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Multigene phylogeny and taxonomy of Torula hydei and Dendryphion hydei spp. nov. from herbaceous litter in northern Thailand

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Multigene phylogeny and taxonomy of *Torula hydei* and *Dendryphion hydei* spp. nov. from herbaceous litter in northern Thailand

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Abstract

Asexual fungi are some of the most significant microorganisms involved in decomposition of plants and contribute to nutrient recycling. During our studies on asexual fungi colonizing herbaceous litter in northern Thailand, we discovered two new fungal species, viz. *Torula hydei* and *Dendryphion hydei* spp. nov. The latter are examined, and their morphological characters are described as well as their DNA sequences from ribosomal and protein coding genes are analysed to infer their phylogenetic relationships with extant fungi. *Torula hydei* is different from other similar *Torula* species in having tiny and catenate conidia. *Dendryphion hydei* can be distinguished from other similar *Dendryphion* species in having large conidiophores and subhyaline to pale olivaceous brown, 2–4(–5)-septate conidia. Multigene phylogenetic analyses of a combined LSU, SSU, TEF1-α, RPB2 and ITS DNA sequence dataset generated from maximum likelihood and Bayesian inference analyses indicate that *T. hydei* forms a distinct lineage and basal to *T. fici*. *Dendryphion hydei* forms a distinct lineage and basal to *D. europaeum*, *D. comosum*, *D. aquaticum* and *D. fluminicola* within Torulaceae (Pleosporales, Dothideomycetes).

Keywords – ~~2~~ new species, Dothideomycetes, Hyphomycetes, Pleosporales, Torulaceae

Introduction

The family Torulaceae Corda was ~~validly~~ introduced by Sturm [46] and is typified by *Torula* Pers. Species in Torulaceae are known only by their asexual morphs which are characterized

by micro- or macronematous conidiophores, with or without apical branches. Conidiogenous cells are doliiform to ellipsoid or clavate, brown, smooth to verruculose, mono- to polyblastic, often cupulate. Conidia are subcylindrical, phragmosporous, acrogenous, brown, dry, smooth to verrucose, characteristically produced in branched chains [3,9,20,30,47,48]. Crous et al. [8] investigated phylogenetic relationships of this family with the inclusion of *Torula* species and accepted *Dendryphion* Wallr. and *Torula* within Torulaceae in Pleosporales. Su et al. [47] introduced *Neotorula* Ariyaw., Z.L. Luo & K.D. Hyde and two new *Dendryphion* species in Torulaceae based on molecular data. Li et al. [29] established a novel genus, *Sporidesmioides* Jun F. Li, Phook. & K.D. Hyde. Su et al. [48] examined 21 freshwater taxa in Torulaceae and updated phylogenetic relationships of taxa within the family based on ITS, LSU, TEF1-α and RPB2 genes and accommodated *Rostriconidium* Z.L. Luo, K.D. Hyde & H.Y. Su within Torulaceae. Currently, there are five accepted genera in Torulaceae viz. *Dendryphion, Neotorula, Rostriconidium*, *Sporidesmioides* and *Torula* [20,29,47,48].

Torula is typified by *T. herbarum* Pers. and is morphologically characterized by having terminal or lateral, monoblastic or polyblastic conidiogenous cells with a thickened and heavily melanized wall on the base and thin-walled and frequently collapsing and becoming coronate on the apex [6]. Crane and Schoknecht [7] provided details of conidiogenesis in *Torula* based on light and transmission electron microscopy. Based on their examination, conidiogenesis has provided good taxonomic insights useful to segregate *Torula* and these were also observed by Mason [33], Hughes [19], Subramanian [49] and Ellis [14,15]. However, there was little information regarding the phylogenetic relationships of *Torula* until the studies of Crous et al. [8], Li et al. [30] and Su et al. [47,48]. Based on the LSU rDNA sequence analysis, Crous et al.

[8] reported two new species, *T. fici* Crous [as '*ficus*'] and *T. hollandica* Crous. Li et al. [30] introduced four new species, *T. chiangmaiensis* Jun F. Li, Phook. & K.D. Hyde, *T. chromolaenae* Jun F. Li, Phook., Mapook & K.D. Hyde, *T. mackenziei* Jun F. Li, Phook. & K.D. Hyde and *T. pluriseptata* Jun F. Li, Phook., Camporesi & K.D. Hyde based on the analysis of a combined LSU, SSU, TEF1-α and RPB2 sequence dataset. Su et al. [48] introduced *T. aquatica* Z.L. Luo, K.D. Hyde, X.J. Su & H.Y. Su based on phylogenetic analyses of the combined ITS, LSU, RPB2 and TEF1-α sequence data. Hyde et al. [22] introduced *T. breviconidiophora* C.G. Lin & K.D. Hyde and *T. polyseptata* C.G. Lin & K.D. Hyde based on the analysis of the combined ITS, LSU, SSU and TEF1-α sequence data. To date, only 15 species have their DNA sequence data being analysed to reveal their phylogenetic placements in Torulaceae [21,22,29,30,47,48,52].

