



# Morphology and multi-gene phylogeny reveal a novel *Torula* (Pleosporales, Torulaceae) species from the plateau lakes in Yunnan, China

Sha Luan<sup>‡</sup>, Hong-Wei Shen<sup>‡,§,I</sup>, Dan-Feng Bao<sup>‡,§</sup>, Zong-Long Luo<sup>‡</sup>, Yun-Xia Li<sup>‡</sup>

<sup>‡</sup> College of Agriculture and Biological Science, Dali University, Dali, China

<sup>§</sup> Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand

<sup>I</sup> School of Science, Mae Fah Luang University, Chiang Rai, Thailand

Corresponding author: Yun-Xia Li ([342788514@qq.com](mailto:342788514@qq.com))

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## Abstract

## Background

During an investigation into lignicolous freshwater fungi from the plateau lakes in Yunnan Province, China, two fresh collections of *Torula* taxa were collected and examined morphologically.

## New information

*Torula luguhuensis* is characterised by: conidiophores which are semi-macronematous mononematous, erect, septate, smooth, slightly flexuous and pale brown; conidiogenous cells which are holoblastic, mono- to polyblastic, integrated, terminal, terminal or intercalary in conidial chains, doliiform and pale brown; conidia which are branched chains, acrogenous, straight or slightly curved, dark brown to blackish, pale brown or subhyaline at apex, 1–3 septate, strongly constricted at the septa, verruculose or finely echinulate and rounded at both ends. A new species was introduced, based on morphological and

phylogenetic analysis of combined ITS, LSU, RPB2 and TEF sequence data. Detailed descriptions and illustrations are provided, with an updated phylogenetic tree depicting intergeneric relationships within the *Torulaceae*.

## Keywords

1 new species, lignicolous freshwater fungi, phylogeny, taxonomy

## Introduction

*Torulaceae* was introduced by Corda (Sturm 1829) with *Torula* as the type. The family is known only by the asexual morph which is characterised by: mostly immersed mycelium, erect, micro- or macronematous, straight or flexuous, subcylindrical conidiophores with or without apical branches and doliiform to ellipsoid or clavate, brown, smooth to verruculose and mono- to polyblastic conidiogenous cells and subcylindrical, phragmosporous, acrogenous, brown, dry and smooth to verrucose conidia that are characteristically produced in branched chains (Crous et al. 2015, Su et al. 2016, Hyde et al. 2016, Li et al. 2017, Li et al. 2020). Currently, six genera, viz. *Cylindrotorula*, *Dendryphion*, *Neopodoconis*, *Neotorula*, *Rutola* and *Torula* are accommodated in *Torulaceae* (Crous et al. 2015, Su et al. 2016, Li et al. 2016, Su et al. 2018, Crous et al. 2020, Qiu et al. 2022).

*Torula* was introduced by Persoon (1795) and is typified by *T. herbarum*. Members in this genus are hyphomycetes and characterised by superficial dark colonies, terminal or lateral, monoblastic or polyblastic conidiogenous cells with a basally thickened and heavily melanised wall, a thin-walled apex and medium to dark brown conidia in branched chains (Crane and Miller 2016). *Torula* has been investigated as an interesting source of secondary metabolites. For example, a new dechlorinated aromatic lactone produced by *Torula* sp. (YIM DT 10072) exhibited antibacterial activity against *Staphylococcus aureus* (Chunyu et al. 2018). Herbarin, dehydroherbarin and o-methylherbarin have been extracted from *Torula herbarum* (Narasimhachari and Gopalkrishnan 1974).

Yunnan is an inland province at a low latitude and high elevation, lying between 21°09'–29°15' N and 97°32'–106°12' E in south-western China, an area which is rich in freshwater resources. The nine major plateau lakes represented by Dianchi Lake, Erhai Lake and Fuxian Lake are major features of Yunnan. Abundant freshwater lake resources provide a favourable environment for the occurrence of lignicolous freshwater fungi (Shen et al. 2022). The studies of lignicolous freshwater fungi in Yunnan are mainly focused on lotic habitats (Su et al. 2016, Luo et al. 2019). At present, only a limited number of early studies have explored the diversity of lignicolous freshwater fungi in Dianchi Lake and Fuxian Lake (Cai et al. 2002, Luo et al. 2004). Presently, we are conducting systematic research on lignicolous freshwater fungi from plateau lakes in Yunnan Province. In this study, two *Torula* species were collected from Lugu Lake and their phylogenetic relationships were analysed, based on molecular sequence data.

