

1 Microbial Systematics (Short Communication)

2
3 **The Generic Circumscription of *Kazachstania* Zubkova (1971) (Sacchromycetaceae)**
4 **The Proposal of New Genus *Vanderwaltomyces***

5
6 Taweesak Malimas,¹ Huong Thi Lan Vu,² Pattaraporn Yukphan,³ Somboon Tanasupawat,⁴
7 Yuzo Yamada,^{3,5,6,*}

8
9 ¹Microbial Laboratory Biosafety Level-1, 46 M, 9 Nongphus, Muangsuang, Roi-Et 45220,
10 Thailand

11 ²Department of Microbiology, Faculty of Biology and Biotechnology, University of
12 Science, Vietnam National University-HCM City, 227 Nguyen Van Cu Street, Ward 4,
13 District 5, Hochiminh City, Vietnam

14 ³BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and
15 Biotechnology (BIOTEC), National Science and Technology Development Agency
16 (NSTDA), 113 Thailand Science Park, Phaholyothin Road, Klong 1, Klong Luang,
17 Pathumthani 12120, Thailand

18 ⁴Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences,
19 Chulalongkorn University, 254 Phayathai Road, Wangmai, Pathumwan, Bangkok 10330,
20 Thailand

21 ⁵JICA Senior Overseas Volunteer, Japan International Cooperation Agency, Shibuya-ku,
22 Tokyo 151-8559, Japan

23 ⁶Laboratory of Applied Microbiology (Professor Emeritus), Department of Agricultural
24 Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka
25 422-8529, Japan

26
27 *Corresponding author

28 Yuzo Yamada

29 E-mail: ymdy333@kdt.biglobe.ne.jp

30 ORCID, 0000-0002-2799-7045

31
32 **Keywords:** *Kazachstania*; *Kazachstania viticola*; *Kluyveromyces*; *Vanderwaltomyces* gen.
33 nov; *Vanderwaltomyces africanus* comb. nov.

34
35 **Abstract**

36 The genus *Kazachstania* Zubkova was once introduced as a monotypic genus including
37 *Kazachstania viticola* Zubkova (1971). Then, a large number of species were accom-
38 modated to the genus. However, the phylogenetic analyses indicated that the genus is
39 extremely diverse, since the species concerned, including *Kazachstania viticola*, the type
40 species, had quite long branches in the phylogenetic trees. The calculated pair-wise
41 sequence similarities of *K. viticola* were quite low (89.4 - 94.6%) to the remaining species.

42 The phylogenetic data obtained indicated that the genus *Kazachstania* is subdivided into
43 several taxonomic homogeneous-natured taxa at the generic level. As a first case, the new
44 genus *Vanderwaltomyces* was proposed for *Kazachstania africana* as *Vanderwaltomyces*
45 *africanus*.

46
47

48 The genus *Kazachstania* Zubkova was introduced as a monotypic genus with
49 *Kazachstania viticola* Zubkova (1971). Later, a large number of species were
50 accommodated to the genus (Kurtzman 2003; Vaughan-Martini et al. 2011). However, the
51 several species of the genus had quite long phylogenetic branches, along with the type
52 species, *K. viticola*, in the phylogenetic tree (Vaughan-Martini et al. 2011). Such
53 phenomena suggest that the genus is quite diverse phylogenetically and taxonomically.

54

55 This paper deals with the presently available sequence data and gives the different
56 conclusion that the genus *Kazachstania* has to be subdivided into five or more groups at
57 the generic level since the genus is taxonomic heterogeneous-natured. In this paper, the
58 monotypic new genus *Vanderwaltomyces* is introduced with the new combination,
59 *Vanderwaltomyces africanus*.