Dendryphion Wallr. was introduced by Wallroth [56] to accommodate hyphomycetous species, *D. comosum* Wallr. The genus is commonly known to be saprobic on dead stems of herbaceous plants and decaying wood, and is characterized by having erect, solitary, branched in upper part, polytretic conidiophores, forming septate, pigmented, thick-walled, finely roughened stipe and a distinct conidiogenous apparatus, with dark scars and catenate, in simple or branched chains of brown, septate (didymo- or cheiro) conidia [8,48]. Crous et al. [9] introduced *D. europaeum* Crous & R.K. Schumacher based on morphological characteristics and molecular data and later Crous et al. [8] accommodated the species in Torulaceae and further accepted *Dendryphion* in Torulaceae. Su et al. [47] circumscribed genera of Torulaceae from freshwater habitats and introduced two *Dendryphion* species, *D. aquaticum* Hong Y. Su & K.D. Hyde and *D. submersum* Hong Y. Su & K.D. Hyde and designated a reference specimen

of *D. nanum* (Nees) S. Hughes based on molecular phylogeny. Su et al. [48] also introduced *D. fluminicola* Z.L. Luo, D.J. Bhat & K.D. Hyde. Only seven *Dendryphion* species have DNA sequence data and their phylogenetic affinities to members of the Torulaceae have been investigated.

In this study, a novel *Torula* species was isolated from herbaceous litters collected from northern Thailand. Among collected samples, *Dendryphion hydei* is also recovered as a new species from northern Thailand. These species are described and illustrated. In addition, an updated phylogenetic tree with our new taxon for the family Torulaceae is provided in this paper.

Material and Methods

Isolation and identification

The specimens were collected from herbaceous litters (*Chromolaena odorata* Linn. and *Bidens pilosa* Linn.) in northern Thailand during the year 2015 to 2016. Samples were returned to the laboratory (Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand) for examination and description of morphological characteristics. The specimens were observed under a Motic SMZ 168 series dissecting stereomicroscope. The conidial structures were picked up by a sterilized surgical needle and transferred into 10% lacto-glycerol on a clean slide and examined under a Nikon Eclipse 80i compound microscope and photo-captured with a Canon 600D digital camera using DIC microscopy. Macro- morphological structures were photographed with a Discovery V.8 stereo microscope fitted with a CARL ZEISS Axio Cam ERc5S microscope camera. Tarosoft® Image Frame Work program v.0.9.0.7

and Adobe Photoshop CS5 Extended version 10.0 software (Adobe Systems Inc., The United States) were used for measurements and drawing photographic plates.

Single conidia isolation was carried out to obtain pure cultures as described in Dai et al. [11]. Germinating conidia were transferred aseptically to potato dextrose agar (PDA) and malt extract agar (MEA) plates and grown at 16–30 °C in alternating day and night light. Colony characters were observed and recorded after one week and at weekly intervals [4, 5].

The type specimens were deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), Yunnan, China. Ex-type living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC 18-0250 and MFUCC 18-0236) and Kunming Institute of Botany Culture Collection (KUMCC 16-0037 and KUMCC 18-0009). Faces of Fungi and Index Fungorum numbers are registered as outlined in Jayasiri et al. [24] and Index Fungorum [23]. New species are established based on guidelines of Jeewon and Hyde [26].

DNA extraction, PCR amplification and sequencing

Fungal mycelium was scraped off and transferred to a 1.5 ml micro-centrifuge tube using a sterilized lancet for genomic DNA extraction. The Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) was used to extract fungal genomic DNA, following the protocols in the manufacturer's instructions.

DNA amplification was performed by polymerase chain reaction (PCR) using the following genes (ITS, LSU, SSU, RPB2 and TEF1-α). The primers ITS5 and ITS4 primer pairs were used to amplify the ITS and 5.8S regions of the rDNA gene [58]; The primers LR0R and

LR5 were used to amplify the partial ribosomal RNA for the 28S nuclear large subunit (LSU) [54]; NS1 and NS4 were used to amplify the partial ribosomal RNA for the 18S nuclear small subunit (SSU) [58]; fRPB2-5F and fRPB2-7cR were used to amplify the partial RNA polymerase second largest subunit (RPB2) [32] and EF1-983F and EF1-2218R were used to amplify the translation elongation factor 1-alpha gene (TEF1-α) [38].

The final volume of the PCR reaction was 25 μl, containing 1 μl of DNA template, 1 μl of each forward and reward primer, 12.5 μl of 2×Easy Taq PCR SuperMix (mixture of *EasyTaq*TM DNA Polymerase, dNTPs, and optimized buffer, Beijing TransGen Biotech Co., Ltd., Beijing, P.R. China) and 9.5 μl of ddH_2O. The PCR thermal cycling conditions of ITS, LSU, SSU and TEF1-α were as follows: 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 50 seconds, elongation at 72 °C for 1 minute, and a final extension at 72 °C for 10 minutes. The PCR thermal cycle program for RPB2 was as follows: initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 1 minute, annealing at 52 °C for 2 minutes, elongation at 72 °C for 90 seconds, and final extension at 72 °C for 10 minutes. Purification and sequencing of PCR fragments with PCR primers mentioned above were carried out at Shanghai Majorbio Biopharm Technology Co., Ltd, China.

Sequence alignment and phylogenetic analyses

Phylogenetic analyses were performed from single gene (LSU dataset) as well as based on a combined LSU, SSU, TEF1-α, RPB2 and ITS sequence dataset. Sequences generated from this study were analyzed with other similar sequences obtained from GenBank and those derived from recent publications [2,22,29,30,47,48] (Table 1). The single gene alignment was

155 performed by using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) [27] and manually
156 aligned wherever necessary in MEGA version 7.0 [28]. Further analyses for the combined
157 dataset were analyzed by maximum likelihood (ML) implemented in RAxMLGUI v.0.9b2
158 [42,43,44,45] and Bayesian Inference (BI) criteria [17, 18] following the methodology in Li et
159 al. [30].

