Three novel ascomycetous yeast species of the Kazachstania clade, Kazachstania saulgeensis sp. nov., Kazachstania serrabonitensis sp. nov. and Kazachstania australis sp. nov. Reassignment of Candida humilis to Kazachstania humilis f.a. comb. nov. and Candida pseudohumilis to Kazachstania pseudohumilis f.a. comb. nov.

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Five ascosporogenous yeast strains related to the genus Kazachstania were isolated. Two strains (CLIB 1764T and CLIB 1780) were isolated from French sourdoughs; three others (UFMG-CM-Y273T, UFMG-CM-Y451 and UFMG-CM-Y452) were from rotting wood in Brazil. The sequences of the French and Brazilian strains differed by one and three substitutions, respectively, in the D1/D2 large subunit (LSU) rRNA gene and the internal transcribed spacer (ITS). The D1/D2 LSU rRNA sequence of these strains differed by 0.5 and 0.7% from Kazachstania exigua, but their ITS sequences diverged by 8.1 and 8.3%, respectively, from that of the closest described species Kazachstania barnettii. Analysis of protein coding sequences of RPB1, RPB2 and EF-1α distinguished the French from the Brazilian strains, with respectively 3.3, 6 and 11.7% substitutions. Two novel species are described to accommodate these newly isolated strains: Kazachstania saulgeensis sp. nov. (type strain CLIB 1764T=CBS 14374T) and Kazachstania serrabonitensis sp. nov. (type strain UFMG-CM-Y273T=CLIB 1783T=CBS 14236T). Further analysis of culture collections revealed a strain previously assigned to the K. exigua species, but having 3.8% difference (22 substitutions and 2 indels) in its ITS with respect to K. exigua. Hence, we describe a new taxon, Kazachstania australis sp. nov. (type strain CLIB 1847T=MB 815523).

Abbreviations: EF-1α, translation elongation factor-1α; ITS, internal transcribed spacer; LSU, large subunit.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are KF582608–KF582610, LT158238, LT158239, LT158241, LT158244, LT158246–LT158251, LT158253, LT158254, LT158258, LT158259, LT158261, LT158262, LT158264–LT158271, LT158273–LT158276, LT158278, LT158280, LT158282–LT158284 and LT504908–LT505004, see Table S1 (available in the online Supplementary Material) for details. The Mycobank (http://www.mycobank.org) accession numbers for Kazachstania saulgeensis sp. nov., Kazachstania serrabonitensis sp. nov., Kazachstania australis sp. nov., Kazachstania humilis f.a. comb. nov. and Kazachstania pseudohumilis f.a. comb. nov. are, respectively, MB 815524, MB 815525, MB 815523, MB 815526 and MB 815527.

Two supplementary tables are available with the online Supplementary Material.
On the basis of DNA sequence comparison, we consider two species, C. humilis and C. pseudohumilis are reassigned to the genus Kazachstania as new combinations. On the basis of sequence analysis, we also propose that Candida milleri and Kazachstania humilis comb. nov. are conspecific.

Yeast isolation

All Brazilian strains were obtained from rotting wood samples collected in the Atlantic rainforest site of the Private Natural Heritage Reserve of Serra Bonita (15° 23′ S 39° 33′ W), Bahia state, northeastern Brazil. Fifteen rotting wood samples were collected and the strains UFMG-CM-Y273<sup>T</sup>, UFMG-CM-Y451 and UFMG-CM-Y452 were isolated from three different samples. Strain UFMG-CM-Y273<sup>T</sup> was obtained from one sample cultured on YNB-CMC broth (0.67 %, w/v, yeast nitrogen base, 1 % CM-cellulose, 0.05 % cellobiose, 0.02 % chloramphenicol, 2 %, v/v, ethanol). Strains UFMG-CM-Y451 and UFMG-CM-Y452 were obtained from two different samples cultured on YNB-cellobiose broth (0.67 %, w/v, yeast nitrogen base, 0.5 % cellobiose, 0.02 % chloramphenicol, 2 %, v/v, ethanol). Approximately 1 g of each rotting wood sample was cultured in 50 ml Falcon tubes with 20 ml of the correspondent medium and incubated at 25 °C with shaking at 150 r.p.m. for 3 to 10 days. When the growth was observed, 0.5 ml of the culture was transferred separately to tubes containing 5 ml of the correspondent medium, and the tubes were incubated as described above. For yeast isolation, one loopful of culture from each tube was streaked on yeast extract–malt extract agar (YMA; 1 % glucose, 0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone, 2 % agar and 0.02 % chloramphenicol) plate. The different yeast morphotypes were purified by repeated streaking on YMA plates and preserved at −80 °C for later identification.

