



# *Geosmithia*—widespread and abundant but long ignored bark beetle symbionts

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## Abstract

*Geosmithia* (Ascomycota: Hypocreales) and their associations with bark beetles have long been in the shadow of the more often studied beetle associates: *Ophiostomatales* and *Microascales*. The last 20 years of research have demonstrated that *Geosmithia* species are widespread and abundant in the subcortical habitat and that they show varied degrees of symbiosis with their insect vectors. This article reviews the taxonomic history of the genus, species diversity, host ranges, and biogeography. We also discuss the ecological roles of various *Geosmithia* species in relation to insect vectors and host trees, phytopathogenicity, and their potential for biotechnology. An extensive review of primary data shows that bark beetle species vector predominantly either *Geosmithia* or ophiostomatoid fungi, but not both, and that this dichotomy is due to the tree substrate features, especially water content and decay stage, which a given bark beetle vector exploits. In both *Geosmithia* and the other beetle-associated fungi, coevolution with insect vectors led from phloem inhabiting ancestors to the formation of ambrosia lineages and reciprocal adaptations in the beetles. Lastly, we define knowledge gaps and suggest further research directions.

**Keywords** *Scolytinae* · *Hypocreales* · Symbiosis · Secondary metabolites · *Geosmithia morbida* · Phytopathogenic fungi

## Introduction

Bark and ambrosia beetles (*Coleoptera*: *Curculionidae*: *Scolytinae* and *Platypodinae*) are associated with a diverse set of ecto and endosymbionts, classified among the prokaryotes, filamentous fungi, yeasts, and microinvertebrates. Fungal symbionts are the most studied, and their dependency on the insect vector ranges from obligatory, in strictly entomochoric fungi, to incidental ones, acquired from the environment. Fungal symbionts interact with the host insect and tree, forming mutualistic, commensal, or antagonistic interactions (Beaver 1989; Six 2013; Hofstetter et al. 2015). The best-studied fungal symbionts of bark beetles belong to ophiostomatoid fungi (*Ascomycota*: *Ophiostomatales*) and *Microascales*. However, beetle galleries frequently harbor many other fungal groups, and

many of the non-ophiostomatalean have been historically ignored (Kirschner 2001; Kirschner et al. 2001; Kolařík et al. 2006; Jankowiak and Kolarik 2010). Filamentous fungi placed in 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 fir-infesting bark beetle species in the USA by Wright (1938), but the fungus was misidentified as *Spicaria anomala* (Kolařík 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. 1999, 2004).

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 this are varied. The species that had been identified in early studies, such as *G. putterillii*, 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). Furthermore, *Geosmithia* species strongly resemble certain species of

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*Penicillium*, *Paecilomyces*, or *Mariannaea*, which are common and also widely ignored residents of bark beetle galleries. *Geosmithia* produces masses of dry spores, a typical feature of airborne fungi, but does 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 to isolate *Ophiostomatales*, which are resistant to the compound, from bark beetles. This means *Geosmithia* and their associations with bark beetles were often missed, and there was a skepticism about the significance of the association.

In the more recent years, however, many independent studies confirmed *Geosmithia* species as stable and often dominant symbionts 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 possible mutual coevolution.

Here, we summarize and interpret *Geosmithia* biology based on a review of more than 140 publications (Fig. 1). This review, for the first time, synthesizes the taxonomy, diversity, ecology, biogeography, and biotechnological potential of the genus *Geosmithia*, including a description of the history of research and an outline of future directions. The paper also addresses the question of why some bark beetles are associated with *Geosmithia* and other species are not.

## History of the genus and the main differentiation features

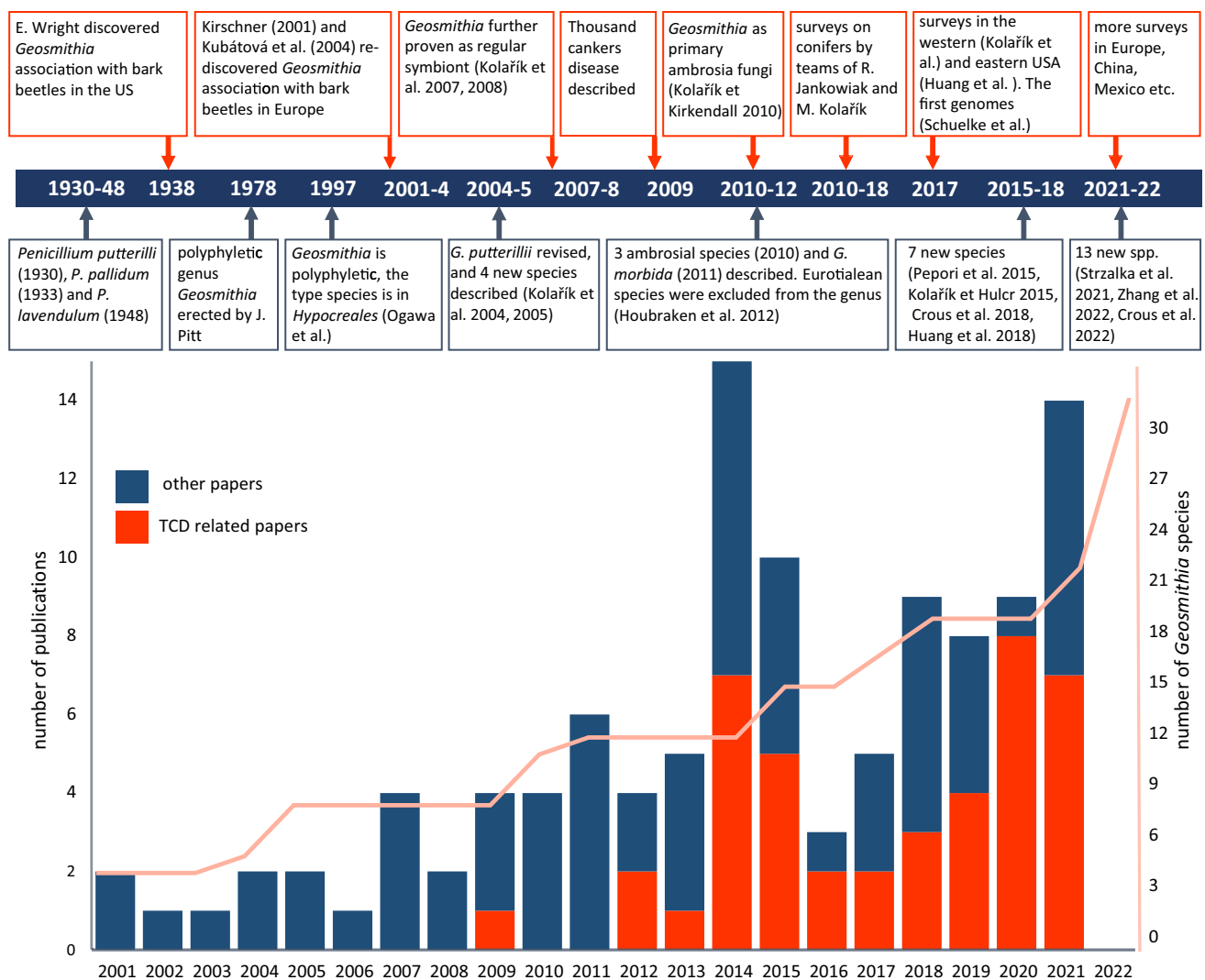
Like in other morpho-genera of anamorphic fungi, the *Geosmithia* generic concept has undergone dramatic changes (Fig. 1). In the current concept, its characteristics include the following: absence of a sexual state, variable colony colors but never green (which diagnoses it as distinct from *Penicillium*), the cylindrical shape of phialides without a prominent neck and with roughened walls, elliptical to cylindrical conidia produced in chains, and the specific initials and base of the conidiophore (Kolařík et al. 2004). The discovery of morphologically unique ambrosia fungi in *Geosmithia* (the identity determined by using gene sequencing) 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* produces the *Penicillium*-like conidiophores, or conidiophores can be much more

complex, irregularly and repeatedly branched. Besides macronematous conidiophores with enteroblastic phialides, semimicronematous or microcrownematous conidiophores can also be formed on aerial or substrate mycelium (Kolařík et al. 2004). Whereas *Penicillium*-like conidiophores produce columns of dry conidia, microcrownematous 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). Another typical *Geosmithia* feature is the conidiophore base, making a so-called peg foot with smooth cell walls and curved shapes (Kolařík et al. 2004) (Fig. 2).

The history of *Geosmithia* taxonomy is linked to the morphologically similar *Penicillium*, and the type species was first described as *Penicillium putterilli* (Thom 1930). Species later classified in *Geosmithia* were first aggregated into the series *P. pallidum* in section *Asymmetrica–Funiculosa* that was established for *P. pallidum*, *P. putterillii*, *P. laven-dulum* (now in *Geosmithia*), and *P. namyslowskii* (now in *Penicillium*, *Eurotiales*) (Raper and Thom 1949). John Pitt (1979) proposed a new genus *Geosmithia*, named in honor of George Smith, to include species from the *P. pallidum* series and some species now 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 began to use the name *Geosmithia* for newly discovered species of similar morphology (Pitt and Hocking 1985; Yaguchi et al. 1993, 1994, 2005). The first studies utilizing molecular data showed that some of the species, including the type species *G. putterillii*, belonged to *Hypocreales*, whereas others belonged to the *Eurotiales* (Ogawa et al. 1997; Ogawa and Sugiyama 2000; Peterson 2000; Iwamoto et al. 2002). 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 affected 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).

## Taxonomy and diversity

The genus possesses relatively high phylogenetic diversity, with over 67 phylogenetic species, of which 32 have been 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.



