

Running title: *Geosmithia* in the western USA.

***Geosmithia* associated with bark beetles and woodborers in the western USA:**

Taxonomic diversity and vector specificity

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Abstract: Fungi in the genus *Geosmithia* (Ascomycota: Hypocreales) are frequent associates of bark beetles and woodborers that colonize hardwood and coniferous trees. One species, *Geosmithia morbida*, is an economically damaging invasive species. We surveyed the *Geosmithia* species of California and Colorado, USA, to: 1) provide baseline data on taxonomy of *Geosmithia* and beetle vector specificity across the western USA; 2) investigate the subcortical beetle fauna for alternative vectors of the invasive *G. morbida*; and 3) interpret the community composition of this region within the emerging global biogeography of *Geosmithia*. *Geosmithia* was detected in 87% of 126 beetle samples obtained from 39 plant species. Twenty-nine species of *Geosmithia* were distinguished of which thirteen may be new species. Bark beetles from hardwoods, *Cupressus*, and *Sequoia* appear to be regular vectors, with *Geosmithia* present in all beetle gallery systems examined. Other subcortical insects appear to vector *Geosmithia* at lower frequencies. Overall, most *Geosmithia* have a distinct level of vector specificity (mostly high, sometimes low) enabling their separation to generalists and specialists. Plant pathogenic *Geosmithia morbida* was not found in association with any other beetle besides *Pityophthorus juglandis*. However, four additional *Geosmithia* species were found in *P. juglandis* galleries. When integrated with recent data from other continents, a global pattern of *Geosmithia* distribution across continents, latitudes, and vectors is emerging: of the 29 *Geosmithia* species found in the western USA, 12 have not been reported outside of the USA. The most frequently encountered species with the widest global distribution also had the broadest range of beetle vectors. Several *Geosmithia* spp. with very narrow vector ranges in Europe exhibited the similar degree of specialization in the USA. Such strong canalization in association could reflect an ancient origin of each individual association, or a recent origin and a subsequent diversification in North America.

Key Words: Entomochoric fungi, *Geosmithia morbida*, *Pityophthorus juglandis*, subcortical beetles, symbiosis

INTRODUCTION

Bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae or Scolytidae, Bright 2014) are ecologically and economically significant herbivores that feed on healthy, decaying,

or dead plant matter. Several aggressive tree-killing species such as *Dendroctonus brevicomis* and *D. ponderosae* in North America and *Ips typographus* in Eurasia are important forest pests. There are at least 565 species of bark and ambrosia beetles in the USA and Canada (Haack 2001, Atkinson 2014, Bright 2014, Seybold et al. 2016) and 273 in Europe (Knížek 2004). Other herbivorous Coleoptera in the subcortical guild belong to the Bostrichidae, Cerambycidae, Buprestidae, and the Curculionidae sensu lato (Schwenke 1974, Bright 2014). Most subcortical beetles are secondary borers of little economic importance, colonizing unhealthy or dying trees, including non-commercial tree species (Ploetz et al. 2013). Research on fungal associates has focused primarily on the few economically important tree killers and invasive species, whereas little is known about fungi associated with the majority of other bark beetles and woodborers.

Most bark and ambrosia beetles interact with subcortical fungi (Beaver 1989), including species that cause vascular wilt and staining (Webber and Gibbs 1989). Most currently known fungal associates of bark beetles belong to the Ophiostomatales (Ascomycota), many of which are completely dependent on their beetle vector and have specialized morphology including sticky entomochoric spores. However, recent research has revealed that several other fungal orders may contain taxonomically diverse and ecologically significant associates of wood-boring insects.

Geosmithia (Ascomycota: Hypocreales) is a genus of mitosporic fungi associated with phloe- and mycophagous bark beetles worldwide (Kolařík and Jankowiak 2013). *Geosmithia* species appear to occur at least as frequently as ophiostomatoid fungal associates of many bark beetles that colonize hardwoods and conifers in the Cupressaceae (Kirschner 2001, Kubátová et al. 2004, Kolařík et al. 2007, 2008, Machingambi et al. 2014). On other conifers (Pinaceae), the typical vectors of *Geosmithia* are bark beetles infesting small diameter branches and twigs of *Pinus*, *Picea*, and *Abies* (Jankowiak and Rossa 2008, Jankowiak and Kolařík 2010, Kolařík and Jankowiak 2013, Jankowiak et al. 2014, Dori-Bachash et al. 2015). *Geosmithia* do not produce sticky spores and in general exhibit various degrees of specificity with their beetle vectors, ranging from specialists with limited distribution on vectors feeding on a single plant genus or family, to generalists associated with numerous vectors, to species not even associated with insects (e.g., living on decaying wood, soil, or cereals, or as endophytes) (Kolařík et al. 2004, Pitt and Hocking 2009, McPherson et al. 2013). The most diverse

group of specialist *Geosmithia* species is associated with beetle vectors that feed on the Pinaceae (Kolařík and Jankowiak 2013, Jankowiak et al. 2014).

The ecological roles of most *Geosmithia* species are poorly understood, with the exception of nutritional symbionts of ambrosia beetles from South America (Kolařík and Kirkendall 2010). Some *Geosmithia* species are plant pathogens and likely increase the fitness of their insect vector, such as in the case of *G. morbida* vectored by *Pityophthorus juglandis* (Kolařík et al. 2011), the causal agent of thousand cankers disease (TCD) of walnut and wingnut in the USA (Tisserat et al. 2009, Hadziabdic et al. 2012, Seybold et al. 2013, Utley et al. 2013, Hishinuma et al. 2016) and recently in Europe (Montecchio and Faccoli 2014). Bark beetle-vectored *G. pallida* has been reported as a causal agent of foamy bark canker in oak in California (Lynch et al. 2014) (but see Discussion). *Geosmithia* species produce numerous biologically active compounds (Stodůlková et al. 2009, 2010, Malak et al. 2013a, b) and several species can inhibit populations of phoretic mites, including parasites of beetle vectors (Machingambi et al. 2014). Some *Geosmithia* species engage in horizontal gene transfer with the pathogenic fungus *Ophiostoma novo-ulmi* (Bettini et al. 2014), but the potential for horizontal acquisition of pathogenicity has not been investigated.

Little is known about the overall diversity and vector spectrum of *Geosmithia* in North America. This is a drawback because: 1) an understanding of the epidemiology and capacity to manage TCD is incomplete without data on alternative vectors of *G. morbida* and 2) the *Geosmithia*-bark beetle symbiosis is a compelling system for research on global patterns in the dynamics and biogeography of symbioses. *Geosmithia* comprises 33 recognized species, of which 14 have been formally described (Kolařík et al. 2004, 2005, 2007, 2008, 2011, 2015, Kolařík and Kirkendall 2010, Kolařík and Jankowiak 2013, Machingambi et al. 2014, Pepori et al. 2015). Six species of *Geosmithia* have been reported from the USA. *Geosmithia lavendula* was first isolated as a laboratory air contaminant in Illinois (Raper and Fennell 1948). Both *G. obscura* and *G. lavendula* were isolated from the camphor shot hole borer (*Cnestus mutilatus*) in Mississippi (Six et al. 2009). McPherson et al. (2013) isolated *G. fassatae* and *G. langdonii* from coast live oak, *Quercus agrifolia*, or bark or ambrosia beetles emerging from this host in Northern California. Finally, Lynch et al. (2014) reported that *G. pallida* was pathogenic on *Q. agrifolia* in Southern California.

The main objective of this study was to provide the first insight into the diversity and vector specificity of *Geosmithia* associated with subcortical insects in the western USA. For this purpose, we sampled a broad vector/host plant spectrum from diverse habitats across California and Colorado. This dataset provides us with the opportunity to examine taxonomic diversity, biogeography, and host specificity of *Geosmithia* from a global perspective by comparing the new data from the USA with published data from Eurasia, thus evaluating the evolutionary stability of *Geosmithia*/bark beetle symbiosis. Our study also aims to explore potential alternative hosts and vectors of the walnut/wingnut pathogen *G. morbida* on trees and other woody plants in the same locations as *Juglans nigra* or other *Juglans* species affected with TCD in California and Colorado. This can have direct impact on the TCD management.

MATERIALS AND METHODS

Sampling and isolation.—We collected 126 samples from 39 plant species at 29 localities in March-May 2009 in California (108 samples/25 localities) and Colorado (19 samples/four localities) (TABLE I, SUPPLEMENTARY TABLE I) and examined 1480 gallery systems of 31 subcortical beetle species (Coleoptera: Cerambycidae, Buprestidae, Bostrichidae, Curculionidae: Cossoninae and Scolytinae). Portions of the host plant infested with beetles were collected and transferred to the laboratory. A sample (twigs, branches, or small diameter stem material) was defined as galleries of one subcortical insect species in the larval feeding stage collected at one sampling date from one site and one host plant. Four to fifteen gallery systems (i.e., larval mines belonging to one parental gallery) were processed from each sample. The sample size was approximately equal for each species of beetle (i.e., a greater portion of a tree part was collected for low-density populations of beetles). Adult insects were excised from the bark and identified. The adults, larvae, eggs, or detritus (boring dust and fungal debris) from each gallery system were spread evenly across the surface of one Petri dish and cultured following Kolařík et al. (2008). Diluted 2% malt extract isolation medium was used in all isolations (MA2; 20 g malt extract (Oxoid, Basingstoke, UK), 15 g agar, 1 L H₂O), and cultures were grown at 24 C for 7-30 d.

Dereplication of *Geosmithia* isolates.—*Geosmithia* isolates were selected for subculturing on the basis of colony morphology. Up to 15 isolates from each sample (425 isolates in total) were further

TABLE I. Samples analyzed in this survey and association with particular *Geosmithia* species

^a The number of samples is given in parentheses.

^b Incidence is given as percentage of samples with presence of particular *Geosmithia* species. Total number of samples was 126.

| Host plant (number of samples) | Associated species | Proportion of gallery systems with the particular fungal species [%] | | | | | | | | | | | | | | | | total number of gallery systems | % of gallery systems with <i>Geosmithia</i> [%] | | | | | | | | | | | | |
|---|---|--|-----------------|---------------------|---------------------|-------------------|----------------------|-------------------|-----------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|---------------------------------|--|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | | <i>G. fassettiae</i> | <i>G. flava</i> | <i>G. langdonii</i> | <i>G. lanandula</i> | <i>G. morbida</i> | <i>G. puterillii</i> | <i>G. ulmacea</i> | <i>G. sp. 2</i> | <i>G. sp. 12</i> | <i>G. sp. 20</i> | <i>G. sp. 21</i> | <i>G. sp. 23</i> | <i>G. sp. 26</i> | <i>G. sp. 27</i> | <i>G. sp. 31</i> | <i>G. sp. 32</i> | | | <i>G. sp. 33</i> | <i>G. sp. 34</i> | <i>G. sp. 35</i> | <i>G. sp. 36</i> | <i>G. sp. 37</i> | <i>G. sp. 38</i> | <i>G. sp. 39</i> | <i>G. sp. 40</i> | <i>G. sp. 41</i> | <i>G. sp. 42</i> | <i>G. sp. 43</i> | <i>G. sp. 44</i> |
| <i>Calocedrus decurrens</i> (4) | <i>Phloeosinus fulgens</i> | | | | | 2 | | 2 | | | | | | | | | | 3 | | | | | | | | | | | 13 | 60 | 20 |
| <i>Cupressus goveniana</i> (5) | <i>Phloeosinus cupressi</i> , <i>Phloeosinus sequoiae</i> | | 21 | 21 | | 76 | | | | 6 | | | | | | | | | | | | | | | | | | | 70 | 100 | |
| <i>Chamaecyparis</i> sp. (1) | <i>Phloeosinus canadensis</i> | | | | | | | | | 47 | | | | | | | | | | | | | | | | 53 | | 15 | 80 | | |
| <i>Juniperus occidentalis</i> var. <i>australis</i> (1) | <i>Phloeosinus punctatus</i> | | | | 53 | | | | | 80 | | | | | | | | | | | | | | | | | | 15 | 100 | | |
| <i>Sequoia sempervirens</i> (12) | <i>Phloeosinus sequoiae</i> , <i>Cryphalus pubescens</i> , Buprestidae sp., Cerambycidae sp. | | 33 | 24 | | 30 | | | | 27 | | | | | 1 | | | | | | | | | | | | | 119 | 100 | | |
| Gymnosperms - Pinaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Abies concolor</i> (1) | <i>Scolytus praeceps</i> | | | | | | | | | | | | | | 7 | 87 | | | | | | | | | 1 | 5 | | 15 | 87 | | |
| <i>Abies concolor</i> (1) | <i>Scolytus ventralis</i> | | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | 0 | | |
| <i>Picea pungens</i> (2) | Buprestidae sp., Cerambycidae sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | 20 | 0 | | |
| <i>Pinus radiata</i> (1) | <i>Ips plastographus</i> | | 15 | | | | | | | | | | | | | | | | | | | | | | 5 | | | 15 | 100 | | |
| <i>Pinus sabiniana</i> (1) | <i>Orthotomicus spinifer</i> | | | | | | | | | 100 | | | | | | | | | | | | | | | | | | 15 | 100 | | |
| <i>Pinus</i> spp. (11) | <i>Pityophthorus</i> spp. | 3 | | | 14 | 6 | | | | 14 | | 10 | 3 | 9 | | | | 5 | | | | | | | 17 | | 13 | 150 | 75 | | |
| <i>Pinus</i> spp. (4) | Cerambycidae spp., Buprestidae sp. | | 38 | | 24 | | | | | 35 | | | | | | | | | | | | | | | | 3 | | 34 | 76 | | |
| <i>Pinus radiata</i> (2) | <i>Pseudips mexicanus</i> | | | | | | | | | | | | | | | | | | | | | | | | 50 | | | 20 | 50 | | |
| <i>Pinus</i> spp. (3) | <i>Orthotomicus latidens</i> | | | | | 9 | | | | 30 | | | | | | | | | | | | | | | | | | 33 | 45 | | |

| | | | | | | | | | | |
|---|--|----|----|----|--|----|---|----|----|-----------|
| <i>Pinus ponderosa</i> (2) | <i>Pityogenes knechteli</i> | 17 | | 17 | | | 7 | 3 | 43 | 30 |
| | | | | | | | | | 8 | 0 |
| <i>Pinus radiata</i> (2) | Cossoninae sp. | | | | | | | | | |
| <i>Pseudotsuga menziesii</i> (2) | <i>Scolytus oregoni</i> | 35 | 26 | 4 | | 13 | 4 | | 23 | 100 |
| <i>P. menziesii</i> (3) | <i>Cryphalus pubescens</i> | 30 | 8 | | | | | 3 | 40 | 40 |
| <i>P. menziesii</i> (1) | Scolytinae sp. | | | | | | | | | |
| | | | | | | | | 33 | | |
| <i>P. menziesii</i> (2) | <i>Carphoborus vandykei</i> | | | | | | | | 5 | 33 29 |
| <i>P. menziesii</i> (4) | Buprestidae sp., Cerambycidae sp. | | 29 | 14 | | | | | | 28 29 |
| <i>P. menziesii</i> (2) | <i>Hylesinus californicus</i> , <i>Scolytus reflexus</i> , <i>Pseudohylesinus nebulosus</i> | | | | | | | | | 30 0 |
| Angiosperms - Anacardiaceae | | | | | | | | | | |
| <i>Pistatia vera</i> (1) | <i>Scobicia</i> sp. | 63 | 75 | | | | | | | 8 100 |
| <i>Toxicodendron diversilobum</i> (3) | <i>Cactopinus rhois</i> , Bostrichidae sp. | | 65 | | | | | 3 | | 30 67 |
| Angiosperms - Asteraceae | | | | | | | | | | |
| <i>Artemisia arborea</i> (1) | Cossoninae sp. | | | | | | | 10 | | 10 10 |
| <i>Baccharis pilularis</i> (1) | Bostrichidae sp. | | 7 | | | | | | | 15 7 |
| Angiosperms - Ericaceae | | | | | | | | | | |
| <i>Vaccinium ovatum</i> (1) | xylophagous bark beetle, old galleries | | 50 | | | | | | | 6 50 |
| Angiosperms - Fagaceae | | | | | | | | | | |
| <i>Notholithocarpus densiflorus</i> (4) | <i>Pseudopityophthorus pubipennis</i> | 11 | 39 | 7 | | 13 | | 62 | | 46 100 |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|--|----|----|----|----|----|----|----|-----|----|----|-----|-----|---|---|---|---|---|---|---|---|---|---|---|----|---|---|---|
| <i>Quercus acrifolia</i> (14) | <i>Pseudopityophthorus pubipennis</i> , <i>Monarthrum scutellare</i> , Buprestidae sp. | 4 | 10 | 19 | 14 | | 3 | | | 1 | 67 | 198 | 100 | | | | | | | | | | | | | | | |
| Angiosperms - Juglandaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Juglans</i> spp. (9) | <i>Pityophthorus juglandis</i> | | 2 | | 23 | 49 | 6 | | | 25 | | 91 | 91 | | | | | | | | | | | | | | | |
| Angiosperms - Lauraceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Umbellularia californica</i> (6) | <i>Scobicia declivis</i> | 23 | | 9 | | | 43 | | | | 13 | 75 | 72 | | | | | | | | | | | | | | | |
| Angiosperms - Oleaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Fraxinus</i> sp. (1) | <i>Hylesinus oregonus</i> | | | | | | | | 80 | | | 10 | 80 | | | | | | | | | | | | | | | |
| Angiosperms - Salixaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Salix</i> sp. (3) | <i>Hylocurus hirtellus</i> , Cerambycidae sp. | 17 | 50 | | | | 23 | | | | | 30 | 80 | | | | | | | | | | | | | | | |
| Angiosperms - Betulaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Alnus</i> sp. (1) | <i>Hylocurus hirtellus</i> | | | | | | | | | | | 10 | 0 | | | | | | | | | | | | | | | |
| Angiosperms - Rosaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Amygdalus communis</i> (2) | <i>Scolytus rugulosus</i> | | | | 17 | | | | 100 | | 10 | 30 | 100 | | | | | | | | | | | | | | | |
| <i>Heteromeles arbutifolia</i> (1) | Scolytinae sp. | | | | | | | | | | 80 | 15 | 80 | | | | | | | | | | | | | | | |
| <i>Prunus</i> spp. (6) | <i>Scolytus rugulosus</i> , <i>Pseudothysanoes hopkinsi</i> | | | | 4 | | 34 | 49 | | | 25 | 80 | 100 | | | | | | | | | | | | | | | |
| Angiosperms - Ulmaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Ulmus</i> spp. (4) | <i>Scolytus multistriatus</i> , <i>Scolytus chevyrewi</i> | | | | | | | | + | 86 | 36 | 36 | 100 | | | | | | | | | | | | | | | |
| Angiosperms - others | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| braodleaved shrub (1) | Bostrichidae sp., xylophagous bark beetle | | | | | 80 | | | | | | 10 | 80 | | | | | | | | | | | | | | | |
| Incidence across all samples^b | | 4 | 12 | 12 | 14 | 4 | 17 | 2 | 1 | 3 | 19 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 2 | 5 | 17 | 4 | 1 | 3 |
| Number of associated host plant families | | 4 | 6 | 5 | 6 | 1 | 7 | 1 | 1 | 1 | 2 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 6 | 3 | 1 | 2 |

selected for dereplication, which was based on morphology of 14 d old colonies (24 C) cultivated on malt extract agar (MEA) and Czapek yeast agar supplemented with trace elements and Czapek-Dox agar (CDA) (Pitt 1979) with particular attention to micromorphology of conidia and conidiophores cultivated on MEA. Selected isolates representing various substrates and locations, from groups of morphologically identical isolates were analyzed with molecular methods.

DNA analyses.— Phenotypic dereplication resulted in 134 isolates that were compared by analysis of nuc rDNA ITS1-5.8S-ITS2 (ITS) sequences. Species within four species-complexes (*Geosmithia* sp. 16 complex, *G. langdonii* complex, *Geosmithia* sp. 26 complex and *G. pallida* complex), are morphologically unique but their ITS sequences are not highly variable. Therefore, representative isolates of the species within these four groups were further characterized by partial sequences of genes for β -tubulin (*TUB2*, 47 sequences), translation elongation factor 1 α (*TEF1*, 51 sequences), and the second-largest subunit of the RNA polymerase II gene (*RPB2*, 45 sequences). The isolate CBS 121749 of *G. obscura* isolated from an ambrosia beetle in Mississippi (Six et al. 2009) and 26 additional isolates obtained from outside of the USA were included in phylogenetic analyses for comparison (SUPPLEMENTARY TABLE II). Finally, all isolates were divided into distinct phylogenetic lineages with uniform and unique phenotypes and assigned putative species numbers. Fifty-seven representative isolates were freeze-dried and deposited in the Culture Collection of Fungi (CCF), Prague, Czech Republic (SUPPLEMENTARY TABLE II) and in the laboratory collection of the first author (abbreviations MK, U, RJ).

