Short Communication

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

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Abstract: The genus *Wickerhamomyces* Kurtzman et al. (2008) was extremely diverse phylogenetically, since a large number of species were equipped with long branches. Of the genus, *Wickerhamomyces anomalus* (= *Hansenula anomala*) and the remaining eight species produced a large cluster with short branches. The pair-wise sequence similarities of *H. anomala* were very high (96.5-99.1%) to the eight species. Thus, the genus *Hansenula* Sydow et Sydow (1919) was revived as a taxonomic homogeneous-natured taxon and phylogenetically and taxonomically separated from the genus *Wickerhamomyces*. In addition, the genus *Wickerhamomyces* was designated as the double generic-structured, along with the genus *Lipomyces* sensu Kurtzman et al. (as the multiple generic-structured) and the genus *Schizosaccharomyces* sensu Kurtzman et Robnett (as the quadruple generic-structured). The three genera actually corresponded to a higher-ranked taxonomic group, i.e., the family.

Keywords: *Wickerhamomyces canadensis*; *Wickerhamomyces anomalus*; *Hansenula anomala*; *Hansenula lynferdii*; *Hansenula myamarensis*.

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

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

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

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

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

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

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

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

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

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

Hat-shaped ascospores, nitarate assimilation for growth, Q-7

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

1. *Hansenula anomala* (Hansen) Sydow et Sydow (1919) (MycoBank146467)
2. *Hansenula ciferrii* Lodder (1932) (MycoBank251417)
3. *Hansenula lynferdii* van der Walt et Johannsen (1975) (MycoBank314872)
4. *Hansenula subpelliculosa* Bedford ex Barnett, Payne et Yarrow (1983) (MycoBank115359)
5. *Hansenula sydowiorum* Scott et van der Walt (1970) (MycoBank314879)
6. *Hansenula myanmarensis* (Nagatsuka, Kawasaki et Seki) comb. nov.
   MycoBank number is 849044.

   Basionym: *Pichia myanmarensis* Nagatsuka, Kawasaki et Seki, Int. J. Syst, Evol. Microbiol. **55**: 1381. 2005.

   The type strain is NBRC11090 = CBS9786.
7. *Hansenula edaphica* (Limtong, Yongmanitchai, Kawasaki et Fujiyama) comb. nov.
   MycoBank number is 849045.

   Basionym: *Wickerhamomyces edaphicus* Limtong, Yongmanitchai, Kawasaki et Fujiyama, FEMS Yeast Res. **9**: 507, 2009.

   The type strain is BCC 21231.
8. *Hansenula siamensis* (Kaewwichian, Kawasaki et Limtong) comb. nov.
   MycoBank number is 849046.

   Basionym: *Wickerhamomyces siamensis* Kaewwichian, Kawasaki et Limtong, Int. J. Syst. Evol. Microbiol. **63**: 1571. 2013.

   The type strain is CBS12570.

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

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

The type strain is CBS12941.

