

The genus *Hansenula* Sydow et Sydow (1919) nom. rev. and the genus *Wickerhamomyces* Kurtzman et al. (2008)

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

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

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

³Microbial Diversity and Utilization Team, Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Phaholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand

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

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

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

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

DOI: <https://doi.org/10.5281/zenodo.8434537>

Published Date: 12-October-2023

Abstract: The genus *Wickerhamomyces* Kurtzman et al. (2008) was extremely diverse phylogenetically, since a large number of species were equipped with long branches. Of the genus, *Wickerhamomyces anomalus* (= *Hansenula anomala*) and the remaining eight species mutually produced a large cluster with short branches. The pair-wise sequence similarities of *H. anomala* were very high (96.5-99.1%) to the eight species. Thus, the genus *Hansenula* Sydow et Sydow (1919) was revived as a taxonomic homogeneous-natured taxon and phylogenetically and taxonomically separated from the genus *Wickerhamomyces*. In addition, the genus *Wickerhamomyces* was designated as the double generic-structured, along with the genus *Lipomyces* sensu Kurtzman et al. (as the multiple generic-structured) and the genus *Schizosaccharomyces* sensu Kurtzman et Robnett (as the quadruple generic-structured). The three genera actually corresponded respectively to a higher-ranked taxon, i.e., the subfamily or the family.

Keywords: *Wickerhamomyces canadensis*; *Wickerhamomyces anomalus*; *Hansenula anomala*; *Hansenula lynferdii*; *Hansenula myamarensis*.

1. INTRODUCTION

The genus *Hansenula* Sydow et Sydow was reported to include 30 species with *Hansenula anomala* (Hansen) Sydow et Sydow (1919), the type species (Kurtzman 1984a). All the thirty were transferred to the genus *Pichia* Hansen (1904) emend. Kurtzman, since the assimilation of nitrate for growth was one of unreliable criteria (Kurtzman 1984b, 1998). Later, the

many species of the genus *Pichia* emend. were reclassified into the newly introduced genus *Wickerhamomyces* Kurtzman et al. with the type species, *Wickerhamomyces canadensis* (Wickerham) Kurtzman et al. (Kurtzman et al. 2008; Kurtzman 2011). However, the new genus *Wickerhamomyces* was still extremely diverse phylogenetically.

Within the newly established genus *Wickerhamomyces*, several species produced one of the largest clusters (Kurtzman 2011). All of them were once classified in the genus *Hansenula* and had nitrate assimilation capability (Kurtzman 1984a, 1998).

This paper deals with the revival of the genus *Hansenula* for the species within the genus *Wickerhamomyces* by phylogenetic analyses (Yamada et al. 2022).

The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences for 36 *Wickerhamomyces* and related species was constructed by the neighbour-joining method. As shown in Fig. 1, the phylogenetic branch of the type species, *W. canadensis*, the type species was extremely long. In addition, most species of the genus represented long branches as well, indicating that the genus *Wickerhamomyces* is considered to be a monotypic genus. Very interesting is that the phylogenetic branch between *W. canadensis* and *W. kurtzmanii* was similar in length to that between *W. canadensis* and *Saccharomyces cerevisiae*. From the phylogenetic data obtained above, the genus *Wickerhamomyces* was regarded as the so-called *Wickerhamomyces* complex.

Within the genus *Wickerhamomyces*, *W. anomalus* (= *Hansenula anomala*, the type species of the genus *Hansenula*) constituted an extremely large cluster with short branches along with the remaining eight species, i.e., *W. ciferrii* (= *H. ciferrii*), *W. siamensis*, *W. edaphicus*, *Pichia myanmarensis*, *W. subpelliculosus* (= *H. subpelliculosa*), *W. lynferdii* (= *H. lynferdii*), *Wickerhamomyces arborarius* f.a. (= *H. arboraria* f.a., Lachance 2012) and *W. sydowiorum* (= *H. sydowiorum*). The location and the constitution of the cluster was not changeable in the phylogenetic trees derived from the neighbour-joining and the maximum parsimony methods, i.e., the nine species within the large cluster were tightly coupled one another (data not shown), differing in this respect from the Lipomycetaceous yeasts (Kurtzman et al. 2007; Jindamorakot et al. 2012; Yamada et al. 2022).