160 The phylogram was represented in Treeview [35] and drawn in Microsoft PowerPoint and
161 converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems Inc., the United
162 States). The new sequences were submitted in GenBank (Table 1). The alignment was
163 deposited in TreeBASE [53] under the accession number 25100.

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in **blue bold** font, while the type strains are in **black bold** font.

Species	Culture collection/ Voucher no.	GenBank accession numbers					References
		ITS	LSU	SSU	RPB2	TEF1-α	
Arthopyrenia salicis	CBS 368.94	KF443410	AY779288	AY538333	KF443397	KF443404	[1]
Cycasicola goaensis	**MFLUCC 17-0754**	**MG828885**	**MG829001**	**MG829112**	**–**	**MG829198**	[57]
Dendryphion aquaticum	**MFLUCC 15-0257**	**KU500566**	**KU500573**	**KU500580**	**–**	**–**	[47]
Dendryphion comosum	**CBS 208.69**	**MH859293**	**MH871026**	**–**	**–**	**–**	[55]
Dendryphion europaeum	CPC 22943	KJ869146	KJ869203	–	–	–	[9]
Dendryphion europaeum	**CPC 23231**	**KJ869145**	**KJ869202**	–	–	–	
Dendryphion fluminicola	KUMCC 15-0321	MG208160	MG208139	–	MG207971	MG207990	
Dendryphion fluminicola	DLUCC 0849	MG208161	MG208140	–	MG207972	MG207991	[48]
Dendryphion fluminicola	**MFLUCC17-1689**	**NR_157490**	**MG208141**	**–**	**–**	**MG207992**	
Dendryphion hydei	**KUMCC 18-0009**	**MN061343**	**MH253927**	**MH253929**	**–**	**MH253931**	**This study**
Dendryphion nanum	HKAS84010	KU500568	KU500575	KU500582	–	–	[47]
Dendryphion nanum	HKAS84012	KU500567	KU500574	KU500581	–	–	
Dendryphion nanum	MFLUCC 16-0987	MG208156	MG208135	–	MG207967	MG207986	[48]
Dendryphion submersum	**MFLUCC15-0271**	KU500565	**KU500572**	**KU500579**	**–**	**–**	[47]
Dendryphion submersum	KUMCC15-0455	MG208159	MG208138	–	MG207970	MG207989	[48]
Hobus wogradensis	**CBS 141484**	**NR_147652**	**KX650546**	**NG_061253**	**KX650575**	**KX650521**	[25]
Liua muriformis	**KUMCC 18-0177**	**MK433599**	**MK433598**	**MK433595**	**MK426799**	**MK426798**	[36]
Neooccultibambusa chiangraiensis	**MFLUCC 12-0584**	**NR_154238**	**KU764699**	**KU712458**	**–**	**–**	[12]
Neoroussoella bambusae	**MFLUCC 11-0124**	**KJ474827**	**KJ474839**	**–**	**KJ474856**	**KJ474848**	[31]
Neotorula aquatica	**MFLUCC 15-0342**	**KU500569**	**KU500576**	**KU500583**	**–**	**–**	[47]
Neotorula submersa	**HKAS 92660**	**NR_154247**	**KX789217**	**–**	**–**	**–**	[20]
Nigrograna mackinnonii	E5202H	JK26415	KJ605422	JK264155	JK264156	JK264154	[40]

