

RESEARCH ARTICLE

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

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Data Availability Statement: All relevant data are within the paper.

Abstract

During our studies on asexual fungi colonizing herbaceous litter in northern Thailand, we discovered two new fungal species, viz. *Dendryphion hydei* and *Torula 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).

Introduction

The family Torulaceae Corda was introduced by Sturm [1] and is typified by *Torula* Pers. Species in Torulaceae are known only by their asexual morphs which are characterized as followed: superficial, effuse, greyish brown to black, powdery colonies; micro- or macronematous conidiophores, with or without apical branches; doliiiform to ellipsoid or clavate, brown, smooth to verruculose, mono- to polyblastic conidiogenous cells which often remaining cupulate; subcylindrical, phragmosporous, acrogenous, brown, dry, smooth to verruculose conidia characteristically produced in branched chains [2,3,4,5,6,7]. Crous et al. [8] investigated phylogenetic relationships of this family with the inclusion of *Torula* species and accepted *Dendryphion* Wallr., besides *Torula* within Torulaceae in Pleosporales. Su et al. [6] introduced *Neotorula* Ariyaw., Z.L. Luo & K.D. Hyde and two new *Dendryphion* species in Torulaceae

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based on molecular data. Li et al. [9] established a novel genus, *Sporidesmioides* Jun F. Li, Phook. & K.D. Hyde. Su et al. [7] examined 21 freshwater taxa and updated phylogenetic relationships of taxa within the family Torulaceae based on ITS, LSU, TEF1- α and RPB2 genes and accommodated *Rostriconidium* Z.L. Luo, K.D. Hyde & H.Y. Su within Torulaceae. Crous et al. [10] designated the epitype of *Rutola* J.L. Crane & Schokn. and accepted the genus in Torulaceae based on LSU phylogeny. Currently, there are six accepted genera in Torulaceae viz. *Dendryphion*, *Neotorula*, *Rostriconidium*, *Rutola*, *Sporidesmioides* and *Torula* [10,4,9,6,7].

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 [11]. Crane and Schoknecht [12] 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 [13], Hughes [14], Subramanian [15] and Ellis [16,17]. However, there was little information regarding the phylogenetic relationships of *Torula* until the studies of Crous et al. [8], Li et al. [5] and Su et al. [6,7]. To date, only 15 species have their DNA sequence data being analysed to reveal their phylogenetic placements in Torulaceae [18,19,9,5,6,7,20].

Dendryphion Wallr. was introduced by Wallroth [21] 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 [8,7]. Crous et al. [3] 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. [6] circumscribed genera of Torulaceae from freshwater. 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 another new species from northern Thailand. These species are described and illustrated. In addition, an updated phylogenetic tree with our new taxa for the family Torulaceae is provided in this study.

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 conidium isolation was carried out to obtain pure cultures as described in Dai et al. [22]. Germinating conidia were transferred aseptically to potato dextrose agar (PDA) and malt extract agar (MEA) plates and grown at room temperature (16–30°C) in alternating day and night light. Colony characters were observed and recorded after one week and at weekly intervals [23,24].

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 were 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. [25] and Index Fungorum [26]. New species are established based on guidelines of Jeewon and Hyde [27].

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 [28]; The primers LR0R and LR5 were used to amplify the partial ribosomal RNA for the 28S nuclear large subunit (LSU) [29]; NS1 and NS4 were used to amplify the partial ribosomal RNA for the 18S nuclear small subunit (SSU) [28]; fRPB2-5F and fRPB2-7cR were used to amplify the partial RNA polymerase second largest subunit (RPB2) [30] and EF1-983F and EF1-2218R were used to amplify the translation elongation factor 1-alpha gene (TEF1- α) [31].

