Mycol. Res. **109** (12): 1323–1336 (December 2005). © The British Mycological Society
doi:10.1017/S0953756205003965 *Printed in the United Kingdom.*

A complex of three new white-spored, sympatric, and host range limited *Geosmithia* species

Miroslav KOLAŘÍK[1,2], Alena KUBÁTOVÁ[1], Ivan ČEPIČKA[3], Sylvie PAŽOUTOVÁ[2] and Petr ŠRŮTKA[4]

[1] *Department of Botany, Faculty of Science, Charles University, Benátská 2, 128 01 Praha 2, Czech Republic.*
[2] *Institute of Microbiology CAS, 142 20 Praha 4, Czech Republic.*
[3] *Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic.*
[4] *Faculty of Forestry and Environment, Czech University of Agriculture, Kamýcká 1176, 165 21 Praha 6, Czech Republic.*

Received 16 December 2004; accepted 29 July 2005.

All hypocrealean species of the genus *Geosmithia* are anamorphic fungi with connections to bark beetles. *G. fassatiae*, *G. langdonii* and *G. obscura* are described as new sympatric species associated with *Scolytus carpini*, *S. intricatus* and *S. rugulosus* in Central Europe. The species represent a complex of three sister taxa with affinities to *G. flava* that may be distinguished by differences in morphology, unique RAPD patterns and by sequences of ITS region rDNA. Intraspecific variability and habitat specificity of new species is described and discussed. The high morphological, genetic and ecological uniformity suggest that these *Geosmithia* spp. are recently derived. A key to all accepted hypocrealean species of the genus is provided.

INTRODUCTION

Geosmithia is a polyphyletic genus with affinities to hypocrealean (*Hypocreales: Bionectriaceae*) and eurotialean (*Eurotiales: Trichocomaceae*) fungi (Ogawa, Yoshimura & Sugiyama 1997, Kolařík *et al.* 2004).

Hypocrealean *Geosmithia* species (*Geosmithia s. str.*, hereafter only as *Geosmithia*) are typical members of the mycobiota inhabiting galleries of many phloem-feeding bark beetles. Phloemophagous bark beetles are typically associated with specialised entomochorous ophiostomatoid fungi. However a large group of bark beetles (especially those infesting deciduous trees) is associated with species of *Geosmithia* while ophiostomatoid fungi are almost lacking (Kirschner 2001, Kubátová *et al.* 2004). Very little is actually known about the ecological role of these fungi. *Geosmithia* spp. are unable to utilize cellulose or lignin, and perhaps are common saprobes adapted to a nutrient-rich but very specific environment (Kubátová *et al.* 2004).

Geosmithia spp. diversified in the bark beetles' habitat (Kolařík 2002, Kolařík *et al.* 2004). Four *Geosmithia* species are known. Pitt's (1979) species *G. putterillii* encompasses three biological species: brown-coloured *G. pallida*, yellow-coloured *G. flava* and white-to yellow-coloured *G. putterillii s. str.* In the galleries of the fig bark beetle *Hypoborus ficus*, lilac-coloured species from the *G. lavendula* aggregate are dominant (M. Kolařík, unpubl.).

In the period 1997–2004, we found many white-spored *Geosmithia* strains in the mycobiota associated with various bark beetles infesting broad-leaved trees from numerous European localities. These strains do not fit any species description and some of them, those morphologically related and sharing beetle vectors, are here described as new species.

MATERIALS AND METHODS

Isolates

We studied 64 *Geosmithia s. str.* isolates (Table 1): (1) isolates of M.K., obtained during this study from six insect species (MK); (2) isolates of A.K. from oak bark beetle (Kubátová *et al.* 2004; AK); and (3) isolates from the Culture Collection of Fungi, Prague (CCF). One isolate from each of the four RAPD-types was used for DNA sequencing. An additional 11 isolates of *G. flava*, *G. lavendula*, *G. pallida*, and *G. putterillii*, listed by Kolařík *et al.* (2004), were used in RAPD analysis.

Cultivation and isolation

Malt agar 2% (MA2) and 4% (MA4) were made from: 1 l brewery malt 2 °Balling, or 1l 4 °Balling and agar 15 g l^{-1}, modified after Fassatiová (1986); malt extract agar (MEA; Pitt 1979); Czapek yeast autolysate agar (CYA; Pitt 1979) supplemented with trace elements

Table 1. *Geosmithia* isolates used in the study.

Species/Original no./ GenBank accession no.[a]	Origin	Collection site/Year isolated (from Czech Republic unless otherwise noted)
Geosmithia fassatiae[b]		
CCF 3334 (=AK 14/93, ex-type, *AJ578482*[c]), AK 31/93	Dead branch of *Quercus pubescens*	Central Bohemia, Bohemian Karst, Srbsko-Pláně, 1993
CCF 3468 (=AK 73/98), AK 37/98, AK 48/98, AK 31/98	*Scolytus intricatus* on *Q. polycarpa*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1998
CCF 3346 (=MK 136), MK 137	*S. intricatus* on *Q. robur*	South Bohemia, Šumava Mts. National Park, Povydří region, near Horní Hrádky, 2001
CCF 3351 (=MK 143), MK 251	*S. intricatus* on *Q. dalechampii*	Slovakia, Muráň plain National Park, near Muráňský hrad castle, 2002
CCF 3469 (=MK 623), MK 627	*S. intricatus* on *Q. cerris*	Hungary, Mecsek Mts., Kőmlő, SW near Szurel, 2003
AK 209/97, AK 210/97, AK 211/97	*S. intricatus* on *Q. petraea*	Central Bohemia, Křivoklát region, Vlastec hill, 1997
AK 43/97	*S. intricatus* on *Q. petraea*	Central Bohemia, Křivoklát region, Zbiroh, Kohoutov Nature Reserve, 1997
AK 106/97, AK 105/97	*S. intricatus* on *Q. petraea*	Central Bohemia, Křivoklát region, Karlova Ves, Mlynářův luh, 1997
AK 147/98, AK 206/98, AK 207/98, AK 208/98, AK 209/98	*S. intricatus* on *Q. robur*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1998
MK 140	*S. rugulosus* on *Malus domestica*	North Bohemia, Louny, Opočno, 2001
MK 388	Cerambycid larva associated with *S. intricatus* feedings on *Fagus sylvatica*	North Bohemia, Louny, near Hřivice, 2003
Geosmithia langdonii, RAPD-type IX 'crustosa'		
CCF 3332 (=AK 142/98, ex-type, *AJ578481*), AK 120/98, AK 203/98, AK 204/98, AK 205/98	*S. intricatus* on *Q. robur*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1998
CCF 3471 (=MK 236)	*S. intricatus* on *Q. dalechampii*	Slovakia, Muráň plain National Park, Muráň, near Muráňský hrad castle, 2002
AK 207/97	*S. intricatus* on *Q. petraea*	Central Bohemia, Křivoklát region, Vlastec hill, 1997
AK 44/97	*S. intricatus* on *Q. petraea*	Central Bohemia, Křivoklát region, Zbiroh, Kohoutov Nature Reserve, 1997
AK 110/97, AK 111/97, AK 128/97	*S. intricatus* on *Q. petraea*	Central Bohemia, Křivoklát region, Karlova Ves, Mlynářův luh, 1997
AK 46/98, AK 47/98, AK 74/98	*S. intricatus* on *Q. polycarpa*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1998
MK 32	*S. intricatus* on *Q. robur*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1999
MK 110	*S. intricatus* on *Q. robur*	North Bohemia, Louny, near Nová Ves, 2000
MK 53, MK 84	Larvae of *Agrilus*. sp. (*Coleoptera: Buprestidae*) associated with *S. intricatus* feedings on *Q. robur*	Eastern Bohemia, Přelouč, near Seník, 2000
MK 116	*S. intricatus* on *Q. robur*	North Bohemia, Louny, near Břinkov, 2000
Geosmithia langdonii, RAPD-type IX 'ramosa'		
CCF 3339 (=AK 141/98), AK 125/98, AK 140/98,	*S. intricatus* on *Q. robur*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1998
CCF 3343 (=MK 111)	*S. intricatus* on *Q. robur*	North Bohemia, Louny, near Nová Ves, 2000
AK 30/98	*S. intricatus* on *Q. polycarpa*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1998
MK 21, MK 41	*S. intricatus* on *Q. robur*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1999
Geosmithia langdonii, RAPD-type X 'albolanosa'		
CCF 3338 (=MK 127, *AJ578480*)	*S. intricatus* on *Q. robur*	North Bohemia, Louny, near Nová Ves, 2000
CCF 3470 (=MK 139)	*S. rugulosus* on *Malus domestica*	North Bohemia, Louny, Opočno, 2001
CCF 3345 (=AK 119/98), AK 143/98	*S. intricatus* on *Q. robur*	Central Bohemia, Velký Osek, Libický luh Nature Reserve, 1998
MK 339, MK 343	*S. carpini* on *Carpinus betulus*	Central Bohemia, Bohemian Karst, SvatVelký Jan pod Skalou, 2003
MK 365, MK 371a	*Taphrorychus bicolor*, associated with *S. intricatus* feedings on *Fagus sylvatica*	North Bohemia, Louny, near Hřivice, 2003
Geosmithia obscura[d]		
CCF 3422 (=MK 86, ex-type, *AJ784999*), MK 73	*S. intricatus* on *Q. robur*	North Bohemia, Louny, near Břinkov, 2000

Table 1. (*Cont.*)

Species/Original no./ GenBank accession no.[a]	Origin	Collection site/Year isolated (from Czech Republic unless otherwise noted)
CCF 3425 (=MK 616)	*S. carpini* on *C. betulus*	Hungary, Bakony range, Vinye near of Fodöfö
CCF 3424 (=MK 391),	*T. bicolor*, associated with *S. intricatus*	
CCF 3423 (=MK 387)	feedings on *Fagus sylvatica*	North Bohemia, Louny, near Hřivice, 2003

[a] Sources: AK, personal collection of A. Kubátová (maintained in CCF), Prague; CCF, Culture Collection of Fungi, Prague; CCM, Czech Collection of Microorganisms, Brno; MK, personal collection of corresponding author (maintained in CCF).