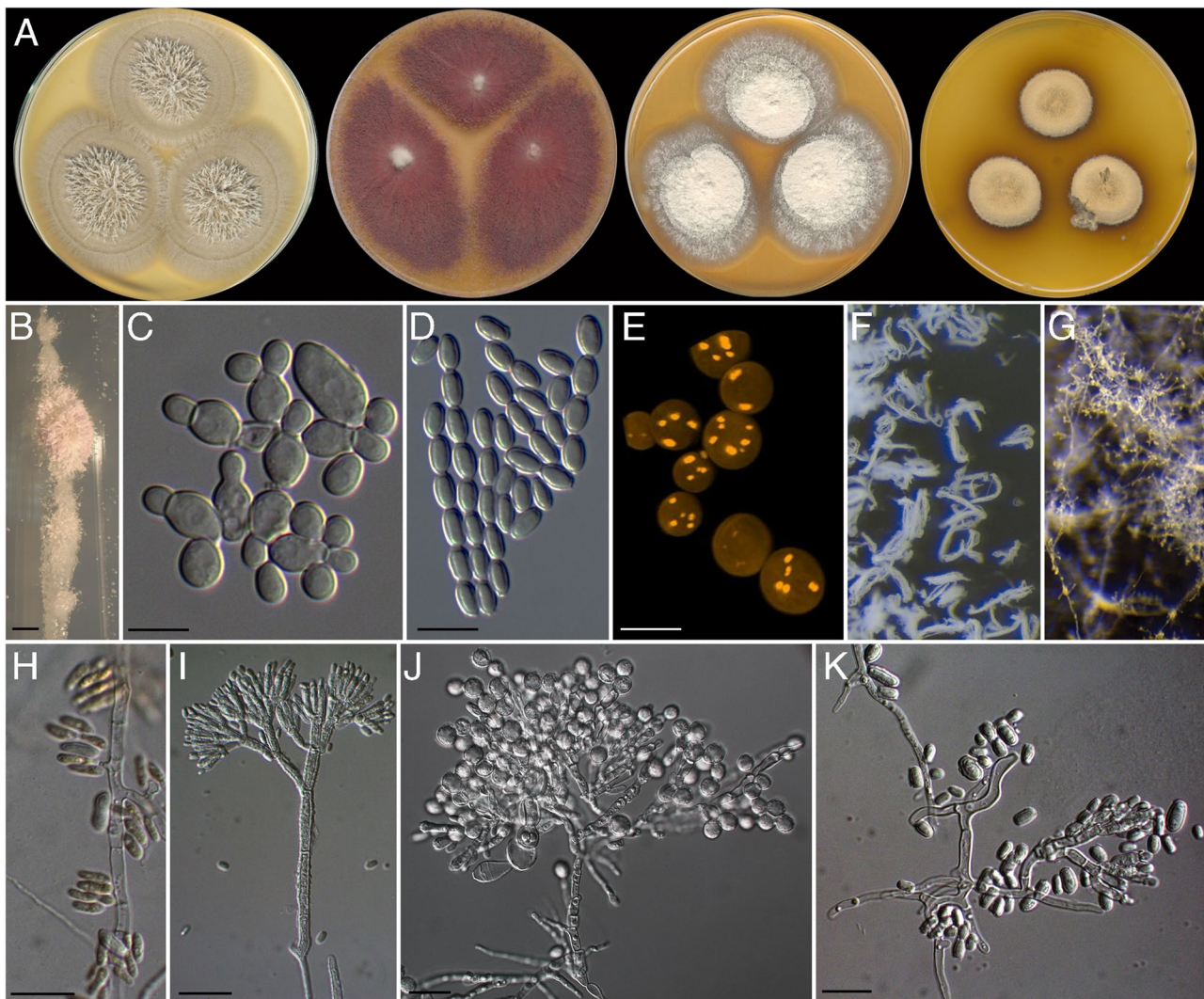
**Fig. 1** Upper part. Chronology of important events related to *Geosmithia* taxonomy (below) and ecology, host range, and biogeography (above). Lower part. Overview of publications on hypocrealean *Geosmithia* species over the last 20 years (2001–2021), with a breakdown of papers focusing on thousand cankers disease (orange bar) and on

other aspects (blue bar). The graph is based on articles extracted from the Scopus database and additional 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)

This numbering system originated in Kolařík et al. (2007, 2008), and species thus labeled are frequently used in literature (Table 1).

Before 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. fassatae*, *G. langdoni*, and *G. obscura* were described from bark beetles in Europe (Kolařík et al. 2005). A large survey of *Geosmithia* in Europe and the Mediterranean basin recognized other 23 undescribed species marked as *Geosmithia* spp. 1–5, 8–13, 16, 19–31 (Kolařík et al. 2007, 2008; Kolařík and

Jankowiak 2013). Subsequent surveys in the USA revealed other 20 species, classified as *Geosmithia* spp. 32–48 (Kolařík et al. 2017; Huang et al. 2019) 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 recognized 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), Israel, and Europe (*G.*



**Fig. 2** Morphological features of *Geosmithia*. **A** Colony morphology on MEA can range from brown (*G. funiculososa*), lilac (*G. carolii*), white to cream (*G. putterillii*), and yellowish (*Geosmithia* sp. 11). **B, C** A yeast-like stage is present 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** Solitarily produced conidia of *G. microcorthyli*. **H** Substrate conidia of *G. carolii*. **I** Penicillate conidiophore in *G. putterillii*. **J** The complex branched conidiophore of *G. eupagioceri*. **K** A simple conidiophore in *Geosmithia* sp. 31. Scale bars **B** 500 µm, **C–E, H, K** 10 µm; **I, J** 20 µm

*cupressina*, *G. fagi*, and *G. pazoutovae*) (Strzałka et al. 2021; Meshram et al. 2022)). Four species, *G. eupagioceri*, *G. microcorthyli*, *G. rufescens*, and *C. cnesini*, were described from ambrosia beetles in Costa Rica (Kolařík and Kirkendall 2010; Kolařík et al. 2015). Other five tentative and undescribed species were recognized during the surveys on bark beetles in South Africa and Israel (Machingambi et al. 2014; Dori-Bachash et al. 2015) or on other substrates (Deka and Jha 2018; Sun et al. 2018) (Table 1). The species *G. tibetensis* (Wu et al. 2013), described from 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, growth at 24–25 °C, the optimal temperature for perhaps all species, and 37 °C, tolerated by few species only (e.g., *G. lavendula* and *G. morbida*) is studied.

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

**Table 1** List of the recognized *Geosmithia* species with geographical distribution and substrate origin. The host spectrum is expressed as a list of host plant families from which the insect vector was collected. Only data confirmed by molecular data are shown

Species <sup>1</sup>	Central and North Eastern Europe (~ temperate Europe)	Mediterranean Basin and Black and Caspian Sea region	Western USA and Northern Mexico	Southeastern and Eastern USA	China	Other substrates or locations	References
<i>G. bombycina</i>	—	—	—	—	Rosaceae	—	Zhang et al. (2022)
<i>G. brevistipitata</i>	—	—	—	—	Cupressaceae	—	Zhang et al. (2022)
<i>G. brunnea</i>	—	—	—	Altingiaceae, Fagaceae	—	—	Huang et al. (2017), Huang et al. (2019)
<i>G. carolliae</i> (G. sp. 19)	—	Moraceae	—	—	—	wing of the bat, Brazil	Kolařík et al. (2007), Crous et al. (2018)
<i>G. cnesini</i>	—	—	—	—	—	ambrosia beetle	Kolařík and Kirkendall (2010), Kolařík et al. (2015)
<i>G. cupressina</i>	—	Cupressaceae	—	—	—	—	Meshram et al. (2022)
<i>G. eupagioceri</i>	—	—	—	—	—	ambrosia beetle	Kolařík and Kirkendall (2010)
<i>G. fagi</i>	Fagaceae, Rosaceae	—	—	—	—	—	Strzałka et al. (2021)
<i>G. fassattiae</i>	Fagaceae, Rosaceae	—	Fagaceae, Lauraceae, Pinaceae, Salicaceae	—	—	—	Kolařík et al. (2008), McPherson et al. (2013), Kolařík et al. (2017)
<i>G. flava</i>	Araliaceae, Betulaceae, Cupressaceae, Fagaceae, Oleaceae, Pinaceae, Rosaceae, Tiliaceae, Ulmaceae	Anacardiaceae, Lauraceae, Moraceae, Rosaceae	Anacardiaceae, Cupressaceae, Fagaceae, Juglandaceae, Pinaceae, Salicaceae	—	—	scolytids from <i>Virgilia</i> spp., South Africa; <i>Ulmus glabra</i> , <i>Hordeum</i> sp. grain, England	Kolařík et al. (2004), Kolařík et al. (2007), Kolařík et al. (2008), Machingambi et al. (2014), Pepori et al. (2015), Kolařík et al. (2017), Strzałka et al. (2021)
<i>G. funiculosa</i> (G. sp. 5)	Fagaceae, Oleaceae, Pinaceae, Rosaceae, Tiliaceae, Ulmaceae	Anacardiaceae	—	—	—	<i>Scolytus</i> beetle, UK	Kolařík et al. (2004), Kolařík et al. (2007), Kolařík et al. (2008), Pepori et al. (2015), Strzałka et al. (2021), Crous et al. (2022)
<i>G. fusca</i>	—	—	—	—	Fabaceae, Phyllanthaceae, Malvaceae	—	Zhang et al. (2022)
<i>G. granulata</i> (= G. sp. 20)	—	Asteraceae, Fabaceae, Moraceae, Oleaceae, Ulmaceae, Anacardiaceae	Cupressaceae, Ulmaceae	—	Fabaceae, Malvaceae	—	Kolařík et al. (2007), Kolařík et al. (2017), Hadj Tateb et al. (2019), Zhang et al. (2022)

Table 1 (continued)

Species <sup>1</sup>	Central and North Eastern Europe (~temperate Europe)	Mediterranean Basin and Black and Caspian Sea region	Western USA and Northern Mexico	Southeastern and Eastern USA	China	Other substrates or locations	References
<i>G. langdonii</i>	<i>Cupressaceae, Betulaceae, Fabaceae, Fagaceae, Tiliaceae, Rosaceae, Ulmaceae</i>	<i>Anacardiaceae, Euphorbiaceae, Fagaceae, Lauraceae</i>	<i>Asteraceae, Cupressaceae, Fagaceae, Lauraceae, Pinaceae</i>	—	—	—	McPherson et al. (2013), Hanzi et al. (2016), Juan Alfredo et al. (2020), Benvenuti et al. (2021), Strzałka et al. (2021), Vitale et al. (2021), Meshram et al. (2022)
<i>G. lavendula</i>	—	<i>Anacardiaceae, Fabaceae, Moraceae, Ulmaceae</i>	<i>Anacardiaceae, Cupressaceae, Fagaceae, Juglandaceae, Pinaceae, Rosaceae</i>	<i>Fagaceae, Vitaceae, unknown</i>	—	laboratory contaminant, U.S; <i>Carya</i> wood, Israel; soil, Venezuela	Pitt (1979), Kolařík et al. (2007), Six et al. (2009), Huang et al. (2017), Kolařík et al. (2017), Hadj Taieb et al. (2019), Huang et al. (2019), Morales-Rodriguez et al. (2021)
<i>G. longisipitata</i> (G. sp. 28)	<i>Pinaceae</i>	—	—	—	—	—	Kolařík and Jankowiak (2013), Jankowiak et al. (2014), Strzałka et al. (2021)
<i>G. luteobrunnea</i>	—	—	—	—	<i>Altingiaceae, Ulmaceae</i>	—	Zhang et al. (2022)
<i>G. microcorthyli</i>	—	—	—	—	—	ambrosia beetle <i>Microcorthylius</i> sp., Costa Rica	Kolařík and Kirkendall (2010)
<i>G. morbida</i>	—	<i>Juglandaceae</i>	<i>Juglandaceae</i>	<i>Juglandaceae</i>	—	—	Hadziabdic et al. (2014b), Montecchio et al. (2015), Kolařík et al. (2017) and others
<i>G. obscura</i>	<i>Betulaceae, Fagaceae</i>	—	—	<i>Cupressaceae, Juglandaceae, Vitaceae</i>	—	—	Kolařík et al. (2008), Six et al. (2009), Huang et al. (2017), Huang et al. (2019)

