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Norsolorinic acid mutants and aflatoxin research

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Vaccaro G. and Bennett J. W. (1999): Norsolorinic acid mutants and aflatoxin research – Czech Mycol. 51: 89–97

Norsolorinic acid is a red polyhydroxyanthraquinone. Mutants of *Aspergillus parasiticus* and *Aspergillus flavus* that accumulate norsolorinic acid, as well as making low levels of aflatoxin, have been used to study aflatoxin biosynthesis and aflatoxin genetics. In physiological studies the red color of norsolorinic acid serves as a visual screen for the putative presence of aflatoxin. In biosynthetic studies using ^{14}C-radioisotope labeling, norsolorinic acid is the first stable intermediate detected in the pathway. By complementation of suitable marked strains, and selection for red pigment, the gene for the enzyme associated with norsolorinic acid became the first gene cloned from the aflatoxin pathway. Gene disruption confirmed the role of norsolorinic acid as an aflatoxin precursor. Several laboratories have partially purified an enzyme that catalyzes the transformation of norsolorinic acid to other pigments. It is proposed that double mutants of *A. nidulans*, lacking both norsolorinic acid production and another secondary metabolite, can be used to screen for regulatory genes of the aflatoxin/sterigmatocystin pathway.

Key words: norsolorinic acid, aflatoxins, polyketides, *Aspergillus flavus*, *Aspergillus parasiticus*

Vaccaro G. a Bennett J. W. (1999): Tvorba kyseliny norsolorinové a aflatoxinu ve zmutovaných kmenech Aspergillus spp. – Czech Mycol. 51: 89–97

Kyselina norsolorinová je červený polyhydroxyantrachinon. Ke studiu biosyntézy aflatoxinu a ke genetickým studiím byly použity mutantní kmeny druhů *Aspergillus parasiticus* a *Aspergillus flavus*, kumulující kyselinu norsolorinovou a mající nízkou produkci aflatoxinu. Červené zbarvení norsolorinové kyseliny slouží ve fyziologických studiích k vizuálnímu vyhledávání domělé přítomnosti aflatoxinu. V biosyntetických studiích, za použití značeného radioisotopu ^{14}C je norsolorinová kyselina detegována jako první stabilní meziprodukt. Pomocí komplementace vybraných značených kmenů, spojené se selekcí na červený pigment bylo možné izolovat první gen metabolické dráhy norsolorinové kyseliny, který kóduje enzym. Mutace genu potvrdila roli norsolorinové kyseliny jako prekurzoru aflatoxinu. Několik laboratoří částečně purifikovalo enzym, který katalyzuje transformaci norsolorinové kyseliny na jiné pigmenty. Navrhuje se, že dvojité mutanty *Aspergillus nidulans*, které jsou defektní jak na produkci kyseliny norsolorinové, tak dalších sekundárních metabolitů, mohou být použity k vyhledávání regulačních genů dráhy aflatoxin/sterigmatocystin.

INTRODUCTION

Mycotoxins are natural products produced by filamentous fungi that evoke a toxic response in vertebrates when introduced in low concentration. Mycotoxins cause both human and veterinary diseases of which the best known is probably St. Anthony's Fire or gangrenous ergotism, a condition documented since medieval times which arises from the ingestion of *Claviceps*-contaminated rye (Bove 1970). In modern times, the mycotoxins which have received the most research attention are the aflatoxins, a family of toxic, mutagenic, and carcinogenic metabolites produced by certain strains of the common molds *Aspergillus flavus* and *A. parasiticus* (Goldblatt 1969, Cast 1989). Under the proper environmental conditions, these fungi can produce aflatoxins on numerous agricultural crops including peanuts, corn, rice, cottonseed and tree nuts. When domestic animals ingest aflatoxin-contaminated feeds, the resulting aflatoxicoses can lead to serious ecomonic losses, especially in poultry and trout (Betina 1989). There is also considerable indirect evidence that aflatoxins are associated with primary liver cancers in human populations (Eaton and Groopman 1994).

It is believed that an understanding of the fundamental mechanisms leading to aflatoxin production may lead to practical methods for protecting human and animal food supplies. To this end, a variety of laboratories have studied the biosynthetic steps in the aflatoxin pathway using both traditional chemical techniques and modern molecular genetic approaches. After several decades of basic research, we are now in the position to dissect the structural and regulatory genes of this complex pathway.

The first known intermediate in the aflatoxin biosynthetic pathway is norsolorinic acid. This red-pigmented polyhydroxyanthraquinone has played an important role in research about aflatoxin biosynthesis, aflatoxin genetics, and aflatoxin molecular biology. This communication reviews the role of norsolorinic acid in the past and outlines experiments for the future. A preliminary version of this paper was presented (Bennett et al. 1994).

Historical background

Norsolorinic acid (2-hexanoyl-1,3,6,8-tetrahydroxyanthraquinone) was named after the lichen *Solorina crocea* from which it was first characterized (Anderson et al. 1966). Norsolorinic acid was later isolated from an ultraviolet light-induced mutant of *A. parasiticus* (Lee et al. 1971). This mutant was easily identified in liquid and agar culture because of its orange-red color and showed an 80 % reduction in aflatoxin production. Continuous illumination, zinc deficiency, and the presence of para-aminobenzoic acid inhibited both norsolorinic acid and aflatoxin production in the mutant while growth in a rich medium containing yeast extract resulted in higher yields of both compounds (Bennett et al. 1971). This correlation was useful because the red pigment was an easy visual screen for the putative presence of aflatoxin. For example, mutants selected for lowered norsolorinic acid production also had lowered aflatoxin production (Bennett and Goldblatt 1973).

A different norsolorinic acid accumulating mutant of *A. parasiticus* was isolated by nitrosoguanidine treatment by Detroy et al. (1973). Later, Papa (1982) used nitrosoguanidine to isolate yet another norsolorinic acid mutant from *Aspergillus flavus*. Bennett and Papa (1988) have reviewed genetic notations used to designate these mutants.

Aflatoxin Biosynthetic Pathway

The polyketide origin of the carbon skeleton of aflatoxin B_1 was established by ^{14}C-labeling experiments by Biollaz et al. (1968ab). During the 1970s, the anthraquinones averufin (Donkersloot et al. 1972) and versicolorin A (Lee et al. 1975) were shown to be aflatoxin precursors. The role of sterigmatocystin as a late intermediate in aflatoxin biosynthesis was also established (Hsieh et al. 1973). Using ^{14}C-labeling, only 2.2 % of label from norsolorinic acid was recovered in aflatoxin B_l, as opposed to 49.4 % for averufin, 45.5 % for versicolorin A, and 65.5 % for sterigmatocystin (Hsieh et al. 1976).

Using these blocked mutants and ^{14}C and ^{13}C-isotope studies, a generally accepted "canonical" scheme for aflatoxin biosynthesis was established: acetate + malonate → anthraquinones → sterigmatocystins → aflatoxin B_1. Details of this pathway have been extensively reviewed; see for example, Turner and Aldridge (1983), Steyn et al. (1980), Bennett and Christensen (1983), Bhatnagar et al. (1992) and Bennett et al. (1997). Norsolorinic acid is the first stable precursor in this polyketide pathway. Theoretically, the product of a polyketide synthase should be an anthrone. The anthrone has never been isolated, presumably due to its rapid oxidation to the more stable norsolorinic acid (Vederas and Nakashima 1980). Townsend et al. (1984, 1988) have proposed and demonstrated that the side chain of norsolorinic acid arises intact from hexanoic acid. After norsolorinic acid, the other pathway intermediates include averantin, averufanin, averufin, versiconal hemiacetal acetate, versiconal, versicolorins A and B, demethyl sterigmatocystin and sterigmatocystin (see Figure 1).

Studying the enzymes that catalyze these polyketide transformations has been difficult (Dutton 1988). The enzyme which is involved in the conversion of norsolorinic acid to averantin is usually called norsolorinic acid dehydrogenase. This enzyme activity was purified by Chuturgoon and Dutton (1991), using reactive green 19-agarose and norsolorinic acid agarose affinity chromatography, and assigned a molecular weight of 140 kD by gel filtration chromatography on a Sephacryl S-300 column.

Further studies on the enzymatic conversion of norsolorinic acid to averufin were conducted in *Emericella heterothallica*, a non-aflatoxigenic species that produces

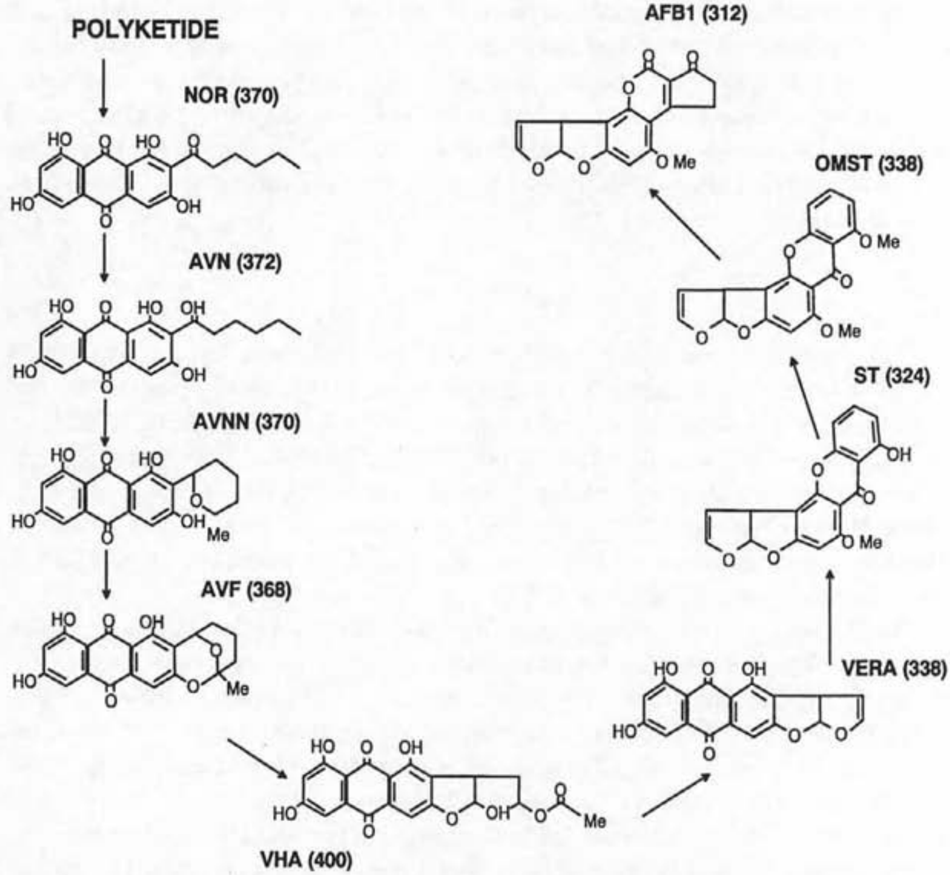

Fig. 1. Proposed scheme for aflatoxin biosynthesis based on mutation analysis, precursor feeding studies, and isotopic labeling. NOR=norsolorinic acid; AVN=averantin; AVNN=averufanin; AVF=averufin; VHA=versiconal hemiacetal acetate, VERA=versicolorin A, ST=sterigmatocystin; OMST=o-methylsterigmatocystin; AFB_1=aflatoxin B_1.

the anthraquinones averantin, averufanin, and averufin (Yabe et al. 1991). This norsolorinic acid dehydrogenase activity was media dependent, present when a complex culture medium containing 2 % yeast extract and 20 % sucrose was used, but absent when the sucrose was replaced with 20 % peptone. In the presence of NAD under yeast extract/peptone culture conditions slight dehydrogenase activity was restored (Yabe et al. 1991).

Bhatnagar and Cleveland (1990) also worked on this enzyme activity, purifying a protein which catalyzed the transformation of norsolorinic acid to averantin from three day old cultures of *A. parasiticus* using a protocol involving ammonium sulfate precipitation and several chromatography steps. The purified enzyme had an estimated molecular weight of 43 kDa.

Genetic analysis

The earliest genetic studies relied on the parasexual cycle in both *A. flavus* and *A. parasiticus*. Pigmented anthraquinones were useful markers in this classical and laborious work (Bennett and Papa 1988). The development of transformation systems for aflatoxigenic species (Woloshuk et al. 1989, Skory et al. 1990) allowed the application of molecular biology approaches and literally revolutionized the field. Using complementation, the norsolorinic acid gene became the first gene cloned from the aflatoxin pathway. Specifically, a genomic library was constructed by inserting wild-type *A. parasiticus* DNA into a cosmid vector containing the nitrate reductase gene, *niaD* and screening based on pigment. The subsequently isolated gene was called *nar-1* for "norsolorinic acid related," but was later renamed *nor-1* in keeping with the classical parasexual notation (Chang et al. 1992). Shortly thereafter, a similar strategy was used to isolate a gene (*ver-1*) that makes the enzyme that converts versicolorin A to sterigmatocystin (Skory et al. 1992). The coordinate appearance of *nor-1* and *ver-1* transcripts in batch cultures suggested that they were controlled by a common regulatory factor (Skory et al. 1993). The deduced amino acid sequence of *nor-1* had a high sequence similarity to other nucleotide binding dehydrogenases/reductases (Trail et al. 1994); and a related *nor-1* gene was isolated from *A. flavus* using gene disruption (Brown-Jenco et al. 1994).

In addition to complementation, genes for the aflatoxin pathway have been isolated after purification of their relevant proteins (*e.g.,* Yu et al. 1993) and by differential expression of transcripts during idiophase (*e.g.,* Feng et al. 1992). Overviews of early aflatoxin molecular biology have been given by Keller et al. (1992) and Trail et al. (1995).

Although parasexual studies had hinted that some of the aflatoxin pathway genes in *A. flavus* might be linked, the data were ambiguous (Bennett and Papa 1988). It was unexpected how much gene clustering was revealed by molecular analysis. Aflatoxin pathway genes in both *A. flavus* and *A. parasiticus* are tightly linked. The use of overlapping clones has made it relatively easy to identify many DNA sequences related to the pathway (Yu et al. 1995; Silva et al. 1996). The clustering of these polyketide pathway genes has been exploited most fully in the cognate sterigmatocystin pathway in *Aspergillus nidulans*. A "genetic workhorse" for over 50 years (Martinelli and Kinghorn 1994), *A. nidulans* is more suited to experimental analysis than either *A. flavus* or *A. parasiticus*. Brown et al. (1996) characterized a 25-gene cluster of approximately 60 kb DNA that contains most of the enzymatic activities required for sterigmatocystin biosynthesis. The

A. nidulans putative reductase gene *stcE* shares 56 % deduced amino acid identity with *nor-1*. The enzymology and molecular biology of aflatoxin biosynthesis, including the steps surrounding norsolorinic acid biosynthesis, have been reviewed by Minto and Townsend (1997).

Finally, another gene from *A. parasiticus* located in the aflatoxin cluster has been confusingly named *norA* because monoclonal antibodies used to isolate the gene inhibited the conversion of norsolorinic acid to averantin (Cary et al 1996) and the purified 43 kDa protein used to generate the monoclonal antibody demonstrated norsolorinic acid reductase activity (Lee et al 1995). *NorA* showed high sequence similarity with *stc V,* an aryl alcohol dehydrogenase from the *A. nidulans* sterigmatocystin gene cluster but only 22 % amino acid sequence similarity to the deduced dehydrogenase translated from *nor-1*. On the other hand, recombinational inactivation has provided direct evidence that *nor*-1 is directly involved in aflatoxin biosynthesis in *A. parasiticus* (Trail et al. 1995).

These data concerning *nor-1* and *norA* underline the importance of multiple approaches to molecular cloning. Complementation (*nor-1*) and reverse genetics (*norA*) identified separate DNA sequences implicated in norsolorinic acid metabolism. Further gene disruption experiments are needed to determine the exact role of *norA*. It is appears that at least some secondary metabolic pathways may have redundant mechanisms. Research underway in our laboratory, in collaboration with Nancy Keller (Texas A&M, College Station, Texas) and N. Ronald Morris (Robert Wood Johnson Medical Center, New Brunswick, N. J.) will use double mutants of *A. nidulans* lacking the red pigment, and another unrelated secondary metabolite, as a screen for presumptive regulatory mutants. The ability to screen colonies with an easily detected color marker remains a cornerstone of genetic analysis, even in this molecular era.

Summary

The first stable intermediate in aflatoxin biosynthesis is the decaketide norsolorinic acid. Mutants of *A. flavus* and *A. parasiticus* that accumulate norsolorinic acid also produce low levels of aflatoxin. Several genes implicated in norsolorinic acid production have been cloned and sequenced from *A. flavus, A. parasiticus* and *A. nidulans*.

An understanding of the regulation and expression of these genes in aflatoxin-producing strains at the molecular level will enlarge our understanding of fungal polyketide synthesis in the laboratory and perhaps improve our ability to control aflatoxin production in the field. The red pigment of norsolorinic acid has proven equally useful in biosynthetic, parasexual and molecular approaches to the aflatoxin problem.

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Hydnaceous fungi of the Czech Republic and Slovakia

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Hrouda P. (1999): Hydnaceous fungi of the Czech Republic and Slovakia – Czech Mycol. 51: 99–155

The paper presents a survey of the results of a study of four hydnaceous genera – *Bankera, Phellodon, Hydnellum* and *Sarcodon* – in the Czech Republic and Slovakia. It is based on material deposited in Czech and Slovak herbaria as well as on literature records of finds of the included species from the studied territory. For each species a short description is provided, highlighting characters distinguishing it from related species. Short notes about its ecology, occurrence and distribution are added. In the latter the actual state is compared with historic and literature data. The study is supplemented with distribution maps of individual species.

Key words: Hydnaceous fungi, occurrence, accompanying trees, distribution, Czech Republic, Slovakia.

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Práce představuje souhrnný přehled výsledků studia čtyř rodů lošákovitých hub – *Bankera, Phellodon, Hydnellum* a *Sarcodon* – na území ČR a SR. Je založena na studiu materiálu uloženého v českých a slovenských herbářích a na literárních záznamech nálezů daných druhů ze studovaného území. U každého druhu je vyhotoven stručný popis, zdůrazněny rozlišovací znaky od podobných druhů a stručně komentována ekologie, výskyt a rozšíření. Současný stav je porovnán s historickými a literárními údaji. Studie je doplněna mapami rozšíření jednotlivých druhů.

INTRODUCTION

Systematic classification of studied genera

The studied genera belong to Holobasidiomycetes, order Thelephorales (according to Pegler et al. 1997), family *Bankeraceae* Donk (genera *Bankera* Coker et Beers ex Pouz., *Phellodon* P. Karst.) and *Thelephoraceae* Chev. (genera *Hydnellum* P. Karst. and *Sarcodon* P. Karst.). The paper includes neither the less relative genera *Hydnum* L.: Fr. and *Auriscalpium* S. F. Gray nor other genera with a hydnoid (spiny) hymenophore (resupinate and gelatinous types).

Ecology and phenology

Species of the genera *Bankera, Phellodon, Sarcodon, Hydnellum* (also *Hydnum*) are living in ectomycorrhiza with trees. This hypothesis is according to Arnolds (1989) based on circumstantial evidences from field observations.

The occurrence of basidiomes is limited to the direct neighbourhood of living trees (also in areas with dispersed growth). Most other known saprophyte basidiomycetes are not limited to the neighbourhood of trees.

Most of the species have a certain degree of host specificity.

Hydnaceous fungi are never found close to trees which usually do not form ectomycorrhiza in Europe (for example *Acer, Aesculus, Ulmus, Fraxinus*), although these trees can grow on otherwise suitable habitats.

Many species prefer soils with only a very thin layer of litter and humus. No intensified decomposition of litter (decomposition of lignine) was observed near the basidiomes. In the habitats in the Czech Republic visited in 1991, the thickness of the surface humus layer ranged from 1 to 7 cm, on average between 2 and 5 cm.

The studied species grow mostly on acid, sandy soils (Pegler et al. 1997) – see also the following table describing chemical properties of soils in habitats visited in 1991:

	humus layer (3–5 cm from surface)			sandy or loamy soil (deeper than 3–5 cm)		
	min.	average	max.	min.	average	max.
pH_{H_2O}	3.1	3.65	3.95	3.75	4	4.2
pH_{KCl}	2.2	2.57	2.9	2.75	3.2	3.75
Ca (mg/100 g)	24.74	63.05	152.97	11.71	13.37	20.4
K (mg/100 g)	9.41	35.11	82.29	3.8	6.79	11.29
Na (mg/100 g)	3.68	9.59	13.95	3.68	5.91	8.41
Mg (mg/100 g)	1.45	7.2	15.5	0.55	1.18	3.58
N (%)	0.39	0.92	1.3	0.09	0.13	0.85
C (%)	25.28	57.71	92.88	0.77	9.29	27.61

The degree of host specificity is different in various species. A small number of the studied species occurs with only one tree species (the species of the genus *Bankera*), some are associated with deciduous trees (*Phellodon confluens, Hydnellum spongiosipes, Sarcodon joeides*), most of them are commonly associated with coniferous trees and there is also quite a number of indifferent species.

The ectomycorrhizal relationship was proved in *Bankera fuligineo-alba*, which was synthetised in pure culture with *Pinus banksiana* (Danielson 1984). The same author performed unsuccessful experiments with growing *Hydnellum peckii, Phellodon melaleucus* and *Sarcodon imbricatus* in pure culture. Other species have not been tested so far.

Hydnaceous fungi are autumn fungi occurring most frequently from August to October. Under extremely favourable conditions (warm spring, sufficient rainfall) these species can be found earlier (in June, the earliest occurrence recorded 12.

VI.), but the number of such records is minimal; also small is the number of later (winter) records (in this case represented by mostly dry, "dead" basidiomes).

MATERIAL AND METHODS

The core of the work represents a revison of material deposited in Czech and Slovak herbaria (BRA, BRNM, BRNU, CB, MJ, OLP, OP, PL, PRC, PRM – abbreviations according to Hradílek et al. 1992). Special thanks belong to J. Herink, who kindly offered not only material from his private herbarium, but also records of more finds not deposited in his herbarium. All unidentified and uncertainly identified specimens and all specimens identified with a forgotten name just as specimens of critical species (not safely to be distinguished by macroscopical characters) were studied microscopically and consulted with Z. Pouzar. The basic characters by which such critical species were distinguished, were spore morphology, presence or absence of clamp-connections on hyphae and colour reaction of the flesh in KOH.

The studied species are treated as follows. The introduction of the special part is formed by the key to the families and genera and by an integral key to all stipitate Hydnums with emphasis on characters for practical identification in field conditions. This key contains stipitate genera including the genera *Auriscalpium* and *Hydnum* (otherwise not included in this study), but excludes resupinate genera or genera with fruticulose basidiomes (*Hericium* type).

In species descriptions practically useful and diagnostic characters were emphasised. Expulsion of liquid mentioned for living basidiomes means guttation, in case of exsiccates it means an expulsion of crystals of unknown composition (Maas Geesteranus 1975). Data from descriptions in literature (Maas Geesteranus 1956, 1957, 1958, 1960, 1975, Nikolaeva 1961, Pegler et al. 1997) were taken over for species which were not even seen as exsiccates; partially the same was done in cases where the number of studied specimens was not regarded sufficiently representative. Spore sizes are given according to Maas Geesteranus (1975).

Changes in the occurrence of each species is commented for the 20th century. A common trend in most species is a strong increase in the number of collections after the Second World War (either caused by a higher number and increased activity of mycologists or by worse documentation of pre-war finds), followed by a smaller or larger decrease in occurrence approximately after the year 1970. The sketched trend can be generalised only for the Czech Republic, where finds have been documented more or less continuously. The situation in Slovakia is different: the finds of Kalchbrenner, Kmeť and Truchlý from the 19th century are followed by a half-century interval of low activity (only 2 records of all studied species are from the years 1901–1950). Later an invasion of mycologists into Slovak forests is noticeable after 1970, manifested by an increased number of collections. This increase can even prevail over the decrease in the number of finds in the Czech Republic and create an erroneous impression of increasing occurrence of certain species at the level of both countries together.

Comments on accompanying trees compare a literature source (Maas Geesteranus 1975) with data from Czechoslovak records. I have also tried to record changes in the representation of accompanying trees for individual species through time. Most of the species do not show any conspicuous change; if some species show a certain shift in the spectrum of accompanying trees, the usual trend is that: the spectrum narrows, i. e. the fungi are more found with one tree.

The distribution maps given for each species are not wholly authoritative, because they illustrate activity of collectors rather than occurrence of individual species; this study is not an exception. The most investigated regions in the Czech Republic are central Bohemia, southern Bohemia, Bohemian-Moravian Highland and the surroundings of Brno, in Slovakia the northern part from Kysuca to Spiš. Greater value can be attributed to a division of records into several time periods showing the disappearing of species from regions where they formerly occurred, respectively new finds in other regions. The records from the periods before 1945, 1945–1970 and after 1970 are differentiated in the distribution maps. The reason for just this differentiation (the years 1945 and 1970 as turning-points) is given above. For all species point mapping was used by reason of the relatively small number of localities. In case of broadly defined localities the centre of each symbol was situated in the centre of the given site (mostly a larger town).

The distribution maps were produced with DMAP for Windows 6.1 (© Alan Morton 1993–97). The following symbols were used:

Accurately defined localities:

documented data:

○ – locality where the species was found before 1945

◍ – locality where the species was found between 1945 and 1970

● – locality where the species was found after 1970

data from literature:

□ – locality where the species was recorded before 1945

▩ – locality where the species was recorded between 1945 and 1970

■ – locality where the species was recorded after 1970

Broadly defined or inaccurately indicated localities:

documented data:

× – sites, where the species was found before 1945

✳ – sites, where the species was found between 1945 and 1970

(No such data after 1970.)

data from literature:

+ – sites, where the species was recorded before 1945

(No such data after 1945.)

If more records were recorded from one locality in different periods, the last collection is always decisive.

In the lists of recorded localities (for rare species only) the localities are arranged according to the phytogeographic divisions of the Czech Republic and Slovakia (Skalický 1988, Futák 1984).

SPECIAL PART

Key to the families and genera of Czecho-Slovak stipitate Hydnums

1) Amyloid spores; basidiome tiny cochleariform with mostly excentric stipe
... *Auriscalpium*, family Auriscalpiaceae

1') Spores non-amyloid; basidiomes of various sizes, minute to relatively large, with ± central stipe

- 2) Spores perfectly smooth, white to ochraceous in mass; basidiome fleshy
... *Hydnum*, family Hydnaceae
- 2') Spores verrucose or spiny; basidiome fleshy or tough
  - 3) Spores short ellipsoid to oval, spinulose with hyaline wall, white in mass; basidiomes with smell of fenugreek especially after drying
  ... family Bankeraceae
    - 4) Basidiome fleshy, never overgrowing branchlets or grass
    ... *Bankera*
    - 4') Basidiome tough, often overgrowing branchlets, grass or other objects
    ... *Phellodon*
  - 3') Spores regular, moderately to roughly verrucose or spiny with coloured wall, brown or brown-ferrugineous in mass; basidiomes without a smell of fenugreek
  ... genera of family Thelephoraceae
    - 5) Basidiome fleshy, never overgrowing branchlets or grass
    ... *Sarcodon*
    - 5') Basidiome tough, often overgrowing branchlets, grass or other objects
    ... *Hydnellum*

In the following table the main characters of the four studied genera are shown.

Genus	Basidiome	Spores	Colour in mass	Smell
Bankera	fleshy, terminated growth	oval, spinulose, wall hyaline	white	fenugreek
Phellodon	tough, indefinite growth			
Hydnellum		irregular, roughly verrucose, wall brownish	brown	different in various species – aromatic, farinaceous, graveolent or without smell
Sarcodon	fleshy, terminated growth			

The key to the genera and the keys to the species of each genus are either based on obvious characters or on characters invisible at first sight, like characters on spores, reaction with KOH etc., which should provide a reliable and precise determination. But as one does not always have a microscope or chemical reagents at one's disposal, one more key based on visual macro-characters only (quite often on colours) is given; this is a complete key for the whole studied group. Herewith, cases can be excluded in which a wrong genus determination automatically leads to an incorrect species determination. This key is only for orientation and sometimes leads to a group of species or to a probable species.

1) Basidiome tiny cochleariform with predominantly lateral stipe
... *Auriscalpium vulgare*

1') Basidiomes of different sizes and shapes with central stipe (distinct or covered by spines running down to its base)

- 2) Basidiome fleshy, never overgrowing branchlets or grass
  - 3) Pileus light orange to orange, with concolorous stipe, spines whitish
    ... species of genus *Hydnum*
  - 3') Pileus with various brown tints
    - 4) Context pink or violet
      ... see *Sarcodon: S. fuligineo-violaceus, S. joeides*
    - 4') Context whitish to brown
      - 5) Base of stipe grey-green (or black-green)
        ... see *Sarcodon: S. fennicus, S. glaucopus, S. scabrosus*
      - 5') Base of stipe differently coloured
        - 6) Surface of pileus breaking up into conspicuous, especially in the centre erect scales at maturity
          - 7) Pileus brown to dark brown; basidiomes lacking smell or aromatic, but never with a smell of fenugreek
            ... *Sarcodon imbricatus*

7') Pileus light brown; basidiomes, especially when dried, with a smell of fenugreek

... *Bankera violascens*

6') Surface of pileus always lacking conspicuous scales or cracked at the centre into areoles, at most breaking up into appressed squamules with raised tips which may continue towards the margin

8) Context whitish (or chrome yellow where the pileus passes into the stipe – not conspicuous in exsiccates); pileus light to orange-brown with appressed squamules

... *Sarcodon versipellis*

8') Context whitish to brown, without yellow colour; pileus light-brown

9) Basidiome, especially when dried, with a smell of fenugreek

10) Surface of pileus covered with putrefying plant remains

... *Bankera fuligineo-alba*

10') Surface of pileus without any putrefying remains

... *Bankera violascens*

9') Basidiome lacking the smell of fenugreek, smell unpleasant

... *Sarcodon leucopus*

2') Tough basidiome, often overgrowing branchlets, grass or other objects

11) Surface of pileus with more or less conspicuous concentric zones

12) Context bright to dark orange

... *Hydnellum auratile*

12') Context whitish to brown

13) Spines light, more or less beige

... *Phellodon tomentosus*

13') Spines brown to dark brown

... *Hydnellum concrescens*

11') Surface of pileus without concentric zones

14) Cross-section of the basidiome showing blue colours (often in zones)

... see *Hydnellum: H. caeruleum, H. suaveolens*

14') Cross-section of the basidiome not showing any blue colours

15) Young basidiomes sulphur yellowish, older ones olive-green

turning black on pressing, mostly more basidiomes grown together; context concolorous

... *Hydnellum geogenium*

15') Basidiomes coloured otherwise

16) Spines grey, grey-brown, grey-beige or dirty white; context black, grey or grey-brown; basidiome, especially when dried, with a smell of fenugreek

... see *Phellodon: P. confluens, P. melaleucus, P. niger*

16') Spines whitish, pink, purple-brown, brown to dark brown; context light-brown, brown to ferrugineous or (at *H. aurantiacum*) whitish and orange in the base of the stipe; basidiome lacking smell of fenugreek

... see *Hydnellum: H. aurantiacum, H. concrescens, H. cumulatum, H. ferrugineum, H. peckii, H. scrobiculatum, H. spongiosipes*

Bankeraceae Donk

Bankera Coker et Beers ex Pouzar

Basidiomes stipitate, pileate. Surface of the pileus at first tomentose, later smooth or broken up into scales, mostly light-brown; stipe concolorous. Spines light-brown to greyish. Context fleshy to tough, non-zoned, white or light coloured, monomitic. Hyphae inflating towards the centre of the pileus, thin-walled, gradually turning to slightly thick-walled and very close together, without clamp-connections. Basidia clavate, 4-spored, without basal clamp-connections. Spores semiglobose to oval, slightly verrucose, colourless. Cystidia absent.

Key to the species:

1) Surface of mature pileus rarely broken into scales, but covered by a thin layer of tomentum, often with remains of overgrown putrefying plant matter; growing under *Pinus*

... *Bankera fuligineo-alba*

1') Surface of mature pileus often broken into scales, not covered with a distinct tomentum and lacking remains of overgrown putrefying plant matter; growing under *Picea*

... *Bankera violascens*

Bankera fuligineo-alba (J. C. Schmidt: Fr.) Pouzar

Pileus about 80 mm in width, velutinous, rarely with appressed squamules, pale, whitish, yellow-brown, flesh-brown, brown, the surface tomentum overgrowing remains of putrefying plant matter. Stipe brown, only pale under the spines. Spines pale brown, greyish, with pink hue in young stages. Context whitish, with age and towards the stipe turning brown, not changing colour in KOH. Expulsion of liquid not observed. Clamp-connections absent. Spores oval with small acute warts, 4.7–5.4 × 2.7–3.6 μm.

Related species. *B. violascens* is often scaly when old and – most of all – never has any putrefying matter on the surface of its pileus, its context turns olive-green in KOH, it grows under *Picea*. The species of the genus *Sarcodon* have no smell of fenugreek and possess roughly verrucose coloured spores of a rather irregular shape.

Occurrence. Less abundant species with a strongly declined occurrence during the last 20 years.

Accompanying trees. Literature sources associate *Bankera fuligineo-alba* with *Pinus*; also in our country *Pinus* occurs in all localities where accompanying trees were recorded, at least as an admixture.

Distribution (Map 1). Formerly less abundant over the whole territory of Bohemia and Moravia, recently rare and confined to isolated localities. The last record from northern Bohemia dates from 1966, in Moravia it was last seen in 1969. The low number of records from Slovakia does not allow to draw conclusions on thedistribution of this species.