The final volume of the PCR reaction was 25 μ l, containing 1 μ l of DNA template, 1 μ l of each forward and reward primer, 12.5 μ l of 2 \times Easy Taq PCR SuperMix (mixture of *Easy-Taq*™ DNA Polymerase, dNTPs, and optimized buffer, Beijing TransGen Biotech Co., Ltd., Beijing, P.R. China) and 9.5 μ l of ddH₂O. 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 [32,10,19,9,5,6,7] (Table 1). The single gene alignment was performed by using MAFFT v. 7 [33] (<http://mafft.cbrc.jp/alignment/server/>) and manually aligned wherever necessary in MEGA version 7.0 [34]. Further analyses for the combined dataset were

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	
<i>Arthopyrenia salicis</i>	CBS 368.94	KF443410	AY779288	AY538333	KF443397	KF443404	[41]
<i>Cycasicola goaensis</i>	MFLUCC 17-0754	MG828885	MG829001	MG829112	-	MG829198	[42]
<i>Dendryphion aquaticum</i>	MFLUCC 15-0257	KU500566	KU500573	KU500580	-	-	[6]
<i>Dendryphion comosum</i>	CBS 208.69	MH859293	MH871026	-	-	-	[43]
<i>Dendryphion europaeum</i>	CPC 22943	KJ869146	KJ869203	-	-	-	[3]
<i>Dendryphion europaeum</i>	CPC 23231	KJ869145	KJ869202	-	-	-	
<i>Dendryphion fluminicola</i>	KUMCC 15-0321	MG208160	MG208139	-	MG207971	MG207990	[7]
<i>Dendryphion fluminicola</i>	DLUCC 0849	MG208161	MG208140	-	MG207972	MG207991	
<i>Dendryphion fluminicola</i>	MFLUCC17-1689	NR_157490	MG208141	-	-	MG207992	
<i>Dendryphion hydei</i>	KUMCC 18-0009	MN061343	MH253927	MH253929	-	MH253931	This study
<i>Dendryphion nanum</i>	HKAS84010	KU500568	KU500575	KU500582	-	-	[6]
<i>Dendryphion nanum</i>	HKAS84012	KU500567	KU500574	KU500581	-	-	
<i>Dendryphion nanum</i>	MFLUCC 16-0987	MG208156	MG208135	-	MG207967	MG207986	[7]
<i>Dendryphion submersum</i>	MFLUCC15-0271	KU500565	KU500572	KU500579	-	-	[6]
<i>Dendryphion submersum</i>	KUMCC15-0455	MG208159	MG208138	-	MG207970	MG207989	[7]
<i>Hobus wogradensis</i>	CBS 141484	NR_147652	KX650546	NG_061253	KX650575	KX650521	[44]
<i>Liua muriformis</i>	KUMCC 18-0177	MK433599	MK433598	MK433595	MK426799	MK426798	[45]
<i>Neooctlibambusa Chiangraiensis</i>	MFLUCC 12-0584	NR_154238	KU764699	KU712458	-	-	[46]
<i>Neorousoella bambusae</i>	MFLUCC 11-0124	KJ474827	KJ474839	-	KJ474856	KJ474848	[47]
<i>Neotorula aquatica</i>	MFLUCC 15-0342	KU500569	KU500576	KU500583	-	-	[6]
<i>Neotorula submersa</i>	HKAS 92660	NR_154247	KX789217	-	-	-	[4]
<i>Nigrograna mackinnonii</i>	E5202H	JK26415	KJ605422	JK264155	JK264156	JK264154	[48]
<i>Nigrograna mackinnonii</i>	CBS 110022	KF015653	KF015609	GQ387553	KF015704	KF407985	[41]
<i>Nigrograna mackinnonii</i>	CBS 674.75	NR_132037	GQ387613	GQ387552	-	-	
<i>Nigrograna marina</i>	CY 1228	-	GQ925848	GQ925835	GU479823	GU479848	[49]
<i>Occultibambusa bambusae</i>	MFLUCC 13-0855	KU940123	KU863112	KU872116	KU940170	KU940193	[22]
<i>Ohleria modesta</i>	WU 36870	KX650562	-	-	KX650582	KX650533	[44]
<i>Ohleria modesta</i>	CBS 141480	KX650563	-	KX650513	KX650583	KX650534	
<i>Parathyridaria ramulicola</i>	CBS 141479	NR_147657	KX650565	KX650514	KX650584	KX650536	[44]
<i>Parathyridaria percutanea</i>	CBS 868.95	NR_147631	NG_058022	NG_062999	KF366452	KF407987	[41]
<i>Parathyridaria robiniae</i>	MFLUCC 14-1119	KY511142	KY511141	-	-	KY549682	[20]
<i>Rousoella Chiangrainia</i>	MFLUCC 10-0556	NR_155712	KJ474840	-	KJ474857	KJ474849	[47]
<i>Rousoella nitidula</i>	MFLUCC 11-0182	KJ474835	KJ474843	-	KJ474859	KJ474852	[47]
<i>Rousoella scabrispora</i>	MFLUCC 11-0624	KJ474836	KJ474844	-	KJ474860	KJ474853	[47]
<i>Rostriconidium aquaticum</i>	KUMCC 15-0297	MG208165	MG208144	-	MG207975	MG207995	[7]
<i>Rostriconidium aquaticum</i>	MFLUCC 16-1113	MG208164	MG208143	-	MG207974	MG207994	
<i>Rostriconidium pandanicola</i>	KUMCC 17-0176	MH275084	MH260318	MH260358	MH412759	MH412781	[50]
<i>Rousoellopsis macrospora</i>	MFLUCC 12-0005	KJ739604	KJ474847	KJ739608	KJ474862	KJ474855	[47]
<i>Rousoellopsis tosaensis</i>	KT1659	-	AB524625	AB524484	AB539104	AB539117	[51]
<i>Rutola graminis</i>	CPC 33267	MN313814	MN317295	-	-	-	[10]
<i>Rutola graminis</i>	CPC 33695	MN313815	MN317296	-	-	-	
<i>Rutola graminis</i>	CPC 33715	MN313816	MN317297	-	-	-	
<i>Sporidesmium australiense</i>	HKUCC 10833	-	DQ408554	-	DQ435080	-	[52]
<i>Sporidesmioides thailandica</i>	MFLUCC 13-0840	MN061347	NG_059703	NG_061242	KX437761	KX437766	[9]
<i>Sporidesmioides thailandica</i>	KUMCC 16-0012	MN061348	KX437758	KX437760	KX437762	KX437767	

