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# The Generic Circumscription of *Kazachstania* Zubkova (1971) (Sacchromycetaceae) The Proposal of the New Genus *Vanderwaltomyces*

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## Abstract

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

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

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

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

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

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

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

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

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

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

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

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

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

The family Saccharomycetaceae

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

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

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

The type species is *Vanderwaltomyces africanus*.

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

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

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

The type strain is CBS 2517.

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

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## Conflict of interest

The authors declare that there are no conflicts of interest.

## Author contributions

T. M., H.T.L. V., P. Y., S. T. and Y. Y. designed the study. T. M. performed the main experiments. H.T.L. V. and P. Y. instructed the experiments. Y. Y. prepared the manuscript. The detailed discussions were made among the five.

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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.

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

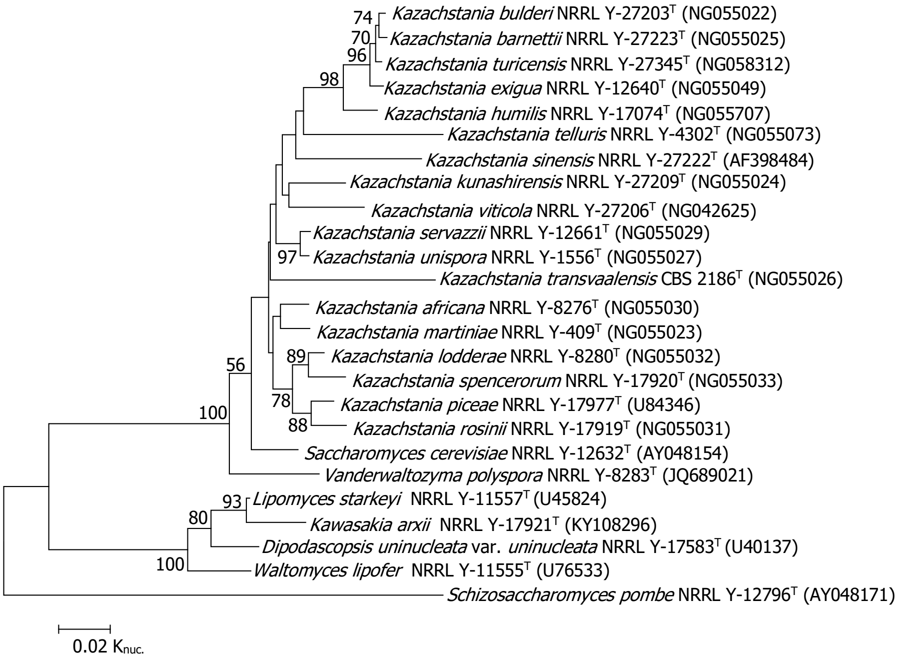
| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 100 | | | | | | | | | | | | | | | | | | | |
| 2 | 98.5 | 100 | | | | | | | | | | | | | | | | | | |
| 3 | 99.0 | 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 | 979 | 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.