The pair-wise 26S rRNA gene D1/D2 domain sequence similarity was calculated in the so-called *Wickerhamomyces* complex. First of all, the sequence similarity was very low (85.8%) between *W. canadensis* and *W. anomalus* (= *H. anomala*) (Table 1). The calculated value was enough to separate the two species at the generic level. Incidentally, the similarities of *W. canadensis* were 83.4, 84.7, 85.6, 86.1, 87.8, 85.6, 83.9, 86.4 and 89.4% respectively, i.e., 83.4-89.4% or below 90%, to *W. kurtzmanii*, *W. queroliae*, *W. mucosus*, *W. strasburgensis*, *W. rabaulensis*, *W. chambardii*, *W. pijperi*, *W. menglaensis* and *W. bisporus* (Table 1). By the way, the calculated similarity between *W. canadensis* and *S. cerevisiae* was 81.6%.

In addition, the sequence similarities of *W. anomalus* were 91.8, 92.6, 92.8, 90.9, 86.5 and 86.0%, i.e., below 93%, to *W. kurtzmanii*, *W. mucosus*, *W. rabaulensis*, *W. chambardii*, *W. pijperi* and *W. bisporus*. (Table 1).

Concerning the candidates of the nine *Hansenula* species, the calculated sequence similarities of *H. anomala* (= *W. anomalus*) were very high (96.5 to *H. ciferrii*, 98.2 to *H. lynferdii*, 98.0 to *H. subpelliculosa*, 96.8 to *H. sydowiorum*, 99.1 to *H. myanmarensis*, 96.8 to *H. edaphica*, 97.0 to *H. siamensis* and 97.9% to *H. arboraria* f.a.), when compared with the remaining *Wickerhamomyces* species (83.4-89.4%) (Table 1). Among the eight species, *H. ciferrii*, *H. sydowiorum*, *H. edaphica* and *H. arboraria* f.a. were not beyond the so-called 98% wall (Yamada et al. 2022). However, the four were also accommodated to the genus *Hansenula*, as shown previously in the genera *Hanseniaspora* and *Kloeckeraspora* (Malimas et al. 2023d).

From the data obtained above, the nine species can be re-classified in the genus *Hansenula* separated from the genus *Wickerhamomyces*.

The genus *Hansenula* Sydow et Sydow (1919) nom. rev. (MycoBank2219)

Hat-shaped ascospores, nitrate assimilation for growth, Q-7

The type species is *Hansenula anomala* (Hansen) Sydow et Sydow.

1. *Hansenula anomala* (Hansen) Sydow et Sydow (1919) (MycoBank146467)

2. *Hansenula ciferrii* Lodder (1932) (MycoBank251417)

International Journal of Novel Research in Life Sciences

Vol. 10, Issue 5, pp: (42-47), Month: September - October 2023, Available at: www.noveltyjournals.com

3. *Hansenula lynferdii* van der Walt et Johannsen (1975) (MycoBank314872)

4. *Hansenula subpelliculosa* Bedford ex Barnett, Payne et Yarrow (1983)
(MycoBank115359)

5. *Hansenula sydowiorum* Scott et van der Walt (1970) (MycoBank314879)

6. *Hansenula myanmarensis* (Nagatsuka, Kawasaki et Seki) comb. nov.
MycoBank number is 849044.

Basionym: *Pichia myanmarensis* Nagatsuka, Kawasaki et Seki, Int. J. Syst. Evol. Microbiol. **55**: 1381. 2005.

The type strain is NBRC11090 = CBS9786.

7. *Hansenula edaphica* (Limtong, Yongmanitchai, Kawasaki et Fujiyama) comb. nov.
MycoBank number is 849045.

