Four new species of Trichomonascaceae (Saccharomycetales, Saccharomycetes) from Central China

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Abstract
Trichomonascaceae is the largest family of ascomycetous yeast in the order Saccharomycetales. In spite of the extensive body of research on Trichomonascaceae in China, there remain new species to be discovered. Here, we describe four new species isolated from several rotting wood samples from Henan Province, Central China. Phylogenetic analysis of a combined ITS and nrLSU dataset with morphological studies revealed four new species in the Trichomonascaceae: Diddensiella luoyangensis, Sugiyamaella cylindrica, Su. robnettiae, and Zygoascus detingensis. Clustering in the Diddensiella clade, D. luoyangensis’ closest neighbour was D. transvaalensis. Meanwhile, Su. cylindrica clustered in the Sugiyamaella clade closest to Su. marilandica and Su. qingdaonensis. Also clustering in the Sugiyamaella clade, Su. robnettiae was most closely related to Su. chuxiongensis. Finally, Z. detingensis occupied a distinct and separated basal branch from the other species of the genus Zygoascus. These results indicate a high species diversity of Trichomonascaceae.

Keywords
New taxa, phylogenetics, taxonomy, Trichomonascaceae, yeasts
Introduction

The family of Trichomonomascaceae was described by Kurtzman and Robnett (2007) to accommodate the genera *Sugiyamaella* Kurtzman and Robnett, *Trichomonascus* (H.S. Jackson) Kurtzman and Robnett, *Wickerhamiella* van der Walt, *Zygoascus* M.Th. Smith and related anamorphs based on multigene phylogenetic analysis (Kurtzman 2011a). Subsequently, two new genera, *Spencermartinsiella* Péter, Dlauchy, Tornai-Lehoczki, M. Suzuki & Kurtzman and *Diddensiella* Péter, Dlauchy and Kurtzman were included based on multi-locus DNA sequences (Péter et al. 2011; Péter et al. 2012). This was followed by Kurtzman and Robnett (2014) in which eight genera were accepted into Trichomonomascaceae while the other anamorphic species such as *Candida glaeosa* clade of the family are currently members of the polyphyletic genus *Candida* (Lachance et al. 2011; Daniel et al. 2014). The majority of taxa included in the family Trichomonomascaceae form septate hyphae, but members of the genus *Wickerhamiella* do not (Kurtzman and Robnett 2007; Lachance and Kurtzman 2011) and instead the genus *Spencermartinsiella* with the type species *Spencermartinsiella europaea* form blastoconidia on small denticles (Péter et al. 2011). With the exception of *Trichomonomascus farinosus* (de Hoog, Rantio-Lehtimäki & M.Th. Smith) Kurtzman & Robnett, all teleomorphic species that form septate hyphae are also heterothallic (Kurtzman and Robnett 2007; Smith et al. 2011a; Péter et al. 2012).

Members of Trichomonomascaceae occur on a wide range of substrates in terrestrial and marine environments worldwide (Sakpuntoon et al. 2020), and some have ecological distribution patterns that may imply close relationships with insects. Species have been isolated either directly from insects or insect related substrates. Furthermore, the species of Trichomonomascaceae are of economic importance to fields of food production, cosmetics, environment, medicine, and agriculture. For instance, several species of *Blastobotrys* von Klopotek play vital roles in production of lipids (Smith et al. 2011b; Thomas et al. 2019), while some species of *Wickerhamiella* are pathogens of humans (Lachance and Kurtzman 2011; Avchar et al. 2019; Belloch et al. 2020). Additionally, some members of *Sugiyamaella*, including *Su. bahiana* L.M. Sena et al., *Su. bonitensis* L.M. Sena et al., *Su. boreocaroliniensis* (Kurtzman) H. Urbina & M. Blackw, *Su. lignohabitans* (Kurtzman) H. Urbina & M. Blackw, *Su. valenteae* L.M. Sena et al., *Su. xylanicola* Morais, Lachance & Rosa and *Su. xylolytica* L.M. Sena et al., possess the ability to ferment D-xylose into ethanol, and three species: *Su. xylanicola*, *Su. lignohabitans*, and *Su. valenteae* are capable of producing highly active xylanases. (Kurtzman 2011b; Morais et al. 2013a, b; Sena et al. 2017). Therefore, the discovery of novel yeasts in Trichomonomascaceae is of both fundamental and applied importance. Moreover, increasing our knowledge and understanding of this group of yeast may provide useful information for their sustainable utilization and conservation of natural resources.

