
LETTERS
TO THE EDITOR

Genus Assignment of Small-Spored Aquatic and Terrestrial Species of the *Metschnikowia* Yeasts

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Recently, interesting data on the differences between certain terrestrial and aquatic *Metschnikowia* Kamienski species in sensitivity to the killer toxins (mycocins) of *Pichia membranifaciens* Hansen have been published [1, 2]. These contain valuable evidence concerning the physiology and ecology of *Metschnikowia*.

Unfortunately, the attempt to use this information for taxonomic purposes, i.e., to divide small-spored aquatic and terrestrial *Metschnikowia* species into different genera, is extremely unsuccessful. The author [1] completely ignores the genetic and molecular data on the species considered in the literature.

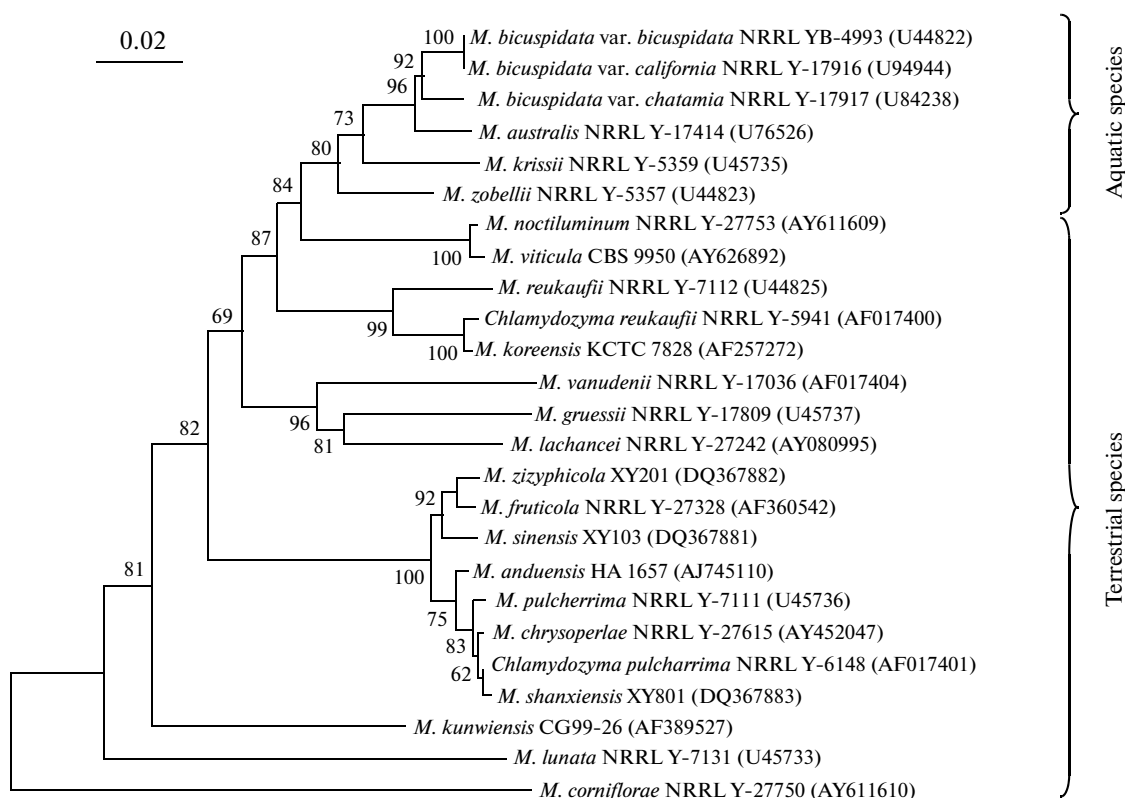
(1) The main conclusion of the author is that “The whole complex of these morphological, biochemical, and molecular biological characteristics suggests that the genus *Metschnikowia* Kamienski with the type species *M. bicuspidata* should be limited to the aquatic species” (p. 156) is incorrect, because the aquatic species *M. bicuspidata* (Metschnikoff) Kamienski var. *bicuspidata*, *M. bicuspidata* var. *californica* Pitt et Miller, *M. bicuspidata* var. *chathamica* Fell et Pitt, *M. australis* (Fell et Hunter) Mendonça-Harler et al., and the terrestrial species *M. pulcherrima* Pitt et Miller, *M. reukaufii* Pitt et Miller, and *M. zygota* Fell et Hunter have the same mating type system that enables them to cross and form diploid interspecies hybrids [3–8]. As far as the “molecular-biological characteristics” are concerned, the author treats the data of phylogenetic analysis very freely [9–11]. The genus *Metschnikowia* is very heterogeneous, and statistical support for many branches is insignificant. Nevertheless, the aquatic species are included in the same clade with *M. reukaufii* and *M. pulcherrima*; moreover, in the same subcluster with *M. reukaufii* [8, 10–19]. The figure shows the phylogenetic tree constructed by us that incorporates all the small-spored *Metschnikowia* species described. Considering the above-mentioned genetic and molecular data, the small-spored aquatic and terrestrial species are undoubtedly assigned to the same genus *Metschnikowia*.

