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Bark beetles and their galleries: well-known niches for little known fungi on the example of Geosmithia

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The oak bark beetle (*Scolytus intricatus, Scolytidae*, Coleoptera) was studied during the years 1997–2003 with respect to the occurrence of microscopic fungi on the surface of its body. Samples were collected in eight localities in the Czech and Slovak Republics. The investigation was focused on all different stages of the beetle's life cycle: eggs, larvae, adults before emergence, adults in generation and maturation feeding (nearly 600 samples), and also on galleries (400 samples). The most frequent fungi associated with *S. intricatus* were yeasts, *Geosmithia* spp. and *Penicillium* spp. Ophiostomatoid fungi were isolated, too. Great attention was paid to the occurrence of *Geosmithia* spp., which were so far recorded rarely. They were frequently found in all stages of the life cycle of *Scolytus intricatus*, except for males in maturation feeding. The ecology of *Geosmithia* spp. in feedings of phloem inhabiting insects is discussed for their negative cellulase production and the ecology of associated insect species. Trees infested with *Scolytus intricatus* represent a major and still little explored niche of *Geosmithia* spp.

Key words: microfungi, *Geosmithia*, *Scolytidae*, ophiostomatoid fungi, yeasts

Kubátová A., Kolařík M., Prášil K. a Novotný D. (2004): Kůrovci a jejich chodbičky – dobře známá nika málo známých hub, příkladem je Geosmithia. – Czech Mycol. 56: 1–18

V letech 1997–2003 byl studován bělokaz dubový (*Scolytus intricatus, Scolytidae*, Coleoptera) s ohledem na výskyt mikroskopických hub na jeho povrchu. Vzorky byly odebírány na osmi lokalitách v České a Slovenské republice. Byla zkoumána všechna stádia životního cyklu bělokaza: vajíčka, larvy, dospělci před vylétnutím, dospělci v generačním a dozrávacím žíru (téměř 600 vzorků) a také chodbičky (400 vzorků). Nejčastěji byly ve spojení s bělokazem dubovým zaznamenány kvasinky, druhy rodu *Geosmithia* a *Penicillium*. Byly izolovány také ophiostomatální houby. Velká pozornost byla zaměřena na výskyt hub rodu *Geosmithia*, které byly dosud u nás

i ve světě zaznamenávány dosti zřídka. Tyto houby byly ve všech stádiích životního cyklu bělokaza velmi časté, s výjimkou samečků v dozrávacím žíru. Ekologie hub rodu *Geosmithia* v požercích lýkožravého hmyzu je diskutována na základě zjištěné negativní produkce celuláz a ekologie jejich hmyzích přenašečů. Dřeviny napadené bělokazem dubovým představují významnou a dosud málo probádanou niku hub rodu *Geosmithia*.

INTRODUCTION

Bark beetles have been monitored by forest pathologists and mycologists on the whole world for several last decennia. Numerous papers dealing with fungi associated with bark beetles were published and still new ecological connections are found and even new species of fungi are described from this niche (e. g. Liou et al. 1999, Kirschner and Oberwinkler 2000, Kirschner et al. 2001).

Subcorticolous insects can be divided into three major groups according to their feeding strategies (Berryman 1989). (1) Saprophages feed on the bark of dead trees or tree parts. Although their diet may be supplemented by microorganisms, strict associations with specific fungi are unlikely to occur. This ecological group of insects is rather unknown because they cause no economic damage. (2) Mycetophages (= xylomycetophages) are known also as "ambrosia beetles". A large part of this group belongs to various Coleopteran families (*Scolytidae, Platipodidae, Lymexilidae*) and to horntail wasps (Hymenoptera: *Siricidae*). Ambrosia beetles feed mostly on mutualistic ambrosial fungi which are cultivated in galleries bored into the sapwood of dead trees or timber. (3) Phytophages (= phloemophages) feed on living tissue of trees and are closely associated with fungi. This group comprises some Scolytids known as bark beetles, some weevils (Coleoptera: *Curculionoidea*) and other insect groups (Coleoptera: *Cerambycidae, Buprestidae*). Their reproduction and survival are apparently not dependent on a specific fungal diet. However, most of them have both specific and coincidental connections with fungi, many of which belong to the ophiostomatoid fungi. These fungi are able to increase the concentration of phloem nutrients (Ayres et al. 2000), they degrade products of host defense chemicals (Beaver 1989) or convert insect pheromones (Leufven 1991). Some of these fungi like *Ceratocystis polonica* (Siemaszko) C. Moreau (Christiansen and Solheim 1990) and *Ophiostoma novo-ulmi* Brasier (Brasier 1991) are dangerous pathogens. For this reason, tree-killing bark beetles are the most intensively studied Scolytids. Other fungi may be parasites of pathogenic invertebrates. For example, *Esteya vermicola* J. Y. Liou et al. is a microfungus associated with beetles that can parasitise nematodes, transferred by the same beetles (Liou et al. 1999, Kubátová et al. 2000). Besides different kinds of parasites, many other microorganisms live in close association with beetles and their galleries: saprotrophs, endophytes, and

other microfungi whose role is not yet known. Several phloemophagous bark beetles are associated with only a small number of ophiostomatoid fungi and with very few and rather unspecific fungal species, among which the anamorphic fungus *Geosmithia putterillii* (Thom) Pitt was found as a dominant species (see Table 1; Kirschner 1998, 2001; Kubátová et al. 1999; Kolařík 2002a, 2002b). On the other hand, *G. putterillii* is regarded a rare taxon on other organic substrates. Likewise, *G. lavendula* (Raper et Fennell) Pitt was yet very rarely isolated from various organic substrates. Recently, this rather little known species was found to be frequent among mycobiota associated with fig bark beetle (Kolařík, unpubl., see Table 1).

The genus *Geosmithia* was erected by Pitt (1979) to accommodate some distinct species of *Penicillium* and at present the genus includes ten species (Pitt 1979; Pitt and Hocking 1985; Yaguchi et al. 1993, 1994). Raper and Thom (1949) and Ramirez (1982) distinguished *Penicillium putterillii* Thom and *P. pallidum* G. Sm. as two different species, based on differences in arrangement of conidial chains, which may occur in well-defined columns in *P. putterillii* or divergent, becoming tangled in age in *P. pallidum*. Pitt (1979) examined several new isolates with intermediate features and placed *P. pallidum* into synonymy of *Geosmithia putterillii*. However this "*G. putterillii* group" has not yet been studied by molecular methods, which could elucidate the position of both species. Recent molecular studies, based on rDNA sequence analyses, showed that *Geosmithia* is a polyphyletic taxon with affinities to Hypocreales and Eurotiales (Iwamoto et al. 2002, Ogawa et al. 1997, Ogawa and Sugiyama 2000, Peterson 2000). The hypocrealean *Geosmithia* species, represented by *G. putterillii* and *G. lavendula*, are closely related to *Acremonium alternatum* Link and to cleisthothecial genera like *Emericellopsis*, and form a separate clade in the family *Bionectriaceae* (Rossman et al. 1999, Rossman et al. 2001).

During our survey of microfungi associated with oak bark beetle (*Scolytus intricatus*) infesting oaks in the Czech Republic and Slovakia, several groups of fungi were found (Kubátová et al. 1999, Kubátová et al. 2002). Among them great attention was paid to ophiostomatoid fungi (Prášil 2000). Within other fungi, *Geosmithia* species were isolated in high frequencies. These results were surprising, because records of this fungus in the Czech Republic were so far very rare (Nováková and Kubátová 1995). Thus, the *Geosmithia* species present an example of fungi living in well-known niche but whose ecology and occurrence of which in the Czech Republic we do not know much of. The main aim of this article is therefore to apprise of these fungi and their niche in detail. We have presented here data about the occurrence of *Geosmithia* fungi on different stages of oak bark beetle. In addition, in order to elucidate the role of *Geosmithia*, we detected the capability of selected strains to utilise cellulose.

Table 1. Insect species associated with *Geosmithia* spp. – summary of yet known data.

Insect species *	Host tree	Geographic origin/References
Cryphalus piceae	*Abies alba*	Germany (Kirschner 1998, 2001)
Ernoporus tiliae	*Tilia cordata*	Czech Republic, Hungary (Kolařík, unpubl.)
Ernoporicus fagi	*Fagus sylvatica*	Slovak Republic (Kolařík, unpubl.)
Hypoborus ficus	*Ficus carica*	Croatia, France (Kolařík, unpubl.)
Leperisinus fraxini	*Fraxinus excelsior*	Germany (Kirschner 1998, 2001), Czech and Slovak Republics, Hungary, France (Kolařík, unpubl.)
Phloesinus thujae	*Chamaecyparis pisifera*	Czech Republic (Kolařík, unpubl.)
Phoracantha semipunctata	–	South Africa (CBS 857.71)
Pityophthorus pityographus	*Picea abies*	Germany (Kirschner 1998, 2001)
Rhaphitropis marchicus (Coleoptera: *Anthribidae*) associated with *S. mali*	*Malus domestica*	Czech Republic (Kolařík, unpubl.)
Scolytus carpini	*Carpinus betulus*	Czech Republic, Hungary (Kolařík, unpubl.)
*Scolytus intricatus***	*Fagus sylvatica, Quercus dalechampii, Q. petraea, Q. polycarpa, Q. robur*	Czech Republic (this study), Czech and Slovak Republics (Kolařík 2002a, Kolařík, unpubl.)
Scolytus mali	*Prunus domestica*	Hungary (Kolařík, unpubl.)
Scolytus multistriatus	*Ulmus carpinifolia*	Czech Republic (Kolařík, unpubl.)
Scolytus ratzeburgi	*Betula pendula*	Czech Republic (Kolařík 2002a)
Scolytus rugulosus	*Prunus domestica, P. spinosa, Frangula alnus, Malus domestica*	Czech and Slovak Republics (Kolařík 2002a), Hungary, France (Kolařík, unpubl.)
Scolytus scolytus	*Ulmus laevis*	Czech Republic (Kolařík 2002a)
Scolytus sp.	*Ulmus* sp.	Netherlands (CBS 248.32)
Scolytus sp.	*Persea gratissima*	Seychelles (IMI 051240b)
Scolytus sp.	–	England (IMI 192499)
Taphrorychus bicolor	*Fagus sylvatica*	Germany (Kirschner 1998)
Xiphydria sp. (Hymenoptera: *Siricidae*)	*Castanea sativa*	Czech Republic (Kolařík 2002a, Kolařík, unpubl.)
undetermined bark beetle	*Cunninghamia konishii*	Taiwan (Kirschner 2001)

* Coleoptera: *Scolytidae*, unless otherwise noted

** *Geosmithia* spp. were also isolated from larvae of *Agrilus* spp. (Coleoptera: *Buprestidae*) and from cerambycid larvae, found near oak bark beetle (*S. intricatus*) feedings.

Table 2. Details on localities and studied material of *Scolytus intricatus* in the Czech and Slovak Republics.

Locality, date of sampling (from the Czech Republic unless otherwise noted)	**Host tree**	**Stage of life cycle**
Central Bohemia, Křivoklát region, Kohoutov near Zbiroh, 49° 55' 25" N, 13°46'14"E, March 1997	*Quercus petraea* (4 trees)	• 50 larvae after wintering (50 galleries) • 20 adults before emergence
Central Bohemia, Křivoklát region, Mlynářův luh near Karlova Ves, 49° 59' 31" N, 13° 51' 06" E, July 1997	*Q. petraea*	• 100 adults in generation feeding (70 galleries) • 30 eggs
Central Bohemia, Křivoklát region, Vlastec near Zbiroh, 49° 55' 40" N, 13° 48' 30" E, October 1997	*Q. petraea*	• 100 larvae before wintering (100 galleries)
Central Bohemia, Libický luh near Velký Osek, 50° 06' 10" N, 15° 10' 15" E, April 1998	*Q. polycarpa*	• 25 larvae after wintering (25 galleries) • 25 adults before emergence (25 galleries)
Central Bohemia, Libický luh near Velký Osek, 50° 06' 10" N, 15° 10' 15" E, July 1998	*Q. robur*	• 25 young larvae (25 galleries) • 25 dead females after oviposition (25 galleries)
Central Bohemia, Libický luh near Velký Osek, 50° 06' 10" N, 15° 10' 15" E, October 1998	*Q. robur*	• 60 larvae before wintering (50 galleries)
Central Bohemia, Libický luh near Velký Osek, 50° 06' 10" N, 15° 10' 15" E, June 1999	*Q. robur*	• 30 males in maturation feeding (30 galleries)
North Bohemia, Louny, near Břinkov, 50° 16' 48" N, 13° 47' 34" E, November 2000	*Q. robur*	• 20 larvae before wintering
South Bohemia, Bohemian Forest, Vydra river region, near Horní Hrádky, 49° 04' 24" N, 13° 30' 30" E, October 2001	*Q. robur*	• 20 larvae before wintering
Slovak Republic, Central Slovakia, Muránska planina Plateau, Šiance hill near Muráň, 48° 46' 10" N, 20° 04' 30" E, July 2002	*Q. dalechampii*	• 35 larvae after wintering • 17 adults before emergence
North Bohemia, Louny, near Hřivice, 50° 17' 03" N, 13° 44' 00" E, January 2003	*Fagus sylvatica*	• 10 wintering larvae • 7 wintering adults

MATERIALS AND METHODS

Localities and material

The investigation was carried out during the years 1997–2003 in eight localities (deciduous forests with prevailing oaks) in the Czech and Slovak Republics (Table 2). The primary sources for the sampling were short parts of branches (c. 10–40 cm long) of four oak species (*Quercus dalechampii, Q. robur, Q. petraea* and *Q. polycarpa*) and beech (*Fagus sylvatica*) infested by oak bark beetle (*Scolytus intricatus, Scolytidae*, Coleoptera) in different stages. Altogether, branches from fourteen trees were sampled. The majority of the branches or trees had recently died off. The study was focused on surface mycobiota of all different stages of the life cycle of *S. intricatus*: eggs, larvae before and after wintering, wintering adults or adults before emergence, and adults in generation and maturation feeding, dead females after oviposition and also on mycobiota in galleries of larvae and adults.

Sampling and isolation

For sampling of adults before emergence, parts of branches were maintained in a laboratory for several days in sterile jars in order to enable maturation of the insects (moist chambers). Males of *S. intricatus* in maturation feeding were caught directly in the localities, whereas larvae, adults and eggs were excised from under the bark by sterile tweezers in the laboratory. Altogether, nearly 600 samples (individuals) of different insect stages (30 eggs, 345 larvae and 224 adults) and 400 samples of galleries (250 of larvae and 150 of adults) were processed. Each insect was individually washed in a tube with 5 ml of sterile water with Tween 80 (0.02 %) using an ultrasonic cleaner (frequency 44 kHz). One ml of suspension and one insect body were separately inoculated on Petri dishes with 1.5 % malt extract agar with streptomycin (0.1 g/l). Material from galleries was inoculated directly onto agar plates.

The Petri dishes were incubated at 25 °C in the dark. During 2–10 weeks, colonies were transferred onto several media: Czapek yeast extract agar (CYA) or 2 % malt extract agar (MEA) after Pitt (1979), and potato-carrot agar (PCA) after Fassatiová (1986). Selected *Geosmithia* strains (over 80 isolates) were lyophilised and are maintained at the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic. One strain was deposited at the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, Czech Republic (CCM 8295).

Testing Geosmithia strains for cellulase production

Testing of cellulolytic enzyme production was carried out not only with our isolates from *Scolytus intricatus*, but also with our isolates from various phloem and sapwood inhabiting insects and other comparative strains from CABI Bioscience, UK and NRRL, USA. Altogether, twenty-one *Geosmithia* isolates were tested: CCF 3333, CCF 3340, CCF 3341, CCF 3344, AK 105/97, AK 125/97, AK 125/98, AK 192/98, MK 97, MK 110, MK 123, MK 124, MK 142, MK 334, MK 353, MK 368, MK 382, and IMI 158645, IMI 191599, IMI 054224, NRRL 2024. A strain of *Pleurotus ostreatus* (Jacq.) P. Kumm. (CCBAS 477, from the Culture Collection of Basidiomycetes, Prague) and *Trichoderma harzianum* Rifai (MK 450) were used as cellulase producing standards. The abbreviation AK stands for strains of A. Kubátová, MK for strains of M. Kolařík.

Two methods were used to detect cellulolytic enzymes (cellulase, endoglucanase) (Pointing 1999). The enriched cellulosis basal medium (CBM) consisted of: $C_4H_{12}N_2O_6$ – 10 g, KH_2PO_4 – 2 g, $MgSO_4 \times 7H_2O$ – 1 g, yeast extract – 1 g, $CaCl_2 \times 2H_2O$ – 0.002 g, distilled water – 1 litre. Inoculum discs (5 mm diam.) were cut from the actively growing edge of colonies grown on CBM supplemented with 2 % w/v glucose. Production of cellulase was tested by degradation of cellulose azure agar. The CBM medium supplemented with 2 % w/v agar transferred in 10 ml glass culture bottles was overlaid by 0.1 ml viscous CBM medium supplemented with 1 % w/v cellulose azure (Sigma). The medium was inoculated with discs of the test fungi and incubated. Migration of dye into the clear lower layers indicates the presence of cellulases (Pointing 1999). Endoglucanases were tested by degradation of carboxymethylcellulose (CMC) agar. The CBM medium was supplemented with 1 % w/v low viscosity CMC, and 2 % w/v agar was added. This medium dispensed into Petri dishes was inoculated with the test fungi and incubated at room temperature. After growth for 14 and 21 days, the plates were flooded with 0.08 % aqueous Congo Red (J. R. Geygi S. A., Bâle, Switzerland), and allowed to sit for 10 minutes. The stain was washed from the agar surface with distilled water and Petri dishes were flooded with 1M NaCl to destain for 15 minutes. The NaCl solution was then removed. CMC degradation around the colonies appears as an yellow-opaque area against a red colour for un-degraded CMC (Pointing 1999).

RESULTS AND DISCUSSION

General results

During this study, several groups of fungi appeared to be frequently associated with *Scolytus intricatus* and its galleries in branches of four species of oak and one species of beech in eight localities studied. The most frequent were yeasts, *Geo-*

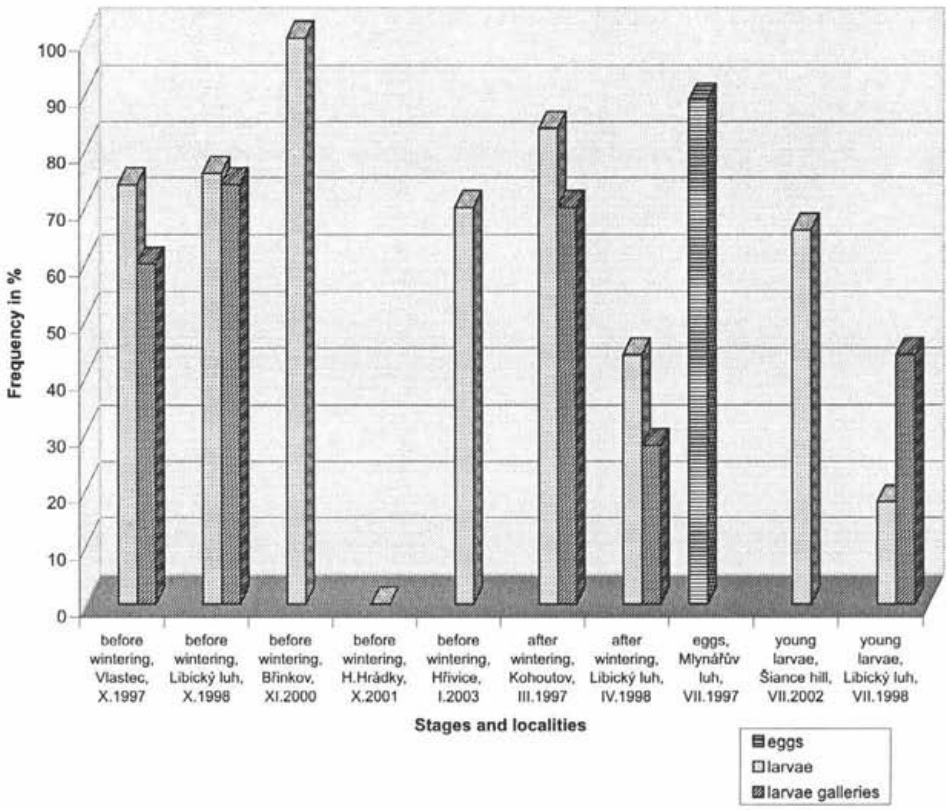


Fig. 1. Occurrence of *Geosmithia* on eggs ond larvae of *Scolytus intricatus* and in larvae galleries in the studied localities.

smithia spp., and *Penicillium* spp. Besides these micromycetes, ophiostomatoid fungi (mainly *Ophiostoma piceae* (Münch) Syd. et P. Syd. s.l.) and other fungi were also isolated. For preliminary results see Kubátová et al. (2002).

A comparison of the occurrence of *Geosmithia* in individual collections is presented in Fig. 1 and 2.

It is noteworthy that *Geosmithia* strains were isolated from *Scolytus intricatus* at seven from eight study sites, from all five tree species (*Quercus dalechampii, Q. petraea, Q. polycarpa, Q. robur,* and *Fagus sylvatica*) and were present in almost all life cycle stages of *Scolytus intricatus* (larvae before and after wintering, adults before emergence, females in generation feeding, dead females after oviposition, eggs) and also in galleries. The exception was the locality in the Bohemian Forest (near Horní Hrádky) where *Geosmithia* strains were not isolated from larvae. However, *Geosmithia* was detected here directly in the larval gallery (on the feeding) incubated in a moist chamber.

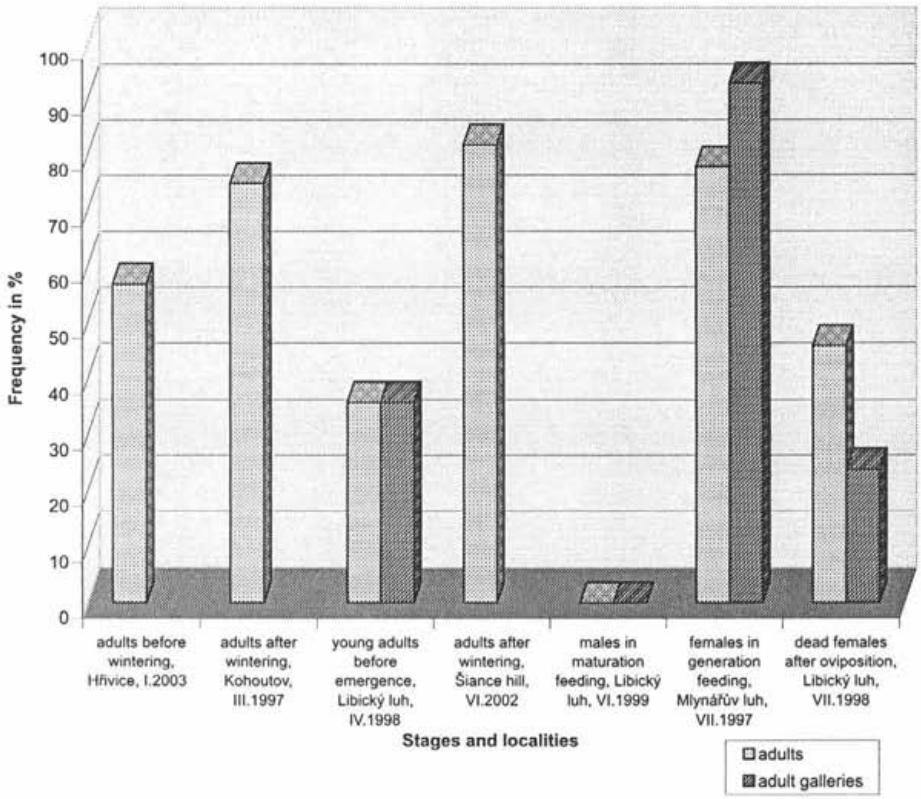


Fig. 2. Occurrence of *Geosmithia* on adults of *Scolytus intricatus* and in their galleries in the studied localities.

Another interesting exception concerned males of *S. intricatus* in maturation feeding (Fig. 2). From these males and in their small boreholes, *Geosmithia* strains were not isolated. However, samples of this stage were collected only once, in the locality Libický luh, in June 1999. Therefore, this result cannot be generalised.

The frequency of *Geosmithia* in other stages of life cycle of *Scolytus intricatus* was in the range 18–89 % in all samples. On samples of larvae, adults and eggs *Geosmithia* was recorded in the ranges 18–84 %, 36–78 %, and 89 %, respectively. *Geosmithia* strains were also isolated from galleries, with frequencies in the range 24–93 %. In galleries of larvae and adults, *Geosmithia* was detected in 28–74 % and 24–93 % of samples, respectively. The highest frequency of occurrence (93 %) was recorded in galleries of adults (females) in generation feeding in the locality Mlynářův luh in July 1997 (Fig. 2).

The abundance of *Geosmithia* depends obviously on many factors, primarily on occurrence of bark beetles in each locality, on the state of health of the trees and

on moisture conditions in samples of tree branches. We did not record a connection of *Geosmithia* strains to any specific stage of the life cycle of *Scolytus intricatus*. Nevertheless, based on the results from all study sites we conclude that our *Geosmithia* strains are closely associated with oak bark beetle *Scolytus intricatus*. It is apparent that the surface of the insect body and galleries are probably one of their major and still not explored niches. Besides association with bark beetles, no close affinity to other niches has yet been reported for this group of *Geosmithia* species in the Czech Republic.

Geosmithia identification

During this study we isolated over 80 strains of *Geosmithia*. Our *Geosmithia* isolates were whitish to light brown and had rough-walled penicillate conidiophores with cylindroidal phialides and predominantly cylindrical conidia. Thus, under the stereomicroscope they can to inexperienced mycologists resemble a whitish *Penicillium*, white *Paecilomyces*, *Mariannaea elegans* (Corda) Samson, *Doratomyces putredinis* (Corda) F. J. Morton et G. Sm. or *Clonostachys rosea* (Link) Schroers et al. All these fungi occur on wood or bark, too. However, *Paecilomyces* differs from our *Geosmithia* isolates in phialides with a distinct long neck, *Mariannaea elegans* is distinguished by verticillate conidiophores (Domsch et al. 1980), *Doratomyces putredinis* has smooth conidiophores and annellophores (Morton and Smith 1963), and *Clonostachys rosea* forms two types of conidiophores. The shape of the conidia of the cited fungi is also different. Interestingly, for all these fungi the name *Penicillium* was used for a certain time. According to Pitt (1979), *Geosmithia* differs from *Penicillium* by its cylindrical conidia and rough-walled cylindroidal phialides. Following re-examinations by Pitt and Hocking (1985) and by Stolk and Samson (1985) have shown that these criteria are not complete and they allow a short but distinct neck on the phialides. Our *Geosmithia* isolates clearly differ from *Penicillium* in their cylindrical conidia and rough-walled cylindroidal phialides without distinct neck.

The genus *Geosmithia* includes ten species at present: *G. argillacea* (Stolk et al.) Pitt, *G. cylindrospora* (G. Sm.) Pitt, *G. eburnea* Yaguchi et al. (teleomorph *Talaromyces eburneus* Yaguchi et al.), *G. emersonii* (Stolk) Pitt (teleomorph *T. emersonii* Stolk), *G. lavendula, G. malachitea* Yaguchi et Udagawa (teleomorph *Chromocleista malachitea* Yaguchi et Udagawa), *G. namyslowskii* (K. M. Zalessky) Pitt, *G. putterillii, G. swiftii* Pitt (teleomorph *T. bacillisporus)*, and *G. viridis* Pitt (Pitt 1979; Pitt and Hocking 1985; Yaguchi et al. 1993, 1994). Our strains lack green pigments in obverse (typical of *G. eburnea, G. namyslowskii,* and *G. viridis*) and reverse (typical of *G. cylindrospora, G. malachitea* and *G. swiftii*), and also violet pigment (*G. lavendula*). Our strains do not grow at 37 °C (*G. argillacea,*

G. eburnea, G. emersonii and *G. swiftii* grow very well). Four species are associated with teleomorphs (see above). In our isolates the teleomorph was not observed. However, the range of morphological features of our isolates is too broad for the remaining tenth species, *Geosmithia putterillii*. Consequently, a more detailed taxonomic study based also on molecular methods is needed.

Morphological examination of our strains led to the distinction of four major groups differing in colony colour (white, whitish, yellowish or buff), colony surface (velutinous, lanose or funiculose), length of conidiophores, shape and size of conidia and arrangement of conidial chains.

The first group (Figs. 3 and 4), represented by strains AK 47/98, AK 119/98, AK 120/98, AK 128/97, and AK 142/98, had whitish colonies, a velutinous to sometimes crustose or slightly lanose surface, monoverticillate to quaterverticillate penicilli, and cylindrical conidia (3.8–5.0 × 2.0–2.5 μm).

The second group, represented by MK 142, was morphologically similar to the first group but sporulated in various shades of yellow. These two groups had an arrangement of conidial chains similar to the ex-neotype strain of *G. putterillii* (NRRL 2024).

The third group, represented by the strains CCF 3340, CCF 3341, CCF 3344, and CCF 3353, had light brown coloured colonies and cylindrical conidia (3.2–4.0 × 1.5–2.0 μm). Its either funiculose or velutinous colonies were closely related to the ex-type strain of *Penicillium pallidum* (NRRL 2037). Although these two species (*Geosmithia putterillii* and *Penicillium pallidum*) are considered by Pitt (1979) conspecific, our preliminary results revealed some differences.

The fourth group (Figs. 5 and 6), represented by the strains AK 105/97, AK 106/97, AK 207/98, AK 48/98 and MK 143, had white, lanose colonies, less branched conidiophores and elliptical to broadly elliptical conidia (3.8–4.5 × × 2.5–3.0 μm). This morphologically well-defined group did not fit to any *Geosmithia* species description in the recent literature (Pitt 1979; Pitt and Hocking 1985; Yaguchi et al. 1993, 1994) and should be described as a new species.

Morphological species may contain cryptic species that are genetically isolated. Species delimitation of mitosporic fungi using only phenotypes is questionable, especially in sympatric living fungi like *Geosmithia* spp. To establish a break of gene flow between sympatrically living cryptic species, the using of molecular markers is necessary. Preliminary results of nucleic acid variation within isolates from *Scolytus intricatus* confirmed our previous morphologically defined grouping, supported the new combination *Geosmithia pallida* (≡ *Penicillium pallidum*) (Kolařík 2002b) and suggested also to distinguish new species in the genus *Geosmithia*. It is evident that *Geosmithia* fungi diversify in bark beetle feedings to many species and this environment represents the home niche of these fungi.

Cellulose degradation

All tested *Geosmithia* isolates were negative for cellulases and endoglucanases. Reference strains of *Pleurotus ostreatus* and *Trichoderma harzianum* were able to degrade both cellulose substrates tested.

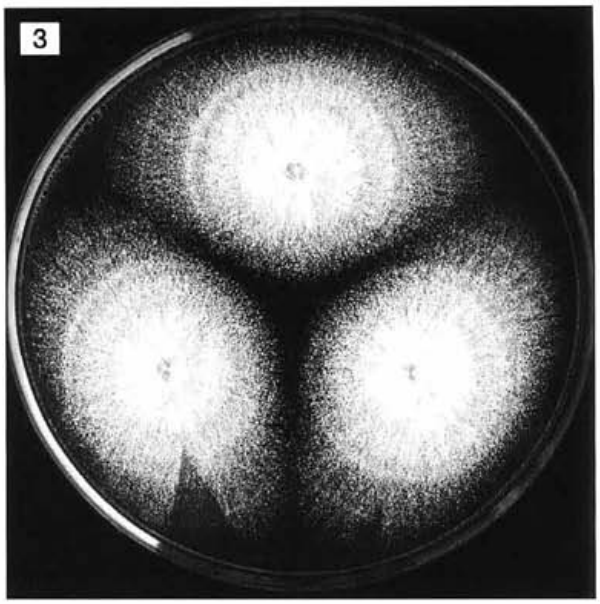
Solid media enzyme assays detecting enzyme synthesis were frequently used in ascomycetes (Untereiner and Malloch 1999, Abdel-Raheem and Shearer 2002). Both methods have their limitations, as reviewed by Pointing (1999) and by Abdel-Raheem and Shearer (2002). Some fungi might be unable to degrade crystaline cellulose (cellulose azure) (Rohrmann and Molitoris 1992, Green III and Highley 1997) or their enzyme production is affected by the mycelial density (Pointing 1999). In spite of these limitations, the cellulose azure method combined with the carboxymethylcellulose agar method is highly recommended as the most reliable qualitative assay for cellulolysis (Pointing 1999).

Ecology and occurrence

Generally, the *Geosmithia* species have so far been encountered rather rarely. In the Czech and Slovak Republics, *G. putterillii* has to date been recorded sporadically: from cereals, out-door air, dust in a flat, leather, and from soil (Nováková and Kubátová 1995, Kubátová et al. 1996). Šrůtka (1996), who studied transmission of fungal spores by *Scolytus intricatus,* did not record *Geosmithia,* but very frequently isolated *Paecilomyces* sp. A personal discussion with him revealed, that his *Paecilomyces* strains could have belonged to *Geosmithia*. Kubátová (2000) published records of *Geosmithia* spp. from *Quercus petraea* and *Q. pubescens* but without data on association with insects. Recently, Kolařík (2002a) started a study on ecology and taxonomy of *Geosmithia* and isolated other *Geosmithia* strains from several bark beetles infesting different trees (Table 1).

The occurrence of *Geosmithia* species in a subcorticolous niche was overlooked for a long time. It was due to: 1) their confusion with morphologically related genera (*Penicillium, Paecilomyces,* etc.); 2) the focusing on ophiostomatoid rather than on auxiliary fungi; 3) the focusing on coniferous bark beetles rather than on those infesting deciduous trees; 4) rare investigations of mycobiota associated with bark beetles without phytopathogenic importance and associated only with unspecific and "unattractive" weed species like *Penicillium*. For these reasons, the only complex data on *Geosmithia* distribution among subcorticolous insects are from Central Europe and Taiwan (Kirschner 1998, 2001; Kolařík 2002a).

Kirschner (2001) mentioned *Geosmithia putterillii* in the group of fungi reported previously from soil and plant remains. Gryndler (1985) studied soil microfungi in Libický luh in the Polabí region (Czech Republic), in one of our localities.



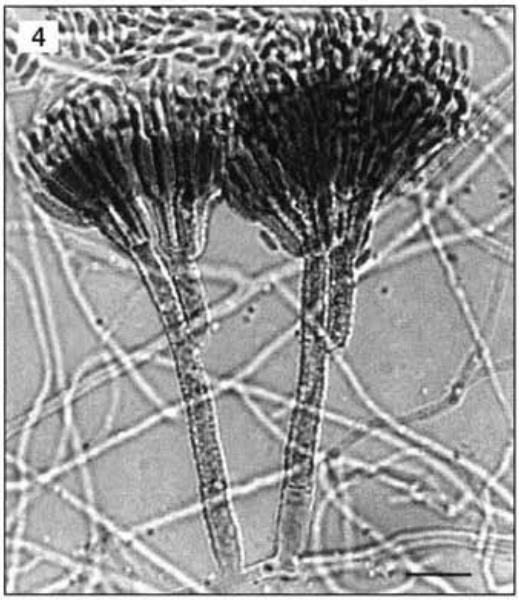
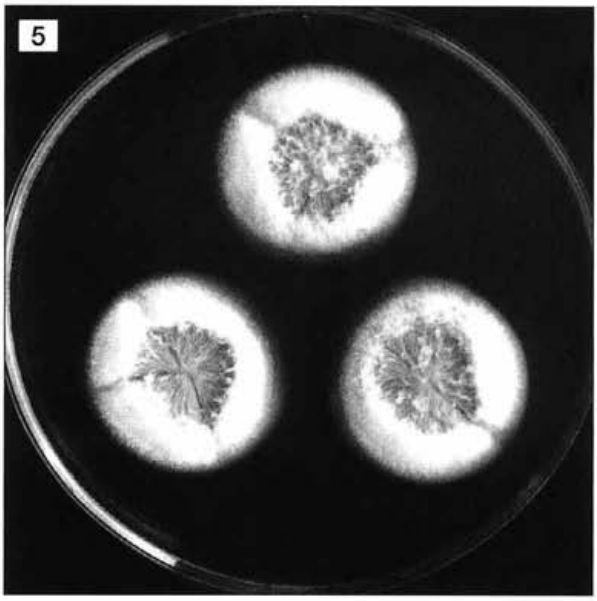


Fig. 3. Colonies on MEA after 14 days at 25 °C, strain AK 128/97.
Fig. 4. Conidiophores with conidia, strain AK 142/98 (Nomarski contrast). Bar = 10 µm.
Figs. 3 and 4. *Geosmithia* sp. (group 1) Photo A. Kubátová



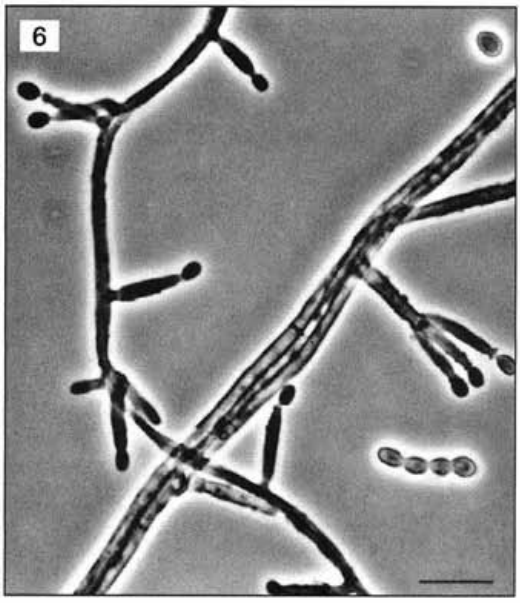


Fig. 5. Colonies on MEA after 14 days at 25 °C, strain AK 48/98.
Fig. 6. Poorly branched conidiophore and single phialides with conidia, strain AK 48/98 (phase contrast). Bar = 10 µm. Photo A. Kubátová
Figs. 5 and 6. *Geosmithia* sp. (group 4)

He did not isolate any strain of *Geosmithia*. During a study of oak endophytes and mycobiota of oak roots Novotný (2003) recorded *Geosmithia* very rarely. We therefore suppose that soil is not the primary niche of *Geosmithia putterillii*.

The mycobiota of *Scolytus intricatus* could be regarded as unspecific, as is typical of other phloem feeding bark beetles associated with *Geosmithia* (Kirschner 2001). The same fungal community was found in other bark beetles listed in the Table 1. An exception was the occurrence of *Geosmithia* on elm beetle (*Scolytus scolytus*), which was associated with the entomophilous species *Ophiostoma novo-ulmi* and on a siricid wasp *Xiphydria* sp., which is typically associated with a mycangial basidiomycete fungus.

Geosmithia was found in most stages of the life cycle of *Scolytus intricatus* under oak bark. For its distribution within localities, the young males of *S. intricatus* in maturation feeding could play a major role. However, *Geosmithia* strains were not found in this stage of its life cycle. In spite of this negative result, it is evident that fungi from the *G. putterillii* complex are facultatively entomophilous, as records from the whole world support it.

Our preliminary data from the most studied insect species (*Scolytus intricatus* and *S. rugulosus*), which were sampled in seven localities in the Czech and Slovak Republics from various host trees, showed that each bark beetle species was characterised by a *Geosmithia* with its own RAPD type specific pattern (Kolařík, unpubl.). This means that they do not transmit a random spectrum of *Geosmithia* fungi, which is suggested by the entomophilous life style of these fungi (Kolařík 2002a). Insect species associated with *Geosmithia* belong to various insect groups but they are nearly uniform in their ecological demands. These insects feed on phloem or inner bark of weakened or recently died deciduous trees. They do not transmit strictly entomochorous species of ophiostomatoid fungi and lack mycetangia or prominent exoskeleton convolutions. It is possible that the adaptation to insect dispersal has parallelled the increase of diaspore production, hence enabling *Geosmithia* to spread by these insects.

In view of the fact that these insect species form very long galleries, which indicates a low nutrition value of the phloem, *Geosmithia* is probably not able to increase nitrogen and phosphorus contents as some ophiostomatoid fungi do (Ayres et al. 2000). Data about entomopathogenicity, conidia resistance against UV-light, diaspore viability after passage through the digestive tract, and ability to degrade chemicals produced by the "tree immunity system" are not known. The profit of *Geosmithia* fungi to insects is not clear and may be only secondary as is the occupation of the niche, which can otherwise be seized by entomopathogenic fungi. Tests of the ability to degrade amorphous and crystalline cellulose show no production of extracellular cellulases. The inability to degrade cellulose limits these fungi to living as primary colonisers in fresh phloem, which is rich in simple sugars. In other habitats these fungi occur rarely, probably because of their

disability to compete with other fungi. It is possible that *Geosmithia* benefits from transportation by bark beetles more than these insects.

Many questions about facultative entomophilous *Geosmithia* species are still open. One of the most interesting one at the end: why do they occur rarely outside the subcorticolous niche, although they produce prodigious numbers of dry conidia? This and other questions remain unanswered and will be addressed in the future.

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Ophiostoma stenoceras and O. grandicarpum (Ophiostomatales), first records in the Czech Republic

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Novotný D. and Šrůtka P. (2004): Ophiostoma stenoceras and O. grandicarpum (Ophiostomatales), first records in the Czech Republic. – Czech Mycol. 56: 19–32

Two species of ophiostomatoid fungi were observed in oaks. *Ophiostoma stenoceras* was isolated during a study of endophytic mycobiota of the roots and seedlings of a sessile oak (*Quercus petraea*). *Ophiostoma grandicarpum* was recorded in the stem of a pedunculate oak (*Q. robur*). These fungi have not yet been reported from the Czech Republic. The knowledge on the occurrence of ophiostomatoid fungi in the Czech Republic is reviewed.

Key words: ophiostomatoid fungi, distribution, oak, roots, bark, *Ceratocystis*, *Quercus petraea*, *Quercus robur*

Novotný D. a Šrůtka P. (2004): Ophiostoma stenoceras a O. grandicarpum (Ophiostomatales), první nálezy v České republice. – Czech Mycol. 56: 19–32

Během studia mykobioty dubů byly pozorovány dva druhy ophiostomatálních hub. Druh *Ophiostoma stenoceras* byl izolován při studiu endofytické mykobioty kořenů dubů a mladých dubových semenáčků (*Quercus petraea*). Druh *Ophiostoma grandicarpum* byl nalezen na kmeni dubu letního (*Q. robur*). V případě obou druhů se jedná o první nálezy z České republiky. V článku je uveden přehled dosud zjištěných druhů ophiostomatálních hub z České republiky.

INTRODUCTION

Ophiostomatoid fungi are associated with different species of bark beetles or plants (especially trees). During the last 25 years, great attention has been paid to these fungi because they cause plant diseases (Upadhyay 1981, Wingfield et al. 1993, Jacobs and Wingfield 2001). This group includes nine genera, which are classified in the orders Microascales and Ophiostomatales (Kirk et al. 2001). Species of *Ceratocystis* and *Ophiostoma* are studied most frequently.

Ophiostomatoid fungi have been known in the Czech Republic since the nineteenth century. The species recorded in the Czech Republic are summarised in Table 1. Corda (1837) described *Graphium penicillioides* on wood of poplar near the city of Prague. Great attention has been paid to the occurrence of ophiostomatoid fungi in the Czech Republic since the 1930s, especially since 1970s, because of a great dieback of elms caused by *Ophiostoma ulmi* ("Dutch

Table 1. Survey of ophiostomatoid fungi recorded in the Czech Republic.

Species	Substrate
Ceratocystis autographa Bakshi	*Tomicus piniperda* (Kotýnková-Sychrová 1966)
Ceratocystis polonica (Siemaszko) C. Moreau	*Ips typographus* (Jankovský et al. 2001)
Ophiostoma bicolor Davidson et Wells	*Ips typographus* (Kotýnková-Sychrová 1966, Jankovský et al. 2001)
Ophiostoma canum (Münch) H. et P. Sydow	*Tomicus minor* (Kotýnková-Sychrová 1966)
Ophiostoma cuculatum H. Solheim	*Ips typographus* (Jankovský et al. 2001)
Ophiostoma minus (Hedgcock) H. et P. Sydow	*Myelophilus piniperda* (Kotýnková-Sychrová 1966)
Ophiostoma minutum Siemaszko	*Ips typographus* (Kotýnková-Sychrová 1966, Jankovský et al. 2001)
Ophiostoma penicillatum (Grosm.) Siemaszko	*Ips typographus* (Kotýnková-Sychrová 1966)
Ophiostoma piceae (Münch) H. et P. Sydow	bark beetles (Kotýnková-Sychrová 1966), cave (Hajdušková 2000), roots of *Quercus robur* and *Quercus petraea* (Novotný 2001), *Scolytus intricatus* (Kubátová et al. 2002), *Ips typographus* (Jankovský et al. 2001)
Ophiostoma piceaperdum (Rumbold) Arx	*Ips typographus*, *Pityogenes chalcographus*, *Xyloterus lineatus* (Kotýnková-Sychrová 1966), *Ips typographus* (Jankovský et al. 2001)
Ophiostoma piliferum (Fr.) H. et P. Sydow	*Pinus* sp., *Picea* sp. (Kotýnková-Sychrová 1966), wood (Páčová et al. 1999)
Ophiostoma serpens (Goidanich) von Arx	*Ips typographus*, *Pityogenes chalcographus* (Kotýnková-Sychrová 1966)
Ophiostoma tetropii Mathiesen	*Picea* sp. (Kotýnková-Sychrová 1966)
Graphium penicillioides Corda	*Populus* sp. (Corda 1837), soil of peat-bog (Kubátová 1998), *Populus nigra* cv. *Italica* (Okada et al. 2000)
Graphium pycnocephalum Grosm.	*Ips typographus* (Kotýnková-Sychrová 1966)
Leptographium lundebergii Lagerberg et Melin	*Hylurgops palliatus*, *Xyloterus lineatus* (Kotýnková-Sychrová 1966)

elm disease" – in the Czech Republic in the periods 1932–1935 and 1972–1983; see Jančařík 1981, 1992) and because *Ophiostoma* species were considered to be the reason of the dieback of oaks ("oak decline") and other trees in this region (Jančařík 1992).

Ophiostoma piceae s. l., *Ophiostoma* spp., *Graphium* sp. and *Leptographium* sp. were isolated from several tree species (e.g. oaks, spruces, pines; see Kubátová and Prášil 1995; Novotný 2001, 2003).

A lot of ophiostomatoid fungi are associated with bark beetles. Kotýnková-Sychrová (1966) studied mycobiota of eight species of beetles (including *Ips typographus*) and recorded 13 species of these fungi. Jankovský et al. (2001) found eight taxa of these micromycetes in mycobiota associated with *Ips typographus* from the Šumava Mts. *Ophiostoma piceae* s.l. and *Ophiostoma* spp. were found on the surface of *Scolytus intricatus* and in its galleries (Kubátová et al. 2002).

O. piceae s.l. was detected by wiping off the surface of a floor of the limestone cave Ostrovské síně (near town Blansko – South Moravia) with cotton swab. The cave is used for speleotherapy (Hajdušková 2000).