Rotting wood, which contains diverse and abundant assimilable carbon compounds, is known to be a rich habitat for yeast species. In the past few years, thirteen species of Trichomonomascaceae, including *Blastobotrys*, *Spencermartinsiella*, and *Sugiyamaella*, were obtained from rotting wood in China, which includes six new species and seven
known species (Wang et al. 2010; Guo et al. 2012; Huang et al. 2018; Chai et al. 2020; Shi et al. 2021). Although the samples of rotting wood were collected in a relatively small geographical area in China, the Trichomonascaceae species are diverse in this rich ecological environment.

During extensive investigations on the diversity of yeast inhabiting rotting wood from China, several unknown yeast strains were collected from Henan Province, and their morphology suggested species of Diddensiella, Sugiyamaella, and Zygoascus. To investigate their taxonomy further, phylogenetic analyses, based on combined ITS and nrLSU sequences, were carried out. Both morphological characteristics and molecular evidence demonstrate that these yeasts represent four new species of Trichomonascaceae, which are described here.

**Materials and methods**

**Sample collection and yeast isolation**

Samples of rotting wood were collected in the Tianchi Mountain National Forest Park (34°33’N, 112°28’E) located near Luoyang City, Henan Province, China. The national forest park is at 850 m above sea level (MASL) and has a continental monsoon climate. The average annual temperature is between 14 °C and 16 °C, and the average annual rainfall is greater than 800 mm. Forty samples of decaying wood were collected between September and October in 2020. Samples were stored in sterile plastic bags and transported under refrigeration to the laboratory within 24 hours. Yeast strains were isolated from rotting wood samples according to previously described methods (Huang et al. (2018) and Shi et al. (2021). One gram of each sample was added to 20 mL sterile yeast extract-malt extract (YM) broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, pH 5.0 ± 0.2), supplemented with 0.025% sodium propionate and 200 mg/L chloramphenicol in a 150 mL Erlenmeyer flask, and then cultured for 3–10 days at 180 rpm on a rotary shaker. Subsequently, 0.1 mL aliquots of the enrichment culture and appropriate decimal serial dilutions were plated on YM agar plates and incubated at 25 °C for 3–4 days. Different yeast colony morphotypes were then isolated via repeated plating on YM agar. Isolates were stored on YM agar slants at 4 °C or in 15% glycerol at −80 °C. All isolates were stored in Microbiology Lab of Nanyang Normal University (NYNU; Nanyang, China), and ex-type cultures of novel yeast were deposited in the fungal collection at Westerdijk Fungal Biodiversity Institute (CBS; Utrecht, The Netherlands). Species nomenclature and descriptions were registered in MycoBank (www.mycobank.org, accessed on February 9, 2022).

**Morphological and physiological investigation**

Morphological and physiological properties were determined according to methods previously described in Kurtzman et al. (2011). Carbon and nitrogen assimilation
tests were performed using liquid media and growth was observed for up to 4 weeks. Carbon fermentation was tested in yeast extract peptone (YP) base media (1% yeast extract and 2% peptone, pH 5.0 ± 0.2), and Durham tubes were used to visualise carbon dioxide production. Growth rates at a range of temperatures (30 °C, 35 °C, 37 °C, and 40 °C) were assessed by streaking cells on to yeast extract peptone glucose (YPD) agar (1% yeast extract, 2% peptone, 2% glucose, 2% agar, pH 5.0 ± 0.2) plates and incubating them for 2 weeks. Formation of true hyphae and pseudohyphae were investigated using the Dalmatian plate method on both cornmeal (CM) and 5% malt extract (ME) agar plates. Induction of the sexual stage was tested by incubating single or mixed cultures of the each of the two strains on PDA agar, cornmeal (CM) agar, 5% malt extract (ME) agar, V8 (1:9) agar, Gorodkowa agar, or yeast carbon base plus 0.01% ammonium sulfate (YCBAS) agar at 25 °C for 2 months (Kurtzman 2011b; Péter et al. 2012; Nagatsuka et al. 2016).

**DNA amplification and sequencing**

Genomic DNA was extracted from each of the yeasts using the Ezup Column Yeast Genomic DNA Purification Kit according to the manufacturer’s protocol (Sangon Biotech, China). The rDNA ITS1-5.8S-ITS2 (ITS) region was amplified using the primer pair ITS1/ITS4 (White et al. 1990). The D1/D2 domain of nrLSU rDNA (nrLSU) was amplified using the primer pair NL1/NL4 (Kurtzman and Robnett 1998). The following parameters were used to amplify the ITS and nrLSU regions: an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 51 °C, and 40 s at 72 °C, and a final extension of 10 min at 72 °C (Shi et al. 2021). PCR products were directly purified and sequenced by Sangon Biotech Inc. (Shanghai, China). The identity and quality of the newly-obtained sequences were assessed by comparing them to sequences in GenBank and assembling them with BioEdit (Hall 1999). Sequences were then submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/; Table 1).

