Geosmithia Species Associated With Bark Beetles From China, With the Description of Nine New Species

Xiuyu Zhang†, You Li†, Hongli Si†, Guoyan Zhao†, Miroslav Kolařík†, Jiri Hulcr†, Xiaqian Jiang†, Meixue Dai* and Runlei Chang*†

†College of Life Sciences, Shandong Normal University, Jinan, China, ‡Vector-Borne Virus Research Center, Fujian Province Key Laboratory of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou, China, §College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, China, ¶School of Forests, Fisheries and Geomatics Sciences, University of Florida, Gainesville, FL, United States, #Institute of Microbiology, Czech Academy of Sciences, Prague, Czechia

Fungi of the genus Geosmithia are frequently associated with bark beetles that feed on phloem on various woody hosts. Most studies on Geosmithia were carried out in North and South America and Europe, with only two species being reported from Taiwan, China. This study aimed to investigate the diversity of Geosmithia species in China. Field surveys in Fujian, Guangdong, Guangxi, Hunan, Jiangsu, Jiangxi, Shandong, Shanghai, and Yunnan yielded a total of 178 Geosmithia isolates from 12 beetle species. The isolates were grouped based on morphology. The internal transcribed spacer, β-tubulin, and elongation factor 1-α gene regions of the representatives of each group were sequenced. Phylogenetic trees were constructed based on those sequences. In total, 12 species were identified, with three previously described species (Geosmithia xerotolerans, G. putterillii, and G. pallida) and nine new species which are described in this paper as G. luteobrunnea, G. radiata, G. brevistipitata, G. bombycina, G. granulata (Geosmithia sp. 20), G. subfulva, G. pulverea (G. sp. 3 and Geosmithia sp. 23), G. fusca, and G. pumila sp. nov. The dominant species obtained in this study were G. luteobrunnea and G. pulverea. This study systematically studied the Geosmithia species in China and made an important contribution to filling in the gaps in our understanding of global Geosmithia species diversity.

INTRODUCTION

Members of Geosmithia are widely distributed fungal associates of phloem- and xylem-feeding beetles (Pitt, 1979; Kolařík et al., 2007, 2017; Lin et al., 2016), such as species in Bostrichidae and Curculionidae-Scolytinae (Coleoptera) (Juzwik et al., 2015; Kolařík et al., 2017). Geosmithia species are predominantly isolated from phloem-feeding bark beetles on broadleaved and conifer trees although they have been documented from many other substrates including soil...
(Kolářík et al., 2004), seed-feeding beetles (Huang et al., 2017), animal skin (Crous et al., 2018), indoor environment (Crous et al., 2018), insect-free plant tissues (McPherson et al., 2013), and food materials (Pitt and Hocking, 2009). To date, almost 60 phylogenetic and 21 formally described Geosmithia species have been recognized (Strzalka et al., 2021).

Geosmithia is similar to Penicillium and Paecilomyces in morphology, but it can be distinguished by the combination of stipe with or without a curved basal cell, verrucose conidiophores (including phialide), cylindrical phialide shape with a very short and cylindrical neck (collula), and ellipsoidal or cylindrical conidia (except globose conidia in Geosmithia eupagioceri and G. microcarpophylly). The colony color could be in shades of white, yellow, brown, or red but never bluish-green or green (Kolářík et al., 2004; Kolářík and Kirkendall, 2010).

The spores of Geosmithia may be transmitted by attaching to the surface of the beetle vector, but the ecological role of most Geosmithia species in symbiosis with bark beetles is still unclear. Some species serve as the main food source or supplementary nutrition for the beetles (Kolářík and Kirkendall, 2010; Machingambi et al., 2014), but most are probably commensals with minimal or no benefit to the beetle (Veselská et al., 2010; Machingambi et al., 2014), but most are probably commensals with minimal or no benefit to the beetle (Veselská et al., 2019) because the vector beetles show neither any apparent morphological adaptation nor nutrient dependence (Huang et al., 2017, 2019). Some Geosmithia species exhibit extracellular antimicrobial and antifungal metabolites, but their ecological implications are unknown (Stodůlková et al., 2009; Veselská et al., 2019).

Some Geosmithia species can cause serious tree diseases. One example is the thousand cankers disease (TCD) of walnuts caused by G. morbida (Kolářík et al., 2011). Following high-density colonization by its beetle vector, the walnut twig beetle (Pityophthorus juglandis), in the phloem of walnut (Juglandis spp.) or wingnut (Pterocarya spp.) trees, G. morbida causes numerous small lesions which eventually girdle the vascular tissue (Tisserat et al., 2009; Kolářík et al., 2011; Utley et al., 2012; Seybold et al., 2013; Hishinuma et al., 2015). TCD has affected many walnut trees in North America, especially in the western United States (Tisserat et al., 2009, 2011), and has recently been detected in Europe (Montecchio et al., 2014). Another mildly pathogenic species Geosmithia sp. 41 causes mild pathogenicity in Quercus argifolia (Kolářík et al., 2017), originally reported as G. pallida (Lynch et al., 2014).

After the discovery of the Geosmithia–beetle association (Kirschner, 2001), there has been an accumulation of reports describing Geosmithia fungi from phloem-feeding bark beetles around the world (Kolářík et al., 2004, 2007, 2008; Kubátová et al., 2004; Kolářík et al., 2005; Kolářík and Jankowiak, 2013; McPherson et al., 2013; Jankowiak et al., 2014; Machingambi et al., 2014; Pepori et al., 2015; Huang et al., 2019; Strzalka et al., 2021). Fungal communities associated with phloem-infected bark beetles are formed by a variety of biological and abiotic factors. The tree host is one of the most important selection factors (Skelton et al., 2018). Like other beetle-vectored fungi such as the ophiostomatoid fungi (Seifert et al., 2013), Geosmithia species display variable degrees of specificity to their beetle vectors and tree hosts, ranging from generalists to single-species specialists (Kolářík et al., 2007, 2008; Kolářík and Jankowiak, 2013; Jankowiak et al., 2014; Veselská et al., 2019). Other factors affecting the fungal community structure include beetle ecology, surrounding host tree community, and climatic factors (Six and Bentz, 2007; Jankowiak et al., 2014). These factors also influence the communities of Geosmithia, most notably by the fact that different beetles infesting the same host tree have similar Geosmithia assemblages (Kolářík et al., 2008; Machingambi et al., 2014).

At present, most of the studies of Geosmithia were conducted in North and South America and Europe, but the mycoflora of Asian bark beetles remain understudied. This study investigated the Geosmithia species from China using phylogenetic analyses and morphological and physiological features, and nine Geosmithia new species are described to fill the gap in our understanding of the global Geosmithia diversity.

**MATERIALS AND METHODS**

**Sampling, Isolating, and Preserving Fungal Isolates**

The beetle gallery samples were collected in Fujian, Guangdong, Guangxi, Hunan, Jiangsu, Jiangxi, Shandong, Shanghai, and Yunnan Province from plant hosts of Altingia gracilipes (Altingiaceae), Gnetum luofuense (Gnetaceae), Lauraceae sp., Liquidambar formosana (Altingiaceae), L. styriaciflua (Altingiaceae), Erriobotrya japonica (Rosaceae), Acacia pennata (Mimosaceae), Rhus chinensis (Anacardiaceae), Cupressus funebris (Cupressaceae), and Ulmus spp. (Ulmaceae) and kept individually in sealable bags (Table 1). The adult beetles were individually placed in Eppendorf tubes. Both galleries and adult beetles were kept at 4°C for further isolation. The beetle vectors included three groups: (1) Curculionidae-Scolytinae: Acanthothomus suscei, Scolytus jiulianshanensis (Curculionidae-Scolytinae), S. semenovi, Microperus sp. L589, Cryptalus eriobotryae, C. kyotoensis, and Phloeosinus sp. and P. cf. hopei, (2) Curculionidae-Platypodinae: Crossoptarsus emancipates, and (3) Bostrichoidea: Dinoderus sp. L489, Sinoxyylon cf. cumumellae and Xylocis torticollis (Table 1). The fungal isolates were obtained by scraping wood tissue from the beetle galleries and crushing the beetle coating. The isolates were inoculated on 2% malt extract agar [MEA: 20 g agar (Solarbio, China), 20 g malt extract (Hopebio, China), and 1 L deionized water] amended with 0.05% streptomycin. The cultures were purified by hyphal-tip subculturing and incubated at 25°C. All the cultures obtained in this study were deposited in the culture collection (SNM) of Shandong Normal University, Jinan, Shandong Province, China. Isolates linked to type specimens of the fungal species were deposited in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. The holotype specimens (dry cultures) were deposited in the Herbarium Mycologicum, Academiae Sinicae (HMAS), Beijing, China (Table 2).
**TABLE 1** | Distribution and number of species of *Geosmithia* among 178 isolated strains.

| *Geosmithia* species | Location | Tree host | Beetle species | Beetle groups | Gallery/beetle | No. |
|----------------------|----------|-----------|----------------|---------------|----------------|-----|
| G. bombycina (2)     | Fujian   | *Eriobotrya japonica* | *Cryphalus eriobotryae* | Curculionidae-Scolytinae | Gallery | 2 |
| G. brevistiptata (18) | Shandong | *Cupressus funebris* | *Phloeosinus cf. hopehi* | Curculionidae-Scolytinae | Gallery | 18 |
| G. fusca (26)        | Yunnan   | *Acacia penna* | *Sinoxylo* cf. *cucumella* | Bostrichidae | Gallery | 8 |
|                     | Guangdong | *Phylanthus emblica* | *Xylocis tortilicornis* | Bostrichidae | Gallery | 10 |
|                     |           | *Hibiscus tiliceus* | *Epomoropus japonicus* | Curculionidae-Scolytinae | Gallery | 8 |
| G. granulata (30)    | Yunnan   | *Acacia penna* | *Sinoxylo* cf. *cucumella* | Bostrichidae | Gallery | 2 |
|                     | Guangdong | *Hibiscus tiliceus* | *Epomoropus japonicus* | Curculionidae-Scolytinae | Gallery | 26 |
|                     | Jiangsu  | *Ulmus sp.* | *Scolytus semenovi* | Curculionidae-Scolytinae | Gallery | 2 |
| G. luteobrunnea (39) | Jiangxi  | *Liquidambar formosana* | *Acanthotomicus suncei* | Curculionidae-Scolytinae | Gallery | 25 |
|                     |         |           | *Ulmus sp.* | *Scolytus julianrhanensis* | Curculionidae-Scolytinae | Gallery | 5 |
|                     |         |           | *Liquidambar styraciflua* | *Acanthotomicus suncei* | Curculionidae-Scolytinae | Gallery | 8 |
| G. pallida (2)       | Yunnan   | *Acacia penna* | *Sinoxylo* cf. *cucumella* | Bostrichidae | Gallery | 2 |
| G. pulverea (33)     | Guangdong | *Gnetum luofuense* | *Dinoderus sp.* | Bostrichidae | Gallery | 1 |
|                     | Shanghai | *Liquidambar styraciflua* | *Acanthotomicus suncei* | Curculionidae-Scolytinae | Gallery | 1 |
|                     | Yunnan   | *Acacia penna* | *Sinoxylo* cf. *cucumella* | Bostrichidae | Gallery | 1 |
|                     | Guangxi  | Unknown    | *Crososatarsus emanciopatus* | Curculionidae-Platypodinae | Gallery | 2 |
|                     | Hunan    | Unknown    | *Microperus sp.* L589 | Curculionidae-Scolytinae | Gallery | 1 |
| G. putterilli (6)    | Jiangxi  | *Eriobotrya japonica* | *Cryphalus eriobotryae* | Curculionidae-Scolytinae | Gallery | 4 |
|                     | Jiangsu  | *Rhus chinensis* | *Cryphalus kyotoensis* | Curculionidae-Scolytinae | Gallery | 1 |
|                     |         | *Ulmus sp.* | *Scolytus semenovi* | Curculionidae-Scolytinae | Gallery | 4 |
|                     |         | *Liquidambar formosana* | *Acanthotomicus suncei* | Curculionidae-Scolytinae | Gallery | 1 |
|                     |         | Unknown    | *Phloeosinus sp.* | Curculionidae-Scolytinae | Gallery | 6 |
|                     |         | *Ulmus sp.* | *Scolytus julianrhanensis* | Curculionidae-Scolytinae | Gallery | 1 |
|                     |         | *Altingia gracilipes* | *Acanthotomicus suncei* | Curculionidae-Scolytinae | Gallery | 4 |
| G. purpurea (2)      | Jiangxi  | *Ulmus sp.* | *Scolytus semenovi* | Curculionidae-Scolytinae | Gallery | 2 |
| G. putterillii (6)   | Jiangxi  | *Laureaceae* | *Phloeosinus sp.* | Curculionidae-Scolytinae | Gallery | 6 |
| G. radiata (14)      | Jiangxi  | *Liquidambar formosana* | *Acanthotomicus suncei* | Curculionidae-Scolytinae | Gallery | 7 |
|                     |         | Unknown    | *Phloeosinus sp.* | Curculionidae-Scolytinae | Gallery | 1 |
|                     |         | *Ulmus sp.* | *Scolytus julianrhanensis* | Curculionidae-Scolytinae | Gallery | 1 |
|                     |         | *Altingia gracilipes* | *Acanthotomicus suncei* | Curculionidae-Scolytinae | Gallery | 6 |
| G. subfulva (5)      | Guangdong | *Hibiscus tiliceus* | *Epomoropus japonicus* | Curculionidae-Scolytinae | Gallery | 2 |
|                     | Fujian   | *Rhus chinensis* | *Hypothenemus sp.* L636 | Curculionidae-Scolytinae | Gallery | 2 |
|                     | Shandong | *Rhus chinensis* | *Cryphalus kyotoensis* | Curculionidae-Scolytinae | Gallery | 1 |
| G. xerotolerans (1)  | Shandong | *Cupressus funebris* | *Phloeosinus cf. hopehi* | Curculionidae-Scolytinae | Gallery | 1 |