(Continued)

Table 1. (Continued)

Species	Culture collection/ Voucher no.	GenBank accession numbers					References
		ITS	LSU	SSU	RPB2	TEF1- α	
<i>Thyridaria broussonetiae</i>	CBS 141481	NR_147658	KX650568	NG_063067	KX650586	KX650539	[44]
<i>Thyridaria broussonetiae</i>	CBS 121895	KX650567	KX650567	–	KX650585	KX650538	
<i>Thyridariella mahakashae</i>	NFCCI 4215	MG020435	MG020438	MG020441	MG020446	MG023140	[53]
<i>Thyridariella mangrovei</i>	NFCCI 4213	MG020434	MG020437	MG020440	MG020445	MG020443	[53]
<i>Torula acaciae</i>	CPC 29737	NR_155944	NG_059764	–	KY173594	–	[54]
<i>Torula aquatica</i>	DLUCC 0550	MG208166	MG208145	–	MG207976	MG207996	[7]
<i>Torula aquatica</i>	MFLUCC16-1115	MG208167	MG208146	–	MG207977	–	
<i>Torula breviconiophora</i>	KUMCC 18–0130	MK071670	MK071672	MK071697	–	MK077673	[19]
<i>Torula camporesii</i>	KUMCC 19–0112	MN507400	MN507402	MN507401	MN507404	MN507403	[55]
<i>Torula chiangmaiensis</i>	KUMCC 16–0039	MN061342	KY197856	KY197863	–	KY197876	[5]
<i>Torula chromolaenae</i>	KUMCC 16–0036	MN061345	KY197860	KY197867	KY197873	KY197880	[5]
<i>Torula fici</i>	CBS 595.96	KF443408	KF443385	KF443387	KF443395	KF443402	[8]
<i>Torula fici</i>	KUMCC 15–0428	MG208172	MG208151	–	MG207981	MG207999	[7]
<i>Torula fici</i>	KUMCC 16–0038	MN061341	KY197859	KY197866	KY197872	KY197879	[5]
<i>Torula gaodangensis</i>	MFLUCC 17–0234	MF034135	NG_059827	NG_063641	–	–	[18]
<i>Torula goaensis</i>	NFCCCL 4040	NR_159045	NG_060016	–	–	–	[56]
<i>Torula herbarum</i>	CPC 24414	KR873260	KR873288	–	–	–	[8]
<i>Torula hollandica</i>	CBS 220.69	NR_132893	NG_064274	KF443389	KF443393	KF443401	[8]
<i>Torula hydei</i>	KUMCC 16–0037	MN061346	MH253926	MH253928	–	MH253930	This study
<i>Torula mackenziei</i>	MFLUCC 13–0839	MN061344	KY197861	KY197868	KY197874	KY197881	[5]
<i>Torula masonii</i>	CBS 245.57	NR_145193	NG_058185	–	–	–	[8]
<i>Torula masonii</i>	DLUCC 0588	MG208173	MG208152	–	MG207982	MG208000	[6]
<i>Torula masonii</i>	KUMCC 16–0033	MN061339	KY197857	KY197864	KY197870	KY197877	[5]
<i>Torula pluriseptata</i>	MFLUCC 14–0437	MN061338	KY197855	KY197862	KY197869	KY197875	[5]
<i>Torula polyseptata</i>	KUMCC 18–0131	MK071671	MK071673	MK071698	–	MK077674	[19]
<i>Torula</i> sp.	CBS 246.57	KF443411	KR873290	–	–	–	[8]

