

The insights into the evolutionary history of *Translucidithyrium*: based on a newly-discovered species

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Academic editor: N. Wijayawardene | Received 15 September 2020 | Accepted 25 November 2020 | Published 17 December 2020

Citation: Li X, Wu H-X, Li J, Chen H, Wang W (2020) The insights into the evolutionary history of *Translucidithyrium*: based on a newly-discovered species. MycoKeys 76: 1–16. <https://doi.org/10.3897/mycokeys.76.58628>

Abstract

During the field studies, a *Translucidithyrium*-like taxon was collected in Xishuangbanna of Yunnan Province, during an investigation into the diversity of microfungi in the southwest of China. Morphological observations and phylogenetic analysis of combined LSU and ITS sequences revealed that the new taxon is a member of the genus *Translucidithyrium* and it is distinct from other species. Therefore, *Translucidithyrium chinense* **sp. nov.** is introduced here. The Maximum Clade Credibility (MCC) tree from LSU rDNA of *Translucidithyrium* and related species indicated the divergence time of existing and new species of *Translucidithyrium* was crown age at 16 (4–33) Mya. Combining the estimated divergence time, paleoecology and plate tectonic movements with the corresponding geological time scale, we proposed a hypothesis that the speciation (estimated divergence time) of *T. chinense* was earlier than *T. thailandicum*. Our findings provided new insights into the species of *Translucidithyrium* about ecological adaptation and speciation in two separate areas.

Keywords

Divergence time, morphological characteristics, new species, Phaeothecoidiaceae, phylogeny, speciation, taxonomy

Introduction

The sooty blotch and flyspeck fungi are widespread species and commonly occur on the surface of leaves, stems and fruits in tropical and subtropical zones (Yang et al. 2010; Gleason et al. 2011; Hongsanan et al. 2017; Zeng et al. 2018). Although these

fungi do not directly harm host plants, they may affect the economic value of fruit sales ability and reduce photosynthesis in plants (Gleason et al. 2011). Sooty blotch fungi can form dark mycelial mats, whereas flyspeck fungi lack mycelial mats, form shiny and small, black spots (Batzer et al. 2005; Yang et al. 2010; Gleason et al. 2011; Zhang et al. 2015; Singtripop et al. 2016; Hongsanan et al. 2017). However, these fungi are poorly known, because of the difficulty in obtaining the strain which grows slowly (Yang et al. 2010; Hongsanan et al. 2017; Zeng et al. 2018).

Phaeothecoidiaceae K.D. Hyde & Hongsanan was introduced by Hongsanan et al. (2017) and accommodated three genera *Chaetothyria*, *Houjia* and *Phaeothecoidiella* in the order Capnodiales. Currently, it includes eight genera: *Chaetothyria*, *Exopassalora*, *Houjia*, *Nowamyces*, *Phaeothecoidiella*, *Rivilata*, *Sporidesmajora* and *Translucidithyrium* (Hongsanan et al. 2020). Members of Phaeothecoidiaceae are related to sooty blotch and flyspeck fungi and characterised by thyrithecia with setae, bitunicate asci and 1-septate ascospores (Singtripop et al. 2016; Hongsanan et al. 2017; Zeng et al. 2019; Hongsanan et al. 2020). *Chaetothyria* is morphologically similar to the family Micropeltidaceae (Reynolds and Gilbert 2005), but is distinguishable by its brown upper wall of ascomata (Wu et al. 2019; Zeng et al. 2019). The genus *Rivilata* is placed in this family on the basis of morphological characters by Doilom et al. (2018). The *Nowamyces* was introduced as a new genus in the new family Nowamycetaceae by Crous et al. (2019) and Hongsanan et al. (2020) placed this genus into Phaeothecoidiaceae by phylogenetic analysis. Hongsanan et al. (2020) listed *Houjia*, *Exopassalora*, *Sporidesmajora* and *Phaeothecoidiella* as asexual genera in Phaeothecoidiaceae.

Translucidithyrium X.Y. Zeng & K.D. Hyde (2018) was introduced as a monotypic genus in Phaeothecoidiaceae, which is represented by *T. thailandicum* X.Y. Zeng & K.D. Hyde (2018). It was characterised by epiphytes on the reverse of living leaves, semi-transparent ascomata, globose to subglobose asci and fusiform ascospores with verrucose and appendages. Ascospores germinated on MEA (Malt Extract Agar Medium) within 24 h. The colonies slowly grow on media, white to grey, circular and villiform (Zeng et al. 2018).

Liu et al. (2017) used the molecular clock approach to estimate the divergence time of the order Capnodiales crown age at 151–283 Mya (million years ago). Zeng et al. (2019) estimated the divergence time of the family Phaeothecoidiaceae crown age at 40–60 Mya. The molecular clock approach for estimating divergence time might be used to predict speciation, historical climate change or other environmental events (Hélène and Arne 2014; Louca and Pennell 2020).

In this study, we collected an extraordinary new species of *Translucidithyrium* in Xishuangbanna, Yunnan Province, China. We described the morphological characteristics and built a phylogenetic tree to determine the classification of the new taxon. We compared and analysed the estimated divergence time of *Translucidithyrium* with the environmental changes around the corresponding time range to propose the evolutionary history hypothesis of *Translucidithyrium* distributed in two different regions (China and Thailand).

Methods

Morphological

Fresh living leaves with olivaceous dots were collected at Xishuangbanna, China (21°55'51"N, 101°15'08"E, 540 m alt.) and delivered to the laboratory for observation. According to Wu et al. (2014), the collected samples were processed and examined by microscopes: the photos of ascomata were taken by using a compound stereomicroscope (KEYENCE CORPORATION V.1.10 with camera VH-Z20R). Hand sections were made under a stereomicroscope (OLYMPUS SZ61) and mounted in water and blue cotton and photomicrographs of fungal structures were taken with a compound microscope (Nikon ECLIPSE 80i). The single spore isolation was implemented by the methods of Choi et al. (1999) and Chomnunti et al. (2014). Germinated spores were individually transferred to PDA (Potato Dextrose Agar Medium) and incubated at 26 °C for 48 h. Colony characteristics were observed and measured after 4 weeks at 26 °C. Images used for figures were processed with Adobe Photoshop CC v. 2015.5.0 software (Adobe Systems, USA). The holotype was deposited at the herbarium of IFRD (International Fungal Research & Development Centre; Research Institute of Resource Insects, Kunming), reference number IFRD 9208. The ex-type strain was deposited at IFRDCC, reference number IFRDCC 3000.

DNA isolation, amplification and sequencing

According to the manufacturer's instructions, genomic DNA was extracted from mycelium growing on PDA at room temperature by using the Forensic DNA Kit (OMEGA, USA). The primer pair LR0R and LR5 was used to amplify the large subunit (LSU) rDNA (Vilgalys and Hester 1990). The primer pair ITS5 and ITS4 was used to amplify the internal transcribed spacer (ITS) rDNA (White et al. 1990). The primer pair NS1 and NS4 was used to amplify the partial small subunit (SSU) rDNA (White et al. 1990). The PCR reactions were in accordance with instructions from Golden Mix, Beijing TsingKe Biotech Co. Ltd, Beijing, China: initial denaturation at 98 °C for 2 min, then 30 cycles of 98 °C denaturation for 10 s, 56 °C annealing for 10 s and 72 °C extension for 10 s (ITS and SSU) or 20 s (LSU) and a final extension at 72 °C for 1 min. All PCR products were sequenced by Biomed (Beijing, China).

