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# Three Novel Ascomycetous Yeast Species Isolated from Plant Leaves and Wild Mushrooms Collected in Thailand

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**ABSTRACT.–** Four yeast strains representing three novel species were isolated from two sources in Thailand, one from the leaf surface of a tall-stilt mangrove (*Rhizophora apiculata*, strain DMKU-RG45) and three from the fruiting bodies of wild mushrooms (strains DMKU-TM03, DMKU-SM11, and DMKU-SM28) collected in Thailand. Sequence analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene and the internal transcribed spacer (ITS) region revealed that these four strains differed by 0.87–8.68% and 2.07–13.55% in their nucleotide divergence from their closest described species in the sequences of the D1/D2 domain of the LSU rRNA gene and the ITS region, respectively. In phylogenetic analyses, the strain DMKU-RG45 belonged to the genus *Cyberlindnera* and was distinct from other recognized species in this genus, whereas the strains DMKU-TM03, DMKU-SM11, and DMKU-SM28 were members of the genus *Blastobotrys*. However, they were in two different positions, each of which was distinct from closely related species. Based on molecular analyses and phenotypic characteristics, the strain DMKU-RG45 is proposed as *Cyberlindnera sirindhorniae* f.a., sp. nov. (the holotype is TBRC 19863$^T$; MycoBank No. is MB 858763). The other three strains (DMKU-TM03, DMKU-SM11, and DMKU-SM28) are proposed as two novel *Blastobotrys* species. *Blastobotrys princeps* f.a., sp. nov. is proposed to accommodate the strain DMKU-TM03 (the holotype is TBRC 19382$^T$; MycoBank No. is MB 858764). *Blastobotrys siamensis* f.a., sp. nov. is proposed to accommodate both strains DMKU-SM11 and DMKU-SM28 (the holotype is TBRC 19380$^T$; MycoBank No. is MB 858765).

**KEYWORDS:** ***Blastobotrys princeps*, *Blastobotrys siamensis*, *Cyberlindnera sirindhorniae*, Mangrove Forest, Mushroom**

## INTRODUCTION

The genus *Blastobotrys* was originally described by von Klopotek in 1967 as anamorphic members of the order *Saccharomycetales*, with *Blastobotrys nivea* as the type species (Smith et al., 2011a). A phylogenetic study using the D1/D2 domain of the large subunit (LSU) rRNA gene revealed that members of the genus *Blastobotrys* were closely related to the anamorphic genera *Arxula*, *Sympodiomyces*, and many species of the anamorphic genus *Candida* (Kurtzman and Robnett 1998; Chai et al., 2020). Later, the phylogenetic relationships among these genera were re-examined using multigene phylogenetic analysis of the mitochondrial small subunit (mtSSU) rRNA, cytochrome oxidase II (*COXII*), and the LSU rRNA genes (Kurtzman and Robnett, 2007). This multigene phylogenetic analysis demonstrated that *Blastobotrys*, *Arxula*, *Sympodiomyces*, and several species of *Candida* formed a clade with teleomorphic species in the *Trichomonascus* clade (Kurtzman and Robnett, 2007; Barretto et al., 2018), and *Trichomonascus mycophagus* was the type species (Smith et al., 2011b). Among these genera, the genus *Blastobotrys* has taxonomic priority over *Sympodiomyces* (Fell and Statzell, 1971) and *Arxula* (van der Walt et al. 1990). Consequently, anamorphic species formerly assigned to the latter two genera were transferred to the genus *Blastobotrys* to meet the rule of "one fungus, one name". This is the most important requirement for fungi, in keeping with the *International Code of Nomenclature for algae, fungi, and plants (ICNafp)* (McNeill et al., 2012). Although *Trichomonascus* (Jackson, 1947) has taxonomic priority over *Blastobotrys*, the use of *Blastobotrys* as a generic name for the *Trichomonascus*/*Blastobotrys* clade was favoured by participants of a workshop on yeast taxonomy in 2015 at Utrecht, The Netherlands (Nouri et al., 2018; Chai et al., 2020). In the latest edition of *The Yeasts: A Taxonomic Study*, 21 species were accepted as members of the genus *Blastobotrys* (Smith et al., 2011a). Later, several additional species of the genus were reported (Barretto et al., 2018; Nouri et al., 2018; Thomas et al., 2019; Visagie et al., 2022; Visagie et al., 2024). At present, there are 38 species in the genus *Blastobotrys.*

The genus *Lindnera* was proposed by Kurtzman et al. (2008) based on a multigene phylogenetic analysis (the nuclear SSU rRNA gene, the D1/D2 domain of the LSU rRNA, and the translation elongation factor-1α genes), to accommodate 11 species previously classified under the genera *Pichia* and *Williopsis* (Kurtzman et al. 2008). Among them, *L. americana* (formerly *Pichia americana*) was assigned as the type species (Kurtzman et al. 2008, Kurtzman, 2011). Later, the term *Lindnera* was found to have been previously used for the genus name *Lindnera* in a validly published

plant genus. Consequently, the name *Cyberlindnera* was proposed, and the recognized *Lindnera* species were transferred to *Cyberlindnera* as new combinations (Minter, 2009). The members of the genus are diverse in terms of their sexual cycle. Some species are homothallic while others are heterothallic (Brysch-Herzberg et al. 2021). The genus shows noticeable differences in ascospore morphology, such as hat-shaped, spheroidal, or spheroidal with an equatorial ledge (Barros et al. 2021). The number of ascospores formed in each ascus is variable and ranges from one to four (Barros et al. 2021; Brysch-Herzberg et al. 2021). According to the *ICNafp* (McNeill et al., 2012), which permits the assignment of related anamorphic or teleomorphic species to the same genus, several additional members of the genus *Cyberlindnera* were later described for a species without a teleomorphic state. Some *Candida* species were reassigned to this genus as a new combination (Brysch-Herzberg et al., 2021). At the time of writing, the *Cyberlindnera* clade consists of 46 species, including 36 *Cyberlindnera* species and 10 anamorphic *Candida* species. Among the 36 *Cyberlindnera* species, 27 are teleomorphic, including both heterothallic and homothallic species. The heterothallic species are *C. americana*, *C. bimundalis*, *C. dasilvae*, *C. euphorbiae*, *C. euphorbiiphila*, *C. fabianii*, *C. lachancei, C. maclurae*, *C. meyerae*, *C. mississippiensis*, *C. misumaiensis*, *C. rhodanensis* and *C. xylosilytica*, (Smith et al., 2011b; Barros et al., 2021; Liu et al., 2024), whereas *C. amylophila*, *C. jadinii*, *C. japonica*, *C. mrakii*, *C. petersonii*, *C. rhizosphaerae*, *C. sargentensis*, *C. saturnus*, *C. suaveolens*, *C. subsufficiens*, *C. sylvatica*, *C. veronae*, *C. wuzhiensis* and *C. xylebori* are homothallic (Smith et al., 2011b; Brysch-Herzberg et al., 2021; Liu et al., 2024).

Plant leaves are important and interrelated habitats of yeasts. Yeast detected on plant leaves can also be either ascomycetous or basidiomycetous species (Khunnamwong et al., 2018; Into et al., 2020). The presence of yeast in plant leaves depends on various factors, e.g., plant species, leaf age, rainfall and climate (Lindow and Brandl, 2003; Khunnamwong et al., 2018). Mangrove trees, primarily members of the family *Rhizophoraceae*, occur in intertidal wetlands of tropical and subtropical regions. Mangrove ecosystems and their associated fungal communities are key components of ecologically and economically significant food webs (Chang et al., 2021). Previously, the yeast community in the mangrove ecosystem has been examined and indicated that mangrove forests serve as favorable habitats for yeasts, and various novel species, such as *Candida taylorii*, *Candidozyma chanthaburiensis*, *Nakaseomyces kungkrabaensis*, *Sungouiella suratensis*, *Vishniacozyma changhuana*, *V. siamensis*, *V. taiwanica* and Y*arrowia phangngaensis* were proposed (Limtong et al., 2008; Limtong et al., 2010; Statzell-Tallman et al., 2010; Kunthiphun et al., 2018; Hoondee et al., 2019; Chang et al., 2021; Gungprakhon et al., 2025).

