

1 Microbial Systematics (Short Communication)

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3 **The Reinstatement of the Genus *Kloeckeraspora* Niehaus (1932) (Apiculate Yeast)**

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31 **Keywords:** *Kloeckeraspora osmophila*; *Kloeckeraspora vineae*; *Kloeckeraspora*
32 *occidentalis*; *Hanseniaspora valvyensis*; *Hanseniaspora uvarum*.

33
34 **Abstract**

35 The genus *Kloeckeraspora* Niehaus was introduced with *Kloeckeraspora osmophila*
36 Niehaus. However, the genus has been neither accepted nor recognized for a long time due
37 to the unclear generic concept. In the phylogenetic tree based on the 26S rRNA gene
38 D1/D2 domain sequences derived from the neighbour-joining method for the seven
39 species, the three species assigned to the genus *Kloeckeraspora* represented an extremely
40 long branch to the *Hanseniaspora* species. The calculated pair-wise sequence similarities
41 were extremely low (86.2 - 88.5%). Incidentally, the sequence similarity between

42 *Vanderwatozyma polyspora* and *Saccharomyces cerevisiae* used as reference standards
43 was 94.0%. Within the respective genus, the similarities were 93.6 - 98.5% in the genus
44 *Kloeckeraspora* and 96.3 - 99.1% in the genus *Hanseniaspora*. From the experimental
45 data, the genus *Kloeckeraspora* should be unequivocally accepted phylogenetically and
46 taxonomically. However, the two genera were not yet perfectly taxonomic homogeneous-
47 natured, since there were a few species showing below 98% sequence similarity and
48 longer phylogenetic branches in the two genera *Kloeckeraspora* and *Hanseniaspora*.

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51 The genus *Kloeckeraspora* Niehaus was introduced along with the species,
52 *Kloeckeraspora osmophila* Niehaus [= *Hanseniaspora osmophila* (Niehaus) Phaff et al.
53 (1984)] (Niehaus 1932). However, the name was not accepted for a long time in the yeast
54 systematics (Cadez and Smith 2011).

55 In the partial base sequencing (Yamada et al. 1992a), the genus *Hanseniaspora* was
56 divided into two groups, i.e., groups *a* and *b*, which were then subdivided into four
57 subgroups, i.e., subgroups *a-1* and *a-2* and subgroups *b-1* and *b-2*.

58 In the dendrogram based on partial base sequences in positions 493 - 622, 130 bases of
59 26S rRNA (designated as region α), the sequence similarity between group *a* (*Hansenia-*
60 *spora osmophila*, *Hanseniaspora vineae* and *Hanseniaspora occidentalis*) and group *b*
61 (*Hanseniaspora valbyensis*, *Hanseniaspora guilliermondii* and *Hanseniaspora uvarum*)
62 was 85%. Within the respective groups *a* and *b*, the similarities were high (97% in
63 subgroup *a-1* and 95% in subgroup *b-1*), respectively. However, *Hanseniaspora*
64 *occidentalis* (subgroup *a-2*) and *Hanseniaspora valbyensis* (subgroup *b-2*) gave low
65 similarities (87% and 85%) to the subgroups *a-1* and *b-1*, respectively.

66 In the dendrogram based on partial base sequencing in positions 1611 - 1835, 225
67 bases of 26S rRNA (designated as region β), the calculated base substitutions between
68 group *a* and group *b* were very high (15). Within the respective groups *a* and *b*, the base
69 substitutions were very low (one and zero). However, *H. occidentalis* (subgroup *a-2*) and
70 *H. valbyensis* (subgroup *b-2*) gave high base substitutions (five and four).

71 In the dendrogram based on partial base sequencing in positions 1451 - 1618, 168
72 bases of 18S rRNA (designated as region γ), the calculated base substitutions between
73 group *a* and group *b* were “six”. Within the respective groups *a* and *b*, the base
74 substitutions were zero and one, respectively. However, the base substitutions of *H.*
75 *occidentalis* (subgroup *a-2*) to subgroup *a-1* and *H. valbyensis* (subgroup *b-2*) to subgroup
76 *b-1* were zero and four, respectively.

77 When compared with *Saccharomyces cerevisiae*, the base substitutions between group
78 *b* and *S. cerevisiae* were surprisingly only “five” in spite of being “six” (the five and
79 additional one) base substitutions between groups *a* and *b* in region γ . Thus, the
80 phylogenetic data obtained above indicated that the two groups *a* and *b* should be
81 recognized as separate genera, i.e., *Kloeckeraspora* and *Hanseniaspora* (Yamada et al.
82 1992b).