List of recorded localities – Czech Republic:

Sadská, sandy *Pinus* wood, IX. 1932, leg. O. Zvěřinová, det. A. Pilát ut *Sarcodon fuligineo-albus* (PRM)

Strážnice, Přívoz, sandy *Pinus* wood, 9. X. 1955, leg. et det. F. Šmarda (BRNM)

Ruda near Nové Strašecí, "Leontýnino polesí" SW of Ruda, between the site "V chaloupkách" and První luh valley, *Picea* wood with *Pinus*, 17. X. 1937, leg. J. Herink sen., det. J. Herink ut *Calodon laevigatus*, rev. Z. Pouzar (PRM)

Plzeň-Bolevec, *Pinus* wood, 10. X. 1906, leg. et det. F. Maloch ut *Hydnum fuligineo-album* (PL)

Blanský les Mts., SE slope of Mt. Kleť, *Pinus* wood, 26. VIII. 1934, leg. Josef et Jan Herink, det. A. Pilát ut *Calodon laevigatus*, rev. Z. Pouzar (PRM)

Cep near Suchdol nad Lužnicí, margin of lake Cepský nový, sandy *Pinus* wood with *Vaccinium*, 30. IX. 1978, leg. J. Kubička, det. Z. Pouzar (PRM)

Chlum u Třeboně, near lake Vydymač, *Pinus* wood with *Vaccinium*, 30. VIII. 1935, leg. et det. K. Kavina ut *Sarcodon fuligineo-albus*, rev. Z. Pouzar (PRM)

Soběslav, *Pinus* wood, 20. IX. 1946, leg. R. Veselý, det. Z. Pouzar (PRM)

Soběslav, Karvánky, *Pinus* wood, VII. 1932, leg. et det. A. Pilát ut *Sarcodon fuligineo-albus* (PRM)

Vlastiboř, Jezárka forest, *Pinus* wood with *Vaccinium* and *Calluna*, 2. X. 1954, leg. F. Kotlaba, det. Z. Pouzar (PRM)

Vlastiboř, Padělky forest, *Pinus* wood with *Vaccinium* and *Calluna*, 24. X. 1954, leg. F. Kotlaba, det. Z. Pouzar (PRM)

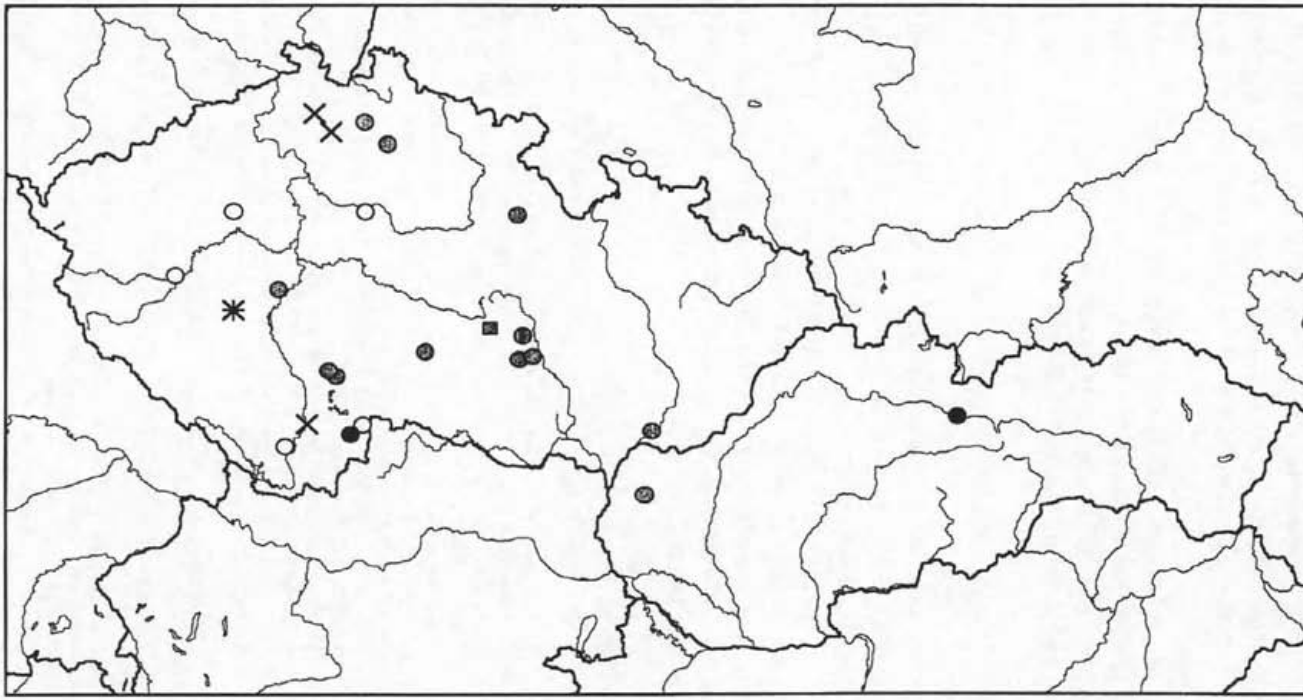
Žebrák, *Pinus* wood, 26. IX. 1954, leg. B. Hřebíková, det. Z. Pouzar (PRM)

Vrtky, west of the village, planted *Pinus* wood, 4. IX. 1965, leg. et det. J. Herink, 2. X. 1966, leg. J. Herink, det. Z. Pouzar (both Herb. Herink)

Podkost, Žehrovský forest, near the road Dobšice - Kamenice, *Pinus nigra* wood with *Vaccinium* and *Calluna*, 330 m, 24. IX. 1952, leg. et det. J. Herink (Herb. Herink)

Kostelec nad Orlicí, coniferous wood, IX. 1950, leg. M. Svrček, det. Z. Pouzar ut *Sarcodon fuligineo-albus* (PRM)

Map No. 1 - Bankera fuligineo-alba



Documented data:

○ - locality where the species was found before 1945

◍ - locality where the species was found between 1945 and 1970

● - locality where the species was found after 1970

Data from literature:

■ - locality where the species was recorded between 1945 and 1970

Broadly defined localities:

✕ - locality where the species was found before 1945

✳ - locality where the species was found between 1945 and 1970

Cejle, Mt. Čeřínek, mixed wood (*Picea abies, Pinus silvestris, Fagus silvatica*), 700 m, 11. IX. 1966, Jihlava mushroom exhibition (BRNM)
Ostrov nad Oslavou, 13. IX. 1960, leg. et det. F. Šmarda (Kříž, Svrček et Šmarda 1961)
Vlkov, forest between Vlkov and Katov, *Pinus* wood, 1. IX. 1957, 16. IX. 1962, leg. et det. F. Valkoun (BRNM)
Věžná, Teplá forest, *Pinus* wood, 500 m, 27. IX. 1946, leg. et det. F. Šmarda (BRNM)
Věžná, forest between Věžná and Střítež, 450 m, 27. IX. 1946, leg. F. Šmarda, det. Z. Pouzar (BRNM)
Dolní Loučky, Falcův mlýn, *Picea* wood with admixed *Pinus*, 17. VII. 1969, leg. B. Kasala, det. K. Kříž (BRNM)
Vidnava, Kohoutí hill, X. 1911, leg. J. Hruby, det. Z. Pouzar (BRNM)

Broadly defined localities:
Near České Budějovice, planted *Pinus* wood, leg.?, det. J. Herink (Herb. Herink)
Doksy, *Pinus* wood, 1936, leg. G. Japp, det. A. Pilát ut *Sarcodon fuligineo-albus* (PRM)
Česká Lípa, 1935, leg. G. Japp, det. Z. Pouzar ut *Sarcodon fuligineo-albus* (PRM)
Rožmitál pod Třemšínem, 10. VIII. 1952, leg. et det. A. Pilát ut *Sarcodon fuligineo-albus* (PRM)

Slovakia:
Bílkove Humence, 15. IX. 1970, leg. Z. Novák, det. I. Fábry (BRA)
Kráľova Lehota, between K. L. and Svarín, calcareous soil, *Pinus* wood, 500 m, 13. VIII. 1982, leg. et det. J. Kuthan (BRA)

Bankera violascens (Alb. et Schw.: Fr.) Pouzar

Pileus about 80 mm in width, surface smooth or broken into concentrically arranged scales, whitish, yellow-, grey- or flesh-brown, never covered with putrefying plant rests. Stipe brown, without a whitish zone under the spines. Spines pale brown, greyish, pinkish, white or bluish in young stages. Context whitish, brown to grey-brown, turning olive-green in KOH when cut. Expulsion of liquid not observed. Clamp-connections absent. Spores oval with small acute warts, 4.5–5.4 × 4.3–4.5 μm.

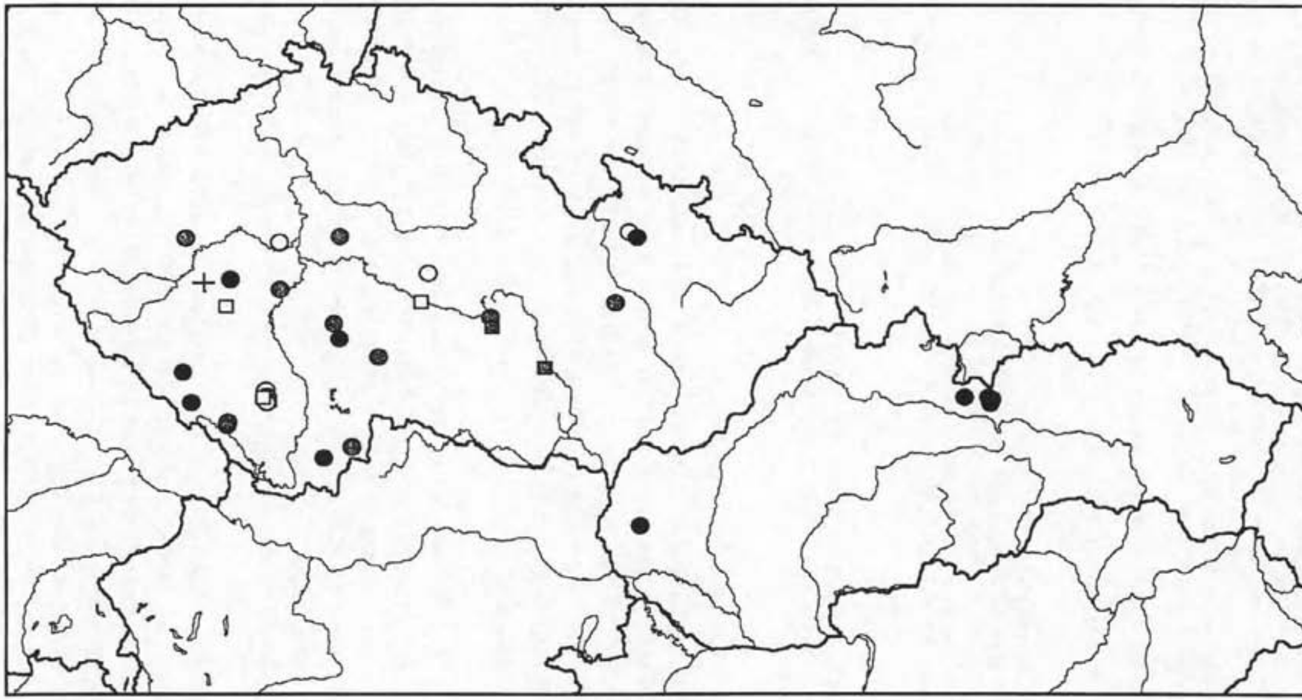
Related species. *B. fuligineo-alba* never has a pileus broken into erect scales and – most of all – the surface of its pileus is covered with putrefying plant matter, its context does not change colour in KOH, it grows under *Pinus*. The species of the genus *Sarcodon* have no smell of fenugreek and possess roughly verrucose coloured spores of rather irregular shape.

Occurrence. Species of moderate abundance, not showing a very conspicuous decline.

Accompanying trees. According to the literature *Bankera violascens* is associated with *Picea*. This is confirmed by our records with only one exception (Křepice near Vodňany, forest SE of road to Libějovické Svobodné Hory, 500 m a. s. l., planted *Pinus* wood, 29. VIII. 1934, leg. et det. J. Herink ut *Sarcodon infundibulum* – but in this locality also *Picea* occurs).

Distribution (Map 2). Formerly not very abundant species in the Czech Republic, recently rare in isolated localities. From Slovakia only a few collections from recent years (Tatra Mts.) exist, so it is impossible to draw any conclusions.

Map No. 2 - Bankera violascens



Documented data:
- ○ - locality where the species was found before 1945
- ◉ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Data from literature:
- □ - locality where the species was recorded before 1945
- ■ - locality where the species was recorded between 1945 and 1970

Broadly defined localities:
- + - locality where the species was recorded before 1945

Phellodon P. Karst.

Basidiome pileate, stipitate; stipe sometimes shortened because of the spines running down to the base. Predominating colours of the basidiome grey or brown. Surface of the pileus tomentose at first, then fibrillose, ridged, slightly scrobiculate, variously coloured. Stipe concolorous with pileus or darker. Spines variously coloured in different species. Context fibrillose, soft or tough to woody, zoned, monomitic, pale or coloured. Hyphae cylindrical (not inflating), always thin-walled, without clamp-connections in the European species. Hyphae in spines similar, somewhat narrower, also without clamp-connections. Basidia clavate, 4-spored, without basal clamp-connections. Spores semiglobose to oval, slightly verrucose, hyaline. Cystidia absent.

Key to the species:

1) Basidiome with conspicuously black context also in the pileus, with violet hue when young, grey-black to grey at maturity
... *P. niger*

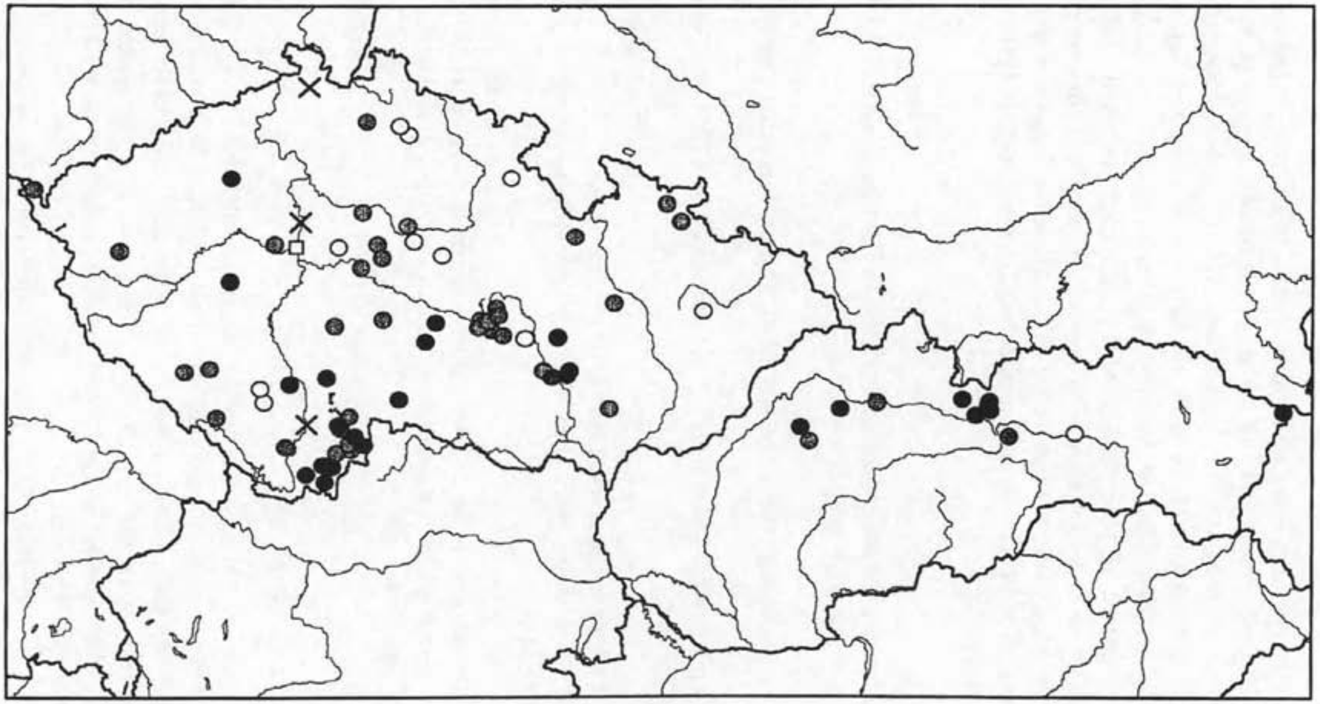
1') Context of the pileus not conspicuously black (but ochraceous, greyish or white)
   2) Surface of pileus with darker brown concentric zones on a light-ochraceous to brown ground
   ... *P. tomentosus*
   2') Surface of pileus without conspicuous concentric zones
      3) Small basidiomes with dark pileus, light spines and dark, thin, smooth stipe
      ... *P. melaleucus*
      3') Basidiomes with beige to brown pileus, concolorous or darker spines, often decurrent to the tomentose base of the stipe; associated with deciduous trees
      ... *P. confluens*

Phellodon niger (Fr.: Fr.) P. Karst.

Pileus about 40 mm in width, young velutinous, older smooth to rough, light-grey or violet in young stages, turning dark to black with age. Stipe tomentose (especially when young), dark. Spines white to blue-grey, grey when old. Context black (dark slate grey after drying), turning blue-green in KOH when cut. Expulsion of liquid not observed. Clamp-connections absent. Spores oval with small acute warts, 3.6–4.5 × 2.7–3.5 μm.

Related species. The context of *P. confluens* does not turn blue-green in KOH; this species grows in deciduous woods only. *P. melaleucus* is smaller, has conspicuously light spines contrasting to the dark stipe and does not have a

Map No. 3 - Phellodon niger



Documented data:

○ - locality where the species was found before 1945

◍ - locality where the species was found between 1945 and 1970

● - locality where the species was found after 1970

Data from literature:

□ - locality where the species was recorded before 1945

Broadly defined localities:

✕ - locality where the species was found before 1945 velutinous pileus. Principal difference: none of the related species have such a black context.

Occurrence. Although showing decline, this species is still relatively abundant.

Accompanying trees. Literature sources mention its occurrence in deciduous, coniferous and mixed woods, under *Fagus, Quercus, Picea* and *Pinus*; a small number of our collections come from deciduous woods, a large majority comes from coniferous and mixed woods. *Picea* occurs in 66 %, *Pinus* in 43 % of localities, but this rate has changed during the last 20 years (*Pinus* present in 62 %, *Picea* in 58 % of localities).

Distribution (Map 3). Relatively most finds are concentrated in southern Bohemia, in the area between Žďár n. Sáz. and Brno in Moravia and in the Tatra-Fatra region in Slovakia. But relatively recent collections from northern and western Bohemia testify that the main reason of the large number of collections in the first mentioned areas is their high rate of investigation.

Phellodon melaleucus (Sw. in Fr.: Fr.) P. Karst.

Pileus about 25 mm in width, rough to wrinkled particularly in old specimens, whitish, ash-grey, grey to blackish or with brown hue. Stipe smooth, thin (1–5 mm), dark brown, grey, black. Spines whitish to pale grey or brown. Context grey to brown, turning green in KOH when cut. Expulsion of liquid not observed. Clamp-connections absent. Spores oval with small acute warts, 3.6–4.5 × 3–4 μm.

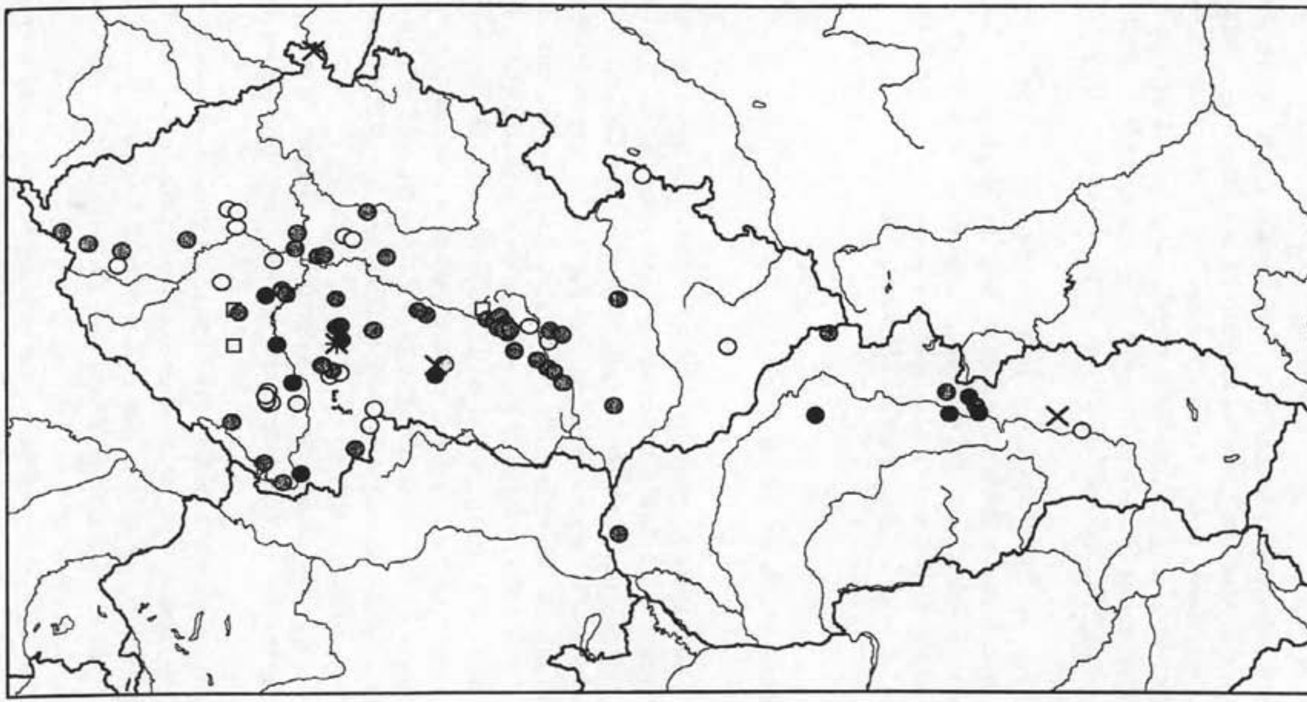
Related species. *P. confluens* does not have a stipe with colours different from the spines, its context does not turn green in KOH and grows under deciduous trees only. *P. niger* has a clearly black context. Both these species lack such a subtle stipe as *P. melaleucus* has. *P. tomentosus* is brown coloured with conspicuous concentric zones (*P. melaleucus* has such zones too, but less conspicuous) and its context does not turn green in KOH.

Occurrence. This species showed a large decline during the last 20 years, but was found in two places in the Czech Republic in 1991. (Has this fungus been overlooked?)

Accompanying trees. In the literature its occurrence in both deciduous and coniferous woods is mentioned; a small number of our collections came from deciduous woods, a large majority from coniferous and mixed woods. *Picea* occurs in 74 %, during the last 30 years in 86 % of localities. The past 10 years this species was not found under any other tree.

Distribution (Map 4). Formerly in the whole of Bohemia and Moravia except for the northern parts; during the last 20 years found almost only in southern Bohemia. In Slovakia most collections are from the Tatra Mts.

Map No. 4 - Phellodon melaleucus



Documented data:
- ○ - locality where the species was found before 1945
- ◍ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Data from literature:
- □ - locality where the species was recorded before 1945

Broadly defined localities:
- ✕ - locality where the species was found before 1945
- ✳ - locality where the species was found between 1945 and 1970

Phellodon tomentosus (L.: Fr.) Banker

Pileus about 35 mm in width, velutinous, concentrically wrinkled, brown, ochraceous, yellow-brown or grey-brown, mostly with conspicuous darker concentric zones. Stipe smooth, more or less concolorous with the pileus. Spines white, then pale ochre-greyish, sometimes pinkish in young stages. Context pale, ochraceous, brown in the stipe, not changing colour in KOH. Expulsion of liquid not observed. Spores oval with small acute warts, 3.1–3.6 × 2.7–3 μm.

Related species. *P. melaleucus* does not have a velutinous pileus with such conspicuous concentric zones and its context turns green in KOH. *Hydnellum concrescens* is sienna to umbra brown with dark brown spines and has a context concolorous with the surface of the basidiome. All species of the genus *Hydnellum* have more roughly verrucose spores lack the smell of fenugreek after drying.

Occurrence. Abundant species with a relatively constant occurrence.

Accompanying trees. In the literature its occurrence in coniferous and mixed woods is mentioned; data from the Czech Republic confirm this with a few exceptions. *Pinus* occurs in 61 % of localities, *Picea* on 48 %. The rate of collections under *Picea* grew during the last 20 years (*Picea* being present in 64 % and *Pinus* in 46 % of localities).

Distribution (Map 5). The species occurs in a great part of the Czech Republic and Slovakia, the highest density of collections is found in the zone western Bohemia – southern Bohemia – Bohemian-Moravian Highland. Remarkable is the absence of collections from such a well-investigated area as the surroundings of Brno is. It is possible that this species was so abundant there that it was not collected and documented (like for example *Sarcodon imbricatus*, which has a small number of records from the surroundings of Brno, too). Just as likely is that the species really does not grow in this area.

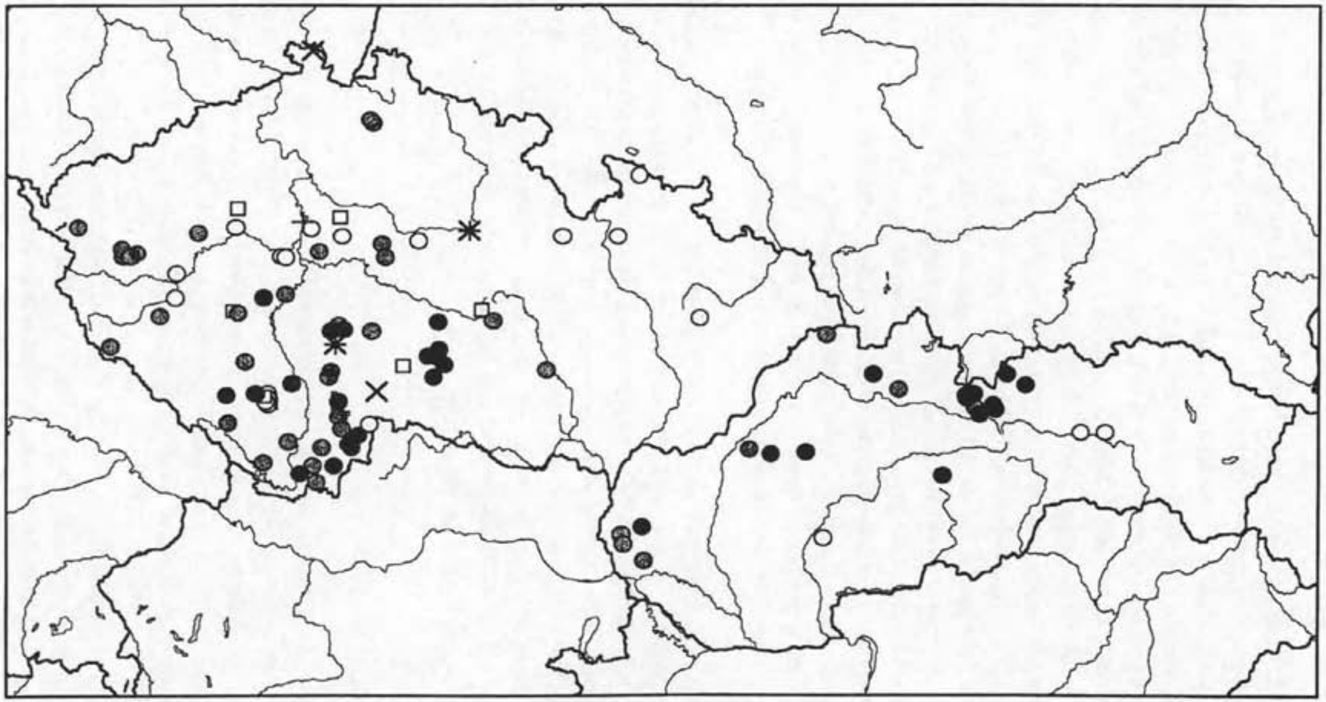
Phellodon confluens (Pers.) Pouzar

Pileus about 40 mm in width, surface at first tomentose, then rough to broken, whitish, greyish, yellow-brown. Stipe tomentose at base, more or less concolorous with the pileus. Spines pale to grey, in fresh young specimens light-blue. Context pale to grey-brown in the stipe, not changing colour in KOH. Expulsion of liquid not observed. Clamp-connections absent. Spores oval with small acute warts, 3.5–4.5 × 3–4 μm.

Related species. *P. niger* has a clearly black context turning blue-green in KOH. *P. melaleucus* is more subtle, has a thin, dark, smooth stipe (conspicuously differing from the spines) and its context turns green in KOH.

Occurrence. Relatively rare species, showing a strong decline during the last 20 years.

Map No. 5 - Phellodon tomentosus



Documented data:
- ○ - locality where the species was found before 1945
- ◉ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Data from literature:
- □ - locality where the species was recorded before 1945

Broadly defined localities:
- ✕ - locality where the species was found before 1945
- ✳ - locality where the species was found between 1945 and 1970
- ＋ - locality where the species was recorded before 1945

Accompanying trees. Literature sources mention its occurrence under *Fagaceae* (*Fagus, Castanea,* mostly *Quercus*), more rarely in mixed woods with *Picea* and *Pinus*; records from the Czech Republic and Slovakia confirm this.

Distribution (Map 6). Rare occurrence, mostly in warm regions; the distribution of this species is limited by the distribution of its accompanying trees, but it was never found in deciduous woods of higher altitudes.

List of recorded localities – Czech Republic:

Praha-Vokovice, valley of Šárecký stream, foot of the slope on the right bank of the stream between Džbán and Dívčí skok, mixed wood (*Pinus sylvestris, Quercus* sp., *Robinia pseudoacacia*) with grassy undergrowth, 17. VIII. 1939, leg. J. Herink, det. Z. Pouzar (PRM)

Poříčany, Kersko, *Quercus* wood, IX. 1936, leg. J. Sýkora, det. A. Pilát (PRM), X. 1937, leg. J. Sýkora, det. Z. Pouzar (PRC), 11. X. 1955, leg. et det. Z. Pouzar, 22. VIII. 1965, leg. et det. E. Wichanský (both PRM), 8. X. 1967, leg. et det. Z. Pouzar (Kotlaba 1968); mixed wood, 30. IV. 1944, leg. M. V. Svrček, det. Z. Pouzar, 22. VIII. 1965, leg. E. Wichanský, det. Z. Pouzar (both PRM)

Chudoplesy near Bakov nad Jizerou, western margin of Baba hill, deciduous wood (*Quercus petraea, Carpinus betulus, Tilia cordata, Betula* sp., *Populus tremula, Crataegus* sp.), 10. IX. 1966, leg. et det. J. Herink (Herb. Herink)

Obora near Obrubce, Obrubce forest, deciduous wood (*Quercus petraea, Betula* sp., *Tilia cordata, Frangula alnus, Carpinus betulus, Populus tremula, Crataegus* sp., *Molinia caerulea*), 240 m, 6. VIII. 1955, 16. VIII. 1958, 16. VIII. 1966, 5. IX. 1970, 5. X. 1974, all leg. et det. J. Herink (Herb. Herink)

Sukorady near Hořice, deciduous wood, 2. VIII. 1965, 25. VII. 1966, both leg. L. Rychtera, det. Z. Pouzar (PRM)

Frahelž, dam of lake Naděje, amongst grass, mosses and fallen leaves of *Quercus* sp., *Populus tremula* and *Salix* sp., 415 m, 20. VIII. 1988, 19. IX. 1988, both leg. T. Papoušek, det. F. Tondl (CB)

Slovakia:

Svätý Jur, *Fagus* wood, 21. IX. 1965, 7. IX. 1966, both leg. et det. I. Fábry ut *P. amicus*, 22. IX. 1966, leg. et det. I. Fábry ut *Hydnellum zonatum*, rev. Z. Pouzar (all BRA)

Malé Karpaty Mts., Kuchyňa, Vývrať, *Quercus-Fagus-Carpinus* wood, 8. VIII. 1972, leg. A. Dermek, det. Z. Pouzar (BRA, Dermek 1973)

Horné Orešany, 30. VII. 1972, leg. B. Matoušek, det. P. Hrouda (BRA)

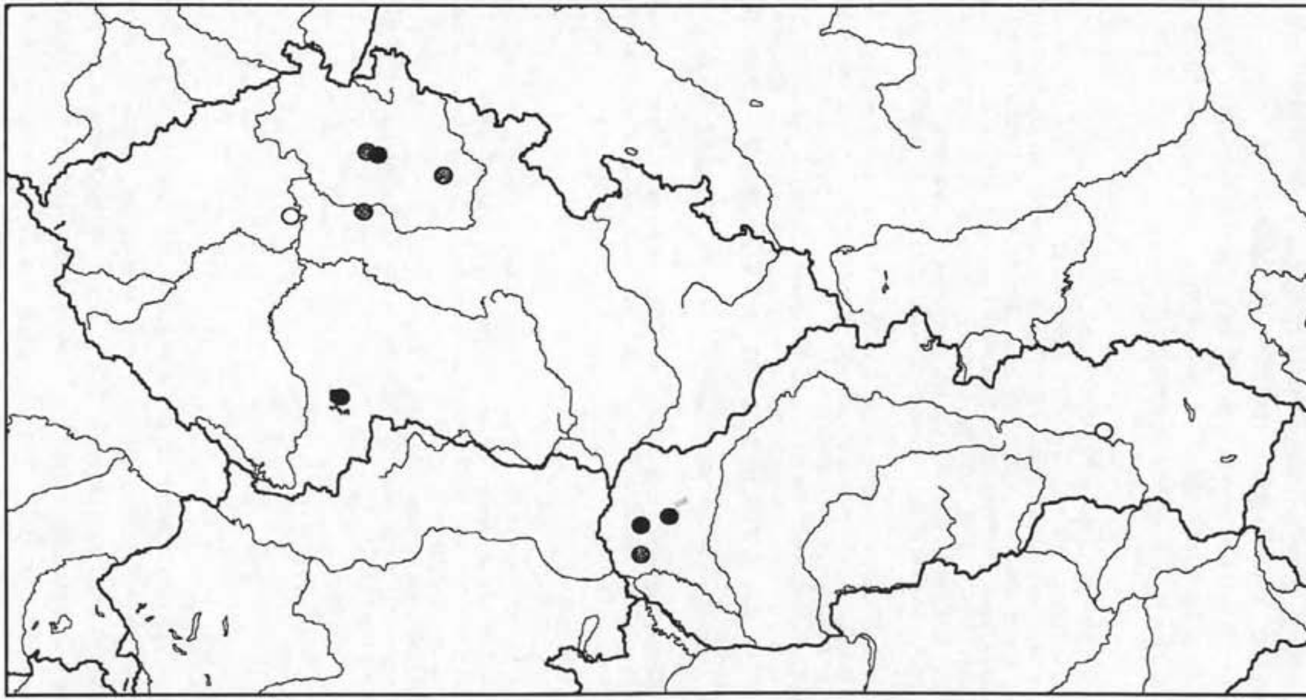
Kluknava, Predná dolina, mossy *Pinus* wood, X. 1862, leg. et det. K. Kalchbrenner ut *Hydnum hepaticum*, rev. Z. Pouzar (BRA)

Thelephoraceae Chev.

Hydnellum P. Karst.

Basidiomes pileate, stipitate; stipe sometimes shortened, because of the spines running down to the base. Surface of the pileus tomentose, fibrillose, ridged, rough or scrobiculate, variously coloured. Stipe tough, concolorous with pileus or not. Spines brown at maturity in most of the species. Context fibrillose, soft or tough to woody, zoned, variously coloured, monomitic. Hyphae rarely inflating, thin-walled to thick-walled, with or without clamp-connections. Hyphae in spines similar, but remaining thin-walled; the presence or absence of clamp-connections

Map No. 6 - Phellodon confluens



Documented data:

○ - locality where the species was found before 1945

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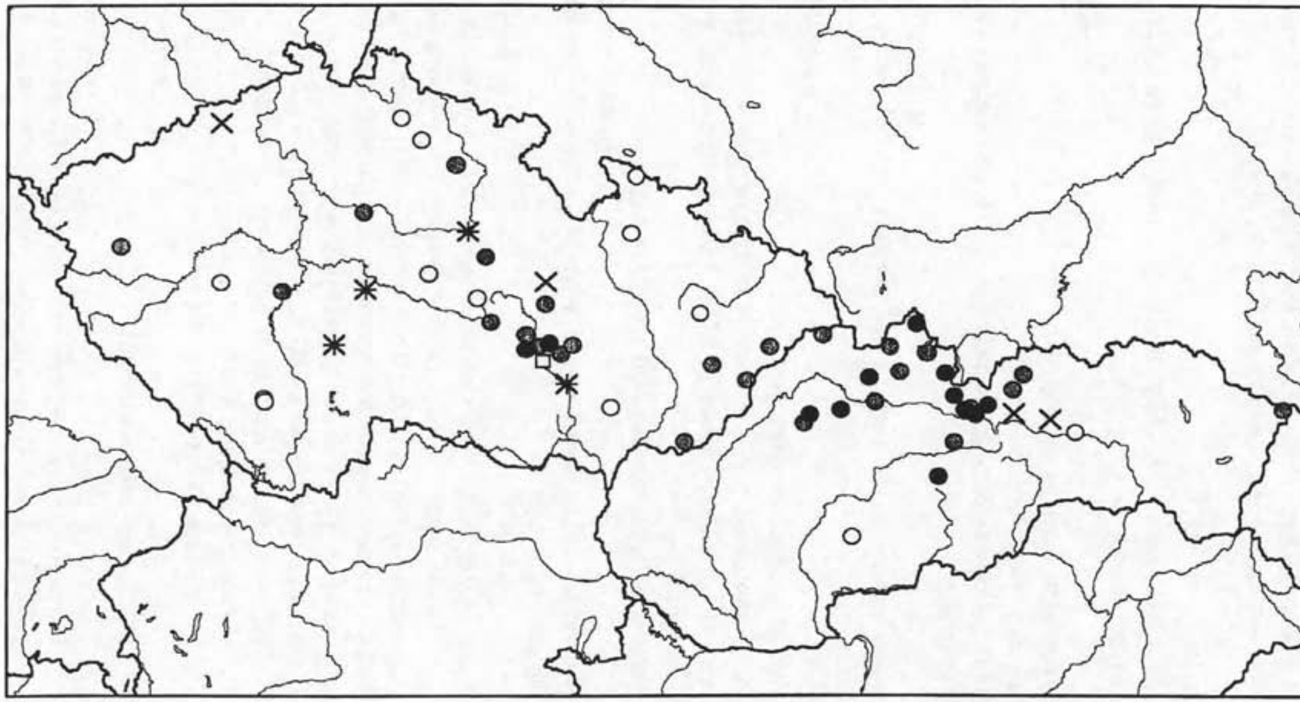
● - locality where the species was found after 1970

on these hyphae and at the base of the clavate 4-spored basidia is connected with their presence or absence in the hyphae of the whole basidiome. Spores of irregular shape, verrucose, tuberculiform or spiny, brownish. Cystidia absent.