[b] The species identity as revealed by RAPD analysis in the course of this study.

[c] GenBank accession nos are printed in italics.

[d] Strain MK 952 *G. obscura* (on *S. carpini* on *C. betulus*, Mirošovice near Prague, Central Bohemia, Sept. 2004, *M. Kolařík*), is not included in RAPD analysis, but morphologically is identical to the ex-type strain.

(0.001 % $ZnSO_4 . 7H_2O$ and 0.0005 % $CuSO_4 . 5H_2O$); and potato carrot agar (PCA; Fassatiová 1986) were used.

Broadleaved trees infested by subcorticolous insects were sampled during 1997–2003 and fungal isolates from 13 localities in the Czech Republic, one Slovakia, and two in Hungary were used (Table 1). Adult subcorticolous insects (i.e. living adults before emergence and adults in maturation feeding stage) or their larvae were excised from bark. Five unwashed living or dead adults, larvae or detritus from each sample were placed directly onto each of 15 Petri dishes (MA2). The wood specimens were incubated in moist chambers for one month at room temperature and fungal growth was observed directly in the insect galleries. Isolated fungi were inoculated on CYA and MEA for identification. We determined the percentage of each white-spored *Geosmithia* to elucidate substrate specificity.

Dried specimens of the holotypes of the new species are deposited in the Mycological Department, National Museum, Prague (PRM). Ex-holotype and other representative strains were freeze-dried in skimmed milk and are deposited in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague (Kubátová & Kolařík 2005).

Cultural and morphological characteristics, DNA analysis and phylogenetic analysis

The procedures of Kolařík *et al.* (2004) were used. The identification media were MEA and CYA. Conidiophore and substrate conidia ontogenesis was observed in plate cultures (Cole, Nag Raj & Kendrick 1969) that were incubated in daylight for the best development of conidiophore roughness. Micromorphology was studied on 7 d old colonies grown on MEA, the conidiophores were taken from margins and near colony centres, as well as from areas differing in texture. Older conidiophores from the 10 d colonies were observed. 20 randomly selected conidia were measured from each strain. The substrate mycelium from the colony margins was studied for presence of substrate conidia. Colour names are given from Kornerup & Wanscher (1981).

DNA isolation, RAPD analysis and ITS region amplification have been described (Kolařík *et al.* 2004). The best reproducibility of PCR reactions was obtained using DNA from 1d old mycelium (0.1–0.5 g).

A data set containing 14 ITS region sequences obtained from seven *Geosmithia* species was created. Sequences were aligned using ClustalX 1.81 (Thompson *et al.* 1997) and the alignment was then manually edited in the BioEdit 5.0.9 (Hall 1999).

Phylogenetic trees were constructed using the maximum likelihood, maximum parsimony, and Fitch-Margoliash with Logdet distance methods implemented in PAUP* 4.0b10 (Swofford 1998), and by the Bayesian method implemented in the program MrBayes 3.0 (Huelsenbeck & Ronquist 2001). The best substitution model for maximum likelihood, i.e. $TrN+\Gamma$ with gamma shape parameter 0.0074, was determined by the hierarchical nested likelihood ratio test implemented in Modeltest 3.06 (Posada & Crandall 1998). A heuristic search with ten replicates of starting tree construction by random taxon addition followed by the TBR branch swapping was used. Bootstrapping was performed with 100 replicates. For the maximum parsimony and distance methods, the heuristic searches with 10 replicates and random taxon addition were used. Bootstrapping was performed with 1000 replicates. In order to lower the violation of the rate homo geneity across sites assumptions, constant positions were excluded from the alignment before performing the Logdet distance analysis (Waddell & Steel 1997). In MrBayes, base frequencies, rates for six different types of substitutions, number of invariant sites, and shape parameter of the gamma correction for rate heterogeneity with four discrete categories were allowed to vary. 5×10^6 generations of the Markov Chain Monte Carlo were run with four simultaneous chains and heating temperature 0.2. First 500 trees were discarded as the burn in.

RESULTS

Morphology

The presence of a peg foot and substrate conidia, as well as a typical sour-cream odour on MEA, is similar to other *Geosmithia* species (Kolařík *et al.* 2004).

Likewise, the odour on CYA is earthy and strong. A typical feature of *G. langdonii*, and especially of *G. obscura*, is the multiply branched conidiophores (6–8 branch points between stipes and conidium) that are all roughened (Figs 42, 46). However, in both species some multiply branched conidiophores occur, where their elements are thinner and smooth. These 'false-branched' conidiophores are aggregations of single bi- or terverticillate conidiophores, connected by their peg foot (Figs 25, 29, 43, 52). The peg foot is homologous with vegetative hypha and so this element has smooth and thinner cell wall. The real nature of conidiophore elements is best recognizable on very young conidiophores, preferably in slide culture. In older conidiophores, it is hard to distinguish between naturally branched and false-branched conidiophores (Figs 43, 52).

Phylogenetic analysis

RAPD analysis

A total of 307 reproducible fragments were amplified with four primers from 79 *Geosmithia* isolates.

Associations among the fungal isolates as revealed by UPGMA cluster analysis of the genetic distances are given in Fig. 1. Isolates were grouped into ten RAPD-types (groups where 90% of bands are constant). RAPD-types I–VIII (numbered according to Kolařík *et al*. 2004) represent currently accepted *Geosmithia* species. RAPD-types IX and X are grouped together with 100% bootstrap support. RAPD pattern generated with the primer OPA 02 did not show significant a difference between these two RAPD-types. These two RAPD-types have identical ITS sequence and are here treated as *G. langdonii*. RAPD-type XII showed significant genetical similarity to *G. langdonii* notably with the primer OPA 02 (25% shared bands). This RAPD-type is treated here as *G. obscura*. RAPD-type XI, *G. fassatiae* is most genetically distant and has low intraspecific variability.

ITS region analysis

Fig. 2 shows the maximum likelihood tree relating representatives of RAPD-types to the other accepted species of *Geosmithia*. All methods produce consistent and well-supported results and topology of trees constructed by different methods differ only in the internal branching of the *Geosmithia pallida* clade. Isolates of *G. lavendula*, *G. langdonii*, *G. obscura*, *G. flava*, and *G. fassatiae* created a robust clade. Sequences of CCF 3338 and CCF 3332 representing RAPD-types of *G. langdonii* are identical. All white-spored species are grouped together with *G. flava* in a strongly supported clade. In all trees, *G. fassatiae* is significantly grouped with two isolates of *G. flava*. Genetic distance between these two species calculated by Kimura two parameter model was 0.01471; this value was similar or smaller than those between *G. pallida* populations (0.00558–0.02243).

Substrate specificity

All three newly described species are sympatric and known only from bark beetles. We have studied phloem feeding bark beetles, from many populations during the seven years (Table 2), with *Geosmithia fassatiae, G. langdonii* and *G. obscura* isolated mainly from *Scolytus carpini*, *S. intricatus* and, more rarely, from *S. rugulosus*. *G. fassatiae* was also isolated from *S. mali*, which was studied only once. All three species (and most notably *G. langdonii*) are typical of *S. intricatus* and *S. carpini* galleries (together with *G. pallida*). These species occur rarely or not at all, in galleries of *S. rugulosus*, whereas *G. flava*, *G. pallida*, and the white-spored RAPD-type characterized by strains IMI 194089 and CCF 3335, prevail there.

TAXONOMY

Geosmithia langdonii Kolařík, Kubátová & Pažoutová, **sp. nov.** (Figs 3–14, 23–29, 36–39)

Etym.: Named for Raymond F. N. Langdon, of Australian mycologist (b. 1916), in recognition of his work.

Pars aversa coloniae culturarum CYA et MEA velutina vel lanosa pro parte; in 4% MEA temperatura 24 °C 40–60 mm diametro post 2 hebdomades crescentes, reversus luteus, luteo-brunneus vel ferrugineus; mycelium ex hyphis hyalinis, vel ferrugineus ad centra; conidiophorae superficie agari vel mycelio aerio exorientes, hyaliniae, penicilli fere biverticillati et terverticillati, symmetrice, raro asymmetrice et multo ramosus; conidiogenesis abundus vel moderatus, albidus; conidia cylindrica vel ellipsoidea, *ca* 4–5 × 2–2.5 μm, portata in longus ordinate catenis.

Typus: **Czech Republic**: *Bohemia*: Velký Osek, alt. 190 m, Libický luh Nature Reserve, ex dead adult of *Scolytus intricatus* in the inner bark of *Quercus robur*, 1998 July, *A. Kubátová* (PRM 901867 – holotypus; CCF 3332 (= AK 142/98) – ex-type culture; PRM 901868-901872 – isotypi). GenBank sequence ex-type AJ578481.