Over the past decade, studies on yeasts associated with fruiting bodies of mushrooms collected from agricultural and natural ecosystems have led to the discovery of several novel species such as *Kodamaea kaohsiungensis*, *K. lidongshanica*, *K. samutsakhonensis*, *K. schenbergiae*, *Kloeckera taiwanica*, *Nakazawaea tricholomae*, *Rhodotorula tropicalis*, *Suhomyces schwaniae*, *Wickerhamiella nakhonpathomensis*, *Wickerhamomyces corioli*, *Yamadazyma sisaketensis* and *Yamadazyma koratensis* (Hsieh et al., 2010; Chang et al., 2012; Khunnamwong et al., 2022; Khunnamwong et al., 2023; Nualthaisong et al., 2023; Liu et al. 2024a; Liu et al. 2024b; Santa-Brigída et al., 2024; Khunnamwong et al., 2025). As mentioned above, mushrooms appear to be a promising source for yeasts, as well as for the discovery of novel yeast species.

Thailand is located in the tropical region and characterized by diverse landscapes, including mountains, plateaus, lowlands, coastal zones, and agricultural areas. This geographical heterogeneity supports a wide range of ecosystems, such as evergreen forests, deciduous forests, and mangrove forests, making the country one of the regions with high biological richness. Although studies on yeast biodiversity have increased in recent years, knowledge of yeast diversity in Thailand remains relatively limited and insufficiently explored. During surveys of yeasts associated with wild mushrooms and a tall-stilt mangrove (*Rhizophora apiculata*) leaves collected in Thailand, one strain (DMKU-RG45) was isolated from a tall-stilt mangrove leaf sample, and three strains (DMKU-TM03, DMKU-SM11, and DMKU-SM28) were isolated from three fruiting body samples of different wild mushrooms. Based on molecular analysis of the D1/D2 domain of the LSU rRNA and ITS sequences, as well as physiological testing, the strain DMKU-RG45 represents a novel ascomycetous yeast species in the genus *Cyberlindnera*, for which the name *Cyberlindnera sirindhorniae* f.a., sp. nov. is proposed. While the strains DMKU-TM03, DMKU-SM11, and DMKU-SM28 were classified as two novel ascomycetous yeast species in the genus *Blastobotrys*, for which the name *Blastobotrys princeps* f.a., sp. nov. (strain DMKU-TM03) and *Blastobotrys siamensis* f.a., sp. nov. (strains DMKU-SM11 and DMKU-SM28) are proposed to accommodate them. Sexual spore formation of the proposed novel yeast species was not observed in this study. Thus, the designation *forma asexualis*

(f.a.) was included following the recommendation of Lachance (2012).

## MATERIALS AND METHODS

### Sample collection and yeast isolation

Fruiting body samples of wild mushrooms were collected from Phu Pha Thoep National Park, Mukdahan Province, Thailand, and a coconut plantation in Ban Phaeo District, Samut Sakhon Province, Thailand (Table 1). Sterile sampling bags and sterile gloves were used to collect samples to minimize the risk of contamination. Three strains (DMKU-TM03, DMKU-SM11, and DMKU-SM28) were isolated using the method described by Khunnamwong et al. (2023). Three grams of mushroom gills were aseptically suspended in 10 ml of a sterile saline solution (0.85%) in a 250 ml Erlenmeyer flask and incubated on a rotary shaker at 25 °C for 30 h to detach yeast cells from the gill surfaces. The suspension was serially diluted tenfold (1:10 to 1:100). A 0.1 ml aliquot of the washing solution was then spread on yeast extract peptone dextrose (YPD) agar (0.1% yeast extract; 2% peptone; 2% glucose and 2% agar) supplemented with 0.02% chloramphenicol and incubated at 25 °C until yeast colonies appeared. *Rhizophora apiculata* leaves were collected from the International Mangrove Botanical Garden Rama IX, Chanthaburi Province, Thailand, on 12 June 2023 (Table 1). The strain DMKU-RG45 was isolated using the method described by Gungprakhon et al. (2025). Briefly, ten leaves of each sample were immersed in 100 ml of a 0.85% saline solution (NaCl) in a 500 ml Erlenmeyer flask and incubated on a rotary shaker at room temperature (30±2ºC) for 60 min to detach yeast cells from the leaves. A 0.1 ml aliquot of the washing solution was then spread onto YPD agar supplemented with 0.02% chloramphenicol and 0.025% sodium propionate. Yeast colonies of different morphologies were selected and purified by cross-streaking on YPD agar. All purified yeast strains were suspended in yeast extract–malt extract (YM) broth (0.3% yeast extract; 0.3% malt extract; 0.5% peptone; 1.0% dextrose; 2.0% agar) supplemented with 20% glycerol and stored at -80 °C. Information of yeasts used in this study is shown in Table 1.

### DNA sequencing and phylogenetic analysis

Genomic DNA was extracted from yeast cells obtained from two-day-old colonies grown on YM agar. The methods used for DNA extraction and amplification were described by Limtong et al. (2007). Sequences of the D1/D2 domain of the LSU rRNA gene and the ITS region were determined from polymerase chain reaction (PCR) products amplified from genomic DNA. Amplification of the D1/D2 domain of the LSU rRNA gene was done by PCR with the forward primer NL1 (5′-CATATCAATAAGCG GAGGAAAAG-3′) and reverse primer NL4 (5′-GGT CCGTGTTTCAAGACGG-3′) (Kurtzman and Robnett, 1998). The ITS region was amplified with the forward primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and reverse primer ITS4 (5′-TCCTCCGCTTATTGAT ATGC-3′) (White et al., 1990). The PCR products were examined and purified using agarose gel electrophoresis. Purified products were commercially sequenced by APICAL SCIENTIFIC Laboratory (Selangor, Malaysia) using primers NL1 and NL4 for the D1/D2 domain and primers ITS1 and ITS4 for the ITS region. Sequences generated from the forward and reverse primers were aligned and assembled using MEGA software, Version 11 (MEGA11) (Tamura et al., 2021) to obtain complete gene sequences. Then, the assembled sequences were compared with the GenBank database using the BLASTn search tool (Altschul et al., 1997). Phylogenetic trees based on the combined sequences of the ITS region and the D1/D2 domain of the LSU rRNA gene were reconstructed using the neighbour-joining (NJ) method in MEGA 11 software. Confidence levels of the clades were estimated from bootstrap analysis (1000 replicates) (Felsenstein, 1985). Only values greater than 50% were recorded on the resulting trees. Reference sequences were retrieved from GenBank under the accession numbers indicated on the phylogenetic tree.

### Morphological and physiological characterization

Morphological, biochemical, and physiological characterization of four yeast strains (DMKU-TM03, DMKU-SM11, DMKU-SM28, and DMKU-RG45) obtained in this study was performed in accordance with the standard methods described by Kurtzman et al. (2011). Mycelium formation was investigated by cultivation on corn meal agar (2% corn meal infusion; 1.5% agar) and potato dextrose agar (PDA; 20% potato infusion, 2% glucose, and 1.5% agar) using a slide culture technique and incubated at 25 °C for 4 weeks. Sexual processes were investigated for individual strains and strain pairs on corn meal agar, 5% malt extract agar (5% malt extract and 1.5% agar), Fowell's acetate agar (0.5% sodium acetate; 2% agar), Gorodkowa agar (0.1% glucose; 0.5% sodium chloride; 1% peptone; 2% agar) and YM agar at 15 and 25 °C for 6–8 weeks. The colony and cell morphologies were examined under a stereo microscope (Nikon) and a light microscope equipped with Nomarski differential interference contrast optics (Carl Zeiss), respectively. Carbon and nitrogen source assimilation tests were conducted in liquid medium, and starved inocula were

