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New records of micromycetes from the Czech Republic. IV. Acrodontium salmoneum, Chaunopycnis alba and Cylindrocarpostylus gregarius, and notes on Dactylaria lanosa and Trichoderma saturnisporum

ALENA KUBÁTOVÁ[1], MICHAL ČERNÝ[2] and ALENA NOVÁKOVÁ[3]

[1] Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ 128 01 Praha 2, Czech Republic
[2] Institute of Landscape Ecology, Academy of Sciences of the Czech Republic, Na Sádkách 7, CZ 370 05 České Budějovice, Czech Republic
[3] Institute of Soil Biology, Academy of Sciences of the Czech Republic, Na Sádkách 7, CZ 370 05 České Budějovice, Czech Republic

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Five saprotrophic mitotic microfungi, *Acrodontium salmoneum, Chaunopycnis alba, Cylindrocarpostylus gregarius, Dactylaria lanosa,* and *Trichoderma saturnisporum,* were found during studies of micromycete diversity in the Šumava Mts., Krkonoše Mts., Krušné hory Mts., Prague and Jevany near Prague in the Czech Republic. The microfungi were isolated from soil, except *Cylindrocarpostylus gregarius,* which was found on bark, and *Acrodontium salmoneum* isolated from air, seeds and mites. Three of them had not yet been reported from the Czech Republic and are considered to be the first records for this country. Descriptions and illustrations are given. All the fungi are maintained in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague.

Key words: conidial fungi, saprotrophs, biologically active metabolites, Bohemia

Kubátová A., Černý M. a Nováková A. (2001): Nové nálezy mikromycetů pro Českou republiku. IV. Acrodontium salmoneum, Chaunopycnis alba a Cylindrocarpostylus gregarius, a poznámky k Dactylaria lanosa a Trichoderma saturnisporum. – Czech Mycol. 53: 237–255

Během studia biodiverzity saprotrofních mikromycetů na Šumavě, v Krkonoších, Krušných horách, v Praze a Jevanech byly v posledních letech nalezeny tyto vzácnější houby: *Acrodontium salmoneum, Chaunopycnis alba, Cylindrocarpostylus gregarius, Dactylaria lanosa a Trichoderma saturnisporum.* Většinou byly izolovány z půdy, pouze *Cylindrocarpostylus gregarius* byl izolován z borky a *Acrodontium salmoneum* z ovzduší, semen a roztočů. Tři z uvedených hub jsou prvními nálezy těchto druhů z území České republiky. Jsou uvedeny popisy a vyobrazení. Kultury všech mikromycetů jsou uchovávány ve Sbírce kultur hub (CCF) katedry botaniky na Přírodovědecké fakultě UK v Praze.

INTRODUCTION

The rare microfungi treated below were recovered during several different surveys of the diversity of saprotrophic micromycetes in the Czech Republic carried out by the authors in the past years. These micromycetes are neither mentioned in Řepová (1989a, 1989b, 1990a, 1990b), who compiled a list of soil micromycetes known from the formerly Czechoslovakia, nor in other papers by our mycologists (with the exception of *Dactylaria lanosa* and *Trichoderma saturnisporum*). They appear to be new records for the Czech Republic.

MATERIALS AND METHODS

Surveys of micromycete diversity were made in the following areas:

— soil microfungi: Šumava Mts. (National Park and Protected Landscape Area) in the south of the Czech Republic (1993–96, see Kubátová et al. 1998), locality Jevany near Prague (1993–95), and Krkonoše Mts. (National Park) in the northern part of the Czech Republic (2000);
— microfungi in mycorhizosphere: Krušné hory Mts. in the north-western part of the Czech Republic (1984–1991, see Černý and Cudlín 1989), and Krkonoše Mts. (National Park) (1991–93)
— air-borne microfungi: outdoor air of Prague (1995–97);
— seed-borne microfungi: Prague (2000–01).

The strains were isolated using a soil dilution method, serial washing of roots or by a Harvard impactor. Isolation media used were soil extract agar with glucose, Rose Bengal and streptomycin (SEGA), malt-extract agar (MEA), wort-beer agar (WBA), or Sabouraud agar (SAB). For identification, cornmeal agar (CMA), Czapek yeast extract agar (CYA), malt-extract agar (MEA), potato-carrot agar (PCA), and potato glucose agar (PGA) were used.

Majority of the strains were lyophilised in skim milk. They are maintained in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague. Brief descriptions of morphological features and additional information follow.

Descriptions of the fungi based on our isolates and other notes.

Acrodontium salmoneum de Hoog 1972 (Fig. 1)

Systematic position: Mitotic fungus, teleomorph not known.

Examined strains

CCF 3106: outdoor air, Prague, Czech Republic, isol. A. Kubátová on WBA by Harvard impactor, IX.1996, as No.160/96, LYO.