The two French strains, CLIB 1764<sup>T</sup> and CLIB 1780, were isolated from sourdough samples obtained from baker Michel Perrin at Ferme des plants, Saulgé, France (46° 20′ 27.08″ N 0° 53′ 12.21″ E), as described in Lhomme et al. (2016). The strains CLIB 1764<sup>T</sup> and CLIB 1780 were isolated from homogenized sourdough in tryptone salt solution (0.85 % NaCl, 0.1 % tryptone) and purified on yeast extract medium plates (1 % yeast extract, 2 % glucose, 1.7 % agar). The yeast cultures were preserved at −80 °C for later identification.

Phenotypic characterization

Morphological observations and physiological tests were performed according to established methods (Yarrow, 1998; Barnett et al., 2000). Sugar fermentations were carried out in Durham fermentation tubes in media containing...
0.5% yeast extract and 1% of each tested sugar. The ID 32C systems (bioMérieux) were used to assess growth on various carbon sources after incubation at 28°C for 2 days. Assimilation of nitrogen compounds was assessed on yeast carbon base minimal medium (Difco) plate supplemented with 1% standard nitrogen source (van der Walt & Yarrow, 1984). Growth at various temperatures was determined by cultivation of the strains in YPD (1% yeast extract, 1% bacto peptone, 1% glucose). Sporulation capacity was assessed on 5% malt agar and McClary’s acetate agar at 20°C. Strains were made to sporulate and spores were isolated by micromanipulation and germinated. The resulting cells were shown to be subsequently capable of sporulation. Mycelium formation was investigated on corn meal agar (Becton Dickinson) in slide culture at 20°C. Morphological properties were studied under a Laborlux S light microscope (Leica Microsystems) coupled to a digital camera.

**DNA sequencing and phylogenetic analysis**

Nucleic acids were extracted and purified following the procedures of Hoffman & Winston (1987). The D1/D2 LSU rRNA gene and internal transcribed spacer (ITS), which includes ITS1–5.8S–ITS2, were amplified by PCR in a final reaction mixture of 50 µl containing between 25 and 50 ng of genomic DNA, 0.8 mM dNTPs, 0.4 µM forward and reverse primers in the recommended buffer and 1 U TaKaRa Ex Taq. Primers used for symmetrical amplifications were NL1 and NL4 (O’Donnell, 1993) for the D1/D2 LSU rRNA gene; ITS1 and ITS4 (White et al., 1990) for the ITS region; RPB1-5F and RPB1-5R (Matheny et al., 2002) for RPB1; RPB2-6F and RPB2-7R (Liu et al., 1999) for RPB2; YTEF-1 and YTEF-6A (Kurtzman & Robnett, 2003) for EF-1α. Amplification reactions were run on a 2720 thermal cycler (Applied Biosystems) as follows: 4 min at 94°C, followed by 30 cycles of 30 s at 94°C, 40 s at the annealing temperature (54°C for the D1/D2, 48°C for ITS, 50°C for RPB1, 51°C for RPB2, 50°C for EF-1α) and 90 s at 72°C, with a final extension step of 7 min at 72°C. PCR products were separated by electrophoresis using a 1% agarose gel.

The resulting amplicons were sequenced on both strands by Eurofins MWG Operon (Eurofins Genomics). Sequencing primers were those used for PCR amplification. Sequences were assembled with the phred/phrap/consed package and compared to sequences in databases, such as GenBank (www.ncbi.nlm.nih.gov/pubmed) and YeastIP (Weiss et al., 2013), using the BLAST program (Altschul et al., 1990). Sequence alignments were generated using MAFFT7 (Katoh & Standley, 2016) and were adjusted manually. Phylogenetic trees were reconstructed with the maximum-likelihood program implemented in MEGA6 (Tamura et al., 2013). Phylogenetic trees were visualized with NJ plot (Perrière & Gouy, 1996).