Genomic DNA was isolated from 3- to 7-d-old cultures. Cell walls were partially disintegrated through pre-incubation with Lytic Enzyme Solution (5 PRIME, Hamburg, Germany) at 37 C for several hours to overnight at 500 rpm on mixing thermoblock MB-102 (Bioer Technology, Hangzhou, China). The DNA was then isolated with ArchivePure DNA Yeast & Gram-+ Kit (5 PRIME, Hamburg). Nuclear rDNA containing the ITS and partial 28S sequence (ca 900 bp) was amplified with primer pair ITS1F (Gardes and Bruns 1993) and NL4 (O'Donnell 1993) and sequenced by using the same primers together with the primer ITS4 (White et al. 1990) and NL1 (O'Donnell 1993). *TUB2* (ca 750 bp) was amplified with primer pair T1/T10 or T1/Bt2b (Glass and Donaldson 1995, O'Donnell and Cigelnik 1997) and the same primers were used for sequencing. Primers EF1-983F/EF1-2218R

(Rehner and Buckley 2005) were used for amplification of *TEF1* (ca 1000 bp), and the same primers together with EF1-728F (Carbone and Kohn 1999) were used for sequencing. *RPB2* was amplified and sequenced by using the primer pair fRPB2-5F/fRPB2-7cR (Liu et al. 1999). The reaction mixtures and amplification protocols are described in Kolařík and Jankowiak (2013). Purification and sequencing of PCR products was performed at Macrogen Inc. (Seoul, South Korea). The EMBL accession numbers are listed in SUPPLEMENTARY TABLE II. Sequences for the ITS region only were combined with published data and aligned in MAFFT 6 (Kato and Toh 2008). The ITS sequences of *Geosmithia* sp. 26 and *Geosmithia* sp. 44 were very variable and difficult to align with sequences of other species, and thus were analyzed separately (see below for additional details). For the three other species-complexes, the dataset consisted of the concatenated *TUB2*, *TEF1* and *RPB2* sequences (SUPPLEMENTARY TABLE II). Alignments generated using MAFFT 6 were submitted to maximum likelihood (ML) phylogenetic analyses in PhyML 3.0 (Guindon et al. 2010). Node support was obtained by using a bootstrap analysis with 1000 replications. A complementary Bayesian phylogenetic inference was performed by using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with the metropolis-coupled Markov chain Monte Carlo search algorithm run for 2,000,000 generations. Burn-in was determined with Tracer 1.4 (<http://tree.bio.ed.ac.uk/software/tracer>) and discarded. Evolutionary models used in phylogenetic analyses were determined by using MEGA 5.05 (Tamura et al. 2011): T92+G for *G. langdonii* and *Geosmithia* sp. 26 species complexes and TN93+G for the other four datasets. *Acremonium alternatum*, a sister group to *Geosmithia* (Rossman et al. 2001), was chosen as the outgroup for the ITS phylogeny. The phylogenetic datasets are deposited in Dryad (doi:10.5061/dryad.56cg8).

The highly variable and unalignable ITS sequences from the *Geosmithia* sp. 26 species complex were further characterized by using the approach recommended by Feliner and Rosselló (2007), which also tested the hypothesis that these sequences represent pseudogenes. The nucleotide composition of the ITS-28S rDNA alignment truncated to equal length (1000 bp) was calculated with DAMBE 5.2.56 (Xia and Xie 2001). The number of variable positions in 5.8S and 28S rDNA was calculated in MEGA 5.05. Structural predictions of the ITS sequences were obtained by using secondary structure models of previously published *Geosmithia* species, including *Geosmithia* sp. 9

and 16, in the ITS2 Database (Wolf et al. 2005). Additional predictions were obtained by using RNA Folding Form 2.3 running on Mfold server (Zuker 2003). The free energy of the model was used as the predictor of the structure stability and compared across the genus.

RESULTS

Identification of *Geosmithia*.— Phenotypic dereplication resulted in 134 isolates that were compared by molecular analysis. The subsequent combined analyses of their phenotypes with ITS and multilocus sequence comparisons delimited 29 *Geosmithia* species in the western USA, of which 13 are putative new species (*Geosmithia* spp. 32-44).

The main ITS sequence dataset (FIG. 1A) excluded the highly divergent sequences of the *Geosmithia* sp. 26 species complex. The latter sequences were characterized separately (below, FIG. 1B). The main dataset contained 210 sequences and 556 positions, from which 161 were variable and 51 were singletons. Based on ITS sequences data, several U.S. isolates were placed into well-supported species-level clades containing previously described species, identity of which was confirmed by phenotypic comparisons: *G. fassatae*, *G. flava*, *G. langdonii*, *G. lavendula*, *G. morbida*, *G. obscura*, *G. putterillii*, *G. ulmacea*, *Geosmithia* sp. 12, and *Geosmithia* sp. 21. Other species that were clearly distinguishable based on morphology and strong ITS phylogenetic support were the red-spored *Geosmithia* sp. 39 and 40. The rest of the isolates belonged to the four species complexes with lower variability in ITS sequences. Their taxonomic identity was assessed by using multigene phylogenetic analysis (FIG. 2).

The material from the western USA contained a large number of brown-spored isolates ascribable to the *G. pallida* species complex. A multigene alignment contained 34 sequences, and had 2841 positions of which 692 were variable and 502 parsimony-informative. Phylogenetic analysis supported the position of isolates from the USA in the already known *Geosmithia* sp. 2, 23, 27, as well as the newly recognized *Geosmithia* spp. 41-43 (FIG. 2). The type of *G. pallida* IMI 040214 (cotton yarn, England) and the strain IMI 054224 (soil, Nigeria) grouped in the same lineage representing *G. pallida* sensu stricto, and this species was absent among the material from the USA.

FIG. 1. A 50% majority-rule consensus tree obtained by Bayesian inference showing phylogeny of *Geosmithia* ITS sequences obtained during this study (printed in bold) and all other published species. A. Dataset without *Geosmithia* sp. 26 complex.

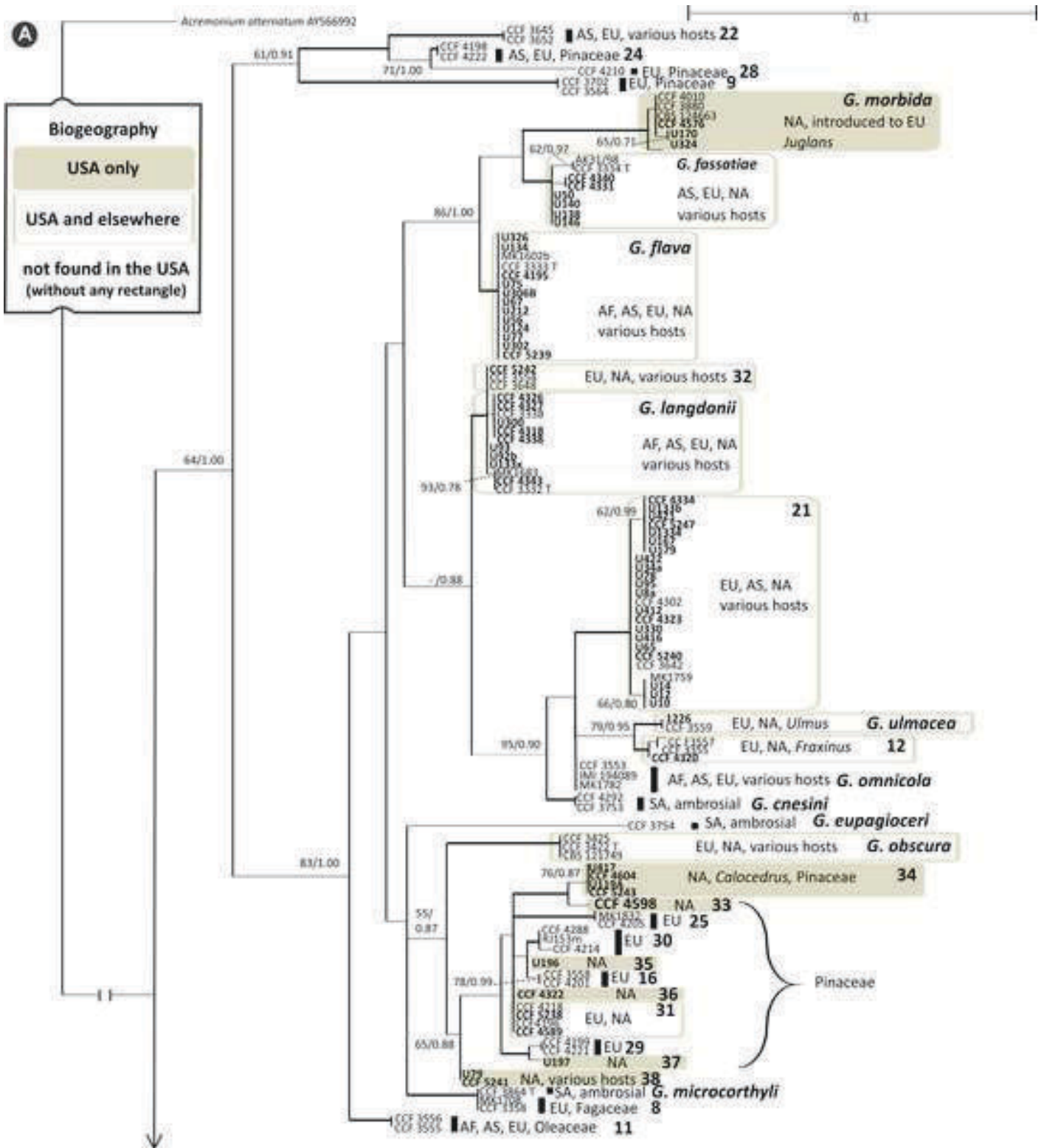
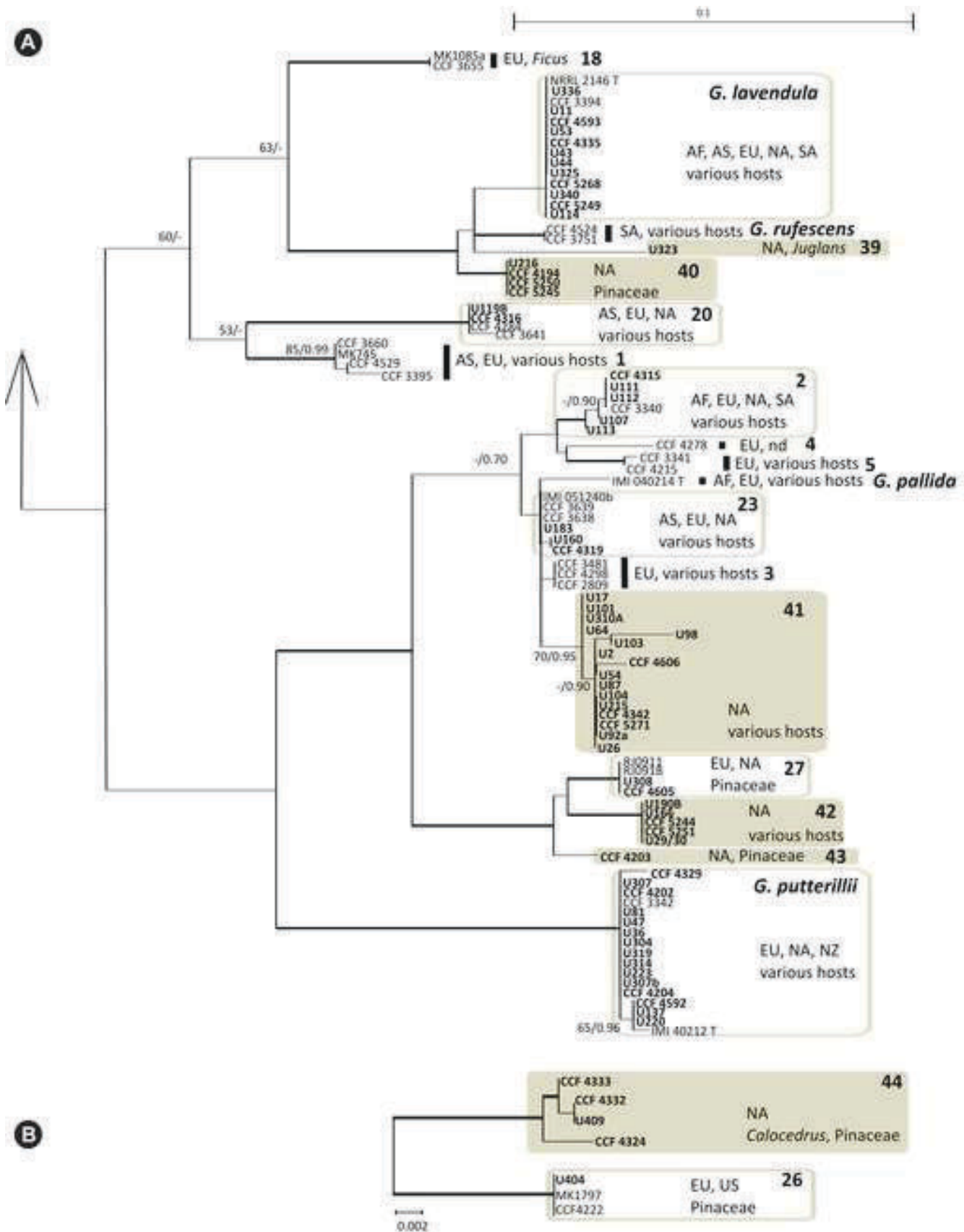


FIG. 1. B The dataset with only the *Geosmithia* sp. 26 complex. Numbers at internal nodes are the Bayesian MCMC posterior probability (≥ 0.7) and PhyML maximum likelihood bootstrap values (≥ 50). Strongly supported branches ($\geq 1.00/95$) are doubled in width. The *Acremonium alternatum* branch is artificially shortened to one-fifth of the actual length. Species numbering is from Kolařík et al. (2007, 2008) and Kolařík and Jankowiak (2013). Area and host range are provided for each species (see SUPPLEMENTARY TABLE IV for details). Species occurring in the USA only (marked by shaded rectangles) or in the USA and other regions (marked by empty rectangles) are highlighted. Abbreviations are: AF – Africa, AS – Asia, EU – Europe, NA – North America, NZ – New Zealand, SA – South America, USA – United States of America, nd – not determined

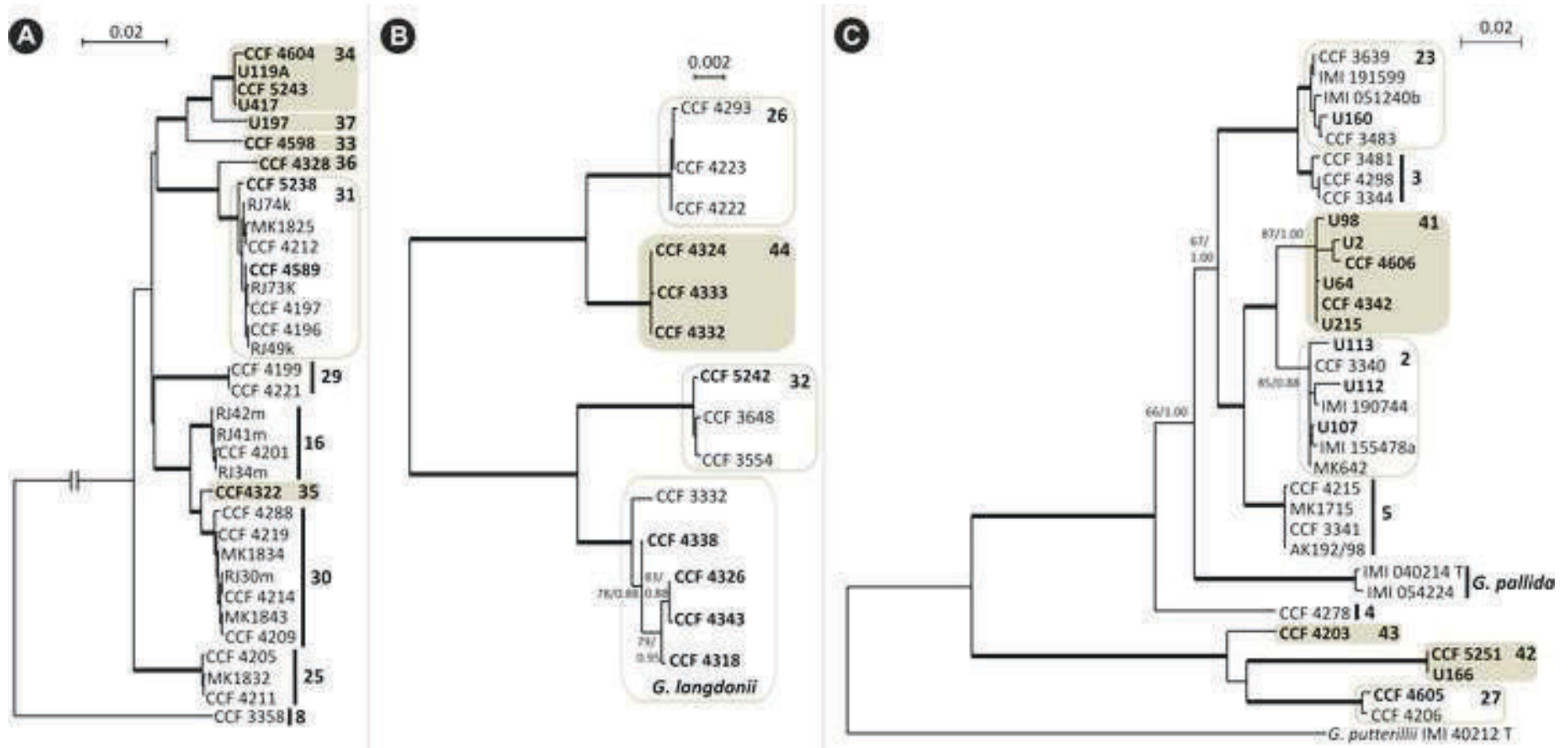


Multigene alignment of the *Geosmithia langdonii* and the *Geosmithia* sp. 26 species complex contained 14 sequences, and had 2775 positions of which 279 were variable and 257 parsimony-informative. In the *G. langdonii* complex, the isolate CCF 5242 and the European strains CCF 3554 and CCF 3648 belong to a separate species *Geosmithia* sp. 32, which is sister to *G. langdonii* (FIG. 2) but is indistinguishable by using ITS sequences. Given all *Geosmithia* species sampled to date its closest relatives are isolates from the *Geosmithia* sp. 26 species complex, where two lineages can be recognized (*Geosmithia* sp. 26 and *Geosmithia* sp. 44) (FIG. 2).

In the *Geosmithia* sp. 16 species complex the alignment contained 34 sequences, and had 3818 positions of which 477 were variable and 272 parsimony-informative. Several isolates clustered in *Geosmithia* sp. 31, or as new independent lineages numbered here as undescribed species 34-37. Two of these species, *Geosmithia* sp. 31 and 36 represented by isolates CCF 4328 and CCF 5238, were indistinguishable by using ITS sequences but differed in other markers: 0.8% positions in the *RPB2* gene (1040 bp), and 2.6% positions in the *TUB2* gene (757 bp) (FIG. 2).

Characterization of ITS sequence from the *Geosmithia* sp. 26 complex.— The ITS sequences of the *Geosmithia* sp. 26 species complex were distinct from the rest of the genus, and clustered on a long branch in the phylogenetic analysis (SUPPLEMENTARY FIGURE 1). The ITS sequences of the complex varied in sequence length, nucleotide composition, and structure when compared to all other isolates examined in this survey. The ITS1 length is 120-137 bp in the *Geosmithia* sp. 26 complex, but ranges from 162 to 185 bp for all other members of *Geosmithia*. Similarly, the ITS2 length is 157-159 bp in the *Geosmithia* sp. 26 complex, but ranges from 171 to 178 bp among other *Geosmithia* species. The lengths of highly conserved 5.8S and partial 28S rDNA are 157 bp and 374 bp, respectively, in the *Geosmithia* sp. 26 complex, whereas other species consistently have lengths of 158 bp and 376 bp, respectively. The 5.8S rDNA has six variable positions across the genus, three of which were due to variability in the *Geosmithia* sp. 26 complex. Similarly, from the 16 variable positions of 28S-rDNA, five were attributable to variability in *Geosmithia* sp. 26 complex. The GC content of the ITS-28S sequence for the *Geosmithia* sp. 26 complex is 51.97-52.01%, whereas it ranges from 53.16 to 55.38% in all the other species. (SUPPLEMENTARY TABLE III).

FIG. 2. Phylogenies of selected species complexes based on a combined *RPB2/TUB2/TEF1* dataset. Relationships in A. *Geosmithia* sp. 16 complex; B. *Geosmithia langdonii* and *Geosmithia* sp. 26 complex; and C. *Geosmithia pallida* complex. The 50% majority consensus tree resulting from analysis in MrBayes is presented. Numbers beside the internal nodes are the Bayesian MCMC posterior probability (≥ 0.7) values and PhyML maximum likelihood bootstrap values (≥ 50). Strongly supported branches ($\geq 1.00/95$) are doubled in width. The trees are unrooted. The *Geosmithia* sp. 8 branch (A.) is artificially shortened to one-tenth of the actual length. The biogeography of the species relative to the USA is highlighted by the rectangles (see FIG. 1). Isolates obtained during this study are printed in bold



The ITS2 database enables predictions of folding of the coded RNA by using secondary structure models. These models were applicable to the ITS of all our species but not to the *Geosmithia* sp. 26 complex. Therefore, for the *Geosmithia* sp. 26 complex, we enforced RNA folding without models (in the ITS2 Database with Mfold). This approach generated a hypothetical structure homologous to other species with four helices and with the presence of specific motif sequences. However, the free energy of this enforced ITS2 secondary structure in the *Geosmithia* sp. 26 complex ranged from -51.7 to -54.7 kcal/mol, which was much higher than that of other species (-70 to -80 kcal/mol) (SUPPLEMENTARY FIGURE II, SUPPLEMENTARY TABLE III).