60

61 The phylogenetic trees based on the 26S rRNA gene D1/D2 domain sequences (LSU
62 D1/D2) was constructed by the neighbour-joining method (Fig. 1). First of all,
63 *Kazachstania viticola*, the type species of the genus showed a very long phylogenetic
64 branch, along with the several representative species, i.e., *K. telluris*, *K. sinensis*, *K.*
65 *kunashirensis* and *K. transvaalensis*. In fact, the pair-wise sequence similarities of *K.*
66 *viticola* were quite low (89.4 - 94.6%) to the remaining species (Table 1). Differing from
67 *Lipomyces starkeyi* and its relatives (Lipomycetaceae) (Yamada et al. 2022) as well as
68 *Octosporomyces octosporus* and *Octosporomyces osmophilus* (Schizosaccharo-
69 mycetaceae) (Vu et al. 2022a), *K. viticola* should constitute the monotypic genus
70 *Kazachstania* again. And the several species mentioned above had to make their own
71 respective genera. The construction of respective genera was supported by the calculated
72 sequence similarities (89.4 - 94.6%). Namely, the similarities did not exceed 98% or more
73 (Yamada et al. 2022; Vu et al. 2022a, b; Yamada 2023; Malimas et al. 2023a, b). Inci-
74 dentally, the sequence similarity was 94.1% between *Vanderwaltozyma polyspora* and
75 *Saccharomyces cerevisiae* used as reference standards as well as the similarities were 94.0
76 - 97.5% among the four Lipomycetaceous yeast genera.

77 *Kazachstania bulderi*, *K. barnettii*, *K. turicensis* and *K. exigua* constituted a common
78 cluster, designated as cluster α . The calculated sequence similarities were 98.5 - 99.4%,
79 the values of which allow to produce an additional independent taxonomic homogeneous-
80 natured genus. *Kasachstania humilis* (= *Candida humilis* = *Kazachstania humilis* f.a.;
81 Lachance 2012) was connected to the member of cluster α with the similarities 96.9 -
82 97.8% (Table 1).

83 *Kazachstania servazzii* and *K. unispora* produced a cluster, designated as cluster β
84 with the similarity 98.9%, the value of which is also able to produce an independent and
85 taxonomic homogeneous-natured genus.

86 Concerning the remaining four species, *K. lodderae*, *K. spenserorum*, *K. piceae* and *K.*
87 *rosinii* made a cluster, designated as cluster γ , whose sequence similarities were 95.3 -
88 97.8%. Finally, the calculated similarity between *K. africana* and *K. martiniae* was 96.6%.

89
90 In the phylogenetic tree based on the 18S rRNA gene sequences (SSU) derived from
91 the neighbour-joining method (Fig. 2), the phylogenetic branches of the representative
92 species were very short as already reported (Malimas et al. 2023a), when compared with
93 those of LSU D1/D2. The cluster of *V. polyspora* and *S. cerevisiae* was surprisingly
94 migrated within the genus *Kazachstania*, indicating that the species was not tightly
95 coupled to one another in the genus, due to a taxonomic heterogeneous-natured taxon.

96 The calculated sequence similarities were widely distributed and very high (96.1 -
97 99.8%) within the genus (Table 2). Incidentally, the similarity was 98.8% between *V.*
98 *polyspora* and *S. cerevisiae*.

99
100 In the phylogenetic tree based on the concatenated sequences from the 18S rRNA
101 genes and 26S rRNA gene D1/D2 domains derived from neighbour-joining (Fig. 3), the
102 reference standards or controls, *V. polyspora* and *S. cerevisiae* were also included in the
103 genus *Kzachstania*. Such a phenomenon was due to the phylogenetic diversity of the
104 genus. In fact, the calculated sequence similarities were 95.2 - 99.8%. (Table 3).
105 Incidentally, the similarity was 97.6% between *V. polyspora* and *S. cerevisiae*.

106 Considering the phylogeny, it is reasonable that the phylogenetic position of the
107 genus *Kazachstania* was located between the genera *Sacchromyces* and *Vanderwaltozyma*
108 (Kurtzman 2003) and the two species used as reference standards migrated easily within
109 the genus *Kazachstania* that was quite diverse phylogenetically.

110
111 *Kazachstania africana* (van der Walt) Kurtzman (2003) was first described as
112 *Kluyveromyces afrinanus* van der Walt (1956). However, the species quite differed in its
113 phylogenetic position from the type species, *Kluyveromyces marxianus* (Hansen) van der
114 Walt (1971), the genus *Kluyveromyces* van der Walt (Kurtzman 2003; Vaughan-Martini et
115 al. 2011). In the present study, the species was located at a special position phylogenet-
116 cally (Fig. 1) and the calculated sequence similarity of *K. africana* was 96.6%, i.e., below
117 98%, to *Kazachstania martiniae* (Table 1) (Yamada et al. 2022; Vu et al. 2022a, b; Yamada
118 2023; Malimas et al. 2023a, b). Thus, it is obvious that a different genus can be given to
119 *Kazachstania africana*.

