

Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces* and other members of the Saccharomycetaceae, and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygotorulaspora*

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Abstract

Genera currently assigned to the Saccharomycetaceae have been defined from phenotype, but this classification does not fully correspond with species groupings determined from phylogenetic analysis of gene sequences. The multigene sequence analysis of Kurtzman and Robnett [FEMS Yeast Res. 3 (2003) 417–432] resolved the family Saccharomycetaceae into 11 well-supported clades. In the present study, the taxonomy of the Saccharomycetaceae is evaluated from the perspective of the multigene sequence analysis, which has resulted in reassignment of some species among currently accepted genera, and the proposal of the following five new genera: *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygotorulaspora*.

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1. Introduction

The name *Saccharomyces* was proposed for bread and beer yeasts by Meyen in 1838 [1], but it was Reess in 1870 [2] who first defined the genus. As additional species were discovered and assigned to *Saccharomyces*, subgroups differing in morphology and physiology were recognized. The presence of these subgroups led to the description of *Zygosaccharomyces* by Barker in 1901 [3] and to *Torulaspora* by Lindner in 1904 [4]. Stelling-Dekker [5] accepted *Torulaspora* and recognized *Zygosaccharomyces* as a subgenus of *Saccharomyces*, but the distinction between these taxa was not always clear because some species have intermediate phenotypes. Lodder and Kreger-van Rij [6], as well as van der Walt [7], argued that it was not possible to separate *Torulaspora* and *Zygosaccharomyces* from *Saccharomyces* until additional taxonomic characters were found to

support the maintenance of three distinct genera. Yarrow [8–10] revived the concept of three genera and separated *Torulaspora* and *Zygosaccharomyces* from *Saccharomyces*, although species assignments were often difficult. One of the most apparent morphological characters among species of the ‘*Saccharomyces* complex’ is the ascus. Some species have persistent asci whereas others have deliquescent asci that release their ascospores at maturity. Van der Walt [11] described the genus *Kluyveromyces* based on *K. polysporus*, later expanding the genus to include all members of the ‘*Saccharomyces* complex’ that produce deliquescent asci [12].

With the introduction of nuclear-DNA reassociation techniques, a number of studies demonstrated that species demarcation from phenotype was often incorrect. Applying this method, Price et al. [13] found nine species variously assigned to *Torulaspora* or *Saccharomyces* to be conspecific with *Torulaspora delbrueckii*, and Vaughan-Martini and Kurtzman [14] showed that 16 previously described *Saccharomyces* species were conspecific with *S. cerevisiae*. With the foregoing precedent, it is not surprising that gene sequence comparisons have shown that

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species assignments among genera of the family Saccharomycetaceae are often incorrect. From 18S rDNA analyses, species of *Kluyveromyces* and *Zygosaccharomyces* were seen to be interspersed with *Saccharomyces* species [15]. Comparisons from cytochrome oxidase II (COX II) [16] and from domains 1 and 2 (D1/D2) of large-subunit (26S) rDNA [17] showed the same heterogeneity. However, none of these single-gene sequence analyses provided strong support for basal lineages, leaving in doubt relationships among more divergent species. Kurtzman and Robnett [18] analyzed relationships among species of the ‘*Saccharomyces* complex’ from sequences of 18S, ITS, 5.8S and 26S rDNAs, translation elongation factor 1- α (EF1- α), mitochondrial small-subunit rDNA and COX II. As with previous studies, single-gene phylogenies did not resolve divergent lineages, but analysis of the combined sequences resolved the ca. 80 species compared into 14 well-supported clades. Support for basal branches leading to these 14 clades was generally not strong, but was suggestive that the clades could be assigned to three families, the Saccharomycetaceae, the Eremotheciaceae, and the Saccharomycodaceae.

Examination of the 11 clades that comprise the Saccharomycetaceae shows that most presently accepted genera include species from other genera (Fig. 1). Most notably, *Kluyveromyces* species are found in six clades, demonstrating that the key character for this genus, ascus deliquescence, has no phylogenetic basis. This is not the first time that ascus deliquescence was shown to be phylogenetically incongruent. Species of *Debaryomyces* characteristically have persistent asci, but *D. udenii* is an exception, which has led to concerns of misclassification. Placement of *D. udenii* in *Debaryomyces*, however, has been supported by rDNA sequence analysis [17,19].

A long-standing goal of yeast systematists has been to develop a classification system based on natural relationships, thus providing genetic homogeneity and predictiveness to taxon names. This has not been possible when using phenotypic characters, but the opportunity to achieve this goal now appears attainable through phylogenetic analysis of gene sequences. A major problem in utilizing this new information is determining the basis for defining taxa. Avise and Johns [20] proposed a standardized scheme of biological classification based on temporal emergence of taxa. They acknowledged, however, that there is neither sufficient well-dated fossil evidence nor are there sufficient gene sequences to accurately date evolutionary events to provide the time scale necessary for this proposal. Another issue is that of missing taxa. The vast majority of yeast species, as well as other microorganisms, are yet to be discovered, and this limited sampling impacts the interpretation of present taxonomic groupings. One likely outcome is that somewhat divergent phylogenetically defined genera will be further divided as additional species are discovered, and that monotypic genera established for isolated species will expand in size as more

species are found. Consequently, genera defined phylogenetically from presently known species will be subject to future modification, but establishing a phylogenetic framework now will provide direction to future work.

Kurtzman and Robnett [18] observed that the extent of resolution from different gene sequences varied among clades of the Saccharomycetaceae with the primary effect being strength of branch support on phylogenetic trees rather than disparate evolutionary histories. Phylogenetic trees constructed from multiple genes have far greater bootstrap support than do single-gene trees, which indicates that each gene sequence is conveying the same evolutionary history and contributing to the strength of the signal. Combining data has been predicted to increase phylogenetic accuracy by increasing signal and dispersing noise [21], and any informational conflicts between genes are not expected to increase statistical support for affected nodes [22]. An alternate approach would be to use whole-genome sequence comparisons to achieve more robust species phylogenies, which should be possible in the near future for taxonomic groups of the size compared here. However, because multigene phylogenies are likely to be an accurate reflection of evolutionary history, whole-genome comparisons would be expected to provide a refinement of the present work rather than result in major changes.

Analysis of the multigene dataset presented by Kurtzman and Robnett [18] showed each of the 11 clades of the Saccharomycetaceae to be similarly diverged from one another. Some of the clades, such as *Saccharomyces*, *Torulaspora* and *Zygosaccharomyces*, as well as *Eremothecium* from the Eremotheciaceae, are recognized from phenotype as well as from phylogenetic analysis. Using these genera as exemplars, the remaining phylogenetically defined clades have been interpreted as genera. To apply the new gene sequence data to development of a phylogenetic system for classification, five new genera and various new combinations are proposed.

2. Materials and methods

2.1. Organisms

The species compared are represented by their type strains or equivalent authentic strains when type material was a drawing or a herbarium specimen. The strains compared are listed in Table 1 with culture collection accession numbers.