Nigrograna mackinnonii	CBS 110022	KF015653	KF015609	GQ387553	KF015704	KF407985	[1]
Nigrograna mackinnonii	**CBS 674.75**	**NR_132037**	**GQ387613**	**GQ387552**	**–**	**–**	
Nigrograna marina	**CY 1228**	**–**	**GQ925848**	**GQ925835**	**GU479823**	**GU479848**	[50]
Occultibambusa bambusae	**MFLUCC 13-0855**	**KU940123**	**KU863112**	**KU872116**	**KU940170**	**KU940193**	[11]
Ohleria modesta	WU 36870	KX650562	–	–	KX650582	KX650533	[25]
Ohleria modesta	CBS 141480	KX650563	–	KX650513	KX650583	KX650534	
Parathyridaria ramulicola	**CBS 141479**	**NR_147657**	**KX650565**	**KX650514**	**KX650584**	**KX650536**	[25]
Parathyridaria percutanea	**CBS 868.95**	**NR_147631**	**NG_058022**	**NG_062999**	**KF366452**	**KF407987**	[1]
Parathyridaria robiniae	**MFLUCC 14-1119**	**KY511142**	**KY511141**	**–**	**–**	**KY549682**	[52]
Roussoella chiangraina	**MFLUCC 10-0556**	**NR_155712**	**KJ474840**	**–**	**KJ474857**	**KJ474849**	[31]
Roussoella nitidula	**MFLUCC 11-0182**	**KJ474835**	**KJ474843**	**–**	**KJ474859**	**KJ474852**	[31]
Roussoella scabrispora	MFLUCC 11-0624	KJ474836	KJ474844	–	KJ474860	KJ474853	[31]
Rostriconidium aquaticum	KUMCC 15-0297	MG208165	MG208144	**–**	MG207975	MG207995	[48]
Rostriconidium aquaticum	**MFLUCC 16-1113**	**MG208164**	**MG208143**	**–**	**MG207974**	**MG207994**	
Roussoellopsis macrospora	**MFLUCC 12-0005**	**KJ739604**	**KJ474847**	**KJ739608**	**KJ474862**	**KJ474855**	[31]
Roussoellopsis tosaensis	KT1659	–	AB524625	AB524484	AB539104	AB539117	[51]
Sporidesmium australiense	HKUCC 10833	–	DQ408554	–	DQ435080	–	[41]
Sporidesmioides thailandica	**MFLUCC 13-0840**	**MN061347**	**NG_059703**	**NG_061242**	**KX437761**	**KX437766**	[29]
Sporidesmioides thailandica	KUMCC 16-0012	MN061348	KX437758	KX437760	KX437762	KX437767	
Thyridaria broussonetiae	**CBS 141481**	**NR_147658**	**KX650568**	**NG_063067**	**KX650586**	**KX650539**	[25]
Thyridaria broussonetiae	CBS 121895	KX650567	KX650567	–	KX650585	KX650538	
Thyridariella mahakashae	**NFCCl 4215**	**MG020435**	**MG020438**	**MG020441**	**MG020446**	**MG023140**	[13]
Thyridariella mangrovei	**NFCCl 4213**	**MG020434**	**MG020437**	**MG020440**	**MG020445**	**MG020443**	[13]
Torula acaciae	**CPC 29737**	**NR_155944**	**NG_059764**	**–**	**KY173594**	**–**	[10]
Torula aquatica	DLUCC 0550	MG208166	MG208145	–	MG207976	MG207996	[48]
Torula aquatica	**MFLUCC16-1115**	**MG208167**	**MG208146**	**–**	**MG207977**	**–**	
Torula breviconidiophora	**KUMCC 18-0130**	**MK071670**	**MK071672**	**MK071697**	**–**	**MK077673**	[22]
Torula chiangmaiensis	**KUMCC 16-0039**	**MN061342**	**KY197856**	**KY197863**	**–**	**KY197876**	[30]

Torula chromolaenae	**KUMCC 16-0036**	**MN061345**	**KY197860**	**KY197867**	**KY197873**	**KY197880**	[30]
Torula fici	**CBS 595.96**	**KF443408**	**KF443385**	**KF443387**	**KF443395**	**KF443402**	[8]
Torula fici	KUMCC 15-0428	MG208172	MG208151	–	MG207981	MG207999	[48]
Torula fici	KUMCC 16-0038	MN061341	KY197859	KY197866	KY197872	KY197879	[30]
Torula gaodangensis	**MFLUCC 17-0234**	**MF034135**	**NG_059827**	**NG_063641**	**–**	**–**	[21]
Torula goaensis	**NFCCL 4040**	**NR_159045**	**NG_060016**	**–**	**–**	**–**	[37]
Torula herbarum	**CPC 24414**	**KR873260**	**KR873288**	**–**	**–**	**–**	[8]
Torula hollandica	**CBS 220.69**	**NR_132893**	**NG_064274**	**KF443389**	**KF443393**	**KF443401**	[8]
Torula hydei	**KUMCC 16-0037**	**MN061346**	**MH253926**	**MH253928**	**–**	**MH253930**	**This study**
Torula mackenziei	**MFLUCC 13-0839**	**MN061344**	**KY197861**	**KY197868**	**KY197874**	**KY197881**	[30]
Torula masonii	**CBS 245.57**	**NR_145193**	**NG_058185**	**–**	**–**	**–**	[8]
Torula masonii	DLUCC 0588	MG208173	MG208152	–	MG207982	MG208000	[47]
Torula masonii	KUMCC 16-0033	MN061339	KY197857	KY197864	KY197870	KY197877	[30]
Torula pluriseptata	**MFLUCC 14-0437**	**MN061338**	**KY197855**	**KY197862**	**KY197869**	**KY197875**	[30]
Torula polyseptata	**KUMCC 18-0131**	**MK071671**	**MK071673**	**MK071698**	**–**	**MK077674**	[22]
Torula sp.	CBS 246.57	KF443411	KR873290	–	–	–	[8]
Torula sp.	KUMCC 19-0112	MN507400	MN507402	MN507401	MN507404	MN507403	In prep.

Abbreviations: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CPC**: Collection of Pedro Crous housed at CBS; **DLUCC**: Dali University Culture Collecting Center, Dali, Yunnan, China. **HKAS**: Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China; **HKUCC**: University of Hong Kong Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; **KUMCC**: Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NFCCI**: National Fungal Culture Collection of India; **KT**: K. Tanaka.

Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS ONE article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to Index Fungorum from where they will be made available to the Global Names Index. The unique Index Fungorum number can be resolved and the associated information viewed through any standard web browser by appending the Index Fungorum number contained in this publication to the prefix www.indexfungorum.org/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)]. All PLOS ONE articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article. A complete explanation of our guidelines for publishing new species can be found on our website: http://www.plosone.org/static/guidelines#fungal

Compliance with Ethical Standards

There is no conflict of interest (financial or non-financial) and all authors have agreed to submission of paper. The authors also declare that they have no conflict of interest and confirm

that the field studies did not involve endangered or protected species.

