

The Subdivision of the Genus *Eremothecium* Borzi emend. Kurtzman (1995)

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Abstract: In the family Saccharomycetaceae, the genus *Eremothecium* Borzi emend. Kurtzman (1995) included the five species that were on the whole characterized by the complex distribution of isoprenoid quinone homologues (Q-5, Q-6, Q-7, Q-8 and Q-9) as well as of needle-shaped ascospore ornamentation within the emended genus. However, the calculated pair-wise sequence similarities among the five species were very low (94.7 - 96.5%, i.e., below 98%) in the 26S rRNA gene D1/D2 domain sequences. The experimental data obtained indicated that the emended genus was not taxonomic homogeneous- but heterogeneous-natured, showing that the five species appropriately accommodated to their own separate five genera as *Eremothecium cymbalariae*, *Nematospora coryli*, *Ashbya gossypii*, *Crebrothecium ashbyi* and *Holleya sinecauda*. In addition, the taxonomic reliability of isoprenoid quinone homologues was discussed in detail.

Keywords: *Crebrothecium ashbyi*; *Nematospora coryli*; *Eremothecium cymbalariae*; *Ashbya gossypii*; *Holleya sinecauda*.

1. SUPPLEMENTARY ABSTRACT

The subfamily Eremothecioideae	
Genus I <i>Eremothecium</i> sensu stricto	<i>Eremothecium cymbalariae</i> Borzi (1888)
Genus II <i>Nematospora</i>	<i>Nematospora coryli</i> Peglion (1897)
Genus III <i>Ashbya</i>	<i>Ashbya gossypii</i> (Ashby et Nowell) Guillielmond (1928)
Genus IV <i>Crebrothecium</i>	<i>Crebrothecium ashbyi</i> Routien (1949)
Genus V <i>Holleya</i>	<i>Holleya sinecauda</i> (Holley) Yamada (1986)
The family Eremotheciaceae	
Genus <i>Eremothecium</i> emend.	<i>Eremothecium cymbalariae</i> Borzi (1888)
	<i>Eremothecium coryli</i> (Peglion) Kurtzman (1995)
	<i>Eremothecium gossypii</i> (Ashby et Nowell) Kurtzman (1995)
	<i>Eremothecium ashbyi</i> (Guillielmond) Kurtzman (1995)
	<i>Eremothecium sinecaudum</i> (Holley) Kurtzman (1995)

The genus *Holleya* was introduced on the basis of the exceptional distribution of ubiquinone-9 (Q-9) as respiratory quinone with the needle-shaped ascospore-forming species, *Holleya sinecauda* (= *Nematospora sinecauda* Holley) (Yamada 1986).

In the phylogenetic analysis based on the partial base sequencing (Yamada and Nagahama 1991), *Holleya sinecauda* (Q-9) represented one base substitution in positions 1685 - 1835, 151 bases of 26S rRNA but considerable base substitutions, i.e., seven bases, in positions 1451 - 1618, 168 bases of 18S rRNA, when compared with *Nematospora coryli* (Q-6 or Q-5, Yamada et al. 1981), demonstrating that the genus *Holleya* was not similar phylogenetically but separable from the genus *Nematospora*. Incidentally, the base substitutions were only four between *Nematospora coryli* and *Saccharomyces cerevisiae* but eight between *Holleya sinecauda* and *Saccharomyces cerevisiae*.

Kurtzman (1995) compared the genera *Ashbya*, *Eremothecium*, *Holleya* and *Nematospora* by use of the 580 base-sequences near the 5'-end of 26S rDNA. The experimental results showed that the five species of the four genera were closely related and the resulting taxa were of little divergence. As a conclusion, all the species concerned were placed in the single genus *Eremothecium* Borzi emend. Kurtzman.

This paper deals with the presently available sequence data and gives the different conclusion that the five species are not closely related phylogenetically but independent from one another to constitute their own taxonomic homogeneous-natured genera respectively (Yamada 2023).

The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences (LSU D1/D2) was constructed by the neighbour-joining method for the five species (Fig. 1). The phylogenetic branches of the five species were somewhat shorter than that (94.0%; Table 1) between *Vanderwaltozyma polyspora* and *Saccharomyces cerevisiae* in the family Saccharomycetaceae. However, the branch lengths were almost the same as that (95.7%) between *Dipodascopsis uninucleata* and *Lipomyces starkeyi* in the family Lipomycetaceae used as reference standards. Among the five species, the calculated pair-wise sequence similarities were 94.7 - 96.5% and of course below 98% (Table 1). By the way, the 98% or more sequence similarities were necessary to constitute the taxonomic homogeneous-natured genera (Yamada et al. 2022; Vu et al. 2022a, b; Yamada 2023; Malimas et al. 2023a).

