MYCOSPHERE 16(1): 4723–4782 (2025) www.mycosphere.org ISSN 2077 7019
https://doi.org/10.5943/mycosphere/16/1/35

ARTICLE

High yeast diversity in primeval forest of Shennongjia, including 21 new species characterized by morphological, phylogenetic, and genomic analyses

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Citation – Qiu YJ, Zhu HY, Zhu QY, Yang LX, Mao YC, Wen Z, Chen SX, Qiu JZ, Bai FY, Han PJ 2025 – High yeast diversity in primeval forest of Shennongjia, including 21 new species characterized by morphological, phylogenetic, and genomic analyses. Mycosphere 16(1): 4723–4782, https://doi.org/10.5943/mycosphere/16/1/35

Abstract

Shennongjia, located in the northwestern of Hubei Province, China, has a northern subtropical monsoon climate, along with vast areas of primeval forest. Its unique geography and altitudinal variation have fostered a rich and rare array of natural resources, making it a biodiversity hotspot. While most biodiversity studies in Shennongjia focus on animals and plants, research on microorganisms, particularly yeasts, remains limited. To address this gap, we collected 624 samples from the region, applied enrichment cultures at different temperatures aimed at isolating primarily ascomycetous yeasts, and ultimately obtained 4,451 yeast isolates. Phylogenetic analysis identified these strains as 161 species, belonging to 69 genera, 28 families, 20 orders, and 9 classes within the Ascomycota and Basidiomycota, including 6 species of *Saccharomyces*. Importantly, we formally describe 21 new species across 13 genera, namely *Ambrosiozyma apicoglobosa* sp. nov., *Cyberlindnera hubeiensis* sp. nov., *Cyberlindnera juglandicorticola* sp. nov., *Cyberlindnera shennongjiaensis* sp. nov., *Hyphopichia shennongjiaensis* sp. nov., *Kluyveromyces shennongjiaensis* sp. nov., *Kregervanrija quercicorticola* sp. nov., *Kregervanrija shennongjiaensis* sp. nov., *Middelhovenomyces xyloputricola* sp. nov., *Nakazawaea arboricola* sp. nov., *Nakazawaea pterocaryicorticola* sp. nov., *Nakazawaea shennongjiaensis* sp. nov., *Pichia shennongjiaensis* sp. nov., *Pichia xylocola* sp. nov., *Saccharomycopsis shennongjiaensis* sp. nov., *Starmera prunicorticola* sp. nov., *Sugiyamaella irregularis* sp. nov., *Suhomyces quercicorticola* sp. nov., *Suhomyces shennongjiaensis* sp. nov., *Yamadazyma betulicola* sp. nov., and *Yamadazyma gelatinotortuosa* sp. nov., with detailed morphological images. Furthermore, 43 new taxonomic combinations were proposed within these genera to accommodate closely related *Candida* species. Our study reveals that Shennongjia harbors a rich and diverse yeast community, including many previously unreported species. This research contributes to the global record of yeast diversity and distribution, which will inspire further investigation and conservation efforts in this valuable region.

Key words – yeast diversity – novel ascomycetous yeast species – phylogeny – taxonomy

Submitted: 03 April 2025; **Accepted:** 16 December 2025; **Published:** 29 December 2025
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Accepted by: Qi-Ming Wang

INTRODUCTION

Shennongjia, officially known as the Shennongjia Forestry District, is located in the northwestern border region of Hubei Province, China, spanning longitudes 109°56′ E to 110°58′ E and latitudes 31°15′ N to 31°57′ N, covering an area of approximately 3,253 square kilometers. The region's terrain is characterized by a higher southwest and lower northeast, with deep, intersecting valleys. Its lowest elevation is 398 meters, while the highest peak reaches 3,105 meters, the highest point in both Hubei Province and Central China. The climate, influenced by subtropical circulation, is classified as northern subtropical monsoon, with year-round warmth and humidity. Average annual temperatures range from 12 to 17 °C, with extremes from -31 to 43 °C, and annual precipitation ranges from 700 to 2,700 mm. The region harbors well-preserved evergreen and deciduous broad-leaved mixed forests and exhibits a complete altitudinal vegetation spectrum. From low to high elevations, this vertical zonation includes evergreen broad-leaved forest, mixed evergreen and deciduous broad-leaved forest, broad-leaved deciduous forest, broadleaf–conifer mixed forest, coniferous forest, and mountain shrub–meadow. These conditions support a diverse ecosystem and the largest area of primary forest in Central China, making Shennongjia a refuge for many ancient relic species as well as rare, endangered, and endemic species unique to China (Zhao et al. 2005, Xie et al. 2017). In addition, the region contains several conservation areas, including the renowned Shennongjia National Nature Reserve, listed as a UNESCO Biosphere Reserve. Its unique geographical features, combined with significant altitudinal variation, have fostered abundant natural resources, making Shennongjia a promising hotspot. However, most biodiversity studies in Shennongjia have concentrated on animals and plants (Zhou et al. 2018, Wu et al. 2021, Zhang & Cai 2021, Zhang et al. 2024), while research on microorganisms (Zhang et al. 2015), particularly yeasts, remains limited.

Yeasts are often defined as predominantly unicellular fungi that reproduce asexually through budding or fission and lack fruiting bodies (Kurtzman et al. 2011a). They are widely distributed worldwide, exhibit remarkable diversity, and have played significant roles in human history and interactions. Some yeasts have been integral to food fermentation, industrial production, agricultural practices, and advancements in science and technology, benefiting humankind for millennia (Johnson & Echavarri-Erasun 2011). While others are among the most prominent disease-causing pathogens, especially those of the genus *Candida*, which pose a threat to global health due to their high number of life-threatening infections and mortality rates (Cooper 2011, Brown et al. 2012, WHO 2022). Despite their importance to food, industry, agriculture, science, technology, and human health, the ecology and distribution of most yeast species are poorly understood (Spurley et al. 2021). Following advancements in high-throughput sequencing technologies, more and more researchers are using culture-independent metagenomics to investigate environmental fungal communities (Samarasinghe et al. 2021, Li et al. 2024). Nonetheless, the isolation and cultivation of strains remain indispensable, providing foundational material for further research and practical applications. As a result, most research on wild ascomycetous yeasts relies on environmental sampling, enrichment, isolation, and purification (Wang et al. 2012, Liti et al. 2017, Boynton et al. 2019). These traditional approaches are admittedly labor-intensive and low-throughput, requiring considerable time and effort compared with modern high-throughput methods. However, a broad and dense sampling strategy and a well-designed isolation approach can help elucidate the distribution and diversity of natural yeasts (Spurley et al. 2021, Mozzachiodi et al. 2022, Rosa et al. 2023, David et al. 2024). For example, extensive environmental sampling has revealed that *Saccharomyces cerevisiae* is not merely a domesticated species, deepening our understanding of its global distribution and diversity (Duan et al. 2018, Peter et al. 2018, Han et al. 2021). Interestingly, strains recognized as the oldest lineage (CHN IX) of *Saccharomyces cerevisiae* were isolated from the primeval forests of Shennongjia (Duan et al. 2018, Han et al. 2021), suggesting that this region may harbor a rich diversity of *Saccharomyces*, and possibly a broader diversity of yeasts in general.

To better understand the diversity of wild yeasts in Shennongjia, focusing on ascomycetous yeasts and particularly *Saccharomyces*, we extensively collected 624 samples of natural substrates between 2023 and 2024. Through an enrichment method at different temperatures, the study yielded

a total of 4,451 isolates, contributing significantly to global yeast distribution records. Our research documented 161 distinct species across 69 genera, 28 families, 20 orders, and 9 classes within the Ascomycota and Basidiomycota, including 6 species of *Saccharomyces*, a rare finding within a single region. Importantly, supported by whole-genome sequencing, this study formally described 21 new species from 13 genera within the Ascomycota, accompanied by detailed morphological images. In addition, 43 new combinations were proposed to reduce the heterogeneity of the ascomycetous genus *Candida*. By highlighting these findings, we hope to inspire further investigation and conservation efforts for the valuable region of Shennongjia.

MATERIALS AND METHODS

Sample collection and yeast isolation

A total of 624 samples of natural substrates were collected from a variety of environments within the Shennongjia Forestry District and its surrounding areas, Hubei Province, China, between 2023 and 2024. These environments included primeval forests, streams, mountainous regions, wetlands, among others (Fig. 1). The majority of the samples were bark from the Fagaceae family. If applicable, GPS locations and plant species were recorded during sampling. The samples were collected using 50 mL or 15 mL centrifuge tubes, or sterile bags, and transported to the laboratory for yeast isolation.

Yeasts were isolated using the enrichment method designed primarily for ascomycetous yeasts, with yeast extract–malt extract (YM, w/v, 1% glucose, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract) or yeast extract peptone dextrose (YPD, w/v, 2% glucose, 2% peptone, and 1% yeast extract) medium supplemented with 200 μg/mL chloramphenicol, and 7% ethanol was added in some cases. Samples were divided into two portions, each placed in 50 mL centrifuge tubes, mixed with medium, and then incubated at 10 °C and 25 °C until evidence of growth or fermentation was observed (generally 2–3 weeks at 25 °C and more than 1 month at 10 °C). Enriched cultures were diluted and spread onto corresponding agar plates, which were then incubated at the same temperatures for 3–4 days. The discrete colonies on the plates were inspected, and “yeast-like” colonies were selected, while those with “mold-like” morphologies were typically avoided. Single colonies were picked for identification, purified, and preserved in 25% glycerol at -80 °C.

Comprehensive details of all samples are provided in Supplementary Table 1.

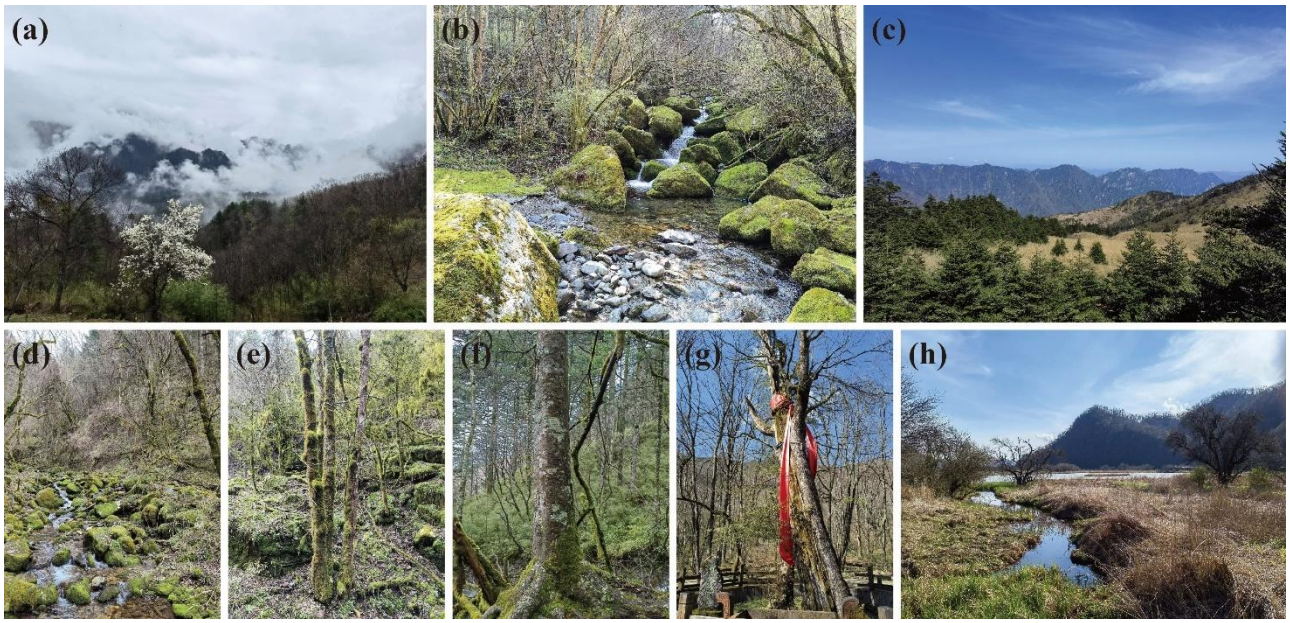

Figure 1 – Sampling environments in Shennongjia. (a) Duidongping. (b) Shennongyuan. (c) Shennongding, the highest peak at 3,105 meters. (d–f) Jinhouling primeval forest. (g) Kumufengchun (in Dajiuhu Lake), featuring a *Quercus aliena* var. *acutiserrata* tree aged over a thousand years. (h) Dajiuhu Lake, a national wetland park of international importance.

DNA extraction, sequencing and phylogenetic analyses

Genomic DNA was extracted using the method described by Zhu et al. (2025). The internal transcribed spacer (ITS) region, including the 5.8S rDNA, was amplified using the primers ITS1 and ITS4, while the 26S rDNA D1/D2 domains were amplified using the primers NL1 and NL4, with the procedure described previously (Bai et al. 2002). The D1/D2 sequences of the obtained strains were subjected to BLASTn searches against the NCBI nucleotide database to identify species. Strains of the same species isolated from the same sample were regarded as clones in the analysis; they, as well as *Mucoromycota* species, were excluded from further analyses.

When a strain exhibited more than 1% sequence divergence from the type material of the most closely related known species in either the D1/D2 or ITS sequence, it was considered a candidate for a novel species. Then, their D1/D2, 5.8S, and ITS sequences were assembled into contigs using SeqMan (Lasergene 7, DNASTAR Inc., Madison, WI, USA) to determine their taxonomic status. Sequences of closely related strains or species were retrieved from GenBank, released genomes, or the Westerdijk Fungal Biodiversity Institute website (https://wi.knaw.nl) as references. These sequences were aligned using MAFFT 7 (Katoh & Standley 2013) and manually trimmed. For the analysis of single D1/D2 or ITS sequences, evolutionary distance data were calculated using Kimura's two-parameter model and the neighbor-joining method in MEGA 7 (Kimura 1980, Kumar et al. 2016). For the analysis of concatenated sequences, Maximum Likelihood (ML) phylogenetic trees were inferred using RAXML 8 (Stamatakis 2014) under the GTR+I+G model, with bootstrap values calculated from 1000 replicates (Felsenstein 1985). A bootstrap percentage (BP) above 50% was considered significantly supported.

Complete information on all yeast isolates is provided in Supplementary Table 2. All strains and GenBank accession numbers used for phylogenetic analysis are listed in Supplementary Table 3.

Phenotypic characterization

New species strains were characterized according to the standard methods described by Kurtzman et al. (2011b), but with modifications for *Saccharomycopsis shennongjiaensis* sp. nov. Specifically, for the carbon assimilation test, casamino acids were added to achieve a final nitrogen concentration of 0.108 mg/mL. Similarly, for the nitrogen and amino acid assimilation tests, compounds were added to achieve the same final nitrogen concentration.

Ascosporulation was investigated using yeast carbon base agar (YCB, w/v, 1.17% yeast carbon base and 2% agar), corn meal agar (CMA, w/v, 2.5% corn starch and 2% agar), potato dextrose agar (PDA, w/v, 20% potato infusion, 2% glucose, and 2% agar), and V8 agar (w/v, 10% V8 juice and 2% agar). The plates were incubated at 25 °C for up to 2 months and examined periodically.

For colony morphology, strains were diluted and spread onto YM agar plates using glass beads, incubated at 25 °C, and photographed with a Leica M125 C stereo microscope.

Assessment of predation

The predation tests for *Saccharomycopsis shennongjiaensis* sp. nov. were conducted as described by Santos et al. (2023). Approximately equal amounts of freshly grown cultures of the *Saccharomycopsis shennongjiaensis* sp. nov. HBP0845D3-3^{T} and prey (*Kluyveromyces starmeri* HBP0845D3-1, *Saccharomycopsis fibuligera* HBP0845D3-2, *Saturnispora dispora* HBP0845E3-3, *Saturnispora zaruensis* HBP0845E3-4, and *Candida nitratophila* HBP0845E4-5, which were isolated from the same sample as the tested isolate HBP0845D3-3^{T}, and *Saccharomyces cerevisiae* HBP0231D4C1, isolated from a different sample) were mixed on glucose–yeast nitrogen base (YNB) without amino acids agar plates (w/v, 0.67% YNB without amino acids, 2% glucose, 2% agar) and incubated at 25 °C. Mixtures were examined daily by microscopy for signs of predation (infection pegs) during 2 days, with predation typically observed after 18 hours.

Whole-genome sequencing, assembly, and assessing

The genomic DNA from the type material of the new species described in this study was sequenced using the DNBSEQ-T7 platform with 2 × 150 bp paired-end reads (Supplementary Table

7). Raw reads were quality-filtered, and adapters were trimmed using fastp 0.23.4 (Chen et al. 2018) with the following parameters: --cut_front --cut_front_window_size 1 --cut_front_mean_quality 20 --cut_tail --cut_tail_window_size 1 --cut_tail_mean_quality 20 --cut_right --cut_right_window_size 4 --cut_right_mean_quality 20 --qualified_quality_phred 20 --unqualified_percent_limit 40 --n_base_limit 5 --length_required 75. De novo assembly was performed using SPAdes 3.15.5 (Prjibelski et al. 2020) with the --careful parameter. The quality of genome assemblies was evaluated using QUAST 5.3.0 (Mikheenko et al. 2023) with the following parameters: --gene-finding --fungus.

All whole-genome data sequenced in this study were submitted to the NCBI BioProject database (http://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA1234233.

ANI analysis

The average nucleotide identity (ANI) was used as a useful criterion for species delimitation in prokaryotes due to its correlation with DNA-DNA hybridization values (Goris et al. 2007, Riesco & Trujillo 2024). ANI values were calculated using pyani 0.2.12 (Pritchard et al. 2016) with the ANIb method. A 95% threshold was tested for delineating the new species described in this study following earlier reported values for pairs of sister species (Yoon et al. 2017, Lachance et al. 2020, Libkind et al. 2020, Peña et al. 2024).

Information on assembled genomes used in this study is listed in Supplementary Table 8. All ANI calculation results are presented in Supplementary Table 9.

RESULTS

Between 2023 and 2024, a total of 624 samples of wild substrates were collected from Shennongjia (Supplementary Table 1), the majority of which were bark from Fagaceae trees. The locations of the sample sites were marked on the map (Fig. 2a). The altitudes of the samples primarily ranged from 1700 m to 2300 m (Fig. 2b), falling within the Deciduous Broadleaf Forest zone. Using the enrichment method at 10 °C and 25 °C, all samples, except for 54, yielded at least one species, with the maximum of 10 species from a single sample (Fig. 2c).

Altogether, 4,451 isolates from Shennongjia samples were assigned to 9 classes, 20 orders, 28 families, 69 genera, and 161 species, including 147 ascomycetous species and 14 basidiomycetous species (although basidiomycetous yeasts were not the primary targets of this study), based on a BLASTn search against the NCBI nucleotide database (Supplementary Table 2). Of the 161 species, 69 were isolated from only a single sample (singletons), while 16 species were found in more than 30 samples making them the most common yeast species in the region (Supplementary Table 4).

The 10 genera with the highest number of species obtained, in descending order, were *Candida* (14), *Cyberlindnera* (9), *Pichia* (9), *Nakazawaea* (8), *Lachancea* (6), *Saccharomyces* (6), *Hanseniaspora* (5), *Kluyveromyces* (5), *Wickerhamomyces* (5), and *Kregervanrija*, *Saccharomycopsis*, and *Saturnispora* (4 each) (Fig. 3a). Excluding identical species isolated from the same samples resulted in 1,710 unique strains (Supplementary Table 4). Among these, the following 10 genera with the highest number of strains obtained, in descending order, were *Saccharomyces* (377), *Lachancea* (201), *Saturnispora* (161), *Torulaspora* (119), *Candida* (98), *Hanseniaspora* (98), *Pichia* (78), *Kluyveromyces* (77), *Huiozyma* (75), and *Zygotorulaspora* (36) (Fig. 3b). Interestingly, six *Saccharomyces* species have been found, *Saccharomyces cerevisiae*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces kudriavzevii*, *Saccharomyces arboricola*, and *Saccharomyces eubayanus*, indicating notable diversity of this genus in the region Shennongjia (Fig. 3a, Supplementary Table 4).

Based on phylogenetic analyses of D1/D2 and ITS sequences, along with phenotypic and morphological characterization, 21 new species were formally described and illustrated in this study. These species belong to the genera *Ambrosiozyma*, *Cyberlindnera*, *Hyphopichia*, *Kluyveromyces*, *Kregervanrija*, *Middelhovenomyces*, *Nakazawaea*, *Pichia*, *Saccharomycopsis*, *Starmera*, *Sugiyamaella*, *Suhomyces*, and *Yamadazyma*. Among them, two species, *Ambrosiozyma apicoglobosa* sp. nov. and *Nakazawaea pterocaryicorticola* sp. nov., were based on a single isolate (Supplementary Table 2). Additionally, based on their clustering in the corresponding clades with

strong bootstrap support in the phylogenetic trees, eight *Candida* species were reassigned to the genus *Cyberlindnera*, three *Candida* and one *Pichia* species to the genus *Starmera*, and 31 *Candida* species to the genus *Yamadazyma*, resulting in a total of 43 new combinations proposed.

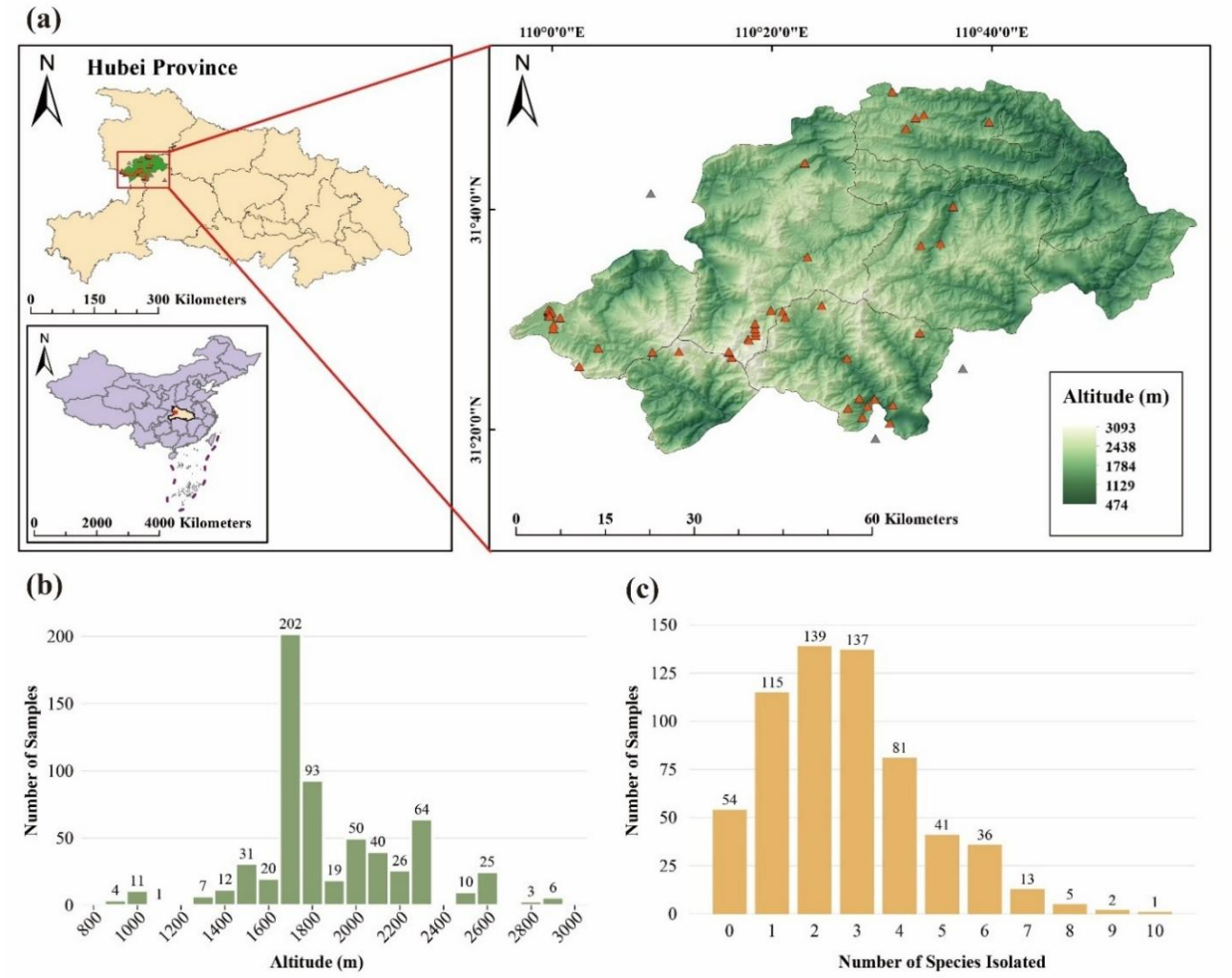

Figure 2 – (a) Map indicating the geographic locations of all sampling sites in the Shennongjia Forestry District, Hubei Province, China. (b) Altitude distribution of the samples collected in this study. (c) Distribution of the number of species isolated from the samples in this study.

Taxonomy

***Ambrosiozyma* (Pichiaceae, Pichiales, Pichiomycetes)** Van der Walt (1972)

Strain HBP0440D4C1 formed a distinct branch within the genus *Ambrosiozyma*, not clustering with any other strains (Fig. 4, Supplementary Figs. 1 and 2). It clustered with four known species—*Ambrosiozyma philentoma*, *Ambrosiozyma oregonensis*, *Ambrosiozyma platypodis*, and *Ambrosiozyma ambrosiae*—in the D1/D2, ITS, and combined D1/D2-ITS trees (Fig. 4, Supplementary Figs. 1 and 2). The strain exhibited 3 to 8 (0.6%–1.7%) nucleotide differences in the D1/D2 domains and 10 to 23 (1.9%–4.3%) nucleotide differences in the ITS region compared to other species in this group (Supplementary Table 5a). Based on whole-genome sequences, the ANI values between strain HBP0440D4C1 and four species—*Ambrosiozyma ambrosiae*, *Ambrosiozyma oregonensis*, *Ambrosiozyma philentoma*, and *Ambrosiozyma platypodis*—were calculated. The highest ANI value (88%) was observed between HBP0440D4C1 and *Ambrosiozyma platypodis* (Supplementary Table 9). These results indicate that strain HBP0440D4C1 represents a novel species within the genus *Ambrosiozyma*.

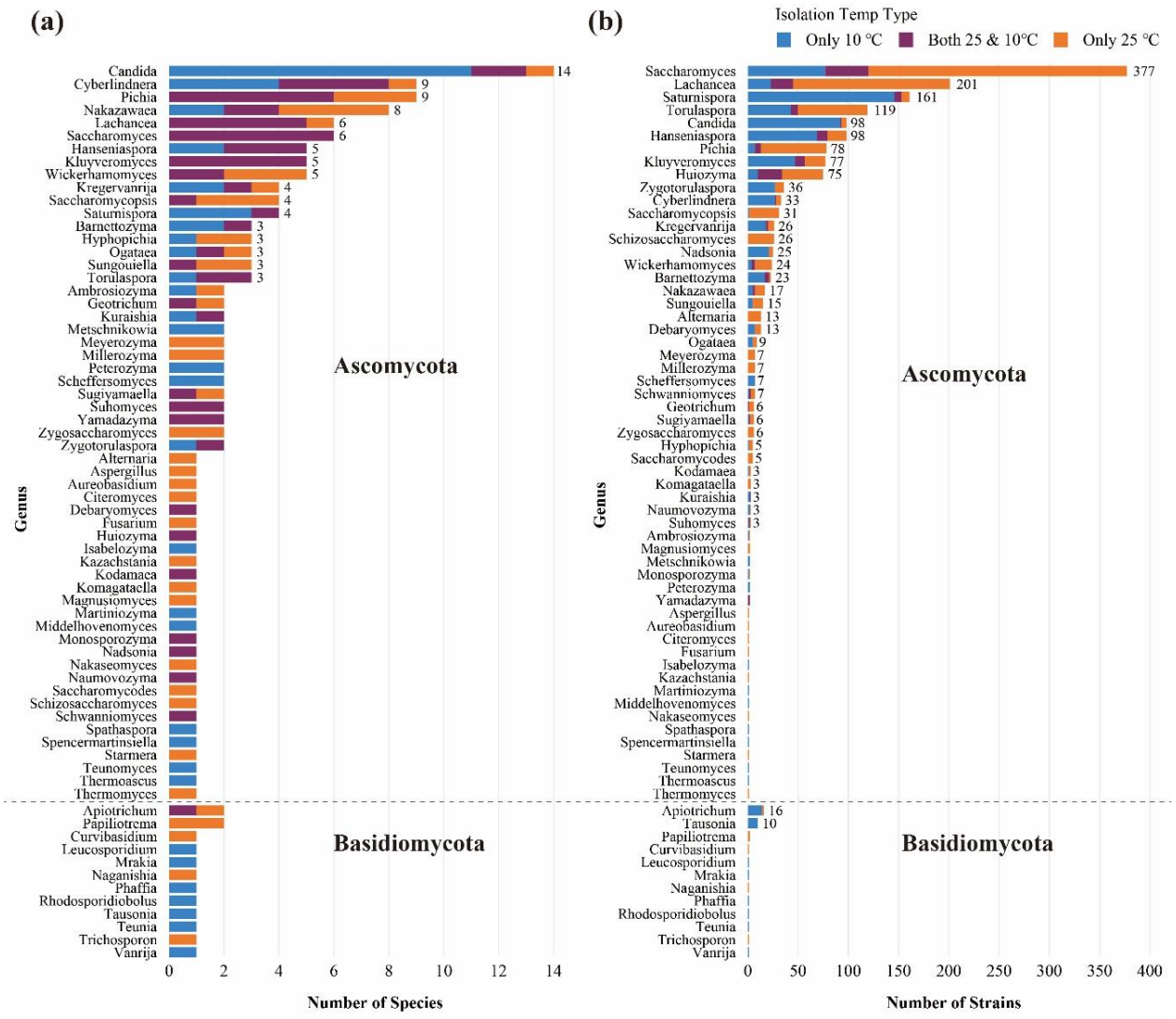

Figure 3 – Sorted bar chart showing the number of species and strains isolated from each genus in this study. (a) Number of species isolated from each genus. (b) Number of strains isolated from each genus. The colors represent the isolation temperature: orange for isolates obtained only at 25 °C, purple for isolates obtained at both 25 °C and 10 °C, and blue for isolates obtained only at 10 °C.

