

1 Microbial Systematics

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3 **The Revision of Schizosaccharomycetaceae***

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33
34 **Abstract**

35 Although the genus *Hasegawaea* was introduced along with the recognition of the
36 genus *Octosporomyces* in the classification of fission yeasts, the two additional genera
37 were neither accepted nor recognized. However, the genus *Schizosaccharomyces* sensu
38 Kurtzman et Robnett was taxonomically heterogeneous-natured and corresponded to a
39 higher-ranked taxon, i.e., a monotypic family. Thus, the following three genera were
40 confirmed in the family Schizosaccharomycetaceae. The genus *Schizosaccharomyces*
41 sensu stricto was comprised of *Schizosacchchromyces pombe*, the genus *Octosporomyces*

42 was of the three species, *Schizosaccharomyces octosporus*, *Schizosaccharomyces*
 43 *osmophilus*, and *Schizosaccharomyces cryophilus* as *Octosporomyces octosporus*,
 44 *Octosporomyces osmophilus*, and *Octosporomyces cryophilus*, and the genus
 45 *Hasegawaea* was of *Schizosaccharomyces japonicus* as *Hasegawaea japonica*. In
 46 conclusion, the precise classification of microorganisms will not be able to be expected
 47 in the generic designation without the presence of taxonomic homogeneous-natured
 48 taxa. The phylogenetic distances have to be absolutely considered. Namely, the longer
 49 the distances are, the more taxonomic heterogeneous natures will be increased in the
 50 resulting genus.

51

52 **Supplementary Abstract**

The family Schizosaccharomycetaceae Beijerinck et Klöcker	
Genus	Species
<i>Schizosaccharomyces</i> Lindner (1893) sensu stricto	<i>S. pombe</i> Lindner (1893)
<i>Octosporomyces</i> Kudriavzev (1960)	<i>O. octosporus</i> (Beijerinck) Kudriavzev (1960)
	<i>O. osmophilus</i> (Brysch-Herzberg et al.) Vu et al. comb. nov.
	<i>O. cryophilus</i> (Helston et al.) Vu et al. comb. nov.
<i>Hasegawaea</i> Yamada et Banno (1987)	<i>H. japonica</i> (Yukawa et Maki) Yamada et Banno (1987)

The family Schizosaccharomycetaceae Beijerinck et Klöcker	
Genus	Species
<i>Schizosaccharomyces</i> Lindner (1893) sensu Kurtzman et Robnett (1991)	<i>S. pombe</i> Lindner (1893), <i>S. octosporus</i> Beijerinck (1894), <i>S. japonicus</i> Yukawa et Maki (1931), <i>S. cryophilus</i> Helston et al. (2010), <i>S. osmophilus</i> Brysch-Herzberg et al. (2019)

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56 In the family Schizosaccharomycetaceae Beijerinck ex Klöcker, the genus *Hasegawaea*
 57 Yamada et Banno was introduced for *Schizosaccharomyces japonicus*, along with the
 58 recognition of the genus *Octosporomyces* Kudriavzev, based on the differences in
 59 ubiquinone isoprenologues as one of the most decisive criteria as well as in ascospore

60 morphology (Yamada and Banno 1987, 1989; Yamada *et al.* 1973; Mikata and Banno
61 1987). The resulting three genera *Schizosaccharomyces*, *Octosporomyces*, and
62 *Hasegawaea* were thus taxonomically homogeneous-natured, i.e., morphologically,
63 physiologically, chemotaxonomically, phylogenetically, and genetically. However, the
64 two generic names were neither accepted nor recognized taxonomically (Kurtzman and
65 Robnett 1991, 1998; Vaughan-Martini and Martini 2011).

