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Tetrapisispora fleetii sp. nov., a new member of the *Saccharomycetaceae*

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Abstract: A new yeast species, *Tetrapisispora fleetii* (ex-type strain NRRL Y-27350, CBS 8957, ML 4554), is proposed based on an isolate from a food-processing plant in Georgia, U.S.A. Genus assignment and distinction from recognized species is based on phylogenetic analysis of nucleotide sequences from ITS and domains D1/D2 of the large subunit (26S) rDNA.

Taxonomic novelty: *Tetrapisispora fleetii* Kurtzman, Statzell-Tallman & Fell sp. nov.
Key words: molecular systematics, new yeast species, *Tetrapisispora fleetii.*

INTRODUCTION

The genus *Tetrapisispora* Ueda-Nishim. & Mikata was proposed by Ueda-Nishimura & Mikata (1999) to accommodate *Kluyveromyces phaffii* van der Walt and three related new species: *Tetrapisispora arboricola* Ued.-Nishim. & Mikata, *T. iriomotensis* Ued.-Nishim. & Mikata, and *T. nanseiensis* Ued.-Nishim. & Mikata. The four species form a distinct clade within the *Saccharomyces* Meyen ex E.C. Hansen complex of species when analyzed from nucleotide divergence in the small subunit (18S) rDNA. The close relationship of these four species was verified from a multigene analysis of the *Saccharomyces* complex (Kurtzman & Robnett 2003), which also showed that *Kluyveromyces blattae* Henninger & Windisch is a basal member of the *Tetrapisispora* clade. For this reason, *K. blattae* was transferred to the genus *Tetrapisispora* (Kurtzman 2003).

In the present work, we describe a new species of *Tetrapisispora*, which was recognized from sequence analysis of ITS and the D1/D2 domains of large subunit (26S) rDNA. This species was isolated from a food-processing plant in northeastern Georgia and sent to the University of Miami for identification. We propose the name *Tetrapisispora fleetii* for this new ascosporogenous species.

MATERIALS AND METHODS

Phenotypic characterizations followed the procedures listed by Yarrow (1998). D1/D2 and ITS rDNA molecular sequencing employed methods presented by Fell et al. (2000) and Kurtzman & Robnett (1998). The ITS and D1/D2 sequences were analyzed phylogenetically by maximum parsimony and neighbour-joining with the Kimura 2-parameter distance correction using the programmes of PAUP 4.0 (v. 63a) (Swofford 1998). Sequence data for *Tetrapisispora fleetii* (NRRL Y-27350, CBS 8957, ML 4554) were deposited with GenBank: D1/D2 = AY645662; ITS = AY645663. The D1/D2 sequences of the other species included in the analysis were from the studies of Kurtzman & Robnett (1998, 2003).

RESULTS

The proposed new species of *Tetrapisispora* was determined to be novel from phylogenetic analysis of nucleotide sequences from the domains D1/D2 of the large subunit rDNA. The dataset used in the analysis included all known ascomycetous yeast species (Kurtzman & Robnett 1998, and subsequent GenBank entries), and the analysis placed the species in the genus *Tetrapisispora* near *T. phaffii* (Fig. 1). Both maximum parsimony and neighbour-joining analyses gave essentially the same tree. A further analysis compared ITS sequences, but because of the large number of indels in the dataset, about half of the nucleotides in ITS1 and ITS2 had to be removed to achieve a reliable alignment. Both maximum parsimony and neighbour-joining analyses gave ITS trees congruent with the D1/D2 trees.

Tetrapisispora fleetii Kurtzman, Statzell-Tallman & Fell, **sp. nov.** MycoBank MB500099. Figs 2–6.

Etymology: The species is named in honor of Prof. dr Graham Fleet, University of New South Wales, Aus-

tralia, for his extensive and outstanding research with yeasts, food microbiology and biotechnology.