(2) The statement “Based on the high level of DNA homology (about 90%) (Mendonça-Harler et al. 1985), the name of the species *M. kamienski* is now considered to be a synonym of *M. bicuspidata* var. *bicuspidata* (Miller, Phaff 1984); however, it should be noted that no cell hybridization between them was observed (Pitt, Miller 1970)” (p. 156) is incorrect. In fact, the cited authors [6] did not study the cell hybridization of “*M. kamienski*,” because cells with mating types were not revealed in this homothallic yeast. Although the attempt to cross “*M. kamienski*” spores with *M. bicuspidata* var. *bicuspidata*, *M. bicuspidata* var. *californica*, and *M. bicuspidata* var. *chathamica* cells or spores was not successful, similar control intraspecies crossings with the involvement of spores were not carried out. The quoted 90% level of DNA homology (in the absence of the fertility data of the corresponding hybrids) does not allow the creation of the variety *M. bicuspidata* var. *kamienski*.

(3) The absence in the experiments of a dozen new, terrestrial, small-spored species, as well as of a big group [19] of large-spored terrestrial *Metschnikowia* species, is an inexcusable omission of the pretentious article [1]. According to the literature molecular-genetic data, the latter yeasts are actually a taxonomically isolated group. Therefore, at least three ecological groups of *Metschnikowia* should have been mentioned on page 156 of the article [1].

(4) Attention should be drawn to the killer testers assigned by the author [1] to one species *Pichia membranifaciens*. The species identity of the two strains, VKM Y-843 and VKM Y-284, whose mycocins virtually did not act upon the *Metschnikowia* yeasts, could be questioned; their molecular-genetic identification was not earlier carried out [20–23]. The strains VKM Y-843 and VKM Y-284, isolated from fallen-off, decomposed plum fruits (Southern Armenia) and a blend of sparkling wine (USSR) were identified by V.I. Kudryavtsev [24, 25] as *Zygopichia chevalieri* (Guilliermond) Klöcker and *Pichia alcoholophila* Klöcker, respectively. The type culture *Zygopichia chevalieri* CBS 240 (VKM Y-840) is known to be

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Phylogenetic analysis of the 26S rRNA D1/D2 nucleotide sequences of the small-spored *Metschnikowia* yeast species [8]. The bootstrap values >50% are given. The scale corresponds to 20 nucleotide substitutions per 1000 nucleotide positions.

assigned to the species *Pichia manshurica* Saito [21, 23].

(5) In the introduction [1], the difference between the aquatic and terrestrial *Metschnikowia* species in sensitivity to the mycocins synthesized by *P. punctispora* (Mélard) Dekker strains is discussed. In contrast to the aquatic species, the terrestrial species are resistant to them [26]. However, it should be taken into account that, according to the molecular karyotypes of the six strains reidentified by the authors [27], only one (the type culture VKM Y-312) is assigned to the renewed species *P. punctispora*; the rest are identified with the species *P. manshurica* [21]. As for the author's [1] statement that *P. membranifaciens* and *P. punctispora* are taxonomically close (p. 154), to our knowledge, no such molecular-genetic data are available in the literature.

In conclusion, it is necessary to carry on the experimental studies [1] with regard to these critical remarks.

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