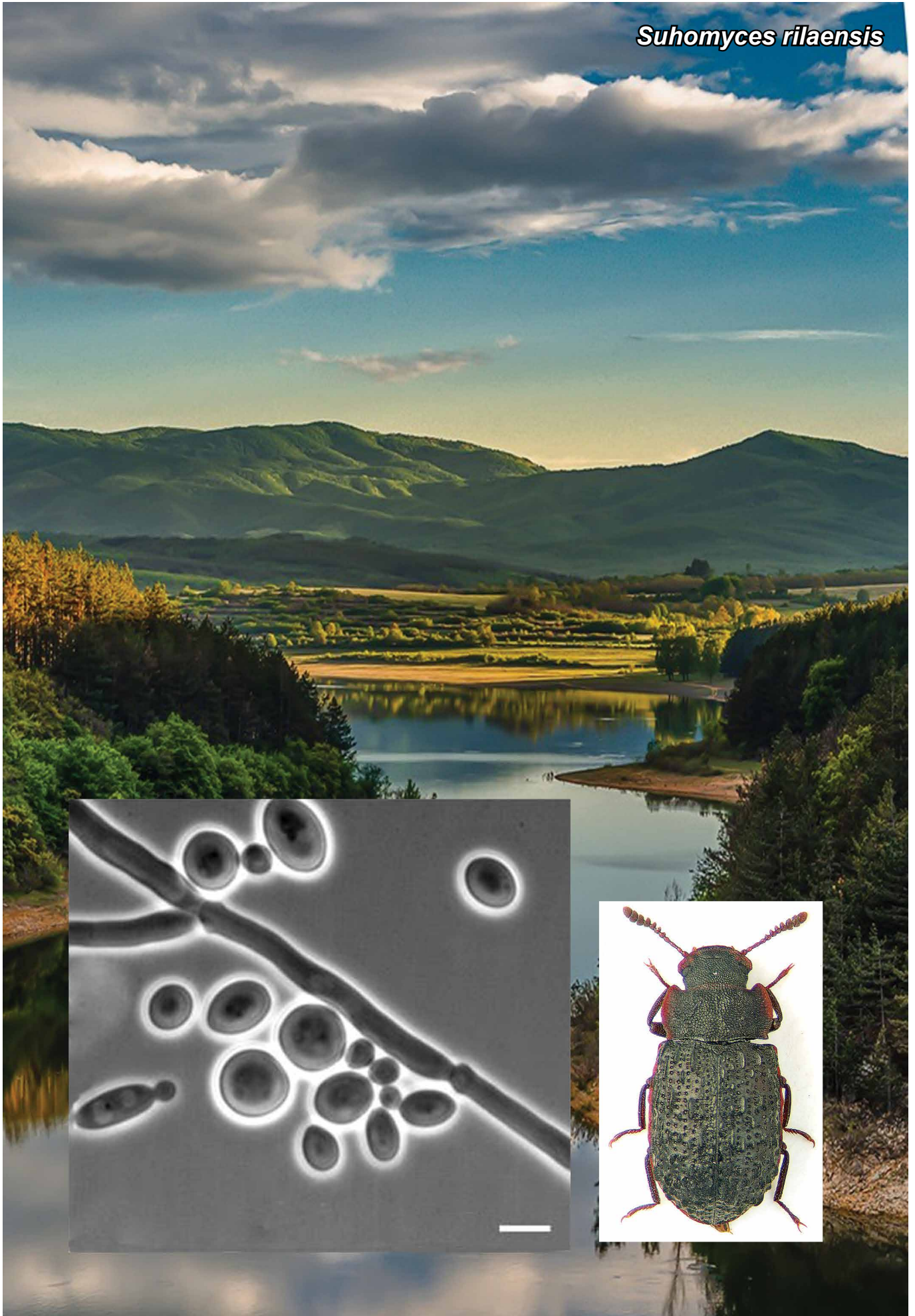


*Suhomyces rilaensis*



Fungal Planet 1174 – 19 December 2020

***Suhomyces rilaensis*** R.A. Dimitrov & Gouliamova, *sp. nov.*

*Etymology.* *Ri-la-en-sis*, referring to the locality *Rila National Park* from which this species was isolated.