For example, the calculated sequence similarity between *Holleya sinecauda* and *Eremothecium cymbalariae* was 95.9%, which was almost the same as that between *Dipodascopsis uninucleata* and *Lipomyces starkeyi* (Yamada et al. 2022). In addition, the similarity (96.5%) between *Holleya sinecauda* and *Nematospora coryli* was lower than that (97.5%) between *Kawasakiarxii* and *Lipomyces starkeyi*. The results obtained above represented that the five species within the genus *Eremothecium* emend. Kurtzman (1995) should be unequivocally divided into five genera respectively (Table 1).

In the phylogenetic tree based on the 18S rRNA gene sequences (SSU) derived from the neighbour-joining method for the five species (Fig. 2), the phylogenetic branches of the five species were on the whole shorter, when compared with those of the phylogenetic tree based on LSU D1/D2. The pair-wise sequence similarities were high (98.4 - 99.4%) (Table 2). On the other hand, the sequence similarity between *Vanderwaltozyma polyspora* and *Saccharomyces cerevisiae* utilized as reference standards was also high as well (98.8%). The phylogenetic data obtained above suggested that the base substitutions in SSU were abnormally slow when compared with those of LSU, and such a curious nature appeared to be distributed widely in the members of the family Saccharomycetaceae, since the reference standards also showed very high sequence similarity (98.8%) (Table 2). The two experimental data indicated that the five species appropriately classified in the separate five genera.

Kurtzman and de Hoog (2011) represented the phylogenetic tree of the genus *Eremothecium* emend. based on the concatenated gene sequences from LSU rRNA, SSU rRNA, 5.8S/alignable ITS rRNA, mitochondrial SSU rDNA, cytochrome oxidase II and translation elongation factor-1a. Since there was no information about the total number of used bases, it was temporally designated as 5500. The phylogenetic branch-lengths were measured by use of a ruler, and the pair-wise sequence similarities were calculated among the five species. The calculated results gave almost the same sequence similarity, i.e., 93.8 - 96.7% and of course below 98% (Table 3).

As described above, the five species assigned to the genus *Eremothecium* emend. were adequate to be placed in the five genera respectively. Chemotaxonomically, the five genera were on the whole complicated (Q-5 - Q-9) in contrast to other genera (Q-6) of the family Saccharomycetaceae. In addition, the five genera showed their own isoprenoid quinone composition, as shown below. To distinguish such taxonomic small and unique groups, the subfamily Eremothecioideae was appropriately given.

The subfamily Eremothecioideae subfam. nov. (Saccharomycetaceae)

Cells are globose or cylindrical. Multilateral budding. Pseudohyphae and true hyphae are present. Asci form 8-32 ascospores, which are generally acicular (Kurtzman and de Hoog, 2011). Ubiquinone-5 - ubiquinone-9 are present.

Mycobank number is 848430.

The type genus is *Eremothecium* Borzi.

Genus I *Eremothecium* Borzi MB1883

Eremothecium cymbalariae Borzi (1888) MB235811

Q-7(Q-6)-equipped (Yamada et al. 1987).

Genus II *Nematospora* Peglion MB3441

Nematospora coryli Peglion (1897) MB222583

Synonym: *Eremothecium coryli* (Peglion) Kurtzman (1995)

Q-6-equipped (Q-5 only in the type strain) (Yamada et al. 1977, 1981).

Genus III *Ashbya* Guilielmond MB389

Ashbya gossypii (Ashby et Nowell) Guilielmond (1928) MB266255

Synonym: *Eremothecium gossypii* (Ashby et Nowell) Kurtzman (1995)

Q-6-equipped (Yamada et al. 1987).

Genus IV *Crebrothecium* Routien MB1283

Crebrothecium ashbyi Routien (1949) MB266255

Synonym: *Eremothecium ashbyi* (Guilielmond) Kurtzman (1995)

Q-6(Q-7)-equipped (Yamada et al. 1987).

Genus V *Holleya* Yamada MB25105

Holleya sinecauda (Holley) Yamada (1986) MB131133

Synonym: *Eremothecium sinecaudum* (Holley) Kurtzman (1995)

Q-9(Q-8)-equipped (Yamada 1986).

