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Two novel species of the genus *Trichosporon* isolated from a cave environment

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Based on morphological and physiological properties and ITS and LSU rDNA sequences, two novel species of the anamorphic basidiomycetous genus *Trichosporon* (Tremellomycetes, Agaricomycotina) are described.

Key words: Agaricomycotina, Tremellomycetes, morphology, DNA sequence data.

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Nováková A., Savická D., Kolařík M. (2015): Dva nové druhy rodu *Trichosporon* izolované z jeskynního prostředí. – Czech Mycol. 67(2): 233–239.

Dva nové druhy anamorfního rodu *Trichosporon* (Tremellomycetes, Agaricomycotina) jsou popsány na základě morfologických a fyziologických vlastností a sekvenčních dat (ITS a LSU rDNA).

SHORT TAXONOMIC REPORT

Material and methods. Strains were cultivated on yeast malt extract agar (YM), malt extract agar (MEA), and Sabouraud glucose agar (SGA) (Atlas 2010) at 25 °C in the dark. For the following examination of growth at various temperature (5, 10, 15, 20, 25, 28, 30, and 37 °C), only cultivation on MEA was used. Estimation of carbon and nitrogen assimilation was carried out in liquid nutrient media according to Kurtzman et al. (2011). Colour determination was performed according to the ISCC-NBS Centroid Color 27 Charts (Kelly 1964). Lactophenol with cotton blue was used for microscopy, a Zeiss Scope A1 light microscope with a ProgRess SpeedXT^{core} 5 camera was used for microphotograph preparation. DNA isolation, PCR of the complete ITS and D1/D2 region of the LSU rDNA sequence, alignment and phylogenetic analysis were performed according to Šutara et al. (2014). The ITS rDNA sequence dataset was created based on sequenced species from the *Trichosporon cutaneum* species group

(Motaung et al. 2013) and the most similar sequences deposited in NCBI GenBank.

Trichosporon aggtelekiense A. Nováková, Savická & M. Kolařík, **sp. nov.** Fig. 1
(MycoBank MB814052)

Holotype. Slovakia, Slovak Karst National Park, Domica Cave, ex cave sediment, 2014, isolated by A. Nováková, dried specimen PRM 934001 derived from living culture CCF 5142 is designated as ex-type.

Gene sequence accession number. EMBL LN866278.

Etymology. The species epithet, "aggtelekiense", is derived from the name of the type locality, the Domica-Baradla cave system, belonging to the Slovak and Aggtelek Karsts, in which abundant occurrence of *Trichosporon* spp. was found in previous studies.

Description. Colonies on MEA 15–17 mm in diam. after 10 days at 25 °C, yellowish white (cream-white), plane and shiny in marginal parts, colony centre umbonate, with mat surface, strongly wrinkled. Colonies on SGA 22–25 mm in diam. after 10 days at 25 °C, yellowish white, plane and shiny in 2–3 mm broad margin, surface delicately wrinkled with cerebriform colony centre (3–5 mm). Septate hyphae with arthroconidia present, arthroconidia non-septate, 11.8 × 3.4 µm or 1–2(3) septate, 13.3–22.4 × 3.4–5.5 µm. Yeast cells ovoid, 3.8–12.6 × 3.3–5.5 µm, single or in short chains.

Distribution and ecology. Cave sediment (Slovakia). Other isolates originate from a bat-inhabited cave in the USA and from a historic tunnel in Portugal (see below). The species seems to prefer underground spaces.

Fermentation. Unknown.

Utilisation in biotechnology, agriculture and food; clinical importance. Unknown.

Notes. It is a member of the *Trichosporon cutaneum* species group, sister to *T. spelunceum* sp. nov. (Fig. 3). The ITS rDNA sequence of strain CCF 5142 is identical with GenBank entries JX675055, KC009041, KC008936 (sediment of bat-inhabited cave, USA; Zhang et al. 2014) and HE646879 (biofilm from black ferromanganese coatings, Portugal; Miller et al. 2012). The strongest similarity was found with *T. spelunceum* CBS 9979^{T} (447/453 bp, 99%) and *T. jirovecii* CBS 6864^{T} (NR073252, 497/508, 98%). The LSU rDNA sequence showed a 99% similarity with *T. oleaginosus* ATCC 20508 (HM802132, 528/536 bp) and a 98% similarity with *T. spelunceum* CBS 9979^{T} (524/535 bp).