In agaro malti post dies 3 ad 25 ºC, cellulae vegetativae ellipsoideae (1.5–3.5 × 2.8–6 µm) ad elongatae (1.8–3 × 3–7 µm), singulae aut binae. Gemmatio multilateralis. Raro pseudomycelium tenuiter formatur. Asci per conjugationem cellularum distinctarum vel e cellula cum gemma, 2 ascosporas continentes. Ascosporae sphaericae vel ellipsoideae. Species homothallica.
Glucosum et galactosum fermentantur. Sucrosum, maltosum, lactosum, raffinosum, et trehalosum non fermentantur. Glucosum, galactosum, ribitolum (lente) et D-gluconas assimilantur. Non assimilantur L-sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, methanolum, ethanolum, glycerolum, erythritolum, galactitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, salicinum, 2-keto-D-gluconas, 5-keto-D-gluconas, D-glucuronas, saccharatum, DL-acidum lacticum, acidum succinicum, acidum citricum, inositolum, hexadecanum et potassii nitratum. Non crescit in substrato 10 % sal / 5 % glucosi continente. Amylum non formatur. Non crescit in 50 % glucoso addito. Vitamina externa crecentiae necessaria. Temperatura 37 ºC cressit. Species nova a speciebus aliis sequentiis nucleotidicis D1/D2 26S rDNA et ITS rDNA distinguenda.
Typus: NRRL Y-27350 (CBS 8957, ML 4554) designat stirpem typicam, isolatus in Georgia, U.S.A., lyophilus depositus in Collectione Culturarum ARS (NRRL), Peoria, Illinois U.S.A.

Growth on 5 % malt extract agar: After 3 d at 25 ºC, the cells are ellipsoidal (1.5–3.5 × 2.8–6 µm) to short-elongate (1.8–3 × 3–7 µm), and occur singly or in pairs (Fig. 2). Budding is multilateral. Growth is tannish-white, semiglistening and butyrous.

Dalmau plate culture on morphology agar: After 7 d at 25 ºC, true hyphae were not formed under the coverglass, but occasional poorly differentiated strands of pseudohyphae were detected (Fig. 3). Aerobic growth is tannish-white, semiglistening and butyrous in texture. Colonies are low convex with a depressed centre. Margins are smooth to finely lobed.

Ascospore formation. Ascospore formation occurred on YM and yeast morphology agars after 7–10 d at 25 ºC. Ascosporulation was not abundant on these two media but was absent on 5 % ME and McClary's acetate agars. Asci, which become deliquescent at maturity, may be unconjugated or show conjugation between independent cells or between a cell and its bud (Fig. 4). Only two ascospores are formed in each ascus. The ascospores are either spherical (Fig. 5) or short-ellipsoidal (Fig. 6). The species may be homothallic as indicated by the presence of conjugation between a cell and its bud. To further test this

Table 1. Fermentation, assimilation and other growth reactions of *Tetrapisispora fleetii.**

Fermentation:					
Glucose	+	Maltose	–	Trehalose	–
Galactose	+	Lactose	–		
Sucrose	–	Raffinose	–		
Assimilation:					
Glucose	+	L-Arabinose	–	D-Mannitol	–
Galactose	+	D-Arabinose	–	D-Glucitol	–
L-Sorbose	–	D-Ribose	–	α-Methyl-D-glucoside	–
Sucrose	–	L-Rhamnose	–	Salicin	–
Maltose	–	D-Glucosamine	–	D-Gluconate	
		N-Acetyl-D-glucosamine			+
Cellobiose	–	Methanol	–	DL-Lactate	–
Trehalose	–	Ethanol	–	Succinate	–
Lactose	–	Glycerol	–	Citrate	–
Melibiose	–	Erythritol	–	Inositol	–
Raffinose	–	Ribitol	+	Hexadecane	–
Melezitose	–	Galactitol	–	Nitrate	–
Inulin	–			Vitamin-free	–
Soluble starch	–				
D-Xylose	–				

Additional assimilation tests and other growth characteristics:			
2-Keto-D-gluconate	–	DBB	–
5-Keto-D-gluconate	–	Gelatin liquefaction	–
Saccharate	–	Growth at 37°C	–
10 % NaCl + 5% glucose	–	D-Glucuronate	–
Starch formation	–	Urease	–
50 % (w/w) glucose-yeast extract agar	–		

* + = positive, – = negative, w = weak.

