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Geosmithia – a widespread, abundant, but long-time ignored symbionts of subcortical insects

by

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HABILITATION THESIS

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CONTENT

1.1 ACKNOWLEDGEMENT

At this point, I would like to thank the people who have made my scientific career possible. Alena Kubátová (Dept. Botany. Charles University) in her research on *Scolytus intricatus*-associated fungi (1993-2000), together with Karel Prášil (Dept. Botany. Charles University) and Petr Šrůtka (Czech Agricultural University), came across a large number of *Penicillium*-like fungi. Alena Kubátová, as a great specialist in the genus *Penicillium*, identified them as a *Geosmithia* members and got in touch with an expert in molecular genetics, Dr. Sylvia Pažoutová (Institute of Microbiology of the Czech Academy of Science, IMIC). Subsequently, they proposed the topic of the diploma thesis, which I worked on and they obtained the grant support from the Czech Science Foundation, which accelerated further study. In 1999, I started the first work on *Geosmithia* and stayed with this topic during my PhD thesis (defended 2007), until now. Alena and Sylvia supported and influenced me throughout the following years. In addition, thanks to very important interactions with Miroslav Flieger (IMIC), the study of *Geosmithia* secondary metabolites became possible. A number of colleagues in the field of entomology, namely Petr Šrůtka, Miloš Knížek (Forestry and Game Management Research Institute), Lawrence Kirkendall (University of Oslo) trained me about insects and provided me with a lot of material. Jiří Hulcr (University of Florida) and Robert Jankowiak (University of Agriculture in Krakow) have played a very important role in my research and are working closely with me on this topic. Furthermore, I would like to thank my *Geosmithia* research team (Milada Chudíčková, Eva Stodůlková, Martin Kostovčík, Tereza Veselská, Karel Švec and others) and the co-authors of the publications who helped me to accomplish the whole research.

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I am indebted to Jiri Hulcr for editing the text and numerous comments on this Thesis. Finally, I would like to thank my mother Ludmila, father Miroslav and wife Zuzana for all-round support.

1.2 OUTLINE OF THE THESIS AND SUMMARY OF THE RESULTS

The work is a continuation of my PhD thesis, submitted in 2006, which dealt with the study of the host spectrum, geography and taxonomy of the genus *Geosmithia.* At that time, the association of *Geosmithia* with subcortical insects (mostly bark beetles), was very little known and mostly considered incidental, not specific, similar to many of the moulds we find in bark beetle galleries. At the time of PhD thesis origin, only the species *G. putterilllii* and *G. lavendula* were recognized, isolated from various non-specific substrates such as soil or cereals. This, together with the absence of slime spores, a typical entomochoric adaptation, made the mycologists sceptical to the importance of this association. The thesis gave an overview of the composition of the communities of the genus *Geosmithia* on bark beetles in temperate Europe and showed that these fungi make communities that are host-specific and considerably stable over

large geographical distances. This pattern indicates entomochory and long-term association. The work was published in 2008 (Kolařík et al., 2008) and became a baseline study for the symbiosis of *Geosmithia* with bark beetles. The PhD thesis also published a taxonomic revision, which led to the revision of old taxa (*Penicillium pallidum*, *G. putterillii*) and the description of four new species (Kolařík et al., 2005; Kolařík et al., 2004). These results raised a number of additional questions that constitute the Aims of the habilitation thesis.

1.2.1 Aim 1. To assess *Geosmithia* host range and community pattern at the global scale

The aim was to expand sampling in terms of number of vectors and geography.

Outputs. *Geosmithia* communities were studied on diverse insect hosts in Central Europe (Jankowiak and Kolarik, 2010; Jankowiak et al., 2014; Kolařík and Jankowiak, 2013; Pepori et al., 2015; Strzałka et al., 2021), Mediterranean basin (Kolařík et al., 2007), Western (Kolařík et al., 2017; Pietsch et al., 2022) and Eastern USA (Huang et al., 2017; Huang et al., 2019), Costa Rica (Kolařík and Kirkendall, 2010) and China (Zhang et al., 2022). The survey showed that *Geosmithia* is worldwide distributed on numerous bark beetles and other phloem- and wood boring insects. Surprisingly, *Geosmithia* were found also as primary ambrosia fungi living in obligate symbiosis with ambrosia beetles (Kolařík and Kirkendall, 2010). The vectors differed in the frequency of association with *Geosmithia* and can be roughly separated to regular and moderate vectors, and to insect species that have very accidental or do not have an association with *Geosmithia*. The degree of association with *Geosmithia* across vectors is determined by the combination of organ preference and host tree species evidently primarily shaped by the substrate quality type, mainly correlated with host tree mass and bark thickness (see chapter Host range and strength of the association with bark beetles). Based on the host range, *Geosmithia* can be divided into generalist species that are very common across many vectors, and to specialist species that occur on vectors inhabiting? host plants of the same plant family. Consequently, the host preference influences the *Geosmithia* community composition of the particular beetle species. It is obviously shaped by the degree of spatial isolation of given bark beetle species in a given area. If there are only host-specific species on a given host plant, more sharply delimited communities are formed. If there are more polyphagous vectors, the differences are erased. See chapters Vector specificity, community composition and Biogeography.

1.2.2 Aim 2. To evaluate diversity and describe newly found *Geosmithia* species

The first study in Central Europe (Kolařík et al., 2008), showed that most of the presented *Geosmithia* strains cannot be ascribed to the known species. Such a large proportion of unknown diversity called for its study and for a formal description of the species found, which is a necessary step for their next study.

Outputs. The global survey (Aim 1) revealed presence of more than 69 phylogenetic *Geosmithia* species. Seven of them belonged to the known taxa (*G. putterillii*, *G. pallida*, *G. flava*, *G. obscura*, *G. lavendula*, *G. fassatiae*, *G. langdonii*) and for the others, a serial number designation was introduced. In my study, I focused on the description of the most important species such as ambrosia species (*Geosmithia*

eupagioceri, *G. microcorthyli*, *G. rufescens*, *C. cnesini*) (Kolařík et al., 2015; Kolařík and Kirkendall, 2010), phytopathogenic *G. morbida* (Kolařík et al., 2011), *G. ulmacea* and *G. omnicola*, both species frequently associated with Dutch elm disease (Pepori et al., 2015), *G. proliferans* and *G. brunea* (Huang et al., 2017), *G. fagi*, *G. longistipitata* and *G. pazoutovae* (Strzałka et al., 2021). I collaborated on the description of nine other species, some of which belong to species isolated also from my collections, i.e. *Geosmithia* sp. 2 - *G. pumila*, *Geosmithia* sp. 3 - *G. pulverea*, *Geosmithia* sp. 20 - *G. granulata* (Zhang et al., 2022), *Geosmithia* sp. 5 – *G. funiculosa* (Crous et al., 2022), and some were newly described based on new collections of the colleagues from China - *G. luteobrunnea*, *G. radiata*, *G. brevistipitata*, *G. bombycina*, *G. subfulva* and *G. fusca* (Zhang et al., 2022) (see chapter Taxonomy and Diversity). Some species common in particular areas (e.g *G*. sp. 41 in North America, *G. funiculosa* in Europe), are absent in others, showing clear biogeographical pattern. Thus, study of unexplored areas and vectors has a great chance to substantially increase our knowledge about *Geosmithia* diversity. See chapter Vector specificity, community composition and Biogeography.

1.2.3 Aim 3. To evaluate the biotechnological potential

Fungi from the order Hypocreales are known producers of various secondary metabolites, but nothing was known in case of *Geosmithia* at the beginning of my research. *Geosmithia spp.* are easily cultivable with a rapid growth, which makes them attractive also for biotechnological purposes. Another advantage is that these fungi are regularly in contact with bark beetles, and thus should have minimal cytotoxicity to animal cells, but still have to compete with co-occurring mites, nematodes, fungi and bacteria. This makes them good target for bioprospecting related with new drug and pesticide discovery. In addition to the biotechnological significance, information on the biological activity of extrolytes is interesting from an ecological point of view, helping us to understand interactions between the fungus and other members of the bark beetle holobiont.

Outputs. Our study on crude extracts shows the huge potential of antibacterial and antifungal activity across the whole genus (Veselská et al., 2019). From the ecological point of view, *Geosmithia* species in the bark beetle galleries have ability to compete with various moulds and bacteria, including insect-associated fungi. Prominent yellow, orange, and red pigments produced by *G. lavendula* and other species were identified as set of anthraquinones, often novel to science, several of them with antibacterial or anti-inflammatory activity (Stodůlková et al., 2009; Stodůlková et al., 2010) and with a potential as highly persistent textile dyes or mordants (Flieger et al., 2009). We also developed a UPLC-MS based method for the separation of *Geosmithia* secondary metabolites (Tylová et al. 2011). See chapter Secondary metabolite production and biotechnological potential.

During the study of the violet-coloured G. *lavendula*, I came across another so far neglected bark beetle symbiont, *Quambalaria cyanescens*. This fungus belongs to a very little explored fungal lineage of Basidiomycota (Exobasidiomycetes, Microstromatales), again with missing knowledge about its secondary

metabolite production. A subsequent study showed that it is a producer of various naphthoquinones, together with newly described quambalarine A-D, which have antimicrobial potential and negligible cytotoxicity to healthy human cells (Prochazkova et al., 2020; Stodůlková et al., 2015; Stodulkova et al., 2008). In addition, they have antiviral activity and selective toxicity to the human carcinoma cells which stimulated a subsequent exploration of its mode of action. That outputs are not part of the Thesis and are summarized in several studies (Grobárová et al., 2016; Matoušková, 2020; Vališ et al., 2017; Zima et al., 2020).

1.2.4 Aim 4. To understand *Geosmithia* ecology. How do they interact with bark beetles or host trees?

There are several types of interactions known between bark beetles and their associated fungi. It can therefore be expected that some of these interactions will also occur in the genus *Geosmithia*.

Outputs. Bark beetle associated fungi are known to have diverse symbiotic (i.e. mutualistic, neutral or antagonistic) interactions with its environment. The most straightforward is the ability to invade healthy plant tissues, exploit these protected nutrient sources by the fungus, which enables to increase it's the beetle´s fitness. Studied strains typically show no signs of phytopathogenicity (Jankowiak and Kolarik, 2010; Strzałka et al., 2021). Mild, but significant lesions were found in case of two strains by Li et al. (2022). The only undisputed case where the fungus makes significant necrosis, is the pathogenic complex of walnut twig beetle, *Pityophthorus juglandis*/ *G. morbida* responsible for the Thousand Cancers Disease of black walnut, *Juglans nigra* (Kolařík et al., 2011). The massive dieback of black walnut has promoted *Geosmithia* research worldwide, and is behind the wider recognition of *Geosmithia* as entomochoric and symbiotic fungi. The *G. mobida* related research, to which I furher contributed, involved study of its genetic variability (Hadziabdic et al., 2014a). Walnut twig beetle transmits other *Geosmithia* species (Kolařík et al., 2017), and as can be seen from preliminary results with infection experiments with *G. obscura* (Pietsch et al., 2022), it needs to be studied whether these species can contribute to necrosis formation. See chapter Phytopathogenic potential and TCD.

1.2.5 Aim 5. To understand *Geosmithia* evolution and biology

As we accumulated knowledge about the host range and pathogenicity, it became obvious that the genus comprised species with variable life strategies, host specificity, degree of affinity to the host beetle and pathogenicity to the host plant. We thus decided to study evolution of the genus by incorporating information about phylogeny, genome size and various phenotype traits (morphology, enzymatic capacity, antibiosis etc.).

Outputs. The study showed the limits of rDNA for robust phylogenetic hypotheses testing, for genus *Geosmithia*. The observed incongruence between rDNA and protein coding genes was attributable to GC content and heterotachy-based artifacts (Kolařík et al., 2017; Veselská et al., 2019). That seems to be a consequence of mechanisms such as the fluctuations in the effective population change, bottlenecks,

usually related to the life history changes, especially those related to the switch between free living style to host-associated life strategy (Kolařík and Vohník, 2017; Kolařík et al., 2021) (papers not included into the Thesis). The study of the evolution of the genus showed that the ancestral species were generalists and later specialized in several lineages to their host vectors. All that shifts were accompanied by loss of metabolic capacity and genome size inflation. We identified three independent origins of primary ambrosia fungi (Kolařík et al., 2015; Kolařík and Kirkendall, 2010), which was accompanied by the cell and genome size inflation and production of particular fatty acids (Veselská and Kolařík, 2015; Veselská et al., 2019). One lineage, *G. morbida*, became plant pathogen, with the unique feature to digest all components of lignocellulose, what feature can be supposed as *G. morbida* virulence factor (Veselská and Kolařík, 2015; Veselská et al., 2019). The genome size and DNA content of the cells were measured by flow cytometry. This method has so far been little used in fungi, mainly due to their very small genomes. Therefore, the method needed to be optimized and suitable standards for determining genome size were sought. This led to the first ever methodological work on flow cytometry in mycology (Veselská et al., 2014). The study of genus evolution and the use of flow cytometry was the subject of Tereza Veselská's master and doctoral thesis (see Student´s theses related to the topic of the habilitation thesis). My study further contributed to knowledge about hydrophobins, which showed that ability to adhesion is important in the evolution of the genus (Frascella et al., 2014). Beside of them, there is a good evidence that *Geosmithia* obtained hyrophobin, cerato-ulmin, by the horizontal transfer from *Ophiostoma novo-ulmi* (Bettini et al., 2014). See chapter Evolution and biology.

1.3 PAPERS INCLUDED IN THE THESIS

(the most important studies are underlined)

1. **Kolařík M**, Kostovčík, M Pažoutová S (2007) Host range and diversity of the genus *Geosmithia* (: Hypocreales) living in association with bark beetles in the Mediterranean area. *Mycological Research* 101: 1298-1310.

2. Stodůlková E, **Kolařík M,** Křesinová Z, Kuzma M, Šulc M, Man P, Novák P, Maršík, P, Landa, P, Olšovská, J, Chudíčková M, Pažoutová S, Černý J, Bella J, Flieger M (2009) Hydroxylated anthraquinones produced by *Geosmithia* species. *Folia Microbiologica* 54: 179-187.

3. Jankowiak R, **Kolařík M** (2010) Fungi associated with the fir bark beetle *Cryphalus piceae* in Poland. *Forest Pathology* 40: 133-144.

4. **Kolařík M,** Kirkendall LR (2010) Evidence for a new lineage of primary ambrosia fungi in *Geosmithia* Pitt (Ascomycota: Hypocreales). *Fungal Biology* 114: 676-689.

5. Stodůlková E, Man P, **Kolařík M**, Flieger M (2010) High-performance liquid chromatography-off line mass spectrometry analysis of anthraquinones produced by *Geosmithia lavendula*. *Journal of Chromatography A* 1217: 6296-6302.

6. **Kolařík M,** Freeland E, Utley C, Tisserat N (2011) *Geosmithia morbida* sp. nov. a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis*) on *Juglans* in the USA. *Mycologia* 103: 325-32.