**Phylogenetic analyses**

Species in the family Trichomonomascaceae with high similarity to the new species described here were selected as references in the phylogenetic analyses. *Tortispora caseinolytica* CBS 7781T and *Tor. ganteri* CBS 12581T were used as outgroup. NCBI accession numbers of sequences used in the phylogenetic tree are listed in Table 1. Initial alignment of the combined ITS + nrLSU dataset was performed using the online version MAFFT 6.0 (Katoh and Toh 2010) followed by manual evaluations and adjustments in BioEdit as needed to obtain reliable and high quality results (Hall 1999). The best-fit nucleotide substitution models for separate and combined nucleotide sequences were selected using jModelTest v2.1.7 (Darriba et al. 2012) according to the Akaike Information Criterion (AIC). The final concatenated sequence alignment was deposited in TreeBase (http://www.treebase.org; submission ID S29358).
Table 1. DNA sequences used in the molecular phylogenetic analysis.

| Species                        | Strain     | Locality            | Sample          | ITS             | D1/D2          |
|-------------------------------|------------|---------------------|-----------------|-----------------|----------------|
| Blastobotrys indiannensis     | CBS 9600   | USA                 | White fungus    | NR_153638       | NG_055333      |
| D. santijacobensis             | CBS 8183   | USA                 | Fallen trunk    | NR_151808       | NG_058985      |
| D. transvaeldenis              | CBS 6663   | South Africa        | Forest litter   | N/A             | DQ442702       |
| D. luoyangensis               | NYNU 201062 | China            | Rotten wood     | MW374289        | MW362346       |
| Middelhovenomyces petrohuensis| CBS 8173   | Chile               | Rotten trunk    | NR_156314       | NG_052111      |
| Middelhovenomyces tepae       | CBS 5115   | Chile               | Decaying tepe tree | NR_154200   | NG_051811      |
| Spencermartinsiella cellulosica| CBS 11952  | China               | Rotten wood     | NR_151783       | NG_052070      |
| Sp. europa                     | CBS 11730  | Hungary             | Rotten wood     | NR_111481       | NG_042528      |
| Sp. lignipatridi               | CBS 12585  | Hungary             | Rotten trunk    | NR_155842       | NG_055382      |
| Sp. silvicola                  | CBS 11952  | Brazil              | Rotting wood    | KT222943        | KC062454       |
| Sugiyamaella americana        | CBS 10352  | USA                 | Frass           | NR_137759       | DQ438193       |
| Su. Ayubii                     | CBS 14108  | Brazil              | Rotting wood    | NR_155796       | KR184132        |
| Su. Bahiana                    | CBS 13474  | Brazil              | Rotting wood    | NR_155810       | KC959941       |
| Su. Bonitensis                 | CBS 14270  | Brazil              | Rotting wood    | NR_155798       | KT006004       |
| Su. Boreocarolinensis          | NRRL YB-1835| USA               | Frass           | NR_165963       | DQ438232       |
| Su. Bullunensis                | CBS 11840  | USA                 | Insect          | NR_111543       | HM208601       |
| Su. Calostasis                 | NRRL Y-17329 | Chile            | Rotting wood    | NR_111229       | DQ438195       |
| Su. Carusensis                 | CBS 14107  | Brazil              | Rotting wood    | NR_155808       | KX501111       |
| Su. Chiloensis                 | CBS 8168   | Chile               | Rotten wood     | DQ911454        | DQ438217       |
| Su. Ciliengensis               | NYNU 181038| China              | Rotting wood    | MK682800        | MK682795       |
| Su. Cylindrica                 | NYNU 201067| China              | Rotting wood    | MW368732        | MW368731       |
| Su. Floridaensis               | NRRL YB-3827 | USA            | Frass           | NR_111230       | DQ438222       |
| Su. grinnbergii                | NRRL Y-27117 | Chile           | Insect          | KY102116        | DQ438199       |
| Su. japonica                   | CBS 10354  | Japan               | Frass           | NR_111239       | DQ438202       |
| Su. Ligni                      | CBS 13482  | Brazil              | Rotting wood    | KX550112        | KX550112       |