Conidiophores on MEA arising from the subsurface, surface or aerial mycelium, hyaline; *stipes* determinate, erect, (8–)20–100(–220) × 3–4 μm, or indeterminate, verrucose, septate, arising from peg foot or from initials suggesting foot-cells of *Aspergillus*; penicilli terminally bearing, biverticillate, more frequently terverticillate, quaterverticillate, or even more branched (up to six branch points between stipes and conidium), often symmetric, single conidiophores are sometimes aggregated to false-branched conidiophores with even more complexity (eight times branched); *rami* (first branch) of different size, often 14–17 × 3–4 μm; metulae in well defined verticils of 2–5, 7–10 × 3–4 μm, verrucose. *Conidiogenous cells* phialides, 9–12 × 2–3 μm, 3–15 per metula, typically cylindroidal without distinct neck, walls verruculose to verrucose, sometimes

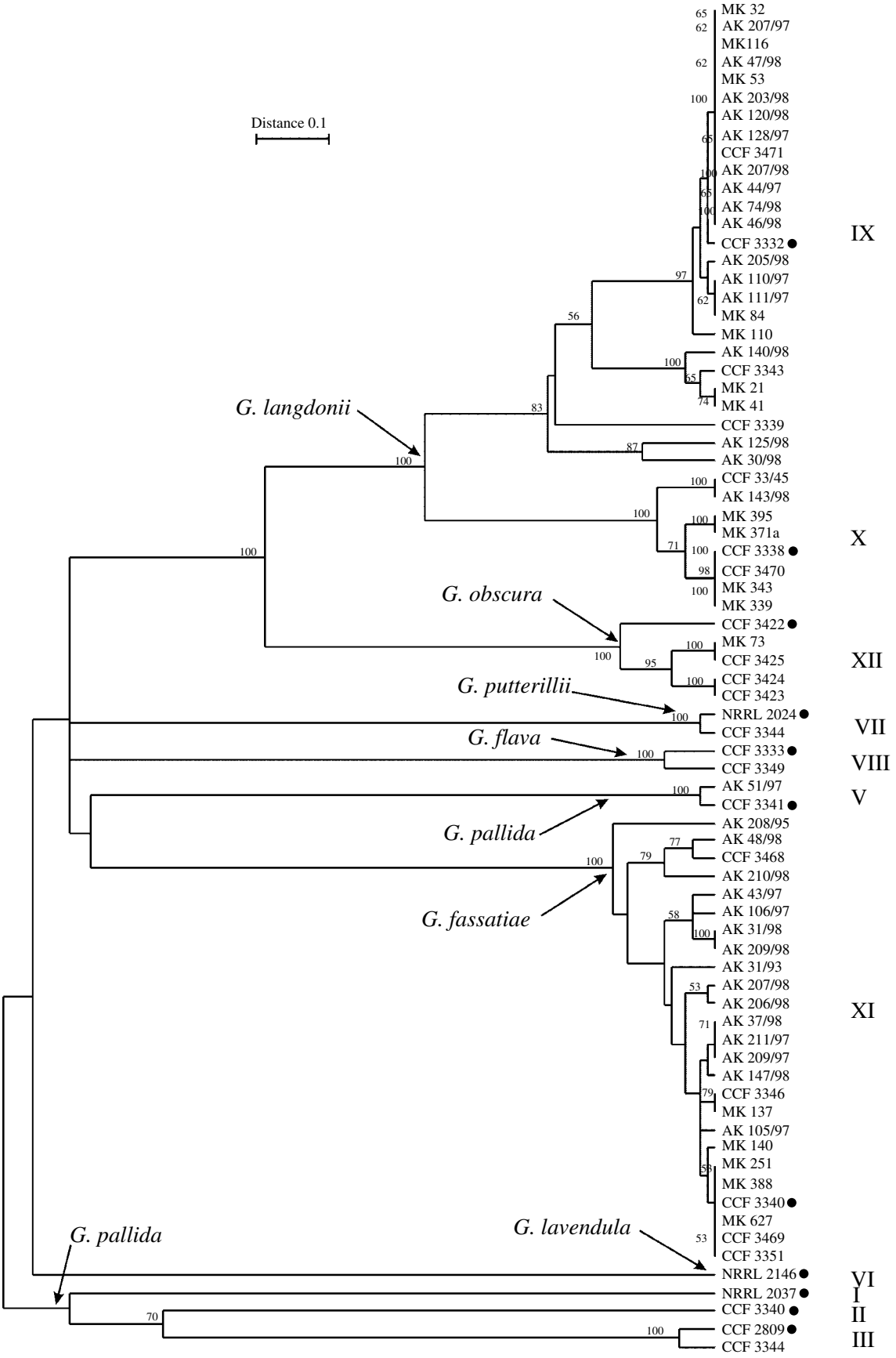


Fig. 1. UPGMA phenogram based on RAPD data from isolates belonging to *Geosmithia s. str*. Clades with less than 50% bootstrap support were collapsed to polytomies. **Bold** dots indicate taxa included in the rDNA tree (Fig. 2). The stability of these branches was evaluated by bootstrap analysis with 100 replications. Numbering of the RAPD-types is according Kolařík *et al*. (2004).

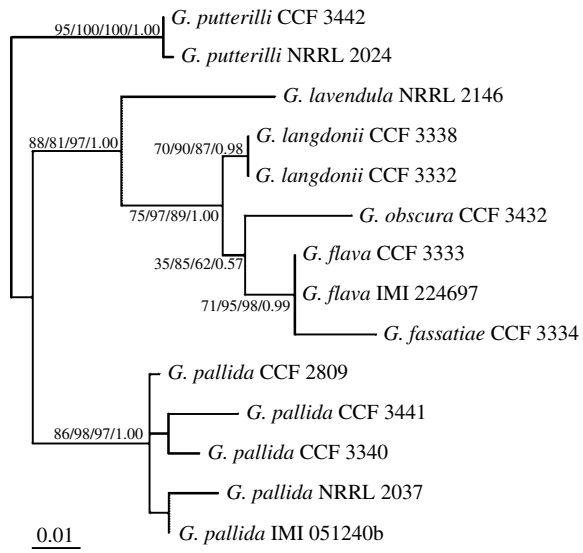


Fig. 2. Phylogenetic tree of the genus *Geosmithia* based on sequences of the ITS region. The alignment contained 562 positions from which 488 were constant. The tree was constructed by the maximum likelihood method using TrN+Γ model of substitution and is unrooted. Bootstrap values from LogDet, maximum parsimony, maximum likelihood, and Bayesian posterior probabilities, respectively, are shown at the nodes. Bootstrap values inside the *G. pallida* clade not shown. The scale bar represents one change per 100 positions.

proliferating; conidia cylindrical, ellipsoidal or broadly ellipsoidal, mostly (3–)4–5(–5.5) × (1.5)2–2.5(–3.5) μm; conidial chains to 500 μm in length, in well defined, persistent, parallel columns. *Conidia* on substrate very abundant, cylindrical, ellipsoidal or clavate, often truncate basally, 3–8 × 2–3 μm.

MEA, 24 °, 14 d: Colonies 40–60 mm diam, plane or radially furrowed, centre low or slightly raised, surface texture velutinous, with masses of penicilli forming crust of conidia to 500 μm deep, or in some areas overlaid by an aerial mycelium also bearing penicilli, or almost floccose without crustose pattern in some strain; margins narrow or lobate, submerged (to 5 mm broad); aerial and substrate mycelium hyaline or pale yellow (2A3), rusty (6E8) or olivaceous (notably in the colony centre by some isolates); substrate mycelium sparse, not forming tough basal felt; conidiogenesis heavy to moderate, uncoloured to pale (2A2) in older areas; exudate absent or clear and uncoloured; soluble pigment absent; reverse pale yellow (2A3), amber yellow (4B6) to rusty (6E8), sometimes with shades of rose or orange.

MEA, 37 °, 14 d: No growth.

CYA, 24 °, 14 d: Colonies 50–60(–70) mm diam, plane or radially furrowed, with centre raised (area to 50 mm height), often closely wrinkled in a cerebriform pattern especially in the centre; surface texture mostly velutinous, with areas consisting of thin felt-like network of hyphae in some isolates, crust of conidia is not so deep and crumbly as those on MEA; mycelium white; conidiogenesis depends on isolate, heavy in velutinous areas, irregular in felt-like areas, white to cream (4A3); exudate absent; soluble pigment absent; reverse dull yellow (3B3), pale orange (5A3) to orange-yellow or amber yellow (4B6).

Habitat: In bark beetle galleries and in surroundings phloem and wood. Known associations see Table 1. Typical fungus for *S. intricatus* galleries.

Distribution: Czech Republic, Slovakia, and Hungary.

Teleomorph: Unknown.

Intraspecific variability: *G. langdonii* is very complex species. Our isolates belong to two major RAPD-types, which show some phenotypic differences, but they lack ecological specificity and their ITS-rDNAs are identical.

G. langdonii RAPD-type IX: Isolates divided according to their phenotype (not supported by RAPDs) to two groups ('crustosa' and 'ramosa'), but strains showing intermediate characters between these two subgroups occur.

The first group, 'crustosa', has a generally velutinous colony texture on MEA, developing a continuous crust of conidial chains which break off as irregular masses when the culture dish is struck or tapped; mycelium hyaline; colony on CYA velutinous (but sometimes showing rudimentary fascicles at the colony margin), sporulation very heavy, white. The conidiophores are borne from subsurface hyphae, stipes only 8–50 × 3.5–4 μm, a biverticillate to terverticillate *penicillus*, regular, often symmetric, 20–50 × 10–20 μm, and *conidia* cylindrical to ellipsoidal, mostly 4 × 2 μm.

On MEA, the second group, 'ramosa', has a colony texture which is nearly velutinous, or velutinous with abundant aerial mycelium but often nearly floccose, the mycelium hyaline at the margins, becoming rusty (6E8) centrally, colony margin often submerged and large (to 15 mm wide), sporulation heavy; the conidial crust is not so deep and does not adhere in masses as those in previous group. On CYA the colony is similar to that of the previous group or, floccose in some areas. Conidia *en masse* cream (4A3). Conidiophores arise the surface or aerial hyphae, varying in the relation to their origin, some similar to the previous group, others are characteristically of indeterminate growth, multiply branched penicillus (to eight branch points) with higher number of elements in clusters, 'false branched' conidiophores often occur; the stipes are 30–150 × 3–4 μm; and the phialides originating at different level of the penicillus; *conidia* cylindrical or ellipsoidal, 4–4.5 × 2 μm.

