

1 Short Communication

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

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30 **Abstract:** The genus *Wickerhamomyces* Kurtzman et al. (2008) was extremely diverse  
31 phylogenetically, since a large number of species were equipped with long branches. Of the  
32 genus, *Wickerhamomyces anomalus* (= *Haansenula anomala*) produced a large cluster with  
33 short branches along with the remaining eight species. The pair-wise sequence similarities  
34 of *H. anomala* were very high (96.5-99.1%) to the eight species. Thus, the genus *Hansenula*  
35 Sydow et Sydow (1919) was revived as a taxonomic homogeneous-natured taxon and  
36 phylogenetically and taxonomically separated from the genus *Wickerhamomyces*.

37

38 **Keywords:** *Wickerhamomyces canadensis*; *Wickerhamomyces anomalus*; *Hansenula ano-*  
39 *mala*; *Hansenula lynferdii*; *Hansenula myamarensis*.

40

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43 The genus *Hansenula* Sydow et Sydow was reported to include 30 species with *Hansenula*  
44 *anomala* (Hansen) Sydow et Sydow (1919), the type species (Kurtzman 1984a). All the  
45 thirty were transferred to the genus *Pichia* Hansen (1904) emend. Kurtzman, since the  
46 assimilation of nitrate for growth was one of unreliable criteria (Kurtzman 1984b, 1998).  
47 Later, the many species of the genus *Pichia* emend. were reclassified into the newly  
48 introduced genus *Wickerhamomyces* Kurtzman et al. (Kurtzman et al. 2008; Kurtzman  
49 2011). However, the new genus *Wickerhamomyces* was still extremely diverse phylogeni-  
50 cally.

51

52 Within the newly established genus *Wickerhamomyces*, several species produced one of the  
53 largest clusters (Kurtzman 2011). All of them were once classified in the genus *Hansenula*  
54 and had nitrate assimilation capability (Kurtzman 1984a, 1998). This paper is concerned  
55 with the revival of the genus *Hansenula* for the species within the genus.

56

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

66

67 Within the genus *Wickerhamomyces*, *W. anomalus* (= *Hansenula anomala*, the type species  
68 of the genus *Hansenula*) constituted an extremely large cluster with short branches along  
69 with the eight species, i.e., *W. ciferrii* (= *H. ciferrii*), *W. siamensis*, *W. edaphicus*, *Pichia*  
70 *myanmarensis*, *W. subpelliculosus* (= *H. subpelliculosa*), *W. lynferdii* (= *H. lynferdii*),  
71 *Wickerhamomyces arborarius* f.a. (= *H. arboraria* f.a.) and *W. sydowiorum* (= *H.*  
72 *sydowiorum*). The location and the constitution of the cluster was not changeable in the  
73 phylogenetic trees derived from the neighbour-joining and the maximum parsimony  
74 methods, i.e., the nine species within the large cluster were tightly coupled one another (data  
75 not shown), differing in this respect from the Lipomycetaceous yeasts (Kurtzman et al.  
76 2007; Jindamorakot et al. 2012; Yamada et al. 2022).

77

78 In the phylogenetic tree based on the concatenated sequences of the 26S rRNA genes, 18S  
79 rRNA genes and translocation elongation factor-1-alpha derived from the neighbour-joining  
80 method, the cluster of the five species concerned gave the same topology (Fig. 2).

81

82 The pair-wise 26S rRNA gene D1/D2 domain sequence similarity was calculated in the so-  
83 called *Wickerhammyces* complex. First of all, the sequence similarity was very low (85.8/%)  
84 between *W. canadensis* and *W. anomalus* (Table 1). The calculated value was enough to

85 separate the two species at the generic level. Incidentally, the similarities of *W. canadensis*  
86 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%  
87 or below 90%, to *W. kurtzmanii*, *W. queroliae*, *W. mucosus*, *W. strasburgensis*, *W.*  
88 *rabaulensis*, *W. chambardii*, *W. pijperi*, *W. menglaensis* and *W. bisporus* (Table 1). By the  
89 way, the calculated similarity between *W. canadensis* and *S. cerevisiae* was 81.6%.

90

91 In addition, the sequence similarities of *W. anomalus* were 91.8, 92.6, 92.8, 90.9, 86.5 and  
92 86.0%, i.e., below 93%, to *W. kurzmanii*, *W. mucosus*, *W. rabaulensis*, *W. chambardii*, *W.*  
93 *pijperi* and *W. bisporus*. (Table 1).

94

95 Concerning the candidates of the nine *Hansenula* species, the calculated sequence  
96 similarities of *H. anomala* (= *W. anomalus*) were very high (96.5 to *H. ciferrii*, 98.2 to *H.*  
97 *lynferdii*, 98.0 to *H. subpelliculosa*, 96.8 to *H. sydowiorum*, 99.1 to *H. myanmarensis*, 96.8  
98 to *H. edaphica*, 97.0 to *H. siamensis* and 97.9% to *H. arboraria* f.a.), when compared with  
99 the remaining *Wickerhamomyces* species (83.4-89.4%) (Table 1). Among the eight species,  
100 *H. ciferrii*, *H. sydowiorum*, *H. edaphica* and *H. arboraria* f.a. were not beyond the so-called  
101 98% wall (Yamada et al. 2022). However, the four were also accommodated to the genus  
102 *Hansenula*, as shown in the genera *Hanseniaspora* and *Kloeckeraspora* (Malimas et al.  
103 2023d).