Results

Phylogenetic analyses

The combined LSU, SSU, TEF1-α, RPB2 and ITS sequence dataset comprises 65 taxa with *Occultibambusa bambusae* (MFLUCC 13-0855) and *Neooccultibambusa chiangraiensis* (MFLUCC 12-0559) as the outgroup taxa. Bayesian Inference (BI) and maximum likelihood (ML) analyses of the combined dataset were performed to determine the placement of our new taxa and infer relationships at the intrageneric level as well as resolving the phylogenetic relationships of the core families in Pleosporales. The phylogenetic trees obtained from BI and ML analyses resulted in trees with largely similar topologies and also similar to those generated from previous studies based on maximum likelihood analysis [21,30,48]. The best scoring RAxML tree is shown in Figure 1, with the final ML optimization likelihood value of -31463.916972 (ln). The dataset consists of 4053 total characters including gaps (LSU: 1–840 bp, SSU: 841–1776 bp, TEF1-α: 1777–2566 bp, RPB2: 2567–3418 bp，ITS: 3419–4053). RAxML analysis yielded 1568 distinct alignment patterns and 32.43% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.246541, C = 0.258447, G = 0.270790, T = 0.224222, with substitution rates AC = 1.436632, AG = 3.543120, AT = 1.440155, CG = 0.960003, CT = 6.670420, GT = 1.000000. The proportion of invariable sites I = 0, the gamma distribution shape parameter alpha = 0.180447 and the Tree-Length = 3.140857. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008264.

Most of the core genera of Torulaceae and other representative genera in Nigrograna ceae, Ohleriaceae, Roussoellaceae and Thyridariaceae are included in our phylogenetic analysis (Fig. 1). Torulaceae formed a well-resolved clade (100% ML and 1.00 PP) with a close relationship to Roussoellaceae and Thyridariaceae. Species of different genera currently accommodated in Torulaceae formed well-resolved subclades except with *Sporidesmioides* which is recovered as basal to other genera with significant Bayesian support (1.00 PP) but with low support in ML analysis (48% ML, data not shown). *Torula* is recovered as a strongly monophyletic genus in Torulaceae. *Torula hydei* is sister to *T. fici* with high support (100% ML and 1.00 PP). *Dendryphion hydei* forms a distinct lineage and related to *D. europaeum*, *D. comosum*, *D. aquaticum*, *D. fluminicola* and *D. submersum* with significant support in BI analysis (0.95 PP).

Fig. 1 Phylogenetic construction using RAxML-based analysis of a combined LSU, SSU, TEF1-α, RPB2 and ITS DNA sequence dataset. Bootstrap support values for maximum likelihood (ML) equal to or greater than 70% and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are shown as "ML/PP" above the nodes. The tree is rooted to *Occultibambusa bambusae* (MFLUCC 13-0855) and *Neooccultibambusa chiangraiensis* (MFLUCC 12-0559). The type strains are in black bold and the newly generated sequences are indicated in blue bold.

Taxonomy

Dendryphion hydei J.F. Li, Phookamsak & Jeewon, *sp. nov.* Fig. 2

[urn:lsid:indexfungorum.org:names:556746]

Facesoffungi number: FOF0457

Etymology – Named in honour of Kevin D. Hyde for his excellent contribution to mycology and on his 65th birthday celebration.

Holotype – KUN-HKAS 97502

Saprobic on a branch litter of *Bidens pilosa* Linn. (Asteraceae). **Sexual morph**: Undetermined. **Asexual morph**: *Colonies* on the substratum superficial, effuse, gregarious, hairy, brown to dark brown. *Mycelium* composed of branched, septate, pale brown to brown hyphae. *Conidiophores* 260–380 µm long × 7–14 µm diam. (13–17 µm diam. at the base) ($\bar{x}$ = 356.7 × 9.9 µm, n = 10) macronematous, mononematous, septate, verrucose, thick-walled, branching simple or penicillate at the tip of primary branches, brown, flexuous. *Conidiogenous cells* 6–10 µm long × 3–5 µm diam. ($\bar{x}$ = 8 × 3.8 µm, n = 20) terminal, integrated, pale brown, polytretic. *Conidia* (17–)20–30(–35) µm long × 4–7 µm diam. ($\bar{x}$ = 26.5 × 5.6 µm, n = 30) single, subhyaline to pale olivaceous brown, slightly paler at the end cells, dry, verrucose, monilioid, 2–4(–5)-septate, constricted at the septa. Conidial secession schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 21 days at 16–30 °C, mycelium partly superficial, partly immersed, slightly effuse, hairy, vertical, with regular edge, white to grayish-brown, not produced pigmentation on media agar.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng District, Mushroom Research Centre, on a branch litter of *Bidens pilosa* Linn., 12 July 2016, J.F. Li, FHP3 (HKAS 97502, **holotype**), ex-type living culture, MFLUCC 18-0236, KUMCC 18-0009.

Notes – *Dendryphion hydei* resembles *D. aquaticum* and *D. europaeum* in morphology.

