

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

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**Abstract:** The genus *Kloeckeraspora* Niehaus (1932) was not accepted for a long time. This paper has tried to reinstate the genus. Two phylogenetic trees were constructed for three *Kloeckeraspora* and four representative *Hanseniaspora* species. In addition, the pair-wise sequence similarities were calculated. In the phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences derived from the neighbour-joining method, the three species of the genus *Kloeckeraspora* represented an extremely long branch. The calculated pair-wise sequence similarities were abnormally low (86.2 - 88.5%) between the two genera. Incidentally, the sequence similarity between *Vanderwatozyma polyspora* and *Saccharomyces cerevisiae* used as references was 94.0%. The genus *Kloeckeraspora* should be unequivocally accepted and retained. However, the two genera were not yet perfectly taxonomic homogeneous-natured.

**Keywords:** *Kloeckeraspora osmophila*; *Kloeckeraspora vineae*; *Kloeckeraspora occidentalis*; *Hanseniaspora valbyensis*; *Hanseniaspora uvarum*; *Hanseniaspora guilliermondii*.

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## I. INTRODUCTION

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

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

In the dendrogram based on partial base sequences in positions 493 - 622, 130 bases of 26S rRNA (designated as region a), the sequence similarity between group *a* (*Hanseniaspora osmophila*, *Hanseniaspora vineae* and *Hanseniaspora occidentalis*) and group *b* (*Hanseniaspora valbyensis*, *Hanseniaspora guilliermondii* and *Hanseniaspora uvarum*) was very

low (85%). Within the respective groups *a* and *b*, the similarities were high (97% in subgroup *a-1* and 95% in subgroup *b-1*), respectively. However, *Hanseniaspora occidentalis* (subgroup *a-2*) and *Hanseniaspora valbyensis* (subgroup *b-2*) gave low similarities (87% and 85%) to the subgroups *a-1* and *b-1*, respectively.

In the dendrogram based on partial base sequencing in positions 1611 - 1835, 225 bases of 26S rRNA (designated as region *b*), the calculated base substitutions between group *a* and group *b* were very high (15). Within the respective groups *a-1* and *b-1*, the base substitutions were very low (one and zero). However, *H. occidentalis* (subgroup *a-2*) and *H. valbyensis* (subgroup *b-2*) gave high base substitutions (five and four) respectively to subgroups *a-1* and *b-1*.

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

When compared with *Saccharomyces cerevisiae*, the base substitutions between group *b* and *S. cerevisiae* were surprisingly only “five” in spite of being “six” (the five and additional one) base substitutions between groups *a* and *b* in region *c*. Thus, the phylogenetic data obtained above indicated that the two groups *a* and *b* should be separated at the generic level, i.e., *Kloeckeraspora* and *Hanseniaspora* (Yamada et al. 1992b).

In the phylogenetic tree based on the concatenated gene sequences (Kurtzman 2003), there were abnormally long branches within the genus *Hanseniaspora*.

This paper deals with the presently available gene sequence data and gives the different conclusion that the genera *Hanseniaspora* Zikes should be subdivided into two genera.

## II. BODY OF ARTICLE

*Kloeckeraspora osmophila*, the type species (Yamada et al. 1992b), *Kloeckeraspora vineae* and *Kloeckeraspora occidentalis* were examined for phylogenetic analyses (Yamada et al. 2022). In the genus *Hanseniaspora*, *Hanseniaspora valbyensis*, *Hanseniaspora uvarum*, *Hanseniaspora guilliermondii* and *Hanseniaspora lindneri* f.a. (= *Kloeckera lindneri*; Lachance 2012) were tested as representative species. *Vandervaltozyma polyspora* and *Saccharomyces cerevisiae* as well as the four species in the family Lipomycetaceae were used as reference standards.

The DNA sequence data were cited from the Gene Bank. The two phylogenetic trees based on the 26S rRNA gene D1/D2 domain and the 18S rRNA gene sequences were respectively constructed by the neighbour-joining method. The accession numbers of the gene sequences used were listed in the resulting phylogenetic trees. In addition, the pair-wise sequence similarities were calculated among the species examined.

