

# The Generic Circumscription of *Tetrapisispora* Ueda-Nishimura et Mikata (1999) (Saccharomycetaceae): The Proposal of *Nishimuraea* gen. nov.

Taweesak Malimas,<sup>1</sup> Huong Thi Lan Vu,<sup>2</sup> Pattaraporn Yukphan,<sup>3</sup>  
Somboon Tanasupawat,<sup>4</sup> Yuzo Yamada,<sup>3,5,6</sup>

<sup>1</sup>Microbial Laboratory Biosafety Level-1, 46 M, 9 Nongphus, Muangsuang, Roi-Et 45220, Thailand

<sup>2</sup>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

<sup>3</sup>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

<sup>4</sup>Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 254 Phayathai Road, Wangmai, Pathumwan, Bangkok 10330, Thailand

<sup>5</sup>JICA Senior Overseas Volunteer, Japan International Cooperation Agency, Shibuya-ku, Tokyo 151-8559, Japan

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

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**Abstract:** The genus *Tetrapisispora* was introduced with the following four species; *T. arboricola*, *T. iriomotensis*, *T. nanseiensis* and *T. phaffii* (the type species). Later, the three species were additionally accommodated to the genus; *T. blattae*, *T. fleetii* and *T. namnaonensis*. Of all the seven species, *Tetrapisispora blattae* represented an abnormally long branch in the phylogenetic trees based on the 26S rRNA gene D1/D2 domain and the 18S rRNA gene sequences derived from the neighbour-joining method. The calculated pair-wise 26S rRNA gene D1/D2 domain sequence similarities were extremely low (81.2 - 95.7%) within the genus. Except for *T. blattae*, the similarities were 90.3 - 95.7% among them. From the phylogenetic data obtained, *Nishimuraea* gen. nov. was introduced along with *Nishimuraea blattae* comb. nov.

**Keywords:** *Tetrapisispora phaffii*; *Tetrapisispora blattae*; *Tetrapisispora namnaonensis*; *Nishimuraea* gen. nov.; *Nishimuraea blattae* comb. nov.

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## 1. INTRODUCTION

The genus *Tetrapisispora* Ueda-Nishimura et Mikata was introduced for the following four species, *T. arboricola*, *T. iriomotensis*, *T. nanseiensis* and *T. phaffii* (= *Kluyveromyces phaffii*), the type species (Ueda-Nishimura and Mikata 1999). Subsequently, *T. blattae*, (= *Kluyveromyces blattae*), *T. fleetii* and *T. namnaonensis* were included in the genus (Kurtzman 2003; Kurtzman et al. 2004; Sumpradit et al. 2005).

Of the seven species, *T. blattae* was characteristic of an extremely long branch in the phylogenetic trees (Sumpradit et al. 2005). Especially, *T. blattae* was located outside the cluster comprised of the remaining six species and then connected surprisingly to that of *Lachancea thermotolerans* (= *Zygosaccharomyces thermotolerans*) and *Zygosaccharomyces rouxii* in the phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences derived from the neighbour-joining method (Sumpradit et al. 2005).

This paper deals with the presently available sequence data and gives the different conclusion that *T. blattae* constitutes an independent taxon at the generic level since the species has an abnormally long branch and the phylogenetic position of the species is not found inside the cluster of *T. phaffii*, the type species and the remaining five species by phylogenetic analyses (Yamada et al. 2022).

The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences (LSU D1/D2) was constructed by the neighbour-joining method (Fig. 1). *Tetrapisispora phaffii*, the type species was connected to *T. namnaonensis* and *T. fleetii* first and then to the remaining three species, *T. iriomotenensis*, *T. nanseiensis* and *T. arboricola*. The phylogenetic branches of the six species were very long within the genus. The lengths of the species almost corresponded to those of the nine species in the different genera (Fig. 1). *Tetrapisispora blattae*, the remaining one was surprisingly located outside the six species as well as the nine species used as reference standards.

The calculated pair-wise sequence similarities were 81.2 - 95.7% within the genus and 90.3 - 95.7% among the five species except for *T. blattae* (Table 1). The calculated values were neither '98% or more' nor 'beyond the so-called 98% wall' (Yamada et al. 2022; Vu et al. 2022a, b; Malimas et al. 2023a, b, c, d). In contrast, the sequence similarity between *T. blattae* and *T. phaffii* was quite low (82.6%), the value of which was much lower than that (88.8%) between *T. blattae* and *Saccharomyces cerevisiae* and that (87.7%) between *T. blattae* and *Vanderwaltozyma polyspora*. Incidentally, the sequence similarity between *V. polyspora* and *S. cerevisiae* was 93.8%. From the phylogenetic data obtained above, *T. blattae* was quite unique.

