

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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32

33 **Abstract**

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

46

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

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50

51 The genus *Hansenula* Sydow et Sydow was reported to include 30 species with *Hansenula*
52 *anomala* (Hansen) Sydow et Sydow (1919), the type species (Kurtzman 1984a). All the
53 thirty were transferred to the genus *Pichia* Hansen (1904) emend. Kurtzman, since the
54 assimilation of nitrate for growth was one of unreliable criteria (Kurtzman 1984b, 1998).
55 Later, the many species of the genus *Pichia* emend. were reclassified into the newly
56 introduced genus *Wickerhamomyces* Kurtzman et al. with the type species,

57 *Wickerhamomyces canadensis* (Wickerham) Kurtzman et al. (Kurtzman et al. 2008;
58 Kurtzman 2011). However, the new genus *Wickerhamomyces* was still extremely diverse
59 phylogenetically.

60

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

64

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

67

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

77

78 Within the genus *Wickerhamomyces*, *W. anomalus* (= *Hansenula anomala*, the type species
79 of the genus *Hansenula*) constituted an extremely large cluster with short branches along
80 with the remaining eight species, i.e., *W. ciferrii* (= *H. ciferrii*), *W. siamensis*, *W. edaphicus*,
81 *Pichia myanmarensis*, *W. subpelliculosus* (= *H. subpelliculosa*), *W. lynferdii* (= *H. lynferdii*),
82 *Wickerhamomyces arborarius* f.a. (= *H. arboraria* f.a., Lachance 2012) and *W. sydowiorum*
83 (= *H. sydowiorum*). The location and the constitution of the cluster was not changeable in
84 the phylogenetic trees derived from the neighbour-joining and the maximum parsimony

85 methods, i.e., the nine species within the large cluster were tightly coupled one another (data
86 not shown), differing in this respect from the Lipomycetaceous yeasts (Kurtzman et al.
87 2007; Jindamorakot et al. 2012; Yamada et al. 2022).

88

89 The pair-wise 26S rRNA gene D1/D2 domain sequence similarity was calculated in the so-
90 called *Wickerhammyces* complex. First of all, the sequence similarity was very low (85.8%)
91 between *W. canadensis* and *W. anomalus* (= *H. anomala*) (Table 1). The calculated value
92 was enough to separate the two species at the generic level. Incidentally, the similarities of
93 *W. canadensis* were 83.4, 84.7, 85.6, 86.1, 87.8, 85.6, 83.9, 86.4 and 89.4% respectively,
94 i.e., 83.4-89.4% or below 90%, to *W. kurtzmanii*, *W. queroliae*, *W. mucosus*, *W.*
95 *strasburgensis*, *W. rabaulensis*, *W. chambardii*, *W. pijperi*, *W. menglaensis* and *W. bisporus*
96 (Table 1). By the way, the calculated similarity between *W. canadensis* and *S. cerevisiae* was
97 81.6%.

98

99 In addition, the sequence similarities of *W. anomalus* were 91.8, 92.6, 92.8, 90.9, 86.5 and
100 86.0%, i.e., below 93%, to *W. kurzmanii*, *W. mucosus*, *W. rabaulensis*, *W. chambardii*, *W.*
101 *pijperi* and *W. bisporus*. (Table 1).

102

103 Concerning the candidates of the nine *Hansenula* species, the calculated sequence
104 similarities of *H. anomala* (= *W. anomalus*) were very high (96.5 to *H. ciferrii*, 98.2 to *H.*
105 *lynferdii*, 98.0 to *H. subpelliculosa*, 96.8 to *H. sydowiorum*, 99.1 to *H. myanmarensis*, 96.8
106 to *H. edaphica*, 97.0 to *H. siamensis* and 97.9% to *H. arboraria* f.a.), when compared with
107 the remaining *Wickerhamomyces* species (83.4-89.4%) (Table 1). Among the eight species,
108 *H. ciferrii*, *H. sydowiorum*, *H. edaphica* and *H. arboraria* f.a. were not beyond the so-called
109 98% wall (Yamada et al. 2022). However, the four were also accommodated to the genus
110 *Hansenula*, as shown previously in the genera *Hanseniaspora* and *Kloeckeraspora*
111 (Malimas et al. 2023d).

112

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

115

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

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

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

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

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

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

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

123 (MycoBank115359)

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

125 6. *Hansenula myanmarensis* (Nagatsuka, Kawasaki et Seki) comb. nov.

126 MycoBank number is 849044.

127 Basionym: *Pichia myanmarensis* Nagatsuka, Kawasaki et Seki, Int. J. Syst, Evol.

128 Microbiol. **55**: 1381. 2005.

129 The type strain is NBRC11090 = CBS9786.

130 7. *Hansenula edaphica* (Limtong, Yongmanitchai, Kawasaki et Fujiyama) comb. nov.

131 MycoBank number is 849045.

132 Basionym: *Wickerhamomyces edaphicus* Limtong, Yongmanitchai, Kawasaki et

133 Fujiyama, FEMS Yeast Res. **9**: 507, 2009.

134 The type strain is BCC 21231.

135 8. *Hansenula siamensis* (Kaewwichian, Kawasaki et Limtong) comb. nov.

