

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* (= *Hansenula anomala*) and the remaining eight
33 species produced a large cluster with short branches. The pair-wise sequence similarities of
34 *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*. Thus, the
37 genus *Wickerhamomyces* was designated as the double generic structured, along with the
38 *Lipomyces* sensu Kurtzman et al. (as the multi-generic structured) and *Schizosaccharo-*
39 *myces* sensu Kurtzman et Robnett (as the triple generic structured).

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41 **Keywords:** *Wickerhamomyces canadensis*; *Wickerhamomyces anomalus*; *Hansenula ano-*
42 *mala*; *Hansenula lynferdii*; *Hansenula myamarensis*.

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46 The genus *Hansenula* Sydow et Sydow was reported to include 30 species with *Hansenula*
47 *anomala* (Hansen) Sydow et Sydow (1919), the type species (Kurtzman 1984a). All the
48 thirty were transferred to the genus *Pichia* Hansen (1904) emend. Kurtzman, since the
49 assimilation of nitrate for growth was one of unreliable criteria (Kurtzman 1984b, 1998).
50 Later, the many species of the genus *Pichia* emend. were reclassified into the newly
51 introduced genus *Wickerhamomyces* Kurtzman et al. (Kurtzman et al. 2008; Kurtzman
52 2011). However, the new genus *Wickerhamomyces* was still extremely diverse phylogenet-
53 ically.

54

55 Within the newly established genus *Wickerhamomyces*, several species produced one of the
56 largest clusters (Kurtzman 2011). All of them were once classified in the genus *Hansenula*

57 and had nitrate assimilation capability (Kurtzman 1984a, 1998). This paper is concerned
58 with the revival of the genus *Hansenula* for the species within the genus.

59

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

69

70 Within the genus *Wickerhamomyces*, *W. anomalus* (= *Hansenula anomala*, the type species
71 of the genus *Hansenula*) constituted an extremely large cluster with short branches along
72 with the eight species, i.e., *W. ciferrii* (= *H. ciferrii*), *W. siamensis*, *W. edaphicus*, *Pichia*
73 *myanmarensis*, *W. subpelliculosus* (= *H. subpelliculosa*), *W. lynferdii* (= *H. lynferdii*),
74 *Wickerhamomyces arborarius* f.a. (= *H. arboraria* f.a.) and *W. sydowiorum* (= *H.*
75 *sydowiorum*). The location and the constitution of the cluster was not changeable in the
76 phylogenetic trees derived from the neighbour-joining and the maximum parsimony
77 methods, i.e., the nine species within the large cluster were tightly coupled one another (data
78 not shown), differing in this respect from the Lipomycetaceous yeasts (Kurtzman et al.
79 2007; Jindamorakot et al. 2012; Yamada et al. 2022).

80

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

84

85 The pair-wise 26S rRNA gene D1/D2 domain sequence similarity was calculated in the so-
86 called *Wickerhamomyces* complex. First of all, the sequence similarity was very low (85.8/%)
87 between *W. canadensis* and *W. anomalus* (Table 1). The calculated value was enough to
88 separate the two species at the generic level. Incidentally, the similarities of *W. canadensis*
89 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%
90 or below 90%, to *W. kurzmanii*, *W. queroliae*, *W. mucosus*, *W. strasburgensis*, *W.*
91 *rabaulensis*, *W. chambardii*, *W. pijperi*, *W. menglaensis* and *W. bisporus* (Table 1). By the
92 way, the calculated similarity between *W. canadensis* and *S. cerevisiae* was 81.6%.

93

94 In addition, the sequence similarities of *W. anomalus* were 91.8, 92.6, 92.8, 90.9, 86.5 and
95 86.0%, i.e., below 93%, to *W. kurzmanii*, *W. mucosus*, *W. rabaulensis*, *W. chambardii*, *W.*
96 *pijperi* and *W. bisporus*. (Table 1).

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

107

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

110

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

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

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

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

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

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

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

118 (MycoBank115359)

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

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

121 MycoBank number is 849044.

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

123 Microbiol. **55**: 1381. 2005.

124 The type strain is NBRC11090 = CBS9786.

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

126 MycoBank number is 849045.

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

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

129 The type strain is BCC 21231.

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

131 MycoBank number is 849046.

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

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

134 The type strain is CBS12570.

135 9. *Hansenula arboraria* f.a. (James, Carvajal, Barahona, Harrington, Lee, Bond et