## Materials and methods

### Isolation and morphological study of strain

Submerged decaying woods were collected from Luguhu Lake, Yunnan Province and brought to the laboratory in zip-lock plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for one week and examined by methods following Luo et al. (2018). Micromorphological characters were observed using an Optec SZ 760 compound stereomicroscope. Temporarily prepared microscope slides were placed under a Nikon ECLIPSE Ni-U compound stereomicroscope for observation and micro-morphological-photography. The morphology of colonies on native substrates were photographed with a Nikon SMZ 1000 stereo zoom microscope.

Single spore isolations were carried out following the methods described by Senanayake (2020). Germinating conidia were transferred aseptically to PDA plates supplemented with 0.5 mg/l of Amoxicillin and grown at room temperature.

Specimens were deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS), Kunming, China. The cultures were deposited in China General Microbiological Culture Collection Center (CGMCC) and Kunming Institute of Botany Culture Collection (KUNCC). The MycoBank number was registered at <https://www.mycobank.org>.

### DNA extraction, PCR and sequencing

Fungal mycelium was scraped from the surface of colonies grown on PDA at room temperature. The Treliet<sup>TM</sup> Plant Genomic DNA Kit (TSP101-50) was used to extract DNA from the ground mycelium according to the manufacturer's instructions. The primers used for PCR amplification were ITS = ITS5/ITS4 (White et al. 1990), LSU = LR0R/LR5 (Vilgalys and Hester 1990), TEF- $\alpha$  = 983F/2218R and RPB2 = fRPB2-5F/fRPB2-7cR (Liu et al. 1999). The final volume of the PCR reaction was 25  $\mu$ l and contained 12.5  $\mu$ l of 2 $\times$  Power Taq PCR MasterMix, (20 mM Tris-HCl pH 8.3, 100 mM KCl, 3 mM MgCl<sub>2</sub>, stabiliser and enhancer), 1  $\mu$ l of each primer (10  $\mu$ M), 1  $\mu$ l genomic DNA extract and 9.5  $\mu$ l deionised water. The PCR of ITS genes was processed as follows: 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 50 seconds, elongation at 72°C for 60 seconds and final extension at 72°C for 10 minutes. The LSU and TEF genes were processed 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 60 seconds and final extension 72°C for 10 minutes. The RPB2 gene region was amplified with an initial denaturation of 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 60 seconds, annealing at 52°C for 2 minutes, elongation at 72°C for 90 seconds and final extension at 72°C for 10 minutes.

PCR amplification was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were sent for sequencing

at Tsingke Biological Engineering Technology and Services Company, Yunnan, China. The sequences were deposited in the GenBank database at the National Center for Biotechnology Information (NCBI) and the accession numbers are listed in Table 1.

Table 1.

Taxa used in the phylogenetic analysis and their corresponding GenBank accession numbers. The newly-generated sequences and the ex-type strains are in bold.

