

**Bark beetles and their galleries: well-known niches for little known fungi on the example of *Geosmithia***ALENA KUBÁTOVÁ<sup>1</sup>, MIROSLAV KOLAŘÍK<sup>1,3</sup>, KAREL PRÁŠIL<sup>1</sup>  
and DAVID NOVOTNÝ<sup>2</sup><sup>1</sup> Department of Botany, Faculty of Science, Charles University, Benátská 2,  
128 01 Praha 2, Czech Republic, e-mail: kubatova@natur.cuni.cz<sup>2</sup> Research Institute of Crop Production, Drnovská 507, 161 06 Praha 6,  
Czech Republic<sup>3</sup> Laboratory of Physiology and Genetics of Fungi, Institute of Microbiology,  
Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Praha 4,  
Czech Republic

Kubátová A., Kolařík M., Prášil K. and Novotný D. (2004): Bark beetles and their galleries: well-known niches for little known fungi on the example of *Geosmithia*. – Czech Mycol. 56: 1–18

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

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

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

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

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

## INTRODUCTION

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

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

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

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

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

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

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

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

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

Locality, date of sampling (from the Czech Republic unless otherwise noted)	Host tree	Stage of life cycle
Central Bohemia, Křivoklát region, Kohoutov near Zbiroh, 49° 55' 25" N, 13° 46' 14" E, March 1997	<i>Quercus petraea</i> (4 trees)	<ul style="list-style-type: none"> <li>• 50 larvae after wintering (50 galleries)</li> <li>• 20 adults before emergence</li> </ul>
Central Bohemia, Křivoklát region, Mlynářův luh near Karlova Ves, 49° 59' 31" N, 13° 51' 06" E, July 1997	<i>Q. petraea</i>	<ul style="list-style-type: none"> <li>• 100 adults in generation feeding (70 galleries)</li> <li>• 30 eggs</li> </ul>
Central Bohemia, Křivoklát region, Vlastec near Zbiroh, 49° 55' 40" N, 13° 48' 30" E, October 1997	<i>Q. petraea</i>	<ul style="list-style-type: none"> <li>• 100 larvae before wintering (100 galleries)</li> </ul>
Central Bohemia, Libický luh near Velký Osek, 50° 06' 10" N, 15° 10' 15" E, April 1998	<i>Q. polycarpa</i>	<ul style="list-style-type: none"> <li>• 25 larvae after wintering (25 galleries)</li> <li>• 25 adults before emergence (25 galleries)</li> </ul>
Central Bohemia, Libický luh near Velký Osek, 50° 06' 10" N, 15° 10' 15" E, July 1998	<i>Q. robur</i>	<ul style="list-style-type: none"> <li>• 25 young larvae (25 galleries)</li> <li>• 25 dead females after oviposition (25 galleries)</li> </ul>
Central Bohemia, Libický luh near Velký Osek, 50° 06' 10" N, 15° 10' 15" E, October 1998	<i>Q. robur</i>	<ul style="list-style-type: none"> <li>• 60 larvae before wintering (50 galleries)</li> </ul>
Central Bohemia, Libický luh near Velký Osek, 50° 06' 10" N, 15° 10' 15" E, June 1999	<i>Q. robur</i>	<ul style="list-style-type: none"> <li>• 30 males in maturation feeding (30 galleries)</li> </ul>
North Bohemia, Louny, near Břínkov, 50° 16' 48" N, 13° 47' 34" E, November 2000	<i>Q. robur</i>	<ul style="list-style-type: none"> <li>• 20 larvae before wintering</li> </ul>
South Bohemia, Bohemian Forest, Vydra river region, near Horní Hrádky, 49° 04' 24" N, 13° 30' 30" E, October 2001	<i>Q. robur</i>	<ul style="list-style-type: none"> <li>• 20 larvae before wintering</li> </ul>
Slovak Republic, Central Slovakia, Muránska planina Plateau, Šiance hill near Muráň, 48° 46' 10" N, 20° 04' 30" E, July 2002	<i>Q. dalechampii</i>	<ul style="list-style-type: none"> <li>• 35 larvae after wintering</li> <li>• 17 adults before emergence</li> </ul>
North Bohemia, Louny, near Hřivice, 50° 17' 03" N, 13° 44' 00" E, January 2003	<i>Fagus sylvatica</i>	<ul style="list-style-type: none"> <li>• 10 wintering larvae</li> <li>• 7 wintering adults</li> </ul>

