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Integrating Different Lines of Evidence to Establish a Novel Ascomycete Genus and Family (*Anastomitrabeculia*, *Anastomitrabeculiaceae*) in *Pleosporales*

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Abstract: A novel genus, *Anastomitrabeculia,* is introduced herein for a distinct species, *Anastomitrabeculia didymospora*, collected as a saprobe on dead bamboo culms from a freshwater stream in Thailand. *Anastomitrabeculia* is distinct in its trabeculate pseudoparaphyses and ascospores with longitudinally striate wall ornamentation. A new family, *Anastomitrabeculiaceae,* is introduced to accommodate *Anastomitrabeculia*. *Anastomitrabeculiaceae* forms an independent lineage basal to *Halojulellaceae* in *Pleosporales* and it is closely related to *Neohendersoniaceae* based on phylogenetic analyses of a combined *LSU, SSU* and *TEF1α* dataset. In addition, divergence time estimates provide further support for the establishment of *Anastomitrabeculiaceae.* The family diverged around 84 million years ago (MYA) during the Cretaceous period, which supports the establishment of the new family. The crown and stem age of *Anastomitrabeculiaceae* was also compared to morphologically similar pleosporalean families.

Keywords: BEAST; *Dothideomycetes*; *Pleosporales*; *Poaceae*; taxonomy; three new taxa; trabeculate pseudoparaphyses

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1. Introduction

Pleosporales is the largest order within *Dothideomycetes* (*Ascomycota*) [1]. The taxonomic and phylogenetic relationships of families and genera within this order are well documented [1–7]. *Pleosporales* comprises two suborders, *Massarineae* and *Pleosporineae* [1]. *Pleosporineae* includes economically important plant pathogens and *Massarineae* includes mainly saprobes from terrestrial or aquatic environments [1,3]. Zhang et al. [1] revised 174 genera and accepted 26 families in *Pleosporales*. The suborder *Massarineae* was resurrected to accommodate five families, the *Lentitheciaceae*, *Massarinaceae*, *Montagnulaceae* (*Didymosphaeriaceae*), *Morosphaeriaceae* and *Trematosphaeriaceae* [1]. Hyde et al. [2] correlated morphology with phylogenetic evidence and accepted 41 families in this order. Tanaka et al. [3] introduced two new families, *Parabambusicolaceae* and *Sulcatisporaceae*, accepting 12 families in *Massarineae*. The family *Longipedicellataceae* was introduced, and the divergence time in *Pleosporales* was estimated with emphasis on *Massarineae* [4]. The crown age of *Pleosporales* was dated to 211 MYA and *Massarineae* was dated to 130 MYA [4]. Species boundaries in *Cucurbitariaceae* were revised [5] and the family, *Lentimurisporaceae*, was introduced in *Pleosporales* [6].

Species in this order are abundant and occur in terrestrial, marine and freshwater habitats [7–9]. The species can be epiphytes, endophytes or parasites of living leaves or stems, hyperparasites on fungi or insects, lichenized, or saprobes of dead plant stems, leaves or bark [7–9]. Currently, about 400 genera in 64 families are known in *Pleosporales* [1,2,7,10–13], with numerous coelomycetous and hyphomycetous taxa as their asexual morphs [1,13–15].

Several pleosporalean taxa are pathogens associated with a broad range of hosts including bamboo. Bamboo (*Poaceae*) comprises over 115 genera with around 1500 species [16–18], can be found in diverse climates [17], and are widely distributed in various forest types in Thailand [18,19]. It has been estimated that around 1100 fungal species belonging to over 200 genera have been described or recorded worldwide on bamboo and most of these bamboo-associated fungi are ascomycetes [20,21].

Divergence time estimates using molecular clock methodologies have been widely used in fungal taxonomy [4,11,22–27]. Several studies have applied molecular dating to provide additional evidence for higher taxa ranking in *Pleosporales* [4,6,7,11]. In this study, we introduce a novel bambusicolous species, *Anastomitrabeculia didymospora* within *Anastomitrabeculia*, which is accommodated in a new family, *Anastomitrabeculiaceae*, based on morphology, multi-loci phylogeny and divergence times estimates.

2. Materials and Methods

2.1. Sample Collection, Isolation and Identification

Dead bamboo culms were collected from a freshwater stream from Krabi province, Thailand, in 2015. The samples were incubated in plastic boxes with sterile and moist tissue at 25–30 °C for 3 days. Pure fungal colonies were obtained using single-spore isolation [28]. Germinating spores were transferred aseptically to potato dextrose agar (PDA) and malt extract agar (MEA) (Difco™). The cultures were incubated at 25 °C with frequent observations. Fungal characters were observed using a stereo microscope (Zeiss SteREO Discovery v. 8) fitted with an Axio Cam ERc5S and a Leica DM2500 compound microscope attached with a Leica MC190 HD camera. All microscopic measurements were carried out using Tarosoft (R) Image Frame Work program and the images were processed with Adobe Photoshop CS6 version 13.0 software (Adobe Systems, San Jose, CA, USA). The type specimens were deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand, and pure cultures were deposited at the Mae Fah Luang University Culture Collection (MFLUCC). The new taxon was linked with Facesoffungi numbers (FoF) [29] and Index Fungorum (Index Fungorum 2020, http://www.indexfungorum.org/, accessed on 2 December 2020) and established based on guidelines recommended by Jeewon and Hyde [30].

2.2. DNA Extraction, PCR Amplification and DNA Sequencing

DNA extraction, PCR amplification, DNA sequencing and phylogenetic analysis were carried out as detailed in Dissanayake et al. [31]. Total genomic DNA was extracted from fresh mycelium with a Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) (Hangzhou, P.R. China) following the manufacturer's protocol. The nuclear ribosomal large subunit 28S rRNA gene (*LSU*) [32], the nuclear ribosomal small subunit 18S rRNA gene (*SSU*) [33] and the translation elongation factor 1-alpha gene (*TEF1α*) [34] were amplified using primers (*LSU*: LROR/LR5, *SSU*: NS1/NS4 and *TEF1α*: 983F/2218R). Polymerase chain reaction (PCR) was performed using PCR mixtures containing 5–10 ng DNA, 1X PCR buffer, 0.8 units Taq polymerase, 0.3 µM of each primer, 0.2 mM dNTP and 1.5 mM $MgCl_2$. PCR conditions were set at an initial denaturation for 3 min at 94 °C, followed by 40 cycles of 45 s of denaturation at 94 °C, annealing for 50 s at 56 °C for *LSU*, *SSU* and 52 °C for *TEF1α* and extension for 1 min at 72 °C, with a final extension of 10 min at 72 °C. All the PCR products were visualised on 1% Agarose gels with added 6 µL of 4S green dyes, per each 100 mL. Successful PCR products were purified and sequencing was performed by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China). All sequences generated in this study were submitted to GenBank

(Table 1) and the ITS region of *Anastomitrabeculia didymospora* was deposited with the accession number MW413900 (MFLUCC 16-0412) and MW413897 (MFLUCC 16-0417).

Table 1. DNA sequences and GenBank numbers used for the phylogenetic analyses in this study. The ex-type strains are in bold and the new taxon introduced in this study is indicated in blue.

Taxon	Strain Number	GenBank Accession Numbers		
		LSU	*SSU*	*TEF1α*
Acrocalymma aquatica	**MFLUCC 11-0208**	JX276952	JX276953	-
Acrocalymma fici	**CBS 317.76**	KP170712	-	-
Acrocalymma medicaginis	**CPC 24340**	KP170713	-	-
Acrocalymma medicaginis	CPC 24341	KP170714	-	-
Acrocalymma medicaginis	CPC 24345	KP170718	-	-
Acrocalymma pterocarpi	**MFLUCC 17-0926**	MK347949	MK347840	-
Aigialus grandis	BCC 20000	GU479775	GU479739	GU479839
Aigialus mangrovis	BCC 33563	GU479776	GU479741	GU479840
Aigialus parvus	BCC 18403	GU479778	GU479743	GU479842
Aigialus rhizophorae	BCC 33572	GU479780	GU479745	GU479844
Aliquandostipite khaoyaiensis	CBS 118232	GU301796	-	GU349048
Amniculicola immersa	**CBS 123083**	FJ795498	GU456295	GU456273
Amniculicola lignicola	**CBS 123094**	EF493861	EF493863	-
Amniculicola parva	**CBS 123092**	GU301797	GU296134	GU349065
Amorosia littoralis	NN 6654	AM292055	AM292056	-
Anastomitrabeculia didymospora	**MFLUCC 16-0412**	MW412978	MW412977	MW411338
Anastomitrabeculia didymospora	MFLUCC 16-0417	MW413899	MW413898	MW411339
Angustimassarina populi	**MFLUCC 13-0034**	KP888642	KP899128	KR075164
Angustimassarina quercicola	**MFLUCC 14-0506**	KP888638	KP899124	KR075169
Anteaglonium abbreviatum	**ANM 925a**	GQ221877	-	-
Anteaglonium globosum	SMH 5283	GQ221911	-	GQ221919
Anteaglonium parvulum	MFLUCC 14-0821	KU922915	KU922916	-
Antealophiotrema brunneosporum	**CBS 123095**	LC194340	LC194298	LC194382
Aquasubmersa japonica	HHUF 30468	LC061586	LC061581	-
Aquasubmersa japonica	**HHUF 30469**	LC061587	LC061582	-
Aquasubmersa mircensis	**MFLUCC 11-0401**	JX276955	JX276956	-
Arthonia dispersa	UPSC 2583	AY571381	AY571379	-
Ascocratera manglicola	BCC 09270	GU479782	GU479747	GU479846
Ascocylindrica marina	MD6011	KT252905	KT252907	-
Ascocylindrica marina	MD6012	KT252906	-	-
Ascocylindrica marina	MF416	MK007123	MK007124	-
Bahusandhika indica	GUFCC 18001	KF460274	-	-
Bambusicola massarinia	**MFLUCC 11-0389**	JX442037	JX442041	-
Berkleasmium micronesicum	BCC 8141	DQ280272	DQ280268	-
Berkleasmium nigroapicale	BCC 8220	DQ280273	DQ280269	-
Bimuria novae-zelandiae	CBS 107.79	AY016356	AY016338	DQ471087
Botryosphaeria dothidea	**CBS 115476**	AY928047	EU673173	AY236898
Brevicollum hyalosporum	MAFF 243400	LC271239	LC271236	LC271245
Brevicollum hyalosporum	**MFLUCC 17-0071**	MG602200	MG602202	MG739516
Brevicollum hyalosporum	PUFNI 17628	MH918671	-	-
Brevicollum versicolor	HHUF 30591	LC271240	LC271237	LC271246
Capnodium salicinum	**CBS 131.34**	DQ678050	DQ677997	-
Cladosporium cladosporioides	CBS 170.54	DQ678057	DQ678004	-
Clematidis italica	MFLUCC 15-0084	KU842381	KU842382	-
Corynespora cassiicola	CBS 100822	GU301808	GU296144	GU349052
Corynespora smithii	CABI 5649b	GU323201	-	GU349018
Crassiparies quadrisporus	HHUF 30590	LC271241	LC271238	LC271248
Crassiparies quadrisporus	**HHUF 30409**	LC100025	LC100017	-
Crassiperidium octosporum	**KT 2144**	LC373108	LC373084	LC373120
Crassiperidium octosporum	KT 2894	LC373109	LC373085	LC373121
Crassiperidium octosporum	KT 3008	LC373110	LC373086	LC373122
Crassiperidium octosporum	KT 3029	LC373111	LC373087	LC373123