Table 1 (continued)

Species <sup>1</sup>	Central and North Eastern Europe (~ temperate Europe)	Mediterranean Basin and Black and Caspian Sea region	Western USA and Northern Mexico	Southeastern and Eastern USA	China	Other substrates or locations	References
<i>G. omnicola</i> (G. sp. 10)	<i>Araliaceae, Betulaceae, Cupressaceae, Fagaceae, Oleaceae, Rosaceae, Salicaceae, Tiliaceae, Ulmaceae</i>	<i>Anacardiaceae, Cupressaceae, Fabaceae, Lauraceae, Moraceae, Rosaceae, Ulmaceae</i>	—	<i>Fagaceae, unknown</i>	—	scolytids from <i>Virgilia</i> spp., South Africa; air, Israel	Kolařík et al. (2007), Kolařík et al. (2008), Machingambi et al. (2014), Pepori et al. (2015), Huang et al. (2017), Kolařík et al. (2017), Huang et al. (2019), Meshram et al. (2022)
<i>G. pallida</i> s. s.	—	—	—	<i>Arecaceae, Fagaceae, Juglandaceae, unknown</i>	<i>Fabaceae</i>	cotton yarn, England; soil, Nigeria	Pitt (1979), Huang et al. (2017), Kolařík et al. (2017), Huang et al. (2019), Zhang et al. (2022)
<i>G. pazoutovae</i>	<i>Fagaceae</i>	—	—	—	—	—	Strzałka et al. (2021)
<i>G. proliferans</i>	—	—	—	<i>Sapindaceae</i>	—	—	Huang et al. (2017), Huang et al. (2019)
<i>G. pulverea</i> (G. sp. 3)	<i>Betulaceae, Fagaceae, Rosaceae</i>	—	—	—	<i>Anacardiaceae, Altingiaceae, Fabaceae, Gneta-ceae, Rosaceae, Ulmaceae</i>	roots of <i>Quercus robur</i> , Czechia; soil, Czechia	Kolařík et al. (2004), Kolařík et al. (2008), Strzałka et al. (2021), Zhang et al. (2022)
<i>G. pumila</i> (G. sp. 2)	<i>Fagaceae, Oleaceae, Rosaceae, Ulmaceae</i>	<i>Fagaceae, Lauraceae, Ulmaceae</i>	<i>Rosaceae</i>	<i>Cupressaceae, Juglandaceae, Oleaceae, Ulmaceae, unknown</i>	<i>Ulmaceae</i>	scolytids from <i>Virgilia</i> , South Africa; apple tree, Cyprus; <i>Cucumis melo</i> , Peru	Kolařík et al. (2004), Kolařík et al. (2007), Kolařík et al. (2008), Machingambi et al. (2014), Pepori et al. (2015), Hanzl et al. (2016), Huang et al. (2017), Kolařík et al. (2017), Huang et al. (2019), Morales-Rodríguez et al. (2021), Strzałka et al. (2021), Zhang et al. (2022)
<i>G. putterillii</i>	<i>Rosaceae</i>	<i>Lauraceae</i>	<i>Cupressaceae, Eri-caceae, Fagaceae, Juglandaceae, Lauraceae, Pinaceae, Salicaceae</i>	—	<i>Lauraceae</i>	<i>Beilschmiedia tawa</i> wood, New Zealand	Kolařík et al. (2004), Kolařík et al. (2007), Kolařík et al. (2008), Kolařík et al. (2017), Zhang et al. (2022)

Table 1 (continued)

Species <sup>1</sup>	Central and North Eastern Europe (~temperate Europe)	Mediterranean Basin and Black and Caspian Sea region	Western USA and Northern Mexico	Southeastern and Eastern USA	China	Other substrates or locations	References
<i>G. radiata</i>	—	—	—	—	<i>Atingiaceae, Ulmaceae</i>	—	Zhang et al. (2022)
<i>G. rufescens</i>	—	—	—	—	—	two ambrosia beetle species, Costa Rica	Kolařík and Kirkendall (2010)
<i>G. subfulva</i>	—	—	—	—	<i>Anacardiaceae, Rosaceae</i>	—	Zhang et al. (2022)
<i>G. xerotolerans</i> (= <i>G.</i> sp. 21)	—	<i>Fabaceae, Moraceae, Oleaceae</i>	<i>Cupressaceae, Fagaceae, Pinaceae, Rosaceae</i>	<i>Cupressaceae, Fagaceae</i>	<i>Cupressaceae</i>	house wall, Spain	Huang et al. (2017), Kolařík et al. (2017), Crous et al. (2018), Huang et al. (2019), Juan Alfredo et al. (2020)
<i>Geosmithia</i> sp. 1	<i>Cupressaceae, Ranunculaceae, Ulmaceae</i>	<i>Fabaceae, Moraceae</i>	—	—	—	—	Kolařík et al. (2007), Kolařík et al. (2008), Hanzi et al. (2016)
<i>Geosmithia</i> sp. 4	<i>Ulmaceae</i>	—	—	—	—	—	Kolařík et al. (2008)
<i>Geosmithia</i> sp. 8	<i>Fagaceae</i>	—	—	unknown	—	—	Kolařík et al. (2008), Huang et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. 9	<i>Pinaceae</i>	—	—	—	—	—	Kolařík et al. (2008), Kolařík and Jankowiak (2013), Jankowiak et al. (2014), Jankowiak and Bilanski (2018)
<i>Geosmithia</i> sp. 11	<i>Fagaceae</i>	<i>Oleaceae</i>	—	<i>Fagaceae</i>	—	endophyte of <i>Adansonia gregorii</i> , Australia, based on sequence similarity (99%, GU19942)	Kolařík et al. (2007), Kolařík et al. (2008), Sakalidis et al. (2011), Huang et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. 12	<i>Fagaceae, Oleaceae</i>	—	<i>Oleaceae</i>	<i>Juglandaceae, Fagaceae, Oleaceae</i>	—	—	Kolařík et al. (2008), Huang et al. (2017), Kolařík et al. (2017), Huang et al. (2019), Surzałka et al. (2021)
<i>Geosmithia ulmacea</i> ( <i>G.</i> sp. 13)	<i>Ulmaceae</i>	—	<i>Ulmaceae</i>	—	—	—	Kolařík et al. (2008), Pepori et al. (2015), Kolařík et al. (2017)



Table 1 (continued)

Species <sup>1</sup>	Central and North Eastern Europe (~ temperate Europe)	Mediterranean Basin and Black and Caspian Sea region	Western USA and Northern Mexico	Southeastern and Eastern USA	China	Other substrates or locations	References
<i>Geosmithia</i> sp. 16	<i>Pinaceae</i>	—	—	—	—	—	Kolařík et al. (2008), Jankowiak and Kolařík (2010), Kolařík and Jankowiak (2013), McPherson et al. (2013), Jankowiak et al. (2014), Jankowiak and Bilanski (2018) and Kolařík et al. (2007)
<i>Geosmithia</i> sp. 22	—	<i>Fagaceae, Moraceae, Oleaceae, Rosaceae</i>	—	—	—	—	Kolařík et al. (2007), Kolařík et al. (2008), Kolařík et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. 23	<i>Betulaceae</i>	<i>Moraceae, Rosaceae</i>	<i>Ulmaceae</i>	<i>Ulmaceae</i> , unknown	—	scolytid on <i>Persea gratissima</i> , Seychelles; <i>Malus pumila</i> branches, Cyprus	Dori-Bachash et al. (2015)
<i>Geosmithia</i> sp. 24	<i>Pinaceae</i>	<i>Pinaceae</i>	—	—	—	—	Kolařík and Jankowiak (2013)
<i>Geosmithia</i> sp. 25	<i>Pinaceae</i>	—	—	—	—	—	Kolařík and Jankowiak (2013), Jankowiak et al. (2014), Kolařík et al. (2017)
<i>Geosmithia</i> sp. 26	<i>Pinaceae</i>	—	<i>Pinaceae</i>	—	—	—	Kolařík and Jankowiak (2013), Jankowiak et al. (2014), Kolařík et al. (2017)
<i>Geosmithia</i> sp. 27	<i>Pinaceae</i>	—	<i>Pinaceae</i>	—	—	—	Kolařík and Jankowiak (2013), Jankowiak et al. (2014), Kolařík et al. (2017)
<i>Geosmithia</i> sp. 29	<i>Pinaceae</i>	—	—	—	—	—	Kolařík and Jankowiak (2013)
<i>Geosmithia</i> sp. 30	<i>Pinaceae</i>	—	—	—	—	—	Kolařík and Jankowiak (2013), Jankowiak et al. (2014)
<i>Geosmithia</i> sp. 31	<i>Pinaceae</i>	—	<i>Pinaceae</i>	—	—	—	Kolařík and Jankowiak (2013), Jankowiak et al. (2014)

Table 1 (continued)

Species <sup>1</sup>	Central and North Eastern Europe (~ temperate Europe)	Mediterranean Basin and Black and Caspian Sea region	Western USA and Northern Mexico	Southeastern and Eastern USA	China	Other substrates or locations	References
<i>Geosmithia</i> sp. 32	Cupressaceae	Oleaceae	Cupressaceae	—	—	—	Kolařík et al. (2008), Kolařík et al. (2017), Juan Alfredo et al. (2020)
<i>Geosmithia</i> sp. 33	—	—	Pinaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 34	—	—	Cupressaceae— <i>Calocedrus</i> , Pinaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 35	—	—	Pinaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 36	—	—	Pinaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 37	—	—	Pinaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 38	—	—	Fagaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 39	—	—	Juglandaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 40	—	—	Pinaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 41	—	—	Anacardiaceae, Asteraceae, Fagaceae, Lauraceae, Pinaceae, Rosaceae	Juglandaceae, Fagaceae, unknown	—	—	Huang et al. (2017), Kolařík et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. 42	—	—	Cupressaceae— <i>Calocedrus</i> , Pinaceae, Rosaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 43	—	—	Pinaceae	—	—	—	Kolařík et al. (2017)
<i>Geosmithia</i> sp. 44	—	—	Cupressaceae, Pinaceae	Pinaceae	—	—	Huang et al. (2017), Kolařík et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. 45	—	—	—	Pinaceae	—	—	Huang et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. 46	—	—	—	Juglandaceae, Fagaceae	—	—	Huang et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. 47	—	—	—	Juglandaceae, Fagaceae	—	—	Huang et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. 48	—	—	—	Cupressaceae	—	—	Huang et al. (2017), Huang et al. (2019)
<i>Geosmithia</i> sp. ( <i>G. pallida</i> complex)	—	—	—	—	—	<i>Scolytoplatypus fasciatus</i> from <i>Virgilia</i> , South Africa	Machingambi et al. (2014)

