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Torula aquilariae sp. nov. (Torulaceae, Pleosporales), a new species associated with *Aquilaria sinensis* from Yunnan, China

Xiao-Hong Li[1,2,3], Rungtiwa Phookamsak[1,2,3*], Fang-Qi Sun[1,2,3,4,5,6], Hong-Bo Jiang[1,2,3], Jian-Chu Xu[1,2,3,7] and Jun-Fu Li[1,2,3*]

[1] *Key Laboratory of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*
[2] *Honghe Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, Honghe County 654400, Yunnan, China*
[3] *Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China*
[4] *Research Center of Microbial Diversity and Sustainable Utilization, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[5] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[6] *Master of Science Program in Applied Microbiology (International Program), Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[7] *World Agroforestry Centre, East and Central Asia Office, 132 Lanhei Road, Kunming 650201, Yunnan, China*
* Corresponding authors, E-mail: phookamsak@mail.kib.ac.cn; lijunfu@mail.kib.ac.cn

Abstract

Torula aquilariae sp. nov. was isolated from a fallen fruit pod of *Aquilaria sinensis* in Yunnan Province, China. Preliminary identification based on morphological characteristics, the new species is typical of *Torula* forming effuse, dark brown to black colonies, with monoblastic or polyblastic, doliiform to ellipsoid or cupulate conidiogenous cells, and acrogenous, phragmosporous, brown, septate, smooth-walled to verrucose conidia, in short, branched chains. Multigene phylogenetic analyses of a concatenated ITS, LSU, SSU, *tef1-α,* and *rpb2* sequence data demonstrated that *Torula aquilariae* sp. nov. is a sister taxon of *T. mackenziei*, and nested between *T. breviconidiophora* and *T. chromolaenae* within *Torula* (Torulaceae, Pleosporales). Morphologically, the new species resembles *T. mackenziei*, but is different in the size of the conidiophores and conidia. Besides, the nucleotide pairwise comparison of ITS, *tef1-α,* and *rpb2* gene regions also supports their distinction. Based on multigene phylogeny coupled with morphological traits and nucleotide polymorphism analyses, *Torula aquilariae* sp. nov. is introduced in this study. Detailed descriptions, illustrations, and an updated phylogeny are provided.

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Introduction

Torulaceae was introduced by Sturm[1] with *Torula* as the type genus. Members of the family are commonly known only by their anamorph as dematiaceous hyphomycetes, producing erect, micro- or macro-nematous conidiophores, with or without apical branches, doliiform to ellipsoid or clavate, brown, smooth to verruculose, mono- to poly-blastic, conidiogenous cells, often with cupulate cells, and acrogenous, phragmosporous, brown, dry, subcylindrical, smooth to verrucose conidia, frequently in branched chains[2–7] Initiative modern taxonomic treatment of Torulaceae based on a morpho-molecular approach was carried out by Crous et al.[2] who investigated the familial phylogenetic affinity, based on molecular data of five representative *Torula* species and *Dendryphion europaeum*; of which only *Dendryphion* and *Torula* were initially accepted in the family. Comprehensive taxonomic studies of Torulaceae have been further carried out by various subsequent authors; Su & Hyde[3] introduced the new genus *Neotorula* and two new *Dendryphion* species and consequently, Su et al.[8] established the genus *Rostriconidium*. Li et al.[9] introduced the monotypic genus *Sporidesmioides* and many new species in *Dendryphion* and *Torula*[5,6]. Crous et al.[10] accepted *Rutola* in Torulaceae and later, Boonmee et al.[11] introduced a monotypic genus *Cylindrotorula* in this family based on phylogenetic evidence. In a recent taxonomic classification, *Rostriconidium* and *Sporidesmioides* were treated as synonyms of *Neopodoconis* by Qiu et al.[12] based only morphological resemblance and molecular data of LSU and SSU. Although, their phylogenetic analyses demonstrated that *Neopodoconis* clade I (= *Rostriconidium*) and *Neopodoconis* clade II (= *Sporidesmioides*) did not form a well-resolved monophyletic clade, concurring with Wang et al.[13]. Subsequently, He et al.[14] clarified the phylogenetic affinities of *Rostriconidium* and *Sporidesmioides* (current name: *Neopodoconis*) in Torulaceae, demonstrating that these two genera formed well-resolved distinct subclades within Torulaceae and three representative species of *Neopodoconis* were excluded from Torulaceae. Therefore, the congeneric status among *Neopodoconis*, *Rostriconidium* and *Sporidesmioides* is questionable, pending further study.

Species of Torulaceae have been naturally found as saprobes on a wide range of hosts in both aquatic and terrestrial habitats; of which most species were isolated from submerged wood, dead branches, and herbaceous litter of various vascular plants worldwide such as in plant families Asteraceae, Brassicaceae, Cyperaceae, Fabaceae, Iridaceae, and Ranunculaceae in Asia (China, India, and Thailand), Europe (France, Germany, and Italy), North America (Canada), and others[2,3,5–8,14–19]. In contrast, a few species were reported as pathogens such as

Torula herbarum was reported to cause stem blight on *Ziziphus mauritiana*, leaf and stem spots in plants and foot-rot of coriander. The species was also isolated from nasal swabs of *Equus equus*[20,21].