Species	Culture/Voucher	GenBank accession numbers			
		ITS	LSU	RPB2	TEF
<b><i>Torula acaciae</i></b>	CPC 29737	NR 155944	NG 059764	<b>KY173594</b>	-
<i>Torula aquatica</i>	DLUCC 0550	<b>MG208166</b>	<b>MG208145</b>	<b>MG207976</b>	<b>MG207996</b>
<b><i>Torula aquatica</i></b>	MFLUCC16-1115	<b>MG208167</b>	<b>MG208146</b>	<b>MG207977</b>	-
<b><i>Torula luguhuensis</i></b>	<b>KUNCC 22-12427</b>	<b>OQ729758</b>	<b>OQ947766</b>	<b>OQ999002</b>	<b>OQ999004</b>
<b><i>Torula breviconidiophora</i></b>	KUMCC 18-0130	<b>MK071670</b>	<b>MK071672</b>	-	<b>MK077673</b>
<i>Torula camporesii</i>	KUMCC 19-0112	<b>MN507400</b>	<b>MN507402</b>	<b>MN507404</b>	<b>MN507403</b>
<b><i>Torula chiangmaiensis</i></b>	KUMCC 16-0039	<b>MN061342</b>	<b>KY197856</b>	-	<b>KY197876</b>
<b><i>Torula chromolaenae</i></b>	KUMCC 16-0036	<b>MN061345</b>	<b>KY197860</b>	<b>KY197873</b>	<b>KY197880</b>
<b><i>Torula fici</i></b>	CBS 595.96	<b>KF443408</b>	<b>KF443385</b>	<b>KF443395</b>	<b>KF443402</b>
<i>Torula fici</i>	KUMCC 15-0428	<b>MG208172</b>	<b>MG208151</b>	<b>MG207981</b>	<b>MG207999</b>
<i>Torula fici</i>	KUMCC 16-0038	<b>MN061341</b>	<b>KY197859</b>	<b>KY197872</b>	<b>KY197879</b>
<b><i>Torula gaodangensis</i></b>	MFLUCC 17-0234	<b>MF034135</b>	NG 059827	-	-
<b><i>Torula goaensis</i></b>	NFCCL 4040	NR 159045	NG 060016	-	-
<b><i>Torula herbarum</i></b>	CPC 24414	<b>KR873260</b>	<b>KR873288</b>	-	-
<b><i>Torula hollandica</i></b>	CBS 220.69	NR 132893	NG 064274	<b>KF443393</b>	<b>KF443401</b>
<b><i>Torula hydei</i></b>	KUMCC 16-0037	<b>MN061346</b>	<b>MH253926</b>	-	<b>MH253930</b>
<b><i>Torula mackenziei</i></b>	MFLUCC 13-0839	<b>MN061344</b>	<b>KY197861</b>	<b>KY197874</b>	<b>KY197881</b>
<b><i>Torula masonii</i></b>	CBS 245.57	NR 145193	NG 058185	-	-
<i>Torula masonii</i>	DLUCC 0588	<b>MG208173</b>	<b>MG208152</b>	<b>MG207982</b>	<b>MG208000</b>
<i>Torula masonii</i>	KUMCC 16-0033	<b>MN061339</b>	<b>KY197857</b>	<b>KY197870</b>	<b>KY197877</b>
<b><i>Torula pluriseptata</i></b>	MFLUCC 14-0437	<b>MN061338</b>	<b>KY197855</b>	<b>KY197869</b>	<b>KY197875</b>
<b><i>Torula polyseptata</i></b>	KUMCC 18-0131	<b>MK071671</b>	<b>MK071673</b>	-	<b>MK077674</b>
<i>Torula sp.</i>	CBS 246.57	<b>KF443411</b>	<b>KR873290</b>	-	-
<b><i>Torula lancangjiangensis</i></b>	HKAS 112709	NR 175706	<b>MW879526</b>	<b>MW729780</b>	<b>MZ567104</b>
<b><i>Torula thailandica</i></b>	GZCC 20-0011	<b>MN907426</b>	<b>MN907428</b>	-	-
<b><i>Torula canangae</i></b>	MFLUCC 21-0169	<b>OL966950</b>	<b>OL830816</b>	-	<b>ON032379</b>
<b><i>Torula chinensis</i></b>	UESTCC 22.0085	<b>OQ127986</b>	<b>OQ128004</b>	-	-
<b><i>Torula longiconidiophora</i></b>	UESTCC 22.0088	<b>OQ127983</b>	<b>OQ128001</b>	<b>OQ158967</b>	<b>OQ158972</b>
<i>Torula longiconidiophora</i>	UESTCC 22.0125	<b>OQ127984</b>	<b>OQ128002</b>	<b>OQ158972</b>	<b>OQ158972</b>

