

1 Microbial Systematics (Short Communication)

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

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20
21 *This paper is dedicated to Professor Dr. Johannes van der Walt, South Africa for his
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23 *The preceding paper entitled “The Revision of Lipomycetaceae” was opened in Jxiv
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33 Keywords: *Kockiozyma suomiensis*; *Myxozyma geophila*. *Kockiozyma geophila* f.a.;
34 *Kockiozyma sirexii* f.a.; *Zygozyma oligophaga*.

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37 **Abstract**

38 The genus *Kockiozyma* was introduced as the ninth and the monotypic genus in the family
39 Lipomycetaceae. On the other hand, a number of anamorphic species were reported as
40 *Myxozyma*. This paper deals with the phylogenetic relationships between the teleomorphic
41 species, *Kockiozyma suomiensis* and the anamorphic species. To constitute a taxonomic-

42 homogeneous-natured genus, 1) the several species concerned have to be tightly coupled
43 with one another phylogenetically. 2) To make the several species tightly coupled, the
44 branch lengths have to be short or the calculated sequence similarities have to be 98% or
45 more in the 18S rRNA gene sequences among the species. Thus, the following seven
46 *Myxozyma* species were accommodated to the genus *Kockiozymba* as *Kockiozymba geophila*
47 f.a., *Kockiozymba sirexii* f.a., *Kockiozymba neotropica* f.a., *Kockiozymba vanderwaltii* f.a.,
48 *Kockiozymba mucilagina* f.a., *Kockiozymba neglecta* f.a. and *Kockiozymba melibiosi* f.a.

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50 **Supplementary Abstract**

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

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

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

- 1) *Kockiozymba suomienensis* (Smith, van der Walt et Yamada) Jindamorakot, Yukphan et Yamada (2012)

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

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

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The three phylogenetic trees based on the concatenated four-gene sequences comprised of 18S rRNA, 26S rRNA, mitochondrial small subunit rRNA and *EF-1 α* were

56 constructed for the Lipomycetaceous yeasts by the neighbour-joining, the maximum
57 parsimony and the maximum likelihood methods (Jindamorakot et al. 2012). In the three,
58 the *Lipomyces* species and the *Dipodascopsis* species newly designated by Kurtzman et al.
59 (2007) were not tightly coupled with one another but loosely coupled and changed their
60 phylogenetic positions in the trees. In contrast, the phylogenetic positions of *Zygozoma*
61 *suomiensis* (= *Lipomyces suomiensis*) and its related anamorphic species were not changed
62 in their topology, as found in the species within the genus *Lipomyces* sensu stricto.
63

64 In the five phylogenetic trees based on the 18S rRNA gene (1,627 bases), 26S rRNA
65 gene (3,147 bases), mitochondrial small subunit rRNA gene (155 bases), *EF-1 α* gene
66 (662 bases) and concatenated four-gene sequences for *Zygozoma* and *Myxozyma* species
67 derived from the neighbour-joining method, *Zygozoma suomiensis* and its related seven
68 *Myxozyma* species produced a common cluster, respectively (Jindamorakot et al. 2012).
69

70 The genus *Kockiozoma* Jindamorakot et al. was introduced in the family Lipomyceta-
71 ceae as the ninth and monotypic genus for *Zygozoma suomiensis* in the family Lipo-
72 mycetaceae (Jindamorakot et al. 2012). The only teleomorphic species, *Kockiozoma*
73 *suomiensis* was characterized by ubiquinone-8 (Q-8 or Q8), along with *Zygozoma*
74 *oligophaga* (van der Walt et al. 1987; Smith et al. 1989). On the other hand, the several
75 anamorphic species that were equipped with Q-8 were additionally reported within the
76 genus *Myxozyma* (Kurtzman and Smith 2011).
77

78 This paper is concerned with the phylogenetic relationships between *Kockiozoma*
79 *suomiensis* and the several anamorphic *Myxozyma* species using the 18S rRNA gene
80 sequences. As a result, the generic name of *Kockiozoma* was given to the seven *Myxozyma*
81 species equipped with Q-8 and showing 98% or more pair-wise sequence similarities.
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83 All the sequence data were cited from Kurtzman et al. (2007) and Kurtzman (2003).
84 The 18S rRNA gene sequences were aligned either indivisually or totally, as described
85 previously (Jindamorakot et al 2012).
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87 In the 18S rRNA gene sequences, the seven *Myxozyma* species showed very high pair-
88 wise sequence similarities (99.8 - 98.7%) to *Kockiozoma suomiensis*; *Myxozyma geophila*,
89 *Myxozyma sirexii*, *Myxozyma neotropica*, *Myxozyma vanderwiltii*, *Myxozyma mucilagina*,
90 *Myxozyma neglecta* and *Myxozyma melibiosi* (Table 1). They were all Q8-equipped
91 without any exception and were included in the cluster of *Kockiozoma suomiensis* (Fig. 1).
92