Key to the species:

1) Young basidiome sulphurous yellow, older olive-green, turning black on pressing, the context concolorous
   ... *H. geogenium*
1') Basidiome differently coloured, never yellow
   2) Context showing blue colours when cut
      3) Base of the stipe conspicuously orange (on the surface and especially in the context)
         ... *H. caeruleum*
      3') No orange colour in the base of the stipe nor elsewhere on the basidiome
         ... *H. suaveolens*
   2') Context without blue colour when cut
      4) Surface of basidiome and also context orange or pale with orange hue; context not turning violet in KOH; base of stipe orange as in *H. caeruleum*
         5) Context pale, surface of pileus pale or orange
            ... *H. aurantiacum*
         5') Context orange to orange-brown, more or less concolorous with the pileus
            ... *H. auratile*
      4') Basidiome (respectively pileus) pale, ferrugineous or brown, context brown to ferrugineous; context quickly turning violet (sometimes changing to olive-green) in KOH; if not turning violet, taste pungent
         6) Surface of pileus brown, non-tomentose, rough, wrinkled, zoned or covered with irregular outgrowths already when young
            7) Pileus rather thin, wrinkled and concentrically zoned; sometimes covered with irregular outgrowths; spores with acute or truncate, angular warts
               8) Spores with truncate, angular warts
                  ... *H. concrescens*
               8') Spores with acute warts
                  ... *H. cumulatum*
            7') Pileus rather massive, nearly always covered with irregular outgrowths (but the previous two species may look similar); spores with rounded warts
               ... *H. scrobiculatum*

Map No. 7 - Hydnellum suaveolens



Documented data:

- ○ - locality where the species was found before 1945
- ◉ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Data from literature:

- □ - locality where the species was recorded before 1945

Broadly defined localities:

- ✕ - locality where the species was found before 1945
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6') Surface of pileus pale and tomentose when young, later changing to ferrugineous and the tomentum falling off
  9) Growing in deciduous woods; tomentum of the stipe smooth ... *H. spongiosipes*
  9') Growing in coniferous woods; tomentum on the stipe rough
    10) Taste pungent; context not turning violet in KOH; hyphae with clamp-connections ... *H. peckii*
    10') Taste mild; context immediately turning violet (sometimes changing to olive-green) in KOH; hyphae without clamp-connections ... *H. ferrugineum*

Hydnellum suaveolens (Scop.: Fr.) P. Karst.

Pileus about 75 mm in width, velutinous in young stages, rough, wrinkled when old, whitish, with bluish hue when young, turning yellow to brown with age. Stipe whitish, sometimes with translucent blue tones. Spines whitish to lightly bluish when young, soon turning pink to brown. Context whitish, brightly blue zoned when cut, a thin cut turning blue-green in KOH. Expulsion of liquid not observed. Clamp-connections present. Spores irregularly tuberculiform, not verrucose, 4–5 × 3–3.6 μm.

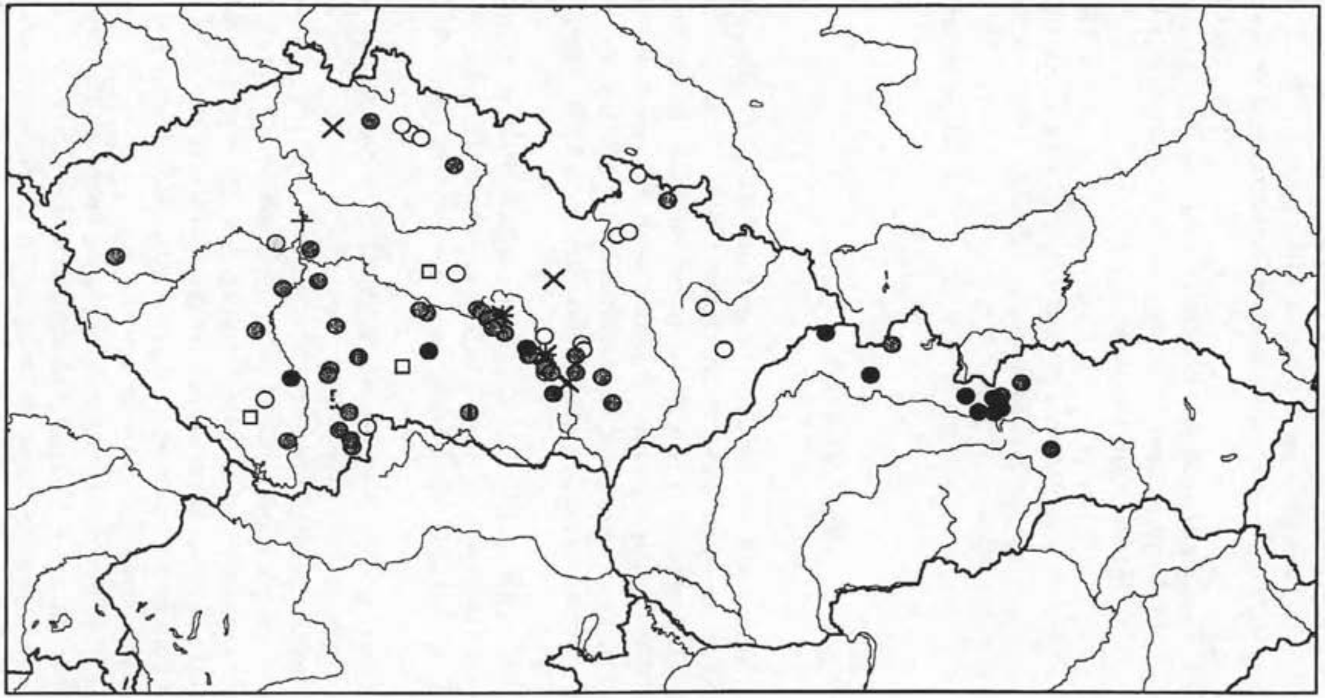
Related species. No other species than *H. caeruleum* havs the blue colouring of the context, but that species has a brightly orange stipe base. The odour of *H. suaveolens* is strong like aniseed (even after 40 years in herbarium – according to J. Herink).

Occurrence. Formerly abundant species which has recently became rare; not found in the Czech Republic during the last 10 years.

Accompanying trees. The literature mentions coniferous trees (*Picea* woods, more rarely other or mixed woods); this is fully confirmed by the Czech and Slovak collections. *Picea* occurs in 84 % of localities, but the rate of localities with occurrence of other trees is neither negligible. Also the collection from Kersko between Praha and Poděbrady is probably connected with *Pinus*. During the last 25 years the species was found only in localities with *Picea*.

Distribution (Map 7). More abundant occurrence in the Bohemian Massif (area of the Czech Republic without the Carpathian Mountains) between Žďár n. Sáz. and Brno, anywhere else rare in isolated localities. In the Carpathians since the 1970s abundant, collected here almost exclusively at mountain and sub-mountain elevations.

Map No. 8 - Hydnellum caeruleum



Documented data:
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- ● - locality where the species was found after 1970

Data from literature:
- □ - locality where the species was recorded before 1945

Broadly defined localities:
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- ✳ - locality where the species was found between 1945 and 1970
- + - locality where the species was recorded before 1945

Hydnellum caeruleum (Hornem.) P. Karst.

Pileus about 60 mm in width, without corrugate formations, young specimens softly velutinous, sometimes with light-blue hue, white, turning light-orange to brown when old. Stipe pale, turning dark when old like the pileus. Spines whitish, old brownish. Context pale, blue coloured in the pileus, brightly orange to ferrugineous in the base of the stipe, a thin cut turning (blue-)green in KOH. Context sometimes with zonations of blue lines when cut, blue colours may also be seen on the surface of the basidiome where damaged. Expulsion of liquid not observed. Clamp-connections present, scattered on old hyphae. Spores with conspicuous angular warts, 5.4–6(-6.3) × 3.4–4.3 μm.

Related species. The similar *H. aurantiacum* has no blue colours in cross-section. *H. suaveolens* has no orange or ferrugineous colours in its context (which is characteristic for the stipe base of *H. caeruleum*).

Occurrence. Formerly an abundant species, but rapidly declining since the 1970s.

Accompanying trees. The literature mentions mostly coniferous trees, more rarely deciduous woods (*Fagus*); in our country the species is known almost exclusively from coniferous woods. *Picea* occurs in 68 %, *Pinus* in 44 % of localities.

Distribution (Map 8). The species was abundant in southern Bohemia, south of Prague, in the Bohemian-Moravian Highland and in southern Moravia (from Žďárské vrchy to Ždánický les) until the 1950s, the last record from the surroundings of Brno dates from 1975, in northern and eastern Bohemia it was last seen in 1963, in northern Bohemia in 1951. In Slovakia the species has been found in the region of the Tatras, where it does not show any sign of decline.

Hydnellum aurantiacum (Batsch: Fr.) P. Karst.

Pileus about 50 mm in width, at first pale beige to white, turning bright to dark orange. Stipe orange to orange-brown. Spines whitish when young, brown at maturity. Context pale in the pileus, orange in the stipe (especially its base), a thin cut turning olive-green in KOH. Expulsion of liquid not observed. Clamp-connections absent. Spores with conspicuous angular warts, (5.8-)6–6.7 × (4-) 4.3–4.9 μm.

Related species. *H. auratile* has a dark orange context concolorous with the pileus surface (the context of *H. aurantiacum* is pale). *H. caeruleum* shows (conspicuous) blue zones in cross-section.

Occurrence. Moderately abundant species, in the Czech Republic rare in the last decades.

Accompanying trees. The literature mentions different trees, both coniferous and deciduous. Our collections from all localities where the accompanying trees were written down, are associated with coniferous trees. *Picea* occurs in 66 %, *Pinus* in 43 % of localities. In collections of the last 20 years *Picea* dominated (73 %).

Distribution (Map 9). Formerly an abundant species in southern Bohemia and the region of the river Sázava, scatteredly growing elsewhere in Bohemia. During the last 30 years it was found only three times in Bohemia. The last record from Moravia dates from 1960. In Slovakia it is relatively abundant at higher altitudes, not showing any signs of decline.

Hydnellum auratile (Britzelm.) Maas G.

Pileus about 25 mm in width, rough, wrinkled to concentrically ridged, orange to light orange-brown, sometimes with concentric, not clearly delimited zones of appressed squamules of a bright orange (to red) colour on a darker (browner) ground; basidiomes subtle. Stipe concolorous with the pileus. Spines mostly brown. Context more or less concolorous with pileus, a thin cut turning olive-green in KOH. Expulsion of liquid not observed. Clamp-connections absent. Spores with conspicuous angular warts, 4.9–5.8 × 3.6–4.5 μm.

Related species. *H. aurantiacum* has the context paler than the surface (except for young specimens) and lacks the mentioned squamules on the pileus surface. The shape is similar to *H. concrescens*, but this species is brown on the surface and also inside, without any orange hue.

Occurrence. Very rare species.

Accompanying woods. The literature mentions both coniferous and deciduous trees (*Picea, Fagus*); our collections are mostly from *Picea* woods.

Distribution (Map 10). Rare occurrence in isolated localities.

List of recorded localities – Czech Republic:

Vlastiboř, forest part of V Horkách, sandy *Pinus* wood with *Calluna* and *Vaccinium*, 430 m, 20. IX. 1991, leg. P. Hrouda, det. Z. Pouzar (PRM)

Mnichovice, VII. 1936, leg. J. Velenovský, det. P. Hrouda (PRC)

Partutovice, coniferous wood, IX. 1936, leg. et det. F. Petrak ut *H. aurantiacum*, rev. R. A. Maas Geesteranus (Maas Geesteranus 1964, Kubička 1965), rev. P. Hrouda (PRM)

Slovakia:

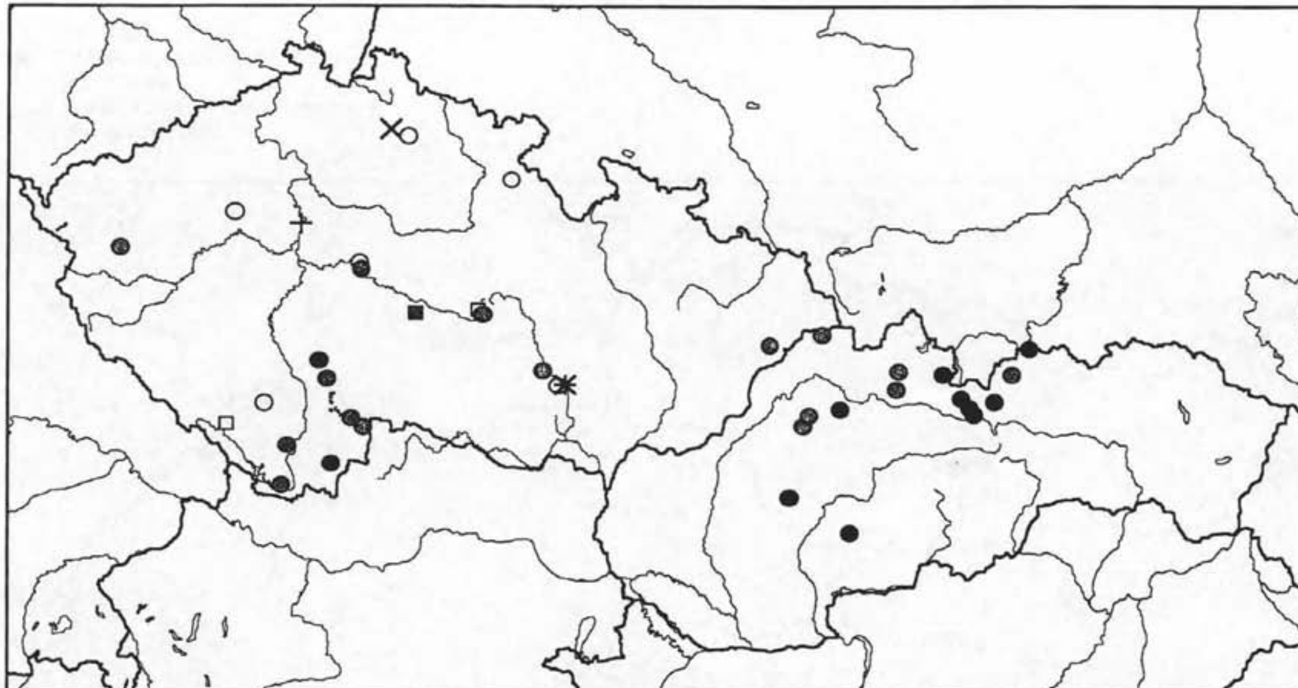
Važec, Važecká poľana (Slamená), mixed *Picea* wood, 1000 m, 12. IX. 1988, leg. F. Kotlaba, det. F. Kotlaba et J. Lazebníček ut *H. aurantiacum*, rev. P. Hrouda (PRM)

Vavrišovo near Liptovský Hrádok, *Picea* wood, 15. VIII. 1974, leg. A. Dermek, det. Z. Pouzar (PRM, BRA)

Hydnellum peckii Banker in Peck

Pileus about 50 mm in width, at first whitish and velutinous, later turning dark, ferrugineous to brown and soon becoming roughly fibrillose. Stipe concolorous. Spines whitish when young, turning brown with age. Context pale to brown,

Map No. 9 - Hydnellum aurantiacum



Documented data:

○ - locality where the species was found before 1945

◍ - locality where the species was found between 1945 and 1970

● - locality where the species was found after 1970

Data from literature:

□ - locality where the species was recorded before 1945

▣ - locality where the species was recorded between 1945 and 1970

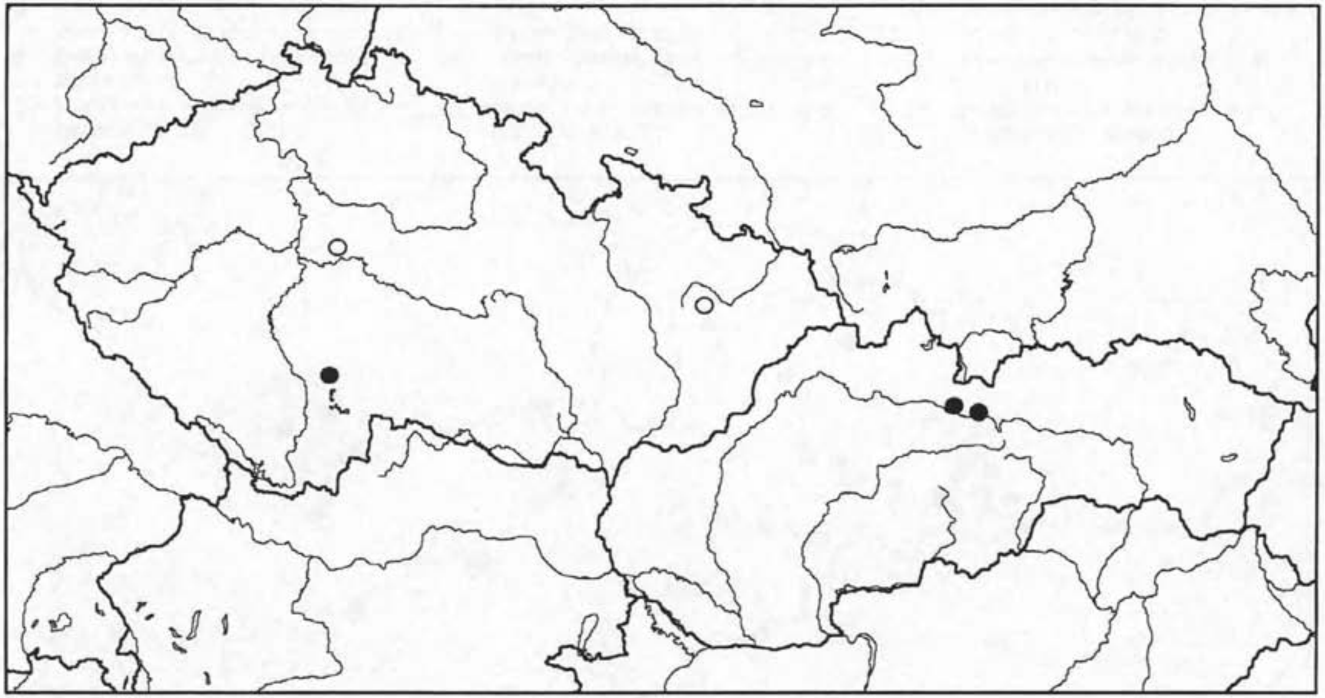
Broadly defined localities:

✕ - locality where the species was found before 1945

✳ - locality where the species was found between 1945 and 1970

+ - locality where the species was recorded before 1945

Map No. 10 - Hydnellum auratile



Documented data:

○ - locality where the species was found before 1945

● - locality where the species was found after 1970

darker in the stipe, not changing colour in KOH. Red drops of expulsed liquid may appear on the surface of the young pileus. Clamp-connections present. Spores with conspicuous angular warts, 4.9–5.4 × 3.8–4 μm.

Related species. *H. ferrugineum* has a mild taste (*H. peckii* is sharp even as an exsiccate), its context turns violet in KOH and it lacks clamp-connections. This also counts for *H. spongiosipes*, which in addition grows only in deciduous woods. *H. concrescens* and related species have their pileus, stipe, spines and context approximately equally brown, darker than the pileus of *H. peckii*, and they also lack clamp-connections.

Occurrence. Moderately abundant species, showing gradual decline.

Accompanying trees. The literature mentions coniferous trees (*Picea, Pinus*); Czech and Slovak records confirm this, coniferous trees are present in all localities. *Pinus* occurs in 67 %, *Picea* in 55 % of localities.

Distribution (Map 11). The species has two centres in the former Czechoslovakia – southern Bohemia and the central part of the Slovak Carpathians – where it still occurs. The last record from Moravia dates from 1974, in northern Bohemia it was last found in 1966.

Hydnellum ferrugineum (Fr.: Fr.) P. Karst.

Pileus about 60 mm in width, whitish and velutinous when young, less velutinous to rough and turning ferrugineous to brown with age. Stipe at its base, particularly in young stages, white to ferrugineous, velutinous, more or less concolorous with the pileus (but not always so), surface tomentose and rather scrobiculate. Spines light brown-violet when young, turning brown with age to dark brown when old. Context ferrugineous brown, a thin cut turning dark carmine in KOH. Red drops of expulsed liquid may appear on the surface of young pilei. Clamp-connections absent. Spores with conspicuous angular warts, (5.4-)5.8–6.3 × 3.6–4.5 μm.

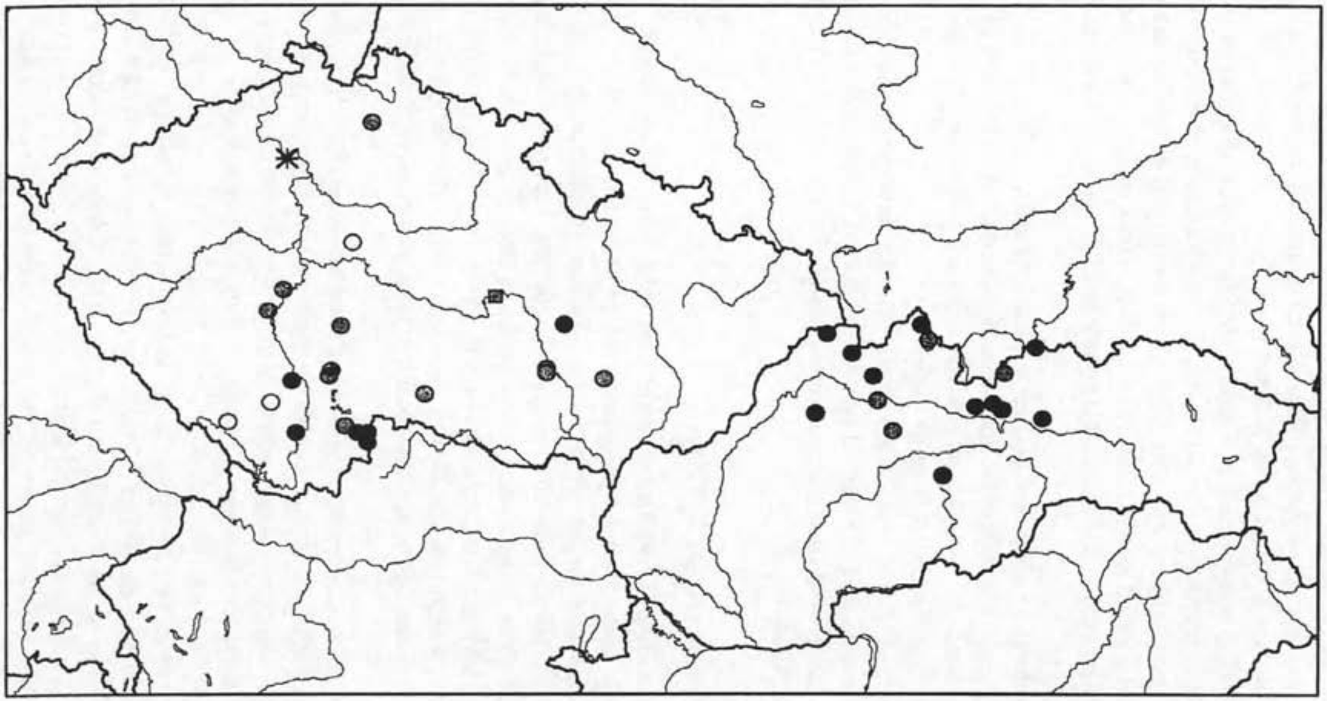
Related species. The context of *H. peckii* is pungent even after drying, does not turn violet in KOH, and has clamp-connections. *H. spongiosipes* has a homogeneous tomentum on the stipe, nearly spiny spores (the warts on the spores of *H. ferrugineum* have truncate apices) and grows in deciduous woods.

Occurrence. Still relatively abundant.

Accompanying trees. The literature mentions coniferous trees (*Picea, Pinus, Abies*), rarely mixed and deciduous woods; our collections confirm this. *Pinus* occurs in 66 %, *Picea* in 38 % of localities; the rate of localities where *Pinus* occurs grew to 79 % during the last 30 years.

Distribution (Map 12). The centre of its distribution is situated in southern Bohemia; the last record from central Bohemia is from 1957, from northern Bohemia 1966, and from Moravia 1960 (near Žďár n. Sáz.). In Slovakia this species

Map No. 11 - Hydnellum peckii



Documented data:
- ○ - locality where the species was found before 1945
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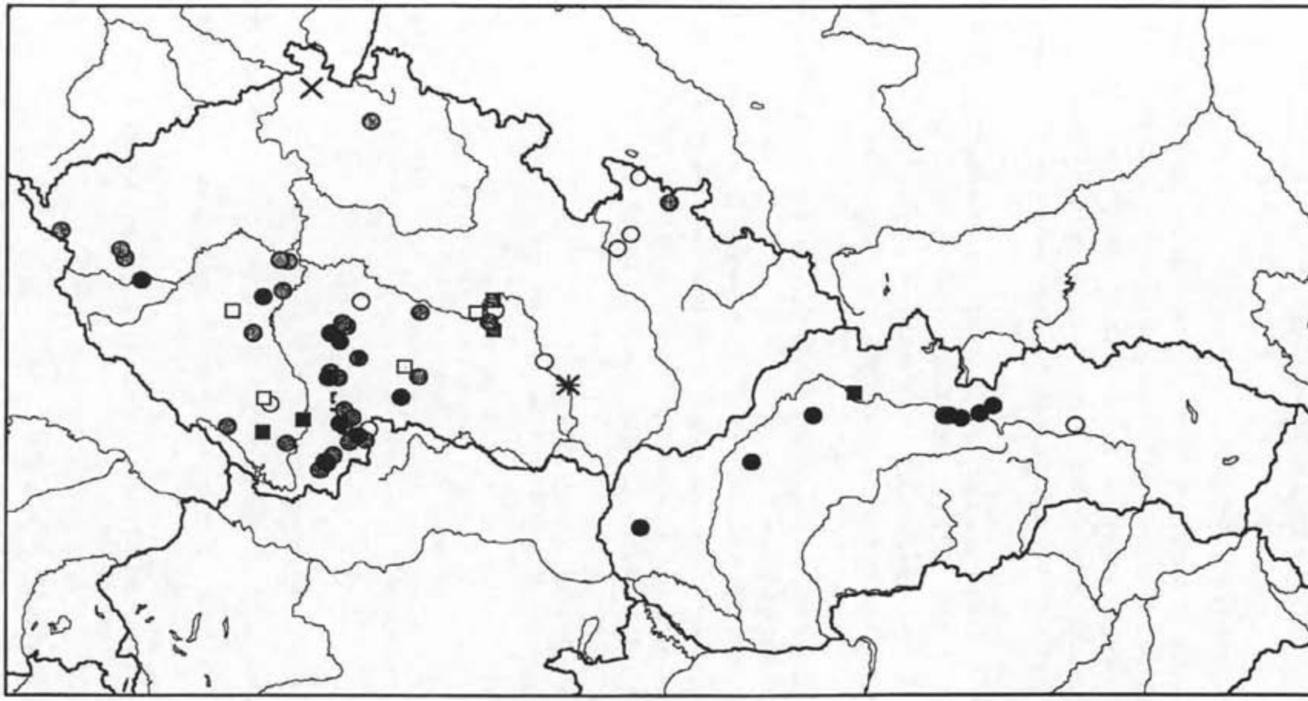
- ● - locality where the species was found after 1970

Data from literature:
- ▩ - locality where the species was recorded between 1945 and 1970

Broadly defined localities:
- ✳ - locality where the species was found between 1945 and 1970

Map No. 12 - Hydnellum ferrugineum



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Data from literature:
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Broadly defined localities:
- ✕ - locality where the species was found before 1945
- ✳ - locality where the species was found between 1945 and 1970

is not very abundant, it was particularly found below the Tatra Mountains, but it is evident that higher altitudes are not typical of it.

Hydnellum spongiosipes (Peck) Pouzar

Pileus about 50 mm in width, velutinous, at first whitish, then fleshy, turning brown with age, often cinnamomeous. Stipe velutinous, concolorous with the pileus or darker, its surface evenly tomentose. Spines at first whitish, then turning brown. Context concolorous with pileus surface, a thin cut turning dark carmine in KOH. Expulsion of liquid not observed. Spores with very conspicuous acute warts, (5.4-) 6.3–7.2 × 4.4–5.4 μm.

Related species. *H. ferrugineum* and *H. peckii* are species of coniferous woods, their spores have truncate warts and an expulsion of red drops may appear in young specimens of these species. Other characters of *H. peckii* are its pungent taste, the presence of clamp-connections and the context not changing colour in KOH.

Occurrence. Rare species, recently found in Slovakia, but not found in the Czech Republic during the last 20 years.

Accompanying trees. The literature mentions *Fagaceae*, most often *Quercus*. Indeed, *Quercus* is present in all Czech and Slovak localities, where the accompanying trees were recorded, in the remaining cases its presence is possible.

Distribution (Map 13). Rare occurrence in isolated localities.

List of recorded localities – Czech Republic:

Poříčany, Kersko, *Quercus* wood, 30. VII. 1944, leg. V. Vacek, det. R. A. Maas Geesteranus, 11. X. 1955, leg. et det. Z. Pouzar (both PRM, Pouzar 1956), 31. VIII. 1960, leg. et det. Z. Pouzar (BRNM), 8. X. 1967, leg. et det. Z. Pouzar (Kotlaba 1968), 7. IX. 1969, leg. P. Krampera, det. K. Kunc ut *H. velutinum = spongiosum* (Krampera 1970); mixed wood, 22. VIII. 1965, leg. et det. E. Wichanský ut *H. velutinum* (PRM)

Obora, Obrubce forest, deciduous wood (*Quercus petraea, Betula* sp., *Tilia cordata, Frangula alnus, Molinia caerulea*), 250 m, 5. IX. 1970, leg. et det. J. Herink (Herb. Herink)

Slovakia:

Žemberovce, slope of Husárka hill, *Quercus* wood, 400 m, 23. IX. 1987, leg. et det. J. Kuthan (BRA)

Gbely, Dúbravka, *Quercus* wood, 17. IX. 1972, 7. VIII. 1974, both leg. et det. A. Dermek (Dermek 1978)

Kuchyňa, Vývrať, 3. VIII. 1972. leg. et det. I. Fábry ut *H. scrobiculatum*, rev. Z. Pouzar, 20. VIII. 1972, leg. A. Horváthová, det. Z. Pouzar (both BRA)

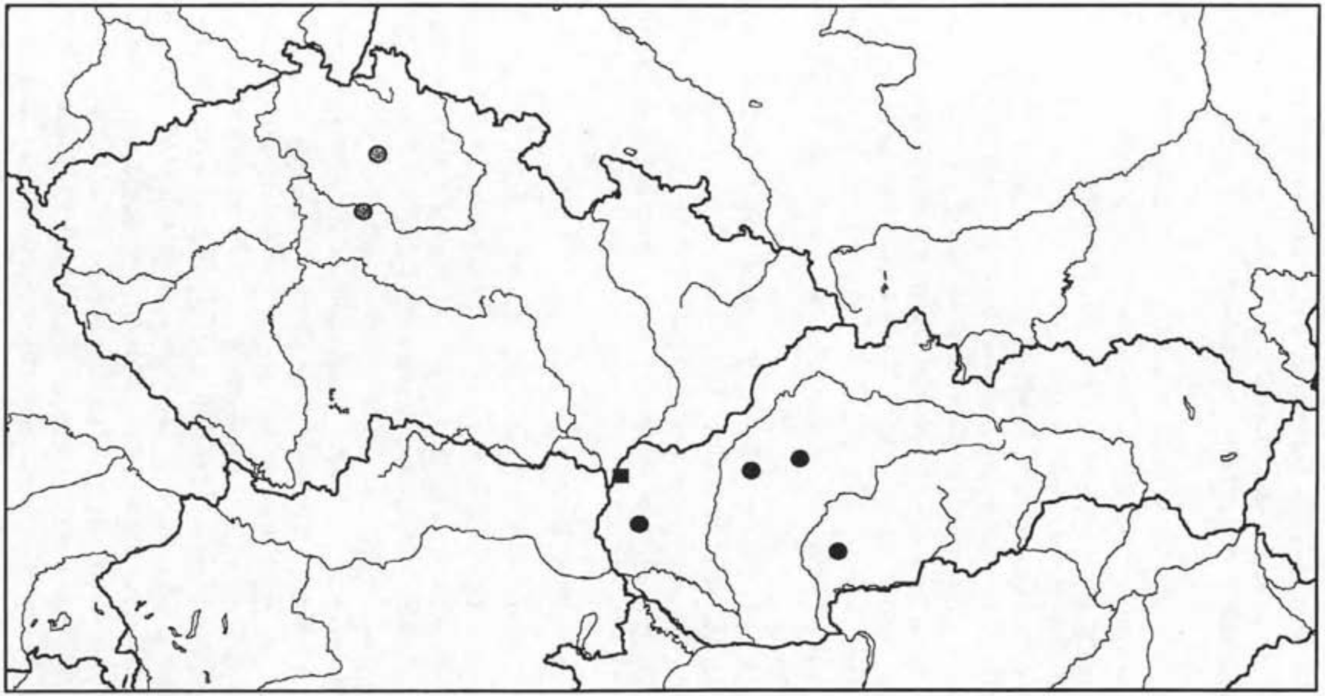
Zlatníky, *Quercus* wood, 400 m, 11. VII. 1971, leg. et det. J. Kuthan (BRA)

Mačov near Diviaky nad Nitricou, deciduous to mixed wood (*Quercus, Pinus*), 420 m, 14. IX. 1980, leg. et det. J. Kuthan (BRA)

Hydnellum scrobiculatum (Fr.) P. Karst.

Pileus about 40 mm in width, rough, wrinkled, mostly covered with many irregular outgrowths or little secondary pileoli; concrescence of basidiomes frequent.

Map No. 13 - Hydnellum spongiosipes



Documented data:

- ◍ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Entire basidiome – both pileus and stipe – brown, margin of the pileus sometimes a little lighter. Spines concolorous, context too, a thin cut turning dark carmine in KOH. Yellowish drops of expulsed liquid may appear on the surface of the pileus. Clamp-connections absent. Spores with conspicuous rounded warts, 5.6–7 × 4.5–4.9 μm.