Species	Culture/Voucher	GenBank accession numbers			
		ITS	LSU	RPB2	TEF
<i>Torula phytolaccae</i>	ZHKUCC 22-0107	<a href="#">ON611796</a>	<a href="#">ON611800</a>	<a href="#">ON660879</a>	<a href="#">ON660881</a>
<i>Torula phytolaccae</i>	ZHKUCC 22-0108	<a href="#">ON611795</a>	<a href="#">ON611799</a>	<a href="#">ON660878</a>	<a href="#">ON660880</a>
<i>Torula sichuanensis</i>	UESTCC 22.0087	<a href="#">OQ127981</a>	<a href="#">OQ127999</a>	-	-
<i>Torula submersa</i>	UESTCC 22.0086	<a href="#">OQ127985</a>	<a href="#">OQ128003</a>	<a href="#">OQ158968</a>	<a href="#">OQ158972</a>
<i>Torula submersa</i>	KUNCC 22-12426	<a href="#">OQ991910</a>	<a href="#">OQ991917</a>	-	<a href="#">OQ999003</a>
<i>Cylindrotorula indica</i>	NFCCI 4836	NR 175156	NG 081308	<a href="#">MT321490</a>	<a href="#">MT321492</a>
<i>Cylindrotorula indica</i>	NFCCI 4837	<a href="#">MT339445</a>	<a href="#">MT339443</a>	<a href="#">MT321491</a>	<a href="#">MT321493</a>
<i>Dendryphion aquaticum</i>	MFLUCC 15-0257	<a href="#">KU500566</a>	<a href="#">KU500573</a>	-	-
<i>Dendryphion comosum</i>	CBS 208.69	<a href="#">MH859293</a>	<a href="#">MH871026</a>	-	-
<i>Dendryphion europaeum</i>	CPC 22943	<a href="#">KJ869146</a>	<a href="#">KJ869203</a>	-	-
<i>Dendryphion europaeum</i>	CPC 23231	<a href="#">KJ869145</a>	<a href="#">KJ869202</a>	-	-
<i>Dendryphion fluminicola</i>	KUMCC 15-0321	<a href="#">MG208160</a>	<a href="#">MG208139</a>	<a href="#">MG207971</a>	<a href="#">MG207990</a>
<i>Dendryphion fluminicola</i>	DLUCC 0849	<a href="#">MG208161</a>	<a href="#">MG208140</a>	<a href="#">MG207972</a>	<a href="#">MG207991</a>
<i>Dendryphion fluminicola</i>	MFLUCC17-1689	NR 157490	<a href="#">MG208141</a>	-	<a href="#">MG207992</a>
<i>Dendryphion hydei</i>	KUMCC 18-0009	<a href="#">MN061343</a>	<a href="#">MH253927</a>	-	<a href="#">MH253931</a>
<i>Dendryphion nanum</i>	HKAS84010	<a href="#">KU500568</a>	<a href="#">KU500575</a>	-	-
<i>Dendryphion nanum</i>	HKAS84012	<a href="#">KU500567</a>	<a href="#">KU500574</a>	-	-
<i>Dendryphion nanum</i>	MFLUCC 16-0987	<a href="#">MG208156</a>	<a href="#">MG208135</a>	<a href="#">MG207967</a>	<a href="#">MG207986</a>
<i>Dendryphion submersum</i>	MFLUCC15-0271	<a href="#">KU500565</a>	<a href="#">KU500572</a>	-	-
<i>Dendryphion submersum</i>	KUMCC15-0455	<a href="#">MG208159</a>	<a href="#">MG208138</a>	<a href="#">MG207970</a>	<a href="#">MG207989</a>
<i>Neotorula aquatica</i>	MFLUCC 15-0342	<a href="#">KU500569</a>	<a href="#">KU500576</a>	-	-
<i>Neotorula submersa</i>	HKAS 92660	NR 154247	<a href="#">KX789217</a>	-	-
<i>Neopodoconis aquaticum</i>	KUMCC 15-0297	<a href="#">MG208165</a>	<a href="#">MG208144</a>	<a href="#">MG207975</a>	<a href="#">MG207995</a>
<i>Neopodoconis aquaticum</i>	MFLUCC 16-1113	<a href="#">MG208164</a>	<a href="#">MG208143</a>	<a href="#">MG207974</a>	<a href="#">MG207994</a>
<i>Neopodoconis pandanicola</i>	KUMCC 17-0176	<a href="#">MH275084</a>	<a href="#">MH260318</a>	<a href="#">MH412759</a>	<a href="#">MH412781</a>
<i>Neopodoconis cangshanense</i>	MFLUCC 20-0146	<a href="#">MW010284</a>	<a href="#">MW010281</a>	<a href="#">MW012636</a>	-
<i>Neopodoconis cangshanense</i>	MFLUCC 20-0147	<a href="#">MW010285</a>	-	-	-
<i>Rutola graminis</i>	CPC 33267	<a href="#">MN313814</a>	<a href="#">MN317295</a>	-	-
<i>Rutola graminis</i>	CPC 33695	<a href="#">MN313815</a>	<a href="#">MN317296</a>	-	-
<i>Rutola graminis</i>	CPC 33715	<a href="#">MN313816</a>	<a href="#">MN317297</a>	-	-
<i>Neopodoconis thailandica</i>	MFLUCC 13-0840	<a href="#">MN061347</a>	NG 059703	<a href="#">KX437761</a>	<a href="#">KX437766</a>
<i>Neopodoconis thailandica</i>	KUMCC 16-0012	<a href="#">MN061348</a>	<a href="#">KX437758</a>	<a href="#">KX437762</a>	<a href="#">KX437767</a>

