Microbial Systematics (Short Communication)

The Revision of Schizosaccharomycetaceae*

Taweesak Malimas[1], Huong Thi Lan Vu[2], Pattaraporn Yukphan[3], Somboon Tanasupawat[4], Kozaburo Mikata[5], Yuzo Yamada**[3,6,7]

[1]Microbial Laboratory Biosafety Level-1, 46 M, 9 Nongphus, Muangsuang, Roi-Et 45220, Thailand
[2]Department of Microbiology, Faculty of Biology and Biotechnology, University of Science, Vietnam National University-HCM City, 227 Nguyen Van Cu Street, Ward 4, District 5, Hochiminh City, Vietnam
[3]Microbial Diversity and Utilization Team, Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Phaholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand
[4]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 254 Phayathai Road, Wangmai, Pathumwan, Bangkok 10330, Thailand
[5]Institute for Fermentation, Osaka, 2-17-85 Juso-honmachi, Yodogawa-ku, Osaka 532-8686, Japan
[6]JICA Senior Overseas Volunteer, Japan International Cooperation Agency, Shibuya-ku, Tokyo 151-8559. Japan
[7]Laboratory of Applied Microbiology (Professor Emeritus), Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

*A preliminary report was presented (Yamada et al. 2022a)

**Corresponding author
E-mail: yamada-yuzo@nifty.com

Keywords: *Schizosaccharomyces*; *Octosporomyces*; *Hasegawaea*; fission yeasts

Abstract

Although the genus *Hasegawaea* was introduced along with the recognition of the genus *Octosporomyces* in the classification of fission yeasts, the two additional genera were neither accepted nor recognized. However, the genus *Schizosaccharomyces* sensu Kurtzman et Robnett was taxonomically heterogeneous-natured and corresponded to a higher-ranked taxon, i.e., a monotypic family. Thus, the following three genera were confirmed in the family Schizosaccharomycetaceae. The genus *Schizosaccharomyces* sensu stricto was comprised of *Schizosacchromyces pombe,* the genus *Octosporomyces* was of the four species, *Octosporomyces octosporus*, *Octosporomyces osmophilus*, *Octosporomyces lindneri* and *Octosporomyces cryophilus*, and the genus *Hasegawaea* was of *Hasegawaea japonica*. In conclusion, the precise classification of microorganisms will not be able to be expected in the generic designation without the presence of taxonomic homogeneous-natured taxa. The phylogenetic distances have to be absolutely considered. Namely, the longer the distances are, the more taxonomic heterogeneous-natures will be increased in the resulting genus.

In the family Schizosaccharomycetaceae Beijerinck ex Klöcker, the genus *Hasegawaea* Yamada et Banno was introduced for *Schizosaccharomyces japonicus*, along with the recognition of the genus *Octosporomyces* Kudriavzev, based on the differences in ubiquinone isoprenologues as one of the most decisive criteria as well as in ascospore morphology (Yamada and Banno 1987, 1989; Yamada et al. 1973; Mikata and Banno 1987). The resulting three genera *Schizosaccharomyces*, *Octosporomyces* and

Hasegawaea were thus taxonomically homogeneous-natured, i.e., morphologically, physiologically, chemotaxonomically, phylogenetically and genetically. However, the two generic names were neither accepted nor recognized taxonomically (Kurtzman and Robnett 1991, 1998; Vaughan-Martini and Martini 2011).

This paper describes the historical surveys in the classification of fission yeasts to retrieve the generic names of *Hasegawaea* and *Octosporomyces* from the phylogenetic and the phenotypic points of view.

The family Schizosaccharomycetaceae Beijerinck ex Klöcker

The type genus is *Schizosaccharomyces*. The three genera are included.

Genus I. *Schizosaccharomyces* Lindner (MB 4905)

Four-spored, warty ascospores and ubiquinone-10 (Q-10)

Schizosaccharomyces pombe Lindner (1893) (MB 212377)

The type strain is NBRC 1628^T (= CBS 356^T = CLIB 833^T).

