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Anthophilous cryptic yeasts: an integrative polyphasic approach reveals novel taxa in northern Thailand

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Abstract

The diversity of this cryptic yeast group is important for understanding the microbial diversity and ecology of tropical ecosystems. This study focuses on anthophilous cryptic yeasts isolated from flowers in northern Thailand. A total number of 187 yeast strains (73 species) were isolated from 63 flower samples. Analysis of the sequences of the D1/D2 domain of the large subunit (LSU), and the internal transcribed spacer (ITS) regions of ribosomal DNA gene of all yeast strains allowed classification into the phyla Ascomycota (22.46%), and Basidiomycota (77.54%). Additionally, new taxa higher than species level were proposed based on the multi-locus phylogenetic analyses including the D1/D2 domain, the ITS, the small subunit rDNA (SSU), the largest subunit of RNA polymerase II (*rpb1*), the second largest subunit of RNA polymerase II (*rpb2*), and translation elongation factor 1 alpha (*tef1-α*). This study provides morphological descriptions, physiological characteristics, and phylogenetic positions for one new order (*Thailandicolales*), one new family (*Thailandicolaceae*), one new genus (*Thailandicola*), and 33 new species, including three new species belonging to three genera (*Priceomyces*, *Starmerella*, and *Wickerhamiella*) in Ascomycota, and 30 new species occurring in 17 genera (*Boekhoutia*, *Filobasidium*, *Curvibasidium*, *Cystobasidium*, *Halobasidium*, *Kwoniella*, *Meira*, *Papiliotrema*, *Parajaminaea*, *Pseudozyma*, *Rhodotorula*, *Saitozyma*, *Sporobolomyces*, *Symmetrospora*, *Sympodiomycopsis*, *Thailandicola*, and *Vishniacozyma*) in Basidiomycota. Fourteen new geographical records, one new habitat, and 37 new records from flower species across 36 genera, 22 families, 17 orders and 9 classes were identified. Furthermore, the invalidly described *Entelexis stigmatis* and *Starmerella orientalis* are validated here as two additional new species. These findings significantly expand our knowledge of anthophilous yeast diversity and distribution in tropical flowers.

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Introduction

Flowers play a crucial role in the reproductive cycle of flowering plants, which attract pollinating insects with their bright hues and unique scents. The pollinators accidentally transmit pollen to the flowers and transfer it to subsequent flowers. These pollinators carry a variety of microorganisms, particularly bacteria, filamentous fungi, and yeasts. The term 'anthophilous' has been used for microorganisms that are flower-associated, suggesting that they may play a role in pollination ecology or plant-microbe interactions[1,2]. Anthophilous microorganisms can be dispersed through the nectar and structures of different flowers, such as petals, stamens, and pistils, which provide a nutrient-rich environment including pollen rich in proteins and amino acids, nectar containing 15 to 75% sugar, and flower tissue. These flower structures support a unique anthophilous yeast community that can adapt to its specific host flower. In addition, the relationship between flowers and their associated anthophilous yeasts represents an important ecological interaction, in which yeasts can influence pollination dynamics, floral scent, and plant reproduction[2–6]. However, variations in floral types and growing sites may influence anthophilous yeast communities, potentially leading to the discovery of valuable, previously undiscovered cryptic taxa. Several studies have established flowers as important habitats for anthophilous yeasts, with the genus *Metschnikowia* being particularly prominent[2,3,7–9]. Additional anthophilous yeast, and yeast-like fungi, commonly detected in nectar and flower surfaces include the genera *Candida*, *Clavispora*, *Cryptococcus*, *Debaryomyces*, *Filobasidium*, *Hanseniaspora*, *Hannaella*, *Kodamaea*, *Kwoniella*, *Meyerozyma*, *Metschnikowia*, *Operculina*, *Papiliotrema*, *Pichia*, *Rhodotorula*, *Sporobolomyces*, *Starmerella*, *Sympodiomycopsis*, *Ustilago*, *Wickerhamiella*, and *Yamadazyma*[3,8–14]. Furthermore, this list of yeast genera is expected to expand as ongoing research continues to uncover the diversity of anthophilous yeasts and yeast-like fungi.

Thailand, located in a tropical region with diverse ecosystems and climates, has been identified by numerous mycologists as a hotspot for the discovery of new filamentous fungal and yeast taxa. The country's rich biodiversity, including its tropical forests, wetlands, and agricultural areas, offers a unique environment for the growth of a wide range of filamentous fungi and yeasts. Interestingly, the variety of floral species in Thailand likely supports a corresponding diversity of anthophilous yeast. For example, novel anthophilous yeasts include *Pichia siamensis* from flowers of *Justicia fragilis* and *Ervatamia coronaria* in Kanchanaburi Province[15], *Candida jaroonii* from unidentified flowers in northeastern Thailand[16], *C. ratchasimensis* and *C. khaoyaiensis* from unidentified flowers in Khao Yai National Park in Nakhon Ratchasima Province[17], *C. wangnamkhiaoensis* from Ukshi (*Calycoopteris floribunda*) flowers[18], *C. konsanensis* isolated from princess jasmine (*Jasminum adenophyllum*) flowers[19], and *Metschnikowia lannaensis*, *Wickerhamiella camelliae*, and *W. thailandensis* isolated from Assum tea (*Camellia sinensis* var. *assamica*) flowers in northern Thailand[9]. According to preliminary investigations, the discovery of novel anthophilous yeast is still limited, particularly in northern Thailand, a region that

represents a significant reservoir of microbial diversity[20]. This area features unique topography, with elevations ranging from 400 to 2,500 meters above sea level, a tropical climate, various forest ecosystems, and rich botanical diversity, all of which create numerous specialized microhabitats conducive to yeast colonization. Over the past decade, several yeast species have been discovered in this region that were isolated from soil, plants, and various substrates, including genera *Curvibasidium*, *Cyberlindnera*, *Cystobasidium*, *Debaryomyces*, *Galactomyces*, *Kazachstania*, *Lipomyces*, *Meyerozyma*, *Naganishia*, *Papiliotrema*, *Rhodosporidiobolus*, *Rhodotorula*, *Saitozyma*, *Saturnispora*, *Schwanniomyces*, *Sporidiobolus*, *Trichosporon*, and *Wickerhamomyces*[9,21–26]. Research on anthophilous yeasts in northern Thailand has further revealed a wide variety of species with potential applications, including plant growth promotion, enzyme production, and the synthesis of volatile compounds. Therefore, this study aimed to isolate anthophilous yeasts associated with various flowers in northern Thailand. Yeast identification was carried out using a polyphasic approach that included morphological, biochemical, and physiological characterization, along with multi-locus phylogenetic analyses. The classification of the anthophilous yeasts obtained in this study is presented in Table 1.

Materials and methods

Sample collection and yeast isolation

Fresh flower samples were collected from Chiang Mai, Chiang Rai, and Phayao Provinces, Thailand between July to October 2024 (Figs 1, 2 and Table 2). Each fresh flower sample was placed in a sterile plastic bag, put on ice and transported to the laboratory within 24 h before sample preparation and subsequent yeast isolation. The excised internal parts of the flowers including carpel stamen, pollen, and nectary were soaked in 5 ml of sterilized 0.85% (w/v) NaCl for 15 min. Then, solution was spread onto yeast malt agar (YMA) plates (1.0% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, and 2.0% agar) supplemented with 50 mg/L chloramphenicol, and incubated at 25 °C for 3 d. Each yeast strain was subcultured on YMA, and deposited at −80 °C in the Sustainable Development of Biological Resources (SDBR) Laboratory's Culture Collection within the Faculty of Science at Chiang Mai University, Thailand, the Thailand Bioresource Research Center (TBRC), Thailand, and Guizhou Medical University Culture Collection (GMBCC), Guiyang, China. The holotype of new taxa was permanently preserved in a metabolically inactive state in the Chiang Mai University Biology Department's Herbarium (CMUB), Chiang Mai University, Chiang Mai, Thailand.

Morphological characterization

The morphological characteristics were examined according to the standard methods described by Kurtzman[27], de Vega et al.[11], and Shibayama et al.[13]. Colony morphology of each yeast strain was examined on YMA after incubation at 25 °C for 5 d. The formation of pseudohyphae and true hyphae was assessed through slide culture on potato dextrose agar (PDA) incubated at 25 °C for one month. Ascospore formation was investigated for individual strains and strain pairs on corn meal agar (CMA), PDA, 5% malt extract agar (5% malt extract and 1.5% agar; 5% MEA), V8 agar, and YMA at 25 °C for one month. Micromorphological characteristics were observed using a light microscope (Nikon Eclipse Ni-U, Tokyo, Japan). The sizes of structures such as cells, pseudohyphae, and true hyphae were measured using the Tarosoft® Image Framework program, based on at least 50 measurements for each structure.

Biochemical and physiological characterization

All strains of the new yeast taxa were characterized biochemically and physiologically, according to the standard methods described by Kurtzman[27]. Carbon and nitrogen source assimilation tests were conducted in liquid medium. Fermentation of carbohydrates was carried out in a liquid medium using Durham fermentation tubes. Cycloheximide resistance was also performed in a liquid medium. Acid production and urea hydrolysis were investigated using solid media. The effect of temperature on yeast growth was determined by using YMA at various temperatures ranging from 10 to 40 °C. The ability to grow in media of high osmotic pressure was performed using 50% and 60% glucose agar, and 10% and 16% sodium chloride (NaCl) plus 5% glucose medium. The physiological status was used to normalize and compare results across previously published data with varying scales. Positive carbon fermentation was indicated by '+', whereas negative fermentation was indicated by '–'. For carbon and nitrogen assimilations and growth characteristics, '–' indicated no growth, 'w' weak growth, 's' slow growth, 'l' latent growth, 'v' variable growth, '+' strong growth, and 'nd' not determined.

Molecular study

Yeast strains were cultivated in 5 mL of yeast malt extract broth (YMB) in 18 × 180 mm test tubes, incubated at 25 °C with shaking at 110 rpm for 48 h. Yeast cells were harvested by centrifugation at 11,000 rpm, and washed three times with sterile distilled water. Genomic DNA was extracted from yeast cells using a DNA Extraction Mini Kit (FAVORGEN, Taiwan, China) following the manufacturer's protocol. Amplification of the D1/D2 domain of the LSU was carried out by PCR with the forward primer NL1, and reverse primer NL4[28]. The ITS region was amplified with the forward primer ITS1, and reverse primer ITS4[29], and SSU was amplified with the forward primer NS1 and reverse primer NS4, the process previously described by Kumla et al.[23]. Additionally, the largest subunit of RNA polymerase II (*rpb1*) was amplified using the forward primer RPB1-Af, and reverse primer RPB1-Cr; the second-largest subunit (*rpb2*) was amplified using the forward primer fRPB2-5F, and reverse primer fRPB2-7cR; and the translation elongation factor 1 alpha (*tef1-α*) was amplified using the forward primer EF1-983F, and reverse primer EF1-2218R. Amplification of these three protein-coding genes was performed as described in Wang et al.[30]. PCR products were checked and then purified using a NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Germany). The purified PCR products were directly sequenced at the 1st Base Company (Kembangan, Malaysia). The obtained sequences were used to query GenBank via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html, accessed on 25 August, 2024).

Phylogenetic analyses

Sequence generated from this study were analyzed with the use of similarity searches retrieved from GenBank based on BLASTn searches of the NCBI nucleotide database (http://blast.ncbi.nlm.nih.gov). Reference sequences were selected based on BLAST results and recent publications. Sequence alignments were aligned with MAFFT v.7 (http://mafft.cbrc.jp/alignment/server, accessed on 27 August, 2024)[31], and the alignments were improved where necessary in BioEdit V.7.0.9.1[32]. Maximum likelihood (ML), and Bayesian inference (BI) analyses were conducted using single-locus and concatenated datasets to generate phylogenetic trees.

The ML tree analyses with 1,000 bootstrap iterations were conducted via the CIPRES Science Gateway platform[33], using

Table 1. Content of anthophilous yeasts in this study.

Phylum: Ascomycota Caval. -Sm.	
Subphylum: Saccharomycotina O.E. Erikss. & Winka	
Class: Dipodascomycetes M. Groenew., Hittinger, Opulente & A. Rokas	
Order: *Dipodascales* M. Groenew., Hittinger, Opulente & A. Rokas	
Family: *Trichomonascaceae* Kurtzman & Robnett	
1.	***Entelexis stigmatis*** Sipiczki ex Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov., (validation and first record in Thailand)
2.	***Starmerella etchellsii*** (Lodder & Kreger-van Rij) C.A. Rosa & Lachance, *Int. J. Syst. Evol. Microbiol.* 68: 1341 (2018)
3.	***Starmerella orientalis*** Alimad., Soudi, F.Y. Bai, S.A. Wang & Q.M. Wang ex Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov., (validation and first record in Thailand)
4.	***Starmerella thailandica*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
5.	***Wickerhamiella pollinicola*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Class: Pichiomycetes M. Groenew., Hittinger, Opulente & A. Rokas	
Order: *Serinales* M. Groenew., Hittinger, Opulente & A. Rokas	
Family: *Debaryomycetaceae* Kurtzman & M. Suzuki	
6.	***Candida tropicalis*** (Castell.) Berkhout, *De Schimmelgesl. Monilia, Oidium, Oospora en Torula, Disset*. Ultrecht: 44 (1923)
7.	***Kodamaea ohmeri*** (Etchells & T.A. Bell) Y. Yamada, Tom. Suzuki, M. Matsuda & Mikata, *Biosc., Biotechn., Biochem.* 59: 1174 (1995)
8.	***Kodamaea restingae*** (C.A. Rosa, Lachance, Starmer, J.S.F. Barker, J.M. Bowles & Schlag-Edl.) C.Y. Chai & F.L. Hui, *MycoKeys* 89: 133 (2022)
9.	***Meyerozyma caribbica*** (Vaughan-Mart., Kurtzman, S.A. Mey. & E.B. O'Neill) Kurtzman & M. Suzuki, *Mycoscience* 51: 8 (2010)
10.	***Priceomyces siamensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Family: *Metschnikowiaceae* T. Kamieński ex Doweld	
11.	***Metschnikowia cibodasensis*** Sjamsur., Oetari, C. Nakash., A. Kanti, Saraswati, & K. Ando, *J. Microbiol. Biotechnol.* 23: 909 (2013), (first record in Thailand)
12.	***Metschnikowia koreensis*** S.G. Hong, J. Chun, H.W. Oh & Bae, *Int. J. Syst. Evol. Microbiol.* 51: 1928 (2001)
Class: Saccharomycetes G. Winter	
Order: *Phaffomycetales* M. Groenew., Hittinger, Opulente & A. Rokas	
Family: *Phaffomycetaceae* Y. Yamada, H. Kawas., Nagats., Mikata & T. Seki	
13.	***Cyberlindnera fabianii*** (Wick.) Minter, *Mycotaxon* 110: 474 (2009)
Order: *Saccharomycodales* M. Groenew., Hittinger, Opulente & A. Rokas	
Family: *Saccharomycodaceae* Kudryavtsev	
14.	***Hanseniaspora lachancei*** Čadež, Poot, Raspor & M.T. Sm., *Int. J. Syst. Evol. Microbiol.* 53: 1679 (2003), (first record in Thailand)
Phylum: Basidiomycota R.T. Moore	
Subphylum: Agaricomycotina Doweld	
Class: Tremellomycetes Doweld	
Order: *Filobasidiales* Jülich	
Family: *Filobasidiaceae* L.S. Olive	
15.	***Filobasidium lannaense*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
16.	***Naganishia albida*** (Saito) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 118 (2015)
17.	***Naganishia diffluens*** (Zach) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 119 (2015)
18.	***Naganishia liquefaciens*** (Saito & M. Ota) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 119 (2015)
Order: *Tremellales* Fr.	
Family: *Bulleribasidiaceae* Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout	
19.	***Hannaella pagnoccae*** Landell, L.R. Brandão, A.C. Barbosa, J.P. Ramos, Safar, F.C.O. Gomes, F.M.P. Sousa, P.B. Morais, Broetto, Leoncini, J.R.A. Ribeiro, Fungsin, M. Takash., Nakase, C.F. Lee, Vainstein, Fell, Scorzetti, Vishniac, C.A. Rosa & P. Valente, *Int. J. Syst. Evol. Microbiol.* 64: 1975 (2013)
20.	***Hannaella phyllophila*** Suruss. & Limtong, *Int. J. Syst. Evol. Microbiol.* 65: 2138 (2014)
21.	***Vishniacozyma marinae*** Q.M. Wang, *Stud. Mycol.*109: 119 (2024)
22.	***Vishniacozyma pollinicola*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Family: *Cryptococcaceae* Kütz. ex Castell. & Chalm.	
23.	***Kwoniella bestiolae*** (Thanh, Hai & Lachance) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 137 (2015), (first record in Thailand)
24.	***Kwoniella heveanensis*** Metin, K. Findley & Heitman, *Mycotaxon* 116: 227 (2011)
25.	***Kwoniella limtongiae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
26.	***Kwoniella saisamorniae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Family: *Rhynchogastremaceae* Oberw. & B. Metzler	
27.	***Papiliotrema aspenensis*** (Ferreira-Paim, T.B. Ferreira, Andrade-Silva, D.J. Mora, D.J. Springer, Heitman, F.M. Fonseca, D. Matos, Melhem & Silva-Verg.) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 96: 135 (2020)
28.	***Papiliotrema flavescens*** (Saito) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 126 (2015)
29.	***Papiliotrema chiangmaiensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
30.	***Papiliotrema pollinicola*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
31.	***Papiliotrema tectonae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Family: *Trimorphomycetaceae* Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout	
32.	***Saitozyma thailandensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Order: *Trichosporonales* Boekhout & Fell	
Family: *Trichosporonaceae* Nann.	
33.	***Trichosporon asahii*** Akagi ex Sugita, A. Nishikawa & Shinoda, *J. Gen. Appl. Microbiol.*, Tokyo 40: 405 (1994)
Subphylum: Pucciniomycotina R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.	
Class: Agaricostilbomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.	
Order: *Agaricostilbales* Oberw. & R. Bauer	
Family: *Chionosphaeraceae* Oberw. & Bandoni	
34.	***Boekhoutia pollinicola*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.

(to be continued)

Table 1. (continued)

Class: Cystobasidiomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.
Order: *Cystobasidiales* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.
Family: *Cystobasidiaceae* Gäum.
35. ***Cystobasidium benthicum*** (Nagah., Hamam., Nakase & Horikoshi) Yurkov, Kachalkin, H.M. Daniel, M. Groenew., Libkind, V. de García, Zalar, Gouliam., Boekhout & Begerow, *Antonie van Leeuwenhoek* 107: 180 (2014), (new habitat record and first record in Thailand)
36. ***Cystobasidium keelungense*** C.F. Chang & S.M. Liu, *Arch. Microbiol.* 201: 31 (2018), (first record in Thailand)
37. ***Cystobasidium minutum*** (Cif. & Redaelli) Yurkov, Kachalkin, H.M. Daniel, M. Groenew., Libkind, V. de García, Zalar, Gouliam., Boekhout & Begerow, *Antonie van Leeuwenhoek* 107: 180 (2014)
38. ***Cystobasidium thailandicum*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
39. ***Halobasidium lannaense*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Class: Cystobasidiomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.
Order: *Erythrobasidiales* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.
Family: *Erythrobasidiaceae* Denchev
40. ***Erythrobasidium primogenitum*** Y.P. Tan, Bishop-Hurley & R.G. Shivas, *Index of Australian Fungi* 1: 1 (2022), (first record in Thailand)
Families *Incertae sedis*
Family: *Symmetrosporaceae* Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout
41. ***Symmetrospora chiangmaiensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
42. ***Symmetrospora hydei*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Class: Microbotryomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.
Order: *Incertae sedis*
Families *Incertae sedis*
43. ***Curvibasidium chiangmaiense*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Order: *Thailandicolales* Kodchasee, Senwanna, J. Kumla & N. Suwannar.
Family: *Thailandicolaceae* Kodchasee, Senwanna, J. Kumla & N. Suwannar.
44. ***Thailandicolales*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., ord. nov.
45. ***Thailandicolaceae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., fam. nov.
46. ***Thailandicola*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., gen. nov.
47. ***Thailandicola hydei*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
48. ***Thailandicola limtongiae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Order: *Sporidiobolales* Doweld
Family: *Sporidiobolaceae* R.T. Moore
49. ***Rhodosporidiobolus fluvialis*** (Fell, Kurtzman, Tallman & J.D. Buck) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 181 (2015)
50. ***Rhodosporidiobolus ruineniae*** (Holzschu, Tredick & Phaff) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 182 (2015)
51. ***Rhodotorula paludigena*** (Fell & Tallman) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 181 (2015)
52. ***Rhodotorula toruloides*** (I. Banno) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, *Stud. Mycol.* 81: 181 (2015)
53. ***Rhodotorula thailandensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
54. ***Sporobolomyces thailandensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Subphylum: Ustilaginomycotina Doweld
Class: Exobasidiomycetes Begerow, M. Stoll & R. Bauer
Order: *Exobasidiales* Henn.
Family: *Brachybasidiaceae* Gäum.
55. ***Meira argovae*** Boekhout, Scorzetti, Gerson & Sztejnb. ex Denchev & T. Denchev, *Mycobiota* 11: 3 (2021), (first record in Thailand)
56. ***Meira plantarum*** Q.M. Wang, Y.Y. Li, M. Groenew. & M.M. Wang, *Frontiers Microbiol.* 12: 16 (2022), (first record in Thailand)
57. ***Meira chiangmaiensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
58. ***Meira limtongiae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
59. ***Meira pollinicola*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Family: *Laurobasidiaceae* Pinruan, Sommai, Suetrong, Somrith. & E.B.G. Jones
60. ***Laurobasidium hachijoense*** (Y. Otani, Kakish. & Iijima) Kakish., Nagao & Denchev, *Phytotaxa* 303: 97 (2017)
Order: *Microstromatales* R. Bauer & Oberw.
Families *Incertae sedis*
61. ***Jaminaea lantanae*** Q.M. Wang, Y.Y. Li, M. Groenew. & M.M. Wang, *Frontiers Microbiol.* 12: 22 (2022), (first record in Thailand)
62. ***Parajaminaea hydei*** Kodchasee, Senwanna, J. Kumla & N. Suwannarach, sp. nov.
63. ***Sympodiomycopsis europaea*** Q.M. Wang, Y.Y. Li, M. Groenew. & F.Y. Bai, *Frontiers Microbiol.* 12: 22 (2022), (first record in Thailand)
64. ***Sympodiomycopsis paphiopedili*** Sugiy., Tokuoka & Komag., in Sugiyama, Tokuoka, Suh, Hirata & Komagata, *Antonie van Leeuwenhoek* 59: 101 (1991), (first record in Thailand)
65. ***Sympodiomycopsis hydei*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
66. ***Sympodiomycopsis limtongiae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
67. ***Sympodiomycopsis saisamorniae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
Class: Ustilaginomycetes Warm.
Order: *Ustilaginales* Bek.
Family: *Ustilaginaceae* Tul. & C. Tul.
68. ***Anthracocystis heteropogonicola*** (Mundk. & Thirum.) McTaggart & R.G. Shivas, *Persoonia* 29: 123 (2012), (first record in Thailand)
69. ***Moesziomyces antarcticus*** (Goto, Sugiy. & Iizuka) Q.M. Wang, Begerow, F.Y. Bai & Boekhout, *Stud. Mycol.* 81: 81 (2015)
70. ***Moesziomyces bullatus*** (J. Schröt.) Vánky, *Bot. Notiser* 130: 133 (1977)
71. ***Moesziomyces parantarcticus*** (Sugita, M. Takash., Mekha & Poonwan) Q.M. Wang, Begerow, F.Y. Bai & Boekhout, *Stud. Mycol.* 81: 81 (2015)
72. ***Pseudozyma chiangmaiensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.

(to be continued)

Table 1. (continued)

73.	***Pseudozyma lannaensis*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
74.	***Pseudozyma limtongiae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
75.	***Pseudozyma pollinicola*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.
76.	***Pseudozyma saisamorniae*** Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov.

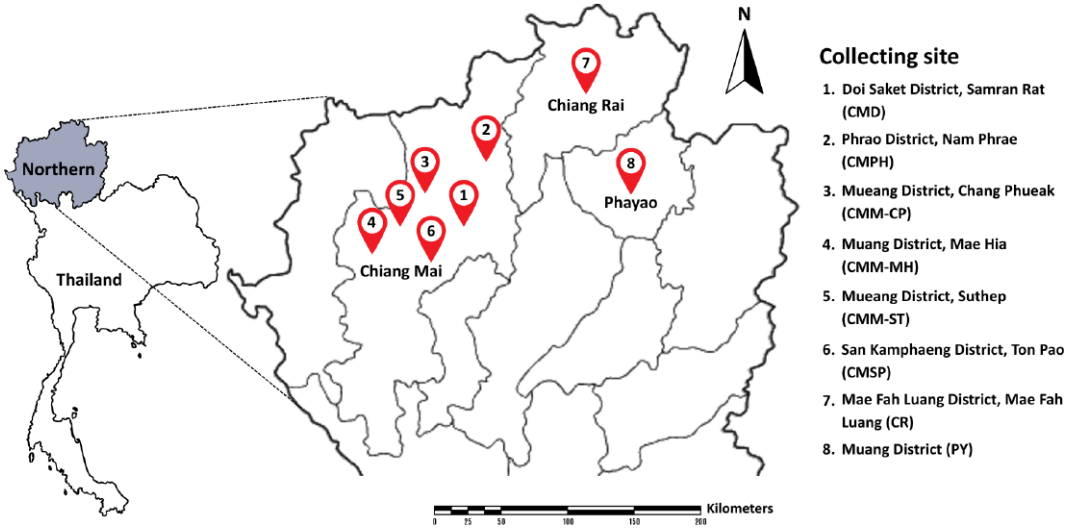

Fig. 1 Location of sampling sites in northern Thailand.

RAxML-HPC v.8 on ACCESS (v.8.2.12)[34], and employing the GTRGAMMA model of evolution. For BI, analyses were performed with MrBayes v.3.2.6[35] to estimate Bayesian posterior probabilities (BYPP)[36,37] by Markov Chain Monte Carlo sampling (BMCMC). MrModeltest 2.3[38] was used to determine the model of nucleotide substitution for each locus. Two independent runs of four simultaneous Markov chains were executed for 1 to 60 million generations (depending on individual settings for the yeast group), trees sampled every 100 generations. When the average standard deviation of split frequencies dropped below 0.01, the analysis was terminated, and the first 25% of the trees, representing the burn-in phase, were discarded. The phylogenetic trees were visualized using FigTree v.1.4.3[39], and edited with Adobe Illustrator CC 2019 (version 23.0.3.585), and Adobe Photoshop CS6 (version 13.0) (Adobe Systems, USA). All sequences generated in this study were submitted to GenBank. All entries are depicted in the phylogenetic tree, along with their corresponding descriptions. New yeast taxa were registered in the MycoBank database[40].

Results

Yeast isolation and diversity

Sixty-three flower samples from northern Thailand were used for the isolation of anthophilous yeasts. A total of 187 strains were identified to the species level, based on the threshold of more than 99% sequence identity with the type strain of a described species in ITS region or D1/D2 domain. The classification of taxa in this study follows the Outline of Fungi and Fungus-Like Taxa[41,42], and updated based on recent relevant literature. Based on morphology and multi-locus phylogeny, 89 strains were identified as 38 previous known yeast species. Details on their host, locality, and sequence accession numbers are provided in Table 3, and a phylogenetic tree of each species are presented in the Supplementary File 1. Whereas 98 strains were determined to represent undescribed species, including two with invalidly published names. They were classified into the phyla Ascomycota (22.46%), and Basidiomycota (77.54%), comprising 36 genera in 22 families (Fig. 3). Five families belonging to the phylum Ascomycota were identified, including *Debaryomycetaceae* (*Candida*, *Kodamaea*, *Meyerozyma*, and *Priceomyces*), *Metschnikowiaceae* (*Metschnikowia*), *Phaffomycetaceae* (*Cyberlindnera*), *Saccharomycodaceae* (*Hanseniaspora*), and *Trichomonascaceae* (*Entelexis*, *Starmerella*, and *Wickerhamiella*). While 15 families, and two *incertae sedis* belonging to the phylum Basidiomycota were identified, including *Brachybasidiaceae* (*Meira*), *Bulleribasidiaceae* (*Hannaella* and *Vishniacozyma*), *Chionosphaeraceae* (*Boekhoutia*), *Cryptococcaceae* (*Kwoniella*), *Cystobasidiaceae* (*Cystobasidium* and *Halobasidium*), *Erythrobasidiaceae* (*Erythrobasidium*), *Filobasidiaceae* (*Filobasidium* and *Naganishia*), *Laurobasidiaceae* (*Laurobasidium*), *Microbotryomycetes* genera *incertae sedis* (*Curvibasidium*), *Microstromatales* genera *incertae sedis* (*Jaminaea*, *Parajaminaea*, and *Sympodiomycopsis*), *Rhynchogastremaceae* (*Papiliotrema*), *Sporidiobolaceae* (*Rhodosporidiobolus*, *Rhodotorula*, and *Sporobolomyces*), *Symmetrosporaceae* (*Symmetrospora*), *Thailandicolaceae* (*Thailandicola*), *Trichosporonaceae* (*Trichosporon*), *Trimorphomycetaceae* (*Saitozyma*), and *Ustilaginaceae* (*Acaromyces*, *Moesziomyces*, and *Pseudozyma*) (Figs 3 and 4).

The distribution of each yeast species is presented in Fig. 4. The majority of isolated anthophilous yeasts had low to moderate frequency of occurrence (1% to 3%), represented by blue to light green shades, with only a few taxa exceeding 5% frequency of occurrence (Fig. 4a). The most frequent ascomycetous yeast was *Metschnikowia koreensis* (8.56% frequency of occurrence), which was found in 11 flower samples in five site locations, and the most frequent basidiomycetous yeast was *Pseudozyma saisamorniae* (4.28% frequency of occurrence), found in eight flower samples presented at three site locations. Other species were found with frequencies of occurrence ranging from 0.53% to 2.67% in each flower species. In addition, *Plumeria pudica* (PY) harbored the high-

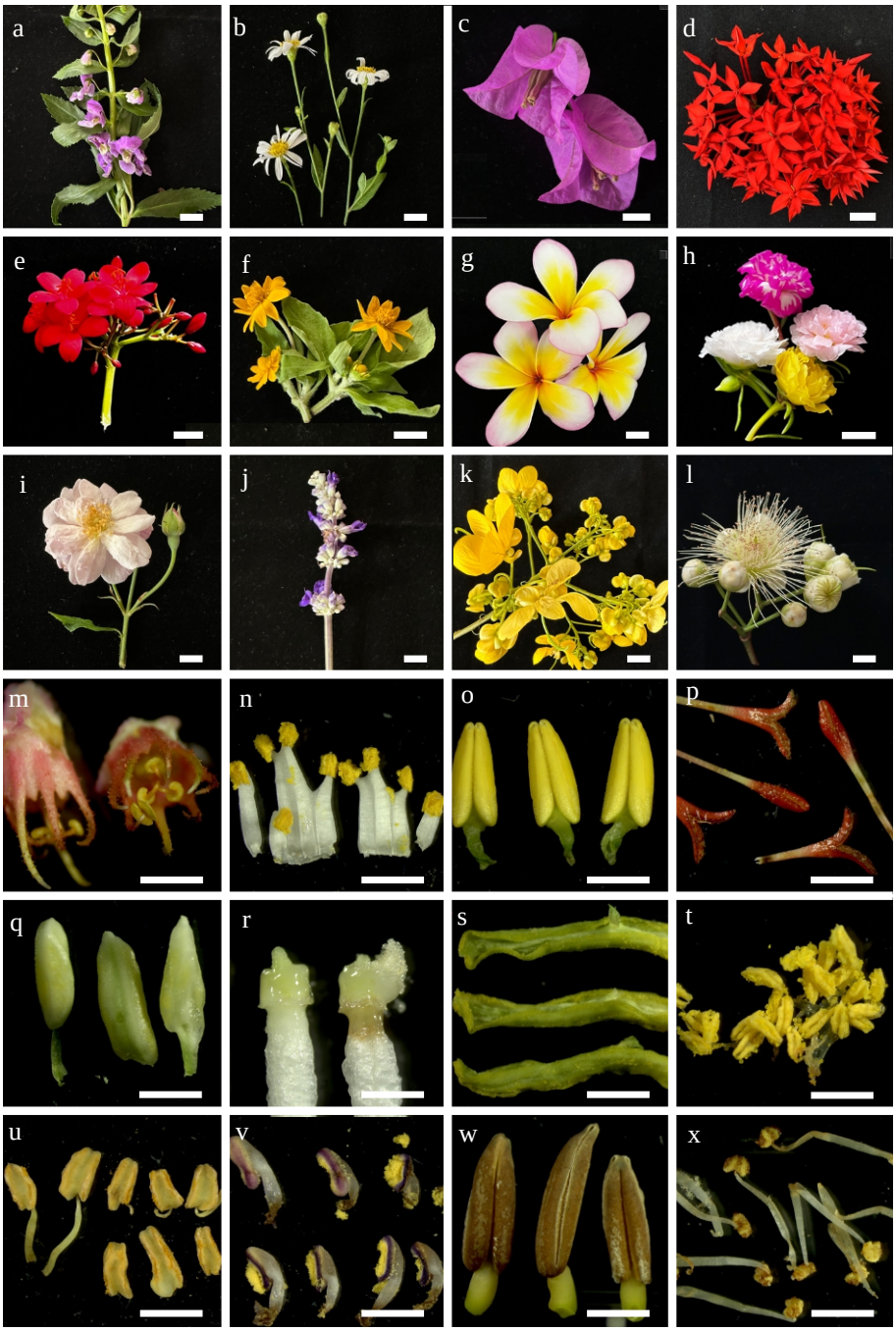

Fig. 2 (a)–(l) Some flower samples collected from northern Thailand, and their (m)–(x) internal parts. (a) *Angelonia goyazensis,* (b) *Bellis perennis,* (c) *Bougainvillea hybrid,* (d) *Ixora chinensis,* (e) *Jatropha integerrima,* (f) *Melampodium divaricatum,* (g) *Plumeria rubra,* (h) *Portulaca grandiflora,* (i) *Rosa* sp., (j) *Salvia farinacea,* (k) *Senna spectabilis,* (l) *Syzygium jambos*. The internal parts of (m) *Antigonon leptopus,* (n) *Citrus japonica,* (o) *Exacum affine,* (p) *Ixora chinensis,* (q) *Jasminum sambac,* (r) *Nerium oleander,* (s) *Passiflora caerulea,* (t) *Portulaca grandiflora,* (u) *Rosa* sp., (v) *Salvia farinacea,* (w) *Senna spectabilis,* and (x) *Syzygium jambos*. Scale bars (a)–(l) = 1 cm, and (m)–(x) = 1 mm.

est number of yeast species, with nine species recorded, followed by *Antigonon leptopus* (CMM-CP), *Curcuma sessilis* (CMM-MH), and *Tectona grandis* (CMM-ST), each of which contained six yeast species (Fig. 4b).

Additionally, 38 known species (Table 3), and two newly validated species (*E. stigmatis* and *S. orientalis*) were recorded for the first time from flower species. Notably, *Cys. benthicum* was newly recorded from a plant habitat. Among these, 14 species were recorded for the first time in Thailand including *An. heteropogonicola*, *Cys. benthicum*, *Cys. keelungense*, *E. stigmatis*, *Er. primogenitum*, *H. lachancei*, *J. lantanae*, *Kw. bestiolae*, *Me. cibodasensis*, *Mei. argovae*, *Mei. plantarum*, *S. orientalis*, *Sym. europaea*, and *Sym. paphiopedili*, the detail is listed in the Supplementary File 1.

Taxonomy:

Phylum: Ascomycota Caval.-Sm.

Subphylum: Saccharomycotina O.E. Erikss. & Winka

Class: Dipodascomycetes M. Groenew., Hittinger, Opulente & A. Rokas

Order: *Dipodascales* M. Groenew., Hittinger, Opulente & A. Rokas

Family: *Trichomonascaceae* Kurtzman & Robnett

Trichomonascaceae are found in a wide range of habitats, some

Table 2. Location of sampling sites and flower species used in this study.

Location sites		Flower species
Chiang Mai Province	Doi Saket District, Samran Rat (CMD)	*Combretum indicum*
	Phrao District, Nam Phrae (CMPH)	*Boesenbergia rotunda*, *Cananga odorata*, *Canna indica*, *Crinum asiaticum*, *Globba winitii*, *Ixora* sp., *Musa sapientum*, *Oncidium* sp., *Phyllanthus pulcher*, *Syzygium jambos*, *Zamioculcas zamiifolia*, and *Zephyranthes minuta*
	Mueang District, Chang Phueak (CMM-CP)	*Adenium obesum*, *Angelonia goyazensis*, *Antigonon leptopus*, *Argyranthemum frutescens*, *Bidens pilosa*, *Capsicum* sp., *Citrus japonica*, *Dolichandrone serrulata*, *Exacum affine*, *Jasminum sambac*, *Mecardonia procumbens*, *Melampodium divaricatum*, *Millingtonia hortensis*, *Murraya paniculata*, *Nerium oleander*, *Physalis minima*, *Plumbago auriculata*, *Plumeria obtusa*, *Portulaca grandiflora*, *Rosa* sp., and *Salvia farinacea*
	Mueang District, Mae Hia (CMM-MH)	*Allamanda cathartica*, *Curcuma sessilis*, *Hamelia patens*, *Plumeria rubra*, *Ruellia tuberosa*, and *Tecoma stans*
	Mueang District, Suthep (CMM-ST)	*Alstonia scholaris*, *Bougainvillea hybrid*, *Carmona retusa*, *Ixora chinensis*, *Passiflora caerulea*, *Senna spectabilis*, and *Tectona grandis*
	San Kamphaeng District, Ton Pao (CMSP)	*Lagerstroemia speciosa*, *Momordica charantia,* and *Morinda citrifolia*
Chiang Rai Province	Mae Fah Luang District, Mae Fah Luang (CR)	*Pentas lanceolata* and *Thunbergia erecta*
Phayao Province	Mueang District (PY)	*Catharanthus roseus*, *Cnidoscolus aconitifolius*, *Dichorisandra thyrsiflora*, *Hibiscus rosa-sinensis*, *Jatropha integerrima*, *Melampodium divaricatum*, *Nerium oleander*, *Ocimum tenuiflorum*, *Pachystachys lutea*, *Plumeria pudica*, and *Thryallis glauca*

Table 3. Known yeast species obtained in this study, with their hosts, localities, and GenBank accession numbers.