Materials and methods

Strains of *Ophiostoma stenoceras* were isolated in November 1999 from peridermal and subperidermal bark of thick roots (2–5 cm) and from fine roots (0.1–0.3 cm) of sessile oak (*Quercus petraea*) from an oak stand locality near Dřevíč in the Křivoklát region in Central Bohemia, Czech Republic. This species was also detected in May 1999 in stems of oak seedlings (*Quercus petraea*) in the village Jíloviště-Strnady (Central Bohemia, near the city of Prague, Czech Republic).

The roots and seedlings were brushed under running water, their surface sterilised (96 % ethanol 1 min., sodium hypochlorite (NAClO) 3 min., 96 % ethanol 0.5 min.) and cut. The thick roots were separated into wood, subperidermal bark and peridermal bark. The seedlings were divided into leaves, stem and roots and were then cut. Pieces of tissues or organs were laid on 2 % malt extract agar and incubated at room temperature up to four weeks.

A strain of *Ophiostoma grandicarpum* was isolated in November 2000 from a branch of *Quercus robur* from the dam of pond Koclířov, near the town of Lomnice nad Lužnicí, Třeboň region, South Bohemia, Czech Republic. Samples of branches were cut into slices 0.5–2 cm thick, which were brushed under running water and then put in sterile, glass moist chambers with sterile cotton wool and sterile filter paper. They were incubated at room temperature for 4–7 weeks.

The isolated strains were deposited in two culture collections of fungi in the Czech Republic [Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, and the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague].

Strains were freeze-dried, preserved under mineral oil or saved agar slant. The strains of *O. stenoceras* were deposited as CCM 8317, CCM 8329, CCF 3261, and the strain of *O. grandicarpum* as CCM 8331.

Growth of the isolated strain was tested on 2 % malt extract agar (MA2), potato-dextrose agar (PDA), potato-carrot agar (PCA) and oatmeal agar (OA). Mycelium of the tested strain was transferred to three Petri dishes per medium.

The identification was based on morphological and cultural features.

RESULTS AND DISCUSSION

Ophiostoma stenoceras (Robak) Melin et Nannf. 1934

= *Ophiostoma albidum* Math.-Käärik 1953. ≡ *Ceratocystis albida* (Math.-Käärik) Hunt 1956. = *Ceratocystis gossypina* Davidson 1971. ≡ *Ophiostoma gossypinum* (Davidson) J. Taylor 1976. = *Ceratocystis eucastaneae* Davidson 1971.

Studied strains (three strains of this species were isolated):

CCM 8317: peridermal bark of thick root of *Quercus petraea*, Dřevíč, Křivoklátsko region, Czech Republic, isol. and det. D. Novotný as no. FF/T/V3, XI. 1999
CCM 8329: oak seedling (*Quercus petraea*), Jíloviště-Strnady (Central Bohemia, near the city of Prague), Czech Republic, isol. and det. D. Novotný as no. D2K3/2TN, V. 1999
CCF 3261: fine root of *Quercus petraea*, Dřevíč, Křivoklátsko region, Czech Republic, isol. and det. D. Novotný as no. FB/L/11, XI. 1999

Macroscopic description

MA2, 28 days, 25 °C: colonies white to luteous, low, exudate absent, reverse pale luteous, pigment absent.

PDA, 28 days, 25 °C: colonies luteous, flat or elevated, exudate absent, reverse pale luteous, pigment absent.

PCA, 28 days, 25 °C: colonies white, flat, low, exudate absent, reverse pale luteous, pigment absent.

OA, 28 days, 25 °C: colonies white, flat, low, exudate absent, reverse white to pale luteous, pigment absent.

The studied strains grow most quickly on OA. The slowest growth was on PDA. Daylight induced formation of perithecia. Perithecia developed earliest and most abundant on OA and PCA medium.

Table 2. Growth of *Ophiostoma stenoceras* on different media at 25 °C

Medium	Colony diam. (mm)		
	7 days	14 days	28 days
MA2	5–7	9.5–11	29–30
PCA	8–9	13–14.5	35–36
OA	12–13	18–22	39–41
PDA	6–9	11–12	18–21

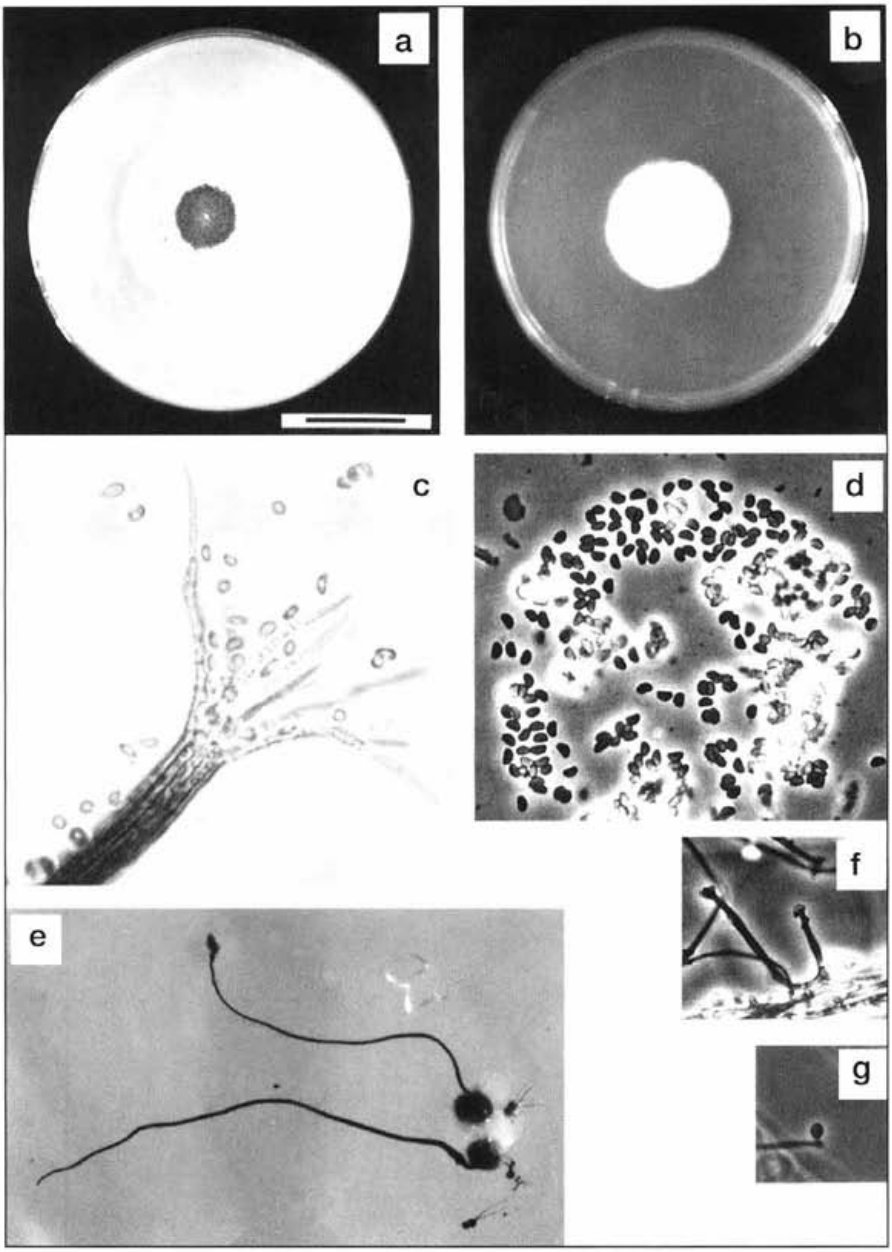

Fig. 1. *Ophiostoma stenoceras* – **a:** four week old colony on OA. **b:** four week old colony on MA2. **c:** apex of neck with ostiolar hyphae. **d:** ascospores. **e:** polyblastic conidiogenous cells. **f:** conidia. **g:** perithecia of *O. grandicarpum* (larger bases with very long necks) and *O. stenoceras* (small perithecia with necks at the bases of *O. grandicarpum*). Scale bar for a, b = 23 mm, c-e = 20 μm, g = 1500 μm.

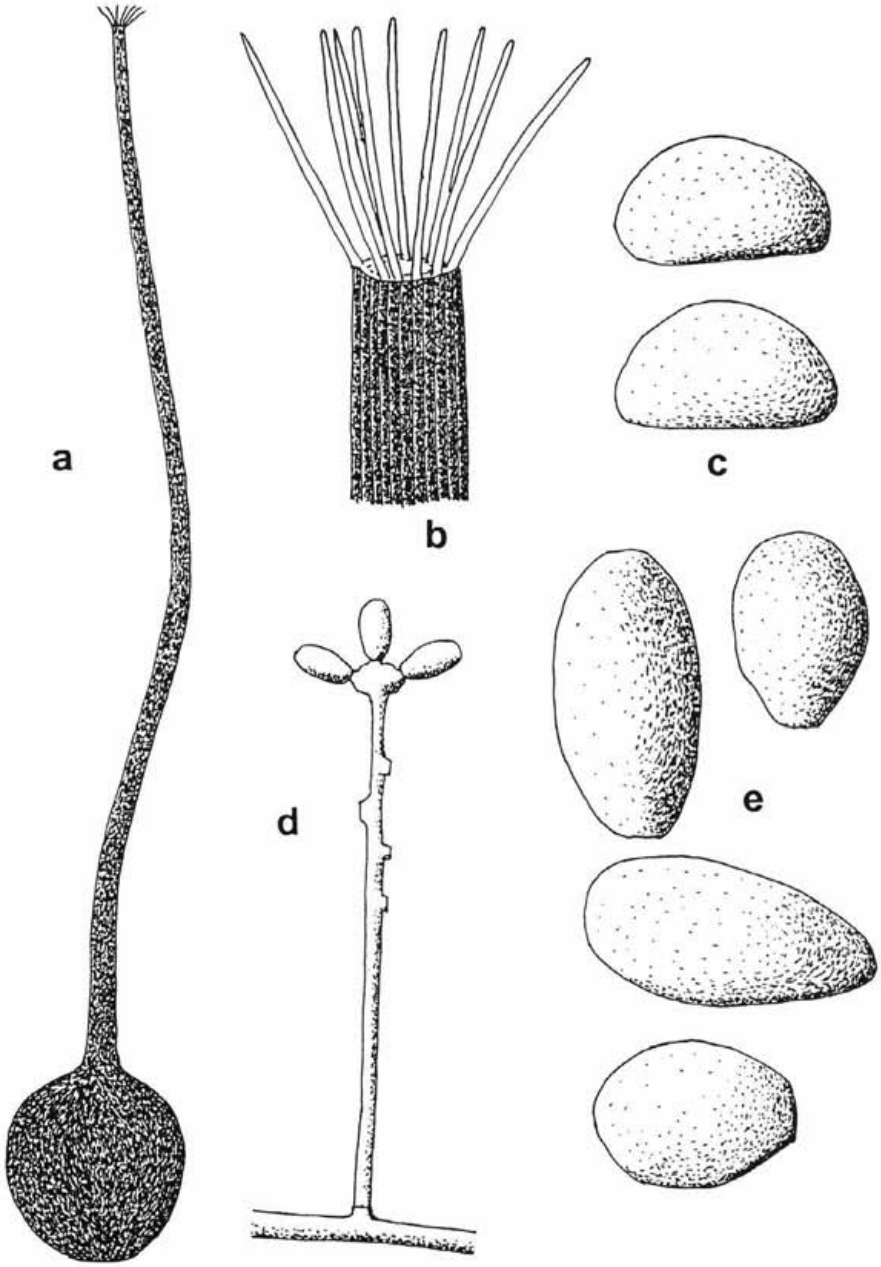

Fig. 2. *Ophiostoma stenoceras* – **a**: perithecium. **b**: apex of neck. **c**: ascospores. **d**: conidiophores. **e**: conidia. Scale bar for a = 80 μm, b = 15 μm, c = 2 μm, d = 10 μm, e = 2 μm. Del. D. Novotný

Microscopic features

Hyphae hyaline, septate, 1–1.5 μm wide, smooth. In culture, perithecia develop superficially on the substrate within 3–4 weeks. Bases globose, 102–132 μm in diameter, black or dark brown. Necks straight or curved, brown to black in colour, 419–756 μm in length, 20–30 μm wide at base, 9–10 μm wide at the tip immediately below the apex. Ostiolar hyphae present, hyaline, septate, divergent, 20–30 μm long and 1.5–2 μm wide at the base, tapering to apex. Ascospores hyaline, one-celled, orange-section shaped in side view, ellipsoid in face view and globose in the end view, 3 × 1.7–2 μm, sheath absent, emerging from the ostiole and forming a spore ball at tip.

Anamorph: *Sporothrix* sp. Conidiophores mononematous or semi-macronematous, hyaline, 1–1.5 μm wide. Conidiogenous cells polyblastic, integrated or discrete, terminal to intercalary, sympodial, denticulate 15–45 μm. Conidia produced sympodially upon the denticles, hyaline, one-celled, obovoid, ellipsoid to globose, tapering towards the base, 3–6 × 2–2.5 μm, solitary or aggregated in a head.

Discussion

So far, this species has been isolated from oaks, pines, chestnut, fir, soil, seawater, *Erica gracilis* and man (Anonymus 2001, Kowalski and Butin 1989, Przybyl 1991, Upadhyay 1981). It has been recorded in oaks in Poland, Austria and Germany. It was found in discoloured or necrotic spots of bark or wood of oaks with oak decline symptoms (Balder 1991, Cech 1991, Kowalski and Butin 1989, Przybyl 1991). In the present study, Novotný isolated this fungus from two healthy roots (without any discoloured or necrotic spots) of two oak trees (*Quercus petraea*) without or with a low degree of oak decline and in a healthy oak seedling. This report is the first one from the Czech Republic.

There were differences observed in growth rates between the strains from the Czech Republic and the strains studied by Kowalski and Butin (1989) and Upadhyay (1981). The strains from the Czech Republic grow slower than those recorded by other mycologists (Kowalski and Butin 1989, Upadhyay 1981).

The ascospores of strains from the Czech Republic (the present study) and Poland (Kowalski and Butin 1989) are broader than those recorded by Upadhyay (1981). The anamorphic state of this species is similar to that of *Sporothrix schencki* Hektoen et Perkins. The conidia of both species are ellipsoid or subglobose (Summerbell et al. 1993).

In the present study, the identification of *O. stenoceras* was based on differences in ascospore, anamorph and perithecium morphology (length of necks and presence of ostiolar hyphae). Five *Ophiostoma* species [*O. epigloeum* (Guerrero) de Hoog, *O.*

grandicarpum (Kowalski et Butin) Rulamort, *O. introcitrinum* (Olchow. et J. Reid) Georg Hausner, J. Reid et Klassen, *O. megalobrunneum* (Davidson et Toole) de Hoog et Scheffer and *O. stenoceras*] are similar in having orange-section shaped ascospores without a gelatinous sheath (Kowalski and Butin 1989, Upadhyay 1981).

In the length of perithecial necks, *Ophiostoma stenoceras* (400–1400 μm) resembles *Ophiostoma megalobrunneum* (950–1500 μm), *O. introcitrinum* (337–510 μm) and *O. epigloeum* (170–700 μm), but differs in other perithecial characters (size of perithecia, presence of ostiolar hyphae, colony colour). Perithecia of *O. megalobrunneum* are 327–500 μm in diameter, necks have ostiolar hyphae and colonies are buff to dark brown or black (Upadhyay 1981). The necks of *O. introcitrinum* and *O. epigloeum* lack ostiolar hyphae and their perithecial bases are 117–225 μm and 115–170 μm in diameter, respectively. Colonies of *O. stenoceras* are white to dull grey, necks have ostiolar hyphae and perithecial bases are 65–200 μm in diameter (Upadhyay 1981). The necks of *O. grandicarpum* have no ostiolar hyphae and are several times longer than the necks (with ostiolar hyphae) of *O. stenoceras* (Kowalski and Butin 1989).

Ophiostoma stenoceras, *O. epigloeum* and *O. megalobrunneum* are associated with *Sporothrix* anamorphs, but the anamorphic states of *O. grandicarpum* and *O. introcitrinum* belong to the genera *Hyalorhinocladiella, Hyalodendron* and *Pesotum* (Upadhyay 1981, as *Hyalopesotum*), respectively. Further, *Ophiostoma megalobrunneum* is associated with a yeast anamorph (Kowalski and Butin 1989, Upadhyay 1981). Conidiogenous cells of the *Sporothrix* anamorph of *O. epigloeum* differ in shape from conidiogenous cells of the *Sporothrix* of *O. stenoceras* (Upadhyay 1981).

Ophiostoma grandicarpum (Kowalski et Butin) Rulamort 1990

≡ *Ceratocystis grandicarpa* Kowalski et Butin 1989

Representative strain (a single strain of this fungus was isolated only):

CCM 8331: branch of *Quercus robur*, dam of pond Koclířov – near the town of Lomnice nad Lužnicí, Třeboň region, South Bohemia, Czech Republic, isol. P. Šrůtka as no. Oph 24, XI. 2000, det. D. Novotný

Macroscopic description:

MA2, 28 days, 25 °C: colonies cream, low, exudate absent, reverse cream, pigment absent.

PDA, 28 days, 25 °C: colonies luteous, low, exudate absent, reverse pale luteous, pigment absent.

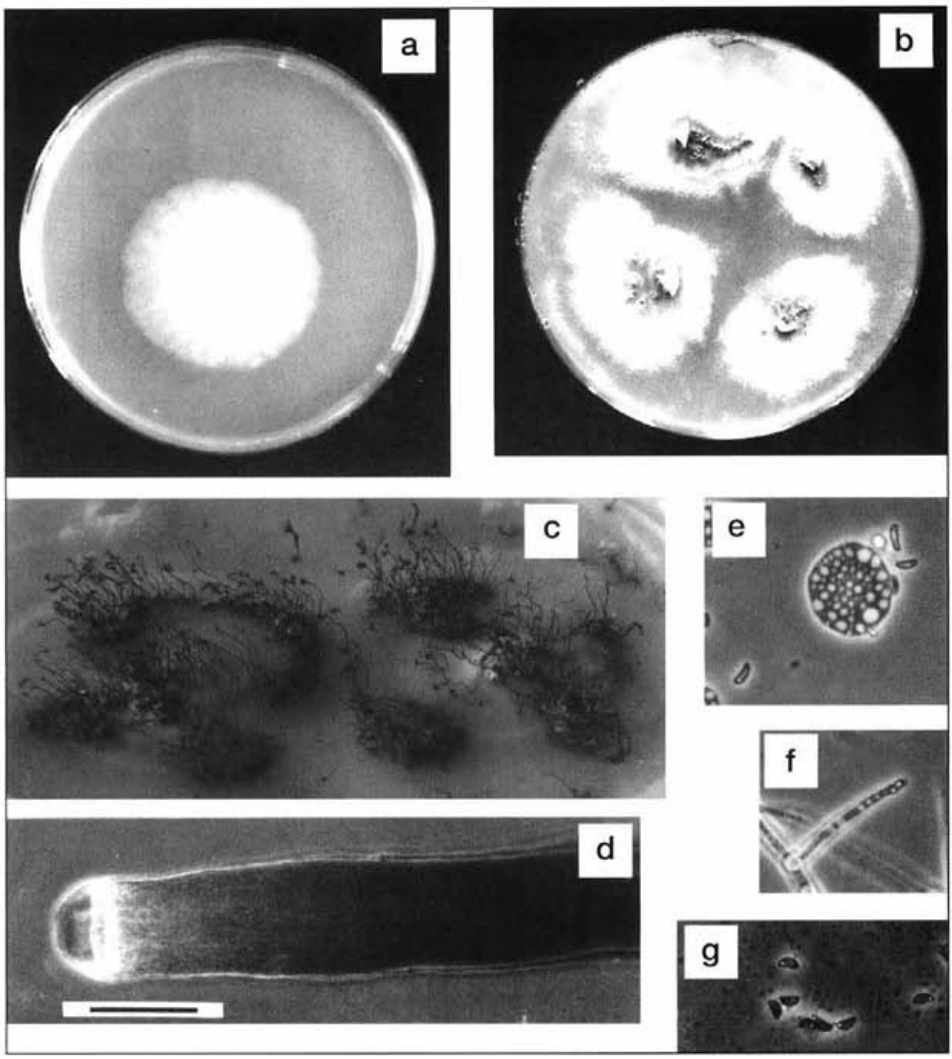

Fig. 3. *Ophiostoma grandicarpum* – **a:** four week old colony on PCA. **b:** three month old colonies on MA2. **c:** perithecia of *O. grandicarpum* in six month old colony on MA2. **d:** apex of neck. **e:** ellipsoid slightly curved conidia. **f:** conidiogenous cells. **g:** ascospores. Scale bar for a, b = 23 mm, c = 12 mm, d = 50 µm, e-g = 20 µm.

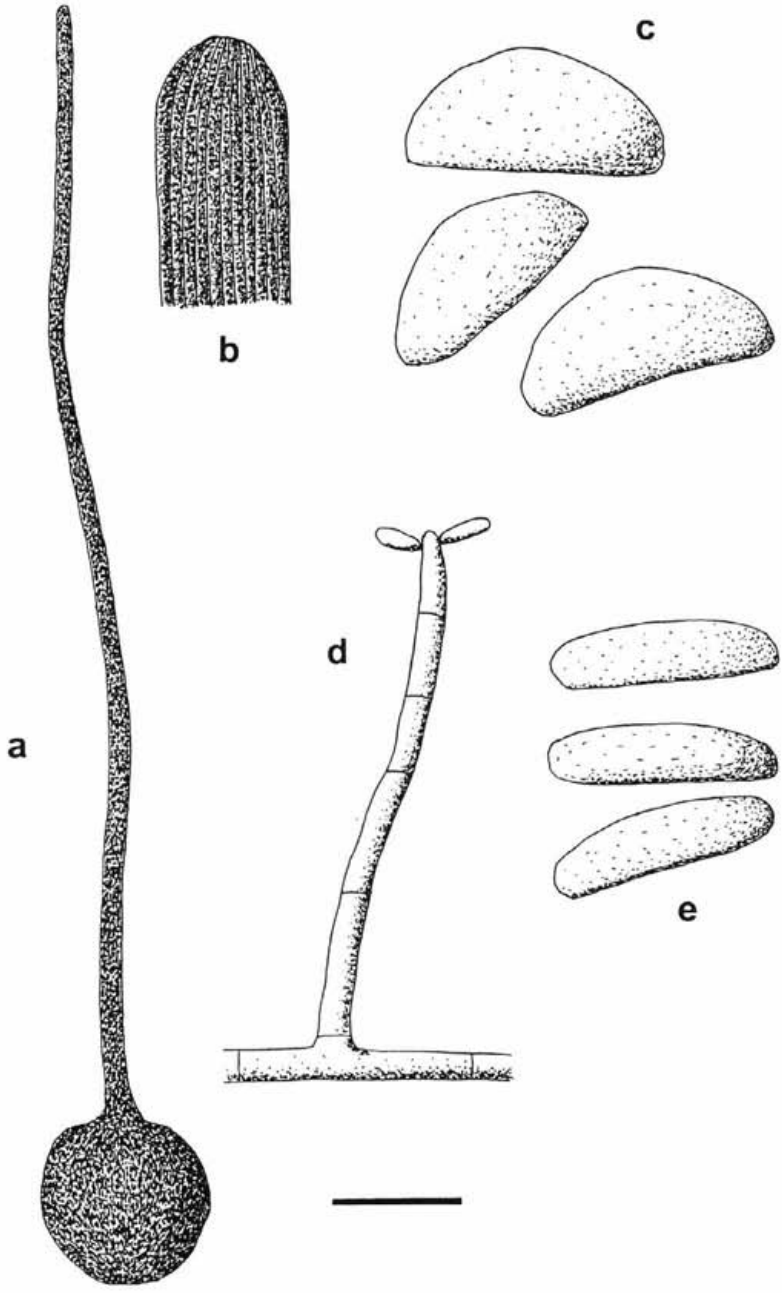

Fig. 4. *Ophiostoma grandicarpum* – **a**: perithecium. **b**: apex of neck. **c**: ascospores. **d**: conidiogenous cells. **e**: conidia. – Scale bar for a = 500 μm, b = 50 μm, c = 2 μm, d = 10 μm, e = 2 μm. Del. D. Novotný

PCA, 28 days, 25 °C: colonies white, exudate absent, reverse white to cream, pigment absent.

OA, 28 days, 25 °C: colonies white, flat, exudate absent, reverse white to cream, pigment absent.

Table 3. Growth of *Ophiostoma grandicarpum* on different media at 25 °C

Medium	Colony diam. (mm)		
	7 days	14 days	28 days
MA2	9–12	13–16.5	24–30
PCA	9–10	12–13	39–42
OA	8–10	19–20	40–44
PDA	8–9	12–14	22–24

The studied strain grows most quickly on OA and PCA. The slowest growth was on PDA. During the present study, perithecia developed on the MA2 medium, but they did not arise in the dark and on OA, PDA and PCA media. The formation of perithecia was induced by daylight.

Microscopic characters

Hyphae hyaline, 1–2 μm wide, smooth. In culture, perithecia develop superficially in the agar substrate within 4–5 months. Bases globose, 496–734 μm in diameter, black. Necks straight or curved, black in colour, 3470–7300 μm in length, 117.3–146.4 μm wide at base, 43.9–58.6 μm wide at the tip immediately below the apex. Ostiolar hyphae absent. Apex of necks of the 6–8 month old perithecia with brown to black hyphae. Asci globose, broadly ellipsoid, hyaline, 6–9 × 7–10 μm. Ascospores hyaline, one-celled, orange-section shaped in side view and ellipsoid in plane view, 3.5–4 × 1.5–2 μm, sheath absent, emerging from the ostiole. The forming of a spore ball at the tip was not observed.

Anamorph: *Hyalorhinocladiella* state. Conidiophores mononematous, hyaline, terminal, 13–39 × 1.5–2 μm. Conidia in heads, hyaline, one-celled, ellipsoid, the ends obtuse, slightly curved, 3.5–5 × 0.9–1.4 μm.

Discussion

The second author observed this species several times during his study of fungi associated with oak branches by using the moist chamber method, but

no other Czech mycologist recorded *Ophiostoma* species with such extremely long perithecial necks. This species was so far detected in oaks in Poland and Germany (Kowalski and Butin 1989, Balder 1991 etc.). Kowalski and Butin (1989) observed 1.6–1.8 μm wide conidia and two anamorph states: *Hyalorhinocladiella* and *Hyalodendron*.

In the present study, the *Hyalorhinocladiella* state producing 0.9–1.4 μm wide conidia appeared. The *Hyalodendron* state was not detected. The strain from the Czech Republic grows slower than strains obtained by Kowalski and Butin (1989).

Four *Ophiostoma* species with necks longer than 5000 μm are known (Grylls and Seifert 1993). *Ophiostoma novae-zelandiae* (Hutchinson et Reid) Rulamort has ostiolar hyphae at the apex of the neck and its ascospores are narrower than those of *O. grandicarpum* (Hutchinson and Reid 1988). Ascospores of *Ophiostoma nothofagi* (Butin) Rulamort are broader (2.5 μm, Butin and Aquilar 1984) than those of *O. grandicarpum*. The anamorphic state of *O. nothofagi* is *Sporothrix* (Butin and Aquilar 1984), but anamorphs of *O. grandicarpum* are *Hyalorhinocladiella* and *Hyalodendron*. The neck of *Ophiostoma multiannulatum* has divergent ostiolar hyphae, and ascospores are reniform or broadly elliptical in side view (Upadhyay 1981). Ascospores of *O. grandicarpum* are orange-section shaped in side view and the neck lacks ostiolar hyphae (Kowalski and Butin 1989). The strain from the Czech Republic has no ostiolar hyphae and the ascospores are orange-section shaped in side view.

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CZECH MYCOL. 56 (1–2), 2004

Intact leaves as substrate for fungi: distribution of endophytes and phylloplane fungi in rattan palms

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Girivasan K. P. and Suryanarayanan T. S. (2004): Intact leaves as substrate for fungi: distribution of endophytes and phylloplane fungi in rattan palms. – Czech Mycol. 56: 33–43

Leaves of twelve species of *Calamus* from southern India were screened simultaneously for the presence of phylloplane and endophytic fungi. Sampling of 2400 leaf segments yielded 824 endophyte isolates belonging to 34 species. Thirty species of phylloplane fungi were recorded. Several fungal species were found to be shared as endophytes by different hosts. However, the overlap between endophyte assemblage and phylloplane fungi of each host was low, suggesting that these two distinct groups of fungi occupy different niches, thereby avoiding competition.

Key words: phylloplane fungi, endophytes, *Calamus*, India

Girivasan K. P. a Suryanarayanan T. S. (2004): Listy jako substrát pro houby: výskyt endofytů a fyloplánních hub u ratanových palem. – Czech Mycol. 56: 33–43

U listů dvanácti druhů ratanových palem rodu *Calamus* z jižní Indie byl sledován výskyt fyloplánních a endofytických hub. Z 2400 segmentů listů bylo získáno 824 izolátů endofytů patřících do 34 druhů. Bylo zaznamenáno celkem 13 duhů fyloplánních hub. Několik druhů hub se v podobě endofytů vyskytovalo zároveň u několika různých hostitelů. Podobnost druhového složení endofytů a fyloplánních hub byla u všech hostitelů malá, což naznačuje, že tyto dvě odlišné skupiny hub využívají rozdílné niky a tím se vyhýbají vzájemné kompetici.

INTRODUCTION

A leaf, even before abscission, is a suitable substrate for a wide variety of fungi. Apart from pathogenic fungi that infect a leaf and cause disease, a leaf supports two other groups of fungi – symptomless phylloplane fungi and symptomless endophytes. Phylloplane fungi are saprotrophs and are confined to the surfaces of living leaves, while the endophytes are biotrophic mutualists, benign commensals or latent pathogens and reside within the leaf tissues. The endophytes infect leaves of a wide variety of plants and remain without producing symptoms or any negative effects (Stone et al. 2000). There are several studies on fungal endophytes including their association with temperate trees (Carroll and Carroll 1978, Petrini 1986) and, more recently, with tropical plant hosts (Rodrigues and Samuels 1990, Rodrigues

1994, Fröhlich et al. 2000, Rajagopal and Suryanarayanan 2000, Kumaresan and Suryanarayanan 2001, Suryanarayanan and Vijaykrishna 2001, Devarajan et al. 2002, Suryanarayanan et al. 2002, 2003). There are, however, fewer studies on phylloplane fungi than on endophytes (Lee and Hyde 2002) and most of these are focused on pathogens of crops or economically important trees (Pugh and Williams 1968, Bainbridge and Dickinson 1972, Dickinson 1973, Vardavakis 1988, Carris 1992). Also, very few studies compare phylloplane and endophytic fungal communities of the leaf (Petrini 1991) and none exist with reference to tropical hosts. Simultaneous sampling of leaf is essential to understand the occurrence and distribution of these fungal groups in a host leaf. Therefore, we studied the phylloplane and endophytic fungi associated with leaves of 12 rattan palms of southern India.

Rattans (canes) belong to the genus *Calamus* in the family *Arecaceae* (*Palmae*). India has about 60 species of rattan (Renuka 1999) distributed in moist forests of the Western Ghats, sub-Himalayan hills and Andaman and Nicobar Islands (Ravikanth et al. 2002). Rattans are economically important palms and support over 300,000 people in rural India (Ravikanth et al. 2002). Natural populations of rattan are threatened due to habitat destruction and the increasing demand for rattan products (Biswas 1991).

MATERIALS AND METHODS

Collection Site

Rattans were collected from the rattan germplasm collection at Kerala Forest Research Institute, Peechi, Kerala State, southern India (10° 32' N lat. and 76° 32' E lon.). This site is characterised by a tropical warm humid climate. The vegetation is of the moist deciduous type. The mean annual rainfall during the year of collection (Jan 2002 to Dec 2002) was 2849.4 mm. The monthly maximum temperature varied from 37.2 °C (April) to 25.1 °C (July). The collections were made during the southwest monsoon period (mid May to August).

Collection of samples

Twelve species of *Calamus* were selected for this study (Table 1). Twenty-five healthy leaflets from each host were collected and brought to the laboratory in sterilised polyethylene bags. The samples were processed within 24 hours after collection. Twenty-two leaflets were sampled for endophytes and the rest was used for isolating phylloplane fungi.

Table 1. Rattan species studied for phylloplane and endophytic fungi.

Host	Code
Calamus hookerianus Becc.	CH
Calamus thwaitesii Becc. et Hook. f.	CTH
Calamus rotang L.	CR
Calamus metzianus Schlecht.	CM
Calamus travancoricus Bedd. ex Becc. et Hook. f.	CTR
Calamus nagabettai Fernandez et Dey	CN
Calamus vattayila Renuka	CV
Calamus pseudotenuis Becc. ex Becc. et Hook. f.	CP
Calamus tenuis Roxb.	CTN
Calamus tetradactylus Hance.	CTE
Calamus dransfieldii Renuka	CD
Calamus andamanicus Kurz.	CAN

Isolation of endophytes

From each leaflet, ten segments (including midvein and lamina) of approximately 0.5 cm^2 were randomly excised using a pair of sterile scissors. The 220 segments thus obtained were surface sterilised by consecutive immersion for 60 seconds in 75 % ethanol, 180 seconds in NaOCl (4 % available chlorine) and 30 seconds in 75 % ethanol (Fisher et al. 1993). From this, 200 segments were randomly selected and plated on potato dextrose agar medium amended with an antibiotic (150 $mg.l^{-1}$) in Petri dishes (9 cm diam.). The efficacy of surface sterilisation was confirmed by the method of Schulz et al. (1998).

Isolation of phylloplane fungi

All 12 species of *Calamus* were also examined simultaneously for the presence of phylloplane fungi using the leaf washing method (Gunasekera et al. 1997). This method is not quantitative since fungi that adhere firmly to the leaf surface may not be isolated (Lee and Hyde 2002). Ten segments of approximately 0.5 cm^2 in size were randomly excised (including lamina and midvein) and were shaken for 20 minutes in 20 ml of autoclaved sterile water containing 0.05 % Tween 20. A dilution series was prepared from the washings. The aliquots were then plated on PDA medium and observed for the growth of fungi. A 1:10 dilution was found to be the most suitable and this was used for all the hosts studied.

The Petri dishes were incubated at 26 °C for 21 days in a light chamber for endophytes and 7 days for phylloplane fungi. The light regime was a 12 h light:

12 h dark cycle. Endophytic fungi were isolated and colonies were transferred to PDA slants and identified. The sterile isolates were given code numbers based on cultural characteristics (Suryanarayanan et al. 1998).

Analysis of results

Colonisation frequency of endophytes was calculated following the method of Hata and Futai (1995). Colonisation frequency (CF) = $N_{col}/N_t \times 100$, where N_{col} is the number of segments colonised by a particular fungus and N_t is the number of segments observed. For comparing the various groups of fungi (phylloplane vs. endophyte, endophyte vs. endophyte), Jaccard's similarity coefficient was used. Jaccard's similarity index = (c/ (a+b-c)) × 100, where a is the number of fungal species present in host 1; b is the number of fungal species present in host 2 and c is the number of common fungi. Relative percentage of occurrence (RPO) of a group of fungi was calculated using the formula, RPO = (colonisation frequency of one group of fungi/ colonisation frequency of all groups of fungi) × 100.

RESULTS AND DISCUSSION

Sampling of 2400 leaf segments from 12 different *Calamus* species yielded 824 isolates of endophytes belonging to 34 species (Table 2). *Calamus vattayila* and *C. andamanicus* showed the highest densities of colonisation by endophytes. In all other cases, the CF % was rather low (Table 2). Previous studies on *Licuala* sp. (Rodrigues and Samuels 1990), *Euterpe oleracea* (Rodrigues 1994), *Sabal bermudana* and *Livistona chinensis* (Southcott and Johnson 1997), and *Trachycarpus fortunei* (Taylor et al. 1999) have also shown that palms are less densely colonised by endophytes. Generally, tropical dicotyledonous trees show higher densities of endophyte colonisation (typically above 80 %) (Lodge et al. 1996; Suryanarayanan et al. 2002, 2003) and hence further studies are needed to explain the low frequency of endophyte colonisation in members of *Arecaceae*.

Coelomycete fungi dominated the endophyte assemblages in ten host species studied (Fig. 1). Coelomycetes are ubiquitous and dominant endophytes in many tropical dicotyledonous trees (Suryanarayanan et al. 1998, 2002). In other palms, the most frequently isolated endophytes were *Idriella* sp., *Glomerella cingulata* (Stonem.) Spauld et Schrenk. and *Letendraeopsis palmarum* K. F. Rodrigues et Samuels (Rodrigues and Samuels 1990, Rodrigues 1994, Taylor et al. 1999). Xylariaceous fungi are common endophytes of tropical plants including palms (Rodrigues 1994, Lodge et al. 1996). In the present study also, a xylariaceous form (form 3) occurred in 8 of the 12 rattan species screened. This fungus was the dominant endophyte in *Calamus thwaitesii* and *Calamus rotang* (Table 2).

Table 2. Colonisation frequency of endophytic fungi isolated from leaves of rattan plants. See Table 1 for host codes.

Fungus	CH	CTH	CR	CM	CTR	CN	CV	CP	CTN	CTE	CD	CAN
Ascomycetes												
Xylariaceous form 1		4.0										
Xylariaceous form 2		6.0										
Xylariaceous form 3	8.0	10.5	16.5	1.0	7.5		1.0	1.5		1.0		
Xylariaceous form 4			2.5									
Xylariaceous form 5			0.5									0.5
Xylariaceous form 6			0.5									
Coelomycetes												
Colletotrichum sp. 1									1.5	0.5		
Colletotrichum sp. 2		1.5										
Phoma sp. 1				0.5							1.0	
Phomopsis sp. 1	4.5	7.5	4.5		0.5		15.5	0.5		1.0		1.0
Phyllosticta sp. 1	27.5	6.0	11.5	24.5	33.5	6.5	60.0	4.5	18.5	26.0	4.5	65.5
Hyphomycetes												
Aspergillus flavus Link : Fr.											1.5	
Aspergillus niger van Tieghem						0.5						
Aureobasidium pullulans (de Bary) Arnaud	0.5											
Cladosporium sp. 1			0.5								0.5	
Cladosporium sp. 2		0.5	0.5									
Corynespora sp. 1	0.5											
Fusarium sp. 1		1.0										
Fusarium sp. 2					0.5							
Nigrospora sp. 1		1.0										
Zygomycetes												
Mortierella sp. 1		0.5										
Rhopalomyces sp. 1								1.0				
Yeast form												
Yeast sp. 1	0.5											
Sterile forms												
Sterile form 1			0.5				0.5					
Sterile form 2							0.5			0.5	1.5	0.5
Sterile form 3											1.5	
Sterile form 4		1.0										
Sterile form 5	0.5											
Sterile form 6				1.5								
Sterile form 7		1.0										
Sterile form 8								1.0				
Sterile form 9						0.5						
Sterile form 10	0.5			0.5								
Sterile form 11		1.0										
Total CF %	**42.5**	**41.5**	**37.5**	**28**	**42**	**7.5**	**77.5**	**8.5**	**20**	**29**	**10.5**	**67.5**
Total no. of isolates	**85**	**83**	**75**	**56**	**84**	**15**	**155**	**17**	**40**	**58**	**21**	**135**
Total no. of species	**8**	**13**	**9**	**5**	**4**	**3**	**5**	**5**	**2**	**5**	**6**	**4**

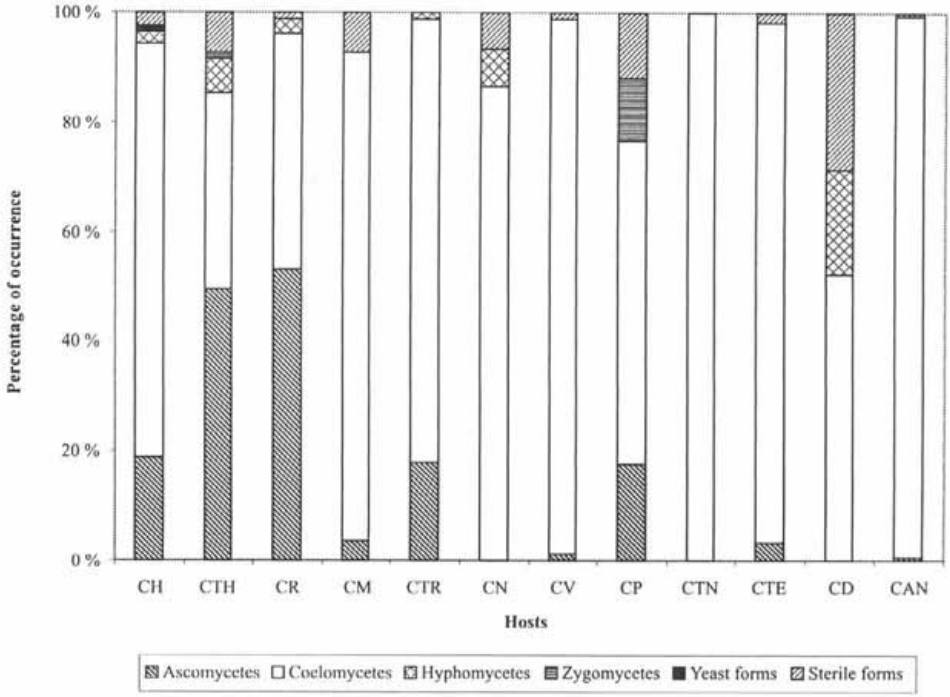

Fig. 1 Relative occurrence of different groups endophytic fungi in rattan palms. See Table 1 for host codes.

Thirty species of phylloplane fungi were recovered in this study (Table 3). These included common phylloplane fungi such as species of *Cladosporium*, *Aureobasidium*, *Aspergillus*, *Pestalotiopsis* and *Colletotrichum* (Cabral 1985, Lee and Hyde 2002).

The overlap between the endophyte assemblages of the different hosts was calculated (Table 4). Out of the sixty six combinations, sixty one had an overlap of more than 10 %. The maximum overlap was between *Calamus vattayila* and *Calamus tetradactylus* (67 %). However, the overlap between the phylloplane and endophytic fungi of each host was low (Table 5). There was absolutely no overlap between these two groups of fungi in seven of the rattan hosts. The maximum overlap was only 17 % (Table 5). Thus, although the leaves of the 12 different species of rattans harboured phylloplane fungi and endophytic fungi, these two groups were distinct. Only a few fungi, such as *Aureobasidium* and *Cladosporium* occurred both as endophytes and phylloplane fungi. Even these fungi may not be 'true' endophytes (Petrini 1991), because phylloplane fungi, in addition to residing on the surface of the leaf, are known to penetrate occasionally the wax layer or

Table 3. Phylloplane fungi isolated from the host species. See Table 1 for host codes.

FUNGUS	CH	CTH	CR	CM	CTR	CN	CV	CP	CTN	CTE	CD	CAN
Ascomycetes												
Talaromyces sp. 1										+		
Coelomycetes												
Pestalotiopsis sp. 1	+	+		+	+		+				+	+
Phomopsis sp. 1							+		+			
Hyphomycetes												
Aspergillus flavus Link : Fr.												+
Aspergillus niger van Tieghem		+	+	+		+		+	+	+		+
Aspergillus ochraceus Wilhelm	+											
Aspergillus sp. 1						+						
Aureobasidium pullulans (de Bary) Arnaud	+			+	+	+						
Cladosporium sp. 1	+	+	+	+	+	+		+				
Cladosporium sp. 2	+	+	+	+	+						+	
Curvularia lunata (Wakker) Boedijn	+		+	+	+							
Drechslera sp. 1	+											
Drechslera hawaiiensis (Bugnicourt) Subram. et Jain ex M. B. Ellis					+							
Fusarium sp. 1									+			
Fusarium sp. 2										+		+
Fusarium sp. 3								+				
Monodictys levis (Wiltshire) Hughes	+											
Nigrospora sp. 1	+											
Trichoderma sp. 1												+
Penicillium sp. 1	+	+			+							
Penicillium sp. 2			+									
Penicillium sp. 3												+
Zygomycetes												
Mucor racemosus Fresen.	+			+								
Yeast forms												
Yeast sp. 2	+	+	+	+	+		+					
Yeast sp. 3	+	+	+									
Yeast sp. 4		+										
Sterile forms												
Sterile form 12					+							
Sterile form 13					+							
Sterile form 14					+							
Sterile form 15			+									
Total no. of species	**13**	**8**	**8**	**8**	**11**	**4**	**3**	**3**	**3**	**3**	**2**	**6**

Table 4. Similarity coefficients (%) between the endophyte assemblages of different rattan palm species. See Table 1 for host codes.

Host	CH	CTH	CR	CM	CTR	CN	CV	CP	CTN	CTE	CD	CAN
CH	100	17	21	30	33	10	30	30	11	30	8	20
CTH		100	22	13	21	7	20	20	7	20	5	13
CR			100	17	30	9	40	27	10	27	15	30
CM				100	29	14	25	25	17	25	22	13
CTR					100	17	50	50	20	50	11	33
CN						100	14	14	25	14	13	17
CV							100	43	17	67	22	50
CP								100	17	43	10	29
CTN									100	40	14	20
CTE										100	22	50
CD											100	25
CAN												100

Table 5. Similarity coefficients (%) between phylloplane fungi and endophyte assemblage of a rattan host. See Table 1 for host codes.

Host	Similarity coefficient
CH	5
CTH	5
CR	13
CM	0
CTR	0
CN	17
CV	14
CP	0
CTN	0
CTE	0
CD	0
CAN	0

cuticle on the surface of the leaf. When they do so, they escape the effects of surface sterilisation and grow on agar plates (Verhoeff 1974); such fungi would fall within the ambit of endophytes by definition.

It is thought that phylloplane and endophytic fungi are involved in different physiological and ecological phenomena unique to the phyllosphere ecosystem

(Petrini 1991). Phylloplane fungi and endophytic fungi are exposed to different environments. Phylloplane fungi are exposed to rapid environmental changes and have a remarkable ability to withstand periodic wetting and drying (Park 1982), while the endophytes have to defend themselves against the defense reactions of the host (Petrini 1991). Our results indicate that these two distinct groups of fungi (phylloplane and endophytic) may avoid competition by occupying different niches offered by a leaf. Such a compartmentalisation could be advantageous to both groups although a brief period of encounter between the two groups on the leaf surface (when the endophyte propagules fall on the leaf surface and enter the host) is unavoidable.

Computation of the similarity index showed that 5–67 % of the endophyte assemblage was shared by all the hosts indicating that certain fungi such as *Phyllosticta*, xylariaceous forms and sterile form 2 occurred as endophytes in many species of rattan. *Phyllosticta* and xylariaceous forms occur as endophytes in many plant species (Rodrigues 1994, Suryanarayanan et al. 2002). Recently, Suryanarayanan et al. (2002) – while studying the distribution of endophytes in different tropical forests – reported that some endophytes were ubiquitous and could be recovered from host species belonging to different families. Such a lack of host specificity among endophytes could depress fungal diversity in a plant community. Thus, it is clear that certain fungal genera could infect unrelated host species and hence there is no direct relationship between the taxonomy of the hosts and that of their endophytes.