Sequences alignments and phylogenetic analysis

BioEdit version 7.0.5.3 (Hall 1999) was used to re-assemble sequences generated from forward and reverse primers for obtaining the integrated sequences. Sequences were downloaded from GenBank using data from the publications of Zeng et al.

(2018), Crous et al. (2019), Hongsanan et al. (2020) and Renard et al. (2020) and aligned using BioEdit version 7.0.5.3 (Hall 1999): in addition, sequences were adjusted manually.

Maximum Likelihood (ML) analysis was conducted by using RAxMLGUI v.1.0 (Silvestro and Michalak 2012). Aligned sequences were input into the software and *Dothidea sambuci* was selected as the outgroup taxon. One thousand non-parametric bootstrap iterations were employed with the “ML + rapid bootstrap” tools and “GTR-GAMMA” arithmetic.

For Bayesian analysis, MrModeltest 2.3 (Nylander 2004) was used to estimate the best-fitting model for the combined LSU and ITS genes. Posterior probabilities (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo (MCMC) sampling in MrBayes v.3.2 (Ronquist and Huelsenbeck 2003). Six simultaneous Markov chains were run for 2,000,000 generations; trees were printed every 1,000 generations; trees were sampled every 100 generations. The first 5,000 trees submitted to the burn-in phase and were discarded; the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (Cai et al. 2006, 2008; Liu et al. 2012).

Fossil calibrations and divergence time estimations

The fossil *Protographum luttrellii* (Renard et al. 2020) was used to calibrate the divergence time of Asterotexiales and Aulographaceae (normal distribution, mean = 119.0, SD = 3.7). The secondary calibration from the family Phaeothecoidiaceae with a crown age of 58 Mya (normal distribution, mean = 50.0, SD = 6.1) was used (Zeng et al. 2019). The additional secondary calibration of Capnodiales was used, based on the result from Liu et al. (2017) (normal distribution, mean = 217.0, SD = 40.0).

Divergence time analysis was carried out using BEAST v1.8.4 (Drummond et al. 2012). Aligned LSU sequence data were loaded into the BEAUti v1.10.4 for generating an XML file. An uncorrelated relaxed clock model (Drummond et al. 2006) with a lognormal distribution of rates was used for the analysis. We used a Yule Process tree prior (Yule 1925; Gernhard 2008), which assumes a constant speciation rate per lineage and a randomly-generated starting tree. The length of chain was set as 50 million generations and sampling parameters were set at every 5,000 generations in MCMC. Subsequent divergence time analysis was carried out using BEAST v.1.10.4 (Drummond et al. 2012). Tracer v.1.7.1 was used to check the effective sample sizes (ESS) and acceptable values were higher than 200. The .log files and .tree files generated by BEAST were combined in LogCombiner v1.10.4 after removing a proportion of states as burn-in. The Maximum Clade Credibility (MCC) tree was given by obtained data and was estimated in TreeAnnotator v.1.10.4 (Liu et al. 2017; Zeng et al. 2019, 2020; Renard et al. 2020).

The phylogenetic tree and MCC tree were visualized in FigTree v.1.4.3 (Rambaut 2012) and Adobe Illustrator CS6 v. 16.0.0 (Adobe Systems, USA).

Table I. Selected taxa in this study with their corresponding GenBank accession numbers. The newly-generated sequences are shown in bold.

No.	Species	Voucher /strain no.	LSU	ITS
1	<i>Acidomyces acidophilus</i>	MH1085	JQ172741	JQ172741
2	<i>Asterina phenacis</i>	TH 589	GU586217	–
3	<i>Asterotectiaceae</i> sp.	VUL.535	MG844162	–
4	<i>Aulographum</i> sp.	VUL.457	MG844158	–
5	<i>Batcheloromyces proteae</i>	CBS 110696	JF746163	JF746163
6	<i>Baudoinia compniacensis</i>	CBS 123031	GQ852580	–
7	<i>Brunneosphaerella protearum</i>	CPC 16338	GU214397	GU214626
8	<i>Buellia minima</i>	Lendemmer 42237(NY)	KX244961	–
9	<i>Camarosporula persooniae</i>	CBS 116258	JF770461	JF770449
10	<i>Capnobotryella renispora</i>	CBS 214.90	GU214398	AY220612
11	<i>Capnodium coffeae</i>	CBS 147.52	GU214400	DQ491515
12	<i>Catenulostroma protearum</i>	CPC 15368	GU214402	GU214628
13	<i>Chaetothyrina guttulata</i>	MFLUCC15–1080	KU358917	KX372277
14	<i>Chaetothyrina guttulata</i>	MFLUCC15–1081	KU358914	KX372276
15	<i>Chaetothyrina musarum</i>	MFLUCC 15–0383	KU710171	–
16	<i>Cladosporium herbarum</i>	CBS 121621	KJ564331	EF679363
17	<i>Cladosporium hillianum</i>	CBS 125988	KJ564334	HM148097
18	<i>Cladosporium ramotenellum</i>	CBS 170.54	DQ678057	AY213640
19	<i>Colletogloeum</i> sp.	NY1_3.2F1c	FJ031986	FJ425193
20	<i>Conidiocarpus(Phragmocarpius) betle</i>	MFLUCC 10–0050	JN832605	–
21	<i>Devriesia staurophora</i>	ATCC 200934	KF901963	AF393723
22	<i>Dissoconium aciculare</i>	CBS 204.89	GU214419	AY725520
23	<i>Dothidea sambuci</i>	AFTOL-ID 274	AY544681	DQ491505
24	<i>Dothistroma pini</i>	CBS 121011	JX901821	JX901734
25	<i>Elasticomyces elasticus</i>	CCFEE 5547	KF309991	–
26	<i>Exopassalora zambiae</i>	YHJN13	GQ433631	GQ433628
27	<i>Extremus adstrictus</i>	TRN96	KF310022	–
28	<i>Friedmanniomyces endolithicus</i>	CCFEE 5199	KF310007	JN885547
29	<i>Hispidoconidioma alpinum</i>	L2–1/2	FJ997286	FJ997285
30	<i>Hortaea werneckii</i>	CBS 100496	GU301817	AY128703
31	<i>Houjia yanglingensis</i>	YHJN13	GQ433631	GQ433628
32	<i>Lecanosticta pini</i>	CBS 871.95	GQ852598	–
33	<i>Lembosia albersii</i>	MFLUCC 13–0377	KM386982	–
34	<i>Lembosina</i> sp.	VUL.644	MG844165	–
35	<i>Leptoxypium cacuminum</i>	MFLUCC 10–0049	JN832602	–
36	<i>Melanodothis caricis</i>	CBS 860.72	GU214431	GU214638
37	<i>Microcyclosporella mali</i>	CPC 16171	GU570545	GU570528
38	<i>Microxypium citri</i>	CBS 451.66	KF902094	–
39	<i>Morenoina calamicola</i>	MFLUCC 14–1162	NG059779	NR154210
40	<i>Mycosphaerella pneumatophorae</i>	AFTOL-ID 762	KJ176856	–
41	<i>Neodevriesia coryneliae</i>	CPC 23534	KJ869211	KJ869154
42	<i>Neodevriesia hilliana</i>	CPC 15382	GU214414	GU214633
43	<i>Neodevriesia xanthorrhoeae</i>	CBS 128219	HQ599606	HQ599605
44	<i>Neopseudocercospora capsellae</i>	CBS 127.29	KF251830	KF251326
45	<i>Nowamycetes globulus</i>	CBS 144598	MN162196	MN161935
46	<i>Nowamycetes piperitae</i>	CBS 143490	MN162200	MN161944
47	<i>Parapenidiella tasmaniensis</i>	CBS 124991	KF901844	KF901522
48	<i>Passalora eucalypti</i>	CBS 111318	KF901938	KF901613
49	<i>Penidiella columbiana</i>	CBS 486.80	EU019274	KF901630
50	<i>Periconiella velutina</i>	CBS 101950	EU041840	EU041783
51	<i>Petrophila incerta</i>	TRN 77	GU323963	–
52	<i>Phaeophleospora eugeniae</i>	CPC 15159	KF902095	KF901742

