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Barnettozyma vustinii A. Yurkov, A.M. Schäfer & Begerow, sp. nov.

Cultura in striis in agaro cum dextroso et peptono et extracto levidinis (GPY) post unum mensem candida ad cremea, obscura, laevis, margine integri. In agaro cum extracto malti (YM) post 3-5 dies ad 25 °C cellulae sphaeroideae ad ovoideae $(2-3 \times 2.5-5 \mu m)$, singulae, binae vel aggregatae, multilateraliter gemmantes. Post hebdomades tres in agaro cum extractis levidinis et malti (YM) vel in agaro cum extracto Solani tuberosi et dextroso (PD) ad 20-25 °C pseudohyphae vel hyphae verae desunt. Status teleomorphicus post cultivationem tres dies in agaro cum dextroso et peptono et extracto levidinis dein post cultivationem tres dies in agaro cum extracto malti et peptono (MYP) dein post cultivationem hebdomadum unum in agaro cum dextroso et peptono et extracto levidinis observatus, homothallicus. Asci conjugatione cellularum gemmarumque oriuntur. Asci continentens 2–4 pileiformes sporas. Glucosum fermentatur. D-Glucosum, L-sorbosum, D-xylosum, L-rhamnosum, cellobiosum, salicinum, glycerolum, ethanolum, D-glucitolum, D-mannitolum, acidum succinicum et acidum DL-lacticum assimilantur at non D-galactosum, D-glucosaminum, L-arabinosum, D-arabinosum, sucrosum, D-maltosum, a,atrehalosum, lactosum, raffinosum, melezitosum, inulinum, amylum solubile, erythritolum, ribitolum, myo-inositolum nec acidum citricum. Assimilatio kalii nitrati, natrii nitrosi, L-lysini et ethylamini. Materia amyloidea non formatur. Ureum non finditur. Vitamina externa ad crescendum necessaria. Temperatura maxima crescentiae: 28 °C, 30 °C (lente).

Etymology. The specific epithet 'vustinii' is in honour of the Russian zymologist Dr Michael M. Vustin for his contributions to the studies of Williopsis species and his suggestions concerning the observation of sexual stages of this novel species.

On Glucose Peptone Yeast extract Agar (GPYA), after 1 mo at 25 °C, the streak culture is white to cream, dull and smooth. The margin is entire. After growth on Yeast extract Malt extract malt (YM) agar for 3-5 d at 25 °C, cells are spheroidal to short ovoidal $(2-3\times2.5-5~\mu m)$, occurring singly, in pairs or in small clusters, and proliferating by multilateral budding. Pseudohyphae and true hyphae are not observed after 3 wk in plate culture, neither on YM nor on potato-dextrose (PDA, Difco) agar at 20-25 °C. The sexual stage was obtained for all studied strains after consequent incubation of the culture on GPYA, Malt extract Yeast extract Peptone (MYP) agar and GPYA (3–7 d on each medium). Ascus formation preceded by conjugation between a parent cell and a bud. Asci contain 2-4 hat-shaped or saturn-shaped ascospores.

Glucose is fermented. Assimilation of carbon compounds: D-glucose, L-sorbose, D-xylose, L-rhamnose, cellobiose, glycerol, ethanol, D-glucitol, D-mannitol, DL-lactate and succinate. No growth occurs on D-galactose, D-glucosamine,

L-arabinose, D-arabinose, sucrose, D-maltose, a,a-trehalose, lactose, raffinose, melezitose, inulin, soluble starch, erythritol, ribitol, myo-inositol, D-glucoronate, citrate, D-glucarate and L-Tartaric acid. Assimilation of nitrogen compounds: potassium nitrate, sodium nitrite, L-lysine and ethylamine. Starch-like compounds are not produced. Urease activity is negative. Growth on vitamin-free medium is negative. Maximal growth temperature: 28 °C (normal growth), 30 °C (weak).

Typus. Germany, Thuringia, National Park Hainich, soil collected at the forest, 51.3558 N, 10.517 E, Apr. 2008, HEW-8-BEB; holotype, culture extype HEW-8-5 = CBS 11554, A. Yurkov, GenBank FN555431 (ITS-region), FN428955 (D1/D2 domain of 26S rRNA gene), FN555430 (16S rRNA gene), MycoBank MB515234.

Notes — Yeasts with saturn-shaped ascospores, formerly accommodated in the genus *Williopsis*, have been observed in soils and rhizospheres in different regions of the world¹. Several attempts have been made to reclassify this genus due to certain heterogeneity in their physiology¹, life cycle², distribution in different soil types³ and ribosomal gene sequence analyses¹.⁴. Recently, on the basis of multigene phylogenetic analysis, the genera *Pichia* and *Williopsis* were re-classified⁴. *Williopsis californica*, *W. pratensis* and several members of the genus *Pichia* were transferred to the novel genus *Barnettozyma*⁴.

During a project aimed to study yeasts in soils under different land use regimes, multiple cultures resembling morphological properties of *Williopsis californica* were isolated by plating soil suspensions on GPYA plates. Detailed physiological and phylogenetic analyses showed that the strains belong to the genus *Barnettozyma*, *Saccharomycetales* (*Ascomycota*, *Saccharomycotina*), but did not correspond to any of the hitherto recognised species.

For phylogenetic tree see MycoBank MB515234.

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Colour illustrations. Plot in the National Park Hainich and soil core sample from which the ex-type strain was isolated; differential interference contrast micrographs of Barnettozyma vustinii: vegetative cells and asci with saturn-shaped ascospores. Scale bars = 10 μ m.

References. ¹Kurtzman CP, Fell JW (eds). 1998. The yeasts. A taxonomic study: 413–419. Elsevier, Amsterdam. ²Naumov GI, Vustin MM, Babjeva IP. 1980. Sexual divergence of yeasts of the genera Williopsis Zender, Zygowilliopsis Kudriavzev and Hansenula H. & P. Sydow. Doklady Akademii Nauk SSSR 255: 468–471. ³Vustin MM, Babjeva IP. 1981. Natural habitats of the yeast of the genera Williopsis Zender, Zygowilliopsis Kudriavzev. Microbiology 50: 1088–1091. ⁴Kurtzman CP, Robnett CJ, Basehoar-Powers E. 2008. Phylogenetic relationships among species of Pichia, Issatchenkia and Williopsis determined from multigene sequence analysis, and the proposal of Barnettozyma gen. nov., Lindnera gen. nov. and Wickerhamomyces gen. nov. FEMS Yeast Research 8: 939–954.