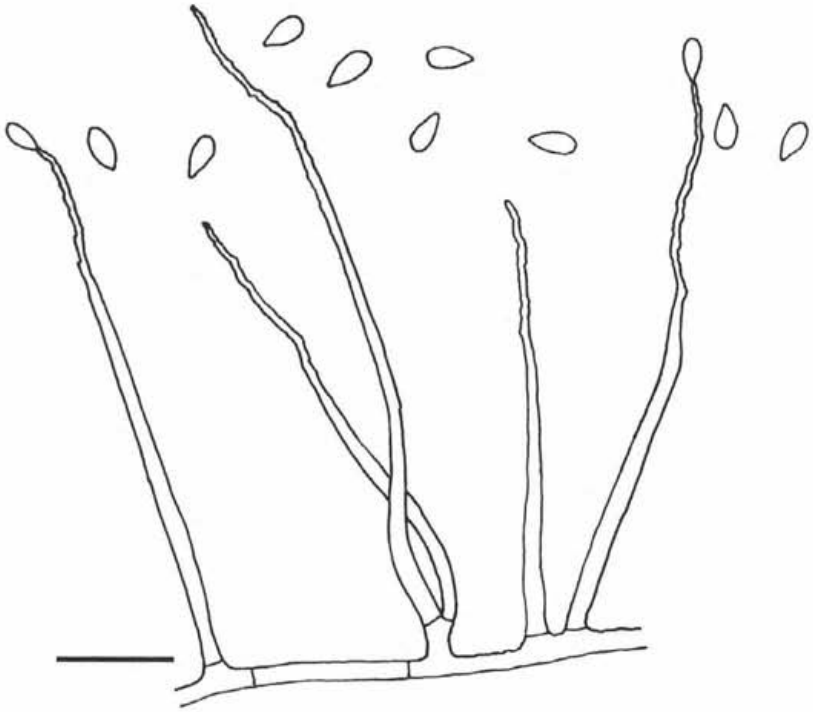

Fig. 1. *Acrodontium salmoneum*, strain CCF 3106, conidiophores with conidia (bar = 10μm). A. Kubátová del.

CCF 3174: digestion tract of mite *Acarus siro*, seed storehouse, Research Institute of Crop Production, Prague, Czech Republic, isol. J. Hubert on MEA, X.2000, as No. 40, det. A. Kubátová, LYO.

CCF 3175: seeds of *Lactuca sativa*, seed storehouse, Research Institute of Crop Production, Prague, Czech Republic, isol. J. Hubert on MEA, X.2000, as No. 8, det. A. Kubátová, LYO.

CCF 3220: digestion tract of mite *Caloglyphus* sp., mouldy wheat, Buštěhrad, Czech Republic, isol. J. Hubert on MEA, VI.2001, as No. 241, det. A. Kubátová, LYO.

Description

Colonies on CMA powdery, peach coloured, growing rather slowly, reaching 13–15 mm, 20–24 mm and 28–33 mm diam. after 7, 10 and 14 days at 25 °C, respectively. Growth on MEA and CYA is similar in growth rate, however sporulation on MEA is somewhat poor and the colonies on CYA are yellowish

peach and funiculose. Colonies at 30 °C after 7 days on CYA reaching 7–8 mm. Growth at 35 °C is nil.

Conidiogenous cells (see Fig. 1) long, growing from undifferentiated hyphae, sometimes in groups of 2–3, slightly tapering to a well-developed rachis, somewhat flexuous, c. 35–95 μm long (including rachis), 1.5–2 μm wide in the lower part and c. 1.2 μm wide in the rachis.

Conidia hyaline, smooth-walled, obovoid with apiculate base, 3.4–5.6 × 2.2–2.8 μm diam.

Our observations are generally in accordance with de Hoog (1972). However, no chlamydospores were observed and growth is somewhat faster in our strains.

Similar species

After de Hoog (1972) this species resembles pink-coloured species of *Nodulisporium* or *Geniculosporium*. They differ by either truncate conidia or short conidiogenous cells.

Habitats and distribution

The fungus was isolated from human sputum (The Netherlands), found as a culture contaminant (U. S. A.), isolated from soil of a beech forest in West Germany, and one strain is from an unknown source (de Hoog 1972). Two other strains were found in the soil of grotto in France (Seigle-Murandi et al. 1980). Steiman et al. (1995) have mentioned a strain isolated from decayed wood and from crab shell. These data do not allow to estimate the typical habitat of this fungus.

Notes: The strain of *Acrodontium salmoneum* found in grotto soil in France is known to produce acrodontiolamide, a secondary metabolite with antifungal properties (Buarque de Gusmão et al. 1993). After Steiman et al. (1995) the acrodontiolamide of this strain inhibits especially phytopathogenic and entomopathogenic fungi.

Chaunopycnis alba W. Gams 1980 (Figs. 2 and 3)

Systematic position: Mitotic fungus, teleomorph not known. The genus includes two species only (Möller and Gams 1993).

Examined strains

CCF 3119: soil in beech forest (*Fagus sylvatica*) near lake Čertovo jezero, Šumava Mts., southern Bohemia, Czech Republic, isol. A. Kubátová on SAB, VI.1996, as No. 94/96.