No.	Species	Voucher /strain no.	LSU	ITS
53	<i>Phaeothecoidea eucalypti</i>	CBS 120831	KF901848	KF901526
54	<i>Phaeothecoidiella illinoisensis</i>	CBS 125223	GU117901	GU117897
55	<i>Phaeothecoidiella missouriensis</i>	CBS 125222	AY598917	AY598878
56	<i>Phloeospora maculans</i>	CBS 115123	GU214670	GU214670
57	<i>Piedraia hortae</i>	CBS 480.64	GU214466	GU214647
58	<i>Piedraia quintanilhae</i>	CBS 327.63	GU214468	–
59	<i>Pseudocercospora vitis</i>	CPC 11595	GU214483	GU269829
60	<i>Pseudoramichloridium henryi</i>	CBS 124775	KF442561	KF442521
61	<i>Pseudotaeniolina globosa</i>	CCFEE 5734	KF310010	KF309976
62	<i>Pseudoveronaea obclavata</i>	CBS 132086	JQ622102	–
63	<i>Racodium rupestre</i>	L346	EU048583	GU067666
64	<i>Racodium rupestre</i>	L424	EU048582	GU067669
65	<i>Ramichloridium apiculatum</i>	CBS 156.59	EU041848	EU041791
66	<i>Ramularia endophylla</i>	CBS 113265	AY490776	AY490763
67	<i>Ramularia pusilla</i>	CBS 124973	KP894141	KP894248
68	<i>Ramulispora sorgbi</i>	CBS 110578	GQ852653	–
69	<i>Readeriella mirabilis</i>	CBS 125000	KF251836	KF251332
70	<i>Recurvomyces minabilis</i>	CBS 119434	GU250372	FJ415477
71	<i>Repetophragma zygopetali</i>	VIC42946	KT732418	–
72	<i>Schizothyrium pomi</i>	CBS 486.50	EF134948	EF134948
73	<i>Scolecogimna mangiferae</i>	CBS 125467	GU253877	GU269870
74	<i>Scorias spongiosa</i>	CBS 325.33	GU214696	GU214696
75	<i>Septoria cytisi</i>	USO 378994	JF700954	JF700932
76	<i>Septoria lysimachiae</i>	CBS 123794	KF251972	KF251468
77	<i>Sonderbenia eucalyptorum</i>	CBS 120220	KF901822	KF901505
78	<i>Sphaerulina myriadea</i>	CBS 124646	JF770468	JF770455
79	<i>Sporidesmajira pennsylvaniensis</i>	CBS 125229	MH874965	MF951287
80	<i>Stenella araguata</i>	CBS 105.75	EU019250	EU019250
81	<i>Teratoramularia kirschneriana</i>	CBS 113093	GU214669	GU214669
82	<i>Teratosphaeria fibrillosa</i>	CBS 1217.07	GU323213	KF901728
83	<i>Toxicocladosporium irritans</i>	CBS 185.58	EU040243	EU040243
84	<i>Toxicocladosporium rubrigenum</i>	CBS 124158	FJ790305	FJ790287
85	<i>Translucidithyrium chinense</i>	IFRDCC 3000	MT659404	MT659671
86	<i>Translucidithyrium thailandicum</i>	MFLUCC 16–0362	MG993048	MG993045
87	<i>Tripospermum myrti</i>	CBS 437.68	GU323216	–
88	<i>Trochophora simplex</i>	CBS 124744	GU253880	GU269872
89	<i>Uwebraunia communis</i>	CBS 114238	EU019267	AY725541
90	<i>Vermiconia foris</i>	CCFEE 5459	GU250390	KF309981
91	<i>Xenoconiothyrium catenatum</i>	CMW 22113	JN712570	JN712502
92	<i>Zasmidium cellare</i>	CBS 146.36	EU041878	EU041821
93	<i>Zygothiala cryptogama</i>	OH4_1A1a	FJ147157	FJ425208
94	<i>Zygothiala tardicrescens</i>	MWA1a	EF164901	AY598856
95	<i>Zygothiala wisconsinensis</i>	OH4_9A1c	FJ147158	FJ425209

Results

Phylogenetic study

The dataset of combined LSU and ITS sequences comprised 1350 characters after alignment. Bayesian Inference, in total, generated 20,001 trees and the average standard deviation of split frequencies reached 0.0096. A total of 15,001 trees were finally used to calculate posterior probabilities. Phylogenetic analysis showed that the new collection clusters with *T. thailandicum* with 100% Maximum Likelihood bootstrap support and 1.00 posterior probabilities (Fig. 1).

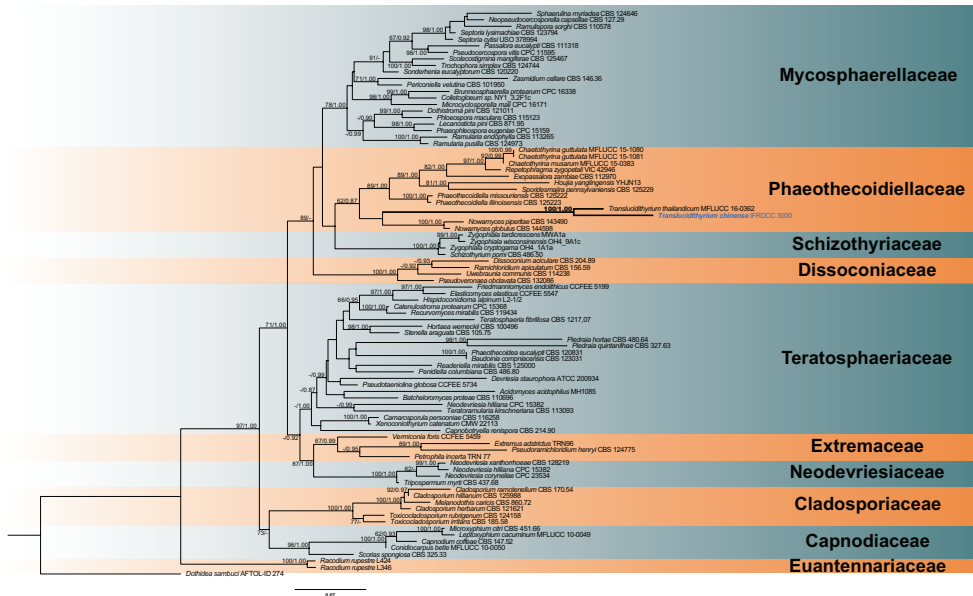


Figure 1. The topology shows family relationships of Capnodiales, based on combined LSU and ITS dataset analysis. Bootstrap values of Maximum Likelihood higher than 60% are shown on the left, while values of Bayesian posterior probabilities above 80% are shown on the right. New species is given in bold. Clades of the key species or family are given in bold. The tree is rooted with *Dothidea sambucci* (Dothideaceae, Dothideales).