Kurtzman and Robnett (1991) described that *Schizosaccharomyces pombe* and *Schizosaccharomyces octosporus* were more closely related to one another than were *Saccharomyces kluyveri* and *Saccharomyces unisporus* and that *Schizosaccharomyces octosporus* and *Schizosaccharomyces japonicus* were separated by a distance only 25% greater than found for outlying *Saccharomyces* species based on their phylogenetic tree. However, the two *Saccharomyces* species were later transferred to the separate two genera *Lachancea* and *Kazachstania* as *Lachancea kluyveri* and *Kazachstania unispora* (Kurtzman 2003), indicating that the *Schizosaccharomyces* species should also share the same fate. Therefore, the authors' opinion rejecting the two generic names of fission yeasts completely lost its validity due to the mistakes in reference standard or reference control selection.

Yamada et al. (1993) determined the partial base sequences of fission yeasts in the three regions of 26S rRNA (positions 493-622, 130 bases, designated as region *a*) and positions 1611-1835, 225 bases, designated as region *b*) and 18S rRNA (positions 1451-1618, 168 bases, designated as region *c*). In the partial base sequencings, *Schizosaccharomyces octosporus* represented one base substitution, when compared with *Schizosaccharomyces pombe*, in region *c*. However, the maximum homologies were very low (75-77%) in region *a* and the base substitutions were very high (45-16) in region *b* compared with the remaining two species. In addition, *Schizosaccharomyces japonicus* represented very low maximum homologies and very high base substitutions in all the three regions, i.e., 75-84% maximum homologies in regions *a*, 45-37 base substitutions in region *b*, and 7-6 base substitutions in region *c*.

Actually, the phylogenetic branches among *Schizosaccharomyces pombe*, *Octosporomyces octossporus* and *Hasegawaea japonica* were much longer than that between *Vanderwaltozyma polyspora* (Kurtzman 2003) and *Saccbaromyces cerevisiae* used as reference standards (Fig. 1).

Additionally, the calculated sequence similarities (84.4-90.4%) among the three species were lower than that (91.7%) (Vu et al. 2021; Yamada et al. 2022b) between *Babjevia anomala* (= *Dipodascopsis anomala*, Q-9) and *Lipomyces starkeyi* (Q-9) in the 26S rRNA gene D1/D2 domain sequences (Table 1). By the way, the sequence similarity between *Vanderwaltozyma polyspora* (Q-6) and *Saccharomyces cerevisiae* (Q-6) was 93.8% (unpublished data).

Thus, the genera *Hasegawaea* and *Octosporomyces* should be enough to be unequivocally accepted and retained in the classification of fission yeasts.

Genus II. *Octosporomyces* Kudriavzev (MB 3551)

Basically eight-spored, smooth ascospores and ubiquinone-9 (Q-9)

The type species is *Octosporomyces octosporus*.

Four species are included.

1. *Octosporomyces octosporus* (Beijerinck) Kudriavzev (1960) (MB 335285)

Basionym: *Schizosaccharomyces octosporus* Beijerinck (1894).

The type strain is NBRC 10373^{T} (= CBS 371^{T} = CLIB 832^{T}).

2. *Octosporomyces osmophilus* (Brysch-Herzberg, Tobias, Seidel, Wittmann, Fischer, Dlauchy et Péter) Vu, Yukphan, Tanasupawat, Mikata et Yamada comb. nov.

Basionym: *Schizosaccharomyces osmophilus* Brysch-Herzberg, Tobias, Seidel, Wittmann, Fischer, Dlauchy et Péter, FEMS Yeast Res 19; foz038-10: 2019.

The type strain is CBS 15793^{T} (= CLIB 3267^{T}).

MycoBank number is 846278.

3. *Octosporomyces lindneri* (Brysch-Herzberg, Sipiczki, Jia, Seidel et Du) Malimas, Vu, Yukphan, Tanasupawat et Yamada comb. nov.

Basionym: *Schizosaccharomyces lindneri* Brysch-Herzberg, Sipiczki, Jia, Seidel et Du, Yeast Wiley 13: 2023.

The type strain is CBS 18203^{T}.

MycoBank number is 849741.

4. *Octosporomyces cryophilus* (Helston, Box, Tang et Baumann) Vu, Yukphan, Tanasupawat, Mikata et Yamada comb. nov.