Torula is the most speciose genus in Torulaceae, and 543 epithets are listed in Index Fungorum[22]. However, less than a half-quarter of the known species have molecular data to clarify their phylogenetic affinities. Of these, other related taxa previously described as *Torula* have also been synonymized to many genera in Sordariomycetes[22]. *Torula* was introduced by Persoon[23] and was initially typified by *Torula monilis*. However, *T. monilis* was treated as a synonym of *T. herbarum* and hitherto Crous et al.[2] treated *T. herbarum* as the type species of *Torula*. The genus is characterized by dark brown to black, discrete, dry, effuse, velvety colonies, and mostly immersed mycelium, with one brown supporting cell of conidiophores, or reduced to the conidiogenous cells, that are mono- to polyblastic, solitary, brown, doliiform to ellipsoidal or clavate, smooth to verruculose conidiogenous cells, with a basally thickened and heavily melanized wall, frequently collapsing and becoming coronate, and acrogenous, phragmosporous, dry, brown, smooth to verrucose, globose to subglobose celled conidia, with strongly constricted at the septa and frequently in branched chains[2,5–7,24]. In accordance with Crous et al.[2] who introduced three new *Torula* species based on a morpho-molecular approach, further studies based on the multigene phylogenetic analyses were carried out by various subsequent authors[3,5–8,14,17,19,25–31]. Of these, the species were reported from different varieties of plant hosts in Asia (China, India, and Thailand), Europe (Italy, the Netherlands, and the UK), and South America (Cuba). To date, about 29 *Torula* species have been confirmed with molecular data[14,31,32].

During our survey of fungal diversity in Honghe Dry-hot Valley, Yunnan Province, China, a novel species *Torula aquilariae* was isolated from a fallen fruit pod of *Aquilaria sinensis* (Thymelaeaceae). The species is described and illustrated with phylogenetic support of a concatenated ITS, LSU, SSU, *tef1-α,* and *rpb2* sequence dataset. Also, the discussion in terms of its morphological differences compared with the closely related species in the genus *Torula*, and an updated phylogenetic tree are provided in this paper.

Materials and methods

Isolation and identification

The specimen was collected from a fallen fruit pod of *Aquilaria sinensis* (Thymelaeaceae) in a terrestrial habitat, in Yunnan Province, China during the wet season of the year 2023. The sample was returned to the mycological laboratory at the Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences for examination, isolation, and description of morphological characteristics. The specimen was observed under a Motic SMZ 168 series dissecting stereomicroscope and photographed with a Discovery V.8 stereomicroscope fitted with a CARL ZEISS Axio Cam ERc5S microscope camera. Micro-morphological features were picked up by a surgical needle and placed onto 10% lacto-glycerol on a clean slide. Micro-morphological features were examined and captured under a Nikon ECLIPSE Ni-U compound microscope connected with a Nikon DS-Ri2 camera using DIC microscopy. The Tarosoft (R) Image Frame Work program and Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems Inc., USA) were used for measurements and drawing photographic plates. Single spore isolation was carried out to obtain pure cultures as described in Li et al.[5,6,33]. Germinating conidia were transferred aseptically to potato dextrose agar (PDA) and malt extract agar (MEA) plates and grown at 25 °C in alternating day and night light. Colony characteristics were observed and recorded after one week and at weekly intervals.

The type specimen is deposited in the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China. Ex-type living culture is preserved in Kunming Institute of Botany Culture Collection (KUNCC). Faces of Fungi number and MycoBank number are registered for the new taxon[34,35].

DNA extraction, PCR amplification, and sequencing

Fungal mycelium was scraped off and stored in a 1.5 ml microcentrifuge tube using a sterilized lancet for genomic DNA extraction. The Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, PR China) was used to extract fungal genomic DNA, following the protocols in the manufacturer's instructions. The DNA amplification was performed by polymerase chain reaction (PCR) using the following genes (ITS, LSU, SSU, *tef1-α*, and *rpb2*). The ITS5 and ITS4 primer pairs were used to amplify the ITS and 5.8S regions of the rDNA gene[36]; the primers LR0R and LR5 were used to amplify the partial ribosomal RNA for the nuclear large subunit (28S, LSU)[37]; the primers NS1 and NS4 were used to amplify the partial ribosomal RNA for the nuclear small subunit (18S, SSU)[36] the primers EF1-983F and EF1-2218R were used to amplify the protein coding region for translation elongation factor 1-alpha gene (*tef1-α*)[38] and the primers fRPB2-5F and fRPB2-7cR were used to amplify the partial ribosomal RNA for the partial RNA polymerase second largest subunit (*rpb2*)[39].

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 × Power*Taq* PCR Master Mix (mixture of EasyTaqTM DNA Polymerase, dNTPs, and optimized buffer, Beijing Bio Teke Corporation (Bio Teke), China) and 9.5 μl of sterilized double-distilled water (ddH_2O). The PCR thermal cycling conditions of ITS, LSU, SSU, and *tef1-α* were processed by initialization at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 50 s, elongation at 72 °C for 1 min, and a final extension at 72 °C for 10 min, and final hold at 4 °C; while the PCR thermal cycle program for *rpb2* was followed as initially 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 2 min, elongation at 72 °C for 90 s, and final extension at 72 °C for 10 min[40]. Purification and sequencing of PCR products were carried out at Shanghai Majorbio Biopharm Technology Co., Ltd, China using the same primers as defined above.

Sequence alignment and phylogenetic analyses

Phylogenetic analyses were performed based on a concatenated ITS, LSU, SSU, *tef1-α,* and *rpb2* sequence data. Sequences generated from this study were aligned with similar sequences obtained from GenBank and those derived from recent publications[14,19] (Table 1). Single gene alignment was performed via the online platform, MAFFT v. 7.511 (http://mafft.cbrc.jp/alignment/server/)[41]. Ambiguous sites were trimmed and manually edited where necessary in MEGA version 6.0[42].

Further analyses were executed based on maximum likelihood (ML) and Bayesian inference (BI) criteria, following the methodology as described in Li et al.[5].

