

1 Microbial Systematics (Short Communication)

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

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

34  
35 **Abstract**

36 The genus *Tetrapisispora* was introduced with the following four species; *T. arboricola*, *T.*  
37 *iriomotensis*, *T. nanseiensis* and *T. phaffii* (the type species). Then, the three species  
38 were additionally classified in the genus; *T. blattae*, *T. fleetii* and *T. namnaonensis*. Of the  
39 seven species, *Tetrapisispora blattii* represented an abnormally long branch in the  
40 phylogenetic trees based on the 26S rRNA gene D1/D2 domain and the 18S rRNA gene  
41 sequences derived from the neighbour-joining method. The calculated pair-wise 26S

42 rRNA gene D1/D2 domain sequence similarities were extremely low (81.2 - 95.7%)  
43 within the genus. Except for *T. blattae*, the similarities were 90.3 - 95.7% among the  
44 remaining five species. From the data obtained, *Nishimuraea* gen. nov. was proposed  
45 along with *Nishimuraea blattii* comb. nov.

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47 The genus *Tetrapisispora* Ueda-Nishimura et Mikata was introduced for the following  
48 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  
50 (Kurtzman 2003; Kurtzman et al. 2004; Sumpradit et al. 2005).

51  
52 Of the seven species, *T. blattae* was characteristic of an extremely long branch in the  
53 phylogenetic trees (Lachance 2011; Sumpradit et al. 2005). Especially, *T. blattae* was  
54 located outside the cluster comprised of the remaining six species and then connected  
55 surprisingly to the cluster *Lachancea thermotolerans* (= *Zygosaccharomyces thermo-*  
56 *tolerans*)/*Zygosaccharomyces rouxii* in the phylogenetic tree based on the D1/D2 domain  
57 of the LSU rDNA derived from the neighbour-joining method (Sumpradit et al. 2005).

58  
59 This paper deals with the presently available sequence data and gives the different  
60 conclusion that *T. blattae* constitutes an independent taxon at the generic level since the  
61 species has an abnormally long branch and the phylogenetic position of the species is not  
62 found inside the cluster of *T. phaffii*, the type species and the remaining five.

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

72 The calculated pair-wise sequence similarities were 81.2 - 95.7% within the genus and  
73 90.3 - 95.7% among the five species except for *T. blattae* (Table 1). The calculated values  
74 were neither '98% or more' nor 'beyond the 98% wall' (Yamada et al. 2022; Vu et al.  
75 2022a, b; Malimas et al. 2023a - d). In contrast, the sequence similarity between *T. blattae*  
76 and *T. phaffii* was quite low (82.6%), the value of which was much lower than that  
77 (88.8%) between *T. blattae* and *Saccharomyces cerevisiae* and to that (87.7%) between *T.*  
78 *blattae* and *Vanderwaltozyma polyspora*. Incidentally, the sequence similarity between *V.*  
79 *polyspora* and *S. cerevisiae* was 93.8%. From the phylogenetic data obtained above, *T.*  
80 *blattae* was quite unique.

81

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

88 The calculated pair-wise sequence similarities were very high (99.3 - 99.8%) among  
89 the five species except for *T. blattae* (Table 2). In contrast, the sequence similarity between  
90 *T. blattae* and *T. phaffii* was quite low (94.4%), the value of which was the same as that  
91 (94.4%) between *T. blattae* and *S. cerevisiae* and also to that (94.4%) between *T. blattae*  
92 and *V. polyspora*. Incidentally, the sequence similarity between *V. polyspora* and *S.*  
93 *cerevisiae* was 98.9%. From the phylogenetic data obtained above, *T. blattae* was quite  
94 unique.

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

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100 The family Saccharomycetaceae

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

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

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

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

116 Basionym: *Kluyveromyces blattae* Henninger et Windisch, Arch. Microbiol. 109: 155.  
117 1976.

118 The type strain is CBS 8765.

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

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

123

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130

131 Conflict of interest

132 The authors declare that there are no conflicts of interest.

133

134 Author contributions

135 T. M., H.T.L. V., P. Y., S. T. and Y. Y. designed the study. T. M. performed the main  
136 experiments. H.T.L. V. and P. Y. instructed the experiments. Y. Y. prepared the manuscript.  
137 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. <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.

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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.

