Microbial Systematics (Short Communication)

The Generic Circumscription of *Tetrapisispora* Ueda-Nishinura et Mikata (1999) (Saccharomycetaceae): The Prposal of Nishimuraea gen. nov.

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

Abstract

The genus *Tetrapisispora* was introduced with the following four species; *T. arboricola*, *T. iriomotenensis*, *T. nanseiensis* and *T. phaffii* (the type species). Then, the three species were additionally classified in the genus; *T, blattae*, *T. fleetii* and *T. namnaonensis*. Of the seven species, *Tetrapisispora blattii* 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 the remaining five species. From the data obtained, *Nishimuraea* gen. nov. was proposed along with *Nishimuraea blattii* comb. nov.

The genus *Tetrapisispora* Uedaa-Nishimura et Mikata was introduced for the following four species, *T. arboricola*, *T. iriomotenensis*, *T. nanseiensis* and *T. phaffii* (= *Kluyveromyces phaffii*) (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 characterstic of an extremely long branch in the phylogenetic trees (Lachance 2011; Sumpradit et al. 2005). Especially, *T. blattae* was located outside the cluster comprised of the remaining six species and then connected surprisingly to the cluster *Lachancea thermotolerans* (= *Zygosaccharomyces thermotolerans*)/*Zygosaccharomyces rouxii* in the phylogenetic tree based on the D1/D2 domain of the LSU rDNA 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.

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. iriomotensis*, *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 for 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 98% wall' (Yamada et al. 2022; Vu et al. 2022a, b; Malimas et al. 2023a - 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 to 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 phylogenetic branches were on the whole shorter among the species tested, as shown and discussed previously (Malimas et al. 2003a - 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 in *T. blattae*.

The calculated pair-wise 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 also to 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

Nishimuraea Malimas, Vu, Yukpnan, 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).

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

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

The type strain is CBS 8765.

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

As described above, *Nishimuraea blattii* is extremely unique phylogenetically. The descriptions of additional species are expected.

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.

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

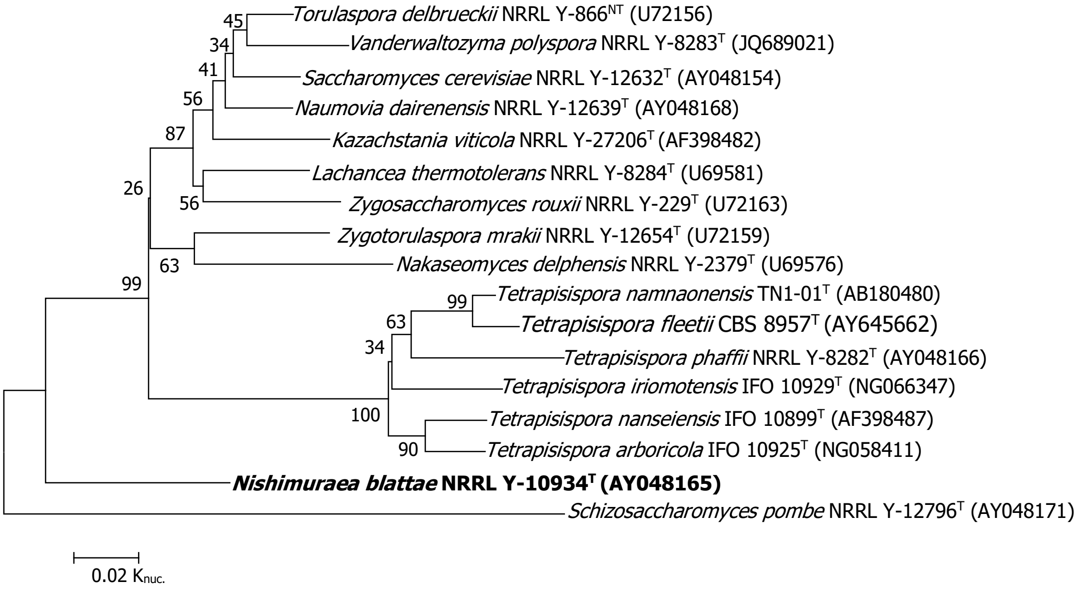
Species	1	2	3	4	5	6	7	8
1. *T. phaffii*	100							
2. *T. iriomotensis*	90.3	100						
3. *T. nanseiensis*	90.7	91.9	100					
4. *T. arboricola*	91.2	93.2	95.7	100				
5. *T. namnaonensis*	91.3	92.4	92.1	93.1	100			
6. *N. blattae*	82.6	81.6	81.8	82.3	81.2	100		
7. *S. cerevisiae*	81.9	84.2	83.9	84.8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

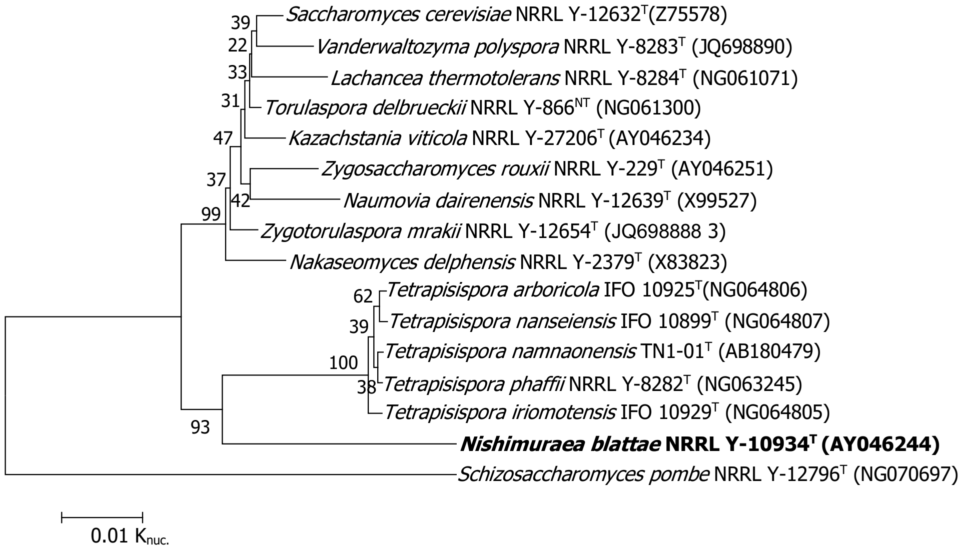
Table 2. The pair-wise sequence similarity in the 18S rRNA gene sequences in *Tetrapisispora* species.

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