120
121 The family Saccharomycetaceae

122
123 *Vanderwaltomyces* Malimas, Vu, Yukphan, Tanasupawat et Yamada gen. nov.

124

125 *Vanderwaltomyces* (Van.der.wal'to.my.ces. N. L. masc. n. *Vaanderwaltomyces*, van
126 der Walt fungus, in honour of Professor Dr. Johannes P. van der Walt, South African yeast
127 taxonomist, who contributed greatly to the yeast systematics)

128 Cells occur singly, in pairs or short chain (Vaughan-Martini et al. 2011). Conjugating
129 cells may be present. Growth is butyrous. Asci arise directly from diploid cells. One to 16
130 or more oblong or reniform ascospores were formed. The spores are liberated from the
131 ascus soon after formation. Glucose and galactose are fermented. Growth occurs on
132 glucose, galactose, DL-lactate and D-gluconate (Vaughan-Martini et al. 2011).

133 MycoBank number is ///////////////.

134 The type species is *Vanderwaltomyces africanus*.

135

136 *Vanderwaltomyces africanus* (van der Walt) Malimas, Vu, Yukphan, Tanasupawat et
137 Yamada comb. nov.

138 Basionym: *Kluyveromyces africanus* van der Walt, Antonie van Leeuwenhoek 22: 325,
139 1956.

140 Synonym: *Kazachstania africana* (van der Walt) Kurtzman (2003).

141 The type strain is CBS 2517.

142 MycoBank number is ////////.//

143

144 As described above, the genus *Kazachstania* is taxonomically and phylogenetically
145 quite diverse. The remaining species of the genus are to be candidates assignable to new
146 genera.

147

148 Acknowledgements

149 The present authors express their sincere thanks for citing the experimental data of the
150 authors.

151

152 Funding information

153 The present authors received no grant from any funding agency.

154

155 Conflict of interest

156 The authors declare that there are no conflicts of interest.

157

158 Author contributions

159 T. M., H.T.L. V., P. Y., S. T. and Y. Y. designed the study. T. M. performed the main
160 experiments. H.T.L. V. and P. Y. instructed the experiments. Y. Y. prepared the manuscript.

161 The detailed discussions were made among the five.

162

163 **References**

164 Kurtzman, C.P. (2003) Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces*
165 and other members of the Saccharomycetaceae and the proposal of the new genera
166 *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygotoruspora*.
167 *FFEMS Yeast Res* **4**: 233-245.

168 Lachance, M.A. (2012) In defense of sexual life cycles: The forma asexualis – an informal
169 proposal. *Yeast Newsletter* **61**: 24-25.

170 Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. and Yamada, Y. (2023a) The
171 subdivision of the genus *Eremolthecum* Borzi emend. Kurtzman. *Jxiv* (DOI:
172 <https://doi.org/10.51094/jxiv.285>).

173 Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. and Yamada, Y. (2023b) The
174 reinstatement of the genus *Kleckeraspora* Niehaus (1932) (apiculate yeasts). *Jxiv*
175 (DOI: <https://doi.org/10.51094/jxiv.317>).

176 Vaughan-Martini, A., Lachance, M.-A., Kurtzman, C.P. (2011) *Kazachstania* Ziubkova
177 (1971) In: Kurtzman, C.P., Fell, J.W. and Boekhout, T. (ed). *The Yeasts: A Taxonomic*
178 *Study*, 5th edition, vol. 2. London: Elsevier, p. 439-470.

179 Vu, H.T.L., Yukphan, P., Tanasupawat, S., Mikata, K. and Yamada, Y. (2022a) The
180 revision of Schizosacchomycetaceae. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.188>).

181 Vu, H.T.L., Yukphan, P., Tanasupawat, S. and Yamada, Y. (2022b) The generic
182 circumscription of *Kockiozyma* (Lipomycetaceae). *Jxiv* (DOI:
183 <https://doi.org/10.51094/jxiv.221>).