83 In the phylogenetic tree based on the concatenated gene sequences (Kurtzman 2003),
84 there was an abnormally long phylogenetic branch within the genus *Hanseniaspora*.

85
86 This paper deals with the presently available sequence data and gives the different
87 conclusion that the genera *Hanseniaspora* Zikes has to be subdivided into two at the
88 generic level.

89
90 The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences (LSU
91 D1/D2) was constructed by the neighbour-joining method for seven species. As shown in
92 Fig. 1, the phylogenetic branches between group *a* (*K. osmophila*, *K. vineae* and *K.*
93 *occidentalis*) and group *b* [*H. uvarum*, *H. guilliermondii*, *H. valbyensis* and *H. lindneri* f.a.
94 (= *Kloeckera lindneri*; Lachance 2012)] were much longer than that between
95 *Vanderwaltozyma polyspora* and *Saccharomyces cerevisiae* used as reference standards as
96 well as the four species assigned to the family Lipomycetaceae.

97 The resulting two groups *a* and *b* were further subdivided into two subgroups,
98 respectively, i.e., subgroups *a-1* (*K. osmophila* and *K. vineae*) and subgroup *a-2* (*K.*
99 *occidentalis*) and subgroup *b-1* (*H. uvarum* and *H. guilliermondii*) and subgroup *b-2* (*H.*
100 *valbyensis* and *H. lindneri* f.a.). The phylogenetic branch between subgroups *a-1* and *a-2*
101 was also longer than that between *V. polyspora* and *S. cerevisiae*, indicating that the two
102 subgroups (in total four) should be also distinguished at the generic level.

103 The pair-wise sequence similarities were calculated, and the calculated similarities
104 were extremely low (86.2 - 88.5%) between groups *a* and *b*, i.e., the two genera (Table 1).
105 On the other hand, the sequence similarities were very high within the respective sub-
106 groups; 98.5% between *K. osmophila* and *K. vineae* (subgroup *1-a*), 99.1% between *H.*
107 *uvarum* and *H. guilliermondii* (subgroup *b-1*) and 98.6% between *H. valbyensis* and *H.*
108 *lindneri* f.a. (subgroup *b-2*) (Table 1). The 98% or more sequence similarities were enough
109 to constitute genera (Yamada et al. 2022; Vu et al. 2022a, b; Yamada 2023; Malimas et al.
110 2023).

111 In contrast, between *K. osmophila* (subgroup *a-1*), the type species of the genus
112 *Kloeckeraspora* (Yamada et al. 1992b) and *H. valbyensis* (subgroup *2-b*), the type species
113 of the genus *Hanseniaspora*, the calculated sequence similarity was 88.5% (Table 1),
114 which was extremely lower than that (94.0%) between *V. polyspora* and *S. cerevisiae*, The
115 calculated similarity was enough to constitute the genus *Kloeckeraspora* apart from the
116 genus *Hanseniaspora*.

117
118 In the genus *Kloeckeraspora*, the sequence similarity was 94.0% between *K. osmo-*
119 *phila* (subgroup *a-1*) and *K. occidentalis* (subgroup *a-2*) (Table 1). In addition, the
120 similarity was 96.5% between *H. valbyensis* (subgroup *b-2*) and *H. uvarum* (subgroup *b-1*)
121 in the genus *Hanseniaspora*. The two calculated values suggested that additional two
122 genera, in total four, would be introduced.

123 However, the additional two were not acceptable, since there were obscure and unclear
124 reasons, as discussed previously in the problem of fission yeast, *Octosporomyces cryo-*
125 *philus* (= *Schizosaccharomyces cryophilus*) (Vu et al. 2022a).

126

127 In the phylogenetic tree based on the 18S rRNA gene sequences (SSU) derived from
128 the neighbour-joining method for the seven species, the cluster between groups *a* group *b*
129 was abnormally long as well (Fig. 2). Additionally, the cluster between subgroups 2-*a* (*H.*
130 *uvarum* and *H. guilliermondii*) and 2-*b* (*H. valbyensis* and *H. lindneri* f.a.) was
131 interestingly much longer than that between *V. polyspora* and *S. cerevisiae*.

132 The calculated sequence similarity (99.0 - 99.8%) was very high in group *a* (Table 2).
133 Concerning group *b*, the sequence similarities were widely distributed (98.3 - 99.8%).
134 Incidentally, it was 98.9% between *V. polyspora* and *S. cerevisiae* (Malimas et al. 2023).

