

1 Microbial Systematics (Short Communication)

2  
3 **The Generic Circumscription of *Kockiozyma* (Lipomycetaceae)\***

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21 \*This paper is dedicated to Professor Dr. Johannes van der Walt, South Africa for his  
22 excellent works in yeast systematics, especially findings of the four *Zygozyma* species.

23 \*The preceding paper entitled “The Revision of Lipomycetaceae” was opened in Jxiv  
24 (DOI: <https://doi.org/10.51094/jxiv.189>).

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31 Keywords: *Kockiozyma suomiensis*; *Myxozyma geophila*. *Kockiozyma geophila* f.a.;  
32 *Kockiozyma melibiosi* f.a.; *Zygozyma oligophaga*.

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35 **Abstract**

36 The genus *Kockiozyma* was introduced as the ninth and the monotypic genus in the family  
37 Lipomycetaceae. On the other hand, a number of anamorphic species were reported as  
38 *Myxozyma*. This paper deals with the phylogenetic relationships between the teleomorphic  
39 species, *Kockiozyma suomiensis* and the anamorphic species. To constitute a taxonomic  
40 homogeneous-natured genus, 1) the several species concerned have to be tightly coupled  
41 with one another phylogenetically. 2) To make the several species tightly coupled, the

42 branch lengths have to be short or the calculated sequence similarities have to be 98% or  
43 more among the species in the 18S rRNA gene sequences. Thus, the following seven  
44 *Myxozyma* species were accommodated to the genus *Kockiozyma* as *Kockiozyma geophila*  
45 f.a., *Kockiozyma sirexii* f.a., *Kockiozyma neotropica* f.a., *Kockiozyma vanderwaltii* f.a.,  
46 *Kockiozyma mucilagina* f.a., *Kockiozyma neglecta* f.a. and *Kockiozyma melibiosi* f.a.

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#### 48 **Supplementary Abstract**

The genus *Kockiozyma* Jindamorakot, Yukphan et Yamada (2012)

- 1) *Kockiozyma suomiensis* (Smith, van der Walt et Yamada) Jindamorakot, Yukphan et Yamada (2012)
- 2) *Kockiozyma melibiosi* f.a. (Shifrine et Phaff) Vu, Yukphan, Tanasupawat et Yamada (2022)
- 3) *Kockiozyma mucilagina* f.a. (Phaff, Starmer, Miranda et Miller) Vu, Yukphan, Tanasupawat et Yamada (2022)
- 4) *Kockiozyma geophila* f.a. (van der Walt, Yamada et Nakase) Yamada, Vu, Yukphan et Tanasupawat (2022)
- 5) *Kockiozyma sirexii* f.a. (Spaaij et Weber) Vu, Yukphan, Tanasupawat et Yamada (2022)
- 6) *Kockiozyma neotropica* f.a. (Spaaij et Weber) Vu, Yukphan, Tanasupawat et Yamada (2022)
- 7) *Kockiozyma vanderwaltii* f.a. (Spaaij, Weber et Smith) Vu, Yukphan, Tanasupawat et Yamada (2022)
- 8) *Kockiozyma neglecta* f.a. (Spaaij, van der Walt et Weber-Spaaij) Vu, Yukphan, Tanasupawat et Yamada (2022)

The genus *Kockiozyma* Jindamorakot, Yukphan et Yamada (2012)

- 1) *Kockiozyma suomiensis* (Smith, van der Walt et Yamada) Jindamorakot, Yukphan et Yamada (2012)

The genus *Myxozyma* van der Walt, Weijman et von Arx (1981)

- 2) *Myxozyma melibiosi* (Shifrine et Phaff) van der Walt, Weijman et von Arx (1981)
- 3) *Mixozyma mucilagina* (Phaff, Starmer, Miranda et Miller) van der Walt, Weijman et von Arx (1981)
- 4) *Myxozyma geophila* van der Walt, Yamada et Nakase (1987)
- 5) *Myxozyma sirexii* Spaaij et Weber (1990)
- 6) *Myxozyma neotropica* Spaaij et Weber (1992)
- 7) *Myxozyma vanderwaltii* Spaaij, Weber et Smith (1992)
- 8) *Myxozyma neglecta* Spaaij, van der Walt et Weber-Spaaij (1998)