The fact that certain genera of fungi occur invariably as endophytes in leaf tissues of taxonomically unrelated (Suryanarayanan et al. 2002, 2003) and geographically isolated (Suryanarayanan and Kumaresan 2000) host plants strongly suggests that these fungi have evolved strategies to lead an endophytic mode of life. Such fungi could well constitute an ecological group dominating the niche created by the internal tissues of plant hosts. Community ecology studies on tropical endophytes are very few (Arnold et al. 2000, 2001; Suryanarayanan et al. 2002, 2003) and there is much room for further studies on the ecology of fungal endophytes and phylloplane fungi especially since we have very limited knowledge of the spread and stabilisation of tropical endophytes as well as the interaction between these two groups of fungi.

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The first record of Cryphonectria parasitica in the Czech Republic

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Jankovský L., Haltofová P., Juhásová G., Kobza M., Adamčíková K. and Palovčíková D. (2004): The first record of Cryphonectria parasitica in the Czech Republic. – Czech Mycol. 56: 45–51

The causal agent of chestnut blight *Cryphonectria parasitica* (Murrill) M. E. Barr is a quarantine pest that has been recorded for the first time on the territory of the Czech Republic. *Cryphonectria parasitica* was observed in a sweet chestnut in the town of Uherský Brod. Infected tree was imported as a two-year-old seedling from Bratislava (Slovakia), 25 years ago. The isolate of *Cryphonectria parasitica* has been compatible with European vc type 13 (EU 13). *Castanea sativa* Mill. occurs in more than 293 localities in the Czech Republic. Its state of health was checked in 232 localities.

Key words: *Castanea sativa*, chestnut blight, Czech Republic, *Cryphonectria parasitica*, quarantine pest

Jankovský L., Haltofová P., Juhásová G., Kobza M., Adamčíková K. a Palovčíková D. (2004): První nález Cryphonectria parasitica v České republice. – Czech Mycol. 56: 45–51

Původce rakoviny kůry kaštanovníku *Cryphonectria parasitica* (Murrill) M. E. Barr byl v ČR zjištěn poprvé. Infikovaný kaštanovník v Uherském Brodě byl dovezen ze Slovenska, konkrétně z Bratislavy, před 25 lety jako dvouletá sazenice. Izolát *Cryphonectria parasitica* byl kompatibilní s evropským kmenem EU 13. Kaštanovník jedlý se vyskytuje v ČR na více než 293 lokalitách. Kontrola zdravotního stavu byla provedena na 232 z nich.

INTRODUCTION

Chestnut blight fungus *Cryphonectria parasitica* (Murrill) M. E. Barr (syn. *Endothia parasitica* (Murrill) P. J. Anderson et H. W. Anderson) is one of the most serious threats to sweet chestnut. The disease comes from Asia where it occurs on local species of chestnut such as *Castanea crenata* Sieb. et Zucc., *C. mollissima* Bl., *C. seguinii* Dode, *C. henryi* (Skan) Rehder et Wilson, and on representatives of the genus *Castanopsis* (D. Don) Spach. Its unintentional introduction to the American continent is dated back to 1902 or 1904. The infection spread rapidly throughout the

North-American continent. On the European *Castanea sativa*, however, the disease was noticed in about 1880, in the region of the Caucasus (Pridnya et al. 1996). In the region of Western Europe the first data come from Belgium and England in 1925 (Anonymus 1950 ex Juhásová 1999). In France, it was identified on a chestnut tree of Asian origin in 1936 (Darpoux 1948). In 1938, the chestnut blight was detected near Genoa in Italy (Biraghi 1946 ex Juhásová 1999). Damage to the European chestnut *Castanea sativa* has not reached such an extent as the damage to the American chestnut *Castanea dentata*. One of the factors in slowing down the progress of infection in Europe is the simultaneous occurrence of hypovirulent strains of the fungus (Heiniger and Stadler 1991, Seemann and Unger 1993).

In the region of the former Czechoslovakia, chestnut blight was identified at the locality of Prašice – Duchonka, district Topoľčany, Slovakia in 1976 (Juhásová 1999), about 60 km from the Czech border. In the region of the Czech Republic, the disease had not been observed until 2002.

The objective of our research was to assess the state of health of chestnuts with the aim to exclude or confirm the occurrence of chestnut blight fungus (*Cryphonectria parasitica*) in the Czech Republic.

MATERIAL AND METHODS

The distribution and state of health of *Castanea sativa* Mill. in the Czech Republic (CR) was monitored in 232 localities (about 600 trees) out of 293 well-known (Haltofová and Jankovský 2003, 2004). This is about 80 % of the Czech population of chestnuts older than 20 years. Included were different plantings in villages or in forest stands where individual examinations could not be carried out in each of the trees. The tree position was surveyed using GPS for further processing in a GIS.

Voucher specimens are deposited in the herbarium of the Department of Forest Protection, Faculty of Forestry and Wood Technology, Mendel University of Agriculture and Forestry Brno (BRNL).

Isolation of strains

The strain from the first find of *Cryphonectria parasitica* in the CR was used for study. Samples of bark were washed in sterile water, surface-sterilised (96 % ethanol 1 min., sterile water, dipping in ethanol), placed on 3 % (weight) malt extract agar (MEA 3) and incubated at room temperature.

Growth of the isolated strain was tested on 3 % malt extract agar (MA3) at five different temperatures (10, 15, 20, 25 and 30 °C). Mycelium of the tested strains

was transferred to Petri dishes in three replicates per temperature. The cultures were cultivated in the dark.

Beginning with the fourth day after inoculation, the growth of the strains was observed regularly every day. The aspect of colonies was recorded using a digital camera, and pictures were further analysed using a system of image analyses in which the surface of the colonies was measured. The period of fructification was also monitored.

The isolated strain has been deposited in the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, CR under no. CCM 8354.

Determination of vegetative compatibility with EU vc types

The vegetative compatibility (hereafter vc) test for *C. parasitica* was performed according to Cortesi, Milgroom and Bisiach (1996). We used PDAg (Potato dextrose agar green) medium described by Powell (1995): 24 g Difco potato dextrose broth, 2 g yeast extract, 7 g malt extract, 0.8 g tannic acid, 2 mg biotin, 2 mg thiamin, 100 mg methionine, 20 g agar per litre of distilled water and the pH indicator bromocresol green 50 $mg.l^{-1}$. Instead of Difco potato dextrose broth we used broth prepared as follows. 200 g skinned potatoes were cooked in one litre of distilled water for one hour. Then they were percolated through gauze. The broth was filled up to one litre with distilled water, and 20 g glucose was added.

Mycelial plugs were removed from the margin of actively growing colonies of *C. parasitica* from 2 % malt agar. Pairs of plugs were placed in contact with the mycelial surfaces on the medium. Each of six pairs of plugs were placed in each of the Petri dishes (9 cm in diameter) with 20 ml of PDAg per plate, about 5 mm from the edge of the dish. The dishes were incubated at 24 °C in the dark and then scored after 5–6 days of cultivation (Cortesi, Milgroom and Bisiach 1996). Each vc test was replicated in a different dish. Incompatible reactions exhibited a demarcation, a dark discoloration in the reaction zone between the colonies in agar, as viewed on the bottom of a Petri dish. Compatible reactions were distinguished by merged colonies with no detectable line (Powell 1995). The isolate of *C. parasitica* CCM 8354 was compared with European testers (EU) of *C. parasitica*. Vc types were labelled with the acronym EU, followed by progressing numbers and may constitute the base for a common European nomenclature. EU testers were established to compare the distribution of vc types of *C. parasitica* in Europe. The original European testers of *C. parasitica* are deposited in ATCC (American Type Culture Collection, www.ATCC.org).

RESULTS

From the total number of assessed trees the causal agent of chestnut blight *Cryphonectria parasitica* was observed only on single, 27 years old tree which was imported as a two-year-old plant from Slovakia, namely from Bratislava, 25 years ago. Localisation: Uherský Brod (eastern Moravia), Za Humny st., co-ordinates: 49° 01' 33" N, 17° 39' 11" E, in a private garden; 27-year-old tree; height 5 m, girth 95 cm (measured at the ground), date: 19^{th} July 2002, rev. 16^{th} October 2002, leg. Pavlína Haltofová, det. Haltofová, Jankovský, Palovčíková, rev. Juhásová. The infected chestnut tree was conspicuous by a fissured longitudinally scaling of the bark and orange-coloured pycnidia of the fungus. Under the bark, a characteristic fan-shaped mycelium was found. In addition to *Cryphonectria parasitica*, fruit bodies of other fungi such as *Coryne sarcoides* (Jacq.) Tul. et C. Tul. and *Stereum hirsutum* (Willd.: Fr.) Pers. were identified in the attacked tree. According to the decision of the State Phytopathological Administration, the infected chestnut tree was felled and removed in March 2003. The isolate of *Cryphonectria parasitica* has been compatible with European vc type 13 (EU 13).

Characteristics of the strain behaviour in a culture

At the beginning, *Cryphonectria parasitica* forms a white mycelium on MA3. Later on, orange fruit bodies are formed. The temperature optimum for the process was 25 °C (Fig. 2). The mycelium, however, fully colonised the surface of the Petri dishes in the course of 3 weeks even at a temperature ranging between 15 and 25 °C. At a temperature of 30 °C, the growth soon stopped. However, the mycelium continued to grow even at a temperature of 10 °C. The culture anamorph fructified first at a temperature of 20 °C, viz. 9 days after inoculation. At a temperature of 25 °C fruit bodies were formed beginning with the 10th day, and one day later the formation of fruit bodies was noticed in cultures cultivated at a temperature of 15 °C. At marginal temperatures of 30 and 10 °C, the formation of fruit bodies was not detected in the course of 22 days.

DISCUSSION AND CONCLUSION

Although the spread of the chestnut blight causal agent by wind is quite common, the occurrence of the disease in an absolutely isolated tree is rather interesting. No more chestnut trees were found in the vicinity. The character of branching of the chestnut near the ground surface could indicate that the infection had taken place long ago. Thus, it is not possible to exclude that the infection was

Fig. 1. The localisation of the find of *Cryphonectria parasitica* (star point). The points indicate the sites with trees of *Castanea sativa* checked from 2001 to 2003.

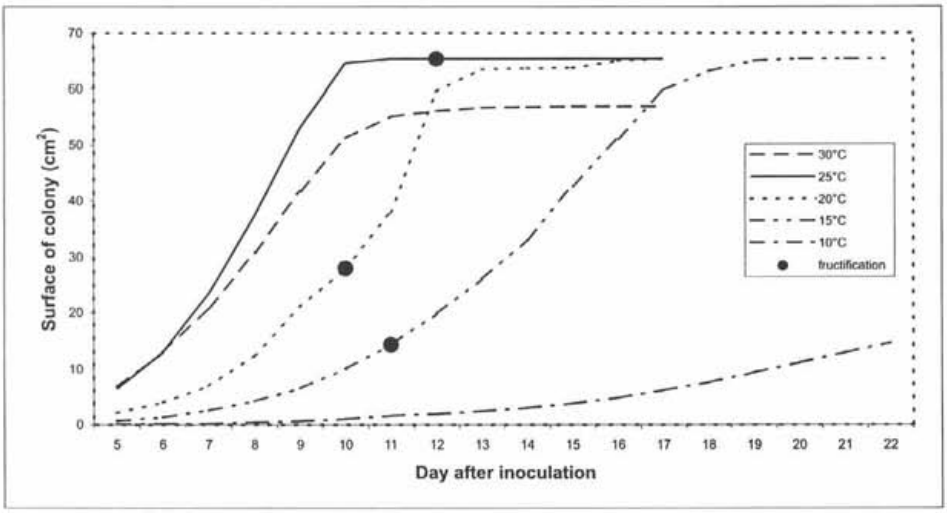

Fig. 2. Growth curves of *Cryphonectria parasitica*, strain no. CCM 8354 at various temperatures.

already transported with a two-year-old plant 25 years ago, i.e. at the time when the first data on the disease were known from Slovakia (1976; Juhásová 1999). The disease may occur in other, unknown and unchecked localities close to the border with Slovakia.

The vegetative compatible group EU 13 (Allemann et al. 1999) has been detected in Slovakia in all three sub-regions of the distribution of *Castanea sativa* where *C. parasitica* was recorded, viz. at 9 localities. In the Malé Karpaty Mts. sub-region, which is geographically the nearest one to the Czech Republic, 44.5 % of examined isolates of *C. parasitica* belonged to the vc group EU 13. The infected tree plant came from Bratislava. However, it is of interest that in Bratislava (Koliba) this vc group has not been identified. On the other hand, in Bratislava – Rača, the vc group includes 28.6 % of the tested isolates. Just in the vicinity of Bratislava (in the Malé Karpaty Mts.), there are localities where the EU 13 group includes even more than 90 % of the tested isolates (Pezinok 96.1 %, Limbach 94.2 %, Griňava 91.1 %). At another localities in Slovakia, the vc group is less numerous. In the region of the Inovec-Tríbeč Mts., it has been recorded at two localities (Lipovník and Radošina), thus representing 13.6 % of the tested isolates. In the third sub-region (Štiavnica-Krupina) which is the most distant from the borderline with the Czech Republic, the EU 13 group includes an even lower number of isolates (5.6 %) (Juhásová and Bernadovičová 2001).

EU 13 was one of the four most frequent vc types in the Carpathian basin (Radócz 2001). In north Italy (Corniglio), 20 % of the samples consisted of vc type EU 13. This vc type was found several times in Bregalia in Switzerland (Bazzigher, Kanzler and Kübler 1981). EU 13 was identified as one of the dominant vc types in Austria (Robin and Heiniger 2001). This vc type is rare in the remaining part of Europe.

The presented occurrence of *Cryphonectria parasitica* is the first documented record of the pathogenic fungus on the territory of the Czech Republic. It is necessary to emphasise that it is so far the only case at more than 232 localities visited. This is roughly about 80 % of the total population of chestnut trees in the CR.

From the epidemiological poit of view, however, new focuses cannot be excluded when their source can only be represented by chestnut trees imported from abroad. With respect to the patchy character of the chestnut distribution in the CR the probability of a rapid spread and of potential economic damage is minimal. However, according to scenarios connected with global climate change it is necessary to consider potential disturbances of natural geographical barriers preventing the spread of some diseases and thus further progress of chestnut blight northwards cannot be eliminated.

In the Czech Republic, chestnuts suffer particularly from non-specific decline at some localities. Numerous factors, both abiotic and biotic, participate in the decline.

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CZECH MYCOL. 56 (1–2), 2004

Contamination of meat stored in home refrigerators in Qena (Egypt)

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Farghaly R. M., Gherbawy Y. A. M. H. and Yosef M. S. (2004): Contamination of meat stored in home refrigerators in Qena (Egypt) – Czech Mycol. 56: 53–62

Eighty samples were collected from different parts of home-refrigerators and meat stored herein, in the province of Qena, Egypt. Quantitative and qualitative estimations of moulds were carried out by conventional methods and the identified *Aspergillus* spp. were confirmed by the RAPD-PCR technique in the Institute of Applied Microbiology (IAM), University of Agricultural Sciences, Vienna, Austria. The obtained results revealed that the highest mould count was 3.9×10^4 CFU/cm^2 in the chest of the refrigerators, followed by 3.2×10^4, 2.6×10^3 and 2.5×10^3 CFU/cm^2 in samples of air and freezer of refrigerators and stored meat, respectively. Eleven mould genera could be identified, the most common of which were *Aspergillus, Penicillium* and *Cladosporium*. The counts and relative frequencies for these genera were 31 (25.4 %), 17 (13.9 %) and 16 (13.1 %), respectively. Five *Aspergillus* species were identified; mainly *A. flavus* 13 (42.0 %), *A. niger* 5 (16.1 %) and *A. nidulans* 5 (16.1 %). The isolated *Aspergillus* species were subjected to further identification by random amplified polymorphic DNA (RAPD) by using type strains from IAM. RAPD-analysis indicated that the *Aspergillus* strains isolated during this study were completely identical with the corresponding type strains from IAM. Public health hazard and significance of mould contamination in home-refrigerators, as well as hygienic measures and recommendations are fully discussed to prevent or minimise such contamination.

Key words: microscopic fungi, stored meat, refrigerators, *Aspergillus*, RAPD-PCR.

Farghaly R. M., Gherbawy Y. A. M. H. and Yosef M. S. (2004): Kontaminace jídla uchovávaného v domácích chladničkách v provincii Qena (Egypt) – Czech Mycol. 56: 53–62

Z různých míst chladniček používaných v domácnostech a z jídla tam uchovávaného bylo odebráno 80 vzorků (provincie Qena, Egypt). Kvalitativní a kvantitativní stanovení mikromycetů bylo provedeno běžnými metodami a určení druhů rodu *Aspergillus* bylo ověřováno molekulárními technikami (RAPD-PCR) v Institutu aplikované mikrobiologie (IAM) na Zemědělské univerzitě ve Vídni. Nejvyšší zjištěné množství mikromycetů bylo 3.9×10^4 CFU/cm^2 ve vnitřním prostoru chladniček, dále pak 3.2×10^4, 2.6×10^3 and 2.5×10^3 CFU/cm^2 ve vzorcích vzduchu, mrazicího boxu chladniček a v uchovávaném jídle. Bylo zjištěno 11 rodů mikromycetů, z nichž nejběžnější byli zástupci z rodů *Aspergillus, Penicillium* a *Cladosporium*. Absolutní a relativní frekvence pro tyto rody byla 31 (25.4 %), 17 (13.9 %) a 16 (13.1 %). Bylo určeno 5 druhů rodu *Aspergillus*, nejčastěji *A. flavus*: 13 (42.0 %), *A. niger*: 5 (16.1 %) a *A. nidulans*: 5 (16.1 %). Určení druhů z rodu *Aspergillus* bylo dále ověřováno metodou amplifikace náhodně zmnožených fragmentů DNA (RAPD) za použití ověřených kmenů z IAM. Tato analýza ukázala, že kmeny druhů z rodu *Aspergillus* izolované během této studie byly zcela identické s ověřenými kmeny z IAM. Závěrem je diskutováno zdravotní riziko, význam kontaminace chladniček v domácnostech a také hygienické podmínky a doporučení směřující k minimalizace kontaminace.

Introduction

Fungi and their mycotoxins are considered a major potential threat to public health and continue to have an extensive impact on the welfare of human and animal populations by affecting not only the quality of meat, but also the availability of clean products. Thus, mycotoxin residues have had a major impact on food industries of the world, which is reflected in the controlled movement of some foodstuffs across international borders. The ultimate concern is that some mycotoxins are highly carcinogenic, mutagenic and teratogenic for humans and animals (Thomas and Lawrence 1977, Bullerman et al. 1986).

Meat begins to be contaminated with moulds in slaughter-houses, mainly with the intestinal contents of slaughter animals, air and dust. The main isolated genera are *Aspergillus, Penicillium* and *Mucor* (Abdel-Rahman et al. 1985, El-Dally et al. 1988, Farghaly 1993). They found that *Aspergillus* species were the most frequent fungi in meat contamination and toxin production, especially *A. flavus* and *A. parasiticus,* which could produce aflatoxins (Ellis et al. 1991, Hamdy et al. 1993).

Some moulds can grow at temperatures in refrigerators typically used in houses and deep freezing has no significant destructive effect upon moulds (Kotinek et al. 1996). Therefore, several mould genera could be isolated from chilled meat, mainly *Aspergillus, Alternaria, Cladosporium, Fusarium, Mucor* and *Penicillium.* These fungi are responsible for spoilage and constitute a real risk to public health due to the production of mycotoxins (Refai and Loot 1969, Mislivec and Tuite 1970, Giradin 1997).

Detection of mould contamination in different parts of refrigerators and meat stored herein is essential to ensure safe and high quality of food. Direct microscopic observation of fungi or cultural methods are frequently used to assess such type of contamination. But as these conventional methods of detection have suffered from several drawbacks, new methods of identification based on PCR techniques have been developed (Messner et al. 1994, Giradin 1997).

Random PCR approaches are being increasingly used to generate molecular markers which are useful for taxonomy and for characterising fungal populations. RAPD-PCR assays have been used extensively to define fungal populations at species, intraspecific, race and strain levels. In general, most studies have been concentrated on intraspecific grouping, although other have been directed at the species level. RAPD-PCR has also been applied in the identification of individual strains within a particular population, some examples being toxin-producing strains of *Aspergillus flavus* (Bayman and Cotty 1993).

Therefore, this study was undertaken to assess mould contamination in home-refrigerators in Upper Egypt and to confirm the isolated strains of *Aspergillus* by using the RAPD-PCR method.

Materials and methods

The conventional techniques

Preparation and cultivation

The materials collected from the province of Qena (10 households) in Egypt were composed of 80 samples: 40 surface swabs taken from chest and freezer of the home-refrigerators, 20 samples of the meat stored (less than 4 weeks) herein, and 20 samples from air inside home-refrigerators. Air samples were obtained by exposing the plates to air for 15 min. while the surface area used for sample collection was 10 cm^2. Specimens were sent to the laboratory, without delay, for preparation of ten-fold dilution up to 10^6 by using sterile peptone water (1 %). One ml of each dilution was poured in duplicated sterile Petri-dishes (12 cm diam.) and one of each covered evenly with sterile melted Malt-extract agar and the other one with Czapek's-Dox agar (pH 4.5). Petri-dishes were then incubated at 25 °C for one week, and examined daily for detection of star-like mould colonies.

Isolation and identification

Detected colonies were picked up with mycological needles and inoculated into sterile slope Malt-extract agar (pH 7) and then incubated at 25 °C for 5 days. The sum of inoculated Malt-extract slope multiplied by the corresponding dilutions represented the total mould count (CFU/cm^2). Then, the isolated cultures in slope agar were further inoculated into Malt-extract agar and Czapek's-Dox agar (pH 7.5) by using 3-point techniques. The identification of mould genera and their species based on taxonomic information permits a general view of most known fungi. This depends mainly on the morphology of the colony, pigmentation of the reverse surface and fungus structures according to Raper and Fennel (1965), Hesseltine and Ellis (1973), Samson et al. (1976) and Rippon (1982).

The molecular technique (PCR)

All *Aspergillus* strains isolated during this study were subjected to further identification by using RAPD-PCR techniques as follows:

DNA extraction

Aspergillus strains were cultured in 100 ml Erlenmeyer-flasks containing 20 ml Mandles Andreoti Medium (per litre, 10 g glucose, 2 g peptone (Difco), 2.8 g ammonium sulphate, 4 g KH_2PO_4, 10 g Na_2HPO_4, 10 ml of a simplified Czapek concentrated (7 g $MgSO_4$, 0.05 g $CuSO_4$ $5H_2O$, 0.1 g $FeSO_4$ $7H_2O$, 0.1 g $ZnSO_4$ $7H_2O$, the final pH was adjusted to 5.0) for five days using a rotary shaker (30 °C, 150 rpm).

The mycelium was collected by filtration and ground to fine powder in liquid nitrogen. Fifty mg of the ground was transferred to a 1.5 ml Eppendorf tube and mixed with 700 μl 2 × CTAB buffer. Eppendorf tubes were incubated at 65 °C for 30 min., then 700 μl of chloroform was added and the mixture was vortexed briefly. The resulting mixture was centrifuged at a maximum speed of 5000 rpm for 30 min. and the clear supernatant was mixed with 600 μl isopropanol chilled to –20 °C. The mixture was centrifuged at a maximum speed for 5 min and the resulting pellet was washed twice with 1 ml of 70 % ethanol. The pellet was dried under vacuum and dissolved in 100 μl TE (10 mM Tris, 1 mM EDTA, pH 7.5) buffer. The DNA concentrations were evaluated by agarose gel electrophoresis.

RAPD – analysis

PCR conditions and separation of RAPD-PCR fragments were made according to Messner et al. (1994). The primers V1 (5' dACGGTCTTGG), V5 (5' dTGC-CGAGCTG) and V6 (5' dTGCAGCGTGG) were used. Synthesis of primers was performed with Codon Genetic systems (Vienna, Austria) using a model 392 DNA synthesizer (Applied Biosystems, Foster City, CA, USA). The temperature profile for primers was as follows: denaturation at 98 °C for 15 s; annealing at 40 °C for 90 s and extention at 72 °C for 100 s for a total of 40 cycles. Amplification products were electrophoresed in 1.4 % agarose gels with 10 × Trisborate-EDTA buffer and were stained with ethidium bromide.

Table 1. Mould counts of 20 contaminated samples from different places in refrigerators and stored meat.

Sources	Chest (CFU/cm^2)	Freezer (CFU/cm^2)	Air (CFU/cm^3)	Meat (CFU/cm^2)
Minimum	33000	220	2400	2000
Maximum	45000	280	4000	3200
Mean	39000	250	3200	2600

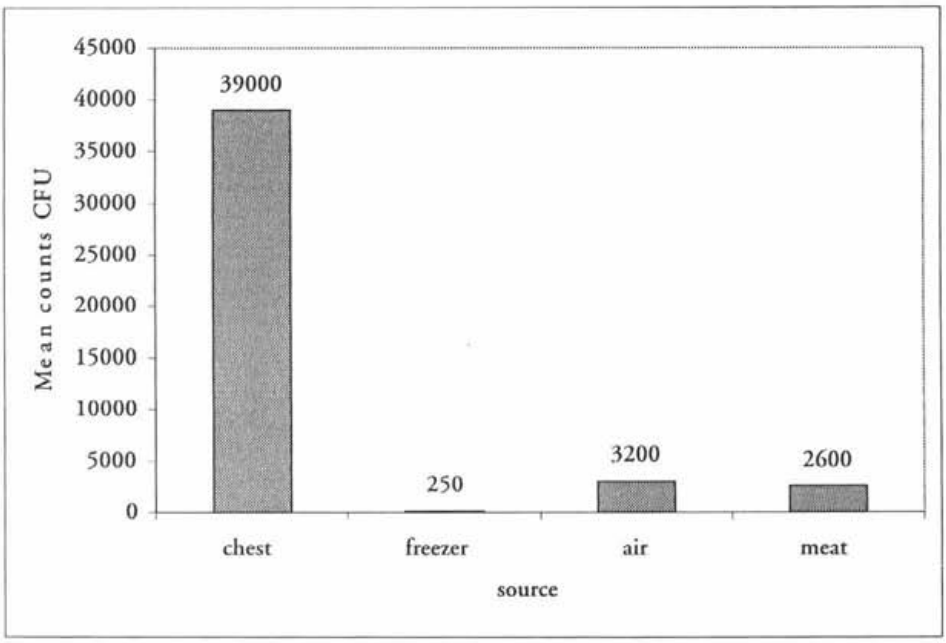

Fig. 1. Mean mould counts at different places in refrigerators and stored meat.

Table 2. Isolated mould genera from different places in refrigerators and stored meat.

Samples	Chest		Freezer		Air		Meat		Total	
	No.	F%	No.	F%	No.	F%	No.	F%	No.	F%
Mould genera										
Alternaria	3	2.5	2	1.6	4	3.3	2	1.6	11	9.0
Aspergillus	10	8.2	6	4.9	8	6.6	7	5.7	31	25.4
Botrytis	3	2.5	2	1.6	0	0.0	2	1.6	7	5.7
Cladosporium	4	3.3	5	4.1	4	3.3	3	2.5	16	13.1
Fusarium	2	1.6	1	0.8	0	0.0	0	0.0	3	2.5
Mucor	4	3.3	0	0.0	3	2.5	1	0.8	8	6.6
Paecilomyces	2	1.6	3	2.0	2	1.6	2	1.6	9	7.4
Penicillium	6	4.9	3	2.5	6	4.9	2	1.6	17	13.9
Rhizopus	0	0.0	2	1.6	2	1.6	2	1.6	6	4.9
Thamnidium	2	1.6	0	0.0	3	2.5	4	3.3	9	7.4
Trichoderma	3	2.5	1	0.8	0	0.0	1	0.8	5	4.1
Total	39	32.0	25	20.5	32	26.2	26	21.3	122	100

No. = number of cases of isolation out of 20 samples.
F% = Frequencies of occurrence in the total fungal isolates.

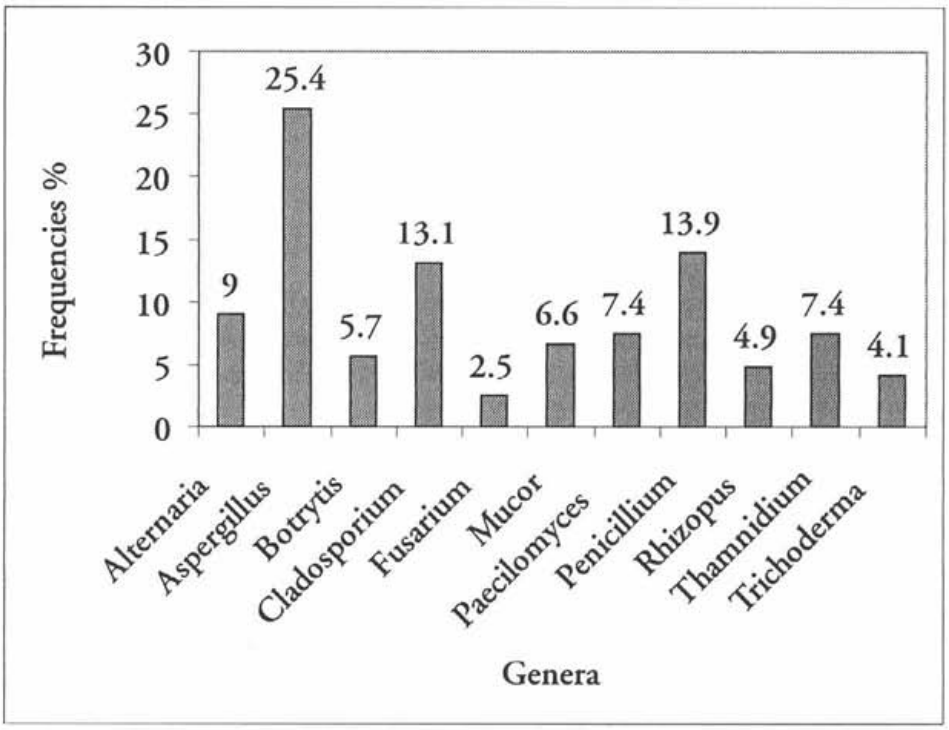

Fig. 2. Isolated mould genera from different places in refrigerators and stored meat.

RESULTS AND DISCUSSION

The obtained results in Table 1 and Fig. 1 show that the averages of total mould counts in the chest, freezer and air of the refrigerators and meat stored herein, were 3.9×10^4, 2.6×10^3, 3.2×10^4 and 2.5×10^2 CFU/cm^2, respectively. These results are more or less similar to those reported by Hamdy et al. (1993), but lower than those detected by Roushdy et al. (1996) and Saad et al. (1998).

Table 2 and Fig. 2 show that eleven mould genera were isolated from different parts of home refrigerators and meat stored herein, the main of which were *Aspergillus, Penicillium*, *Cladosporium* and *Alternaria*. The counts and relative frequencies for these genera were 31 (25.4 %), 17 (13.9 %), 16 (13.1 %) and 11 (9.0 %), respectively. *Paecilomyces, Thamnidium, Mucor*, *Botrytis, Rhizopus, Trichoderma* and *Fusarium* were isolated in lower percentages. For the genera of *Aspergillus* and *Penicillium*, similar results were tabulated by Gill and Lowry (1982) and Abdel-Rahman et al. (1985). Also, Mizakova el al. (2002) studied the occurrence of moulds in fermented raw meat products and reported

Table 3. Isolated *Aspergillus* species from different places in refrigerators and stored meat.

Sources	Chest		Freezer		Air		Meat		Total	
	No.	F%	No.	F%	No.	F%	No.	F%	No.	F%
Aspergillus spp.										
A. flavus	5	16.1	3	9.6	2	9.6	3	9.6	13	42.0
A. fumigatus	1	3.2	1	3.2	1	3.2	1	3.2	4	12.9
A. nidulans	2	6.4	1	3.2	1	3.2	1	3.2	5	16.1
A. niger	2	6.4	1	3.2	2	6.4	0	0.0	5	16.1
A. vitis	0	0.0	0	0.0	2	6.4	2	6.4	4	12.9
Total	10	32.2	6	19.4	8	25.8	7	22.6	31	100

No. = number of cases of isolation out of 20 samples.
F% = Frequencies of occurrence in the total fungal isolates.

that *Penicillium, Acremonium, Mucor, Cladosporium* and *Aspergillus* were the most frequently isolated genera of moulds. The genus *Cladosporium* responsible for black spots in chilled meat were previously detected by Gill et al. (1981) and Roushdy et al. (1996), but with higher frequency. The family *Mucoraceae,* which includes genera *Thamnidium*, *Mucor* and *Rhizopus,* was reported by Hadlok and Schipper (1974) and Refai el al. (1993). Other genera was isolated by Mislivec et al. (1970), Booth (1971) and Mansour (1986).

Table 3 and Fig. 3 indicate that *Aspergillus flavus* was the most predominant species followed by *A. niger, A. nidulans* (= *Emericella nidulans*), *A. fumigatus* and *A. amstelodami* (= *A. vitis*). These results are similar to those obtained by Mislivec et al. (1970) and Mansour (1986), but slightly different from those reported by Hamdy et al. (1993). Hamdy et al. (1993) recorded mean mould counts of surface swab samples obtained from 50 samples fresh and 25 cold stored meat collected from butcher's shops in Giza City of 9.4×10^2 and 8.9×10^3 CFU/cm^2, respectively. The isolated mould genera from the surface of cold stored meat were *Aspergillus, Alternaria, Cladosporium, Mucor, Penicillium* and *Rhizopus* with frequencies of 45.3, 1.9, 13.2, 5.7, 30.2 and 4.3 %, respectively.

Fig. 4 indicate that *Aspergillus* species (*A. flavus, A. fumigatus, A. niger, A. nidulans* and *A. amstelodami)* isolated from home-refrigerators in Qena, Egypt showed RAPD patterns identical with their corresponding type strains from IAM. These results indicate clearly that the results obtained by classical methods for identification of these strains were confirmed with the molecular method.

The results obtained in the present study indicate that moulds are capable of growing in home-refrigerators where they can tolerate lower temperatures. This contributes to various sources of mould contamination such as bad hygiene in abattoirs, meat transportation and personal hygiene in homes. The predominance

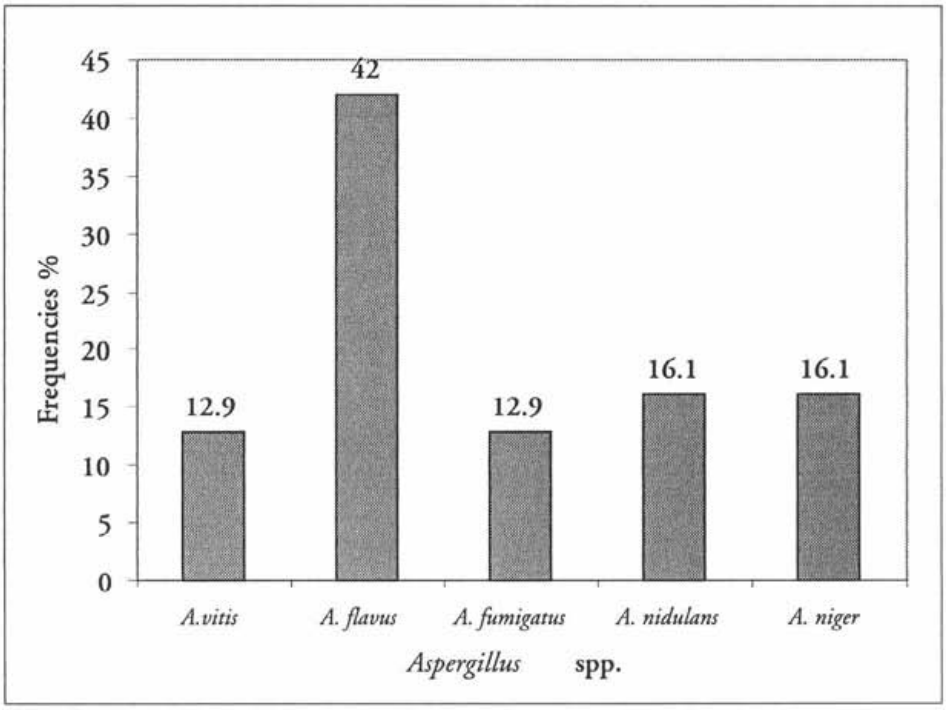

Fig. 3. Isolated *Aspergillus* species from different places in refrigerators and stored meat.

of *Aspergillus* and *Penicillium* species constitutes a real risk to human health due to their ability of mycotoxin production. Van Walbeek et al. (1969) reported that a strain of *Aspergillus flavus* produced aflatoxin at 7.5 °C and 10 °C in 4 weeks. Also, Kiermeier and Behringer (1977) noticed aflatoxin formation in moistened milk powder at temperatures between 1 and 5 °C and at 10 °C. Park and Bullerman (1983) reported that trace levels of aflatoxin (10 to 60 ppb) were detected on summer sausages produced by *Aspergillus flavus* at 5 °C. Hamdy et al. (1993) reported that different *Aspergillus* strains as well as aflatoxins B1, B2, G1 and G2 produced by *A. flavus* could be obtained in variable amounts from fresh and cold meat samples. Also, according to Ostrý (2001), mycotoxins in meat and meat products are produced at temperatures between 4 °C and 40 °C. There are several ways to minimise mould growth in refrigerators. Clean the inside of the refrigerator every month with one table spoon of backing soda dissolved in a quarter of a litre of water. Rinse with clean water, then dry. Mouldy food should be put in plastic bags for disposal in a covered trash can, so that animals and children cannot get into it. To remove mould odour, use 20 ml vinegar / l of water or 40 ml/ l chlorine bleach of water. Rinse with clean water and dry.

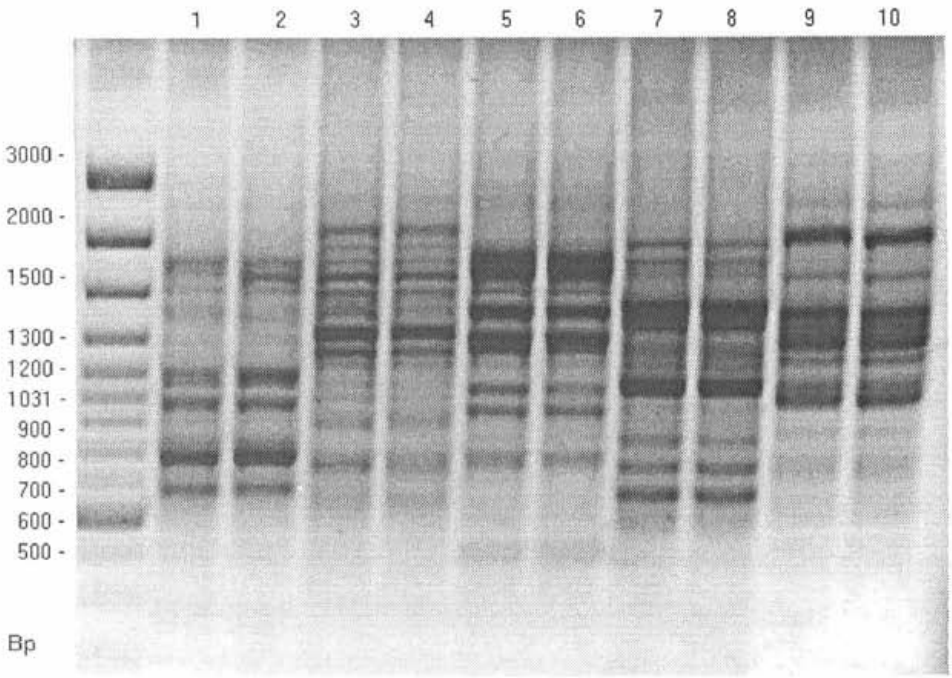

Fig. 4. Pattern of fragments from RAPD analysis of different *Aspergillus* species, printed by M13 oligonucleotide (GAGGGTGGCGGTTCT).

Lane 1 *Aspergillus flavus* from our study
Lane 2 *Aspergillus flavus* MA 86 from IAM (Institute of Applied Microbiology, Vienna, Austria)
Lane 3 *Aspergillus fumigatus* from our study
Lane 4 *Aspergillus fumigatus* MA 148 from IAM
Lane 5 *Aspergillus niger* from our study
Lane 6 *Aspergillus niger* MA 1922 from IAM
Lane 7 *Aspergillus nidulans* from our study
Lane 8 *Aspergillus nidulans* MA 337 from IAM
Lane 9 *Aspergillus vitis* from our study
Lane 10 *Aspergillus vitis* MA 1068 from IAM

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CZECH MYCOL. 56 (1–2), 2004

Fungi on Juncus trifidus in the Czech Republic. I.

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Suková M. (2004): Fungi on Juncus trifidus in the Czech Republic. I. – Czech Mycol. 56: 63–84

Fungi on *Juncus trifidus* were collected and studied during the years 1998–2003, most intensively in 2002. Almost all known localities of this relict plant in the Czech Republic were visited. In this first contribution, 14 species of ascomycetes and anamorphic fungi are mentioned. Populations of *Juncus trifidus* in the Sudetes and Hercynian mountains are small in comparison with populations in the Alps and Carpathians. However, three species of arcto-alpine fungi (*Hysteronaevia minutissima*, *Hysteropezizella diminuens*, *Naeviella paradoxa*) and *Lachnum roseum* have been found there as new records for the Czech Republic. The richest localities of fungi on *Juncus trifidus* are Mt. Sněžka (Krkonoše Mts., Sudetes) and Jezerní stěna rock wall in the cirque of Černé jezero lake (Šumava Mts., Hercynicum).

Key words: Ascomycetes, anamorphic fungi, *Hysteronaevia minutissima*, *Hysteropezizella diminuens*, *Mycosphaerella perexigua* var. *minima*, *Naeviella paradoxa*, *Septoria*, taxonomy, ecology.

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Houby na sítině trojklanné (*Juncus trifidus*) byly studovány v letech 1998–2003, nejintenzivněji v roce 2002. Téměř všechny lokality této sítiny známé v České republice byly navštíveny. Z nalezených askomycetů a anamorfních hub v tomto prvním příspěvku uvádím 14 druhů. Populace sítiny trojklanné v sudetských a hercynských pohořích jsou oproti alpským a karpatským malé, nicméně byly v nich nalezeny významné arktoalpinské druhy (*Hysteronaevia minutissima*, *Hysteropezizella diminuens*, *Naeviella paradoxa*) a druh *Lachnum roseum*, které nebyly dosud z území České republiky publikovány. K lokalitám nejbohatším na juncikolní houby patří Sněžka v Krkonoších a Jezerní stěna v karu Černého jezera na Šumavě.

INTRODUCTION

In Central Europe fungi on *Juncus trifidus* have been studied especially in the Eastern Alps, Karkonosze Mts., Wysokie and Zachodnie Tatry Mts. and Babia Góra mountain range (Scheuer 1988, 1996, 1999; Scheuer and Chlebicki 1997; Chlebicki 2002). Altogether 10 species of ascomycetes, one hyphomycete (*Arthrinium cuspidatum*) and four coelomycetous species were given. Of the ascomycetes, *Bricookea sepalorum*, *Lophodermium juncinum* and *Phacosphaeria juncicola* are not known from the Czech Republic. Five species (*Brunnipila calycioides*, *Hysteropezizella diminuens*, *Hysteronaevia minutissima*, *Lachnum*

roseum and *Naeviella paradoxa*) were found during the recent study. Two species (*Cistella fugiens* and *Diplonaevia emergens*) are known from other *Juncus* species in the Czech Republic (Suková 2003).

In the Czech Republic, *Juncus trifidus* occurs in the Šumava Mts., Krkonoše Mts., Králický Sněžník mountain range and Hrubý Jeseník Mts. (Dostál 1989, Jeník 1961, Procházka and Štech 2002). No attention to fungi on *Juncus trifidus* was paid and almost no data were published. Only one species (*Brunnipila calycioides*) was mentioned by Svrček (1993) from plant communities which are known from the Carpathians (Slovakia) and also from the Krkonoše Mts. (Czech Republic). One specimen of the species collected in Hrubý Jeseník by A. Pilát is housed in PRM and one locality was published by Suková (2003) from the Šumava Mts. Fungi on *Juncus trifidus* on the Polish side of Mt. Sněžka (Polish part of the Krkonoše Mts.) were investigated by Chlebicki (1990a, 2002).

METHODS

Almost all known localities of *Juncus trifidus* in the Czech Republic were recurrently visited. From Mt. Šerák in the Hrubý Jeseník Mts. only a herbarium specimen was seen. More (mycologically not yet studied) localities of *Juncus trifidus* are known from the Krkonoše Mts. and the Hrubý Jeseník Mts. (see Jeník 1961: 32, 309–314). Unless stated otherwise, dried material was prepared in water under a stereomicroscope and studied under a light microscope, using Nomarski contrast. The amyloid reaction of the asco-apical apparatus (I+, I–) was examined in Melzer's reagent (MLZ). Descriptions are based on studied material, which is deposited in the Herbarium of the Mycological Department, National Museum, Praha (PRM 618868, 896497, 900000, 901483–901552) and the Herbarium of the Department of Botany, Charles University, Praha (PRC). Unless stated otherwise, the specimens were collected and identified by M. Suková. The abbreviation "not." was used for scanty material, not worth to be kept in herbarium, but sufficient for microscopic study, identification and confirmation of the occurrence at the locality. For morphological terms used for the occurrence of fungi on various parts of *Juncus trifidus* see Fig. 1.

Localities and character of studied Juncus trifidus populations

1: Western Bohemia/Germany, Šumava Mts./Bayerischer Wald Mts., 6.5 km SW of the village of Zelená Lhota, Mt. Velký Ostrý/Gr. Osser and rock ridge going SE from the peak, alt. 1280–1290 m; tufts and stands on rocks of various orientation. – **2:** Western Bohemia, Šumava Mts., 6 km NW of the village of Železná Ruda, Jezerní stěna rock wall, on and under SW edge of cirque of Černé jezero lake,

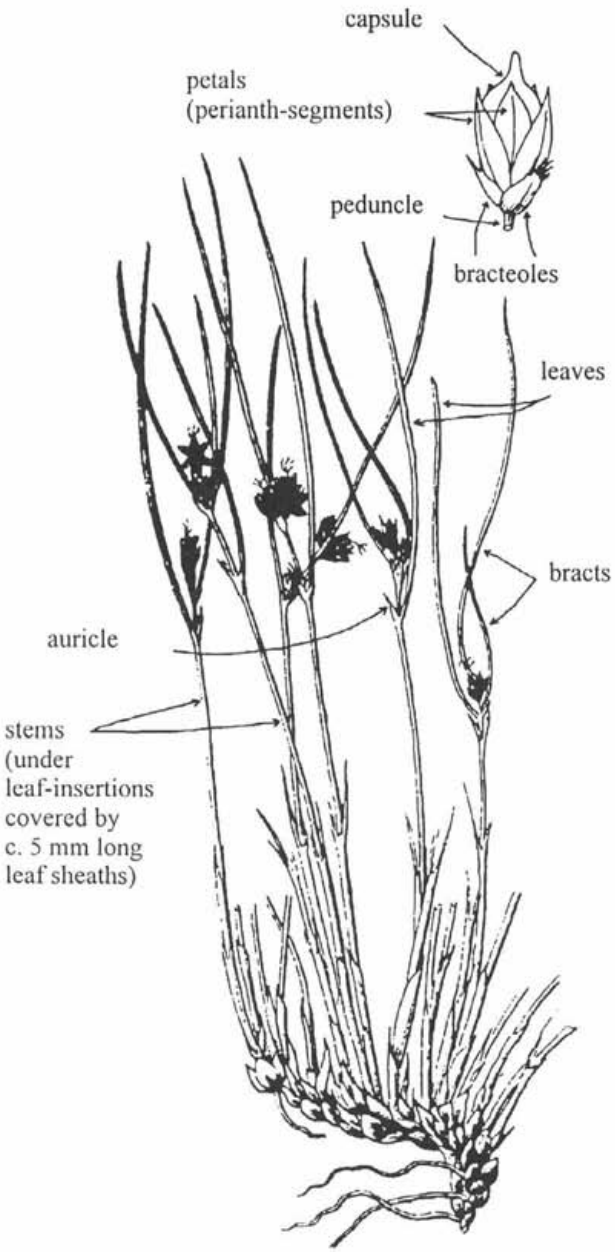

Fig. 1. Morphological terms used for the location of occurrence of fungi on various parts of shoots (aboveground parts of plants) of *Juncus trifidus*. Lower leaves and their long sheaths were often missing in collections, so especially upper leaves with sheaths about 5 mm long were examined. The schematic drawing of a living plant is taken over from Dostál (1989).

