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The Revision of Schizosaccharomycetaceae*

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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 three species, *Schizosaccharomyces octosporus*, *Schizosaccharomyces osmophilus*, and *Schizosaccharomyces cryophilus* as *Octosporomyces octosporus*, *Octosporomyces osmophilus*, and *Octosporomyces cryophilus*, and the genus *Hasegawaea* was of *Schizosaccharomyces japonicus* as *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.

GRAPHICAL ABSTRACT

The family Schizosaccharomycetaceae Beijerinck et Klöcker

Genus	Species
Schizosaccharomyces Lindner (1893) sensu stricto	*S. pombe* Lindner (1893)
Octosporomyces Kudriavzev (1960)	*O. octosporus* (Beijerinck) Kudriavzev (1960) *O. osmophilus* (Brysch-Herzberg et al.) Vu et al. com. nov. *O. cryophilus* (Helston et al.) Vu et al. com. nov.
Hasegawaea Yamada et Banno (1987)	*H. japonica* (Yukawa et Maki) Yamada et Banno (1987)

The family Schizosaccharomycetaceae Beijerinck et Klöcker

Genus	Species
Schizosaccharomyces Lindner (1893) sensu Kurtzman et Robnett (1991)	*S. pombe* Lindner (1893), *S. octosporus* Beijerinck (1894), *S. japonicus* Yukawa et Maki (1931), *S. cryophilus* Helston et al. (2010), *S. osmophilus* Brysch-Herzberg et al. (2019)

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*; 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 (84.9-91.5%) among the three species were lower than that (91.7%) (Vu *et al.* 2021) 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*.
Three 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 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).

Concerning *Schizosaccharomyces cryophilus*, the phylogenetic position of the species was not so closely related (Fig. 1). The sequence similarities were 95.2% and 96.3% 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 relationship between *Babjevia anomala* and *Neoaidaea tothii*, in which the calculated sequence similarity was 95.6% (Vu *et al*. 2021). 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). There is, however, a certain risk that a heterogeneous-natured taxon is sometimes given taxonomically in the generic designation (Vu *et al*. 2021), when based on the monophyly only, and the resulting genus corresponds to a higher-ranked taxon, e.g., the family Schizosaccharomycetaceae. In the monotypic family Schizosaccharomycetaceae, the genus *Schizosaccharomyces* sensu Kurtzman et Robnett 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*, and *Schizosaccharomyces cryophilus*, indicating that the existence of three genera was confirmed and the latter three species were adequate to be accommodated to the genus *Octosporomyces* (Fig. 2).

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 heterogeneities will be increased.

Thus, the genus *Schizosaccharomyces* Lindner sensu Kurtzman et Robnett (1991) would correspond 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).

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Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

Y.Y., H.T.L.V., P.Y., S.T., and K.M designed the study. H.T.L.V. 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., and K.M.

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

Species	Similarity (%)				
	S. pombe	*O. octosporus*	*O. osmophilus*	*O. cryophilus*	*H. japonica*
Schizosaccharomyces pombe CBS 356^{T}	100	90.5	90.7	91.5	89.6
Octosporomyces octosporus CBS 371^{c}		100	98.1	95.2	84.9
Octosporomyces osmophilus SZ134-FG-A^{T}			100	96.3	86.1
Octospormyces cryophilus NRRLY-48691^{T}				100	86.1
Hasegawaea japonica CBS 354^{T}					100

The pair-wise 26S rRNA gene D1/D2 domain sequence similarities were calculated for 590-604 bases with the program BioEdit (version 7.2.5) (Hall, BioEdit: An important software for molecular biology. *GERF Bull Biosci* 2011; **2**: 60).