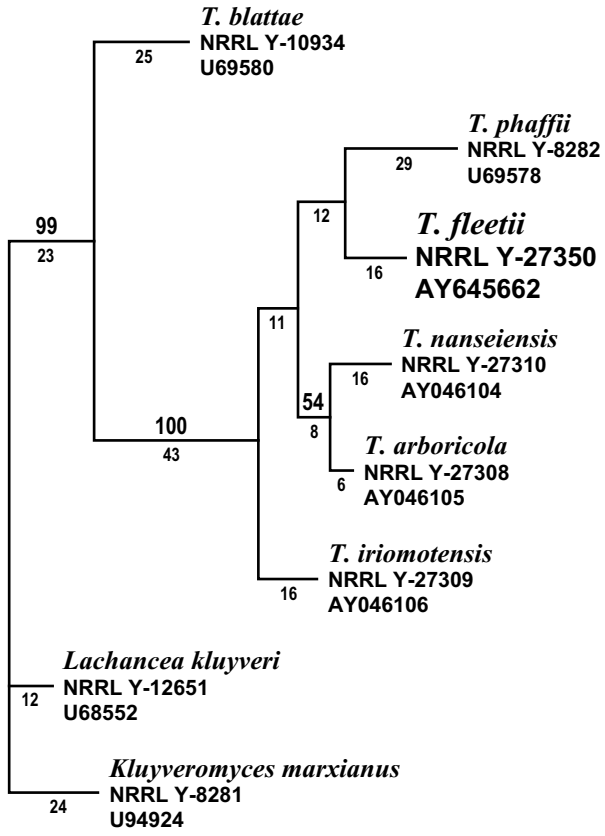


Fig. 1. Phylogenetic tree showing placement of *Tetrapisispora fleetii* among species of the genus *Tetrapisispora* with reference species *Lachancea kluyveri* and *Kluyveromyces marxianus* (outgroup species in the analysis) as represented by the single most parsimonious tree derived from maximum parsimony analysis of nucleotide sequences from 26S rDNA domains D1/D2. Branch lengths, proportional to nucleotide substitutions, are given below the branches and bootstrap values, based on 1000 replicates, are given above the branches. Frequencies under 50 % are not presented. Tree length = 241, consistency index = 0.768, retention index = 0.643, parsimony informative characters = 87. All taxa are represented by ex-type strains.

possibility, 24 single-ascospore isolates were obtained by micromanipulation. Six of the spores germinated and produced colonies that formed two-spored asci. These results suggest that the species is homothallic, but because the asci form only two ascospores, it is not certain that ascosporulation was preceded by meiosis.