136 Roberts) comb. nov. MycoBank number is 849049.

137 Basionym: *Wickerhamomyces arborarius* f.a. James, Carvajal, Barahona, Harrington,

138 Lee, Bond et Roberts, Int. J. Syst. Evol. Microbiol. **64**: 1060. 2014.

139 The type strain is CBS12941.

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

145

146 **Conclusion**

147 The generic compositions of *Lipomyces* sensu Kurtzman et al (2007) and
148 *Schizosaccharomyces* sensu Kurtzman et Robnett (1991) were very similar to that of
149 *Wickerhamomyces* Kurtzman et al. (2008). In the three genera, there were extremely long
150 branch-equipped taxa, e.g., the *Lipomyces* sensu stricto included *L. starkeyi* (Q-9), *L.*
151 *kononenkoe* (Q-9), *L. yamadae* (Q-9) and so on, the genus *Waltomyces* did *W. lipofer* (Q-
152 10), the genus *Zygozoma* did *Z. oligophaga* (Q-8), the genus *Babjevia* did *B. anomala* (Q-
153 9), the genus *Smithiozyma* did *S. japonica* (Q-9), the genus *Kawasakia* did *K. arxii* (Q-9),
154 the genus *Limtongia* did *L. smithiae* (Q-9) and the genus *Kockiozyma* did *K. suomiensis* (Q-
155 8), *K. geophila* f.a. (Q-8) and *K. yamanashienais* f.a. within the *Lipomyces* sensu Kurtzman
156 et al. (Yamada et al. 2022) and the genus *Schizosaccharomyces* sensu stricto included *S.*
157 *pombe* (Q-10), the genus *Octosporomyces* did *O. octosporus* (Q-9), *O. osmophilus*, *O.*
158 *cryophilus* (Q-9) and *O. lindnerii* (= *S. lindnerii*; Brysch-Herzberg et al. 2023) and the genus
159 *Hasegawaea* did *H. japonica* (no Q or trace amount of Q-10) within the genus
160 *Schizosaccharomyces* sensu Kurtzman et Robnett (Vu et al. 2022a). The many species of
161 the genus *Lipomyces* sensu stricto and the four species of the genus *Octosporomyces*
162 actually corresponded phylogenetically to the nine species (Q-7) of the genus *Hansenula*
163 within the genus *Wickerhamomyces* or the so-called *Wickerhamomyces* complex. As
164 described above, the genus *Wickerhamomyces* was comprised of the double generic
165 structured in the taxonomic point of view, as well as the genera *Lipomyces* sensu Kurtzman
166 et al. (2007) (the multi-generic structured) and *Schizosaccharomyces* sensu Kurtzman et
167 Robnett (1991) (the triple generic structured). In addition, the three were not the taxonomic
168 homogeneous-natured taxa in common.

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177 **Conflicts of interest**

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

179

180 **Author contributions**

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

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

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246 The genus *Wickerhamomyces* Kurtzman et al. (2008) は系統学的に極めて diverse である。
247 すなわち、多くの種が極めて長い系統枝をもっているがゆえである。その中にあつ
248 て、*Wickerhamomyces anomalus* (= *Hansenula anomala*) は、唯一、大きなクラスター
249 を与え、その数、9 種に及ぶ。そのクラスター間の系統枝は極めて短く、このクラ
250 スターは、分類学的均一性を保持していることを示す。*Hansenula anomala* と他の
251 8 種との sequence similarity は非常に高く、96.5-99.1% を示した。かくて、the genus

252 *Hansenula* Sydow et Sydow (1919) nom. rev.が与えられ、the genus *Wickerhamomyces*
 253 とは、系統学的にも、また、分類学的にも区別されることが判明した。このような
 254 異例な属の二重構造について、分類学的に *Lipomyces* および *Schizosaccharomyces*
 255 についても論述した。