**DNA Extraction, Amplification, and Sequencing**

DNA was extracted by scraping fresh fungal tissue from pure cultures and adding to 50 µl extraction solution of the PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA, United States). The samples were vortexed for 1 min, incubated at 100°C for 10 min, and then centrifuged at 5,000 rpm (MiniSpin Plus Centrifuge, Eppendorf 5453, Germany) for 5 min. The supernatant was transferred to a new Eppendorf tube and used as the template for polymerase chain reaction (PCR) amplification.

The rDNA region of ITS1-5.8S-ITS2, internal transcribed spacer (ITS), was amplified using the primer pair of ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). The translation elongation factor 1-α gene (TEF1-α) was amplified using the primer pair of EF1-983F and EF1-2218R (Rehner and Buckley, 2005). β-Tubulin (TUB2) was amplified by using T10 and Bt2b (Glass and Donaldson, 1995; O’Donnell and Cigelnik, 1997). The second-largest subunit of the RNA polymerase II gene (RPB2) was amplified using the primer pair of RPB2-5F/RPB2-7cR (Liu et al., 1999). The PCR amplifications were carried out in a final 25-µl PCR reaction mixture consisting of 50–100 ng template DNA, 1.25 U Taq polymerase (Vazyme Biotech Co., Ltd., Nanjing, China), 200 µM dNTP, 0.5 µM of each primer, and 5% (v/v) dimethyl sulfoxide. The PCR conditions were as follows: 95°C for 3 min, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The final extension step was 72°C for 10 min. The amplified products were sequenced in Sangon Biotech, Qingdao, Shandong Province, China.

**DNA Sequence Analyses**

The sequences obtained using the forward and reverse primers were aligned in Geneious version 10.2.2 (Biomatters, Auckland, New Zealand). The reference sequences of *Geosmithia* species
TABLE 2 | Cultures examined in this study and their GenBank accession numbers.

| Species          | Isolation no. | Beetle vectors | Tree host                  | ITS            | TEF1-α        | TUB2          | RPB2          | References                  |
|------------------|---------------|----------------|---------------------------|----------------|---------------|---------------|---------------|------------------------------|
| G. bombycine     | SNM934        | C. erobotryae   | Eriobotrya japonica       | MZ519396       | MZ514871      | MZ514862      | OL825679      | Present study                |
|                  | SNM933        | C. erobotryae   | E. japonica               | MZ519395       | MZ514870      | MZ514861      | OL825678      | Present study                |
| G. brevistipitata| SNM1616       | Phloeosinus cf. hopehi | Cupressus funebris      | OK584392       | OK832357      | OK632375      | OL825675      | Present study                |
|                  | SNM1610       | Phloeosinus cf. hopehi | C. funebris              | OK584393       | OK832356      | OK632373      | OL825677      | Present study                |
|                  | SNM1611       | Phloeosinus cf. hopehi | Cupressus funebris      | OK584394       | OK832355      | OK632374      | OL825676      | Present study                |
| G. brunnea       | CBS 142634    | Xylosandrus compactus | Liquidambar styraciflua | KY872741       | KY872747      | KY872751      | KY882266      | Huang et al., 2017           |
|                  | CBS 142635    | X. compactus    | L. styraciflua            | KY872742       | KY872747      | KY872752      | KY882268      | Huang et al., 2017           |
| G. cnesini       | CCF 3753      | Cnesinus lecontei | Croton draco              | AM947670       |                |               |               | Kolarik and Kirkendall, 2010 |
|                  | MK 1820       | C. lecontei     | C. draco                  | AM947671       |                |               |               | Kolarik and Kirkendall, 2010 |
| G. eupagioceri   | MKA1-1-b      | Eupagiocerus dentipes | Paulinia renesi         | AM947666       |                |               |               | Kolarik and Kirkendall, 2010 |
|                  | CCF 3754      |                |                           |                | LRS55705      | LRS55704      |               | Kolarik et al., 2019*        |
| G. fagi          | CCF 6235      | Taphrochryxus bicolor | Fagus sylvatica         | LR812775       | LR813193      | LR813119      |               | Strzalka et al., 2021        |
|                  | 21114TBb      | T. bicolor      | F. sylvatica              | LR812776       | LR813120      |               |               | Strzalka et al., 2021        |
|                  | CCF 6234      | T. bicolor      | F. sylvatica              | LR812785       | LR813141      | LR813129      |               | Strzalka et al., 2021        |
| G. fassatiae     | AK 31/98      | S. intricatus   | Quercus sp.               | AM421039       | MH850557      |               |               | Kolarik et al., 2008         |
|                  | CCF 4331      |                |                           | HFS54239       | KFS58984      |               |               | Kolarik et al., 2012*        |
|                  | CCF 4340      |                |                           | HFS54284       | KFS58985      |               |               | Kolarik et al., 2012*        |
|                  | CCF 3334      |                | Quercus pubescens         | MH850530       |               |               |               | Kolarik et al., 2005         |
| G. flava         | CCF 3335      | Xyphidia sp.    | Castanea sativa           | AJ578483       | MH850541      |               |               | Kolarik et al., 2004         |
|                  | CCF4347      | Cerambycidae sp. | Pseudotsuga menziesii     | HFS54244       | MH850542      | KFS58987      |               | Kolarik et al., 2004         |
|                  | CCF3354      |                |                           |                | LRS55685      |               |               | Kolarik et al., 2019*        |
| G. fusca         | SNM1577       | Phylanthus emblica | Xylocis tortilicornis   | OK584387       | OK632359      | OK832371      | OL825662      | Present study                |
|                  | SNM1578       | Phy. Emblica    | Xylocis tortilicornis    | OK584388       | OK632358      | OK632370      | OL825661      | Present study                |
|                  | SNM17102      | Sinoxylon cf. cucumella | Acacia penata           | MZ519390       | MZ514866      | MZ514857      | OL825664      | Present study                |
| G. granulate     | SNM1015       | Sin. cf. cucumella | Ac. penata               | MZ519392       | MZ514865      | MZ514856      | OL825663      | Present study                |
|                  | SNM1013       | Sin. cf. cucumella | Ac. penata               | MZ519398       | MZ514873      | MZ514864      | OL825667      | Present study                |
| G. lavendulan    | CCF 3051      |                | Laboratory contamination | AF033885       |               |               |               | Kolarik et al., 2004         |
|                  | CCF 3394      | Carphoborus vestitus | Pistacia terebinthus    | AM421098       |               | MH850484      |               | Kolarik et al., 2007         |
|                  | CCF 4336      |                |                           |                | KFS53902      |               |               | Hamelin et al., 2013         |
| G. langdonii     | CCF 3332      | Scolytus intricatus | Quercus rubur           | KFS58297       | HG799876      | HG799887      | HG799928      | Kolarik et al., 2005; Kolarik et al., 2017 |
|                  | CCF 4338      | C. pubescens    | Sequoia sempervirens     | HFS54245       | HG799877      | HG799881      | HG799929      | Kolarik et al., 2017         |
| G. longistipitata| RJ278m        | Pityophthorus pityographus | Picea abies          | HE604124       |               |               |               | Strzalka et al., 2021        |
|                  | CCF 4210      | P. pityographus | P. abies                | HE604154       | LRS813194     | LRS813140     |               | Strzalka et al., 2021        |

(Continued)
| Species            | Isolation no. | Beetle vectors          | Tree host            | GenBank accession no. | References                        |
|--------------------|---------------|-------------------------|----------------------|-----------------------|-----------------------------------|
| G. luteobrunnea    | **SNM261**    | S. jiulianshanensis     | Ulmus sp.            | MW222399              | Present study                     |
|                    | **SNM226**    | A. suncei               | L. styraciflua       | MW222404              | Present study                     |
|                    | **SNM287**    | A. suncei               | L. styraciflua       | MW222393              | Present study                     |
|                    | **SNM256**    | A. suncei               | L. formosana         | MW222401              | Present study                     |
| G. microcorythyl    | **CCF 3861**  | Microcorythylus sp.     | Cassia grandis       | FM986796              | Kolarík and Kirkendall, 2010      |
| G. moribida        | **CBS 124664**| Jugalgen nigra          |                      | FN434081              | Kolarík and Kirkendall, 2010      |
|                    | CCF 3881      | Pythophorus juglandis   | J. nigra             | FN434082              | Kolarík and Kirkendall, 2010      |
| G. obscura         | **CCF 3422**  | S. intricatus           | Q. robur             | AJ784999              | Kolarík et al., 2005              |
|                    | **CCF 3425**  | S. carpini              | Carpinus betulus     | AM181460              | Kolarík et al., 2005              |
| G. omnicolor       | **MK 1707**   | Hylesinus orni          | Fraxinus sp.         | AM181452              | Kolarík et al., 2008              |
|                    | CCF 11015     | H. orni                 | Fraxinus sp.         | AM181450              | Kolarík et al., 2008              |
|                    | CNR 5         | H. orni                 | Fraxinus sp.         | AM181433              | Kolarík et al., 2008              |
| G. pallida         | **CCF 3053**  | Cotton yarn             |                      | AJ578486              | Kolarík et al., 2004, 2017        |
|                    | CCF 3324      | Soil                    |                      | HG799846              | Kolarík et al., 2004, 2017        |
|                    | **SNM1165**   | Sin. cf. cucumella      | Aca. pennata         | MZ519393              | Present study                     |
|                    | **SNM1166**   | Sin. cf. cucumella      | Aca. pennata         | MZ519394              | Present study                     |
| G. pazoutovae      | 22015aSI      | S. intricatus           | Q. robur             | LR812786              | Strzałka et al., 2021             |
|                    | 24Wa14S1      | S. intricatus           | Q. robur             | LR812787              | Strzałka et al., 2021             |
| G. proliferans     | **CBS 142636**| Phloeotribus frontalis  | Acer negundo         | KY872744              | Huang et al., 2017                |
|                    | CBS 142637    | P. frontalis            | A. negundo           | KY872745              | Huang et al., 2017                |
| G. pulvere          | **SNM885**    | Dinoderus sp.           | Gnetum lochusense    | MW222410              | Present study                     |
|                    | **SNM270**    | A. suncei               | L. formosana         | MW222398              | Present study                     |
|                    | **SNM248**    | A. suncei               | L. styraciflua       | MW222402              | Present study                     |
|                    | **SNM886**    | Crossotarsus emancipatus|                    | MW222411              | Present study                     |
|                    | **SNM887**    | C. emancipatus          |                    | MW222412              | Present study                     |
|                    | **SNM888**    | Microperus sp.          | Choerospondias axillaris | MW222409              | Present study                     |
| G. pulvere         | **SNM1653**   | Ulmus pumila            |                      | OK584389              | Present study                     |
|                    | **SNM1657**   | Ulmus pumila            |                      | OK584390              | Present study                     |
| G. puttleri        | **CCF 3052**  | Beltschromia tawa       | B. tawa              | AF033384              | Kolarík et al., 2004, 2017        |
|                    | U 307         | Beltschromia tawa       | B. tawa              | HF546306              | Kolarík et al., 2004, 2017        |
| G. radiata         | **SNM279**    | Phloeosinus sp.         |                    | MW584874              | Present study                     |
|                    | **SNM884**    | A. suncei               | L. formosana         | MW592407              | Present study                     |