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

69

70 The family Schizosaccharomycetaceae Beijerinck ex Klöcker

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

72

73 Genus I. *Schizosaccharomyces* Lindner (MB 4905)

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

75 *Schizosaccharomyces pombe* Lindner (1893) (MB 212377)

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

77

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

89 Yamada *et al.* (1993) determined the partial base sequences of fission yeasts in the
90 three regions of 26S rRNA (positions 493-622, 130 bases, designated as region *a*) and
91 positions 1611-1835, 225 bases, designated as region *b*) and 18S rRNA (positions 1451-
92 1618, 168 bases, designated as region *c*). In the partial base sequencings, *Schizosac-*
93 *charomyces octosporus* represented one base substitution, when compared with
94 *Schizosaccharomyces pombe*, in region *c*. However, the maximum homologies were
95 very low (75-77%) in region *a* and the base substitutions were very high (45-16) in
96 region *b* compared with the remaining two species. In addition, *Schizosaccharomyces*
97 *japonicus* represented very low maximum homologies and very high base substitutions
98 in all the three regions, i.e., 75-84% maximum homologies in regions *a*, 45-37 base
99 substitutions in region *b*, and 7-6 base substitutions in region *c*.

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

104 Additionally, the calculated sequence similarities (84.9-91.5%) among the three
105 species were lower than that (91.7%) (Vu *et al.* 2021; Yamada *et al.* 2022b) between
106 *Babjevia anomala* (= *Dipodascopsis anomala*, Q-9) and *Lipomyces starkeyi* (Q-9) in the
107 26S rRNA gene D1/D2 domain sequences (Table 1). By the way, the sequence simi-
108 larity between *Vanderwaltozyma polyspora* (Q-6) and *Saccharomyces cerevisiae* (Q-6)
109 was 93.8% (unpublished data).

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

112
113 Genus II. *Octosporomyces* Kudriavzev (MB 3551)

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

115 The type species is *Octosporomyces octosporus*.

116 Three species are included.

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

118 Basionym: *Schizosaccharomyces octosporus* Beijerinck (1894).

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

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

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

125 The type strain is CBS 15793^T (= CLIB 3267^T)

126 MycoBank number is 846278.

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

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

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

132 MycoBank number is 846279.

133
134 In the fission yeasts, the two new *Schizosaccharomyces* species were described
135 (Helston *et al.* 2010; Brysch-Herzberg *et al.* 2019).

136 Of the two, *Schizosaccharomyces osmophilus* was phylogenetically very closely
137 related to *Octosporomyces octosporus* (Fig. 1). The sequence similarity was 98.1%
138 between the two species (Table 1), indicating that the new species was obviously
139 classified in the genus *Octosporomyces* but not in the genus *Schizosaccharomyces* sensu

140 stricto. The phylogenetic data obtained above was supported by Brysch-Herzberg *et al.*
141 (2019) who calculated 1.8% base substitution (= 98.2% sequence similarity). However,
142 the authors accommodated the new species to the genus *Schizosaccharomyces* sensu
143 Kurtzman et Robnett, which is taxonomic-heterogeneous natured, but not to the genus
144 *Octosporomyces* Kudriavzev, which is phylogenetically close-related and taxonomic-
145 homogeneous natured. The calculated sequence similarity mentioned above was almost
146 the same as that (98.0%) between *Kockiozma suomiensis* (= *Zygozoma suomiensis*, Q-8)
147 and *Myxozyma geophila* (Q-8) (Vu *et al.* 2021; Yamada *et al.* 2022b).

148 Concerning *Schizosaccharomyces cryophilus*, the phylogenetic position of the
149 species was not so closely related (Fig. 1). The sequence similarities were 95.2% and
150 96.3% respectively to *Schizosaccharomyces octosporus* and *Schizosaccharomyces*
151 *osmophilus* (Table 1). The calculated sequence similarities were not so high, so that the
152 species may be able to be accommodated to a different genus, as shown in the
153 relationship between *Babjevia anomala* and *Neoaidea tothii*, in which the calculated
154 sequence similarity was 95.6% (Vu *et al.* 2021; Yamada *et al.* 2022b). However, it is
155 adequate at the present time to be temporarily classified in the genus *Octosporomyces*,
156 since the species had ubiquinone-9 (Q-9) (Kaino *et al.* 2018) and an almost identical
157 length of ITS1 (307 bp, internal transcribed spacer 1) to that (343 bp) of *Schizo-*
158 *saccharomyces octosporus* (Helston *et al.* 2010). Incidentally, *Schizosaccharomyces*
159 *pombe* had 417 bp ITS1 and *Schizosaccharomyces japonicus* had 183 bp ITS1.