Species	Strain	Host	Locality	GenBank accession number			
				D1/D2	ITS	*rpb1*	*tef1-α*
Ascomycota, Saccharomycotina							
Dipodascomycetes, *Dipodascales, Trichomonascaceae*							
Starmerella etchellsii	SDBR-CMU641	*Passiflora caerulea*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834436	PV834623	–	–
Pichiomycetes, *Serinales, Debaryomycetaceae*							
Candida tropicalis	SDBR-CMU677	*Melampodium divaricatum*	Thailand, Phayao Province, Mueang District	PV834442	PV834627	–	–
Kodamaea ohmeri	SDBR-CMU607	*Boesenbergia rotunda*	Thailand, Chiang Mai Province, Phrao District, Nam Phrae	PV834443	PV834628	–	–
	SDBR-CMU626	*Jasminum sambac*	Thailand, Chiang Mai Province Mueang District, Chang Phueak	PV834444	PV834629	–	–
	SDBR-CMU660	*Catharanthus roseus*	Thailand, Phayao Province, Mueang District	PV834445	PV834630	–	–
	SDBR-CMU663	*Catharanthus roseus*	Thailand, Phayao Province, Mueang District	PV834446	PV834631	–	–
	SDBR-CMU670	*Pentas lanceolata*	Thailand, Phayao Province, Mueang District	PV834447	PV834632	–	–
Kodamaea restingae	SDBR-CMU640	*Bougainvillea hybrid*	Thailand, Chiang Mai Province, Mueang District	PV834448	PV834633	–	–
	SDBR-CMU672	*Crinum asiaticum*	Thailand, Chiang Mai Province, Phrao District, Nam Phrae	PV834449	PV834634	–	–
Meyerozyma caribbica	SDBR-CMU644	*Pentas lanceolata*	Thailand, Chiang Rai Province, Mae Fah Luang District, Mae Fah Luang	PV834450	PV834635	–	–
	SDBR-CMU676	*Melampodium divaricatum*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834451	PV834636	–	–
Metschnikowiaceae							
Metschnikowia cibodasensis	SDBR-CMU554	*Tecoma stans*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834454	PV834639	–	–
Metschnikowia koreensis	SDBR-CMU553	*Plumeria obtusa*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834455		–	–
	SDBR-CMU555	*Syzygium jambos*	Thailand, Chiang Mai Province, Phrao District, Nam Phrae	PV834456	PV834640	–	–
	SDBR-CMU558	*Allamanda cathartica*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834457		–	–
	SDBR-CMU559	*Allamanda cathartica*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834458	–	–	–
	SDBR-CMU625	*Jasminum sambac*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834459	–	–	–
	SDBR-CMU643	*Pentas lanceolata*	Thailand, Chiang Rai Province, Mae Fah Luang, Mae Fah Luang	PV834460	–	–	–
	SDBR-CMU646	*Cnidoscolus aconitifolius*	Thailand, Phayao Province, Mueang District	PV834461	–	–	–
	SDBR-CMU649	*Jatropha integerrima*	Thailand, Phayao Province, Mueang District	PV834462	–	–	–
	SDBR-CMU651	*Dichorisandra thyrsiflora*	Thailand, Phayao Province, Mueang District	PV834463	–	–	–
	SDBR-CMU655	*Pentas lanceolata*	Thailand, Chiang Rai Province, Mae Fah Luang, Mae Fah Luang	PV834464	–	–	–
	SDBR-CMU657	*Nerium oleander*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834465	–	–	–
	SDBR-CMU658	*Nerium oleander*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834466	–	–	–
	SDBR-CMU669	*Pentas lanceolata*	Thailand, Chiang Rai Province, Mae Fah Luang, Mae Fah Luang	PV834467	–	–	–
	SDBR-CMU671	*Bougainvillea hybrid*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834468	–	–	–

(to be continued)

Table 3. (continued)

Species	Strain	Host	Locality	GenBank accession number			
				D1/D2	ITS	*rpb1*	*tef1-α*
	SDBR-CMU673	*Ixora chinensis*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834469	–	–	–
	SDBR-CMU674	*Ixora chinensis*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834470	–	–	–
Saccharomycetes, *Phaffomycetales, Phaffomycetaceae*							
Cyberlindnera fabianii	SDBR-CMU563	*Rosa* sp.	Thailand, Chiang Mai Province, Mueang District, Chang Phueak,	PV834471	PV834641	–	–
Saccharomycodales, *Saccharomycodaceae*							
Hanseniaspora lachancei	SDBR-CMU560	*Antigonon leptopus*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834472	PV834642	–	–
	SDBR-CMU598	*Antigonon leptopus*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834473	PV834643	–	–
Basidiomycota, **Agaricomycotina** **Tremellomycetes, *Filobasidiales, Filobasidiaceae***							
Naganishia albida	SDBR-CMU556	*Nerium oleander*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834476	PV834646	–	–
Naganishia diffluens	SDBR-CMU712	*Argyranthemum frutescens*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak,	PV834477	PV834647	–	–
	SDBR-CMU716	*Argyranthemum frutescens*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak,	PV834478	PV834648	–	–
Naganishia liquefaciens	SDBR-CMU609	*Rosa* sp.	Thailand, Chiang Mai Province, Mueang District, Chang Phueak,	PV834479	PV834649	–	–
Tremellales, Bulleribasidiaceae							
Hannaella pagnoccae	SDBR-CMU576	*Curcuma sessilis*	Thailand, Chiang Mai Province, Mueang District, Mae Hia	PV834480	PV834650	–	–
	SDBR-CMU653	*Dichorisandra thyrsiflora*	Thailand, Phayao Province, Mueang District	PV834481	PV834651	–	–
	SDBR-CMU683	*Plumeria pudica*	Thailand, Phayao Province, Mueang District	PV834482	PV834652	–	–
	SDBR-CMU688	*Plumeria pudica*	Thailand, Phayao Province, Mueang District	PV834483	PV834653	–	–
Hannaella phyllophila	SDBR-CMU583	*Oncidium* sp.	Thailand, Chiang Mai Province, Phrao District, Nam Phrae	PV834484	PV834654	–	–
Vishniacozyma marinae	SDBR-CMU692	*Plumeria pudica*	Thailand, Phayao Province, Mueang District	PV834485	PV834655	–	–
Cryptococcaceae							
Kwoniella bestiolae	SDBR-CMU642	*Passiflora caerulea*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834488	PV834658	–	–
	SDBR-CMU699	*Bougainvillea hybrid*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834489	PV834659	–	–
Kwoniella heveanensis	SDBR-CMU633	*Tectona grandis*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834490	PV834660	–	–
	SDBR-CMU726	*Alstonia scholaris*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834491	PV834661	–	–
Rhynchogastremaceae							
Papiliotrema aspenensis	SDBR-CMU652	*Plumeria pudica*	Thailand, Phayao Province, Mueang District	PV834496	PV834666	–	–
Papiliotrema flavescens	SDBR-CMU668	*Pachystachys lutea*	Thailand, Phayao Province, Mueang District	PV834499	PV834669	–	–
Trichosporonales*, *Trichosporonaceae							
Trichosporon asahii	SDBR-CMU549	*Bidens Pilosa*	Thailand, Chiang Mai Province, Mueang District, Chang Phuea	PV834507	PV834677	–	–
	SDBR-CMU656	*Nerium oleander*	Thailand, Phayao Province, Mueang Districtin	PV834508	PV834678	–	–
Pucciniomycotina **Cystobasidiomycetes, *Cystobasidiales*, *Cystobasidiaceae***							
Cystobasidium benthicum	SDBR-CMU720	*Lagerstroemia speciosa*	Thailand, Chiang Mai Province, San Kamphaeng District, Ton Pao	PV834511	PV834681	–	–
Cystobasidium keelungense	SDBR-CMU585	*Mecardonia procumbens*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834512	PV834682	–	–
	SDBR-CMU713	*Argyranthemum frutescens*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834513	PV834683	–	–
Cystobasidium minutum	SDBR-CMU608	*Rosa* sp.	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834514	PV834684	–	–
	SDBR-CMU721	*Lagerstroemia speciosa*	Thailand, Chiang Mai Province, San Kamphaeng District, Ton Pao	PV834515	PV834685	–	–
Cystobasidiomycetes, ***Erythrobasidiales, Erythrobasidiaceae***							
Erythrobasidium primogenitum	SDBR-CMU689	*Plumeria pudica*	Thailand, Phayao Province, Mueang District	PV834525	PV834695	–	–
Microbotryomycetes, *Sporidiobolales, Sporidiobolaceae*							
Rhodosporidiobolus fluvialis	SDBR-CMU562	*Rosa* sp.	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834532	PV834702	–	–
	SDBR-CMU568	*Citrus japonica*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834533	PV834703	–	–
	SDBR-CMU659	*Nerium oleander*	Thailand, Phayao Province, Mueang District	PV834534	PV834704	–	–

(to be continued)

Table 3. (continued)

Species	Strain	Host	Locality	GenBank accession number			
				D1/D2	ITS	*rpb1*	*tef1-α*
Rhodosporidiobolus ruineniae	SDBR-CMU593	*Zamioculcas zamiifolia*	Thailand, Chiang Mai Province, Phrao District, Nam Phrae	PV834535	PV834705	–	–
	SDBR-CMU665	*Ocimum tenuiflorum*	Thailand, Phayao Province, Mueang District	PV834536	PV834706	–	–
	SDBR-CMU724	*Combretum indicum*	Thailand, Chiang Mai Province, Doi Saket District, Samran Rat	PV834537	PV834707	–	–
Rhodotorula paludigena	SDBR-CMU580	*Exacum affine*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834538	PV834708	–	–
Rhodotorula toruloides	SDBR-CMU678	*Melampodium divaricatum*	Thailand, Phayao Province, Mueang District	PV834542	PV834712	–	–
Ustilaginomycotina							
Exobasidiomycetes, *Exobasidiales, Brachybasidiaceae*							
Meira argovae	SDBR-CMU709	*Morinda citrifolia*	Thailand, Chiang Mai Province, San Kamphaeng District, Ton Pao	PV834546	PV834716	–	–
	SDBR-CMU729	*Alstonia scholaris*	Thailand, Chiang Mai Province Mueang District, Suthep	PV834547	PV834717	–	–
	SDBR-CMU730	*Alstonia scholaris*	Thailand, Chiang Mai Province Mueang District, Suthep	PV834548	PV834718	–	–
Meira plantarum	SDBR-CMU687	*Plumeria pudica*	Thailand, Phayao Province, Mueang District	PV834555	PV834725	–	–
Exobasidiales, Laurobasidiaceae							
Laurobasidium hachijoense	SDBR-CMU615	*Syzygium jambos*	Thailand, Chiang Mai Province, Phrao District, Nam Phrae	PV834559	PV834729	–	–
	SDBR-CMU616	*Syzygium jambos*	Thailand, Chiang Mai Province, Phrao District, Nam Phrae	PV834560	PV834730	–	–
Microstromatales, Microstromatales genera incertae sedis							
Jaminaea lantanae	SDBR-CMU693	*Hibiscus rosa-sinensis*	Thailand, Phayao Province, Mueang District	PV834561	PV834731	–	–
Sympodiomycopsis europaea	SDBR-CMU605	*Plumeria obtusa*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834564	PV834734	PV941870	PV947475
	SDBR-CMU637	*Bougainvillea hybrid*	Thailand, Thailand, Chiang Mai Province, Mueang District, Suthep	PV834565	PV834735	–	–
Sympodiomycopsis paphiopedili	SDBR-CMU567	*Hamelia patens*	Thailand, Chiang Mai Province, Mueang District, Mae Hia	PV834572	PV834742	–	–
	SDBR-CMU708	*Morinda citrifolia*	Thailand, Chiang Mai Province, San Kamphaeng District, Ton Pao	PV834573	PV834743	–	–
Ustilaginomycetes, *Ustilaginales, Ustilaginaceae*							
Anthracocystis heteropogonicola	SDBR-CMU551	*Bidens pilosa*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834576	PV834746	–	–
	SDBR-CMU589	*Antigonon leptopus*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834577	PV834747	–	–
Moesziomyces antarcticus	SDBR-CMU548	*Zephyranthes minuta*	Thailand, Chiang Mai Province, Phrao District, Nam Phrae	PV834578	PV834748	–	–
	SDBR-CMU634	*Senna spectabilis*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834579	PV834749	–	–
	SDBR-CMU647	*Hibiscus rosa-sinensis*	Thailand, Phayao Province, Mueang District	PV834580	PV834750	–	–
	SDBR-CMU706	*Momordica charantia*	Thailand, Chiang Mai Province, San Kamphaeng District, Ton Pao	PV834581	PV834751	–	–
Moesziomyces bullatus	SDBR-CMU571	*Citrus japonica*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834582	PV834752	–	–
	SDBR-CMU582	*Exacum affine*	Thailand, Chiang Mai Province, Mueang District, Chang Phueak	PV834583	PV834753	–	–
	SDBR-CMU645	*Cnidoscolus aconitifolius*	Thailand, Phayao Province, Mueang District	PV834584	PV834754	–	–
	SDBR-CMU675	*Melampodium divaricatum*	Thailand, Phayao Province, Mueang District	PV834585	PV834755	–	–
	SDBR-CMU679	*Cnidoscolus aconitifolius*	Thailand, Phayao Province, Mueang District	PV834586	PV834756	–	–
Moesziomyces parantarcticus	SDBR-CMU654	*Pentas lanceolata*	Thailand, Phayao Province, Mueang District	PV834587	PV834757	–	–
	SDBR-CMU733	*Ixora chinensis*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834588	PV834758	–	–
	SDBR-CMU734	*Ixora chinensis*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834589	PV834759	–	–
	SDBR-CMU732	*Ixora chinensis*	Thailand, Chiang Mai Province, Mueang District, Suthep	PV834590	PV834760	–	–

'–', undetermined sequence.

with ecological distribution patterns indicating close interactions with insects, nectar, and decaying plant matter. Furthermore, species in *Trichomonascaceae* are economically important in industries (such as food production and cosmetics), medicine, and agriculture. Members of this family typically pyriform to oval in shape, and while some genera form septate hyphae, *Wickerhamiella* does not[43,44]. Currently, 12 genera are listed in this family including *Blastobotrys*, *Crinitomyces*, *Deakozyma*, *Diddensiella*, *Groenewaldozyma*, *Limtongella*, *Spencermartinsiella*, *Starmerella*, *Sugiyamaella*, *Trichomonascus*, *Wickerhamiella*, and *Zygoascus*[41]. In this study, five yeast species were presented, including *Entelexis stigmatis* (two strains), *Starmerella etchellsii* (one strain), *S. orientalis* (two strains), *S. thailandica* sp. nov. (two strains), and *Wickerhamiella pollinicola* sp. nov. (three strains).

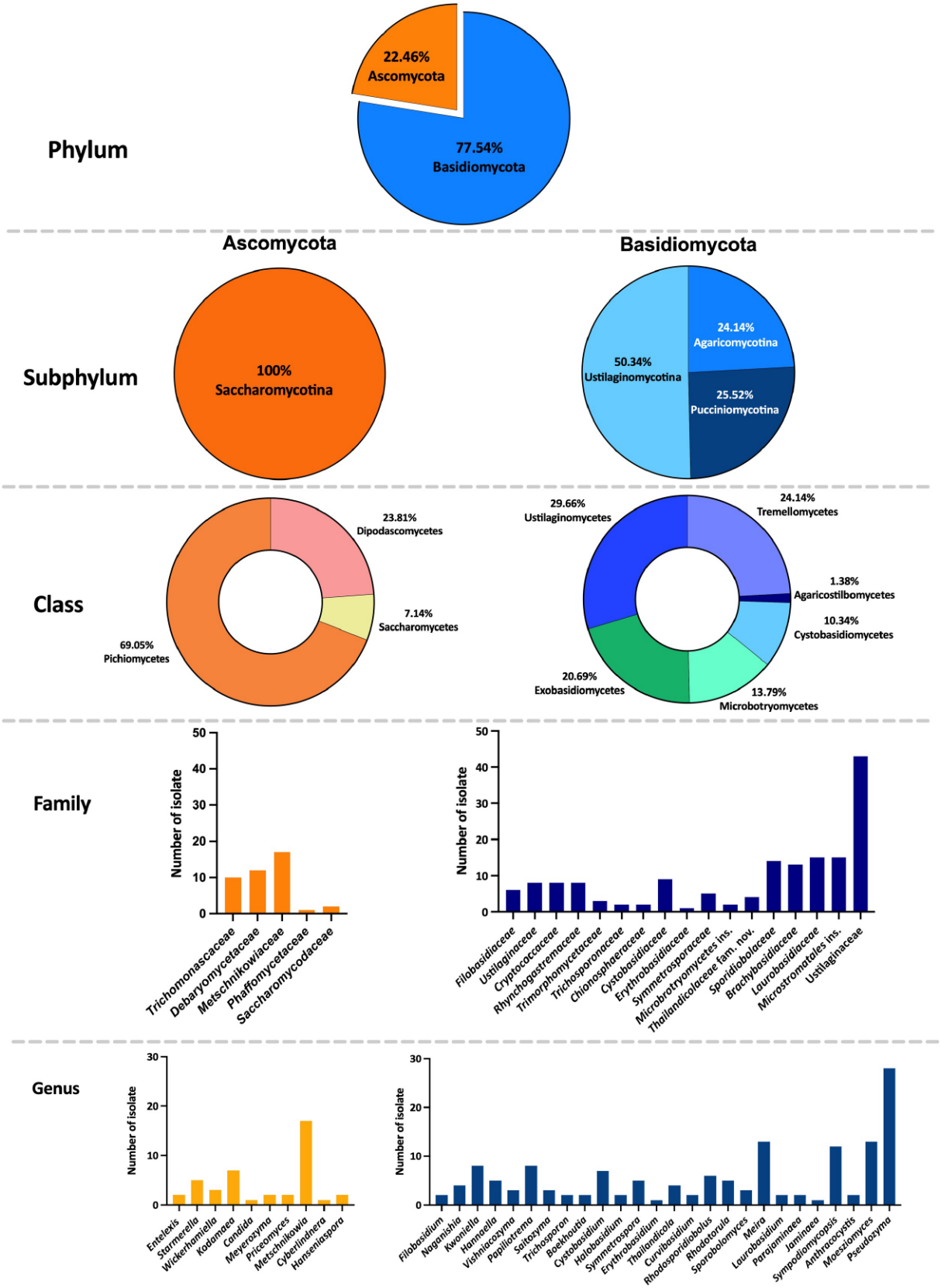

Fig. 3 Classification of anthophilous yeasts isolated from flowers collected in northern Thailand in this study.

Entelexis stigmatis Sipiczki ex Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Supplementary File 1: Fig. S1a)

MycoBank number: MB 860217

≡ *Starmerella stigmatis* (Sipiczki) ex C.A. Rosa & Lachance, *Int. J. Syst. Evol. Microbiol.* 68(4): 1342 (2018)

≡ *Candida stigmatis* Sipiczki. *FEMS Yeast Res.* 10(3): 364 (2010)

Description – Sipiczki[45]

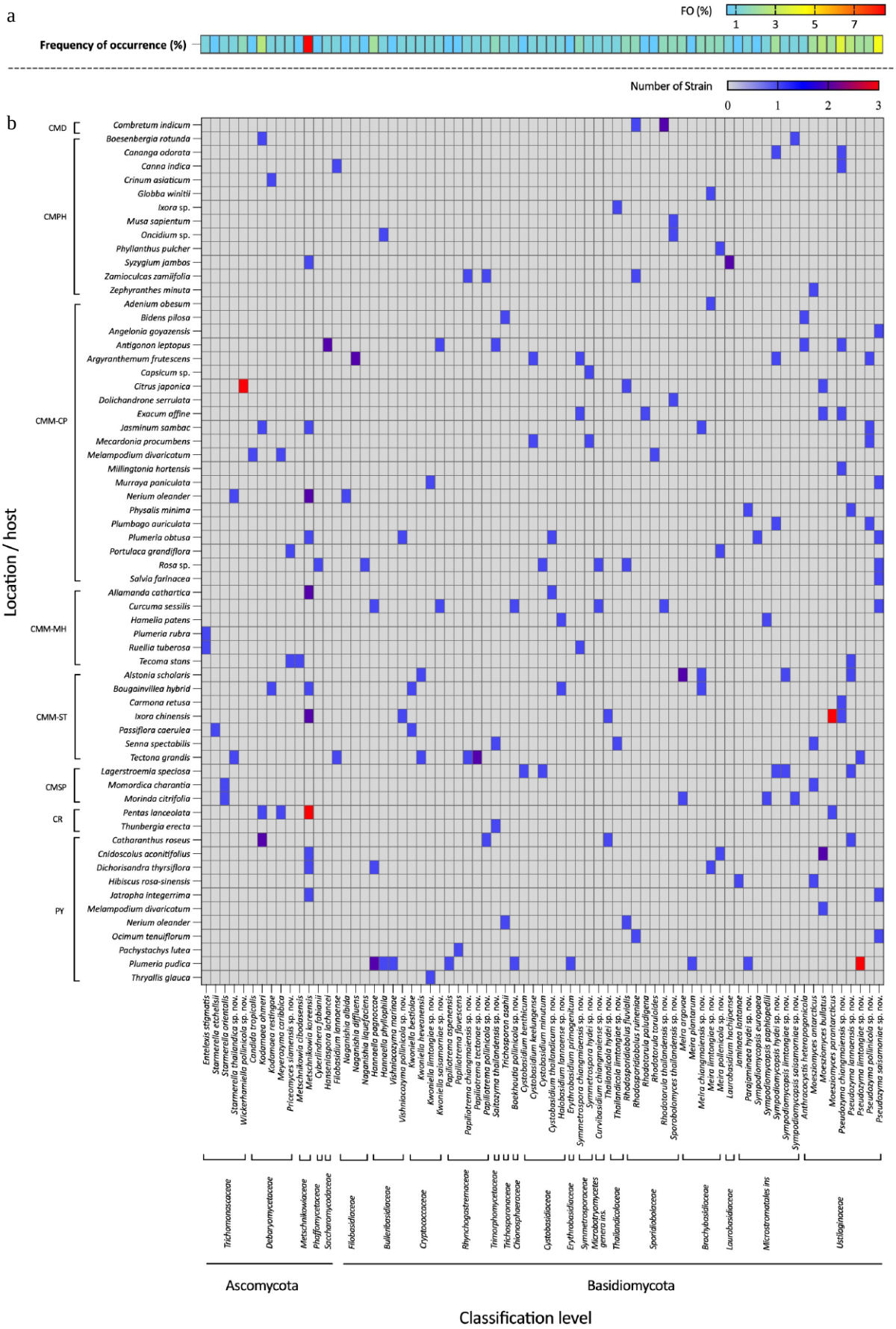

Fig. 4 Heatmap showing the consensus species diversity of anthophilous yeasts isolated from flowers collected in northern Thailand. (a) Frequency of occurrence (FO %) = number of samples, (b) where a particular species was observed, as a proportion of the total number of samples.

Holotype – INDIA, Hyderabad District, flower of *Magnolia* sp., 2007, sin. coll., CBS 11464 holotype, preserved in a metabolically inactive state; isotype CCY 29-179-1 = CBS 11462.

(*Candida stigmatis* Sipiczki, *FEMS Yeast Res.* 10[3]: 364 [2010] [nom. inval., Melbourne Code, Art., 40.7]), *Starmerella stigmatis* C.A. Rosa & Lachance, in Santos, Leon, Barros, Freitas, Hughes, Morais, Lachance & Rosa, *Int. J. Syst. Evol. Microbiol.* 68[4]: 1342 [2018] [nom. inval., Melbourne Code, Art., 40.7])

Material examined – THAILAND, Chiang Mai Province, Mueang District, Mae Hia in watrakanu flower (*Ruellia tuberosa*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU591, Chang Phueak, in frangipani flower (*Plumeria rubra*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU604. GenBank numbers SDBR-CMU591: PV834437 (D1/D2); SDBR-CMU604: PV834438 (D1/D2).

Ecology and distribution – *Magnolia* sp. flower in India[45], and flowers of *Plumeria rubra* and *Ruellia tuberosa* in Thailand (this study).

Notes – Phylogenetic analyses based on combined D1/D2 domain and ITS sequence data (Fig. 5) showed that *Entelexis* forms a distinct clade, separate from *Starmerella*, which is concurred with the results of previous studies[46]. *Entelexis stigmatis* strains SDBR-CMU591 and SDBR-CMU604 were clustered with the type of species (CBS 11464), and another representative strain (GY9L07). A comparison of D1/D2 domain and ITS indicates that the present strains are not significantly different from *E. stigmatis* (only one and four differentiated nucleotides, respectively). However, the species of *E. stigmatis* is currently considered Nom. inval. under Art. 40.7 of the Melbourne Code as listed in Index Fungorum[47]. Therefore, *E. stigmatis* is validated as a member of *Entelexis* by providing a registration identifier and presenting corrected type citations, along with references to the original descriptions. The morphological characteristics of the yeast strains in this study are similar to those previously reported, which were identified as *E. stigmatis*. Additionally, we hereby represent its first isolation from flowers of *Plumeria rubra* and *Ruellia tuberosa*, and new geological distribution in Thailand.

Starmerella orientalis (Alimad., Soudi, F.Y. Bai, S.A. Wang & Q.M. Wang) ex Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Supplementary File 1: Fig. S1c)

MycoBank number: MB860216

Description – Alimadadi et al.[48]

Holotype – IRAN, East Azerbaijan Province, flower of *Salsola* sp., October 2012, sin. coll., SAM09 holotype, preserved in a metabolically inactive state; isotype IBRC-M 30204 = CBS 14142.

(*Starmerella orientalis* Alimad., Soudi, F.Y. Bai, S.A. Wang & Q.M. Wang, *Int. J. Syst. Evol. Microbiol.* 66[3]: 1478 [2016] [nom. inval., Shenzhen Code, Art., 40.7])

Material examined – THAILAND, Chiang Mai Province, San Kamphaeng District, Ton Pao, in balsum pear flower (*Momordica charantia*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU707, Indian mulberry flower (*Morinda citrifolia*), August 2024, P. Kodchasee living culture SDBR-CMU711. GenBank numbers SDBR-CMU707: PV834432 (D1/D2), PV834619 (ITS); SDBR-CMU711: PV834433 (D1/D2), PV834620 (ITS).

Ecology and distribution – *Salsola* sp. flower in Iran[48], and flowers of *Momordica charantia* and *Morinda citrifoli* in Thailand (this study).

Notes – *Starmerella orientalis* was introduced by Alimadadi et al.[48] based on physiological characteristics and phylogeny of rRNA gene sequences. However, the species is considered Nom. inval., Art. 40.7 (Shenzhen) in Index Fungorum[47]. Phylogenetic analyses of a combined D1/D2 domain, and ITS sequence dataset (Fig. 5) show that the present sequence strains, SDBR-CMU707 and SDBR-CMU711, form a clade, clustering with *S. orientalis* with 78% MLBS and 0.99 BYPP support value. A comparison of D1/D2 domain and ITS region shows that the present strains, SDBR-CMU707 and SDBR-CMU711, are not significantly different from *S. orientalis* (only one nt substitutions in both regions). Therefore, *S. orientalis* is validated as a member of *Starmerella* by providing a registration identifier and presenting corrected type citations, along with references to the original descriptions. In addition, morphological comparison reveals that the present yeast strains correspond to previously characterized strains of *S. orientalis*[48], representing a new geographical distribution in Thailand, and marking the first isolation from the flowers of *Momordica charantia* and *Morinda citrifolia*.

Starmerella thailandica Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Fig. 6)

MycoBank number: MB860170

Etymology – The species name '*thailandica*' refers to Thailand, the country where the type strain was isolated.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak in oleander flower (*Nerium oleander*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, holotype, CMUB40094 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU592 = GMBCC2395 = TBRC21393. GenBank numbers PV834434 (D1/D2), PV834621 (ITS), PX582293 (*rpb1*), PX582313 (*rpb2*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1.5–2 mm in diameter), yellowish white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are ovoid to ellipsoidal (1.92–3.47 × 3.44–4.92 µm, n = 50), budding is polar. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Ascospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, D-galactose, sorbose, ribose, xylose, sucrose, raffinose, glycerol, D-glucitol, D-mannitol, D-glucono-1,5-lactone, D-gluconate, succinate, citrate, ethanol, and xylitol are assimilated, but *N*-acetyl glucosamine, L-arabinose, D-arabinose, L-rhamnose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, salicin, melibiose, lactose, melizitose, inulin, soluble starch, erythritol, ribitol, galactitol, *myo*-inositol, D-glucuronate, D-galacturonic acid, DL-lactate, and methanol are not assimilated. Ammonium sulfate, ethylamine, L-lysine (weak) are assimilated as sole nitrogen source, but potassium nitrate, and sodium nitrite are not assimilated. Cadaverine is variable. Growth occurs on media containing 50% glucose, 60% glucose, 10% NaCl/5% glucose, and 16% NaCl/5% glucose. No growth occurs on media containing, 0.01% cycloheximide and 0.1% cycloheximide. Urease reaction and acid formation are negative. Growth at 10, 20, 25, 35, 37 °C, but not at 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Suthep, in teak flower (*Tectona grandis*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU631. GenBank numbers PV834435 (D1/D2), PV834625 (ITS).

Notes – Phylogenetic analyses based on a combined dataset of the D1/D2 domain and ITS sequences revealed that *S. thailandica* (SDBR-CMU592 and SDBR-CMU631) is closely related to *S. fangiana* strains 11-1462 and 11-1463. Together, they form a sister clade to *S. fangiana*, including strains JCM36912 (type strain), QFM-Y-5, QFM-Y-6, D5S-2, QFM-Y-10, DMKU-DWEN32-1, QFM-Y-6, and 16S1[49], with

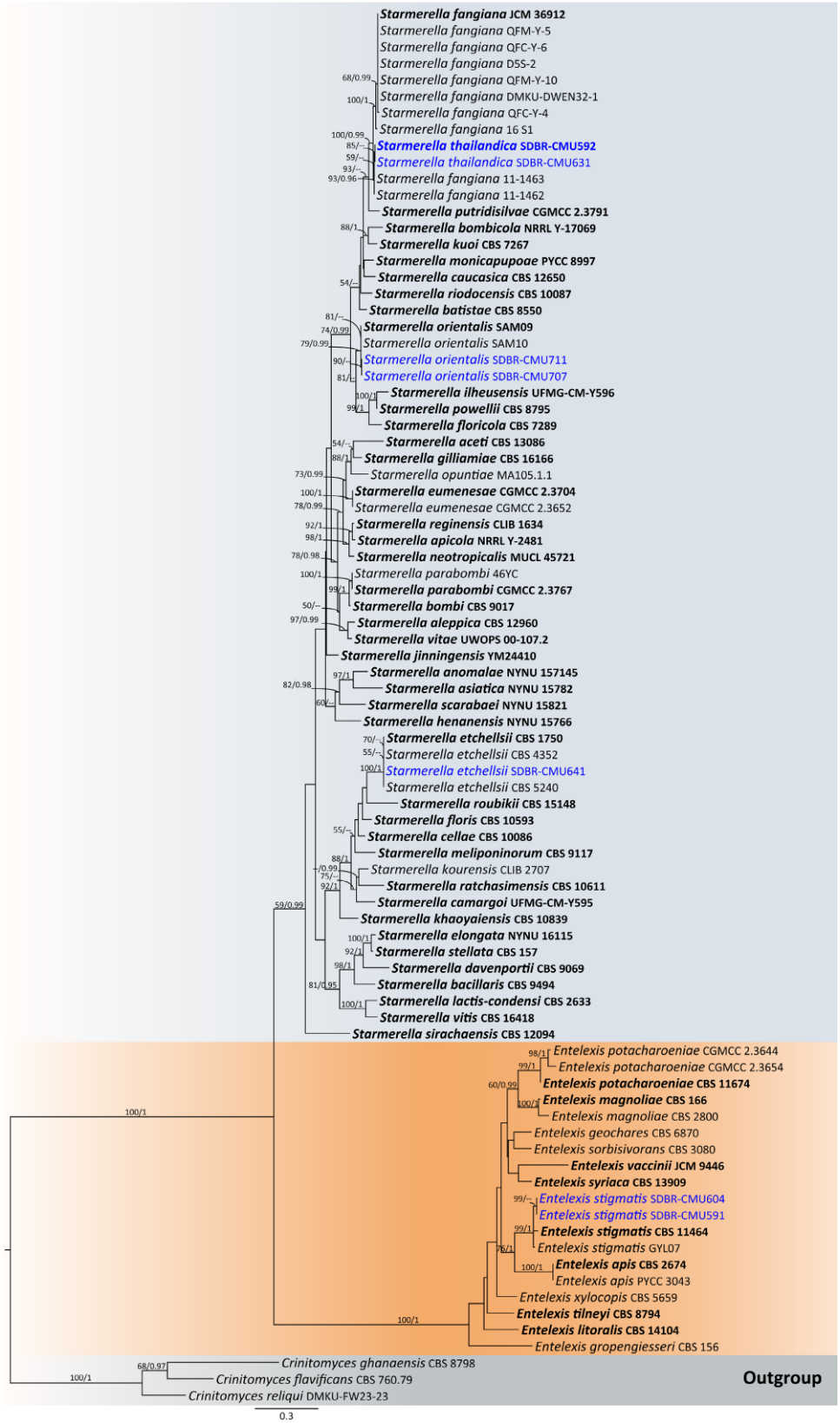

Fig. 5 Phylogram generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing genera *Entelexis* and *Starmerella* in *Trichomonascaceae*. The tree is rooted to *Crinitomyces flavificans* (CBS 760.79), *C. ghanaensis* (CBS 8798), and *C. reliqui* (DMKU-FW23-23). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Eighty-seven strains are included in the combined sequence analysis, which comprise 1,111 characters with gaps. Bootstrap support values for maximum likelihood (≥ 50%, ML, left) and Bayesian posterior probabilities (≥ 0.95, PP, right) are indicated above the nodes. Double dashes (--) denote support values below 50% ML and 0.95 PP. The scale bar represents 0.3 nucleotide substitutions per site. Ex-type strains are shown in bold, and sequences generated in this study are highlighted in blue.

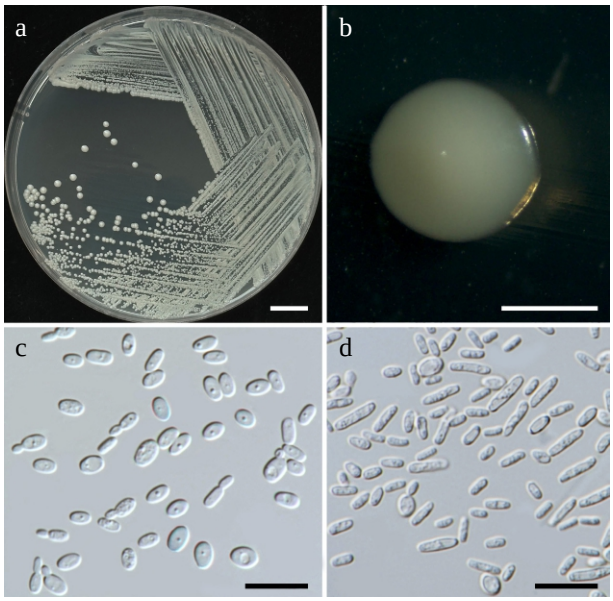

Fig. 6 Morphological characteristics of *Starmerella thailandica* (SDBR-CMU592, ex-type). (a) Culture, (b) single colony, (c) budding cells, on YMA at 25 °C for 5 d, and (d) elongated cells on PDA at 25 °C after 2 weeks. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

100% BSML and 0.99 BYPP support (Fig. 5). *Starmerella thailandica* and *S. fangiana* strains 11-1462 and 11-1463 differ 2 nucleotide substitutions in the ITS region, respectively, suggesting that they may represent the same species. However, further investigation, including detailed morphological and physicochemical characterization of *S. fangiana* strains 11-1462 and 11-1463, is required. In contrast, *S. thailandica* SDBR-CMU592 and SDBR-CMU631 differ from *S. fangiana* clade (including type strain) by 2 nucleotide substitutions and 1 gap (0.22%) in the D1/D2 domain, and by 10–25 nucleotide mismatches (~2.40%–5.84%), including substitutions and deletions, in the ITS region. Likewise, *S. fangiana* strains 11-1462 and 11-1463 differ from *S. fangiana* clade by 2 nucleotide substitutions, and 1 gap (0.22%) in the D1/D2 domain, and by 7–25 nucleotide mismatches (~1.88%–5.85%) in the ITS region. Based on the ITS region mismatches, *S. thailandica* and *S. fangiana* strains 11-1462 and 11-1463 represent distinct taxa from *S. fangiana*. *Starmerella thailandica* can be distinguished from *S. fangiana* JCM36912 by its ability to assimilate xylose, L-arabinose, D-glucono-1,5-lactone, D-gluconate, and creatine, as well as by its growth in 16% NaCl/5% glucose medium. Moreover, *S. fangiana* was able to grow at 40 °C, whereas *S. pollenicalo* cannot[49]. Based on ITS sequence data and phenotypic characteristics, strains SDBR-CMU592 and SDBR-CMU631 are therefore proposed to represent a novel *Starmerella* species.

Wickerhamiella pollinicola Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Fig. 7)

MycoBank number: MB860171

Etymology – The specific epithet, '*pollinicola*' refers to the substrate origin of the type strain, pollen structure.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak in kumquat flower (*Citrus japonica*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, holotype CMUB40088 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU569 = GMBCC2396 = TBRC21388. GenBank numbers PV834439 (D1/D2), PV834624 (ITS), PX582314 (*rpb2*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1.5–2 mm in diameter), yellowish white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are ovoid to ellipsoidal (2.31–5.42 × 5.15–8.53 μm, n = 50), budding is polar. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Ascospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is positive. D-Glucose, galactose, sorbose, xylose (weak), DL-arabinose (weak), sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, melizitose, glycerol, erythritol, ribose (weak), D-glucitol, mannitol, and D-glucono-1,5-lactone are assimilated, but *N*-acetyl glucosamine, L-rhamnose, cellobiose, salicin, melibiose, lactose, raffinose, inulin, soluble starch, ribitol, galactitol, *myo*-inositol, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, methanol, ethanol, and xylitol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite (weak), ethylamine (weak), L-lysine (weak), and cadaverine are assimilated as sole nitrogen source. Growth occurs on media containing 50% glucose and 60% glucose. No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is positive. Growth on 10, 15, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak in kumquat flower (*Citrus japonica*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU617 and SDBR-CMU618. GenBank numbers SDBR-CMU617: PV834440 (D1/D2), PV834625 (ITS); SDBR-CMU618: PV834441 (D1/D2), PV834626 (ITS).

Notes – *Wickerhamiella pollinicola* (SDBR-CMU569, SDBR-CMU617 and SDBR-CMU618) formed a strong clade with 100 MLBS, 0.99 PP support value and clustered as sister to *W. azyma* (CBS 6826, type species) and *W. azymoides* (UFMG-R287, type species; Fig. 8). A comparison of D1/D2 domain revealed that *W. pollinicola* differed from *W. azyma* and *W. azymoide*s by 4 nucleotide substitutions (0.7% nucleotide divergence) and 9 nucleotide substitutions with 1 gap (1.57% nucleotide divergence), respectively. While *W. pollinicola* differs by 15 nucleotide substitutions, and 7 gaps (3.54%) from *W. azyma*, and by 16 nucleotide substitutions and 8 gaps (3.76%)

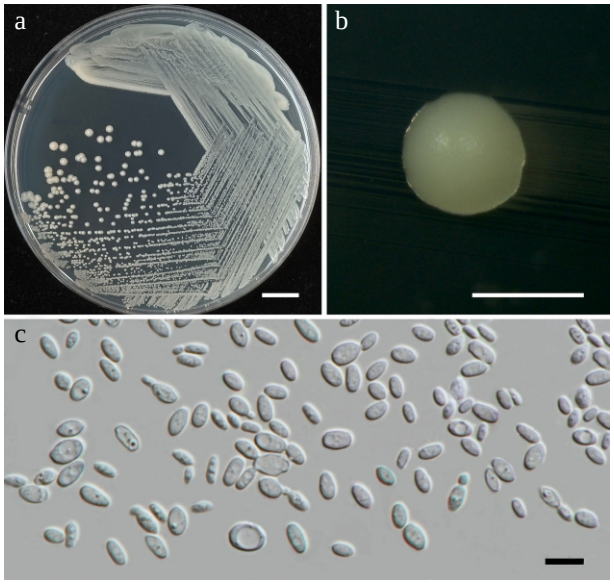

Fig. 7 Morphological characteristics of *Wickerhamiella pollinicola* (SDBR-CMU569, ex-type). (a) Culture, (b) single colony, (c) cells and budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, and (c) = 10 μm.

from *W. azymoides* in ITS region. These differences indicate that the strain represents a new member of the genus *Wickerhamiella*. *Wickerhamiella pollinicola* can be distinguished from *W. azyma* by its ability to assimilate maltose, α-α-trehalose, methyl-α-D-glucoside, melizitose, D-Glucono-1,5-lactone, citrate, ammonium sulfate, and potassium. *Wickerhamiella pollinicola* can grow at 30 °C, while *W. azyma* shows maximum growth temperature at 37 °C[50]. *Wickerhamiella pollinicola* can ferment glucose, whereas *W. azymoides* cannot, allowing for clear differentiation between the two species[51]. Moreover, *W. pollinicola* can assimilate D-arabinose, melezitose, potassium nitrate, and sodium nitrite, in contrast to *W. azymoides*[51].

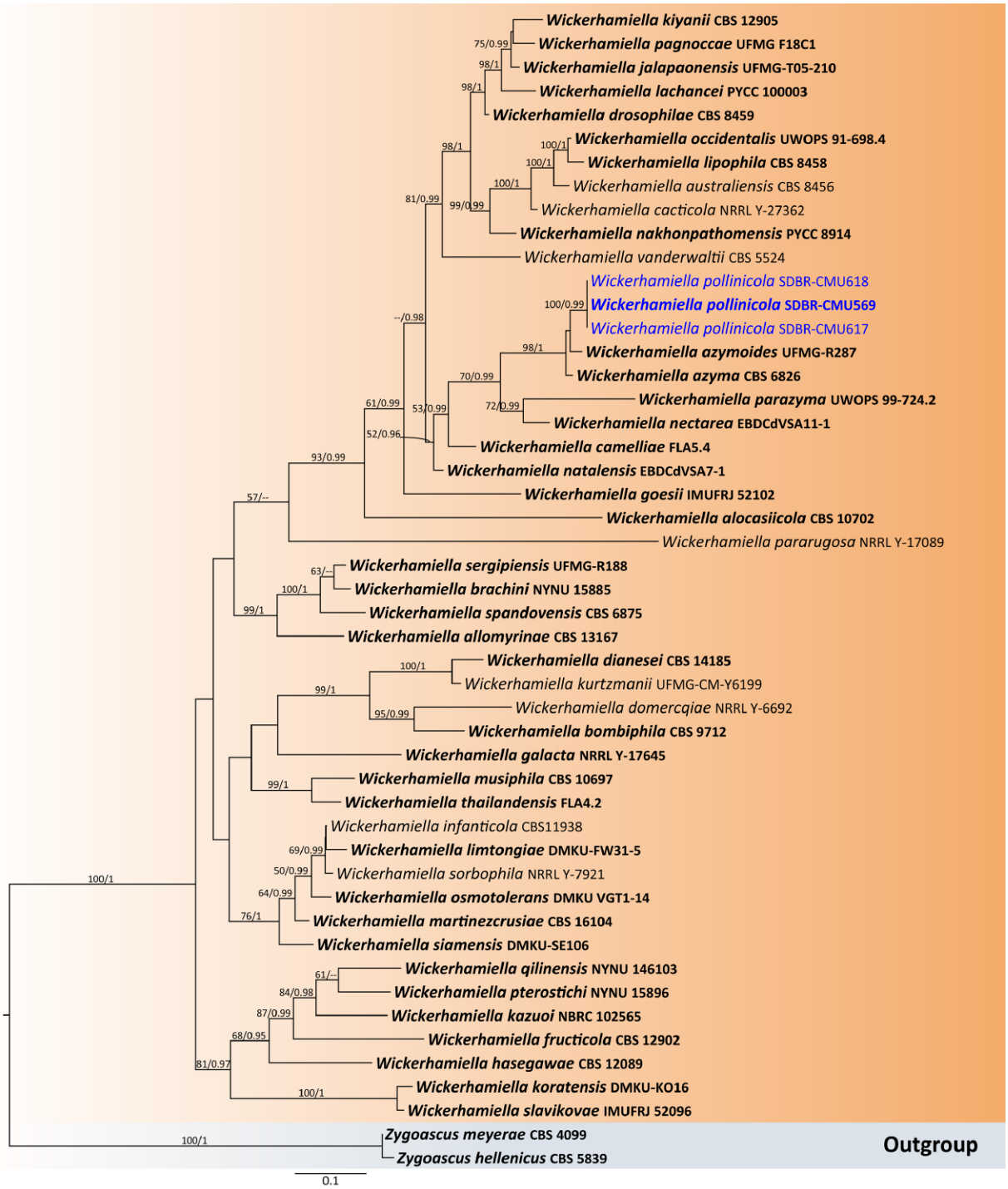

Fig. 8 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing genus *Wickerhamiella* in *Trichomonascaceae*. The tree is rooted to *Zygoascus hellenicus* (CBS 5839) and *Z. meyerae* (CBS 4099). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Forty-nine strains are included in the combined sequence analysis, which comprise 1,190 characters with gaps. Bootstrap support values for maximum likelihood (≥ 50%, ML, left) and Bayesian posterior probabilities (≥ 0.95, PP, right) are indicated above the nodes. Double dashes (--) denote support values below 50% ML and 0.95 PP. The scale bar represents 0.1 nucleotide substitutions per site. Ex-type strains are shown in bold, and sequences generated in this study are highlighted in blue.

