Microbial Systematics

The Subdivision of the Genus *Eremothecium* Borzi emend. Kurtzman

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Abstract

In the family Saccharomycetaceae, the genus *Eremothecium* Borzi emend. Kurtzman included the five species that were on the whole characterized by the complex distribution of isoprenoid quinone homologues (Q-5, Q-6, Q-7 and Q-9) as well as of needle-shaped ascospore ornamentation. However, the calculated pair-wise sequence similarities among the five species were very low (94.7 - 96.5%) in the 26S rRNA gene D1/D2 domain sequences and so on. The experimental data obtained indicated that the emended genus was not taxonomic homogeneous- but heterogeneous-natured, indicating that the five

species should be accommodated to their own separate five genera as *Eremothecium cymbalariae*, *Nematospora coryli*, *Ashbya gossypii*, *Crebrothecium ashbyi* and *Holleya sinecauda*.

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 sinecaauda* (= *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 did considerable base substitutions, i.e., seven bases, when compared with *Nematospora coryli* (Q-6 or Q-5, Yamada et al. 1981) in positions 1451 - 1618, 168 bases of 18S rRNA, demonstrating that the genus *Holleya* was not similar phylogenetically but separable from the genus *Nematospor*a. 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 taxa was 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 but phylogenetically independent from one another to constitute their own taxonomic homogeneous-natured genera, as previously reported (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. As shown in Fig. 1, the phylogenetic branches of the five species were somewhat shorter than that (94.0%) between *Vanderwaltozyma polyspora* and *Saccharomyces cerevisiae* in the family Sacharomycetaceae (Table 1). 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 below 98% (Table 1).

For example, the calculated sequence similarity between *Holleya sinecauda* and *Eremothecium cymbalariae* was 95.9%, which was almost the same as that (95.7%) 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 *Kawasakia arxii* and *Lipomyces starkeyi*. The results obtained above represented that the five species within the genus *Eremothecium* emend. 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 short, 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 should be classified in the separate five genera.

The phylogenetic tree based on the concatenated sequences from the 18S rRNA genes and 26S rRNA gene D1/D2 domains was constructed by the neighbour-joining method for the five species. As shown in Fig. 3, the phylogenetic branches of the five species were longer when compared with those of SSU. The calculated pair-wise sequence similarities were 97.6 - 98.4% (Table 3). On the other hand, the sequence similarity between *Vanderwaltozyma polyspora* and *Saccharomyces cerevisiae* utilized as reference standards gave a similar sequence similarity (97.6%). The two experimental data suggested that the five species should be classified in the separate five genera.

Kurtzman and de Hoog (2011) represented the phylogenetic tree of the genus *Eremothecium* emend. Kurtzman based on the concatenated gene sequences from LSU rRNA, SSU rRNA, 5.85/alignable ITS rRNA, mitochondrial SSU rDNA, cytochrome oxidase II and translation elongation factor-1-α. 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 4).

As described above, the five species assigned to the genus *Eremothecium* emend. were adequate to be subdivided into five genera respectively. Chemotaxonomically and morphologically, the five species were very complicated in contrast to other members (Q-6) of the family Saccharomycetaceae (Yamada et al. 1977, 1981, 1987; Kurtzman 1995).

To distinguish the taxonomic small and unique group, the subfamily Eremothecioideae was appropriately given.

The subfamily Eremothecioideae subfam. nov., the family Saccharomycetaceae

Genus I *Eremothecium* Borzi MB1883
Eremothecium cymbalariae Borzi (1888) MB235811
Q-7(Q-6)-equipped.

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).

Genus III *Ashbya* Guillielmond MB389
Ashbya gossypii (Ashby et Nowell) Guillielmond (1928) MB266255
Synonym: *Eremothecium gossypii* (Ashby et Nowell) Kurtzman (1995)
Q-6-equipped.

Genus IV *Crebrothecium* Routien MB1283
Crebrothecium ashbyi Routien (1949) MB266255
Synonym: *Eremothecium ashbyi* (Guillielmond) Kurtzman (1995)
Q-6(Q-7)-equipped.

