

The Generic Circumscription of *Kockiozyma* (Lipomycetaceae)*

¹Taweesak Malimas, ²Huong Thi Lan Vu, ³Somboon Tanasupawat, ⁴Yuzo Yamada

¹Microbial Laboratory Biosafety Level-1, 46 M, 9 Nongphus, Muangsuang, Roi-Et 45220, Thailand

²Department 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

³Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 254 Phayathai Road, Wangmai, Pathumwan, Bangkok 10330, Thailand

⁴Laboratory of Applied Microbiology (Professor Emeritus), Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

*This article is dedicated to Professor Dr. Johannes P. van der Walt, South Africa for his excellent works in yeast systematics, especially findings of the four *Zygozyma* species, which was reclassified as *Zygozyma*, *Kawsakia*, *Limtongia* and *Kockiozyma* species respectively.

⁴Corresponding: Yuzo Yamada (ORCID ID: 0002-0002-2799-7045), yamada-yuzo@nifty.com

DOI: <https://doi.org/10.5281/zenodo.13736058>

Published Date: 09-September-2024

Abstract: The genus *Kockiozyma* was introduced as the ninth and the monotypic genus in the family Lipomycetaceae. On the other hand, a number of anamorphic species were reported in the genus *Myxozyma*. This paper deals with the phylogenetic relationships between the teleomorphic species, *Kockiozyma suomiensis* and the anamorphic species. To constitute a taxonomic homogeneous-natured genus, 1) the several species concerned have to be tightly coupled with one another phylogenetically. 2) To make the several species tightly coupled, the branch lengths have to be short or the calculated sequence similarities have to be 98% or more among the species in the 18S rRNA gene sequences. Thus, the following seven *Myxozyma* species were accommodated to the genus *Kockiozyma* as *Kockiozyma geophila* f.a., *Kockiozyma sirexii* f.a., *Kockiozyma neotropica* f.a., *Kockiozyma vanderwaltii* f.a., *Kockiozyma mucilagina* f.a., *Kockiozyma neglecta* f.a. and *Kockiozyma melibiosi* f.a.

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

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) Malimas, Vu, Tanasupawat et Yamada (2024)

3) *Kockiozyma mucilagina* f.a. (Phaff, Starmer, Miranda et Miller) Malimas, Vu, Tanasupawat et Yamada (2024)

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) Malimas, Vu, Tanasupawat et Yamada (2024)

6) *Kockiozyma neotropica* f.a. (Spaaij et Weber) Malimas, Vu, Tanasupawat et Yamada (2024)

7) *Kockiozyma vanderwaltii* f.a. (Spaaij, Weber et Smith) Malimas, Vu, Tanasupawat et Yamada (2024)

8) *Kockiozyma neglecta* f.a. (Spaaij, van der Walt et Weber-Spaaij) Malimas, Vu, Tanasupawat et Yamada (2024)

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)

1. INTRODUCTION

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

The three phylogenetic trees based on the concatenated four-gene sequences comprised of 18S rRNA, 26S rRNA, mitochondrial small subunit rRNA and *EF-1a* were constructed for the Lipomycetaceous yeasts by the neighbour-joining, the maximum parsimony and the maximum likelihood methods (Jindamorakot et al. 2012). In the phylogenetic trees, the *Lipomyces* species and the *Dipodascopsis* species newly designated by Kurtzman et al. (2007) were not tightly coupled respectively with one another but loosely coupled and changed their phylogenetic positions. In contrast, the phylogenetic positions of *Zygozyma suomiensis* (= *Lipomyces suomiensis*) and its related anamorphic species were not changed in their topology, as found in the species within the genus *Lipomyces* sensu stricto.

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

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

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

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

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. *Zygozoma oligophaga*.

