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# *Trichoderma fassatiae*, a new species from the section *Pachybasium* isolated from soil in the Czech Republic

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*Trichoderma fassatiae* sp. nov. isolated from forest soil in the Czech Republic is described based on phenotype and DNA sequence data (ITS rDNA, RPB2, and TEF1α). It belongs to the *Semiorbis* clade in the section *Pachybasium*. Characters distinguishing it from similar species are provided.

**Key words:** Ascomycota, *Hypocreales*, *Trichoderma*, morphology, DNA sequence data.

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Na základě fenotypových znaků a sekvenčních dat (ITS rDNA, RPB2 a TEF1α) je popsán nový druh *Trichoderma fassatiae*. Patří do kladu *Semiorbis* v sekci *Pachybasium*. V článku jsou uvedeny znaky odlišující jej od podobných druhů.

## SHORT TAXONOMIC REPORT

M e t h o d s follow Kopchinskiy et al. (2005) and Samuels et al. (2015). The culture was grown on PDA (potato dextrose agar) and SNA (low nutrient agar; Nirenberg 1976) at 15, 20, 25 and 30 °C in the dark. Microscopic observations were made in Melzer's reagent. Colour determination of colonies was performed according to the ISCC-NBS Centroid Color Charts (Foster 2004).

***Trichoderma fassatiae*** A. Nováková, Kubátová, Valinová, Hubka & M. Kolařík, **sp. nov.** Fig. 1
(MycoBank MB814051)

Holotype. PRM 933821 (isotype PRM 933822). Czech Republic, South Bohemia, near the villages of Albeř and Klášter, alt. 650 m, 49°01'39'' N, 15°09'07'' E. Isolated from soil of mixed forest under *Fagus sylvatica*, A. Kubátová, 11 Sept 2000; ex-type culture CCF 5000 (= CBS 140105 = AK 115/00).

Gene sequence accession number. EMBL LN866275 (ITS rDNA), LN866276 (RPB2), and LN866277 (TEF1α).

Etymology. Named after Czech mycologist Olga Fassatiová (1924–2011), acclaimed specialist in microscopic fungi.

Description of ex-type culture. Colonies on PDA at 25 °C at first white, after 10 days Moderate Yellowish Green (#679267) to Dark Yellowish Green (#355E3B). Reverse colourless; no distinctive odour produced. Colony radius on PDA and SNA after 3 days: see Tab. 1. Conidia formed within 4–5 days.

Conidiophores colourless, smooth-walled, with "*Pachybasium*-like" branching, with short lateral branches in whorls. Phialides ampulliform, (4.0)6.3–7.7(9.6) µm long and (2.2)2.6–3.7(4.6) µm wide. Conidia ellipsoidal to oblong, smooth, (3.0)4.5–5.4(6.4) × (2.2)2.5–3.1(3.3) µm, green to dark green. Chlamydospores absent.

**Tab. 1.** Colony radius (mm) of *Trichoderma fassatiae* CCF 5000 at five temperatures after 3 days (n = 5).

| Temperature and medium | 15 °C | 20 °C | 25 °C | 30 °C | 35 °C |
|---|---|---|---|---|---|
| PDA | 3–10 | 20–25 | 30–40 | 4–8 | no growth |
| SNA | 4–5 | 12–20 | 15–25 | 5–10 | no growth |

Taxonomic position. According to Roskov et al. (2014), the genus *Trichoderma* (Ascomycota, Sordariomycetes, *Hypocreales*) includes 143 species. In addition, 46 species of *Hypocrea* were combined into *Trichoderma* by Jaklitsch & Voglmayr (2013). These were not yet incorporated in Roskov et al. (2014).

Morphologically similar species are *Trichoderma fertile* and *T. oblongisporum* (Bissett 1991, Samuels et al. 2012). Both species produce chlamydospores.

Four genetic regions were used for identification and phylogenetic positon assessment of strain CCF 5000. The nuclear ribosomal internal transcribed spacer (ITS rDNA) region and partial large ribosomal subunit (LSU rDNA) sequence were amplified using the ITS1/NL4 primer set. The RNA polymerase II gene (RPB2) was amplified with the fRPB2-5F/fRPB2-7cR primer set. DNA isolation, PCR conditions and sequencing of both DNA regions followed Kolařík & Jankowiak (2013). The translation elongation factor-1α (TEF1α) gene, including