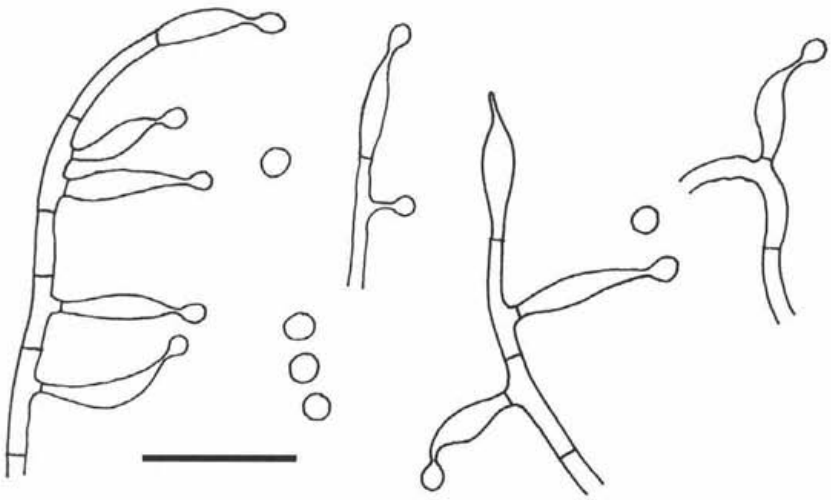

Fig. 2. *Chaunopycnis alba*, strain CCF 3119, conidiogenous cells with conidia (bar = 20μm). A. Kubátová del.

CCF 3176: soil in beech forest *(Fagus sylvatica)*, near Jevany, central Bohemia, Czech Republic, isol. A. Nováková on SEGA, I.1994, det. A. Kubátová, LYO.

CCF 3185: soil of the peat-bog Úpské rašeliniště, Krkonoše Mts., northern Bohemia, Czech Republic, isol. A. Kubátová on SEGA with LiCl, XI.2000, as No. 25/01, LYO.

Description

Colonies on PCA and CMA white, floccose, with yellow tint in reverse, growing rather slowly, reaching 15–22 mm, 25–28 mm and 31–35 mm diam. after 7, 10 and 14 days at 25 °C, respectively. Growth on WBA somewhat faster, colonies reaching ca 19–23 mm, 29–33 mm and 43–45 mm diam. after 7, 10 and 14 days, respectively. Growth at 37 °C is nil.

Conidiomata (Fig. 3c) were observed after 10 days or more on PCA and CMA (sometimes however even after 3 or 4 weeks), forming small globose granules on medium. They were 80–380 μm diam., with little-differentiated filamentous walls, and filled with a dense mass of small conidia. Sporulation on MEA was very poor. The sporulation of the strains CCF 3176 and CCF 3185 is much better than of strain CCF 3119.

Conidiogenous cells (phialides according to Gams 1980) (Figs. 2 and 3) were visible after disruption of the conidiomata. They were better observable in Melzer's reagent (potassium iodide, iodine and chloralhydrate) than in lactic acid stained by methylene blue. Phialides are solitary or irregularly arranged, grow from

hyaline, septate, smooth-walled hyphae c. 0.7–1.5 μm wide. They are cylindrical or inflated, straight or slightly curved, with narrow neck, 6–11 × 1.6–1.9 μm (terminal phialides sometimes longer) and are remarkably similar to those of *Tolypocladium geodes*. Below the terminal phialide, a short lateral neck is sometimes formed like in *Sesquicillium*.

Conidia small, hyaline, globose, smooth-walled, 1.6–1.9 μm diam.

Our observations of microscopic and macroscopic features are in accordance with Gams (1980).

Similar species

The other species of this genus, *Chaunopycnis ovalispora*, isolated from an antarctic lichen, differs from *C. alba* in size and shape of the conidia and phialides (see Möller and Gams 1993). Besides, *C. ovalispora* is not able to grow at temperatures above 20 °C. *Tolypocladium geodes* resembles *Chaunopycnis alba* in its conidiogenous cells and conidia, but it does not form individual conidiomata. *Sesquicillium microsporum,* another similar species, has irregularly branched conidiophores and has affinity to myxomycetes.

Habitats and distribution

Gams (1980) studied many strains from different types of soil (e.g. forest soil, greenhouse soil, páramo soil) and plant material (flower buds, needles etc.) from different parts of the world (Colombia, France, Scotland, Sri Lanka, Sweden, The Netherlands). Nilsson et al. (1992) found *C. alba* to be very frequent in a mire in Sweden. Other large collections of isolates are from lichens and mosses of Antarctica, Spitsbergen and the Swiss Alps (Möller et al. 1995). These authors also studied strains from Brazil, Germany, Great Britain, Madagascar, Malaysia, Singapore, U. S. A. etc. The fungus is considered to be cosmopolitan. In our region it was probably neglected due to its minute conidiogenous structures.

Notes: *Chaunopycnis alba* is interesting by its unusual conidioma type enclosed in a wall of loosely intertwined hyphae, somewhat resembling pycnidia. Thus, it is intermediate between Hyphomycetes and Coelomycetes.

Screenings of many strains made in the 1990s revealed high genetic, metabolic and physiological diversity within this species (Möller et al. 1995, 1996). Some isolates are interesting by its production of cyclosporin A, an immunosuppressive compound (Möller et al. 1995), and of pyridoxatin, which has a high cytotoxicity against human cancer cell lines and inhibits gelatinase A, playing an important role in cancer invasion (Lee et al. 1996).