**Species delineation**

Isolated from French sourdough, strains CLIB 1764T and CLIB 1780 had D1/D2 LSU rRNA gene and ITS sequences which differed by one base pair out of 544 bp and two base pairs out of 589 bp, respectively. UFMG-CM-Y273T, UFMG-CM-Y451 and UFMG-CM-Y452 had the same D1/D2 LSU rRNA gene sequence; it differed by a single base substitution out of 544 bp from that of CLIB 1764T, while the ITS sequences that these three strains shared differed by three substitutions out of 608 bp. CLIB 1780 has a D1/D2 LSU rRNA gene sequence identical to that of strain UFMG-CM-Y273T and the other strains isolated from rotten wood in Brazil and two base pair difference out of 575 bp in ITS sequences (Table S2). These results may indicate that all these strains belong to the same taxon.

Pairwise comparisons of CLIB 1764T D1/D2 LSU rRNA gene sequence with that of the type strains of the most similar species showed three substitutions out of 535 bp with K. exigua CBS 379NT, four substitutions out of 536 bp with Kazachstania turicensis CBS 8665T, five substitutions out of 509 bp with K. wufongensis BCRC 23138T, six substitutions out of 536 bp with K. bulderi CBS 8638T and seven substitutions out of 536 bp with K. barnetti CBS 5648. Sequence divergence of the ITS between the French sourdough strain CLIB 1764T and related species was much greater, as there were 45 substitutions out of 431 bp between the ITS of CLIB 1764T and that of the type strain K. exigua CBS 379NT. Likewise, we observed 55 substitutions out of 431 bp between the Brazilian strains and the K. exigua CBS 379NT ITS sequence. CLIB 1764T and UFMG-CM-Y273T ITS sequences differed from K. barnetti CBS 5648 by 8.1 and 8.3% differences, respectively (Table S2). These results indicate that the French and Brazilian strains may belong to an as yet undescribed species. The use of sequences from rRNA genes for species delineation may be misleading because they may provide poor discrimination, as already observed for the genus Debaryomyces for example (Groenewald et al., 2008; Jacques et al., 2009; Nguyen et al., 2009). This observation and the need for a more robust phylogeny between these species in this part of the Kazachstania clade prompted us to analyse other sequences, such as the protein coding gene sequences RBPI, RBP2 and EF-1α.

Unlike the almost identical D1/D2 LSU rRNA gene and ITS sequences in the French and Brazilian strains, the divergences between the sequences of CLIB1764T and UFMG-CM-Y273T were 22 (3.3%), 45 (6%) and 113 (11.7%), respectively, when RBPI, RPB2 and EF-1α sequences were compared (Table S2). These results indicate that the two taxa are clearly distinct. Strains CLIB 1764T and CLIB 1780 had identical sequences for these three genes. Strains UFMG-CM-Y273T and UFMG-CM-Y451 have an identical RPB2 sequence; UFMG-CM-Y452 sequence diverged by two base pairs, whereas the three Brazilian strains shared the same RBPI sequence. Although the French and the Brazilian strains have almost identical D1/D2 and ITS sequences, the two groups of strains belong to two distinct taxa on
the basis of the RPB1, RPB2 and EF-1α coding genes. We propose to describe two novel species to accommodate these strains: Kazachstania saulgeensis sp. nov. (type strain CLIB 1764T and CLIB 1780) and Kazachstania serrabonitensis sp. nov. (type strain UFMG-CM-Y273T, UFMG-CM-Y451 and UFMG-CM-Y452). Type strain of K. saulgeensis differed from its closest relatives and K. saulgeensis CLIB 1780 by its inability to ferment glucose and galactose. On the other hand, K. saulgeensis and K. serrabonitensis, albeit weakly for the latter, are the only species which can utilize cadaverine as the sole nitrogen source (Table 1).

