://www.ncbi.nlm.nih.gov). We also included the strains from Tanaka et al. (2015), Sharma et al. (2015), Boonmee et al. (2016), Mapook et al. (2016) and Hongsanan et al. (2016) and these are listed in Table 1. Sequences were aligned with MUSCLE in MEGA 6 (Tamura et al. 2013). The alignments were checked visually and improved manually where the ambiguous nucleotides presented by using Bioedit 7.2 (Hall 1999). Leading or trailing gaps exceed from primer binding site were trimmed from the alignments prior to tree building. Maximum likelihood analyses (ML), including 1000 bootstrap replicates, was performed using RAxML (Stamatakis 2014) as implemented in raxmlGUI version v.1.3.1 (Silvestro & Michalak 2012). The search strategy was set to rapid bootstrapping. The analysis was carried out with the general time reversible (GTR) model for nucleotide substitution and a discrete gamma-distributed with four rate categories (O'meara et al. 2006, Stamatakis et al. 2008, Guindon et al. 2010). The bootstrap replicates were summarized on to the best scoring tree. Maximum likelihood bootstrap values equal or greater than 70 % are given in black below or above each node (Fig. 1).

The best model for different genes partition in the concatenated data set was determined in MrModeltest 2.3 (Nylander 2004) for posterior probability (PP). In our analysis, GTR+I+G model was used for each partition. The posterior probability (PP) distribution (Zhaxybayeva & Gogarten 2002) was estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.2 (Huelsenbeck & Ronquist 2001). Four simultaneous Markov chains were run for 10,000,000 generations and trees were sampled every 1000th generation, thus 10,000 trees were obtained. The suitable burn-in phase were determined by traces inspected in Tracer version 1.6 (Rambaut et al. 2014). Based on the tracer analysis, the first 1,000 trees representing 10% of burn-in phase of the analyses were discarded. While the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01). Bayesian Posterior Probabilities (PP) equal or greater than 0.90 for the clades in the ML tree are given above each node in black (Fig. 1).

Fossil calibration and Divergence time estimates

Divergence time estimation was carried out in BEAST 1.8.0 (Drummond et al. 2012). We performed two different dating scenarios. Scenario 1 used a normal posterior distribution for the divergence between Arthoniomycetes and Dothideomycetes and the crown age of Capnodiales. The split between Arthoniomycetes (outgroup) and Dothideomycetes was given a secondary calibration point according to Gueidan et al. (2011), Prieto & Wedin (2013), Beimforde et al. (2014) and Pérez-Ortega et al. (2016), with a mean of 300 Mya and a standard deviation (SD) of 50 Mya, which give a lower boundary of 382 Mya on the 95% credibility interval (CI; node 1, Fig. 2). The crown age of Capnodiales was set based on the fossil of Metacapnodiaceae and given a mean of 100 Mya, and SD of 150 Mya, giving a lower boundary of 346 Mya (Gueidan et al. 2011, Prieto & Wedin 2013, Pérez-Ortega et al. 2016, Hongsanan et al. 2016; node 2, Fig. 2). For scenario 2, we also set a constraint for the *Aigialus* (Aigialaceae) crown based on the fossil *Margaretbarromyces dictyosporus* (Mindell et al. 2007, Berbee & Taylor 2010, Taylor et al. 2015). For this calibration

we used a gamma distribution, with an offset of 35 Mya based on the minimal ages of amber (Mindell et al. 2007), and a shape of 1.0 and scale of 25, giving a lower boundary of the 95% CI of 110 Mya (node 3, Fig. 2).

Nucleotide substitution models determined by jModelTest version 2.1.10 (Darriba et al. 2012). The GTR+I+G nucleotide substitution model were applied to LSU, SSU, and RPB2 partitions based on the results from jModelTest 2.1.10. The BEAST run was prepared in BEAUti 1.8.0 (BEAST package). The data partitions were set with unlinked substitution, linked clock models and linked tree. Calibration points were set as described for the two scenarios above. A lognormal distribution was used for the relaxed clock applied for the datasets. The Yule speciation process was used as tree prior, which specifies a constant rate of speciation divergence (Gernhard et al. 2008). The value prior was carried out using mean 1.00 in units of substitutions per site per time unit. The analyses were performed for 100 million generations. Tracer version 1.6 was used to check the effective sample sizes (ESS), assuring values more than 200. To validate the analysis, we performed two independent analyses, sampling parameters every 10,000th generation. Based on the tracer analysis the first 1,000 trees, i.e. 10%, were discarded as burn-in. The remaining trees were combined by using LogCombiner 1.8.0 (BEAST package). The maximum clad creditability (MCC) was calculated in TreeAnnotator 1.8.0 (BEAST package).

The geological time scale in this study were retrieved from the International Commission on Stratigraphy (ICS) website (www.stratigraphy.com). The chronostratigraphy chart version 2016/04 were applied to estimate the geological time scale at the base of Fig. 2.

Phylogenetic trees and data files were visualized in FigTree v. 1.4 (Rambaut & Drummond 2008). The phylograms with bootstrap values and/or posterior probabilities on the branches are presented in Fig. 1 and 2 by using graphical options available in Adobe Illustrator CS v. 6. All sequences generated in this study were submitted to GenBank.

Table 1 GenBank, culture collection code and accession numbers used in this study.

Taxon[1]	Strain number	GenBank accession numbers		
		LSU	SSU	RPB2
Aigialus grandis	BCC 20000	GU479775	GU479739	GU479814
Aigialus mangrovei	BCC 33563	GU479776	GU479741	GU479815
Aigialus parvus	BCC 18403	GU479778	GU479743	GU479817
Aigialus rhizophorae	BCC 33572	GU479780	GU479745	GU479819
Aliquandostipite khaoyaiensis	CBS 118232	GU301796	NG_016494	–
Alternaria alternata	**CBS 916.96** [T]	DQ678082	KC584507	KC584375
Amniculicola lignicola	**CBS 123094** [T]	EF493861	EF493863	EF493862
Anteaglonium abbreviatum	ANM 925a	GQ221877	–	–
Anteaglonium globosum	GKML101N	GQ221875	–	–
Antennariella placitae	**CBS 124785** [T]	GQ303299	–	–
Aquastroma magniostiolata	**CBS 139680** [T]	AB807510	AB797220	–
Aquaticheirospora lignicola	**RK-2006a** [T]	AY736378	AY736377	–
Aquilomyces patris	**CBS 135661** [T]	KP184041	KP184077	–
Aquilomyces rebunensis	**CBS 139684** [T]	AB807542	AB797252	–
Arthonia dispersa	UPSC2583	AY571381	AY571379	
Ascocratera manglicola	CBS 120023	GU301799	GU296136	GU371763
Asteromassaria pulchra	CBS 124082	GU301800	GU296137	GU371772
Bactrodesmium cubense	CBS 680.96	AB807508	AB797218	–
Bambusicola bambusae	**MFLUCC 11-0614** [T]	JX442035	JX442039	–
Bambusicola massarinia	**MFLUCC 11-0389** [T]	JX442037	JX442041	–
Bambusicola splendida	**MFLUCC 11-0439** [T]	JX442038	JX442042	–
Bambusistroma didymosporum	**MFLUCC 13-0862** [T]	KP761730	KP761737	KP761721
Botryosphaeria dothidea	**CBS 115476** [T]	NG_027577	DQ677998	DQ677944
Capnodium salicinum	**CBS 131.34** [T]	DQ678050	DQ677997	–
Clypeoloculus akitaensis	**CBS 139681** [T]	AB807543	AB797253	–
Cucurbitaria berberidis	CBS 363.93	GQ387606	GQ387545	–
Cucurbitaria berberidis	CBS 394.84	GQ387605	GQ387544	–
Cystocoleus ebeneus	L161	EU048578	EU048571	–
Delitschia didyma	UME 31411	DQ384090	NG_016519	–