Basionym: *Wickerhamomyces edaphicus* Limtong, Yongmanitchai, Kawasaki et Fujiyama, FEMS Yeast Res. **9**: 507, 2009.

The type strain is BCC 21231.

8. *Hansenula siamensis* (Kaewwichian, Kawasaki et Limtong) comb. nov.
MycoBank number is 849046.

Basionym: *Wickerhamomyces siamensis* Kaewwichian, Kawasaki et Limtong, Int. J. Syst. Evol. Microbiol. **63**: 1571. 2013.

The type strain is CBS12570.

9. *Hansenula arboraria* f.a. (James, Carvajal, Barahona, Harrington, Lee, Bond et Roberts) comb. nov.
MycoBank number is 849049.

Basionym: *Wickerhamomyces arborarius* f.a. James, Carvajal, Barahona, Harrington, Lee, Bond et Roberts, Int. J. Syst. Evol. Microbiol. **64**: 1060. 2014.

The type strain is CBS12941.

All the nine species in the genus *Hansenula* were positive in the nitrate assimilation for growth except for *H. siamensis*. The nine were taxonomic homogeneous-natured, since the calculated similarities were beyond or around the so-called 98% wall (Yamada 2023; Yamada et al. 2022; Vu et al. 2022a, b; Malimas et al. 2023a, b, c, d).

2. CONCLUSION

It is noticeable that the genus *Wickerhamomyces* Kurtzman et al. (2008) phylogenetically included a different genus, i.e., the genus *Hansenula* Sydow et Sydow (1919).

The generic compositions of *Lipomyces* sensu Kurtzman et al (2007) and *Schizosaccharomyces* sensu Kurtzman et Robnett (1991) were very similar to that of *Wickerhamomyces*. In the three genera mentioned above, there were extremely long branch-equipped taxa, e.g., the genus *Lipomyces* sensu stricto including *L. starkeyi* (Q-9), *L. kononenkoe* (Q-9), *L. yamadae* (Q-9) and so on, the genus *Waltomyces* doing *W. lipofer* (Q-10), the genus *Zygozoma* doing *Z. oligophaga* (Q-8), the genus *Babjevia* doing *B. anomala* (Q-9), the genus *Smithiozoma* doing *S. japonica* (Q-9), the genus *Kawasakia* doing *K. arxii* (Q-9), the genus *Limtongia* doing *L. smithiae* (Q-9) and the genus *Kockiozoma* doing *K. suomiensis* (Q-8), *K. geophila* f.a. (Q-8) and *K. yamanashienais* f.a. (Yamada et al. 2022) within the *Lipomyces* sensu Kurtzman et al. and the genus *Schizosaccharomyces* sensu stricto including *S. pombe* (Q-10), the genus *Octosporomyces* doing *O. octosporus* (Q-9), *O. osmophilus*, *O. cryophilus* (Q-9) and *O. lindnerii* (= *S. lindnerii*; Brysch-Herzberg et al. 2023) and the genus *Hasegawaea* doing *H. japonica* (no Q or trace amount of Q-10) (Vu et al. 2022a) within the genus *Schizosaccharomyces* sensu Kurtzman et Robnett. The many species of the genus *Lipomyces* sensu stricto and the four species of the genus *Octosporomyces* were very similar to the nine species (Q-7) of the genus *Hansenula* within the genus *Wickerhamomyces* phylogenetically.