Class: Pichiomycetes M. Groenew., Hittinger, Opulente & A. Rokas

Order: *Serinales* M. Groenew., Hittinger, Opulente & A. Rokas

Family: *Debaryomycetaceae* Kurtzman & M. Suzuki

Debaryomycetaceae, the members of this family exhibit polyphyllous budding as their primary form of asexual reproduction. Cells of this family are generally ovate to elliptic, smooth in surface, and of medium size. That widespread ecological distribution and considerable industrial relevance include soil, plants, food products, animal tissues insects, which may function as either beneficial symbionts or occasional opportunistic pathogens[52,53]. Currently, 23 genera are listed in this family including *Aciculoconidium*, *Candida*, *Danielozyma*, *Debaryomyces*, *Diutina*, *Hemisphaericaspora*, *Hyphopichia*, *Kodamaea*, *Kurtzmaniella*, *Limtongozyma*, *Lodderomyces*, *Metahyphopichia*, *Meyerozyma*, *Millerozyma*, *Nematodospora*, *Priceomyces*, *Scheffersomyces*, *Schwanniomyces*, *Spathaspora*, *Suhomyces*, *Teunomyces*, *Wickerhamia*, and *Yamadazyma*[41]. In this study, five yeast species were presented, including *Kodamaea ohmeri* (five strains), *K. restingae* (two strains), *Candida tropicalis* (one strain), *Meyerozyma caribbica* (two strains), and *Priceomyces siamensis* sp. nov. (two strains).

Priceomyces siamensis Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Fig. 9)

MycoBank number: MB860172

Etymology – '*siamensis*' referring to Siam, the old name of Thailand, where the new species was found.

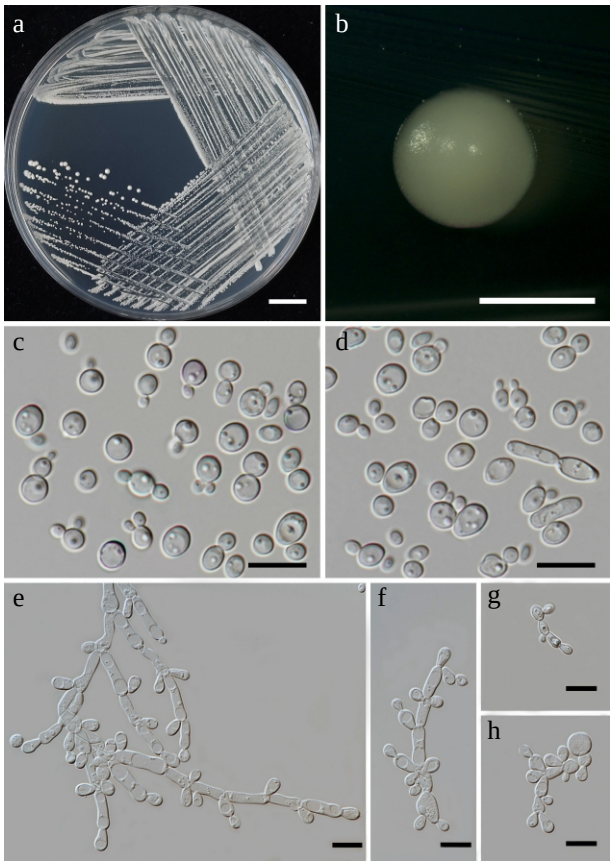

Fig. 9 Morphological characteristics of *Priceomyces siamensis* (SDBR-CMU614, ex-type). (a) Culture, (b) single colony, (c), (d) cells and budding cells on YMA after 5 days at 25 °C. (e)–(h) Pseudohyphae and blastoconidia on PDA after 2 weeks at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, and (c)–(h) = 10 µm.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Mae Hia in Yellow elder flower (*Tecoma stans*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, holotype, CMUB40099 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU614 = GMBCC2397 = TBRC21399. GenBank numbers PV834452 (D1/D2), PV834637 (ITS), PX622318 (SSU), PX582294 (*rpb1*), PX582315 (*rpb2*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1.5–2 mm in diameter), white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are spherical, ovoid, ellipsoidal (2.95–4.88 × 3.28–9.5 µm, n = 50). Budding is polar. Blastoconidia in Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae are formed. Ascospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is positive. D-Glucose, galactose, sorbose, *N*-acetyl glucosamine (weak), ribose, xylose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, melizitose, glycerol, erythritol, ribitol, D-glucitol, mannitol, D-glucono-1,5-lactone, D-gluconate, succinate, citrate, and xylitol are assimilated, but L-arabinose, D-arabinose, L-rhamnose, salicin, melibiose, lactose, raffinose, inulin, soluble starch, galactitol, *myo*-inositol, D-glucuronate, methanol, D-galacturonic acid, DL-lactate, and ethanol are not assimilated. Ammonium sulfates and ethylamine hydrochloride are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, L-lysine, and cadaverine are not assimilated. Growth occurs on media containing 50% glucose and 60% glucose, 10% NaCl/5% glucose, and 16% NaCl/5% glucose. Not growth occurs on media containing 0.01% cycloheximide and 0.1% cycloheximide. Acid formation is negative. Growth on 10, 15, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak in portulaca flower (*Portulaca grandiflora*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU701. GenBank numbers PV834453 (D1/D2) PV834638 (ITS), PX622319 (SSU).

Notes – In the phylogenetic analyses, two new strains (SDBR-CMU614 and SDBR-CMU701) of *P. siamensis* formed a separate lineage, sister to *P. melissophilus* with 100% MLBS and 0.99 PP support values (Fig. 10) and clustered with *P. fermenticarens* CBS7040 and *P. castillae* CBS 6053. The present strains differed from those strains by 0.86% nucleotide divergence (5 nucleotide substitutions), 1.36% (8 nucleotide substitutions and 2 gaps) and 3.51% (20 nucleotide substitutions and 2 gaps), respectively, in D1/D2. The ITS sequences of the three strains by 1.59% nucleotide divergence (10 nucleotide substitutions and 4 gaps), 0.79% (5 nucleotide substitutions and 4 gaps), and 4.90% (28 nt substitutions and 16 gaps), respectively, which indicated that strain represents a new member of *Priceomyces*. The phenotypic comparisons between *P. siamensis* and *P. melissophilus*, *P. fermenticarens*, and *P. castillae* are shown in Table 4.

Family: *Metschnikowiaceae* T. Kamieński ex Doweld

The members of this family generally have elongated, elliptic, or needle-shaped cell shapes developed within elongated asci, especially in *Metschnikowia*[56]. Many species in this group are found in habitats associated with fruits due to their ability to ferment with high glucose and to thrive in high-sugar environments. It is noteworthy that it produces antimicrobial compounds, especially against other fungi, which contribute to the ecosystem[57]. Hyde et al.[41] mention 15 genera are listed in this family including *Australozyma*, *Candidozyma*, *Clavispora*, *Danielia*, *Gabaldonia*,

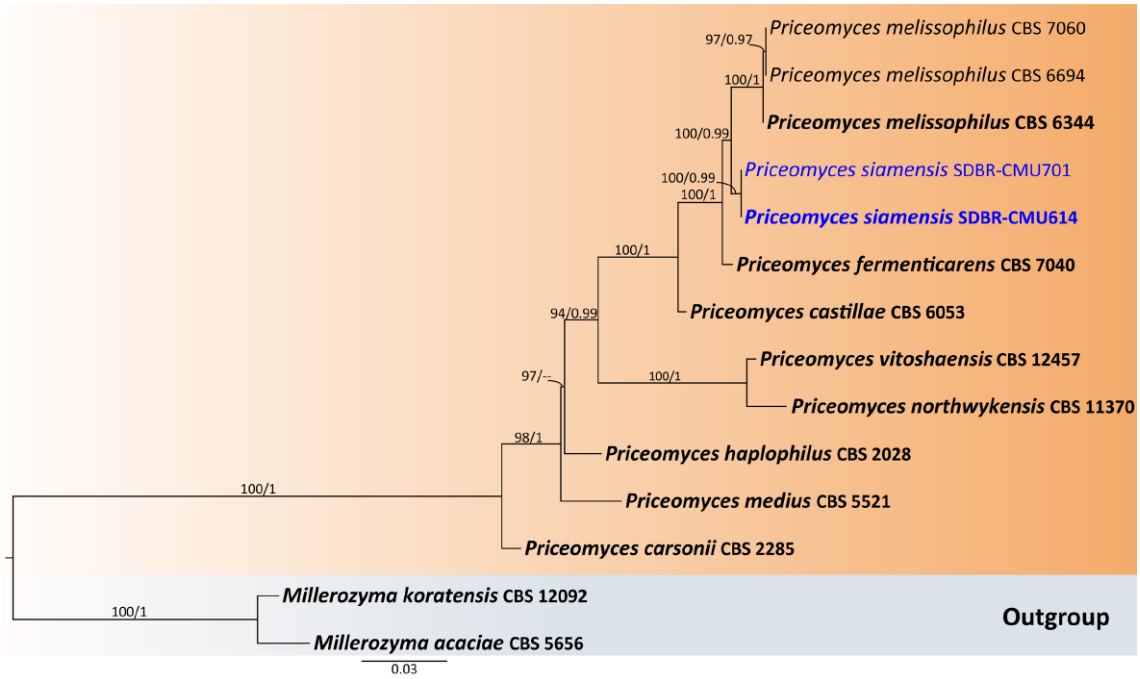

Fig. 10 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Priceomyces*. The tree is rooted to *Millerozyma acaciae* (CBS 5656) and *M. koratensis* (CBS 12092). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Fourteen strains are included in the combined sequence analysis, which comprise 1,121 characters with gaps. Bootstrap support values for maximum likelihood (≥ 50%, ML, left), and Bayesian posterior probabilities (≥ 0.95, PP, right) are indicated above the nodes. Double dashes (--) denote support values below 50% ML and 0.95 PP. The scale bar represents 0.03 nucleotide substitutions per site. Ex-type strains are shown in bold, and sequences generated in this study are highlighted in blue.

Gaillardinia, *Helenozyma*, *Hermanozyma*, *Isabelozyma*, *Metschnikowia*, *Osmozyma*, *Soucietia*, *Sungouiella*, *Tanozyma*, and *Wilhelminamyces*. In this study, two yeast species were presented, including one strain of *Metschnikowia cibodasensis*, and 16 strains of *Me. koreensis* (Supplementary File 1).

Class: Saccharomycetes G. Winter

Order: *Phaffomycetales* M. Groenew., Hittinger, Opulente & A. Rokas

Table 4. Phenotypic characteristics differentiating *Priceomyces siamensis* from closely related *Priceomyces* species.

Characteristics		1	2	3	4
Fermentation	Glucose	+	–	–	–
	D-Ribose	+	–	+	v
	D-Xylose	+	–	w	+
	L-Arabinose	w	–	w	v
	L-Rhamnose	–	–	–	+
	Sucrose	+	+	–	+
	Maltose	+	+	–	+
	α-α-Trehalose	+	–	–	+
	Cellobiose	+	w	–	v
Carbon assimilation	Salicin	–	–	+	+
	Melibiose	–	+	–	v
	Lactose	–	–	–	+
	Raffinose	–	–	–	v
	Melizitose	+	–	–	+
	Soluble starch	–	–	–	+
	Erythritol	+	+	+	–
	Succinate	+	–	+	+
	Ethanol	–	+	+	+
Nitrogen assimilation	Potassium nitrate	–	–	–	nd
Growth characteristics	Growth at 37 °C	–	+	+	+

Strains 1: *P. siamensis* sp. nov, 2: *P. melissophilus*[54], 3: *P. fermenticarens*[55], and 4: *P. castillae*[27].

Family: *Phaffomycetaceae* Y. Yamada, H. Kawas., Nagats., Mikata & T. Seki

Phaffomycetaceae represents exhibit spherical to ellipsoidal cell morphology with vegetative reproduction occurring through multilateral budding[58]. Members of this family exhibit moderate fermentative abilities and can assimilate various carbon compounds. Some species show adaptations to specific environments and are also found in plants, insects, or soil habitats[59]. Currently, four genera are recorded in this family including *Barnettozyma*, *Cyberlindnera*, *Phaffomyces,* and *Starmera*[41]. In this study, *Cyberlindnera fabianii* (one strain) was identified (Supplementary File 1).

Order: *Saccharomycodales* M. Groenew., Hittinger, Opulente & A. Rokas

Family: *Saccharomycodaceae* Kudryavtsev

Saccharomycodaceae is characterized by its unique vegetative reproduction via bipolar budding, where buds form on broad bases at the cell poles, resulting in the characteristic lemon or apiculate cell shape that distinguishes many members of this group[27]. The members of this family exhibit varying degrees of fermentative capability, with some species being strongly fermentative. Many species demonstrate notable tolerance to acidic environments, explaining their prevalence in fruit-associated habitats and fermentation processes[58]. Currently, two genera are listed in this family including *Hanseniaspora* and *Saccharomycodes*[41]. In this study, *Hanseniaspora lachancei* (two strains) are presented (Supplementary File 1).

Phylum: Basidiomycota R.T. Moore

Subphylum: Agaricomycotina Doweld

Class: Tremellomycetes Doweld

Order: *Filobasidiales* Jülich

Family: *Filobasidiaceae* L.S. Olive

Filobasidiaceae, their basidial morphology consists of long, tubular, and often holobasidia-like structures (filobasidia) that develop terminal basidiospores through lateral budding rather than on sterigmata. These basidiospores are typically produced in a row along the elongated basidium[60]. Vegetative reproduction occurs through budding, the cells generally appearing spherical to oval. Additionally, some members produce extracellular polysaccharide capsules and exhibit distinctive carotenoid pigmentation, resulting in orange or red colony coloration. The cell wall composition includes xylose and mannose as predominant carbohydrates[61]. Physiologically, *Filobasidiaceae* do not ferment sugars but can assimilate various carbon compounds. Currently, six genera are listed in this family including *Filobasidium*, *Goffeauzyma*, *Heterocephalacria*, *Naganishia*, *Syzygospora*, and *Zyzygomyces*[41]. In this study *Filobasidium lannaense* sp. nov. (two strains), *Naganishia albida* (one strain), *N. diffluens* (two strains), and *N. liquefaciens* (one strain) were presented.

Filobasidium lannaense Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Fig. 11)

MycoBank number: MB860174

Etymology – '*lannaense*' referring to the Kingdom of Lanna, the historic name of northern Thailand, where the new species was found.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Suthep, in teak flower (*Tectona grandis*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, holotype, CMUB40102 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU628 = GMBCC2398 = TBRC21403. GenBank numbers PV834474 (D1/D2), PV834644 (ITS), PX622320 (SSU), PX582295 (*rpb1*), PX582334 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (3–3.5 mm in diameter), white to pale yellow, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are globosal and ellipsoidal (5.06–12.55 × 6.4–13.31 μm, n = 50), budding is polar. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

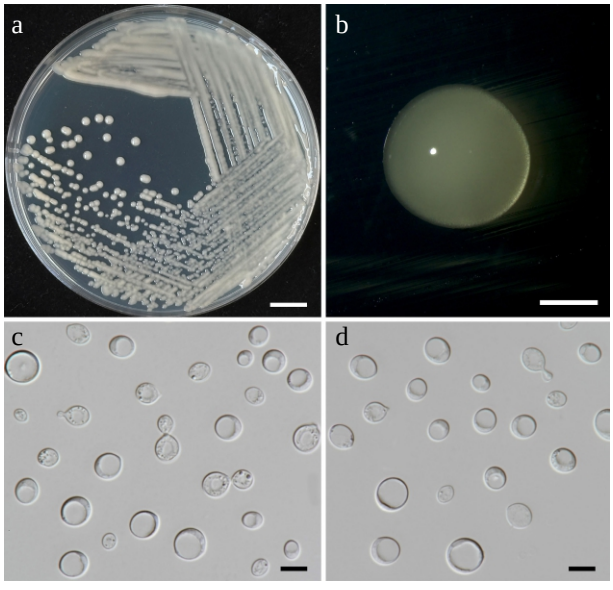

Fig. 11 Morphological characteristics of *Filobasidium lannaense* (SDBR-CMU628, ex-type). (a) Culture, (b) single colony, (c), (d) budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Fermentation of glucose is negative. D-Glucose, D-galactose, ribose, xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, salicin, melibiose lactose, raffinose, melizitose, glycerol (or weak), D-mannitol, galactitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate (or weak), D-galacturonic acid, citrate, succinate, ethanol (or weak), and xylitol are assimilated, but L-sorbose, *N*-acetyl glucosamine, inulin, soluble starch, erythritol, ribitol, D-glucitol, DL-lactate, and methanol are not assimilated. Ammonium sulfates, potassium nitrate (or weak), sodium nitrite, ethylamine hydrochloride and L-lysine are assimilated as sole nitrogen source, but cadaverine are not assimilated. No growth occurs on media containing 50% glucose, and 60% glucose. No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth at 10, 15, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in canna lily flower (*Canna indica*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU681. GenBank numbers PV834475 (D1/D2), PV834642 (ITS), PX622321 (SSU), PX582296 (*rpb1*), PX582335 (*tef1-α*).

Notes – A phylogenetic analysis of the combined D1/D2 domain and ITS sequence dataset shows that SDBR-CMU628 and SDBR-CMU681 strains are closely related to *Filobasidium pseudomali* (NYNU 2111105 and NYNU 22986), *F. mali* CGMCC 2.4012, *F. castaneae* NYNU 2111105 and *F. globosum* (CGMCC 2.5680 and CGMCC 2.5656) (Fig. 12). The D1/D2 sequences differed by 0.16%–1% nucleotide divergence (2–6 nt substitutions) and 2.10%–8.75% (13–53 nt substitutions) in the ITS region. Therefore, a new species, *Filobasidium lannaense* is introduced in *Filobasidiaceae*. Additionally, phenotypic differences between *F. lannaense* and *F. pseudomali*, *F. castaneae*, *F. mali*, and *F. globosum* are shown in Table 5.

Order: *Tremellales* Fr.

Family: *Bulleribasidiaceae* Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout

Bulleribasidiaceae are found on the surfaces of plants, particularly as organisms that reside on the leaf blades. Numerous species exhibit psychrotolerant traits, which enable them to thrive in cold climates[64]. Characterized by this group, colonies can be white, cream, pink, or orange due to the production of carotenoid pigments. The cells are spherical, oval, or elongated. Physiologically, most species do not ferment sugars but display a wide range of abilities to assimilate various carbon compounds. Most are urea positive and can use inositol as a carbon source, a property that aids in species identification[65]. Currently, six genera are listed in this family including *Bulleribasidium*, *Derxomyces*, *Dioszegia*, *Hannaella*, *Nielozyma*, and *Vishniacozyma*[41]. In this study, *Hannaella pagnoccae* (four strains), *Ha. phyllophila* (one strain), *Vishniacozyma marinae* (one strain), and *V. pollinicola* sp. nov. (two strains) were presented (Fig. 13, Supplementary File 1).

Vishniacozyma pollinicola Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Fig. 14)

MycoBank number: MB860175

Etymology – The specific epithet '*pollinicola*' refers to the substrate origin of the type strain, pollen structure.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in frangipani flower (*Plumeria obtusa*), July 2024, P.

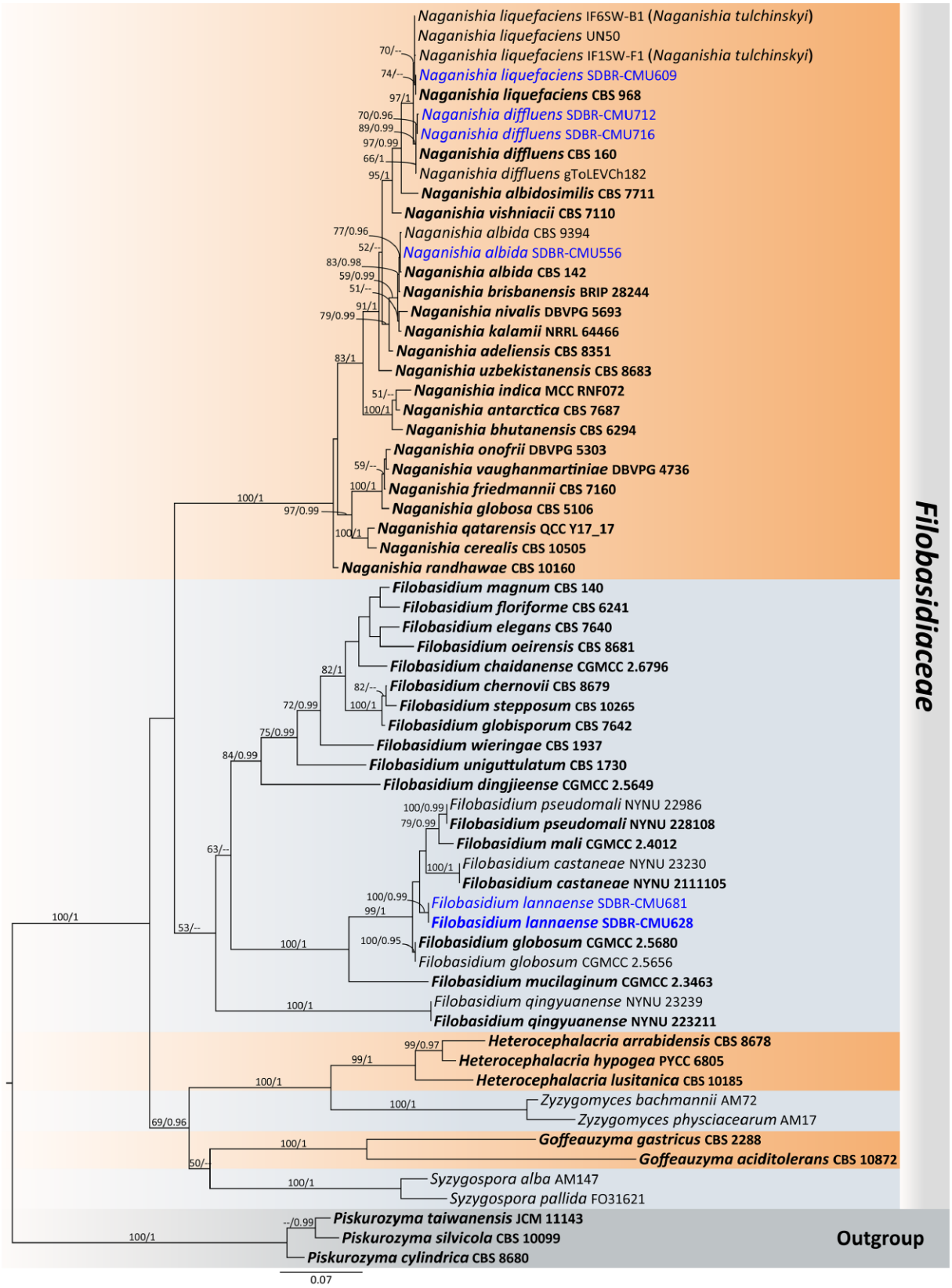

Fig. 12 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Filobasidiaceae*. The tree is rooted to *Piskurozyma cylindrica* (CBS 8680), *Pi. silvicola* (CBS 10099), and *Pi. taiwanensis* (JCM 11143). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Sixty-four strains are included in the combined sequence analysis, which comprise 1,398 characters with gaps. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

Table 5. Phenotypic characteristics differentiating *Filobasidium lannaense* from closely related *Filobasidium* species.

Characteristics		1	2	3	4	5
Carbon assimilation	L-Sorbose	–	+	–	+	+
	N-Acetyl glucosamine	–	+	–	–	–
	D-Ribose	+	+	–	–	–
	D-Xylose	+	+	w	w	–
	L-Arabinose	+	+	+	w	+
	D-Arabinose	+	–	–	–	+
	L-Rhamnose	+	+	w	w	v
	Methyl-α-D-glucoside	+	+	w	w	+
	Cellobiose	+	+	+	w	+
	Salicin	+	+	–	w	–
	Melibiose	+	+	+	w	+
	Lactose	+	+	+	–	+
	Raffinose	+	+	+	w	+
	Inulin	–	+	+	–	+
	Glycerol	w	+	–	–	–
	Ribitol	w	+	–	–	+
	D-Glucitol	+	+	–	–	+
	Galactitol	+	+	–	+	+
	myo-Inositol	+	+	w	w	+
	Succinate	+	+	w	w	+
	Citrate	+	+	–	–	+
	Ethanol	w	–	–	w	+
	Sodium nitrite	+	+	–	–	+
Nitrogen assimilation	Ethylamine HCl	w	+	+	–	+
	Cadaverine	–	–	–	+	–
	Growth at 25 °C	+	+	+	+	+
Growth characteristics	Growth at 30 °C	+	–	–	v	+
	Growth at 35 °C	–	–	–	v	–

Strains 1: *F. lannaense* sp. nov., 2: *F. castaneae*[62], 3: *F. globosum*, 4: *F. mali*[63], and 5: *F. pseudomali*[62].

Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40097 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU603 = GMBCC2399 = TBRC21396. GenBank numbers PV834487 (D1/D2), PV834657 (ITS), PV941856 (*rpb1*), PV947458 (*rpb2*), PV947490 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1.0–1.5 mm in diameter), yellowish white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are spheroid to ellipsoid (2.55–4.95 × 3.03–5.75 μm, $n = 50$), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, D-galactose, *N*-acetyl glucosamine, ribose, xylose, L-arabinose, D-arabinose, L-rhamnose (or slow), sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, salicin (or weak), melibiose (or weak), lactose, raffinose, melizitose, D-mannitol, galactitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid (or weak), citrate (or slow), succinate, ethanol, and xylitol are assimilated, but L-sorbose, inulin, soluble starch, glycerol, erythritol, ribitol, D-glucitol, DL-lactate, and methanol are not assimilated. Ammonium sulfates, ethylamine hydrochloride, and L-lysine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, and cadaverine are not assimilated. No growth occurs on media containing 50% glucose and 60% glucose. No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth occurs at 10, 15, 25, 30, but is absent at 35, 37, and 40 °C.

Additional strains examined: THAILAND, Chiang Mai Province, Mueang District, Suthep, in Ixora flower (*Ixora* sp.), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU599. GenBank numbers PV834486 (D1/D2), PV834656 (ITS).

Notes – Phylogenetic analyses of a concatenated D1/D2 domain and ITS sequence dataset show that the present strains (SDBR-CMU599 and SDBR-CMU603) form a separate clade, clustering with *V. melezitolytica* CBS 15490, *V. nebularis* CBS 12283, and *V. floricola* NCAIMY.02320 (98% MLBS and 0.99 BYPP; Fig. 13). The D1/D2 sequences of the present strains differed 1.13%–1.94 % nucleotide divergence with 7 nt substitutions from *V. melezitolytica*, 12 nt substitutions from *V. nebularis* and 11 nt substitutions from *V. floricola*. The ITS sequences also demonstrated divergence 1.79%–2.83% (9–13 nt substitutions and 16–17 gaps) of three species, supporting the recognition of the present strains as a novel species within *Vishniacozyma*. Furthermore, *V. pollinicola* can be differentiated from *V. melezitolytica* with the ability to assimilate melibiose, melizitose, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, citrate, and xylitol[63]. In contrast, *V. pollinicola* does not assimilate in sorbose, inulin, glycerol, ribitol, and D-glucitol, while *V. melezitolytica* can grow. Unfortunately, the phenotypic characteristics of *V. nebularis* and *V. floricola* were not observed[66], and thus comparisons across the species could not be made.

Family: *Cryptococcaceae* Kütz. ex-Castell. & Chalm.

Cryptococcaceae, this family can produce polysaccharide capsules surrounding the yeast cells, particularly evident in pathogenic and environmental survival[67,68]. The vegetative reproduction occurs primarily through budding, the cells generally appearing spherical to oval, and some species exhibit distinctive colony morphologies ranging from mucoid due to capsule production to dry[69]. Physiologically, *Cryptococcaceae* species typically do not ferment sugars but demonstrate varied abilities to assimilate different carbon and nitrogen compounds. Some species produce urease, which serves as a diagnostic. The family includes both environmental saprophytes and opportunistic pathogens of medical significance[70]. Currently, three genera are listed in this family including *Cryptococcus*, *Kwoniella*, and *Teunia*[41]. In this study, *Kwoniella bestiolae* (two strains), *Kw. heveanensis* (two strains), *Kw. limtongiae* sp. nov (two strains), and *Kw. saisamorniae* sp. nov. (two strains) were presented (Fig. 15).

Kwoniella limtongiae Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Fig. 16)

MycoBank number: MB860178

Etymology – '*limtongiae*' named in honor of mycologist Savitree Limtong, for her contributions to yeast systematics.

Holotype – THAILAND, Phayao Province, Mueang District, in galphimia flower (*Thryallis glauca*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, holotype, CMUB40107 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU667 = GMBCC2400 = TBRC21409. GenBank numbers PV834493 (D1/D2), PV834663 (ITS), PX622323 (SSU), PX582317 (*rpb2*), PX570015 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.8–1.2 mm in diameter), yellowish white, smooth surface, entire margin, and convex elevation. The cells are globosal and ellipsoidal, (6.11–8.97 × 5.94–10.38 μm, $n = 50$), budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2

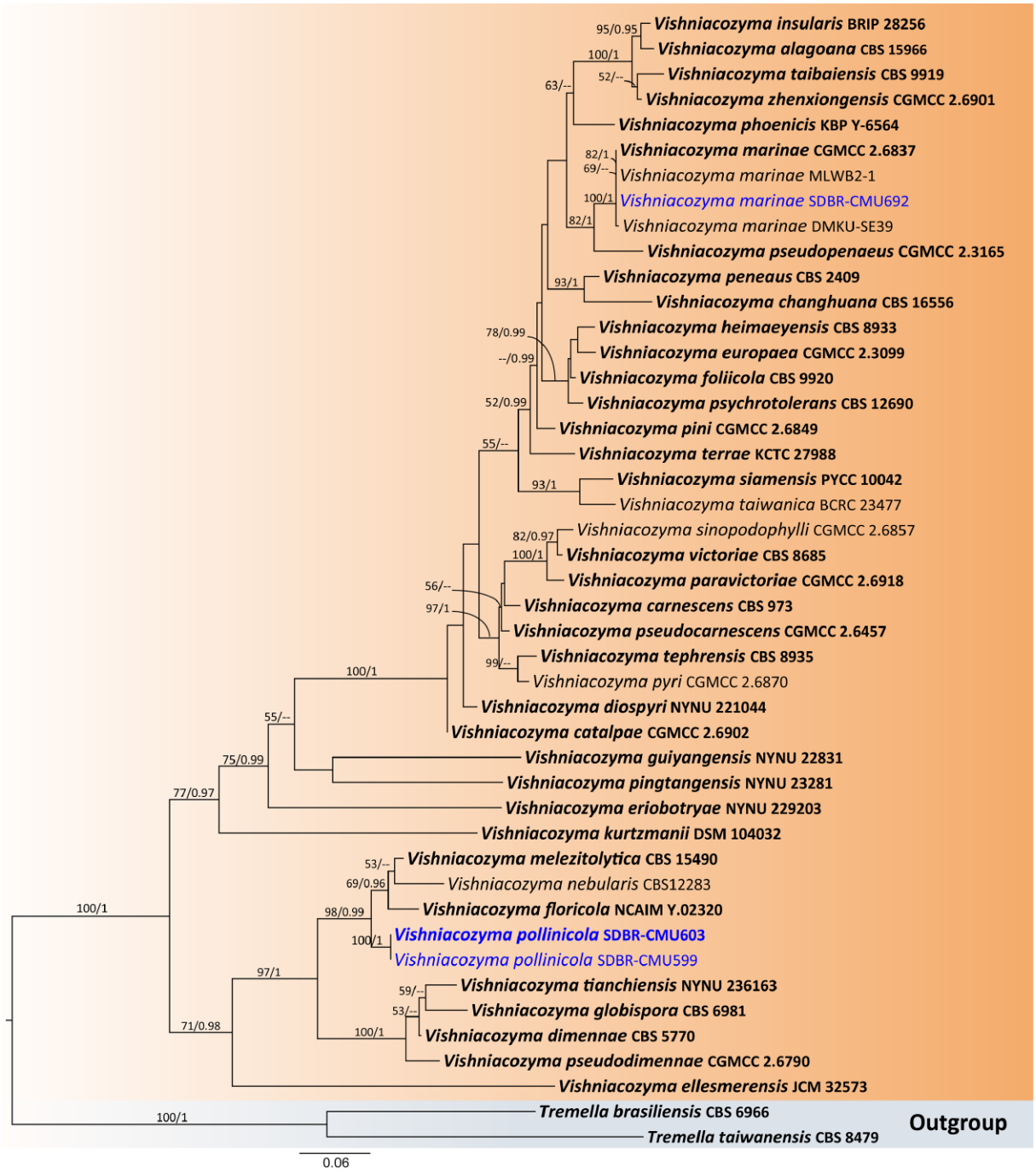

Fig. 13 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Vishniacozyma*. The tree is rooted to *Tremella brasiliensis* (CBS 6966) and *T. taiwanensis* (CBS 8479). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Fourty-five strains are included in the combined sequence analysis, which comprise 1,204 characters with gaps. The average standard deviation of the split frequencies of the BI analysis was 0.004360. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, ribose (weak), xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, salicin (weak), lactose, raffinose, melizitose, glycerol (weak), ribitol, D-glucitol, mannitol, galactitol, *myo*-inositol, D-gluconate, D-galacturonic acid, DL-lactate, succinate, and xylitol are assimilated, but *N*-acetyl glucosamine, melibiose, inulin, soluble starch, erythritol, D-glucono-1,5-lactone, D-glucuronate (weak), citrate, methanol, and ethanol are not assimilated. Ammonium sulfates, ethylamine hydrochloride, L-lysine, and cadaverine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, and creatine are not assimilated. Growth occurs on media

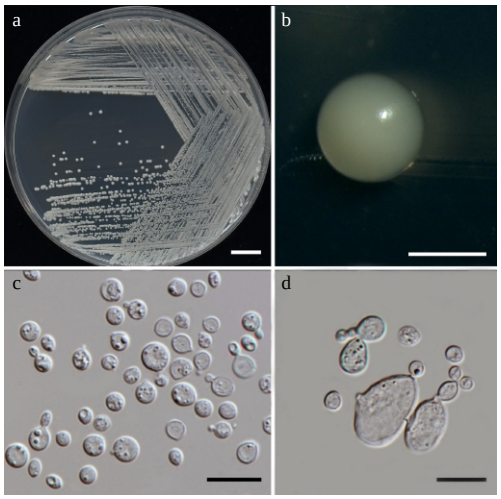

Fig. 14 Morphological characteristics of *Vishniacozyma pollinicola* (SDBR-CMU603, ex-type). (a) Culture, (b) single colony, (c) cells and budding cells on YMA after 5 d at 25 °C. (d) Rudimentary pseudohypha on YMA after 3 weeks at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

containing 50% glucose and 60% glucose (weak). No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction and acid formation are positive. Growth was observed at 10, 15, 25, 35, and 37 °C but not at 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in Orange jasmine flower (*Murraya paniculata*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU588. GenBank numbers PV834492 (D1/D2), PV834662 (ITS), PX622322 (SSU), PX582316 (*rpb2*), PX570014 (*tef1-α*).

Notes – Phylogenetic analyses of a combined D1/D2 domain and ITS sequence data demonstrated that two strains of *Kwoniella limtongiae* (SDBR-CMU667 and SDBR-CMU588) formed a robust clade and clustered with *Kw. heveanensis*, and *Kw. saisamorniae* with 98% MLBS and 1 BYPP statistic support (Fig. 15). The D1/D2 sequences of the two strains differed by 0.32% nucleotide divergence (2 nt substitutions), and 2.07% (12 nt substitutions) in ITS region. Furthermore, *Kw. limtongiae* differed from *Kw. saisamorniae* by 1.90% nucleotide divergence (11 nt substitutions), indicating that these strains represent a novel species within *Kwoniella*.

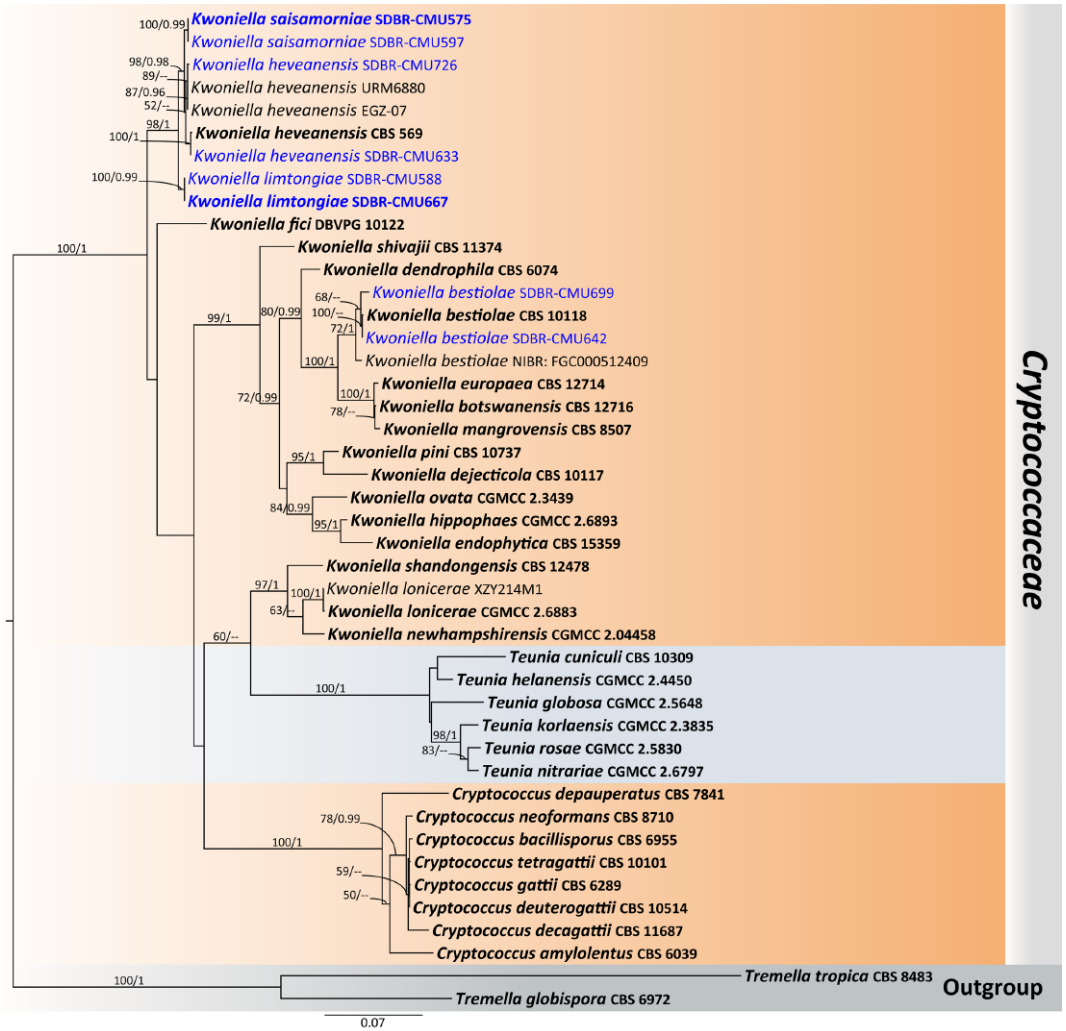

Fig. 15 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Cryptococcaceae*. The tree is rooted to *Tremella globispora* (CBS 6972) and *T. tropica* (CBS 8483). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Fourty-four strains are included in the combined sequence analysis, which comprise 1,274 characters with gaps. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

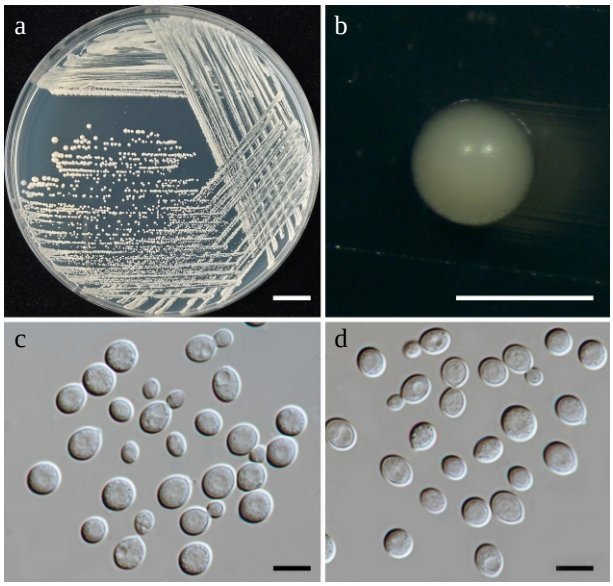

Fig. 16 Morphological characteristics of *Kwoniella limtongiae* (SDBR-CMU667, ex-type). (a) Culture, (b) single colony, (c), (d) cells and budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Additionally, phenotypic differences between *Kw. limtongiae*, *Kw. heveanensis*, and *Kw. saisamorniae* are shown in Table 6.

