

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*. In  
37 addition, the genus *Wickerhamomyces* was designated as the double generic-structured,  
38 along with the genus *Lipomyces* sensu Kurtzman et al. (as the multiple generic-structured)  
39 and the genus *Schizosaccharomyces* sensu Kurtzman et Robnett (as the quadruple generic-  
40 structured).

41

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

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

55

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

60

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

70

71 Within the genus *Wickerhamomyces*, *W. anomalus* (= *Hansenula anomala*, the type species  
72 of the genus *Hansenula*) constituted an extremely large cluster with short branches along  
73 with the eight species, i.e., *W. ciferrii* (= *H. ciferrii*), *W. siamensis*, *W. edaphicus*, *Pichia*  
74 *myanmarensis*, *W. subpelliculosus* (= *H. subpelliculosa*), *W. lynferdii* (= *H. lynferdii*),  
75 *Wickerhamomyces arborarius* f.a. (= *H. arboraria* f.a.) and *W. sydowiorum* (= *H.*  
76 *sydowiorum*). The location and the constitution of the cluster was not changeable in the  
77 phylogenetic trees derived from the neighbour-joining and the maximum parsimony  
78 methods, i.e., the nine species within the large cluster were tightly coupled one another (data  
79 not shown), differing in this respect from the Lipomycetaceous yeasts (Kurtzman et al.  
80 2007; Jindamorakot et al. 2012; Yamada et al. 2022).

81

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

85

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

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

98

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

108

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

111

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

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

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

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

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

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

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

119 (MycoBank115359)

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

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

122 MycoBank number is 849044.

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

124 Microbiol. **55**: 1381. 2005.

125 The type strain is NBRC11090 = CBS9786.

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

127 MycoBank number is 849045.

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

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

130 The type strain is BCC 21231.

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

132 MycoBank number is 849046.

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

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

135 The type strain is CBS12570.

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

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

140 The type strain is CBS12941.

141

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

146

## 147 **Conclusion**

148 The generic compositions of *Lipomyces* sensu Kurtzman et al (2007) and  
149 *Schizosaccharomyces* sensu Kurtzman et Robnett (1991) were very similar to that of  
150 *Wickerhamomyces* Kurtzman et al. (2008). In the three genera, there were extremely long  
151 branch-equipped taxa, e.g., the genus *Lipomyces* sensu stricto included *L. starkeyi* (Q-9), *L.*  
152 *kononenkoae* (Q-9), *L. yamadae* (Q-9) and so on, the genus *Waltomyces* did *W. lipofer* (Q-  
153 10), the genus *Zygozoma* did *Z. oligophaga* (Q-8), the genus *Babjevia* did *B. anomala* (Q-  
154 9), the genus *Smithiozyma* did *S. japonica* (Q-9), the genus *Kawasakia* did *K. arxii* (Q-9),  
155 the genus *Limtongia* did *L. smithiae* (Q-9) and the genus *Kockiozyma* did *K. suomiensis* (Q-  
156 8), *K. geophila* f.a. (Q-8) and *K. yamanashienais* f.a. within the *Lipomyces* sensu Kurtzman  
157 et al. (Yamada et al. 2022) and the genus *Schizosaccharomyces* sensu stricto included *S.*  
158 *pombe* (Q-10), the genus *Octosporomyces* did *O. octosporus* (Q-9), *O. osmophilus*, *O.*  
159 *cryophilus* (Q-9) and *O. lindnerii* (= *S. lindnerii*; Brysch-Herzberg et al. 2023) and the genus  
160 *Hasegawaea* did *H. japonica* (no Q or trace amount of Q-10) within the genus  
161 *Schizosaccharomyces* sensu Kurtzman et Robnett (Vu et al. 2022a). The many species of  
162 the genus *Lipomyces* sensu stricto and the four species of the genus *Octosporomyces*  
163 actually corresponded phylogenetically to the nine species (Q-7) of the genus *Hansenula*

164 within the genus *Wickerhamomyces* or the so-called *Wickerhamomyces* complex. As  
165 described above, the genus *Wickerhamomyces* was comprised of the double generic-  
166 structured in the taxonomic point of view, as well as the genera *Lipomyces* sensu Kurtzman  
167 et al. (2007) (the multiple generic-structured) and *Schizosaccharomyces* sensu Kurtzman et  
168 Robnett (1991) (the quadruple generic-structured). In addition, the three were not the  
169 taxonomic homogeneous-natured taxa in common.