Related species. The species is macroscopically not reliably distinguishable from *H. concrescens* and *H. cumulatum*. Under the microscope the spores of *H. scrobiculatum* are found to have rounded warts, *H. concrescens* has spores with truncate and *H. cumulatum* with acute warts.

Occurrence. Less abundant species, showing a growing decline.

Accompanying trees. The literature mentions coniferous trees (mostly *Pinus*), but also mixed woods (with representatives of the family *Fagaceae*); Czech and Slovak collections come from coniferous (the majority), mixed and deciduous woods. *Pinus* occurs in 54 %, *Quercus* in 29 % of localities. There is a shift from deciduous woods (most collections in the 1930s and 1940s) to coniferous woods (most collections in the 1950s); the species was not found under deciduous tree during the last decade.

Distribution (Map 14). Formerly locally abundant species in the whole of Bohemia, recently limited to a few localities in the area between Prague and České Budějovice. The last record from the northern part of Bohemia is from 1946, in Moravia it was last seen in 1970. In Slovakia it occurs rarely in isolated localities.

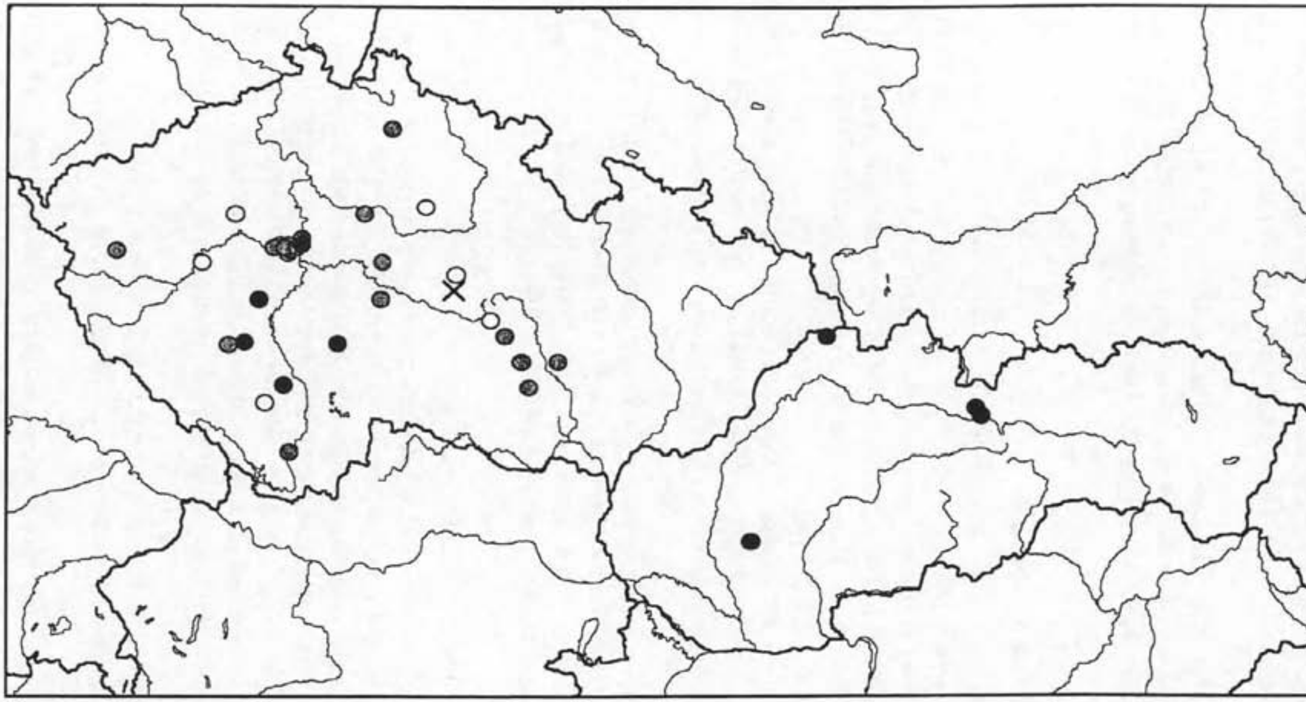
Hydnellum concrescens (Pers.) Banker

Pileus about 40 mm in width, brown, wrinkled, with concentric zones, if not destroyed by irregular outgrowths on the surface of the pileus. Stipe concolorous with the pileus. Spines sometimes paler than pileus in young stages, turning dark brown at maturity. Context brown, concolorous with the surface of the basidiome, a thin cut turning dark carmine in KOH. Yellowish drops of expulsed liquid may appear on the surface of the pileus. Clamp-connections absent. Spores with conspicuous angular warts, 5.4–6.1 × (3.6-)4–4.5 μm.

Related species. *H. scrobiculatum* and *H. cumulatum* are macroscopically indistinguishable from this species; basidiomes of *Hydnellum* with a conspicuously concentrically zoned pileus and without surface outgrowths, very probably concern *H. concrescens*, but a microscope is needed to check this: the spores of *H. cumulatum* have acute warts, those of *H. scrobiculatum* rounded warts; the spores of *H. concrescens* have warts with truncate apices (like molar teeth). It can also be confused with *H. auratile* with an orange context and *Phellodon tomentosus* with light, beige to ochraceous spines, oval spores with small apices and a smell of fenugreek when dried.

Occurrence. Formerly abundant, showing strong decline.

Map No. 14 - Hydnellum scrobiculatum



<u>Documented data:</u>

○ - locality where the species was found before 1945

◉ - locality where the species was found between 1945 and 1970

● - locality where the species was found after 1970

<u>Broadly defined localities:</u>

✕ - locality where the species was found before 1945

Accompanying trees. According to the literature this species grows under both coniferous and deciduous trees, which is confirmed in our country. The spectrum of accompanying trees is very wide. The most frequent are *Picea* (52 %) and *Quercus* (35 % of localities).

Distribution (Map 15). The species occurs almost in the whole area of the former Czechoslovakia. It does not have distinct distribution centres. It is currently not abundant in Slovakia and rare in the Czech Republic, but is still found in isolated localities.

Hydnellum cumulatum K. Harrison

Pileus about 25 mm in width, scrobiculate, sulcate to radially ridged, brown, often more basidiomes grown together. Stipe concolorous with the pileus, spines more or less so. Colour of the context similar to the surface of the pileus, a thin cut turning dark carmine in KOH. Yellowish drops of expulsed liquid may appear on the surface of the basidiome. Clamp-connections absent. Spores with conspicuous acute warts, 4.3–5.6 × 3.6–4.3 μm.

Related species. *H. cumulatum* is macroscopically indistinguishable from *H. concrescens* and *H. scrobiculatum*. It differs from these species by its acutely spiny spores; *H. concrescens* has spores with truncate, angular warts, *H. scrobiculatum* with rounded warts.

Accompanying trees. The literature mentions coniferous trees (*Picea, Pinus*).

Czech find of *Hydnellum cumulatum* (Map 16): Šalmanovice (southern Bohemia), *Pinus sylvestris*, 2. IX. 1960, leg. C. Bas, det. R. A. Maas Geesteranus (preserved in L; Maas Geesteranus 1975, Kubička 1981).

Hydnellum geogenium (Fr.) Banker

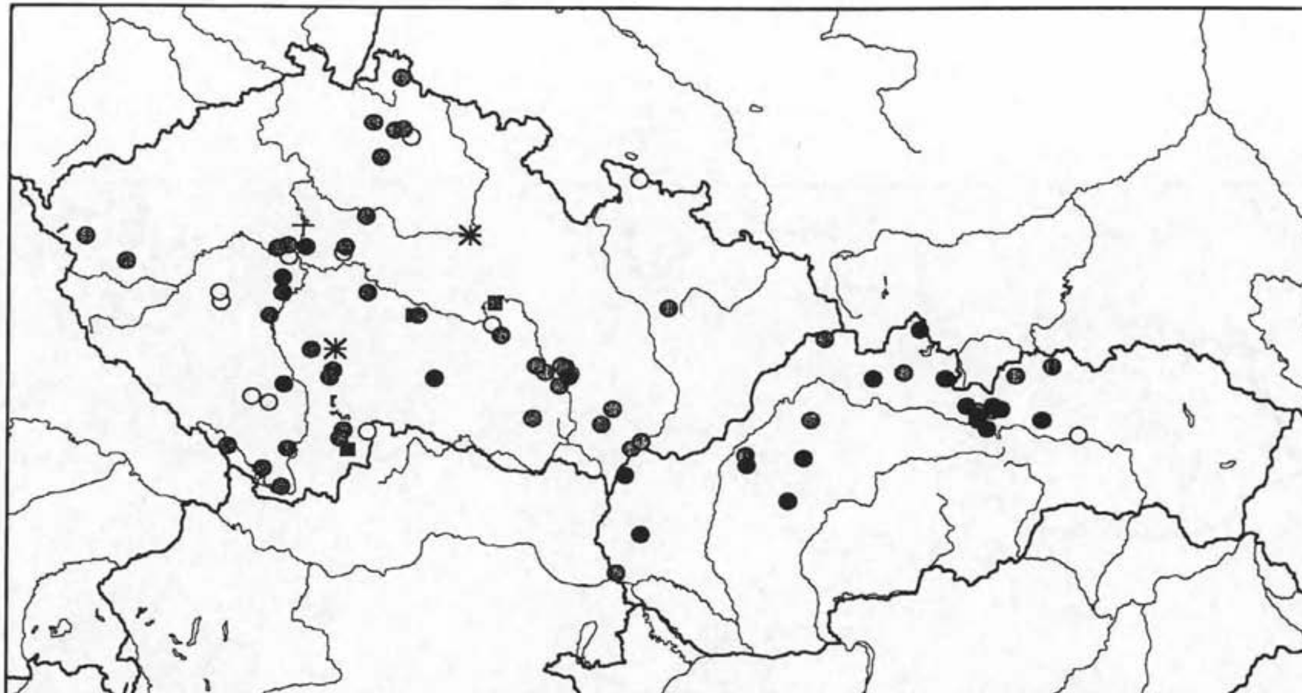
Pileus about 25 mm in width, at first sulphurous yellow, with age and by pressing turning olive-green to black, with irregular surface, wrinkled, with outgrowths or secondary pileoli, basidiomes often grown together. Stipe (often indistinguishable when basidiomes are grown together) concolorous. Spines sulphurous yellow when young, then turning brown. Context yellow when young, gradually turning to olive-green, a thin cut turning olive in KOH. Expulsion of liquid not observed. Clamp-connections present. Spores with not very conspicuous angular warts, 4.5–5.2 × 3.1–3.6 μm.

Related species. Not to be confused with any other species.

Occurrence. Rare species, still found in Slovakia, almost disappeared from the Czech Republic.

Accompanying trees. Literature sources mention coniferous trees. This species seems to be associated with *Picea*, which occurs (with one exception – a collection from the 19th century) in all localities where the trees were recorded.

Map No. 15 - Hydnellum concrescens

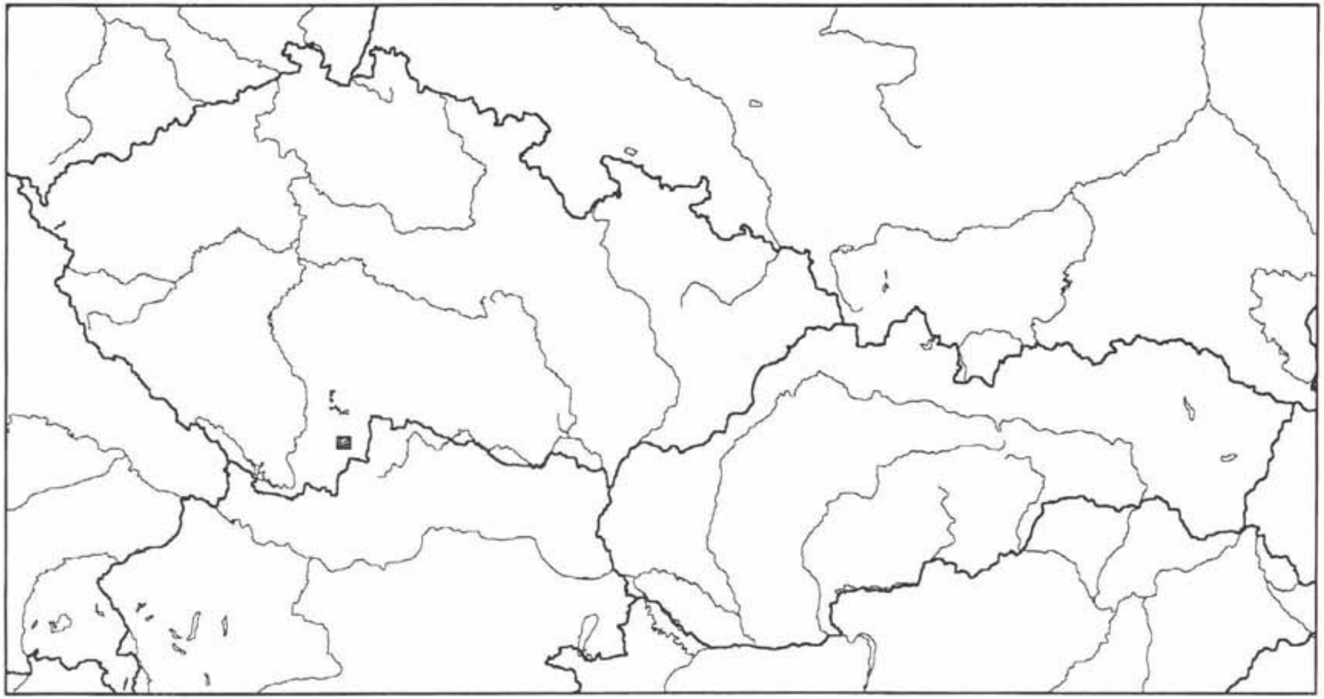


Documented data:
- ○ - locality where the species was found before 1945
- ◍ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Data from literature:
- ▨ - locality where the species was recorded between 1945 and 1970
- ■ - locality where the species was recorded after 1970

Broadly defined localities:
- ✳ - locality where the species was found between 1945 and 1970
- + - locality where the species was recorded before 1945

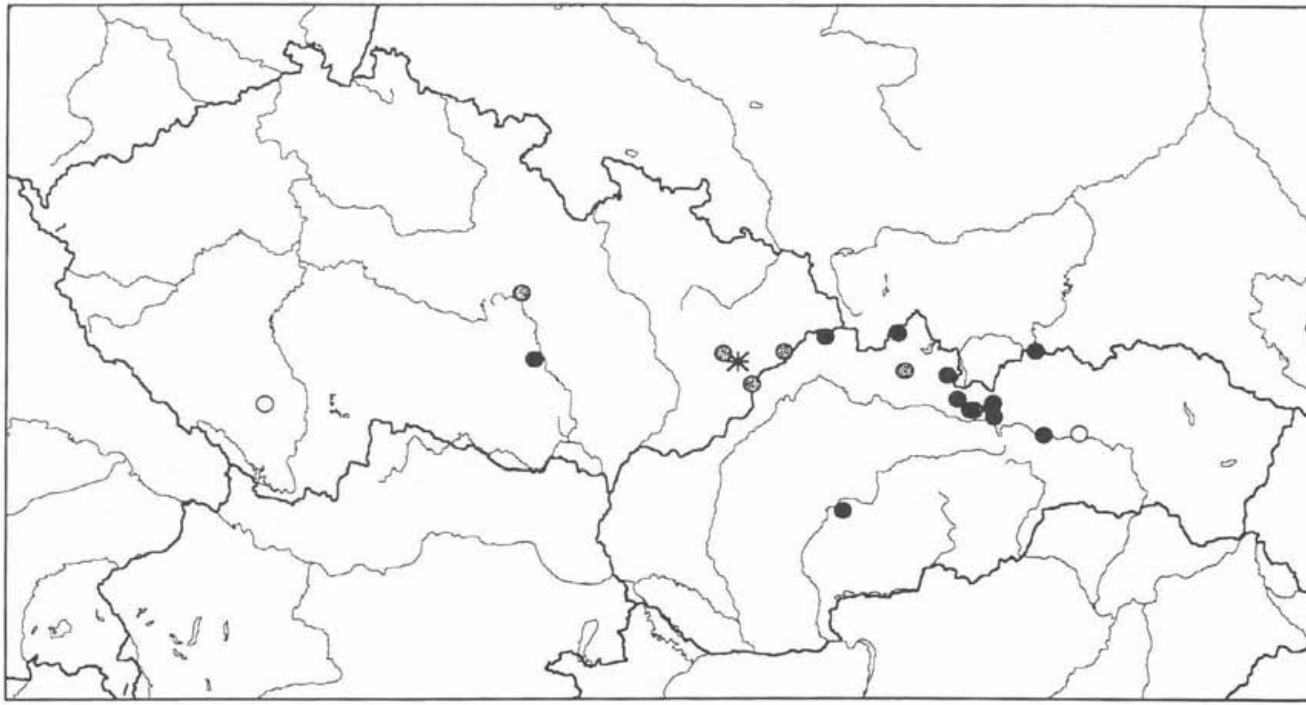
Map No. 16 - Hydnellum cumulatum



Data from literature:

▤ - locality where the species was recorded between 1945 and 1970

Map No. 17 - Hydnellum geogenium



Documented data:

○ - locality where the species was found before 1945

◉ - locality where the species was found between 1945 and 1970

● - locality where the species was found after 1970

Broadly defined localities:

✳ - locality where the species was found between 1945 and 1970

Distribution (Map 17). Isolated occurrence in the Bohemian Massif, somewhat more abundant in the Carpathians (the belt Beskydy – Kysuca – Orava – Tatry – Spiš); prefers higher altitudes.

List of recorded localities – Czech Republic:

Libějovické Svobodné Hory, forest on Holička hill, planted *Picea* wood, 550 m, VII. 1936, leg. et det. J. Herink (PRM)

Sádek, *Picea* wood, 17. IX. 1950, leg. E. Horníček, det. J. Herink (Herb. Herink)

Kuřimské Jestřabí, Falcův mlýn, *Picea* wood with admixed *Pinus*, 11. IX. 1974, leg. B. Kasala, det. K. Kříž (BRNM)

Kateřinice, central-western part of Mt. Dubcová mountain, planted *Picea* wood, 480 m, 31. VII. 1944, leg. V. Pospíšil, det. F. Šmarda (BRNM), 2. IX. 1948, leg. et det. V. Pospíšil (PRM)

Pošle, Poschla forest, Piceetum nudum, 3. IX. 1948, 13. VII. and 26. VII. 1953, all leg. V. Pospíšil, det. F. Šmarda, rev. F. Kotlaba (BRNM)

Velké Karlovice, Babské valley, *Picea-Fagus* wood, 4. IX. 1948, leg. V. Pospíšil, det. F. Šmarda (BRNM)

Broadly defined locality:

Vsetín, 15. IX. 1946, leg. V. Pospíšil, det. F. Šmarda (BRNM)

Slovakia:

Sklenné Teplice near Banské Štiavnica, *Picea* wood, 600 m, 28. VIII. 1974, leg. et det. J. Kuthan (BRA)

Spišské Vlachy, *Pinus* wood, IX. 1859, leg. et det. K. Kalchbrenner ut *Hydnum sulphureum* (BRA)

Čingov, Hradisko hill (3 km west of Spišská Nová Ves), *Picea* wood, 560 m, 17. IX. 1985, leg. et det. J. Kuthan (BRA)

Nižná Šuňava, *Picea* wood, 850 m, 16. VII. 1977, leg. et det. J. Kuthan (BRA)

Mt. Osobitá, slope, *Picea* wood, 1200 m, 7. VIII. 1977, leg. et det. J. Kuthan (BRA)

Štrbské Pleso, Uhliščata, slope of Spálený vrch, *Picea* wood, 1000 m, 14. IX. 1989, leg. et det. J. Kuthan (BRA)

Červený Kláštor, *Picea* wood (with *Abies*), 550 m, 10. VII. 1971, leg. et det. J. Kuthan (BRA)

Račkova valley, right bank of Račková stream, 3 km north of Pribylina, *Picea* wood, 850 m, 17. VIII. 1974, leg. A. Dermek, det. P. Hrouda (BRA)

Východná, Krátke forest, *Picea* wood, 950 m, 2. IX. 1978, leg. J. Kuthan, Jos. Herink et Jan Herink, det. Jos. Herink (Herb. Herink)

Važecké louky, north of the road Východná - Važec, *Picea* wood, 850 m, 23. VIII. 1970, leg. et det. J. Kuthan (BRA)

Tatranská Štrba, *Picea* wood, 900 m, 20. IX. 1977, leg. et det. J. Kuthan (BRA)

Raková, Korcháň valley, *Picea* wood, 550 m, 22. IX. 1974; 650 m, 5. X. 1974, both leg. et det. J. Kuthan (BRA)

Oravský Podzámok, forest in the direction of Hruštín, old *Picea* wood, 550 m, 12. VIII. 1959, 23. VIII. 1960, both leg. et det. I. Fábry (BRA)

Mútne, *Picea* wood, 650 m, 3. VIII. 1973, leg. et det. J. Kuthan (BRA)

Sarcodon P. Karst.

Basidiomes pileate, stipitate. Surface of pileus at first tomentose, then glabrescent, with cuticle sooner or later breaking up into areoles or scales, mostly brown, sometimes with yellow hue; stipe similarly coloured. Spines brown. Context fleshy to tough, non-zoned, most often whitish to brown (different colours are characteristic of some species or groups of species), monomitic. Hyphae in context broadening towards the centre of pileus, thin-walled to slightly thick-walled, with or without clamp-connections. Hyphae in spines similar. Basidia with or without basal clamp-connections, corresponding to their presence or absence in the context, clavate, 4-spored. Spores of irregular shape, tuberculiform, verrucose, brownish. Cystidia absent.

Key to the species:

1) Context pink or violet; hyphae without clamp-connections
   2) Pileus turning dark (to black) with age; species of coniferous woods ... *S. fuligineo-violaceus*
   2') Pileus brown, rather with a red or pink hue; species of deciduous woods ... *S. joeides*

1') Context not pink or violet
   3) Base of the stipe grey– (to black-) green; hyphae without clamp-connections
      4) Pileus brown, cinnamomeous or purple-brown, sometimes broken up into dark brown scales on a somewhat lighter brown ground, stipe concolorous with pileus; context turning blue-green in KOH; spores rough with angular warts ... *S. scabrosus*
      4') Pileus yellow-brown to ochraceous, sometimes broken up into scales, which may be come dark-brown, although the ground remains yellow-brown; stipe yellow-brown, ochraceous to ferrugineous; spores with small rounded warts
         5) Both pileus and stipe more or less equally ochraceous, pileus sometimes broken up into a slightly darker scales on a lighter ground; context not changing colour in KOH ... *S. fennicus*
         5') Pileus and stipe differently coloured, cuticle of the pileus breaking up into brown areoles in the centre and scales at the margin (darker towards the centre) on a yellowish ground; stipe dirty pale to purple-brown; context turning blue-green in KOH ... *S. glaucopus*
   3') Base of the stipe not differently coloured; hyphae with clamp-connections
      6) Context whitish, chrome-yellow where the pileus passes into stipe (not clear in exsiccates); spores without distinct warts, mnot larger than $5.5 \times 4.5\ \mu m$ ... *S. versipellis*
      6') Context whitish to brown, without yellow colours; spores with mangular warts, not smaller than $7 \times 4.5\ \mu m$

7) Pileus fleshy to dark brown, breaking up into large scales, erect in the centre of pileus and adjacent on its margin, and deep fissures

... *S. imbricatus*

7') Pileus pale, yellow– to light-brown, breaking up in the centre into areoles or scales with slightly raised tips

... *S. leucopus*

Sarcodon imbricatus (L.: Fr.) P. Karst.

Pileus about 100 mm in width, fleshy brown, red-brown to dark brown, with age breaking up into conspicuous scales, erect in the centre of the pileus, appressed towards its margin, on a lighter ground. Stipe lighter, turning brown towards the pileus. Spines pale to purple-brown. Context whitish in the pileus, brown in the base of the stipe, not changing colour in KOH. Expulsion of liquid not observed. Clamp-connections present. Spores with conspicuous angular warts, 7.2–8.2 × 4.9–5.4 μm.

Related species. The pileus of *S. leucopus* is areolate or possesses only appressed squamules. *S. scabrosus* has a grey-green stipe base and a conspicuously bitter taste (the taste of *S. imbricatus* is neutral or only slightly bitterish). The context of *S. joeides* and *S. fuligineo-violaceus* is pink or violet.

Occurrence. Formerly abundant species showing a relatively conspicuous decline.

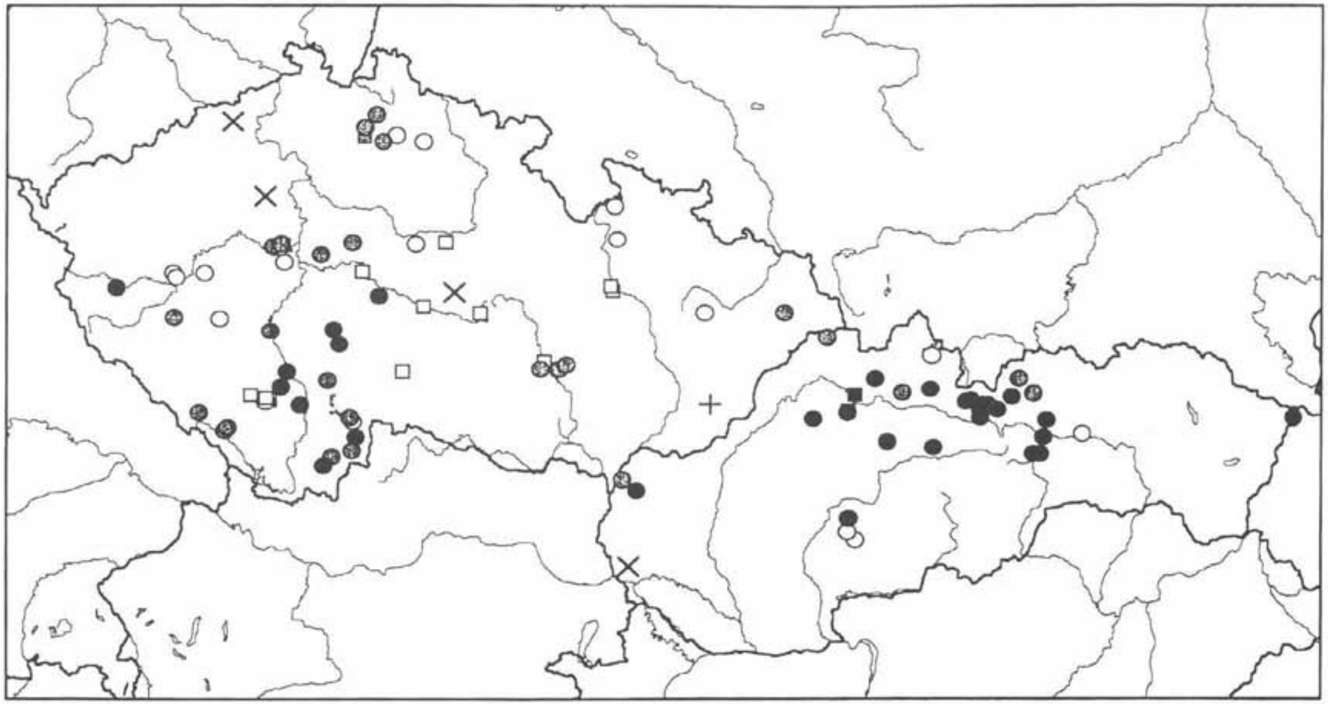
Accompanying trees. The literature mentions coniferous trees, which is confirmed in our countries with a few exceptions. The most frequent accompanying tree is *Picea*, which occurs in 79 % of localities.

Distribution (Map 18). According to J. Herink *S. imbricatus* was so abundant in the past that it was not documented, so its earlier occurrence was probably denser than the map shows. The last record from northern Bohemia dates from the year 1965, from the surroundings of Prague 1968, in Moravia it was last seen in 1957. The only part of Bohemia where it is still commonly collected is southern Bohemia. In Slovakia this species is still abundant, especially at higher altitudes.

Sarcodon leucopus (Pers.) Maas G. et Nannf.

Pileus about 100 mm in width, light– to dark brown, at first tomentose, later radially fibrillose towards the margin and areolate or with appressed squamules in the centre; the scales are darker on a lighter (to yellow-brown) ground. Stipe concolorous, mainly in the lower part paler, appressed squamulose with age. Spines at first whitish, later purple-brown. Context whitish with a brown or violet, after some time sometimes also light-green hue, not changing colour in

Map No. 18 - Sarcodon imbricatus



Documented data:

- ○ - locality where the species was found before 1945
- ◍ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Data from literature:

- □ - locality where the species was recorded before 1945
- ▤ - locality where the species was recorded between 1945 and 1970
- ■ - locality where the species was recorded after 1970

Broadly defined localities:

- × - locality where the species was found before 1945
- + - locality where the species was recorded before 1945

KOH. Expulsions of liquid not observed. Clamp-connections present. Spores with conspicuous angular warts, (6.7-)7.2-7.6(-9) × 4.5-5.6 μm.

Related species. *S. imbricatus* has pronounced scales with raised tips or completely erect at least in the centre of the pileus and does not have such an unpleasant smell as *S. leucopus*. Fresh basidiomes of *S. versipellis* are brightly orange and the spores of this species have broad rounded warts. *S. glaucopus* has a similarly light and areolate pileus, but the base of its stipe is grey-green.

Occurrence. Rare species, which has not been found in Slovakia during the last 10 years and in the Czech Republic during the last 30 years.

Accompanying trees. The literature mentions coniferous trees. In the former Czechoslovakia *S. leucopus* was found also in deciduous woods. The most frequent accompanying tree is *Picea*, which occurs in 64 % of localities.

Distribution (Map 19). Rare occurrence in isolated localities. The last record from Bohemia dates from 1955, in Moravia it was last seen in 1960.

List of recorded localities – Czech Republic:

Floodplain of the Vltava river between Zvíkov and Červená – valley of Kučeřský stream, *Picea* wood, 17. VIII. 1955, leg. et det. M. Svrček ut *S. laevigatus* (PRM)

Svojanov, *Picea-Abies-Betula* wood, 21. VI. 1949, leg. J. Kubička det. Z. Pouzar ut *S. laevigatus* (PRM)

Veverská Bítýška, Hranečník forest along the road to Lažánky, *Picea* wood with *Abies* undergrowth, 17. X. 1960, leg. et det. F. Šmarda ut *S. laevigatus* (BRNM)

Semetín near Vsetín, 20. VII. 1953, leg. F. Šmarda, det. Z. Pouzar ut *S. laevigatus* (BRNM)

Javorníky Mts., Nový Hrozenkov, cirque, *Picea-Abies* wood, 600 m, 23. VII. 1953, leg. F. Šmarda, det. Z. Pouzar ut *S. laevigatus* (BRNM)

Slovakia:

Brodské, *Pinus-Quercus* wood, 9. IX. 1973, leg. et det. A. Dermek ut *Hydnum leucopus* (BRA)

Svätý Jur, *Fagus* wood, 21. IX. 1965, leg. et det. I. Fábry ut *S. laevigatus* (BRA)

Kuchyňa, Vývrať, *Quercus-Fagus-Carpinus* wood, 8. VIII. 1972, leg. A. Dermek (Dermek 1973 ut *Hydnum laevigatum*), 20. IX. 1980, leg. R. Režďovič, det. A. Dermek ut *S. laevigatus* (BRA)

Važecké lúky, north of the road Východná - Važec, *Picea* wood, 950 m, 14. IX. 1970, leg. J. Kuthan, det. Z. Pouzar ut *S. laevigatus* (PRM, BRA)

Raková, Korcháň valley, *Picea* wood, 19. VII. 1964, leg. et det. J. Veselský ut *S. laevigatus* (BRNM)

Oravská priehrada, Ústie, Jedličník hill, west of Ústie, 750m, 25. VII. 1894, leg. et det. S. Truchlý ut *Hydnum laevigatum* (BRA)

Lendak, *Picea* wood, 800 m, 11. VIII. 1957, leg. et det. B. Ježek, J. Kubička et K. Kříž ut *S. laevigatus* (BRNM)

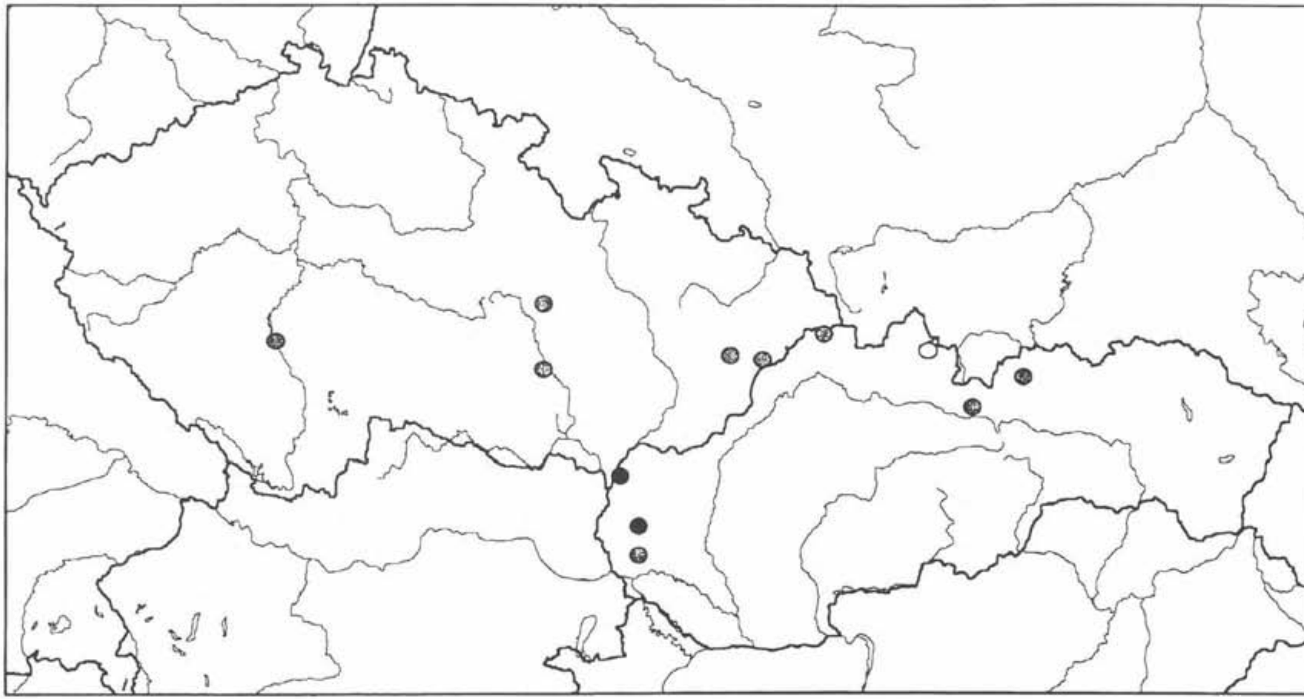
Not localised site:

Nitov, Pýšna hill, 1018 m, 2. IX. 1892, leg. et det. S. Truchlý ut *Hydnum laevigatum* (BRA)

Sarcodon versipellis (Fr.) Quél.

Pileus about 80 mm in width, orange-brown, lighter towards the margin, dried brownish (or yellowish to greyish) coloured, appressed squamulose to fibrillose towards the margin, the squamules and fibrils being darker brown. Stipe concolorous

Map No. 19 - Sarcodon leucopus



Documented data:

○ - locality where the species was found before 1945

◍ - locality where the species was found between 1945 and 1970

● - locality where the species was found after 1970

or lighter. Spines whitish to purple-brown. Context white, greyish only in the base of the stipe and where the pileus passes into the stipe; not changing colour in KOH. Expulsion of liquid not observed. Clamp-connections present. Spores irregularly tuberculiform, 4.5–5.5 × 3.5–4.5 μm.