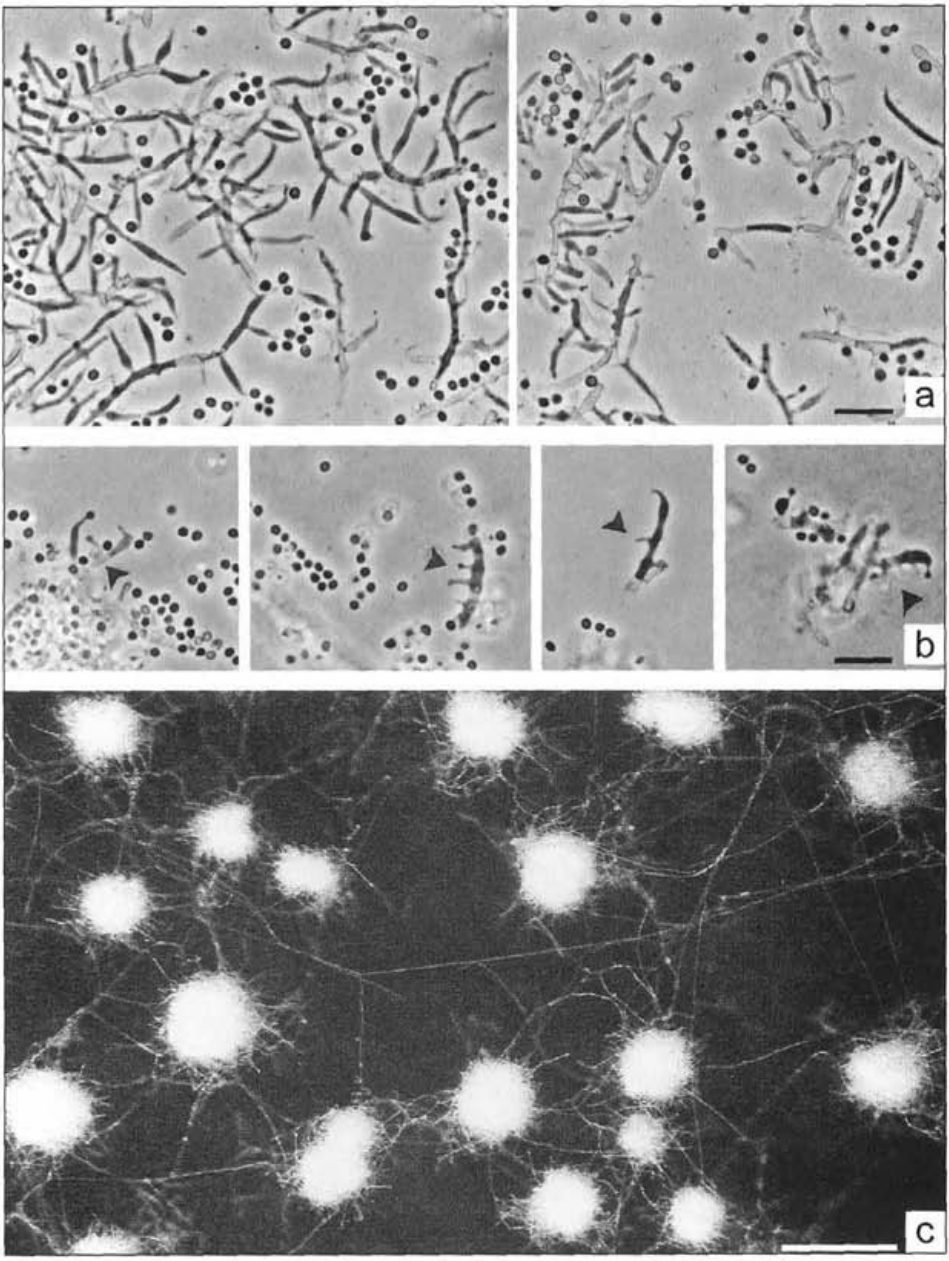

Fig. 3. *Chaunopycnis alba*: **a** – conidiogenous cells and conidia, strain CCF 3185 (CMA, 1 month), phase contrast (bar = 10μm); **b** – conidiogenous cells with terminal and lateral necks (arrows) and conidia, strain CCF 3176 (PCA, 10 days), phase contrast (bar = 10μm); **c** – conidiomata, strain CCF 3185 (PCA, 1 month) (bar = 250μm). Photo A. Kubátová

Cylindrocarpostylus gregarius (Bres.) Kirschner et Oberw. 1999 (Figs. 4 and 5)

Bas. *Diplocladium gregarium* Bres. 1903
Syn. *Cylindrocladium gregarium* (Bres.) de Hoog 1978

Systematic position: Anamorphic *Hypocreales* (Hawksworth et al. 1995). Teleomorph not known. The genus is monospecific.

Examined strain

CCF 2751: ex bark of stump of *Picea abies*, locality Kalek, Krušné hory Mts., Czech Republic, isol. M. Černý on WBA, X.1991.

Description

Colonies on MEA with sparse, whitish mycelium, reaching 15–18 mm, 23–26 mm and 32–37 mm diam. at 25 °C after 7, 10 and 14 days, respectively. Growth on CMA is somewhat faster. Colonies after 10 days have a light brown tint. Sporulation is better after 10–14 days on both media.

Conidiophores (Fig. 4a, b and 5a, c) mononematous, erect, c. 160–560 μm long, c. 15–30 μm wide in the basal part, penicillately branched at the top. Young stipes smooth, hyaline, mature ones warted and brownish, especially at the base. Conidiophores three to five-stage branched, metulae with warted walls (Fig. 5b).

Conidiogenous cells (phialides) cylindrical, hyaline, smooth, c. 25–33 × 2.5–3.5 μm.