Maximum likelihood (ML) analysis was implemented by the Randomized Axelerated Maximum Likelihood program on raxmlGUI v. 7.4.2 graphical interface[43] with the GTR + GAMMAI model of nucleotide substitution and run for 1000 rapid bootstrap replicates[44]. The best-fit model of nucleotide substitution of the combined dataset was determined by MrModeltest v. 2.3[45]; of which GTR + I + G was the best-fit model for a combined dataset under the Akaike Information Criterion (AIC) and incorporated into the analysis. Bayesian inference (BI) was analyzed by MrBayes v.3.1.2[46]. Bayesian posterior probabilities (PP) were evaluated by Markov chain Monte Carlo sampling (BMCMC)[46–48]. Two parallel runs with six simultaneous Markov chains were run for one million generations, and trees were sampled every 100th generation (resulting in 10,001 trees). The first 2,000 trees were set as burn-in and were discarded. The remaining trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree.

The phylograms were represented in FigTree v1.4.0 program[49], drawn in PowerPoint 2016 (Microsoft Inc., Redmond, WA, USA), and converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems Inc., USA). The new sequences were submitted in GenBank (Table 1). The alignment was deposited in TreeBASE (2024) under the accession number 31909. New species is established as per recommendations proposed by Jeewon & Hyde[50].

Results

Taxonomy

Torula aquilariae X.H. Li, Phookamsak & J.F. Li, sp. nov.

MycoBank number: MB 857067, **Facesoffungi number:** FOF 17237; Fig. 1.

Etymology: Refers to the host genus, *Aquilaria*, of which the holotype was collected.

Holotype: KUN-HKAS 145332

Saprobic on fallen fruit pod of *Aquilaria sinensis* (Thymelaeaceae). **Teleomorph**: Undetermined. **Anamorph**: *Colonies* sporadic on host, black, powdery. *Mycelium* immersed on the substrate, composed of septate, branched, smooth, light brown hyphae. *Conidiophores* 6–20 μm long × 3.5–5 μm wide ($\bar{x}$ = 15.6 × 4.6 μm, n = 10), macronematous, mononematous, solitary, erect, subcylindrical, subhyaline to light brown, minutely verruculose, thick-walled, consisting of 1–4 cells, without apical branches, or reduced to conidiogenous cells, with 1–2-celled, ellipsoid to subglobose or protuberance cells, arising from hyphae. *Conidiogenous cells* 3.9–4.7 μm long × 4.2–5 μm wide ($\bar{x}$ = 4.5 × 4.8 μm, n = 10), polyblastic, terminal, dark brown to black, smooth to minutely verruculose, thick-walled, globose to ellipsoid or sometimes coronate at apex. *Conidia* 12.5–15 μm long × 3.8–5 μm wide ($\bar{x}$ = 14.5 × 4.4 μm, n = 20) catenated, acrogenous, phragmosporous, light brown to greyish-brown, smooth to minutely verruculose, 1–3-septate, rounded at both ends or with a dark, terminal, coronate, cupulate cell at apex, composed of moniliform cells, slightly constricted at some septa, chiefly subcylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from the apical cell. Colonies growing on PDA, reaching 5 cm in 10 d at 28 °C, mycelium partly immersed to superficial, slightly effuse, cottony, with a regular edge, surface greyish-white to brown, reverse pale black, dense, circular, slightly raised, smooth, entire, wrinkled folded, producing brown pigmentation in agar; teleomorph not formed within 60 d.

Material examined: CHINA, Yunnan Province, Yuan Jiang, on a fallen fruit pod of *Aquilaria sinensis*. (Thymelaeaceae), 12 July 2023, J-F. Li, TA-01 (KUN-HKAS 145332, holotype), ex-type living culture KUNCC 24-18640.

Notes: *Torula aquilariae* resembles *T. masonii* in having brown, verruculose, conidia and with constricted at the septa and a dark terminal coronate cell at the apex, but differs in having smaller (*T. aquilariae*, 14.5 × 4.4 μm vs 21.6 × 9.5 μm, in *T. masonii*) conidia, shorter (*T. aquilariae*, 15.6 × 4.6 μm vs 16.8 × 4.5 μm, in *T. masonii*) conidiophores with smaller conidiogenous cells (*T. aquilariae*, 4.5 × 4.8 μm vs 6.6 × 5.3 μm, in

Table 1. Taxa used in the phylogenetic analysis and their corresponding GenBank accession numbers.

Taxa	Culture Collection	GenBank accession numbers					Ref.
		ITS	LSU	SSU	*rpb2*	*tef1-α*	
Arthopyrenia salicis	CBS 368.94	KF443410	AY538339	AY538333	KF443397	KF443404	[51,52]
Cycasicola goaensis	MFLUCC 17-0754^T	MG828885	NG_059057	NG_061287	N/A	MG829198	[53]
Cylindrotorula indica	NFCCI 4836^T	NR_175156	NG_081308	N/A	MT321490	MT321492	[11]
C. indica	NFCCI 4837	MT339445	MT339443	N/A	MT321491	MT321493	[11]
Dendryphion aquaticum	MFLUCC 15-0257^T	KU500566	KU500573	KU500580	N/A	N/A	[3]
D. comosum	CBS 208.69^T	MH859293	MH871026	N/A	N/A	N/A	[54]
D. fluminicola	MFLUCC 17-1689^T	MG208162	MG208141	N/A	N/A	MG207992	[8]
Mauritiana rhizophorae	BCC 28866^T	N/A	GU371824	GU371832	GU371796	GU371817	[55]
M. rhizophorae	BCC 28867	N/A	GU371825	GU371833	GU371797	GU371818	[55]
Neooccultibambusa thailandensis	MFLUCC 16-0274^T	MH275074	MH260308	MH260348	MH412758	MH412780	[56]
Neopodoconis jiangxiensis	HJAUP C0947^T	N/A	ON693849	ON693843	N/A	N/A	[12]
N. meilingensis	HJAUP C0905^T	N/A	ON693846	ON693847	N/A	N/A	[12]
N. obclavata	HJAUP C0829^T	N/A	ON693848	ON693844	N/A	N/A	[12]
N. saprophyticus	HJAUP C0830^T	N/A	ON693851	ON705129	N/A	N/A	[12]
N. sinensis	HJAUP C0909^T	N/A	ON693845	ON693850	N/A	N/A	[12]
N. yunnanensis	KUNCC 22-10737^T	OP359401	OP359410	OP369295	OP476726	OP471613	[57]
Neotorula aquatica	MFLUCC 15-0342^T	KU500569	KU500576	KU500583	N/A	N/A	[3]