Taxon[1]	Strain number	GenBank accession numbers		
		LSU	SSU	RPB2
Delitschia winteri	**CBS 225.62** [T]	DQ678077	DQ678026	DQ677975
Deniquelata barringtoniae	**MFLUCC 11-0422** [T]	NG_042696	JX254656	–
Dictyosporium alatum	**ATCC 34953** [T]	DQ018101	DQ018080	–
Dictyosporium elegans	NBRC 32502	DQ018100	DQ018079	–
Dictyosporium strelitziae	**CBS 123359** [T]	FJ839653	–	–
Didymella exigua	**CBS 183.55** [T]	EU754155	EU754056	–
Didymosphaeria rubi-ulmifolii	**MFLUCC 14-0024** [T]	KJ436585	KJ436587	–
Digitodesmium bambusicola	**CBS 110279** [T]	DQ018103	–	–
Erythrodecton granulatum	Ertz 9908 (BR)	EU704090	–	EU704022
Extremus antarcticus	**CCFEE5312**	KF310020	–	KF310086
Falciformispora lignatilis	BCC 21117	GU371826	GU371834	–
Falciformispora senegalensis	**CBS 196.79** [T]	KF015631	KF015636	KF015717
Fissuroma maculans	**MFLUCC 10-0886**	NG_042598	JN846738	–
Fuscostagonospora sasae	**CBS 139687** [T]	**AB807548**	**AB797258**	–
Halomassarina thalassiae	JK 5262D	GU301816	–	–
Halotthia posidoniae	**BBH 22481**	GU479786	GU479752	–
Helicascus elaterascuss	HKUCC 7769	AY787934	AF053727	–
Helicascus kanaloanus	A237	–	AF053729	–
Hysterium angustatum	**CBS 236.34** [T]	FJ161180	GU397359	FJ161117
Inflatispora pseudostromatica	**CBS 123110** [T]	JN231131	JN231132	JN231133
Jahnula seychellensis	**SS2113**	EF175665	EF175643	–
Jalapriya toruloides	CBS 209.65	DQ018104	DQ018081	–
Keissleriella cladophila	**CBS 104.55** [T]	GU301822	GU296155	GU371735
Latorua caligans	**CBS 576.65** [T]	KR873266	–	–
Latorua grootfonteinensis	**CBS 369.72** [T]	KR873267	–	–
Lentithecium fluviatile	CBS 122367	GU301825	GU296158	–
Lentithecium lineare	IFRD 2008	FJ795435	FJ795478	–
Leptosphaeria doliolum	**CBS 505.75** [T]	GQ387576	GQ387515	KT389640
Leptoxyphium cacuminum	**MFLUCC 10-0049** [T]	JN832602	JN832587	–
Lindgomyces ingoldianus	**ATCC 200398** [T]	AB521736	AB521719	–
Longipedicellata aptrootii	MFLUCC 16-0384	KY066738	KY066740	–
Longipedicellata aptrootii	MFLUCC 16-0244	KY066739	KY066741	KY066737
Longipedicellata aptrootii	**MFLUCC 10-0297** [T]	KU238894	KU238895	KU238891
Lophiostoma macrostomum	JCM 13544	AB619010	AB618691	JN993491
Lophiotrema lignicola	**CBS 122364** [T]	FJ795445	GU296166	FJ795462
Lophiotrema nucula	**CBS 627.86** [T]	GU301837	–	GU371792
Lophium mytilinum	**AFTOL-ID 1609** [T]	DQ678081	DQ678030	DQ677979
Macrodiplodiopsis desmazieri	CBS 221.37	JX681100	–	–
Macrodiplodiopsis desmazieri	**CBS 140062** [T]	KR873272	–	–
Magnicamarosporium iriomotense	**KT 2822** [T]	AB807509	AB797219	–
Massaria gigantispora	CBS 125593	HQ599397	HQ599447	–
Massaria inquinans	CBS 125591 [ET]	HQ599400	HQ599442	–
Massarina eburnea	CBS 473.64	GU301840	GU296170	GU371732
Matsushimamyces bohaniensis	**CBEC 001** [T]	KR350633	–	–
Matsushimamyces venustum	CBS 140212	KT428158	–	–
Mauritiana rhizophorae	BCC 28866	GU371824	GU371832	–
Melanomma pulvis-pyrius	CBS 371.75	GU301845	FJ201989	GU371798
Monodictys capensis	CBS 134928	AB807551	AB797261	–
Montagnula aloes	**CPC 19671** [T]	JX069847	–	–
Morosphaeria ramunculicola	BCC 18404	GQ925853	GQ925838	–
Morosphaeria velatispora	**BCC 17059** [T]	GQ925852	GQ925841	–
Multilocularia bambusae	**MFLUCC 11-0180** [T]	KU693438	KU693442	–
Multiseptospora thailandica	**MFLUCC 11-0183** [T]	KP744490	KP753955	–
Murilentithecium clematidis	**MFLUCC 14-0561** [T]	KM408758	KM408760	KM454446
Myriangium duriaei	CBS 260.36	NG_027579	AF242266	KT216528
Myriangium hisparanicum	CPC 18561	JN940391	JN940562	–
Mytilinidion rhenanum	**CBS 135.34** [T]	FJ161175	FJ161136	FJ161115
Neoastrosphaeriella krabiensis	**MFLUCC 11-0025** [T]	JN846729	JN846739	–
Neobambusicola strelitziae	**CBS 138869** [T]	KP004495	–	–
Neokalmusia brevispora	CBS 120248	AB524600	AB524459	AB539099

