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Pigment production in incompatibility zones of Trametes versicolor is in correlation with the laccase activity of the dikaryons involved

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Tomšovský M. and Homolka L. (2003): Pigment production in incompatibility zones of Trametes versicolor is in correlation with the laccase activity of the dikaryons involved. – Czech Mycol. 55: 155–160

A correlation between the extracellular laccase activity (estimated by a drop test using ABTS) and the presence of a dark pigmented zone in the pairing of different *Trametes (Coriolus) versicolor* dikaryons in vitro was studied. Altogether 24 dikaryotic strains from different substrates and distant localities of three European countries were paired to each other and the pairings were checked for the presence of dark pigment in the contact zone. Using the χ^2 test, a positive correlation between the laccase activity and the presence of pigment was found.

Key words: Basidiomycetes, *Trametes versicolor*, laccase, pigment, χ^2 test

Tomšovský M. a Homolka L. (2003): Produkce pigmentu v nekompatibilních zónách outkovky pestré (Trametes versicolor) souvisí s lakázovou aktivitou přilehlých dikaryonů. – Czech Mycol. 55: 155–160

Závislost mezi aktivitou extracelulární lakázy (zjišťovanou kapkovacím testem pomocí ABTS) a tvorbou tmavé pigmentované zóny byla sledována při párování dikaryotických kmenů outkovky pestré – *Trametes (Coriolus) versicolor* – in vitro. Celkem 24 dikaryotických kmenů sebraných na různých substrátech a pocházejících ze tří evropských zemí bylo vzájemně párováno ve všech kombinacích. Následně byla zjišťována přítomnost pigmentu v kontaktní zóně vytvořené mezi kmeny. Závislost mezi tvorbou pigmentu a lakázovou aktivitou byla byla potvrzena pomocí χ^2 testu.

INTRODUCTION

Pairing tests between different dikaryotic basidiomycete strains in vitro often take place in experimental studies on fungal ecology. These studies describe fungal ecological strategies in interactions of the respective strain with other individuals. A combat between two fungal individuals (of the same or a different species) can result in either a deadlock (no fungus is able to invade the other) or in replacement of one individual by the other. Interspecific interactions of various basidiomycetes were described in several studies (Boddy and Rayner 1983, Holmer et al. 1997, Iakovlev and Stenlid 2000, White and Boddy 1992).

Trametes versicolor (L.: Fr.) Pilát, a common circumglobal polypore species, is often used in such experiments. The following interactions between *T. versicolor* and other basidiomycete species were found: *T. versicolor* replaced *Phanerochaete magnoliae* (Ainsworth and Rayner 1991) or *Phlebia rufa* (Boddy and Rayner 1983) and the species reached a deadlock with *Phlebia radiata* (Boddy and Rayner 1983, White and Boddy 1992). On the other hand, *T. versicolor* was often replaced by *Lenzites betulina*, which is able to destroy *Trametes* mycelium (Rayner et al. 1987).

During pairing between dikaryons in vitro, pigmented zones are often formed. These zones resemble the narrow, dark zones that separate genetically different individuals in wood (Rayner and Todd 1977). The formation of pigmented zones is a result of enzymatic action: darkening of the fungal tissues results from oxidation of phenolic substances by extracellular phenoloxidases (such as laccase) and peroxidases. These enzymes produced by basidiomycetes decompose nutrient substrates. The products of oxidation are usually *o*-quinones, which are highly unstable and undergo polymerisation to yield dark melanin-like pigments (Collins et al. 1963). Depending on the cultivation conditions and interactions with antagonists, the activity of the respective enzymes changes. Li (1981) compared phenoloxidase activity in pigmented zone lines with that found in adjacent mycelial tissues of *Phellinus weirii*. The zone lines exhibited stronger enzymatic reactions than the adjacent tissues.

The aim of the study was to find out if there is any correlation between the production of dark pigment and laccase activity of different *T. versicolor* dikaryotic strains.

MATERIAL AND METHODS

The strains used were obtained by isolation from the fresh sporocarps under sterile conditions. The sporocarps of *Trametes versicolor* from 15 different hardwood species of 13 genera were collected in distant localities in the Czech Republic, Bulgaria and Montenegro (former Yugoslavia) (Tab. 1). The strains were maintained on MEGA medium (malt extract 1 %, glucose 1 %, agar Difco 1.6 %) and incubated at 23 °C. All cultures are deposited in the Culture Collection of Basidiomycetes (CCBAS), Prague, Czech Republic. The sporocarps are deposited in the herbarium of the National Museum (PRM), Prague, Czech Republic.

Pairings between the strains were made by placing mycelial plugs (9 mm diameter), cut from the margins of actively growing colonies, approximately 3 cm apart in the centre of Petri dishes (6 cm diameter) containing MEGA medium.

Table 1. Ecological characteristics and laccase activity of the tested *Trametes versicolor* strains.

Strain	Substrate	Country	Locality	Latitude N	Longitude E	Laccase activity	Herbarium number
V01	*Rosa* sp.	Czech Republic	Havlíčkův Brod	49°36' 27.43"	15°35' 25.94"	++++	PRM 900581
V02	*Prunus domestica*	Czech Republic	Mírovka	49°33' 43.78"	15°36' 56.99"	+++	PRM 900612
V03	*Alnus glutinosa*	Czech Republic	Velemín	50°32' 18.49"	13°56' 56.99"	++++	PRM 900592
V04	*Fagus sylvatica*	Czech Republic	Mt. Milešovka	50°33' 20.85"	13°56' 55.00"	+++	PRM 900600
V05	*Corylus avellana*	Czech Republic	Srbsko	49°55' 52.20"	14°06' 56.76"	++++	PRM 900594
V06	*Fagus sylvatica*	Czech Republic	Srbsko	49°55' 52.20"	14°06' 56.76"	+++	PRM 900587
V07	*Fagus sylvatica*	Czech Republic	Prague	50°04' 08.30"	14°25' 16.94"	++++	PRM 900608
V08	*Malus domestica*	Czech Republic	Havlíčkův Brod	49°36' 24.17"	15°35' 20.21"	++	PRM 900580
V09	*Vitis vinifera*	Czech Republic	Brno	49°13' 31.95"	16°35' 13.34"	++++	PRM 900591
V10	*Fagus sylvatica*	Czech Republic	Brumov-Bylnice	49°02' 42.79"	18°02' 06.67"	++++	PRM 900614
V11	*Robinia pseudacacia*	Czech Republic	Prague	50°02' 27.45"	14°26' 53.66"	+++	PRM 900595
V12	*Carpinus betulus*	Czech Republic	Nové Město nad Metují	50°20' 58.94"	16°09' 27.00"	++++	PRM 900604
V13	a hardwood	Czech Republic	Jílové	49°54' 50.88"	14°30' 11.27"	+++	PRM 900602
V14	*Fagus sylvatica*	Czech Republic	Žofín primeval forest	48°40' 00.13"	14°42' 32.76"	+	PRM 900583
V15	*Acer platanoides*	Czech Republic	Potštejn	50°04' 13.81"	16°18' 44.53"	+	PRM 900599
V16	*Fagus* sp.	Montenegro	Lovčen Mts.	42°25' 35.0"	18°51' 00"	+++	PRM 900613
V17	*Fagus* sp.	Montenegro	Bjelasica Mts.	42°54' 00"	19°37' 00"	++++	PRM 900576
V18	*Fagus orientalis*	Bulgaria	Rhodopes Mts.	42°01' 30"	24°15' 00"	++++	PRM 900575
V19	*Quercus* sp.	Bulgaria	Tulovo primeval forest	42°34' 59"	25°33' 00"	++++	PRM 900609
V20	*Quercus* sp.	Bulgaria	Tulovo primeval forest	42°34' 59"	25°33' 00"	+++	PRM 900577
V21	*Betula* sp.	Czech Republic	Albeř	49°01' 35.64"	15°09' 10.02"	++++	PRM 900593
V22	*Salix* sp.	Czech Republic	Mirochov	49°00' 24.84"	14°56' 37.42"	++	PRM 900607
V23	*Corylus avellana*	Czech Republic	Bečov nad Teplou	50°05' 03.38"	12°49' 22.36"	+++	PRM 900579
V24	*Alnus incana*	Czech Republic	Šilhéřovice	49°53' 24.31"	18°16' 18.31"	++++	PRM 900597

The cultures were incubated in darkness at 23 °C for three weeks. The dishes were then checked for the presence of the pigmented zone.

Laccase activity was estimated by a spot test using ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) according to Niku-Paavola et al. (1990). The mycelium was removed from the 2-week old cultures on Petri dishes (9 cm diameter) by scraping off with a scalpel; then 3 ml of a fresh staining ABTS solution (5 mg/ml distilled H_2O) was poured over the agar plate, which was then incubated for 6 hours. The colour intensity (indicating extracellular laccase activity) was classified using a four-point scale as follows: weak (+), medium (++), strong (+++), very strong (++++).

The correlation between pigment production in pairings and laccase activity of the strains paired was estimated by the χ^2 test, which is the most common test for significance of relationship between categorical variables (according to Lepš 1996).

RESULTS AND DISCUSSION

The results of the pairings are summarised in Table 2. Altogether 126 pairings (60 %) out of 210 resulted in antagonism accompanied by pigment production. The remaining 84 pairings (40 %) exhibited antagonism without pigmentation. In case of pigment production, 37 % of pairings formed pigment only in a small part of the contact zone between the mycelia, and 63 % pairings formed pigment in the entire zone. All pairings between the same strains resulted in complete fusion of isolates.

Intensity of the enzymatic reaction of the paired isolates (Tab. 1) was classified as weak in 2, medium in 2, strong in 8, and very strong in 12 out of 24 strains. Relations among the four above-mentioned values of laccase activity and three values of pigment occurrence (no pigment; pigment in the part of the zone; pigment in the entire zone) were then tested. The χ^2 test revealed a significant relation between the tested variables ($\chi^2 = 47.61$, df = 6, $P<0.05$). Positive correlation between laccase activity and presence of pigment was found at *Trametes versicolor* pairings. These results do not correspond with those of Iakovlev and Stenlid (2001), who did not find any relation between pigmentation and laccase activity. The above-mentioned authors studied interspecific matings of several basidiomycete species (*Antrodiella citrinella, Flammulina velutipes, Fomitopsis pinicola* etc.). It is likely that pigment production is also influenced by other factors. For example, the presence of cadmium in the media can induce a dark pigmentation in *T. versicolor* (Baldrian and Gabriel 1997). Todd and Rayner (1978) found that pigment production diminishes with increased relatedness of the isolates. To decrease the effect of relatedness, isolates from distant localities were tested in this study. In three cases the couples of strains (V01, V08; V05, V06; V19, V20) were collected in the same locality, but all three couples were genetically distinct

Tab 2. Pairing among different dikaryotic isolates of *Trametes versicolor* (V01-V24). ○: antagonism; △: antagonism accompanied by pigment production, △N: pigment producted in small part of contact zone; •: complete fusion of isolates.

	V24	V23	V22	V21	V20	V19	V18	V17	V16	V15	V14	V13	V12	V11	V10	V09	V08	V07	V06	V05	V04	V03	V02	V01
V01	△N	○	△N	△N	△N	△	○	○	△	○	○	△	○	○	△	△	○	○	△	○	○	△	○	•
V02	○	○	△N	○	△N	○	△	○	△	△	△	○	△	△	△	△	△	△	△	△	△	△	•	
V03	△N	△	○	△	△N	△	△	○	△N	△	△	△	○	△	△	△	○	○	△	△	△	•		
V04	△N	○	△	○	△N	△N	○	○	△	△	○	△	○	○	△	△	○	△	○	○	•			
V05	△	○	△	△	△	△N	△	○	△N	△	○	△	○	○	△	△	△	△	○	•				
V06	○	○	○	△N	○	△N	△	△	○	△N	○	○	○	○	○	○	○	○	•					
V07	△N	△N	△N	△N	△N	△N	△N	○	△N	△N	○	○	○	○	△	△	○	•						
V08	○	○	○	○	○	△N	○	○	△N	△N	○	○	△	○	○	○	•							
V09	△	△N	△	△	△	△	△N	○	△	△	△	△	△	△	○	•								
V10	○	△N	△N	△N	△N	△	△	○	○	△	△N	△	○	△	•									
V11	△N	△	△	○	△N	△N	○	△N	△N	△N	○	○	△	•										
V12	○	△	△N	△	○	△N	△	○	△N	△N	△	△	•											
V13	○	○	○	○	○	△N	△N	○	○	○	△	•												
V14	△N	○	○	○	○	△N	○	○	△N	△N	•													
V15	△N	△N	△N	△	○	△N	○	△N	△N	•														
V16	○	△N	○	△	○	△	△	○	•															
V17	△	○	○	△	○	○	△	•																
V18	○	○	△N	△N	△N	△N	•																	
V19	△N	△N	△N	△N	△N	•																		
V20	○	△N	○	○	•																			
V21	○	△N	△N	•																				
V22	○	○	•																					
V23	○	•																						
V24	•																							

and their pairings turned out as expected (i.e., contact zones were formed). The communication between different fungal individuals is a complex process requiring further investigations.

Finally, we believe that this study can help understanding intraspecific ecological processes.

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Gymnopilus igniculus – find from the Czech Republic and notes on its variability

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Holec J., Antonín V., Graca M. and Moreau P.-A. (2003): Gymnopilus igniculus – find from the Czech Republic and notes on its variability. – Czech Mycol. 55: 161–172

Gymnopilus igniculus Deneyer, P.-A. Moreau et Wuilbaut (Agaricales, *Cortinariaceae*), a violet-coloured species described in 2002 from Belgium, was found in the city of Ostrava, part Radvanice, Czech Republic. The fruitbodies grew on decaying wood of *Fraxinus* in a unique habitat – a burning coal mine dump, the surface of which reached about 45 °C. The most important characters of the fruitbodies are the purplish to vinaceous- or reddish-brown colour of the pileus covering which is tomentose-fibrillose when young and fibrillose-squamulose to distinctly scaly at maturity, the yellow pileus ground, the absence of any ring, the whitish membranaceous to fibrillose veil, the stipe distinctly longitudinally purplish brown fibrillose on the dirty white or slightly violaceous ground, the context yellowish with reddish-violaceous tinge and fungoid smell, the relatively large spores [8.0–9.5(–11) × 6.0–6.8(–7.2) μm] with rough verrucose to verrucose-rugulose ornamentation, the variable shape of cheilocystidia and the absence of pleurocystidia. A detailed description of macro- and microcharacters, colour photographs of fresh fruitbodies and line drawings are provided. Some characters deviating from the Belgian collections are discussed and remarks on other European finds of *Gymnopilus* with violet colours are added.

Key words: fungi, Agaricales, *Cortinariaceae*, violet-coloured *Gymnopilus*, Europe, taxonomy, ecology, coal mine dumps.

Holec J., Antonín V., Graca M. a Moreau P.-A. (2003): Gymnopilus igniculus – nález v České republice a poznámky k jeho variabilitě. – Czech Mycol. 55: 161–172

Gymnopilus igniculus Deneyer, P.-A. Moreau et Wuilbaut (Agaricales, *Cortinariaceae*), druh s fialovými odstíny na plodnici popsaný v roce 2002 z Belgie, byl nalezen v Ostravě-Radvanicích. Plodnice rostly na tlejícím dřevě jasanu v neobvyklém prostředí – na hořící hornické haldě, kde teplota povrchu dosahovala až 45 °C. Nejdůležitějšími znaky nalezených plodnic jsou: purpurová až vínově nebo červeně hnědá barva klobouku, který je v mládí plstnatě vláknitý, v dospělosti vláknitě šupinatý až zřetelně šupinatý na žlutém podkladu, nepřítomnost prstenu, bílé blanité až vláknité velum, třeň zřetelně podélně purpurově hnědě vláknitý na špinavě bílém nebo lehce nafialovělém podkladu, žlutavá dužnina s červenavě fialovým odstínem a houbovou vůní, poměrně velké výtrusy [8.0–9.5(–11) × 6.0–6.8(–7.2) μm] s hrubě bradavčitou až bradavčitě vráščitou ornamentikou, proměnlivý tvar cheilocystid a nepřítomnost pleurocystid. Je publikován podrobný popis makro- a mikroznaků, barevné fotografie čerstvých plodnic a kresba a fotografie mikroznaků. Jsou diskutovány některé odlišnosti od belgických sběrů a připojeny poznámky k dalším evropským nálezům fialově zbarvených druhů z rodu *Gymnopilus*.

INTRODUCTION

In spring 1999, M. Graca found an interesting *Gymnopilus* species growing in a unique habitat – a burning coal mine dump in the city of Ostrava, part Radvanice, Czech Republic. The fruitbodies were remarkable by the purplish to vinaceous brown colours on the pileus and stipe surface, a character which is unique in *Gymnopilus* species collected in Europe. M. Graca photographed the fruitbodies on site and sent them immediately to the Department of Botany, Moravian Museum, Brno, where V. Antonín and A. Vágner made two independent and thorough descriptions of macrocharacters. Later, V. Antonín handed the dried material over to Jan Holec for detailed elaboration, who included it in his taxonomic study of the genus *Gymnopilus* in Central Europe (see e.g. Holec 2001).

Gymnopilus species with purplish, violaceous or vinaceous tinged fruitbodies are rare in Europe. They mostly represent species imported from the tropics or subtropics. It is e.g. *Gymnopilus purpuratus* (Cooke et Massee) Singer, described from tree fern stems in the Royal Botanic Gardens, Kew (Cooke and Massee, Grevillea 18: 73, 1890; Cooke 1883: 375; coloured picture: Cooke 1881–1891: plate 964). Fruitbodies identified as *G. purpuratus* were also found in the greenhouse of the Botanical Garden in Zürich (Breitenbach and Kränzlin 2000: 140). The name *G. purpuratus* was further used for collections from compost heaps of wood and bark remnants in the Ribnitz-Damgarten district in Germany (Kreisel and Lindequist 1988, Ludwig 2001: 154, coloured picture by Ludwig 2000: p. 45). Röllin (1998) published finds identified as *Gymnopilus* cf. *peliolepis* from a base of a palm tree in a bureau in Genève, Switzerland. Find of *Gymnopilus dilepis* (Berk. et Broome) Singer from a pot with *Philodendron* purchased from a supermarket in Great Britain was published by Watling (1998); a more recent, abundant find, on heap of woodchips, has been illustrated by T. Leech in Henrici (2002: back cover).

Recently, Deneyer et al. (2002) described the new species *Gymnopilus igniculus* Deneyer, P.-A. Moreau et Wuilbaut found at two burning schist dumps in Belgium. Coloured photographs of this species were published in the work by Bon and Roux (2002: pl. 1-B) and by Wuilbaut (2002: p. 31). Finally, Bon and Roux (2002) used the name *Gymnopilus luteifolius* (Peck) Singer for *G. purpuratus* s. Breitenbach et Kränzlin (2000) and the name *Gymnopilus peliolepis* (Speg.) Singer for *G. purpuratus* s. Ludwig (2000, 2001).

A comparison of our finds with purplish to violaceous coloured *Gymnopilus* species known from Europe showed that our fungus is identical with *Gymnopilus igniculus*. Detailed data on characters and ecology of the finds from the Czech Republic are presented here.

MATERIAL AND METHODS

Herbarium specimens are kept in the Mycological Department, National Museum, Prague (PRM) and Department of Botany, Moravian Museum, Brno (BRNM). The colour codes are according to Kornerup and Wanscher (1981). Microcharacters were studied in a 5 % KOH solution. The pigmentation of the pileus and stipe cuticle was studied in pure water. Iodine reactions were studied in Melzer's reagent prepared according to the formula given by Moser (1983). For spore size measurements, 20 spores from each collection were randomly selected.

Abbreviations: E = length/width ratio of the spores, Q = mean value of E for all spores studied.

RESULTS

Gymnopilus igniculus Deneyer, P.-A. Moreau et Wuilbaut

Doc. Mycol., vol. 32, no. 125: 11, 2002 (the species was at first published without Latin description and type designation in Bon and Roux 2002: p. 4, p. 15–16)

Illustrations: Bon and Roux (2002: pl. 1-B), Wuilbaut (2002: p. 31).

Description of the fruitbodies from the Czech Republic (see collections studied):

Basidiocarps single or in small groups, never cespitose, growing on decaying wood of *Fraxinus*.

Pileus (7-)15–40 mm broad, broadly conical to convex, involute at margin when young, then convex with applanate centre and inflexed margin, almost applanate with slightly inflexed margin at the end, entirely distinctly tomentose when young, then except for centre ± adpressed radially fibrillose, almost pyramidal fibrillose-squamulose at centre and radially adpressed fibrillose towards margin when old, vesture purplish or vinaceous brown (9–11E6–7, 10F7), with paler margin (9E7) when young, then vinaceous only at centre and paler, purplish ochraceous brown (8–9D7–8) towards margin, when old reddish brown (9D-E7) at centre and paler (8–9D6) towards margin, ground yellowish to pale dirty yellow; margin sometimes decorated with almost membranaceous velar remnants.

Lamellae rather distant, L = 18–24, l = 2–3, broadly adnate or emarginate and shortly decurrent with tooth, ± horizontal, light yellow (3A4) to orange-yellow (4–5A5), with concolorous, irregularly serrulate, finely pubescent edge.

Stipe 20–80 × 2–6 mm, cylindrical, sometimes slightly clavate (up to 7 mm) or attenuated towards base, often curved, distinctly longitudinally purplish brown (10D5–6, 10–11E6–7) fibrillose or fibrillose-squamulose on dirty white or slightly violaceous ground, whitish or with violaceous tinge, less distinctly fibrillose (paler than other parts of stipe) and sometimes striate (decurrent lamellar tooth) at apex; with dirty whitish basal mycelium.

Velum membranaceous, dirty whitish or pale dirty brownish, at margin yellowish when young, its remnants sometimes distinct at pileus margin and near stipe apex (when young) and only as indistinct fibrils on stipe surface (when old).

Context hollow in stipe, whitish to yellowish, in stipe apex and above lamellae more distinctly yellow, pale violaceous under pileipellis, slightly violaceous (reddish) in stipe middle; with fungoid or indistinct smell and bitterish taste.

Spores 8.0–9.5(-11) × 6.0–6.8(-7.2) μm, E = 1.25–1.50, Q = 1.39, broadly ellipsoid, with small but distinct suprahilar depression visible in side view, rusty yellow in KOH with darker, rusty brown wall which is slightly thick-walled, surface densely covered with low but small to large (up to 1.5 μm broad) and irregular verrucose to rugulose-verrucose ornamentation, hilar appendix tiny, spore interior distinctly dextrinoid (staining vinaceous reddish brown) in Melzer's reagent with the wall remaining rusty yellow.

Basidia 24–28 × 8–9 μm, mostly 4-spored, rarely 2– or 1-spored, clavate to subutriform, sometimes with a slight medial constriction, clamped. Basidioles 10–30 × 3–9 μm, cylindrical when young, then distinctly clavate, some of them filled with a homogeneous yellow-rusty pigment (in 5 % KOH), clamped.

Cheilocystidia 24–35 × 6–10 μm, arranged in "nests" on edge or mixed with basidioles, narrowly clavate to fusiform-lageniform when young, then typically lageniform to subutriform with 3–4 μm broad neck and more or less distinct globose head 5–8 μm in diameter, thin-walled, hyaline, clamped. Pleurocystidia not observed.

Lamellar trama regular to subregular, consisting of parallel hyphae 3–20 μm broad, narrower hyphae located near the subhymenium, cells cylindrical, slightly fusiform to narrowly ellipsoid, with hyaline interior and yellowish wall, non-dextrinoid, clamped, subhymenium of densely arranged hyphae.

Pileus cuticle a cutis, 60–70 μm thick, 2-layered, upper layer thin, dark reddish to violet brown in KOH, of densely and radially arranged (parallel in a section) hyphae 4–20 μm broad, cells cylindrical to narrowly fusiform or narrowly ellipsoid, with distinct violet to reddish brown incrustations arranged in a zebra to tiger pattern, terminal cells indistinct, subfusoid to narrowly clavate, lower layer thick, less coloured, yellow in KOH, of loosely arranged parallel to slightly interwoven hyphae with less distinct incrustations, this layer gradually passing into the pileus context made up of cylindrical, narrowly fusiform to narrowly ellipsoid hyphae up to 25 μm broad, hyaline or pale brownish in KOH. When a pileus scalp is observed, the pileus surface is covered with fascicles or a sparse net of cells forming the upper layer of the pileus cuticle.

Stipe cuticle a cutis of densely arranged, parallel, cylindrical hyphae 3–8 μm broad, yellow-brown with violet tinge, cells with yellow-brown to rusty brown incrustations, slightly thick-walled, clamped, terminal cells indistinct, cylindrical;

Fig. 1. *Gymnopilus igniculus*, Czech Republic, Ostrava-Radvanice, burning coal mine dump, on decaying wood of *Fraxinus*, 1 May 1999, found and photographed by M. Graca (PRM 900986). Young and mature fruitbodies.

Fig. 2. *Gymnopilus igniculus*, for explanations see Fig. 1. Mature fruitbodies.

Fig. 3. Collecting site of *Gymnopilus igniculus*: flat part of the burning coal mine dump in Ostrava-Radvanice, Czech Republic. The fungus grew on decayed wood of *Fraxinus* among the moss *Aulacomnium palustre* (left bottom part of the photograph). Photo M. Graca, August 2003.

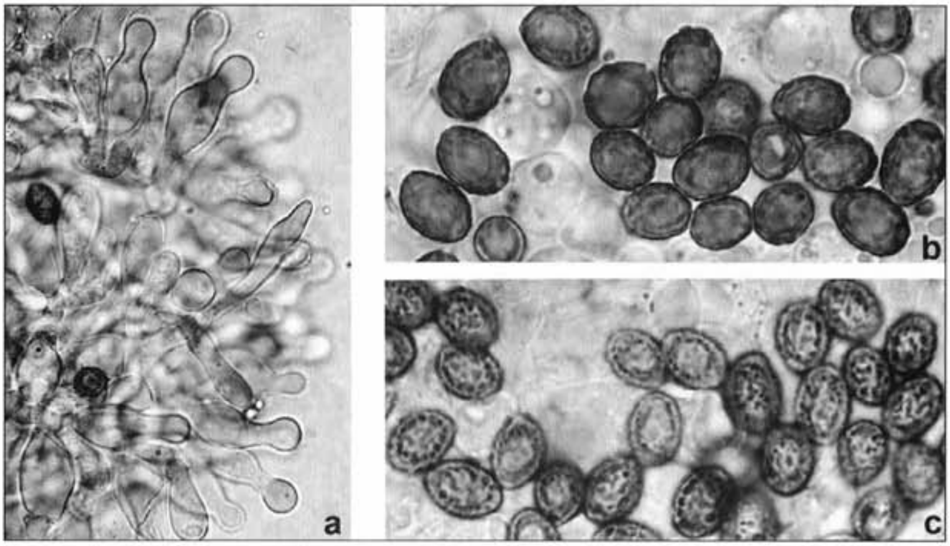

Fig. 4. *Gymnopilus igniculus* (PRM 900986). a: cheilocystidia, b: spores – outline, c: spores – ornamentation. Photo J. Holec.

caulocystidia not observed, but a sparse net of interwoven, yellow-brown incrusted veil hyphae 2–8 μm broad covering the cuticle.

Stipe context made up of cylindrical to subellipsoid, slightly thick-walled, yellowish hyphae up to 15 μm broad, mixed with 5–10 μm broad branched hyphae with yellow content in KOH. Clamp connections present in all tissues.

Fragments of lamellae exuding a bright yellow pigment when mounted in 5 % KOH.

Collecting site

Localisation: Czech Republic, northeastern part: Silesia region, city of Ostrava, 5.4 km E of the Central Bus Station, 0.5 km SE of dead-end street called "Slunná" in Ostrava-Michálkovice, coal mine dump situated S of Ostrava-Michálkovice, NE of Ostrava-Radvanice and W of Petřvald, 270 m a.s.l. The dump belonged to the former coal mine "Hedvika" in Petřvald (concern "Julius Fučík").

Habitat: coal mine dump formed by rocks typical of the Carboniferous of the Czech part of the "Hornoslezská pánev" basin. The dump is composed of silty shales, claystones, siltstones and fossil soil with Stigmaria; to a lesser extent of fine-grained sandstones. At present, most parts of the dump are reclaimed and planted mainly with *Betula* stands (30–40 years), further mixed stands (*Betula, Tilia, Populus, Quercus, Fagus*) and somewhere also with stands of *Pinus nigra* or *Quercus robur*. In some places, heat and gasses escape from lower parts of the dump containing coal with a relatively high content of sulphur. The soil of the collecting site is rather warm. During collecting days, its surface reached a temperature of about 45 °C. Although the site is insolated, it is moist due to the escaping humid heat and gasses. Both collections are from the same site (about 10 m^2) in the flat part of the burning mine heap.

Vegetation: The surface is overgrown by the moss *Aulacomnium palustre* (det. V. Plášek; a moss species mostly growing in moist meadows and spring areas), the grass *Setaria pumila* and a tree stand of *Fraxinus excelsior*. The herb *Solidago canadensis* and trees like *Betula pendula* and a young individual of *Quercus robur* occur at the margins of the collecting site. Concerning other species of fungi, *Leucoagaricus meleagris* and *Polyporus ciliatus* were observed.

Collections studied

Czech Republic, northeastern part: Silesia region, Ostrava-Radvanice, burning coal mine dump, on decaying wood of *Fraxinus*, 24 April 1999, leg. M. Graca (BRNM 686264); ibid., 1 May 1999 (PRM 900986). – Belgium, Cuesmes, "Terril du Levant", among *Campylopus retroflexus* on hot mineral ground (charcoal), 9 Dec. 2001, leg. Y. Deneyer, P.-A. Moreau, J. Nuytinck and J. J. Wuilbaut (herb.

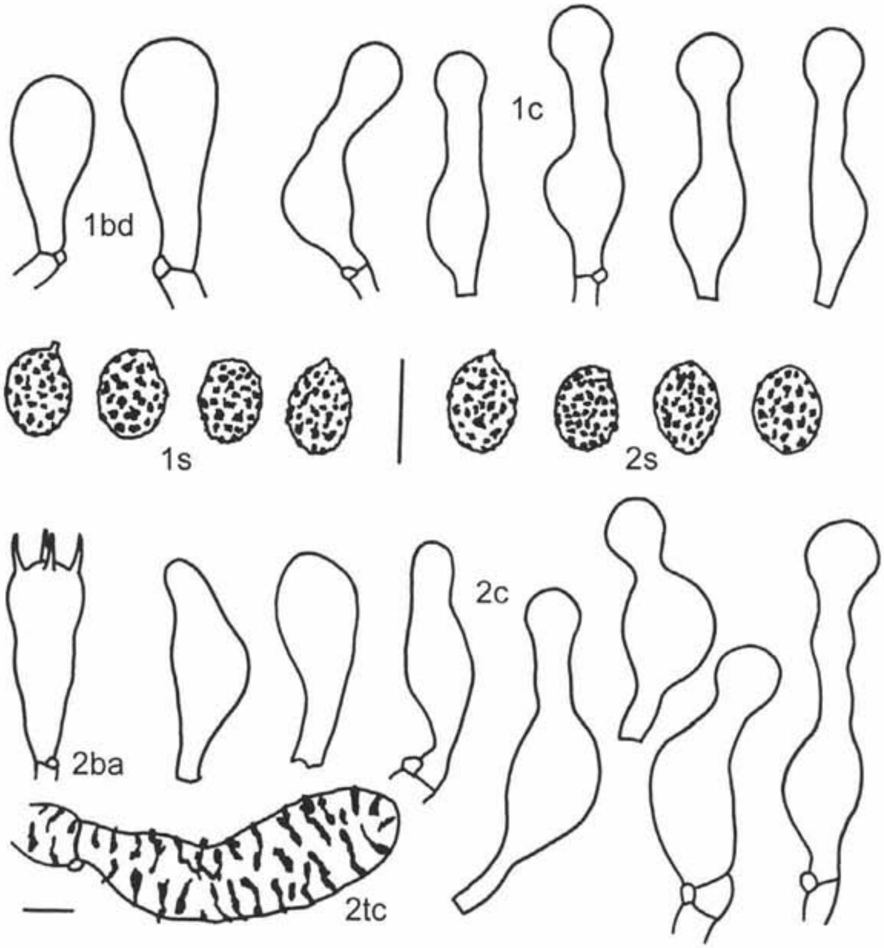

Fig. 5. *Gymnopilus igniculus*, Czech Republic, Ostrava-Radvanice, microcharacters. 1: collection from 24 April 1999 (BRNM 686264), 2: collection from 1 May 1999 (PRM 900986); ba: basidia, bd: basidioles, c: cheilocystidia, s: spores, tc: terminal cells of hyphae from upper layer of pileus cuticle. Scale bars = 10 μm. Del. J. Holec.

PAM 01120901: fruitbodies not formally designated as isotype, but originating from the 30 original fruitbodies from which the holotype deposited in BR was selected; all these specimens were collected on the same surface having about 20 m^2).

DISCUSSION

Gymnopilus igniculus and its variability

The fruitbodies found at Ostrava-Radvanice in the Czech Republic in agree in most essential characters with those of *Gymnopilus igniculus* described from Belgium (some differences are discussed below). This also concerns the habitat. In both countries the species grows in a unique artificial habitat – burning coal dumps, the surface of which is warm even in winter and spring (Belgium: 40 °C on surface, see Bon and Roux 2002: 16, Czech Republic: 45 °C). The conspecifity was also confirmed by a study of microcharacters of the original material of *G. igniculus* from Belgium (see Collections studied).

The most important characters of finds of *G. igniculus* from the Czech Republic are the purplish to vinaceous or reddish brown colour of the pileus covering (which is tomentose-fibrillose when young and fibrillose-squamulose to distinctly scaly at maturity, especially at centre), the yellow pileus ground, the absence of any ring, the whitish veil, the stipe distinctly longitudinally purplish brown fibrillose on a dirty white or slightly violaceous ground, the context yellowish with reddish-violaceous tinge and fungoid smell, relatively large spores (8.0–9.5(-11) × 6.0–6.8(-7.2) μm) with rough verrucose to verrucose-rugulose ornamentation, the variable shape of its cheilocystidia and the absence of pleurocystidia.

However, there are also differences in macrocharacters between the fruitbodies from Belgium and the Czech Republic. According to P.-A. Moreau, who saw the Belgian population in the field, the fruitbodies from Belgium are slender and possess small purplish scales on a yellow ground, and are not completely purple-fibrillose like the fruitbodies from Ostrava. All the collections Y. Deneyer made in the two localities mentioned in the original description of *G. igniculus* (at least 10 times) were similar. At first view, the fruitbodies from the two countries are different. However, when the descriptions are compared, the differences with the original *G. igniculus* are only quantitative: the Belgian fruitbodies have a pileus cuticle thin and soon differentiated into scales, with very few fibrils; those from the Czech Republic have a thicker pileus cuticle which dissociates into scales less or only with age. When the young fruitbodies are compared, they are almost identical. Possibly, Deneyer et al. (2002) described in fact local populations with smaller and slender fruitbodies with an underdeveloped pileus cuticle, whilst the material from the Czech Republic represents robust and more coloured fruitbodies. In addition, such more robust fruitbodies were also found in France (burning dump Pinchonvalles, Avion, Pas-de-Calais) by J. Vast and R. Courtecuisse (see note by R. Courtecuisse at the end of the paper by Deneyer et al. 2002: p. 16). In this case, the pileus surface was also fibrillose but later divided (broken) into appressed scales (we saw a photograph kindly provided by R. Courtecuisse). Maybe this aspect was caused by insolation, as the fruitbodies were collected in June.

The fruitbodies from the Czech Republic and France also differ from the Belgian ones by their smell and taste. The smell of the first ones was not very distinctive while a prominent farinaceous-herbaceous smell and taste was noted in the Belgian material. External conditions (temperature or drought?) may be responsible for this discrepancy, similarly as in the previous case.

A trophic difference can also been pointed out between the original localities and French and Czech sites: all collections from Belgium, small and slender, grow between mosses (*Campylopus*), the mycelium growing from a layer of moss litter. The robust fruitbodies collected by Courtecuisse and Graca are associated with wood remnants, perhaps a more favourable substrate for their development.

This analysis can be concluded from the result that *G. igniculus* obviously has a greater variability of macrocharacters than was observed in the original collecting sites in Belgium. The conspecifity of Belgian and Czech finds is confirmed mainly by agreement in habitat, microcharacters and appearance of young fruitbodies. The more robust forms from the Czech Republic and France having a fibrillose pileus covering which later separates into scales may be somewhat different due to fructification in spring (the Belgian fruitbodies were collected from October to February). In this period the insolation is higher and air humidity lower which may cause a different development of the pileus cuticle.

Remarks on other European finds of Gymnopilus with violet colours

All purplish or violet coloured finds of *Gymnopilus* hitherto known from Europe (see Introduction) differ from *Gymnopilus igniculus* in smaller spores mostly measuring 6–8.5 × 4–6 μm ("average" spores without the extremely large ones which are often present in *Gymnopilus*) and in more distinct, mostly erect scales covering the whole pileus surface. Moreover, *Gymnopilus purpuratus* s. Kreisel and Lindequist (1988) differs in the presence of abundant pleurocystidia and in blue to blue-greenish colour changes on the stipe surface and in the context.

No traces of greenish, bluish-greenish or grey-blue were observed on fruitbodies of *Gymnopilus igniculus* from both Belgium and the Czech Republic. Such colour changes are known in *Gymnopilus purpuratus* s. Kreisel and Lindequist (1988), and Ludwig (2000, 2001) from Germany, especially in context and lower part of stipe. Kreisel and Lindequist (1988) and Gartz (1989) proved that this coloration is caused by the alkaloids psilocybin, psilocin and baeocystin. As green or blue colours are neither described nor depicted by Cooke (1883, 1881–1891: pl. 964) in *Gymnopilus purpuratus*, it is rather problematic whether the German finds are conspecific with the original *G. purpuratus* of Cooke and Massee. We tried to loan the type of *G. purpuratus* from Kew for comparison, but, unfortunately, it has been sent on loan abroad. However, in our opinion, the name *G. purpuratus* seems to be inapplicable to the German material for the reasons mentioned above.

Bon and Roux (2002: 4, 14) consider *Gymnopilus purpuratus* an invalid name. This is not true as the basionym (*Agaricus purpuratus* Cooke et Masee 1890) fulfil all conditions for valid publication and its combination into *Gymnopilus* made by Singer (1951: 561) is valid as well. They use the name *Gymnopilus peliolepis* (Speg.) Singer for *G. purpuratus* s. Ludwig, i.e. for the German finds discussed above. This is also debatable as no greenish or bluish-greenish tinges are mentioned by Spegazzini or Hesler (1969) in *G. peliolepis*. However, we cannot solve this problem at the moment. A thorough study based on fresh collections of violet coloured *Gymnopilus* species (both from the tropics and Europe) as well as type studies of *G. purpuratus* and *G. peliolepis* would be desirable to judge the identity of these species.

Gymnopilus purpuratus s. Breitenbach and Kränzlin (2000) found on peat in the tropical greenhouse of the Botanical Garden in Zürich is obviously not identical with *G. purpuratus* s. Kreisel and Lindequist (1988) and Ludwig (2000, 2001) as it has no pleurocystidia and no traces of bluish or blue-greenish colours. However, its conspecifity with the original *G. purpuratus* by Cooke and Massee is also debatable. The habitat is similar in both cases (tropical greenhouses), but the original *G. purpuratus* has a completely purple or purple-brown pileus covered with minute floccose scales of the same colour, whereas the find from Zürich is characterised by a yellow pileus background covered with wine-red to red-violet scales. This is such a distinct difference (compare e.g. plate 964 by Cooke 1881–1891 with photo no. 148 by Breitenbach and Kränzlin 2000) that the two finds can hardly be conspecific. A type study of *G. purpuratus* could help to prove this opinion more exactly, but, as shown above, the type was not accessible during our study. Bon and Roux (2002) are of the opinion that the find from Zürich is conspecific with the American species *Gymnopilus luteifolius* (Peck) Singer (a name also known under the grammatically incorrect form *G. luteofolius*). However, the original *G. luteifolius* also has some characters different from *G. purpuratus* s. Breitenbach and Kränzlin (2000): presence of pleurocystidia, context at first reddish (*G. purpuratus* s. Breitenbach and Kränzlin: no pleurocystidia, context yellow). However, the discussion is rather problematic without knowledge of the variability of both taxa and without a type study. What seems to be clear is the identity of *G. purpuratus* s. Breitenbach and Kränzlin (2000) and the find from Sicily named *G. luteifolius* by Bon and Roux (2002: p. 3, 15–16, plate 1-A). The finds are identical in most microcharacters and in the appearance of the fruitbodies. According to Roberts in Henrici (2002: 30), these collections might belong to *G. dilepis* (Berk. et Broome) Singer. Roberts studied types of both *G. dilepis* and *G. purpuratus* housed in Kew and found that the species are undoubtedly not identical. The recent British material identified as *G. dilepis* (Watling 1998, Henrici 2002) really agrees with the type of *G. dilepis* (Roberts in Henrici 2002: 30).