(to be continued)

Table 1. (continued)

Taxa	Culture Collection	GenBank accession numbers					Ref.
		ITS	LSU	SSU	*rpb2*	*tef1-α*	
N. submersa	HKAS 92660^{T}	NR_154247	NG_059727	N/A	N/A	N/A	[4]
Occultibambusa bambusae	MFLUCC 13-0855^{T}	KU940123	KU863112	N/A	KU940170	KU940193	[58]
Pseudocoleodictyospora tectonae	MFLUCC 12-0385^{T}	NR_154338	KU764709	NG_061232	KU712491	N/A	[59]
P. tectonae	MFLUCC 12-0387	KU712444	KU764704	KU712462	KU712492	N/A	[59]
Pseudothyridariella mahakoshae	NFCCI 4215^{T}	MG020435	MG020438	MG020441	MG020446	MG023140	[60]
Rostriconidium aquaticum	MFLUCC 16-1113^{T}	MG208164	MG208143	N/A	MG207974	MG207994	[8]
R. aquaticum	KUMCC 15-0297	MG208165	MG208144	N/A	MG207975	MG207995	[8]
R. cangshanense	MFLUCC 20-0147^{T}	MW010285	MW010281	N/A	MW012636	N/A	[61]
R. pandanicola	KUMCC 17–0176^{T}	MH275084	MH260318	MH260358	MH412759	MH412781	[56]
Roussoella hysterioides	HH 26988	N/A	AB524622	AB524481	AB539102	AB539102	[55,62]
R. pustulans	KT 1709	N/A	AB524623	AB524482	AB539103	AB539116	[55,62]
Roussoellopsis macrospora	MFLUCC 12-0005^{T}	KJ739604	KJ474847	KJ739608	KJ474862	KJ474855	[63,64]
R. tosaensis	KT 1659	N/A	AB524625	AB524484	AB539104	AB539117	[55,62]
Rutola graminis	CBS 145906^{T}	NR_175150	NG_078685	N/A	N/A	N/A	[10]
R. graminis	CPC 33695	MN313815	MN317296	N/A	N/A	N/A	[10]
Sporidesmioides thailandica	MFLUCC 13-0840^{T}	MN061347	NG_059703	KX437759	KX437761	KX437766	[9]
S. thailandica	KUMCC 16-0012	MN061348	KX437758	KX437760	KX437762	KX437767	[9]
Thyridariella mangrovei	NFCCI 4213^{T}	MG020434	MG020437	MG020440	MG020445	MG020443	[60]
Torula acaciae	CPC29737^{T}	NR_155944	NG_059764	N/A	KY173594	N/A	[2]
T. aquatica	DLUCC 0550	MG208166	MG208145	N/A	MG207976	MG207996	[8]
T. aquatica	MFLUCC 16-1115^{T}	MG208167	MG208146	N/A	MG207977	N/A	[8]
T. aquilariae	**KUNCC 24-18640^{T}**	**PQ788522**	**PQ788524**	**N/A**	**PQ810570**	**PQ810572**	**Present study**
T. aquilariae	**HKAS 145332^{T}**	**PQ788521**	**PQ788523**	**N/A**	**PQ810569**	**PQ810571**	**Present study**
T. breviconidiophora	KUMCC18-0130^{T}	MK071670	MK071672	MK071697	N/A	MK077673	[28]
T. calceiformis	HKAS 125551^{T}	OP751054	OP751052	OP751050	OQ630510	OQ630512	[65]
T. calceiformis	HKAS 125552	OP751055	OP751053	OP751051	OQ630511	OQ630513	[65]
T. camporesii	KUMCC19-0112^{T}	MN507400	MN507402	MN507401	MN507404	MN507403	[28]
T. canangae	MFLUCC 21-0169^{T}	OL966950	OL830816	N/A	N/A	ON032379	[29]
T. chiangmaiensis	KUMCC16-0039^{T}	MN061342	KY197856	KY197863	N/A	KY197876	[5]
T. chinensis	UESTCC 22.0085^{T}	OQ127986	OQ128004	OQ127995	N/A	N/A	[32]
T. chromolaenae	KUMCC16-0036^{T}	MN061345	KY197860	KY197867	KY197873	KY197880	[5]
T. fici	CBS 595.96^{T}	KF443408	KF443385	KF443387	KF443395	KF443402	[2]
T. fici	KUMCC16-0038	MN061341	KY197859	KY197866	KY197872	KY197879	[5]
T. gaodangensis	MFLUCC17-0234^{T}	MF034135	NG_059827	NG_063641	N/A	N/A	[26]
T. goaensis	MTCC 12620^{T}	NR_159045	NG_060016	N/A	N/A	N/A	[27]
T. herbarum	CPC24414^{T}	KR873260	KR873288	N/A	N/A	N/A	[2]
T. hollandica	CBS 220.69^{T}	NR_132893	NG_064274	KF443389	KF443393	KF443401	[2]
T. hydei	KUMCC16-0037^{T}	MN061346	MH253926	MH253928	N/A	MH253930	[6]
T. lancangjiangensis	HKAS 112709^{T}	NR_175706	NG_081516	NG_078759	MW729780	MW729785	[11]
T. longan	ZHKUCC 22-0121^{T}	OR194035	OR194027	OR194032	OR228535	OR228537	[66]
T. luguhuensis	CGMCC 3.24256^{T}	OQ729758	OQ947766	N/A	OQ999002	OQ999004	[57]
T. mackenziei	MFLUCC 13-0839^{T}	MN061344	KY197861	KY197868	KY197874	KY197881	[5]
T. mackenziei	HKAS 112705	MW723058	MW879525	MW774581	N/A	N/A	[11]
T. masonii	CBS 245.57^{T}	NR_145193	NG_058185	N/A	N/A	N/A	[2]
T. phytolaccae	ZHKUCC 22-0107^{T}	ON611796	ON611800	ON611798	ON660879	ON660881	[19]
T. pluriseptata	MFLUCC 14-0437^{T}	MN061338	KY197855	KY197862	KY197869	KY197875	[5]
T. polyseptata	KUMCC 18-0131^{T}	MK071671	MK071673	MK071698	MT235830	MT235791	[28]
T. sichuanensis	UESTCC 22.0087^{T}	OQ127981	OQ127999	OQ127990	N/A	N/A	[32]
T. suae	CGMCC 3.24259^{T}	OP359406	OP359415	OP369300	OP476730	OP471618	[13]
T. submersa	UESTCC 22.0086^{T}	OQ127985	OQ128003	OQ127994	OQ158968	OQ158978	[32]
T. sundara	MFLU 21-0089	OM276824	OM287866	N/A	N/A	N/A	[31]
T. sundara [as *T. longiconidiophora*]	UESTCC 22.0088^{T}	OQ127983	OQ128001	OQ127992	OQ158967	OQ158977	[32]
T. sundara [as *T. longiconidiophora*]	UESTCC 22.0125	OQ127984	OQ128002	OQ127993	OQ158972	OQ158976	[32]
T. sundara	HKAS 124486	OR470708	OR470713	OR470703	OR753781	OR753786	[14]
T. sundara	HKAS 124487	OR470709	OR470714	OR470704	OR753782	OR753787	[14]
T. sundara	KUNCC 22–12430	OP359403	OP359412	OP369297	N/A	OP471615	[13]
T. sundara	KUNCC 22–13431	OP359404	OP359413	OP369298	OP476728	OP471616	[13]
T. thailandica	GZCC20-0011^{T}	MN907426	MN907428	MN907427	N/A	N/A	[7]