Ambrosiozyma apicoglobosa Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 5

MycoBank number: MB857095

Etymology – Referring to the globose-shaped cells at the apex of the hyphae.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies appear cream-colored, with a hard texture and are deeply embedded in the medium. They have a hirsute surface with fringed margins formed by hyphae or pseudohyphae (Fig. 5a). Vegetative cells are spherical to elongate, 5.3–8.5 × 4.6–7.7 µm ($\overline{x}$ = 6.6 × 5.7 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 5b–c). In YM broth, after 1 month at room temperature, a white sediment formed, and a dry, white pellicle appeared. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, abundant true hyphae developed under the coverglass (Fig. 5d–f). Asci containing four ascospores are also observed in Dalmau plate cultures on CMA (Fig. 5g), and a released hat-shaped ascospore is observed on YM agar after 2 days (Fig. 5h). On YCB agar, after 1 month at 25 °C, Pseudohyphae are formed (Fig. 5i), and four endogenous conidia are observed in true hyphae (Fig. 5j–l), while two endogenous conidia are observed in the globose-shaped cells at the apex of the hyphae (Fig. 5m–o).

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, sucrose, maltose, cellobiose, trehalose, melezitose, soluble starch (weak), D-xylose (slow), D-ribose (latent), L-

rhamnose, D-glucosamine, ethanol, glycerol, erythritol, ribitol, D-mannitol (latent), D-glucitol, methyl-α-D-glucoside, salicin, DL-lactate (weak), succinate, citrate (weak), N-acetyl-D-glucosamine and xylitol are assimilated as sole carbon sources, while galactose, L-sorbose, lactose, melibiose, raffinose, inulin, L-arabinose, D-arabinose, methanol, galactitol, D-glucuronate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate (slow), potassium nitrate, sodium nitrite, L-lysine (weak), ethylamine (slow) and cadaverine (slow) are assimilated as sole nitrogen sources. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, collected from moss on a *Quercus* tree at 2250 m, August 3, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8534^{T} (= HBP0440D4C1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37603 (=CGMCC 2.8534) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

GenBank accession number – The ITS-5.8S region and D1/D2 domains of the LSU sequence of strain HBP0440D4C1 is PQ568179.

Notes – Physiologically, the new species differentiated from its closely related species, *Ambrosiozyma philentoma* and *Ambrosiozyma ambrosiae*, by its ability to assimilate nitrate (Supplementary Table 6a). It is also distinguished from *Ambrosiozyma oregonensis* and *Ambrosiozyma platypodis* by its inability to ferment glucose (Supplementary Table 6a).

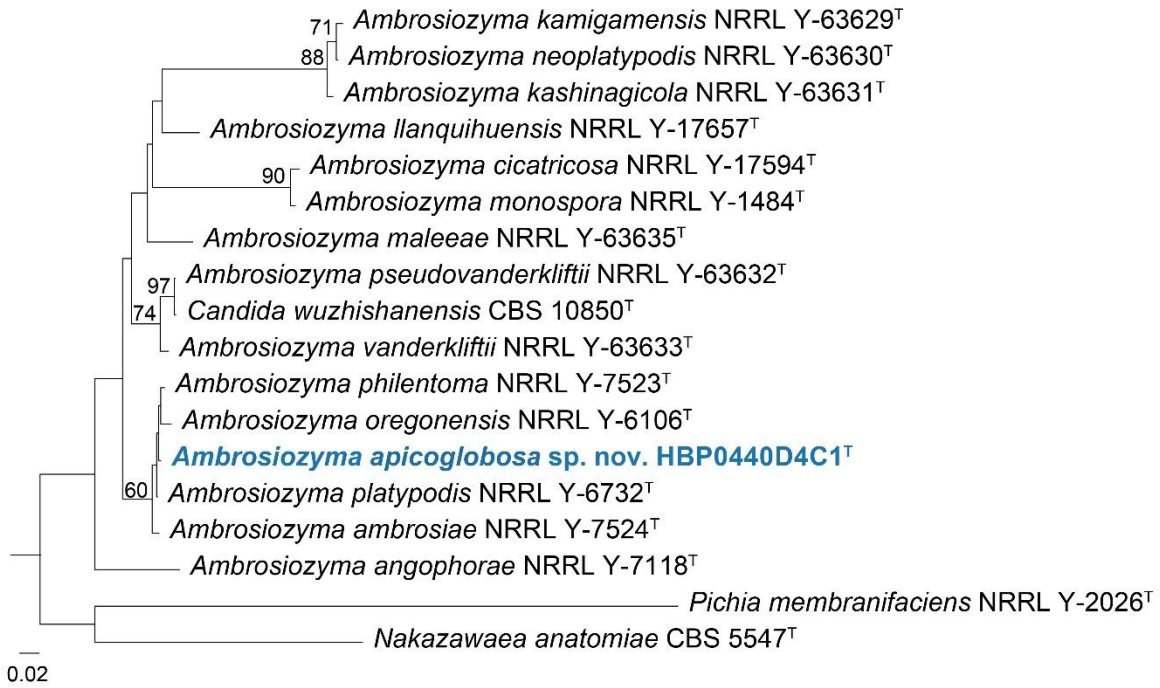

Figure 4 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Ambrosiozyma apicoglobosa* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Pichia membranifaciens* and *Nakazawaea anatomiae* is used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

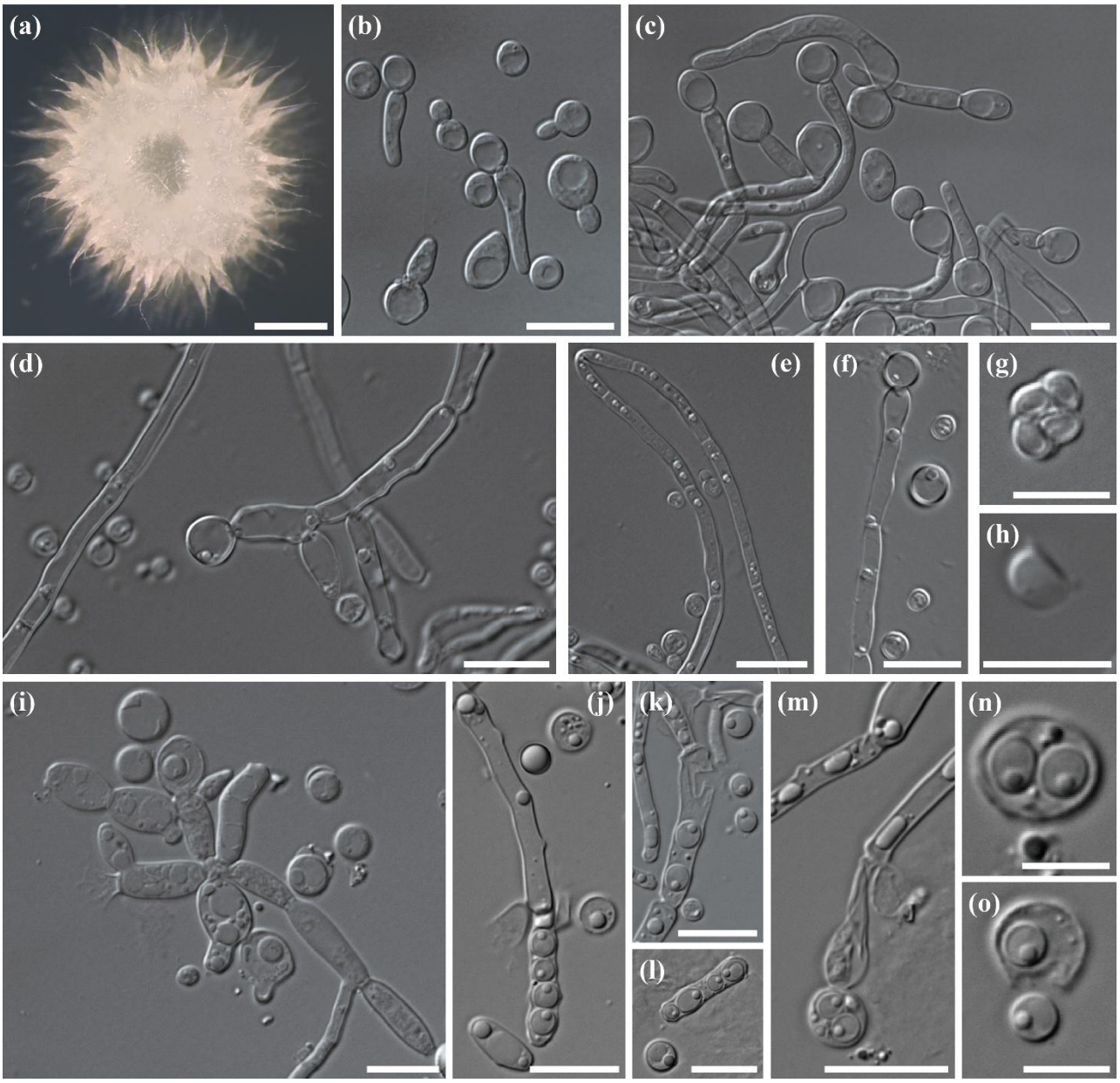

Figure 5 – Morphology of *Ambrosiozyma apicoglobosa* sp. nov. (strain HBP0440D4C1). (a) Colony on YM agar after 3 days. (b–c) Vegetative cells on YM agar after 2 days. (d–f) True hyphae growth under the coverglass on CMA after 2 weeks. (g) An ascus with four ascospores on CMA after 2 weeks. (h) A released hat-shaped ascospore on YM agar after 2 days. (i) Pseudohyphae on YCB agar after 1 month. (j–o) Endogenous conidia in true hyphae and in the cell at the apex of the hyphae on YCB agar after 1 month. All at 25 °C. Scale bars: a = 500 µm, b–f = 10 µm, g–h = 5 µm, i–m = 10 µm, n–o = 5 µm.

***Cyberlindnera* (Phaffomycetaceae, Phaffomycetales, Saccharomycetes)** Minter (2009)

Six strains from this study within the *Cyberlindnera* clade were grouped into three distinct clades (Fig. 6, Supplementary Figs. 3 and 4).

Three strains—HBP0767E3-1, HBP1019E3-1, and HBP0414E4D2—shared identical D1/D2 and ITS sequences, forming a separate branch and suggesting that they were conspecific. The ANI values between strain HBP0767E3-1 and the three closely related species *Cyberlindnera mycetangii*, *Cyberlindnera maritima*, and *Cyberlindnera sylvatica* were 78.8%, 81.3%, and 81.3% (Supplementary Table 9), respectively. These findings strongly suggest that HBP0767E3-1 represents a novel species within the genus *Cyberlindnera*.

Strain HBP0706E4-1 formed a distinct branch and did not cluster with any other strains within

the genus *Cyberlindnera*. The ANI values between strain HBP0706E4-1 and three known species—*Cyberlindnera americana*, *Cyberlindnera bimundalis*, and *Cyberlindnera nakhonratchasimensis*—ranged from 76% to 77% (Supplementary Table 9), suggesting that it represents a novel species within the genus *Cyberlindnera*.

Two strains, HBP0222F4D1 and HBP0214C4C1, clustered with two unidentified strains, 25-ESXF-17-3 and 2-17-ESXF-17-1, which were isolated from rotten wood in Hubei Province, forming a distant branch (Fig. 6, Supplementary Figs. 3 and 4). These strains shared similar D1/D2 sequences with no more than two nucleotide differences, and similar ITS sequences with no more than three nucleotide differences, suggesting that they were conspecific. The ANI value between strain HBP0214C4C1 and strain HBP0706E4-1 was 74.7% (Supplementary Table 9), indicating that HBP0214C4C1 represents another novel species.

Cyberlindnera hubeiensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 7

MycoBank number: MB857099

Etymology – Referring to the place where the species was isolated, Hubei Province.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, glistening, smooth, raised and dome-like, with a hyphal margin (Fig. 7a). After 7 days, the hyphal margin has expanded (Fig. 7b). Vegetative cells are ovoid to ellipsoidal, 3.1–5.5 × 2.3–4.0 μm ($\overline{x}$ = 4.3 × 3.0 μm, n = 20), reproduce by multilateral budding and through formation of blastoconidia, and occur singly or in pairs (Fig. 7c–d). In YM broth, after 1 month at room temperature, a white sediment formed, and a white ring, sometimes with islets, is present. In Dalmau plate culture on CMA, after 1 weeks at 25 °C, pseudohyphae are developed under the coverglass (Fig. 7e–f). Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose is positive, while galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, sucrose, maltose, cellobiose, trehalose (latent), raffinose (weak), melezitose, soluble starch (weak), D-xylose, L-arabinose (weak), D-arabinose (weak), L-rhamnose (slow), ethanol, glycerol, D-mannitol, D-glucitol (positive/slow), methyl-α-D-glucoside (slow), salicin, DL-lactate (slow), succinate, citrate and xylitol are assimilated as sole carbon sources, while galactose, L-sorbose, lactose, melibiose, inulin, D-ribose, D-glucosamine, methanol, erythritol, ribitol, galactitol, D-glucuronate, myo-inositol, hexadecane and N-acetyl-D-glucosamine are not assimilated. Ammonium sulfate, L-lysine, ethylamine (positive/slow) and cadaverine are assimilated as sole nitrogen sources, while potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive (slow). Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C and 37 °C is positive, while growth at 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Tanjiawan, 31.4575°N, 110.0704°E, collected from bark of a tree at 2347.8 m, July 11, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8539^{T} (= HBP0214C4C1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37608 (= CGMCC 2.8539) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratype – *ibid.* Wujiaya, 31.3183°N, 110.4659°E, collected from bark of a *Castanea mollissima* tree at 2050 m, July 11, 2023, HBP0222F4D1 (= CGMCC 2.8540 = JCM 37609).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences of strains HBP0214C4C1 and HBP0222F4D1 are PQ568156 and PQ568157, respectively.

Notes – Physiologically, the new species differs from its closely related species, *Cyberlindnera juglandicorticola* sp. nov., by its ability to grow at 37 °C and assimilate citrate. The four strains representing *Cyberlindnera hubeiensis* sp. nov. were isolated from rotten wood and bark in Hubei Province by different researchers, suggesting a narrow distribution of the species in China.

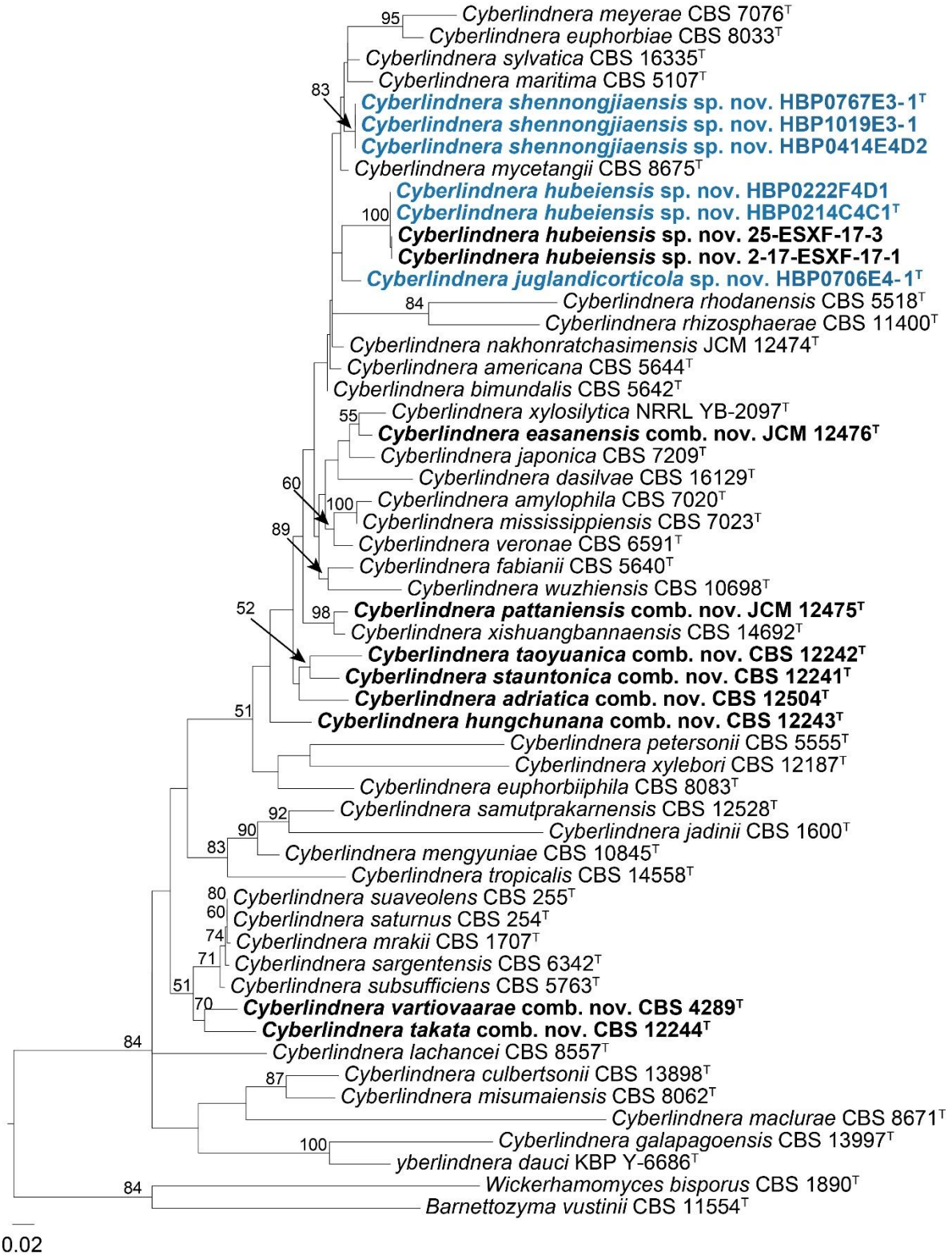

Figure 6 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Cyberlindnera hubeiensis* sp. nov., *Cyberlindnera juglandicorticola* sp. nov., and *Cyberlindnera shennongjiaensis* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Wickerhamomyces bisporus* and *Barnettozyma vustinii* are used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

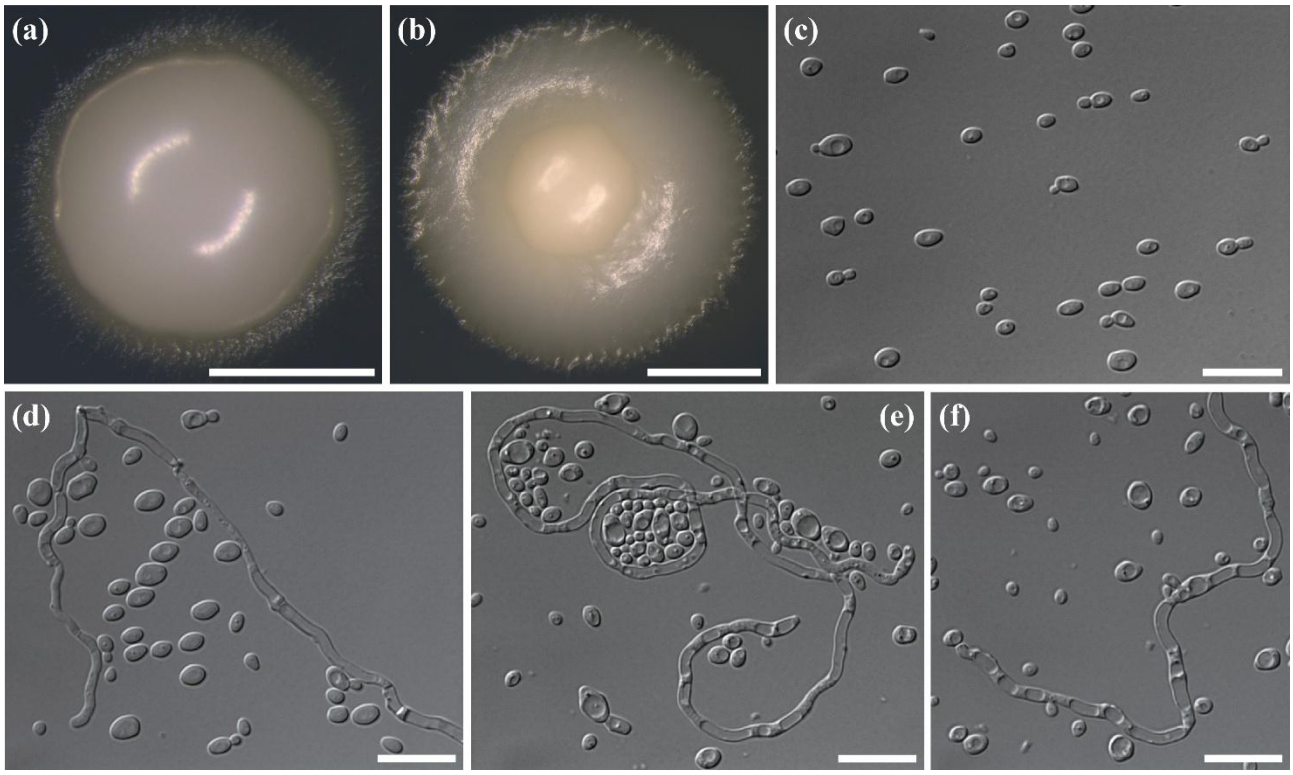

Figure 7 – Morphology of *Cyberlindnera hubeiensis* sp. nov. (strain HBP0214C4C1). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c–d) Vegetative cells on YM agar after 2 days. (e–f) Pseudohyphae growth under the coverglass on CMA after 1 weeks. All at 25 °C. Scale bars: a = 500 µm, b = 1 mm, c–f = 10 µm.

Cyberlindnera juglandicorticola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 8

MycoBank number: MB857100

Etymology – Referring to the species isolated from the bark of *Juglans*.

Culture characteristics – On YM agar, after 7 days at 25 °C, the colonies are butyrous, cream-colored to tannish-white, dull, with a smooth to slightly rough surface, broadly conical, and with an entire margin (Fig. 8a). Vegetative cells are oval to ellipsoidal, 3.8–7.1 × 3.0–6.3 µm ($\overline{x}$ = 5.3 × 4.2 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 8b). In YM broth, after 1 month at room temperature, a white sediment formed. On YCB agar, after 1 month at 25 °C, pseudohyphae developed (Fig. 8c), and released hat-shaped ascospores are observed (Fig. 8d–f).

Physiological and biochemical characteristics – In sugar fermentation tests, glucose is positive, while galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, sucrose, maltose, cellobiose, trehalose (weak), melezitose, soluble starch (weak), D-xylose, L-rhamnose, ethanol, glycerol, D-mannitol, D-glucitol, methyl-α-D-glucoside (weak), salicin, DL-lactate (weak), succinate (weak) and xylitol (weak) are assimilated as sole carbon sources, while galactose, L-sorbose, lactose, melibiose, raffinose, inulin, L-arabinose, D-arabinose, D-ribose, D-glucosamine, methanol, erythritol, ribitol, galactitol, D-glucuronate, citrate, myo-inositol, hexadecane and N-acetyl-D-glucosamine are not assimilated. Ammonium sulfate, potassium nitrate, L-lysine, ethylamine (latent) and cadaverine (slow) are assimilated as sole nitrogen sources, while sodium nitrite is not assimilated. Growth in vitamin-free medium is positive (weak). Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Duidongping, 31.6126°N, 110.5588°E, collected from bark of a *Juglans* tree at 1651.9 m, April 11, 2024, by Y.J. Qiu, Q.Y.

Zhu & F.Y. Bai; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8538^T (= HBP0706E4-1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37607 (= CGMCC 2.8538) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

GenBank accession number – The ITS-5.8S region and D1/D2 domains of the LSU sequence of strain HBP0706E4-1 is PQ568187.

Notes – Physiologically, the new species differs from its closely related species, *Cyberlindnera hubeiensis* sp. nov., by its inability to grow at 37 °C and assimilate citrate.

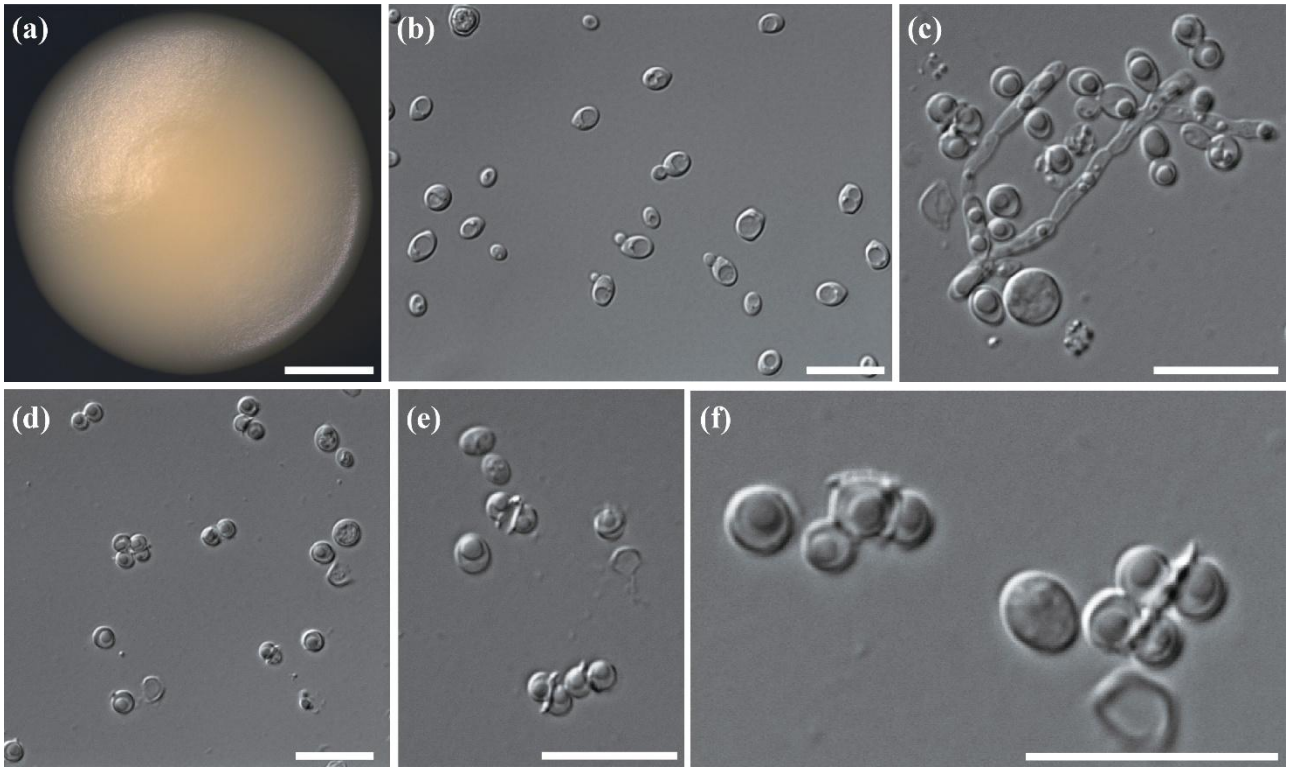

Figure 8 – Morphology of *Cyberlindnera juglandicorticola* sp. nov. (strain HBP0706E4-1). (a) Colony on YM agar after 7 days. (b) Vegetative cells on YM agar after 2 days. (c) Pseudohyphae on YCB agar after 1 month. (d-f) Released hat-shaped ascospores on YCB agar after 1 month. All at 25 °C. Scale bars: a = 1 mm, b–f = 10 µm.

Cyberlindnera shennongjiaensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 9

MycoBank number: MB857102

Etymology – Referring to the place where the species was isolated, Shennongjia.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, cream-colored, glistening, smooth, flat, with an entire margin (Fig. 9a). After 7 days, the colonies become semi-glistening, broadly conical, with a slight development of a hyphal margin (Fig. 9b). Vegetative cells are oval to ellipsoidal, 4.0–7.1 × 2.5–5.2 µm ($\overline{x}$ = 5.7 × 3.5 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 9c). In YM broth, after 1 month at room temperature, a white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, pseudohyphae formed under the coverglass (Fig. 9d). On CMA, after 1 month at 25 °C, asci and released hat-shaped ascospores are observed (Fig. 9e–h).

Physiological and biochemical characteristics – In sugar fermentation tests, glucose is positive, while galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented, except that strain HBP1019E3-1 is weakly positive for galactose. Glucose, sucrose, maltose (positive/slow), cellobiose (positive/slow), trehalose (positive/slow), melezitose, soluble starch (weak), D-xylose (positive/slow), L-arabinose (slow/weak), D-arabinose (weak/negative), L-rhamnose (positive/slow), D-glucosamine (weak/negative), ethanol, glycerol (positive/slow), D-mannitol (positive/slow), D-

glucitol (positive/slow), methyl-α-D-glucoside (weak/negative), salicin, DL-lactate (positive/weak), succinate (positive/slow), citrate (slow/weak), xylitol (positive/slow) are assimilated as sole carbon sources, while galactose, L-sorbose, lactose, melibiose, raffinose, inulin, D-ribose, methanol, erythritol, ribitol, galactitol, D-glucuronate, myo-inositol, hexadecane and N-acetyl-D-glucosamine are not assimilated. Ammonium sulfate, potassium nitrate (slow/weak), L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources, while sodium nitrite is not assimilated. Growth in vitamin-free medium is positive (weak). Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Shennong Tianchi, 31.8102°N, 110.5636°E, collected from pieces of wood/bark from a tree at 1710 m, April 11, 2024, by Z. Wen & P.J. Han; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8543^{T} (= HBP0767E3-1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37612 (= CGMCC 2.8543) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratypes – *ibid.* Sanchagou, 31.8457°N, 110.5165°E, collected from humus at 1970 m, August 2, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0414E4D2 (= CGMCC 2.8542 = JCM 37611); *ibid.* Renhehu (Lake No. 7 of Dajiuhu Lake), 31.5088°N, 110.9960°E, collected from bark of a *Malus hupehensis* tree at 1721 m, by Y.J. Qiu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP1019E3-1 (= CGMCC 2.8597); *ibid.* Gongjiaping, 31.4264°N, 110.6229°E, collected from bark of a *Quercus spinosa* tree at 1850 m, July 11, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0249E3D1 (= CGMCC 2.8544 = JCM 37613).