**TABLE 1.** Details of yeast strains examined in this study.

| Strain | Isolation source | Collection site | GenBank accession no.* | | Date of sample collection |
|---|---|---|---|---|---|
| | | | ITS | LSU | |
| *Blastobotrys princeps* f.a., sp. nov. | | | | | |
| DMKU-TM03 (TBRC 19381$^T$= PYCC 10051$^T$) | Gills of the fruiting body of *Russula* sp. | Phu Pha Thoep National Park, Mukdahan Province | PQ579848 | PQ881991 | 15 August 2022 |
| *Blastobotrys siamensis* f.a., sp. nov. | | | | | |
| DMKU-SM11 (TBRC 19380$^T$= PYCC 10050$^T$) | Gills of the fruiting body of *Russula* sp. | Coconut plantation, Samut Sakhon Province | PP832912 | PP832906 | 18 July 2022 |
| DMKU-SM28 | Gills of the fruiting body of unidentified mushroom | Coconut plantation, Samut Sakhon Province | PP833612 | PP832936 | 18 July 2022 |
| *Cyberlindnera sirindhorniae* f.a., sp. nov. | | | | | |
| DMKU-RG45 (TBRC 19863$^T$=PYCC 10336$^T$) | Leaf of *Rhizophora apiculata* | International Mangrove Botanical Garden Rama IX, Chanthaburi Province | PQ896807 | PQ892153 | 12 June 2023 |

*Gene sequences: ITS, internal transcribed spacer region; LSU, D1/D2 domain of the nuclear large subunit rRNA gene.

used in nitrogen assimilation tests (Kurtzman et al., 2011). Fermentation of carbohydrates was done in a liquid medium using Durham fermentation tubes. Cycloheximide resistance was also performed in a liquid medium, while urea hydrolysis was conducted on agar slants. Acid production and the diazonium blue B (DBB) reaction were investigated on a solid medium in Petri dishes. Growth at various temperatures (15, 30, 35, and 37 °C) was determined by cultivation on YM agar.

### Abbreviations

DBB, diazonium blue B; ITS, internal transcribed spacer; LSU, large subunit; NJ, neighbor-joining; PDA, potato dextrose agar; YM, yeast extract–malt extract; YPD, yeast extract–peptone–glucose; SSU, small subunit.

## RESULTS

### Novel species delineation and phylogenetic analysis of the genus *Blastobotrys*

All yeast strains were identified to the species level following the guidelines for yeast identification based on nucleotide sequence divergence proposed by Kurtzman and Robnett (1988), which recommend that strains exhibiting more than 1% sequence divergence within the ~600 bp D1/D2 domain of the LSU rRNA gene be regarded as distinct species. In addition, the nucleotide similarity thresholds for species delimitation in the D1/D2 domain and ITS region, as suggested by Vu et al. (2016), were also applied in this study.

Analysis of the D1/D2 domain of the LSU rRNA gene and the ITS region sequences showed that the strain DMKU-TM03 was closely related to *Blastobotrys elegans* NRRL Y-17572$^T$ and *B. aristatus* NRRL Y-17579$^T$. However, it had 2.53% nucleotide divergence (15 nucleotide (nt) substitutions) and 3.20% nucleotide divergence (19 nt substitutions) in the D1/D2 domain of the LSU rRNA gene, respectively. The strain DMKU-TM03 differed from *B. elegans* and *B. aristatus* by 9.43% nucleotide divergence (48 nt substitutions) and 4.73% nucleotide divergence (21 nt substitutions) in the ITS region, respectively. The two strains (DMKU-SM11 and DMKU-SM28) were found to share identical sequences in both the D1/D2 domain of the LSU rRNA gene and the ITS region in terms of pairwise sequence similarity. The closely related species were *B. peoriensis* CBS 14259$^T$, *B. adeninivorans* NRRL Y-17692$^T$, *B. raffinosifermentans* NRRL Y-27150$^T$, and *B. americana* CBS 10337$^T$. They had 4.73, 5.83, 6.52, and 8.68% nucleotide divergence (34, 34, 38, and 50 nt substitutions), respectively. In the ITS region, the difference from the above four closely related species was 10.61, 11.05, 12.86, and 13.55% nucleotide divergence (76, 57, 59, and 72 nt substitutions), respectively.

For phylogenetic analysis, NJ phylograms were constructed based on the concatenated sequences of the ITS region and the D1/D2 domain of the LSU rRNA gene of the strains DMKU-TM03, DMKU-SM11, and DMKU-SM28, and all recognised species of the genus *Blastobotrys* to confirm the placement of these three strains. The NJ tree indicated that the strain DMKU-TM03 was placed in the *Trichomonascus/Blastobotrys* clade (Fig. 1) but separated from other recognised species of the genus *Blastobotrys*. Two strains, DMKU-SM11 and DMKU-SM28, formed a subclade that contains the type strains, *B. adeninivorans*, *B. raffinosifermentans*, *B. americana*, and *B. peoriensis*. However,

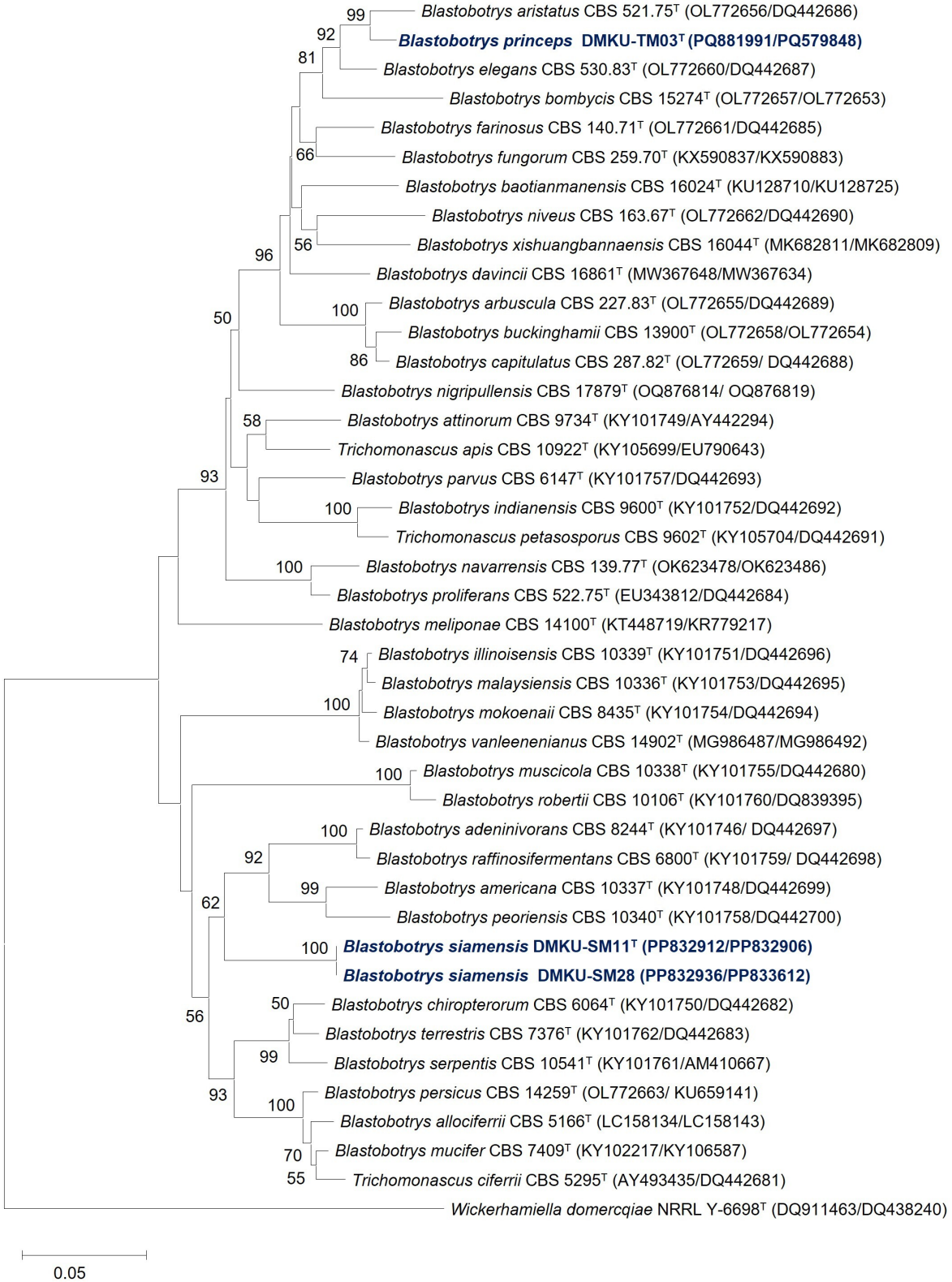


**FIGURE 1.** Phylogenetic tree based on the concatenated sequences of the ITS region and the D1/D2 domain of the LSU rRNA gene, showing positions of the two novel species with respect to closely related species. The tree was constructed with the neighbor-joining (NJ) method using MEGA 11 software. The alignment included 937 bp. Bootstrap support values above 50% are given at nodes based on 1000 replications. The strain number is indicated after the species name. The strains in this study are indicated in bold blue text. Superscript "T" indicates a type strain. The numbers in parentheses are GenBank accession numbers of the ITS/D1/D2 sequences. *Wickerhamiella domercqiae* NRRL Y-6698$^{T}$ (DQ911463/DQ438240) was used as an outgroup in these analyses. *Bar,* patristic distance of 0.05.