## MATERIALS AND METHODS

## Localities and material

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

## Sampling and isolation

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

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

Testing *Geosmithia* strains for cellulase production

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

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

## RESULTS AND DISCUSSION

## General results

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

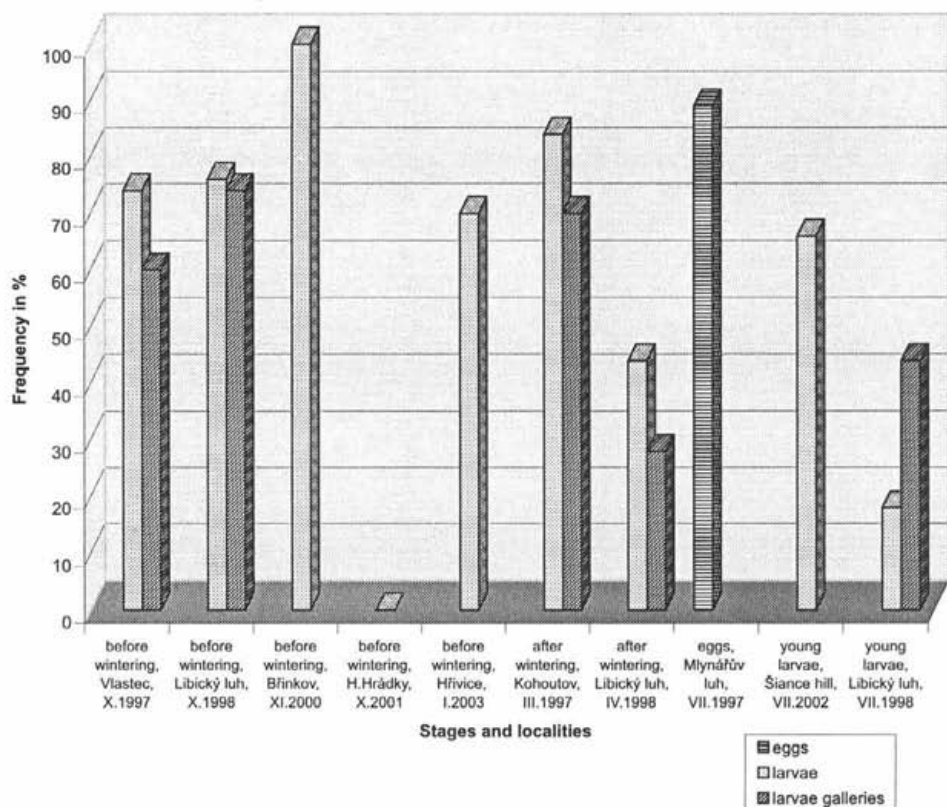


Fig. 1. Occurrence of *Geosmithia* on eggs and larvae of *Scolytus intricatus* and in larvae galleries in the studied localities.