Kwoniella saisamorniae Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Fig. 17)

MycoBank number: MB860182

Etymology – '*saisamorniae*' referring to the name of mycologist Saisamorm Lumyong in honor of her 75th birthday.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Mae Hia, in Siam tulip flower (*Curcuma sessilis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, holotype CMUB40090 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU575 = GMBCC2401 = TBRC21390. GenBank numbers PV834494 (D1/D2), PV834664 (ITS), PX622324 (SSU), PX570009 (*rpb1*), PX582318 (*rpb2*), PX582336 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.8–1.2 mm in diameter), yellowish white, smooth surface, entire margin, and convex elevation. The cells are broadly ellipsoidal, (4.49–9.29 × 5.5–12.1 μm, *n* = 50), budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, ribose, xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, lactose, raffinose, melizitose, glycerol, erythritol, ribitol, D-glucitol, mannitol, galactitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate (weak), D-galacturonic acid, DL-lactate, succinate, and xylitol are assimilated, but *N*-acetyl glucosamine, salicin, melibiose, inulin, soluble starch, citrate, methanol, and ethanol are not assimilated. Ammonium sulfates, ethylamine hydrochloride, L-lysine, and cadaverine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite and creatine are not assimilated. Growth occurs on media containing 50% glucose and 60% glucose (weak). No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction and acid formation are positive. Growth was observed at 10, 15, 25 °C but not at 37 and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in confederate vine flower (*Antigonon leptopus*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU597. GenBank numbers PV834495 (D1/D2), PV834665 (ITS), PX622325 (SSU), PX570010 (*rpb1*), PX582319 (*rpb2*), PX582337 (*tef1-α*).

Notes – Multi-locus analyses revealed that two strains of SDBR-CMU575 and SDBR-CMU597 formed a clade sister to *Kw. heveanensis* (Fig. 15). Based on the BLASTn search, the closest match using D1/D2 and ITS sequences is *Kw. heveanensis*, showing 0.32% nucleotide divergence (1 nt substitutions) and 1.08% (6 nt substitutions), respectively. Moreover, *Kw. saisamorniae* is different from *Kw. heveanensis* by 3.27 and 6.09% nucleotide divergence (27 and 56 nt substitutions) in *rpb1* and *tef1-α*, respectively. Thus, we introduce a new species, *Kw. saisamorniae*, isolated from flowers of *Antigonon leptopus* and *Curcuma sessilis*, Thailand. In addition, phenotypic differences between *Kw. saisamorniae*, *Kw. heveanensis*, and *Kw. limtongiae* are shown in Table 6.

Table 6. Phenotypic characteristics differentiating *Kwoniella limtongiae* and *Kw. saisamorniae* from closely related *Kwoniella* species.

Characteristics		1	2	3
Carbon assimilation	D-Ribose	w	+	+
	D-Arabinose	+	+	–
	Salicin	w	–	+
	Glycerol	w	+	–
	Erythritol	–	+	+
	D-Glucono-1,5-lactone	–	+	nd
	D-Glucuronate	–	w	+
	DL-Lactate	+	+	w
	Citrate	–	–	w
	Ethanol	–	–	+
Nitrogen assimilation	Creatine	–	–	+
Growth characteristics	Growth at 35 °C	+	w	nd
	Growth at 37 °C	+	–	+

Strains 1: *Kw. limtongiae* sp. nov., 2: *Kw. saisamorniae* sp. nov., and 3: *Kw. heveanensis*[69].

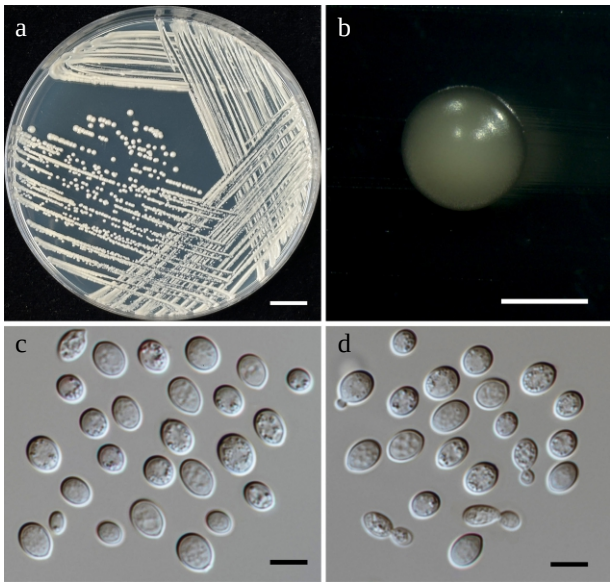

Fig. 17 Morphological characteristics of *Kwoniella saisamorniae* (SDBR-CMU575, ex-type). (a) Culture, (b) single colony, (c), (d) cells and budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Family: *Rhynchogastremaceae* Oberw. & B. Metzler

The family was established by Metzler et al.[71] to accommodate the unique dimorphic mycoparasite *Rhynchogastrema coronatum*. The type species demonstrates both yeast-like and filamentous forms depending on environmental conditions and life cycle stage. The vegetative morphology includes both unicellular yeast-like forms and filamentous forms. The cell is spherical to oval and hyphal forms exhibit typical basidiomycetous characteristics including septate hyphae and specialized reproductive structures[71,72]. The ecology of *Rhynchogastremaceae* is primarily that of mycoparasites, serving important functions in fungal community dynamics. Their parasitic activities can influence host population dynamics and contribute to biodiversity maintenance in fungal ecosystems[73]. Currently, two genera are listed in this family including *Papiliotrema* and *Rhynchogastrema*[41]. In this study, *Papiliotrema aspenensi* (one strain), *Pa. chiangmaiensis* sp. nov. (two strains), *Pa. pollinicola* sp. nov. (two strains), and *Pa. tectonae* sp. nov. (two strains) were presented.

Papiliotrema chiangmaiensis Kodchasee, Senwanna, J. Kumla & N. Suwannar., sp. nov. (Fig. 18)

MycoBank number: MB860184

Etymology – '*chiangmaiensis*' referring to Chiang Mai Province, where the new species was found.

Holotype – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in Zamioculcas flower (*Zamioculcas zamiifolia*) July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40095 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU594 = GMBCC2402 = TBRC21394. GenBank numbers PV834497 (D1/D2), PV834667 (ITS), PX622326 (SSU), PV941857 (*rpb1*), PV947469 (*rpb2*), PV844824 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1–2 mm in diameter), white to pale yellow, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are ellipsoidal and cylindrical (2.67– 4.27 × 3.84–5.76 μm, *n* = 50), Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose (or weak), ribose, xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, *α*-*α*-trehalose, methyl-*α*-D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose, melizitose, erythritol, ribitol, D-glucitol, D-mannitol, galactitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, succinate, ethanol (or slow), and xylitol are assimilated, but *N*-acetyl glucosamine, inulin, soluble starch, glycerol, DL-lactate, citrate, and methanol are not assimilated. Ammonium sulfate, L-lysine, and ethylamine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, and cadaverine are not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, 10% NaCl/5% glucose. No growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction and acid formation are positive. Growth was observed at 10, 20, and 25 °C but 35, and 37 °C are variable.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Suthep, in teak flower (*Tectona grandis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU627. GenBank numbers PV834498 (D1/D2), PV834668 (ITS), PX622327 (SSU).

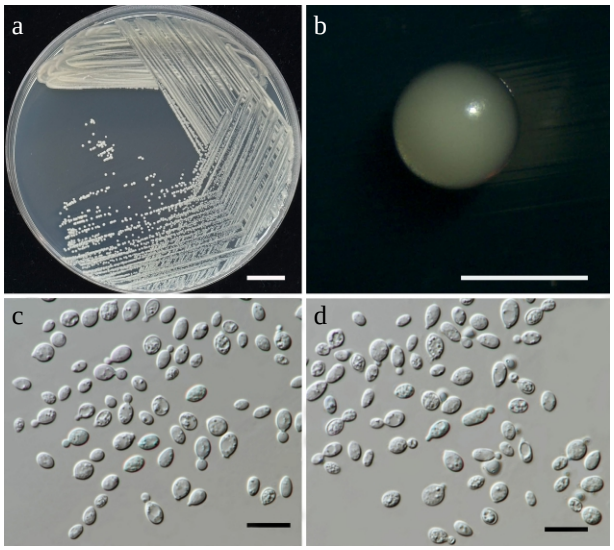

Fig. 18 Morphological characteristics of *Papiliotrema chiangmaiensis* (SDBR-CMU594, ex-type). (a) Culture, (b) single colony, (c), (d) cells and budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Notes – The new species, *Papiliotrema chiangmaiensis*, strains SDBR-CMU594 and SDBR-CMU627 formed a distinct lineage, closely related to *Pa. leoncinii* CBS 13918 and *Pa. tapputiae* BRIP 75038 (Fig. 19). *Papiliotrema chiangmaiensis* differed from *Pa. leoncinii* and *Pa. tapputiae* by 1.04% nucleotide divergence (6 nt substitutions and 1 gap), and 0.82% (5 nt substitutions), respectively, in the D1/D2 domain. While the ITS regions showed 0.2%–1.2% mismatches compared to those known species. Moreover, *Pa. chiangmaiensis* can be distinguished from *Pa. leoncinii* with its ability to assimilate methyl-*α*-D-glucoside, galactitol, and ethylamine. But cannot grow inulin, citrate, and sodium nitrite[74].

Papiliotrema pollinicola Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 20)

MycoBank number: MB860186

Etymology – The specific epithet '*pollinicola*' refers to the substrate origin of the type strain, pollen structure.

Holotype – THAILAND, Phayao Province, Mueang District, in vinca flower (*Catharanthus roseus*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40106 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU664 = GMBCC2403 = TBRC21408. GenBank numbers PV834501 (D1/D2), PV834671 (ITS), PX622329 (SSU), PX582297 (*rpb1*), PV947470 (*rpb2*), PV844825 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.8–1.5 mm in diameter), yellowish white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are globose to ellipsoidal (3.1–5.58 × 3.64–5.9 μm, *n* = 50), occur singly in pairs, or in chains budding. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, D-galactose, sorbose, *N*-acetyl glucosamine, ribose, xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, *α*-*α*-trehalose, methyl-*α*-D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose, melizitose, glycerol, erythritol, ribitol, D-glucitol, mannitol, galactitol, *myo*-inositol,

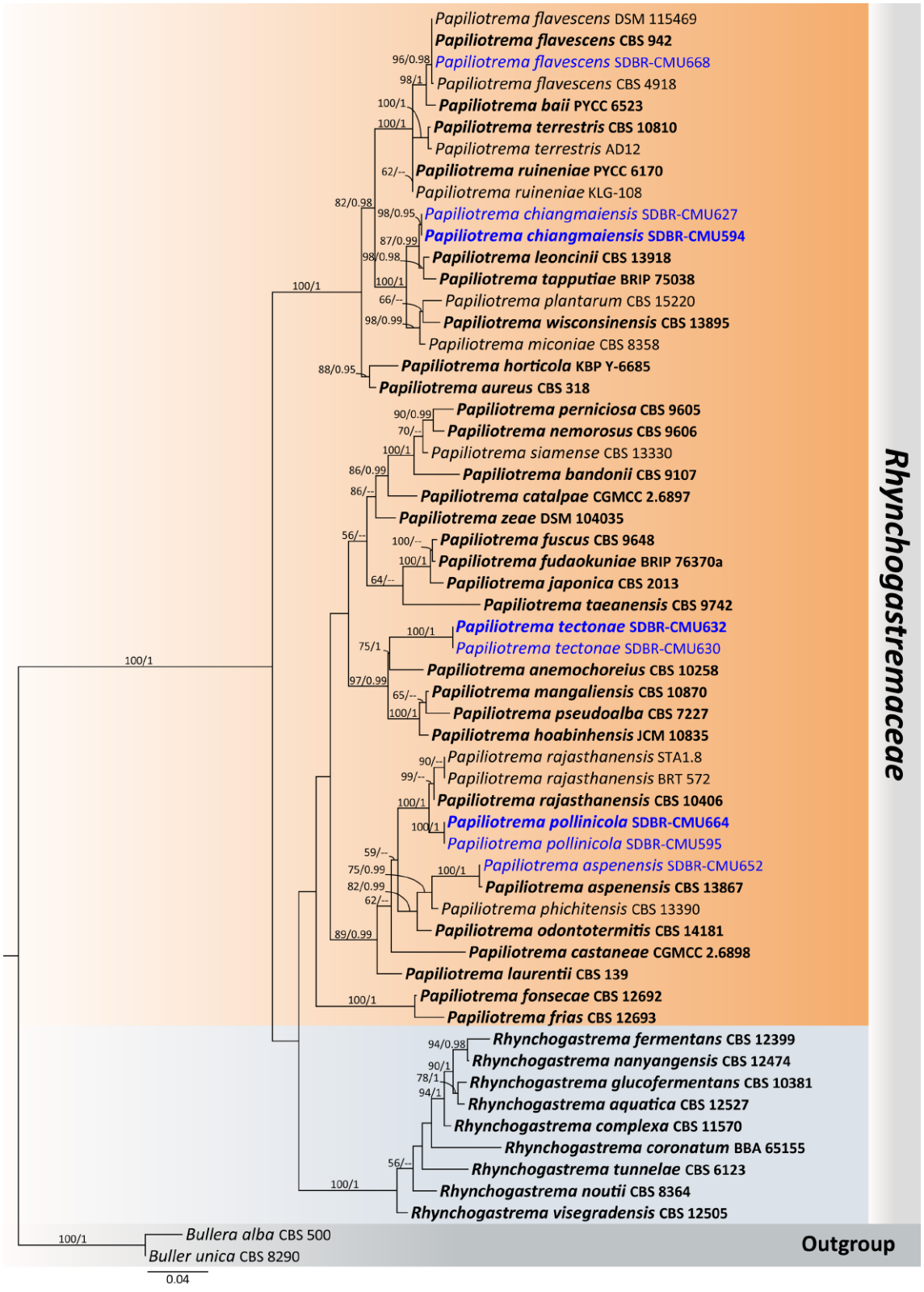

Fig. 19 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Rhynchogastremaceae*. The tree is rooted to *Bullera alba* (CBS 500) and *B. unica* (CBS 8290). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Fifty-eight strains are included in the combined sequence analysis, which comprise 1,218 characters with gaps. The average standard deviation of the split frequencies of the BI analysis was 0.009286. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

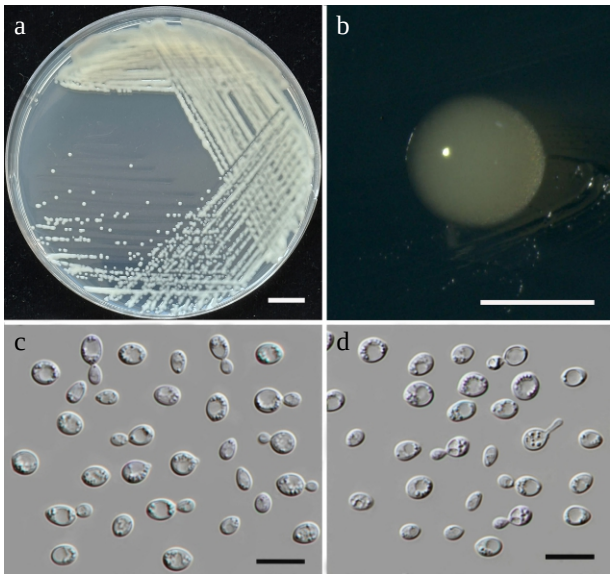

Fig. 20 Morphological characteristics of *Papiliotrema pollinicola* (SDBR-CMU664, ex-type). (a) Culture, (b) single colony, (c), (d) cells and budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

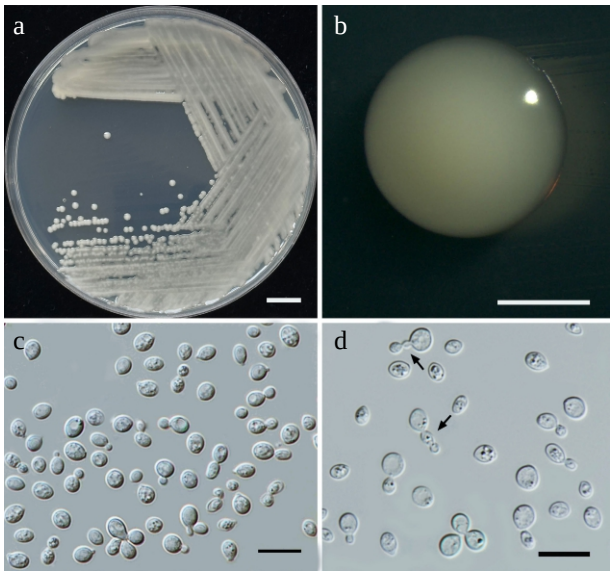

Fig. 21 Morphological characteristics of *Papiliotrema tectonae* (SDBR-CMU632, ex-type). (a) Culture, (b) single colony, (c), (d) budding cells, and chains of cells (indicated by arrows) on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, ethanol, and xylitol are assimilated, but methanol, inulin, and soluble starch are not assimilated. Ammonium sulfate, L-lysine, and ethylamine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, and cadaverine are not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose (weak). No growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth at 10, 20, 25, and 30 °C but absent at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in Zamioculcas (*Zamioculcas zamiifolia*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU595. GenBank numbers PV834500 (D1/D2), PV834670 (ITS), PX622328 (SSU).

Notes – The new species, *Papiliotrema pollinicola* (SDBR-CMU664 and SDBR-CMU595) formed a distinct clade with 100 MLBS, 1 BYPP support value and clustered as sister to *Pa. rajasthanensis* (CBS 10406; type strain, STA1.8 and BRT 572; Fig. 19). A sequence comparison revealed that *Pa. pollinicola* differed from the type strain of *Pa. rajasthanensis* by 3.70% nucleotide divergence (38 substitutions and 1 gap) in the D1/D2 domain, 0.56% (3 substitutions) in the ITS region, and 1.96% nucleotide divergence (21 substitutions and 6 gaps) in the *rpb2* gene. Based on physiological test, *Pa. pollinicola* can be distinguished from *Pa. rajasthanensis* by its ability to assimilate glycerol and DL-lactate. Likewise, *Pa. pollinicola* was able to grow at 50% glucose, 60% glucose, and 10% NaCl/5% glucose[75].

Papiliotrema tectonae Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 21)

MycoBank No: MB860185

Etymology – The specific epithet '*tectonae*' refers to *Tectona*, the plant genus from which the new species was found.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Suthep, in teak flower (*Tectona grandis*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach holotype, CMUB40103 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU632 = GMBCC2404 = TBRC21404. GenBank numbers PV834503 (D1/D2), PV834673 (ITS), PX622331 (SSU), PV941859 (*rpb1*), PV947472 (*rpb2*), PV844827 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1–2 mm in diameter), yellowish white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are globose to ellipsoidal (2.25– 4.75 × 2.85–5.56 μm, *n* = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, *N*-acetyl glucosamine (or slow), ribose (or weak), xylose, L-arabinose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside (or slow), cellobiose, salicin (or slow), melibiose (or slow), lactose, raffinose, melizitose, ribitol (or slow), D-glucitol, mannitol, galactitol, *myo*-inositol (or weak), D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, and xylitol (or slow) are assimilated, but L-sorbose, methanol, ethanol, inulin, soluble starch, glycerol, erythritol, D-arabinose, and L-rhamnose are not assimilated. Ammonium sulfate and L-lysine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, ethylamine and cadaverine are not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose (weak). No growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction and acid formation are positive. Growth at 10, 20, 25, and 30 °C, but absent at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Suthep, in teak flower (*Tectona grandis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU630. GenBank numbers PV834502 (D1/D2), PV834672 (ITS), PX622330 (SSU), PV941858 (*rpb1*), PV947471 (*rpb2*), PV844826 (*tef1-α*).

Notes – In the phylogenetic analyses, the strains SDBR-CMU632 and SDBR-CMU630 of *Papiliotrema tectonae* formed a separate

lineage, sister to *Pa. anmochoreius* (CBS 10258) with 75% MLBS and 1 BYPP support values (Fig. 19). The present strains differed from the latter by 2.86% nucleotide divergence (18 nt substitutions) in the D1/D2 domain and 4.75% (25 nt substitutions and 8 gaps) in the ITS region. In addition, *Pa. tectonae* differs from *Pa. anemochoreia* by its inability to assimilate inulin glycerol, erythritol, D-arabinose, and L-rhamnose, whereas *Pa. anemochoreia* can utilize these substrates. Additionally, *Pa. tectonae* was able to grow on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose[76].

Family: *Trimorphomycetaceae* Xin Zhan Liu, F.Y. Bai, M. Groenew, & Boekhout

Trimorphomycetaceae represents a morphologically and ecologically distinctive family within the including mixed conidial-basidial fruiting bodies, distinctive twinned conidia in the type genus, and mycoparasite. *Trimorphomycetaceae* can exist in a variety of growth forms, including the distinctive filamentous and basidiocarp structures and yeast stages. Currently, four genera are listed in this family including *Carlosrosaea*, *Saitozyma*, *Sugitazyma*, and *Trimorphomyces*[41]. In this study, *Saitozyma thailandensis* sp. nov. (three strains) was presented.

Saitozyma thailandensis Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 22)

MycoBank number: MB860188

Etymology – The species name '*thailandensis*' refers to Thailand, the country where the type strain was isolated.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in confederate vine flower (*Antigonon leptopus*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40092 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU590 = GMBCC2405 = TBRC21392. GenBank numbers PV834504 (D1/D2), PV834674 (ITS).

Description – The culture on YMA after 5 days at 25 °C, colonies are circular form (0.8–1.0 mm in diameter), pale yellow, smooth surface, entire margin, and convex elevation. The cells are ellipsoidal to cylindrical (1.84–4.6 × 3.65–6.07 μm, $n = 50$), occur singly, in pairs or in chains budding. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiosporess were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for 1 month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, *N*-acetyl glucosamine, ribose, xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, salicin, melibiose, lactose (weak), raffinose, melizitose, erythritol, ribitol (weak), D-glucitol, mannitol, galactitol, *myo*-inositol (weak), D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, and xylitol are assimilated, but inulin, soluble starch, glycerol, methanol, and ethanol are not assimilated. Ammonium sulfates, ethylamine hydrochloride, L-lysine, and cadaverine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, and creatine are not assimilated. Growth occurs on media containing 50% glucose (weak). No growth occurs on media containing 60% glucose, 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is positive (weak). Growth at 10, 20, 25, 35, and 37 °C but absent at 40 °C

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Suthep, in American cassia flower (*Senna spectabilis*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU636; Chiang Rai Province, Mae Fah Luang District, Mae Fah Luang, in bush clock vine flower (*Thunbergia erecta*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU698. GenBank numbers SDBR-CMU636: PV834506 (D1/D2), PV834676 (ITS); SDBR-CMU698: PV834505 (D1/D2), PV834675 (ITS).

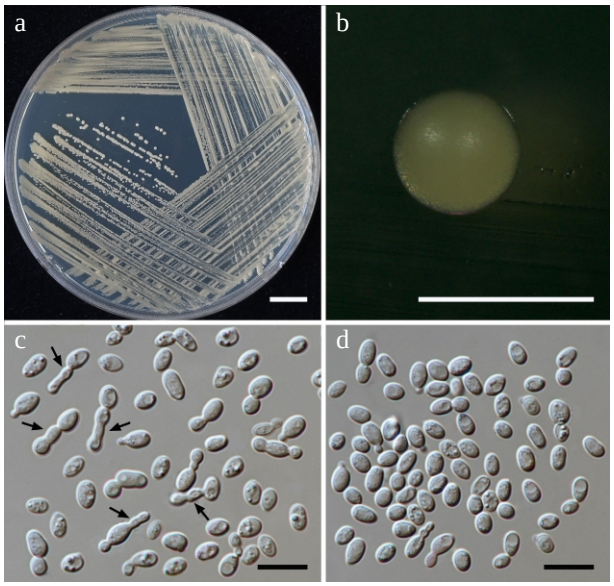

Fig. 22 Morphological characteristics of *Saitozyma thailandensis* (SDBR-CMU590, ex-type). (a) Culture, (b) single colony, (c),chains of cells (indicated by arrows), (d) budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Notes – *Saitozyma thailandensis* strains SDBR-CMU590, SDBR-CMU636 and SDBR-CMU698 were placed in the *Saitozyma* clade (Fig. 23), and closely related to *Sa. flava* CBS 331, *Sa. pseudoflava* CBS 15576 and *Sa. paraflava* CBS 10100. These strains showed which 0.16%, 0.49%, and 1.16% nucleotide divergence (1, 3, and 8 nt substitutions) in the D1/D2 domain, respectively. The ITS region had differences by 1.17%, 1.93%, and 4.15% (6, 10, and 20 nt substitutions). Additionally, after 2 weeks on PDA, the cell presented a few chains and elongated formed and elongated but not eveloped to pseudohyphae and true hyphae (Fig. 22c), and a distinct comparison of the phenotypic characteristics between *Sa. thailandensis*, *Sa. flava*, *Sa. paraflava*, and *Sa. pseudoflava* are shown in Table 7. Thus, *Sa. thailandensis* is introduced as a new species.

Order: *Trichosporonales* Boekhout & Fell

Family: *Trichosporonaceae* Nann.

Trichosporonaceae is found in a wide range of habitats, including soil, water, and plant surfaces, and are constituents of the normal microbiota of humans and other animals. Members of *Trichosporonaceae* exist primarily as yeasts but can produce various hyphal structures. The characteristic and taxonomically is the production of arthroconidia - asexual propagules that form through the disarticulation of septate hyphae at the septal junctions. These arthroconidia are typically unicellular, ranging from cubical to barrel-shaped or elongate[60,79]. Currently, nine genera are listed in this family, including *Apiotrichum*, *Cutaneotrichosporon*, *Effuseotrichosporon*, *Haglerozyma*, *Nothotrichosporon*, *Pascua*, *Prillingera*, *Trichosporon*, and *Vanrija*[41]. In this study, *Trichosporon asahii* (one strain) is presented (Supplementary File 1).

Subphylum: Pucciniomycotina R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.

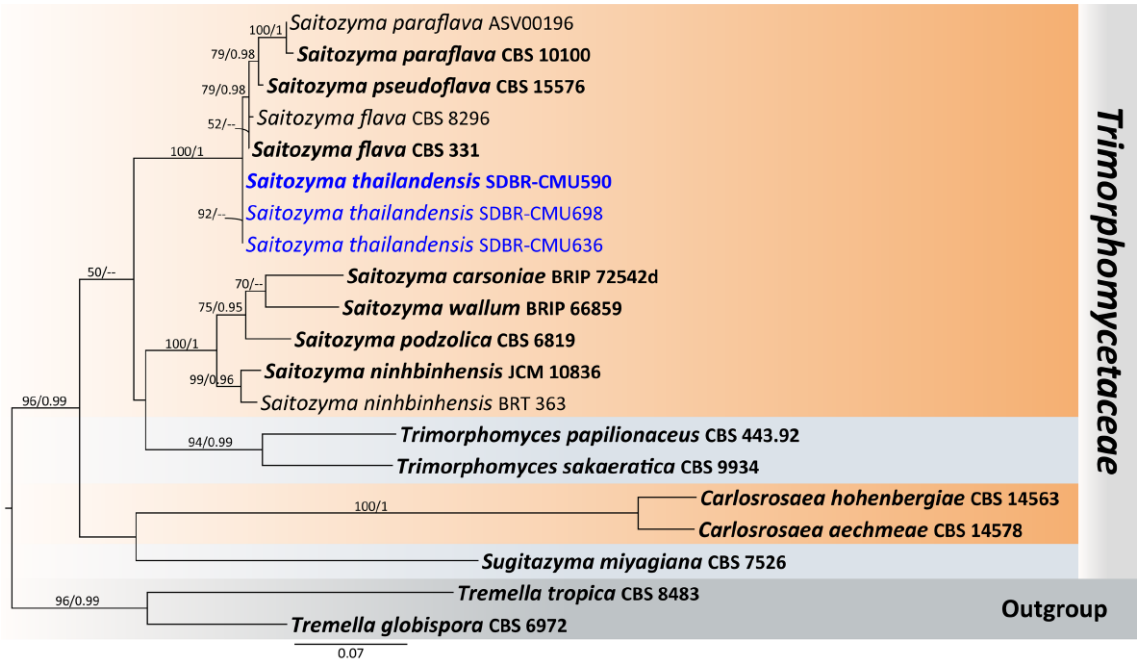

Fig. 23 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Trimorphomycetaceae*. The tree is rooted to *Tremella globispora* (CBS 6972) and *T. tropica* (CBS 8483). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Twenty strains are included in the combined sequence analysis, which comprise 1,149 characters with gaps. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

Table 7. Phenotypic characteristics differentiating *Saitozyma thailandensis* from closely related *Saitozyma* species.

Characteristics		1	2	3	4
Carbon assimilation	L-Sorbose	+	–	–	–
	N-Acetyl glucosamine	+	+	+	l/w
	D-Arabinose	+	w	+/w	+/w
	L-Rhamnose	+	+	+/w	l/w
	α-*α*-Trehalose	+	+	+	–
	Salicin	+	+	–	l/w
	Melibiose	+	+	+	+/w
	Lactose	w	+	+	–
	Inulin	–	w	–	+/w
	Soluble starch	–	+	+/s	–
	Glycerol	–	–	+/w	–
	Erythritol	+	+	–	–
	Ribitol	w	w	+	l/w
	D-Glucitol	+	+	–/+	l/w
	D-Mannitol	+	+	–/w	l/w
	Galactitol	+	w	–/w	+
	myo-Inositol	w	+	+	+
	DL-Lactate	+	w	+/s	–
	Succinate	+	+	+/s	–
	Citrate	+	w	+/s	–
	Ammonium sulfate	+	–	–	+
Nitrogen assimilation of	Ethylamine HCl	+	nd	–	+
	L-Lysine	+	–	–	l/w
Growth characteristics	Growth at 30 °C	+	+	–	+
	Growth at 35 °C	+	–	nd	–
	Growth at 37 °C	+	–	nd	–
	Growth on 50% Glucose	w	–	–	nd

Strains 1: *Sa. thailandensis* sp. nov, 2: *Sa. flava*[77], 3: *Sa. paraflava*[78], and 4: *Sa. pseudoflava*[63].

Class: Agaricostilbomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.

Order: *Agaricostilbales* Oberw. & R. Bauer

Family: *Chionosphaeraceae* Oberw. & Bandoni

Chionosphaeraceae demonstrate a wide range of habitats ranging from saprotrophic decomposition to mycoparasitic relationships with other fungi. Characteristics of this family exhibit unicellular yeasts or gasteroid basidia with simultaneous basidiospore production per basidium, that allow these fungi to exploit different ecological and environmental[65,80]. Currently, six genera are listed in this family including *Ballistosporomyces*, *Boekhoutia, Chionosphaera*, *Cystobasidiopsis*, *Kurtzmanomyces,* and *Stilbum*[41]. In this study, *Boekhoutia pollinicola* sp. nov. (two strains) were presented.

Boekhoutia pollinicola Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 24)

MycoBank number: MB860189

Etymology – the specific epithet '*pollinicola*' refers to the substrate origin of the type strain, pollen structure.

Holotype – THAILAND, Phayao Province, Mueang District, Baan Tom, in bridal bouquet flower (*Plumeria pudica*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40109 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU684 = GMBCC2406 = TBRC21411. GenBank numbers PV834510 (D1/D2), PV834680 (ITS), PX622333 (SSU), PV941873 (*rpb1*), PV947459 (*rpb2*), PV947486 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1–2 mm in diameter), light orange, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are spheroidal to short ovoidal (1.65–3.54 × 3.77–6.17 μm, n = 50), occur singly or in pairs budding on sterigma. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

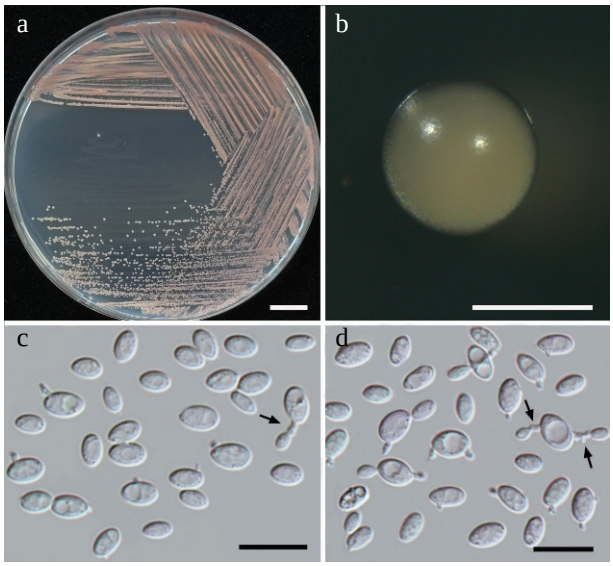

Fig. 24 Morphological characteristics of *Boekhoutia pollinicola* (SDBR-CMU684, ex-type). (a) Culture, (b) single colony, (c), (d) cells and budding cells on sterigma (indicated by arrows) on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, ribose, xylose, sucrose, maltose, α-α-trehalose (or slow), methyl-α-D-glucoside (or weak), cellobiose (or slow), melibiose (or latent), lactose (or latent), raffinose (or latent), melizitose, erythritol (or latent or slow), ribitol (or slow), glucitol (weak or slow), mannitol (weak or slow), D-gluconate, DL-lactate, and succinate (weak or latent) are assimilated, but *N*-acetyl glucosamine, L-rhamnose, salicin, inulin, soluble starch, glycerol, galactitol, *myo*-inositol, D-glucuronate, D-galacturonic acid, citrate, methanol, ethanol, and xylitol are not assimilated. Assimilation is variable for D-glucono-1,5-lactone, and D-arabinose. Ammonium sulfate, L-lysine, ethylamine hydrochloride, and cadaverine are assimilated as the sole nitrogen source. Potassium nitrate and sodium nitrite are not assimilated. No growth occurs on media containing 50% glucose, 60% glucose, 10% NaCl/5% glucose, 15% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Growth in vitamin-free medium is negative. Acid formation is negative. Urease reaction is positive. Growth at 10, 20, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Mae Hia, in Siam tulip flower (*Curcuma sessilis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, living culture SDBR-CMU621. GenBank numbers PV834509 (D1/D2), PV834679 (ITS), PX622332 (SSU).

Notes – In molecular phylogenetic analysis, two strains, SDBR-CMU621 and SDBR-CMU684, formed a distinct clade, sister to *Boekhoutia foliicola* JCM 36090 and *B. sterigmata* CBS 15553 (Fig. 25). The D1/D2 sequences of the two strains differed by 4.80% (27 nt substitutions and 6 gaps) from *B. foliicola* and 4.55% nucleotide divergence (28 nt substitutions and 6 gaps) from *B. sterigmata*. The ITS sequences of the strains demonstrated divergence with 8.19% (28 nt substitutions and 23 gaps) from *B. foliicola* and 7.97% (33 nt substitutions and 29 gaps) from *B. sterigmata*. These findings suggest that the two strains represent a novel species in the *Boekhoutia*. Furthermore, they can be differentiated by the ability of *B. pollinicola* to grow in ribose, melibiose, lactose, erythritol, glucitol, D-gluconate, DL-lactate, and succinate. Additionally, *B. pollinicola* was able to grow at 30 °C, whereas the maximum growth temperature for *B. foliicola* and *B. sterigmata* is 25 °C[63,81].

Class: Cystobasidiomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.

Order: *Cystobasidiales* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.

Family: *Cystobasidiaceae* Gäum.

Member of *Cystobasidiaceae* have been isolated from diverse environments, including cold climates, Arctic regions, various plant-associated habitats, dung-inhabiting habitats, and mycoparasitic habitats. In yeast form characterized by nano-meterfusion myco-parasitism with tremelloid haustorial cells and septal pores with cystosomes[65,80] the colony have pastel red or orange colors that produce bioactive compounds like carotenoids with antioxidant properties[82]. Currently, six genera are listed in this family, including *Cystobasidium*, *Begerowomyces*, *Cystastrum*, *Halobasidium*, *Robertozyma*, and *Queiroziella*[83]. In this study, *Cystobasidium benthicum* (one strain), *Cys. keelungense* (two strains), *Cys. minutum* (two strains), *Cys. thailandicum* sp. nov. (two strains), and *Halobasidium lannaense* sp. nov. (two strains) were presented (Fig. 26).

Cystobasidium thailandicum Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 27)

MycoBank number: MB860191

Etymology – The species name '*thailandicum*' refers to Thailand, the country where the type strain was isolated.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Mae Hia, in Golden trumpet flower (*Allamanda cathartica*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40111 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU697 = GMBCC2407 = TBRC21414. GenBank numbers PV834516 (D1/D2), PV834686 (ITS), PX622334 (SSU), PX582338 (*tef1-α*).

Description – Colonies on YMA after five days at 25 °C are circular form (1.5–2 mm in diameter), pastel red, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are spheroidal to ovoidal (4.51–6.89 × 5.47–8.24 μm, n = 50), occur singly and polar budding. In Dalmau plates after 2 weeks on corn-meal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for 1 month.

Fermentation of glucose is negative. D-Glucose, galactose, xylose, L-arabinose, sucrose, α-α-trehalose, cellobiose, salicin, lactose, melizitose glycerol, D-glucono-1,5-lactone, D-gluconate, D-galacturonic acid, and succinate are assimilated, but sorbose (or weak), *N*-acetyl glucosamine, D-arabinose (or weak), maltose, D-ribose, L-rhamnose, methyl-α-D-glucoside, melibiose, raffinose, inulin, soluble starch, erythritol, ribitol, D-glucitol, mannitol, galactitol, *myo*-inositol, D-glucuronate, DL-lactate, citrate, methanol, ethanol, and xylitol are not assimilated. Ammonium sulfates are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, ethylamine hydrochloride, L-lysine, and cadaverine are not assimilated. No growth occurs on media containing 50% glucose, 60% glucose, 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth on 10, 15, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in frangipani flower (*Plumeria obtusa*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU565. GenBank numbers PV834517 (D1/D2), PV834687 (ITS), PX622335 (SSU), PX582339 (*tef1-α*).