However, these species can be distinguished based on the size of the conidiophores, conidiogenous cells and conidia, as well as the conidial septation and habitats (see Table 2). *Dendryphion hydei* has 2–4(–5)-septate conidia and inhabit in a terrestrial environment, similar to *D. europaeum*. However, *D. europaeum* has smaller conidiophores and conidia, and the conidia of *D. europaeum* are (2–)3(–5)-septate while *D. aquaticum* inhabit in a freshwater environment and has 3–6-septate conidia [9,47]. In the phylogenetic tree, *D. hydei* forms a separate lineage and clustered with *D. europaeum*, *D. comosum*, *D. aquaticum* and *D. fluminicola* with significant support in Bayesian inference analysis (0.95 PP). In this study, we collected *D. hydei* from *Bidens pilosa*, which is a new host record for this species. A morphometric comparison of the new taxon with other similar taxa of *Dendryphion* provide in Table 2.

Fig. 2 *Dendryphion hydei* (HKAS 97479, holotype) a Colonies on branch of *Bidens pilosa*. b, c Apex of conidiophores with conidial structures. d, e Conidiophores. f–i Conidiogenous cells. j–q Conidia. Scale bars: a = 100 µm, d, e = 50 µm, b, f–i = 20 µm, b, c, f–q = 10 µm.

275 **Table 2** Synopsis of morphological features of *Dendryphion* species discussed in this study

Species	Size (µm) Conidiophores	Size (µm) Conidiogenous cells	Size (µm) Conidia	Conidial septation	Host/substrate and habitat	Distribution	Reference
Dendryphion aquaticum	250–285 × 7.5–11.5	5–9 × 4–6	22–33 × 6.5–7.5	3–6	Decaying wood submerged in stream	China (Yunnan)	[47]
Dendryphion comosum	Up to 400 × 9–14	Up to16 × 5–8	9–65 × 5–9	1–5(−9)	Various hosts and substrates	Cosmopolitan distribution	[16, 39]
Dendryphion europaeum	180–250 × 8–10	6–10 × 5–7	(15–)20–28(–33) × (6–)7	(2–)3(–5)	*Hedera helix*, *Heracleum sphondylium*	Germany, Netherlands	[9]
Dendryphion fluminicola	114–176 × 7–10	N/A	31–46 × 8–9	2–6	Decaying wood submerged in a stream in Cangshan Mountain, Lancang River and Jinsha River	China (Yunnan)	[48]
Dendryphion hydei	260–380 × 7–14	6–10 × 3–5	(17–)20–30(–35) × 4–7	2–4(–5)	Branch litter of of *Bidens pilosa*	Thailand	This study
Dendryphion nanum	52–64 × 6.5–8.5	13–19 × 6–8	56.7–74.5 × 10–12	3–11	Various hosts and substrates	Cosmopolitan distribution	[16,47]
Dendryphion submersum	210–335 × 3.5–4.5	11–15 × 4.5–6.5	15–25 × 5–7	2–5	Decaying wood submerged in stream	China (Yunnan)	[47]

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Torula hydei J.F. Li, Phookamsak & Jeewon, *sp. nov.* Fig. 3

[urn:lsid:indexfungorum.org:names:556747]

Facesoffungi number: FoF 04573

Etymology – Named in honour of Kevin D. Hyde for his excellent contribution to mycology and on his 65th birthday celebration.

Holotype – HKAS 97478

Saprobic on an aerial dead branch of *Chromolaena odorata* Linn. **Sexual morph**: Undetermined. **Asexual morph**: *Colonies* discrete on host, black, powdery. *Mycelium* immersed on the substrate, composed of septate, branched, smooth, light brown hyphae. *Conidiophores* (1.5–)2–3 µm long × 1.5–2 µm diam. ($\bar{x}$ = 2.2 × 1.8 µm, n = 10), macronematous, mononematous, solitary, erect, light brown, verruculose, thick-walled, consist of one cell or reduced to conidiogenous cells, without apical branches, subcylindrical to subglobose, arising from hyphae. *Conidiogenous cells* 3–5.5 µm long × 4.3–5 µm diam. ($\bar{x}$ = 3.8 × 4.5 µm, n = 20), polyblastic, terminal, dark brown to black, smooth to minutely verruculose, thick-walled, doliiform to ellipsoid. (7.5–)8–14 µm long × 2–4 µm diam.($\bar{x}$ = 10.4 × 3.4 µm, n = 30), solitary to catenate, acrogenous, simple, phragmosporous, light brown to brown, minutely verruculose, 2–3-septate, rounded at both ends, composed of subglobose cells, slightly constricted at some septa, chiefly sub-cylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 10 days at 16–30 °C, mycelium partly superficial, partly immersed, slightly effuse, hairy, vertical, with regular edge, light brown to brown, not produced pigmentation on media agar; not sporulated on media agar

within 2 months.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng District, on an aerial dead branch of *Chromolaena odorata* Linn. (Asteraceae), 26 December 2015, J.F. Li, MRC2 (HKAS 97478, **holotype**), ex-type living culture, MFLUCC 18-0250, KUMCC 16-0037.

Notes – *Torula hydei* resembles *T. herbarum* and *T. fici* in having 2–3-septate, catenated, brown, verruculose conidia, but differs in having smaller conidia [9]. Phylogenetic analyses showed that *T. hydei* constitutes an independent lineage basal to *T. fici* (100% ML and 1.00 BYPP).