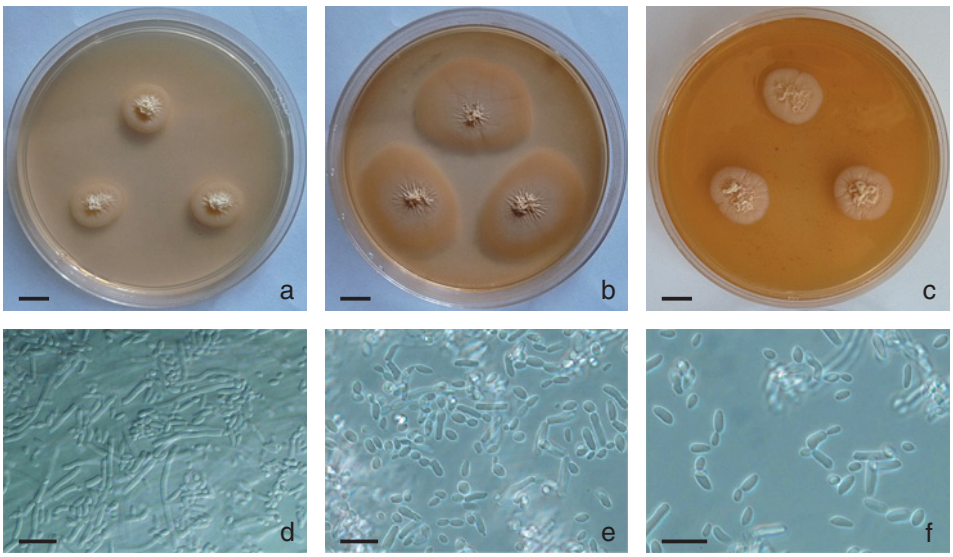

Fig. 1. *Trichosporon aggtelekiense*, colonies of ex-type culture: **a** – on MEA after 10 days, **b** – on MEA after 21 days, **c** – on SGA after 10 days; **d** – arthroconidia and yeast cells; **e**, **f** – yeast cells. Scale bars = 1 cm (colonies) and 10 µm (microphotographs). Photo A. Nováková.

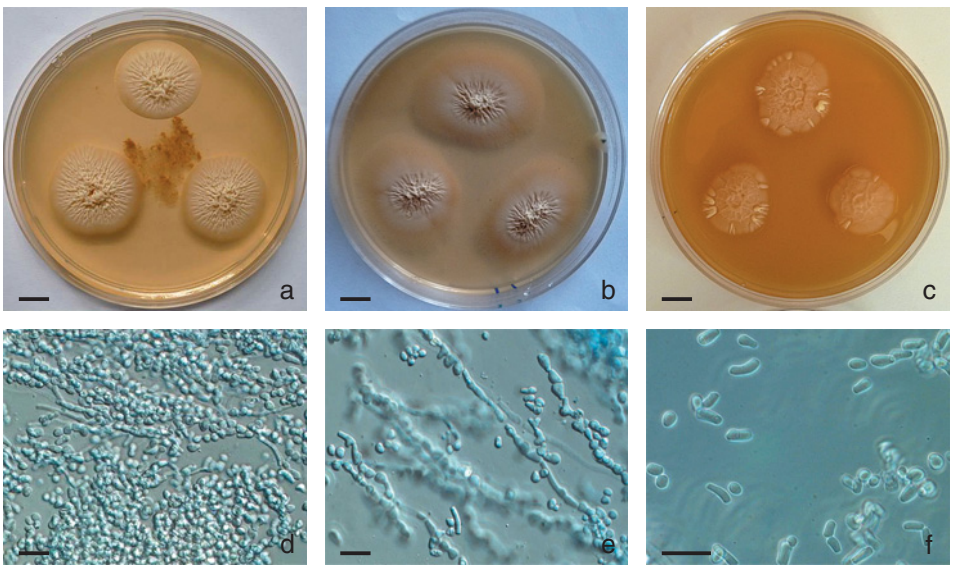

Fig. 2. *Trichosporon spelunceum*, colonies of ex-type culture: **a** – on MEA after 10 days, **b** – on MEA after 21 days, **c** – on SGA after 10 days; **d**, **e** – arthroconidia and yeast cells; **f** – yeast cells. Scale bars = 1 cm (colonies) and 10 µm (microphotographs). Photo A. Nováková.