Conidia (Fig. 4c and 5d) hyaline, smooth, slightly curved, 2–4-celled, c. 14–40 × × 4.2–5.2 μm, forming a drop at the tip of the conidiophore. Anastomoses were frequently observed between hyphae and even between conidia.

The above mentioned data correspond with measurements by Kirschner and Oberwinkler (1999). Conidiophore branching of *C. gregarium* resemble that of *Leptographium*, however *Leptographium* has one-celled conidia only. Morphology and habitat of *C. gregarium* are also very close to *Stilbella fusca*. However, the latter species forms predominantly synnemata (Seifert 1985).

Habitat and distribution

The species was first described (as *Diplocladium gregarium*) from bark of *Pinus sylvestris* in Poland (Bresadola 1903). Other known records are from Germany, where the fungus was found in bark beetle galleries in *Pinus sylvestris* and *Picea abies* and was also isolated from the beetles themselves (Kirschner and Oberwinkler 1999). Our find supports the probable affinity of this fungus for bark of conifers.

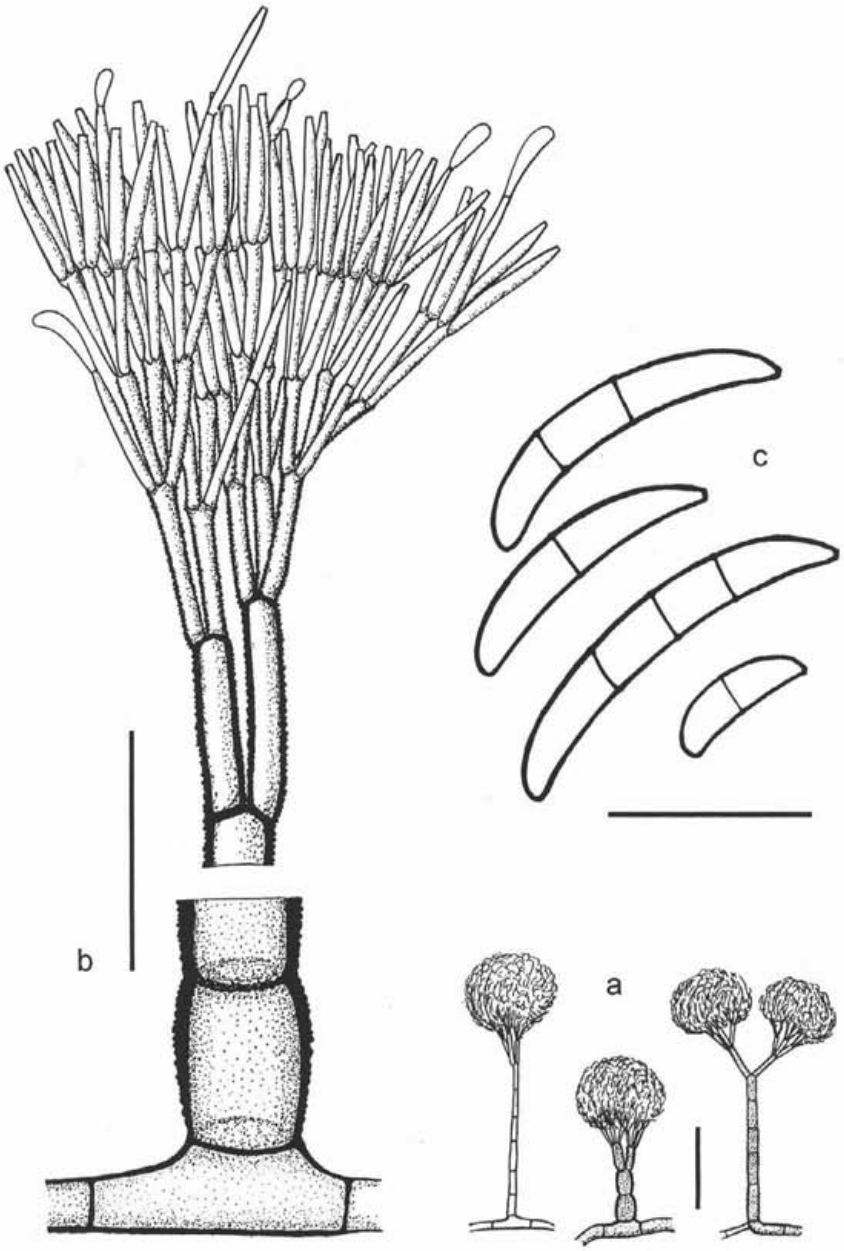

Fig. 4. *Cylindrocarpostylus gregarius*, strain CCF 2751: **a** – habitus of conidiophores (bar = 100μm); **b** – base of conidiophore and conidiophore branching (bar = 50μm); **c** – conidia (bar = 20μm). M. Černý del.