Taxon[1]	Strain number	GenBank accession numbers		
		LSU	SSU	RPB2
Neomassariosphaeria typhicola	**CBS 609.86 T**	EF165033	EF165037	EF165041
Neottiosporina paspali	CBS 331.37	EU754172	EU754073	GU371779
Parabambusicola bambusina	KT 2637	AB807538	AB797248	–
Parameliola acaciae	**MFLU 15-0378**	KU285142	–	–
Parameliola dimocarpi	**MFLU 15-0045**	KU285142	–	–
Periconia homothallica	**CBS 139698 T**	AB807565	AB797275	–
Periconia pseudodigitata	**CBS 139699 T**	AB807564	AB797274	–
Phaeodimeriella cissampeli	MFLU 16-0558	KU746806	KU746808	KU746810
Phaeodimeriella dilleniae	MFLU 14-0013	KU746805	KU746807	KU746809
Phyllosticta ampelicida	**CBS 111645 T**	DQ377876	EU673223	–
Piedraia hortae	**CBS 480.64 T**	GU214466	AY016349	KF902289
Pleomassaria siparia	**AFTOL-ID 1600 T**	DQ678078	DQ678027	DQ677976
Plespora herbarum	**CBS 191.86 T**	GU238160	GU238232	DQ247794
Polyschema congolensis	**CBS 542.73 T**	EF204502	–	EF204486
Polyschema larviformis	CBS 463.88	EF204503	–	–
Polyschema sclerotigenum	UTHSC:DI14-305	KP769976	–	–
Polyschema terricola	**CBS 301.65 T**	EF204504	EF204519	EF204487
Preussia funiculata	**CBS 659.74 T**	GU301864	GU296187	GU371799
Pseudoasteromassaria fagi	**MAFF 245222 T**	LC061589	LC061584	–
Pseudoasteromassaria fagi	MAFF 245221	LC061590	LC061585	–
Pseudocoleophoma calamagrostidis	**CBS 139700 T**	LC014609	LC014604	–
Pseudocoleophoma polygonicola	**CBS 139701 T**	AB807546	AB797256	–
Pseudodictyosporium elegans	**CBS 688.93 T**	DQ018106	DQ018084	–
Pseudodictyosporium wauense	NBRC 30078	DQ018105	DQ018083	–
Pseudomassariosphaeria grandispora	CBS 613.86	EF165034	GU296172	GU371725
Pseudomonodictys tectonae	**MFLUCC 12-0552 T**	KT285573	KT285574	KT285572
Pseudostrickeria muriformis	**MFLUCC 13-0764 T**	KT934254	KT934258	–
Pseudoxylomyces elegans	**MAFF 243852 T**	AB807598	AB797308	–
Psiloglonium clavisporum	**CBS 306.38 T**	FJ469672	GU296191	–
Ramularia endophylla	**CBS 113265 T**	KF251833	–	KF252332
Rasutoria pseudotsugae	rapssd	EF114704	EF114729	–
Rasutoria tsugae	ratstk	EF114705	EF114730	GU371809
Rimora mangrovei	JK 5246A	GU301868	GU296193	GU371759
Roccella fuciformis	Tehler 8171	FJ638979	–	FJ639038
Schismatomma decolorans	Ertz 5003 (BR)	NG_027622	NG_013155	–
Schizothyrium pomi	CBS 406.61	EF134949	–	–
Sigarispora arundinis	**CBS 621.86 T**	DQ782384	DQ782383	DQ782386
Stagonospora pseudocaricis	**CBS 135132 T**	KF251762	–	KF252264
Sulcatispora acerina	**KT 2982 T**	LC014610	LC014605	–
Sulcatispora berchemiae	**KT 1607 T**	AB807534	AB797244	–
Teratosphaeria fibrillosa	CBS 121707	GU323213	GU296199	–
Tetraploa aristata	CBS 996.70	AB524627	AB524486	–
Tetraplosphaeria sasicola	**JCM 13167 T**	AB524631	AB524490	–
Trematosphaeria grisea	**CBS 332.50 T**	KF015614	KF015641	KF015720
Trematosphaeria pertusa	**CBS 122368 T**	FJ201990	FJ201991	FJ795476
Tubeufia chiangmaiensis	**MFLUCC 11-0514 T**	KF301538	KF301543	–
Tubeufia javanica	**MFLUCC 12-0545 T**	KJ880036	KJ880035	–
Uwebraunia commune	CBS 114238	EU019267	GU214526	–

1 The ex-type strains are in bold, the new family introduced in this study is indicated in red.

Results

Topology of phylogenetic analysis

The final alignment of the combined dataset consisted of 137 strains and 3002 characters (LSU 903 characters, SSU 1033 characters, and RPB2 1066 characters). The tree topology for LSU was compared visually to confirm that it is correlated with the overall tree topology obtained from the combined alignment at the family level. The topology at family levels is similar to previous studies (Sharma et al. 2015, Boonmee et al. 2016, Li et al. 2016). The Bayesian analysis and maximum likelihood topology are comparable, except for *Fuscostagonospora sasae* (CBS 139687)