(Continued)
| Species           | Isolation no. | Beetle vectors | Tree host | ITS         | TEF1-α     | TUB2       | RPB2       | References                          |
|------------------|---------------|----------------|-----------|-------------|------------|------------|------------|-------------------------------------|
| *Geosmithia*     |               |                |           |             |            |            |            | Kolarík and Kirkendall, 2010        |
|                  |               |                |           |             |            |            |            |                                    |
| **TABLE 2**      | **Continued** |                |           |             |            |            |            |                                    |
| **G. rufescens** | MK 1800       | C. lecontei    | C. draco  | AM947667    |            |            |            | Kolarík and Kirkendall, 2010        |
|                  | MK 1803       | C. lecontei    | C. draco  | AM947668    |            |            |            | Kolarík and Kirkendall, 2010        |
|                  | CCF 3752      | C. lecontei    | C. draco  | AM947669    |            |            |            | Kolarík and Kirkendall, 2010        |
|                  |                |                |           |            |            |            |            | Kolarík et al., 2019*               |
| **G. subfulva**  | SNM1304<sup>T</sup> | H. tiliae | E. japonicus | OK584385 | OK584386 | OK632363 | OK632362 | Present study                        |
|                  | SNM1298       | H. tiliae      | E. japonicus | OK583436 | OK583437 | OK632362 | OK632369 | Present study                        |
|                  | CCF 3559      | S. multistriatus | U. sp.   | AM181439    | MHS0535   |            |            | Kolarík et al., 2008                |
|                  | 1226          | S. schevyrewi  | U. sp.    | KJ716463    |            |            |            | Zerillo et al., 2014*               |
|                  | CNR23         |                | U. minor  | KP990560    |            |            |            | Pepori et al., 2015                 |
|                  | CNR24         |                | U. minor  | KP990561    |            |            |            | Pepori et al., 2015                 |
| **G. ulmacea**   | CCF 3559      | S. multistriatus | U. sp.   | AM181439    | MHS0535   |            |            | Kolarík et al., 2008                |
|                  |                |                |           |            |            |            |            |                                    |
| **G. xerotolerans** | CCF 5270    | S. oregoni     | P. menziesii | NR_169923  | MHS0534   |            |            | Kolarík et al., 2017                |
|                  | FMR 17085<sup>T</sup> |            |           |            |            |            |            |                                    |
|                  | CCF 4280      | H. ficus       | F. carica | AM421049    | MHS0533   |            |            | Kolarík et al., 2007                |
|                  |                |                |           |            |            |            |            |                                    |
| **SNM1618**      | CCF 4334      | Phloeosinus cf. hopehi | Cupressus funebris | OK584391 | OK632354 | OK632372 | - | Present study                        |
| **G. sp. 2**     | U107          | Scolytus rugulosus | Prunus sp. | HFS54256   | HG799855  | HG799818  | HG799910  | Kolarík et al., 2017                |
|                  | MK 642        | H. ohi         | Fraxinus ormus | HG799852   | HG799906  |            |            | Kolarík et al., 2017                |
| **G. sp. 3**     | CCF 4298      | S. intricatus  | Quercus daecheampi | AM181436  | HG799851  | HG799814  | HG799905  | Kolarík et al., 2008, 2017          |
|                  | CCF 3481      | Scolytus carpin | C. betulius | AM181467   | HG799842  | HG799805  | HG799896  | Kolarík et al., 2017                |
| **G. sp. 4**     | CCF 4278      | Pteleobius vittatus | U. laevis | AM181466   | HG799850  | HG799813  | HG799904  | Kolarík et al., 2008, 2017          |
| **G. sp. 5**     | CCF 3341      | S. intricatus  | Quercus petraea | AJ578487  | HG799837  | HG799801  | HG799891  | Kolarík et al., 2004, 2017          |
|                  | CCF 4215      | P. ptygographus | P. abies  | HE604117   |            | HGS999835 |            | Kolarík et al., 2013                |
|                  | AK192/96      | S. intricatus  | Q. robur  | HE604117   |            |            |            | Kolarík et al., 2017                |
| **G. sp. 6**     | CCF 3358      | S. intricatus  | Q. petraea | AM181421   | MHS0559   | FM986788  |            | Kolarík et al., 2007                |
| **G. sp. 9**     | CCF 3564      |                |           | AM181428   | AM746018  |            |            |                                    |
| **G. sp. 11**    | CCF 3702      |                |           | AM181428   | AM746018  |            |            |                                    |
|                  | RJ0266        |                |           | AM181419   | MHS0551   |            |            | Kolarík et al., 2010*               |
| **G. sp. 12**    | CCF 3555      | S. intricatus  | Q. pubescens | AM181419  | MHS0545   | KF853931  |            | Kolarík et al., 2008                |
|                  | CCF 3556      | S. intricatus  | Q. pubescens | AM181418  | MHS0545   |            |            | Kolarík et al., 2008                |
| **G. sp. 14**    | CCF 4320      | Hylaeinus orogens | Fraxinus sp. | HFS56229  | MHS0532   | KF853932  |            | Kolarík et al., 2017                |
|                  | CCF 3557      | Lepersinus orni | F. excelsior | AM181431  | MHS0531   |            |            | Kolarík et al., 2017                |
| **G. sp. 16**    | CCF 4201      | P. ptygographus | P. abies  | HE604146   | HE604206  | HE601818  | HE604234  | Kolarík et al., 2013                |
|                  | RJ34m         | P. ptygographus | P. abies  | HE604146   | HE604206  | HE604528  |            | Kolarík et al., 2013                |
| **G. sp. 19**    | CCF 3858      | Hypoborus ficus | Ficus carica | AM421085  | MHS0546   |            |            | Kolarík et al., 2007                |
|                  | CCF 3855      | H. ficus       | F. carica | AM421075   | MHS0547   |            |            | Kolarík et al., 2007                |
| **G. sp. 20**    | CCF 4316      | Phloeosinus fulgens | Calocedrus decurrens | HFS56226  | MHS0547   |            |            | Kolarík et al., 2007                |
|                  | U193          | Scolytus schevyrewi | U. pumila | HFS56287   | MHS0548   |            |            |                                    |

(Continued)
| Species | Isolation no. | Beetle vectors | Tree host | ITS | TEF1-α | TUB2 | RPB2 | References |
|---------|--------------|----------------|-----------|-----|--------|------|------|------------|
| Geosmithia sp. 22 | CCF 3645 | Phloeotribus scarabeoides | Olea europaea | AM421061 | MHS80552 | KF853941 | | Kolařík et al., 2007 |
| Geosmithia sp. 23 | CCF 3652 | P. scarabeoides | O. europaea | AM421062 | MHS80553 | | | Kolařík et al., 2007 |
| Geosmithia sp. 24 | MB136 | Scolytoid beetles | P. scarabeoides | AJ758489 | HG799808 | HG799899 | | Kolařík et al., 2004, 2017 |
| Geosmithia sp. 25 | MB242 | Pityogenes calcaratus | Prunus armeniaca | AM421068 | HG799838 | HG799892 | | Kolařík et al., 2004, 2017 |
| Geosmithia sp. 26 | MB322 | O. erosus | Prunus sylvestris | AM421069 | HG799840 | HG799893 | | Kolařík et al., 2017 |
| Geosmithia sp. 27 | CCF 4294 | Pityogenes quadridentis | P. sylvestris | AM421070 | HG799841 | HG799894 | | Kolařík et al., 2017 |
| Geosmithia sp. 28 | MK1772 | P. pityographus | P. sylvestris | AM421071 | HG799842 | HG799895 | | Kolařík et al., 2017 |
| Geosmithia sp. 29 | CCF 4205 | Cryphalus abietis | Abies alba | AM421072 | HG799843 | HG799896 | | Kolařík et al., 2017 |
| Geosmithia sp. 30 | MK1832 | C. piceae | A. alba | AM421073 | HG799844 | HG799897 | | Kolařík et al., 2017 |
| Geosmithia sp. 31 | CCF 4206 | Pityogenes bidentatus | A. alba | AM421074 | HG799845 | HG799898 | | Kolařík et al., 2017 |
| Geosmithia sp. 32 | CCF 4605 | Pityophthorus sp. | Pinus ponderosa | AM421075 | HG799846 | HG799899 | | Kolařík et al., 2017 |
| Geosmithia sp. 33 | CCF 4211 | C. piceae | A. alba | AM421076 | HG799847 | HG799900 | | Kolařík et al., 2017 |
| Geosmithia sp. 34 | CCF 4288 | L. decidua | A. alba | AM421077 | HG799848 | HG799901 | | Kolařík et al., 2017 |
| Geosmithia sp. 35 | CCF 4191 | P. pityographus | A. alba | AM421078 | HG799849 | HG799902 | | Kolařík et al., 2017 |
| Geosmithia sp. 36 | CCF 4598 | Scolytus praeceps | Abies concolor | AM421079 | HG799850 | HG799903 | | Kolařík et al., 2017 |
| Geosmithia sp. 37 | CCF 4204 | Ips plasticographus | C. decurrens | AM421080 | HG799851 | HG799904 | | Kolařík et al., 2017 |
| Geosmithia sp. 38 | U417 | S. praeceps | A. concolor | AM421081 | HG799852 | HG799905 | | Kolařík et al., 2017 |
| Geosmithia sp. 39 | U196 | Pityophthorus sp. | P. menziesii | AM421082 | HG799853 | HG799906 | | Kolařík et al., 2017 |
| Geosmithia sp. 40 | CCF 4328 | Pityophthorus sp. | P. menziesii | AM421083 | HG799854 | HG799907 | | Kolařík et al., 2017 |
| Geosmithia sp. 41 | MK1814 | Cedrus atlantica | | AM421084 | HG799855 | HG799908 | | Kolařík et al., 2017 |
| Geosmithia sp. 42 | U197 | Pityophthorus sp. | P. menziesii | AM421085 | HG799856 | HG799909 | | Kolařík et al., 2017 |
| Geosmithia sp. 43 | U79 | Pseudopityophthorus pubipennis | Notothalictocarpus densiflorus | AM421086 | HG799857 | HG799910 | | Kolařík et al., 2017 |
| Geosmithia sp. 44 | CCF 5241 | P. pubipennis | Quercus acutifolia | AM421087 | HG799858 | HG799911 | | Kolařík et al., 2017 |
| Geosmithia sp. 45 | U233 | P. juglandis | Juglans hindsii | AM421088 | HG799859 | HG799912 | | Kolařík et al., 2017 |
| Geosmithia sp. 46 | CCF 5250 | Pityophthorus sp. | Pinus ponderosa | AM421089 | HG799860 | HG799913 | | Kolařík et al., 2017 |
| Geosmithia sp. 47 | CCF 5245 | P. radiata | Pinus radiata | AM421090 | HG799861 | HG799914 | | Kolařík et al., 2017 |
| Geosmithia sp. 48 | U235 | Cossoninae sp. | Artemisia arborea | AM421091 | HG799862 | HG799915 | | Kolařík et al., 2017 |
| Geosmithia sp. 49 | U432 | Bostrichidae sp. | Toxicodendron diversilobum | AM421092 | HG799863 | HG799916 | | Kolařík et al., 2017 |
| Geosmithia sp. 50 | U64 | Scobicia declivis | Umbellularia californica | AM421093 | HG799864 | HG799917 | | Kolařík et al., 2017 |
| Geosmithia sp. 51 | U166 | P. canadensis | Chamaecyparis pisifera | AM421094 | HG799865 | HG799918 | | Kolařík et al., 2017 |
| Geosmithia sp. 52 | CCF 5251 | S. rugulosus | Prunus sp. | AM421095 | HG799866 | HG799919 | | Kolařík et al., 2017 |

