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

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

Supplementary Abstract

The family Mrakiaceae Liu et al.	
Genus	Species
Mrakia Yamada et Komagata sensu stricto	*Mrakia frigida*[T] (Fell et al.) Yamada et Komagata (1987)
	Mrakia gelida (Fell et al.) Yamada et Komagata (1987)
	Mrakia psychrophila Xin et Zhou (2007)
	Mrakia robertii Thomas-Hall et Turchetti (2010)
	Mrakia blollopsis Thomas-Hall et Turchetti (2010)
Mrakiella Margesin et Fell sensu stricto	*Mrakiella crioconiti*[T] Margesin et Fell (2008)
	Mrakiella aquatica (Jones et Slooff) Margesin et Fell (2008)
	Mrakiella nicombsii Thomas-Hall (2010)
	Mrakiella arctica (Tsuji) comb. nov.
	Mrakiella hoshinonis (Tsuji et al.) comb. nov.
	Mrakiella fibulata (Yuekov et Turchetti) comb. nov.
	Mrakiella panshiensis (Jia et Hui) comb. nov.
	Mrakiella stelviica (Turchetti et Buzzini) comb. nov.
	Mrakiella montana (Turchetti et Buzzini) comb. nov.
	Mrakiella terrae (Park et al.) comb. nov.
	Mrakiella soli (Park et al.) comb. nov.
Krasilnikovozyma Liu et al. emend.	*Krasilnikovozyma curviuscula*[T] (Bavjeva et al.) comb. nov.
	Krasilnikovozyma huempii f.a. (Ramirez et Gonzalez) Liu et al. (2015)
	Krasilnikovozyma tahquamenonensis f.a. (Wang et al.) Liu et al. (2015)

The family Mrakiaceae Liu et al.	
Genus	Species
Mrakia Yamada et Komagata emend. Liu et al.	*Mrakia frigida*[T] (Fell et al.) Yamada et Komagata (1987)
	Mrakia gelida (Fell et al.) Yamada et Komagata (1987)
	Mrakia psychrophila Xin et Zhou (2007)
	Mrakia robertii Thomas-Hall et Turchetti (2010)
	Mrakia blollopsis Thomas-Hall et Turchetti (2010)
	*Mrakia crioconiti*n (Margesin et Fell) Liu et al. (2015)
	Mrakia aquatica (Jones et Slooff) Liu et al. (2015)
	Mrakia nicombsii (Thomas-Hall) Liu et al. (2015)
	Mrakia arctica Tsuji (2017)
	Mrakia hoshinonis Tsuji et al. (2019)
	Mrakia fibulata Yurkov et Turchetti (2019)
	Mrakia panshiensis Jia et Hui (2020)
	Mrakia stelviica Turchetti et Buzzini (2020)
	Mrakia montana Turchetti et Buzzini (2020)
	Mrakia terrae Park et al. (2021)
	Mrakia soli Park et al. (2021)
Krasilnikovozyma Liu et al.	*Krasilnikovozyma huempii*[T] (Ramirez et Gonzalez) Liu et al. (2015)
	Krasilnikovozyma tahquamenonensis (Wang et al.) Liu et al. (2015)

The genus *Mrakia* Yamada et Komagata was separated from the genus *Leucosporidium* Fell et al. and introduced with *Mrakia frigida* as the type species based on the characteristic isoprenoid quinone-8 (Q-8) (Yamada and Komagata 1987). Up to now, five species have been reported: *Mrakia frigida*, *Mrakia gelida*, *Mrakia psychrophile*, *Mrakia robertii* and *Mrakia bololopsis*. In contrast, the anamorphic genus *Mrakiella* Margesin et Fell was proposed with the type species, *Mrakiella cryoconiti* (Margesin and Fell 2008), and the 10 species have been reported.

Later, the genus *Mrakiella* was transferred taxonomically to the teleomorphic genus *Mrakia* with emendation (Liu et al. 2015). The genus *Mrakia* Yamada et Komgata emend. Liu et al. formed the monophyletic group along with *Krasilnikovozym*a, *Phaffia*, *Udeniomyces*, *Itersonilia* and *Tausonia* (Liu et al. 2915).

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

The family Mrakiaceae Liu, Bai, Groenew et Boekhout, the order Cystofilobasidiales Fell, Roeijman et Boekhout:

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

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

The type species is *Mrakia frigida*.