## Sequencing and sequence alignment

Sequences were assembled with BioEdit (Hall 1999) and those with high similarity indices were determined from a BLAST search to find the closest matches with taxa in *Torula* and from recently-published data (Li et al. 2020, Li et al. 2023, Tian et al. 2023). Aligned sequences of each loci (ITS, LSU, RPB2 and TEF) were combined and manually improved using BioEdit v.7.0.5.2 (Hall 1999). All consensus sequences and the reference sequences were automatically aligned with MAFFT (Katoh and Standley 2013). Additionally, sequence trimming was performed with trimAl v.1.2 with default parameters (<http://trimal.cgenomics.org> for specific operation steps) (Capella-Gutiérrez et al. 2009) and combined using SequenceMatrix (Vaidya et al. 2011). Ambiguous regions were excluded from the analysis and gaps were treated as missing data. FASTA alignment formats were changed to PHYLIP and NEXUS formats using the website Alignment Transformation Environment (ALTER) (<http://sing.ei.uvigo.es/ALTER/>).

## Phylogenetic analyses

Maximum Likelihood (ML) analysis was performed by setting RAxML-HPC2 on XSEDE (8.2.12) (Stamatakis 2006, Stamatakis et al. 2008) in the CIPRES Science Gateway (Miller et al. 2010) (<http://www.phylo.org/portal2>) using the GTR+GAMMA model with 1000 bootstrap repetitions. Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist et al. 2012) and the best-fitting model of sequences evolution was estimated via Capella-Gutiérrez 2.2 (Guindon and Gascuel 2003, Darriba et al. 2012, Ronquist et al. 2012). The Markov Chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP) (Rannala and Yang 1996). Bayesian analyses of six simultaneous Markov chains were run for 5 M generations and trees were sampled every thousand generations. Phylogenetic trees were visualised using FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>), while editing and typesetting were achieved using Adobe Illustrator (AI) (Adobe Systems Inc., United States).

## Taxon treatments

### *Torula luguhuensis* S. Luan, H.W. Shen & Z.L. Luo sp. nov.

- MycoBank [MB 848773](#)

#### Material

##### Holotype:

- a. scientificName: *Torula luguhuensis*; kingdom: fungi; phylum: Ascomycota; class: Dothideomycetes; order: Pleosporales; family: Torulaceae; genus: *Torula*; waterBody: Luguhu Lake; locationRemarks: China, Yunnan Province, submerged decaying wood in Luguhu Lake; verbatimLatitude: 27°44'13.59"N; verbatimLongitude: 100°49'04.72"E; habitat: freshwater, submerged decaying wood; recordedBy: Sha Luan; identifiedBy: Sha Luan; collectionID: LGH H 6-43-1; collectionCode: L335; occurrenceID: B75E1EC7-0546-585A-A29A-1577106D0D0B

## Description

Saprobic on submerged decaying wood (Fig. 1a). **Sexual morph:** Undetermined. **Asexual morph:** Colonies effuse on nature substrate, scattered, velutinous, dark brown to black. Mycelium immersed to superficial, composed of hyaline, becoming brown closer to fertile region, septate, branched hyphae. Conidiophores semi-macronematous mononematous, erect, septate, smooth, slightly flexuous, pale brown (Fig. 1b and c). Conidiogenous cells holoblastic, mono- to polyblastic, integrated, terminal, terminal or intercalary in conidial chains, doliiiform, pale brown. Conidia in branched chains, dry, acrogenous, straight or slightly curved, more or less cylindrical, dark brown to blackish, pale brown or subhyaline at apex, 1–3 septate, strongly constricted at the septa, verruculose or finely echinulate, rounded at both ends, easily separating, 12–18 µm (= 15 µm, SD = 3, n = 60) long, 6–8 µm (= 7 µm, SD = 1, n = 60) wide (Fig. 1d-m).