**TABLE 2.** Phenotypic characteristics of *Blastobotrys princeps* f.a., sp. nov., *Blastobotrys siamensis* f.a., sp. nov. and their closely related species.

| Characteristics | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| **Fermentation of carbohydrates** | | | | | | | | |
| Glucose | + | + | + | + | + | + | - | + |
| Galactose | - | - | + | + | - | - | - | v |
| Maltose | - | - | + | + | - | - | - | + |
| Sucrose | - | - | + | + | - | - | - | - |
| α-α-Trehalose | - | - | + | + | w | + | - | + |
| Lactose | - | - | - | - | nd | - | - | - |
| Raffinose | - | - | + | + | - | - | - | - |
| **Assimilation of carbon compounds** | | | | | | | | |
| D-Arabinose | + | + | + | + | + | + | - | + |
| L-Rhamnose | - | + | + | - | - | - | - | v |
| Sucrose | - | - | + | + | - | + | - | + |
| Maltose | + | + | + | + | - | + | + | + |
| α-α-Trehalose | + | + | + | - | + | - | + | + |
| Methyl-α-D-glucoside | - | - | + | + | + | + | - | v |
| Melibiose | + | - | + | + | + | + | - | v |
| Lactose | s | + | + | - | - | + | + | + |
| Raffinose | + | - | + | + | + | + | - | v |
| Melezitose | + | - | + | + | - | + | - | - |
| Inulin | + | nd | - | + | - | - | v | v |
| Soluble starch | + | nd | + | + | - | + | v | + |
| Galactitol | + | nd | - | + | + | + | v | + |
| *myo*-Inositol | + | + | + | + | + | + | - | v |
| DL-Lactate | + | + | - | + | w | - | - | - |
| Citrate | + | + | + | + | - | - | + | v |
| Methanol | - | - | - | - | nd | - | nd | nd |
| **Assimilation of nitrogen compounds** | | | | | | | | |
| Potassium nitrate | - | - | + | + | - | + | - | - |
| Sodium nitrite | - | - | nd | nd | nd | nd | nd | nd |
| **Growth characteristics** | | | | | | | | |
| Growth w/o vitamins | + | + | - | - | - | - | - | - |
| Growth at 35ºC | + | + | + | + | nd | nd | - | nd |
| Growth at 37ºC | + | + | + | + | - | nd | - | - |
| Growth in 0.01% Cycloheximide | - | - | + | + | nd | + | nd | nd |
| Growth in 0.1% Cycloheximide | - | - | + | nd | nd | + | nd | nd |
| Growth on 50% Glucose | + | + | + | nd | nd | nd | nd | nd |
| Growth on 60% Glucose | - | - | nd | nd | nd | nd | nd | nd |
| Growth on 10% NaCl + 5% glucose | + | + | nd | nd | nd | nd | - | nd |
| Growth on 15% NaCl + 5% glucose | - | - | nd | nd | nd | nd | - | nd |

Growth reactions: –, no growth; w, weak growth; s, slow growth; v, variable; +, strong growth; nd, not determined.

Species: 1, *B. siamensis* f.a., sp. nov.; 2, *B. princeps* f.a., sp. nov.; 3, *B. adeninivorans*; 4, *B. raffinosifermentans*; 5, *B. americana*; 6, *B. peoriensis*; 7, *B. elegans*; 8, *B. aristate*.

Data for species 1 and 2 are from this study, for species 3 is from Visagie et al. (2022), for species 4–6 are from Kurtzman (2007), and for species 7–8 are from Smith et al. (2011a).

they were placed at a different position with 62% bootstrap support (Fig. 1). The findings indicated that these three strains could be classified into two novel species of the genus *Blastobotrys*. These strains could be distinguished from their phylogenetically closest recognized neighbour not only by the analysis of nucleotide divergence and phylogenetic placement, but also by phenotypic characteristics, as shown in Table 2.

## Taxonomy

***Blastobotrys princeps* sp. nov.**

MycoBank number: MB858764

(Fig. 2)

**Etymology.**– The species epithet *princeps* (prin.ceps′. L. fem. n.), a Latin word meaning "the first, the foremost, the most eminent, or the first person". The name is given in honour of Her Royal Highness Princess Maha Chakri Sirindhorn of Thailand, who has long been interested in the biodiversity and conservation of Thailand's natural resources. She has encouraged studies on biodiversity and natural resource conservation in Thailand.

**Holotype.**– TBRC 19381 is designated as the holotype of *Blastobotrys princeps* f.a., sp. nov. It was isolated from fruiting body of a *Russula* sp. collected from Phu