Despite these factors, the identity of this species complex as a member of the *Geosmithia* genus was verified by sequences of other marker genes. The 28S rDNA sequence of the strain CCF 4324 (HF546232) showed the highest similarity (99%) to various *Geosmithia* species (e.g. *Geosmithia* sp. 21, HF546329), whereas the closest non-*Geosmithia* species were less similar (e.g. 98%, *Emericellopsis terricola*, HQ698592). The *TEF1* gene (CCF 4324, LN907600) of this species showed similarity to various *Geosmithia* species ranging from 96% (*G. langdonii*, HG799876) to 93% (*G. putterillii*, HG799853), whereas it was less than 90% similar to other genera such as *Clonostachys* and *Myrothecium*. The *RPB2* (CCF 4324, LN907604) and *TUB2* sequences (CCF 4324, LN907591) were most similar to *G. langdonii* (94% in the *RPB2*, 87% in *TUB2*), whereas non-*Geosmithia* genera only showed similarities of 75% (*RPB2*) and 82% (*TUB2*) or lower.

***Geosmithia*-beetle-tree specificity.**—*Geosmithia* was isolated from 109 beetle samples (out of the total 126) from 39 plant species infested by 26 species of bark beetles and other subcortical insects (Cerambycidae, Bostrichidae, Buprestidae) (TABLE I, SUPPLEMENTARY TABLE I).

Several bark beetle species appear to associate regularly with *Geosmithia*: fungi in this genus were present in all of their galleries in all sampled populations. In such samples, *Geosmithia* was often visible in the galleries without any magnification after removal of the bark, appearing as a white, yellow, or violet surface in galleries and pupal chambers. The following beetle species appear to be regular *Geosmithia* vectors (only species with more than 3 collections were assessed): *Phloeosinus* spp. and *Cryphalus pubescens* on *Cupressus* and *Sequoia*; *Pityophthorus juglandis* on *Juglans*; *Pseudopityophthorus pubipennis* on *Quercus* and *Notholithocarpus*; *Scolytus rugulosus* on *Amygdalus*

and *Prunus*; and *Scolytus multistriatus* or *schevyrewi* on *Ulmus*. *Geosmithia* was also present in all gallery systems colonized by *Scolytus oregoni* on *Pseudotsuga menziesii*, but only two samples were available.

In the following bark beetle species, *Geosmithia* was isolated from more than half (67-80%) of the sampled galleries, but not from all: *Cactopinus rhois* on *Toxicodendron*; *Hylocurus hirtellus* on *Salix*; *Pityophthorus* spp. on *Pinus*; and *Scolytus praeceps* on *Abies*. A common non-bark beetle wood borer associated with *Geosmithia* is the lead cable borer, *Scobicia declivis* (Col., Bostrichidae), collected on *Umbellularia californica*. *Geosmithia* was found in 72% of galleries of this species, which bores into xylem to oviposit. Its galleries are filled with frass and other detritus.

Insects with the least strict associations, where *Geosmithia* was isolated from 5-50% of galleries, included *Phloeosinus fulgens* on *Calocedrus*, several species on *Pseudotsuga* (*Cryphalus pubescens*, *Carphoborus vandykei*) and several species on *Pinus* (*Orthotomicus latidens*, *Pityogenes knechteli*, *Pseudips mexicanus*). No *Geosmithia* were observed in several other samples from *Calocedrus*, *Pseudotsuga*, and *Pinus* (associated with various bark beetles, Cossoninae spp., Buprestidae spp. and Cerambycidae spp.). We also did not observe *Geosmithia* in any sample from *Picea* (associated with Buprestidae sp., Cerambycidae sp. and an undetermined bark beetle) and *Alnus* (*Hylocurus hirtellus*) (TABLE I, SUPPLEMENTARY TABLE I).

***Geosmithia* community structure.**—The most common *Geosmithia* species isolated from the broadest vector spectrum (beetles on 8-10 host plant genera) were *Geosmithia* sp. 21, *G. putterillii*, *G. lavendula*, *G. langdonii*, *G. flava*, and *Geosmithia* sp. 41. Less widespread species included *Geosmithia* sp. 42 and *G. fassatae* isolated from vectors feeding on four plant genera, and the remaining species were isolated from only one or two plant genera. From these taxa, two species were isolated in higher frequencies: *Geosmithia* sp. 40 appears to be specific to Pinaceae and *G. morbida* was only found on *Juglans*. The substrates of several rarely encountered species (*Geosmithia* sp. 27 and *Geosmithia* sp. 26 from Pinaceae, *Geosmithia* sp. 12 from *Fraxinus*, and *G. ulmacea* from *Ulmus*) agree with host ranges reported from Europe, and these species seem to be restricted to the vectors feeding on these plants. Phylogenetically, Pinaceae-specific *Geosmithia* spp. 33-36 clustered with European species with the same host range, and thus may share the limited vector range typical for this

whole phylogenetic lineage. The same is true for *Geosmithia* sp. 44, which is sister to Pinaceae-specific *Geosmithia* sp. 26 and seems to have the same ecological restriction. In other rare cases we cannot distinguish if these *Geosmithia* spp. are truly species restricted to limited range of vectors and plants, or widespread but undersampled species (FIG. 1, 2, TABLE I, SUPPLEMENTARY TABLE I, IV).

Most of the putatively host-specific *Geosmithia* species are restricted to the vectors feeding on Pinaceae (9 species), or Pinaceae plus *Calocedrus* (2 species). Consequently, *Geosmithia* communities on these hosts were more diverse than those on other host plants. These vectors were associated with at least twice as many *Geosmithia* species as insects on other host plants with similar sample sizes (e.g. compare the number of *Geosmithia* species on *Pinus* (15 associated *Geosmithia* spp.) versus *Quercus* (7 *Geosmithia* spp.), *Pseudotsuga* versus *Cupressus* (4 *Geosmithia* spp.) or *Umbellularia* (4 *Geosmithia* spp.), and *Calocedrus* versus *Ulmus* (3 *Geosmithia* spp.) (TABLE I).

DISCUSSION

The ITS marker in *Geosmithia*.—Although the ITS “fungal DNA barcode” is a suitable marker for species-level identification in most *Geosmithia* species, it is not sufficiently informative in *Geosmithia* sp. 32, *Geosmithia* sp. 36, *G. microcorthyli* (Kolařík and Kirkendall 2009) and *Geosmithia* sp. 24 species complex (Dori-Bachash et al. 2015). Similar phylogenetic conservatism has been reported for other genera such as *Aspergillus* (Geiser et al. 2007) and *Penicillium* (Skouboe et al. 1999). Phylogenetic analysis of ITS data placed *Geosmithia* sp. 26 and sp. 44 on long branches, incongruent with results from protein-coding genes. Paralogy, incomplete lineage sorting, horizontal gene transfer, hybridization, heterogeneity of nucleotide composition, and different rates of evolution (heterotachy) can result in incongruence between single-gene topologies (Feliner and Rosselló 2007; Degnan et al. 2009; Knowles 2009). Numerous copies of rDNA may be present in Ascomycota as paralogous copies, and typically have less than 3% intragenomic variability in ITS region (Simon and Weiss 2008). In comparison to such paralogues, rRNA in pseudogenes mutates without functional constraints, exhibits higher variability in conserved regions, differs in length, and possesses a number of methylation-induced substitutions, which in turn lead to reduced GC content and reduced stability

of the secondary structure that would be ensured normally by GC-rich helices (Buckler-IV et al., 1997; Bailey et al. 2003; Feliner and Rosselló 2007). Limited attention has been paid to the recognition of pseudogenes in fungi, yet they are probably widespread (Rooney and Ward 2005). Species in the *Geosmithia* sp. 26 complex exhibited all of the above-mentioned deviations in the length, GC content, secondary structure stability and sequence variability in rDNA, suggesting the presence of a pseudogene or a deep paralogue. Nevertheless, an alternative rDNA copy was not detected, even when the alternative PCR primers and numerous strains of *Geosmithia* sp. 26 complex were used. Genomic data from *Geosmithia* are necessary to clarify the mechanisms giving rise to such distinctive ITS sequences.

Vector range.— Specificity of associations of *Geosmithia* fungi with particular host tree taxa is similar in Europe and in North America. In general, *Geosmithia* is more common on hardwoods than on most conifers, especially Pinaceae (although *Geosmithia* is moderately abundant in association with beetles from *Pinus*). Our survey demonstrates that *Geosmithia* is regular associate of phloem-feeding bark beetles on hardwoods in the western USA. This association was previously suggested for hardwood-associated bark beetles in California (McPherson et al. 2013, Lynch et al. 2014), Africa, Asia, and Europe (Kolařík et al. 2007, 2008; Machingambi et al. 2014).

Interestingly, bark beetles specialized on Cupressaceae (e.g., *Sequoia* and *Cupressus*) have the same regular association with *Geosmithia* and carry the same set of *Geosmithia* species as beetles on most hardwoods. This similarity between hardwoods and Cupressaceae, but not Pinaceae, has been reported from Europe previously (Kolařík et al. 2007, 2008). Only one genus in the Cupressaceae – *Calocedrus* – did not conform to this pattern. Instead, beetles from *Calocedrus* (i.e., *Phloeosinus fulgens*) carried *Geosmithia* species identical to those on beetles living on trees from the Pinaceae. The reason for this is not clear, but it may reflect the geographic overlap of the *Calocedrus* and Pinaceae that we sampled. All of our *Calocedrus* localities were located in higher elevations (above 1000 m) in forests dominated by *Pinus* and *Pseudotsuga*.

In the Pinaceae, namely in *Pinus* and *Picea*, *Geosmithia* appear to be associated primarily with beetles feeding under thin bark, such as on branches. In contrast, bark beetles that prefer moist substrate covered by thick bark (e.g., the trunk) more frequently carry ophiostomatoid fungi (Kolařík

and Jankowiak 2013; Jankowiak et al. 2014). Similarly, among conifers in the western USA, *Pinus*-infesting bark beetles (*Pityophthorus* spp.) collected from twigs and small diameter branches had the strongest association with *Geosmithia* (present in 75-100% of gallery systems). Less frequent association, where *Geosmithia* occupied fewer than 30-50% galleries, was found in *Orthotomicus latidens*, *Pityogenes knechteli*, or *Pseudips mexicanus*, which prefer to colonize stems and larger diameter branches (Bright and Stark 1973; Wood 1982).

Abies was sampled only sporadically in our study. Wright (1938) reported *Geosmithia* fungi (there identified as *Spicaria anomala*, SUPPLEMENTARY MATERIAL I) from *Scolytus subscaber* and *S. praeceps* from *Abies concolor* in California, and suggested that this fungus kills the *Abies* cambium. Wright concluded *Geosmithia* was a predominant associate of *S. praeceps* and *S. subscaber* as it was isolated from 72-89% of emerging adults or gallery systems (SUPPLEMENTARY MATERIAL I). We confirmed abundant occurrence of *Geosmithia* on *S. praeceps* from *Abies concolor*, albeit with only a single collection. Wright (1938) further reports absence of *Geosmithia* in *Scolytus ventralis* galleries, concordant with our observations. This raised the question: is there a similar pattern in the frequency of *Geosmithia* association in *Abies*-infesting bark beetles, as was observed in *Pinus* and *Piceae* (see above)? *Scolytus praeceps*, *S. subscaber*, and the fir-invading bark beetle *Cryphalus piceae* from Europe (Jankowiak and Kolařík 2010) have same strong association with *Geosmithia*, and both prefer branches and thin-barked portions of the trunk (Bright and Stark 1973; Pfeffer 1995). Contrary to that, *Scolytus ventralis* attack trunks that are at least 10 cm in diameter (Bright and Stark 1973). This suggests some link between beetle ecology and the degree of its association with *Geosmithia*, as in associates of *Pinus* and *Picea*. Nevertheless, the degree of the association of fir-infesting bark beetles with *Geosmithia* needs to be investigated through a greater sampling effort.

Our study confirms that Bostrichidae are also effective vectors of *Geosmithia* in the western USA, as they are in the Mediterranean basin (Kolařík et al. 2007). In contrast, wood borers in the beetle families Buprestidae, Cerambycidae or Cossoninae were not associated with *Geosmithia* in our survey of the western USA. This suggests that *Geosmithia* is vectored by insects that enter the phloem or xylem as adults and reproduce within the tree hosts (Scolytinae, Bostrichidae), but not by wood

borers where adults do not enter the tissues and only oviposit from the bark surface (Buprestidae, Cerambycidae, Curculionidae sensu lato).

Specificity to the tree species and microhabitat.—Although the majority of *Geosmithia* species isolated during this and previous studies were found only in bark beetle galleries, several species appear to be generalists with the potential to live also outside of bark beetle galleries: specifically, *G. flava*, *G. putterillii*, *Geosmithia* sp. 23, *G. langdonii* and *G. lavendula* (TABLE I, SUPPLEMENTARY TABLE IV). *Geosmithia langdonii* was found to be a bark or ambrosia beetle associate, as well as an endophyte on the same oak trees (McPherson et al. 2013). These globally distributed generalists, together with *Geosmithia* sp. 21 and 41, were the most frequent *Geosmithia* in our U.S. material, recovered from numerous vectors. How the life cycles of the environmental generalists and specialists differ is unknown. Many of the generalists may live as plant endophytes as reported for *G. langdonii* (McPherson et al. 2013).

Geosmithia specialists are species recorded repeatedly from a very limited vector and tree species range. Examples include *Geosmithia ulmacea* (bark beetles on *Ulmus*), *Geosmithia* sp. 12 (*Fraxinus*), *G. morbida* (*Juglans*) and *Geosmithia* sp. 31, 27, and 26 (bark beetles specific to Pinaceae), which show the same high specificity in the USA as in Europe (FIG. 1, SUPPLEMENTARY TABLE IV). *Geosmithia* species specific to Pinaceae in the USA belong to several phylogenetic lineages that have been recognized as pine-specialists in Europe (Kolařík and Jankowiak 2013) or belong to new phylogenetic lineages of a Pinaceae specialists (e.g., the red-spored *Geosmithia* sp. 40).

***Geosmithia morbida* host range.** The phytopathogenic species *G. morbida* was found to be associated with a single beetle species, *Pityophthorus juglandis*. Four additional *Geosmithia* species were found frequently associated with this beetle. The apparent dependence of *G. morbida* on insects from *Juglans* (represented here only by *P. juglandis*) is an encouraging finding from a phytosanitary perspective, since it is unlikely that non-*Juglans* host trees (besides *Pterocarya*) and their associated insects represent a source of *G. morbida* inoculum. Although *G. morbida* potentially can be vectored passively by scolytids with broad host ranges such as *Xyleborinus saxeseni* (Tisserat et al. 2009) or *Hylocurus hirtellus* (Dallara et al. 2012) emerging from infected black walnut trees, this specialized

fungus does not occur significantly on the other hosts. *Geosmithia morbida* can be recognized easily on MEA by velvety, yellowish (sometimes turning to salmon-colored shades), sporulating colonies with lobate margins (often with yellowish pigment diffusing into the medium), by the ability to grow at 37 C, and the absence of growth on CDA (Kolařík et al. 2011). The only other species in our western American sampling with velvety, yellowish and lobate colonies on MEA was *Geosmithia ulmacea*, but it grows more slowly, does not grow at 37 C, and does grow on CDA (Pepori et al. 2015). We conclude that *G. morbida* has a unique phenotype amongst the *Geosmithia* spp. of the western USA and can be easily diagnosed.

***Geosmithia* biogeography.** Over ten years of *Geosmithia* research combining molecular identification and diverse substrate sampling enabled us to summarize the biogeography, vector affinity, and host range of this genus in the Northern Hemisphere (SUPPLEMENTARY TABLE IV). Twelve of 29 species reported from the USA have not yet been found outside of this region. Among them, *Geosmithia* sp. 41 was the most frequently isolated species in the western USA. Our analysis also showed that the previously reported occurrence of *G. pallida* s. s in the USA, thought to cause foamy bark canker (Lynch et al. 2014), is incorrect. *Geosmithia pallida* was absent among our samples, which included galleries from oaks and western oak bark beetles (*Pseudopityophthorus pubipennis*), and the sequences deposited by Lynch et al. (2014) are identical to *Geosmithia* sp. 41 (e.g., HF546292 with KJ468687).

Surprisingly, some of the most abundant, generalist species of *Geosmithia* taxa are not shared between North America and Eurasia. For example, *G. omnicola* (*Geosmithia* sp. 10) and *Geosmithia* sp. 1, both widespread in Eurasia and Africa, were not recovered in the U.S. samples (FIG. 1, SUPPLEMENTARY TABLE IV). Even though our sampling was limited, it appears that some aspect of the ecology of the generalist *Geosmithia* species may have enforced geographic isolation between the continents.

This contrasts with the intercontinental similarity of *Geosmithia* specialists (e.g., *G. morbida*, *G. ulmacea*, and *Geosmithia* sp. 12, 26, 27 and 31), a phenomenon that may reflect the relative uniformity of the bark beetle fauna in the Holarctic region in terms of genera, but not in terms of species or populations (Horn et al. 2006, 2009, Painter et al. 2007, Jordal and Kambestad 2014).

Various *Geosmithia* specialists may have coevolved with their beetle vectors in parallel in Nearctic and Palearctic regions since the split of various beetle genera. A second factor that may have contributed to the similarity of *Geosmithia* specialists between the two continents is the increasing level of homogenization of bark beetle fauna around the world, and the corresponding homogenization of their fungal associates (Brasier 2000, Harrington et al. 2011, Montecchio and Faccoli 2014). We do not know for sure which *Geosmithia* species are native to North America and which are recently established. In most cases, immigrant fungi appear to lose strict specificity and join the local pool of bark beetle-associated fungi (Jacobi et al. 2007, Kim et al. 2011, Taerum et al. 2013, Kostovčík et al. 2015, Carrillo et al., 2014). Both scenarios -- long-term maintenance of specificity from the time prior to the split of vector lineages, as well as a series of recent introductions -- point to the fact that *Geosmithia* specialists maintain conservative associations with their vectors regardless of the region.

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FOOTNOTES

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SUPPLEMENTARY FIG. I. Maximum likelihood phylogenetic tree constructed in PhyML 3.0 (Guindon 2010) showing phylogeny of *Geosmithia* isolates obtained during this study and all other *Geosmithia* published species. Numbers at internal nodes are maximum likelihood bootstrap supports. The alignment of ITS sequences was generated in MAFFT 6 (Kato and Toh 2008) and consisted of 418 characters from which 127 were variable and 96 parsimony informative. The *Acremonium alternatum* branch and branch leading to the *Geosmithia* sp. 26 complex are artificially shortened to one-fifth of the actual length. Species numbering is from Kolařík et al. (2007, 2008) and Kolařík and Jankowiak (2013). Additional information about the strains is provided in SUPPLEMENTARY TABLE II.

SUPPLEMENTARY FIG. II. Secondary structure of the *Geosmithia* sp. 26 ITS2 rDNA compared with five other *Geosmithia* species. The structures were modeled in Mfold (*Geosmithia* sp. 26) and the ITS Database (other species).

SUPPLEMENTARY TABLE I. Complete list of the samples analyzed and their association with *Geosmithia*.

SUPPLEMENTARY TABLE II. List of the isolates analyzed, their taxonomic identity and EMBL sequence accession codes. The list includes 134 isolates obtained during this study and 26 isolates from the *G. pallida* complex, *Geosmithia langdonii*, *Geosmithia* sp. 26, and *Geosmithia* sp. 32 from various sources, used for comparison. This set of sequences was used in the phylogenetic analyses (ITS and multigene dataset).

SUPPLEMENTARY TABLE III. Summary of the nucleotide composition; length of ITS segments; and free energy of the ITS2 secondary structure across all *Geosmithia* species.

SUPPLEMENTARY TABLE IV. Overview of area and host range of all published *Geosmithia* spp.