| Su. lignohabitans              | NRRL YB-1473 | USA            | Decayed log     | NR_119622       | DQ438198       |
| Su. maritensis                 | NRRL YB-1336 | USA            | Decayed log     | NR_111237       | DQ438197       |
| Su. marilandica                | NRRL YB-1847 | USA            | Frass           | NR_165965       | DQ438219       |
| Su. mastotermitis              | CBS 14182  | Berlin              | Termite         | NR_156606       | KU883286       |
| Su. neomexicana                | CBS 10349  | USA                 | Frass           | NR_165966       | DQ438201       |
| Su. novakii                    | NRRL Y-27346 | Hungary         | Rotting wood    | NR_111235       | DQ438196       |
| Su. paludigena                 | NRRL Y-12697 | Russia         | Peat            | NR_111236       | DQ438194       |
| Su. pineola                    | CBS 10348  | USA                 | Frass           | NR_165967       | DQ438200       |
| Su. qingdaonensis              | CBS 11390  | China               | Rotting wood    | NR_151806       | FJ613527       |
| Su. robbii                     | NYNU 201066 | China            | Rotting wood    | MW368730        | MW368701       |
| Su. robbii                     | NYNU 201005 | China            | Rotting wood    | OM501585        | OM501589       |
| Su. smithiae                   | CBS 7522.2 | Brazil              | Soil            | DQ911455        | DQ438218       |
| Su. tyrpani                    | CBS 15876  | Poland              | Soil            | MK388412        | MK387312       |
| Su. valdiviana                 | NRRL Y-7791 | Chile           | Rotting wood    | NR_111544       | DQ438220       |
| Su. valenteae                  | CBS 14109  | Brazil              | Rotting wood    | NR_155797       | KT005999       |
| Su. xiangenensis               | NYNU 161041 | China            | Rotting wood    | KY213802        | KY213817       |
| Su. xylanicola                 | CBS 12683  | Brazil              | Rotting wood    | KC493642        | KC493642       |
| Su. xyloxytica                 | CBS 13493  | Brazil              | Rotting wood    | KU214874        | KF889433       |
| Su. yunnanensis                | NYNU 161059 | China            | Rotting wood    | MT257259        | MT257257       |
| Tortispora ganteri             | CBS 12581  | Mexico              | Necrotic plant tissue | NR_154483   | KC681893       |
| Tortispora caseinolitica        | CBS 7781   | USA                 | Necrotic plant tissue | NR_154482   | NG_055343       |
| Trichomonascus petasorus       | CBS 9602   | USA                 | Frass           | NR_155940       | NG_055332       |
| Zygoguscia biomembranitola      | CBS 14157  | Japan               | Viscous gel     | NR_156007       | LC060997       |
| Z. bituminiphila               | CBS 8813   | Canada              | Tar             | NR_137545       | NG_055308       |
| Z. hellenicus                  | CBS 5839   | Germany             | Mastic bovine udder | NR_11258   | NG_055323       |
Maximum likelihood (ML) and Bayesian inference (BI) analyses were used for the phylogenetic analyses. The ML analysis was carried out using RAxML v.7.2.8 with a GTR + G + I, model of site substitution including estimation of Gamma-distributed rate heterogeneity and a proportion of invariant sites (Stamatakis 2006). Branch support was evaluated using bootstrapping with 1000 replicates (Hillis and Bull 1993). The BI analysis was performed using MrBayes v3.2 (Ronquist et al. 2012), for two independent runs, each with four Markov chains Monte Carlo (MCMC) independent runs for 5 ×10⁶ generations (split frequencies = 0.011). The first 25% of trees were discarded as “burn-in” of each analysis and the remaining 75% were then used to calculate Bayesian posterior probabilities of the majority rule consensus tree.

Phylogenetic trees from the ML and BI analyses were visualised with FigTree v1.4.3 (Rambaut 2016) and edited in Adobe Illustrator CS6. Branches that received bootstrap support for maximum likelihood (BS) and Bayesian posterior probabilities (BPP) greater than or equal to 50% (BS) and 0.95 (BPP) were considered to be significantly supported.