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

Conclusion

The generic compositions of *Lipomyces* sensu Kurtzman et al (2007) and *Schizosaccharomyces* sensu Kurtzman et Robnett (1991) were very similar to that of *Wickerhamomyces* Kurtzman et al. (2008). In the three genera, there were extremely long branch-equipped taxa, e,g., the genus *Lipomyces* sensu stricto included *L. starkeyi* (Q-9), *L. kononenkoae* (Q-9), *L. yamadae* (Q-9) and so on, the genus *Waltomyces* did *W. lipofer* (Q-10), the genus *Zygozyma* did *Z. oligophaga* (Q-8), the genus *Babjevia* did *B. anomala* (Q-9), the genus *Smithiozyma* did *S. japonica* (Q-9), the genus *Kawasakia* did *K. arxii* (Q-9), the genus *Limtongia* did *L. smithiae* (Q-9) and the genus *Kockiozyma* did *K. suomiensis* (Q-8), *K. geophila* f.a. (Q-8) and *K. yamanashienais* f.a. within the *Lipomyces* sensu Kurtzman et al. (Yamada et al. 2022) and the genus *Schizosaccharomyces* sensu stricto included *S. pombe* (Q-10), the genus *Octosporomyces* did *O. octosporus* (Q-9), *O. osmophilus*, *O. cryophilus* (Q-9) and *O. lindnerii* (= *S. lindnerii*; Brysch-Herzberg et al. 2023) and the genus *Hasegawaea* did *H. japonica* (no Q or trace amount of Q-10) within the genus *Schizosaccharomyces* sensu Kurtzman et Robnett (Vu et al. 2022a). The many species of the genus *Lipomyces* sensu stricto and the four species of the genus *Octosporomyces* actually corresponded phylogenetically to the nine species (Q-7) of the genus *Hansenula* within the genus *Wickerhamomyces* or the so-called *Wickerhamomyces* complex. As describeded above, the genus *Wickerhamomyces* was comprised of the double generic-structured in the taxonomic point of view, as well as the genera *Lipomyces* sensu Kurtzman et al. (2007) (the multiple generic-structured) and *Schizosaccharomyces* sensu Kurtzman et Robnett (1991) (the quadruple generic-structured). In addition, the three were not the taxonomic homogeneous-natured and actually corresponded to a higher-ranked taxonomic group, i.e., the family.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

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

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The genus *Hansenula* Sydow et Sydow (1919) nom. rev. and the genus *Wickerhamomyces* 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。

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*.