SUPPLEMENTARY MATERIAL I. Identity of the fungus published by Wright (1938) as *Spicaria anomala*.

| | | | | | | | | | |
|-------------------------------|--|--|-----|-----|----|-----|-----|-----|----|
| <i>S. sempervirens</i> | <i>Cerambycidae</i> sp., <i>Phloesinus</i> | CA, Jack London State historic park, 38°20'51.2"N 122°33'44.0"W | 100 | | | 7 | 100 | | |
| <i>S. sempervirens</i> | <i>Cryphalus pubescens</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | 80 | 53 | | 15 | 100 | | |
| <i>S. sempervirens</i> | <i>Phloesinus sequoiae</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | 100 | | | 15 | 100 | | |
| <i>S. sempervirens</i> | <i>Phloesinus sequoiae</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | | 100 | | 10 | 100 | | |
| <i>S. sempervirens</i> | <i>Phloesinus sequoiae</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | 100 | | 7 | 8 | 100 | | |
| <i>S. sempervirens</i> | <i>Phloesinus sequoiae</i> | CA, Salt Poin, 38°33'59.4"N 123°19'51.5"W | | 67 | 67 | 15 | 100 | | |
| <i>S. sempervirens</i> | <i>Buprestidae</i> sp., <i>Cerambycidae</i> sp. | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | 100 | | | 7 | 100 | | |
| Gymnosperms - Pinaceae | | | | | | | | | |
| <i>Abies concolor</i> | <i>Scolytus praeceps</i> | CA, Grover Hot Springs SP, 38°41'46.5"N 119°50'41.0"W | | | 7 | 87 | 15 | 87 | |
| <i>Abies concolor</i> | <i>Scolytus ventralis</i> | CA, Jack London State historic park, 38°20'51.2"N 122°33'44.0"W | | | | | 10 | 0 | |
| <i>Picea pungens</i> | <i>Buprestidae</i> sp., <i>Cerambycidae</i> sp. | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | | | | 10 | 0 | |
| <i>Picea pungens</i> | <i>Scolytinae</i> sp. | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | | | | 10 | 0 | |
| <i>P. angustifolia</i> | <i>Orthotomicus latidens</i> | CA, Jonkinson lake, 38°42'52.9"N 120°33'44.5"W | | | | | 10 | 0 | |
| <i>Pinus angustifolia</i> | <i>Orthotomicus latidens</i> , | CA, Union Dam, 38°54'16.9"N 120°22'36.6"W | | 67 | | | 15 | 67 | |
| <i>P. longaeva</i> | <i>Pityophthorus</i> sp. | CA, White Mts., Ancient Bristlecone Pine Forest, 37°23'09.8"N 118°10'45.8"W | 53 | 100 | | | 15 | 100 | |
| <i>P. monophylla</i> | <i>Pityophthorus</i> sp. | CA, White Mts., 37°18'06.8"N 118°10'07.7"W | 7 | 100 | | | 15 | 100 | |
| <i>P. muricata</i> | <i>Pityophthorus</i> sp. | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | 20 | | | 7 | 15 | 20 | |
| <i>P. muricata</i> | <i>Pityophthorus</i> sp. | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | | | 53 | 100 | 15 | 100 | |
| <i>P. nigra</i> | <i>Pityophthorus</i> sp. | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | 80 | | | 10 | 80 | |
| <i>P. ponderosa</i> | <i>Pityophthorus</i> sp. | CA, Salt Poin, 38°33'59.4"N 123°19'51.5"W | | | | 100 | 10 | 100 | |
| <i>P. ponderosae</i> | <i>Orthotomicus latidens</i> , | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | 38 | | | | 8 | 38 | |
| <i>P. ponderosae</i> | <i>Pityophthorus</i> sp. | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | | | | 10 | 50 | |
| <i>P. ponderosae</i> | <i>Pityogenes knechteli</i> , | CO, Rocky Mts., Roosevelt National Forest, Donner pass, 40° 32' 21.86"N 105° 28' 8.98" W | 33 | 20 | | 13 | 7 | 15 | 53 |

| | | | | | | | | | |
|------------------------|---|--|-----|-----|----|-----|----|----|-----|
| <i>P. ponderosae</i> | <i>Pityogenes knechteli</i> | CO, Rocky Mts., Roosevelt National Forest, 40°27'35.6"N 105°25'18.9"W | | 13 | | | | 15 | 13 |
| <i>P. ponderosae</i> | <i>Pityophthorus</i> sp. | CO, Rocky Mts., Roosevelt National Forest, 40°27'35.6"N | 40 | | 33 | | | 15 | 53 |
| <i>P. radiata</i> | <i>Ips plastographus</i> | CA, Monterey, 36° 35' 21.27" N121° 57' 35.24" W | 100 | | | 33 | | 15 | 100 |
| <i>P. radiata</i> | <i>Pseudips mexicanus</i> , <i>Pityophthorus</i> sp. | CA, Monterey, 36° 35' 21.27" N121° 57' 35.24" W | | | | 100 | | 10 | 100 |
| <i>P. sabineana</i> | Buprestidae sp., Cerambycidae sp. | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | 100 | | | 50 | | 4 | 100 |
| <i>P. ponderosae</i> | Cerambycidae sp. | CO, Rocky Mts., Roosevelt National Forest, 40°27'35.6"N 105°25'18.9"W | | | | | | 10 | 0 |
| <i>P. angustifolia</i> | Buprestidae sp., Cerambycidae sp. | CA, Jonkinson lake, 38°42'52.9"N 120°33'44.5"W | | | | | | 3 | 0 |
| <i>P. sabineana</i> | <i>Orthotomicus spinifer</i> , <i>Pityophthorus</i> sp. | CA, Cache creek Canyon, 38°54'31.3"N 122°18'45.8"W | | 100 | | | | 15 | 100 |
| <i>P. sabineana</i> | <i>Pityophthorus</i> sp. | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | | | | | 47 | 15 | 47 |
| <i>P. sabiniana</i> | <i>Pityophthorus</i> sp. | CA, Alder Creek (Alta Sierra) - USFS campground, 35°43'11.3"N 118°36'43.2"W | 33 | 80 | 7 | 40 | | 15 | 100 |
| <i>P. sabiniana</i> | <i>Pityophthorus</i> sp. | CA, Bodfish, Miracle hot Springs, 35°35'53.1"N 118°30'10.9"W | | | | | 80 | 15 | 80 |
| <i>P. radiata</i> | <i>Pseudips mexicanus</i> , <i>Pityophthorus</i> sp. | CA, Monterey, 36° 35' 21.27" N121° 57' 35.24" W | | | | | | 10 | 0 |
| <i>P. radiata</i> | Cossoninae sp. | CA, Monterey, 36° 35' 21.27" N121° 57' 35.24" W | | | | | | 5 | 0 |
| <i>P. radiata</i> | Cossoninae sp. | CA, Monterey, 36° 35' 21.27" N121° 57' 35.24" W | | | | | | 3 | 0 |
| <i>Ps. menziesii</i> | <i>Hylesinus californicus</i> , <i>Scolytus reflexus</i> | CO, Rocky Mts., Roosevelt National Forest, 40°27'35.6"N 105°25'18.9"W | | | | | | 10 | 0 |
| <i>Ps. menziesii</i> | Buprestidae sp., Cerambycidae sp. | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | | | | | | 10 | 0 |
| <i>Ps. menziesii</i> | <i>Pseudohylesinus nebulosus</i> | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | | | | | | 10 | 0 |
| <i>Ps. menziesii</i> | <i>Carphoborus vandykei</i> | CA, Jonkinson lake, 38°42'52.9"N 120°33'44.5"W | | | | | | 15 | 0 |
| <i>Ps. menziesii</i> | Cerambycidae sp., <i>Carphoborus vandykei</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | | | | | 33 | 4 | 33 |

| | | | | | | | | | |
|---------------------------------------|--|--|-----|----|----|----|----|----|-----|
| <i>Ps. menziesii</i> | Buprestidae sp., Cerambycidae sp. | CA, Lodi, Armstrong, 38° 520.42°N 121°16'25.30"W | | | | 13 | | 8 | 13 |
| <i>Ps. menziesii</i> | Buprestidae sp., Cerambycidae sp. | CA, Bohemian river, 38° 27' 18.64°N 123° 0' 13.89"W | | | | | | 10 | 0 |
| <i>Pseudotsuga menziesii</i> | Buprestidae sp., Cerambycidae sp. | CA, Lodi, Armstrong, 38° 520.42°N 121°16'25.30"W | 100 | | 50 | | | 8 | 100 |
| <i>Ps. menziesii</i> | <i>Cryphalus pubescens</i> | CA, Fort Ross, 38°30'51.3"N 123°14'20.7"W | 87 | | | | | 15 | 87 |
| <i>Ps. menziesii</i> | <i>Cryphalus pubescens</i> | CA, Fort Ross, 38°30'51.3"N 123°14'20.7"W | 53 | | | | | 15 | 53 |
| <i>Ps. menziesii</i> | <i>Cryphalus pubescens</i> | CA, Fort Ross, 38°30'51.3"N 123°14'20.7"W | | | | | | 10 | 0 |
| <i>Ps. menziesii</i> | <i>Cryphalus pubescens</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | 27 | 20 | | 7 | | 15 | 80 |
| <i>Ps. menziesii</i> | Scolytinae sp. | CA, Union Dam, 38°54'16.9"N 120°22'36.6"W | | | | | 33 | 15 | 33 |
| <i>Ps. menziesii</i> | <i>Scolytus oregoni</i> | CA, Bohemian river, 38° 27' 18.64°N 123° 0' 13.89"W | 100 | | | | | 8 | 100 |
| <i>Ps. menziesii</i> | <i>Pityophthorus</i> sp., <i>Scolytus oregoni</i> , <i>Cryphalus pubescens</i> | CO, Rocky Mts., Cameron Pass, 40°31'15"N 105°53'33"W | 40 | | 7 | | 20 | 7 | 15 |
| Angiosperms - Anacardiaceae | | | 63 | 75 | | | | 8 | 100 |
| <i>Pistatia vera</i> | <i>Scobicia</i> sp. | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | 40 | | | | | 10 | 40 |
| <i>Toxicodendron diversilobum</i> | <i>Cactopinus rhois</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | 100 | | | | | 15 | 100 |
| <i>T. diversilobum</i> | <i>Cactopinus rhois</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | | | | | 20 | 5 | 20 |
| <i>T. diversilobum</i> | Bostrichidae sp. | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | | | | | | | |
| Angiosperms - Asteraceae | | | | | | | 10 | 10 | 10 |
| <i>Artemisia arborea</i> | Cossoninae sp. | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | 7 | | | | | 15 | 7 |
| <i>Baccharis pilularis</i> | Bostrichidae sp. | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | | | | | | | |
| Angiosperms - Ericaceae | | | 50 | | | | | 6 | 50 |
| <i>Vaccinium ovatum</i> | xylophagous bark beetle, old galleries | CA, Salt Poin, 38°33'59.4"N 123°19'51.5"W | | | | | | | |

Angiosperms - Fagaceae

| | | | | | | | |
|-------------------------------------|---------------------------------------|--|-----|-----|-----|----|-----|
| <i>Notholithocarpus densiflorus</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Lodi, Armstrong, 38° 520.42"N 121°16'25.30"W | 53 | | 60 | 15 | 100 |
| <i>N. densiflorus</i> | <i>P. pubipennis</i> | CA, Lodi, Armstrong, 38° 520.42"N 121°16'25.30"W | | 100 | | 6 | 100 |
| <i>N. densiflorus</i> | <i>P. pubipennis</i> | CA, Jack London State historic park, 38°20'51.2"N 122°33'44.0"W | 33 | | 100 | 15 | 100 |
| <i>N. densiflorus</i> | <i>P. pubipennis</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | 100 | 33 | | 10 | 100 |
| <i>Quercus acrifolia</i> | <i>P. pubipennis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | | | 100 | 15 | 100 |
| <i>Q. acrifolia</i> | <i>P. pubipennis</i> | CA, Fairfield Osborn Preserve, 38° 20' 36.17" N 122° 35' 41.24" W | 40 | | 27 | 15 | 100 |
| <i>Q. acrifolia</i> | <i>Monarthrum</i> | CA, Fairfield Osborn Preserve, 38° | 67 | | 47 | 15 | 100 |
| <i>Q. acrifolia</i> | <i>P. pubipennis</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | 60 | 80 | 7 | 15 | 100 |
| <i>Q. acrifolia</i> | <i>P. pubipennis</i> | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | 80 | 87 | 33 | 15 | 100 |
| <i>Q. berberidifolia</i> | <i>P. pubipennis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N | 100 | | | 15 | 100 |
| <i>Q. berberidifolia</i> | <i>P. pubipennis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N | 47 | | 100 | 15 | 100 |
| <i>Q. berberidifolia</i> | <i>P. pubipennis</i> | CA, Lodi, Armstrong, 38° 520.42"N 121°16'25.30"W | | | 100 | 15 | 100 |
| <i>Q. berberidifolia</i> | Buprestidae sp. | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | | | 66 | 3 | 66 |
| <i>Q. kelloggii</i> | <i>P. pubipennis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N | | | 100 | 15 | 100 |
| <i>Q. kelloggii</i> | <i>P. pubipennis</i> | CA, Lodi, Armstrong, 38° 520.42"N 121°16'25.30"W | | | 100 | 15 | 100 |
| <i>Quercus</i> sp. | <i>P. pubipennis</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | 53 | | 67 | 15 | 100 |
| <i>Quercus</i> sp. | <i>P. pubipennis</i> | CA, Eldorado National Forest, | | | 100 | 15 | 100 |
| <i>Quercus</i> sp. | <i>P. pubipennis</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | | | 100 | 15 | 100 |

Angiosperms - Juglandaceae

| | | | | | | | |
|--------------------------|--------------------------------|--|----|-----|----|----|-----|
| <i>Juglans australis</i> | <i>Pityophthorus juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), | 60 | 100 | | 5 | 100 |
| <i>J. californica</i> | <i>P. juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | 80 | | | 10 | 80 |
| <i>J. hindsii</i> | <i>P. juglandis</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | | | 53 | 15 | 53 |

| | | | | | | | | | |
|---------------------------------|----------------------------|--|----|-----|-----|-----|----|----|-----|
| <i>J. hindsii</i> | <i>P. juglandis</i> | CA, Davis, Putah creek, 38°31'1.03"N, 122°12'49.68"W | | | 100 | | | 15 | 100 |
| <i>J. hindsii</i> | <i>P. juglandis</i> | CA, Davis, Putah creek, | | | 87 | | | 15 | 87 |
| <i>J. hindsii</i> | <i>P. juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), | 50 | | | 100 | | 10 | 100 |
| <i>J. nigra</i> | <i>P. juglandis</i> | CA, Rio Oso, 38°57'40.5"N | | | 100 | | | 3 | 100 |
| <i>J. nigra</i> | <i>P. juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), | 25 | 25 | 63 | | | 8 | 100 |
| <i>Juglans sp.</i> | <i>P. juglandis</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | | | 80 | | | 10 | 80 |
| Angiosperms - Lauraceae | | | | | | | | | |
| <i>Umbellularia</i> | <i>Scobicia declivis</i> | CA, Winters, Lake Beryessa, Quail | | | | | 13 | 15 | 13 |
| <i>U. californica</i> | <i>S. declivis</i> | CA, Lodi, Armstrong, 38° 52'0.42"N 121°16'25.30"W | | 50 | | | | 10 | 50 |
| <i>U. californica</i> | <i>S. declivis</i> | CA, Lodi, Armstrong, 38° 52'0.42"N 121°16'25.30"W | 70 | | | | | 10 | 70 |
| <i>U. californica</i> | <i>S. declivis</i> | CA, Fairfield Osborn Preserve, 38° 20' 36.17" N 122° 35' 41.24" W | | 70 | | | 80 | 10 | 100 |
| <i>U. californica</i> | <i>S. declivis</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | | | 100 | | | 15 | 100 |
| <i>U. californica</i> | <i>S. declivis</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | 67 | | 80 | | | 15 | 100 |
| Angiosperms - Oleaceae | | | | | | | | | |
| <i>Fraxinus sp.</i> | <i>Hylesinus oregonus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | | 80 | | | 10 | 80 |
| Angiosperms - Salixaceae | | | | | | | | | |
| <i>Salix sp.</i> | <i>Hylocurus hirtellus</i> | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | 50 | | 30 | | | 10 | 50 |
| <i>Salix sp.</i> | <i>Hylocurus hirtellus</i> | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | | 100 | | | | 15 | 100 |
| <i>Salix sp.</i> | Cerambycidae sp. | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | | | 80 | | | 5 | 80 |
| Angiosperms - Rosaceae | | | | | | | | | |
| <i>Amygdalus communis</i> | <i>Scolytus rugulosus</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | | | 100 | | | 15 | 100 |
| <i>A. communis</i> | <i>Scolytus rugulosus</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | 33 | | 100 | | 20 | 15 | 100 |
| <i>Heteromeles</i> | <i>Pseudopityophthor</i> | CA, Winters, Lake Beryessa, Quail | | | | | 80 | 15 | 80 |
| <i>Prunus persica</i> | <i>Scolytus rugulosus</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | | | 100 | | | 15 | 100 |

| | | | | | | |
|----------------------|--|---|-----|-----|-----|--------|
| <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CA, Davis, Putah creek, 38°31'1.03"N, 122°12'49.68"W | 20 | 100 | 15 | 100 |
| <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> , <i>Pseudothysanoes hopkinsi</i> | CA, Lodi, Armstrong, 38° 520.42"N 121°16'25.30"W | 80 | 20 | 15 | 100 |
| <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CA, Lodi, Armstrong, 38° 520.42"N 121°16'25.30"W | 100 | 40 | 15 | 100 |
| <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | | 100 | 10 100 |
| <i>P. virginiana</i> | <i>Scolytus rugulosus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | | 100 | 10 100 |

Angiosperms - Ulmaceae

| | | | | | | |
|---------------------|-------------------------------|---|--|-------|----|-----|
| <i>Ulmus pumila</i> | <i>Scolytus multistriatus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | 67 87 | 15 | 100 |
| <i>Ul. pumila</i> | <i>Scolytus schevyrewi</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | 100 | 15 | 100 |
| <i>Ul. pumila</i> | <i>Scolytus schevyrewi</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | 100 | 6 | 100 |
| <i>Ulmus</i> sp. | <i>Scolytus schevyrewi</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | "+" | | |

Angiosperms - Betulaceae

| | | | | | | |
|--------------------|----------------------------|---|--|--|----|---|
| <i>Alnus rubra</i> | <i>Hylocurus hirtellus</i> | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | | | 10 | 0 |
|--------------------|----------------------------|---|--|--|----|---|

Angiosperms - others

| | | | | | | |
|-------------------|---------------------------------------|---|----|--|----|----|
| broadleaved shrub | Bostrichidae sp., xylophagous bark | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | 80 | | 10 | 80 |
|-------------------|---------------------------------------|---|----|--|----|----|

Incidence across all samples^a 4 12 12 14 17 4 2 1 0 3 19 1 1 1 2 1 1 1 2 1 1 1 2 2 5 17 4 1 3 1480

Supplementary table 2. List of the isolates used in the study, their taxonomic identity and EMBL sequence accession codes. The list includes 134 isolates isolated and sequenced during this study and 26 isolates from *G. pallida* species complex, *Geosmithia langdonii*, *Geosmithia* sp. 26 and *Geosmithia* sp. 32 from various sources, which were sequenced for comparison. Additional published isolates were used in the phylogenetic analyses (ITS rDNA and multigene datasets). Species numbering is according to Kolařík et al. (2008) and Kolařík et Jankowiak (2013). The EMBL accession codes generated during this study are printed in bold.

Title of the paper: Geosmithia associated with bark beetles and woodborers in the western USA: Taxonomic diversity and vector specificity

Authors: Kolařík M., Hulcr J., Tisserat N., De Beer W., Kostovčík M., Kolaříková Z., Seybold S. J., Rizzo D. M.

| Species | Collection no. | Host plant or Reference | Vector species | Locality | Sequence Accession No. | | | | Reference |
|-------------------------------|----------------------------------|---------------------------------|--------------------------------|--|------------------------|----------------|------|------|------------------------------|
| | | | | | ITS rDNA | TEF-1 α | RPB2 | Tub2 | |
| <i>G. cnesini</i> | CCF 3753 | viz reference | | | AM947670 | | | | Kolařík et Kirkendall (2010) |
| <i>G. cnesini</i> | CCF 4292 | viz reference | | | AM947671 | | | | Kolařík et Kirkendall (2010) |
| <i>G. eupagioceri</i> | CCF 3754 | viz reference | | | AM947666 | | | | Kolařík et Kirkendall (2010) |
| <i>G. fassatae</i> (=sp. 14) | CCF 3334 ^T (=AK14/93) | viz reference | | | AJ578482 | | | | Kolařík et al. (2005) |
| <i>G. fassatae</i> (=sp. 14) | AK31/98 | viz reference | | | AM421039 | | | | Kolařík et al. (2008) |
| <i>G. fassatae</i> (= sp. 14) | CCF 4331 (=U406) | <i>Pinus sabiniana</i> | <i>Pityophthorus</i> sp. | CA, Alder Creek (Alta Sierra) - USFS campground, 35°43'11.3"N 118°36'43.2"W | HF546239 | | | | this study |
| <i>G. fassatae</i> (= sp. 14) | CCF 4340 (=U72), U140 | <i>Salix</i> sp. | <i>Hylocurus hirtellus</i> | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | HF546247 | | | | this study |
| <i>G. fassatae</i> (=sp. 14) | U140 | <i>Salix</i> sp. | <i>Hylocurus hirtellus</i> | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | HF546272 | | | | this study |
| <i>G. fassatae</i> (= sp. 14) | U138 | <i>Umbellularia californica</i> | <i>Scobicia declivis</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | HF546270 | | | | this study |
| <i>G. fassatae</i> (= sp. 14) | U146 | <i>Umbellularia californica</i> | <i>Scobicia declivis</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | HF546275 | | | | this study |
| <i>G. fassatae</i> (= sp. 14) | U50 | <i>Umbellularia californica</i> | <i>Scobicia declivis</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546338 | | | | this study |
| <i>G. flava</i> (sp. 7) | CCF 3333 ^T (=MK101) | viz reference | | | AJ578483 | | | | Kolařík et al. (2004) |
| <i>G. flava</i> (=sp. 7) | MK1602b | viz reference | | | AM181457 | | | | Kolařík et al. (2007) |
| <i>G. flava</i> (= sp. 7) | U302 | <i>Cyperus groverianus</i> | <i>Phloeosinus cupressi</i> | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546221 | | | | this study |
| <i>G. flava</i> (= sp. 7) | CCF 4195 (=U303) | <i>Cyperus groverianus</i> | <i>Phloeosinus cupressi</i> | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546302 | | | | this study |
| <i>G. flava</i> (= sp. 7) | CCF 4602 (=U326) | <i>Juglans nigra</i> | <i>Pityophthorus juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546317 | | | | this study |