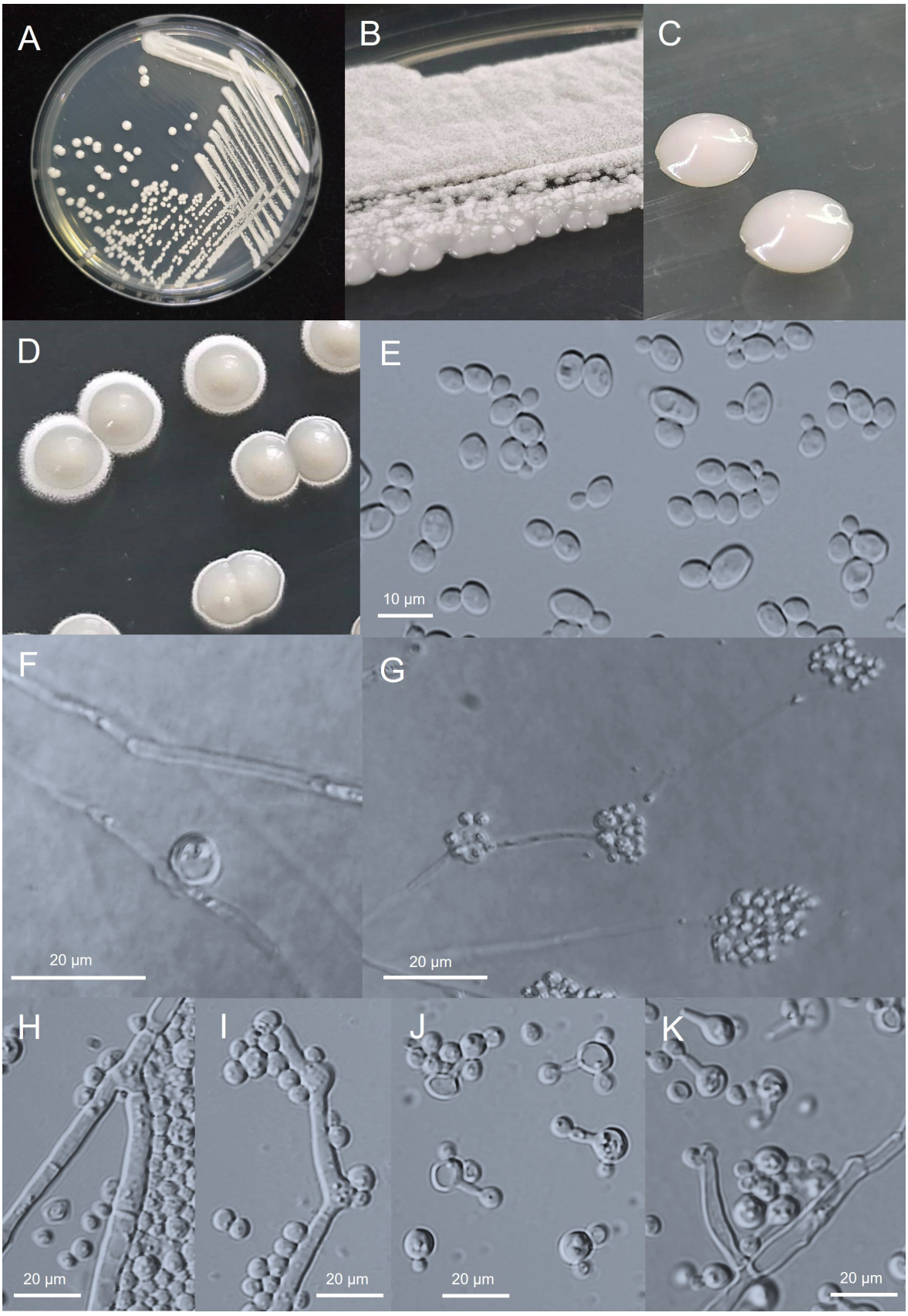


**FIGURE 2.** *Blastobotrys princeps* f.a., sp. nov. TBRC 19381$^{T}$. **A–C.** colonies on YM agar after 7 days at 25 °C; **D.** colonies on YM agar after 2 weeks at 25 °C; **E.** budding cells on YM agar after 3 days at 25 °C; **F.** hypha with spherical cells borne on denticles after 7 days on corn meal agar at 25 °C; **G.** conidiophores with primary conidia bearing secondary conidia after 7 days on corn meal agar at 25 °C; **H, I.** true hypha with blastoconidia after 7 days on corn meal agar at 25 °C; **J, K.** formation of protuberances after 7 days on Fowell's acetate agar at 25 °C.

Pha Thoep National Park in Mukdahan Province, Thailand. It is permanently preserved in a metabolically inactive state in the Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology, Thailand. The isotype culture has been preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, as PYCC 10051.

**Cell morphology and sexual state.**– After 7 days at 25 °C on YM agar, the streak culture and colonies are white to cream in colour, butyrous with raised and entire margins. Some areas of the streak culture are white with an almost powdery appearance (Figs. 2A–C). After 2 weeks, the colony margin is filamentous with a snow-white colour (Fig. 2D). After 3 days at 25 °C on YM agar, cells are subglobose to ovoid (3.5–4.9×5.3–8.4 µm) and occur singly or in pairs. Budding is multilateral (Fig. 2E). After 7 days at 25 °C, growth under the cover glass of a Dalmau plate culture with corn meal agar shows abundant true hyphae (Figs. 2F–I). Hyphal cells also form denticles with blastoconidia (Figs. 2F, G). Protuberances are produced on Fowell's acetate agar after 14 days of incubation (Fig. 2J, K). Ascospores are not observed on corn meal agar, 5% malt extract agar, Fowell's acetate agar, Gorodkowa agar and YM agar after 2 months at either 15 or 25 °C.

**Phenotypic and growth characteristics.**– Fermentation of glucose is negative. D-Glucose, galactose, sorbose, *N*-acetyl glucosamine, ribose (or weak), xylose, D-arabinose, L-arabinose, rhamnose, maltose, α-α-trehalose, cellobiose, salicin, lactose, glycerol, erythritol, ribitol, D-glucitol, D-mannitol, salicin, melibiose, lactose, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate and xylitol are assimilated. However, sucrose, methyl α-D-glycoside, melibiose, raffinose, and melezitose are not assimilated. Nitrogen assimilation is positive for ammonium sulfate, ethylamine, cadaverine, and L-lysine, but potassium nitrate and sodium nitrite are negative. Growth in a vitamin-free medium is negative. Growth occurs on 50% (w/v) and 10% (w/v) sodium chloride plus 5% (w/v) glucose. No growth occurs in the presence of 0.01 or 0.1% cycloheximide, and 15% (w/v) sodium chloride plus 5% (w/v) glucose, and 60% (w/v) glucose. Growth is observed at 15, 30, 35, and 37 °C, but not at 40 °C. Soluble starch-like extracellular carbohydrates are not produced. Acid formation is negative. Hydrolysis of urea and the DBB reaction are negative.

***Blastobotrys siamensis* sp. nov.**
MycoBank number: MB858765
(Fig. 3)

**Etymology.**–The species epithet *siamensis* (si.am. en´sis. N.L. fem. adj.), refers to Siam, the old name of Thailand, where the strains were isolated.

**Holotype.**– TBRC 19380 is designated as the holotype of *Blastobotrys siamensis* f.a., sp. nov. It was isolated from gills of fruiting body of a *Russula* sp. collected from a coconut plantation in Ban Phaeo District, Samut Sakhon province, Thailand. It is permanently preserved in a metabolically inactive state in the Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology, Thailand. The isotype culture has been preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, as PYCC 10050.

**Cell morphology and sexual state.**– After 7 days at 25 °C on YM agar, the streak culture and colonies are white to cream in colour, powdery and butyrous with lobed to filamentous margins (Figs. 3A–C). After 3 days at 25 °C on YM agar, the cells are globose to subglobose (1.1–1.7×1.9–3.2µm). Budding is multilateral (Figs. 3D, E). Elongated cells with terminal denticles are formed (Fig. 3F). Growth under the cover glass of a Dalmau plate culture with corn meal agar is quite restricted and shows true hyphae bearing blastoconidia after 7 days at 25 °C (Figs. 3G–J). Ascospores are not observed on corn meal agar, 5% malt extract agar, Fowell's acetate agar, Gorodkowa agar, or YM agar after 2 months at either 15 or 25 °C.

**Phenotypic and growth characteristics.**– Fermentation of glucose is positive. D-Glucose, D-galactose, L-sorbose, *N*-acetyl glucosamine, D-ribose (or slow), D-xylose, L-arabinose, D-arabinose, L-rhamnose (or latent), maltose, α-α-trehalose, cellobiose, salicin, lactose, glycerol, erythritol, ribitol, D-glucitol, D-mannitol, galactitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, succinate, citrate, ethanol and xylitol are assimilated. However, sucrose, methyl-α-D-glucoside, melibiose, raffinose, melezitose, inulin, soluble starch, DL-lactate, and methanol are not. Nitrogen assimilation is positive for ammonium sulfate, ethylamine, cadaverine, and L-lysine, but potassium nitrate and sodium nitrite are negative. Growth occurs on 50% (w/v) and 10% (w/v) sodium chloride plus 5% (w/v) glucose. No growth occurs in the presence of 0.01 or 0.1% cycloheximide, and 15% (w/v) sodium chloride plus 5% (w/v) glucose, and 60% (w/v) glucose. Growth is observed at 15, 30,