Related species. *S. fennicus* is similarly orange-ochraceous, but does not have scales on its pileus, the base of the stipe is grey-green and it does not have clamp-connections. A grey-green stipe base and the absence of clamp-connections is characteristic of *S. glaucopus*, too. The fresh pileus of *S. leucopus* is brown (not brightly orange as the pileus of *S. versipellis*) and its at least 7 × 4.5 μm large spores have conspicuous angular warts.

Occurrence. Rare species, not found in the Czech Republic for almost 50 years.

Accompanying trees. The literature mentions its occurrence in coniferous (*Picea*) and mixed (*Abies, Fagus*) woods; it was always found under coniferous trees in Czechoslovakia, *Picea* occurs everywhere with one exception (*Abies*).

Distribution (Map 20). Collected (except for one collection from southwest Bohemia) in a few isolated localities in submountainous areas of the Carpathians. J. Kuthan's note on the label of the collection documented in PRM as *Hydnum balsamiolens* from Fačkov (north-western Slovakia), 2. VIII. 1970, adds: "It is interesting that it occurs on basic substrate, although in Raková (other locality, also north-western Slovakia) are zones with calcareous breccia. May be it is a calciphilous species."

List of recorded localities – Czech Republic:

Libějovické Svobodné Hory, forest on Holička hill, *Picea* wood, 550 m, VII. 1936, leg. J. Herink, det. Z. Pouzar (PRM)

Kateřinice near Vsetín, Dubcové kopce, 31. VII. 1944, leg. V. Pospíšil (Kubička 1971)

Slovakia:

Fačkov, valley of Rybná stream under Čierna skala, *Picea-Juniperus* wood, 2. VIII. 1970, leg. J. Kuthan, det. J. Kuthan et Z. Pouzar ut *Hydnum balsamiolens* (PRM); *Pinus-Picea* wood, 500 m, 16. VIII. 1973, leg. et det. J. Kuthan ut *Hydnum balsamiodorum* (BRA)

Sklenné Teplice, *Picea-Pinus-Abies* wood, 500 m, 15. VII. 1971, leg. et det. J. Kuthan ut *Hydnum balsamiodorum* (BRA)

Liptovský Ján, Jánska valley, under *Picea* in *Pinus-Picea* wood, 700 m, 14. VII. 1985, leg. et det. J. Kuthan (BRA)

Mt. Osobitá, slope, *Abies* wood, 1200 m, 6. VIII. 1977, leg. et det. J. Kuthan (BRA)

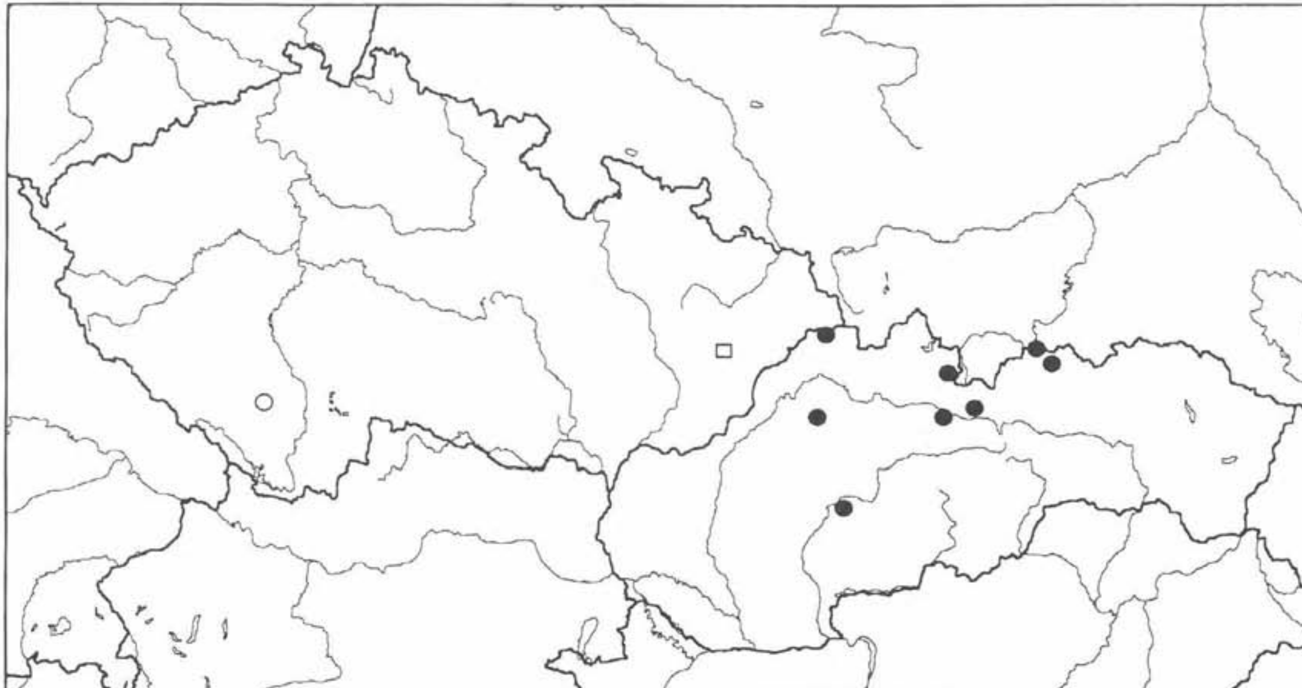
Červený Kláštor, *Picea-Abies* wood, 550 m, 17. VII. 1971, leg. et det. J. Kuthan ut *Hydnum balsamiodorum* (BRA)

Važecké lúky, north of the road Východná - Važec, *Pinus-Picea* wood, 850 m, 20. IX. 1970, leg. et det. J. Kuthan ut *Hydnum balsamiodorum*; 700 m, 17. IX. 1972, leg. et det. J. Kuthan (both BRA)

Raková, Korcháň valley, *Picea-Pinus-Abies* wood, 650 m, 15. VII. 1967, leg. J. Kuthan, det. Z. Pouzar ut *Hydnum balsamiolens* (PRM), 28. VII. 1974, leg. et det. J. Kuthan ut *Hydnum balsamiodorum; Picea* wood, 650 m, 14. VII. 1968, leg. et det. J. Kuthan ut *Hydnum balsamiodorum* (both BRA); *Picea-Abies-Fagus* wood, leg. J. Kuthan, det. Z. Pouzar ut *Hydnum balsamiodorum* (PRM)

Vyšné Ružbachy, *Picea-Pinus-Larix* wood, 650 m, 17. VII. 1971, leg. et det. J. Kuthan ut *Hydnum balsamiodorum* (BRA)

Map No. 20 - Sarcodon versipellis



Documented data:

○ - locality where the species was found before 1945

● - locality where the species was found after 1970

Data from literature:

□ - locality where the species was recorded before 1945

Sarcodon scabrosus (Fr.) P. Karst.

Pileus about 75 mm in width, soon breaking up into scales appressed on the margin, erect in the centre, red-brown, brown to black-brown, contrasting with the pale ground. Stipe fleshy-brown or concolorous with the scales of the pileus, turning to grey-, blue- or black-green, covered by whitish mycelium towards its base. Spines pale, slowly turning brown. Context whitish, grey-green in the base of the stipe, turning blue-green in KOH. Expulsion of liquid not observed. Clamp-connections absent. Spores with conspicuous prolonged angular warts, (5.4-)6.3–7.3 × (3.6-) 4–5 μm.

Related species. The scales of *S. glaucopus* are appressed also in the centre of the pileus (erect in *S. scabrosus*) and its spores have round warts. The stipe of *S. imbricatus* lacks the grey-green base and has a brown context; the taste of *S. imbricatus* is at most slightly bitterish (clearly acrid-bitter in *S. scabrosus*). The context of *S. joeides* and *S. fuligineo-violaceus* is pink or violet.

Occurrence. Relatively abundant species, showing a slight decline during the last decades.

Accompanying trees. The literature mentions both coniferous (mostly *Pinus*) and deciduous (*Fagaceae* – *Quercus, Castanea*) trees. There are collections from deciduous woods in our country too, but collections from coniferous woods dominate. The most frequent accompanying tree is *Pinus*, which occurs in 70 % of localities.

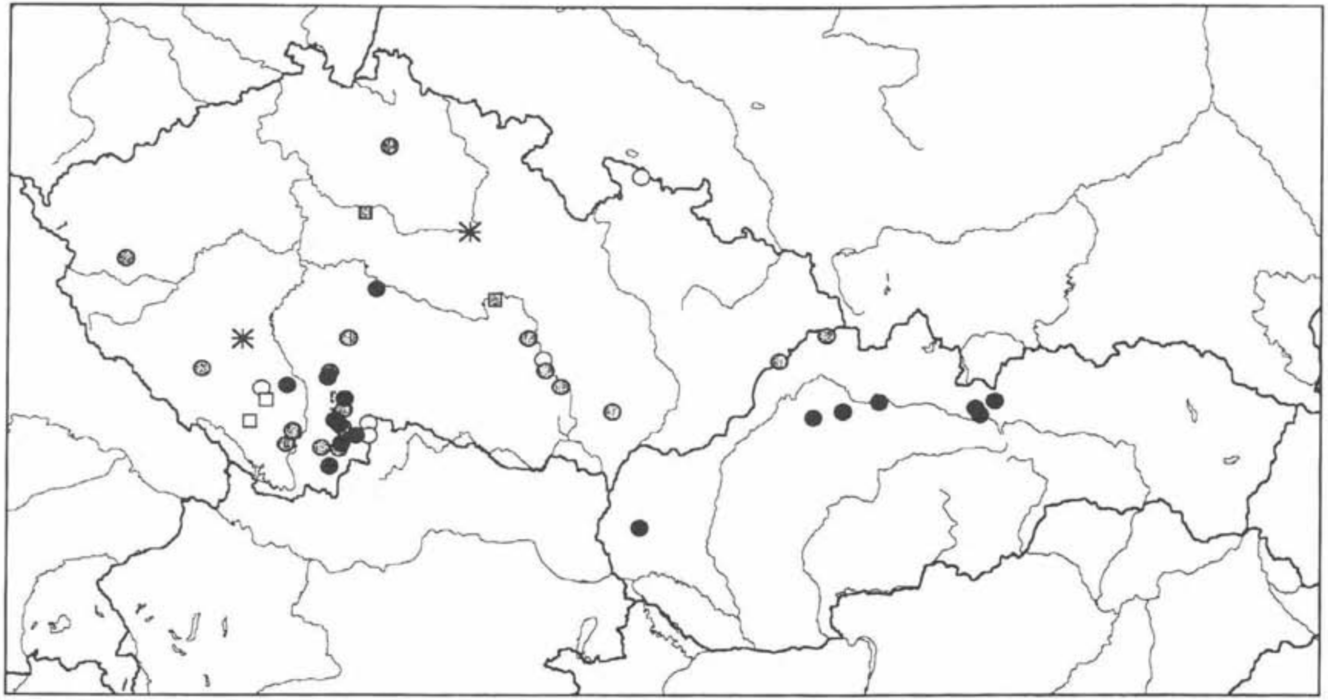
Distribution (Map 21). Abundant occurrence in southern Bohemia, where the species is still collected relatively often, in other regions are only isolated localities or groups of localities. The last record from western Bohemia is from 1966, from northern Bohemia 1952, and in Moravia it was recorded until 1970.

Sarcodon glaucopus Maas G. et Nannf.

Pileus about 50 mm in width, pale, yellowish to brown, here and there with greyish hue, areolate in the centre, scaly towards the margin (brown scales on a lighter ground), darker in the centre. Stipe brown in the upper part, grey-green in the lower part, base whitish. Spines whitish, later purple-brown. Context whitish, grey-green in the base of the stipe, turning blue-green in KOH. Yellowish dots of expulsed substance may appear on the pileus surface after drying. Clamp-connections absent. Spores with not very conspicuous rounded warts, (5-)5.4–5.8 (-6.3) × (3.6-)4–4.5 μm.

Related species. *S. scabrosus* has a grey-green stipe base too, but the surface of its pileus is darker brown (light brown in *S. glaucopus*) with conspicuously ascendent or erect scales in its centre. The only other species with a grey-green stipe base and whitish context is *S. fennicus*, but this has an ochraceous pileus without scales and its context does not change colour in KOH. The similarly

Map No. 21 - Sarcodon scabrosus



Documented data:

○ - locality where the species was found before 1945

◍ - locality where the species was found between 1945 and 1970

● - locality where the species was found after 1970

Data from literature:

□ - locality where the species was recorded before 1945

▣ - locality where the species was recorded between 1945 and 1970

Broadly defined localities:

✳ - locality where the species was found between 1945 and 1970

light coloured *S. leucopus* does not have a grey-green stipe base. The spores of *S. scabrosus* and *S. leucopus* have angular warts.

Occurrence. Rare species, not found in the Czech Republic for more than 20 years.

Accompanying trees. The literature mentions coniferous trees which is confirmed by our records. The number of records is, however, too small to make any detailed conclusions.

Distribution (Map 22). Rare occurrence in isolated localities.

List of recorded localities – Czech Republic:

Žebrák, *Pinus* wood, 16. VIII. 1953, leg. B. et F. Hřebíkovi, det. Z. Pouzar ut *S. fennicus*, rev. Z. Pouzar (PRM)

Čechtice, Dvorce forest, *Picea* wood, 15. IX. 1968, leg. V. Brambora, det. Z. Pouzar (PRM)

Veverská Bítýška, Hranečník forest along the road to Lažánky, *Picea* wood with *Abies* undergrowth, 2. IX. 1951, leg. K. Kříž, 28. IX. 1960, leg. F. Šmarda, both det. F. Šmarda ut *S. amarescens* (BRNM)

Zdravá Voda near Žarošice, *Pinus-Picea* wood, 27. VIII. 1949, leg. et det. V. Vacek (PRM)

Velká Losenice, 1912 (Holub 1926, ut *Hydnum amarescens*)

Slovakia:

Štrbské Pleso, Uhliščata, slope of Spálený vrch, *Picea* wood, 1000 m, 14. IX. 1989, leg. et det. J. Kuthan (BRA)

Raková, Korcháň valley, *Pinus-Picea-Abies* wood, 11. VIII. and 8. IX. 1968, leg. J. Kuthan, det. Z. Pouzar ut *S. amarescens; Picea* wood, 11. X. 1970, leg. et det. J. Kuthan ut *S. amarescens* (all BRA)

Sarcodon fennicus (P. Karst.) P. Karst.

Pileus about 50 mm in width, ochraceous, yellow-brown, without scales, fibrillose, or with darker scales on a pale ground. Stipe concolorous in upper part, grey-green in below, with whitish mycelium on its base. Spines whitish to purple-brown. Context whitish, grey-green in the base of the stipe, not changing colour in KOH. Expulsion of liquid not observed. Clamp-connections absent. Spores with not very conspicuous rounded warts, 6.3–7.6 × 4.5–5.2 μm.

Related species. *S. versipellis* is similarly orange-brown and almost scaleless, but this species does not have a grey-green stipe base. The grey-green stipe base is also characteristic of *S. glaucopus*, but its pileus is almost always areolate or (at least appressed) squamulose and its context turns green to blue-green in KOH.

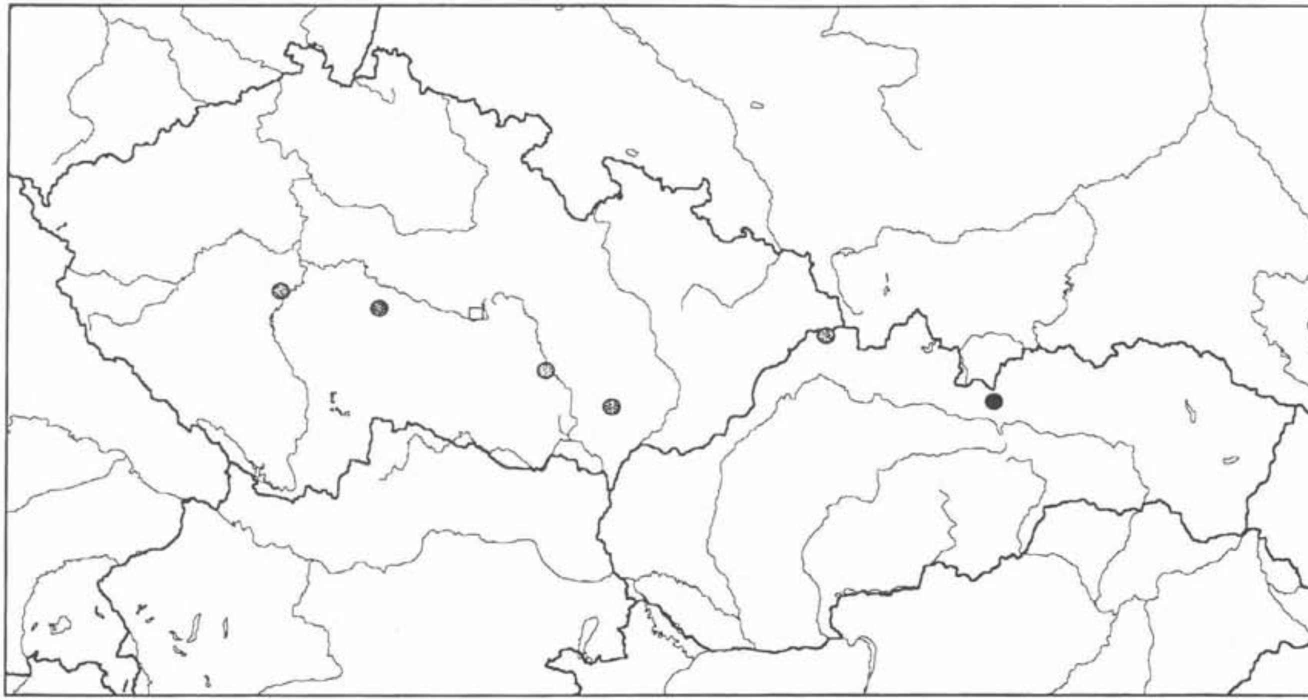
Occurrence. Very rare species.

Accompanying trees. The literature mentions coniferous trees, which is confirmed in the former Czechoslovakia.

Distribution (Map 23). Rare occurrence in isolated localities; the species is not known from Moravia and Slovakia.

List of recorded localities – Czech Republic:

Map No. 22 - Sarcodon glaucopus



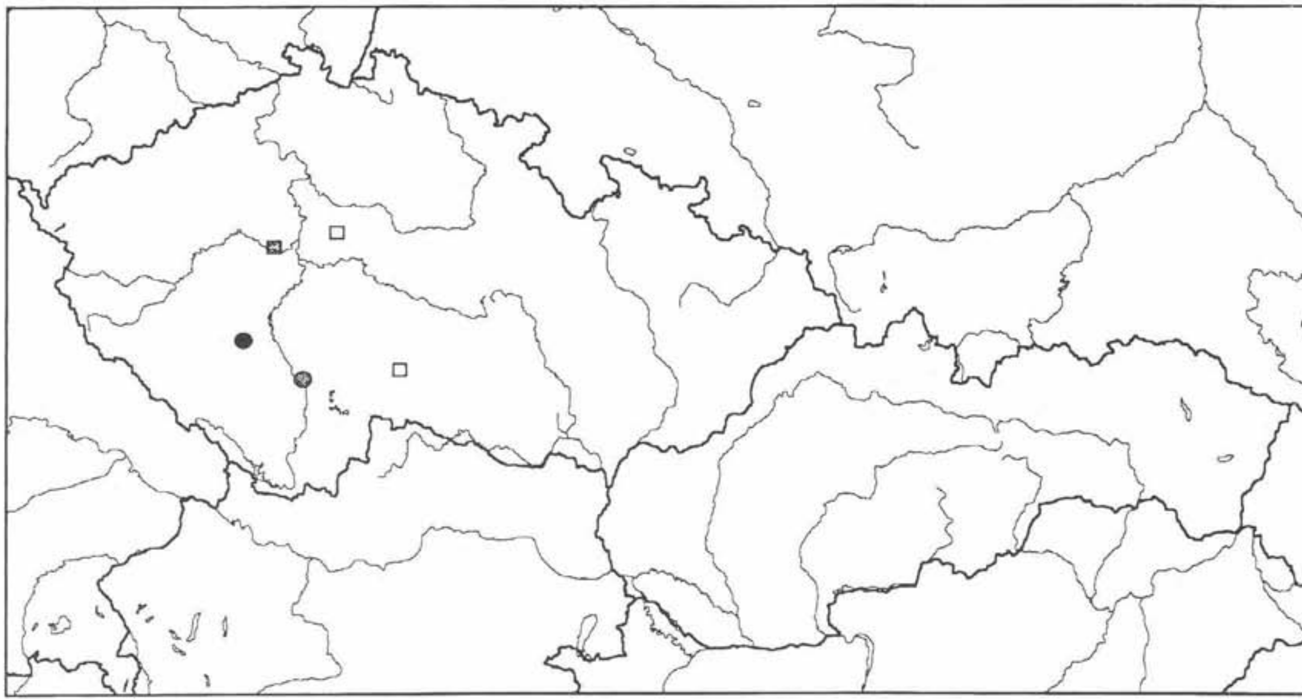
Documented data:

- ◍ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Data from literature:

- □ - locality where the species was recorded before 1945

Map No. 23 - Sarcodon fennicus



Documented data:

- ◍ - locality where the species was found between 1945 and 1970
- ● - locality where the species was found after 1970

Data from literature:

- □ - locality where the species was recorded before 1945
- ▣ - locality where the species was recorded between 1945 and 1970

Karlštejn, forest in the direction of Mořina, occurrence in the 1950s (verbal report of Z. Pouzar)
Buzice, hamlet Buzičky, Buzíček forest, *Picea* wood, 450 m, 5. VIII. 1974, leg. J. Herink, det. Z. Pouzar (Herb. Herink)
Týn nad Vltavou, Bedrník forest, *Picea* wood, 4. VII. 1965, leg. B. Karlasová, det. M. Svrček (PRM)
Babice, old *Pinus* wood, VIII. 1919, leg. O. Zvěřinová (Velenovský 1922)
Počátky, Válcha, 1929 (Sak 1930)

Sarcodon joeides (Pass.) Bataille

Pileus about 60 mm in width, sinuous, areolate or appressed scaly, pale brown to fleshy brown, more ochre after drying. Stipe concolorous with the pileus, sometimes grey-green at its base. Spines at first pale, then brown. Context at first pink, later violet in the pileus above the spines and in the stipe, grey in the base of the stipe, turning blue-green in KOH. Yellowish dots of expulsed substance may appear on the pileus surface after drying. Clamp-connections absent. Spores with conspicuous angular warts, 5.4–5.8 × 3.6–4.2 µm.

Related species. *S. joeides* is distinguished from all other species except *S. fuligineo-violaceus* by the pink or violet context, but the latter grows only in coniferous woods.

Accompanying trees. According to the literature deciduous trees, mostly *Quercus*, but also *Castanea* and *Fagus*.

Slovak find of *Sarcodon joeides* (Map 24): Malé Karpaty (western Slovakia), Vývrať near Kuchyňa, *Quercus-Fagus-Carpinus* wood, 8. VIII. 1972, leg. et det. A. Dermek ut *Hydnum commutatum* (Dermek 1973).

Sarcodon fuligineo-violaceus (Kalchbr. in Fr.) Pat.

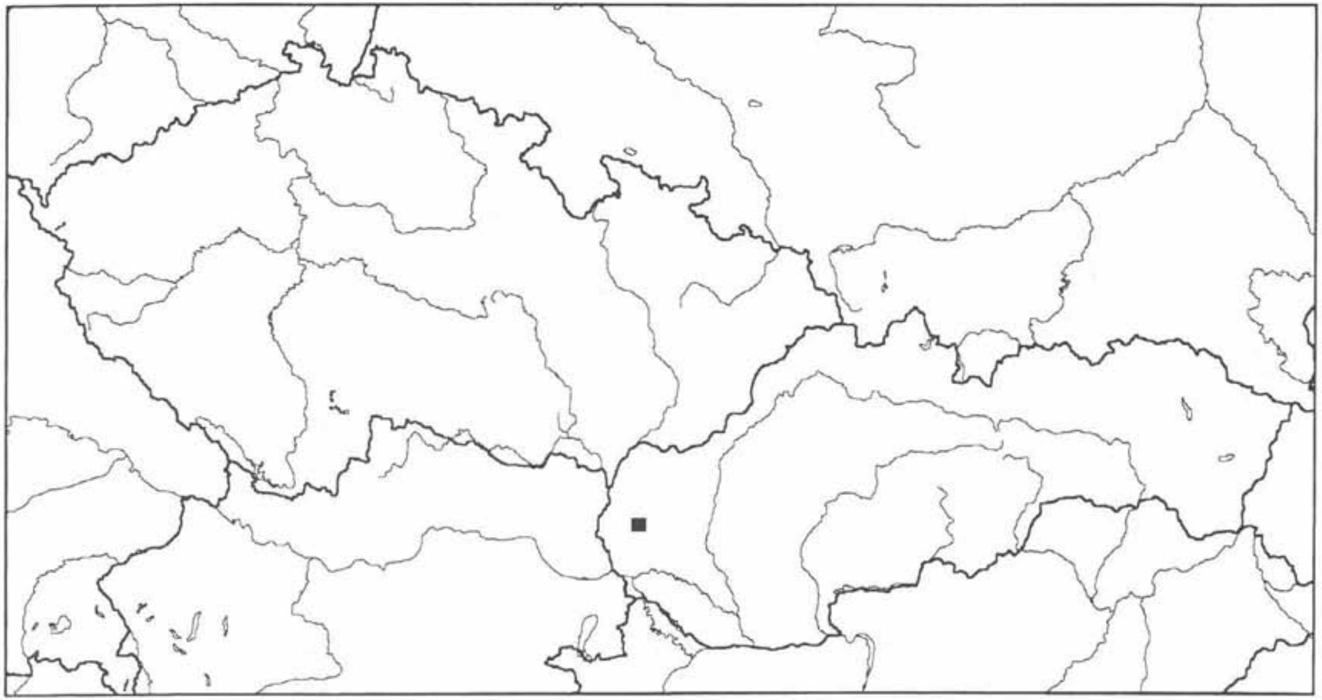
Pileus about 70 mm in width, red-brown to dark brown, sometimes with blackish hue, innately squamulose. Stipe concolorous with the pileus, paler when young. Spines brown. Context at first pink, later blue-grey-violet in the pileus, with red hue in the stipe and grey-green in its base, turning blue-green in KOH. Yellowish dots of expulsed substance may appear on the surface of the pileus after drying. Clamp-connections absent. Spores with more or less conspicuous acute warts, 5.4–6.5 × 4–4.7(-5.4) µm.

Related species. The pink or violet context distinguishes *S. fuligineo-violaceus* from all other species except *S. joeides*, which grows only in deciduous woods.

Accompanying trees. The literature mentions coniferous trees (*Abies, Picea, Pinus*).

Slovak find of *Sarcodon fuligineo-violaceus* (type locality, Map 25): Near Spišské Vlachy (eastern Slovakia), *Pinus sylvestris* [in pinetis Carpatorum ad Olaszi], IX. 1870, leg. et det. K. Kalchbrenner ut *Hydnum fuligineo-violaceum* (preserved in UPS; Maas Geesteranus 1960, 1975).

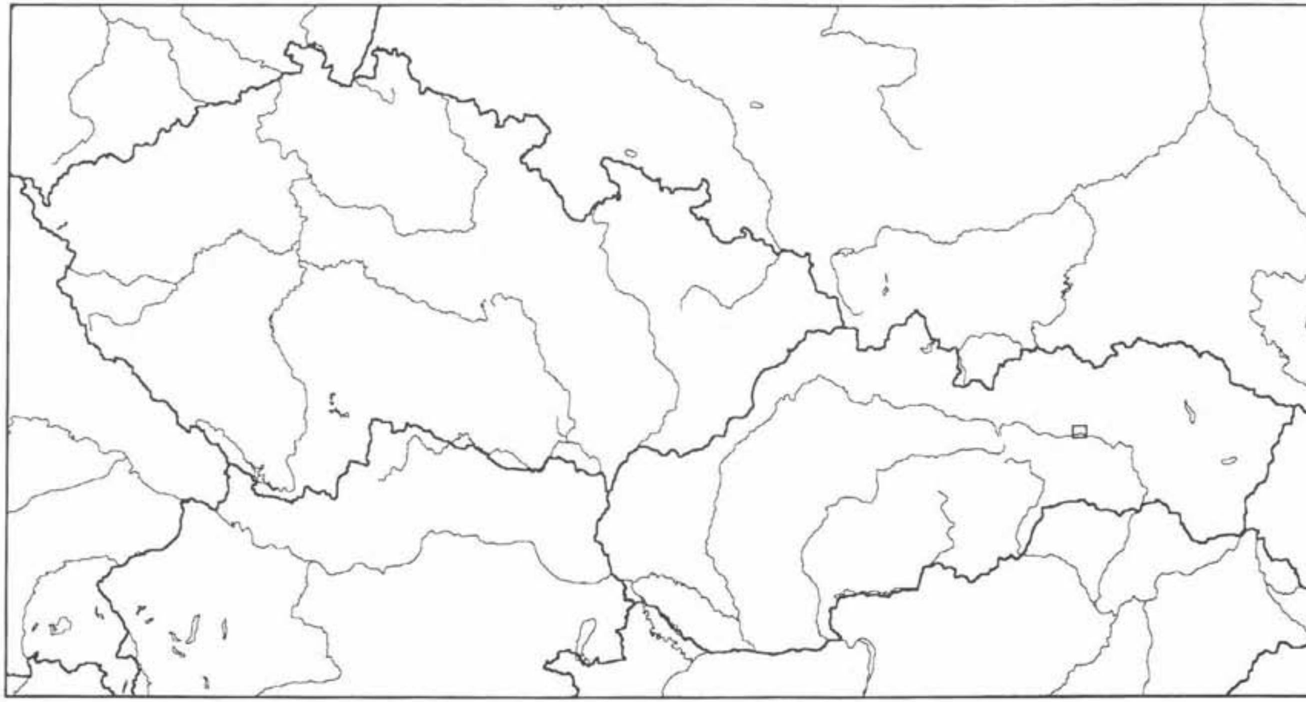
Map No. 24 - Sarcodon joeides



Data from literature:

■ - locality where the species was recorded after 1970

Map No. 25 - Sarcodon fuligineo-violaceus



Data from literature:

□ - locality where the species was recorded before 1945

Conclusion

Twenty-five species of hydnaceous fungi were recorded from the area of the Czech Republic and Slovakia. Their occurrence, ecology and distribution is commented and documented with distribution maps. A considerable decline in occurrence during the last decades was found for almost all species. Quantification of this decline is possible only in the Czech Republic, where a sufficient number of records from a longer period is available.

Acknowledgements

I wish to thank prom. biol. Z. Pouzar, CSc. for his consultancy, the revision of critical specimens and lending a lot of literature, the late doc. RNDr. V. Skalický, CSc. for consultancy especially on the concept of this paper and chorological problems, MUDr. J. Herink for lending specimens from his private herbarium and providing records about his collections and literature, RNDr. F. Kotlaba, CSc. for help with the identification of localities, lending literature and providing information about the recent occurrence of some species. I also thank PhDr. R. Fellner, CSc. for lending literature, Dr. M. Beran, E. Lippert, Ing. V. Pravda, P. Vampola and Ing. J. Valter for information about the recent occurrence of species or sending collections, I. Ostrý for making soil analyses and employees of the above mentioned herbaria for lending material for revision.

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CZECH MYCOL. 51 (2-3), 1999

Isolation of fungi from tomato rhizosphere and evaluation of the effect of some fungicides and biological agents on the production of cellulase enzymes by Nectria haematococca and Pythium ultimum var. ultimum

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Gherbawy Y. A. M. H. and Abdelzaher H. M. A. (1999): Isolation of fungi from tomato rhizosphere and evaluation of the effect of some fungicides and biological agents on the production of cellulase enzymes by Nectria haematococca and Pythium ultimum var. ultimum – Czech Mycol. 51: 157–170

Forty-five species and two species varieties belonging to twenty-six genera of fungi were isolated from 30 soil samples from the rhizosphere of tomato plants. The fungi most frequently isolated were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Gibberella fujikuroi*, *Nectria haematococca* and *Rhizopus stolonifer*.

Ridomil and Vitavax-captan (10, 50 and 100 ppm) had no significant effects on the activity of C_1 and C_x enzymes of *Nectria haematococca*. C_x enzyme activity was slightly increased at 10 and 50 ppm, but slightly decreased at 100 ppm. Vitavax-captan (10, 50 and 100 ppm) significantly decreased C_1 enzyme activities of *N. haematococca*. C_x enzyme activity was slightly increased at 10 and 50 ppm, but at 100 ppm it showed a slightly inhibitory effect. Ridomil caused a slight increase in the activity of C_x and C_1 enzymes by *Pythium ultimum* var. *ultimum* at low and moderate doses but the highest dose of Ridomil caused a slight reduction. Vitavax-captan slightly increased the activity of C_x and C_1 enzymes in *P. ultimum* var. *ultimum*.

Normal and sterilised filtrates of *Myrothecium verrucaria*, *Penicillium oxalicum* and *Trichoderma harzianum* induced a small decrease in C_1 enzyme activity of *Nectria haematococca*. The sterilised filtrates of the three fungi tested caused greater inhibition compared to the normal filtrate. The production of C_x enzyme was slightly increased with normal and sterilised filtrates of *Penicillium oxalicum* and *Trichoderma harzianum*, but was significantly increased by both types of filtrates of *Myrothecium verrucaria*. The two types of filtrate of all fungi tested did not significantly affect the activity of C_1 and C_x enzymes by *Pythium ultimum* var. *ultimum*.

Production of extracellular protein by *Nectria haematococca* was not significantly affected by any dose of the tested fungicides. It was slightly increased by the two types of filtrate of the three tested fungi but significantly increased by the normal filtrate of *Myrothecium verrucaria*. The normal filtrate of all the fungi tested enhanced extracellular protein production to a greater extent than the sterilised filtrate. Extracellular proteins of *Pythium ultimum* var. *ultimum* were slightly increased by all doses of Vitavax-captan and low doses only of Ridomil, also two types of filtrate of all tested fungi caused a slightly increasing effect.

Key words: Biological control, root-rot, fungicides, *Nectria haematococca*, *Pythium ultimum* var. *ultimum*.

Gherbawy Y. A. M. H. and Abdelzaher H. M. A. (1999): Izolace hub z rhizosféry rajčat a hodnocení účinku některých fungicidů a biologických agens na produkci celulázových enzymů druhů Nectria haematococca a Pythium ultimum var. ultimum. – Czech Mycol. 51: 157–170

Z rhizosféry rajčat bylo izolováno 45 druhů hub, z nichž se nejčastěji vyskytovaly druhy *Aspergilus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Gibberella fujikuroi*, *Nectria haematococca* a *Rhizopus stolonifer*. U druhů *Nectria haematococca* a *Pythium ultimum* var. *ultimum* byl hodnocen účinek fungicidů Ridomilu a Vitavaxu – captanu na aktivitu celulázových enzymů; u druhů *Nectria haematococca* nebyl zjištěn žádný významný účinek těchto fungicidů na produkci C_1 a C_x enzymů. Nižší dávky (10 a 50 ppm) spíše zvyšovaly aktivitu enzymů, vyšší dávky (100 ppm) měly spíš inhibiční efekt. U druhu *Pythium ultimum* var. *ultimum* pouze Vitavax způsoboval slabé zvýšení aktivity C_1 a C_x enzymů. Studiem účinku filtrátů hub *Myrothecium verrucaria, Penicillium oxalicum* a *Trichoderma harzianum* bylo zjištěno, že tyto způsobily zvýšení aktivity C_x enzymu a snížení aktivity C_1 enzymu druhu *Nectria haematococca*, ale neměly žádný významný účinek na aktivitu celulázových enzymů druhu *Pythium ultimum* var. *ultimum*.