Taxonomy

Translucidithyrium chinense H. X. Wu & X. H. Li, sp. nov.

Index Fungorum number: IF 557843

Facesoffungi number: FoF 09429

Figures 2, 3

Etymology. Refer to the location of species, China.

Holotype. IFRD9208

Description. *Epiphytic* on living leaves, ascomata with papillate. *Superficial hyphae* absent. **Sexual morph:** *Ascomata* solitary or scattered, 480–870 µm diam. (\bar{x} = 741 µm, n = 6), 65–82 µm high (\bar{x} = 72 µm, n = 8), olivaceous to brown, slightly semi-transparent under highlighted background, circular to suborbicular, with slightly prominent papilla, membranous, without ostiole (Fig. 2A–C). *Peridium* 8.3–10 µm thick, (\bar{x} = 9 µm, n = 11), composed of irregular, meandering, interwoven arranged cells, two layers: from brown to hyaline, outer layer composed of closely-arranged cells, brown; inner layer composed of hyaline, oblong, subdense arranged cells, poorly developed at the base (Fig. 2D–F). *Asci* evenly distributed and parallel arranged in hamathecium (Fig. 2D–F), 65–90 × 51–81 µm (\bar{x} = 77 × 60 µm, n = 10), 8-spored, bitunicate, hyaline, with an ocular chamber, ovoid at immature state, globose to subglobose at mature

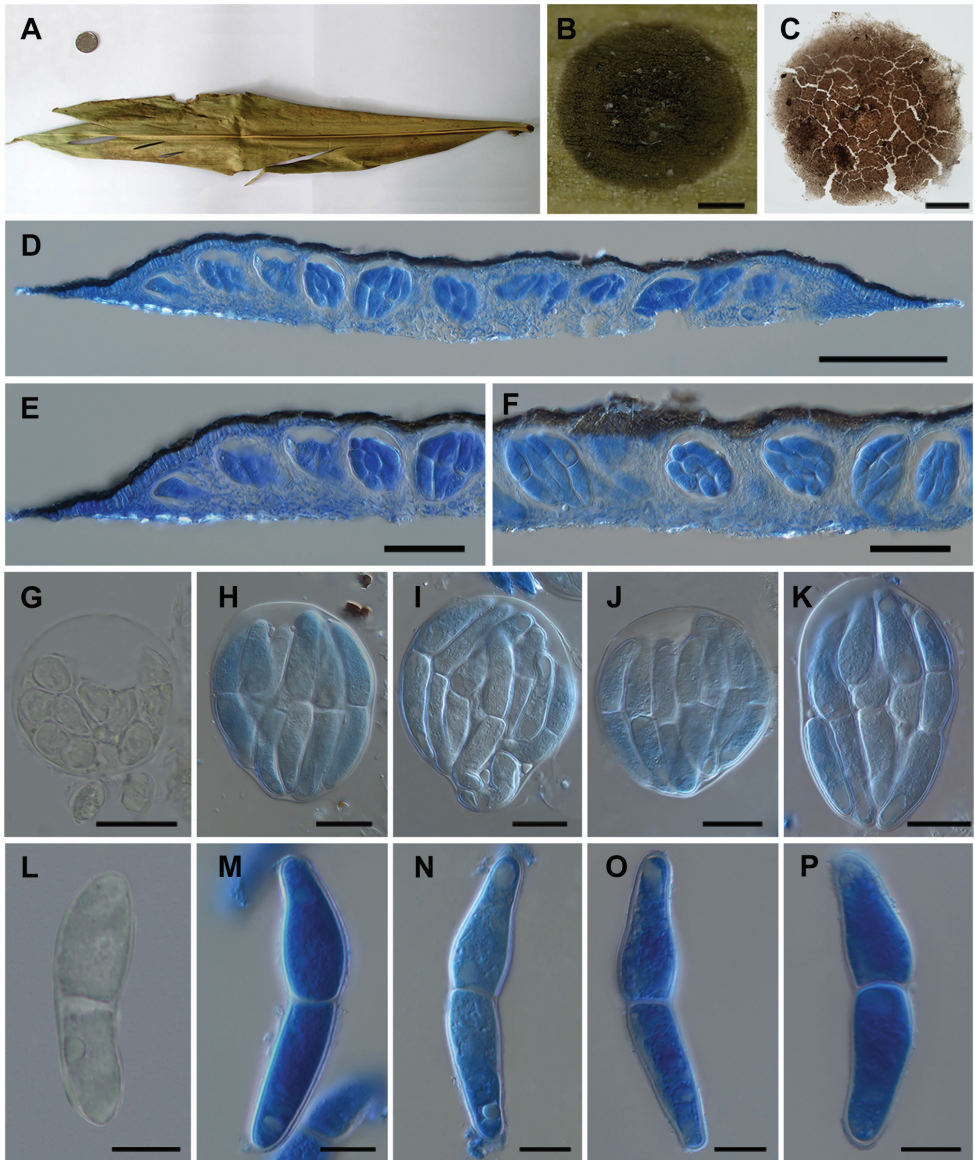


Figure 2. *Translucidithyrium chinense* (IFRD 9208, holotype) **A** plant leaves **B** ascoma on leaves surface **C** squash of ascoma at 20 times amplification **D** cross section of ascoma in blue cotton at 20 times amplification **E, F** cross section of ascoma in blue cotton at 40 times amplification **G** asci at 100 times amplification **H–K** asci in blue cotton at 100 times amplification **L** ascospore at 100 times amplification **M–P** ascospore in blue cotton at 100 times amplification. Scale bars: 200 μm (**B**); 100 μm (**C, D**); 50 μm (**E, F**); 20 μm (**G–K**); 10 μm (**L–P**). We slightly adjusted the contrast, saturation and hue of images and removed the contaminants around main object in images in PS software without obscuration, erasure or distortion of any information existing in the original document.