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52 The three phylogenetic trees based on the concatenated four-gene sequences comprised  
53 of 18S rRNA, 26S rRNA, mitochondrial small subunit rRNA and *EF-1 $\alpha$*  were  
54 constructed for the Lipomycetaceous yeasts by the neighbour-joining, the maximum  
55 parsimony and the maximum likelihood methods (Jindamorakot et al. 2012). In the

56 phylogenetic trees, the *Lipomyces* species and the *Dipodascopsis* species newly designated  
57 by Kurtzman et al. (2007) were not tightly coupled with one another but loosely coupled  
58 and changed their phylogenetic positions. In contrast, the phylogenetic positions of  
59 *Zygozoma suomiensis* (= *Lipomyces suomiensis*) and its related anamorphic species were  
60 not changed in their topology, as found in the species within the genus *Lipomyces* sensu  
61 stricto.

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63 In the five phylogenetic trees based on the 18S rRNA gene (1,627 bases), 26S rRNA  
64 gene (3,147 bases), mitochondrial small subunit rRNA gene (155 bases), *EF-1 $\alpha$*  gene  
65 (662 bases) and concatenated four-gene sequences for *Zygozoma* and *Myxozyma* species  
66 derived from the neighbour-joining method, *Zygozoma suomiensis* and its related seven  
67 *Myxozyma* species produced a common cluster, respectively (Jindamorakot et al. 2012).

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69 The genus *Kockiozyma* Jindamorakot et al. was introduced as the ninth and monotypic  
70 genus for *Zygozoma suomiensis* in the family Lipomycetaceae (Jindamorakot et al. 2012).  
71 The only teleomorphic species, *Kockiozyma suomiensis* was characterized by ubiquinone-  
72 8 (Q-8 or Q8), along with *Zygozoma oligophaga* (van der Walt et al. 1987; Smith et al.  
73 1989). On the other hand, the several anamorphic species that were equipped with Q-8  
74 were additionally reported within the genus *Myxozyma* (Kurtzman and Smith 2011).

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76 This paper is concerned with the phylogenetic relationships between *Kockiozyma*  
77 *suomiensis* and the several anamorphic *Myxozyma* species using the 18S rRNA gene  
78 sequences. As a result, the generic name of *Kockiozyma* was given to the seven *Myxozyma*  
79 species equipped with Q-8 and showing 98% or more pair-wise sequence similarities.

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81 All the sequence data were cited from Kurtzman et al. (2007) and Kurtzman (2003).  
82 The 18S rRNA gene sequences were aligned either individually or totally, as described  
83 previously (Jindamorakot et al. 2012).

84  
85 In the 18S rRNA gene sequences, the seven *Myxozyma* species showed very high pair-  
86 wise sequence similarities (99.8 - 98.7%) to *Kockiozyma suomiensis*; *Myxozyma geophila*,  
87 *Myxozyma sirexii*, *Myxozyma neotropica*, *Myxozyma vanderwiltii*, *Myxozyma mucilagina*,  
88 *Myxozyma neglecta* and *Myxozyma melibiosi* (Table 1). They were all Q8-equipped  
89 without any exception and included in the cluster of *Kockiozyma suomiensis* (Fig. 1).

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91 The other Q8-equipped *Myxozyma* species were three; *Myxozyma monticola* (95.7%),  
92 *Myxozyma kluyveri* (96.50%) and *Myxozyma udenii* (96.5%) (data not shown). The  
93 remaining two *Myxozyma* species were Q9-equipped; *Myxozyma lipomycoides* (96.3%)  
94 and *Myxozyma nipponensis* (95.0%) (data not shown). *Zygozoma oligophaga* and  
95 *Myxozyma monticola* constituted a different cluster from *Kockiozyma suomiensis*. The  
96 calculated pair-wise sequence similarity was 95.0% between the two species (data not

97 shown), indicating that the species could not constitute the common teleomorphic genus  
98 *Zygozoma* along with *Zigozoma oligophaga*.