Several species of Kazachstania (Kazachstania unispora, Kazachstania servazzii, K. bulderi, K. barnettii, K. exigua, C. humilis and K. saulgeensis) have been isolated from fermented products, such as fermented onion, kefir grains or bread sourdoughs (Vardjan et al., 2013; Cheng et al., 2014; De Vuyst et al., 2014). K. saulgeensis was only detected in one of the 15 French natural wheat sourdoughs that were investigated at the same time (Lhomme et al., 2016). However, K. exigua, the most closely related species to K. saulgeensis on the basis of D1/D2 LSU rRNA gene, has been found in rye, teff and wheat sourdoughs (De Vuyst et al., 2014). This species has been isolated from wheat sourdoughs of geographically distant countries (China, USA, Ethiopia, Germany, Italy and France). Given the poor discrimination provided by the D1/D2 LSU rRNA gene sequence in this Kazachstania subclade, it is possible that some of the K. exigua strains may have been misidentified.

Several species of Kazachstania have been recovered from plant materials (Vaughan-Martini et al., 2011). K. serrabonitensis was isolated from rotting wood samples collected from an Atlantic rainforest site in Brazil. However, in extensive sampling in other Atlantic rainforest sites, we have isolated other strains of this new species also associated with rotting wood (C. A. Rosa, unpublished results). Probably, this species is a minor component of the yeast community associated with this substrate. K. serrabonitensis probably grows in the rotting wood assimilating simple sugars, such as glucose, released by the microbial hydrolysis of cellulose. This species could be vectored by insects that visit rotting wood for feeding, such as beetles.

C. humilis and C. milleri were considered to be conspecific (Vaughan-Martini, 1995; Kurtzman & Robnett, 1998), since C. humilis CBS 5658T and C. milleri CBS 6897T.

### Table 1. Comparison of selected phenotypic properties of species assigned to Kazachstania with the three novel species

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Species: 1, K. barnettii; 2, K. bulderi; 3, K. exigua; 4, K. turicensis; 5, K. rupicola; 6, K. wufongensis; 7, C. humilis; 8, C. pseudohumilis; 9, C. milleri; 10, K. saulgeensis sp. nov.; 11, K. serrabonitensis sp. nov.; 12, K. australis sp. nov. CLIB 162T. Data for K. barnettii, K. bulderi, K. exigua, K. turicensis, C. humilis and C. milleri are from Vaughan-Martini et al. (2011), for K. rupicola are from Safar et al. (2013), for K. wufongensis are from Lee et al. (2009) and for C. pseudohumilis are from Wang et al. (2009). All species, but C. humilis and some K. turicensis strains, assimilate sucrose. In addition, K. turicensis and C. humilis cannot assimilate raffinose. Assimilation of glycerol varies according to species. K. turicensis and some strains of K. bulderi cannot ferment galactose. Three species cannot ferment raffinose: K. turicensis, K. wufongensis and C. milleri. K. bulderi is the only species capable of assimilating maltose, xylose and mannose. C. humilis is the only species which does not grow at 30°C; the others do not grow at 37°C, but K. bulderi can grow up at 40°C together with some strains from K. turicensis. +, Positive; –, negative; V, variable; W, weak; s, slow; ND, not determined.
differed by only one substitution in the D1/D2 LSU rRNA gene. Kurtzman & Robnett (2003) detected 10 differences in the ITS and 11 differences in the EF-1α gene sequences of these two strains and they were designated as separated species on the basis of this sequence comparison (Kurtzman & Robnett, 2003; Lachance et al., 2011). However, in contrast to the claim by Kurtzman & Robnett (2003), we found only three differences out of 1127 bp in the EF-1α gene sequence (0.2%). By comparing the sequences of the genes coding for \( \text{RPB1} \) and \( \text{RPB2} \) of the type strains of these two species, we found a divergence amounting to nine substitutions (1.5%) and eight substitutions (1.2%), respectively. A number of unresolved base pairs in the sequence of \( C. \text{humilis} \) CLIB 1323\(^T \), six out of the nine differences observed between CLIB 1323\(^T \) and CBS 6897\(^T \) in \( \text{RPB1} \) and seven out of eight differences in \( \text{RPB2} \), may indicate a certain level of heterozygosity in this strain, which therefore amplifies the divergence between the two type strains. Interestingly, the level of intra-specific diversity among \( C. \text{milleri} \) strains was shown to be higher than the divergence between \( C. \text{humilis} \) CBS 5658\(^T \) and \( C. \text{milleri} \) CBS 6897\(^T \) (Vigentini et al., 2014), suggesting that these isolates may be part of a species complex. Species complexes comprising haploid, aneuploid and diploid mosaic lineages are common, as shown for \( \text{Debaryomyces} \text{hansenii} \) (Jacques et al., 2010), \( \text{Milloecyma} \text{farinosa} \) (Louis et al., 2012; Mallet et al., 2012) and \( \text{Zygosachymomyces} \text{rouxii} \) (Gordon & Wolfe, 2008; Solieri et al., 2013). Heterozygosity within the \( C. \text{humilis} \) strain CBS 5658\(^T \) markers that we observed is in agreement with this hypothesis, although our measures of DNA content of \( C. \text{humilis} \) CBS 5658\(^T \) using flow cytometry indicated a 1n content (data not shown). Despite physiological differences between the two type strains, such as the inability of \( C. \text{milleri} \) CBS 6897\(^T \) to utilize sucrose and raffinose, we chose to be conservative and considered, as proposed by Vaughan-Martini (1995), that the two taxa are conspecific on the basis of a limited sequence divergence.