The genus *Crebrothecium* Routien was synonymous with the genus *Eremothecium* Borzi (Kurtzman and de Hoog 2011). However, the present experimental data has shown that it is phylogenetically deniable, since the calculated sequence similarity between *Crebrothecium* and *Eremothecium* was low but not high [96.5%, Table 1; 95.9%, Table 3; 96.6%, Yamada 2023). Additionally, the isoprenoid quinone homologue distributions were different from each other; Q-6(Q-7) in the genus *Crebrothecium* and Q-7(Q-6) in the genus *Eremothecium* (Yamada et al. 1987).

The five species classified in the subfamily Eremothecioideae were quite unique, differing from other members (Q-6) of the family Saccharomycetaceae. The complex distributions of isoprenoid quinone homologues as well as of needle-shaped ascospore ornamentation in the genus *Eremothecium* emend. was reasonable, since the emended genus was a taxonomic heterogeneous-natured taxon.

2. CONCLUSION

1. Kurtzman (1995, 2003) and Kurtzman and Robnett (1998) should notice the branch lengths (= the so-called evolutionary distances) in the phylogenetic trees in the generic designation or the generic concept. Namely, the longer the branches were, the more taxonomic heterogeneous-natured taxa would be born.

According to the present authors' experiences, the calculated sequence similarities have to be 98% or more (beyond the so-called 98% wall) in the species concerned to constitute a taxonomic homogeneous-natured genus (Yamada et al. 2022; Vu et al. 2022a, b; Yamada 2023; Malimas et al. 2023a).

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The experimental data obtained above indicated that the present authors' generic concept, i.e., the existence of taxonomic homogeneous-natured genera was phylogenetically and taxonomically reasonable.

2. The unreliability of the isoprenoid quinone homologues as a taxonomic tool proposed by Kurtzman (1995, 2003) and Kurtzman and Robnett (1998) was completely resolved in the present study.

Namely, their generic definition in the genus *Eremothecium* Borzi emend. Kurtzman (1995) was not perfect. In fact, the emended genus was subdivided into the five monotypic genera as mentioned above and also previously (Yamada 2023), in which a single isoprenoid quinone homologue was respectively distributed, except for the genus *Nematospora*, in which only the type strain had Q-5 and others did Q-6 (Yamada et al. 1977, 1981). The exceptional distribution of Q-5 was thus only one, and there was not any exception in its distribution among a large number of yeasts as well as among the gram-negative and gram-positive bacteria (Yamada et al. 1969; Collins and Jones 1981).

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Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

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

REFERENCES

- [1] Collins, M.D. & Jones, D. 1981. Distribution of isoprenoid quinone structural type in bacteria and their taxonomic implications. *Mycrobiol. Rev.* June: 316-354.
- [2] Kurtzman, C.P., 1995. Relationships among the genera *Ashbya*, *Eremothecium*, *Holleya* and *Nematospora* determined from rDNA sequence divergence. *J. Industr. Microbiol.* **14**: 523-530.
- [3] Kurtzman, C.P., 2003. Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces* and other members of the Saccharomycetaceae and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygorhizula*. *FEMS Yeast Res.* **4**: 233-245.
- [4] Kurtzman, C.P. & Robnett, C.J., 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* **73**: 331-371.
- [5] Kurtzman, C.P. & de Hoog, G.S., 2011. *Eremothecium* Borzi emend. Kurtzman (1995). In: Kurtzman, C.P., Fell, J.W. and Boekhout, T. (ed). *The Yeasts: A Taxonomic Study*, 5th edition, vol. 2. London: Elsevier, p. 405-412.
- [6] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. & Yamada, Y., 2023a. The reinstatement of the genus *Kloeckeraspora* Niehaus (1932) (apiculate yeasts). *Jxiv* (DOI: <https://org/10.51094/jxiv.317>).
- [7] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupwat, S. & Yamada, Y., 2023b. The subdivision of the genus *Eremothecium* Borzi emend. Kurtzman (1995). *Jxiv* (DOI: <https://org/10.51094/jxiv.285>).
- [8] Vu, H.T.L., Yukphan, P., Tanasupawat, S., Mikata, K. & Yamada, Y., 2022a. The revision of Schizosaccharomycetaceae. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.188>).
- [9] Vu, H.T.L., Yukphan, P., Tanasupawat, S. & Yamada, Y., 2022b. The generic circumscription of *Kockiozyma* (Lipomycetaceae). *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.221>).
- [10] Yamada, Y., 1986. *Holleya* gen. nov., an ascosporegenous yeast genus for the Q9-equipped organism whose ascospores are needle-shaped with smooth surfaces in their anterior half and concentric ridges in their posterior half and without appendages. *J. Gen. Appl. Microbiol.* **32**: 447-449.