Table 1. *Cont.*

Taxon	Strain Number	GenBank Accession Numbers		
		LSU	*SSU*	*TEF1α*
Crassiperidium octosporum	KT 3046	LC373112	LC373088	LC373124
Crassiperidium octosporum	KT 3188	LC373113	LC373089	LC373125
Crassiperidium octosporum	KT 3468	LC373114	LC373090	LC373126
Crassiperidium octosporum	KT 3604	LC373115	LC373091	LC373127
Crassiperidium octosporum	KT 3605	LC373116	LC373092	LC373128
Crassiperidium octosporum	MM 9	LC373117	LC373093	LC373129
Crassiperidium quadrisporum	**KT 27981**	LC373118	LC373094	LC373130
Crassiperidium quadrisporum	KT 27982	LC373119	LC373095	LC373131
Cryptoclypeus oxysporus	**HHUF 30507**	LC194345	LC194303	LC194390
Cryptocoryneum akitaense	**MAFF 245365**	LC194348	LC194306	LC096136
Cryptocoryneum japonicum	**MAFF 245370**	LC194356	LC194314	LC096144
Cryptocoryneum longicondensatum	**MAFF 245374**	LC194360	LC194318	LC096148
Cyclothyriella rubronotata	**CBS 141486**	KX650544	KX650507	KX650519
Cyclothyriella rubronotata	CBS 121892	KX650541	-	KX650516
Cyclothyriella rubronotata	CBS 385.39	MH867543	-	-
Cyclothyriella rubronotata	CBS 419 85	GU301875	-	GU349002
Delitschia didyma	UME 31411	DQ384090	AF242264	-
Delitschia winteri	CBS 225.62	DQ678077	DQ678026	DQ677922
Dendrographa decolorans	Ertz 5003	AY548815	AY548809	-
Dendrographa leucophaea f. minor		AF279382	AF279381	-
Dendryphion europaeum	CPC 22943	KJ869203	-	-
Dendryphion europaeum	**CPC 23231**	NG_059120	-	-
Dendryphion nanum	MFLUCC 16-0975	MG208132	-	MG207983
Didymosphaeria rubi-ulmifolii	**MFLUCC 14-0023**	KJ436586	KJ436588	-
Dissoconium aciculare	CBS 204.89	GU214419	GU214523	-
Ernakulamia cochinensis	PRC 3992	LT964670	-	-
Flavomyces fulophazii	CBS 135761	KP184040	KP184082	-
Fuscostagonospora cytisi	**MFLUCC 16-0622**	KY770978	KY770977	KY770979
Fuscostagonospora sasae	**CBS 139687**	AB807548	AB797258	-
Fusculina eucalyptorum	**CBS 145083**	MK047499	-	-
Gordonomyces mucovaginatus	**CBS 127273**	JN712552		
Halojulella avicenniae	JK 5326A	GU479790	GU479756	-
Halojulella avicenniae	BCC 20173	GU371822	GU371830	GU371815
Halojulella avicenniae	PUFD542	MK026757	MK026754	-
Halojulella avicenniae	BCC 18422	GU371823	GU371831	GU371816
Halojulella avicenniae	BCC28357	KC555567	KC555565	-
Halojulella avicenniae	GR00584	KC555568	KC555566	-
Halotthia posidoniae	**BBH 22481**	GU479786	GU479752	-
Helminthosporium aquaticum	**MFLUCC 15-0357**	KU697306	KU697310	-
Helminthosporium velutinum	MAFF 243854	AB807530	AB797240	-
Helminthosporium velutinum	MFLUCC 13-0243	KU697305	-	-
Helminthosporium velutinum	**MFLUCC 15-0423**	KU697304	-	-
Hermatomyces iriomotensis	**HHUF 30518**	LC194367	LC194325	LC194394
Hermatomyces tectonae	**MFLUCC 14-1140**	KU764695	KU712465	KU872757
Hermatomyces thailandica	**MFLUCC 14-1143**	KU764692	KU712468	KU872754
Hobus wogradensis	**TI**	KX650546	KX650508	KX650521
Hysterium angustatum	**CBS 236.34**	FJ161180	GU397359	FJ161096
Hysterium angustatum	MFLUCC 16-0623	MH535893	MH535885	MH535878
Jahnula seychellensis	**SS2113**	EF175665	EF175643	-
Latorua caligans	**CBS 576.65**	KR873266	-	-
Latorua grootfonteinensis	**CBS 369.72**	KR873267	-	-
Lentimurispora urniformis	**MFLUCC 18-0497**	MH179144	MH179160	MH188055
Leptosphaeria doliolum	**CBS 505.75**	GQ387576	GQ387515	GU349069
Leptoxyphium cacuminum	**MFLUCC 10-0049**	JN832602	JN832587	-
Leucaenicola phraeana	**MFLUCC 18-0472**	MK348003	MK347892	-
Lignosphaeria fusispora	**MFLUCC 11-0377**	KP888646	-	-
Lignosphaeria thailandica	**MFLUCC 11-0376**	KP888645	-	-

Table 1. *Cont.*

Taxon	Strain Number	GenBank Accession Numbers		
		LSU	*SSU*	*TEF1α*
Lindgomyces ingoldianus	**ATCC 200398**	AB521736	AB521719	-
Longiostiolum tectonae	**MFLUCC 12 0562**	KU764700	KU712459	-
Lophiotrema eburnoides	HHUF 30079	LC001707	LC001706	-
Lophiotrema nucula	**CBS 627.86**	GU301837	GU296167	GU349073
Macrodiplodiopsis desmazieri	**CBS 140062**	KR873272	-	-
Magnicamarosporium diospyricola	MFLUCC 16-0419	KY554212	KY554211	KY554209
Massarina eburnea	CBS 473.64	GU301840	GU296170	-
Massariosphaeria phaeospora	**CBS 611.86**	GU301843	GU296173	-
Mauritiana rhizophorae	BCC 28866	GU371824	GU371832	GU371817
Medicopsis romeroi	CBS 122784	EU754208	EU754109	KF015679
Medicopsis romeroi	**CBS 252.60**	EU754207	EU754108	KF015678
Medicopsis romeroi	CBS 132878	KF015622	KF015648	KF015682
Murispora rubicunda	IFRD 2017	FJ795507	GU456308	GU456289
Neoastrosphaeriella krabiensis	**MFLUCC 11-0025**	JN846729	JN846739	-
Neohendersonia kickxii	**CBS 112403**	KX820266	-	-
Neohendersonia kickxii	CBS 122938	KX820268	-	-
Neohendersonia kickxii	CBS 114276	KX820267	-	-
Neohendersonia kickxii	CPC 24865	KX820270	-	-
Neohendersonia kickxii	CBS 122941	KX820269	-	-
Neomassaria fabacearum	**MFLUCC 16-1875**	KX524145	KX524147	KX524149
Neomassaria formosana	**NTUCC 17-007**	MH714756	MH714759	MH714762
Neomassarina chromolaenae	**MFLUCC 17-1480**	MT214466	MT214419	MT235785
Neomassarina pandanicola	**MFLUCC 16-0270**	MG298945	-	MG298947
Neomassarina thailandica	**MFLUCC 10-0552**	KX672157	KX672160	KX672163
Neomassarina thailandica	MFLUCC 17-1432	MT214467	MT214420	MT235786
Neotorula aquatica	**MFLUCC 150342**	KU500576	KU500583	-
Neotorula submersa	**KUMCC 15-0280**	KX789217	-	-
Occultibambusa bambusae	**MFLUCC 13-0855**	KU863112	KU872116	-
Occultibambusa pustula	**MFLUCC 11-0502**	KU863115	KU872118	-
Ohleria modesta	MGC	KX650562	-	KX650533
Ohleria modesta	**CBS 141480**	KX650563	KX650513	KX650534
Paradictyoarthrinium diffractum	MFLUCC 13-0466	KP744498	KP753960	-
Paradictyoarthrinium diffractum	MFLUCC 12-0557	KP744497	-	-
Paradictyoarthrinium hydei	MFLUCC 13-0465	MG747497	-	-
Paradictyoarthrinium tectonicola	MFLUCC 13-0465	KP744500	KP753961	-
Paradictyoarthrinium tectonicola	**MFLUCC 12-0556**	KP744499	-	-
Periconia thailandica	**MFLUCC 17-0065**	KY753888	KY753889	-
Phaeoseptum aquaticum	**CBS 123113**	JN644072	-	-
Phaeoseptum terricola	MFLUCC 10-0102	MH105779	MH105780	MH105781
Phyllosticta capitalensis	CBS 226.77	KF206289	KF766300	-
Piedraia hortae	**CBS 480.64**	GU214466	-	-
Polyplosphaeria fusca	**CBS 125425**	AB524607	AB524466	AB524822
Preussia lignicola	**CBS 363.69**	DQ384098	DQ384087	-
Preussia lignicola	CBS 264.69	GU301872	GU296197	GU349027
Pseudoastrosphaeriella bambusae	**MFLUCC 11-0205**	KT955475	KT955455	KT955437
Pseudoastrosphaeriella longicolla	**MFLUCC 11-0171**	KT955476	KT955456	KT955438
Pseudoastrosphaeriella thailandensis	**MFLUCC 10-0553**	KT955477	KT955456	KT955439
Pseudolophiotrema elymicola	**HHUF 28984**	LC194381	LC194339	LC194418
Pseudomassariosphaeria bromicola	**MFLUCC 15-0031**	KT305994	KT305996	KT305999
Pseudotetraploa curviappendiculata	**CBS 125426**	AB524610	AB524469	AB524825
Quadricrura septentrionalis	CBS 125428	AB524617	AB524476	AB524832
Racodium rupestre	L346	EU048583	EU048575	-
Racodium rupestre	L424	EU048582	EU048577	-
Ramusculicola thailandica	**MFLUCC 13-0284**	KP888647	KP899131	KR075167
Rimora mangrovei	JK 5246A	GU301868	GU296193	
Roccella fuciformis	Tehler 8171	FJ638979	-	-
Rostriconidium aquaticum	**KUMCC 15-0297**	MG208144	-	MG207995