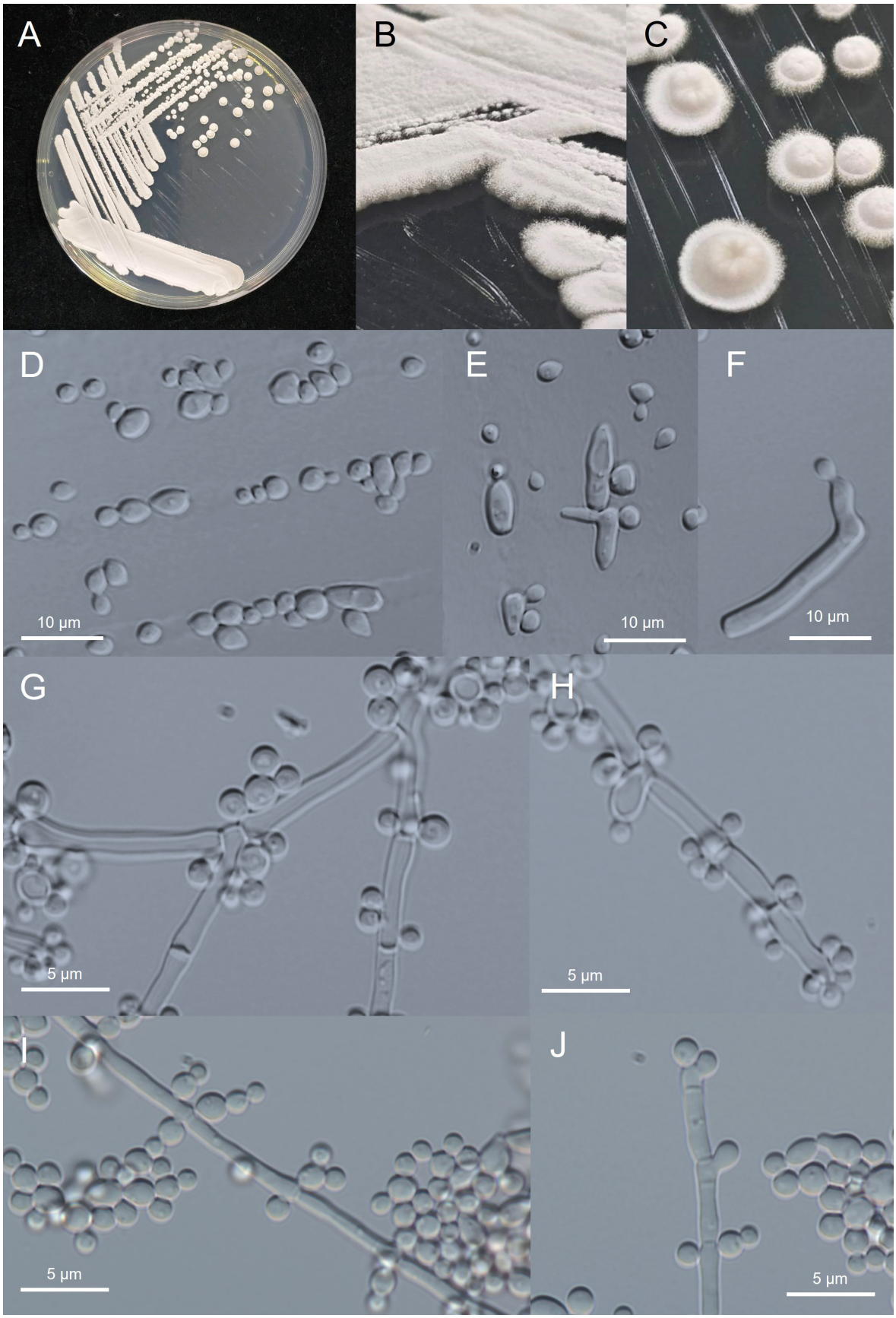


**FIGURE 3.** *Blastobotrys siamensis* f.a., sp. nov. TBRC 19380$^{T}$. **A–C.** colonies on YM agar after 7 days at 25 °C; **D, E.** budding cells on YM agar after 3 days at 25 °C; **F.** elongated cell with a terminal denticle on YM agar after 3 days at 25 °C; **G–J.** true hyphae bearing blastoconidia after 7 days at 25 °C on corn meal agar.

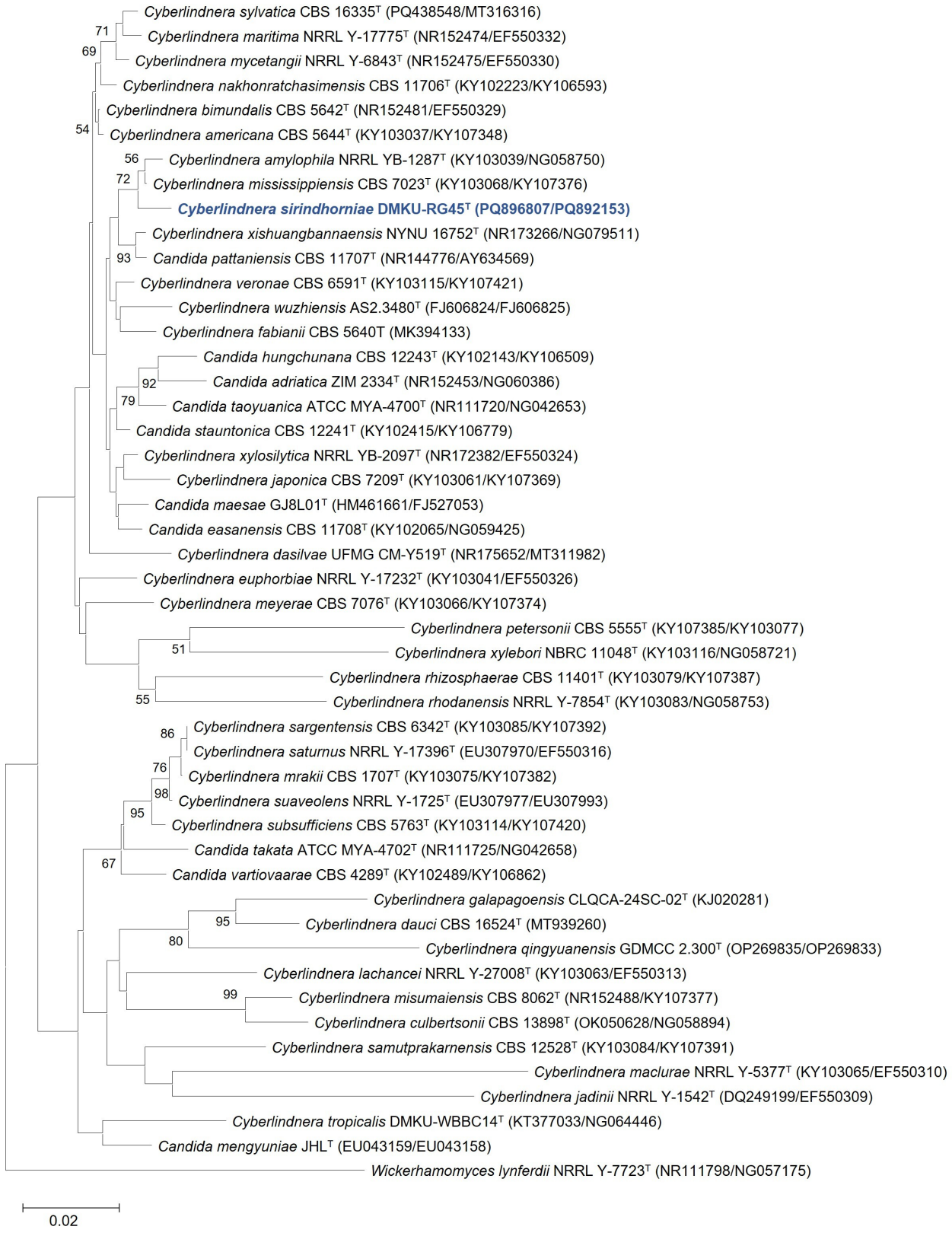


**FIGURE 4.** Phylogenetic tree based on the concatenated sequences of the ITS region and the D1/D2 domain of the LSU rRNA gene, showing positions of *Cyberlindnera sirindhorniae* f.a., sp. nov. with respect to closely related species. The tree was constructed with the neighbor-joining (NJ) method using MEGA 11 software. The alignment included 728 bp. Bootstrap support values above 50% are given at nodes based on 1000 replications. The strain number is indicated after the species name. The strain in this study is indicated in bold blue text. Superscript "T" indicates a type strain. The numbers in parentheses are GenBank accession numbers of the ITS/D1/D2 sequences. *Wickerhamomyces lynferdii* NRRL Y-7723T (NR111798/NG057175) was used as an outgroup in these analyses. *Bar,* patristic distance of 0.02.

35, and 37 °C, but not at 40 °C. Soluble starch-like extracellular carbohydrates are not produced. Acid formation is negative. Hydrolysis of urea and the DBB reaction are negative.