Results

Molecular phylogenetic analysis

The combined ITS and nrLSU dataset was analysed to infer the phylogenetic relationships of the family Trichomonomascaceae and the new Chinese isolates. The dataset consisted of 59 sequences including the outgroup, Tortispora caseinolytica CBS 7781T and Tor. ganteri CBS 12581T. A total of 943 characters including gaps (376 for ITS and 567 for nrLSU) were included in the phylogenetic analysis. GTR + I + G was inferred as the best-fit model for the combined nrLSU and ITS nucleotide sequences according to the AIC in jModelTest v2.1.7 (Darriba et al. 2012). The topologies of the phylogenetic tree of ML and BI analyses are identical, and only the ML tree with a final optimisation likelihood value of −12097.50 is shown in Fig. 1. RAxML bootstrap support values (BS) ≥ 50% and Bayesian posterior probability values (BPP) ≥ 0.95 are shown above the branches and indicated with bolded lines.
Figure 1. Maximum-likelihood phylogenetic tree based on ITS and nrLSU nucleotide sequences. Bootstrap values (BP) \(\geq 50\%\) from ML analysis and Bayesian posterior probabilities (BPP) \(\geq 0.95\) are shown on the branches. Newly described species are indicated in bold and their metabolically inactive ex-type strains are indicated by “T” after the species name.
In the phylogeny (Fig. 1), newly generated strains in this study nested in the genera *Diddensiella*, *Sugiyamaella*, and *Zygoascus* within the Trichomonascaceae. *D. luoyangensis* clustered in the *Diddensiella* clade with an affinity to *D. santjacobensis* (C. Ramírez & A. González) Péter, Dlauchy & Kurtzman and *D. transvaalensis* (Kurtzman) Péter, Dlauchy & Kurtzman. *Su. cylindrica* and *Su. robnettiae* clustered in the *Sugiyamaella* clade with close similarity to the type species *Su. smithiae* (Giménez-Jurado) Kurtzman and Robnett (2007), and to other related species with high bootstrap support (BS = 94%; BPP = 1.0). Additionally, *Su. cylindrica* clustered together with *Su. marilandica* (Kurtzman) H. Urbina & M. Blackw and *Su. qingdaoensis* (F.L. Li & S.A. Wang) Handel, Wang, Yurkov & König with strong bootstrap support (BS 100%, BPP 1.0), while *Su. robnettiae* formed a separate lineage within *Sugiyamaella* that included *Su. ayubii* L.M. Sena et al., *Su. chuxiongensis* C.Y. Chai & F.L. Hui, and *Su. valenteae* L.M. Sena et al. *Z. detingensis* formed a unique branch of the tree which was clearly distinct and diverged from other species of *Zygoascus*.

**Taxonomy**

*Diddensiella luoyangensis* C.Y. Chai & F.L. Hui, sp. nov.

MycoBank No: 842899

**Etymology.** The specific epithet *luoyangensis* refers to the geographic origin of the type strain: Luoyang City, Henan.

**Type.** China, Henan Province, Luoyang City, Song County, the Tianchi Mountain National Forest Park, in rotting wood, October 2020, J.Z. Li & Z.T. Zhang (holotype NYNU 201062T, ex-type CBS 16659 = CICC 33512, holotype and ex-type are preserved in a metabolically inactive state).

**Description.** In YM broth after 3 days at 25 °C, cells are ovoid (2–3 × 3–5 μm) and occur singly or in pairs. Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. On YM agar after 3 days at 25 °C, colonies are white to cream-coloured, convex, butyrous, and smooth with entire margins. In Dalmau plate culture on corn meal agar, pseudohyphae and true hyphae are formed. Asci or signs of conjugation are not observed on sporulation media. Fermentation of sugars is absent. Glucose, galactose, l-sorbose, glucosamine, d-ribose, d-xylose, l-arabinose, d-arabinose, l-rhamnose, sucrose, maltose, trehalose, methyl D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose, melezitose, inulin, glycerol, erythritol, ribitol, d-glucitol, d-mannitol, galactitol, myo-inositol, d-glucono-1, 5-lactone, 2-keto-d-gluconate, 5-keto-d-gluconate, d-gluconate, d-gluconate, d-lactate succinate, citrate, and ethanol are assimilated as sole carbon sources. Methanol is not assimilated. L-lysine, creatine, glucosamine, and D-tryptophan are assimilated as sole nitrogen sources, while nitrate, nitrite, ethylamine, cadaverine, creatinine, and imidazole are not assimilated. Minimum growth temperature is 15 °C, and maximum growth temperature is 37 °C. Growth in the presence of 0.1% cycloheximide is present, but growth in the presence...
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of 10% NaCl plus 5% glucose and 1% acetic acid is absent. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are negative.

Additional isolate examined. CHINA, Henan Province, Luoyang City, Song County, the Tianchi Mountain National Forest Park, in rotting wood, October 2020, J.Z. Li & Z.T. Zhang (NYNU 201074).