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100 Previously, the pair-wise sequence similarity between *Myxozyma geophila* and  
101 *Kockiozoma suomiensis* was calculated to be 98.0% in the 26S rRNA gene D1/D2 domain  
102 sequences (Vu et al. 2021; Yamada et al. 2022). Between *Octosporomyces osmophilus* (= *Schizosaccharomyces osmophilus*) and *Octosporomyces octosporus*, a similar sequence  
103 similarity (98.1%) was given (Vu et al. 2022). On the other hand, the calculated sequence  
104 similarity was 85.9% between *Zygozoma oligophaga* and *Kockiozoma suomiensis*  
105 (Yamada et al. 2022).

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108 In the assimilation of carbon sources for growth, *Kockiozoma suomiensis* and the seven  
109 *Myxozyma* species on the whole represented a similar pattern to one another, except for  
110 *Myxozyma neotropica*. Especially, there were few species assimilating trehalose (Table 1).  
111 In contrast, *Myxozyma monticola* utilized a relatively large number of carbon sources, e.g.,  
112 trehalose and L-rhamnose. In contrast, there were a few of carbon sources assimilated by  
113 *Zygozoma oligophaga*, e.g., glucose, galactose, glycerol, galactitol and D-glucitol.

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115 To constitute a taxonomic homogeneous-natured genus, 1) the several species  
116 concerned have to be tightly coupled with one another phylogenetically. 2) To make the  
117 several species tightly coupled, the branch lengths have to be short in the phylogenetic tree  
118 or the calculated sequence similarities have to be 98% or more among the species in the  
119 18S rRNA gene sequences.

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121 From the results obtained above, the following new combinations are introduced in the  
122 genus (Lachance 2012; Yamada et al. 2022).

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124 The genus *Kockiozoma* Jindamorakot, Yukphan et Yamada (MB 587737)

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126 1. *Kockiozoma suomiensis* (Smith, van der Walt et Yamada) Jindamorakot, Yukphan et  
127 Yamada (2012) MB 587754

128 Basionym: *Zygozoma suomiensis* Smith, van der Walt et Yamada (1990)

129 Synonym. *Lipomyces suomiensis* (Smith, van der Walt et Yamada) Kurtzman,  
130 Albertyn et Basehoar-Powers (2007).

131 2. *Kockiozoma melibiosi* f.a. (Shifrine et Phaff) comb. nov.

132 Basionym: *Torulopsis melibiosum* Shifrine et Phaff, Mycologia, 41: 49, 1956.

133 Synonym: *Myxozyma melibiosi* (Shifrine et Phaff) van der Walt, Weijman et von Arx  
134 (1981)

135 The type strain is CBS 2102.

136 3. *Kockiozoma mucilagina* f.a. (Phaff, Starmer, Miranda et Miller) comb. nov.

137 Basionym: *Candida mucilagina* Phaff, Starmer, Miranda et Miller, Int. J. Syst.  
138 Bacteriol. 30: 596, 1980.

139 Synonym: *Myxozyma mucilagina* (Phaff, Starmer, Miranda et Miller) van der Walt,  
140 Weijman et Miller (1981).

141 The type strain is CBS 7071.

142 4. *Kockiozyma geophila* f.a. (van der Walt, Yamada et Nakase) Yamada, Vu, Yukphan et  
143 Tanasupawat (2022)

144 Basionym: *Myxozyma geophila* van der Walt, Yamada et Nakase (1987).

145 5. *Kockiozyma sirexii* f.a. (Spaaij et Weber) comb. nov.

146 Basionym: *Myxozyma sirexii* Spaaij et Weber, Syst. Appl. Microbiol. 15: 428, 1992.  
147 The type strain is UOFS Y-2054 = NRRL Y-27626

148 6. *Kockiozyma neotropica* f.a. (Spaaij et Weber) comb. nov.