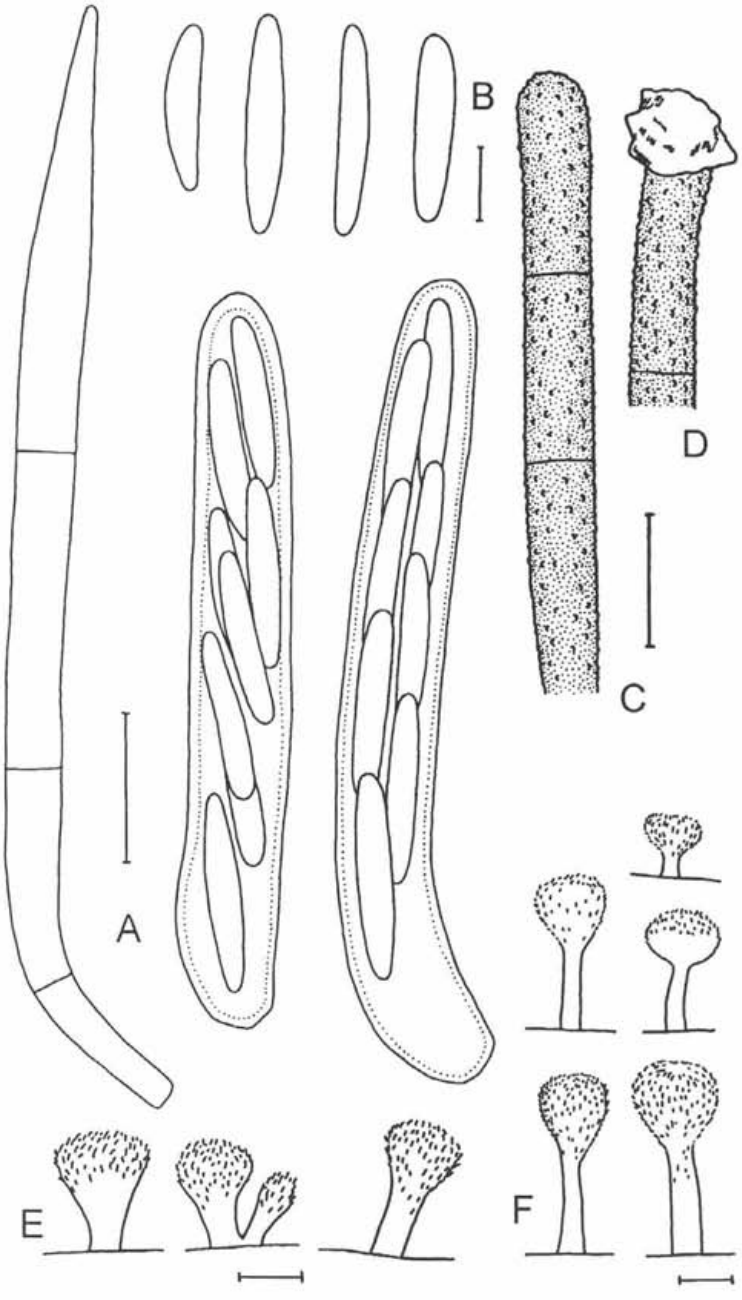

Fig. 2. *Brunnipila calycioides* (Rehm) Baral (in MLZ): A: paraphyse and asci; B: ascospores; C-D: hairs (C: PRM 901518, D: PRM 618868); E-F: shape of dried apothecia (E: PRM 618868, F: PRM 901519). Scale bars: A: 10 μm; B: 5 μm; C-D: 10 μm; E-F: 100 μm.

alt. 1300–1315 m, 49° 10' 12.5" N, 13° 10' 21" E; stands and isolated tufts on rocks. – **3:** Eastern Bohemia/Poland, Krkonoše Mts./Karkonosze Mts., SE and E side of peak of Mt. Sněžka/Śnieżka, alt. 1560–1590(–1600) m; large terrestrial stands with scattered stones. – **4:** Eastern Bohemia, Králický Sněžník mountain range, Vlaštovčí skály rocks c. 850 m SW of peak of Mt. Králický Sněžník, alt. 1260–1290 m; scattered tufts on rocks. – **5:** Northern Moravia, Hrubý Jeseník Mts., 4.5 km ESE of the village of Ramzová, Mt. Keprník, alt. 1415–1423 m, 50° 10' 13" N, 17° 06' 59.5" E; mostly terrestrial stands, less frequent stands on small rocks. – **6:** Northern Moravia, Hrubý Jeseník Mts., 5.5 km SE of the village of Ramzová, Mt. Vozka, alt. 1360–1370 m, 50° 08' 47" N, 17° 08' 11" E; stands on rocks and among stones. – **7:** Northern Moravia, Hrubý Jeseník Mts., Mt. Červená hora, small E oriented rock between Červená hora peak and Kamenné okno rock, alt. c. 1300 m, 50° 08' 44.5" N, 17° 08' 09.5" E; scattered (not numerous) tufts on the rock. – **8:** Northern Moravia, Hrubý Jeseník Mts., c. 5.3 km W of the village of Karlova Studánka, Petrovy kameny rock, alt. 1430 m; two tufts on ENE slope of the rock.

Results and discussion

Fungi collected on Juncus trifidus

ASCOMYCETES

Brunnipila calycioides (Rehm) Baral et Krieglst., Beih. Zeitschr. Mykol., 6: 49, 1985.

Syn.: *Lachnum calycioides* (Rehm) Rehm, Ascomyceten in Rabenhorst's Krypt.-Fl. Deutschl., Oest. und Schweiz, 1/3: 909, 1893.

Bas.: *Dasyscypha calycioides* Rehm, Ber. Naturhist. Ver. Augsburg 26: 42, 1881.

Fig. 2.

Description: Apothecia stipitate, brown with brown hairs and whitish-greyish yellow discs, 180–450 μm in diam. Stipes at least in lower parts dark brown, up to 400 μm long. Hairs 56–72 × 4.3–6.3 μm (width measured in apical parts), cylindrical, brown, incrustated, mostly 2–3-septate, lateral wall of cells of hairs about 0.5–0.7 μm thick (thicker in young hairs). Asci (35-)50–57 × 5.5–6 μm, asco-apical apparatus I– in MLZ, I+ in MLZ only after pretreatment in 5% KOH solution. Ascospores biseriate, one-celled, 9–12 × 2–2.5 μm, straight, rarely slightly curved, hyaline. Paraphyses lanceolate, 3.3–4 μm wide, distinctly exceeding asci by 8.5–19.5 μm.

Habitat: On dead stems, leaves, bracts and peduncles of *Juncus trifidus*. It was observed almost in all studied parts of the plants except of the capsules. It was rarely observed also on lying old, conglomerated stems.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1), 31 May 2003, PRM; Šumava Mts., Jezerní stěna (loc. 2), 14 May 2002, PRM. – Krkonoše Mts., Sněžka (loc. 3), 5 June 2002 (PRM 901552) and 13 July 2002 (PRM 901518, 901519). – Králický Sněžník (loc. 4): 23 July 1999, PRC; 19 May 2002 and 7 July 2002, PRM. – Hrubý Jeseník Mts.: Mt. Šerák, July 1947, leg. A. Pilát, det. M. Svrček, rev. M. Suková, PRM 618868; Keprník (loc. 5), 5 July 2002, PRM; Vozka (loc. 6), 5 July 2002, PRM; Červená hora (loc. 7), 5 July 2002, not. M. Suková (one apothecium only); Petrovy kameny (loc. 8), 21 May 2002 and 4 July 2002, PRM.

Comments: In the collected material, apices of hairs of young apothecia were often paler than other parts of hairs. The stipe or base of the stipe is usually brown to dark brown, the cup (or also upper part of the stipe) is paler and covered by hairs. Older, dark brown, long stalked apothecia with only a palisade of 3-celled hairs at the margin were found on Mt. Sněžka (PRM 901518). Other outer parts than the margin were dark brown, with blackened surface cells, without hairs.

In material from the Hrubý Jeseník Mts. and Králický Sněžník mountain range also some apothecia bearing hairs with a hyaline refractive matrix on hair apices (resembling primordia of apical crystals known e.g. in *Brunnipila clandestina*) and sometimes also well-developed apical crystals were observed. It was frequent especially in the material from Mt. Šerák and Mt. Vozka in the Hrubý Jeseník Mts. The presence of an amorphous matrix was already described by Scheuer (1988), and the crystals were reported by Breitenbach and Kränzlin (1984).

Brunnipila calycioides is the most frequent and abundant fungus species on *Juncus trifidus* in the Czech Republic. It is also known from other *Juncus* species, especially *Juncus filiformis* (Suková et al. 2003), *J. squarrosus* and rarely also *J. effusus* in the Czech Republic, where it is distributed from the supramontane to the alpine belt. It has been recorded on *Juncus trifidus* at the Polish side of Mt. Sněžka in the Karkonosze Mts. (Chlebicki 1990a) and on the Jezerní stěna rock wall (loc. 2) in the Šumava Mts. (Suková 2003). In Central Europe, the species has been reported on *Juncus trifidus* also from the Tatry Mts. (e.g. Velenovský 1934, Scheuer and Chlebicki 1997, Chlebicki 2002), Nízke Tatry Mts. (Svrček 1962), Babia Góra mountain range (Chlebicki 1990b) and from the Eastern Alps (Scheuer 1988). It has been reported from various *Juncaceae* and *Cyperaceae* (Scheuer 1988).

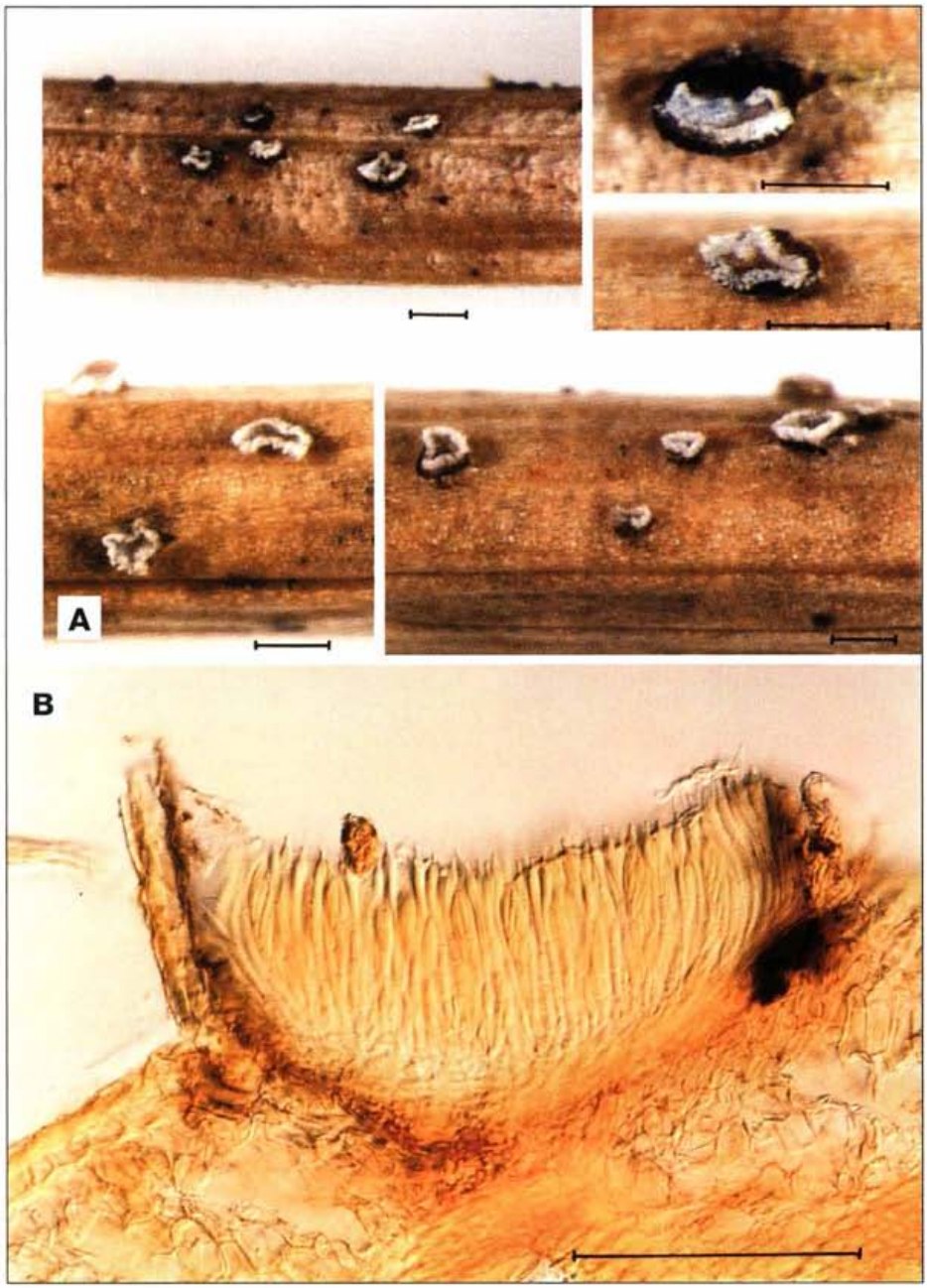

Fig. 3. A: *Hysteronaevia minutissima* (Rehm) Nannf.: apothecia; B: *Hysteropezizella diminuens* (P. Karst.) Nannf.: apothecium in longitudinal section (in MLZ). Scale bars: A: 250 µm; B: 100 µm.

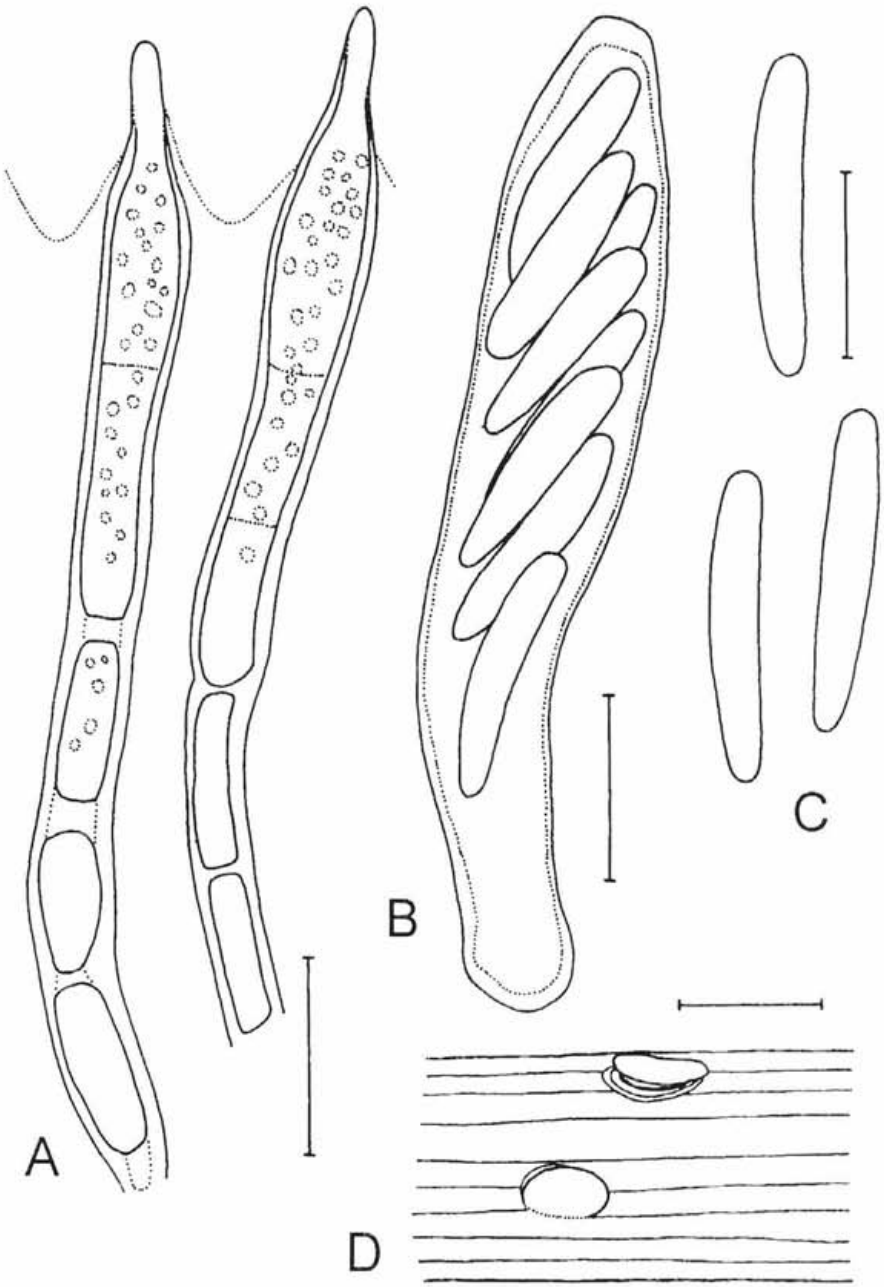

Fig. 4. A-D: *Hysteropezizella diminuens* (P. Karst.) Nannf. (in MLZ): A: paraphyses; B: ascus; C: ascospores; D: apothecia. Scale bars: A-C: 10 μm; D: 500 μm.

Hysteronaevia minutissima (Rehm) Nannf., Nord. J. Bot. 4: 242, 1984.

Bas.: *Belonidium juncisedum* var. *minutissimum* Rehm, Ascomyceten in Rabenhorst's Krypt.-Fl. Deutschl., Oest. und Schweiz, 1/3: 568, 1891.

Fig. 3A.

Description: Apothecia erumpent by a longitudinal slit in the surface of plant tissues, black with white fringed margin and grey surface of the hymenium, rounded, slightly elongated in the direction of the stem, (140-)170–200(-340) μm long when fresh, remaining protruded on drying. Microcharacters observed in MLZ in slides from fresh material and in water from dried material: Excipulum in water composed of light brown, elongated, thin-walled cells, 9.5–12.5 × 6.5–10 μm. Surface layer of excipulum dark brown, marginal hyphae brown to light beige-brown, apical cells of the hyphae slightly tapering to the rounded tips. Asci I–, 74–88 × × 10–12 μm in MLZ, 8.5–9 μm wide in water, clavate, apically conical. Ascospores biseriate, hyaline, one-celled, almost straight, (20-)21–23 × 3–3.7(-4) μm in MLZ, 17–20 × 3 μm in water, guttulate. Paraphyses hyaline, filiform with rounded to slightly acute tips, 2–3.3 μm wide.

Habitat: On dead, overwintered stems and bracts of *Juncus trifidus*.

Material studied: Šumava Mts., Jezerní stěna (loc. 2), 28 July 2002, leg. et det. M. Suková, rev. C. Scheuer, PRM 900000.

Comments: An ascospores size of 15–21(-23) × 2–2.5(-3) μm is given in Nannfeldt's description (Nannfeldt 1984). Wider ascospores were seen in material from the Czech Republic, however, it was in the range of variability of this species.

I found *Hysteronaevia minutissima* only on two tufts of *J. trifidus* in a gorge between rocks (loc. 2). The shoots of the tufts hung down over a slanting stone. Probably it also occurs in other (not yet examined) parts of the population of *J. trifidus* at the locality.

Hysteronaevia minutissima is an arcto-alpine species, reported here for the first time from the Czech Republic. Nannfeldt (1984) reported the species on *Juncus trifidus* from Mt. Arber (Javor) in the German part of the Šumava Mts. (Bayerischer Wald). It is known on *Juncus trifidus* also from Scandinavia, from the Alps in Austria (Nannfeldt 1984, Scheuer 1988) and from the Chornohora Mts. in Ukraine (Chlebicki 2002).

Hysteronaevia minutissima has been reported from *Juncus gerardii*, *J. hostii*, *J. jacquinii*, *J. trifidus* and *J. monanthos* (Rehm 1891, Nannfeldt 1984, Scheuer 1988).

Hysteropezizella diminuens (P. Karst.) Nannf., Nova Acta Reg. Soc. Sci. Upsal. ser. 4, 8(2): 114, 1932.

Bas.: *Trochila diminuens* P. Karst., F. fenn. n. 851, 1869 (not seen).

Figs. 3B, 4A-D.

Description: Apothecia black or dark grey with white margin, 150–350 μm in diam., often slightly elongated in the direction of the stem or bract, immersed, exposed by 'cutting off' a lid (as wide as the apothecium) composed of plant-tissue. Excipulum most well-developed in lateral wall of apothecium, composed of elongated, pale greyish brown, thin-walled cells. Asci 47–60 × (7.5-)9.5–12 μm, 8-spored, clavate, asco-apical apparatus distinctly I+ in MLZ after pretreatment in 5% KOH solution, only slightly I+ without pretreatment. Ascospores biseriate, one-celled, 13.5–17.5 × 2.5–3 μm, straight, rarely slightly curved, hyaline, often biguttulate. Paraphyses lanceolate, 4.2–6 μm wide, exceeding asci by 16–24 μm, apical parts embedded in a hyaline, slightly granular substance.

Habitat: On dead stems (also basal parts of stems) and bracts of *Juncus trifidus*.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1, both Czech and German side), 31 May–1 June 2003, PRM, young and old material; Jezerní stěna (loc. 2), 28 July 2002, PRM. – Karkonosze Mts. (Poland), Snieżka (loc. 3), 21 October 1996, leg. et det. A. Chlebicki, rev. M. Suková, KRAM F, old material. – Králický Sněžník (loc. 4): 23 July 1999, PRC; 7 July 2002, PRM. – Hrubý Jeseník Mts.: Vozka (loc. 6), 5 July 2002, PRM; Červená hora (loc. 7), 5 July 2002 and 17 May 2003, PRM.

Comments: The asco-apical apparatus was only slightly amyloid in Melzer's reagent, when dried material had been rehydrated in water before studying. After pretreatment in 5 % KOH solution it was distinctly amyloid in MLZ. Défago (1968) and Scheuer (1988) described amyloid reaction in Lugol's solution.

Hysteropezizella diminuens is known from various *Poaceae*, *Cyperaceae* and *Juncaceae* (Défago 1968, Scheuer 1988). I have collected this species only on *Juncus trifidus* in the Czech Republic. It is fructifying in July and August. However, young apothecia still covered by a continuous layer of plant-tissues are present already in the spring. The hymenium in these young apothecia contains only septate hyphae with rounded to slightly conical tips. Lanceolate paraphyses and asci are not developed at this stage.

The species has been reported on various host plants from the Eastern Alps (Scheuer 1988, Magnes and Hafellner 1991), Sudetes (Schröter 1893–1908, Chlebicki 2002), Tatra Mts. (Hruby 1932, Scheuer and Chlebicki 1997), Greenland (Défago 1968), Faeroe Islands, Alaska, Iceland and Canada (see Chlebicki 2002). From the Czech Republic, this arcto-alpine species is reported for the first time. Schröter (1893–1908) published it from the Polish side of the Krkonoše Mts. on

leaves of *Carex*. Chlebicki (2002) reported it on *Juncus trifidus* from the Polish side of Mt. Sněžka. I have collected only material that is too young for identification on Mt. Sněžka (loc. 3).

Lachnum roseum (Rehm) Rehm, Ascomyceten in Rabenhorst's Krypt.-Fl. Deutschl., Oest. und Schweiz, 1/3: 882, 1893.
Bas.: *Dasyscypha rosea* Rehm, Ber. Naturhist. Ver. Augsburg 26: 41, 1881.

Description: Apothecia subsessile or shortly stipitate, 270–300 μm in diam., light-coloured. Hairs hyaline or with slight brown-rose tint, thick-walled, incrustate, 37–43 × 3–3.7 μm, mostly 1-septate, with apical octahedral crystals (5–10 μm high, 9–11 μm wide). Paraphyses lanceolate, 2.2–2.8 μm wide, up to 5 μm longer than immature asci.

Habitat: On old, dead, conglomerated stems of *Juncus trifidus* lying in litter.

Material studied: Šumava Mts., Jezerní stěna (loc. 2), 14 May 2002, PRM 896497, two apothecia only.

Comments: The presence of distinct octahedral crystals on the apices of hairs agrees with the description in Scheuer (1988). Colour and shape of the apothecia is in agreement with Rehm (1893), Raitviir (1970) and Scheuer (1988). The smaller diameter of the apothecia, shorter, narrower and only one-septate hairs and the narrower paraphyses only slightly exceeding asci are probably a consequence of studying too young, not yet developed material.

In the Czech Republic it was found only at one locality in the Šumava Mts. The nearest known localities are in the Alps (Scheuer 1988, Nograsek and Matzer 1994). It is known also from the Vysoké Tatry Mts., Nízke Tatry Mts. (Svrček 1962) and Kremnické vrchy Mts. (Mihál sec. Škubla 2003) in Slovakia. Scheuer (1988) reported it from *Juncus trifidus*, *Carex ferruginea* and *Trichophorum cespitosum*, Nograsek and Matzer (1994) from *Sesleria varia* and Svrček (1962) from *Nardus stricta*.

Mycosphaerella perexigua (P. Karst.) Johanson var. **minima** Johanson, Öfvers. af Kongl. Vetenskaps-Akad. Förh. 41/9[1884]: 166, 1885. Fig. 5A.

Description: Ascomata immersed, (35-)45–50(-60) μm in diam. Wall blackish brown to brown or light brown, textura angularis composed of cells 5.5–8 × 5–6.2 μm in surface view. Ascoma containing 5–9 mature asci and 2–6 young asci (without spores and mostly without developed thickened wall in upper part). Asci bitunicate with wall thickened in upper part, as typical in *Mycosphaerella*, 23–27(-31) × × (7.5-)9–11.5(-13) μm, (5-)8-spored. Ascospores hyaline, two-celled, mostly without

constriction at septum or slightly constricted, 12–17.5 × 2.5–3.3 μm (in asci 10–15 × 1.5–2.8 μm), often 4-guttulate.

Habitat: On dead stems, sheaths, leaves, bracts, peduncles and only rarely on petals of *Juncus trifidus*.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1, both Czech and German side), 31 May 2003, PRM; Jezerní stěna (loc. 2), 14 May 2002, PRM. – Karkonosze Mts. (Poland), Snieżka (loc. 3), 5 June 2002, PRM. – Králický Sněžník (loc. 4), 18 May 2002, PRM. – Hrubý Jeseník Mts.: Kepmík (loc. 5), 17 May 2003, PRM; Vozka (loc. 6), 21 March 2004, not. M. Suková (too young material); Petrovy kameny (loc. 8), 21 May 2002, PRM.

Comments: It is a common fungus on *Juncus trifidus* and *Juncus filiformis* (see Suková et al. 2003) in the Czech Republic. The Czech material was identified according to Johanson (1885), Tomilin (1979) and Scheuer (1988), and agrees especially with Scheuer's description, including the fact that the fungus mostly occurs in parts of plants with a well-developed cuticula. This variety is known from various *Cyperaceae* and *Juncus* spp. (Scheuer 1988, Magnes and Hafellner 1991, Suková et al. 2003). In comparison with my material from *J. trifidus*, Magnes and Hafellner (1991) observed longer asci and ascospores more distinctly constricted at the septa in material from *Cyperaceae*. Johanson (1885) mentioned ascospores not being constricted at the septa in the original description based on material from *Scirpus caespitosus*, syn. *Trichophorum cespitosum* (see Tutin et al. 1980). According to my observation, at least the non-constricted and slightly constricted ascospores are in the range of variability of the variety.

Ascomata located strictly under stomata of *Juncus trifidus* were observed in the material from Králický Sněžník (loc. 4).

Naeviella paradoxa (Rehm) Clem., Gen. fung., p. 174, 1909.
Bas.: *Naevia paradoxa* Rehm, Ber. Naturhist. Ver. Augsburg 26: 102, 1881.

Description: Apothecia in dried material dark brownish black, rounded, slightly elongated in the direction of the *Juncus trifidus* bracts, 100–180 μm long, 70–120 μm wide, erumpent through surface plant tissues by several irregular valves. Asci I–, 42.5–55 × 7.5–9.7 μm, 8-spored, clavate. Ascospores biseriate, one-celled, 11–13 × 3–4.2 μm (measured only in asci), straight or almost straight, hyaline, with two big and several smaller guttules. Paraphyses filiform, hyaline, septate, branched, with slightly enlarged, light brown (1.2–2.5 μm wide) to conspicuously enlarged, rounded, brown-coloured apices (4–4.5 μm wide), apices sometimes surrounded by a brown, finely granular extracellular matrix 5.5–8(-9) μm wide.

Habitat: On dead, overwintered bracts of *Juncus trifidus*.

Material studied: Krkonoše Mts., Sněžka (loc. 3), 5 June 2002, PRM.

Comments: In the Czech Republic, this arcto-alpine species was found only at the locality with the highest altitude, Mt. Sněžka. The nearest known locality is Liliowe pass between the Zachodnie Tatry Mts. and the Wysokie Tatry Mts. (Scheuer and Chlebicki 1997). It has also been reported from the Alps (Nannfeldt 1982, Scheuer 1988), Scandinavia and Greenland (Nannfeldt 1982). According to Nannfeldt (1982) it is known only from *Juncus trifidus* and *J. monanthos*. Scheuer (1988, 1999) recorded a very similar fungus (*Naeviella* cf. *paradoxa*) with slightly shorter ascospores on *Elyna myosuroides*.

Niptera eriophori (Opiz) Rehm, Ber. Bayer. Bot. Ges. 14: 103, 1914.
Bas.: *Peziza eriophori* Opiz, Lotos 5: 86, 1855.

Description: Apothecia sessile, 180–400 μm in diam., brown, erumpent through surface tissues of the plant during their ontogenesis, finally superficial. Excipulum of a textura angularis, cells isodiametric, light brown to brown, 6.5–10 × 6–7.5 μm in view on surface. Marginal cells brown, smooth, 7.5–10.5 × 5.5–7.5 μm. Asci 72–80 × 10.5–15.5 μm, 8-spored, asco-apical apparatus I+ in MLZ. Ascospores biseriate, two-celled, hyaline, 15–17.5 × 4.8–5.5 μm. Paraphyses filiform, hyaline, septate, branched, in upper part slightly enlarged, 1.8–3 μm wide.

Habitat: On dead stems (especially in their upper part) and bracts of *Juncus trifidus*.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1), 1 June 2003, PRM; Jezerní stěna (loc. 2), 28 July 2002, PRM. – Krkonoše Mts., Sněžka (loc. 3), 5 June and 13 July 2002, PRM. – Králický Sněžník (loc. 4): 23 July 1999, PRC; 18 May 2002 and 7 July 2002, PRM. – Hrubý Jeseník Mts.: Keprník (loc. 5), 5 July 2002, PRM; Vozka (loc. 6), 5 July 2002, PRM.

Comments: Common species on various *Juncaceae* and *Cyperaceae*. On *Juncus trifidus* it occurred on stems and especially bracts lying for example on or in moss cushions. On the Jezerní stěna rock wall, which possesses good air-humidity conditions, it was found on stems and bracts caught in *Calluna vulgaris* growing on a rock.

In a previous work focusing on *Juncus filiformis* (Suková et al. 2003), *Niptera eriophori* was published with an erroneous citation of the authors' names (as *Niptera eriophori* (L. A. Kirchn.) Rehm). The species was not mentioned in Kirchner's publications (Kirchner 1856). The protologue is in Opiz (1855) and the correct author's citation is *Niptera eriophori* (Opiz) Rehm (see also Magnes and Hafellner 1991). The incorrect basionym *Peziza eriophori* L. A. Kirchn. was given by Rehm (1891) for the first time and taken over by later authors cited in Nannfeldt (1983).

COELOMYCETES

Dinemasporium strigosum (Pers.: Fr.) Sacc., Michelia 2: 281, 1881.
Bas.: *Peziza strigosa* Pers., Syn. method. fung., p. 648, 1801. Fig. 5B.

Description: Conidiomata black, densely setose. Setae 150–340 × 6–9 μm, dark brown, septate, medially thick-walled, gradually attenuated to tips. Conidia one-celled, hyaline, nearly straight to slightly curved, with appendages at each end, 10.5–13 × 2.3–3 μm (measured without appendages), appendages 6.5–8.5 μm long.

Habitat: On dead stems, leaves, bracts, peduncles, petals and rarely also on capsules of *Juncus trifidus*.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1, both Czech and German side), 31 May–1 June 2003, PRM; Jezerní stěna (loc. 2), 14 May 2002, PRM; 28 July 2002, PRM, old material. – Krkonoše Mts., Sněžka (loc. 3), 5 June 2002, PRM. – Králický Sněžník (loc. 4), 7 July 2002, PRM. – Hrubý Jeseník Mts.: Kepmík (loc. 5), 5 July 2002, PRM; Vozka (loc. 6), 5 July 2002, PRM.

Comments: Common species occurring on various host plants from *Poaceae* to *Juncaceae* and *Cyperaceae*, from lowlands to mountains. It is frequent on *Juncus trifidus*.

Septoria sp. Fig. 5C.

Description: Conidiomata immersed, 40–77 × 40–56 μm, about 45–50 μm high, in upper part with a large, irregularly rounded opening (21.5–23.5 × 17.5–22 μm) as typical of *Septoria*. Wall composed of a textura angularis with greyish-brown, angular cells (5.5–7.5 × 3.3–5.7 μm) in surface view; in longitudinal section composed of oval, elongated cells (4.7–7.5 × 1.3–1.8 μm). Conidia hyaline, smooth, straight to slightly curved, attenuated at apical ends, slightly tapering to truncate basal ends, 1–2-celled, two-celled: 23–25.5 × 1.8–2.1 μm, one-celled: 16.5–22.5 × 1.7–2.1 μm.

Habitat: On withering bracts of living plants of *Juncus trifidus*.

Material studied: Krkonoše Mts., Sněžka (loc. 3), 13 July 2002, PRM.

Comments: My collection differs from *Septoria* sp. 1 and *Septoria* sp. 2 reported from *Juncus trifidus* by Chlebicki (2002). It is more similar to *Septoria* sp. 2, which has one-septate conidia, but it differs in having distinctly smaller conidiomata and shorter conidia. Identification according to Teterevnikova-Babajan (1987: 232–233) and Saccardo (1892: 381–387) was not successful.

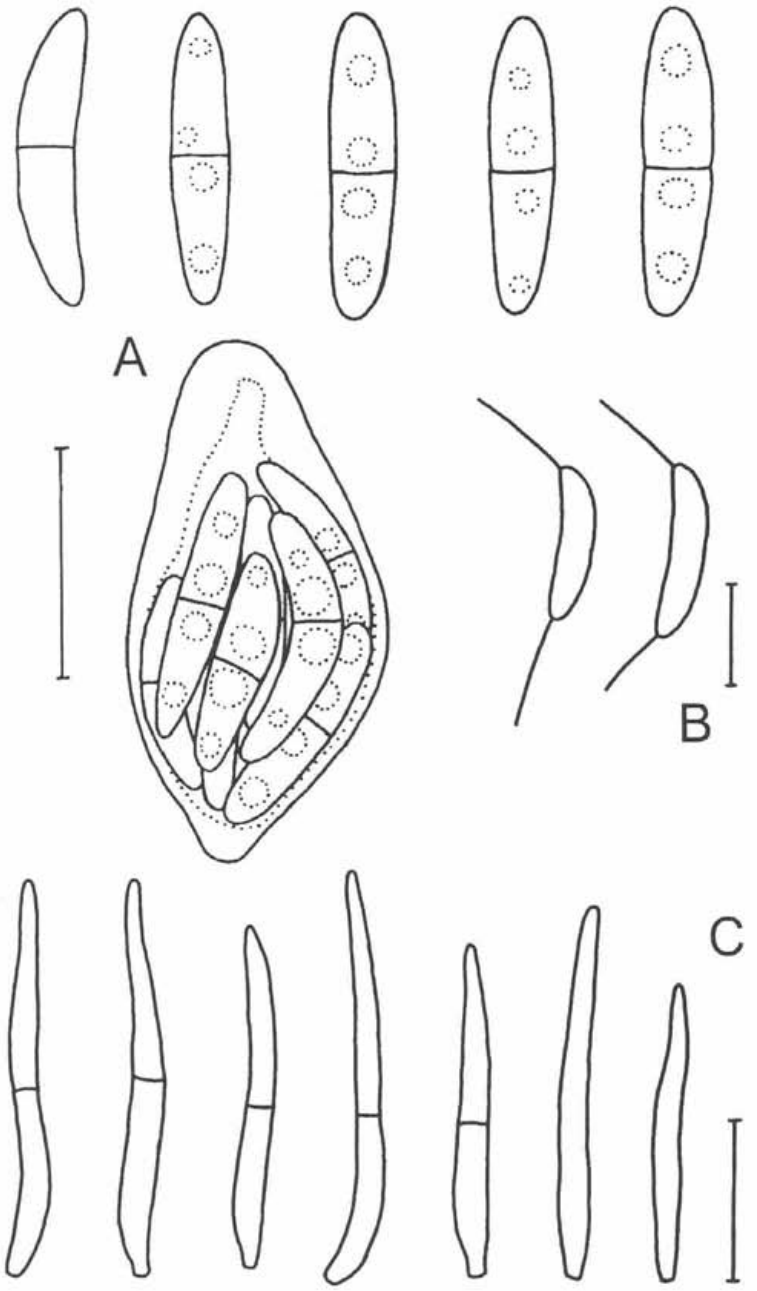

Fig. 5. Conidia: A: *Mycosphaerella perexigua* (P. Karst.) Johanson var. *minima* Johanson (in water): ascus and ascospores; B: *Dinemasporium strigosum* (Pers.: Fr.) Sacc. (in MLZ); C: *Septoria* sp. (in water). Scale bars: A: 10 μm; B: 5 μm; C: 10 μm.

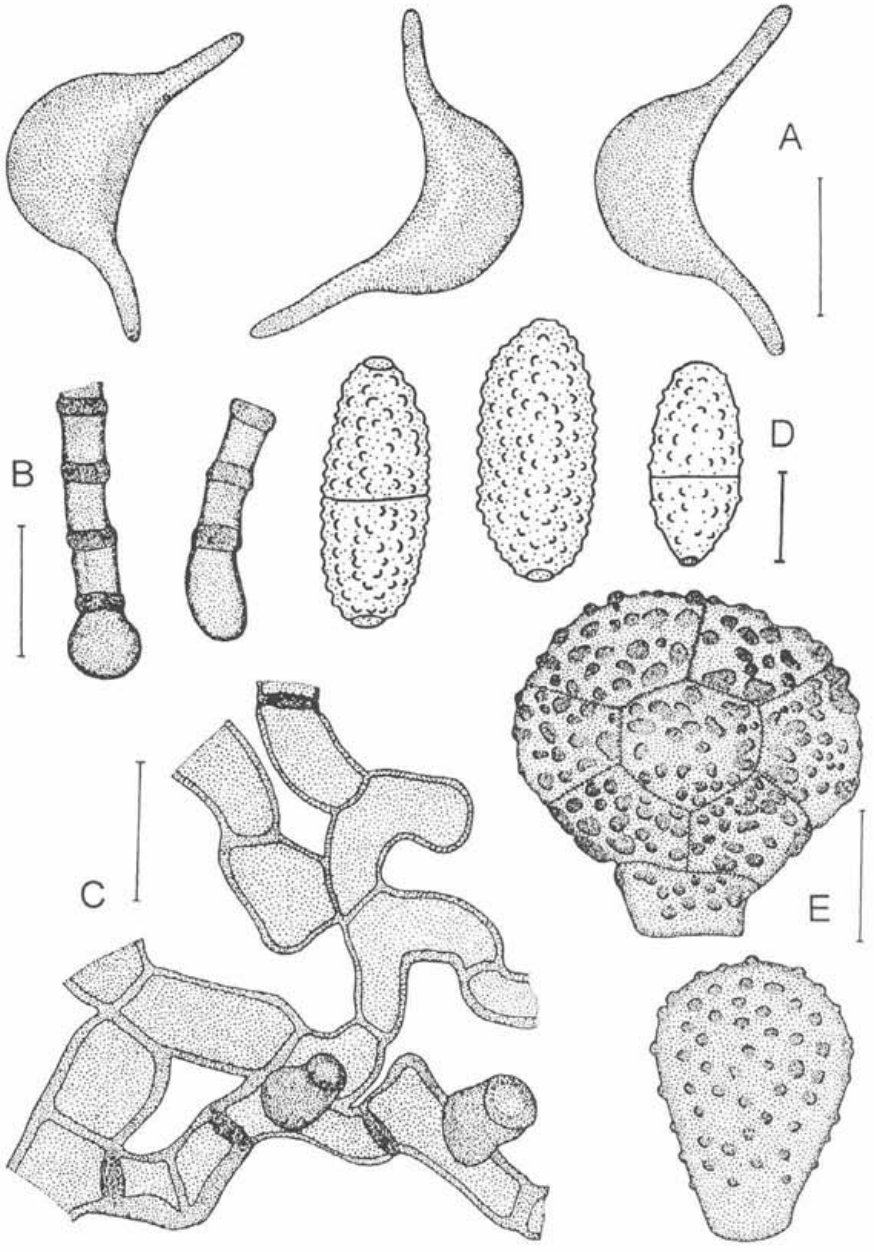

Fig. 6. A-C: *Arthrinium cuspidatum* (Cooke et Harkn.) Höhn. (in MLZ): A: conidia; B: conidiophores; C: hyphae with mother cells of conidiophores; D: *Cladosporium herbarum* (Pers.: Fr.) Link (in MLZ): conidia; E: *Epicoccum nigrum* Link (in MLZ): conidia. Scale bars: A-C: 10 μm; D: 5 μm; E: 10 μm.

HYPHOMYCETES

Arthrinium cuspidatum (Cooke et Harkn.) Höhn., Mitt. Bot. Inst. Tech. Hochsch. Wien 2: 15, 1925.
Bas.: *Camptoum cuspidatum* Cooke et Harkn., Grevillea 12: 33, 1883.
Syn.: *Arthrinium bicorne* Rostr., Bot. Tidsskr. 15: 235, 1886. Fig. 6A-C.

Description: Colonies on surface of plant tissues brownish black to black, rounded to ellipsoid, 400–700 μm in diam. Hyphae 3.5–6 μm wide, pale brown, septate, branched and anastomosing. Mother cells of conidiophores 5.5–10 × 4.5–7 μm, ampulliform, dark brown. Conidiophores 18–30 × 4.5–5.5 μm, light brown, with distinct, thick, dark brown septa, unbranched. Conidia brown, curved, with two outward curved horns, 22–31 (measured incl. horns) × 8.5–9 μm, horns 8–12 μm long.

Habitat: On dead stems, sheaths, leaves, bracts and peduncles of *Juncus trifidus*. It was also abundant on long leaf sheaths at the base of plants.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1, both Czech and German side), 31 May 2003, PRM; Jezerní stěna (loc. 2), 14 May 2002, PRM. – Krkonoše Mts., Sněžka (loc. 3, both Czech and Polish side), 5 June 2002, PRM. – Králický Sněžník (loc. 4), 18 May 2002, PRM. – Hrubý Jeseník Mts.: Keprník (loc. 5), 17 May 2003, PRM; Vozka (loc. 6), 17 May 2003, PRM; Vozka (loc. 6), 21 March 2004, leg. et det. M. Suková et A. Chlebicki, PRM; Petrovy kameny (loc. 8), 21 May 2002, PRM.

Comments: Common species on *Juncus* spp., occurring from the supramontane to the alpine belt in the Czech Republic (Suková et al. 2003) and sporulating on *Juncus trifidus* especially from March to May. The occurrence of *Arthrinium cuspidatum* especially at bases of shoots and that of *Brunnipila calycioides* in upper parts of shoots was observed at the locality Sněžka (loc. 3).

It was reported on various *Juncus* species, e.g. by Lindau (1906, 1910) from Switzerland and Scandinavia, and a collection on *Juncus filiformis* from Sweden was distributed by Kabát et Bubák (Fungi imperfecti exsiccati, fasc. 7, no. 340, 1906), both under the name *Arthrinium bicorne* Rostr. Cooke (1954) reported it also from *Cyperaceae*. Recently *A. cuspidatum* has been published on *Juncus trifidus* from the Eastern Alps (Scheuer 1996), Western Sudetes (Chlebicki 2002) and Carpathians (Scheuer and Chlebicki 1997).

Botrytis cinerea Pers.: Fr., Syst. mycol., vol. 3(2), p. 396, 1832. *Botrytis cinerea* Pers., Syn. method. fung., p. 690, 1801.

Description: Sclerotia black, immersed, erumpent through surface plant tissues or only conidiophores growing through the tissues. Conidiophores brown, septate in intervals of 48–90 μm, branched, cells c. 18–24 μm wide, usually

constricted at the septa to 16.5–19 μm. Conidia 9.7–15.5 × 6–8.5(-10) μm, hyaline to subhyaline, smooth, ellipsoid to obovate, rounded at upper end, tapering to basal end, produced holoblastically at apices of conidiophores and branches.

Habitat: On dead stems and inflorescences of *Juncus trifidus*.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1, both Czech and German side), 31 May 2003, PRM. – Krkonoše Mts.: Sněžka (loc. 3), 25 September 1999, cultivated in moist chamber culture from 27 September to 19 October, PRC; Sněžka (loc. 3, both Czech and Polish side), 5 June 2002, PRM. – Králický Sněžník (loc. 4), 18–19 May 2002, PRM. – Hrubý Jeseník Mts.: Keprník (loc. 5), 5 July 2002, PRM; Červená hora (loc. 7), 5 July 2002 and 17 May 2003, PRM.

Comments: Common plurivorous species growing especially on dicotyledonous plants. Not so common on *Juncus trifidus*, but more frequent there than on other *Juncus* species.

Cladosporium herbarum (Pers.: Fr.) Link, Mag. Ges. naturf. Freunde 7: 37, 1815. Bas.: *Dematium herbarum* Pers., Usteri's Ann. Bot. 11: 32, 1794. Fig. 6D.

Description: Conidiophores brown, nodose, 80–120 μm long, 5.5–7 μm wide, often arranged in tufts or in rows in the direction of the stem. Ramo-conidia rare. Conidia pale brown, verrucose, 0–1(-3)-septate, mostly 1-septate, (10-)11.5–18.5 × × 4–6.5(-8.5) μm, with scars on one or both ends.