7. Tylová T, **Kolařík M**, Olšovská J (2011) The UHPLC-DAD fingerprinting method for analysis of extracellular metabolites of fungi of the genus *Geosmithia* (Acomycota: Hypocreales). *Analytical and Bioanalytical Chemistry* 400: 2943-2952.

8. Kolařík M, Jankowiak R (2013) Vector affinity and diversity of *Geosmithia* fungi living on subcortical insects inhabiting Pinaceae species in Central and Northeastern Europe. *Microbial Ecology* 66: 682-700.

9. Hadziabdic D, Vito L, Windham M, Pscheidt J, Trigiano R, **Kolařík M** (2014) Genetic differentiation and spatial structure of *Geosmithia morbida*, the causal agent of thousand cankers disease in black walnut (*Juglans nigra*). *Curr Genet* 60: 75–87.

10. Bettini PP, Frascella A, **Kolařík M**, Comparini C, Pepori AL, Santini A, Scala F, Scala A (2014) Widespread horizontal transfer of the cerato-ulmin gene between *Ophiostoma novo-ulmi* and *Geosmithia* species. *Fungal Biology* 118: 663-674.

11. Jankowiak R, **Kolařík M**, Bilańskic P (2014) Association of *Geosmithia* fungi (Ascomycota: Hypocreales) with pine- and spruce-infesting bark beetles in Poland. *Fungal Ecology* 11: 71–79.

12. Veselská T, Svoboda J, Růžičková Z, **Kolařík M** (2014) Application of flow cytometry for genome size determination in *Geosmithia* fungi: A comparison of methods. *Cytometry Part A*. 85: 4–861.

13. Frascella A, Bettini PP, **Kolařík M**, Comparini C, Pazzagli L, Luti S, Scala F, Scala A (2014) Interspecific variability of class II hydrophobin GEO1 in the genus *Geosmithia*. *Fungal Biology* 118: 862-871.

14. Veselská T, **Kolařík M** (2015) Application of flow cytometry for exploring the evolution of *Geosmithia* fungi living in association with bark beetles: the role of conidial DNA content. *Fungal Ecology* 13: 83-92.

15. Stodůlková E, Císařová I, **Kolařík M**, Chudíčková M, Novák P, et al. (2015) Biologically active metabolites produced by the basidiomycete *Quambalaria cyanescens*. *PLoS ONE* 10(2): e0118913.

16. Pepori AL, **Kolařík M**, Bettini PP, Vettraino AM, Santini A (2015) Morphological and molecular characterisation of *Geosmithia* species on European elms. *Fungal Biology* 119:1063-1074.

17. **Kolařík M**, Hulcr J, Kirkendall LR. (2015) New species of *Geosmithia* and *Graphium* associated with ambrosia beetles in Costa Rica. *Czech Mycology* 67: 29-35.

18. **Kolařík M**, Hulcr J, Tisserat N, De Beer W, Kostovčík M, Kolaříková Z, Seybold SJ, Rizzo DM (2017) *Geosmithia* associated with bark beetles and woodborers in the western USA: taxonomic diversity and vector specificity. *Mycologia* 109:185-199

19. Huang Y-T, **Kolařík M**, Kasson M, Hulcr J (2018) Two new *Geosmithia* species in *G. pallida* species complex from bark beetles in eastern USA. *Mycologia* 109: 790-803.

20. Huang Y.-T, Skelton J, Johnson AJ, **Kolařík M**, Hulcr J (2019) *Geosmithia* species in southeastern USA and their affinity to beetle vectors and tree hosts. *Fungal Ecology* 39: 168-183.

21. Veselská T, Skelton J, Kostovčík M, Hulcr J, Baldrian P, Chudíčková M, Cajthaml T, Vojtová T, Garcia-Fraile P, **Kolařík M** (2019) Adaptive traits of bark and ambrosia beetle-associated fungi. *Fungal Ecology* 41:165-176.

22. Procházková E, Kucherak O, Stodůlková E, Tošner Z, Císařová I, Flieger M, **Kolařík M,** Baszczyňski O (2021) NMR structure elucidation of naphthoquinones from *Quambalaria cyanescens*. *Journal of Natural Products* 84: 46–55.

23. Strzałka B, **Kolařík M**, Jankowiak R (2021) *Geosmithia* associated with hardwood-infesting bark and ambrosia beetles, with the description of three new species from Poland. *Antonie van Leeuwenhoek* 114: 169-194.

24. Li Y, Bateman C, Skelton J, Wang B, Black A, Huang Y-T, Gonzalez A, Jusino MA, Nolen ZJ, Freeman S, Mendel Z, **Kolařík, M**., Knížek, M, Park J-H, Sittichaya W, Pham T-H, Ito S, Torii M, Gao L, Johnson AJ, Lu M, Sun J, Zhang Z, Adams DC, Hulcr J (2022) Preinvasion assessment of exotic bark beetle-vectored fungi to detect tree-killing pathogens. *Phytopathology* 112: 261-270.

25. Zhang X, Li Y, Dai M, Si H, Zhao G, **Kolařík M**, Hulcr J, Jiang X, Chang R (2022) *Geosmithia* species associated with bark beetles from Southern China, with the description of four new Species. *Frontiers in Microbiology* 13: 10.3389/fmicb.2022.820402

26. Pietsch GM, Gazis R, Klingeman WE, Huff ML, Staton ME, **Kolařík M**, Hadziabdic D (2022) Characterization and microsatellite marker development for a common bark and ambrosia beetle associate, *Geosmithia obscura*. *MicrobiologyOpen* 11:e1286

27. Crous PW, Boers J, Holdom D, Steinrucken T, Tan Y, Vitelli J, Shivas R, Barrett M, Boxshall A-G, Broadbridge J et al. (2022) Fungal Planet description sheets: 1383–1435. *Persoonia-Molecular Phylogeny and Evolution of Fungi* 48: 261-371

1.4 STUDENT´S THESES RELATED TO THE TOPIC

In the course of the *Geosmithia* research, six students had the opportunity to work on attractive and unexplored topics what resulted in ten Theses defended at three departments of Faculty or Sciences at

Charles University and University of Chemistry and Technology, Prague. The list of theses, elaborated with my contribution (supervisor, official or non-official consultant), is provided below.

1. Veselská T (2022) Comparative ecophysiology as a tool for the study of adaptive traits of fungal symbionts and pathogens. Doctoral thesis (PhD. degree). Department of botany, Faculty of Sciences, Charles University. Supervisor: M. Kolařík

2. Lovás D (2022) Identifikace bioaktivních metabolitů hub asociovaných s kůrovci. Bachelor Thesis (Bc. Degree). University of Chemistry and Technology, Prague, Department of Biotechnology. Bachelor thesis (Bc. degree). Supervisor prof. Ing. Jan Masák, CSc.

3. Fabryová A (2016) Study of culturable anaerobic bacterial communities living in symbiosis with bark beetles; its isolation, taxonomy and biotechnical potential. Diploma thesis (MSc. degree). Department of genetics and microbiology, Faculty of Sciences, Charles University. Supervisor: MSc. Paula García Fraile, Ph.D.

4. Veselská T (2013) Evoluční ekologie rodu *Geosmithia*. Diploma thesis (MSc. degree). Department of botany, Faculty of Sciences, Charles University. Supervisor: M. Kolařík

5. Musil K, (2013) Studium biologicky aktivních sekundárních metabolitů produkovaných vybraným kmenem hub rodu *Geosmithia* metodou UPLC-DAD-TOF-MS. Bachelor thesis (Bc. degree). Department of analytical chemistry and microbiology, Faculty of Sciences, Charles University. Supervisor: RNDr. Mgr. Jana Olšovská, Ph.D.

6. Tylová T (2013) Metody kapalinové chromatografie pro analýzu biologicky aktivních mikrobiálních sekundárních metabolitů. Doctoral thesis (Ph.D. degree). Department of analytical chemistry and microbiology, Faculty of Sciences, Charles University. Supervisor: RNDr. Mgr. Jana Olšovská, Ph.D.

7. Veselská T (2010) Genetika hub, evoluce genomu a vyuziti prutokoveho cytometru pri studiu DNA. Bachelor thesis (Bc. degree). Department of botany, Faculty of Sciences, Charles University. Supervisor: M. Kolařík

8. Křesinová Z (2007) Studium sekundárních metabolitů houby *Geosmithia lavendula*. Doctoral thesis (RNDr. degree). Department of analytical chemistry and microbiology, Faculty of Sciences, Charles University.

9. Křesinová Z (2007). Studium sekundárních metabolitů houby *Geosmithia lavendula*. Diploma thesis (MSc. degree). Department of analytical chemistry and microbiology, Faculty of Sciences, Charles University. Supervisor: prof. RNDr. Zuzana Bosáková, CSc.

10. Kostovčík M (2006) Molekulárne genetická a morfologická analýza komplexu *Geosmithia lavendula*. Diploma thesis (MSc. degree). Department of genetics and microbiology, Faculty of Sciences, Charles University. Supervisor: RNDr. Sylva Pažoutová, CSc.

1.5 EXTENDED REVIEW OF THE GEOSMITHIA TAXONOMY, GEOGRAPHY, DIVERSITY, ECOLOGY AND BIOTECHNOLOGICAL POTENTIAL

1.5.1 Introduction

Bark and ambrosia beetles (*Coleoptera: Curculionidae: Scolytinae, Platypodinae*) are associated with a diverse set of ecto- and endosymbionts, classified among the prokaryotes, filamentous fungi, yeasts, and microinvertebrates. Fugal symbionts are the most studied and their dependency on the insect vector ranges from obligatory, in strictly entomochoric fungi, to incidental, acquired from the environment. Fungal symbionts interact with the host insect and tree, forming mutualistic, commensal or antagonistic interactions (Beaver, 1989; Hofstetter et al., 2015; Six, 2013). The best studied fungal symbionts of bark beetles belong to ophiostomatoid fungi (*Ascomycota: Ophiostomatales, Microascales*). However, beetle galleries harbour many other fungal families equally frequently, but many of the non-ophiostomatalean have been historically ignored (Jankowiak and Kolarik, 2010; Kirschner, 2001; Kirschner et al., 2001; Kolařík et al., 2006). Filamentous fungi placed into the genus *Geosmithia* (*Ascomycota: Hypocreales*, *Bionectriaceae*) used to be sporadically reported as plant or soil saprobes (Pitt, 1979; Pitt and Hocking, 2009). The very first record of *Geosmithia* from the bark beetle niche, and a suggestion of its phytopathogenicity, was from the fir bark beetles in the USA by Wright (1938), but the fungus was misidentified as *Spicaria anomala* (Kolarik et al. 2017). The regular association of *Geosmithia* fungi with bark beetles was simultaneously discovered in Germany (Kirschner, 1998, 2001) and Czechia (Kubátová et al., 2004; Kubátová et al., 1999). During the first decade of the new millennium, the question of the tightness of the association of *Geosmithia* with bark beetles was not yet settled. The reasons for these doubts were numerous and relevant. The identified species, such as *G. putterilllii* have been known from various non-specific substrates such as soil or cereals (Kolařík et al., 2004; Pitt and Hocking, 2009). In addition, the generic concept of *Geosmithia* before 2012 included species of Hypocreales (*Geosmithia* in the current definition) but also Eurotiales, which have no connection to insects (Houbraken et al., 2012). Further, *Geosmithia* strongly resembles *Penicillium*, *Paecilomyces* or *Mariannaea*, which are common and widely ignored contaminants of bark beetle galleries. *Geosmithia* produces masses of dry spores, a typical feature of airborne fungi, but do not form slimy spores, a typical entomochoric adaptation. In addition, *Geosmithia* is typically found on hardwoods and conifers of the cypress family, associated with little-studied secondary bark beetles of minor economic importance. Finally, *Geosmithia* is highly sensitive to cycloheximide, an antifungal agent often used in the study of ophiostomatalean fungi which are resistant to it. Thus, the *Geosmithia* presence has been frequently missed and there was a scepticism about the significance of the association.

In the following years, however, many independent studies confirmed *Geosmithia* as a stable, and often dominant symbiont of many bark beetles worldwide, forming fungal communities specific to the host trees frequented by the vector beetles. The subsequent discovery of a phytopathogenic species *G. morbida* (Kolařík et al., 2011), and also species living as primary ambrosia fungi (Kolařík and Kirkendall, 2010),

resulted in the recognition of *Geosmithia* as a genus containing regular bark beetle symbionts with apparent long-term coevolution. Here we summarize and interpret *Geosmithia* biology based on a review more than 140 publications (Fig. 1).

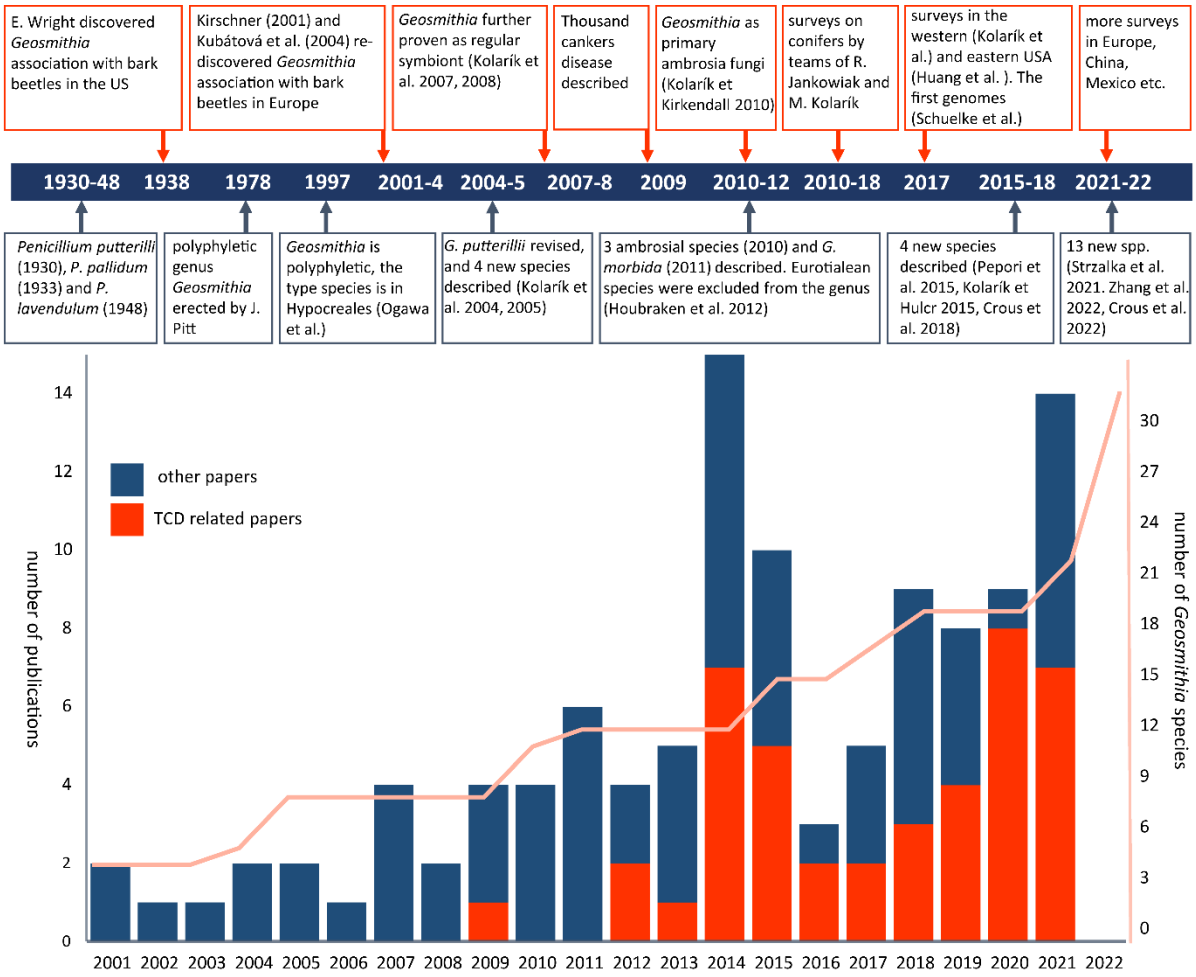

Fig. 1 Upper part. Chronology of important events related to *Geosmithia* taxonomy (below) and ecology, host range and biogeography (above). Lower part. Overview of publications on Hypocrealean *Geosmithia* species over the last 20 years (2001-2021), with a breakdown of papers focusing on Thousand Cankers Disease (orange bar) and on other aspects (blue bar). The graph is based on articles excerpted by the Scopus database and few other important papers. The chart does not include the numerous papers that focus primarily on the biology of the walnut twig beetle, a TCD vector. The graph also presents the increase of described species within the genus (pink line).