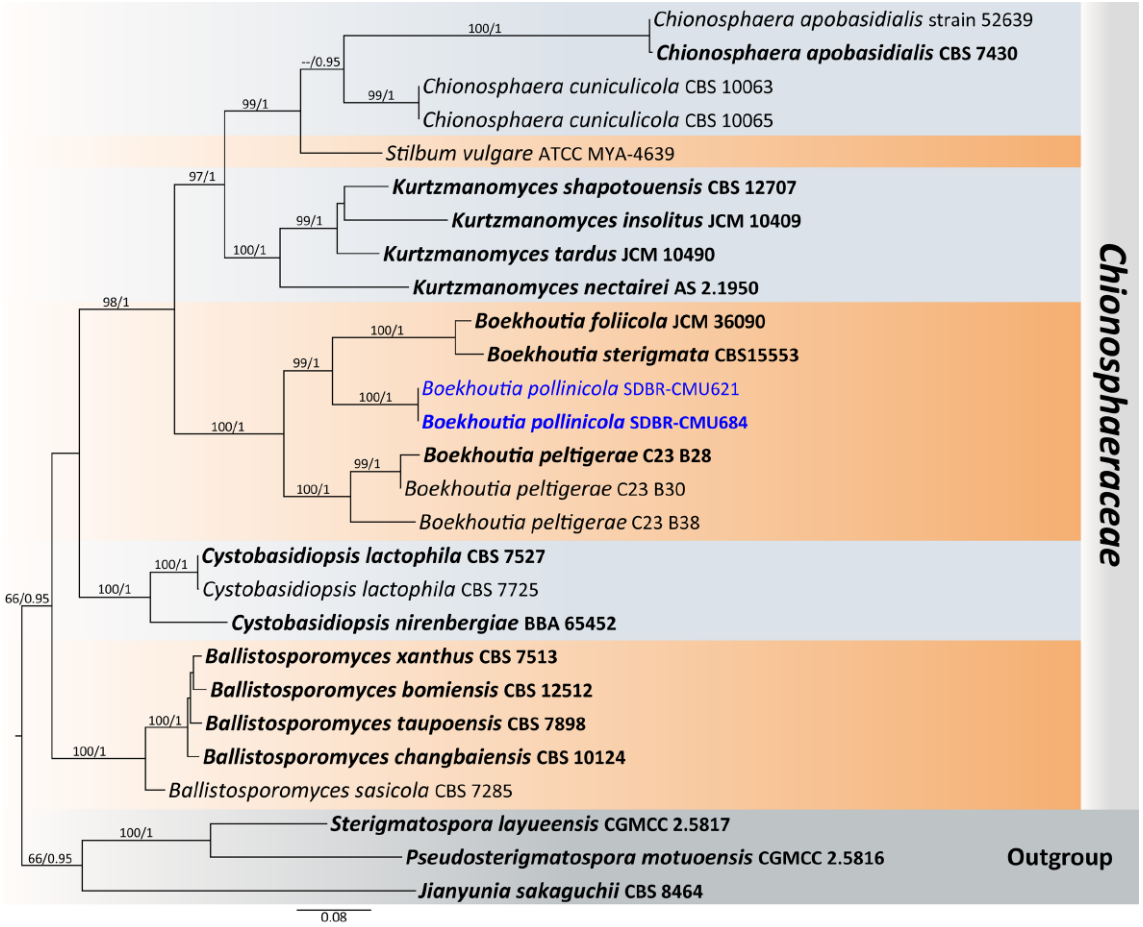

Fig. 25 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Chionosphaeraceae*. The tree is rooted to *Jianyunia sakaguchii* (CBS 8464), *Pseudosterigmatospora motuoensis* (CGMCC 2.5816), and *Sterigmatospora layueensis* (CGMCC 2.5817). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Twenty-seven strains are included in the combined sequence analysis, which comprise 1,594 characters with gaps. The average standard deviation of the split frequencies of the BI analysis was 0.001890. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

Notes – *Cystobasidium thailandicum* SDBR-CMU697 and SDBR-CMU565 formed a distinct lineage, closely related to *Cys. calyptogenae* CBS 9125, CBS 11058, and CBS 11318 in the *Cystobasidium* clade (Fig. 26). The present strains differed from *Cys. calyptogenae* by 0.33% nucleotide divergence (2 substitutions) in the D1/D2 domain, 0.89% (5 substitutions) in the ITS region, and 1.96% nucleotide divergence (21 substitutions and 6 gaps) in the *tef1-α* gene. Based on physiological test, *Cys. thailandicum* can be distinguished from *Cys. calyptogenae* by its ability to assimilate L-sorbose, D-arabinose, maltose, raffinose, D-glucitol, D-mannitol, DL-lactate, and citrate. In contrast, *Cys. thailandicum* does not assimilate cellobiose, lactose, D-glucuronate, and D-galacturonic acid. Likewise, *Cys. thailandicum* can grow in 50% and 60% glucose medium, whereas *Cys. calyptogenae* cannot grow under these conditions[84].

Halobasidium lannaense Kodchasee, Senwanna, J. Kumla. & N. Suwannar., sp. nov. (Fig. 28)

MycoBank number: MB860192

Etymology – '*lannaense*' referring to the Kingdom of Lanna, the historic name of northern Thailand, where the new species was found.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Mae Hia, in coloradillo flower (*Hamelia patens*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40098 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU612 = GMBCC2408 = TBRC21398. GenBank numbers PV834518 (D1/D2), PV834688 (ITS), PX622336 (SSU), PV941860 (*rpb1*), PV947457 (*rpb2*), PV947487 (*tef1-α*).

Description – Colonis on YMA after 5 d at 25 °C is circular form (1.0–1.5 mm in diameter), pale orange, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are spheroidal to ovoidal (1.69–3.35 × 2.29–5.6 μm, $n = 50$), occur singly, in pairs, or in chains budding. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose (or weak), xylose, L-arabinose, D-arabinose (or weak), sucrose, maltose, *α*-*α*-trehalose, salicin, raffinose, melizitose, glycerol, D-glucitol (or slow), mannitol, D-glucono-1,5-lactone, D-gluconate, DL-lactate, succinate, and citrate are assimilated, but *N*-acetyl glucosamine, D-ribose, L-rhamnose, methyl-*α*-D-glucoside, cellobiose, melibiose, lactose, inulin, soluble starch, erythritol, ribitol, galactitol, *myo*-inositol, D-glucuronate, D-galacturonic acid, methanol, ethanol, and xylitol are not assimilated. Ammonium

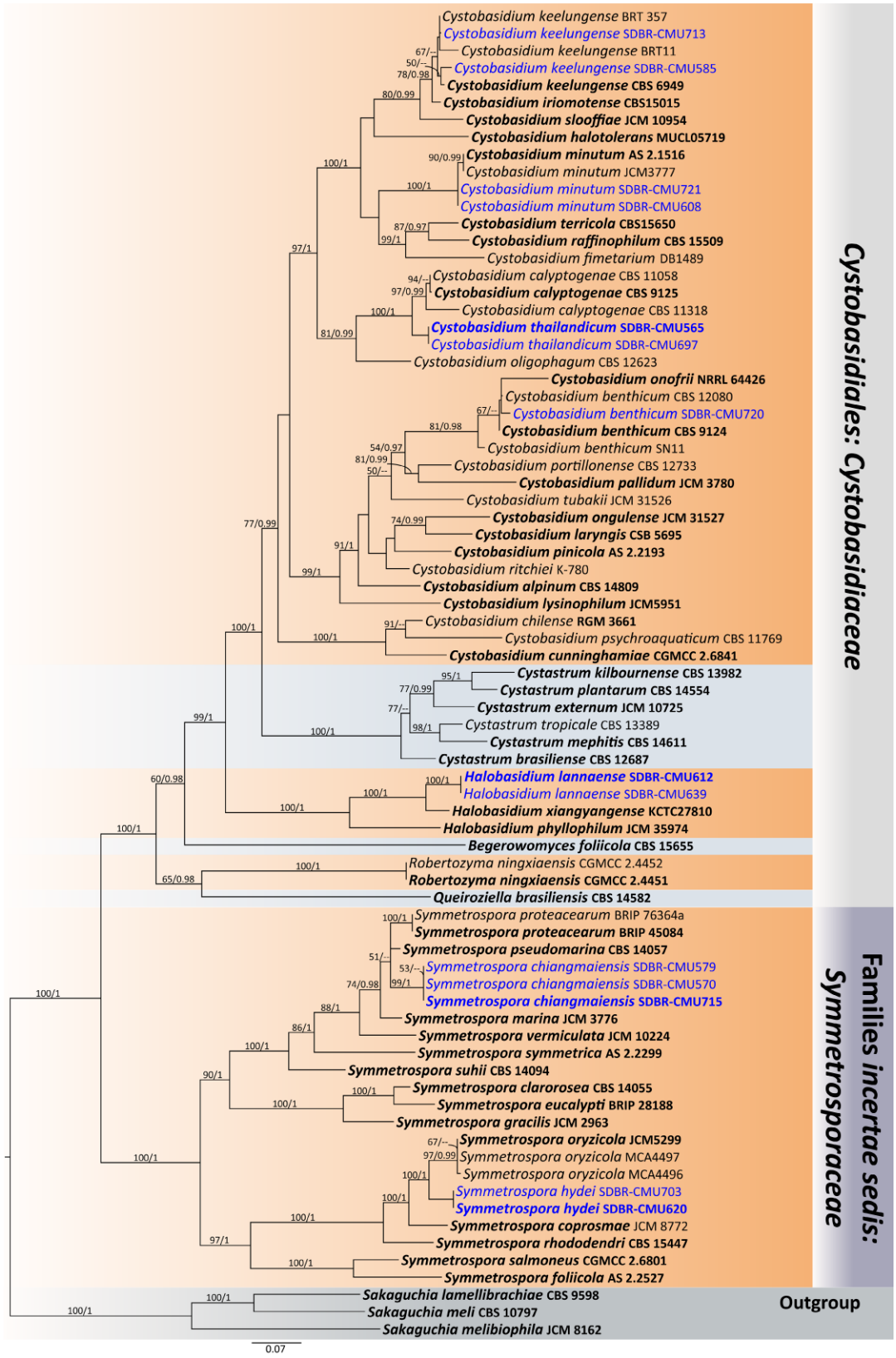

Fig. 26 Phylogram generated by maximum likelihood analysis of the combined D1/D2 domain of LSU, ITS, SSU, *rpb1*, *rpb2*, and *tef1-α* sequence data representing *Cystobasidiaceae* (*Cystobasidiales*) and *Symmetrosporaceae* (*Incertae sedis*) in Cystobasidiomycetes. The tree is rooted to *Sakaguchia lamellibrachiae* (CBS 9598), *S. meli* (CBS 10797), and *S. melibiophila* (JCM 8162). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Seventy-seven strains are included in the combined sequence analysis, which comprise 7,211 characters with gaps. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

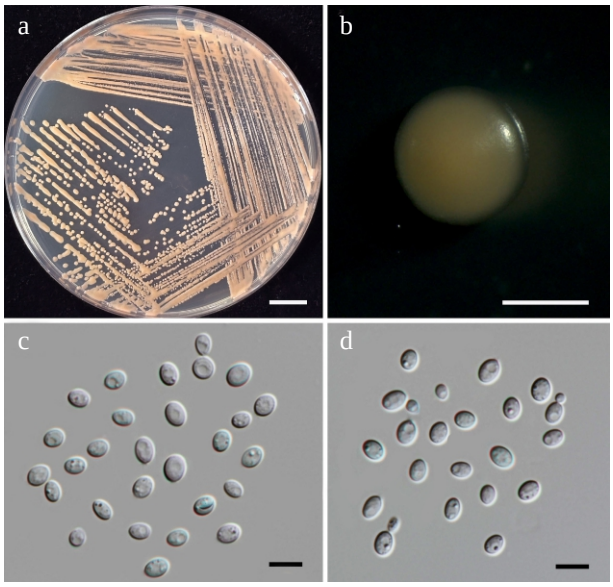

Fig. 27 Morphological characteristics of *Cystobasidium thailandicum* (SDBR-CMU697, ex-type). (a) Culture, (b) single colony, (c), (d) cells and budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

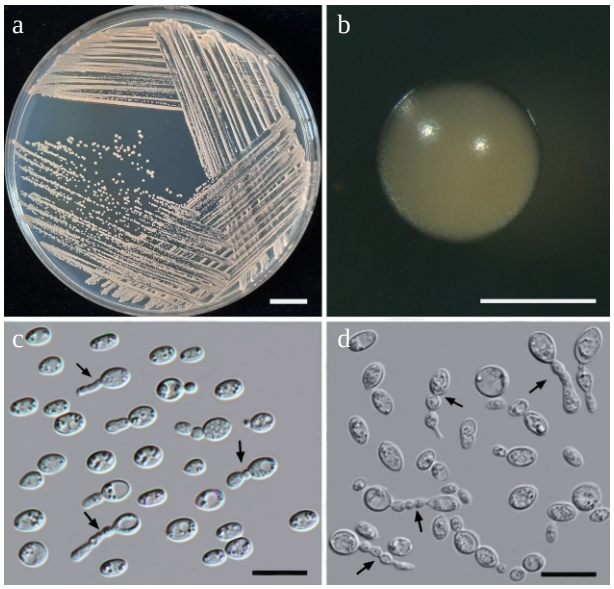

Fig. 28 Morphological characteristics of *Halobasidium lannaense* (SDBR-CMU612, ex-type). (a) Culture, (b) single colony, (c) chains of cells on YMA after 5 d at 25 °C, and (d) chains of cells (indicated by arrows) on YMA after 3 weeks at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

sulfate and L-lysine are assimilated as sole nitrogen sources, but potassium nitrate, sodium nitrite, ethylamine hydrochloride, and cadaverine are not assimilated. Growth occurs on media containing 50% glucose and 60% glucose, but not on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth at 10, 20, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Suthep, in bougainvillea (*Bougainvillea hybrid*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU639. GenBank numbers PV834519 (D1/D2), PV834689 (ITS), PX622337 (SSU), PX582320 (*rpb2*), PX582340 (*tef1-α*).

Notes – Two strains, SDBR-CMU612 and SDBR-CMU639, introduced as a new member of *Halobasidium*, formed a distinct lineage closely related to *Hal. xiangyangense* KCTC27810 (Fig. 26). The present strains differed from *Hal. xiangyangense* by 0.33% nucleotide divergence (2 substitutions) in the D1/D2 domain, 1.28% (7 substitutions) in the ITS region, 6.55% (62 substitutions) in the *rpb2* gene, and 2.62% nucleotide divergence (21 substitutions) in the *tef1-α* gene. *Halobasidium lannaense* can be distinguished from *Hal. xiangyangense* with its ability to assimilate raffinose, melizitose, D-glucitol, D-glucono-1,5-lactone, and DL-lactate as well as by its ability to grow on media containing 50% and 60% glucose[85]. Additionally, *Hal. lannaense* also produced chains of two or three buds on YMA at 25 °C for 5 d (Fig. 28c), and developed elongated buds after 3 weeks (Fig. 28d), which is the unique character of *Halobasidium*, but not present Y-shaped groups of cells like *Hal. xiangyangense*[85].

Class: Cystobasidiomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss, & Oberw.

Order: *Erythrobasidiales* R. Bauer, Begerow, J.P. Samp., M. Weiss, & Oberw.

Family: *Erythrobasidiaceae* Denchev

Erythrobasidiaceae represents a small but distinct family of basidiomycetous yeasts that are primarily characterized by a pink to red color, which is reflected in the name 'Erythro' (meaning red). Cells are ellipsoidal and cylindrical. They are primarily of the genus type found in the phyllosphere[65,86]. Currently, two genera are listed in this family, including *Bannoa* and *Erythrobasidium*[41]. In this study, *Erythrobasidium primogenitum* (one strain) is presented (Supplementary File 1).

Families *Incertae sedis*

Family: *Symmetrosporaceae* Q.M. Wang, F.Y. Bai, M. Groenew, & Boekhout

Symmetrospora species have been isolated from soil environments and leaf surfaces and other habitats, indicating broad ecological adaptation across terrestrial ecosystems[87]. The colonies produce entire margins and colony color varies from pink to brick-red, budding cells present. Currently, one genus is *Symmetrospora* listed in this family[41]. In this study, *Sy. chiangmaiensis* sp. nov. (three strains), and *Sy. hydei* sp. nov. (two strains) are presented (Fig. 26).

Symmetrospora chiangmaiensis Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 29)

MycoBank number: MB860193

Etymology – '*chiangmaiensis*' referring to Chiang Mai Province, where the new species was found.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in marguerite daisy flower (*Argyranthemum frutescens*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40114 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU715 = GMBCC2409 = TBRC21417. GenBank numbers PV834522 (D1/D2), PV834692 (ITS), PX622340 (SSU), PX582341 (*tef1-α*).

Description – Colonies on YMA after 5 d at 25 °C are circular form (0.8–1.2 mm in diameter), reddish orange, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are spheroidal to ovoidal (3.43–6.72 × 6.17–11.48 μm, n = 50), occur singly and multilateral budding. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual

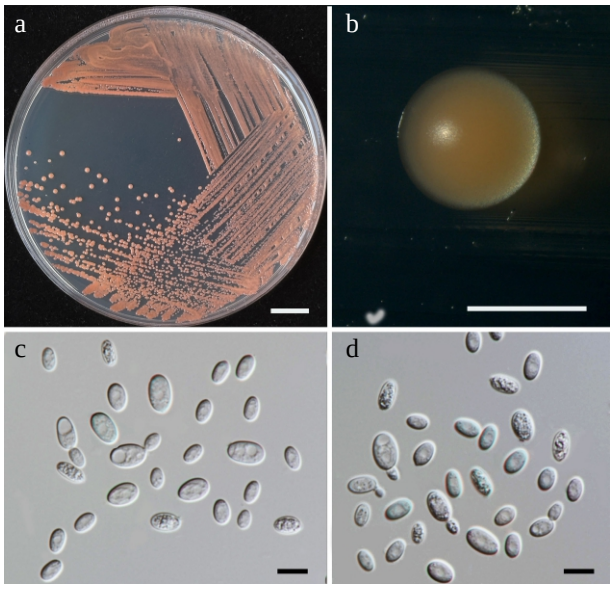

Fig. 29 Morphological characteristics of *Symmetrospora chiangmaiensis* (SDBR-CMU715, ex-type). (a) Culture, (b) single colony, and (c), (d) cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, D-galactose, sorbose, ribose, xylose, DL-arabinose, L-rhamnose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, lactose, raffinose, melizitose, glycerol, ribitol, D-glucitol, mannitol, D-glucono-1,5-lactone (weak), D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, and xylitol are assimilated, but *N*-acetyl glucosamine, salicin, melibiose, inulin, soluble starch, erythritol, galactitol, myo-inositol, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine, L-lysine, and cadaverine (weak) are assimilated as sole nitrogen sources. No growth occurs on media containing 50% glucose, 60% glucose, 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth at 10, 15, 25, and 30 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Mae Hia, in watrakanu flower (*Ruellia tuberosa*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU570; Chang Phueak, in Persian gentian flower (*Exacum affine*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla. and N. Suwannarach, living culture SDBR-CMU579. GenBank numbers SDBR-CMU570: PV834520 (D1/D2), PV834690 (ITS), PX622338 (SSU); SDBR-CMU579: PV834521 (D1/D2), PV834691 (ITS), PX622339 (SSU).

Notes – *Symmetrospora chiangmaiensis*, SDBR-CMU715, SDBR-CMU570 and SDBR-CMU579, formed a sister clade, with *Sy. proteacearum*, BRIP 45084, BRIP 763649a, and *Sy. pseudomarina*, CBS 14057 (Fig. 26). The D1/D2 sequences of these three strains were differed by 0.18%–0.34% nucleotide divergence (1–2 nt substitutions) and the ITS sequences of the three strains demonstrated divergence (7–9 nt substitutions) more than 1.23%–1.62%. Additionally, a distinct comparison of the phenotypic characteristics between *Sy. chiangmaiensis* and *Sy. pseudomarina* are shown in Table 8.

Symmetrospora hydei Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 30)

Table 8. Phenotypic characteristics differentiating *Symmetrospora chiangmaiensis* and *Sy. hydei* from closely related *Symmetrospora* species.

Characteristics		1	2	3	4	5	6
Carbon assimilation	L-Sorbose	+	w	l/w	+	–	w
	D-Ribose	+	+	l/w	+	+	–
	D-Xylose	+	+	+	+	+	–
	D-Arabinose	+	+	l/w	+	+	–
	L-Rhamnose	+	–	l/w	+	v	–
	Maltose	+	+	+	+	–	–
	Methyl-α-D-glucoside	+	+	l/w	+	+	–
	Salicin	–	w	w	+	nd	–
	Melibiose	–	–	–	+	–	+
	Lactose	+	+	–	–	+	–
	Raffinose	+	+	l/w	+	nd	–
	Melizitose	+	+	l/w	+	+	–
	D-Glucuronate	+	–	+	+	nd	nd
	D-Galacturonic acid	+	–	+	–	nd	nd
	DL-Lactate	+	+	l/w	+	nd	–
	Citrate	+	–	+	+	nd	nd
	Xylitol	+	w	nd	nd	nd	nd
	Potassium nitrate	+	w	–	–	v	+
	Sodium nitrite	+	w	nd	nd	nd	–
Nitrogen assimilation	Ethylamine HCl	+	w	–	–	nd	–
	L-Lysine	+	w	–	–	+	+
	Cadaverine	w	w	–	–	v	–
Growth characteristics	Growth at 30 °C	+	–	–	+	v	–
	Growth at 35 °C	–	–	–	+	–	–

Strains 1: *Sy. chiangmaiensis* sp. nov, 2: *Sy. hydei* sp. nov, 3: *Sy. coprosmae*, 4: *Sy. oryzicola*[88], 5: *Sy. pseudomarina*[87], and 6: *Sy. rhododendri*[63].

MycoBank number – MB860194

Etymology – '*hydei*' referring to the name of Kevin D. Hyde in honor of his 70th birthday.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in Yellow-flowered waterhyssop (*Mecardonia procumbens*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40100 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU620 = GMBCC2410 = TBRC21401. GenBank numbers PV834523 (D1/D2), PV834693 (ITS), PX622341 (SSU), PX582321 (*rpb2*), PX582342 (*tef1-α*).

Description – Colonies on YMA after 5 d at 25 °C are circular form (0.8–1.2 mm in diameter), orange red or yellowish red, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are spheroidal to ovoidal (6.02–9.31 × 7.01–13.35 μm, *n* = 50), occur singly and polar budding. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, D-galactose, sorbose (weak), ribose, xylose, DL-arabinose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, salicin (weak), lactose, raffinose, melizitose, glycerol, ribitol, D-glucitol, mannitol, D-gluconate, DL-lactate, succinate, and xylitol (weak) are assimilated, but *N*-acetyl glucosamine, L-rhamnose, melibiose, inulin, soluble starch, erythritol, galactitol, *myo*-inositol, D-glucono-1,5-lactone D-glucuronate, D-galacturonic acid, citrate, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate (weak), sodium nitrite (weak), ethylamine (weak), L-lysine (weak), and cadaverine (weak) are assimilated as sole nitrogen source. No growth occurs on media containing 50% glucose, 60% glucose, 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth on 10, 15, and 25 °C.

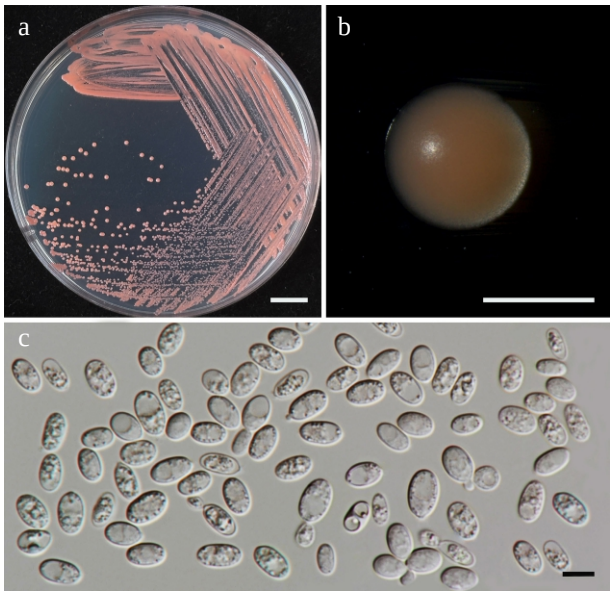

Fig. 30 Morphological characteristics of *Symmetrospora hydei* (SDBR-CMU620, ex-type). (a) Culture, (b) single colony, and (c) cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, and (c) = 10 μm.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in chili flower (*Capsicum* sp.), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU703. GenBank numbers PV834524 (D1/D2), PV834694 (ITS), PX622342 (SSU), PX582322 (*rpb2*), PX582343 (*tef1-α*).

Notes – *Symmetrospora hydei* formed a distinct lineage and was sister to *Sy. coprosmae*, *Sy. oryzicola*, and *Sy. rhododendri* with 100% BSML and 1 BYPP (Fig. 26). However, *Sy. hydei* differed from those three species by 0.64%, 0.79%, and 0.95% nucleotide divergence (4, 5, and 6 nt substitutions) in the D1/D2 domain, respectively, and by 0.87, 0.70, and 1.91% nucleotide divergence (5, 4, and 11 nt substitutions), respectively, in the ITS region. Additionally, the present strain is different from *Sy. coprosmae* and *Sy. oryzicola* by 4.77%–6.69% and 4.05%–5.72% nucleotide divergence in *rpb2* and *tef1-α*, respectively. Moreover, distinguishing characteristics between *Sy. hydei* and *Sy. coprosmae*, *Sy. oryzicola*, and *Sy. rhododendri* are given in Table 8.

Class: Microbotryomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw.

Order *incertae sedis*

Families *incertae sedis*

Currently, 17 genera are listed in this *Microbotryomycetes* genera *incertae sedis*, including *Atractocolax*, *Baiomyces*, *Curvibasidium*, *Fengyania*, *Litoriozyma*, *Nakaseozyma*, *Oberwinklerozyma*, *Pseudohyphozyma*, *Pseudoleucosporidium*, *Reniforma*, *Sampaiozyma*, *Slooffia*, *Spencerozyma*, *Trigonosporomyces*, *Udeniozyma*, *Vonarxula*, and *Yunzhangia*[41,81]. In this study, *Curvibasidium chiangmaiense* sp. nov. (two strains) was presented.

Curvibasidium chiangmaiense Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 31)

MycoBank number: MB860195

Etymology – The specific epithet '*chiangmaiense*' referring to Chiang Mai Province, where the new species was found.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Mae Hia, in Siam tulip flower (*Curcuma sessilis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40089

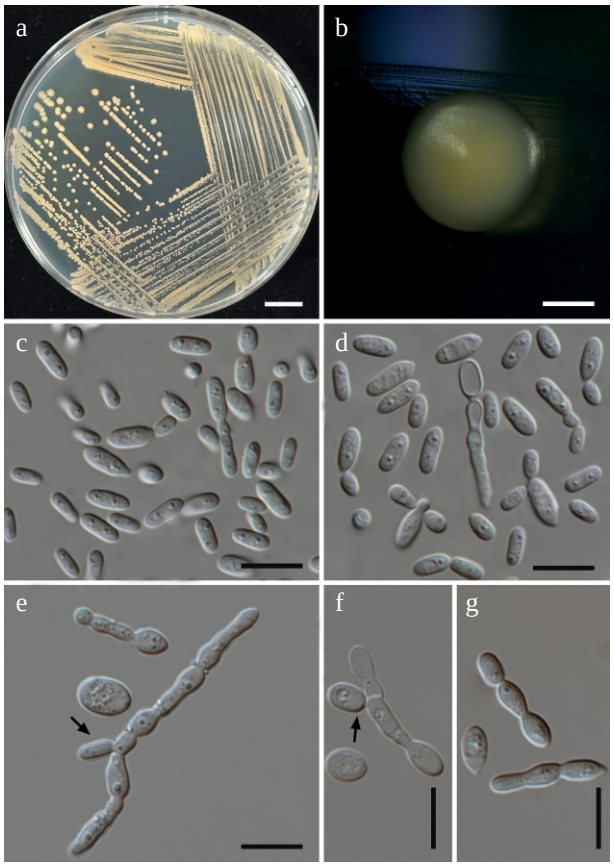

Fig. 31 Morphological characteristics of *Curvibasidium chiangmaiense* (SDBR-CMU574, ex-type). (a) Culture, (b) single colony, and (c), (d) cells on YMA after 5 d at 25 °C. (e), (f) Pseudohyphae and blastoconidia (indicated by arrows) on PDA after 2 weeks at 25 °C, and (g) elongate cells. Scale bars: (a) = 10 mm, (b) = 1 mm, and (c)–(g) = 10 μm.

(preserved in metabolically inactive state), ex-type living culture SDBR-CMU574 = GMBCC2411 = TBRC21389. GenBank numbers PV834526 (D1/D2), PV834696 (ITS), PV819892 (SSU), PX582298 (*rpb1*), PX582323 (*rpb2*), PV947473 (*tef1-α*).

Description – Colonies on YMA after five days at 25 °C are circular form (1.8–2 mm in diameter), light orange, smooth surface, smooth, butyrous, with margin slightly fringed. The cells are spheroidal to ovoidal (1.82–3.59 × 4.9–8.89 μm, *n* = 50), occur singly and polar budding. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, L-arabinose, sucrose, maltose, α-α-trehalose, cellobiose, salicin, melizitose, ribitol, D-glucitol, mannitol, galactitol, D-glucono-1,5-lactone, D-gluconate, and succinate are assimilated, but xylose, *N*-acetyl glucosamine, D-arabinose, D-ribose, L-rhamnose, methyl-α-D-glucoside, melibiose, lactose, raffinose, inulin, soluble starch, glycerol, erythritol, *myo*-inositol, D-glucuronate, D-galacturonic acid, DL-lactate, citrate, methanol, ethanol, and xylitol are not assimilated. Ammonium sulfates, ethylamine hydrochloride, and L-lysine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, and cadaverine are not assimilated. Growth on media containing 50% but not on media containing glucose, 60% glucose, 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is positive. Growth on 10, 15, 25, 30, and 35 °C but absent at 37 and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in rose flower (*Rosa* sp.), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU578. GenBank numbers PV834527 (D1/D2), PV834697 (ITS), PV819893 (SSU), PX582299 (*rpb1*), PX582324 (*rpb2*), PV947474 (*tef1-α*).

Notes – Phylogenetic analyses of a concatenated D1/D2 domain, ITS, SSU, *rpb1*, *rpb2*, and *tef1-α* sequence dataset revealed that *Curvibasidium chiangmaiense*, SDBR-CMU574 and SDBR-CMU578, formed a separate clade, clustering with *Cu. nothofaggi* CBS 8166 and *Cu. pallidicorallinum* CBS9091 (Fig. 32). A comparison of D1/D2 domain and ITS sequences indicated that our strains are not significantly different from *Cu. nothofaggi* (2 nt substitutions in D1/D2 and 1 nt substitutions in ITS), and *Cu. pallidicorallinum* (5 nt substitutions, including 2 gaps in D1/D2 and 0 nt substitutions in ITS). However, *Cu. chiangmaiense* is different from *Cu. nothofaggi* by 7.86% nucleotide divergence (38 nt substitutions) in *rpb1*, 7.32% nucleotide divergence (78 nt substitutions) in *rpb2*, and 3.86% nucleotide divergence (55 nt substitutions) in *tef1-α*, respectively. Likewise, *Cu. chiangmaiense* is different from *Cu. pallidicorallinum* by 9.14.% nucleotide divergence (37 nt substitutions) in *rpb1*, 7.73% nucleotide divergence (84 nt substitutions) in *rpb2*, and 3.84% nucleotide divergence (64 nt substitutions) in *tef1-α*, respectively. Thus, the novel species, *Cu. chiangmaiense*, is introduced from *Curcuma sessilis* and *Rosa* sp. in Thailand. Additionally, distinguishing characteristics between *Cu. chiangmaiense* and another related species are given in Table 9.

Thailandicolales Kodchasee, Senwanna, J. Kumla, & N. Suwannar., ord. nov.

MycoBank number: MB860196

Etymology – '*Thailandicolales*' reference to the name of the host country Thailand.

Description – Member of Microtryomycetes. The diagnosis of the order *Thailandicolales* is based on the description of the genus *Thailandicola*. The nomenclature of the order is based on the genus *Thailandicola*.

Type family – *Thailandicolaceae* Kodchasee, Senwanna, J. Kumla, & N. Suwannar.

Thailandicolaceae Kodchasee, Senwanna, J. Kumla, & N. Suwannar., fam. nov.

MycoBank number: MB860197

Etymology – '*Thailandicolaceae*' refers to the name of the host country Thailand.

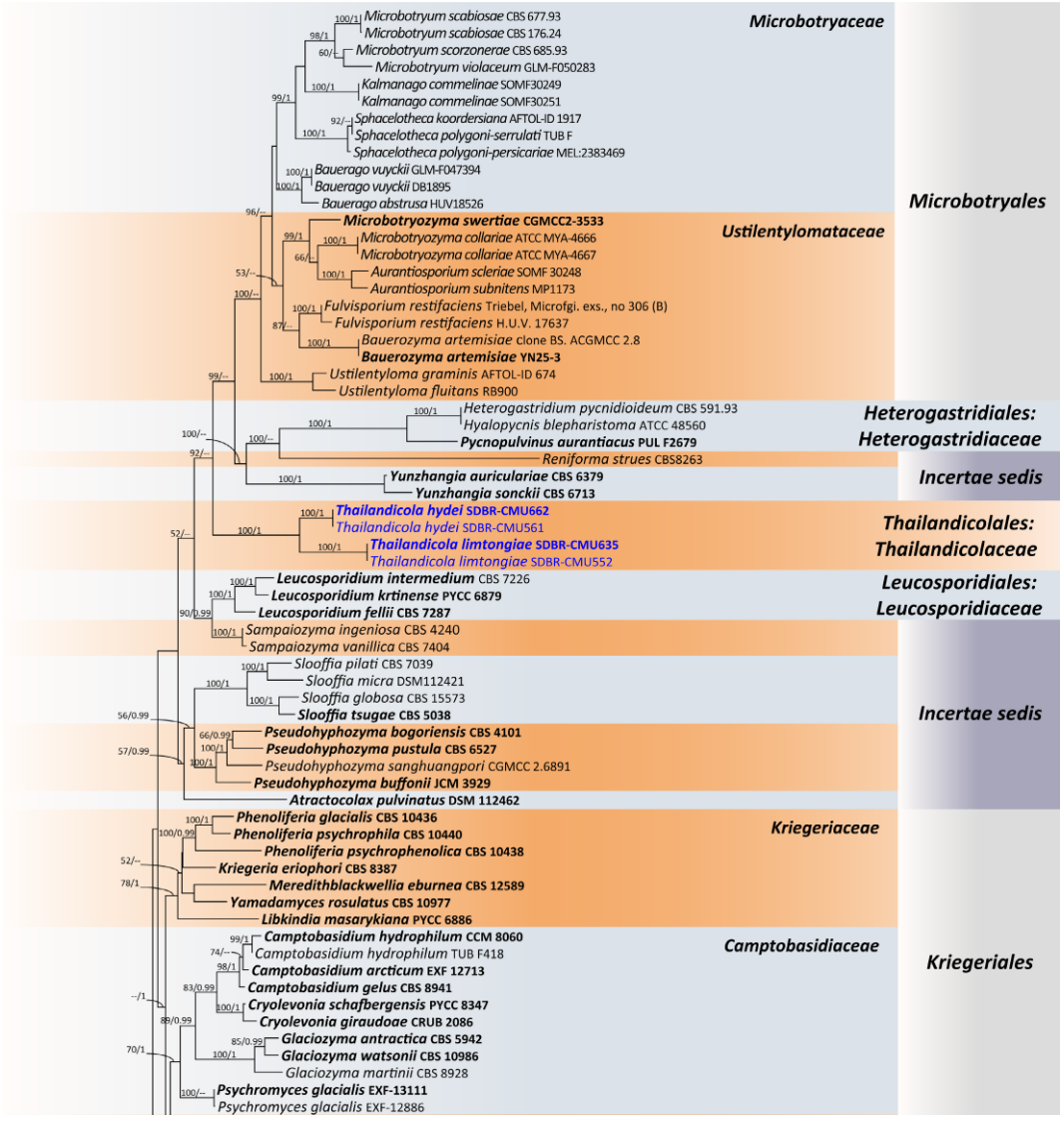

Fig. 32 (to be continued)

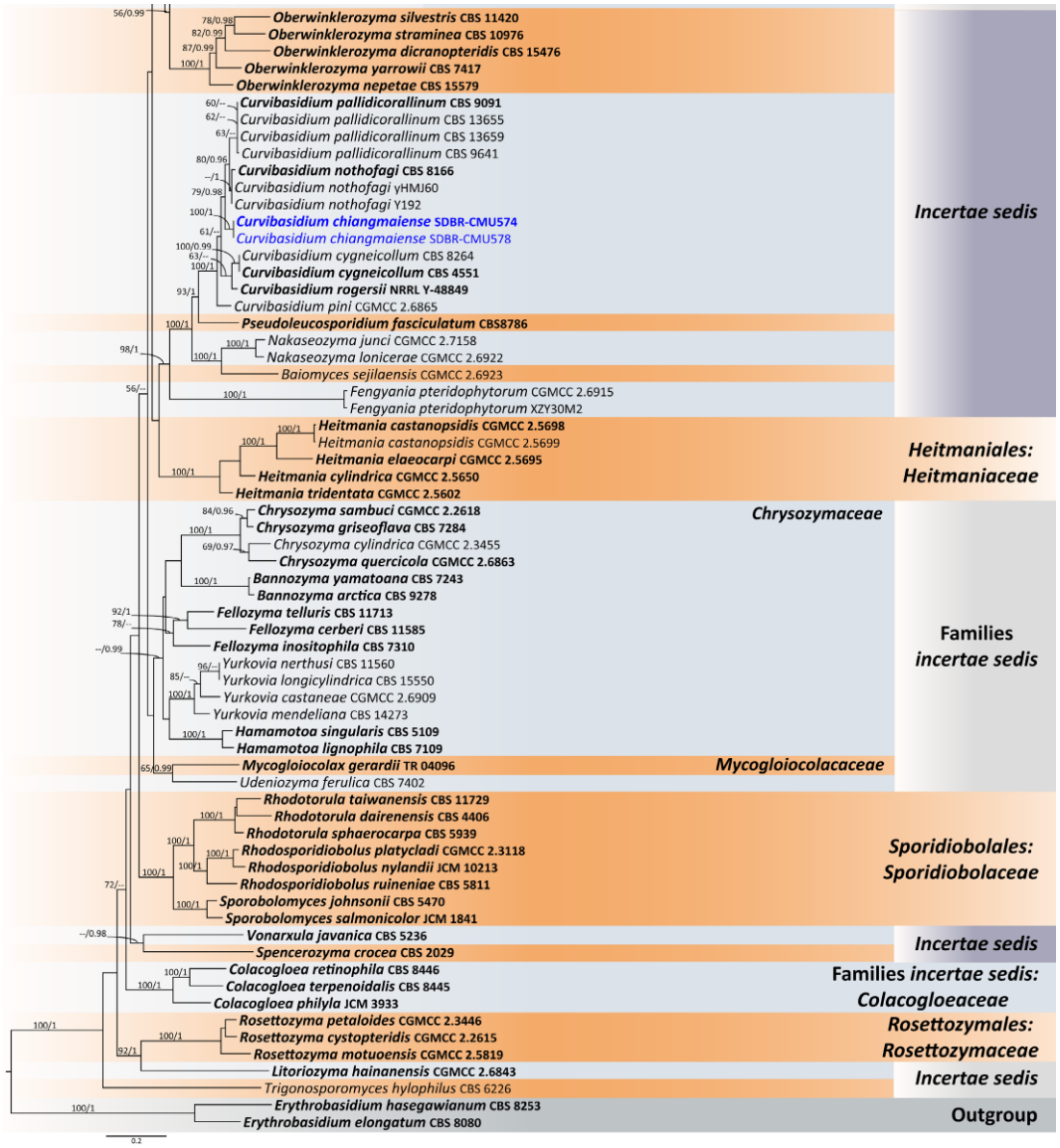

Fig. 32 Phylogenetic tree by maximum likelihood analysis of the combined D1/D2 domain of LSU, ITS, SSU, *rpb1*, *rpb2*, and *tef1-α* sequence data representing Microbotryomycetes. The tree is rooted to *Erythrobasidium elongatum* (CBS 8080) and *E. hasegawianum* (CBS 8253). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. One hundred and thirty-one strains are included in the combined sequence analysis, which comprise 12,715 characters with gaps. The average standard deviation of the split frequencies of the BI analysis was 0.009417. Bootstrap support values for maximum likelihood (≥ 50%, ML, left) and Bayesian posterior probabilities (≥ 0.95, PP, right) are indicated above the nodes. Double dashes (--) denote support values below 50% ML and 0.95 PP. The scale bar represents 0.3 nucleotide substitutions per site. Ex-type strains are shown in bold, and sequences generated in this study are highlighted in blue.

Description – Member of *Thailandicolales* (Microtryomycetes). The diagnosis of the family *Thailandicolaceae* is based on the description of the genus *Thailandicola*. The nomenclature of the family is based on the genus *Thailandicola*.

Type genus – *Thailandicola* Kodchasee, Senwanna, J. Kumla & N. Suwannar., gen nov.

Thailandicola Kodchasee, Senwanna, J. Kumla, & N. Suwannar., gen. nov.

MycoBank number: MB860198

Etymology – '*Thailandicola*' refers to the name of the host country Thailand.

Type species – *Thailandicola limtongiae* Kodchasee, Senwanna, J. Kumla, & N. Suwannar.

Description – The colonies are circular pale yellow, and smooth surface. The cells are ellipsoidal, budding is polar. Pseudohyphae are produced. Basidiospores have not been observed in individual or in mixed cultures. Fermentation ability is absent. Nitrate and nitrite are not assimilated.

Notes – *Thailandicola* is introduced as a new genus based on morphology and multi-locus phylogenetic support. A BLASTn search using the D1/D2 sequence of SDBR-CMU552, SDBR-CMU561, SDBR-CMU635, and SDBR-CMU662 had a less than 90% similarity with other taxa in the Microbotryomycetes and 80%–90% (60%–100% coverage) in the ITS region. These differences indicate that the strains represent a new member of the Microbotryomycetes. Based on the combined multi-locus dataset (D1/D2, ITS, SSU, *rpb1*, *rpb2*, and *tef1-α*; Fig. 32), demonstrated that *Thailandicola* formed an independent clade basal to *Microbotryales*, *Kriegeriales*, *Heterogastridiales*, and *Leucosporidiales* with 100% MLBS and 1 BYPP support values.