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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	83.4	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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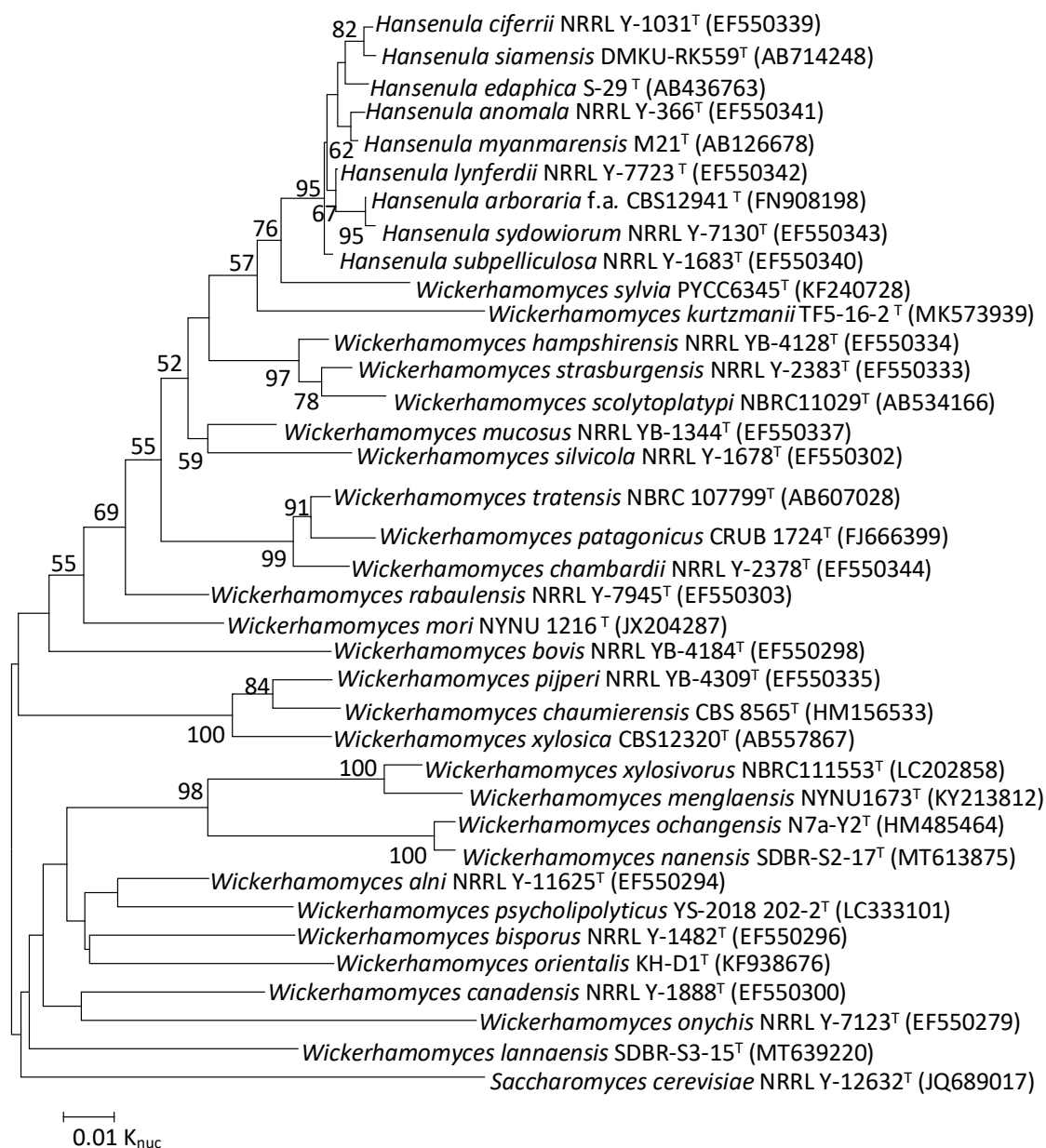


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 491 bases for the nine species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.

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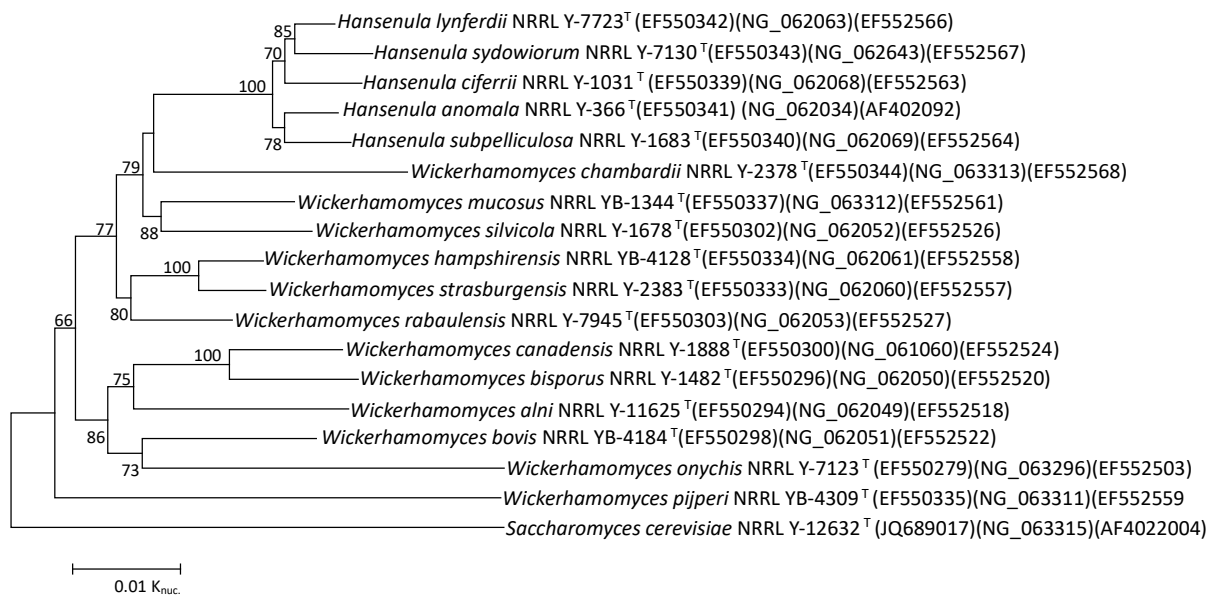


Fig. 2. The phylogenetic tree based on the concatenated sequences (5528 bases) of 26S rRNA genes, 18S rRNA genes and translation elongation factor-1-alpha for the five species of the genus *Hansenula* derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate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

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