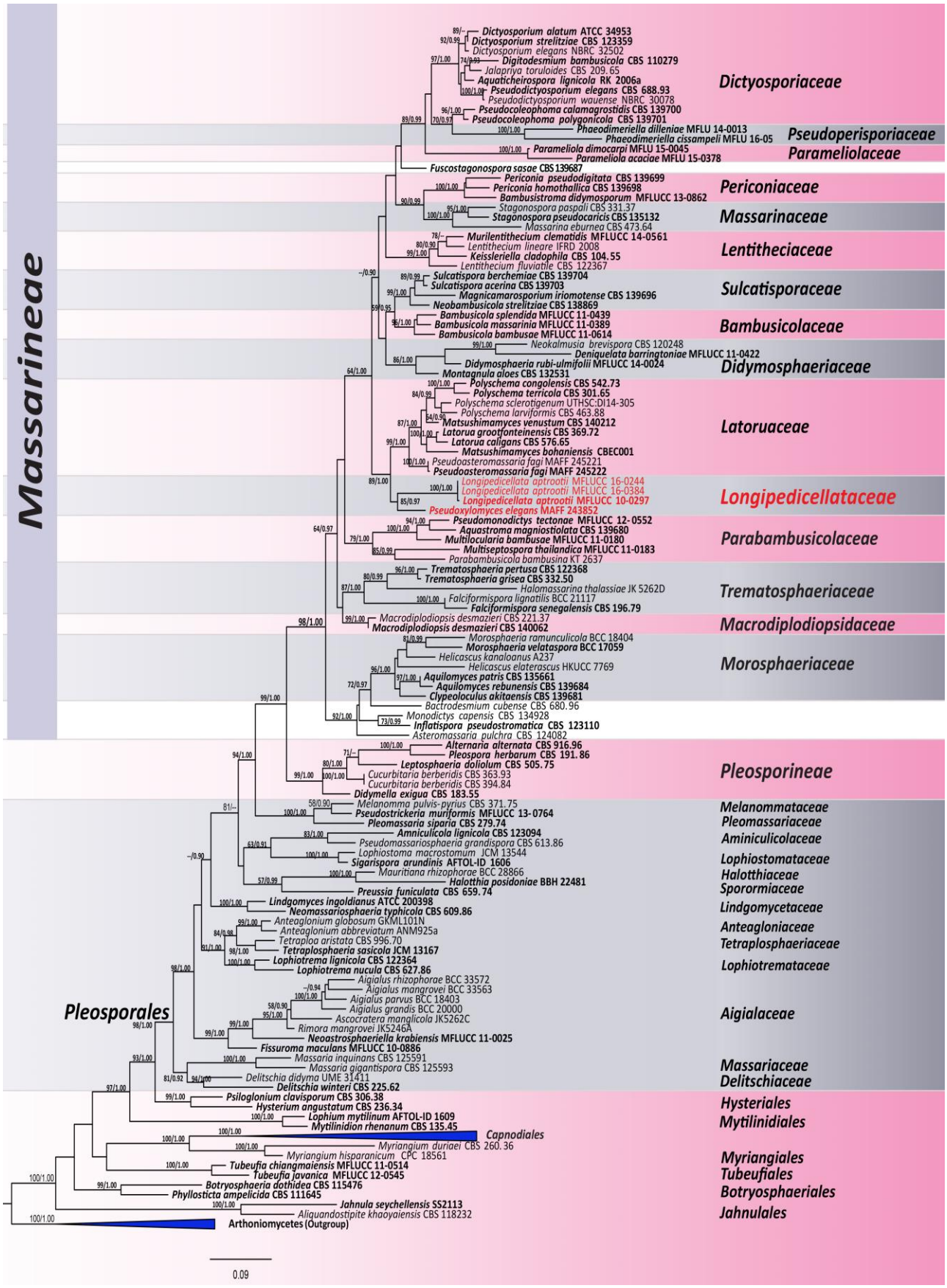

Fig. 1 – The best scoring RAxML tree based on a combined partial LSU, SSU, and RPB2 gene datasets. Bootstrap values ≥70% from the maximum likelihood (ML) analysis are followed by Bayesian posterior probabilities (PP) values ≥0.90. The tree is rooted with Arthoniomycetes as the outgroup taxa. The family Longipedicellataceae which determined in this study is indicated in red. The ex-type and references strains are indicated in black bold. Hyphen (-) represents support values ≤70%/0.90.

being a sister taxon to Periconiaceae and Massarinaceae in the Bayesian analysis (data not shown). The best scoring RAxML tree had a final likelihood value of -49637.912100 and is shown in Fig. 1.

Our analysis included 109 strains from Pleosporales, which forms a sister group to Hysteriales with high support (93 % ML/1.00 PP). Three strains of *Longipedicellata aptrootii* form a sister group to *Pseudoxylomyces elegans* (MAFF 243852) with strong support (85 % ML/0.97 PP) and we introduce the family Longipedicellataceae for these two genera. Longipedicellataceae clusters in the suborder Massarineae (Pleosporales). Longipedicellataceae further clusters with Latoruaceae with high support (89 % ML/1.00 PP).

Furthermore, in our analysis *Phaeodimeriella* (Pseudoperisporiaceae) (Mapook et al. 2016) and two strains of *Parameliola* (Li et al. 2016) form a distinct lineage, sister to Dictyosporiaceae. *Phaeodimeriella dilleniae* and *Ph. cissampeli* form a sister clade with *Pseudocolephoma* species (70% ML/ 0.97 PP) which Tanaka et al. (2015) placed in Dictyosporiaceae. *Parameliola* formed a clade with *Phaeodimeriella* and *Pseudocolephoma,* however there is not good statistic support for the internal clade. Thus, our results provide evidence for the use of Pseudoperisporiaceae and Parameliolaceae. *Pseudocolephoma* may need transferring to Pseudoperisporiaceae following further studies. *Fuscostagonospora sasae* may be a new family, but we refrain from introducing it here as there is only one representative strain.

Divergence time estimates

The topologies from the BEAST analysis are congruent with the result from the maximum likelihood analysis (Fig. 1). The divergence time estimates based on scenarios 2 (one secondary and two fossil calibrations) are shown in Fig. 2, the calibration points are indicated with red dots. The horizontal bars at the nodes represents the 95% highest posterior probability (HPD) intervals for the age of that node. The mean dates estimated in this study mostly rely on highest posterior density of previous studies (Beimforde et al. 2014, Pérez-Ortega et al. 2016). The divergence times

Table 2 Divergence time estimates of Dothideomycetes lineages obtained from BEAST analysis using calibrations from amber (*Aigialus* sp., Metacapnodiaceae). For each divergence, the median and the 95% Highest Posterior Density are provided. Divergence times are provided in millions of years (Mya). The geological time scales are given. The node numbers correspond to numbers used in Fig. 2 to show placement in the chronogram.

Nodes		**This study**				**Gueidan et al. (2011)**	**Prieto & Wedin (2013)**	**Beimforde et al. (2014) (Cali 2)**	**Pérez-Ortega et al. (2016)**
		Scenario 1 (1 fossil)	**Geological time scales**	**Scenario 2 (2 fossil)**	**Geological time scales**				
1	Dothideomycetes-Arthoniomycetes	270 (167-369)	Permian	317 (236-397)	Carboni-ferous	362	302	362	313
2	Capnodiales crown group	122 (65-181)	Middle Cretaceous	147 (102-202)	Late Jurassic	-	-	~160	-
3	Aigialaceae – *Aigialus sp.*	22 (10-39)	Miocene	39 (35-49)	Eocene	-	-	-	-
4	Dothideomycetes crown group	244 (148-347)	Middle Triassic	293 (213-371)	Permian	338	174	350	290
5	Pleosporales crown group	170 (95-247)	Middle Jurassic	211 (153-277)	Late Triassic	~190	-	~150	-
6	Massarineae-Plesporineae	115 (65-168)	Cretaceous	144 (104-191)	Early Cretaceous	-	-	-	-
7	Massarineae crown group	104 (57-151)	Cretaceous	130 (96-174)	Cretaceous	-	-	-	-
8	Latoruaceae-Longipedicellataceae	59 (30-94)	Paleocene	75 (48-107)	Cretaceous	-	-	-	-
9	Longipedicellataceae crown group	44 (15-77)	Eocene	56 (25-89)	Eocene	-	-	-	-
10	Latoruaceae crown group	42 (22-70)	Eocene	53 (31-78)	Eocene	-	-	-	-