Basionym: *Schizosaccharomyces cryophilus* Helston, Box, Tang et Baumann, FEMS Yeast Res 10; 784: 2010.

The type strain is NRRL Y-48691^{T} (= CBS 11777^{T} = NBRC 106824^{T}).

MycoBank number is 846279.

In the fission yeasts, the two new *Schizosaccharomyces* species were described (Helston et al. 2010; Brysch-Herzberg et al. 2019).

Of the two, *Schizosaccharomyces osmophilus* was phylogenetically very closely related to *Octosporomyces octosporus* (Fig. 1). The sequence similarity was 98.1% between the two species (Table 1), indicating that the new species was obviously classified in the genus *Octosporomyces* but not in the genus *Schizosaccharomyces* sensu stricto. The phylogenetic data obtained above was supported by Brysch-Herzberg et al. (2019) who calculated 1.8% base substitution (= 98.2% sequence similarity). However, the authors accommodated the new species to the genus *Schizosaccharomyces* sensu Kurtzman et Robnett, which is taxonomic-heterogeneous natured, but not to the genus *Octosporomyces* Kudriavzev, which is phylogenetically close-related and taxonomic-homogeneous natured. The calculated sequence similarity mentioned above was almost the same as that (98.0%) between *Kockiozma suomiensi*s (= *Zygozyma suomiensis*, Q-8) and *Myxozyma geophila* (Q-8) (Vu et al. 2021; Yamada et al. 2022b).

Recently, the new species, *Schizosaccharomyces lindneri* was described (Brysch-Herzberg et al. 2023). The species represented an extremely short phylogenetic branch to *Octosporomyces octosporus* (Fig. 1). The calculated sequence similarity was very high (99.4%), which was higher than that (98.1%) of *Octosporomyces osmophilus* (Table 1). Thus, it is reasonable that the species should be accommodated to the genus *Octosporomyces*.

Concerning *Schizosaccharomyces cryophilus*, the phylogenetic position of the species was not so closely related (Fig. 1). The sequence similarities were 95.2% and 96.4% respectively to *Schizosaccharomyces octosporus* and *Schizosaccharomyces osmophilus* (Table 1). The calculated sequence similarities were not so high, so that the species may be able to be accommodated to a different genus, as shown in the relationships between *Babjevia anomala* and *Neoaidaea tothii*, in which the calculated sequence similarity was 95.6% (Vu et al. 2021; Yamada et al. 2022b) and between *Kloeckeraspora osmophila* and *Kloeckeraspora occidentalis*, in which the calculated sequence similarity was 94.0% (Malimas et al. 2023a). However, it is adequate at the present time to be temporarily classified in the genus *Octosporomyces*, since the species had ubiquinone-9 (Q-9) (Kaino et al. 2018) and an almost identical length of ITS1 (307 bp, internal transcribed spacer 1) to that (343 bp) of *Schizosaccharomyces octosporus* (Helston et al. 2010). Incidentally, *Schizosaccharomyces pombe* had 417 bp ITS1 and *Schizosaccharomyces japonicus* had 183 bp ITS1.

Genus III. *Hasegawaea* Yamada et Banno (MB 25179)

Basically eight-spored, smooth ascospores without papillae, dimorphic growth, respiration deficiency and no detectable ubiquinone (a trace amount of Q-10)

Hasegawaea japonica (Yukawa et Maki) Yamada et Banno (1987) (MB 132784)

Basionym: *Schizosaccharomyces japonicus* Yukawa et Maki (1931).

Synonym: *Octosporomyces japonicus* (Yukawa et Maki) Kudriavzev (1960).

The type strain is NBRC 1609^{T} (= CBS 354^{T} = CLIB 3267^{T})

Schizosaccharomyces japonicus is especially distant phylogenetically, i.e., the species is the furthest removed from other species among the fission yeasts (Liu et al. 2009; Rhind et al. 2011; Aoki et al. 2017).