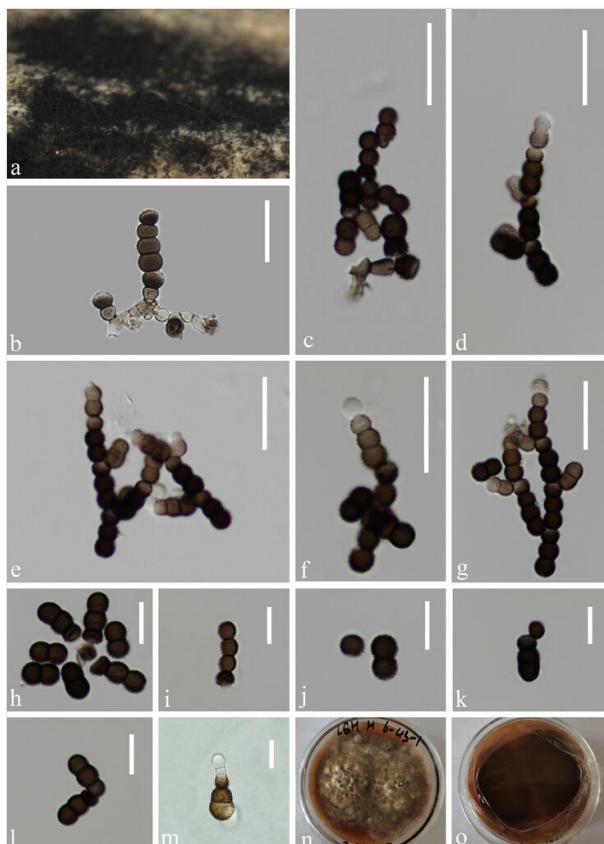


Figure 1. [doi](#)

*Torula luguhuensis* (KUN-HKAS 124588, holotype). **a** Colonies on decaying wood; **b, c** Conidiophores with conidia; **d-l** Conidia; **m** Germinating conidium; **n, o** Colonies on PDA from surface and reverse. Scale bars: b-g 20 µm, h-m 10 µm.

**Culture characteristics:** Conidia germinating on PDA within 12 hours and germ tubes produced from the apex. Colonies growing on PDA, reaching 10 cm in 15 days at 24°C, mycelium partly superficial, partly immersed, hairy, with regular edge, maroon to yellowish-brown (Fig. 1o and p).

**Material examined:** China, Yunnan Province, submerged decaying wood in Luguhu Lake, 100°49'04.72"E, 27°44'13.59"N, March 2021, Sha Luan, *Torula luguhuensis* (KUN-HKAS 124588, holotype), ex-type culture, CGMCC 3.24256 = KUNCC 22-12427.

### Etymology

Referring to Luguhu Lake, China, where the fungus was collected.

### Notes

In the multigene phylogenetic analysis, *Torula luguhuensis* clustered with *T. aquatica* (MFLUCC 16-1115, DLUCC 0550) with 100% ML and 1.00 PP support. *Torula luguhuensis* resembles *T. aquatica* in having macronematous or semi-macronematous, erect conidiophores and verruculose conidia (Su et al. 2018). However, *Torula luguhuensis* differs from *T. aquatica* in having larger conidia (12–18 × 6–8 vs. 9–14 × 5–6 µm). A comparison of RPB2, ITS and LSU nucleotides between *T. luguhuensis* and *T. aquatica* showed 48/775 bp (6.2%), 5/433 bp (1.2%) and 3/796 bp (0.3%) differences with no gaps, respectively. Based on morphological and phylogenetic analysis, we introduce *T. luguhuensis* as a new species.

## *Torula submersa* W.H. Tian, Y.P. Chen & Maharachch J. Fungi 2023

- MycoBank [MB 847013](#)

### Description

Saprobic on submerged decaying wood (Fig. 2a, b). **Sexual morph:** Undetermined. **Asexual morph:** Colonies effuse on nature host, black, friable. Mycelium immersed to superficial, composed of septate, pale brown, branched hyphae. Conidiophores macronematous, mononematous, erect, smooth, straight or slightly flexuous, dark brown to pale brown (Fig. 2c). Conidiogenous cells polyblastic, terminal, dark brown, pale brown at apex, on conidiophores, minutely verruculose, doliform to subglobose. Conidia solitary or in branched chains, acrogenous, simple, phragmosporous, dark brown, pale brown at apex, 2–4-septate, constricted at the septa, verruculose or finely echinulate, rounded at both ends, easily separating; 14–20 µm (= 17 µm, SD = 3, n = 60) long, 6–8 µm (= 7 µm, SD = 1, n = 60) wide (Fig. 2d-n).