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

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

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



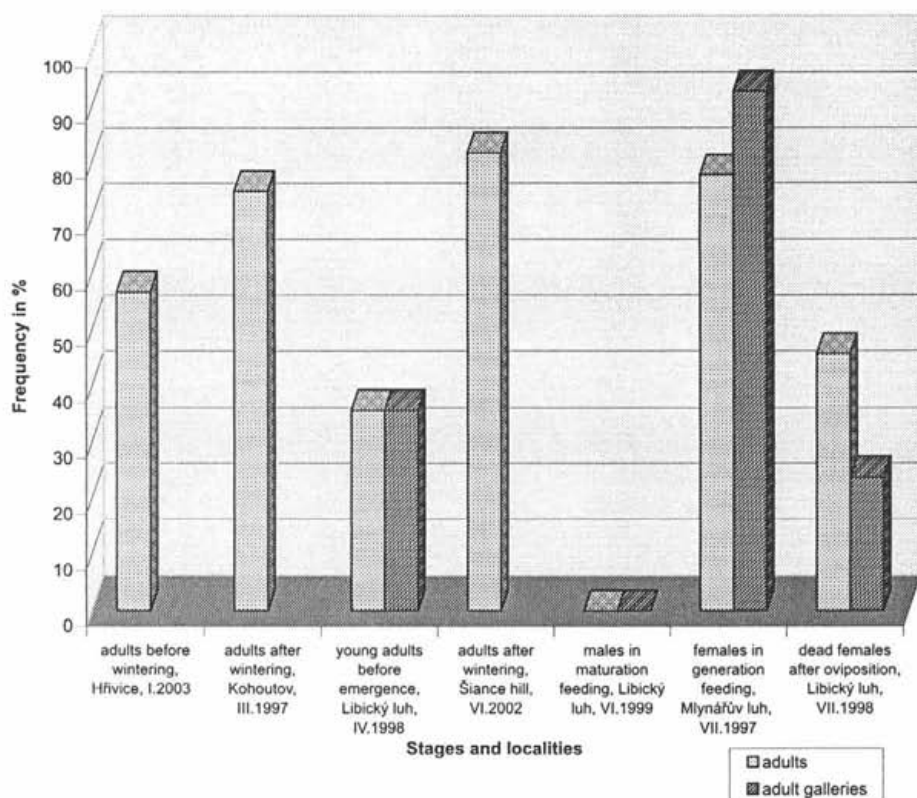


Fig. 2. Occurrence of *Geosmithia* on adults of *Scolytus intricatus* and in their galleries in the studied localities.

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

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

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

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

### Geosmithia identification

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

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

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

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

The first group (Figs. 3 and 4), represented by strains AK 47/98, AK 119/98, AK 120/98, AK 128/97, and AK 142/98, had whitish colonies, a velutinous to sometimes crustose or slightly lanose surface, monoverticillate to quaterverticillate penicilli, and cylindrical conidia ( $3.8-5.0 \times 2.0-2.5 \mu\text{m}$ ).

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

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

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

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

## Cellulose degradation

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

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

## Ecology and occurrence

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

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

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

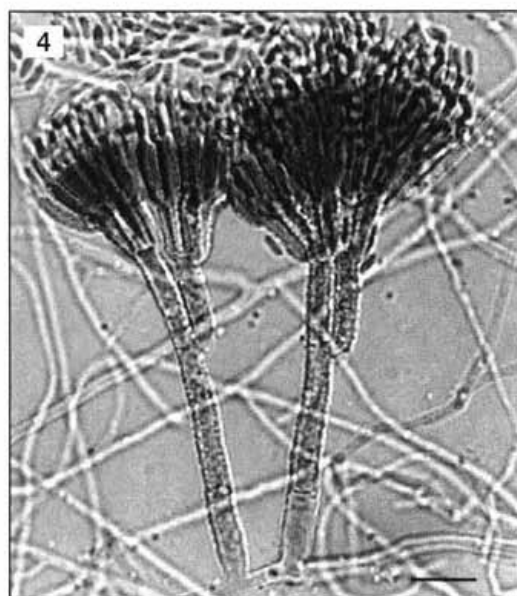
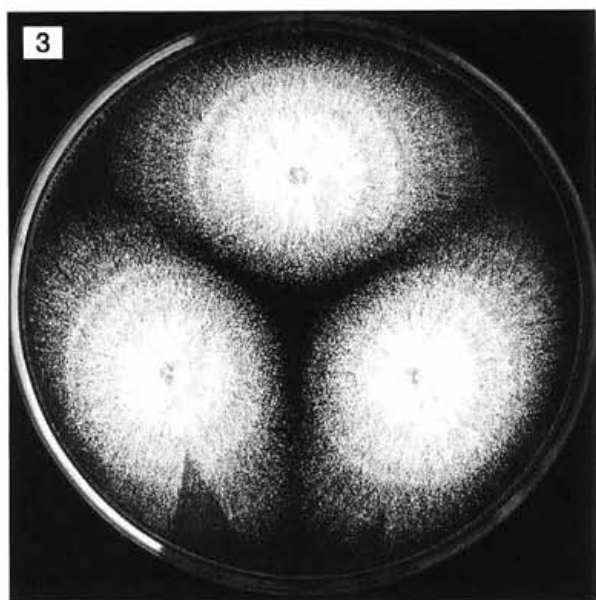


Fig. 3. Colonies on MEA after 14 days at 25 °C, strain AK 128/97.