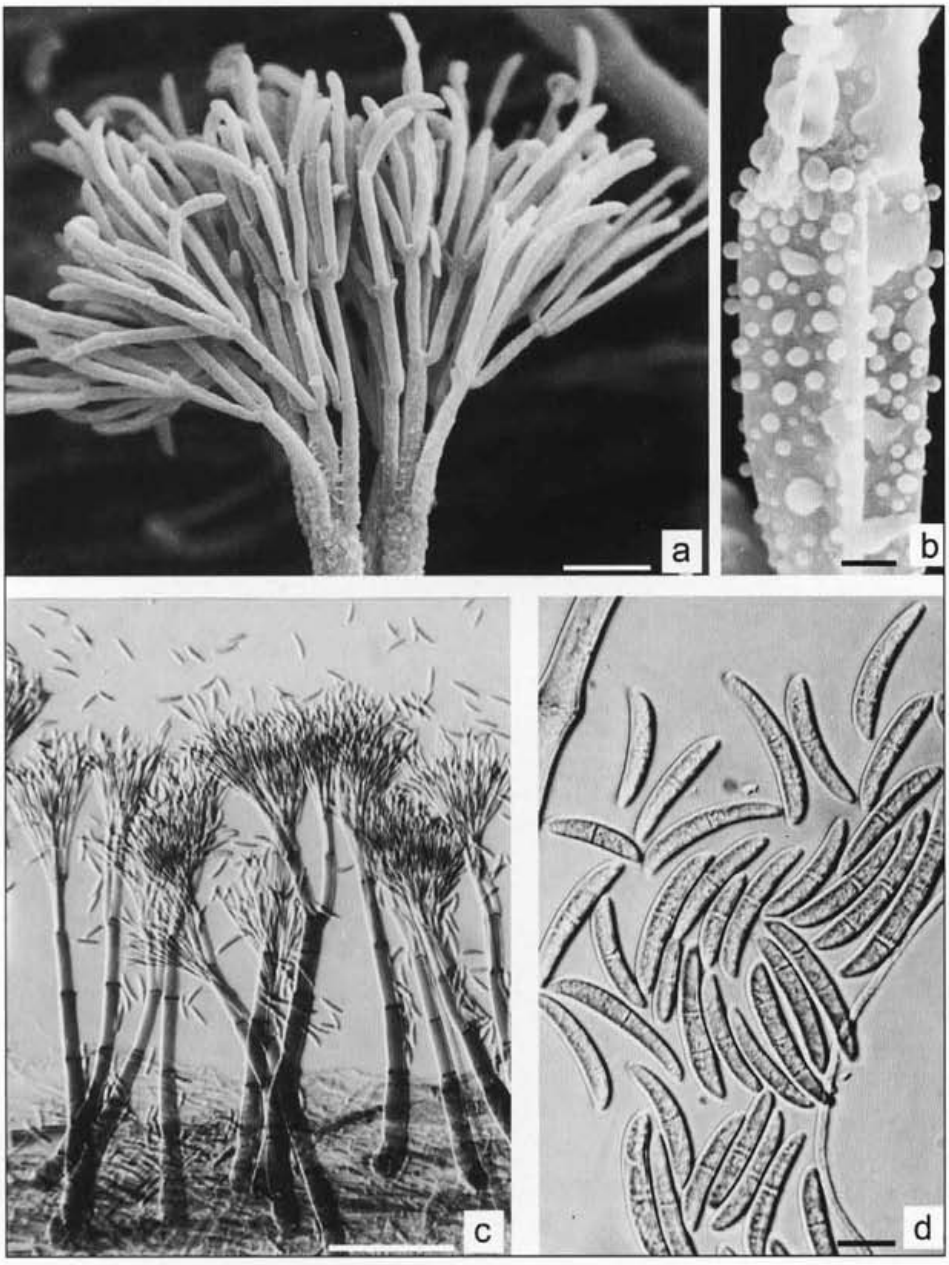

Fig. 5. *Cylindrocarpostylus gregarius*, strain CCF 2751: **a** – penicillately branched conidiophore tip, SEM (bar = 20μm); **b** – part of metula with warted walls, SEM (bar = 5μm); **c** – habitus of conidiophores (bar = 100μm); **d** – conidia (bar = 10μm).

Photos J. Nebesařová (a, b) and M. Černý (c, d)

Notes: The generic classification of this species has changed during the last decades. Hughes (1958) reduced *Diplocladium* Bonord. to a synonym of *Cladobotryum* Nees. De Hoog (1978) revised some fungicolous Hyphomycetes including *Cladobotryum*. He studied the type specimen of *Diplocladium gregarium* Bres. and due to its hyaline conidiophores, elongated 1–2-celled conidia and resemblance to *Cylindrocladium* he recombined it as *Cylindrocladium gregarium* (Bres.) de Hoog. Contrary to this, Crous and Wingfield (1994), who revised *Cylindrocladium*, excluded *C. gregarium* from this genus, emphasizing the resemblance of the conidiophore branching pattern to *Leptographium* Lagerb. et Melin and *Phialocephala* W. B. Kendr. Kirschner et Oberwinkler (1999) rediscovered fresh material of a fungus almost matching the description of *Diplocladium gregarium*. The only difference was the 0–1–septate conidia mentioned in Bresadola's description *versus* 0–3-septate conidia seen in their own material. Therefore they re-examined the type material of *D. gregarium*, found a few conidia with two septa and concluded that their fungus was conspecific with *Diplocladium gregarium*. As there was no appropriate genus for that species, they established the new genus *Cylindrocarpostylus* Kirschner et Oberwinkler with the single species *Cylindrocarpostylus gregarius* (Bres.) Kirschner et Oberwinkler. It is of interest to note, that M. Černý, who collected this fungus in Czechoslovakia in 1991, also observed bi- to tri-septate conidia in his material, which otherwise corresponded to the description of *Cylindrocladium gregarium*. Kirschner and Oberwinkler (1999) explain this phenomenon by the age of the colony: young colonies produce more conidia with three septa, whereas in old cultures single-septate conidia predominate.