In the above-mentioned three genera, i.e., *Lipomyces* sensu stricto, *Octosporomyces* and *Hansenula*, the phylogenetic branches were quite short and the calculated sequence similarities were also very high (Fig. 1 & Table 1). As described above, the genus *Wickerhamomyces* Kurtzman et al. was comprised of the double generic-structured (*Hansenula/Wickerhamomyces*) in the taxonomic point of view, as well as the genera *Lipomyces* sensu Kurtzman et al. was of the multiple generic-structured (*Lipomyces* sensu stricto/*Waltomyces/Zygozoma/Babjevia/Smithiozyma/Limtongia/Kockiozyma/Lipomyces* sensu Kurtzman et al.) and *Schizosaccharomyces* sensu Kurtzman et al. and Robnett was of the quadruple generic-structured (*Schizosaccharomyces* sensu stricto/*Octosporomyces/Hase-gawaea/Schizosaccharomyces* sensu Kurtzman et al. and Robnett). In addition, the three genera *Wickerhamomyces* Kurtzman et al., *Lipomyces* sensu Kurtzman et al. and *Schizosaccharomyces* sensu Kurtzman et al. and Robnett were not taxonomic homogeneous but heterogeneous-natured and corresponded respectively to a higher-ranked taxon, i.e., the subfamily or the family (Malimas et al. 2023c).

ACKNOWLEDGEMENTS

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

Funding information

The present authors received no grant from any funding agency.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

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

REFERENCES

- [1] Brysch-Herzberg, M., Jia, G.-S., Sipiczki, M., Seidel, M., Li, W., Assali, I. & Du, L.-L. 2023. *Schizosaccharomyces lindnerii* sp. nov., a fission yeast occurring in honey. *Yeast Wiley* 1-17.
- [2] Jindamorakot, S., Yukphan, P. & Yamada, Y. 2012. *Kockiozyma* gen. nov., for *Zygozoma suomiensis*: The phylogeny of the Lipomycetaceous yeasts (Lipomycetaceae). *Ann Microbiol* **62**: 1831-1840.
- [3] Kurtzman, C.P., 1984a. *Hansenula* H. et P. Sydow. In: Kregg-van Rij, N.J.W. (ed). The Yeasts: A Taxonomic Study, 3rd edition, Amsterdam: Elsevier, p. 165-213.
- [4] Kurtzman, C.P., 1984b. Synonymy of the yeast genera *Hansenula* and *Pichia* demonstrated through comparisons of deoxyribonucleic acid relatedness. *Antonie van Leeuwenhoek* **50**: 209-217.
- [5] Kurtzman, C.P., 1998. *Pichia* E.C. Hansen emend. Kurtzman In: Kurtzman, C.P. and Fell, J.W. (ed). The Yeasts: A Taxonomic Study, 4th edition, Amsterdam: Elsevier, p. 273-352.
- [6] Kurtzman, C.P., 2011. *Wickerhamomyces* Kurtzman, Robnett & Basehoar-Powers (2008). In: Kurtzman, C.P., Fell, J.W. and Boekhout, T. (ed). The Yeasts: A Taxonomic Study, 5th edition, vol. 2. London: Elsevier, p. 899-917.
- [7] Kurtzman, C.P., Albertyn, J. & Basehoar-Powers, E., 2007. Multigene phylogenetic analysis of the Lipomycetaceae and proposed transfer of *Zygozoma* species to *Lipomyces* and *Babjevia anomala* to *Dipodascopsis*. *FEMS Yeast Res* **7**: 1027-1034.
- [8] Kurtzman, C.P., Robnett, C.J., & Basehoar-Powers, E., 2008. Phylogenetic relationships among species of *Pichia*, *Issatchenkia* and *Williopsis* determined from multigene sequence analysis and the proposal of *Barnettozyma* gen. nov., *Lindnera* gen. nov. and *Wickerhamomyces* gen. nov. *FEMS Yeast Res* **8**: 939-954.
- [9] Lachance, M.A., 2012. In defense of sexual life cycles: The forma sexualis – an informal proposals. *Yeast Newsletter* **61**: 24-25.

International Journal of Novel Research in Life Sciences

Vol. 10, Issue 5, pp: (42-47), Month: September - October 2023, Available at: www.noveltyjournals.com

[10] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. & Yamada, Y., 2023a. The subdivision of the genus *Eremothecium* Borzi emend. Kurtzman. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.285>).

[11] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. & Yamada, Y., 2023b. The generic circumscription of *Kazachstania* Zubkova (1971) (Saccharomycetaceae): The proposal of the new genus *Vanderwaltomyces*. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.328>).