160

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

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

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

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

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

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

168

169 *Schizosaccharomyces japonicus* is especially distant phylogenetically, i.e., the
170 species is the furthest removed from other species among the fission yeasts (Liu *et al.*
171 2009; Rhind *et al.* 2011; Aoki *et al.* 2017).

172 Liu *et al.* (2009) made phylogenomic analyses on the basis of the monophyly of
173 Taphrinomycotina, including *Schizosaccharomyces* fission yeasts. In the phylogenetic
174 tree, *Schizosaccharomyces japonicus* evolved first and separated from the remaining
175 two species, *Schizosaccharomyces pombe* and *Schizosaccharomyces octosporus*, as
176 described above (Aoki *et al.* 2017). There is, however, a certain risk that a
177 heterogeneous-natured taxon is sometimes given taxonomically in the generic
178 designation (Vu *et al.* 2021; Yamada *et al.* 2022b), when based on the monophyly only,
179 and the resulting genus corresponds to a higher-ranked taxon, e.g., the family
180 Schizosaccharomycetaceae. In the monotypic family Schizosaccharomycetaceae, the

181 genus *Schizosaccharomyces* sensu Kurtzman et Robnett actually had quite hetero-
182 geneous natures genetically (Helston et al. 2010), chemotaxonomically (Yamada et al.
183 1973; Mikata and Yamada 1999; Kaino et al. 2018), and physiologically (Vaughan-
184 Martini and Martini 2011; Sipiczki et al. 1998; Aoki et al. 2017). In addition, it is of
185 interest that the branch between *Schizosaccharomyces japonicus* and *Schizosaccharo-*
186 *myces pombe* was much longer than that between *Saccharomyces* (Q-6) and *Pichia* (Q-
187 7) in the phylogenetic tree based on the concatenated sequences of 13 proteins (*cox1*,
188 *cox2*, *cox3*, *cob*, *atp6*, *atp9* and *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*) encoded
189 by mtDNA. The experimental data obtained above indicated that the genus *Hasegawaea*
190 could not be rejected but retained.

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

196 In the comparative study on *wtf* genes in the family Schizosaccharomycetaceae by
197 De Carvalho (2020), it was emphasized that the only *Schizosaccharomyces japonicus* did
198 not have such genes. And the evolutionary analysis indicated that a very similar
199 topology to those described above was found in the resulting phylogenetic tree.
200 *Schizosaccharomyces japonicus* was first distantly removed from the others, and then
201 *Schizosaccharomyces pombe* was. In contrast, the branches were quite short among
202 *Schizosaccharomyces octosporus*, *Schizosaccharomyces osmophilus*, and *Schizo-*
203 *saccharomyces cryophilus*, indicating that the existence of three genera was confirmed
204 and the latter three species were adequate to be accommodated to the genus
205 *Octosporomyces* (Fig. 2).

206
207 Kurtzman (2003) introduced ‘clade’ or ‘phylogenetically circumscribed genus’ as
208 generic concept. However, it was not yet perfect to get the taxonomic homogeneous-
209 natured taxon for the genus. Namely, the branch lengths should be additionally
210 considered in the phylogenetic trees.

211
212 In the seven ranks used for the systematics of plants and animals, the taxonomically
213 homogeneous taxa can be available only when the lower-ranked two taxa, i.e., species
214 and genus are used. Therefore, the present authors naturally selected a taxonomic
215 homogeneous-natured taxon in the generic designation, since the longer the phylo-
216 genetic distances are the more taxonomic heterogeneities will be increased.

217
218 Thus, the genus *Schizosaccharomyces* Lindner sensu Kurtzman et Robnett (1991)
219 would correspond to the monotypic family that actually includes the phylogenetic-
220 distant and the phenotypic-distinct species, as far as the genus is not divided into three,

221 i.e., *Schizosaccharomyces* Lindner (1893) sensu stricto, *Octosporomyces* Kudriavzev
222 (1960), and *Hasegawaea* Yamada et Banno (1987).