184 Yamada, Y., Vu, H.T.L, Yukphan, P. and Tanasupawat, S. (2022) The revision of
185 Lipomycetaceae. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.189>).

186 Yamada, Y. (2023) The generic circumscription of *Eremothecium* emend. Kurtzman, *Jxiv*
187 (DOI: <https://doi.org/1051094/jxiv.270>)

188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204

205
206

Table 1. The pair-wise sequence similarity in the 26S rRNA gene D1/D2 domain sequences in *Kazachstania* species.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	100																			
2	96.6	100																		
3	95.7	96.4	100																	
4	95.0	95.0	97.6	100																
5	95.8	96.2	97.5	96.6	100															
6	94.8	95.3	96.6	95.3	97.8	100														
7	92.1	91.2	90.5	90.3	91.0	91.4	100													
8	95.8	97.1	96.2	95.0	95.8	95.3	91.9	100												
9	96.6	96.7	96.4	95.1	96.7	95.8	91.6	98.9	100											
10	94.1	94.4	94.8	93.9	94.2	93.4	90.8	95.9	96.0	100										
11	91.2	91.4	92.5	92.0	91.2	91.6	88.2	92.8	92.8	92.1	100									
12	94.1	94.6	94.6	94.4	94.2	93.0	89.6	94.2	94.8	94.4	91.8	100								
13	90.3	90.9	91.4	90.1	90.1	90.3	89.4	92.5	92.1	91.4	89.5	89.4	100							
14	92.5	93.0	94.1	92.5	92.5	93.0	89.8	94.1	93.5	94.9	92.7	92.1	91.9	100						
15	93.0	93.0	92.6	92.1	92.3	92.1	89.4	93.0	93.2	94.9	91.1	91.9	91.0	96.9	100					
16	92.6	92.5	92.8	92.5	92.5	92.6	90.0	93.5	93.4	95.1	91.8	91.9	91.2	97.1	98.9	100				
17	92.3	92.5	92.8	92.5	92.1	92.3	89.6	93.5	93.0	94.8	91.8	91.9	91.6	97.8	98.5	99.2	100			
18	92.5	92.6	93.0	92.6	92.3	92.5	89.8	93.5	93.2	94.9	91.6	91.8	91.4	97.3	98.7	99.4	99.4	100		
19	95.8	95.7	95.0	94.4	94.6	94.2	90.3	95.5	95.8	93.5	92.1	93.7	90.5	92.8	93.5	93.4	92.6	92.8	100	
20	94.0	93.0	92.6	91.2	92.8	92.8	88.3	92.6	93.2	92.8	89.3	92.1	88.7	91.4	91.7	91.9	91.2	91.4	94.1	100
Species	21	22	23	24																
21	100																			
22	95.2	100																		
23	95.7	94.0	100																	
24	97.5	94.1	95.4	100																

1. *K. africana*; 2. *K. martiniae*; 3. *K. lodderae*; 4. *K. spencerorum*; 5. *K. piceae*; 6. *K. rosinii*; 7. *K. transvaalensis*; 8. *K. servazzii*; 9. *K. unispora*; 10. *K. kunashirensis*; 11. *K. sinensis*; 12. *K. viticola*; 13. *K. telluris*; 14. *K. humilis*; 15. *K. exigua*; 16. *K. turicensis*; 17. *K. barnettii*; 18. *K. bulderi*; 19. *Saccharomyces cerevisiae*; 20. *Vanderwaltozyma polyspora*; 21. *Lipomyces starkeyi*; 22. *Waltomyces lipofer*; 23. *Dipoascopsis uninucleata*; 24. *Kawasakia arxii*. The total sequences were of 557 - 568 bases. For the strains used, refer to Figs.