Table 1. *Cont.*

Taxon	Strain Number	GenBank Accession Numbers		
		LSU	*SSU*	*TEF1α*
Rostriconidium aquaticum	MFLUCC 16-1113	MG208143	-	MG207994
Salsuginea ramicola	KT 2597.1	GU479800	GU479767	GU479861
Salsuginea ramicola	CBS 125781	MH877872	-	-
Scorias spongiosa	CBS 325.33	MH866910	GU214696	-
Seriascoma didymospora	MFLUCC 11-0179	KU863116	KU872119	-
Sigarispora arundinis	JCM 13550	AB618998	AB618679	LC001737
Sigarispora ravennica	**MFLUCC 14-0005**	KP698414	KP698415	-
Splanchnonema platani	CBS 222.37	KR909316	KR909318	KR909319
Sporidesmioides thailandica	KUMCC 16-0012	KX437758	KX437760	KX437767
Sporidesmioides thailandica	**MFLUCC 13-0840**	NG_059703	NG_061242	KX437766
Sporormia fimetaria	UPS:Dissing Gr.81.194	GQ203729	-	-
Sporormiella minima	**CBS 52450**	DQ468046	-	DQ468003
Stagonospora pseudocaricis	**CBS 135132**	KF251762	KF251259	KF252741
Stemphylium vesicarium	**CBS 191.86**	DQ247804	DQ247812	DQ471090
Stemphylium vesicarium	CBS 714.68	DQ678049	DQ767648	DQ677888
Sulcatispora acerina	**KT2982**	LC014610	LC014605	LC014615
Sulcosporium thailandicum	**MFLUCC 12-0004**	KT426563	KT426564	-
Teichospora quercus	**CBS 143396**	MH107966	-	MH108030
Tetraplosphaeria sasicola	**KT 563**	AB524631	AB524490	AB524838
Torula gaodangensis	**MFLUCC 17-0234**	NG_059827	NG_063641	-
Torula herbarum	CBS 111855	KF443386	KF443391	KF443403
Triplosphaeria maxima	**MAFF 239682**	AB524637	AB524496	-
Tubeufia chiangmaiensis	**MFLUCC 11-0514**	KF301538	KF301543	KF301557
Tubeufia javanica	**MFLUCC 12-0545**	KJ880036	KJ880035	KJ880037
Vargamyces aquaticus	CBS 639.63	KY853539	-	-
Vargamyces aquaticus	HKUCC 10830	DQ408575	-	-
Versicolorisporium triseptatum	**HHUF 28815**	AB330081	AB524501	-
Westerdykella dispersa	CBS 297.56	MH869191	-	-
Westerdykella ornata	**CBS 379.55**	GU301880	GU296208	GU349021
Xenomassariosphaeria rosae	**MFLUCC 15-0179**	MG829092	MG829192	-

2.3. Phylogenetic Analysis

The sequence data were assembled using BioEdit v. 7.2.5 [35] and subjected to a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest matches with taxa in *Pleosporales*. Reference sequence data of this order and some representatives of other orders of *Dothideomycetes* were downloaded from previously published studies [1,6,36–39]. The sequences were automatically aligned using default settings in MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) [40]. A combined dataset of three gene regions (*LSU*, *SSU* and *TEF1α*) was prepared and manually adjusted using BioEdit and AliView [41]. Phylogenetic analyses of the combined dataset were performed using maximum likelihood, maximum parsimony and Bayesian inference method. Maximum likelihood analyses (ML), including 1000 bootstrap pseudoreplicates, were performed at the CIPRES web portal [42] using RAxML v. 8.2.12 [43]. Maximum parsimony analysis was conducted using PAUP v.4.0b 10 [44] with the heuristic search option and number of replications 1000 each. The Tree Length (TL), Consistency Indices (CI), Retention Indices (RI), Rescaled Consistency Indices (RC) and Homoplasy Index (HI) were documented.

The best model for different genes partition was determined in JModelTest version 2.1.10 [45] for posterior probability (PP). The general time reversible (GTR) model with a discrete gamma distribution plus invariant site (GTR+I+G) substitution model was used for the combined dataset. Posterior probabilities [46] were estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.6 [47]. Four simultaneous Markov chains were run for 10 million generations and trees were sampled every 1000th generation, thus resulting in 10,000 trees. The suitable burn-in phase was determined by inspecting traces

in Tracer version 1.7 [48]. The first 10% of generated trees representing the burn-in phase of the analyses were discarded, while the remaining trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree. The phylograms were visualized with FigTree v1.4.0 program [49] and edited using Adobe Illustrator CS6 v15.0 (Adobe Systems, USA).

2.4. Fossil Calibration and Divergence Time Estimates

Divergence times were estimated with BEAST 2.6.2 [50] based on the methodology described in Phukhamsakda et al. [4]. The aligned sequence dataset (*LSU*, *SSU* and *TEF1α*) used for the phylogenetic analyses were loaded into BEAUTI 2.6.2 to prepare the XML file. Nucleotide substitution models were determined using JModelTest version 2.1.10. The GTR+I+G nucleotide substitution model was applied to *LSU* and *TEF1α* partitions. The symmetrical (SYM) model with a discrete gamma distribution plus invariant site (SYM+I+G) substitution model was applied to the SSU partition. The data partitions were set with unlinked substitution, linked clock model and linked tree. An uncorrelated relaxed clock model with lognormal distribution was used. The Yule speciation process, which assumes a constant rate of speciation divergence, was used as the tree prior [51]. The analysis was performed in BEAST 2.6.2 for 100 million generations, sampling every 1000 generations. The effective sample size (ESS) was analysed with Tracer version 1.7 to check that the values were greater than 200, as recommended by Drummond et al. [52]. The first 20% trees were discarded as the burn-in phase and the remaining trees were combined in LogCombiner 2.6.2. The maximum clade credibility was calculated in TreeAnnotator v 2.6.2. The phylograms were visualized with FigTree v.1.4.0 program.

To estimate the divergence time for Anastomitrabeculiaceae, the fossil *Metacapnodium succinum* (*Metacapnodiaceae*) was used to set the crown age of *Capnodiales* using a normal distribution, mean of 100 MYA, SD of 150 MYA, giving 95% credibility interval of 346 MYA [4,23,53,54]. The fossil *Margaretbarromyces dictyosporus* was used to calibrate the crown age of *Aigialus* (*Aigialaceae*) using a gamma distribution, with an offset of 35 MYA, a shape of 1.0, scale of 25, providing 95% credibility interval of 110 MYA [4,55–57]. The split between *Arthoniomycetes* (outgroup) and *Dothideomycetes* was used as the secondary calibration using a normal distribution, mean of 300 MYA, SD of 50 MYA, giving 95% credibility interval of 382 MYA [22,36,53,54].

3. Results

3.1. Phylogenetic Analyses

The combined gene alignment comprised 196 strains and 2800 characters (*LSU*: 860 characters, *SSU*: 1039 characters and *TEF1α*: 901 characters). Among the 2800 characters, there were 1492 conserved sites (53%), 364 variable sites (13%) and 944 parsimony informative sites (34%). The parsimony analysis of the data matrix yielded one most parsimonious tree out of 1000 (CI = 0.265, RI = 0.659, RC = 0.175, HI = 0.735, Tree Length = 7606). Based on BLAST search in the NCBI GenBank of the *LSU* gene, the newly generated taxon MFLUCC 16-0412 and MFLUCC 16-0417 show 95% similarity to *Crassiperidium quadrisporum* (KT 27981 and KT 27982). The topology of the phylogenetic tree based on the *LSU* gene was generally congruent with the overall topology of the tree based on the combined dataset. Phylogenetic trees generated from maximum likelihood, maximum parsimony and Bayesian analysis of the combined dataset resulted in similar topologies with some exception. The position of *Cyclothyriellaceae* and *Longiostiolaceae* differed between the three methods. The best scoring RAxML tree had a final likelihood value of −40,523.297855 (Figure 1). The new taxon formed an independent lineage basal to the *Halojulellaceae* with strong Bayesian inference support and moderate support from maximum likelihood (0.99 PP/65% MLBT). A new genus *Anastomitrabeculia* is therefore introduced within *Anastomitrabeculiaceae* to accommodate the new species.