INTRODUCTION

Root rots are an enormously diverse group of diseases caused mainly by soil-borne pathogens. Some *Fusarium* and *Pythium* species that are not vascular pathogens produce toxins and enzymes particularly those degrading cell walls (Endo and Colt 1974; Wood and Jellis 1984). In the root-rot of many plants cellulases play an important part in pathogenesis (Mehrotra 1980).

Direct penetration of susceptible hosts by phytopathogenic fungi has been regarded as a mechanical process. However, it has been stated (Strobel and Mathre 1970; Agrios 1978) that extracellular enzymes produced by pathogenic fungi may help in the softening and disintegration of the host's cell wall to facilitate the penetration of the pathogen. Such enzymes might play an effective role in resistance or susceptibility to the disease initiation (Ferraris and Matta 1977; Misaghi 1982; Yehia et al. 1992).

The effect of fungicides on certain microbial processes such as decomposition of cellulose has been reviewed by several authors (Abdel-Kader et al. 1989; Ismail et al. 1989; El-Zayat et al. 1991; Hemida et al. 1993). Two of the most important fungicides that are used in Egypt for controlling serious tomato pathogens are Vitavax-captan and Ridomil.

As far as we know, little research has been done to evaluate the influence of microbial biocontrollers on the production and activity of cellulase enzymes by pathogenic fungi. Therefore , this work was conducted to evaluate the effect of some fungicides (Vitavax-captan and Ridomil) and biocontrol agent (*Myrothecium verrucaria, Penicillium oxalicum* and *Trichoderma harzianum*) on the production of cellulase enzymes by the causal agents of tomato root-rot (*Pythium ultimum* var. *ultimum* and *Nectria haematococca*) at Qena Governorate (Egypt), as an initial step in understanding this chemical and biological control system mechanism.

Materials and methods

I – Soil mycoflora

1 – Estimation of rhizosphere fungi

This part was carried out to make isolation for some fungi that are known as biological agents and grow saprophytically on tomato field soil as well.

Samples of rhizosphere soil were collected from the root system of 30 healthy tomato plants grown at the Farm of South Valley University, Qena, Egypt during March, 1997. From these soil samples, fungi were isolated on potato-dextrose agar (PDA) by the dilution plate method as described by Abdel-Hafez et al. (1990a). There were five replicate plates for each sample and the developing fungi were identified by reference to standard texts. Average total count (ATC; calculated per g dry soil in all samples), number of cases of isolation (NCI; out of 30 cases), occurrence remarks (OR) and total count percentage (TC %) of various fungal genera and species recovered from 20 rhizosphere soil samples were calculated.

2 – Isolation of *Pythium* species from tomato plant rhizosphere

Two methods were employed for isolation of *Pythium* spp. from tomato rhizospheric soil samples as follows:

1 – Particles of soil were placed in Petri-dishes containing VP3 medium (Ali-Shtayeh 1986) for selective isolation of *Pythium* species. The emerging hyphal tips were transferred to water agar medium for further purification from bacterial contamination (Abdelzaher et al 1994).

2 – Autoclaved segments of *Zea mays* leaf blades and autoclaved cucumber seed were used as baits. Five grams of rhizosphere soil were placed in a Petri dish. Ten ml of sterilised distilled water (Willoughby 1956, with modifications and Abdelzaher et al. 1995) were added to enable the baits to float on the surface. After five days of incubation at 25 °C the baits were removed, washed thoroughly with sterilised distilled water and blotted dry with sterile filter paper. Four baits were then placed at the edge a of Petri dish containing VP3 medium for selective isolation of *Pythium* spp. The plates were incubated at 20 °C for three days or until colonies appeared. The emerging hyphal tips were transferred to water agar medium for further purification from bacterial contamination (Abdelzaher et al. 1994).

Identification of *Pythium* species isolated in this investigation

Principally the key of Plaats-Niterink (1981) and Dick (1990) were principally used for identification, and descriptions by Waterhouse (1967) and Middleton (1943) were consulted for comparison or confirmation of the identifications!

II – Pathological study

Fusarium solani (anamorph of *Nectria haematococca*) was found to be the most prevalent species among the other *Fusarium* species in soil samples. *Pythium ultimum* var. *ultimum* was the only *Pythium* species from the tested soil samples. Therefore they were subjected to further experimentation.

Pathogenicity test (root-rot test)

The pathogenicity of *Nectria haematococca* and *Pythium ultimum* var. *ultimum* were tested on tomato seeds (cultivar Maramande). *Nectria haematococca* was grown for 14 days and *Pythium ultimum* var. *ultimum* for 10 days in 500 ml Erlenmeyer flasks containing a sterile mixture of cornmeal and sand (30:70, v / v) to which 50 ml of distilled water was added. Soil (loamy clay) was sterilised at 20 psi for 2 hours and then aerated for at least 2 weeks at room temperature before 100 ml of inoculum was incorporated into 900 g of sterilised soil. Control soils were inoculated with the sand-cornmeal mixture free from the fungus. The seeds were surface sterilised using 0.1 % mercuric chloride for 2 minutes, rinsed several times with sterilised water and then planted in infested soils in 100 g plastic pots (three seeds per pot). Non infested controls were included in all tests. The pathogenicity of each *Nectria* and *Pythium* isolate on tomato roots was based on a root-rot index (RRI) from 0 to 4, where 0 = healthy roots, 1 = 1 – 10 %, 2 = 11 – 25 %, 3 = 26 – 50 % and 4 = more than 50 % necrosis of the root system. The soils were watered to saturation every 2 – 3 days. The experiments were carried out in a illuminated growth cabinet (Precision, U. S. A.) at 30 °C and 5,000 Lux.

III – Enzymatical studies

1 – Fungicides

The two different fungicides used in this work were Vitavax-captan 75 % wp (Vitavax; 5,6-dihydro-2-methyl-1,4-Oxathiin-3-carboxanilido Captan; N –[Trichloromethylthio]-4-cyclohexene-1,2-dicarboximide) produced by Uniroyal International Division of Uniroyal Inc. Amity Road Bethany, Connecticut 06525 U. S. A., and Ridomil MZ 72 WP (8 % methyl D,L-N-[2,6 dimethyl phenyl]-N-[2 methoxy-acetyl]-alaninate) produced by Novartis Limited, Basle, Switzerland.

2 – Biological agents

Myrothecium verrucaria, Penicillium oxalicum and *Trichoderma harzianum* were isolated from rhizosphere samples and maintained on potato dextrose agar

(PDA) plates. Aliquots of 50 ml CMC-based liquid media (g/L of the following $NaNO_3$ 2.0, $KHPO_4$ 1.0, $MgSO_4 . 7H_2O$ 0.5, KCl 0.5 and carboxymethylcellulose CMC 10.0) were dispensed into 250 ml conical flasks. Each flask was inoculated with an agar mycelial disc (10-mm diameter) of the tested mould obtained from 7 days old cultures grown on a solid basal media. Cultures in flasks were incubated at 28 °C for two weeks and filtered. The culture filtrate was sterilised with Zites's bacterial filter. Some of the sterilised filtrate was autoclaved for 15 minutes at 120 °C, while the rest was considered normal filtrate.

Effect of fungicides and biological agents on endo-and exo-1,4 β-D-glucanase production.

Aliquots of 30 ml CMC-based liquid medium were dispensed into 100 ml conical flasks. After cooling, the tested fungicides (10,50 and 100 ppm, active ingredient/kg) and biocontrol agents (fresh and autoclaved) were added to the sterilised liquid medium. Flasks without fungicides and biocontrol agents were used as control. They were all inoculated with 1 ml of spore suspension of *Nectria haematococca* or *Pythium ultimum* var. *ultimum* and incubated at 28 °C without shaking.

Mycelial dry weight

After two weeks mycelia were collected by filtration and placed in an oven at 70 °C until constant weight. The dry weight of each treatment was calculated and is expressed as mg/30 ml media.

Enzymatic activity

Cellulase (C_x-cellulase) activity was measured by incubating a mixture of 1 ml of the culture filtrate and 0.5 ml of 0.5 % CMC in 50 mM citrate buffer (pH 5.2) at 37 °C for 30 minutes (Mandels et al. 1976).

C_1-cellulase activity was also measured in terms of filter paper activity by incubating a mixture containing 50 mg strip (10×10 cm) of Whatman No. 1 filter paper, 1 ml of 50 mM citrate buffer (pH 5.2) and 1 ml of the culture filtrate at 37 °C for 30 minutes (Chandrashekar and Kaveriappa 1988). The reaction was terminated by boiling in a water bath for 5 minutes and reducing sugars were determined photometrically according to Nelson (1944). Results were calculated using a glucose standard curve.

Production of extracellular protein

Extracellular protein production was also assessed (Lowry et al. 1951).

RESULTS AND DISCUSSION

Rhizosphere fungi

Forty-five species and 2 species varieties belonging to 26 genera were isolated from the rhizosphere soil of tomato plants.

Aspergillus was the most common genus isolated (Table 1). It was recovered from all samples and amounted to 39.5 % of total fungi. Of the nine species isolated *Aspergillus flavus, A. fumigatus, A. niger* and *A. terreus* were the most prevalent. They were recovered from 50 – 80 % of the samples comprising 5.4 – 15.4 % of total fungi. The species of *Aspergillus* isolated during the study were previously encountered in various types of Egyptian soil as reported by Abdel-Hafez et al. (1990a,b,c, 1995) and Abdelzaher et al. (1997b).

Gibberella, Nectria and *Rhizopus* were also isolated with high frequencies of occurrence. They were recovered from 56.7 %, 60 % and 63.3 % of the samples comprising 3.9 %, 7.8 % and 9.9 % of total fungi, respectively. They were represented by *Gibberella fujikuroi, G. gordonia, G. intricans, Nectria haematococca* and *Rhizopus stolonifer*. Abdel-Hafez et al. (1995) isolated the three genera previously from sugarcane rhizosphere at Qena Governorate. They found the three genera constituting 16.7 %, 25 % and 41.7 % of the samples matching 1.3 %, 3.1 % and 0.9 % of total fungi. Abdelzaher et al. (1997b) also isolated the three previously genera from the rhizosphere of a maize plant in El – Minia Governorate (Egypt). They recovered these genera from 40 %, 55 % and 30 % of the samples matching 2.6 %, 7.8 % and 1.5 % of total fungi.

Cochliobolus spicifer, Curvularia ovoidea, Mucor hiemalis, Mycosphaerella tassiana and *Myrothecium verrucaria* were isolated in moderate frequency of occurrence. They represented 26.7 – 43.3 % of the samples matching 1.7 – 3.7 % of total fungi. Most of the above fungal species were previously isolated, but with different incidences, from the rhizosphere of several plants cultivated or growing in Egypt (Abu El-Souod et al. 1988; Abdel-Hafez et al. 1990 a,c, 1995; Abdelzaher et al. 1997a) or other parts of the world (Nagaraja 1990, 1991; Rajendra and Saxena 1991). The remaining genera and species were isolated rarely or in small quantities (Table 1).

Occurrence of *Pythium* species in rhizosphere soil of tomato

The results of *Pythium* isolation from the tomato rhizosphere indicated that *Pythium ultimum* var. *ultimum* was present in the tomato rhizosphere of the field studied. *P. ultimum* var. *ultimum* was isolated from Egypt as a causal agent of wheat damping – off (Abdelzaher et al. 1997b). It was previously mentioned from Egyptian soil (El-Helaly et al. 1972; Abdelzaher et al. 1997a).

Table 1 Average total count (ATC), number of cases of isolation (NCL), occurrence remarks (OR) and percentage of total count of various fungal genera and species recovered from 20 rhizosphere soil samples of tomato plants on PDA medium at 28 °C.

Genera & species	ATC	NCI & OR	TC %
Acremonium strictum	4400	5L	1.8
Alternaria	3200	7L	1.3
A. alternata	2600	5L	1.1
A. tenuissima	600	2R	0.3
Aspergillus	94200	30H	39.5
A. candidus	1800	3R	0.8
A. flavus	19800	21H	8.3
A. fumigatus	17600	15H	7.4
A. niger	36800	24H	15.4
A. ochraceus	1800	5L	0.8
A. sydowii	1200	4L	0.5
A. terreus	12800	17H	5.4
A. ustus	1400	4L	0.6
A. violaceus	1000	3R	0.4
Botryotrichum atrogriseum	600	2R	0.3
Chaetomium globosum	2400	4L	1.0
Cladosporium cladosporioides	800	2R	0.3
Cochliobolus	8400	12M	2.5
C. lunatus	3200	5L	1.3
C. spicifer	5200	9M	2.2
Cunninghamella echinulata	4200	7L	1.8
Curvularia ovoidea	8200	13M	3.4
Emericella	4600	6L	1.9
E. nidulans	2000	4L	0.8
E. nidulans var. *dentata*	1800	3R	0.8
E. nidulans var. *lata*	800	2R	0.3
Eurotium amstelodami	2000	2R	0.8
Fusarium oxysporum	7200	7L	3.0
Gibberella	9200	17H	3.9
G. fujikuroi	4400	11M	1.8
G. gordonia	1400	3R	0.6
G. intricans	3400	8M	1.4
Humicola grisea	2400	2R	1.0
Mucor	8800	8M	3.7
M. circinelloides	1200	2R	0.5
M. hiemalis	4000	8M	1.7
M. racemosus	3600	6L	1.5
Mycosphaerella tassiana	5400	10M	2.3
Myrothecium verrucaria	8800	8M	3.7
Nectria haematococca	18600	18H	7.8
Papulaspora immersa	1000	2R	0.4
Penicillium	11000	7L	4.6
P. aurantiogriseum	1200	4L	0.5
P. chrysogenum	4200	7L	1.8

Table 1 cont.

Genera & species	ATC	NCI & OR	TC %
P. funiculosum	2200	2R	0.9
P. oxalicum	1000	3R	0.4
P. puberulum	1200	4L	0.5
P. purpurogenum	1200	2R	0.5
Phoma glomerata	1400	3R	0.6
Rhizopus stolonifer	23600	19H	9.9
Setospheria rostrata	1600	4L	0.7
Stachybotrys chartarum	2000	4L	0.8
Trichoderma harzianum	1600	3R	0.7
Ulocladium chartarum	3200	7L	1.3
Gross total counts	238800		
Number of genera	26		
Number of species	45 + 2 Var.		

Occurrence remarks: H = high occurrence, 15 – 30 cases (out of 30); M = moderate occurrence, between 8 – 14 cases; L = low occurrence, 4 – 7 cases; R = rare occurrence, 1 – 3 cases.

Table 2 Root rot index (RRI) of tomatoes infected with *Nectria haematococca* and *Pythium ultimum* var. *ultimum* after 24 days of sowing in infested soil.

Fungi	No. of germinated seeds out of 30 seeds	No. of damped – off seedlings	No. of root rotted seedlings	RRI*
Nectria haematococca	30	5	25	4
Pythium ultimum var. *ultimum*	28	8	20	4

* Root-rot index results are attributed to tomato plants that escaped from the damping-off.

Pathogenicity test (root – rot test)

Nectria haematococca and *Pythium ultimum* var. *ultimum* isolated from the tomato rhizosphere were used in this study. Isolates of *Nectria haematococca* and *Pythium ultimum* var. *ultimum* were highly pathogenic to the root of tomato seedlings in greenhouse pathogenicity trials when soil was infested with the cormeal-sand inoculum (Table 2).

Khallil and Ammar (1994) reported that both normal and autoclaved culture filtrates of *Fusarium solani* caused upper yellowing and epinasty of tomato leaf cuttings (var. Marmande). The culture filtrate was less effective by either autoclaving or minimising concentration. Also, they reported that the total death due to *F. solani* application into the soil was 63.04 %. Such results make clear that the tested isolate of *F. solani* was highly pathogenic.

Pythium ultimum var. *ultimum* can become a severe parasite of many plants, it is a causal agent of damping-off and root-rots of many crops (Plaats–Niterink 1981).

Table 3 Effect of Ridomil and Vitavax-captan on the production of biomass, exo-(C_1) and endo-C_x β–1,4-glucanase and extracellular protein by *Nectria haematococca* and *Pythium ultimum* var. *ultimum*.

Organism	Fungicides	Doses (ppm)	Dry weight (mg / 30 ml media)	Cellulase production (mg reducing sugars / ml crude enzyme / 30 min / 30 ml media)		Extracellular protein (mg egg albumin protein / 30 ml media)
				C_1	C_x	
Nectria haematococca	Control		62.1	3.3	3.6	0.48
	Ridomil	10	80.3*	1.2	7.6	0.43
		50	88.6*	1.8	7.0	0.28
		100	49.2*	1.2	0.6	0.7*
	Vitavax-captan	10	48.3*	0.6*	7.6	0.4
		50	45.5*	0.2*	4.2	0.63
		100	47.9*	0.8*	3.0	0.65
Pythium ultimum var. *ultimum*	Control		32.6	0.13	0.18	0.19
	Ridomil	10	50.2*	0.16	0.26	0.28
		50	37.7*	0.29	0.08	0.17
		100	18.4*	0.06	0.06	0.11
	Vitavax-captan	10	23.5*	0.19	1.36	0.20
		50	18.5*	0.56	1.14	0.25
		100	10.8*	0.63	1.18	0.30

* Significantly different from the control at 5 % level. Each value is the average of three replicates.

Enzymatic activities

1 – Fungicides

The mycelial dry weight of *Nectria haematococca* and *Pythium ultimum* v. *ultimum* were significantly increased by low and moderate doses of Ridomil but at high doses the effect became inhibitory. On the contrary the dry weight of both fungal species were significantly decreased by all doses of Vitavix-captan (Table 3 and 4). Abdalla and Manci (1979) reported that 50 to 300 ppm of the herbicide Stomp reduces the mycelial dry weight of *Pythium* using liquid media.

On the other hand, the mycelial dry weight of *Nectria haematococca* and *Pythium ultimum* var. *ultimum* was significantly increased by the untreated (normal) filtrate of *Myrothecium verrucaria, Penicillium oxalicum* and *Trichoderma harzianum*. The autoclaved filtrate of the previous biological agents caused a slight decrease in the dry weight of both tested fungi, but the aufiltrate of *Trichoderma. harzianum* caused a slight increase (Table 3 and 4).

Table 3 reveals that the Ridomil effect on C_1 cellulase of the culture filtrate of *Nectria haematococca* was slightly inhibitory. Its activity against CMC was slightly promoting at low and moderate doses, but it was non-significantly inhibited at

Table 4 Effect of *Myrothecium verrucaria, Penicillium oxalicum* and *Trichoderma harzianum* filtrate on the production of biomass, exo-(C_1) and endo-(C_x) β-1,4-glucanase and extracellular protein by *Nectria haematococca* and *Pythium ultimum* var. *ultimum*

Organism	Biocontrol agents	Filtrate type	Dry weight (mg / 30 ml media)	Cellulase production (mg reducing sugars / ml crude enzyme / 30 min / 30 ml media)		Extracellular protein (mg egg albumin protein / 30 ml media)
				C_1	C_x	
Nectria haematococca	Control		62.1	3.3	3.6	0.48
	Myrothecium verrucaria	Normal	78.3*	2.3	8.1*	0.7*
		Sterilised	60.5	1.7	5.9	0.63
	Penicillium oxalicum	Normal	66.2	2.0	5.8	0.65
		Sterilised	60.1	1.5	4.6	0.55
	Trichoderma harzianum	Normal	83.2*	2.1	5.4	0.68
		Sterilised	64.5	2.0	4.2	0.5
Pythium ultimum var. *ultimum*	Control		32.6	0.13	0.18	0.19
	Myrothecium verrucaria	Normal	38.2*	0.49	0.82	0.28
		Sterilised	32.0	0.45	0.16	0.25
	Penicillium oxalicum	Normal	35.4	0.78	3.2*	0.27
		Sterilised	30.3	0.17	0.40*	0.22
	Trichoderma harzianum	Normal	43.5*	0.09	0.28	0.20
		Sterilised	36.7	0.63	0.24	0.26

* Significantly different from the control at 5 % level. Each value is the average of three replicates. Normal = Untreated

high doses. This results indicate that *Nectria haematococca* was able to tolerate the lower concentrations of Ridomil.

Table 3 shows that the Ridomil effect on C_1 production by *Pythium ultimum* var. *ultimum* was slightly promoting at low and moderate doses but was slightly inhibited at high doses. In the case of C_x it was slightly promoting at low doses only and slightly decreasing at both moderate and high doses. Adaptation of fungi to low doses of toxicants has been demonstrated by many investigators (El-Khadem et al. 1979; Kataria and Dodan 1982; Sayed et al. 1990).

Vitavax-captan caused a significant inhibition of C_1 enzyme activity of *Nectria haematococca* at the different experimental doses. It was also slightly promoting at its low and moderate doses with C_x enzyme production, but its high doses had a slightly inhibitory effect.

Vitavax-captan showed a slightly promotive effect on the production of C_1 and C_x enzymes by *Pythium ultimum* var. *ultimum* (Table 3).

These results were basically similar to those obtained by Gupta and Prasad (1969). They reported that fungicides may inhibit or reduce the production and activity of various enzymes from several fungi. Sporulation of the fusaria was reduced by 50 % by Vitavax at 50 ppm (Mathure et at. 1971). Lukens and Sisler (1958) and Owens and Novotny (1959) suggested that captan acts by inhibiting a number of enzymes in phosphorus metabolism, certain oxidases and dehydrogenases, carboxylase and coenzyme-A. Soil drenched with 25 ml of 0.2 % Vitavax suspension per 2000 g soil/pot significantly reduced the post-emergence damping-off of tomato seedlings caused by *Pythium aphandermatum* (Nene and Thapliyal 1979).

Hemida et al. (1993) found that Tilt induced a significant decreasing inhibitory effect on activities of C_x produced by *Fusarium solani*. On the other hand, they reported that the activity of C_x produced by the same fungus was significantly increased with doses of Primextra, but the insecticide Polytrin was the only pesticide to show a significant toxicity on the activity of C_1 produced by *F. solani*.

Production of extracellular proteins by *Nectria haematococca* was not significantly affected by the low or moderate doses of Ridomil, but the highest dose significantly increased their production. On the other hand, only the low doses of Vitavax-captan slightly decreased the production of extracellular protein, but the moderate and high doses slightly increased them (Table 3).

With regard to the production of extracellular protein by *Pythium ultimum* var. *ultimum* under the effect of Ridomil, it was found that production was inhibited at moderate and high doses but enhanced at low doses. On the other hand, Vitavax-captan showed a slightly promotive effect on the production of extracellular protein at all experimental doses (Table 3).

El-Abyad et al. (1988) reported that Prometryn at high doses (128 and 256 ppm) disturbed membrane permeability and increased the production of extracellular protein of two *Fusarium* spp. which cause wilt. Abdel-Basset et al. (1992) reported that the significant inhibitory effect of Selecron on the extracellular protein produced by *Fusarium solani* was confined to low doses after 12 and 16 days and to high doses after 8 – 20 days.

2 – Biological agents

Table 4 shows that C_1 cellulase of *Nectria haematococca* was inhibited by both types of filtrate (normal & sterilised) of all tested biological agents. The sterilized filtrate of all tested fungi showed strong inhibition in comparison to the normal filtrate. On the other hand the activity of the filtrate of tested fungi on C_x production was slightly promotive with normal and sterilised filtrates of *Penicillium oxalicum* and *Trichoderma harzianum*, but significantly promotive at both types of *Myrothecium verrucaria* filtrate.

Culture filtrates of *Penicillium oxalicum* induced a significant increase in C_x activity of *Pythium ultimum* var. *ultimum* (Table 4) but had no effect on C_1 activity. Culture filtrates of the other two fungi had no effect on C_x and C_1 activity.

Howell (1991), Ghisalberti and Sivasithamparan (1991), and Jensen and Wolffhechel (1995) reported that strains of *Trichoderma* and *Gliocladium* produce many different secondary metabolites. Some of these seem to play a key role in many interactions causing antibiosis and lysis of the pathogen. Bertagnolli et al. (1996) reported that a greater degree of inhibition of growth of the fungal pathogen *Rhizoctonia solani* was observed when culture filtrate from *Trichoderma harzianum* was added to the medium than when the culture filtrate from *Rhizoctonia solani* was added to *Trichoderma harzianum* cultures. Extracellular enzymes including β–1,3-glucanase, chitinase and cellulase (Cruz et al. 1993; Lorito et al. 1994; Harman et al. 1995) are effective in disrupting the mycelium of the pathogen.

The untreated filtrate of *Myrothecium verrucaria* had a significant effect on the production of extracellular proteins by *Nectria haematococca*, all other filtrates did not show a significant effect in comparison with control (Table 4).

The two types of filtrate of all tested fungi showed a not significant increasing effect on the production of extracellular protein by *Pythium ultimum* var. *ultimum* (Table 4).

Further studies concerning substances involved in the inhibition of cellulase enzyme activity by *Nectria haematococca* under the effect of the tested biological agents should be studied.

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Polycoccum minutulum (Dothideales, Ascomycetes), a new lichenicolous fungus on Trapelia placodioides

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Kocourková J. and Berger F. (1999): *Polycoccum minutulum* (Dothideales, Ascomycetes), a new lichenicolous fungus on *Trapelia placodioides* – Czech Mycol. 51: 171–177

A new lichenicolous fungus species, *Polycoccum minutulum* Kocourková et F. Berger is described from Central Europe. The combination of features of this species, such as discoloration of infected parts of the host, the formation of galls, the size of the halonate spores provided with verruculose epispore and also the host *Trapelia placodioides* Coppins et P. James, distinguishes it from other species of the genus *Polycoccum* Sauter ex Körb.

Key words: Lichenicolous fungi, Ascomycetes, Dothideales, *Polycoccum*, *Trapelia placodioides*, flora of Austria, flora of the Czech Republic.

Kocourková J. and Berger F. (1999): *Polycoccum minutulum* (Dothideales, Ascomycetes), nová lichenikolní houba na *Trapelia placodioides* – Czech Mycol. 51: 171–177

Nová lichenikolní houba *Polycoccum minutulum* Kocourková et F. Berger je popsána ze střední Evropy. Kombinace charakteristických znaků tohoto druhu jako jsou odbarvení napadených částí stélky, tvorba hálek, velikost halonátních spór s bradavičnatým episporem a hostitel *Trapelia placodioides* Coppins et P. James odlišuje tento druh od příbuzných druhů rodu *Polycoccum* Sauter ex Körb.

INTRODUCTION

In 1997 independently of each other both of us collected a lichenicolous fungus on *Trapelia placodioides*, which proved to be a still undescribed species of *Polycoccum*. The features of this new species are fitting well in the concept of the genus (Hawksworth and Diederich 1988). This species so far has not been found on other *Trapelia* species. It is supposed to be widespread but overlooked.

RESULTS AND DISCUSSION

Diagnosis

Polycoccum minutulum Kocourková et F. Berger sp. nov. Fig. 1–8.

Fungus lichenicolus, in thallo Trapeliae placodioides vigens, cecidia supra thallum crescentes, areolas lichenis decolorantes. Ascomata singularia (dispersa) vel plerumque congregata, primum in cecidia immersa, maturitate erumpentia, obpyriformia, ostiolata, atra, 90–130 μm alta et (60-) 70–120 μm lata, (singularia majora). Paries e cellulis pseudoparenchymaticis applanatis compositus, in regione ostioli incrassatus et atrofusce pigmentatus, 18–20 μm crassus, ad basim subfuscus, 5 μm crassus; e 3 stratis cellularum formatus. Pseudoparaphyses parce ramosae et anastomosantes, septatae, 1–1.5 μm latae. Asci fissitunicati, clavati, basim breviter stipitati, 8-spori, 50–62 × 14–17 μm magni; in solutione iodina (sec. Lugol) non caerulescentes. Ascosporae 1-septatae, ellipsoideae, in asco irregulariter distichae, olivaceofuscae usque fuscae, ad septum leniter constrictae, parce halonatae, (11-) 12–16 × 5–6 (-7) μm; cum episporo verruculoso. Pycnidia desunt.

Typus: Bohemia centr., regio protecta Křivoklátsko, distr. Rakovník, prope pag. Roztoky, in valle rivuli Klucná, in clivo lapidoso ad merid.– occid. versus, ad saxa rhyolitica, matrix: *Trapelia placodioides*, alt. 280 m s.m., MTB 5949, 31.8.1997, leg. P. Kocourek et J. Kocourková (Holotypus – PRM 842975, isotype herb. Berger 11830).

Description

Lichenicolous fungus forming discoloured galls on infected areoles of *Trapelia placodioides*.

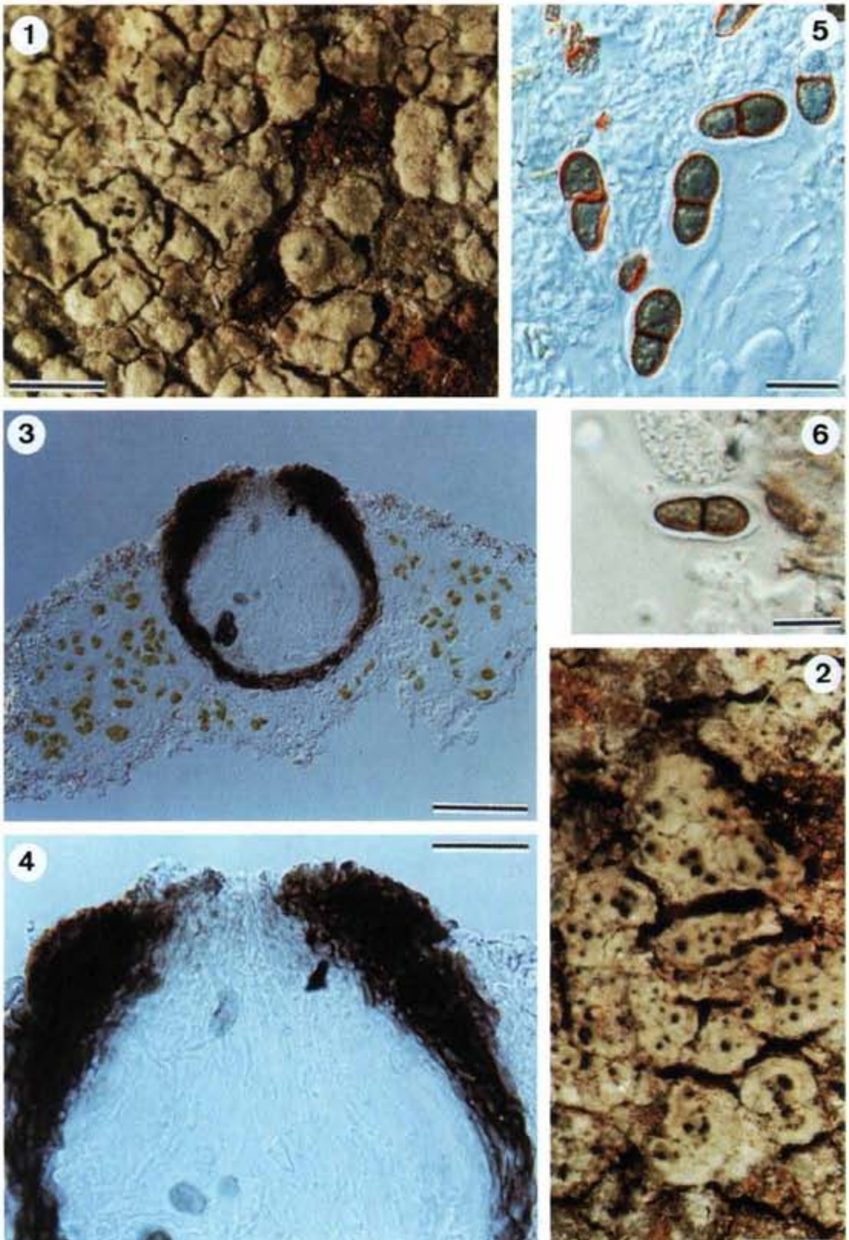
Ascomata arising singly or in groups of up to 20, usually about 5–10 in the infected areole, immersed to finally erumpent, at maturity only the upper part exposed, black, obpyriform, (60-) 70–120 μm wide, 90–130 μm tall; single ascomata always larger, ostiolum somewhat papillate. Wall composed of about 3 layers of flattened pseudoparenchymatous cells, pale olivaceous-brown below, 5 μm thick, around the ostiole expanded to 18–20 μm, dark brown.

Hamathecium of sparsely branched and anastomosing pseudoparaphyses, 1–1.5 μm wide, centrum I-, KI-, without periphyses.

Asci elongate clavate, short-stalked, 8-spored, 50–65 × 14–17 μm, thick-walled, with fissitunicate discharge, KI– apical dome, content of asci KI+ orange.

Ascospores irregularly to distichously arranged in the asci, 1-septate, ellipsoid, slightly constricted at the septum, with cells of somewhat different size, rounded at apices, olivaceous to dark brown at maturity, (12-) 13–16 (-17) × 5–6 (-7) μm; wall of ascospores composed of several layers, epispore coarsely verruculose, perispore developed as a gelatinous sheath usually 0.5–1.5 μm wide. Conidiomata not observed.

Etymology: The specific epithet "minutulum" is derived from the character of the ascomata, which are those of the most minute in the genus *Polycoccum*.



Figs 1–6. *Polycoccum minutulum* Kocourková et F. Berger (Holotype).
1–2. Habitus of infected host thallus of *Trapelia placodioides* with immersed ascomata. Scale = 0,5 mm. – **3.** Vertical section of ascoma immersed in host thallus. Scale = 50 μm. – **4.** Detail of ascoma showing structure of the ascomatal wall, ostiolum and hamathecium formed of paraphysoids. Scale = 20 μm. – **5.** Halonate ascospores with verruculose epispore. Scale = 10 μm. – **6.** Ascospore with extremely wide gelatinous sheath. Scale = 10 μm. (All preparations in water.)

Host and distribution

The only host lichen so far known is *Trapelia placodioides*.

This fungus was found in several localities in Central Europe (Austria, Czech Republic). It is restricted to areas with siliceous rocks, where *Trapelia placodioides* is abundant. Once recognized, we have been able to collect it in further 6 localities all together without great effort.

Observations

There is an interesting, not yet fully understood difference in the formation of galls between Czech and Austrian material. In the Bohemian and Moravian material white sphaerical galls can be seen without a lens on the grey thalli, giving them a powdered appearance. In the Austrian specimens the galls are very flat and inconspicuous also under a dissecting microscope. The anatomical features of both populations are identical. Generally the fungus seems to be a very aggressive commensalist, already colonizing thalli with a diameter of only 3 mm. In an early stage of development only a hue of white pruina can be seen on the infected, not yet discoloured thallus. Heavy infection causes a nodose appearance and a white discoloration of the host. This is not a bleaching process but is caused by confluence of pruinose areas. According to our observations no other damage of the host thallus occurs. On fertile specimens of *Trapelia placodioides*, found in two of the Austrian localities, *P. minutulum* has not been observed.

The host lichen has been recognized and described rather recently (Coppins and James 1984). Before obviously no one had paid much attention to searching lichenicolous fungi on this widespread, easily recognizable, mostly sterile and unattractive lichen. So we were able to find only two references in literature [*Pyrenidium actinellum* Nyl. (published by Hafellner and Mauer 1994: 129) and *Bispora lichenum* Diederich (Hawksworth 1994: 337)].

Discussion

The combination of features of *P. minutulum* such as the discolouration of infected parts of the host, the formation of galls, the size of the halonate spores with a verruculose epispore and also the host *Trapelia placodioides* Coppins et P. James, distinguishes it from other species of the genus *Polycoccum* Sauter ex Körb.