223

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231 Conflict of interest

232 The authors declare that there are no conflicts of interest.

233

234 Author contributions

235 Y.Y., H.T.L.V., P.Y., S.T., and K.M designed the study. H.T.L.V. performed the main
236 experiments. P.Y. instructed how to make the experiments. Y.Y. prepared the
237 manuscript. The detailed discussion was made among Y.Y., H.T.L.V., P.Y., S.T., and
238 K.M.

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286 organisms whose asexual reproduction is by fission and whose ascospores have
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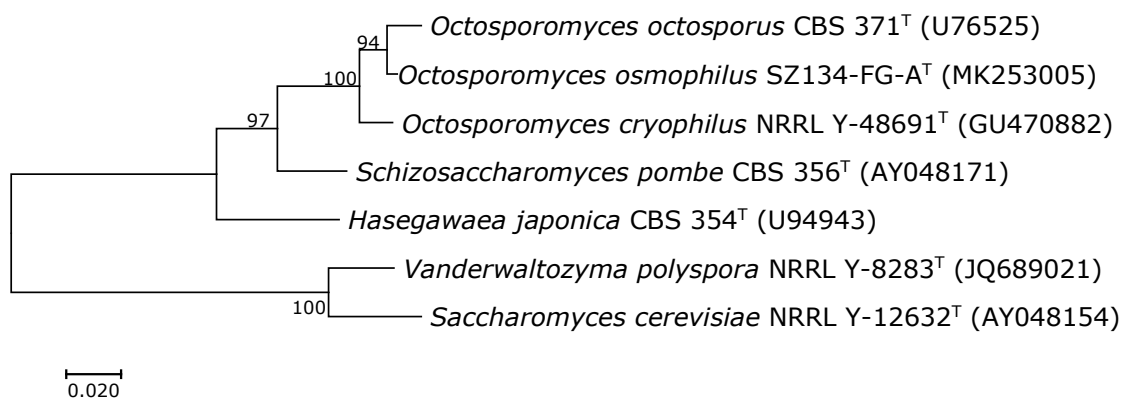
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Table 1. The pair-wise 26S rRNA gene D1/D2 domain sequence similarity.

Species	Similarity (%)				
	<i>S. pombe</i>	<i>O. octosporus</i>	<i>O. osmophilus</i>	<i>O. cryophilus</i>	<i>H. japonica</i>
<i>Schizosaccharomyces pombe</i> CBS 356 ^T	100	90.5	90.7	91.5	89.6
<i>Octosporomyces octosporus</i> CBS 371 ^c		100	98.1	95.2	84.9
<i>Octosporomyces osmophilus</i> SZ134-FG-A ^T			100	96.3	86.1
<i>Octosporomyces cryophilus</i> NRRL Y-48691 ^T				100	86.1
<i>Hasegawaea japonica</i> 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).