93 The other Q8-equipped *Myxozyma* species were three; *Myxozyma monticola* (95.7%),
94 *Myxozyma kluiveri* (96.50%) and *Myxozyma udenii* (96.5%) (data not shown). The
95 remaining two *Myxozyma* species were Q9-equipped; *Myxozyma lipomycoides* (96.3%)
96 and *Myxozyma nipponensis* (95.0%) (data not shown). *Zygozoma oligophaga* and

97 *Myxozyma monticola* constituted a different cluster from *Kockiozyma suomiensis*. The
98 calculated pair-wise sequence similarity was 95.0% between the two species (data not
99 shown), indicating that the species could not constitute the common teleomorphic genus
100 *Zygozyma* along with *Zigozyma oligophaga*.

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102 In the assimilation of carbon sources for growth, *Kockiozyma suomiensis* and the seven
103 *Myxozyma* species on the whole represented a similar pattern to one another, except for
104 *Myxozyma neotropica*. Especially, there were few species assimilating trehalose (Table 1).
105 In contrast, *Myxozyma monticola* utilized a relatively large number of carbon sources, e.g.,
106 trehalose and L-rhamnose. On the other hand, there were a few of carbon sources
107 assimilated by *Zygozyma oligophaga*, e.g., glucose, galactose, glycerol, galactitol and D-
108 glucitol.

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110 To constitute a taxonomic-homogeneous natured genus, 1) the several species
111 concerned have to be tightly coupled with one another phylogenetically. 2) To make the
112 several species tightly coupled, the branch lengths have to be short in the phylogenetic tree
113 or the calculated sequence similarities have to be 98% or more among the species in the
114 18S rRNA gene sequences (Yamada et al. 2022; Vu et al. 2021, 2022).

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

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119 The genus *Kockiozyma* Jindamorakot, Yukphan et Yamada (MB 587737)

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121 1. *Kockiozyma suomiensis* (Smith, van der Walt et Yamada) Jindamorakot, Yukphan et
122 Yamada (2012) MB 587754

123 Basionym: *Zygozyma suomiensis* Smith, van der Walt et Yamada (1990)

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

126 2. *Kockiozyma melibiosi* f.a. (Shifrine et Phaff) comb. nov.

127 Basionym: *Torulopsis melibiosum* Shifrine et Phaff, Mycologia, 41: 53, 1956.

128 Synonym: *Myxozyma melibiosi* (Shifrine et Phaff) van der Walt, Weijman et von Arx
129 (1981)

130 The type strain is CBS 2102.

131 MycoBank number is //////////////.

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

133 Basionym: *Candida mucilagina* Phaff, Starmer, Miranda et Miller, Int. J. Syst.
134 Bacteriol. 30: 598 (1980).

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

137 The type strain is CBS 7071.

138 MycoBank number is /////
139 4. *Kockiozyma geophila* f.a. (van der Walt, Yamada et Nakase) Yamada, Vu, Yukphan et
140 Tanasupawat (2022)
141 Basionym: *Myxozyma geophila* van der Walt, Yamada et Nakase (1987).
142 5. *Kockiozyma sirexii* f.a. (Spaaij et Weber) Yamada, Vu, Yukphan et Tanasupawat
143 (2022)
144 Basionym: *Myxozyma sirexii* Spaaij et Weber (1992).
145 6. *Kockiozyma neotropica* f.a. (Spaaij et Weber) comb. nov.
146 Basionym: *Myxozyma neotropica* f.a. Spaaij et Weber, Antonie van Leeuwenhoek,
147 62: 263, 1992
148 The type strain is CBS 7953.
149 MycoBank number is /////
150 7. *Kockiozyma vanderwaltii* f.a. (Spaaij, Weber et Smith) comb. nov.
151 Basionym: *Myxozyma vanderwaltii* Spaaij, Weber et Smith, Antonie van
152 Leeuwenhoek 63: 19, 1993.
153 The type strain is CBS 7793.
154 MycoBank number is /////
155 8. *Kockiozyma neglecta* f.a. (Spaaij, van der Walt et Weber-Spaaij) comb. nov.
156 Basionym: *Myxozyma vanderwaltii* Spaaij, van der Walt et Weber-Spaaij, Antonie
157 van Leeuwenhoek 73: 144, 1998.
158 The type strain is CBS 7058.
159 MycoBank numbrt is /////
160
161 Yamazaki et al. (2020) reported 12 anamorphic *Lipomyces* species. Considering the
162 phylogenetic positions of the species, the six species are to be accommodated to the genus
163 *Kockiozyma* as *Kockiozyma kiyosumica* f.a., *Kockiozyma yaeyamensis* f.a., *Kockiozyma*
164 *takeshimensis* f.a., *Kockiozyma haiminakanus* f.a., *Kockiozyma chibensis* f.a. and
165 *Kockiozyma iriomotensis* f.a. (Yamada et al. 2022).