The discussion on violet-coloured species of *Gymnopilus* in Europe clearly shows how poor our knowledge of this group in Europe is and how difficult it is to identify the finds. The reasons are the rarity of such finds, evident tropical or subtropical origin of collections from indoor or greenhouses and the difficulty to judge the variability of European records with respect to species described from other continents. A thorough world-wide study based on good knowledge of the variability of fresh material and on type studies would be desirable to clear up the taxonomy of this group.

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Distribution and ecology of Armillaria species in some habitats of southern Moravia, Czech Republic

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Jankovský L. (2003): Distribution and ecology of Armillaria species in some habitats of southern Moravia, Czech Republic. – Czech Mycol. 55: 173–186

In forest ecosystems of southern Moravia, five species of annulate *Armillaria* species and the exannulate species *Armillaria socialis* were observed. *Armillaria ostoyae* shows its ecological optimum in the forest type group *Querceto-Fagetum* where it represents an important parasite of spruce. *Armillaria gallica* is a dominant species of floodplain forests and thermophilic oak communities where *A. ostoyae* is lacking. *Armillaria mellea* occurs on broadleaved species and fruit trees. *Armillaria cepistipes* and *A. borealis* were detected in the Drahanská vrchovina Highlands only, *A. socialis* occurs rarely on stumps and bases of dead oak trees in a hard-wooded floodplain forest along the Dyje river. It is one of the northernmost localities in Europe. *Armillaria* spp. were identified in 79 hosts, 33 of which were coniferous species. The main role of *Armillaria* spp. consists in the decomposition of wood in soil (stumps, roots) and in the species spectrum regulation in the course of succession.

Key words: *Armillaria*, root rots, hosts, ecology

Jankovský L. (2003): Rozšíření a ekologie druhů rodu václavka (Armillaria) na některých lokalitách jižní Moravy. – Czech Mycol. 55: 173–186

V lesních ekosystémech jižní Moravy bylo zjištěno 5 druhů prstenatých václavek a bezprstenný druh *Armillaria socialis*. *Armillaria ostoyae* má ekologické optimum ve skupině lesních typů *Querceto-Fagetum*, kde je významným parazitem na smrku. Dominantním druhem lužních lesů a termofilních doubrav je *A. gallica*, kde naopak zcela scházela *A. ostoyae*, *A. mellea* se vyskytuje ohniskovitě na listnatých a ovocných dřevinách. Václavky *A. cepistipes* a *A. borealis* byly zjištěny pouze na Drahanské vrchovině. *Armillaria socialis* se vyskytuje vzácně na pařezech a bázích odumřelých dubů v tvrdém luhu podél Dyje. Jde o jednu z nejseverněji položených lokalit v Evropě. Václavky byly identifikovány na 79 hostitelích, z toho bylo 33 druhů jehličnanů. Hlavní funkce václavek spočívá v dekompozici dřevní hmoty v půdě (pařezy, kořeny) a v regulaci druhového spektra dřevin v průběhu sukcese.

INTRODUCTION

The species of the genus *Armillaria* are mentioned from all continents with the exception of the Antarctic. They are part of a number of ecosystems, from tundra across mixed forests of the temperate zone and tropical forests to sclerophyllous formations of semideserts. An overview of the present knowledge on the distribution of the *Armillaria* species in particular continents is given e. g. by Kile et al. (1994).

In Europe, on the basis of genetic studies and intersterility tests by Korhonen (1978) and other nomenclatural and taxonomic papers (e.g. Marxmüller 1982, 1987, 1992; Termorshuizen and Arnolds 1987), five species of annulate *Armillaria* species are distinguished: *Armillaria borealis* H. Marxmüller et Korhonen, *A. cepistipes* Velen., *A. ostoyae* (Romagn.) Herink, *A. mellea* (Vahl: Fr.) Kumm. and *Armillaria gallica* H. Marxmüller et Romagn. In addition, two exannulate *Armillaria* species occur in Europe, viz. *A. socialis* (DC.: Fr.) Fayod and *A. ectypa* (Fr.) Emel.

The geographical distribution of *Armillaria* spp. in particular countries of Europe is described in a number of papers (Guillaumin et al. 1993; Sicoli, Luisi and Manicone 1994; Tsopelas 1994; Żółciak 1989 etc.). The distribution of *Armillaria mellea* s. l. in the Czech Republic or Czechoslovakia is descibed in a number of papers concerning particularly forest pathology aspects (Málek 1966, 1973; Lazebníček 1973; Černý 1973, 1985; Jančařík and Jankovský 1999; Jankovský 1995 etc.). Ecology, distribution and host spectrum of *Armillaria mellea* (Vahl: Fr.) P. Kumm. is given by Antonín (1988).

Armillaria spp. were noticed in more than 600 woody species and herbs (Raab 1962, Guillaumin et al. 1985, Kile et al. 1991) from all climatic zones. Among the hosts, we can find representatives of trees, shrubs, herbs and grasses. *Armillaria* spp. attacks also fruit trees and in some cases even agricultural crops. There are also mycorrhizal associations of *Armillaria* spp. with some non-green orchids such as *Galeola septentrionalis* and *Gastrodia cunningamii* (Hamada 1940, Terashita and Chuman 1989, Cambell 1962). *Armillaria* spp. also cause damage to plantings of ornamental and fruit species and other agricultural crops. In Europe, *Armillaria mellea* is frequently mentioned as a causal agent of dying lemon-trees in the Mediterranean, almonds, apricot trees, peach trees and other stone fruits, grape-vine, walnuts etc. (Guillaumin and Lung-Escarmant 1985). As causal agents of diseases, *Armillaria* spp. are mentioned even in strawberry, prickly pear, cotton-plant and various species of vegetables. In the Czech Republic, *Armillaria* spp. cause occasionally damage to fruit orchards and gardens.

As primary parasites, *Armillaria* spp. occur in the Czech Republic mainly in secondary stands of conifers, particularly of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). The main predisposition factor is a disorder in the water regime of a host plant. There is a marked water deficit in secondary spruce stands at lower altitudes and highlands (Málek 1966, Jankovský 1995).

The aim of the paper is to determine the species spectrum, distribution and phytopathological aspects of *Armillaria* spp. in forest ecosystems of southern Moravia represented by forest stands in the hills in the vicinity of Brno, thermophilic oak communities and floodplain forests in the alluvia of the Dyje and Morava rivers.

Fig. 1 The monitored areas. I: The Křtiny Training Forest Enterprise (TFE), II: The Bobravská pahorkatina hilly country, III: The park plantings and gardens in Brno, IV: The Lednice-Valtice area.

MATERIAL AND METHODS

Distribution, species and host spectrum were studied in 4 areas:

I. The Křtiny Training Forest Enterprise (TFE), represented by the southern edge of the Drahanská vrchovina Highlands. The TFE region is situated at the northern edge of Brno. Autochthonous oak/beech, beech and fir/beech stands in the 3rd-4th forest vegetation zones (oak/beech and beech zones) were replaced here by secondary spruce stands in the past. The studied area is situated on the border between southern and central Moravia in the region of the Drahanská vrchovina Highlands geomorphological unit formed by highlands on monotonous sediments of culm. The biota belong to the 3rd oak/beech to the 5th fir/beech forest vegetation zone and only along edges, thermophilic elements occur more frequently. Potential vegetation consists of beech communities of the *Luzula* type, in broken relief floriferous beech communities. Biodiversity is increased by contact of the Drahanská vrchovina Highlands with the north-Pannonian and Carpathian subprovince. Considerable remnants of beech and cultivated spruce stands occur on slopes. The natural flora is markedly affected by anthropogenic activities particularly by extensive plantings of exotic woody species. These are then potential hosts of fungal parasites including *Armillaria* sp. The altitude of the region ranges between 350 and 600 m.

II. The Bobravská pahorkatina hilly country (the Bobrava Uplands), NW, W and SW of Brno separates the Drahanská vrchovina Highlands from the central Bohemian-Moravian Highlands. Forest communities are formed there similarly as in the Křtiny TFE by oak/beech and beech stands which are markedly affected by human activities. Particularly at higher altitudes, natural communities have been replaced by spruce plantations.

III. Park plantings and gardens in part of the built-up area of Brno include generally secondary park plantings aged 40 to 250 years. They represent only a fragment of the autochthonous flora and the occurrence of *Armillaria* sp. was monitored above all on a number of exotic species there. Urban alley plantings and gardens are also included.

IV. The ecosystems of a floodplain forest and thermophilic oak communities of the Lednice-Valtice area, including forest ecosystems of the Dolnomoravský úval ravine. Floodplain forests are represented by soft-wooded and hard-wooded floodplain stands along the Dyje and Morava rivers. Křivé jezero National Nature Reserve (NNR) near Bulhary as well as Randšpurk NNR and Cahnov NNR in Soutok game preserve can serve as examples of the locality. Thermophilic oak communities are natural forest ecosystems on gravel alluvia in the Dyje river floodplain represented for example by the locality Rendezvous. *Quercus cerris,* among others, appears to be a stand-forming species.

The results are presented as the summary of records from 1988–1999.

Species identification was carried out primarily according to morphology of fruitbodies. In problematic cases, the identification was verified by intersterility tests. The occurrence of infection by *Armillaria* spp. was also indicated according to symptoms of infection such as the presence of rot, mycelial fans (syrrocia), rhizomorphs etc. Species identification was in some cases only based on the morphology of vegetative mycelium in vitro. The mycelium was cultivated on malt-extract agar (MEA 3). For the purpose of physiological tests, also other media were used, e.g. Sabouraud's agar, Czapek-Dox agar and also other organic substrates.

Armillaria spp. was considered to be a parasite when its fruitbodies or direct symptoms of infection were found on a living individual.

Isolated strains (44) and specimens (86) are deposited in the collection of cultures of the Department of Forest Protection and Game Management, MUAF Brno.

RESULTS

In the studied regions of southern Moravia, five annulate species of *Armillaria* and the exannulate species *A. socialis* (Tab. 1) were found. *Armillaria* spp. were determined in 79 woody species, 44 of which were broadleaved species and 33

Table 1. Summary of frequencies of *Armillaria* records according to their relation to a host (1989–1997).

Region		*A. borealis*		*A. cepistipes*		*A. ostoyae*		*A. mellea*		*A. gallica*		*A. socialis*		*Armillaria* sp.		*Armillaria* total	
		sapr.	par.	sapr.	par.	sapr.	par.	sapr.	par.	sapr.	par.	sapr.	par.	sapr.	par.	**sapr.**	**par.**
Drahany Highlands	conif.	4	1	6	–	763	144	–	–	23	3	–	–	63	88	**859**	**236**
	broadl.	3	1	20	2	40	21	1	2	64	23	–	–	–	46	**128**	**95**
	total	7	2	26	2	803	165	1	2	87	26	–	–	63	129	**981**	**326**
Bobrava Uplands	conif.	–	–	2	–	157	39	–	–	14	6	–	–	16	24	**189**	**69**
	broadl.	–	–	–	–	11	8	28	4	26	4	–	–	–	33	**65**	**49**
	total	–	–	2	–	168	47	28	4	40	10	–	–	16	57	**254**	**118**
Park and garden plantings in Brno	conif.	–	–	–	–	3	13	–	–	2	5	–	–	1	7	**6**	**25**
	broadl.	–	–	–	–	–	–	1	5	4	25	–	–	–	8	**5**	**38**
	total	–	–	–	–	3	13	1	5	6	30	–	–	1	15	**11**	**63**
Lednice-Valtice area	conif.	–	–	–	–	–	–	–	–	5	13	–	–	–	–	**5**	**13**
	broadl.	–	–	–	–	–	–	1	2	48	26	1	–	2	1	**52**	**29**
	total	–	–	–	–	–	–	1	2	53	30	1	–	2	1	**57**	**33**
Total for all localities	conif.	4	1	8	–	923	196	–	–	44	20	–	–	80	131	**1059**	**348**
	broadl.	3	1	20	2	51	29	31	13	142	78	1	–	2	88	**250**	**211**
	total	**7**	**2**	**28**	**2**	**974**	**225**	**31**	**13**	**186**	**98**	**1**	–	**82**	**207**	**1309**	**547**

coniferous species (Tab. 2). Besides of woody species, *Armillaria* spp. were observed on two species of herbs – *Fragaria* sp. and *Pelargonium* sp. As for quantity, most finds came from conifers (about 70 % collections), particularly *Picea abies* (55 %). The majority of collections of fruitbodies originated from stumps, dead wood or dead standing trees (70 %) where *Armillaria* decomposes wood as a saprophyte. This stage generally follows a parasitic stage in which the species colonises particular parts of a host. After its death, the fungus quickly colonises wood.

Except for *Armillaria mellea* and *A. socialis*, all species were observed both in broadleaves and conifers. None of the species showed either a saprophyte or parasite character (Tab. 1). Only *Armillaria ostoyae* can be regarded a predominant parasite in secondary spruce plantations. The same observation is valid for *A. mellea* in the warmest regions. *Armillaria gallica* and *A. borealis* are mainly saprophytes and occasionally function as necrotrophic parasites. *Armillaria cepistipes* and *A. socialis* are mentioned as saprophytes. However, *A. cepistipes* was observed on root systems of dying trees in the same areas with beech decline as in the Bílé Karpaty Mts. In none of the species, it is possible to speak about an exclusive saprophyte or parasite.

Table 2. Survey of number of *Armillaria* hosts observed in southern Moravia

		Armillaria borealis	*Armillaria cepistipes*	*Armillaria ostoyae*	*Armillaria mellea*	*Armillaria gallica*	*Armillaria socialis*	*Armillaria total*
Number of hosts	Conifers	2	2	28	0	13	0	33
	Broadleaves and shrubs	2	4	8	9	37	1	44
	Herbs	–	–	–	–	–	–	2
	Total	4	6	36	9	50	1	79

Infection is primarily dependent on the predisposition of a host. Pathogenicity of particular species or virulence of strains are only secondary. The lower proportion of parasitism in *A. gallica* is caused by the fact that its ecological optimum occurs on wet sites of floodplain forests and on the bottoms of valleys along water courses. Generally, autochthonous stands are tolerant to infection by *Armillaria*. On the other hand, there exist plantings of some conifers in the Lednice park in SE Moravia which are heavily damaged by *Armillaria*. *Armillaria ostoyae* shows its ecological optimum in the nutrient-rich series of lower and medium altitudes (forest type group *Querceto-Fagetum*), i.e. in regions where the original species composition (*Fagus sylvatica*, *Quercus petraea*, *Abies alba* etc.) has been radically changed in favour of more vulnerable spruce plantations.

Survey of the host spectrum of Armillaria in Southern Moravia

Coniferous hosts of *Armillaria* species (records on the basis of vegetative traits without species identification are also included)

Abies alba, A. concolor, A. grandis, A. nordmanniana, Cryptomeria japonica, Chamaecyparis lawsoniana, Chamaecyparis sp., *Juniperus virginiana, J. chinensis, J. communis, Larix decidua, Picea abies, Picea glauca, P. mariana, P. omorica, P. orientalis, P. pungens, Pinus aristata, P. banksiana, P. koraiensis, P. contorta, P. monticola, P. nigra, P. mugo, P. flexilis, P. rotundata, P. strobus, P. sylvestris, P. wallichiana, Pseudotsuga menziesii, Taxus baccata, Thuja plicata, Thuja occidentalis, Tsuga* sp.

Broadleaved hosts *Armillaria* species

Acer campestre, A. platanoides, A. pseudoplatanus, A. saccharinum, Aesculus hippocastanum, Alnus glutinosa, Alnus incana, Armeniaca vulgaris, Betula sp., Carpinus betulus, Castanea sativa, Cerasus avium, Cornus mas, Corylus avellana, C. colurna, Fagus sylvatica, Fraxinus excelsior, F. angustifolia, Gleditschia triacanthos, Gymnocladus dioica, Juglans nigra, J. regia, Malus domestica, Padus serotina, Persica vulgaris, Populus sp., *Prunus domestica, Pyrus communis,* *P. domestica, Quercus cerris, Q. petraea, Q. pubescens, Q. robur, Q. rubra, Q. virgiliana, Robinia pseudacacia, Rosa* sp., *Sambucus nigra, S. racemosa, Salix* sp., *Sorbus aucuparia, S. torminalis, Syringa vulgaris, Tilia* sp., *Ulmus* sp., *Vitis vinifera.*

Herbs as hosts of *Armillaria* species

Fragaria vesca, Pelargonium sp.

Hosts of *Armillaria borealis*

Picea abies, Pinus sylvestris, Betula sp., *Fagus sylvatica.*

Hosts of *Armillaria cepistipes*

Abies alba, Picea abies, Alnus glutinosa, A. incana, Betula sp., *Fagus sylvatica.*

Hosts of *Armillaria ostoyae*

Conifers: *Abies alba, A. grandis, A. concolor, A. nordmanniana, Cryptomeria japonica, Chamaecyparis lawsoniana, Juniperus chinensis, J. communis, Picea abies, P. glauca, P. mariana, P. omorica, P. pungens, Pinus contorta, P. flexilis, P. koraiensis, P. monticola, P. banksiana, P. nigra, P. mugo, P. aristata, P. rotundata, P. strobus, P. sylvestris, P. wallichiana, Pseudotsuga menziesii, Thuja plicata, T. occidentalis.*

Broadleaves: *Betula* sp., *Carpinus betulus, Fagus sylvatica, Castanea sativa, Quercus petraea, Q. robur, Q. rubra, Sorbus torminalis.*

Hosts of *Armillaria mellea*

Acer platanoides, Armeniaca vulgaris, Cerasus avium, Fraxinus excelsior, Juglans regia, Malus domestica, Persica vulgaris, Quercus cerris, Quercus petraea.

Hosts of *Armillaria gallica*

Conifers: *Abies alba, Juniperus virginiana, J. chinensis, J. communis, Larix decidua, Picea abies, Picea omorica, Picea orientalis, Picea pungens, Pinus contorta, Pinus sylvestris, Pseudotsuga menziesii, Thuja plicata.*

Broadleaves and shrubs: *Acer campestre, A. platanoides, A. pseudoplatanus, A. saccharinum, Aesculus hippocastanum, Alnus glutinosa, Armeniaca vulgaris, Betula* sp., *Carpinus betulus, Cerasus avium, Cornus mas, Corylus* *avellana, C. colurna, Fagus sylvatica, Fraxinus excelsior, F. angustifolia, Gleditschia triacanthos, Malus domestica, Padus serotina, Pelargonium* sp., *Populus* sp., *Prunus domestica, Quercus cerris, Q. petraea, Q. pubescens, Q. robur, Q. rubra, Q. virgiliana, Robinia pseudoacacia, Rosa* sp., *Salix* sp., *Sambucus nigra, S. racemosa, Sorbus aucuparia, Syringa vulgaris, Tilia* sp., *Ulmus* sp.

Distribution and pathology of Armillaria species

Secondary spruce stands growing on *Querceto-Fagetum* nutrient-rich forest sites in the Drahanská vrchovina Highlands, represented especially by the Křtiny TFE, are seriously damaged by *Armillaria ostoyae*. The main reason is a precipitation deficit: the total amount of annual precipitation is only about 600 mm and above all summer droughts act as an initiational stressor (Manion 1991). It occurs as an opportunist parasite in declining firs, oaks and other autochthonous tree species there. *Armillaria* species participate significantly in the decomposition of stumps and dead roots. *Armillaria gallica* is predominantly a saprophyte of decomposed wood in moist habitats of the alluvia of streams and slope bases. As a parasite, it occurs occasionally both in broadleaves and conifers. *Armillaria cepistipes* and *A. borealis* are relatively rare species being of no major economic importance. *Armillaria mellea* was found in *Fraxinus excelsior* and on stumps of *Acer platanoides* in the lowest part (alt. 280 m) of the Křtiny TFE on the border of Brno. In this area, *Armillaria* species were found in 43 host species, twenty of which are coniferous species.

In predominatly broadleaved oak/beech stands of the Brno Uplands, *Armillaria* spp. occasionally parasitise in coppices. The following species were noticed: *Armillaria ostoyae*, *A. mellea* and *A. gallica*. In secondary spruce stands, the situation is similar as in the Drahanská vrchovina Highlands, where the stands are stressed by low altitude. Moreover, a number of the stands were established on loess. *Armillaria mellea* occurs in focuses and causes locally also damages to pole stands of *Quercus petraea*. *Armillaria ostoyae* parasitise in oak trees only occasionally at higher altitudes (alt. 300–450 m). In the region, *Armillaria* species were found in 12 host species, six of which are coniferous species.

In park plantings and gardens in Brno, *Armillaria* spp. are quite common. Fruit trees are particularly damaged by *A. mellea* but also by *A. gallica*, which is commonly distributed in Brno parks in a number of broadleaves and exotic conifers. *Armillaria ostoyae* occurs only at higher situated border parts in conifers, broadleaves and fruit trees. In addition to fruit trees *A. mellea* was recorded in *Quercus robur* and *Quercus petraea* only. The occurrence of mycelial fans in grapevine (*Vitis vinifera*) is also of interest. From 30 host species 13 are conifers.

The prevailing *Armillaria* species of floodplain forests and thermophilic oak stands in the Lednice-Valtice area including the Lednice park is *A. gallica*. In the

Lednice park, it infects also conifers predisposed to wilting in the period of summer droughts. It was found there in *Abies alba, Picea abies, P. orientalis, P. pungens, Taxus baccata*, *Thuja plicata* and other species. *Armillaria mellea* occurs as an opportunist parasite in oak (*Quercus petraea*, *Q. robur*, *Q. cerris*). Other species of *Armillaria* were not found. *Armillaria* spp. were identified in 10 broadleaved species and 5 coniferous species.

Notes to the ecology of particular species of Armillaria in the region

Armillaria gallica occurs as the only *Armillaria* species in all regions under study. It is an absolutely dominant species in floodplain forest ecosystems and in lowland oak communities of the Lednice-Valtice area. As a saprophyte, it is common particularly on broadleaves of lower-altitude regions. In impaired individuals, however, it changes into a parasite. In conifers, it parasitise in park plantings in the Lednice park and also in alluvia along streams of the Drahanská vrchovina Highlands. Symptoms of damage to conifers are similar as in *A. ostoyae*, however, long strand-like rhizomorphs form abundantly under bark and in soil. In broadleaves, it is the causal agent of closed and open stem cavities, where it moves up to a height of 3–6 m. It was detected in 30 broadleaved species. The spectrum of hosts of the fungus was the largest of all *Armillaria* species (50 host species.). A record on *Syringa vulgaris* and *Pelargonium* sp. is also of interest.

Armillaria mellea is a thermophilic species which occurs in the region of southern Moravia on various species of broadleaves. Right in the Brno agglomeration, it is a frequent parasite on fruit trees in gardens (e.g. *Cerasus avium, Armeniaca vulgaris, Persica vulgaris, Juglans regia, Malus domestica, Pyrus communis*). It was detected in 10 broadleaved species. Its occurrence is also mentioned on *Thuja* sp. in Brno gardens (Antonín 1988). This corresponds to the ecology of the fungus in southern Europe (Guillaumin et al. 1985).

Armillaria ostoyae is the most important species of *Armillaria* causing considerable economic damage in secondary spruce stands. It absolutely predominates particularly in upland regions and, on the contrary, at the lowest altitudes it was not found. The majority of records originate from spruce (55 %). In total, it was identified in 36 tree species, 3 of which are broadleaved species. In addition to native species, it was noticed in most of exotic conifers in glades and arboreta in the region of the Křtiny TFE. It is of interest that the ecological optimum of *A. ostoyae* is in many respects quite identical with the ecological optimum of silver fir (*Abies alba*). Also its southern boundary distribution in Europe corresponds to the range of silver fir (Greece, SW France, Corsica, the Apennines). In remnants of natural stands in the Křtiny TFE, *A. ostoyae* is primarily a saprophyte. It goes over to parasitism in silver fir (*Abies alba*) and oak (*Quercus petraea*). However, it never causes such damage as in spruce, and infection by *Armillaria* is limited only to peripheral parts of the root system. The cause of infection of living trees is again their predisposition due to drought stress (particularly summer droughts). Oak trees are infected mainly at higher elevations (about 400–500 m alt.) where they grow on the margin of the ecological optimum.

Armillaria cepistipes was detected particularly as a saprophyte on buried wood of broadleaves and more rarely also conifers. It is rather problematic to distinguish this species from *A. gallica*. A simple identification according to the morphology of fruitbodies and cultures is not reliable. To a certain extent, it differs ecologically because *A. cepistipes* is distributed and replaces *A. gallica* at higher altitudes and in the north. Fruitbodies corresponding to *A. cepistipes* f. *pseudobulbosa* Romagn. et H. Marxmüller (1983) were also found on damaged stems of living alders (Křtiny TFE, Forest District Řečkovice). *A. cepistipes* was noticed on roots of declining or dead beech trees at the localities of Radějov (White Carpathians), Tetčice (Bobrava Upland) and the Drahanská vrchovina Highlands. In this case, identification was verified by PCR tests. The status of the species in the conditions of the Czech Republic requires further research. *Armillaria cepistipes* appears to be more abundant than previously expected. It is more frequent particularly in beech stands at medium altitudes.

Armillaria borealis is probably a less frequent *Armillaria* species in the Czech Republic so far. In the vicinity of Brno, it was noticed in pine, birch and also spruce. Similarly as *A. ostoyae*, it can be a causal agent of root rots of spruce. Unlike that species, *A. borealis* pervades into sapwood soon after the host's death, forming fruitbodies on the stem at a height of 2–5 m. The formation of rhizomorphs is not abundant as compared with *A. gallica* and *A. cepistipes*. The fungus was detected only in the Drahanská vrchovina Highlands.

Armillaria socialis was found only on stumps of oak trees and on bases of dead standing trees of *Quercus robur* in the floodplain of the Lanžhot virgin forest and National Nature Reserve (NNR) Cahnov. It occurs also in the Ranšpurk NNR and in the Křivé jezero NNR (Antonín, personal communication). It is a markedly thermophilic species.

DISCUSSION

Although *Armillaria* species are intensively studied in many aspects, more detailed data on the distribution of particular species in the Czech Republic have not been published so far. Černý (1989) mentioned a differentiation of species based on their ecological requirements. The occurrence and ecology of *Armillaria* species in the region of southern Moravia generally correspond to these characteristics.

As for European species, the broadest spectrum of hosts is given for *Armillaria mellea*. Based on data from France, England and Italy, Guillaumin et al. (1993) mention 142 host species from 30 families. The list includes also introduced species. An absolute majority is formed by broadleaved trees and shrubs, as for conifers 14 representatives of *Pinaceae* are mentioned, 2 representatives of *Taxodiaceae* (*Cryptomeria japonica*, *Sequiadendron giganteum*) and 10 representatives of *Cupressaceae*. It is also of interest that some monocotyledonous plants are mentioned as hosts of *Armillaria mellea*, e.g. *Arundo donax*, *Strelitzia reginae*, *Musa chinensis*. *Armillaria socialis* is mentioned from 12 host species of 4 families, however, not even in one case a find from conifers is mentioned. *Armillaria ostoyae* was found in 38 plant species of 9 families, 21 of which are coniferous species (*Pinaceae* and *Cupressaceae* families). *Armillaria gallica* was observed on 40 host species of 14 families, 10 of which were coniferous species (*Pinaceae* and *Cupressaceae* families). *Armillaria borealis* is given from two hosts only and *Armillaria cepistipes* from *Tilia platyphyllos* only.

From the Czech Republic, Lazebníček (1973) mentions 26 host species of 23 genera on which fruitbodies of *Armillaria mellea* s. l. were recorded. The greatest proportion belongs to *Picea abies*, followed by *Fagus sylvatica*, *Quercus* sp. div., *Abies alba*, *Carpinus betulus*, *Betula* sp. div. and *Alnus* sp. div. In living hosts, fruitbodies were found only in 5 % of finds. As for fruit trees, only apple (*Malus domestica*) and cherry (*Cerasus avium*) are mentioned.

Antonín (1988) mentions 15 host species (14 broadleaves, 1 conifer – *Thuja* sp.) of *Armillaria mellea* from the Czech Republic.

A great number of host species also comes from agricultural crops, particularly various fruit trees and grapevine. Damage caused by *Armillaria* species are of significant economic importance particularly to stone fruits like almonds (*Amygdalus communis*), plums (*Prunus domestica*), cherries (*Cerasus avium*), apricots (*Armeniaca vulgaris*) and grapevine (*Vitis vinifera*) in the southern part of Europe (Guillaumin 1985). In the tropics, *Armillaria* species parasitise even on banana trees (*Musa* sp.), cacao trees (*Theobroma cacao*) and rubber trees (*Hevea brasilensis*).

At present, a significant increase in *Armillaria* spp. as a mortality stressor can be observed in some localities of the Czech Republic. The fungus reacts primarily to an increased predisposition of spruce due to climatic extremes (Holuša and Liška 2002). *Armillaria* spp. can occur as an important biotic agent destabilising spruce stands under the impact of climatic changes (Jankovský et al. 2003, Jankovský 2003).

CONCLUSIONS

The diversity of ecological conditions of forests in southern Moravia is also confirmed by observation of the complete species spectrum of annulate *Armillaria* species. The occurrence of *A. mellea* and particularly the exannulate *A. socialis* represents the northern boundary of their distribution in the region. The vicinity of Brno is also important for its plantings of exotic species in neighbouring arboreta and urban parks. A number of them was quite commonly attacked by *Armillaria* spp.

In total, 5 species of annulate *Armillaria* and the exannulate *A. socialis* were found. *Armillaria ostoyae* shows its ecological optimum in the forest type group *Querceto-Fagetum* where it is an important parasite of spruce. It also attacks *Abies alba*, *Quercus petraea* and other species. *Armillaria gallica* is a dominant species of floodplain forests and thermophilic oak communities. On the contrary, *A. ostoyae* was almost missing there. *Armillaria mellea* occurs on broadleaves and fruit trees. *Armillaria cepistipes* and *A. borealis* were found only in the Křtiny TFE. *Armillaria socialis* occurs rarely on stumps and bases of dead oak trees in a floodplain forest along the Dyje river (NNR Cahnov, Ranšpurk and Křivé jezero). It is one of its northernmost localities. Such a complete *Armillaria* species spectrum in the relatively small area of southern Moravia particularly in the Drahanská vrchovina Highlands is not common in Europe.

The main role of *Armillaria* spp. consists in the decomposition of wood in soil such as stumps and roots. In case of any physiological weakening of host species, *Armillaria* spp. begin to fulfil this role already on living trees such as in the case of spruce which is grown under conditions at the margin of its ecological optimum.

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New, rare and less known macromycetes in Slovakia I

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Ripková S. and Hagara L. (2003): New, rare and less known macromycetes in Slovakia I. – Czech Mycol. 55: 187–200

Data on ecology, Slovak and European occurrence and endangerment of six new, rare and/or less known macromycetes collected in Slovakia are given. *Phlebia ryvardenii* was found for the first time in Slovakia, *Hyphodontia latitans* was collected for the first time after more than one hundred years and a new locality of *Spongipellis fractipes* is reported, too. In addition, new localities and ecological data on *Hypoxylon ticinense*, *Pluteus aurantiorugosus* and *Rhodotus palmatus* are presented.

Key words: *Hyphodontia latitans*, *Hypoxylon ticinense*, *Phlebia ryvardenii*, *Pluteus aurantiorugosus*, *Rhodotus palmatus*, *Spongipellis fractipes*, occurrence, ecology.

Ripková S. a Hagara L. (2003): Nové, zriedkavé a málo známe makromycéty na Slovensku I. – Czech Mycol. 55: 187–200

Na Slovensku sme zaznamenali šesť nových, vzácnych a/alebo menej známych makromycétov. Uvádzame nové poznatky o ich ekológii, výskyte na Slovensku a v Európe, tiež údaje o ich vzácnosti a ohrozenosti. *Phlebia ryvardenii* sme našli prvýkrát na území Slovenska, *Hyphodontia latitans* po viac ako sto rokoch a *Spongipellis fractipes* na novej (dosiaľ druhej) lokalite. Pre *Hypoxylon ticinense*, *Pluteus aurantiorugosus* a *Rhodotus palmatus* sme doplnili nové lokality a ekologické údaje.

INTRODUCTION

From Slovakia, 2609 taxa of macromycetes are reported (Adamčík et al. 2003), and more than 300 taxa are included in the Red list of Slovak fungi (Lizoň 2001). Škubla (1989) estimated that 5300 taxa of macromycetes should occur in Slovakia. This means that more than 50 % of them need to be "discovered". Most mycologists would like to collect and present new, rare and less known taxa, learn more about their taxonomy, biology, ecology and distribution. Our contribution is also focused on such taxa. On the other hand, we must keep in mind that there is urgent need to collect data on so-called common macromycetes as well. For complex knowledge and better understanding of the regional mycoflora both rare and common taxa have to be studied.

MATERIAL AND METHODS

The presented data on ecology are mostly based on the second author's herbarium specimens (the abbreviation of L. Hagara's herbarium is LH), specimens from SLO [the first author's (née Jančovičová) specimens], BRA, PRM and private herbaria. The abbreviations of herbaria are cited in accordance with the Index Herbariorum (Holmgren et al. 1990). Data on specimens are updated and explanatory notes are given in brackets. Names of phytogeographical units of Slovakia are according to Futák (1966). Data on endangerment in European countries are given according to the following sources: Austria (Krisai-Greilhuber 1999), Czech Republic (Antonín and Bieberová 1995), Denmark (Vesterholt 1998), Germany (Benkert 1992), Hungary (Siller and Vasas 1993), Netherlands (Arnolds 1989), Norway (Bendiksen and Høiland 1992), Poland (Wojewoda and Ławrynowicz 1986), Slovakia (Lizoň 2001), Slovenia (Anonymus 1994), Sweden (Gärdenfors 2000), Switzerland (Senn-Irlet et al. 1998).

RESULTS AND DISCUSSION

Hyphodontia latitans (Bourdot et Galzin) Ginns et Lefebvre

Descriptions and/or illustrations: Eriksson and Ryvarden (1976), Ryvarden and Gilbertson (1993), Langer (1994), Vampola and Vágner (1995).

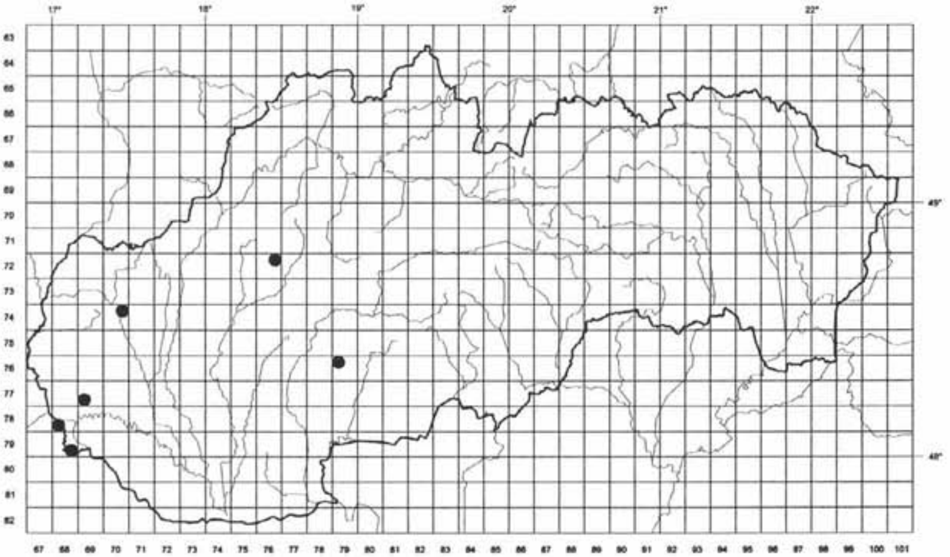
Ecology: *Hyphodontia latitans* is a saprophyte producing one-year-old fruitbodies on stumps and trunks of coniferous and deciduous trees, probably from July to November.

In Slovakia, Kmeť collected *Hyphodontia latitans* for the first time in 1889. He found the species on *Acer* sp. in July (Kotlaba 1984). After more than one hundred years Kotlaba found the species on a fallen trunk of *Fagus sylvatica* in October (specimen by Kotlaba from 1988 deposited in PRM). We have collected the species at three other localities on fallen decaying trunks of *Alnus glutinosa*, *Negundo aceroides* and *Salix fragilis* in September and November.

Other known hosts of the species in Europe are *Picea abies* and *Pinus* sp. (Kotlaba 1984, Vampola and Vágner 1995).

Notes: Eriksson and Ryvarden (1976) had no doubts about relationships between the genera *Chaetoporellus* Bondartsev et Singer and *Hyphodontia* J. Erikss. However, as the species of these genera differ in spore shape, they kept *Chaetoporellus* as a genus of its own. Other authors, e.g. Domański (1974), Kotlaba (1984) and Ryvarden and Gilbertson (1993), classified the species into the genus *Chaetoporellus* too.

We have accepted the taxonomic concept by Ginns and Lefebvre (1993) who assigned the species to the genus *Hyphodontia*. Langer (1994) transferred the



Map 1. Occurrence of *Hyphodontia latitans* in Slovakia.

species to the genus *Hyphodontia* independent of Ginns and Lefebvre (1993), but his combination is superfluous.

According to Langer (1994), *Hyphodontia latitans* was found by Kmeť in Germany ("Sachsen, Chemnitz") on 4 July 1889, but according to Kotlaba (1984), Kmeť found the species in the Štiavnické vrchy Mts. in Slovakia on the same day. The similarity of the names of the towns of Schemnitz (old name of the town of Banská Štiavnica in Slovakia) and Chemnitz (town in Saxony) probably caused this mistake. On Kmeť's herbarium labels is printed out "Fungi Schemnitzienses". However, Langer (1994) made Chemnitz from Schemnitzienses and also incorrectly added Sachsen (Saxony). Kmeť did not use the term of Štiavnické vrchy; only Kotlaba (1984) used it.

Occurrence in Slovakia: six localities: three in the Podunajská nížina Lowland (3 specimens), one locality in the Malé Karpaty Mts. (1 specimen), one locality in the Štiavnické vrchy Mts. (1 specimen) and one locality in the Strážovské and Súľovské vrchy Mts. (Map 1).

Occurrence in Europe: Czech Republic, England, France, Poland, Slovakia and Yugoslavia (Kotlaba 1984, Vampola and Vágner 1995).

Endangerment: *Hyphodontia latitans* is listed in the Red list of fungi in Poland and Slovakia.

Material studied: Podunajská nížina Lowland: 7868c: the city of Bratislava, the municipal part of Karlova Ves, Sihoť Island, flood plain forest, 138 m a. s. l., on wood of fallen decaying trunk of *Negundo aceroides*, 23 Sept. 1997, leg. S. Jančovičová (SLO). – 7968d: the city of Bratislava, the municipal part of Rusovce, flood plain forest, 130 m a. s. l., on wood of fallen trunk cf. *Ulmus laevis*, 1 May 2002, leg. L. Hagara (LH). – 7769c: the National Nature Reserve of Šúr, the village of Svätý Jur, 130 m a. s. l., on wood of fallen rotten trunk of *Alnus glutinosa*, 21 Nov. 2002, leg. L. Hagara (LH). – Malé Karpaty Mts.: 7470b: the village of Buková, the isolated settlement of "Nespalovci", 240 m a. s. l., on fallen trunk of *Salix fragilis*, 8 Nov. 1997, leg. L. Hagara (LH). – Strážovské and Súľovské vrchy Mts.: 7276b: the village of Uhrovské Podhradie, Rokoš hill, in valley above the village, on fallen trunk of *Fagus sylvatica*, 18 Oct. 1988, leg. F. Kotlaba (PRM).

Literary data: Štiavnické vrchy Mts.: 7679a: the village of Prenčov, the locality of "Čierne blato", ca. 580 m a. s. l., on *Acer* sp., 4 July 1889, leg. A. Kmeť (Kotlaba 1984).

Hypoxylon ticinense L. E. Petrini

Descriptions and/or illustrations: Petrini and Müller (1986), Petrini-Klieber (1985), Cetto (1993), Ju and Rogers (1996), Leroy and Surault (1999).

Ecology: *Hypoxylon ticinense* is a saprophyte producing fruitbodies on branches and trunks of deciduous trees, often damaged by fire, usually from June to December. It seems that the species prefers flood plain forests.

We have found the species in Slovakia on wood and bark of fallen decaying branches and trunks of *Acer* sp., *Crataegus oxyacantha*, *Fraxinus* sp., *Negundo aceroides*, *Padus avium* and *Swida sanguinea* from June to October. Some of these specimens were presented by Jančovičová and Glejdura (1999).

Numerous specimens of *H. ticinense* from Slovakia, collected by Pouzar on *Fraxinus excelsior*, *Populus alba*, *Salix* sp. and *Swida sanguinea*, are deposited in PRM.