Table 1 (continued)

Species <sup>1</sup>	Central and North Eastern Europe (~temperate Europe)	Mediterranean Basin and Black and Caspian Sea region	Western USA and Northern Mexico	Southeastern and Eastern USA	China	Other substrates or locations	References
<i>Geosmithia</i> sp. ( <i>G. pallida</i> complex)	—	—	—	—	—	endophyte of <i>Brucea mollis</i> , India, unique lineage based on KU693285	Deka and Jha (2018)
<i>Geosmithia</i> sp. ( <i>G. pallida</i> complex)	—	—	—	—	—	sea sediment, China, unique lineage based on MK047400	Sun et al. (2018)
<i>Geosmithia</i> sp. ( <i>G. sp. 24</i> complex)	—	—	—	—	—	scolytids from <i>Pinus</i> , Israel, sister to <i>Geosmithia</i> sp. 24	Dori-Bachash et al. (2015)
<i>Geosmithia</i> sp. ( <i>G. sp. 8</i> complex)	—	—	—	—	—	<i>Phloeosinus</i> spp. from <i>Cupressus</i> , Izrael	Meshram et al. (2022)

<sup>1</sup>The numbering for species no. 1–31 follows Kolařík et al. (2007, 2008), Kolařík and Jankowiak (2013), 33–44 (Kolařík et al. 2017), 45–48 (Huang et al. 2019). *Geosmithia pulvereae* and *Geosmithia* sp. 23 may represent the same species (Zhang et al. 2022)

species. Therefore, alternative markers are needed for better resolution in some species complexes. Other commonly used markers include the genes for the RNA polymerase II second largest subunit (*RPB2*, region defined by the primers fRPB2-5F/fRPB2-7R),  $\beta$ -tubulin (*TUB2*, primers T10/Bt2b) and translation elongation factor 1- $\alpha$  (*TEF-1 $\alpha$* ) 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, but 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. langdonii* species complexes (Kolařík et al. 2017).

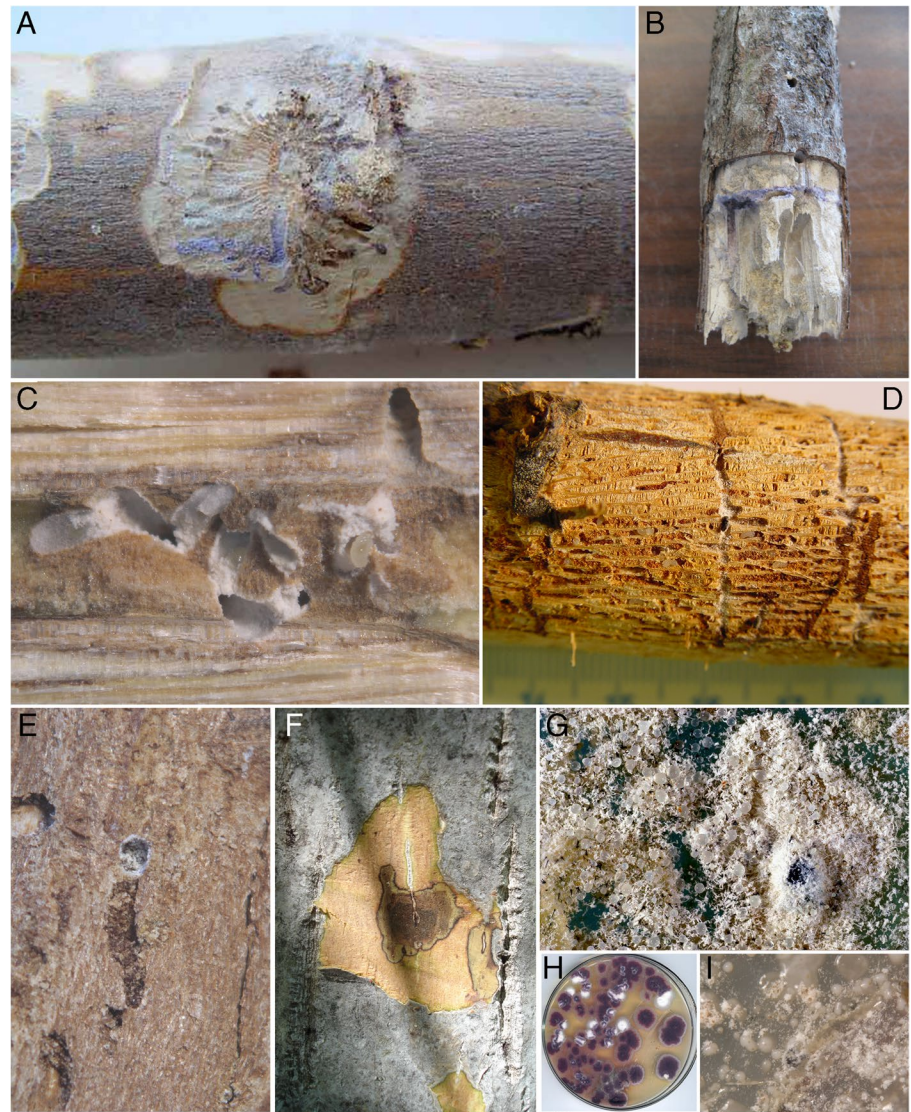
### Host range and intimacy of the association with bark beetles

*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* fungi are usually isolated from all the gallery throughout the insect's life cycle and can be visually conspicuous, particularly in pupal chambers and detritus in larval passages (Fig. 3).

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 the ubiquitous, non-indigenous fast-growing molds, a rinse in a modified White solution can be used, particularly when isolating from beetles captured in the environment outside of the galleries (Barras 1972; Kolařík et al. 2008). *Geosmithia* 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*, and *Platypodinae*: 140; other *Curculionidae*: 5; *Cerambycidae*: 2; *Bostrichidae*: 6) have been studied for the presence of *Geosmithia*; this fungus was found on 119 of

**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. microcorthyli* 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*. **G** *G. flava* and *Ophiostoma novo-ulmi* (white droplets) growing on agar plated with *Scolytus multistriatus* 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)



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 has also been found on seed-feeding *Coccotrypes* species (*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, China, and Taiwan (Fig. 4, Tables 1 and 2).

The degree of *Geosmithia* association with tree hosts or 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 studies. We recommend using basic measures such as the proportion of gallery systems (e.g., Kolařík et al. 2017), of numbers of insect bodies (adults, larvae), or gallery segments (e.g., Jankowiak et al. 2014; Dori-Bachash et al. 2015) with *Geosmithia* present, out of all sampled. A more quantitative estimate of prevalence is the percentage of CFU counts belonging to *Geosmithia* within the entire fungus community (Skelton et al. 2018).

Already Roland Kirschner (2001) noted that bark beetles differ in their degree of association with *Geosmithia*. Subcortical insects (mostly bark beetles) can be divided into those with whom *Geosmithia* is associated strongly,

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

Region	Host plant	Insect vector	Tree part and ecology <sup>1</sup>	Relative <i>Geosmithia</i> abundance <sup>2</sup>	Total number of <i>Geosmithia</i> spp.	References
Costa Rica	<i>Cassia</i>	<i>Microcorthyli</i> sp.	T, B/AMB	100%	1	Kolařík and Kirkendall (2010)
	<i>Croton</i>	<i>Cnesinus lecontei</i>	T, B/AMB	100%	2	Kolařík and Kirkendall (2010)
China	<i>Paulinia</i>	<i>Eupagiocerus dentipes</i>	T, B/AMB	100%	2	Kolařík and Kirkendall (2010)
	<i>Pinus</i>	<i>Dendroctonus armandi</i>	T/PHL	0%	0	Hu et al. (2015)
	various host	<i>Acanthotomicus suncei</i> , <i>Crossotarsus emancipatus</i> , <i>Cryphalus eriobotryae</i> , <i>C. kyotoensis</i> , <i>Dinoderus</i> sp., <i>Ernoporus japonicus</i> , <i>Hypothenemus</i> sp., <i>Microperus</i> sp., <i>Phloeosinus cf. hopehi</i> , <i>Phloeosinus</i> sp., <i>Scolytus julianshananensis</i> , <i>S. semenovi</i> , <i>Sinoxylon</i> cf. <i>cucumella</i> ( <i>Bostrichidae</i> ), <i>Xylocis tortilicornis</i> ( <i>Bostrichidae</i> )	-	+	3	Zhang et al. (2022)
Europe, Mediterranean Basin	<i>Abies</i>	<i>Cryphalus piceae</i>	T, B, L/PHL	37-82%	6	Kirschner (2001), Kolařík et al. (2008), Jankowiak and Kolařík (2010), Jankowiak and Bilanski (2018)
		<i>Orthotomicus laricis</i>	T/PHL	0%	0	Jankowiak and Bilanski (2018)
		<i>Pissodes piceae</i> ( <i>Curculionidae</i> , <i>Molytinae</i> )	L, T/PHL, XYL	0%	0	Jankowiak and Bilanski (2018)
		<i>Pityokteines curvidens</i>	L, T/PHL	24%	1	Jankowiak and Bilanski (2018)
		<i>Pityokteines vorontzovi</i>	B, L/PHL	70%	1	Jankowiak and Bilanski (2018)
		<i>Pityophthorus pityographus</i>	T, B/PHL	80%	2	Jankowiak and Bilanski (2018)
		<i>Trypodendron lineatum</i>	T/PHL	0%	0	Jankowiak and Bilanski (2018)
		<i>Xyleborinus saxesenii</i>	T/PHL	0%	0	Jankowiak and Bilanski (2018)
		<i>Dryocoetes alni</i>	B, L/PHL	5%	1	Strzałka et al. (2021)
		<i>Scolytus ratzeburgii</i>	L, T/PHL	0%	0	Kolařík et al. (2008), Linnakoski et al. (2008), Strzałka et al. (2021)
		<i>Scolytus carpini</i>	B, T/PHL	100%	5	Kolařík et al. (2008)
		<i>Xylocleptes bispinus</i>	liana stem/PHL	20%	1	Kolařík et al. (2008)
	<i>Phloeosinus armatus</i> , <i>P. bicolor</i>	T, B/PHL	100%	4	Meshram et al. (2022)	
	<i>Phloeosinus henschi</i> , <i>P. thujae</i>	T, B/PHL	100%	4	Kolařík et al. (2007), Kolařík et al. (2008)	
	<i>Phloeophthorus cristatus</i> , <i>Phloeotribus rhododactylus</i>	B, L/PHL	50-100%	1	Kolařík et al. (2007), Kolařík et al. (2008)	
	<i>Aphanarthrum</i> sp.	stem/PHL	100%	1	Kolařík et al. (2007)	
	<i>Ernoporus fagi</i> , <i>Taphrorhynchus bicolor</i>	T, B inner bark of trunk/PHL	27-29%	4	Kolařík et al. (2008), Strzałka et al. (2021)	
	<i>Trypodendron domesticum</i> , <i>T. signatum</i>	L/AMB	0%	0	Strzałka et al. (2021)	
	<i>Hypoborus ficus</i>	B, L, T/PHL	98%	9	Kolařík et al. (2007)	
	<i>Hylesinus crenatus</i>	T/PHL	24%	1	Strzałka et al. (2021)	
	<i>Hylesinus varius</i> , <i>H. orni</i> , <i>H. toranio</i>	B, L/PHL	90-100%	5	Kolařík et al. (2008), Strzałka et al. (2021)	