It was also proposed that \( K. \text{exigu}a \) type strain CBS 379\(^{NT} \) may also be alloplid or hybrid on the basis of the level of gene redundancy deduced from partial genome sequencing (Bon et al., 2000). The \( \text{RPB1} \) gene of CBS 379\(^{NT} \) that we have sequenced carried a deletion of 216 bp (Table S2). This could indicate that the gene that we sequenced may not be functional. If this is the case, \( \text{RPB1} \) being an essential gene, another \( \text{RPB1} \) gene exists in CBS 379\(^{NT} \), in congruence with an eventual allopolyploid or a hybrid nature of this strain.

Considering the diversity of the \( \text{Kazachstania} \) subclade harbouring \( K. \text{exigu}a \), we analysed a number of strains maintained in various collections. One of them, strain CLIB 162\(^T \) (=CBS 2141\(^T \)) isolated from soil in South Africa, was previously assigned to the species \( K. \text{exigu}a \). The rDNA sequence divergence when CLIB 162\(^T \) and \( K. \text{exigu}a \) CBS 379\(^{NT} \) were compared is 4 bp for D1/D2 LSU rRNA gene and 22 substitutions and 2 indels for ITS (Table S2). These observations indicated that CLIB 162\(^T \) may belong to a novel taxon. This was confirmed by divergence in the \( \text{RPB1}, \text{RPB2} \) and \( \text{EF-1α} \) genes with 8, 15 and 36 substitutions, respectively. We propose the novel species \( \text{Kazachstania australis} \) (type strain CLIB 162\(^T \)=CBS 2141\(^T \)) to accommodate this strain.

The large majority of the species of the \( \text{Kazachstania} \) clade have been isolated from soil in various areas of the globe (Vaughan-Martini et al., 2011). Some of the species, such as \( \text{Kazachstania picea} \), have been specifically associated with rhizosphere, but for most of them, their association with a specific soil substratum is unknown.

The phylogenetic placement in the \( \text{Kazachstania} \) subclade, including \( K. \text{rupicola}, K. \text{exigu}a, K. \text{wufongensis}, K. \text{bulderi}, K. \text{turicensis}, K. \text{barnettii}, C. \text{pseudohumilis}, C. \text{humilis} \) and \( C. \text{milleri} \), of the three novel species that we describe here is shown in a phylogenetic tree reconstructed from the concatenation of the markers D1/D2 LSU rRNA gene, \( \text{RPB1}, \text{RPB2} \) and \( \text{EF-1α} \) gene sequences (Fig. 1).