2.2. Phylogenetic analysis

The phylogenetic analysis used for the taxonomic proposals presented is represented by figure 9 of Kurtzman and Robnett [18] and reproduced here as Fig. 1. As described in that study, the phylogenetic tree was derived

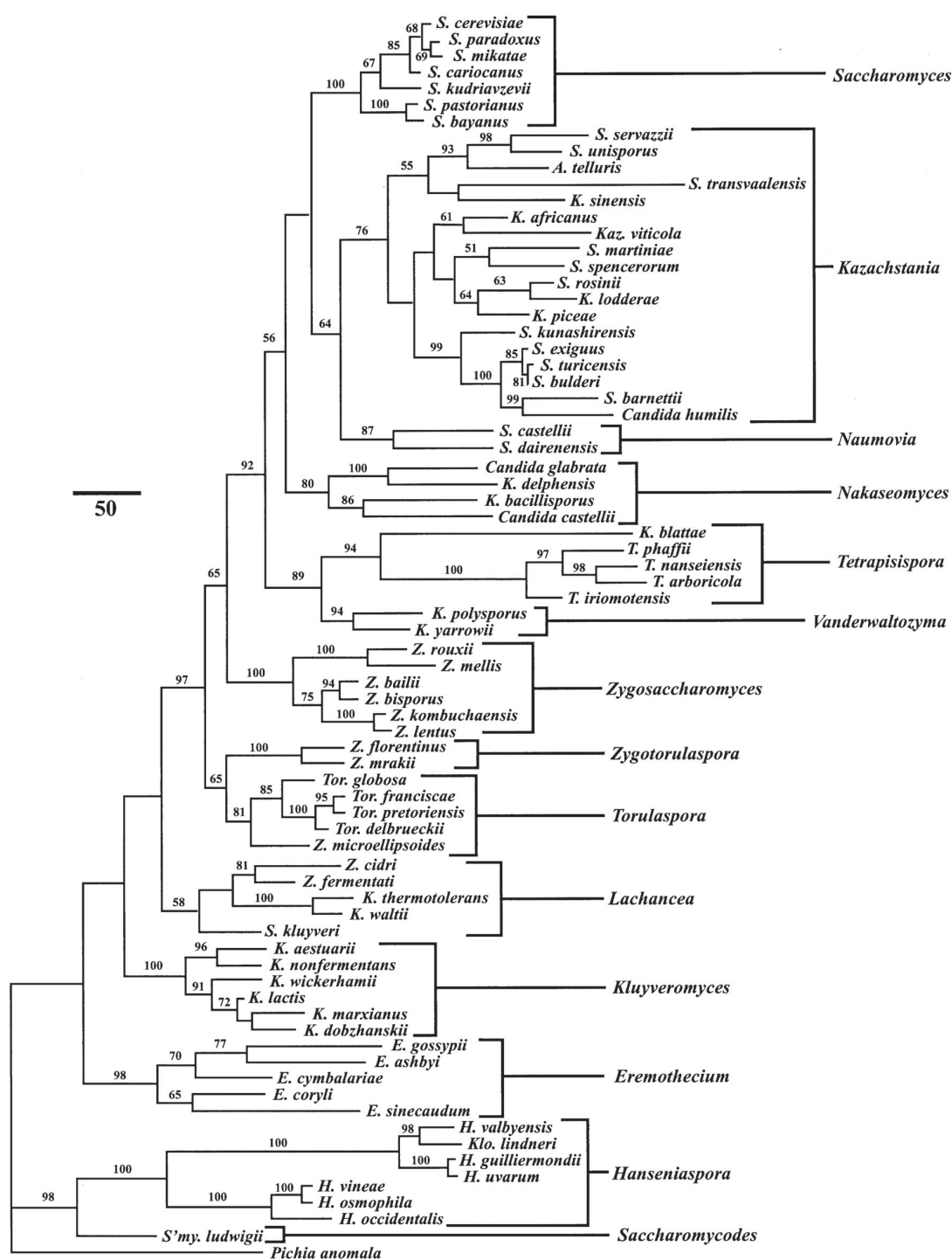


Fig. 1. Phylogenetic tree resolving species of the 'Saccharomyces complex' into clades, which are proposed as phylogenetically circumscribed genera. This is one of three most parsimonious trees derived from maximum-parsimony analysis of a dataset comprised of nucleotide sequences from 18S, 5.8S/alignable ITS, and 26S (three regions) rDNAs, EF-1 α , mitochondrial small-subunit rDNA and COX II [18]. Branch lengths, based on nucleotide substitutions, are indicated by the bar. Bootstrap values $\geq 50\%$ are given. *Pichia anomala* is the outgroup species, and all species are analyzed from type strains.

Table 1
Species compared

Species ^a	Accession numbers ^{b,c}	
	NRRL	Other
<i>Arxiozyma telluris</i>	YB-4302 ^T	CBS 2685
<i>Candida castellii</i>	Y-17070 ^T	CBS 4332
<i>C. glabrata</i>	Y-65 ^T	CBS 138
<i>C. humilis</i>	Y-17074 ^T	CBS 5658
<i>Eremothecium ashbyi</i>	Y-1363 ^A	
<i>E. (Nematospora) coryli</i>	Y-12970 ^T	CBS 2608
<i>E. cymbalariae</i>	Y-17582 ^A	CBS 270.75
<i>E. (Ashbya) gossypii</i>	Y-1056 ^A	CBS 109.51
<i>E. (Holleya) sinicaudum</i>	Y-17231 ^T	CBS 8199
<i>Hanseniaspora guilliermondii</i>	Y-1625 ^T	CBS 465
<i>H. (Kloeckeraspora) occidentalis</i>	Y-7946 ^T	CBS 2592
<i>H. (Kloeckeraspora) osmophila</i>	Y-1613 ^T	CBS 313
<i>H. uvarum</i>	Y-1614 ^T	CBS 314
<i>H. valbyensis</i>	Y-1626 ^T	CBS 479
<i>H. (Kloeckeraspora) vineae</i>	Y-17529 ^T	CBS 2171
<i>Kazachstania viticola</i>	Y-27206 ^T	CBS 6463
<i>Kloeckera lindneri</i>	Y-17531 ^T	CBS 285
<i>Kluyveromyces aestuarii</i>	YB-4510 ^T	CBS 4438
<i>K. africanus</i>	Y-8276 ^T	CBS 2517
<i>K. bacillisporus</i>	Y-17846 ^T	CBS 7720
<i>K. blattae</i>	Y-10934 ^T	CBS 6284
<i>K. delphensis</i>	Y-2379 ^T	CBS 2170
<i>K. dobzhanskii</i>	Y-1974 ^T	CBS 2104
<i>K. lactis</i> var. <i>lactis</i>	Y-8279 ^T	CBS 683
<i>K. lodderae</i>	Y-8280 ^T	CBS 2757
<i>K. marxianus</i>	Y-8281 ^T	CBS 712
<i>K. nonfermentans</i>	Y-27343 ^T	JCM 10232
<i>K. piceae</i>	Y-17977 ^T	CBS 7738
<i>K. polysporus</i>	Y-8283 ^T	CBS 2163
<i>K. sinensis</i>	Y-27222 ^T	CBS 7660
<i>K. thermotolerans</i>	Y-8284 ^T	CBS 6340
<i>K. waltii</i>	Y-8285 ^T	CBS 6430
<i>K. wickerhamii</i>	Y-8286 ^T	CBS 2745
<i>K. yarrowii</i>	Y-17763 ^T	CBS 8242
<i>Saccharomyces barnettii</i>	Y-27223 ^T	CBS 6946
<i>S. bayanus</i>	Y-12624 ^T	CBS 380
<i>S. bulderi</i>	Y-27203 ^T	CBS 8638
<i>S. cariocanus</i>	Y-27337 ^T	NCYC 2890
<i>S. castellii</i>	Y-12630 ^T	CBS 4309
<i>S. cerevisiae</i>	Y-12632 ^{NT}	CBS 1171
<i>S. dairenensis</i>	Y-12639 ^T	CBS 421
<i>S. exiguus</i>	Y-12640 ^{NT}	CBS 379
<i>S. humaticus</i>		IFO 10673 ^T
<i>S. kluyveri</i>	Y-12651 ^T	CBS 3082
<i>S. kudriavzevii</i>	Y-27339 ^T	IFO 1802
<i>S. kunashirensis</i>	Y-27209 ^T	CBS 7662
<i>S. martiniae</i>	Y-409 ^T	CBS 6334
<i>S. mikatae</i>	Y-27341 ^T	IFO 1815
<i>S. naganishii</i>		IFO 10181 ^T
<i>S. paradoxus</i>	Y-17217 ^{NT}	CBS 432
<i>S. pastorianus</i>	Y-27171 ^{NT}	CBS 1538
<i>S. rosinii</i>	Y-17919 ^T	CBS 7127
<i>S. servazzii</i>	Y-12661 ^T	CBS 4311
<i>S. spencerorum</i>	Y-17920 ^T	CBS 3019
<i>S. (Pachytichospora) transvaalensis</i>	Y-17245 ^T	CBS 2186
<i>S. turicensis</i>	Y-27345 ^T	CBS 8665
<i>S. unisporus</i>	Y-1556 ^T	CBS 398
<i>S. yakushimaensis</i>		IFO 1889 ^T
<i>Saccharomycodes ludwigii</i>	Y-12793 ^T	CBS 821
<i>Tetrapisispora arboricola</i>	Y-27308 ^T	IFO 10925
<i>T. iriomotensis</i>	Y-27309 ^T	IFO 10929
<i>T. nanseiensis</i>	Y-27310 ^T	IFO 10899