Table 9. Phenotypic characteristics differentiating *Curvibasidium chiangmaiense* and closely related species.

Characteristics	1	2	3	4	5	6	7	8	9
Carbon assimilation									
D-Galactose	+	+	+	s	+	–	–	–	–
L-Sorbose	+	+	+	+	+	+	+	–	–
D-Ribose	–	+	–	w	–	–	–	+	w
D-Xylose	–	+	+	s	+	–	+	+	–
L-Arabinose	+	+	–	s	+	+	–	+	+
D-Arabinose	–	+	–	w	s	–	–	+	w
Sucrose	+	–	–	–	–	+	+	+	+
Maltose	+	–	–	–	–	–	+	+	+
α-α-Trehalose	+	–	–	–	–	+	v	–	+
Methyl-α-D-glucoside	–	–	–	–	–	+	+	w	w
Salicin	+	+	+	+	+	+	–	+	+
Melibiose	–	–	–	–	–	+	–	nd	–
Raffinose	–	–	–	+	–	–	–	–	–
Melizitose	+	–	–	–	nd	+	+	nd	+
Inulin	–	–	–	–	nd	+	+	nd	+
Soluble starch	–	–	–	–	–	+	w	nd	+
Glycerol	–	–	–	s	nd	w	+	–	–
D-Glucitol	+	+	+	+	+	+	–	–	–
Galactitol	+	+	+	+	+	–	–	–	–
DL-Lactate	–	–	–	s	–	+	+	+	+
Succinate	+	+	+	+	+	+	+	+	+
Citrate	–	+	+	s	–	+	–	–	–
Ethanol	–	+	+	+	+	+	+	+	+
Nitrogen assimilation									
Potassium nitrate	–	–	+	–	–	+	+	+	+
Sodium nitrite	–	–	–	–	nd	+	w	–	w
L-Lysine	+	+	+	+	+	–	+	+	+
Cadaverine	–	+	–	–	–	+	+	w	+
Growth characteristics									
Growth at 15 °C	+	nd	nd	nd	nd	17 °C	nd	nd	17 °C
Growth at 25 °C	+	+	+	+	+	nd	nd	+	nd
Growth at 30 °C	+	–	–	–	–	–	nd	–	–
Growth at 35 °C	+	–	nd	–	nd	nd	nd	nd	nd
Growth in 0.01% Cycloheximide	–	nd	+	+	–	nd	nd	nd	nd

Strains 1: *Cu. chiangmaiense* sp. nov, 2: *Cu. cygneicollum*, 3: *Cu. nothofagi*, 4: *Cu. pallidicollinum*[89], 5: *Cu. rogersii*[90], 6: *Baiomyces sejilaensis*, 7: *Fengyania pteridophytorum*, 8: *Nakaseozyma junci*, and 9: *N. lonicerae*[81].

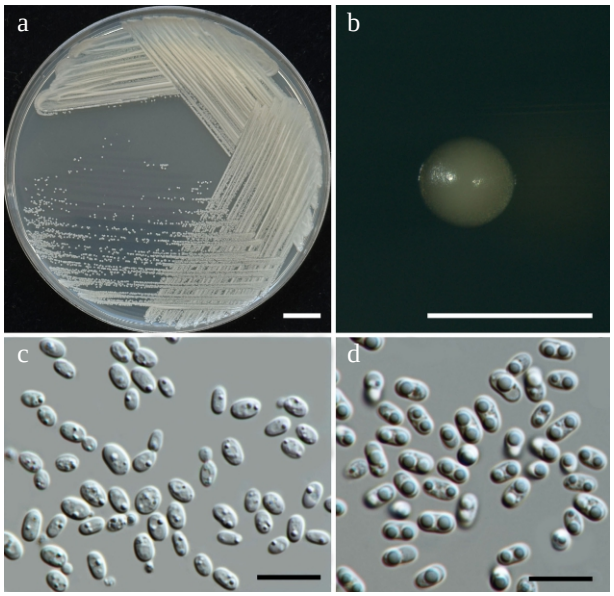

Fig. 33 Morphological characteristics of *Thailandicola hydei* (SDBR-CMU662, ex-type). (a) Culture, (b) single colony, (c), (d) cells and budding cells on YMA after 5 d at 25 °C. (d) Vegetative cells with one or two large globules on PDA at 25 °C for 2 weeks. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Thailandicola hydei Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 33)

MycoBank number: MB860199

Etymology – '*hydei*' referring to the name of Kevin D. Hyde in honor of his 70th birthday.

Holotype – THAILAND, Phayao Province, Mueang District, in vinca flower (*Catharanthus roseus*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40105 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU662 = GMBCC2412 = TBRC21407. GenBank numbers PV834529 (D1/D2), PV834699 (ITS), PV819895 (SSU), PV941862 (*rpb1*), PV947463 (*rpb2*), PV947480 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.8–1.0 mm in diameter), pale yellow, smooth surface, glistening apperance, entire margin, and convex elevation. The cells are ellipsoidal (2.41–4.18 × 3.61–5.3 μm, n = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced pseudohypae, and true hyphae were observed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, *N*-acetyl glucosamine (or weak), sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, raffinose, melizitose, D-glucono-1,5-lactone, and DL-lactate (or weak or slow) are assimilated, but D-galactose, sorbose, ribose, xylose, L-arabinose, D-arabinose, L-rhamnose, melibiose, lactose, inulin, soluble starch, glycerol, erythritol, ribitol, D-glucitol, mannitol, galactitol, *myo*-inositol, D-gluconate, citrate, methanol, ethanol, and xylitol are not assimilated. Assimilation is variable for salicin, D-glucuronate, D-galacturonic acid, and succinate. Ammonium sulfate and lysine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, ethylamine, and cadaverine are not assimilated. Growth occurs on media containing 50% glucose. No growth occurs on media containing 60% glucose, 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth was observed at 10, 20, 25, 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Suthep, in Chinese ixora flower (*Ixora chinensis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU561. GenBank numbers PV834528 (D1/D2), PV834698 (ITS), PV819894 (SSU), PV941861 (*rpb1*), PV947462 (*rpb2*), PV947479 (*tef1-α*).

Notes – In a BLASTn search revealed that the D1/D2 sequence of the *Th. hydei* SDBR-CMU662 and SDBR-CMU561 had 90% similarity with *Oberwinklerozyma yarrowii* CBS 7417, type strain whereas the ITS sequences were closely related to *Fellozyma inositophila* CBS 7310. Based on multi-locus phylogenetic analyses (Fig. 32), *Th. hydei* formed a sister clade to *Th. limtongiae*. Comparing the phenotypic characteristics of *Th. hydei*, *Th. limtongiae*, and another related specie are given in Table 10.

Thailandicola limtongiae Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 34)

MycoBank number: MB860200

Etymology – '*limtongiae*' named in honor of mycologist Savitree Limtong, for her contributions to yeast systematics.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Suthep, in American cassia flower (*Senna spectabilis*), July 2024, P.

Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40104 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU635 = GMBCC2413 = TBRC21405. GenBank numbers PV834531 (D1/D2), PV834701 (ITS), PV819897 (SSU), PV941864 (*rpb1*), PV947465 (*rpb2*), PV947482 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.8–1.0 mm in diameter), yellowish white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are ellipsoidal and ovoid (1.98–4.29 × 2.73–5.98 µm, *n* = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. Pseudohyphae were observed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose (or weak or slow), *N*-acetyl glucosamine, L-rhamnose, sucrose, maltose, *α*-*α*-trehalose, methyl-*α*-D-glucoside, cellobiose (or weak), salicin, melizitose, glycerol (or weak), mannitol, D-glucono-1,5-lactone, D-gluconate (or weak), D-glucuronate, D-galacturonic acid (or slow), DL-lactate (or slow), succinate, and citrate (or slow) are assimilated, but sorbose, ribose, xylose, L-arabinose, D-arabinose, melibiose, lactose, raffinose, inulin, soluble starch, erythritol, ribitol, D-glucitol, galactitol, *myo*-inositol, methanol, ethanol, and xylitol are not assimilated. Ammonium sulfate and lysine are assimilated as sole nitrogen source, but potassium nitrate, sodium nitrite, ethylamine, and cadaverine are not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose (weak). No

Table 10. Phenotypic characteristics differentiating *Thailandicola hydei*, *Th. limtongiae* and closely related species in order *Heterogastridiales Kriegeriales*, *Leucosporidiales*, and *Microbotryales*.

Characteristics/order		*Thailandicolales*		*Heterogastridiales*	*Kriegeriales*		*Leucosporidiales*			*Leucosporidiales*		*Incertae sedis*		
		1	2	3	4	5	6	7	8	9	10	11	12	13
Carbon assimilation	D-Galactose	s/w	–	–	+	w	–	+	+	–	–	–	+	–
	L-Sorbose	–	–	–	+	w	+	+	s	w	s	–	+	+
	N-Acetyl glucosamine	+	w/+	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	nd
	D-Ribose	–	–	–	+	+	+	+/l	s	–	s/w	–	–	+
	D-Xylose	–	–	–	+	w	+	+/l	+	–	+	–	+	+
	L-Arabinose	–	–	–	+	w	+	–	+	–	–	–	+/–	–
	D-Arabinose	–	–	–	v	+	+	+/l	–	–	–	–	–	+
	L-Rhamnose	+	–	–	+	w	+	–	–	–	–	–	–	–
	Sucrose	+	+	–	+	+	+	–	+	+	+	+	–	+
	Maltose	+	+	–	+	+	+	–	+	+	+	+/l	–	+
	α-*α*-Trehalose	+	+	–	+	+	+	+/l	+	+	+	+/l	+	+
	Methyl-*α*-D-glucoside	+	+	nd	+	+	nd	–	–	+	+	–	–	+
	Cellobiose	w/+	+	v	+	+	+	+/l	–	+	+	–	–	+
	Salicin	+	v	nd	+	+	+	–	–	–	+	–	–	+
	Melibiose	–	–	–	–	–	+	–	–	–	–	–	–	–
	Lactose	–	–	–	–	–	+	–	–	–	+	–	–	+
	Raffinose	–	+	–	–	–	+	–	–	+/l	–	–	–	+
	Melizitose	+	+	–	+	+	+	–	+	+	+	+	–	+
	Inulin	–	–	nd	–	–	+	–	+	+	v	v	–	–
	Soluble starch	–	–	–	–	–	+	–	–	w	–	–	–	+
	Glycerol	w	–	–	+	+	+	+	+	w	+	+	+	+
	Erythritol	–	–	–	–	nd	+	–	–	–	–	–	–	–
	Ribitol	–	–	–	+	w	+	+/l	+	+/l	s/w	nd	–	+
	D-Glucitol	–	–	nd	+	–	+	+	+	–	+	nd	+	+
	D-Mannitol	+	–	+	+	+	+	+	+	+	+	+	+	+
	Galactitol	–	–	nd	+	–	+	–	–	–	–	nd	–	–
	myo-Inositol	–	–	–	–	–	+	–	–	–	–	nd	–	–
	D-Glucono-1,5-lactone	+	+	nd	nd	+	+	+/l	+	nd	nd	nd	+/l	+
	D-Gluconate	w	–	nd	+	nd	+	nd	+	nd	–	+	nd	+
	D-Glucuronate	+	v	nd	+	nd	+	nd	+	–	nd	nd	nd	+
	D-Galacturonic acid	s	v	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+
	DL-Lactate	s	s/w	nd	+	+	+	–	s	w	–	–	nd	+
	Succinate	+	v	nd	+	–	+	+	+	+	s/w	–	+	+
	Citrate	s	–	+	+	–	+	+	+	+	–	–	–	+
	Methanol	–	–	nd	–	–	–	nd	nd	–	–	+	–	–
	Ethanol	–	–	nd	+	w	+	+	+	–	–	nd	nd	–
	Xylitol	–	–	nd	v	w	nd	nd	–	nd	nd	nd	nd	+
Nitrogen assimilation	Ammonium sulfate	+	+	nd	nd	nd	nd	+	nd	+	+	nd	nd	nd
	Potassium nitrate	–	–	–	+	–	+	–	+	+	+	–	+	+
	Sodium nitrite	–	–	nd	+	–	+	–	–	+	–	nd	nd	+
	Ethylamine HCl	–	–	nd	nd	+	+	+	+	+	+	nd	nd	–
	L-Lysine	+	+	nd	nd	w	+	l	+	+	–	nd	nd	+
	Cadaverine	–	–	nd	nd	nd	+	+	+	+	–	nd	nd	–
Growth characteristics	Growth at 25 °C	+	+	nd	+	+	+	+	+	+	+	+	+	+
	Growth at 30 °C	+	+	nd	–	+	nd	nd	+	+	+	+	+	+

Strains 1: *Th. hydei* sp. nov, 2: *Th. Limtongiae* sp. nov., 3: *Reniforma strues*[93], 4: *Kriegeria eriophori*, 5: *Meredithblackwellia eburnean*[94], 6: *Leucosporidium fellii*[95], 7: *Leu. Intermedium*[96], 8: *Leu. Krtinense*[97], 9: *Bauerozyma artemisiae*[81], 10: *Microbotryozyma swertiae*[63], 11: *Yunzhangia auriculariae*, 12: *Yu. sonckii*, and 13: *Sampaiozyma vanillic*[65].

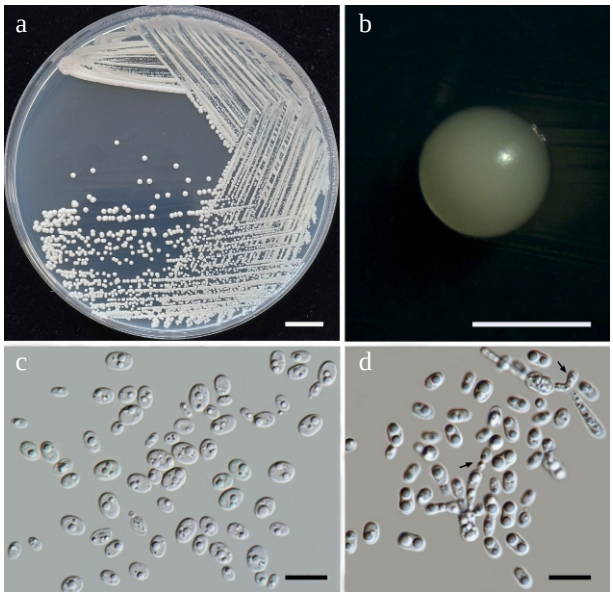

Fig. 34 Morphological characteristics of *Thailandicola limtongiae* sp. nov. (SDBR-CMU635, ex-type). (a) Culture, (b) single colony, (c) cells and budding cells on YMA after 5 d at 25 °C. (d) Vegetative cells with one or two large globules and pseudohyphae (indicated by arrows) on PDA at 25 °C for 2 weeks. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth was observed at 10, 20, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in West Indian jasmine (*Ixora* sp.), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU552. GenBank numbers PV834530 (D1/D2), PV834700 (ITS), PV819896 (SSU), PV941863 (*rpb1*), PV947464 (*rpb2*), PV947481 (*tef1-α*).

Notes – In a BLASTn search revealed that the D1/D2 sequence of the *Th. limtongiae* SDBR- SDBR-CMU635 and SDBR-CMU552 had 90% similarity with *Rhodotorula mucilaginosa* CBS 316, type strain whereas the ITS sequences were closely related to *Meredithblackwellia eburnea* CBS 12589. Based on phylogenetic analysis of a multilocus dataset (Fig. 32), *Th. limtongiae* from a sister clade to *Th. hydei*. Likewise, the phenotypic differences of *Th. limtongiae* can be distinguished from *Th. hydei* (Table 10).

Order: *Sporidiobolales* Doweld

Family: *Sporidiobolaceae* R.T. Moore

Sporidiobolacae species occur worldwide and have been isolated from a wide variety of substrates, including air, tree leaves, and organic matter. Members of this family are known as decomposers and for their ability to thrive in diverse environmental conditions. Additionally, some species in this family are economically important due to their role as sources of biotechnological products. Their ability to utilize waste materials in the synthesis of carotenoids, lipids, and enzymes makes them valuable for applications in the food, pharmaceutical, and industrial applications[91,92]. *Sporidiobolacae* represents exhibit spherical to elongate, reproduces by budding, some species form pseudohyphae and produce basidiospores. The colony present salmon-pink to red[82]. Currently, three genera are listed in this family including *Rhodosporidiobolus*, *Rhodotorula*, and *Sporobolomyces*[41]. In this study, *Rhodosporidiobolus fluvialis* (three strains), *R. ruineniae* (three strains), *Rhodotorula paludigena* (one strain), *Rh. toruloides* (one strain), *Rh. thailandensis* sp. nov. (three strains), and *Sporobolomyces thailandensis* sp. nov. (three strains) were presented (Fig. 35).

Rhodotorula thailandensis Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 36)

MycoBank number: MB 860201

Etymology – The species name '*thailandensis*' refers to Thailand, the country where the type strain was isolated.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Suthep in Fukien tea flower (*Carmona retusa*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40087 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU566 = GMBCC2414 = TBRC21387. GenBank numbers PV834539 (D1/D2), PV834709 (ITS), PX622343 (SSU), PV941865 (*rpb1*), PV947466 (*rpb2*), PV947483 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1.0–2.0 mm in diameter), light orange or orange, white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are ellipsoidal and cylindrical (1.66–3.09 × 2.75–5.90 μm, n = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, ribose, xylose, L-arabinose (or slow), L-rhamnose (or weak), sucrose, α-α-trehalose, cellobiose, salicin, raffinose, glycerol, ribitol, D-glucitol (or slow), mannitol (or slow), galactitol (or slow), D-glucono-1,5-lactone, succinate, citrate, and xylitol are assimilated, but D-galactose, sorbose, *N*-acetyl glucosamine, melibiose, melizitose, inulin, soluble starch, erythritol, myo-Inositol, D-gluconate, D-glucuronate, D-galacturonic acid, methanol, and ethanol are not assimilated. Assimilation is variable for DL-lactate, D-arabinose, maltose, methyl-α-D-glucoside, and lactose. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine, and L-lysine are assimilated as sole nitrogen sources, but cadaverine is not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose (weak). No growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth was observed at 10, 20, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Mae Hia, in Siam tulip flower (*Curcuma sessilis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU577; Doi Saket District, Samran Rat, in Chinese honey suckle (*Combretum indicum*) flower, August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU723. GenBank numbers SDBR-CMU577: PV834540 (D1/D2), PV834710 (ITS), PX622344 (SSU), PV941866 (*rpb1*), PV947467 (*rpb2*), PV947484 (*tef1-α*); SDBR-CMU723: PV834541 (D1/D2), PV834711 (ITS), PX622345 (SSU), PV941867 (*rpb1*), PV947468 (*rpb2*), PV947485 (*tef1-α*).

Notes – *Rhodotorula thailandensis* SDBR-CMU566, SDBR-CMU577 and SDBR-CMU723 were placed in the *Rhodotorula* clade (Fig. 35), and were closely related to *Rh. tropicalis* PYCC 8913 by 4.32% nucleotide divergence (26 nt substitutions) in D1/D2 regions and 5.92% nucleotide divergence (35 nt substitutions and 11 gaps) in the ITS region, which indicated that they represent a new species of *Rhodotorula*. Physiologically, *Rh. thailandensis* differs from *Rh. tropicalis* in its inability to galactose and sorbose. Additionally, *Rh. thailandensis* was able to grow on 50% and 60% glucose, while *Rh. tropicalis* could not grow at that medium[98].

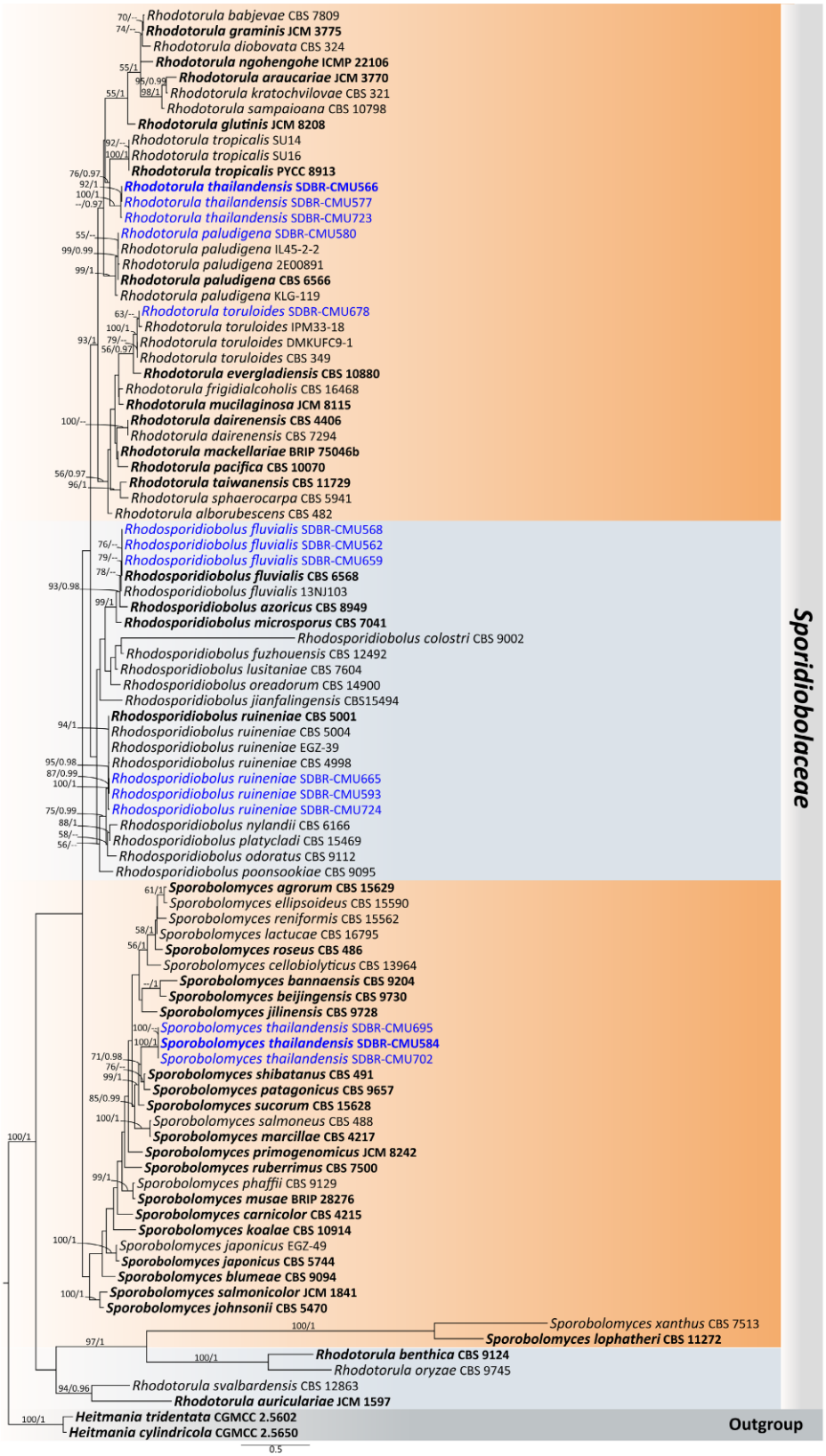

Fig. 35 Phylogram generated by maximum likelihood analysis of the combined D1/D2 domain of LSU, ITS, SSU, *rpb1*, *rpb2*, and *tef1-α* sequence data representing *Sporidiobolaceae*. The tree is rooted to *Heitmania cylindrica* (CGMCC 2.5650) and *H. tridentata* (CGMCC 2.5602). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Ninety-two strains are included in the combined sequence analysis, which comprise 7,114 characters with gaps. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

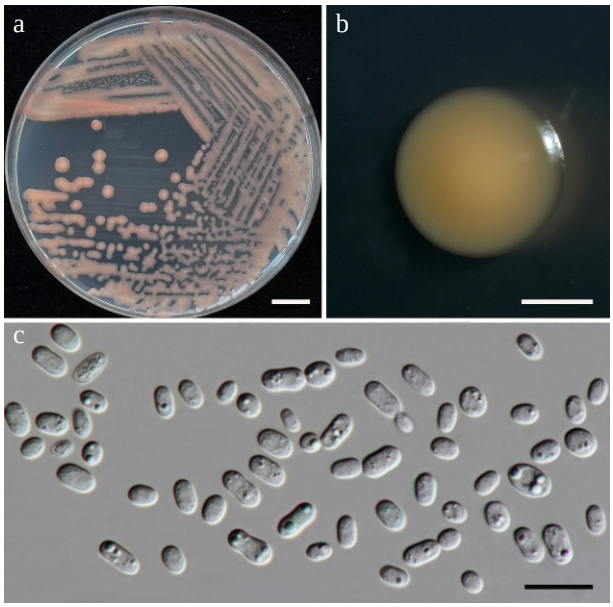

Fig. 36 Morphological characteristics of *Rhodotorula thailandensis* (SDBR-CMU566, ex-type). (a) Culture, (b) single colony, (c) cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, and (c) = 10 μm.

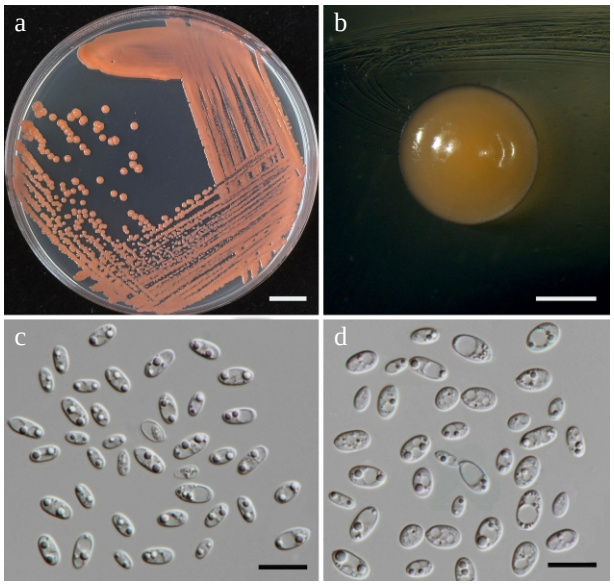

Fig. 37 Morphological characteristics of *Sporobolomyces thailandensis* (SDBR-CMU584, ex-type). (a) Culture, (b) single colony, (c) cells, and (d) budding cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Sporobolomyces thailandensis Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 37)

MycoBank number: MB860203

Etymology – The species name '*thailandensis*' refers to Thailand, the country where the type strain was isolated.

Holotype – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in Oncidium flower (*Oncidium* sp.), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40091 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU584 = GMBCC2415 = TBRC21391. GenBank numbers PV834543 (D1/D2), PV834713 (ITS), PX622346 (SSU), PX582300 (*rpb1*), PX582325 (*rpb2*), PX582344 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1.8–2.0 mm in diameter), reddish orange, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are ellipsoidal and cylindrical (2.38–4.55 × 4.4–8.66 μm, n = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, sorbose, xylose, sucrose, maltose, α-α-trehalose, raffinose, melizitose, glycerol (weak), ribitol, D-glucitol, mannitol, D-glucono-1,5-lactone, succinate, and citrate are assimilated, but D-galactose, *N*-acetyl glucosamine, ribose, D-arabinose, L-arabinose, L-rhamnose, methyl-α-D-glucoside, cellobiose, salicin, melibiose, lactose, inulin, soluble starch, erythritol, galactitol, myo-Inositol, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, methanol, ethanol, and xylitol are not assimilated. Ammonium sulfate, potassium nitrate (weak), sodium nitrite (weak), ethylamine, and L-lysine are assimilated as sole nitrogen source, but cadaverine is not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose (weak). No growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is positive. Growth on 10, 15, 25, 30, and 35 °C but not at 37 and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in banana flower (*Musa sapientum*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU695; Mueang District, Chang Phueak, in khae na flower (*Dolichandrone serrulate*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU702. GenBank numbers SDBR-CMU695: PV834544 (D1/D2), PV834714 (ITS), PX622347 (SSU), PX582301 (*rpb1*), PX582326 (*rpb2*), PX582345 (*tef1-α*); SDBR-CMU702: PV834545 (D1/D2), PV834715 (ITS), PX622348 (SSU), PX582302 (*rpb1*).

Notes – Based on phylogenetic analyses (Fig. 35), *Sporobolomyces thailandensis* SDBR-CMU584, SDBR-CMU695, and SDBR-CMU702 found to be closely related to *Sp. sucorum* CBS 15628, *Sp. shibatanus* CBS 491, *Sp. patagonicus* CBS 9657 *Sp. sucorum* CBS 15628, and *Sp. salmoneus* CBS 488. These species are differed by 0.37%, 0.68%, 0.85%, and 1.23% nucleotide divergence (2, 4, 5, and 7 nt substitutions), respectively, in the D1/D2 domain. Meanwhile the ITS region revealed differences of 1.41%, 1.04%, 1.08%, and 2.48% (7, 6, 6, and 14 nt substitutions), respectively. Additionally, *Sp. thailandensis* is different from *Sp. patagonicus* and *Sp. salmoneus* by 12.92%–13.14% and 4.10%–7.38% nucleotide divergence in *rpb2* and *tef1-α*, respectively. The phenotypic characteristics of *Sp. thailandensis* can be distinguished from *Sp. patagonicus*, *Sp. salmoneus*, *Sp. shibatanus*, and *Sp. sucorum* are provided in Table 11.

Subphylum: Ustilaginomycotina Doweld

Class: Exobasidiomycetes Begerow, M. Stoll, & R. Bauer

Order: *Exobasidiales* Henn.

Family: *Brachybasidiaceae* Gäum.

Brachybasidiaceae represents a group of plant-parasitic basidiomycetes that decay dead organic matter, including wood and leaves. The vegetative morphology presents dimorphism, producing a saprobic haploid and a parasitic dikaryotic form. The cellular structure exhibits septate hyphae and specialized reproductive structures[102–104]. Currently, nine genera are listed in this family including *Brachybasidium*, *Dicellomyces*, *Eidernor*, *Kordyana*, *Lelum*, *Marantokordyana*, *Meira*, *Proliferobasidium*, and *Yunzhangomyces*[41]. In this study, *Meira argovae* (three strains), *Mei. plantarum* (one

Table 11. Phenotypic characteristics differentiating *Sporobolomyces thailandensis* from closely related *Sporobolomyces* species.

	Characteristics	1	2	3	4	5
Carbon assimilation	D-Galactose	–	s	v	+	w
	D-Ribose	–	+	v	+/s	–
	Sucrose	+	+	+	+	–
	Maltose	+	v	+	+	–
	α-α-Trehalose	+	v	+	+	–
	Methyl-α-D-glucoside	–	v	+	+	nd
	Cellobiose	–	–	+	+	–
	Salicin	–	–	+	+	nd
	Melibiose	–	–	+	–	–
	Raffinose	+	+	+	+	–
	Melizitose	+	+	+	+	–
	Soluble starch	–	+	l	+	–
	Glycerol	w	s	–	+	w
	Ribitol	+	–	–	v	nd
	D-Glucitol	+	v	+	–	nd
	D-Mannitol	+	s	+	+	–
	D-Gluconate	–	+	+	+	nd
	Citrate	+	–	v	+	–
	Ethanol	–	–	–	+	+
Nitrogen assimilation	Potassium nitrate	w	+	v	–	+
	Sodium nitrite	w	–	v	–	–
	Ethylamine HCl	+	+	v	nd	w
	L-Lysine	+	+	v	nd	–
Growth characteristics	Growth at 30 °C	+	+	v	+	+
	Growth at 35 °C	–	–	–	–	+
	Growth at 37 °C	–	–	–	nd	w
	Growth in 0.1% Cycloheximide	v	–	nd	–	+

Strains 1: *Sp. thailandensis* sp. nov. 2: *Sp. patagonicus*[99], 3: *Sp. salmoneus* 4: *Sp. shibatanus*[88,100], and 5: *Sp. sucorum*[101].

strain), *Mei. chiangmaiensis* sp. nov. (three strains), *Mei. limtongiae* sp. nov. (three strains), and *Mei. pollinicola* sp. nov. (three strains) are presented. (Fig. 38).

Meira chiangmaiensis Kodchasee, Senwanna, J. Kumla. & N. Suwannar., sp. nov. (Fig. 39)

MycoBank number: MB860204

Etymology – '*chiangmaiensis*' referring to Chiang Mai Province, where the new species was found.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in jasmine flower (*Jasminum sambac*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40101 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU624 = GMBCC2416 = TBRC21402. GenBank numbers PV834549 (D1/D2), PV834719 (ITS), PX622349 (SSU), PV941868 (*rpb1*), PV947460 (*rpb2*), PV947488 (*tef1-α*).

Description – Colonies on YMA after 3 d at 25 °C is circular form (1–2 mm in diameter) pale yellow smooth, after 5 d rigid, wrinkled, warty, velvety, and presence of thin aerial mycelia. After growth on YMA at 25 °C for 3 d, the cells are ellipsoidal to fusiform (0.95–1.84 × 5.42–18.1 μm, *n* = 50), occur polar budding. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, *N*-acetyl glucosamine (or weak), ribose, xylose, L-arabinose, D-arabinose (or weak), sucrose, maltose, α-α-trehalose, cellobiose, salicin (or weak), melibiose, raffinose, melizitose, erythritol, ribitol (or weak), D-glucitol, mannitol, *myo*-inositol (or weak), D-glucono-1,5-lactone, succinate, citrate (or weak), and xylitol (or weak) are assimilated, but sorbose, L-rhamnose, methyl-α-D-glucoside, lactose, inulin, soluble starch, glycerol (or weak), galactitol, methanol, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, and ethanol are not assimilated. Ammonium sulfates, potassium nitrate (or weak), and ethylamine hydrochloride are assimilated as sole nitrogen sources, L-lysine are variable, but sodium nitrite and cadaverine are not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose (or weak). Not growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth on 10, 15, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Suthep, in bougainvillea (*Bougainvillea hybrid*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU638; White cheesewood (*Alstonia scholaris*) flower, September 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU731. GenBank numbers SDBR-CMU638: PV834550 (D1/D2), PV834720 (ITS), PX622350 (SSU), PV941869 (*rpb1*), PV947489 (*tef1-α*); SDBR-CMU731: PV834551 (D1/D2), PV834721 (ITS), PX622351 (SSU).

Notes – *Meira chiangmaiensis* SDBR-CMU624, SDBR-CMU638 and SDBR-CMU731 were related to *Mei. pollinicola* by 0.98% nucleotide divergence (6 nt substitutions) in the D1/D2 domain and 3.08% (24 nt substitutions, including 19 gaps) in the ITS region. In addition, *Mei. chiangmaiensis* and *Mei. pollinicola* differed from *Mei. argovae* CBS 110053 by 0.5% and 0.67% nucleotide divergence (3 nt substitutions and 4 nt substitutions, including 1 gap, respectively,) in the D1/D2 regions and 2.3% and 2.79% (14 nt substitutions and 17 nt substitutions including 20 gaps, respectively) in the ITS region. Moreover, *Mei. chiangmaiensis* is different from *Mei. argovae* by 3.67% nucleotide divergence (27 substitutions and 1 gap) in *rpb1*, 4.56% nucleotide divergence (49 substitutions and 1 gap) in *rpb2* and 3.52% nucleotide divergence (31 substitutions and 1 gap) in *tef1-α* (Fig. 38). Based on physiological tests, *Mei. chiangmaiensis* can distinguish it from *Mei. argovae*[105] and *Mei. pollinicola*, which are shown in Table 12.

Meira limtongiae Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 40)

MycoBank number: MB860178

Etymology – '*limtongiae*' named in honor of mycologist Savitree Limtong, for her contributions to yeast systematics.

Holotype: THAILAND, Phayao Province, Mueang District, in blue ginger flower (*Dichorisandra thyrsiflora*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40110 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU696 = GMBCC2417 = TBRC21413. GenBank numbers PV834554 (D1/D2), PV834724 (ITS), PX622354 (SSU), PX570013 (*rpb2*).

Description – Colonies on YMA after 3 d at 25 °C are circular form (1–2 mm in diameter) pale yellow smooth, after 5 d rigid, wrinkled, warty, velvety, and presence of thin aerial mycelia. After growth on YMA at 25 °C for 3 d, the cells are ellipsoidal to fusiform (1.08–2.24 × 4.01–16.92 μm, *n* = 50), occur polar budding. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, ribose, xylose, L-arabinose, D-arabinose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, salicin, raffinose, melizitose, glycerol (weak), erythritol, ribitol, D-glucitol, mannitol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, DL-lactate, succinate, citrate,

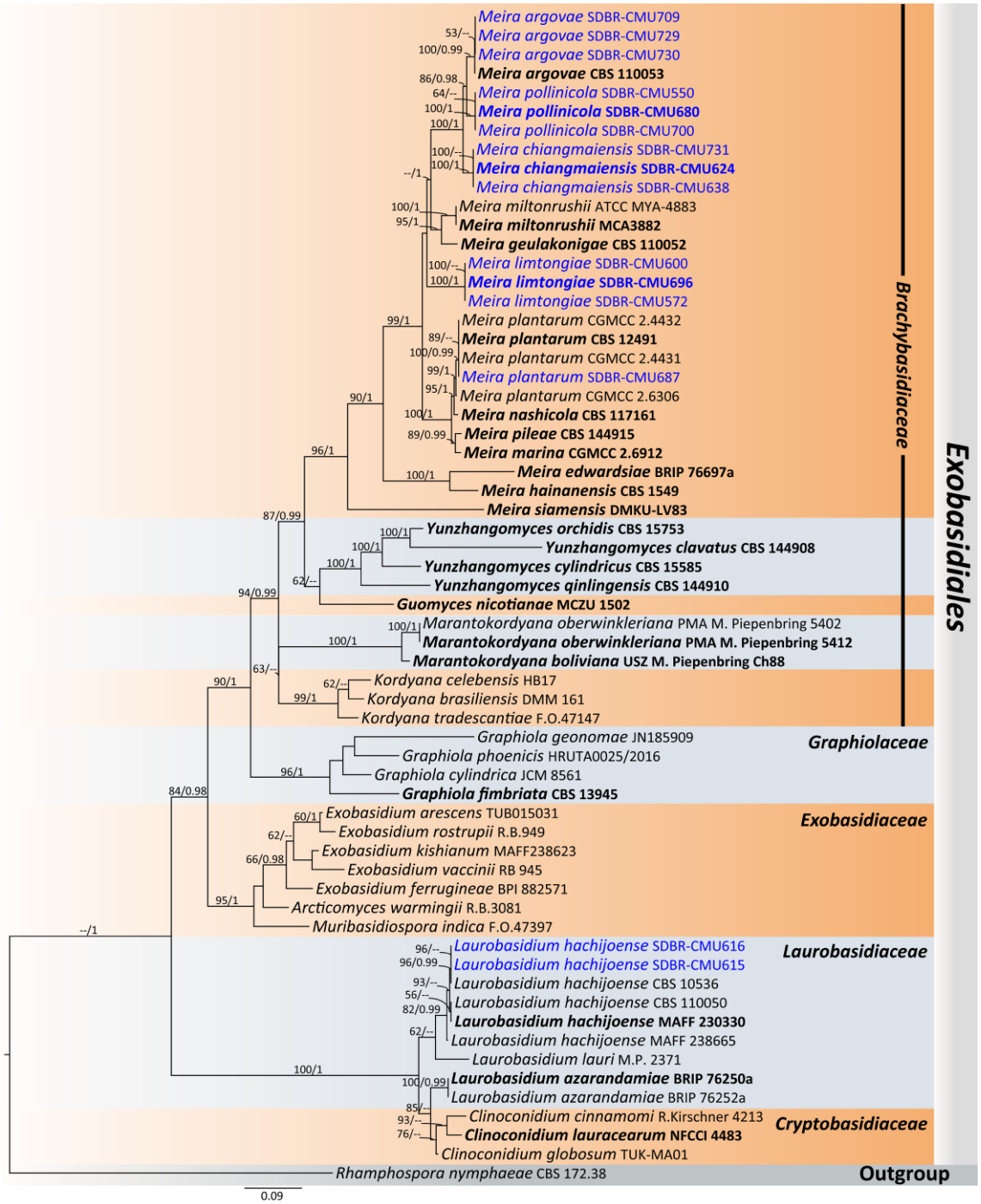

Fig. 38 Phylogram generated by maximum likelihood analysis of the combined D1/D2 domain of LSU, ITS, SSU, *rpb1*, *rpb2*, and *tef1-α* sequence data representing *Meira* and *Laurobasidium* in *Exobasidiales*. The tree is rooted to *Rhamphospora nymphaeae* (CBS 172.38). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Sixty-two strains are included in the combined sequence analysis, which comprise 5,633 characters with gaps. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

and xylitol are assimilated, but sorbose, *N*-acetyl glucosamine L-rhamnose, melibiose, lactose, inulin, soluble starch, galactitol, *myo*-inositol, methanol, D-galacturonic acid, and ethanol are not assimilated. Ammonium sulfates, potassium nitrate, sodium nitrite, ethylamine hydrochloride (weak), and L-lysine (weak) are assimilated as sole nitrogen source, but cadaverine are not assimilated. Growth occurs on media containing 50% glucose and 60% glucose (weak). Not growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth on 10, 15, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in adenium flower (*Adenium obesum*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N.