Habitat: On dead stems, leaves, bracts, peduncles, bracteoles and petals of *Juncus trifidus*.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1, both Czech and German side), 31 May–1 June 2003, PRM; Jezerní stěna (loc. 2), 14 May 2002, PRM. – Krkonoše Mts., Sněžka (loc. 3), 19 September 1998, PRC. – Králický Sněžník (loc. 4): 23 July 1999, PRC; 7 July 2002, PRM. – Hrubý Jeseník Mts., Vozka (loc. 6), 5 July 2003, PRM.

Comments: Common plurivorous species, frequent on *Juncus trifidus* especially at the end of summer. In the beginning of the next season usually only dead material of the fungus can be found.

Epicoccum nigrum Link, Mag. Ges. Naturf. Freunde 7: 32, 1815. Fig. 6E.

Description: Conidia brown, roughly verrucose, broadly obovate with truncate base, 14–17.5 high, 12–18 μm wide, arranged in rounded clusters ('tufts') 75–100 μm in diam. on surface of stems.

Habitat: On dead stems of *Juncus trifidus*.

Material studied: Bayerischer Wald Mts.: Gr. Osser (loc. 1, German side), 31 May 2003, PRM. – Krkonoše Mts., Sněžka (loc. 3), 19 September 1998, PRC. – Hrubý Jeseník Mts., Červená hora (loc. 7), 17 May 2003, PRM, old material.

Comments: Plurivorous species, occasionally found on *Juncus trifidus*. It was found together with *Cladosporium herbarum* at the end of summer.

Periconia atra Corda, Icon. fung. 1: 19, 1837.

Description: Conidiophores about 250–300 μm high (including heads), 16.5–18.5 μm wide in lower half, brown, septate, in upper part with branches arranged in verticils. Rarely divided into two branches, each of them with verticils. Conidia brown, rounded, verrucose, 5.5–9(-10) μm in diam.

Habitat: On dead stems, leaves, bracts, peduncles and petals of *Juncus trifidus*.

Material studied: Šumava Mts.: Velký Ostrý (loc. 1, both Czech and German side), 31 May–1 June 2003, PRM. – Krkonoše Mts., Sněžka (loc. 3), 13 July 2002, PRM. – Králický Sněžník (loc. 4), 19 May 2002, PRM. – Hrubý Jeseník Mts., Vozka (loc. 6), 21 March 2004, PRM.

Comments: Species known from various grasses, *Cyperaceae* and *Juncaceae* (Ellis 1971). In the Czech Republic it is common on *Juncaceae* from lowlands to mountains.

Conclusions

Distribution of fungi in studied localities (Tab. 1): Mt. Sněžka in the Krkonoše Mts. and Jezerní stěna rock wall in the cirque of Černé jezero lake in the Šumava Mts. are localities richest in fungi on *Juncus trifidus*. The population of *Juncus trifidus* on Mt. Sněžka is the biggest one in comparison with other localities in the Czech Republic and is situated at the highest altitude. Only there the arcto-alpine species *Naeviella paradoxa* was found, earlier known from the Alps, Tatra Mts. (Carpathians), Scandinavia and Greenland. The cirque of Černé jezero lake has specific climatic conditions (higher air humidity and less strong winds) whereas other studied Czech localities are mostly situated on open peaks or slopes. Three species (*Hysteronaevia minutissima*, *Hysteropezizella diminuens* and *Naeviella paradoxa*) considered arcto-alpine fungi by Chlebicki (2002) have been found in the Czech Republic for the first time. *Brunnipila calycioides* (see Suková et al. 2003), considered an arcto-alpine species by Chlebicki (2002), is distributed in the Czech Republic also at lower altitudes (from the supramontane to the alpine belt) than the above mentioned species.

Substrate specificity: *Botrytis cinerea*, *Cladosporium herbarum*, *Dinemasporium strigosum*, *Epicoccum nigrum* and *Periconia atra* are common plurivorous

Tab. 1. Occurrence of fungi at the studied localities: Šumava Mts.: Velký Ostrý (loc.1), Jezerní stěna (loc. 2); Krkonoše Mts.: Sněžka (loc. 3); Králický Sněžník mountain range: Králický Sněžník (loc. 4); Hrubý Jeseník Mts.: Keprník (loc. 5), Vozka (loc. 6), Červená hora (loc. 7), Petrovy kameny (loc. 8). The polish side of Mt. Sněžka and the German side of Mt. Velký Ostrý were also included. The relative size of *Juncus trifidus* populations is indicated.

	Hercynicum		Western Sudetes	Eastern Sudetes				
	Šumava Mts.		Krkonoše Mts.	Králický Sněžník	Hrubý Jeseník Mts.			
Locality	1	2	3	4	5	6	7	8
Size of population of *Juncus trifidus*	++++	++++	+++++	+++	++++	++++	++	+
Arthrinium cuspidatum	*	*	*	*	*	*		*
Botrytis cinerea	*		*	*	*		*	
Brunnipila calycioides	*	*	*	*	*	*	*	*
Cladosporium herbarum	*	*	*	*		*		
Dinemasporium strigosum	*	*	*	*	*	*		
Epicoccum nigrum	*		*				*	
Hysteronaevia minutissima		*						
Hysteropezizella diminuens	*	*	*	*		*	*	
Lachnum roseum		*						
Mycosphaerella perexigua var. *minima*	*	*	*	*	*	*		*
Naeviella paradoxa			*					
Niptera eriophori	*	*	*	*	*	*		
Periconia atra	*		*	*				
Septoria sp.			*					

species. *Hysteropezizella diminuens*, *Lachnum roseum* and *Niptera eriophori* are ascomycetes inhabiting various grasses, sedges and rushes. *Arthrinium cuspidatum*, *Brunnipila calycioides* and *Mycosphaerella perexigua* var. *minima* are known from *Juncaceae* and *Cyperaceae* only. *Hysteronaevia minutissima* is a species specific to *Juncus*. *Naeviella paradoxa* is known from *Juncus trifidus* and *J. monanthos* according to Nannfeldt (1982).

Acknowledgements

I would like to thank Dr. Christian Scheuer (Graz, Austria) for revision of the material of *Hysteronaevia minutissima*. My thanks are expressed to Dr. Jan Kirschner (Průhonice, Czech Republic) for consultation concerning the morphology of *Juncus trifidus*. I wish to thank Drs. Zdeněk Pouzar, Jan Holec (Praha, Czech Republic) and Andrzej Chlebicki (Kraków, Poland) for critically reading the manuscript. The research was supported by grants of the Ministry of Culture of the Czech Republic (projects no. MK0CEZ99F0201, RK04PO3OMG010).

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Marasmius species (Tricholomataceae) found in man-influenced habitats in the vicinity of Yaoundé, Cameroon

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Mossebo D. C. and Antonín V. (2004): Marasmius species (Tricholomataceae) found in man-influenced habitats in the vicinity of Yaoundé, Cameroon. – Czech Mycol. 56: 85–111

The authors describe collections of 13 taxa of *Marasmius* species made in the vicinity of the Cameroon capital, Yaoundé, in stands under the influence of man. One of them, *Marasmius luteostipitatus*, is described as a new species of sect. *Sicci*.

Key words: *Marasmius*, *M. luteostipitatus*, *Tricholomataceae*, Cameroon, Africa, new species.

Mossebo D. C. a Antonín V. (2004): Druhy rodu Marasmius (Tricholomataceae) sbírané na člověkem ovlivněných stanovištích v okolí Yaoundé, hlavního města Kamerunu. – Czech Mycol. 56: 85–111

Autoři popisují nálezy 13 taxonů špiček z rodu *Marasmius* na člověkem ovlivněných stanovištích v okolí Yaoundé, hlavního města Kamerunu. Jeden z nich, *Marasmius luteostipitatus*, je popsán jako nový druh ze sekce *Sicci*.

During his field studies of the Cameroonian mycoflora, one of us (D. C. M.) collected some interesting *Marasmius* species in the vicinity of the Cameroon capital, Yaoundé, in 1997–2000. In 2001, the same localities were visited by both authors. All of the studied localities were ± strongly influenced by man (plantations, small fields, etc.) with scattered trees (as remnants of the original forest). The collections of the *Marasmius* species in those habitats are summarised in this paper.

Macroscopic descriptions and photographs were made by the collector. Microscopic descriptions were made by the second author (V. A.) and based on dried material or material preserved in a conservation liquid using an Olympus BX50 light microscope with a magnification of 1000×. A drawing tube was used for

the drawing of microscopic features. Observations were made on mounts in the following reagents: Congo Red, 10 % KOH and Melzer's reagent. Indications of colours follow Kornerup and Wanscher (1983).

The following abbreviations are used: av. = mean value of the sizes of basidiospores, E = quotient of length and width of the basidiospores, Q = mean value of E in all collections studied, L = number of entire lamellae, l = number of lamellulae between each pair of entire lamellae. Authors of fungal names are cited according to Kirk and Ansell (1992), abbreviations of herbaria follow Holmgren and Keuken (1974).

Marasmius atrorubens (Berk.) Mont., Ann. Sci. Nat., Bot., sér. 4, 1: 118. 1854. (Fig. 1, Pl. 1)

Agaricus atrorubens Berk., Journ. Bot. 1: 138. 1842.

Description of collected carpophores

Pileus 7–15 mm broad, conical-convex with small central papilla when young, then applanate, finely tomentose, radially weakly striate up to 1/2 or 2/3 (never sulcate), yellow-brown to ferrugineous-yellow with dark ferrugineous-brown centre. Lamellae ± close, L = 20–23, l = 2–3, narrow (± 1 mm broad), emarginate and attached with a tooth, dirty whitish, with pubescent, yellow-brown edge. Stipe 45–55 × up to 0.5 mm, filiform, cylindrical, entirely distinctly strigose-hairy, slightly lustrous, whitish at apex, up to brown or dirty brown towards base; basal mycelium long, strigose, ochraceous.

Basidiospores 11–13.5 × 3.7–4.5 μm, av. = 12.1 × 4.1 μm, E = 2.4–3.3, Q = 3.0, (sub)fusoid, thin-walled, hyaline, non-dextrinoid, smooth. Basidia 20–24 × 6.0–8.0 μm, 4-spored, clavate. Basidioles 12–23 × 4.0–9.0 μm, clavate, subcylindrical. Cheilocystidia in the form of broom-cells, 10–20 × 6.0–8.0 μm, clavate to subcylindrical, with thin or apically slightly thickened walls; thick-walled parts brown in KOH. Pleurocystidia 22–32 × 5.0–9.0 μm, ± fusoid, sometimes with mucronate apex, refractive, hyaline, thin-walled. Trama hyphae ± cylindrical, ± thin-walled, dextrinoid, hyaline, up to 10 μm wide. Pilcipellis a hymeniderm made up of broom-cells of the Siccus-type, 10–25 × 5.0–8.0 μm, clavate to subcylindrical, usually slightly thick-walled at apex, with 8–25, obtuse to subacute, slightly thick-walled, nodulose, digitate to narrowly conical, up to 10 × 1.5 μm projections, mixed with scattered thick-walled broom-cells; walls of both types ± brown in KOH. Stipitipellis a cutis consisting of cylindrical, parallel, slightly thick-walled, dextrinoid, up to 5.0 μm wide hyphae with ochraceous yellow walls in KOH. Caulocystidia numerous, 55–240 × 10–15 μm, subulate to lageniform, rostrate, simple, (sub)acute, slightly thick-walled (up to 1.0 μm), hyaline to pale ochraceous. Clamp-connections present in all tissues.

Pl. 1. *Marasmius atrorubens* (BRNM 666058), photo V. Antonín.
Pl. 2. *Marasmius* cf. *bingaensis* (BRNM 686392; herb. Mossebo), photo D. C. Mossebo.
Pl. 3. *Marasmius haediniformis* (herb. Mossebo), photo D. C. Mossebo.

Pl. 4. *Marasmius grandisetulosus* (BRNM 666054), photo V. Antonín.
Pl. 5. *Marasmius luteostipitatus* (BRNM 666062), photo V. Antonín.

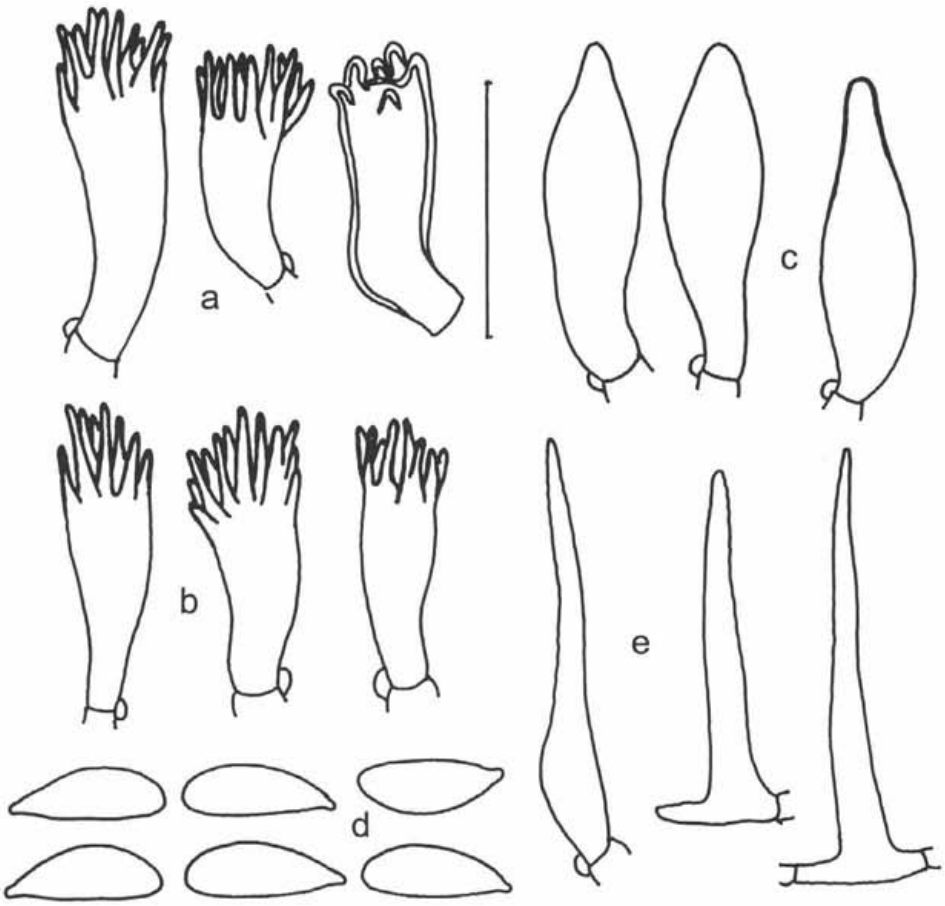

Fig. 1. *Marasmius atrorubens* (BRNM 666058). a. pileipellis cells, b. cheilocystidia, c. pleurocystidia, d. basidiospores, e. caulocystidia. Scale bar = 50 μm for caulocystidia, 20 μm for other structures.

Ecology and locality: Yaoundé, Mt. Eloundem, single on fallen leaves and detritus, 30 March 2001 leg. V. Antonín (Cm01.09) and D. C. Mossebo (BRNM 666058).

Notes: *Marasmius atrorubens* is characterised by having an often papillate, orange-brown (when young), then darker orange-brown pileus at centre, which is paler towards margin, rather close lamellae, a reddish brown stipe with orange tinge, moderately large basidiospores, well-developed, often mucronate, short pleurocystidia and numerous, ± slightly thick-walled caulocystidia. It belongs to sect. *Sicci* Singer, subsect. *Siccini* Singer, ser. *Atrorubentes* Desjardin et E. Horak. It seems to be widely distributed in tropical Africa (Benin, Burundi, Cameroon, Democratic Republic of Congo, Tanzania, Uganda; Antonín 2004a).

Marasmius nummularius Berk. et Broome differs by its concolorous lamellar edges, smaller basidiospores [(11–)12–15 × (3–)3.5–5 μm (Desjardin et al. 2000) or 10–12 × 3–3.5 μm (Pegler 1986)], well-developed caulosetae and the absence of pleurocystidia. Moreover, Desjardin et al. (2000) mentioned two types of cheilocystidia. *Marasmius glaucopus* (Pat.) Sacc. et D. Sacc. has a dark purplish brown pileus, dark purple lamellae and smaller basidiospores (8.3–9.3 × 3.8–5 μm) (Pegler 1983, Singer 1976).

Marasmius cf. bingaensis Singer, Bull. Jard. Bot. Brux. 34: 382. 1964.
(Fig. 2, Pl. 2)

Description of collected carpophores

Pileus 5–10 mm broad when young, 15–30 mm when old, campanulate or subhemispherical, then convex to plano-convex, rarely applanate, radially sulcate except for 1–3 mm broad glabrous centre, with slightly denticulate margin, reddish orange or pale orange brownish. Lamellae distant, L = ca. 14–18, l = 0, adnexed, up to 3.5 mm broad, slightly ventricose, white, with entire, concolorous edge. Stipe 40–70 × 1–2 mm, cylindrical, fistulose, thin, white at apex, orange-brown to dark violaceous brown towards base. Context membranaceous, white. Spore print white.

Basidiospores (15–)20–25 × 3.5–6.0 μm, av. = 22.3 × 5.3 μm, E = 3.8–4.9, Q = 4.2, fusoid to (sub)lacrimoid or subclavate, thin-walled, hyaline, non-dextrinoid. Basidia 25–28 × 7.5–8.0 μm, 4-spored, clavate. Basidioles 15–28 × 4.0–8.0 μm, clavate, (sub)fusoid. Cheilocystidia 15–18 × 6.0–7.0 μm, in the form of broom-cells of the Siccus-type, clavate to subcylindrical, thin-walled, with up to 10 × 1.0 μm, digitate, nodulose, obtuse projections. Pleurocystidia numerous, 33–65 × 9.0–15 μm, fusoid, clavate-fusoid to clavate, thin-walled, with slightly refractive contents. Trama hyphae cylindrical to subinflated, ± thin-walled, branched, hyaline, dextrinoid, up to 15 μm wide. Pileipellis a hymeniderm made up of broom-cells of the Siccus-type, clavate, often irregular, thin-walled and hyaline at base, slightly thick-walled and ochraceous yellow with olivaceous tinge above, with 8–17 digitate, obtuse, irregular to regular, thick-walled, up to 10 × 2.0 μm projections with ochraceous yellow and olivaceous tinged walls; mixed with scattered, mostly larger, 13–31 × 5.0–10 μm, more distinctly thick-walled broom-cells, with a few more robust, conical obtuse projections. Stipitipellis a cutis consisting of parallel, cylindrical, slightly thick-walled, smooth, dextrinoid, up to 5.0 μm wide hyphae with ochraceous-olivaceous walls in KOH. Caulocystidia absent; scattered broom-cells present at apex. Clamp-connections present in all tissues.

Ecology and locality: Cameroon, Yaoundé, in a secondary forest at the foot of Mt. Eloundem (alt. 1600 m), fasciculate to cespitose, on dead twigs and branches, 25 Aug.1999 leg. D. C. Mossebo M234 (BRNM 686392 and herb. Mossebo).

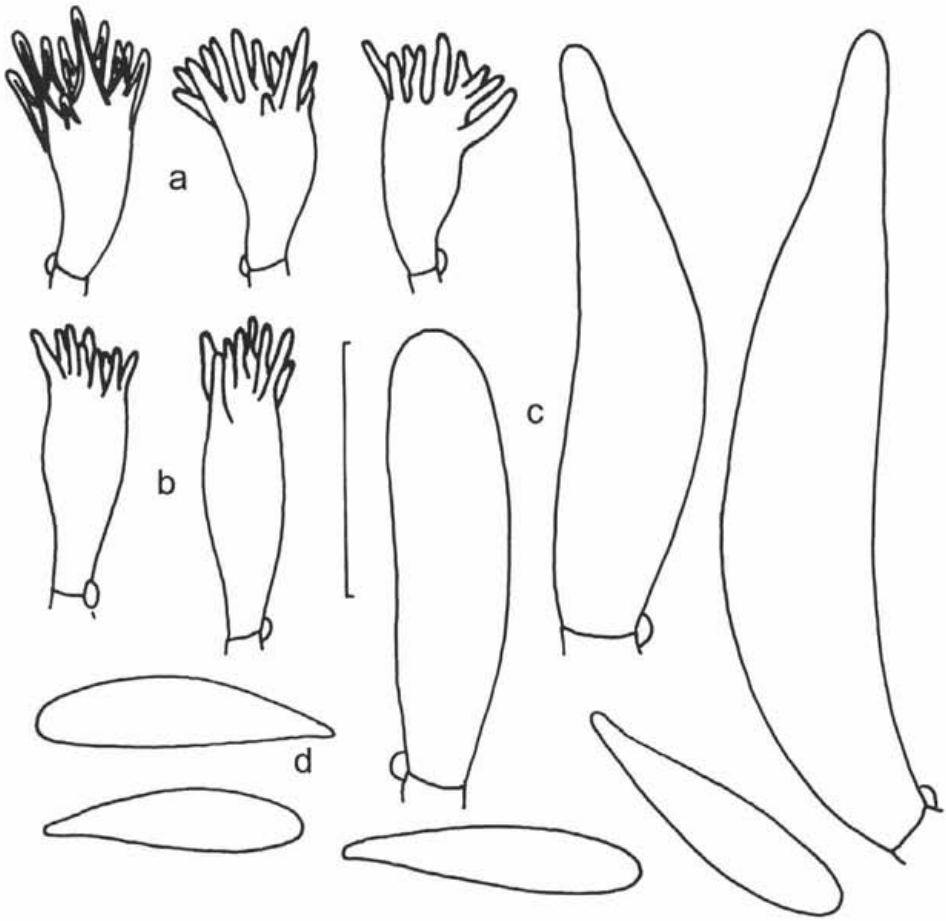

Fig. 2. *Marasmius* cf. *bingaensis* (BRNM 686392; herb. Mossebo). a. pileipellis cells, b. cheilocystidia, c. pleurocystidia, d. basidiospores. Scale bar = 20 μm.

Notes: This species is characterised by having a rather small reddish orange or pale orange-brownish pileus, distant lamellae, an orange-brown to dark violaceous brown stipe towards base, large basidiospores, well-developed pleurocystidia and lacking caulocystidia. Having those microscopic features, it belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Haematocephali* Singer.

This fungus is similar to *M. bingaensis* Singer but the latter differs by its slightly darker pileus (± reddish brown (8–9D6), pinkish brown), stipe without an orange tinge (brown or chestnut brown) and smaller basidiospores (14–21 × 3.8–5.5(–6.0) μm). However, we do not exclude that collection M234

fits into the variability of *M. bingaensis*. Therefore, we refrain to describe it as a new taxon.

Marasmius camerunensis Antonín et Mossebo in Antonín, Mycotaxon 85: 113. 2003.

Ecology and locality: Cameroon, Littoral Prov., near the village of Poola'a, c. 5 km from Nkongsamba, on dead branches and trunks, 20 Aug. 1998 leg. D. C. Mossebo M196(1) (holotype BRNM 670732 and isotype herb. Mossebo).

Notes: A detailed description, drawings of microscopic characters and a discussion were published by Antonín (2003).

Marasmius confertus Berk. et Broome, Journ. Linn. Soc., Bot. 14: 34. 1873. (Fig. 3)

Description of collected carpophores

Pileus 20–35 mm broad, subhemispherical, convex to plano-convex, sometimes obtusely conical with regular margin, smooth, non-striate, glabrous, reddish orange, darker at centre, sometimes covered with patches of a vanishing whitish substance. Lamellae moderately crowded, L = c. 30–40, l = 2–3, emarginate, ± horizontal, narrow (1–2 mm broad), whitish to yellowish, with concolorous entire edge. Stipe 40–55 × 1–1.5 mm, cylindrical, thin, filiform, fistulose, whitish to yellowish at apex, orange to dark orange towards base, with rich basal tomentum. Context thin to membranaceous, white. Spore print whitish.

Basidiospores (8.0-)10–13 × 4.0–5.5 μm, av. = 11.5 × 4.7 μm, E = 2.2–2.7, Q = 2.5, pip-shaped, lacrimoid, subellipsoid, thin-walled, hyaline, non-dextrinoid. Basidia 4-spored, clavate. Basidioles 15–25 × 3.0–7.5 μm, cylindrical to clavate. Cheilocystidia 11–20 × 5.0–8.0 μm, in the form of broom-cells of the Siccus-type, clavate to cylindrical, thin-walled, hyaline; projections up to 10(–14) × 1.0(-1.5) μm, digitate to subconical, nodulose, slightly thick-walled, with pale brownish walls in KOH. Pleurocystidia 35–55 × 9.0–12 μm, cylindrical, subfusoid, subclavate, often rostrate, obtuse, thin-walled, hyaline, with refractive contents. Trama hyphae cylindrical, thin-walled, subhyaline, dextrinoid, up to 12 μm wide. Pileipellis a hymeniderm consisting of two types of broom-cells of the Siccus-type: (1) 8.0–12 × 6.0–9.0 μm, clavate to cylindrical cells, thin-walled and hyaline at base, slightly thick-walled and brown at apex; projections up to 8.0(-10) × 1.0(-1.5) μm, numerous (10–40), digitate, obtuse to subacute, nodulose, with brown walls in KOH; (2-)11–21 × 8.0–11 μm, setoid, clavate to subcylindrical cells, thick-walled (up to 2.0 μm, except for their only slightly thick-walled base), with

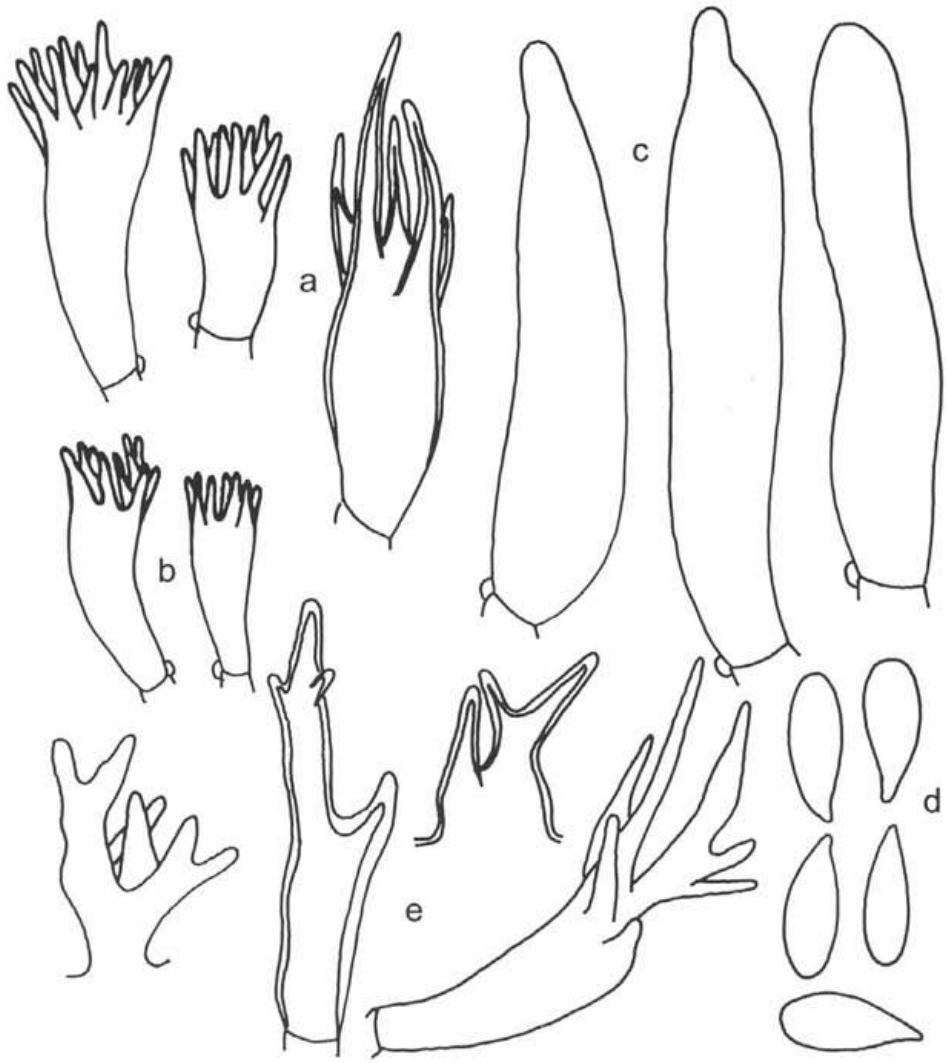

Fig. 3. *Marasmius confertus* (BRNM 686391; herb. Mossebo). a. pileipellis cells, b. cheilocystidia, c. pleurocystidia, d. basidiospores, e. caulocystidia. Scale bar = 20 μm.

brown walls; with projections up to 45 × 3.0 μm, ± scattered (1–8), conical, smooth to nodulose, subacute to acute, with brown walls in KOH. Stipitipellis a cutis consisting of cylindrical, parallel, thick-walled (up to 2 μm), dextrinoid, up to 10 μm wide hyphae with (ochraceous)olivaceous walls in KOH. Caulocystidia in the form of broom-cells of the Siccus-type, 11–22 × 6.0–12 μm, cylindrical, clavate to fusoid, slightly to distinctly thick-walled (0.5–2.0 μm), sometimes irregular,

adpressed to erect, sometimes transient to a setoid form (with one projection, then 32–48 μm long), with pale to dark brown walls in KOH; projections up to 30 × 3.0 μm, ± conical, obtuse to subacute, rarely acute, thick-walled (0.5–2.0 μm), scattered (1–7), with pale to dark brown walls in KOH; rarely the entire cell ± thin-walled and then only pale brownish. Clamp-connections present in all tissues.

Ecology and locality: Yaoundé, foot of Mt. Eloundem in the vicinity of the capital, alt. 1600 m, fasciculate on litter lying on soil, 25 Aug. 1999 leg. D. C. Mossebo M229 (BRNM 686391 and herb. Mossebo).

Notes: A very distinct species by its moderately large, smooth, reddish orange pileus, rather smaller basidiospores, very distinct, non-setoid pleurocystidia, two types of pileipellis cells and caulocystidia in the form of ± thick-walled broom-cells. Having those features, it belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Haematocephali* Singer.

Compared with the literature, Antonín (2004a) mentioned more distant lamellae (L = 16–19) and slightly larger basidiospores (11.5–15(-17) × 4.0–5.0(-6.0) μm).

It represents a fungus widely distributed in tropical Africa (Cameroon, Democratic Republic of Congo, Kenya, Nigeria and Uganda; Antonín 2004a).

Marasmius aff. corrugatiformis Singer, Bull. Jard. Bot. Brux. 34: 374. 1964.
(Fig. 4)

Description of collected carpophores

Pileus 5–30 mm broad, conical when young, then plano-convex, rarely applanate, with distinct, 4–6 mm broad umbo, regular at margin, reddish brown to dark brown at centre, paler, ochraceous yellow towards margin. Lamellae crowded, L = ca. 80, l = 3–4, almost free to adnexed to an adpressed small collarium, narrow (0.5–2 mm), ± horizontal, white, with concolorous, entire edge. Stipe 30–90 × 1.5–3 mm, cylindrical, fistulose, pruinose, whitish at apex, ochraceous to pale violaceous brown towards base, usually with a ± tomentose basal mycelium. Context very thin to membranaceous, whitish. Spore print white to whitish.

Basidiospores 5.0–7.0 × 2.0–3.0 μm, av. = 5.9 × 2.6 μm, E = 1.8–2.7, Q = 2.1–2.3, ellipsoid-fusoid, thin-walled, hyaline, non-dextrinoid. Basidia 14–19 × × 4.5–6.0 μm, 4-spored, clavate. Basidioles 8.0–19 × 3.0–7.0 μm, cylindrical, clavate or fusoid. Cheilocystidia in the form of broom-cells of the Siccus-type, 10–18 × 5.0–8.0 μm, cylindrical to clavate, entirely thin-walled or slightly thick-walled above, hyaline, with moderately numerous (4–20), up to 8.0(-10) × × 1.0(-1.5) μm, digitate, nodulose, obtuse projections; mixed with basidioles. Pleurocystidia absent. Trama hyphae consisting of cylindrical to ellipsoid, thin-walled, hyaline, up to 20 μm wide cells, sometimes mixed with cylindrical

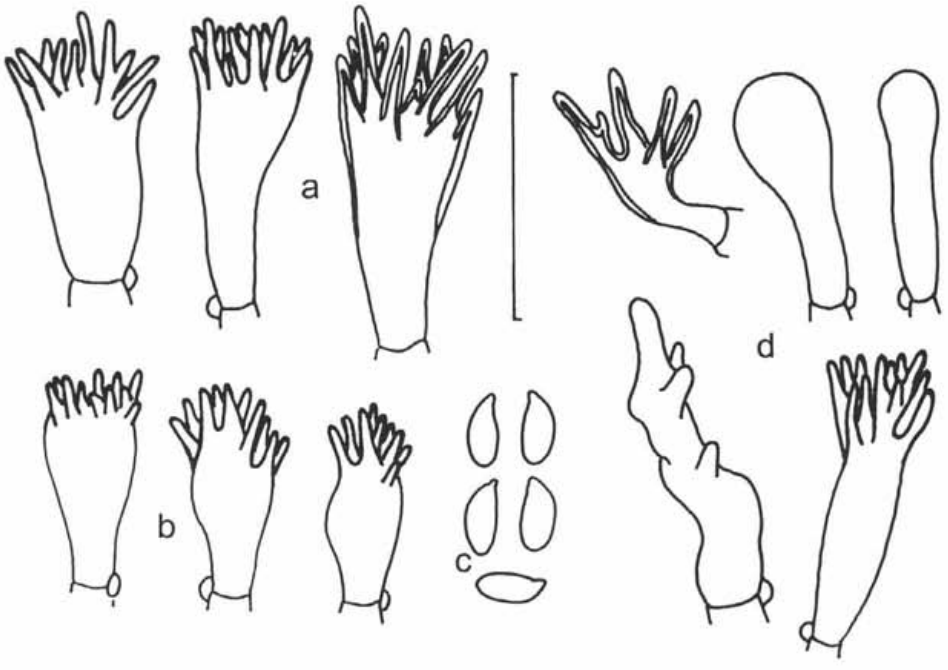

Fig. 4. *Marasmius* aff. *corrugatiformis* (herb. Mossebo). a. pileipellis cells, b. cheilocystidia, c. basidiospores, d. caulocystidia. Scale bar = 20 μm.

or slightly inflated, slightly thick-walled, 2.0–5.0 μm wide hyphae made up of long articles, which are more distinctly dextrinoid than other hyphae. Pileipellis a hymeniderm made up of broom-cells of the Siccus-type, 10–25 × 5.5–10 μm, (sub)cylindrical to clavate, thin-walled at base and slightly thick-walled above or entirely thick-walled, non-dextrinoid, with less numerous to numerous (4–20(-30)), up to 15 × 2.0 μm, digitate to subconical, ± obtuse to subacute, ± smooth to nodulose, thick-walled projections; thick-walled parts with distinctly ochraceous yellow walls in KOH. Stipitipellis a cutis consisting of cylindrical, parallel, slightly thick-walled, dextrinoid, up to 5.0 μm wide hyphae with subhyaline to yellowish walls in KOH. Caulocystidia in the form of broom-cells of the Siccus-type, predominantly present at apex, scattered towards base, 10–30 × 4.0–9.0 μm, cylindrical to clavate, either entirely thin-walled or entirely thick-walled or slightly thick-walled at apex only, non-dextrinoid, subhyaline, with up to 10 × 1.0 μm, thin– to slightly thick-walled, obtuse, nodulose, digitate to conical projections; mixed with less numerous, 17–26 × 4.5–7.0 μm, adpressed to erect, clavate to cylindrical, ± slightly thick-walled terminal cells (present also towards base). Clamp-connections present in all tissues.

Ecology and locality: Littoral Province, Banla'a, about 10 km from Nkongsamba, on litter, 18 Aug. 1998 leg. D. C. Mossebo M192 (herb. Mossebo). – Littoral Province, Poola'a, about 5 km from Nkongsamba, fasciculate on litter in a coffee plantation, 20 Aug. 1998 leg. D. C. Mossebo M195 (herb. Mossebo).

Notes: This fungus is characterised by having a rather large, centrally reddish brown to dark brown and marginally ochraceous yellow pileus, crowded lamellae, a long, pruinose stipe which is ochraceous to pale violaceous-brown towards base, small basidiospores, short basidia and basidioles, and caulocystidia in the form of broom-cells of the Siccus-type and clavate to cylindrical cells. It belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Atrorubentes* Desjardin et E. Horak. It belongs to the *M. corrugatiformis/katangensis* group.

The true *Marasmius corrugatiformis* Singer has the same pileus colour and size of basidiospores but it differs by the presence of only one type of caulocystidia (lacking typical broom-cell caulocystidia). *Marasmius katangensis* Singer has a dark brown pileus with a pale brown margin and larger basidiospores (7.0–12.5 × 3.0–5.5 μm). *Marasmius subarborescens* Singer has a paler, white, only at centre pale ochraceous pileus, inconspicuous (sometimes scattered or absent) cheilocystidia which are smooth or with 1–6 thin-walled projections and only simple, clavate, subcylindrical or fusoid caulocystidia (sometimes mixed with scattered broom-cells). *Marasmius confertus* Berk. et Broome, belonging to ser. *Haematocephali* Singer, has a ± uniformely brown, orange or brownish orange pileus, only one type of caulocystidia (broom-cells only) and possesses pleurocystidia. Both collections of this fungus are preserved only in a conservation liquid, which involves some problems, and this is one of the reasons for refraining to describe it as a new taxon.

Marasmius cf. ferruginoides Antonín, Mycotaxon (in press). (Fig. 5)

Marasmius cf. *gardneri* Singer s. Pegler, Kew Bull. Addit. Ser. 6: 194. 1977.

Description of collected carpophores

Pileus 5–17 mm broad, convex, sometimes slightly umbonate, with regular margin, smooth, glabrous, greyish brown, darker at centre. Lamellae moderately distant, L = c. 30, l = 2–3, adnate, narrow (1 mm), horizontal, whitish, with concolorous entire edge. Stipe 25–40 × 0.9–1 mm, cylindrical, filiform, fistulose, almost concolorous with pileus. Context very thin to membranaceous, whitish.

Basidiospores 11–13 × 3.0–4.0 μm, fusoid to clavate-fusoid, thin-walled, hyaline, non-dextrinoid. Basidia 20 × 8.0 μm (only one found), 4-spored, clavate. Basidioles 13–26 × 3.0–8.0 μm, clavate to cylindrical. Cheilocystidia 18–22 × 7.0–8.5 μm, clavate, thin-walled, with less numerous, robust, conical, obtuse, thin-walled projections. Pleurocystidia 30–35 × 8.0–10 μm, (sub)cylindrical, (sub)fusoid, subclavate, thin-walled, with slightly refractive contents. Trama hyphae cylindrical, thin-walled,

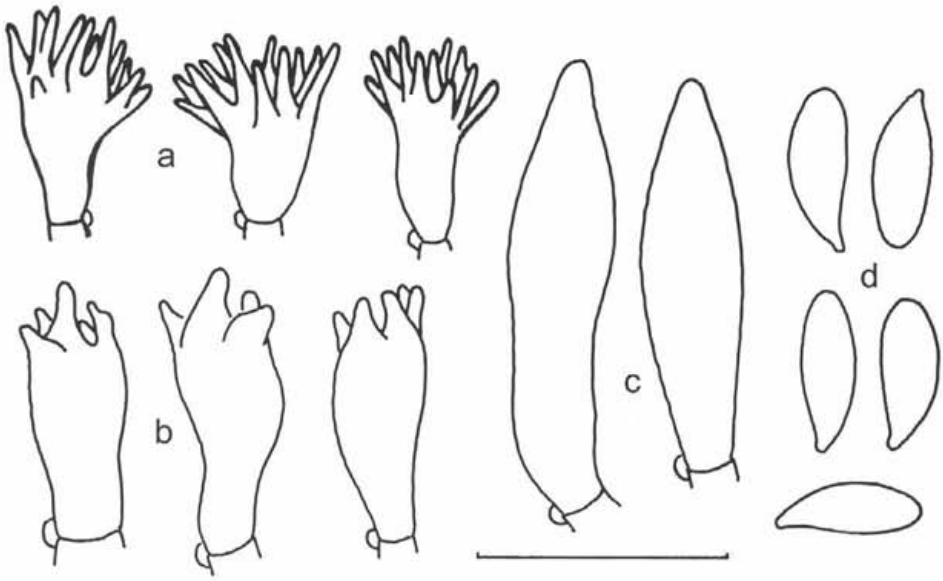

Fig. 5. *Marasmius* cf. *ferruginoides* (BRNM 686390; herb. Mossebo). a. pileipellis cells, b. cheilocystidia, c. pleurocystidia, d. basidiospores. Scale bar = 20 μm.

subhyaline, dextrinoid, up to 20 μm wide. Pileipellis a hymeniderm formed of broom-cells of the Siccus-type, 7.0–13 × 4.0–9.0 μm, clavate, subcylindrical, sometimes branched, thin-walled and hyaline at base, thin– to slightly thick-walled and hyaline to ochraceous yellow above; projections up to 8.0 × 1.5 μm, numerous [(8-)11–25(-30)], mostly conical, less frequently digitate, obtuse to subacute, slightly nodulose, slightly thick-walled, with ochraceous yellow walls in KOH. Stipitipellis a cutis consisting of cylindrical, parallel, slightly thick-walled, dextrinoid, up to 5.0 μm wide hyphae, with ochraceous olivaceous (yellow) walls in KOH. Caulocystidia absent. Clamp-connections present in all tissues.

Ecology and locality: about 2 km from the airport Nsimalen, about 20 km from Yaoundé, in a banana plantation, in ± large groups on dead branches lying on soil, 14 Oct. 1997 leg. D. C. Mossebo M117 (BRNM 686390 and herb. Mossebo).

Notes: This species is characterised by having a rather small, smooth pileus, rather close lamellae, moderately large fusoid or clavate-fusoid basidiospores, well-developed but rather short pleurocystidia, and lacking caulocystidia. It belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Haematocephali* Singer.

This collection differs from descriptions in literature by more close lamellae and a differently coloured pileus; microscopic characters agree. Antonín (2004a, b) mentioned a deep yellow, yellowish orange or orange pileus, and the first author described the pileus colour as greyish brown. However, a photograph of

it shows fungi with ± pale orange-brown pileus! Therefore it may really belong to *M. ferrugineoides*.

Marasmius grandisetulosus Singer, Bull. Jard. Bot. Brux. 34: 379. 1964.
(Fig. 6, Pl. 4)

Description of collected carpophores

Pileus 13–27 mm broad, campanulate-convex, with applanate to slightly depressed centre, crenulate at margin, sulcate-striate except for the slightly rugulose centre, finely tomentose, yellow-brown (± rather dark when young), then brownish yellow in striae but remaining yellow-brown at centre and on sulci. Lamellae distant, L = 16–18, l = 0–1, rather broad, shortly adnate, slightly intervenose when old, whitish, then cream-coloured, with finely pubescent, pale yellow-brown, rarely ± concolorous edge. Stipe 25–45 × 0.5–1 mm, cylindrical, mostly curved, slightly lustrous, smooth, glabrous, whitish at apex and brownish towards base in young carpophores, then cream at apex and through an (orange-) brown zone up to dark brown towards base; at base with whitish to pale ochraceous basal mycelium. Context membranaceous, without special smell.

Basidiospores 15–17.5 × 3.5–4.5 μm, av. = 16.3 × 3.9 μm, E = 3.6–5.0, Q = 4.3, clavate, clavate-fusoid, thin-walled, smooth, non-dextrinoid, hyaline. Basidia 27–29 × 9.0–10 μm, 4-spored, clavate. Basidioles 15–29 × 4.0–9.0 μm, clavate, fusoid or subcylindrical. Cheilocystidia in the form of broom-cells of the Siccus-type, 11–16 × 6.0–7.0 μm, clavate or subcylindrical, thin-walled or with slightly thick-walled apex, with digitate, nodulose, slightly thick-walled projections; thick-walled parts and projections pale ochraceous in KOH. Pleurocystidia 33–60 × 7.0–11 μm, fusoid, subcylindrical, sublageniform, often rostrate, thin-walled, with refractive contents. Trama hyphae cylindrical to subinflated, thin-walled, dextrinoid, hyaline, up to 12 μm wide. Pileipellis a hymeniderm formed of broom-cells of the Siccus-type, 14–21 × (5.0–)8.0–11 μm, clavate to subcylindrical, thin-walled with slightly thick-walled apex, with 10–18(-21) nodulose, slightly thick-walled, subacute to obtuse, up to 10 × 1.5 μm projections; thick-walled parts and projections with (yellow-)ochraceous walls in KOH. Stipitipellis a cutis consisting of cylindrical, parallel, slightly thick-walled, dextrinoid, up to 5.0 μm wide hyphae with pale ochraceous walls in KOH. Caulocystidia absent except for scattered broom-cells at apex. Clamp-connections present in all tissues.

Ecology and locality: Yaoundé, Mt. Eloundem, single on dead stem, 30 March 2001 leg. V. Antonín Cm01.05 and D. C. Mossebo (BRNM 666054).

Notes: *Marasmius grandisetulosus* is characterised by having a moderately broad, campanulate-convex, yellow-brown and brownish yellow striped pileus,

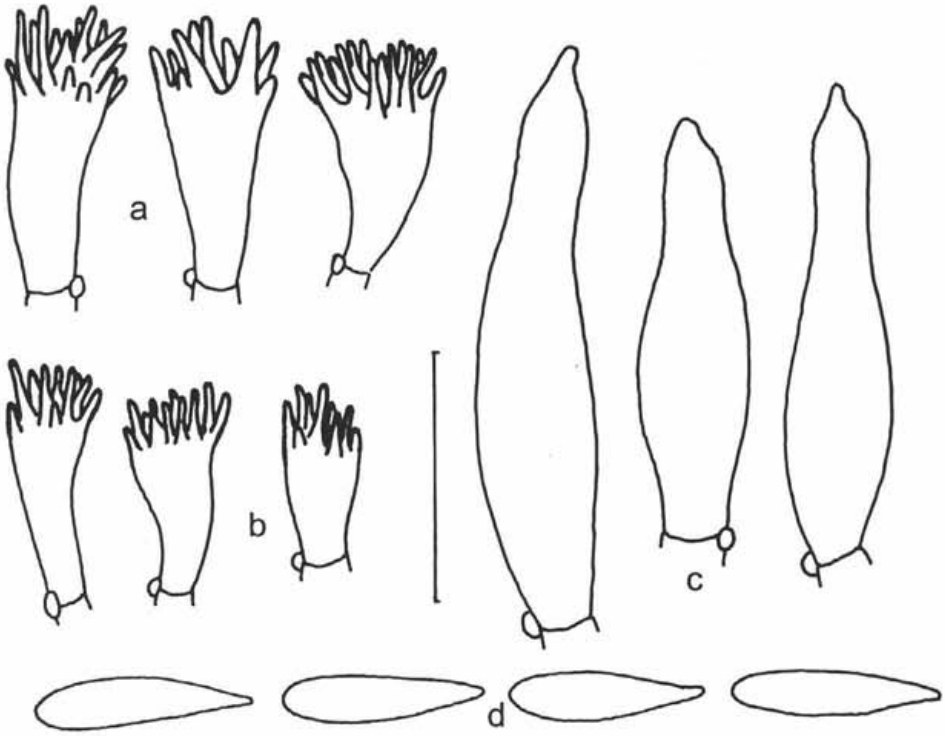

Fig. 6. *Marasmius grandisetulosus* (BRNM 666054). a. pileipellis cells, b. cheilocystidia, c. pleurocystidia, d. basidiospores. Scale bar = 20 μm.

distant lamellae with a mostly yellow-brown edge, a brown stipe becoming almost black when old, rather large basidiospores, well-developed pleurocystidia and lacking caulocystidia. Having those microscopic features, it belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Haematocephali* Singer. Our collection (BRNM 666054) differs only by slightly smaller basidiospores [16.9–21.5(-23) × 3.5–5.4 μm, Antonín 2004a; compared to 18–21 × 3.5–4.5 μm, Singer 1964, respectively].