The anatomical features of *Polycoccum minutulum* well agree with those of the type species *P. trypethelioides* (Th. Fr.) R. Sant. *Polycoccum* is a genus with species characteristic in having a narrow host spectrum. Most of them, with the exception of *P. cladoniae* Diederich et D. Hawksw., *P. innatum* (Müll.

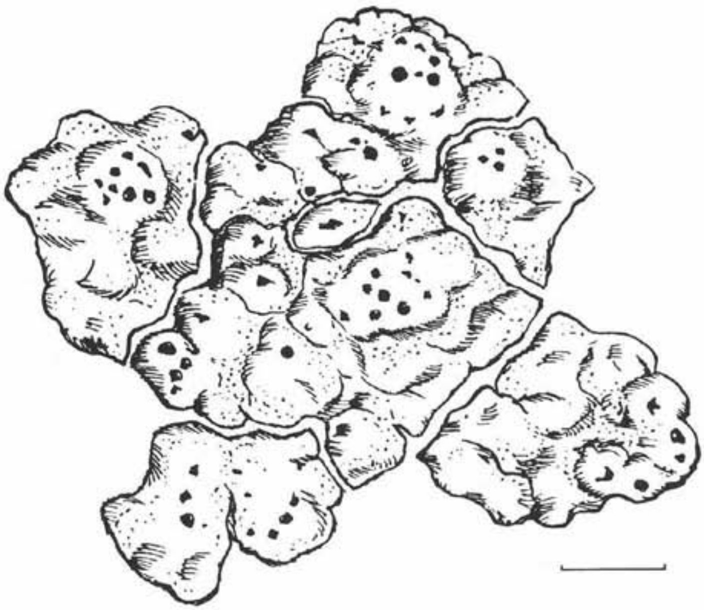


Fig. 7. *Polycoccum minutulum* Kocourková et F. Berger (Holotype). Detail of infected areoles of *Trapelia placodioides* enclosing ascomata in galls. Scale = 0,5 mm. Drawing by P. Kocourek.

Arg.) D. Hawksw., *P. montis-wilhelmii* Diederich, *P. peltigerae* (Fuckel) Vězda, *P. slaptoniense* D. Hawksw., *P. superficiale* D. Hawksw. et Miadl., *P. trypethelioides, P. umbilicariae* (Lindsay) D. Hawksw. and *P. vermicularium* (Lindsay) D. Hawksw., grow on saxicolous crustose lichens, just as the new species.

Among the 31 currently accepted species of *Polycoccum* 9 species are regularly causing the formation of galls on their hosts: *P. innatum, P. jamesii* D. Hawksw., *P. kerneri* Steiner, *P. peltigerae*, *P. pulvinatum* (Eitner) R. Sant., *P. serusiauxii* Matzer, *P. slaptoniense*, *P. trypethelioides* and *P. umbilicariae*. Also *P. sporastatiae* (Anzi) Arnold occasionally causes the formation of them. A verruculose spore ornamention is known in *P. bryonthae* (Arnold) Vězda, *P. cladoniae, P. evae* Calatayud et Rico, *P. marmoratum* (Kremp.) D. Hawksw., *P. microsticticum* (Leight. ex Mudd) Arnold, *P. montis-wilhelmi, P. rugulosarium* (Lindsay) D. Hawksw., *P. slaptoniense, P. trypethelioides* and *P. vermicularium*. The size of the ascospores is very similar to that of *P. rugulosarium* known from the Antarctic region and Tasmania only (Diederich and Hawksworth 1988: 304) and of *Polycoccum montis-wilhelmii* described from Papua New Guinea (Aproot et al. 1997: 141). Regarding the length, width and also the L/W ratio of the spore dimensions, none of the corresponding species can be identical with *P. minutulum*. *P. bryonthae* and *P. peltigerae* have monostichously arranged spores. *P. rugulosarium*, growing on several closely related species of *Caloplaca* in the southern hemisphere, does not form galls. *P. umbilicariae* has equal-sized but smooth ascospores and grows only on *Lasallia pustulata*.

Additional specimens:

(all collections quoted are from *Trapelia placodioides*; J. K. = J. Kocourková, P. K. = P. Kocourek, F. B. = F. Berger)

Austria: Upper Austria, Donautal, Schlögener Schlinge, Steiner Fels, on granite rocks near the ground in *Cytiso-Pinetum*, 480 m, MTB 7549; 3.XII.1997, coll. F. B., Be 11754. - Donautal, Freizell, on granite rocks near the ground in *Cytiso-Quercetum*, 440 m, MTB 7549; 29.X.1997, coll. F. B., Be 11673. - Bezirk Grieskirchen, Natternbach, Leitenbachtal, SW exposed rock stream, 420 m, on granite boulders, 26.X.1997, coll. F. B., Be 11650.

Czech Republic: Central Bohemia, Distr. Rakovník, Protected Landscape Area Křivoklátsko, near the village of Roztoky and the settlement of Višňová, on rhyolite rock near road by the river Berounka, 250 m, MTB 5949; 24.IX.1997, coll. J. K. and P. K. (PRM 891427). - Distr. Rakovník, Protected Landscape Area Křivoklátsko, nature reserve Stříbrný luh, on W slope in mixed forest, on shale rocks, 280 m, MTB 5949; 10.I.1998, coll. J. K. and P. K. (PRM 758281). - South-western Moravia, Distr. Třebíč, near confluence of the rivers Chvojnice and Oslava, below the castle Ketkovický hrad, on NE exposed slope with boulder scree, on granulite boulder, 360 m, MTB 6863; 5.X.1998, coll. J. K. (PRM 892553).

Ecology and associated lichens

The Austrian material has been found on siliceous stones on the ground in broadleaved forests at colline and submontane elevations. Associated species in the Austrian localities are: *Rhizocarpon obscuratum, Trapelia obtegens, Porpidia soredizodes, Scoliciosporum umbrinum, Lepraria caesioalba* and *Acarospora fuscata*.

The type locality, Klucná valley in the Czech Republic, is situated in a rhyolite boulder field (*Aceri-Carpinetum*). *Porpidia tuberculosa, Rhizocarpon obscuratum, Trapelia obtegens, Lepraria* sp., *Lecanora polytropa, Scoliciosporum umbrinum, Rhizocarpon lavatum, Rhizocarpon oederi, Micarea sylvicola, Amandinea punctata, Psilolechia lucida* and *Baeomyces rufus* were found in close vicinity of the infected thalli (species are listed according to frequency). *Rhizocarpon lecanorinum, Porpidia crustulata, Lecanora soralifera, Miriquiridica leucophaea, Acarospora fuscata, Ochrolechia lactea* and *Diploschistes scruposus* were growing on adjacent boulders. *Trapelia obtegens, Placynthiella icmalea* and *Melanelia verruculifera* were associated with infected thalli of *Trapelia placodioides* on the

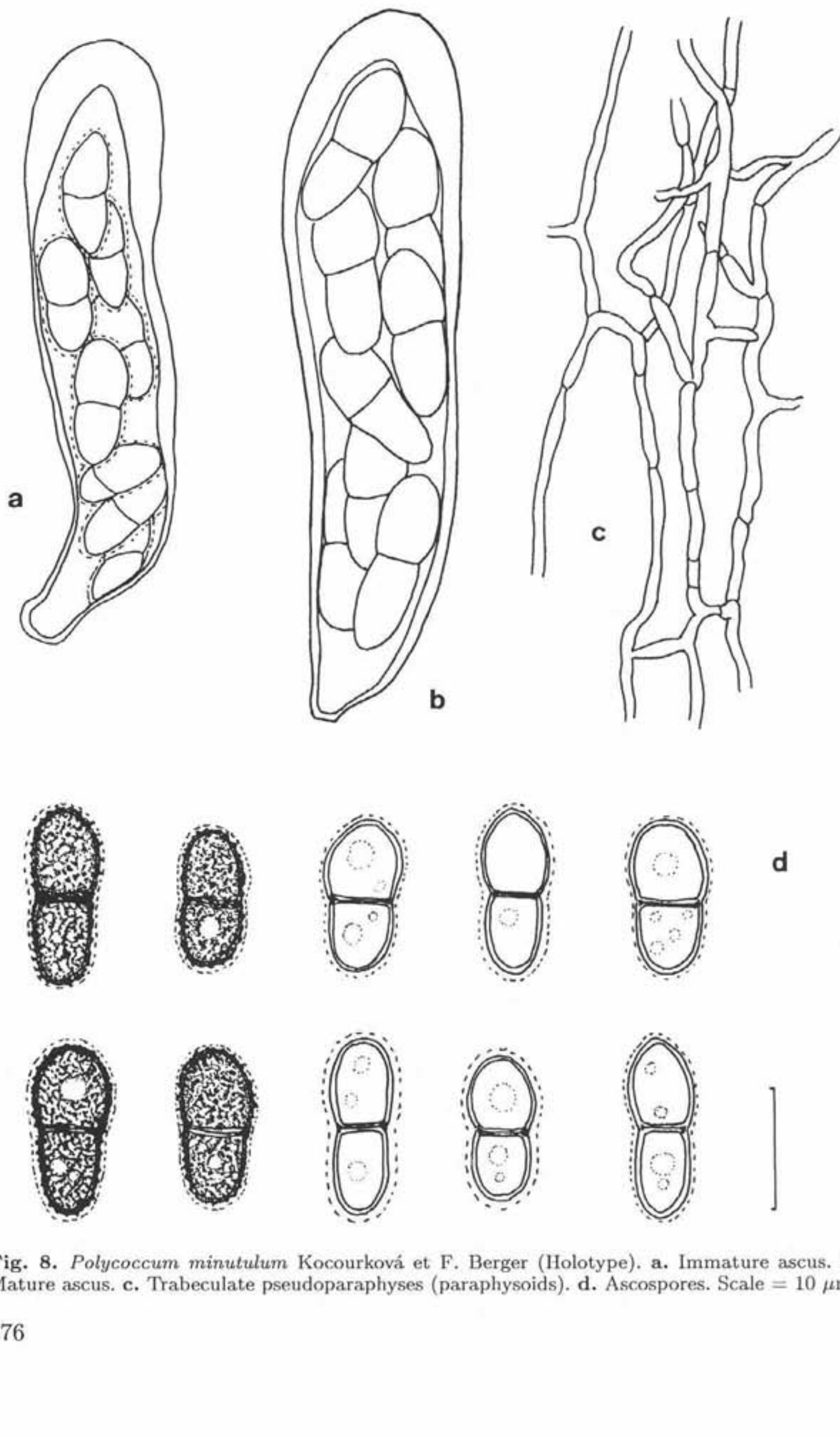


Fig. 8. *Polycoccum minutulum* Kocourková et F. Berger (Holotype). **a.** Immature ascus. **b.** Mature ascus. **c.** Trabeculate pseudoparaphyses (paraphysoids). **d.** Ascospores. Scale = 10 μm.

vertical wall of a rhyolite rock near the ground in the locality Višňová (*Sorbo-Quercetum*). *Rhizocarpon obscuratum, Melanelia verruculifera, Scoliciosporum umbrinum* and *Porpidia soredizodes* were found in the locality Stříbrný luh on small rocks of metamorphic shale along a forest path (*Sorbo-Quercetum*). All Czech and Moravian localities are situated on slopes of foggy river valleys or the cold and wet brook valleys (type locality) in an area otherweise rich in thermophytic flora with the occurrence of some montane elements.

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CZECH MYCOL. 51 (2–3), 1999

Lichenicolous fungi from the Czech Republic. 1. Weddellomyces xanthoparmeliae Calatayud et Nav.-Ros.

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Kocourková J. (1999): Lichenicolous fungi from the Czech Republic. 1. Weddellomyces xanthoparmeliae Calatayud et Nav.-Ros.– Czech Mycol. 51: 179–184

The parasitic lichenicolous fungus *Weddellomyces xanthoparmeliae* Calatayud et Nav.-Ros., recently described from *Xanthoparmelia* cf. *protomatra* (Calatayud et Navarro-Rosinés 1998) is now reported also from central Europe from the Czech Republic. It has been collected in several localities on *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale and *X. somloensis* (Gyelnik) Hale. *Weddellomyces xanthoparmeliae* occurs in great quantity in all the Czech and Moravian localities and therefore it seems possible to find it without difficulties also in other warm areas in situations with rather xerothermic habitats where the hosts are commonly present.

Key words: Dothideales, Dacampiaceae, *Weddellomyces xanthoparmeliae* Calatayud et Nav.-Ros., lichenicolous fungi, taxonomy, *Xanthoparmelia*.

Kocourková J. (1998): Lichenikolní houby České republiky. 1. *Weddellomyces xanthoparmeliae* Calatayud et Nav.-Ros.– Czech Mycol. 51: 179–184

Parazitická lichenikolní houba *Weddellomyces xanthoparmeliae* Calatayud et Nav.-Ros. (Ascomycetes, Dothideales) nedávno popsaná z *Xanthoparmelia* cf. *protomatra* (Calatayud et Navarro-Rosinés 1998) je nyní známa také ze střední Evropy z České republiky. Na českých a moravských lokalitách se vyskytuje na *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale a *X. somloensis* (Gyelnik) Hale vždy v hojném množství. Je pravděpodobné, že bez větších obtíží bude nalezena i v jiných teplých oblastech, především na lokalitách s poněkud xerothermním rázem, kde jsou oba hostitelské lišejníky rozšířené.

INTRODUCTION

Nine species are currently known in the genus *Weddellomyces* D. Hawksw. Navarro-Rosinés and Roux (1995, 1997), who described four species of them as new to science delimited this genus, originally established by Hawksworth (1986) as monotypic for *Weddellomyces epicallopisma* (Weddell) D. Hawksw., only for those species which are characterized above all in having cephalothecioid plates on the upper part of the ostiolate ascomata, (4-)6–8-spored bitunicate asci, a hamathecium of pseudoparaphyses with lacking periphysoids and transversely septate brown spores fitted usually with distinct pores at the septa. Until the description of *W. xanthoparmeliae* (Calatayud and Nav.-Ros. 1998) all the species have been found only on crustaceous or crustaceous placodioid lichens of the genera *Caloplaca, Aspicilia* and *Protoblastenia* on calcareous substrates.

Material and Methods

Macroscopic features were examined with a MST 131 stereomicroscope (up to 48x). Microscopic characters were studied in squash preparations and hand cut sections in water, lactophenol Cotton Blue, 10 % KOH, Lugol's iodine solution and BCr under an Olympus BX-50 microscope (up to 1000x) fitted with Nomarski differential interference contrast. Macrophotographs and microphotographs were taken with an Olympus PM 10 and Fuji 200 ASA film; macrophotographs on an Olympus SZH 10 stereomicroscope and microphotographs on an Olympus BX-50 microscope.

Weddellomyces xanthoparmeliae Calatyud et Nav.-Ros. Figs. 1–10

Description

Mycelium immersed in host thallus, pale yellow-brown, but hyphae emerging from ascomata red-brown, 3–4 μm thick.

Ascomata perithecioid, arising singly or in small groups from the thallus and the thalline margin of the apothecia of the host, at first immersed, later lifting the cortical layer, splitting it and making the upper part of the ascoma becoming reflexed as black stars, subglobose, 150–230 μm diam., ostiolate, ostiolum 15–20 μm wide in transversal section. Ascomatal wall formed of blocks of cephalothecioid plates clearly visible in surface view but only at low magnification (200×) under a light microscope in the upper half of the ascoma only. Peridium in vertical section dark red-brown, 25–30 μm thick, composed of 5–8 layers of compressed pseudoparenchymatous cells with lumina 8–12 × 2–5 μm, below ostiolum only around 20 μm thick and composed of subglobose or irregularly shaped cells with lumina 4–6 × 2–4 μm. Pigment of the cells finely granulose when observed in KOH solution.

Hamathecium of two different types of interascal anastomosed and ramificated filaments, 1–1.5 and 3–4 μm thick respectively, I+ yellow. Periphyses s.l. lacking. Only these thick and short-celled filaments were seen to occur in primordia of ascomata without asci. Like Navarro-Rosinés and Roux (1995) in a related species. I also was unhappy to be unsuccessful in finding of the origin of these filaments generally seen as attached to the base and to the upper part of the ascomatal cavity only finally observed free in the upper part. Later with developing asci also the other thin and long-celled filaments becoming visible. These are often growing irregularly through the ascomatal cavity up into the ostiolum and resemble paraphysoids in the sense of Hawksworth et al. (1995). They were found in ascomata in both fresh and dried material in contrast to the observations by Navarro-Rosinés et Roux (1995), who found them only in dried herbarium material of a related species.

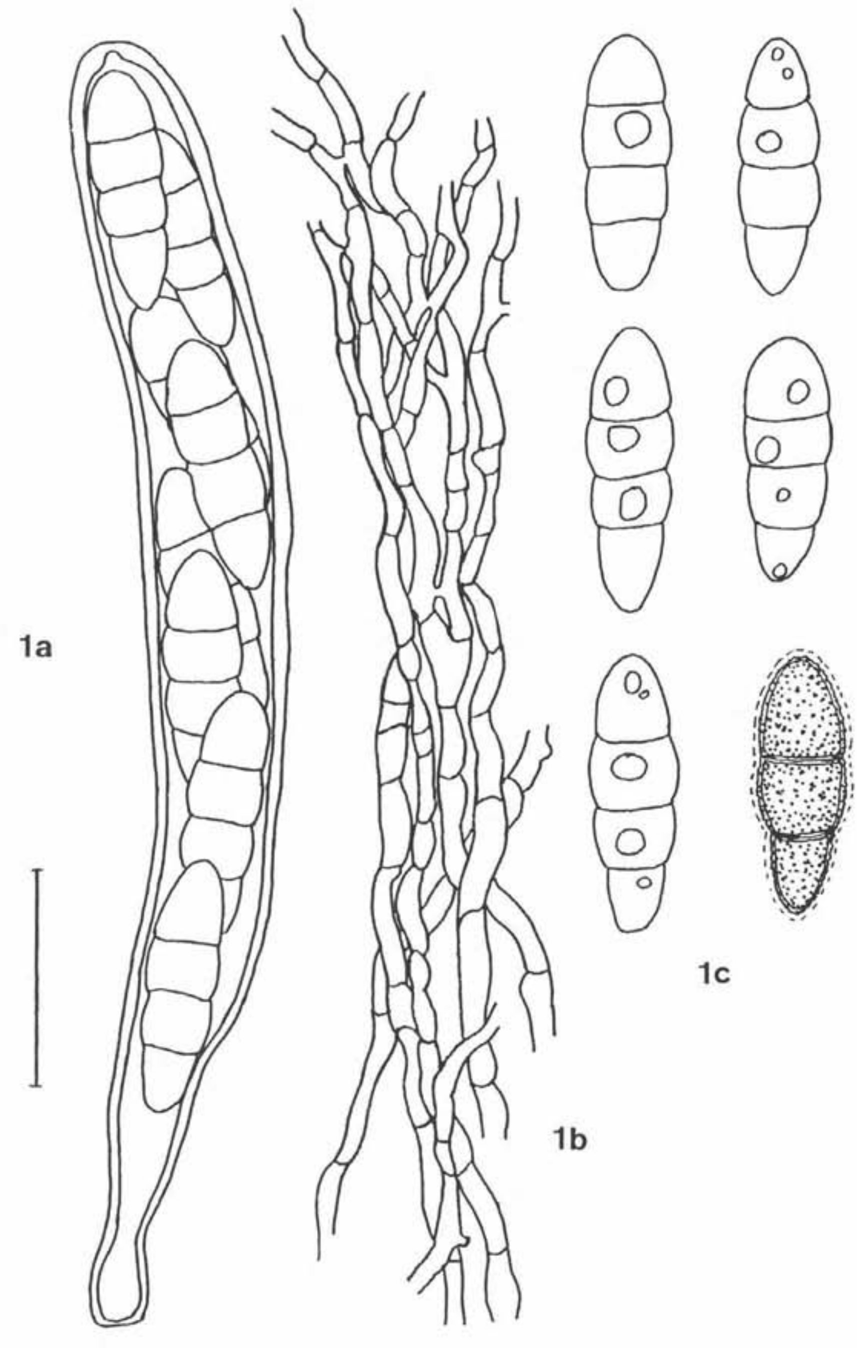


Fig. 1. *Weddellomyces xanthoparmeliae* **a.** Ascus. **b.** Thick short-celled interascal filaments. **c.** Ascospores (Scale = 20 μm.)

Asci subcylindrical to elongate-clavate, (4-)6–8-spored, 100–140 × 14–18 μm, with truncate base, I+ orange.

Ascospores irregularly to distichously arranged in the asci, ellipsoid, tending to taper towards the base, with the upper end rounded and lower end attenuated, with developed outer perispore as distinct gelatinous sheath 0.5–1.5 μm, (1-)3-septate, slightly constricted at the septa, central pore in the septa almost invisible, gold brown, verruculose, guttulate, 23–29 × 8–9(-10) μm, inner perispore I+ and BCr+ blue.

Observations

In contrast to our observations Calatayud and Nav.-Ros. (1998) did not observe any positive reaction of inner perispore with I and BCr, which was present in our specimens when the reagents were several times readded.

According to Calatayud and Navarro-Rosinés this species is most simmilar to *W. erythrocarpae* by a very fine ornamentation of inner perispore and prominent torus of young spores.

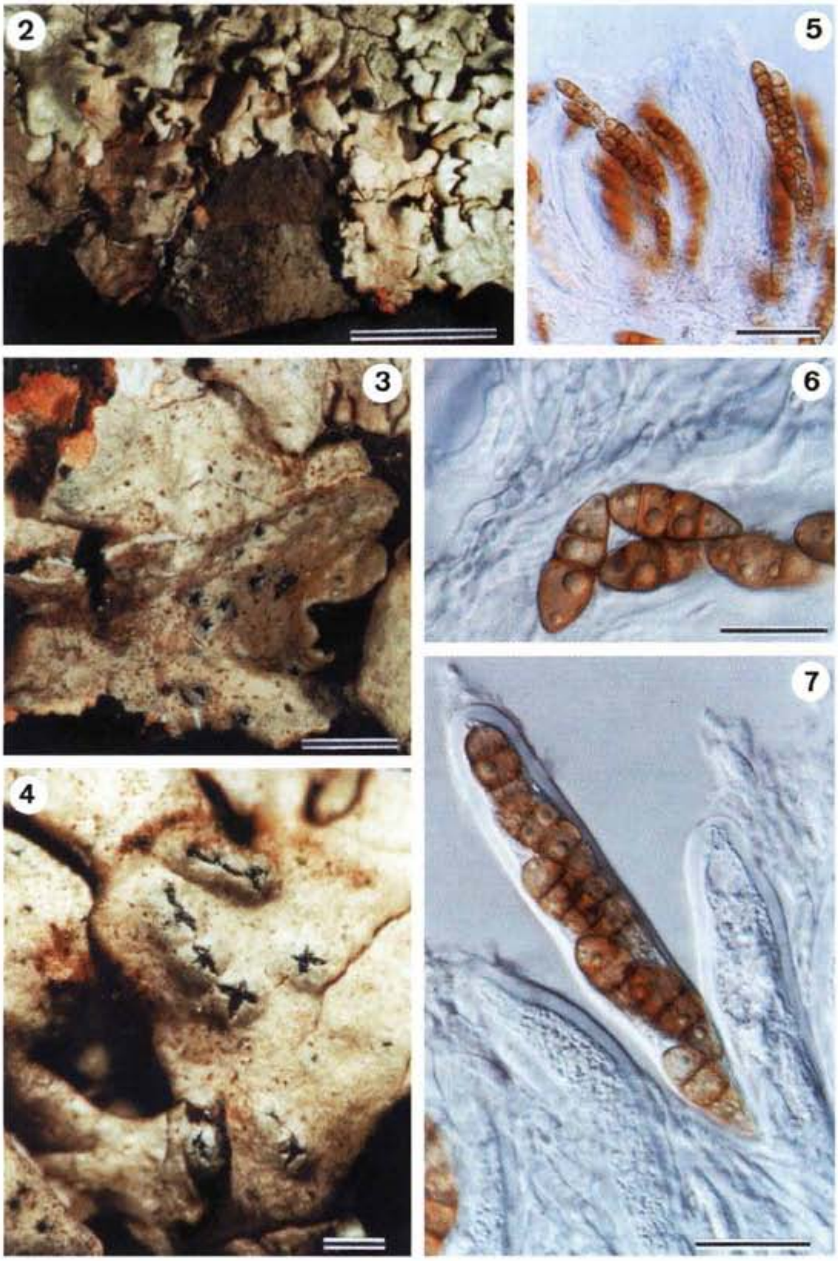
It strongly resembles also the type species *Weddellomyces epicallopisma* in the similar size of spores, which are slightly wider and more broadly rounded at both ends, its ascomata being much larger and asci wider. The last species occurs on calcicolous lichens as *Caloplaca* gr. *aurantia*, *Caloplaca* gr. *variabilis* and *Protoblastenia rupestris* in contrast to the occurrence of *Weddellomyces xanthoparmeliae* on *Xanthoparmelia conspersa* and *X. somloensis* which are growing on acid substrates.

Biology

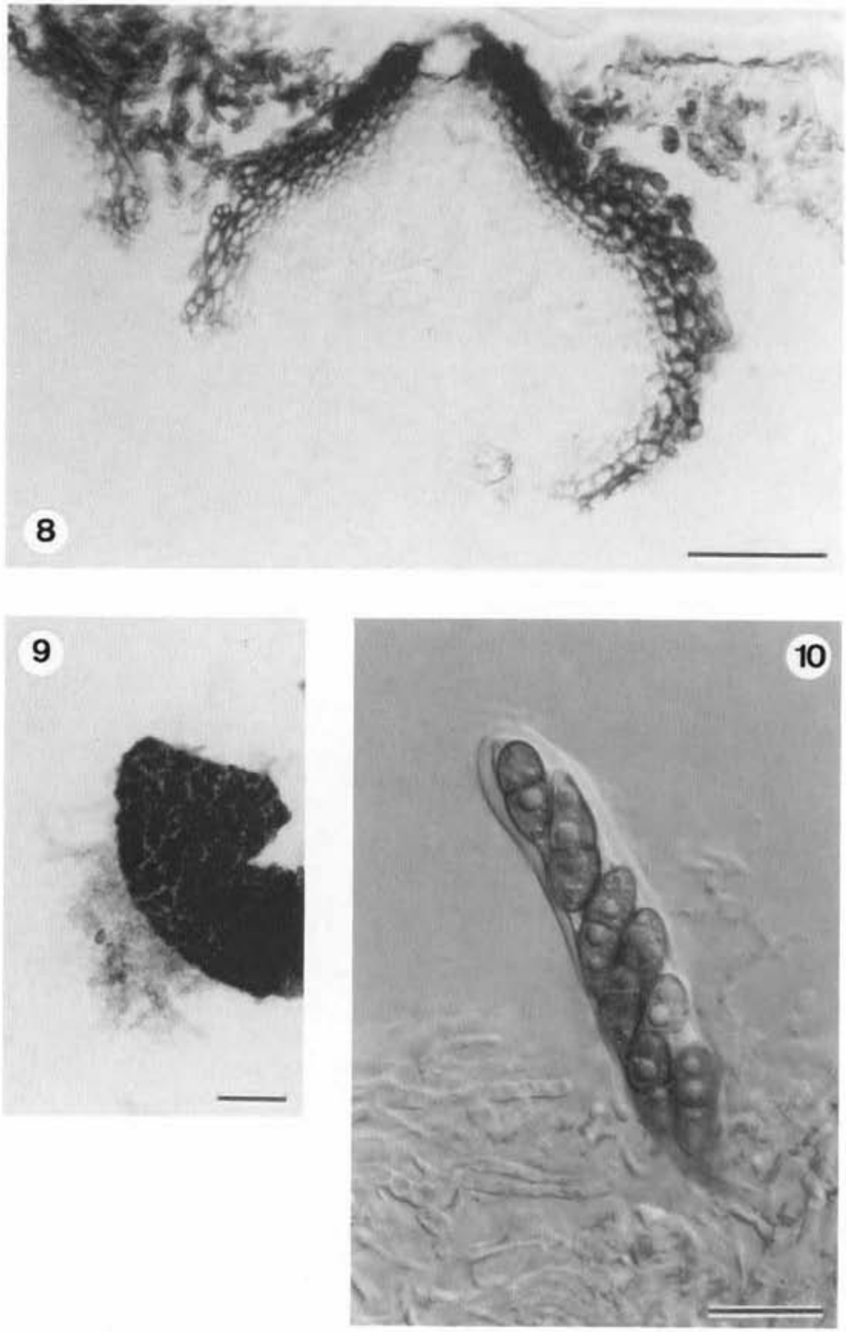
Weddellomyces xanthoparmeliae is parasitic fungus as regarding its biology and has a clearly destructive effect on the host thallus. The infection spots are very conspicuous, pink-orange, at first 0.5–1 cm large. They develop in the middle of the thallus or at its margin. The medulla of the infected host becoming intensive orange, the apothecia of the hosts are reduced in size. Later the spots extend concentrically up to 2–3 cm, the central necrotic part becoming fragmented and soon breaking off.

Distribution

So far the species has been known from Spain only now it is reported also from the Central Europe from the Czech Republic.



Figs 2–7. *Weddellomyces xanthoparmeliae.* **2.** Concentric expansion of infection spot, destroyed part of thallus is broken off. Scale = 100 mm. **3.** Intensively orange coloured medulla of the infected part of the host thallus showing ascomata splitting cortex. Scale = 100 mm. **4.** Ascomata splitting cortex of *Xanthoparmelia conspersa* at higher magnification. Scale = 25 mm. **5.** Part of hamathecium and asci. Scale = 50 μm. **6.** Thick interascal filaments and slightly verruculose spores. Scale = 20 μm. **7.** Asci in different stages of development. Scale = 20 μm. (All preparations in water.)



Figs 8–10. *Weddellomyces xanthoparmeliae*. **8.** Vertical section of ascoma. Pseudoparenchymatic ascomatal wall. Scale = 50 μm. **9.** Surface view showing cephalothecioid plates at lower magnification. Scale = 50 μm. **10.** Ascus showing the bitunicate structure and internal apical beak. Scale = 20 μm. (All preparations in water.)

Ecology

The fungus was present in all the studied localities in rich quantities. All collections were made in warm or thermophytic areas in Central Bohemia and Southern Moravia in localities with increased air humidity due to the proximity of water sources but exposed to the sun from the south or west or at most partly shaded. It was several times found in a mixed infection with *Echinothecium reticulatum* auct., *Abrothallus caerulescens* Kotte, *Stigmidium xanthoparmeliarum* Hafellner, *Lichenoconium usneae* (Anzi) D. Hawksw. and *Cornutispora* sp.

Possible confusion

Lichenoconium usneae and *Cornutispora* sp. were seen to cause similar symptoms of infection on the thalli of *Xanthoparmelia* in our collections. Thalli damaged by *Lichenoconium usneae* are bleached for a great part and also become pale orange. The coelomycete fungus *Cornutispora* sp. with irregularly formed conidia and mostly more than three appendages was also found to cause bleaching of large parts of the thallus of *Xanthoparmelia conspersa*. But *W. xanthoparmeliae* is easily recognized already in the field by occurrence of very large black ascomata. When the large ascomata are developed and are also splitting the thallus and reflexing as black stars than the fungus may also resemble another lichenicolous fungus *Neolamya xanthoparmeliae* Kocourková ined., but here the colour of affected parts of thalli never turns to orange. The last new species will be described soon. A bit similar type of infection is caused also by *Marchandiomyces corallinus* in forming concentric expanding spots similar to those caused by *W. xanthoparmeliae*, but the symptoms are only similar until the easily recognized deep pink sclerotia are developed.

Specimens collected

Central Bohemia, Distr. Rakovník, LPA Křivoklátsko, Krakovec, on SW slope below the Krakovec castle, on shale rocks, 430 m, on *Xanthoparmelia conspersa*, MTB 5947; 10. IV.1999, coll. P. K. (PRM 758559). – Distr. Rakovník, LPA Křivoklátsko, on slope of Čertova skála rock, on spilite, on *Xanthoparmelia* sp. (th.), 290 m, MTB 6048; 28. IX.1996, coll. J. H. (PRM 892559, together with *Echinothecium reticulatum* auct.). – Distr. Rakovník, LPA Křivoklátsko, between the villages of Skryje and Šlovice, on spilite by the river Berounka, on *Xanthoparmelia somloensis*, 290 m, MTB 6048; 26. VII.1998, coll. J. K. and P. K. (PRM 892558). – Distr. Rakovník, LPA Křivoklátsko, nature reserve Stříbrný luh, on W slope of rhyolite rocks above the river Berounka, on *Xanthoparmelia conspersa* (th.), 280 m, MTB 5949; 13. VII.1998, coll. J. K. and P. K. (PRM 892556). – Distr. Beroun, LPA Křivoklátsko, below the castle Točník, on porphyritic rock, on

Xanthoparmelia somloensis, 370 m, MTB 6149; 6. VII.1998, coll. J. K. and P. K. (PRM 892557). – Ibid.: on *X. somloensis* (PRM 758514, specimen of *Stigmidium xanthoparmeliarum*, together with *Weddellomyces xanthoparmeliae* and *Lichenoconium usneae*). – The city of Praha, near Pitkovice, in the valley of the brook Pitkovický potok, nature reserve Pitkovická stráň, on shale, on *Xanthoparmelia somloensis*, 280 m, MTB 5953; 11. IX.1998, coll. J. K. (PRM 892560, together with *Abrothallus caerulescens* and *Echinothecium reticulatum* auct.). – Southern Moravia, Distr. Znojmo, Chvalatice, the Vranov reservoir, near Chvalatická zátoka, on the S slope, on granite boulder scree; on *Xanthoparmelia conspersa*, 360 m, MTB 7060; 6. IX.1998, coll. J. K. (PRM 758529). – Distr. Znojmo, National Park Podyjí, Vranov n. Dyjí ca 4 km SE of the village, ca 4 km SE of the village, on the top of ridge above the river, on shale rocks, on *Xanthoparmelia conspersa*, 490 m, MTB 7160; 4. IX.1998, coll. J. K. (PRM 757414).

ACKNOWLEDGEMENTS

I am indebted to Dr. Z. Pouzar for valuable comments on the manuscript. I am also grateful to Olympus s. r. o., especially to Ing. J. Čerepjuk, for their kind help in introducing us to special problems of microscopic photography. The work was supported by grants from the Grant Agency of the Czech Republic (projects no. 206/96/0709 and 526/96/0250).

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CZECH MYCOL. 51 (2–3), 1999

Peronospora swinglei – ein neuer Falscher Mehltaupilz für die Tschechische Republik

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Müller J. (1999): Peronospora swinglei – a new downy mildew for the Czech Republic. – Czech Mycol. 51: 185–191

In 1984–1995 *Peronospora swinglei* was found in the Moravian Carst on *Salvia verticillata* and *S. pratensis* and in Brno on cultivated *Salvia officinalis*. The description of the downy mildew on *Salvia verticillata* is given, the host plants and the world distribution according to the host plants with remarks about the ecology of the occurrence are stated.

Key words: *Peronospora swinglei*, description, hosts, distribution.

Müller J. (1999): Peronospora swinglei – nová plíseň pro Českou republiku. – Czech Mycol. 51: 185–191

V letech 1984–1995 byla nalezena *Peronospora swinglei* v Moravském krasu na *Salvia verticillata* a *S. pratensis* a v Brně na pěstované *Salvia officinalis*. Je podán popis plísně na *Salvia verticillata*, uvedeny hostitelské rostliny a světové rozšíření podle hostitelských rostlin s poznámkami o ekologii výskytu.