Notes. Two strains were collected from two different substrates, representing D. luoyangensis, clustered in the Diddensiella clade which is sister to species D. transvaalensis. D. luoyangensis differed from D. transvaalensis by 1.6% substitutions in the D1/D2 domain. Furthermore, we were unable to align the ITS sequence of D. luoyangensis with the D. transvaalensis type strain, because the ITS sequence of D. transvaalensis is not currently available from either the NCBI GenBank or CBS databases. Physiologically, D. luoyangensis differs from its closely related species, D. transvaalensis (Lachance et al. 2011), based on growth in L-rhamnose, lactose, inulin, D-gluconate and growth at 37 °C, which are present for D. luoyangensis and absent for the latter species. Moreover, D. transvaalensis ferments glucose and galactose, while this new species does not.

Sugiyamaella cylindrica C.Y. Chai & F.L. Hui, sp. nov.
MycoBank No: 842900
Fig. 3

Etymology. The specific epithet cylindrica refers to the cylindrical vegetative cells of the type strain.

Type. CHINA, Henan Province, Luoyang City, Song County, the Tianchi Mountain National Forest Park, in rotting wood, October 2020, J.Z. Li & Z.T. Zhang

Figure 2. Morphology of D. luoyangensis (NYNU 201062, holotype) A budding cells were indicated by arrows in YM broth after 3 d B pseudohyphae and true hyphae on CM agar after 14 d. Scale bars: 10 μm.
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(holotype NYNU 201067T, ex-type CBS 16662 = CICC 33514, holotype and ex-type are preserved in a metabolically inactive state).

**Description.** In YM broth after 3 days at 25 °C, cells are cylindrical (2–3 × 5–7 μm) and occur singly or in pairs. Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. On YM agar after 3 days at 25 °C, colonies are white to cream-coloured, butyrous, convex and smooth with entire margins. In Dalmau plate culture on corn meal agar, rudimentary pseudohyphae are formed. Ascii or signs of conjugation are not observed on sporulation media. Glucose and trehalose are weakly fermented, but, galactose, maltose sucrose, melibiose, lactose, cellobiose, melezitose, raffinose, inulin and xylose are not fermented. Glucose, galactose, L-sorbitose, glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, salicin, melibiose, raffinose, melezitose, inulin, glycerol, erythritol, ribitol, D-glucitol, D-mannitol, galactitol, myo-inositol, D-glucono-1, 5-lactone, 2-keto-D-glucoside, 5-keto-D-glucuronic acid, D-glucuronic acid, D-lactate succinate, and ethanol are assimilated as sole carbon sources. Lactose, D-gluconate, citrate and methanol are not assimilated. Nitrate, nitrite, L-lysine, creatine, glucosamine, and D-tryptophan are assimilated as sole nitrogen sources. Ethylamine, cadaverine, creatinine, and imidazole are not assimilated. Minimum growth temperature is 15 °C, and maximum growth temperature is 35 °C. Growth in the presence of 0.1% cycloheximide is present, but growth in the presence of 1% acetic acid and 10% NaCl plus 5% glucose is absent. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are negative.

**Additional isolate examined.** China, Henan Province, Luoyang City, Song County, the Tianchi Mountain National Forest Park, in rotting wood, October 2020, J.Z. Li & Z.T. Zhang (NYNU 201034).

**Notes.** Two strains were collected from two different substrates, representing *Su. cylindrica*, clustered in the *Sugiyamaella* clade and are closely related to

![Figure 3. Morphology of Su. cylindrica (NYNU 201067, holotype) A budding cells were indicated by arrows in YM broth after 3 d B rudimentary pseudohyphae on CM agar after 14 d. Scale bars: 10 μm.](image-url)
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Su. marilandica and Su. qingdaonensis. The nucleotide differences between the new species and the close relatives Su. marilandica and Su. qingdaonensis are 1.1–1.4% substitutions in the D1/D2 domain and 5.0–5.9% substitutions in the ITS region, respectively. Physiologically, Su. cylindrica differs from the closely related species Su. marilandica and Su. qingdaonensis (Wang et al. 2010; Kurtzman 2011b) in its ability to assimilate glycerol and dL-lactate and to grow at 35 °C. Additionally, the new species ferments trehalose, while Su. marilandica and Su. qingdaonensis do not.

Sugiyamaella robnettiae C.Y. Chai & F.L. Hui, sp. nov.  
MycoBank No: 842901
Fig. 4

Etymology. The specific epithet robnettiae named in honour of Christie J. Robnett for her proposal of the genus Sugiyamaella.

