Short Communication

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

Taweesak Malimas,[1] Huong Thi Lan Vu,[2] Pattaraporn Yukphan,[3] Somboon Tanasupawat,[4]
Yuzo Yamada,[3,5,6,]**

[1]Microbial Laboratory Biosafety Level-1, 46 M, 9 Nongphus, Muangsuang, Roi-Et 45220, Thailand

[2]Department of Microbiology, Faculty of Biology and Biotechnology, University of Science, Vietnam National University-HCM City, 227 Nguyen Van Cu Street, Ward 4, District 5, Hochiminh City, Vietnam

[3]Microbial Diversity and Utilization Team, Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Phaholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand

[4]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 254 Phayathai Road, Wangmai, Pathumwan, Bangkok 10330, Thailand

[5]JICA Senior Overseas Volunteer, Japan International Cooperation Agency, Shibuya-ku, Tokyo 151-8559, Japan

[6]Laboratory of Applied Microbiology (Professor Emeritus), Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

*A preliminary report was presented (Yamada, 2023).

**Corresponding author E-mail: ymdy333@kdt.biglobe.ne.jp

ABSTRACT

In the family Saccharomycetaceae, the genus *Eremothecium* Borzi emend. Kurtzman (1995) included the five species, which 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, showing 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*.

Keywords: *Crebrothecium ashbyi*; *Nematospora coryli*; *Eremothecium cymbalariae*; *Ashbya gossypii*; *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 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, 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 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 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 (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 Sacharomycetaceae. 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%; i.e., 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).

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. 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 should be 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, 5.85/alignable ITS rRNA, mitochondrial SSU rDNA, cytochrome oxidase II and translation elongation factor-1α. 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 five genera respectively. Chemotaxonomically and morphologically, the five species were complex in contrast to other members (Q-6) of the family Saccharomycetaceae (Yamada et al. 1977, 1981, 1987; Yamada 1986; Kurtzman 1995). To distinguish the taxonomic small and unique group, 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). MycoBank number is 848430.

The type grnus 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* Guillielmond MB389

Ashbya gossypii (Ashby et Nowell) Guillielmond (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* (Guillielmond) 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 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 diverse and 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 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% 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 phylogenetically and taxonomically reasonable.

ACKNOWLEDGEMENTS

The present authors express their sincere thanks for citing the experimental data of the authors.

Funding information

The present authors received no grant from any funding agency.

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

Kurtzman, C.P., 1995. Relationships among the genera *Ashbya*, *Eremothecium*, *Holleya* and *Nematospora* determined from rDNA sequence divergence. J. Industr. Microbiol. **14**: 523-530.

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 *Zygotorulaspora*. FEMS Yeast Res. **4**: 233-245.

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.

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.

Vu, H.T.L., Yukphan, P., Tanasupawat, S., Mikata, K. & Yamada, Y., 2022a. The revision of Schizosacchromycetaceae. Jxiv (DOI: https://doi.org/10.51094/jxiv.188).

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).

Yamada, Y., 1986. *Holleya* gen. nov., an ascosporogenous 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.

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.

Yamada, Y., Arimoto, M. & Kondo, K., 1977. Coenzyme Q system in the classification of some ascosporogenous genera in the families Saccharomycetaceae and Spermophthoraceae. Antonie van Leeuwebhoek **43**: 65-71.

Yamada, Y., Sasaki, J., Hou, C.F., Tahara, Y., Miranda, M. & Phaff, H.J., 1981. A new natural quinone, ubiquinone-5 isolated from an ascosporogenous yeast species, *Nematospora coryli*. *Agric. Biol. Chem.* **45**: 2671-2673.

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.

Yamada, Y., Vu, H.T.L, Yukphan, P. & Tanasupawat, S., 2022. The revision of Lipomycetaceae. Jxiv (DOI: https://doi.org/10.51094//jxiv.189).