Table 1 (Continued).

Species ^a	Accession numbers ^{b,c}	
	NRRL	Other
<i>T. phaffii</i>	Y-8282 ^T	CBS 4417
<i>Torulaspora delbrueckii</i>	Y-866 ^T	CBS 1146
<i>T. franciscae</i>	Y-17532 ^T	CBS 2926
<i>T. globosa</i>	Y-12650 ^T	CBS 764
<i>T. pretoriensis</i>	Y-17251 ^T	CBS 2187
<i>Zygosaccharomyces bailii</i>	Y-2227 ^T	CBS 680
<i>Z. bisporus</i>	Y-12626 ^T	CBS 702
<i>Z. cidri</i>	Y-12634 ^T	CBS 4575
<i>Z. fermentati</i>	Y-1559 ^T	CBS 707
<i>Z. florentinus</i>	Y-1560 ^T	CBS 746
<i>Z. kombuchaensis</i>	YB-4811 ^T	CBS 8849
<i>Z. lentus</i>	Y-27276 ^T	CBS 8574
<i>Z. mellis</i>	Y-12628 ^T	CBS 736
<i>Z. microellipsoides</i>	Y-1549 ^T	CBS 427
<i>Z. mrakii</i>	Y-12654 ^T	CBS 4218
<i>Z. rouxii</i>	Y-229 ^T	CBS 732
Reference species		
<i>Pichia anomala</i>	Y-366 ^{NT}	CBS 5759

^aCommonly recognized synonym names are given in parentheses.

^bT = type strain, NT = neotype strain, A = authentic strain, the reference strain used when there is no living type or ex-type strain.

^cNRRL = ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA; CBS = Centraalbureau voor Schimmelmecultures, Utrecht, The Netherlands; JCM = Japan Collection of Microorganisms, Saitama, Japan; IFO = Institute for Fermentation, Osaka, Japan; NCYC = National Collection of Yeast Cultures, Norwich, UK.

from maximum-parsimony analysis of a dataset comprised of nucleotide sequences from 18S, 5.8S/alignable ITS, and 26S (three regions) rDNAs, translation elongation factor EF-1 α , mitochondrial small-subunit rDNA and COX II. Analyses were made using PAUP* 4.063a [23], and bootstrap values were based on 1000 replications. GenBank accession numbers for all nucleotide sequences analyzed were previously reported [18].

Three recently described species of *Saccharomyces*, i.e. *S. humaticus*, *S. naganishii*, and *S. yakushimaensis* were not included in the work of Kurtzman and Robnett [18], but are included in the present study. Phylogenetic placement of these three new species near *Saccharomyces transvaalensis* and *Kluyveromyces sinensis* was determined from maximum-parsimony analysis of D1/D2 26S rDNA sequences that were provided in the original descriptions of these species [24].

3. Results and discussion

The 11 clades of the Saccharomycetaceae resolved from multigene phylogenetic analysis are shown in Fig. 1 with proposed species assignments to phylogenetically circumscribed genera. Table 2 is a compilation of intra- and intergeneric divergence among the species compared. Not unexpectedly, the clades vary in size with intrageneric distances often reflecting the number of species in each clade.

The proposed genus *Zygorulasporea* has just two species, which are separated by 52 nucleotide differences, whereas *Zygosaccharomyces* has six species with a divergence of 154 nucleotides and *Eremothecium* has five species with a divergence of 331 nucleotides. Do these clades represent genera? When phylogenetically circumscribed, the genera *Saccharomyces*, *Torulaspora*, *Zygosaccharomyces* and *Eremothecium* can also be recognized from phenotype. Several of the other clades are less easily recognized from available phenotypic data, but genetically, they are just as well defined as *Saccharomyces*. Consequently, these clades, although phenotypically somewhat heterogeneous, appear to be phylogenetically circumscribed genera. The following proposals of phylogenetically circumscribed genera also include a phenotypic description of the taxa. Because some of the genera are difficult to recognize from phenotype, a key is provided. Individual species descriptions that include known synonyms are given in *The Yeasts*, A Taxonomic Study, 4th edition [25–32] and in *Yeasts of the World* [33].

3.1. Accepted taxa and proposed new genera and new combinations for species of the *Saccharomycetaceae*

3.1.1. *Kazachstania* Zubkova (1971)

3.1.1.1. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Pseudo-

hyphae may be formed, but true hyphae are not produced.

Ascospore formation. Asci may be unconjugated or show conjugation between independent cells or between a cell and its bud. Asci may be deliquescent or persistent and produce 1–16 or more ascospores that are spherical, ovoidal or elongate. Ascospore surfaces may be roughened or smooth.

Physiology/biochemistry. Glucose is fermented and most species ferment and assimilate galactose. Cadaverine, L-lysine and ethylamine are seldom utilized as nitrogen sources. Nitrate is not utilized. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. The *Kazachstania* clade, although moderately well supported basally, has a relatively large number of poorly supported internal nodes. Besides the genes analyzed for Fig. 1, Kurtzman and Robnett [18] also sequenced actin-1 and RNA polymerase II in an unsuccessful attempt to better resolve internal lineages. The species *Kluyveromyces africanus*, *Kazachstania viticola* and *Saccharomyces martiniae* are particularly subject to movement within the clade, depending on the outgroup used in phylogenetic analysis. For this reason, the entire clade is treated as a single genus, but it seems likely that the clade will resolve into three main lineages if a larger number of gene sequences are included in the phylogenetic analysis.