Genus V *Holleya* Yamada MB25105
Holleya sinecauda (Holley) Yamada (1986) MB131133
Synonym: *Eremothecium sinecaudum* (Holley) Kurtzman (1995)
Q-9(Q-8)-equipped.

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 was low but not high (96.5%, Table 1; 95.9%., Table 4; 96.6%, Yamada 2023).

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

Conclusions

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, namely, the longer the branches were, the more taxonomic heterogeneous-natured genera would be born.

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

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

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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.

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

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

The percent similarity was calculated by use of 573 - 630 bases. All the strains used were the type strains or the authentic strains (See Fig. 1).

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

Species	1	2	3	4	5	6	7
1. *Crebrothecium ashbyi*	100						
2. *Nematospora coryli*	98.6	100					
3. *Eremothecium cymbalaraie*	98.6	99.0	100				
4. *Ashbya gossipii*	98.8	99.4	99.1	100			
5. *Holleya sinecauda*	98.4	99.1	98.7	99.1	100		
6. *Saccharomyces cerevisiae*	96.9	97.2	97.0	97.5	97.1	100	
7. *Vanderaltozyma polyspora*	96.7	96.9	96.8	97.1	96.7	98.8	100
Species	8	9	10	11			
8. *Lipomyces starkaeyi*	100						
9. *Waltomyces lipofer*	95.4	100					
10. *Dipodascopsis uninucleata*	95.6	97.5	100				
11. *Kawasakia arxii*	96.0	96.8	97.5	100			

The percent similarity was calculated by use of 1746 - 1790 bases. All the strains used were the type strains or the authentic strains (See Fig. 2).

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Table 3. The pair-wise sequence similarity in the concatenated sequences from the 18S rRNA genes and 26S rRNA gene D1/D2 domains in the five species.

Species	1	2	3	4	5	6	7
1. *Crebrothecium ashbyi*	100						
2. *Nematospora coryli*	97.6	100					
3. *Eremothecium cymbalaraie*	98.1	98.3	100				
4. *Ashbya gossipii*	97.8	98.4	98.4	100			
5. *Holleya sinecauda*	97.6	98.5	98.0	98.1	100		
6. *Saccharomyces cerevisiae*	94.5	94.3	94.5	94.8	94.2	100	
7. *Vanderaltozyma polyspora*	94.6	94.3	94.6	94.7	94.4	97.6	100
Species	8	9	10	11			
8. *Lipomyces starkaeyi*	100						
9. *Waltomyces lipofer*	95.4	100					
10. *Dipodascopsis uninucleata*	95.6	96.6	100				
11. *Kawasakia arxii*	96.3	96.1	97.0	100			

The percent similarity was calculated by use of 2278 - 2280 bases. All the strains used were the type strains or the authentic strains (See Fig. 3).

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Table 4. The pair-wise sequence similarity in the five species.

Species	1	2	3	4	5
1. *Ashbya gossypii*	100				
2. *Crebrothecium ashbyi*	96.3	100			
3. *Eremothaecium cymbalariae*	96.1	95.9	100		
4. *Nematospora cryli*	95.1	95.4	96.7	100	
5. *Holleya sinecauda*	93.8	94.1	95.4	96.5	100

Data was cited from the phylogenetic tree based on the concatenated sequences from LSU, SSU, ITS, mitochondrial SSU, cytochrome oxidase II and elongation factor-1α (Kurtzman and de Hoog 2011). The percent similarities were calculated by use of the total 5500 bases.

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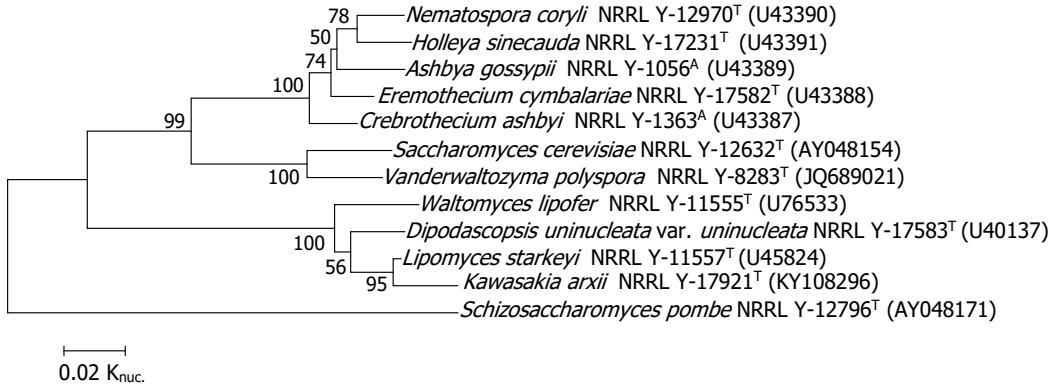

Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 559 bases for the five species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.

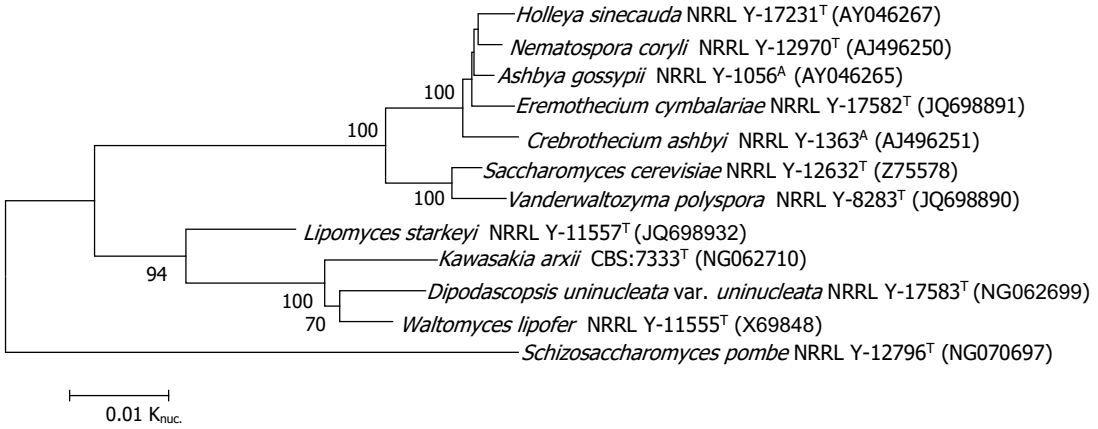

Fig. 2. The phylogenetic tree based on the 18S rRNA gene sequences with 1661 bases for the five species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.

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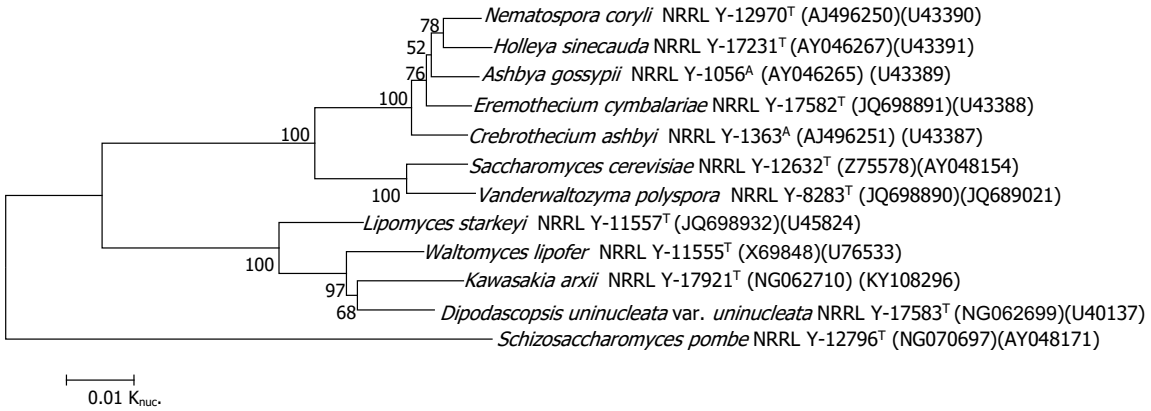

Fig. 3. The phylogenetic tree based on the concatenated sequences from the 18S rRNA genes and 26S rRNA gene D1/D2 domains with 2220 bases for the five species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.