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

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Abstract

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

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

The genus *Kloeckeraspora* Niehaus was introduced along with the species, *Kloeckeraspora osmophil*a Niehaus [= *Hanseniaspora osmophila* (Niehaus) Phaff et al. (1984)] (Niehaus 1932). However, the name was not accepted for a long time in the yeast systematics (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 subgroups, 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 α), the sequence similarity between group *a* (*Hanseniaspora osmophila*, *Hanseniaspora vineae* and *Hanseniaspora occidentalis*) and group *b* (*Hanseniaspora valbyensis*, *Hanseniaspora guilliermondii* and *Hanseniaspora uvarum*) was 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 β), the calculated base substitutions between group *a* and group *b* were very high (15). Within the respective groups *a* and *b*, 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).

In the dendrogram based on partial base sequencing in positions 1451 - 1618, 168 bases of 18S rRNA (designated as region γ), the calculated base substitutions between group *a* and group *b* were “six”. Within the respective groups *a* and *b*, the base substitutions were zero and one, respectively. 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 γ. Thus, the phylogenetic data obtained above indicated that the two groups *a* and *b* should be recognized as separate genera, i.e., *Kloeckeraspora* and *Hanseniaspora* (Yamada et al. 1992b).

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

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

The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences (LSU D1/D2) was constructed by the neighbour-joining method for seven species. As shown in 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.; Lachance 2012) were much longer than that between *Vanderwaltozyma 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 pair-wise sequence similarities were calculated, and the calculated 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 genera (Yamada et al. 2022; Vu et al. 2022a, b; Yamada 2023; Malimas et al. 2023).

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

In the genus *Kleockeraspora*, 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) derived from the neighbour-joining method for the seven species, the cluster between groups *a* group *b* was abnormally long as well (Fig. 2). 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 in group *a* (Table 2). Concerning group *b*, the sequence similarities were widely distributed (98.3 - 99.8%). Incidentally, it was 98.9% between *V. polyspora* and *S. cerevisiae* (Malimas et al. 2023).

On the whole, the base substitutions were 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. 2023).

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

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

From the experimental data obtained above, the introduction of the two genera *Kloeckeraspora* and *Haseniaspora* was unequivocally accepted and recognized phylogenetically in the family Saccharomycetaceae. 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. 2023).

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

The family Saccharomycetaceae

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).

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

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

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

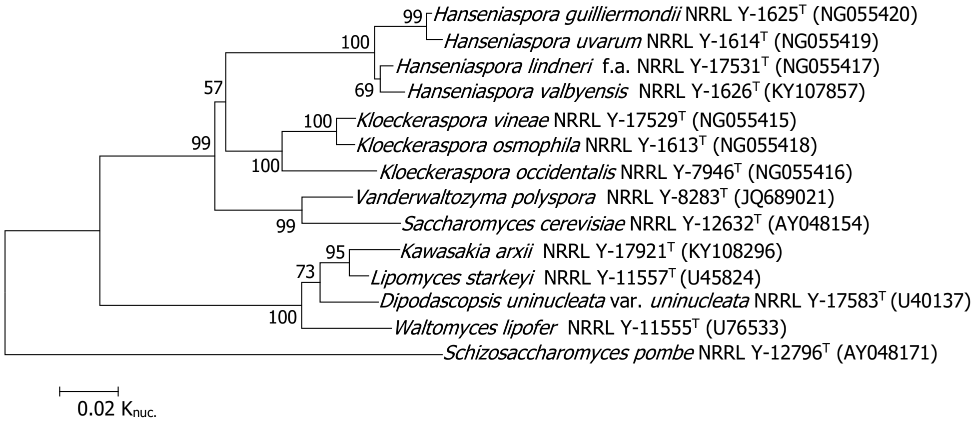
The total sequences were of 1705 - 1732 bases.

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.

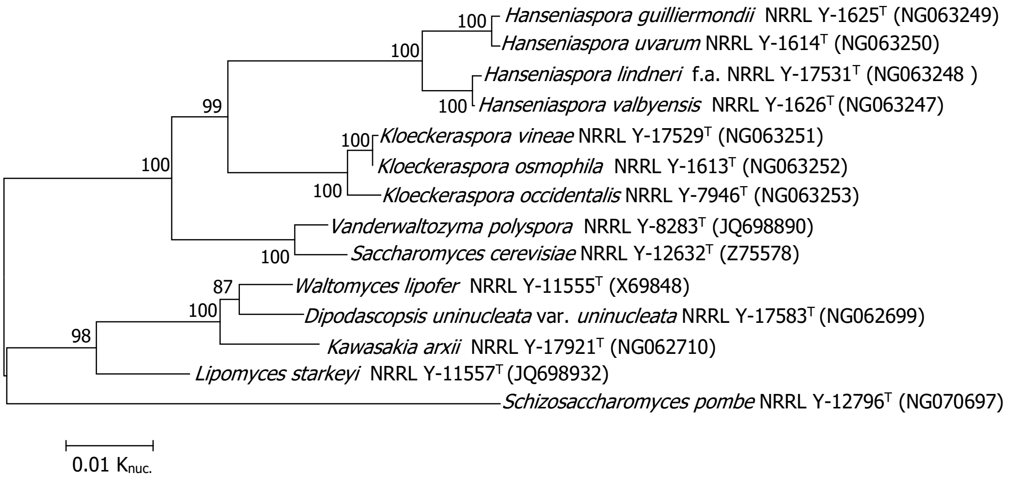
Species	1	2	3	4	5	6	7	8	9
1. *K. osmophila*	100								
2. *K. vineae*	99.5	100							
3. *K. occidentalis*	97.8	97.7	100						
4. *H. valbyensis*	93.1	93.0	92.8	100					
5. *H. uvarum*	92.5	92.4	92.1	97.8	100				
6. *H. guilliermondii*	92.5	92.5	92.3	97.8	99.5	100			
7. *H. lindneri* f.a.	93.1	93.0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

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

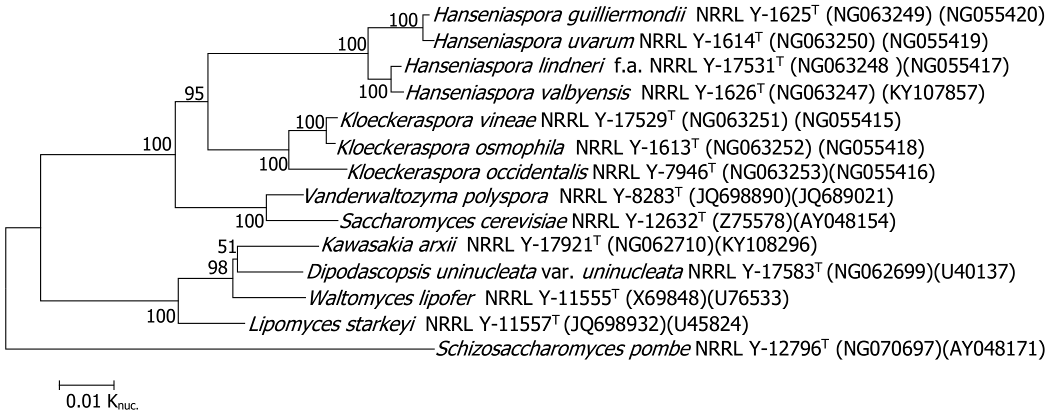
The total sequences were of 2256 - 2274 bases.



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.



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.



Fig, 3. The phylogenetic tree based on the concatenated sequences from the18S 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.