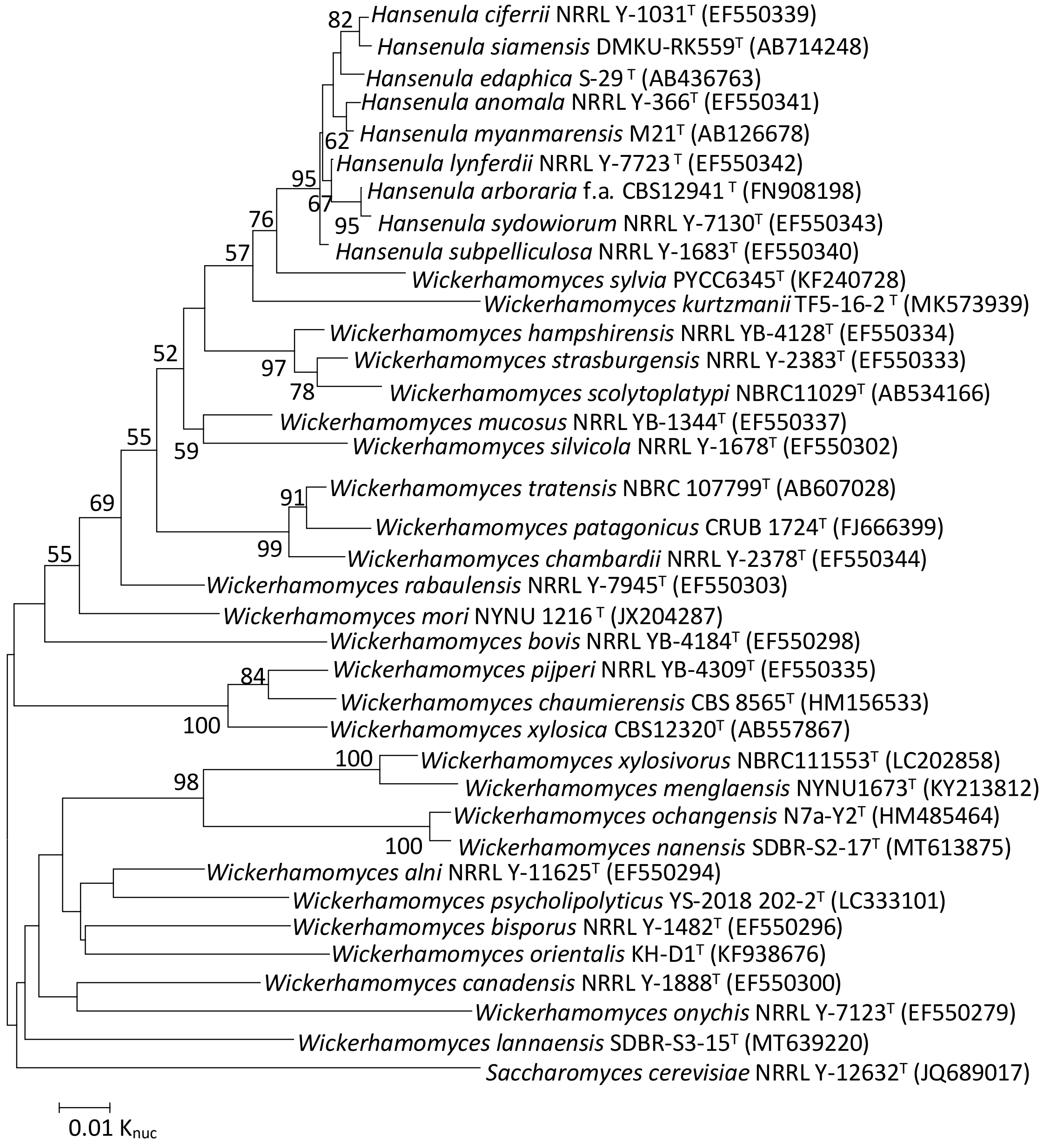

Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 491 bases for the nine species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.

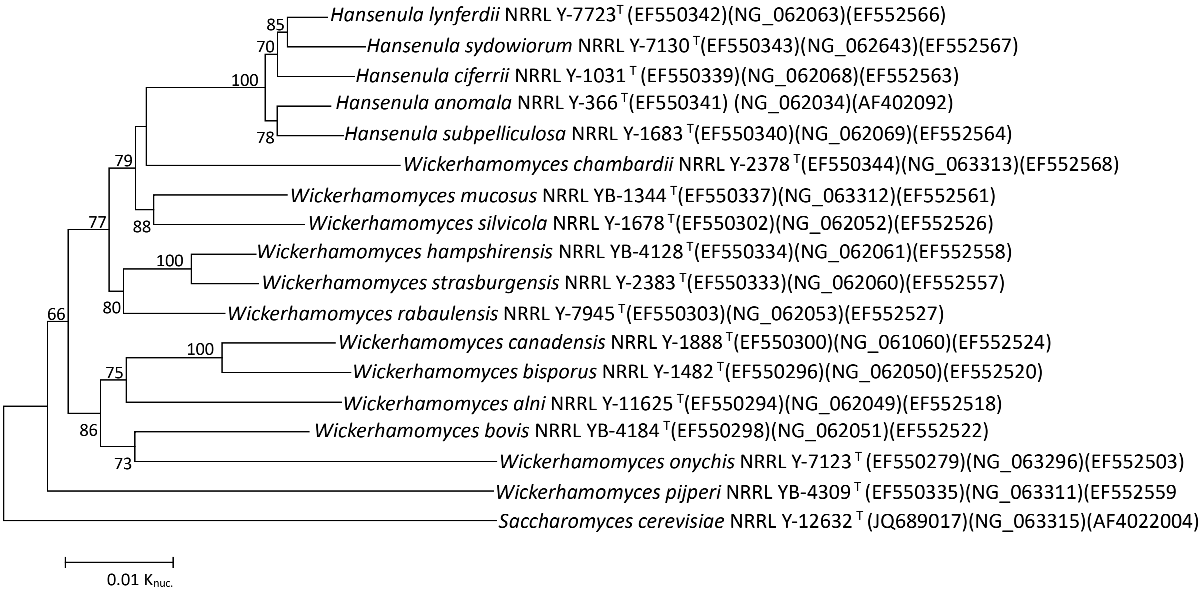

Fig. 2. The phylogenetic tree based on the concatenated sequences (5528 bases) of 26S rRNA genes, 18S rRNA genes and translation elongation factor-1-alpha for the five species of the genus *Hansenula* derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate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