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

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analyzed by maximum likelihood (ML) implemented in RAXMLGUI v.0.9b2 [35,36,37,38] and Bayesian Inference (BI) criteria [39,40] following the methodology in Li et al. [5].

The phylogram was represented in Treeview [57] and drawn in Microsoft PowerPoint and converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems Inc., the United States). The new sequences were submitted in GenBank (Table 1). The alignment was deposited in TreeBASE [58] under the accession number 25462.

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: PubMed Central and LOCKSS.

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 71 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 [18,5,7]. The best scoring RAxML tree is shown in Fig 1, with the final ML optimization likelihood value of -32357.090382 (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 1585 distinct alignment patterns and 33.97% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.246366, C = 0.258260, G = 0.271248, T = 0.224126, with substitution rates AC = 1.424215, AG = 3.485957, AT = 1.457990, CG = 0.955364, CT = 6.607514, GT = 1.000000. The proportion of invariable sites I = 0, the gamma distribution shape parameter alpha = 0.180234 and the Tree-Length = 3.299994. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008574.

Most of the core genera of Torulaceae and other representative genera in Nigrogranaceae, 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 for *Sporidesmioides* which is recovered as basal to other genera with significant Bayesian support (1.00 PP) but with low support in ML analysis (56% ML). *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 (1.00 PP).