166

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174 **Conflicts of interest**

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

176

177 **Author contributions**

178 Y.Y., H.T.L.V., P.Y., and S.T. designed the study. H.T.L.V. performed the main
179 experiments. P.Y. instructed how to make the experiments. Y.Y. prepared the manuscript.
180 The detailed discussion was made among Y.Y., H.T.L.V., P.Y., and S.T.

181

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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 ^{2*}										
Glucose	+	+	+	+	+	+	+	+	+	+
Inulin	-	-	-	-	-	-	-	-	-	-
Sucrose	-	+/w	-	+	+	+	-	-	+	-
Raffinose	-	-	-	-	-	-	-	-	v	-
Melibiose	-	-	-	+	-	-	-	+	+	-
Galactose	+	+	+	+	+	+	+	+	+	+
Lactose	+	v	-	+	v	-	-	v	+	-
Trehalose	-	-	-	+	s	v	-	-	+	-
Maltose	-	-	-	+	+	+	-	-	+	-
Methyl- α -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	+	+

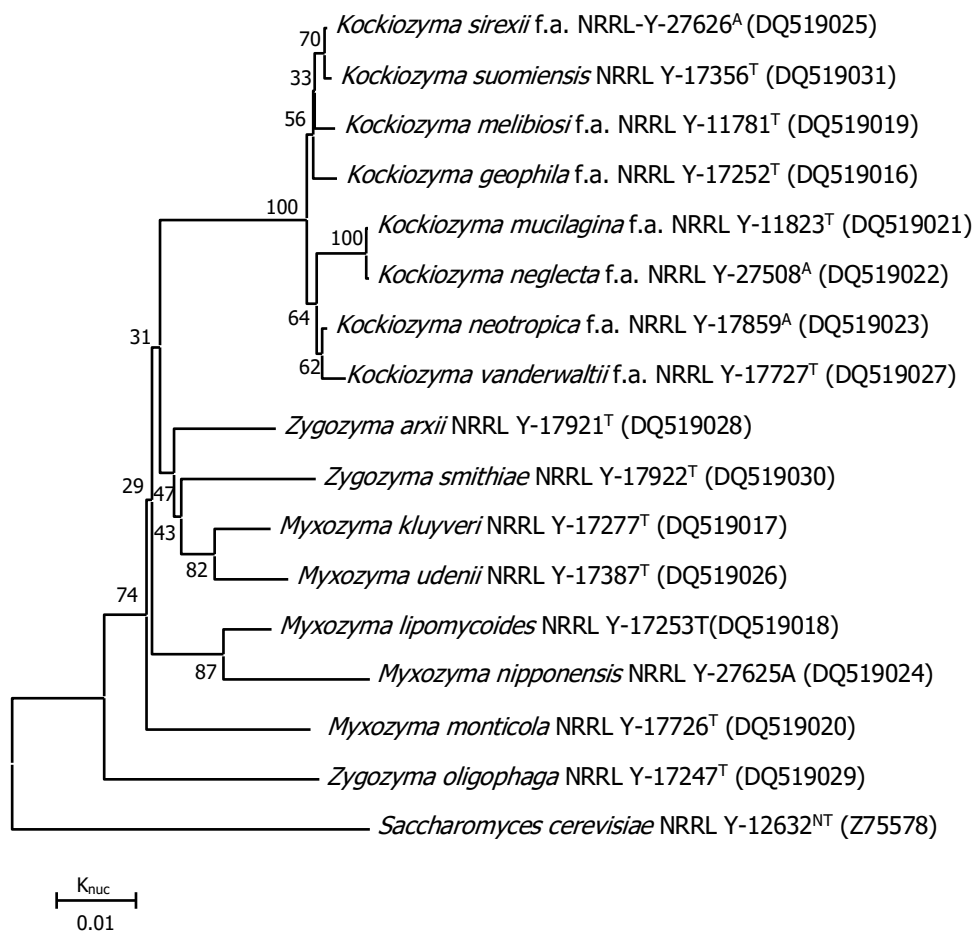
Pair-wise sequence similarities between *K. suomiensis* and the respective species in the 18S rRNA gene sequences (1,627 bases) (Jindamraakot et al. 2012)

^{2*}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 melibiosi*; 9. *Myxozyma monticola*; 10. *Zygozima 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).