| | | | | | | |
|--------------------------------|-----------------------|-----------------------------------|---------------------------------------|--|----------|------------------------------|
| <i>G. lavendula</i> (= sp. 18) | CCF 3394 | viz reference | | | AM421098 | Kolafik et al. (2007) |
| <i>G. lavendula</i> (= sp. 18) | U11 | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CA, Davis, Putah creek, 38°31'1.03"N, 122°12'49.68"W | HF546257 | this study |
| <i>G. lavendula</i> (= sp. 18) | U114 | <i>Quercus acrifolia</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | HF546261 | this study |
| <i>G. lavendula</i> (= sp. 18) | U336 | <i>J. hindsii</i> | <i>Pityophthorus juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546320 | this study |
| <i>G. lavendula</i> (= sp. 18) | CCF 4325 (=U30) | <i>A. communis</i> | <i>Scolytus rugulosus</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | HF546233 | this study |
| <i>G. lavendula</i> (= sp. 18) | U325 | <i>J. nigra</i> | <i>Pityophthorus juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546316 | this study |
| <i>G. lavendula</i> (= sp. 18) | CCF 4335 (=U42) | <i>Toxicodendron diversilobum</i> | <i>Cactopinus rhois</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546243 | this study |
| <i>G. lavendula</i> (= sp. 18) | CCF 5268 (=U337) | <i>J. californica</i> | <i>Pityophthorus juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546321 | this study |
| <i>G. lavendula</i> (= sp. 18) | U340 | <i>Pistatia vera</i> | <i>Scobicia</i> sp. | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546322 | this study |
| <i>G. lavendula</i> (= sp. 18) | CCF 4593 (=U405) | <i>P. sabiniana</i> | <i>Pityophthorus</i> sp. | CA, Alder Creek (Alta Sierra) - USFS campground, 35°43'11.3"N 118°36'43.2"W | HF546326 | this study |
| <i>G. lavendula</i> (= sp. 18) | U43 | <i>Toxicodendron diversilobum</i> | <i>Cactopinus rhois</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546335 | this study |
| <i>G. lavendula</i> (= sp. 18) | U44 | <i>Q. berberidifolia</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546336 | this study |
| <i>G. lavendula</i> (= sp. 18) | U53 | <i>Pseudotsuga menziesii</i> | Buprestidae sp., Cerambycidae sp. | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546339 | this study |
| <i>G. lavendula</i> (= sp. 18) | CCF 5249 (=U55) | <i>Pinus sabiniana</i> | Buprestidae sp., Cerambycidae sp. | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546341 | this study |
| <i>G. microcorthyli</i> | CCF 3864 ^T | viz reference | | | FM986798 | Kolafik et Kirkendall (2010) |
| <i>G. morbida</i> | CCF 3880 | viz reference | | | FN434072 | Kolafik et al. (2011) |
| <i>G. morbida</i> | CCF 4010 | viz reference | | | FN434077 | Kolafik et al. (2011) |
| <i>G. morbida</i> | CBS 124664 | viz reference | | | FN434081 | Kolafik et al. (2011) |
| <i>G. morbida</i> | CBS 124663 | viz reference | | | FN434082 | Kolafik et al. (2011) |
| <i>G. morbida</i> | CCF 4576 (=U173) | <i>Juglans nigra</i> | <i>Pityophthorus juglandis</i> | CA, Rio Oso, 38°57'40.5"N 121°32'32.8"W | HF546282 | this study |

| | | | | | | | | | |
|---------------------------------|--|--|--------------------------------|--|-----------------|-----------------|-----------------|-----------------|-----------------------------------|
| <i>G. morbida</i> | U170 | <i>Juglans nigra</i> | <i>Pityophthorus juglandis</i> | CA, Rio Oso, 38°57'40.5"N 121°32'32.8"W | HF546283 | | | | this study |
| <i>G. morbida</i> | U324 | <i>Juglans nigra</i> | <i>Pityophthorus juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546315 | | | | this study |
| <i>G. obscura</i> (=sp. 17) | CCF 3422 ^T | viz reference | | | AJ784999 | | | | Kolařík et al. (2005) |
| <i>G. obscura</i> (=sp. 17) | CCF 3425 | viz reference | | | AM181460 | | | | Kolařík et al. (2007) |
| <i>G. obscura</i> (=sp. 17) | DLS1629 | viz reference | | | EU807967 | | | | Six et. al. (2009) |
| <i>G. omnicola</i> (=sp. 18) | MK1782 | viz reference | | | AM421060 | | | | Kolařík et al. (2007) |
| <i>G. omnicola</i> (=sp. 10) | CCF 3553 | viz reference | | | AM181433 | | | | Kolařík et al. (2008) |
| <i>G. omnicola</i> (=sp. 10) | IMI 194089 | viz reference | | | AM181450 | | | | Kolařík et al. (2008) |
| <i>G. pallida sensu stricto</i> | IMI 040214 ^T (=NRRL 2037, CCF 3053) | ex cotton yarn | | UK, England, 1931 | AJ578486 | HG799908 | HG799817 | | Kolařík et al. (2004), this study |
| <i>G. pallida sensu stricto</i> | IMI 054224 (=CCF 3324) | ex soil | | Nigeria, izol. R.M. Jackson, 1954 | - | HG799846 | HG799900 | HG799809 | Kolařík et al. (2004), this study |
| <i>G. pallida sensu stricto</i> | IMI 051240b (=CCF 3318) | viz reference | | | AJ578489 | | | | Kolařík et al. (2004), this study |
| <i>G. putterillii</i> | IMI 40212 ^T (=NRRL 2024, CCF 3052) | ex discoloured timber of <i>Beilschmiedia tawa</i> | | New Zealand, izol. J.C. Neill, 1946 | AF033384 | HG799853 | HG799907 | HG799816 | Kolařík et al. (2004), this study |
| <i>G. putterillii</i> (=sp. 6) | CCF 3342 | viz reference | | | AM421042 | | | | Kolařík et al. (2004) |
| <i>G. putterillii</i> (=sp.6) | CCF 4592 (=U83) | <i>Umbellularia californica</i> | <i>Scobicia declivis</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | HF546348 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U307 | <i>Pinus ponderosa</i> | <i>Pityophthorus</i> sp. | CO, Rocky Mts., Roosevelt National Forest, 40°27'35.6"N 105°25'18.9"W | HF546306 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | CCF 4202 (=U131a) | <i>Cy. macrocarpa</i> | <i>Phloeosinus sequoiae</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | HF546222 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U137 | <i>U. californica</i> | <i>Scobicia declivis</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | HF546269 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U220 | <i>Salix</i> sp. | <i>Hylocurus hirtellus</i> | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | HF546297 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U223 | <i>Calocedrus decurrens</i> | <i>Phloeosinus fulgens</i> | CA, Union Dam, 38°54'16.9"N 120°22'36.6"W | HF546298 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U304 | <i>Cy. monterey</i> | <i>Phloeosinus cupressi</i> | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546303 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U307b | <i>Pinus muricata</i> | <i>Pityophthorus</i> sp. | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546307 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | CCF 4329 (=U327) | <i>Juglans australis</i> | <i>Pityophthorus juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546237 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U314 | <i>Cy. monterey</i> | <i>Phloeosinus cupressi</i> | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546312 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U319 | <i>Cy. groverianus</i> | <i>Phloeosinus cupressi</i> | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546313 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U36 | <i>Sequoia sempervirens</i> | <i>Phloeosinus sequoiae</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546324 | | | | this study |

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| <i>G. putterillii</i> (=sp.6) | U47 | <i>U. californica</i> | <i>Scobicia declivis</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546337 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | CCF 4204 (=U48) | <i>U. californica</i> | <i>Scobicia declivis</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546224 | | | | this study |
| <i>G. putterillii</i> (=sp.6) | U81 | <i>Lithocarpus densiflorus</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546347 | | | | this study |
| <i>G. rufescens</i> | CCF 3751 | viz reference | | | AM947667 | | | | Kolařík et Kirkendall (2010) |
| <i>G. rufescens</i> | CCF 4524 | viz reference | | | AM947668 | | | | Kolařík et Kirkendall (2010) |
| <i>G. rufescens</i> | MK1821 | viz reference | | | AM947669 | | | | Kolařík et Kirkendall (2010) |
| <i>G. ulmacea</i> (=sp. 13) | 1226 | <i>Ulmus</i> sp. | <i>Scolytus schevyrewi</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | KJ716463 | | | | Zerillo et al. (2014) |
| <i>G. ulmacea</i> (=sp. 13) | CCF 3559 | viz reference | | | AM181439 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 1 | CCF 3395 | viz reference | | | AM181472 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 1 | MK745 | viz reference | | | AM421081 | | | | Kolařík et al. (2007) |
| <i>Geosmithia</i> sp. 1 | CCF 4529 | viz reference | | | AM421094 | | | | Kolařík et al. (2007) |
| <i>Geosmithia</i> sp. 1 | CCF 3660 | viz reference | | | AM421520 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 2 | CCF 4315 (=U109) | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> , <i>Pseudothyanoes hopkinsi</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546225 | | | | this study |
| <i>Geosmithia</i> sp. 2 | U111a | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> , <i>Pseudothyanoes hopkinsi</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546258 | | | | this study |
| <i>Geosmithia</i> sp. 2 | U113 | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> , <i>Pseudothyanoes hopkinsi</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546259 | HG799857 | | | this study |
| <i>Geosmithia</i> sp. 2 | U112 | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> , <i>Pseudothyanoes hopkinsi</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546260 | HG799856 | HG799819 | | this study |
| <i>Geosmithia</i> sp. 2 | U107 | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546256 | HG799855 | HG799910 | HG799818 | this study |
| <i>Geosmithia</i> sp. 2 | MK642 | <i>Fraxinus ornus</i> | <i>Hylesinus orni</i> | Hungary, Villány region, Vokány, izol. M. Kolařík, 2003 | - | HG799852 | HG799906 | HG799815 | this study |
| <i>Geosmithia</i> sp. 2 | IMI 155478a (=CCF 3319) | ex trees in apple orchard | | Cyprus, izol. J.P. Zyngas, 1971 | - | HG799840 | HG799894 | HG799803 | this study |
| <i>Geosmithia</i> sp. 2 | CCF 3340 (=MK115) | <i>Quercus</i> sp. | <i>Agriilus</i> sp. | Czech R., Louny, Nová Ves, izol. M. Kolařík, 2000 | AJ578485 | HG799836 | HG799890 | | Kolařík et al. (2004), this study |
| <i>Geosmithia</i> sp. 2 | IMI1 90744 (=CCF 3320) | ex <i>Cucumis melo</i> | | Peru, izol. A. Wallbridge | - | HG799841 | HG799895 | HG799804 | this study |
| <i>Geosmithia</i> sp. 3 | CCF 2809 (=AK132/93) | viz reference | | | AJ578488 | | | | Kolařík et al. (2004) |
| <i>Geosmithia</i> sp. 3 | CCF 3344 (=MK114) | <i>Quercus robur</i> | <i>Scolytus intricatus</i> | Czech R., Břínkov near Louny, izol. 2003, M. Kolařík | - | HG799848 | HG799902 | HG799811 | this study |

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| <i>Geosmithia</i> sp. 3 | CCF 4298 (=MK250) | <i>Q. dalechampii</i> | <i>Scolytus intricatus</i> | Slovakia, Muranska planina, Muransky hrad, izol. M. Kolařík, 2002 | AM181436 | HG799851 | HG799905 | HG799814 | Kolařík et al. (2008), this study |
| <i>Geosmithia</i> sp. 3 | CCF 3481 (=MK612) | <i>Carpinus betulus</i> | <i>Scolytus carpini</i> | Hungary, Bakony range, Vinye, izol. 2003, M. Kolařík | AM181467 | HG799842 | HG799896 | HG799805 | Kolařík et al. (2008), this study |
| <i>Geosmithia</i> sp. 4 | CCF 4278 (=MK1722) | <i>Ulmus laevis</i> | <i>Pteleobius vittatus</i> | Czech R., Břeclav, izol. M. Kolařík, 2004 | AM181466 | HG799850 | HG799904 | HG799813 | Kolařík et al. (2008), this study |
| <i>Geosmithia</i> sp. 5 | MK1715 | <i>Tilia</i> sp. | <i>Ernoporus tiliae</i> | Bulgaria, Rodopy, Bačkovovo, izol. M. Kolařík, 2005 | - | HG799849 | HG799903 | HG799812 | Kolařík et al. (2008), this study |
| <i>Geosmithia</i> sp. 5 | CCF 4215 (=MK137m) | <i>Picea abies</i> | <i>Pityophthorus pityographus</i> | Poland, Szydłowiec, izol. R. Jankowiak, 2007 | HE604117 | HG799854 | HG799909 | | Kolařík et Jankowiak (2013), this study |
| <i>Geosmithia</i> sp. 5 | AK192/98 | <i>Quercus robur</i> | <i>Scolytus intricatus</i> | Czech Republic, Libický luh, 1998 | - | HG799835 | HG799889 | HG799800 | this study |
| <i>Geosmithia</i> sp. 5 | CCF 3341 (=AK108/97) | <i>Quercus petraea</i> | <i>Scolytus intricatus</i> | Czech R., Krivoklátsko region, Mlynářův luh, izol. A. Kubátová, 1997 | AJ578487 | HG799837 | HG799891 | HG799801 | Kolařík et al. (2004), this study |
| <i>Geosmithia</i> sp. 8 | CCF 3358 | viz reference | | | AM181421 | FN429955 | | FM986788 | Kolařík et Kirkendall (2009) |
| <i>Geosmithia</i> sp. 8 | MK1708 | viz reference | | | AM181444 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 9 | CCF 3564 | viz reference | | | AM181428 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 9 | CCF 3702 | viz reference | | | AM746018 | | | | Jankowiak et Kolařík (2011) |
| <i>Geosmithia</i> sp. 11 | CCF 3556 | viz reference | | | AM181418 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 11 | CCF 3555 | viz reference | | | AM181419 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 12 | CCF 3355 (=MK551) | viz reference | | | AM181430 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 12 | CCF 3557 (=MK661) | viz reference | | | AM181431 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 12 | CCF 4320 (=U164) | <i>Fraxinus</i> sp. | <i>Hylesinus oregonus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | | HF546229 | | | this study |
| <i>Geosmithia</i> sp. 16 | CCF 3558 | viz reference | | | AM181422 | | | | Kolařík et al. (2008) |
| <i>Geosmithia</i> sp. 16 | CCF 4196 (22k) | viz reference | | | HE604142 | | | | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 16 | CCF 4201 (=RJ08m) | viz reference | | | HE604146 | HE604206 | HE604234 | HE604181 | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 16 | RJ34m | viz reference | | | | HE604207 | HE604259 | HE604182 | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 16 | RJ41m | viz reference | | | | HE604209 | HE604260 | | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 16 | RJ42m | viz reference | | | | HE604208 | HE604261 | HE604183 | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 16 | | | | | AM421075 | | | | Kolařík et al. (2007) |
| <i>Geosmithia</i> sp. 19 | CCF 3655 | viz reference | | | AM421085 | | | | Kolařík et al. (2007) |
| <i>Geosmithia</i> sp. 19 | CCF 3658 (=MK1085a) | viz reference | | | AM421073 | | | | Kolařík et al. (2007) |
| <i>Geosmithia</i> sp. 20 | CCF 3641 | viz reference | | | | | | | |

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| <i>Geosmithia</i> sp. 20 | CCF 4284 | viz reference | | | AM421074 | Kolařik et al. (2007) |
| <i>Geosmithia</i> sp. 20 | CCF 4316 (=U119b) | <i>Calocedrus decurrens</i> | <i>Phloeosinus fulgens</i> | CA, Jonkinson lake, 38°42'52.9"N 120°33'44.5"W | HF546226 | this study |
| <i>Geosmithia</i> sp. 20 | U193 | <i>Ulmus pumila</i> | <i>Scolytus schevyrewi</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | HF546287 | this study |
| <i>Geosmithia</i> sp. 21 | CCF 3642 | viz reference | | | AM421047 | Kolařik et al. (2007) |
| <i>Geosmithia</i> sp. 21 | MK1759 | viz reference | | | AM421048 | Kolařik et al. (2007) |
| <i>Geosmithia</i> sp. 21 | CCF 4302 | viz reference | | | AM421053 | Kolařik et al. (2007) |
| <i>Geosmithia</i> sp. 21 | CCF 4323 (=U21) | <i>Amygdalus communis</i> | <i>Scolytus rugulosus</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | HF546296 | this study |
| <i>Geosmithia</i> sp. 21 | U167 | <i>Chamaecyparis</i> sp. | <i>Phloeosinus canadensis</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | HF546280 | this study |
| <i>Geosmithia</i> sp. 21 | CCF 4334 (=U411) | <i>Juniperus occidentalis</i> var. <i>australis</i> | <i>Phloeosinus punctatus</i> | CA, White Mts., 37°18'06.8"N 118°10'07.7"W | HF546242 | this study |
| <i>Geosmithia</i> sp. 21 | U412 | <i>Juniperus occidentalis</i> var. <i>australis</i> | <i>Phloeosinus punctatus</i> | CA, White Mts., 37°18'06.8"N 118°10'07.7"W | HF546328 | this study |
| <i>Geosmithia</i> sp. 21 | U416 | <i>Juniperus occidentalis</i> var. <i>australis</i> | <i>Phloeosinus punctatus</i> | CA, White Mts., 37°18'06.8"N 118°10'07.7"W | HF546329 | this study |
| <i>Geosmithia</i> sp. 21 | U421 | <i>Pinus longaeva</i> | <i>Pityophthorus</i> sp. | CA, White Mts., Ancient Bristlecone Pine Forest, 37°23'09.8"N 118°10'45.8"W | HF546334 | this study |
| <i>Geosmithia</i> sp. 21 | U422 | <i>Pinus longaeva</i> | <i>Pityophthorus</i> sp. | CA, White Mts., Ancient Bristlecone Pine Forest, 37°23'09.8"N 118°10'45.8"W | HF546334 | this study |
| <i>Geosmithia</i> sp. 21 | CCF 5240 (=U23) | <i>Pinus sabiniana</i> | <i>Orthotomicus spinifer</i> , <i>Pityophthorus</i> sp. | CA, Cache creek Canyon, 38°54'31.3"N 122°18'45.8"W | HF546299 | this study |
| <i>Geosmithia</i> sp. 21 | CCF 5269 (=U330) | <i>Prunus persica</i> | <i>Scolytus rugulosus</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546318 | this study |
| <i>Geosmithia</i> sp. 21 | U8a | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CA, Davis, Putah creek, 38°31'1.03"N, 122°12'49.68"W | HF546252 | this study |
| <i>Geosmithia</i> sp. 21 | U10 | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CA, Davis, Putah creek, 38°31'1.03"N, 122°12'49.68"W | HF546263 | this study |
| <i>Geosmithia</i> sp. 21 | U12 | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CA, Davis, Putah creek, 38°31'1.03"N, 122°12'49.68"W | HF546350 | this study |
| <i>Geosmithia</i> sp. 21 | CCF 5270, CCF 5247 (=U198) | <i>Pseudotsuga menziesii</i> | <i>Pityophthorus</i> sp., <i>Scolytus oregoni</i> , <i>Cryphalus pubescens</i> | CO, Rocky Mts., Cameron Pass, 40°31'15"N 105°53'33"W | HF546289 | this study |
| <i>Geosmithia</i> sp. 21 | U65 | <i>Pseudotsuga menziesii</i> | Buprestidae sp., Cerambycidae sp. | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546343 | this study |
| <i>Geosmithia</i> sp. 21 | U133b | <i>Sequoia sempervirens</i> | <i>Phloeosinus sequoiae</i> | CA, Salt Poin, 38°33'59.4"N 123°19'51.5"W | HF546266 | this study |