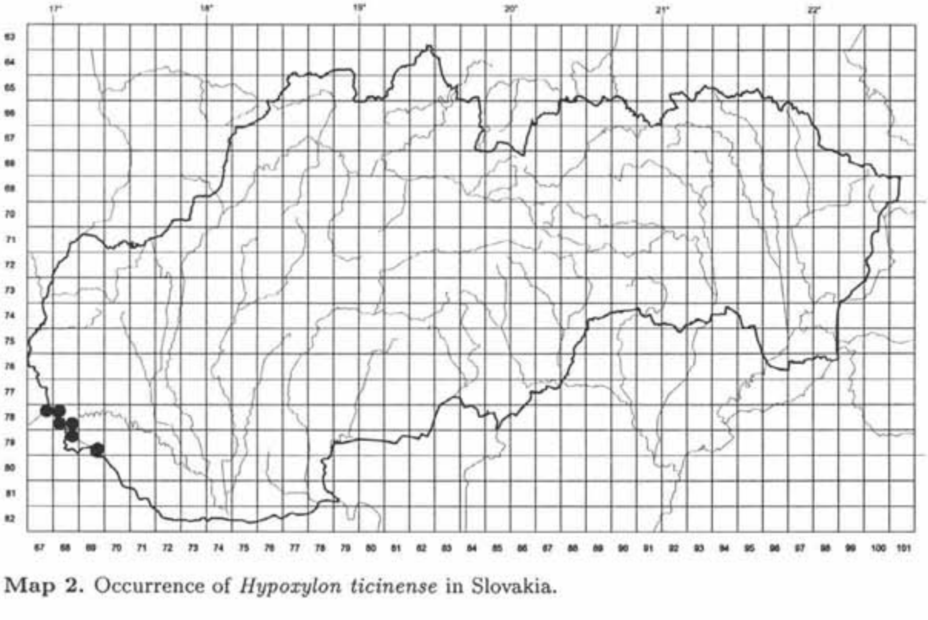
The species has been collected on fallen branches of *Fraxinus* sp. in Austria (Krisai-Greilhuber 1992), on *Crataegus* sp. in Croatia (specimen by Tortić from 1971 deposited in PRM), on bark of *Fraxinus excelsior*, on *Alnus* sp. and *Sambucus nigra* in France (Petrini-Klieber 1985, Ju and Rogers 1996, Leroy and Surault 1999), on *Crataegus* sp. in Italia (Cetto 1993), and on bark of *Crataegus oxyacantha* in Switzerland (Petrini-Klieber 1985, Ju and Rogers 1996).

Occurrence in Slovakia: six localities in the Podunajská nížina Lowland (33 specimens; Map 2).

Occurrence in Europe: Austria (Krisai-Greilhuber 1992), Croatia (specimen by Tortić from 1971 deposited in PRM), France (Petrini-Klieber 1985, Ju and Rogers 1996), Italia (Cetto 1993), Slovakia and Switzerland (Petrini-Klieber 1985, Ju and Rogers 1996).

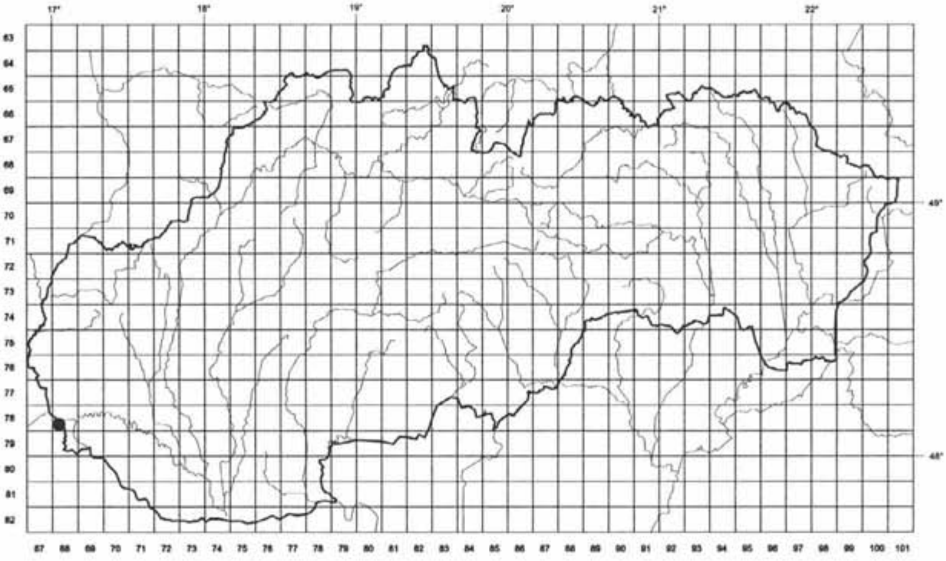
Endangerment: *Hypoxylon ticinense* is listed in the Red list of fungi in Austria and Slovakia.

Material studied: Podunajská nížina Lowland: 7867b: the city of Bratislava, the municipal part of Devín, Sedláčkov ostrov Island, flood plain forest, ca. 135 m a. s. l., on wood of fallen decaying trunk of *Swida sanguinea*, 26 June 1997, leg. S. Jančovičová (SLO). – Ibidem, on wood of fallen decaying trunk of *Crataegus oxyacantha*, 2 July 1997 (SLO). – Ibidem, on bark of fallen



Map 2. Occurrence of *Hypoxylon ticinense* in Slovakia.

decaying branch of *Fraxinus* sp., 8 Jan. 1998 (SLO). – Ibidem, on wood of fallen decaying trunk of deciduous tree, 20 July 1998 (SLO). – Ibidem, on wood and bark of fallen decaying branch of *Fraxinus* sp., 16 July 1999 (SLO). – Ibidem, on bark of fallen trunk of *Crataegus oxyacantha*, 4 July 1999, leg. L. Hagara (LH). – 7868a: the city of Bratislava, the municipal part of Karlova Ves, Sihoť Island, flood plain forest, ca. 135 m a. s. l., on wood of fallen decaying branch of *Padus avium*, 29 July 1998, leg. S. Jančovičová (SLO). – Ibidem, on wood of fallen decaying branch of *Fraxinus* sp., 16 Oct. 1998 (SLO). – 7868c: the city of Bratislava, the municipal part of Karlova Ves, Sihoť Island, flood plain forest, ca. 135 m a. s. l., on wood of fallen decaying trunk of *Negundo aceroides*, 23 June 1998, leg. S. Jančovičová (SLO). – Ibidem, on bark of branch of standing tree of *Padus avium*, 24 Sept. 1998 (SLO). – Ibidem, on wood of fallen decaying branch of *Fraxinus* sp., 24 Aug. 1999 (SLO). – Ibidem, on wood of fallen decaying branch of deciduous tree, 24 Aug. 1999 (SLO). – 7868c (7868a, lack of detailed information does not allow an accurate localisation): the city of Bratislava, the municipal part of Karlova Ves, Veľký ostrov Island (Sihoť Island), on fallen trunk of *Swida sanguinea*, 22 Oct. 1979, leg. Z. Pouzar (PRM). – Ibidem, on fallen branch of *Swida sanguinea* (PRM). – Ibidem, on fallen trunk of *Fraxinus excelsior* (PRM). – Ibidem, on fallen trunk of *Fraxinus excelsior* (PRM). – Ibidem, on fallen branch of decidous tree cf. *Fraxinus* sp. (PRM). – 7968b: the city of Bratislava, the municipal part of Rusovce, the island near the port, on fallen trunk of *Swida sanguinea*, 21 Oct. 1979, leg. Z. Pouzar (PRM, 5 specimens). – Ibidem, on fallen trunk of *Salix* sp. (PRM). – 7968b: the city of Bratislava, the municipal part of Rusovce, flood plain forest near gamekeeper's cottage, 130 m a. s. l., on fallen branch of *Acer* sp., 16 July 1995, leg. L. Hagara (LH). – 7968b (7969a, lack of detailed information does not allow an accurate localization): the city of Bratislava, the municipal part of Podunajské Biskupice, the Nature Reserve of Ostrov Kopáč, on fallen trunk of *Swida sanguinea*, 12 Oct. 1979, leg. P. Lizoň (PRM). – Ibidem, 27 Aug. 1974, leg. Z. Pouzar (PRM). – Ibidem, on dry trunk of *Swida sanguinea*, 27 Aug. 1974, leg. Z. Pouzar (PRM). – 7868d: the city of Bratislava, the municipal part of Petržalka, the island near the racecourse, on fallen trunks and branches of *Swida sanguinea*, 20 Oct. 1979, leg. Z. Pouzar (PRM). – Ibidem, on half-fallen trunk of *Swida sanguinea* (PRM, 4 specimens). – 7969d: the village of Šamorín, the



Map 3. Occurrence of *Phlebia ryvardenii* in Slovakia.

Kalinkovské rameno branch, in the forest by the riverside, on fallen trunk of deciduous tree cf. *Populus alba*, 12 Oct. 1979, leg. Z. Pouzar (PRM). – Switzerland: Kt. Tessin, Locarno, Boscone di Moleno, on *Crataegus oxycantha*, Dec. 1983, leg. Zenone (Isotypus, PRM). – Croatia, Čička Poljana, S of Zagreb, on *Crataegus* sp., 10 Oct. 1971, leg. M. Tortić (PRM).

Phlebia ryvardenii Hallenb. et Hjortstam

Description and illustration: Hallenberg and Hjortstam (1988).

Ecology: We have found the species on wood of a fallen rotten trunk of *Salix* cf. *alba* in May.

Hallenberg and Hjortstam (1988) described the species on the basis of a specimen from Sweden producing fruitbodies on *Picea abies* in September. The second known specimen of *Phlebia ryvardenii* is from Spain, found on *Pinus* sp. in November (Hallenberg and Hjortstam 1988).

Occurrence in Slovakia: *Phlebia ryvardenii* is new for Slovakia, known only from one locality in the Podunajská nížina Lowland (1 specimen; Map 3).

Occurrence in Europe: Slovakia, Spain and Sweden (Hallenberg and Hjortstam 1988).

Endangerment: *Phlebia ryvardenii* is listed in the Red list of fungi in Slovakia.

Material studied: Podunajská nížina Lowland: 7868c: the city of Bratislava, the municipal part of Karlova Ves, Sihoť Island, flood plain forest, 136 m a. s. l., on wood of fallen rotten trunk of *Salix* cf. *alba*, 21 May 1998, leg. S. Jančovičová (SLO).

Pluteus aurantiorugosus (Trog) Sacc.

Descriptions and/or illustrations: Antonín et al. (1995), Wilhelm (1992), Vellinga (1990), Hagara et al. (1999).

Ecology: in Slovakia, *Pluteus aurantiorugosus* is known as a saprophyte producing fruitbodies on wood of fallen decaying to rotten trunks, branches and stumps of *Aesculus hippocastanum*, *Fagus sylvatica*, *Fraxinus* sp., *Quercus cerris*, *Quercus* sp. and *Ulmus* sp. from June to October.

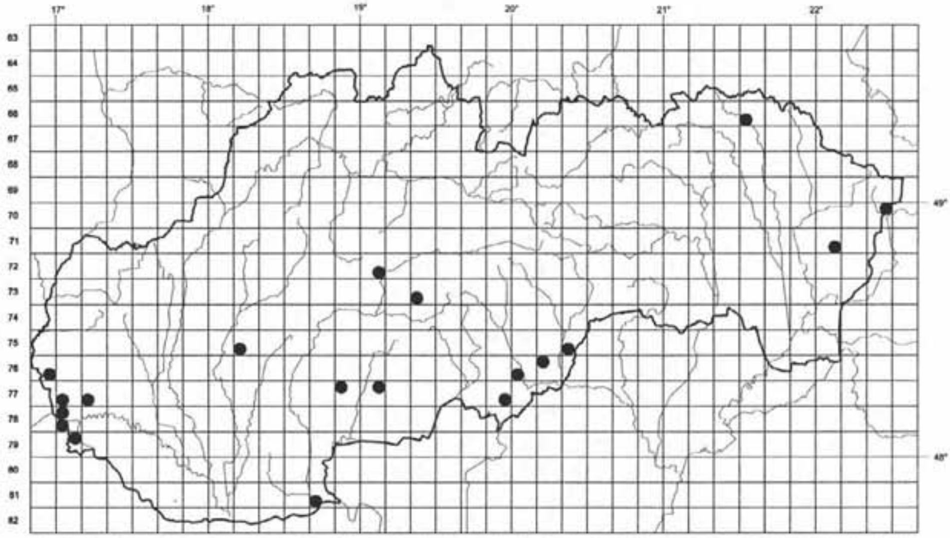
Other known hosts of the species in Europe are *Acer pseudoplatanus*, *Alnus* sp., *Carpinus betulus*, *Populus* sp., *Quercus petraea*, *Salix* sp., *Tilia cordata* and *Ulmus minor* (Antonín et al. 1995, Wilhelm 1992).

Occurrence in Slovakia: twenty-two localities: four in the Ipeľsko-rimavská brázda Furrow (4 specimens), one locality in the Záhorská nížina Lowland (1 specimen), six localities in the Podunajská nížina Lowland (8 specimens), one locality in the Malé Karpaty Mts. (1 specimen), one locality in the Tríbeč Mts. (1 specimen), one locality in the Kremnické vrchy Mts. (1 specimen), one locality in the Poľana Mts. (1 specimen), two localities in the Štiavnické vrchy Mts. (3 specimens), one locality in the Vihorlatské vrchy Mts. (1 specimen), one locality in the Nízke Beskydy Mts. (1 specimen) and one locality in the Bukovské vrchy Mts. (1 specimen; Map 4).

Occurrence in Europe: Austria, Czech Republic, Denmark, France, Germany, Great Britain, Hungary, Netherlands, Poland, Russia, Slovakia, Sweden and Switzerland (Antonín et al. 1995, Senn-Irlet et al. 1998).

Endangerment: *Pluteus aurantiorugosus* is classified as an endangered fungus in Austria, the Czech Republic, Denmark, Germany, the Netherlands, Poland, Slovakia, Sweden and Switzerland.

Material studied: Ipeľsko-rimavská brázda Furrow: 7687a: ca. 2.7 km NNE of the village of Figa, the locality of Farská studňa, in the pheasantry of "Stránska", 230 m a. s. l., on decaying stump of *Quercus cerris*, 27 June 1982, leg. L. Hagara (BRA). – 7588c: the town of Tornaľa, ca. 3.6 km E of the part of Starňa, in the management-plan area of "Hubovo", 280 m a. s. l., on decaying stump of *Quercus cerris*, 1 July 1982, leg. L. Hagara (BRA). – 7686c: the Bučenská vrchovina Mts., the Nature Reserve of Kurinecká dubina, ca. 0.5 km S of the village of Kurinec, ca. 220 m a. s. l., on wood of fallen rotten trunk of *Quercus* sp., 24 Oct. 2002, leg. K. Skokanová (SLO). – 7785d: the Cerová vrchovina Mts., the SE hillslope of the Steblová skala hill, ca. 1 km E of the village of Gortva, ca. 400 m a. s. l., on wood of fallen rotten trunk of *Quercus* sp., 25 Oct. 2002, leg. M. Vašutová (SLO). – Podunajská nížina Lowland: 7868a: the city of Bratislava, the municipal part of Karlova Ves, Sihoť Island, flood plain forest, 137 m a. s. l., on wood of fallen decaying trunk of *Fraxinus* sp., 22 Oct. 1997, leg. S. Jančovičová (SLO). – 7868c: the city of Bratislava, the municipal part of Petržalka, Pečňa Island, 136 m a. s. l., 1 Oct. 1994, leg. V. Kabát (BRA). – 7968b: the city of Bratislava, the municipal part of Rusovce, grove near main road, 135 m a. s. l., 29 Sept. 1994, leg. V. Kabát (BRA). – 7968b: the city of Bratislava, the municipal part of Rusovce, flood plain forest near the Rusovské rameno branch, 128 m a. s. l., on fallen trunk of *Aesculus hippocastanum*, 8 Oct. 1996, leg. L. Hagara (LH). – 7968b: the city of Bratislava, the municipal part of Rusovce, park near a manor, 132 m a. s. l., on stump of *Aesculus hippocastanum*, 27 Sept. 1997, leg. D. Krajný and M. Hagarová (LH). – 7968b (7969a,



Map 4. Occurrence of *Pluteus aurantiorugosus* in Slovakia.

lack of detailed information does not allow an accurate localization): the city of Bratislava, the municipal part of Podunajské Biskupice, the Nature Reserve of Ostrov Kopáč, on decaying trunk, 21 Oct. 1995, leg. I. Kautmanová (BRA). – 7769c: the city of Bratislava, the municipal part of Vajnory, the Panónsky háj grove, 5 Oct. 1995, leg. V. Kautman (BRA). – 8178c: near the town of Štúrovo, 220 m a. s. l., on stump of deciduous tree cf. *Fraxinus* sp., 15 Aug. 1970, leg. J. Kuthan (BRA). – Malé Karpaty Mts.: 7768c: the city of Bratislava, the municipal part of Lamač, mixed forest, on decaying stump, 7 July 1996, leg. M. Pokorná (BRA). – Tríbeč Mts.: 7575c: the village of Jelenec, Dúň hill, 260 m a. s. l., on fallen branch of *Quercus* sp., 18 July 1998, leg. L. Hagara (LH). – Poľana Mts.: 7382c: the Nature Monument of Kalamárka, Kalamárka hill, 800 m a. s. l., on wood of standing trunk of *Fagus sylvatica*, 9 June 1996, leg. S. Glejdura (LDM, the herbarium of the Forestry and Wood-Technology Museum in the town of Zvolen in Slovakia). – Štiavnické vrchy Mts.: 7779a: near the village of Ladzany, ca. 400 m a. s. l., on decaying stump of *Ulmus* sp., 14 June 1984, leg. J. Kuthan (BRA). – 7779a: ca. 4 km NW of the village of Ladzany, 450 m a. s. l., on decaying stump of *Quercus* sp., 9 Aug. 1988, leg. P. Škubla (BRA). – 7780b: ca. 1 km SE of the village of Čabradský Vrbovok, 280 m a. s. l., on decaying stump of *Quercus* sp., 17 June 1988, leg. P. Škubla (BRA). – Vihorlatské vrchy Mts.: 7198d: the National Nature Reserve of Jovsianska hrabina, ca. 1 km NE of the church in the village of Jovsa, deciduous forest (*Carpinus betulus*, *Quercus* sp.), 170 m a. s. l., on wood of fallen rotten stump of *Quercus* sp., 10 June 2002, leg. Z. Argalášová (SLO). – Bukovské vrchy Mts.: 7000b: the National Nature Reserve of Rožok, on stump of *Fagus sylvatica*, 5 Oct. 1992, leg. S. Adamčík (BRA).

Literary data: Záhorská nížina Lowland: 7667d: the village of Vysoká pri Morave (Antonín et al. 1995). – Kremnické vrchy Mts.: 7280d: the locality of Suchý vrch (Antonín et al. 1995). – Nízke Beskydy Mts.: 6695c: the town of Svidník (Antonín et al. 1995).

Rhodotus palmatus (Bull.) Maire

Descriptions and/or illustrations: Dermek (1985), Antonín and Vágner (1993), Antonín et al. (1995), Noordeloos (1995), Krieglsteiner (2001).

Ecology: *Rhodotus palmatus* is known in Slovakia as a saprophyte producing fruitbodies on wood of fallen decaying trunks and branches of *Alnus* sp., *Acer campestre*, *Fraxinus* sp., *Negundo aceroides* and *Ulmus* sp. from August to November.

Other known hosts of this species in Europe are species of the genera *Acer* (Dermek 1985), *Aesculus*, *Malus*, *Quercus* (Lizoň 1985) and *Populus* (Antonín et al. 1995).

Notes: The first known find of *Rhodotus palmatus* in Slovakia, published by Fábry (1977) and Dermek (1985), is from the National Nature Reserve of Šúr (specimen by Fábry from 10 Sept. 1969 deposited in BRA). Záhorovská (1984, 1997) and Lizoň (1985) incorrectly located the first find of this species on Sihoť Island and Sedláčkov ostrov Island.

The second find of the species is from Sedláčkov ostrov Island (specimen by Feráková and Schwarzová from 29 Sept. 1982). This find was published by Záhorovská (1984), Lizoň (1985) and Záhorovská et al. (1996). Because the herbarium specimen of this find does not exist, some authors, such as Dermek (1985), Záhorovská (1997) and Antonín et al. (1995), incorrectly located the second find of *Rhodotus palmatus* on Sihoť Island.

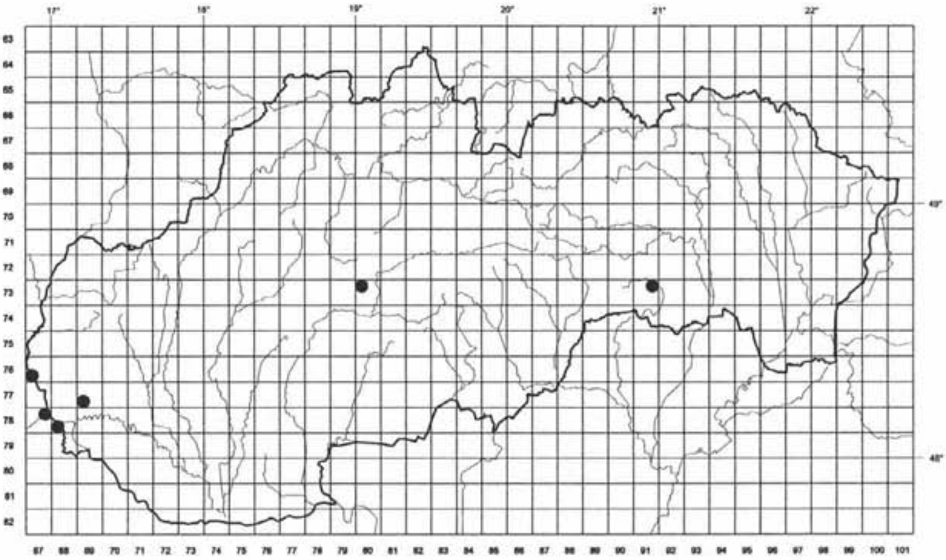
Occurrence in Slovakia: six localities: one in the Slovenský kras Karst (1 specimen), one locality in the Záhorská nížina Lowland (1 specimen), three localities in the Podunajská nížina Lowland (8 specimens) and one locality in the Kremnické vrchy Mts. (1 specimen; Map 5).

Occurrence in Europe: Austria, Czech Republic, France, Germany, Great Britain, Hungary, Italy, Lithuania, Netherlands, Norway, Romania, Slovakia, Spain, Sweden and Switzerland (Antonín et al. 1995, Krieglsteiner 2001).

Endangerment: *Rhodotus palmatus* is classified as an endangered fungus in Austria, the Czech Republic, Germany, Hungary, the Netherlands, Norway, Slovakia, Slovenia and Sweden.

Material studied: Záhorská nížina Lowland: 7667c: the village of Vysoká pri Morave, inundation area of the Morava river, ca. 4.3 km SSE of the church (in the village), remnants of flood plain forest (*Fraxinus* sp., *Acer campestre* etc.), ca. 140 m a. s. l., on wood of fallen decaying branch of *Fraxinus* sp., 10 Oct. 2001, leg. S. Ripková (SLO). – Podunajská nížina Lowland: 7867b: the city of Bratislava, the municipal part of Devín, Sedláčkov ostrov Island, flood plain forest, 138 m a. s. l., on wood of fallen decaying trunk of *Negundo aceroides*, 21 Oct. 1998, leg. S. Jančovičová (SLO). – Ibidem, on wood of fallen decaying branch of *Fraxinus* sp., 25 Aug. 1999, leg. S. Jančovičová and J. Ripka (SLO). – 7868c: the city of Bratislava, the municipal part of Karlova Ves, Sihoť Island, flood plain forest, 137 m a. s. l., on wood of fallen decaying trunk of *Fraxinus* sp., 7 Aug. 1997, leg. S. Jančovičová (SLO). – 7769c: the National Nature Reserve of Šúr, 10 Sept. 1969, leg. I. Fábry (BRA). – Ibidem, ca. 1.5 km S of the village of Jur pri Bratislave (the village of Svätý Jur), 130 m a. s. l., on fallen branch of *Alnus* sp., 2 Oct. 1988, leg. L. Hagara (BRA). – Kremnické vrchy Mts.: 7380a: the village of Badín, the Badínsky prales virgin forest, 720 m a. s. l., on fallen trunk of deciduous tree, 30 Sept. 1994, leg. Ľ. Varjú and L. Hagara (LH).

Literary data: Podunajská nížina Lowland: 7867b: the city of Bratislava, Slovanský ostrov Island (Sedláčkov ostrov Island), on stump of *Ulmus* sp.?, 29 Sept. 1982, leg. V. Feráková and



Map 5. Occurrence of *Rhodotus palmatus* in Slovakia.

T. Schwarzová (cf. Dermek 1985, Lizoň 1985, Záhorovská 1984). – 7868c: the city of Bratislava, Sihoť Island, under deciduous trees, 26 Oct. 1994, leg. V. Kabát (Škubla 1995). – 7868c: the city of Bratislava, Sihoť Island, 17 Nov. 1994 (Kabát 1995). – Slovenský kras Karst: 7391b (7391c, lack of detailed information does not allow an accurate localization): the village of Zádiel, the Zádielska dolina valley (Antonín et al. 1995).

Spongipellis fractipes (Berk. et M. A. Curtis) Kotl. et Pouzar

Descriptions and/or illustrations: Kotlaba and Pouzar (1976), Antonín et al. (1995), Krieglsteiner (2000), Ryvarden and Gilbertson (1993).

Ecology: *Spongipellis fractipes* is a saprophyte producing fruitbodies on wood of deciduous trees from June to October.

The National Nature Reserve of Šúr (flood plain forest with dominance of *Alnus glutinosa*) was the only locality of *Spongipellis fractipes* in Slovakia so far. Fruitbodies were produced only on wood of fallen trunks and branches of *Alnus glutinosa* (Kotlaba and Pouzar 1976).

We have confirmed the occurrence of the species in the National Nature Reserve of Šúr, where we collected fruitbodies on a fallen branch and stump of *Alnus glutinosa*. In addition, we have found a second locality in Slovakia, too. It is Sedláčkov ostrov Island (flood plain forest with dominance of *Populus* × *canescens*, *P. nigra* and *Salix alba*), where we have found the fruitbodies on wood and bark of fallen trunks of *Fraxinus* sp., *Negundo aceroides*, *Populus nigra* and *Populus* sp. and on wood and bark of a stump of *Salix alba*.

Other known hosts of the species are *Carpinus betulus* and *Fraxinus excelsior* in Europe, *Acer saccharophorum*, *Acer* sp., *Betula pendula*, *Betula* sp., *Fagus sylvatica* and *Padus avium* in the USA (Kotlaba and Pouzar 1976, Krieglsteiner 2000).

Notes: Kotlaba and Pouzar (1976) discussed the systematic position of this species. They stated that according to the main characters (duplex context, thick-walled spores and presence of pseudoskeletal hyphae) it should belong to the genus *Spongipellis* Pat. Because of its having cystidia, pseudoskeletal hyphae only in stipitate fruitbodies, the plasticity of the fruitbodies and the distinct shape of the spores they placed it in a new subgenus *Spongipellis* subg. *Loweomyces* Kotl. et Pouzar.

Later, Jülich (1984) accepted *Spongipellis* subg. *Loweomyces* as an autonomous genus and presented the species as *Loweomyces fractipes* (Berk. et M. A. Curtis) Jülich.

Ryvarden and Gilbertson (1993) had no doubts about the relationship of the species to *Spongipellis*, but the plasticity of the fruitbodies, duplex context and slightly thick-walled spores place it, in their opinion, closer to *Abortiporus biennis* (Bull.) Singer (the type of *Abortiporus*) than to *Spongipellis spumeus* (Sowerby) Pat. (the type of *Spongipellis*). They therefore placed it in the genus *Abortiporus* Murrill.

The main argument of Kotlaba and Pouzar (1976) for treating the species as *Spongipellis fractipes* was that it differs from *Abortiporus biennis* in its presence of cystidia and the lack of gloeocystidia and chlamydospores. They pointed out that the delimitation of *Abortiporus* is vague and that it should be transferred to *Spongipellis* subg. *Abortiporus*.

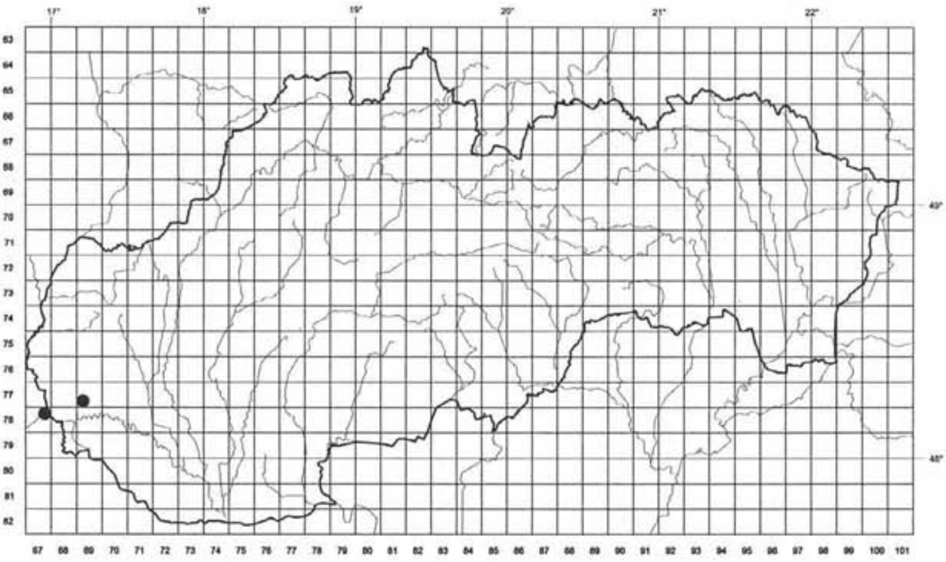
Occurrence in Slovakia: two localities in the Podunajská nížina Lowland (19 specimens; Map 6).

Occurrence in Europe: Austria, Belarus, Croatia, France, Georgia and Germany (Antonín et al. 1995, Krieglsteiner 2000).

Endangerment: *Spongipellis fractipes* is listed in the Red list of fungi in Slovakia.

Material studied: Podunajská nížina Lowland: 7769c: the National Nature Reserve of Šúr, the village of Svätý Jur, on stump of *Alnus* sp., 2 Oct. 1993, leg. L. Hagara (LH). – Ibidem, on fallen branch of *Alnus* sp., 12 Oct. 1995, leg. L. Hagara (LH). – 7867b: the city of Bratislava, the municipal part of Devín, Sedláčkov ostrov Island, flood plain forest, ca. 135 m a. s. l., on wood of fallen decaying trunk of *Negundo aceroides*, 13 Aug. 1997, leg. S. Jančovičová (SLO). – Ibidem, 20 July 1998 (SLO). – Ibidem, on wood of fallen decaying trunk of *Populus nigra*, 27 July 1998 (SLO). – Ibidem, on wood of fallen decaying trunk of *Populus* sp., 6 Aug. 1998 (SLO). – Ibidem, on wood and bark of decaying stump of deciduous tree, 16 July 1999 (SLO). – Ibidem, on wood and bark of fallen decaying trunk of deciduous tree, 16 July 1999 (SLO). – Ibidem, on wood of fallen decaying trunk of *Fraxinus* sp., 5 Aug. 1999 (SLO). – Ibidem, on wood and bark of decaying stump of *Salix* sp., 5 Aug. 1999 (SLO).

Literary data: Podunajská nížina Lowland: 7769c: the National Nature Reserve of Šúr, near the village of Jur pri Bratislave (Svätojurský Šúr) (the village of Svätý Jur), in *Alnetea*



Map 6. Occurrence of *Spongipellis fractipes* in Slovakia.

glutinosae, on wood in cavity of dead trunk of cf. *Quercus* sp., 24 June 1972, leg. I. Fábry (PRM) (Kotlaba and Pouzar 1976). – Ibidem, on rotten wood of *Alnus glutinosa*, 25 Oct. 1972 (PRM). – Ibidem, on fallen trunk of *Alnus glutinosa*, 25 Oct. 1972, leg. Z. Pouzar (PRM). – Ibidem, on fallen branch and trunk of *Alnus glutinosa*, 24 Aug. 1974, leg. Z. Pouzar, V. Holubová and Z. Heinrich (PRM). – Ibidem, on fallen trunk of *Alnus glutinosa*, 9. Sept. 1974, leg. F. Kotlaba (PRM). – Ibidem, on fallen branch of *Alnus glutinosa*, 9 Sept. 1974, leg. F. Kotlaba (PRM). – Ibidem, on fallen branch of *Alnus glutinosa*, 10 Sept. 1974, leg. F. Kotlaba (PRM). – Ibidem, on fallen branch of *Alnus glutinosa*, 15 Sept. 1975, leg. F. Kotlaba (PRM). – Ibidem, on fallen trunk of *Alnus glutinosa*, 15 Sept. 1975, leg. F. Kotlaba (PRM).

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State of diversity research on macrofungi in Slovakia

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Adamčík S., Kučera V., Lizoň P., Ripka J. and Ripková S. (2003): State of diversity research on macrofungi in Slovakia. – Czech Mycol. 55: 201–213

Available published data on occurrence and distribution of macrofungi in Slovakia were collected and transferred into a database. Data reported in the checklist of Slovak fungi were complemented with additional data from published papers and books previously not included in the survey. The degree of knowledge of our mycoflora was extrapolated by comparing data in selected taxonomic groups and phytogeographical units, and is presented in schematic maps.

Key words: field research, overview, number of taxa, phytogeographical units, Slovakia

Adamčík S., Kučera V., Lizoň P., Ripka J. a Ripková S. (2003): Stav výskumu diverzity makromycétov na Slovensku. – Czech Mycol. 55: 201–213

Pre poznanie diverzity makroskopických húb na Slovensku sme použili databázu zostavenú z dát excerpovaných z dostupnej literatúry. Údaje v zozname húb Slovenska sme doplnili o ďalšie, predtým nezaznamenané taxóny. Databáza obsahujúca všetky spracované dáta umožnila vyhodnotiť počet taxónov a porovnať zastúpenie taxonomických skupín v rámci jednotlivých fytogeografických celkov Slovenska. Poznanie diverzity makroskopických húb na Slovensku je interpretované na schematických mapách.

INTRODUCTION

The current knowledge of fungal diversity in Slovakia reflects the intensity of mycological research from the past to the present. Although the number of fungal taxa probably exceeds many times that of phanerogams, algae and bryophytes, there have been available fewer records, which does not reflect a real picture of the richness of Slovak mycoflora.

Svrček (1965a, 1965b) was the first and only one who completed a comprehensive overview of the research on fungi in former Czechoslovakia. His overview was based on published data, field notes and partly also on herbarium collections.

The aim of our contribution is to present the state of diversity research on macrofungi in Slovakia using data in the database created for the Checklist of Slovak fungi (Lizoň and Bacigálová 1999). The original database was built on

Tab. 1. Data included in the database.

source of information	number of taxa	number of publications	number of entries*
Fungi (Lizoň and Bacigálová 1999)	2471	358	8979
additional data	138	68	3153
total	2609	426	12132

* entry = taxon, incl. information on phytogeographical unit, source of data; one entry may include several records for the same phytogeographical unit in the same source of data

excerpts from 358 publications resulting in 8979 entries for 2471 taxa (species and infraspecific taxa) of macrofungi. A later excerption of 68 publications (most of them published after 1997, see references 2) added 138 more taxa (species and infraspecific taxa) to the database (Tab. 1). The current database has only few data taken from herbarium collections. Our next step will be to include into the database information from both institutional and private mycological collections.

MATERIAL AND METHODS

Delimitation of taxonomic groups (Ascomycota, Discomycetes, Basidiomycota, Aphyllophorales s. l., Boletales s. l., Agaricales s. l., Gasteromycetes) follows the Dictionary of the fungi (Kirk et al. 2001).

Maps represent the numbers of recorded taxa of a particular taxonomic group in the phytogeographical units of Slovakia (Futák 1966). For numbers of the units see Tab. 2.

RESULTS

Explanation of numbers

For example Ascomycota: 496 (19 %) – total number of recorded taxa in Slovakia, the percentage expresses the number of recorded taxa of particular taxonomic group compared with a total number of recorded taxa in Slovakia.

For example Nízke Tatry Mts. (136, 50 %) – number of recorded taxa in the unit, the percentage expresses the number of recorded taxa of particular taxonomic group compared with a total number of recorded taxa in the phytogeographical unit.

Macrofungi: 2609 (100 %) Map 1, Tab. 2

Highest number of taxa: Bukovské vrchy Mts. (1163).

High number of taxa: Malé Karpaty Mts. (644), Záhorská nížina Lowland (568), Tríbeč Mts. (497), Podunajská nížina Lowland (479).

Tab. 2. Number of taxa hitherto known in the phytogeographical units of Slovakia.

	M	A	di	B	aph	ag	bo	ga
Slovakia	**2609**	**496**	**416**	**2113**	**603**	**1295**	**112**	**103**
1 Burda Mts.	27	0	0	27	9	3	11	4
2 Ipeľsko-rimavská brázda Furrow	242	32	17	210	98	73	18	21
3 Slovenský kras Karst	172	10	10	162	90	48	14	10
4 Záhorská nížina Lowland	568	53	47	515	123	283	68	41
5 Devínska Kobyla Mts.	173	25	23	148	40	76	12	20
6 Podunajská nížina Lowland	479	65	55	414	133	208	28	45
7 Košická kotlina Basin	78	6	6	72	54	1	2	15
8 Východoslovenská nížina Lowland	83	1	1	82	63	1	1	17
9 Biele Karpaty Mts. (southern part)	25	10	10	15	5	1	2	7
10 Malé Karpaty Mts.	644	110	83	534	173	280	52	29
11 Považský Inovec Mts.	89	7	6	82	55	18	7	2
12 Tribeč Mts.	497	41	34	456	107	290	40	19
13 Strážovské and Súľovské vrchy Mts.	120	35	34	85	23	40	11	11
14a Pohronský Inovec Mts.	7	0	0	7	3	1	2	1
14b Vtáčnik Mts.	30	3	2	27	12	7	4	4
14c Kremnické vrchy Mts.	148	21	10	127	82	33	3	9
14d Poľana Mts.	360	48	37	312	28	241	31	12
14e Štiavnické vrchy Mts.	355	43	24	312	151	117	25	19
14f Javorie Mts.	8	2	2	6	4	1	1	0
15 Slovenské rudohorie Mts.	332	40	35	292	118	127	31	16
16 Muránska planina Plateau	52	9	5	43	35	7	0	1
17 Slovenský raj Mts.	59	29	29	30	19	9	0	2
18 Stredné Pohornádie Valley	14	3	3	11	6	1	1	3
19 Slanské vrchy Mts.	151	8	7	143	111	6	1	25
20 Vihorlatské vrchy Mts.	36	14	12	22	15	4	2	1
21a Malá Fatra (Lúčanská Fatra) Mts.	156	28	28	128	15	93	14	6
21b Malá Fatra (Krivánska Fatra) Mts.	160	21	18	139	52	63	12	12
21c Veľká Fatra Mts.	284	32	28	252	109	112	16	15
21d Chočské vrchy Mts.	25	6	6	19	10	6	1	2
22 Nízke Tatry Mts.	270	136	126	134	43	65	9	17
23a Západné Tatry Mts.	242	46	42	196	50	126	16	4
23b Vysoké Tatry Mts.	395	48	46	347	151	147	39	10
23c Belianske Tatry Mts.	321	77	74	244	62	151	17	14
24 Pieniny Mts.	6	0	0	6	2	1	1	2
25 Turčianska kotlina Basin	41	4	4	37	7	23	5	2
26a Liptovská kotlina Basin	323	26	24	297	65	195	28	9
26b Spišské kotliny Basins	135	41	38	94	58	24	5	7
27a Biele Karpaty Mts. (northern part)	5	3	3	2	0	2	0	0
27b Javorníky Mts.	7	2	2	5	3	1	0	1
28 Západné Beskydy Mts.	42	8	8	34	11	18	1	4
29 Spišské vrchy Mts.	109	11	11	98	87	6	1	4
30a Šarišská vrchovina Mts.	33	18	17	15	9	1	1	4
30b Čergov Mts.	84	4	2	80	20	52	2	6
30c Nízke Beskydy Mts.	79	6	5	73	36	31	5	1
31 Bukovské vrchy Mts.	1163	110	86	1053	278	713	40	22

M – macrofungi, **A** – Ascomycota, **di** – Discomycetes, **B** – Basidiomycota, **aph** – Ahyllophorales s. l., **bo** – Boletales s. l., **ag** – Agaricales s. l., **ga** – Gasteromycetes.

Less than 100 taxa per unit: 21 units.

Ascomycota: 496 (19 %) Map 2, Tab. 2

Highest number of taxa: Nízke Tatry Mts. (136, 50 %), Malé Karpaty Mts. (110, 17 %), and Bukovské vrchy Mts. (110, 9 %).
High number of taxa in a relatively small unit: Belianske Tatry Mts. (77, 24 %).
Less than 30 taxa per unit: 27 units.
Ratio Ascomycota/all fungi per unit: 0–60 %

Discomycetes: 416 (16 %) Map 3, Tab. 2

Highest number of taxa: Nízke Tatry Mts. (126, 50 %).
High number of taxa: Bukovské vrchy Mts. (86, 7 %), Malé Karpaty Mts. (83, 13 %).
Less than 25 taxa per unit: 25 units.
Ratio Discomycetes/all fungi per unit: 0–60 %.

