

1 Microbial Systematics (Short Communication)

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3 **The Generic Circumscription of *Mrakia* and Related Taxa (Psychrophilic Yeasts)**

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27 **Keywords:** *Mrakia*; *Mrakia frigida*; *Mrakiella*; *Mrakiella cryoconiti*; *Krasilnikovozyma*  
28 *curviuscula* comb. nov..

29  
30 **Abstract**

31 In the family Mrakiaceae, the type genus *Mrakia* sensu stricto included five teleomorphic  
32 species with the type species, *Mrakia frigida*. In contrast, the anamorphic genus *Mrakiella*  
33 sensu stricto did 11 species with the type species, *Mrakiella cryoconiti*. Between the two  
34 genera, the completely separated clusters were shown in the phylogenetic tree (LSU  
35 D1/D2) derived from the maximum parsimony method. The pair-wise sequence similarity  
36 between *Mrakia frigida* and *Mrakiella cryoconiti* and *Mrakiella aquatica* were 98.2% and  
37 96.8% respectively. The calculated pair-wise sequence similarities were 100-99.3% among  
38 the five *Mrakia* species and were 97.1% between *Mrakiella cryoconiti* and *Mrakiella*  
39 *aquatica*. The teleomorphic genus *Krasilnikovozyma* emend. contained three species with  
40 the type species, *Krasilnikovozyma curviuscula*. Thus, the two teleomorphic genera were  
41 taxonomic homogeneous-natured, and the three were characteristic of Q-8.



45 The genus *Mrakia* Yamada et Komagata was separated from the genus *Leucospori-*  
46 *dium* Fell et al. and introduced with *Mrakia frigida* as the type species based on the  
47 characteristic isoprenoid quinone-8 (Q-8) (Yamada and Komagata 1987). Up to now, five  
48 species have been reported: *Mrakia frigida*, *Mrakia gelida*, *Mrakia psychrophila*, *Mrakia*  
49 *robertii* and *Mrakia bolollopsis*. In contrast, the anamorphic genus *Mrakiella* Margesin et  
50 Fell was proposed with the type species, *Mrakiella cryoconiti* (Margesin and Fell 2008),  
51 and the 11 species have been reported.

52 Later, the genus *Mrakiella* was transferred taxonomically to the teleomorphic genus  
53 *Mrakia* with emendation (Liu et al. 2015). The genus *Mrakia* Yamada et Komagata emend.  
54 Liu et al. formed the monophyletic group along with *Krasilnikovozyma*, *Phaffia*,  
55 *Udeniomyces*, *Itersoniella* and *Tausonia* (Liu et al. 2015).

56 This paper is concerned with the revival of the genus *Mrakiella* on the basis of the  
57 phylogenetic separation within the genus *Mrakia* emend. i.e., the teleomorphic and the  
58 anamorphic groups, the former of which was especially taxonomic homogeneous-natured  
59 again.

60

61 The family Mrakiaceae Liu, Bai, Groenew et Boekhout, the order Cystofilobasidiales  
62 Fell, Roeyman et Boekhout:

63

64 Genus I. *Mrakia* Yamada et Komagata sensu stricto (MB25264)

65

66 One to three-celled metabasidium with basidiospores is shown (Fell 2011), extremely  
67 short phylogenetic branches are produced within the genus in a phylogenetic tree (LSU  
68 D1/D2) and ubiquinone-8

69 The type species is *Mrakia frigida*.

70

71 1. *Mrakia frigida* (Fell, Stanzell, Hunter et Phaff) Yamada et Komagata (1987)  
72 (MB135389)

73 Basionym: *Leucosporidium frigidum* Fell, Stanzell, Hunter et Phaff (1969)

74

75 2. *Mrakia gelida* (Fell, Stanzell, Hunter et Phaff) Yamada et Komagata (1987)  
76 (MB135390)

77 Basionym: *Leucosporidium gelidum* Fell, Stanzell, Hunter et Phaff (1969)

78

79 3. *Mrakia psychrophila* Xin et Zhou (2007) (MB508500)