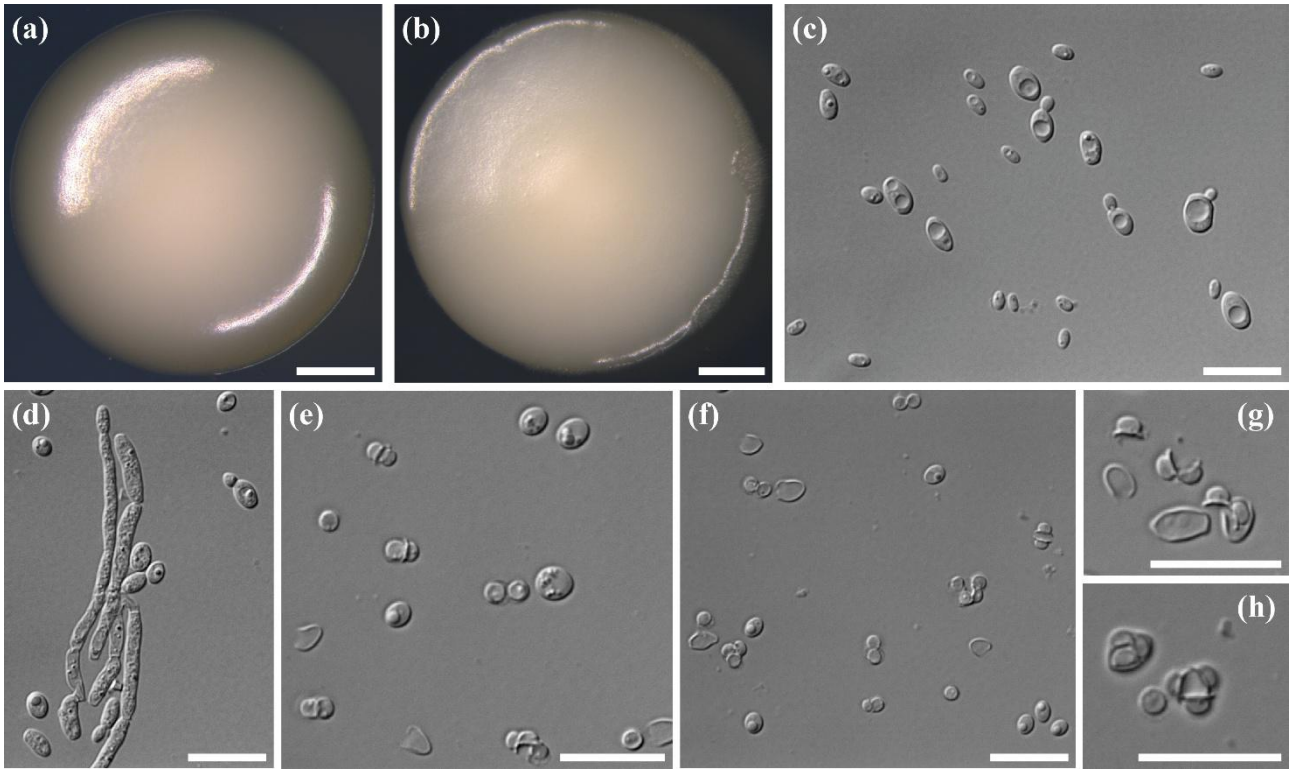

Figure 9 – Morphology of *Cyberlindnera shennongjiaensis* sp. nov. (strain HBP0767E3-1). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. (d) Pseudohyphae growth under the coverglass on CMA after 2 weeks. (e-h) Asci and released hat-shaped ascospores on CMA after 1 month. All at 25 °C. Scale bars: a = 500 μm, b = 1 mm, c–h = 10 μm.

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequence are as follows: HBP0767E3-1 (PQ568200), HBP0414E4D2 (PQ568175), HBP1019E3-1 (PQ568217) and HBP0249E3D1 (PQ568163).

Notes – Physiologically, the new species differs from its closely related species, *Cyberlindnera maritima*, in its ability to slowly or weakly assimilate nitrate.

New combinations

Cyberlindnera adriatica (Čadež, Cardinali, Ciafardini & G. Péter) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857124

Holotype: CBS 12504 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida adriatica* Čadež, Cardinali, Ciafardini & G. Péter, Int. J. Syst. Evol. Microbiol. 62(9): 2299 (2012)

Cyberlindnera easanensis (Jindam., Thuy & Nakase) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857125

Holotype: JCM 12476 preserved in a metabolically inactive state in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Basionym – *Candida easanensis* Jindam., Thuy & Nakase, J. gen. appl. Microbiol., Tokyo 50(5): 263 (2004)

Cyberlindnera hungchunana (C.F. Lee) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857126

Holotype: CBS 12243 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida hungchunana* C.F. Lee, Antonie van Leeuwenhoek 102(1): 17 (2012)

Cyberlindnera pattaniensis (Jindam., Duy & Nakase) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857127

Holotype: JCM 12475 preserved in a metabolically inactive state in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Basionym – *Candida pattaniensis* Jindam., Duy & Nakase, J. gen. appl. Microbiol., Tokyo 50(5): 265 (2004)

Cyberlindnera stauntonica (C.F. Lee) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857128

Holotype: CBS 12241 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida stauntonica* C.F. Lee, Antonie van Leeuwenhoek 102(1): 19 (2012)

Cyberlindnera takata (C.F. Lee) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857129

Holotype: CBS 12244 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida takata* C.F. Lee, Antonie van Leeuwenhoek 102(1): 14 (2012)

Cyberlindnera taoyuanica (C.F. Lee) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857130

Holotype: CBS 12242 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida taoyuanica* C.F. Lee, Antonie van Leeuwenhoek 102(1): 16 (2012)

Cyberlindnera vartiovaarae (Capr.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857131

Holotype: CBS 4289 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionyms – *Torulopsis vartiovaarae* Capr., Can. J. Microbiol. 7: 683 (1961)

≡ *Candida vartiovaarae* (Capr.) Uden & H.R. Buckley, Yeasts, a taxonomic study, 2nd Edn (Amsterdam): 1068 (1970)

≡ *Candida vartiovaarae* (Capr.) Uden & H.R. Buckley, Mycotaxon 17: 298 (1983)

Hyphopichia **(Debaryomycetaceae, Serinales, Pichiomycetes)** Arx & Van der Walt (1976)

Two strains, HBP0412E4D1 and HBP0412E4D2, formed a distinct branch and did not cluster with any other strains in the genus *Hyphopichia* (Fig. 10, Supplementary Figs. 5 and 6). They shared identical D1/D2 sequences and similar ITS sequences, differing by only one nucleotide substitution, indicating that they were conspecific. The group represented by HBP0412E4D1 was distinct from its closest relative, *Hyphopichia pseudoburtonii*, with 27 nucleotide mismatches (6.4%, 18 substitutions, and 9 gaps over 424 nucleotides) in the D1/D2 regions and 8 nucleotide differences (3.4%, 6 substitutions, and 2 gaps over 232 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI value between strain HBP0412E4D1 and *Hyphopichia pseudoburtonii* was 78%, while it was 74% compared to *Hyphopichia wangnamkhiaoensis* (Supplementary Table 9). These results suggest that HBP0412E4D1 represents a novel species within the genus *Hyphopichia*.

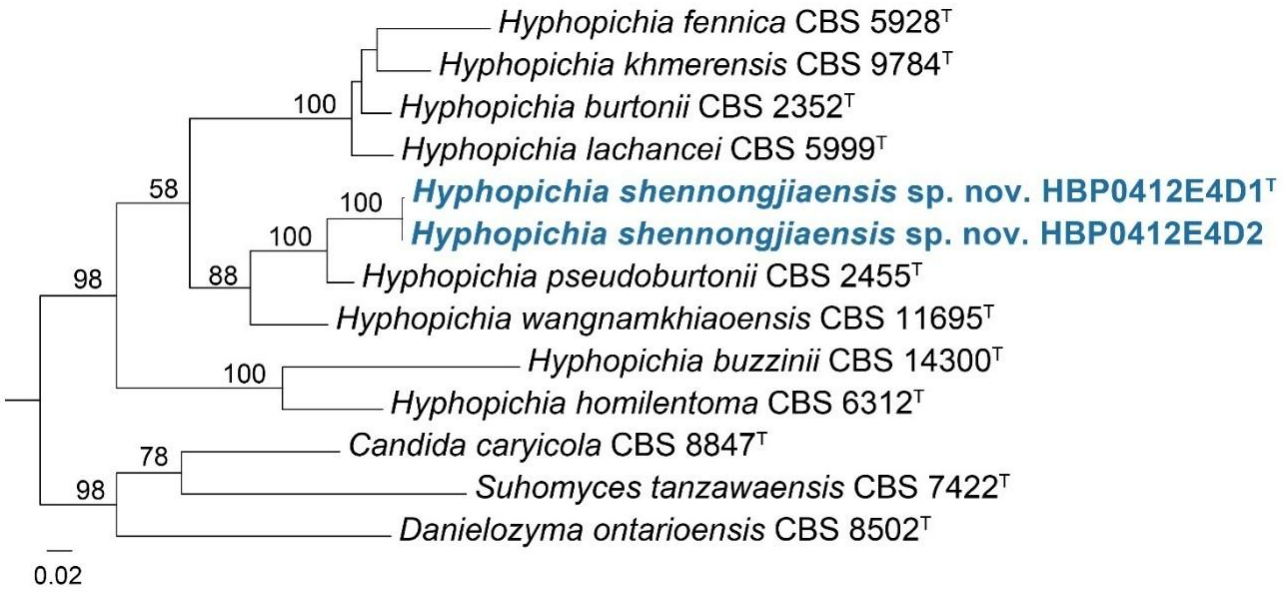

Figure 10 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Hyphopichia shennongjiaensis* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Suhomyces tanzawaensis*, *Schizosaccharomyces pombe*, and *Candida caryicola* are used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

Hyphopichia shennongjiaensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 11

MycoBank number: MB857103

Etymology – Referring to the place where the species was isolated, Shennongjia.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, dull, with a smooth to slightly rough surface, dome-like and raised, and spread an extremely extensive and abundant hyphal margin (Fig. 11a–b). Vegetative cells are oval to ellipsoidal, 3.8–7.8 × 2.6–5.6 µm ($\overline{x}$ = 6.4 × 4.0 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs, occasionally in trios (Fig. 11c). In YM broth, after 1 month at room temperature, a white sediment formed, and a very thin white, powdery pellicle floated on the surface. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, abundant true hyphae developed under the coverglass (Fig. 11d). Notably, numerous denticulate blastoconidiogenous cells are prominently observed on the hyphae (Fig. 11e–j). Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

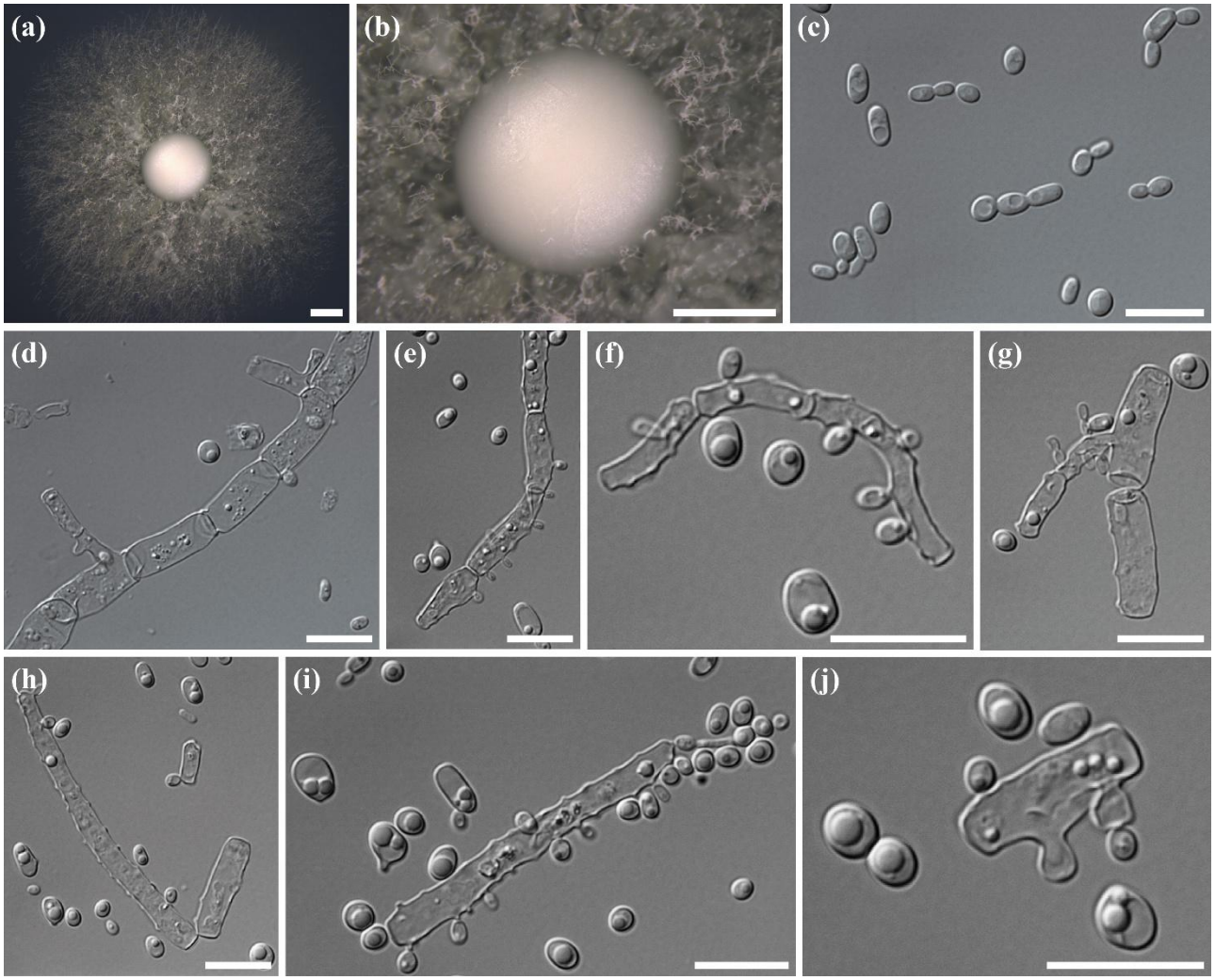

Figure 11 – Morphology of *Hyphopichia shennongjiaensis* sp. nov. (strain HBP0412E4D1). (a–b) Colony on YM agar after 3 days. (c) Vegetative cells on YM agar after 2 days. (d) True hyphae growth under the coverglass on CMA after 2 weeks. (e–j) Numerous denticulate blastoconidiogenous cells on hyphae, YM agar, 7 days. All at 25 °C. Scale bars: a–b = 1 mm, c–j = 10 µm.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, sucrose (latent), maltose and trehalose (slow) are positive, while galactose, lactose and raffinose are not fermented. Glucose, galactose, sucrose, maltose, cellobiose, trehalose, melezitose, soluble starch, D-arabinose (weak), D-ribose (slow), D-glucosamine (weak), ethanol, glycerol, erythritol, ribitol, D-

mannitol, D-glucitol, methyl-α-D-glucoside, salicin, succinate, citrate, N-acetyl-D-glucosamine and xylitol are assimilated as sole carbon sources, while L-sorbose, lactose, melibiose, raffinose, inulin, D-xylose, L-arabinose, L-rhamnose, methanol, galactitol, D-glucuronate, DL-lactate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources, while potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is positive (weak). Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is positive. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Sanchagou, 31.8457°N, 110.5165°E, collected from pieces of wood and moss from a *Quercus* tree at 1970 m, August 2, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8545^{T} (= HBP0412E4D1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37614 (= CGMCC 2.8545) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratype – *ibid.* HBP0412E4D2 (= CGMCC 2.8573).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences are PQ568172 for strain HBP0412E4D1 and PQ568173 for strain HBP0412E4D2.

Notes – Physiologically, the new species differs from its closely related species, *Hyphopichia pseudoburtonii*, in its ability to ferment maltose.

Kluyveromyces **(Saccharomycetaceae, Saccharomycetales, Saccharomycetes)** Van der Walt (1956)

Five strains—HBP0519F4D1, HBP0252F4D1, HBSP735-3, HBP0234F4D1, HBP0252E4D4, and HBP0582F4D1—formed a distinct branch and did not cluster with any other strains in the genus *Kluyveromyces* (Fig. 12, Supplementary Figs. 7 and 8). The group represented by HBSP735-3 shared similar D1/D2 sequences with only one nucleotide gap, and ITS sequences with two nucleotide gaps, indicating that they were conspecific. It differed from its closest relative, *Kluyveromyces dobzhanskii*, by six nucleotide mismatches (1.1%, 6 substitutions over 523 nucleotides) in the D1/D2 domains and 11 nucleotide differences (1.8%, 10 substitutions and 1 gap over 598 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI value between strain HBSP735-3 and *Kluyveromyces dobzhanskii* was 86%, while it was 77% compared to *Kluyveromyces lactis* and 74% to *Kluyveromyces marxianus* (Supplementary Table 9). These results suggest that HBSP735-3 represents a novel species within the genus *Kluyveromyces*.

Kluyveromyces shennongjiaensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 13

MycoBank number: MB857104

Etymology – Referring to the place where the species was isolated, Shennongjia.

Culture characteristics – On YM agar, after 7 days at 25 °C, the colonies are butyrous, tannish-white with a slight pink, due to pulcherrimin production, which leaves a diffusible dark red mark into the medium. The colonies have a slightly rough surface, are conically raised, and have an entire margin (Fig. 13a). Vegetative cells are oval to ellipsoidal, 4.5–6.3 × 2.9–5.0 μm ($\overline{x}$ = 5.5 × 3.8 μm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 13b). In YM broth, after 1 month at room temperature, a white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not observed. Asci are formed through conjugation between haploid cells or between bud and parent cells, or arise directly from diploid cells. One to four globose ascospores are formed per ascus (Fig. 13c–h).

Physiological and biochemical characteristics – In sugar fermentation tests, glucose (positive/weak), galactose, sucrose, maltose, and raffinose (weak/negative) are positive, while lactose and trehalose are not fermented. Glucose, galactose, L-sorbose (weak/negative), sucrose, maltose, cellobiose (weak/negative), trehalose (positive/latent), raffinose, melezitose (positive/latent), inulin

(weak), soluble starch (weak/negative), D-xylose (weak/negative), ethanol, glycerol (slow/negative), D-mannitol (weak/negative), D-glucitol (positive/slow), methyl-α-D-glucoside, salicin (positive/slow), DL-lactate, succinate (positive/slow), citrate (weak/negative) and xylitol (weak/negative) are assimilated as sole carbon sources, while lactose, melibiose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, erythritol, ribitol, galactitol, D-glucuronate, myo-inositol, hexadecane and N-acetyl-D-glucosamine are not assimilated. Ammonium sulfate, potassium nitrate (weak/negative), L-lysine, ethylamine (positive/slow) and cadaverine are assimilated as sole nitrogen sources, while sodium nitrite is not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C is weak or negative, and no growth is observed at 40 °C.

Typus – China, Hubei Province, Shennongjia Forestry District, collected from bark of a *Ulmus pumila* tree, August 2023, by Q.Y. Zhu; isolated by S. Hu. Holotype: CGMCC 2.7485^{T} (= HBSP735-3) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 36900 (= CGMCC 2.7485) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratypes – *ibid.* Niujiaowan, 31.7375°N, 110.3833°E, collected from pieces of wood from a *Quercus* tree at 1870 m, July 11, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0234F4D1 (= CGMCC 2.8614); *ibid.* Gongjiaping, 31.4264°N, 110.6229°E, collected from *Umbilicaria* sp. at 1850 m, July 11, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0252E4D4 (= CGMCC 2.8570), (HBP0252F4D1 = CGMCC 2.8571); *ibid* Wenshui River, 31.5956°N, 110.3872°E, collected from bark of a *Quercus* tree at 1750 m, July 17, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0519F4D1 (= CGMCC 2.8615); *ibid.* Huaxuechang, 31.4418°N, 110.4472°E, collected from bark of a *Quercus* tree at 2350 m, July 19, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0582F4D1 (= CGMCC 2.8606).

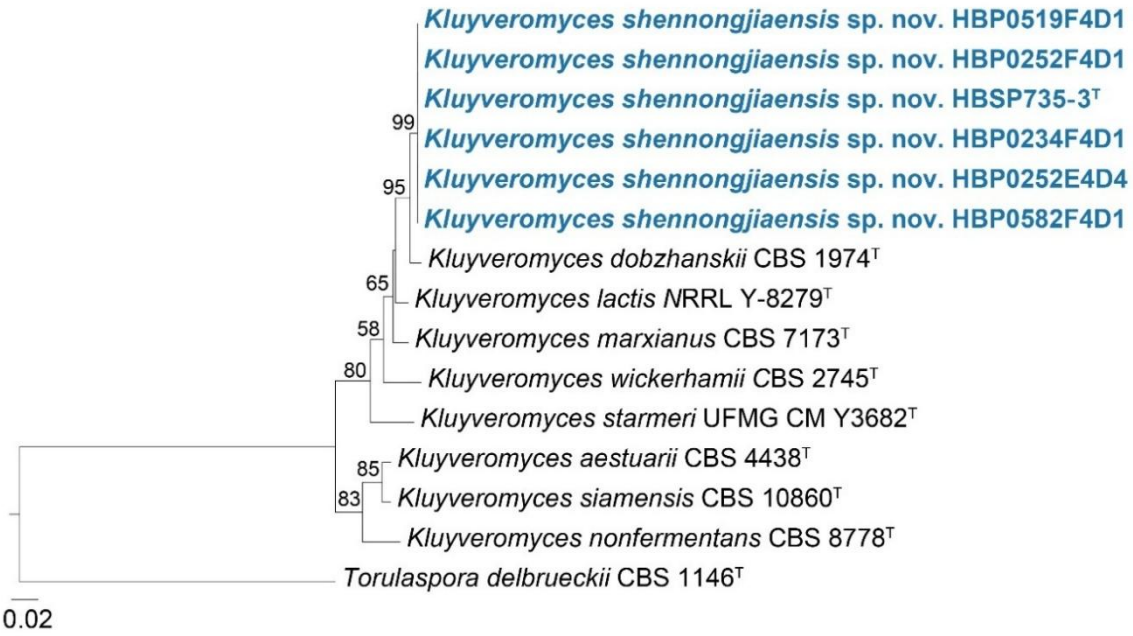

Figure 12 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Kluyveromyces shennongjiaensis* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Torulaspora delbrueckii* is used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

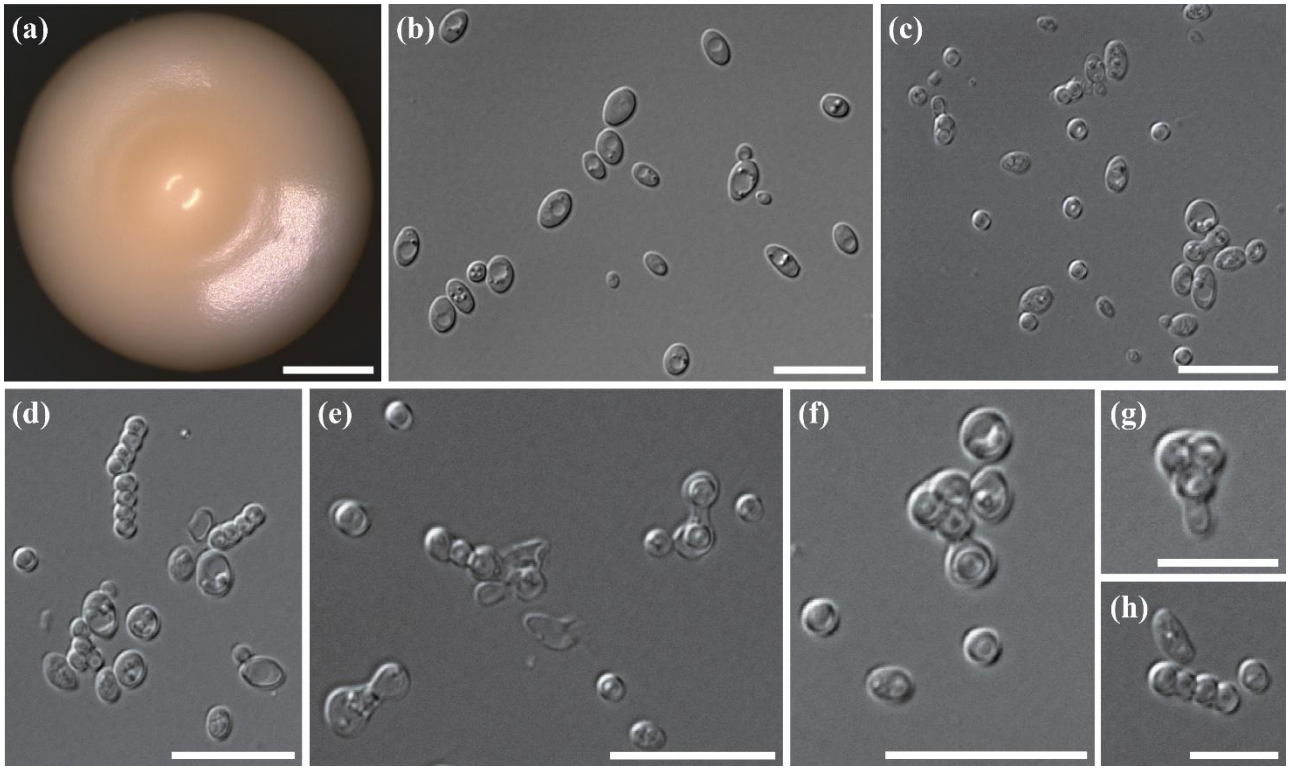

Figure 13 – Morphology of *Kluyveromyces shennongjiaensis* sp. nov. (strain HBSP735-3). (a) Colony on YM agar after 7 days. (b) Vegetative cells on YM agar after 2 days. (c–h) Asci with globose ascospores under the coverglass on CMA after 2 weeks. All at 25 °C. Scale bars: a = 1 mm, b–f = 10 µm, g–h = 5 µm.

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequence are as follows: HBSP735-3 (PQ568227), HBP0234F4D1 (PQ568160), HBP0252E4D4 (PQ568164), HBP0252F4D1 (PQ568165), HBP0519F4D1 (PQ568182) and HBP0582F4D1 (PQ568184).

Notes – Physiologically, the new species differs from its closely related species, *Kluyveromyces dobzhanskii*, in its inability to ferment raffinose and trehalose.

***Kregervanrija* (Pichiaceae, Pichiales, Pichiomycetes)** Kurtzman (2006)

Nine strains grouped together within the *Kregervanrija* clade (Fig. 14, Supplementary Figs. 9 and 10) and were separated into two distinct branches. Six strains—HBP1076D3-1, HBSY774-2, HBP1031D4-1, HBP0903D3-3, HBP0434D4C2, and HBP0347D4C3—shared similar ITS and D1/D2 sequences with both no more than three nucleotide differences, indicating that they were conspecific. The group represented by HBSY774-2 differed from its closest relative, *Kregervanrija fluxuum*, by nine nucleotide mismatches (1.5%, 9 substitutions over 572 nucleotides) in the D1/D2 domains and 26 nucleotide differences (6.7%, 16 substitutions and 10 gaps over 390 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI values between strain HBSY774-2 and three known species—*Kregervanrija delftensis*, *Kregervanrija fluxuum*, and *Kregervanrija pseudodelftensis*—ranged from 84% to 86% (Supplementary Table 9), suggesting that HBSY774-2 represents a novel species.

Three strains—HBP0248E4D1, HBP0720E3-5, and HBP0901E4-5—shared identical ITS and D1/D2 sequences, indicating that they were conspecific. Pairwise comparisons showed that HBP0720E3-5 exhibited 1.7% sequence divergence in the D1/D2 domains from the other four *Kregervanrija* species (Supplementary Table 5b). In the ITS region, HBP0720E3-5 differed from the other four *Kregervanrija* species by 17 to 35 nucleotide mismatches (4.6%–8.7%) (Supplementary Table 5b). The ANI values between strain HBP0720E3-5 and three known species—*Kregervanrija delftensis*, *Kregervanrija fluxuum*, and *Kregervanrija pseudodelftensis*—ranged from 85% to 88%,

while it was 87% in comparison to HBSY774-2 (Supplementary Table 9). These results suggest that HBP0720E3-5 represents another novel species within the genus *Kregervanrija*.

