

Description of *Diutina* gen. nov., *Diutina siamensis*, f.a. sp. nov., and reassignment of *Candida catenulata*, *Candida mesorugosa*, *Candida neorugosa*, *Candida pseudorugosa*, *Candida ranongensis*, *Candida rugosa* and *Candida scorzettiae* to the genus *Diutina*

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Three strains (DMKU-RE28, DMKU-RE43^T and DMKU-RE123) of a novel anamorphic yeast species were isolated from rice leaf tissue collected in Thailand. DNA sequence analysis demonstrated that the species forms a sister pair with *Candida ranongensis* CBS 10861^T but differs by 24–30 substitutions in the LSU rRNA gene D1/D2 domains and 30–35 substitutions in the ITS region. A phylogenetic analysis based on both the small and the large rRNA gene subunits confirmed this connection and demonstrated the presence of a clade that also includes *Candida catenulata*, *Candida mesorugosa*, *Candida neorugosa*, *Candida pseudorugosa*, *Candida rugosa* and *Candida scorzettiae*. The clade is not closely affiliated to any known teleomorphic genus, and forms a well-separated lineage from currently recognized genera of the Saccharomycetales. Hence, the genus *Diutina* gen. nov. is proposed to accommodate members of the clade, including *Diutina siamensis* f.a. sp. nov. and the preceding seven *Candida* species. The type strain is DMKU-RE43^T (=CBS 13388^T=BCC 61183^T=NBRC 109695^T).

Abbreviations: f.a., *forma asexualis* (asexual form); ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit.

The GenBank/EMBL/DDBJ accession numbers of the SSU and the LSU rRNA gene sequences of *Diutina siamensis* f.a. DMKU-RE43^T, *Diutina mesorugosa* CBS 12656^T, *Diutina rugosa* CBS 613^T, *Diutina pseudorugosa* CBS 10433^T, *Diutina neorugosa* CBS 12627^T, *Diutina ranongensis* CBS 10861^T, *Diutina catenulata* CBS 565^T and *Diutina scorzettiae* CBS 10107^T are KT336715–KT336722, respectively. The MycoBank numbers of *Diutina rugosa* comb. nov., *Diutina catenulata* comb. nov., *Diutina mesorugosa* comb. nov., *Diutina neorugosa* comb. nov., *Diutina pseudorugosa* comb. nov., *Diutina ranongensis* comb. nov., *Diutina scorzettiae* comb. nov. and *Diutina siamensis* f.a., sp. nov. are MB 813768, MB 813778, MB 813786, MB 813787, MB 813788, MB 813874, MB 813789 and MB 813790, respectively.

A supplementary figure is available with the online Supplementary Material.

One mystery of yeast biodiversity is the role played by phylogenetically isolated species that are recovered only infrequently and from an eclectic array of substrates. One rightly wonders whether the remote position of rare species in the tree of life is an indication of impending extinction, or alternatively a sign that their true habitat remains elusive. In the last monograph on *Candida* (Lachance *et al.*, 2011), *Candida catenulata*, *Candida rugosa* and *Candida scorzettiae* were reported to form an unaffiliated clade with an unconvincing connection to the genus *Kodamaea*. The species are known from a few isolates from clinical specimens, insects, rotting wood, dung, or spoiled food. Three more relatives not included in the monograph, *Candida mesorugosa*, *Candida neorugosa* and *Candida pseudorugosa*, have been isolated from clinical materials (Li *et al.*, 2006; Paredes *et al.*,

2012; Chaves *et al.*, 2013) and another, *Candida ranongensis*, was found in a small number of estuarine water samples in Thailand (Am-In *et al.*, 2011). Addition of new taxa did little to provide a more satisfactory placement of the clade from sequences of the D1/D2 domains of the large subunit rRNA gene. These are usually sufficient for the identification of existing yeast species and the discovery of new species, but a more reliable phylogenetic classification often requires a broader sequence sampling, as exemplified in some recent studies that used multilocus approaches for classification at the level of higher categories (Deák & Péter, 2013; Kurtzman & Robnett, 2013; Sousa *et al.* 2014).

Our interest in the placement of the *C. catenulata* clade arose from an investigation of endophytic yeasts in rice leaf tissue in Thailand. Endophytic yeasts colonize healthy plant tissue without causing any apparent damage (Petrini, 1991; Azevedo *et al.*, 2000; Oliveira *et al.*, 2012). Examples of endophytic species in the Ascomycota include *Meyerozyma guilliermondii*, *Candida oleophila*, *Candida railenensis*, *Cyberlindnera saturnus*, *Metschnikowia pulcherrima*, *Wickerhamomyces anomalus*, and *Wickerhamiella siamensis* (Nassar *et al.*, 2005; Isaeva *et al.*, 2010; Rodríguez *et al.*, 2011; Oliveira *et al.*, 2012; Khunnamwong *et al.*, 2014). Endophytes with affinities in the Basidiomycota include *Cryptococcus albidus*, *Cryptococcus flavescens*, *Cryptococcus laurentii*, *Cryptococcus magnus*, *Papiliotrema siamense*, *Occultifur tropicalis*, *Rhodotorula graminis*, *Rhodotorula mucilaginosa*, *Rhodotorula pinicola* and *Rhodotorula rubra* (Gai *et al.*, 2009; Abdel-Motaal *et al.*, 2009; Isaeva *et al.*, 2010; Khan *et al.*, 2012; Akhtyamova & Sattarova, 2013; Khunnamwong *et al.*, 2015; Surussawadee *et al.*, 2014).

Here, we report on three endophytic isolates that represent a sister species to *C. ranongensis*. The previously simple matter of providing a phylogeny and a standard description of the new species is now complicated by the recent abolition of the dual nomenclature hitherto used in mycology to designate asexual forms in a system where generic assignments were based on sexual cycle morphology (Miller *et al.*, 2011; McNeill *et al.*, 2012). Whereas existing members of the *C. catenulata* clade were formally assigned to the polyphyletic genus *Candida*, new rules dictate that a novel species should only be assigned to *Candida* if it belongs to a clade that includes *Candida albicans* and is currently typified by *Candida tropicalis* (Daniel *et al.*, 2014). We therefore propose the genus *Diutina* gen. nov. to accommodate the novel species *Diutina siamensis* f.a., sp. nov. and the transfer of other clade members to the new genus. Our attempts to place the genus *Diutina* into an existing family were fraught with difficulty and we choose to leave the family assignment open until the existing family structure within the order Saccharomycetales is better clarified based on eventual phylogenomic data.