136 MycoBank number is 849046.

137 Basionym: *Wickerhamomyces siamensis* Kaewwichian, Kawasaki et Limtong, Int. J.

138 Syst. Evol. Microbiol. **63**: 1571. 2013.

139 The type strain is CBS12570.

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

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

144 The type strain is CBS12941.

145

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

150

151 **Conclusion**

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

154

155 The generic compositions of *Lipomyces* sensu Kurtzman et al (2007) and
156 *Schizosaccharomyces* sensu Kurtzman et Robnett (1991) were very similar to that of
157 *Wickerhamomyces*. In the three genera mentioned above, there were extremely long branch-
158 equipped taxa, e.g., the genus *Lipomyces* sensu stricto including *L. starkeyi* (Q-9), *L.*
159 *kononenkoae* (Q-9), *L. yamadae* (Q-9) and so on, the genus *Waltomyces* doing *W. lipofer*
160 (Q-10), the genus *Zygozoma* doing *Z. oligophaga* (Q-8), the genus *Babjevia* doing *B.*
161 *anomala* (Q-9), the genus *Smithiozoma* doing *S. japonica* (Q-9), the genus *Kawasakia* doing
162 *K. arxii* (Q-9), the genus *Limtongia* doing *L. smithiae* (Q-9) and the genus *Kockiozoma*
163 doing *K. suomiensis* (Q-8), *K. geophila* f.a. (Q-8) and *K. yamanashienais* f.a. (Yamada et al.
164 2022) within the *Lipomyces* sensu Kurtzman et al. and the genus *Schizosaccharomyces*
165 sensu stricto including *S. pombe* (Q-10), the genus *Octosporomyces* doing *O. octosporus*
166 (Q-9), *O. osmophilus*, *O. cryophilus* (Q-9) and *O. lindnerii* (= *S. lindnerii*; Brysch-Herzberg
167 et al. 2023) and the genus *Hasegawaea* doing *H. japonica* (no Q or trace amount of Q-10)

168 (Vu et al. 2022a) within the genus *Schizosaccharomyces* sensu Kurtzman et Robnett. The
169 many species of the genus *Lipomyces* sensu stricto and the four species of the genus
170 *Octosporomyces* were very similar to the nine species (Q-7) of the genus *Hansenula* within
171 the genus *Wickerhamomyces* phylogenetically.

172

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

187

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195 **Conflicts of interest**

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

197

198 Author contributions

199 Y.Y., T.M., H.T.L.V., P.Y. and S.T. designed the study. T.M. performed the main
200 experiments. P.Y. instructed how to make the experiments. Y.Y. prepared the manuscript.

201 The detailed discussions were made among Y.Y., T.M., H.T.L.V., P.Y., and S.T.

202

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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. 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*.

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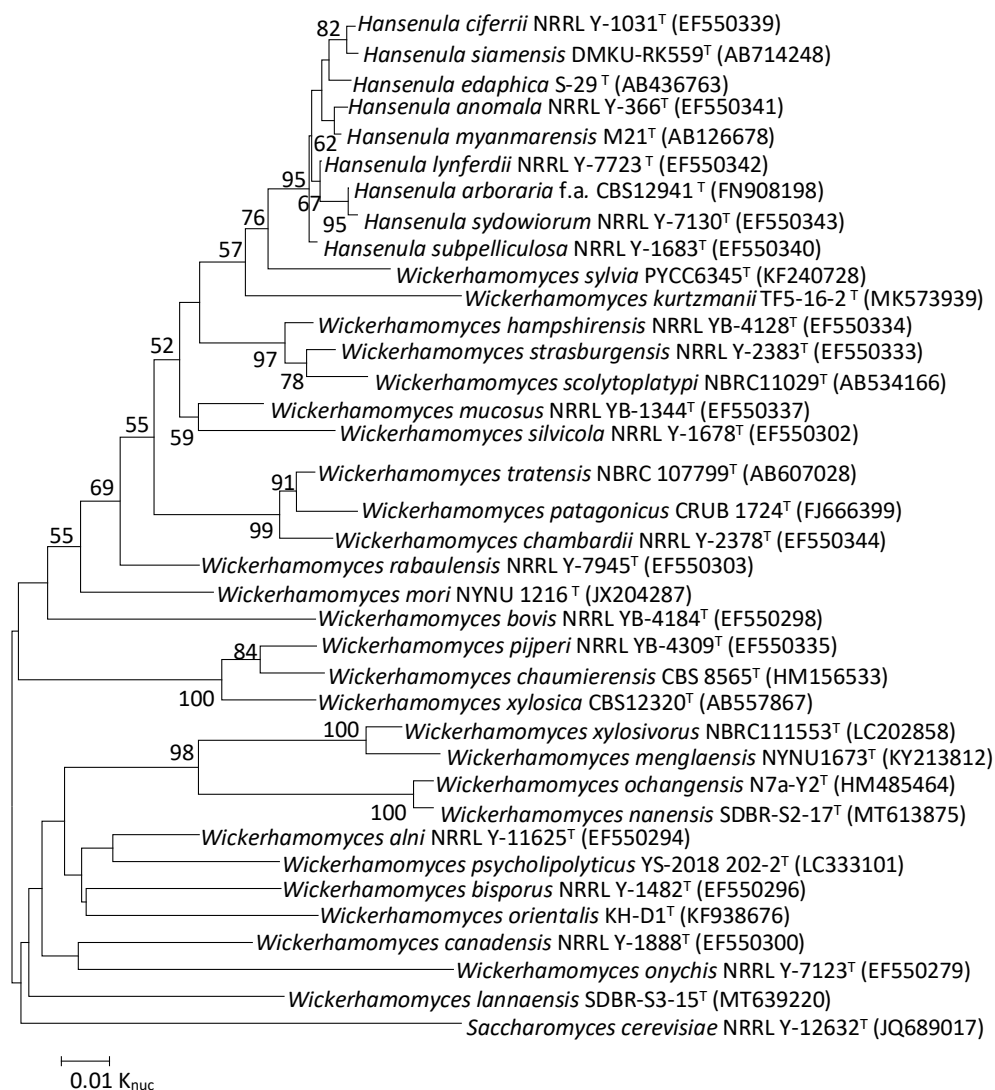


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 491 bases for the nine *Hansenula* species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.

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