1.5.2 History of the genus definition and the main differentiation features

Like in other morpho-genera of anamorphic fungi, the *Geosmithia* generic concept has undergone a dramatic changes (Fig. 1). In the current concept, its characteristics include the following: absence of sexual state, the presence of many colony color but not green color (which diagnoses it as distinct from *Penicillium*), presence of cylindrical shape of phialides without prominent neck and with roughened walls, elliptical to cylindrical conidia produced in chains and presence of specific cellular initials and the conidiophore basis (Kolařík et al., 2004). The discovery of the morphologically unique ambrosia fungi

expands this morphological concept to also include solitary and globose conidia (Kolařík and Kirkendall, 2010). The colony color ranges from white to cream, to various shades of yellow, brown, rusty or red. *Geosmithia* produce the *Penicillium*-like type conidiophore, or conidiophores can be much more complex, irregularly and repeatedly branched. Besides macronematous conidiophores with enteroblastic phialides, microcronematous conidiophores can also be formed on aerial or substrate mycelium (Kolařík et al., 2004). Whereas *Penicillium*-like conidiophores produce columns of dry conidia, microcronematous conidiophores form holoblastic, solitary conidia in slimy droplets. This conidial type, referred to as substrate conidia, is another feature found in related genera such as *Gliocladium* and *Nalanthamala* (Schroers et al., 2005). Other typical *Geosmithia* feature is conidiophore basis, making so-called "peg foot" with smooth cell wall and curved shapes (Kolařík et al., 2004) (Fig. 2).

The type species, *G. putterillii*, was first described as a *Penicillium putterillii* and the history of *Geosmithia* is linked to the morphologically similar *Penicillium* genus. Species of *Geosmithia* was first aggregated into the series *P. pallidum* in *Asymmetrica–Funiculosa* section that was established for *P. pallidum*, *P. putterillii*, *P. lavendulum* (now in *Geosmithia*). *P. namyslowskii* (now in *Penicillium*, Eurotiales) (Raper and Thom, 1949). John Pitt (1979) proposed a new genus *Geosmithia*, named in hour of George Smith, to include species from *P. pallidum* series and some species nowadays classified in Eurotiales. Although at first the concept was not accepted by some authors (Ramirez, 1982; Stolk and Samson, 1986) it was soon solidified in taxonomic lists (Pitt and Samson, 1993; Pitt et al., 2000) and other authors begun to use the name *Geosmithia* for newly discovered species of similar morphology (Pitt and Hocking, 1985; Yaguchi et al., 1993; Yaguchi et al., 1994; Yaguchi et al., 2005). The first studies utilizing molecular data showed that, while some of the species originally placed in the genus, including the type species *G. putterilli,* belonged to Hypocreales, others were in fact within the Eurotiales (Iwamoto et al., 2002; Ogawa and Sugiyama, 2000; Ogawa et al., 1997; Peterson, 2000). An eventual revision resulted in the creation of the monophyletic *Geosmithia* within Hypocreales, and placed other species into the genera of *Penicillium, Rasamsonia* and *Talaromyces* within Eurotiales (Houbraken et al., 2012). These changes also affect the classification of *Rasamsonia argillacea*, a fungus of clinical importance (Giraud et al., 2013),

which is still sometimes incorrectly identified by the old name *Geosmithia argillacea* (Giordano et al., 2021).

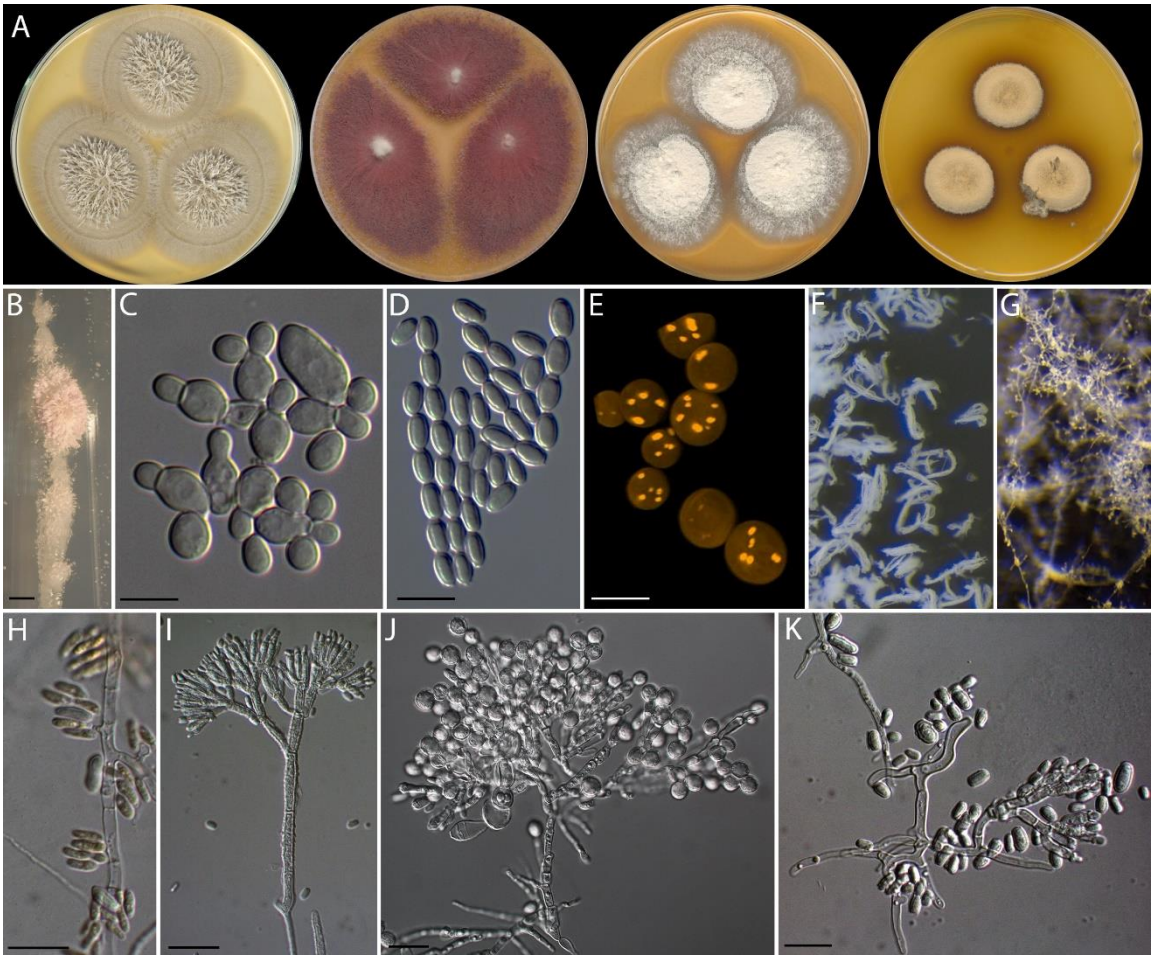

Fig. 2 Morphological features of *Geosmithia*. **A** Colony morphology on MEA can range from brown (*G. funiculosa*), lilac (*G. carolii*), white to cream (*G. putterilli*) and yellowish (*Geosmithia* sp. 11). **B, C** Yeast-like stage is presented in some species during the initial growth phase. *Geosmithia carolii* on MEA, 1 day, 24 °C. **D** Oblong and catenate conidia of *G. carolii*. **E** Globose and multinucleate conidia of *G. eupagioceri* stained by propidium iodide and observed under confocal microscope. **F** Long conidial chains of *Geosmithia* sp. 8 CCF4528. **G** Solitary produced conidia of *G. microcorthyli*. **H** Substrate conidia of *G. carolii.* **I** Penicillate conidiophore in *G. putterillii*. **J** Complexly branched conidiophore of *G. eupagioceri.* **K** Simple conidiophore in *Geosmithia* sp. 31. Scale bars **B** 500 µm, **C-E, H, K** 10 µm, **I, J** 20 µm

1.5.3 Taxonomy and Diversity

The genus possess relatively high phylogenetic diversity, with over 67 phylogenetic species, from which 32 were formally described (Fig. 1, Table 1). Most of the remaining species have been studied to a degree that allows diagnosis to the species level, but they have not been described formally. These species are

informally identified by numbers. This numbering system originated in Kolařík et al. (2007; 2008) and species thus labeled are frequently used in literature (Table 1).

Before the year 2004, only two species, *G. lavendula* and *G. putterillii* (incl. its synonym, *P. pallidum*), were formally accepted. Later, *G. putterillii* was found to be a complex of three species, *G. putterillii*, *G. pallida* (it itself consisting of five phylogenetic species) and *G. flava* (Kolařík et al. 2004). Three other species, *G. fassatiae*, *G. langdoni* and *G. obscura* were described from bark beetles in Europe (Kolařík et al., 2005). A large survey of *Geosmithia* in Europe and Mediterranean basin recognised other 23 undescribed species marked as *Geosmithia* sp. 1-5, 8-13, 16, 19-31 (Kolařík and Jankowiak, 2013; Kolařík et al., 2007; Kolařík et al., 2008). Subsequent surveys in the USA revealed other 20 species, classified as *Geosmithia* sp. 32-48 (Huang et al., 2019; Kolařík et al., 2017) or described as *G. morbida* (Kolařík et al., 2011), *G. proliferans* and *G. brunea* (Huang et al., 2017). Recently, several numbered species were formally described: *G. ulmacea* (sp. 13) and *G. omnicola* (sp. 10) (Pepori et al., 2015), *G. xerotolerans* (sp. 21), *G. carolliae* (sp. 19) (Crous et al., 2018) and *G. longistipitata* (sp. 28) (Strzałka et al., 2021). Some of the previously recognised taxa (sp. 2 - *G. pumila*, sp. 3, 23 - *G. pulverea*, sp. 20 - *G. granulata*), and others newly found (*G. luteobrunnea*, *G. radiata*, *G. brevistipitata*, *G. bombycina*, *G. subfulva* and *G. fusca*) were described from China (Zhang et al., 2022) and Europe (*G. cupressina*, *G. fagi* and *G. pazoutovae*) (Meshram et al., 2022; Strzałka et al., 2021)). Four species, *G. eupagioceri*, *G. microcorthyli*, *G. rufescens* and *C. cnesini* were described from ambrosia beetles in Costa Rica (Kolařík et al., 2015; Kolařík and Kirkendall, 2010). Other five tentative and undescribed species were recognized during the surveys on bark beetles in South Africa and Israel (Dori-Bachash et al., 2015; Machingambi et al., 2014) or on other substrates (Deka and Jha, 2018; Sun et al., 2018) (Table 1). The species *G. tibetensis* (Wu et al., 2013), described from the soil in Tibet, may not be a true *Geosmithia*; no molecular data were provided and its morphology fits that of Eurotiales.

The methods used to characterize *Geosmithia* species follow those used in studies on the genus *Penicillium* and *Aspergillus*. The most commonly used culture substrates are two nutrient-rich media, Malt extract agar (MEA) and Czapek Yeast Autolysate Agar (CYA), and the basal medium Czapek Dox Agar (CZD), the combination of which provides good resolution between most species. Regarding the cultivation temperature, studying growth at 24-25 °C, optimal temperature for perhaps all species, and 37 °C, tolerated by few species only (e.g. *G. lavendula* and *G. morbida*) is used.

The ITS rDNA marker, commonly used to delimit species across fungi, is used to characterize *Geosmithia* species, but it has its limits, especially among closely related species. Therefore, alternative markers are needed for better resolution in some species complexes. Other commonly used markers include RNA polymerase II second largest subunit (RPB2, region defined by the primers fRPB2-5F/fRPB2-7R), β-tubulin gene (TUB2, primers T10/Bt2b) and translation elongation factor 1-α gene (TEF-1α) including the large exon part (primers EF1- 983F/EF1-2218R) and the intron area (EF1-728F/EF1-986R). The latter

shows by far the greatest variability among *Geosmithia* species (Strzałka et al., 2021). The discriminatory power of the alternative markers can be assessed by studying groups of species that are clearly distinguishable morphologically and ecologically, yet have identical ITS sequences, such as *G. microcorthyli* (Kolařík and Kirkendall, 2010), *G. longistipitata* (Strzałka et al., 2021), *Geosmithia* sp. 24 (Dori-Bachash et al., 2015), *Geosmithia* sp. 16 (Kolařík and Jankowiak, 2013) and *G. langdoni* species complexes (Kolařík et al., 2017).

1.5.4 Host range and strength of the association with bark beetles

Geosmithia species are most commonly isolated from the subcortical niche created by bark beetles. The materials which yield most colony forming units are the internal surfaces of galleries, particularly the pupal chambers, but also the surface of eggs, larvae, and adults, and the gallery detritus. Adults captured outside of galleries, prior to the gallery initiation or after emergence from pupation, also frequently yield *Geosmithia* cultures. *Geosmithia* are usually isolated from all the gallery throughout its life cycle and can be visually conspicuous, particularly in pupal chambers and detritus in larval passages (Fig. 3). They are best isolated from active gallery systems, but also found in abandoned galleries for some time, as are ophiostomatoid fungi.

Each of the above substrates requires a different approach for optimal *Geosmithia* recovery. Spores attached to surfaces of beetle adults and larvae are cultured using a wash on standard agar media MEA and PDA, and spore load is quantified by serial dilution. Fungi from gallery detritus or walls can be cultured by directly spreading this material onto agar plates. This method readily yields *Geosmithia*, but it is not quantitative. To reduce contaminating fast-growing molds from adults trapped outside of galleries, a rinse in a modified White solution can be used (Kolařík et al., 2008). *Geosmithina* communities can be documented without culturing by using DNA metabarcoding with the standard ITS rDNA primers (Morales-Rodríguez et al., 2021).