International Journal of Novel Research in Life Sciences

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- [11] Yamada, Y. & Nagahama, T., 1991. The molecular phylogeny of the ascomycetous yeast genus *Holleya* Yamada based on the partial sequences of 18S and 26S ribosomal ribonucleic acids. *J. Gen. Appl. Microbiol.* **37**: 199-206.
- [12] Yamada, Y., Aida, K. & Uemura, T. 1969. Enzymatic studies on the oxidation of sugar and sugar alcohol. V. Ubiquinone of acetic acid bacteria and its relation to classification of genera *Gluconobacter* and *Acetobacter*, especially of the so-called intermediate strains. *J. Gen. Appl. Microbiol.* **15**: 181-196.
- [13] Yamada, Y., Arimoto, M. & Kondo, K., 1977. Coenzyme Q system in the classification of some ascosporegenous genera in the families Saccharomycetaceae and Spermophthoraceae. *Antonie van Leeuwenhoek* **43**: 65-71.
- [14] Yamada, Y., Sasaki, J., Hou, C.F., Tahara, Y., Miranda, M. & Phaff, H.J., 1981. A new natural quinone, ubiquinone-5 isolated from an ascosporegenous yeast species, *Nematospora coryli*. *Agric. Biol. Chem.* **45**: 2671-2673.
- [15] Yamada, Y., Banno, I., von Arx, J.A. & van der Walt, J.P., 1987. Taxonomic significance of the coenzyme Q system in yeasts and yeast-like fungi. In: de Hoog, G.S., Smith, M.Th. and Weijman, A.C.M. (ed). *The Expanding Realm of Yeast-like Fungi*. Amsterdam: Elsevier, p. 299-308.
- [16] Yamada, Y., Vu, H.T.L, Yukphan, P. & Tanasupawat, S., 2022. The revision of Lipomycetaceae. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.189>).
- [17] Yamada, Y., 2023. The generic circumscription of *Eremothecium* emend. Kurtzman. *Jxiv* (DOI: <https://doi.org/1051094/jxiv.270>)

Preliminary reports were opened [7 17].

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Table 1. The pair-wise sequence similarity in the 26S rRNA gene D1/D2 domain sequences for the five species.

Species	1	2	3	4	5	6	7
1. <i>Crebrothecium ashbyi</i>	100						
2. <i>Nematospora coryli</i>	94.7	100					
3. <i>Eremothecium cymbalaraie</i>	96.5	96.3	100				
4. <i>Ashbya gossipii</i>	94.7	95.8	96.1	100			
5. <i>Holleya sinecauda</i>	95.2	96.5	95.9	94.9	100		
6. <i>Saccharomyces cerevisiae</i>	86.9	85.4	86.6	86.9	85.4	100	
7. <i>Vanderaltosyza polyspora</i>	87.5	85.9	87.1	86.8	86.4	94.0	100
Species	8	9	10	11			
8. <i>Lipomyces starkaeyi</i>	100						
9. <i>Waltomyces lipofer</i>	95.2	100					
10. <i>Dipodascopsis uninucleata</i>	95.7	94.0	100				
11. <i>Kawasakia arxii</i>	97.5	94.1	95.4	100			

The percent similarity was calculated by use of 573 - 630 bases. For the calculation of sequence similarities, refer to Yamada et al. (2022).

Table 2. The pair-wise sequence similarity in the 18 rRNA gene sequences for the five species.

Species	1	2	3	4	5	6	7
1. <i>Crebrothecium ashbyi</i>	100						
2. <i>Nematospora coryli</i>	98.5	100					
3. <i>Eremothecium cymbalaraie</i>	98.6	99.1	100				
4. <i>Ashbya gossipii</i>	98.8	99.4	99.2	100			
5. <i>Holleya sinecauda</i>	98.3	99.1	98.8	99.1	100		
6. <i>Saccharomyces cerevisiae</i>	96.9	97.2	97.0	97.5	97.1	100	
7. <i>Vanderaltosyza polyspora</i>	96.6	96.9	96.8	97.1	96.7	98.8	100
Species	8	9	10	11			
8. <i>Lipomyces starkaeyi</i>	100						
9. <i>Waltomyces lipofer</i>	95.4	100					
10. <i>Dipodascopsis uninucleata</i>	95.6	97.5	100				
11. <i>Kawasakia arxii</i>	96.0	96.8	97.5	100			

The percent similarity was calculated by use of 1745 - 1790 bases. For the calculation of sequence similarities, refer to Yamada et al. (2022).