Marasmius tenuisetulosus (Singer) Singer and *M. montagneanus* Singer are very closely related species. The first species differs especially by always having concolorous lamellar edges and hyaline cheilocystidia. The second one has concolorous lamellar edges, shorter pleurocystidia (27–43 × 5.5–11 μm), and grows on dead leaves (Singer 1976).

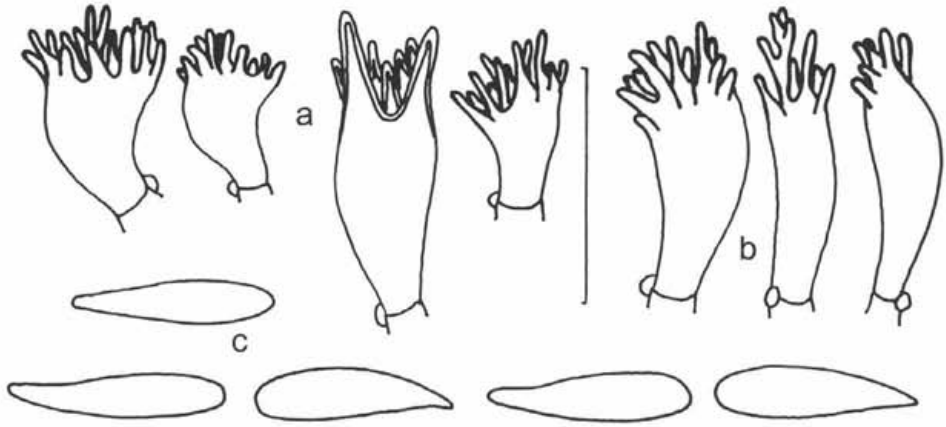

Fig. 7. *Marasmius haediniformis* (herb. Mossebo). a. pileipellis cells, b. cheilocystidia, c. basidiospores. Scale bar = 20 μm.

Marasmius haediniformis Singer, Bull. Jard. Bot. Brux. 34: 363. 1964.

(Fig. 7, Pl. 3)

Description of collected carpophores

Pileus 10–40 mm broad, conical to campanulate, then convex to plano-convex, almost applanate when old, with regular to slightly denticulate margin, entirely plicate-striate except for the glabrous, 3–5 mm broad centre, whitish to ochraceous yellow. Lamellae moderately crowded, L = c. 23–28, l = 2, ± horizontal, 1–4 mm broad, white to off-white, with concolorous entire edge. Stipe 50–90 × 1–1.5 mm, cylindrical, slightly broadened at base, fistulose, concolorous with lamellae at apex, orange-brown to brown towards base. Context very thin to membranaceous, concolorous with pileus. Spore print whitish to ochraceous.

Basidiospores 15–17 × 3.5–4.5 μm, av. = 15.6 × 3.8 μm, E = 3.6–5.0, Q = 4.2, narrowly clavate, clavate-fusoid to narrowly lacrimoid, thin-walled, hyaline, non-dextrinoid. Basidia 25 × 7.0 μm (only one found), 4-spored, clavate. Basidioles 15–33 × 3.0–8.0 μm, clavate, cylindrical to fusoid. Cheilocystidia in the form of broom-cells of the Siccus-type, 11–18 × 5.0–9.0 μm, clavate to cylindrical, thin-walled, hyaline; projections up to 8.0 × 1.0(-1.5) μm, digitate, obtuse, nodulose to coralloid, ± slightly thick-walled, hyaline. Pleurocystidia absent. Trama hyphae cylindrical, thin-walled, hyaline, dextrinoid, up to 15 μm wide. Pileipellis a hymeniderm formed of broom-cells of the Siccus-type, 10–22 × 5.0–12 μm, cylindrical to clavate, entirely thin-walled or slightly thick-walled at apex, with numerous (10–30), up to 8.0 × 1.0 μm, digitate, obtuse to subacute, nodulose, slightly thick-walled projections; thick-walled parts with pale yellowish-ochraceous

walls. Stipitipellis a cutis consisting of cylindrical, parallel, thick-walled (up to 1.0 μm), dextrinoid, up to 7.0 μm wide hyphae with yellow ochraceous (olivaceous) walls in KOH. Caulocystidia absent; scattered broom-cells of the Siccus-type present at apex. Clamp-connections present in all tissues.

Ecology and locality: Yaoundé, foot of Mt. Eloundem in the vicinity of Yaoundé, secondary forest, 1600 m alt., fasciculate to cespitose on litter and dead branches, 25 Aug. 1999 leg. D. C. Mossebo M65E (BRNM 686388 and herb. Mossebo). – ? Nsimalen (about 20 km from Yaoundé), about 2 km from the airport, in a banana plantation, fasciculate to cespitose on litter lying on soil, 14 Oct. 1997 leg. D. C. Mossebo M65C (herb. Mossebo).

Notes: This species is characterised by having a moderately large, very pale, plicate-striate pileus, a long stipe, rather large basidiospores, and lacking pleurocystidia and caulocystidia. It belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Leonini* Singer.

Collection M65C is included with a question mark. It differs by a larger, up to 50 mm broad pileus, a dark violaceous tinged stipe at base and cheilocystidia in the form of broom-cells mixed with irregular to coralloid cells; other characters agree with collection M65E.

Marasmius haediniformis probably represents a pantropical species (Singer 1976, Pegler 1997). In Africa, it has been collected in Cameroon, Democratic Republic of Congo, Ghana, Malawi, Nigeria, Sierra Leone, Uganda and Zimbabwe (Antonín 2004a).

Marasmius aff. haediniformis Singer, Bull. Jard. Bot. Brux. 34: 363. 1964.
(Fig. 8)

Description of collected carpophores

Pileus 10–20 mm broad, hemispherical to convex, then plano-convex, membranaceous, slightly radially striate, whitish when young, then beige to yellowish, becoming ochraceous when dry. Lamellae moderately distant, L = ca. 13–18, l = 2(–3), adnate, straight, narrow (less than 1 mm broad), white to cream, with entire concolorous edge. Stipe 50–80 × 1–1.5 mm, cylindrical, fistulose, smooth, glabrous, beige to yellowish at apex, orange-brown or violaceous red towards base. Context membranaceous, whitish.

Basidiospores 15–21 × 3.0–4.0 μm, av. = 18.2 × 3.7 μm, E = 4.3–6.7, Q = 4.9, narrowly clavate, sometimes clavate-cylindrical or subfusoid, thin-walled, hyaline, non-dextrinoid. Basidia 28–33 × 7.0–8.5 μm, 4-spored, clavate. Basidioles 20–35 × 3.0–8.0 μm, clavate, cylindrical, subfusoid. Cheilocystidia 16–20 × × 6.0–9.0 μm, in the form of broom-cells of the Siccus-type, clavate to subcylindrical, thin-walled; projections up to 5.0 × 1.0 μm, digitate, nodulose,

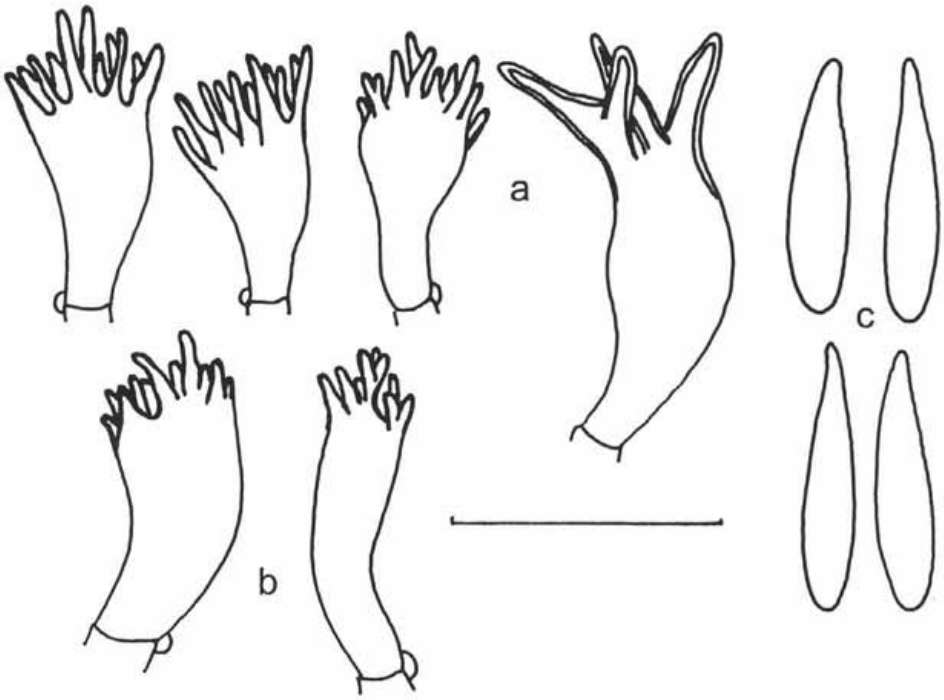

Fig. 8. *Marasmius* aff. *haediniformis* (BRNM 686389; herb. Mossebo). a. pileipellis cells, b. cheilocystidia, c. basidiospores. Scale bar = 20 μm.

obtuse. Pleurocystidia absent. Trama hyphae ± cylindrical, thin-walled, branched, dextrinoid, up to 15 μm wide. Pileipellis a hymeniderm formed of broom-cells of the Siccus-type, 11–22 × 7.0–12 μm, clavate, thin-walled at base, slightly thick-walled at apex, rarely entirely slightly thick-walled, with 2–10 × 0.75–1.5 μm, ± numerous (10–20), digitate, nodulose, slightly thick-walled, obtuse to subacute projections; thick-walled parts of all cells yellowish in KOH. Stipitipellis a cutis consisting of parallel, cylindrical, slightly thick-walled, smooth, dextrinoid, up to 4.0 μm wide hyphae, with ochraceous and olivaceous tinged walls in KOH. Caulocystidia absent. Clamp-connections present in all tissues.

Ecology and locality: Nsimalen (20 km from Yaoundé), about 2 km from the airport, in a banana plantation, in small groups on dead trunks and stumps, 14 Oct. 1997 leg. D. C. Mossebo M116 (BRNM 686389 and herb. Mossebo).

Notes: This collection is published separately from the typical *M. haediniformis*. It differs especially by its orange-brown to violaceous red stipe at base, distinctly longer basidiospores and longer basidia and basidioles. It may represent either a new taxon (variety) or fall within a wider variability of *M. haediniformis*.

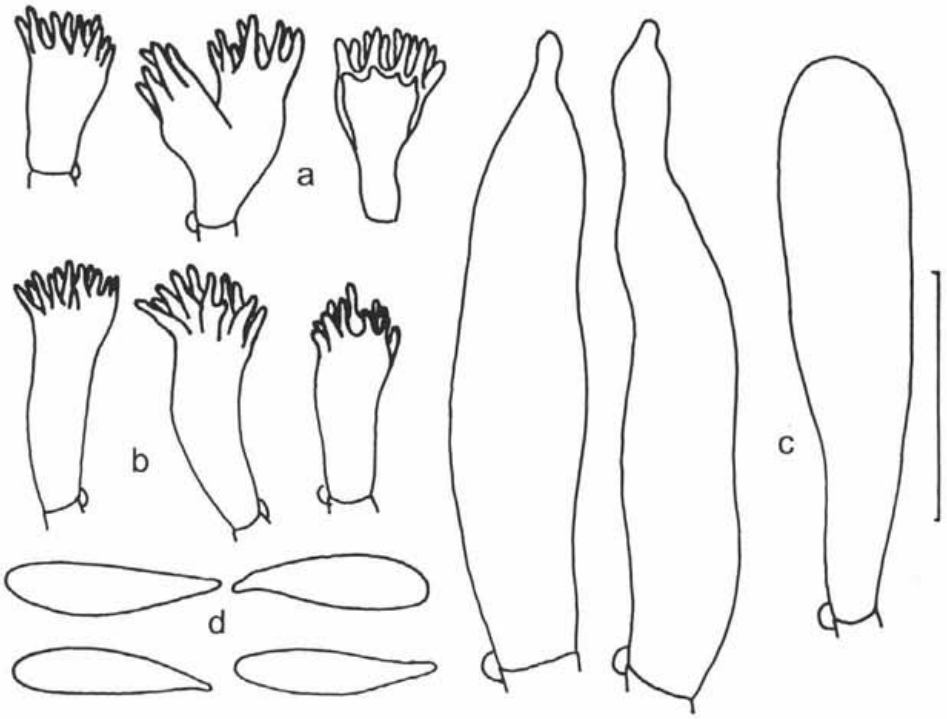

Fig. 9. *Marasmius haematocephalus* (BRNM 686387; herb. Mossebo). a. pileipellis cells, b. cheilocystidia, c. pleurocystidia, d. basidiospores. Scale bar = 20 μm.

Marasmius haematocephalus (Mont.) Fr., Epicr. Syst. Mycol.: 382. 1838.

(Fig. 9)

Agaricus haematocephalus Mont., Ann. Sci. Nat. sér. 2, 8: 369. 1837; *Androsaceus haematocephalus* (Mont.) Pat., J. Bot. 3: 336. 1889.

Description of collected carpophores

Pileus 2–5 mm broad when young, 7–12 mm when old, campanulate or obtusely conical, depressed at centre, with denticulate margin, sulcate-striate, dark violaceous. Lamellae distant, L = ca. 13–15, l = 0(–1), adnate, narrow (1–2 mm), horizontal to slightly ventricose, white-violaceous, with concolorous entire edge. Stipe 30–40 × 0.5–0.6 mm, very thin, filiform, fistulose, ± dark violaceous, with distinct whitish basal tomentum. Context membranaceous, whitish. Spore print whitish to ochraceous.

Basidiospores (14-)15–18.5 × 3.5–5.0 μm, av. = 15.7 × 4.1 μm, E = 3.0–4.3, Q = 3.8, narrowly fusoid, lacrimoid, narrowly clavate, thin-walled, hyaline, non-dextrinoid. Basidia 24 × 6.5 μm, 4-spored, clavate. Basidioles 13–28 ×

× 3.0–8.0 μm, clavate, subfusoid. Cheilocystidia in the form of broom-cells of the Siccus-type, 11–20 × 4.5–7.0 μm, clavate to cylindrical, thin-walled; projections up to 6.0 × 1.0(-1.5) μm, ± digitate, slightly thick-walled, obtuse to subacute, nodulose. Pleurocystidia 37–65 × 9.0–14 μm, numerous, fusoid, clavate, rostrate, thin-walled, with refractive contents, hyaline. Pileipellis a hymeniderm formed of broom-cells of the Siccus-type, (8.0-)11–18 × 5.0–10 μm, cylindrical to clavate, thin- to slightly thick-walled at base, slightly to distinctly thick-walled above, non-dextrinoid, with thick-walled parts grey-brown to brown-black in KOH; projections up to 6.0 × 1.0(-1.5) μm, digitate to narrowly conical, obtuse to subacute, mostly distinctly nodulose, moderately numerous (8–16(-20)), slightly to distinctly thick-walled, with walls grey-brown to brown-black in KOH (the coloration of pileipellis cells and projections is very distinct and dark). Stipitipellis a cutis consisting of cylindrical, parallel, thick-walled, dextrinoid, up to 5 μm wide hyphae with scattered short lateral projections towards apex and olivaceous walls in KOH. Caulocystidia absent. Clamp-connections present in all tissues.

Ecology and locality: Yaoundé, Campus of the University of Yaoundé, mostly in ± large groups, on dead fallen leaves and dead twigs, 8 June 2000 leg. D. C. Mossebo M272 (BRNM 686387 and herb. Mossebo).

Notes: *Marasmius haematocephalus* is characterised by having a small, ± dark violaceous pileus, distant, violaceous lamellae, rather large basidiospores, distinct pleurocystidia, and by the absence of caulocystidia. It belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Haematocephali* Singer.

Marasmius haematocephalus represents a rather common pantropical and subtropical species known from the U. S. A. (e.g. Desjardin and Horak 1997), Central and South America (Courtecuisse 1996, Dennis 1951, Desjardin and Horak 1997, Patouillard 1889, Pegler 1977, 1988, Pegler and Calonge 1997, Singer 1976), Asia (Desjardin and al. 2000, Pegler 1977, Petch 1948, Wen Hua-An and Sun Shu-Xiao 1999), and New Zealand and Papua New Guinea (Desjardin and Horak 1997). In tropical Africa, it has been found in Cameroon, Democratic Republic of Congo, Gabon, Ghana, Ivory Coast, Kenya, Nigeria, Republic of the Congo, Sierra Leone, Tanzania, Uganda and Zimbabwe (Hennings 1897, Nicholson 1989, Pegler 1977, Patouillard 1928).

Marasmius aff. katangensis Singer, Bull. Jard. Bot. Brux. 34: 375. 1964.

(Fig. 10)

Description of collected carpophores

Pileus 3–30 mm broad, subhemispherical to campanulate and slightly umbonate when young, then obtusely conical to plano-convex or applanate, regular at margin, slightly striate, grey-orange to dark orange, darker at centre. Lamellae

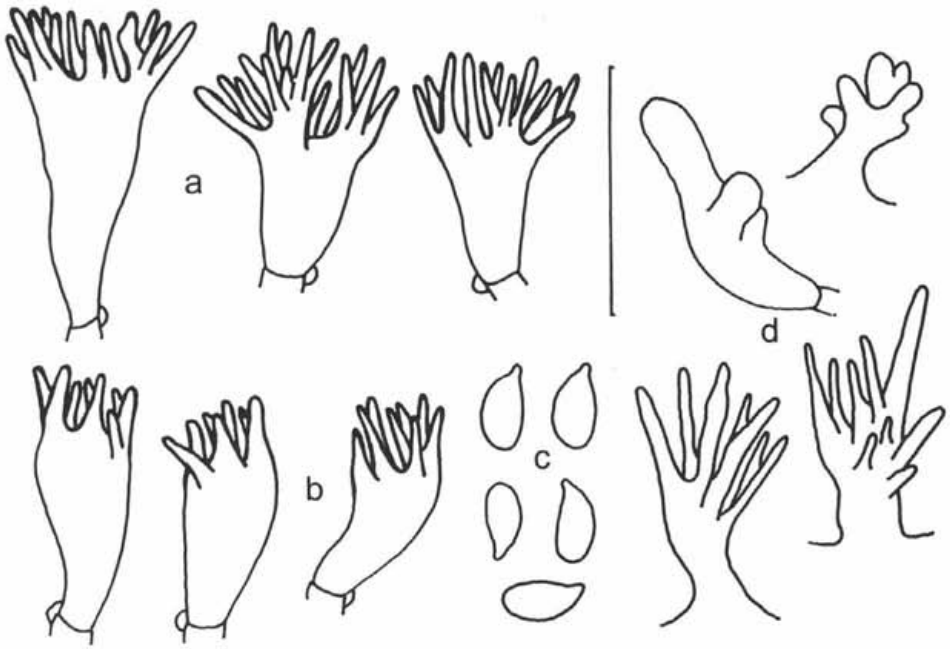

Fig. 10. *Marasmius* aff. *katangensis* (BRNM 686393; herb. Mossebo). a. pileipellis cells, b. cheilocystidia, c. basidiospores, d. caulocystidia. Scale bar = 20 μm.

rather crowded, L = c. 22–30, l = 3, narrow, 1–2 mm broad, horizontal to slightly ventricose, ochraceous to yellowish, with entire, concolorous edge. Stipe 30–55 × 1–1.5 mm, cylindrical, slightly broadened at base, fistulose, thin, whitish in the upper part, through brown-orange up to dark brown towards base and turning yellowish at the basal point. Context very thin to membranaceous (up to 1 mm above stipe insertion with pileus), whitish. Spore print whitish.

Basidiospores 6.2–7.5 × 2.7–3.5 μm, av. = 6.9 × 3.1 μm, E = 1.9–2.5, Q = 2.3, pip-shaped, ellipsoid-lacrimoid, thin-walled, hyaline, non-dextrinoid, mostly in tetrads in preparatum. Basidia 17–21 × 5.5–7.0 μm, 4-spored, clavate. Basidioles 13–32 × 2.5–7.0 μm, cylindrical, clavate, subfusoid. Cheilocystidia 11–15 × 5.0–8.0 μm, in the form of broom-cells of the Siccus-type, clavate, thin-walled, at apex sometimes slightly thick-walled, (sub)hyaline; projections up to 10 × 1.5 μm, digitate, ± obtuse, slightly thick-walled, slightly nodulose. Pleurocystidia absent. Trama hyphae cylindrical, thin-walled, hyaline to pale yellowish in KOH, dextrinoid, up to 10 μm wide. Pileipellis a hymeniderm formed of broom-cells of the Siccus-type, clavate to subcylindrical, thin-walled at base, thin– to slightly thick-walled above, with 8–18 digitate, obtuse to subacute, slightly nodulose, mostly slightly thick-walled, 4.0–10 × up to 1.0(–1.5) μm,

subhyaline to pale yellowish-greyish projections. Stipitipellis a cutis consisting of parallel, cylindrical, slightly thick-walled, smooth, up to 5.0 μm wide hyphae, non-dextrinoid and yellowish in KOH. Caulocystidia in the form of broom-cells of the Siccus-type similar to those in the pileipellis, 5.0–20 × 3.0–6.0 μm, entirely slightly thick-walled, with up to 15 × 2.0 μm, digitate projections, mixed with ± clavate, simple to coralloid cells. Clamp-connections present in all tissues.

Ecology and locality: Yaoundé, Campus of the University of Yaoundé, in groups, on fallen twigs and leaves on soil, 17 May 1999 leg. D. C. Mossebo M264 (BRNM 686393 and herb. Mossebo).

Notes: This fungus is characterised by having a grey-orange to dark orange pileus, rather crowded lamellae, a brown-orange to dark brown stipe towards base, small basidiospores, short basidia, two types of caulocystidia and lacking pleurocystidia. It belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Atrorubentes* Desjardin et E. Horak.

The most closely related species is *Marasmius katangensis* Singer with a similar type of caulocystidia. However, it has a dark brown pileus with pale brown margin and larger basidiospores (7.0–12.5 × 3.0–5.5 μm); *M. corrugatiformis* Singer differs especially by a brownish orange, ochraceous orange or bright orange pileus only at centre, its paler, yellow-orange or almost white margin, and by possessing only one type of caulocystidia (lacking typical broom-cell caulocystidia). *Marasmius confertus* Berk. et Broome has only broom-cell caulocystidia and possesses pleurocystidia (it belongs to ser. *Haematocephali* Singer).

Marasmius luteostipitatus Mossebo et Antonín sp. nov. (Fig. 11, Pl. 5)

Pileo 15–30 mm lato, late convexo, centro applanato, sulcato-striato, luteolo-albido, centro pallide ochraceo. Lamellis distantibus, L = 16–17, intervenosis, luteolo-albidis. Stipite 30–60 × 1–2 mm, cylindraceo, glabro, apicem luteolo vel luteo, ad basim obscure brunneo vel nigro-brunneo. Basidiosporis 16–18(-20) × × 4.0–5.0 μm, clavatis vel anguste lacrimoideis, hyalinis, inamyloideis. Basidiis tetrasporis. Cheilocystidiis e cellulis similibus cellulis typo Marasmii sicci, 16–20 × × 5.0–6.5 μm, clavatis vel subfusiformibus, tenuitunicatis. Pileipellis hymeniformis, e cellulis similibus cellulis hymenodermatis Marasmii sicci, 12–20 × 6.0–12 μm, clavatis vel subcylindraceis, tenuitunicatis vel crassitunicatis. Caulocystidiis absentibus. Hyphis fibulatis, in stipite et medulla dextrinoideis. Ad detritum.

HOLOTYPUS: Cameroon, Provincia Central, Nsimalen, 3. IV. 2001 leg. V. Antonín Cm01.13 et D. C. Mossebo (holotypus in herbario BRNM 666062 asservatur).

Description of collected carpophores

Pileus 15–30 mm broad, broadly convex, then ± applanate at centre, without a distinct central umbo, entirely striate-plicate, somewhat undulate at mar-

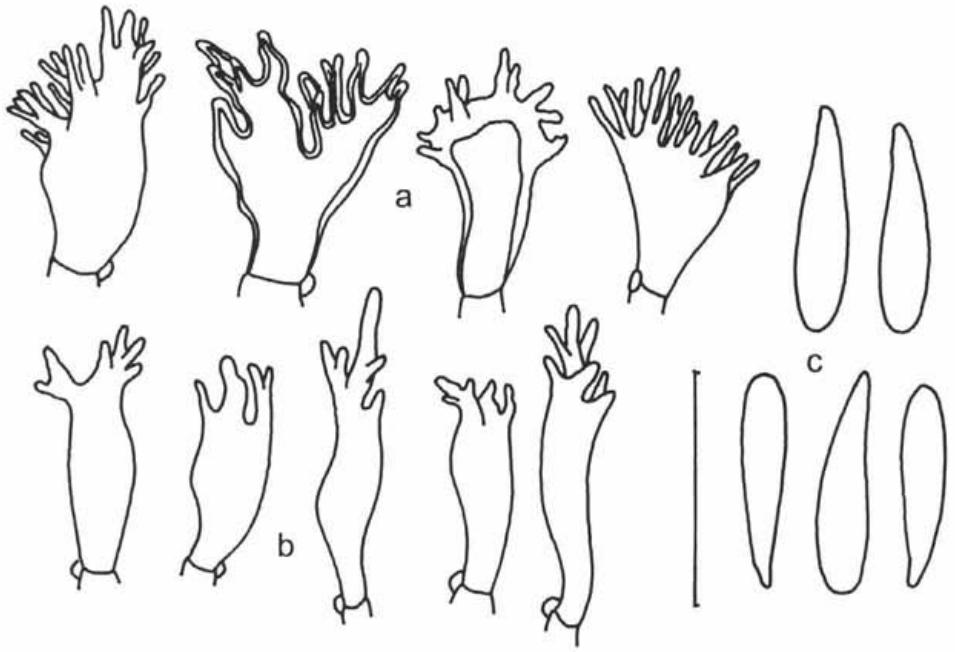

Fig. 11. *Marasmius luteostipitatus* (BRNM 666062). a. pileipellis cells, b. cheilocystidia, c. basidiospores. Scale bar = 20 μm.

gin, which is slightly reflexed when old, minutely tomentose, cream, more ochraceous-yellowish at centre, whitish cream at margin. Lamellae distant, L = 16–17, l = 2(–3), broadly adnate to a pseudocollarium, irregularly intervenose, moderately broad (up to 2–3 mm), cream, with concolorous pubescent edge. Stipe 30–60 × 1–2 mm, cylindrical, slightly broadened at apex, sometimes slightly broadened at base, curved, hollow, lustrous, smooth, glabrous, distinctly yellow at apex, dark brown towards base when young, then yellowish at apex, through a pale brown zone up to black-brown towards base.

Basidiospores 16–18(-20) × 4.0–5.0 μm, av. = 17.9 × 4.6 μm, E = 3.3–4.6, Q = 3.9, clavate, narrowly lacrimoid, thin-walled, non-dextrinoid, hyaline. Basidia 4-spored, clavate. Basidioles 13–47 × 3.0–9.0 μm, cylindrical, clavate. Cheilocystidia in the form of broom-cells of the Siccus-type transient to coralloid cells, 16–20 × 5.0–6.5 μm, (narrowly) clavate to subfusoid, thin-walled, with infrequent, nodulose, obtuse, ± thin-walled, up to 10 × 2.0 μm projections. Pleurocystidia absent. Trama hyphae cylindrical to subinflated, ± thin-walled, hyaline (pale yellowish in subpileipellis), dextrinoid, up to 15 μm wide. Pileipellis a hymeniderm made up of broom-cells of the Siccus-type, 12–20 × 6.0–12 μm, clavate or subcylindrical, often branched, thin– to distinctly thick-walled, with

10–35 nodulose, obtuse to subacute, thin– to thick-walled, up to 15.0 × 2.0 μm projections; thick-walled parts yellow in KOH. Stipitipellis a cutis consisting of cylindrical, parallel, slightly thick-walled, dextrinoid, up to 5.0 μm wide hyphae with yellowish (apex) or pale olivaceous (base) walls in KOH. Caulocystidia absent; scattered broom-cells present at apex. Clamp-connections present in all tissues.

Ecology and locality: Nsimalen, c. 20 km S of Yaoundé, single on detritus, 3 Apr. 2001 leg. V. Antonín Cm01.13 and D. C. Mossebo (holotype, BRNM 666062).

Notes: *Marasmius luteostipitatus* is characterised by having a pale coloured pileus, ochraceous-yellowish at centre, and whitish cream at margin, distant, irregularly intervenose lamellae, a distinctly yellow stipe at apex, moderately large basidiospores, cheilocystidia in the form of broom-cells with transient forms to coralloid cells and often branched or irregular pileipellis broom-cells; pleuro– and caulocystidia are lacking. Having those microscopic features, it belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Leonini* Singer.

Except for the presence of the yellow colour, it is very close to *M. haediniformis* Singer, with slightly smaller basidiospores (12.0–16.5 × (3.0-)3.5–5.0 μm), smaller cheilocystidia (13–16(-20) × 5.4–10 μm) and very short projections of the pileipellis broom-cells (1.0–5.0 × 1.0 μm). The apically yellow stipe represents a very distinct character in ser. *Leonini*. Only *Marasmius berteroi* var. *major* Singer, described from Argentina, has a similar stipe colour. However, it differs in having a larger, 10–56 mm broad, orange-fulvous, orange, orange-red or ferrugineous pileus, a reddish brown to chestnut brown stipe at base, smaller ((8-)9–15.3 × 2.7–4 μm) basidiospores and 20–30 × 5.5–7 μm basidia (Singer 1976).

Marasmius cf. sierraleonis Beeli, Bull. Jard. Bot. État Brux. 15: 36. 1938.
(Fig. 12)

Description of collected carpophores

Pileus ± 5 mm broad, convex, slightly depressed at centre, sulcate-striate, finely tomentose, rusty (cinnamomeous) brown. Lamellae moderately distant, L = 15, l = 0(–1), free, cream, with finely pubescent, cinnamomeous-brown edge. Stipe 35 × ± 0.5 mm, filiform, cylindrical, glabrous, smooth, lustrous, brownish at apex, through brown up to black brown towards base.

Basidiospores (14-)15–16.5 × 3.5–4.5 μm, av. = 15.5 × 3.9 μm, E = 3.4–4.5, Q = 4.0, fusoid, lacrimoid, clavate, thin-walled, non-dextrinoid, hyaline. Basidia 26–27 × 8.0–9.5 μm, 4-spored, clavate. Basidioles 15–28 × 5.0–9.0 μm, clavate, cylindrical or fusoid. Cheilocystidia in the form of broom-cells of the Siccus-type, 10–17 × 6.0–10 μm, clavate or subcylindrical, ± thin-walled with slightly thick-walled apex, with nodulose, slightly thick-walled, ± obtuse projections; thick-walled parts yellow-ochraceous in KOH. Pleurocystidia absent.

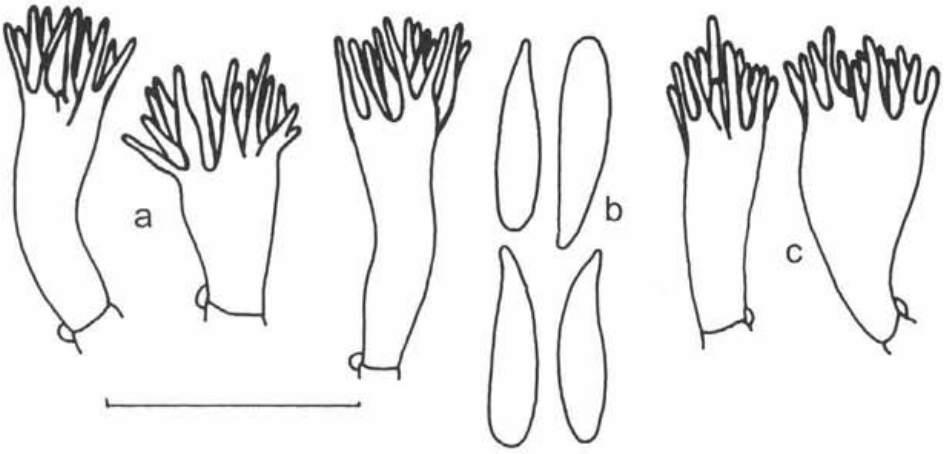

Fig. 12. *Marasmius* cf. *sierraleonis* (BRNM 666053). a. pileipellis cells, b. basidiospores, c. cheilocystidia. Scale bar = 20 μm.

Trama hyphae cylindrical to subinflated, thin-walled, dextrinoid, hyaline, up to 15 μm wide. Pileipellis a hymeniderm formed of broom-cells of the Siccus-type, 12–25 × 5.0–10 μm, clavate to (sub)cylindrical, thin-walled at base and slightly thick-walled at apex, with 9–20 slightly thick-walled, obtuse to subacute, nodulose, up to 15 × 1.5(–2.0) μm projections; thick-walled parts with ochraceous or yellow-brown walls in KOH. Stipitipellis a cutis consisting of cylindrical, parallel, slightly thick-walled, dextrinoid, up to 5.0 μm wide hyphae with ochraceous walls in KOH. Caulocystidia absent, except for scattered broom-cells at apex. Clamp-connections present in all tissues.

Ecology and locality: Yaoundé, Mt. Eloundem, single on detritus, 30 March 2001 leg. V. Antonín Cm01.04 and D. C. Mossebo (BRNM 666053).

Notes: *Marasmius sierraleonis* is characterised by having a dull yellowish to rusty (purplish) brown pileus, white to cream lamellae with hyaline then darkening edge, without (or with only one) lamellulae, a thin, black stipe, large, fusoid, sublacrimoid or narrowly clavate basidiospores and by lacking pleuro– and caulocystidia. Having those microscopic features, it belongs to sect. *Sicci*, subsect. *Siccini* Singer, ser. *Leonini* Singer. The collection published here differs by a smaller pileus and a slightly smaller stipe (pileus 7–25 mm broad and stipe 25–70 × 0.5–1 mm in typical forms) and smaller basidiospores (16–23(-25) × 3.5–6.0 μm in typical forms).

So far, *Marasmius sierraleonis* has been collected in Cameroon, the Democratic Republic of Congo, Kenya, Nigeria, Sierra Leone, Tanzania and Zimbabwe (Antonín 2004a).

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CZECH MYCOL. 56 (1–2), 2004

Effect of temperature on the production of sclerotia by the psychrotrophic fungus Typhula incarnata in Poland

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Hoshino T., Prończuk M., Kiriaki M. and Yumoto I. (2004): Effect of temperature on the production of sclerotia by the psychrotrophic fungus Typhula incarnata in Poland. – Czech Mycol. 56: 113–120

Isolates of the snow mold fungus *Typhula incarnata* from Radzików, Błonie, near Warsaw in Poland formed many small-sized sclerotia (< 1 mm) at 0 °C. This phenomenon was not observed in other isolates from different locations that have regions with annual snow cover. The formation of small-sized sclerotia by Polish isolates decreased with rising temperature, but the formation of large-sized sclerotia increased.

Key words: sclerotium size, snow mold fungi, *Typhula incarnata*

Hoshino T., Prończuk M., Kiriaki M. and Yumoto I. (2004): Vliv teploty na vývin sklerocií u psychrotrofního druhu Typhula incarnata v Polsku. – Czech Mycol. 56: 113–120

Kmeny druhu *Typhula incarnata* pocházející z Radzikówa u Blonia poblíž Varšavy v Polsku tvořily při teplotě 0 °C mnoho malých sklerocií (< 1 mm). Tento jev nebyl pozorován u kmenů pocházejících z oblastí, kde sněhová pokrývka vytrvává po delší období. Tvorba malých sklerocií u polských kmenů se snižovala s rostoucí teplotou, zatímco tvorba velkých sklerocií se zvyšovala.

INTRODUCTION

Snow mold fungi are psychrophilic or psychrotrophic fungal pathogens of perennial grasses and winter cereals in the Northern Hemisphere (Hsiang et al. 1999, Smith 1986). The genus *Typhula* (Basidiomycota) includes five species of snow mold fungi, *T. incarnata* Lasch ex Fr., *T. ishikariensis* S. Imai, *T. phacorrhiza* (Rich.: Fr.) Fr., *T. trifolii* Rostr. and *T. variabilis* Riess. The first two species have serious economic impact, and *T. incarnata* requires less than three months of snow cover to cause a serious injury to fodder grasses and winter cereals. Injuries caused

by this fungus have been observed even without snow cover (Årsvoll 1973, Bruehl and Cunfer 1971, Detiffe et al. 1981). *T. ishikariensis* is a psychrophilic fungus and is found mainly in areas with more than 5 months of snow cover (Årsvoll 1973, Bruehl and Cunfer 1971).

It has been reported that *T. incarnata* is a versatile pathogen with a different ecological behaviour in different environments (Matsumoto et al. 1995). In contrast, *T. ishikariensis* has evolved several infraspecific taxa adapted to different winter climate (Matsumoto 1992, 1994). Size variation of sclerotia in *T. ishikariensis* is as great as that in *T. incarnata*, but this is due to strain variability and the character is stable within the strain (Matsumoto et al. 2001). Matsumoto and Tajimi (1990) reported that there is a correlation between winter climate and sclerotium size in *T. ishikariensis*. Isolates from snowy regions had large sclerotia, while those from regions with less snow had small sclerotia. This has not been found in *T. incarnata*.

Dynowska (1983, 1984, 1992) reported *T. incarnata*, *T. ishikariensis*, *T. phacorrhiza*, *T. sclerotioides* (Pers.) Fr., *T. subvariabilis* Berthier and *T. variabilis* from Olsztyn in northern Poland. In April 2000, we found *T. incarnata* in Radzików, Błonie, *T. phacorrhiza* and *T. variabilis* in Bartążek, Bartąg in Poland. The winter climate in Poland is variable, and consequently the periods of snow cover are variable (Dynowska 1983, Prończuk and Zagdańska 1993). Polish isolates of *T. incarnata* seem to have adapted to shorter periods of snow cover compared with strains from other regions. In this study, we tried to elucidate the variation in morphological characteristics of *T. incarnata* from Poland, which are considered adaptations to shorter periods of snow cover.

MATERIALS AND METHODS

Isolation of Typhula incarnata from overwintering grass leaves in Poland

Fungal sclerotia were collected from decayed leaves or stems of perennial ryegrass (*Lolium perenne* L.) from the Plant Breeding and Acclimatization Institute in Radzików, Błonie, 30 km east of Warsaw, Poland on May 19–21, 2000. The fungal sclerotia were placed in paper envelopes and dried at room temperature during transportation. In the laboratory (AIST, Japan), the fungal sclerotia were surface-sterilized in 70 % (v/v) ethanol and 0.5 % (as active chlorine) sodium hypochlorite solution and thoroughly washed with sterilized distilled water. They were then cut with sterilized razor blades, placed on potato dextrose agar (PDA, Difco, Becton Dickinson Microbiology Systems, MD, USA) so that cut surfaces were in contact with the agar, and incubated at 5 °C. Mycelia from growing margins of the colonies were transferred to new PDA plates (each 9 cm in diameter).

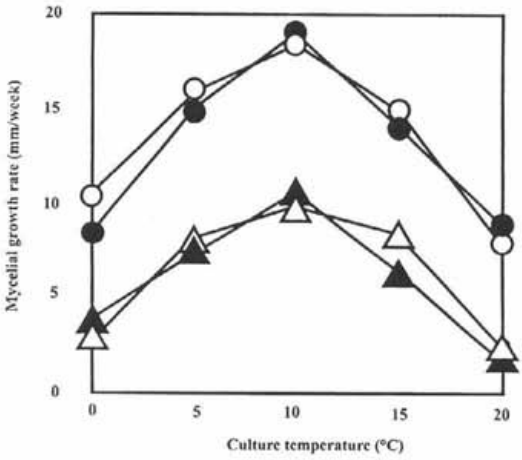

Fig. 1. Thermal dependence of mycelial growth rate of *Typhula incarnata* from Poland and Russia. Open circles: strain R-1, closed circles: strain R-2 from Poland (Radzików, Błonie), open triangles: strain SPB-1, closed triangles: strain SPB-2 from Russia (St. Petersburg).

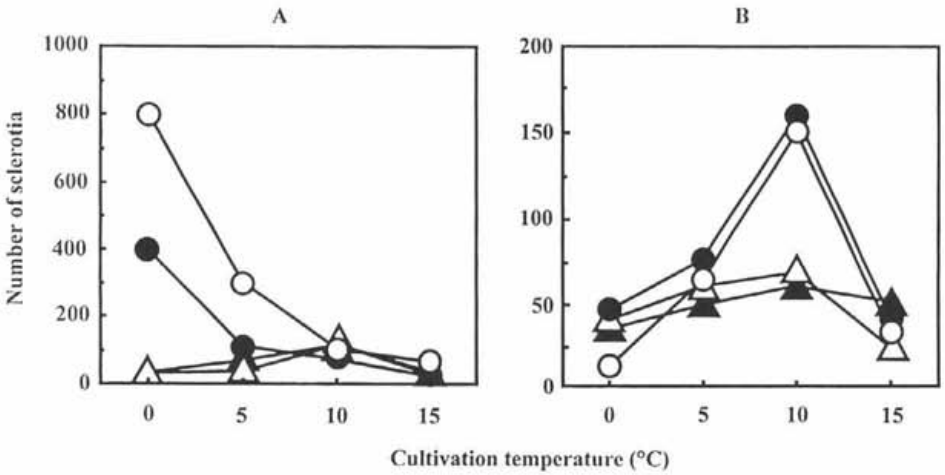

Fig. 2. Effects of temperature on sclerotium formation of cultures of *Typhula incarnata* in Poland and Russia. A. Number of small-sized sclerotia ($<$ 1 mm). B. Number of normal-sized sclerotia ($>$ 1 mm). Open and closed circles: isolates from Poland (Radzików, Błonie), open and closed triangles: isolates from Russia (St. Petersburg).

Fungal strains in this research

Two strains of *T. incarnata* from different sclerotia collected in Radzików, Poland were used in our study. Another two strains of *T. incarnata* from St. Petersburg in Russia were used for comparison in our research. Isolates from St. Petersburg were prepared from fungal sclerotia in winter wheat from the experimental field of N. I. Vavilov Research Institute of Plant Industry. We obtained these fungal sclerotia on May 2000. All isolates were maintained on PDA slant cultures at 0 °C.

Growth temperature of mycelia and sclerotium formation

Mycelial discs of 5 mm diameter were cut from the margin of an actively growing colony, transferred to the centre of PDA plates (I. D., 9 cm), and inoculated in duplicate at five different temperatures from 0 to 20 °C. During mycelial growth, the colony diameter was observed daily for up to 21 days after inoculation. The linear mycelial growth rate per week was calculated after the initial log period. After 2 months of incubation at 0, 5, 10 and 15 °C, the number and diameters of sclerotia on each plate were measured, in duplicate.

RESULTS AND DISCUSSION

Four pathogenic *Typhula* species, *T. incarnata*, *T. ishikariensis*, *T. phacorrhiza* and *T. variabilis*, have been found in Olsztyn in northern Poland (Dynowska 1983, 1984). We found a few infections of *T. incarnata* in overwintering leaves of perennial ryegrass (*Lolium perenne* L.) in Radzików, Błonie, in the central part of Poland. We also collected sclerotia of *T. phacorrhiza* and *T. variabilis* from perennial ryegrass in Bartążek, Bartąg, northern Poland. However, *T. ishikariensis* was not found in those two areas, probably because it needs a longer period (more than 5 months) of snow cover (Årsvoll 1973, Bruehl and Cunfer 1971). Therefore, *T. ishikariensis* might not have adapted to the current warm climate in Poland (Prończuk and Zagdańska 1993).

The sclerotia of *T. incarnata* collected from Radzików, Błonie were red to dark brown in colour and globose to oval in shape and were formed on the surface and inside leaves. The average size of 100 sclerotia was 0.51–0.79 (av. 0.62) × 0.39–0.47 (av. 0.44) mm. The morphology of sclerotia was almost the same as that described by Dynowska (1983, 0.45–3.5 × 0.5–2 mm), but the average sclerotium size was smaller than those in other localities (3–4 mm: Berthier 1976 from unknown localities, 0.5–4.5 × 0.5–2 mm: Ito 1955 from Japan, 0.5–2 × 1.5–4 mm: Remsberg

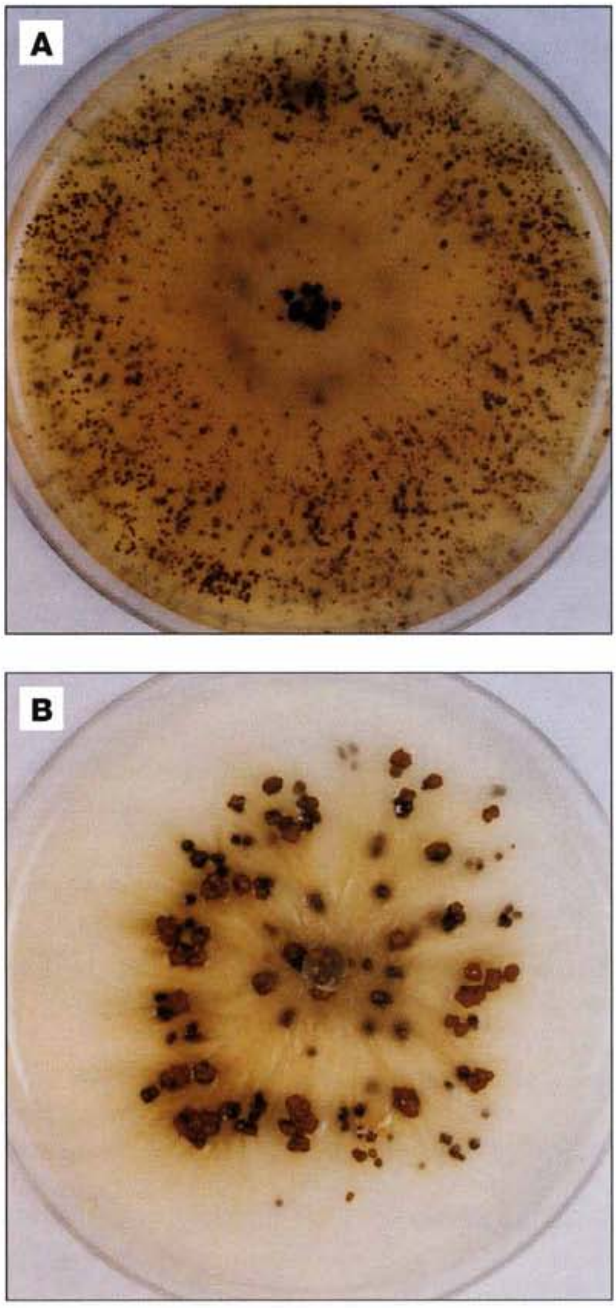

Fig. 3. Morphology of colonies of *Typhula incarnata* on PDA, grown at 0 °C over a period of 1 month. A. Strain R-1 from Poland (Radzików, Blonie). B. Strain SPB-1 from St. Petersburg, Russia.