135 On the whole, the base substitutions were very slow in the 18S rRNA gene sequences,
136 as found in the five species classified in the subfamily Eremothecioideae, the family
137 Saccharomycetaceae (Malimas et al. 2023).

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139 The phylogenetic tree based on the concatenated sequences from the 18S rRNA genes
140 and 26S rRNA gene D1/D2 domains (SSU+ LSU D1/D2) was constructed by the
141 neighbor-joining method for the seven species. As shown in Fig. 3, the topology of the
142 phylogenetic tree was on the whole almost similar to that of SSU. Namely, there was an
143 abnormally long branch between groups *a* and *b*, as shown in SSU and LSU D1/D2.
144 Differing from SSU, the branch between subgroups *a-1* (*K. osmophila* and *K. vineae*) and
145 *a-2* (*K. occidentalis*) was relatively long.

146 The calculated sequence similarities were 97.7 - 99.5% within group *a* (*K. osmophila*,
147 *K. vineae* and *K. occidentalis*) (Table 3). Concerning group *b*, the sequence similarities
148 were also widely distributed (97.8 - 99.5%). Incidentally, the similarity was 97.6%
149 between *V. polyspora* and *S. cerevisiae* (Malimas et al. 2023).

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151 From the experimental data obtained above, the introduction of the two genera
152 *Kloeckeraspora* and *Haseniaspora* was unequivocally accepted and recognized
153 phylogenetically in the family Saccharomycetaceae. However, the two genera were not yet
154 taxonomic homogeneous-natured, since the calculated sequence similarities were
155 sometimes below 98% (Yamada et al. 2022; Vu et al. 2022a, b; Yamada 2023; Malimas et
156 al. 2023).

157

158 The phylogenetic data obtained above confirmed that the following three species were
159 unequivocally accommodated to the genus *Kloeckeraspora* as shown previously (Yamada
160 et al. 1992b).

161

162 The family Saccharomycetaceae

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164 Genus *Kloeckeraspora* Niehaus MB2571
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166 The type species is *Kloeckeraspora osmophila* Niehaus
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168 *Kloeckeraspora osmophila* Niehaus (1932) MB273111
169 The type strain is CBS 313.
170
171 *Kloeckeraspora vineae* (van der Walt et Tscheuschner) Yamada, Maeda et Banno
172 (1992) MB456345
173 The type strain is CBS 2171.
174
175 *Kloeckeraspora occidentalis* (Smith) Yamada, Maeda et Banno (1992) MB456344
176 The type strain is CBS 2592.
177
178 According to Cadez and Smith (2011), the phenotypic differentiation of the two genera
179 *Kloeckeraspora* and *Hanseniaspora* was made by ascospore morphology and growth in
180 the presence of 0.01% cycloheximide; spherical ascospores in the former but hat-shaped
181 ascospores in the latter and no growth in the former but growth in the latter.
182
183 For the generic differentiations, the ascospore morphology was utilized in the apiculate
184 yeasts as mentioned above as well as the needle-shaped ascospore-forming yeasts as
185 reported previously (Yamada 2023).
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194 Conflict of interest
195 The authors declare that there are no conflicts of interest.
196
197 Author contributions
198 T. M., H.T.L. V., P. Y., S. T. and Y. Y. designed the study. T. M. performed the main
199 experiments. H.T.L. V. and P. Y. instructed the experiments. Y. Y. prepared the manuscript.
200 The detailed discussions were made among the five.
201
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 234
 235
- 236 Appendix
- 237 Jindamorakot et al. (2009) described two new species in the apiculate yeasts, *Hansenia-*
 238 *spora thailandica* and *Hanseniaspora singularis*. According to the present authors'
 239 grouping, the former is of subgroup *b-3* (a new subgroup) and the latter is of subgroup *b-*
 240 *2*, showing the phylogenetic and taxonomic diversity of the genus.
- 241 [Jindamorakot, S., Ninomiya, S. et al. (2009) Three new species of bipolar budding yeasts
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Table 1. The pair-wise sequence similarity in the 26S rRNA gene D1/D2 domain sequences in *Kloeckeraspora* and *Hanseniaspora* species.