G. langdonii RAPD-type X '*albolanosa*': Colony texture on MEA generally velutinous to floccose, sometimes with tall funicules centrally, mycelium white, sporulation moderate to low; colony on CYA zonate, with sandy yellow sporulating areas and areas consisting of a thin, felt-like network of sterile mycelium; *conidiophores* borne from the surface or aerial hyphae. The stipes are of irregular and often indeterminate length, 50–220 × 3–4 μm; the penicillus is monoverticillate, biverticillate or terverticillate, rarely

Table 2. Bark beetles examined for presence (+, or number) or absence (−) of *Geosmithia* fungi and percentage occurrence of *G. fassatiae* (GF), *G. langdonii* (GL), and *G. obscura* (GO).

Host tree and geographical origin[a]	Bark beetle species (number of populations studied)[b]	GF[c]	GL[c]	GO[c]	Other *Geosmithia* spp.
C. betulus (CZ)	*Scolytus carpini* (2)	1 50%	1 50%	1 50%	+ (100%)
C. betulus (HU)	*S. carpini* (1)	−	−	+	+
Quercus spp., *Fagus sylvatica* (CZ, SK)	*S. intricatus* (16)	8 50%	15 94%	1 6%	+ (100%)
Quercus spp. (HU)	*S. intricatus* (3)	0	3 100%	0	+ (100%)
Various trees and shrubs from *Rosaceae* (CZ, SK)	*S. rugulosus* (10)	3 30%	1 10%	0 0%	+ (100%)
Cerasus sp., *Prunus* spp. (HU)	*S. rugulosus* (4)	1 25%	0	0	+ (100%)
Armeniaca vulgaris, Prunus sp. (CR)	*S. rugulosus* (2)	−	−	−	+ (100%)
Malus domestica (CZ)	*S. mali* (1)	+	−	−	+
Amygdalus communis (CR, SY)	*S. amygdali* (2)	−	−	−	+ (100%)
Ulmus spp. (CZ, IT)	*Pteleobius vittatus* (4), *S. ensifer* (4), *S. multistriatus* (7), *S. pygmaeus* (1) and *S. scolytus* (3)	−	−	−	+ (100%)
Tilia spp. (CZ, SK, HU)	*Ernoporus tiliae* (12)	−	−	−	+ (100%)
Fraxinus spp. (CZ, HU, SK, FR, TU)	*Hylesinus fraxini* (13) (syn. *Leperisinus fraxini*)	−	−	−	+ (100%)
	H. orni (3) (syn. *L. orni*)	−	−	−	+ (100%)
	H. crenatus (2)	−	−	−	−
	H. toranio (syn. *H. oleiperda*) (2)	−	−	−	+ (100%)
Abies alba (SK)	*Cryphalus piceae* (1)	−	−	−	+
Fagus sylvatica (CZ, SK)	*Ernoporicus fagi* (4)	−	−	−	+ (25%)
	Taphrorychus bicolor (1)	−	−	−	−
Chamaecyparis pisifera, *Juniperus* spp. (CZ, CR, SL, IT)	*Phloesinus thujae* (7)	−	−	−	+ (100%)
Sarothamnus scoparius (CZ)	*Phloeophthorus rhododactylus* (1)	−	−	−	+
Laurus nobilis (FR)	*Lyparthrum colchicum* (1)	−	−	−	+
Olea europea (MED)	*Phloeophthorus pubifrons* (1)	−	−	−	−
	Phloeotribus scaraboides (5)	−	−	−	+ (60%)
Ficus carica (MED)	*Hypoborus ficus* (40)	−	−	−	+ (97%)
Pistacia spp. (MED)	*Chaetoptelius vestitus* (4)	−	−	−	+ (100%)
	Carphoborus perrisi (3)	−	−	−	+ (100%)
Hedera helix (CZ, IT)	*Kissophagus hedereae* (4)	−	−	−	+ (50%)
Clematis vitalba (HU)	*Xylocleptes bispinus* (5)	−	−	−	−
Euphorbia characias, *E. dendroides* (CR, SP)	*Thamnurgus* spp. (2)	−	−	−	−

[a] CZ, Czech Republic; CR, Croatia; IT, Italy; FR, France; HU, Hungary; SK, Slovak Republic; SL, Slovenia; SP, Spain; SY, Syria; TU, Turkey; and MED, various countries in Mediterranean.

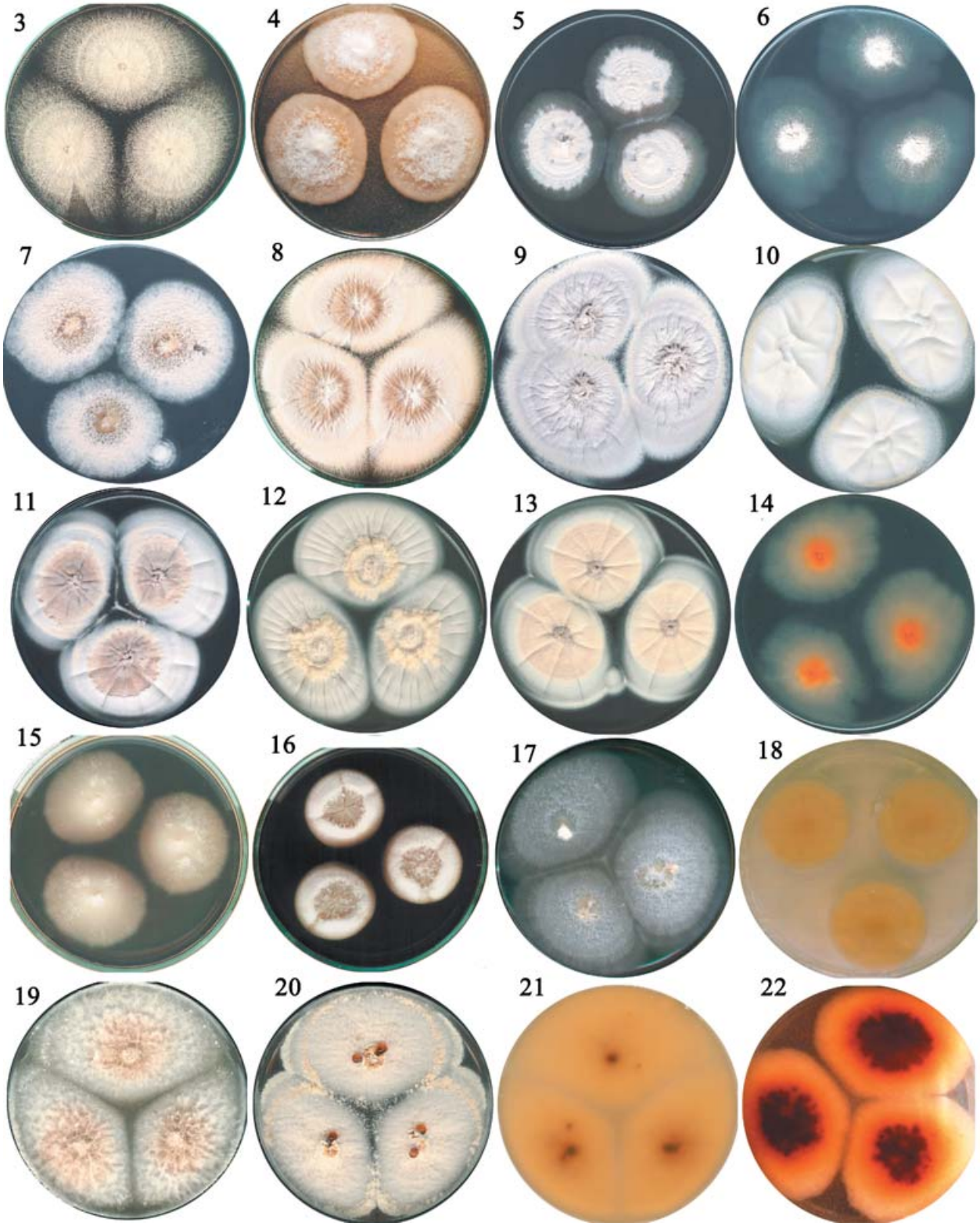
[b] The total number of bark beetles populations studied. Each population represents beetles from one tree sampled at one time in one sampling site.

[c] Number and percentage indicate the proportion of samples where the fungus was found. Only samples with a sufficient number of insect samples (at least 75 adults or larvae from one sample) were used for semi-quantitative analysis of GF, GL and GO host specificity.