[12] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. & Yamada, Y., 2023c. The subdivision of the genus *Kazachstania* Zubkova sensu Kurtzman (2003) (Saccharomycetaceae). *Jxiv* (DOI: <https://org/10.51094/jxiv.334>).

[13] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. & Yamada, Y., 2023d. The reinstatement of the genus *Kloeckeraspora* Niehaus (1932) (apiculate yeasts). *Int J Novel Res Life Sci* **10**: 32-37 (DOI: <https://doi.org/10.5281/zenodo.8409923>).

[14] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. & Yamada, Y., 2023e. The genus *Hansenula* Sydow et Sydow (1919) nom. rev. and the genus *Wickerhamomyces* Kurtzman et al. (2008). *Jxiv* (DOI: <https://org/10.51094/jxiv.397>).

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

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

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

[18] Yamada, Y., 2023. The generic circumscription of *Eremothecium* emend. Kurtzman. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.270>).

A preliminary report was opened [14]. Corresponding: Yuzo Yamada (0000-0002-2799-7045), e-mail: yamada-yuzo@nifty.com

Table 1. The pair-wise sequence similarity in the sequences of 26S rRNA genes D1/D2 domain in the *Wickerhamomyces* and *H. Hansenula* species.

Sp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	100																			
2	83.4	100																		
3	84.7	88.2	100																	
4	85.6	89.4	84.9	100																
5	86.1	89.3	87.1	93.6	100															
6	87.8	89.2	87.2	93.9	91.8	100														
7	85.6	90.9	86.1	91.9	91.9	90.4	100													
8	83.9	85.1	82.0	89.9	89.2	87.3	87.5	100												
9	86.4	82.6	83.0	83.0	83.5	85.6	82.5	84.3	100											
10	89.4	84.5	86.6	85.6	86.8	88.6	85.4	84.9	85.3	100										
11	85.8	91.8	89.7	92.6	92.3	92.8	90.9	86.5	83.0	86.0	100									
12	84.2	91.4	89.2	91.9	91.5	90.4	90.7	85.7	82.6	84.2	96.5	100								
13	85.4	92.5	89.2	93.3	93.6	92.0	92.2	86.8	82.6	85.8	98.2	97.3	100							
14	85.1	92.8	89.2	93.5	93.1	91.8	92.2	86.6	83.0	85.3	98.0	97.5	99.4	100						
15	84.9	91.6	88.4	92.8	93.5	91.1	91.7	85.9	82.3	85.1	96.8	97.1	98.5	98.4	100					
16	85.4	91.8	89.9	92.5	92.3	92.7	90.7	86.2	83.5	86.0	99.1	96.6	98.0	97.9	96.6	100				
17	84.8	91.9	89.4	93.8	91.9	90.8	91.2	85.5	82.6	85.3	96.8	97.1	97.5	97.7	96.4	97.7	100			
18	84.4	91.6	90.1	92.4	92.6	91.1	91.5	86.3	82.8	85.1	97.0	98.0	97.7	97.8	97.0	97.9	97.8	100		
19	85.4	91.4	89.2	92.6	92.8	91.5	90.7	85.3	82.6	85.5	97.9	96.1	97.9	97.7	97.9	98.4	97.2	97.0	100	
20	81.6	83.5	97.4	82.3	84.9	83.3	85.3	83.0	79.1	82.2	83.4	82.5	83.7	83.2	83.3	83.4	83.4	83.4	82.6	100

1. *W. canadensis*; 2. *W. kurtzmanii*; 3. *W. queroliae*; 4. *W. mucosus*; 5. *W. strasburgensis*; 6. *W. rabaulensis*; 7. *W. chambardii*; 8. *W. pijperi*; 9. *W. menglaensis*; 10. *W. bisporus*; 11. *H. anomala*; 12. *H. ciferrii*; 13. *H. lynferdii*; 14. *H. subpelliculosa*; 15. *H. sydowiorum*; 16. *H. myanmarensis*; 17. *H. edaphica*; 18. *H. siamensis*; 19. *H. arboraria* f.a.; 20. *Sccharomyces cerevisiae*.