207
208
209
210

Table 2. The pair-wise sequence similarity in the 18S rRNA gene sequences in *Kazachstania* species.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	100																			
2	98.5	100																		
3	99.0	99.0	100																	
4	98.8	99.1	99.5	100																
5	99.0	98.8	99.4	99.2	100															
6	99.0	98.7	99.2	99.1	99.7	100														
7	97.5	98.0	97.8	97.8	97.8	97.8	100													
8	97.3	97.8	97.4	97.5	97.5	97.5	98.1	100												
9	97.3	97.8	97.4	97.5	97.5	97.5	98.3	99.7	100											
10	98.8	99.3	99.5	99.4	99.3	99.1	97.6	97.5	97.5	100										
11	98.1	98.2	98.0	97.8	98.1	98.2	98.0	98.3	98.5	98.1	100									
12	98.5	98.6	98.8	98.7	98.8	98.7	97.4	97.8	97.6	99.0	98.4	100								
13	96.8	97.0	96.9	96.6	96.9	96.9	97.1	97.9	97.9	96.8	97.6	97.1	100							
14	98.5	98.9	99.0	98.8	98.8	98.8	97.4	97.3	97.5	99.4	98.2	98.8	96.6	100						
15	98.5	98.9	99.0	98.8	98.8	98.8	97.3	97.3	97.4	99.4	98.4	98.8	96.6	99.5	100					
16	98.5	99.0	99.1	98.9	98.8	98.7	97.4	97.3	97.4	99.5	98.4	98.8	96.7	99.5	99.6	100				
17	98.4	98.8	98.9	98.8	98.7	98.5	97.2	97.1	97.3	99.3	98.2	98.7	96.5	99.3	99.8	99.7	100			
18	98.6	99.0	99.1	99.0	98.9	98.8	97.4	97.4	97.5	99.5	98.4	98.9	96.7	99.5	99.8	99.9	99.7	100		
19	98.7	99.2	99.1	98.9	99.0	98.8	97.8	97.7	97.7	99.4	98.2	98.8	97.1	98.8	98.8	98.9	98.7	99.0	100	
20	98.0	98.9	98.2	98.3	98.4	98.1	97.4	97.4	97.4	98.5	97.8	98.2	96.8	98.1	98.2	98.3	98.1	98.4	98.8	100
Species	21	22	23	24																
21	100																			
22	95.5	100																		
23	95.7	97.6	100																	
24	95.9	96.7	97.5	100																

1. *K. africana*; 2. *K. martiniae*; 3. *K. lodderae*; 4. *K. spencerorum*; 5. *K. piceae*; 6. *K. rosinii*; 7. *K. transvaalensis*; 8. *K. servazzii*; 9. *K. unispora*; 10. *K. kunashirensis*; 11. *K. sinensis*; 12. *K. viticola*; 13. *K. telluris*; 14. *K. humilis*; 15. *K. exigua*; 16. *K. turicensis*; 17. *K. barnettii*; 18. *K. bulderi*; 19. *Saccharomyces cerevisiae*; 20. *Vanderwaltozyma polyspora*; 21. *Lipomyces starkeyi*; 22. *Waltomyces lipofer*; 23. *Dipoascopsis uninucleata*; 24. *Kawasakia arxii*. The total sequences were of 1704 - 1709 bases. For the strains used, refer to Figs.

211
212
213
214
215
216
217

218
219

Table 3. The pair-wise sequence similarity in the concatenated sequences from the 18S rRNA genes and 26S rRNA gene D1/D2 domains in *Kazachstania* species.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	100																			
2	98.0	100																		
3	98.2	98.4	100																	
4	97.9	98.0	99.1	100																
5	98.2	98.1	98.9	98.5	100															
6	97.9	97.8	98.6	98.1	99.2	100														
7	96.2	96.3	96.0	96.0	96.1	96.2	100													
8	96.9	97.6	97.1	96.9	97.1	96.9	96.6	100												
9	96.9	97.6	97.1	96.9	97.1	96.9	96.7	99.8	100											
10	97.7	98.2	98.5	98.0	98.1	97.7	95.9	97.1	97.1	100										
11	96.4	96.5	96.6	96.4	96.4	96.6	95.5	97.0	97.1	96.6	100									
12	97.4	97.6	97.8	97.6	97.7	97.3	95.4	96.9	96.8	98.0	96.7	100								
13	95.2	95.4	95.5	95.0	95.2	95.3	95.2	96.6	96.6	95.5	95.6	95.2	100							
14	97.0	97.5	97.9	97.3	97.3	97.4	95.5	96.5	96.7	98.3	96.9	97.3	95.4	100						
15	97.2	97.5	97.6	97.2	97.2	97.2	95.4	96.2	96.3	98.3	96.6	97.2	95.2	98.9	100					
16	97.1	97.4	97.6	97.3	97.3	97.3	95.5	96.4	96.5	98.4	96.7	97.3	95.3	98.9	99.6	100				
17	96.9	97.3	97.5	97.3	97.1	97.0	95.3	96.3	96.4	98.2	96.6	97.1	95.3	98.9	99.3	99.6	100			
18	97.1	97.5	97.7	97.4	97.3	97.3	95.5	96.4	96.5	98.4	96.7	97.3	95.4	99.0	99.6	99.8	99.6	100		
19	98.0	98.3	98.0	97.8	97.9	97.7	95.9	97.1	97.1	98.0	96.7	97.5	95.4	97.4	97.6	97.6	97.3	97.5	100	
20	97.0	97.5	96.9	96.5	97.0	96.8	95.2	96.2	96.2	97.2	95.7	96.7	94.8	96.5	96.7	96.8	96.5	96.7	97.6	100
Species	21	22	23	24																
21	100																			
22	95.4	100																		
23	95.7	96.7	100																	
24	96.3	96.1	97.0	100																