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### 178 **Conflicts of interest**

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

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### 181 **Author contributions**

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

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

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

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The genus *Wickerhamomyces* Kurtzman et al. (2008) は系統学的に極めて diverse である。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。その中にあって、*Wickerhamomyces anomalus* (= *Hansenula anomala*) は、唯一、大きなクラスターを与え、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 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 *Hansenula* Sydow et Sydow (1919) nom. rev. が与えられ、the genus *Wickerhamomyces* 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。このような異例な属の二重構造について、分類学的に *Lipomyces* および *Schizosaccharomyces* 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	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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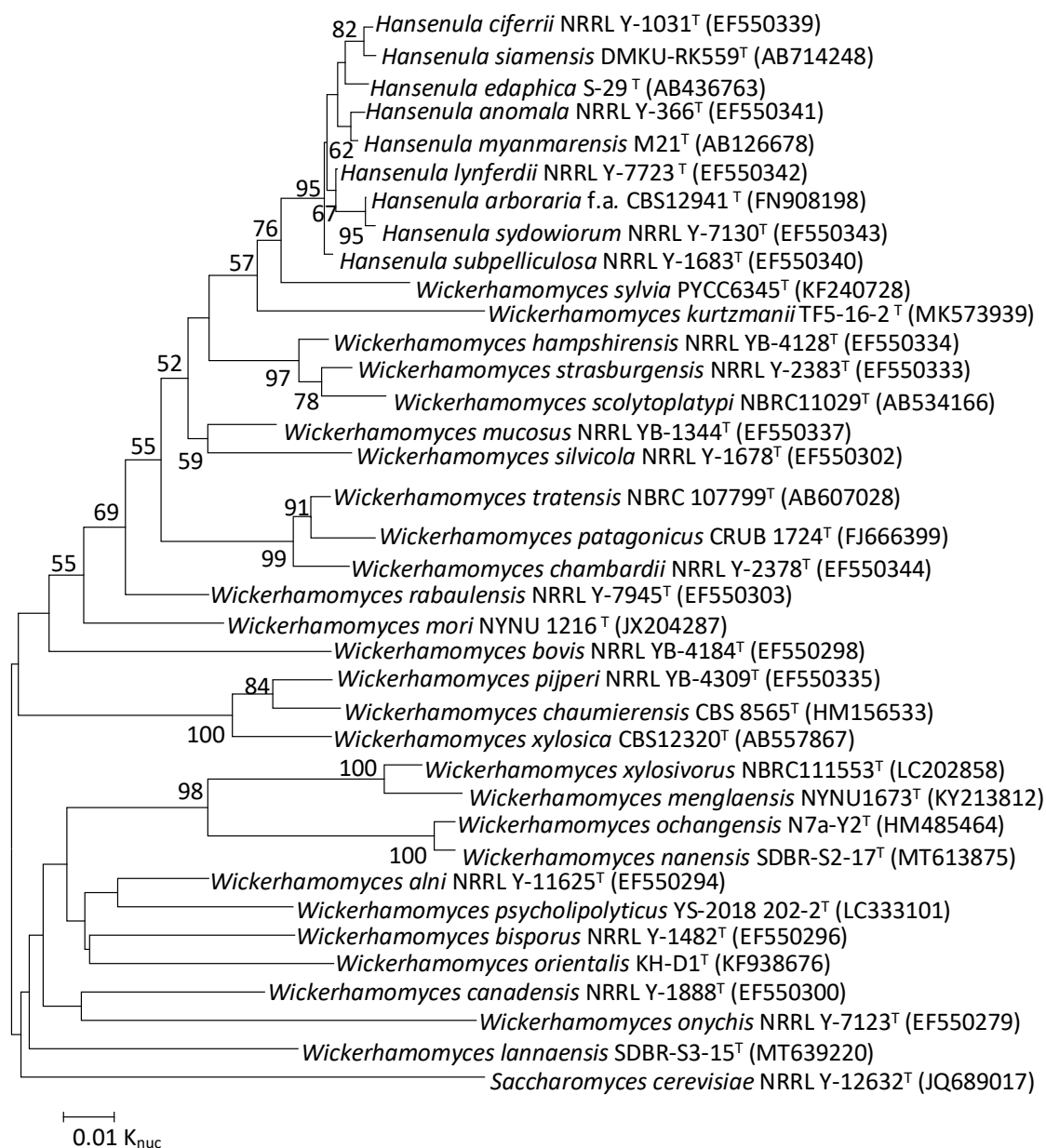