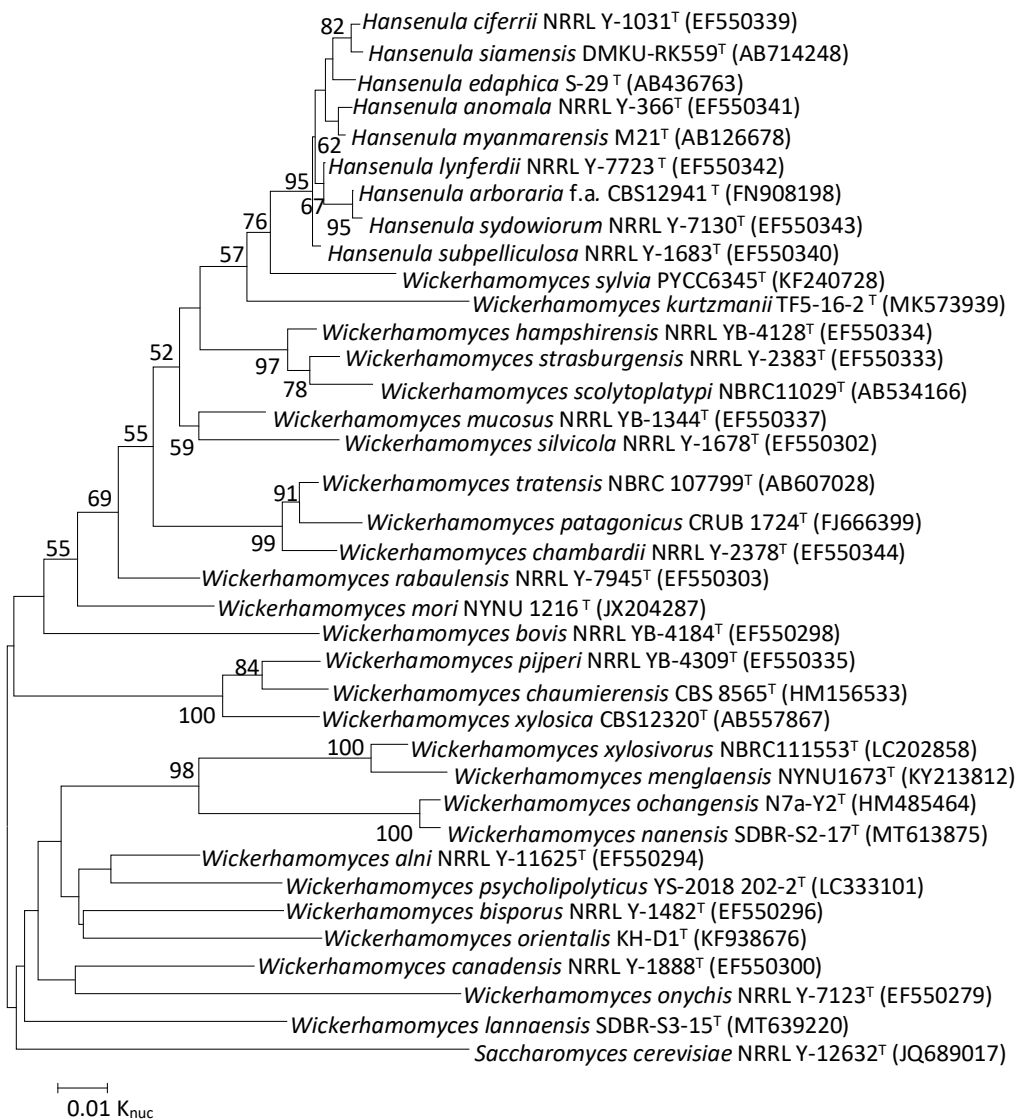


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 491 bases for the nine *Hansenula* species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.