Table 3. The pair-wise sequence similarity in the five species.

Species	1	2	3	4	5
1. <i>Ashbya gossypii</i>	100				
2. <i>Crebrothecium ashbyi</i>	96.3	100			
3. <i>Eremothaecium cymbalariae</i>	96.1	95.9	100		
4. <i>Nematospora coryli</i>	95.1	95.4	96.7	100	
5. <i>Holleya sinecauda</i>	93.8	94.1	95.4	96.5	100

The original data was cited from the phylogenetic tree based on the concatenated sequences from LSU, SSU, ITS, mitochondrial SSU, cytochrome oxidase II and elongation factor-1 α (Kurtzman and de Hoog 2011). The percent similarities were calculated by use of the total 5500 bases. For the calculation of sequence similarities, refer to Yamada et al. (2022).

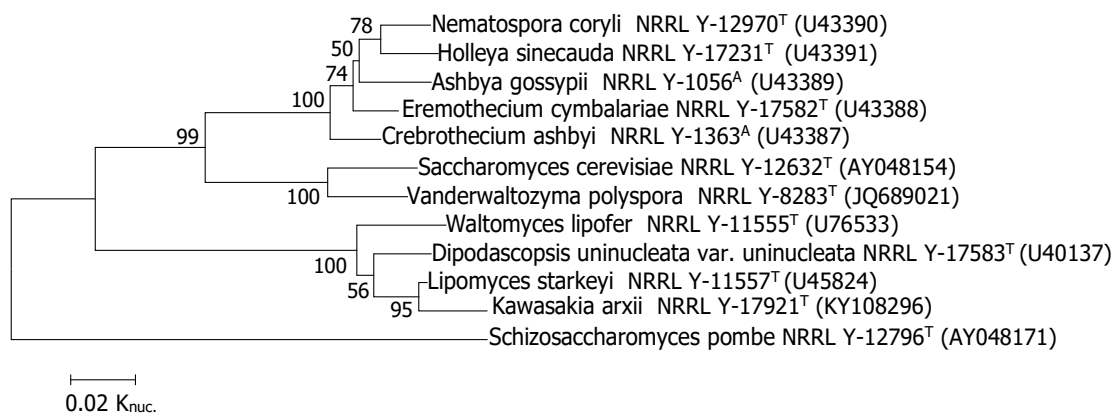


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 559 bases for the five species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicated bootstrap values (%) deduced from 1000 replications. For the construction of the phylogenetic tree, refer to Yamada et al. (2022).

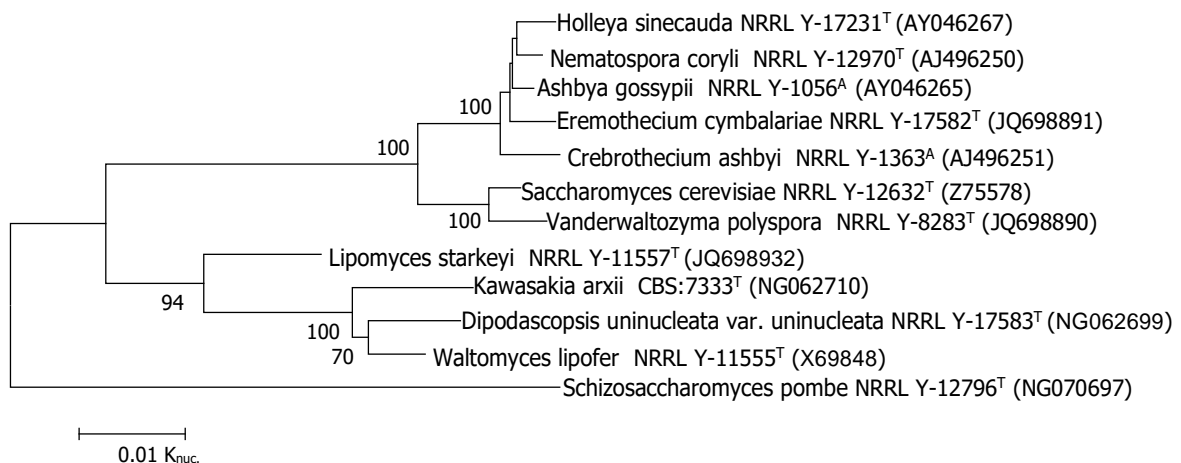


Fig. 2. The phylogenetic tree based on the 18S rRNA gene sequences with 1661 bases for the five species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicated bootstrap values (%) deduced from 1000 replications. For the construction of the phylogenetic tree, refer to Yamada et al. (2022).