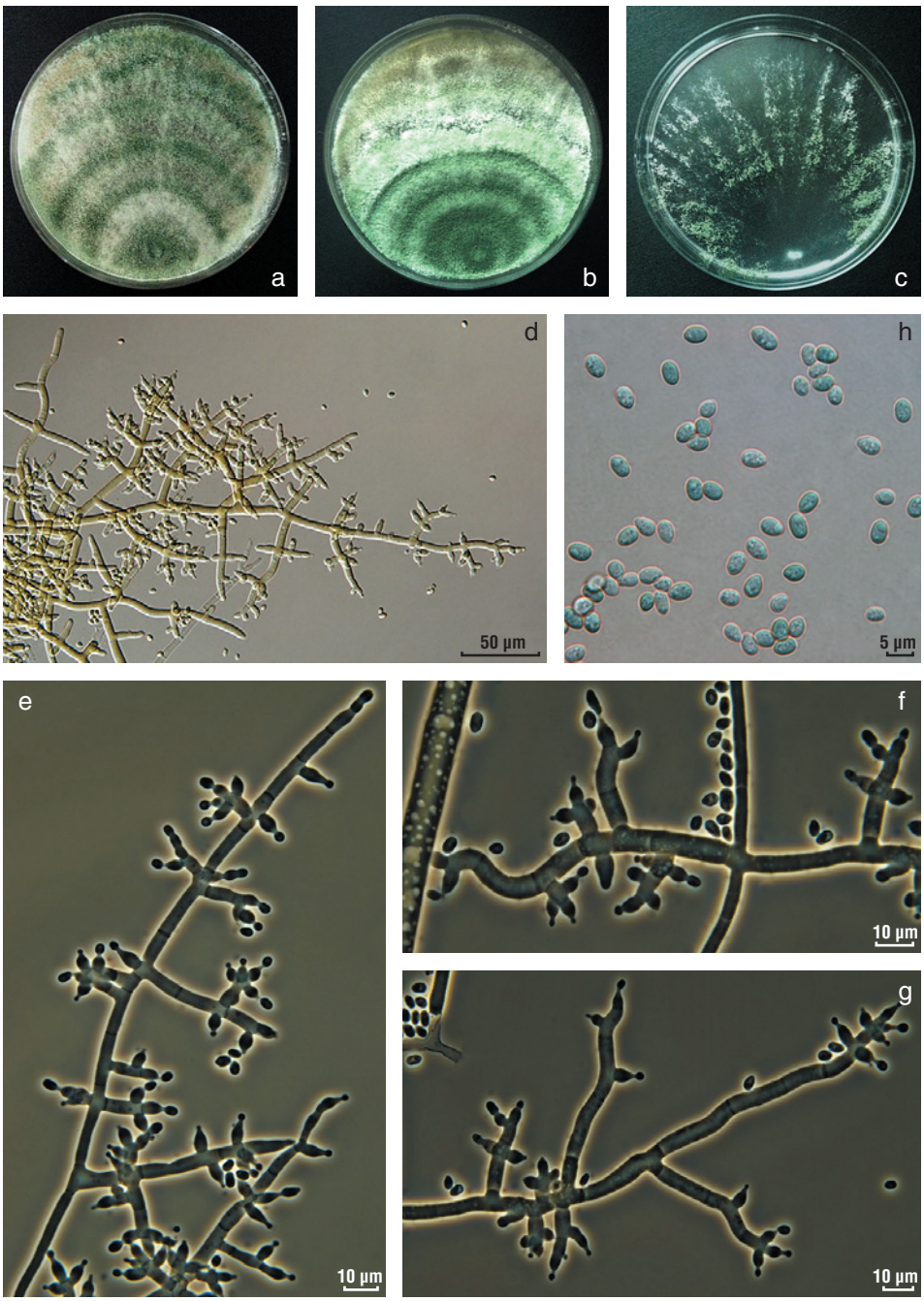


**Fig. 1.** *Trichoderma fassatiae*, ex-type culture. **a** – colony on PDA, 7 d at 25 °C; **b** – colony on PDA, 14 d at 25 °C; **c** – colony on SNA, 14 d at 25 °C; **d, e, f, g** – conidiophores; **h** – conidia. Scale bars: d = 50 µm; e, f, g = 10 µm; h = 5 µm. Photo Š. Valinová (a–c), A. Kubátová (d–h).

the variable introns 4 and 5, was amplified and sequenced using the tef85f/tef954r primer set as described by Hoyos-Carvajal et al. (2009). One dataset containing RPB2 sequences and two separate datasets for each TEF1α intron were constructed using representative sequences published by Jaklitsch (2011) and the most similar sequences from NCBI GenBank. The alignment and subsequent phylogenetic analyses conducted in MEGA6 (Tamura et al. 2013) placed our strain into the *Semiorbis* clade of the section *Pachybasium*, sister to *Trichoderma hunua* and *T. oblongisporum*, followed by *T. semiorbis*, *T. fertile*, *Hypocrea moravica* and *T. fomiticola*. Based on ITS rDNA sequences, TrichoKEY ver. 2.0 identified our strain as an undescribed species of *Trichoderma*. The TrichoBLAST tool, which uses reference sequences from the ISTH database, showed *T. hunua* CBS 238.63 as the most similar (451/456 bp, 99%), followed by *T. semiorbis* DAOM 167636 (449/455 bp, 99%). The same tool showed *T. oblongisporum* DAOM 167085 as the most similar (879/894 bp, 98%), followed by *T. fertile* DAOM 167070 (869/894 bp, 97%) and *T. semiorbis* DAOM 167636 (851/894 bp, 95%) when the RPB2 gene was used. The same search using the TEF1α sequence of intron 4 showed *Hypocrea moravica* DAOM 216461 (235/265 bp, 89%) as the best match. In the case of the TEF1α intron 5 sequence, the closest match was *T. oblongisporum* CBS 343.93 (136/142 bp, 96%). Blast similarity search of the TEF1α and RPB2 genes in the whole nucleotide NCBI database did not result in closer matches than was obtained from the curated ISTH sequence database.

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