Yeasts

Yeast strains used in this study are listed in Table 1. Three endophytic strains (DMKU-RE28, DMKU-RE43^T and

DMKU-RE123) were isolated from tissue of surface-sterilized rice leaves collected in Thailand by the method of Khunnamwong *et al.* (2014). Briefly, three grams of leaf tissue were cut into fragments (3 × 3 cm) and surface-sterilized by immersion in 70 % ethanol for 3 min and 5 % sodium hypochlorite for 3 min, followed by rinsing five times for 5 min in sterile deionized water. The sterilized leaves were cut into smaller fragments (0.5 × 0.5 cm), ground in a sterile mortar, and placed onto YM agar supplemented with 0.02 % chloramphenicol. Plates were incubated at 25 °C until yeast colonies appeared. Strain DMKU-RE28 came from a sample collected in Bang Len district, Nakhon Pathom province (14° 3' 0" N 100° 10' 60" E) on 12 January 2012. Strains DMKU-RE43^T and DMKU-RE123 were obtained from samples collected 2 March 2012 in U Thong district, Suphan Buri province (14° 24' 39" N 99° 53' 22" E) and Kao Liao district, Nakhon Sawan province (15° 53' 16" N 100° 5' 47" E), respectively. The ex-type strains of *C. catenulata* CBS 565^T, *C. mesorugosa* CBS 12656^T, *C. neorugosa* CBS 12627^T, *C. pseudorugosa* CBS 10433^T, *C. rugosa* CBS 613^T, and *C. scorzettiae* CBS 10107^T were obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands. Strains UWOPS 91-643.2 and UWOPS 91-647.1 had been recovered from *Drosophila* sp. feeding on soapberries (*Sapindus saponaria*) in Hawaii Volcanoes National Park, Hawaii, in 1991. Two strains (RS16 and DMKU-Y-24-3) are from estuarine water in Ranong, Thailand and peat swamp forest soil in Narathiwat, Thailand, respectively.

DNA sequencing and phylogenetic analysis

The sequences of the internal transcribed spacer (ITS) (ITS1-5.8S-ITS2) region and the D1/D2 region of the large subunit (LSU) rRNA gene of strains DMKU-RE28, DMKU-RE43^T and DMKU-RE123 were determined from PCR products amplified from genomic DNA, using primers ITS1 and ITS4 (White *et al.*, 1990), and NL1 and NL4 (Kurtzman & Robnett, 1998), respectively. DNA extraction and amplification of the ITS and D1/D2 regions were performed as described previously (Limtong *et al.*, 2007). The PCR product was checked by agarose gel electrophoresis and purified using a HiYield Gel/PCR DNA Fragments Extraction kit (RBC Bioscience), according to the manufacturer's protocol. The purified product was submitted to Macrogen (Korea) for sequencing with the same primers. The sequence of the region spanning the small-subunit (SSU) rRNA gene through the LSU rRNA gene was determined by PCR amplification using the primers NS7A, NL5A (Kurtzman & Robnett, 2003), SSU1f, SSU4r, SSU3f, SSU2r, LSU3f, LSU4r, LSU5f and LSU2r (Rosa *et al.*, 2007). Sequencing templates were amplified directly from whole yeast cells and sequenced as described by Lachance *et al.* (1999). Amplified DNA was analysed in an ABI sequencer at the John P. Robarts Research Institute, London, ON, Canada. The sequences were assembled, edited and aligned with MEGA software version 6.06 (Tamura *et al.*, 2013). Phylogenetic trees were

Table 1. Properties of yeast strains examined in this study^T, Type strain.

Strain	Locality	Source of sample	Reference
<i>Diutina rugosa</i> comb. nov. CBS 613 ^T =ATCC 10571 ^T	Unknown, USA	Faeces of human	Lachance <i>et al.</i> (2011)
<i>Diutina catenulata</i> comb. nov. CBS 565 ^T	Puerto Rico, USA	Faeces of a dysentery patient	Lachance <i>et al.</i> (2011)
<i>Diutina pseudorugosa</i> comb. nov. XH 1164 ^T =AS 2.3107 ^T =CBS 10433 ^T	Beijing, China	Sputum of an ICU patient with acute pneumonia	Li <i>et al.</i> (2006)
<i>Diutina scorzettiae</i> comb. nov. QuB-82 ^T =CBS 10107 ^T =NRRL Y-27665 ^T	Wageningen, Netherlands	Rotten oak wood (<i>Quercus robur</i> L.)	Middelhoven & Kurtzman (2007)
RS16	Ranong, Thailand	Estuarine water in a Thai mangrove forest	This study
DMKU-Y24-3	Narathiwat, Thailand	Peat swamp forest soil	This study
UWOPS 91-497.1	Hawaii, USA	<i>Drosophila</i> sp. on soapberry fruit	This study
UWOPS 91-493.2	Hawaii, USA	<i>Drosophila</i> sp. on soapberry fruit	This study
<i>Diutina ranongensis</i> comb. nov. RS28 ^T =BCC 25964 ^T =NBRC 103861 ^T =CBS 10861 ^T	Ranong, Thailand	Estuarine water in a Thai mangrove forest	Am-In <i>et al.</i> (2011)
<i>Diutina neorugosa</i> comb. nov. UTHSC 10-2054 ^T =CBS 12627 ^T	San Antonio, Texas, USA	Leg wound of female patient	Paredes <i>et al.</i> (2012)
<i>Diutina mesorugosa</i> comb. nov. L69D ^T =CBS 12656 ^T	Sao Paulo, Brazil	Blood of comatose ICU patient	Chaves <i>et al.</i> (2013)
<i>Diutina siamensis</i> f.a., sp. nov. DMKU-RE28	Nakhon Pathom, Thailand	Rice leaf tissue (<i>Oryza sativa</i>)	This study
DMKU-RE43 ^T =BCC 61183 ^T =NBRC 109695 ^T =CBS 13388 ^T	Suphan Buri, Thailand	Rice leaf tissue (<i>Oryza sativa</i>)	This study
DMKU-RE123	Nakhon Sawan, Thailand	Rice leaf tissue (<i>Oryza sativa</i>)	This study