**Ecology**

Species of the genus *Blastobotrys* have been isolated from different sources, such as soil (*B. adeninivorans*, *B. mokoenaii*, *B. persicus*, *B. terrestris*, *B. vanleenenianus*), cave soil (*B. malaysiensis*), rotting wood (*B. xishuangbannaensis*), plants (*B. navarrensis*, *B. nigripullensis*, *B. robertii*, *B. muscicola*, *B. capitulata*), mushrooms (*B. buckinghamii*), fungi (*B. attinorum*, *B. indianensis*), insects (*B. baotianmanensis*, *B. xishuangbannaensis*), livers (*B. chiropterorum*, *B. mucifer*), the guts of animals (*B. baotianmanensis*, *B. serpentis*), indoor air (*B. arbuscula*, *B. elegans*), and house dust (*B. nigripullensis*, *B. davincii*) (Kurtzman CP, 2007; Smith et al., 2011a; Visagie et al., 2024). In Thailand, only two species of the genus *Blastobotrys* have been documented. *Blastobotrys arbuscular* was isolated from the phylloplane of rice (Into et al., 2020), and *B. chiropterorum* was isolated from sediments in mangrove forest (Kunthiphun et al., 2018). In the current study, two novel species, *B. princeps* and *B. siamensis*, were isolated from another source *viz.* fruiting bodies of wild mushrooms collected from a coconut plantation and a dry dipterocarp forest in Thailand. More research is needed to fully understand the ecological peculiarities of the species in this genus.

**Novel species delineation and phylogenetic analysis of the genus *Cyberlindnera***

Based on Blastn results, the D1/D2 domain of the LSU rRNA gene and the ITS region sequences of strain DMKU-RG45 indicated that it belongs to the genus *Cyberlindnera*. The most closely related species of this strain was *C. amylophila* NRRL YB-1287$^T$ and *C. mississippiensis* CBS 7023$^T$ in terms of pairwise sequence similarity. They had 0.87% nucleotide divergence (5 nt substitutions) and 1.38% nucleotide divergence (8 nt substitutions) in the D1/D2 domain of the LSU rRNA gene, respectively. The ITS sequence of the strain DMKU-RG45 differed by 2.16% (12 nt substitutions) and 2.07% (12 nt substitutions and 15 gaps) from *C. amylophila* NRRL YB-1287$^T$ and *C. mississippiensis* CBS 7023$^T$, respectively. Phylogenetic analysis was done based on the concatenated sequences of the ITS and the D1/D2 domain of the LSU rRNA genes of the strain DMKU-RG45 and the type strains of the recognized species in the *Cyberlindnera* clade. The NJ tree showed that the strain DMKU-RG45 was clustered with the type strains of *C. amylophila* and *C. mississippiensis*, but placed at a different position with 72% bootstrap support (Fig. 4). This confirmed its recognition as a distinct species of the genus. The strain DMKU-RG45 could be distinguished from its closely related phylogenetic species by analyses of nucleotide divergence and phylogenetic placement, as well as by phenotypic characteristics, as shown in Table 3. Based on the above data, assigning this strain as a novel species in the genus *Cyberlindnera* is justified. In this study, we describe the novel species, *Cyberlindnera sirindhorniae* f.a., sp. nov. based on a single strain, as it will add to the understanding of yeast phylogeny and species diversity (Kurtzman, 2010).

***Cyberlindnera sirindhorniae* sp. nov.**

MycoBank number: MB 858763

(Fig. 5)

**Etymology.**– The species epithet *sirindhorniae* (si.rin.dhor.niae′. N.L. fem. adj.), a yeast named after Her Royal Highness Maha Chakri Sirindhorn on the occasion of her 70$^{th}$ birthday. She has been supportive of natural heritage studies in Thailand. This species is named as a token of respect and recognition of the great interest shown by Her Majesty in the natural history and conservation of natural resources in Thailand.

**Holotype.**– TBRC 19863 is designated as holotype of *Cyberlindnera sirindhorniae* f.a., sp. nov. It was isolated from *Rhizophora apiculata* leaf surfaces collected from the International Mangrove Botanical Garden Rama IX, Chanthaburi Province, Thailand. It is permanently preserved in a metabolically inactive state in the Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology, Thailand. The isotype culture has been preserved in a metabolically inactive state in the Portuguese Yeast Culture Collection, as PYCC 10336.

**Cell morphology and sexual state.**– After 7 days at 25 °C on YM agar, the streak culture and colonies are white-cream in colour, butyrous, circular, smooth, glistening, and convex with entire margins (Figs. 5A–C). After 3 days at 25 °C on YM agar, the cells are ovoid (4.8–10.2×6.8–13.3µm) and budding is multilateral (Fig. 5D). After 7 days in Dalmau plate culture on corn meal agar at 25 °C, pseudohyphae with blastoconidia are present (Figs. 5E–G). Ascospores are not observed on corn meal agar, 5% malt extract agar, Fowell's acetate agar, Gorodkowa agar or YM agar after 6 weeks at either 15 or 25 °C.

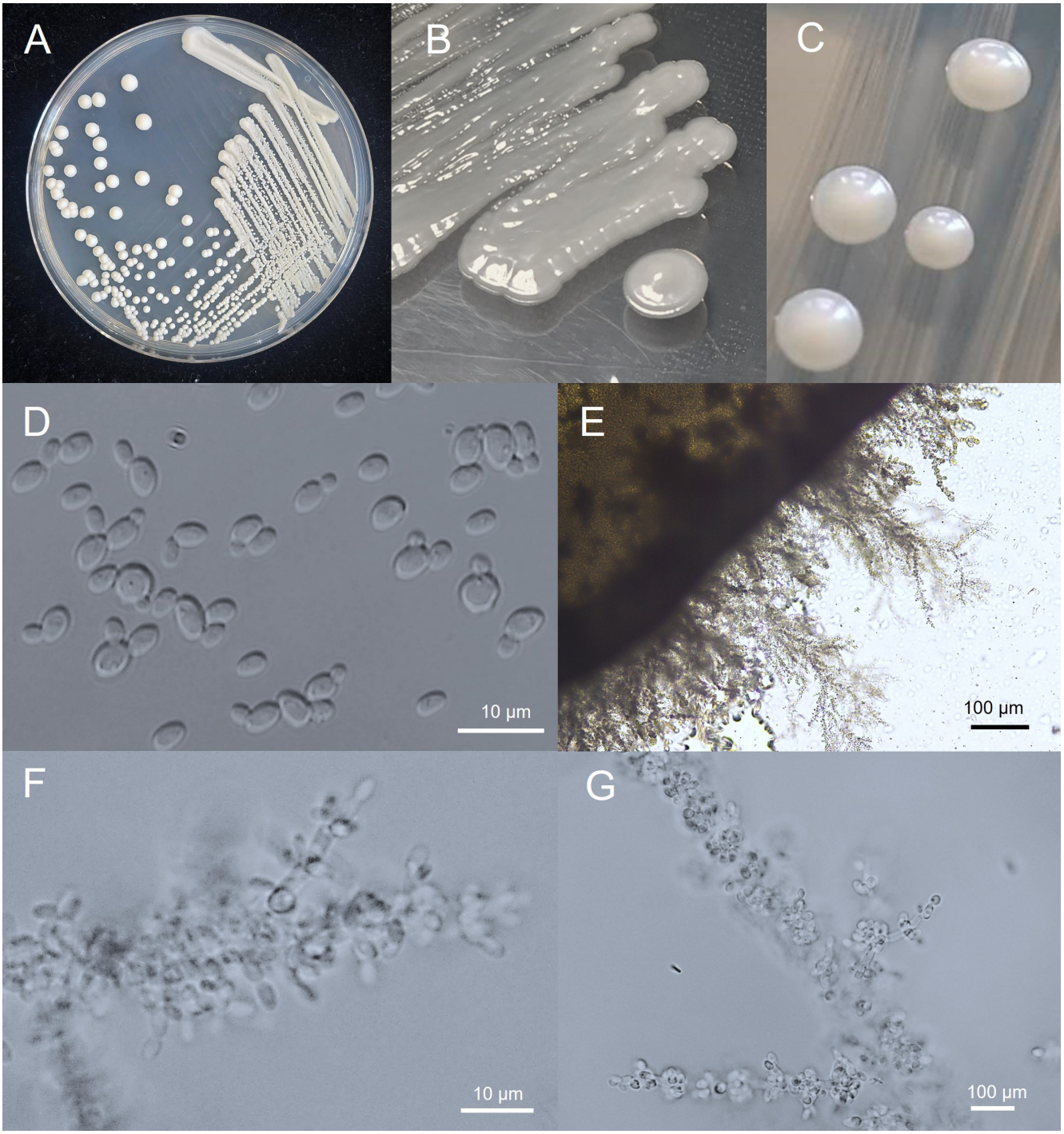


**FIGURE 5.** *Cyberlindnera sirindhorniae* f.a., sp. nov. TBRC 19863$^{T}$. **A–C.** colonies on YM agar after 7 days at 25 °C; **D.** budding cells on YM agar after 3 days at 25 °C (bar, 10 µm); **E–G.** pseudohyphae with blastoconidia grown on corn meal agar after 7 days at 25 °C.