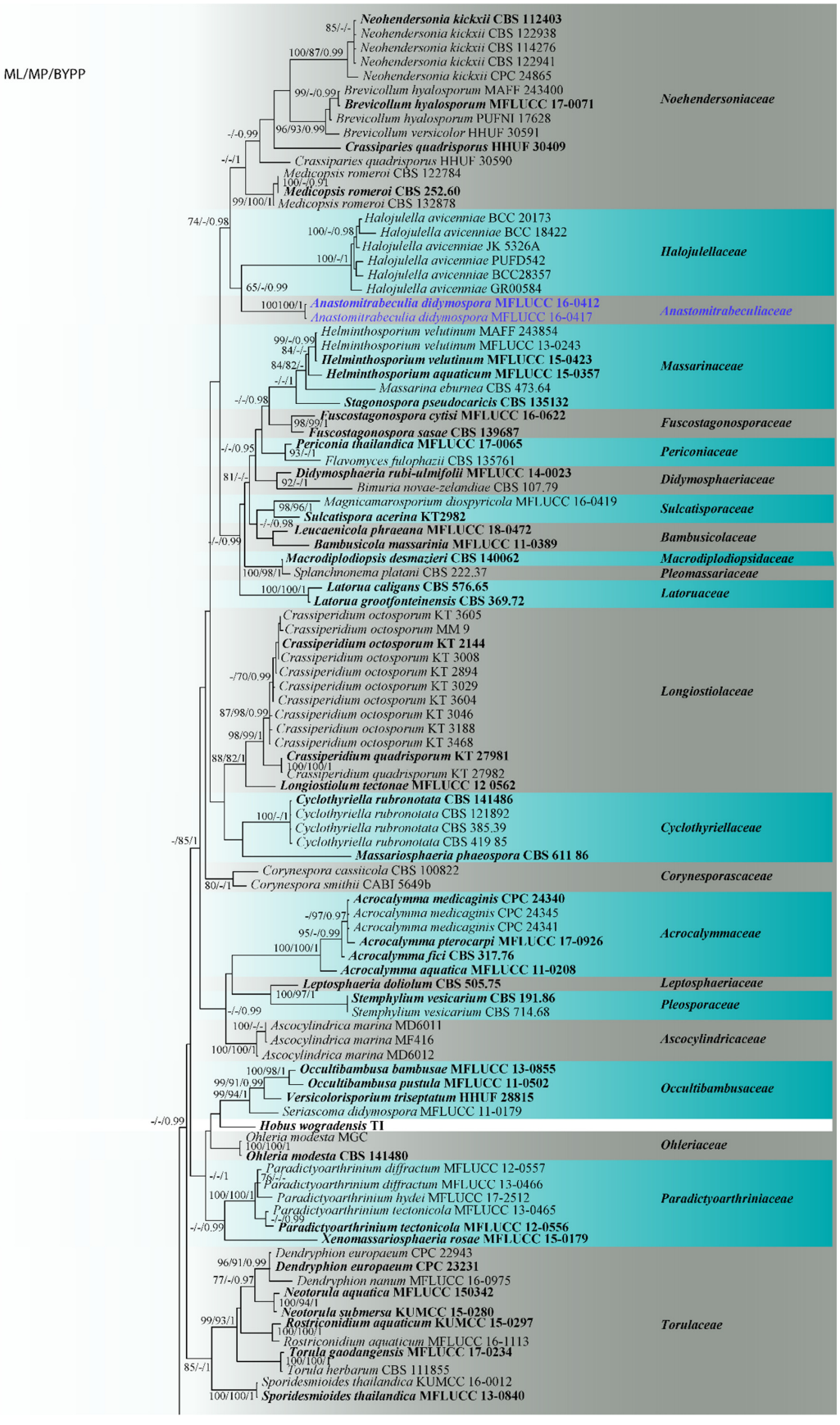

Figure 1. *Cont.*

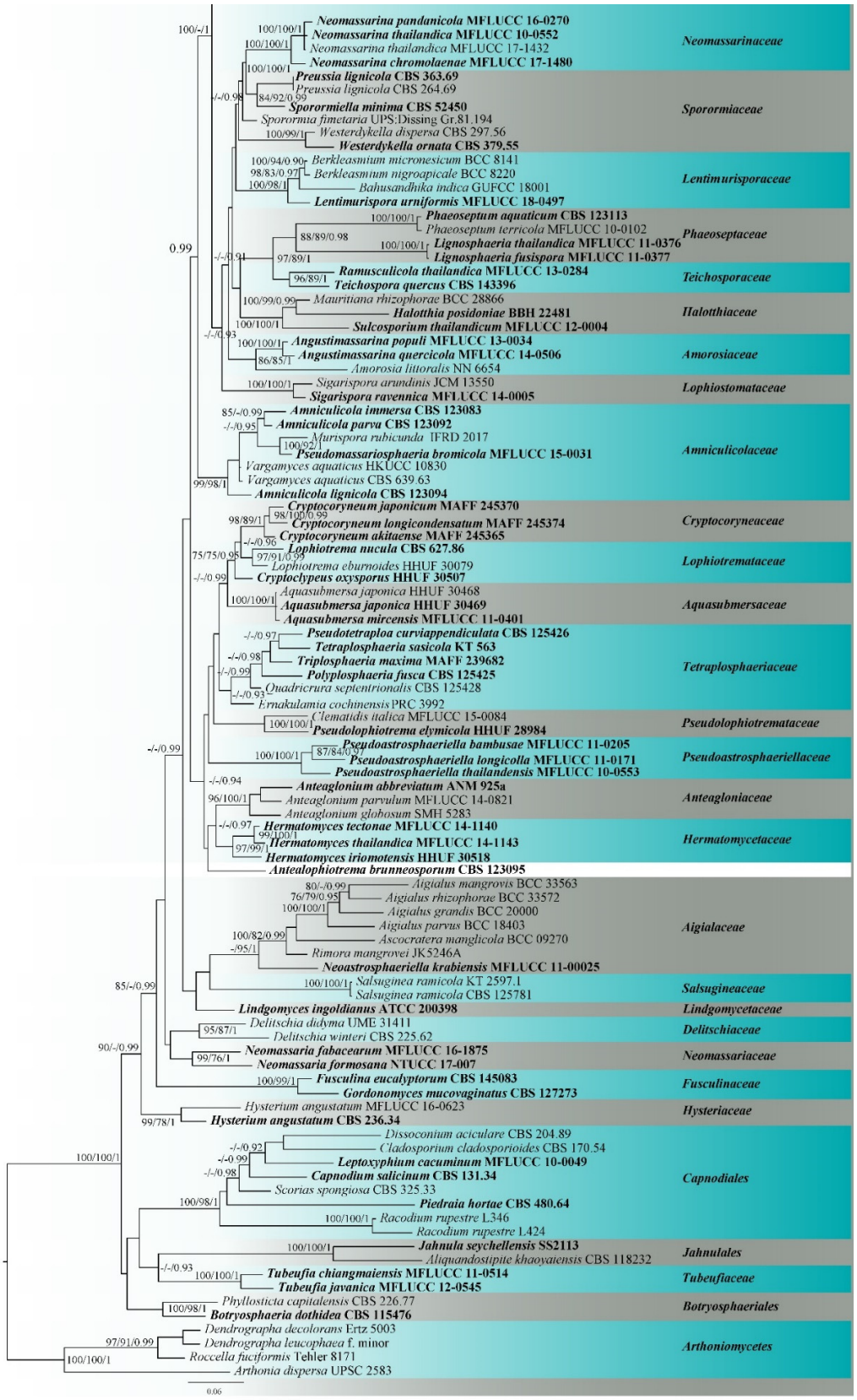

Figure 1. The best scoring RAxML tree based on a combined *LSU*, *SSU* and *TEF1α* dataset. RAxML bootstrap support and maximum parsimony values ≥60% (BT), as well as Bayesian posterior probabilities ≥0.90 (BYPP) are shown, respectively, near the nodes. The ex-type strains are in bold and the scale bar indicates 0.06 changes per site. The tree is rooted with species of *Arthoniomycetes* and the new taxon is indicated in blue.

3.2. Fossil Calibration and Divergence Time Estimates

The topology of the maximum clade credibility (MCC) tree (Figure 2) was congruent with the tree obtained from the Bayesian inference analysis and the maximum likelihood analysis. The divergence times of the dating analysis are listed in Table 2. The crown age of *Dothideomycetes* is estimated at 263 MYA during the Permian period based on the MCC tree. The split of *Arthoniomycetes* and *Dothideomycetes* occurred around 323 MYA during the Carboniferous period. The crown age of *Pleosporales* is estimated at 206 MYA, and *Hysteriales* diverged from *Pleosporales* approximately 236 MYA during the Triassic period. The crown age of *Anastomitrabeculiaceae* is estimated at around 2.6 MYA, and it diverged from *Halojulellaceae* at around 84 (52–116) MYA. *Anastomitrabeculiaceae* formed an independent lineage with close relationship to *Halojulellaceae* with strong posterior probability in the MCC tree (0.99 BYPP). The divergence time of *Anastomitrabeculiaceae* was compared to Pleosporalean families with trabeculate pseudoparaphyses, cylindrical asci and ascospores with a sheath (Table 3). The divergence time of *Anastomitrabeculiaceae* was also compared to *Didymosphaeriaceae* as they are morphologically similar by having trabeculate pseudoparaphyses and cylindrical asci.

Table 2. Divergence time estimates obtained from BEAST analysis. The median and the 95% Highest Posterior Density are provided in million years ago (MYA). The geological time scales are given based on the median node age.

Nodes	Node Age	Geological Time Period
Arthoniomycetes–Dothideomycetes	323 (310–349)	Carboniferous
Dothideomycetes crown group	263 (216–313)	Permian
Hysteriales–Pleosporales	236 (188–300)	Triassic
Pleosporales crown group	206 (171–254)	Triassic
Capnodiales crown group	147 (99–200)	Jurassic
Anastomitrabeculiaceae stem group	84 (52–116)	Cretaceous
Aigialaceae–Aigialus sp.	37 (18–56)	Eocene
Anastomitrabeculiaceae crown group	2.6 (0.19–6.61)	Neogene

Table 3. Divergence time estimates obtained from BEAST analysis for families with similar morphology to *Anastomitrabeculiaceae*. The crown age and the stem age are provided in million years ago (MYA).