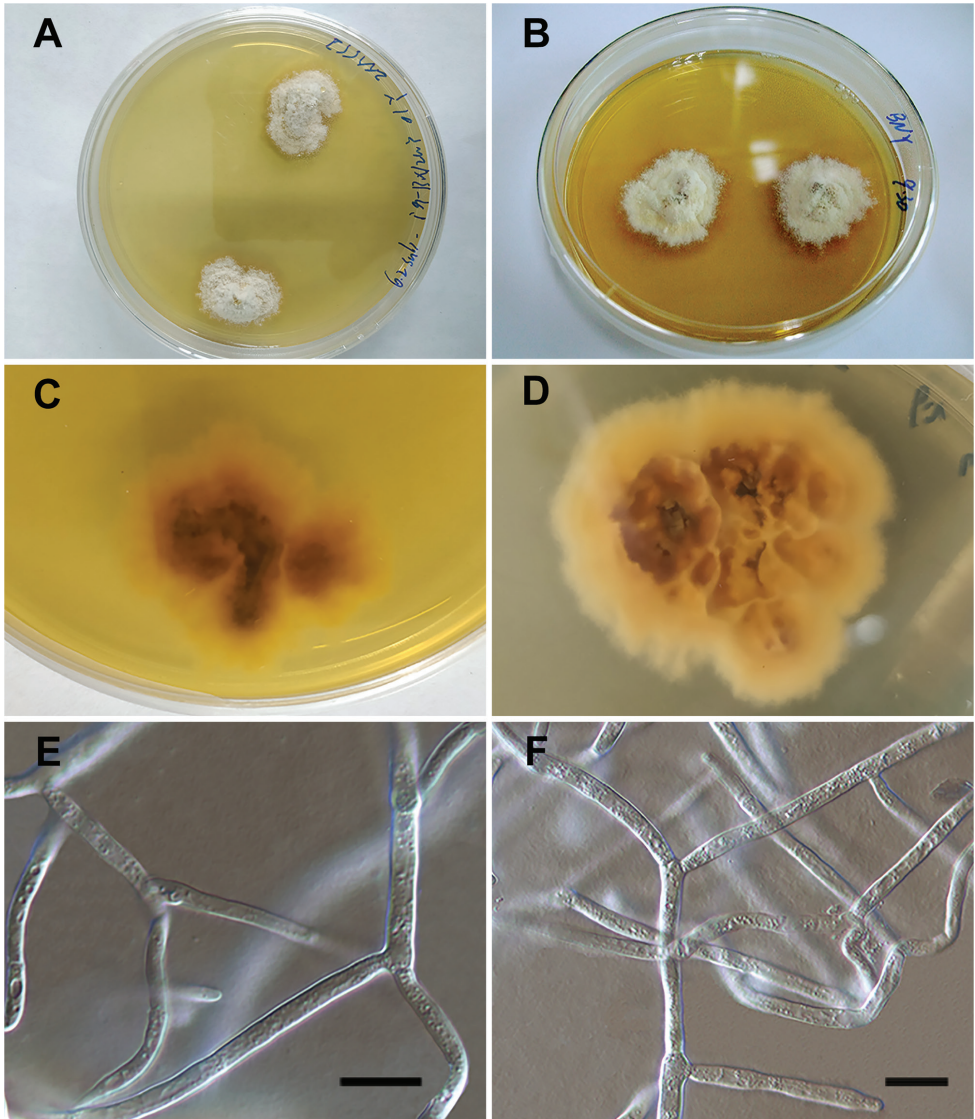


Figure 3. Culture of *Translucidithyrium chinense* (IFRDCC3000) **A, B** culture growing on the medium **C, D** the bottom of the medium with culture growing **E, F** the mycelium of culture at 100 times amplification. Scale bars: 10 μm (**E, F**).

state, lacking pedicel, paraphyses absent (Fig. 2G–K). *Ascospores* 41–65 \times 10–13 μm (\bar{x} = 50 \times 11 μm , n = 20), irregularly overlapping, hyaline, ovoid at young state, fusi-form with both ends tapered at mature state, 1-septate, constricted at the septum, upper cell a little larger than lower, with guttules at both ends, verrucose (Fig. 2L–P). **Asexual morph:** Undetermined.

Culture characteristics. Ascospores germinating on MEA at 36 h after spore-isolation, germinating on PDA at 48 h after spore-isolation. Colonies slow growing on MEA and PDA, irregular, villiform, convex, white on surface, yellow to brown at base. After a long period of growth, the pigments produced by culture discolour the medium, roots generate at the bottom (Fig. 3A–D). Culture hyphae hyaline, branched, constricted at the septum, 3 μm wide (Fig. 3E, F).

Material examined. CHINA, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Xishuangbanna Botanical Garden; 21°55'51"N, 101°15'08"E, 540 m alt.; 21 Apr 2019; Haixia Wu and Xinhao Li leg; collected on living leaves of *Alpinia blepharocalyx* (IFRD 9208, holotype), ex-type living culture (IFRDCC 3000).

Notes. This new species is morphologically similar to *Translucidthyrium thailandicum* in having semi-transparent and largish ascomata, globose asci and hyaline ascospores with 1-septate. However, *Translucidthyrium chinense* has a slightly papilla thyrsothecium with weaker transmittance and ascospores with guttules at both ends, while *T. thailandicum* has a flattened thyrsothecium with higher transmittance and ascospores with appendages at both ends; besides, the size of ascomata and asci of *T. chinense* are slightly larger than those of *T. thailandicum* (795 μm vs. 621 μm; 77 μm vs. 64 μm). The culture characteristics of both species are different: the culture of *T. chinense* grows more slowly, has roots inserting into medium and turn the bottom brown. Phylogenetically, *T. chinense* clusters with *T. thailandicum* as a distinct clade with high support (100% ML / 1.00 PP, Fig. 1).

Divergence times estimates. The Maximum Clade Credibility (MCC) tree was similar to the major lineages in the Bayesian and ML trees. The crown age of

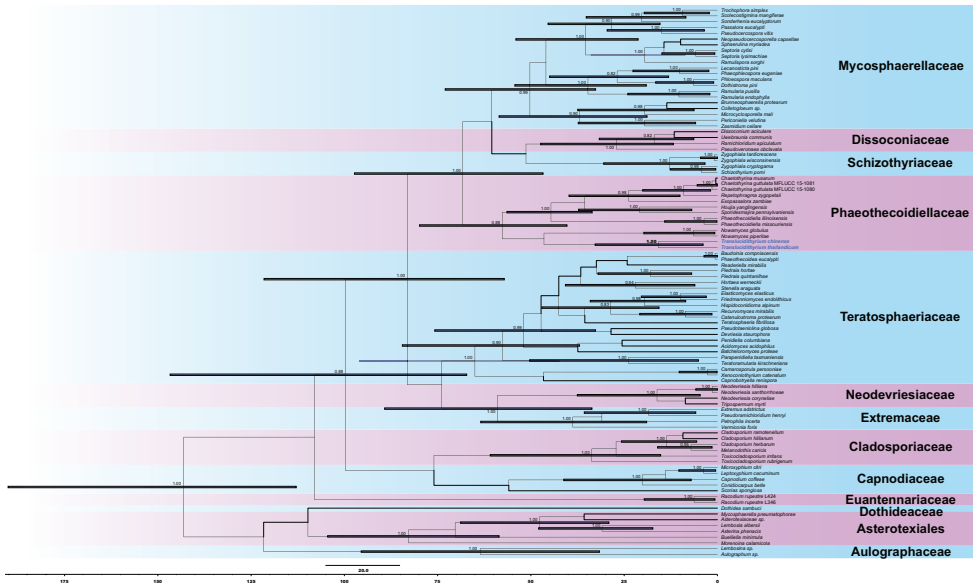


Figure 4. The MCC tree with divergence times estimates of Phaeothecoidiaceae obtained from a Bayesian approach (BEAST). Numbers at nodes indicate posterior probabilities (pp) for node support; bars correspond to the 95% highest posterior density (HPD) intervals. The key species are given in blue.