Table 2 (continued)

Region	Host plant	Insect vector	Tree part and ecology <sup>1</sup>	Relative <i>Geosmithia</i> abundance <sup>2</sup>	Total number of <i>Geosmithia</i> spp.	References
	<i>Hedera</i>	<i>Kissaphagus hederæ</i>	liana stem/PHL	33%	2	Kolařík et al. (2008)
	<i>Larix</i>	<i>Ips cembrae</i>	L, T/PHL	0%	0	Jankowiak and Rossa (2007)
		<i>Orthotomicus laricis</i>	T/PHL	0%	0	Kirschner (2001)
		<i>Trypodendron lineatum</i>	T/PHL	0%	0	Kirschner (2001), Jankowiak and Bilanski (2018)
	<i>Laurus</i>	<i>Liparthrum colchicum</i>	B, L/PHL	100%	3	Kolařík et al. (2007), Benvenuti et al. (2021), Vitale et al. (2021)
	<i>Olea</i>	<i>Phloeotribus scarabaeoides</i>	B, L/PHL	100%	4	Kolařík et al. (2007)
	<i>Picea</i>	<i>Cryphalus abietis</i>	B, L/PHL	100%	3	Kolařík and Jankowiak (2013)
		<i>Crypturgus cinereus</i>	L, T/PHL	0%	0	Kirschner (2001)
		<i>Crypturgus pusillus</i>	B/PHL	0%	0	Kirschner (2001)
		<i>Dendroctonus micans</i>	L, T/PHL	0%	0	Kolařík and Jankowiak (2013), Dohet et al. (2016)
		<i>Dryocoetes autographus</i>	T/PHL	0-6%	2	Kirschner (2001), Jankowiak et al. (2014)
		<i>Hylurgops palliatus</i>	T/PHL	0-2%	1	Kirschner (2001), Jankowiak (2006b), Jankowiak et al. (2014)
		<i>Ips amitinus</i>	L/PHL	2%	1	Jankowiak et al. (2014)
		<i>Ips duplicatus</i>	L, T/PHL	0%	0	Kolařík and Jankowiak (2013)
		<i>Ips typographus</i>	T/PHL	0-1%	2	Kirschner (2001), Jankowiak and Hilszczański (2005), Persson et al. (2009)
		<i>Pityogenes chalcographus</i>	L/PHL	0-24%	6	Kirschner (2001), Jankowiak et al. (2014)
		<i>Pityophthorus pityographus</i>	B, L/PHL	58%	7	Jankowiak et al. (2014)
		<i>Polygraphus poligraphus</i>	L/PHL	24%	3	Kirschner (2001), Jankowiak et al. (2014)
		<i>Trypodendron lineatum</i>	T/PHL	0%	0	Kirschner (2001), Jankowiak and Bilanski (2018)
	<i>Pinus</i>	<i>Acanthocinus aedilis</i> (Cerambycidae)	L, T/PHL, XYL	0%	0	Jankowiak and Rossa (2007)
		<i>Crypturgus cinereus</i>	L, T/PHL	0%	0	Kirschner (2001)
		<i>Hyllobius abietis</i> (Curculionidae, Molytinae)	T/PHL, XYL	0%	0	Kolařík and Jankowiak (2013)
		<i>Hylurgus ligniperda</i>	T/PHL	0%	0	Davydenko et al. (2014)
		<i>Ips acuminatus</i>	L/PHL	0%	0	Davydenko et al. (2017)
		<i>Ips sexdentatus</i>	T/PHL	0%	0	Kirschner (2001), Kolařík and Jankowiak (2013), Davydenko et al. (2021)
		<i>Monoctonus galloprovincialis</i> (Cerambycidae)	L/PHL, XYL	3.30%	1 (?)	Jankowiak and Rossa (2007)
		<i>Orthotomicus erosus</i>	L/PHL	23%	1	Dori-Bachash et al. (2015)
		<i>Orthotomicus laricis</i>	T/PHL	0%	0	Jankowiak and Bilanski (2018), Kirschner (2001)

**Table 2** (continued)

Region	Host plant	Insect vector	Tree part and ecology <sup>1</sup>	Relative <i>Geosmithia</i> abundance <sup>2</sup>	Total number of <i>Geosmithia</i> spp.	References
		<i>Pissodes castaneus</i> , <i>P. piniphilus</i> ( <i>Curculionidae</i> , <i>Molytinae</i> )	T/PHL, XYL	0%	0	Kolařík and Jankowiak (2013)
		<i>Pityogenes bidentatus</i>	B, L/PHL	41–82%	6	Jankowiak and Rossa (2008), Jankowiak et al. (2014)
		<i>Pityogenes calcaratus</i>	T, B, L/PHL	84%	1	Dori-Bachash et al. (2015)
		<i>Pityogenes chalcographus</i>	L/PHL	24%	4	Jankowiak et al. (2014)
		<i>Pityogenes quadrifidens</i>	B, L/PHL	86%	2	Kolařík and Jankowiak (2013)
		<i>Pityophthorus pityographus</i>	B, L/PHL	69%	7	Jankowiak et al. (2014)
		<i>Tomicus destruens</i>	L/PHL	0%	0	Dori-Bachash et al. (2015), Muñoz-Adalia et al. (2017)
		<i>Tomicus minor</i>	L, T/PHL	0%	0	Jankowiak (2008)
		<i>Tomicus piniperda</i>	T/PHL	0%	0	Jankowiak (2006a), Jankowiak and Bilański (2007), Silva et al. (2015), Muñoz-Adalia et al. (2017)
		<i>Trypodendron lineatum</i>	T/PHL	0%	0	Kirschner (2001), Jankowiak and Bilański (2018)
<i>Pistacia</i>		<i>Chaetoptelium perrisi</i> , <i>Ch. vestitus</i>	B, L/PHL	20–100%	3	Kolařík et al. (2007), Hadj Taieb et al. (2019)
<i>Populus</i>		<i>Trypophloeus</i> spp.	T, B/PHL	33%	1	Kolařík et al. (2008)
<i>Rosaceae</i>		<i>Scolytus mali</i>	L, T/PHL	42%	3	Strzałka et al. (2021)
		<i>Scolytus rugulosus</i>	B, L/PHL	75–100%	8	Kolařík et al. (2007), Kolařík et al. (2008), Strzałka et al. (2021)
<i>Quercus</i>		<i>Dryocoetes villosus</i>	thick bark of B, L/PHL	0%	0	Strzałka et al. (2021)
		<i>Scobicia pustulata</i> ( <i>Bostrichidae</i> )	B, T/XYL	100%	1	Kolařík et al. (2007)
		<i>Scolytus intricatus</i>	T, B/PHL	15–100%	11	Kubátová et al. (2004), Kolařík et al. (2008), Strzałka et al. (2021)
		<i>Platypus cylindrus</i>	T, B/AMB	+	1	Belhoucine et al. (2011)
		<i>Xyleborus monographus</i>	B, L/AMB	0%	0	Strzałka et al. (2021)
<i>Spartium</i>		<i>Liparthrum genistae</i> , <i>Phloeotribus rhododactylus</i> , <i>Phloeophthorus cristatus</i>	B, L/PHL	50–100%	1	Kolařík et al. (2007), Kolařík et al. (2008)
<i>Tilia</i>		<i>Ernoporus tiliae</i>	B, T/PHL	73–100%	4	Kolařík et al. (2008), Strzałka et al. (2021)
<i>Ulmus</i>		<i>Magdalis armigera</i> ( <i>Curculionidae</i> , <i>Magdalinae</i> )	B, L/PHL, XYL	+	1	Kolařík et al. (2008)
		<i>Pteleobius vittatus</i> , <i>Scolytus multistriatus</i> , <i>S. pygmaeus</i> , <i>S. kirschii</i>	B, L/PHL	47–100%	7	Kolařík et al. (2007), Kolařík et al. (2008), Strzałka et al. (2021)
		<i>Scolytus scolytus</i>	L, T/PHL	22%	3	Strzałka et al. (2021)
various hard-woods		<i>Anisandrus dispar</i>	V/AMB	4%	1	Strzałka et al. (2021)

Table 2 (continued)