reconstructed using the maximum-likelihood (ML) algorithm included in MEGA software and the MrBayes plugin (Huelsenbeck & Ronquist, 2001) of the Geneious (Biomatters) program. *Schizosaccharomyces pombe* CBS 356^T (GenBank accession no. Q68936/ JQ689077) was used as outgroup in these analyses.

Growth characteristics

The growth characteristics (Table 1) were determined by replica plating following standard methods (Kurtzman *et al.*, 2011). Casein hydrolysis was determined using a modification (Phaff *et al.*, 1994) of the medium proposed by Ahearn *et al.* (1968). The medium contained 2.4 % skimmed milk powder, 0.3 % beef extract, 0.5 % tryptone, 0.1 % glucose and 1.5 % agar. Lipid hydrolysis was determined on a medium containing 0.4 % Tween 80, 1 % peptone, 0.1 % yeast extract, 0.5 % NaCl, 0.02 % CaCl₂ and 2 % agar. Growth at various temperatures was determined

with replica plate cultures on YM agar incubated at 4 to 12 °C at every even-numbered temperature and at one-degree intervals from 28 to 41 °C, at the microbial temperature facility of the University of Western Ontario Biotron. Ubiquinones were extracted from cells cultured in 500 ml Erlenmeyer flasks containing 250 ml yeast extract-peptone-dextrose (YPD) broth (1 % yeast extract, 2 % peptone and 2 % dextrose) on a rotary shaker at 28 °C for 48 h and purified according to the methods of Yamada & Kondo (1973) and Kuraishi *et al.* (1985). Isoprenologues were identified by HPLC as described previously (Limtong *et al.*, 2007). Formation of true hyphae or pseudohyphae of strains DMKU-RE28, DMKU-RE43^T and DMKU-RE123 was investigated by cultivation for up to 7 days on potato dextrose agar (PDA; 20 % potato infusion, 2 % dextrose and 1.5 % agar) in slide culture at 25 °C. Ascospore formation was investigated by growing strains individually or in pairs on PDA, corn meal agar, 5 % malt extract agar (5 % malt extract and 1.5 % agar), YM agar, and Yeast Carbon Base agar supplemented with

0.01 % yeast extract (YCBY) at 25 °C for up to 6 weeks with periodic examination under the microscope.

Species delineation and phylogenetic placement

Analysis of the sequences of the ITS/5.8S rRNA gene region and the D1/D2 region of the LSU rRNA gene indicated that the three endophytic yeast strains represent a single species. Strains DMKU-RE43^T and DMKU-RE28 were identical and differed from strain DMKU-RE123 by three nucleotide substitutions in the D1/D2 region. The ITS regions of strains DMKU-RE43^T and DMKU-RE123 were identical and differed by a single substitution and an indel from that of strain DMKU-RE28. A phylogenetic analysis of concatenated small and large subunit rRNA genes (Fig. 1) placed the species next to *C. ranongensis* CBS 10861^T but the two differ by 24–30 substitutions (4.7–5.9 %) and 9–10 indels in the D1/D2 region (511 nt) and by 30–35 substitutions (7.1–8.3 %) and 21–38 gaps in the ITS region (414 nt). We therefore conclude that the endophytes represent a distinct sister species to *C. ranongensis*. The analysis further shows that these species have no close affinity to the genus *Candida* as it is to be redefined (Daniel *et al.*, 2014) and represented in Fig. 1 by *Lodderomyces elongisporus*. Instead, the two species form a well-defined clade with *Candida catenulata*, *C. mesorugosa*, *C. neorugosa*, *C. pseudorugosa*, *C. rugosa*, and *C. scorzettiae*. Consistent with this, we propose the genus *Diutina* gen. nov. to accommodate members of the clade.

As the reality and placement of the genus *Diutina* gen. nov. is of some importance, we also subjected the data to a Bayesian analysis (Fig. S1, available in the online Supplementary Material), which confirmed the conclusions drawn from the maximum-likelihood tree presented in Fig. 1. The cohesiveness of the clade is supported by a posterior probability of 1 in one case and a bootstrap value of 92 % in the other. Next arises the question of family assignment. Our analyses include representatives of all currently proposed families of the order Saccharomycetales (Kurtzman & Robnett, 2013), but fails to link the genus *Diutina* gen. nov. reliably to an existing family. As it happens, our analysis puts in question the integrity of the family Metschnikowiaceae *sensu* Kurtzman (2011a), which is poorly supported by either approach. The maximum-likelihood tree presented in Fig. 1 has bootstrap values less than 50 % for clades that contain the four genera currently assigned to the family Metschnikowiaceae (*Metschnikowia*, *Clavispora*, *Kodamaea* and *Aciculoconidium*) or these together with *Diutina* gen. nov. Moreover, the Bayesian analysis (Fig. S1) suggests a poorly supported (0.71) family Metschnikowiaceae with a paraphyletic structure with respect to *Diutina* gen. nov. In the interest of nomenclatural stability, which is a fundamental but too often neglected objective of good systematics, we choose to leave the family unassigned until a reliable genome-based phylogeny is in hand. The weakness of current

family assignments based on phylogeny is substantiated by the uncertainty of placement of certain genera. For example, the genus *Hyphopichia*, represented here by *Hyphopichia heinii*, pairs up with *Wickerhamia fluorescens* in our maximum-likelihood analysis (Fig. 1), well within the family Debaryomycetaceae. At variance with this, our Bayesian analysis (Fig. S1) positions the genus *Hyphopichia* in an early emerging position with respect to a clade that combines the family Metschnikowiaceae with *Diutina* gen. nov., but with low support. In reply to the eventual objection that ribosomal genes are not sufficiently reliable, it should be noted that two published multilocus analyses position *Hyphopichia* as a sister taxon to the Metschnikowiaceae in one instance (Kurtzman, 2011b) and well inside the Debaryomycetaceae in the other (Kurtzman and Robnett, 2013). The old cliché, ‘more work is needed’, appears entirely appropriate.