The newly generated sequences are shown in bold and the ex-type strains are indicated in superscript 'T'. N/A means the data is not available in GenBank.

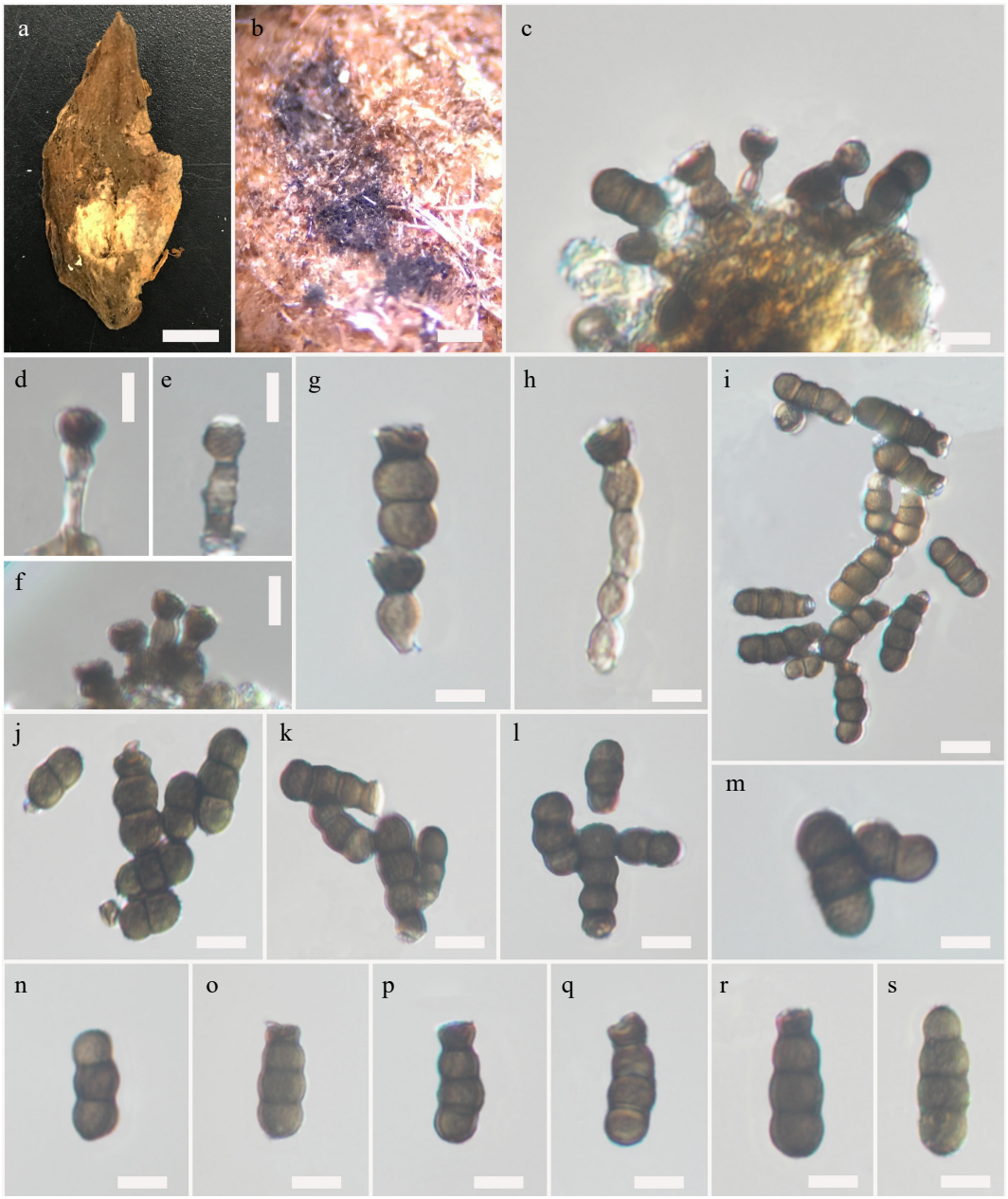

Fig. 1 *Torula aquilariae* (KUN-HKAS 145332, holotype). (a) A fallen fruit pod of *Aquilaria sinensis*. (b) Colonies on a fallen fruit pod of *A. sinensis*. (c) Conidial structure on the substrate. (d)–(h) Conidiophores with conidiogenous cell. (i)–(m) Conidial masses. (n)–(s) Conidia. Scale bars: (a) 0.5 cm, (b) 200 μm, (c)–(h) and (j)–(s) 5 μm, (i) 10 μm.

T. masonii)[2,5]. Phylogenetic analyses showed that *T. aquilariae* constitutes an independent branch and is sister to *T. mackenziei*. Morphologically, *T. aquilariae* differs from *T. mackenziei* in having conidia with a dark terminal coronate cell at the apex, which is rounded and paler in *T. mackenziei*; and longer conidiophores (*T. aquilariae*, 15.6 × 4.6 μm vs 3.8 × 3.5 μm, in *T. mackenziei*) while other morphological characters are difficult to use to distinguish these two species[5]. Moreover, the comparison of nucleotide pairwise differences indicated that *T. aquilariae* differs from *T. mackenziei* (MFLUCC 13-0839, ex-type strain) in 10/488 bp (2%) difference across the ITS gene region, 52/857 bp (6%) difference across the *tef1-α* gene region and 53/895 bp (5.9% difference, across the *rpb2* gene region. Based on morphological characteristics and phylogenetic support coupled with the difference in nucleotide polymorphism, *T. aquilariae* is introduced as a new species in this study.

Phylogenetic analyses

The concatenated ITS, LSU, SSU, *tef1-α,* and *rpb2* dataset comprise 75 taxa with *Occultibambusa bambusae* (MFLUCC 13–0855) and *Neooccultibambusa thailandensis* (MFLUCC 16–0274) 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 species and clarified relationships at the interspecific level. 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. The best-scoring RAxML tree