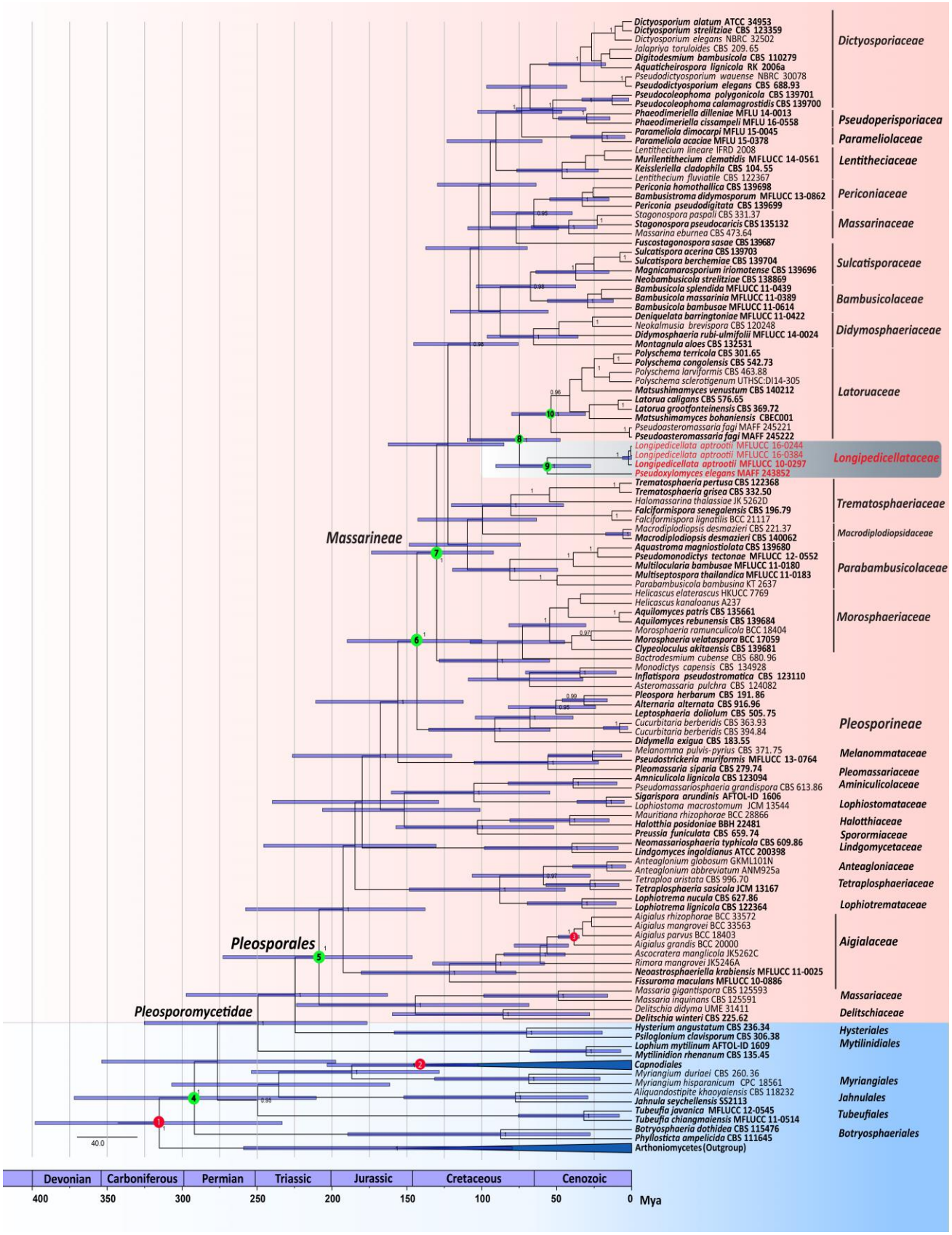

Fig. 2 – Maximum clade credibility (MCC) tree with divergence times estimates for main groups of the Dothideomycetes obtained from a Bayesian approach (BEAST) using four fossil calibrations. Numbers at nodes indicate posterior probabilities (PP) for node support. Bars correspond to the 95% highest posterior density (HPD) intervals. The fossil minimum age constraints and second calibrations used in this study are marked with red dots. Geological time scales are given at the base, together with scale in million years ago (Mya). The nodes specially discussed in this study are marked with green dots. Longipedicellataceae is highlighted in gray. For estimated median ages of numbered nodes, see Table 2.

of the dating analysis are listed in Table 2. The crown group discussed below refers to all the descendants of the last common ancestor living in the group. The maximum clade creditability tree indicates that the split of Dothideomycetes and Arthoniomycetes occurred around 317 (236−397) Mya, in the Carboniferous. The split between Massarineae and Pleosporineae, two major suborders within Pleosporales occurred approximately 144 (104−191) Mya, in the Cretaceous. The Pleosporales crown group evolved around 211 (153−277) Mya during the Triassic. In this study, we focus on the suborder Massarineae which evolved around 130 (96−174) Mya, during the Cretaceous. Based on our analysis, the families within Massarineae mostly evolved in the Cenozoic era (present around 66 Mya), with the exception of Trematosphaeriaceae, which evolved in the late Cretaceous (80 Mya). Latoruaceae and Longipedicellataceae diverged around 75 (48−107) Mya in the late Cretaceous period. The common ancestor of living representative of Longipedicellataceae strains collected from freshwater (crown group) occurred around 56 (25−89) Mya, in the Cenozoic. Latoruaceae, is a related family, known from soil and hot springs with a crown age around 53 (31−78) Mya, in the Eocene.

Taxonomy

Longipedicellataceae Phukhams., J. Bhat & K.D. Hyde, *fam. nov.*

Index Fungorum Number: IF552532, Facesoffungi number: FoF 01408

Type genus – *Longipedicellata* Zhang, K.D. Hyde & J.K. Liu Phytotaxa 247 (2): 104 (2016)

Saprobic on dead and submerged woody material. Sexual morph *Ascomata* semi-immersed to erumpent in host tissue, coriaceous, solitary, scattered, sometimes embedded under a pseudoclypeus, subglobose to ellipsoidal, black to brown, ostiolate. *Peridium* multi-layered, of black to brown cells of *textura angularis*, sometimes *textura prismatica*, somewhat carbonaceous, thin, easy to break. *Hamathecium* comprising few, long, broad, septate, branched, cellular pseudoparaphyses, surrouding asci and along the inner layer of peridium. *Asci* 8-spored, bitunicate, fisitunicate, clavate, long-pedicellate, bulbous, thin-walled, with an apical ocular chamber. *Ascospores* bi-seriate, overlapping, ellipsoidal, narrowly subfusiform, conical at apex, hyaline, 1-septate, constricted at septum, guttulate, smooth-walled. Asexual morph: Hyphomycetous *Colonies* black to dark-brown, circular, effuse. *Mycelium* composed of smooth, hyaline to dark brown, septate, branched, hyphae, swollen in ovoid cells. *Chlamydospores* acropetal, catenate, doliiform, branched, subglubose to oval, rough-walled, in long chains, brown to reddish-brown.

Notes – Longipedicellataceae includes the genera *Longipedicellata* and *Pseudoxylomyces* which are reported from aquatic habitats, as saprobes on woody substrates. Longipedicellataceae is characterized by semi-immersed or erumpent, clypeate ascomata on the host tissues, with black to brown ostiolates, clavate asci with long pedicels, and 2-celled, hyaline ascospores. Chlamydospore formation in *Longipedicellata* and *Pseudoxylomyces* is also significant. All the strains formed a well-supported clade (89%ML/1.00PP) basal to Latoruaceae within the suborder Massarineae (Pleosporales). *Longipedicellata* and *Pseudoxylomyces* clustered together with good support (85 % ML/0.97 PP).

Longipedicellata Zhang, K.D. Hyde & J.K. Liu Phytotaxa 247 (2): 104 (2016)

Index Fungorum Numbers: IF551685, *Facesoffungi number*: FoF 02665

Type species – *Longipedicellata aptrootii* (Hyde & Wong) Zhang, K.D. Hyde & J.K. Liu

Saprobic on dead and submerged woody material in freshwater. Sexual morph: *Ascomata* lenticular, immersed, beneath a blackened pseudoclypeus, scattered or clustered, coriaceous, black to brown, sometimes ostiolate. *Peridium* comprising multi-layered, black to brown cells of *textura angularis*, outer layer somewhat carbonaceous, inner layer composed of subhyaline gelatinous cells, easy to break. *Hamathecium* comprising few, long, broad, transversely septate, branched, cellular pseudoparaphyses, surrounding asci and along the inner layer of peridium. *Asci* 8-spored, bitunicate, fisitunicate, clavate, long-pedicellate, thin-walled, with an ocular chamber. *Ascospores* 8-spored, bi-seriate, overlapping, ellipsoid, narrowly subfusiform, conical at apex, hyaline, with 1-transverse-septa, constricted at the septum, guttulate at the centre of each cell, surrounded by

mucilaginous sheath (adaptation from Zhang et al. 2016). Asexual morph: Hyphomycetous. *Colonies* dark-brown to black, circular, effuse, with serrate margin. *Mycelium* composed of smooth, hyaline to dark brown, septate, branched hyphae, flexuous, slightly filiform on agar suface, septate, branched, thick-walled, smooth, pale brown, septate, integrated, terminal or sometimes intercalary, gangliar-type (Bhat 2010), smooth to verrucose, hyaline to pale brown, tranformed into chlamydospores. *Conidia* chlamydosporous monilioid, in acropetal chains, doliiform, subglubose to oval, rough-walled, brown to reddish-brown.