(Continued)
TABLE 2

| GenBank accession no. | α | Species | Isolation no. | Beetle vectors | Tree host | ITS | TEF1-α | TUB2 | RPB2 | References |
|-----------------------|---|---------|---------------|----------------|-----------|-----|--------|------|------|------------|
| Geosmithia sp. 43     |   | G. eucalyptus | CCF 4203     | Pityogenes knechteli | *P. ponderosae* (HF546223) | HG799864 | HG799863 | HG799916 | Kolarčík et al., 2017 |
| Geosmithia sp. 44     |   | G. eucalyptus | CCF 4333     | Pityophthorus sp. | *P. sabiniana* (LN907598) | Kolarčík et al., 2017 |
| Geosmithia sp. 45     |   | G. eucalyptus | CCF 4332     | Pityophthorus sp. | *P. sabiniana* (LN907599) | Kolarčík et al., 2017 |
| Geosmithia sp. 46     |   | G. eucalyptus | Hulcr 17004  | Pityophthorus annectens | *P. taeda* (MH580482) | Huang et al., 2019 |
| Geosmithia sp. 47     |   | G. eucalyptus | Hulcr 17006  | Pityophthorus annectens | *P. taeda* (MH580487) | Huang et al., 2019 |
| Geosmithia sp. 48     |   | G. eucalyptus | Hulcr 18823  | Pityophthorus pulicarius | *P. taeda* (MH580505) | Huang et al., 2019 |
| Geosmithia sp. 49     |   | G. eucalyptus | Hulcr 11575  | Pseudopityophthorus minutissimus | *Q. laurifolia* (MH426748) | Huang et al., 2019 |
| Geosmithia sp. 50     |   | G. eucalyptus | Hulcr 18077  | Pseudopityophthorus minutissimus | *J. nigra* (MH426766) | Huang et al., 2019 |
| Geosmithia sp. 51     |   | G. eucalyptus | Hulcr 18201  | Pseudopityophthorus minutissimus | *J. nigra* (MH426776) | Huang et al., 2019 |
| Geosmithia sp. 52     |   | G. eucalyptus | Hulcr 19182  | Pseudopityophthorus minutissimus | *C. illinoinensis* (MH426789) | Huang et al., 2019 |
| Geosmithia sp. 53     |   | G. eucalyptus | Hulcr 19190  | Pseudopityophthorus minutissimus | *P. dentatus* (MH426797) | Huang et al., 2019 |
| Geosmithia sp. 54     |   | Emericellopsis pallida | CBS 490.71   | Emericellopsis pallida | *P. ponderosae* (NR_145052) | KC998998 |

The isolates recovered in the present study are in bold. Emericellopsis pallida was selected as the outgroup of phylogenies. Strains in italics were screened for morphological studies. *The sequences are available on NCBI but have not been published.*

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Morphological Study

Morphological characters were observed and recorded using Olympus BX61 microscope (Olympus Corporation, Tokyo, Japan). The images were analyzed using ImageJ. At least 50 measurements for each of the structures were measured. The results of the calculation are expressed as (minimum -) mean minus standard deviation – mean plus standard deviation – (maximum). One-way ANOVA in SPSS version 26.0 was used to evaluate the morphological differences of the different species, with a significance level of 0.05 (Supplementary Figure S5).

Growth Study

Three independently isolated strains of each novel taxon were randomly selected for the growth experiments. The actively growing edge mycelia were inoculated at the center of 90-mm Petri dishes containing 2% MEA and incubated in darkness at temperatures ranging from 5 to 35°C at 5°C intervals and 37°C for 8 days; each temperature had three duplicates. The colony diameters were measured every 2 days, and then the optimum temperature of growth for each species and the high- and low-temperature conditions of growth were calculated.

RESULTS

In total, 125 samples (N) were collected (Table 1). A total of 178 strains in the genus *Geosmithia* were isolated from 12 beetle species. The sequences were retrieved from GenBank (Table 2). *Emericellopsis pallida* CBS 490.71 was chosen as the phylogenetic outgroup. The sequences were aligned by using the online version of MAFFT v. 7 (Katoh and Standley, 2013) with the default settings. The best nucleotide substitution model for each partition was determined in jModelTest v. 2.1.1 (Darriba et al., 2012). Maximum parsimony (MP) analyses were performed using MEGA v.10.2.0 with 1,000 bootstrap replicates; gaps were treated as a fifth-state character. Maximum likelihood (ML) phylogenetic analyses were conducted in the CIPRES Science Gateway (Miller et al., 2010) using RAxML v. 8.2.2 (Stamatakis, 2014) with the recommended partition parameters to assess the tree topology and bootstrap values from 1,000 replicate searches. Bayesian inference (BI) was estimated in the CIPRES Science Gateway (Miller et al., 2010) using MrBayes 3.2.7a (Ronquist et al., 2012). The MCMC runs of four chains were executed simultaneously from a random starting tree for 20 million generations, and every 100 generations were sampled, resulting in 200,000 trees. Chain convergence was determined with Tracer 1.7, and the effective sample size values over 200 are considered adequate. A total of 50,000 trees were discarded during burn-in. Posterior probabilities were estimated from the retained 150,000 trees. Phylogenetic trees were visualized and edited in FigTree v. 1.4.3. The final alignments used in this study have been submitted to TreeBase (nos.: 28242).

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1. http://tree.bio.ed.ac.uk/software/tracer
2. https://www.treebase.org/
3. https://imagej.net/
4. https://www.ibm.com/analytics/spss-statistics-software
species and their galleries. One hundred fifty-eight strains were from the galleries and 20 strains were from the beetles. There were 63 strains from Jiangxi, 47 from Guangdong, 23 from Shandong, 20 from Yunnan, 9 from Shanghai, 8 from Jiangsu, 5 from Fujian, 2 from Guangxi, and 1 from Hunan (Table 1).

**Phylogenetic Analysis**

The preliminary classification was carried out by BLAST on NCBI GenBank using the ITS marker (Supplementary Table S1). Subsequently, 32 representative strains were selected for multi-gene phylogenetic analysis, and 24 strains were screened for morphological studies (Table 2). The aligned sequences, including gaps, yielded 555 characters for ITS where 124 were parsimony informative, 899 characters for TEF1-α where 209 were parsimony informative, 1,066 characters for RPB2 where 380 were parsimony informative, and 653 characters for TUB2 where 321 were parsimony informative. The concatenated dataset comprised 162 sequences covering 3,173 characters where 1,028 were parsimony informative. The final average standard deviation of split frequency of MCMC analysis was 0.009591 for the concatenated dataset, 0.004862 for ITS, 0.006573 for TEF1-α, 0.008026 for RPB2, and 0.007595 for TUB2. The best substitution model for ITS, TEF1-α, RPB2, TUB2, and combined alignment was GTR + I + G. For all datasets (ITS, TUB2, TEF1-α, and RPB2), ML, MP, and Bayesian inference produced nearly identical topologies, with slight variations in the statistical support for each of the individual sequence datasets (Figure 1 and Supplementary Figures S1–S4). Phylograms obtained by ML are presented for all the individual datasets.

**Morphological Statistical Analysis**

The results of the morphological comparison of the different species are presented in Supplementary Figure S5. The values are mean of 50 measurements (±) SD, and significant differences according to Dunnnett-t3′ multiple-range tests at $p < 0.05$ levels were indicated and followed by different letters.

**Taxonomy**

Among the 178 strains obtained in this study, 12 species were identified. Nine of these species are new to science and are described as follows:

**Geosmithia luteobrunnea** R. Chang and X. Zhang, sp. nov.

Etymology: *luteobrunnea*, referring to the yellowish-brown appearance of the colony on MEA.

Diagnosis: The stipe of *G. luteobrunnea* is slightly thicker and shorter than that in other species. *Geosmithia luteobrunnea* can grow at 5 and 35°C, even grows slowly at 37°C (Figure 2).

Type: China, Jiangxi Province, Ganzhou City, Longnan County, Jiulianshan National Nature Reserve (24°34′1″ N, 114°30′ E), from the gallery of *Scolytus jiulianshanensis* on *Ulmus* sp., 5 May 2020, S. Lai, Y. Xu, S. Liao, Y. Wen and T. Li (HMAS 249919 – holotype, SNM261 = CGMCC3.20252 – ex-holotype culture).
Description: Sexual state not observed. Asexual state penicillium-like and (19.0–) 29.6–61.5 (–85.0)-µm long. Conidiophores borne mostly from aerial fungal hyphae, erect, determine, solitary, sometimes funiculose, with all parts verrucose; base often consisting of a curved and atypically branched cell, stipe (6.4–) 11.3–40.1 (–78.4)-µm long, (1.5–) 1.7–3.2 (–6.0)-µm wide; penicillus, monoverticillate to tetraverticillate (penicilli of conidiophores on aerial funiculose mycelia are monoverticillate or biverticillate), symmetric or asymmetric, often irregularly branched, rami (1st branch) in whorls of 1–3, (4.1–) 5.2–7.0 (–8.7) × (1.2–) 1.7–2.5 (–3.2) µm, metulae (last branch) in whorls of 1 to 2, (4.0–) 4.9–6.5 (–7.6) × (1.4–) 1.8–2.3 (–2.6) µm; phialides in whorls of 1–3, cylindrical, without or with short cylindrical neck and smooth to verrucose walls, (4.2–) 5.1–7.5 (–10.2) × (1.1–) 1.5–2.3 (–2.7) µm. Conidia hyaline to subhyaline, smooth, narrowly cylindrical to ellipsoidal, (2.3–) 2.9–4.0 (–4.7) × (0.9–) 1.2–1.7 (–2.2) µm, produced in non-persistent conidial chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 50–64 mm at 20°C, 58–78 mm at 25°C, and 44–70 mm at 30°C. The hyphae grow slowly at 5 and 35°C. After 8 days of culture, the colony diameter was 1.5–4 mm and 11–14 mm, respectively. The optimal temperature for growth was 25°C. Colonies at 25°C, 8 days, were oppressed, velutinous, or floccose with raised mycelial cords; colony margin smooth, filamentous, diffuse; aerial mycelium sparse; substrate mycelium sparse; conidiogenesis moderate; milky white to light yellow; reverse lighter brown; absence of exudate; no soluble pigment. When incubated at 35°C, colonies were raised, slightly depressed at the center, rugose, or irregularly furrowed; margin undulate somewhat erose; aerial mycelia sparse to moderate; substratum mycelia dense, forming a tough basal felt; the colony was darker and yellowish-brown; reverse brown; soluble pigment was brown. MEA, 37°C, 8 days, germinating only.

Host: Liquidambar formosana, Liquidambar styraciflua, Ulmus sp.

Beetle vectors: Acanthotomicus suncei, Scolytus jiulianshanensis.