Basidiomycota: 2113 (81 %) Map 4, Tab. 2

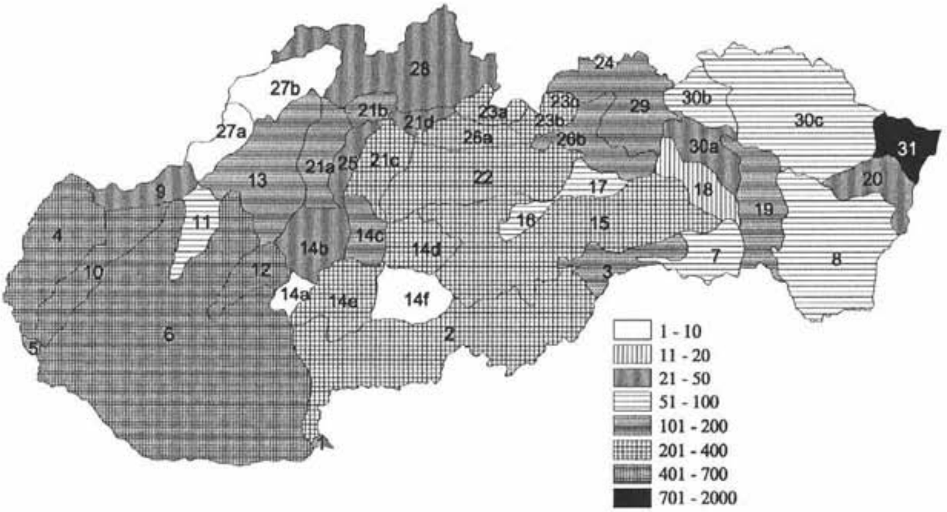
Highest number of taxa: Bukovské vrchy Mts. (1053, 91 %).
High number of taxa: Malé Karpaty Mts. (534, 83 %), Záhorská nížina Lowland (515, 91 %), Tríbeč Mts. (456, 92 %), Podunajská nížina Lowland (414, 86 %).
Less than 100 taxa per unit: 21 units.
Ratio Basidiomycota/all fungi per unit: 40–100 %.

Aphyllophorales s. l.: 603 (23 %) Map 5, Tab. 2

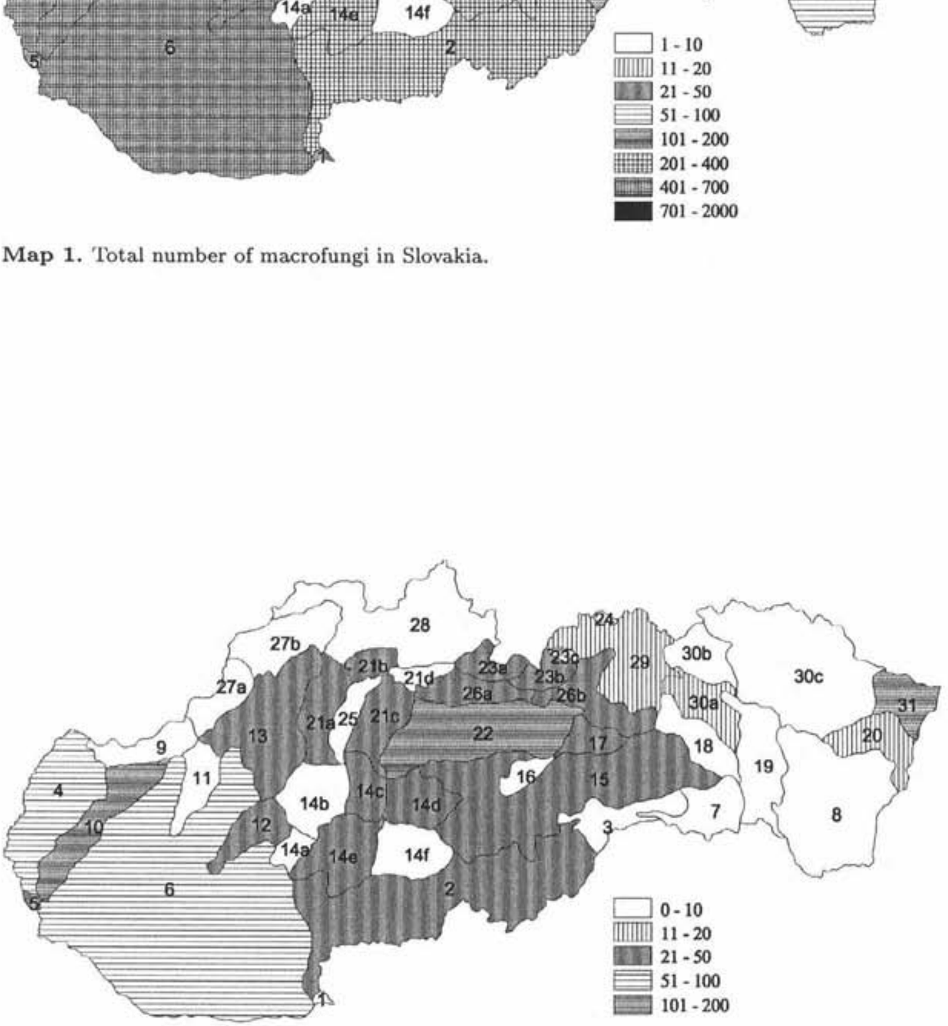
Highest number of taxa: Bukovské vrchy Mts. (278, 24 %).
High number of taxa: Malé Karpaty Mts. (173, 27 %), Štiavnické vrchy Mts. (151, 43 %), Vysoké Tatry Mts. (151, 38 %).
Less than 30 taxa per unit: 19 units.
Ratio Aphyllophorales s. l./all fungi per unit: 0–80 %.

Boletales s. l.: 112 (4 %) Map 6, Tab. 2

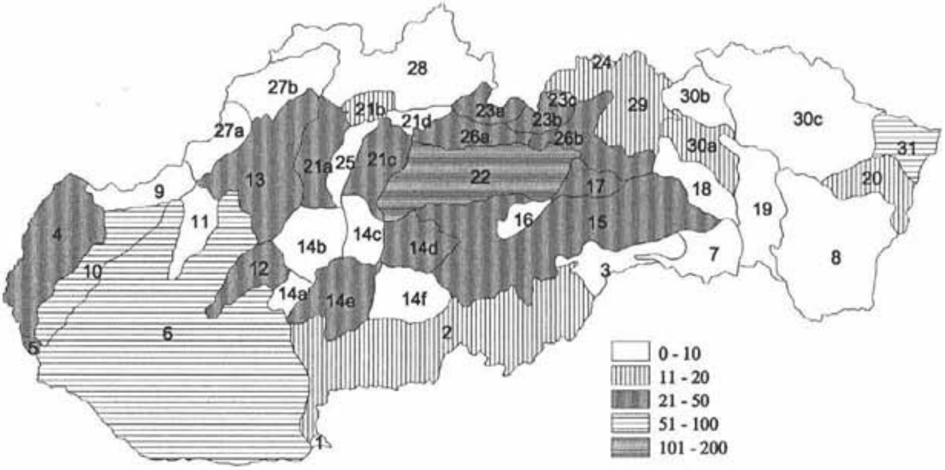
Highest number of taxa: Záhorská nížina Lowland (68, 12 %).
High number of taxa: Malé Karpaty Mts. (52, 8 %), Tríbeč Mts. (40, 8 %), Bukovské vrchy Mts. (40, 3 %), Vysoké Tatry Mts. (39, 10 %).
Less than 10 taxa per unit: 25 units.
Ratio Boletales s. l./all fungi per unit: 0–41 %.



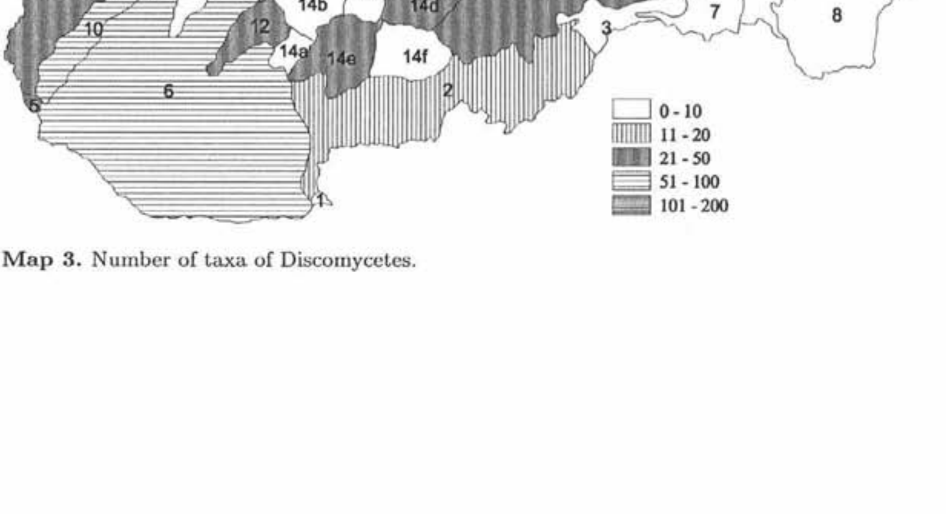
Map 1. Total number of macrofungi in Slovakia.



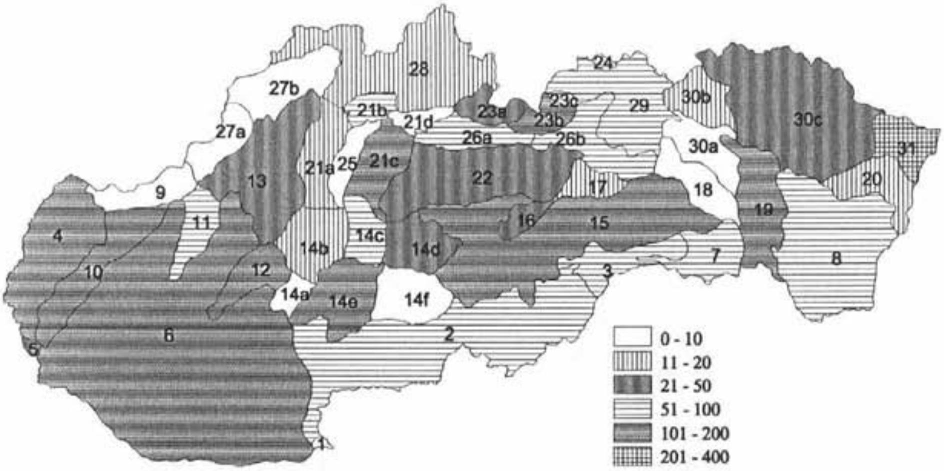
Map 2. Number of taxa of Ascomycota.



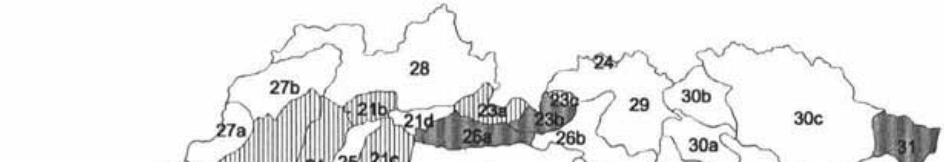
Map 3. Number of taxa of Discomycetes.



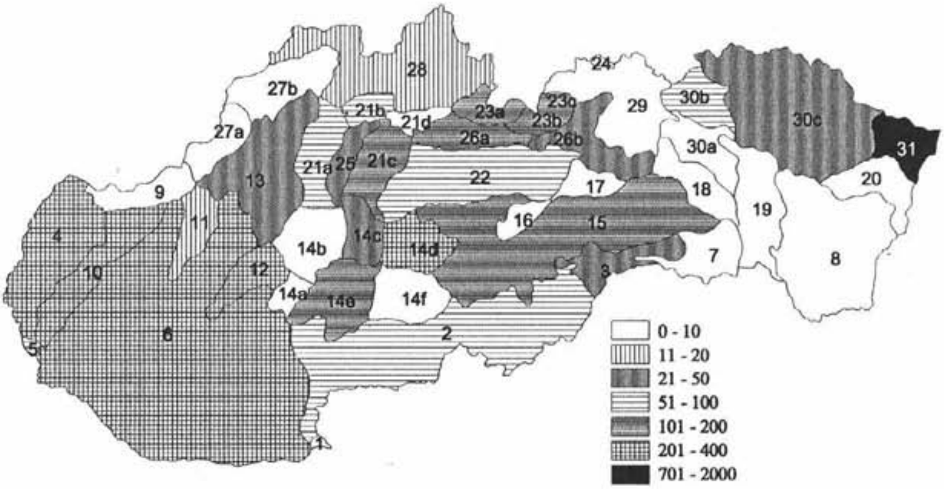
Map 4. Number of taxa of Basidiomycota.



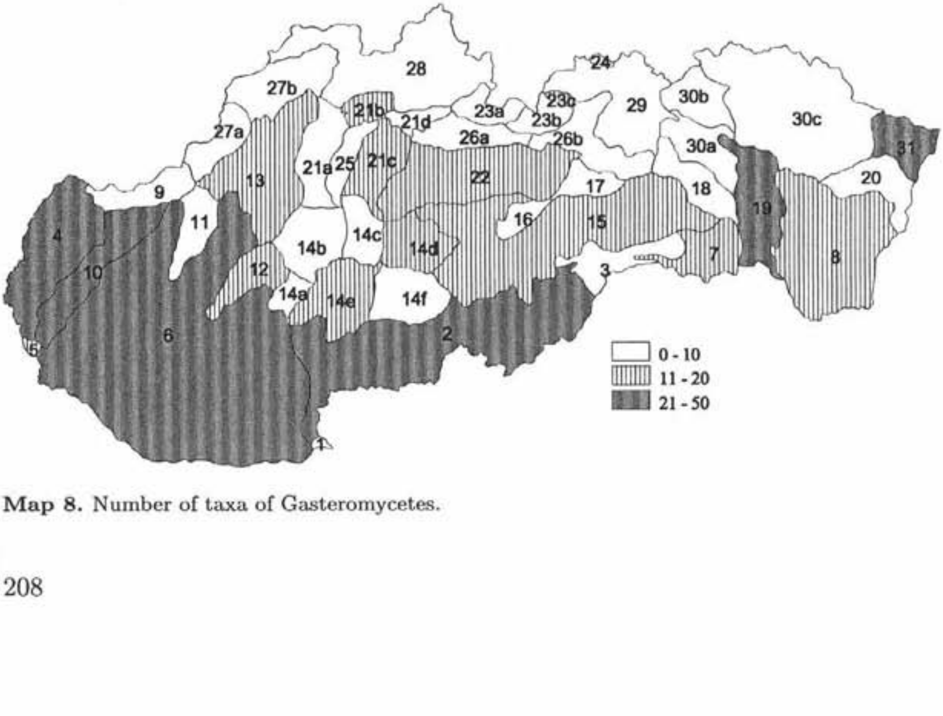
Map 5. Number of taxa of Aphyllophorales s. l.



Map 6. Number of taxa of Boletales s. l.



Map 7. Number of taxa of Agaricales s. l.



Map 8. Number of taxa of Gasteromycetes.

Agaricales s. l.: 1295 (50 %) Map 7, Tab. 2

Highest number of taxa: Bukovské vrchy Mts. (713, 61 %).

High number of taxa: Tríbeč Mts. (290, 59 %), Záhorská nížina Lowland (283, 50 %), Malé Karpaty Mts. (280, 43 %), Poľana Mts. (241, 67 %), Podunajská nížina Lowland (208, 43 %), Liptovská kotlina Basin (195, 60 %).

Less than 50 taxa per unit: 26 units.

Ratio Agaricales s. l./all fungi per unit: 1–67 %.

Gasteromycetes: 103 (4 %) Map 8, Tab. 2

Highest number of taxa: Podunajská nížina Lowland (45, 9 %).

High number of taxa: Záhorská nížina Lowland (41, 7 %), Malé Karpaty Mts. (29, 5 %), Slanské vrchy Mts. (25, 17 %).

Less than 10 taxa per unit: 25 units.

Ratio Gasteromycetes/all fungi per unit: 0–33 %.

CONCLUSIONS

The number of professional and amateur mycologists who have published data on Slovak macrofungi is limited. Most of them are specialised in particular taxonomic groups and/or regions, which has also caused a lack of data both in taxonomic groups and phytogeographical units.

The best known unit is Bukovské vrchy Mts. Long-term field research of Slovak and Czech mycologists resulted in a publication presenting information on 1163 taxa (Kuthan et al. 1999). Collecting sites within Záhorská nížina Lowland, Podunajská nížina Lowland and Malé Karpaty Mts. are situated close to the capital city of Bratislava and are frequently visited by mycologists and thus "produced" high numbers of records. Few records (less than 10) have been published from Pohronský Inovec Mts., Javorie Mts., Pieniny Mts., Biele Karpaty Mts. (northern part) and Javorníky Mts. There is urgent need for extensive field research in those units.

The majority of recorded taxa in Ascomycota (496) are represented by Discomycetes (416). There is little chance of gathering more data in other groups of Ascomycota because of a lack of specialists.

The number of recorded taxa of Aphyllophorales s. l. compared with the total number of recorded taxa in the phytogeographical units Spišské vrchy Mts. (80 %), Východoslovenská nížina Lowland (76 %), Slanské vrchy Mts. (74 %) and Košická kotlina Basin (69 %) is relatively high. This was probably caused by insufficient research on other taxonomic groups of fungi.

Members of Agaricales s. l. are the most popular macrofungi and the number of recorded taxa (1295) covers ca. 50 % of all macrofungi. For example in Bukovské vrchy Mts. there were recorded 713 taxa, representing 55 % of all Agaricales s. l. recorded in Slovakia.

Boletales s. l. are also very popular but 112 taxa represent only 4 % of all recorded macrofungi in Slovakia. There are even units, such as Pohronský Inovec Mts., Javorie Mts., Muránska planina Plateau, Vihorlatské vrchy Mts., Biele Karpaty Mts. (northern part), Javorníky Mts., Nízke Beskydy Mts., with one or without any records. On the contrary, good collecting sites of Boletales s. l. in Záhorská nížina Lowland, Malé Karpaty Mts. and Vysoké Tatry Mts. show high numbers of published data.

This preliminary report illustrates that (1) published data are an important source of data that reflect the intensity of field research, (2) comparison of geographical and/or phytogeographical units and biotopes requires (even in such a small country as Slovakia) thousands and thousands of records, and (3) a well-established network of field co-workers focusing on all groups of macrofungi should be organised.

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CZECH MYCOL. 55 (3–4), 2003

Taxonomic revision of the genus Cheilymenia – 10. Cheilymenia apiculispora spec. nov., a new species of the section Coprobia

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Moravec J. (2003): Taxonomic revision of the genus Cheilymenia – 10. Cheilymenia apiculispora J. Moravec spec. nov., a new species of the section Coprobia. – Czech Mycol. 55: 215–222

Cheilymenia apiculispora J. Moravec spec. nov., a new species of the genus *Cheilymenia* Boud., section *Coprobia* (Boud.) J. Moravec, is described according to a collection from the Russian Far East, and compared to related taxa.

Key words: *Cheilymenia apiculispora* spec. nov., section *Coprobia*, Pezizales, *Pyronemataceae*.

Moravec J. (2003): Taxonomická revize rodu Cheilymenia – 10. Cheilymenia apiculispora J. Moravec spec. nov., nový druh sekce Coprobia (Pezizales, Pyronemataceae). – Czech Mycol. 55: 215–222

Cheilymenia apiculispora J. Moravec spec. nov., nový druh rodu *Cheilymenia* Boud. sekce *Coprobia* (Boud.) J. Moravec, je popsán podle nálezu z ruského Dálného Východu a porovnán s příbuznými druhy.

The present paper follows the previously published contributions within the framework of the author's taxonomic revision of the genus *Cheilymenia* Boud. Ahead of a monograph of the genus, which is currently being prepared, a new species of the section *Coprobia* is described here.

MATERIAL AND METHODS

The ascospores were stained with "cotton blue" [Geigy s. 123 or 0.5 % methyl blue (R. A. L.) in lactic acid] called here "C^4B" which stains directly without heating the slides and does not destroy the separable perispore. For other examinations see Moravec (2003).

Acronyms of the herbaria:

BPI – U. S. National Fungus Collections, Beltsville, Maryland, U. S. A.;
BRNM – Botanical Department of the Moravian Museum, Brno, Czech Republic;
CUP – Department of Plant Pathology, Cornell University, Ithaca, New York, U.S.A.;
PAN – Herbarium, Botany Department, Panjab University, Chandigarh, Punjab, India;
TAA – Institute of Zoology and Botany, Tartu, Estonia;

UPS – Institute of Systematic Botany, University of Uppsala, Sweden;
J. Mor. – private herbarium (Discomycetes), Jiří Moravec, Adamov, Czech Republic.

RESULTS AND DISCUSSION

Only two sections of the genus *Cheilymenia*, the section *Coprobia* (Boud.) J. Moravec and the section *Striatisporae* J. Moravec accommodate species characterised by ascospores possessing rib-like striation on the separable perispore. The cyanophilic striation is observable when the ascospores are stained with C^4B in lactic acid without heating the slides (seen without any staining in several species only) and is perfectly distinguishable in SEM photomicrographs. The latter section was subdivided to two series, the series *Striatisporae* and series *Tenuistriatae* (Moravec 1990).

In species of the section *Coprobia*, the apothecia are "hairless", possessing only sparse or extremely rare, hyaline, thin-walled, hyphoid lateral hairs (occasionally with slightly thickened walls), while those of the section *Striatisporae* may possess both hyphoid or true subacuminate to acuminate marginal and lateral hairs. The shape of apothecial hairs did not prove to be decisive for recognising separate genera within *Cheilymenia* (see also the discussion in Moravec 1990) as there are intermediate shapes of hairs in species of the genus and this is especially illustrative in these two sections. Even in the type species of the section *Striatisporae*, *C. theleboloides* (Alb. et Schwein.: Fr.) Boud., which normally possesses apothecia with sparsely distributed but well developed true (subacute to acute) hairs, these hairs may be extremely sparse, and in a number of collections the apothecia entirely lack such true hairs and possess only sparse hyaline hyphoid hairs (I consider such collections to represent merely a "hairless" form of *C. theleboloides*). Species of the series *Tenuistriatae* possess robust marginal hairs in combination with well differentiated excipulum.

The excipular structure and the shape of paraphyses are more important for a delimitation of these two sections. Species of the section *Coprobia*, with the type species *Cheilymenia granulata* (Bull.: Fr.) J. Moravec, possess a simpler excipular structure and thick paraphyses. These two characters distinguish them from species of the section *Striatisporae* which possess thinner (filiform) paraphyses and more frequent hyphae in the medulla, often forming a textura subintricata in species of the series *Striatisporae*, or textura intricata in species of the series *Tenuistriatae* (compare fig. 2–2a to fig. 3a-3b in Moravec 1990). Nevertheless, the excipulum in the section *Coprobia* is not unequivocally simple ("undifferentiated" as commonly interpreted) as the cells in the medullary layer are much smaller and hyphal elements, though less frequent, also occur, especially in the area under the hypothecium (see also fig. 38 in Le Gal 1954 and fig. 1 in Moravec 1990).

As the intermediate shapes and variable frequency of hairs as well as transitional patterns of structures in the medullary layer within these two sections exist, the species accommodated in them are obviously members of only one genus, *Cheilymenia* as emended in Moravec (1990).

The new species described below belongs to the section *Coprobia*.

Cheilymenia apiculispora J. Moravec spec. nov. (Figs. 1–4)

Diagnosis. Apothecia 1–4 mm diam., gregaria, sessilia, primum molliter patellaria, orbicularia dein discoidea et explanata, molliter et tenuiter carnosa, tota laete flavo-ochracea usque ochracea; pars exterior apothecii minute granulato-furfuracea; parte basali rarissime cum pilis sparsis, hyphoideis hyalinis, septatis. Excipulum externum e textura globulosa, usque globuloso-angulare, cum cellulis (15-)25–-75(-90) μm in diam. Excipulum internum (medulla) e textura globuloso-angulare, cum cellulis parvis (8–25 μm in diam.), subglobosis, sed etiam cum hyphis irregularibus, sparsis, aliquando vesiculare-inflatis constat; hypothecium e cellulis et hyphis irregularibus, minoribus (4–8 μm in diam.), cyanophilis constat. Asci 100–140 × 7.5–12.5 μm, cylindracei, octospori. Ascosporae (11.0-)12.5–16.5(-18.2) × × (6.0-)6.3–7.9 (-9.0) μm, plerumque 14.3 × 7.1 μm (ornamento excluso), eguttulatae, ellipsoideae seu elongato-ellipsoideae, perisporio separabile, crasse et sparse longitudinale striato cum costis (0.3-)0.6–0.9(-1.3) μm crassis, cyanophilis, simplicibus vel anastomosantibus, cum apiculis (0.3-)0.5–2.1(-2.6) μm longis ad polis donatae. Paraphyses crasse filiformes, (3-)4–5(-6) μm crassae, apice sensim clavato-incrassatae (6–9(-12) μm).

Holotypus. In excremento vaccino, Russia: Far East, Primorsk Region, in jugo montium Pidan, mons Hualaza, 1. VIII. 1970, leg. B. Kullman et Ain Raitviir. Holotypus in herbario TAA (No 61438) et isotypi in BRNM 686284 et in herbario privato J. Mor. asservantur.

Apothecia (Fig. 1) 1–4 mm in diam., sparsely gregarious, sessile, first shallowly saucer-shaped, orbicular, then expanded to flattened, thin-fleshy and soft, pale yellow-ochraceous to ochraceous, external surface finely scurfy, hairless.

Apothecial structure (Fig. 4). Hymenium 110–150 μm thick. Hypothecium indistinctly differentiated from medullary layer, about 30–45 μm thick, composed of small (4–8 mm in diam.) irregular cyanophilic cells. Medullary excipulum about 60–120 μm thick, composed of irregular globose to subangular cells, 8–25 μm in diam., forming a textura globulosa to angularis, but occasionally mixed with 7–12 μm thick hyphae with cyanophilic septa which are often vesicular-inflated (up to the diameter of the globose cells), in some areas inconsistently forming an indefinite texture, especially under hypothecium. Ectal excipulum about 80–180 μm thick, slightly differentiated from medulla, composed of much larger globose or globose-ellipsoid cells (15-)25–75(-90) μm in diam, forming textura globulosa.

Hairs absent on the margin and flanks. Hyaline superficial hyphoid hairs (Fig. 4) extremely rarely occur near receptacular base; they are hyaline, 25–70 × 7–12 μm, aseptate or sparsely septate, thin-walled.

Asci (Fig. 2.) 100–140 × 7.5–12.5 μm, cylindrical with blunt apex, eight-spored. Ascospores (Fig. 3 and SEM Fig. 5) eguttulate, with thick homogenous content (more distinct than in *C. granulata*), ellipsoid or ovoid or elongate ellipsoid, (11.0-)12.5–16.5(-18.2) × (6.0-)6.3–7.9(-9.0) μm, mostly 14.3 × 7.1 μm (ornamentation and apiculi excluded); perispore possessing conspicuously coarse and strongly cyanophilic longitudinal ribs (when stained with C^4B in lactic acid without heating); the ribs are continuous or usually obliquely anastomosing, (0.3-)0.6–0.9(-1.3) μm thick and 0.3–0.6(-1.0) μm high, forming conspicuous, blunt or mostly subacute and elongate, (0.3-)0.5–2.1(-2.6) μm high apiculi on ascospore poles. Paraphyses (Fig. 2) thickly filiform to cylindrical, (3-)4–5(-6) μm thick, apices moderately or more distinctly enlarged to 6–9(-12) μm, with subhyaline (in rehydrated apothecia) content.

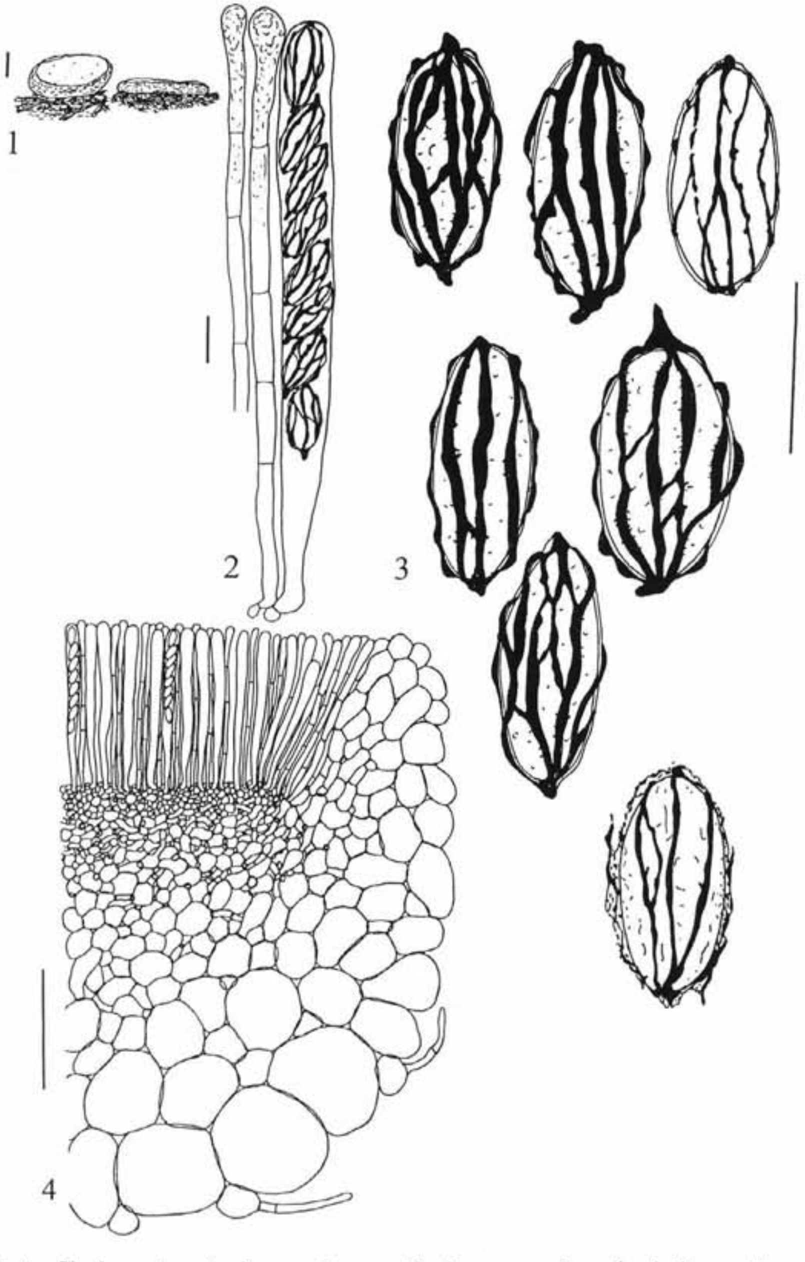
Habitat and distribution. On cow dung. Known only from the type locality in the Far East.

Type material examined. Russia: Far East, Primorsk Region, Pidan mountain range, Mt. Hualaza, on cow dung, 1. VIII. 1970, leg. B. Kullman and Ain Raitviir. Holotype in TAA 61438, isotypes in BRNM 686284 and in herb. J. Mor.

Etymology. Derived from the Latin "apiculus" and "spora", referring to the distinct apiculi on the ascospore poles in this new species.

Remarks. *Cheilymenia apiculispora* spec. nov., although macroscopically resembling *C. granulata*, is a very conspicuous and easily distinguishable species, especially for its constantly apiculate and coarsely longitudinally costate ascospore perispore. The ornamentation of the perispore consists of extremely thick and strongly cyanophilic ribs forming an irregularly uneven ascospore outline. The perispore loosens but rarely separates entirely from the epispore, as the rigid ribs stiffen the wall.

No species of *Cheilymenia* possesses such thick rib-like ascospore ornamentation as that in the new species. It is even much coarser than that in *Cheilymenia crassistriata* (J. Moravec) J. Moravec (compare illustrations in Moravec 1987, 1990). Another taxon commonly occurring in Asia, *Cheilymenia striata* (K. S. Thind, E. K. Cash et Pr. Singh) J. Moravec, described from India by Thind, Cash and Singh (1977) as *Ascophanus striatus* [= *Coprobia striata* (K. S. Thind, E. K. Cash et Pr. Singh) Waraitch], distinctly differs in having smaller ascospores with much finer and denser ascospore striation – the striation is the same as that in *C. granulata* (or even finer), so the name does not reflect any difference between these two taxa. After examination of the isotype (BPI ex PAN) of *Ascophanus striatus* and of the



Figs 1–4. *Cheilymenia apiculispora*: 1. – apothecia on cow dung (scale bar = 1 mm); 2. – paraphyses and ascus (scale bar = 10 μm); 3. – seven mature ascospores, oil immersion, C^4B, note one ascospore (underneath) with loosening perispore (scale bar = 10 μm); 4. – median section through apothecial margin, with rare hyphoid hairs (scale bar = 100 μm). Isotype J. Mor. (ex holotype TAA 61438).

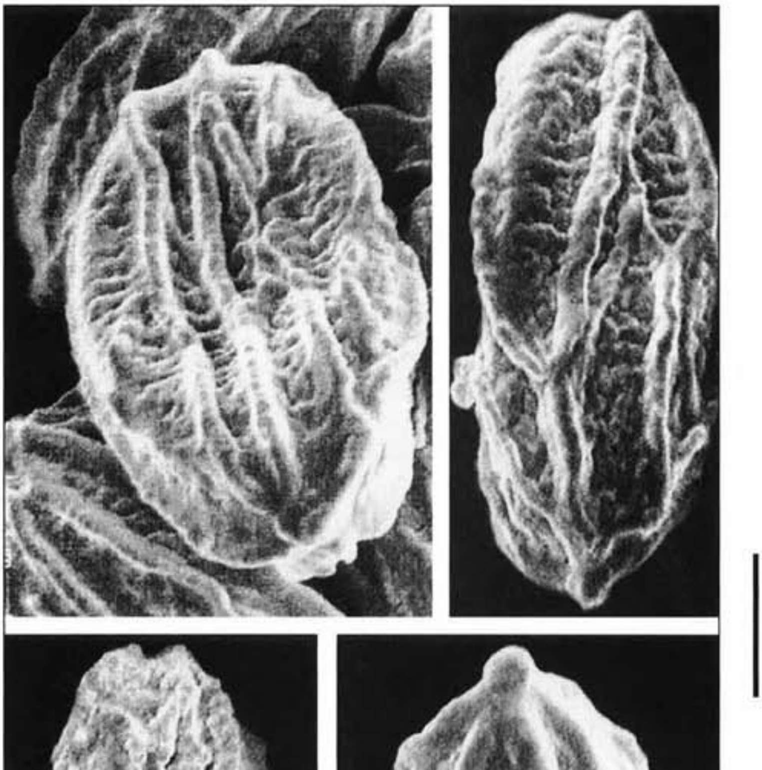


Fig. 5. *Cheilymenia apiculispora.* SEM photomicrographs of ascospores (scale bar = 3 μm). Isotype J. Mor. (ex holotype TAA 61438).

other material from India treated by Thind and Kaushal (1978) [but also from Kyrghystan (TAA), Nepal (BPI) and China (J. Mor.)], I consider *Cheilymenia striata* merely an infraspecific taxon of *C. granulata*. It should be noted that the collection (UPS, CUP) reported from Macaronesia by Korf and Zhuang (1991) as *C. striata* is another, very different species, possessing true apothecial hairs and is close to *C. lemuriensis* Heim ex Le Gal (section *Striatisporae*) – the hairs were overlooked by the cited authors.

The elongate apiculi on the ascospore poles in *C. apiculispora* are unique in *Cheilymenia*. Within the genus, only *C. polaripustulata* possesses apiculate ascospores but the apiculi are blunt as they are formed by rounded pustules and the ascospore striation is much finer and denser (Moravec 1998). Moreover, *C. polaripustulata*, which belongs to the section *Striatisporae* series *Tenuistriatae*, differs in having apothecia with robust marginal hairs.

I have placed *C. apiculispora* into the section *Coprobia* especially for its shape of the hairless apothecia (with extremely sparse hyphoid hairs occurring only near the apothecial bases), and less differentiated medullary layer, which, however, comprises somewhat more frequent hyphal elements (but hyphae mostly of an indefinite shape and arrangement). Moreover, the thickly filiform paraphyses (though comparatively slender and less enlarged than those in *C. granulata*) better correspond with those in the species of the section *Coprobia*.

Regarding the other species of these two sections, I have treated them in detail (including their infraspecific taxa), and the results will be published in the upcoming monograph of the genus *Cheilymenia*.

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CZECH MYCOL. 55 (3–4), 2003

New records and ecology of Naeviopsis carneola in Central Europe with notes on other fungi growing on Juncus filiformis

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Suková M., Scheuer C. and Buryová B. (2003): New records and ecology of Naeviopsis carneola in Central Europe with notes on other fungi growing on Juncus filiformis. – Czech Mycol. 55: 223–239

Naeviopsis carneola (Ascomycetes, Helotiales, *Dermateaceae*), earlier known only from three localities in Sweden and Poland, is reported from further localities in the Czech Republic, Poland, Germany and Austria. The ecology of the species in Central Europe is evaluated (habitat, altitude, period of fructification, occurrence on various parts of *Juncus filiformis* shoots and presence of associated juncicolous fungi). Known localities are mapped. A description based on recent collections, a drawing and for the first time also microphotographs (TLM) are provided.

Key words: fungi on *Juncus filiformis*, *Naeviopsis carneola*, ecology, distribution, taxonomy, Central Europe, Czech Republic.

Suková M., Scheuer C. a Buryová B. (2003): Nové nálezy a ekologie druhu Naeviopsis carneola ve střední Evropě s poznámkami k dalším houbám rostoucím na Juncus filiformis. – Czech Mycol. 55: 223–239

Druh *Naeviopsis carneola* (Ascomycetes, Helotiales, *Dermateaceae*), dříve známý jen ze tří lokalit ve Švédsku a Polsku, byl nalezen na dalších lokalitách v České republice, Polsku, Německu a Rakousku. Byla studována ekologie tohoto druhu ve střední Evropě (prostředí, nadmořská výška, období fruktifikace, výskyt na různých částech prýtů *Juncus filiformis* a ostatní juncikolní houby přítomné na studovaných prýtech). Všechny dosud známé lokality jsou vyznačeny na schematické mapce. Je uveden popis doplněný perokresbou a fotografiemi apothecií a důležitých mikroznaků.

INTRODUCTION

Hein (1976) introduced the genus *Naeviopsis* for 12 species occurring on herbs and on leaves of trees. Most of them are known only from Europe. Since that time only one more species has been described – *Naeviopsis carneola*. It was published by Hein and Nannfeldt (in Holm and Nannfeldt 1992) with a detailed description. The specimens from two localities in Sweden were distributed in Fungi exsiccati suecici praesertim Upsalienses. Scheuer and Chlebicki (1997) published the second record from the Tatra Mts. (Poland) together with a drawing and SEM photograph of an apothecium. The collection was later distributed in Mycotheca Graecensis and

Dupla Fungorum (Scheuer and Poelt 1997, Scheuer 1997). A small collection from the Niedere Tauern Mts. in Styria (Austria) previously unpublished by the second author also proved to contain this fungus. Recently *N. carneola* was found in 1999 and 2001 in the Šumava Mts. in the Czech Republic (Suková 2003). An investigation was made in the season of 2002 with the aim to find it in other localities, mainly in the Czech Republic, and to give more information about its ecology (habitat, altitude, period of fructification, occurrence on various parts of *Juncus*-shoots, presence of other juncicolous fungi on the shoots and substrate specificity).

METHODS

Localities of *Juncus filiformis* favourable for the occurrence of *Naeviopsis carneola* (especially in the Czech Republic) were systematically visited, their habitat conditions and presence of *Naeviopsis carneola* and other fungi were recorded. Both fresh (specimens PRM 896491, 900920, 900922, 900933, 900942) and dried (other specimens and associated fungi) material was studied in water and Melzer's reagent under a TLM microscope using Nomarski contrast. 1 % and 5 % solutions of KOH were also used (PRM 895088). The description is based on the first author's recent collections. Herbarium specimens are deposited in the herbaria PRM (Mycological Department, National Museum, Praha) and GZU (Institut für Botanik, Universität Graz).

RESULTS AND DISCUSSION

Naeviopsis carneola B. Hein et Nannf., in L. Holm et Nannf., Thunbergia 16: 12, 1992.