As part of this study, we examined four strains, from our collections, that exhibited an affinity to *Diutina* gen. nov. (DMKU-Y24-3, RS16, UWOPS 91-643.2 and UWOPS 91-647.1). The four strains were identical in their ITS and D1/D2 sequences and differed from *C. scorzettiae* by six substitutions in the ITS region and four in the D1/D2 region. This degree of divergence sits at the very limit of what might be considered enough to warrant the description of a novel species. A neighbour-joining analysis based on the sequences of the ITS region and the D1/D2 region of the five strains (data not shown) showed that there is no phylogenetic justification for separation, and so the isolates are assigned to *C. scorzettiae*. The growth profiles of the strains supports the species assignment.

Ecology

Members of the proposed genus originate from a diversity of sources (Table 1), but an ecological generalization does not seem possible in view of the small number of known specimens. Isolation substrates include a leg wound (*C. neorugosa*), faeces (*C. rugosa* and *C. catenulata*), sputum (*C. pseudorugosa*), blood (*C. mesorugosa*), rotten oak wood (*C. scorzettiae*), and estuarine water (*C. ranongensis*), as well as soil and spoiled food (Li *et al.*, 2006; Middelhoven & Kurtzman, 2007; Am-In *et al.*, 2011; Lachance *et al.*, 2011; Paredes *et al.*, 2012; Chaves *et al.*, 2013). The present study adds three strains of a novel species isolated from rice leaf tissue and four strains of *C. scorzettiae* recovered from estuarine water, soil, and fruit flies. None of the isolates are thought to be the causative agent of disease, although some have been recognized as opportunistic pathogens (Li *et al.*, 2006; Lachance *et al.*, 2011; Chaves *et al.*, 2013; Kobayashi *et al.*, 2013). Limtong & Kaewwichian (2015) have reported the isolation of *C. rugosa* and *C. catenulata* from the surface of rice leaves and the latter was once recovered from a stingless bee in Brazil (Lachance *et al.* 2011). The question of the significance of rare, phylogenetically isolated species in the want of a defined habitat remains open.