**Culture characteristics:** Conidia germinating on PDA within 10 hours and germ tubes produced from the apex. Colonies growing on PDA, reaching 10 cm in 15 days at

24°C. Colonies were raised in the middle, velvety on the surface and had a white centre fading to yellowish-brown, reverse, yellowish-brown in centre and white edges.



Figure 2. [doi](#)

*Torula submersa* (HKAS 126510). **a-b** Colonies on decaying wood; **c** Conidiophores with conidia; **d-n** Conidia. Scale bars: c-e 20 µm, f-n 10 µm.

**Material examined:** China, Yunnan Province, submerged decaying wood in Luguhu Lake, 100°49'08.33"E, 27°39'39.24"N, March 2021, Yan Tao, L147 (KUNCC 22–12426).

#### Notes

*Torula submersa* was introduced by Tian et al. (2023), collected on a submerged decaying branch from Sichuan Province. In this study, a fresh collection was made on submerged decaying wood in Luguhu Lake, Yunnan Province. Phylogenetic analysis showed that our collection (KUNCC 22–12426) sistered with *T. submersa* (Fig. 3). Morphological characteristics of our new collection are consistent with *Torula submersa* (Tian et al. 2023). Based on morphological characteristics and phylogenetic analysis,

we, therefore, identified our new collection as *Torula submersa*, which was collected from a lentic freshwater habitat for the first time.