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| <i>Geosmithia</i> sp. 21 | U1334 | <i>Sequoia sempervirens</i> | <i>Phloeosinus sequoiae</i> | CA, Salt Poin, 38°33'59.4"N 123°19'51.5"W | HF546267 | | | | | this study |
| <i>Geosmithia</i> sp. 21 | U14 | <i>Sequoia sempervirens</i> | Buprestidae sp. | CA, Davis, 38°32'55.19"N 121°44'24.64"W | HF546271 | | | | | this study |
| <i>Geosmithia</i> sp. 21 | U78 | <i>Sequoia sempervirens</i> | <i>Cryphalus pubescens</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | HF546345 | | | | | this study |
| <i>Geosmithia</i> sp. 21 | U34a | <i>Amygdalus communis</i> | <i>Scolytus rugulosus</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | HF546323 | | | | | this study |
| <i>Geosmithia</i> sp. 22 | CCF 3645 | viz reference | | | AM421061 | | | | | Kolařík et al. (2007) |
| <i>Geosmithia</i> sp. 22 | CCF 3652 | viz reference | | | AM421062 | | | | | Kolařík et al. (2007) |
| <i>Geosmithia</i> sp. 23 | IMI 051240b (=CCF 3318) | <i>Persea gratissima</i> | scolytid beetles | Seychelles, izol. G.S. Brown, 1952 | AJ578489 | HG799845 | HG799899 | HG799808 | | Kolařík et al. (2004), this study |
| <i>Geosmithia</i> sp. 23 | IMI 191599 (=CCF 3322) | ex branches of <i>Malus pumila</i> | | Cyprus, izol. J.P. Zyngas, 1975 | - | HG799847 | HG799901 | HG799810 | | Kolařík et al. (2004), this study |
| <i>Geosmithia</i> sp. 23 | CCF3639 (=MK781) | <i>Prunus armeniaca</i> | <i>Scolytus rugulosus</i> | Turkey, Silifke, Demircili, izol. M. Kolařík, 2004 | AM421068 | HG799838 | HG799892 | HG799802 | | Kolařík et al. (2004), this study |
| <i>Geosmithia</i> sp. 23 | CCF 3638 | viz reference | | | AM421067 | | | | | Kolařík et al. (2007) |
| <i>Geosmithia</i> sp. 23 | CCF 3483 (=MK762) | <i>Prunus armeniaca</i> | <i>Scolytus rugulosus</i> | Turkey, Silifke, Demircili, izol. M. Kolařík, 2004 | - | HG799843 | HG799897 | HG799806 | | Kolařík et al. (2004), this study |
| <i>Geosmithia</i> sp. 23 | U183 | <i>Ulmus pumila</i> | <i>Scolytus multistriatus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | HF546228 | | | | | this study |
| <i>Geosmithia</i> sp. 23 | CCF 4319 (=U159) | <i>Ulmus pumila</i> | <i>Scolytus multistriatus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | HF546278 | | | | | this study |
| <i>Geosmithia</i> sp. 23 | U160 | <i>Ulmus pumila</i> | <i>Scolytus multistriatus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | HF546284 | HG799859 | HG799911 | HG799820 | | Kolařík et Jankowiak (2016), this study |
| <i>Geosmithia</i> sp. 24 | CCF 4222 | viz reference | | | HE604158 | | | | | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 24 | CCF 4198 | viz reference | | | HE604161 | | | | | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 25 | MK1829b | viz reference | | | HE604217 | HE604249 | HE604185 | | | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 25 | MK1832 | viz reference | | | HE604128 | HE604218 | HE604250 | HE604186 | | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 25 | CCF 4211 (=MK1829a) | viz reference | | | HE604127 | HE604219 | HE604253 | HE604187 | | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 25 | CCF 4205 (=MK1835) | viz reference | | | HE604158 | LN907595 | LN907602 | LN907594 | | Kolařík et Jankowiak (2016), this study |
| <i>Geosmithia</i> sp. 26 | CCF 4222 (=RJ26) | <i>Pinus sylvestris</i> | <i>Pityogenes bidentatus</i> | Poland, Babimost, izol. R. Jankowiak, 2006 | HE604112 | LN907596 | LN907601 | LN907593 | | Kolařík et Jankowiak (2016), this study |
| <i>Geosmithia</i> sp. 26 | CCF 4223 (=MK1796) | <i>Pinus sylvestris</i> | <i>Pityophthorus pityographus</i> | Czech R., Senik near Prelouc, izol. M. Kolařík, 2006 | HE604112 | LN907597 | LN907603 | LN907592 | | Kolařík et Jankowiak (2016), this study |
| <i>Geosmithia</i> sp. 26 | CCF 4293 (=MK1828) | <i>Pinus sylvestris</i> | <i>Pityophthorus pityographus</i> | Czech R., Sedlčany, izol. M. Kolařík, 2008 | HE604112 | LN907597 | LN907603 | LN907592 | | Kolařík et Jankowiak (2016), this study |
| <i>Geosmithia</i> sp. 26 | U404 | <i>Pinus monophylla</i> | <i>Pityophthorus</i> sp. | CA, White Mts., 37°18'06.8"N 118°10'07.7"W | HF546325 | | | | | this study |
| <i>Geosmithia</i> sp. 27 | CCF 4206 (=RJ0919) | <i>Pinus sylvestris</i> | <i>Pityogenes bidentatus</i> | Poland, Żurada, izol. R. Jankowiak, 2006 | HE794978 | HG799839 | HG799893 | | | Kolařík et Jankowiak (2016), this study |
| <i>Geosmithia</i> sp. 27 | U308 | <i>Pinus ponderosa</i> | <i>Pityophthorus</i> sp. | CO, Rocky Mts., Roosevelt National Forest, 40°27'35.6"N 105°25'18.9"W | HF546308 | | | | | this study |
| <i>Geosmithia</i> sp. 27 | CCF 4605 (=U308b) | <i>Pinus ponderosa</i> | <i>Pityophthorus</i> sp. | CO, Rocky Mts., Roosevelt National Forest, 40°27'35.6"N 105°25'18.9"W | HF546309 | HG799867 | HG799919 | HG799827 | | Kolařík et Jankowiak (2016), this study |
| <i>Geosmithia</i> sp. 28 | CCF 4210 | viz reference | | | HE604154 | | | | | Kolařík et Jankowiak (2013) |
| <i>Geosmithia</i> sp. 29 | CCF 4221 | viz reference | | | HE604125 | HE604233 | HE604248 | HE604184 | | Kolařík et Jankowiak (2013) |

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|--------------------------|---------------------|-------------------------------|----------------------------------|---|-----------------|-----------------|-----------------|-----------------------------|-----------------------------------|
| <i>Geosmithia</i> sp. 29 | CCF 4199 | viz reference | | | HE604126 | HE604232 | HE604243 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 30 | CCF 4214 (=RJ156m) | viz reference | | | HE604160 | HE604214 | HE604239 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 30 | CCF 4209 (=RJ09m) | viz reference | | | | HE604210 | HE604235 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 30 | MK1843 | viz reference | | | | HE604222 | HE604255 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 30 | CCF 4288 (=MK1801) | viz reference | | | HE604132 | HE604216 | HE604242 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 30 | MK1834 | viz reference | | | | HE604220 | HE604252 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 30 | CCF 4219 (=MK1836) | viz reference | | | | HE604221 | HE604254 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | CCF 4212 (=MK1811b) | viz reference | | | | HE604225 | HE604244 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | CCF 4197 (=RJ15ak) | viz reference | | | | HE604229 | HE604240 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | MK1825 | viz reference | | | | HE604227 | HE604246 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | CCF 4196 (=RJ22k) | viz reference | | | | HE604230 | HE604256 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | RJ73k | viz reference | | | | HE604231 | HE604263 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | RJ49k | viz reference | | | | HE604228 | HE604262 | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | CCF 4218 | viz reference | | | HE604141 | | | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | RJ74k | viz reference | | | HE604143 | | | Kolařík et Jankowiak (2013) | |
| <i>Geosmithia</i> sp. 31 | CCF 4589 (=U309) | <i>Pinus nigra</i> | <i>Pityophthorus</i> sp. | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | HF546310 | | | this study | |
| <i>Geosmithia</i> sp. 31 | CCF 5238 (=U407) | <i>Pinus sabiniana</i> | <i>Pityophthorus</i> sp. | CA, Alder Creek (Alta Sierra) - USFS campground, 35°43'11.3"N 118°36'43.2"W | HF546327 | HG799921 | HG799829 | this study | |
| <i>Geosmithia</i> sp. 32 | CCF 3554 (=MK538) | <i>Chamaecyparis pisifera</i> | <i>Phloeosinus thujae</i> | Czech R., Kácov, izol. M. Kolařík and P. Šrůtka, 2003 | AM181426 | HG799874 | HG799926 | HG799885 | Kolařík et al. (2008), this study |
| <i>Geosmithia</i> sp. 32 | CCF 3648 (=MK1625) | <i>Olea europea</i> | <i>Phloeotribus scarabeoides</i> | Spain, Yator, near Cadiar, izol. M. Kolařík, 2005 | AM421038 | HG799875 | HG799927 | HG799886 | Kolařík et al. (2008), this study |
| <i>Geosmithia</i> sp. 32 | CCF 5242 (=U130) | <i>Sequoia sempervirens</i> | <i>Phloeosinus sequoiae</i> | CA, Bohemian river, 38° 27' 18.64"N 123° 0' 13.89"W | HF546265 | HG799873 | HG799925 | HG799888 | Kolařík et al. (2008), this study |
| <i>Geosmithia</i> sp. 33 | CCF 4598 (=U418) | <i>Abies concolor</i> | <i>Scolytus praeceps</i> | CA, Grover Hot Springs SP, 38°41'46.5"N 119°50'41.0"W | HF546331 | HG799869 | HG799923 | HG799831 | this study |
| <i>Geosmithia</i> sp. 34 | U119a | <i>Calocedrus decurrens</i> | <i>Ips plastographus</i> | CA, Jonkinson lake, 38°42'52.9"N 120°33'44.5"W | HF546262 | | | | this study |
| <i>Geosmithia</i> sp. 34 | CCF 4604 (=U218) | <i>Calocedrus decurrens</i> | <i>Ips plastographus</i> | CA, Jonkinson lake, 38°42'52.9"N 120°33'44.5"W | HF546295 | HG799866 | HG799918 | HG799826 | this study |
| <i>Geosmithia</i> sp. 34 | U417 | <i>Abies concolor</i> | <i>Scolytus praeceps</i> | CA, Grover Hot Springs SP, 38°41'46.5"N 119°50'41.0"W | HF546330 | HG799868 | HG799922 | HG799830 | this study |

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| <i>Geosmithia</i> sp. 34 | CCF 5243 (=U419) | <i>Abies concolor</i> | <i>Scolytus praeceps</i> | CA, Grover Hot Springs SP, 38°41'46.5"N 119°50'41.0"W | HF546332 | | | | this study |
| <i>Geosmithia</i> sp. 35 | CCF 4322 (=U196) | <i>Pseudotsuga menziesii</i> | <i>Pityophthorus</i> sp., <i>Scolytus oregoni</i> , <i>Cryphalus pubescens</i> | CO, Rocky Mts., Cameron Pass, 40°31'15"N 105°53'33"W | HF546231 | HG799914 | HG799823 | | this study |
| <i>Geosmithia</i> sp. 36 | CCF 4328 (=U316) | <i>Pinus muricata</i> | <i>Pityophthorus</i> sp. | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546236 | HG799920 | HG799828 | | this study |
| <i>Geosmithia</i> sp. 37 | U197 | <i>Pseudotsuga menziesii</i> | <i>Pityophthorus</i> sp., <i>Scolytus oregoni</i> , <i>Cryphalus pubescens</i> | CO, Rocky Mts., Cameron Pass, 40°31'15"N 105°53'33"W | HF546288 | HG799862 | HG799915 | HG799824 | this study |
| <i>Geosmithia</i> sp. 38 | U79 | <i>Notholithocarpus densiflorus</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546346 | | | | this study |
| <i>Geosmithia</i> sp. 38 | CCF 5241 (=U95) | <i>Quercus acrifolia</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | HF546251 | | | | this study |
| <i>Geosmithia</i> sp. 39 | U323 | <i>Juglans hindsii</i> | <i>Pityophthorus juglandis</i> | CA, Winters, Juglans Germplasm Collection (USDA/ARS), 38°30'06.9"N 121°58'44.8"W | HF546314 | | | | this study |
| <i>Geosmithia</i> sp. 40 | CCF 4194 (=U307a) | <i>Pinus muricata</i> | <i>Pityophthorus</i> sp. | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546220 | | | | this study |
| <i>Geosmithia</i> sp. 40 | CCF 5250 (=U143) | <i>Pinus ponderosa</i> | <i>Pityophthorus</i> sp. | CA, Salt Poin, 38°33'59.4"N 123°19'51.5"W | HF546273 | | | | this study |
| <i>Geosmithia</i> sp. 40 | CCF 5245 (=U306a) | <i>Pinus radiata</i> | <i>Ips plastographus</i> | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546304 | | | | this study |
| <i>Geosmithia</i> sp. 40 | U216 | <i>Pseudotsuga menziesii</i> | <i>Cryphalus pubescens</i> | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | HF546293 | | | | this study |
| <i>Geosmithia</i> sp. 41 | U215 | <i>Artemisia arborea</i> | Cossoninae sp. | CA, Salmon Creek, 38°21'18.3"N 123°03'56.2"W | HF546292 | HG799865 | HG799917 | HG799825 | this study |
| <i>Geosmithia</i> sp. 41 | CCF 5291(=U92a) | <i>Lithocarpus densiflorus</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Jack London State historic park, 38°20'51.2"N 122°33'44.0"W | HF546351 | | | | this study |
| <i>Geosmithia</i> sp. 41 | U54 | <i>Pinus sabiniana</i> | Buprestidae sp., Cerambycidae sp. | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546340 | | | | this study |
| <i>Geosmithia</i> sp. 41 | CCF 4606 (=U144b) | <i>Pseudotsuga menziesii</i> | Scolytinae sp. | CA, Union Dam, 38°54'16.9"N 120°22'36.6"W | HF546274 | HG799858 | | | this study |
| <i>Geosmithia</i> sp. 41 | U101 | <i>Quercus acrifolia</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Fairfield Osborn Preserve, 38° 20' 36.17" N 122° 35' 41.24" W | HF546253 | | | | this study |
| <i>Geosmithia</i> sp. 41 | U2 | <i>Quercus acrifolia</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546290 | HG799863 | | | this study |
| <i>Geosmithia</i> sp. 41 | U310a | <i>Quercus acrifolia</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Monterey, 36° 35' 21.27" N 121° 57' 35.24" W | HF546311 | | | | this study |
| <i>Geosmithia</i> sp. 41 | U98 | <i>Quercus acrifolia</i> | <i>Monarthrum scutellare</i> | CA, Fairfield Osborn Preserve, 38° 20' 36.17" N 122° 35' 41.24" W | HF546352 | HG799872 | | HG799834 | this study |
| <i>Geosmithia</i> sp. 41 | U104 | <i>Quercus berberidifolia</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546255 | | | | this study |
| <i>Geosmithia</i> sp. 41 | U103 | <i>Quercus kelloggii</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Lodi, Armstrong, 38° 5'20.42"N 121°16'25.30"W | HF546254 | | | | this study |
| <i>Geosmithia</i> sp. 41 | CCF 5271 (=U15) | <i>Quercus kelloggii</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546276 | | | | this study |

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| <i>Geosmithia</i> sp. 41 | U26 | <i>Quercus</i> sp. | <i>Pseudopityophthorus pubipennis</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | HF546300 | | | | | this study |
| <i>Geosmithia</i> sp. 41 | CCF 4342 (=U86) | <i>Toxicodendron diversilobum</i> | Bostrichidae sp. | CA, Berkeley, 37°57'24.5"N 122°16'07.4"W | HF546249 | HG799871 | HG799924 | HG799833 | | this study |
| <i>Geosmithia</i> sp. 41 | U64 | <i>Umbellularia californica</i> | <i>Scobicia declivis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546342 | HG799870 | HG799930 | HG799832 | | this study |
| <i>Geosmithia</i> sp. 41 | U17 | <i>Heteromeles arbutifolia</i> | <i>Pseudopityophthorus pubipennis</i> | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546281 | | | | | this study |
| <i>Geosmithia</i> sp. 41 | U87 | <i>Umbellularia californica</i> | <i>Scobicia declivis</i> | CA, Fairfield Osborn Preserve, 38° 20' 36.17" N 122° 35' 41.24" W | HF546349 | | | | | this study |
| <i>Geosmithia</i> sp. 42 | U29/30 | <i>Amygdalis communis</i> | <i>Scolytus rugulosus</i> | CA, Davis, 38°32'55.19"N 121°44'24.64"W | HF546301 | | | | | this study |
| <i>Geosmithia</i> sp. 42 | U166 | <i>Chamaecyparis</i> sp. | <i>Phloeosinus canadensis</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | HF546279 | HG799860 | HG799912 | HG799821 | | this study |
| <i>Geosmithia</i> sp. 42 | CCF 5251 (=U185b) | <i>Prunus</i> sp. | <i>Scolytus rugulosus</i> | CO, Fort Collins, 40°34'30.5"N 105°05'07.6"W | HF546285 | HG799861 | HG799913 | HG799822 | | this study |
| <i>Geosmithia</i> sp. 42 | CCF 5244 (=U154) | <i>Pinus ponderosa</i> | <i>Pityogenes knechteli</i> , <i>Pityophthorus</i> sp. | CO, Rocky Mts., Roosevelt National Forest, Donner pass, 40° 32' 21.86"N 105° 28' 8.98" W | HF546277 | | | | | this study |
| <i>Geosmithia</i> sp. 43 | CCF 4203 (=U205) | <i>Pinus ponderosa</i> | <i>Pityogenes knechteli</i> , <i>Pityophthorus</i> sp. | CO, Rocky Mts., Roosevelt National Forest, Donner pass, 40° 32' 21.86"N 105° 28' 8.98" W | HF546223 | HG799864 | HG799916 | | | this study |
| <i>Geosmithia</i> sp. 44 | CCF 4333 (=U410) | <i>Calocedrus decurrens</i> | <i>Phloeosinus fulgens</i> | CA, Grover Hot Springs SP, 38°41'46.5"N 119°50'41.0"W | HF546241 | LN907598 | | LN907590 | | this study |
| <i>Geosmithia</i> sp. 44 | CCF 4332 (=U408) | <i>Pinus sabiniana</i> | <i>Pityophthorus</i> sp. | CA, Bodfish, Miracle hot Springs, 35°35'53.1"N 118°30'10.9"W | HF546240 | LN907599 | | | | this study |
| <i>Geosmithia</i> sp. 44 | CCF 4324 (=U25) | <i>Pinus sabiniana</i> | <i>Pityophthorus</i> sp. | CA, Winters, Lake Beryessa, Quail Ridge Reserve, 38°31'1.03"N 122°12'49.68"W | HF546232 | LN907600 | LN907604 | LN907591 | | this study |

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Supplementary table 3. Summary of the nucleotide composition, length of ITS rDNA segments and free energy of the ITS2 secondary structure across all *Geosmithia* species.

Title of the paper: *Geosmithia* associated with bark beetles and woodborers in the western USA: Taxonomic diversity and vector specificity

Authors: Kolařík M., Hulcr J., Tisserat N., De Beer W., Kostovčík M., Kolaříková Z., Seybold S. J., Rizzo D. M

| Species | Culture collection | ITS-LSU rDNA | %T | %A | %C | %G | %GC | ITS1 length [bp] | 5.8S length [bp] | ITS2 length [bp] | LSU length [bp] | ITS2 secondary structure energy kcal/mol |
|-------------------------------------|--------------------|--------------------|-------|-------|-------|-------|-------|------------------|------------------|------------------|-----------------|--|
| <i>Acremonium alternatum</i> | CBS 233.70 | AY566992, GQ377488 | 22.94 | 23.9 | 26.1 | 27.06 | 53.16 | 156 | 158 | 172 | 376 | -58.9 |
| <i>G. cnesinii</i> | MK1820 | AM947671 | 21.58 | 24.2 | 25.89 | 28.33 | 54.22 | 175 | 158 | 175 | 376 | -71.6 |
| <i>G. eupagioceri</i> | CCF3754 | AM947666 | 21.07 | 24.25 | 26.78 | 27.9 | 54.68 | 177 | 158 | 175 | 376 | -75.5 |
| <i>G. fassatiae</i> (sp. 14) | CCF3334 | AJ578482 | 20.99 | 24.46 | 26.34 | 28.21 | 54.55 | 175 | 158 | 176 | 376 | -68 |
| <i>G. flava</i> (sp. 7) | MK1602b | AM181457 | 21.02 | 24.48 | 26.17 | 28.33 | 54.5 | 175 | 158 | 175 | 376 | -56.5 |
| <i>G. langdonii</i> (sp. 15) | MK1683a | AM181458 | 21.31 | 24.69 | 25.73 | 28.26 | 53.99 | 174 | 158 | 175 | 376 | -67.3 |
| <i>G. langdonii</i> (sp. 15) | CCF3338 | AJ578480 | 21.31 | 24.69 | 25.73 | 28.26 | 53.99 | 174 | 158 | 175 | 376 | -67.3 |
| <i>G. lavendula</i> (sp. 18) | CCF3394 | AM421098 | 20.88 | 24.18 | 26.81 | 28.13 | 54.94 | 172 | 158 | 175 | 376 | -77.3 |
| <i>G. microcorthyli</i> | CCF3864 | FM986798 | 21.21 | 24.67 | 26.17 | 27.95 | 54.12 | 175 | 158 | 175 | 376 | -75.8 |
| <i>G. morbida</i> | CBS 124663 | FN434080 | 20.98 | 23.8 | 26.61 | 28.61 | 55.22 | 181 | 158 | 175 | 376 | -75.8 |
| <i>G. obscura</i> (sp. 17) | CCF3425 | AM181460 | 20.65 | 24.39 | 26.92 | 28.01 | 54.96 | 179 | 158 | 175 | 376 | -75.8 |
| <i>G. pallida sensu stricto</i> | NRRL2037 | AJ578486 | 20.48 | 24.25 | 26.98 | 28.29 | 55.28 | 175 | 158 | 175 | 376 | -82.8 |
| <i>G. putterillii</i> (sp. 6) | CCF3342 | AM421042 | 22.08 | 24.43 | 25.38 | 28.11 | 53.49 | 173 | 158 | 171 | 376 | -70.4 |
| <i>G. rufescens</i> | CCF3751 | AM947667 | 21.5 | 24.15 | 26.6 | 28.2 | 54.8 | 172 | 158 | 176 | 376 | -80.2 |
| <i>Geosmithia</i> sp. 1 | MK745 | AM421081 | 21.75 | 24.86 | 25.52 | 27.87 | 53.39 | 172 | 158 | 174 | 376 | -75.7 |
| <i>Geosmithia</i> sp. 1 | MK1790 | AM421094 | 21.68 | 24.69 | 25.64 | 27.99 | 53.63 | 172 | 158 | 174 | 376 | -75.7 |
| <i>Geosmithia</i> sp. 2 | CCF3340 | AJ578485 | 20.64 | 24.41 | 26.48 | 28.46 | 54.94 | 175 | 158 | 175 | 376 | -82.3 |
| <i>Geosmithia</i> sp. 3 | CCF2809 | AJ578488 | 20.55 | 24.22 | 26.67 | 28.56 | 55.23 | 175 | 158 | 175 | 376 | -83.2 |
| <i>Geosmithia</i> sp.5 | CCF4215 | HE604117 | 21.02 | 24.13 | 26.39 | 28.46 | 54.85 | 175 | 158 | 175 | 376 | -76.6 |
| <i>Geosmithia</i> sp. 5 | CCF3341 | AJ578487 | 21.01 | 24.13 | 26.48 | 28.37 | 54.85 | 175 | 158 | 175 | 376 | -80.8 |
| <i>Geosmithia</i> sp. 8 | CCF3358 | AM181421 | 21.21 | 24.67 | 26.17 | 27.95 | 54.12 | 175 | 158 | 175 | 376 | -75.8 |
| <i>Geosmithia</i> sp. 9 | CCF3564 | AM181428 | 20.58 | 24.44 | 26.69 | 28.29 | 54.98 | 175 | 158 | 178 | 376 | -90.1 |
| <i>Geosmithia omnicola</i> (sp. 10) | MK1782 | AM421060 | 21.18 | 24.37 | 25.96 | 28.49 | 54.45 | 176 | 158 | 175 | 376 | -71.6 |
| <i>Geosmithia</i> sp. 11 | CCF3555 | AM181419 | 21.35 | 24.46 | 25.87 | 28.32 | 54.19 | 173 | 158 | 174 | 376 | -71.7 |
| <i>Geosmithia</i> sp. 12 | CCF3355 | AM181430 | 20.61 | 24.23 | 26.46 | 28.69 | 55.15 | 185 | 158 | 176 | 376 | -71.6 |
| <i>Geosmithia ulmacea</i> (sp. 13) | CCF3559 | AM181439 | 20.81 | 24.46 | 26.34 | 28.25 | 54.74 | 176 | 158 | 175 | 376 | -76.4 |
| <i>Geosmithia</i> sp. 16 | CCF4201 | HE604146 | 21.56 | 24.37 | 25.87 | 28.21 | 54.08 | 177 | 158 | 175 | 376 | -70.7 |
| <i>Geosmithia</i> sp. 20 | CCF3641 | AM421073 | 21.37 | 24.93 | 25.77 | 27.93 | 53.7 | 175 | 158 | 176 | 376 | -64.1 |
| <i>Geosmithia</i> sp. 21 | MK592 | AM421053 | 21.11 | 24.5 | 25.9 | 28.3 | 54.31 | 175 | 158 | 175 | 376 | -72.8 |