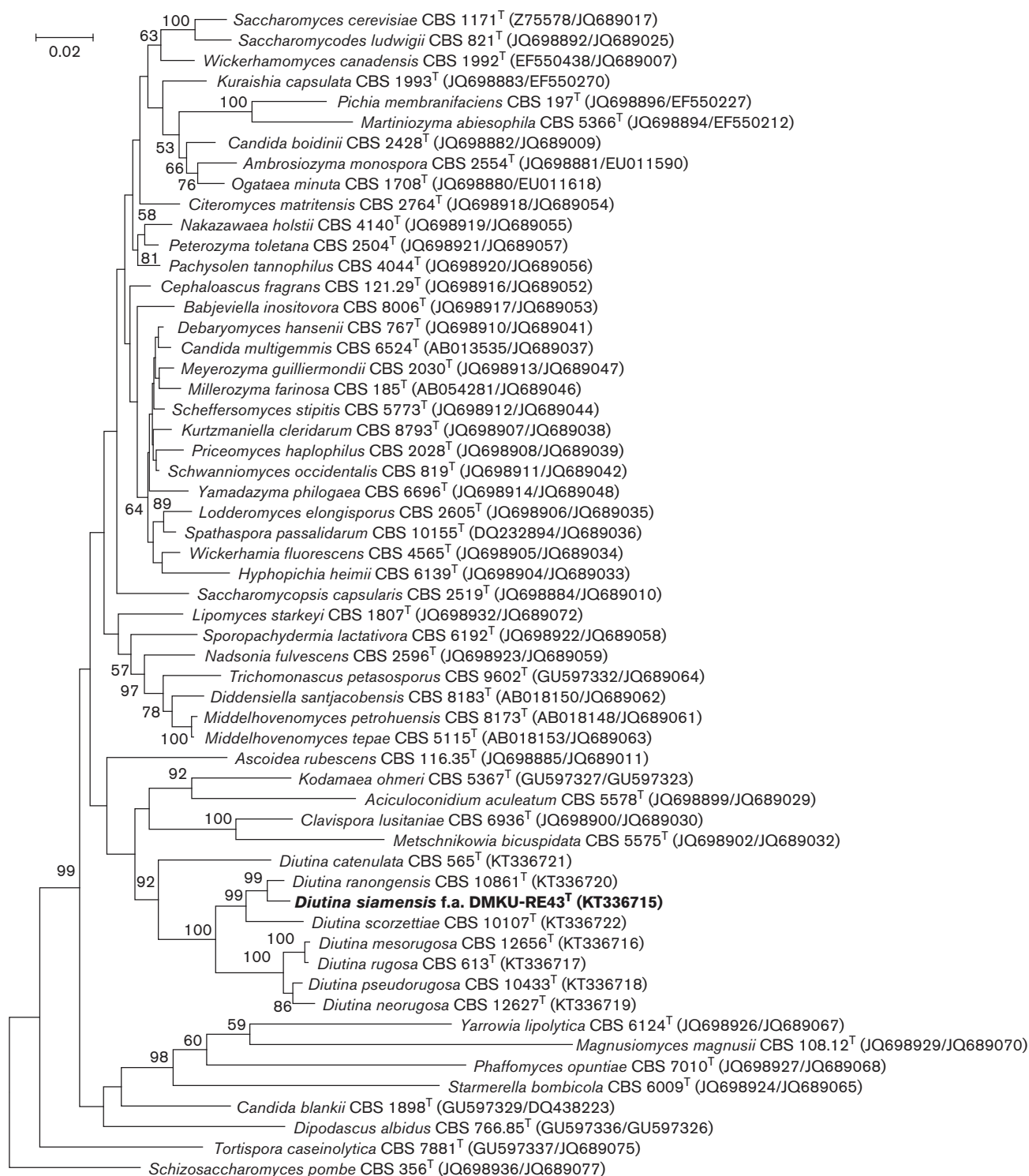


Fig. 1. Phylogenetic tree based on concatenated SSU and LSU rRNA genes showing the placement of *Diutina siamensis* f.a. sp. nov. with respect to related species and genera. The tree was reconstructed using the maximum-likelihood method in MEGA 6.0 software package. Distances were fitted to Kimura's two-parameter model. Numbers at the nodes are percentage bootstrap values from 1000 pseudoreplicates. GenBank accession numbers are given in parentheses. *Schizosaccharomyces pombe* CBS 356^T (GenBank accession nos Q698936/JQ689077) was used as the outgroup. Bar, patristic distance of 0.02.

Taxonomy

Description of *Diutina* Khunnamwong, Jindamorakot, Limtong & Lachance gen. nov.

Diutina (Di.u'ti.na. L. fem. n. *Diutina* long-lasting, expressing the hope that the genus is being thus named in perpetuity and will not fall victim to frivolous nomenclatural changes).

Growth is by multilateral budding. Cells are ovoid to ellipsoidal. Pseudohyphae are produced. Ascospore formation has not been observed. Sugars may or may not be fermented. Glucose, galactose, ethanol, glucitol, succinic acid, *N*-acetylglucosamine and hexadecane are assimilated. Ethylamine, lysine and cadaverine are utilized as sole nitrogen sources, but nitrate and nitrite are not. DBB and urease reactions are negative. Starch-like compounds are not produced.

Phylogenetic placement: Saccharomycetales, Saccharomycotina, Ascomycota.

The type species of the genus is *Diutina rugosa* (Anderson) Khunnamwong, Jindamorakot, Limtong & Lachance.

The MycoBank number is MB 813756.

Description of *Diutina siamensis* Khunnamwong, Jindamorakot, Limtong & Lachance sp. nov.

Diutina siamensis (si.am.en'sis. N.L. fem. adj. *siamensis* of Siam, the old name of Thailand, where the type strain was isolated).

Growth occurs in YM broth. After 3 days at 25 °C, cells are subglobose to elongate (2–3 × 3–18.5 µm) and occur singly or in pairs. Budding is multilateral (Fig. 2a). After 3 days on YM agar at 25 °C, the streak culture is white-cream, soft with a smooth surface and has an entire margin. Pseudohyphae and true hyphae are formed abundantly in slide culture on PDA after 7 days at 25 °C (Fig. 2b). Ascospore formation has not been observed in any of the strains singly or in pairs on PDA agar, corn meal agar, 5 % malt extract agar or YM agar at 15 °C and 25 °C for up to 6 weeks. Fermentation is absent. The major ubiquinone is Q-9. The growth characteristics are given in Table 2.

The type strain is DMKU-RE43^T, which was isolated from rice leaf tissue (*Oryza sativa*) collected from U Thong district, Suphan Buri province, Thailand. The type strain has been deposited at the Centraalbureau voor Schimmelcultures (CBS), Netherlands, as strain CBS 13388^T (=BCC 61183^T=NBRC 109695^T).

The MycoBank number is MB 813790.

New species combinations

Diutina rugosa (H. W. Anderson) Khunnamwong, Jindamorakot, Limtong & Lachance comb. nov.

Basionym: *Mycoderma rugosum*, H. W. Anderson. *J Infect Dis* 21, 341 (1917).

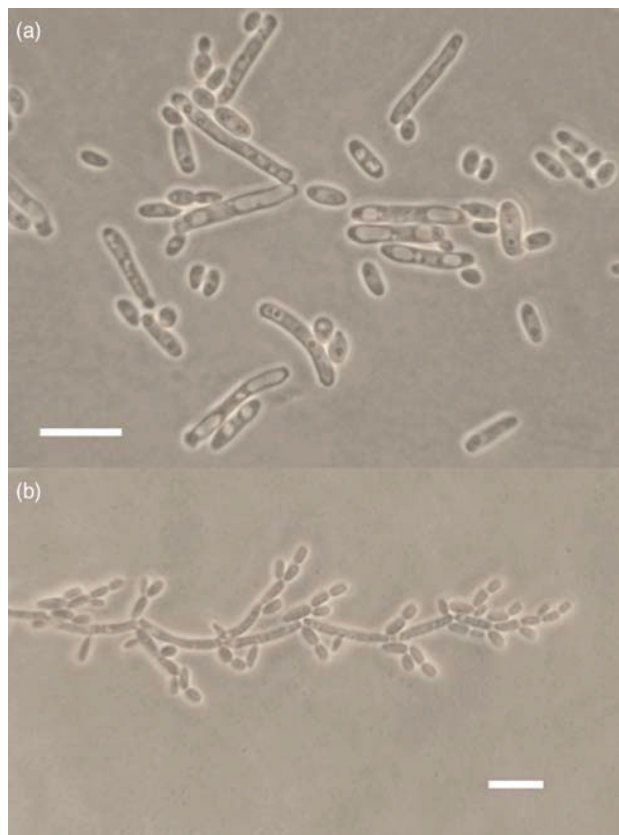


Fig. 2. Micrographs of *Diutina siamensis* f.a., sp. nov. DMKU-RE43^T. (a) Budding cells on YM agar after 3 days at 25 °C. (b) Pseudohyphae formed on PDA after 7 days at 25 °C. Bar, 10 µm.

Type strain: CBS 613^T, from human faeces (Table 1).

The MycoBank number is MB 813768.

Diutina catenulata (H. A. Diddens & J. Lodder) Khunnamwong, Jindamorakot, Limtong & Lachance comb. nov.