Type. CHINA, Henan Province, Luoyang City, Song County, the Tianchi Mountain National Forest Park, in rotting wood, October 2020, J.Z. Li & Z.T. Zhang (holotype NYNU 201066, ex-type CBS 16663 = CICC 33513, holotype and ex-type are preserved in a metabolically inactive state).

Description. In YM broths after 3 days at 25 °C, the cells are ellipsoidal to elongate (2–4 × 2–8 μm) and occur singly or in pairs. Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. On YM agar after 3 days at 25 °C, colonies are white to cream-coloured, convex, buttery and smooth with entire margins. In Dalmau plate culture on corn meal agar, pseudohyphae and true hyphae are formed. Asci or signs of conjugation are not observed on sporulation media. Fermentation of sugars is absent. Glucose, galactose, L-sorbose, glucosamine, D-xylene, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, salicin, arbutin, lactose, inulin, glycerol, erythritol, ribitol, xylitol, D-glucitol, D-mannitol, galactitol, D-glucono-1, 5-lactone, 2-keto-D-gluconate, 5-keto-D-gluconate, succinate, citrate, and ethanol are assimilated as sole carbon sources. D-ribose, melibiose, raffinose, melezitose, myo-inositol, D-gluconate, D-lactate, and methanol are not assimilated. Nitrate, nitrite, creatine, glucosamine, and D-tryptophan are assimilated as sole nitrogen sources. Ethylamine, L-lysine, creatinine, and imidazole are not assimilated. Minimum growth temperature is 15 °C, and maximum growth temperature is 35 °C. Growth in the presence of 0.1% cycloheximide is present, but growth in the presence of 10% NaCl plus 5% glucose and 1% acetic acid is absent. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are negative.

Additional isolates examined. CHINA, Henan Province, Luoyang City, Song County, the Tianchi Mountain National Forest Park, in rotting wood, October 2020, J.Z. Li & Z.T. Zhang (NYNU 201005).

Notes. Two strains were collected from two different substrates, formed a well-supported group related to Su. chuxiongensis, representing a new species, Su. robnettiae. Su. robnettiae differs from Su. chuxiongensis by 1.9% substitutions in the D1/D2 domain.
and 6.4% substitutions in the ITS region. Physiologically, unlike *Su. chuxiongensis* (Shi et al., 2021), *Su. robnettiae* is unable to assimilate d-ribose, melibiose, raffinose, or melezitose but is able to assimilate glycerol and lactose.

### *Zygoascus detingensis* C.Y. Chai & F.L. Hui, sp. nov.

MycoBank No: 842902  
Fig. 5

**Etymology.** The specific epithet *detingensis* refers to the geographic origin of the type strain, Deting Town, Henan.

**Type.** China, Henan Province, Luoyang City, Song County, the Tianchi Mountain National Forest Park, in rotting wood, October 2020, J.Z. Li & Z.T. Zhang (holotype NYNU 201087, ex-type CBS 16667 = CICC 33516, holotype and ex-type preserved in a metabolically inactive state).

**Description.** In YM broth after 3 days at 25 °C, cells are subglobsoidal to globoidal (2–3 × 2–4 μm) and occur singly or in pairs. Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. On YM agar after 3 days at 25 °C, colonies are cream, smooth, opalescent, convex and glistening. In Dalmau plate culture on corn meal agar, pseudohyphae and true hyphae are formed. Asci or signs of conjugation are not observed on sporulation media. Fermentation of sugars is absent. Glucose, galactose (weak), glucosamine, d-ribose (weak), d-xylose, d-arabinose (weak), l-arabinose (weak), l-rhamnose (weak), sucrose (weak), maltose (weak), trehalose, methyl α-d-glucoside (weak), cellobiose (weak), salicin, melibiose, lactose (weak), raffinose, melezitose (weak), inulin (weak), glycerol (weak),
Four new species of Trichomonascaceae

Four new species of Trichomonascaceae are described, named *Z. detingensis*. 

**Additional isolate examined.** China, Henan Province, Luoyang City, Song County, the Tianchi Mountain National Forest Park, in rotting wood, October 2020, J.Z. Li & Z.T. Zhang (NYNU 201011).

**Notes.** Two strains were collected from two different substrates, both representing *Z. detingensis*, branched separately from the *Zygoascus* clade. *Z. detingensis* differed from the other *Zygoascus* species by more than 9.7% substitutions in the D1/D2 domain and 11.5% substitutions in the ITS region, respectively. Physiologically, *Z. detingensis* differs from its closely related species, *Z. bituminiphila* (V. Robert, B. Bonjean, Karutz, Paschold, W. Peeters & Wubbolts) Nagatsuka, Kiyuna & Sugiyama (Nagatsuka et al. 2016), in its inability to assimilate L-sorbose and its ability to assimilate L-rhamnose, methyl α-D-glucoside, melibiose, lactose, inulin melezitose, erythritol, and 2-keto-D-gluconate. Moreover, *Z. bituminiphila* ferments glucose, galactose, trehalose, and cellobiose, while *Z. detingensis* does not.