Since the pioneering work of Wright (1938), 153 species of subcortical insects (Curculionidae, Scolytinae, Platypodinae: 140; other Curculionidae: 5; Cerambycidae: 2; Bostrichidae: 6) have been studied for the presence of *Geosmithia*; this fungus was found on 119 of them (Table 2). Within scolytine beetles, it was common on phloem-feeding species (111 out of 140 species) but also on ambrosia beetles (10 species out of 14). It also has been found on seed-feeding *Coccotrypes* (Scolytinae). *Geosmithia* vectors from other beetle groups include the Bostrichidae (6 out of 6 studied species) and Cerambycidae (2 of 2 studied species). It was absent in conifer-associated weevils of the genera *Hylobius* and *Pissodes* but it was isolated from another subcortical weevil, *Magdalis armigera* from elm. Surveys focused specifically on *Geosmithia*, or comprehensively documenting fungal communities of subcortical beetles, have been carried out mainly in Europe, the Mediterranean basin, and North America, with fewer studies from the rest of the world, such as from South America, South Africa and China (Fig. 4, Table 1, 2).

The degree of *Geosmithia* association with tree hosts of with beetle vectors can be determined by various quantitative approaches. Unfortunately, different approaches have been used by different authors, including nonstandard definitions of a sample and of sample size, making it difficult to compare between studies. We recommend using basic measures such as the proportion of gallery systems (e.g. (Kolařík et al., 2017), insect bodies (adults, larvae), or gallery segments (eg. (Dori-Bachash et al., 2015; Jankowiak et al., 2014), with *Geosmithia* out of all sampled. A more quantitative estimate of prevalence is the percentage of CFU counts belonging to *Geosmithia* within the whole sampled fungus community (Skelton et al., 2018).

Already Roland Krischner (2001) noted that bark beetles differ in their degree of association with *Geosmithia*. He also noted that beetles frequently transmitting *Geosmithia* tend to carry lower frequency and diversity of ophiostomatoid fungi, and called *Geosmithia* an ecological replacement for ophiostomatoids. Subsequent studies confirmed this pattern. Subcortical insects (mostly bark beetles) can be divided into those with whom *Geosmithia* is associated strongly, moderately, or not at all (Table 2, Fig. 5). Several beetle-tree networks are regular *Geosmithia* vectors: 1) broad leaved shrubs and trees, except of *Betula* and *Alnus*, and beetle species preferring trunk bases, 2) trees in the family *Cupressaceae*, except for *Calocedrus*, 3) trees in the family *Pinaceae*, mostly on beetles that colonize parts with the thinner bark. *Geosmithia* beetle vectors associated with hardwoods include subcortical *Curculionidae* (several subfamilies: *Cossoninae, Scolytinae*, and *Mesoptiliinae*), and *Bostrichidae*. Wood borers which occur under bark only as larvae but not as adults (*Cerambycidae* and *Buprestidae*) do not serve as reliable vectors, and therefore are not typically associated with *Geosmithia*. *Geosmithia* are rare or absent on insects colonizing large limbs and trunks of *Pinaceae* and *Betula*. Within *Pinaceae*, *Geosmithia* abundance and diversity is negatively correlated with thickness of the wood substrata preferred by the insects (Jankowiak and Bilanski, 2018; Kolařík and Jankowiak, 2013). Similarly on *Betula*, the bark beetle *Scolytus ratzeburgi* feeds in very moist substrate, under the impermeable bark, and hosts an abundance of ophiostomatoid fungi, but no *Geosmithia* (Linnakoski et al., 2008). On most other hardwoods, such as *Fraxinus*, *Ulmus* and woody plants from the *Rosaceae* family, vectors specific to trunk bases has much less frequent association. Most conifers within *Cupressaceae* support diverse communities of *Geosmithia*, with the exception of *Calocedrus*. Isolations from the beetle *Phloesinus fulgens*, specific to *Calocedrus,* typical yield low abundance of *Geosmithia*, and mostly *Pinaceae*-specific species. This may reflect the larger size of the tree and more humid environment than in most other *Cupressaceae* (Table 2).

Fig. 3 *Geosmithia* on native samples and cultivations plates **A** *G. lavendula* (lilac) and *G. radiata* (white) in *Hypoporus ficus* galleries (*Ficus*, Croatia). **B** *G. lavendula* in bostrichid gallery (*Toxicodendron,* California). **C** *G. microcorthili* in galleries of ambrosia beetle *Microcorhyllus* sp. (Costa Rica). **D** *G. flava* in galleries of *Ernoporus tiliae* (*Tilia*, Czechia). **E** *G. flava* in pupal chamber of *Cryphalus piceae* (*Abies*, Czechia). **F** Necrosis caused by *G. morbida* in the phloem of *Juglans* (testing hole of *Pityophthorus juglandis*, U.S). **G** *G. flava* and *Ophiostoma novo-*

ulmi (white droplets) growing on agar plated with *Scolytus multistiatus* adults (*Ulmus*, Czechia). **H** Agar plate with *Geosmithia* colonies obtained from *H. ficus* galleries (*Ficus*, Croatia). **I**. *Pityophthorus pityographus* adult and detritus from the gallery overgrown by yeasts and *Geosmithia* sp. 24 (*Pinus*, Czechia).

1.5.5 Vector specificity, community composition and Biogeography

The recent twenty years of research on *Geosmithia* worldwide has finally enabled the first attempt at a synthesis of the ecology and distribution of these fungi (Table 1, 2, Fig. 4). *Geosmithia* can be divided into generalist species that are common across vectors worldwide, and can also be found outside of the subcortical habitat, such as in decaying wood, soil, cereals and foodstuffs (Kolařík et al., 2004; Labuda and Tancinová, 2006; Pitt and Hocking, 2009), sea sediments (Ameen et al., 2014; Sun et al., 2018), cave environment (Bastian et al., 2009; Crous et al., 2018), or as plant endophyte (Deka and Jha, 2018; McPherson et al., 2013; Sakalidis et al., 2011) (Table 1). This is typical of species in the *G. pallida* complex (*G. pumilla*, *G. pulverea*), then *G. fassatiae*, *G. flava*, *G. granulata*, *G. langdonii*, *G. obscura, G. omnicola*, *G. putterilli, G. xerotolerans* and *Geosmithia* sp. 1. In contrast, specialists species occur on vectors sharing the host plants of the same plant family. These include species that are restricted in occurrence to *Pinaceae* hosts, then *G. morbida* (*Juglans*, Europe, North America), *G. ulmacea* (*Ulmus*, Europe, North America). Sometimes the host preference is maintained in the particular geographical area with occurrence on other hosts in different areas (e.g. *Geosmithia* sp. 12 - *Fraxinus*, *G*. sp. 32 - *Cupressaceae*, *G*. sp. 11 - *Olea*, *G. carolliae – Ficus*). Some species common in some areas (e.g *G*. sp. 41 in North America, *G. funiculosa* in Europe), are absent in others, suggesting biogeographical patterns independent of the tree vector/host distribution (Fig. 4, Table 1). We do not have enough data for ambrosial *Geosmithia* species, but a high vector specificity can be expected. This appears to be the case with *G. eupagioceri*, which has so far only been found on the beetle *Eupagiocerus dentipes* in two separate collections in Central America (Kolařík and Kirkendall, 2010, J. Hulcr, unpublished).

Geosmithia communities in any given locality is structured by the influence of the local host tree availability, biogeographical limits, and the presence of suitable vector beetles. The strongest predictors is the host plant. Increasingly it is becoming evident that the beetle vectors are passive (with exception of primary ambrosia species), not actively involved with *Geosmithia*, and that these apparent fungus-beetle associations are derived from the underlying patterns of the tree host use by *Geosmithia.* As *Geosmithia* depend on host trees for development, and on beetles frequenting those trees for transmission, their ecological specialization is best understood on the level of tree-beetle networks. Consequently, insect vector species who regularly co-occur in the same tree part consequently have similar communities of fungi without being able to actively select them. For example, *Pinus* trees support the same community across different vectors beetles, and the community is different from those associated with *Picea* (Jankowiak et al., 2014; Kolařík and Jankowiak, 2013) and *Abies* (Jankowiak and Bilanski, 2018; Jankowiak and Kolarik,

2010), and even further distant from those specific to angiosperms and *Cupressaceae* (except of *Calocedrus*). As an example, polyphagous vectors such as *Pityogenes chalcographus* and *Pityophthorus pityographus* carry fungi specific to *Pinus* or *Picea*, depending on the substrate from which were collected (Jankowiak et al., 2014). This same pattern of *Geosmithia* community structure has been observed in angiosperms in temperate Europe and USA (Huang et al., 2019; Kolařík et al., 2008; Strzałka et al., 2021). Community composition is also shaped by the degree of specificity of the bark beetle vectors available in a given area. If there are only host-specific beetles on a given host plant, more sharply delimited *Geosmithia* communities are formed. Conversely, polyphagous beetle vectors create more diffuse fungus communities (Kolařík et al., 2017; Kolařík et al., 2007). In turn, this regional species pool and its dynamics also influences the richness of *Geosmithia* in individual beetle galleries: in mixed forest, *Geosmithia* communities are more diverse and then in conifer monocultures (Jankowiak et al., 2014).

Some *Geosmithia* species may be primarily endophytic, and only secondarily associated with bark beetle galleries. In California, *G. langdonii* was isolated from both bark beetle galleries as well as an end, whereas two other only isolated from bark beetle galleries (McPherson et al., 2013).

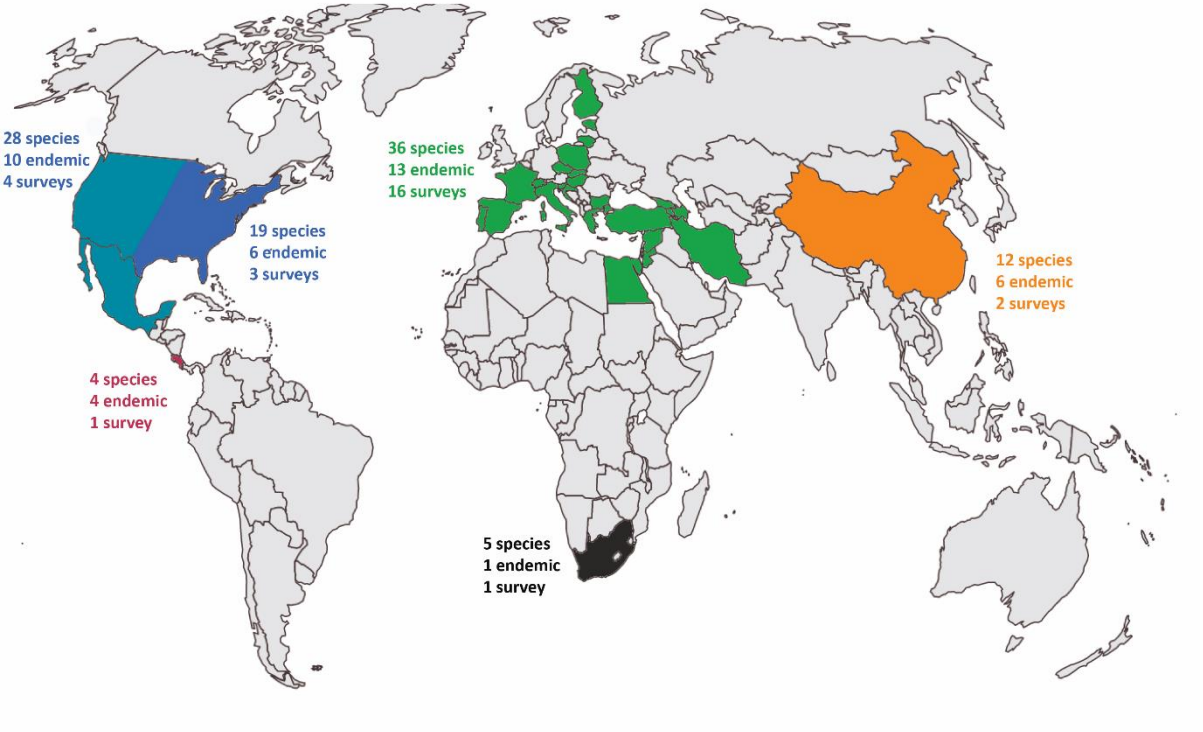

Fig. 4 Map showing the locations where *Geosmithia* species spectrum and diversity has been studied and indicates the total number of species found in each area and the number of species not yet found outside that area ("endemic" species). The map is based on Table 2.

1.5.6 Geosmithia interactions with host insect and plant

It is not completely clear how adult bark beetles transport *Geosmithia*. The majority of known and reliable vectors lack mycangia or any other organs adapted to fungus dispersal, and propagules appear to be transmitted passively in the gut or opportunistically attached to crevices and punctures of the exoskeleton. Several reports show *Geosmithia* presence in mycangia (Belhoucine et al., 2011; Kolařík et al., 2017; Six et al., 2009). Phoretic mites are also able to vector *Geosmithia* fungi (Machingambi et al., 2014).

Bark beetle associated fungi are known to have diverse symbiotic (i.e. mutualistic, neutral or antagonistic) interactions with their beetle vectors. The most straightforward is commensalism or by-product mutualism, in which the fungus benefits from the beetle's ability to invade fresh plant tissues, which enables the fungus to exploit these nutrient sources, but the fungus does not necessarily benefit the beetle vector (Six, 2020; Six and Wingfield, 2011). Ambrosial *Geosmithia* species are mutualistic, as they provide nutrition to the beetle hosts, but it remains unknown whether the non-ambrosia *Geosmithia* also provide any benefit. Most species are good degraders of hemicellulose, and some are able to also degrade cellulose and lignin; which may benefit the beetle directly or indirectly. Some are able to utilize uric acid as nitrogen sources (Veselská et al., 2019), and thus recycling of nitrogen from the beetle waste product may be a benefit to their hosts that has not been tested. *Geosmithia* can further interact with the insect via volatile chemicals. Volatiles of *G. morbida* attract its insect vector and may synergize beetle aggregation (Blood et al., 2018).

Geosmithia also interacts with other fungi in the beetle galleries. For example, mycoparasitism by *Geosmithia* was observed on *Ophiostoma novo-ulmi*, the fungus responsible for the Dutch elm disease (Pepori et al., 2018). *Geosmithia* produces variety of biologically active compounds, through which they can interact with the ambient microbial community. Antibiosis towards fungi and bacteria has been found in many *Geosmithia* species (Veselská et al., 2019), and tested most extensively in *G. lavendula* (Hadj Taieb et al., 2019; Malak et al., 2013a; Stodůlková et al., 2009) and *G. pallida* KU693285 (Deka and Jha, 2018). Machingambi et al. (2014) have suggested that mites (bark beetle parasites) were unable to feed or reproduce in the presence of *Geosmithia* associates; the miticidal potential of *Geosmithia* should be studied in detail. *G. lavendula* and other species produce hydroxylated anthraquinones (hAQs) with many bioactive properties (Ganapaty et al., 2004; Hilker and Köpf, 1994; Poche, 1998; Stodůlková et al., 2009); the role of hAQs in the bark beetle ecosystem has not been evaluated.