Translucidithyrium showed 16 Mya (4–33), which was earlier than the divergence time of most genera in Phaeothecoidiaceae. The estimated divergence time of Phaeothecoidiaceae from Zeng et al. (2019) is 58 Mya, which corresponds to our results.

Discussion

Translucidithyrium thailandicum was found in the north of Thailand (Zeng et al. 2018). *Translucidithyrium chinense* was found in the Xishuangbanna Region, southwest of China, which lies on the northern border of a rainforest with rich microfungus resources. The new species is characterised by brown to olivaceous ascomata and slightly semi-transparent, subglobose asci without pedicel and fusiform ascospores with verrucose and guttules (Fig. 2). *T. chinense* is introduced as a new species in *Translucidithyrium* by morphological and phylogenetic studies (Figs 1–3).

The ascomata of *Translucidithyrium* are different from related genera of Phaeothecoidiaceae: *Nowamyces* has immersed ascomata, *Chaetothyria* has ascomata with setae and *Rivulata* has subcuticular ascomata (Singtripop et al. 2016; Doilom et al. 2018; Zeng et al. 2018; Crous et al. 2019; Hongsanant et al. 2020). *Translucidithyrium* is similar to the family Schizothyriaceae in having semi-transparent ascomata, globose to subglobose asci and hyaline ascospores with guttules. Schizothyriaceae includes *Schizothyrium*, *Plochmopeltis*, *Hexagonella*, *Lecideopsella*, *Mycerema*, *Kerniomyces*, *Metathyriella*, *Myriangiella*, *Amazonotheca* and *Vonarxella* (Phookamsak et al. 2016; Wijayawardene et al. 2020). The morphology of *T. chinense* is most similar to *Lecideopsella* by having globose asci and 1-septate ascospores, but *Lecideopsella* has a short pedicel at the bottom of the asci (Phookamsak et al. 2016; Zeng et al. 2018). Phylogenetically, *Translucidithyrium* formed a long clade and clustered within the family Phaeothecoidiaceae. It indicated the existing certain genetic distance amongst *Translucidithyrium*, Phaeothecoidiaceae and Schizothyriaceae. Phaeothecoidiaceae and Schizothyriaceae are poorly studied families (Batzer et al. 2008; Phookamsak et al. 2016; Singtripop et al. 2016; Hongsanant et al. 2017; Zeng et al. 2018). Therefore, more fresh specimens with molecular data are needed to confirm the classification of *Translucidithyrium*, Phaeothecoidiaceae and Schizothyriaceae.

Zuckermandl and Pauling (1962) suggested that the number of differences amongst amino acids was proportional to species divergence time. We estimated the divergence time using BEAST analysis. The divergence time of *Translucidithyrium* crown age was estimated at 16 Mya (4–33), which was earlier than the crown ages of *Chaetothyria* at 2 Mya (0–5), the crown ages of *Repetophragma* at 9 Mya (2–20), the crown ages of *Nowamyces* at 7 Mya (1–20) and the crown ages of *Phaeothecoidiella* at 4 Mya (0–14) within Phaeothecoidiaceae (Fig. 4). The divergence time of *Translucidithyrium* is earlier than other genera in Phaeothecoidiaceae. We estimate that the long divergence time should affect the genetic variation (Pauling 1964; Hall and Hallgrímsson 2008). Additionally, the evolutionary molecular clock approach confirmed the long clades of *Translucidithyrium* in the phylogenetic tree (Fig. 1).

Historical events amongst different biological groups could then be compared with the dates of plate tectonic movements and paleoecology, according to the corresponding geological time scale (Lomolino et al. 2006; Berbee and Taylor 2010). Through relevant studies on the Qinghai-Tibet Plateau, it was found that the time of intense tectonic uplift and denudation is concentrated in 60–35 Mya, 25–17 Mya, 12–8 Mya and 5 Mya. Global cooling might have an impact on climate change in East Asia, especially at 15 Mya and 8 Mya (Lu et al. 2010). Rising plateaus and global cooling were drying up Asia (Liu 2000; Garziona et al. 2015). The time of the Qinghai-Tibet Plateau uplift and global cooling corresponded to the interval of the species in *Translucidithyrium* divergence time. We predict that the speciation of *T. chinense* was earlier than the speciation of *T. thailandicum*, as the divergence of *Translucidithyrium* was related to the Qinghai-Tibet Plateau uplift and global cooling. According to the evolution history of *Translucidithyrium*, it could be speculated that the speciation of *T. chinense* was earlier than *T. thailandicum*. With the climate becoming colder and with increased drought, *T. chinense* migrated from China to Thailand gradually to find a suitable area, then *T. thailandicum* formed. Due to the end of global cooling, the distribution pattern of *Translucidithyrium* in two different countries formed. Increasing fresh collections and application of new methodologies may result in modified conclusions.

Acknowledgements

Funds for research were provided by the Grant for Essential Scientific Research of National Nonprofit Institute (no. CAFYBB2019QB005), the Yunnan Province Ten Thousand Plan of Youth Top Talent Project (no. YNWR-QNBJ-2018-267) and the Yunnan Fundamental Research Projects (grant NO. 202001AT070014). The authors are deeply grateful to Prof. K.D. Hyde (Mae Fah Luang University, Thailand, MFU) for editing the English language of the manuscript, to Dr. Xiang-Yu Zeng and Dr. Nawaz Haider for revising this manuscript and to Dr. Rungtiwa Phookamsak for guiding experiment operation.

References

- Batzer JC, Gleason ML, Harrington TC, Tiffany LH (2005) Expansion of the sooty blotch and flyspeck complex on apples based on analysis of ribosomal DNA gene sequences and morphology. *Mycologia* 97(6): 1268–1286. <https://doi.org/10.1080/15572536.2006.11832735>
- Batzer JC, Arias MMD, Harrington TC, Gleason ML, Groenewald JZ, Crous PW (2008) Four species of *Zygothiala* (Schizothyriaceae, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. *Mycologia* 100: 246–258. <https://doi.org/10.1080/15572536.2008.11832480>
- Berbee ML, Taylor JW (2010) Dating the molecular clock in fungi – how close are we? *Fungal Biology Reviews* 24: 1–16. <https://doi.org/10.1016/j.fbr.2010.03.001>