The genus *Kazachstania* was validly described by Zubkova in 1971 [34] and therefore has taxonomic priority

Table 2

Extent of intragenomic and intergeneric nucleotide changes among members of the *Saccharomycetaceae*, *Eremotheciaceae* and *Saccharomycodaceae* from analysis of a multigene dataset^a

Genus	Intragenomic nucleotide changes	Intergeneric nucleotide changes												
		Kaz.	Nau.	Nak.	Tet.	Van.	Zyg.	Z'tor.	Tor.	Lac.	Klu.	Ere.	Han.	S'my.
<i>Saccharomyces</i> (7) ^b	88	101	104	96	181	160	166	206	177	194	232	270	416	412
<i>Kazachstania</i> (21)	345		75	115	200	179	185	225	196	213	251	289	435	431
<i>Naumovia</i> (2)	106			118	203	182	188	228	199	216	254	292	438	434
<i>Nakaseomyces</i> (4)	197				155	134	140	180	151	168	206	244	390	386
<i>Tetrapispora</i> (5)	340 ^c					77	181	221	192	209	247	285	431	427
<i>Vanderwaltozyma</i> (2)	99						160	200	171	188	226	264	410	406
<i>Zygosaccharomyces</i> (6)	154							138	109	126	164	202	348	344
<i>Zygorulasporea</i> (2)	52								69	132	170	208	354	350
<i>Torulaspora</i> (5)	105									103	141	179	325	321
<i>Lachancea</i> (5)	115										94	132	278	274
<i>Kluyveromyces</i> (6)	129											124	270	266
<i>Eremothecium</i> (5)	331												246	242
<i>Hanseniaspora</i> (7)	297 ^d													150
<i>Saccharomycodes</i> (1)	(1 sp.)													(1 sp.)

^aThe multigene dataset used is comprised of nucleotide sequences from 18S, 5.8S/alignable ITS, and 26S (three regions) rDNAs, EF-1 α , mitochondrial small-subunit rDNA and COX II. The dataset was pruned of all potentially ambiguously aligned regions resulting in 4962 characters of which 929 were parsimony informative. Distances are summations of branch lengths given on a phylogenetic tree derived from maximum-parsimony analysis (dataset used for figure 9 of Kurtzman and Robnett [18]). Intragenomic distances are based on the two most distantly related species of each genus. Intergeneric distances are the sum of nucleotide changes separating the basal nodes of the genera compared. All species are represented by type, neotype, or authentic strains as listed in Table 1.

^bNumber of recognized species is given in parentheses and includes associated anamorphic species (*Candida*, *Kloeckera*).

^cIntragenomic divergence in *Tetrapispora* was 107 nucleotide changes with the exclusion of *T. blattae*.

^dDivergence in the *H. valbyensis* clade is 65 nucleotide changes, and 54 changes in the *H. vineae* clade.

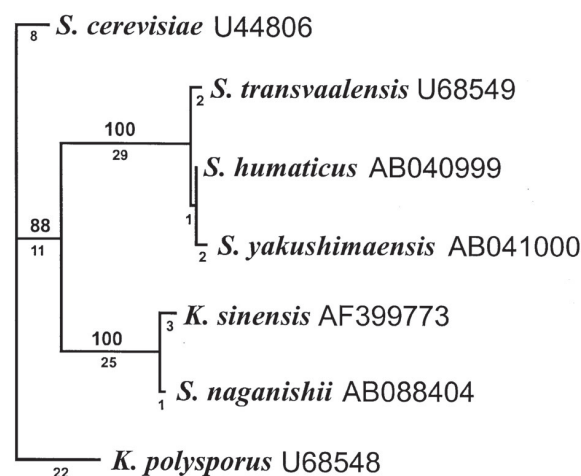


Fig. 2. Phylogenetic tree showing placement of *Saccharomyces humaticus*, *S. naganishii* and *S. yakushimaensis* with representative species of the *Kazachstania* clade. Represented by one of three most parsimonious trees derived from maximum-parsimony analysis of D1/D2 26S rDNA sequences. Tree length = 104, consistency index = 0.923, retention index = 0.917 and rescaled consistency index = 0.846. Type strains were analyzed for all species. GenBank accession numbers follow species names. Branch lengths, based on nucleotide substitutions, are the lower numbers and bootstrap values $\geq 50\%$ are given above the branches. *Kluyveromyces polysporus* was the outgroup species in the analysis.

over *Arxiozyma* van der Walt & Yarrow (1984) [35] and *Pachytichospora* van der Walt (1978) [36], two closely related monotypic genera also included in this clade. Species of this clade that are currently assigned to *Saccharomyces* or *Kluyveromyces* must be transferred to *Kazachstania* as new combinations because they are not members of either *Saccharomyces* or *Kluyveromyces* as now defined. Recognition of the genus *Kazachstania* from phenotype alone is difficult because the species assigned have little definitive group-specific morphology and their restricted responses on the standard tests used in yeast taxonomy do not reliably separate them from certain species in other genera. Lack of phenotypic identity is not peculiar to *Kazachstania* species, but is characteristic of many species in the ‘*Saccharomyces* complex’, which has led to past uncertainties about genus assignments. Assignment to *Kazachstania* of the three newly described species *Saccharomyces humaticus*, *S. naganishii* and *S. yakushimaensis* was made from phylogenetic analysis of D1/D2 26S rDNA sequences, which places these three species near ‘*Saccharomyces transvaalensis*’ and ‘*Kluyveromyces sinensis*’ in the *Kazachstania* clade (Fig. 2).

3.1.1.2. Species accepted.

1. *Kazachstania africana* (van der Walt) Kurtzman comb. nov.
Basionym: *Kluyveromyces africanus* van der Walt. Antonie van Leeuwenhoek 22, 325. 1956.
2. *Kazachstania barnettii* (Vaughan-Martini) Kurtzman comb. nov.

Basionym: *Saccharomyces barnettii* Vaughan-Martini. Antonie van Leeuwenhoek 68, 116. 1995.

3. *Kazachstania bulderi* (Middelhoven, Kurtzman & Vaughan-Martini) Kurtzman comb. nov.
Basionym: *Saccharomyces bulderi* Middelhoven, Kurtzman & Vaughan-Martini. Antonie van Leeuwenhoek 77, 224. 2000.
4. *Kazachstania exigua* (Reess ex E.C. Hansen) Kurtzman comb. nov.
Basionym: *Saccharomyces exiguus* Reess ex E.C. Hansen. C.R. Trav. Lab. Carlsberg 2, 146. 1888.
5. *Kazachstania humatica* (Mikata & Ueda-Nishimura) Kurtzman comb. nov.
Basionym: *Saccharomyces humaticus* Mikata & Ueda-Nishimura. Int. J. Syst. Evol. Microbiol. 51, 2193. 2001.
6. *Kazachstania kunashirensis* (James, Cai, Roberts & Collins) Kurtzman comb. nov.
Basionym: *Saccharomyces kunashirensis* James, Cai, Roberts & Collins. Int. J. Syst. Bacteriol. 47, 458. 1997.
7. *Kazachstania lodderae* (van der Walt & Tscheuschner) Kurtzman comb. nov.
Basionym: *Saccharomyces lodderae* (as *S. lodderi*) van der Walt & Tscheuschner. Antonie van Leeuwenhoek 23, 188. 1957.
8. *Kazachstania martiniae* (James, Cai, Roberts & Collins) Kurtzman comb. nov.
Basionym: *Saccharomyces martiniae* James, Cai, Roberts & Collins. Int. J. Syst. Bacteriol. 47, 458. 1997.
9. *Kazachstania naganishii* (Mikata, Ueda-Nishimura & Hisatomi) Kurtzman comb. nov.
Basionym: *Saccharomyces naganishii* Mikata, Ueda-Nishimura & Hisatomi. Int. J. Syst. Evol. Microbiol. 51, 2191. 2001.
10. *Kazachstania piceae* (Weber & Spaaij) Kurtzman comb. nov.
Basionym: *Kluyveromyces piceae* Weber & Spaaij. Antonie van Leeuwenhoek 62, 240. 1992.
11. *Kazachstania rosinii* (Vaughan-Martini, Barcaccia & Pollacci) Kurtzman comb. nov.
Basionym: *Saccharomyces rosinii* Vaughan-Martini, Barcaccia & Pollacci. Int. J. Syst. Bacteriol. 46, 616. 1996.
12. *Kazachstania servazzii* (Capriotti) Kurtzman comb. nov.
Basionym: *Saccharomyces servazzii* Capriotti. Ann. Microbiol. Enzimol. 17, 83. 1967.
13. *Kazachstania sinensis* (Li, Fu & Tang) Kurtzman comb. nov.
Basionym: *Kluyveromyces sinensis* Li, Fu & Tang. Acta Microbiol. Sin. 30, 96. 1990.
14. *Kazachstania spencerorum* (Vaughan-Martini) Kurtzman comb. nov.
Basionym: *Saccharomyces spencerorum* Vaughan-Martini. Antonie van Leeuwenhoek 68, 116. 1995.