Description (Figs. 1, 2, 3)

Fresh apothecia ochraceous, rounded, slightly elongated in direction of the *Juncus filiformis* shoot (stem, bract or leaf), (270-)400–820 × (230-)270–600 μm (measured at Loc. 17a, PRM 900932 and 900933), erumpent by a longitudinal slit in the surface tissues of the shoot or by 4 valves. While the apothecia are still young, the slit closes on drying, later the sides of the slit crack, forming valves which stay open in mature apothecia. Sometimes the disc may be completely exposed as the surface tissues are destroyed. Dried apothecia are mostly brownish orange, up to 450 μm in length.

Microcharacters. Unless stated otherwise, the data in this description are based on dried material examined in Melzer's reagent (MLZ). Paraphyses filiform, 2 μm wide, hyaline, smooth, septate, forked, in the upper part conspicuously enlarged to

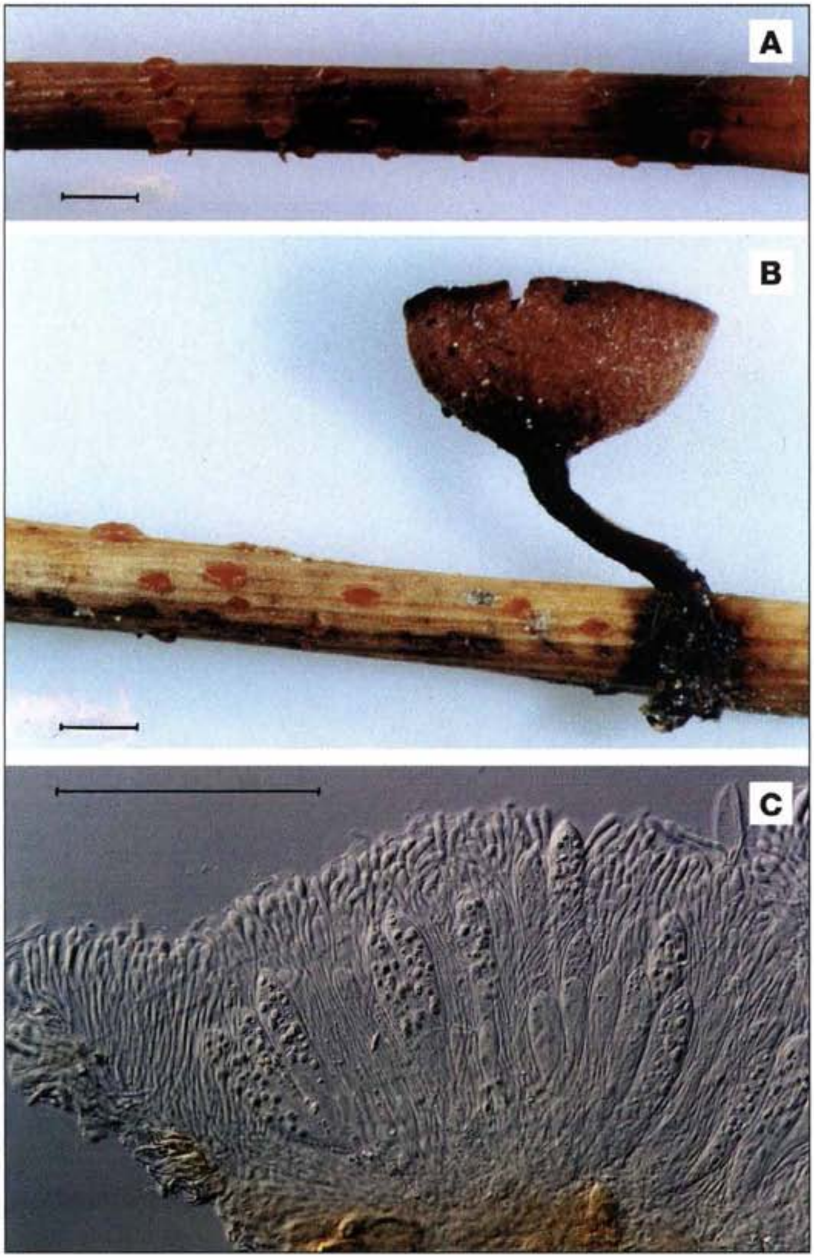

Fig. 1. *Naeviopsis carneola* B. Hein et Nannf.: A-B: apothecia on shoots of *Juncus filiformis* (in B also an apothecium of *Myriosclerotinia curreyana*), Poland, Karkonosze Mts., SW edge of cirque of Mł. Staw (PRM 900922); C: apothecium in longitudinal section, dried material (PRM 895088) studied in 1 % KOH solution. Scale bars A-B: 1 mm; C: 100 μm.

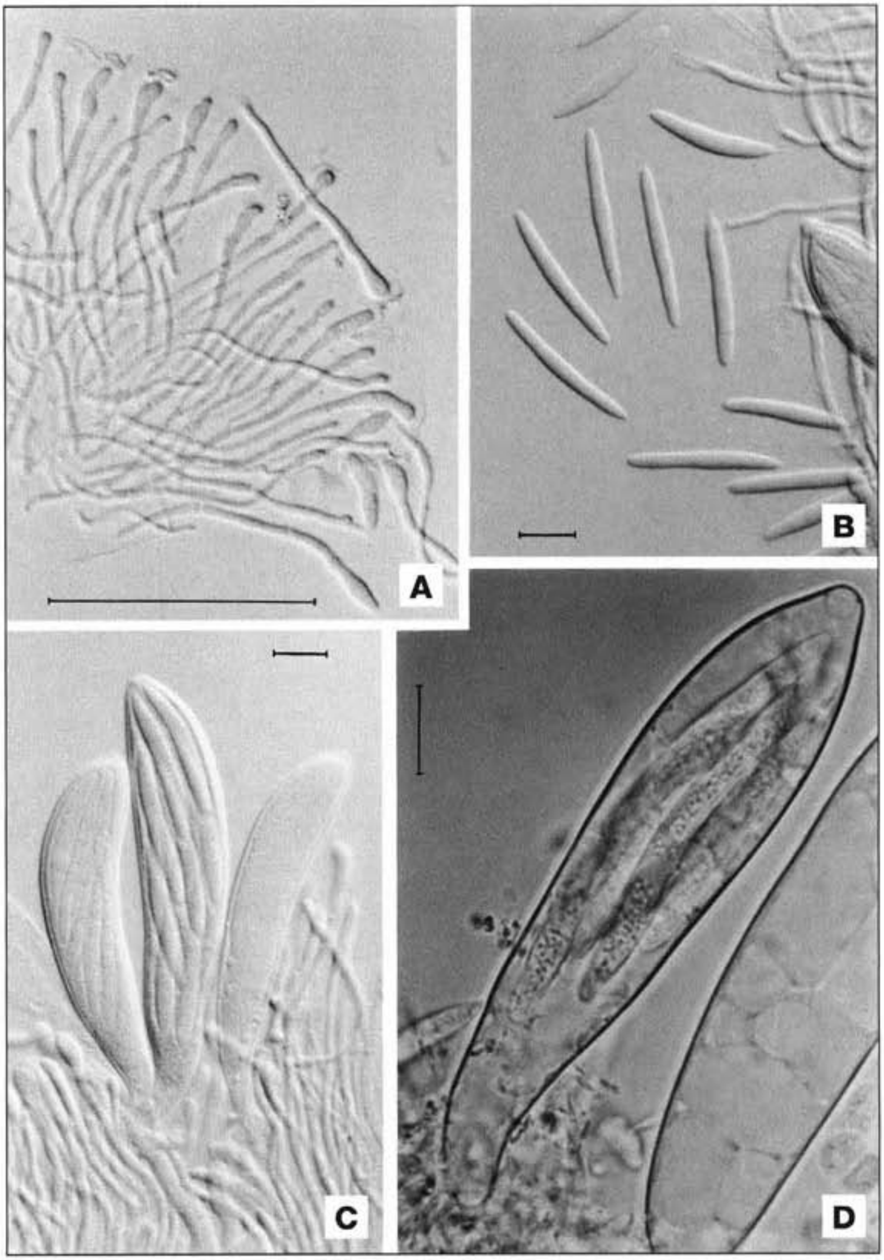

Fig. 2. *Naeviopsis carneola* B. Hein et Nannf.: A: paraphyses; B: ascospores; C: asci; D: living ascus. A-C: dried material (PRM 895088) studied in Melzer's reagent using Nomarski contrast; D: fresh material (PRM 900942) studied in water in TLM. Scale bars A: 50 µm; B-C: 10 µm; D: 10 µm.

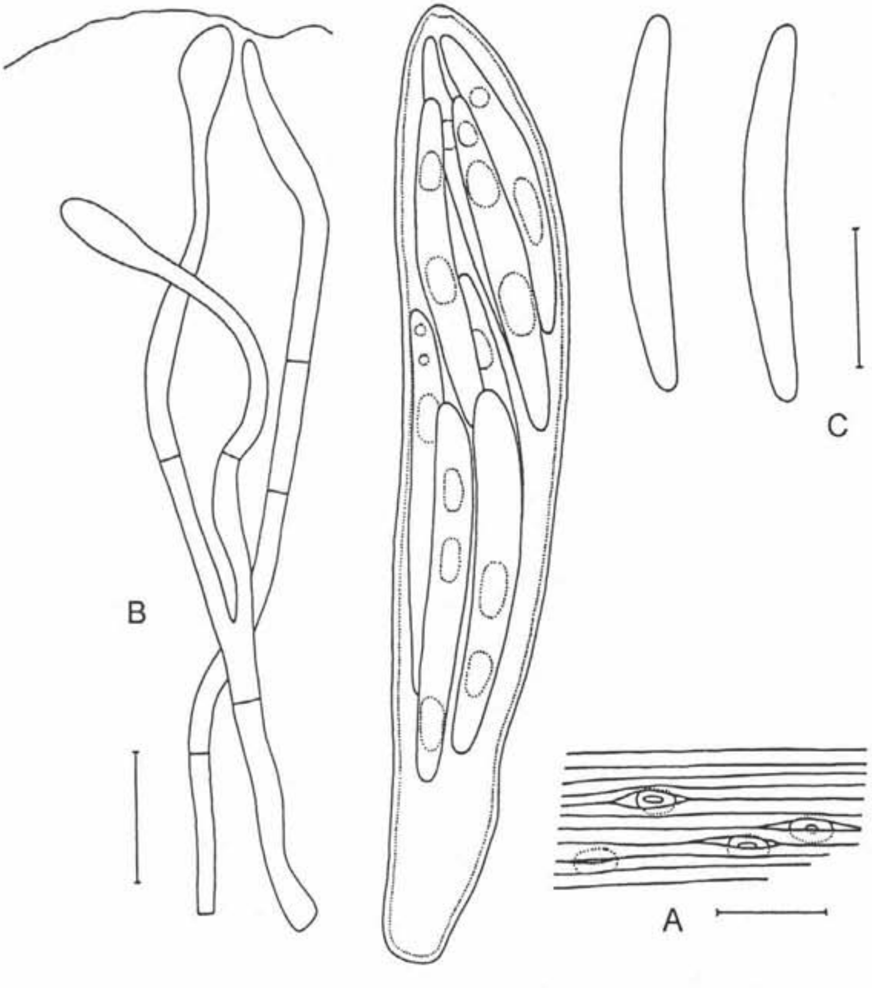

Fig. 3. *Naeviopsis carneola* B. Hein et Nannf. (PRM 895092): A: apothecia on bract of *Juncus filiformis*; B: paraphyses and ascus; C: ascospores. B-C: dried material studied in Melzer's reagent. Scale bars A: 500 μm; B-C: 10 μm.

2.5–4.7 μm, in fresh material studied in water to (2.5-)3.5–5(-5.5) μm, embedded in a hyaline gelatinous substance. Asci inoperculate, octosporic, (57-)60–74(-78) × (12-)13–16(-17), fresh in water 70–90(-96) × (12.5-)14.5–18.5(-20) μm, clavate, upper part characteristically conical especially in MLZ or water (in KOH more rounded), porus not amyloid in MLZ (also after pretreatment in 5 % KOH). Ascospores mostly triseriate, one-celled, (23-)26.5–31 × (3-)3.5–4 μm, in fresh material studied in water (30-)33–39(-43) × (3-)4–4.5 μm, narrowly fusiform, only slightly curved, slightly gradually pointed towards each end, smooth, nearly hyaline with slight lemon tint. Rarely one or several septa in some ascospores were seen in the collections PRM 895092 (observed in a slide prepared from dried material) and PRM 900942 (observed in a slide from fresh material).

Material studied, habitat characteristics and character of localities

Localities are marked in a schematic map (Fig. 4) using numbers indicated in the following survey. Vegetation units (esp. associations), when stated, are used according to Neuhäuslová (2001) for the Šumava Mts. and according to Chytrý et al. (2001) for the other mountains of the Czech Republic. In the following survey all specimens were collected on dead overwintered shoots or parts of shoots of *Juncus filiformis*. The morphological term "shoots" means aboveground parts of plants (sheaths, leaves, stems, inflorescences and bracts); "stem" = part of shoot under inflorescence, "bract" = part of shoot above inflorescence in case of *Juncus filiformis*. We use the term "shoots" especially when it is not possible to specify, whether *Naeviopsis carneola* is located on stems, bracts or leaves. The abbreviation "not." = "notavit" (Kotlaba 1999) is used in case the material of *Naeviopsis carneola* was old or scanty, not worth to be housed in PRM, but sufficient for microscopic study and confirmation of the occurrence in the locality. Unless stated otherwise, the specimens were collected and identified by M. Suková. Bryophytes were identified by B. Buryová. The term "associated fungi" is used for fungi which were found on the same shoots as *N. carneola*, although they often occurred also on other shoots in the locality or in the tuft.

Sweden: 1) Gästrikland, Hille parish, "Tolfforsskogen", c. 200 m NW of Tolffors, on fallen last year's culms (in the specimen are fragments of shoots), 7 July 1951, leg. J. A. Nannfeldt, Fungi exsiccati suecici praesertim Upsalienses no. 3428 (isotype), PRM 877293, lit.: Holm and Nannfeldt (1992). – **2)** Småland, Lidhult parish, N of "Knotsnabben", on last year's culms (in the specimen are fragments of shoots), 2 July 1929, leg. J. A. Nannfeldt, l. c. no. 3429, PRM 877346, lit.: Holm and Nannfeldt (1992). – **Poland: 3a)** Karkonosze (Krkonoše) Mts., WNW of Śnieżka (Sněžka) Mt., SW edge of cirque of "Mł. Staw" lake, open area with *Nardus stricta*, *Deschampsia cespitosa*, *Juncus filiformis*, *Sphagnum girgensohnii* and *S. capillifolium* between tourist trail and growth of *Pinus mugo*, *Vaccinium myrtillus* and *Homogyne alpina*, alt. 1390 m, 2 June 2002, PRM 900922, associated with *Arthrinium cuspidatum*, *Myriosclerotinia curreyana* and *Niptera eriophori*. – **3b)** Karkonosze Mts., same locality, site with *Juncus squarrosus*, *J. filiformis* and tuft of *Molinia caerulea* in open area (mosaic of *Eriophorum vaginatum*, *E. angustifolium*, *Calluna* *vulgaris*, *Nardus stricta* and *Deschampsia cespitosa*) between tourist trail and growth of *Pinus mugo*, alt. 1390 m, on stems and bracts, 2 June 2002, PRM 900921, associated with *Arthrinium cuspidatum*. – **4)** Tatra Mts., Tatra National Park, N-exposed slopes of the Przełęcz Liliowe pass down to the "Dolina Gąsienicowa" valley, mosaic of *Pinetum mugi*, small patches of tall herb vegetation and small boggy depressions with *Juncus filiformis* and *Eriophorum vaginatum*, alt. 1600 m, on dead stems, 9 July 1993, leg. et det. C. Scheuer (no. 3075, 3145; Mycotheca Graecensis no. 99, Dupla Fungorum 1997), GZU, lit.: Scheuer and Chlebicki (1997), associated with e.g. *Arthrinium cuspidatum*, *Brunnipila calycioides*, *Micropeltopsis nigro-annulata* var. *papillosa*. – **Czech Republic: 5a)** Krušné hory Mts., 7 km NE of the village of Stříbrná, nature reserve "Velké Jeřábí jezero", bog with *Pinus* ×*pseudopumilio*, *Vaccinium myrtillus*, *V. uliginosum*, *Empetrum nigrum*, *Oxycoccus* sp., *Eriophorum vaginatum*, *E. angustifolium*, *Calluna vulgaris*, *Polytrichum commune* and scattered *Carex nigra*, at margin of the bog are patches of *Juncus filiformis* and *Avenella flexuosa*, patches of *Molinia caerulea*, also *Trientalis europaea* and some spruces are present in the bog, the bog is surrounded by spruce forests and by a clearing at the W side, alt. 940 m, on bracts lying parallelly over *Sphagnum fallax* tufts, 24 June 2002, PRM 900943. – **5b)** Krušné hory Mts., 7 km NE of the village of Stříbrná, nature reserve "Velké Jeřábí jezero", sloping open boggy area (SSE of Loc. 5a) with growth of *Carex rostrata* and *Sphagnum flexuosum* with scattered patches of *Juncus filiformis*, *Carex nigra* and *C. canescens*, alt. 925 m, on shoots lying parallelly on *Sphagnum flexuosum* tufts, 23 June 2002, PRM 900936, associated with *Arthrinium cuspidatum*. – **6)** Krušné hory Mts., "Božídarské rašeliniště" bog, 700 m SW of the village of Boží dar, moist meadows in close vicinity of the bog (*Pinus* ×*pseudopumilio*, *Betula nana*, *Vaccinium myrtillus*, *V. uliginosum*, *V. vitis-idaea*, *Eriophorum vaginatum*, *Oxycoccus* sp.) with *Bistorta major*, *Potentilla erecta*, *Galium* sp., *Comarum palustre*, *Carex rostrata*, *C. nigra*, *C. canescens*, *Eriophorum angustifolium*, *Juncus filiformis* and *Sphagnum fallax*, alt. 1005 m, on bracts lying in tufts of *Deschampsia cespitosa*, *Molinia caerulea* or among *Sphagnum fallax* plants, 22 June 2002, PRM 900935, associated with *Arthrinium cuspidatum*, *Niptera eriophori*. – **7)** Jizerské hory Mts., N of village of Josefův důl, 2.75 km WSW of settlement Smědava, nature reserve "U Posedu", small open place among young spruces with *Juncus filiformis*, *Eriophorum vaginatum*, *Carex canescens*, *Avenella flexuosa* and *Vaccinium myrtillus*, alt. 1000 m, on stems and bracts lying among *Sphagnum girgensohnii* plants, on *Polytrichastrum formosum* tufts, on *Cephalozia bicuspidata* growths and on decaying decorticated log, 30 June 2002, leg. M. Suková et D. Novotný, rev. C. Scheuer, PRM 900940, associated with *Arthrinium cuspidatum*, *Niptera eriophori* (PRM 900941), *Mollisia* cf. *palustris*. – **8)** Krkonoše Mts. (Giant Mts.), Labský důl valley, NW edge of "Harrachova jáma" cirque, small tufts of *Juncus filiformis* in open area with *Nardus stricta*, in close vicinity of rocks, snowfield and growths of *Pinus mugo*, *Vaccinium myrtillus* and *Athyrium* sp., the locality is not far above a spruce forest with birch and *Sorbus aucuparia*, alt. 1220 m, on lying stem and leaf, 31 May 2002, PRM 900920. – **9)** Krkonoše Mts., NW of Luční bouda chalet, "Čertova louka" bog (*Eriophorum angustifolium*, *E. vaginatum*, *Trichophorum* sp., *Carex pauciflora*, *Andromeda polifolia*, *Pinus mugo*, *Vaccinium myrtillus*), area with *Molinia caerulea*, scattered *Juncus filiformis* and *Carex nigra*, *Sphagnum* spp., *Polytrichum commune*, *Homogyne alpina* and *Nardus stricta*, alt. 1385 m, on stem and bract among *Sphagnum* plants, 14 July 2002, not. M. Suková. – **10a)** Krkonoše Mts., "Úpské rašeliniště" bog, 1 km ENE of Luční bouda chalet, mosaic of growths of *Pinus mugo* and open areas with *Eriophorum angustifolium*, *E. vaginatum*, *Trichophorum* sp. and *Andromeda polifolia*. *Juncus filiformis* grows along the tourist trail on a mesic place with moss, *Deschampsia cespitosa*, *Homogyne alpina*, *Taraxacum* sp. and *Viola* sp., alt. 1430 m, on stems and bracts, 5 June 2002, PRM 900924 (the material of *N. carneola* was relatively old), associated with *Arthrinium cuspidatum* (PRM 900925). – **10b)** Krkonoše Mts., same locality and similar place with *Juncus filiformis* by the trail, 5 June 2002, PRM 900926, associated with *Arthrinium cuspidatum* and *Brunnipila calycioides*. – **11)** Krkonoše Mts., "Obří důl" valley, bog 250 m N of chalet Bouda v Obřím dole, open area (surrounded by spruce forest) with *Sphagnum fallax*, *Trientalis europaea*, *Potentilla erecta*, *Vaccinium vitis-idaea*, *V. myrtillus*, *Calamagrostis* sp., *Anthoxanthum odoratum*, *Nardus stricta*, *Juncus effusus* and *J. filiformis*, alt. 920 m, on languid (flattened, not terete) stems, bracts and leaves lying among *Sphagnum fallax* plants, 4 June 2002, PRM 900923, associated with *Arthrinium cuspidatum* and *Niptera eriophori*. – **12)** Krkonoše Mts., nature reserve "Černohorské rašeliniště" bog (*Pinus mugo*, *Vaccinium myrtillus*, *V. uliginosum*, *Eriophorum vaginatum*, *Trichophorum cespitosum* and *Andromeda polifolia*), WNW margin of the reserve (spruce forest with *Vaccinium myrtillus*, *V. vitis-idaea* and *Trientalis europaea*, and open areas with *Vaccinium uliginosum*, *Eriophorum vaginatum* and *Oxycoccus* sp.); *Juncus filiformis* grows by a drainage ditch (*Sphagnum fallax*, *S. cuspidatum*, *S. russowii* and *Polytrichum commune*) along a tourist trail, alt. 1180–1190 m, on shoots lying parallelly over tufts of peat-mosses (*Sphagnum* spp.), 6 June 2002, PRM 900927, associated with *Arthrinium cuspidatum* and *Brunnipila calycioides*. – **13a)** Hrubý Jeseník Mts., Mt. "Velký Jezerník", nature reserve Slatě, in saddle between the peaks of Velký Jezerník and Malý Děd, bog (*Eriophorum vaginatum*, *Vaccinium uliginosum*, *V. vitis-idaea*, *V. myrtillus*, *Oxycoccus* sp., *Andromeda polifolia*, *Sphagnum* spp., *Polytrichum commune*, *Empetrum nigrum*, *Calluna vulgaris*, also *Melampyrum pratense*, *Avenella flexuosa* and *Trientalis europaea*) surrounded by spruce forest (*Sphagno-Piceetum*) with peat-mosses (*Sphagnum* spp.); *Juncus filiformis* grows sporadically in the bog and more frequent along a wooden pathway where also *Deschampsia cespitosa*, *Nardus stricta*, *Anthoxanthum* sp., *Carex canescens* and *C. echinata* occur, alt. 1300 m, on stems and bracts lying among dead *Nardus* shoots or lying on wood of the path, 5 July 2002, PRM 900942, associated with *Arthrinium cuspidatum* and *Niptera eriophori*. – **13b)** Hrubý Jeseník Mts., same locality, small site with *Juncus filiformis*, brownish green coloured peat-moss and *Polytrichum commune* (surrounded by *Nardus stricta*, *Deschampsia cespitosa*, *Molinia caerulea*, *Juncus effusus* and *Homogyne alpina*) between the wooden pathway and the spruce forest, alt. 1300 m, on shoots lying on tufts and among plants of *Sphagnum* sp. and *Polytrichum commune*, 20 May 2003, PRM 900919, associated with *Arthrinium cuspidatum*, *Brunnipila calycioides* and *Mycosphaerella perexigua* var. *minima*. – **14)** Šumava Mts. (Bohemian Forest), 2.5 km N of the village of Kvilda, "Jezerní slať" bog, *Sphagno-Pinetum mugi* with *Pinus ×pseudopumilio*, *Betula nana*, *Calluna vulgaris*, *Vaccinium myrtillus*, *V. uliginosum*, *V. vitis-idaea*, *Eriophorum vaginatum*, *E. angustifolium*, *Carex nigra*, *C. canescens* and *C. rostrata*; *Juncus filiformis* occurs only in a small area between *Pinus ×pseudopumilio* and a wooden pathway with *Polytrichum commune*, *Sphagnum flexuosum*, *Nardus stricta*, *Melampyrum pratense*, *Eriophorum angustifolium* and *Carex nigra*, alt. 1075 m, 49° 02' 30" N, 13° 34' 40" E, on bracts and leaves lying among *Sphagnum flexuosum* plants and among *Polytrichum commune* stems, 12 June 2002, rev. C. Scheuer, PRM 896488, associated with *Arthrinium cuspidatum* (PRM 896490), *Brunnipila calycioides*, *Mycosphaerella perexigua* var. *minima* and *Niptera eriophori*. – **15)** Šumava Mts., 2.75 km NE of the site Březník, "Ptačí nádrž", at left bank of Ptačí potok brook, open area with *Carex rostrata*, *Juncus filiformis*, *Sphagnum fallax*, *Polytrichum commune* and less frequent *Carex nigra*, at the margin of the area near a spruce forest also occur *Vaccinium myrtillus*, *Trientalis europaea* and *Calamagrostis* sp., alt. 1130 m, on dead bracts lying on tufts and among plants of *Sphagnum fallax*, 13 June 2002, PRM 896498, associated with *Arthrinium cuspidatum* and *Brunnipila calycioides* (PRM 896499). – **16)** Šumava Mts., S of Modrava village, "Luzenské údolí" valley (with bogs surrounded by uniform spruce forest), c. 1 km S of Březník, on left side of the road Hraniční louka – Březník, by drainage ditch (*Juncus filiformis*, *Carex nigra*, *C. canescens*, *Sphagnum fallax*, *Polytrichum commune* and *Deschampsia cespitosa*) between road and bog (mosaic of *Pinus ×pseudopumilio* and *Eriophorum vaginatum*, *Vaccinium uliginosum*, *Trichophorum cespitosum*, *Oxycoccus* sp. and *Andromeda polifolia*), alt. 1147 m, on stems, bracts and leaves lying among *Sphagnum fallax* plants or lying parallelly on lying decorticated fragment of spruce trunk, 7 June 2001, rev. C. Scheuer, PRM 895088, lit.: Suková (2003), associated with *Arthrinium cuspidatum*, *Brunnipila calycioides*, *Mycosphaerella perexigua* var. *minima* and *Phyllachora therophila*. – **17a)** Šumava Mts., ESE bank of "Plešné jezero" lake, vegetation with *Juncus filiformis* and *Sphagnum girgensohnii* between bank and spruce forest (*Dryopterido dilatatae-Piceetum*) with *Sorbus aucuparia*, *Vaccinium myrtillus*, *V. vitis-idaea* and *Dryopteris dilatata* (sporadically *Betula*, *Pinus ×pseudopumilio* and *Salix* sp. by the bank), alt. 1090 m, on stems and especially on bracts lying parallelly on root of a tree, on leaves of birch, on tufts of *Sphagnum girgensohnii* and *Polytrichum commune*, 10 June 2002, PRM 900932 and 900933, associated with *Arthrinium cuspidatum*, *Brunnipila calycioides*, *Cistella fugiens* (base of stem among *Sphagnum* plants, PRM 900934) and *Lachnum diminutum*. – **17b)** Šumava Mts., SW bank of "Plešné jezero" lake, vegetation with *Calamagrostis* sp., *Carex rostrata*, *Sphagnum girgensohnii* and *Polytrichum commune*, alt. 1090 m, on stems, bracts and leaves lying on tufts and among plants of *Sphagnum girgensohnii*, 30 June 1999, det. C. Scheuer, PRM 895092. – **18a)** Šumava Mts., 450 m W of Mt. Plechý, "Rakouská louka" bog, central part of the bog (*Eriophorum vaginatum*, *Vaccinium uliginosum*, *Melampyrum pratense*, *Andromeda polifolia*, *Oxycoccus* sp.), alt. 1345 m, on a few separate shoots lying among *Sphagnum russowii* plants, 26 June 2002, not. M. Suková. – **18b)** Šumava Mts., 450 m W of Mt. Plechý, "Rakouská louka" bog, margin (*Sphagno-Piceetum* with *Vaccinium myrtillus*, *Molinia caerulea*, *Eriophorum vaginatum*, *Polytrichum commune*, *Sphagnum fallax*) of the bog near spruce forest, alt. 1345 m, on separate shoots lying on tufts and among plants of *Sphagnum fallax*, 26 June 2002, PRM 896491, associated with *Arthrinium cuspidatum* and *Brunnipila calycioides*. – **18c)** Šumava Mts., 400 m WSW of Mt. Plechý, SSE of "Rakouská louka" bog, open area between spruce forest and tourist trail Plechý – Trojmezí, *Juncus filiformis* areas (with *Carex nigra* and *C. canescens*) in stands of *Nardus stricta*, alt. 1345 m, 26 June 2002, PRM 900938, associated with *Arthrinium cuspidatum* and *Brunnipila calycioides*. – **19)** Novohradské hory Mts., 800 m SE of Pohořský rybník pond, N of nature reserve "Pohořské rašeliniště" (bog with *Pinus* ×*pseudopumilio*, *Vaccinium myrtillus*, *V. uliginosum*, *Eriophorum vaginatum*), open area (*Eriophorum angustifolium*, *E. vaginatum*, *Carex rostrata*, *C. nigra*, *C. canescens*, *Juncus filiformis*, *Avenella flexuosa*, *Nardus stricta*, *Potentilla erecta*, *Sphagnum* spp. and *Polytrichum commune*) between the reserve, spruce forest and road, alt. 888 m, on stems and bracts lying among plants of *Sphagnum fallax* and *S. girgensohnii* or lying parallelly over *Polytrichum commune* tufts, 9 June 2002, not. M. Suková, associated with *Arthrinium cuspidatum* (PRM 900929), *Brunnipila calycioides* (PRM 900930) and *Niptera eriophori* (PRM 900931). – **Germany: 20)** Bayern, Bayerischer Wald Mts., "Rachelsee" (Roklanské jezero) lake, site with *Warnstorfia exannulata*, *Sphagnum fallax*, *Polytrichum* sp., fallen leaves of *Fagus*, *Juncus filiformis*, *Carex canescens*, *C. rostrata* and *C. echinata* at waterside near dike, alt. 1072 m, on bracts lying on *Warnstorfia exannulata* growths or among *Sphagnum fallax* plants, 27 June 2002, PRM 900939. – **Austria: 21)** Oberösterreich, Bayerischer Wald Mts., 400 m SWW of Plöckenstein (Plechý) Mt., SSE of "Rakouská louka" bog, growth of *Juncus filiformis*, *Avenella flexuosa*, *Nardus stricta* and *Polytrichum* sp. between tourist trail and spruce forest (*Calamagrostio villosae-Piceetum*), alt. 1345 m, on shoots lying parallelly on soil and on *Dicranella heteromalla*, 26 June 2002, PRM 900937, associated with *Arthrinium cuspidatum* and *Brunnipila calycioides*. – **22)** Steiermark, Niedere Tauern Mts., Wölzer Tauern Mts., c. 19 km SSW of the town of Liezen, 9 km SE of the village of Donnersbach, SSW above the resort Planneralm, in a boggy area named "Plannerkessel", close to the small lake Kothüttensee, along the path to the peak Karlspitze, small depression in *Pinus mugo* bog (characteristic plants in the bog: *Carex magellanica*, *C. echinata*, *Eriophorum vaginatum*, *Andromeda polifolia*; in wetter places in the depressions also *Carex limosa*, *C. canescens*, *Juncus filiformis*, *Trichophorum cespitosum*), alt. c. 1800 m, 47° 23' N, 14° 11' E, 25 August 1981, leg. et det. C. Scheuer (Nr. 917), GZU.

Ecology

Habitat of *Juncus filiformis* shoots. *Naeviopsis carneola* was usually found on terete *Juncus* shoots lying among brownish green (*Sphagnum* sect. *Cuspidata* – *S. fallax*, *S. flexuosum*) or green coloured peat-mosses (*S. girgensohnii*), less frequently in *Polytrichum commune*. It was seldom found on or among other bryophytes (*Cephalozia bicuspidata*, *Warnstorfia exannulata*, *Polytrichastrum formosum*) or in tufts of grasses. *Naeviopsis carneola* occurred also on shoots lying over mosses or other materials (wood, roots of trees, fallen leaves), e.g. in a rich growth of *Juncus filiformis* at the ESE side of Plešné jezero lake (Loc. 17a, locality with high air humidity). It was also found on shoots lying on decorticated prostrate trunks in Luzenské údolí valley (Loc. 16) in the Šumava Mts. and in the Jizerské hory Mts. (Loc. 7).

Changes during the vegetation season were studied in Hrubý Jeseník (Loc. 13a-b) and at the ESE side of Plešné jezero lake (Loc. 17a). The shoots of *J. filiformis* are first (in the beginning of the season) situated above the peat-mosses and of light colour. After that the peat-mosses (similarly in case of *Polytrichum commune*) grow and the shoots become more and more lodged. Subsequently they are enclosed by *Sphagnum* cushions and sometimes the surface tissues of the plants can gradually be destroyed (e.g. collection PRM 895092, Loc. 17b). Fructification of *Naeviopsis* begins at Plešné jezero already on shoots above the *Sphagnum* tufts (e.g. collections PRM 900932–3 from 10 June 2002, Loc. 17a), later (investigated on 26 June 2002) – when lying within *Sphagnum* cushions – only *Lachnum diminutum* was found frequently. In Hrubý Jeseník fructification begins when the shoots are lying partly on and partly among the peat-mosses. Parts of shoots lying in *Sphagnum* are frequently more or less brown-coloured thanks to the water, other, not so moist parts are light-coloured. The brown colour disappears soon after drying. Apothecia of the *Naeviopsis* are usually more frequent on the brown parts (observed during the collecting of specimen PRM 900919, Loc. 13b). Brown-coloured parts of shoots with apothecia were seen also in localities 4, 5a and 8.

Other fungi found on the same shoots as *Naeviopsis carneola*: *Arthrinium cuspidatum* (Cooke et Harkn.) Höhn.; *Brunnipila calycioides* (Rehm) Baral; *Cistella fugiens* (Buckn.) Matheis; *Lachnum diminutum* (Roberge) Rehm; *Micropeltopsis nigro-annulata* (Webster) Spooner et Kirk var. *papillosa* (Scheuer) Magnes et Hafellner; *Mollisia* cf. *palustris* (Roberge ex Desm.) P. Karst. – species with grey-brown apothecia and one-celled ascospores slightly attenuated towards one end; *Mycosphaerella perexigua* (P. Karst.) Johans. var. *minima* Johans. – identified according to Tomilin (1979) and Scheuer (1988); *Myriosclerotinia curreyana* (Berk.) N. F. Buchw.; *Niptera eriophori* (L. A. Kirchn.) Rehm; *Phyllachora therophila* (Desm.) Arx et E. Müll.

Naeviopsis carneola was most frequently observed together with *Arthrinium cuspidatum* and *Brunnipila calycioides*. *Arthrinium cuspidatum* was located at the bases and lower parts of shoots, *Naeviopsis carneola* on stems near inflorescences and especially on bracts (except of their tips), only rarely on leaves. *Brunnipila calycioides* was relatively often found in upper parts of bracts and leaves. Also other fungi were relatively frequent – *Mycosphaerella perexigua* var. *minima* occurring on various parts of shoots, *Niptera eriophori* on stems and especially on bracts (in many cases alternating with *Naeviopsis carneola*) and *Mollisia* cf.

palustris located usually on inflorescences and adjacent parts of stems and bracts. Clusters of perithecia of *Phyllachora therophila* and the superficial catathecia of *Micropeltopsis nigro-annulata* var. *papillosa* (Loc. 4) were scattered over various parts of the shoots. *Cistella fugiens* was recorded only once, on the base of a stem. This species occurs rather on bases of not so old shoots standing or less frequently lying in conditions different from those favourable for *Naeviopsis carneola*. It is more common on sheaths and basal parts of stems of *Juncus effusus*. Apothecia of *Myriosclerotinia curreyana* were found on various parts of stems only at Mł. Staw lake (Loc. 3a). Sclerotia (probably of *M. curreyana*) occurred at Černohorské rašeliniště bog (Loc. 12) on *Juncus filiformis* stems without *Naeviopsis carneola*. *Lachnum diminutum* occurs on various parts of shoots later in the season, so it was not often found together with *Naeviopsis carneola*.

Period of fructification of *Naeviopsis carneola* and associated fungi in the Czech Republic and close vicinity (border mountains). *Naeviopsis carneola* can be found mature from the last week of May to the first half of June, depending on altitude, local climatic conditions and habitat of *Juncus filiformis* shoots. After that period, in the second half of June, it is more or less old, but it can be recognised by more or less open slits or valves, and by mature asci at least present in some apothecia. The latest collections containing mature apothecia in good condition were made at the beginning of July in the Hrubý Jeseník Mts. (Loc. 13a).

Arthrinium cuspidatum sporulates on *Juncus filiformis* at the same time as *Naeviopsis carneola*. Apothecia of *Brunnipila calycioides* are present longer and are usually found young or mature together with mature *Naeviopsis carneola*. The pseudothecia of *Mycosphaerella perexigua* var. *minima* are mature at the end of May and in the first half of June. *Phyllachora therophila* is usually old in June. Apothecia of *Niptera phaea* and *Mollisia* cf. *palustris* usually mature in June (recent finds on *Juncus filiformis*) and later (according to experience from the Czech Republic and other *Juncaceae*, it is known also from summer and autumn). *Lachnum diminutum* appears already in the first half of June, but it is more frequent later (Plešné jezero lake, Loc. 17a). *Myriosclerotinia curreyana* (see also Suková and Svrček 2001) matures on *Juncus filiformis* in the subalpine belt in the first half of June.

Substrate specificity. *Naeviopsis carneola* is strictly specific to *Juncus filiformis* whereas other fungi found on *J. filiformis* (*Arthrinium cuspidatum*, *Brunnipila calycioides*, *Lachnum diminutum*, *Mycosphaerella perexigua* var. *minima*, *Myriosclerotinia curreyana*) occur also on other species of *Juncus*. *Niptera eriophori* is known also from other graminaceous marsh plants from the families *Juncaceae* and *Cyperaceae*. *Micropeltopsis nigro-annulata* var. *papillosa* is probably rather unspecific, but apparently has a preference for such substrata. *Mollisia palustris* is given from various graminaceous plants in the literature, but our identifications are still tentative.

Altitude. *Naeviopsis carneola* occurs in open areas in the supramontane (Hejný and Slavík 1988) and subalpine belt in Central Europe. In the subalpine belt it was found in the Krkonoše Mts. (Mł. Staw, Čertova louka, Úpské rašeliniště, altitudes 1385–1430 m), and Tatra Mts. (Dolina Gąsienicowa, alt. 1550–1650 m), and in the Eastern Alps (Niedere Tauern, alt. 1800 m). Other recently studied localities are in the supramontane belt (altitude 888–1005 m in Krušné hory, Jizerské hory, Novohradské hory; 1070–1150 m in the Šumava Mts.; (920-)1180–1345 m in Krkonoše and Hrubý Jeseník). The species does not occur at lower altitudes characterised by *Juncus filiformis* growing in communities with *Comarum palustre*, peat-mosses (*Sphagnum* spp.) and various other marsh plants. Such localities were investigated in the Novohradské hory Mts. (Mlýnský rybník pond, alt. 760 m and Huťský rybník dam lake, alt. 800 m) and in the Žďárské vrchy hills (Velké Dářko pond, alt. 615 m). *Comarum palustre* (species typical of submontane and lower altitudes) has been found together with *Naeviopsis carneola* at the only one studied locality (Božídarské rašeliniště, Loc. 6).

Concerning associated fungi, *Arthrinium cuspidatum*, *Brunnipila calycioides* (see also Chlebicki 1990), and *Micropeltopsis nigro-annulata* var. *papillosa* are distributed in the montane (sensu lato), subalpine and alpine belt (where they occur on *Juncus trifidus*). *Myriosclerotinia curreyana* has been reported from subalpine (on *Juncus filiformis*), montane and lower altitudes (on *J. effusus*) in the Czech Republic (Suková and Svrček 2001). *Mollisia* cf. *palustris* occurs in montane and lower altitudes. *Niptera eriophori* and *Cistella fugiens* are common fungi at various altitudes. *Lachnum diminutum* is known from montane and especially lower altitudes.