Region	Host plant	Insect vector	Tree part and ecology <sup>1</sup>	Relative <i>Geosmithia</i> abundance <sup>2</sup>	Total number of <i>Geosmithia</i> spp.	References
North America	<i>Abies</i>	<i>Xyleborinus saxeseni</i>	B, L/AMB	0%	0	Strzalka et al. (2021)
		<i>Scolytus subscaber</i>	B/PHL	72%	1 (?)	Wright (1938)
	<i>Acer</i>	<i>Scolytus ventralis</i>	T/PHL	0%	0	Wright (1938), Kolařík et al. (2017)
		<i>Scolytus praeceps</i>	B, L/PHL	87–88%	4	Wright (1938), Kolařík et al. (2017)
	<i>Calocedrus</i>	<i>Phloeotribus frontalis</i>	V, inner bark/PHL	33%	1	Huang et al. (2017)
		<i>Phloeosinus fulgens</i>	B, L/PHL	20%	4	Kolařík et al. (2017)
	<i>Carya</i>	<i>Hypothenemus dissimilis</i>	T, B/PHL	50–100%	2	Huang et al. (2017), Huang et al. (2019)
		<i>Hypothenemus rotundicollis</i>	T, B/PHL	100%	2	Huang et al. (2019)
	<i>Celtis</i>	<i>Xylobiops basilaris</i> (Bostrichidae)	V/XYL	0–100%	3	Huang et al. (2019)
		<i>Chramesus chapuisii</i>	T, B/PHL	10–11%	2	Huang et al. (2017), Huang et al. (2019)
	<i>Cinnamomum</i>	<i>Phloeotribus texanus</i>	B, L/PHL	17%	1	Huang et al. (2017), Huang et al. (2019)
	<i>Cupressaceae</i>	<i>Cnesinus strigicollis</i>	T, B, L/PHL	0%	0	Huang et al. (2017)
		<i>Phloeosinus canadensis</i> , <i>P. cupressi</i> , <i>P. punctatus</i> , <i>P. sequoia</i>	V/PHL	80–100%	6	Kolařík et al. (2017)
	<i>Fraxinus</i>	<i>Phloeosinus serratus</i> , <i>P. deleari</i>	V/PHL	100%	2	Juan Alfredo et al. (2020)
		<i>Phloeosinus dentatus</i>	V/PHL	50–60%	4	Huang et al. (2017), Huang et al. (2019)
	<i>Juglans</i>	<i>Hylesinus aculeatus</i>	L/PHL	17%	2	Huang et al. (2017)
		<i>Hylesinus oregonus</i>	L, T/PHL	80%	1	Kolařík et al. (2017)
	<i>Juniperus</i>	<i>Pityophthorus juglandis</i>	T, B/PHL	90–100%	5	Kolařík et al. (2017), various others studies
		<i>Ambrosiodmus lecontei</i>	V/AMB	0%	0	Huang et al. (2017), Huang et al. (2019)
	<i>Notholithocarpus</i>	<i>Pseudopityophthorus pubipennis</i>	T, B, L/PHL	100%	7	Kolařík et al. (2017)
<i>Phoenix</i>	<i>Coccotrypes dactyliperda</i>	seeds	75%	1	Huang et al. (2017)	
	<i>Dendroctonus frontalis</i>	T/PHL	0%	0	Dighton et al. (2021)	
<i>Pinus</i>	<i>Dendroctonus ponderosae</i>	T/PHL	0%	0	Lim et al. (2005), Lee et al. (2006)	
	<i>Dendroctonus punctatus</i> , <i>D. valens</i>	T/PHL	0%	0	Dohet et al. (2016)	
<i>Pinus</i>	<i>Dendroctonus rhizophagus</i>	T, seedlings/PHL	0%	0	Gonzalez-Escobedo et al. (2019)	
	<i>Ips avulsus</i>	L, T/PHL	0%	0	Huang et al. (2017), Huang et al. (2019)	
<i>Pinus</i>	<i>Ips pini</i>	T, L/PHL	0%	0	Lim et al. (2005)	
	<i>Ips plastrographus</i>	T/PHL	100%	2	Kolařík et al. (2017)	
<i>Pinus</i>	<i>Orthotomicus latidens</i>	L/PHL	45%	2	Kolařík et al. (2017)	
	<i>Orthotomicus spinifer</i>	T/PHL	100%	1	Kolařík et al. (2017)	
<i>Pinus</i>	<i>Pityogenes knechtli</i>	B, L/PHL	30%	4	Kolařík et al. (2017)	
	<i>Pityophthorus confusus</i>	little known, B, L/PHL	29–50%	1	Huang et al. (2017), Huang et al. (2019)	
<i>Pinus</i>	<i>Pityophthorus pulicarius</i>	T/PHL	0–12.5%	1	Huang et al. (2017), Huang et al. (2019)	

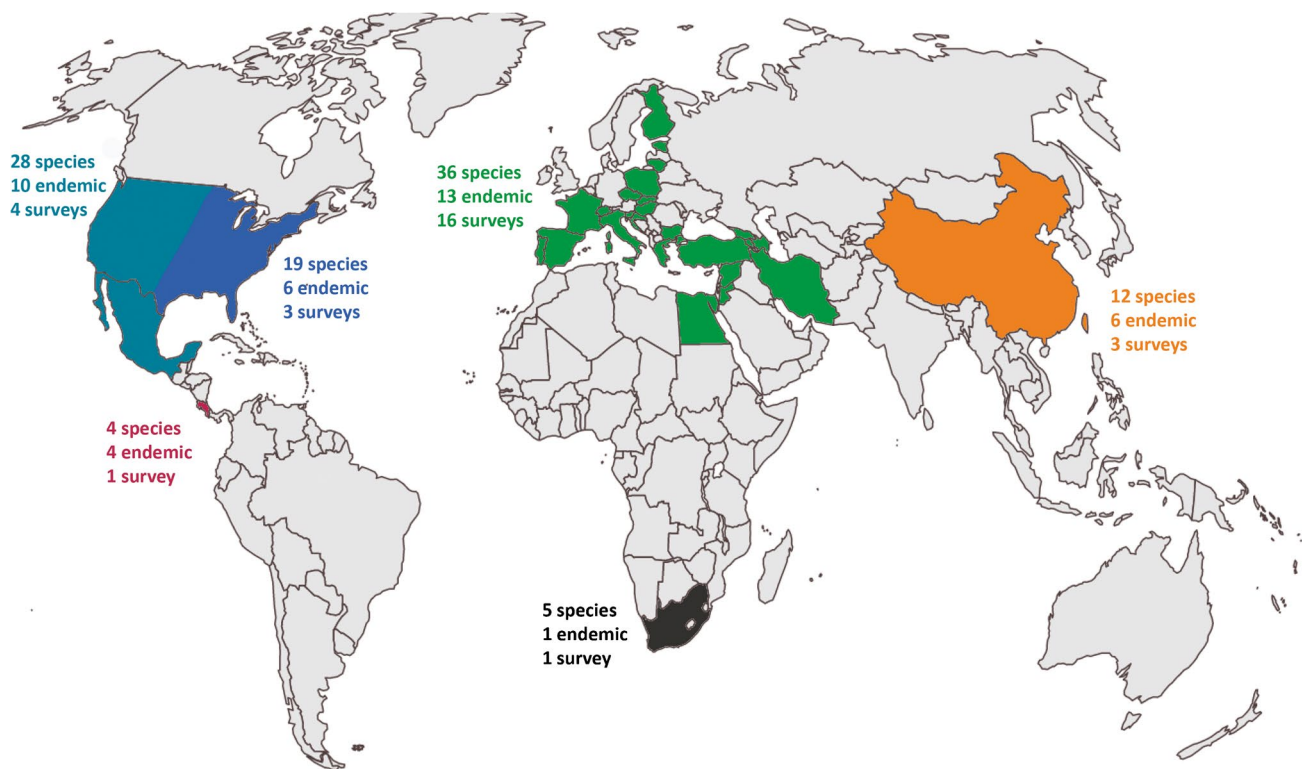


**Table 2** (continued)

Region	Host plant	Insect vector	Tree part and ecology <sup>1</sup>	Relative <i>Geosmithia</i> abundance <sup>2</sup>	Total number of <i>Geosmithia</i> spp.	References
		<i>Pseudips mexicanus</i>	T/PHL	50%	1	Kolařík et al. (2017)
		<i>Pityophthorus annectens</i>	little known, B/PHL	10%	1	Huang et al. (2019)
	<i>Pistacia</i>	<i>Scobicia</i> sp. ( <i>Bostrichidae</i> )	V/XYL	100%	2	Kolařík et al. (2017)
	<i>Prunus</i>	<i>Phloeotribus liminaris</i>	L/PHL	25%	1	Huang et al. (2017)
	<i>Pseudotsuga</i>	<i>Carphoborus vandykei</i>	unknown/PHL	15%	1	Kolařík et al. (2017)
		<i>Cryphalus pubescens</i>	L, seedlings/PHL	40%	3	Kolařík et al. (2017)
		<i>Scolytus oregoni</i>	L/PHL	100%	5	Kolařík et al. (2017)
	<i>Rosaceae</i>	<i>Scolytus rugulosus</i>	B, L/PHL	100%	5	Kolařík et al. (2017)
	<i>Quercus</i>	<i>Micracisella namula</i>	T, PHL	50%	1	Huang et al. (2017), Huang et al. (2019)
		<i>Pseudopityophthorus minutissimus</i>	B, L/PHL	28-56%	1	Huang et al. (2017), Huang et al. (2019)
		<i>Pseudopityophthorus pubipennis</i>	T, B, L/PHL	100%	7	McPherson et al. (2013), Kolařík et al. (2017)
	<i>Toxicodendron</i>	<i>Cactopinus rhois</i>	liana stem/PHL	67%	2	Kolařík et al. (2017)
	<i>Ulmus</i>	<i>Scolytus multistriatus</i> , <i>S. scheyvrewi</i>	B, L/PHL	100%	3	Kolařík et al. (2017)
	<i>Umbellularia</i>	<i>Scobicia declivis</i> ( <i>Bostrichidae</i> )	V/XYL	72%	4	Kolařík et al. (2017)
	various hard-woods or unknown	<i>Chaetophloeus</i> sp.	T, B/PHL	100%	1	Huang et al. (2017)
		<i>Cryptocarenum seriatus</i>	T/PHL	100%	1	Huang et al. (2017)
		<i>Xyleborus celsus</i>	T/AMB	100%	1	Huang et al. (2017)
		<i>Hyllocurus hirtellus</i>	T, B/PHL	80%	3	Kolařík et al. (2017)
		<i>Hypothenemus eruditus</i>	V, mostly T, B/PHL	0-25%	1	Huang et al. (2017), Huang et al. (2019)
		<i>Xylosandrus compactus</i>	T/AMB	0-50%	1	Huang et al. (2017), Huang et al. (2019)
		<i>Xylosandrus crassiusculus</i>	V/AMB	0-50%	1	Huang et al. (2017), Huang et al. (2019)
		<i>Xylosandrus crassiusculus</i> , <i>Xylosandrus compactus</i>	V, mostly T, B/AMB	0%	0	Huang et al. (2017), Huang et al. (2019)
South Africa	<i>Virgilia</i>	<i>Xylosandrus mutilatus</i>	V/AMB	+	2	Six et al. (2009)
		<i>Scolytoplatypus fasciatus</i>	V/AMB	100%	1	Machingambi et al. (2014)
		<i>Cryphalini</i> sp. 1, <i>Hapalogenitus fuscipennis</i> , <i>Liparthrum</i> sp. 1	B/PHL	100%	5	Machingambi et al. (2014)
Taiwan		<i>Cnestus nitidipennis</i> , <i>Hadrodemius comans</i> , <i>H. globus</i>	AMB	+	1	Lin et al. (2016)

<sup>1</sup>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 and Zabecki (2000) for European; Wood (1982), Bright and Stark (1973), and Smith and Cognato (2014) for American; and Machingambi et al. (2014) for South African species

<sup>2</sup>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



**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

moderately, or not at all (Table 2, Fig. 5). Strongly associated vectors include beetles infesting broad-leaved shrubs and trees, with two exceptions: (1) beetles on *Betula* and *Alnus* and (2) beetle species preferring trunk bases. Beetles on conifers with thin bark are also strongly associated vectors, particularly the many twig- and branch-beetles on *Pinaceae* and *Cupressaceae*. Most conifers within *Cupressaceae* support diverse communities of *Geosmithia* fungi, with the exception of *Calocedrus*. Isolations from the *Calocedrus*-specific beetle *Phloeosinus fulgens* typically yield a low abundance of *Geosmithia* strains and mostly *Pinaceae*-specific species. This may reflect the larger size of the tree and a more humid environment than in most other *Cupressaceae* (Table 2). Other than bark beetles, additional beetle vectors of *Geosmithia* include several subcortical non-scolytine weevil subfamilies (*Cossoninae* 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*.