Families	Crown Age	Stem Age
Aigialaceae	102	141
Amniculicolaceae	90	177
Anastomitrabeculiaceae	2.6	84
Anteagloniaceae	52	98
Bambusicolaceae	29	57
Cyclothyriellaceae	66	95
Delitschiaceae	78	131
Didymosphaeriaceae	47	81
Fuscostagonosporaceae	26	63
Lindgomycetaceae	31	92
Neomassariaceae	82	131
Pseudoastrosphaeriellaceae	56	147
Tetraplosphaeriaceae	91	189

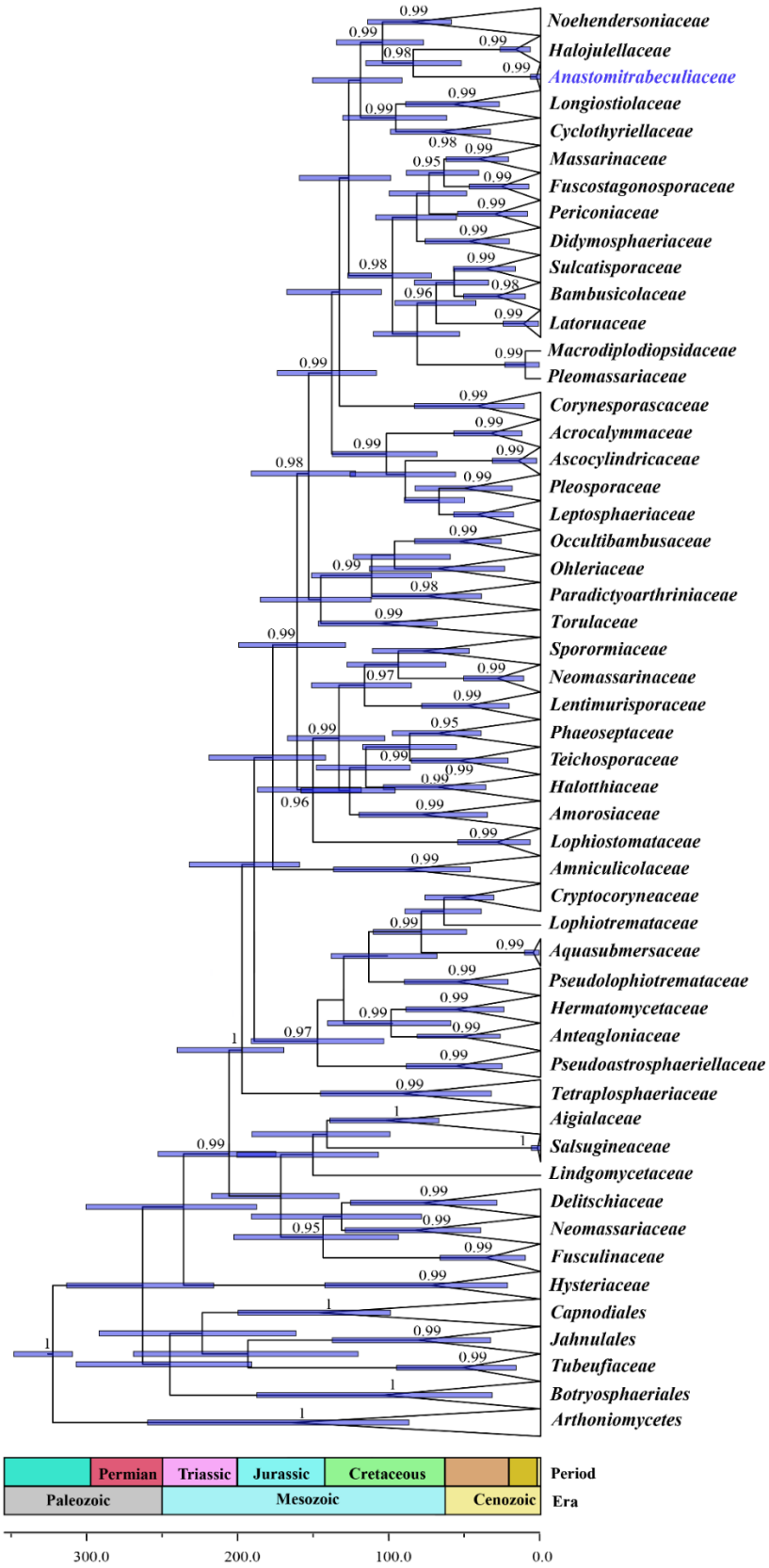

Figure 2. Maximum clade credibility (MCC) tree of families in *Dothideomycetes* using BEAST. Numbers at nodes indicate posterior probabilities (PP) for node support. Bars correspond to the 95% highest posterior density (HPD) intervals. Posterior probabilities greater than 0.95 are given near the nodes. The new taxon is indicated in blue. Geological time scales are given at the base together with scale in million years ago (MYA) [58].

3.3. Taxonomy

Anastomitrabeculiaceae Bhunjun, Phukhams and K.D. Hyde, *fam. nov.*

Index Fungorum number: IF556817, Facesoffungi number: FoF 09521.

Etymology: Referring to the name of the type genus.

Saprobic on dead bamboo culms submerged in freshwater. **Sexual morph**: *Ascomata* immersed under a clypeus to semi-immersed, gregarious, uniloculate, globose to subglobose, carbonaceous, black. *Ostiole* central, apex well developed. *Peridium* multi-layered, sub-carbonaceous or coriaceous, with dark brown to hyaline cells arranged in a *textura angularis*. *Hamathecium* composed of numerous, filamentous, trabeculate pseudoparaphyses, septate, anastomosing between the asci and at the apex. *Asci* bitunicate, fissitunicate, broad cylindrical to cylindrical-clavate, bulbous pedicel, with an ocular chamber. *Ascospores* biseriate, broadly fusiform, septate, smooth-walled, with wall ornamentation, surrounded by mucilaginous sheath.

Note: *Anastomitrabeculiaceae* is introduced to include *Anastomitrabeculia*, which is reported as a saprobe on bamboo culms. *Anastomitrabeculiaceae* is characterised by semi-immersed, coriaceous or carbonaceous ascomata with septate, trabeculate pseudoparaphyses and hyaline ascospores with longitudinally striate wall ornamentation, surrounded by mucilaginous sheath. *Anastomitrabeculiaceae* formed a well-supported independent lineage closely related to *Halojulellaceae*, but *Halojulellaceae* differs by its cellular pseudoparaphyses and golden-brown ascospores.

Type genus: *Anastomitrabeculia* Bhunjun, Phukhams and K.D. Hyde.

Anastomitrabeculia Bhunjun, Phukhams. and K.D. Hyde, *gen. nov.*

Index Fungorum number: IF556560, Facesoffungi number: FoF 09522.

Etymology: Referring to the trabeculate pseudoparaphyses anastomosing between the asci and at the apex.

Colonies on natural substrate umbonate at the centre, circular, black shiny dots are visible on the host surface. *Ascomata* on surface of the host, immersed under a clypeus, gregarious, uniloculate, subglobose, carbonaceous. *Ostiole* orange pigment near ostiole. *Peridium* comprising multilayers of brown to hyaline cells of *textura angularis*, inner layers composed of thin, hyaline cells. *Asci* 8–spored, bitunicate, fissitunicate, broad cylindrical to cylindrical-clavate, with a bulbous pedicellate, rounded at the apex, with an ocular chamber. *Ascospores* biseriate, broadly fusiform, tapering towards the ends, hyaline, with guttules in each cell, constricted at the septa, with longitudinally striate wall ornamentation, surrounded by mucilaginous sheath.

Note: *Anastomitrabeculia* is established as a monotypic genus. It is characterised by the presence of carbonaceous ascomata, with orange pigment near ostiole and ascospores with longitudinally striate wall ornamentation. *Anastomitrabeculia* is morphologically similar to members of *Pleosporales* in having perithecioid ascomata, bitunicate asci and hyaline ascospores.

Type species: *Anastomitrabeculia didymospora* Bhunjun, Phukhams and K.D. Hyde.

Anastomitrabeculia didymospora Bhunjun, Phukhams and K.D. Hyde, sp. nov.

Index Fungorum number: IF556559; Facesoffungi number: FoF 09523 Figure 3.

Etymology: Referring to the didymosporous ascospores.

Holotype–MFLU 20-0694.

Saprobic on dead bamboo culms submerged in freshwater. **Sexual morph**: *Ascomata* 430–460 μm high, 435–575 μm diam., immersed under a clypeus to semi-immersed, gregarious, uniloculate, globose to subglobose, carbonaceous, rough, black, ostiolate. *Ostiole* 160 μm high, 270 μm diam., central, apex well developed, papillate, with pore-like opening, with periphyses filling the ostiolar canal, dark brown to black, orange pigment near ostiole. *Peridium* 6–18 μm wide, comprising 3–5 layers of brown to hyaline cells of *textura angularis*, inner layers composed of thin, hyaline cells. *Hamathecium* of dense, long, 0.8–1.25 μm wide ($\overline{x}$ = 1 μm, n = 50), filiform, filamentous, trabeculate pseudoparaphyses, septate, branched,

embedded in a gelatinous matrix, anastomosing between the asci and at the apex. *Asci* 125–160 × 15–20 µm ($\overline{x}$ = 145 × 17 µm, *n* = 20), 8–spored, bitunicate, fissitunicate, broad cylindrical to cylindrical-clavate, with bulbous pedicellate, rounded at the apex, with an ocular chamber. *Ascospores* 18–28 × 7–10 µm ($\overline{x}$ = 22.5× 9 µm, *n* = 20), biseriate, broadly fusiform, tapering towards the ends, hyaline, 1-septate at the centre, constricted at the septum, cell above septate enlarged, straight, smooth-walled, with longitudinally striate wall ornamentation, surrounded by mucilaginous sheath. **Asexual morph:** Undetermined.