1940 from Finland). Host plants of this fungus had been only slightly damaged by fungal infections, suggesting that snow mold diseases caused by *T. incarnata* do not have a great impact on overwintering grasses in Radzików, Błonie.

Figure 1 shows the thermal dependence of mycelial growth rates of several isolates collected in Poland and one other locality (St. Petersburg, Russia). Isolates from Poland and Russia showed the same optimum growth temperature, 10 °C. The same results were obtained from isolates from other countries (e.g. Smith 1986). However, mycelial growth rates of Polish isolates were faster than those of Russian isolates. In addition, Polish isolates formed many small-sized sclerotia (< 1 mm) on PDA at 0 °C (Fig. 2A and Fig. 3A). The same results were obtained by using other cultural media such as corn meal agar, lima bean agar, malt extract agar, oat meal agar and tomato juice agar (data not shown). We did not observe a high production of small-sized sclerotia from other isolates of *T. incarnata* in our cultural collection from various localities such as Japan, Russia and Nordic countries (Faroe Islands, Greenland, Iceland and Norway). Wu and Hsiang (1999) and Kim et al. (1992) reported that *T. incarnata* produced abundant sclerotia at temperatures ranging from 0 to 15 °C. A similar pattern was observed in the formation of normal-sized sclerotia (> 1 mm) of Polish isolates (Fig. 2B). However, the formation of small-sized sclerotia decreased with increasing cultivation temperature.

T. ishikariensis biotype C, which is highly adapted to the condition of short snow cover in Japan, is also known to produce small-sized sclerotia (Honkura et al. 1986). However, the sclerotium size of *T. ishikariensis* is not dependent on environmental conditions including culture temperature. On the other hand, sclerotium production of Polish isolates of *T. incarnata* was greatly changed by low temperatures. Small and large sclerotia of *T. incarnata* from Poland did not show any differences in morphological characteristics (rind pattern and basidiocarp formation, data not shown). Therefore, small-sized sclerotia of *T. incarnata* probably had the same ecological role as that of large-sized sclerotia.

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Checklist of downy mildews, rusts and smuts of Moravia and Silesia

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Kokeš P. and Müller J. (2004): Checklist of downy mildews, rusts and smuts of Moravia and Silesia. – Czech Mycol. 56: 121–148

This checklist includes 736 taxa of downy mildews, rusts and smuts reported from Moravia and Czech Silesia, Czech Republic. There are 114 species parasiting on crops and other cultivated plants. The list includes the frequency of occurrence, i. e. commonness or rarity of individual taxa. The work is based on literature data.

Key words: plant-parasitic fungi, occurrence, regions of the Czech Republic, Peronosporales, Sclerosporales, Urediniomycetes, Ustilaginomycetes.

Kokeš P. a Müller J. (2004): Seznam fytopatogenních plísní, rzí a snětí Moravy a Slezska. – Czech Mycol. 56: 121–148

Seznam fytopatogenních plísní, rzí a snětí Moravy a českého Slezska obsahuje 736 taxonů. Mezi nimi je 114 druhů, které parazitují na kulturních rostlinách. U jednotlivých taxonů je uvedena frekvence výskytu, tj. jejich hojnost resp. vzácnost. Práce vychází téměř výhradně z literárních údajů.

INTRODUCTION

This work is the first complete checklist of downy mildews (Peronosporales s. str. and Sclerosporales), rusts (Urediniomycetes) and smuts (Ustilaginomycetes) of Moravia and Czech Silesia (eastern part of the Czech Republic). The first and last flora of Moravian and Silesian fungi was written by Niessl (1865). His work includes also downy mildews, rusts and smuts, but this work is now very outdated. Picbauer (1927) published a summary of Moravian rusts with their hosts and localities which were known up to that date. In the last 77 years many new species have been found in the area, new knowledge of the biology of rusts was obtained, and the species concept and nomenclature were changed.

Our checklist is based on 259 mycological works. Downy mildews are divided into the orders Sclerosporales and Peronosporales from the class Oomycetes according to Dick et al. (1984) and Dick (2001). Rusts and smuts are defined according to Vánky (2001), and Cummins and Hiratsuka (1984). The orders Microstromatales and Exobasidiales are newly classified as smuts, the order Microbotryales is reclassified from smuts into rusts. The taxa in these groups are arranged alphabetically. Doubtful data, only quoted by Hruby (1927, 1929,

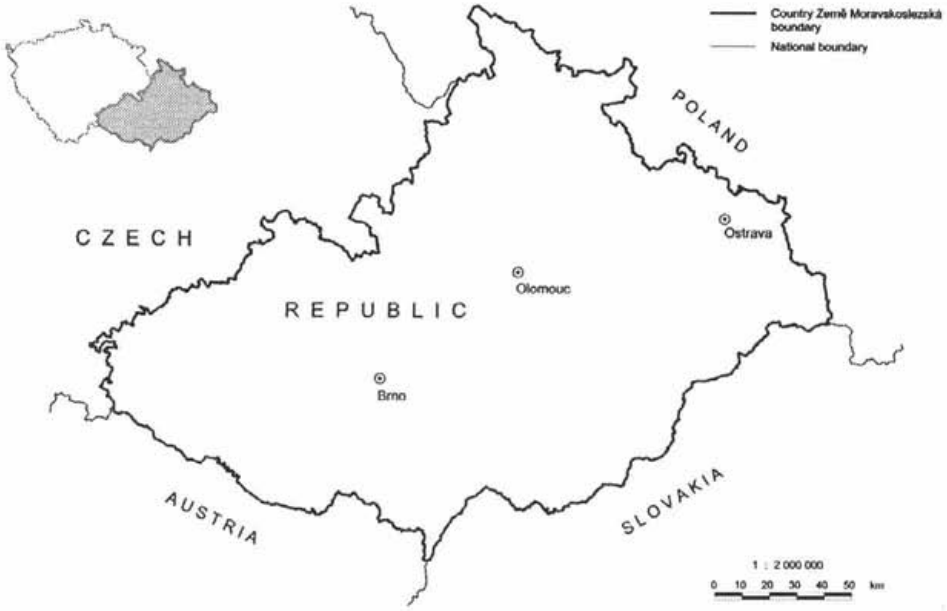

Fig. 1. Map of the studied area (country Země Moravskoslezská).

1930) and later not verified, are placed separately at the end of the checklist. Some of these data are incorrect. See the criticism of Picbauer (1928b), the notes by Skalický (1953b, 1954a, 1983) and by Vánky (1994).

The nomenclature of Sclerosporales and Peronosporales is according to Constantinescu (1991), Constantinescu and Fatehi (2002), Wrońska (1986), Brandenburger (1985), smuts according to Vánky (1994, 1998a, 1998b, 2000), Nannfeldt (1981), Brandenburger (1985), and rusts mostly according to Brandenburger (1994), partially according to Zwetko (2000), rusts on sedges according to Poelt and Zwetko (1997), and rusts on grasses according to Urban and Marková (1987, 1994a, 1994b, 1995, 1999), Urban (1969, 1995, 1997) and Marková and Urban (1997, 1998). Author's names are abbreviated according to Brummitt and Powell (2004).

The studied area was set according to the border of the country Země Moravskoslezská, which was effective in the periods 1928–1939 and 1945–1948 (Fig. 1). This area occupies about 26.8 thousand km^2. There are two geographical units in the area, the Czech Highlands and the Carpathian Mountains. A small part of the Pannonian Lowlands is situated in the south.

As basic information we quote the frequency of occurrence for individual taxa: 0 – extinct or missing, 1 – rare, 2 – rather rare, 3 – scattered, 4 – rather common, 5 – common. These rates are based on 55 years experience of Jiří Müller, taking

account of literature data. Some species of the mentioned parasitic fungi occur on several species of hosts in different frequencies of occurrence. For example *Albugo candida* is common on *Capsella bursa-pastoris* (L.) Medik., but it is rare on *Lunaria annua* L. In this case the taxon is quoted as common (5). The frequency of occurrence of the fungus is consequently rated by the highest level reached on any host. Taxa are rated according to the present situation. For example *Urocystis occulta* was still scattered in the 1930s, but now it is rated as missing.

In cases, when it was not clear to which taxon the literature record belongs, we used the species in a wider sense: *Plasmopara umbelliferarum* s. lat. (on *Angelica palustris* (Besser) Hoffm., *Heracleum sphondylium* L., *Levisticum officinale* W. D. J. Koch, *Torilis japonica* (Houtt.) DC.), *Microbotryum violaceum* s. lat. (on *Dianthus pontederae* A. Kern.), *Puccinia arenariicola* s. lat. (on *Carex ovalis* Gooden.), *Puccinia graminis* (on *Polypogon monspeliensis* (L.) Desf.), *Puccinia urticata* (on *Carex hordeistichos* Vill., *Urtica dioica* L., *Urtica urens* L.).

Puccinia dactylidina Bubák is not mentioned in the checklist. Its occurrence in Moravia was published by Zimmermann (1914: 82) and Picbauer (1927b: 473, 1929: 14, 1942a: 77, 1942b: 189), but Urban (1966a: 212) discovered that all records concern *Puccinia graminis* subsp. *graminicola*.

RESULTS

Peronosporales s. str.

Albugo *amaranthi* (Schwein.) Kuntze – 5, *A. candida* (Pers. ex Hook.) Kuntze – 5, *A. caryophyllacearum* (Wallr.) Cif. et Biga – 0, *A. portulacae* (DC.) Kuntze – 1, *A. tragopogonis* (Pers.) Gray – 3.

Basidiophora *entospora* Roze et Cornu – 1.

Bremia *lactucae* Regel s. lat. – 5.

Bremiella *baudysii* (Skalický) Constant. et Negrean – 1.

Hyaloperonospora *lunariae* (Gäum.) Constant. – 4, *H. niessleana* (Berl.) Constant. – 3, *H. parasitica* (Pers.: Fr.) Constant. – 5.

Paraperonospora *leptosperma* (de Bary) Constant. – 4, *P. tanaceti* (Gäum.) Constant. – 1.

Perofascia *lepidii* (McAlpine) Constant. – 1.

Peronospora *aestivalis* Syd. – 3, *P. affinis* Rossmann – 4, *P. agrestis* Gäum. – 5, *P. agrostemmatis* Gäum. – 0, *P. alchemillae* G. H. Otth – 2, *P. alpicola* Gäum. – 1, *P. alsinearum* Casp. – 5, *P. alta* Fuckel – 5, *P. androsaces* Niessl – 1, *P. antirrhini* J. Schröt. – 1, *P. aparines* (de Bary) Gäum. – 5, *P. aquatica* Gäum. – 2, *P. arborescens* (Berk.) Casp. – 5, *P. arenariae* (Berk.) Tul. – 5,

P. arthurii Farl. – 1, *P. arvensis* Gäum. – 5, *P. asperuginis* W. G. Schneid. – 2, *P. astragalina* Syd. – 1, *P. boni-henrici* Gäum. – 3, *P. bulbocapni* Beck – 3, *P. calotheca* de Bary – 5, *P. campestris* Gäum. – 1, *P. candida* Fuckel – 2, *P. chenopodii* Schltdl. – 5, *P. chenopodii-glauci* Gäum. – 1, *P. chenopodii-polyspermi* Gäum. – 3, *P. chrysosplenii* Fuckel – 3, *P. conglomerata* Fuckel – 5, *P. consolidae* Lagerh. ex Jacz. et P. A. Jacz. – 1, *P. coronillae* Gäum. – 2, *P. corydalis* de Bary – 5, *P. corydalis-intermediae* Gäum. – 1, *P. cyparissiae* de Bary – 1, *P. debaryi* E. S. Salmon et Ware – 2, *P. destructor* (Berk.) Casp. ex Berk. – 3, *P. digitalidis* Gäum. – 1, *P. dipsaci* Tul. ex de Bary – 1, *P. echinospermi* (Swingle) Swingle – 1, *P. effusa* (Grev.) Rabenh. – 3, *P. erodii* Fuckel – 2, *P. ervi* A. Gustavsson – 3, *P. erythraeae* J. G. Kühn ex Gäum. – 1, *P. euphorbiae* Fuckel – 1, *P. ficariae* Tul. ex de Bary – 5, *P. flava* Gäum. – 1, *P. fulva* Syd. – 3, *P. galii* Fuckel – 3, *P. gei* Syd. – 1, *P. glechomae* Oescu et Rădul. – 1, *P. grisea* (Unger) Unger – 3, *P. herniariae* de Bary – 1, *P. hiemalis* Gäum. – 4, *P. holostei* Casp. ex de Bary – 2, *P. hyoscyami* de Bary – 1, *P. knautiae* Fuckel ex J. Schröt. – 3, *P. kochiae-scopariae* Kochman et T. Majewski – 2, *P. lamii* A. Braun – 5, *P. lathyri-verni* A. Gustavsson – 2, *P. lentis* Gäum. – 1, *P. lepigoni* Fuckel – 1, *P. linariae* Fuckel – 2, *P. linariae-genistifoliae* Săvul. et Rayss – 1, *P. lini* J. Schröt. – 1, *P. lithospermi* Gäum. – 1, *P. lotorum* Syd. – 1, *P. lychnitis* Gäum. – 1, *P. manshurica* (Naumov) Syd. – 2, *P. mayorii* Gäum. – 2, *P. melampyri* (Buchholz) Davis – 1, *P. melandrii* Gäum. – 2, *P. meliloti* Syd. – 4, *P. minor* (Casp.) Gäum. – 5, *P. myosotidis* de Bary – 3, *P. obovata* Bonord. – 2, *P. oerteliana* J. G. Kühn – 2, *P. omphalodis* Gäum. – 3, *P. parva* Gäum. – 1, *P. paula* A. Gustavsson – 1, *P. phacae* Gäum. – 2, *P. phyteumatis* Fuckel – 1, *P. pisi* Syd. – 2, *P. plantaginis* Underw. – 1, *P. polygoni* (Thüm.) A. Fisch. – 3, *P. polygoni-convolvuli* A. Gustavsson – 3, *P. potentillae* de Bary – 1,

Fig. 1. *Plasmopara halstedii* on *Helianthus annuus* L.; Vyškov, Marefy, field above the slope Ševy.
Fig. 2. *Coleosporium inulae* (III) on *Inula ensifolia* L.; Vyškov, Nevojice, Malhotky.
Fig. 3. *Endophyllum euphorbiae-sylvaticae* (I) on *Euphorbia amygdaloides* L.; Vyškov, Kožušice, Strabišov.
Fig. 4. *Frommeëlla mexicana* var. *indicae* (II) on *Duchesnea indica* (Andrews) Focke; Brno - město, Veveří, Botanic Gardens of Masaryk University.
Fig. 5. *Melampsora galanthi-fragilis* (0, I) on *Galanthus nivalis* L.; Vyškov, Doubrava, valley of Velká Haná - Kamenná chaloupka.
Fig. 6. *Microbotryum scorzonerae* on *Scorzonera humilis* L.; Vyškov, Stříbrná, Romanovické louky.
Notes to the photographs:
0 = spermogonia
I = aecia
II = uredia
III = telia
All photographs were taken by Petr Kokeš in the period 2001–2003. The localities are structured as follows: district, municipality, name of the locality.


1


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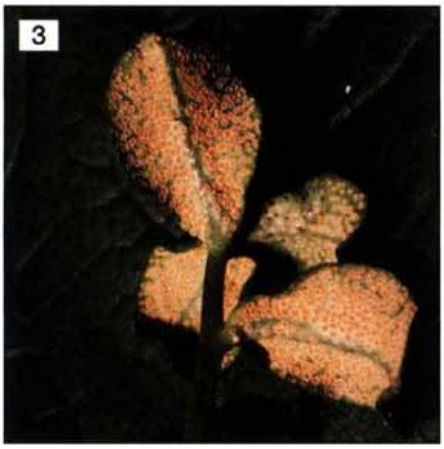
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P. potentillae-reptantis Gäum. – 1, *P. pulveracea* Fuckel – 1, *P. radii* de Bary – 1, *P. ranunculi* Gäum. – 5, *P. romanica* Săvul. et Rayss – 3, *P. rubi* Rabenh. ex J. Schröt. – 1, *P. ruegeriae* Gäum. – 1, *P. rumicis* Corda – 3, *P. sanguisorbae* Gäum. – 1, *P. saxifragae* Bubák – 1, *P. schachtii* Fuckel – 3, *P. scleranthi* Rabenh. ex J. Schröt. – 4, *P. senneniana* Gonz. Frag. et Sacc. – 3, *P. sepium* Gäum. – 3, *P. sherardiae* Fuckel – 2, *P. silenes* G. W. Wilson – 1, *P. sordida* Berk. et Broome – 3, *P. sparsa* Berk. – 4, *P. stachydis* Syd. – 2, *P. statices* Lobik – 2, *P. swinglei* Ellis et Kellerm. – 1, *P. symphyti* Gäum. – 3, *P. tabacina* D. B. Adam – 0, *P. tomentosa* Fuckel – 1, *P. trifolii-arvensis* Syd. – 1, *P. trifolii-hybridi* Gäum. – 4, *P. trifoliorum* de Bary – 4, *P. trivialis* Gäum. – 3, *P. valerianellae* Fuckel – 3, *P. verbasci* Gäum. – 3, *P. viciae* (Berk.) Casp. – 3, *P. violacea* Berk. – 2, *P. violae* de Bary ex J. Schröt. – 4.

Plasmopara *angelicae* (Casp.) Trotter – 2, *P. angustiterminalis* Novot. – 1, *P. caucalis* Săvul. et O. Săvul. – 0, *P. chaerophylli* (Casp.) Trotter – 4, *P. conii* (Casp.) Trotter – 1, *P. dauci* Săvul. et O. Săvul. – 2, *P. densa* (Rabenh.) J. Schröt. – 2, *P. epilobii* (G. H. Otth) Sacc. et P. Syd. – 1, *P. geranii-sylvatici* Săvul. et O. Săvul. – 4, *P. halstedii* (Farl.) Berl. et de Toni – 2, *P. isopyri-thalictroidis* (Săvul. et Rayss) Săvul. – 3, *P. obducens* (J. Schröt.) J. Schröt. – 2, *P. pastinacae* Săvul. et O. Săvul. – 2, *P. petroselini* Săvul. et O. Săvul. – 2, *P. pimpinellae* var. *maioris* Wrońska – 3, *P. pimpinellae* Săvul. et O. Săvul. var. *pimpinellae* – 3, *P. pusilla* (de Bary) J. Schröt. – 5, *P. pygmaea* (Unger) J. Schröt. s. lat. – 4, *P. ribicola* J. Schröt. – 1, *P. selini* Wrońska – 1, *P. silai* Săvul. et O. Săvul. – 1, *P. umbelliferarum* var. *hacquetiae* Skalický – 1, *P. umbelliferarum* (Casp.) J. Schröt. ex. Wartenw. s. str. var. *umbelliferarum* – 5, *P. umbelliferarum* s. lat. – 1, *P. viticola* (Berk. et M. A. Curtis ex de Bary) Berl. et de Toni – 5.

Pseudoperonospora *cubensis* (Berk. et M. A. Curtis) Rostovzev – 5, *P. humuli* (Miyabe et Takah.) G. W. Wilson – 4, *P. urticae* (Lib. ex Berk.) E. S. Salmon et Ware – 2.

Fig. 7. *Microbotryum silenes-inflatae* on *Lychnis viscaria* L.; Vyškov, Kotáry, U tří jedlí.
Fig. 8. *Microbotryum stellariae* on *Stellaria graminea* L.; Vyškov, Pulkava, valley of Brodečka - Obrova noha.
Fig. 9. *Milesina feurichii* (II) on *Asplenium septentrionale* (L.) Hoffm.; Vyškov, Opatovice, valley of Malá Haná - village chapel of St. Jan Nepomucký.
Fig. 10. *Phragmidium rosae-pimpinellifoliae* (I) on *Rosa spinosissima* L.; Brno - venkov, Kanice, Hády.
Fig. 11. *Puccinia betonicae* (III) on *Betonica officinalis* L.; Vyškov, Kotáry, U tří jedlí.
Fig. 12. *Puccinia galanthi* (III) on *Galanthus nivalis* L.; Vyškov, Němčany, Němčanský háj.
Notes to the photographs:
0 = spermogonia
I = aecia
II = uredia
III = telia
All photographs were taken by Petr Kokeš in the period 2001–2003. The localities are structured as follows: district, municipality, name of the locality.

Sclerosporales

Sclerospora *graminicola* (Sacc.) J. Schröt. – 1.

Urediniomycetes

Aecidium *senecionis-crispati* J. Schröt. – 1, *A. teodorescui* Săvul. et O. Săvul. – 1.

Chrysomyxa *abietis* (Wallr.) Unger – 1, *C. empetri* J. Schröt. ex Cummins – 1, *C. ledi* (Alb. et Schwein.) de Bary – 1, *C. pyrolata* G. Winter s. str. – 1, *C. ramischiae* Lagerh. – 1.

Coleosporium *cacaliae* G. H. Otth – 4, *C. campanulae* (F. Strauss) Tul. – 5, *C. cerinthes* J. Schröt. – 1, *C. doronici* Namysł. ex Syd. et P. Syd. – 1, *C. euphrasiae* G. Winter – 2, *C. inulae* Rabenh. – 2, *C. martianoffianum* Syd. – 1, *C. melampyri* (Rebent.) P. Karst. – 4, *C. petasitis* Cooke – 5, *C. pulsatillae* (F. Strauss) Fuckel – 1, *C. senecionis* (Pers.) J. Kickx f. – 5, *C. sonchi* (F. Strauss) Tul. – 3, *C. tussilaginis* (Pers.) Berk. s. str. – 5.

Cronartium *flaccidum* (Alb. et Schwein.) G. Winter – 5, *C. ribicola* J. C. Fisch. – 5.

Cumminsiella *mirabilissima* (Peck) Nannf. – 5.

Endophyllum *euphorbiae-sylvaticae* (DC.) G. Winter – 2, *E. sempervivi* (Alb. et Schwein.) de Bary – 3.

Frommeëlla *mexicana* var. *indicae* J. W. McCain et J. F. Hennen – 1, *F. tormentillae* (Fuckel) Cummins et Y. Hirats. – 2.

Gymnosporangium *clavariiforme* (Pers.) DC. – 2, *G. cornutum* Arthur ex F. Kern – 3, *G. sabinae* G. Winter – 5, *G. tremelloides* R. Hartig – 1.

Hyalopsora *aspidiotus* (Magnus) Magnus – 3, *H. polypodii* (Dietel) Magnus – 4.

Kuehneola *uredinis* (Link) Arthur – 3.

Leucotelium *cerasi* (Bérenger) Tranzschel – 1.

Melampsora *allii-fragilis* Kleb. s. str. – 2, *M. allii-populina* Kleb. – 3, *M. amygdalinae* Kleb. – 3, *M. ari-salicina* A. Raabe – 1, *M. caprearum* Thüm. – 5, *M. euonymi-caprearum* Kleb. – 1, *M. euphorbiae* (C. Schub.) Castagne s. str. – 5, *M. euphorbiae-amygdaloidis* W. Muell. – 1, *M. euphorbiae-dulcis* G. H. Otth – 4, *M. euphorbiae-gerardianae* W. Muell. – 2, *M. galanthi-fragilis* Kleb. – 1, *M. helioscopiae* G. Winter s. str. – 5, *M. hypericorum* G. Winter – 3, *M. lapponum* Lindf. – 1, *M. larici-epitea* Kleb. – 3, *M. larici-populina* Kleb. – 4, *M. laricis* R. Hartig ex Kleb. – 4, *M. lini* (Ehrenb.) Desm. – 3, *M. liniperda* (Körn.) Palm – 1, *M. magnusiana* G. Wagner ex Kleb. – 2, *M. pinitorqua* Rostr. – 1, *M. repentis* Plowr. – 0, *M. ribesii-purpureae* Kleb. – 1, *M. ribesii-viminalis*

Kleb. – 1, *M. rostrupii* G. Wagner ex Kleb. – 3, *M. salicis-albae* Kleb. – 3, *M. vernalis* Niessl ex G. Winter – 1.

Melampsorella *caryophyllacearum* J. Schröt. – 3.

Melampsoridium *betulinum* (Fr.) Kleb. – 5, *M. carpini* (Fuckel) Dietel – 1, *M. hiratsukanum* S. Ito ex Hirats. – 4.

Microbotryum *anomalum* (J. Kunze ex G. Winter) Vánky – 2, *M. bistortarum* (DC.) Vánky – 1, *M. cordae* (Liro) G. Deml et Prillinger – 2, *M. dianthorum* (Liro) H. Scholz et I. Scholz – 2, *M. duriaeanum* (Tul. et C. Tul.) Vánky – 1, *M. holostei* (de Bary) Vánky – 1, *M. lychnidis-dioicae* (DC. ex Liro) G. Deml et Oberw. – 3, *M. majus* (J. Schröt.) G. Deml et Oberw. – 2, *M. marginale* (DC.) Vánky – 1, *M. parlatorei* (A. A. Fisch. Waldh.) Vánky – 1, *M. reticulatum* (Liro) R. Bauer et Oberw. – 3, *M. scabiosae* Vánky – 5, *M. scorzonerae* (Alb. et Schwein.) G. Deml et Prillinger – 1, *M. silenes-inflatae* (DC. ex Liro) G. Deml et Oberw. – 2, *M. stellariae* (Liro) G. Deml et Oberw. – 1, *M. stygium* (Liro) Vánky – 1, *M. tragopogonis-pratensis* (Pers.) R. Bauer et Oberw. – 3, *M. violaceum* (Pers.: Pers.) G. Deml et Oberw. s. str. – 2, *M. violaceum* s. lat. – 4.

Milesina *blechni* (P. Syd. et Syd.) P. Syd. et Syd. – 1, *M. dieteliana* (P. Syd. et Syd.) Magnus – 1, *M. feurichii* (Magnus) Magnus – 2, *M. kriegeriana* (Magnus) Magnus – 1, *M. murariae* (Faull) P. Syd. et Syd. ex Hirats. f. – 2, *M. vogesiaca* (Faull) P. Syd. et Syd. ex Hirats. f. – 1.

Naohidemyces *vaccinii* (G. Winter) S. Sato, Katsuya et Y. Hirats. – 3.

Nyssopsora *echinata* (Lév.) Arthur – 1.

Ochropsora *ariae* (Fuckel) Ramsb. – 3.

Phragmidium *bulbosum* (F. Strauss) Schltdl. – 5, *P. candicantium* (Vleugel) Dietel – 2, *P. fragariae* (DC.) Rabenh. – 3, *P. fusiforme* J. Schröt. – 3, *P. mucronatum* (Pers.) Schltdl. – 4, *P. potentillae* (Pers.) P. Karst. – 3, *P. rosae-pimpinellifoliae* Dietel – 1, *P. rubi-idaei* (DC.) P. Karst. – 3, *P. sanguisorbae* (DC.) J. Schröt. – 4, *P. tuberculatum* Jul. Müll. – 4, *P. violaceum* (Schultz) G. Winter – 4.

Puccinia *abrotani* Fahrend. – 1, *P. absinthii* (R. Hedw. ex DC.) DC. – 3, *P. acetosae* Körn. – 4, *P. adoxae* R. Hedw. ex DC. – 3, *P. aecidii-leucanthemi* E. Fisch. – 1, *P. aegopodii* Röhl. – 5, *P. albescens* Plowr. – 2, *P. allii* F. Rudolphi – 1, *P. alpina* Fuckel – 1, *P. angelicae* (Schumach.) Fuckel s. str. – 1, *P. angelicae-mamillata* Kleb. – 2, *P. antirrhini* Dietel et Holw. – 3, *P. apii* Desm. – 1, *P. arenariae* (Schumach.) G. Winter – 5, *P. arenariicola* Plowr. var. *caricis-montanae* (E. Fisch.) Zwetko – 2, *P. arenariicola* s. lat. – 2, *P. argentata* (Schultz) G. Winter – 4, *P. aromatica* Bubák – 4, *P. arrhenathericola* E. Fisch. – 1, *P. artemisiella* P. Syd. et Syd. – 5, *P. artemisiicola* P. Syd. et Syd. – 1, *P. asarina* Kunze – 5, *P. asparagi* DC. – 2, *P. asperulae-aparines* Picb. – 2, *P. asperulae-cynanchicae* Wurth – 3, *P. asperulae-odoratae* Wurth – 3, *P. asteris* Duby – 1, *P. astrantiae* Kalchbr. s. str. – 2, *P. australis* Körn. – 1, *P. balsamitae* (F. Strauss) Röhl. – 2,

P. bardanae (Wallr.) Corda – 5, *P. barkhausiae-rhoeadifoliae* Bubák – 3, *P. behenis* G. H. Otth – 3, *P. betonicae* (Alb. et Schwein.) DC. – 2, *P. bistortae* (F. Strauss) DC. – 4, *P. brachycyclica* E. Fisch. – 2, *P. brachypodii* G. H. Otth s. str. – 5, *P. bromina* Erikss. subsp. *bromina* var. *bromina* – 5, *P. bromina* subsp. *symphyti-bromorum* (Fr. Müll.) Z. Urb. et J. Marková var. *paucipora* (Z. Urb.) Z. Urb. et J. Marková – 3, *P. bupleuri-falcati* G. Winter – 4, *P. calthae* Link – 3, *P. calthicola* J. Schröt. – 1, *P. campanulae* Carmich. ex Berk. s. lat. – 1, *P. carduorum* Jacky – 4, *P. caricicola* Fuckel – 1, *P. caricina* DC. s. str. – 4, *P. caricina* var. *caricina* – 1, *P. caricina* var. *pringsheimiana* (Kleb.) D. M. Hend. – 2, *P. caricina* var. *ribesii-pendulae* (Hasler) D. M. Hend. – 1, *P. caricina* var. *ribis-nigri-paniculatae* (Kleb.) D. M. Hend. – 1, *P. carlinae* Jacky – 2, *P. carthami* Corda – 1, *P. centaureae* DC. – 4, *P. centaureae-vallesiacae* Hasler – 3, *P. cervariae* Lindr. – 4, *P. cesatii* J. Schröt. – 4, *P. chaerophylli* Purton – 5, *P. chamaedryos* Ces. – 3, *P. chondrillae* Corda – 5, *P. chondrillina* Bubák et P. Syd. – 3, *P. chrysanthemi* Roze – 0, *P. chrysosplenii* Grev. – 3, *P. cichorii* Bellynck – 4, *P. cicutae* Lasch – 1, *P. circaeae* Pers. – 3, *P. cnici* H. Mart. var. *cnici* – 5, *P. cnici* var. *crassiuscula* Savile – 2, *P. coaetanea* Bubák – 2, *P. conglomerata* (F. Strauss) Röhl. – 2, *P. conii* Fuckel ex Lagerh. – 3, *P. constricta* (Lagerh.) Bubák – 1, *P. convolvuli* Castagne – 3, *P. coronata* var. *avenae* W. P. Fraser et Ledingham – 4, *P. coronata* Corda var. *coronata* – 5, *P. coronata* var. *intermedia* Z. Urb. – 1, *P. crepidis* J. Schröt. s. str. – 2, *P. crepidis-grandiflorae* Hasler – 2, *P. cruchetii* Hasler – 2, *P. cyani* Pass. – 2, *P. cynodontis* Lacroix ex Desm. – 1, *P. dentariae* (Alb. et Schwein.) Fuckel – 3, *P. deschampsiae* Arthur – 2, *P. difformis* Kunze – 1, *P. digraphidis* Soppitt – 3, *P. dioicae* Magnus s. str. – 2, *P. divergens* Bubák – 3, *P. doronicella* P. Syd. et Syd. – 4, *P. dracunculina* Fahrend. – 1, *P. echinopis* DC. – 4, *P. epilobii* DC. – 1, *P. erikssonii* Bubák – 3, *P. extensicola* Plowr. var. *extensicola* – 0, *P. extensicola* var. *linosyridi-caricis* (E. Fisch.) Zwetko – 3, *P. fergussonii* Berk. et Broome – 1, *P. ferruginosa* P. Syd. et Syd. – 2, *P. festucae* Plowr. – 3, *P. fuckelii* P. Syd. et Syd. – 1, *P. galanthi* Unger – 2, *P. galii-verni* Ces. – 3, *P. gentianae* (F. Strauss) Röhl. – 1, *P. geranii-sylvatici* P. Karst. – 1, *P. gibberosa* Lagerh. – 2, *P. glechomatis* DC. – 4, *P. globulariae* DC. – 2, *P. graminis* Pers. – 1, *P. graminis* subsp. *graminicola* Z. Urb. – 5, *P. graminis* subsp. *graminis* – 5, *P. helianthi* Schwein. – 3, *P. hieracii* H. Mart. – 5, *P. hierochloina* Kleb. – 1, *P. holcina* Erikss. – 2, *P. hordei* G. H. Otth s. str. – 5, *P. hordei-murini* N. F. Buchw. – 3, *P. horiana* Henn. – 1, *P. humilicola* Hasler – 3, *P. hypochoeridis* Oudem. – 3, *P. hysterium* (F. Strauss) Röhl. – 3, *P. intybi* (Juel) Syd. et P. Syd. – 1, *P. iridis* Wallr. – 2, *P. isiacae* G. Winter – 1, *P. jaceae* G. H. Otth – 4, *P. komarovii* Tranzschel – 5, *P. lactucarum* Syd. et P. Syd. – 2, *P. lagenophorae* Cooke – 3, *P. lapsanae* Fuckel – 5, *P. laschii* Lagerh. var. *laschii* – 5, *P. laschii* var. *palustris* Savile – 2, *P. laschii* var. *pannonici* Savile – 2, *P. laserpitii* Lindr. – 1, *P. leontodontis* Jacky – 5, *P. libanotidis* Lindr. – 1, *P. liliacearum*

Duby – 3, *P. linosyridis-vernae* Gäum. – 1, *P. littoralis* Rostr. – 1, *P. lojkaiana* Thüm. – 1, *P. longissima* J. Schröt. – 2, *P. luzulae* Lib. – 3, *P. luzulae-maximae* Dietel – 3, *P. maculosa* (F. Strauss) Röhl. s. str. – 4, *P. magelhaenica* Peyr. ex Magnus – 3, *P. magnusiana* Körn. s. str. – 3, *P. major* (Dietel) Dietel s. str. – 3, *P. malvacearum* Bertero ex Mont. – 5, *P. mei-mamillata* Semadeni – 2, *P. melicae* (Erikss.) P. Syd. et Syd. – 3, *P. menthae* Pers. – 5, *P. microsora* Körn. – 1, *P. millefolii* Fuckel – 3, *P. mixta* Fuckel – 4, *P. moliniae* Tul. – 2, *P. montivaga* Bubák – 2, *P. mougeotii* Lagerh. – 1, *P. mulgedii* P. Syd. et Syd. – 2, *P. nigrescens* L. A. Kirchn. – 4, *P. nitida* (F. Strauss) Röhl. s. str. – 4, *P. obscura* J. Schröt. s. str. – 3, *P. opizii* Bubák – 4, *P. orchidearum-phalaridis* Kleb. – 1, *P. oreoselini* (F. Strauss) Fuckel – 2, *P. paludosa* Plowr. – 2, *P. pelargonii-zonalis* Doidge – 3, *P. perplexans* Plowr. – 4, *P. persistens* Plowr. subsp. *agropyri* (Ellis et Everh.) Z. Urb. et J. Marková var. *agropyri* – 2, *P. persistens* subsp. *agropyri* var. *agropyrina* (Erikss.) Z. Urb. et J. Marková – 3, *P. persistens* subsp. *agropyri* var. *cerinthes-agropyrina* (Tranzschel) Z. Urb. et J. Marková – 2, *P. persistens* subsp. *agropyri* var. *milii-effusi* (Dupias) Z. Urb. et J. Marková – 1, *P. persistens* subsp. *persistens* var. *heteroecica* Z. Urb. et J. Marková – 2, *P. persistens* subsp. *persistens* var. *persistens* – 5, *P. persistens* subsp. *triticina* (Erikss.) Z. Urb. et J. Marková – 5, *P. peucedani-alsatici* Picb. – 2, *P. phlei-pratensis* Erikss. et Henning – 2, *P. phragmitis* (Schumach.) Körn. – 5, *P. picridis* Hazsl. – 4, *P. piloselloidarum* Probst – 2, *P. pimpinellae* (F. Strauss) Röhl. – 4, *P. poae-nemoralis* G. H. Otth s. str. – 5, *P. poarum* var. *petasiti-pulchellae* (Lüdi) Z. Urb. et J. Marková – 3, *P. poarum* Nielsen var. *poarum* – 5, *P. podospermi* DC. – 2, *P. polygoni* Alb. et Schwein. – 5, *P. polygoni-amphibii* Pers. s. str. – 4, *P. praecox* Bubák – 5, *P. pratensis* A. Blytt s. str. – 1, *P. ptarmicae* P. Karst. – 1, *P. pulsatillae* Kalchbr. – 3, *P. pulverulenta* Grev. – 3, *P. punctata* Link s. str. – 5, *P. punctiformis* (F. Strauss) Röhl. – 5, *P. pygmaea* Erikss. s. str. – 4, *P. pyrethri* C. Schub. – 3, *P. recondita* Roberge ex Desm. s. str. – 5, *P. retifera* Lindr. – 2, *P. ribis* DC. – 1, *P. rivalis* Gäum. – 2, *P. ruebsaamenii* Magnus – 1, *P. salviae* Unger – 2, *P. saniculae* Grev. – 2, *P. saxifragae* Schltdl. – 2, *P. schirajewskii* Tranzschel – 2, *P. schismi* Bubák var. *schismi* – 1, *P. schmidtiana* Dietel – 0, *P. schneideri* J. Schröt. – 1, *P. schroeteriana* Kleb. – 1, *P. scillae* Linh. – 1, *P. scillae-rubrae* P. Cruchet – 1, *P. scirpi* DC. – 0, *P. scorzonerae* Juel – 1, *P. semadenii* Gäum. – 1, *P. senecionis* Lib. – 1, *P. sesleriae* Reichardt – 1, *P. sii-falcariae* J. Schröt. – 5, *P. silai* Fuckel – 1, *P. singularis* Magnus – 0, *P. soldanellae* Fuckel – 1, *P. sorghi* Schwein. – 5, *P. stachydis* DC. – 2, *P. stipae* Arthur var. *stipina* (Tranzschel ex Kleb.) H. C. Greene et Cummins – 2, *P. striiformis* Westend. var. *striiformis* – 4, *P. sylvatica* J. Schröt. – 4, *P. tanaceti* DC. s. str. – 4, *P. taraxaci* Plowr. – 5, *P. taraxaci-serotini* Picb. – 1, *P. tenuistipes* Rostr. – 1, *P. thesii* Chaillet – 2, *P. thlaspeos* C. Schub. – 1, *P. tinctoriicola* Magnus – 2, *P. tirolensis* Zwetko – 1, *P. triseti* Erikss. – 4, *P. uliginosa* Juel – 1, P. *urticata* F. Kern – 5, *P. urticata* var. *biporula* Zwetko – 3, *P. urticata* var.

urticae-acutae (Kleb.) Zwetko – 5, *P. urticata* var. *urticae-acutiformis* (Kleb.) Zwetko – 5, *P. urticata* var. *urticae-hirtae* (Kleb.) Zwetko – 5, *P. urticata* var. *urticae-inflatae* (Hasler) Zwetko – 1, *P. urticata* var. *urticae-pilosae* (Hasler) Zwetko – 4, *P. urticata* var. *urticae-ripariae* (Hasler) Zwetko – 4, *P. urticata* var. *urticae-umbrosae* (Hasler) Zwetko – 1, *P. urticata* var. *urticae-vesicariae* (Kleb.) Zwetko – 2, *P. variabilis* Grev. – 1, *P. veratri* Duby – 1, *P. veronicae* J. Schröt. – 2, *P. veronicae-longifoliae* Savile – 1, *P. verruca* Thüm. – 1, *P. violae* DC. – 5, *P. virgae-aureae* (DC.) Lib. – 0, *P. vossii* Körn. – 1, *P. vulpinae* J. Schröt. – 1, *P. winteriana* Magnus (nom. nud.) – 1.

Pucciniastrum *agrimoniae* (Dietel) Tranzschel – 2, *P. circaeae* (G. Winter) Speg. ex de Toni – 3, *P. epilobii* G. H. Otth – 4, *P. pyrolae* Dietel ex Arthur – 2.

Sphacelotheca *hydropiperis* (Schumach.) de Bary – 3.

Thekopsora *areolata* (Fr.) Magnus – 3, *T. goeppertiana* (J. G. Kühn) Hirats. f. – 2, *T. guttata* (J. Schröt.) P. Syd. et Syd. – 3, *T. symphyti* (Bubák) Berndt – 4.

Trachyspora *intrusa* (Grev.) Arthur – 4.

Tranzschelia *discolor* (Fuckel) Tranzschel et M. A. Litv. – 2, *T. fusca* Dietel – 5, *T. pruni-spinosae* (Pers.) Dietel – 5, *T. pulsatillae* (Opiz) Dietel – 1, *T. thalictri* (Chevall.) Dietel – 1.

Triphragmium *filipendulae* (Lasch) Pass. – 1, *T. ulmariae* (DC.) Link – 4.

Uredinopsis *filicina* Magnus – 3.

Uromyces *acetosae* J. Schröt. – 2, *U. acutatus* Fuckel – 3, *U. aecidiiformis* (F. Strauss) C. C. Rees – 2, *U. airae-flexuosae* Ferd. et Winge – 4, *U. ambiguus* (DC.) Fuckel – 3, *U. anthyllidis* J. Schröt. s. str. – 3, *U. appendiculatus* (Pers.) Unger – 3, *U. armeriae* J. Kickx f. – 3, *U. behenis* (DC.) Unger – 2, *U. beticola* (Bellynck) Boerema, Loer. et Hamers – 3, *U. cacaliae* (DC.) Unger – 4, *U. caraganae* Magnus – 1, *U. cristatus* J. Schröt. et Niessl – 3, *U. dactylidis*

Fig. 13. *Puccinia persistens* subsp. *persistens* var. *heteroecica* (I) on *Hepatica nobilis* Mill.; Brno - venkov, Ochoz, Lysá hora.

Fig. 14. *Puccinia peucedani-alsatici* (primary II) on *Peucedanum alsaticum* L.; Vyškov, Milešovice, Milešovická stráň.

Fig. 15. *Puccinia schismi* var. *schismi* (I) on *Muscari comosum* (L.) Mill.; Brno – město, Obřany, slope above the first railway tunnel.

Fig. 16. *Tranzschelia pulsatillae* (III) on *Pulsatilla grandis* Wender.; Vyškov, Mouřínov, Ševy.

Fig. 17. *Triphragmium filipendulae* (primary II) on *Filipendula vulgaris* Moench; Vyškov, Komořany, Malé strany.

Fig. 18. *Uromyces junci* (II) on *Juncus articulatus* L.; Vyškov, Stříbrná, Zadní Lipová.

Notes to the photographs:
0 = spermogonia
I = aecia
II = uredia
III = telia

All photographs were taken by Petr Kokeš in the period 2001–2003. The localities are structured as follows: district, municipality, name of the locality.


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G. H. Otth s. str. – 4, *U. dianthi* (Pers.) Niessl – 2, *U. ervi* Westend. – 3, *U. euphorbiae-corniculati* Jordi – 3, *U. festucae* Syd. et P. Syd. – 2, *U. ficariae* Tul. – 4, *U. fulgens* (Hazsl.) Bubák – 1, *U. gageae* Beck – 5, *U. galegae* (Opiz) Sacc. – 1, *U. genistae* Fuckel – 4, *U. geranii* (DC.) Fr. s. str. – 5, *U. glycyrrhizae* (Rabenh.) Magnus – 1, *U. graminis* (Niessl) Dietel – 2, *U. hedysari-obscuri* (DC.) Carestia et Picc. – 1, *U. heimerlianus* Magnus – 3, *U. inaequialtus* Lasch – 3, *U. jordianus* Bubák – 2, *U. junci* (Desm.) Tul. – 2, *U. kabatianus* Bubák – 3, *U. kalmusii* Sacc. – 1, *U. laburni* (DC.) G. H. Otth s. str. – 1, *U. limonii* (DC.) Berk. – 4, *U. lineolatus* (Desm.) J. Schröt. – 4, *U. lupinicolus* Bubák – 1, *U. magnusii* Kleb. – 1, *U. minor* J. Schröt. – 2, *U. muscari* (Duby) L. Graves – 3, *U. onobrychidis* Bubák – 3, *U. ononidis* Pass. – 2, *U. orobi* (Schumach.) Fuckel – 4, *U. pallidus* Niessl – 1, *U. phyteumatum* (DC.) Unger – 2, *U. pisi* (DC.) G. H. Otth s. str. – 4, *U. poae* Rabenh. s. str. – 5, *U. polygoni-avicullariae* (Pers.) P. Karst. – 4, *U. punctatus* J. Schröt. – 4, *U. renovatus* P. Syd. et Syd. – 1, *U. rumicis* (Schumach.) G. Winter – 5, *U. sarothamni* A. L. Guyot et Massenot – 1, *U. scrophulariae* Fuckel – 2, *U. scutellatus* (Pers.) Lév. – 5, *U. silphii* Arthur – 4, *U. sommerfeltii* Hyl., Jørst. et Nannf. – 1, *U. striatus* J. Schröt. s. str. – 4, *U. tinctoriicola* Magnus – 1, *U. trifolii* (R. Hedw. ex DC.) Fuckel – 4, *U. trifolii-repentis* var. *fallens* (Arthur) Cummins – 4, *U. trifolii-repentis* Liro var. *trifolii-repentis* – 5, *U. trigonellae* Pass. (personal communication of Vladimír Zacha to Jiří Müller) – 1, *U. valerianae* Fuckel – 4, *U. veratri* (DC.) J. Schröt. – 3, *U. verbasci* Niessl – 3, *U. verruculosus* J. Schröt. – 3, *U. viciae-craccae* Const. – 1, *U. viciae-fabae* (Pers.) J. Schröt. s. str. – 5.

Xenodochus *carbonarius* Schltdl. – 2.