Basionym: *Candida catenulata* H. A. Diddens & J. Lodder. Die anaskosporogenen Hefen, Zweite Hälfte, 486 (1942).

Type strain: CBS 565^T, from faeces of a dysentery patient (Table 1).

The MycoBank number is MB 813778.

Diutina mesorugosa (G. M. Chaves, G. R. Terçarioli, A. C. B. Padovan, R. C. Rosas, R. C. Ferreira, A. S. Melo & A. L. Colombo) Khunnamwong, Jindamorakot, Limtong & Lachance comb. nov.

Basionym: *Candida mesorugosa* G. M. Chaves, G. R. Terçarioli, A. C. B. Padovan, R. C. Rosas, R. C. Ferreira, A. S. Melo & A. L. Colombo. *Med Mycol* 51, 231 (2013).

Table 2. Growth characteristics of species of *Diutina* gen. nov.

Species: 1, *Diutina rugosa* comb. nov.; 2, *Diutina catenulata* comb. nov.; 3, *Diutina mesorugosa* comb. nov.; 4, *Diutina neurugosa* comb. nov.; 5, *Diutina pseudorugosa* comb. nov.; 6, *Diutina ranongensis* comb. nov.; 7, *Diutina scorzettiae* comb. nov.; 8, *Diutina siamensis* sp. nov. All species assimilate glucose, galactose, ethanol, glucitol, succinate, N-acetylglucosamine and hexadecane, but not inulin, sucrose, raffinose, melibiose, lactose, melezitose, methyl α -D-glucoside, sorbose, L-arabinose, D-arabinose, methanol, erythritol, xylitol, galactitol, inositol, citrate, malate, tannin, acetone or ethyl acetate. All species utilize ethylamine, lysine and cadaverine as sole nitrogen sources, but not nitrate or nitrite. DBB and urease reactions are negative. Starch-like compounds are not produced. Acid formation is absent. All species grow at 28 and 33 °C. All species grow in the absence of amino acids, but not in the absence of vitamins. All species grow on YM agar with phosphoric acid, pH 2.5, but not pH 2.0. None of the species grow on YM agar with 0.5 % acetic acid or in the presence of 8 % ethanol, or on Yeast Nitrogen Base with 1 % phenylalanine as sole carbon and nitrogen source. None of the species hydrolyse gelatin. +, Positive; –, negative; s, slow; w, weak; v, variable.

Characteristic	1	2	3	4	5	6	7	8
Fermentation of glucose	–	–	–	+	+	–	–	–
Assimilation of carbon compounds								
Trehalose	–	–	–	–	–	+	+ / w	s
Maltose	–	–	–	–	–	+	+	+
Starch	w	–	–	–	–	–	w / s	w / s
Cellobiose	–	–	–	–	–	–	w / + / s	–
Salicin	w	–	–	–	–	–	w / –	–
Rhamnose	w	–	–	–	–	w	–	–
Xylose	+	–	+	+	+	w	w / + / s	w / s
Ribose	w	w	w	w	w	+	+ / w	w
1-Propanol	–	w	+	s	w	+	+ / s	+
2-Propanol	w	–	w	w	w	s	w / –	w
1-Butanol	w	–	w	w	w	+	+ / w	+ / w
Glycerol	+	w	s	w	w	w	w / s	s
Ribitol	–	–	–	w	–	–	w / s	w
Mannitol	+	w	+	+	–	+	+	+
Lactate	–	–	–	–	–	+	–	–
Gluconate	+	w	–	–	–	–	v	w / +
Glucono- Δ -lactone	w	–	–	–	w	s	w / s	+
D-glucuronate	–	–	w	–	–	s	–	w / –
2-Ketogluconate	–	–	–	–	–	s	w	s
Glucosamine	–	s	–	–	–	–	–	–
Hexadecane	s	w	s	w	w	+	+ / s	s
Growth on/at:								
NaCl (5%)	+	+	+	+	+	+	v	w / +
NaCl (10%)	–	–	–	s	w	w	w / –	–
Cycloheximide (10 mg l ⁻¹)	–	+	w	+	+	+	+	+
Cycloheximide (100 mg l ⁻¹)	–	+	–	–	–	+	+	+
Cycloheximide (1000 mg l ⁻¹)	–	s	–	–	–	–	v	–
Digitonin (8 mg l ⁻¹)	+	s	s	–	s	–	–	–
Cetyl trimethylammonium bromide (CTAB) (50 mg l ⁻¹)	–	–	–	–	–	–	+	+
Acetic acid (0.5%)	–	–	–	–	–	+	v	–
Ethanol (6%)	+	–	+	+	–	s	–	–
Growth temperature								
Minimum (°C)	12	8	≥12	6	6	12	8	12
Maximum (°C)	≥41	36	≥41	40	≥41	36	33–34	38–40
Growth at:								
34 °C	+	+	+	+	+	+	v	+
35 °C	+	+	+	+	+	+	–	+
36 °C	+	+	+	+	+	w	–	+
37 °C	+	–	+	+	+	–	–	+
38 °C	+	–	+	+	+	–	–	+
39 °C	+	–	+	+	+	–	–	v
40 °C	+	–	+	+	+	–	–	v
41 °C	+	–	+	–	+	–	–	–
Hydrolysis of:								
Casein	–	+	–	w	–	–	–	–
Tween 80	–	–	–	–	–	–	+	s / –

Type strain: L69D^T (=CBS 12656^T), from blood of coma-tose ICU patient (Table 1).

The MycoBank number is MB 813786.

***Diutina neorugosa* (K. Paredes, D. A. Sutton, J. Cano & J. Guarro) Khunnamwong, Jindamorakot, Limtong & Lachance comb. nov.**

Basionym: *Candida neorugosa* K. Paredes, D. A. Sutton, J. Cano, A. W. Fothergill.

S. D. Lawhon, S. Zhang, J. P. Watkins & J. Guarro. *J Clin Microbiol* 50, 2397 (2012).

Type strain: UTHSC 10-2054^T (=CBS 12627^T), from leg wound of a patient (Table 1).

The MycoBank number is MB 813787.