is shown in Fig. 2, with the final ML optimization likelihood value of –37630.023997 (ln). The dataset consists of 3,952 total characters including gaps (ITS: 1–625 bp, LSU: 626–1,354 bp, SSU: 1,355–2,294 bp, *tef1-α*: 2,295–3,067 bp, *rpb2*: 3,068–3,952 bp). RAxML analysis yielded 960 distinct alignment patterns and 30.36% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.244496, C = 0.260259, G = 0.274215, T = 0.221030, with substitution rates AC = 1.131746, AG = 2.587944, AT = 1.227920, CG = 0.930470, CT = 5.073462, GT = 1.000000. The proportion of invariable sites I = 0, the gamma distribution shape parameter alpha = 0.283343, and the tree length = 4.777392. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with the final average standard deviation of split frequencies = 0.008843.

Seventy-five available ex-type and representative strains of genera in Torulaceae, and other related families (Halotthiaceae, Pseudocoleodictyosporaceae, Roussoellaceae, and Thyridariaceae) are included in the present phylogenetic analyses (Fig. 2). Multigene phylogenetic analyses demonstrated that these families formed well-resolved monophyletic clades in the present study, except Torulaceae that is well-resolved in ML analysis (84% ML), but has low support in BI analysis. Of these, representative genera in Torulaceae also formed well-resolved subclades in Torulaceae, except *Neopodoconis*/*Rostriconidium* that are well-resolved in ML analysis (80% ML), but have low support in BI analysis. *Neopodoconis saprophyticus* (HJAUP C0830) nested among *Rostriconidium* species with significant support in BI analyses (0.99 BYPP). Whereas *N. meilingensis* (HJAUP C0905), *N. sinensis* (HJAUP C0909), and *N. yunnanensis* (KUNCC 22-10737) clustered as basal to *Rostriconidium* species. Besides, *Sporidesmioides thailandica* (MFLUCC 13-0840 and KUMCC 16-0012) clustered with *Neopodoconis jiangxiensis* (HJAUP C0947), and *N. obclavata* (HJAUP C0829) with significant support (83% ML, 0.95 BYPP) and nested between Pseudocoleodictyosporaceae, and Thyridariaceae, distant from Torulaceae.