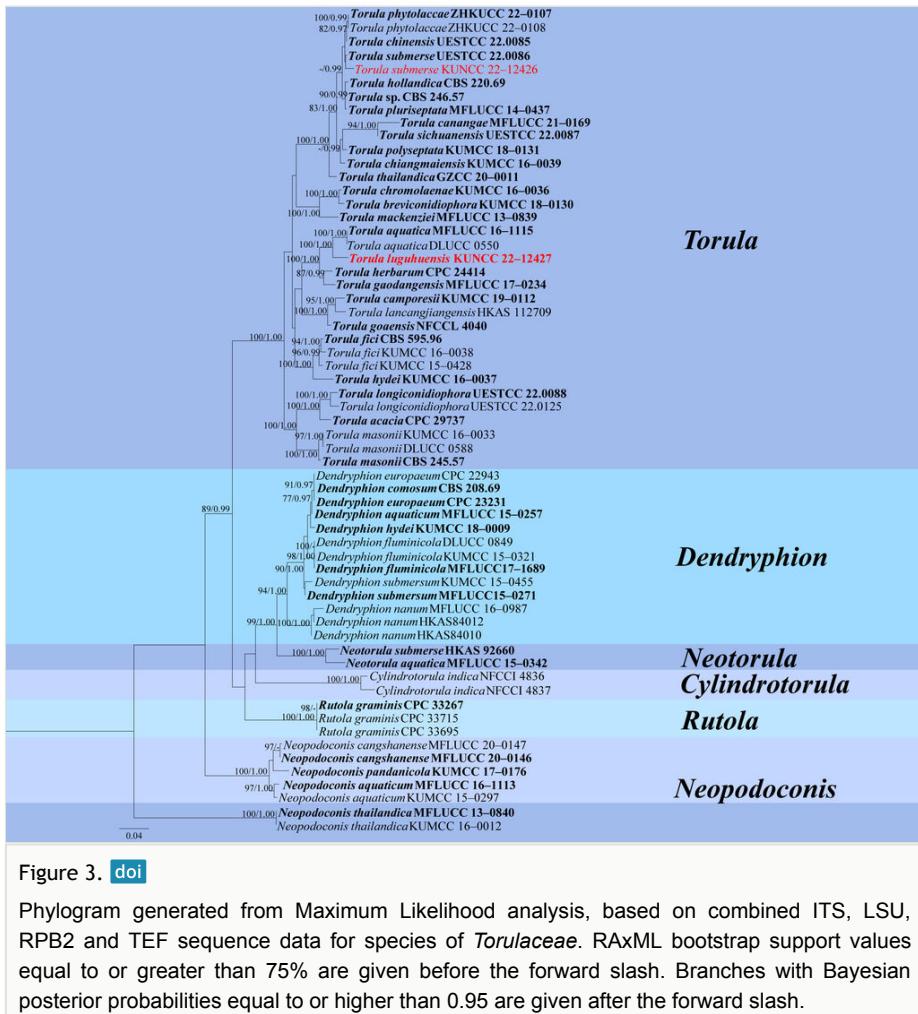


Figure 3. doi: <https://doi.org/10.31233/osf.io/2t34r>

Phylogram generated from Maximum Likelihood analysis, based on combined ITS, LSU, RPB2 and TEF sequence data for species of *Torulaceae*. RAxML bootstrap support values equal to or greater than 75% are given before the forward slash. Branches with Bayesian posterior probabilities equal to or higher than 0.95 are given after the forward slash.

## Discussion

Species of *Torula* are quite similar in morphology and most species lack DNA sequence data to support their phylogenetic relationships (Crous et al. 2015). This causes taxonomic confusion and some species may have been misidentified. Some *Torula* species may be the same or belong to other genera and their taxonomic statuses have to be further investigated (Crane and Miller 2016). In addition to the morphological examination, DNA-based phylogenetic analysis should be performed for more *Torula* species. Herein, we combined multi-loci phylogenetic analysis and morphological characterisation to introduce

one new species which contributes to the taxonomy for the genus and addition of DNA sequence in databases.

Taxonomic research on *Torulaceae* in China is mainly concentrated in the south-western region and commonly found in freshwater habitats (Hyde et al. 2016, Su et al. 2016, Su et al. 2018, Qiu et al. 2022, Tian et al. 2023). There are four genera of *Torulaceae* viz. *Dendryphion*, *Neopodoconis*, *Neotorula* and *Torula* which are reported from freshwater habitats in China. In this study, a checklist of *Torulaceae* species in China is provided. *Torula* is commonly found on submerged decaying wood in freshwater environments with most species having been isolated from lotic water (Table 2). In our study, we found a new species in a lake in Yunnan Province. Presumably there could be other new species in these habitats and it is necessary to investigate lignicolous freshwater fungi in other lakes in Yunnan. A checklist of *Torulaceae* species from freshwater habitats in China is shown in Table 2 below.

Table 2.

Checklist of *Torulaceae* species from freshwater habitats in China.

Species	Distribution	Habitat	New species/record	Reference
<i>Dendryphion aquaticum</i>	Yunnan	Lotic	new species	Su et al. (2016)
<i>Dendryphion fluminicola</i>	Yunnan	Lotic	new species	Su et al. 2018
<i>Dendryphion nanum</i>	Yunnan	Lotic	new record	Su et al. (2016)
<i>Dendryphion submersum</i>	Yunnan	Lotic	new species	Su et al. (2016)
<i>Neotorula aquatica</i>	Yunnan	Lotic	new species	Su et al. (2016)
<i>Neotorula submersa</i>	Yunnan	Lotic	new species	Hyde et al. (2016)
<i>Neopodoconis aquaticum</i>	Yunnan	Lotic	new species	Su et al. (2018)
<i>Neopodoconis cangshanensis</i>	Yunnan	Lotic	new species	Qiu et al. (2022)
<i>Neopodoconis pandanicola</i>	Yunnan	Lotic	new record	Qiu et al. (2022)
<i>Torula aquatica</i>	Yunnan	Lentic and Lotic	new species	Su et al. (2018)
<i>Torula fici</i>	Yunnan	Lotic	new record	Su et al. (2018)
<i>Torula lancangjiangensis</i>	Yunnan	Lotic	new species	Boonmee et al. (2021)
<i>Torula mackenziei</i>	Yunnan	Lotic	new record	Boonmee et al. (2021)
<i>Torula gaodangensis</i>	Guizhou	Lotic	new species	Hyde et al. (2020)
<i>Torula chinensis</i>	Sichuan	Lotic	new species	Tian et al. 2023
<i>Torula longiconidiophora</i>	Sichuan	Lotic	new species	Tian et al. 2023
<i>Torula sichuanensis</i>	Sichuan	Lotic	new species	Tian et al. (2023)
<i>Torula submerse</i>	Sichuan	Lotic	new species	Tian et al. 2023
<i>Torula masonii</i>	Yunnan	Lentic	new record	Su et al. (2018)

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