Dactylaria lanosa Malla et W. Gams 1971 (Fig. 6)

Systematic position: Mitotic fungus, teleomorph not known (Hawksworth et al. 1995). In the genus *Dactylaria*, it is classified under the section *Dactylaria* (de Hoog 1985). The genus includes 82 species (Goh and Hyde 1997).

Examined strains

CCF 2739: ex rhizosphere of *Picea abies*, Kamenný vrch hill, Krušné hory Mts., Czech Republic, isol. M. Černý on WBA by serial washing of roots, XI.1990, as 15aPi-K, LYO.

CCF 2850: ex rhizosphere of *Picea abies*, Modrý důl valley, Krkonoše Mts., Czech Republic, isol. M. Černý on WBA by serial washing of roots, 1993, as No. 219b.

CCF 2982: ex forest soil under *Picea abies*, Svaroh Mt., Šumava Mts., southern Bohemia, Czech Republic, isol. A. Kubátová on SAB, VI.1995, as No. 82/95, LYO.

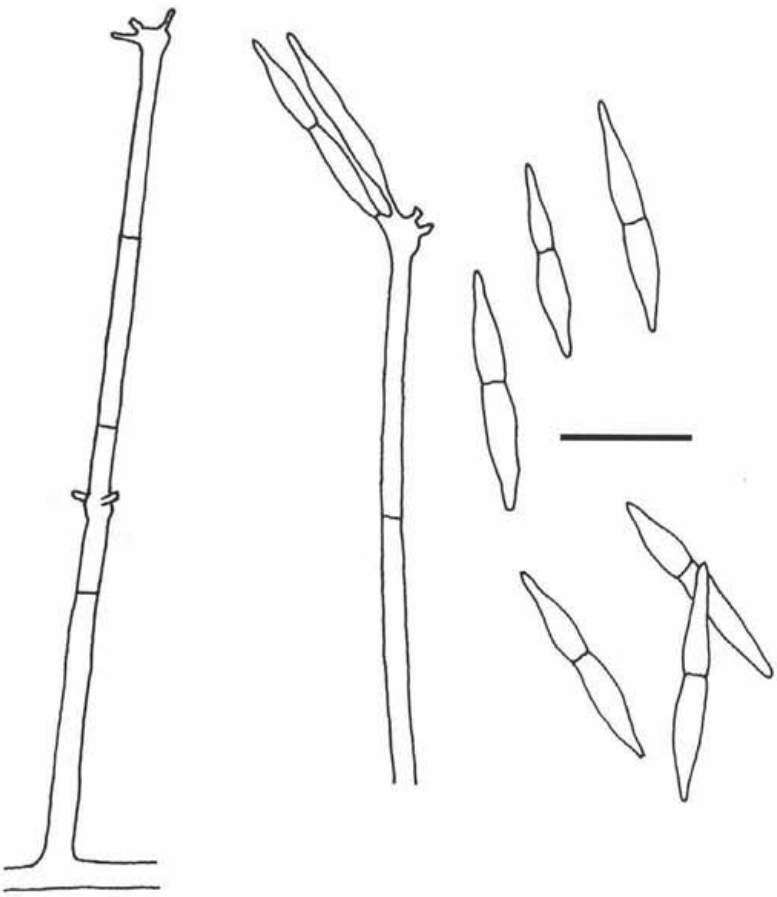

Fig. 6. *Dactylaria lanosa*, strain CCF 2982, conidiophores with conidia (bar = 10μm).
A. Kubátová del.

Other finds:

MC 99U: ex rhizosphere of *Picea abies*, Sluneční údolí valley, Krkonoše Mts., Czech Republic, isol. M. Černý on WBA by serial washing of roots, IX.1991.

GEF 20/95: ex forest soil under *Picea abies*, Mt. Jezerní hora, Šumava Mts., southern Bohemia, Czech Republic, isol. A. Kubátová on SAB, VI.1995.

Description

Colonies on MEA whitish to yellow, lanose, reaching 14–17 mm, 21–24 mm and 28–35 mm diam. after 7, 10 and 14 days at 25 °C, respectively. Reverse brown-orange to brown. Old cultures are deep yellow and produce yellow diffused pigment into the agar. Growth at 30 °C is nil.