| | | | | | | | | | | | | |
|--------------------------|---------|----------|-------|-------------------------|-----------|-----------|--------------|-----|-----|-----|-----|--------------|
| | | | | 8 | 8 | 3 | | | | | | |
| <i>Geosmithia</i> sp. 22 | CCF3645 | AM421061 | 21.78 | 24.1 3 | 26.1 | 27.9 8 | 54.08 | 174 | 158 | 175 | 376 | -72.9 |
| <i>Geosmithia</i> sp. 23 | CCF3638 | AM421067 | 20.55 | 24.1 3 | 26.7 7 | 28.5 6 | 55.33 | 175 | 158 | 175 | 376 | -83.2 |
| <i>Geosmithia</i> sp. 24 | MK1772 | HE604164 | 21.4 | 24.5 1 | 25.9 2 | 28.1 7 | 54.09 | 174 | 158 | 175 | 376 | -72.9 |
| <i>Geosmithia</i> sp. 25 | MK1832 | HE604128 | 21.97 | 24.6 9 | 25.4 5 | 27.8 9 | 53.34 | 177 | 158 | 175 | 376 | -67.3 |
| <i>Geosmithia</i> sp. 27 | RJ091B | HE604115 | 20.71 | 23.9 1 | 26.8 4 | 28.5 4 | 55.38 | 169 | 158 | 174 | 376 | -63.0 |
| <i>Geosmithia</i> sp. 28 | CCF4210 | HE604154 | 20.45 | 24.5 8 | 26.7 4 | 28.2 4 | 54.98 | 177 | 158 | 175 | 376 | -73.7 |
| <i>Geosmithia</i> sp. 29 | CCF4199 | HE604126 | 21.44 | 24.4 4 | 25.9 4 | 28.1 8 | 54.12 | 177 | 158 | 175 | 376 | -57.6 |
| <i>Geosmithia</i> sp. 30 | CCF4219 | HE604134 | 21.46 | 24.4 6 | 25.9 6 | 28.1 2 | 54.08 | 177 | 158 | 174 | 376 | -69.9 |
| <i>Geosmithia</i> sp. 30 | CCF4214 | HE604135 | 21.46 | 24.5 5 | 25.9 6 | 4096 7 | 53.98 | 177 | 158 | 174 | 376 | -68.8 |
| <i>Geosmithia</i> sp. 31 | CCF4218 | HE604141 | 21.54 | 24.4 4 | 25.8 4 | 28.1 8 | 54.02 | 177 | 158 | 175 | 376 | -71.5 |
| <i>Geosmithia</i> sp. 31 | RJ74k | HE604143 | 21.63 | 24.4 4 | 25.7 5 | 28.1 8 | 53.93 | 177 | 158 | 175 | 376 | -71.5 |
| <i>Geosmithia</i> sp. 31 | CCF4218 | HE604141 | 21.54 | 24.4 4 | 25.8 4 | 28.1 8 | 54.03 | 177 | 158 | 175 | 376 | -73.2 |
| <i>Geosmithia</i> sp. 32 | U130 | HF546265 | 21.31 | 24.6 9 | 25.7 3 | 28.2 6 | 53.99 | 177 | 158 | 175 | 376 | -67.3 |
| <i>Geosmithia</i> sp. 33 | U418 | HF546331 | 20.87 | 25.3 3 | 26.4 1 | 27.3 9 | 53.80 | 177 | 158 | 175 | 376 | -74.10 |
| <i>Geosmithia</i> sp. 34 | U119A | HF546262 | 21.44 | 24.4 4 | 26.0 3 | 28.0 9 | 54.12 | 177 | 158 | 175 | 376 | -74.10 |
| <i>Geosmithia</i> sp. 35 | CCF4322 | HF546231 | 21.56 | 24.4 6 | 25.8 7 | 28.1 2 | 53.98 | 177 | 158 | 174 | 376 | -69.9 |
| <i>Geosmithia</i> sp. 36 | CCF4328 | HF546236 | 21.10 | 25.2 2 | 26.0 8 | 27.6 0 | 53.68 | 177 | 158 | 175 | 376 | -73.2 |
| <i>Geosmithia</i> sp. 37 | U197 | HF546288 | 21.25 | 24.4 4 | 26.1 2 | 28.1 8 | 54.31 | 177 | 158 | 175 | 376 | -73.3 |
| <i>Geosmithia</i> sp. 38 | U79 | HF546346 | 21.07 | 24.4 4 | 26.3 1 | 28.1 8 | 54.49 | 177 | 158 | 175 | 376 | -73.2 |
| <i>Geosmithia</i> sp. 39 | U323 | HF546314 | 20.35 | 24.3 7 | 27.3 1 | 27.9 7 | 55.28 | 177 | 158 | 175 | 376 | -74.20 |
| <i>Geosmithia</i> sp. 40 | CCF4194 | HF546220 | 20.35 | 24.9 2 | 26.9 9 | 27.7 5 | 54.73 | 172 | 158 | 175 | 376 | -73.3 |
| <i>Geosmithia</i> sp. 41 | CCF4342 | HF546249 | 20.24 | 24.6 7 | 26.7 4 | 28.3 4 | 55.08 | 171 | 158 | 175 | 376 | -83.2 |
| <i>Geosmithia</i> sp. 41 | U64 | HF546342 | 20.24 | 24.5 8 | 26.7 4 | 28.4 4 | 55.18 | 171 | 158 | 175 | 376 | -83.2 |
| <i>Geosmithia</i> sp. 42 | U190B | HF546286 | 19.96 | 25.1 6 | 26.9 6 | 27.9 2 | 54.88 | 162 | 158 | 174 | 376 | -72.2 |
| <i>Geosmithia</i> sp. 43 | CCF4203 | HF546223 | 20.32 | 24.2 0 | 27.2 2 | 28.2 6 | 55.48 | 168 | 158 | 174 | 376 | -72.7 |
| <i>Geosmithia</i> sp. 26 | MK1797 | HE604105 | 21.69 | 26.3 4 | 25.2 3 | 26.7 4 | 51.97 | 120 | 157 | 157 | 374 | -51.8 |
| <i>Geosmithia</i> sp. 26 | U404 | HF546325 | 21.70 | 26.2 4 | 25.3 3 | 26.7 4 | 52.07 | 119 | 157 | 158 | 374 | -51.8 |
| <i>Geosmithia</i> sp. 44 | CCF4333 | HF546241 | 22.56 | 26.2 4 | 24.3 5 | 26.8 4 | 51.19 | 133 | 157 | 159 | 374 | -54.7 |
| <i>Geosmithia</i> sp. 44 | CCF4332 | HF546240 | 22.87 | 26.0 4 | 24.2 6 | 26.8 3 | 51.09 | 137 | 157 | 159 | 374 | -54.7 |
| <i>Geosmithia</i> sp. 44 | CCF4324 | HF546232 | 21.73 | 26.2 6 | 24.6 1 | 27.3 9 | 52.01 | 133 | 157 | 159 | 374 | -54.7 |

Supplementary table 4. Overview of areal and host range of all published *Geosmithia* spp.

Title of the paper: *Geosmithia* associated with bark beetles and woodborers in the western USA:

Taxonomic diversity and vector specificity

Authors: Kolařík M., Hulcr J., Tisserat N., De Beer W., Kostovčík M., Kolaříková Z., Seybold S. J., Rizzo D. M.

¹ The numbering for species no. 1 —31 follows Kolařík et al. (2007, 2008, 2013).

² The host plant family and a number of analyzed samples (in brackets) from Temperate Europe (extracted from Kolařík et al. (2008, 2013) and Mediterranean area (extracted from Kolařík et al. 2007) is presented. Only samples containing *Geosmithia* fungi were used in the computation.

| Species code ¹ | Species | Distribution and host affinity of <i>Geosmithia</i> | | | |
|---------------------------|---------------------------|--|---|--|---|
| | | On subcortical insects ² | | | Other Substrates or Locations |
| | | Central and North Eastern Europe (~Temperate Europe) (Angiosperms + Cupressaceae = 94 samples (Kolařík et al. 2008) (Pinaceae = 85 samples, (Kolařík and Jankowiak 2013) | Mediterranean Basin (Southern Europe and Western Asia) and Black and Caspian Sea region (Angiosperms + Cupressaceae = 82 samples (Kolařík et al. 2007) | Western U.S. (Angiosperms + Cupressaceae + Pinaceae = 109 samples (this study) | |
| 1 | <i>Geosmithia</i> sp. 1 | Cupressaceae (1), Ranunculaceae (1) | Fabaceae (1), Moraceae (20) | — | |
| 2 | <i>Geosmithia</i> sp. 2 | Fagaceae (4), Oleaceae (3), Rosaceae (6), Ulmaceae (3) | Ulmaceae (3) | Rosaceae (2) | tree in apple orchard (Cyprus), <i>Cucumis melo</i> (Peru) (Kolařík et al. 2004, this study), various scolytid species from <i>Virgilia</i> spp. (South Africa) (Machingambi et al. 2014) |
| 3 | <i>Geosmithia</i> sp. 3 | Betulaceae (2), Fagaceae (16), Rosaceae (2) | — | — | roots of <i>Quercus robur</i> (Czech R.), soil (Czech R.) (Kolařík et al. 2004) |
| 4 | <i>Geosmithia</i> sp. 4 | Ulmaceae (2) | — | — | |
| 5 | <i>Geosmithia</i> sp. 5 | Fagaceae (5), Oleaceae (3), Pinaceae (2), Rosaceae (3), Tiliaceae (3), Ulmaceae (12) | — | — | <i>Scolytus</i> beetle (UK) (Kolařík et al. 2004) |
| 6 | <i>G. puterillii</i> | Rosaceae (1) | Lauraceae (1) | Lauraceae (3), Cupressaceae (8), Pinaceae (3), Fagaceae (2), Ericaceae (1), Juglandaceae (1), Salicaceae (2) | timber of <i>Beilschmiedia tawa</i> (New Zealand) (Pitt 1979) |
| 7 | <i>G. flava</i> | Araliaceae (1), Betulaceae (2), Cupressaceae (1), Fagaceae (2), Oleaceae (14), Pinaceae (1), Rosaceae (8), Tiliaceae (16), Ulmaceae (2) | Anacardiaceae (1), Lauraceae (1), Moraceae (10), Rosaceae (1) | Cupressaceae (5), Pinaceae (5), Fagaceae (2), Anacardiaceae (1), Juglandaceae (1), Salicaceae (1) | <i>Ulmus glabra</i> (England), <i>Hordeum</i> sp. grein (England) (Kolařík et al. 2004), various scolytid species from <i>Virgilia</i> spp. (South Africa) (Machingambi et al. 2014) |
| 8 | <i>Geosmithia</i> sp. 8 | Fagaceae (5) | — | — | Machingambi et al. (2014) isolated species which is sequentially related to <i>G. sp. 8</i> , but the co-specificity should be verified using other markers in this case. |
| 9 | <i>Geosmithia</i> sp. 9 | Pinaceae (10) | — | — | |
| 10 | <i>G. omnicola</i> | Tiliaceae (4), Rosaceae (11), Ulmaceae (12), Salicaceae (1), Cupressaceae (1), Araliaceae (1), Fagaceae (1), Oleaceae (3), Betulaceae (1) | Anacardiaceae (5), Cupressaceae (3), Fabaceae (1), Lauraceae (1), Moraceae (30), Rosaceae (1), Ulmaceae (2) | — | air (Israel) (Kolařík et al. 2008), various scolytid species from <i>Virgilia</i> spp. (South Africa) (Machingambi et al. 2014) |
| 11 | <i>Geosmithia</i> sp. 11 | Fagaceae (2) | Oleaceae – Olea (14) | — | phloem endophyte of <i>Adansonia gregorii</i> (Australia), sequence GU19942 has 3 bp difference from <i>Geosmithia</i> sp. 11 and could represent another species (Sakalidis et al. 2011) |
| 12 | <i>Geosmithia</i> sp. 12 | Fagaceae (1), Oleaceae – Fraxinus (10) | — | Oleaceae — Fraxinus (1) | |
| 13 | <i>Geosmithia ulmacea</i> | Ulmaceae – Ulmus (4) | — | Ulmaceae — Ulmus (1) | |
| 14 | <i>G. fassatae</i> | Fagaceae (11), Rosaceae (3) | — | Pinaceae (1), Fagaceae (1), Salicaceae (1), Lauraceae (2) | <i>Pseudopityophthorus pubipennis</i> on <i>Quercus</i> spp. (U.S.) (McPherson et al. 2013). |
| 15 | <i>G. langdonii</i> | Cupressaceae (3), Betulaceae (2), Fabaceae (1), Fagaceae (17), Tiliaceae (1), Rosaceae (1), Ulmaceae (2) | Anacardiaceae (2), Euphorbiaceae (1) | Lauraceae (1), Cupressaceae (6), Pinaceae (2), Fagaceae (5), Asteraceae (1) | <i>Platypus cylindrus</i> on <i>Quercus suber</i> , Algeria (Belhoucine et al. 2011), <i>Quercus</i> spp., from wood without beetle presence (U.S.) (McPherson et al. 2013). |
| 16 | <i>Geosmithia</i> sp. 16 | Pinaceae (4) | — | — | |
| 17 | <i>G. obscura</i> | Betulaceae (2), Fagaceae (3) | — | — | <i>Xylosandrus mutilatus</i> on <i>Vitis rotundifolia</i> (USA) (Six et al. 2009) |
| 18 | <i>G. lavendula</i> | — | Anacardiaceae (2), Fabaceae (2), Moraceae (18), Ulmaceae (1) | Cupressaceae (1), Pinaceae (5), Anacardiaceae (3), Fagaceae (2), Juglandaceae (4), Rosaceae (2) | laboratory contaminant (USA), wood of <i>Carya illinoensis</i> (Israel), soil (Venezuela), air (India) (Pitt 1979), |

| | | | | | |
|----|---------------------------------|------------------|---|--|---|
| | | | | | <i>Xylosandrus mutilatus</i> (USA) (Six et al. 2009). We revised strains from India and Venezuela and confirmed their identity with <i>G. lavendula</i> (unpublished data). In addition, the specimen labeled as <i>Penicillium lavendulum</i> (syn. <i>G. lavendula</i>), found on bark beetles in Kansas (U.S.) is deposited in the Herbarium of the New York Botanical Garden (isolated in 1957, NY305179). |
| 19 | <i>Geosmithia</i> sp. 19 | — | Moraceae (2) | — | |
| 20 | <i>Geosmithia</i> sp. 20 | — | Asteraceae (1), Fabaceae (1), Moraceae (20), Oleaceae (5), Ulmaceae (2) | Cupressaceae (1), Ulmaceae (3) | |
| 21 | <i>Geosmithia</i> sp. 21 | — | Fabaceae (1), Moraceae (23), Oleaceae (10) | Cupressaceae (7), Pinaceae (9), Fagaceae (2), Rosaceae (6) | |
| 22 | <i>Geosmithia</i> sp. 22 | — | Fagaceae (1), Moraceae (1), Oleaceae (2), Rosaceae (1), | — | |
| 23 | <i>Geosmithia</i> sp. 23 | — | Moraceae (2), Rosaceae (3) | Ulmaceae (1) | Scolytid beetle on <i>Persea gratissima</i> (Seychelles), <i>Malus pumila</i> branches (Cyprus), this study |
| 24 | <i>Geosmithia</i> sp. 24 | Pinaceae (30) | — | — | <i>Pityogenes calcaratus</i> (occurred in 84 % of galleries), <i>Orthotomicus erosus</i> (21% galleries), all on <i>Pinus</i> in Israel. The species evidently contains two cryptic taxa based on <i>TEF1α</i> gene (Dori-Bachash et al. 2015) |
| 25 | <i>Geosmithia</i> sp. 25 | Pinaceae (4) | — | — | |
| 26 | <i>Geosmithia</i> sp. 26 | Pinaceae (14) | — | Pinaceae (1) | |
| 27 | <i>Geosmithia</i> sp. 27 | Pinaceae (2) | — | Pinaceae (1) | |
| 28 | <i>Geosmithia</i> sp. 28 | Pinaceae (1) | — | — | |
| 29 | <i>Geosmithia</i> sp. 29 | Pinaceae (2) | — | — | |
| 30 | <i>Geosmithia</i> sp. 30 | Pinaceae (15) | — | — | |
| 31 | <i>Geosmithia</i> sp. 31 | Pinaceae (8) | — | Pinaceae (1) | |
| 32 | <i>Geosmithia</i> sp. 32 | Cupressaceae (1) | Oleaceae (1) | Cupressaceae (1) | |
| 33 | <i>Geosmithia</i> sp. 33 | — | — | Pinaceae (1) | |
| 34 | <i>Geosmithia</i> sp. 34 | — | — | Cupressaceae — Calocedrus (1), Pinaceae (1) | |
| 35 | <i>Geosmithia</i> sp. 35 | — | — | Pinaceae (1) | |
| 36 | <i>Geosmithia</i> sp. 36 | — | — | Pinaceae (1) | |
| 37 | <i>Geosmithia</i> sp. 37 | — | — | Pinaceae (1) | |
| 38 | <i>Geosmithia</i> sp. 38 | — | — | Fagaceae (2) | |
| 39 | <i>Geosmithia</i> sp. 39 | — | — | Juglandaceae (2) | |
| 40 | <i>Geosmithia</i> sp. 40 | — | — | Pinaceae (6) | |
| 41 | <i>Geosmithia</i> sp. 41 | — | — | Anacardiaceae (1), Lauraceae (1), Asteraceae (1), Pinaceae (3), Fagaceae (14), Lauraceae (2), Rosaceae (1) | |
| 42 | <i>Geosmithia</i> sp. 42 | — | — | Cupressaceae (1), Pinaceae (1), Rosaceae (3) | |
| 43 | <i>Geosmithia</i> sp. 43 | — | — | Pinaceae (1) | |
| 44 | <i>Geosmithia</i> sp. 44 | — | — | Cupressaceae (1), Pinaceae (1) | |
| — | <i>G. microcorthyli</i> | — | — | — | ambrosia beetle <i>Microcorthyli</i> sp. (Costa Rica) (Kolařík and Kirkendall 2010) |
| — | <i>G. eupagioceri</i> | — | — | — | ambrosia beetle <i>Eupagiocerus dentipes</i> (Costa Rica) (Kolařík and Kirkendall 2010) |
| — | <i>G. cnesini</i> | — | — | — | ambrosia beetle <i>Cnesinus lecontei</i> (Costa Rica) (Kolařík and Kirkendall 2010) |
| — | <i>G. morbida</i> | — | — | Juglandaceae (5) | Widespread in whole U.S. (Kolařík et al. 2011, Hadziabdic et al. 2012). Newly recorded from Italy (Montecchio and Faccoli 2014) |
| — | <i>G. rufescens</i> | — | — | — | ambrosia beetles <i>Eupagiocerus dentipes</i> and <i>Cnesinus lecontei</i> (Costa Rica) (Kolařík and Kirkendall 2010) |
| — | <i>Geosmithia pallida</i> s. s. | — | — | — | cotton yarn (England), soil (Nigeria), (Pitt 1979) (this study) |
| — | <i>Geosmithia</i> sp. | — | — | — | Fungal associate of the ambrosia beetle <i>Scolytoplatypus fasciatus</i> from <i>Virgilia oroboides</i> (South Africa). It belongs to <i>G. pallida</i> group based on ITS rDNA sequences (Machingambi et al. 2014) |
| — | | | | | Species sister to <i>Geosmithia</i> sp. 24 from which differs in <i>TEF1α</i> gene. It is an associate of <i>Pinus</i> infesting bark beetles in Israel (Bachash et al. 2015). |
| — | <i>Geosmithia tibetensis</i> | — | — | — | Isolated from soil (Tibet) (Wu et al. 2013). Position in <i>Geosmithia</i> |