Types of localities. *Naeviopsis carneola* was found in various types of localities:

a) Glacial cirques. The cirque of Plešné jezero lake (Loc. 17a-b) in the Šumava Mts. (Bohemian Forest) is considered the most natural locality of *N. carneola* in the Czech Republic. According to Neuhäuslová (2001), its moraine is covered mostly by natural spruce forest on siliceous substrate, particularly *Dryopterido dilatatae-Piceetum* SE of the lake and *Athyrio alpestris-Piceetum* on slopes SW of the lake. *Juncus filiformis* forms a stand with *Sphagnum girgensohnii* at ESE side (Loc. 17a) and a stand with *Calamagrostis* sp., *Carex rostrata*, *Sphagnum girgensohnii* and *Polytrichum commune* (Loc. 17b) in a relatively large horizontal area by the SW bank of the lake. *Carex rostrata* is more frequent in an adjacent wetter area by the water. Only fragmentary occurrence of *Juncus filiformis* with *Naeviopsis carneola* was found in the cirque of Rachelsee lake (Loc. 20) on the

German side of the mountain range (Bayerischer Wald) on a rather man-influenced stand between a path and the bank near the dike.

b) Subalpine bogs with *Pinus mugo* or mosaics of *Pinus mugo* growths, boggy places with typical plants and more mesic open places with prevailing *Nardus stricta* (Locs. 3a-b, 4, 8, 9, 10a-b, 22). Only few small tufts of *Juncus filiformis* were found in the open area with *Nardus stricta* among growths of *Pinus mugo* in Harrachova jáma cirque in Labský důl valley (Loc. 8). The occurrence of *Naeviopsis carneola* there seems to be peripheral and we suppose that *Juncus filiformis* and *Naeviopsis carneola* are more frequent on Pančavská louka bog and adjacent bogs on a plateau W above Labský důl valley.

c) Supramontane bogs (Locs. 5a, 12, 13a-b, 14, 16, 18a-b) often with *Pinus mugo* or *Pinus ×pseudopumilio* (lacking e.g. at Loc. 13a-b), with *Eriophorum vaginatum*, other typical plants (for details see "Material studied and character of localities") and mostly with red coloured peat-mosses. Separate shoots of *Juncus filiformis* among "red coloured" *Sphagnum russowii* in the centre of a bog has been found only once (Loc. 18a). *Juncus filiformis* occurs regularly in wetter places in marginal parts of the bogs, in slightly depressed places along wooden pathways and near drainage ditches e.g. along the roads. Brownish green coloured peat-mosses (e.g. *Sphagnum fallax*, *S. flexuosum*) are often present together with *Juncus filiformis* on such places.

d) Sloping (Locs. 5b, 15) or horizontal (Loc. 11) open boggy areas surrounded by spruce forests in the supramontane belt. Large growths of brownish green coloured peat-mosses (e.g. *Sphagnum fallax*, *S. flexuosum*) prevail there together with *Carex rostrata* and/or *Calamagrostis* sp. scattered regularly over the whole area of the growth. *Juncus filiformis*, *Carex nigra* and *Polytrichum commune* are usually also present.

e) Mostly uniform, large stand of *Juncus filiformis* on a man-influenced stand (Loc. 21, alt. 1345 m) in open area along a tourist trail surrounded by spruce forests. *Naeviopsis carneola* was present only in some parts of the stand where shoots are lying under wet conditions.

Various *Juncus filiformis* stands without occurrence of *Naeviopsis carneola* have been studied too. *Arthrinium cuspidatum* prevails on whole shoots in less humid or mesic localities in the supramontane and subalpine belt (e.g. locality at "Nad Malým kotlem" crossroad, in saddle between Mt. Velký Máj and Mt. Jelení hřbet in the Hrubý Jeseník Mts., Czech Republic, dried up boggy place with *Eriophorum vaginatum* and *Carex* spp., alt. 1330 m). *Arthrinium cuspidatum* has been collected also on several *Juncus filiformis* shoots in a stand affected by human activities (e.g. open area between road and clearing in cultivated spruce forest E of nature reserve Labský důl valley in the Krkonoše Mts., alt. 1170 m).

Distribution (Fig. 4)

According to the present state of knowledge, *Naeviopsis carneola* is distributed in Scandinavia and in mountains of Central Europe. In Scandinavia it is known from Sweden (Holm and Nannfeldt 1992) and its occurrence in Norway is expected (the first author observed old material of the species in the National Parks Rondane and Ormtjernkampen in August 2002). In Central Europe it is known from the Western Sudetes (Jizerské hory Mts., Krkonoše Mts.), Eastern Sudetes (Hrubý Jeseník Mts.), Carpathians (Tatra Mts.), mountains of the Hercynian phytogeographical subprovince (Krušné hory Mts., Šumava Mts., Novohradské hory Mts.) and from the central range of the Eastern Alps (Niedere Tauern). *Naeviopsis carneola* is considered to be a species with a boreal-montane distribution with a tendency to occur in the subalpine belt.

In the Czech Republic, *N. carneola* is distributed in protected areas in border mountains. Altogether 16 localities were found and some further localities can be expected – e.g. Modravské slatě bogs in the Šumava Mts. and Pančavská louka bog in the Krkonoše Mts. Šumava (the richest locality is Plešné jezero lake, Loc. 17a-b) and Krkonoše (the richest locality is Černohorské rašeliniště bog, Loc. 12) are the most important centres of occurrence of the species in the Czech Republic. In the other Czech mountains only few localities of *N. carneola* have been found, mostly in isolated nature reserves. The richest of them is Velké Jeřábí jezero bog (Loc. 5a) in the Krušné hory Mts. The locality Malení on Mt. Smrk (alt. 1125 m) in Rychlebské hory Mts. (Eastern Sudetes) seemed to be favourable for the occurrence of *N. carneola*. However, the area of the bog is too small and the substrate (*Juncus filiformis*) is lacking and consequently, the species was not found there.

The Slovak part of the Tatra Mts. has not yet been investigated. The presence of the species in this territory and probably also in some other Slovak mountains (belonging to the Carpathians) is expected.

CONCLUSIONS

Naeviopsis carneola is considered to be a species with a boreal-montane distribution with a tendency to occur in the subalpine belt. At present it is known from Scandinavia and from mountains of Central Europe. In the Czech Republic, *N. carneola* is related to protected areas in the supramontane and subalpine belt, with centres of occurrence in the National Parks of Šumava Mts. and Krkonoše Mts. *Naeviopsis carneola* is strictly specific to *Juncus filiformis*.

The ecology of the species was investigated mainly in the Czech Republic and its close vicinity (border mountains). Habitat conditions most important for the occurrence of *N. carneola* seem to be high humidity (1), cold climate (2) and subsequently nativeness of vegetation (3). (1) Most frequently it occurs on

Fig. 4. Known localities of *Naeviopsis carneola* B. Hein et Nannf., for localities 1–22 see "Material studied and character of localities".

shoots lying within *Sphagnum* sect. *Cuspidata*, *S. girgensohnii* and less frequently *Polytrichum commune* tufts. (2) It is lacking in localities at lower altitudes even if the plant cover is similar – composed of the same species of plants as in some localities of *Naeviopsis carneola*. (3) In addition to localities with natural vegetation it is known also from man-influenced localities, however, these localities are regularly not far from those with natural vegetation. On shoots of *Juncus filiformis*, *Naeviopsis carneola* was most frequently observed together with *Arthrinium cuspidatum* and *Brunnipila calycioides*. *Arthrinium cuspidatum* was located in lower parts of shoots, *Naeviopsis carneola* on stems near inflorescences and especially on bracts (except of their tips, where *B. calycioides* often occurred). The period of fructification is relatively short (from the last week of May to the first half of June, rarely to the beginning of July) in comparison with most other fungi found on *Juncus filiformis*.

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Endophytic fungal assemblage of two halophytes from west coast mangrove habitats, India

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Maria G. L. and Sridhar K. R. (2003): Endophytic fungal assemblage of two halophytes from west coast mangrove habitats, India. – Czech Mycol. 55: 241–251

Twenty-five endophytic fungi comprising three ascomycetes, 20 mitosporic fungi and two sterile fungi were recovered from two halophytes (*Acanthus ilicifolius* and *Acrostichum aureum*) of a west coast mangrove habitat in India. Overall colonisation of tissue segments by endophytes ranged between 74.5 % (*Acanthus ilicifolius*) and 77.5 % (*Acrostichum aureum*). Analysis using the Jaccard's similarity coefficient revealed 16–25 % similarity in endophyte assemblage among different tissues, and 24.5 % between the two hosts. Out of four tissues screened, species richness and diversity were high in stems of *Acanthus ilicifolius* and roots of *Acrostichum aureum*. The most dominant endophyte was *Colletotrichum* sp. in prop roots of *Acanthus ilicifolius*, and Yeast sp. 1 in rhizomes of *Acrostichum aureum*. Among the dominant endophytes (colonisation frequency >5 %), *Acremonium* and Yeast sp. 1 were common to both hosts. *Acanthus ilicifolius* showed dominance of a single species, (*Colletotrichum* sp.), while in *Acrostichum aureum* multiple species dominance was seen (*Acremonium* sp., *Penicillium* sp. and Yeast sp. 1). Only one typical marine mitosporic fungus (*Cumulospora marina*) was recovered from the roots of *Acanthus ilicifolius*.

Key words: mangroves, halophytes, endophytes, fungi, India

Maria G. L. a Sridhar K. R. (2003): Společenstvo endofytických hub ve dvou druzích halofytů v mangrovech na západním pobřeží Indie. – Czech Mycol. 55: 241–251

V mangrovech na západním pobřeží Indie bylo ze dvou druhů halofytů (*Acanthus ilicifolius* a *Acrostichum aureum*) zjištěno 25 druhů endofytických hub, z toho 3 druhy askomycetů, 20 druhů anamorfních hub a 2 druhy hub ve sterilním stavu. Celková kolonizace segmentů pletiv se pohybovala mezi 74.5 % (*Acanthus ilicifolius*) a 77.5 % (*Acrostichum aureum*). S použitím Jaccardova indexu podobnosti byla zjištěna 16–25-procentní podobnost ve složení společenstva endofytů mezi různými pletivy a 24.5-procentní podobnost mezi oběma hostiteli. Ze 4 studovaných typů pletiv byla nejvyšší diverzita endofytů ve kmenech druhu *Acanthus ilicifolius* a v kořenech *Acrostichum aureum*. Dominantním endofytem byl druh *Colletotrichum* sp. v oporných kořenech *Acanthus ilicifolius* a kvasinka sp. 1 ve rhizomech *Acrostichum aureum*. Z dalších dominantních endofytů (s frekvencí kolonizace >5 %) byly druhy *Acremonium* sp. a kvasinka sp. 1 společné pro oba hostitele. U *Acanthus ilicifolius* dominoval jeden druh (*Colletotrichum* sp.), zatímco u *Acrostichum aureum* bylo dominantních druhů více (*Acremonium* sp., *Penicillium* sp. a kvasinka sp. 1). Byla nalezena pouze jedna typicky mořská anamorfní houba, a sice *Cumulospora marina* v kořenech druhu *Acanthus ilicifolius*.

INTRODUCTION

The term endophyte refers to the fungi and bacteria, which throughout or part of their life cycle invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues, but cause no symptoms of disease (Wilson 1995). Although endophytes have drawn the attention of mycologists for about 80 years (Lewis 1924), serious efforts to study them begun only in the 1970s (Bernstein and Carroll 1977, Carroll et al. 1977, Carroll and Carroll 1978). Fungal endophytes have been isolated from a variety of plant species (Wilson and Carroll 1994), generally from the temperate parts of the world (Petrini 1986, 1991). Studies on fungal endophytes of the tropical region were initiated recently (Rodrigues and Petrini 1997). Endophytic fungi have been studied at different spatial scales: from different parts of a simple leaf to a geographic scale (Carroll 1995, Taylor et al. 1999). The endophytic mycoflora of tropical plants differs from that of temperate plants (Rodrigues and Petrini 1997, Taylor et al. 1999). For instance, higher numbers of xylariaceous fungi were found in endophyte assemblages from tropical palms when compared with temperate palms (Fröhlich 1997, Petrini et al. 1995). Such difference in endophyte assemblages has been connected to climatic factors (Fisher et al. 1995, Taylor et al. 1999). Besides understanding the ecology, distribution and diversity of fungi, endophytic fungi are the centres of attraction for recognising novel metabolites of agricultural and pharmaceutical value (Strobel et al. 1996).

Among 54 mangrove tree species and 60 mangrove associate plant species, up to 55 have been studied for saprophytic fungi (Jones and Alias 1997). Recently a series of papers has been published on the foliar endophytes of mangrove plant species of the east coast of India (Kumaresan and Suryanarayanan 2001, 2002; Suryanryanan et al. 1998; Suryanarayanan and Kumaresan 2000). Ananda and Sridhar (2002) investigated the diversity of root endophytic fungi of mangrove plant species of the west coast of India. In order to fill the gap in studies on endophytes of all mangrove plant species, the present investigation has been concentrated on the endophytic assemblage of an angiosperm mangrove associate (*Acanthus ilicifolius*) and a pteridophyte mangrove associate (*Acrostichum aureum*) established in a mangrove habitat of the west coast of India.

MATERIALS AND METHODS

Sampling

The angiosperm shrub, *Acanthus ilicifolius* L. and a pteridophyte, *Acrostichum aureum* L. grow abundantly in homogeneous stands along the mangrove vegetation in the Nethravathi River mouth located about 4 km south of Mangalore, south-west coast of India. Ten plants (about 5 m apart) of *Acanthus ilicifolius* and *Acrostichum aureum* each growing in pure stands at low-tide levels were chosen for sampling during the summer season (April-May). In April 2001 five healthy mature leaves, five pieces (about 8–10 mm diameter and 3–5 cm length) of stem, prop roots and roots were randomly cut from each plant of *Acanthus ilicifolius* for the study. From each plant of *Acrostichum aureum* five healthy mature leaves, five pieces (about 1 cm thick and 5 cm length) of rhizome and roots were sampled in May 2001. The plant material was brought to the laboratory in sterile polyethylene bags in cold pack and processed within 4 hours after sampling.

Surface sterilisation and incubation

The plant material was rinsed gently in freshwater to remove debris. From each leaf 0.5 × 1 cm segments were prepared and the rest of the material (*Acanthus ilicifolius*: stem, prop root and root; *Acrostichum aureum*: petiole, rhizome and root) was cut into segments of 1 cm length. They were surface sterilised according to the method outlined by Taylor et al. (1999) with a slight modification. Each set of plant material was immersed in 95 % ethanol for 1 min. followed by immersion in 6 % sodium hypochlorite (BDH, UK) for 6 min. and again in 95 % ethanol for 0.5 min. Later the segments were rinsed three times in sterile distilled water before plating on 1.5 % malt extract agar (MEA) medium complemented with terramycin (250 $\mathrm{mg.l^{-1}}$; Sigma, USA). The plates were incubated at $25 \pm 1\,^{\circ}\mathrm{C}$ for up to seven weeks. The light regime was 12 hours light alternated with 12 hours darkness. Periodically the plates were screened for fungal outgrowth from the plant tissue. Wherever growth occurred, the tips of growing mycelia were transferred to fresh antibiotic-free MEA. In most plant segments fungal growth was seen after 7–10 days of incubation.

Data analyses

The colonisation frequency (% CF), number of fungi per segment and contribution of dominant endophytes (% DE) (Kumaresan and Suryanarayanan 2001) were calculated as follows:

$$\text{Colonisation frequency (\% CF)} = \frac{\text{Number of tissue segments colonised by a fungus}}{\text{Total number of tissue segments assessed}} \times 100$$

$$\text{Number of fungi per segment} = \frac{\text{Total number of isolations of fungi}}{\text{Total number of tissue segments assessed}}$$

$$\text{Contribution of dominant endophytes (\% DE)} = \frac{\text{\% CF of the dominant endophyte}}{\text{Sum of \% CF of all endophytes}} \times 100$$

The independent samples t-test (MICROSTAT, Ecosoft Inc. 1984) was applied to determine significant differences in colonisation frequency of endophytes in different tissues of the same host and between the two host plant species. The diversity (Magurran 1988) and evenness (Pielou 1975) of endophytic fungi in each type of plant material was determined. The percent Jaccard's index of similarity (JI) was calculated for all pairs of host tissues and also for the two hosts (Kenkel and Booth 1992).

RESULTS

Out of 200 segments of *Acanthus ilicifolius*, 149 (74.5 %) segments yielded 160 isolates comprising two ascomycetes, 17 mitosporic fungi and two sterile fungi (Table 1). Differences in endophyte colonisation were seen between the four tissues tested (leaf, stem, prop root and root). The highest number of stem segments (88 vs. 62–84 %) of *Acanthus ilicifolius* was colonised by endophytic fungi. The number of endophytes (12 vs. 8–11), number of isolates (51 vs. 26–48) and mean number of endophytes per segment (1.02 vs. 0.52–0.96) were also higher in stem segments than in other tissues. Among the 200 segments of *Acrostichum aureum* analysed, 155 (77.5 %) segments yielded 158 isolates comprising two ascomycetes and 14 mitosporic fungi (Table 1). The highest number of petiole segments (88 vs. 70–78 %) was colonised by endophytic fungi. Species richness was highest in root segments (11 vs. 7–10), while the mean number of endophytes per segment was higher in rhizomes (1 vs. 0.56–0.88) than in other tissues. Twelve species belonging to ten genera of fungi were common to both hosts. The independent samples t-test for difference in two group means revealed no significant difference in total colonisation frequencies between the two hosts irrespective of tissues.

Five endophytes were dominant (CF, 5 % and more) in both plant species (Table 2). *Colletotrichum* sp. was the most dominant endophyte in *Acanthus ilicifolius* (DE, 32.5 %). It showed 64 % CF in prop roots followed by 32 % CF in stems (Table 1). Yeast sp. 1 was dominant in *Acrostichum aureum* (DE, 36.8 %) and colonisation was highest in rhizomes (38 % CF) followed by roots (32 % CF) (Table 2). Among the dominant endophytes, *Acremonium* sp. and Yeast sp. 1 were common to both hosts. Table 3 shows the species richness, diversity and evenness of endophytic fungi in the four tissues of the host plants. *Acanthus ilicifolius* showed the highest species richness (12) and diversity (0.917; 3.585) of endophytic fungi in stems followed by leaves. In *Acrostichum aureum*, roots showed highest species richness (11) and diversity (0.909; 3.459) followed by leaves. The percent Jaccard's index of similarity (JI) between the four tissue types of *Acanthus ilicifolius* revealed a maximum of 25 % similarity between prop roots and roots (Table 4). In the rest of the tissues, it ranged between 16 and 23.1 %. In *Acrostichum aureum* also the maximum similarity was 25 % between leaves and roots; petioles and

Table 1. Colonisation frequency (% CF) of fungal endophytes in different tissues of *Acanthus ilicifolius* and *Acrostichum aureum*.

Endophyte	*Acanthus ilicifolius*					*Acrostichum aureum*				
	Tissues				Total % CF	Tissues				Total % CF
	Leaf	Stem	Prop root	Root		Leaf	Petiole	Rhizome	Root	
Ascomycetes										
Ascotricha chartarum Berk.	0	0	0	0	**0**	4	0	0	0	**1**
Yeast sp. 1	0	0	4	16	**5**	4	10	38	32	**21**
Yeast sp. 2	0	2	0	4	**1.5**	0	0	0	0	**0**
Mitosporic fungi										
Acremonium sp.	4	8	6	6	**6**	6	16	12	12	**11.5**
Alternaria chlamydosporus Mouchacca	2	0	2	0	**1**	0	0	0	0	**0**
Alternaria sp.	0	0	0	0	**0**	8	0	0	2	**2.5**
Aspergillus sp. 1	2	4	0	0	**1.5**	0	0	0	0	**0**
Aspergillus sp. 2	0	0	0	2	**0.5**	12	0	10	10	**8**
Aspergillus sp. 3	6	2	0	0	**2**	0	0	0	2	**0.5**
Cladosporium sp.	18	0	0	0	**4.5**	6	4	4	2	**4**
Colletotrichum sp.	4	32	64	4	**26**	0	0	0	0	**0**
Cumulospora marina J. Schmidt	0	0	0	6	**1.5**	0	0	0	0	**0**
Cytospora sp.	0	26	0	0	**6.5**	4	0	0	0	**1**
Dicyma sp.	0	4	2	0	**1.5**	4	0	0	0	**1**
Fusarium oxysporum	0	8	2	0	**2.5**	0	6	2	2	**2.5**
Fusarium sp.	0	2	14	2	**4.5**	0	2	0	2	**1**
Nigrospora oryzae (Berk. et Br.) Petch	0	0	0	0	**0**	4	4	0	4	**3**
Paecilomyces puntoni (Vuill.) Nannizzi	0	2	0	0	**0.5**	0	0	0	0	**0**
Paecilomyces sp.	0	4	0	0	**1**	0	20	0	0	**5**
Penicillium sp.	10	0	0	0	**2.5**	4	10	28	16	**14.5**
Phoma sp.	2	0	0	0	**0.5**	0	0	0	4	**1**
Pestalotiopsis sp.	2	0	0	4	**1.5**	0	0	0	0	**0**
Trichoderma sp.	0	0	0	0	**0**	0	0	6	0	**1.5**
Sterile mycelia (SM)										
SM 1	10	8	2	0	**5**	0	0	0	0	**0**
SM 2	10	0	0	8	**4.5**	0	0	0	0	**0**
Number of segments assessed	50	50	50	50		50	50	50	50	
Number of segments colonised	32	44	42	31		35	44	37	39	
Number of isolations	35	51	48	26		28	36	50	44	
Number of endophytes	11	12	8	9		10	8	7	11	
Mean number of endophytes per segment	0.7	1.02	0.96	0.52		0.56	0.72	1	0.88	

Table 2. Percent contribution by the dominant endophytes (% DE) to the assemblages in two halophytes (L – leaf; P – petiole; PR – prop root; R – root; RH – Rhizome; S – stem).

Dominant endophyte	% DE	Extent of colonisation
Acanthus ilicifolius		
Colletotrichum sp.	32.5	PR>S>L & R
Cytospora sp.	8.1	S
Acremonium sp.	7.5	S>PR & R>L
Yeast sp. 1	6.3	R>PR
Sterile fungus (SM 1)	6.3	L>S>PR
Acrostichum aureum		
Yeast sp. 1	36.8	RH>R>P>L
Penicillium sp.	25.4	RH>R>P>L
Acremonium sp.	20.2	P>RH & R>L
Aspergillus sp. 2	14.0	L>RH & R
Paecilomyces sp.	8.8	P

Table 3. Species richness, diversity and evenness of endophytic fungi in halophytes.

Host and tissue	Species richness	Diversity		Evenness	
		Simpson	Shannon	Simpson	Shannon
Acanthus ilicifolius					
Leaf	11	0.909	3.459	0.941	0.887
Stem	12	0.917	3.585	0.886	0.805
Prop root	8	0.875	3.000	0.602	0.567
Root	9	0.889	3.170	0.939	0.906
Acrostichum aureum					
Leaf	10	0.900	3.322	0.978	0.963
Petiole	8	0.875	3.000	0.938	0.897
Rhizome	7	0.857	2.807	0.872	0.814
Root	11	0.909	3.459	0.876	0.796

Table 4. Jaccard's similarity coefficients (JI, in %) of endophytes in tissues of halophytes (L – leaf; P – petiole; PR – prop root; R – root; RH – Rhizome; S – stem).

Acanthus ilicifolius		S	PR	R
	L	18.5	17.4	16.7
		S	23.1	16
			PR	25
Acrostichum aureum		P	RH	R
	L	21.7	22.7	25
		P	25	24
			RH	25

rhizomes; rhizomes and roots. The endophyte species composition between the two halophytes did not overlap more than 24.5 % even though the plants were growing in the same geographical area and exposed to similar environmental conditions.

DISCUSSION

Mitosporic fungi were more common than ascomycetes as endophytes in both halophytes studied, just as in foliar endophytes (Kumaresan and Suryanarayanan 2001, 2002; Suryanarayanan et al. 1998; Suryanarayanan and Kumaresan 2000) and root endophytes of mangroves (Ananda and Sridhar 2002) and seagrass (Devarajan et al. 2002). In decomposing mangrove litter, ascomycetes outnumbered mitosporic fungi (Kohlmeyer and Volkmann-Kohlmeyer 1991). Colonisation frequency of endophytes varies with altitude, humidity (Petrini and Carroll 1981), rainfall (Rajagopal and Suryanarayanan 2000, Suryanarayanan et al. 1998) and host susceptibility (Elamo et al. 1999, Petrini and Carroll 1981). In this study, although the overall colonisation (74.5 and 77.5 %) and mean number of endophytes per segment (0.8 and 0.79) were similar in *Acanthus ilicifolius* and *Acrostichum aureum*, about 75 % of the endophyte assemblage differed between them. Such difference between hosts from the same location reveals that endophyte colonisation depends on host species rather than edaphic or environmental factors.

In the present study, *Acanthus ilicifolius* was dominated by a single endophytic fungus *Colletotrichum* sp. (Table 1). The dominance of *Colletotrichum* sp. in prop roots of *Acanthus ilicifolius* drastically decreased the evenness indices (Table 3), whereas lack of dominance of single species in leaves and roots resulted in uniform evenness (Ludwig and Reynolds 1988). However, root endophytes of *Acanthus ilicifolius* showed multiple species dominance (*Cylindrocarpon* sp., *Phoma* sp., Sterile sp. 2 and 4; range 10–30 %). Multiple endophytic species dominance was also common in the roots of *Avicennia officinalis, Rhizophora mucronata* and *Sonneratia caseolaris* (Ananda and Sridhar 2002). Single species dominance in foliar endophytes is seen in many mangrove halophytes: *Avicennia marina* (*Phoma* sp., 15.3 %), *Bruguiera cylindrica* (*Colletotrichum gloeosporioides*, 34 %), *Rhizophora apiculata* (*Sporormiella minima*, 16.7 %), *Rhizophora mucronata* (*Sporormiella minima*, 15.7–19.3 %) and *Suaeda maritima* (*Camarosporium palliatum*, 11.7 %) (Kumaresan and Suryanarayanan 2001, Suryanarayanan et al. 1998, Suryanarayanan and Kumaresan 2000). In *Acrostichum aureum* multiple endophyte dominance was seen. Colonisation frequencies of *Acremonium* sp., *Penicillium* sp. and Yeast sp. 1 were above 10 %. These endophytes are common in all four tissues. Similarly, in leaves of *Lumnitzera racemosa* multiple endophyte dominance was seen (*Alternaria* sp., 8.3 %; *Phomopsis* sp., 10.3 % and *Phyllosticta* sp., 11.7 %) (Kumaresan and Suryanarayanan 2001). High rates of multiple endophyte colonisations have been previously recorded in roots of mangrove plant species (Ananda and Sridhar 2002), leaves of palms (Fröhlich 2000) and temperate deciduous trees (Fisher and Petrini 1990). At the outset, although *Aspergillus* spp., *Cladosporium* sp., *Paecilomyces* spp. and *Penicillium* sp. were seem to be contaminants, they were recovered repeatedly from the surface sterilised segments of halophytes.

Acremonium sp., *Alternaria* sp., *Cladosporium* sp., *Colletotrichum* sp. and *Fusarium* sp. are common foliar endophytes of the beach halophyte *Suaeda fruticosa* (Fisher and Petrini 1987), mangrove plant species (Kumaresan and Suryanarayanan 2001, 2002; Suryanarayanan et al. 1998; Suryanarayanan and Kumaresan 2000) and the seagrass, *Halophila ovalis* (Devarajan et al. 2002). In this study, except for *Colletotrichum* sp. all the above species are common to both hosts and found in almost all tissues screened. In fact, *Acremonium, Alternaria* and *Cladosporium* are not host-specific and hence found in different tissues and hosts (Petrini et al. 1982, Suryanarayanan et al. 2000). Among endophytes, *Colletotrichum* spp. are most frequent in tropical plants, particularly in the *Musa acuminata* species complex in Hong Kong and Australia (Brown et al. 1998), and in mangrove plant communities of India: *Acanthus ilicifolius, Arthrocnemum indicum, Sesuvium portulacastrum, Avicennia marina, Bruguiera cylindrica, Ceriops decandra, Excoecaria agallocha* and *Lumnitzera racemosa* (Kumaresan and Suryanarayanan 2001, Suryanarayanan and Kumaresan 2000). In the present study, *Colletotrichum* sp. is most dominant in *Acanthus ilicifolius* prop roots and stems. This suggests a high adaptability of *Colletotrichum* spp. for an endophytic life style in mangrove halophyte plant communities too. In the present study, *Acremonium* sp. and *Colletotrichum* sp. were dominant endophytes in all tissues of *Acanthus ilicifolius*. Sadaba et al. (1995) observed increased occurrence of *Acremonium* sp. and *Colletotrichum gloeosporioides* in different parts of standing senescent *Acanthus ilicifolius* of Mai Po Mangrove of Hong Kong. Dominance of *Acremonium* and *Colletotrichum* in senescent standing wood of *Acanthus ilicifolius* indicates the role of these endophytic fungi in decomposition (Kumaresan and Suryanarayanan 2002). *Phomopsis* spp. and *Phyllosticta* spp. are common foliar endophytes in many mangrove plant species (Kumaresan and Suryanarayanan 2001, 2002; Suryanarayanan et al. 1998; Suryanarayanan and Kumaresan 2000). *Phomopsis* spp. were also common root endophytes of *Avicennia officinalis* and *Rhizophora mucronata* (Ananda and Sridhar 2002). But in our study, neither *Phyllosticta* nor *Phomopsis* sp. were recovered. Similarly, yeasts were not recorded in mangrove plant species so often as endophytes possibly due to the kind of media employed. Yeast sp. 1 is a dominant endophyte in both halophytes in the current study (Table 2) but we were unable to identify it in our laboratory.

According to Petrini (1986) a few endophytic fungi dominate a single host plant species. The dominant endophytes were different for each host plant species in a mangrove community (Kumaresan and Suryanarayanan 2001). In this study, *Colletotrichum* sp. was dominant in prop roots (64 %) of *Acanthus ilicifolius*, which also colonised other tissues (leaf, stem and roots). Similarly, Yeast sp. 1 was dominant in rhizomes (38 %) followed by roots (32 %) although it was found in leaves and petioles of *Acrostichum aureum*. This shows the preference of specific tissue of host by *Colletotrichum* and Yeast sp. 1. Out of 25 endophytes in the two halophytes, 16 were isolated five or more times (Table 1). Differences in assemblage and frequencies of endophytes in different tissue types of a given host plant have been recorded (Rodrigues 1994, 1996). In fact, different tissues have been considered distinct microhabitats for endophytes (Petrini et al. 1992). The variations in endophytic fungal density in different tissues of the halophytes studied reveal that some selection operates in constituting the endophytic assemblages of tissues besides selection in each mangrove plant species (Kumaresan and Suryanarayanan 2001).

Among the endophytic fungi, the only known marine mitosporic fungus recovered was *Cumulospora marina*, which was isolated from the roots of *Acanthus ilicifolius*. Occurrence of *Cumulospora marina* accounts for 4 % of the total endophytes recovered. Although plant detritus on coastal sand dunes harbour several marine fungi, they were not dominant (13 %) root endophytes of coastal sand dune halophytes (Beena et al. 2000). Similarly, marine fungi were not dominant endophytes of roots of mangrove plant species (Ananda and Sridhar 2002). Due to paucity of information on endophytes of halophytes and mangrove plant species, based on the present pilot study and the available literature, definite conclusions cannot be drawn like that in the case of the palm endophytes (Fröhlich et al. 2000, Taylor et al. 1999).

In summary, root endophytes of 200 segments each of *Acanthus ilicifolius* and *Acrostichum aureum* of the west coast of India yielded 25 fungi in which terrestrial mitosporic fungi dominated. *Acanthus ilicifolius* showed single species dominance (*Colletotrichum* sp.), while *Acrostichum aureum* had multiple species dominance (*Acremonium* sp., *Aspergillus* sp. 2, *Penicillium* sp. and Yeast sp. 1). Except for *Cumulospora marina* (in roots of *Acanthus ilicifolius*) no marine fungi were recovered. It seems the endophytic fungal assemblage of herbaceous plants and tree species of mangroves differs. Mangroves are important forest ecosystems confined to tropics and subtropics. Future studies on different hosts in a wide geographic range, in different seasons, tissue types and age classes might reveal more on the endophytic fungal status and their significance.

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Aerometric study on thermophilous fungi in a farm house, Chennai

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Udaya Prakash N. K. and Vittal B. P. R. (2003): Aerometric study on thermophilous fungi in a farm house, Chennai. – Czech Mycol. 55: 253–259

A survey on airborne thermophilous fungi at a farmhouse in Chennai, India was made using an Andersen 2-stage viable sampler for the period from January 1997 to December 1997 at fortnight intervals. A total of 582 colonies belonging to 17 species were recorded. The species *Emericella nidulans* was dominant with an average of 60.2 CFU/m^3 of air followed by *Aspergillus fumigatus* and *Thermomyces lanuginosus* with 34.7 CFU/m^3 of air and 32.2 CFU/m^3 of air, respectively. The total respirable fraction recorded was 58.4 %.

Key words: airborne fungi, Andersen 2-stage sampler, occupational environment, respirable fraction, India.

Udaya Prakash N. K. a Vittal B. P. R. (2003): Aerometrická studie termofilních hub na statku ve městě Chennai v Indii. – Czech Mycol. 55: 253–259

Termofilní mikroskopické houby vyskytující se v podobě konidií ve vzduchu byly studovány na statku ve městě Chennai v Indii za pomoci dvoustupňového Andersenova aeroskopu, a sice v intervalu 14 dnů od ledna 1997 do prosince roku 1997. Celkem bylo izolováno 582 kolonií patřících 17 druhům hub. Dominantním druhem byla *Emericella nidulans* s průměrným počtem 60.2 CFU/m^3 vzduchu; za ní následovaly druhy *Aspergillus fumigatus* a *Thermomyces lanuginosus* s 34.7 CFU/m^3, resp. 32.2 CFU/m^3 vzduchu. Celková respirabilní frakce činila 58.4 %.

INTRODUCTION

The term thermophilous fungi includes both thermophilic and thermotolerant fungi, terms that have been widely used by different authors (Apinis and Pugh 1967, Evans 1972, Hudson 1973, Kuthubutheen and Pugh 1977, Sandhu et al. 1980, Sandhu and Singh 1985). However, Hedger (1974) stressed that any discussion on thermophilic fungi must first underline the adapted definition of thermophilism (Mouchacca 1985). Hence, in the present study the fungi which have an ability to produce colonies at 50 °C are termed as thermophilous fungi. Thermophilous fungi have been isolated from many sources including air (Abdel-Fattah and Swelim 1982, Evans 1972, Hudson 1973, Hughes and Crosier 1973, Jones and Cookson 1985, Rippon et al. 1980). In India only few reports are available regarding airborne thermophilous fungi (Deshmukh and Shukla 1984, Sandhu and Singh 1985). Thakur (1977) dealt with airborne thermophilic fungi near the farmhouse in Bombay. However, there was no report from Chennai regarding airborne thermophilous fungi. Hence, an aerometric study on thermophilous fungi at a farmhouse in Chennai was conducted.

MATERIALS AND METHODS

The sampler: The 2-stage Andersen microbial air sampler is a portable sampler using a 12V battery (Andersen Samplers, Inc., Atlanta, Georgia). The air inflow rate of the sampler is 0.028 m^3/minute. The sampler is made of aluminium with 200 holes arranged in a radial pattern on each stage. The 50 % effective cut-off diameter is 8 μm. Thus, the microbial particles on stage 1 are large particle fractions and those on stage 2, the small particle fraction, includes the vast majority of respirable particles, i.e. those less than 5 μm in aerodynamic diameter which are deposited in human tracheobronchial and alveolar regions (Jones and Cookson 1983).

Sampling site: The sampling site in Chennai (Madras renamed as Chennai, situated at 13°8' N and 80°19' E on the east coast of India) is a farmhouse located opposite the Basinbridge bus station in the northern part of the city. The length of the farmhouse is about 80 m and the width is 64 m. The samples were taken at the central part within the farmhouse. Nearly 400 people are residing in the environment and the number of dairy animal exceeds 300 at the site.

Sampling procedure: The portable sampler was disinfected by wiping with 70 % alcohol dipped cotton swabs and then loaded with 2 Petri dishes containing YpSs medium (Cooney and Emerson 1964; yeast extract – 4.0 g, K_2PO_4 – 1.0 g, $MgSO_4$ – 0.5 g, soluble starch – 15.0 g and agar – 20.0 g). Streptomycin was added to the medium to arrest the bacterial growth. The sampler was placed at a height of 1 meter and was operated for 5 minutes duration at each sampling. The samples were taken between 10 and 11 o'clock in the morning hours. This was repeated at fortnight intervals starting from January 1997 to December 1997. However, in November only one sample was taken due to flood. After the sampling, the plates were brought to the laboratory and incubated at 50 °C in an incubator. A trough of water was placed within the incubator to avoid dehydration of the media. The developing colonies were counted, isolated and identified after 5 days of incubation.

Data analysis: The data received were analysed and presented as average CFU/m^3 of air, relative contribution, isolation frequency and respirable fractions, as follows.

The colonies isolated were converted to Colony Forming Units (CFU)/m^3 of air as follows:

$$X = \frac{y_1 + y_2}{(0.1415)}$$

Whereby
X = no. of CFU/m^3
y_1 = number of colonies on plate 1
y_2 = number of colonies on plate 2
0.1415 = amount of sampled air (m^3)

$$\text{Average CFU/m}^3 \text{ of an individual species} = \frac{\text{Total CFU/m}^3 \text{ of a species}}{\text{Total number of samplings (23)}}$$

$$\text{Relative contribution} = \frac{\text{Total CFU/m}^3 \text{ of an individual species}}{\text{Total number of CFU/m}^3 \text{ of all species}} \times 100$$

$$\text{Isolation frequency} = \frac{\text{No. of samplings in which the species was isolated}}{\text{Total number of samplings (23)}} \times 100$$

$$\text{Respirable fraction} = \frac{\text{Total no. of colonies recorded on plate 2 of the sampler}}{\text{Total no. of colonies recorded on both plates}} \times 100$$

RESULTS

During the study period (January 1997 to December 1997) 582 colonies of thermophilous fungi belonging to 17 species were recorded. Among the fungi isolated *Emericella nidulans*, *Aspergillus fumigatus* and *Thermomyces lanuginosus* occupied the first, second and third position with 60.1, 34.7 and 32.2 CFU/m^3 of air, respectively, out of a total 178.3 CFU/m^3 of air on average. *Emericella nidulans* contributed to 39.1 % and *Aspergillus fumigatus* contributed to 22.6 % of the total composition.

The isolation frequency of *Emericella nidulans* and *Aspergillus fumigatus* was nearly 70 % and that of *Thermomyces lanuginosus* was 43.5 % of the samplings. The fungi *Aspergillus terreus* and *Myceliophthora thermophila* were isolated from 21.7 % of the samplings. The average CFU/m^3, percent contribution and isolation frequency per species are given in Table 1.

The maximum amount of CFU/m^3 of air was obtained during the month of August followed by the month of March. In February and July a more or less equal amount of CFU was recorded and similarity was also seen among the months of April, June and December. However, there were no thermophilous fungi recorded in November (Fig. 1).

Species such as *Aspergillus fumigatus, Aspergillus terreus* and *Rhizomucor pusillus* had respirable fractions of more than 80 % and the fungi *Emericella*

Table 1. List of thermophilous fungi isolated from a farmhouse in Chennai, their average CFU/m^3 of air, relative contribution and isolation frequency.

No.	Species	Average CFU/m^3	Relative contribution %	Isolation frequency
01	*Absidia corymbifera*	1.53	0.86	17.39
02	*Acremonium* sp.	0.30	0.17	4.34
03	*Aspergillus fumigatus*	34.68	19.58	69.56
04	*Aspergillus terreus*	11.35	6.41	21.73
05	*Chaetomium thermophilum* var. *coprophilum*	0.30	0.17	4.34
06	*Chaetomium thermophilum* var. *dissitum*	0.60	0.34	4.34
07	*Emericella nidulans*	60.16	33.96	73.91
08	*Humicola grisea* var. *thermoidea*	2.76	1.55	13.04
09	*Humicola insolens*	0.30	0.17	4.34
10	*Malbranchea cinnamomea*	1.22	0.69	17.39
11	*Myceliophthora thermophila*	14.73	8.31	21.73
12	*Rhizomucor pusillus*	10.43	5.89	13.04
13	*Rhizopus stolonifer*	0.30	0.17	4.34
14	*Paecilomyces variotii*	6.44	3.63	4.34
15	*Penicillium dupontii*	0.60	0.34	4.34
16	*Thermoascus aurantiacus*	0.60	0.34	8.69
17	*Thermomyces lanuginosus*	32.23	18.19	43.47

nidulans and *Myceliophthora thermophila* nearly 65 %. The total respirable fraction is given in Fig. 2.

DISCUSSION

In farmhouses in general, isolation of thermophilous fungi in higher concentrations depends on the availability of source material within the environment. The huge accumulation of cattle dung, hay material and the urine of animals result in a self-heated pile, which is favourable for the proliferation of thermophilous fungi. Andersen and Coe (1974) reported that moist, sun heated piles of herbivore dung can maintain a temperature suitable for growth of thermophilic fungi. The recovery of *Emericella nidulans* and *Aspergillus fumigatus* in large amounts of CFU/m^3 of air in Chennai is due to their thermotolerant nature and their ability to tolerate wide range of temperatures (Hudson 1973).