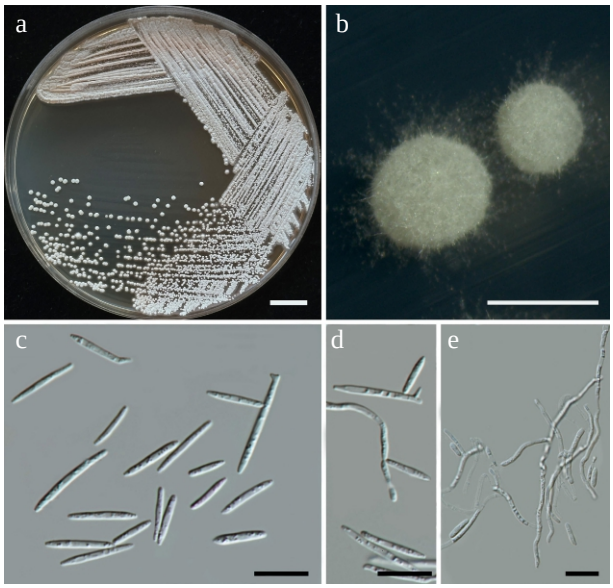

Fig. 39 Morphological characteristics of *Meira chiangmaiensis* (SDBR-CMU624, ex-type). Culture (a), single colony (b) and cells (c, d) on YMA after five days at 25 °C. Pseudohyphae on PDA after 2 weeks at 25 °C (d, e). Scale bars a = 10 mm, b = 1 mm, c and d = 10 µm.

Suwannarach, living culture SDBR-CMU572; Phrao District, Nam Phrae, in dancing ladies ginger (*Globba winitii*) flower, July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU600. GenBank numbers SDBR-CMU572: PV834552 (D1/D2), PV834723 (ITS), PX622352 (SSU), PX570011 (*rpb2*); SDBR-CMU600: PV834553 (D1/D2), PV834715 (ITS), PX622353 (SSU), PX570012 (*rpb2*).

Notes – *Meira limtongiae* formed a distinct lineage and was sister to *Mei. argrovae*, *Mei chiangmaiensis*, *Mei. pollinicola, Mei. miltonrushii* and, *Mei. geulakonigae* (Fig. 38). *Meira limtongiae* differed from these species by 0.83%–1.85% nucleotide divergence (5–10 substitutions, including gaps) in the D1/D2 domain, 4.64%–6.46% nucleotide divergence (28–39 substitutions, including gaps) in the ITS region, and 13.84%–14.69% nucleotide divergence (148–157 substitutions, including gaps) in *rpb2*. Additionally, distinguishing physiological characteristics between *Mei. limtongiae* and other *Maira* species are provided in Table 12.

Meira pollinicola Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 41)

MycoBank number: MB860206

Etymology – The specific epithet '*pollinicola*' refers to the substrate origin of the type strain, pollen structure.

Holotype – THAILAND, Phayao Province, Mueang District, in chaya spinach flower (*Cnidoscolus aconitifolius*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype CMUB40108 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU680 = GMBCC2418 = TBRC21410. GenBank numbers PV834557 (D1/D2), PV834727 (ITS), PX622356 (SSU), PX582304 (*rpb1*), PX582328 (*rpb2*).

Description – Colonis on YMA after 3 d at 25 °C is circular form (1–2 mm in diameter) yellow/orange smooth, after 5 d rigid, wrinkled, warty, velvety, and presence of thin aerial mycelia. After growth on YMA at 25 °C for 5 d, the cells are ellipsoidal to fusiform (1.16–2.84 × 3.81–12.00 µm, *n* = 50), occur polar budding. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained

Table 12. Phenotypic characteristics differentiating *Meira chiangmaiensis* sp. nov., *Mei. pollinicola* sp. nov., and *Mei. limtongiae* sp. nov. from closely related *Meira* species.

Characteristics	1	2	3	4	5	6	7	8	9	10
Carbon assimilation										
L-Sorbose	–	–	–	–	–	w/l	–	–	–	–
D-Arabinose	w/–	–	w	+	+	–	+/w	+	–	w
L-Rhamnose	–	–	–	–	–	–	–/w	nd	–	–
Methyl-α-D-glucoside	–	+	–	–	–	–	w	nd	–	–
Cellobiose	+	+	+	+	w	l	+	+	+	+
Salicin	w/+	+	+	l	l	w/l	w	l	l/w	w
Melibiose	+	–	–	+/–	+	l	+	+	+	+
Melizitose	+	+	+	–	–	+	+	+	+	+
Inulin	–	–	–	w	l	l	–/w	–	–	+
Soluble starch	–	–	–	+/l	l	w/l	–/w	+	–	–
Glycerol	–	w	–	w/l	l	–	–/w	–	–	w
Ribitol	w	+	w	+/l	+/l	–	+/w	–	–	w
D-Glucitol	+	+	+	+	+	+	–/w	+	+	w
Galactitol	–	–	–	+/l	l	–	+	–	v	–
myo-Inositol	w/–	–	–	–	–	–	–/w	–	–	–
D-Glucono-1,5-lactone	+	+	–	+	l	nd	+	–	nd	nd
D-Gluconate	–	w	–	–	–	nd	w	–	nd	nd
DL-Lactate	–	+	w	w/l	l	l	v	w	–	–
Succinate	+	+	w	+	+	l	+/w	+	+	w/l
Citrate	w/–	+	–	+	l	w/l	w	–	–	–
Ethanol	–	–	–	l	l	+	w	+	–	w
Xylitol	w/–	+	–	+/d	+/d	nd	+/w	+	nd	nd
Nitrogen assimilation										
Potassium nitrate	w/–	+	+	+	–	l	+	+	+	+
Sodium nitrite	–	+	+	+	–	l	+	+	+	+
L-Lysine	v	w	w	–	+	l	+	nd	l/w	+
Cadaverine	–	–	–	+	+	+	+	nd	–	+
Growth characteristics										
Growth at 25 °C	+	+	+	+	+	nd	+	+	+	+
Growth at 30 °C	+	+	+	+	+	+	+	nd	nd	nd
Growth at 35 °C	–	–	–	–	+	nd	nd	nd	nd	nd
Growth at 37 °C	–	–	–	–	+	nd	–	–	nd	nd
Growth in 0.01% cycloheximide	–	–	–	–	+	nd	–	nd	nd	nd
Growth in 0.1% cycloheximide	–	–	–	–	+	nd	–	nd	nd	nd
Growth on 50% glucose	+	+	+	–	–	+	+	nd	–	–

Strians 1: *Mei. chiangmaiensis* sp. nov., 2: *Mei. limtongiae* sp. nov, 3: *Mei. pollinicola* sp. nov., 4: *Mei. argovae*, 5: *Mei. geulakonigii*[105], 6: *Mei. marina*[81], 7: *Mei. miltonrushii*[103], 8: *Mei. nashicola*[106], 9: *Mei. plantarum*, and 10: *Mei. pileae*[107].

for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, ribose, xylose, L-arabinose, D-arabinose (weak), sucrose, maltose, α-α-trehalose, cellobiose, raffinose, melizitose, erythritol, ribitol (or weak), D-glucitol, mannitol, DL-lactate (or weak), and succinate, (or weak) are assimilated, but sorbose, *N*-acetyl glucosamine L-rhamnose, methyl-α-D-glucoside, salicin, melibiose, lactose, inulin, soluble starch, glycerol, galactitol, *myo*-inositol, D-glucono-1,5-lactone, citrate, methanol, D-gluconate, D-glucuronate, D-galacturonic acid, ethanol, and xylitol are not assimilated. Ammonium sulfates, potassium nitrate, sodium nitrite, ethylamine hydrochloride, and L-lysine (or weak) are assimilated as sole nitrogen source, but cadaverine are not assimilated. Growth occurs on media containing 50% glucose and 60% glucose (weak). Not growth occurs on media containing 10% NaCl/5% glucose 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth on 10, 15, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in frangipani flower (*Phyllanthus pulcher*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach,

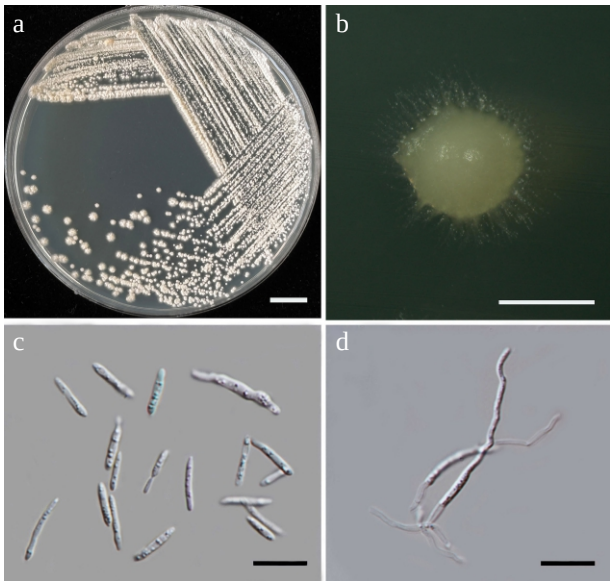

Fig. 40 Morphological characteristics of *Meira limtongiae* (SDBR-CMU696, ex-type). (a) Culture, (b) single colony, and (c) cells on YMA after 5 d at 25 °C. (d) Pseudohyphae on PDA after 2 weeks at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

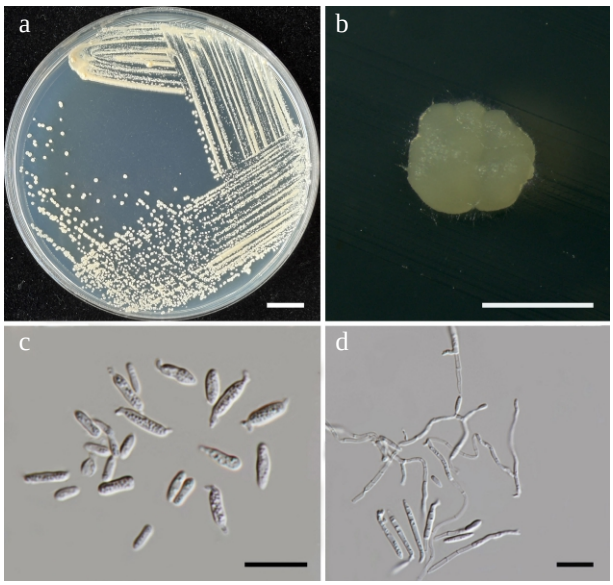

Fig. 41 Morphological characteristics of *Meira pollinicola* (SDBR-CMU680, ex-type). (a) Culture, (b) single colony, and (c) cells on YMA after 5 d at 25 °C. (d) Pseudohyphae on PDA after 2 weeks at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

living culture SDBR-CMU550; Mueang District, Chang Phueak, in purslane flower (*Portulaca grandiflora*), September 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU700. GenBank numbers SDBR-CMU550: PV834556 (D1/D2), PV834714 (ITS), PX622355 (SSU), PX582303 (*rpb1*), PX582327 (*rpb2*); SDBR-CMU700: PV834558 (D1/D2), PV834728 (ITS), PX622357 (SSU), PX582305 (*rpb1*), PX582329 (*rpb2*).

Notes – *Meira pollinicola* SDBR-CMU680, SDBR-CMU550 and SDBR-CMU700 were related to *Mei. argovae* CBS110053 and *Mei. chiangmaiensis* SDBR-CMU624 (Fig. 38). *Meira pollinicola* differed from the to *Mei. argovae* by 0.67% nucleotide divergence (4 nt substitutions and 1 gap) in D1/D2 regions and 2.79% (17 nt substitutions and 20 gaps) in ITS region. Likewise, *Mei. pollinicola* differed from *Mei. chiangmaiensis* with 0.98% nucleotide divergence (6 nt substitutions) in D1/D2 regions and 3.08% (19 nt substitutions and 24 gaps) in ITS region. Moreover, *Mei. pollinicola* is different from *Mei. argovae* and *Mei. chiangmaiensis* by 2.47%–4.26% and 3.09% to 4.22% nucleotide divergence in *rpb1* and *rpb2*, respectively. Based on physiological test, *Mei. pollinicola* can be distinguished from *Mei. argovae*[105] and *Mei. chiangmaiensis* as shown in Table 12.

Family: *Laurobasidiaceae* Pinruan, Sommai, Suetrong, Somrith, & E.B.G. Jones

Laurobasidiaceae morphologically possesses basidial sterigmata, thin-walled basidiospores with an abaxial hilum, and sporulates on the surface of the host tissues that cause to causing aerial-root-like galls on the host plant. Sexual morph present elongated basidium holobasidiate, clavate to cylindrical, with sterigmata; basidiospores ellipsoidal, oblong, or cylindrical, septate, thin-walled, with an abaxial hilum, while asexual morph present thin- and smooth-walled, fusiform, clavate to narrow ellipsoid of conidia hyaline[108]. Currently, one genus is listed in this family, including *Laurobasidium*[41]. *Laurobasidium hachijoense* (two strains) was isolated and identified in this study (Fig. 38, Supplementary File 1).

Order: *Microstromatales* R. Bauer & Oberw.

Families *incertae sedis*

Currently, five genera are listed under *Microstromatales* genera *incertae sedis*, including *Baueromyces*, *Jaminaea*, *Parajaminaea*, *Pseudomicrostroma*, and *Sympodiomycopsis* [41]. In this study, seven yeast species were isolated and presented, including *Jaminaea lantanae* (one strain), *Parajaminaea hydei* sp. nov. (two strains), *Sympodiomycopsis europaea* (two strains), *Sym. paphiopedili* (two strains), *Sym. hydei* sp. nov. (four strains), *Sym. limtongiae* sp. nov. (two strains) and *Sym. saisamorniae* sp. nov. (two strains).

Parajaminaea hydei Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 42)

MycoBank number: MB860207

Etymology – '*hydei*' referring to the name of mycologist Kevin D. Hyde in honor of his 70th birthday.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in ground cherry flower (*Physalis minima*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40096 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU601 = GMBCC2419 = TBRC21395. GenBank numbers PV834562 (D1/D2), PV834732 (ITS), PX622358 (SSU), PV947453 (*rpb2*), PV947477 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.8–1.0 mm in diameter), yellowish white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are ellipsoidal and ovoid (2.25–3.63 × 3.06–6.8 μm, $n = 50$), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, neither pseudohyphae nor true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, ribose (or latent or slow), L-arabinose, sucrose, maltose (or slow), methyl-α-D-glucoside (or latent or slow), raffinose, melizitose, glycerol (or slow), erythritol (or weak), mannitol (or latent), and ethanol are assimilated, but D-galactose, sorbose, xylose, D-arabinose, L-rhamnose, α-α-trehalose, salicin, melibiose, lactose, inulin, soluble starch, ribitol, *myo*-inositol, D-glucono-1,5-lactone, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, methanol, and xylitol are not assimilated. Assimilation is variable for *N*-acetyl glucosamine,

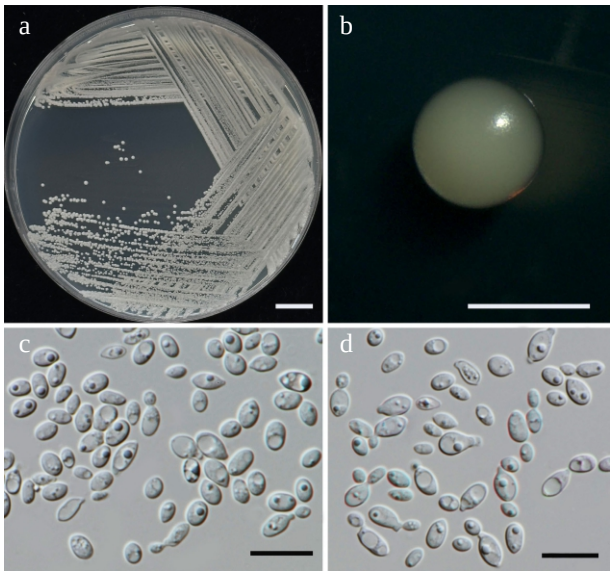

Fig. 42 Morphological characteristics of *Parajaminaea hydei* (SDBR-CMU601, ex-type). (a) Culture, (b) single colony, and (c), (d) cells on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

cellobiose, D-glucitol, galactitol, and D-gluconate. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose (weak). No growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth was observed at 10, 20, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Phayao Province, Mueang District, in bridal bouquet flower (*Plumeria pudica*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU685. GenBank numbers SDBR-CMU685: PV834563 (D1/D2), PV834733 (ITS), PX622359 (SSU), PV947454 (*rpb2*), PV947478 (*tef1-α*).

Notes – *Parajaminaea hydei* SDBR-CMU601 and SDBR-CMU685 were identified as members of *Parajaminaea* and related to *Par. phylloscopi* CBS14087 and *Par. albiziae* CMW 36935 (Fig. 43). The D1/D2 sequences differed by 3.5% nucleotide divergence (18 nt substitutions including 2 gaps), and 8.43% nucleotide divergence (50 nt substitutions including 16 gaps) in the ITS region with *Par. phylloscopi*, while differed from *Par. albiziae* by 3.22% nucleotide divergence (19 nt substitutions including 1 gap) and 8.13% (53 nt substitutions including 17 gaps) in D1/D2 and ITS region, respectively, which indicated that they represent a novel species in *Parajaminaea*. Additionally, *Par. hydei* can be distinguished from *Par. phylloscopi* by its inability to assimilate α-α-trehalose, inulin, succinate and its lack of growth at 35 and 42 °C[109].

Sympodiomycopsis hydei Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 44)

MycoBank number: MB860208

Etymology – '*hydei*' referring to the name of Kevin D. Hyde in honor of his 70th birthday.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak in cape leadwort flower (*Plumbago auriculata*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40112 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU704 = GMBCC2420 = TBRC21415. GenBank numbers PV834567 (D1/D2), PV834737 (ITS), PV941870 (*rpb1*), PV947456 (*rpb2*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.5–0.8 mm in diameter), yellowish white, smooth surface, glistening appearance, entire margin, and convex elevation. The cells are ovoid to obclavate (2.04–3.52 × 3.4–6.68 μm, *n* = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, ribose (or weak), L-arabinose, sucrose, maltose (or weak), methyl-α-D-glucoside, melibiose (or slow), raffinose, melizitose, glycerol, erythritol (or slow), ribitol, D-glucitol, mannitol, and xylitol (or weak) are assimilated, but inulin, soluble starch, lactose, cellobiose, salicin, *N*-acetyl glucosamine, xylose, D-arabinose, L-rhamnose, α-α-trehalose, galactitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, and L-lysine are assimilated as sole nitrogen source, but ethylamine and cadaverine are not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose (weak). No growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth was observed at 10, 20, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in ylang-ylang tree flower (*Cananga odorata*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU694; Mueang District, Chang Phueak, in marguerite daisy flower (*Argyranthemum frutescens*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU717; San Kamphaeng District, Ton Pao, in flosreginae flower (*Lagerstroemia speciosa*), September 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU718. GenBank numbers SDBR-CMU694: PV834566 (D1/D2), PV834736 (ITS); SDBR-CMU717: PV834568 (D1/D2), PV834738 (ITS); SDBR-CMU718: PV834569 (D1/D2), PV834739 (ITS).

Notes – Based on phylogenetic analysis (Fig. 43), *Sym. hydei* (SDBR-CMU704, SDBR-CMU694, SDBR-CMU717, and SDBR-CMU718) formed a separate lineage and clustered as sister to *Sym. europaea*, *Sym. kandeliae*, *Sym. limtongiae*, *Sym. paphiopedili*, and *Sym. yantaiensis*, However, *Sym. hydei* differed from those species by 0.67%–2.10% nucleotide divergence (4–13 nt substitutions including gaps) in the D1/D2 domain and 6.55%–7.91% mismatches (39–51 nt substitutions including 12–20 gaps) in the ITS regions. Furthermore, *Sym. hydei* can be distinguished from those species in its physiological characteristics (Table 13).

Sympodiomycopsis limtongiae Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 45)

MycoBank number: MB860209

Etymology – '*limtongiae*' named in honor of mycologist Savitree Limtong, for her contributions to yeast systematics.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Suthep, in white cheese wood flower (*Alstonia scholaris*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla and N. Suwannarach, holotype, CMUB40115 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU728 = GMBCC2421 = TBRC21418. GenBank numbers PV834570 (D1/D2), PV834740 (ITS), PV941872 (*rpb1*), PV947455 (*rpb2*), PV947476 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.7–1.2 mm in diameter), white glistening appearance,

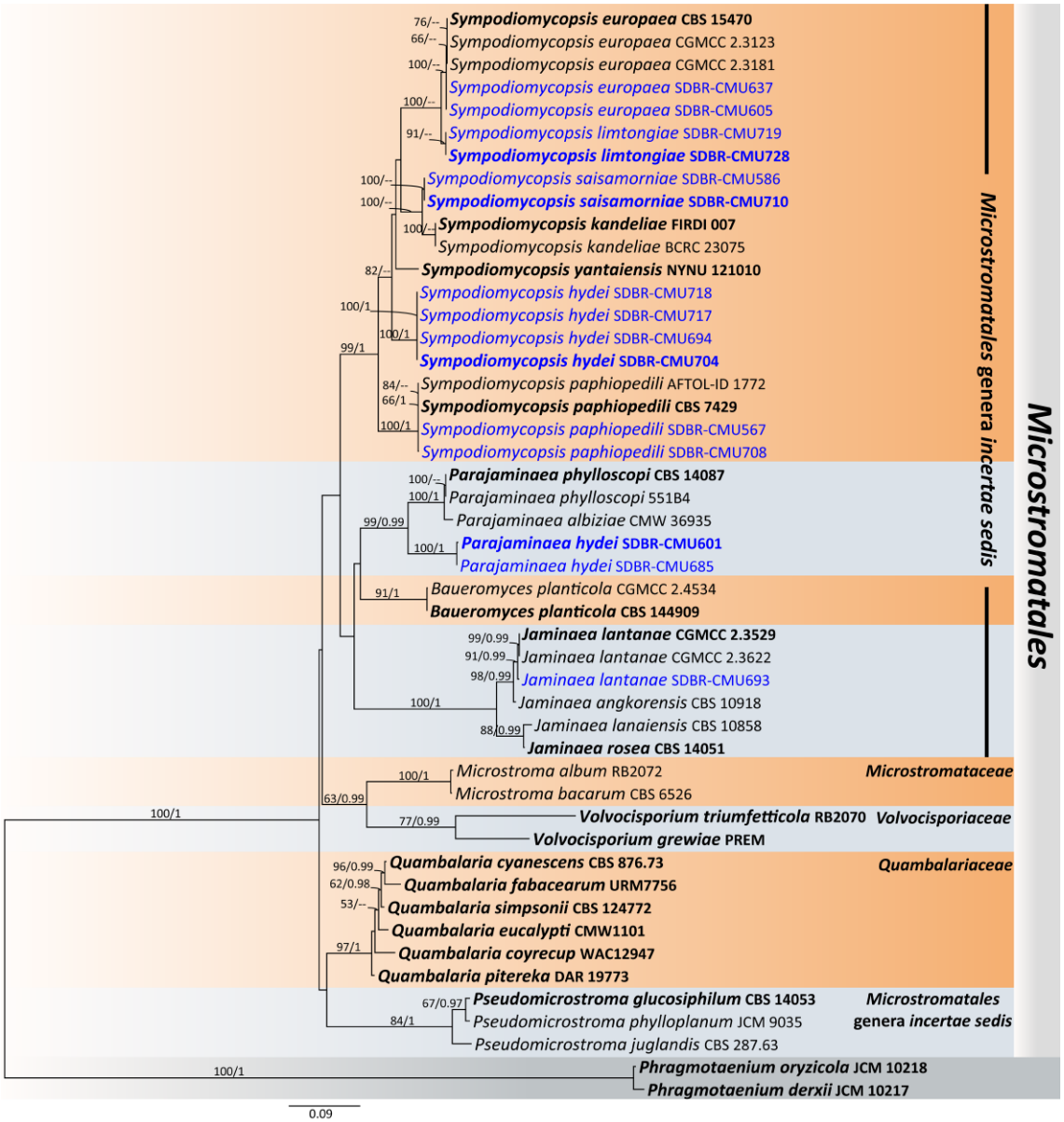

Fig. 43 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Microstromatales*. The tree is rooted to *Phragmotaenium derxii* (JCM 10217) and *P. oryzicola* (JCM 10218). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Fourty-eight strains are included in the combined sequence analysis, which comprise 1,326 characters with gaps. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.95 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.95 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

and eroded margins. The cells are ovoid to obclavate (2.05–3.42 × 3.69–6.43 μm, n = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose (slow), ribose, xylose, L-arabinose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, melibiose (slow), lactose (slow), raffinose, melizitose, glycerol, erythritol, D-mannitol, D-glucono-1,5-lactone (weak), DL-lactate, succinate, and xylitol are assimilated, but *N*-acetyl glucosamine, D-arabinose, L-rhamnose, cellobiose, salicin, inulin, soluble starch, ribitol, D-glucitol, galactitol, *myo*-inositol, D-gluconate, D-glucuronate, D-galacturonic acid, citrate, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, and L-lysine are assimilated as sole nitrogen source, but ethylamine and cadaverine are not assimilated. Growth occurs on media containing 50% glucose, 60% glucose, and 10% NaCl/5% glucose. No growth occurs on media containing 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth on 10, 15, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, San Kamphaeng District, Ton Pao, in flosreginae flower (*Lagerstroemia speciosa*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU719. GenBank numbers PV834571 (D1/D2), PV834741 (ITS).

Notes – *Sympodiomycopsis limtongiae* SDBR-CMU728 and SDBR-CMU719 were identified as members of *Sympodiomycopsis* and

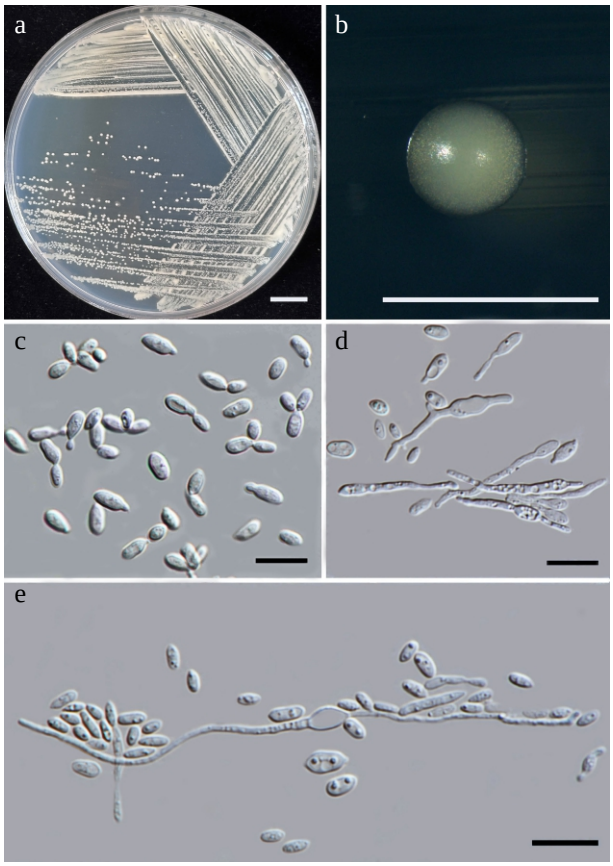

Fig. 44 Morphological characteristics of *Sympodiomycopsis hydei* (SDBR-CMU704, ex-type). (a) Culture, (b) single colony, and (c) cells on YMA at 25 °C for 5 d. (d), (e) Pseudohyphae on PDA after 2 weeks at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, and (c)–(e) = 10 µm.

related to *Sym. europaea* CBS 15470, CGMCC 2.3123, and CGMCC 2.3181 (Fig. 43). A comparison of the D1/D2 and ITS sequences revealed that *Sympodiomycopsis limtongiae* differed from the latter by 0.48% nucleotide divergence (2 nt substitutions) and 1.96% (13 nt substitutions and 1 gap), respectively. In addition, *Sym. limtongiae* is different from *Sym. europaea* by 7.74%, 6.11%, and 2.08% nucleotide divergence (58, 66, and 18 nt substitutions) in *rpb1*, *rpb2*, and *tef1-α*, respectively. Likewise, distinguishing characteristics between *Sym. limtongiae* and *Sym. europaea*[92] are shown in Table 12.

Sympodiomycopsis saisamorniae Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 46)

MycoBank number: MB860210

Etymology – '*saisamorniae*' referring to the name of mycologist Saisamorm Lumyong, in honor of her 75th birthday.

Holotype – THAILAND, Chiang Mai Province, San Kamphaeng District, Ton Pao, in Indian mulberry flower (*Morinda citrifolia*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40113 (preserved in metabolically inactive state), ex-type living SDBR-CMU710 = GMBCC2422 = TBRC21416. GenBank numbers PV834574 (D1/D2), PV834744 (ITS), PX622360 (SSU), PX582306 (*rpb1*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.8–1.2 mm in diameter), pale orange, smooth surface, glistening appearance, circular shape, entire margin, and convex elevation. The cells are ovoid to obclavate (2.01–3.78 × 3.61–7.22 µm, *n* = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, sorbose, ribose (weak), xylose (weak), L-arabinose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, raffinose, melizitose, glycerol, erythritol (weak), and D-mannitol (weak) are assimilated, but galactose, *N*-acetyl glucosamine, D-arabinose, L-rhamnose, cellobiose, salicin, melibiose inulin, lactose, soluble starch, ribitol, D-glucitol, galactitol, *myo*-inositol, D-glucono-1,5-lactone (weak), D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, methanol, ethanol, and xylitol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine (weak), and L-lysine (weak) are assimilated as sole nitrogen source but and cadaverine are not assimilated. Growth occurs on media containing 50% glucose 60% glucose. No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Urease reaction is positive. Acid formation is negative. Growth was observed at 10, 20, 25, 30 °C but not at 35, 37, 40 °C.

Table 13. Phenotypic characteristics differentiating *Sym. hydei*, *Sym. limtongiae*, and *Sym. saisamorniae* from closely related *Sympodiomycopsis* species.

Characteristics	1	2	3	4	5	6	7
Carbon assimilation							
D-Glucose	+	+	+	+	+	+	w
D-Galactose	w	+	–	+	+/s	+	w
L-Sorbose	+	s/+	+	+	+	+	–
D-Arabinose	–	–	–	–	+/s	+	–
α-α-Trehalose	+	v	+	w	+	+	+
Cellobiose	–	–	–	–	+/s	+	–
Salicin	–	–	–	–	w	w	–
Melibiose	w	s	–	+	v	+	–
Lactose	–	v	–	+	+/s	+	–
Inulin	–	–	–	–	w/–	–	–
Soluble starch	–	–	–	+	w/–	–	–
Erythritol	w	w/+	w	+	+	+	+
Ribitol	-	v	–	–	v	+	–
D-Glucitol	w	v	–	+/w	+	+	w
D-Mannitol	+	+	w	+	+	+	+
Galactitol	–	–	–	–	w	–	–
myo-Inositol	–	–	–	w	w/–	+	–
D-Gluconate	v	–	–	nd	s/w	nd	+
Succinate	+	v	–	nd	w	+	+
Citrate	–	–	–	nd	w	+	+
Ethanol	–	–	–	+	s	+	+
Xylitol	–	w/+	–	nd	v	nd	–
Nitrogen assimilation							
Potassium nitrate	+	+	+	v	w	+	+
Sodium nitrite	+	+	+	–	v	+	–
Ethylamine HCl	–	–	w	–	w	+	–
L-Lysine	+	+	w	+	w	–	–
Cadaverine	–	–	–	+	nd	–	–
Growth characteristics							
Growth at 25 °C	+	+	+	+	+	+	+
Growth at 30 °C	+	+	+	–	+	+	+
Growth in 0.01% Cycloheximide	–	–	–	nd	+	nd	+
Growth on 50% Glucose	+	+	+	–	nd	+	+
Growth on 60% Glucose	+	+	+	nd	nd	nd	–
Growth on 10% NaCl + 5% glucose	w	+	–	nd	nd	nd	+

Strian 1: *Sym. hydei* sp. nov, 2: *Sym. limtongiae* sp. nov, 3: *Sym. saisamorniae* sp. nov, 4: *Sym. europaea*[92], 5: *Sym. kandeliae*[110], 6: *Sym. paphiopedili*[111], and 7: *Sym. yantaiensis*[112].

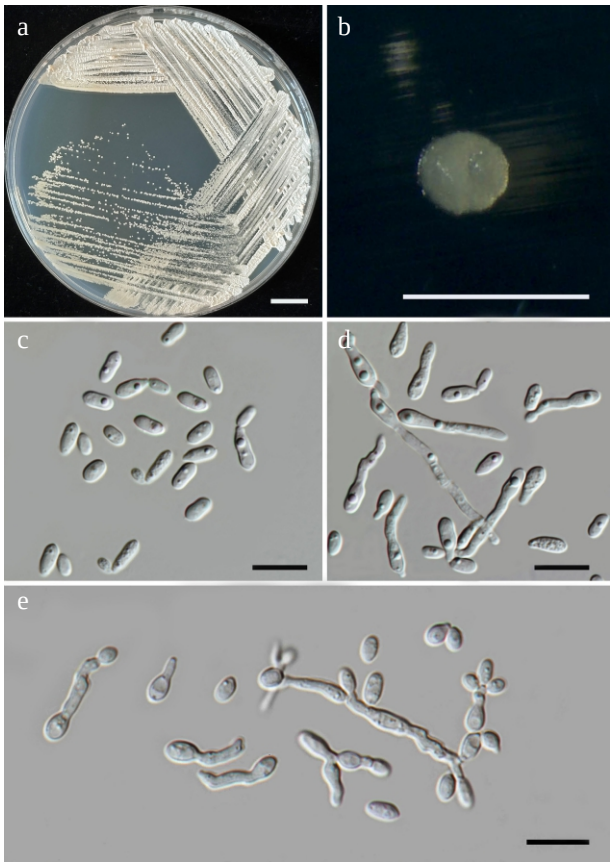

Fig. 45 Morphological characteristics of *Sympodiomycopsis limtongiae* (SDBR-CMU728, ex-type). (a) Culture, (b) single colony, and (c) cells on YMA at 25 °C for 5 d. (d), (e) Pseudohyphae on PDA after 2 weeks at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, and (c)–(e) = 10 μm.

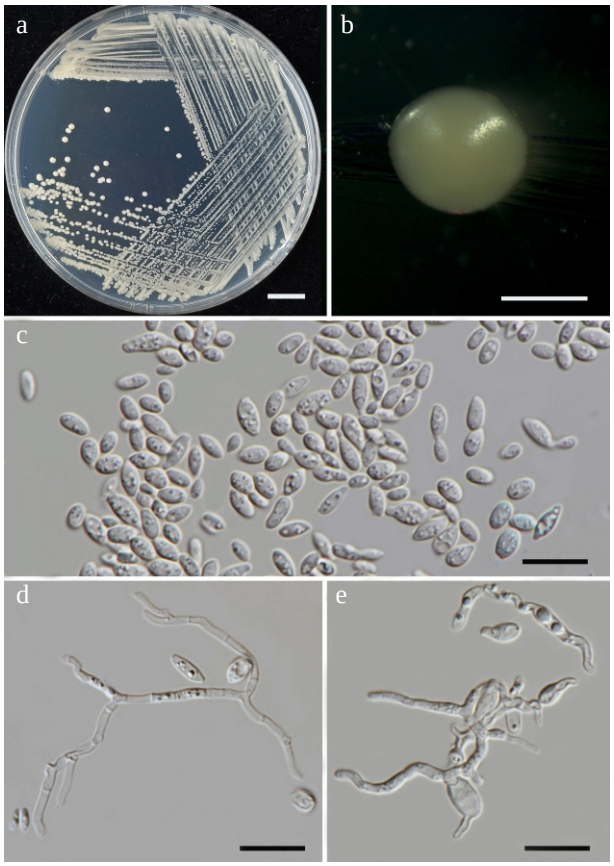

Fig. 46 Morphological characteristics of *Sympodiomycopsis saisamorniae* (SDBR-CMU710, ex-type). (a) Culture, (b) single colony, (c) cells and budding cells on YMA at 25 °C for 5 d. (d), (e) True hyphae and pseudohyphae on PDA after 2 weeks at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c)–(e) = 10 μm.

Additional strains examined – THAILAND, Chiang Mai Province, Phrao District, Nam Phrae, in kaempfer flower (*Boesenbergia rotunda*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU586. GenBank numbers PV834575 (D1/D2), PV834745 (ITS), PX622361 (SSU), PX582307 (*rpb1*).

Notes – Two strains of *Sym. saisamorniae* (SDBR-CMU710 and SDBR-CMU586) introduced as a new member of *Sympodiomycopsis*, formed a distinct lineage, closely related to *Sym. kandeliae* (Fig. 43). *Sympodiomycopsis saisamorniae* different from *Sym. kandeliae* by 0.5% nucleotide divergence (3 nt substitutions) in D1/D2, 2% (13 nt substitutions) in the ITS regions, and 11.40% nucleotide divergence (80 nt substitutions and 21 gaps) in *rpb1*. Moreover, *Sym. saisamorniae* can be distinguished from *Sym. kandeliae* in its physiological tests (Table 13).

Class: Ustilaginomycetes Warm.

Order: *Ustilaginales* Bek.

Family: *Ustilaginaceae* Tul. & C. Tul.

Members of *Ustilaginaceae* are facultative parasites that have a yeast-like or filamentous saprophytic phase, which causes smut in corn, wheat, and grass crops. In biotechnology, *Ustilaginaceae* have the potential applications in the food, pharmaceutical, and chemical industry with substances glycolipids, organic acids, and polyols[113–115]. Almost all *Ustilaginaceae* species have a dimorphic life cycle that includes an asexual, saprophytic yeast-like stage and a filamentous sexual stage, which is required to parasitize a host. The aerial mycelium consists of ramifying, acropetal chains of fusiform conidia. Chlamydospores may be present, but ballistoconidia are absent[92,116]. Currently, 25 genera are listed in this family including *Ahmadiago*, *Aizoago*, *Anomalomyces*, *Anthracocystis*, *Bambusiomyces*, *Centrolepidosporium*, *Dirkmeia*, *Eriocaulago*, *Eriosporium*, *Franzpetrakia*, *Kalmanozyma*, *Langdonia*, *Macalpinomyces*, *Melanopsichium*, *Moesziomyces*, *Parvulago*, *Pattersoniomyces*, *Pseudozyma*, *Shivasia*, *Sporisorium*, *Stollia*, *Tranzscheliella*, *Triodiomyces*, *Ustilago*, and *Yunchangia*[41]. In this study, *Anthracocystis heteropogonicola* (two strains), *Moesziomyces antarcticus* (four strains), *Mo. bullatus* (five strains), *Mo. parantarcticus* (four strains), *Pseudozyma chiangmaiensis* sp. nov. (seven strains), *Ps. lannaensis* sp. nov. (five strains), *Ps. limtongiae* sp. nov. (four strains), *Ps. pollinicola* sp. nov. (four strains), and *Ps. saisamorniae* sp. nov. (eight strains) are presented (Fig. 48, Supplementary File 1)

Pseudozyma chiangmaiensis Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 47)

MycoBank number: MB860211

Etymology – '*chiangmaiensis*' referring to Chiang Mai Province, where the new species was found.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Suthep, in Fukien tea flower (*Carmona retusa*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40082 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU611 = GMBCC2423 = TBRC21397. GenBank numbers PV834594 (D1/D2), PV834764 (ITS), PX622365 (SSU), PX582308 (*rpb1*), PX582346 (*tef1-α*).

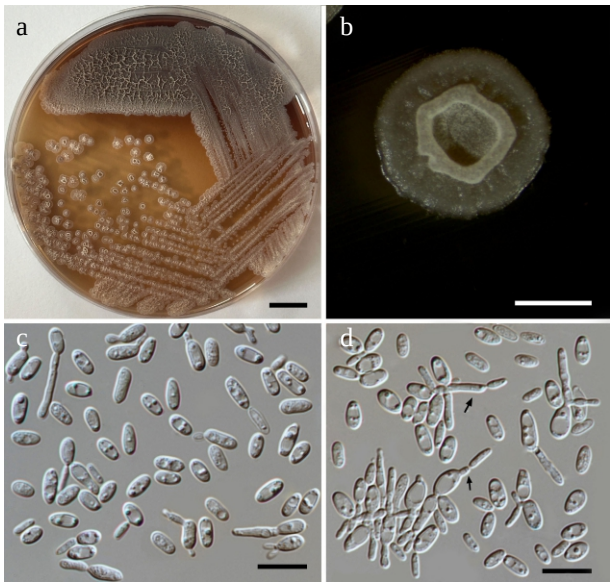

Fig. 47 Morphological characteristics of *Pseudozyma chiangmaiensis* (SDBR-CMU611, ex-type). (a) Culture, (b) single colony, (c), (d) cells rudimentary pseudohyphae (indicated by arrows) on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 µm.