Yamada, Y., 2023. The generic circumscription of *Eremothecium* emend. Kurtzma. Jxiv (DOI: https://doi.org/1051094//jxiv.270)

The genus *Eremothecium* Borzi emend. Kurtzman (1995) (Saccharomycetaceae) の分割

T. Malimas1), H.T.L. Vu2), P. Yukphan3), S. Tanasupawat4), 山田雄三2),5),6)

1)Microbial Laboratory Biosafety Level-1, 46 M, Thailand
2)Vietnam National University-HCM City, Vietnam
3)Thailand Bioresource Research Center (TBRC), Thailand
4)Chulalongkorn University, Thailand
5)JICA Senior Overseas Volunteer, JICA
6)静岡大学農学部 (名誉教授)

The genus *Eremothecium* Borzi emend. Kurtzman (1995) には５種が含まれる．この５種には呼吸鎖キノン系より眺めて，Q-5,Q-6,Q-7,Q-9 と複雑な分布を示し，針状子嚢胞子の ornamentation もまた複雑である．本属内での塩基配列類似度は非常に低く，94.7-96.5%であった．このことは，本属が分類学上極めてheterogeneous な性質を持ち，それぞれが，homogeneousな５個の属 (*Eremothecium cymbalariae*, *Nematospora coryli*, *Ashbya gossypii*, *Crebrothecium ashbyi*, *Holleya sinecauda*) に分割すべきであることが示された．

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).

Table 3. 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.

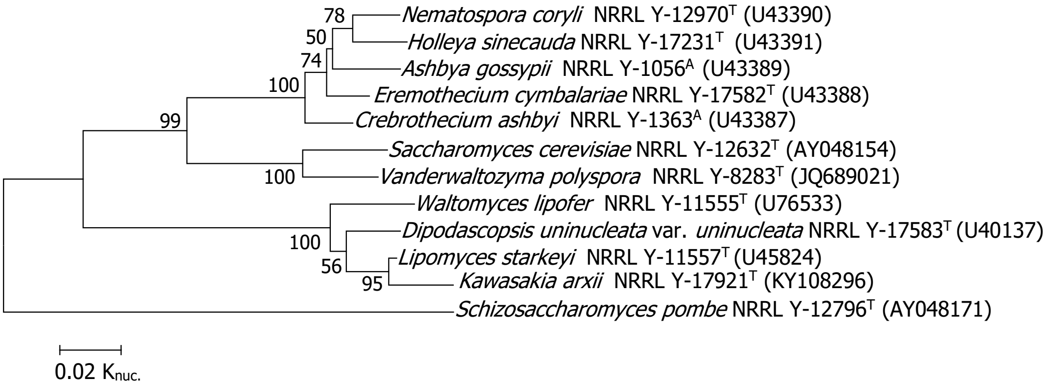

Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 559 bases for the five species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.

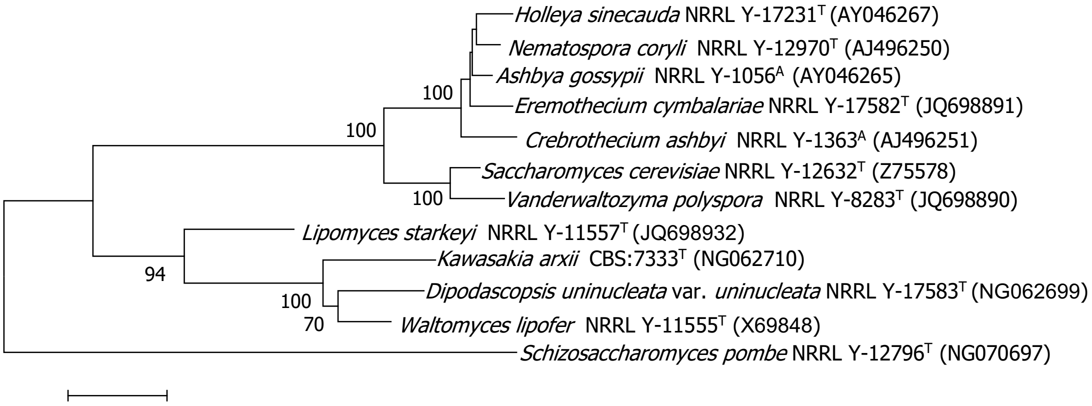

Fig. 2. The phylogenetic tree based on the 18S rRNA gene sequences with 1661 bases for the five species derived from the neighbour-joining method. The numerals at the nodes of the respective branches indicate bootstrap values (%) deduced from 1000 replications.