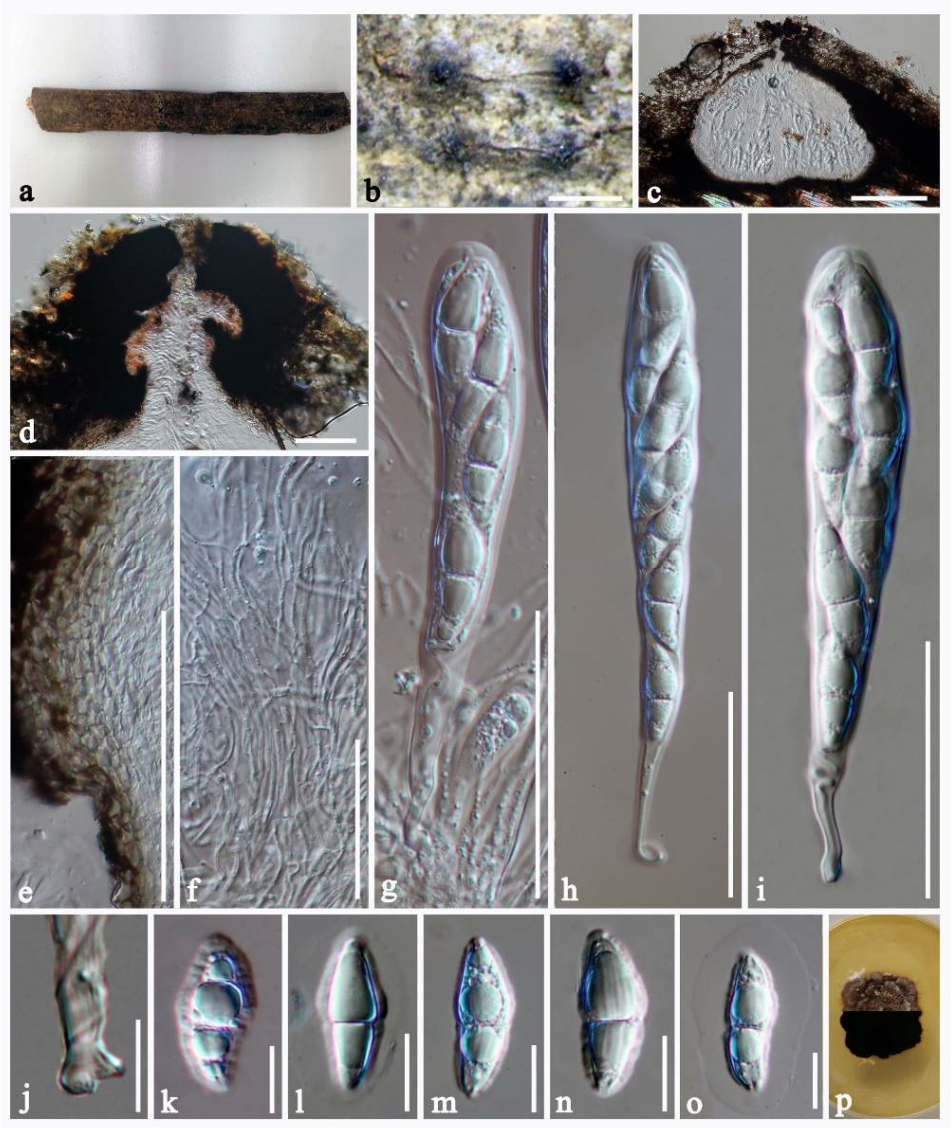

Figure 3. *Anastomitrabeculia didymospora* (MFLU 20-0694, **holotype**). (**a**) Ascomata on bamboo. (**b**) Close-up of ascomata. (**c**) Vertical section of ascoma. (**d**) Ostiolar canal. (**e**) Peridium layer. (**f**) Trabeculate pseudoparaphyses. (**g–i**) Asci. (**j**) Pedicel. (**k–o**) Ascospores showing mucilaginous sheath. (**p**) Culture characteristics on PDA from above and below (9 cm diameter petri dish). Scale bar: (**b**) = 500 µm, (**c**) = 200 µm, (**d–i**) = 50 µm, (**j–o**) = 10 µm.

Culture characters: Ascospores germinating on MEA and PDA within 24 h with germ tubes developing from basal cells. Colonies on MEA and PDA umbonate at the centre, circular, friable, reaching 20 mm diameter after four weeks of incubation at 25 °C. Culture on MEA with white aerial mycelium, dark brown at the centre and paler towards the edge from above and below. Culture on PDA dark brown from above and below.

Material examined: THAILAND, Krabi province (8.1° N, 98.9° E), on dead bamboo culms, 15 December 2015, C. Phukhamsakda, KR001 (MFLU 20-0694, **holotype**), ibid,

18 December 2015 (MFLU 20-0695, **paratype**); ex-type living culture MFLUCC 16-0412; ex-paratype living culture, MFLUCC 16-0417.

4. Discussion

In this study, we introduce a new species, genus and family for a collection of *Pleosporales* found on bamboo. The introduction of new taxa, even at the family level, is not surprising, considering that about 93% of fungi remain unknown to science despite ca. 2000 species described every year [59,60]. Pleosporalean species can occur in terrestrial, marine and freshwater habitats [7–9]. Several studies have reported new pleosporalean taxa from freshwater or marine habitats or from bambusicolous hosts [1,3]. *Pleosporales* have unique characters such as perithecioid ascomata typically with a papilla and bitunicate, generally fissitunicate asci, bearing mostly septate ascospores of different colours and shapes, with or without a gelatinous sheath [7]. The morphology of *Anastomitrabeculiaceae* is similar to members of the *Pleosporales* based on the presence of pseudoparaphyses, perithecioid ascomata, bitunicate asci and hyaline ascospores. *Anastomitrabeculiaceae* is characterised by semi-immersed to superficial ascomata, trabeculate pseudoparaphyses, cylindrical asci and ascospores with longitudinally striate wall ornamentation, surrounded by mucilaginous sheath. The newly discovered species formed a well-supported independent lineage basal to the *Halojulellaceae* based on phylogenetic analyses of the combined dataset (0.99 PP/65% MLBT). *Halojulellaceae* differs by its cellular pseudoparaphyses and golden brown ascospores [2]. The new taxon is also phylogenetically closely related to *Neohendersoniaceae*, which differs by its cellular pseudoparaphyses and smooth-walled ascospore [61]. A novel genus *Anastomitrabeculia* is therefore introduced to accommodate one new species, *Anastomitrabeculia didymospora.* A new family, *Anastomitrabeculiaceae*, is also introduced to accommodate this independent lineage.

Several pleosporalean families such as Aigialaceae, Amniculicolaceae, Anteagloniaceae, Astrosphaeriellaceae, Bambusicolaceae, Biatriosporaceae, Caryosporaceae, Cyclothyriellaceae, Delitschiaceae, Didymosphaeriaceae, Fuscostagonosporaceae, Lindgomycetaceae, Melanommataceae, Neomassariaceae, Pseudoastrosphaeriellaceae, Striatiguttulaceae and Tetraplosphaeriaceae share similar characters to Anastomitrabeculiaceae in having trabeculate pseudoparaphyses, cylindrical asci and ascospores with a sheath [7]. The nature of pseudoparaphyses is often overlooked, but they have taxonomic relevance at the genus and possibly family levels [7], but not at the ordinal level [62]. These families differ from Anastomitrabeculiaceae mainly by their ascospores, for example, Aigialaceae and Amniculicolaceae have brown and muriform ascospores [7]. Anteagloniaceae differs by having a peridium composed of dark brown cells of textura epidermoidea, cellular or trabeculate pseudoparaphyses and small, uniseriate ascospores [2]. Astrosphaeriellaceae differs by its brown, sub-fusiform to fusiform, obclavate to ellipsoidal, or limoniform ascospores [63] and Biatriosporaceae differs by its immersed ascomata and fusiform, dark brown ascospores [2]. Caryosporaceae differs by its broad-fusiform, ovoid or ellipsoid, brown ascospores [64]. Bambusicolaceae species have also been isolated from dead bamboo culms, but they differ from Anastomitrabeculiaceae by their cellular pseudoparaphyses and multi-seriate, smooth-walled ascospores [2]. Cyclothyriellaceae differs by its uniseriate, ellipsoid to fusiform, brown ascospores with several eusepta [65]. Fuscostagonosporaceae differs in having globose to subglobose ascomata, fissitunicate asci with long stipes and narrowly fusiform ascospores [66]. Anastomitrabeculiaceae shares several characters with Didymosphaeriaceae in having immersed ascomata formed under a clypeus, trabeculate pseudoparaphyses and cylindrical asci. Didymosphaeriaceae and Melanommataceae differ in having cellular or trabeculate pseudoparaphyses and brown, multi-septate, muriform ascospores [7]. Lindgomycetaceae differs by the presence of cellular or trabeculate pseudoparaphyses and brown, multi-septate ascospores with bipolar mucilaginous appendages [7]. Neomassariaceae differs by its immersed ascomata and ellipsoid ascospores. Pseudoastrosphaeriellaceae differs by its brown to reddish-brown ascospores with longitudinal ridges towards the ends and Striatiguttulaceae differs in having brown, ellipsoid

ascospores with paler end cells. Tetraplosphaeriaceae differs by its immersed ascomata and slightly curved, pale brown ascospores [7].

Divergence time estimate has been widely used as supporting evidence to clarify taxonomic status of extant or novel families in fungal taxonomy [4,6,23,24,26,27,67]. In this study, the MCC tree was congruent with the topology of the trees generated from Bayesian inference analysis and maximum likelihood analyses. The divergence time estimates for the crown age of *Dothideomycetes* (263 MYA), the split of *Dothideomycetes* and *Arthoniomycetes* (323 MYA), the crown age of *Pleosporales* (206 MYA) and the divergence of *Hysteriales* from *Pleosporales* (236 MYA) are similar to previous studies [4,7,11]. Hyde et al. [27] recommended that the divergence times of families should be between 50 and 150 MYA. The stem age is usually preferred to the crown age in taxa ranking as it is not affected by the sample size of the clade [27]. Based on the MCC tree, *Anastomitrabeculiaceae* and *Halojulellaceae* share the stem age of 84 MYA which supports the establishment of *Anastomitrabeculiaceae*.

The divergence time of *Anastomitrabeculiaceae* was also compared to Pleosporalean families with trabeculate pseudoparaphyses, cylindrical asci and ascospores with a sheath (Table 3). *Cyclothyriellaceae* has an estimated crown age of 66 MYA and it diverged at 95 MYA. *Fuscostagonosporaceae* has a crown age of approximately 26 MYA and it diverged around 63 MYA. *Bambusicolaceae*, which was also isolated from dead bamboo culms, has a crown age of 29 MYA and a stem age of about 57 MYA. The stem age of *Anastomitrabeculiaceae* lies within the range of divergence times of those with similar morphology, but the crown age of *Anastomitrabeculiaceae* (2.6 MYA) is much earlier compared to these families. *Bambusicolaceae* was introduced by Hyde at al. [2] to include three bambusicolous taxa, and it currently has 15 species [7]. *Fuscostagonosporaceae* was introduced by Hyde at al. [66] to accommodate one bambusicolous taxon and it currently has four species [7]. Ariyawansa et al. [64] introduced the pleosporalean family, *Caryosporaceae*, which is morphologically similar to *Astrosphaeriellaceae* and *Trematosphaeriaceae* [7]. Based on Liu et al. [11], the stem age of *Caryosporaceae* (85 MYA) is similar to *Trematosphaeriaceae* (88 MYA) compared to *Astrosphaeriellaceae* (113 MYA), but the crown age of *Caryosporaceae* (2 MYA) is much earlier compared to *Astrosphaeriellaceae* (55 MYA) and *Trematosphaeriaceae* (65 MYA). *Astrosphaeriellaceae* currently has 111 species, and *Trematosphaeriaceae* has 103 species, whereas *Caryosporaceae* has ten species [7]. Compared to their morphologically similar families, the early crown of *Anastomitrabeculiaceae* and *Caryosporaceae* could be due to their smaller sample size. Therefore, further collections are needed for an accurate estimation of the crown age as it is affected by the sample size of the clade [27]. This could also be due to rapid speciation of pleosporalean fungal species given their high adaptation capabilities.

The estimated crown age of *Pleosporales* (206 MYA) lies within the early Triassic period. The origin of monocotyledons is estimated within the late Cretaceous period (around 145 MYA) [68]. This period is associated with the diversification of pleosporalean families, which continued during the early Cretaceous period when there was a major diversification and radiation of angiosperms, which favoured further diversification of Pleosporalean families to adapt to various hosts [69].