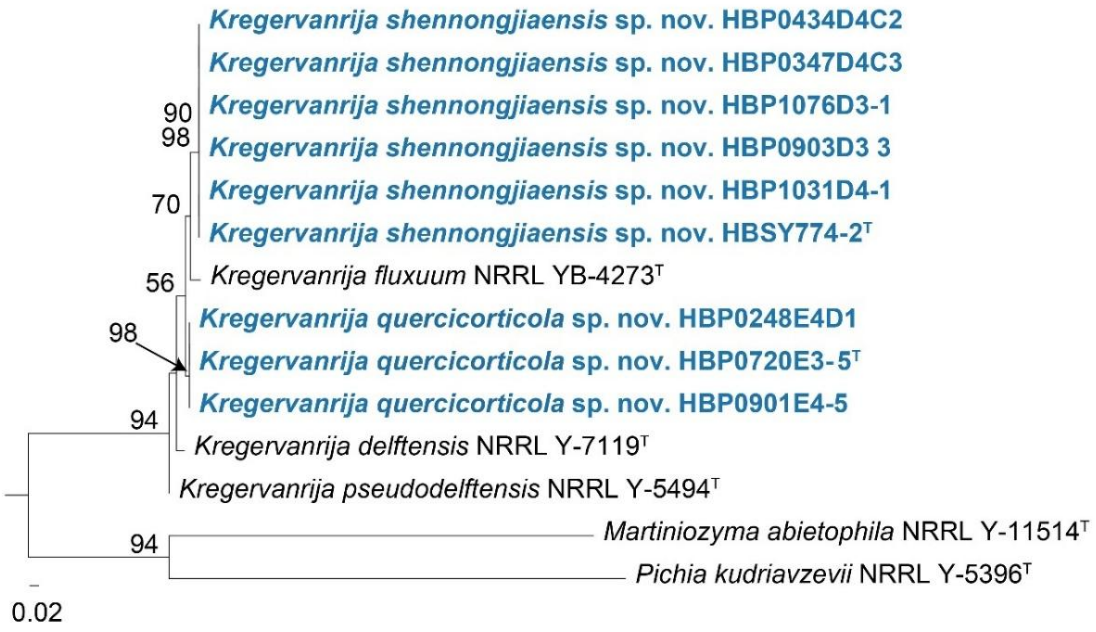

Figure 14 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Kregervanrija quercicorticola* sp. nov. and *Kregervanrija shennongjiaensis* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Martiniozyma abietophila* and *Pichia kudriavzevii* are used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

Kregervanrija quercicorticola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 15

MycoBank number: MB857105

Etymology – Referring to the species isolated from the bark of *Quercus*.

Culture characteristics – On YM agar, after 7 days at 25 °C, the colonies are butyrous, cream-colored, dull, smooth, with a broadly conical elevation, and an entire margin (Fig. 15a). Vegetative cells are ovoid to elongate, 4.4–8.0 × 2.5–3.8 µm ($\overline{x}$ = 6.1 × 3.0 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 15b). In YM broth, after 1 month at room temperature, a white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not observed. On YCB agar, after 1 month at 25 °C, asci are formed through conjugation between complementary mating types and one to two globose ascospores are formed per ascus (Fig. 15c–f).

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, L-sorbose (weak/negative), ethanol, D-mannitol (positive/slow), D-glucitol, DL-lactate (weak/negative) are assimilated as sole carbon sources, while galactose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, glycerol, erythritol, ribitol, galactitol, methyl-α-D-glucoside, salicin, D-glucuronate, succinate, citrate, myo-inositol, hexadecane, N-acetyl-D-glucosamine and xylitol are not assimilated. Ammonium sulfate, potassium nitrate (weak/negative), L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources, while sodium nitrite is not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is positive (weak). Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast

extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Duidongping, 31.6126°N, 110.5588°E, collected from bark of a *Quercu* tree at 1651.9 m, April 11, 2024, by Y.J. Qiu, Q.Y. Zhu & F.Y. Bai; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8546^{T} (= HBP0720E3-5) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37615 (= CGMCC 2.8546) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratypes – *ibid.* Gongjiaping, 31.4264°N, 110.6229°E, collected from bark of a *Quercus spinosa* tree at 1850 m, July 11, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0248E4D1 (= CGMCC 2.8569); *ibid.* around Shennong Hotel, 31.5042°N, 110.3537°E, collected from bark of a *Fagaceae* tree at 1916 m, April 12, 2024, by Y.J. Qiu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP0901E4-5 (= CGMCC 2.8547 = JCM 37616).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequence are as follows: HBP0720E3-5 (PQ568190), HBP0248E4D1 (PQ568162) and HBP0901E4-5 (PQ568213).

Notes – Physiologically, the new species differs from its closely related species, *Kregervanrija fluxuum*, in its ability to grow in a vitamin-free medium. The genus *Kregervanrija* included three species in the fifth edition of *The Yeasts, a Taxonomic Study* (Kurtzman 2011c). No new species of this genus have been reported in nearly 20 years, since Kurtzman (2006) described the third species, *Kregervanrija pseudodelftensis*.

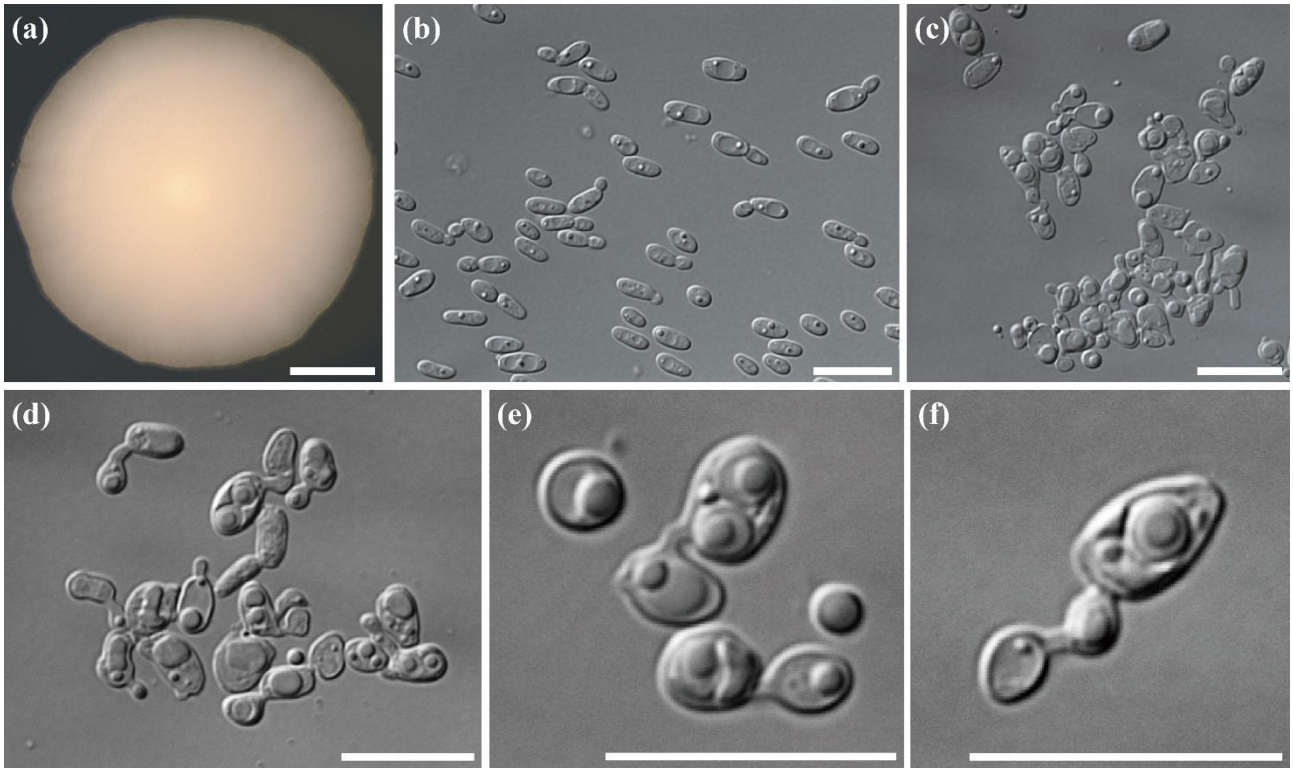

Figure 15 – Morphology of *Kregervanrija quercicorticola* sp. nov. (strain HBP0720E3-5). (a) Colony on YM agar after 7 days. (b) Vegetative cells on YM agar after 2 days. (c–f) Asci with globose ascospores formed from conjugation of complementary mating types on YCB agar after 1 month. All at 25 °C. Scale bars: a = 1 mm, b–f = 10 µm.

Kregervanrija shennongjiaensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 16

MycoBank number: MB857106

Etymology – Referring to the place where the species was isolated, Shennongjia.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, cream-colored, dull, with a central flat area. The surface is covered with numerous thick, columnar, and curved protrusions, radiating from the center of the colony (Fig. 16a). After 7 days, the margin has expanded (Fig. 16b). Vegetative cells are ellipsoidal to oblong, 5.6–11.1 × 2.4–3.5 µm ($\overline{x}$ = 7.7 × 3.0 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 16c). In YM broth, after 1 month at room temperature, a white sediment formed, and thin climbing pellicles are present. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, pseudohyphae developed under the coverglass (Fig. 16d). On YCB agar, after 1 month at 25 °C, asci are formed through conjugation between complementary mating types and one to two globose ascospores are formed per ascus (Fig. 16e–f).

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, D-glucosamine (weak/negative), ethanol, ribitol (weak/negative), D-mannitol (positive/slow), D-glucitol, succinate (weak/negative) and N-acetyl-D-glucosamine (weak/negative) are assimilated as sole carbon sources, while galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, glycerol, erythritol, galactitol, methyl-α-D-glucoside, salicin, D-glucuronate, DL-lactate, citrate, myo-inositol, hexadecane and xylitol are not assimilated. Ammonium sulfate, potassium nitrate (weak/negative), L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources, while sodium nitrite is not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

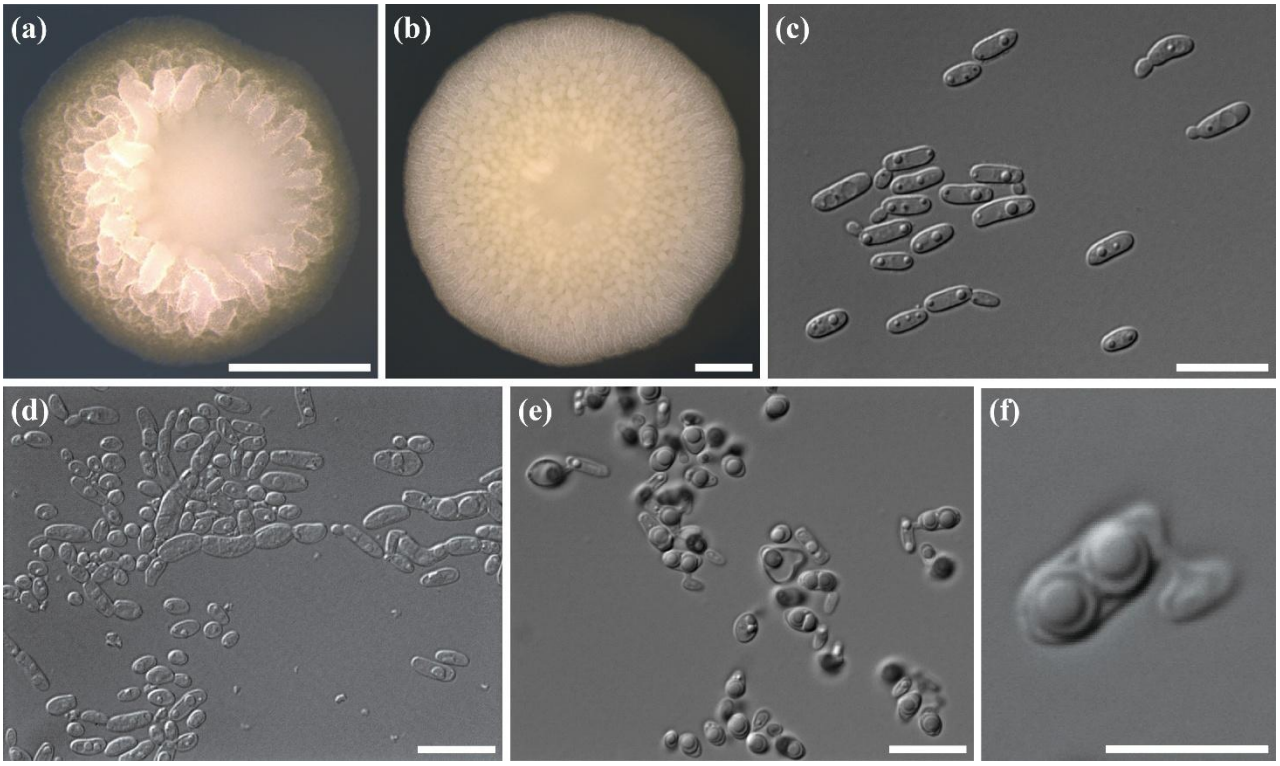

Figure 16 – Morphology of *Kregervanrija shennongjiaensis* sp. nov. (strain HBSY774-2). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. (d) Pseudohyphae growth under the coverglass on CMA after 2 weeks. (e–f) Asci with globose ascospores formed from conjugation of complementary mating types on YCB agar after 1 month. All at 25 °C. Scale bars: a–b = 1 mm, c–e = 10 µm, f = 5 µm.

Typus – China, Hubei Province, Shennongjia Forestry District, collected from bark of a *Emmenopterys henryi* tree, September 2023, by Q.Y. Zhu; isolated by S. Hu. Holotype: CGMCC 2.7453^{T} (= HBSY774-2) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37661 (= CGMCC 2.7453) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratypes – *ibid.* Longmen River Forest Farm, 31.3201°N, 110.4904°E, collected from soil under a *Quercus* tree at 1470 m, July 23, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0347D4C3 (= CGMCC 2.8617); *ibid.* collected from soil under a *Fagaceae* tree at 2250 m, August 3, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0434D4C2 (= CGMCC 2.8574); *ibid.* around Shennong Hotel, 31.5042°N, 110.3537°E, collected from bark of a *Fagaceae* tree at 1916 m, April 12, 2024, by Y.J. Qiu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP0903D3-3 (= CGMCC 2.8595); *ibid.* Viving of the Withered Tree (in Dajiuhu Lake), 31.5159°N, 109.9952°E, collected from bark of a *Fagaceae* tree at 1747 m, April 12, 2024, by Y.J. Qiu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP1031D4-1 (= CGMCC 2.8616); *ibid.* around Dajiuhu Lake, 31.5033°N, 110.0125°E, collected from pieces of wood/bark from a tree at 1827 m, by Z. Wen & P.J. Han, isolated by L.X. Yang & Y.C. Mao, HBP1076D3-1 (= CGMCC 2.8603).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequence are as follows: HBSY774-2 (PQ568228), HBP0347D4C3 (PQ568171), HBP0434D4C2 (PQ568178), HBP0903D3-3 (PQ568214), HBP1031D4-1 (PQ568221) and HBP1076D3-1 (PQ568225).

Notes – Physiologically, the new species differs from its closely related species, *Kregervanrija delftensis* and *Kregervanrija pseudodelftensis*, in its inability to grow in 10% sodium chloride plus 5% glucose medium (Supplementary Table 6b). Furthermore, it is distinguished from *Kregervanrija fluxuum* by its ability to assimilate D-glucitol (Supplementary Table 6b).

Middelhovenomyces **(Dipodascaceae, Dipodascales, Dipodascomycetes)** Kurtzman & Robnett (2014)

Strain HBP0414E4D6 formed a distinct branch within the *Middelhovenomyces* clade and did not cluster with any other strains (Fig. 17, Supplementary Figs. 11 and 12). Currently, the genus *Middelhovenomyces* comprises two known species, *Middelhovenomyces petrohuensis* and *Middelhovenomyces tepae*. Strain HBP0414E4D6 differed from *Middelhovenomyces petrohuensis* by 13 nucleotide differences (2.2%; 10 substitutions and 3 gaps over 578 nucleotides) in the D1/D2 domains and by 59 nucleotide differences (11.4%; 31 substitutions and 28 gaps over 516 nucleotides) in the ITS region. Similarly, it differed from *Middelhovenomyces tepae* by 12 nucleotide differences (2.1%; 9 substitutions and 3 gaps over 578 nucleotides) in the D1/D2 domains and by 62 differences (12.0%; 31 substitutions and 31 gaps over 515 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI value between strain HBP0414E4D6 and *Middelhovenomyces tepae* was 75% (Supplementary Table 9). These results suggest that strain HBP0414E4D6 represents a third novel species within the genus *Middelhovenomyces*.

Middelhovenomyces xyloputricola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 18

MycoBank number: MB857108

Etymology – Referring to the species isolated from decaying wood/humus.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, glistening, with a smooth to slightly rough surface, broadly conical in elevation, and have an entire margin (Fig. 18a). Vegetative cells are ovoid to ellipsoidal, 4.2–6.1 × 3.0–4.5 µm ($\overline{x}$ = 5.1 × 3.6 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 18b–c). In YM broth, after 1 month at room temperature, a white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not observed. Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, galactose (weak), L-sorbose (weak), sucrose, maltose, cellobiose (slow), trehalose (weak), soluble starch (slow), D-xylose

(slow), D-glucosamine (weak), ethanol, glycerol (slow), methyl-α-D-glucoside (slow), salicin (weak), D-glucuronate (weak), DL-lactate (slow), succinate (slow) and N-acetyl-D-glucosamine are assimilated as sole carbon sources, while lactose, melibiose, raffinose, melezitose, inulin, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, citrate, myo-inositol, hexadecane and xylitol are not assimilated. Ammonium sulfate, L-lysine (latent), ethylamine (slow) and cadaverine are assimilated as sole nitrogen sources, while potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C is weak, and no growth is observed at 40 °C.

Typus – China, Hubei Province, Shennongjia Forestry District, Sanchagou, 31.8457°N, 110.5165°E, collected from humus at 1970 m, August 2, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype CGMCC 2.8549^{T} (= HBP0414E4D6) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37618 (= CGMCC 2.8549) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

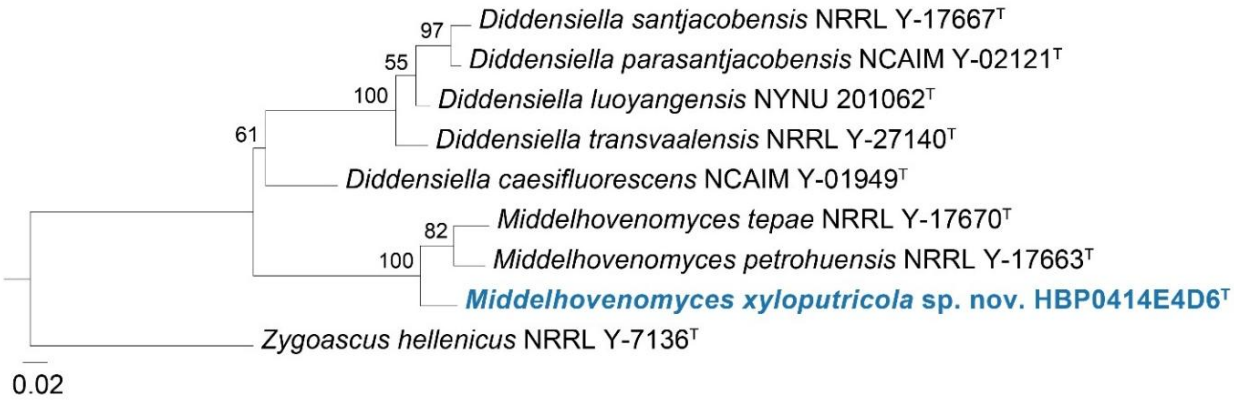

Figure 17 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Middelhovenomyces xyloputricola* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Zygoascus hellenicus* is used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

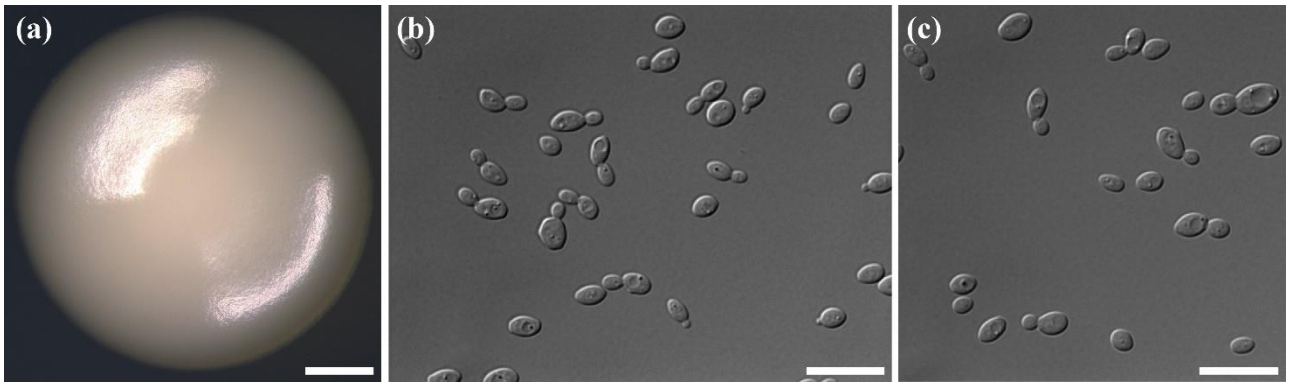

Figure 18 – Morphology of *Middelhovenomyces xyloputricola* sp. nov. (strain HBP0414E4D6). (a) Colony on YM agar after 7 days. (b–c) Vegetative cells on YM agar after 2 days. All at 25 °C. Scale bars: a = 500 µm, b–c = 10 µm.

GenBank accession number – The ITS-5.8S region and D1/D2 domains of the LSU sequence of strain HBP0414E4D6 is PQ568176.

Notes – Physiologically, the new species differs from its closely related species, *Middelhovenomyces petrohuensis* and *Middelhovenomyces tepae*, in its ability to grow at both 30 °C and 37 °C.

Nakazawaea **(Pachysolenaceae, Alaninales, Pichiomycetes)** Y. Yamada, K. Maeda & Mikata (1994)

Seven strains grouped together within the *Nakazawaea* clade and were separated into three distinct groups (Fig. 19, Supplementary Figs. 13 and 14). Five strains—HBP0257C4C3, HBP0768E3-1, HBP0257C4C4, HBP0330E4D1, and HBP0733E4-4—shared identical D1/D2 sequences and similar ITS sequences with only one nucleotide substitution, indicating that they were conspecific. Two undescribed strains, NCAIM Y.0215 and IBUN 03998, shared identical D1/D2 sequences with strain HBP0330E4D1. However, the ITS sequence of NCAIM Y.0215 was unavailable, and IBUN-03998 exhibited five nucleotide substitutions in the ITS region compared to HBP0330E4D1, indicating that both strains may belong to the same species as HBP0330E4D1. The group represented by HBP0330E4D1 was closely related to *Nakazawaea molendinolei*, though it differed by 14 nucleotide mismatches (3.0%, 9 substitutions and 5 gaps over 467 nucleotides) in the D1/D2 domains and 57 nucleotide mismatches (10.9%, 34 substitutions and 23 gaps over 525 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI values between strain HBP0330E4D1 and its closely related species, *Nakazawaea molendinolei* and *Nakazawaea peltata*, were 73% (Supplementary Table 9). The results strongly suggest that strain HBP0330E4D1 represents a novel species within the genus *Nakazawaea*.

Strain HBP0702E3-1 formed a distant branch within the *Nakazawaea* clade (Fig. 19, Supplementary Figs. 13 and 14). Its D1/D2 sequence was most closely related to *Nakazawaea pomicola*, differing by three nucleotide mismatches (0.6%, 3 substitutions over 466 nucleotides) in the D1/D2 domains and 45 nucleotide mismatches (9.1%, 28 substitutions and 17 gaps over 494 nucleotides) in the ITS region. In the ITS region, strain HBP0702E3-1 showed seven nucleotide mismatches (1.5%, 7 substitutions over 464 nucleotides) in the D1/D2 domains and 41 nucleotide mismatches (8.4%, 29 substitutions and 12 gaps over 487 nucleotides) in the ITS region compared to *Nakazawaea holstii*. The ANI values between strain HBP0702E3-1 and its closely related species, *Nakazawaea holstii*, *Nakazawaea molendinolei*, and *Nakazawaea pomicola*, were 73% (Supplementary Table 9). These results suggest that strain HBP0702E3-1 also represents another novel species within the genus *Nakazawaea*.

Strain HBP0745E3-2 formed a distinct branch at the root of the *Nakazawaea* clade in all three trees (Fig. 19, Supplementary Figs. 13 and 14). A BLAST search with both D1/D2 and ITS sequences of strain HBP0745E3-2 showed *Ambrosiozyma kamigamensis* as the top match. Strain HBP0745E3-2 differed from *Ambrosiozyma kamigamensis* by five nucleotide mismatches (5.7%, 5 substitutions over 414 nucleotides) in the D1/D2 domains and 18 nucleotide mismatches (3.8%, 10 substitutions and 8 gaps over 479 nucleotides) in the ITS region. The ANI value between strain HBP0745E3-2 and *Nakazawaea siamensis* was 72.1% (Supplementary Table 9). Therefore, strain HBP0745E3-2 is temporarily identified as a novel species within the genus *Nakazawaea* in this study.

Nakazawaea arboricola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 20

MycoBank number: MB857109

Etymology – Referring to the species isolated from the bark/wood of trees.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, unobtrusively glistening, smooth, and convex, with an entire margin (Fig. 20a). After 7 days, the colonies become cream-colored, glistening, with a flattened center (Fig. 20b). Vegetative cells are ovoid to ellipsoidal, 3.1–4.4 × 2.0–3.5 μm ($\overline{x}$ = 3.9 × 2.6 μm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 20c). In YM broth, after 1 month at room temperature, a

white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not observed. Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose is latent positive, while galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, galactose, sucrose, maltose, cellobiose (slow), trehalose, melezitose (slow), inulin (weak), soluble starch (weak), D-xylose (slow), L-arabinose (weak), D-arabinose (weak), D-ribose (slow), L-rhamnose, ethanol, glycerol, erythritol (weak), ribitol, D-mannitol, D-glucitol, methyl-α-D-glucoside, salicin, succinate, citrate, N-acetyl-D-glucosamine and xylitol are assimilated as sole carbon sources, while L-sorbose, lactose, melibiose, raffinose, D-glucosamine, methanol, galactitol, D-glucuronate, DL-lactate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, L-lysine, ethylamine (slow) and cadaverine (slow) are assimilated as sole nitrogen sources. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is positive (weak). Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C and 37 °C is positive, while growth at 40 °C is weak.

Typus – China, Hubei Province, Shennongjia Forestry District, Shennong Tianchi, 31.8062°N, 110.5513°E, collected from pieces of wood/bark from a tree at 1708 m, April 11, 2024, by Z. Wen & P.J. Han; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8535^{T} (= HBP0745E3-2) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37604 (= CGMCC 2.8535) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

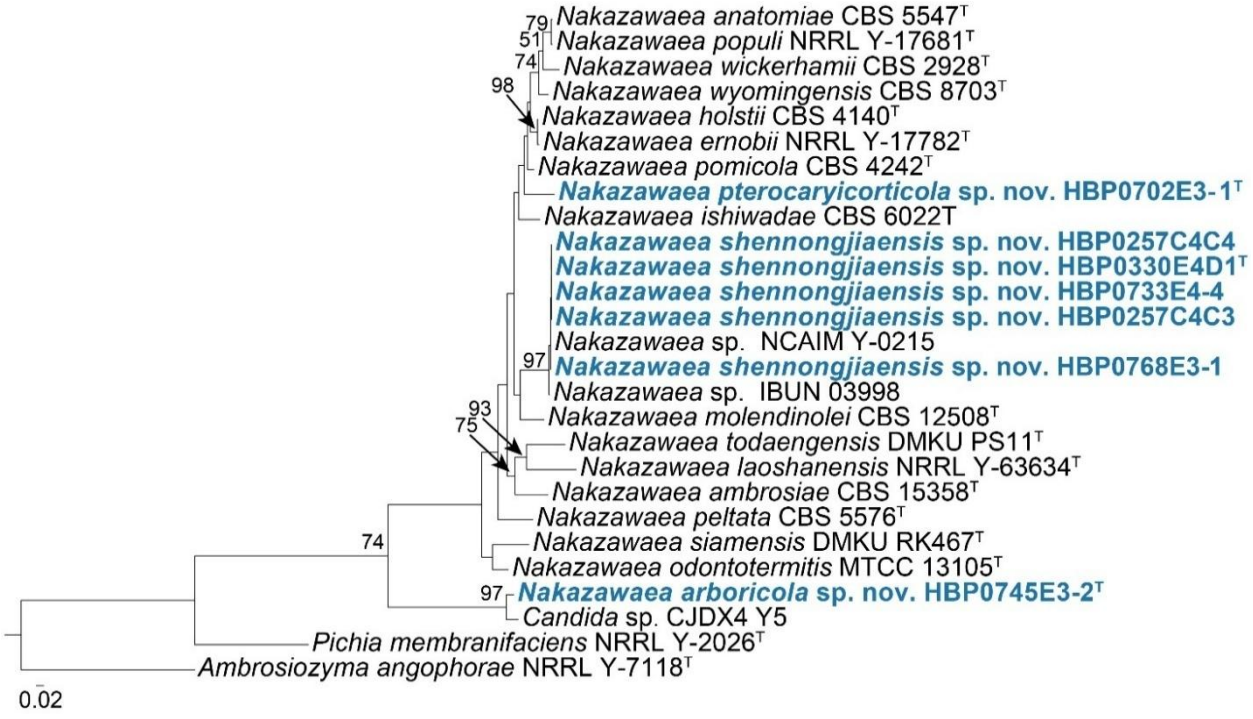

Figure 19 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Nakazawaea arboricola* sp. nov., *Nakazawaea pterocaryicorticola* sp. nov., and *Nakazawaea shennongjiaensis* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Ambrosiozyma angophorae* and *Pichia membranifaciens* are used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

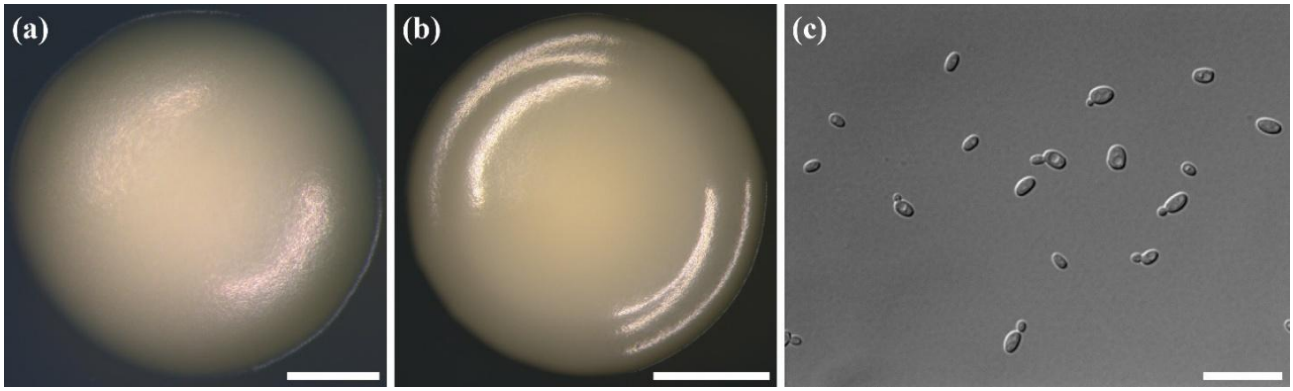

Figure 20 – Morphology of *Nakazawaea arboricola* sp. nov. (strain HBP0745E3-2). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. All at 25 °C. Scale bars: a = 250 µm, b = 1 mm, c = 10 µm.