Distribution: Currently only known from Jiangxi and Shanghai.
Notes: *Geosmithia luteobrunnea* and *G. radiata* are phylogenetically close to each other on ITS, TUB2, RPB2, TEF1-α trees, and combined alignment tree (Figure 1 and Supplementary Figures S1–S4). The colony morphology of *G. luteobrunnea* and *G. radiata* are also similar, but there are many differences among those two species. First of all, their sequences are different (Table 3). Then, under the microscope, the morphological differences between them are more obvious (Supplementary Figure S5). The spore of *G. radiata* is shorter than the other species. The stipe of *G. radiata* is thicker than the other species, and the stipe of *G. luteobrunnea* is slightly shorter than the other two species (Supplementary Figure S5). Moreover, their growths at different temperatures are also different (Table 4). *G. luteobrunnea* can grow at both temperatures, especially at 35°C, even grows slowly at 37°C. *Geosmithia radiata* only grows a little at 5°C and grows slowly at 35°C. The growth speed of *G. radiata* is faster than that of *G. radiata* (Table 4). *Geosmithia luteobrunnea* and *G. radiata* form a species group outstanding by cream to yellow or brown coloration of sporulation accompanied by the darker (brownish to rusty) shades of the substrate mycelium and colony reverse. This feature is shared also by the phylogenetically related *Geosmithia* sp. 11 (Kolářík et al., 2007) which is known from Europe and the Mediterranean (Kolářík et al., 2007, 2008) and seems to be diagnostic for the whole species group.

Additional cultures examined: China, Shanghai, from the gallery of *Acanthotomicus suncei* on *Liquidambar styraciflua*, April 2019, L. Gao (SNM226, SNM287).

**Geosmithia radiata** R. Chang and X. Zhang, sp. nov.

MycoBank MB839257

Etymology: *radiata*, referring to the radial appearance of the colony on MEA.

Diagnosis: The spore and the stipe of *G. radiata* are thicker than closely related species. *Geosmithia radiata* only grows a little at 5 and 35°C (Figure 3).

Type: China, Jiangxi Province, Ganzhou City, Longnan County (24°5′2.4″ N, 114°47′2.4″ E), from the gallery of *Acanthotomicus suncei* on *Liquidambar formosana*, 5 May 2020, S. Lai (HMAS 249920 – holotype, SNM279 = CGMCC3.20253 – ex-holotype culture).

Description: Sexual state not observed. Asexual state penicillium-like and (22.6–) 35.6–85.7 (–119.3)-μm long. *Conidiophores* borne from the substrate or aerial hyphae, sometimes arising laterally from another conidiophore, erect, determinate, solitary, with all parts verrucose; stipe commonly (7.3–) 18.4–63.6 (–115.8)-μm long, (1.6–) 2.1–3.8 (–5.9)-μm wide, penicillus, with walls thick, septate; penicillus terminal, monoverticillate, biverticillate, or triverticillate, mostly symmetrical, rami (1st branch) in whorls of 2 to 3, (4.2–) 5.2–7.8 (–10.6) × (1.3–) 2.1–3.5 (–4.8) μm; metulae (last branch) in whorls of 1 to 2, (2.6–) 3.9–5.8 (–7.3) × (1.3–) 1.7–2.6 (–3.3) μm. Phialides in whorls of 1–5, (3.9–) 4.6–6.2 (–7.7) × (1.5–) 1.9–2.8 (–3.9) μm, cylindrical, without or with short cylindrical neck and smooth to verrucose walls. *Conidia* cylindrical to ellipsoidal, smooth, hyaline to subhyaline, (2.2–) 2.5–3.2 (–4.0) × (0.9–) 1.1–1.5 (–1.8) μm, formed in non-persistent conidial chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 50–58 mm at 20°C, 59–69 mm at 25°C, and 49–60 mm at 30°C. The hyphae grow slowly at 5 and 35°C. After 8 days of culture, the colony diameter was only 1 and 1–4 mm, respectively. The optimal temperature for growth is 25°C. Colonies at 25°C, 8 days, plane, slightly raised centrally, velutinous, with a slight overgrowth of aerial mycelium, with floccose and funiculose areas; substrate mycelium darker, aerial mycelium hyaline; sporulation moderate to heavy, pale cream; vegetative mycelium hyaline; reverse lighter yellow; soluble pigment and exudate absent. When incubated at 35°C, colonies were rising, slightly sunken in the center, furrowed, or irregularly fringed; the substratum hyphae were dense and formed a tough basal felt. The colony is dark and yellowish-brown. MEA, 37°C, 8 days: no growth.

Host: *Liquidambar formosana*, *Ulmus* sp.

Beetle vectors: *Acanthotomicus suncei*, *Scolytus jiulianshanensis*.

Distribution: Jiangxi.

Notes: See comparisons between *Geosmithia luteobrunnea*, *G. radiata* below the description of *G. luteobrunnea*.

Additional cultures examined: China, Jiangxi Province, Ganzhou City, Xunwu County (24°57′ N, 115°38′2″ E), from the gallery of *Acanthotomicus suncei* on *Liquidambar formosana*, 5 May 2020 (SNM884).

**Geosmithia brevistipitata** R. Chang and X. Zhang, sp. nov.

MycoBank MB841503

Etymology: *brevistipitata*, referring to the short conidiophore stipe, a character distinguishing it from other members of the species complex.

Diagnosis: Isolates of *G. brevistipitata* formed a monophyletic clade on all the phylogenetic trees (Figure 4).

Type: China, Shandong Province, Linyi City, Tianfo scenic spot (35°5′ N, 118°2′ E), from the gallery of *Phloeoecinus* cf. *hopehi* on *Cupressus funebris*, 8 August 2021, Y. Cao (HMAS 351566 - holotype, SNM1616 = CGMCC3.20627 – ex-holotype culture).

| Species                  | ITS rDNA (555 bp) | TEF1-α (899 bp) | TUB2 (666 bp) | RPB2 (1066 bp) |
|--------------------------|-------------------|----------------|---------------|----------------|
| *G. radiata*             | 5 (0.90%)         | 8–9 (0.89–1.0%) | 4 (0.60%)     | 6 (0.56%)      |
| *G. luteobrunnea*        |                   |                |               |                |
TABLE 4 | The colony diameter of G. subfulva, G. bombycine, G. luteobrunnea, G. radiata, G. granulata, and G. pallida species complex, G. brevistipitata and G. pumila, at different temperatures after 8 days on malt extract agar medium (unit: millimeter).

| Species/T     | 5°C  | 20°C | 25°C | 30°C | 35°C | 37°C |
|---------------|------|------|------|------|------|------|
| G. bombycine  | 1    | 20–23| 24–31| 22–30| 5–8  | 0    |
| G. brevistipata| 2 to 3| 20–30| 23–34| 8–12 | 0    | 0    |
| G. fusca      | 1–6  | 21–26| 25–36| 26–32| 1–11 | ≈0   |
| G. granulata  | <1   | 27–32| 30–34| 8–12 | 2–4  | 0    |
| G. luteobrunnea| 1–4  | 50–64| 58–78| 44–70| 11–14| ≈0   |
| G. pulvereia  | 0    | 23–29| 30–37| 31–36| 1.5–4| 0    |
| G. pumila     | 7–10 | 25–29| 25–33| 22–26| ≈0   | 0    |
| G. radiata    | 1    | 50–58| 59–69| 49–60| 1–4  | 0    |
| G. subfulva   | 4–6  | 17–26| 24–36| 20–29| 35   | 0    |

Description: Sexual state not observed. Asexual state penicillium-like and (9.5–) 15.5–42.3 (–77.9)–µm long. Conidiophores borne from the substrate or aerial hyphae, sometimes arising laterally from another conidiophore, erect, determinate, solitary, with all parts verrucose; stipe commonly (2.9–) 7.5–30.0 (–56.0) × (1.3–) 1.9–3.0 (–4.1) µm, penicillus, with walls thick, septate; penicillus terminal, monoverticillate or biverticillate, mostly symmetrical, metulae in whors of 2–3, (4.6–) 6.3–9.1 (–11.2) × (1.8–) 2.0–2.7 (–3.2) µm. Phialides in whors of 2–5, (3.2–) 5.0–8.7 (–11.4) × (1.3–) 1.7–2.4 (–2.8) µm, cylindrical, without or with short cylindrical neck and smooth to verrucose walls. Conidia cylindrical to ellipsoidal, smooth, hyaline to subhyaline, (2.2–) 2.4–3.1 (–3.8) × (1.2–) 1.5–1.9 (–2.2) µm, formed in non-persistent conidial chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 24–30 mm at 20°C, 23–34 mm at 25°C, and 8–12 mm at 30°C. The hyphae grow slowly at 5°C. After 8 days of culture, the colony diameter was only 2–3 mm. No growth at 35°C. The optimal temperature for growth is 20–25°C. Colonies at 25°C, 8 days, plane, granular, with a slight growth of aerial mycelium; substrate mycelium white; reverse white; sporulation moderate white; soluble pigment and exudate absent. MEA, 37°C, 8 days: no growth.

Host: Cupressus funebris.

Beetle vectors: Phloeosinus cf. hophei.

Distribution: Shandong.

Notes: Isolates of G. brevistipitata formed a monophyletic clade on both ITS, TUB2, TEF1-α, and RPB2 trees. Its closely related species differ on various trees, such as according to ITS tree, isolates of G. brevistipitata were closely related to G. cnesini, G. xerotolerans, G. omnicola, G. ulmacea, and Geosmithia sp. 12 (Supplementary Figure S5), but according to the TUB2 tree, isolates of G. brevistipitata were connected to other species, e.g., G. microcorthyli and G. obscura (Supplementary Figure S2). Among the other species described, it is outstanding by the combination of slow growth and white colony color and short stipe.

Additional cultures examined: China, Shandong Province, Linyi City, Tianfo scenic spot (118°2′ N, 35°5′ E), from the gallery of Phloeosinus cf. hophei on Cupressus funebris, 8 August 2021, Y. Cao (SNM1610).

Geosmithia granulata R. Chang and X. Zhang, sp. nov. MycoBank MB 840646
FIGURE 4 | Morphological characteristics of Geosmithia brevistipitata sp. nov. (SNM1616 = CGMCC3.20627, SNM1610, SNM1611). (A) Eight-day-old culture on 2% malt extract agar. (B–E) Conidiophores and conidia. The stipe (indicated with arrows) is short and sometimes not smooth. Scale bars: 10 μm (B–E).

FIGURE 5 | Morphological characteristics of Geosmithia granulata sp. nov. (SNM1015 = CGMCC3.20450, SNM1013). (A) Eight-day-old culture on 2% malt extract agar. (B–E) Conidiophores and conidia. Conidia hyaline, smooth, wide oval shape, like an egg. Scale bars: 10 μm (B–E).

Etymology: granulata, referring to the granular appearance of the colony on MEA.

Diagnosis: The conidia of *G. granulata* are shorter than the closely related species (Figure 5).

Type: China, Yunnan Province, Xishuangbanna City, Xishuangbanna Botanical Garden (21°55′1″ N, 101°16′1″ E), from the gallery of *Sinoxylon cf. cucumella* on *Acacia pennata*, 1 May 2021, Y. Dong and Y. Li (HMAS
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FIGURE 6 | Morphological characteristics of Geosmithia subfulva sp. nov. (SNM1304 = CGMCC3.20579, SNM1298). (A) Eight-day-old culture on 2% malt extract agar. (B–E) Conidiophores and conidia. The metulae (indicated with arrows) branches are few and sparse. Scale bars: 10 \( \mu \)m (B–E).

351568 - holotype, SNM1015 = CGMCC3.20450 – ex-holotype culture.