15. *Kazachstania turicensis* (Wyder, Meile & Teuber) Kurtzman comb. nov.
Basionym: *Saccharomyces turicensis* Wyder, Meile & Teuber. Syst. Appl. Microbiol. 22, 423. 1999.
16. *Kazachstania telluris* (van der Walt) Kurtzman comb. nov.
Basionym: *Saccharomyces telluris* (as *S. tellustris*) van der Walt. Antonie van Leeuwenhoek 23, 27. 1957.
17. *Kazachstania transvaalensis* (van der Walt) Kurtzman comb. nov.
Basionym: *Saccharomyces transvaalensis* van der Walt. Antonie van Leeuwenhoek 22, 192. 1956.
18. *Kazachstania unispora* (Jørgensen) Kurtzman comb. nov.
Basionym: *Saccharomyces unisporus* Jørgensen. Die Mikroorganismen der Gärungsindustrie, 5te Aufl., p. 371, 1909. P. Parey, Berlin.
19. *Kazachstania viticola* Zubkova (1971) (type species of the genus *Kazachstania*).
20. *Kazachstania yakushimaensis* (Mikata & Ueda-Nishimura) Kurtzman comb. nov.
Basionym: *Saccharomyces yakushimaensis* Mikata & Ueda-Nishimura. Int. J. Syst. Evol. Microbiol. 51, 2194. 2001.

3.1.2. *Kluyveromyces* Kurtzman, Lachance, Nguyen & Prillinger nom. cons. (2001)

3.1.2.1. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Pseudohyphae may be formed, but true hyphae are not produced.

Ascospore formation. Asci may be unconjugated or show conjugation between independent cells or between a cell and its bud. Asci are deliquescent at maturity and produce 1–4 spherical, ovoidal or reniform ascospores.

Physiology/biochemistry. With the exception of one species, glucose is fermented and all species assimilate galactose. Cadaverine, L-lysine and ethylamine are generally utilized as nitrogen sources. Nitrate is not utilized. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. Species previously described as *Kluyveromyces* are distributed among six clades (Fig. 1), demonstrating the polyphyly of this genus when defined from the character of ascus deliquescence. Lachance [28] has reviewed the history of the genus and discussed possible species groupings based on phenotype, genotype and habitat specificity. In view of molecular, physiological, ecological and biotechnological considerations, Kurtzman et al. [37] proposed to conserve *Kluyveromyces* with *K. marxianus* as the conserved type species. This resulted in assignment of the six known species of the *K. marxianus* clade to the newly conserved *Kluyveromyces*. Naumov [38] and Naumov and Naumova [39] have argued

that these six species should be placed in the genus *Zygo-fabospora*, but the proposal of Kurtzman et al. [37] pointed out that the genus *Zygo-fabospora* was ambiguously conceived, and that changing the genus name of the widely known and biotechnologically important species *K. marxianus* and *K. lactis* after more than 30 years assignment to *Kluyveromyces* is incompatible with Article 14.2 of the International Code of Botanical Nomenclature [40], which proposes nomenclatural stability for well-known species.

3.1.2.2. Species accepted.

1. *Kluyveromyces aestuarii* (Fell) van der Walt (1971).
2. *Kluyveromyces dobzhanskii* (Shehata, Mrak & Phaff) van der Walt (1971).
Kluyveromyces lactis (Dombrowski) van der Walt (1971).
3.1. *Kluyveromyces lactis* (Dombrowski) van der Walt var. *lactis* (1986).
3.2. *Kluyveromyces lactis* var. *drosophilae* (Shehata, Mrak & Phaff) Sidenberg & Lachance (1986).
4. *Kluyveromyces marxianus* (E.C. Hansen) van der Walt (1971) (type species of the genus *Kluyveromyces* nom. cons.).
5. *Kluyveromyces nonfermentans* Nagahama, Hamamoto, Nakase & Horikoshi (1999).
6. *Kluyveromyces wickerhamii* (Phaff, M. W. Miller & Shifrine) van der Walt (1971).

3.1.3. *Lachancea* Kurtzman gen. nov.

3.1.3.1. Latin diagnosis of *Lachancea* Kurtzman gen. nov. *Asci conjugati vel inconjugati, habentes 1–4 ascosporae globosae et rumpuntur vel non rumpuntur. Cellulae vegetativae globosae, ellipsoideae aut elongatae. Pseudohyphae fiunt raro; non fiunt hyphae verae. Glucosum et alius saccharas fermentantur. Cadaverinum, L-lysinum et ethylaminum plerumque assimilantur. Nitrates kalicos non assimilantur. Systema coenzymatis Q-6 adest. Diazonium caerulian B non respondens. Species typica: *Lachancea thermotolerans* (Filippov) Kurtzman comb. nov.*

3.1.3.2. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Pseudohyphae may be formed, but true hyphae are not produced.

Ascospore formation. Asci may be unconjugated or show conjugation between independent cells or between a cell and its bud. Asci may be deliquescent or persistent and produce 1–4 spherical ascospores.

Physiology/biochemistry. Glucose and at least one other sugar are fermented. Galactose is assimilated by nearly all species. Cadaverine, L-lysine and ethylamine are generally utilized as nitrogen sources, but nitrate is not utilized.

Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. The five species assigned to this newly described genus were formerly members of *Kluyveromyces*, *Saccharomyces* and *Zygosaccharomyces*. Despite differences in the morphology of their ascospore states, the species share many similarities in physiology and habitat. The somewhat low bootstrap support for the basal node of this genus results from inclusion of the former *Saccharomyces kluyveri*, which may eventually serve as type species for a sister genus.

The genus is named in honor of Dr. Marc-André Lachance, University of Western Ontario, London, Ontario, Canada, for his many contributions to yeast systematics and ecology.

3.1.3.3. *Species accepted*.

1. *Lachancea cidri* (Legakis) Kurtzman comb. nov.
Basionym: *Saccharomyces cidri* Legakis. A contribution to the study of the yeast flora of apples and apple wine. Thesis. University of Athens, Greece. 1961 (cf. van der Walt, p. 609, 1970 [7]).
2. *Lachancea fermentati* (H. Naganishi) Kurtzman comb. nov.
Basionym: *Zygosaccharomyces fermentati* H. Naganishi. J. Zymol. 6, 1. 1928.
3. *Lachancea kluyveri* (Phaff, M. W. Miller & Shifrine) Kurtzman comb. nov.
Basionym: *Saccharomyces kluyveri* Phaff, M. W. Miller & Shifrine. Antonie van Leeuwenhoek 22, 159. 1956.
4. *Lachancea thermotolerans* (Filippov) Kurtzman comb. nov. (type species of the genus *Lachancea*).
Basionym: *Zygosaccharomyces thermotolerans* Filippov. Arb. Zentr. Biochem. Forsch. Inst. Nahrungs-u. Genussmittel-Ind. 2, 26. 1932.
5. *Lachancea waltii* (K. Kodama) Kurtzman comb. nov.
Basionym: *Kluyveromyces waltii* K. Kodama. J. Ferm. Technol. 52, 609. 1974.