GenBank accession number – The ITS-5.8S region and D1/D2 domains of the LSU sequence of strain HBP0745E3-2 is PQ568195.

Notes – The new species is located at the base of the *Nakazawaea* clade (Fig. 19, Supplementary Figs. 13 and 14) with strong support, indicating its placement within the *Nakazawaea* genus.

Nakazawaea pterocaryicorticola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 21

MycoBank number: MB857110

Etymology – Referring to the species isolated from the bark of *Pterocarya*.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, cream-colored, glistening, smooth, flat, with an entire margin (Fig. 21a). After 7 days, the colonies become slightly convex and unobtrusively glistening (Fig. 21b). Vegetative cells are subglobose to ellipsoidal, 2.6–5.1 × 2.1–3.5 µm ($\overline{x}$ = 3.8 × 2.9 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 21c). In YM broth, after 1 month at room temperature, a white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not observed. Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose and trehalose (latent) are positive, while galactose, sucrose, maltose, lactose and raffinose are not fermented. Glucose, galactose, L-sorbose (weak), sucrose, maltose, cellobiose (latent), trehalose (slow), lactose (latent), melezitose (latent), soluble starch, D-xylose (latent), D-arabinose (weak), D-ribose (weak), L-rhamnose (weak), ethanol, glycerol (latent), ribitol (weak), D-mannitol (latent), D-glucitol (slow), methyl-α-D-glucoside (weak), salicin (latent), succinate (slow), citrate (latent), N-acetyl-D-glucosamine (weak) and xylitol (latent) are assimilated as sole carbon sources, while melibiose, raffinose, inulin, L-arabinose, D-glucosamine, methanol, erythritol, galactitol, D-glucuronate, DL-lactate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, L-lysine (slow), ethylamine and cadaverine are assimilated as sole nitrogen sources. Growth in vitamin-free medium is positive (slow). Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C is weak, and no growth is observed at 40 °C.

Typus – China, Hubei Province, Shennongjia Forestry District, around the Songluo Township Government, 31.6719°N, 110.6087°E, collected from bark of a *Pterocarya stenoptera* tree at 869 m, April 11, 2024, by Y.J. Qiu, Q.Y. Zhu & F.Y. Bai; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8550^{T} (= HBP0702E3-1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37619 (= CGMCC 2.8550) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

GenBank accession number – The ITS-5.8S region and D1/D2 domains of the LSU sequence of strain HBP0702E3-1 is PQ568186.

Notes – Physiologically, the new species differs from its closely related species, *Nakazawaea pomicola*, by its inability to assimilate L-arabinose. Furthermore, it is distinguished from *Nakazawaea holstii* by its inability to grow in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium.

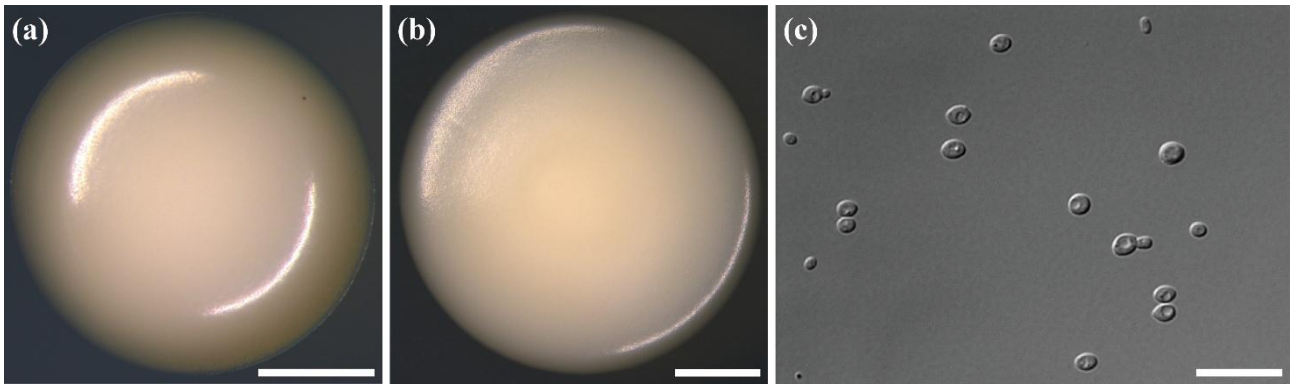

Figure 21 – Morphology of *Nakazawaea pterocaryicorticola* sp. nov. (strain HBP0702E3-1). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. All at 25 °C. Scale bars: a = 500 μm, b = 1 mm, c = 10 μm.

Nakazawaea shennongjiaensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 22

MycoBank number: MB857111

Etymology – Referring to the place where the species was isolated, Shennongjia.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, glistening, with a slightly rough surface, broadly conical, and an entire margin (Fig. 22a). After 7 days, the colonies turn cream-colored and appear dull (Fig. 22b). Vegetative cells are subglobose to ellipsoidal, 2.2–4.1 × 1.7–2.9 μm ($\overline{x}$ = 2.9 × 2.2 μm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 22c). In YM broth, after 1 month at room temperature, a white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not observed. Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose and trehalose are positive, while galactose, sucrose, maltose, lactose and raffinose are not fermented. Glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melezitose (positive/latent), inulin (weak/negative), soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, methyl-α-D-glucoside (weak/negative), salicin, DL-lactate (variable), succinate, citrate, N-acetyl-D-glucosamine, xylitol are assimilated as sole carbon sources, while lactose, melibiose, raffinose, methanol, erythritol, galactitol, D-glucuronate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is positive (weak). Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C and 37 °C is positive, while growth at 40 °C is negative, except that strain HBP0330E4D1^{T} is weakly positive at 40 °C.

Typus – China, Hubei Province, Shennongjia Forestry District, Niwotang, collected from pieces of wood from a *Quercus* tree at 1650 m, July 29, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8552^{T} (= HBP0330E4D1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37621 (= CGMCC 2.8552) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratypes – *ibid.* Damochang, 31.3712°N, 110.5168°E, collected from bark of a *Quercus* tree at 2227 m, July 11, 2023, HBP0257C4C3 (= CGMCC 2.8551 = JCM 37620), HBP0257C4C4 (= CGMCC 2.8572); *ibid.* Duidongping, 31.6126°N, 110.5588°E, collected from bark of a *Castanea mollissima* tree at 1651.9 m, April 11, 2024, by Y.J. Qiu, Q.Y. Zhu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP0733E4-4 (= CGMCC 2.8581); *ibid.* Shennong Tianchi, 31.8103°N, 110.5636°E, collected from pieces of wood/bark from a tree at 1710 m, April 11, 2024, by Z. Wen & P.J. Han, isolated by L.X. Yang & Y.C. Mao, HBP0768E3-1 (= CGMCC 2.8587).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequence are as follows: HBP0330E4D1 (PQ568169), HBP0257C4C3 (PQ568166), HBP0257C4C4 (PQ568167), HBP0733E4-4 (PQ568193) and HBP0768E3-1 (PQ568201).

Notes – Physiologically, the new species differs from its closely related species, *Nakazawaea molendinolei*, by its ability to assimilate galactose, L-sorbose, sucrose, and maltose.

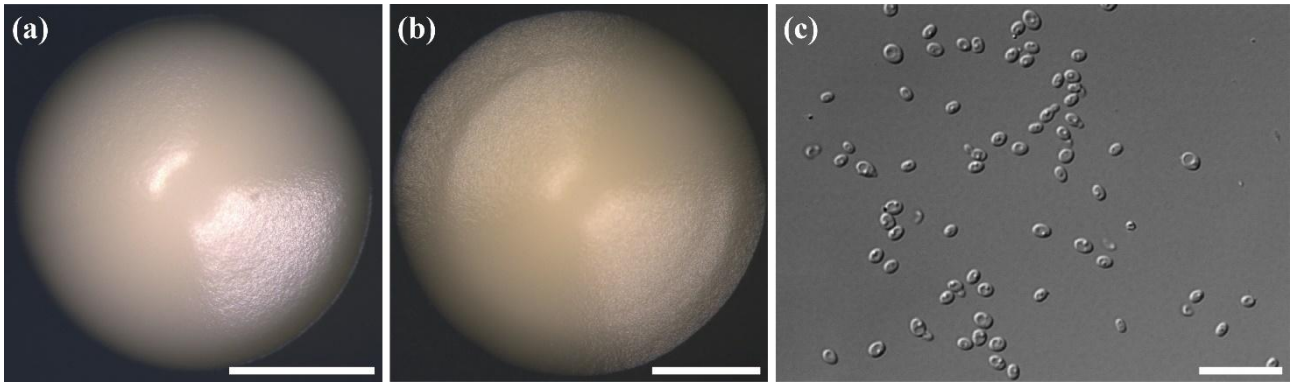

Figure 22 – Morphology of *Nakazawaea shennongjiaensis* sp. nov. (strain HBP0330E4D1). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. All at 25 °C. Scale bars: a–b = 1 mm, c = 10 μm.

***Pichia* (Pichiaceae, Pichiales, Pichiomycetes)** E.C. Hansen (1904)

Eighteen strains clustered within the *Pichia* clade, forming two distinct groups (Fig. 23, Supplementary Figs. 15 and 16). Fifteen strains—HBP0720D4-1, HBP0720D4-3, HBP1042D4-1, HBP0723D4-1, HBP0604D4-5, HBP0573F4D3, HBP0507F4D1, HBP0507D4C2, HBP0230C4C1, HBP0788E3-2, HBP1016D3-1, HBP1030D3-1, HBP0780D4-3, HBP1032D4-2, and HBP1040D3-1—formed a cohesive cluster with similar D1/D2 sequences, differing by no more than four nucleotide differences, and similar ITS sequences, differing by no more than three nucleotide substitutions, suggesting that they were conspecific. The group represented by strain HBP0780D4-3 was distinct from its closely related species, *Pichia membranifaciens*, exhibiting 10 nucleotide mismatches (1.8%, 2 substitutions and 8 gaps over 566 nucleotides) in the D1/D2 domains and 43 nucleotide mismatches (10.2%, 12 substitutions and 31 gaps over 420 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI value between strain HBP0780D4-3 and *Pichia membranifaciens* was 84% (Supplementary Table 9), indicating that strain HBP0780D4-3 represents a novel species within the genus *Pichia*.

Three strains—HBP0772E3-2, HBP0772D4-1, and HBP0414D4C1—formed a separate group (Fig. 23, Supplementary Figs. 15 and 16) with similar D1/D2 and ITS sequences, differing by only one nucleotide substitution, suggesting that they were conspecific. This group was closely related to *Pichia garciniae*, with 12 nucleotide mismatches (2.1%, 11 substitutions and 1 gap over 560 nucleotides) in the D1/D2 domains and 47 nucleotide mismatches (11.8%, 22 substitutions and 25 gaps over 398 nucleotides) in the ITS region. It also differed from *Pichia manshurica* by six nucleotide mismatches (1.1%, 6 substitutions over 560 nucleotides) in the D1/D2 domains and 57 nucleotide mismatches (14.2%, 33 substitutions and 24 gaps over 402 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI value between strain HBP0772D4-1 and *Pichia manshurica* was 77% (Supplementary Table 9), suggesting that strain HBP0772D4-1 represents

another novel species within the genus *Pichia*.

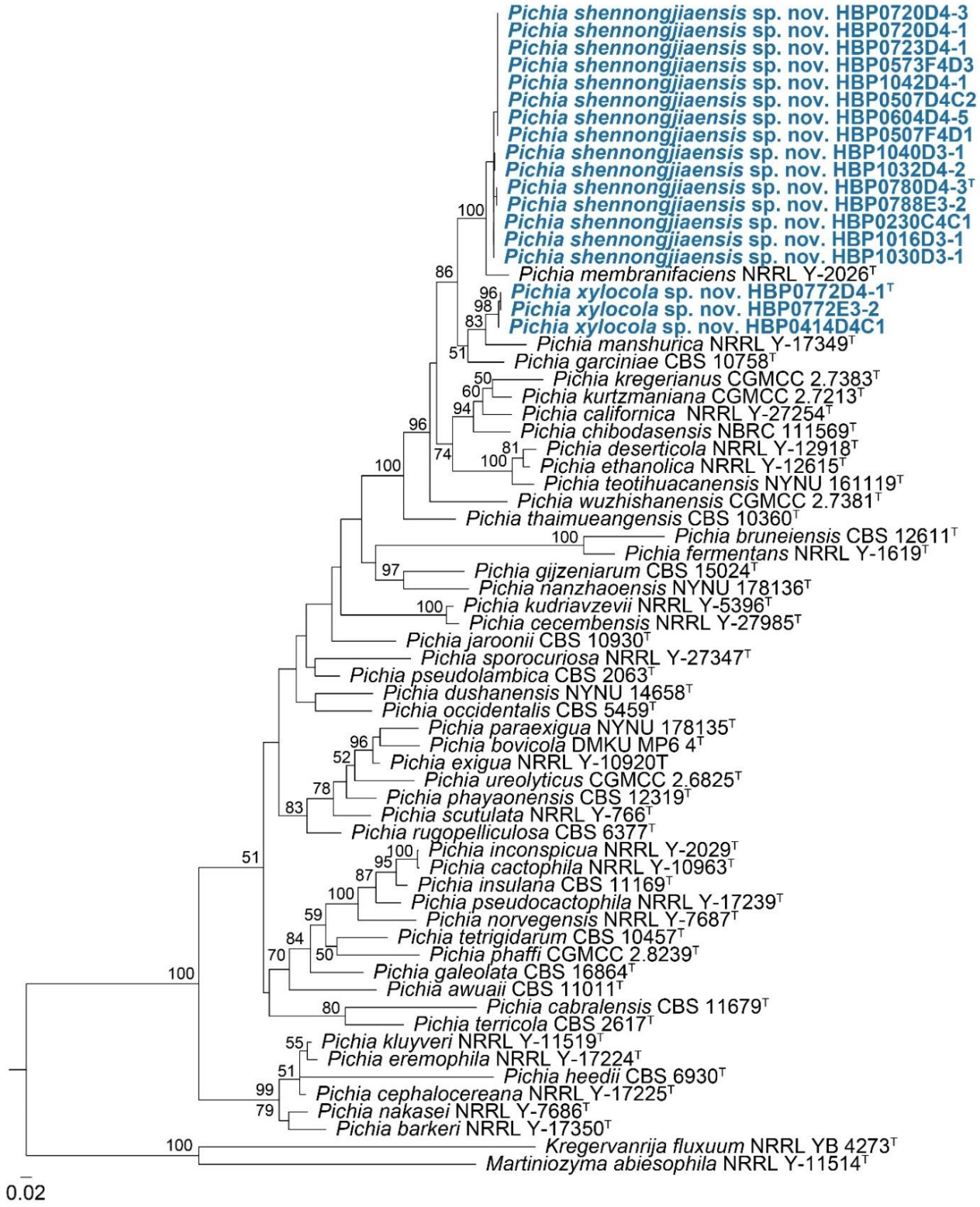

Figure 23 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Pichia shennongjiaensis* sp. nov. and *Pichia xylocola* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Kregervanrija fluxuum* and *Martiniozyma abiesophila* are used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

Pichia shennongjiaensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 24

MycoBank number: MB857112

Etymology – Referring to the place where the species was isolated, Shennongjia.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, dull, with a rough and irregular surface, exhibiting folds, wrinkles, and a coralloid appearance (Fig. 24a). After 7 days, the colonies turn reddish, with the coralloid margin expanding radially (Fig. 24b). Vegetative cells are subglobose, ellipsoidal, and elongate, 4.1–8.5 × 2.7–4.9 μm ($\overline{x}$ = 6.5 × 3.8 μm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 24c). In YM broth, after 1 month at room temperature, a white sediment formed, and a very thin white, powdery pellicle floated on the surface, climbing up the walls of the tube. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not developed, while asci are observed (Fig. 23d). Additionally, asci are also observed on YCB agar after 1 month (Fig. 24e–g). One to four globose ascospores are formed per ascus.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, L-sorbose, D-glucosamine (weak/negative), ethanol, glycerol (weak/negative), succinate (weak), N-acetyl-D-glucosamine (positive/latent) and xylitol (positive/slow) are assimilated as sole carbon sources, while galactose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, methyl-α-D-glucoside, salicin, D-glucuronate, DL-lactate, citrate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources, while potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is positive. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C is weak or negative, and no growth is observed at 40 °C.

Typus – China, Hubei Province, Shennongjia Forestry District, Shennong Tianchi, 31.8103°N, 110.5636°E, collected from pieces of wood/bark from a tree at 1710 m, April 11, 2024, by Z. Wen & P.J. Han; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8555^{T} (= HBP0780D4-3) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37693 (= CGMCC 2.8555) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratypes – *ibid.* HBP0788E3-2 (= CGMCC 2.8593); *ibid.* Wujiaya, 31.3183°N, 110.4659°E, collected from bark of a *Betula schmidtii* tree at 2050 m, July 11, 2023, HBP0230C4C1 (= CGMCC 2.8568); *ibid.* Duanjiangping, 31.3252°N, 110.8979°E, collected from moss on a *Castanea mollissima* tree at 1680 m, July 15, 2023, HBP0507D4C2 (= CGMCC 2.8575), HBP0507F4D1 (= CGMCC 2.8576); *ibid.* Huaxuechang, 31.4418°N, 110.4472°E, collected from pieces of wood from a *Quercus* tree at 2350 m, July 19, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0573F4D3 (= CGMCC 2.8577); *ibid.* Dajiuhu Lake, 31.5033°N, 110.0125°E, collected from soil at 1730 m, by D.Y. Han, isolated by Y.J. Qiu, HBP0604D4-5 (= CGMCC 2.8578); *ibid.* Duidongping, 31.6126°N, 110.5588°E, collected from bark of a *Quercus* tree at 1651.9 m, April 11, 2024, by Y.J. Qiu, Q.Y. Zhu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP0720D4-1 (= CGMCC 2.8553 = JCM 37622), HBP0720D4-3 (= CGMCC 2.8579); *ibid.* collected from bark of a *Castanea mollissima*, HBP0723D4-1 (= CGMCC 2.8580); *ibid.* Renhehu (Lake No. 7 of Dajiuhu Lake), 31.5066°N, 110.9957°E, collected from bark of a *Malus hupehensis* tree at 1733 m, by Y.J. Qiu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP1016D3-1 (= CGMCC 2.8596); *ibid.* Viving of the Withered Tree (in Dajiuhu Lake), 31.5159°N, 109.9952°E, collected from bark of a *Fagaceae* tree at 1747 m, April 12, 2024, by Y.J. Qiu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP1030D3-1 (= CGMCC 2.8599), collected from bark of a *Quercus* tree, HBP1032D4-2 (= CGMCC 2.8600); *ibid.* Meirenshu (in Dajiuhu Lake), 31.5132°N, 109.9977°E, collected from bark of a *Malus hupehensis* tree at 1729 m, April 12, 2024, by Y.J. Qiu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao,

HBP1040D3-1 (= CGMCC 2.8601); *ibid.* Renshouhu (Lake No. 9 of Dajiuhu Lake), 31.5112°N, 109.9996°E, collected from bark of a *Fagaceae* tree at 1734 m, April 12, 2024, by Y.J. Qiu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP1042D4-1 (= CGMCC 2.8602).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences are as follows: HBP0780D4-3 (PQ568206), HBP0230C4C1 (PQ568158), HBP0507D4C2 (PQ568180), HBP0507F4D1 (PQ568181), HBP0573F4D3 (PQ568183), HBP0604D4-5 (PQ568185), HBP0720D4-1 (PQ568188), HBP0720D4-3 (PQ568189), HBP0723D4-1 (PQ568192), HBP0788E3-2 (PQ568210), HBP1016D3-1 (PQ568216), HBP1030D3-1 (PQ568220), HBP1032D4-2 (PQ568222), HBP1040D3-1 (PQ568223) and HBP1042D4-1 (PQ568224).

Notes – Physiologically, the new species differs from its closely related species, *Pichia membranifaciens*, by its ability to assimilate L-sorbose.

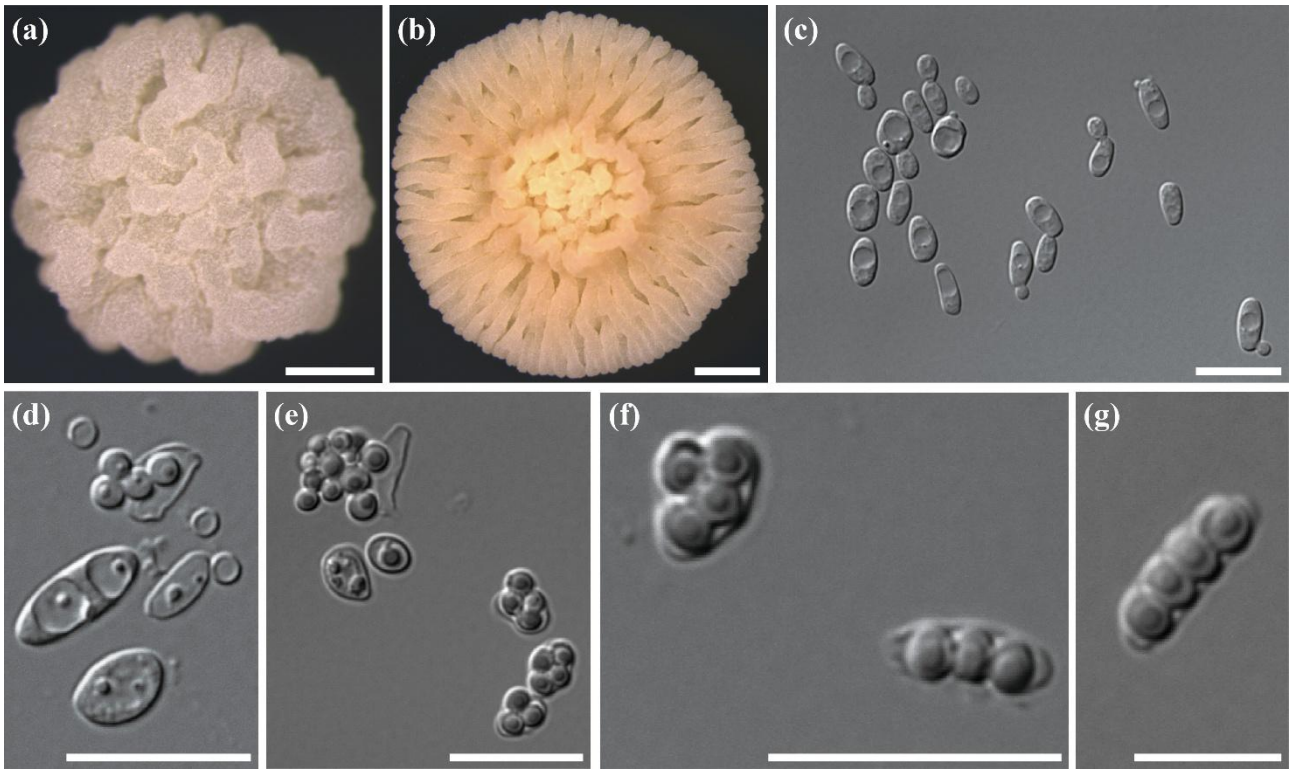

Figure 24 – Morphology of *Pichia shennongjiaensis* sp. nov. (strain HBP0780D4-3). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. (d) An ascus is releasing four globose ascospores under the coverglass on CMA after 2 weeks. (e–g) Asci with globose ascospores on YCB agar after 1 month. All at 25 °C. Scale bars: a = 500 µm, b = 1 mm, c–f = 10 µm, g = 5 µm.

Pichia xylocola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 25

MycoBank number: MB857113

Etymology – Referring to the species isolated from wood/humus.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, dull, with a rough and undulating surface, and flat in the center (Fig. 25a). After 7 days, the colony centers turn reddish, while the margins become entire (Fig. 25b). Vegetative cells are subglobose, ellipsoidal, and elongate, 4.1–11.7 × 3.1–4.9 µm ($\overline{x}$ = 7.7 × 3.9 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 25c). In YM broth, after 1 month at room temperature, a white sediment formed, and white, dry islets are present, climbing up the walls of the tube. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, pseudohyphae and asci are observed under the coverglass (Fig. 25d–e). Additionally, asci are observed on YCB agar after 1 month (Fig. 25f–g). One to four globose ascospores are formed per ascus.

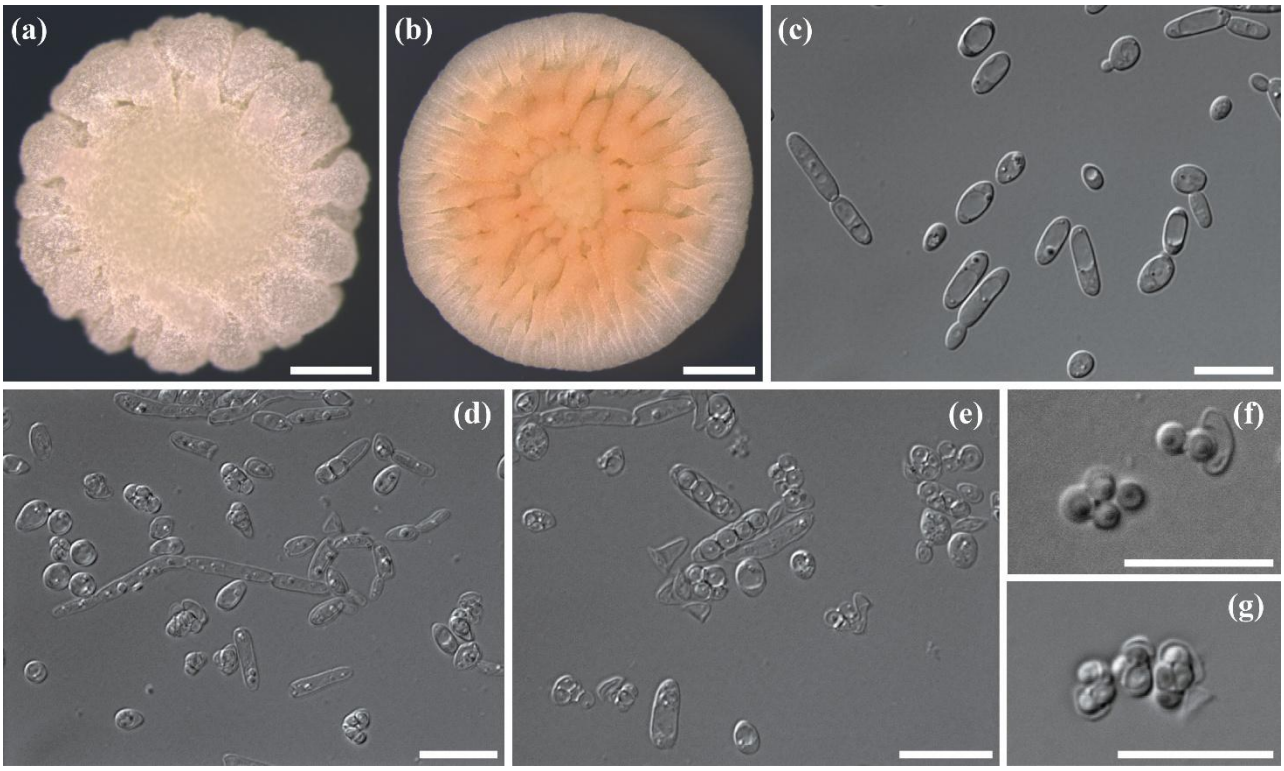

Figure 25 – Morphology of Pichia *xylocola* sp. nov. (strain HBP0772D4-1). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. (d–e) Pseudohyphae and asci growth under the coverglass on CMA after 2 weeks. (f–g) Asci and releasing globose ascospores on YCB agar after 1 month. All at 25 °C. Scale bars: a = 500 μm, b = 1 mm, c–g = 10 μm.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, D-glucosamine (weak/negative), ethanol, glycerol, DL-lactate (weak/negative), succinate (weak) and N-acetyl-D-glucosamine are assimilated as sole carbon sources, while galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, methyl-α-D-glucoside, salicin, D-glucuronate, citrate, myo-inositol, hexadecane and xylitol are not assimilated. Ammonium sulfate, L-lysine, ethylamine (positive/latent) and cadaverine are assimilated as sole nitrogen sources, while potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Shennong Tianchi, 31.8103°N, 110.5636°E, collected from pieces of wood/bark from a tree at 1710 m, April 11, 2024, by Z. Wen & P.J. Han; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8556^{T} (= HBP0772D4-1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37695 (= CGMCC 2.8556) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratypes – *ibid.* HBP0772E3-2 (= CGMCC 2.8589), *ibid.* Sanchagou, 31.8457°N, 110.5165°E, collected from humus at 1970 m, August 2, 2023, by Q.Y. Zhu, isolated by Y.J. Qiu, HBP0414D4C1 (= CGMCC 2.8554 = JCM 37694).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences are as follows: HBP0772D4-1 (PQ568203), HBP0772E3-2 (PQ568204) and HBP0414D4C1

(PQ568174).