*Classification* — Incertae sedis, *Saccharomycetales*, *Saccharomycetes*.

After 7 d at 25 °C in 5 % glucose-yeast extract broth, *cells* are spherical, subglobose, ellipsoidal and oblong, 2–7 × 2–9 µm, occurring singly or in clusters. *Asexual reproduction* is by multilateral budding. After 7 d at 25 °C on 5 % malt extract agar (MEA) the *culture* is cream, butyrous, smooth, glistening, convex and with an entire margin fringed with filaments. Dalmat plate culture after 10 d on yeast morphology agar results in the formation of pseudohyphae. *Aerobic growth* is dimorphic, center is eroded, and the margin is completely eroded, fringed with filaments. *Ascospore production* was not detected either alone or in pairs on yeast extract, malt extract agar (YMA), 5 % MEA, McClary acetate agar, potato dextrose agar (PDA), malt agar (MA2) and diluted V8 agar.

*Fermentation* — Glucose is fermented. Galactose, maltose, sucrose, lactose and raffinose are not fermented.

*Carbon assimilation* — D-glucose, D-galactose, D-glucosamine (+,w), D-ribose, D-xylose, α,α-trehalose, cellobiose (+,w), salicin, arbutin, glycerol, meso-erythritol, ribitol, xylitol (+,w), D-glucitol, D-mannitol, Glucono b-lactone (+,w), 2 keto-D-gluconate, D-gluconate (w,-), succinate, citrate, ethanol and propane 1,2 diol are assimilated. L-Sorbose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, methyl α-glucoside, melibiose, lactose, raffinose, melezitose, inuline, soluble starch, galactitol, myo-inositol, D-gluconate, D-galacturonate, DL-lactate, methanol, butane 2,3 diol, quinic acid, saccharate and galactonic acid are not assimilated.

*Nitrogen assimilation* — Nitrite, ethylamine, L-lysine are assimilated. Nitrate, creatine, creatinine, N-acetyl-glucosamine and imidazole are not assimilated.

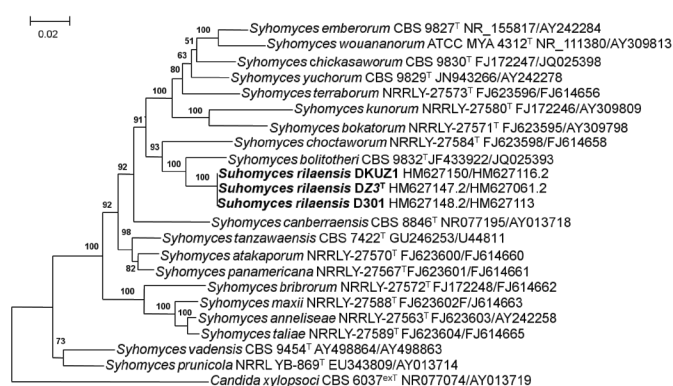
*Other tests* — Growth in medium containing 0.01 and 0.1 % cycloheximide is negative. Growth in medium containing 50 % and 60 % glucose is negative. Starch production, urea and DBB tests are negative. Growth in medium containing 10 % NaCl is positive. Growth in 15 % NaCl is negative. Growth at 25 °C, 30 °C, 35 °C and 37 °C is positive. Growth at 42 °C is negative. Growth without all vitamins test is negative.