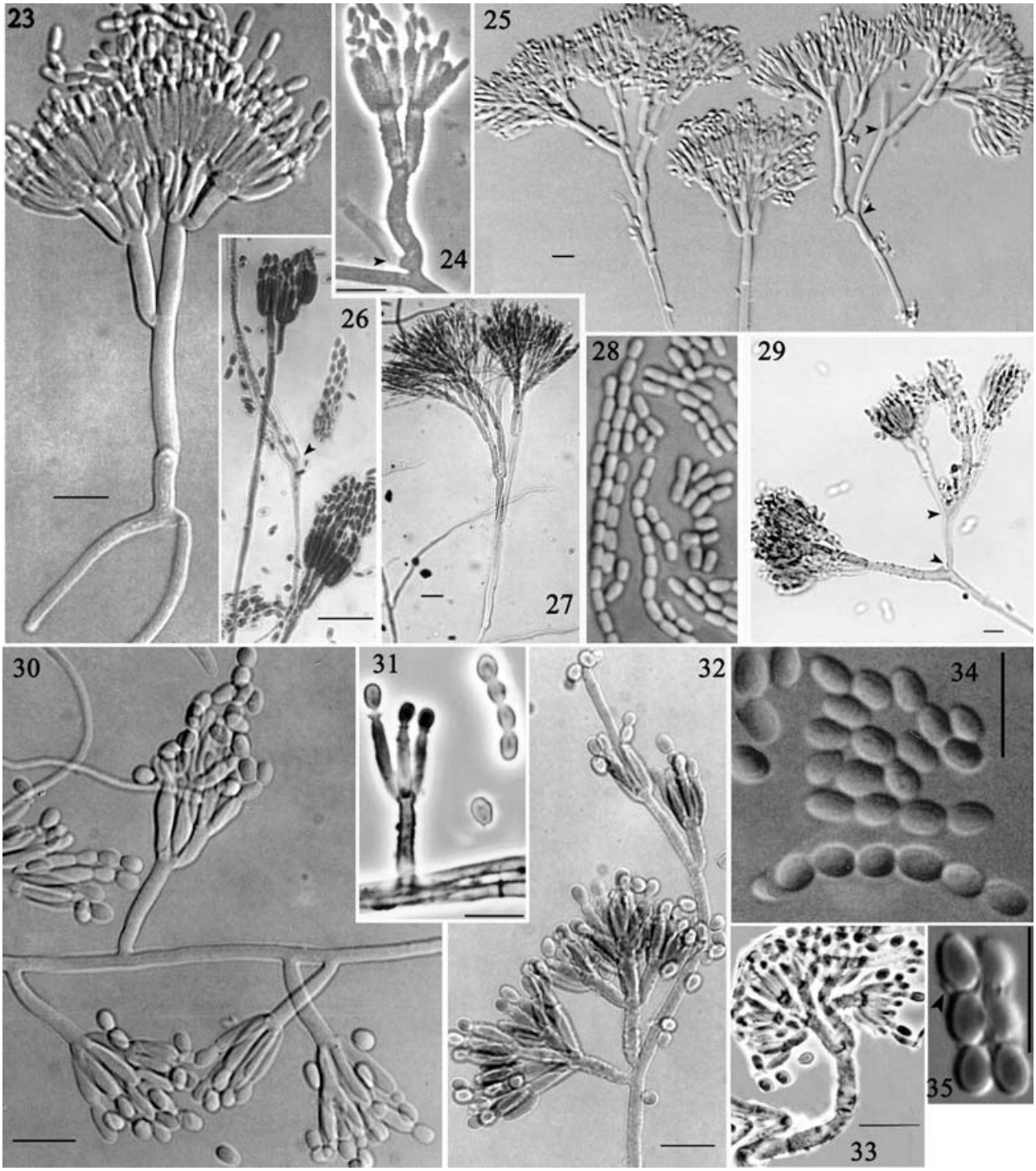
more complex, often asymmetric, with fewer elements less appressed than those of previous groups, sometimes metulae and phialides are mixed. The conidia are cylindrical, ellipsoidal to broadly ellipsoidal, variable in size but broader than those previous RAPD-type, (4)4.5–5(5.5) × 2.0–2.5(4.0) μm. This group can only be recognised by RAPD, the phenotype is highly variable. For cultures examined see Table 1.

Distinctive features: *G. langdonii* is the most difficult of the known *Geosmithia* spp. to define, due to a high intraspecific variability. Whitish colonies, the arrangement of the conidial columns, and the absence of close textured basal felt on both media, are striking characteristics which distinguish *G. langdonii* from *G. pallida*. The absence of yellow shades of sporulation on both media is distinct from the colouration of *G. flava* colonies.

In its typical crustose form, *G. langdonii* is readily recognisable; conidial chains form a whitish crust of conidia adherent in masses on MEA, but on CYA produce typically a cerebriform pattern of colonies and the conidia are mostly cylindrical, 4 × 2 μm. Some strains with less branched penicilli form the group 'ramosa' and resemble *G. putterillii*, by colony character. But *G. putterillii* has conidiophore less branched, and longer and thinner conidia (4–4.5 × 1.5 μm).



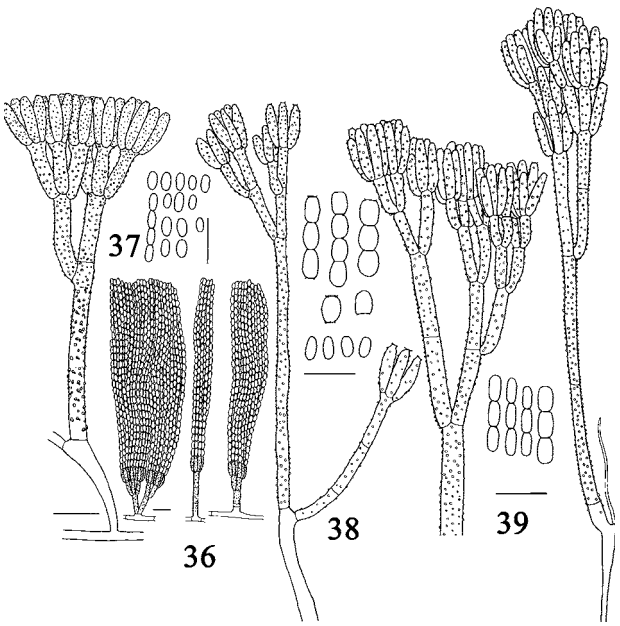
Figs 3–14. *Geosmithia langdonii*. Colonies on MEA and CYA at 24 °C, after 14 d in the dark. **Fig. 3.** CCF 3332 (MEA). **Fig. 4.** CCF 3338 (MEA). **Fig. 5.** MK 371 (MEA). **Fig. 6.** MK 117 (MEA). **Fig. 7.** AK 207/97 (MEA). **Fig. 8.** CCF 3332 (CYA). **Fig. 9.** AK 207/97 (CYA). **Fig. 10.** CCF 3338 (CYA). **Fig. 11.** MK 365 (CYA). **Fig. 12.** AK 30/98 (CYA). **Fig. 13.** AK 125/98 (CYA). **Fig. 14.** CCF 3343 reverse, (MEA). **Figs 15–18.** *Geosmithia fassatiae*. Colonies on MEA and CYA at 24 °C, after 14 d in the dark. **Fig. 15.** CCF 3334 (MEA). **Fig. 16.** CCF 3334 (CYA). **Fig. 17.** CCF 3470 (MEA). **Fig. 18.** CCF 3334 (MEA), reverse. **Figs 19–22.** *Geosmithia obscura* CCF 3422. Colonies on MEA and CYA at 24 °C, after 14 d in the dark. **Fig. 19.** MEA. **Fig. 20.** CYA. **Fig. 21.** MEA, reverse. **Fig. 22.** MEA, reverse of the freshly isolated strain.



Figs 23–35. Light micrograph of penicilli and conidia on MEA at 24 °C, 7 d in the dark, mounted in Melzer's reagent (Figs 23, 25, 36) or in lactic acid with cotton blue (others). **Figs 23–29.** *Geosmithia langdonii*. **Figs 23, 24.** Penicilli of CCF 3332, showing collapsing hypha (arrow) arising from peg foot. **Figs 25, 29.** Penicilli of CCF 3343, false branched penicilli consisted of entire penicilli are connected with their peg foots (arrows). **Fig. 26.** Penicilli of CCF 3338, showing the peg foot (arrow). **Fig. 27.** Atypical monilioid penicillus of CCF 3332. **Fig. 28.** Conidia of CCF 3332. Bar = 10 μm. **Figs 30–35.** *G. fassatiae*. **Figs 30–32.** Penicillus of CCF 3334. **Fig. 33.** Terverticillate penicillus of AK 48/98. **Figs 34–35.** Conidia of CCF 3334, showing collarettes (arrow). Bar = 10 μm.

G. langdonii 'ramosa' is morphologically similar to *G. obscura* and the delimiting characters of this two species are summarized below.

The RAPD-type X 'albolanosa' is morphologically similar to *G. fassatiae*. These two species both produce a similar colony texture, the conidia and also relatively simple branched conidiophores. However, in colonies of *G. langdonii* 'albolanosa', velutinous areas occur. The micromorphological features are more distinctive: the stipes are longer the penicillus is often more branched, and the conidia are less oval than those of *G. fassatiae*. Under low magnifications, the longer and



Figs 36–39. *Geosmithia langdonii*. **Figs 36, 38.** Penicilli, conidia and conidial chains of *G. langdonii* 'crustosa' (CCF 3332). **Figs 38, 39.** Penicilli and conidia of *G. langdonii* 'albolanosa'. **Fig. 38.** CCF 3345. **Fig. 39.** CCF 3338. Bars = 10 μm.

often parallel conidial chains of *G. langdonii* are also distinctive.

Substrate conidia were found only in *G. langdonii* isolates. Their occurrence can vary by strain (e.g. in *G. flava*, Kolařík *et al*. 2004) and taxonomical value of this character may be narrow, but we use it to set *G. langdonii* apart from *G. fassatiae* and *G. obscura*.