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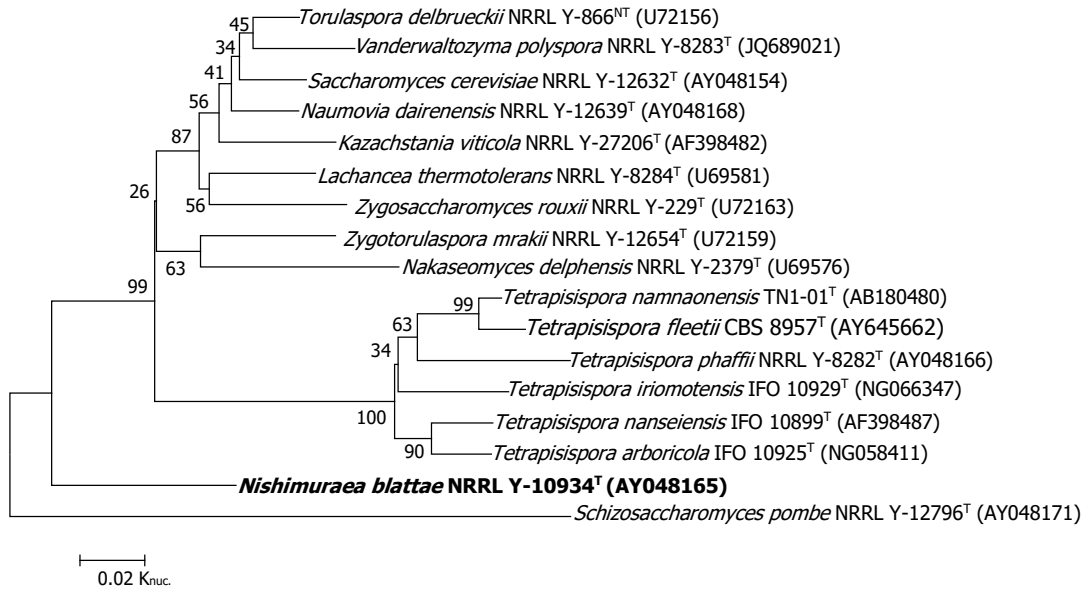


Fig. 1. The phylogenetic tree based on the 26S rRNA gene D1/D2 domain sequences with 524 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

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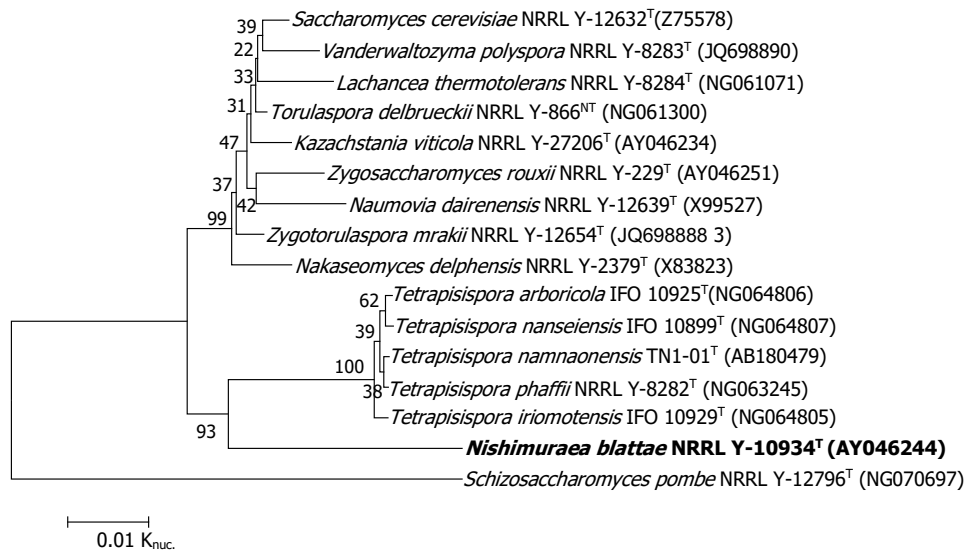


Fig. 2. The phylogenetic tree based on the 18S rRNA gene sequences with 1672 bases derived from the neighbour-joining method. The numerals at the nodes of respective branches indicate bootstrap values (%) deduced from 1000 replications.

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