Hosts and their symbionts can speciate in parallel, which relates to a high level of congruence between the phylogeny of the hosts and their symbionts [70,71]. Therefore, studies focusing on divergence time is important for a better understanding of host–pathogen interaction as well as co-evolutionary interactions [72]. This study uses a polyphasic approach based on morphology, multi-locus phylogenetic analyses and divergence time estimates. By implementing a polyphasic approach, we provide strong evidence for introducing the new family based on congruent results supporting the establishment of a new family.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zhang, Y.; Crous, P.W.; Schoch, C.L.; Hyde, K.D. Pleosporales. *Fungal Divers.* **2012**, *53*, 1–221. [CrossRef] [PubMed]
2. Hyde, K.D.; Jones, E.B.G.; Liu, J.K.; Ariyawansa, H.; Boehm, E.; Boonmee, S.; Braun, U.; Chomnunti, P.; Crous, P.W.; Dai, D.Q.; et al. Families of *Dothideomycetes*. *Fungal Divers.* **2013**, *63*, 1–313. [CrossRef]
3. Tanaka, K.; Hirayama, K.; Yonezawa, H.; Sato, G.; Toriyabe, A.; Kudo, H.; Hashimot, A.; Matsumura, M.; Harada, Y.; Kurihara, Y.; et al. Revision of the *Massarineae* (*Pleosporales*, *Dothideomycetes*). *Stud. Mycol.* **2015**, *82*, 75–136. [CrossRef] [PubMed]
4. Phukhamsakda, C.; Hongsanan, S.; Ryberg, M.; Ariyawansa, H.A.; Chomnunti, P.; Bahkali, A.H.; Hyde, K.D. The evolution of *Massarineae* with *Longipedicellataceae* fam. nov. *Mycosphere* **2016**, *7*, 1713–1731. [CrossRef]
5. Jaklitsch, W.M.; Checa, J.; Blanco, M.N.; Olariaga, I.; Tello, S.; Voglmayr, H. A preliminary account of the *Cucurbitariaceae*. *Stud. Mycol.* **2018**, *90*, 71–118. [CrossRef] [PubMed]
6. Liu, N.G.; Lin, C.G.; Liu, J.K.; Samarakoon, M.C.; Hongsanan, S.; Bhat, D.J.; Hyde, K.D.; McKenzie, E.H.; Jumpathong, J. *Lentimurisporaceae*, a new Pleosporalean family with divergence times estimates. *Cryptogam. Mycol.* **2018**, *39*, 259–283. [CrossRef]
7. Hongsanan, S.; Hyde, K.D.; Phookamsak, R.; Wanasinghe, D.N.; Mckenzie, E.; Sarma, V.V.; Boonmee, S.; Lücking, R.; Pem, D.; Bhat, D.J.; et al. Refined families of *Dothideomycetes*: *Dothideomycetidae* and *Pleosporomycetidae*. *Mycosphere* **2020**, *11*, 1553–2107. [CrossRef]
8. Ramesh, C. Loculoascomycetes from India. *Front. Fungal Divers. India* **2003**, 457–479.
9. Kruys, Å.; Eriksson, O.E.; Wedin, M. Phylogenetic relationships of coprophilous *Pleosporales* (*Dothideomycetes*, *Ascomycota*), and the classification of some bitunicate taxa of unknown position. *Mycol. Res.* **2006**, *110*, 527–536. [CrossRef]
10. Zhang, Y.; Schoch, C.L.; Fournier, J.; Crous, P.W.; De Gruyter, J.; Woudenberg, J.H.C.; Hirayama, K.; Tanaka, K.; Pointing, S.B.; Spatafora, J.W.; et al. Multi-locus phylogeny of *Pleosporales*: A taxonomic, ecological and evolutionary re-evaluation. *Stud. Mycol.* **2009**, *64*, 85–102. [CrossRef]
11. Liu, J.K.; Hyde, K.D.; Jeewon, R.; Phillips, A.J.; Maharachchikumbura, S.S.; Ryberg, M.; Liu, Z.Y.; Zhao, Q. Ranking higher taxa using divergence times: A case study in *Dothideomycetes*. *Fungal Divers.* **2017**, *84*, 75–99. [CrossRef]
12. Wijayawardene, N.N.; Hyde, K.D.; Lumbsch, H.T.; Liu, J.K.; Maharachchikumbura, S.S.N.; Ekanayaka, A.H.; Tian, Q.; Phookamsak, R. Outline of *Ascomycota*: 2017. *Fungal Divers.* **2018**, *88*, 167–263. [CrossRef]
13. Wijayawardene, N.N.; Hyde, K.D.; Al-Ani, L.K.T.; Tedersoo, L.; Haelewaters, D.; Rajeshkumar, K.C.; Zhao, R.L.; Aptroot, A.; Leontyev, D.V.; Saxena, R.K.; et al. Outline of Fungi and fungus-like taxa. *Mycosphere* **2020**, *11*, 1060–1456. [CrossRef]
14. Wijayawardene, N.N.; Hyde, K.D.; Wanasinghe, D.N.; Papizadeh, M.; Goonasekara, I.D.; Camporesi, E.; Bhat, D.J.; Mckenzie, E.H.C.; Phillips, A.J.L.; Diederich, P.; et al. Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Divers.* **2016**, *77*, 1–316. [CrossRef]
15. Wijayawardene, N.N.; Hyde, K.D.; Rajeshkumar, K.C.; Hawksworth, D.L.; Madrid, H.; Kirk, P.M.; Braun, U.; Singh, R.V.; Crous, P.W.; Kukwa, M.; et al. Notes for genera: *Ascomycota*. *Fungal Divers.* **2017**, *86*, 1–594. [CrossRef]
16. Gratani, L.; Crescente, M.F.; Varone, L.; Fabrini, G.; Digiulio, E. Growth pattern and photosynthetic activity of different bamboo species growing in the Botanical Garden of Rome. *Flora-Morphol. Distrib. Funct. Ecol. Plants* **2008**, *203*, 77–84. [CrossRef]
17. Kelchner, S.A.; Group, B.P. Higher level phylogenetic relationships within the bamboos (*Poaceae*: *Bambusoideae*) based on five plastid markers. *Mol. Phylogenet. Evol.* **2013**, *67*, 404–413. [CrossRef]
18. Dai, D.Q.; Phookamsak, R.; Wijayawardene, N.N.; Li, W.J.; Bhat, D.J.; Xu, J.C.; Taylor, J.E.; Hyde, K.D.; Chukeatirote, E. Bambusicolous fungi. *Fungal Divers.* **2016**, *82*, 1–105. [CrossRef]
19. Kirschner, R.; Yang, Z.L.; Zhao, Q.; Feng, B. *Ovipoculum album*, a new anamorph with gelatinous cupulate bulbilliferous conidiomata from China and with affinities to the *Auriculariales* (*Basidiomycota*). *Fungal Divers.* **2009**, *43*, 55–65. [CrossRef]
20. Hyde, K.D.; Zhou, D.; McKenzie, E.; Ho, W.; Dalisay, T. Vertical distribution of saprobic fungi on bamboo culms. *Fungal Divers.* **2002**, *11*, 109–118.
21. Hyde, K.D.; Zhou, D.; Dalisay, T. Bambusicolous fungi: A review. *Fungal Divers.* **2002**, *9*, 1–14.
22. Prieto, M.; Wedin, M. Dating the diversification of the major lineages of *Ascomycota* (Fungi). *PLoS ONE* **2013**, *8*, e65576. [CrossRef] [PubMed]