Notes – Physiologically, the new species differs from its closely related species, *Pichia manshurica*, by its inability to grow in 10% sodium chloride plus 5% glucose medium. Furthermore, it is distinguished from *Pichia garciniae* by its inability to assimilate xylitol.

***Saccharomycopsis* (Saccharomycopsidaceae, Ascoideales, Saccharomycetes)** Schiønning (1903)

Two strains, HBP0845D3-3 and HBP084504-1, formed a separate branch and did not cluster with any other strains in the genus *Saccharomycopsis* (Fig. 26, Supplementary Figs. 17 and 18). The group represented by HBP0845D3-3 exhibited identical D1/D2 and ITS sequences, suggesting that they were conspecific. It was closely related to *Saccharomycopsis microspora* and *Saccharomycopsis synnaedendra*. Strain HBP0845D3-3 differed from *Saccharomycopsis microspora* by 28 nucleotide mismatches (5.4%, 27 substitutions and 1 gap over 518 nucleotides) in the D1/D2 domains, and 26 (10.7%, 20 substitutions and 6 gaps over 244 nucleotides) nucleotide mismatches in the ITS region. It differed from *Saccharomycopsis synnaedendra* by 34 nucleotide mismatches (6.7%, 33 substitutions and 1 gap over 518 nucleotides) in the D1/D2 domains, and 27 nucleotide mismatches (10.4%, 19 substitutions and 8 gaps over 260 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI values between strain HBP0845D3-3 and its two closest relatives, *Saccharomycopsis microspora* and *Saccharomycopsis synnaedendra*, were both at 71% (Supplementary Table 9), strongly indicating that HBP0845D3-3 represents a novel species within the genus *Saccharomycopsis*.

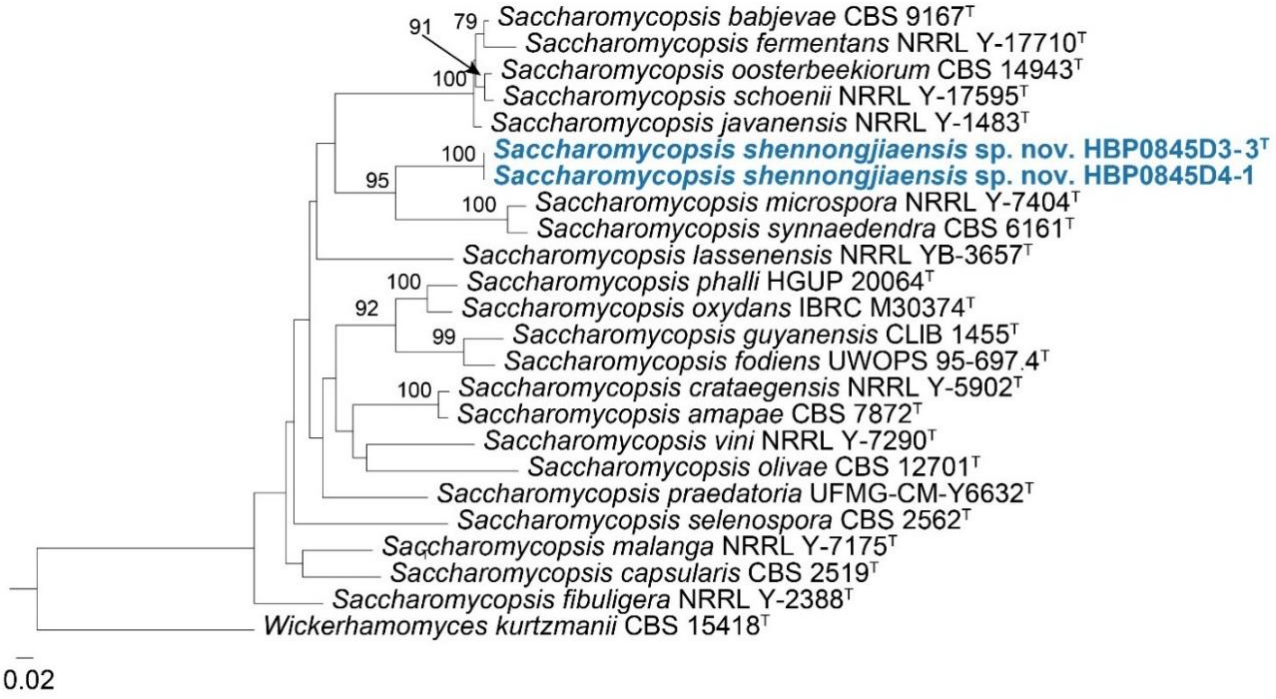

Figure 26 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Saccharomycopsis shennongjiaensis* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Wickerhamomyces kurtzmanii* is used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

Saccharomycopsis shennongjiaensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 27

MycoBank number: MB857114

Etymology – Referring to the place where the species was isolated, Shennongjia.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, glistening, with a rough surface and a hyphal margin (Fig. 27a). After 10 days, the colonies turn light yellow, develop a spiny surface, and the texture becomes flexible, non-viscous, and indispersible (Fig. 27b). Vegetative cells are ellipsoidal, elongate, and occasionally tapered on one end, 4.5–7.9 × 2.0–3.2 µm ($\overline{x}$ = 6.1 × 2.6 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 26c–d). In YM broth, after 1 month at room temperature, a white sediment formed, and white, patchy islets is present. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, abundant true hyphae are developed under the coverglass (Fig. 27e). Asci are spheroidal to ovoid, arose laterally or terminally on the hyphae. One to four hat-shaped ascospores are formed per ascus (Fig. 27f–h).

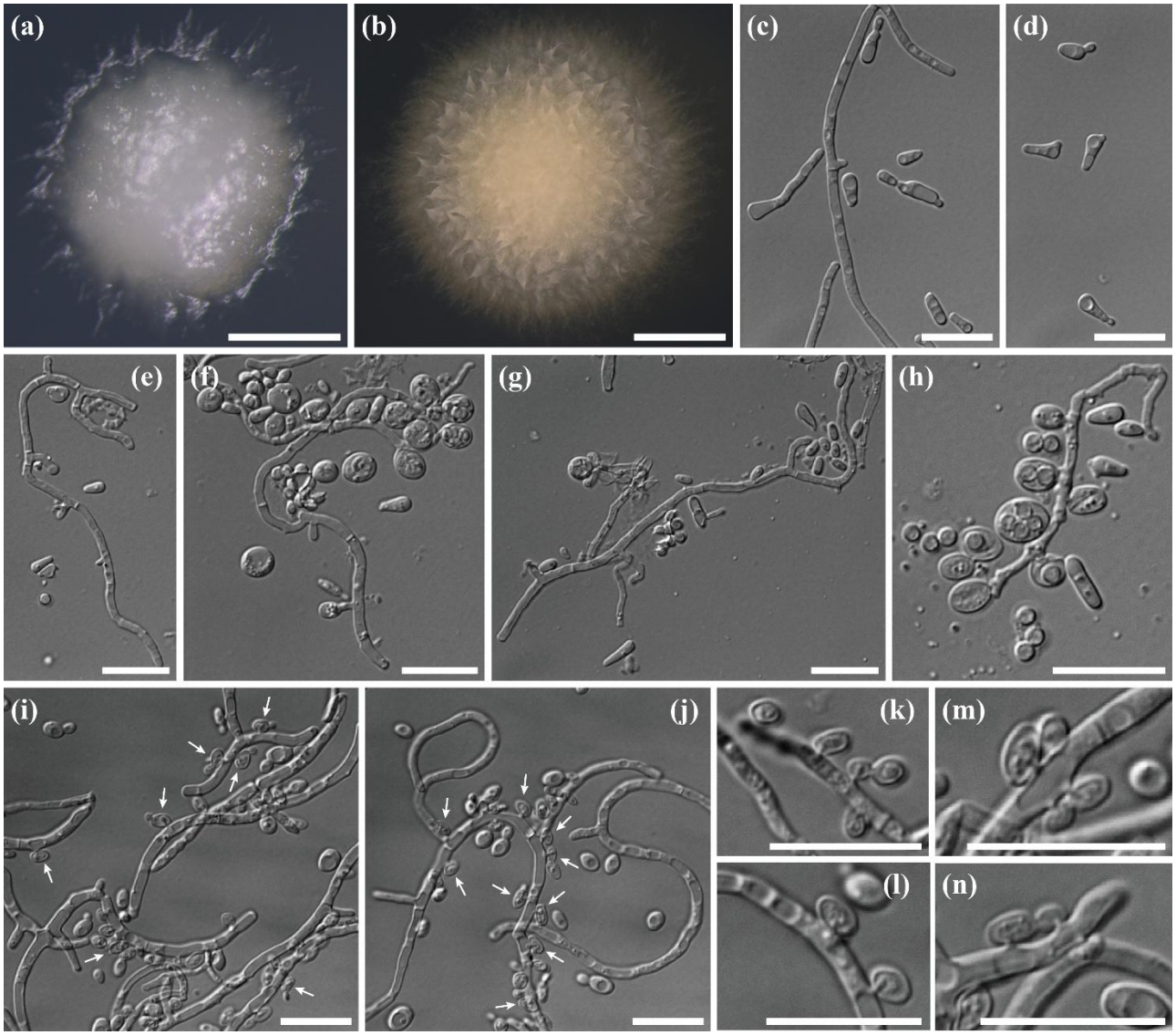

Figure 27 – Morphology of *Saccharomycopsis shennongjiaensis* sp. nov. (strain HBP0845D3-3). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 10 days. (c–d) Vegetative cells on YM agar after 2 days. (e) True hyphae growth under the coverglass on CMA after 2 weeks. (f–h) Spheroidal to ovoid asci usually arose laterally or terminally on the hyphae, and the released ascospores were hat-shaped, under the coverglass on CMA after 2 weeks. (i–n) Predation against *Candida nitratophila* (strain HBP0845E4-5). Infection pegs inside prey cells after 18 hours of incubation of the predator-prey mixture on glucose–YNB agar (without amino acids). White arrows show infection pegs inside prey cells. All at 25 °C. Scale bars: a = 500 µm, b = 1 mm, c–n = 10 µm.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose,

sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, maltose (weak), trehalose, soluble starch, D-ribose, L-rhamnose, ethanol, glycerol, erythritol, ribitol, D-mannitol (weak), D-glucitol (weak), succinate (weak) and xylitol are assimilated as sole carbon sources, while galactose, L-sorbose, sucrose, cellobiose, lactose, melibiose, raffinose, melezitose, inulin, D-xylose, L-arabinose, D-arabinose, D-glucosamine, methanol, galactitol, methyl-α-D-glucoside, salicin, D-glucuronate, DL-lactate, citrate, myo-inositol, hexadecane and N-acetyl-D-glucosamine are not assimilated. L-asparagine (slow), L-glutamine, L-methionine (weak), ammonium chloride, ammonium bicarbonate (weak) and aasamino acids are assimilated as sole nitrogen sources, while ammonium sulfate, ammonium citrate, potassium nitrate, sodium nitrite, ethylamine, cadaverine, L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine are not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Shennongyuan, 31.4517°N, 110.1925°E, collected from pieces of wood/bark from a tree at 2057 m, April 11, 2024, by Z. Wen & P.J. Han; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8557^{T} (= HBP0845D3-3) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37623 (= CGMCC 2.8557) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratype – *ibid.* HBP0845D4-1 (= CGMCC 2.8594).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences are PQ568211 for strain HBP0845D3-3 and PQ568212 for strain HBP0845D4-1.

Notes – After 18 hours on glucose–YNB agar plates without amino acids, predation was observed in mixtures with *Candida nitratophila*, which was isolated from the same samples, with infection pegs inside the prey cells (Fig. 27i–n). However, no predation was observed on four other species isolated from the same sample, namely *Khveromyces starmeri*, *Saccharomycopsis fibuligera*, *Saturnispora dispora*, and *Saturnispora zaruensis*, as well as *Saccharomyces cerevisiae*, isolated from a different sample. Physiologically, the new species differs from *Saccharomycopsis microspora* by its inability to assimilate D-xylose and from *Saccharomycopsis synnaedendra* by its inability to assimilate D-arabinose.

Starmera **(Phaffomycetaceae, Phaffomycetales, Saccharomycetes)** Y. Yamada, Higashi, S. Ando & Mikata (1997)

Two strains, HBP1023D3-1 and HBP1023D3-2, formed a distinct branch within the genus *Starmera* and did not cluster with any other strains (Fig. 28, Supplementary Figs. 19 and 20). The group represented by HBP1023D3-2 exhibited identical D1/D2 sequences and similar ITS sequences, differing by only two nucleotide substitutions and one gap, suggesting that they were conspecific. It was closely related to *Starmera quercuum*, differing by 10 nucleotide mismatches (2.0%, 7 substitutions and 3 gaps over 512 nucleotides) in the D1/D2 domains. Based on whole-genome sequences, the ANI value between strain HBP1023D3-2 and *Starmera quercuum* was 87% (Supplementary Table 9), strongly indicating that strain HBP1023D3-2 represents a novel species within the genus *Starmera*.

Starmera prunicorticola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 29

MycoBank number: MB857115

Etymology – Referring to the species isolated from the bark of *Prunus*.

Culture characteristics – On YM agar, after 7 days at 25 °C, the colonies are butyrous, cream-colored, glistening, with a smooth to slightly rough surface, a flat center, and an entire margin (Fig. 29a). Vegetative cells are globose to subglobose, 2.9–4.9 × 2.5–4.0 μm ($\overline{x}$ = 4.0 × 3.4 μm, n = 20),

reproduce by multilateral budding, and occur singly or in pairs (Fig. 29b). In YM broth, after 1 month at room temperature, a white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not observed. Asci are dumbbell-shaped and contain one to four hat-shaped ascospores, observed on CMA, YCB, and V8 agars (Fig. 29c–f).

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, cellobiose (weak), ethanol, glycerol, D-mannitol (slow), D-glucitol (slow), salicin (slow), succinate (weak), citrate (weak) and xylitol (weak) are assimilated as sole carbon sources, while galactose, L-sorbose, sucrose, maltose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, erythritol, ribitol, galactitol, methyl-α-D-glucoside, D-glucuronate, DL-lactate, myo-inositol, hexadecane and N-acetyl-D-glucosamine are not assimilated. Ammonium sulfate, potassium nitrate (latent), L-lysine, ethylamine (latent) and cadaverine (latent) are assimilated as sole nitrogen sources, while sodium nitrite is not assimilated. Growth in vitamin-free medium is positive (slow). Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C is weak, and no growth is observed at 40 °C.

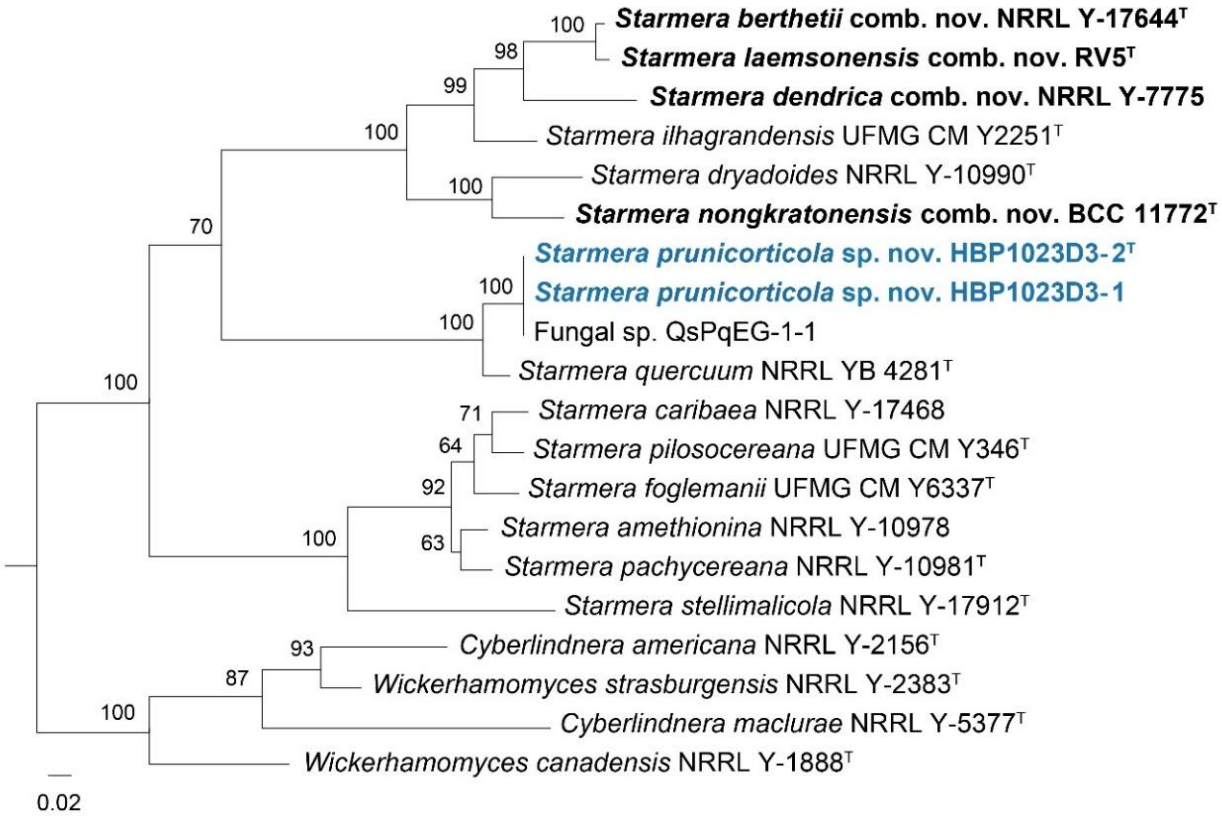

Figure 28 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Starmera prunicorticola* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Wickerhamomyces canadensis*, *Cyberlindnera americana*, *Wickerhamomyces strasburgensis*, and *Cyberlindnera maclurae* are used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

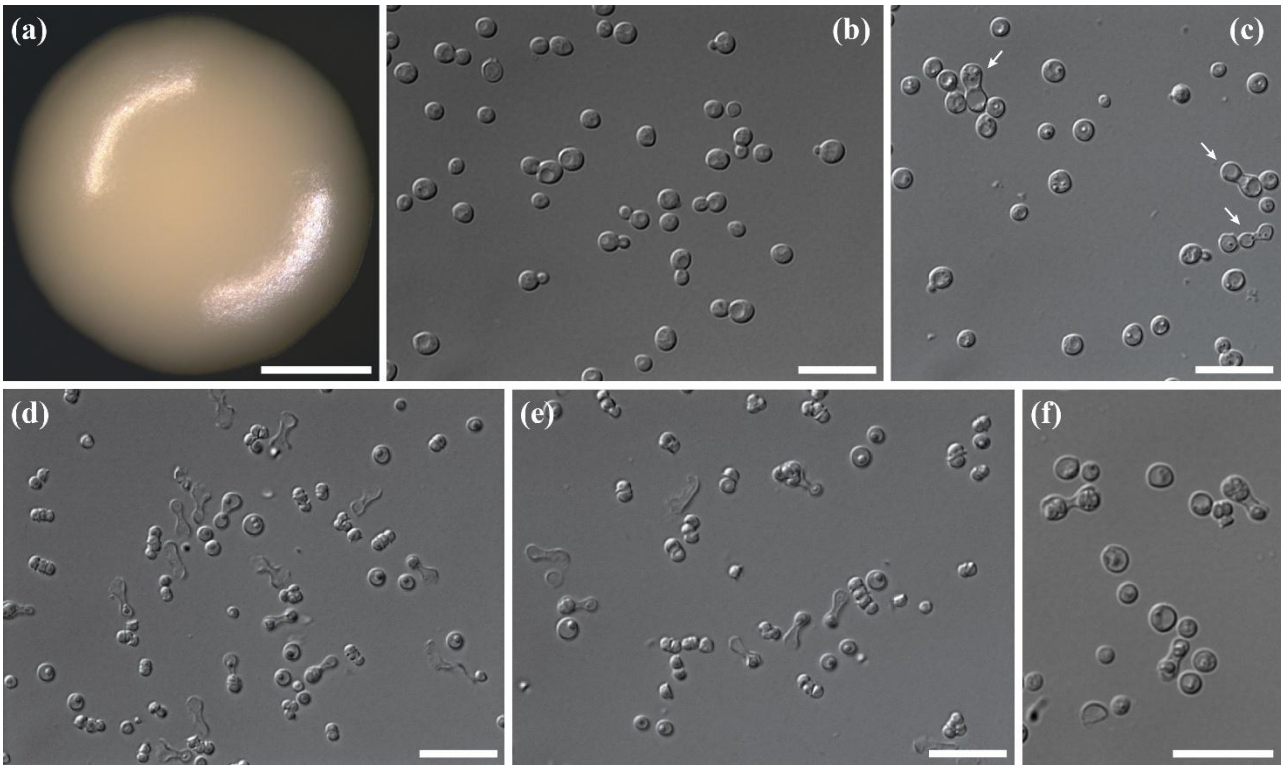

Figure 29 – Morphology of *Starmera prunicorticola* sp. nov. (strain HBP1023D3-2). (a) Colony on YM agar after 7 days. (b) Vegetative cells on YM agar after 2 days. (c) Cells forming asci under the coverglass on CMA after 2 weeks. (d–e) Asci and releasing hat-shaped ascospores on YCB agar after 1 month. (f) Dumbbell-shaped Asci on V8 agar after 1 month. All at 25 °C. Scale bars: a = 1 mm, b–f = 10 μm.

Typus – China, Hubei Province, Shennongjia Forestry District, Tangchaogumu (in Dajiuhu Lake), 31.5132°N, 109.9953°E, collected from bark of a *Prunus brachypoda* tree at 1739 m, April 12, 2024, by Y.J. Qiu & F.Y. Bai; isolated by L.X. Yang & Y.C. Mao. Holotype: CGMCC 2.8558^{T} (= HBP1023D3-2) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37624 (= CGMCC 2.8558) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratype – *ibid.* HBP1023D3-1 (= CGMCC 2.8598).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences are PQ568219 for strain HBP1023D3-2 and PQ568218 for strain HBP1023D3-1.

Notes – Physiologically, the new species differs from its closely related species, *Starmera quercuum*, by its inability to assimilate DL-lactate.

New combinations

Starmera berthetii (Boidin, Pignal, Mermiér & Arpin) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857132

Holotype: NRRL Y-17644 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionym – *Candida berthetii* Boidin, Pignal, Mermiér & Arpin, Cahiers de La Maboké 1: 100 (1963)

Starmera dendrica (Van der Walt, Klift & D.B. Scott) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857133

Holotype: NRRL Y-7775 preserved in a metabolically inactive state in the Agricultural Research

Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionyms – *Torulopsis dendrica* Van der Walt, Klift & D.B. Scott, Antonie van Leeuwenhoek 37(3): 461 (1971)

≡ *Candida dendrica* (Van der Walt, Klift & D.B. Scott) S.A. Mey. & Yarrow, Int. J. Syst. Bacteriol. 28(4): 612 (1978)

Starmera laemsonensis (Am-In, Limtong, Yongman. & Jindam) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857134

Holotype: BCC 35154 (= RV5) preserved in a metabolically inactive state in the BIOTEC Culture Collection (BCC), Pathum Thani, Thailand.

Basionym – *Candida laemsonensis* Am-In, Limtong, Yongman. & Jindam., Int. J. Syst. Evol. Microbiol. 61(2): 458 (2011)

Starmera nongkratonensis (Nakase & Jindam.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857135

Holotype: BCC 117721 preserved in a metabolically inactive state in the BIOTEC Culture Collection (BCC), Pathum Thani, Thailand.

Basionym – *Pichia nongkratonensis* Nakase & Jindam., Mycoscience 46(3): 193 (2005)

Sugiyamaella **(Trichomonascaceae, Dipodascales, Dipodascomycetes)** Kurtzman & Robnett (2007)

Two strains isolated in this study, HBP0208C3C5 and HBP0208C4C3, along with another strain from China, GY43S04, formed a distinct branch within the genus *Sugiyamaella* (Fig. 30, Supplementary Figs. 21 and 22). These three strains exhibited similar D1/D2 sequences with only one nucleotide difference, and similar ITS sequences with no more than three nucleotide differences, suggesting that they were conspecific. The group represented by strain HBP0208C3C5 was closely related to a cluster comprising seven known species: *Sugiyamaella americana*, *Sugiyamaella carassensis*, *Sugiyamaella bullrunensis*, *Sugiyamaella amazoniana*, *Sugiyamaella bonitensis*, *Sugiyamaella bielyi*, and *Sugiyamaella ligni* (Fig. 30, Supplementary Figs. 21 and 22). Strain HBP0208C3C5 differed from these species by 55 to 72 nucleotide mismatches (11.7% to 15.0%) in the D1/D2 domains and 37 to 60 nucleotide mismatches (12.5% to 19.0%) in the ITS region (Supplementary Table 5c). The ANI value between strain HBP0208C3C5 and *Sugiyamaella americana* was 75% (Supplementary Table 9). These results suggest that strain HBP0208C3C5 represents a novel species within the genus *Sugiyamaella*.