3.1.4. *Nakaseomyces* Kurtzman gen. nov.

3.1.4.1. *Latin diagnosis of Nakaseomyces* Kurtzman gen. nov.. Asci conjugati vel inconjugati, rumpuntur, habentes 1–8 ascosporae reniformes aut bacilliformes. Cellulae vegetativae globosae, ellipsoideae aut elongatae. Non fiunt pseudohyphae et hyphae verae. Glucosum fermentantur. Cadaverinum, L-lysinum et ethylaminum assimilantur raro. Nitras kalicus non assimilantur. Systema coenzymatis Q-6 adest. Diazonium caeruleum B non respondens. Species typica: *Nakaseomyces delphensis* (van der Walt & Tscheuschner) Kurtzman comb. nov.

3.1.4.2. *Genus description. Vegetative reproduction.* Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Neither pseudohyphae nor true hyphae are produced.

Ascospore formation. Asci may be unconjugated or show conjugation between independent cells. The asci are deliquescent and produce 1–8 reniform or bacilliform ascospores.

Physiology/biochemistry. Glucose is fermented, but galactose is neither fermented nor assimilated. Cadaverine, L-lysine and ethylamine are seldom utilized as nitrogen sources. Nitrate is not utilized. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. This genus is phylogenetically well separated from other clades of the ‘*Saccharomyces* complex’. Two species of the anamorphic genus *Candida*, *C. glabrata* and *C. castellii*, are members of this clade.

The genus is named in honor of Dr. Takashi Nakase, formerly Director of the Japan Collection of Microorganisms, Saitama, Japan, for his many contributions to yeast systematics including the early application of molecular methods to the study of relationships among yeasts.

3.1.4.3. *Species accepted*.

1. *Nakaseomyces bacillisporus* (Lachance, Phaff & Starmer) Kurtzman comb. nov.
Basionym: *Kluyveromyces bacillisporus* Lachance, Phaff & Starmer. Int. J. Syst. Bacteriol. 43, 116. 1993.
2. *Nakaseomyces delphensis* (van der Walt & Tscheuschner) Kurtzman comb. nov. (type species of the genus *Nakaseomyces*).
Basionym: *Saccharomyces delphensis* van der Walt & Tscheuschner. Antonie van Leeuwenhoek. 22, 165. 1956.

3.1.5. *Naumovia* Kurtzman gen. nov.

3.1.5.1. *Latin diagnosis of Naumovia* Kurtzman gen. nov.. Asci non conjugati et non rumpuntur. Habentes 1–4 ascosporae globosae. Cellulae vegetativae globosae, ellipsoideae aut elongatae. Non fiunt pseudohyphae et hyphae verae. Glucosum et galactosum fermentantur. Cadaverinum, L-lysinum, ethylaminum et nitras kalicus non assimilantur. Systema coenzymatis Q-6 adest. Diazonium caeruleum B non respondens. Species typica: *Naumovia dairienensis* (H. Naganishi) Kurtzman comb. nov.

3.1.5.2. *Genus description. Vegetative reproduction.* Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Neither pseudohyphae nor true hyphae are produced.

Ascospore formation. Asci are unconjugated and persistent. Each produces 1–2, rarely 4, spherical ascospores.

Physiology/biochemistry. Glucose and galactose are fermented. Cadaverine, L-lysine, ethylamine and nitrate are not utilized as nitrogen sources. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. *Naumovia* is phylogenetically well separated from other members of the ‘*Saccharomyces* complex’, including *Kazachstania* to which it assumes a

basal position. The two assigned species, *N. castellii* and *N. dairenensis*, can be separated from one another only using molecular methods.

The genus is named in honor of Drs. Gennadi I. Naumov and Elena S. Naumova, State Institute for Genetics and Selection of Industrial Microorganisms, Moscow, Russia, for their extensive studies on genetically defining biological yeast species, most notably those assigned to *Saccharomyces*.

3.1.5.3. Species accepted.

1. *Naumovia castellii* (Capriotti) Kurtzman comb. nov.
Basionym: *Saccharomyces castellii* Capriotti. Ann. Fac. Agric. Sassari 14, 7. 1966.
2. *Naumovia dairenensis* (H. Naganishi) Kurtzman comb. nov. (type species of the genus *Naumovia*).
Basionym: *Saccharomyces dairenensis* (as *S. dairenensis*) H. Naganishi. Bot. Mag. Tokyo 31, 107. 1917.

3.1.6. *Saccharomyces Meyen ex Reess* (1870)

3.1.6.1. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Pseudohyphae may be formed, but true hyphae are not produced.

Ascospore formation. Asci are unconjugated, persistent, and produce 1–4 spherical to ovoidal ascospores.

Physiology/biochemistry. Glucose, raffinose and usually sucrose are fermented, often vigorously. Cadaverine, L-lysine, ethylamine and nitrate are not utilized as nitrogen sources. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. On the basis of the multigene study of Kurtzman and Robnett [18], the genus *Saccharomyces* is restricted to members of the *S. cerevisiae* clade. This now includes seven species, six heterothallic biological species and the hybrid species *S. pastorianus* [41]. Circumscription of the genus from this clade brings a return to the earlier concept of *Saccharomyces*, which was based on those species giving a vigorous alcoholic fermentation, and subsequently termed the sensu stricto species [7,29]. The seven species accepted are regarded as genetically isolated from one another on the basis of genetic crosses [41] as well as from molecular comparisons [14,18]. Although *S. cariocanus*, *S. cerevisiae* and *S. paradoxus* appear to be separate biological species from genetic crosses [41], they show relatively little gene sequence divergence [18].

3.1.6.2. Species accepted.

1. *Saccharomyces bayanus* Saccardo (1895).
2. *Saccharomyces cariocanus* Naumov, James, Naumova, Louis & Roberts (2000).
3. *Saccharomyces cerevisiae* Mayen ex E.C. Hansen (1883) (type species of the genus *Saccharomyces*).

4. *Saccharomyces kudriavzevii* Naumov, James, Naumova, Louis & Roberts (2000).
5. *Saccharomyces mikatae* Naumov, James, Naumova, Louis & Roberts (2000).
6. *Saccharomyces paradoxus* Bachinskaya (1914).
7. *Saccharomyces pastorianus* E.C. Hansen (1904).

3.1.7. *Tetrapisispora* Ueda-Nishimura & Mikata (1999)

3.1.7.1. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Neither pseudohyphae nor true hyphae are produced.

Ascospore formation. Asci are unconjugated, deliquescent or persistent, and produce 1–8, rarely more, spheroidal, ovoidal, reniform or bacilliform ascospores.

Physiology/biochemistry. Glucose and galactose are fermented. Cadaverine, L-lysine, ethylamine, and nitrate are not utilized as nitrogen sources. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. The genus *Tetrapisispora* was recognized from the isolation of its members on a phylogenetic tree derived from 18S rDNA sequences [42]. Multigene sequence analysis has confirmed that the *Tetrapisispora* species form a distinct clade [18]. *Kluyveromyces blattae* is a markedly basal member of this clade (Fig. 1) and was not included in *Tetrapisispora* by Ueda-Nishimura and Mikata [42]. In this study, *K. blattae* has been transferred to *Tetrapisispora* because its association with the primary members of the clade shows strong bootstrap support and there seems little reason at present to maintain this species in a separate monotypic genus. The genus name *Tetrapisispora* refers to the four ‘pea-like’ ascospores as formed by the species *T. arboricola*, *T. iriomotensis*, and *T. nanseiensis*. However, the ascospores formed by *T. phaffii*, which was included in the original description of the genus as type species, and the newly assigned *T. blattae*, are reniform [28,42]. Two species have persistent asci (*T. arboricola*, *T. nanseiensis*) whereas the others are deliquescent.