23. Hongsanan, S.; Sánchez-Ramírez, S.; Crous, P.W.; Ariyawansa, H.A.; Zhao, R.L.; Hyde, K.D. The evolution of fungal epiphytes. *Mycosphere* **2016**, *7*, 1690–1712. [CrossRef]
24. Hongsanan, S.; Maharachchikumbura, S.S.N.; Hyde, K.D.; Samarakoon, M.C.; Jeewon, R.; Zhao, Q.; Al-Sadi, A.M.; Bahkali, A.H. An updated phylogeny of *Sordariomycetes* based on phylogenetic and molecular clock evidence. *Fungal Divers.* **2017**, *84*, 25–41. [CrossRef]
25. Samarakoon, M.C.; Hyde, K.D.; Promputtha, I.; Hongsanan, S.; Ariyawansa, H.A.; Maharachchikumbura, S.S.N.; Daranagama, D.A.; Stadler, M.; Mapook, A. Evolution of *Xylariomycetidae* (*Ascomycota*: *Sordariomycetes*). *Mycosphere* **2016**, *7*, 1746–1761. [CrossRef]
26. Samarakoon, M.C.; Hyde, K.D.; Hongsanan, S.; McKenzie, E.H.; Ariyawansa, H.A.; Promputtha, I.; Zeng, X.Y.; Tian, Q.; Liu, J.K. Divergence time calibrations for ancient lineages of *Ascomycota* classification based on a modern review of estimations. *Fungal Divers.* **2019**, *96*, 285–346. [CrossRef]
27. Hyde, K.D.; Maharachchikumbura, S.S.; Hongsanan, S.; Samarakoon, M.C.; Lücking, R.; Pem, D.; Harishchandra, D.; Jeewon, R.; Zhao, R.L.; Xu, J.C.; et al. The ranking of fungi: A tribute to David L. Hawksworth on his 70th birthday. *Fungal Divers.* **2017**, *84*, 1–23. [CrossRef]
28. Senanayake, I.C.; Rathnayaka, A.R.; Marasinghe, D.S.; Calabon, M.S.; Gentekaki, E.; Lee, H.B.; Hurdeal, V.G.; Pem, D.; Dissanayake, L.S.; Wijesinghe, S.N.; et al. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere* **2020**, *11*, 2678–2754. [CrossRef]
29. Jayasiri, S.C.; Hyde, K.D.; Ariyawansa, H.A.; Bhat, J.; Buyck, B.; Cai, L.; Dai, Y.C.; Abd-Elsalam, K.A.; Ertz, D.; Hidayat, I.; et al. The faces of fungi database: Fungal names linked with morphology, molecular and human attributes. *Fungal Divers.* **2015**, *74*, 3–18. [CrossRef]
30. Jeewon, R.; Hyde, K.D. Establishing species boundaries and new taxa among fungi: Recommendations to resolve taxonomic ambiguities. *Mycosphere* **2016**, *7*, 1669–1677. [CrossRef]
31. Dissanayake, A.J.; Bhunjun, C.S.; Maharachchikumbura, S.S.N.; Liu, J.K. Applied aspects of methods to infer phylogenetic relationships amongst fungi. *Mycosphere* **2020**, *11*, 2652–2676. [CrossRef]
32. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [CrossRef] [PubMed]
33. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols*; Elsevier: Amsterdam, The Netherlands, 1990; pp. 315–322. [CrossRef]
34. Rehner, S.A.; Buckley, E. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences, evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **2005**, *97*, 84–98. [CrossRef] [PubMed]
35. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic Acids Symposium Series*; Information Retrieval Ltd.: London, UK, 1999; Volume 41, pp. 95–98.
36. Pinnoi, A.; Jeewon, R.; Sakayaroj, J.; Hyde, K.D.; Jones, E.B.G. *Berkleasmium crunisia* sp. nov. and its phylogenetic affinities to the *Pleosporales* based on 18S and 28S rDNA sequence analyses. *Mycologia* **2007**, *99*, 378–384. [CrossRef]
37. Beimforde, C.; Feldberg, K.; Nylinder, S.; Rikkinen, J.; Tuovila, H.; Dörfelt, H.; Gube, M.; Jackson, D.J.; Reitner, J.; Seyfullah, L.J.; et al. Estimating the Phanerozoic history of the Ascomycota lineages: Combining fossil and molecular data. *Mol. Phylogenet. Evol.* **2014**, *78*, 386–398. [CrossRef]
38. Pratibha, J.; Prabhugaonkar, A.; Hyde, K.D.; Bhat, D.J. Phylogenetic placement of *Bahusandhika*, *Cancellidium* and *Pseudoepicoccum* (asexual *Ascomycota*). *Phytotaxa* **2014**, *176*, 68–80. [CrossRef]
39. Thambugala, K.M.; Hyde, K.D.; Tanaka, K.; Tian, Q.; Wanasinghe, D.N.; Ariyawansa, H.A.; Jayasiri, S.C.; Boonmee, S.; Camporesi, E.; Hashimoto, A.; et al. Towards a natural classification and backbone tree for *Lophiostomataceae*, *Floricolaceae*, and *Amorosiaceae* fam. nov. *Fungal Divers.* **2015**, *74*, 199–266. [CrossRef]
40. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **2019**, *20*, 1160–1166. [CrossRef]
41. Larsson, A. AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* **2014**, *30*, 3276–3278. [CrossRef]
42. Miller, M.A.; Pfeiffer, W.; Schwartz, T. Creating the CIPRES science gateway for inference of large phylogenetic trees. In Proceedings of the 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, 14 November 2010; pp. 1–8.
43. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef]
44. Swofford, D.L. *PAUP: Phylogenetic Analysis Using Parsimony, Version 4.0 b10*; Sinauer Associates: Sunderland, UK, 2002.
45. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772. [CrossRef] [PubMed]
46. Zhaxybayeva, O.; Gogarten, J.P. Bootstrap, Bayesian probability and maximum likelihood mapping: Exploring new tools for comparative genome analyses. *BMC Genom.* **2002**, *3*, 4. [CrossRef]
47. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef] [PubMed]

48. Rambaut, A.; Drummond, A.J.; Xie, D.; Baele, G.; Suchard, M.A. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* **2018**, *67*, 901. [CrossRef] [PubMed]
49. Rambaut, A. FigTree v1.4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 15 August 2020).
50. Bouckaert, R.; Vaughan, T.G.; Barido-Sottani, J.; Duchêne, S.; Fourment, M.; Gavryushkina, A.; Heled, J.; Jones, G.; Kühnert, D.; De Maio, N.; et al. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **2019**, *15*, e1006650. [CrossRef]
51. Gernhard, T.; Hartmann, K.; Steel, M. Stochastic properties of generalised Yule models, with biodiversity applications. *J. Math. Biol.* **2008**, *253*, 769–778. [CrossRef]
52. Drummond, A.J.; Suchard, M.A.; Xie, D.; Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **2012**, *29*, 1969–1973. [CrossRef]
53. Gueidan, C.; Ruibal, C.; De Hoog, G.S.; Schneider, H. Rock-inhabiting fungi originated during periods of dry climate in the late Devonian and middle Triassic. *Fungal Biol.* **2011**, *115*, 987–996. [CrossRef]
54. Pérez-Ortega, S.; Garrido-Benavent, I.; Grube, M.; Olmo, R.; de los Ríos, A. Hidden diversity of marine borderline lichens and a new order of fungi: *Collemopsidiales* (*Dothideomyceta*). *Fungal Divers.* **2016**, *80*, 285–300. [CrossRef]
55. Mindell, R.A.; Stockey, R.A.; Beard, G.; Currah, R.S. *Margaretbarromyces dictyosporus* gen. sp. nov.: A permineralized corticolous ascomycete from the Eocene of Vancouver Island, British Columbia. *Mycol. Res.* **2007**, *111*, 680–684. [CrossRef]
56. Berbee, M.; Taylor, J.W. Dating the molecular clock in fungi-how close are we? *Fungal Biol. Rev.* **2010**, *24*, 1–16. [CrossRef]
57. Taylor, T.N.; Krings, M.; Taylor, E.L. Ascomycota. In *Fossil fungi*; Academic Press: San Diego, CA, USA, 2015.
58. Cohen, K.M.; Finney, S.C.; Gibbard, P.L.; Fan, J.X. The ICS International Chronostratigraphic Chart. *Episodes* **2013**, *36*, 199–204. [CrossRef] [PubMed]
59. Willis, K.J. *State of the World's Fungi 2018*; Royal Botanic Gardens, Kew: London, UK, 2018.
60. Hyde, K.D.; Jeewon, R.; Chen, Y.J.; Bhunjun, C.S.; Calabon, M.S.; Jiang, H.B.; Lin, C.G.; Norphanphoun, C.; Sysouphanthong, P.; Pem, D.; et al. The numbers of fungi: Is the descriptive curve flattening? *Fungal Divers.* **2020**, *103*, 219–271. [CrossRef]
61. Giraldo, A.; Crous, P.W.; Schumacher, R.K.; Cheewangkoon, R.; Ghobad-Nejhad, M.; Langer, E. The Genera of Fungi—G3: *Aleurocystis*, *Blastacervulus*, *Clypeophysalospora*, *Licrostroma*, *Neohendersonia* and *Spumatoria*. *Mycol. Prog.* **2017**, *16*, 325–348. [CrossRef]
62. Liew, E.C.Y.; Aptroot, A.; Hyde, K.D. Phylogenetic significance of the pseudoparaphyses in *Loculoascomycete* taxonomy. *Mol. Phylogenet. Evol.* **2000**, *16*, 392–402. [CrossRef]
63. Phookamsak, R.; Norphanphoun, C.; Tanaka, K.; Dai, D.Q.; Luo, Z.L.; Liu, J.K.; Su, H.Y.; Bhat, D.J.; Bahkali, A.H.; Mortimer, P.E.; et al. Towards a natural classification of *Astrosphaeriella*-like species; introducing *Astrosphaeriellaceae* and *Pseudoastrosphaeriellaceae* fam. nov. and *Astrosphaeriellopsis*, gen. nov. *Fungal Divers.* **2015**, *74*, 143–197. [CrossRef]
64. Ariyawansa, H.A.; Hyde, K.D.; Jayasiri, S.C.; Buyck, B.; Chethana, K.W.T.; Dai, D.Q.; Dai, Y.C.; Daranagama, D.A.; Jayawardena, R.S.; Lücking, R.; et al. Fungal diversity notes 111–252: Taxonomic and phylogenetic contributions to fungal taxa. *Fungal Divers.* **2015**, *75*, 27–274. [CrossRef]
65. Jaklitsch, W.M.; Voglmayr, H. Hidden diversity in *Thyridaria* and a new circumscription of the *Thyridariaceae*. *Stud. Mycol.* **2016**, *85*, 35–64. [CrossRef]
66. Hyde, K.D.; Norphanphoun, C.; Abreu, V.P.; Bazzicalupo, A.; Chethana, K.W.T.; Clericuzio, M.; Dayarathne, M.C.; Dissanayake, A.J.; Ekanayaka, A.H.; He, M.Q.; et al. Fungal diversity notes 603–708: Taxonomic and phylogenetic notes on genera and species. *Fungal Divers.* **2017**, *87*, 1–235. [CrossRef]
67. Zhang, S.N.; Hyde, K.D.; Jones, E.B.G.; Jeewon, R.; Cheewangkoon, R.; Liu, J.K. *Striatiguttulaceae*, a new pleosporalean family to accommodate *Longicorpus* and *Striatiguttula* gen. nov. from palms. *MycoKeys* **2019**, *49*, 99–129. [CrossRef]
68. Chaw, S.M.; Chang, C.C.; Chen, H.L.; Li, W.H. Dating the Monocot–Dicot divergence and the origin of core Eudicots using whole chloroplast genomes. *J. Mol. Evol.* **2004**, *58*, 424–441. [CrossRef] [PubMed]
69. Phillips, A.J.; Hyde, K.D.; Alves, A.; Liu, J.K. Families in *Botryosphaeriales*: A phylogenetic, morphological and evolutionary perspective. *Fungal Divers.* **2019**, *94*, 1–22. [CrossRef]
70. De Vienne, D.M.; Refrégier, G.; López-Villavicencio, M.; Tellier, A.; Hood, M.E.; Giraud, T. Cospeciation vs host-shift speciation: Methods for testing, evidence from natural associations and relation to coevolution. *New Phytol.* **2013**, *198*, 347–385. [CrossRef] [PubMed]
71. Phukhamsakda, C.; McKenzie, E.; Phillips, A.J.L.; Jones, E.B.G.; Bhat, D.J.; Marc, S.; Bhunjun, C.S.; Wanasinghe, D.N.; Thongbai, B.; Camporesi, E.; et al. Microfungi associated with *Clematis* (*Ranunculaceae*) with an integrated approach to delimiting species boundaries. *Fungal Divers.* **2020**, *102*, 1–203. [CrossRef]
72. Zeng, X.Y.; Jeewon, R.; Hongsanan, S.; Hyde, K.D.; Wen, T.C. Unravelling evolutionary relationships between epifoliar *Meliolaceae* and angiosperms. *J. Syst. Evol.* **2020**, in press. [CrossRef]