In the phylogenetic tree based on the 18S rRNA gene sequences (SSU) (Fig. 2), the branches were on the whole shorter among the species examined, as discussed previously (Malimas et al. 2003a, b, c, d). The five species of the genus *Tetrapisispora* represented quite short phylogenetic branches in contrast to those of LSU D1/D2. However, it is of great interest that there was almost no change in the branch length of *T. blattae*.

The calculated pair-wise 18S rRNA gene sequence similarities were very high (99.3 - 99.8%) among the five species except for *T. blattae* (Table 2). In contrast, the sequence similarity between *T. blattae* and *T. phaffii* was quite low (94.4%), the value of which was the same as that (94.4%) between *T. blattae* and *S. cerevisiae* and that (94.4%) between *T. blattae* and *V. polyspora*. Incidentally, the sequence similarity between *V. polyspora* and *S. cerevisiae* was 98.9%. From the phylogenetic data obtained above, *T. blattae* was quite unique.

*Tetrapisispora blattae* was different phylogenetically from the remaining six species of the genus. Therefore, the species is adequate to be taxonomically distinguished at the generic level.

The family Saccharomycetaceae Winter

*Nishimuraea* Malimas, Vu, Yukphan, Tanasupawat et Yamada gen. nov.

*Nishimuraea* (Ni.shi'mu.ra.e.a. N. L. fem. n. *Nishimuraea*, Nishimura, in honour of Dr. K. Ueda-Nishimura, Institute for Fermentation, Osaka, Japan, who contributed greatly in the yeast systematics)

Growth is butyrous, glossy and white to cream coloured (Lachance 2011). Cells are globose to ellipsoid and occur singly, in pairs or in chains. Pseudohyphae and true hyphae are not formed. Ascus arises from diploid cells, and one to eight or more spherical to ellipsoidal ascospores are produced. The spores are liberated from the ascus soon (Lachance 2011). Glucose and galactose are fermented. Growth occurs on glucose, galactose and glycerol. Q-6 is present (Lachance 2011).

MycoBank number is 848291.

*Nishimuraea blattae* (Henninger et Windisch) Malimas, Vu, Yukphan, Tanasupawat et Yamada comb. nov.

Basionym: *Kluyveromyces blattae* Henninger et Windisch, Arch. Microbiol. 109: 155. 1976.

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The type strain is CBS 8765. Mycobank number is 848292.

Synonym: *Tetrapisispora blattae* (Henninger et Windisch) Kurtzman (2003).

As described above, *Nishimuraea blattae* is extremely unique phylogenetically. The additional species are expected to be isolated and described in the genus *Nishimuraea*.

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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] 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 *Zygorulasporea*. *FEMS Yeast Res.* **4**: 233-245.
- [2] Kurtzman, C.P., Statzell-Tallman, A. & Fell, J. W., 2004. *Tetrapisispora fleetii* sp. nov., a new member of the Saccharomycetaceae. *Stud. Mycol.* **50**: 397-400.
- [3] Lachance, M.-A., 2011. *Tetrapisispora* Ueda-Nishimura & Mikata emend. Kurtzman (2003) In: Kurtzman, C.P., Fell, J.W. and Boekhout, T. (ed). *The Yeasts: A Taxonomic Study*, 5th edition, vol. 2. London: Elsevier, p. 859-866.
- [4] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupawat, S. & Yamada, Y., 2023a. The subdivision of the genus *Eremothecium* Borzi emend. Kurtzman. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.285>).
- [5] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupawat, S. & Yamada, Y., 2023b. The reinstatement of the genus *Kleckerasporea* Niehaus (1932) (apiculate yeasts). *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.317>).
- [6] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupawat, S. & Yamada, Y., 2023c. The generic circumscription of *Kazachstania* Zubkova (1971) (Saccharomycetaceae): The proposal of the new genus *Vanderwaltomyces*. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.328>).
- [7] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupawat, S. & Yamada, Y., 2023d. The subdivision of the genus *Kazachstania* Zubkova (1971) (Saccharomycetaceae). *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.334>).
- [8] Malimas, T., Vu, H.T.L., Yukphan, P., Tanasupawat, S. & Yamada, Y., 2023e. The generic circumscription of *Tetrapisispora* Ueda-Nishimura et Mikata (1999) (Saccharomycetaceae): The proposal of *Nishimuraea* gen. nov. *Jxiv* (DOI: <https://doi.org/10.51094/jxiv.337>).
- [9] Sumpradit, T., Limtong, S., Yongmanitchai, W., Kawasaki, H. & Seki, T., 2005. *Tetrapisispora namnaonensis* sp. nov., a novel ascomycetous yeast species isolated from forest soil of Nam Nao National Park, Thailand. *Int. J. Syst. Evol. Microbiol.* **55**:1735-1738.
- [10] Ueda-Nishimura, K. & Mikata, K., 1999. A new yeast genus, *Tetrapisispora* gen. nov. *Tetrapisispora iriomotensis* sp. nov., *Tetrapisispora nanseiensis* sp. nov. and *Tetrapisispora arboricola* sp. nov., from the Nansei Islands and reclassification of *Kluyveromyces phaffii* (van der Walt) van der Walt as *Tetrapisispora phaffii* comb. nov. *Int. J. Syst. Bacteriol.* **49**: 1915-1924.