***Diutina pseudorugosa* (J. Li, Y. Xu & F. Y. Bai) Khunnamwong, Jindamorakot, Limtong & Lachance comb. nov.**

Basionym: *Candida pseudorugosa* J. Li, Y. Xu & F. Y. Bai. *J Clin Microbiol* 44, 4486 (2006).

Type strain: XH 1164^T (AS 2.3107^T=CBS 10433^T), from sputum of an ICU patient (Table 1).

The MycoBank number is MB 813788.

***Diutina ranongensis* (S. Am-In, S. Limtong, W. Yongmanitchai & S. Jindamorakot) Khunnamwong, Jindamorakot, Limtong & Lachance comb. nov.**

Basionym: *Candida ranongensis* S. Am-In, S. Limtong, W. Yongmanitchai & S. Jindamorakot. *Int J Syst Evol Microbiol* 61, 454 (2011).

Type: RS28^T (=BCC 25964^T=NBRC 103861^T=CBS 10861^T), from estuarine.

Water, Thailand (Table 1).

The MycoBank number is MB 813874.

***Diutina scorzettiae* (W. J. Middelhoven & C. P. Kurtzman) Khunnamwong, Jindamorakot, Limtong & Lachance comb. nov.**

Basionym: *Candida scorzettiae* W. J. Middelhoven & C. P. Kurtzman. *Antonie van Leeuwenhoek* 92, 233 (2007).

Type strain: CBS 10107^T (=NRRL Y-27665^T), from rotten oak wood (Table 1).

The MycoBank number is MB 813789.

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References

- Abdel-Motaal, F. F., El-Zayat, S. A., Kosaka, Y., El-Sayed, M. A., Nassar, M. S. M. & Ito, S. (2009). Four novel ustilaginomycetous anamorphic yeast species isolated as endophytes from the medicinal plant *Hyoscyamus muticus*. *Asian J Plant Sci* 8, 526–535.
- Ahearn, D. G., Meyers, S. P. & Nichols, R. A. (1968). Extracellular proteinases of yeasts and yeastlike fungi. *Appl Microbiol* 16, 1370–1374.
- Akhtyamova, N. & Sattarova, R. K. (2013). Endophytic yeast *Rhodotorula rubra* strain TG-1: antagonistic and plant protection activities. *Biochem Physiol* 2, 1–5.
- Am-In, S., Limtong, S., Yongmanitchai, W. & Jindamorakot, S. (2011). *Candida andamanensis* sp. nov., *Candida laemsonensis* sp. nov. and *Candida ranongensis* sp. nov., anamorphic yeast species isolated from estuarine waters in a Thai mangrove forest. *Int J Syst Evol Microbiol* 61, 454–461.
- Azevedo, J. L., Maccheroni, W. Jr, Pereira, J. O. & de Araújo, W. L. (2000). Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electron J Biotechnol* 3, 1–4.
- Chaves, G. M., Terçarioli, G. R., Padovan, A. C. B., Rosas, R. C., Ferreira, R. C., Melo, A. S. & Colombo, A. L. (2013). *Candida mesorugosa* sp. nov., a novel yeast species similar to *Candida rugosa*, isolated from a tertiary hospital in Brazil. *Med Mycol* 51, 231–242.
- Daniel, H. M., Lachance, M. A. & Kurtzman, C. P. (2014). On the reclassification of species assigned to *Candida* and other anamorphic ascomycetous yeast genera based on phylogenetic circumscription. *Antonie van Leeuwenhoek* 106, 67–84.
- Deák, T. & Péter, G. (2013). Developments in yeast taxonomy. *Acta Aliment* 42, 55–68.
- Gai, C. S., Lacava, P. T., Maccheroni, W. Jr, Glienke, C., Araújo, W. L., Miller, T. A. & Azevedo, J. L. (2009). Diversity of endophytic yeasts from sweet orange and their localization by scanning electron microscopy. *J Basic Microbiol* 49, 441–451.
- Huelsenbeck, J. P. & Ronquist, F. (2001). mrbayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Isaeva, O. V., Glushakova, A. M., Garbuz, S. A., Kachalkin, A. V. & Chernov, I. Iu. (2010). [Endophytic yeast fungi in plant storage tissues]. *Izv Akad Nauk Ser Biol* 37, 34–43 (In Russian).
- Khan, Z., Guelich, G., Phan, H., Redman, R. & Doty, S. (2012). Bacterial and yeast endophytes from poplar and willow promote growth in crop plants and grasses. *ISRN Agronomy* 2012, 1–11.
- Khunnamwong, P., Surussawadee, J., Jindamorakot, S. & Limtong, S. (2014). *Wickerhamiella siamensis* f.a., sp. nov., an endophytic and epiphytic yeast species isolated from sugar cane leaf. *Int J Syst Evol Microbiol* 64, 3849–3855.
- Khunnamwong, P., Surussawadee, J., Jindamorakot, S., Ribeiro, J. R. A., Hagler, A. N. & Limtong, S. (2015). *Occultifur tropicalis* f.a., sp. nov., a novel cystobasidiomycetous yeast species isolated from tropical regions. *Int J Syst Evol Microbiol* 65, 1578–1582.