Description: Sexual state not observed. Asexual state penicillium-like, (9.6–) 11.6–26.0 (–50.6) \( \mu \)m in length. Conidiophores emerging from hyphae, smooth, septate; stipe (4.0–) 4.8–8.3 (–14.3) \( \times \) (1.1–) 1.4–2.1 (–2.6) \( \mu \)m; penicilli typically longer than the stipe, terminal, monoverticillate, biverticillate, or terverticillate, symmetric or asymmetric, often irregularly branched, rarely more; metulae in whorls of 1–2, (5.2–) 5.7–8.1 (–11.3) \( \times \) (1.0–) 1.3–1.7 (–2.0) \( \mu \)m; phialides in whorls of 1–4, smooth, (3.3–) 4.9–7.1 (–8.8) \( \times \) (1.0–) 1.3–1.8 (–2.2) \( \mu \)m. Conidia hyaline, smooth, wide oval shape, like an egg, (1.5–) 1.8–2.2 (–2.5) \( \times \) (0.8–) 1.0–1.4 (–1.8) \( \mu \)m. Conidia formed in long, non-persistent conidial chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 27–32 mm at 20\( ^\circ \)C, 30–34 mm at 25\( ^\circ \)C, and 8–12 mm at 30\( ^\circ \)C. At 5\( ^\circ \)C: germinating only. At 35\( ^\circ \)C, the mycelia grew slowly. After 8 days of culture, the diameter of the colony was 2–4 mm. The optimal growth temperature is 20–25\( ^\circ \)C. At 25\( ^\circ \)C, 8 days: Colonies were flat and radiated, surface texture powdery; central hyphae slightly raised and wrinkled, conidiogenesis heavy; marginal colonies were similar to annual rings, slightly flocculent, hyphae were sparse, milky white, reverse creamrige white; without exudate and insoluble pigment. MEA, 37\( ^\circ \)C, 8 days, no growth.

Host: Acacia pennata, Hibiscus tiliaceus, Ulmus sp.

Beetle vectors: Sinoxylon cf. cumuluma, Enroporus japonicus, Scolytus semenovi.

Distribution: Guangdong, Jiangsu, Yunnan.

Notes: According to the tree made by ITS and TEF1-\( \alpha \) sequence, SNM1015 and SNM1013 were clustered with Geosmithia sp. 20 (Supplementary Figures S1, S3). TUB2 and RPB2 sequences of Geosmithia sp. 20 were not available on GenBank; therefore, Geosmithia sp. 20 was not included in TUB2 and RPB2 trees. These results suggested that our isolates and Geosmithia sp. 20 belonged to the same species, described as G. granulata sp. nov. This extends the geographical distribution of this species to the Mediterranean Basin (Kolařík et al., 2007) and western part of the United States (Kolařík et al., 2017) where it was found in association with many bark beetle species feeding on plants from the families Asteraceae, Fabaceae, Moraceae, Oleaceae, Ulmaceae (Mediterranean Basin), or Cupressaceae, Ulmaceae (Western United States).

Additional cultures examined: China, Yunnan Province, Xishuangbanna City, Xishuangbanna Botanical Garden (21°55′1″ N, 101°16′1″ E), from the gallery of Sinoxylon cf. cumuluma on Acacia pennata, 1 May 2021, Y. Dong and Y. Li (SNM1013).

Geosmithia subfulva R. Chang and X. Zhang, sp. nov.

MycoBank MB 841505

Etymology: subfulva, referring to the beige appearance of the colony on MEA.

Diagnosis: Isolates of G. subfulva formed a monophyletic clade on all the phylogenetic trees (Figure 6).

Type: China, Guangdong Province, Zhuhai City (22°16′48″ N, 113°30′28″ E), from the gallery of Ernoporus japonicus in the
twig of *Hibiscus tiliaceus*, 21 June 2021, W. Lin (HMAS 351569 - holotype, SNM1304 = CGMCC3.20579 – ex-holotype culture).

Description: Sexual state not observed. Asexual state penicillium-like and (13.3–) 21.0–43.5 (–62.5)-μm long. Conidiophores arising from substrate or aerial mycelium with all parts verrucose; stipe (5.3–) 9.3–26.4 (–36.6) × (0.9–) 1.2–1.6 (–1.8) μm; penicillus, biverticillate to quaterverticillate, symmetric or asymmetric, often irregularly branched, rarely more, rami (1st branch) in whors of 1–2, (4.8–) 5.6–7.4 (–8.4) × (1.0–) 1.3–1.8 (–2.0) μm, metulae (last branch) in whors of 1–3, (4.0–) 4.6–5.9 (–6.9) × (0.9–) 1.2–1.6 (–1.8) μm; phialides 1–3, cylindrical or ellipsoidal, without or with short cylindrical neck and smooth to verrucose walls, (3.6–) 4.8–6.9 (–10.0) × (0.8–) 1.1–1.4 (–1.6) μm. Conidia hyaline, smooth, wide oval shape, (1.1–) 1.5–2.2 (–2.2) × (1.0–) 1.1–1.5 (–1.7) μm. Conidia formed in long, non-persistent conidial chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 17–26 mm at 20°C, 24–36 mm at 25°C, and 20–29 mm at 30°C. At 5 and 35°C, the mycelia grew slowly. After 8 days of culture, the colony diameter was 4–6 and 3–5 mm. The optimal growth temperature is 25°C. Colonies at 25°C, 8 days, plane with radial rows and slightly raised centrally, texture velutinous (powdery); beige to off-white; reverse milky white; soluble pigment and exudate absent. When incubated at 35°C, the colonies are the same as described above. MEA, 37°C, 8 days: no growth.

Host: *Hibiscus tiliaceus*, *Rhus chinensis*.

Beetle vectors: *Cryphalus kyotoensis*, *Ernoporus japonicus*, *Hypothenemus* sp. L636.

Distribution: Fujian, Guangdong, Shandong.

Notes: Isolates SNM1304 and SNM1298 formed a monophyletic clade on both ITS, TUB2, TEF1-α, and RPB2 trees (Supplementary Figures S1–S4). On the ITS tree, SNM1304 and SNM1298 were distinct from all other species. On the TUB2 tree, SNM1304 and SNM1298 are linked to *G. pulverea* but have no strong support. On TEF1-α and RPB2 trees, they are nested in a clade including not only *G. pulverea* but also several other species.

Additional cultures examined: China, Guangdong Province, Zhuhai City (22°16′48″ N, 113°30′28″ E), from the gallery of *Ernoporus japonicus* LW320 in the twig of *Hibiscus tiliaceus*, 21 June 2021, W. Lin (SNM1298).

**Geosmithia pulverea** R. Chang and X. Zhang, sp. nov.

MycoBank MB839259

Etymology: *pulverea*, powdery in Latin. On MEA medium, *G. pulverea* has powdery sporulation.

Diagnosis: *Geosmithia pulverea* produces long spore chains, while its closely related species does not (Figure 7).

Type: China, Guangdong Province, Shenzhen City (21°55′12″ N, 110°16′12″ E), from the gallery of *Dinoderus* sp. L489 in the vine of *Gnetum luofuense*, 12 April 2018, Y. Li (HMAS 249922 – holotype, SNM885 = CGMCC3.20255 – ex-holotype culture).

Description: Sexual state not observed. Asexual state penicillium-like and (17.5–) 30.9–84.3 (–120.1)-μm long. *Conidiophores* arising from substrate or aerial mycelium with all parts verrucose; base often consisting of curved and atypically branched cell; stipe (16.2–) 32.7–85.7 (–153.9) × (1.9–) 2.5–3.7 (–4.7) μm; penicillus, biverticillate to quaterverticillate, symmetric or asymmetric, often irregularly branched, rarely more, rami (1st branch) in whors of 2–4, (8.2–) 10.2–14.4 (–18.9) × (2.2–) 2.5–3.3 (–3.9) μm, metulae (last branch) in whors of 2–3, (6.3–) 7.5–10.9 (–15.8) × (1.8–) 2.1–2.8 (–3.5) μm; phialides 1–3, cylindrical or ellipsoidal, without or with short cylindrical neck and smooth to verrucose walls, (5.3–) 7.0–9.6 (–12.3) × (1.5–) 1.8–2.5 (–3.0) μm. Conidia hyaline, smooth, narrowly cylindrical to ellipsoidal, (2.1–) 2.5–3.4 (–5.1) × (1.1–) 1.2–1.6 (–2.0) μm.
µm. Conidia formed in long, non-persistent conidial chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 23–29 mm at 20°C, 30–37 mm at 25°C, and 31–36 mm at 30°C. No growth at 5°C. At 35°C, the mycelia grew slowly. After 8 days of culture, the colony diameter was 1.5–4 mm, with a yellow soluble pigment. The optimal growth temperature is 25–30°C. Colonies at 25°C, 8 days, plane with radial rows and slightly raised centrally, texture velutinous (powdery); sporulation abundant, light brownish yellow to buff; reverse yellowish to slightly avellaneous brown; soluble pigment and exudate absent. When incubated at 35°C, the colonies are the same as described above. MEA, 37°C, 8 days: no growth.

Host: *Acacia pennata*, *Gnetum luofuense*, *Liquidambar formosana*, *L. styraciflua*, *Choeirospondias axillaris*, *Lauraceae*, *Eriobotrya japonica*, *Rhus chinensis*, *Ulmus spp.*

Beetle vectors: *Sinoxylon cf. cuminumella*, *Acanthotomicus suncei*, *Crotosartus emancipatus*, *Dinoderus sp.* L489, *Microperus sp.* L589, *Phloeosinus sp.*, *Scolytus semenovi*, *Scolytus jiulianshanensis*, *Cryphalus kyotoensis*, *Cryphalus eriobotryae*.

Distribution: Fujian, Guangdong, Guangxi, Hunan, Jiangxi, Yunnan, Shandong, Shanghai.

Notes: *Geosmithia fusca* colony was powdery and brown-yellow. One of the most obvious features is the long spore chain. According to the tree made by ITS sequence, SNM888, SNM885, and SNM248 were clustered with *Geosmithia* sp. 3, and SNM866, SNM887, and SNM270 were clustered with *Geosmithia* sp. 23 (Supplementary Figure S1). However, in the trees with TUB2, TEF1-α, and RPB2, these strains did not have a clear subclassification (Supplementary Figures S2–S4). It was consequently recognized, using multigene phylogeny, together with *Geosmithia* sp. 23, as a well-defined phylogenetic species inside the *G. pallida* species complex (Huang et al., 2017; Kolařík et al., 2017). The colony of *G. pulvereosa* was very similar to *Geosmithia* sp. 3 on MEA, but *Geosmithia* sp. 3 was darker and wrinkled (Kolařík et al., 2004). *Geosmithia pulvereosa* seems to have a smaller stipe size, but other features fit the morphology of *Geosmithia* sp. 3 (Kolařík et al., 2004). In this study, we are providing a formal description for the Chinese strains related to *Geosmithia* sp. 3 and sp. 23 which are known to be distributed over various bark beetle hosts in Temperate Europe in the case of *Geosmithia* sp. 3 (Kolařík et al., 2004, 2008; Strzalka et al., 2021) or seems to have a global distribution and many bark beetle hosts across Temperate Europe (Strzalka et al., 2021), the Mediterranean Basin (Kolařík et al., 2007), *Northern America* (Huang et al., 2017, 2019; Kolařík et al., 2017), and *Seychelles* (Kolařík et al., 2017). Further study is needed to assess the taxonomic relationships between *G. pulvereosa*, *Geosmithia* sp. 3, and *Geosmithia* sp. 23.

Additional cultures examined: China, Guangxi Province, Shangsi City, Shiwanodashan Mt. (21°54′12″ N, 107°54′14″ E), from the body surface of *Crotosartus emancipatus*, 27 March 2018, Y. Li (SNM887).

China, Hunan Province, Changsha City, Yuelushan Mt. (28°10′56″ N, 112°55′41″ E), from the gallery of *Microperus* sp. L589 on the trunk of *Choeirospondias axillaris*, 15 July 2019, Y. Li (SNM888).

### Geosmithia fusca R. Chang and X. Zhang, sp. nov.

**MycoBank**: MB841506

**Etymology**: *fusca*, referring to the brown appearance of the colony on MEA.

**Diagnosis**: The difference with closely related species *G. cucumellae* is reflected in such a way that the conidia of *G. fusca* are smooth and do not produce long spore chains (Figure 8).

**Type**: China, Guangdong Province, Zuhui City, Agricultural Science Research Center (22°18′9″ N, 113°31′40″ E), from the gallery of *Xylocis tortilicornis* on *Phyllanthus emblica*, 6 July 2021, W. Lin (HMAS 351570 - holotype, SNM1578 = CGMCC3.20626 – ex-holotype culture).