Kirschner (2001) 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. *Geosmithia* are rare or absent on insects colonizing large limbs and trunks of *Pinaceae* and *Betula*. Within *Pinaceae*, *Geosmithia* abundance and diversity are negatively correlated with the thickness of the wood substrata preferred by the insects (Kolařík and Jankowiak 2013; Jankowiak and Bilanski 2018). Similarly, on *Betula*, the bark beetle *Scolytus ratzeburgi* feeds in a 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 the trunk show much less frequent association with *Geosmithia*. This pattern 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 competitiveness under drought conditions, as found in *G. morbida* (Williams and Ginzel 2021). Other abiotic variables, such as oxygen level and resin concentration, on the growth of *Geosmithia* should be tested, as they 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).

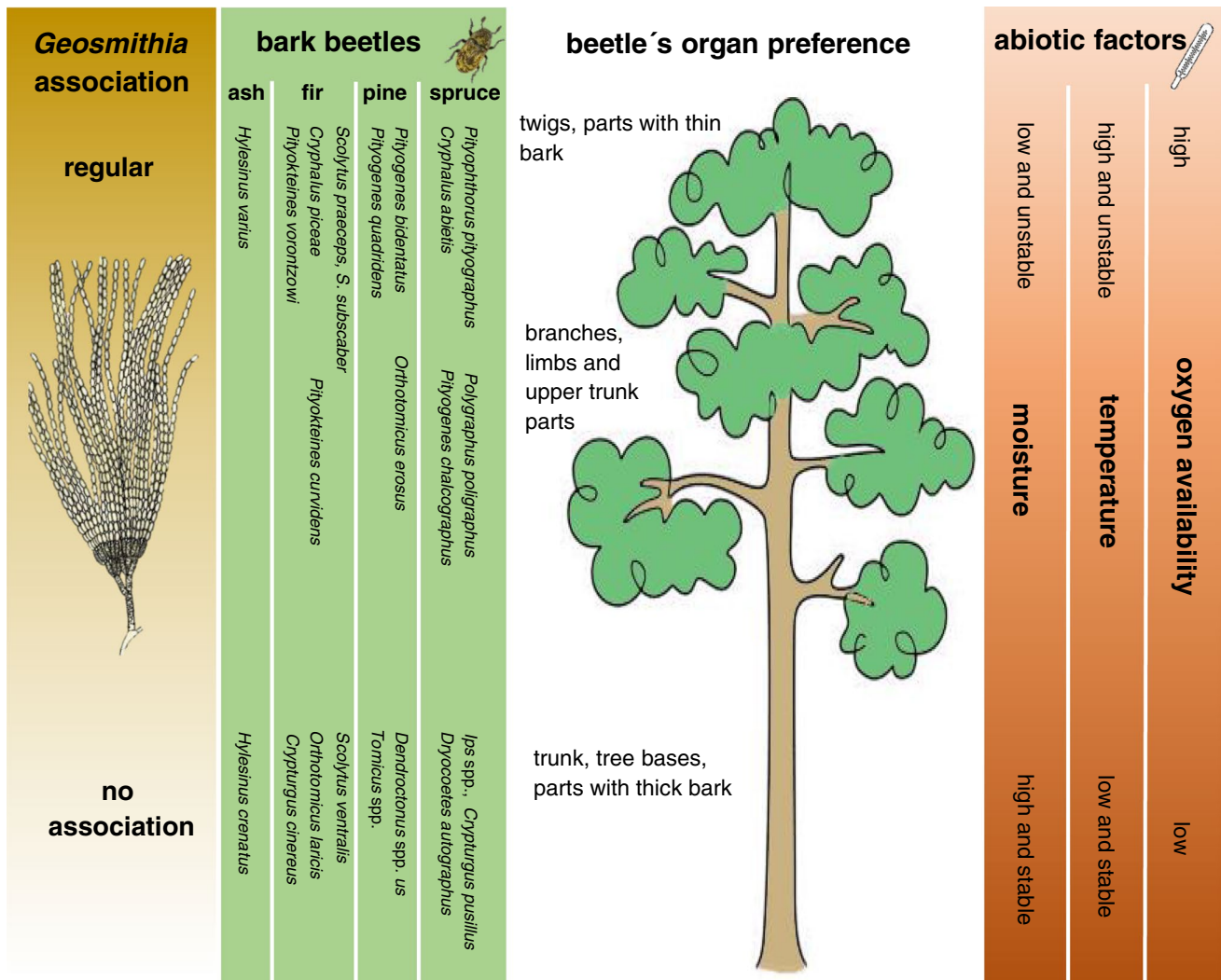


Fig. 5 Schematic presentation of the *Geosmithia* association across bark beetles of different organ preference. The abiotic factors of different beetle substrates are shown

### Vector specificity, community composition, and biogeography

The recent 20 years of research on *Geosmithia* worldwide have finally enabled the first attempt at a synthesis of the ecology and distribution of these fungi (Tables 1 and 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 (Sakalidis et al. 2011; McPherson et al. 2013; Deka and Jha 2018) (Table 1). This is typical of species in the *G. pallida* complex (*G. pumila*, *G. pulverea*),

then *G. fassatae*, *G. flava*, *G. granulata*, *G. langdonii*, *G. obscura*, *G. omnicola*, *G. putterillii*, *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), and *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 host and vector 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 are structured by the influence of the local host tree availability, biogeographical limits, and the presence of suitable vector beetles. The strongest predictor 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 the respective *Geosmithia* species. As *Geosmithia* fungi 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 that 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* (Kolařík and Jankowiak 2013; Jankowiak et al. 2014) and *Abies* (Jankowiak and Kolarik 2010; Jankowiak and Bilanski 2018) 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 either to *Pinus* or to *Picea*, depending on the substrate from which they were collected (Jankowiak et al. 2014). This same pattern of *Geosmithia* community structure has been observed in angiosperms in temperate Europe and the USA (Kolařík et al. 2008; Huang et al. 2019; 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. 2007, 2017). In turn, this regional species pool and its dynamics also influence the richness of *Geosmithia* species 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 endophyte, whereas two other species were only isolated from bark beetle galleries (McPherson et al. 2013).

## ***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 (Six et al. 2009; Belhoucine et al. 2011; Kolařík et al. 2017). 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, 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 and Wingfield 2011; Six 2020). 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 beetle associates that has not been tested. *Geosmithia* fungi 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* spp. also interact with other fungi in the beetle galleries. For example, mycoparasitism by *Geosmithia* species was observed on *Ophiostoma novo-ulmi*, the fungus responsible for the Dutch elm disease (Pepori et al. 2018). *Geosmithia* spp. produce a 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* (Stodůlková et al. 2009; Malak et al. 2013a; Hadj Taieb et al. 2019) 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 (Hilker and Köpf 1994; Poche 1998; Ganapaty et al. 2004; Stodůlková et al. 2009); the role of hAQs in the bark beetle ecosystem has not been evaluated.

## Evolution and biology

The reconstruction of phylogenetic relationships among *Geosmithia* should have been conducted primarily using protein-coding genes. Ribosomal DNA markers, typically used in other fungi, have several limitations in *Geosmithia*. Specifically, *Geosmithia* sp. 26 is a species complex that has rDNA sequences very different from other species 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 size and bottlenecks, possibly related to the switch between free-living to host-associated life strategy (see 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 are sometimes found also outside the bark beetle habitat. At least six lineages convergently evolved specificity to the *Pinaceae* (Veselská et al. 2019; Strzałka et al. 2021; 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 grow 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). The ambrosia species also produce large conidia, a phenotype seen in other ambrosia fungi (Kolařík and Kirkendall 2010; Kolařík et al. 2015).

One lineage, *G. morbida*, became a plant pathogen, with the unique ability to digest all components of lignocellulose, a feature that can serve as its virulence factor (Veselská et al. 2019), as in other plant pathogenic fungi (Doehlemann et al. 2017; Jagadeeswaran et al. 2021). In general, specialists, such as those on *Pinaceae* and the *Juglans*-specific *G. morbida*, have a reduced metabolic breadth in comparison to generalists.

The genome size in *Geosmithia* spp. correlates with cell size (e.g., conidia), as in most eukaryotes (Gregory 2001), 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 behavior 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 *G. morbida*, this is manifested by non-coalescent necrotic lesions in the host tree tissues (Montecchio et al. 2015). The sexual stage has never been observed, and the only population genetics study in the genus suggested the absence of recombination in *G. morbida* (Zerillo et al. 2014). Both mating gene idiomorphs (MAT1-1, MAT1-2) are present across the genus (M. Kolařík, unpublished), and targeted crossing experiments should be carried out to induce the sexual stage, as has been done in other molds 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* (Rossman et al. 1999; Plishka et al. 2009). 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 polyploidization (Veselská and Kolařík 2015). The genome size values, measured by flow cytometry in *G. morbida*, *G. flava*, and *G. putterillii* (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) (Veselská and Kolařík 2015; Schuelke et al. 2017). The number of genes is around 6000, and only 73–146 were found to be species-specific. Between 300 and 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* fungi, like other Dikarya, have hyphae coated with hydrophobins, small proteins that form a hydrophobic membrane and play a crucial role in interactions with hydrophobic substrates such as plant or insect cuticle. Species of *Geosmithia* have class II hydrophobins, called GEO1 (Bettini et al. 2012; Frascella et al. 2014). They possess an intragenic tandem repeat sequence involved 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. Phylogenetic relationships reconstructed from GEO1 and those from classical gene barcodes are highly incongruent. This suggests that GEO1 evolves through multiple horizontal transfers and/or by birth–death evolution (Frascella et al. 2014). The same mechanisms are known to affect other genes under high selection pressure, such as secondary metabolism gene clusters in the genus *Fusarium* (Proctor et al. 2018). There is also evidence that at least six *Geosmithia* species obtained another hydrophobin, 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 present only in *Geosmithia* strains from elms (Scala et al. 2007), but not in those from other tree hosts (Bettini et al. 2014).