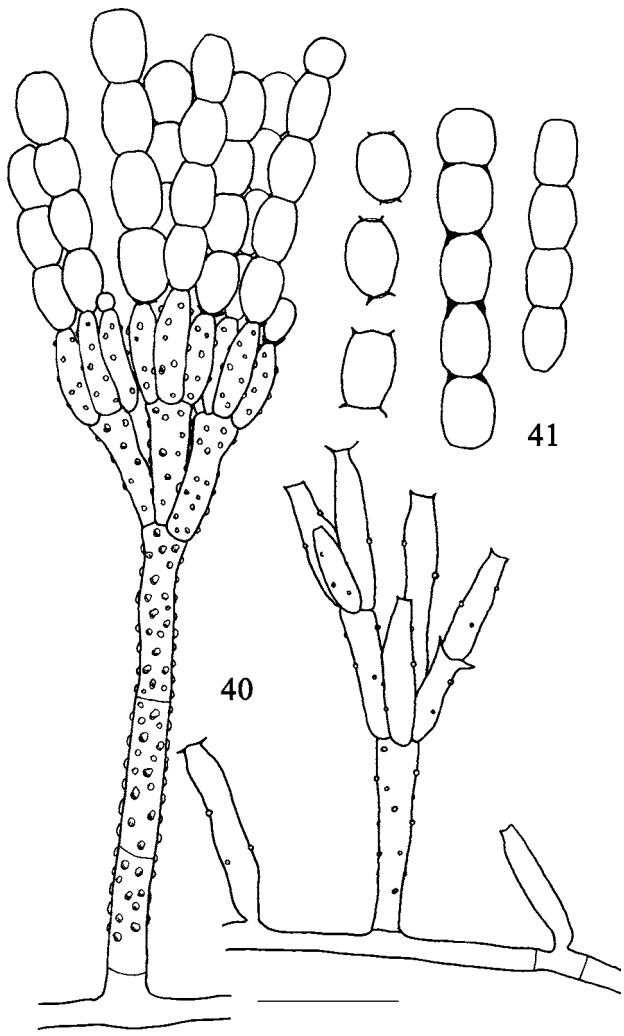
Geosmithia fassatiae Kubátová, Kolařík & Pažoutová, **sp. nov.** (Figs 15–18, 30–35, 40–41)

Etym.: Named for Olga Fassatiov, Czech mycologist (b. 1924), whose research on filamentous fungi contributed greatly to the development of Czech mycological science.

Pars aversa coloniae culturarum CYA et MEA gravis albolanosa; in 4% MEA temperatura 24 °C 45–50 mm diametro post 2 hebdomades crescentes, reversus palidus luteus; conidiogenesis sparsus, albidus, conidiophora brevis, in mycelio aerio formata; penicilli fere monoverticillati vel biverticillati, raro diferent; conidia ellipsoidea vel plusminusve subspaerica, *ca* 3.5 × 2.5–3 μm.

Typus: **Czech Republic**: *Bohemia*: Bohemian Karst, Srbsko-Pláně, alt 280 m, ex branch of *Q. pubescens*, 1993, *A. Kubátová* (PRM 901861 – holotypus; CCF 3334 (= AK 14/93) ex-type culture; PRM 901862–901866 – isotypi). GenBank sequence ex-type AJ578482.

Conidiophores on MEA arising from aerial hyphae, stipes erect, 8–50 × 2–5 μm, rarely taller, smooth to verrucose, septate, without a peg foot; penicilli terminally bearing a small number of elements, typically monoverticillate to biverticillate, penicilli arising near the colony base sometimes more branched



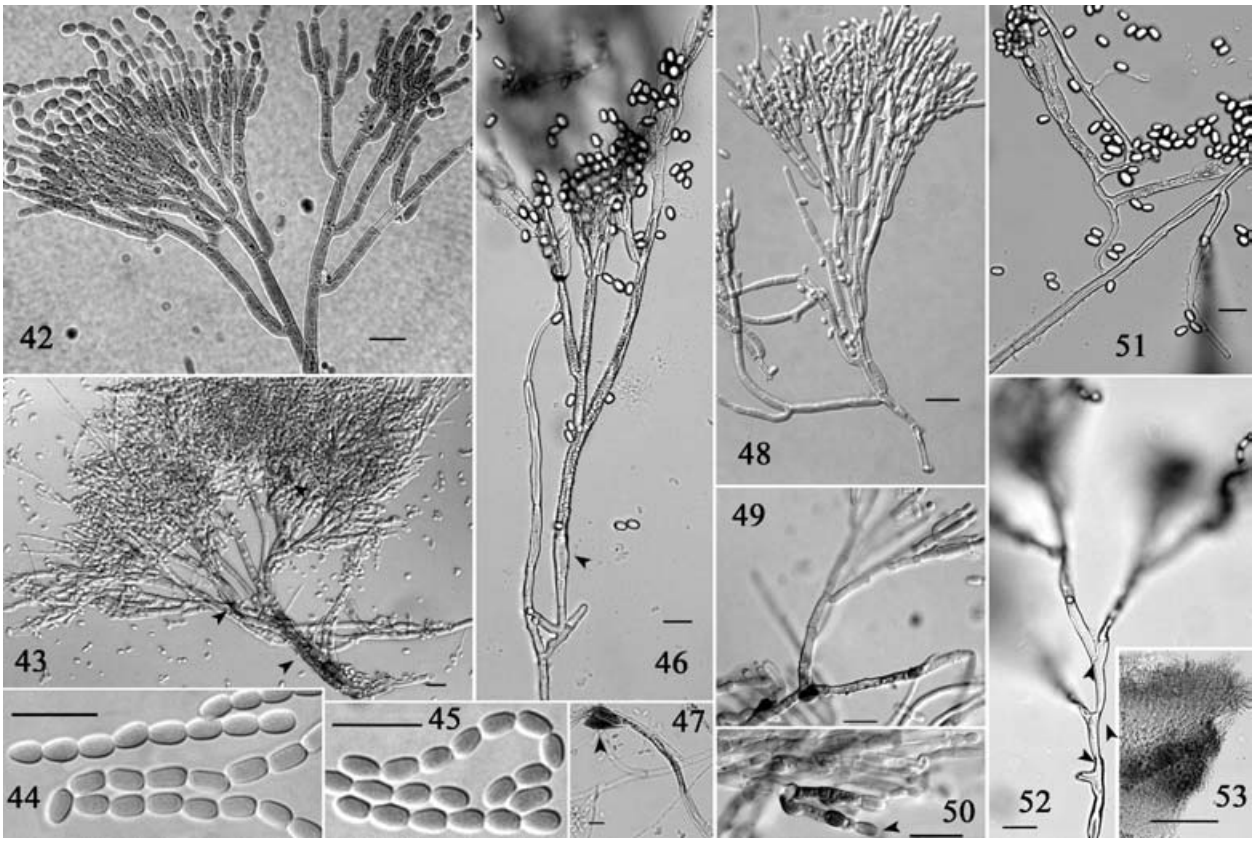
Figs 40, 41. *Geosmithia fassatiae* (CCF 3334). **Fig. 40.** Penicilli. **Fig. 41.** Conidia. Bar = 10 μm.

(terverticillate), often symmetric; metulae 7–13 × 2–3 μm, 2–4 per branch, smooth to verrucose; *conidiogenous cells* phialides, 9–12(–15) × 1.5–3 μm, 3–8 per metula, sometimes solitarly (monophialidic conidiophores) and then longer and slender, cylindrical, clavate or obclavate, often with a clear collar around the apical pore, walls smooth to verruculose, often proliferating to another phialide; conidia smooth, ellipsoidal to broadly ellipsoidal, subglobose or barrel-shaped, (3–)3.5–4.5(5.5) × (2–)2.5–3(4) μm, conidial chains in well defined and persistent columns; substrate conidia absent.

MEA, 24 °, 14 d: Colonies 45–50 mm diam, plane or slightly radially furrowed, sometimes raised centrally; surface texture deeply floccose; margins submerged (to 5 mm broad); mycelium white; substrate mycelium forming tough basal felt; conidiogenesis sparse, white; exudate lacking; soluble pigment absent; reverse white, pale yellow (2A3) or sometimes pale orange (5A3).

MEA, 37 °, 14 d: No growth (microcolonies only).

CYA, 24 °, 14 d: Colonies 35–40 mm diam, plane or lightly radially furrowed, low or raised centrally; surface texture mostly deeply floccose or aerial



Figs 42–53. *Geosmithia obscura*. Light micrograph of the type strain CCF 3422 (as otherwise noted); penicilli and conidia on MEA at 24 °C, 7 d in the dark or from the center of 10 d old colony (Figs 43, 49, 50), mounted in Melzer's reagent (Figs 42, 44, 48) or in lactic acid with cotton blue (others), or observed in slide culture after 4 d on MEA on the daylight (Figs 46, 51, 52). **Fig. 42.** Penicillus. **Fig. 43.** False branched penicillus with melanized elements (arrows) from the center of 10 d old colony. **Fig. 44.** Conidia (CCF 3424). **Fig. 45.** Conidia. **Fig. 46.** Penicillus of CCF 3422 with the peg foot (arrow). **Fig. 47.** Melanized aerial mycelium with the typical thickening (arrow) from the center of 10 d old colony. **Fig. 48.** Penicillus (CCF 3425). **Fig. 49.** Penicillus with the melanized base and cell wall thickenings from the center of 10 d old colony. **Fig. 50.** Detail of melanized phialides and conidia (arrow) from the center of 10 d old colony. **Fig. 51.** Simply branched penicilli connected with peg foots. **Fig. 52.** Penicilli connected with peg foots, forming young false branched conidiophore. **Fig. 53.** Sporodochium with pigmented base. Bar = 50 μm (Fig. 53). Bar = 10 μm (all others).

mycelium form only thin, felt-like network of hyphae; mycelium white, conidiogenesis sparse, white; exudate absent or dull and white; soluble pigment absent; reverse uncoloured or pale yellow (2A3).

Habitat: In bark beetle galleries and surroundings phloem and wood. For known associates see Table 1.

Distribution: Known from the Czech Republic, Slovakia, Hungary, and Croatia.

Teleomorph: Unknown.

Intraspecific variability: The isolates examined are phenotypically and genotypically homogenous.