### Description of \( \text{Kazachstania saulgeensis} \)

**Jacques, Sarilar, Urien, Perrin, Tinsley, Sicard & Casaregola sp. nov.**

The species epithet \( \text{saulgeensis} \) (saul.ge.en’sis. N.L. fem. adj. \( \text{saulgeensis} \) refers to the town Saulgé, Poitou-Charentes region, France, from where the type strain was isolated).

After 2 days on YPD agar at 25 °C, the colonies are white to cream coloured, smooth and semi-glossy with faint striations, and with an undulating margin. Cells are globose or slightly ovoid (5–7×6–9 μm) and occur singly or in pairs. Budding is multipolar. After 7 days at 20 °C on corn meal agar, neither pseudomycelium nor true mycelium is formed. Asc containing one or two globose ascospores were observed on McClary’s yeast agar after 7 days at 20 °C (Fig. 2). Maximum growth temperature is 30 °C. D-Galactose, glucose, sucrose and trehalose are fermented, but cellobiose, lactose, maltose, melezitose and melibiose are not fermented. Fermentation of raffinose is variable. D-Galactose, D-glucose, raffinose, sucrose and trehalose are assimilated. L-Arabinose, cellobiose, erythritol, glycerol, N-acetylglucosamine, 2-keto-D-gluconate, glucosamine, D-glucuronate, inositol, DL-lactate, lactose, maltose, mannitol, melezitose, melibiose, methyl α-D-glucoside, L-rhamnose, ribose, D-sorbitol, L-sorbose, sodium glucuronate and D-xylose are not assimilated. Potassium nitrate, sodium nitrite, glucosamine, L-lysine, cadaverine, creatine and creatinine are assimilated. Ethylamine is not assimilated. Growth in 10% (w/v) NaCl and in 50% glucose is positive. Growth in 0.01% cycloheximide and in 60% glucose is negative. Production of acetic acid is negative. Urea hydrolysis is negative. Physiological data are from strains CLIB 1764\(^T \) and CLIB 1780.

The type culture, strain CLIB 1764\(^T \), is preserved as a cryopreparation and a lyophilized preparation in the Biological Resource Center CIRM-Levures, INRA/ArboParisTech, 78350 Jouy-en-Josas, France and has also been deposited in...
the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, under the number CBS 14374T. MycoBank accession number: MB 815524.

Description of Kazachstania serrabonitensis Lopes, C. G. Morais, Uetanabaro, Jacques, Casaregola & Rosa sp. nov.

Kazachstania serrabonitensis (ser. ra. bo. ni. ten'sis N.L. fem. adj. serrabonitensis refers to the Serra Bonita reserve located in southern Bahia, Brazil, from where the type strain was isolated).

After 2 days on YPD agar at 25°C, the colonies are cream coloured, smooth and semi-glossy with faint striations, and with an undulating margin. Cells are globose or slightly ovoid (4–5×5–6 μm) and occur singly or in pairs. Budding is multipolar. After 7 days at 20°C on corn meal agar, neither pseudomycelium nor true mycelium is formed. Asci containing one or four globose ascospores were observed on McClary’s acetalte agar after 7 days at 20°C (Fig. 2). Maximum growth temperature is 30°C. D-Galactose (delay), D-glucose, raffinose, sucrose and trehalose are fermented. Cellobiose, lactose, maltose, melezitose and melibiose are not fermented. D-Galactose, D-glucose, raffinose, sucrose and trehalose are assimilated. L-Arabinose, cellobiose, erythritol, N-acetylglucosamine, 2-keto-D-gluconate, glucosamine, D-glucuronate, glycerol, inositol, D-lactate, malate, mannitol, melezitose, melibiose, methyl α-D-glucoside, D-l-rhamnose, D-ribose, D-sorbitol, L-sorbose, sodium gluconate and D-xylose are not assimilated. Potassium nitrate (weak), sodium nitrite (weak), glucosamine (weak), L-lysine, cadaverine (weak), creatine (weak) and creatinine (weak) are assimilated. Ethylamine is not assimilated. Growth in 10% NaCl (w/v) is positive. Growth in 0.01% cycloheximide and in 50% glucose is negative. Production of acetic acid is negative. Urea hydrolysis is negative. Physiological data are from strains UFMG-CM-Y273T, UFMG-CM-Y451 and UFMG-CM-Y452. The type culture, strain UFMG-CM-Y273T, is preserved as a cryo-preparation and a lyophilized preparation in the Biological Resource Center CIRM-Levures, INRA/AgroParisTech, 78350 Jouy-en-Josas, France under the number CLIB 1783T and has also been deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, under the number CBS 14236T. MycoBank accession number: MB 815525.