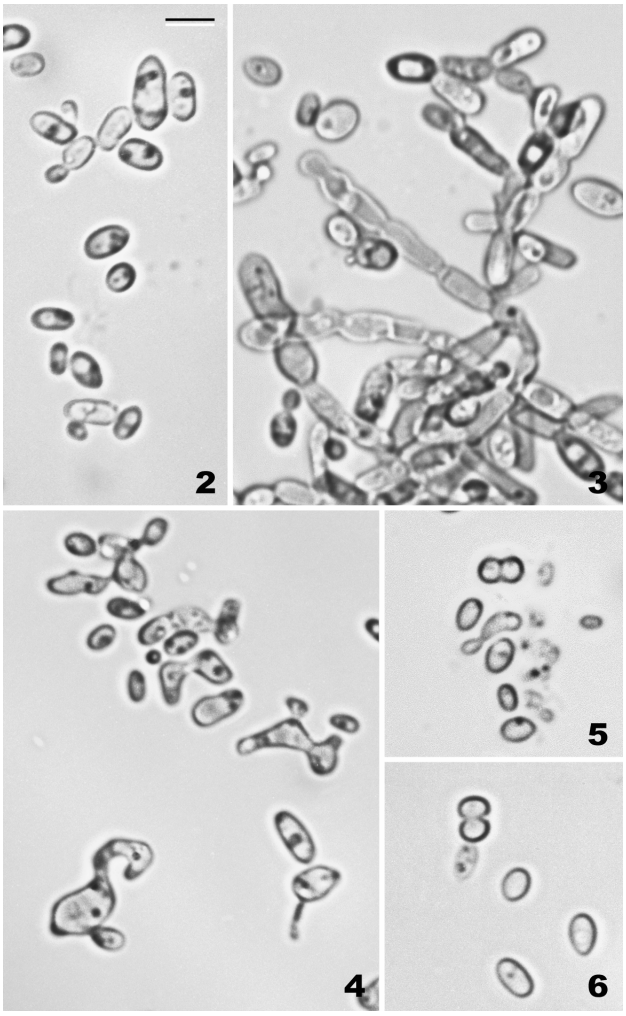
Fermentation, assimilation and other growth characteristics: Table 1.

Type strain: The ex-type strain was isolated in 1999 by an anonymous collector as a random culture swipe from equipment in a food-processing plant located in northeastern Georgia, U.S.A. The strain was deposited at CBS, NCAUR and the University of Miami as CBS 8957, NRRL Y-27350, ML 4554, respectively.

DISCUSSION

The approximately 70 species placed in the Saccharomycetaceae have been assigned to 11 phylogenetically circumscribed genera on the basis of multigene sequence analyses (Kurtzman 2003, Kurtzman & Robnett 2003). Some of these genera, such as *Zygosaccharomyces* B.T.P. Barker and *Torulaspora* Lindner, can be recognized from phenotype, but others, such as *Tetrapisispora* and *Kazachstania* Zubkova, cannot be differentiated from phenotype.

Species of the latter two genera differ from one another in ascospore morphology, as well as in persistence or deliquescence of the asci. Many of the species ferment and assimilate few carbon compounds, further limiting diagnostic characters. For *Tetraspisispora*, individual species can be recognized through a combination of growth reactions and morphology, and these diagnostic characters are given in Table 2.



Figs 2–6. *Tetraspisispora fleetii* NRRL Y-27350. 2. Budding cells, 5 % ME agar after 3 d. 3. Sparingly differentiated pseudohyphae, aerobic growth, yeast morphology agar after 7 d. 4. Conjugating cells, 5 % ME agar after 4 d. 5. Pair of spherical ascospores, YM agar after 10 d. 6. Pair of ovoid ascospores, YM agar after 10 d. Incubation was at 25 ºC for all cultures. Scale bar = 5 µm for all figures.

Table 2. Diagnostic characteristics for species of *Tetrapisispora.**

	Species					
Growth/Morphology	***T. arboricola***	***T. blattae***	***T. fleetii***	***T. iriomotensis***	***T. nanseiensis***	***T. phaffii***
Trehalose	+	–	–	–	–	–
Glycerol	+	v	–	+	+	+
Ribitol	–	–	+	–	–	–
D-Gluconate	v	–	+	+	–	+
Vitamin-free	–	–	–	w	–	–
37 °C	–	–	+	–	–	–
Ascus	per	del	del	del	per	del

* + = positive, – = negative, v = strain variable (+/-), w = weakly positive, per = persistent, del = deliquescent.

Phylogenetically, *T. fleetii* is strongly supported within the genus *Tetrapisispora* (Fig. 1) and shares a branch with *T. phaffii*. However, internal branch support is weak, and branch swapping within the genus can be anticipated with the addition of more species and the inclusion of additional genes in an analysis.

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