- Cai L, Jeewon R, Hyde KD (2006) Phylogenetic investigations of Sordariaceae based on multiple gene sequences and morphology. *Mycological Research* 110: 137–150. <https://doi.org/10.1016/j.mycres.2005.09.014>
- Cai L, Guo XY, Hyde KD (2008) Morphological and molecular characterisation of a new anamorphic genus *Cheirosporium*, from freshwater in China. *Persoonia* 20: 53–58. <https://doi.org/10.3767/003158508X314732>
- Choi Y, Hyde KD, Ho W (1999) Single spore isolation of fungi. *Fungal Diversity* 3: 29–38.
- Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q, Alias AS (2014) The sooty moulds. *Fungal Diversity* 66: 1–36. <https://doi.org/10.1007/s13225-014-0278-5>
- Crous PW, Wingfield MJ, Cheewangkoon R, Carnegie AJ, Burgess TI, Summerell BA, Edwards J, Taylor PWJ, Groenewald JZ (2019) Foliar pathogens of eucalypts. *Studies in Mycology* 94, 125–298. <https://doi.org/10.1016/j.simyco.2019.08.001>
- Doilom M, Hyde KD, Phookamsak R, Dai DQ, Tang LZ, Hongsanan S, Chomnunti P, Boonmee S, Dayarathne MC, Li WJ, Thambugala KM, Perera RH, Daranagama DA, Norphanphoun C, Konta S, Dong W, Ertz D, Phillips AJL, McKenzie EHC, Vinit K, Ariyawansa HA, Jones EBG, Mortimer PE, Xu JC, Promputtha I (2018) Mycosphere Notes 225–274: types and other specimens of some genera of *Ascomycota*. *Mycosphere* 9(4): 647–754.
- Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4: e88. <https://doi.org/10.1371/journal.pbio.0040088>
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29(8): 1969–1973. <https://doi.org/10.1093/molbev/mss075>
- Garziona CN, Quade J, Decelles PG, English NB (2015) Predicting paleo-elevation of Tibet and the Himalaya from $\delta^{18}\text{O}$ vs. altitude gradients in meteoric water across the Nepal Himalaya. *Earth & Planetary Science Letters* 183: 215–229. [https://doi.org/10.1016/S0012-821X\(00\)00252-1](https://doi.org/10.1016/S0012-821X(00)00252-1)
- Gernhard T (2008) The conditioned reconstructed process. *Journal of Theoretical Biology* 253: 769–778. <https://doi.org/10.1016/j.jtbi.2008.04.005>
- Gleason ML, Batzer JC, Sun GY, Zhang R, Arias MMD, Sutton TB, Crous PW, Ivanović M, McManus PS, Cooley DR, Mayr U, Weber RWS, Yoder KS, Ponte EMD, Biggs AR, Oertel B (2011) A new view of sooty blotch and flyspeck. *Plant Disease* 95(4): 368–383. <https://doi.org/10.1094/PDIS-08-10-0590>
- Hall BK, Hallgrímsson B (2008) Strickberger's evolution. Sudbury, Jones and Bartlett.
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hélène M, Arne M (2014) Phylogenetic approaches for studying diversification. *Ecology Letters* 17: 508–525. <https://doi.org/10.1111/ele.12251>
- Hongsanan S, Hyde KD, Phookamsak R, Wanasinghe DN, McKenzie EHC, Sarma VV, Boonmee S, Lücking R, Bhat DJ, Liu NG, Tennakoon DS, Pem D, Karunarathna A, Jiang SH, Jones EBG, Phillips AJL, Manawasinghe IS, Tibpromma S, Jayasiri SC, Sandamali DS, Jayawardena RS, Wijayawardene NN, Ekanayaka AH, Jeewon R, Lu YZ, Dissanayake AJ, Zeng XY, Luo ZL, Tian Q, Phukhamsakda C, Thambugala KM, Dai DQ, Chethana KWT, Samarakoon MC, Ertz D, Bao DF, Doilom M, Liu JK, Pérez-Ortega S, Suija A, Senwana

- C, Wijesinghe SN, Konta S, Niranjana M, Zhang SN, Ariyawansa HA, Jiang HB, Zhang JF, Norphanphoun C, de Silva NI, Thiagaraja V, Zhang H, Bezerra JDP, Miranda-González R, Aptroot A, Kashiwadani H, Harishchandra D, Sérusiaux E, Aluthmuhandiram JVS, Abeywickrama PD, Devadatha B, Wu HX, Moon KH, Gueidan C, Schumm F, Bundhun D, Mapook A, Monkai J, Chomnunti P, Suetrong S, Chaiwan N Dayarathne MC, Yang J, Rathnayaka AR, Bhunjun CS, Xu JC, Zheng JS, Liu G, Feng Y, Xie N (2020) Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. *Mycosphere* 11(1): 1553–2107. <https://doi.org/10.5943/mycosphere/11/1/13>
- Hongsanan S, Zhao RL, Hyde KD (2017) A new species of *Chaetothyria* on branches of mango, and introducing Phaeothecoidiaceae fam. nov. *Mycosphere* 8: 137–146.
- Liu JK, Hyde KD, Jeewon R, Phillips AJL, Maharachchikumbura SSN, Ryber, M, Liu ZY, Zhao Q (2017) Ranking higher taxa using divergence times: a case study in Dothideomycetes. *Fungal Diversity* 84(1): 75–99. <https://doi.org/10.1007/s13225-017-0385-1>
- Liu JK, Phookamsak R, Doilom M, Wikee S, Li YM, Ariyawansa H, Boonmee S, Chomnunti P, Dai DQ, Bhat JD, Romero AI, Zhuang WY, Monkai J, Jones EBG, Chukeatirote E, Ko TWK, Zhao YC, Wang Y, Hyde KD (2012) Towards a natural classification of Botryosphaerales. *Fungal Diversity* 57: 149–210. <https://doi.org/10.1007/s13225-012-0207-4>
- Liu X (2000) Sensitivity of East Asian monsoon climate to the uplift of the Tibetan Plateau. *Palaeogeography Palaeoclimatology Palaeoecology* 183: 223–245. [https://doi.org/10.1016/S0031-0182\(01\)00488-6](https://doi.org/10.1016/S0031-0182(01)00488-6)
- Lomolino MV, Riddle BR, Whittaker RH, Brown JH (2006) *Biogeography*. Inc. Publishers, Sunderland.
- Louca S, Pennell MW (2020) Extant timetrees are consistent with a myriad of diversification histories. *Nature* 580: 502–505. <https://doi.org/10.1038/s41586-020-2176-1>
- Lu H, Wang X, Li L (2010) Aeolian sediment evidence that global cooling has driven late Cenozoic stepwise aridification in central Asia. *Geological Society London Special Publications* 342: 29–44. <https://doi.org/10.1144/SP342.4>
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Pauling L (1964) Molecular disease and evolution. *Bulletin of the New York Academy of Medicine* 40(5): 334–342. <https://doi.org/10.1159/000405851>
- Phookamsak R, Boonmee S, Norphanphoun C, Wanasinghe D, de Silva N, Dayarathne M, Hongsanan S, Bhat DJ, Hyde KD (2016) Schizothyriaceae. *Mycosphere* 7: 154–189. <https://doi.org/10.5943/mycosphere/7/2/7>
- Rambaut A (2012) FigTree version 1.4.0. Computer program and documentation distributed by the author. <http://treebioedacuk/software/figtree>
- Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311. <https://doi.org/10.1007/BF02338839>
- Renard LL, Stockey RA, Upchurch G, Berbee ML (2020) A new epiphyllous fly-speck fungus from the Early Cretaceous Potomac group of Virginia (125–112 Ma): *Protographum lutrellii*, gen. et sp. nov. *Mycologia* 112: 504–518. <https://doi.org/10.1080/00275514.2020.1718441>