**Figure 5.** Morphology of *Z. detingensis* (NYNU 201087, holotype) A budding cells were indicated by arrows in YM broth after 3 d B pseudohyphae and true hyphae on CM agar after 14 d. Scale bars: 10 μm.
Discussion

In the present study, we collected rotting wood from the Tianchi Mountain National Forest Park located near Luoyang City in Henan Province of China. From these samples, we isolated several yeast strains. Some of these yeasts are known species, such as *Metschnikowia henanensis*, *Saturnispora galanensis*, *Wickerhamomyces menglaensis* and *Deakozyma yunnanensis*. Here, we recovered eight isolates from eight rotting woods of *Trichomonascaceae* yeast representing four new species belonging to the genera *Diddensiella*, *Sugiyamaella*, and *Zygoascus*. We described these new species as *D. luoyangensis*, *Su. cylindrica*, *Su. Robnettiae*, and *Z. detingensis* based on molecular phylogenetic and morphological evidence. A thorough and comprehensive phylogenetic analysis of the family *Trichomonascaceae* based on the combined ITS and the D1/D2 domains of the LSU rRNA gene sequences is provided, including almost all GenBank representatives and newly generated sequences, which may serve as a reference for the field. This study provides information on the species delimitation of the family *Trichomonascaceae* based on morphological and phylogenetic evidence.

Our phylogenetic analyses, based on ITS and the D1/D2 domains of the LSU rRNA gene sequences, are in concordance with previous studies (Morais et al. 2013b; Sena et al. 2017; Shi et al. 2021). However, the genus *Sugiyamaella* of *Trichomonascaceae* is not a monophyletic group. Morais et al. (2013b) indicated that *Sugiyamaella* is polyphyletic, where the species are intertwined with representatives of the genera *Diddensiella* and *Spencermartinsiella*. From the latter study, the genus could be divided into two main clades, which were later supported by Sena et al. (2017) and Shi et al. (2021). In this study, all species of *Sugiyamaella* and related genera were used to refine our understanding of the evolutionary relationships of this family, based on the ITS and nrLSU dataset. As shown in Fig. 1, all genera of *Trichomonascaceae* formed monophyletic groups with the exception of *Sugiyamaella* in which two main clades were reconstructed: (i) *Su. smithiae* (the type species), *Su. lignonhabitans*, and *Su. valdiviana* and their related species and (ii) *Su. americana*, *Su. bullrunensis*, (S.O. Suh, Houseknecht & J.J. Zhou) H. Urbina & M. Blackw, *Su. carassensis* L.M. Sena et al. and *Su. ligni* L.M. Sena et al.

In recent years, more than 40 yeast species have been identified from rotting wood in China (Wang et al. 2010; Guo et al. 2012; Gao et al. 2017; Zheng et al. 2017; Huang et al. 2018; Chai et al. 2020; Lv et al. 2020; Shi et al. 2021). Among them, at least 16 species of *Trichomonascaceae* have been isolated from rotting wood in China, including six new species previously obtained from China (*Bla. xishuangbannaensis*, *Sp. cellulosicola*, *Su. qingdaonensis*, *Su. xiaquanensis*, *Su. Chuxiong*, and *Su. yunnanensis*) (Wang et al. 2010; Guo et al. 2012; Huang et al. 2018; Chai et al. 2020; Shi et al. 2021), new records of six species not known to occur in China (*Su. americana*, *Su. ayubii*, *Su. novakii*, *Su. paludigena*, *Su. Valenteae*, and *Su. valdiviana*) (Shi et al. 2021), and four novel species identified in this study (*D. luoyangensis*, *Su. cylindrica*, *Su. robnettiae*, and *Z. detingensis*). In China, there remain species to be discovered, such as those sequences of the D1/D2 domains of the LSU rRNA gene listed under GenBank accessions JN581115 and JN581116. To date, including the four new...
Four new species of *Trichomonascaceae* worldwide (www.mycobank.org). Although the taxonomy of *Trichomonascaceae* has been a focus of research in the past, many regions are under-sampled and more novel indigenous *Trichomonascaceae* species will undoubtedly be discovered in the future.

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