Taxonomy

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

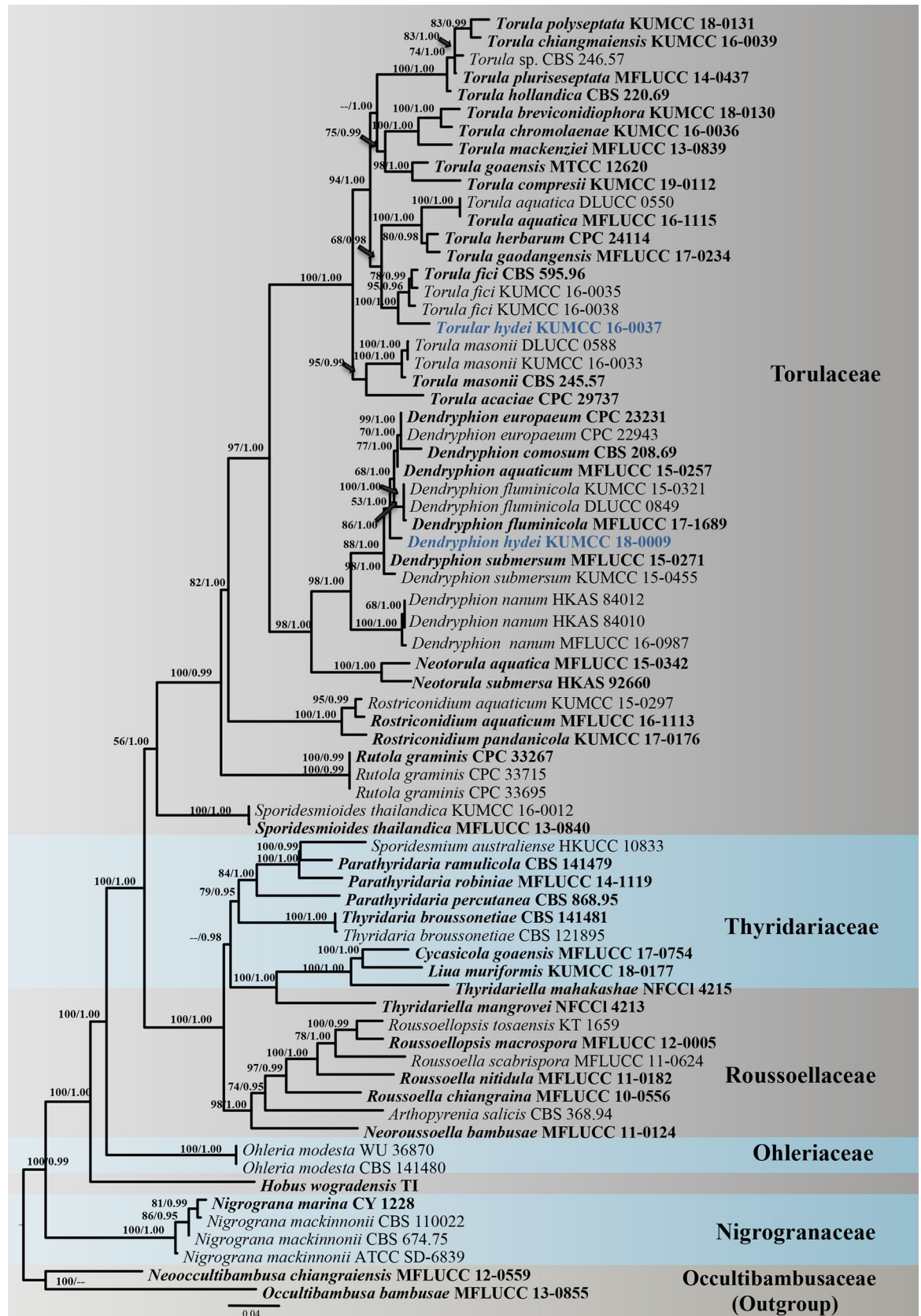


Fig 1. Phylogenetic construction using RAxML-based analysis of a combined LSU, SSU, TEF1- α , RPB2 and ITS DNA sequence dataset. Bootstrap support values for maximum likelihood (ML) equal to or greater than 50% and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are shown as "ML/PP" 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.

<https://doi.org/10.1371/journal.pone.0228067.g001>

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

Facesoffungi number: FoF04574

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 \times 7–14 μm diam. (13–17 μm diam. at the base) (\bar{x} = 356.7 \times 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 \times 3–5 μm diam. (\bar{x} = 8 \times 3.8 μm , n = 20) terminal, integrated, pale brown, polytretic. Conidia (17–)20–30(–35) μm long \times 4–7 μm diam. (\bar{x} = 26.5 μ 5.6 μm , n = 30) single, subhyaline to pale olivaceous brown, slightly paler at the end cells, dry, verrucose, moniloid, 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* 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*. *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 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* inhabits in a freshwater environment and has 3–6-septate conidia [3,7]. 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 (1.00 PP). A comparison of TEF1- α nucleotides shows that *D. hydei* differs from *D. fluminicola* in 20/852 bp (2.3% difference, no gap) and from *D. submersum* in 30/902 bp (3.3% difference, no gap). A comparison of ITS nucleotides shows that *D. hydei* differs from *D. europaeum* in 19/553 bp (3.4% difference, no gap) and differs from *D. aquaticum* in 6/398 bp (1.5% difference, no gap). Phylogenetic analyses support *D. hydei* as a new species in *Dendryphion*. These tally with recommendations outlined by Jeewon and Hyde [27] to establish our new species. 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.

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

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



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 μ m, d, e = 50 μ m, b, f-i = 20 μ m, b, c, f-q = 10 μ m.

<https://doi.org/10.1371/journal.pone.0228067.g002>

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

Species	Size (µm)			Conidial septation	Host/substrate and habitat	Distribution	Reference
	Conidiophores	Conidiogenous cells	Conidia				
<i>Dendryphion hydei</i>	260–380 × 7–14	6–10 × 3–5	(17–)20–30(–35) × 4–7	2–4(–5)	Branch litter of <i>Bidens pilosa</i>	Thailand	This study
<i>Dendryphion aquaticum</i>	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)	[6]
<i>Dendryphion comosum</i>	Up to 400 × 9–14	Up to 16 × 5–8	9–65 × 5–9	1–5(–9)	Various hosts and substrates	Cosmopolitan distribution	[59, 60]
<i>Dendryphion europaeum</i>	180–250 × 8–10	6–10 × 5–7	(15–)20–28(–33) × (6–)7	(2–)3(–5)	<i>Hedera helix</i> , <i>Heracleum sphondylium</i>	Germany, Netherlands	[3]
<i>Dendryphion fluminicola</i>	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)	[7]
<i>Dendryphion nanum</i>	52–64 × 6.5–8.5	13–19 × 6–8	56.7–74.5 × 10–12	3–11	Various hosts and substrates	Cosmopolitan distribution	[59,6]
<i>Dendryphion submersum</i>	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)	[6]