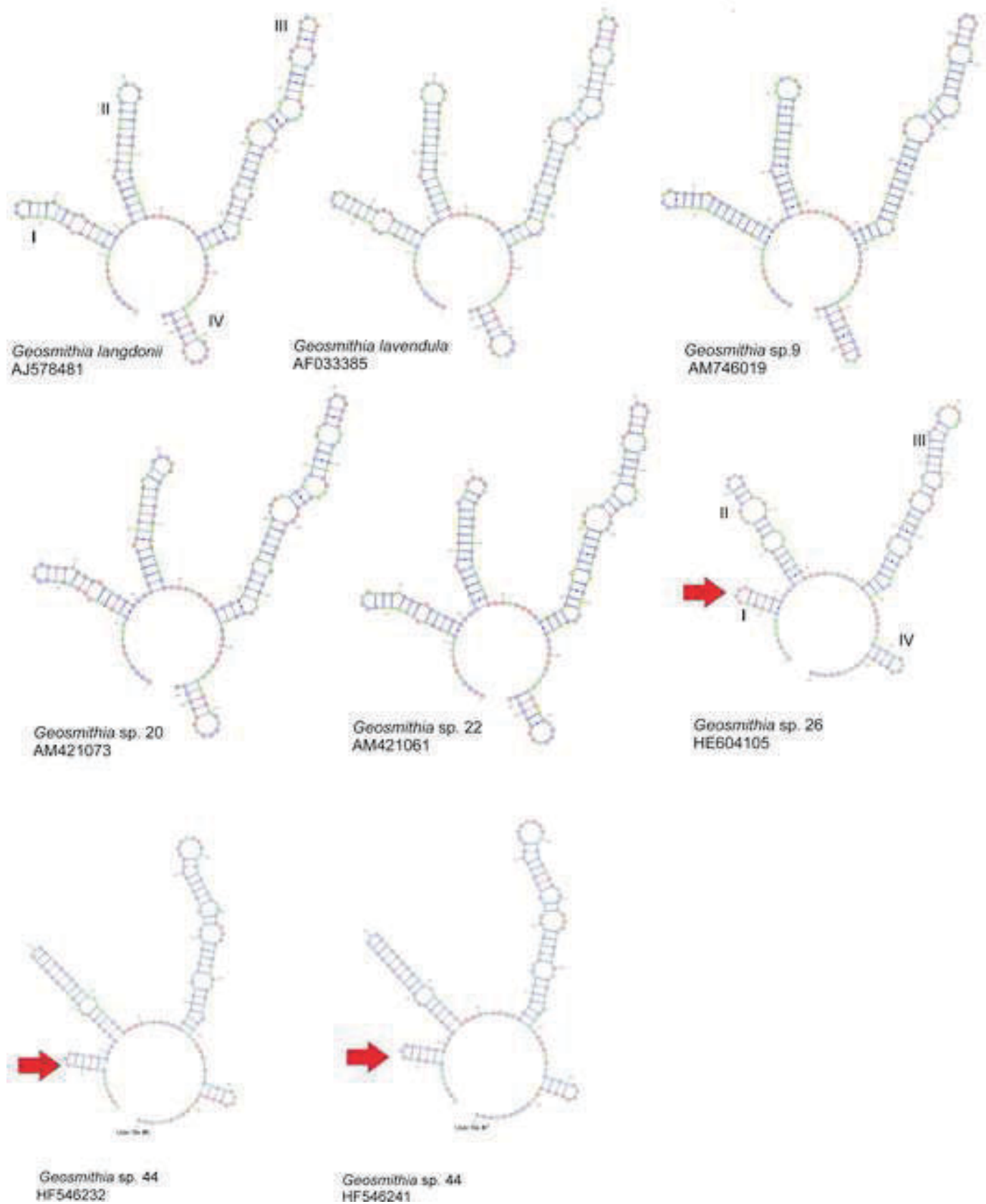
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Supplementary figure 2.

Secondary structure of the ITS2 rDNA of *Geosmithia* sp. 26 compared with five other *Geosmithia* species. The structures were modelled and driven in Mfold (*Geosmithia* sp. 26) and the ITS Database (other species).



Supplementary material I. Identity of the fungus published by Wright (1938) as *Spicaria anomala*.

Title of the paper: *Geosmithia* associated with bark beetles and woodborers in the western USA:

Taxonomic diversity and vector specificity

Authors: Kolařík M., Hulcr J., Tisserat N., De Beer W., Kostovčík M., Kolaříková Z., Seybold S. J., Rizzo D. M.

Ernest Wright published the finding of a hyphomycete isolated from the galleries of *Scolytus praeceps* and *Scolytus subscaber* collected from *Abies concolor* in California. This fungus was determined by him as *Spicaria anomala*, but it is *Geosmithia* in fact. Thus, the Wright's study is the first published report about *Geosmithia* bark beetle association.

Abundance of association (Wright 1938)

It was isolated from 66 *S. praeceps* adults from the total number of 75 individuals captured into the cages after the beetle emergence (i.e. 88% of adults). Further it was isolated from the stained wood and bark surrounding attacks of *Scolytus praeceps* (68 out of 76 attacks; 89%) and *S. subscaber* (13 out of 18 attacks; 72%). No adults were studied in the case of *S. subscaber*. Wright reports that: "Fructification of the *Spicaria* staining fungus occurs regularly with the respective beetle galleries in the form of small, whitish masses of spores. These spores aggregations can be readily mistaken from fresh *Scolytus* beetle frass." He concluded that the fungus was the predominant fungus associated with *S. praeceps*. He also reports that this fungus was isolated from the tops of Douglas fir infested with smaller *Scolytus* species (p. 764)

Original description of *S. anomala* provided by Wright (1938)

His description of the fungus is as follows: "Under the low power of a binocular microscope (20 X), the upright stalks with the accompanying chains of spores resemble miniature, white, bushy trees. Under higher power (40 X) the branches are seen to occur verticillately in threes around the conidiophore stalks with ultimate ramuli also generally in threes. The spores are borne in great profusion as single chains from the apex of each ramulus. Individual spores are hyaline, ovate, and 5 μ to 6 μ in diameter. In potato-dextrose agar (pH 5.5) the submerged hyphae of *S. anomala* are plainly septate, and in old cultures (5 months) attain a maximum width of 5.5 μ , averaging approximately 3.0 μ . Aerial conidiophore stalks average about 3.5 μ . in diameter, with branches occurring at a height of 20 μ . to 40 μ . The stalks are usually not more than five-septate. The total height of the conidiophores with chains attached is approximately 150 μ (fig. 2)".

Spicaria anomala formed white to cream-colored, zonate colonies and readily produced conidiophores and chains of conidia on all the media on which it was grown (malt, potato-dextrose, corn-meal, and Czapek's agars) (fig. 3). A brown coloration was imparted to the medium, particularly to potato-dextrose agar, increasing in intensity with the age of the culture. Since the hyphae remained hyaline, the coloration of the agar probably was due to the break-down of certain nutrients. Coloration was less intense in the other agars used. It may be significant that *Trichosporium symbioticum* hyphae were colored brown most readily in malt agar and less readily in potato dextrose agar. Microscopic examinations of sections of infected wood stained by the Cartwright method (1) showed the hyphae of *Spicaria anomala* in the tracheids and medullary my cells. No conidiophore formation was seen, however, within the tracheids all reported for *Trichosporium symbioticum*. The hyphae were very delicate as compared with those of other staining fungi, but were well distributed throughout the stained areas. They regularly entered the medullary rays and tracheids through the pits. They did not appear, however, to concentrate in the ray parenchyma to the same extent as do the blue staining fungi (*Ceratostomella* spp.). No method of differential staining that clearly showed the presence of the fungus hyphae in the phloem was found; therefore isolations proved the only reliable evidence of their presence in these tissues.

Figure 1. Micromorphology of *S. anomala* reprinted from the Wright (1938). The shape and septation of the conidiophore basis with prominent scar, typical for *Geosmithia*, is highlighted by arrows.

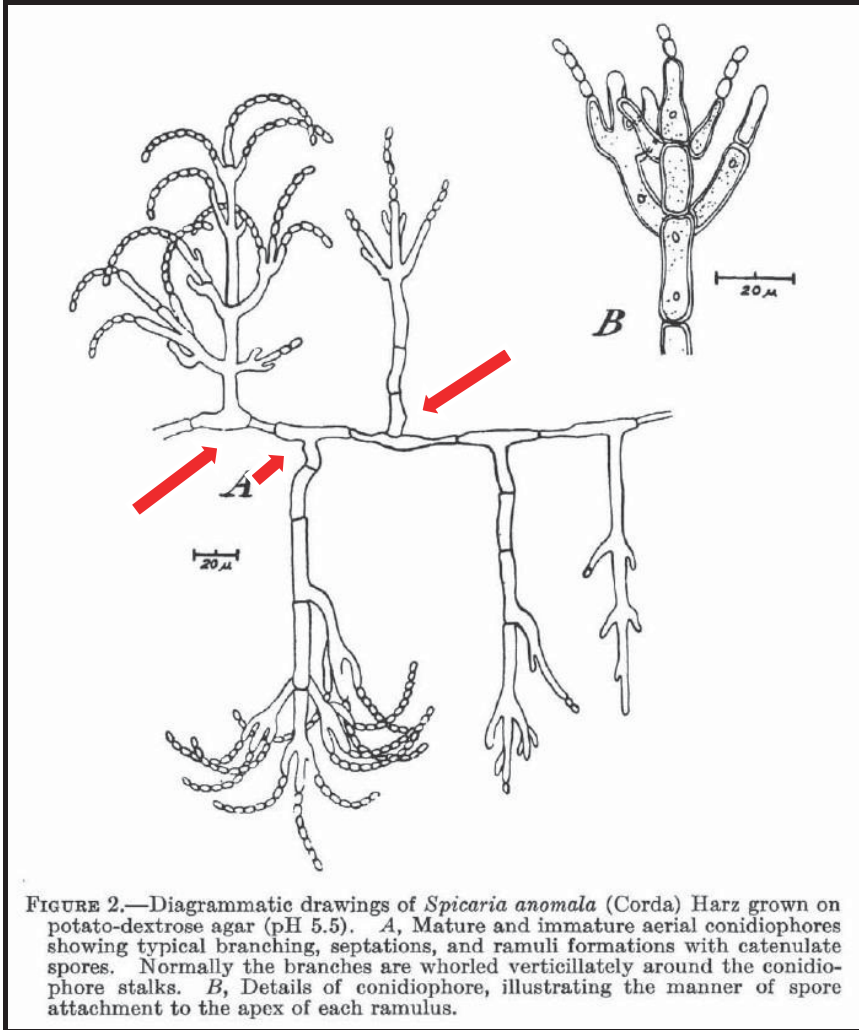
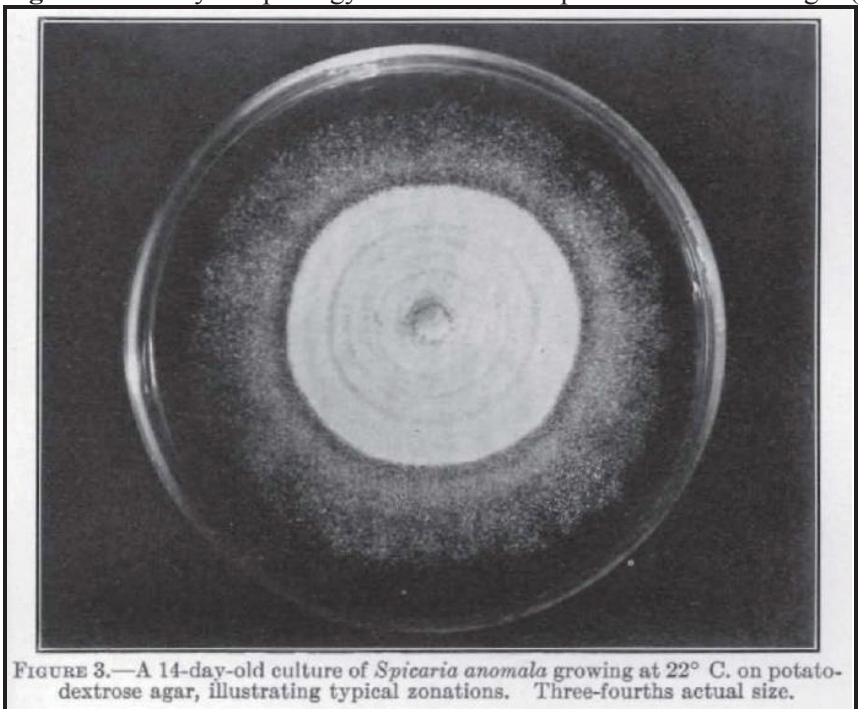


Figure 2. Colony morphology of *S. anomala* reprinted from the Wright (1938).



Comments on the identity of *S. anomala*

Wright's description of *S. anomala* fits to general morphology of *Geosmithia*. We do not know any other fungus, especially those from bark beetle galleries (eg. *Clonostachys rosea* or *Nectria mariannaeae*) similar to this description. Colony morphology of both fungi is characterized by cream white sporulation, zonation, smooth surface, abundant production of conidiophores with long and persistent chains of dry conidia. Apparent production of the pigment into the medium is also shared by both taxa (Figure 2, 3).

Concerning micromorphology. Wright depicted the typical *Geosmithia* shape of conidiogenous cells, which are cylindrical abruptly tapering towards the apex (without long neck). This character together with cylindrical to ellipsoidal shape of conidia is an outstanding character for *Geosmithia* (Pitt 1979). Harwood associated *Geosmithia* species such as *G. langdonii* or *G. putterillii* have regularly and often symmetrically branched penicillus with roughened cell walls (Kolařík et al. 2004, 2005). No such regular organization of the conidiophores is typical for the most of *Geosmithia* from Pinaceae. These species typically have smooth cell walls (or only inconspicuously roughened), disorganized conidiophores which are less sporulating, having more rounded conidia and with small number of hyphal elements (such as rami or ramiculi and phialides) at each branching point. Other typical feature of *Geosmithia*, seen also in Wright's description (Figure 1, marked here by arrows), is the specific shape and septation of the conidiophore basis. In *Geosmithia*, the septa delimiting stipes from fertile hypha are located randomly, very often not immediately in the ramification and then superficially resembling *Aspergillus* foot cells. In addition, the conidiophore basis is often peg shaped or irregularly curved, bearing the scar after the other collapsed hyphal elements (Kolařík et al. 2004, 2005, 2011) (Figure 4). All these characters, including dimension of conidia (5-6 µm) and conidiophores are consistent with Wright description and we conclude that his fungus was *Geosmithia* in fact. This is the first published record about the association of *Geosmithia* with bark beetles.

Figure 3. Examples of colony morphology of *Geosmithia* species specifically associated with bark beetles feeding on Pinaceae.

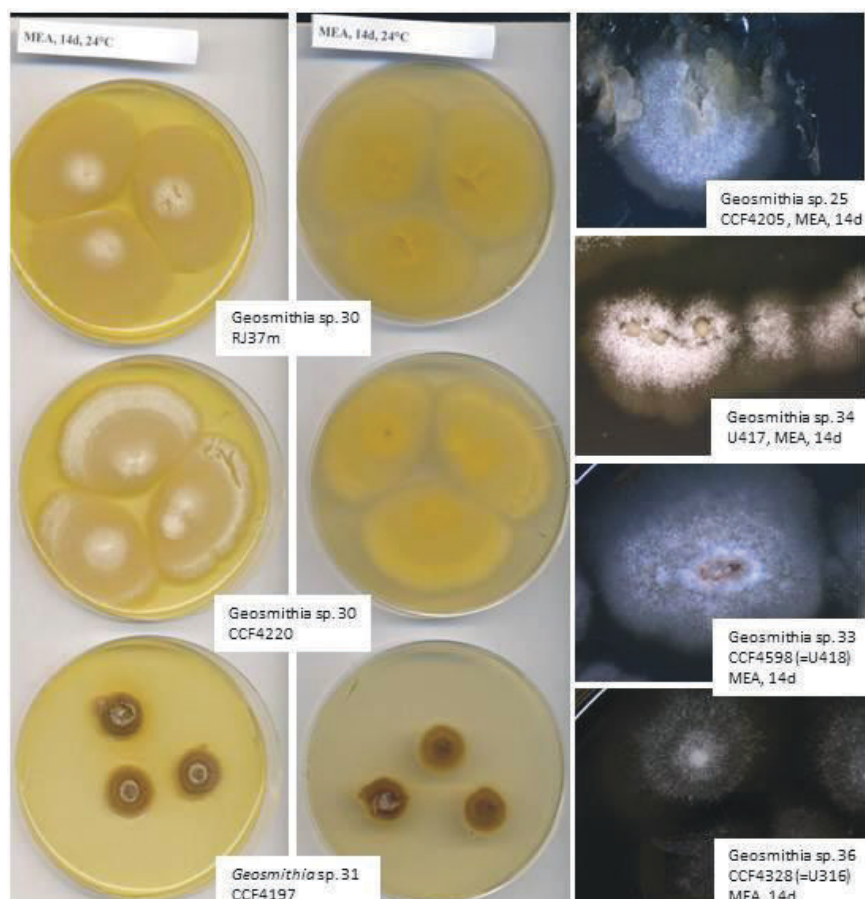
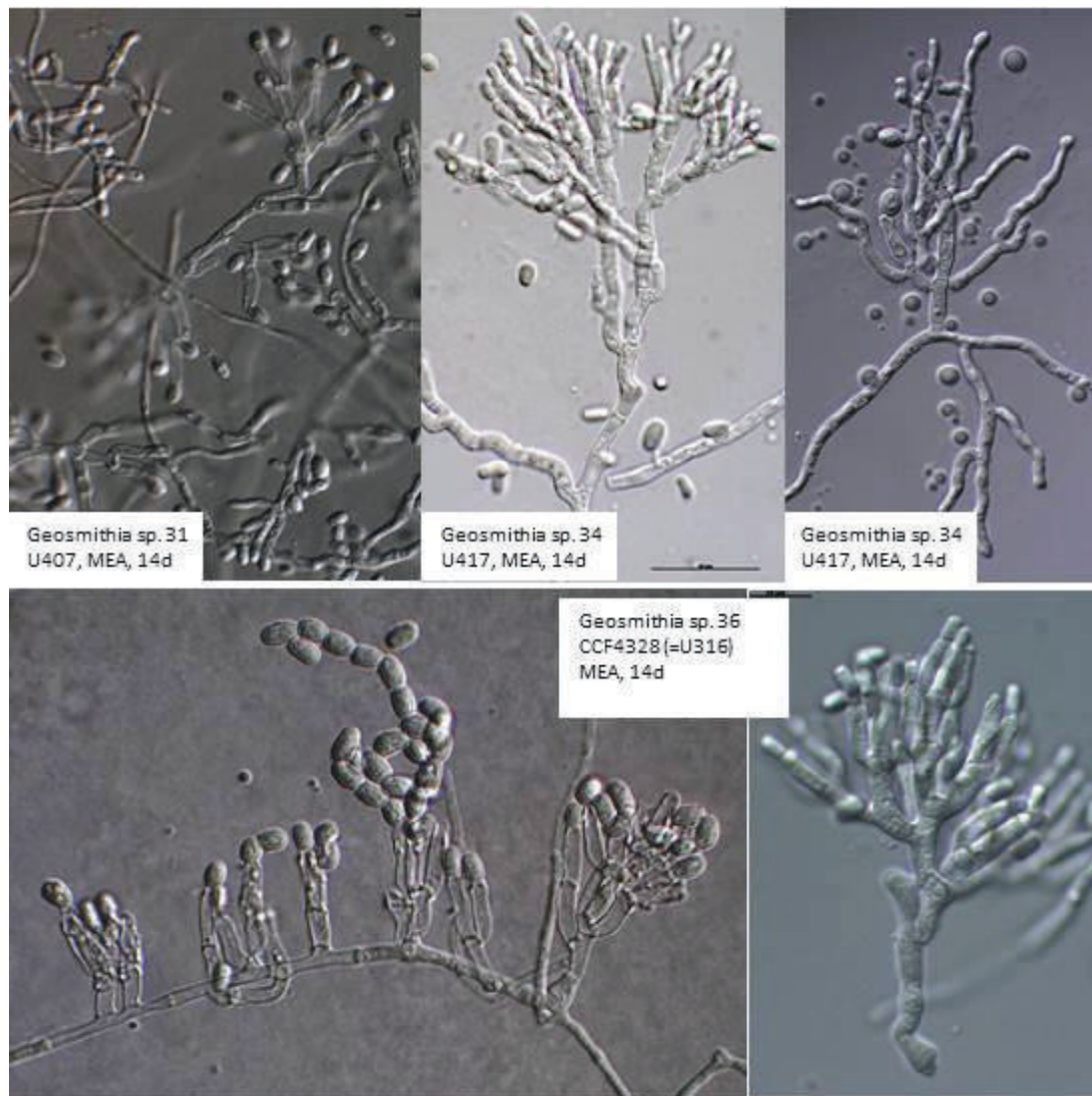


Figure 4. Examples of micromorphology of *Geosmithia* species specifically associated with bark beetles feeding on Pinaceae.



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