Distinctive features: Production of deeply lanose and slow growing colonies on both media, conspicuous conidiophores, and broadly ellipsoidal conidia which are hyaline *en masse*. Sparse sporulation and a slightly yellowish reverse are also typical. *G. fassatiae* resembles RAPD-type X 'albolanosa' of *G. langdonii* in colony texture and conidium character. The differences between these two groups are explained above.

Geosmithia obscura Kolařík, Kubátová & Pažoutová, **sp. nov.** (Figs 19–22, 42–54)

Etym. obscurus (dark), refering to the dark soluble pigment and darkly melanized conidiophore elements.

Pars aversa coloniae culturarum CYA et MEA lanose vel velutina pro parte; in 4% MEA temperatura 24 °C ad 70 mm diametro post 2 hebdomades crescentes, reversum brunneus, bruneo-purpureus vel nigrescens; mycelium basalis et conidiophorae pallidea, melanescentiae, penicilli fere biverticillati et terverticillati vel asymmetrice multiplico ramosi; conidiogenesis moderatus vel abundus, albidus usque subalbidus; conidia cylindrica, 4.5–5 × (2–)2.5(–3) μm, portata in longus ordinate catenis.

Typus: **Czech Republic**: *Bohemia*: Louny: forest near Břinkov, alt. 260 m, ex dead adult of *Scolytus intricatus* in the mother gallery, in the inner bark of *Quercus robur*, Nov. 2000, *M. Kolařík* (PRM 901873 – holotypus; CCF 3422 (= MK 86) ex-type culture; PRM 901874–901878 – isotypi). GenBank sequence ex-type AJ784999.

Conidiophores on MEA arising from the subsurface, surface or aerial mycelium, stipes determinate, erect, (20–)40–60(–120) × 3.5–6.5 μm, smooth to verrucose, septate, hyaline or melanized, arising from a long peg foot or from initials recalling foot-cells of the

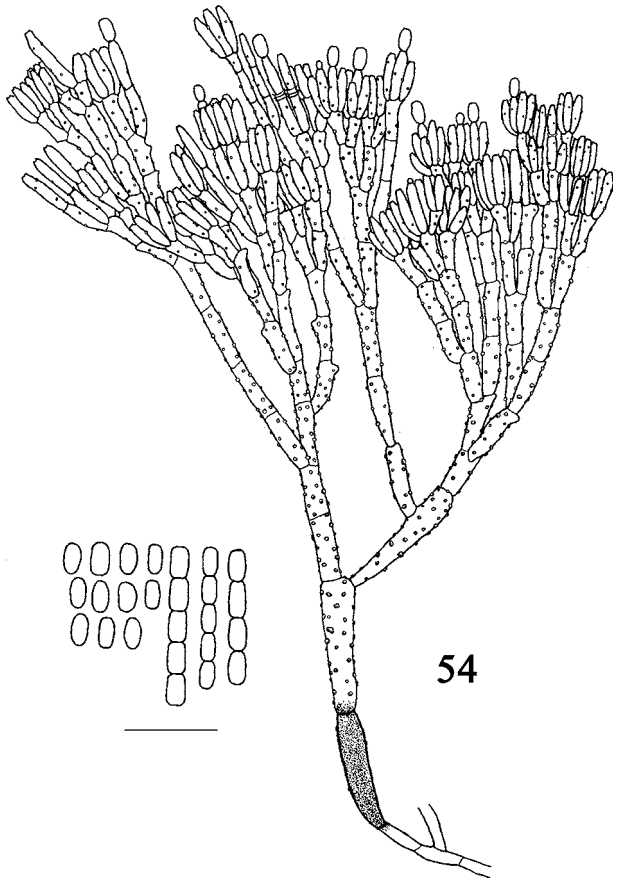


Fig. 54. *Geosmithia obscura* (CCF 3422), penicillus and conidia. Bar = 10 μm.

Aspergillus type; penicilli terminal, 100–150 × 100–150 μm, highly septate, biverticillate to quaterverticillate in conidiophores arising from the aerial mycelium or even more branched in those arising from substrate or surface mycelium (even 9 branch levels between stipes and conidium), conidiophores often asymmetric, proliferating and irregular, with phialides or metulae arising in the different level of the penicillus, single conidiophores connected by peg foot are sometimes aggregated to clusters; rami or other branches of different size; metulae in verticils of 1–4, smooth to verrucose. *Conidiogenous cells* phialides, 6–12 × 2–3 μm, 3–6 per metula, typically cylindroidal without distinct neck, walls smooth to verruculose, often with a slight colarette, sometimes proliferating. *Conidia* cylindrical, (4–) 4.5(–5) × (2–)2.5 μm; conidial chains to 500 μm in length in velutinous areas, in well defined, persistent and parallel columns; substrate conidia absent.

MEA, 24 °, 14 d: Colonies 65–70 mm diam, plane, low or slightly raised centrally, surface texture floccose (striking in freshly isolated strains), with velutinous areas overlaid by aerial mycelium; margins narrow or slightly lobate; mycelium hyaline, or pale (2A2) to rusty (6E8); substrate mycelium sparse, does not form tough basal felt; conidiogenesis moderate to heavy (in velutinous areas), uncoloured to cream (4A3) with light shades of brown in older areas; exudate clear, *terra cotta* (7D7); soluble pigment absent; reverse amber yellow (4B6) to rusty (6E8) or darker in the colony centre.

MEA, 37 °, 14 d: No growth.

CYA, 24 °, 14 d: Colonies 70–80 mm diam, plane or radially furrowed, narrow; surface texture velutinous in the central area, marginal area consisting of thin felt-like network of hyphae without sporulation; margins narrow; mycelium white; conidiogenesis moderate in velutinous areas, white to cream (4A3) with slightly brown tones during ageing; exudate clear, *terra cotta* (7D7); soluble pigment absent; reverse brownish orange (6C7).

Habitat: In bark beetle galleries and surroundings phloem and wood. For known associates see Table 1.

Distribution: Known from the Czech Republic and Hungary.

Teleomorph: Unknown.

Intraspecific variability: Examined isolates exhibit high phenotypic and genotypic homogeneity.

Distinctive features: *G. obscura* typically has rapidly growing colonies, highly branched conidiophores, and large cylindrical conidia. The vegetative hyphae, stipes, and various penicillus elements are gradually melanized in age. Conidiophores, including some conidia, can be melanized after 3 wk. *G. obscura* resembles *G. langdonii* 'ramosa': both species have a similar colony pattern, highly branched conidiophores and presence of melanized hyphae. However, *G. langdonii* grows slowly, has smaller conidia and conidiophores are never melanized. *G. obscura* seems to be a rare species.

DISCUSSION

RAPD technique was used to recognise groups within large sets of morphologically similar isolates. These groups (RAPD-types) can be interpreted as genetically isolated biological units, with a more or less constant pattern (set of alleles) across wide geographical areas and habitats. We describe these lineages as new species because their unique phenotypes and specific habitats make them easily recognisable.

The relatedness of *G. langdonii* and *G. obscura*, according to their RAPD pattern is significant, and is reflected also in their phenotypes. Isolates of *G. langdonii* 'ramosa' have a similar phenotype but of lesser intensity than *G. obscura*. In *G. langdonii*, only the vegetative mycelium is melanized and the conidiophores are less branched. Similarly, there is a visible phenotype transition between *G. fassatiae* and *G. langdonii*. The series of character intensity can range from *G. fassatiae* to *G. langdonii* 'albolanosa', to *G. langdonii* 'crustosa' and 'ramosa' to *G. obscura*. In this series, the conidiophore pattern varied from simple and open to highly branched and compact, and the mycelium from hyaline to melanized substrate and fertile hyphal elements, and in the production of soluble pigments and a colour light to dark reverse. This case of intergrading phenotypes, where differences

Key to *Geosmithia s. str.* species

The following provisional key is based on our observations of the 157 hypocrealean *Geosmithia* strains listed in Table 1, in Kolařík *et al.* (2004), and of *G. lavendula* strains CCF 3475, CCF 3476, CBS 582.67 and IMI 207686. The delimitation of eurotialean *Geosmithia* spp. is based upon ex-type cultures (*G. argillacea* CCF 2544, *G. viridis* CCF 3048, *G. namyslowskii* CCF 3049) or upon their original protologues (*G. cylidrospora*, *G. eburnea*, *G. emersonii*, *G. swifti*, *G. malachitea*). Colony features are based on colonies on MEA and CYA at 10–14 d at 24 ° unless otherwise stated. Micromorphology is derived from colonies on MEA at 7 d, 24 ° unless otherwise noted. For terms and other details see Kolařík *et al.* (2004).

1 Phialides cylindrical to acerose, with a distinct neck; peg foot and substrate conidia absent; never associated with bark beetles . . . . . [eurotialean *Geosmithia* spp.; not treated]
Phialides cylindrical, without a distinct neck (abruptly tappering to an apical pore) or with a small cylindrical collar; peg foot usually present; substrate conidia in some species; typically associated with bark beetles . . . . . [hypocrealean *Geosmithia* spp.] 2

2(1) Conidia *en masse* lilac, reddish lilac or violet to violet brown; in some isolates growth at 37 ° . . **lavendula** aggr.
Conidia *en masse* coloured differently; no growth at 37 ° 3

3(2) Conidia *en masse* on CYA in shades of brown, 3–4 × 1.5–2 μm . . . . . **pallida**
Conidia *en masse* on CYA hyaline, cream, yellowish white or yellow, conidia mostly larger 4

4(3) Conidiogenesis sparse; conidia *en masse* hyaline, broadly ellipsoidal, 3.5–4.5 × 2.5–3 μm; conidiophores typically monoverticillate to biverticillate, shorter than 80 μm; colonies not exceeding 50 mm diam at 14 d . . **fassatiae**
Conidiogenesis profine; conidia *en masse* hyaline to cream or yellow, ellipsoidal to cylindrical; conidiophores more branched and larger; colonies growing rapidly, to 50 mm at 14 d 5

5(4) Conidia *en masse* (especially on CYA) yellow or salmon, 3.5–4 × 2–2.5 μm . . . . . **flava**
Conidia *en masse* hyaline, cream or yellowish white, 4–5 μm in length 6

6(5) Conidia *en masse* cream or yellowish white, 4–4.5 × 1.5–2 μm; conidiophores biverticillate to terverticillate; vegetative hyphae not melanized . . . . . **putterillii**
Conidia *en masse* hyaline or cream, 4–5 × 2–2.5 μm; conidiophores often more branched; vegetative hyphae sometimes melanized 7

7(6) Conidiophores after 10 d not melanized; colonies not exceeding 70 mm; substrate conidia abundant . **langdonii**
Some conidiophores after 10 d melanized; colonies exceeding 70 mm; substrate conidia absent . . **obscura**

between species are quantitative, is characteristic for closely related anamorphic species like those in *Penicillium*.