- Reynolds DR, Gilbert GS (2005) Epifoliar fungi from Queensland, Australia. *Australian Systematic Botany* 18(3): 265–289. <https://doi.org/10.1071/SB04030>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335–337. <https://doi.org/10.1007/s13127-011-0056-0>
- Singtripop C, Hongsanun S, Li J, De Silva NI, Phillips AJL, Jones GEB, Bahkali AH, Hyde KD (2016) *Chaetothyria mangiferae* sp. nov., a new species of *Chaetothyria*. *Phytotaxa* 255(1): 21–33. <https://doi.org/10.11646/phytotaxa.255.1.2>
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246. <https://doi.org/10.1128/JB.172.8.4238-4246.1990>
- von Höhnelt FXR (1909) Fragmente zur Mykologie: VIII. Mitteilung (Nr. 354 bis 406). *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math.-naturw. Klasse Abt. I.* 118: 1157–1246.
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) *PCR protocols: a guide to methods and applications*. Academic Press, New York, 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wijayawardene NN, Hyde KD, Al-Ani LKT, Tedersoo L, Haelewaters D, Rajeshkumar KC, Zhao RL, Aptroot A, Leontyev DV, Saxena RK, Tokarev YS, Dai DQ, Letcher PM, Stephenson SL, Ertz D, Lumbsch HT, Kukwa M, Issi IV, Madrid H, Phillips AJL, Selbmann L, Pfliegler WP, Horváth E, Bensch K, Kirk PM, Kolaříková K, Raja HA, Radek R, Papp V, Dima B, Ma J, Malosso E, Takamatsu S, Rambold G, Gannibal PB, Triebel D, Gautam AK, Avasthi S, Suetrong S, Timdal E, Fryar SC, Delgado G, Réblová M, Doilom M, Dolatabadi S, Pawłowska JZ, Humber RA, Kodsueb R, Sánchez-Castro I, Goto BT, Silva DKA, de Souza FA, Oehl F, da Silva GA, Silva IR, Błaszowski J, Jobim K, Maia LC, Barbosa FR, Fiuza PO, Divakar PK, Shenoy BD, Castañeda-Ruiz RF, Somrithipol S, Lateef AA, Karunarathna SC, Tibpromma S, Mortimer PE, Wanasinghe DN, Phookamsak R, Xu J, Wang Y, Tian F, Alvarado P, Li DW, Kušan I, Matočec N, Mešić A, Tkalčec Z, Maharachchikumbura SSN, Papizadeh M, Heredia G, Wartchow F, Bakhshi M, Boehm E, Youssef N, Hustad VP, Lawrey JD, Santiago ALC, Bezerra JDP, Souza-Motta CM, Firmino AL, Tian Q, Houbraken J, Hongsanan S, Tanaka K, Dissanayake AJ, Monteiro JS, Grossart HP, Suija A, Weerakoon G, Etayo J, Tsurykau A, Vázquez V, Mungai P, Damm U, Li QR, Zhang H, Boonmee S, Lu YZ, Becerra AG, Kendrick B, Brearley FQ, Motiejūnaitė J, Sharma B, Khare R, Gaikwad S, Wijesundara DSA, Tang LZ, He MQ, Flakus A, Rodriguez-Flakus P, Zhurbenko MP, McKenzie EHC, Stadler M, Bhat DJ, Liu JK, Raza M, Jeewon R, Nasonova ES, Prieto M, Jayalal RGU, Erdoğan M, Yurkov A, Schnittler M, Shchepin ON, Novozhilov YK, Silva-Filho AGS, Gentekaki E, Liu P, Cavender JC, Kang Y, Mohammad S, Zhang LF, Xu RF, Li YM, Dayarathne MC, Ekanayaka AH, Wen TC, Deng CY, Pereira OL, Navathe S, Hawksworth DL, Fan XL, Dissanayake LS, Kuhnert E, Grossart HP, Thines M (2020) Outline of Fungi and fungi-like taxa. *Mycosphere* 11(1): 1060–1456. <https://doi.org/10.5943/mycosphere/11/1/8>

- Wu HX, Chen H, Rong K, Yi CS (2019) Type studies in Micropeltidaceae and suggested transfer of three excluded genera. *Nova Hedwigia* 108: 517–526. https://doi.org/10.1127/nova_hedwigia/2019/0517
- Wu HX, Li Y, Ariyawansa HA, Li W, Yang H, Hyde KD (2014) A new species of *Microthyrium* from Yunnan, China. *Phytotaxa* 176(1): 213–218. <https://doi.org/10.11646/phytotaxa.176.1.21>
- Yang HL, Sun GY, Batzer JC, Crous PW, Groenewald JZ, Gleason ML (2010) Novel fungal genera and species associated with the sooty blotch and flyspeck complex on apple in China and the USA. *Persoonia* 24: 29–37. <https://doi.org/10.3767/003158510X492101>
- Yule GU (1925) A mathematical theory of evolution, based on the conclusions of Dr. J.C. Willis. *Philosophical Transactions of the Royal Society B – Biological Sciences* 213: 21–87. <https://doi.org/10.1098/rstb.1925.0002>
- Zeng XY, Hongsanan S, Hyde KD, Putarak C, Wen TC (2018) *Translucidithyrium thailandicum* gen. et sp. nov.: a new genus in Phaeothecoidiaceae. *Mycological Progress* 17: 1087–1096. <https://doi.org/10.1007/s11557-018-1419-0>
- Zeng XY, Jeewon R, Hongsanan S, Hyde KD, Wen TC (2020) Unravelling evolutionary relationships between epifoliar Meliolaceae and angiosperms. *Journal of Systematics and Evolution*. <https://doi.org/10.1111/jse.12643>
- Zeng XY, Wu HX, Hongsanan S, Jeewon R, Wen TC, Maharachchikumbura SSN, Chomnunti P, Hyde KD (2019) Taxonomy and the evolutionary history of Micropeltidaceae. *Fungal Diversity* 97: 393–436. <https://doi.org/10.1007/s13225-019-00431-8>
- Zhang M, Gao L, Shang S, Han X, Zhang R, Latinović J, Latinović N, Batzer JC, Gleason ML, Sun GY (2015) New species and record of *Zygophiala* (Capnodiales, Mycosphaerellaceae) on apple from Montenegro. *Phytotaxa* 195: 227–235. <https://doi.org/10.11646/phytotaxa.195.3.2>
- Zhaxybayeva O, Gogarten JP (2002) Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* 3: e4. <https://doi.org/10.1186/1471-2164-3-4>
- Zuckerkindl E, Pauling LB (1962) Molecular disease, evolution, and genetic heterogeneity. In: Kasha M, Pullman B (Eds) *Horizons in Biochemistry*. Academic Press, New York, 189–225.