Fig. 19. *Anthracoidea limosa* on *Carex limosa* L.; Jeseník, Ostružná, Trojmezí.
Fig. 20. *Anthracoidea subinclusa* on *Carex melanostachya* M. Bieb. ex Willd.; Břeclav, Lednice, Pastvisko.
Fig. 21. *Exobasidium oxycocci* on *Oxycoccus palustris* Pers.; Jeseník, Rejvíz, Močály.
Fig. 22. *Urocystis miyabeana* on *Polygonatum multiflorum* (L.) All.; Vyškov, Letonice, Dražovický háj - Kyhelec.
Fig. 23. *Urocystis muscaridis* on *Muscari comosum* (L.) Mill.; Vyškov, Moravské Prusy, Zouvalka.
Fig. 24. *Ustilago trichophora* on *Echinochloa crus-galli* (L.) P. Beauv.; Vyškov, Brankovice, valley of Pohraniční potok below the wood Chroustová.
Notes to the photographs:
0 = spermogonia
I = aecia
II = uredia
III = telia
All photographs were taken by Petr Kokeš in the period 2001–2003. The localities are structured as follows: district, municipality, name of the locality.

Ustilaginomycetes

Anthracoidea *arenaria* (Syd.) Nannf. – 4, *A. caricis* (Pers.) Bref. – 2, *A. caryophylleae* Kukkonen – 2, *A. echinospora* (Lehtola) Kukkonen – 1, *A. heterospora* (B. Lindeb.) Kukkonen – 1, *A. humilis* Vánky – 1, *A. karii* (Liro) Nannf. – 1, *A. limosa* (Syd.) Kukkonen – 1, *A. michelii* Vánky – 3, *A. paniceae* Kukkonen – 1, *A. pilosae* Vánky – 2, *A. pratensis* (Syd.) Boidol et Poelt – 1, *A. subinclusa* (Körn.) Bref. – 1, *A. tomentosae* Vánky – 3.

Doassansia *alismatis* (Nees) Cornu – 1, *D. limosellae* (J. Kunze) J. Schröt. – 1, *D. niesslii* de Toni – 1, *D. sagittariae* (Fuckel) C. Fisch – 1, *D. sparganii* Vánky – 1.

Doassansiopsis *occulta* (H. Hoffm.) Dietel – 1.

Entorrhiza *aschersoniana* (Magnus) Lagerh. – 1, *E. casparyana* (Magnus) Lagerh. – 1.

Entyloma *achilleae* Magnus – 2, *E. bellidiastri* Maire – 1, *E. calendulae* (Oudem.) de Bary – 3, *E. chrysosplenii* J. Schröt. – 3, *E. corydalis* de Bary – 1, *E. dahliae* Syd. et P. Syd. – 2, *E. erigerontis* Syd. et P. Syd. ex Cif. – 1, *E. eryngii* (Corda) de Bary – 3, *E. fergussonii* (Berk. et Broome) Plowr. – 1, *E. ficariae* Thüm. ex A. A. Fisch. Waldh. – 3, *E. fuscum* J. Schröt. – 1, *E. gaillardianum* Vánky – 3, *E. hieracii* Syd. et P. Syd. ex Cif. – 3, *E. leontodontis* Syd. et P. Syd. ex Cif. – 1, *E. linariae* J. Schröt. – 2, *E. magnusii* (Ule) Woronin – 1, *E. matricariae* Rostr. – 4, *E. microsporum* (Unger) J. Schröt. – 3, *E. picridis* Rostr. – 2, *E. plantaginis* A. Blytt – 1, *E. ranunculi-repentis* Sternon – 2, *E. serotinum* J. Schröt. – 3, *E. tanaceti* Syd. – 1, *E. tragopogonis* Lagerh. – 1, *E. urocystoides* Bubák – 1, *E. verruculosum* Pass. – 2, *E. zacintha* Vánky – 1.

Exobasidium *arescens* Nannf. (a so far not published record; Brno - venkov, Tišnov, Klucanina; 23. 6. 1928 leg. P. Coufalová, BRNM) – 1, *E. japonicum* Shirai – 3, *E. karstenii* Sacc. et Trotter (identified and published as *E. oxycocci*; Šumperk, Kouty nad Desnou, Velký Jezerník; 27. 9. 1927 leg. R. Picbauer, BRNM) – 2, *E. myrtilli* Siegm. – 1, *E. oxycocci* Rostr. ex Shear – 1, *E. pachysporum* Nannf. – 2, *E. rhododendri* (Fuckel) C. E. Cramer – 1, *E. rostrupii* Nannf. – 2, *E. vaccinii* (Fuckel) Woronin s. str. – 3.

Farysia *thuemenii* (A. A. Fisch. Waldh.) Nannf. – 1.

Glomosporium *leptideum* (Syd.) Kochman – 2.

Graphiola *phoenicis* (Moug.) Poit. – 1.

Melanotaenium *ari* (Cooke) Lagerh. – 2, *M. cingens* (Beck) Magnus – 1, *M. endogenum* (Unger) de Bary – 1.

Microstroma *album* (Desm.) Sacc. – 1, *M. juglandis* (Bérenger) Sacc. – 3.

Moesziomyces *bullatus* (J. Schröt.) Vánky – 2.

Moreaua *aterrima* (Tul. et C. Tul.) Vánky – 1.

Schizonella *cocconii* (Morini) Liro – 2, *S. intercedens* Vánky et A. Nagler – 3, *S. melanogramma* (DC.) J. Schröt. – 5.

Sporisorium *andropogonis* (Opiz) Vánky – 3, *S. destruens* (Schltdl.) Vánky – 1, *S. neglectum* (Niessl) Vánky – 3, *S. sorghi* Ehrenb. ex Link – 1.

Thecaphora *affinis* W. G. Schneid. ex A. A. Fisch. Waldh. – 1, *T. saponariae* (F. Rudolphi) Vánky s. lat. – 2, *T. seminis-convolvuli* (Desm.) S. Ito – 5.

Tilletia *caries* (DC.) Tul. et C. Tul. – 4, *T. contraversa* J. G. Kühn – 3, *T. laevis* J. G. Kühn – 2, *T. olida* (Riess) J. Schröt. – 3, *T. secalis* (Corda) Körn. – 0, *T. separata* J. Kunze ex G. Winter – 1, *T. sphaerococca* (Wallr.) A. A. Fisch. Waldh. – 2.

Tolyposporium *junci* (J. Schröt.) Woronin ex J. Schröt. – 1.

Urocystis *agropyri* (Preuss) A. A. Fisch. Waldh. – 3, *U. agrostidis* (Lavrov) Zundel – 1, *U. anemones* (Pers.: Pers.) G. Winter – 3, *U. avenae-elatioris* (Kochman) Zundel – 2, *U. avenastri* (Massenot) Nannf. – 1, *U. bromi* (Lavrov) Zundel – 1, *U. colchici* (Schltdl.) Rabenh. – 3, *U. ficariae* (Liro) Moesz – 2, *U. filipendulae* (Tul.) J. Schröt. – 1, *U. fischeri* Körn. ex G. Winter – 1, *U. johansonii* (Lagerh.) Magnus – 1, *U. junci* Lagerh. – 1, *U. leimbachii* Oertel – 1, *U. magica* Pass. s. lat. – 2, *U. miyabeana* Togashi et Onuma – 2, *U. muscaridis* (Niessl) Moesz – 2, *U. occulta* (Wallr.) Rabenh. ex Fuckel – 0, *U. ornithogali* Körn. – 1, *U. paridis* (Unger) Thüm. – 1, *U. poae* (Liro) Padwick et A. Khan – 1, *U. primulae* (Rostr.) Vánky – 1, *U. pulsatillae* (Bubák) Moesz – 3, *U. ranunculi* (Lib.) Moesz – 4, *U. ranunculi-auricomi* (Liro) Zundel – 1, *U. syncocca* (L. A. Kirchn.) B. Lindeb. – 1, *U. trientalis* (Berk. et Broome) B. Lindeb. – 1, *U. ulei* Magnus – 1, *U. violae* (Sowerby) A. A. Fisch. Waldh. – 1.

Ustilago *avenae* (Pers.) Rostr. – 3, *U. bullata* Berk. s. lat. – 1, *U. calamagrostidis* (Fuckel) G. P. Clinton – 3, *U. echinata* J. Schröt. – 1, *U. filiformis* (Schrank) Rostr. – 5, *U. grandis* Fr. – 1, *U. hordei* (Pers.) Lagerh. – 2, *U. hypodytes* (Schltdl.) Fr. – 4, *U. luzulae* Sacc. – 2, *U. maydis* (DC.) Corda – 5, *U. nuda* (J. L. Jensen) Kellerm. et Swingle – 4, *U. ornithogali* (J. C. Schmidt et Kunze) Magnus – 3, *U. oxalidis* Ellis et Tracy – 5, *U. serpens* (P. Karst.) B. Lindeb. – 2, *U. spermophora* Berk. et M. A. Curtis ex de Toni – 2, *U. striiformis* (Westend.) Niessl s. lat. – 3, *U. syntherismae* (Schwein.) Peck – 2, *U. trichophora* (Link) Körn. – 2, *U. tritici* (Pers.) Rostr. – 3, *U. vaillantii* Tul. et C. Tul. – 3.

Doubtful data by Hruby

Paraperonospora sulphurea (Gäum.) Constant. [as *Peronospora sulphurea* Gäum.], *Peronospora agrimoniae* Syd., *P. amaranthi* Gäum., *P. calaminthae* Fuckel, *P. chelidonii* Miyabe ex Jacz. et P. A. Jacz., *P. chenopodii-rubri* Gäum., *P. cynoglossi* Burrill ex Swingle, *P. cytisi* Rostr., *P. fragariae* Roze et Cornu, *P. gigantea* Gäum., *P. lagerheimii* Gäum., *P. ononidis* G. W. Wilson, *P. potentillae-anserinae* Gäum., *P. stigmaticola* Raunk., *P. tetragonolobi* Gäum.;

Gymnosporangium torminali-juniperinum E. Fisch. ex F. Kern, *Melampsora abieti-caprearum* Tubeuf, *M. larici-pentandrae* Kleb. [as *M. minutissima* Bubák], *Microbotryum aviculare* (Liro) Vánky [as *Ustilago avicularis* Liro], *M. kuehneanum* (R. Wolff) Vánky [as *Ustilago kuehneana* R. Wolff], *M. pustulatum* (DC.) R. Bauer et Oberw. [as *Ustilago pustulata* (DC.) G. Winter], *Milesina carpatorum* Hyl., Jørst. et Nannf. [as *M. carpatica* Wróbl.], *Phragmidium acuminatum* (Fr.) Cooke [as *P. rubi-saxatilis* Liro], *Puccinia cnici-oleracei* Pers. ex Desm. [as *P. andersonii* Berk. et Broome], *P. pallidefaciens* Lindr., *P. retecta* Syd., *P. rubefaciens* Johanson, *P. urticata* F. Kern var. *urticae-flaccae* (Hasler) Zwetko [as *P. caricis* (Schumach.) Rebent. on *Carex flacca* Schreb.], *P. vesiculosa* Schltdl., *Uredinopsis atkinsonii* Magnus, *Uromyces lapponicus* Lagerh. [as *U. carneus* (Nees) Har.]; *Anthracoidea fischeri* (P. Karst.) Kukkonen [as *A. caricis* (Pers.) Bref. on *Carex vulpina* L.], *Ustilago davisii* Liro, *U. scrobiculata* Liro.

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CZECH MYCOL. 56 (1–2), 2004

Zur Systematik von Bacidia permira (foliicole Flechte, Ascomycotina)

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Vězda A. (2004): Zur Systematik von Bacidia permira (foliicole Flechte, Ascomycotina). – Czech Mycol. 56: 149–150

Die systematische Stellung der foliicolen *Bacidia permira* Vězda 1975 wird näher behandelt. Nach der Struktur der Apothecien und der borstenförmigen vegetativen Vermehrungsorgane (Hyphophoren) gehört die Art in die später definierte Familie *Gomphillaceae* (Poelt und Vězda 1987). Aus taxonomischen Gründen wird für sie ein neuer Gattungsnamen *Uluguria* gen. nov. vorgeschlagen.

Key words: foliicolous lichens, *Uluguria* gen. nov., Ascomycotina, Tansania

Vězda A. (2004): K systematice druhu Bacidia permira (foliikolní lišejník, Ascomycotina) – Czech Mycol. 56: 149–150

Systematické postavení foliikolního lišejníku *Bacidia permira* Vězda 1975 je upřesněno. Podle stavby apothecií a vegetativních rozmnožovacích orgánů (hyfoforů) přísluší tento druh k později definované čeledi *Gomphillaceae* (Poelt et Vězda 1987). Z taxonomických důvodů je pro něj navržen nový rod *Uluguria* gen. nov.

Im Jahre 1975 wurde dem Verfasser von Dr. T. Pócs (Eger, Ungarn) reiches Material von foliicolen Flechten aus Tansania zur Bearbeitung anvertraut. Darunter fand sich eine kuriose foliicole (epiphylle) Art mit gestielten relativ grossen Apothecien, die von zahlreichen borstenförmigen schwarzen Vermehrungsorganen, s. g. Hyphophoren, begleitet werden. Diese tragen je ein Büschel von zusammen-geklebten fadenförmigen Konidien s.g. Diahyphen.

Nach dem damaligen Stand der Systematik der foliicolen Flechten war es nicht möglich eine richtige Zuordnung dieser Flechte zu den damals bekannten Genera zu treffen. Die Flechte wurde daher unter dem Namen *Bacidia* s. ampl. beschrieben: *Bacidia permira* Vězda sp. nov.; dazu darf bemerkt werden, dass in der Diskussion zu dieser Art (Vězda 1975: 421) bereits auf eine verwandschftliche Beziehung zu den Gattungen *Tricharia* Fée em. R. Sant. und *Echinoplaca* Fée (*Gomphillaceae*) hingewiesen wurde.

Die Systematik der foliicolen Flechten hat sich in den daraufkommenden Jahrzehnten weiter entwickelt, niemand hat sich aber mit der systematischen Zugehörigkeit dieser Art näher beschäftigt.

Die innere Struktur der Apothecien und das Vorkommen einer vegetativen Vermehrung durch die borstenförmigen Organe – die Hyphophoren – , zeigt eine klare Zugehörigkeit dieser Art zu den später definierten *Gomphillaceae* (Vězda

und Poelt 1987). Die Innenstruktur der gestielten Apothecien, vor allem die nadelförmigen, querseptierten Askosporen, und die Ausbildung der Hyphophoren (Vězda 1975: 420) erlaubt aber eine Zuordnung den bisher bekannten Gattungen der Gomphillaceen nicht.

Da es sich heute offensichtlich um eine neue Gattung der *Gomphillaceae* sensu Vězda und Poelt (1975) handelt, ist es notwendig, statt dem provisorischen Klassifikation zu *Bacidia* eine neue Gattung aufzustellen:

Uluguria Vězda. gen. nov. familiae *Gomphillaceae*

Praesertim ascosporis acicularibus hyphophorisque bacilliformibus simplicibus ab alia genera familiae *Gomphillaceae* (sensu Vězda et Poelt 1975) differt.

Etymologie: Nach dem Fundort (Uluguru-Gebirge in Tansania).

Typus generis:

Uluguria permira (Vězda) Vězda comb. nova

Basionym: *Bacidia permira* Vězda, Folia Geobot. Phytotax., Praha, 10: 419, 1975.

LITERATUR

VĚZDA A. (1975): Foliicole Flechten aus Tansania (Ost-Africa). – Folia Geobot. Phytotax. 10: 383–432.

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Notes on the exsiccatum "Vězda: Lichenes rariores" with Index to fascicles 1–50 (Nos. 1–500)

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Key words: Lichenes rariores exsiccati, Antonín Vězda, date of publication, new taxa, combinations, types

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Contributing collectors

The exsiccatum contains lichens from many parts of the world. Fascicles 1–50 are based on collections made or supplied by the following botanists:

Aptroot A. et M., Barreno Eva, Bolognini Gloria, Boom Van den P. P. G., Breuss O., Brodo F. et L. M., Calatayud V., Ceni F., Chuva S., Codogno M., Coppins B., Culberson Chicita, Culberson W. L, Čuba J., Elix J. A., Etayo J. A., Evers W. H., Farkas Edit, Feige C. B. et G., Giralt M., Hafellner J., Horáková Jana, James P., Jarman J., Kalb Astrid, Kalb K., Kantvilas J., Krog Hildur, Knox E., Koch M., Kockinger, Lazzarin G., Liška J., Lókós L., Lumbsch T., Lücking R., Malcolm W., Mayhofer H., Metzer M., Meurk C. D., Miadlikowska Jolanta, Naoloma V. R., Nimis P. J., Obermayer W., Orbán S., Ornduff R., Otonello D., Palice Z., Pinna D., Pittoni H., Pócs T., Poelt J., Prügger J., Puntillo D., Pogers R., Roivanen H., Scutri N., Soják J., Streimann H., Tretiach M., Troneček J., Türk R., Vašák V., Vězda A., Vivant J., Wetmore C., Williams J., Wippel Anita and Wittmann H.

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Identification of the taxa

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Authors' abbreviations

In this list, authors' abbreviations were corrected according to the current standard. Consequently, the abbreviations used here can differ from those used on the original labels. I thank Jana Kocourková (Prague) for her help in this respect.

New taxa and combinations

The labels of this exsiccatum are also distributed separately in the form of booklets to many botanists and botanical libraries. New combinations made in the labels and new taxa descibed there are, therefore, validly published. The following new combinations and new taxa appeared in the series:

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Seminar "Mycoremediation 2003", Prague, Czech Republic, October 9^{th}–10^{th}, 2003

The seminar was organised by joint Commission for Experimental Mycology of the Czechoslovak Microbiological Society and the Czech Scientific Society for Mycology together with a group of experts collaborating under NATO project No. 978297 "Evaluation of composting and fungal treatment technology for remediation of PAH-contaminated soil". The purpose of the seminar was to provide insight into the complexity of application of fungi in remediation of polluted soils. Only 17 participants took part in the seminar representing 6 countries (Czech Republic, Estonia, Germany, Italy, Norway, Slovak Republic).

Application of fungi in soil remediation (mycoremediation) is a very complex issue that comprises many different aspects, both theoretical and practical. During the first day of the seminar, two plenary lectures and seven specialised communications were presented. In the opening plenary lecture V. Šašek (Czech Republic) tried to sum up current state and perspectives of application of fungi in remediation of contaminated soils pointing out both positive results and several drawbacks. All these aspects were supported by the contribution by A. Majcherczyk (Germany), who explained why the sophisticated technology developed at the University of Göttingen, Germany as a pilot-scale treatment of contaminated soil by cultures of ligninolytic fungi, has not been brought real practice. On the other hand, T. Eggen (Norway) showed a successful (allthough lab-scale) application of spent oyster mushroom (*Pleurotus ostreatus*) substrate in remediation of industrial soil highly polluted with polycyclic aromatic hydrocarbons. Vanessa Leonardi (Italy) draw the attention to one important point in soil bioremediation, i.e. improvement of mycoremediation efficacy by pre-treatment of long-term contaminated soil with surfactants. A comparison of mycoremediation with the method of composting in the clean-up of soils contaminated with polycyclic aromatic hydrocarbons (PAHs) was presented by T. Cajthaml (Czech Republic); both the processes were proved to be successful, composting being more efficient.

The second part of the first day was opened by a plenary lecture presented by Č. Novotný (Czech Republic). The lecture dealt with one of the crucial topics in the research of the biodegrading potential of ligninolytic basidiomycetes, i.e., the correlation between activities of individual ligninolytic enzymes and the potential of the fungus to degrade organopollutants. This lecture was followed by a contribution presented by T. Cajthaml, who documented integrated research of both ligninolytic enzyme activities and degrading potential in the model white rot fungus *Irpex lacteus* (Fr.: Fr.) Fr. The last two contributions concerned another important factor of the bioremediation business. The goal of the treatments is a decrease in toxicity of the contaminated matrix. Anne Kahru (Estonia)

compared changes in toxicity and mutagenicity of soil polluted with polycyclic aromatic hydrocarbons after treatment by mycoremediation and composting. Tomáš Hubálek (Czech Republic) pointed out the problems of ecotoxicological evaluation, when different methods of bioremediation are applied. Abstracts of all the presentations are part of this report.

During the second day of the meeting the participants visited two localities close to the town of Soběslav (South Bohemia). The first one was a site where remediation of aged-contaminated soil took place (the soil was polluted during long-term timber preservation), the other place was an oyster mushroom farm. The reason of the visit was that the large-scale production of lignocellulosic material colonised by fungal mycelium (that can be applied in field bioremediation trials) is basically the same as the preparation of compost for growing oyster mushrooms. The trip was concluded with a short mushroom foray in south Bohemian forests.

ACKNOWLEDGEMENT

The organisation of the seminar was supported by NATO grant EST CLG No. 978297, the field trip to South Bohemia by the Czechoslovak Microbiological Society.

Václav Šašek and Jiří Kunert

Applications of mycoremediation in practice

Použití mykoremediace v praxi

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Ligninolytic fungi belonging to the class of Basidiomycetes have developed unique mechanisms for the degradation of recalcitrant compounds such as lignin. Due to the non-specific character of the radical-mediated reaction of ligninolytic enzymes, the biodegradation of a wide variety of xenobiotic compounds, having an aromatic structure like lignin, have become subjects of extensive research. Most important environmental pollutants, such as chlorophenols, polycyclic aromatic hydrocarbons, polychlorinated biphenyls and dioxins, trinitrotoluene and other nitroaromatic explosives, different synthetic dyes and pesticides, have proven to be degradable by ligninolytic fungi. Most of the experiments were performed using liquid culture media. In soil conditions the fungal degrading potential is only one prerequisite, and other factors influence the degradation process. Many of the parameters (chemical, physiological and biological soil properties, chemical structure and bioavailability of the pollutant) are similar to those generally influencing any soil bioremediation process. Other conditions (ability to colonise the soil matrix and compete with the indigenous soil microflora, as well as the resistance to toxic compounds present in the polluted soil) are more or less specific for the application of ligninolytic fungi.

Field applications of fungi in soil remediation (mycoremediation) were not always successful. This indicates that more research is needed to establish mycoremediation as an effective and reliable soil-remediation technology. Both positive and negative field-scale experiences of fungal treatment of polluted soils using mycelia of *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm., *Phanerochaete chrysosporium* Burds. and other *Phanerochaete* species that have been performed in the Czech Republic and the USA were described in the lecture. Individual requirements (decolourising potential, growth parameters, ability to degrade respective pollutants, ability to colonise non-sterile toxic soil) that the fungus has to meet on the way from the first screening to application in field remediation trials were evaluated in the lecture.

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Application of fungal technology in remediation of polluted soil in Germany

Použití houbové technologie při čištění kontaminované půdy v Německu

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Lignin degrading white rot fungi have been demonstrated to be able to degrade numerous recalcitrant environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins, DDT, chlorophenols, nitrotoluene, and different pesticides, in sterile, liquid culture as well as in complex soil systems. Application of these wood-inhabiting fungi for soil bioremediation requires methods that establish a growth of fungus in soil by adding lignocellulosic substrates or mixing soil with already fungus-colonised materials. However, contaminated soils originating from former industrial sites are usually not polluted in a uniform way and display areas of high concentration of chemicals prohibiting any microbial activity. Mixing of soil samples and disrupting of contaminated aggregates is in many cases unavoidable but also difficult to realise, e.g. in case of wet soil or a high clay content.

We developed a large-scale soil preparation system based on: 1. preparation of soil slurry by addition of water, 2. supplementation of the slurry with additives (e.g. potato pulp, tensides), 3. solidification of the slurry by adding wood chips, and 4. inoculation of the solid mixture with a millet culture of white rot fungi. The method overcame any problems of soil/contaminant inhomogenity and composition, delivered a uniform size of soil particles as wood chips covered with soil, and resulted in a homogenous inoculation with the fungus. This treatment was successfully applied for bioremediation of soil contaminated with PAHs and PCBs in scales ranging from 1 dm^3 to 1 m^3. The growth of fungus was extremely uniform within the soil particles and the degradation rates obtained at a large scale were corresponding to the laboratory experiments showing up to 80 % degradation of PAHs after 3 months. However, in several cases the degradation was not successful due to a low availability of the contaminants. This problem could not be overcome by addition of surfactants, emulsifiers, solvents or modification of the treatment.

Use of spent mushroom substrate for bioremediation of polluted soil

Využití vyplozeného houbového substrátu k bioremediaci znečištěných půd

TRINE EGGEN

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In the middle of the 1980s it was demonstrated that some ligninolytic basidiomycetes were able to degrade recalcitrant environmental pollutants (polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, synthetic dyes and dioxins). This was the start of an effort to apply these fungi for remediation of soil contaminated with hazardous organic compounds. In the beginning most studied fungus was *Phanerochaete chrysosporium*, but soon other fungi were evaluated for their potential to degrade organopollutants, among others also edible fungi such as *Pleurotus ostreatus* (oyster mushroom) and *Lentinula edodes* (shiitake).

Edible mushroom production is a large industry in several countries, and use of spent fungal substrate, which still contains active fungal mycelium, represents an agroindustrial byproduct and is available nearly for free. Experiments with spent fungal substrate from oyster mushroom production were performed to investigate its capability as an inoculum in bioremediation processes.

Experimental design and initial PAH concentration have shown to be influencing factors. In soil with a high initial PAH concentration (e.g. 16,000 ppm of 16 PAHs) a low reduction of PAHs with 4 or more rings was observed. On the contrary, fungal treatment of soil with a lower initial PAH concentration (e.g. 2,000 ppm of 16 PAH), the 2-ring PAHs were reduced to less than 1 % of the original total concentration; simultaneously a significant reduction of 4-ring compounds and also of some 5-ring compounds was documented. Additional reinoculation of fungal substrate into already mycoremediated soil stimulated further PAH degradation.

The effects of surfactans on mycoremediation of aged PAH-contaminated soil

Vliv surfaktantů na mykoremediaci půd ze starých zátěží kontaminovaných polycyklickými aromatickými uhlovodíky

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The use of white rot fungi for decontamination of PAH-contaminated soils has been studied for many years. Ligninolytic fungi posses a great potential for

PAH removal. However, bioremediation of aged contaminated soils is often limited by low bioavailability of pollutants. The addition of surfactants to increase the diffusive mass-transfer rate of soil pollutants has received considerable attention in the last years.

Pretreatment of aged PAH-contaminated soil with four types of surfactants: soya oil, Tween 80, Tween 20 and olive-mill wastewater, was studied at laboratory scale in Erlenmeyer flasks thus simulating an on-site mycoremediation treatment. Two white rot fungi, *Irpex lacteus* strain 617/93 and *Pleurotus ostreatus* strain 3004 from the Culture Collection of Basidiomycetes, Institute of Microbiology, Prague were selected for their efficiency in degrading PAHs. Contaminated soil originated from a site of a former gasholder in Prague-Měcholupy, Czech Republic and contained PAH sum 2526 ppm. Before use in experiments the soil was pretreated with 5 % water dispersion of individual surfactants and left for 6 days at 4 °C.

Experimental setup. The fungi were grown in Erlenmeyer flasks on moistened and sterilised wheat straw for 21 days and after that the pretreated contaminated soil was put on top of the culture. The flasks were incubated at 24 °C for 6 weeks during which the humidity of the flask contents was maintained by regular addition of distilled water, and fungal development documented by photography. At the end of the experiment the material (straw grown mycelium + contaminated soil) of each flask was harvested and the contents of PAHs was estimated by a standard HPLC analysis (see the following abstract).

The results showed that: (i) in flasks with soil without any pretreatment (non-treated controls) a good growth of fungal mycelium appeared only on straw, not on contaminated soil; (ii) soil pretreated with surfactants was colonised by mycelium, the most pronounced colonisation was observed in soils pretreated with soya oil or olive-mill waste water; (iii) in flasks with pretreated soil a more abundant mycelial growth was observed also on straw; (iv) air mycelium over the pretreated soil turned to a greyish colour indicating that some material was translocated from soil into fungal hyphae; (v) both fungi under study behaved in the same, above described way.

The performance of fungi in soil bioremediation depends not only on their capacity to degrade respective pollutants but also on the capability of the fungal mycelium to colonise the soil matrix.

Mycoremediation versus composting of soil polluted with PAHs

Srovnání mykoremediace a kompostování při remediaci půd obsahujících polycyklické aromatické uhlovodíky

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Composting has been used to treat solid waste such as agricultural wastes, sewage sludge and food wastes. The technique was also used for bioremediation of contaminated soils originating from different industrial sites. Composting matrices are rich sources of microorganisms including bacteria, actinomycetes and fungi that can degrade pollutants.

Another perspective method is the application of fungal technology (mycoremediation) for the cleanup of contaminated soils. Several strains of white rot fungi have proven to attack many organopollutants including PAHs. However, most of the studies have been carried out using *Phanerochaete chrysosporium* and out of many hundreds of species possessing ligninolytic activity, only few have been studied in detail. That is why fungal remediation techniques have only slowly been brought into practice.

In our investigation we tried to compare the efficiency of degradation of polycyclic aromatic hydrocarbons (PAHs) in four different industrial, contaminated soils by composting and mycoremediation. The soils originated from a former gas-works in Prague-Michle (total PAH content 1466 mg/kg dry soil), a former tar-producing plant in Ostrava (total PAH content 2832 mg/kg dry soil), a former gasholder in Prague-Měcholupy (total PAH content 2526 mg /kg dry soil), and a wood-treatment plant in Soběslav (total PAH content 1987 mg /kg dry soil). All the sites are situated in the Czech Republic.

The results showed that both techniques are promising and both were able to reduce contamination during the several-month treatment. However, we found that after long-term post-composting maturation the level of contamination dropped significantly more than mycoremediation could reach. Average degradation (sum of PAHs) was 50 % by fungi but in the case of composting it was 75 % and in soil from the wood-treatment plant even 95 %.

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Correlation of ligninolytic enzyme activities with fungal capacity to degrade recalcitrant pollutants

Vztah mezi aktivitou ligninolytických enzymů a schopností houby degradovat rekalcitrantní polutanty

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Pollution with recalcitrant xenobiotic chemicals has become one of the major environmental problems. Some of these chemicals are highly resistant to biodegradation by native microflora. Ligninolytic fungi responsible for the white rot of wood have proven to decompose and mineralise a broad range of persistent chemicals as a result of the non-specificity of their extracellular enzyme system. Many of those chemicals are major pollutants: ammunition waste, pesticides, organochlorines, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), synthetic dyes, wood preservatives and synthetic polymers.

Ligninolytic fungi (LF) can be used for the biodegradation of pollutants in both contaminated water and soil. These fungi secrete one or more of the three major ligninolytic enzymes, lignin peroxidase (LiP, EC 1.11.1.14), Mn-dependent peroxidase (MnP, EC 1.11.1.13) and phenol oxidase (laccase) (LAC, EC 1.10.3.2). Each fungus has its typical enzyme pattern. Extracellular peroxidases and laccases have repeatedly shown to oxidise recalcitrant compounds in vitro but the importance of in vivo enzyme levels for biodegradation efficiency remains unclear. The question of correlability of enzyme activities, responsible for degradation of recalcitrant pollutants, with the degradation rate is thus relevant as other factors can become rate limiting in the biodegradation process due to its complexity.

Our study documented levels of MnP, LiP and LAC in various LF species cultivated in liquid media or colonising soil with explorative mycelium. Their effect on degradation of PAHs, PCBs and synthetic dyes was studied. Submerged and stationary cultures of *Irpex lacteus* were compared with respect to extracellular enzyme synthesis and the corresponding capability of decolorisation of RO16 azo dye and RBBR anthraquinone dye. In the former cultures, productions of MnP, LiP and LAC were significantly reduced. The difference in enzyme activities correlated with a lower rate of decolorisation of RO16, but not of RBBR.

A comparison of cultures of *I. lacteus* immobilised on polyurethane or wood showed differences between the production of extracellular ligninolytic activities, comparable to those observed in stationary and submerged cultures. The decolorisation of RBBR was similar in both immobilised cultures, which was in accordance with the observation in liquid cultures where a significant reduction in the synthesis of MnP did not result in a decrease of the RBBR decolorisation rate. A similar decolorisation efficiency of the two immobilised cultures was also

observed in the case of textile colouring bath liquids containing the dye mixtures Drimaren Blue and Drimaren Red. The respective decolorisation rates measured in the polyurethane culture after 7 days were 83 ± 6 and 94 ± 4 % of the initial absorbance value, compared to 99 ± 1 and 82 ± 9 % in the wood growing culture. In contrast, the ability to decolorise the Acid Black dye-containing colouring bath liquid strongly correlated with a higher synthesis of MnP in the polyurethane culture, where 95 ± 3 % of the initial dye absorbance was decolorised within 7 days, compared to only 18 ± 9 % in the wood culture. Decolorisation of the textile dye mixture Remazol Green was rather low in both immobilised cultures, irrespective of the MnP level produced.

Soil cultures of *Pleurotus ostreatus*, *Phanerochaete chrysosporium* and *Trametes versicolor*, where pre-sterilised soil was spiked with PAHs and colonised by explorative mycelium of a fungal organism growing from wheat straw, were used and degradation of PAH was investigated. The fungal explorative mycelium was able to secrete ligninolytic enzymes into soil. Correlability between the enzyme levels in soil and PAH degradation was very poor, probably due to other factors such as a low bioavailability of PAH molecules due to sorption to soil particles, hydrophobicity of the pollutant molecule, etc.

The study showed that the importance of high enzyme levels for efficient degradation of recalcitrant chemicals was better demonstrable in liquid medium cultures compared to cultures growing in soil.

Biodegradation of selected PAHs by the ligninolytic fungus Irpex lacteus

Biodegradace vybraných polycyklických aromatických uhlovodíků lygninolytickou houbou Irpex lacteus

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The study of metabolites of polycyclic aromatic hydrocarbons (PAHs) degraded by ligninolytic fungi is a prerequisite for the application of fungal technology in practice because some of their metabolites can also represent serious environmental pollutants which may have mutagenic and carcinogenic potential.

In our work we demonstrated that representatives of PAHs (benzo[a]anthracene, benzo[a]pyrene, benzo[g,h,i]perylene) were degraded by the ligninolytic fungus *Irpex lacteus* in liquid nutrient medium. The products were analysed by GC-Ion

trap mass spectrometry. The combination of full scan mass spectra, product ion scans (MS-MS) and derivatisation of the degradation products provided further insight in the degradation mechanism initiated by *I. lacteus*. Particularly the daughter ion scans enabled the interpretation of unknown degradation products, even though they were only produced at trace level. Most of the structures suggested were later confirmed with authentic standards.

The results indicated that besides a strong potential of the fungus to degrade PAHs no dead-end metabolites were accumulated. We proposed a pathway for the degradation of benzo[a]anthracene, corresponding with the decomposition of anthracene where, except for anthraquinone, we detected 7,12-benzo[a]anthracenedione. Another parallel pathway appeared via 6-hydroxy-1,2-naphthalenedione.

Toxicity and mutagenicity of PAH-polluted soils during composting and fungal treatment

Sledování toxicity a mutagenicity půd kontaminovaných polycyklickými aromatickými uhlovodíky v průběhu kompostování a mykoremediace

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Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental contaminants because some of them are toxic, (pro)mutagenic and resist biodegradation. It has also been shown that some of the decomposition products of PAHs are more toxic or mutagenic than the parental compounds. In this work the toxicity and mutagenicity of PAHs-polluted soils during composting and fungal bioremediation was studied. The soils (1987 mg PAHs per kg of soil) originated from the territory of a wood-treatment plant in Soběslav, Czech Republic. The comparative remediation study was carried out in Norway (small-scale composting and fungal treatment) and also in Prague (pilot scale composting). The change of (geno)toxicity during bioremediation was studied analysing the samples in the beginning, middle and at the end of the treatment process. Two different extractants were applied for the extraction of toxicants from the soil: extraction of the soil with water (to mimic the hazard via the soil-water path) and extraction with methanol (to predict the potential hazard of less soluble and soil-bound pollutants). The ratio of the soil and extractant was 1+10 in both cases. For toxicity testing the photobacterial (*Vibrio fischeri*) bioassays (Microtox and Solid-Phase Flash-Assay) were used. Mutagenicity was studied using Ames assay (*Salmonella typhimurium* TA98) with and without metabolic activation (S9).

In all samples analysed (initial soil and all treatment samples, altogether 10 samples) the water-extracted toxicity was absent or low and no mutagenicity was observed. The methanol-extracted toxicity exceeded water-extractable toxicity about 70-fold. Mutagenicity was observed only in the case of methanol extracted samples of pilot-scale composting (Prague): mutagenicity was developed in the middle of composting and was not removed by the end of the treatment (after maturation). It was shown that the change of mutagenicity and toxicity during the treatments was dependent on the technique applied and was not directly correlated to the removal of PAHs.

Problems in ecotoxicological estimation of soils after bioremediation

Otázky ekotoxikologického měření půd po bioremediaci

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Toxicity tests characterising ecotoxicity of bioremediated soil are sometimes not in accordance with each other. We faced such a situation in the case of pilot-scale composting when we used soil containing 1987 mg PAHs per kg, originating from the territory of a wood-treatment plant in Soběslav, Czech Republic. We observed a decrease in ecotoxicity according to the bioluminescence test with *Vibrio fischeri* (EC_{20}= 49 % at start versus EC_{20}= 80 % after composting). The small decline in ecotoxicity (around 10 %) was seen also with the seed germination test using *Sinapis alba*. However, the results from the test with earthworms of the species *Eisenia foetida* showed a lasting ecotoxicity effect. There are other drawbacks in application of ecotoxicity tests for evaluation of bioremediation of soil. Using the seed germination test for estimating actual soil ecotoxicity before and after composting was found to be unsuitable. Already the original fresh substrate used for a compost-soil mixture showed toxicity to plant seeds, however, this was not related to toxicity of the polluted soil. This phenomenon was even more pronounced in tests with earthworms when no earthworms survived in freshly mixed substrate. If the substrate was left to dry for 2 weeks prior to performance of the earthworm test, ecotoxicity decreased dramatically (the earthworm survival was around 90 %). Also the part of the compost pile from which the samples were taken for testing played a significant role. The necessity to perform different ecotoxicity tests at the beginning of remediation and creating a suitable battery of ecotests can result in a better evaluation of the ecotoxicity during bioremediation.

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Book Review

S. E. LINDOW, E. I. HECHT-POINAR AND V. J. ELLIOTT (EDS.)

Phyllosphere microbiology

American Phytopathological Society Press, St. Paul, Minnesota, 2002, xii + 395 p.
ISBN 0–089054–286–4. Price US$ 69.

Very impressive cover photo of the leaf surface of *Phaseolus* invites readers.

The book is based on lectures given at the 7th International Symposium on the Microbiology of Aerial Plant Surfaces held at the University of California, Berkeley, in August 2000. The contributions cover many aspects of the phyllosphere study, connect bacteriology, mycology, ecology, plant pathology, molecular biology, population biology and aerobiology.

The book is divided to six parts: The physical and chemical environment of plant surfaces (3 chapters), Interactions between epiphytes and their hosts (6), Interactions among microbes on plant surfaces (5), Plant surface microbes: Agricultural practices and food quality (3), Modelling of interactions and movement of microbes on plant surfaces (5), and Contributions of phyllosphere microbiology to science and agriculture (1).

The first part is dealing especially with effect of leaf surface waxes and UV radiation on colonization by microorganisms and responses of microbial communities to these stress factors. Very interesting is chapter on leaf surface topography made by atomic force microscopy.

Part II is focused on interactions between epiphytes and hosts, mainly on examples of bacteria and yeasts.

Part III covers interactions among phylloplane microbes alone. One provides information on biofilms on leaf surfaces, which are sites of high bacterial density. Another theme of this part is defenses of marine seaweeds against bacterial colonization. For mycologists could be very interesting the chapter of Richard R. Bélanger and Tyler J. Avis on ecological processes and interactions in phyllosphere fungi. Authors treated here the main life strategies of fungi (competition, parasitism and antibiosis) with several examples. Other chapters are dealing with horizontal gene pool in bacterial colonization in the phyllosphere and the bacterial genus *Xanthomonas*, which is encountered in phylloplane either as pathogen, or as saprotroph.

Part IV has close affinity to agriculture and food safety. Emphasis is placed on biological control of fire blight, disease of pear and apple caused by *Erwinia amylovora*. Another interesting chapter presents sources of human pathogens (e.g. *Listeria*, *Escherichia coli* and *Salmonella)* on plants. Attention is also given to bacterial blight of rice caused *Xanthomonas oryzae*.

In part V, for mycologists is very useful chapter by John C. Zak on fungal community structure and function on leaf surface and chapter by Donald E. Aylor on aerobiology of fungi and relation to capture and release by plants.

Sixth part gives an overview on advances in phyllosphere microbiology during the last fifty years, since the first publications on phyllosphere of Last (1955) and Ruinen (1956). It is very valuable for all interested in this field.

Very useful is index of names and terms at the end of this book.

I have only little criticism to this work. It is dealing with some orthographical errors, e.g. *Erispihe graminis* (p. 11, instead of *Erysiphe*), *Monolinia fructicola* and *Oidendron* (p. 30, instead of *Monilinia, Oidiodendron*), and *Deschslera* (p. 306, instead of *Drechslera*).

In conclusion, I consider this book of a great value not only for specialists in the phyllosphere but also for other microbiologists, mycologists, ecologists and plant pathologists.

Alena Kubátová

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75th anniversary of Professor Bronislav Hlůza

JIŘÍ LAZEBNÍČEK

Professor RNDr. Bronislav Hlůza, CSc., has been involved in many activities during his scientific life. He was an excellent botany, mycology, mycotoxicology and ecology teacher. In many publications, lectures and at field excursions he also proved to be a great populariser of botanical, mycological and nature conservation work, not only to the benefit of students but also of members of the Natural History Museum Society in Olomouc and the general public. He was a specialist in didactics and environmental education.

Hlůza's most important publications include e.g. dissertations entitled "The distribution of several species of the genus *Amanita* in Czechoslovakia" (1976), and "The ecological study of *Amanita citrina* and *A. porphyria* and their distribution in Czechoslovakia" (1967) and several studies on *Amanita phalloides* (1982), *A. pantherina* (1985), *A. gemmata* (1986), *A. regalis* (1987), *A. muscaria* (1987) and other poisonous fungi *(Inocybe erubescens,* 1988; *Boletus satanas,* 1986) etc.

Prof. Hlůza was head of the mycological advice bureau for Olomouc and its surroundings for more than 30 years. He established a mycological working group of students and inspired many students of the Pedagogical Faculty of Palacký University to do research work and encouraged them to publish their results in this field.

Prof. Hlůza is a member of the Czechoslovak Biological Society, Czech Botanical Society, Czech Scientific Society for Mycology (he was a member of its Committee in the years 1997–2000), Czech Mycological Society, Slovak Mycological Society, and Natural History Museum Society in Olomouc (he was head of its Biological Department for 25 years). He collaborated for 40 years with scientists at several foreign universities – Halle and Kiel (Germany), and Lublin (Poland).

He has also been working as a judicial expert in mycotoxicology. He was the co-ordinator of the project "Mapping of poisonous fungi in the Czech Republic".

B. Hlůza has been working in mycofloristic research in the Litovelské Pomoraví Protected Landscape Area for more than 35 years, in the Jeseníky Protected Landscape Area, in the Military Training Area Libavá and in other regions of the Czech Republic.

Professor Hlůza has received more than twenty honourable commendations, diplomas, awards and many medals in the last thirty-two years – from the Czech Scientific Society for Mycology (of which he is an honorary member), the Natural History Museum Society in Olomouc (of which he is an honorary member, too), Palacký University in Olomouc, the Regional Pedagogic Institute Olomouc, and also from the Ministry of Education of the Czech Republic.

On behalf of all Czech and Slovak mycologists and friends, colleagues, collaborators and students from the Pedagogical Faculty of Palacký University in Olomouc we send cordial wishes and regards to Prof. Bronislav Hlůza to his 75th birthday. We wish him good health and pleasant years to come, even if we know that these years will be filled with further intensive work in botany and mycology – work that will certainly gratify him.

Mycological bibliography of B. Hlůza (1999–2004)

(the first two parts of B. Hlůza's mycological bibliography were published in Česká Mykologie 43(2), 1989, and in Czech Mycology 52(1), 1999)

1999

Příspěvek k ekologii a fenologii muchomůrky šedivky Amanita spissa (Fr.) Opiz. – In: Jankovský L., Krejčíř R. and Antonín V. (eds.), Houby a les, Proceedings of the conference, 3–5 June 1999, p. 32–34, Brno.

Ing. Jiří Lazebníček – 65 let. – Mykol. Listy, no. 70: 19–20.

Za Jaroslavem Kupkou. – Mykol. Listy, no. 70: 24–26.

Lazebníček J. and Hlůza B.: Výstava hub v Tovačově. – Mykol. Sborn. 76(2): 78–79.

Za profesorem B. Salatou. – Žurnál UP, Olomouc, vol. 8, no. 24: 4.

2000

Pozvánka na výstavy hub. – Žurnál UP, Olomouc, vol. 10, no. 2: 2.

2001

Lazebníček J. and Hlůza B.: Tři houbařské výstavy na střední a severní Moravě v září 2000. – Mykol. Sborn. 78(1): 54–55.

2002

Index Mykologických listů 71–80. – Mykol. Listy, no. 81: 14–32.

2003

Vejmutovky onemocněly. – Šternberské listy, no. 9/2003: 6.

Jaké bylo houbařské jaro? – Šternberské listy, no. 12/2003: 5.

Houbaři se snad letos ještě dočkají. – Šternberské listy, no. 16/2003: 5.

Podzim – čas václavek. – Šternberské listy, no. 17/2003: 5.

Císařová O., Zedníková K., Kubištová Z. and Hlůza B.: Intoxikace houbami v ÚSL FN Olomouc. – In: Súhrn prednášok 44. Májová súdnolekárska konferencia, Bratislava 27.–29. mája 2003. Ed. Lekárska fakulta Univerzity Komenského, Bratislava.

2004

První letošní houby. – Šternberské listy, no. 3/2004: 6.

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Preparation of manuscripts. Manuscripts are to be submitted in English, German or French. The text of the manuscript should be written on one side of white paper (A4, 210 × 297 mm) with broad margins (maximum 30 lines per page). Each manuscript must include *an abstract* (in English) not exceeding 100 words and a maximum of five key words. The paper will be followed by an abstract in Czech (or Slovak). The journal is responsible, however, for the translation of abstracts into Czech for foreign authors. Please send *two copies* of the typescript. The authors are asked to submit diskettes with *the accepted manuscripts* prepared on personal computers. The files should be in ASCII format, graphs in Excel. Avoid any special type of text formatting except for italic and bold options.

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References. References are to be listed in alphabetical order according to the surnames of the first authors. The bibliography should be written as follows:

Moravec J. (1984): Two new species of Coprobia and taxonomic remarks on the genera Cheilymenia and Coprobia (Discomycetes, Pezizales). – Čes. Mykol. 38: 146–155. (*journal article*)

Ryvarden L. (1978): The Polyporaceae of North Europe, Vol. 2. Inonotus-Tyromyces. – 507 p. Oslo. (*book*)

Tommerup I. C., Kuek C. and Malajczuk N. (1987): Ectomycorrhizal inoculum production and utilization in Australia. – In: Sylvia D. M., Hung L. L., and Graham J. H. (eds.), Proceedings of the 7th North American Conference on Mycorrhizae, p. 93–295, Gainesville. (*book chapter, abstract, article in proceedings*)

The references *in text* should be Moravec (1984), or (Moravec 1984); or Kühner and Romagnesi (1974). When there are three or more authors use the form Tommerup et al. (1987).

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