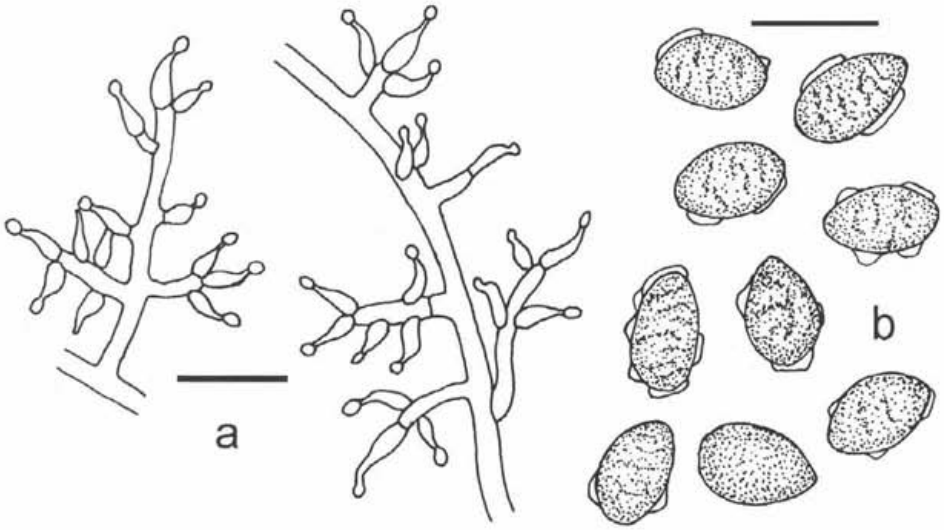

Fig. 7. *Trichoderma saturnisporum*, strain CCF 2983: a – conidiophores (bar = 20μm); b – conidia (bar = 5μm). A. Kubátová del.

Conidiophores (Fig. 6) mononematose, erect, long, simple or rarely with a branch, 1.8–2.3 μm wide, similar to vegetative hyphae, bearing 1–4 μm long conidiiferous denticles, which are often scattered at the terminal part of the conidiophores or arise below the apex. Mycelial hyphae sometimes with golden-yellow encrustations.

Conidia hyaline, smooth, narrow, fusiform, predominantly two-celled, c. 14–20.2 × 1.8–2.3 μm.

Macroscopic and microscopic features correspond to those reported by Malla and Gams (1971) and de Hoog (1985).

Habitats and distribution

The type strain was isolated from roots of *Picea abies* in Denmark (Malla and Gams 1971), other specimens were found in forest soil in Sweden, on a decaying *Picea* trunk in Germany, and on *Pseudohydnum gelatinosum* in The Netherlands (de Hoog 1985). These data and our own finds suggest a certain affinity of *Dactylaria lanosa* to *Picea* itself as a substrate or to habitats with *Picea* trees. A nematophagous character, observed in several *Dactylaria* species, was not recorded in this species.

The first three above cited strains were published by Kubátová et al. (1997) in the new catalogue of filamentous fungi and two of them also by Kubátová et al. (1998) in an article on soil microfungi of the Šumava Mts.

Trichoderma saturnisporum Hammill 1970 (Fig. 7)

Systematic position: Anamorphic *Hypocreales* (Hawksworth et al. 1995). Teleomorph is not known. In the genus *Trichoderma* it is classified in the section *Longibrachiatum* (Samuels et al. 1998).

Examined strain

CCF 2983: ex soil in beech forest (*Fagus sylvatica*), Mt. Ždanidla, Šumava Mts., southern Bohemia, Czech Republic, isol. A. Kubátová on SAB, VI.1996, as No. 106/96, LYO.

Description

Colonies on PGA, MEA and CMA fast growing, covering a 9 cm Petri dish in 3–4 days at 25 °C. Mycelium sparse, inconspicuous, whitish. On CMA yellow pigment diffusing into agar was observed. Fruiting areas dark olive green. At 37 °C, colonies on MEA cover the Petri dish in 7 days, mycelium inconspicuous. At present, our strain shows poor sporulation.

Conidiophores (Fig. 7a) are aggregated into tufts, irregularly branched.

Phialides solitary, paired or in verticils of 3, ampulliform or lageniform, often curved, ca 10–12 μm long.

Conidia (Fig. 7b) green, ovoid to ellipsoidal, 4.4–5.4 × 3.1–3.8 μm, mostly with distinct hyaline wing-like outgrowths c. 0.7 μm high, sometimes without wings.

Our observations are in accordance with data of Hammill (1970), Doi et al. (1987), and Gams and Bissett (1998). In contrast to Doi et al. (1987), no chlamydospores were detected.

A similar species is *Trichoderma ghanense* (Doi et al. 1987). Its conidia have irregular extensions on the surface, but are longer (up 8.2 μm).

Habitat and distribution

Trichoderma saturnisporum was first isolated from forest soil in Georgia, United States (Hammill 1970). After Samuels et al. (1998) it is known from Australia, Italy, South Africa, Turkey and the United States. The species was isolated predominantly from soil, in some cases from the rhizosphere of *Triticum* sp., straw of *Triticum* sp. and roots of *Pseudotsuga menziesii*, Alexandrova and Velikanov (1999) isolated an other strain from steppe soil in Kalmykia, southwestern Russia.

The first report of the above cited strain (CCF 2983) in the Czech Republic was published by Kubátová et al. (1997). The strain is mentioned by Kubátová et al. (1998), too. Noteworthy, Marvanová (1999) isolated this fungus at the same

time (June 1996) from alluvial meadow soil in southern Moravia, Czech Republic. She isolated it on soil agar at 42 C and characterised it therefore as thermotolerant.

Notes: Doi et al. (1987) placed *T. saturnisporum* in a new section, *Trichoderma* sect. *Saturnisporum*. However, molecular studies of Kuhls et al. (1997) showed a close relationship to section *Longibrachiatum*, therefore Gams and Bisset (1998) merged the section *Saturnisporum* with the section *Longibrachiatum*.

T. saturnisporum is known to produce some biologically active compounds. Rebuffat et al. (1993) isolated the peptides saturnisporins, peptaibols with antibiotic activity against *Staphylococcus aureus*. Ritieni et al. (1995) reported production of the antibiotics paracelsin A, B, C, D and E.

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