Longipedicellata aptrootii (Hyde & Wong) Zhang, K.D. Hyde & J.K. Liu, Phytotaxa 247(2): 104 (2016) Fig. 3

Index Fungorum number: IF551686 *Facesoffungi number*: FoF: 01273

≡ *Didymella aptrootii* Hyde & S.W. Wong, Australas. Mycol. 18(3): 54 (1999)

Holotype – IFRD (HKU(M) 3333)!)

Saprobic on dead and submerged stem of Bambusodeae. Sexual morph: *Ascomata* 115–143 μm high × 182–269 μm diam. ($\bar{x}$ = 125 × 235 μm, n = 10), semi-immersed to erumpent in the host tissue, coriaceous, solitary, scattered or in small groups, sometimes under the clypeus, subglobose to ellipsoidal, rough-walled, black to brown, sometimes ostiolate. *Peridium* 7–15(–25) μm wide, composed of 2–4 layers of black to brown cells of *textura angularis* and *textura prismatica*, outer layer somewhat carbonaceous, inner layer composed of subhyaline gelatinous cells, up to 10 μm, thin, easy to break. *Hamathecium* comprising few, 1.1–2.8 μm wide ($\bar{x}$ = 2 μm, n = 20), long, broad, septate, branched, cellular pseudoparaphyses, surrounding asci and along the inner layer of peridium. *Asci* 63–170 × 14–25 μm ($\bar{x}$ = 81 × 18 μm, n = 70), 8-spored, bitunicate, fisitunicate, clavate, long-pedicellate, 28–38 μm, bulbous, thin-walled, shallow ocular chamber up to 1.8 μm wide × 2 μm high. *Ascospores* 19–26 μm × 7–11 μm ($\bar{x}$ = 23 × 9 μm, n = 70), overlapping bi-seriate, ellipsoid, narrowly subfusiform, conical at the apex, hyaline, 1-septate, constricted at the septum, guttulate in the centre of cells, smooth-walled. Asexual morph: Hyphomycetous. Mycelium producing chalmydospores and chlamydospore-like structures after two months, smooth-walled, hyaline to dark brown, septate, branched, hyphae, with ovoid cells 0.7–2(–3) μm diam.. *Colonies* black to dark-brown, circular, effuse, flexuous, slightly filiform on agar suface, septate, branched, thick-walled, smooth, pale brown, gangliar-type, integrated, terminal or sometimes intercalary, smooth to verrucose, hyaline to pale brown,.tranformed into chlamydospores. *Conidia* chlamydosporous 4–14 × 5–11 μm diam. ($\bar{x}$ = 9 × 11 μm, n = 80), monilioid, in long acropetal chains, catenate, doliiform, branched, subglubose to oval, rough-walled, brown to reddish-brown.

Culture characteristics – Ascospores germinating on PDA within 24 h, with germ tubes developing from apical and basal cells. Colonies on PDA reaching 20 mm in diameter after four weeks at 25°C. Colonies black, with arial white mycelium, umbonate at the centre, with circular, friable, dark brown margin; reverse dark brown to black, with a horizontal, deep slit in the agar. Chlamydospore-like structures formed in culture after two months of incubation.

Material examined – Hong Kong, New Territories, Plover Cove Reservoir, on submerged bamboo, 15 November 1996, K.D. Hyde (IFRD (HKU(M) 3333)!); Thailand, Chiang Rai, Hui Kang Pla Waterfall, on submerged bamboo (Poaceae), 18 January 2010, Huang Zhang (MFLU 10–0162!, reference specimen), ex-culture: MFLUCC 10–0297; *ibid* (MFLU 10–0163!, MFLU 10–0164!, MFLU 16–0032!); Chiang Rai Province, on dead and submerged stem of Bambusodeae (Poaceae), 14 June 2015, C. Phukhamsakda, CP015 (MFLU 16–0032, paratype), *ibid*., 15 January 2016, ex-paratype living culture, MFLUCC 16–0384, KMUCC 15–0552.

Notes – The genus *Longipedicellata* was introduced by Zhang et al. (2016) based on a collection of *Didymella aptrootii* K.D. Hyde & S.W. Wong, from a freshwater stream in Thailand. In their phylogenetic analyses, the genus showed a close relationship to Bambusicolaceae with moderate support (65 % ML/0.99 PP). Therefore, Zhang et al. (2016) transferred *Didymella aptrootii* to *Longipedicellata aptrootii* and placed it in Bambusicolaceae. We also collected fresh material of *Longipedicellata aptrootii*. Our collection (MFLUCC 16–0384) was morphologically similar with the type material of *L. aptrootii*. In our analyses, the genus formed a close relationship

with *Pseudoxylomyces elegans* (Goh et al.) Kaz. Tanaka & K. Hiray. basal to Latoruaceae (85 % ML/0.97 PP). The *L. aptrootii* strain (MFLUCC 16–0384) formed chlamydospore-like structures in culture, and produced conidia in chains at the apex which were similar to *Pseudoxylomyces elegans. Pseudoxylomyces elegans* also produces chlamydospores in chains at the apex of hypha (Goh et al. 1997).