Fig. 3 *Torula hydei* (HKAS 97478, holotype). a Colonies on dead branch of Chromolaena odorata. b–e Conidiophores with conidiogenous cell. f–j Budding on conidia. k, l Conidia in chain. m–t Conidia. Scale bars: a = 100 µm, b, k–l = 5 µm, c, f–j, q–t = 2 µm, d, e, m–p = 1 µm

Discussion

Taxonomic characterizations of taxa in Torulaceae have been well-studied since Crous et al. [8] re-classified *Torula* and *Dendryphion* in Torulaceae (Pleosporales, Dothideomycetes) based on phylogenetic analyses of LSU sequence data. Subsequent authors introduced the new genera and species in this family based on multigene phylogenetic analyses coupled with morphological characteristics (see Table 3) [21, 29, 30, 47, 48, 52]. However, there are more than 520 epithets under the genus *Torula* and 85 epithets under *Dendryphion* available in Index Fungorum [23], but these described species lack DNA sequence data to verify their

phylogenetic placement and affinities with other related fungi. Nevertheless, many species previously described as *Torula* and *Dendryphion* have also been synonymized to many genera in Sordariomycetes [23]. Taxa in these genera need to be clarified based on molecular data.

Torula and *Dendryphion* are widespread on hosts and habitats and commonly found as saprobes in both terrestrial and aquatic habitats from temperate to tropical regions [9,16,21,29, 30,47,48,52]. In this study, species in Torulaceae collected from herbaceous plants in northern Thailand were examined. Our new taxon, *Torula hydei* is characterized by morphs that correspond to those outlined by Li et al. [30]. However, *T. hydei* is unique in having very tiny conidia as compared to other similar species. We also note distinct nucleotide base pair differences between *T. hydei* and *T. fici* across TEF1-α gene region analysed (43/760 bp, 5.7% difference).

Dendryphion hydei is unique in having large conidiophores and subhyaline to pale olivaceous brown, 2–4(–5)-septate conidia to compare with other related species in *Dendryphion* (Table 2). Our multiloci phylogeny also positions our new taxon as independent lineage and phylogenetically apart from other species (Fig. 1). A comparison of TEF1-α nucleotides shows that *Dendryphion hydei* differs from *D. fluminicola* in 20/852 bp (2.3% difference), from *D. submersum* in 30/902 bp (3.3% difference). A comparison of ITS nucleotides shows that *D. hydei* differs from *D. europaeum* in 19/553 bp (3.4% difference) and differs from *D. aquaticum* in 6/398 bp (1.5% difference). Phylogenetic analyses support *D. hydei* as a new species in *Dendryphion*. These tally with recommendations outlined by Jeewon and Hyde [26] to establish our new species.

It is interesting to note that species of Torulaceae have been found to be mostly associated

with the host family Asteraceae. In this study our new strains were collected from Asteraceae and Li et al. [30] also reported two novel *Torula* species, *T. chromolaenae* and *T. mackenziei* from Asteraceae, indicating that Asteraceae harbors a diversity of these fungi. *Dendryphion hydei* collected from an herbaceous host collected in northern Thailand is also the first record on the host (*Bidens pilosa*) and location. Our combined LSU, SSU, TEF1-α, RPB2 and ITS phylogenetic analyses also support *D. hydei* as a new species.

Table 3 Synopsis of morphological features of the genera in Torulaceae.

Genus	Morphological features			Reference
	Conidia	**Conidiophores**	**Conidiogenous cells**	
Dendryphion	Acropleurogenous, catenate or solitary, simple or branched, cylindrical to obclavate, or cheiroid, pale to mid brown or olivaceous brown, multi-septate, smooth or verrucose	Macronematous, mononematous, branched at the apex, brown to black, smooth or with verruculose at the upper part, with paler branches	Mono- or polytretic, integrated, terminal and intercalary on branches, sympodial, clavate, cylindrical or doliiform, cicatrized, with large and dark scars.	[47,48]
Neotorula	Acrogenous, in chains, clavate to subcylindrical, septate, dark bands at the septa, pale green when young, brown when mature, verruculose	Macronematous, mononematous, cylindrical, 3–6-septate, with one or severa short branches near the apex, smooth, dark brown, paler towards the apex,	Tretic, with a distinct pore, integrated, terminal, pale brown or subhyaline, doliiform or lageniform	[47]
Rostriconidium	Solitary, pyriform to rostrate, dark brown to black, with a thick, black truncate scar at the base and pale pigment cell above the scar, narrowly cylindrical and obtuse at the apex	Macronematous, mononematous, single or caespitose, septate, smooth, brown or dark brown, unbranched, thick-walled, cylindrical, arising from a stromatic base.	Monotretic or polytretic, integrated, terminal, cylindrical, dark brown	[48]
Sporidesmioides	Acrogenous, solitary, pyriform to rostrate, ampulliform to obclavate, truncate at the base, septate, brown to dark brown, with paler at the upper end cells, smooth or verruculose to echinulate	Macronematous, mononematous, scattered, unbranched, straight to curved, sometimes percurrently proliferating	Polyblastic, integrated, indeterminate or percurrent, terminal, sometimes intercalary sympodial, dark and prominent, cylindrical or doliiform.	[29]
Torula	Acrogenous, in branched chains, subcylindrical to cylindrical, brown, constricted at septa, smooth to verrucose, conidial cells subglobose	Micronematous, reduced to conidiogenous cells, or with a brown supporting cell	Mono- to polyblastic, solitary on mycelium, doliiform to ellipsoid or clavate, cupulate, brown, smooth to verruculose,	[8,30,47]

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545 Supporting Information

546 **Fig. 1** Phylogenetic construction using RAxML-based analysis of a combined LSU, SSU,
547 TEF1-α, RPB2 and ITS DNA sequence dataset. Bootstrap support values for maximum

likelihood (ML) equal to or greater than 70 % and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are shown as "ML/PP" above the nodes. The tree is rooted to *Occultibambusa bambusae* (MFLUCC 13-0855) and *Neooccultibambusa chiangraiensis* (MFLUCC 12-0559). The type strains are in black bold and the newly generated sequences are indicated in blue bold.