149 Basionym: *Myxozyma neotropica* f.a. Spaaij et Weber, Antonie van Leeuwenhoek,  
150 62: 262, 1992.

151 The type strain is CBS 7953.

152 7. *Kockiozyma vanderwaltii* f.a. (Spaaij, Weber et Smith) comb. nov.

153 Basionym: *Myxozyma vanderwaltii* Spaaij, Weber et Smith, Antonie van  
154 Leeuwenhoek 63: 18, 1993.

155 The type strain is CBS 7793.

156 8. *Kockiozyma neglecta* f.a. (Spaaij, van der Walt et Weber-Spaaij) comb. nov.

157 Basionym: *Myxozyma neglecta* Spaaij, van der Walt et Weber-Spaaij, Antonie van  
158 Leeuwenhoek 73: 144, 1998.

159 The type strain is CBS 7058.

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161 Yamazaki et al. (2020) reported 12 anamorphic *Lipomyces* species. Considering the  
162 phylogenetic positions of the species, the six species of the twelve were to be  
163 accommodated to the genus *Kockiozyma* as *Kockiozyma kiyosumica* f.a., *Kockiozyma*  
164 *yaeyamensis* f.a., *Kockiozyma takeshimensis* f.a., *Kockiozyma haiminakana* f.a.,  
165 *Kockiozyma chibensis* f.a. and *Kockiozyma iriomotensis* f.a., all of which were  
166 characteristic of Q-8.

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168 In conclusion, it is of great interest that the only genus *Kockiozyma* includes such  
169 anamorphic species within the genus, being quite different in this respect from the genera  
170 *Lipomyces* sensu stricto and *Babjevia* in the family Lipomycetaceae.

171

172 Acknowledgements

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

175

176 Funding information

177 The authors received no specific grant from any funding agency.

178

179 Conflicts of interest

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

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182 Author contributions

183 Y.Y., H.T.L.V., P.Y., and S.T. designed the study. H.T.L.V. performed the main  
184 experiments. P.Y. instructed how to make the experiments. Y.Y. prepared the manuscript.

185 The detailed discussion was made among Y.Y., H.T.L.V., P.Y., and S.T.

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Table 1. The phylogenetic and the phenotypic characteristics of *Kockiozyma suomiensis* and its related *Myxozyma* species.

Species	1	2	3	4	5	6	7	8	9	10
Sequence similarity (%)*	100	99.4	99.8	99.5	99.2	98.7	98.7	99.6	95.7	94.7
Assimilation of carbon source <sup>2*</sup>										
Glucose	+	+	+	+	+	+	+	+	+	+
Inulin	-	-	-	-	-	-	-	-	-	-
Sucrose	-	+/w	-	+	+	+	-	-	+	-
Raffinose	-	-	-	-	-	-	-	-	v	-
Melibiose	-	-	-	+	-	-	-	+	+	-
Galactose	+	+	+	+	+	+	+	+	+	+
Lactose	+	v	-	+	v	-	-	v	+	-
Trehalose	-	-	-	+	s	v	-	-	+	-
Maltose	-	-	-	+	+	+	-	-	+	-
Methyl- $\alpha$ -D-glucoside	-	-	-	+	+	+	-	-	+	-
Cellobiose	+	-	-	+	+	+	+	+	-	-
L-Sorbose	+	+	+	+	+	+	+	v	+	v
L-Rhamnose	-	-	-	+	-	+	-	-	+	-
D-Xylose	+	+	+	+	+	+	+	+	+	v
L-Arabinose	+	+	+	+	+	+	+	+	+	-
D-Arabinose	+	+	+	+	+	v	+	+	+	-
D-Ribose	-	+	+	+	v	v	-	+	+	-
Glycerol	+	+	+	+	+	+	+	+	+	+
Erythritol	-	-	+	-	-	-	-	-	+	-
Ribitol	+	+	+	+	+	+	+	+	+	-
Galactitol	-	+	-	-	-	-	-	-	-	+
D-Mannitol	+	s	+	+	-	s	-	w/-	s	-
D-Glucitol	+	+	+	+	+	+	-	s	+	+