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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), semi-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 prostrate 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. *Conidia* (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, brown to dark brown, minutely verruculose, 2–3-septate, rounded at both ends, composed of subglobose cells, slightly constricted at some septa, chiefly subcylindrical. *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 [3]. Phylogenetic analyses showed that *T. hydei* constitutes an independent lineage basal to *T. fici* (100% ML and 1.00 BYPP). Morphologically *T. hydei* differs from *T. fici* in having smaller conidia (*T. hydei*, (7.5–) 8–14 × 2–4 µm versus (12–)13–17(–19) × 5(–6) µm, *T. fici*) and the conidia are also brown to dark brown, paler at the apex where branching occurs [8]. Whereas, *T. fici* has brown conidia,



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.

<https://doi.org/10.1371/journal.pone.0228067.g003>

with a pale brown apex and the fertile cells in the conidial chain, where branching occurs, are darker brown than other cells [8]. The conidiogenous cells of *T. fici* are slightly larger than *T. hydei* and frequently clavate (*T. fici*, (5–)6(–8) × 5(–7) μm versus 3–5.5 × 4.3–5 μm, *T. hydei*), whereas, *T. hydei* has doliiform to ellipsoid conidiogenous cells [8]. We also note distinct nucleotide base pair differences between *T. hydei* and *T. fici* (CBS 595.96, type strain) across the ITS gene region (8/479 bp, 1.7% difference, no gap) and TEF1-α gene region analysed (43/760 bp, 5.7% difference, no gap). Based on distinct morphological characteristics and phylogenetic support, *T. hydei* is introduced as a new species in this study.

Discussion

Taxonomic characterizations of taxa in Torulaceae have been well-studied since Crous et al. [8] who re-classified *Torula* and *Dendryphion* in Torulaceae (Pleosporales, Dothideomycetes) based on phylogenetic analyses of LSU sequence data. Subsequent authors introduced new genera and species in this family based on multigene phylogenetic analyses coupled with morphological characteristics (see Table 3) [10,18, 9, 5, 6, 7, 20]. Recently, there are more than 520 epithets in the genus *Torula* and 85 epithets in *Dendryphion* listed in Index Fungorum [26]. However, most of the 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 [26]. Taxa in these genera need to be clarified based on molecular data.

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

Genus	Morphological features			Reference
	Conidia	Conidiophores	Conidiogenous cells	
<i>Dendryphion</i>	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.	[6,7]
<i>Neotorula</i>	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 several 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	[6]
<i>Rostriconidium</i>	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	[7]
<i>Rutola</i>	Acrogenous, simple to branched chains, phragmosporous, brown, verruculose, aseptate to multi-septate, fragmenting into segments	Micronematous, appressed to substrate, branched, septate, pale brown	Monoblastic, integrated, terminal or intercalary, pale brown	[10]
<i>Sporidesmioides</i>	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.	[9]
<i>Torula</i>	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,	[8,5,6]

<https://doi.org/10.1371/journal.pone.0228067.t003>

Torula and *Dendryphion* have a wide host range in various habitats and are commonly found as saprobes in both terrestrial and aquatic habitats in temperate to tropical regions [10,3,59,18,9, 5,6,7,20]. It is interesting to note that many *Torula* species have been found to be associated with the host family Asteraceae [59,5]. In this study, our new strains were collected from Asteraceae and Li et al. [5] also reported two novel *Torula* species, *T. chromolaenae* and *T. mackenziei* from Asteraceae, indicating that Asteraceae harbors a diversity of these taxa. *Dendryphion hydei* was also collected from *Bidens pilosa* (Asteraceae) and is the first record from northern Thailand.

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Methodology: Junfu Li, Rungtiwa Phookamsak.

Project administration: Rungtiwa Phookamsak.

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Writing – review & editing: Rajesh Jeewon, Peter E. Mortimer, Itthayakorn Promputtha.

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