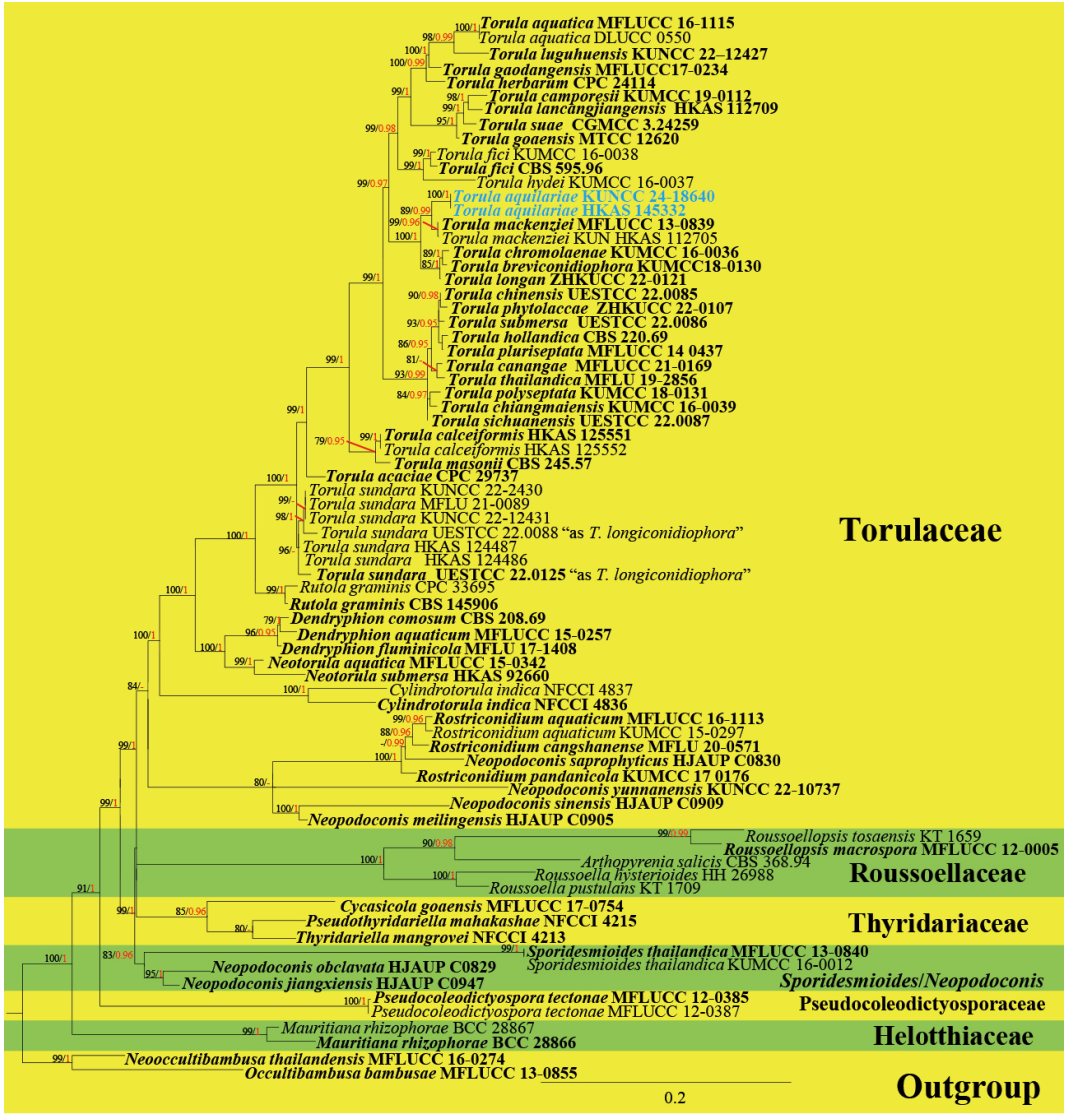

Fig. 2 Phylogenetic construction using RAxML-based analysis of a combined ITS, LSU, SSU, *tef1-α*, and *rpb2* DNA sequence dataset. Bootstrap support values for maximum likelihood (ML) equal to or greater than 70% and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are shown as 'ML/PP' at the nodes. The tree is rooted to *Occultibambusa bambusae* (MFLUCC 13-0855) and *Neooccultibambusa thailandensis* (MFLUCC 16–0274). The type strains are in black bold and the newly generated sequences are indicated in blue bold.

In the present study, most of the *Torula* species formed well-supported branches within *Torula* (≥ 70% ML, and 0.95 BYPP), except for *T. canangae*, *T. hollandica*, *T. pluriseptata*, *T. submersa*, *T. sundara*, and *T. thailandica*. *Torula canangae* is sister to *T. thailandica* with 80% ML support but with low support in BI analysis. *Torula hollandica* is sister to *T. pluriseptata*. These two species clustered with *T. submersa*, *T. phytolaccae*, and *T. chinensis* with significant support (93% ML, and 0.95 BYPP). *Torula sundara* formed a well-resolved subclade in ML analysis (96% ML) but had low support in BI analysis. Two new strains (KUNCC 24-18640 and HKAS 145332) formed a robust subclade, sister to *T. mackenziei* (MFLUCC 13-0839 and KUN-HKAS 112705) with significant support (89% ML and 0.99 BYPP, Fig. 2) and closely related to *T. breviconidiophora*, *T. chromolaenae,* and *T. longan*.

Discussion

Torula have a wide host range in various habitats and are commonly found as saprobes in both aquatic and terrestrial habitats in temperate to tropical climatic zones[2,5,6,56,65,67]. Even though, more than 540 species epithets were listed under the genus *Torula*, most of which contain ambiguous species that were emphasized by only morphological characteristics. These ambiguous species need to be clarified based on type specimen using a polyphasic taxonomic approach. This study reveals a *Torula* species isolated from a fallen fruit pod of *Aquilaria sinensis* in Honghe Dry-hot Valley, Yunnan, China, which morphologically fits well among extant species in having conidia with dark terminal coronate cells at the apex and long, subcylindrical conidiophore with coronate conidiogenous cells. Multigene phylogenetic analyses demonstrated that the new species, *Torula aquilariae* (KUNCC 24-18640 and KUN-HKAS 145332) clustered with *T. mackenziei*, but phylogenetic analyses fully support it as the distinct species, constitute an independent lineage with high statistical support (Fig. 2). *Torula aquilariae* is also the first record on host *Aquilaria sinensis* from Honghe Dry-hot Valley.

Despite some morphological differences that segregate each species in *Torula*, the morphology of *Torula* species are similar and is difficult to use to distinguish them. However, multigene phylogenetic analyses can be utilized in clarifying the interspecific relationships of these species, of which *tef1-α* and *rpb2* genes are reliable phylogenetic markers for delineating *Torula* species. For instance, Li et al.[5] introduced *T. chromolaenae* and *T. mackenziei* that resemble *T. herbarum* (the type species) in having solitary to catenate, septate, brownish and round-ended conidia, mononematous conidiophores and polyblastic conidiogenous cells. However, these three species formed well-resolved distinct subclades in Li et al.[5,6] and are clearly distinguished when the number of taxon samples were increased[13,14,19,68].