Fig. 2 *Dendryphion hydei* (HKAS 97479, holotype) a Colonies on branch of *Bidens pilosa*. b, c Apex of conidiophores with conidial structures. d, e Conidiophores. f–i Conidiogenous cells. j–q Conidia. Scale bars: a = 100 µm, d, e = 50 µm, b, f–i = 20 µm, b, c, f–q = 10 µm

Fig. 3 *Torula hydei* (HKAS 97478, holotype). a Colonies on dead branch of Chromolaena odorata. b–e Conidiophores with conidiogenous cell. f–j Budding on conidia. k, l Conidia in chain. m–t Conidia. Scale bars: a = 100 µm, b, k–l = 5 µm, c, f–j, q–t = 2 µm, d, e, m–p = 1 µm

Fig. 1 Phylogenetic tree

Click here to download Figure Fig. 1 ML tree.jpg

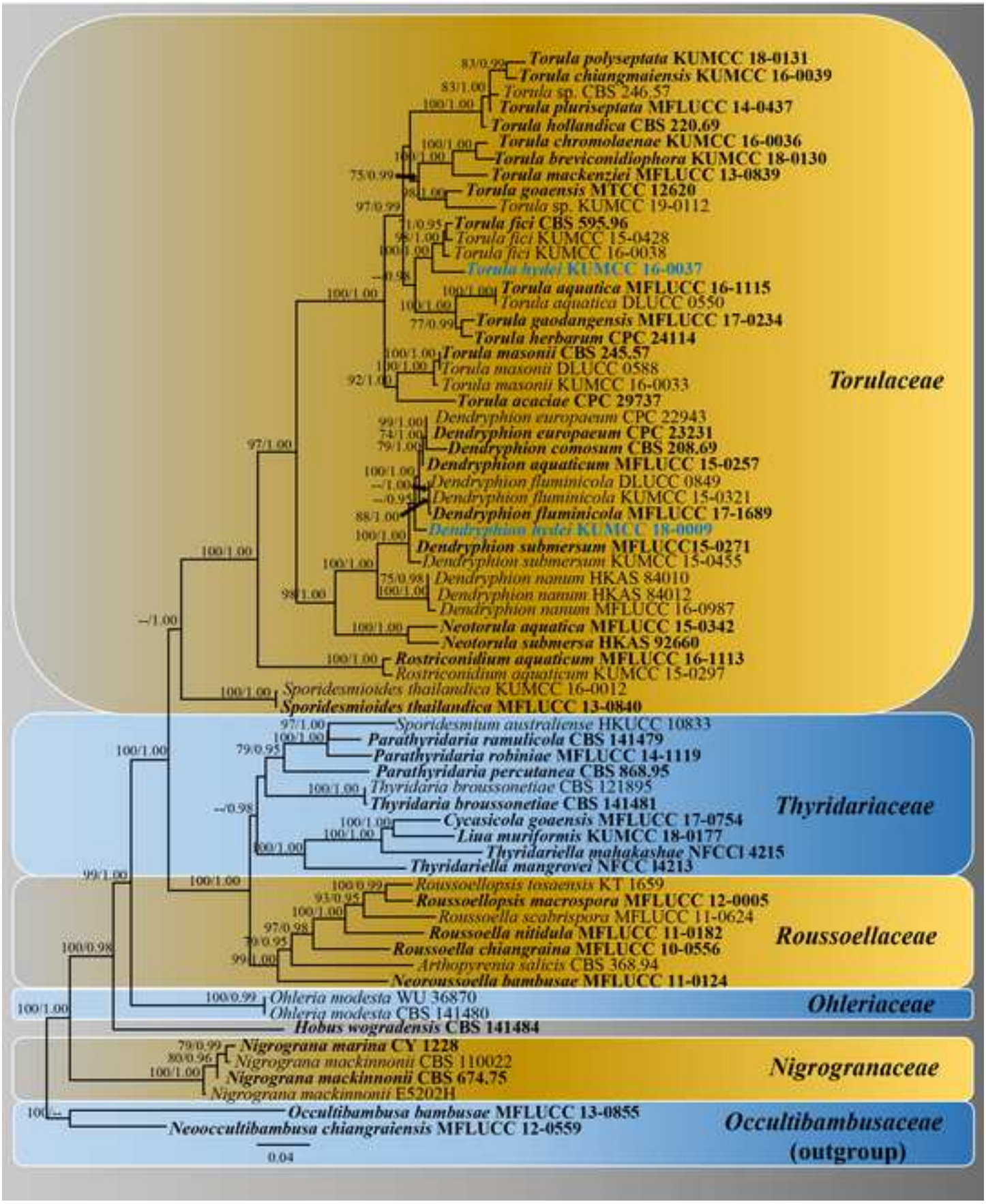

Fig. 2

Click here to download Figure Fig. 2 Dendryphion hydei.jpg

Fig. 3

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