104

105 From the data obtained above, the nine species can be classified in the genus *Hansenula*.

106

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

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

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

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

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

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

113 4. *Hansenula subpelliculosa* Bedford ex Barnett, Payne et Yarrow (1983)  
114 (MycoBank115359)  
115 5. *Hansenula sydowiorum* Scott et van der Walt (1970) (MycoBank314879)  
116 6. *Hansenula myanmarensis* (Nagatsuka, Kawasaki et Seki) comb. nov.  
117 MycoBank number is 849044.  
118 Basionym: *Pichia myanmarensis* Nagatsuka, Kawasaki et Seki, Int. J. Syst, Evol.  
119 Microbiol. **55**: 1381. 2005.  
120 The type strain is NBRC11090 = CBS9786.  
121 7. *Hansenula edaphica* (Limtong, Yongmanitchai, Kawasaki et Fujiyama) comb. nov.  
122 MycoBank number is 849045.  
123 Basionym: *Wickerhamomyces edaphicus* Limtong, Yongmanitchai, Kawasaki et  
124 Fujiyama, FEMS Yeast Res. **9**: 507, 2009.  
125 The type strain is BCC 21231.  
126 8. *Hansenula siamensis* (Kaewwichian, Kawasaki et Limtong) comb. nov.  
127 MycoBank number is 849046.  
128 Basionym: *Wickerhamomyces siamensis* Kaewwichian, Kawasaki et Limtong, Int. J.  
129 Syst. Evol. Microbiol. **63**: 1571. 2013.  
130 The type strain is CBS12570.  
131 9. *Hansenula arboraria* f.a. (James, Carvajal, Barahona, Harrington, Lee, Bond et  
132 Roberts) comb. nov. MycoBank number is 849049.  
133 Basionym: *Wickerhamomyces arborarius* f.a. James, Carvajal, Barahona, Harrington,  
134 Lee, Bond et Roberts, Int. J. Syst. Evol. Microbiol. **64**: 1060. 2014.  
135 The type strain is CBS12941.  
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137 All the nine species in the genus *Hansenula* were positive in the nitrate assimilation for  
138 growth except for *H. siamensis*. The nine were taxonomic homogeneous-natured, since the  
139 calculated similarities were beyond or around the so-called 98% wall (Yamada 2023;  
140 Yamada et al. 2022; Vu et al. 2022a, b; Malimas et al. 2023a, b, c, d). In addition, the DNA

141 base compositions of the five species were 32.2-39.3 mol% G+C (range, 7.1 mol% G+C),  
142 i.e., below 40 mol% G+C (Kurtzman 1984a), and all the five species, of course, assimilated  
143 nitrate as a nitrogen source for growth (Kurtzman 2011).

144

#### 145 **Acknowledgements**

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

148

#### 149 Funding information

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

151

#### 152 Conflicts of interest

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

154

#### 155 Author contributions

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

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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. cijferrii*; 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*.