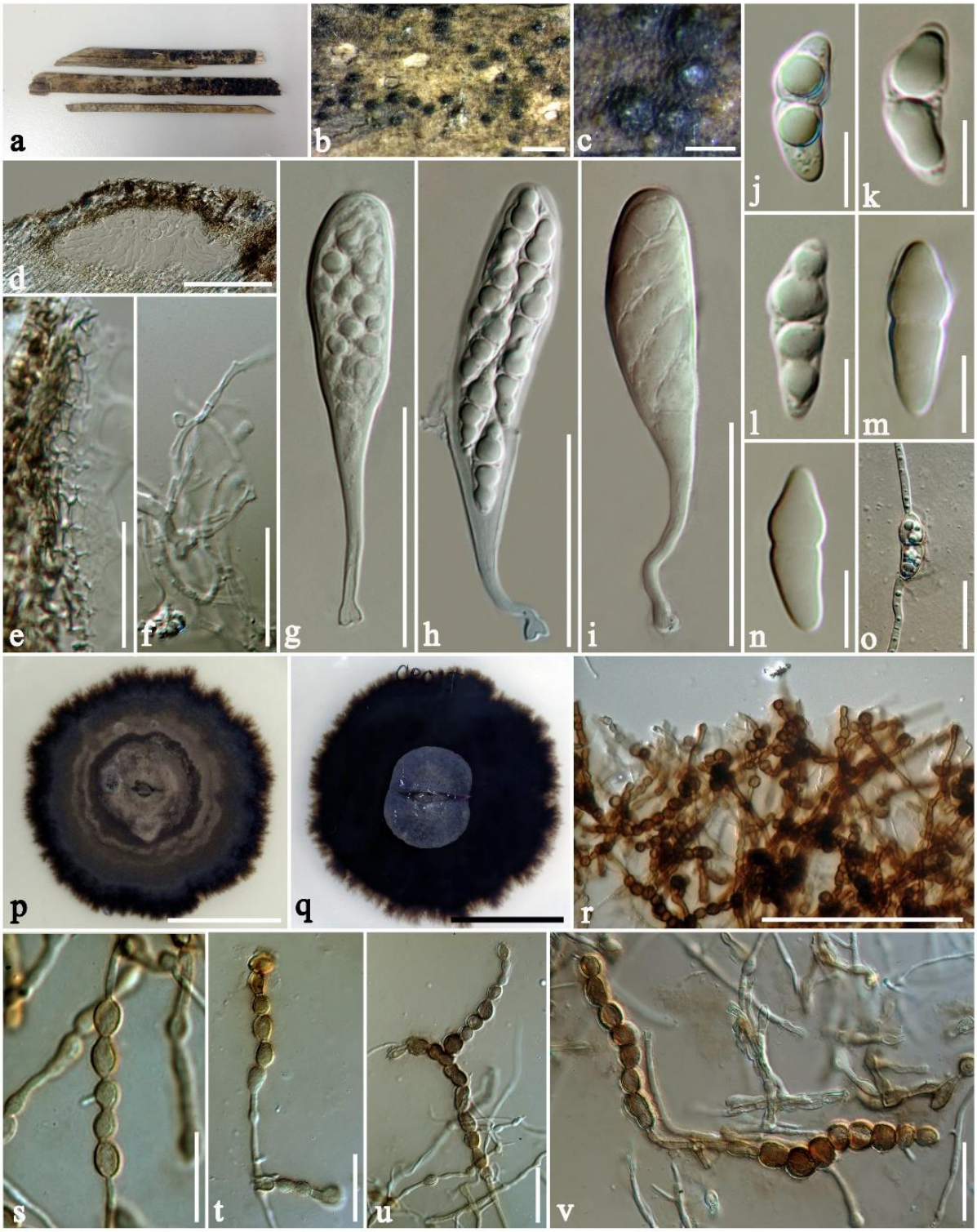

Fig. 3 – *Longipedicellata aptrootii* (MFLU 16–0032, **reference specimen**). **a** Bambusodeae sp. habit. **b**, **c** Appearance of ascomata on the host surface. **d** Vertical section of ascoma. **e** Section of peridium comprising cells of *textura prismatica*. **f** Pseudoparaphyses. **g**–**i** Asci with long pedicels. **j**–**n** Development stages of ascospores. **o** Germinated ascospore. **p**, **q** Culture characters on PDA. **r** Close up of culture. **s** Chlamydospores. **t**–**v** Chlamydospore-like conidia in chains. – Bars: b = 500 µm, c = 200 µm, d = 100 µm, e–f, o, s–v = 20 µm, g–i = 50 µm, j–n = 10 µm, p–q = 20 cm.

Pseudoxylomyces (Goh, W.H. Ho, K.D. Hyde & K.M. Tsui) Tanaka & K. Hirayama., Studies in Mycology 82: 126. 2015.

Type species – *Pseudoxylomyces elegans* (Goh, W.H. Ho, K.D. Hyde & K.M. Tsui) Tanaka & K. Hirayama, Mycol. Res. 101 (11): 1324 (1997)

Holotype – HKU (M) 2777

See Goh et al. 1997 for description, Figs. 15–20.

Notes – *Pseudoxylomyces* was introduced to accommodate *Xylomyces elegans*, that clustered in Pleosporales and not Jahnulales. Thus, *Xylomyces elegans* was synonimyzed as *Pseudoxylomyces elegans* (Tanaka et al. 2015). *Pseudoxylomyces elegans* collections are reported from aquatic habitats on decaying wood. Tanaka et al. (2015) showed that *Pseudoxylomyces elegans* formed a basal clade to Trematosphaeriaceae and then placed it genus *incertae sedis* in the suborder Massarineae (Pleosporales). In our analysis, *P. elegans* is inferred as closely related to *Longipedicellata aptrootii* with strong support (85 % ML/0.97 PP). However, no sexual morph is reported for *P. elegans*. When comparing asexual morphs of the genera, they both form chlamydospores-like structures. *Pseudoxylomyces elegans*, is characterized by dematiaceous mycelia and conidiophores and chlamydospore-like, thick-walled, terminal or intercalary, septate conidia, which are very similar to the chlamydospore structures seen in *Longipedicellata aptrootii.* They may even be the same genus.

Discussion

In this paper, we introduce a new family, Longipedicellataceae, in the suborder Massarineae (Pleosporales). Longipedicellataceae includes the genera *Longipedicellata* (*L. aptrootii*) and *Pseudoxylomyces* (*P. elegans*) which are found on woody debris in freshwater (Hyde & Wong 1999, Zhang et al. 2016).

Divergence time estimates

Pleosporales is the largest order in Dothideomycetes, and species have a highly diverse range of habitat and lifestyles (Zhang et al. 2012, Hyde et al. 2013). Pleosporales is included in the subclass Pleosporomycetidae, and shares a common ancestry with Hysteriales and Mytilinidiales (Boehm et al. 2009, Hyde et al. 2013). The order has unique characteristics in its perithecial ascomata, whereas Hysteriales and Mytilinidiales commonly have hysterothecial, carbonaceous and elongate ascomata (Boehm et al. 2009, Mugambi & Huhndorf 2009, Lumbsch & Huhndorf 2010).

Divergence time estimates in this study are based on calibration points for Dothideomycetes and previous studies (Beimforde et al. 2014, McLaughlin & Spatafora 2015, Pérez-Ortega et al. 2016). In addition, the ages of the order Pleosporales and the divergences time of Longipedicellataceae and related families in Pleoporales were estimated in this study.

Schmidt et al. (2010) reported a Curvularia-like taxon in Ethiopian amber from the Cretaceous period. However, similar dematiaceous hyphomycetes with three horizontal septate conidia can be found in various fungal genera (Ellis 1971, Seifert et al. 2011). Consequently, Beimforde et al. (2014) did not use this fossil for calibration of Pleosporales. Mindell et al. (2007) described *Margaretbarromyces dictyosporus,* a pleosporalean species from Vancouver Island (Canada). The species colonized fossilized woody bark in rocks dated to the Eocene epoch (56–33 Mya, Mindell et al. 2007, Berbee & Taylor 2010). Based on the information from the fossil, Mindell et al. (2007) compared this fungus to *Cucurbitaria* (Cucurbitariaceae, Pleosporales). The morphology is also comparable to extant genera in Pleosporales; *Margaretbarromyces dictyosporus* is similar to the marine fungus *Aigialus* (Aigialaceae, Pleosporales). This intertidal genus is typically associated with bark or dead wood in mangroves (Suetrong et al. 2009, Jones et al. 2015). The morphology is similar based on the flask-shaped pseudothecial, uniloculate, immersed ascomata, with ostioles filled with periphyses, a peridium of *textura intricata*, overlapping asci, and dictyospores with lighter or globose, hyaline, end cells (Kohlmeyer & Schatz 1985, Hyde 1992, Schmit & Shearer 2003, Suetrong et al. 2009, Jones et al. 2015).