Sugiyamaella irregularis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 31

MycoBank number: MB857116

Etymology – Referring to the irregular colony morphology.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, glistening, with a bumpy, rough, undulating surface and a hyphal margin (Fig. 31a). After 7 days, the colonies turn yellow, develop folds, wrinkles, and a brain-like surface, with the margin becoming extremely irregular in shape (Fig. 31b). Vegetative cells are ovoid to ellipsoidal, 4.6–7.6 × 3.1–5.7 μm ($\overline{x}$ = 6.3 × 4.5 μm, n = 20), reproduce by multilateral budding and through formation of blastoconidia, and occur singly or in pairs (Fig. 31c). In YM broth, after 1 month at room temperature, a white sediment formed. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae and pseudohyphae are developed under the coverglass (Fig. 31d–g). Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, galactose, L-sorbose (slow), sucrose (weak), cellobiose, trehalose, melibiose, melezitose (weak), inulin (weak), D-xylose, L-arabinose, D-arabinose (weak), D-ribose (weak), D-glucosamine (weak), ethanol (slow), erythritol

(slow), ribitol (weak), D-mannitol, D-glucitol (slow), salicin, D-glucuronate, DL-lactate (weak), succinate, N-acetyl-D-glucosamine and xylitol (slow) are assimilated as sole carbon sources, while maltose, lactose, raffinose, soluble starch, L-rhamnose, methanol, glycerol, galactitol, methyl-α-D-glucoside, citrate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate (slow), potassium nitrate, sodium nitrite, L-lysine, ethylamine (slow) and cadaverine (weak) are assimilated as sole nitrogen sources. Growth in vitamin-free medium is positive (slow). Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

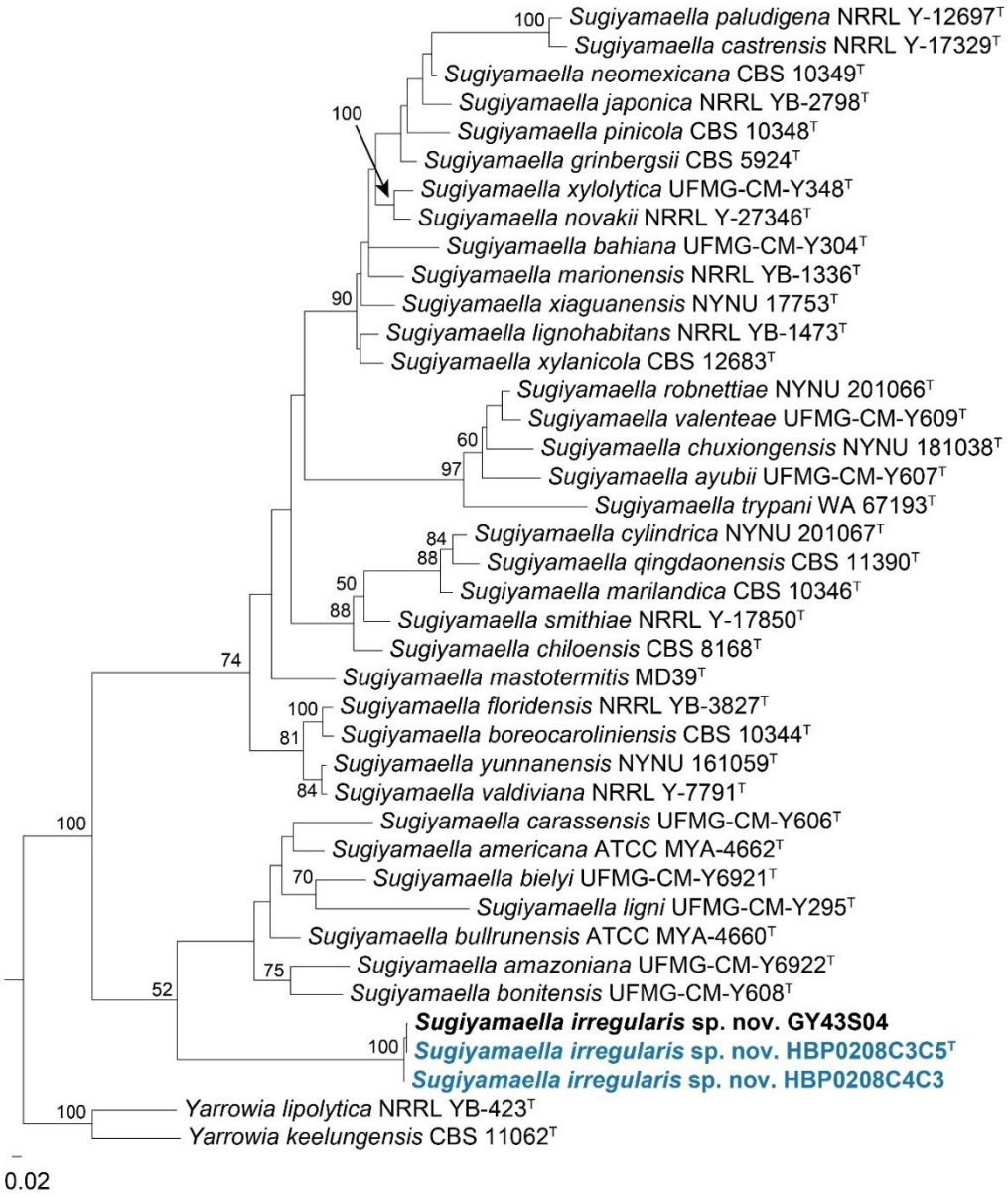

Figure 30 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Sugiyamaella irregularis* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Yarrowia keelungensis* and *Yarrowia lipolytica* are used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

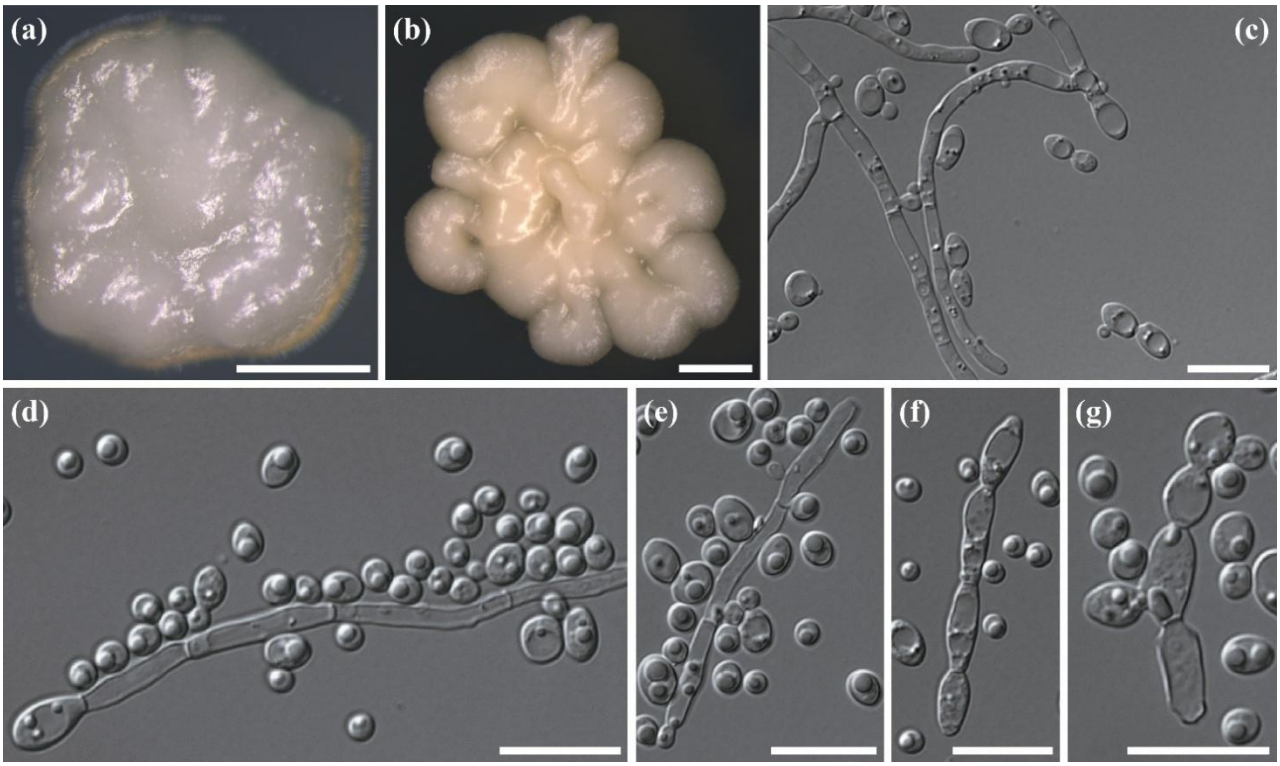

Figure 31 – Morphology of *Sugiyamaella irregularis* sp. nov. (strain HBP0208C3C5). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. (d–e) True hyphae growth under the coverglass on CMA after 2 weeks. (f–g) Pseudohyphae growth under the coverglass on CMA after 2 weeks. All at 25 °C. Scale bars: a = 500 µm, b = 1 mm, c–g = 10 µm.

Typus – China, Hubei Province, Shennongjia Forestry District, Tanjiawan, 31.4575°N, 110.0704°E, collected from bark of a *Quercus spinosa* tree at 2347.8 m, July 11, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8559^{T} (= HBP0208C3C5) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37625 (= CGMCC 2.8559) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratype – *ibid.* HBP0208C4C3 (= CGMCC 2.8566).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences are PQ568151 for strain HBP0208C3C5 and PQ568152 for strain HBP0208C4C3.

Notes – Physiologically, the new species differs from its closely related species, *Sugiyamaella amazoniana*, *Sugiyamaella bielyi*, *Sugiyamaella bonitensis*, *Sugiyamaella ligni*, *Sugiyamaella carassensis*, and *Sugiyamaella bullrunensis*, by its ability to assimilate ribose. Furthermore, it is distinguished from *Sugiyamaella americana* by its inability to grow at 37 °C (Supplementary Table 5c). The three strains representing *Sugiyamaella irregularis* sp. nov. are derived from bark in Hubei Province and unknown sources in the Taiwan region of China, indicating a limited distribution of the species in China.

***Suhomyces* (Debaryomycetaceae, Serinales, Pichiomycetes)** M. Blackw. & Kurtzman (2016)

Four strains grouped within the *Suhomyces* clade were divided into two distinct groups (Fig. 32, Supplementary Figs. 23 and 24). Strain HBP0247C4C1 and other 75 uncultured *Candida* clone formed a unique branch within the *Suhomyces* clade in the D1/D2 tree with only one nucleotide difference (Supplementary Fig. 23). It was closely related to *Suhomyces panamericanus*, differing by 10 nucleotide mismatches (2.1%, 10 substitutions over 476 nucleotides) in the D1/D2 domains and by 10 nucleotide mismatches (3.3%, 6 substitutions and 4 gaps over 300 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI values between strain HBP0247C4C1 and seven

known species—*Suhomyces atakaporum*, *Suhomyces bolitotheri*, *Suhomyces bribrorum*, *Suhomyces chickasaworum*, *Suhomyces panamericanus*, *Suhomyces tanzawaensis*—ranged from 74% to 77% (Supplementary Table 9), suggesting that strain HBP0247C4C1 represents a novel species within the genus *Suhomyces*.

Three strains—HBP0139A3AB, HBP0139A4AB, and HBP0722E4-2—grouped with three additional strains, including strain 335407 from a mushroom in the USA, strain KBP:Y-5640 from leaves of *Impatiens glandulifera* in Russia, and strain XZFM12-1 from China, exhibiting similar D1/D2 sequences with only one nucleotide difference and similar ITS sequences with no more than two nucleotide differences, suggesting that they were conspecific. Two unidentified strains, GY44S04 from China and BG99-11-14-1-4-2 from the gut of a mycetophagid beetle, shared similar D1/D2 sequences, differing by one nucleotide gap with strain HBP0139A3AB, although their ITS sequences were unavailable. One unidentified strain CBS 12740 and six other strains identified as *Suhomyces tanzawaensis*, shared similar ITS sequences, differing by no more than two nucleotide mismatches with strain HBP0139A3AB, although their D1/D2 sequences were unavailable. This suggests that these strains may also belong to the same species as HBP0139A3AB.

A BLAST search using the D1/D2 sequences of strain HBP0139A3AB identified *Suhomyces bolitotheri* as the closest match, showing 25 nucleotide mismatches (5.7%, 14 substitutions and 11 gaps over 442 nucleotides) in the D1/D2 domains and 39 nucleotide mismatches (13.0%, 21 substitutions and 18 gaps over 300 nucleotides) in the ITS region. In contrast, when the ITS sequences were used, the top match was *Suhomyces chickasaworum*, with 99 nucleotide mismatches (20.8%, 57 substitutions and 42 gaps over 477 nucleotides) in the ITS region and 33 nucleotide mismatches (11.3%, 24 substitutions and 9 gaps over 291 nucleotides) in the D1/D2 domains. The ANI values between strain HBP0139A3AB and seven known species—*Suhomyces atakaporum*, *Suhomyces bolitotheri*, *Suhomyces bribrorum*, *Suhomyces chickasaworum*, *Suhomyces panamericanus*, *Suhomyces tanzawaensis*—ranged from 73.6% to 74.4% (Supplementary Table 9). These results strongly indicate that strain HBP0139A3AB represents another novel species.

Suhomyces quercicorticola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 33

MycoBank number: MB857117

Etymology – Referring to the species isolated from the bark of *Quercus*.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, glistening, smooth, and flat, with a hyphal margin (Fig. 33a). After 7 days, the hyphal margin expands, forming two different concentric rings. The inner ring appears hirsute and butyrous, while the outer ring is white and fluffy (Fig. 33b–c). Vegetative cells are globose, ovoid, or ellipsoidal, 2.6–6.5 × 2.0–3.3 μm ($\overline{x}$ = 3.8 × 2.7 μm, n = 20), reproduce by multilateral budding and through formation of blastoconidia, and occur singly or in pairs (Fig. 33d). In YM broth, after 1 month at room temperature, a white sediment formed, and a thick, white, moist pellicle is present. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae and pseudohyphae are developed under the coverglass (Fig. 33e–f). Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose and trehalose (latent) are positive, while galactose, sucrose, maltose, lactose and raffinose are not fermented. Glucose, galactose, sucrose, maltose, cellobiose, trehalose, melezitose, soluble starch (weak), D-xylose, D-ribose, D-glucosamine, ethanol, glycerol, erythritol, ribitol, D-mannitol, D-glucitol, methyl-α-D-glucoside, salicin (weak), succinate (weak), citrate (weak) and N-acetyl-D-glucosamine, xylitol (slow) are assimilated as sole carbon sources, while L-sorbose, lactose, melibiose, raffinose, inulin, L-arabinose, D-arabinose, L-rhamnose, methanol, galactitol, D-glucuronate, DL-lactate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate (weak), L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources, while sodium nitrite is not assimilated. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is positive (weak). Growth on

50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

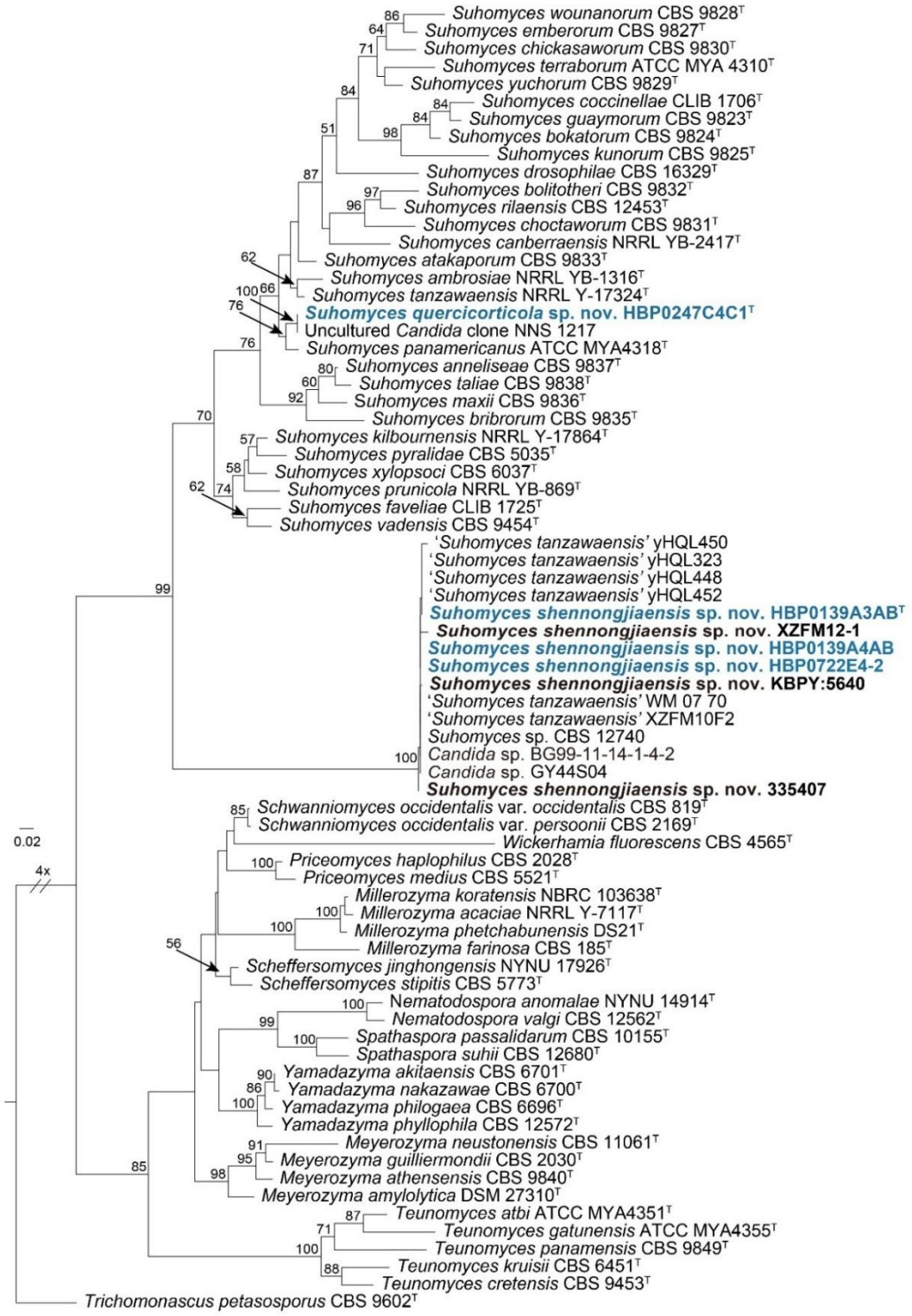

Figure 32 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Suhomyces quercicorticola* sp. nov. and *Suhomyces shennongjiaensis* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Trichomonascus petasosporus* is used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

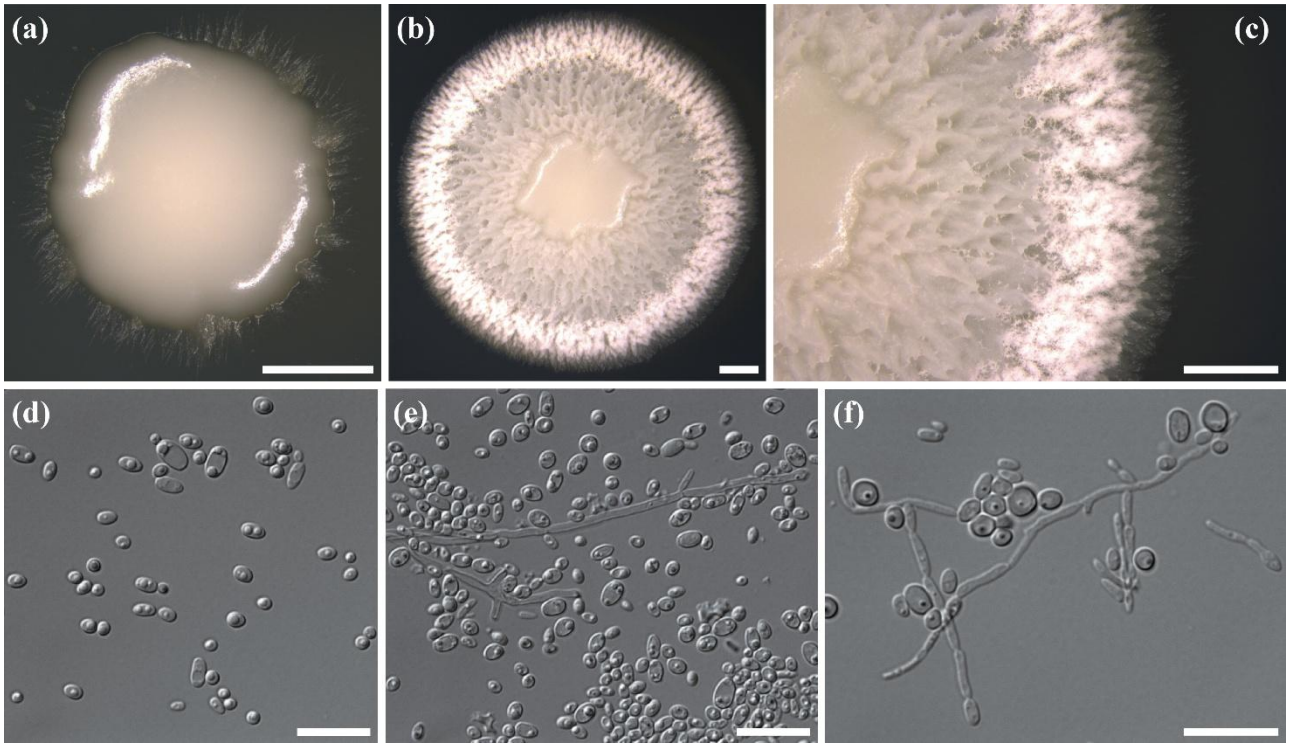

Figure 33 – Morphology of *Suhomyces quercicorticola* sp. nov. (strain HBP0247C4C1). (a) Colony on YM agar after 3 days. (b–c) Colony on YM agar after 7 days. (d) Vegetative cells on YM agar after 2 days. (e) True hyphae on PDA after 1 month. (f) Pseudohyphae growth under the coverglass on CMA after 2 weeks. All at 25 °C. Scale bars: a–c = 1 mm, d–f = 10 µm.

Typus – China, Hubei Province, Shennongjia Forestry District, Gongjiaping, 31.4264°N, 110.6229°E, collected from bark of a *Quercus* tree at 1850 m, July 11, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8560^{T} (= HBP0247C4C1) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37626 (= CGMCC 2.8560) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

GenBank accession number – The ITS-5.8S region and D1/D2 domains of the LSU sequence of strain HBP0247C4C1 is PQ568161.

Notes – Physiologically, the new species differs from its closely related species, *Suhomyces panamericanus* and *Suhomyces atakaporum*, by its ability to assimilate ethanol.

Suhomyces shennongjiaensis Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 34

MycoBank number: MB857118

Etymology – Referring to the place where the species was isolated, Shennongjia.

Culture characteristics – On YM agar, after 7 days at 25 °C, the colonies are butyrous, cream-colored, dull, smooth, and flat, with an entire margin (Fig. 34a). Vegetative cells are ovoid to ellipsoidal, 3.1–7.6 × 2.2–4.7 µm ($\overline{x}$ = 5.6 × 3.2 µm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 34b–c). In YM broth, after 1 month at room temperature, a white sediment formed, and a thin white, powdery pellicle floated on the surface. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae and pseudohyphae, sometimes with blastoconidia produced laterally, are developed under the coverglass (Fig. 34d–f). Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose is positive, while galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, galactose, L-sorbose (weak), sucrose, maltose, cellobiose, trehalose, melezitose, D-xylose

(positive/latent), D-glucosamine, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, methyl-α-D-glucoside, salicin, succinate, citrate, N-acetyl-D-glucosamine, xylitol (weak) are assimilated as sole carbon sources, while lactose, melibiose, raffinose, inulin, soluble starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, erythritol, galactitol, D-glucuronate, DL-lactate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources. Growth in vitamin-free medium is positive. Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is positive (weak). Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive, while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Yunpan, 31.4295°N, 110.0413°E, collected from bark of a *Quercus* tree at 1750 m, June 30, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8561^{T} (= HBP0139A3AB) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37627 (= CGMCC 2.8561) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratypes – *ibid.* HBP0139A4AB (= CGMCC 2.8607); *ibid.* Duidongping, 31.6126°N, 110.5588°E, collected from bark of a *Castanea mollissima* tree at 1651.9 m, April 11, 2024, by Y.J. Qiu, Q.Y. Zhu & F.Y. Bai, isolated by L.X. Yang & Y.C. Mao, HBP0722E4-2 (= CGMCC 2.8562 = JCM 37628).

GenBank accession numbers – GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences are as follows: HBP0139A3AB (PQ568149), HBP0139A4AB (PQ568150) and HBP0722E4-2 (PQ568191).

Notes – Physiologically, the new species differs from its closely related species, *Suhomyces bolitotheri*, by its ability to assimilate melezitose. Furthermore, it is distinguished from *Suhomyces chickasaworum* by its inability to ferment galactose and trehalose.

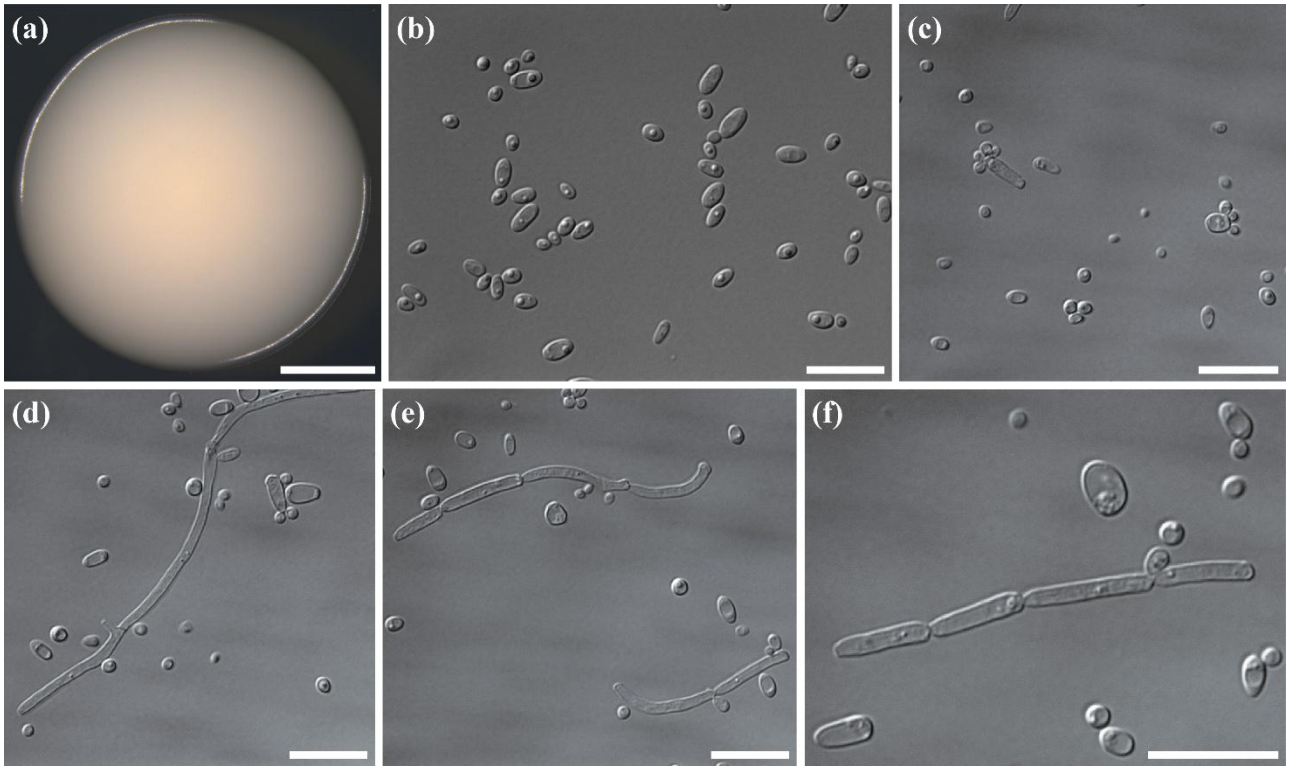

Figure 34 – Morphology of *Suhomyces shennongjiaensis* sp. nov. (strain HBP0139A3AB). (a) Colony on YM agar after 7 days. (b) Vegetative cells on YM agar after 2 days. (c) Budding cells on V8 agar after 1 month. (d) True hyphae on V8 after 1 month. (e–f) Pseudohyphae on V8 after 1 month. All at 25 °C. Scale bars: a = 2 mm, b–f = 10 μm.

***Yamadazyma* (Debaryomycetaceae, Serinales, Pichiomycetes)** Billon-Grand (1989)

Three strains clustered within the *Yamadazyma* clade (Fig. 35, Supplementary Figs. 25 and 26), forming two distinct branches. Two of these strains, HBP0209C4C1 and HBP0209E4D2, grouped together with identical D1/D2 and ITS sequences, confirming that they were conspecific. A BLAST search using the D1/D2 sequence of strain HBP0209E4D2 identified *Yamadazyma mexicana* as the closest match, differing by 19 nucleotide mismatches (3.8%, 17 substitutions and 2 gaps over 504 nucleotides) in the D1/D2 domains and 47 nucleotide mismatches (9.9%, 34 substitutions and 13 gaps over 474 nucleotides) in the ITS region. When the ITS sequence was used, the closest match was *Yamadazyma gorgasii* comb. nov., with 25 nucleotide mismatches (5.0%, 20 substitutions and 15 gaps over 504 nucleotides) in the D1/D2 domains and 23 nucleotide mismatches (4.9%, 12 substitutions and 11 gaps over 472 nucleotides) in the ITS region. Based on whole-genome sequences, the ANI values between strain HBP0209E4D2 and the two closely related species, *Yamadazyma mexicana* and *Yamadazyma gorgasii* comb. nov., were 72% and 76%, respectively (Supplementary Table 9). These results suggest that strain HBP0209E4D2 represents a novel species within the genus *Yamadazyma*.

Strain HBP0209C4C2 formed a distinct branch and did not cluster with any other strains (Fig. 35, Supplementary Figs. 25 and 26). A BLAST search using the D1/D2 sequence of strain HBP0209C4C2 identified *Yamadazyma dushanensis* as the closest match, differing by 5 nucleotide mismatches (1.0%, 5 substitutions over 487 bp) in the D1/D2 domains and 19 nucleotide mismatches (4.1%, 16 substitutions and 3 gaps over 461 bp) in the ITS region. When the ITS sequence was used, the closest match was *Yamadazyma luoyangensis*, with 10 nucleotide mismatches (2.1%, 20 substitutions over 487 bp) in the D1/D2 domain and 12 nucleotide mismatches (2.6%, 8 substitutions and 4 gaps over 464 bp) in the ITS region. The ANI value between strain HBP0209C4C2 and *Yamadazyma dushanensis* was 77% (Supplementary Table 9), indicating that strain HBP0209C4C2 represents another novel species within the genus *Yamadazyma*.

Yamadazyma betulicola Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 36

MycoBank number: MB857119

Etymology – Referring to the species isolated from *Betula*.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, glistening, smooth, flat, with an entire margin (Fig. 36a). After 7 days, the colonies become cream-colored, dull to semi-glistening (Fig. 36b). Vegetative cells are ovoid to ellipsoidal, 2.7–4.3 × 1.6–2.9 μm ($\bar{x}$ = 3.7 × 2.1 μm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 36c). In YM broth, after 1 month at room temperature, a white sediment formed, and a white ring is present. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae or pseudohyphae are not developed. Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose and trehalose (slow) are positive, while galactose, sucrose, maltose, lactose and raffinose are not fermented. Glucose, galactose, sucrose, maltose (latent), cellobiose, trehalose, melezitose, soluble starch (weak), D-xylose, L-arabinose (latent), D-arabinose (weak), L-rhamnose (slow), ethanol, glycerol, erythritol (latent), ribitol, D-mannitol (slow), D-glucitol, methyl-α-D-glucoside, salicin (slow), succinate (slow), citrate (slow), N-acetyl-D-glucosamine (latent) and xylitol (latent) are assimilated as sole carbon sources, while L-sorbose, lactose, melibiose, raffinose, inulin, D-ribose, D-glucosamine, methanol, galactitol, D-glucuronate, DL-lactate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, L-lysine (latent), ethylamine and cadaverine are assimilated as sole nitrogen sources, while potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive (slow). Starch-like substances are not produced. Urease activity is negative. Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C and 37 °C is positive, while growth at 40 °C is negative.