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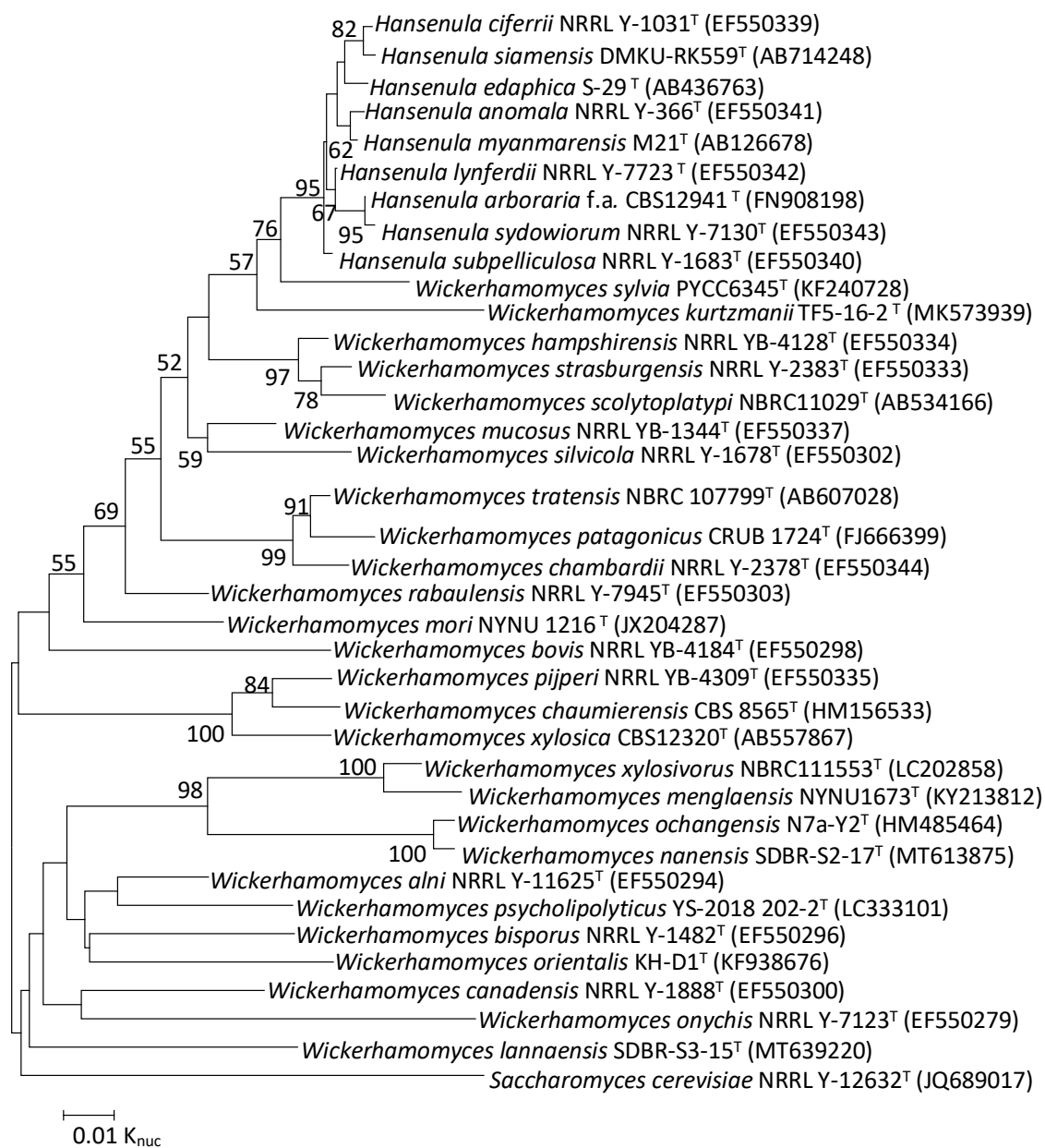


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 491 bases for the nine species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.

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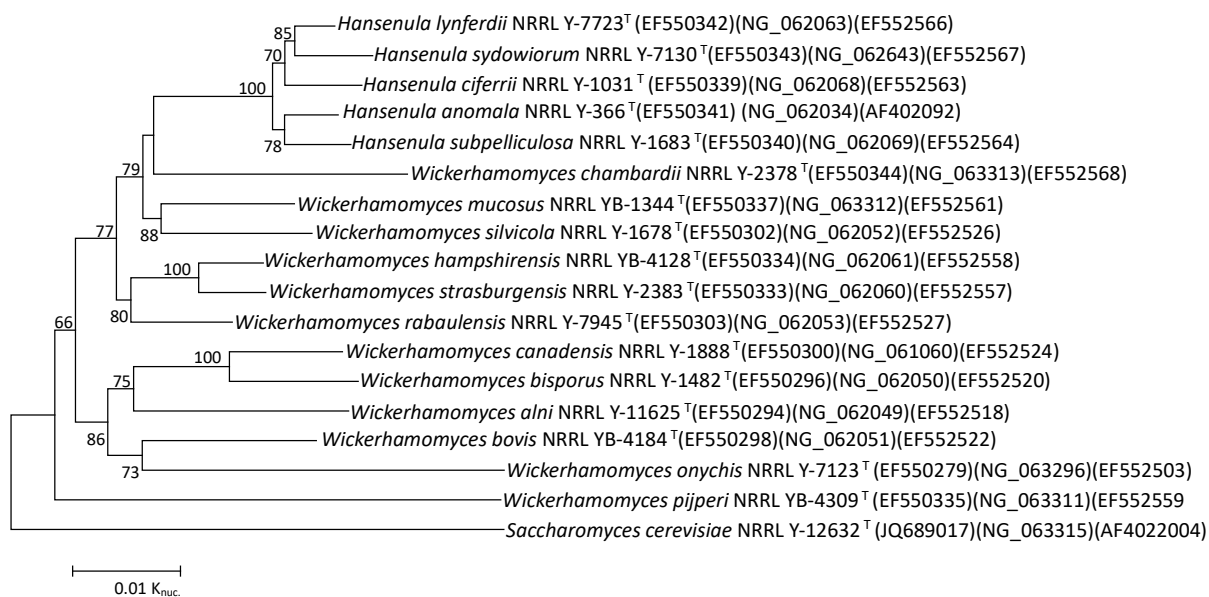


Fig. 2. The phylogenetic tree based on the concatenated sequences (5528 bases) of 26S rRNA genes, 18S rRNA genes and translation elongation factor-1-alpha for the five species of the genus *Hansenula* derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

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