In the phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences (LSU D1/D2) (Fig. 1), the phylogenetic branches between group *a* (*K. osmophila*, *K. vineae* and *K. occidentalis*) and group *b* (*H. uvarum*, *H. guilliermondii*, *H. valbyensis* and *H. lindneri* f.a.) were much longer than that between *Vandervaltozyma polyspora* and *Saccharomyces cerevisiae* used as reference standards as well as the four species assigned to the family Lipomycetaceae.

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

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

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

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

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

In the phylogenetic tree based on the 18S rRNA gene sequences (SSU) (Fig. 2), the cluster between groups *a* and group *b* was abnormally long as well. Additionally, the cluster between subgroups *2-a* (*H. uvarum* and *H. guilliermondii*) and *2-b* (*H. valbyensis* and *H. lindneri* f.a.) was interestingly much longer than that between *V. polyspora* and *S. cerevisiae*.

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

It is of interest that the base substitutions were on the whole very slow in the 18S rRNA gene sequences, as found in the five species classified in the subfamily Eremothecioideae, the family Saccharomycetaceae (Malimas et al. 2023a). In the genus *Tetrapisispora*, the base substitutions were very slow (Malimas et al. 2023c). In fact, the calculated sequence similarities were very high (99.3 - 99.8%).

From the experimental data obtained above, the introduction of the two genera *Kloeckeraspora* and *Hanseniaspora* was unequivocally accepted and recognized from the phylogenetic point of view. However, the two genera were not yet taxonomic homogeneous-natured, since the calculated sequence similarities were sometimes below 98% (Yamada et al. 2022; Vu et al. 2022a, b; Yamada 2023; Malimas et al. 2023a, c).

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

The family Saccharomycetaceae

The genus *Kloeckeraspora* Niehaus MB2571

The type species is *Kloeckeraspora osmophila* Niehaus

*Kloeckeraspora osmophila* Niehaus (1932) MB273111

The type strain is CBS 313.

*Kloeckeraspora vineae* (van der Walt et Tscheuschner) Yamada, Maeda et Banno  
(1992) MB456345

The type strain is CBS 2171.

*Kloeckeraspora occidentalis* (Smith) Yamada, Maeda et Banno (1992) MB456344

The type strain is CBS 2592.

According to Cadez and Smith (2011), the phenotypic differentiation of the two genera *Kloeckeraspora* and *Hanseniaspora* was made by ascospore morphology and growth in the presence of 0.01% cycloheximide; spherical ascospores in the former but hat-shaped ascospores in the latter and no growth in the former but growth in the latter.

For the generic differentiations, the ascospore morphology was utilized in the apiculate yeasts as mentioned above as well as the needle-shaped ascospore-forming yeasts as reported previously (Yamada 2023).

### III. CONCLUSION

The genus *Kloeckeraspora* Niehaus (1932) should be recognized and accepted, along with the genus *Hanseniaspora* Zikes (1912) in the yeast systematics. However, the two genera are not yet taxonomic homogeneous-natured completely.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

T. M., H.T.L. V., P. Y., S. T. and Y. Y. designed the study. T. M. performed the main experiments. H.T.L. V. and P. Y. instructed the experiments. Y. Y. prepared the manuscript. The detailed discussions were made among the five.

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APPENDIX

Jindamorakot et al. (2009) described two new species in the apiculate yeasts, *Hanseniaspora thailandica* and *Hanseniaspora singularis*. According to the present authors' grouping, the former is of subgroup *b-3* (a new subgroup) and the latter is of subgroup *b-2*, showing the phylogenetic and taxonomic diversity of the genus.

[14] S. Jindamorakot, S. Ninomiya et al. Three new species of bipolar budding yeasts of the genus *Hanseniaspora* and its anamorph *Kloeckera* isolated in Thailand. FEMS Yeast Res 9: 1327-1337, 2009.

A preliminary report was opened (Malimas et al. 2023b). Corresponding: Y. Yamada, [yamada-yuzo@nifty.com](mailto:yamada-yuzo@nifty.com)

TABLE I: The pair-wise sequence similarity in the 26S rRNA gene D1/D2 domain sequences in *Kloeckeraspora* and *Hanseniaspora* species.