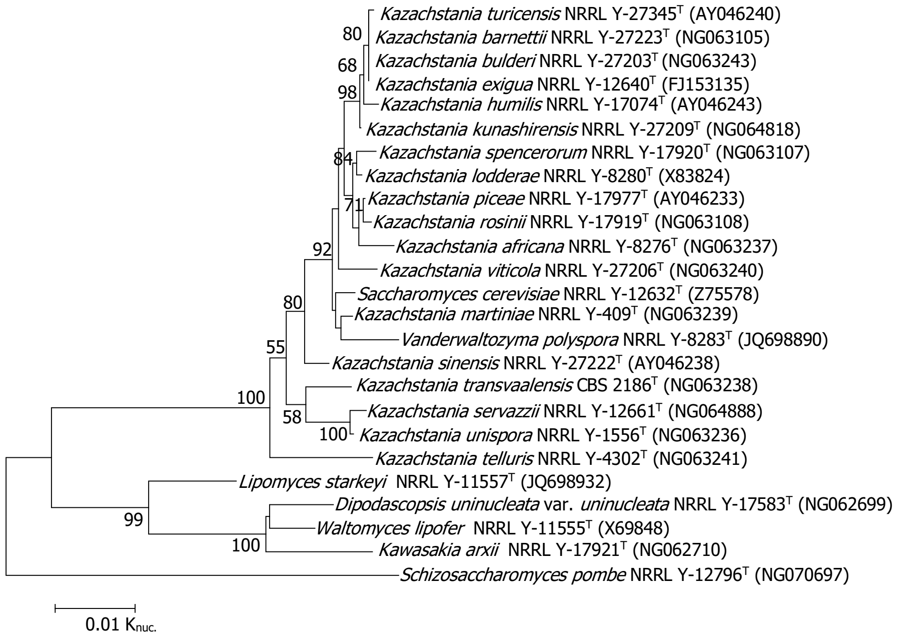
Table 3. The pair-wise sequence similarity in the concatenated sequences from the 18S rRNA genes and 26S rRNA gene D1/D2 domains in *Kazachstania* species.

| 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 | | | | | | | | | | | | | | | | |

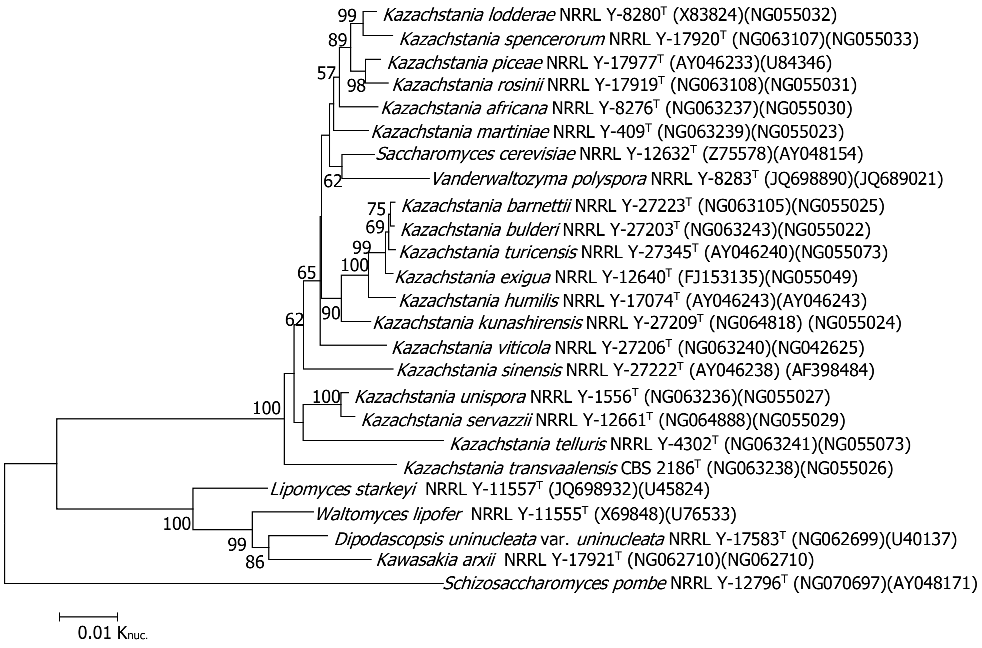
1. *K. africana*; 2. *K. martiniae*; 3. *K. lodderae*; 4. *K. spencerorum*; 5. *K. piceae*; 6. *K. rosinii*; 7. *K. transvaalensis*; 8. *K. servazzii*; 9. *K. unispora*; 10. *K. kunashirensis*; 11. *K. sinensis*; 12. *K. viticola*; 13. *K. telluris*; 14. *K. humilis*; 15. *K. exigua*; 16. *K. turicensis*;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



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.



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.



Fig, 3. The phylogenetic tree based on the concatenated sequences from the18S 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.