## Phytopathogenic potential and thousand cankers disease

While most *Geosmithia* spp. appear to be saprophytes, the pathogenicity capabilities of some species deserve closer scrutiny. Already the first study on *Geosmithia* (Wright 1938) showed 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 belonged to *Geosmithia* sp. 33 or sp. 34 (Kolařík et al. 2017), and the pathogenicity observations, while convincing, require further verification.

Pathogenicity has been 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. omnicola*, *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* (Strzałka et al. 2021; Crous et al. 2022), *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 *Geosmithia* species do induce phloem necrose, 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, lesions 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) and *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 an 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 need 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. (Scala et al. 2007; Bettini et al. 2014), while no active role of the respective *Geosmithia* species in the disease has been demonstrated yet.

The only species that creates significant necroses, the accumulation of which may kill 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 cankers disease (TCD) of black walnut, *Juglans nigra* (Tisserat et al. 2009; Kolařík et al. 2011). *G. morbida* and its vector beetle *P. juglandis* are native to the West of Northern America, and recently, they dispersed to other parts of the continent, as well as to Italy (reviewed in Daniels et al. 2016) and France (Saurat et al. 2023). For several years following this expansion and a drought, there was a notable dieback of black walnut across the USA. 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. morbida* involved its genetic variability (Hadziabdic et al. 2014a; Zerillo et al. 2014), host tree range and virulence (Sitz et al. 2017, 2021; Hefty et al. 2018), vectors (Chahal et al. 2019), migration (Hadziabdic et al. 2014b; Montecchio and Faccoli 2014; Moricca et al. 2020; Marchioro and Faccoli 2022), detection (Stackhouse et al. 2021), eradication (Dal Maso et al. 2019; Seabright et al. 2019; Juzwik et al. 2021), and competition with co-occurring fungi (Gazis et al. 2018). The walnut twig beetle transmits other *Geosmithia* species (Kolařík et al. 2017), 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).

## 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* show 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 with antibacterial or anti-inflammatory activity (Stodůlková et al. 2009, 2010; Malak et al. 2013c) 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, 2018). Their biological activities include antimicrobial, cytotoxic, angiotensin-converting enzyme inhibitory, antileishmanial, or nematocidal properties (Table 3).

While these first studies 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*—have only 14 to 19 secondary metabolite gene clusters, which contrasts with related filamentous fungi having four times higher numbers of similar gene 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.

## 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 the beetle-tree ecosystem observed in *Geosmithia* is in many aspects similar to that observed in ophiostomatoid fungi. In both groups, association with beetles led to the evolution of ambrosia lineages from phloem inhabiting ancestors and a coevolutionary response from the beetles, e.g., by the evolution of mycangia (although on a greater scale in the ophiostomatoid lineages). What benefits *Geosmithia* spp. present to their vectors needs further study in light of similar studies on ophiostomatoid fungi. Another question is the spatial distribution of *Geosmithia* spp. around galleries. Due to their hyaline hyphae and lack of pigment production, it is unclear how far they penetrate the surrounding tissues and whether they thus contribute to wood decomposition or interact with fungal species that decompose dead wood, as is known for ophiostomatoid fungi (Skelton et al. 2020).

One of the most important paradigms that has emerged from the surge of studies on *Geosmithia* is that these fungi 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. However, *Geosmithia* 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 (both lineages), in turn, dominate phloem and sapwood, which retains moisture longer, and therefore are associated with bark beetles in the trunk and roots.

In terms of pathogenicity, some *Geosmithia* cause discoloration of the phloem around the beetle vector 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.

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, and sometimes closely related species display dramatically different ecology. *Geosmithia* shows a similar pattern and is an excellent model for the study of adaptive traits related to species interactions. The evolutions of these traits in *Geosmithia* have 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 need to emulate the methods from better-studied

**Table 3** List of secondary metabolites reported from *Geosmithia*

Compound	Chemical class	Activity	Occurrence	Reference
Rheomodol (1,3,6,8-tetrahydroxyanthraquinone)	Anthraquinone	Inflammatory activity (10 µg/mL), <i>Acinetobacter baumannii</i> (MIC 12.5 µg/mL)	<i>G. lavendula</i>	Stodůlková et al. (2009), Wang et al. (2019)
Rhodolamprometrin (1-acetyl-2,4,5,7-tetrahydroxyanthraquinone)	Anthraquinone	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> (MIC 64–512 µg/mL), affects morphology of mammalian cells	<i>G. lavendula</i>	Stodůlková et al. (2009), Malak et al. (2013c)
1-acetyl-2,4,5,7,8-pentahydroxyanthraquinone	Anthraquinone	<i>S. aureus</i> , <i>B. subtilis</i> (MIC 64–512 µg/mL), inflammatory activity (10 µg/mL), affects cell cycle mammalian cell	<i>G. lavendula</i>	Stodůlková et al. (2009), Malak et al. (2013c)
2,4,5,7-tetrahydroxy anthraquinone -1-carboxylic acid methyl ester	Anthraquinone	n.a.	<i>G. lavendula</i>	Stodůlková 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 AQ	Anthraquinone	Methicillin resistant <i>S. aureus</i> (IC50 16.1 µg/mL)	<i>G. lavendula</i>	Malak et al. (2013c)
2-acetyl-1,4,5,7-tetrahydroxy-9,10-anthraquinone	Anthraquinone	n.a.	<i>G. lavendula</i>	Malak et al. (2013c)
1-acetyl-2,4,5,6,7-pentahydroxy-9,10-anthraquinone	Anthraquinone	n.a.	<i>G. lavendula</i>	Malak et al. (2013c)
4-[2,4'-dihydroxy-6'-(hydroxymethyl)benzyl]benzene-1,2-diol	Benzhydriyl	n.a.	<i>G. langdonii</i>	Malak et al. (2013c)
p-hydroxybenzyl alcohol	Benzyl alcohol	Broad application in human medicine, mostly neuroactive (Zhu et al. 2018)	<i>G. lavendula</i>	Malak et al. (2013c)
4-hydroxybenzyl alcohol	Benzyl alcohol	Antioxidant and anti-inflammatory activities (Kumar et al. 2017)	<i>G. langdonii</i>	Malak et al. (2014)
(1S,2R,3R,4R,5R)-2,3,4-trihydroxy-5-methylcyclohexyl-2',5'-dihydroxybenzoate	Carbasugar	Antileishmanial (IC50 100 µM)	<i>G. langdonii</i>	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)	<i>G. langdonii</i>	Malak et al. (2018)
3,4-dihydroxytoluene	Catechol	n.a.	<i>G. langdonii</i>	Malak et al. (2014)
Bisdethiobis(methylthio)gliotoxin	Cyclic dipeptide	Antibacterial (Ratnaweera et al. 2016)	<i>G. pallida</i> MK047400	Sun et al. (2018)
6-acetylbis(methylthio)gliotoxin	Cyclic dipeptide	No antibacterial or cytotoxic (Liang et al. 2014)	<i>G. pallida</i> MK047400	Sun et al. (2018)
6-deoxy-5a,6-didehydrogliotoxin	Cyclic dipeptide	Cytotoxic (Sun et al. 2012)	<i>G. pallida</i> MK047400	Sun et al. (2018)



Table 3 (continued)

Compound	Chemical class	Activity	Occurrence	Reference
5a,6-didehydrogliotoxin	Cyclic dipeptide	n.a.	<i>G. pallida</i> MK047400	Sun et al. (2018)
6-(phenylmethyl)-(3R,6R)-2,5-piperazinedione	Cyclic dipeptide	n.a.	<i>G. pallida</i> MK047400	Sun et al. (2018)
3-(hydroxymethyl)-3,6-bis(methylthio)-6-(phenylmethyl)-(3R,6R)-2,5-piperazinedione	Cyclic dipeptide	n.a.	<i>G. pallida</i> MK047400	Sun et al. (2018)
3-(hydroxymethyl)-6-(methoxyl)-6-(phenylmethyl)-(3R,6R)-2,5-piperazinedione	Cyclic dipeptide	n.a.	<i>G. pallida</i> MK047400	Sun et al. (2018)
5a,6-anhydrobisdehydrobis(methylthio)gliotoxin	Cyclic dipeptide	n.a.	<i>G. pallida</i> MK047400	Sun et al. (2018)
Geospallin A, Geospallin B, Geospallin C	Cyclic dipeptide	Angiotensin-converting enzyme	<i>G. pallida</i> MK047400	Sun et al. (2018)
(+)-epiepoformin	Cyclohexane epoxide	Antifungal, zootoxic and phytotoxic (Cala et al. 2018)	<i>G. langdonii</i>	Malak et al. (2014)
(-)-dihydroepiepoformin	Cyclohexane epoxide	n.a.	<i>G. langdonii</i>	Malak et al. (2014)
(4S,5S)-4,5-dihydroxy-2-methylcyclohex-2-enone	Cyclohexene and cyclohexenone	Potato microtuber induction (Salvatore et al. 2020)	<i>G. langdonii</i>	Malak et al. (2014)
(4R,5R,6R)-4,5-dihydroxy-6-(6'-methylsalicyloxy)-2-methyl-2-cyclohexen-1-one	Cyclohexene and cyclohexenone	n.a.	<i>G. langdonii</i>	Malak et al. (2014)
Didodecyl thiodipropionate	Dicarboxylic acid	Antioxidant	<i>G. lavendula</i>	Malak et al. (2013c)
6-methylsalicylic acid	Hydroxybenzoate	n.a.	<i>G. langdonii</i>	Malak et al. (2014)
3-hydroxytoluene (m-Cresol)	Phenol derivative	n.a.	<i>G. langdonii</i>	Malak et al. (2014)
2,5-dihydroxybenzaldehyde	Phenolic aldehyde	Nematicidal, cytotoxic (Kim et al. 2021)	<i>G. langdonii</i>	Malak et al. (2014)
Genitsylquinone	Quinone	Antibiotic, herbicide (Buckingham 1996)	<i>G. langdonii</i>	Malak et al. (2014)
(22E)-ergosta-6,22-diene-3 $\beta$ ,5 $\alpha$ ,8 $\alpha$ -triol	Sterol	n.a.	<i>G. lavendula</i>	Malak et al. (2013c)

taxa such as *Penicillium*, *Aspergillus*, or *Fusarium*. More variable DNA markers are needed to answer taxonomic, evolutionary, and molecular biology questions. Similarly, a broader array of differentiated media (DG18, G25N, MY70S, CREA) is needed for morphological and metabolic characters. The whole genus 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. They do not 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 are a promising target for fungus-based 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. Kolařík unpublished).

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**Availability of data and material** All important data are provided directly in the paper. The primary data used to create Fig. 1 are available from the corresponding author on request.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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