- Kobayashi, H., Kawakami, S., Ogawa, Y., Shibata, N. & Suzuki, S. (2013). Isolation of mannooligosaccharides corresponding to antigenic determinants of pathogenic yeast *Candida catenulata* cell wall mannan. *Adv Microbiol* 3, 222–226.
- Kuraishi, H., Katayama-Fujimura, Y., Sugiyama, J. & Yokoyama, T. (1985). Ubiquinone systems in fungi. I. Distribution of ubiquinones in the major families of ascomycetes, basidiomycetes, and deuteromycetes, and their taxonomic implications. *Trans Mycol Soc Japan* 26, 383–395.
- Kurtzman, C. P. (2011a). Discussion of teleomorphic and anamorphic ascomycetous yeasts and yeast-like taxa. In *The Yeasts, a Taxonomic Study*, 5th edn., pp. 293–307. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.
- Kurtzman, C. P. (2011b). Phylogeny of the ascomycetous yeasts and the renaming of *Pichia anomala* to *Wickerhamomyces anomalus*. *Antonie van Leeuwenhoek* 99, 13–23.
- Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* 73, 331–371.
- Kurtzman, C. P. & Robnett, C. J. (2003). Phylogenetic relationships among yeasts of the ‘*Saccharomyces* complex’ determined from multigene sequence analyses. *FEMS Yeast Res* 3, 417–432.
- Kurtzman, C. P. & Robnett, C. J. (2013). Relationships among genera of the Saccharomycotina (Ascomycota) from multigene phylogenetic analysis of type species. *FEMS Yeast Res* 13, 23–33.
- Kurtzman, C. P., Fell, J. W., Boekhout, T. & Robert, V. (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In *The yeasts, a taxonomic study*, 5th edn., pp. 87–110. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.
- Lachance, M. A., Bowles, J. M., Starmer, W. T. & Barker, J. S. F. (1999). *Kodamaea kakaduensis* and *Candida tolerans*, two new ascomycetous yeast species from Australian Hibiscus flowers. *Can J Microbiol* 45, 172–177.
- Lachance, M. A., Boekhout, T., Scorzettii, G., Fell, J. W. & Kurtzman, C. P. (2011). *Candida* Berkhout (1923). In *The Yeasts, a Taxonomic Study*, pp. 987–1278. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. 5th edn.
- Li, J., Xu, Y. C. & Bai, F. Y. (2006). *Candida pseudorugosa* sp. nov. a novel yeast species from sputum. *J Clin Microbiol* 44, 4486–4490.
- Limtong, S. & Kaewwichian, R. (2015). The diversity of culturable yeasts in the phylloplane of rice in Thailand. *Ann Microbiol* 65, 667–675.
- Limtong, S., Yongmanitchai, W., Tun, M. M., Kawasaki, H. & Seki, T. (2007). *Kazachstania siamensis* sp. nov., an ascomycetous yeast species from forest soil in Thailand. *Int J Syst Evol Microbiol* 57, 419–422.
- McNeill, J., Barrie, F. R., Buck, W. R., Demoulin, V., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Marhold, K., other authors, (2012). *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011 (Regnum Vegetabile 154)*. Koenigstein: Koeltz Scientific Books.
- Middelhoven, W. J. & Kurtzman, C. P. (2007). Four novel yeasts from decaying organic matter: *Blastobotrys robertii* sp. nov., *Candida cretensis* sp. nov., *Candida scorzettiae* sp. nov. and *Candida vadensis* sp. nov. *Antonie van Leeuwenhoek* 92, 233–244.
- Miller, J. S., Funk, V. A., Wagner, W. L., Barrie, F., Hoch, P. C. & Herendeen, P. (2011). Outcomes of the 2011 botanical nomenclature section at the XVIII international botanical congress. *PhytoKeys* 5, 1–3.
- Nassar, A. H., El-Tarabily, K. A. & Sivasithamparam, K. (2005). Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots. *Biol Fertil Soils* 42, 97–108.
- Oliveira, A. C. D., Watanabe, F. M. F., Vargas, J. V. C., Rodrigues, M. L. F. & Mariano, A. B. (2012). Production of methyl oleate with a lipase from an endophytic yeast isolated from castor leaves. *Biocatal Agric Biotechnol* 1, 295–300.
- Paredes, K., Sutton, D. A., Cano, J., Fothergill, A. W., Lawhon, S. D., Zhang, S., Watkins, J. P. & Guarro, J. (2012). Molecular identification and antifungal susceptibility testing of clinical isolates of the *Candida rugosa* species complex and proposal of the new species *Candida neorugosa*. *J Clin Microbiol* 50, 2397–2403.
- Petrini, O., Andrews, J. H. & Hirano, S. S. (1991). Fungal endophytes of tree leaves. In *Microbial Ecology of Leaves*, pp. 179–197. New York: Springer.
- Phaff, H. J., Starmer, W. T., Lachance, M. A. & Ganter, P. F. (1994). *Candida caseinolytica* sp. nov., a new species of yeast occurring in necrotic tissue of *Opuntia* and *Stenocereus* species in the southwestern United States and Baja California, Mexico. *Int J Syst Bacteriol* 44, 641–645.
- Rodríguez, P., Reyes, B., Barton, M., Coronel, C., Menéndez, P., Gonzalez, D. & Rodríguez, S. (2011). Stereoselective biotransformation of α -alkyl- β -keto esters by endophytic bacteria and yeast. *J Mol Catal, B Enzym* 71, 90–94.
- Rosa, C. A., Lachance, M. A., Teixeira, L. C. R. S., Pimenta, R. S. & Morais, P. B. (2007). *Metschnikowia cerradonensis* sp. nov., a yeast species isolated from ephemeral flowers and their nitidulid beetles in Brazil. *Int J Syst Evol Microbiol* 57, 161–165.
- Sousa, F. M. P., Morais, P. B., Lachance, M. A. & Rosa, C. A. (2014). *Hagleromyces* gen. nov., a yeast genus in the Saccharomycetaceae, and description of *Hagleromyces aureorens* sp. nov., isolated from water tanks of bromeliads. *Int J Syst Evol Microbiol* 64, 2915–2919.
- Surussawadee, J., Khunnamwong, P., Srisuk, N. & Limtong, S. (2014). *Papiliotrema siamense* f.a., sp. nov., a yeast species isolated from plant leaves. *Int J Syst Evol Microbiol* 64, 3058–3062.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). *MEGA6: molecular evolutionary genetics analysis version 6.0*. *Mol Biol Evol* 30, 2725–2729.
- White, T. J., Bruns, T., Lee, S., Taylor, J. W., Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A Guide to Methods and Applications*, pp. 315–322. New York: Academic Press.
- Yamada, Y. & Kondo, K. (1973). Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus*, and the yeast-like genera *Sporobolomyces* and *Rhodospiridium*. *J Gen Appl Microbiol* 19, 59–77.