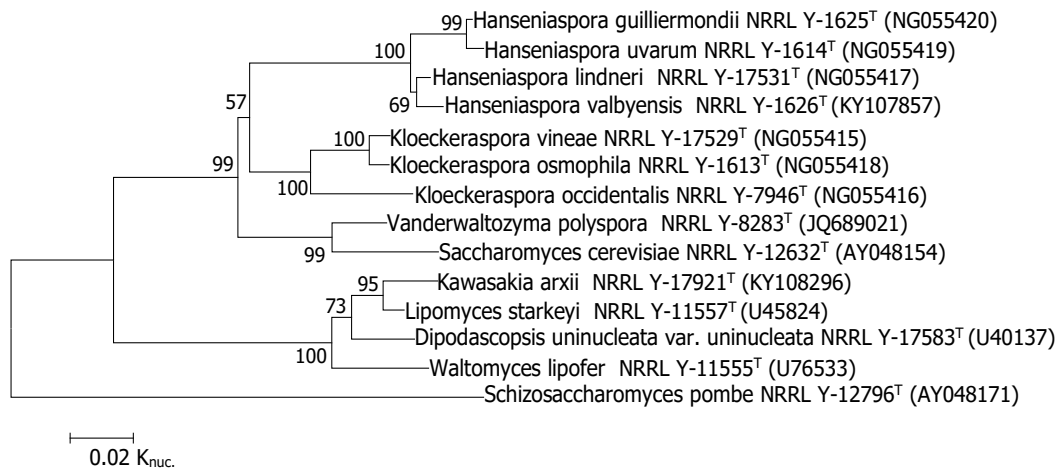
Species	1	2	3	4	5	6	7	8	9
1. <i>K. osmophila</i>	100								
2. <i>K. vineae</i>	98.5	100							
3. <i>K. occidentalis</i>	94.0	93.6	100						
4. <i>H. valbyensis</i>	88.5	88.5	87.5	100					
5. <i>H. uvarum</i>	87.3	87.3	86.2	96.5	100				
6. <i>H. guilliermondii</i>	87.6	87.6	86.8	96.3	99.1	100			
7. <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.*, *Kawasaki*. The total sequences were of 568 - 570 bases.

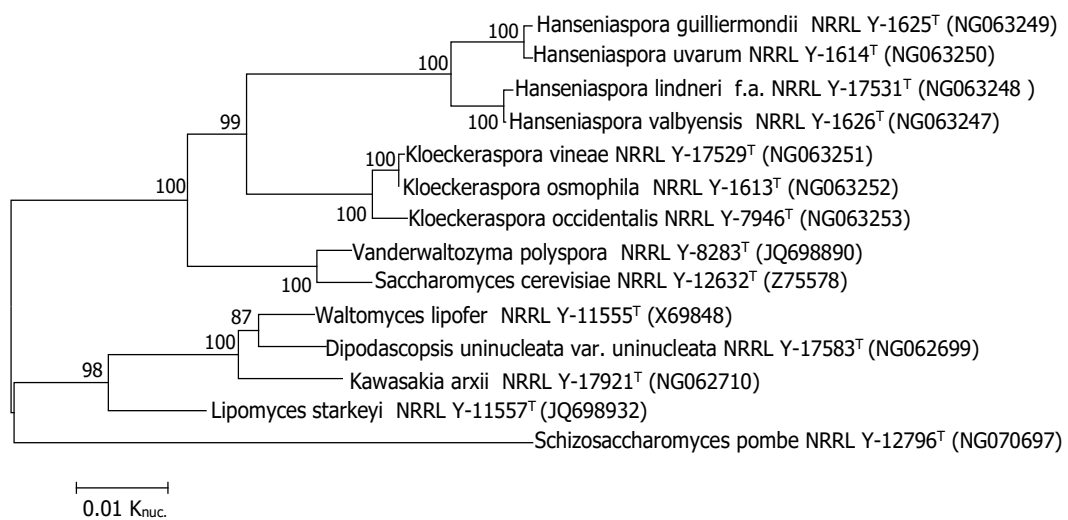
TABLE II: The pair-wise sequence similarity in the 18S rRNA gene sequences in *Kloeckera-*spora** and *Hanseniaspora* species.

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					

The total sequences were of 1705 - 1732 bases. For the abbreviations of generic names, refer to Table I.



**Fig. 1.** The phylogenetic tree of *Kloeckeraspora* species 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.



**Fig. 2.** The phylogenetic tree of *Kloeckeraspora* species 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.