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

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

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

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

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

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

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

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

The pair-wise sequence similarities between the type speccies, *Mrakia frigida* and the remaining four *Mrakia* species were extremely high (100 - 99.5%) (Table 1). In contrast, the sequence similarities between *Mrakia frigida* and *Mrakiella cryyoconiti* and *Mrakiella aquatica* were low (98.2 and 96.8%). Among the five *Mrakia* species, the calculated pair-wise sequence similarities were 100 - 99.3% (data not shown).

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

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

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

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

The type species is *Mrakiella crioconiti*

1. *Mrakiella crioconiti* Margesin et Fell (2008) (MB537002)

2. *Mrakiella aquatica* (Jones et Slooff) Margesin et Fell (2008) (MB514705)
Basionym: *Candida aquatica* Jones et Slooff (1966)

3. *Mrakiella nicombsii* Thomas-Hall (2010) (MB514692)

4. *Mrakiella arctica* (Tsuji, Tanabe, Vincent et Uchida) comb. nov.
Basionym: *Mrakia arctica* Tsuji, Tanabe, Vincent et Uchida (2017) (MB821502)
The type strain is JCM32070^T

5. *Mrakiella hoshinonis* (Tsuji, Tanabe, Vincent et Uchida) comb. nov.
Basionym: *Mrakia hoshinonis* Tsuji, Tanabe, Vincent et Uchida (2019) (MB825484)
The type strain is JCM 32575^{T}.

6. *Mrakiella fibulata* (Yuekov et Turchetti) comb. nov.
Basionym: *Mrakia fibulata* Yuekov et Turchetti (2019) (MB 830398)
The type strain is DSM 103931^{T}.

7. *Mrakiella panshiensis* (Jia et Hui) comb. nov.
Basionym: *Mrakia panshiensis* Jia et Hui (2020) (MB834813)
The type strain is NYNU 18562^{T}.

8. *Mrakiella stelviica* (Turchetti et Buzzini) comb. nov.
Basionym: Mrakia *stelviica* Turchetti et Buzzini (2020) (MB835624)
The type strain is DBVPG10734^{T}

9. *Mrakiella montana* (Turchetti et Buzzini) comb. nov.
Basionym: *Mrakia montana* Turchetti et Buzzini (2020) (MB835626)
The type strain is CBS 16462^{T}.

10. *Mrakiella terrae* (Park, Maeng et Sathiyaraj) comb. nov.
Basionym: *Mrakia* Park, Maeng et Sathiyaraj (2021) (MB836844)
The type strain is YP416^{T}.

11. *Mrakiella soli* (Park, Maeng et Sathiyaraj) comb. nov.
Basionym: *Mrakia soli* Park, Maeng et Sathiyaraj (2021) (MB836847)
The type strain is YP421^{T}.

In contrast to the teleomorphic species of the genus *Mrakia*, the anamorphic *Mrakiella* species represented relatively long phylognetic branches, indicating that the evolutionary stages may be distinct from one another. Within the genus *Mrakiella*, there is none of teleomorphic species, being different from the relationship between *Myxozyma* and *Kockiozyma* species (Lipomycetaceae) (Yamada et al. 2022).

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

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

Genus III. *Krasilnikovozyma* Liu et al. emend. (MB812178)

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

The type species is *Krasilnikovozyma curviuscula.*

1. *Krasilnikovozyma curviuscula* (Bavjeva et al.) comb. nov.

Basionym: *Mraka curviuscula* Bavjeva, Lisichkina, Reshetova et Danilevitch (2002) (MB529873)

The type strain is CBS 9136^{T}.

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

3. *Krasilnikovozyma tahquamenonensis* f.a. (Wang et al.) Liu et al. (2015) (MB813656)

According to Fell (2011), *Mrakia curviuscula* (= *Krasilnikovozyma curviuscula*) produced a non-septate tubular metabasidium with one to two sporidia, which differed morphologically from those of *Mrakia frigida* and *Mrakia gelida*.

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

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

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

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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^{T}, 2. *Mrakia gelida* CBS 5272^{T}, 3. *Mrakia robertii* 8912^{T}, 4. *Mrakia blollopsis* CBS 8921^{T}, 5. *Mrakia psychrophila* CBS 10829^{T}, 6. *Mrakiella cryoconiti* CBS 10834^{T}, 7. *Mrakiella aquatica* CBS 5443^{T}, 8. *Mrakiella niccombsii* CBS 8917^{T}, 9. *Mrakiella hoshinonis* JCM 32575^{T}, 10. *Mrakiella arctica* JCM 32070^{T}.