80

81 4. *Mrakia robertii* Thomas-Hall et Turchetti (2010) (MB514690)

82

83 5. *Mrakia blollopsis* Thomas-Hall et Turchetti (2010) (MB514691)

84

85 In the phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences, the  
86 clusters of the teleomorphic and the anamorphic species were completely separated from  
87 each other (Thomas-Hall et al. 2010). In addition, the phylogenetic branches of the five  
88 *Mrakia* species were abnormally short, when compared with those of the anamorphic  
89 representative species, *Mrakiella cryoconiti* and *Mrakiella aquatica*, suggesting that the  
90 appearance of *Mrakia* species on the earth was relatively new from the view-point of  
91 evolutionary aspect.

92 The pair-wise sequence similarities between the type species, *Mrakia frigida* and the  
93 remaining four *Mrakia* species were extremely high (100 - 99.5%) (Table 1). In contrast,  
94 the sequence similarities between *Mrakia frigida* and *Mrakiella cryoconiti* and *Mrakiella*  
95 *aquatica* were low (98.2 and 96.8%), indicating that the two genera were completely  
96 separated from each other phylogenetically. Among the five *Mrakia* species, the calculated  
97 pair-wise sequence similarities were 100 - 99.3% (data not shown).

98 In the ITS region, the calculated sequence similarities were 98.7 - 97.0% among the  
99 five *Mrakia* species. Between *Mrakia frigida* and *Mrakiella cryoconiti* and *Mrakiella*  
100 *aquatica*, they were 94.9 and 92.3% (Tsuji et al. 2019).

101 To introduce the taxonomic homogeneous-natured genus, the calculated pair-wise  
102 sequence similarities were 98% or more between *Kockiozyma suominensis* and *Myxozyma*  
103 *geophila* (= *Kockiozyma geophila* f.a.; Lipomycetaceae) (Yamada et al. 2022) and between  
104 *Octosporomyces octosporus* (= *Schizosaccharomyces octosporus*) and *Octosporomyces*  
105 *osmophilus* (= *Schizosaccharomyces osmophilus*; Schizosaccharomycetaceae) (Vu et al.  
106 2022a) in the 26S rRNA gene D1/D2 domain sequences. In the 18S rRNA gene sequences,  
107 98% or more sequence similarities were also calculated to accommodate seven *Myxozyma*  
108 species to the teleomorphic genus *Kockiozyma* (Lipomycetaceae) (Vu et al. 2022b).

109 From the data obtained above, the teleomorphic genus *Mrakia* sensu stricto should be  
110 accepted, since the sequence similarities were extremely high (99.5% or more) in the  
111 family Mrakiaceae.

112

113 Genus II. *Mrakiella* Margesin et Fell sensu stricto (MB536881)

114

115 No metabasidium is shown (Fell and Margesin 2011), long phylogenetic branches are  
116 produced within the genus in a phylogenetic tree (LSU D1/D2) and ubiquinone-8

117 The type species is *Mrakiella cryoconiti*

118

119 1. *Mrakiella cryoconiti* Margesin et Fell (2008) (MB537002)

120

121 2. *Mrakiella aquatica* (Jones et Slooff) Margesin et Fell (2008) (MB514705)

122 Basionym: *Candida aquatica* Jones et Slooff (1966)

123

124 3. *Mrakiella niccombsii* Thomas-Hall (2010) (MB514692)