Description: Sexual state not observed. Asexual stage penicillium-like and (16.3–) 20.2–55.8 (–94.3)-µm long. Conidiophores variable in shape and size, emerging from a surface mycelium, with all segments smooth or minutely verrucose to distinctly verrucose, stipe, stipe (8.6–) 10.1–38.5 (–70.1) × (1.2–) 1.5–2.1 (–2.6) µm; penicilli typically shorter than the stipe, terminal, monovernicillate or bivernicillate, or symmetric or asymmetric, irregularly branched; metulae in whorls of 2–3, (4.9–) 6.0–8.3 (–9.9) × (1.1–) 1.3–1.8 (–2.1) µm; phialides in whors of 1–3, smooth, (4.6–) 5.8–8.1 (–9.4) × (1.0–) 1.3–1.7 (–1.9) µm. Conidia cylindrical to ellipsoid, (1.5–) 2.0–2.7 (–3.4) × (0.9–) 1.1–1.7 (–1.7) µm. Conidia formed in long, non-persistent conidial chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 21–26 mm at 20°C, 25–36 mm at 25°C, and 26–32 mm at 30°C. At 5 and 35°C, the mycelia grew slowly. After 8 days of culture, the colony diameter was 1–3 and 7–11 mm, respectively. The optimal growth temperature is 25–30°C. At 25°C, 8 days: Colonies flat with radial rows, surface texture powdery; sporulation abundant, brown; central hyphae were raised and white flocculent; reverse yellowish to brown; without exudate and insoluble pigment. MEA, 37°C, 8 days: germinating only.

Host: *Hibiscus tiliacus*, *Phyllanthus emblica*, *Acacia pennata*.

Beetle vectors: *Ernoporus japonicus*, *Xylocis tortilicornis*, *Sinoxylon cf. cuminumella*.

Distribution: Guangdong, Yunnan.

Notes: In the phylogenetic tree, SNM1012, SNM1067 and SNM1577, SNM1578 formed very close separate branches (Figure 1 and Supplementary Figures S1–S4), but combined with morphological analysis, it was found that SNM1012, SNM1067 and SNM1577, SNM1578 had no significant difference except for a small difference in spore length (Supplementary Figure S5). So, they are described as the same species.

Additional cultures examined: China, Guangdong Province, Zuhui City, Agricultural Science Research Center (22°30′25″ N, 113.5277° E), from the gallery of *Xylocis tortilicornis* LW319 on *Phyllanthus emblica*, 6 July 2021, W. Lin (SNM1577).

China, Yunnan Province, Xishuangbanna City, Xishuangbanna Botanical Garden (21°55′1″ N, 101°16′1″ E), from the gallery of *Sinoxylon cf. cuminumella* on the trunk of *Acacia pennata*, 1 May 2021, Y. Dong and Y. Li (SNM1012, SNM1167).

### Geosmithia pumila R. Chang and X. Zhang, sp. nov.

**MycoBank**: MB841507
Etymology: *pumila*, referring to the tree host of *Ulmus pumila* where this species has been isolated.

Diagnosis: Isolates of *G. pumila* formed a monophyletic clade on all the phylogenetic trees (Figure 9).

Type: China, Jiangsu Province, Nanjing City, Nanjing Forestry University (32°3′36″ N, 118°48′36″ E), from the gallery of *Scolytus semenovi* in the branch of *Ulmus* sp., 25 August 2021, S. Lai (HMAS 351571 -
FIGURE 10 | Morphological characteristics of Geosmithia bombycina sp. nov. (SNM933 = CGMCC3.20578, SNM934). (A) Eight-day-old culture on 2% malt extract agar. (B–E) Conidiophores and conidia. The penicilli (indicated with arrows) are typically shorter than the stipe, terminal, monoverticillate, biverticillate or terverticillate, symmetric or asymmetric. Scale bars: 10 μm (B–E).

holotype, SNM1653 = CGMCC3.20630 – ex-holotype culture).

Description: Sexual state not observed. Asexual state penicillium-like and (12.9–) 35.9–72.7 (–109.4)–μm long. Conidiophores arising from substrate or aerial mycelium with all parts verrucose; stipe (9.9–) 19.7–51.5 (–77.9) × (1.2–) 1.4–2.2 (–2.6) μm; penicillus, monoverticillate or biverticillate, mostly monoverticillate, symmetric or asymmetric, often irregularly branched, rarely more, metulae in whorls of 2 to 3, (5.1–) 6.3–8.9 (–10.5) × (1.1–) 1.4–2.0 (–2.3) μm; phialides 1–3, smooth to verrucose walls, (5.0–) 5.7–7.3 (–8.5) × (1.1–) 1.2–1.6 (–1.8) μm. Conidia hyaline, smooth, ellipsoidal, (1.5–) 1.9–2.5 (–2.9) × (0.9–) 1.1–1.5 (–1.9) μm. Conidia formed in long, non-persistent conidial chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 25–29 mm at 20°C, 25–33 mm at 25°C, and 22–26 mm at 30°C. At 35°C: germinating only. At 5°C, the mycelia grew slowly. After 8 days of culture, the colony diameter was 7–10 mm. The optimal growth temperature is 20–25°C. Colonies at 25°C, 8 days, plane with radial rows, texture velutinous (powdery), slightly funiculus centrally; sporulation medium, light yellow to rice white; reverse milk-white; soluble pigment and exudate absent. MEA, 37°C, 8 days: no growth.

Host: Ulmus sp.

Beetle vectors: Scolytus semenovi.

Distribution: Jiangsu.

Notes: Based on ITS, TUB2, TEF1-α, and RPB2 trees (Supplementary Figures S1–S4), SNM1653 and SNM1657 were grouped with Geosmithia sp. 2. Therefore, we considered that SNM1653, SNM1657, and Geosmithia sp. 2 were the same species. This extends the geographical range to Europe, the Mediterranean Basin (Kolařík et al., 2007, 2008), the whole United States (Huang et al., 2017, 2019; Kolařík et al., 2017), Peru (Kolařík et al., 2004), and South Africa (Machingambi et al., 2014), which is reported to be in association with a large number of insect and tree hosts.

Additional cultures examined: China, Jiangsu Province, Nanjing City, Nanjing Forestry University (32°3′36″ N, 118°48′36″ E), from the gallery of Scolytus semenovi in the branch of Ulmus sp., 25 August 2021, S. Lai (SNM1657).

Geosmithia bombycina R. Chang and X. Zhang, sp. nov.

MycoBank MB 840535

Etymology: bombycina, referring to the cotton appearance of the colony on MEA.

Diagnosis: Isolates of G. bombycina formed a monophyletic clade on all the phylogenetic trees (Figure 10).

Type: China, Fujian Province, Fuqing City (25°71′ N, 119°15′ E), from the gallery of Cryphalus eriobotryae on Eriobotrya japonica, 8 April 2021, Y. Li (HMAS 350284 – holotype, SNM933 = CGMCC3.20578 – ex-holotype culture).

Description: Sexual state not observed. Asexual state penicillium-like, (14.0–) 20.2–41.0 (–62.6) μm in length. Conidiophores emerging from hyphae, smooth, septate; stipe (5.4–) 9.4–30.0 (–47.5) × (0.9–) 1.4–2.0 (–2.4) μm; penicilli typically shorter than the stipe, terminal, monovermicillate, biverticillate or terverticillate, symmetric or asymmetric, often irregularly branched, rarely more; metulae in whorls of 1–2, (5.1–) 5.9–8.3 (–10.5) × (1.0–) 1.2–1.7 (–2.1) μm; phialides in whorls of 2–4, smooth, (4.9–) 5.8–9.4 (–12.6) × (0.9–) 1.3–1.7 (–2.0) μm.
μm. Conidia hyaline, smooth, narrow, and oval, (2.1–) 2.4–3.3 (–4.1) × (0.8–) 0.9–1.3 (–1.5) μm, produced in non-persistent chains. Substrate conidia absent.

MEA, 8 days: Colony diameter 20–23 mm at 20°C, 24–31 mm at 25°C, and 22–30 mm at 30°C. The hyphae grow slowly at 5 and 35°C. After 8 days of culture, the colony diameter was less than 1 and 5–8 mm, respectively. The optimal temperature for growth was 25–30°C. At 25°C, 8 days: The colonies were flat, like annual rings; central hyphae were raised and white flocculent; filamentous, diffuse, basal mycelium sparse; conidiogenesis moderate, milk-white; reverse creamy white; no exudate and insoluble pigment. When incubated at 35°C, the colonies grew, and the mycelia were sparse and snowflake-shaped, with no soluble pigment. MEA, 37°C, 8 days, no growth.

Host: *Eriobotrya japonica*.

Beetle vectors: *Cryphalus eriobotryae*.

Distribution: Currently only known from Fujian.

Notes: According to ITS, TUB2, and TEF1-α sequences (Supplementary Figures S1–S4), SNM933 and SNM934 formed a monophyletic clade and nested with *Geosmithia* sp. 22, *Geosmithia* sp. 24, *G. longistipitata*, *G. pazoutovae*, and *G. fagi*. The RPB2 sequences for these species were not available on GenBank. Therefore, SNM933 and SNM934 formed a distinct clade that was far away from all the known species on the RPB2 tree.

Additional cultures examined: China, Fujian Province, Fuqing City (25°71’ N, 119°15’ E), from the gallery of *Cryphalus eriobotryae* on *Eriobotrya japonica*, 8 April 2021, Y. Li (SNM934).

**DISCUSSION**

This is the first relatively comprehensive study of *Geosmithia* species associated with bark beetle in China. The samples were collected from 9 provinces, 12 tree hosts, and 12 bark and ambrosia beetles. A total of 178 strains of *Geosmithia* were isolated in this study. The analyses by ITS, RPB2, TUB2, and TEF1-α showed that those isolates were separated into 12 taxa, with three strains previously described, *G. xerotolerans*, *G. putterillii*, and *G. pallida*, and the other nine were novel species, described as *G. luteobrunnea*, *G. radiata*, *G. brevistipitata*, *G. bombycina*, *G. granulata* (*Geosmithia* sp. 20), *G. subfulva*, *G. pulvere* (*Geosmithia* sp. 3 and *Geosmithia* sp. 23), *G. fusca*, and *G. pumila* in this study. Those species were isolated from larvae, frass, and wood dust in the beetle galleries of dying, stressed, or weakened broad-leaf and conifer tree hosts, such as *Liquidambar* spp., *Ulmus* sp., and *Cupressus* sp.

The dominant species obtained in this study were *G. luteobrunnea* and *G. pulvere*, with 39 and 33 strains, respectively (Table 1). The reason for their abundance in our dataset is the fact that our study focused on sampling from *Altinginaceae*. Two species, *G. putterillii* and *G. radiata*, have only been isolated in Jiangxi (Table 1). The samples collected from Guangxi and Hunan only yielded *G. pulvere*.

*Geosmithia putterillii* was isolated from bark beetles feeding on plants from the family of Rosaceae (Kolařík et al., 2008) and Lauraceae in Europe (Kolařík et al., 2004) and on various families of angiosperms and gymnosperms in the Western United States (Kolařík et al., 2017). The type strain was isolated from timber in New Zealand (Pitt, 1979). In this study, *G. putterillii* was isolated from the gallery of *Phloeosinus* sp. on Lauraceae log (Jiangxi). This study is the first report of *G. putterillii* in China. It is becoming clear that *G. putterillii* is widely distributed globally, across many beetle hosts.

Another known species collected in this study is *G. pallida*, originally isolated from cotton yarn and soil (Kolařík et al., 2004). Later, it was found to be associated with beetles, such as ambrosia beetle *Xylosandrus compactus* (Vannini et al., 2017), and plants such as *Brueca mollis* (Deka and Jha, 2018). *G. pallida* was previously reported to induce dieback poisoning on coast live oak (*Quercus agrifolia*) by Lynch et al. (2014). Later, it was proved that the identification was incorrect, and the causal agent of this disease was confirmed to be *Geosmithia* sp. 41 (Kolařík et al., 2017). Two isolates were obtained from the gallery of *Sinoxylon cf. cucumella* on *Acacia pennata* in this study, which is the first report of *G. pallida* in China.