1.5.7 Evolution and biology

The reconstruction of phylogenetic relationships among *Geosmithia* has been conducted primarily using protein-coding genes, as the ribosomal DNA markers, typically used in other fungi, genes have several limitations in *Geosmithia*. Specifically, *Geosmithia* sp. 26 is a species complex that has very different rDNA sequences from others and a very low GC content, preventing a quality alignment (Kolařík et al.,

2017). Subsequently, phylogenies inferred from rDNA and from protein coding genes are in conflict (Veselská et al., 2019). The rapid rDNA sequence evolution and GC content deviation in *Geosmithia* sp. 26 may be a consequence of fluctuations in the effective population change and bottlenecks, possibly related to the switch between free living to host associated life strategy (Kolařík and Vohník, 2017; Kolařík et al., 2021).

Geosmithia species feature many life history traits distributed across the phylogeny, making the genus an ideal model for studying the evolution of individual life styles and associated phenotypic traits (Veselská et al., 2019). Basal *Geosmithia* lineages are generalists, with broad host ranges across Angiosperms and Gymnosperms and sometimes found also outside of bark beetle habitat. At least six lineages convergently evolved specificity to the *Pinaceae* family (Strzałka et al., 2021; Veselská et al., 2019; Zhang et al., 2022). The shifts were accompanied by losses of metabolic capacity and by genome size inflation. In vitro, this is apparent by the inability to growth on basal CZD agar, which lacks important nutrient supplements such as vitamins (in particular the B group). Three other derived lineages converged on the ambrosia strategy, providing nutrition to specific beetle vectors. This was accompanied by the cell and genome size inflation and the production of large amounts of oleic fatty acid, likely associated with the nutritive function (Veselská and Kolařík, 2015; Veselská et al., 2019). In terms of morphology, ambrosia species produce large conidia, a phenotype seen in other ambrosia fungi (Kolařík et al., 2015; Kolařík and Kirkendall, 2010). One lineage, *G. morbida*, became plant pathogen, with the unique ability to digest all components of lignocellulose, what can serve as its virulence factor (Veselská et al., 2019), similarly as in other plant pathogenic fungi (Doehlemann et al., 2017; Jagadeeswaran et al., 2021). In general, specialists, such as those on *Pinaceae* and the *Junglans*-specific *G. morbida*, have a reduced metabolic breadth in comparison to generalists. The genome size in *Geosmithia*, correlates with cell size (e.g., conidia), as in most eukaryotes, and is related to the ecology of the species. Specifically, species specialized to a narrow host range (including *G. morbida*) have relatively large genomes, compared to generalists. The largest genomes are present in the ambrosial species (Veselská and Kolařík, 2015).

Relatively little research has been done on the genetics and mating behaviour of these fungi. As with other filamentous ascomycetes, there is a system of vegetative incompatibility that leads to some isolates making mycelial fusions with each other but not with others. In practice, this is manifested by the presence of non-coalescent lesions in the case of *G. mrobida* (Montecchio et al., 2015). The sexual stage has never been observed, and the only population genetics study in the genus suggested absence of recombination in *G. morbida* (Zerillo et al., 2014). Both mating gene idiomorphs (MAT1-1, MAT1-2) are present across the genus (M.K. unpublished) and targeted crossing experiments should be carried out to induce the sexual stage, as has been done in other moulds where the sexual stage was unknown (O'Gorman et al., 2008). In *Geosmithia*, a cleistothecial type of sexual state can be expected, as is the case with related fungi such as *Nigrosabulum*, *Mycoarachis* or *Hapsidospora* (Plishka et al., 2009; Rossman et al., 1999). The genome size, determined by flow cytometry, is 20.5 to at least 54 Mb. The largest genomes are those

of ambrosial species, probably due to the ancient polyplodisation (Veselská and Kolařík, 2015). The genome size values measured by flow cytomertry in *G. morbida*, *G. flava* and *G. putterilli* (24.4-24.7, 25.5-25.8, 26-26.3 Mbp) agree with those measured by whole genome sequencing (26.5, 29.6, 30.0 Mbs) (Schuelke et al., 2017). The number of genes is around 6,000 and only 73-146 were found to be species-specific. Between 300-400 (349-403) protein-coding genes belong to secreted proteins. There are few genes involved in secondary metabolism compared to related taxa such as *Acremonium chrysogenum* and *Stanjemonium grisellum*. In *G. morbida*, 26 genes have homologs with known involvement in interactions with the plant host and thus a potential role in pathogenesis (Schuelke et al., 2017).

Geosmithia, like other Dikarya, have hyphae coated with hydrophobins, that are small proteins, forming and hydrophic membrane and have a crucial role in interactions with hydrophobic substrates such as plant or insect cuticle. *Geosmithia* have class II hydrophobins, called GEO1 (Bettini et al., 2012; Frascella et al., 2014). They possess intragenic tandem repeat sequence implicated in the rapid generation of variation and subsequent adaptation. GEO1 is also under strong selection pressure, suggesting that the capacity for adhesion is important in the evolution of the genus. The cluster is evolving either via multiple horizontal transfer events and/or birth-and-death evolution. There is also good evidence that at least six *Geosmithia* obtained another hyrophobin, cerato-ulmin, by a horizontal transfer from *Ophiostoma novo-ulmi*. Cerato-ulmin is involved in the virulence of *O. novo-ulmi*, a causal agent in Dutch elm disease of elms, and is presented only in *Geosmithia* strains from elms, but not in those from other tree hosts (Bettini et al., 2014).

1.5.8 Phytopathogenic potential and TCD

While most *Geosmithia* appear to be saprophytes, the pathogenicity capabilities of some species deserves closer scrutiny. Already the first study on *Geosmithia* (Wright, 1938) studied the infectious potential of *Geosmithia* from *Scolytus praeceps* and *S. subscaber.* When inoculated into a live plant host, these strains were able to cause significant necrosis in the cambium of *Abies concolor* trunk. Based on the morphology, the strain used in the study probably belongs to *Geosmithia* sp. 34 or sp. 34 (Kolařík et al., 2017), and the pathogenicity observations, while convincing, require further verification.

Until now, the pathogenicity was studied by inoculating the phloem of seedlings or adult tree branches in more than 20 *Geosmithia* species and mostly showed no evidence of pathogenicity. In particular, no pathogenic effect was found in *Geosmithia* sp. 16 on *Abies alba* (Jankowiak and Kolarik, 2010), two species from *Geosmithia* sp. 24 species complex on *Pinus* spp. in Israel (Dori-Bachash et al., 2015), four species (*G. cupressina*, *G. langdonii, G. omnicola*, *G.* sp. 708) on *Cupressus* (Meshram et al., 2022), five species (*G. flava*, *G. ominicola*, *G. pumila* (=*G.* sp. 2), *G.* sp. 8, *G.* sp. A) on *Virgilia* (Machingambi et al., 2014), six *Geosmithia* spp. (*G. fagi*, *G. flava*, *G. langdonii*, *G. ulmacea*, *G. pulverea* (= sp. 3) and *G. funiculosa* (= sp. 5)) on *Acer*, *Fagus*, *Quercus*, *Tilia* and *Ulmus* (Crous et al., 2022; Strzałka

et al., 2021), *G. luteobrunnea* on *Liquidambar* (Gao et al., 2021; Zhang et al., 2022) and 11 *Geosmithia* strains originating from Czechia, Korea, Vietnam, China, Papua New Guinea, Taiwan on *Quercus shumardii,* and *Q. virginiana* (Li et al., 2022).

A few *Geosmitia* do induce phloem necroses, however, and several are involved in plant diseases. Pathogenicity assays performed using the excised shoot method showed ability of tissue lesion formation in *G. granulata* (= sp. 20), *G. lavendula*, *G. omnicola* and *G. pallida* on *Pistacia vera* (Hadj Taieb et al., 2019). However, testing pathogenicity on detached shoots is questionable, as the results cannot be extrapolated to natural field conditions. Mild, but significant, lession were created by *Geosmithia* sp. 12568 (*Cryphalus piceus*, South Korea) on *Pinus* spp. (Li et al., 2022) and by *Geosmithia* sp. on an artificially inoculated *Olea europea* trunk (van Dyk et al., 2021). Čížková et al. (2005) have shown that *G. pumila* (= sp. 2) *G. langdonii* inhibited the growth of garden cress *Lepidium sativum*.

Several tree diseases are caused by bark beetles which carry *Geosmithia* species, and the fungi may form discolored areas around the beetle galleries, but are not pathogenic themselves. In the so-called Foamy Bark Canker of *Quercus agrifolia* in California (USA), the disease appears to be caused by infestation by the bark beetle *Pseudopityophthorus pubipennis*. The beetle vectors *Geosmithia* sp. 41 and other species ((Kolařík et al., 2017)). This fungus produces significant lesions on artificially inoculated excised oak shoots (Lynch et al., 2014), but both the disease and its causal agent needs further study. Large mortality of American sweetgum (*Liquidambar styraciflua*) planted in China caused by the bark beetle *Acanthotomicus suncei* also involves several species of *Geosmithia*, most commonly *G. luteobrunnea*, around the beetle galleries, but again, the fungus is not pathogenic on its own (Gao et al., 2021; Zhang et al., 2022). Similarly puzzling is the presence of the Dutch elm disease pathogenicity factor cerato-ulmin in *Geosmithia* spp. (Bettini et al., 2014; Scala et al., 2007) while the no active role of the fungus in the disease has been demonstrated yet.

The only case where the fungus makes significant necrosis and together with its vector kills the host plant, is *Geosmithia morbida*. Together with its vector, the walnut twig beetle *Pityophthorus juglandis*, the two organisms contributed to the phenomenon of the Thousand Cancers Disease (TCD) of black walnut, *Juglans nigra* (Kolařík et al., 2011; Tisserat et al., 2009). While *G. morida* and its vector beetle *P. juglandis* are to West of the Norther America, and recently dispersed to other parts of the continent, as well as to Europe (reviewed in Daniels et al. (2016). For several years following this expansion and a drought, there was a notable dieback of black walnut across the U.S. The dieback has recently subsided, with the exceptions of locations where black walnut is planted outside of its typical growing conditions (i.e., California), suggesting that the disease has been largely a symptom of drought. While temporary, the impact of TCD spurred research on *Geosmithia* and its symbiosis with bark beetles (Fig. 1). Research on *G. mobida* involved its genetic variability (Hadziabdic et al., 2014a; Zerillo et al., 2014), host tree range a virulence (Hefty et al., 2018; Sitz et al., 2017; Sitz et al., 2021), vectors (Chahal et al., 2019), migration

(Hadziabdic et al., 2014b; Marchioro and Faccoli, 2022; Montecchio and Faccoli, 2014; Moricca et al., 2020), detection (Stackhouse et al., 2021), eradication (Dal Maso et al., 2019; Juzwik et al., 2021; Seabright et al., 2019) and competition with co-occurring fungi (Gazis et al., 2018). Walnut twig beetle transmits other *Geosmithia* species (Kolařík et al., 2017), and as can be seen from preliminary results with infection experiments with *G. obscura* (Pietsch et al., 2022).

A cross-phylogeny comparison of pathogenic and non-pathogenic species at the genome (Schuelke et al., 2017)) and phenotype level has shown that *G. morbida* is unique among *Geosmithia* species in producing an enzyme that breaks down both cellulose and lignin (Veselská et al., 2019). This capacity can be considered one of the virulence factors responsible for the ability to necrotize the phloem of walnut (Veselská et al., 2019). An interesting avenue of research is the study of the presence of viruses in *G. morbida* that may modulate virulence (Montecchio et al., 2015).

1.5.9 Secondary metabolite production and biotechnological potential

The order Hypocreales is known for the ability to produce a variety of secondary compounds, including toxins. Even crude extracts from *Geosmithia* shows the potential for antibacterial and antifungal activity across the genus (Deka and Jha, 2018; Veselská et al., 2019). Aside from common fungal metabolites, 48 secondary metabolites were found uniquely in *Geosmithia* (Table 3). Prominent yellow, orange, and red pigments produced by *G. lavendula* represent more than twenty different hydroxylated anthraquinones, often novel to science, several of them with antibacterial or anti-inflammatory activity (Malak et al., 2013c; Stodůlková et al., 2009; Stodůlková et al., 2010) and with the potential as persistent textile dyes or mordants (Flieger et al., 2009). *Geosmithia pallida* complex strain FS140 (Table 2) produced 12 different thiodiketopiperazines, including three previously unknown ones (Sun et al., 2018). A single strain identified morphologically as *G. langdonii* yielded 14 metabolites, including four new ones (Malak et al., 2013b; Malak et al., 2018). Their biological activities include antimicrobial, cytotoxic, angiotensin-converting enzyme inhibitory, antileishmanial or nemacitidal (Table 3).

While these first studied on secondary metabolites in *Geosmithia* yielded a large proportion of novel compounds and broad biological activity, the chemical arsenal is rather limited in terms of biosynthetic pathways, yielding mostly low molecular weight, structurally simple metabolites. The three species studied - *G. morbida, G. putterilli* and *G. flava* - produce only 14 to 19 secondary metabolite gene clusters only, which contrasts with related filamentous fungi having four-time greater number of similar genes clusters (Schuelke et al., 2017). However, the genetics of secondary metabolite production was explored in these three species only, all belonging to a single phylogenetic lineage, and the novelty of these products bids for further bioprospecting.

1.5.10 Conclusion and future research

Geosmithia has been in the spotlight only for the last decade, and so it is not surprising that many questions, long studied in ecologically similar taxa, are still unanswered. The broad evolutionary direction towards long-term and stable adaptation to beetle vectors observed in *Geosmithia* is the same as that observed in ophiostomatoid fungi. In both groups, it culminated in the evolution of ambrosia lineages from phloem inhabiting ancestors, and a coevolutionary response from the beetles. One of the most important paradigms that has emerged from the surge of studies on *Geosmithia* is that *Geosmithia* are an ecological complement to the ophiostomatoid fungi (Kirschner, 2001). We suggest here the terms *Geosmithia*-type and ophiostomatoid fungi-type association. Both fungal groups are dependent on bark beetle vectors for their dispersal and reproduction. *Geosmithia*, however is almost exclusively found in phloem that is drier, and typically more advanced in decay, and as a result are associated with bark beetle communities utilizing upper and thinner parts of trees. Ophiostomatoid fungi, in turn, dominate phloem which retains moisture longer, and therefore are associated with bark beetles on the trunk and roots. This patterns is replicated all around the world, but the factors responsible for it remain unclear. One of these factors could be the relatively greater tolerance to desiccation and osmotolerance in *Geosmithia*, as is known in *G. xerotolerans* (Crous et al., 2018), and greater competitivenes under drought conditions, as found in *G. morbida* (Williams and Ginzel, 2021). Other abiotic variables such as oxygen level and resin concentration have been identified as distinguishing the growth of *Edoconidiophora polonica*, living in the fresh phloem of the tree trunks, from *Ophiostoma* species living in the dead phloem and in thinner tree parts (Solheim, 1991). Their effect on the growth of *Geosmithia* should be tested (Fig. 5). In terms of pathogenicity, many bark beetle-associates cause discoloration of the phloem around the beetle gallery, but *bona fide* pathogenicity in the absence of the beetle is rare, truly present only in *G. morbida*. Several species, such as those on fir in North America, are good candidates for verification of possible weak pathogenicity (Wright, 1938).