The conspecific status of *Torula longiconidiophora* and *T. sundara* is uncertain. *Torula sundara* was introduced by Jayawardena et al.[31] (published in February) to accommodate the hyphomycete species previously described as *Dwayabeeja sundara*. The new collection was isolated from bamboo culms in Chiang Mai Province, Thailand, and has shown morphological resemblance with *D. sundara*. Although, the type specimen of *D. sundara* was not examined, phylogenetic analyses demonstrated that the new collection belongs to *Torula* and is sister to *T. acaciae*. Therefore, the new combination, *Torula sundara* was proposed by Jayawardena et al.[31]. In the same year, Tian et al.[32] introduced *T. longiconidiophora* for the new taxon isolated from decaying wood in a damp environment in Sichuan Province, China (published in January). Phylogenetic analyses also demonstrated that *T. longiconidiophora* formed a sister clade with *T. acaciae* in Tian et al.[32]. Recent taxonomic work on Torulaceae was carried out by He et al.[14], who investigated phylogenetic relationships of the genera in Torulaceae and reported a new record for *T. sundara* on decaying wood submerged in freshwater from Yunnan, China. Based on morphological resemblance and phylogenetic evidence, He et al.[14] treated *T. longiconidiophora* as a synonym of *T. sundara*, even though *T. longiconidiophora* was published earlier. Besides, the type strain of *T. sundara* is unavailable, of which only ITS and LSU genes are available for *T. sundara* (MFLUCC 21-0067; reference strain for the combination). In the present study, five representative strains of *T. sundara* (including two strains previously named as *T. longiconidiophora*) do not form a well-resolved subclade in *Torula*. Hence, the conspecific status between *T. longiconidiophora* and *T. sundara* as well as the intraspecific relationships between representative strains of *T. sundara* need to be clarified using sufficient phylogenetic markers.

Qiu et al.[12] synonymized *Rostriconidium* and *Sporidesmioides* under the genus *Neopodoconis* based on their morphological resemblance and phylogenetic analyses of combined LSU and SSU gene regions. Qiu et al.[12] introduced five new species in *Neopodoconis*, namely *N. jiangxiensis*, *N. meilingensis*, *N. obclavata*, *N. saprophyticus,* and *N. sinensis*. In their phylogenetic analyses, *N. meilingensis* and *N. saprophyticus* clustered with *Rostriconidium* in Torulaceae. Whereas *N. jiangxiensis*, *N. obclavata,* and *N. sinensis* formed a sister subclade with *Sporidesmioides*, basal to Rousoellaceae. Unfortunately, the type of *Neopodoconis*, *N. ampullacea*, lacks molecular data to clarify its generic placement. Besides, *Sporidesmium*-like taxa are well-known to be morphologically similar; however, these taxa can be segregated into different genera and families based on molecular data[3,8,9,69]. Even though, the representative *Neopodoconis* species is phylogenetically separated into two distinctive clades in Qiu et al.[12], *Rostriconidium* and *Sporidesmioides* were treated as synonyms of *Neopodoconis* based solely on morphological characteristics. Subsequently, Wang et al.[13] demonstrated that *Neopodoconis* formed two distinct clades: clade I comprised *N. saprophyticus* and other *Rostriconidium* species in Torulaceae and clade II comprised *N. jiangxiensis*, *N. meilingensis*, *N. obclavata*, *N. sinensis,* and *Sporidesmioides thailandica*, as basal to Thyridariaceae. He et al.[14] updated the phylogenetic relationship of the genera in Torulaceae based on multigene phylogenetic analyses of a combined ITS, LSU, SSU, *tef1-α,* and *rpb2* genes. Their phylogenetic results demonstrated that *Rostriconidium* and *Sporidesmioides* formed well-resolved distinct subclades in Torulaceae. In the present study, *Neopodoconis meilingensis* (HJAUP C0905), *N. sinensis* (HJAUP C0909), *N. saprophyticus* (HJAUP C0830), and *N. yunnanensis* (KUNCC 22-10737) clustered with *Rostriconidium* species in Torulaceae. Whereas, *N. jiangxiensis* (HJAUP C0947) and *N. obclavata* (HJAUP C0829) clustered with *Sporidesmioides* and is basal to Thyridariaceae. Based on phylogenetic evidence and delimitation on molecular data of the type species of *Neopodoconis*, we therefore, tentatively resurrect the genera *Rostriconidium* and *Sporidesmioides* until the generic placement of *Neopodoconis* is clarified based on type study.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization, data curation, and formal analysis: Li XH, Li JF, Phookamsak R; funding acquisition: Xu JC, Li JF; investigation, methodology, and writing—original draft: Li XH, Sun FQ, Jiang HB, Li JF, Phookamsak R; project administration: Li XH, Sun FQ, Li JF; supervision: Xu JC, Li JF, Phookamsak R. writing—review and editing: Li XH, Sun FQ, Li JF, Jiang HB, Phookamsak R. All authors contributed to the article and approved the submitted version.

Data availability

The information of the new species introduced in this study can be found in online repositories. The name is registered for Mycobank repository under MycoBank number: MB 857067. The final tree and sequence matrix are deposited in TreeBASE (2024) under the accession number 31909 and GenBank accession number(s) can be found below: www.ncbi.nlm.nih.gov/genbank/ as ITS: PQ788521, PQ788522; LSU: PQ788523, PQ788524; *tef1-α*: PQ810569, PQ810570; *rpb2*: PQ81057, PQ810572.

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Conflict of interest

The authors declare that they have no conflict of interest.

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