**Phenotypic and growth characteristics.**– Fermentation of glucose is positive. D-Glucose, sucrose, maltose, xylose, cellobiose, salicin, melibiose, D-arabinose, L-arabinose, rhamnose, D-gluconate, (or weak), α-α-trehalose, methyl α-D-glycoside, DL-lactate, sorbitol (or slow positive), raffinose, melezitose, glycerol, D-glucitol, D-mannitol, D-glucono-1,5-lactone, succinate, citrate, ethanol, xylitol, D-arabitol and D-fructose are assimilated. However, galactose, sorbose, *N*-acetyl glucosamine, ribose, lactose, inulin, erythritol, ribitol, galactitol, *myo*-inositol, D-glucuronate, D-galacturonic acid, and methanol are not. Nitrogen assimilation is positive for ammonium sulfate, ethylamine, cadaverine and L-lysine. Potassium nitrate and sodium nitrite are not assimilated. Growth in a vitamin-free medium is negative. Growth occurs on media containing 50% (w/v) and 60% (w/v) glucose, and 10% (w/v) or 15% (w/v) sodium chloride plus 5% (w/v) glucose. No

**TABLE 3.** Phenotypic characteristics of *Cyberlindnera sirindhorniae* f.a., sp. nov. and its closely related species.

| Characteristics | 1 | 2 | 3 |
|---|---|---|---|
| **Fermentation of carbohydrates** | | | |
| Glucose | + | + | + |
| Galactose | - | - | - |
| Maltose | - | + | + |
| Sucrose | + | + | + |
| α-α-Trehalose | - | - | - |
| Lactose | - | - | - |
| Raffinose | - | - | - |
| **Assimilation of carbon compounds** | | | |
| D-Xylose | + | + | w/- |
| L-Arabinose | w | + | w/- |
| D-Arabinose | w | - | w/- |
| L-Rhamnose | w | - | +/- |
| Methyl-α-D-glucoside | s | + | +/-/w |
| Cellobiose | + | + | w/- |
| Salicin | + | - | +/-/w |
| Melibiose | + | - | - |
| Raffinose | + | - | - |
| Melezitose | + | + | w/- |
| Soluble starch | - | + | - |
| Glycerol | + | + | +/-/w |
| D-Glucitol | + | + | +/-/w |
| D-Mannitol | + | + | +/-/w |
| Galactitol | - | + | - |
| D-Glucono-1,5-lactone | + | nd | nd |
| DL-Lactate | s | + | +/- |
| Succinate | + | + | +/- |
| Citrate | + | + | +/- |
| Methanol | - | nd | nd |
| Ethanol | + | + | +/-/w |
| **Growth characteristics** | | | |
| Growth w/o vitamins | - | - | - |
| Growth at 35ºC | + | + | + |
| Growth at 37ºC | + | nd | nd |
| Growth in 0.01% Cycloheximide | - | nd | nd |
| Growth in 0.1% Cycloheximide | - | nd | nd |
| Growth on 50% Glucose | + | nd | nd |
| Growth on 60% Glucose | + | nd | nd |
| Growth on 10% NaCl + 5% glucose | + | nd | nd |
| Growth on 15% NaCl + 5% glucose | + | - | - |

Growth reactions: –, no growth; w, weak growth; s, slow growth; v, variable; +, strong growth; nd, not determined.
Species: 1, *C. sirindhorniae* f.a., sp. nov.; 2, *C. amylophila*; 3, *C. mississippiensis*.
Data for species 1 is from this study, and for species 2–3, it is from Smith et al. (2011b)

growth occurs in the presence of 0.01 or 0.1% cycloheximide. Growth is observed at 15, 30, 35, and 37 °C, but not at 40 °C. Soluble starch-like extracellular carbohydrates are not produced. Acid formation is positive. Hydrolysis of urea and DBB reaction are negative.

## Ecology

Member species of the genus *Cyberlindnera* have been found in diverse habitats, including rotten wood (*C. dasilvae*, *C. galapagoensis*, *C. qingyuanensis*, *C. xishuangbannaensis*), carrots (*C. dauci*), slime flux of *Fagus sylvatica* (*C. sylvatica*), insect frass (*C. nakhonratchasimensis*), ambrosia beetle galleries (*C. xylebori*), and soil (*C. culbertsonii*) (Ninomiya et al., 2013; Guamán-Burneo et al., 2015; Zheng et al. 2017, Crous et al. 2020, Brysch-Herzberg et al. 2021, Barros et al. 2021, Liu et al. 2024). In Thailand, several species of the genus *Cyberlindnera* have been reported from diverse habitats. *Cyberlindnera fabianii* was found on the sugarcane phylloplane (Limtong et al., 2014) and in mangrove forest water (Bonthong et al., 2025). *Cyberlindnera jadinii* was isolated from pia samples (Angchuan et al., 2021), while *C. rhodanensis* has been isolated from various sources, including traditional fermented tea leaves (*Camellia sinensis* var. *assamica*) (Kanpiengjai et al., 2016), the phylloplane of sugarcane

(Limtong et al., 2014), and rice leaf tissues (Khunnamwong et al., 2018). *Cyberlindnera samutprakarnensis* was recovered from cosmetic industrial wastes (Poomtien et al., 2013), whereas *C. subsufficiens* was obtained from peat samples (Boonmak et al., 2020). In addition, *C. tropicalis* was isolated from soil (Boontham et al., 2017). In the present study, the novel species was isolated from another habitat *viz.* leaf surfaces of *R. apiculata* collected from a mangrove forest in Thailand. This confirmed that species in the genus *Cyberlindnera* could be present in diverse habitats. However, no conclusion could be reached concerning the habitat of the genus.

## DISCUSSION

Previous reports on yeast diversity in Thailand indicated that only two species of the genus *Blastobotrys* (Kunthiphun et al., 2018; Into et al., 2020), and six species of the genus *Cyberlindnera* had been documented (Poomtien et al., 2013; Limtong et al., 2014; Kanpiengjai et al., 2016; Boontham et al., 2017; Khunnamwong et al., 2018; Boonmak et al., 2020; Angchuan et al., 2021; Bonthong et al., 2025).

In the present study, two novel species of *Blastobotrys* (namely *Blastobotrys princeps* and *Blastobotrys siamensis*) and one novel species of *Cyberlindnera* (namely *Cyberlindnera sirindhorniae*) were discovered and formally proposed. These findings considerably expand our knowledge of yeast diversity in Thailand and contribute new taxa to the global framework of yeast classification. They also highlight the pressing need for continued exploration of the country's yeast diversity, distribution, and ecological roles.

In the present study, novel species exhibit distinctive characteristics from their closely related species (Table 2 and 3). The two novel *Blastobotrys* species exhibited remarkable osmotolerance, growing on media containing up to 60% (w/v) glucose and 10% (w/v) sodium chloride plus 5% (w/v) glucose, whereas the *Cyberlindnera sirindhorniae* DMKU-RG45 grew on media containing 50% (w/v) glucose and 15% (w/v) sodium chloride plus 5% (w/v) glucose. All novel species showed thermophile growth at 37 °C. These distinctive physiological traits highlight the potential of these novel yeasts as promising resources for diverse industrial and biotechnological applications.

## ACKNOWLEDGEMENTS

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