Fig. 4. Conidiophores with conidia, strain AK 142/98 (Nomarski contrast). Bar = 10 µm.

Figs. 3 and 4. *Geosmithia* sp. (group 1)

Photo A. Kubátová

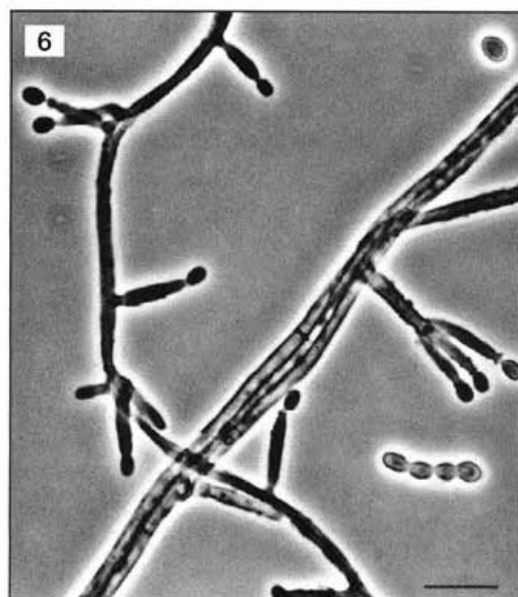
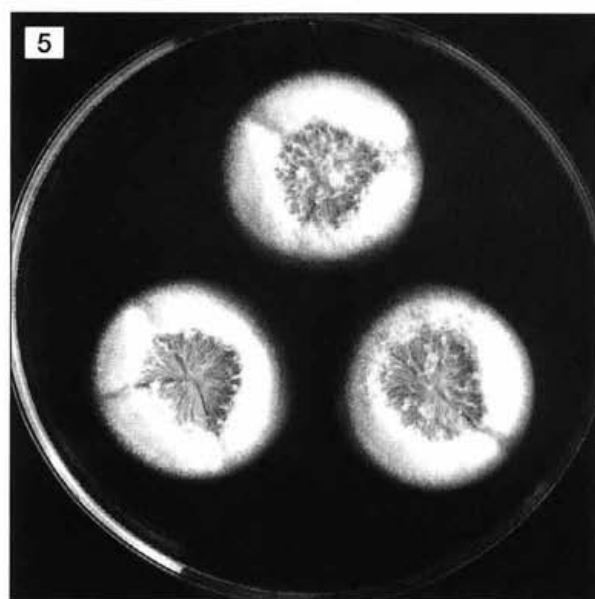


Fig. 5. Colonies on MEA after 14 days at 25 °C, strain AK 48/98.

Fig. 6. Poorly branched conidiophore and single phialides with conidia, strain AK 48/98 (phase contrast). Bar = 10 µm.

Figs. 5 and 6. *Geosmithia* sp. (group 4)

Photo A. Kubátová

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

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

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

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

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

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

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

#### ACKNOWLEDGEMENTS

The authors thank P. Šrůtka (Czech University of Agriculture, Prague, Czech Republic) for providing infested material of oaks and for help with sample collecting, Z. Kozakiewicz (CABI Bioscience, United Kingdom), D. Tuthill and M. Christensen (University of Wyoming, USA) and M. Tomšovský (CCBS, Prague, Czech Republic) for providing reference fungal strains, and J. Strejček (Czech Entomological Society) for identification of the beetle *Rhaphitropis marchicus*.

The study was supported by the Grant Agency of the Czech Republic (Projects no. 203/97/0037 and no. 522/02/1206).

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