3.1.7.2. Species accepted.

1. *Tetrapisispora arboricola* Ueda-Nishimura & Mikata (1999).
2. *Tetrapisispora blattae* (Henninger & Windisch) Kurtzman comb. nov.
Basionym: *Kluyveromyces blattae* Henninger & Windisch. Arch. Microbiol. 109, 155. 1976.
3. *Tetrapisispora iriomotensis* Ueda-Nishimura & Mikata (1999).
4. *Tetrapisispora phaffii* (van der Walt) Ueda-Nishimura & Mikata (1999) (type species of the genus *Tetrapisispora*).
5. *Tetrapisispora nanseiensis* Ueda-Nishimura & Mikata (1999).

3.1.8. *Torulaspora* Lindner (1904)

3.1.8.1. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Pseudohyphae, if formed, are not well differentiated, and true hyphae are not produced.

Ascospore formation. Asci may be unconjugated or show conjugation between independent cells or between a cell and its bud. Ascospore-producing cells often have a genus-diagnostic tapered protuberance that may represent a modified conjugation tube or a distorted bud. Asci are persistent and produce 1–4 spherical ascospores that may appear roughened under the light microscope.

Physiology/biochemistry. Glucose and at least one other sugar are fermented. Galactose is assimilated by most species. Cadaverine, L-lysine and ethylamine are variously utilized as nitrogen sources by some of the species, but nitrate is not utilized as a sole source of nitrogen. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. Asci of *Torulaspora* species often have a small tapered protuberance that is diagnostic for the genus. The species *T. microellipsoides* was previously transferred to *Torulaspora*, then reassigned to *Zygosaccharomyces* [10], and now reinstated in *Torulaspora* on the basis of multigene sequence analysis [18].

3.1.8.2. Species accepted.

1. *Torulaspora delbrueckii* (Lindner) Lindner (1904) (type species of the genus *Torulaspora*).
2. *Torulaspora globosa* (Klöcker) van der Walt & E. Johannsen (1975).
3. *Torulaspora franciscae* Capriotti (1958).
4. *Torulaspora microellipsoides* (Osterwalder) van der Walt & E. Johannsen (1975).
5. *Torulaspora pretoriensis* (van der Walt & Tscheuschner) van der Walt & E. Johannsen (1975).

3.1.9. *Vanderwaltozyma* Kurtzman gen. nov.

3.1.9.1. Latin diagnosis of *Vanderwaltozyma* Kurtzman gen. nov.. Asci conjugati vel inconjugati, rumpuntur, habentes 1–100 ascosporae globosae, elongatae aut reniformes. Cellulae vegetativae globosae, ellipsoideae aut elongatae. Pseudohyphae fiunt raro; non fiunt hyphae verae. Glucosum, galactosum et alius saccharas fermentantur. Cadaverinum, L-lysinum, ethylaminum et nitras kalici non assimilantur. Systema coenzymatis Q-6 adest. Diazonium caerulian B non respondens. Species typica: *Vanderwaltozyma polyspora* (van der Walt) Kurtzman comb. nov.

3.1.9.2. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Pseudohyphae, if formed, are not well differentiated. True hyphae are not produced.

True hyphae are not produced.

Ascospore formation. Asci, which are deliquescent at maturity, may be unconjugated or show conjugation between independent cells. Depending on the species, asci produce 1–100 spheroidal, oblong or reniform ascospores.

Physiology/biochemistry. Glucose, galactose and occasionally other sugars are fermented. Cadaverine, L-lysine, ethylamine and nitrate are not utilized as nitrogen sources. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. The two species assigned to *Vanderwaltozyma*, *V. polyspora* and *V. yarrowii*, form a clade basal to the genus *Tetrapisispora*. *Vanderwaltozyma polyspora* represented the type species of *Kluyveromyces* prior to conservation of that genus with a new type [37]. The species name *polysporus* was originally selected to indicate that asci form large numbers of ascospores, which is in contrast to the closely related *V. yarrowii* that produces just four ascospores per ascus.

The genus is named in honor of Dr. Johannes P. van der Walt, formerly of the Microbiology Research Group, Council of Scientific and Industrial Research, Pretoria, South Africa, for his many contributions to yeast taxonomy.

3.1.9.3. Species accepted.

1. *Vanderwaltozyma polyspora* (van der Walt) Kurtzman comb. nov. (type species of the genus *Vanderwaltozyma*).
Basionym: *Kluyveromyces polysporus* van der Walt. Antonie van Leeuwenhoek 22, 271. 1956.
2. *Vanderwaltozyma yarrowii* (van der Walt) Kurtzman comb. nov.
Basionym: *Kluyveromyces yarrowii* van der Walt. Syst. Appl. Microbiol. 8, 210. 1986.

3.1.10. *Zygosaccharomyces* Barker (1901)

3.1.10.1. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Pseudohyphae, if formed, are generally not well differentiated, and true hyphae are not produced.

Ascospore formation. Asci are generally conjugated, with the conjugants frequently presenting a 'dumbbell' configuration. Asci are persistent and produce 1–4 ascospores, often equally distributed between the two conjugants.

Physiology/biochemistry. Glucose is fermented, but not galactose. Species variously utilize cadaverine, L-lysine and ethylamine as nitrogen sources, but nitrate is not assimilated. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus. The six species retained in *Zygosaccharomyces* represent a well-supported clade (Fig. 1) that can also be recognized from phenotype. *Zygosacchar-*

omyces barkeri has been designated as the type species of *Zygosaccharomyces* [10]. This species was described by Barker [3], but named by Saccardo and Sydow [43]. Type material for this species no longer exists, but it was believed to be the same species as *Z. rouxii* [6,10]. In view of the absence of an extant type species, *Zygosaccharomyces rouxii* (Boutroux) Yarrow is proposed as the neotype species of the genus *Zygosaccharomyces* with type material represented by the culture CBS 732 (NRRL Y-229), isolated from grape must in Italy.

3.1.10.2. Species accepted.

1. *Zygosaccharomyces bailii* (Lindner) Guilliermond (1912).
2. *Zygosaccharomyces bisporus* H. Naganishi (1917).
3. *Zygosaccharomyces kombuchaensis* Kurtzman, Robnett & Basehoar-Powers (2001).
4. *Zygosaccharomyces lentus* Steels, Bond, Collins, Roberts, Stratford & James (1999).
5. *Zygosaccharomyces mellis* Fabian & Quinet (1928).
6. *Zygosaccharomyces rouxii* (Boutroux) Yarrow (1977) (neotype species of the genus *Zygosaccharomyces*).

3.1.11. *Zygotulasporea* Kurtzman gen. nov.

3.1.11.1. Latin diagnosis of *Zygotulasporea* Kurtzman gen. nov.. *Asci conjugati vel inconjugati, non rumpuntur, habentes 1–4 ascospores globosae vel subglobosae. Cellulae vegetativae globosae, ellipsoideae aut elongatae. Pseudohyphae fiunt raro; non fiunt hyphae verae. Glucosum, galactosum et alius saccharas fermentantur. Cadaverinum, L-lysimum et ethylaminum plerumque assimilantur. Nitras kalicus non assimilantur. Systema coenzymatis Q-6 adest. Diazonium caeruleum B non respondens. Species typica: *Zygotulasporea mrakii* (Capriotti) Kurtzman comb. nov.*

3.1.11.2. Genus description. Vegetative reproduction. Asexual reproduction is by multilateral budding on a narrow base. Cells are spheroidal, ovoidal or elongate. Pseudohyphae may be present but are usually not well differentiated. True hyphae are not produced.