Species	1	2	3	4	5	6	7	8	9
1. <i>K. osmophila</i>	100								
2. <i>K. vineae</i>	98.5	100							
3. <i>K. occidentalis</i>	94.0	93.6	100						
4. <i>H. valbyensis</i>	88.5	88.5	87.5	100					
5. <i>H. uvarum</i>	87.3	87.3	86.2	96.5	100				
6. <i>H. guilliermondii</i>	87.6	87.6	86.8	96.3	99.1	100			
7. <i>H. lindneri</i> f.a.	88.5	88.5	87.6	98.6	97.2	96.6	100		
8. <i>S. cerevisiae</i>	89.8	89.8	89.5	86.3	85.6	86.3	86.8	100	
9. <i>V. polyspora</i>	90.7	90.4	91.5	88.3	87.0	87.3	88.7	94.0	100
Species	10	12	13	14					
10. <i>L. starkeyi</i>	100								
11. <i>W. lipofer</i>	95.2	100							
12. <i>D. uninucleata</i>	95.7	94.0	100						
13. <i>K. arxii</i>	97.5	94.1	95.4	100					

K., *Kloeckeraspora*; *H.*, *Hanseniaspora*; *S.*, *Saccharomyces*; *V.* *Vanderwaltozyma*; *L.* *Lipomyces*; *W.*, *Waltomyces*; *D.*, *Dipoascopsis*; *K.*, *Kawasakia*.

The total sequences were of 568 - 570 bases.

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Table 2. The pair-wise sequence similarity in the 18S rRNA gene sequences in *Kloeckeraspora* and *Hanseniaspora* species.

Species	1	2	3	4	5	6	7	8	9
1. <i>K. osmophila</i>	100								
2. <i>K. vineae</i>	99.8	100							
3. <i>K. occidentalis</i>	99.0	99.0	100						
4. <i>H. valbyensis</i>	94.7	94.6	94.6	100					
5. <i>H. uvarum</i>	94.7	94.6	94.5	98.3	100				
6. <i>H. guilliermondii</i>	94.5	94.5	94.6	98.4	99.7	100			
7. <i>H. lindneri</i> f.a.	94.7	94.6	94.6	99.8	98.3	98.4	100		
8. <i>S. cerevisiae</i>	94.7	94.6	94.3	93.9	93.6	93.4	93.8	100	
9. <i>V. polyspora</i>	94.9	94.8	94.5	94.3	94.0	93.8	94.2	98.9	100
Species	10	11	12	13					
10. <i>L. starkeyi</i>	100								
11. <i>W. lipofer</i>	95.5	100							
12. <i>D. uninucleata</i>	95.7	97.6	100						
13. <i>K. arxii</i>	95.9	96.7	97.5	100					

K., *Kloeckeraspora*; *H.*, *Hanseniaspora*; *S.*, *Saccharomyces*; *V.* *Vanderwaltozyma*; *L.* *Lipomyces*; *W.*, *Waltomyces*; *D.*, *Dipoascopsis*; *K.*, *Kawasakia*.

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Table 3. The pair-wise sequence similarity in the concatenated sequences from the 18S rRNA genes and 26S rRNA gene D1/D2 domains in *Kloeckeraspora* and *Hanseniaspora* species.

Species	1	2	3	4	5	6	7	8	9
1. <i>K. osmophila</i>	100								
2. <i>K. vineae</i>	99.5	100							
3. <i>K. occidentalis</i>	97.8	97.7	100						
4. <i>H. valbyensis</i>	93.1	93.0	92.8	100					
5. <i>H. uvarum</i>	92.5	92.4	92.1	97.8	100				
6. <i>H. guilliermondii</i>	92.5	92.5	92.3	97.8	99.5	100			
7. <i>H. lindneri</i> f.a.	93.1	93.0	92.9	99.5	98.0	97.9	100		
8. <i>S. cerevisiae</i>	94.0	93.9	93.6	91.9	91.4	91.5	92.0	100	
9. <i>V. polyspora</i>	94.4	94.2	94.4	92.6	92.0	92.0	92.7	97.6	100
Species	10	11	12	13					
10. <i>L. starkeyi</i>	100								
11. <i>W. lipofer</i>	95.3	100							
12. <i>D. uninucleata</i>	95.6	96.7	100						
13. <i>K. arxii</i>	96.3	96.0	96.9	100					

K., *Kloeckeraspora*; *H.*, *Hanseniaspora*; *S.*, *Saccharomyces*; *V.* *Vanderwaltozyma*; *L.* *Lipomyces*; *W.*, *Waltomyces*; *D.*, *Dipodascopsis*; *K.*, *Kawasakia*.

The total sequences were of 2256 - 2274 bases.