Fig. 1. Phylogenetic placement of K. saulgeensis sp. nov., K. serrabonitensis sp. nov. and K. australis sp. nov. among related species with the maximum-likelihood analysis of concatenated D1/D2 LSU rRNA, RPB1, RPB2 and EF-1α genes. The evolutionary distances were computed using the General Time Reversible model. The analysis involved 13 nucleotide sequences with a total of 2360 positions. Z. rouxii CBS 732NT was used as the outgroup. All positions containing gaps and missing data were eliminated. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Percentage bootstrap values are given for 1000 replicates. Bar, 5 changes per 100 nucleotides.
Description of Kazachstania australis Jacques, Sarilar & Casaregola sp. nov.

The species epithet australis (aus.tralis. N.L. fem. adj. australis refers to South Africa, from where the type strain was isolated).

After 2 days on YPD agar at 25 °C, the colonies are cream coloured, smooth and semi-glossy with faint striations, and with a slightly undulating margin. Cells are globose to ellipsoid (6–7×8–10 µm) and occur singly or in mother-bud pairs. Budding is multipolar. After 7 days at 20 °C on corn meal agar, neither pseudomycelium nor true mycelium is formed. Asci containing one to four globose ascospores were observed on McClary’s acetate agar after 7 days at 20 °C (Fig. 2). Maximum growth temperature is 35 °C. D-Galactose, D-glucose, raffinose, sucrose and trehalose are fermented. Cellobiose, lactose, maltose, melezitose and melibiose are not fermented. D-Galactose, D-glucose, raffinose, sucrose, trehalose and ethanol are assimilated. L-Arabinose, cellobiose, erythritol, N-acetylglucosamine, 2-keto-D-gluconate, glucosamine, D-gluconate, glycerol, inositol, DL-lactate, lactose, mannose, mannan, mannitol, melezitose, melibiose, methyl α-D-glucoside, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, sodium gluconate, D-xylose and methanol are not assimilated. Ethylamine, Potassium nitrate, sodium nitrite, glucosamine, L-lysine, cadaverine, creatine and creatinine are not assimilated. Growth in 10 % NaCl (w/v) is positive. Growth in 0.01 % cycloheximide and in 50 % glucose is negative. No starch-like substance is produced. Production of acetic acid is negative. Urea hydrolysis and diazonium blue B reactions are negative. Physiological data are from strain CLIB 162T.

The type culture, strain CLIB 162T, is preserved as a cryopreparation and a lyophilized preparation in the Biological Resource Center CIRM-Levures, INRA/AgroParisTech, 78350 Jouy-en-Josas, France and also in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, where it was originally deposited under the number CBS 2141T. Mycobank number: MB 815523.

New combinations

Yarrow et al. (1978) transferred all the species of the genus Torulopsis to the genus Candida because the distinction between Torulopsis and Candida, which was based on the absence or presence of pseudohyphae, was not satisfactory. This included Candida humilis described earlier by Nel & van der Walt (1968). Candida pseudohumilis did not show sporulation (Wang et al., 2009). The International Code of Nomenclature for algae, fungi and plants (Melbourne Code) permits related species, including anamorphic, to be assigned to the same genus (McNeill, 2012). Phylogenetic placement of these two species clearly indicate they belong to the Kazachstania clade on the basis of D1/D2
Kazachstania humilis (E.E. Nel & van der Walt) Jacques, Sarilar & Casaregola comb. nov.

Type strain: CBS 5658T (CLIB 1323T).
MycoBank number: MB 815526.


Type strain: CBS 11404T (CLIB 1974T).
MycoBank number: MB 815527.

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