Liu et al. (2009) made phylogenomic analyses on the basis of the monophyly of Taphrinomycotina, including *Schizosaccharomyces* fission yeasts. In the phylogenetic tree, *Schizosaccharomyces japonicus* evolved first and separated from the remaining two species, *Schizosaccharomyces pombe* and *Schizosaccharomyces octosporus*, as described above (Aoki et al. 2017). However, there was a certain risk that a heterogeneous-natured taxon was sometimes given taxonomically in the generic designation (Vu et al. 2021; Yamada et al. 2022b), when based on the monophyly only, and the resulting genus corresponded to a higher-ranked taxon, i.e., the monotypic family Schizosaccharomycetaceae, as discussed in the genus *Wickerhamomyces* Kurtzman et al. (2008), in which the genus *Hansenula* Sydow et Sydow (1919) was actually included (Malimas et al. 2023b).

In the monotypic family Schizosaccharomycetaceae, the genus *Schizosaccharomyces* sensu Kurtzman et Robnett (1991) actually had quite heterogeneous natures genetically (Helston et al. 2010), chemotaxonomically (Yamada et al. 1973; Mikata and Yamada 1999; Kaino et al. 2018), and physiologically (Vaughan-Martini and Martini

2011; Sipiczki et al. 1998; Aoki et al. 2017). In addition, it is of interest that the branch between *Schizosaccharomyces japonicus* and *Schizosaccharomyces pombe* was much longer than that between *Saccharomyces* (Q-6) and *Pichia* (Q-7) in the phylogenetic tree based on the concatenated sequences of 13 proteins (*cox1*, *cox2*, *cox3*, *cob*, *atp6*, *atp9* and *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*) encoded by mtDNA. The experimental data obtained above indicated that the genus *Hasegawaea* could not be rejected but retained.

A maximum-likelihood phylogeny of the fission yeasts from the concatenation-based analysis of 815 BUSCO amino acid genomic sequences (total 56,2376 sites) was studied by Rhind et al. (2011). The resulting phylogenetic tree was very similar in topology to that of Liu et al. (2009) as well as to that mentioned above based on the 26S rRNA gene D1/D2 domain sequences (Fig. 1).

In the comparative study on *wtf* genes in the family Schizosaccharomycetaceae by De Carvalho (2020), it was emphasized that the only *Schizosccharomyces japonicus* did not have such genes. And the evolutionary analysis indicated that a very similar topology to those described above was found in the resulting phylogenetic tree. *Schizosaccharomyces japonicus* was first distantly removed from the others, and then *Schizosaccharomyces pombe* was. In contrast, the branches were quite short among *Schizosaccharomyces octosporus*, *Schizosaccharomyces osmophilus*, *Schizosaccharomyces lindneri* and *Schizosaccharomyces cryophilus*, indicating that the existence of three genera was confirmed and the latter four species were adequate to be accommodated to the genus *Octosporomyces*.

Kurtzman (2003) introduced ‘clade’ or ‘phylogenetically circumscribed genus’ as generic concept. However, it was not yet perfect to get the taxonomic homogeneous-natured taxon for the genus. Namely, the branch lengths should be additionally considered in the phylogenetic trees.

In the seven ranks used for the systematics of plants and animals, the taxonomically homogeneous taxa can be available only when the lower-ranked two taxa, i.e., species and genus are used. Therefore, the present authors naturally selected a taxonomic homogeneous-natured taxon in the generic designation, since the longer the phylogenetic distances are the more taxonomic heterogeneous-natures will be increased.

Thus, the genus *Schizosaccharomyces* Lindner sensu Kurtzman et Robnett (1991) corresponded to the monotypic family that actually includes the phylogenetic-distant and the phenotypic-distinct species, as far as the genus is not divided into three, i.e., *Schsizosaccharomyces* Lindner (1893) sensu stricto, *Octosporomyces* Kudriavzev (1960) and *Hasegawaea* Yamada et Banno (1987).

Acknowledgements

The authors express their sincere thanks for citing a number of articles for detailed discussions.

Funding information

The authors received no specific grant from any funding agency.

Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

Y.Y., H.T.L.V., P.Y., T.M., K.M. 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 discussion was made among Y.Y., H.T.L.V., P.Y., S.T., K.M., and T.M.

References

Aoki K, Furuya K, Niki H. *Schizosaccharomyces japonicus*: A distinct dimorphic yeast among the fission yeasts. *Cold Spring Harb Protoc* 2017; doi: 10.1101/pdb. top082651.