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (1.8–3.0 mm in diameter), yellowish white, umbonate, zonate towards the margin, and with the margin fringed and producing brown pigment into agar. The cells are ovoid to ellipsoidal (2.04–3.25 × 4.31–8.15 µm, *n* = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained for individual strain and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, *N*-acetyl glucosamine, ribose, xylose, L-arabinose, D-arabinose, sucrose, maltose, *α*-*α*-trehalose, methyl-*α*-D-glucoside, cellobiose (weak), melibiose, lactose, raffinose, melizitose, glycerol, erythritol, ribitol, D-glucitol, mannitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate (weak), succinate (weak), citrate, and xylitol are assimilated, but L-rhamnose, salicin, inulin, soluble starch, galactitol, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine, L-lysine, and cadaverine are all assimilated as sole nitrogen source. Growth occurs on media containing 50% glucose and 60% glucose (weak). No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth is present at 10, 15, 25, and 30 °C, but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in Persian gentian flower (*Exacum affine*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU581; confederate vine flower (*Antigonon leptopus*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU596; Indian cork flower (*Millingtonia hortensis*), September 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU725; Suthep, in Ixora flower (*Ixora chinensis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU587; Phrao District, Nam Phrae, in ylang-ylang tree flower (*Cananga odorata*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU648; canna lily flower (*Canna indica*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU682. GenBank numbers SDBR-CMU581: PV834591 (D1/D2), PV834761 (ITS), PX622362 (SSU); SDBR-CMU587: PV834592 (D1/D2), PV834762 (ITS), PX622363 (SSU); SDBR-CMU596: PV834593 (D1/D2), PV834763 (ITS), PX622364 (SSU); SDBR-CMU648: PV834595 (D1/D2), PV834765 (ITS), PX622366 (SSU); SDBR-CMU682: PV834596 (D1/D2), PV834766 (ITS), PX622367 (SSU); SDBR-CMU725: PV834597 (D1/D2), PV834767 (ITS), PX622368 (SSU).

Notes – *Pseudozyma* was introduced by Bandoni, based on the type species, *Pseudozyma prolifica*[117]. Currently the genus comprises 13 epithets; however, only eight epithets are supported by molecular data[47]. Due to their uncertain phylogenetic positions, the taxonomic placement of some *Pseudozyma* species remains unresolved. Thus, the term 'pro tempore (pro tem.)' was purposed to denote single-species lineages that are temporarily maintained, viz. *Ps. alboarmeniaca pro tem.*, *Ps. thailandica pro tem.*, *Ps. hubeiensis pro tem.*, and *Ps. pruni pro tem.*[65]. In a BLASTn search of NCBI GenBank, the closest match of the D1/D2 domain and ITS sequences of *Pseudozyma* strains isolated in this study was *P. hubeiensis* with 98%–100% and 96%–100% similarity, respectively. Based on a concatenated D1/D2 domain and ITS sequence dataset, these strains clearly formed five distinct subclades, each corresponding to a separate species (Fig. 48, Supplementary File 1). A comparison of D1/D2 and ITS sequences among the species indicates that each species differs from the others (Table 14). Likewise, they can also be distinguished by colony characteristics, cells, and pseudohypha characters (Figs 47, 49–52). Although many strains on various substrates worldwide have been identified as *Ps. hubeiensis*, molecular evidence suggests that certain strains (i.e., AD_L20, AD_L22, DMic 154889, PYCC 9351, and Py1C; Fig. 48) may represent separate species. These strains are tentatively named as *Ps. hubeiensis* until more collections and sequence data clarify their taxonomic status. Additionally, phenotypic differences between *Ps. chiangmaiensis*, *Ps. lannaensis*, *Ps. limtongiae*, *Ps. pollinicola*, *Ps. saisamorniae*, and *Ps. hubeiensis* are shown in Table 15.

Phylogenetic analysis (Fig. 48) shows that *Ps. chiangmaiensis* is sister to *Ps. lannaensis*. *Pseudozyma chiangmaiensis* is similar to *Ps. lannaensis* in having ovoid to ellipsoidal cells (Figs 47, 49). Budding cells of *Ps. chiangmaiensis* are polar, while *Ps. lannaensis* are polar on short stalk. Furthermore, *Ps. chiangmaiensis* forms colonies with umbonate elevation and produces a brown pigment diffusing into the agar. Whereas *Ps. lannaensis* forms colonies with umbonate elevation and filamentous margins. Physiologically, *Ps. chiangmaiensis* differs from *Ps. lannaensis* and *Ps. saisamorniae* in its ability to utilize gluconate. Compared to *Ps. pollinicola*, it shows a different ability to assimilate ribitol. Additionally, *Ps. chiangmaiensis* differs from *Ps. hubeiensis* in its ability to utilize sorbose, *myo*-inositol, and citrate, whereas *Ps. hubeiensis* cannot grow on these substrates. Moreover, *Ps. chiangmaiensis* is capable of growing in the presence of 50% glucose, while *Ps. hubeiensis* cannot grow[65] (Table 15).

Pseudozyma lannaensis Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 49)

MycoBank number: MB860212

Etymology – '*lannaensis*' referring to the Kingdom of Lanna, the historic name of northern Thailand, where the new species was found.

Holotype – THAILAND, Phayao Province, Mueang District, in vinca flower (*Catharanthus roseus*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40084 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU661 = GMBCC2424 = TBRC21406. GenBank numbers

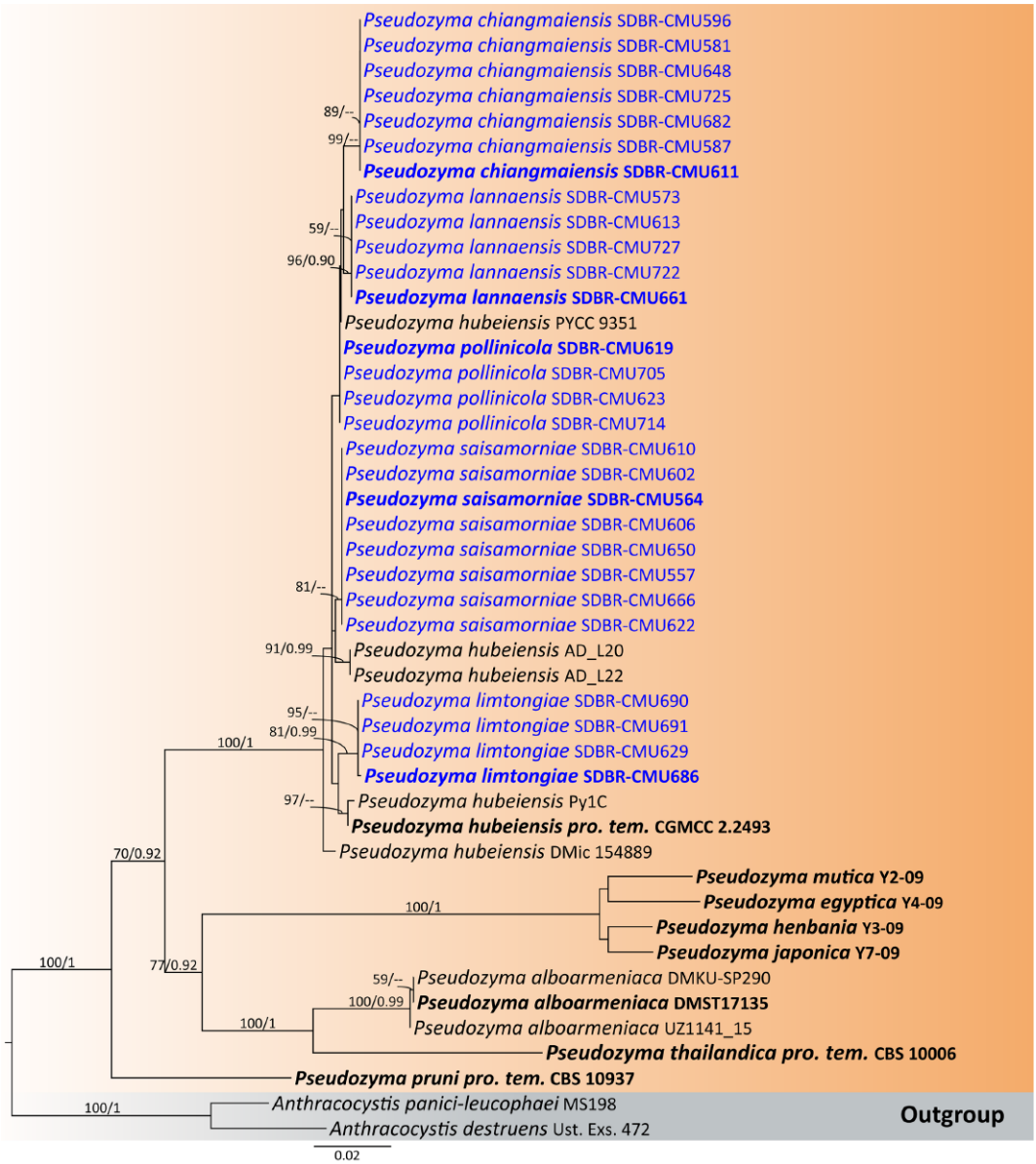

Fig. 48 Phylogenetic tree generated by maximum likelihood analysis of the combined D1/D2 domain of LSU and ITS sequence data representing *Pseudozyma*. The tree is rooted to *Anthracocystis destruens* (Ust. Exs. 472) and *A. panici-leucophaei* (MS198). Single-locus analyses were also performed, and topology and clade stability were compared from combined gene analyses. Fourty-five strains are included in the combined sequence analysis, which comprise 1,704 characters with gaps. The average standard deviation of the split frequencies of the BI analysis was 0.004541. Bootstrap support values for maximum likelihood ≥ 50% (ML, left) and Bayesian posterior probabilities ≥ 0.90 (PP, right) are indicated above the node. Double dashes (--) represent support values less than 50% ML/0.90 PP. The scale bar represents the expected number of nucleotide substitutions per site. The ex-type strains are in bold, and the newly generated sequences in this study are in blue.

Table 14. Percentage of nucleotide divergence in the ITS region among the species most closely related to *Pseudozyma hubeiensis*.

Taxa	*Ps. chiangmaiensis*	*Ps. lannaensis*	*Ps. limtongiae*	*Ps. pollinicola*	*Ps. saisamorniae*	*Ps. hubeiensis*
Ps. chiangmaiensis	–	1.20% (9 nt)	3.28% (25 nt)	1.07% (8 nt)	1.98% (15 nt)	2.73% (19 nt)
Ps. lannaensis	1.20% (9 nt)	–	3.27% (25 nt)	1.07% (8 nt)	1.19% (19 nt)	1.87% (13 nt)
Ps. limtongiae	3.28% (25 nt)	3.13% (25 nt)	–	2.62% (20 nt)	2.48% (19 nt)	2.97% (21 nt)
Ps. pollinicola	1.07% (8 nt)	1.07% (8 nt)	2.62% (20 nt)	–	1.72% (13 nt)	1.87% (13 nt)
Ps. saisamorniae	1.98% (15 nt)	2.51% (19 nt)	2.48% (19 nt)	1.72% (13 nt)	–	2.29% (16 nt)
Ps. hubeiensis	2.73% (19 nt)	1.87% (13 nt)	3.03% (21 nt)	1.87% (13 nt)	2.29% (16 nt)	–

Each value indicates that nucleotide divergence (%) between pairs of species, the number of nucleotide substitutions (nt) between the indicated species pairs.

PV834600 (D1/D2), PV834770 (ITS), PX622371 (SSU), PX582309 (*rpb1*), PX582330 (*rpb2*), PX582347 (*tef1-α*).

Description – The culture on YMA after 5 days at 25 °C, colonies are circular from (0.8–1.5 mm in diameter), pale brownish-yellow or brownish, initially dull, smooth with entire margin, becoming umbonate with undulate and filamentous margins. The cells are ovoid and ellipsoidal (2.02–3.55 × 4.65–9.12 μm, $n = 50$), occur singly or in pairs. Budding is polar on a short stalk. Ballistoconidia were not

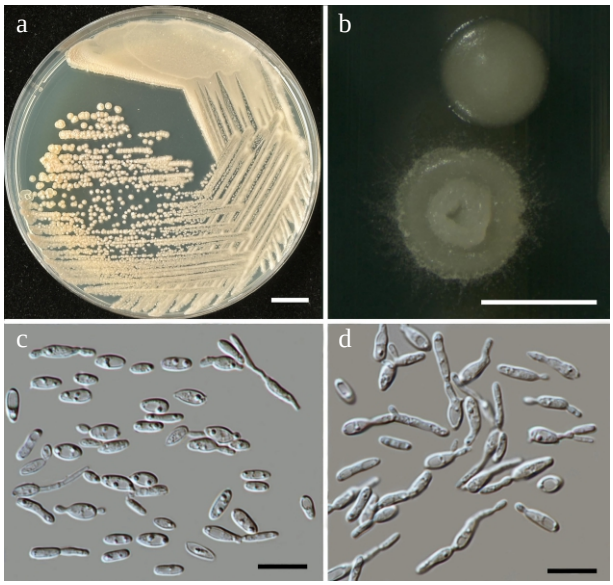

Fig. 49 Morphological characteristics of *Pseudozyma lannaensis* (SDBR-CMU661, ex-type). (a) Culture, (b) single colony, (c) cells, and (d) rudimentary pseudohyphae on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, *N*-acetyl glucosamine, ribose, xylose, L-arabinose (weak), D-arabinose (weak), sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose (weak), salicin (weak), melibiose, lactose, raffinose, melizitose, glycerol (weak), erythritol (weak), ribitol (weak), D-glucitol, mannitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-galacturonic acid (weak), DL-lactate, succinate, citrate, and xylitol are assimilated, but L-rhamnose, inulin, soluble starch, galactitol, D-glucuronate, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine, L-lysine, and cadaverine are all assimilated as sole nitrogen source. Growth occurs on media containing 50% glucose and 60% glucose (weak). No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth is present at 10, 15, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in ground cherry flower (*Physalis minima*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU573; Suthep, in White cheesewood flower (*Alstonia scholaris*), September 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU727; Mae Hia, in yellow trumpetbush flower (*Tecoma stans*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU613; San Kamphaeng District, Ton Pao, in Queen's flower (*Lagerstroemia speciosa*), September 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU722. GenBank numbers SDBR-CMU573: PV834598 (D1/D2), PV834768 (ITS), PX622369 (SSU); SDBR-CMU613: PV834599 (D1/D2), PV834769 (ITS), PX622370 (SSU); SDBR-CMU722: PV834601 (D1/D2), PV834771 (ITS), PX622372 (SSU); SDBR-CMU727: PV834602 (D1/D2), PV834772 (ITS), PX622373 (SSU).

Notes – The new species, *Pseudozyma lannaensis* is similar to *Ps. saisamorniae* in having ovoid and ellipsoidal cells that formed polar budding on short stalk. *Pseudozyma lannaensis* forms colonies with umbonate elevation and filamentous margins, while *Ps. saisamorniae* formed wrinkled colonies when mature. In addition, cells of *Ps. lannaensis* are shorter than the latter (2.02–3.55 × 4.65–9.12 μm vs 2.2–3.31 × 4.43–7.83 μm). Physiologically, *Ps. lannaensis* differs from *Ps. hubeiensis* in its ability to utilize sorbose, *myo*-inositol, lactate and citrate, whereas *Ps. hubeiensis* cannot grow on these substrates. Moreover, *Ps. lannaensis* was able to grow on 50% glucose, while *Ps. hubeiensis* could not grow at that medium[65]. Compared to *Ps. chiangmaiensis*, *Ps. limtongiae*, and *Ps. pollinicola*, them shows different ability to assimilate gluconate (Table 15).

Pseudozyma limtongiae Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 50)

MycoBank number: MB860213

Etymology – '*limtongiae*' named in honor of mycologist Savitree Limtong, for her contributions to yeast systematics.

Holotype – THAILAND, Phayao Province, Mueang District, in bridal bouquet flower (*Plumeria pudica*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40085 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU686 = GMBCC2425 = TBRC21412. GenBank numbers PV834616 (D1/D2), PV834786 (ITS), PX622383 (SSU), PX582312 (*rpb1*), PX582333 (*rpb2*), PX582350 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, colonies are circular form (0.8–1.5 mm in diameter), yellowish white, initial smooth, glistening, convex with entire margin, becoming crateriform when mature. The cells are fusiform or cylindrical (1.32–2.87 × 4.33–9.15 μm, *n* = 50), occur singly or in pairs. Budding is polar on short stalks. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained for

Table 15. Phenotypic characteristics differentiating *Pseudozyma chiangmaiensis*, *Ps. lannaensis*, *Ps. pollinicola*, *Ps. limtongiae*, and *Ps. saisamorniae* from closely related *Pseudozyma* species.

Characteristics		1	2	3	4	5	6
Carbon assimilation	L-Sorbose	+	+	+	+	+	–
	N-Acetyl glucosamine	+	+	+	w	+	+
	L-Arabinose	+	w	+	+	+	+
	D-Arabinose	+	w	+	+	+	+
	Cellobiose	+	w	+	w	w	+
	Salicin	w	w	+	w	–	–
	Soluble starch	–	–	–	–	–	+/w
	Glycerol	+	w	+	+	–	+
	Erythritol	+	w	+	w	–	+
	Ribitol	+	w	+	–	w	+
	D-Glucitol	+	+	+	+	w	+
	D-Mannitol	+	+	+	+	w	+
	myo-Inositol	+	+	+	+	+	–
	D-Gluconate	+	–	+	w	–	nd
	D-Galacturonic acid	+	+	w	w	+	nd
	DL-Lactate	w	+	+	w	+	–
	Succinate	w	+	+	+	+	+
	Citrate	+	+	+	+	+	–
Nitrogen assimilation	Sodium nitrite	+	+	+	w	+	+
	Ethylamine HCl	+	+	+	w	+	+
Growth characteristics	Growth at 35 °C	–	w	–	–	–	nd
	Growth at 37 °C	–	w	–	–	–	+
	Growth on 50% Glucose	+	+	+	+	w	–

Strains 1: *Ps. chiangmaiensis* sp. nov., 2: *Ps. lannaensis*, 3: *Ps. limtongiae*, 4: *Ps. pollinicola*, 5: *Ps. saisamorniae*, and 6. *Ps. hubeiensis*[65].

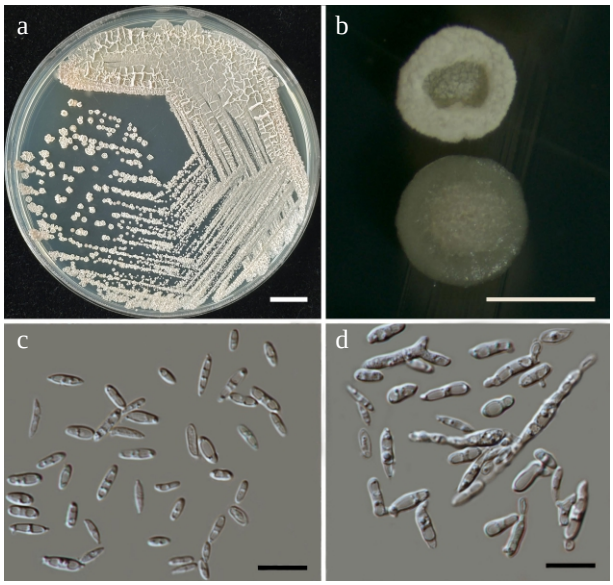

Fig. 50 Morphological characteristics of *Pseudozyma limtongiae* (SDBR-CMU686, ex-type). (a) Culture, (b) single colony, (c) cells, and (d) rudimentary pseudohyphae on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, *N*-acetyl glucosamine, ribose, xylose, L-arabinose, D-arabinose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose, melizitose, glycerol, erythritol, ribitol, D-glucitol, mannitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate, D-galacturonic acid (weak), DL-lactate, succinate, citrate, and xylitol are assimilated, but L-rhamnose, inulin, soluble starch, galactitol, D-glucuronate, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine, L-lysine and cadaverine are all assimilated as sole nitrogen source. Growth occurs on media containing 50% glucose and 60% glucose (weak). No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide and 0.1% cycloheximide. Acid formation is negative. Growth is present at 10, 15, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – THAILAND, Phayao Province, Mueang District, in bridal bouquet flower (*Plumeria pudica*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU690 and SDBR-CMU691; Chiang Mai Province, Mueang District, Suthep, in teak flower (*Tectona grandis*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU629. GenBank numbers SDBR-CMU629: PV834615 (D1/D2), PV834785 (ITS), PX622382 (SSU); SDBR-CMU690: PV834617 (D1/D2), PV834787 (ITS), PX622384 (SSU); SDBR-CMU691: PV834618 (D1/D2), PV834788 (ITS), PX622385 (SSU).

Notes – *Pseudozyma limtongiae* shares morphological similarity with *Ps. hubeiensis* (CGMCC 2.2493) in having cylindrical with polar budding on a short stalk[118]. However, they can be distinguished by their phylogenetic relationships, colony morphology and phenotypic characteristics. Physiologically, *Ps. limtongiae* differs from *Ps. hubeiensis* in its ability to utilize sorbose, salicin, *myo*-inositol, lactate and citrate, whereas *Ps. hubeiensis* cannot assimilated on these substrates. Additionally, *Ps. limtongiae* was able to grow on 50% glucose, while *Ps. hubeiensis* could not grow at that medium[65]. Compared to *Ps. saisamorniae*, that *Ps. limtongiae* ability to utilize glycerol and erythritol but *Ps. saisamorniae* cannot assimilate (Table 15).

Pseudozyma pollinicola Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 51)

MycoBank number: MB860214

Etymology – The specific epithet '*pollinicola*' refers to the substrate origin of the type strain, pollen structure.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in yellow-flowered waterhyssop (*Mecardonia procumbens*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40083 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU619 = GMBCC2426 = TBRC21400. GenBank numbers PV834603 (D1/D2), PV834773 (ITS), PX582310 (*rpb1*), PX582331 (*rpb2*), PX582348 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, the colonies are circular form (0.8–1.2 mm in diameter), brownish-yellow or brownish, butyrous, dull and wrinkled, producing brown pigment in agar. The cells are ellipsoidal to fusoid (1.53–3.14 × 4.29–9.39 μm, n = 50), occur singly or in pairs. Budding is polar. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, *N*-acetyl glucosamine (weak), ribose, xylose, L-arabinose, D-arabinose, sucrose, maltose, α-α-trehalose, methyl-α-D-glucoside, cellobiose (weak), salicin (weak), melibiose, lactose, raffinose, melizitose, glycerol, erythritol (weak), D-glucitol, mannitol, *myo*-inositol, D-glucono-1,5-lactone, D-gluconate (weak), D-glucuronate, D-galacturonic acid (weak), DL-lactate (weak), succinate, citrate, and xylitol are assimilated, but L-rhamnose, inulin, soluble starch, ribitol, galactitol, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine, L-lysine, and cadaverine are all assimilated as sole nitrogen source. Growth occurs on media containing 50% glucose and 60% glucose (weak). No growth occurs on media containing 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is negative. Growth is present at 10, 15, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – Thailand, Chiang Mai Province, Mueang District, Chang Phueak, in jasmine flower (*Jasminum sambac*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU623; cape leadwort (*Plumbago auriculata*) flower, August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU705; marguerite daisy flower (*Argyranthemum frutescens*), September 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU714. GenBank numbers SDBR-CMU623: PV834604 (D1/D2), PV834774 (ITS); SDBR-CMU705: PV834605 (D1/D2), PV834775 (ITS); SDBR-CMU714: PV834606 (D1/D2), PV834776 (ITS).

Notes – *Pseudozyma pollinicola* produces a brown pigment diffusing into the agar similar with *Ps. chiangmaiensis* but can be distinguished by forming round with raise margin colonies and having ellipsoidal to fusoid cells. Moreover, *Ps. pollinicola* can be distinguished from *Ps. hubeiensis* by its ability to assimilate sorbose, *myo*-inositol and citrate as well as by its ability to grow on media containing 50%[65]. Additionally, *Ps. pollinicola* distinguished from *Ps. saisamorniae* by its ability to assimilate salicin and glycerol (Table 15).

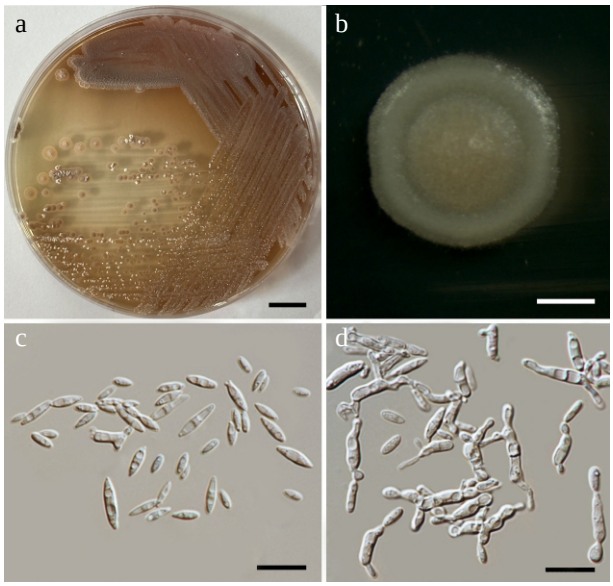

Fig. 51 Morphological characteristics of *Pseudozyma pollinicola* (SDBR-CMU619, ex-type). (a) Culture, (b) single colony, (c) cells, and (d) rudimentary pseudohyphae on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

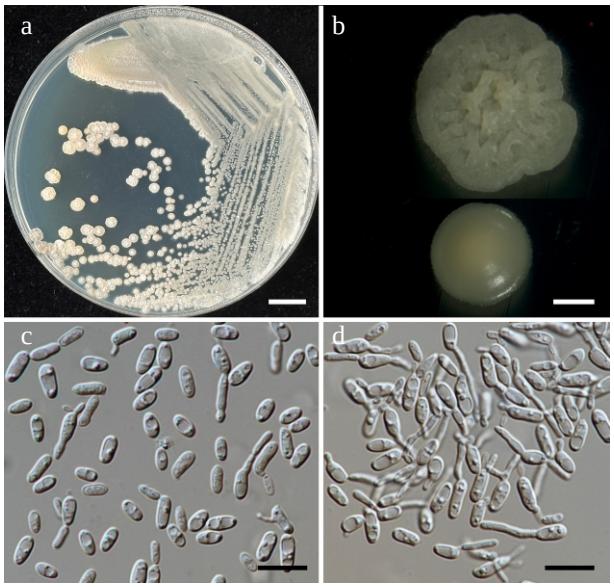

Fig. 52 Morphological characteristics of *Pseudozyma saisamorniae* (SDBR-CMU564, ex-type). (a) Culture, (b) single colony, (c) cells, and (d) rudimentary pseudohyphae on YMA after 5 d at 25 °C. Scale bars: (a) = 10 mm, (b) = 1 mm, (c), (d) = 10 μm.

Pseudozyma saisamorniae Kodchasee, Senwanna, J. Kumla, & N. Suwannar., sp. nov. (Fig. 52)

MycoBank number: MB860215

Etymology – '*saisamorniae*' referring to the name of mycologist Saisamorm Lumyong, in honor of her 75th birthday.

Holotype – THAILAND, Chiang Mai Province, Mueang District, Chang Phueak, in angelonia flower (*Angelonia goyazensis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, holotype, CMUB40116 (preserved in metabolically inactive state), ex-type living culture SDBR-CMU564 = GMBCC2427 = TBRC21386. GenBank numbers PV834608 (D1/D2), PV834778 (ITS), PX622375 (SSU), PX582311 (*rpb1*), PX582332 (*rpb2*), PX582349 (*tef1-α*).

Description – The culture on YMA after 5 d at 25 °C, the colonies are circular form (1.2–3.15 mm in diameter), yellowish white, initially dull, smooth with entire margin, becoming wrinkled with undulate margins. The cells are ovoid and ellipsoidal (2.2–3.31 × 4.43–7.83 μm, *n* = 50), occur singly or in pairs. Budding is polar on a short stalk. Ballistoconidia were not produced. In Dalmau plates after 2 weeks on cornmeal agar and PDA at 25 °C, pseudohyphae and true hyphae are formed. Basidiospores were not obtained for individual strains and strain pairs on YMA, CMA, 5% MEA, PDA, and V8 agar after incubation at 25 °C for one month.

Fermentation of glucose is negative. D-Glucose, galactose, sorbose, *N*-acetyl glucosamine, ribose, xylose, L-arabinose, D-arabinose, sucrose, maltose, *α*-*α*-trehalose, methyl-*α*-D-glucoside, cellobiose (weak), melibiose, lactose, raffinose, melizitose, ribitol (weak), D-glucitol (weak), mannitol (weak), *myo*-inositol, D-glucono-1,5-lactone, D-glucuronate, D-galacturonic acid, DL-lactate, succinate, citrate, and xylitol are assimilated, but L-rhamnose, salicin, inulin, soluble starch, glycerol, erythritol, galactitol, D-gluconate, methanol, and ethanol are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine, L-lysine, and cadaverine are all assimilated as sole nitrogen source. Growth occurs on media containing 50% glucose (weak). No growth occurs on media containing 60% glucose, 10% NaCl/5% glucose, 16% NaCl/5% glucose, 0.01% cycloheximide, and 0.1% cycloheximide. Acid formation is positive (weak). Growth is present at 10, 15, 25, and 30 °C but not at 35, 37, and 40 °C.

Additional strains examined – Thailand, Chiang Mai Province, Mueang District, Chang Phueak, in Orange jasmine flower (*Murraya paniculata*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU557; rose (*Rosa* sp.), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU610; frangipani (*Plumeria obtusa*) flower, July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU602; mealycup sage flower (*Salvia farinacea*) flower, July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU606; Mae Hia, in Siam tulip flower (*Curcuma sessilis*), July 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU622; Phayao Province, Mueang District, in, in holy basil flower (*Ocimum tenuiflorum*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU666; peregrina flower (*Jatropha integerrima*), August 2024, P. Kodchasee, C. Senwanna, J. Kumla, and N. Suwannarach, living culture SDBR-CMU650. GenBank numbers SDBR-CMU557: PV834607 (D1/D2), PV834777 (ITS), PX622374 (SSU); SDBR-CMU602: PV834609 (D1/D2), PV834779 (ITS), PX622376 (SSU); SDBR-CMU606: PV834610 (D1/D2), PV834780 (ITS), PX622377 (SSU); SDBR-CMU610: PV834611 (D1/D2), PV834781 (ITS), PX622378 (SSU); SDBR-CMU622: PV834612 (D1/D2), PV834782 (ITS) PX622379 (SSU); SDBR-CMU650: PV834613 (D1/D2), PV834783 (ITS), PX622380 (SSU); SDBR-CMU666: PV834614 (D1/D2), PV834784 (ITS), PX622381 (SSU).

Notes – *Pseudozyma saisamorniae* can be distinguished from the other new taxa described in this study based on colony characteristics, cell morphology, and phylogenetic analyses, as discussed above. In addition, *Ps. saisamorniae* can be distinguished from *Ps. hubeiensis* with its ability to assimilate sorbose, *myo*-inositol, lactate and citrate, but inability to assimilate soluble, starch glycerol and erythritol. Moreover, its ability to grow on media containing 50%[65]. Compared to *Ps. chiangmaiensis*, *Ps. lannaensis*, *Ps. limtongiae*, and *Ps. pollinicola*, which can utilize salicin, glycerol, and erythritol, *Ps. saisamorniae* cannot assimilate these compounds (Table 15).

Discussion

Yeasts can be isolated from an exceptionally varied range of sources in ecosystem on Earth. Natural environments serve as primary reservoirs, including soil, freshwater and marine systems, air, and plants[23,119–122]. Animal-associated sources are equally important, encompassing the gastrointestinal tracts, skin, and oral cavities of mammals, birds, and insects, as well as various invertebrate hosts[4,123–126]. Traditional fermented foods and beverages represent another major isolation sources, including beer, bread, cheese, kimchi, wine, yogurt, and various cultured products from around the world[26,127–131] Clinical, medical, and industrial environments also offer valuable opportunities for yeast isolation[120,126,132–134]. Moreover, plant-based materials offer rich isolation opportunities from fruits, vegetables, flowers, grains, nectar, plant exudates, and decomposing organic matter[3,6,135–137]. This study, which investigated anthophilous yeasts from flowers in northern Thailand, revealed high diversity, with 187 yeast strains obtained and identified across nine classes, 17 orders, 22 families, 36 genera, and 73 species, including 33 species newly introduced to science. Their identification was based on polyphasic approaches, including morphology, physiology, biochemistry, and muti-locus phylogenetic analysis according to several previous studies[27,60,63,65,81,98]. In addition, some yeast species obtained in this study, including *Curvibasidium chiangmaiensis*, *Cystobasidium thailandicum*, and *Symmetrospora hydei*, could not be distinguished solely using D1/D2 and ITS sequences, therefore, physiological characteristics and additional sequences (SSU, *rpb1*, *rpb2*, and *tef1-α*) were used to clarify their taxonomy from its closet relative. This result was in agreement with previous studies[65,81,138,139] demonstrating that multi-locus sequence data provided a good resolution regarding relationships and resolve species boundaries. This study demonstrates that flowers represent a rich and diverse habitat for anthophilous yeasts, with the potential for the discovery of novel species. Some yeast species obtained in this study have been reported from flowers, such as *Entelexis stigmatis*, *Cyberlindnera fabianii*, *Hannaella pagnoccae*, *Meyerozyma caribbica*, *Metschnikowia koreensis*, *Naganishia diffluens*, *Papiliotrema flavescens*, *Rhodosporidiobolus ruineniae*, *Starmerella etchellsii*, and *Sympodiomycopsis paphiopedi*[3,4,8,9,14,45,140]. The present study reveals a dominance of anthophilous basidiomycetous yeasts (77.54%) over those belonging to Ascomycota (22.46%) with *Pseudozyma* the most frequent basidiomycetous yeast and *Metschnikowia* being the most frequent ascomycetous yeast. Similarly, Han et al.[8] studied the diversity of anthophilous yeasts from wildflowers around Jangseong Lake in Jeollanam-do, located in South Korea, and found that basidiomycetous yeasts were more abundant (78.33%) than ascomycetous yeasts (21.67%), with *Pseudozyma* and *Metschnikowia* being the most frequent basidiomycetous and ascomycetous yeasts, respectively. Canto et al.[3] reported a higher abundance of basidiomycetous yeasts compared to ascomycetous yeasts across a wide diversity of flowers in Mexico with *Ustilago* the most frequent basidiomycetous yeast and *Metschnikowia* being the most frequent ascomycetous yeast. Mittelbach et al.[2] studied the diversity of yeasts in flowers on the island of Tenerife in the eastern Anaga Mountains and found that ascomycetous yeasts were more abundant (58.64%) than basidiomycetous yeasts (41.36%), with *Metschnikowia* being the most frequent ascomycetous yeast and *Cryptococcus* the most frequent basidiomycetous yeast. Additionally, Hyun et al.[141] isolated anthophilous yeasts from wildflowers in Ulleungdo and Yokjido, Gyeongsangbuk-do, South Korea, and showed different ratios of yeast numbers between ascomycetous and basidiomycetous yeasts. In Ulleungdo, they found that ascomycetous yeasts were more abundant (62.50%) than basidiomycetous yeasts (37.50%), while in Yokjido, they found that basidiomycetous yeasts were more abundant (60%) than ascomycetous yeasts (40%). *Rhodotorula* is the most abundant basidiomycetous genus found in both Ulleungdo and Yokjido, whereas the most abundant ascomycetous genera found in Ulleungdo and Yokjido were *Pichia* and *Metschnikowia*, respectively. However, Pozo et al.[7] investigated the yeast communities in flower nectar from Cazorla-Segura-Las Villas Natural Park, southeastern Spain, and found that ascomycetous yeasts were more abundant (92.59%) than basidiomycetous yeasts (7.14%), with *Metschnikowia* being the most frequent ascomycetous yeast and *Rhodotorula* being the most frequent basidiomycetous yeast. Kanpiengjai et al.[9] isolated anthophilous yeasts from the flowers of *Camellia sinensis* var. *assamica* in northern Thailand and found that ascomycetous yeasts (82.93%) were significantly more abundant than basidiomycetous yeasts (17.07%), with *Pseudozyma* being the most frequent basidiomycetous yeast and *Metschnikowia* being the most frequent ascomycetous yeast.

The diversity of anthophilous yeasts is influenced by multiple interrelated factors. Canto et al.[3] suggest that the primary factor influencing yeast community composition is the specific interaction between yeasts and host plants, as different flowers offer unique habitats and resources such as varying nectar compositions and floral structures. Lachance[142] found that only ascomycetes from the order *Metschnikowiaceae* are related to anthophilous yeasts due to their ability to utilize sucrose, the main component of nectar. Mittelbach et al.[2] found that ascomycetous yeasts tend to inhabit sucrose-dominant nectars, whereas basidiomycetous yeasts are more commonly associated with nectars rich in monosaccharides, reflecting the variation in sugar composition among different flower species. Additionally, Peay et al.[143] has shown that nectar habitats stand out by high sugar concentrations, microaerophilic conditions, low nitrogen levels, and the widespread presence of anti-microbial compounds may regulate the colonization of anthophilous yeasts and influence their diversity. The diversity and behavior of pollinators and other insect visitors (such as bees, bumblebees, butterflies, and birds) significantly affect anthophilous yeast dispersal and colonization among flowers[2,4,6]. For example, *Cystobasidium minutum*, *Meira argovae*, and *Rhodotorula toruloides* are anthophilous yeasts that have been found in honeycombs and honeybees, with the pollinator *Apis* species potentially acting as a vector in their dispersal[3,144,145]. Mittelbach et al.[2] found that nectar sugar concentration, and the frequency of flower visitors (such as birds, bees, and bumblebees) influence the diversity of anthophilous yeasts on the Canary Islands. Environmental conditions, including temperature, humidity, rainfall, wind, and geographic location, further impact the survival and proliferation of anthophilous yeasts[120,146]. Therefore, the factors influencing the diversity of anthophilous yeasts in northern Thailand should be further explored in future studies, as understanding these determinants may provide valuable insights into yeast ecology, insect-plant-microbe interactions, and regional biodiversity. The composition of nectar (including sugars, amino acids, and lipids) of each flower species requires further study. Additionally, the relationship between the yeasts detected in this study, and their surrounding environment, warrants further investigation.

Northern Thailand represents one of the most significant global biodiversity hotspots, particularly for fungal diversity (filamentous fungi, mushrooms, and yeasts)[9,20,23,41,147–153]. This study highlights the diversity of anthophilous yeasts isolated from flowers within this region, which can also be considered an important habitat for yeast diversity, and may potentially include new and/or previously undocumented species. Therefore, this study provides valuable information and enhances the understanding of anthophilous yeast diversity. It may also serve as a catalyst, encouraging mycologists to

further investigate the distribution, diversity, and ecological roles of anthophilous yeasts not only in Thailand but throughout Asia and globally. Furthermore, both previously known and novel yeast strains identified in this study represent valuable resources for future biotechnological innovation because these tropical yeasts exhibit unique characteristics (such as osmotolerance, thermal stability, and specialized metabolic pathways) that could support a wide range of industrial applications, including the synthesis of enzymes, bioethanol, natural flavor compounds, and biocontrol agents.

Conclusions

This study investigated anthophilous yeasts isolated from floral samples in northern Thailand. From 63 flower specimens, a total of 187 yeast strains were obtained, representing nine classes, 17 orders, 22 families, 36 genera, and 73 species. Molecular characterization revealed that the majority of isolated yeasts belonged to Basidiomycota (77.54%), with the remainder classified as Ascomycota (22.46%). Interestingly, one new order, one new family, 33 new species, and two validated species were introduced based on a polyphasic approach integrating morphological descriptions, physiological characteristics, and multi-locus phylogenetic analyses. Furthermore, this study documented 14 species with newly recognized geographical distributions, one novel habitat association, and 37 first host records. These findings significantly advance knowledge of the biodiversity and biogeography of anthophilous yeasts in tropical ecosystems, revealing a rich but previously underexplored diversity in Thailand and Southeast Asia.

Ethical statements

Not applicable.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization: Kodchasee P, Kumla J, Suwannarach N; formal analysis, validation, data curation: Kodchasee P, Khunnamwong P, Senwanna C, Kumla J, Suwannarach N; software: Kodchasee P, Senwanna C, Tiemsan S, Kumla J; resources, visualization: Kodchasee P, Senwanna C, Kumla J, Suwannarach N; writing – original draft: Kodchasee P, Khunnamwong P, Senwanna C, Kumla J, Suwannarach N; investigation, methodology, writing – review and editing: Kodchasee P, Khunnamwong P, Senwanna C, Duangkon N, Wongsa K, Tiemsan S, Kaewnunta A, Kumla J, Suwannarach N; supervision, project administration, funding acquisition: Suwannarach N. All authors have read and agreed to the published version of the manuscript.

Data availability

The DNA sequence data obtained from this study have been deposited in GenBank under accession numbers: D1/D2 (PV834432 to PV834618), ITS (PV834619 to PV834788), *rpb1* (PV941856 to PV941873, PX582293 to PX582312, PX570009, PX570010), *rpb2* (PV947453 to PV947472, PX582313 to PX582333, and PX570011 to PX570013), *tef1-α* (PV947473 to PV947490, PV844824 to PV844827, PX582334 to PX582350, PX570014, and PX570015), and SSU (PV819892 to PV819897, and PX622318 to PX622385). All data analyzed during this study are included in this published article and its supplementary information.

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Conflict of interest

The authors declare that they have no conflict of interest.

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