The dissemination theory explained by Maheshwari (1997) explains how spores get into hay, wood chips and agricultural produce through air. It explains their presence in dung of herbivores – the spores of thermophilic fungi present in fodder are eaten by the herbivores and are discharged in dung, which heatsup when accumulated in mass. The isolation of 17 species from the aerial environment of the farmhouse confirms the dissemination of spores of thermophilous fungi by means of air.

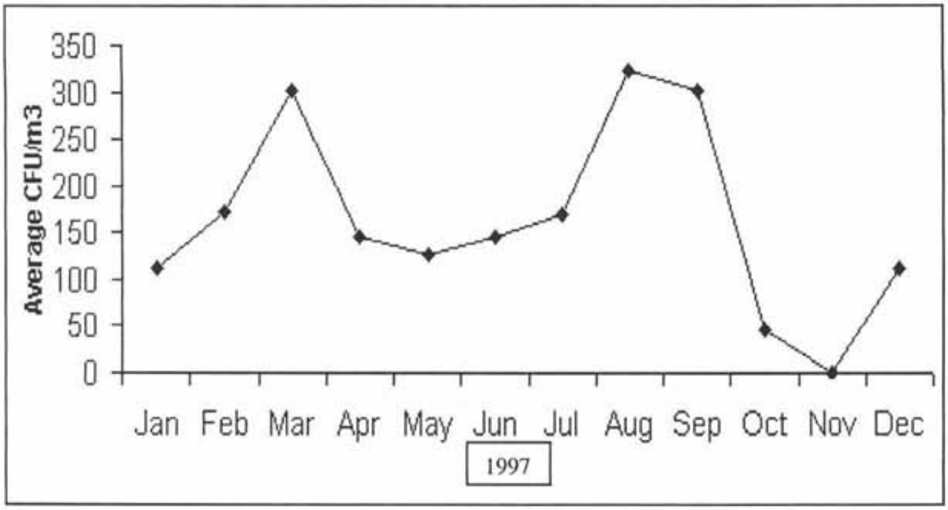

Fig. 1. Seasonal distribution of thermophilous fungi in the atmosphere of the farmhouse.

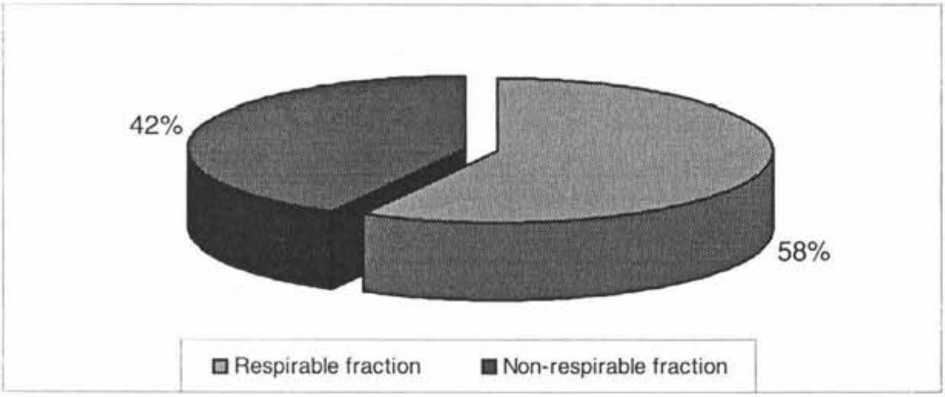

Fig. 2. Total respirable fraction recorded from the farmhouse.

In our study the occurrence of thermophilous fungi reached a peak during the month of August followed by March. The occurrence of the peak in August and the double maxima was already reported by other authors (Evans 1972, Hudson 1973, Sandhu and Singh 1985).

Our study provides year round data on the presence of thermophilous fungi in a farmhouse environment with high human activity. Species such as *Absidia corymbifera, Aspergillus fumigatus, Rhizomucor pusillus* and *Thermomyces lanuginosus* were already reported as opportunistic pathogens (Hughes and Crosier

1973). Thus, the people and the animals in the environment are prone to exposure to such airborne thermophilous fungi which act as a source of antigens for respiratory hypersensitive syndromes such as Farmer's Lung Disease and Allergic Broncho-Pulmonary Aspergillosis (Gregory and Lacey 1963, Lacey and Lacey 1964, Hughes and Crosier 1973, Tansey and Brock 1978). Hence, further study is required to determine the role of thermophilous fungi in relation to human diseases.

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Phytotoxin production and rice sheath blight development by Rhizoctonia solani mutants derived from gamma irradiation

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Babu S., Nandakumar R., Sriram S., Raguchander T., Balasubramanian P. and Samiyappan R. (2003): Phytotoxin production and rice sheath blight development by Rhizoctonia solani mutants derived from gamma irradiation. – Czech Mycol. 55: 261–271

To evaluate the role of the RS-toxin, a phytotoxin produced by the fungus *Rhizoctonia solani* that causes sheath blight disease of rice, varying doses of gamma irradiation were used to generate mutants of the fungus. All the mutants showed reduced mycelial growth compared to the wild isolate RS7 in liquid culture. The mutants exhibited significant differences in virulence on detached leaf sheath and intact rice plants, the toxin produced and sclerotial formation in culture media and infected leaf sheaths. The amount of toxin produced was positively correlated with disease development. SDS-PAGE analysis of mycelial proteins showed that the mutants and wild isolate produced many proteins of different molecular weights at different stages of mycelial growth. In the mutants resulting from higher doses of gamma rays, the amount of toxin produced and their ability to induce disease symptoms were also greatly reduced.

Key words: gamma rays, mutants, phytotoxin, *Rhizoctonia solani*, rice, sheath blight

Babu S., Nandakumar R., Sriram S., Raguchander T., Balasubramanian P. a Samiyappan R. (2003): Produkce fytotoxinu a vývoj onemocnění pochev listů rýže způsobeného mutanty Rhizoctonia solani získanými působením záření gama. – Czech Mycol. 55: 261–271

Produkce a role RS toxinu (fytotoxinu) byla hodnocena u mutantů houby *Rhizoctonia solani*, původce onemocnění pochev listů rýže. Mutanti byli získáni po působení různých dávek záření gama. U všech mutantů byla zjištěna redukce růstu mycelia v tekutém médiu v porovnání s kontrolním izolátem RS7. Všichni mutanti vykazovali výrazné rozdíly ve virulenci na oddělených listových pochvách i na netknutých rostlinách, v produkci toxinů a v tvorbě sklerocií jak v tekutém médiu, tak na infikovaných pochvách listů. Množství produkovaného toxinu bylo v korelaci s rozvojem choroby. Analýza proteinů pomocí SDS-PAGE ukázala, že mutanti i kontrolní izolát produkují celou řadu proteinů s různou molekulární hmotností v různých stádiích růstu mycelia. U mutantů, kteří vznikli po ozáření vyššími dávkami záření gama, byla zjištěna snížená schopnost produkce toxinů a vyvolání příznaku onemocnění.

INTRODUCTION

Studies on non toxigenic mutants of plant pathogenic fungi implicate toxins in phytopathogenesis. Subsequent genetic research probe the secrets of toxin biosynthesis and the regulatory network that links toxin production to expression of plant pathogenicity (Gross 1991).

Previous work demonstrated the production of a phytotoxin (designated RS-toxin) by *Rhizoctonia solani* Kühn, as the cause of sheath blight of rice (Sriram et al. 1997, 2000; Vidhyasekaran et al. 1997). Before the mechanisms by which RS-toxin is produced are understood, the extent to which toxin production contributes to virulence has to be tested. To evaluate the importance of toxin in disease development, toxinless mutants were developed in several fungal pathogens, viz. *Helminthosporium carbonum* producing HC-toxin (Panaccione et al. 1992), *Giberella zeae* producing trichothecene (Proctor et al. 1995), *Mycosphaerella zeae-maydis* producing PM-toxin (Yun et al. 1997) and *Macrophomina phaseolina* producing phaseolinone (Sett et al. 2000).

Hence to establish a role of toxin in the virulence of *R. solani* and to study RS-toxin biosynthesis, it would be useful to have mutants that are altered or blocked in toxin synthesis. The objective of this study was to determine the correlation, if any, between RS-toxin production and rice sheath blight disease by generating mutants of *R. solani* through gamma irradiation.

MATERIALS AND METHODS

Pathogen

The *Rhizoctonia solani* field isolates (RS7 and RS6) used in the study was obtained from the culture collections of Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India (Sriram et al. 1997).

Mutagenesis

Mycelial mats harvested from 24-h-old cultures grown in potato dextrose broth (PDB) were used for gamma irradiation. The mycelia were cut into very small bits (approximately 1 mm) with sterile scalpel under aseptic conditions and dispensed in 1 ml of sterile water in separate sterile test tubes. The mycelial suspensions were exposed to gamma rays by placing the open tubes in a gamma chamber (source of gamma rays – Cobalt 60, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India). Based on the half life period of the radioactive material, the time interval was adjusted to yield doses of 5, 10, 20, 30, 40 and 50 krads of gamma rays exposure per different tubes. The suspension in each tube was diluted to 10^{-3} concentration with sterile water and one ml of suspension from each tube was pipetted into Petri dishes. Warm potato dextrose agar (PDA) medium (20 ml per plate) was poured and allowed to solidify. The plates were incubated at room temperature (28 ± 2 °C) for 2 days. Then individual colonies from single hypha of different gamma treatments were subcultured and maintained on PDA slants.

Purification and quantification of RS-toxin

Isolation and purification of toxins from the mutants and the wild isolates were done as described earlier (Vidhyasekaran et al. 1997; Sriram et al. 1997, 2000). Briefly, the culture filtrate was condensed to one tenth volume in a flash evaporator and precipitated using methanol. The filtrate was further extracted with hexane, ethyl acetate and chloroform. The crude toxin thus obtained was purified by subjecting it to column chromatographic separation. Fractions corresponding to peak absorbance were condensed and used as the toxin.

The toxin was quantified using anthrone reagent (200 mg of anthrone in 100 ml of 95 % sulphuric acid) and expressed in glucose equivalents (Hedge and Hofreiter 1962). The reaction mixture contained 5 μl of the toxin, 995 μl distilled water and 4 ml of the anthrone reagent. The color that developed was read at 630 nm in a Hitachi spectrophotometer.

Sheath blight symptom development

Purified toxin and sclerotia collected from the mutants and the wild isolate were used for studying symptom development on rice sheaths. Rice sheaths of uniform thickness and length (7 cm) collected from 40-day-old rice plants (cultivar IR 50) grown under green house conditions, were cut and surface sterilized in 0.1 % $MgCl_2$ for 30 sec and washed in repeated changes of sterile water. Two sheaths were placed on sterile glass slides kept on 2 layers of moist filter paper inside a 9 cm Petri dish. Uniform sized sclerotia and 10 μl (50 μg) of purified toxin of the mutants and wild isolate were placed separately on leaf sheaths and incubated for 3 days. Symptoms that developed were graded using a 0–5 scale (Sriram et al. 1997, 2000). Sterile water was used as a negative control.

Forty-day-old rice plants of susceptible cultivar IR 50 grown in pots, were inoculated using sclerotia of the mutants and the wild isolate. Two sclerotia per sheath were placed between the stem and sheath, covered with moist cotton and tied with parafilm. Less virulent *R. solani* isolate (RS6) was used for comparison. Uninoculated controls were also maintained. Sheaths were regularly watered by moistening the cotton with equal quantity of sterile water so as to maintain high humidity. The symptom development was observed after 10 days and graded (0–5 scale) based on the lesion size. Virulence index was calculated using the formula (Sriram et al. 1997),

$$\text{Virulence Index (VI)} = \frac{\Sigma \text{ Total grade points}}{\text{Number of sheath observed}} \times \frac{100}{\text{Maximum grade}}$$

The leaf sheaths showing typical blight symptoms were collected and incubated at room temperature for one week in Petri dishes with 3 layers of moist filter paper. The number of sclerotia produced on the rice sheath was recorded and the sclerotia produced per g of the infected rice sheath was calculated.

SDS-PAGE analysis of mycelial proteins

The mutants and the wild isolate were grown on PDB and incubated at 25 °C in a shaker (120 rpm). The mycelia were harvested by filtering through Whatman no. 1 filter paper after 5 and 10 days of incubation. The mycelial mats were ground in a sterile pestle and mortar, adding 0.1 M sodium phosphate buffer (pH 7.0), centrifuged (4 °C, 10,000 rpm) and the supernatant was used for analysis of protein profile. PMSF was added at the concentration of 1 mM to the protein samples to avoid degradation and stored at –20 °C. Sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) was carried out in polyacrylamide gel slabs consisting of 4 % stacking gel and 12 % separating gel using Sigma Aldrich Techware system (Sigma, St. Louis, USA) by the method of Laemmli (1970). The protein content of the mycelial extracts were estimated (Bradford 1976) and loaded at 30 μg per well. The gels were stained with Coomassie brilliant blue (R 250) stain.

Correlation analysis

Correlation coefficients between various parameters, viz. disease development on rice plants, symptom development on detached rice sheath inoculated with sclerotia and toxin, sclerotial development on infected plant tissue and the amount of toxin produced were determined for the *R. solani* mutants and isolates using the IRRISTAT programme (International Rice Research Institute, The Philippines).

RESULTS

The mutant cultures isolated after gamma irradiation of 5, 10, 20, 30, 40 and 50 krads were designated as RSG1, RSG2, RSG3, RSG4, RSG5 and RSG6 respectively. The mutants showed no morphological differences but variation was observed in the dry weight of the mycelia produced in liquid culture. The mycelial

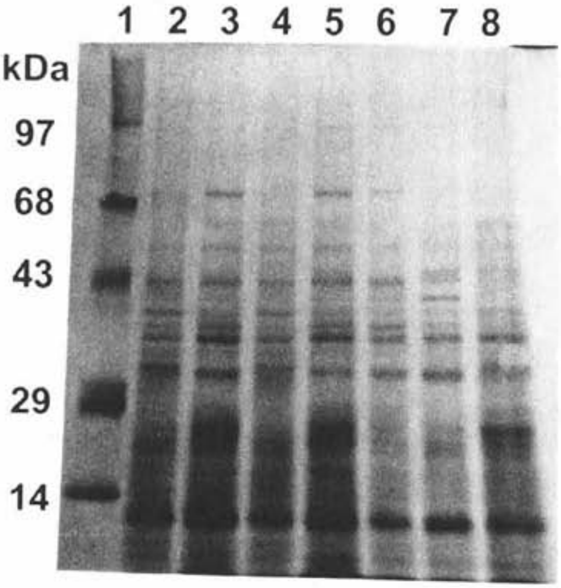

Fig. 1a. SDS-PAGE of 5 day old mycelia of *R. solani* mutants.
Lanes: 1-marker, 2-RSG1, 3-RSG2, 4-RSG3, 5-RSG4, 6-RSG5, 7-RSG6 and 8-RS7 (wild type).

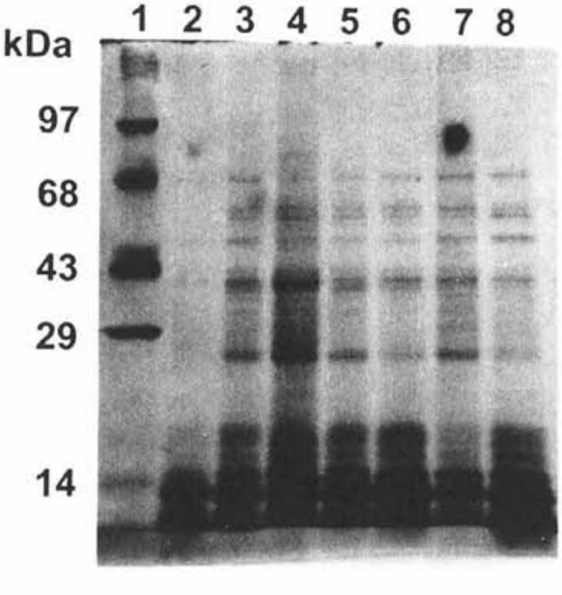

Fig. 1b. SDS-PAGE of 10 day old mycelia of *R. solani* mutants.
Lanes: 1-marker, 2-RSG1, 3-RSG2, 4-RSG3, 5-RSG4, 6-RSG5, 7-RSG6 and 8-RS7 (wild type).

Table 1. Mycelial growth and sheath blight symptom development by *R. solani* isolates and mutants.

Mutants / Isolates	Mean mycelial dry weight (g)	Virulence Index		
		Inoculation of sclerotia on detached sheath	Inoculation of toxin on detached sheath	Sheath blight disease on rice plants
RSG1	0.75 a	53.3 bcd	46.7 b	60.0 cd
RSG2	0.84 ab	53.3 bcd	43.3 b	56.7 cd
RSG3	0.81 ab	73.3 d	56.7 b	63.3 d
RSG4	0.90 b	43.3 b	40.0 b	35.0 c
RSG5	0.77 ab	50.0 bc	40.0 b	36.7 c
RSG6	0.80 ab	40.0 b	36.7 ab	35.0 c
RS7	1.17 c	66.7 cd	50.0 b	70.0 d
RS6	ND	ND	ND	15.0 b
Uninoculated control		13.3 a	13.3 a	0.0 a

RS7 – virulent isolate; RS6 – less virulent isolate
Values in a column followed by common letter are not significantly different (P = 0.05) by Duncan's multiple range test (IRRISTAT software, IRRI, The Philippines).
ND: Not determined

growth of all the mutants were significantly reduced when compared to the wild isolate RS7 (Table 1).

Mutant RSG3 although recorded highest virulence index of 73.3 in sclerotia inoculation studies, was at par with RSG1, RSG2 and wild isolate. The virulence indexes of RSG4 and RSG6 were lesser than the wild isolate (Table 1). There was no difference in virulence among the mutants and wild isolates when the toxin was used for inoculation.

Under greenhouse conditions, three mutants (RSG4, RSG5 and RSG6) were less virulent as compared to RS7 (VI = 70.0) and the other mutants. However, none of the mutants recorded lesser virulence index than the naturally less virulent wild type field isolate, RS6.

The mutants and wild isolates also differed in sclerotial production. The number of sclerotia produced per g of infected rice sheath was much less in RSG5 and RSG6 ($\leq 1/g$ of plant tissue). RSG5 and RSG2 produced significantly fewer numbers of sclerotia/ml of culture media (8 sclerotia/ml) as compared to 18 sclerotia/ml in RS7. Sclerotia produced by other mutants in infected sheaths and culture media did not show any variation from the wild isolate.

The amount of toxin produced by RSG1 and RSG3 was equal to the wild isolate (Table 2). All other mutants showed a reduction in the amount of toxin produced. The amount of toxin produced was more than 30 per cent lower in RSG4 and RSG6 than in the wild isolate.

Table 2. Number of sclerotia and amount toxin produced by mutants of *R. solani*.

Mutants/Isolates	No. of sclerotia/g rice sheath tissue	Amount of toxin*
RSG1	3.3 c	10.9 bcd
RSG2	3.7 c	9.6 abc
RSG3	4.1 c	11.4 cd
RSG4	3.6 c	8.5 ab
RSG5	0.1 a	8.6 ab
RSG6	1.0 b	8.3 ab
RS7	3.7 c	12.5 d
RS6	0.2 a	7.6 a
Uninoculated control	0.0 a	–

RS7 – virulent isolate; RS6 – less virulent isolate

*The amount of toxin expressed in mg glucose equivalents

Values in a column followed by common letter are not significantly different (P = 0.05) by Duncan's multiple range test (IRRISTAT software, IRRI, The Philippines)

Table 3. Correlation coefficients between virulence and toxin production.

	Virulence index on rice plants	Virulence index on detached sheath (sclerotial inoculation)	Virulence index on detached sheath (toxin inoculation)	Sclerotial production on infected rice sheath	Toxin produced
Virulence index on rice plants	1.000	0.919*	0.899*	0.792 ns	0.926*
Virulence index on detached sheath (sclerotial inoculation)		1.000	0.980**	0.710 ns	0.849 ns
Virulence index on detached sheath (toxin inoculation)			1.000	0.744 ns	0.769 ns
Sclerotial production on infected rice sheath				1.000	0.745 ns
Toxin produced					1.000

* Significant at P = (0.05)

ns – non significant

The protein profiles of 5 and 10 day old mycelia of *R. solani* mutants and the wild isolate were different (Fig 1a, 1b). All the mutants and the wild isolate had many proteins in common. Five day old mycelial protein profiles revealed a 40 kDa protein present only in RSG2, RSG4 and RS7 and a 60 kDa protein present only in RSG2 and RSG5. RSG3 was different from other mutants and wild isolate in having a 80 kDa and a 24 kDa protein produced in 10 day old mycelia. RSG2 and RSG6 varied from other mutants by having a 62 kDa protein.

Correlation coefficients between various parameters of toxin production (Table 3) indicate that the development of sheath blight disease in the susceptible rice cultivar (IR 50) is positively correlated with the amount of toxic material produced by a particular mutant/isolate (r = 0.926). The symptom development on rice plants was also significantly correlated with the virulence index of the detached leaf sheath inoculated with sclerotia (r = 0.919) and toxin (r = 0.899). Similarly the symptom development on rice sheath inoculated with sclerotia was highly correlated with inoculation with the toxin (r = 0.980).

DISCUSSION

Genetic manipulation of pathogens is essential for understanding the biochemical basis of disease initiation and development. Mutations are the cornerstones of many genetic, biochemical, physiological and molecular studies (Beremand 1989).

To analyse more critically, the role of the RS-toxin in sheath blight pathogenesis, we investigated *R. solani* mutants altered in toxin production. Mutants with enhanced or reduced toxin production could be successfully used to determine the involvement of toxin in disease development. Further, the study of mutants with altered toxin synthesis could facilitate the application of molecular genetic approaches in the study of toxin biosynthesis.

With the help of mutants altered in toxin production, the role of toxins in plant disease has been confirmed in many plant pathogen interactions viz., *Cochliobolus heterostrophus* producing T-toxin in maize (Leach et al. 1982a, 1982b; Turgeon et al. 1995), *C. carbonum* producing HC-toxin (Panaccione et al. 1992), trichothecene produced by *Gibberella zeae* (Proctor et al. 1995), *Gibberella pullicaris* (Desjardins et al. 1992, Beremand 1989, Hohn and Desjardins 1992), *Fusarium sporotrichioides* (Beremand 1987), *Mycosphaerella zeae-maydis* producing PM-toxin (Yun et al. 1997), *Macrophomina phaseolina* producing phaseolinone (Sett et al. 2000), *Pseudomonas syringae* pathovar *tabaci* producing tabtoxin (Turner and Taha 1984), *Pseudomonas syringae* pathovar *syringae* (Rich et al. 1992), *P. syringae* pathovar *tomato* producing coronatine (Bender et al. 1987), *Streptomyces scabies* producing thaxtomin A (Goyer et al. 1998).

Sclerotia being resistant structures and moreover aggregates of mycelia and the lack of spore structures in *R. solani* necessitated mutagenic conditions with varying intensities of gamma irradiation. Considering the less convenience in the isolation of mutants from multicelled mycelia, the *R. solani* mycelia were irradiated using 5–50 krads of gamma rays. The overall morphology of colony size, surface texture and color did not significantly vary among the mutants and wild type isolate. However, treatment with gamma rays resulted in reduced mycelial growth under liquid culture conditions.

When the sclerotia were inoculated on detached leaf sheath and intact rice plants, mutants exhibited differences in virulence index. Some of the mutants had reduced virulence compared to the wild isolate RS7. This was not unexpected as most mutations are deleterious to the organisms. Similar reduced virulence of mutants also occurs in *Cochliobolus heterostrophus* (Leach et al. 1982a) and *Pseudomonas syringae* pathovar *tomato* (Bender et al. 1987) where the mutants produce lesions smaller than the wild type pathogen. The effect of inoculation of the RS-toxin to detached leaf sheaths, however, failed to establish a difference among the mutant/isolates. This may be attributed to the host factors involved in the natural release of toxin from sclerotia. This was not relevant in the case of purified toxin directly used for inoculation.

The amount of toxin produced by mutants RSG1 and RSG3 was equal to the wild type isolate RS7. Since all the mutants produced toxin, no mutant could be designated as tox$^-$. But the amount of toxin produced by the mutants reduced to more than 30 percent in RSG4 and RSG6. Goyer et al. (1998) reported 2–10 times reduction in thaxtomin produced by the *Streptomyces scabies* mutants.

In addition to mycelial growth, virulence and toxin production, some of the mutants showed reduced sclerotial production in culture media and infected host tissue. It is also interesting to note that none of the mutants were superior to the wild type virulent RS7 and inferior than wild type less virulent RS6 isolates in all the characteristics under study. Among the mutants, those derived from higher doses of gamma rays (30, 40 and 50 krads) were altered remarkably in their virulence and toxin production.

Panaccione et al. (1992) reported that specific proteins secreted inside the mycelium regulated the toxin production in *Cochliobolus carbonum*. SDS-PAGE analysis of mycelial proteins was used as a molecular tool to study the variation among the field isolates of *R. solani* (Sriram et al. 1997). Many mycelial proteins of *R. solani* mutants and wild isolate had the same migration pattern in this study, while some new proteins were present and some wild type proteins were absent in the mutant's mycelia of different ages. Hence from the results obtained it was difficult to determine if a particular protein was involved in toxin synthesis. However changes in mycelial protein pattern seen in the mutants as well as the altered toxin production and virulence indicate that some proteins may play a role in toxin synthesis.

Correlation between the various parameters indicated that the disease development in rice plants inoculated with mutants/isolates was positively correlated with amount of toxin produced. The symptom development on detached sheathes was also positively correlated with disease development.

From all the experiments, it was observed that the mutagen used generated mutation to a lesser extent which resulted in toxin less mutants rather than tox$^-$

mutants. Since the mutation is not site directed, the mutagenesis could not have completely blocked the toxin gene(s) which is evident from that none of the mutant obtained being a non toxin producer. However the study provides evidence of the importance of toxin in the sheath blight disease and the amount of toxin produced by the pathogen playing a significant role in degree of disease development.

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Book Review

Kálmán Vánky

Illustrated genera of smut fungi. Second edition.
APS PRESS, The American Phytopathological Society – St. Paul, Minnesota, 2002,
(I-XIV) 238 p., 98 figures containing 422 illustrations.
ISBN: 0–89054–297-X, price: 69 USD.
The first edition of this book was reviewed in CM 43/4, 1989 by Z. Pouzar. Last year this famous book was newly edited.

Comparing the present edition with that of 1987, it is evident that it has been significantly enlarged and actualised. The author is at present the most recognised specialist and expert on the taxonomy of smut fungi, as his numerous papers especially from the past years show. In this new edition he uses his lifelong experience and knowledge. He characterises the genera with the help of results of the latest studies and completes the description by objective tables. Compared with the previous edition that contained the description of 55 genera, this edition presents 89 genera, 77 of which are accepted and 12 are considered doubtful or have been excluded from the smut fungi.

The book consists of a substantially reworked and extended introduction and a principal part that contains descriptions and illustrations of smut fungi genera in alphabetical order.

Starting with a brief historical review of the classification of smut fungi, the author arrives to the present state of knowledge of this group of parasites. New characters obtained by the study of the ultrastructure of septal pore and host-parasite interaction zone are presented graphically and well-arranged. These characters and the results of molecular analyses of sequence NA are used to construct a phylogenetically based classification of smuts. An incorporation of smut fungi into the new system of classification of basidiomycetous fungi is presented. From the orders of the class Ustilaginomycetes only the smut fungi with teliospores are included in the book. However, the order Microbotryales belonging to the class Urediniomycetes is also presented in the book.

A separate chapter is dedicated to the special problems of classification and nomenclature that have arisen lately. Using the examples of individual groups of smut fungi the author points out that newly obtained data leading to the phylogenetically based classification are permanently accumulating. Several special keys to the genera of the groups under discussion are presented. They are based on the main differentiating characters of these genera. This classification will of course be further developed as new knowledge is obtained.

In the following chapter Vánky summarises the knowledge on specialisation of smuts to the host plants. Most species are parasites on grasses. The chapter enumerates the monocotyledonous and dicotyledonous plant families and the number of smut genera living on them.

Very important is the enlarged key to the genera of smut fungi. Also additional parts of the book as a glossary, abbreviations and a list of references are very helpful.

In the main part of the book, the reproductions of the author's own drawings unfortunately do not have the quality of the previous edition. On the other hand, some microphotographs in this edition were replaced by better ones. Completely new tables illustrating the description of newly included genera are presented.

The book is the best manual of smut fungi, and an essential work for mycologists, plant pathologists and students all over the world.

Jaroslava Marková

CZECH MYCOL. 55 (3–4), 2003

Survival rate of Trichophyton equinum and T. verrucosum mutants at lyophilisation

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Rybnikář A., Hejtmánek M. and Weigl E. (2003): Survival rate of Trichophyton equinum and T. verrucosum mutants at lyophilisation. – Czech Mycol. 55: 273–276

Trichophyton equinum and *T. verrucosum* mutants were prepared from monoconidial wild-type strains by induction with ultraviolet radiation. The percentage of elements surviving at lyophilisation was approximately the same as or higher than that of relative wild-type strain with four of twelve *T. equinum* mutants and five of nine *T. verrucosum* mutants. With remaining eight *T. equinum* mutants and four *T. verrucosum* mutants the ability to survive at lyophilisation was lower in comparison with the wild-type strains.

Key words: lyophilisation, dermatophytes, mutants

Rybnikář A., Hejtmánek M. a Weigl E. (2003): Přežívání mutantů Trichophyton equinum a T. verrucosum při lyofilizaci. – Czech Mycol. 55: 273–276

Mutanti *Trichophyton equinum* a *T. verrucosum* byli připraveni z monokonidiálních divokých kmenů indukcí UV-zářením. U čtyř z dvanácti mutantů *T. equinum* a u pěti z devíti mutantů *T. verrucosum* bylo procento elementů přežívajících při lyofilizaci přibližně stejné nebo i vyšší než u příslušného divokého kmene. U zbývajících osmi mutantů *T. equinum* a čtyř mutantů *T. verrucosum* byla schopnost přežívat lyofilizaci oproti výchozím kmenům nižší.

INTRODUCTION

First studies of preparation of dermatophyte mutants by means of ultraviolet radiation were published long ago by Emmons and Hollaender (1939, 1945). Their studies were above all aimed at taxonomy. Growth, morphological, anatomical and biochemical properties of mutants of dermatophyte fungi induced by ultraviolet radiation were also studied by other authors (Lenhart 1965, 1969; Hejtmánek et al. 1986; Hejtmánek and Geschwinderová 1988). We were interested to know to what extent induced mutation affects the ability of dermatophytes to survive at lyophilisation. We selected 12 *Trichophyton equinum* mutants and 9 *T. verrucosum* mutants prepared by means of ultraviolet radiation to solve these problems. The formation of microconidia in all tested mutants reached the level of wild-type strains.

MATERIALS AND METHODS

Wild-type strains of *Trichophyton equinum* no. 4043 and *T. verrucosum* no. 650 as well as their mutants induced by ultraviolet radiation (Hejtmánek et al. 1986, Weigl and Hejtmánek 1988) were cultivated on malt agar. Some of tested strains (wild-type 650, M-9, M-31, M-141) have been deposited in Czech Collection of Microorganisms Brno, the other strains have been deposited in the collection of microorganisms in Bioveta Ivanovice na Hané company. Cultivation took place in the dark at a temperature of 28 °C for a period of 12–16 days. Grown cultures were homogenised in a physiological saline environment until a homogeneous suspension was formed. As a protective lyophilisation medium a water solution of 5 % gelatine and 7.5 % saccharose was added in the same amount as the saline solution. The suspension formed was dispensed standardly into glass medicine bottles under stable agitation and lyophilised (Rybnikář et al. 1983). The medicine bottles with lyophilised strains were closed with rubber airtight stoppers under vacuum.

The number of viable CFU (colony forming units) was established before lyophilisation and within 5 days after lyophilisation terminated with all strains being investigated. Inoculation of standardly diluted samples on Sabouraud's agar were performed by the plate dilution method (Rybnikář 1981).

RESULTS

If the a number of CFU before lyophilisation is set to 100 %, then 85 % of CFU survive lyophilisation with the wild-type strain of *Trichophyton equinum*. The same or a higher percentage of survival rate at lyophilisation was found at four of twelve *T. equinum* mutants. The number of viable elements after lyophilisation amounted to 32.4–70.9 % (Table 1) with the remaining eight *T. equinum* mutants.

With the wild-type strain of *T. verrucosum* the number of CFU fell to 58.9 % after lyophilisation in comparison with the state before lyophilisation (Table 2).

With five *T. verrucosum* mutants of nine being investigated the fall of relative viability was practically the same or even distinctly lower at lyophilisation. The viability of four *T. verrucosum* mutants was relatively low in comparison with wild-type strain (27.3–49.8 %) after lyophilisation.

DISCUSSION

Lyophilisation presents one of the most utilized methods of long-term preservation of microscopical fungi (Bunse and Steigleder 1991). A fact of common knowledge is that only spore-bearing strains of micromycetes are suitable for this method of preservation. The best results were obtained with cultures forming a great number

Table 1. Survival rate of *Trichophyton equinum* strains at lyophilisation.

Strain number*	CFU number/ml of standard suspension		CFU % surviving at lyophilisation
	before lyophilisation	after lyophilisation	
Wild-type 4043	8,510,000	7,230,000	85.0
M-3	4,125,000	3,550,000	86.1
M-5	4,090,000	2,900,000	70.9
M-48	7,900,000	4,975,000	63.0
M-70	3,775,000	2,425,000	64.2
M-77	5,150,000	4,350,000	84.5
M-85	7,950,000	3,800,000	47.8
M-88	1,064,000	350,000	32.9
M-92	6,975,000	4,125,000	59.1
M-94	3,122,000	1,010,000	32.4
M-141	8,650,000	8,450,000	97.7
M-146	1,851,000	750,000	40.5
M-159	8,700,000	7,900,000	90.8

* Strains M-3 to M-159 are mutants prepared from wild-type no. 4043 by means of ultraviolet radiation.

Table 2. Survival rate of *Trichophyton verrucosum* strains at lyophilisation.

Strain number*	CFU number/ml of standard suspension		CFU % surviving at lyophilisation
	before lyophilisation	after lyophilisation	
Wild-type 650	8,400,000	4,950,000	58.9
M-1	3,175,000	2,410,000	75,9
M-8	4,925,000	3,175,000	64.5
M-9	8,825,000	6,770,000	76.7
M-25	1,685,000	460,000	27.3
M-26	3,250,000	2,338,000	71.9
M-31	10,850,000	6,800,000	62.7
M-39	1,920,000	675,000	35.2
M-41	3,424,000	1,150,000	33.6
M-62	6,150,000	3,063,000	49.8

* Strains M-1 to M-62 are mutants prepared from wild-type no. 650 by means of ultraviolet radiation.

of microconidia (Rybnikář et al. 1983, Rybnikář 1994) at lyophilisation of dermatophytes. Therefore, we selected for our experiments mutants with which the forming of these spores was not expressively reduced in comparison with initial strains.

It is obvious from former studies (Hejtmánek et al. 1986, Hejtmánek and Geschwinderová 1988, Weigl and Hejtmánek 1988) that some biological properties of dermatophyte mutants prepared by means of ultraviolet radiation are in comparison with wild-type strains markedly different. Avirulent mutants or mutants having reduced virulence have originated from wild-type, virulent types of *T. equinum* and *T. verrucosum*. Above all their growth rate, temperature sensitivity, micro- and macromorphology, keratinolytic activity as well as biochemical properties are different. The results of this study show that the induced mutation of dermatophyte strains can strongly decrease or even slightly increase their sporulation. The ability to survive at lyophilisation was relatively decreased in some mutants in comparison with the wild-type strain. However, with other mutants it was not affected negatively. With several strains prepared by ultraviolet radiation even a higher survival rate at lyophilisation was found in comparison with the wild-type strain. These results were unexpected. However, they showed that it is possible to obtain more advantageous properties of new dermatophyte strains prepared by way of mutation.

The methods of induced mutation and selection of dermatophytes are used not only in theoretical studies, but also in industry. The mutant strains of *T. equinum* and *T. verrucosum* prepared by ultraviolet radiation form the basic effective part of freeze-dried antimycotic vaccines produced at Bioveta Ivanovice na Hané (Rybnikář et al. 1990, Rybnikář et al. 1996).

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CZECH MYCOL. 55 (3–4), 2003

Rost-, Brand- und Falsche Mehltaupilze neu für Mähren und tschechisch Schlesien

JIŘÍ MÜLLER

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Müller J. (2003): Rusts, smuts and downy mildews new for Moravia and Czech Silesia. – Czech Mycol. 55: 277–290

27 species and 1 variety (7 species of *Peronosporales* s. str., 17 species and 1 variety of Urediniomycetes and 3 species of Ustilaginomycetes) from Moravia and Czech Silesia so far not published, are reported. From that *Peronospora arthurii*, *P. statices*, *Plasmopara angustiterminalis*, *Coleosporium doronici*, *Frommeëlla mexicana* var. *indicae*, *Melampsoridium hiratsukanum* and *Puccinia ruebsaamenii* are new for the Czech Republic.

Key words: Peronosporales, Urediniomycetes, Ustilaginomycetes, Moravia, Czech Silesia

Müller J. (2003): Rzi, sněti a fytopatogenní plísně nové pro Moravu a české Slezsko. – Czech Mycol. 55: 277–290

Je uvedeno 27 druhů a 1 varieta (7 druhů *Peronosporales* s. str., 17 druhů a 1 varieta Urediniomycetes a 3 druhy Ustilaginomycetes), které nebyly dosud z Moravy a českého Slezska publikovány. Z toho *Peronospora arthurii*, *P. statices*, *Plasmopara angustiterminalis*, *Coleosporium doronici*, *Frommeëlla mexicana* var. *indicae*, *Melampsoridium hiratsukanum* a *Puccinia ruebsaamenii* jsou nové pro Českou republiku.

EINLEITUNG

Bei der Vorbereitung eines Verzeichnisses mährischer und schlesischer Rost-, Brand- und Falscher Mehltaupilze (P. Kokeš und J. Müller in Vorbereitung) stellten wir fest, dass einige Arten, die ich in meinem Herbarium besitze, bisher aus diesem Gebiet nicht publiziert worden sind. Damit diese Nachweise in das Verzeichnis eingereiht werden können, erlaube ich mir, diese Funde zu veröffentlichen. Einige dieser Arten hat aus Mähren und Schlesien lediglich Hruby (1927, 1929, 1930) publiziert. Seine Angaben werden jedoch als sehr zweifelhaft betrachtet (siehe die Kritik Picbauers (1928) und die Bemerkungen Skalickýs (1953: 135, 1954: 133–134, 1983: 79–80) und Vánkys (1994: 107, 187)). Hruby führt z. B. aus den Steppenbeständen beim Ort Pouzdřany in Südmähren *Uromyces lapponicus* Lagerh. an *Astragalus austriacus* auf. Dieser arktisch-alpine Rostpilz parasitiert in Eurasien an *Astragalus alpinus* L., *A. australis* (L.) Lam. und *A. maddenianus* Benth. *Peronospora ranunculi* Gäum. bestimmte er als *Plasmopara pygmaea* (Unger) J. Schröt. (siehe Skalický 1954), *Entyloma calendulae* (Oudem.) de Bary an *Calendula officinalis* L. aus Deutschland als *E. zinniae* Syd., die nur aus Südafrika bekannt ist (Vánky 1994) usw. Einige von Hruby aus Mähren bzw. Schlesien angegebene Arten kann ich bestätigen, wenn auch von anderen Lokalitäten.

MATERIAL UND METHODEN

Mein eigenes Material wurde in der Zeitspanne von 1949–2002 auf dem Gebiet Mährens und auch Schlesiens gesammelt und mittels des Lichtmikroskops Meopta bei der Vergrösserung 100× und 450× bestimmt. Die Gattungen wurden in die Ordnung Peronosporales und die Klassen Urediniomycetes und Ustilaginomycetes nach den neuen Klassifikationen von Dick, Wong et Clark (1984) und Vánky (2001) eingereiht.

Autorennamen der Pilze wurden nach Hooker et Jackson (1996) abgekürzt. Die Nomenklatur der Wirtspflanzen wurde den bisher erschienenen 6 Bänden der "Květena České republiky" von Hejný et Slavík (1988–1992), Slavík (1995–2000), sonst Dostál (1989), entnommen. Die Fundorte der einzelnen Arten sind chronologisch angeordnet. Die Belege befinden sich in meinem Herbarium.

Abkürzungen und Zeichen: N = nördlich, S = südlich, W = westlich, Ö = östlich, NW = nordwestlich usw. Bei den Rostpilzen werden die Entwicklungsstadien (0 = Spermogonien, I = Aezien, II = Uredien, III = Telien) aufgeführt. !! = leg. J. Müller, ! = der Pilz wurde von mir bestimmt oder revidiert.

ERGEBNISSE UND DISKUSSION

Peronosporales s. str.

Peronospora arthurii Farl.