1. *K. africana*; 2. *K. martiniae*; 3. *K. lodderae*; 4. *K. spencerorum*; 5. *K. piceae*; 6. *K. rosinii*; 7. *K. transvaalensis*; 8. *K. servazzii*; 9. *K. unispora*; 10. *K. kunashirensis*; 11. *K. sinensis*; 12. *K. viticola*; 13. *K. telluris*; 14. *K. humilis*; 15. *K. exigua*; 16. *K. turicensis*; 17. *K. barnettii*; 18. *K. bulderi*; 19. *Saccharomyces cerevisiae*; 20. *Vanderwaltozyma polyspora*; 21. *Lipomyces starkeyi*; 22. *Waltomyces lipofer*; 23. *Dipodascopsis uninucleata*; 24. *Kawasakiia arxii*. The total sequences were of 2256 - 2276 bases. For the strains used, refer to Figs.

220
221
222
223
224

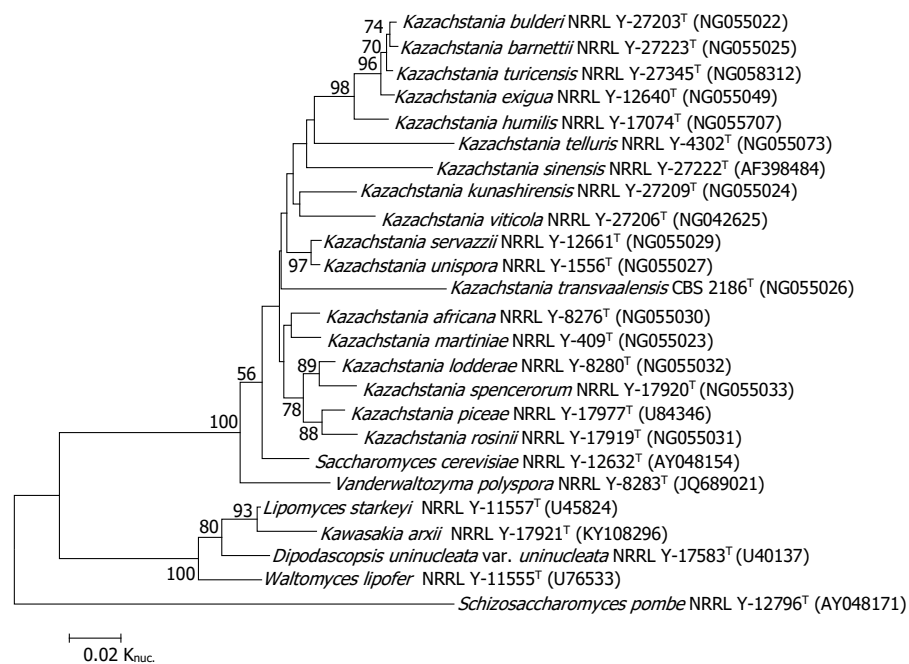


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 548 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