In our study, we applied two dating scenarios to compare the unity of applying the fossil calibration to the order Pleosporales. Scenario 1 without calibration of *Aigialus* crown groups and scenario 2 given the estimates constraint of the *Aigialus* crown group. The fossil ages were used as minimal age constraints. The divergence time estimation resulted in slightly different dates between the two scenarios tested. In general, scenario 2 resulted in older age estimates than scenario 1. However, the result from scenario 2 are rather similar to previous studies (Table. 2, Vijaykrishna et al. 2006, Schmidt et al. 2010, Gueidan et al. 2011, Prieto & Wedin 2013, Beimforde et al. 2014, McLaughlin & Spatafora 2015, Pérez-Ortega et al. 2016). The split of Dothideomycetes and Arthoniomycetes occurred around 270 Mya in scenario 1, versus 317 Mya in scenario 2. The Pleosporales crown group occurred around 170 Mya in the middle Jurassic, versus 211 Mya in the late Triassic. The order Pleosporales split from Hysteriales in the Triassic (227 Mya) in scenario 2 (Fig. 2). The split between suborder Massarineae-Plesporineae is estimated occur around 115 Mya in scenario 1, versus 144 Mya in scenario 2, the suborder Massarineae has estimated crown age around 104 Mya in scenario 1 (late Cretaceous), versus 130 Mya in scenario 2 (Cretaceous), the family Longipedicellataceae evolved around 44 Mya in scenario 1, versus 56 Mya in scenario 2 (Eocene). Although the estimates differ, they provide an idea of the order of evolutionary events and can thus be used to determine the order and rough times when different higher taxa evolved and can be used as evidence to support the status of these taxa (Li et al. 2005, Beimforde et al. 2014, Hongsanan et al. 2016, Samarakoon et al. 2016, Zhao et al. 2016).

Previously dating studies estimated diversification events between Dothideomycetes and Arthoniomycetes to be over 300 million years ago in the Carboniferous period (Gueidan et al. 2011, Beimforde et al. 2014, McLaughlin & Spatafora 2015, Pérez-Ortega et al. 2016). Our analysis demonstrate that applying a constraint at a node close to the tips is informative to the overall divergence times in the analysis. Consequently, the derived age estimates are reliant and affected by the selected fossil calibration point constraint in each analysis (Beimforde et al. 2014, Garnica et al. 2016). We present the scenario with oldest ages of Arthoniomycetes-Dothideomycetes split (scenario 2) which is more similar to previous studies.

Evolution history of Longipedicellataceae

Molecular phylogenetic analysis has been used to distinguish and provide evidence for various taxon levels in fungal systematics (Li et al. 2005, Ertz et al. 2015, Maharachchikumbura et al. 2015). In this study, we applied the relaxed molecular clock to date divergences in Pleosporales. Based on our phylogenetic result, Massarineae and Plesporineae form two major clades in Pleosporales. In recent studies, 14 families and six taxa *incertae sedis* were placed in the suborder Massarineae (Tanaka et al. 2015, Li et al. 2016, Mapook et al. 2016). The split between Massarineae and Plesporineae occurred around 144 Mya. The Massarineae crown age is estimated to be around 130 Mya and the Plesporineae crown age is around 93 Mya. The values for Plesporineae may be underestimated since there may be basal taxa in this suborder that are not included in this study. Species of Pleosporineae are mostly reported as pathogens or endophytes in plants (Ginkel et al. 1998, Doilom et al. 2013, Ariyawansa et al. 2015). On the other hand, Massarineae are frequently saprobes in woody substrates (Barr 1979, Vijaykrishna et al. 2006, Kirk et al. 2008, Zhang et al. 2013, Tanaka et al. 2015). However, some taxa, such as *Deniquelata* (Didymosphaeriaceae), *Setoseptoria* (Lentitheciaceae) and *Stagonospora* (Massarinaceae) are plant pathogens (Ariyawansa et al. 2013, Hyde et al. 2016). Latoruaceae and Longipedicellataceae appear to have evolved around 75 Mya. Based on the molecular clock, Latoruaceae and Longipedicellataceae are warranted as distinct families within Massarineae.

The morphologies of these families correspond to the range found within Massarineae. *Longipedicellata aptrootii* was collected from submerged bamboo debris in different locations (Hyde & Wong 1999, Zhang et al. 2016, this study). *Pseudoxylomyces elegans* was reported from various locations, but all from submerged wood in freshwater habitats (Goh et al. 1997, Prihatini et al. 2008, Tanaka et al. 2015). In our phylogenetic analysis, *Longipedicellata aptrootii* is closely related to *Pseudoxylomyces elegans* with high support (85% ML/0.97 PP). This has been shown in

previous studies with *Pseudoxylomyces elegans* forming a clade within Pleosporales (Prihatini et al. 2008, Tanaka et al. 2015). Morphologically, the genus *Pseudoxylomyces*, typified by *P. elegans*, is characterized by dematiaceous mycelia and gangliar-type of conidia production (Bhat 2010). Chlamydospore-like, thick-walled, terminal or intercalary, septate conidia structures seen in *Longipedicellata aptrootii*. Distinguishable conidia are absent in both species. In the phylogenetic analysis, *Pseudoxylomyces* along with *Longipedicellata* form a separate clade from Latoruaceae. Latoruaceae species were reported from various habitats, mostly from soil and some are thermo-tolerant (Agrawal & Tiwari 1997, Sharma et al. 2015). Longipedicellataceae and Latoruaceae are similar in their hyphomycetes-like morphology of the asexual morph in *Latorua, Matsushimamyces*, and *Polyschema*. Nevertheless, those genera produce distinguishable conidia and not chlamydospore-like structures.

This study is unique in studies on Dothideomycetes as it uses a polyphasic approach in introducing a new family based on morphology, phylogeny and evolutionary divergence times and thus provides strong evidence for the application of Longipedicellataceae. It also provides estimates for the divergence of the subclass Pleosporomycetidae (249 Mya), order Pleosporales (211 Mya), suborders Massarineae (130 Mya) and Pleosporineae (93 Mya), families Parabambusicolaceae (81 Mya), Didymosphaeriaceae (66 Mya), Longipedicellataceae (56 Mya), Dictyosporiaceae (34 Mya) and the youngest family Macrodiplodiopsidaceae (6 Mya). Branch legth may represent unknown taxa that have either not been discovered or they are probably extinct taxa. Therefore, the taxon sampling in a group impacts on the age estimates of the crown nodes. It will be of considerable interest to compare the divergence times of the various higher taxon ranks (phylla, classes, orders, families, genera) across the kingdom fungi and establish if each ranks evolved at roughly the same time period or whether there is considerable disparity in the divergence times (Hedges et al. 2015, Samarakoon et al. 2016) and thus whether mycologists have treated different groups similarly or differently.

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