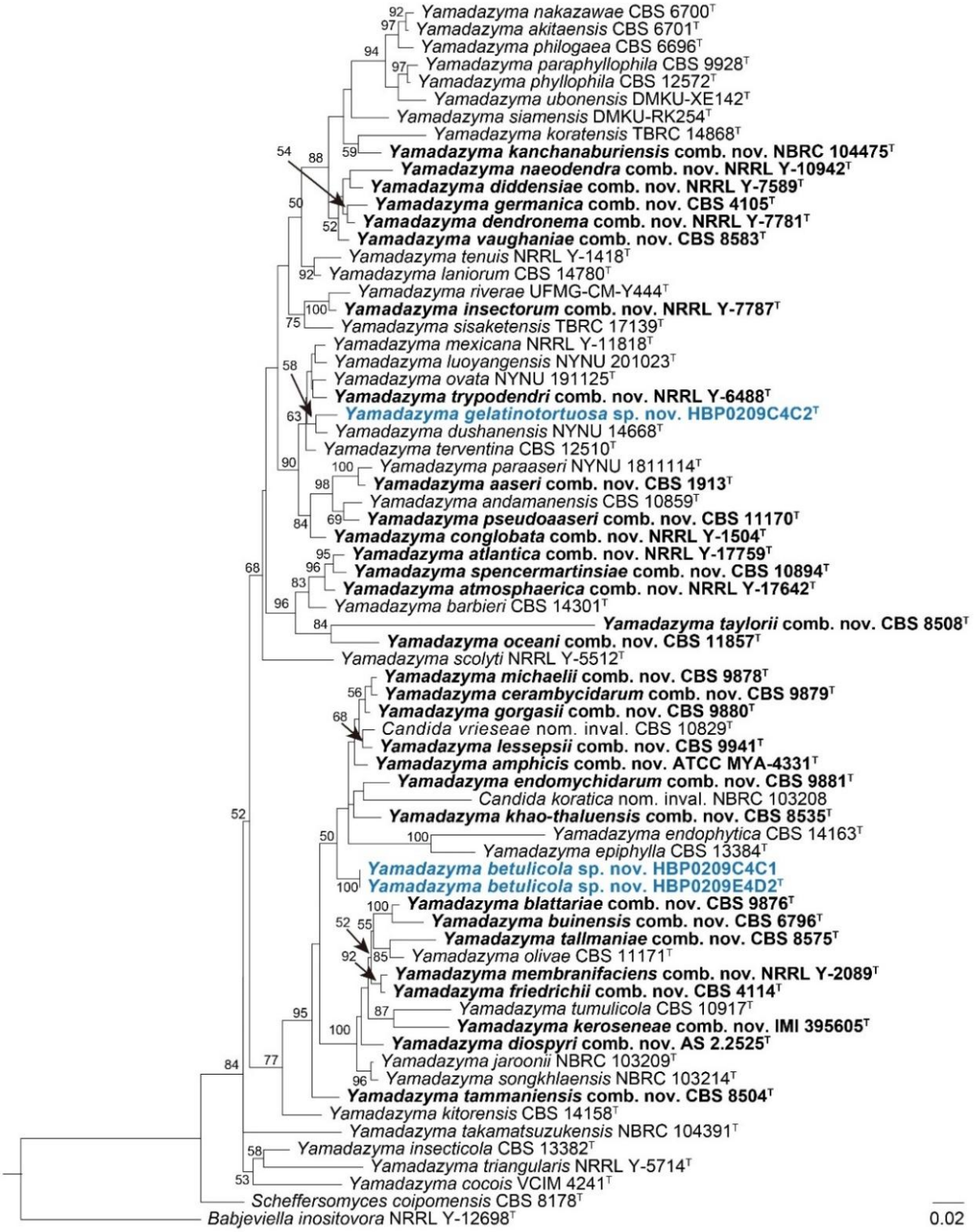

Figure 35 – Maximum-likelihood (ML) tree based on the concatenated ITS-5.8S region and D1/D2 domains of the LSU sequences, showing the phylogenetic position of *Yamadazyma betulicola* sp. nov. and *Yamadazyma gelatinotortuosa* sp. nov. Bootstrap values (≥50%) from 1000 replicates are shown along the branches. *Babjeviella inositovora* and *Scheffersomyces coipomensis* are used as the outgroup. Strains of new species isolated in this study are marked in blue, strains proposed as the same new species or new combinations are in bold, and sequences from type material are indicated with a superscripted 'T'. The scale bar represents 0.02 substitutions per nucleotide position.

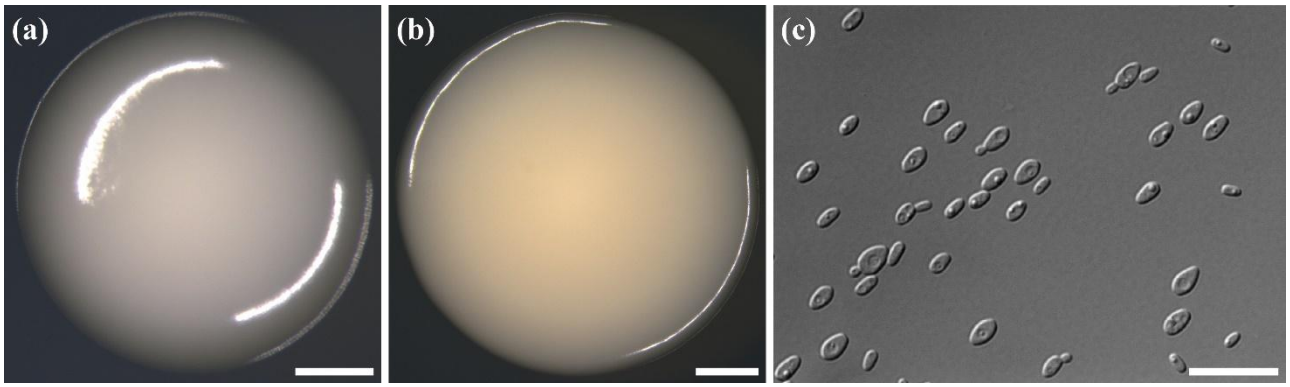

Figure 36 – Morphology of *Yamadazyma betulicola* sp. nov. (strain HBP0209E4D2). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. All at 25 °C. Scale bars: a = 500 μm, b = 1 mm, c = 10 μm.

Typus – China, Hubei Province, Shennongjia Forestry District, Tanjiawan, 31.4575°N, 110.0704°E, collected from pieces of wood from a *Betula schmidtii* tree at 2347.8 m, July 11, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8564^{T} (= HBP0209E4D2) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37630 (= CGMCC 2.8564) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

Paratype – *ibid.* HBP0209C4C1 (= CGMCC 2.8567).

GenBank accession numbers – The ITS-5.8S region and D1/D2 domains of the LSU sequences are PQ568153 for strain HBP0209E4D2 and PQ568155 for strain HBP0209E4D2.

Notes – Physiologically, the new species differs from its closely related species, *Yamadazyma mexicana* and *Yamadazyma gorgasii* comb. nov., by its inability to ferment galactose.

Yamadazyma gelatinotortuosa Y.J. Qiu, H.Y. Zhu & F.Y. Bai sp. nov. Fig. 37

MycoBank number: MB857120

Etymology – Referring to the gelatinous and twisted colony morphology.

Culture characteristics – On YM agar, after 3 days at 25 °C, the colonies are butyrous, white, glistening, with a gelatinous surface exhibiting twisted folds, and an irregularly shaped margin (Fig. 37a). After 7 days, the colonies become cream-colored and expand (Fig. 37b). Vegetative cells are ovoid to ellipsoidal, 4.2–12.3 × 1.9–3.4 μm ($\overline{x}$ = 6.4 × 2.6 μm, n = 20), reproduce by multilateral budding, and occur singly or in pairs (Fig. 37c–e). In YM broth, after 1 month at room temperature, a white sediment formed, and a white ring is present. In Dalmau plate culture on CMA, after 2 weeks at 25 °C, true hyphae and pseudohyphae, sometimes with blastoconidia produced laterally, are developed under the coverglass (Fig. 37f–h). Sexual structures are not observed on YCB agar, CMA, PDA, or V8 agar after 1 month at 25 °C.

Physiological and biochemical characteristics – In sugar fermentation tests, glucose is positive, while galactose, sucrose, maltose, lactose, raffinose and trehalose are not fermented. Glucose, galactose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch (weak), D-xylose, L-arabinose (slow), D-arabinose (weak), D-ribose, L-rhamnose, D-glucosamine (weak), ethanol, glycerol, erythritol, ribitol, D-mannitol, D-glucitol, methyl-α-D-glucoside, salicin (slow), succinate, citrate (weak), N-acetyl-D-glucosamine and xylitol (latent) are assimilated as sole carbon sources, while L-sorbose, lactose, inulin, methanol, galactitol, D-glucuronate, DL-lactate, myo-inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, L-lysine, ethylamine and cadaverine are assimilated as sole nitrogen sources, while sodium nitrite is not assimilated. Growth in vitamin-free medium is positive (slow). Starch-like substances are not produced. Urease activity is positive (weak). Diazonium Blue B reaction is negative. Growth in 10% (w/v) sodium chloride plus 5% (w/v) glucose medium is negative. Growth on 50% (w/v) glucose–yeast extract agar and 60% (w/v) glucose–yeast extract agar is negative. Growth at 30 °C is positive,

while growth at 37 °C and 40 °C is negative.

Typus – China, Hubei Province, Shennongjia Forestry District, Tanjiawan, 31.4575°N, 110.0704°E, collected from pieces of wood from a *Betula schmidtii* tree at 2347.8 m, July 11, 2023, by Q.Y. Zhu; isolated by Y.J. Qiu. Holotype: CGMCC 2.8565^{T} (= HBP0209C4C2) preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Isotype: JCM 37631 (= CGMCC 2.8565) deposited in the Japan Collection of Microorganisms (JCM), Koyadai, Japan.

GenBank accession number – The ITS-5.8S region and D1/D2 domains of the LSU sequence of strain HBP0209C4C2 is PQ568154.

Notes – Physiologically, the new species differs from its closely related species, *Yamadazyma dushanensis* and *Yamadazyma luoyangensis*, by its inability to ferment galactose and trehalose.

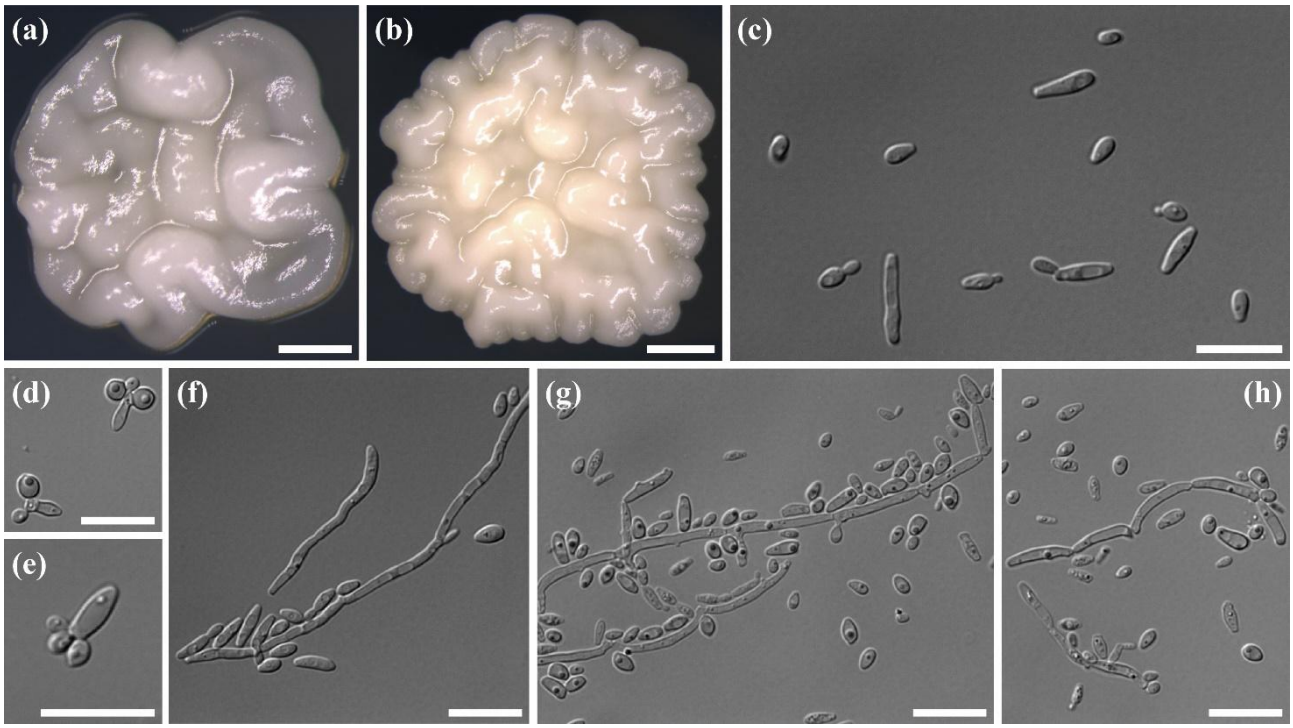

Figure 37 – Morphology of *Yamadazyma gelatinotortuosa* sp. nov. (strain HBP0209C4C2). (a) Colony on YM agar after 3 days. (b) Colony on YM agar after 7 days. (c) Vegetative cells on YM agar after 2 days. (d–e) Budding cells on PDA after 1 month. (f) True hyphae on YM agar after 2 days. (g) True hyphae on V8 after 1 month. (h) Pseudohyphae on V8 after 1 month. (g–h) Budding cells on PDA after 1 month. All at 25 °C. Scale bars: a = 500 µm, b = 1 mm, c–h = 10 µm.

New combinations

Yamadazyma aaseri (Dietrichson ex Uden & H.R. Buckley) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857136

Holotype: CBS 1913 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionyms – *Candida aaseri* Dietrichson, *Annls Parasit. hum. comp.* 29: 476 (1954)

≡ *Azymocandida aaseri* Dietrichson ex E.K. Novák & Zsolt, *Acta bot. hung.* 7: 134 (1961)

≡ *Candida aaseri* Dietrichson ex Uden & H.R. Buckley *Yeasts, a taxonomic study*, 2nd Edn (Amsterdam): 912 (1970)

Synonymy – *Candida butyri* Nakase, *J. gen. appl. Microbiol.*, Tokyo 17(6): 475 (1971)

Yamadazyma amphicis (S.O. Suh, N.H. Nguyen & M. Blackw.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857137

Holotype: ATCC MYA-4331 preserved in a metabolically inactive state in the American Type Culture Collection (ATCC), Manassas, Virginia, USA.

Basionym – *Candida amphicis* S.O. Suh, N.H. Nguyen & M. Blackw. Mycol. Res. 109(9): 1049 (2005)

Yamadazyma atlantica (Siepmann) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857138

Holotype: NRRL Y-17759 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionyms –

Trichosporon atlanticum Siepmann, Veröff. Inst. Meeresf. Bremerhaven 8: 90 (1962)

≡ *Candida atlantica* (Siepmann) S.A. Mey. & Simione, Yeasts, a taxonomic study, 3rd Edn (Amsterdam): 621 (1984)

≡ *Candida atlantica* (Siepmann) S.A. Mey. & Simione, Mycotaxon 66: 100 (1998)

Yamadazyma atmosphaerica (Santa María) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857139

Holotype: NRRL Y-17642 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionym – *Candida atmosphaerica* Santa María, Agric. Calabro-Siculo 8: 801 (1959)

Yamadazyma blattariae (S.O. Suh, N.H. Nguyen & M. Blackw.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857140

Holotype: CBS 9876 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida blattariae* S.O. Suh, N.H. Nguyen & M. Blackw., Mycol. Res. 109(9): 1049 (2005)

Yamadazyma buinensis (Soneda & S. Uchida) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857141

Holotype: CBS 6796 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida buinensis* Soneda & S. Uchida, Bull. natn. Sci. Mus., Tokyo 14(3): 448 (1971)

Yamadazyma cerambycidarum (S.O. Suh, N.H. Nguyen & M. Blackw.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857142

Holotype: CBS 9879 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida cerambycidarum* S.O. Suh, N.H. Nguyen & M. Blackw., Mycol. Res. 109(9): 1051 (2005)

Yamadazyma conglobata (Redaelli) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857143

Holotype: NRRL Y-1504 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionyms – *Torulopsis conglobata* Redaelli, Miceti Associaz. Microbica Tuberculosi Polmonare Cavitaria (Pavia): 58 (1925)

≡ *Candida conglobata* (Redaelli) Cif., Manuale di Micologia Medica, Edn 22: 245 (1960)

Yamadazyma dendronema (Van der Walt, Klift & D.B. Scott) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857144

Holotype: NRRL Y-7781 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionym – *Candida dendronema* Van der Walt, Klift & D.B. Scott, Antonie van Leeuwenhoek 37(4): 451 (1971)

Yamadazyma diddensiae (Phaff, Mrak & O.B. Williams) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857145

Holotype: NRRL Y-7589 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionyms – *Trichosporon diddensiae* Phaff, Mrak & O.B. Williams, Mycologia 44(4): 440 (1952)

≡ *Candida diddensiae* (Phaff, Mrak & O.B. Williams) Fell & S.A. Mey. Mycopath. Mycol. appl. 32: 189 (1967)

Yamadazyma diospyri (F.Y. Bai & H.Z. Lu) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857146

Holotype: AS 2.2525 preserved in a metabolically inactive state in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China.

Basionym – *Candida diospyri* F.Y. Bai & H.Z. Lu, Int. J. Syst. Evol. Microbiol. 54(4): 1412 (2004)

Yamadazyma endomychidarum (S.O. Suh, N.H. Nguyen & M. Blackw.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857147

Holotype: CBS 9881 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida endomychidarum* S.O. Suh, N.H. Nguyen & M. Blackw., Mycol. Res. 109(9): 1052 (2005)

Yamadazyma friedrichii (Uden & Windisch) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857148

Holotype: CBS 4114 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – Candida friedrichii Uden & Windisch, Antonie van Leeuwenhoek 34: 270 (1968)

Yamadazyma germanica (Kurtzman, Robnett & Yarrow) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857149

Holotype: CBS 4105 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida germanica* Kurtzman, Robnett & Yarrow, Antonie van Leeuwenhoek 80(1): 79 (2001)

Yamadazyma gorgasii (S.O. Suh, N.H. Nguyen & M. Blackw.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857150

Holotype: CBS 9880 preserved in a metabolically inactive state in the Westerdijk Fungal

Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida gorgasii* S.O. Suh, N.H. Nguyen & M. Blackw., Mycol. Res. 109(9): 1051 (2005)

Yamadazyma insectorum (D.B. Scott, Van der Walt & Klift) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857151

Holotype: NRRL Y-7787 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionym – *Candida insectorum* D.B. Scott, Van der Walt & Klift, Mycopath. Mycol. appl. 47(3): 229 (1972)

Yamadazyma kanchanaburiensis (Nakase, Jindam., Ninomiya, Imanishi, H. Kawas. & Potach.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857152

Holotype: NBRC 104475 preserved in a metabolically inactive state in the NITE Biological Resource Center (NBRC), Kisarazu, Japan.

Basionym – *Candida kanchanaburiensis* Nakase, Jindam., Ninomiya, Imanishi, H. Kawas. & Potach., J. gen. appl. Microbiol., Tokyo 54(5): 262 (2008)

Yamadazyma keroseneae (Buddie, Bridge, J. Kelley & R.J. Ryan) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857153

Holotype: IMI 395605 preserved in a metabolically inactive state in the CABI Culture Collection (IMI), United Kingdom.

Basionym – *Candida keroseneae* Buddie, Bridge, J. Kelley & R.J. Ryan, Lett. Appl. Microbiol. 52(1): 74 (2011)

Yamadazyma khao-thaluensis (J.Z. Groenew., M.T. Sm. & V. Robert) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857154

Holotype: CBS 8535 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida khao-thaluensis* J.Z. Groenew., M.T. Sm. & V. Robert, Persoonia 26: 43 (2011)

Yamadazyma lessepsii (S.O. Suh, N.H. Nguyen & M. Blackw.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857155

Holotype: CBS 9941 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida lessepsii* S.O. Suh, N.H. Nguyen & M. Blackw., Mycol. Res. 109(9): 1053 (2005)

Yamadazyma membranifaciens (Lodder & Kreger-van Rij) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857156

Holotype: NRRL Y-2089 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionyms – *Candida melibiosi* var. *membranifaciens* Lodder & Kreger-van Rij, Yeasts, a taxonomic study, [Edn 1] (Amsterdam): 673 (1952)

≡ *Candida membranifaciens* (Lodder & Kreger-van Rij) Wick. & Burton, J. Bact. 68: 597 (1954)

Synonymys – *Candida majoricensis* Genestar, Microbiol. esp. 9: 278 (1956)

≡ *Procandida majoricensis* (Genestar) E.K. Novák & Zsolt, Acta bot. hung. 7: 133 (1961)

Candida membranifaciens subsp. *flavinogenes* Lin Wang, Z.M. Chi, Xiang H. Wang, L. Ju & N. Guo, Microbiol. Res.163(3): 264 (2008)

Oospora srinivasii Mahdih., Centbl. Bakt. ParasitKde, Abt. II 78: 254-259 (1929)

Yamadazyma michaelii (S.O. Suh, N.H. Nguyen & M. Blackw.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857157

Holotype: CBS 9878 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionyms – *Candida michaelii* S.O. Suh, N.H. Nguyen & M. Blackw., Mycol. Res. 109(9): 1049 (2005)

Yamadazyma naeodendra (Van der Walt, Johannsen & Nakase) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857158

Holotype: NRRL Y-10942 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionyms – *Candida naeodendra* Van der Walt, Johannsen & Nakase, Antonie van Leeuwenhoek 39(3): 491 (1973)

Yamadazyma oceani (Burgaud & G. Barbier) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857159

Holotype: CBS 11857 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida oceani* Burgaud & G. Barbier, Antonie van Leeuwenhoek 100(1): 79 (2011)

Yamadazyma pseudoaaseri (Pfüller, Y. Gräser, M. Erhard & M. Groenew.) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857160

Holotype: CBS 11170 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida pseudoaaseri* Pfüller, Y. Gräser, M. Erhard & M. Groenew., J. Clin. Microbiol. 49(12): 4199 (2011)

Yamadazyma spencermartinsiae (Statzell, Scorzetti & Fell) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857161

Holotype: CBS 10894 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida spencermartinsiae* Statzell, Scorzetti & Fell, Int. J. Syst. Evol. Microbiol. 60(8): 1980 (2010)

Yamadazyma tallmaniae (J.Z. Groenew., M.T. Sm. & V. Robert) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857162

Holotype: CBS 8575 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida tallmaniae* J.Z. Groenew., M.T. Sm. & V. Robert, Persoonia 26: 44 (2011)

Yamadazyma tammaniensis (Kurtzman & Robnett) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857163

Holotype: CBS 8504 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida tammaniensis* Kurtzman & Robnett, Can. J. Microbiol. 44(10): 968 (1998)

Yamadazyma taylorii (Statzell, Scorzetti & Fell) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857164

Holotype: CBS 8508 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida taylorii* Statzell, Scorzetti & Fell, Int. J. Syst. Evol. Microbiol. 60(8): 1981 (2010)

Yamadazyma trypodendri (Kurtzman & Robnett) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857165

Holotype: NRRL Y-6488 preserved in a metabolically inactive state in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA.

Basionym – *Candida trypodendri* Kurtzman & Robnett, Can. J. Microbiol. 44(10): 971 (1998)

Yamadazyma vaughaniae (J.Z. Groenew., M.T. Sm. & V. Robert) H.Y. Zhu, Y.J. Qiu, P.J. Han & F.Y. Bai comb. nov.

MycoBank number: MB857166

Holotype: CBS 8583 preserved in a metabolically inactive state in the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands.

Basionym – *Candida vaughaniae* J.Z. Groenew., M.T. Sm. & V. Robert, Persoonia 26: 44 (2011)

DISCUSSION

To date, research on yeast diversity in Shennongjia has been limited, with most studies focusing on animals and plants. In this study, we established a dataset of 4,451 yeast isolates, comprising strain taxonomy, sample information, and isolation conditions, to provide deeper insights into the species composition of yeast in Shennongjia. In total, 147 ascomycetous and 14 basidiomycetous yeast species were identified. By 2020, at least 782 yeast species were known in China (Boekhout et al. 2022). Over the subsequent four years, 125 new yeast species were discovered (Zhu et al. 2025). The yeast species identified in this study constitute approximately 17.7% of the total reported in China, highlighting the pressing need for a deeper investigation into the unexplored yeast diversity in Shennongjia. However, systematic studies on the yeast diversity of this region have been lacking, and records of species occurrence in Shennongjia remain limited. Our study is expanding the known diversity and ecology of wild yeasts, and is showing the importance of documenting both novel and known species to advance microbial diversity research.

Notably, six *Saccharomyces* species were found in Shennongjia. The genus *Saccharomyces* currently comprises nine natural species: *Saccharomyces cerevisiae*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces kudriavzevii*, *Saccharomyces arboricola*, *Saccharomyces jurei*, *Saccharomyces eubayanus*, *Saccharomyces uvarum*, and the newly discovered *Saccharomyces chiloensis* (Alsammar & Delneri 2020, Peña et al. 2024). In this study, we isolated six *Saccharomyces* species: *S. cerevisiae* (173 strains), *S. paradoxus* (67 strains), *S. mikatae* (40 strains), *S. kudriavzevii* (41 strains), *S. arboricola* (43 strains), and *S. eubayanus* (13 strains) (Supplementary Table 4). Although these six species have previously been reported from different regions of China (Peris et al. 2023), to our knowledge, there has been no record of all of them occurring in the same geographic area. In addition, Shennongjia is home to the oldest lineage (CHN IX) of *S. cerevisiae* and holds the richest genetic diversity of this species in China (Duan et al. 2018, Peter et al. 2018, Han et al. 2021). These findings underscore the exceptionally high diversity of *Saccharomyces* in Shennongjia, a rarity

on a global scale.

Among the top 20 yeast species isolated in this study (Supplementary Table 4) , many are closely associated with food technology. *Saccharomyces cerevisiae*, the most frequently isolated species, has been central to wine brewing and food fermentation for nearly 10,000 years. The amylolytic yeast *Saccharomycopsis fibuligera*, typically associated with starch-rich fermentation environments (Kurtzman & Smith 2011; Wang et al. 2021), is rarely reported from wild habitats; however, 28 isolates were obtained in this study, expanding our understanding of its natural sources. *Torulaspora delbrueckii*, another widely distributed yeast, is recognized for its contribution to food preservation, effectively inhibiting spoilage microorganisms during early fermentation stages (Simonin et al. 2018). Similarly, *Candida sake* has attracted interest in modern enology, where non-*Saccharomyces* yeasts are used to enhance low-temperature fermentations, reduce ethanol content, and enrich aromatic complexity (Ballester-Tomás et al. 2017). The wide variety of yeast species found in Shennongjia, many of which are already utilized in human applications, suggest that a wealth of untapped natural microbial resources remains to be explored.

Most importantly, based on the phylogenetic tree of combined ITS-D1/D2 sequences and whole-genome sequencing, we formally described 21 novel species and proposed 43 new taxonomic combinations. Numerous high-quality images of morphological characteristics of these new species are provided, representing a valuable reference for taxonomic studies. Interestingly, *Saccharomycopsis shennongjiaensis* sp. nov. was found to prey on *Candida nitratophila* by deploying infection pegs. This predation is a directed physical attack aimed at retrieving nutrients from the prey cell, also found in most *Saccharomycopsis* species (Kurtzman & Smith 2011, Junker et al. 2019, Santos et al. 2023, Dost et al. 2024). Leveraging this necrotrophic mycoparasitic ability, *Saccharomycopsis* species have shown potential in biocontrol applications, such as killing multidrug-resistant *Candida auris* (Junker et al. 2018) and suppressing *Penicillium* species on oranges (Pimenta et al. 2008, 2010). Furthermore, the molecular mechanism underlying this predatory behavior, regulated by a conserved MAP kinase signaling pathway, is triggered by methionine starvation (Rij et al. 2024). Unlike the vast majority of yeasts that can assimilate sulfur in the form of inorganic sulfate, members of the *Saccharomycopsis* clade lack this ability due to the complete absence of genes in the sulfate assimilation pathway (Junker et al. 2017, 2019). This aligns with our finding that *Saccharomycopsis shennongjiaensis* sp. nov. cannot utilize ammonium sulfate. To our knowledge, the only other yeast showing a similar inability is the genus *Yueomyces*, which also lacks the capacity for sulfate assimilation (Yu et al. 2023). Such yeasts with unique metabolic characteristics represent interesting subjects for future molecular and physiological investigations.

However, the exploration of yeast diversity in Shennongjia remains limited, as many forested areas are inaccessible due to conservation regulations and safety concerns. To uncover potential yeast species in Shennongjia, more innovative methods should be incorporated. Traditional hand-based isolation techniques are labor-intensive and can be influenced by subjective factors. Increasing the number of isolations can improve the likelihood of discovering rare or novel species (Lewis et al. 2021). Recently, a high-throughput automated colony isolation platform was developed (Huang et al. 2023), which, although originally designed for gut bacteria, may have potential to be adapted for large-scale environmental yeast surveys in the future. Meanwhile, continued conservation efforts are essential to protect Shennongjia and other similarly valuable regions recognized as national nature reserves or as internationally protected ecological areas, such as World Heritage Sites, Global Geoparks, or Biosphere Reserves.

ACKNOWLEDGEMENTS

This study was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDB0810000), the China Postdoctoral Science Foundation (No. 2025M782844), the National Science and Technology Fundamental Resources Investigation Program of China (No. 2023FY101300), the National Natural Science Foundation of China (Nos. 32170006, 32170011 and 32470007), the Youth Innovation Promotion Association of the Chinese Academy of Sciences (No. 2022087), and the Hubei Provincial Key Research and Development Program (No. 2023BBB004).

DECLARATION OF COMPETING INTEREST

All authors declare that they have no competing interests.

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SUPPLEMENTAL MATERIAL

All supplementary materials are available at https://doi.org/10.6084/m9.figshare.28722251.

Supplementary Figures – Neighbor-Joining tree based on D1/D2 or ITS sequences

Supplementary Table 1 – Information on 624 samples collected in this study

Supplementary Table 2 – Dataset of 4,451 yeast isolates in this study

Supplementary Table 3 – Strains and GenBank accession numbers used for phylogenetic analysis in this study

Supplementary Table 4 – Number of strains for each species isolated in this study

Supplementary Table 5 – Sequence differences between the new species and closely related species in this study

Supplementary Table 6 – Physiological and biochemical differences between the new species and closely related species in this study

Supplementary Table 7 – Information on all strains whose genomes were sequenced in this study

Supplementary Table 8 – Information for all assembled genomes used in this study

Supplementary Table 9 – ANI values calculated in this study