An *Oenothera* sp. cult. Břeclav: Zuchtstation Valtice, ca. 200 m ü. M., 1. VII. 1993 leg. P. Ackermann ! An *Oenothera biennis* L. Brno-Obřany: öde Stelle am Abhang oberhalb der Eisenbahnstrecke nach Bílovice n. Sv., ca. 240 m ü. M., 19. V. 1999 !! In der Slowakei entdeckte diesen Pilz V. Zacha an *Oenothera* sp.: Senica: Waldbaumschule beim Ort Šajdíkove Humence, ca. 200 m ü. M., 23. IX. 1994 ! Dieser Neomyzet ist neu für die ehemalige Tschechoslowakei. In Valtice kam dieser Falsche Mehltaupilz in grossflächigen Kulturen von *Oenothera* vor. Er wurde hier offensichtlich durch befallene Samen eingeschleppt, denn es wurde festgestellt, dass *P. arthurii* durch infizierte Samen verbreitet wird (siehe Neergard 1977). Sie stammt aus Nordamerika, wo sie an den Gattungen *Clarkia*, *Gaura*, *Oenothera* und *Onagra* aus der Familie *Onagraceae* parasitiert. An *Clarkia* kommt sie auch in Palästina und Marokko vor. In Europa kann sie als ein Neomyzet bezeichnet werden. Vor 1970 wurde sie in Deutschland gefunden (Kochman et

Majewski 1970). Aus Deutschland sind noch folgende Aufsammlungen bekannt: Sachsen: Elbe-Mulde-Tiefland: bei Beerendorf, an *Oenothera biennis,* VII. 1996 leg. H. Jage; Kossa, an *O. biennis* und *O. pycnocarpa* G. F. Atk. et Bartlett, X. 1996 leg. H. Jage (Jage 1998). Erzgebirge: Landkreis Marienberg, Pressnitztal NW Boden, ca. 500 m ü. M., an *O. biennis,* 3. V. 1998 leg. W. Dietrich (Triebel 1999). Mecklenburg-Vorpommern: Bahnhof in Greifswald, an *O. parviflora* L. s. l., 5. VII. 1997 leg. M. Scholler (Scheuer 1998).

Peronospora chenopodii-polyspermi Gäum.

An *Chenopodium polyspermum* L. Vsetín: Tal unter dem Zádilský, ca. 400 m ü. M., 4. VIII. 1973 !! Břeclav: Feld SW von Velké Němčice, ca. 180 m ü. M., 27. VI. 1980 !! Wird von Hruby (1930) angegeben: Přerov: Garten in Hranice (Petrak, Flora Bohemiae et Moraviae exsiccata II/1 Pilze, Lf. 39, Nr. 1942). Břeclav: Dyje-Auen bei Strachotín (heute durch die Talsperre vernichtet).

Peronospora dipsaci de Bary

An *Dipsacus laciniatus* L. Hodonín: Feldrand am Wassergraben S Ort Mikulčice, 158 m ü. M., 26. IX. 1989 !! Wird von Hruby (1930) angegeben: an *Dipsacus fullonum* L.: im Flachlande überall häufig. Orte Břeclav, Lednice, Hodonín, Brno, Ivančice, Olomouc. An *D. laciniatus*: Dyje-Auen bei Dolní Věstonice und Lednice. An *Virga pilosa* (L.) Hill: Morava-Auen bei Hodonín, 1923.

Peronospora lychnitis Gäum.

An *Steris viscaria* (L.) Rafin. (*Viscaria vulgaris* Bernh.). Brno: am Felsen im Tal des Flusses Jihlava bei Ivančice, ca. 220 m ü. M., 13. VI. 1954 !! Wird von Hruby (1930) an *Lychnis coronaria* (L.) Desr. aufgeführt: Gärtnerei Kalina in Brno – Královo Pole, 1926.

Peronospora ruegeriae Gäum.

An *Onobrychis viciifolia* Scop. Brno-Bohunice: am Zaun des Krankenhauses, 280 m ü. M., 17. V. 1999 leg. et det. H. Dvořáková ! Wird von Hruby (1930) angegeben: an *O. viciifolia*: in Mittel- und Westmähren verbreitet. Brno, Třebíč, Velké Meziříčí, Náměšť n. Osl., Moravský Krumlov.

Peronospora statices Lobik

An *Goniolimon tataricum* (L.) Boiss. (*Statice tatarica* L.) cult. Hodonín: Feld der Gärtnerei der Landwirtschaftlichen Produktionsgenossenschaft Strážnice, ca. 180 m ü. M., 16. X. 1984 leg. V. Zacha ! Die Wirtspflanze wird bei uns als Zierpflanze für Trockensträusse angebaut. Der Falsche Mehltau kam in den 80er Jahren epiphytotisch vor, aber es ist mir keine Publikation aus Mähren bekannt. Er wurde erstmalig von A. I. Lobik im Juni 1925 an *Limonium gmelinii* (Willd.)

Kuntze in Russland gefunden und 1928 beschrieben. 1932 haben T. Săvulescu und Rayss an *Limonium vulgare* Mill. subsp. *serotinum* (Rchb.) Gams aus Rumänien eine *Peronospora constantineanui* und 1976 Simonian an *Limonium meyeri* (Boiss.) Kuntze aus Armenien *Peronospora limonii* aufgestellt, die jedoch beide nach Constantinescu (1991) mit *P. statices* Lobik identisch sind. *P. statices* ist auch an *Limonium sinuatum* (L.) Mill. in England und den Niederlanden, *L.* × *hybridum* und *L. altaicum* in Italien, *Goniolimon tataricum* in Ungarn, *L. vulgare* subsp. *serotinum* in Frankreich, *L. meyeri* in Aserbaidschan und *L. gmelinii* in Rumänien bekannt (Hall, Lane et Mellor 1997). Die letztgenannten Autoren haben auch Oosporen in den befallenen Blättern gefunden und vermuten, dass der Pilz durch infizierte Setzlinge oder Samen verbreitet wird. Nach den Erfahrungen aus England war Furalaxyl wirksam gegen diese Krankheit.

Plasmopara angustiterminalis Novot.

An *Xanthium strumarium* L. Znojmo: Ackerrain unweit vom Ort Medlice, ca. 350 m ü. M., X. 1960 !! Ackerrain bei Kubšice (unweit Moravský Krumlov), ca. 250 m ü. M., IX. 1966 !! Ackerrain bei Tvořihráz, ca. 200 m ü. M., IX. 1966 !! Dieser Falsche Mehltaupilz parasitiert an *Xanthium strumarium* in Russland, Litauen, der Ukraine, Polen, der Slowakei (Nové Zámky: beim Flusse Hron im Orte Bíňa, V. 1982 leg. V. Zacha !), Ungarn, Rumänien (Kochman et Majewski 1970) und Österreich (Poelt et Remler 1977), an *Bidens frondosa* L., *B. vulgata* Greene und *Ambrosia psilostachya* DC. in Nordamerika.

Urediniomycetes

Coleosporium doronici Namysł., III.

An *Doronicum austriacum* Jacq. Hrubý Jeseník: subalpine Wiese am oberen Rande des Kessels Velká kotlina zusammen mit *Pinus mugo* Turra, ca. 1400 m ü. M., 19. VIII. 1953 !! An *Pinus mugo* entwickelt der Rostpilz wahrscheinlich Spermogonien und Aezien (siehe Müller 1980). An *D. austriacum* wird dieser Rostpilz von Hruby (1927) auch vom Hrubý Jeseník aufgeführt: Weg von Karlova Studánka über Karlov zur Velká kotlina.

Frommeëlla mexicana var. **indicae** J. W. McCain et J. F. Hennen, II, III.

An *Duchesnea indica* (Andrews) Focke. Brno-Veveří: Botanischer Garten der Masaryk-Universität, 245 m ü. M., 29. V. 2002 leg. R. Řepka et J. Müller und 5. XI. 2002 !! Noch Anfang November entwickelte die Art reichlich Uredien, seltener Telien. Die Teliosporen messen 55–78 × 18–22 μm. Diesen Neomyzeten hat Herr Ing. Radomír Řepka entdeckt. Er ist neu für die Tschechische Republik. Er wurde von McCain und Hennen (1990) aufgrund der Aufsammlung der Telien an *Duchesnea indica* aus den USA beschrieben: Alabama, Lee Co., Auburn, XI. 1899 leg. F. S. Earle. Der Rost parasitiert nur an dieser Wirtspflanze. In den USA weist er eine bedeutende Verbreitung auf: District of Columbia, Alabama, Arkansas, Arizona, California, Delaware, Florida, Georgia, Indiana, Kentucky, Louisiana, Maryland, Missouri, Mississippi, New York, North Carolina, Oklahoma, Pennsylvania, South Carolina, Tennessee, Texas, Vermont und Virginia. Aus den Glashäusern ist er noch aus Michigan und Minnesota bekannt. Daneben wurde der Rost in Mexico, Brasilien, an 2 Fundorten in Argentinien und in China in Hunan gefunden (McCain et Hennen 1990). In den USA ist er hauptsächlich im Osten (Vermont – Missouri) und Süden (Florida – California) verbreitet. In Europa wurde er in Frankreich von Viennot-Bourgin (1954) im August 1952 beim Dorfe Denguin NW von Pau (Basses-Pyrénées) gefunden. Der Erstfund aus Mitteleuropa stammt aus Österreich: Oberösterreich: Ried im Innkreis und Steiermark: mehrfach in Graz (Poelt et Zwetko 1997). Nach den letzten Autoren dürfte der Pilz in erster Linie im Uredostadium überdauern. Die Tschechische Republik ist also der dritte Staat in Europa, wo dieser Rostpilz nachgewiesen wurde.

Melampsora ari-salicina A. Raabe, 0, I.

An *Arum alpinum* Schott et Kotschy. Přerov: im jungen, offensichtlich angepflanzten Eichen-Weidenwalde am rechten Ufer des Flusses Bečva zwischen den Orten Teplice nad Bečvou und Černotín, ca. 245 m ü. M., 21. IV. 2002 !! Die Weiden gehören zur Art *Salix fragilis* und am Boden fand ich abgefallene Blätter dieser Weide vom vorigen Jahr mit Telien. Die Spermogonien und Aezien wurden bisher nicht beschrieben, deswegen gebe ich hier die Beschreibung: Spermogonien auf der Blattober– und –unterseite. Aezienlager (Caeoma) auf der Blattunterseite, kreisförmig um die Spermogonien angeordnet, orange, rund bis länglich, mitunter hörnchenförmig gekrümmt, 0,75–2 mm lang und 0,5–1 mm breit, auf der Blattoberseite 5–10 mm grosse, rundliche blassgrüne Flecken verursachend. Aeziosporen unregelmässig, im Umriss oval, eiförmig oder rund und dabei stets schwach eckig, 17,5–24 × 14–18 μm (n = 10), die Wand 2 μm dick, fein und dicht warzig (Warzenabstand ca. 1 μm). *Arum alpinum* ist eine neue Wirtspflanze.

Melampsoridium hiratsukanum S. Ito ex Hirats., II, III.

An *Alnus incana* (L.) Moench und *Alnus glutinosa* (L.) Gaertner. Vyškov: Rand des Mischwaldes im Tal des Baches W vom Ort Studnice, 543 m ü. M., 5. IX. 2002 !! Alnetum oberhalb des Tales der Velká Haná N Rychtářov, 386 m ü. M., 6. IX. 2002 leg. P. Kokeš ! Alnetum am Teich des Hraniční potok SÖ Nové Sady, 475 m ü. M., 8. IX. 2002 leg. P. Kokeš ! Alnetum an der Strasse von Ferdinandsko nach Zelená Hora, 467 m ü. M., 8. IX. 2002 leg. P. Kokeš ! Alnetum im Tal Konůvky S Rašovice, 256 m ü. M., 29. IX. 2002 leg. P. Kokeš ! Alnetum im Tal des Baches Brodečka Ö Kobylničky, 268 m ü. M., 24. X. 2002 leg. P. Kokeš ! An *Alnus incana* und *A. glutinosa* × *incana* (*A.* ×*pubescens* Tausch). Vyškov:

Alnetum an der Quelle des Baches W der Ortschaft Ferdinandsko, 508 m ü. M., 8. und 27. IX. 2002 leg. P. Kokeš ! An *A. incana*. Alnetum am Südhang der Vojenská NW von Drysice, 425 m ü. M., 8. IX. 2002 leg. P. Kokeš ! Linkes Ufer des Baches SÖ Kloboučky, ca. 235 m ü. M., 1. X. 2002 leg. P. Kokeš et J. Müller. An *A. incana*, *A. glutinosa* und *A. ×pubescens*. Alnetum im Tal Žbánovský žleb NÖ Bousín, 452 m ü. M., 19. X. 2002 leg. P. Kokeš ! Alnetum im Tal des Baches Hraniční potok SW Myslejovice, 352 m ü. M., 20. X. 2002 leg. P. Kokeš !

Wie ersichtlich, kam *M. hiratsukanum* im Kreis Vyškov epiphytotisch vor. Im Gegensatz zu den Angaben von Henderson et Bennell (1979), dass der Rost in Britannien vor allem an jungen Trieben und ein– oder zweijährigen Sämlingen von *Alnus* vorkommt und der als *Melampsoridium betulinum* (Fr.) Kleb. bestimmt wurde, kam es bei uns zum Befall erwachsener Bäume. Besonders die Bäume von *Alnus incana* wurden sehr stark befallen, sodass fast alle Blätter dicht von den Uredien bedeckt waren und die Blattspreiten rollten sich aufwärts längs der Mittelrippe. Auf den früher befallenen Blättern bildeten die leeren, hyalinen (wahrscheinlich ausgekeimten) Urediosporen eine weissliche Kruste. Oft bildeten sich die Sporenlager längs der Blattnerven. *Alnus glutinosa* wurde schwächer befallen und immer nur in der Nähe von infizierter *A. incana*. An der ersteren kam es nicht zur Blattrollung und die Telien erschienen früher als an *A. incana*. Ein Strauch von *A. glutinosa* in unmittelbarer Nähe von *A. incana* wurde meistens auf der der *A. incana* angrenzenden Seite befallen, sodass offensichtlich der Rostpilz von *A. incana* auf *A. glutinosa* übergeht. Beschreibung meiner Aufsammlungen: Urediosporen länglich-oval bis elliptisch, farblos bis blass gelblich, 22–33 × 10,5–16 μm (n = 20) gross. Wand ca. 2 μm dick, über die ganze Oberfläche (auch am Scheitel) locker stachelig (Stachelabstand ca. 3 μm). Obere Pseudoperidienzellen von der Fläche gesehen polygonal, 7–13 × 7–11 μm gross; seitliche Pseudoperidienzellen radial gestreckt. Mündungszellen eiförmig-konisch, in einen scharf spitzen, bis 50 μm langen und unten 6 μm breiten Stachel mit vereinzelten kleinen Wärzchen ausgezogen. Teliosporen 51–54 × 13–16 μm, oben und unten gerundet, bräunlich, Wand ca. 1 μm, oben etwa 3 μm dick.

M. hiratsukanum wechselt an *Larix* sp. hinüber. An der nahe verwandten Gattung *Duschekia* Opiz (= *Alnus* sect. *Alnobetula* Koch) ist *Melampsoridium alni* (Thüm.) Dietel bekannt. Seine Urediosporen sind länger: 32–47 × 9–15 μm und haben einen glatten Scheitel (ohne Stacheln). *M. hiratsukanum* wurde 1927 aus Japan beschrieben. Jedoch schon 1921 hat Grove in England ein *Melampsoridium* an *Alnus incana* gefunden, das aber zu *M. betulinum* oder *M. alni* gestellt wurde (siehe Gäumann 1959: 71). *M. betulinum* hat 22–40 × 8–12 μm grosse und am oberen Ende glatte Urediosporen. 1924 berichtet Wilson über Uredien und Telien an Keimpflanzen von *Alnus glutinosa* und *A. incana,* die in Schottland aus importiertem Saatgut gezogen worden waren. Kuprevicz et Tranzschel (1957) halten diesen Rost für *M. hiratsukanum*. Dieser Rostpilz kommt in Japan und im Fernen Osten Russlands vor (hier an *Alnus hirsuta* Turcz., *A. japonica* Sieb. et Zucc. und dem Hybriden *A. hirsuta* × *japonica*). Nach Kuprevicz und Tranzschel (1957) wurde dieser Pilz offensichtlich an *Alnus*-Arten auch in Nord- und Zentralamerika von Kalifornien nach Guatemala und in Südamerika in Ecuador gefunden. Henderson et Bennell (1979) bezweifeln die Existenz von *M. hiratsukanum* in Europa und schreiben, dass die Angaben über den Rost an *Alnus* in Britannien *Melampsoridium betulinum* betreffen. Põldmaa (1997) führt *Melampsoridium* sp. an *Alnus incana* von einigen Lokalitäten in Estland auf.

Den 12. IX. 1999 entdeckte W. Dietrich *Melampsoridium hiratsukanum* an *Alnus incana* in Deutschland: Sachsen, Erzgebirge: NSG Lohenbachtal bei Tannenberg, II ! und den 14. IX. 2002 an *A. incana* und *A. glutinosa* im NSG Am Steinbach, II, III ! An *A. incana* fand W. Dietrich diesen Rost auch auf der böhmischen Seite des Erzgebirges bei Kovářská, 8. X. 2001, II, III ! (Erstfund in der Tschechischen Republik). Endlich sendete mir Herr A. Bolay einen Rost an *Alnus incana* und *A. glutinosa,* den er in der Schweiz, Vaud: Arboretum in Aubonne, 16. X. 2001 gesammelt und als *Melampsoridium hiratsukanum* bestimmt hat, zur Revision. Ich konnte die Richtigkeit der Identifikation bestätigen. Die Merkmale seiner Belege stimmen vollkommen mit meinem Material überein. Nach A. Bolay (in litt.) entwickelte sich der Rost epidemisch in der deutschen und französischen Schweiz. Ende Oktober 2002 sammelte A. Bolay *M. hiratsukanum* im botanischen Garten in Genf an *Alnus incana*, *A. hirsuta*, *A. japonica* und *A. maritima* (Marshall) Nutt. Er beobachtete diesen Rostpilz an *A. incana* auch in Frankreich im Tal der Isère bei Grenoble.

Neuestens (Scheuer 2003) wird *M. hiratsukanum* aus Österreich gemeldet: an *Alnus incana*. Steiermark: östlicher Stadtrand von Graz, Äussere Ragnitz, in einem Garten, 410 m ü. M., 22. X. 2001 leg. W. Obermayer; Gleinalpe, südlicher Stadtrand von Bruck a. d. Mur, ca. 500 m ü. M., 9. VIII. 2002 leg. Ch. Scheuer. An *Alnus* × *pubescens*. Steiermark: Grazer Bergland N von Graz, S von St. Radegund b. Graz, im Strassengraben, 445 m ü. M., 19. IX. 2002 leg. Ch. Scheuer et C. M. Denchev. Nach Scheuer (l. c.) ist *M. hiratsukan*um an *Alnus incana* in den Jahren 1999–2002 anscheinend in weiten Gebieten Österreichs massenhaft aufgetreten.

Microbotryum stellariae (Liro) G. Deml et Oberw.

An *Stellaria graminea* L. Šumperk: *Festucetum rubrae* oberhalb der Bus-Station Květná bei der Stadt Staré Město, 545 m ü. M., 15. VIII. 2001 !! Wiese zwischen den Hügeln Starý und Větrov NÖ von Staré Město, 830 m ü. M., 22. VIII. 2001 !! Králický Sněžník: Wiese unterhalb der Bergbaude Návrší bei Stříbrnice, ca. 780 m ü. M., 25. VIII. 2001 !! Vyškov: feuchte Wiese im Tal des Baches Brodečka W des Hügels Obrova hora bei Otaslavice, 280 m ü. M., 18. X. 2001 leg. P. Kokeš et J. Müller.

Milesina blechni (P. Syd. et Syd.) P. Syd. et Syd., II.

An *Blechnum spicant* (L.) Roth. Schlesische Beskiden: Fichtenwald am Bach oberhalb der Kolonie Folvark beim Ort Jablunkov, ca. 480 m ü. M., 5. X. 1950 !! Im Tal des Baches Radvanov bei Jablunkov, ca. 500 m ü. M., 7. X. 1950 !! Am Waldweg auf der Velká Čantoryje bei Nýdek, ca. 600 m ü. M., 8. X. 1950 !! Auf dem Berg Ostrá hora bei Písek, ca. 600 m ü. M., 10. X. 1950 !! Hrubý Jeseník: Tal des Baches Studený potok unterhalb des Wasserfalls Vysoký vodopád, ca. 750 m ü. M., 12. VIII. 1953 !! Tal des Flusses Bílá Opava, ca. 1200 m ü. M., 17. VIII. 1953 !! Tal Divoký důl, ca. 1200 m ü. M., 21. VIII. 1953 !! Tal des Flusses Divoká Desná, ca. 850 m ü. M., 21. VIII. 1953 !! Wird von Hruby (1929) auch vom Hrubý Jeseník angegeben: Kessel Velká kotlina.

Milesina kriegeriana (Magnus) Magnus, II.

An *Dryopteris dilatata* (Hoffm.) A. Gray. Schlesien: Rychlebské hory: am Wasserfall im Tal des Baches Stříbrný potok, ca. 500 m ü. M., 20. IX. 1955 !! An *D. dilatata* wird von Hruby (1927) vom Hrubý Jeseník aufgeführt: "Paradies" bei Ostružná und "Nesselkoppe" bei Jeseník. An *D. carthusiana* (Vill.) H. P. Fuchs von demselben Autor: Vidnava: Černý potok (Jüppelbach).

Milesina vogesiaca (Faull) Hirats. f., II.

An *Polystichum aculeatum* (L.) Roth. Moravskoslezské Beskydy: Tal des Baches am Nordhang unterhalb des Jägerhauses Salajka SW vom Ort Bílá, ca. 700 m ü. M., 14. VIII. 1952 !! Hruby (1927) führt an *P. aculeatum* vom Hrubý Jeseník auf: Velká kotlina, Ramzová, Ö von Králický Sněžník: "Paradies" nächst den Wiesen Jívina (Saalwiesen) N Velké Vrbno und Moravskoslezské Beskydy: Lysá hora bei Ostravice.

Puccinia hordei-murini N. F. Buchw., II, III.

An *Hordeum murinum* L. Brno: Schuttplatz hinter der juristischen Fakultät der MU, ca. 250 m ü. M., VI. 1949 !! Břeclav: am Weg im Ort Valtice, ca. 195 m ü. M., 15. VI. 1951 !! Brno-Černá Pole: hinter dem Hause No. 76 der Gasse Provazníkova, ca. 240 m ü. M., 28. V. und 28. VI. 1977 !! Brno-Husovice: Parkanlage "Park blahoslavené sestry Marie Restituty" oberhalb der Gasse Hálkova, 210 m ü. M., 3. VII. 2002 !! Brno-Černovice: Gasse Charbulova beim psychiatrischen Krankenhaus, ca. 200 m ü. M., 27. VII. 2002 !!

Puccinia horiana Henn., III.

An *Dendranthema indicum* (L.) Des Moul. cult. wurde der Rost von der Pflanzenärztlichen Staatsadministration auf dem Felde einer Gärtnerei in Südmähren im September 2000 festgestellt. Es handelt sich um eine Quarantäne-Krankheit und deswegen wurden alle befallenen Pflanzen vernichtet. Der Beleg wurde von mir revidiert.

Abb. 1. Blätter von *Alnus incana*, stark befallen von Uredien des *Melampsoridium hiratsukanum*. Foto P. Kokeš.

Abb. 2. Unterseite eines Blattes von *Alnus incana* mit Uredien des *Melampsoridium hiratsukanum* – Detail. Foto P. Kokeš.

Abb. 3. Urediospore von *Melampsoridium hiratsukanum*. Vergrösserung 1250×. Foto M. Hladíková.

Puccinia ruebsaamenii Magnus, III.

An *Origanum vulgare* L. Moravskoslezské Beskydy: an der Mündung des Baches Mazák beim Ort Ostravice, ca. 420 m ü. M., 7. VIII. 1952 !! Der Rostpilz ist neu für die Tschechische Republik.

Puccinia urticata F. Kern var. **urticae-umbrosae** (Hasler) Zwetko, III.

An *Carex umbrosa* Host. Vyškov: Alnetum am Bach Pytlácký potok SW vom Ort Prostějovičky, 434 m ü. M., 4. XI. 2001 leg. et det. P. Kokeš !

Puccinia verruca Thüm., III.

An *Colymbada scabiosa* (L.) Holub (*Centaurea scabiosa* L.). Tišnov: *Arrhenatheretum elatioris* am Gipfel des Hügels Hradisko bei Železné, ca. 340 m ü. M., 30. VII. 1998 leg. P. Kopřivová et J. Müller. An *C. scabiosa* wurde dieser Rost in der Tschechischen Republik noch nicht gesammelt.

Puccinia winteriana Magnus (nom. nud.), 0, I.

An *Allium ursinum* L. Břeclav: am Nordrand des Waldes Kolby in nassen Wiesen "Jezero" bei Pouzdřany, ca. 200 m ü. M., 20. V. 1979 !! Uherské Hradiště: Auenwald Kněžpolský les bei Jarošov, ca. 185 m ü. M., gemeinsam mit *Phalaroides arundinacea* (L.) Rauschert, 24. V. 1980 !! Dačice: Tal Janovské údolí im Wildpark von Mutná Ö Slavonice, 450 m ü. M., IV. 2002 leg. L. Vaněčková !

Tranzschelia discolor (Fuckel) Tranzschel et M. A. Litv., III.

An *Amygdalus nana* L. cult. Brno-Jundrov: im Garten am Flusse Svratka, ca. 220 m ü. M., X. 1983 leg. V. Zacha ! An *Prunus cerasifera* Ehrh. subspont. Brno-Husovice: am Weg in der Parkanlage oberhalb der Gasse Hálkova ul., ca. 210 m ü. M., 10. XI. 1999 !! An *Prunus insititia* L. und *P. domestica* L. Vyškov: Wiesen Hony bei Malínky, ca. 250 m ü. M., 20. IX. 2001 !! An *P. domestica*. Vyškov: Garten in Vinohrady beim Ort Luleč, 373 m ü. M., 19. X. 2001 leg. et det. P. Kokeš ! und an der Strasse zwischen Nové Hvězdlice und Chvalkovice unweit des Hügels Zavanky, 332 m ü. M., 24. X. 2001 leg. et det. P. Kokeš ! An *Armeniaca vulgaris* Lam. Vyškov: Garten in Vinohrady beim Ort Luleč, 372 m ü. M., 19. X. 2001 leg. et det. P. Kokeš !

Uromyces junci (Desm.) Tul., II.

An *Juncus articulatus* L. Brno: am Waldweg im Tälchen des Baches N von Bílovice n. Sv. (rechter Ufer des Flusses Svitava), ca. 300 m ü. M., 13. X. 1973 !! Tišnov: Waldweg, Na vysoké beim Ort Zahrada, 1998 leg. P. Kopřivová ! Vyškov: seichter kleiner Tümpel auf der Wiese SW Hügel Zadní Lipová SÖ Nové Sady, 506 m ü. M., 11. VIII. 2002 leg. P. Kokeš ! Seichter kleiner Tümpel im nördlichen Teil der Wiesen Holásková NW Radslavičky, 458 m ü. M., 24. VIII. 2002 leg.

P. Kokeš ! Seichter kleiner Tümpel auf den Wiesen W des Véspěrk NW Radslavičky, 445 m ü. M., 24. VIII. 2002 leg. P. Kokeš ! Seichter kleiner Tümpel im W-Teil der Wiesen Holásková NW Radslavičky, 481 m ü. M., 24. VIII. 2002 leg. P. Kokeš ! Nasse Wiese im Tal des Baches W vom Ort Studnice, 518 m ü. M., 5. IX. 2002 leg. P. Kokeš !

Uromyces limonii (DC.) Berk., II, III.

An *Goniolimon tataricum* (L.) Boiss. cult. Brno-Pisárky: Parkanlage Riviéra, ca. 200 m ü. M., 28. IX. 1975 !! Brno-Komárov: Feld am Meierhof Ráj, 197 m ü. M., 6. XI. 1976 !! Hodonín: Feld der Gärtnerei der Landwirtschaftlichen Produktionsgenossenschaft Strážnice, ca. 180 m ü. M., 16. X. 1984 leg. V. Zacha ! Břeclav: Gärtnerei im Ort Tvrdonice, ca. 180 m ü. M., VII. 1991 leg. V. Zacha !

Uromyces renovatus P. Syd. et Syd., II.

An *Lupinus albus* L. cult. Kroměříž: Getreideforschungsanstalt, ca. 190 m ü. M., 3. IX. 1955 leg. F. Brückner ! Ich selbst sammelte diesen Rost auch an *L. albus* in Ostböhmen: Pardubice: Feld bei Lipoltice, ca. 280 m ü. M., 23. VIII. 1977 !! Brückner (1958 p. 444) führt eine Photographie von *U. renovatus* an *Lupinus albus* ohne Fundortangabe auf (Photo Getreideforschungsanstalt Kroměříž), wahrscheinlich von der Aufsammlung aus Kroměříž, jedoch es ist nicht namentlich bezeichnet.

Ustilaginomycetes

Sporisorium sorghi Link

An *Sorghum bicolor* (L.) Moench cult. Brno-Černá Pole: Versuchsfeld des Phytopathologischen Institutes, 240 m ü. M., XI. 1951 !! Břeclav: Feld beim Ort Pohořelice, ca. 200 m ü. M., ca. 1955 leg. L. Marvanová ! Daselbst, 1956 leg. V. Zacha ! Feld in Brno-Komárov, ca. 200 m ü. M., VIII. 1958 leg. J. Soušek ! An *Sorghum sudanense* (Piper) Stapf cult. Břeclav: Feld bei Pohořelice, ca. 200 m ü. M., IX. 1952 leg. V. Zacha ! *S. sudanense* ist wahrscheinlich eine neue Wirtspflanze für Europa (wird von Vánky (1994) nicht angegeben). Benada (1958) schreibt, dass *S. sorghi* [ut *Sphacelotheca sorghi* (Link) G. P. Clinton] in der Tschechoslowakei an *Sorghum sudanense* gefunden wurde und bildet ein schönes Photo ab. Der genaue Fundort wird jedoch nicht erwähnt.

Urocystis leimbachii Oertel

An *Adonanthe vernalis* (L.) Spach (*Adonis vernalis* L.). Hodonín: sonniger Westhang Kopcoví bei Milotice, ca. 250 m ü. M., 24. V. 1981 leg. V. Frantík ! Im Ort Čejč, Haus No. 24, kultiviert, ca. 185 m ü. M., 25. V. 1981 leg. V. Frantík ! Den ersten Fund hat Herr Ing. V. Frantík an Sämlingen von *A. vernalis*, die er zu Hause in Čejč aus den am Kopcoví gesammelten Samen gezogen hat, gemacht. Meinen Hinweis folgend fand er dann den Brand auch an dieser natürlichen Lokalität.

Urocystis primulae (Rostr.) Vánky

An *Primula veris* subsp. *canescens* (Opiz) Lüdi. Brno: im Walde am Osthang des Hügels Dřínová bei Tišnov, 354 m ü. M., 18. VI. 2000 leg. P. Kopřivová !

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First finds of "alder-Phytophthora" in the Czech Republic

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Černý K., Gregorová B., Holub V. and Strnadová V. (2003): First finds of "alder-Phytophthora" in the Czech Republic. – Czech Mycol. 55: 291–296

The new hybride "alder-*Phytophthora*" (*P. cambivora* × *P.* cf. *fragariae*) has originated in western Europe and its area has an expanding tendency. This pathogenic fungus was isolated during studies of declining alders in the river basin of the Ohře river at Chodovský potok brook near Karlovy Vary, western Bohemia. The fungus was found in a substrate with damaged roots and in conductive tissues of trunks of declining trees of *Alnus glutinosa*.

Key words: *Phytophthora*, *Alnus glutinosa*, alder decline

Černý K., Gregorová B., Holub V. a Strnadová V. (2003): První nález hybridního taxonu „alder-*Phytophthora*" v České republice. – Czech Mycol. 55: 291–296

Nový hybrid, tzv. „alder-*Phytophthora*" (*P. cambivora* × *P.* cf. *fragariae*), vznikl v západní Evropě a jeho areál se neustále rozšiřuje. Patogen byl izolován během studií chřadnoucích olší v povodí Ohře na Chodovském potoce poblíž Karlových Varů. Houba byla zjištěna v substrátu s poškozenými kořeny a ve vodivých pletivech hynoucích jedinců *Alnus glutinosa*.

INTRODUCTION

In the last decade a massive decline of alder (*Alnus glutinosa*, *A. incana*, and *A. cordata*) has been reported in western and central Europe (e.g. Gibbs et al. 1999). Declining alders have been reported from Great Britain, Ireland, Belgium, the Netherlands, Sweden, Denmark, France, Germany, Switzerland, Austria, Italy and Hungary (e.g. Anselmi et al. 2001, Brasier et al. 1995, Cech 2000, Osswald et al. 2001, Werres 1998). Some species from the genera *Phytophthora* and *Pythium* were found to be the cause of the disease. As the most harmful a new *Phytophthora* hybrid, so called "alder-*Phytophthora*" (Brasier 1995), was found.

In the Czech Republic an increasing decline of alders was noted at the end of the 1980s and at the beginning of the 1990s (Jančařík 1993). However, the possible cause of this disease, activity of pathogenic pythiaceous fungi in roots and trunks of trees, was not found.

During research by Gregorová et al. (2002) many reports of alder decline were obtained, mainly in river basins of the Labe and Ohře rivers. Several sites with alder decline were visited in 2001 and 2002 and samples of soil with damaged roots and of necrotic trunk tissues were collected. One of the isolated fungi, the "alder-*Phytophthora*", was found at two localities by the Chodovský potok brook near Karlovy Vary. More than one hundred trees of different age have been killed at this locality in the past years and the stability of banks of the lower course of this brook disturbed.

MATERIALS AND METHODS

Study site

The strains of "alder-*Phytophthora*" were found at two localities near Karlovy Vary (district Karlovy Vary, north-west Bohemia). The first locality is the left bank of the Chodovský potok brook in Karlovy Vary-Dvory (50° 13' 42" N, 12° 49' 28" E) near the road to Počerny, the second one is the right bank of the Chodovský potok brook, about 150 m upstream of the bridge near Zátiší (50° 14' 14" N, 12°47' 20" E). This fungus was isolated from soil with damaged roots and from conductive trunk tissue of damaged trees of *Alnus glutinosa*.

Methods

The samples were processed the day after taking. The soil samples were cultivated by the baiting method, the samples of conductive tissue and bark were cultivated directly on agar media.

The soil samples with damaged roots were inserted into sterile glass containers and flooded by deionised sterile water. Young, surface-sterilised leaves of *Alnus glutinosa*, *Syringa vulgaris*, and *Rhododendron* sp. were floated over the water surface as baits. Samples were cultivated in the dark at 21 °C. After several days the baits were repeatedly investigated under a dissect microscope. Parts of leaves with characteristic coenocytic mycelium and/or zoosporangia or with expanding necrosis were cut and put on agar plates.

The samples of conductive tissues and bark of trunks were rinsed with sterile water and repeatedly shaken in sterile water with Tween 20. The samples were then cut into small fragments of tissue, which were rinsed again and placed on Petri dishes.

The water agar with benomyl (25 ppm), quintozene (100 ppm), and with penicillin (50 ppm) and the water agar without fungicides were used for isolation. The obtained strains were purified on the water agar medium and then cultivated and retained on oatmeal agar.

The isolated fungus was first identified as *P. cambivora* (Petri) Buisman according to Erwin and Ribeiro (1996). However, later it was shown that our

Fig. 1. "alder-*Phytophthora*". A: coenocytic hyphae, B: young nesting zoosporangium. Bars: 10 µm.

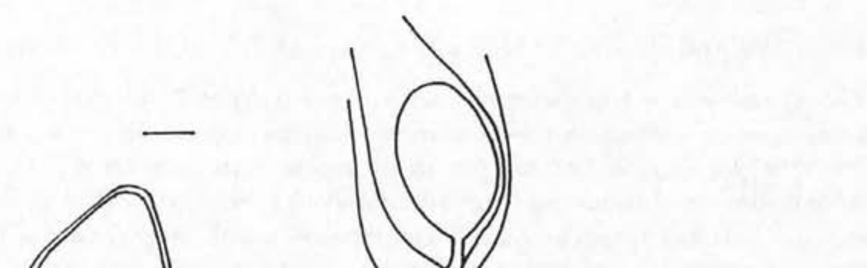

Fig. 2. "alder-*Phytophthora*". A: mature zoosporangium, B: proliferation of zoosporangiophores, C: oogonium and oospore. Bar: 10 µm.

isolates are very close to a fungus associated with alder mortality in Britain, described originally by Brasier et al. (1995). Strains are deposited at Agency for Nature Conservation and Landscape Protection of the Czech Republic.

RESULTS AND DISCUSSION

Frequency of fungus occurrence

The samples were taken throughout the year, but the main part of pythiaceous fungi were found only in summer and autumn. The samples from winter, spring and late autumn were often negative. Five strains of "alder-*Phytophthora*" were isolated in September 2001, the two other were acquired in July and October.

During research of the stands with alder-decline on the Chodovský potok brook several number of samples were taken. 34 samples of soil with roots (only 6 of them positive) and 12 samples of trunk necrosis (1 positive) were acquired. The low frequency of isolation (15.22 %) corresponds with the biology of parasitic pythiaceous fungi and with methodical difficulties of "alder-*Phytophthora*" isolation and corresponds to the obviously mentioned frequencies of isolation (e.g. Jung 1998).

Description and distinguishing characters of "alder-*Phytophthora*"

Colony diameter 6–8 cm/week on oatmeal agar (OA) at 21 °C; the colony is appressed and has sometimes sparse aerial mycelium; mycelium hyaline, coenocytic (older mycelium may be septate), branched, hyphae with diameter 6.6–9.8 μm with numerous small inclusions (Fig. 1A); coralloid hyphae (typical of *P. cambivora*) and chlamydospores absent. Sporangiophores mainly simple, unbranched. Zoosporangia terminal, non-caducous, ellipsoid, sometimes ovoid, non-papillate or with a minute papilla (Fig. 1B, 2A); internally proliferate, often nested within the original zoosporangium (Fig. 1B, 2B); measuring 49–62 × 28–32 μm. Oogonia sparse, their numbers diminishing during the time of cultivation and many of them aborting. Oogonia terminal, sphaerical, thick-walled, tuberculate, diameter 30–44 μm (Fig. 2C), antheridia amphigynous.

This fungus was described in 1995 for the first time (Brasier et al. 1995) and its taxonomic position was discussed. The following genetic study (Brasier et al. 1999) showed that "alder-*Phytophthora*" is not a single taxonomic entity but an array of phenotypically highly diverse heteroploid genotypes, thus the fungus does not have a scientific name at present. The first parent of this fungus is *P. cambivora*, the second one is still unknown, but it is close to *P. fragariae*. Both fungi are thought to be introduced in Europe (Brasier et al. 1999).

The pathogen differs from its parents by pathogenicity; *P. cambivora* and *P. fragariae* do not have the capacity to cause disease in alder (Brasier et al. 1999). The growth optimum of pathogen is lower than the growth optimum of *P. cambivora* (Brasier et al. 1995).

The isolated hybrid "alder-*Phytophthora*" is morphologically very close to its parent species, especially *P. cambivora*. The isolated fungus differs by its self-fertility (its parents are heterothallic), but the production of oogonia may be partially disrupted in many strains (Brasier et al. 1995). The other morphologic characters are similar to the characters of *P. cambivora*. The pathogenic strains are partially different by the felty appearance of their colonies and the sparse aerial mycelium, and by the presence of some smaller oogonia.

Symptoms and disease development

The first symptoms are the presence of small, chlorotic leaves, dying leaves and the appearing of leafless twigs in the crown of alders during summer. The foliage may be sparse and faded in one part or in the entire crown, especially when the disease is acute. Reddish-brown or brown spots and necrosis on bark appear on the basis of stem. Under these spots strips of necrotised conductive tissue projecting from the base of the tree develop. When most tissue around the trunk is killed by the pathogen, the foliage become sparse, the dying of branches is progressive; in a later stage of the disease the tree has a skeletal appearance. Sometimes a secondary overgrow develops. The root system and conductive tissue of these trees are highly reduced and the disease symptoms may look like tracheomycosis.

The symptoms of decline are similar in the whole area of alder decline in Europe, but the impact of the fungus on native alder populations is greatly varying in dependence of race of pathogen and conditions of the stand. The standard variant of "alder-*Phytophthora*" is more damaging to alder than some other variants, e.g. the "Swedish variant" in northern Europe (see Brasier et al. 1999, Werres 1998).

There are some other species of *Phytophthora* which are probably involved in the disease. These include *P. gonapodyides*, *P. citricola*, and *P. syringae* (Jung et al. 2000, Osswald et al. 2001). We found *P. syringae* associated with the alder decline at one other locality near Karlovy Vary.

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