125

- 126 4. *Mrakiella arctica* (Tsuji) comb. nov.  
127 Basionym: *Mrakia arctica* Tsuji, Mycoscience, 59: 57 (2017) (MB821502)  
128 The type strain is JCM32070<sup>T</sup>  
129
- 130 5. *Mrakiella hoshinonis* (Tsuji, Tanabe, Vincent et Uchida) comb. nov.  
131 Basionym: *Mrakia hoshinonis* Tsuji, Tanabe, Vincent et Uchida, Int. J. Syst. Evol.  
132 Microbiol., DOI 10.1099/ijsem.0.003216, 4 (2019) (MB825484)  
133 The type strain is JCM 32575<sup>T</sup>.  
134
- 135 6. *Mrakiella fibulata* (Yurkov et Turchetti) comb. nov.  
136 Basionym: *Mrakia fibulata* Yurkov et Turchetti, Antonie van Leeuwenhoek, 113: 506  
137 (2020) (MB 830398)  
138 The type strain is DSM 103931<sup>T</sup>.  
139
- 140 7. *Mrakiella panshiensis* (Jia et Hui) comb. nov.  
141 Basionym: *Mrakia panshiensis* Jia et Hui, Mycokeys, 74: 82 (2020) (MB834813)  
142 The type strain is NYNU 18562<sup>T</sup>.  
143
- 144 8. *Mrakiella stelviica* (Turchetti et Buzzini) comb. nov.  
145 Basionym: *Mrakia stelviica* Turchetti et Buzzini, Int. J. Syst. Evol. Microbiol. 70:  
146 4707 (2020) (MB835624)  
147 The type strain is DBVPG10734<sup>T</sup>  
148
- 149 9. *Mrakiella montana* (Turchetti et Buzzini) comb. nov.  
150 Basionym: *Mrakia montana* Turchetti et Buzzini, Int. J. Syst. Evol. Microbiol. 70:  
151 4709 (2020) (MB835626)  
152 The type strain is CBS 16462<sup>T</sup>.  
153
- 154 10. *Mrakiella terrae* (Park, Maeng et Sathiyaraj) comb. nov.  
155 Basionym: *Mrakia terrae* Park, Maeng et Sathiyaraj, Mycobiology, 49: 470 (2021)  
156 (MB836844)  
157 The type strain is YP416<sup>T</sup>.  
158
- 159 11. *Mrakiella soli* (Park, Maeng et Sathiyaraj) comb. nov.  
160 Basionym: *Mrakia soli* Park, Maeng et Sathiyaraj, Mycobiology, 49: 472 (2021)  
161 (MB836847)  
162 The type strain is YP421<sup>T</sup>.  
163

164 In contrast to the teleomorphic species of the genus *Mrakia*, the anamorphic  
165 *Mrakiella* species represented relatively long phylogenetic branches, indicating that the  
166 evolutionary stages may be distinct from one another. Within the genus *Mrakiella*, there is

167 none of teleomorphic species, being different from the relationship between *Myxozyma*  
168 and *Kockiozyma* species (Lipomycetaceae) (Yamada et al. 2022).

169 The calculated pair-wise sequence similarities within the genus *Mrakiella* were quite  
170 diverse (97.1 - 98.8%) (Table 1), as observed in the genera *Myxozyma*, *Candida* and  
171 *Cryptococcus*.

172 According to Turchetii et al. (2020), *Mrakia stelviica* and *Mrakia montana* produced  
173 basidiospores from germinating teliospores and to Zhang et al. (2020), *Mrakia pan-*  
174 *schiensis* represented the teleomorphic stage, i.e., teliospores were produced and might  
175 germinate by a bud-like projection.

176

177 Genus III. *Krasilnikovozyrna* Liu et al. emend. (MB812178)

178

179 Non-septate tubular metabasidium with sporidia is shown (Fell 2011), relatively short  
180 phylogenetic branches are produced within the genus in a phylogenetic tree (LSU D1/D2)  
181 and ubiquinone-8

182 The type species is *Krasilnikovozyrna curviuscula*.

183

184 1. *Krasilnikovozyrna curviuscula* (Bav'eva, Lisichkina, Reshetova et Danilevitch)  
185 Yurkov, Kachalkin et Sampaio (2020) (MB829125)