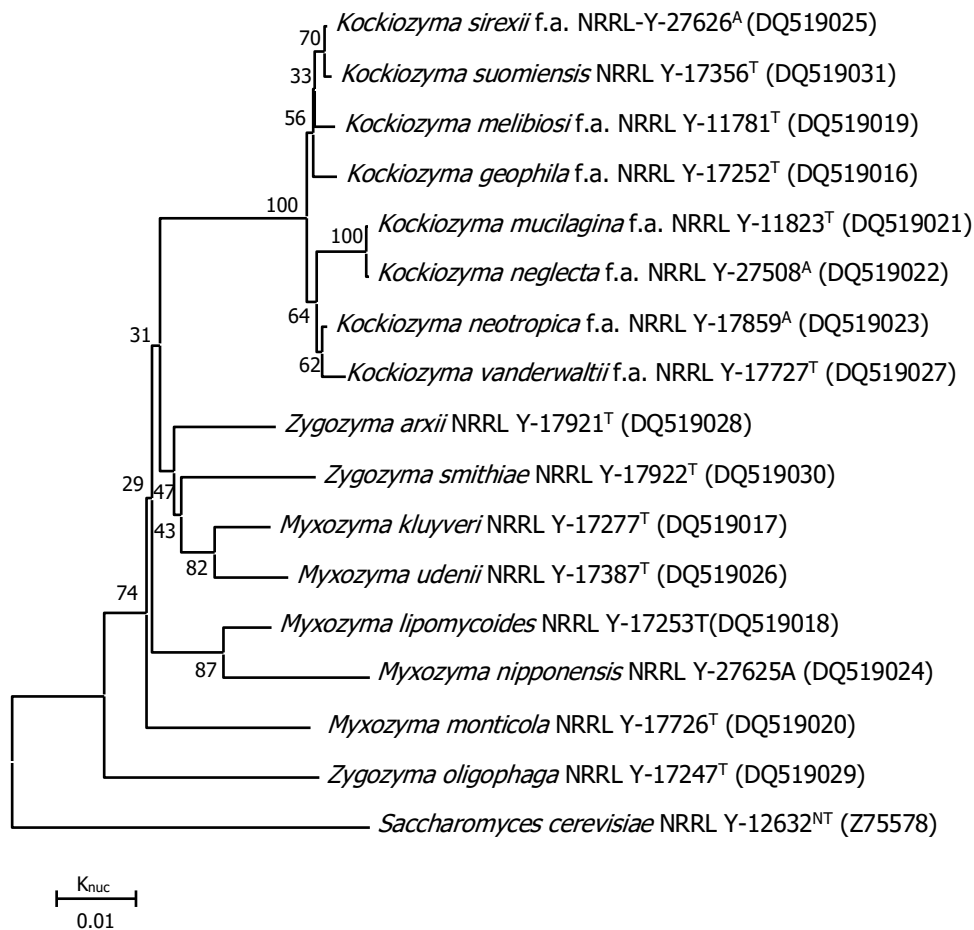
Pair-wise sequence similarities between *K. suomiensis* and the respective species in the 18S rRNA gene sequences (1,627 bases) (Jindamraakot et al. 2012)

<sup>2\*</sup>Assimilation of carbon sources for growth (cited from Smith and Kurtzman 2011 and Kurtzman and Smith 2011).

1. *Kockiozyma suomiensis*; 2. *Myxozyma geophila*; 3. *Myxozyma sirexii*; 4. *Myxozyma neotropica*; 5. *Myxozyma vanderwaltii*; 6. *Myxozyma mucilagina*; 7. *Myxozyma neglecta*; 8. *Myxozyma melibiosii*; 9. *Myxozyma monticola*; 10. *Zygozyma oligophaga*.

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Fig. 1. The phylogenetic tree based on the 18S rRNA gene sequences derived from the neighbour-joining method (Jindamorakot et al. 2012).