G. fassatiae is genetically highly related to *G. flava*. Nevertheless, these two species have distinct RAPD and morphological characters and differ in their insect vectors. *G. flava* is a dominant fungus in *Ernoporus tiliae* and *Hylesinus fraxini* galleries, where *G. fassatiae* is absent (data not shown).

There is a trend to increase the number of diaspores by closely packing conidiophores to form palisades (Kolařík *et al.* 2004). The incorporation of peg foot and vegetative hyphae into the penicillus facilitates the formation of multiple (false-) branched penicilli with many phialides on different levels, so increasing the number of diaspores per stipe at little cost. The biological role of substrate conidia formation is not known, and so far they have only been observed in culture (and aerial mycelium; Kolařík *et al.* 2004).

Bark beetles carrying *Geosmithia* spp. do not transmit strictly entomochorous ophiostomatoid fungi, and lack mycetangia or prominent exoskeleton convolutions. It is possible that the adaptation to insect dispersal has paralleled increasing diaspore production, facilitating spread by insects with no adaptation to fungal transmission. Conidial chains in *G. langdonii* and *G. obscura* are typically persistent and form conidial crusts to 500 μm deep. The poor liberation of solitary conidia from chains prevents an anemochoric life-style in this species.

G. *fassatiae* is morphologically and genetically homogenous, represented probably by several clones spread over a large area. On the other hand, G. *langdonii* was frequently isolated and exhibits a high genotypic and morphological diversity, probably due to recombination events or by range overlaps of previously allopatric populations.

The three new *Geosmithia* species were isolated only from several ecologically related *Scolytus* species. We considered this association to be regular (at least in Central Europe) because of the specificity of *Geosmtihia* species to certain insect vectors (Kolařík, unpubl.). The affinity of certain fungi to specific vectors is the norm in entomochoric fungi like *Ceratocystis*, *Ceratocystiopsis*, *Leptographium*, and *Ophiostoma* (Six & Paine 1999, Jacobs & Wingfield 2001, Kirschner 2001).

G. langdonii and *G. fassatiae* were isolated from the oak bark beetle (*S. intricatus*) which feeds preferentially on oaks, from the fruit bark beetle (*S. rugulosus*) which feeds on trees and shrubs of *Rosaceae*, and from *S. carpini* which feeds mostly on hornbeam (*Carpinus betulus*). *S. intricatus* and *S. carpini* have common alternate hosts (e.g. *Fagus sylvatica*, *Quercus* spp.) and so the entomochoric fungi from these bark beetles are not entirely spatially isolated. The composition of the *Geosmithia* community associated with facultatively sympatric *S. intricatus* and *S. carpini* is nearly the same (they had the same RAPD-types as *G. pallida*). Similarly, *S. mali* and *S. rugulosus* are sympatric, and

all these vectors transmit *G. fassatiae*. The distribution *G. fassatiae* and *G. langdonii* vectors (*S. intricatus* and *S. rugulosus*) seems to be allopatric, but these two bark beetles can rarely encounter each other during larval and generation feeding on trees from the *Prunaceae*, *Salix* spp. etc. (Doganlar & Schopf 1984, Příhoda 1990). The position of these morphologically and genetically similar *Geosmithia* spp. in phylogenetic analyses shows a derived position. These species probably diverged recently and so occur in a small number of ecologically and taxonomically related vectors (nothing is published on the phylogenetic relatedness of *Scolytus* spp.).

The expected distribution of the newly described *Geosmithia* spp. is inferred from the distribution of their beetle vectors. These bark beetles, indigenous to Europe, Asia Minor, and North Africa (Pfeffer 1994), have been introduced into other countries, where the host trees grow. *S. rugulosus* is the most widespread, and is recorded from North and South America (Haack 2001).

At least in central Europe, the three newly described white-spored species can easily be identified. However, there are other white-spored *Geosmithia* spp., associated with vectors of these species, that belong to both local and very rare RAPD-types (e.g. CCF 3350, CCF 3358) and to common, worldwide RAPD-types (e.g. IMI 194089, CCF 3335). Other white-spored *Geosmithia* strains were found associated with bark beetles listed in Table 2. These strains are morphologically very similar, but genetically not related. We have described here three white-spored species that have a limited habitat and are easy to distinguish by phenotype. In general, molecular typing is necessary for identification of white-spored *Geosmithia* spp.

ACKNOWLEDGEMENTS

This project was supported by Czech Grant Agency grant 522/02/1206, 206/03/H137, by the institutional project MSM 0021620828 of the Ministry of Education, Youth and Sports and by the Institutional Research Concept AV0Z5020903. We thank Miloš Knížek (Forestry and Game Management Research Institute, Jíloviště-Strnady, Prague) for the bark beetle identifications and Stephen W. Peterson for the revision of the manuscript.

REFERENCES

Cole, G. T., Nag Raj, T. R. & Kendrick, W. B. (1969) A simple technique for time-lapse photomicrography of microfungi in plate culture. *Mycologia* **61**: 726–730.

Doganlar, M. & Schopf, R. (1984) Some biological aspects of the European oak bark beetle, *Scolytus intricatus* (Ratz.) (Col., Scolytidae) in the northern parts of Germany (BRD). *Zeitschrift für Angewandte Entomologie* **97**: 153–162.

Fassatiová, O. (1986) Moulds and filamentous fungi in technical microbiology. Elsevier, Amsterdam.

Haack, R. A. (2001) Intercepted Scolytidae (*Coleoptera*) at U.S. ports of entry: 1985–2000. *Integrated Pest Management Reviews* **6**: 253–282.

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Huelsenbeck, J. P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Jacobs, K. & Wingfield, M. J. (2001) *Leptographium* species: tree pathogens, insect associated, and agents of blue-stain. – 224 p., APS Press.

Kirschner, R. (1998) Diversität mit Borkenkäfern assoziierter filamentöser Mikropilze. – 573 p., ms. [PhD thesis, depon. in: Fakultät für Biologie, Eberhard-Karls Universität, Tübingen.]

Kirschner, R. (2001) Diversity of filamentous fungi in bark beetle galleries in Central Europe. In *Trichomycetes and Other Fungal Groups: Professor Robert W. Lichtwardt Commemoration Volume* (J. K. Misra & B. W. Horn, eds): 175–196. Science Publishers, Enfield (NH), USA.

Kolařík, M. (2002) Ekologie a taxonomie hypokreálních zástupců rodu *Geosmithia*. – 152 p., ms. [Dipl. thesis, depon. in: Department of Botany, Faculty of Science, Charles University, Prague.]

Kornerup, A. & Wanscher, J. H. (1981) Taschenlexikon der Farben. Muster–Schmidt Verlag, Göttingen.

Kubátová, A. & Kolařík, M. (2005) Culture Collection of Fungi (CCF) in Prague – original isolates accessed in 2003 and 2005. *Novitates Botanicae Universitatis Carolinae* [in press].

Kolařík, M., Kubátová, A., Pažoutová, S. & Šrůtka, P. (2004) Morphological and molecular characterisation of *Geosmithia putterillii*, *G. pallida* comb. nov. and *G. flava* sp. nov., associated with subcorticolous insects. *Mycological Research* **108**: 1053–1069.

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

Ogawa, H., Yoshimura, A. & Sugiyama, J. (1997) Polyphyletic origin of species of the anamorphic genus *Geosmithia* and the relationships of the cleistothecial genera: evidence from 18S, 5S, and 28S rDNA sequence analysis. *Mycologia* **89**: 756–771.

Pfeffer, A. (1994) Zentral- und westpaläarktische Borken- und Kernkäfer (Coleoptera: Scolytidae, Platypodidae). *Entomologica Basiliensia* **17**: 5–310.

Pitt, J. I. (1979) *Geosmithia* gen. nov. for *Penicillium lavendulum* and related species. *Canadian Journal of Botany* **57**: 2021–2030.

Posada, D. & Crandall, K. A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Příhoda, A. (1990) Hynutí dubů ve středních Čechách. *Bohemia Centralis* **19**: 81–89.

Six, D. L. & Paine, T. D. (1999) Phylogenetic comparison of ascomycete mycangial fungi and *Dendroctonus* bark beetles (Coleoptera: Scolytidae). *Annals of the Entomological Society of America* **92**: 159–166.

Swofford, D. L. (1998) *PAUP*. phylogenetic analysis using parsimony* (* *and other methods*). Version 4. Sinauer Associates, Sunderland, MA.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876–4882.

Waddell, P. & Steel, A. M. (1997) General time-reversible distances with unequal rates across sites: mixing gamma and inverse Gaussian distributions with invariant sites. *Molecular Phylogenetics and Evolution* **8**: 398–414.

Corresponding Editor: D. L. Hawksworth