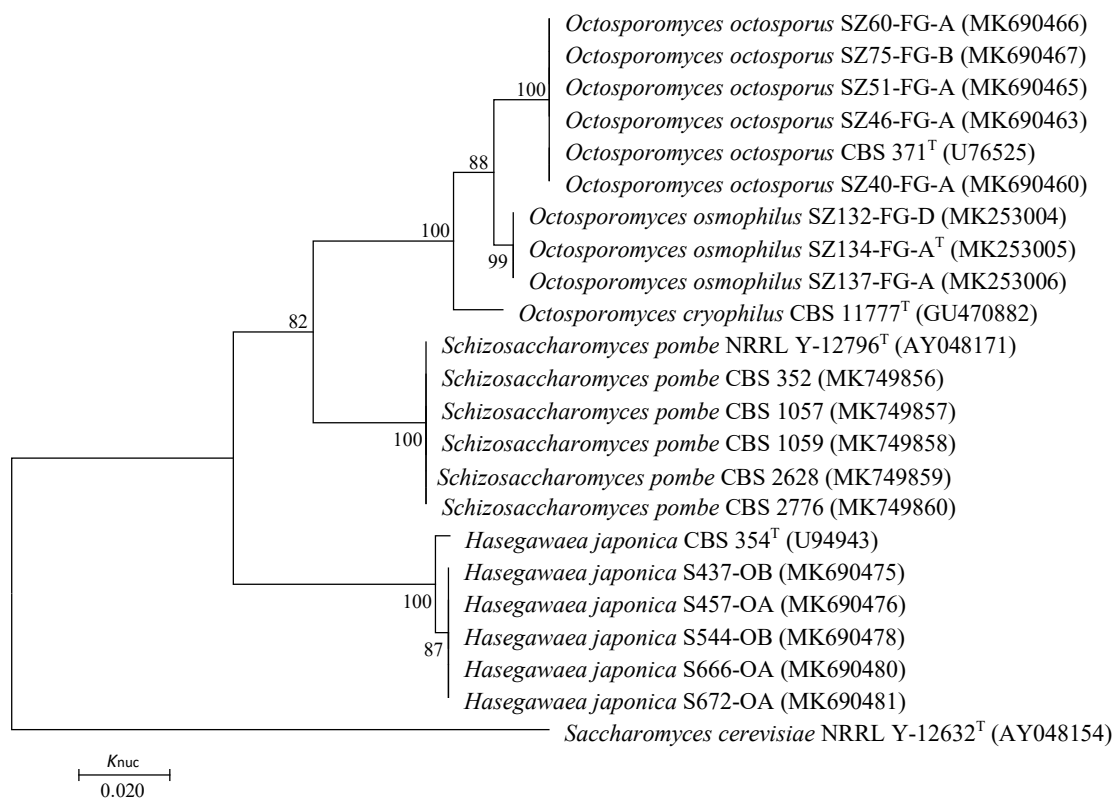
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Fig. 1. The phylogenetic tree of fission yeasts based on the 26S rRNA gene D1/D2 domain sequences. The evolutionary history was inferred using the neighbour-Joining method (Saito and Nei. *Mol Biol Evol* 1987; 4: 406). *Saccharomyces cerevisiae* and *Vanderwaltozyma 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

320 to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-
 321 parameter method (Kimura. *Evolution* 1980; **16**: 111) and are in the units of the number of base
 322 substitutions per site. The analysis involved seven nucleotide sequences. All positions containing
 323 gaps and missing data were eliminated. There was a total of 563 positions of the 26S rRNA gene
 324 D1/D2 domain sequences in the final dataset. Evolutionary analyses were conducted in MEGA7
 325 (Kumar *et al. Mol Biol Evol* 2016; **33**: 1870). According to the theory of Dr. Kurtzman (Kurtzman
 326 and Robnett 1991, 1998; Vaughan-Martini and Martini 2011), all the fission yeasts were designated
 327 as *Schizosaccharomyces pombe*, *Schizosaccharomyces octosporus*, *Schizosaccharomyce japonicus*,
 328 *Schizosaccharomyces cryophilus*, and *Schizosaccharomyces osmophilus*.
 329
 330



331
 332 Fig. 2. The phylogenetic tree of fission yeasts based on the 26S rRNA gene D1/D2 domain
 333 sequences. The evolutionary history was inferred using the neighbour-Joining method.
 334 *Saccharomyces cerevisiae* was used as outgroup. The optimal tree with the sum of branch length =
 335 0.32701129 was shown. The percentage of replicate trees, in which the associated taxa clustered
 336 together in the bootstrap test (1000 replicates) are shown next to the branches. The analysis involved
 337 23 nucleotide sequences. There was a total of 494 positions of the 26S rRNA gene D1/D2 domain
 338 sequences in the final dataset. The phylogenetic tree was constructed under the same condition as in
 339 Fig. 1.

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343

344 **Appendix:**

345 According to Gabor Peter (private communication), the calculated pair-wise 26S
346 rRNA gene D1/D2 domain sequence similarity was 72-78% between *Novakomyces olei*
347 and *Schizosaccharomyces pombe*.

348