The other major lineages of fungi associated with bark beetles – the *Ophiostomatales*, *Microascales*, and several groups within *Fusarium* – also include a range of specificity, from plant pathogens, to soil saprobes, to obligate ambrosia fungi. Sometimes closely related species display dramatically different ecology. *Geosmithia* shows similar patterns, and is an excellent model for the study of adaptive traits related to species interactions. The evolutions of these traits in *Geosmithia* has been documented at the phenotypic level (Veselská et al., 2019), and the next step needs to include a deeper, genomic level.

The main lesson learned from the recent surge of interest in the study of *Geosmithia* is that these fungi are woefully undersampled geographically. A few species are cosmopolitan generalists, but many show considerable specificity to hosts and locations (Fig. 4). Continued studies on this genus needs to emulate the methods from a better studied taxa such as *Penicillium*, *Aspergillus* or *Fusarium*. More variable DNA markers are needed to answer taxonomic, evolutionary and molecular biology questions. Similarly,

and broader array of differentiated media (DG18, G25N, MY70S, CREA) are needed for morphological and metabolic characters. *Geosmithia* also still lacks sufficient genomic data, as only three genomes have been published to date.

Given the many new chemicals isolated from *Geosmithia*, these fungi deserve research also for their biotechnological potential. These fungi do not appear to produce structurally complex substances, and also the diversity of secondary metabolites and biosynthetic pathways is modest. However, the known substances show no or very little cytotoxicity to animal cells, and at the same time they have a number of biological activities. The bioactivity is highly selective, i.e., the fungi do not harm insects, while showing antibacterial antibiosis. Their potential to interact with organisms that are pathogens of bark beetles, such as nematodes and mites, should be tested. Ambrosia species potentially an interesting target for fungal food research, since they provide a complete nutrition to their animal vectors concentrated in enlarged conidia rich in proteins and oils, while being entirely non-toxic and non-melanized (Veselská et al., 2019), M. K. unpublished).

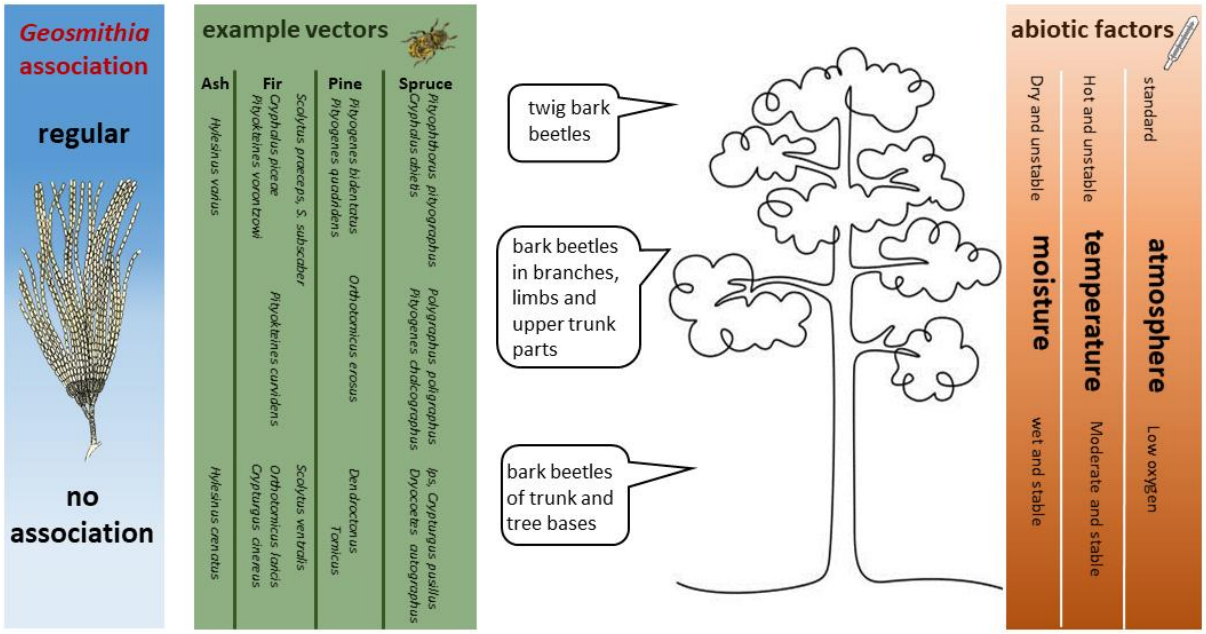

Fig 5 Schematic presentation of the *Geosmithia* association across bark beetles of different organ preference. The abiotic factors of different beetle substrates are shown.

Table 1 List of recognised *Geosmithia* species with geographical distribution and substrate origin. The host spectrum is expressed as list of host plant families from which the insect vector was collected

[1] The numbering for species no. 1 —31 follows Kolařík et al. (2007, 2008,2013), 33-44 (Kolařík et al., 2017), 45-48 (Huang et al., 2019). *Geosmithia pulverea* and *Geosmithia* sp. 23 may represent a same species (Zhang et al., 2022).

Species[1]	**Central and North Eastern Europe (~Temperate Europe)**	**Mediterranean Basin and Black and Caspian Sea region**	**Western U.S. and Northern Mexico**	**South and Eastern U.S.**	**China**	**Other substrates or locations**	**References**
G. bombycina	—	—	—	—	*Rosaceae*	—	(Zhang et al., 2022)
G. brevistipitata	—	—	—	—	*Cupressaceae*	—	(Zhang et al., 2022)
G. brunnea	—	—	—	*Altingiaceae, Fagaceae*	—	—	(Huang et al., 2017; Huang et al., 2019)
G. carolliae (*G.* sp. 19)	—	*Moraceae*	—	—	—	wing of the bat, Brazil	(Crous et al., 2018; Kolařík et al., 2007)
G. cnesini	—	—	—	—	—	ambrosia beetle *Cnesinus lecontei,* Costa Rica	(Kolařík et al., 2015; Kolařík and Kirkendall, 2010)
G. cupressina	—	*Cupressaceae*	—	—	—	—	(Meshram et al., 2022)
G. eupagioceri	—	—	—			ambrosia beetle *Eupagiocerus dentipes,* Costa Rica	(Kolařík and Kirkendall, 2010),
G. fagi	*Rosaceae, Fagaceae*	—	—	—	—	—	(Strzałka et al., 2021)

G. fassatiae	*Fagaceae, Rosaceae*	—	*Pinaceae, Fagaceae, Salicaceae, Lauraceae*	—	—	—	(Kolařík et al., 2017; Kolařík et al., 2008; McPherson et al., 2013)
G. flava	*Araliaceae, Betulaceae, Cupressaceae, Fagaceae, Oleaceae, Pinaceae, Rosaceae, Tiliaceae, Ulmaceae*	*Anacardiaceae, Lauraceae, Moraceae, Rosaceae*	*Anacardiaceae, Cupressaceae, Fagaceae, Juglandaceae, Pinaceae, Salicaceae*	—	—	scolytids from *Virgilia* spp., South Africa; *Ulmus glabra*, *Hordeum* sp. Grain, England	(Kolařík et al., 2017; Kolařík et al., 2007; Kolařík et al., 2008; Kolařík et al., 2004; Machingambi et al., 2014; Pepori et al., 2015; Strzałka et al., 2021),
G. funiculosa (*G.* sp. 5)	*Fagaceae, Oleaceae, Pinaceae, Rosaceae, Tiliaceae, Ulmaceae*	*Anacardiaceae*	—	—	—	*Scolytus* beetle, UK	(Crous et al., 2022; Kolařík et al., 2007; Kolařík et al., 2008; Kolařík et al., 2004; Pepori et al., 2015; Strzałka et al., 2021)
G. fusca	—	—	—	—	*Fabaceae, Phyllanthaceae, Malvaceae*	—	(Zhang et al., 2022)
G. granulata (= *G.* sp. 20)	—	*Asteraceae, Fabaceae, Moraceae, Oleaceae, Ulmaceae, Anacardiaceae*	*Cupressaceae, Ulmaceae*	—	*Fabaceae, Malvaceae*	—	(Hadj Taieb et al., 2019; Kolařík et al., 2017; Kolařík et al., 2007; Zhang et al., 2022)

G. langdonii	*Cupressaceae, Betulaceae, Fabaceae, Fagaceae, Tiliaceae, Rosaceae, Ulmaceae*	*Anacardiaceae, Euphorbiaceae, Fagaceae, Lauraceae*	*Asteraceae, Cupressaceae, Fagaceae, Lauraceae, Pinaceae*	—	—	—	(Benvenuti et al., 2021; Hanzi et al., 2016; Juan Alfredo et al., 2020; McPherson et al., 2013; Meshram et al., 2022; Strzałka et al., 2021; Vitale et al., 2021)
G. lavendula	—	*Anacardiaceae, Fabaceae, Moraceae, Ulmaceae*	*Anacardiaceae, Cupressaceae, Fagaceae, Juglandaceae, Pinaceae, Rosaceae*	*Fagaceae, Vitaceae*, unknown	—	laboratory contaminant, U.S; *Carya* wood, Israel, soi,Venezuela	(Hadj Taieb et al., 2019; Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2017; Kolařík et al., 2007; Morales-Rodríguez et al., 2021; Pitt, 1979; Six et al., 2009)
G. longistipitata (*G.* sp. 28)	*Pinaceae*	—	—	—	—	—	(Jankowiak et al., 2014; Kolařík and Jankowiak, 2013; Strzałka et al., 2021)
G. luteobrunnea	—	—	—	—	*Altingiaceae, Ulmaceae*		(Zhang et al., 2022)
G. microcorthyli	—	—	—	—	—	ambrosia beetle *Microcorthylus* sp., Costa Rica	(Kolařík and Kirkendall, 2010)
G. morbida	—	*Juglandaceae*	*Juglandaceae*	*Juglandaceae*	—	—	(Hadziabdic et al., 2014b; Kolařík et al.,

							2017; Montecchio et al., 2015) and others
G. obscura	*Betulaceae, Fagaceae*		—	*Cupressaceae, Juglandaceae, Vitaceae*	—	—	(Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2008; Six et al., 2009)
G. omnicola (*G.* sp. 10)	*Araliaceae, Betulaceae Cupressaceae, Fagaceae, Oleaceae, Rosaceae, Salicaceae, Tiliaceae, Ulmaceae*	*Anacardiaceae, Cupressaceae, Fabaceae, Lauraceae, Moraceae, Rosaceae, Ulmaceae*	—	*Fagaceae, unknown*	—	scolytids from *Virgilia* spp., South Africa; air, Israel	(Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2017; Kolařík et al., 2007; Kolařík et al., 2008; Machingambi et al., 2014; Meshram et al., 2022; Pepori et al., 2015)
G. pallida s. s.	—	—	—	*Juglandaceae, Fagaceae, unknown, Arecaceae*	*Fabaceae*	cotton yarn, England; soil, Nigeria	(Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2017; Pitt, 1979; Zhang et al., 2022)
G. pazoutovae	*Fagaceae*			—	—	—	(Strzałka et al., 2021)
G. proliferans	—	—	—	*Sapindaceae*	—	—	(Huang et al., 2017; Huang et al., 2019)
G. pulverea (*G.* sp. 3)	*Betulaceae, Fagaceae, Rosaceae*	—	—	—	*Gnetaceae Altingiaceae, Fabaceae, Rosaceae, Anacardiaceae, Ulmaceae*	roots of *Quercus robur*, soil, Czechia	(Kolařík et al., 2008; Kolařík et al., 2004; Strzałka et al., 2021; Zhang et al., 2022)

G. pumila (*G.* sp. 2)	*Fagaceae, Oleaceae, Rosaceae, Ulmaceae*	*Fagaceae, Lauraceae, Ulmaceae*	*Rosaceae*	*Cupressaceae, Fagaceae, Juglandaceae, Oleaceae, Ulmaceae, unknown*	*Ulmaceae*	scolytids from *Virgilia,* South Africa; apple tree, Cyprus, *Cucumis melo,* Peru	(Hanzi et al., 2016; Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2017; Kolařík et al., 2007; Kolařík et al., 2008; Kolařík et al., 2004; Machingambi et al., 2014; Morales-Rodríguez et al., 2021; Pepori et al., 2015; Strzałka et al., 2021; Zhang et al., 2022)
G. putterillii	*Rosaceae*	*Lauraceae*	*Cupressaceae, Ericaceae, Fagaceae, Juglandaceae, Lauraceae, Pinaceae, Salicaceae*	—	*Lauraceae*	*Beilschmiedia tawa* wood, New Zealand	(Kolařík et al., 2017; Kolařík et al., 2007; Kolařík et al., 2008; Kolařík et al., 2004; Zhang et al., 2022)
G. radiata	—	—	—	—	*Altingiaceae, Ulmaceae*	—	(Zhang et al., 2022)
G. rufescens	—	—	—			two ambrosia beetle species, Costa Rica	(Kolařík and Kirkendall, 2010)
G. subfulva	—	—	—	—	*Rosaceae*, Anacardiaceae	—	(Zhang et al., 2022)
G. xerotolerans (= *G.* sp. 21)	—	Fabaceae, Moraceae, Oleaceae	*Cupressaceae*, Fagaceae, *Pinaceae*, *Rosaceae*	*Cupressaceae*, Fagaceae	*Cupressaceae*	house wall, Spain	(Crous et al., 2018; Huang et al., 2017; Huang et al., 2019;

							Juan Alfredo et al., 2020; Kolařík et al., 2017)
Geosmithia sp. 1	*Cupressaceae*, Ranunculaceae, Ulmaceae	Fabaceae, Moraceae	—	—	—	—	(Hanzi et al., 2016; Kolařík et al., 2007; Kolařík et al., 2008)
Geosmithia sp. 4	Ulmaceae	—	—	—	—	—	(Kolařík et al., 2008)
Geosmithia sp. 8	Fagaceae	—	—	unknown	—	—	(Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2008)
Geosmithia sp. 9	*Pinaceae*	—	—	—	—	—	(Jankowiak and Bilanski, 2018; Jankowiak et al., 2014; Kolařík and Jankowiak, 2013; Kolařík et al., 2008)
Geosmithia sp. 11	Fagaceae	Oleaceae	—	Fagaceae	—	endophyte of *Adansonia gregorii,* Australia, based on sequence similarity (99%, GU19942)	(Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2007; Kolařík et al., 2008; Sakalidis et al., 2011)
Geosmithia sp. 12	Fagaceae, Oleaceae	—	Oleaceae	Juglandaceae, Fagaceae, Oleaceae	—	—	(Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2017; Kolařík et al., 2008; Strzałka et al., 2021)