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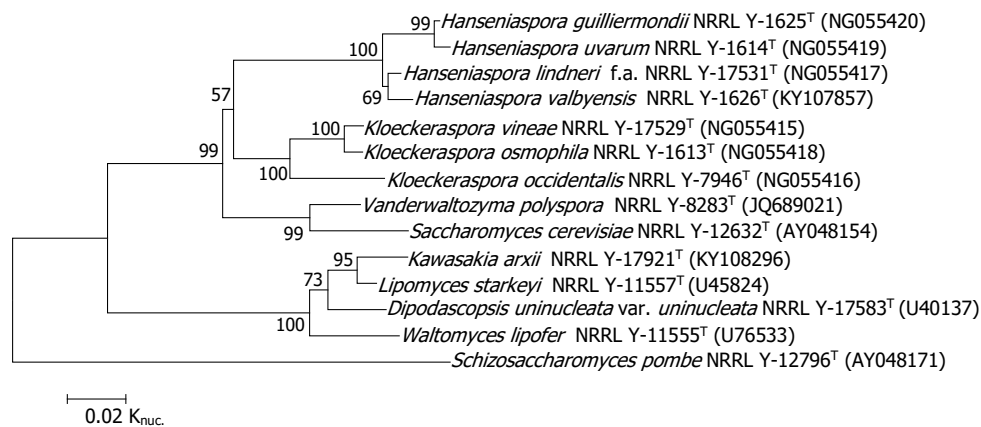


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 554 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

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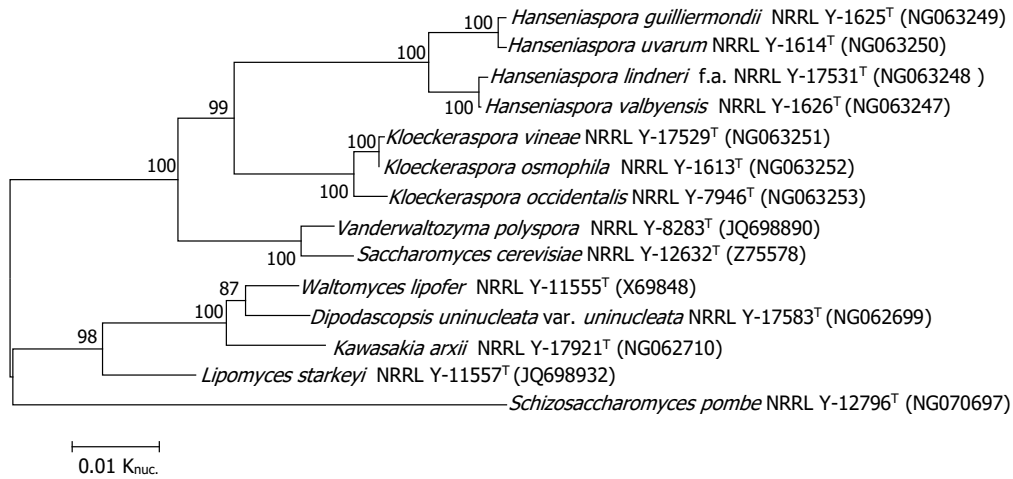


Fig. 2. The phylogenetic tree based on the 18S rRNA gene sequences with 1652 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

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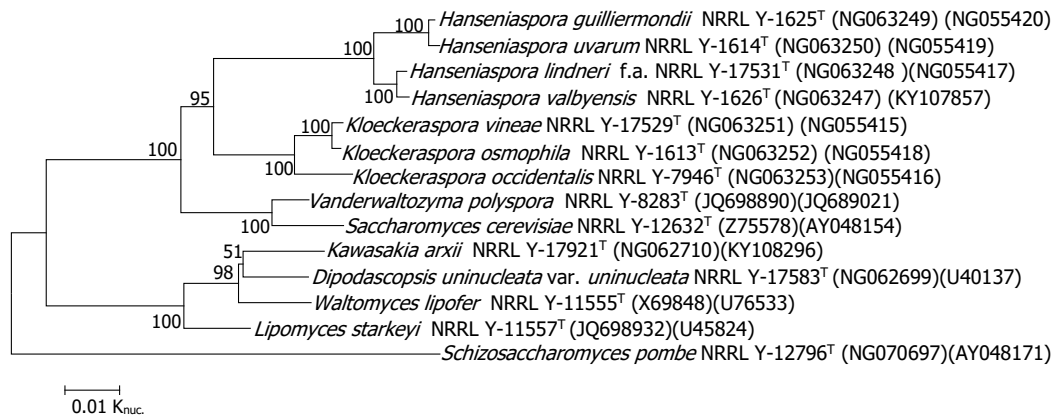


Fig. 3. The phylogenetic tree based on the concatenated sequences from the 18S rRNA genes and 26S rRNA gene D1/D2 domains with 2025 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

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