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- [11] 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>).
- [12] 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.337>).
- [13] Yamada, Y., Vu, H.T.L., Yukphan, P. & Tanaspawat, S., 2022. The revision of Lipomycetaceae. Jxiv (DOI: <https://doi.org/10.51094/jxiv.189>).

A preliminary report was opened [8].

Corresponding: Yuzo Yamada; [yamada-yuzo@nifty.com](mailto:yamada-yuzo@nifty.com).

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

Species	1	2	3	4	5	6	7	8
1. <i>T. phaffii</i>	100							
2. <i>T. iriomotensis</i>	90.3	100						
3. <i>T. nanseiensis</i>	90.7	91.9	100					
4. <i>T. arboricola</i>	91.2	93.2	95.7	100				
5. <i>T. namnaonensis</i>	91.3	92.4	92.1	93.1	100			
6. <i>N. blattae</i>	82.6	81.6	81.8	82.3	81.2	100		
7. <i>S. cerevisiae</i>	81.9	84.2	83.9	84.8	84.1	88.8	100	
8. <i>V. polyspora</i>	81.2	83.0	82.5	83.0	82.3	87.7	93.8	100
Species	9	10	11	12				
9. <i>L. starkeyi</i>	100							
10. <i>W. lipofer</i>	95.2	100						
11. <i>D. uninucleata</i>	95.7	94.0	100					
12. <i>K. arxii</i>	97.5	94.1	95.4	100				

*T*, *Tetrapisispora*; *N*, *Nishimuraea*; *S*, *Saccharomyces*; *V*, *Vanderwaltozyma*; *L*, *Lipomyces*; *W*, *Waltomyces*; *D*, *Dipoascopsis*; *K*, *Kawasakia*. The total sequences were of 549 - 568 bases. The type strains were used.

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

Species	1	2	3	4	5	6	7	8
1. <i>T. phaffii</i>	100							
2. <i>T. iriomotensis</i>	99.6	100						
3. <i>T. nanseiensis</i>	99.5	99.4	100					
4. <i>T. arboricola</i>	99.5	99.3	99.8	100				
5. <i>T. namnaonensis</i>	99.8	99.5	99.7	99.6	100			
6. <i>N. blattae</i>	94.4	94.4	94.3	94.4	94.4	100		
7. <i>S. cerevisiae</i>	96.0	96.1	96.2	96.1	96.1	94.4	100	
8. <i>V. polyspora</i>	96.0	96.1	96.0	96.0	96.1	94.4	98.9	100
Species	9	10	11	12				
9. <i>L. starkeyi</i>	100							
10. <i>W. lipofer</i>	95.5	100						
11. <i>D. uninucleata</i>	95.7	97.6	100					
12. <i>K. arxii</i>	95.9	96.7	97.5	100				

*T*, *Tetrapisispora*; *N*, *Nishimuraea*; *S*, *Saccharomyces*; *V*, *Vanderwaltozyma*; *L*, *Lipomyces*; *W*, *Waltomyces*; *D*, *Dipoascopsis*; *K*, *Kawasakia*. The total sequences were of 1705 - 1734 bases. The type strains were used.