Brysch-Herzberg M, Tobias A, Seidel M, Wittmann R, Wohlmann E, Fischer R, Dlauchy D, Péter G. *Schizosaccharomyces osmophilus* sp. nov., an osmophilic fission yeast occurring in bee bread of different solitary bee species. *FEMS Yeast Res* 2019; **19**: foz038.

Brysch-Herzberg M, Jia G-S, Sipiczki M, Seidel M, Li W, Assali I, Du L-L *Schizosaccharomyces lindneri* sp. nov., a fission yeast occurring in honey. *Yeast Wiley* 2023; 1-17.

De Carvalho M. *wft*: killing gametes for more than 110 million years. *The Open University* 2020.

Helston M, Box JA, Tang W, Baumann P. *Schizosaccharomyces cryophilus* sp. nov., a new species of fission yeast. *FEMS Yeast Res* 2010; **10**: 779-786.

Kaino T, Tonoko K, Mochizuki S, Takashima Y, Kawamukai M. *Schizosaccharomyces japonicus* has low levels of CoQ10 synthesis, respiration deficiency and efficient ethanol production. *Biosci Biotechnol Biochem* 2018; **82**: 1031-1042.

Kurtzman CP. Phylogenetic circumscription of *Saccharomyces* and *Kluyveromyces* and other members of the Saccharomycetaceae, and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygotorulaspora*. *FEMS Yeast Res* 2003; **4**: 233-245.

Kurtzman CP, Robnett CJ. Phylogenetic relationships among species of *Saccharomyces*, *Schizosaccharomyces*, *Debaryomyces* and *Schwanniomyces* determined from partial ribosomal RNA sequences. *Yeast* 1991; **7**: 61-72.

Kurtzman CP, Robnett CJ. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* 1998; **73**: 331-371.

Liu Y, Leigh JW, Brinkmann H, Cushion MT. Rodriguez-Ezpeleta N, Philippe H, Lang BF. Phylogenomic analyses support the monophyly of Taphrinomycotina, including *Schizosaccharomyces* fission yeasts. *Mol Biol Evol* 2009; **26**, 27-34.

Malimas T, Vu HTL, Yukphan P, Tanasupawat S, Yamada Y. The reinsatatement of the genus *Kloeckeraspora* Niehaus (1932) (apiculate yeast). *Jxiv* 2023a (DOI: https://doi.org/10.51094/jxiv.317).

Malimas T, Vu HTL, Yukphan P, Tanasupawat S, Yamada Y. The genus *Hansenula* Sydow et Sydow (1919) nom. rev. and the genus *Wickerhamomyces* Kurtzman et al. (2008). *Jxiv* 2023b (DOI: https://org/10.51094/jxiv.397).

Mikata M, Banno I. Surface structures of ascopsores of the genus *Schizosaccharomyces*. *IFO Res Commun* 1987; **13**: 45-51.

Mikata K, Yamada Y. The ubiquinone system of *Hasegawaea japonica* (Yukawa et Maki) Yamada et Banno: A new method for identifying ubiquinone homologs from yeast cells. *IFO Res Commun* 1999; **19**: 41-46.

Rhind N, Chen Z, Yassour M, Thompson DA, Haas BJ, Habib N, Wapinski I, Roy S, Lin MF, Heiman DI, Young SK, Furuya K. *et al*. Comparative functional genomics of the fission yeasts. *Science* 2011; 332 (6032): 930-936. doi:10.1126/science.1203357.

Sipiczki M, Takeo K, Yamaguchi M, Yoshida S, Miklos I. Environmentally controlled dimorphic cycle in a fission yeast. *Microbiology* 1998; **144**: 1319-1330.

Vaughan-Martini A, Martini A. *Schizosaccharomyces* Lindner (1893) In: Kurtzman CP Fell JW Boekhout T (ed). The Yeasts: A Taxonomic Study, 5th edition. vol. 2. London: Elsevier, 2011, p. 779-784.

Vu HTL, Yukphan P, Tanasupawat S, Yamada Y. The revision of the family Lipomycetaceae. *JSMRS 27th Ann Meet* 2021; *Gen Lect* O-7: p. 15; on line presentation, p. 1-10.