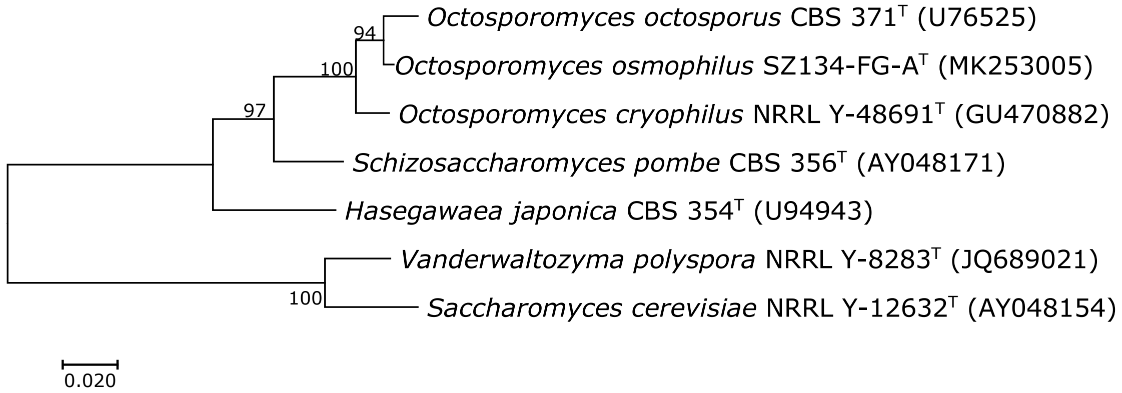

Fig. 1. The phylogenetic tree of fission yeasts based on the 26S rRNA gene D1/D2 domain sequences. The evolutionary history was inferred using the neighbour-Joining method (Saito and Nei. *Mol Biol Evol* 1987; **4**: 406). S*accharomyces cerevisiae* and V*anderwaltozyma polyspora* were used as outgroups. The optimal tree with the sum of branch length = 0.40892606 was shown. The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches (Felsenstein. *Evolution* 1985; **39**: 783. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura. *Evolution* 1980; **16**: 111) and are in the units of the number of base

substitutions per site. The analysis involved seven nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 563 positions of the 26S rRNA gene D1/D2 domain sequences in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*. *Mol Biol Evol* 2016; **33**: 1870). According to the theory of Dr. Kurtzman (Kurtzman and Robnett 1991, 1998; Vaughan-Martini and Martini 2011), all the fission yeasts were designated as *Schizosaccharomyces pombe*, *Schizosaccharomyces octosporus*, *Schizosaccharomyce japonicus*, *Schizosaccharomyces cryophilus*, and *Schizosaccharomyces osmophilus*.

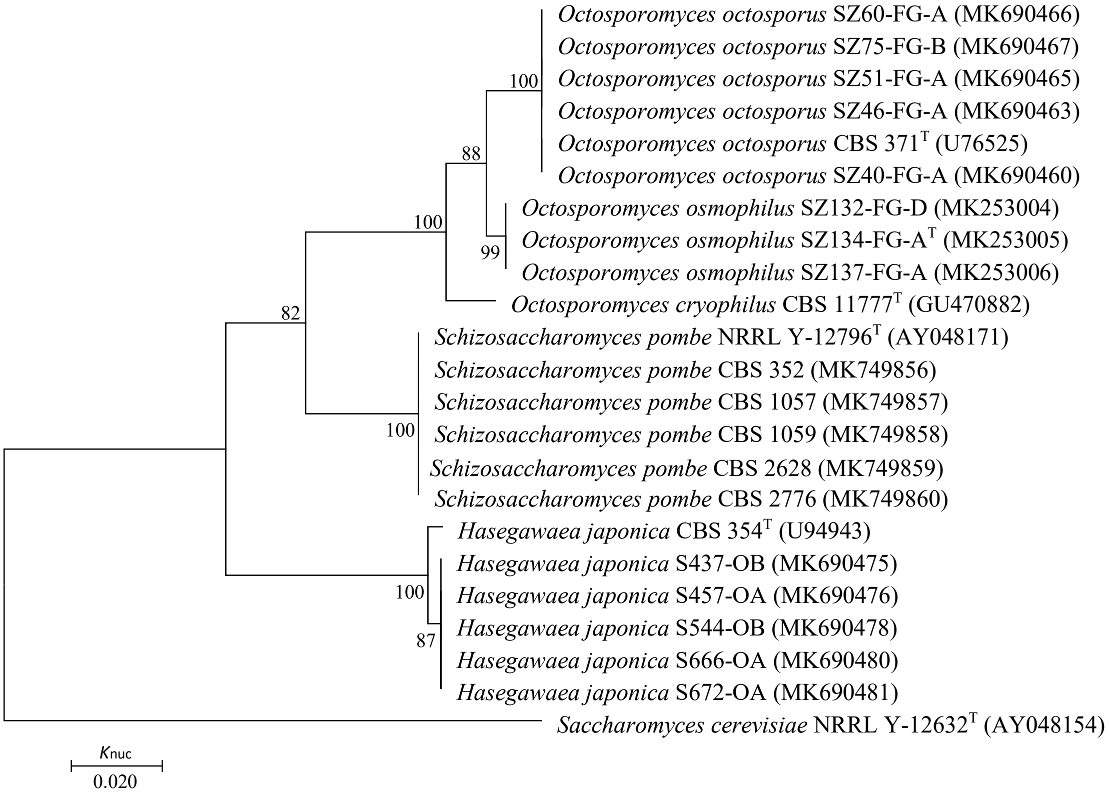

Fig. 2. The phylogenetic tree of fission yeasts based on the 26S rRNA gene D1/D2 domain sequences. The evolutionary history was inferred using the neighbour-Joining method. *Saccharomyces cerevisiae* was used as outgroup. The optimal tree with the sum of branch length = 0.32701129 was shown. The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The analysis involved 23 nucleotide sequences. There was a total of 494 positions of the 26S rRNA gene D1/D2 domain sequences in the final dataset. The phylogenetic tree was constructed under the same condition as in Fig. 1.