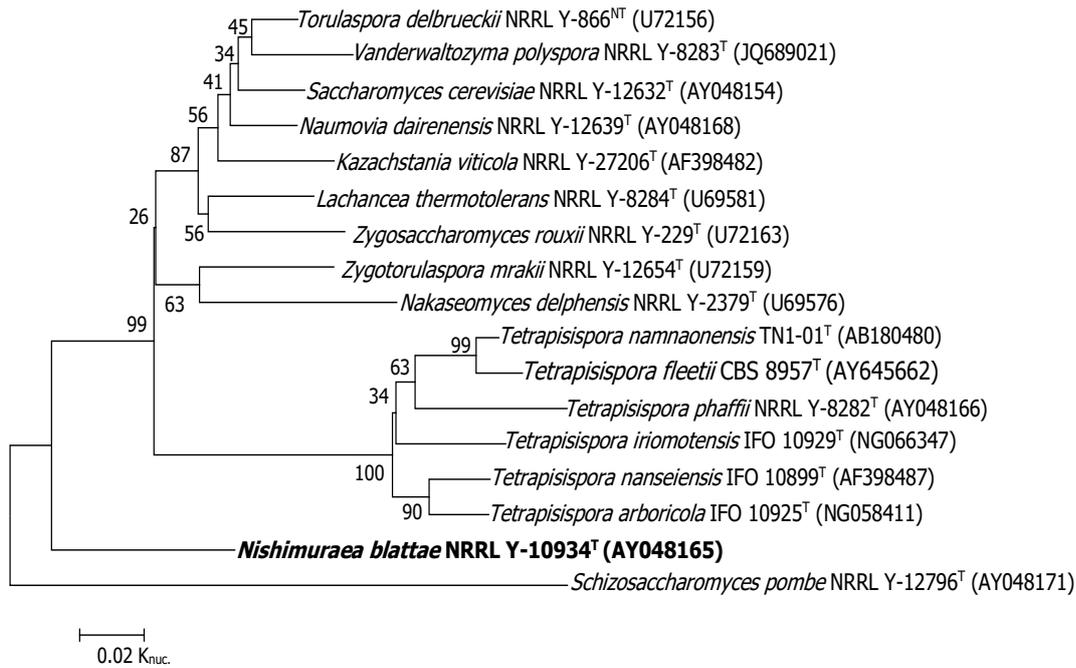


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 524 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

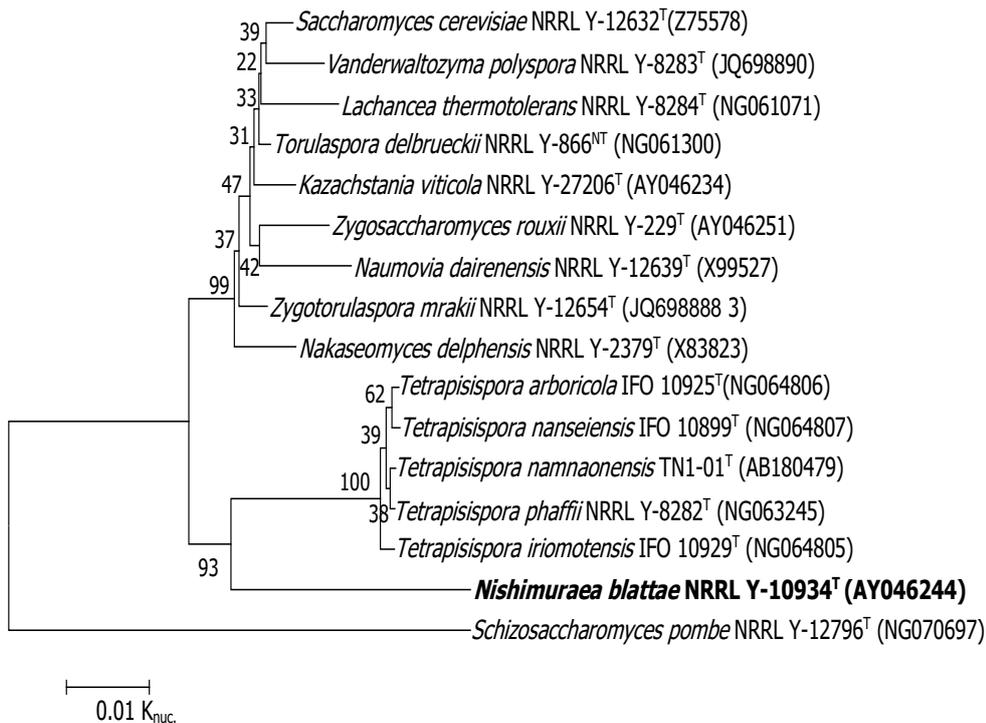


Fig. 2. The phylogenetic tree based on the 18S rRNA gene sequences with 1672 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.