Trichosporon spelunceum A. Nováková, Savická & M. Kolařík, **sp. nov.** Fig. 2
(MycoBank MB814053)

Synonym: *Trichosporon shinodae* Sugita, Takshima & Kikuchi, nom. inval. (MycoBank MB 493532).

Holotype. Japan, Yamaguchi Prefecture, Akiyoshidai, ex bat guano in bat-inhabited limestone cave, 2003, isolated by Sugita, Takshima & Kikuchi, dried specimen PRM 934001 derived from living culture CBS 9979 = CCF 5143 is designated as ex-type.

Gene sequence accession number. EMBL LN866279.

Etymology. The species epithet "spelunceum" is derived from the origin of this type strain, a cave environment; from the Latin noun spelunca – „cave" in English.

Description. Colonies on MEA 17–20 mm in diam. after 10 days at 25 °C, yellowish white, strongly wrinkled and with delicately umbonate colony centre. Colonies on SGA 20–25 mm in diam. after 10 days at 25 °C, cream to beige, cerebriform in centre with wrinkled pustules in margins. Colonies cerebriform, yellowish white to light yellowish pink on MEA at 28 and 30 °C. Septate hyphae with arthroconidia present, arthroconidia non-septate, 12.9–24.5 × 4.2–5.0 µm or 1–2 septate, 19.6–47.3 × 4.6–5.0 µm. Yeast cells oval to ovoid or clavate, 6.5–9.6(14.8) × 5–6.5 µm, single or in short chains.

Distribution and ecology. Bat guano in bat-inhabited limestone caves (Akiyoshi-do and Senbutsu-shonyudo, Japan). Isolated also from aerobic granular biomass from sewage sludge in Croatia (see below).

Fermentation. Unknown.

Utilisation in biotechnology, agriculture and food; clinical importance. Unknown.

Notes. It is a member of the *Trichosporon cutaneum* species group, sister to *T. aggtelekiense* sp. nov. (Fig. 3). Sugita et al. (2005) reported this species as *Trichosporon* sp. 2 from two limestone caves in Japan. Later they registered strain CBS 9979 in the MycoBank database (MB493532) as the type of *Trichosporon shinodae* Sugita, Takshima & Kikuchi but without a valid description. The ITS rDNA sequence of strain CBS 9979 is identical with *Trichosporon* sp. UASWS 0950 (GenBank accession no. KM251238), which was isolated from a sewage sludge in Croatia (Crovacore et al. unpublished). It differs in 2 bp out of 436 bp from strain JCM 12591 (AB180201), which was deposited as *Trichosporon shinodae* by Sugita et al. (2005). It has the strongest similarity with *T. aggtelekiense* CCF 5142^{T} (447/453 bp, 99%) and *T. cutaneum* CBS 2466^{T} (AF444325, 473/485 bp, 98%). The LSU rDNA sequence shows a 1 bp difference from *T. shinodae* JCM 12591 (AB180201, 650 bp), a 98% similarity with *T. aggtelekiense* CCF 5142 (524/535 bp) and a 97% similarity with *T. jirovecii* ATCC 34499 (HM802131, 535/551 bp).