Geosmithia ulmacea (*G*. sp. 13)	Ulmaceae	—	Ulmaceae	—	—	—	(Kolařík et al., 2017; Kolařík et al., 2008; Pepori et al., 2015)
Geosmithia sp. 16	*Pinaceae*	—	—	—	—	—	(Jankowiak and Bilanski, 2018; Jankowiak and Kolarik, 2010; Jankowiak et al., 2014; Kolařík and Jankowiak, 2013; Kolařík et al., 2008; McPherson et al., 2013)
Geosmithia sp. 22	—	Fagaceae, Moraceae, Oleaceae, *Rosaceae*	—	—	—	—	(Kolařík et al., 2007)
Geosmithia sp. 23	Betulaceae	Moraceae, *Rosaceae*	Ulmaceae	Ulmaceae, unknown	—	scolytid on *Persea gratissima*, Seychelles; *Malus pumila* branches, Cyprus	(Huang et al., 2019; Kolařík et al., 2017; Kolařík et al., 2007; Kolařík et al., 2008)
Geosmithia sp. 24	*Pinaceae*	*Pinaceae*	—		—	—	(Dori-Bachash et al., 2015)
Geosmithia sp. 25	*Pinaceae*	—	—	—	—	—	(Kolařík and Jankowiak, 2013)

Geosmithia sp. 26	*Pinaceae*	—	*Pinaceae*	—	—		(Jankowiak et al., 2014; Kolařík et al., 2017; Kolařík and Jankowiak, 2013)
Geosmithia sp. 27	*Pinaceae*	—	*Pinaceae*	—	—	—	(Jankowiak et al., 2014; Kolařík et al., 2017; Kolařík and Jankowiak, 2013)
Geosmithia sp. 29	*Pinaceae*	—	—	—	—	—	(Kolařík and Jankowiak, 2013)
Geosmithia sp. 30	*Pinaceae*	—	—	—	—	—	(Jankowiak et al., 2014; Kolařík and Jankowiak, 2013)
Geosmithia sp. 31	*Pinaceae*	—	*Pinaceae*	—	—	—	(Jankowiak et al., 2014; Kolařík and Jankowiak, 2013)
Geosmithia sp. 32	*Cupressaceae*	Oleaceae	*Cupressaceae*	—	—	—	(Juan Alfredo et al., 2020; Kolařík et al., 2017; Kolařík et al., 2008)
Geosmithia sp. 33	—	—	*Pinaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 34	—	—	*Cupressaceae* — *Calocedrus*, *Pinaceae*	—	—	—	(Kolařík et al., 2017)

Geosmithia sp. 35	—	—	*Pinaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 36	—	—	*Pinaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 37	—	—	*Pinaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 38	—	—	*Fagaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 40	—	—	*Pinaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 41	—	—	*Anacardiaceae, Asteraceae, Fagaceae, Lauraceae, Pinaceae, Rosaceae*	*Juglandaceae, Fagaceae, unknown*	—	—	(Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2017)
Geosmithia sp. 42	—	—	*Cupressaceae - Calocedrus, Pinaceae, Rosaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 43	—	—	*Pinaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 44	—	—	*Cupressaceae, Pinaceae*	*Pinaceae*	—	—	(Huang et al., 2017; Huang et al., 2019; Kolařík et al., 2017)
Geosmithia sp. 45	—	—	—	*Pinaceae*	—	—	(Huang et al., 2017; Huang et al., 2019)
Geosmithia sp. 39	—	—	*Juglandaceae*	—	—	—	(Kolařík et al., 2017)
Geosmithia sp. 46	—	—	—	*Juglandaceae, Fagaceae*	—	—	(Huang et al., 2017; Huang et al., 2019)

Geosmithia sp. 47	—	—	—	*Juglandaceae, Fagaceae*	—	—	(Huang et al., 2017; Huang et al., 2019)
Geosmithia sp. 48	—	—	—	*Cupressaceae*	—	—	(Huang et al., 2017; Huang et al., 2019)
Geosmithia sp. (*G. pallida* complex)	—	—	—	—	—	*Scolytoplatypus fasciatus* from *Virgilia,* South Africa	(Machingambi et al., 2014)
Geosmithia sp. (*G. pallida* complex)	—	—	—	—	—	endophyte of *Brucea mollis*, India, unique lineage based on KU693285	(Deka and Jha, 2018).
Geosmithia sp. (*G. pallida* complex)	—	—	—	—	—	sea sediment, China, unique lineage based on MK047400	(Sun et al., 2018).
Geosmithia sp. (*G.* sp. 24 complex)	—	—	—	—	—	scolytids from *Pinus*, Israel, sister to *Geosmithia* sp. 24	(Dori-Bachash et al., 2015)
Geosmithia sp. (*G.* sp. 8 complex)	—	—	—	—	—	*Phloeosinus* spp. from *Cupresssus*, Izrael	(Meshram et al., 2022)

Table 2 Summary of the insect vectors studied for *Geosmithia* presence and the strength of *Geosmithia*-vector association

[1] The organ preference and feeding habit was classified in the following categories: T – small twigs, B – branches, L – limbs and top of the trunk, thin barked parts, small diameter trunk, T – large diameter trunk, stumps, trunk bases, V – any part, PHL – phloem and bark, AMB – ambrosia beetle, XYL – sapwood; and is based on Postner (1974) and Foit (2010) and Kula et al. (2000) for European, Wood (1982), Bright et Stark (1973) and Smith et al. (2014) for American and Machingambi et al. (2014) for South African species.

[2] Relative frequency is given, depending on the study, as the number of independent gallery systems, or beetle individuals (adults, or larvae) where *Geosmithia* have been found. If *Geosmithia* were found but not quantified, a + symbol is given.

Region	**Host plant**	**Insect vector**	**Tree part and ecology[1]**	**Relative *Geosmithia* abundance[2]**	**Total number of *Geosmithia* spp.**	**References**
Costa Rica	*Cassia*	*Microcorthylus* sp.	T,B/AMB	100%	1	(Kolařík and Kirkendall, 2010)
	Croton	*Cnesinus lecontei*	T,B/AMB	100%	2	(Kolařík and Kirkendall, 2010)
	Paulinia	*Eupagiocerus dentipes*	T,B/AMB	100%	2	(Kolařík and Kirkendall, 2010)
China	*Pinus*	*Dendroctonus armandi*	T/PHL	0%	0	(Hu et al., 2015)

	various host	*Acanthotomicus suncei, Crossotarsus emancipates, Cryphalus eriobotryae, C. kyotoensis, Dinoderus* sp.*, Ernoporus japonicus, Hypothenemus* sp.*, Microperus* sp.*, Phloeosinus cf. hopehi, Phloeosinus sp., Scolytus jiulianshanensis, S. semenovi, Sinoxylon cf. cucumella* (*Bostrichidae*)*, Xylocis tortilicornis* (*Bostrichidae*)	-	+	3	(Zhang et al., 2022)
	Abies	*Cryphalus piceae*	T, B,L/PHL	37-82%	6	(Jankowiak and Bilanski, 2018; Jankowiak and Kolarik, 2010; Kirschner, 2001; Kolařík et al., 2008)
		Orthotomicus laricis	T/PHL	0%	0	(Jankowiak and Bilanski, 2018)
		Pisodes piceae (*Curculionidae*, *Molytinae*)	L, T/PHL,XYL	0%	0	(Jankowiak and Bilanski, 2018)
		Pityokteines curvidens	L,T/PHL	24%	1	(Jankowiak and Bilanski, 2018)
		Pityokteines vorontzowi	B,L/PHL	70%	1	(Jankowiak and Bilanski, 2018)
Europe, Mediterranean		*Pityophthorus pityographus*	T, B/PHL	80%	2	(Jankowiak and Bilanski, 2018)
		Trypodendron lineatum	T/PHL	0%	0	(Jankowiak and Bilanski, 2018)
		Xyleborinus saxesenii	T/PHL	0%	0	(Jankowiak and Bilanski, 2018)
	Alnus	*Dryocoetes alni*	B,L/PHL	5%	1	(Strzałka et al., 2021)
	Betula	*Scolytus ratzeburgi*	L,T/PHL	0%	0	(Kolařík et al., 2008; Linnakoski et al., 2008; Strzałka et al., 2021)
	Carpinus	*Scolytus carpini*	B,T/PHL	100%	5	(Kolařík et al., 2008)
	Clematis	*Xylocleptes bispinus*	liana stem/PHL	20%	1	(Kolařík et al., 2008)
	Cupressaceae	*Phloeosinus armatu, P. bicolor*	T, B/PHL	100%	4	(Meshram et al., 2022)

Cupressaceae	*Phloeosinus henschi, P. thujae*	T, B/PHL	100%	4	(Kolařík et al., 2007; Kolařík et al., 2008)
Cytisus	*Phloeotribus rhododactylus, Phloeophthorus cristatus*	B,L/PHL	50-100%	1	(Kolařík et al., 2007; Kolařík et al., 2008)
Euphorbia	*Aphanarthrum* sp.	stem/PHL	100%	1	(Kolařík et al., 2007)
Fagus	*Ernoporicus fagi, Taphrorychus bicolor*	T,B inner bark of trunk/PHL	27-29%	4	(Kolařík et al., 2008; Strzałka et al., 2021)
Ficus	*Hypoborus ficus*	B,L,T/PHL	98%	9	(Kolařík et al., 2007)
Fraxinus	*Hylesinus crenatus*	T/PHL	24%	1	(Strzałka et al., 2021)
	Hylesinus varius, H. orni, H. toranio	B,L/PHL	90-100%	5	(Kolařík et al., 2008; Strzałka et al., 2021)
Hedera	*Kissophagus hederae*	liana stem/PHL	33%	2	(Kolařík et al., 2008)
Larix	*Ips cembrae*	L,T/PHL	0%	0	(Jankowiak and Rossa, 2007)
	Orthotomicus laricis	T/PHL	0%	0	(Kirschner, 2001)
	Trypodendron lineatum	T/PHL	0%	0	(Jankowiak and Bilanski, 2018; Kirschner, 2001)
Laurus	*Liparthrum colchicum*	B,L/PHL	100%	3	(Benvenuti et al., 2021; Kolařík et al., 2007; Vitale et al., 2021)
Olea	*Phleotribus scarabeiodes*	B,L/PHL	100%	4	(Kolařík et al., 2007)
Picea	*Cryphalus abietis*	B,L/PHL	100%	3	(Kolařík and Jankowiak, 2013)
	Crypturgus cinereus	L,T/PHL	0%	0	(Kirschner, 2001)
	Crypturgus pusillus	B/PHL	0%	0	(Kirschner, 2001)

	Dendroctonus micans	L,T/PHL	0%	0	(Dohet et al., 2016; Kolařík and Jankowiak, 2013)
	Dryocoetes autographus	T/PHL	0-6%	2	(Jankowiak et al., 2014; Kirschner, 2001)
	Hylurgops palliatus	T/PHL	0-2%	1	(Jankowiak, 2006b; Jankowiak et al., 2014; Kirschner, 2001)
	Ips amitinus	L/PHL	2%	1	(Jankowiak et al., 2014)
	Ips duplicatus	L,T/PHL	0%	0	(Kolařík and Jankowiak, 2013)
	Ips typograhpus	T/PHL	0-1%	2	(Jankowiak and Hilszczański, 2005; Kirschner, 2001; Persson et al., 2009)
	Pityogenes chalcographus	L/PHL	0-24%	6	(Jankowiak et al., 2014; Kirschner, 2001)
	Pityophthorus pityographus	B,L/PHL	58%	7	(Jankowiak et al., 2014)
	Polygraphus poligraphus	L/PHL	24%	3	(Jankowiak et al., 2014; Kirschner, 2001)
	Trypodendron lineatum	T/PHL	0%	0	(Jankowiak and Bilanski, 2018; Kirschner, 2001)
	Acanthocinus aedilis (*Cerambycidae*)	L,T/PHL,XYL	0%	0	(Jankowiak and Rossa, 2007)
	Crypturgus cinereus	L,T/PHL	0%	0	(Kirschner, 2001)
Pinus	*Hylobius abietis* (*Curculionidae, Molytinae*)	T/PHL,XYL	0%	0	(Kolařík and Jankowiak, 2013)
	Hylurgus ligniperda	T/PHL	0%	0	(Davydenko et al., 2014)
	Ips acuminatus	L/PHL	0%	0	(Davydenko et al., 2017)

Ips sexdentatus	T/PHL	0%	0	(Davydenko et al., 2021; Kirschner, 2001; Kolařík and Jankowiak, 2013)
Monochamus galloprovincialis (*Cerambycidae*)	L/PHL,XYL	3.30%	1 (?)	(Jankowiak and Rossa, 2007)
Orthotomicus erosus	L/PHL	23%	1	(Dori-Bachash et al., 2015)
Orthotomicus laricis	T/PHL	0%	0	(Jankowiak and Bilanski, 2018), (Kirschner, 2001)
Pissodes castaneus, P. piniphilus (*Curculionidae, Molytinae*)	T/PHL,XYL	0%	0	(Kolařík and Jankowiak, 2013)
Pityogenes bidentatus	B,L/PHL	41-82%	6	(Jankowiak et al., 2014; Jankowiak and Rossa, 2008)
Pityogenes calcaratus	T, B,L/PHL	84%	1	(Dori-Bachash et al., 2015)
Pityogenes chalcographus	L/PHL	24%	4	(Jankowiak et al., 2014)
Pityogenes quadridens	B,L/PHL	86%	2	(Kolařík and Jankowiak, 2013)
Pityophthorus pityographus	B,L/PHL	69%	7	(Jankowiak et al., 2014)
Tomicus destruens	L/PHL	0%	0	(Dori-Bachash et al., 2015; Muñoz-Adalia et al., 2017)
Tomicus minor	L,T/PHL	0%	0	(Jankowiak, 2008)
Tomicus piniperda	T/PHL	0%	0	(Jankowiak, 2006a; Jankowiak and Bilański, 2007; Muñoz-Adalia et al., 2017; Silva et al., 2015)
Trypodendron lineatum	T/PHL	0%	0	(Jankowiak and Bilanski, 2018; Kirschner, 2001)

Pistatia	*Chaetoptelius perrisi, Ch. vestitus*	B,L/PHL	20-100%	3	(Hadj Taieb et al., 2019; Kolařík et al., 2007)
Populus	*Trypophloeus* spp.	T,B/PHL	33%	1	(Kolařík et al., 2008)
Rosaceae	*Scolytus mali*	L,T/PHL	42%	3	(Strzałka et al., 2021)
	Scolytus rugulosus	B,L/PHL	75-100%	8	(Kolařík et al., 2007; Kolařík et al., 2008; Strzałka et al., 2021)
Quercus	*Dryocoetes villosus*	thick bark of B,L/PHL	0%	0	(Strzałka et al., 2021)
	Scobicia pustulata (*Bostrichidae*)	B,T/XYL	100%	1	(Kolařík et al., 2007)
	Scolytus intricatus	T, B/PHL	15-100%	11	(Kolařík et al., 2008; Kubátová et al., 2004; Strzałka et al., 2021)
	Platypus cylindrus	T, B/AMB	Anisandrus dispar	1	(Belhoucine et al., 2011)
Spartium	*Liparthrum genistae, Phloeotribus rhododactylus, Phloeophthorus cristatus*	B,L/PHL	50-100%	1	(Kolařík et al., 2007; Kolařík et al., 2008)
Tilia	*Ernoporus tiliae*	B,T/PHL	73-100%	4	(Kolařík et al., 2008; Strzałka et al., 2021)
	Magdalis armigera (*Curculionidae*, *Magdalinae*)	B,L/PHL,XYL	+	1	(Kolařík et al., 2008)
Ulmus	*Scolytus multistriatus, S. pygmaeus*, *S. kirsch, Pteleobius vittatus*	B,L/PHL	47-100%	7	(Kolařík et al., 2007; Kolařík et al., 2008; Strzałka et al., 2021)
	Scolytus scolytus	L,T/PHL	22%	3	(Strzałka et al., 2021)
various hardwoods	*Anisandrus dispar*	V/AMB	4%	1	(Strzałka et al., 2021)
	Trypodendron domesticum	L/AMB	0%	0	(Strzałka et al., 2021)