*Typus.* BULGARIA, in Podgorie area below Samuilovo village, from the gut of *Bolitophagus interruptus* found on a *Polyporus* sp., *D. Gouliamova* (holotype DZ3 preserved in metabolically inactive state in the yeast collection of the Institute of Microbiology, Sofia, Bulgaria. The ex-type culture is deposited at National bank for microorganisms and cell cultures (NBIMCC), Sofia Bulgaria, and at the CBS-KNAW culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands as NBIMCC 8930 = CBS 12453; D1/D2 LSU and ITS sequences GenBank HM627113 and HM627148, MycoBank MB802451).

*Additional materials examined.* BULGARIA, in vicinity of Rila monastery, strain isolated from the gut of *Bolitophagus reticulatus*, D301 = NBIMCC 8929 = CBS 12443, D1/D2 LSU and ITS sequences GenBank HM627061 and HM627147; DKUZ1 isolated from unidentified grasshopper (*Orthoptera*), NBIMCC 8931 = CBS 12460, D1/D2 LSU and ITS sequences GenBank HM627116 and HM627150.

*Colour illustrations.* A view of reservoir Koprinka in Rose Valey, Bulgaria. Morphology of cells of *Suhomyces rilaensis* DZ3<sup>T</sup> in 5 % glucose broth after 1 wk; *Bolitophagus interruptus* (Photo credits to S. Zayakov and K. Makarov, <https://www.zin.ru/Animalia/Coleoptera/eng/bolintkm.htm>). Scale bar = 5 µm.

*Notes* — *Suhomyces tanzawaensis* was isolated from mosses in Japan (Nakase et al. 1988) and had no known close relatives for a long time. In 2001 six additional species were isolated from mushrooms, plants and insect frass (Kurtzman 2001). Suh et al. (2004) isolated 16 new yeast species belonging to the clade from the gut of mushroom feeding insects (Suh et al. 2004). Kurtzman et al. (2016) proposed a new genus *Suhomyces* to accommodate members of the clade. During a yeast biodiversity survey conducted in Bulgaria in 2008–2011 three conspecific yeast strains (100 % identity in both LSU nrDNA and ITS nrDNA sequences) belonging to the genus *Suhomyces* were isolated from the gut of beetles. Three strains, DZ3, D301 and DKUZ, have the most similar sequences in the database belonging to *S. bolitotheri* (97 % identity in LSU nrDNA sequence) and *S. tanzawaensis* (88 % identity in ITS1+2 nrDNA sequence), thus indicating that the three Bulgarian strains represent a new yeast species. Phylogenetic analysis of combined LSU rDNA and ITS sequences placed the new species and *S. bolitotheri* in a separate subclade (100 % support). Pairwise analysis of the sequences from multiple alignment data showed that the new strains show 73 % similarity (242 identical nt.: 626 subst., 36 gaps) in ITS-LSU nrDNA with *S. bolitotheri* and 75 % similarity (248 identical nt.: 622 subst., 86 gaps) with *S. choktaworum*. The results of the phylogenetic analyses were confirmed by the comparative analysis of physiological profiles of the yeast strains and closest relatives on the phylogenetic tree. The analysis showed that six physiological characteristics distinguish the new strains from *S. bolitotheri*. The new species is not able to ferment galactose, is able to assimilate propane 1,2 diol and is not able to assimilate L-sorbose. Growth in the presence of 16 % NaCl, 50 % and 60 % glucose, and in the presence of 0.01 cycloheximide is negative. Nine characteristics distinguished the new strains from *S. choktaworum*. The new species is not able to ferment galactose, is not able to assimilate L-sorbose, L-arabinose and D-arabinose. Growth in the presence of 16 % NaCl, 50 % and 60 % glucose, and in the presence of 0.01 and 0.1 % of cycloheximide is negative.



Phylogenetic tree obtained by the analysis of combined ITS and LSU nrDNA sequences of *Suhomyces rilaensis* DZ3<sup>T</sup> and related species using a neighbour-joining method (Kimura two-parameter model; MEGA v. 7; 100 bootstrap replicates).