225
226
227

228
229

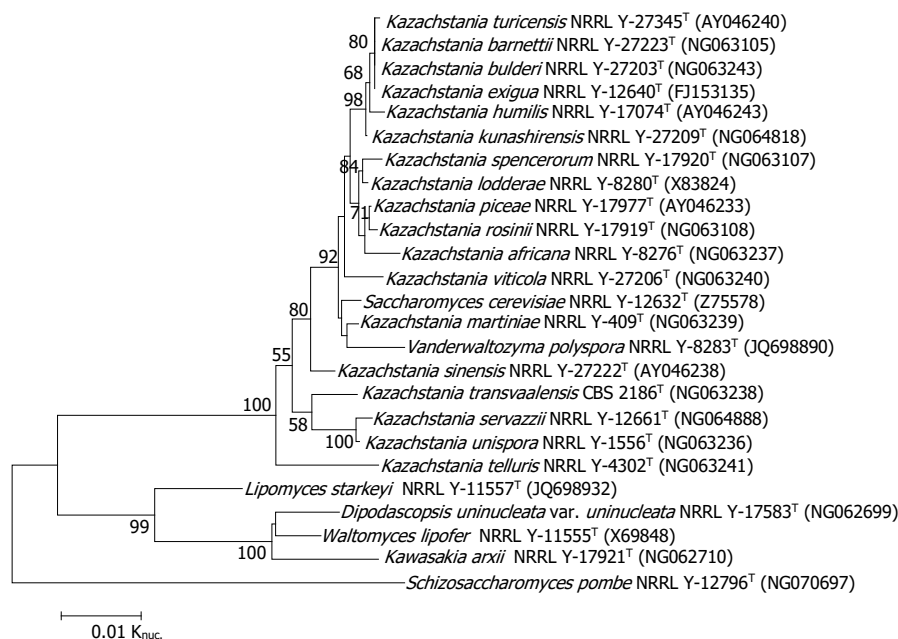


Fig. 2. The phylogenetic tree based on the 18S rRNA gene sequences with 1630 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

230
231

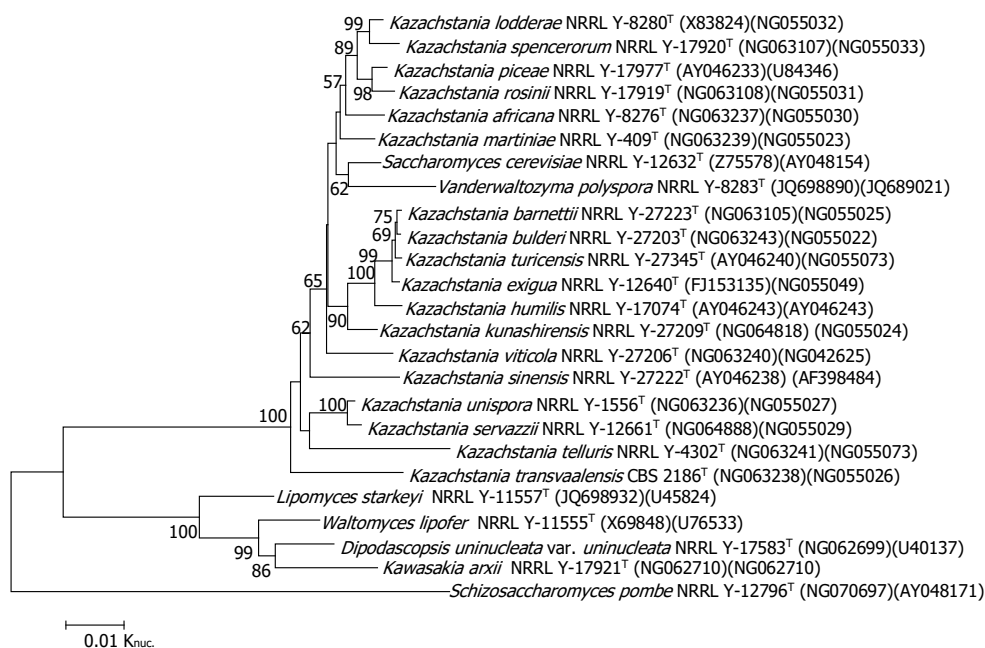


Fig. 3. The phylogenetic tree based on the concatenated sequences from the 18S rRNA genes and 26S rRNA gene D1/D2 domains with 2178 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

232