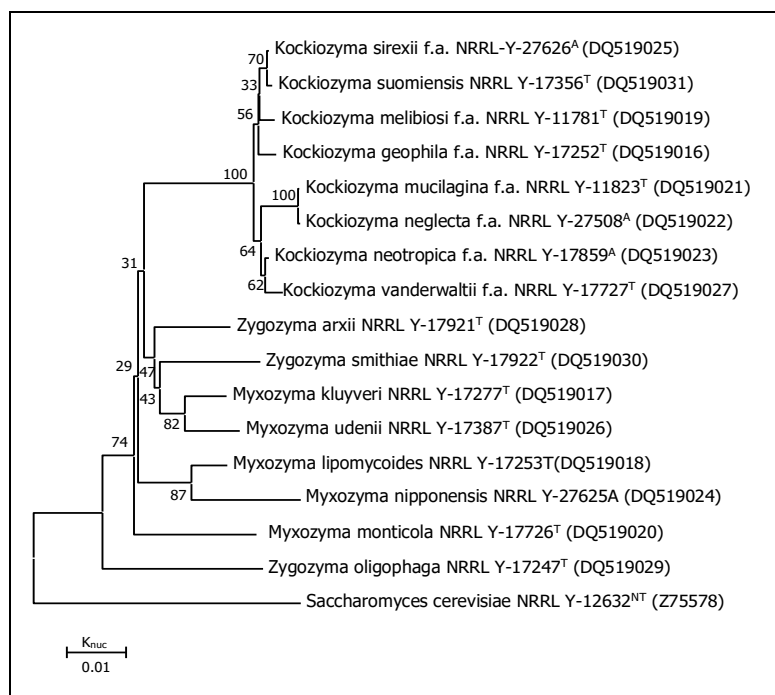


Fig. 1. The phylogenetic tree based on the 18S rRNA gene sequences derived from the neighbour-joining method (Jindamorakot et al. 2012).

The other Q8-equipped *Myxozyma* species were three; *Myxozyma monticola* (95.7%), *Myxozyma kluyveri* (96.50%) and *Myxozyma udenii* (96.5%) (data not shown). The remaining two *Myxozyma* species were Q9-equipped; *Myxozyma lipomycoides* (96.3%) and *Myxozyma nipponensis* (95.0%) (data not shown). *Myxozyma monticola* (Q-8) constituted a different cluster from *Kockiozyma suomiensis*. In fact, the calculated pair-wise sequence similarity was 95.0% between the two species (data not shown), indicating that the species could not be classified in the genus *Kockiozyma* along with *Zygozyma oligophaga* (Q-8).

Previously, the pair-wise sequence similarity between *Myxozyma geophila* and *Kockiozyma suomiensis* was calculated to be 98.0% in the 26S rRNA gene D1/D2 domain sequences (Vu et al. 2021; Yamada et al. 2022). Between *Octosporomyces osmophilus* (= *Schizosaccharomyces osmophilus*) and *Octosporomyces octosporus*, a similar sequence similarity (98.1%) was given (Malimas et al. 2023). On the other hand, the calculated sequence similarity was 85.9% between *Zygozyma oligophaga* and *Kockiozyma suomiensis* (Yamada et al. 2022).

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

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

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

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

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

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

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

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

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

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

The type strain is CBS 2102.

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

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

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

The type strain is CBS 7071.

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

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

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

Basionym: *Myxozyma sirexii* Spaaij et Weber, Syst. Appl. Microbiol. 15: 428, 1992.

The type strain is UOFS Y-2054 = NRRL Y-27626.

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

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

The type strain is CBS 7953.

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

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

The type strain is CBS 7793.

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

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

The type strain is CBS 7058.

Yamazaki et al. (2020) reported 12 anamorphic *Lipomyces* species. Considering the phylogenetic positions of the species, the six species of the twelve should be accommodated to the genus *Kockiozyma* as *Kockiozyma kiyosumica* f.a., *Kockiozyma yaeyamensis* f.a., *Kockiozyma takeshimensis* f.a., *Kockiozyma haiminakana* f.a., *Kockiozyma chibensis* f.a. and *Kockiozyma iriomotensis* f.a. in addition to *Kockiozyma yamanashiensis* f.a. (Yamazaki et al. 2017, Yamada et al.2022), all of which were characteristic of Q-8.

2. CONCLUSION

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

ACKNOWLEDGEMENTS

The present 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.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

Y.Y., H.T.L.V., T.M. and S.T. designed the study. T.M. and H.T.L.V. performed the main experiments. Y.Y. prepared the manuscript. The detailed discussion was made among Y.Y., H.T.L.V., T.M. and S.T.