		Xyleborinus saxeseni	B,L/AMB	0%	0	(Strzałka et al., 2021)
		Xyleborus monographus	B/L/AMB	0%	0	(Strzałka et al., 2021)
		Scolytus subscaber	B/PHL	72%	1 (?)	(Wright, 1938)
	Abies	*Scolytus ventralis*	T/PHL	0%	0	(Kolařík et al., 2017; Wright, 1938)
		Scolytus praeceps	B,L/PHL	87-88%	4	(Kolařík et al., 2017; Wright, 1938)
	Acer	*Phloeotribus frontalis*	V, inner bark/PHL	33%	1	(Huang et al., 2017)
	Calocedrus	*Phloeosinus fulgens*	B,L/PHL	20%	4	(Kolařík et al., 2017)
	Carya	*Hypothenemus dissimilis*	T,B/PHL	50-100%	2	(Huang et al., 2017; Huang et al., 2019)
		Hypothenemus rotundicollis	T,B/PHL	100%	2	(Huang et al., 2019)
North America		*Xylobiops basilaris* (*Bostrichidae*)	V/XYL	0-100%	3	(Huang et al., 2019)
	Celtis	*Chramesus chapuisii*	T,B/PHL	10-11%	2	(Huang et al., 2017; Huang et al., 2019)
		Phloeotribus texanus	B,L/PHL	17%	1	(Huang et al., 2017; Huang et al., 2019)
	Cinnamomum	*Cnesinus strigicollis*	T,B,L/PHL	0%	0	(Huang et al., 2017)
	Cupressaceae	*Phloeosinus cupressi, P. sequoia, P. canadensis, P. punctatus*	V/PHL	80-100%	6	(Kolařík et al., 2017)
		Phloeosinus serratus, P. deleoni	V/PHL	100%	2	(Juan Alfredo et al., 2020)
		Phloeosinus dentatus	V/PHL	50-60%	4	(Huang et al., 2017; Huang et al., 2019)

Fraxinus	*Hylesinus aculeatus*	L/PHL	17%	2	(Huang et al., 2017)
	Hylesinus oregonus	L, T/PHL	80%	1	(Kolařík et al., 2017)
Juglans	*Pityophthorus juglandis*	T,B/PHL	90-100%	5	(Kolařík et al., 2017), various others studies
Juniperus	*Ambrosiodmus lecontei*	V/AMB	0%	0	(Huang et al., 2017; Huang et al., 2019)
Notholithocarpus	*Pseudopityophthorus pubipennis*	T, B,L/PHL	100%	7	(Kolařík et al., 2017)
Phoenix	*Coccotrypes dactyliperda*	seeds	75%	1	(Huang et al., 2017)
Pinus	*Dendroctonus frontalis*	T/PHL	0%	0	(Dighton et al., 2021)
	Dendroctonus ponderosae	T/PHL	0%	0	(Lee et al., 2006; Lim et al., 2005)
	Dendroctonus punctatus, D. valens	T/PHL	0%	0	(Dohet et al., 2016)
	Dendroctonus rhizophagus	T, seedlings/PHL	0%	0	(Gonzalez-Escobedo et al., 2019)
	Ips avulsus	L,T/PHL	0%	0	(Huang et al., 2017; Huang et al., 2019)
	Ips pini	T,L/PHL	0%	0	(Lim et al., 2005)
	Ips plastographus	T/PHL	100%	2	(Kolařík et al., 2017)
	Orthotomicus latidens	L/PHL	45%	2	(Kolařík et al., 2017)
	Orthotomicus spinifer	T/PHL	100%	1	(Kolařík et al., 2017)
	Pityogenes knechteli	B,L/PHL	30%	4	(Kolařík et al., 2017)

	Pityophthorus confusus	little known, B,L/PHL	29-50%	1	(Huang et al., 2017; Huang et al., 2019)
	Pityophthorus pulicarius	T/PHL	0-12.5%	1	(Huang et al., 2017; Huang et al., 2019)
	Pseudips mexicanus	T/PHL	50%	1	(Kolařík et al., 2017)
	Pityophthorus annectens	little known, B/PHL	10%	1	(Huang et al., 2019)
Pistatia	*Scobicia* sp. (*Bostrichidae*)	V/XYL	100%	2	(Kolařík et al., 2017)
Prunus	*Phloeotribus liminaris*	L/PHL	25%	1	(Huang et al., 2017)
	Carphoborus vandykei	unknown/PHL	15%	1	(Kolařík et al., 2017)
Pseudotsuga	*Cryphalus pubescens*	L, seedlings/PHL	40%	3	(Kolařík et al., 2017)
	Scolytus oregoni	L/PHL	100%	5	(Kolařík et al., 2017)
Rosaceae	*Scolytus rugulosus*	B,L/PHL	100%	5	(Kolařík et al., 2017)
	Micracisella nanula	T,PHL	50%	1	(Huang et al., 2017; Huang et al., 2019)
Quercus	*Pseudopityophthorus minutissimus*	B,L/PHL	28-56%	1	(Huang et al., 2017; Huang et al., 2019)
Quercus	*Pseudopityophthorus pubipennis*	T, B,L/PHL	100%	7	(Kolařík et al., 2017; McPherson et al., 2013)
Toxicodendron	*Cactopinus rhois*	liana stem/PHL	67%	2	(Kolařík et al., 2017)
Ulmus	*Scolytus multistriatus, S. schevyrewi*	B,L/PHL	100%	3	(Kolařík et al., 2017)

	Umbellularia	*Scobicia declivis* (*Bostrichidae*)	V/XYL	72%	4	(Kolařík et al., 2017)
	various hardwoods or unknown	*Chaetophloeus* sp.	T,B/PHL	100%	1	(Huang et al., 2017)
		Cryptocarenus seriatus	T/PHL	100%	1	(Huang et al., 2017)
		Xyleborus celsus	T/AMB	100%	1	(Huang et al., 2017)
		Hylocurus hirtellus	T,B/PHL	80%	3	(Kolařík et al., 2017)
		Hypothenemus eruditus	V, mostly T, B/PHL	0-25%	1	(Huang et al., 2017; Huang et al., 2019)
		Xylosandrus compactus	T/AMB	0-50%	1	(Huang et al., 2017; Huang et al., 2019)
		Xylosandrus crassiusculus	V/AMB	0-50%	1	(Huang et al., 2017; Huang et al., 2019)
		Xylosandrus crassiusculus, Xylosandrus compactus	V, mostly T,B/AMB	0%	0	(Huang et al., 2017; Huang et al., 2019)
		Xylosandrus mutilatus	V/AMB	+	2	(Six et al., 2009)
South Africa	*Virgilia*	*Scolytoplatypus fasciatus*	V/AMB	100%	1	(Machingambi et al., 2014)
		Cryphalini sp. 1, *Hapalogenius fuscipennis, Liparthrum* sp. 1	B/PHL	100%	5	(Machingambi et al., 2014)

Table 3 List of secondary metabolites reported from *Geosmithia*

Compound	Chemical class	Activity	Occurrence	Reference
Rheoemodin (1,3,6,8-tetrahydroxyanthraquinone)	anthraquinone	inflammatory activity (10 µg/mL), *Acinetobacter baumannii* (MIC 12.5 µg/mL)	*G. lavendula*	(Stodůlková et al., 2009), (Wang et al., 2019)
Rhodolamprometrin (1-acetyl-2,4,5,7-tetrahydroxyanthraquinone)	anthraquinone	*Staphylococcus aureus*, *Bacillus subtilis* (MIC 64–512 µg/mL, affects morphology of mammalian cells	*G. lavendula*	(Stodůlková et al., 2009), (Malak et al., 2013c)
1-acetyl-2,4,5,7,8-pentahydroxyanthraquinone	anthraquinone	*S. aureus*, *Bacillus subtilis* (MIC 64–512 µg/mL), inflammatory activity (10 µg/mL), affects cell cycle mammalian cell	*G. lavendula*	(Stodůlková et al., 2009), (Malak et al., 2013c)
2,4,5,7-tetrahydroxy AQ–1-carboxylic acid methyl ester	anthraquinone	n.a.	*G. lavendula*	(Stodulkova et al., 2010)
1,x-diacetyl-2,4,5,7-tetrahydroxy AQ				
1,x-diacetyl-2,4,5,7,8-pentahydroxy AQ				
1-acetyl-2,4,5,7,8-pentahydroxy AQ				
1-acetyl-2,4,5,7-tetrahydroxy AQ				
1,x-diacetyl-monomethoxy-trihydroxy AQ				
1,3,6,8-Tetrahydroxy AQ				
1,3,5,6,8-pentahydroxy AQ				
1-acetyl-dimethoxy-dihydroxy AQ				
1,x-diacetyl-dimethoxy-dihydroxy AQ				
1-acetyl-monomethoxy-tetrahydroxy AQ				
1,x-diacetyl-monomethoxy-tetrahydroxy AQ				
1-acetyl-monomethyl-trihydroxy AQ				
1-acetyl-monomethoxy-trihydroxy AQ				
1,x-diacetyl-trimethoxy-hydroxy AQ				

1-acetyl-2,4,6,8-tetrahydroxy-9,10-anthraquinone	anthraquinone	anti methicillin resistant *Staphylococcus aureus* (IC50 16.1 µg/mL)	*G. lavendula*	(Malak et al., 2013c)
2-acetyl-1,4,5,7-tetrahydroxy-9,10-anthraquinone	anthraquinone	n.a.	*G. lavendula*	(Malak et al., 2013c)
1-acetyl-2,4,5,6,7-pentahydrxy-9,10-anthraquinone	anthraquinone	n.a.	*G. lavendula*	(Malak et al., 2013c)
4-(2′,4′-dihydroxy-6′-(hydroxymethyl)benzyl)benzene-1,2-diol	benzhydryl	n.a.	*G. langdonii*	(Malak et al., 2014)
p-hydroxybenzyl alcohol	benzyl alcohol	broad application in human medicine, mostly neuroactive (Zhu et al., 2018)	*G. lavendula*	(Malak et al., 2013c)
4-hydroxybenzyl alcohol	benzyl alcohol	antioxidant and anti-inflammatory activities (Kumar et al., 2017)	*G. langdonii*	(Malak et al., 2014)
(1S,2R,3R,4R,5R)-2,3,4-trihydroxy-5-methylcyclohexyl-2′,5′-dihydroxybenzoate	carbasugar	antileishmanial (IC50 100 µM)	*G. langdonii*	(Malak et al., 2018)
1S,2S,3S,4R,5R)-4-[(2′,5′-dihydroxybenzyl)oxy]-5-methylcyclohexane-1,2,3-triol	carbasugar	antileishmanial (IC50 57 µM)	*G. langdonii*	(Malak et al., 2018)
3,4-dihydroxytoluene (4-methylcatechol)	catechol	n.a.	*G. langdonii*	(Malak et al., 2014)
bisdethiobis (methylthio)gliotoxin	cyclic dipeptide	antibacterial (Ratnaweera et al., 2016)	*G. pallida* MK047400	(Sun et al., 2018)
6-acetylbis(methylthio)gliotoxin	cyclic dipeptide	no antibacterial or cyotoxic (Liang et al., 2014)	*G. pallida* MK047400	(Sun et al., 2018)
6-deoxy-5a,6-didehydrogliotoxin	cyclic dipeptide	cytotoxic (Sun et al., 2012)	*G. pallida* MK047400	(Sun et al., 2018)
5a,6-didehydrogliotoxin	cyclic dipeptide	n.a.	*G. pallida* MK047400	(Sun et al., 2018)
6-(phenylmethyl)-(3R,6R)-2,5-piperazinedione	cyclic dipeptide	n.a.	*G. pallida* MK047400	(Sun et al., 2018)
3-(hydroxymethyl)-3,6-bis(methylthio)-6-(phenylmethyl)-(3R,6R)-2,5-piperazinedione	cyclic dipeptide	n.a.	*G. pallida* MK047400	(Sun et al., 2018)

3-(hydroxymethyl)-6-(methoxyl)-6-(phenylmethyl)-(3R,6R)-2,5-piperazinedione	cyclic dipeptide	n.a.	*G. pallida* MK047400	(Sun et al., 2018)
5a,6-anhydrobisdethiobis(methylthio)gliotoxin	cyclic dipeptide	n.a.	*G. pallida* MK047400	(Sun et al., 2018)
Geospallin A	cyclic dipeptide	angiotensin-converting enzyme	*G. pallida* MK047400	(Sun et al., 2018)
Geospallin B	cyclic dipeptide	angiotensin-converting enzyme	*G. pallida* MK047400	(Sun et al., 2018)
Geospallin C	cyclic dipeptide	angiotensin-converting enzyme	*G. pallida* MK047400	(Sun et al., 2018)
(+)-epiepoformin	cyclohexane epoxide	antifungal, zootoxic and phytotoxic (Cala et al., 2018)	*G. langdonii*	(Malak et al., 2014)
(−)-dihydroepiepoformin	cyclohexane epoxide	n.a.	*G. langdonii*	(Malak et al., 2014)
(4S,5S)-4,5-dihydroxy-2-methylcyclohex-2-enone	cyclohexene and cyclohexenone	potato microtuber induction (Salvatore et al., 2020)	*G. langdonii*	(Malak et al., 2014)
(4R,5R,6R)-4,5-dihydroxy-6-(6′-methylsalicyloxy)-2-methyl-2-cyclohexen-1-one	cyclohexene and cyclohexenone	n.a,	*G. langdonii*	(Malak et al., 2014)
didodecyl thiodipropionate	dicarboxylic acid	antioxidant	*G. lavendula*	(Malak et al., 2013c)
6-methylsalicylic acid	hydroxybenzoate	n.a.	*G. langdonii*	(Malak et al., 2014)
3-hydroxytoluene (m-Cresol)	phenol derivate	n.a.	*G. langdonii*	(Malak et al., 2014)
2,5-dihydroxybenzaldehyde	phenolic aldehyde	nematicidal, cytotoxic (Kim et al., 2021)	*G. langdonii*	(Malak et al., 2014)
gentisylquinone	quinone	antibiotic, herbicide (Buckingham, 1996)	*G. langdonii*	(Malak et al., 2014)
(22E)-ergosta-6,22-diene-3β,5α,8α-triol	sterol	n.a.	*G. lavendula*	(Malak et al., 2013c)

1.5.11. References

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1.6 APPENDIX – SCIENTIFIC PAPER INCLUDED INTO THE HABILITATION THESIS