Yamada Y, Banno I. *Hasegawaea* gen. nov., an ascosporogenous yeast genus for the organisms whose asexual reproduction is by fission and whose ascospores have smooth surfaces without papillae and which are characterized by the absence of coenzyme Q and by the presence of linoleic acid in cellular fatty acid composition. *J Gen App. Microbiol* 1987; **33**: 295-298.

Yamada Y Banno I. A proposal to divide the genus *Schizosaccharomyces* Lindner sensu Yarrow into three separate genera. *Yeast*; 1989: **5**, S393-S398.

Yamada Y, Arimoto M, Kondo K. Coenzyme Q system in the classification of the ascosporogenous yeast genus *Schizosaccharomyces* and the yeast-like genus *Endomyces*. *J Gen Appl Microbiol* 1973; **19**: 353-358.

Yamada Y, Asahi T, Maeda K, Mikata K. The phylogenetic relationships of fission yeasts based on the partial sequences of 18S and 26S ribosomal RNAs: The recognition of *Hasegawaea* Yamada et Banno along with *Schizosaccharomyces* Lindner. *Bull Fac Agric Shizuoka Univ* 1993; **43**: 29-38.

Yamada Y, Vu HTL, Yukphan P, Tanasupawat S, Mikata K. The revision of Schizo-saccharomycetaceae. *JSMRS 28th Ann Meet* 2022a; *Gen Lect* O-2: p. 22.

Yamada Y, Vu HTL, Yukphan P, Tanasupawat S. The revision of Lipomycetaceae. *Jxiv* 2022b; DOI: https://doi.org/10.51094/Jxiv.189.

Table 1. The pair-wise 26S rRNA gene D1/D2 domain sequence similarity.

Species	1	2	3	4	5	6
1 *Schizosaccharomyces pombe* CBS 356^T	100	90.4	90.6	90.1	90.9	89.8
2 *Octosporomyces octosporus* CBS 371^T		100	98.1	99.4	95.2	84.4
3 *Octosporomyces osmophilus* SZ134-FG-A^T			100	97.9	96.4	85.0
4 *Octosporomyces lindneri* NRRL Y-4869^T				100	95.0	84.3
5 *Octospormyces cryophilus* NRRLY-48691^T					100	84.5
6 *Hasegawaea japonica* CBS 354^T						100

The percent sequence similarity was calculated for 589 bases.

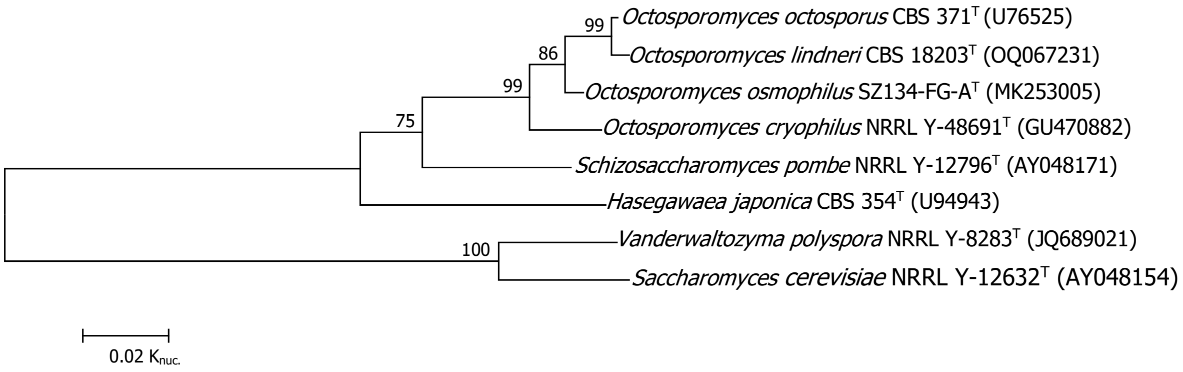

Fig. 1. The phylogenetic tree of fission yeasts based on the 26S rRNA gene D1/D2 domain sequences for 543 bases. The evolutionary history was inferred using the neighbour-Joining method. V*anderwaltozyma polyspora* and S*accharomyces cerevisiae* were used as outgroup. The bootstrap test (1000 replicates) was shown next to the branches.