REFERENCES

- [1] Jindamorakot, S., Yukphan, P. and Yamada, Y. (2012) *Kockiozyma* gen. nov., for *Zygozyma suomiensis*: The phylogeny of the Lipomycetaceous yeasts. *Ann Microbiol* **62**: 1831-1840.
- [2] Kurtzman, C.P. (2003) Phylogenetic circumscription of *Saccharomyces* and *Kluyveromyces* and other members of the Saccharomycetaceae, and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygorulasporea*. *FEMS Yeast Res* **4**: 233-245.
- [3] Kurtzman, C.P., Albertyn, J. and Basehoar-Powers, E. (2007) Multigene phylogenetic analysis of the Lipomycetaceae and the proposed transfer of *Zygozyma* species to *Lipomyces* and *Babjevia anomala* to *Dipodascopsis*. *FEMS Yeast Res* **7**: 1027-1034.
- [4] Kurtzman, C.P. and Smith, M.Th. (2011) *Myxozyma* van der Walt, Weijman et von Arx. In *The Yeasts: A Taxonomic Study*, 5th edition. vol. 2. Kurtzman, C.P., Fell, J.W., Boekhout, T. (eds), London: Elsevier, pp. 1303-1312.
- [5] Lachance, M.A. (2012) In defense of sexual life cycles: The forma asexualis – an informal proposal. *Yeast Newsletter* **61**: 24-25.
- [6] Smith, M.Th., van der Walt, J.P., Yamada, Y. and Batenburg-van der Vegte, W.H. (1989) *Zygozyma suomiensis* sp. nov. (Lipomycetaceae), a new species from Finland. *Antonie van Leeuwenhoek* **56**: 283-288.

International Journal of Novel Research in Life Sciences

Vol. 11, Issue 5, pp: (1-6), Month: September - October 2024, Available at: www.noveltyjournals.com

- [7] Smith, M.Th. and Kurtzman, C.P. (2011) *Lipomyces* Lodder & Kreger-van Rij (1952). In *The Yeasts: A Taxonomic Study*, 5th edition. vol. 2. Kurtzman, C.P., Fell, J.W., Boekhout, T. (eds), London: Elsevier, pp. 545-560.
- [8] van der Walt, J.P., von Arx, J.A., Ferreira, N.P. and Richard, P.D.G. (1987) *Zygozoma* gen. nov., a new genus of the Lipomycetaceae. *Syst Appl Microbiol* **9**: 115-120.
- [9] Vu, H.T.L., Yukphan, P., Tanasupawat, S. and Yamada, Y. (2021) The Revision of the Lipomycetaceae. *27th Ann Meet JSMRS 2021, Gen Lect O-7*: p. 15; on line presentation, p. 1-10.
- [10] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupawat, S., Mikata, K. and Yamada, Y. (2023) The Revision of Schizosacchomycetaceae. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.355>).
- [11] Yamada, Y., Vu, H.T.L., Yukphan, P. and Tanasupawat, S. (2022) The Revision of Lipomycetaceae. *Jxiv* (DOI: <https://doi.org/10.51094/Jxiv.475>).
- [12] Yamazaki A, Yanagiba M, Naganuma T. (2017) Two novel Lipomycetaceous yeast species, *Lipomyces okinawensis* sp. nov. and *Lipomyces yamanashiensis* f.a., sp. nov., isolated from soil in the Okinawa and Yamanashi prefectures, Japan. *Int J Syst Evol Microbiol* **67**: 2941-2946.
- [13] Yamazaki, A., Lorliam, W., Kawasaki, H., Uchino, M. and Suzuki, K. (2020) Fourteen novel lipomycetaceous yeast species isolated from soil in Japan and transfer of *Dipodascopsis anomala* to the genus *Babjevia* based on ascospore production phenotype. *Int J Syst Evol Microbiol* **70**: 1372-1397.
- [14] Vu, H T.L., Yukphan, P., Tanasupawat, S. and Yamada, Y. (2022) The generic circumscription of *Kockiozoma* (Lipomycetaceae) (DOI: <https://doi.org/10.51094/jxiv.221>).

*This work was preliminary reported [14].

APPENDIX

In the yeast systematics, the teleomorph and the anamorph recently have the common generic name. Until the genetic mechanism of sexual reproduction is completely resolved, the so-called one fungus-one name system is very inconvenient and quite obscure in actual situation.