186 Basionym: *Mrakia curviuscula* Bav'eva, Lisichkina, Reshetova et Danilevitch  
187 (2002) (MB529873)

188 The type strain is CBS 9136<sup>T</sup>.

189

190 2. *Krasilnikovozyrna huempii* f.a. (Ramirez et Gonzalez) Liu et al. (2015) (MB812179)

191

192 3. *Krasilnikovozyrna tahquamenonensis* f.a. (Wang et al.) Liu et al. (2015)  
193 (MB813656)

194

195 According to Fell (2011), *Mrakia curviuscula* (= *Krasilnikovozyrna curviuscula*)  
196 produced a non-septate tubular metabasidium with one to two sporidia, which appeared to  
197 differ morphologically from those of *Mrakia frigida* and *Mrakia gelida*.

198 Liu et al. (2015) introduced the genus *Krasilnikovozyrna* as an anamorphic taxon, since  
199 the type species was designated to be *Krasilnikovozyrna huempii* (= *Cryptococcus*  
200 *huempii*). From the view-point of the traditional yeast systematics, it appeared to be  
201 problematic. Namely, the basic characteristics of living things on the earth are based on  
202 their reproduction, especially their sexual reproduction. Therefore, it is general that the  
203 teleomorphic genus has precedence over the anamorphic genus in the yeast systematics,  
204 and the name of the teleomorphic genus *Krasilnikovozyrna* is able to be given to the  
205 anamorphic species (Lachance 2012).

206

207 In the phylogenetic tree based on the concatenated ITS and LSU D1/D2 sequences  
208 derived from the maximum likelihood method (Zhang et al. 2020), the cluster of the genus  
209 *Mrakiella* was divided into two, i.e., one included *Mrakia stelviica* and *Mrakia montana*  
210 and the other did *Mrakia panshiensis*.

211 For the two teleomorphic species, *Mrakia stelviica* and *Mrakia montana* (Turchetii et al.  
212 2020), a new genus will be introduced, and for the one species, *Mrakia panshiensis*  
213 (Zhang et al. 2020), another new genus will be done.

214

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## 223 **Conflicts of interest**

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

225

## 226 **Author contributions**

227 Y.Y., H.T.L.V., P.Y. and S.T. designed the study. H.T.L.V. performed the main experiments.  
228 P.Y. instructed how to make the experiments. Y.Y. prepared the manuscript. The detailed  
229 discussions were made among Y.Y., H.T.L.V., P.Y., and S.T.

230

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264 genus for the Q<sub>8</sub>-equipped, self-sporulating organisms, which produce a unicellular  
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271 teleomorphic-stage of *M. arctica*. *Myckeys* **74**: 75-90.

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Table 1. The pair-wise sequence similarity of D1/D2 in *Mrakia* and *Mrakiella* species\*

Species	1	2	3	4	5	6	7
Sequence similarity (%)	100	100	99.8	99.5	99.5	98.2	96.8
Species	6	7	8	9	10		
Sequence similarity (%)	100	97.1	97.5	97.5	98.8		
Species	7	8	9	10			
Sequence similarity (%)	100	98.2	98.8	97.1			

\*The original data (the number of base substitution) was cited from Tsuji et al. (2019). In this case, the precise length of D1/D2 was not known. It was designated as 560 bases in sequence calculation.

1. *Mrakia frigida* CBS 5270<sup>T</sup>, 2. *Mrakia gelida* CBS 5272<sup>T</sup>, 3. *Mrakia robertii* 8912<sup>T</sup>, 4. *Mrakia blollopsis* CBS 8921<sup>T</sup>, 5. *Mrakia psychrophila* CBS 10829<sup>T</sup>, 6. *Mrakiella cryoconiti* CBS 10834<sup>T</sup>, 7. *Mrakiella aquatica* CBS 5443<sup>T</sup>, 8. *Mrakiella niccombsii* CBS 8917<sup>T</sup>, 9. *Mrakiella hoshinonis* JCM 32575<sup>T</sup>, 10. *Mrakiella arctica* JCM 32070<sup>T</sup>.

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