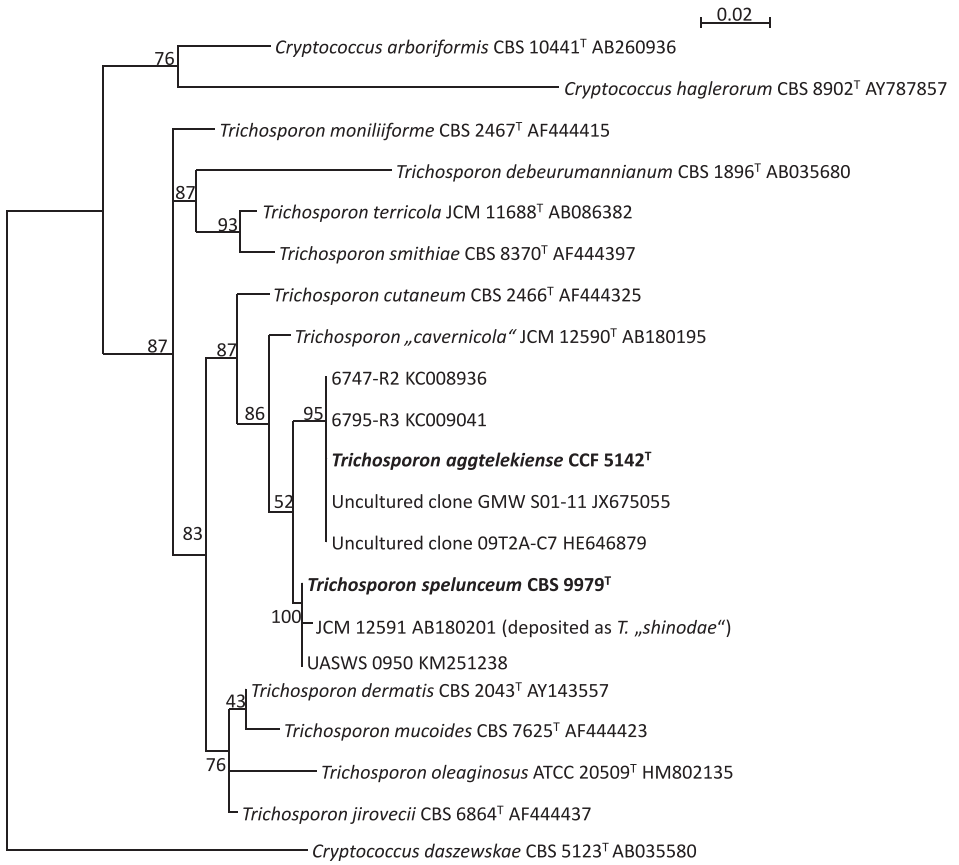

Fig. 3. Maximum likelihood phylogenetic tree constructed using ITS rDNA sequences in PhyML 3.0 using the TN93 substitution model and 500 bootstrap replicates. The tree shows the position of *Trichosporon aggtelekiense* and *T. spelunceum* among members of *T. cutaneum* species group. Taxon names are followed by isolate number and GenBank accession number. *Cryptococcus daszewskae* was used as an outgroup.

A comparison of growth characteristics, assimilation of carbon and nitrogen compounds and other physiological characteristics of *Trichosporon aggtelekiense* and *T. spelunceum* is presented in Tabs. 1 and 2.

Tab. 1. Growth characteristics of *Trichosporon aggtelekiense* and *T. spelunceum*.
Symbol explanation: + strong growth, ± limited growth, – no growth.

	T. aggtelekiense		*T. spelunceum*	
Temperature	Growth on MEA	Colony diameter (mm)	Growth on MEA	Colony diameter (mm)
5 °C	±	5–6	±	3–4
10 °C	±	5–6	±	3–4
15 °C	+	9–11	+	8–9
20 °C	+	13–15	+	12–13
25 °C	+	15–17	+	17–20
28 °C	±	3–5	+	17–20
30 °C	±	1–3	+	9–10
37 °C	–	0	–	0

Tab. 2. Assimilation of carbon and nitrogen compounds in liquid media and other physiological characteristics of *Trichosporon aggtelekiense* and *T. spelunceum*.

	T. aggtelekiense	*T. spelunceum*
Glucose	+	+
Sucrose	+	+
Raffinose	+	+
Galactose	+	+
Lactose	+	+
Trehalose	+	+
Maltose	+	+
Melezitose	+	+
Cellobiose	+	+
Xylose	+	–
Arabinose	+	–
Inositol	+	+
Nitrate	–	–
Urea hydrolysis	+	+
Growth on YM medium (after 10 days, at 25 °C)	creeping pellicle and sediment	a ring and sediment are formed

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