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 491 bases for the nine species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.

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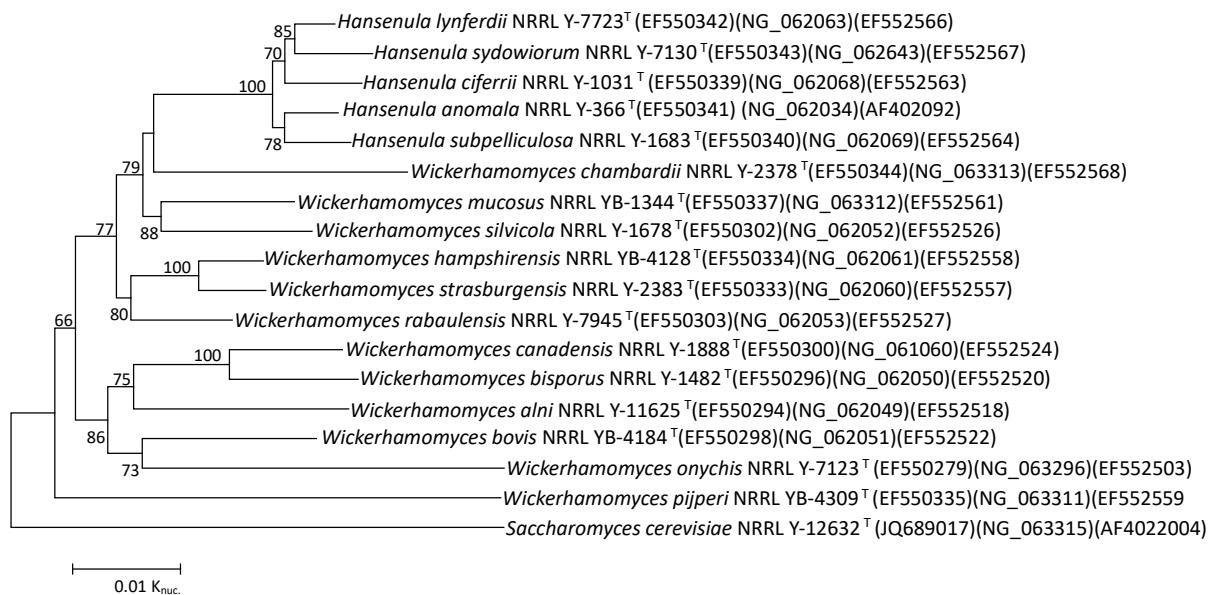


Fig. 2. The phylogenetic tree based on the concatenated sequences (5528 bases) of 26S rRNA genes, 18S rRNA genes and translation elongation factor-1-alpha for the five species of the genus *Hansenula* derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate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

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