EINLEITUNG

Bei der mykofloristischen Erforschung der Rost-, Brand- und Falschen Mehltaupilze im Mährischen Karst fand ich *Peronospora swinglei* 1984 auf *Salvia verticillata* zwischen Lažánky und Jedovnice, 1988 auf *Salvia pratensis* auf der Skalka bei Ochoz und 1995 im Říčkabachtal bei Ochoz. In demselben Jahr entdeckte Frau Ing. H. Tichá diesen parasitischen Pilz auf *Salvia officinalis*, die im Heilpflanzengarten in Brno auf der Kraví hora kultiviert wurde. *Peronospora swinglei* war bei uns bisher nicht bekannt.

MATERIAL UND METHODIK

Von den befallenen Pflanzen wurden im Freien einige Blätter mit dem Konidienträgerrasen auf der Unterseite (mittels einer Lupe festgestellt), abgerissen und in eine Tüte eingelegt. Zu Hause wurden die Blätter flächig ausgebreitet, in Löschpapier gelegt und mässig belastet. Während des Winters wurden die ausgetrockneten Blätter in kryptogamologische Umschläge gegeben, mit Etiketten versehen und der Pilz mittels eines Lichtmikroskops identifiziert. Der Konidienträgerrasen wurde mittels einer Präpariernadel abgekratzt, in einen Tropfen

Laktophenol auf dem Objektträger übertragen, mittels der Präpariernadeln fein zerrissen, mit dem Deckglas bedeckt und mikroskopiert. Die Nomenklatur der Gefässpflanzen wurde von Hejný und Slavík (eds.) (1988–1997) und Dostál (1989) übernommen.

ERGEBNISSE UND DISKUSSION

Peronospora swinglei Ellis et Kellerman,
Journ. Mycol. 3: 104, 1887 (*P. lamii* auct. p.p.)

Beschreibung des Falschen Mehltaupilzes auf *Salvia verticillata* von Lažánky: Konidienträgerrasen auf der Blattunterseite, sehr spärlich, hell bräunlich, locker, auf 3–6 mm grossen, graubraunen, von den Blattadern begrenzten Flecken. Auf der Blattoberseite braungelbliche, später braun werdende, weniger deutlich von Blattadern begrenzte Flecke. – Konidienträger 475–599 μm hoch und 4–8 μm dick, am Grunde auf 8 μm schwach verdickt, 4–7 fach verzweigt, der unverzweigte Teil beträgt 2/3 bis 3/4 der Gesamthöhe. Zweige gebogen, letzte Gabeläste schnabelförmig eingebogen, recht- oder stumpfwinkelig, ca 11 μm lang und an der Einsatzstelle ca 2 μm dick. – Konidien breit ellipsoidisch oder breit eiförmig, beidendig gerundet, graubräunlich, sehr wenig ausgebildet, 21–22 × 17–20 μm. – Weder Oogonien noch Oosporen sind bisher bekannt. Auch auf *Salvia officinalis* wurden die Oosporen nicht festgestellt (mündliche Mitteilung von H. Tichá).

Peronospora swinglei wurde von amerikanischen Mykologen auf *Salvia reflexa* Hornem. (*S. lanceolata* auct.) aus den Vereinigten Staten beschrieben (den Fundort des Typus siehe weiter unten).

Wirtspflanzen:

Salvia officinalis L., *S. pratensis* L., *S. reflexa* Hornem., *S. sclarea* L., *S. verticillata* L., *S. viridis* L. *S. horminum* L. emend. Briq. und *Salvia* sp.

Verbreitung und Ökologie:

Auf *Salvia officinalis:* Nordamerika: USA: Florida: Homestead: an einer Redland vegetable farm, ca 2 m ü. M., VI. 1993 (Mc Millan et Graves 1994 ut *P. lamii* A. Br.). Europa: Tschechische Republik: Brno: botanischer Heilpflanzengarten auf Kraví hora, 280 m ü. M., 18. VIII. 1995 leg. H. Tichá.

Auf *Salvia pratensis:* Europa: Britische Inseln (ohne nähere Angaben), vor dem J. 1891 (Massee 1891 sec. Francis et Waterhouse 1988 ut *P. lamii*). Deutschland: Hessen: Bad Nauheim: bei Schwalheim und Rödgen (Jaap 1914); Thüringen

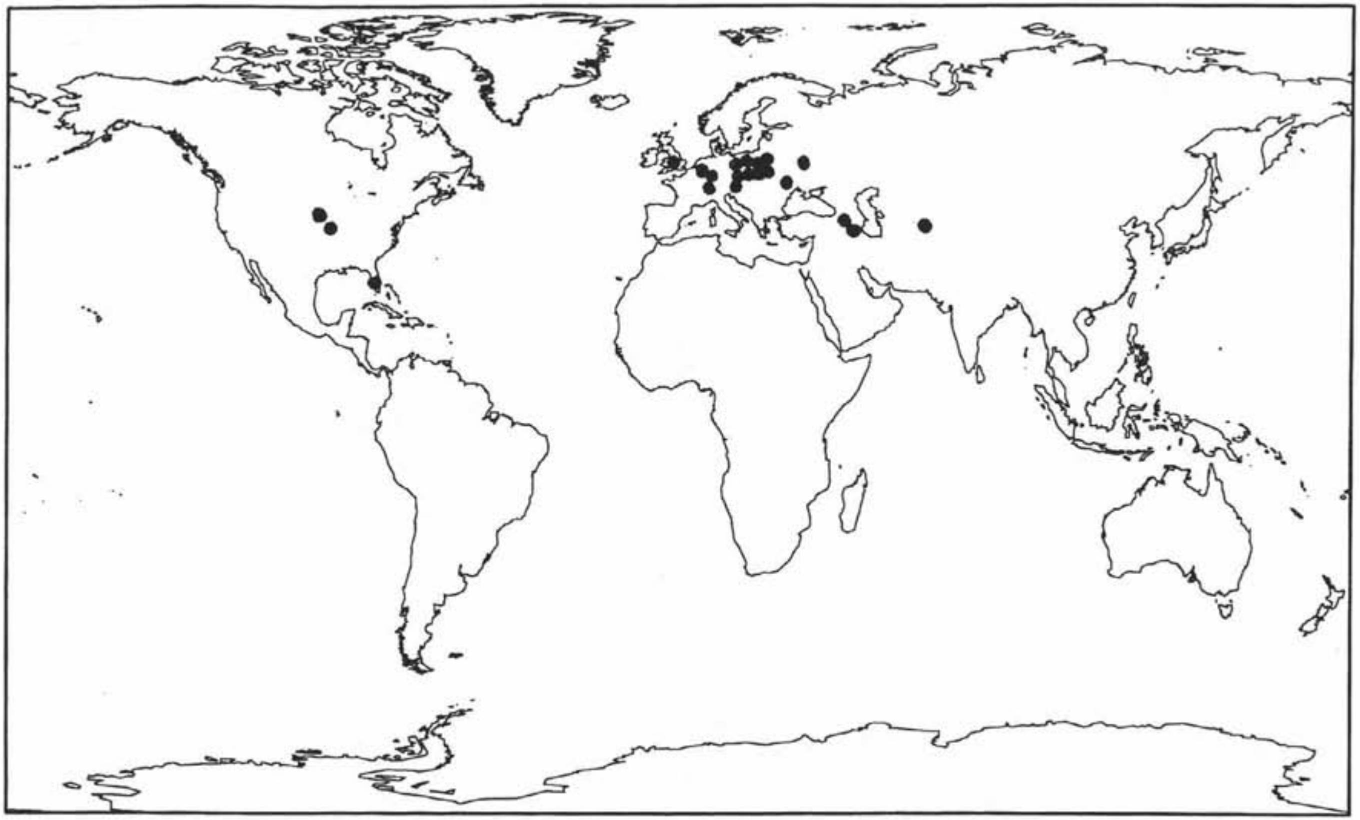

Abb. 1. Verbreitung von *Peronospora swinglei*

(Gäumann 1923); Bayern: Günzburg, Deffingen, Kleinkötz, Mindelaltheim, Offingen, Lkrs. Günzburg; Württemberg (Doppelbaur et Doppelbaur 1972). Polen: Unterschlesien: Legnica: Rudna bei Lubin (Schroeter 1889); Bydgoszcz: Brudzyn, Wybranow und Włoszonow bei Znin, Miradz bei Mogilno (Szulczewski 1910). Ostpolen: Warszawa: Bezirk Nowy Dwór Mazowiecki; Białystok: Bezirk Siemiatycze (Kochman et Majewski 1970), Drohiczyn: Xerothermrasen auf der Góra Zamkowa, Mielnik: Xerothermrasen auf der Góra Rowska, VIII. (Romaszewska-Sałata et Mułenko 1983); Biala Podlaska: Gnojno: trockenes Ufer des Flusses Bug, nicht häufig, VI.-VII. 1980-1982 (Danilkiewicz 1990); Lublin: Bochotnica bei Puławy, Ciecierzyn bei Lublin, Lublin-Czechów, Nałęczów, Rudnik; Zamosc: Izbica, Łabunie, Tarnogóra bei Izbica, ziemlich häufig, V.-VIII. (Romaszewska-Sałata 1977); Tarnobrzeg: Dwikozy bei Sandomierz, nicht häufig im Brachypodietum pinnati, 10. VIII.1980 (Romaszewska-Sałata 1982); Kielce: Naturschutzgebiet Lipny Dół bei Miechów (Romaszewska-Sałata 1977). Tschechische Republik: Brno: Xerothermrasen am Westhang der Skalka bei Ochoz, ca 390 m ü. M., 13. V.1988 leg. J. Müller; Xerothermrasen auf lichter Stelle des Waldes am Südhang des Felsens Bajerova skála im Říčkabachtal bei Ochoz, ca 375 m ü. M., 9. VI.1995 leg. J. Müller. Österreich: Steiermark: südlich Hart bei St. Peter E Graz, 8. V.1983 J. Poelt (Melzer et al. 1984).

Auf *Salvia reflexa:* Nordamerika: USA: Kansas: Manhattan, VI.1887 leg. W. T. Swingle – Typuslokalität, herausgegeben in Ell. et Ev., N. Amer. Fungi 2203 (Ellis et Kellerman 1887, Constantinescu 1991).

Auf *Salvia sclarea:* Europa: Moldavien (Osipjan 1967); Mittelasien: Usbekistan: g. Samarkand: Ferganskaja dolina, VI.-VII. (Gaponenko 1972).

Auf *Salvia verticillata:* Europa: Ostpolen: Zamosc: Brody bei Szczebrzeszyn, häufig, VI.-VII. (Romaszewska-Sałata 1975); Tschechische Republik: Mährischer Karst: strauchiger Rain zwischen Lažánky und Jedovnice bei Blansko, ca 460 m ü. M., 22. IX.1984 leg. J. Müller. Asien: Armenien (Novotelnova et Pystina 1985): Diližan: Mündung des Flusses Bldančaj, 15. VIII.1963, starker Befall (Osipjan 1967).

Auf *Salvia viridis:* Asien: Aserbajdžan (Novotelnova et Pystina 1985): Nagorno-Karabachskaja Avt. obl.: Mardakertskij rajon, im Vorgebirge des Kaukasus auf Trockenhängen, 30. V.1952 Uljaniščev (Uljaniščev 1967).

Auf *Salvia* sp.: Nordamerika: USA: Kansas (Anonymus 1960, Farr et al. 1989). Ehem. SSSR (*Salv. pratensis*, Jačevskij et Jačevskij 1931): Europa: Russland: Voronežskaja oblasť (Osipjan 1967).

Gäumann (1923) bezweifelt die Richtigkeit der Bestimmung der *Peronospora* auf *Salvia pratensis* aus Hessen und Thüringen, doch wurde später *P. swinglei* auf diesem Wirt in Deutschland und anderen Ländern Europas gefunden (siehe Fundortaufzählung), sodass sie auch in Hessen und Thüringen sicher vorkommt. Das Verzeichnis der veröffentlichten Fundorte erfasst offensichtlich nicht die tat-

Abb. 2: Konidienträger von *Peronospora swinglei* von der Skalka bei Ochoz. Vergr. 250
Photo Z. Vícha

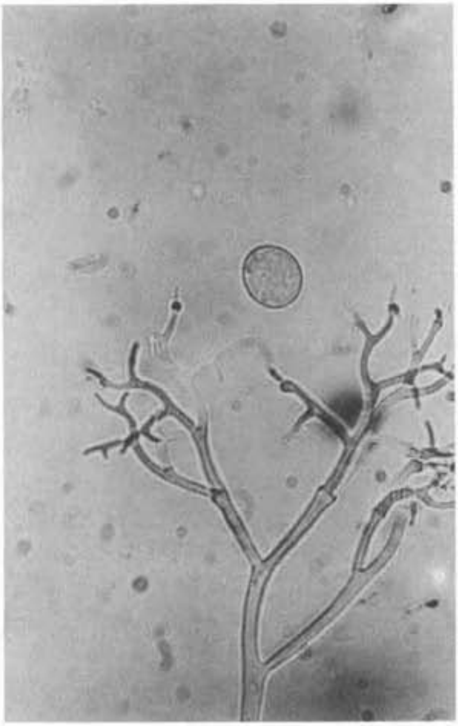

Abb. 3: Konidienträgerzweige und Konidie. Vergr. 500
Photo Z. Vícha

sächliche Verbreitung von *P. swinglei*, sondern stellt eher die Aktivität dortiger Peronosporales-Spezialisten dar (siehe Polen, Deutschland, Tschechische Republik).

Schon Uljaniščev (1967) bezeichnete *P. swinglei* vom phytogeographischen Standpunkt aus als holarktischen Typ. Der Pilz kommt im Gürtel zwischen 25 ° bis 53 ° nördl. Br. vor. Das natürliche Vorkommen ist an die Atlantische südliche Unterregion Nordamerikas gebunden, in Europa und Transkaukasien an die Eurosibirische Unterregion und der östlichste Fundort liegt in der Mittelasiatischen Unterregion. Das südlichste Auftreten ist auf Florida, jedoch auf angepflanzter *Salvia officinalis*. Was die vertikale Verbreitung betrifft, wurde *P. swinglei* in ca 2 m (Florida) bis ca 1280 m ü. M. (Diližan am Malyj Kavkaz) gesammelt, am häufigsten jedoch zwischen 100 bis 500 m. Im Laufe des Jahres kommt sie von Mai bis September vor.

In Europa und am Kleinen Kaukasus tritt *P. swinglei* auf trockenen Hängen mit xerothermer Vegetation auf, z. B. im *Brachypodietum pinnati*. In der

Tschechischen Republik wurde sie im Kalkgebiet des Mährischen Karstes gefunden, z. B. am steinigen, strauchigen Südhang der Bajerova skála, wo sie auf *Salvia pratensis* in Gesellschaft von *Anthericum ramosum* L., *Arabis hirsuta* (L.)Scop., *Bromopsis erecta* (Huds.)Fourr., *Carex michelii* Host, *Cornus mas* L., *Dictamnus albus* L., *Erysimum odoratum* Ehrh., *Gagea villosa* (Bieb.) Duby, *Lactuca quercina* L., *Melica transsilvanica* Schur, *Potentilla arenaria* Borkh., *Pulmonaria mollis* Hornem., *Seseli osseum* Crantz, *Thymus kosteleckyanus* Opiz, *T. marschallianus* Willd., *Tithymalus epithymoides* (L.) Klotzsch et Garcke, *Veronica prostrata* L. u. a. vorkommt. In Florida herrscht zur Fundzeit (im Frühling) ein kühles (7–10 °C nachts und 20–24 °C tagsüber) und trockenes (20–50 % relat. Luftfeuchtigkeit) Klima. Es wird aber Tau gebildet (Mc Millan Jr. in litter.).

Ob dieser Falsche Mehltaupilz auf bestimmte Arten der Gattung *Salvia* spezialisiert ist, ist nicht bekannt, weshalb wir nicht wissen, ob der Pilz von den wildwachsenden Arten auf *Salvia officinalis,* die als Heilpflanze angebaut wird, überzugehen vermag.

Danksagung. Bei der Erarbeitung dieses Artikels wurde ich in freundlicher Weise von Frau Ing. H. Tichá in Brno und den Herren W. Dietrich in Annaberg-Buchholz, Prof. R. T. Mc Millan Jr. in Homestead, Florida, Dr. M. Scholler in Greifswald, Prof. Z. Urban in Prag und Ing. Z. Vícha in Brno unterstützt, wofür ich der genannten Frau und den Herren freundlichst danke.

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Book review

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Hifomicetes demaciáceos de Cuba. Enteroblásticos

Museo regionale di scienze naturali, Monografie XXIII, Torino, 1997. 388 pages.
ISBN 88–86041–19–5. Hardcover price L 140 000.

In mycological literature there is continuing interest in the attractive group of microfungi with dark conidia, conidiophores or mycelium, the so-called dematiaceous hyphomycetes (Deuteromycetes). Information on these fungi given by Hughes, Ellis, Matsushima and Rao in the past are now complemented by a detailed and critical treatment of the enteroblastic dematiaceous fungi of Cuba.

This monograph is the result of several years of extensive research by the authors following up the previous studies by Castañeda Ruiz. It deals with the taxonomy and ecology of 201 species belonging to 68 genera of tretic and phialidic dematiaceous hyphomycetes found in Cuba. All species are described in detail and illustrated with black and white linedrawings on 121 plates; many illustrations are original, some others are taken over the world literature. All data are purposely extensive, as this is the first comprehensive study carried out on Cuba and probably in Latin-America.

Descriptions include macroscopic and microscopic features and valuable comments on taxonomy and ecology of each described taxon. The most important synonyms and where possible the anamorph-teleomorph connection are given. Moreover, notes on habitat, substrate, locality and date of collection and depository herbarium are included. The dichotomic key for all genera treated is based on the ontogeny of reproductive structures and other morphological characters. Further keys on the species level, together with excellent illustrations, enable a successful determination. The book is complemented with a bibliography (almost 140 entries) and a fungus/substrate index.

All material described has been collected on the island of Cuba, mostly in tropical forests, and with respect to this a noteworthy diversity of the fungi studied has been revealed. New or supplementary data on rare or obscure genera, such as *Piricaudilium* Hol.-Jech., *Paraceratocladium* Castañeda, *Craspedodidymum* Hol.-Jech., *Mercadomyces* J. Mena, *Phragmospathulella* J. Mena et Mercado, *Holubovaea* Mercado, *Oramasia* Urries (the correct name of this genus is *Vermiculariopsiella* Bender) and many others, are very useful for current identification and taxonomic revisions in the future.

The treatment is the successful result of international cooperation. The authors come from Cuba and the Czech Republic (Holubová-Jechová, who unexpectedly died in 1993), the book is issued in Italy and written in Spanish (except the sleeve note in English). The monograph is not only an excellent contribution extending our knowledge of microfungi distribution and diversity, but many of the fungi treated here are important phytopathogenic species or cause spoilage of food and deterioration of industrial products. For that reason this book can be of particular interest to specialists on tropical mycology and fungal taxonomy as well as to phytopathologists and biologists in a broad sense.

Karel Prášil

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Míka František, (*1925), Kreuzmannova 2, 318 04 Plzeň. (mycofloristics)

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Moravec Jiří, (*1942), P. O. Box 17/A, 679 04 Adamov u Brna. (taxonomy of operculate Discomycetes)

Motyčková Kamila, (*1977), Střešovická 1014/43, 164 00 Praha 6. (mycofloristics)

Müler Jiří, RNDr., (*1925) Provazníkova 76, 613 00 Brno. (Peronosporales, Uredinales, Ustilaginales-mycofloristics, taxonomy, biology)

Nedělník Jan, RNDr., (*1958), Výzkumný ústav pícninářský, s. r. o., 664 41 Troubsko. (micromycetes, phytopathology)

Němec Robert, (*1979), K Lukám 642, 142 00 Praha 4-Libuš. (Aphyllophorales, *Phellinus*)

NOVÁČEK JIŘÍ, RNDr., (*1962), SZŠ, Sadová 1234, 286 20 Čáslav. (physiology, mycorrhiza, toxicology, coenology)

NOVÁKOVÁ ALENA, RNDr., CSc., (*1955), ÚPB AV ČR, Na sádkách 7, 370 05 České Budějovice. (soil micromycetes)

NOVÁKOVÁ JIŘINA, RNDr., CSc., (*1923), Rezkova 5, 602 00 Brno. (phytopathology, viruses-fungi interaction)

NOVOTNÝ DAVID, Mgr., (*1971), Česká sbírka mikroorganizmů, Tvrdého 14, 602 00 Brno. (Ophiostomatales, Hyphomycetes, phytopathology and ecology)

NOVOTNÝ JIŘÍ, (*1947), Průběžná 18, 370 04 České Budějovice. (mycofloristics, photography of fungi)

ONDŘEJ MICHAL, RNDr., CSc., (*1938), Vikýřovice 416, 788 13 Šumperk. (taxonomy of plant parasitic fungi)

OSTRÝ VLADIMÍR, MVDr., CSc., (*1957), Státní zdravotní ústav, Centrum hygieny potravinových řetězců, Palackého 1–3, 612 42 Brno. (micromycetes in food products, alimentary mycology)

OTČENÁŠEK MILOŠ, RNDr., DrSc., (*1934), Brožíkova 426, 530 09 Pardubice. (experimental medical mycology)

OTTOVÁ VLASTA, RNDr., (*1934), Pětipeského 2, 169 00 Praha 6. (micromycetes of waste waters, selective micromycetes)

PAPOUŠEK TOMÁŠ, Ing., (*1942), Generála Svobody 29, 370 01 České Budějovice. (photography of fungi, macromycetes – floristics and chorology of fungi in southern Bohemia)

PATERA MIROSLAV, Ing., (*1957), Nouzov 863, 273 51 Unhošť. (*Russula*)

PAULECH PETER, RNDr., CSc., (*1963), ÚEFESAV, Nádražna 52, 900 28 Ivanka pri Dunaji, Slovensko. (Ustilaginales, Tilletiaceae, mycofloristics)

PEŠKOVÁ VÍTĚZSLAVA, Ing., (*1966), VÚLHM, Jíloviště-Strnady, 156 04 Praha 5 – Zbraslav. (mycorrhiza)

PIECKOVÁ ELENA, Ing., MPH, PhD., (*1966), ÚPKM, Limbová 14, 833 01 Bratislava, Slovensko. (micromycetes, mycotoxins)

PIKÁLEK PETR, Doc., RNDr., CSc., (*1943), Katedra genetiky a mikrobiologie, PřF UK, Viničná 5, 128 44 Praha 2. (genetics of filamentous fungi, esp. wood-destroying Basidiomycetes)

POLÁK OLDŘICH, (*1921), Jurkovičova 10, 638 00 Brno – Lesná. (popularization of fungi)

POSPÍŠIL VALENTIN, RNDr., CSc., (*1911), Sedlákova 22, 602 00 Brno. (phytogeography of higher fungi)

PRÁŠIL KAREL, prom. biol., CSc., (*1949), Katedra botaniky, PřF UK, Benátská 2, 128 01 Praha. (micromycetes – Ascomycetes)

PROCHÁZKA MILOSLAV, Doc., MUDr., CSc., (*1932), Steinerova 12, 811 07 Bratislava, Slovensko.

Procházková Zdeňka, prom.biol., CSc., (*1949), VÚLHM, Výzkumná stanice Uherské Hradiště, 686 04 Kunovice. (phytopathology)

Prokinová Evženie, Ing., CSc., (*1955), ČZU AF – kat. ochrany rostlin, 165 21 Praha 6 – Suchdol. (phytopathogenous fungi)

Příhoda Antonín, Doc., Ing., (*1919), Nad školou 26, 252 67 Tuchoměřice. (mycofloristics, micromycetes, protection of fungi)

Réblová Martina, Mgr., (*1971), Botanický ústav AV ČR, 252 43 Průhonice. (taxonomy and ecology of Pyrenomycetes and Hyphomycetes)

Rod Jaroslav, Ing., CSc., (*1943), ÚKZUZ-OPMOR, Šlechtitelů 11, 783 71 Olomouc. (phytopathology)

Roth Jiří, PaedDr., (*1940), Jiráskova 4211, 430 03 Chomutov. (mycofloristics, mycocoenology, protection of fungi)

Roziaková Eva, PharmDr., (*1962), Štátny zdravotný ústav, odd. mykológie život. prostredia, Kuzmányho 27, 036 01 Martin, Slovensko. (diagnostics of micromycetes in alimentary products, waters and natural environment, detection of mycotoxins in food)

Rybnikář Alois, RNDr., (*1948), Bioveta a.s., 683 23 Ivanovice na Hané. (pathogenous micromycetes, dermatophytes, immunology of mycoses)

Savická Dana, Ing., (*1963), VŠCHT, Ústav biochemie a mikrobiologie, Technická 5, 166 28 Praha 6. (microscopic filamentous fungi)

Skála Edvard, (*1945), Trnovanská 1297/42, 415 04 Teplice. (mycofloristics, photography of fungi)

Skálová Anna, RNDr., (*1947), U žlebu 1057, Uherský Brod, (microscopic fungi of working environment, imperfect and wood-destroying fungi)

Slavíček Josef, (*1945), Čajkovského 975, 500 09 Hradec Králové. (Corticiaceae s.l., Agaricales – *Entoloma, Psathyrella, Conocybe*, computers in mycology)

Soukup František, RNDr., CSc., (*1948), VÚLHM, Jíloviště-Strnady, 156 04 Praha 5 – Zbraslav. (forest phytopathology, wood-destroying fungi)

Soukup Libor, Mgr., (*1969), Lesní 1555, 347 01 Tachov. (mycofloristics)

Svrčková Jiřina, RNDr., (*1925), Seifertova 48, 130 00 Praha 3 – Žižkov. (mycology of alimentary products)

Sychrová Eliška, RNDr., CSc., (*1941), Michelská 72/1240, 145 00 Praha 4. (phytopathology)

Syrovátko Petr, RNDr., (*1958), U lípy 986, 549 01 Nové Město nad Metují. (parasitic and saprotrophic micromycetes)

Šašek Václav, RNDr., CSc., (*1937), Mikrobiologický ústav AV ČR, Vídeňská 1083, 142 20 Praha 4. (physiology of fungi)

Šimek Martin, (*1964), Vítkovická 2044, 288 03 Nymburk. (Polyporaceae s. l.)

Šimonovičová Alexandra, RNDr., CSc., (*1954), PriF UK, katedra pedológie – B2, Mlynská dolina, 842 15 Bratislava, Slovensko. (micromycetes in soil and natural environment)

ŠIMŮNEK JAN, Doc., MUDr., CSc., (*1954), Húskova 43, 618 00 Brno. (micromycetes, mycotoxins)

ŠKUBLA PAVOL, Ing., CSc., (*1946), J. Palárika 8, 927 00 Šala, Slovensko. (macromycetes, photography of fungi, *Cortinarius, Hygrocybe*)

ŠROBÁROVÁ ANTÓNIA, RNDr., CSc., (*1944), Leningradská 12, 811 01 Bratislava, Slovensko. (phytopathology)

ŠULCOVÁ JANA, RNDr., (*1956), Třeboradická 1075, 182 00 Praha 8, (medical mycology)

ŠUTARA JOSEF, (*1943), Prosetická 239, 415 01 Teplice - jih. (anatomy and taxonomy of Boletaceae, wood-destroying fungi in buildings)

ŠVECOVÁ MILADA, PaedDr., RNDr., CSc., (*1953), PřF UK, Viničná 7, 128 44 Praha 2. (*Lophodermium*, fungi of conducting tissues of woody plants - *Ceratocystis, Ophiostoma*)

ŠVINGR JIŘÍ, (*1935), Tylova 846, 251 01 Říčany u Prahy.

TICHÝ HERBERT, (*1936), Drůbežárna 64, 439 01 Černčice u Loun. (mycofloristics)

TMEJ LIBOR, (*1967), Křetínská 252, 561 12 Brandýs nad Orlicí. (Discomycetes)

TOMŠOVSKÝ MICHAL, (*1977), Žižkov II č. 1258, 580 01 Havlíčkův Brod. (general mycology)

TONDL FRANTIŠEK, prom.biol., (*1939), KHS, Schneiderova 32, 370 71 České Budějovice. (mycofloristics of southern Bohemia)

TÓTHOVÁ LÍVIA, RNDr., (*1961), VÚ vodného hospodárstva, Gen. Svobodu 5, 812 49 Bratislava, Slovensko. (mikromycetes in waters)

URBAN ZDENĚK, Prof., RNDr., DrSc., (*1923), Na vrcholu 18, 130 00 Praha 3. (Uredinales, Ustilaginales, Pyrenomycetes)

VACEK VLADIMÍR, RNDr., CSc., (*1926), Vídeňská 58, 639 00 Brno. (mycofloristics)

VÁGNER ALOIS, (*1939), Moravské zemské muzeum, Zelný trh 6, 659 37 Brno. (mycofloristics, *Tricholoma, Pluteus, Melanoleuca, Peziza, Scutellinia*)

VAHALÍK MIROSLAV, Ing., (*1946), Oseva, Přerovská 41, 772 32 Olomouc. (fungal diseases of fruit trees and leguminous crops)

VALTER JIŘÍ, Ing., (*1930), Tř. Kpt. Jaroše 2411, 390 01 Tábor. (mapping of distribution of fungi in Tábor distr.)

VAMPOLA PETR, (*1952), Žižkova 87, 586 01 Jihlava. (taxonomy, distribution and ecology of Polyporales s.l., protection of fungi with emphasis on European species)

VÁŇOVÁ MARIE, RNDr., CSc., (*1943), Katedra botaniky PřF UK, Benátská 2, 128 81 Praha 2. (taxonomy and ecology of Zygomycetes, lower fungi)

VARJÚ ĽUDOVÍT, (*1951), Gessayova 2, 852 44 Bratislava, Slovensko. (*Polyporales*)

VAŠUT RADIM, (*1976), 5. května 1557, 756 61 Rožnov pod Radhoštěm. (mycofloristics of macromycetes)

VAVERKA SLAVOJ, Doc., Ing., CSc., (*1933), Ústav ochrany rostlin AF MZLU, Zemědělská 1, 613 00 Brno. (agricultural phytopathology)

VENUTA MAREK, (*1976), Kadov 35, Moravský Krumlov, (general mycology, photography of fungi)

VESELÝ DÁŠA, Doc., Ing., DrSc., (*1933), Kapitána Stránského 988, 198 00 Praha 9 – Černý Most II. (phytopathology, mycoparasitic fungi)

VEVERKA KAREL, Ing., DrSc., (*1943), VÚRV, Drnovská 507, 161 06 Praha 6 – Ruzyně. (phytopathology)

VIDLÁKOVÁ ANNA, Ing., (*1945), Šlechtitelská stanice, 751 17 Horní Moštěnice. (phytopathology – leguminous crops, cereals)

VLACH JAN, (*1942), Jiráskovo nám. 19, 301 54 Plzeň. (micromycetes)

VLASÁK JOSEF, RNDr., CSc., (*1948), Horní 120, 373 41 Hluboká nad Vltavou. (floristics of Polypores)

VOBR FRANTIŠEK, (*1912), Vyšehradská 35/428, 128 00 Praha 2.

VOLKOVÁ-NOVOTNÁ JANA, RNDr., (*1963), Sempra a. s., Šlechtitelská stanice, 391 76 Slapy u Tábora. (phytopathology, antagonistic relationship among microorganisms)

VOSTATEK MIROSLAV, RNDr., PhMr., (*1930), Bělehradská 271, 530 09 Pardubice. (toxicology)

VOŽENÍLKOVÁ BOHUMILA, Ing., CSc., (*1943), katedra rostlinné výroby, Zemědělská fak. JU, Studentská 13, 370 05 České Budějovice. (antagonistic fungi against *Trichoderma harzianum*)

ZÁHOROVSKÁ ERIKA, Doc., RNDr., CSc., (*1938), Kuklovská 31, 841 65 Bratislava, Slovensko.

ZEDNÍK JOSEF, (*1947), Máchova alej 9, 568 02 Svitavy. (mycofloristics)

ZEMÁNKOVÁ MICHAELA, Mgr., (*1967), VÚRV, mykologické odd., Drnovská 507, 161 06 Praha 6 – Ruzyně. (mycofloristics, micromycetes – *Fusarium*)

ZÍTA VLADIMÍR, (*1952), Opavská 2626, 415 01 Teplice. (mycofloristics, photography of fungi, Gasteromycetes – Geastraceae)

ZVÁRA JOSEF, Prof., Ing., CSc., Zemědělská fak. JU, Studentská 13, 370 05 České Budějovice.

Book review

ROY WATLING AND EVELYN TURNBULL

British Fungus Flora Agarics and Boleti

Vol. 8. Cantharellaceae, Gomphaceae and amyloid-spored and xeruloid members of Tricholomataceae (excl. Mycena). Royal Botanic Gardens Edinburgh 1998. Price £ 12,50.

A new volume of the popular series of British Fungus Flora monographs deals with two different groups of macromycetes: 1. Cantharelloid and gomphoid fungi (*Cantharellus, Craterellus, Pseudocraterellus* and *Gomphus*) belonging to Aphyllophorales s. l., in which the family Cantharellaceae forms a separate group whilst members of Gomphaceae are relatives to *Ramaria*. 2. White-spored agarics belonging to Tricholomataceae with amyloid spores (except for the genus *Mycena*) and those with xeruloid characters. Species having a sarcodimitic hyphal structure are included in the last mentioned group (14 genera in total). A "Colour identification chart" is enclosed with the book.

In the genus *Cantharellus, C. amethysteus* is considered a separate species, and the name *C. ferrugineus* Orton (in Central Europe rather forgotten) is used for *"C. cibarius"* becoming slowly yellow-red or sienna on surface after bruising. The true *C. cibarius* as well as *C. pallens* are species without colour changes after touching. In the genus *Cystoderma*, the taxon *rugoso-reticulatum*, present in Britain (Bon 1987), is not mentioned. It represents an infraspecific taxon of *C. amianthinum*, not a separate species as proposed by Wasser (1993). The authors recognize *Pseudoomphalina graveolens* and *P. kalchbrenneri* as two clearly separate species (only the first one occurs in Britain), in contrast to Kotlaba and Pouzar (1995), who consider both fungi conspecific. The authors correctly distinguish *Oudemansiella, Xerula* and *Megacollybia* as separate genera.

According to the Code (Art. 13), only the name of Fries – as the author of a sanctioning work – can be written with a colon (: Fr.). Here, also other authors' names are sometimes erroneously written with a colon, e. g. Secretan (p. 42), Persoon (p. 88), Pollini (p. 109) and Mérat (p. 136). The correct combination of authors of *Leucopaxillus gentianeus* is (Quél.) Kotlaba (basionym *Clitocybe gentianea* Quél. 1873). The date of Fries' combination of *Panus ringens* (Fr.) Fr. is 1874 (not 1828, p. 100). In *Cantharellus stevensoni* (a basionym of *Gerronema stevensoni*) the authors' abbreviations (Berk. et Br.) are missing (p. 113).

The book will certainly be very useful to all professional and amateur mycologists for its clearly constructed keys especially in groups which have not been monographed for a long time (e. g. *Hemimycena*).

Vladimír Antonín

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BON M. (1987): The mushrooms and toadstools of Britain and North-western Europe. – Hodder & Stoughton, London – Sydney – Auckland – Toronto.

KOTLABA F. & POUZAR Z. (1995): Pseudoomphalina kalchbrenneri (Agaricales) in the Czech Republic. – Czech Mycol. 48(3): 199–205.

WASSER S. P. (1993): Tribes Cystodermateae Sing. and Leucocoprineae Sing. of the CIS and Baltic States. – Libri Bot. 9: 1–105.

Index to new names and combinations appearing in Czech Mycology 51 (2–3)

Polycoccum minutulum Kocourková et Berger spec. nov. 171

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

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

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

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