Ascospore formation. Asci may be unconjugated, or show conjugation between independent cells or between a cell and its bud. Asci are persistent and produce 1–4 spherical to subspherical ascospores.

Physiology/biochemistry. Glucose, galactose, and often other sugars are fermented. Species variously utilize cadaverine, L-lysine and ethylamine as nitrogen sources, but nitrate is not assimilated. Coenzyme Q-6 is produced. The diazonium blue B reaction is negative.

Comments on the genus: The clade is phylogenetically well supported and represented by two closely related species, *Z. florentinis* and *Z. mrakii*. As Kurtzman and Robnett [18] pointed out, the clade shows affinities with both *Zygosaccharomyces* and *Torulaspora*. When phylogenetically analyzed with *Pichia anomala* as the outgroup, the

clade is basal to and weakly associated with *Torulaspora*, but when the outgroup is *Schizosaccharomyces pombe*, the clade becomes weakly associated with and basal to *Zygosaccharomyces*. This weak association with both genera suggests it to be an intermediate taxon that should be regarded as a separate genus.

3.1.11.3. Species accepted.

1. *Zygotulasporea florentinis* (Castelli ex Kudryavtsev) Kurtzman comb. nov.
Basionym: *Zygosaccharomyces florentinus* Castelli ex Kudryavtsev. Die Systematik der Hefen, p. 275. 1960. Akademi Verlag, Berlin.
2. *Zygotulasporea mrakii* (Capriotti) Kurtzman comb. nov. (type species of the genus *Zygotulasporea*).
Basionym: *Zygosaccharomyces mrakii* Capriotti. Arch. Mikrobiol. 30, 392. 1958.

3.2. Diagnostic key to phylogenetically circumscribed genera of the Eremotheciaceae, Saccharomycodaceae and Saccharomycetaceae

The following is a diagnostic key to the newly circumscribed genera and is based on data given in The Yeasts, A Taxonomic Study, 4th edition [25–32] and in Yeasts of the World [33]. Many of the species assimilate relatively few carbon compounds, markedly limiting the choices for species and genus recognition. Consequently, the key leads to genera, groups of species in particular genera, and to some individual species.

- 1a. Ascospores are elongated, often with pointed ends. Yeast cells, if formed, arise by multilateral budding – Eremotheciaceae/*Eremothecium*
- 1b. Ascospores are not elongated. Vegetative reproduction is by bipolar budding on a broad base – Saccharomycodaceae – 2
- 1c. Ascospores are not elongated. Vegetative reproduction is by multilateral budding – Saccharomycetaceae – 3
- 2 (1b). a. Raffinose, ethanol and DL-lactate are assimilated – *Saccharomyces*
- b. Raffinose, ethanol and DL-lactate are not assimilated – *Hanseniaspora/Kloeckera*
- 3 (1c). a. Asci predominantly arise from conjugation between independent cells with most conjugants forming a ‘dumbbell’ configuration – *Zygosaccharomyces*
- b. Asci often form a tapered protuberance – *Torulaspora*
- c. Asci are persistent, unconjugated and with 1–4 globose to subglobose ascospores. Sucrose, raffinose, maltose and/or melezitose are assimilated. Ethylamine is not utilized – *Saccharomyces*
- d. Not the preceding combination of characters – 4
- 4 (3d). a. Ribitol, mannitol or glucitol are assimilated (*Kluyveromyces*, *Lachancea*, *Zygotulasporea*) – 5
- b. Ribitol, mannitol or glucitol are not assimilated (*Kazachstania*, *Nakaseomyces*, *Naumovia*, *Tetrapisispora*, *Vanderwaltozyma*) – 6
- 5 (4a). a. Asci are persistent; succinate is assimilated; *m*-inositol is not required for growth – *Zygotulasporea*
- b. Asci are deliquescent; *m*-inositol is not required for growth *Kluyveromyces*

- c. Not the above combination of characters (5a, b) – *Lachancea*
- 6 (4b). a. Only glucose is fermented; D-gluconate is assimilated – *Nakaseomyces*
- b. Not the preceding combination of characters (6a) – 7
- 7 (6b). a. Asci are deliquescent – 8
- b. Asci are persistent – 11
- 8 (7a). a. Galactose is fermented – 9
- b. Galactose is not fermented – *Kazachstania sinensis*
- 9 (8a). a. Sucrose is fermented; citrate is assimilated – *Vanderwaltozyma polyspora*
- b. Sucrose is fermented; citrate is not assimilated – *Kazachstania lodderae*
- c. Sucrose is not fermented; D-gluconate or 2-keto D-gluconate are assimilated – *Tetrapisispora* pro parte (*T. blattae*, *T. iriomotensis*, *T. phaffii*)
- d. Sucrose is not fermented; D-gluconate or 2-keto D-gluconate are not assimilated – 10
- 10 (9d). a. Succinate is assimilated – *Kazachstania piceae*
- b. Succinate is not assimilated; asci produce as many as 16 ascospores – *Kazachstania africana*
- c. Succinate is not assimilated; asci produce no more than 4 ascospores per ascus – *Vanderwaltozyma yarrowii*
- 11 (7b). a. Galactose is fermented – 12
- b. Galactose is not fermented – *Kazachstania telluris*
- 12 (11a). a. Sucrose is fermented – *Kazachstania* pro parte (*K. naganishii*, *K. spencerorum*, *K. exigua*, *K. turicensis*, *K. barnettii*, *K. bulderi*)
- b. Sucrose is not fermented – 13
- 13 (12b). a. Growth with ethylamine – *Kazachstania* pro parte (*K. unispora*, *K. humatica*, *K. yakushimaensis*)
- b. Growth absent with ethylamine – 14
- 14 (13b). a. Growth with 0.01% cycloheximide – 15
- b. Growth absent with 0.01% cycloheximide – 16
- 15 (14a). a. Trehalose assimilated – *Kazachstania servazzii*
- b. Trehalose not assimilated – *Tetrapisispora nanseiensis*
- 16 (14b). a. Trehalose assimilated – 17
- b. Trehalose not assimilated; growth at 34°C – *Kazachstania viticola*
- c. Trehalose not assimilated; growth absent at 34°C – *Kazachstania rosinii*
- 17 (16a). a. Trehalose fermented – *Kazachstania martiniae*
- b. Trehalose not fermented – 18
- 18 (17b). a. L-Lysine and cadaverine utilized – *Kazachstania transvaalensis*
- b. L-Lysine utilized; cadaverine not utilized – *Kazachstania kunishirensis*
- c. L-Lysine not utilized; predominantly 2 ascospores/ascus – *Naumovia*
- d. L-Lysine not utilized; predominantly 4 ascospores/ascus – *Tetrapisispora arboricola*

4. Conclusions

The proposal of a new genus is ordinarily based on the presence of a novel phenotype, which has been viewed as indicative of phylogenetic separation from other genera. However, there are now sufficient examples from molecular studies that phenotype is often not predictive of genotype. This concept has become widely recognized by taxonomists describing new yeast species. Reliance is now placed on molecular divergence from known species rather than from phenotypic differences. With this change, stan-

dard fermentation and assimilation tests can no longer be used to define species, but the test results may be used to diagnostically recognize some genetically defined species or species groups, and they serve to present the general physiological properties of the species, which are of value to ecologists, biotechnologists and others needing information on substrate utilization.

Systematics based on phylogenetic analysis of gene sequences provides a taxonomic structure in which the names of genera and higher orders of classification have genetic meaning and predictiveness. This restructuring of systematics is necessary to complement and guide ongoing studies in evolution, genomics and proteomics. Failure to make these taxonomic changes will perpetuate a system of classification with little relevance to other fields of science.

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