Most of *G. luteobrunnea* were isolated from the galleries of *A. suncei* (Table 1). *Acanthotomicus suncei* was recorded on *Liquidambar* in Fujian, Jiangsu, Jiangxi, Zhejiang, and Shanghai, China (Li et al., 2021). The hosts of this beetle were limited to sweet gum trees, such as *L. styaciflua* and *L. formosana*. The beetle was recorded as an agent of great damage to the imported American sweetgum *L. styaciflula* in Shanghai and neighboring Jiangsu Province (Gao and Cognato, 2018). The role of the fungus in this outbreak and the tree pathology remain uninvestigated, although the authors of this paper noted small lesions around the beetle galleries. The other five isolates were isolated from the galleries of *S. jiulianshanensis* on *Ulmus* sp., which suggests that *G. luteobrunnea* might colonize a wide range of tree hosts.

*Geosmithia radiata* was only isolated in samples from Jiangxi Province, from two plant families: *Altinginaceae* and *Ulmaceae* (Table 1). The colony of *G. radiata* is similar to *G. luteobrunnea* in morphology, but the difference can be seen in the micromorphology (Supplementary Figure S5). In addition, *G. luteobrunnea* can grow faster at 35°C, while *G. radiata* grows slower, and *G. luteobrunnea* could grow at 35°C, but *G. radiata* could not (Table 4).

*Geosmithia brevistipitata* and *G. xelotolerans* were isolated from the gallery of *Phloeosinus cf. hopehi*. This is not the first time that *Geosmithia* species were isolated from the gallery of *Phloeosinus* species. According to previous reports, *G. flava*, *G. longdonii*, *G. putterillii*, *G. Lavandula*, etc., are all related to *Phloeosinus* (Kolařík et al., 2017). It is now more certain that *Phloeosinus* and *Geosmithia* are closely related. *Geosmithia xelotolerans* is cosmopolite, known from the Mediterranean on many bark beetle species infecting *Fabaceae*, *Moraceae*, *Oleaceae* (Kolařík et al., 2007), in Western US on *Cupressaceae*, *Pinaceae* *Fagaceae*, *Rosaceae* (Kolařík et al., 2017), and in Eastern US on *Cupressaceae*, *Fagaceae* (Huang et al., 2017, 2019), and wall of the wall (Spain, Crous et al., 2018). Our study expanded the distribution range of *G. xelotolerans*.

*Geosmithia bombycina* was isolated from the gallery of *C. eriobotryae* on *E. japonica*. *Cryphalus eriobotryae* is one of the beetle pests that infest loquat (Zheng et al., 2019). This is the first study about the fungal association of this beetle.
**Geosmithia granulata** was isolated from the gallery of *Sinoxylon cf. cucumella* on *Acacia pennata*, *Ernoporus japonicus* on *Hibiscus tilicuas*, and *Scolytus semenovi* on *Ulmus* sp. in this study. It was reported that it could be vectored by different beetle species which infested several plant hosts (Kolarík et al., 2007). In this study, we expanded the range of its beetle vectors and tree host.

**Geosmithia pulvorea** is a species closely related to Geosmithia sp. 3 and *Geosmithia* sp. 23, which are known from various bark beetle hosts in Europe, United States, and Seychelles (Kolarík et al., 2007, 2008, 2017; Huang et al., 2017, 2019). Further study is needed to clarify the evolutionary relationship among these three lineages. In this study, we isolated *G. pulvorea* from *Aca. gracilipes*, *Alt. gracilipes*, *E. japonica*, *Gne. luofuense*, *L. formosana*, *L. styraciflua*, *Rhus chinensis*, and *Ulmus* sp. (Table 1), which suggested that this species could colonize a very wide variety of plant hosts. It is also the most widely distributed species, isolated from Guangdong, Guangxi, Hunan, Jiangsu, Jiangxi, Shandong, and Shanghai (Table 1) and vectored by several beetle species, such as *S. jiluanshanensis*, *A. suncei*, *C. emancipatus*, *C. kyotoensis*, *Dinoderus* sp., *Microperus* sp., and *Phlocosinus* sp. (Table 1). Moreover, the abundance of Geosmithia species associated with *A. suncei* in the current study was also consistent with the frequent occurrence in Shanghai and Jiangxi (Gao et al., 2021).

In addition to *G. pallida*, *Geosmithia pulvorea*, and *Geosmithia fusca* are the species found in the *G. pallida* species complex in this study. Only eight isolates of *G. fusca* were obtained from the gallery of *Sinoxylon cf. cucumella* on *Acacia pennata*. Two isolates of *G. pallida*, eight isolates of *G. pulvorea*, and two isolates of *G. granulata* were also obtained from this beetle. Information about this beetle was very limited. As far as we know, it was found on Wendlandia tintoria and distributed in the Himalayan mountain area and Burma, Thailand, Laos, and Vietnam (Liu, 2010; Liu and Beaver, 2018; Borowski, 2021). This is the first report in China, and this is the first study on its fungal associations.

**CONCLUSION**

This study does not provide sufficient data to determine the structure of the *Geosmithia* community in China, as was inferred in Europe and United States after a significantly greater sampling effort (Kolarík et al., 2007, 2008, 2017; Kolarík and Jankowiak, 2013; Jankowiak et al., 2014; Huang et al., 2017, 2019). Fungal communities are regulated by several factors, including geographic location, host tree species, and bark beetle vectors. Further sampling is needed to understand the determinants (Veselská et al., 2019). It is clear, however, that the diversity of China’s subcortical fungi is substantial. Fungal communities associated with trees need to be further investigated because many currently unknown species may cause plant diseases.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

RC, MD, and YL designed the research. YL, HS, and GZ collected the samples. XZ, RC, and YL isolated and purified the fungal cultures. XZ, RC, and XJ completed the data acquisition, analyses, and interpretation. XZ and RC completed the writing of the manuscript. MK, JH, and YL revised the text, taxonomy, and phylogeny. All authors approved the manuscript.

**FUNDING**

JH was partially funded by a cooperative agreement with the USDA Forest Service, by the USDA APHIS Plant Protection Act, and by the National Science Foundation.

**ACKNOWLEDGMENTS**

We would like to thank Ling Zhang, Yufeng Cao, Shengchang Lai (Jiangxi Agricultural University), Dr. Yongying Ruan (Shenzhen Polytechnic), Yiyi Dong (University of Florida), and Dr. Lei Gao (Shanghai Academy of Landscape Architecture Science and Planning) for assisting in insect collection, Dr. Sarah Smith and Prof. Anthony Cognato (Michigan State University) and Mr. Wei Lin (Technical Center of Gongbei Customs District People’s Republic of China) for assisting with beetle identification, and Dr. Shuping Wang (Shanghai Entry-Exit Inspection and Quarantine Bureau) and Jue Wang (Beijing Forest University) for assisting with sequencing.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.820402/full#supplementary-material

### REFERENCES

Borowski, T. (2021). World inventory of beetles of the family Bostrichidae (Coleoptera). Part 2. Check list from 1758 to 2007. *World News Nat. Sci.* 9–41.

Crous, P. W., Luangsa-Ard, J. J., Wingfield, M. J., Carneje, A. J., Hernández-Restrepo, M., Lombard, L., et al. (2018). Fungal Planet description sheets: 785-867. Persoonia 41, 238–417. doi: 10.3767/persoonia.2018.41.12

Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9:772. doi: 10.1038/nmeth.2109

Deka, D., and Jha, D. K. (2018). Optimization of culture parameters for improved production of bioactive metabolite by endophytic *Geosmithia pallida*. *Persoonia* 41, 238–417. doi: 10.3767/persoonia.2018.41.12
(KU693285) isolated from Brucella mорris Wall ex. Kurz, an endangered medicinal plant. J. Pure Appl. Microbiol. 12, 1205–1213. doi: 10.22207/jpam.12.3.21

Dori-Bachash, M., Avarhimi-Moyal, L., Protasov, A., Mendel, Z., and Freeman, S. (2015). The occurrence and pathogenicity of Geosmithia spp. and common blue-stain fungi associated with pine bark beetles in planted forests in Israel. Eur. J. Plant Pathol. 143, 627–639.

Gao, L., and Cognato, A. I. (2018). Acanthotomicus suncei, a new sweetgum tree pest in China (Coleoptera: Curculionidae: Scolytinae: Ipini). Zootaxa 447, 595–599. doi: 10.11646/zootaxa.4473.1.13

Gao, L., Li, Y., Wang, Z.-X., Zhao, J., Hulcr, J., Wang, J.-G., et al. (2021). Biology and associated fungi of an emerging bark beetle pest, the sweetgum inscriber Acanthotomicus suncei (Coleoptera: Curculionidae). J. Pure Appl. Microbiol. 145, 508–517. doi: 10.11101/jen.12861

Gardes, M., and Bruns, T. D. (1993). ITS primers with enhanced specificity for filamentous ascomycetes. Appl. Environ. Microbiol. 61, 1233–1230. doi: 10.1128/aem.61.4.1233-1230.1995

Grum-Grzhimaylo, A. A., Georgieva, M. L., Debets, A. J., and Bilanenko, E. N. (2017). Acanthotomicus suncei, a new phytopathogenic species living in symbiosis with the walnut twig beetle (Pityophthorus juglandis) in association with Phytopythorax juglandis (Fungi: Geosmithiales) and the thousand cankers disease pathogen, Geosmithia morbida (Ascomycota: Hypocreales). Can. Entomol. 148, 83–99. doi: 10.1139/ce2015.37

Huang, Y.-T., Kolarik, M., Sasson, M. T., and Hulcr, J. (2017). Two new Geosmithia species in G. pallida species complex from bark beetles in eastern USA. Mycologia 109, 790–803. doi: 10.1002/myc.2017141022

Huang, Y.-T., Skelton, J., Johnson, A. J., Kolarik, M., and Hulcr, J. (2019). Geosmithia species in southeastern USA and their affinity to beetle vectors and tree hosts. Fungal Ecol. 39, 168–183. doi: 10.1016/j.fusene.2019.02.005

Jankowiak, R., Kolarik, M., and Bilanski, P. (2014). Association of Geosmithia fungi (Ascomycota: Hypocreales) with pine- and spruce-infesting bark beetles in Poland. Fungal Ecol. 11, 71–79. doi: 10.1016/j.fusene.2014.04.002

Juzwik, J., Banik, M. T., Reed, S. E., English, J. T., and Ginzle, M. D. (2015). Geosmithia morbida Found on Weevil Species - S10482-x. Mycol. Res. 66, 682–700. doi: 10.1016/j.mycres.2007.06.010

Kolarik, M., Kubatova, A., Pazoutova, S. (2008). Geosmithia fungi are highly diverse and consistent bark beetle associates: evidence from their community structure in temperate europe. Mycol. Res. 108, 1053–1069. doi: 10.1017/S0953755208000796

Kolarik, M., Kubatova, A., Pazoutova, S., and Siklita, P. (2004). Morphological and molecular characterisation of Geosmithia putterillii, G. pallida comb. nov. and G. flava sp. nov., associated with subcorticolous insects. Mycol. Res. 108, 55–68. doi: 10.1017/S0953755200000796

Kubatova, A., Kolarik, M., Prasil, K., and Novotny, D. (2004). Bark beetles and their galleries: well-known niches for little known fungi on the example of Geosmithia. Czech Mycol. 56, 1–18. doi: 10.33585/cmy.56.s101

Li, Y., Wan, Y., Lin, W., Ernstsons, A. S., and Gao, L. (2021). Estimating potential distribution of sweetgum pest Acanthotomicus suncei and potential economic losses in nursery stock and Urban Areas in China. Insects 12:155. doi: 10.3390/insects12020155

Lin, Y.-T., Shih, H.-H., Huang, Y.-T., Lin, C.-S., and Chen, C.-Y. (2016). Two species of beetle-associated Geosmithia in Taiwan. Fungal Sci. 31, 29–29. doi: 10.33585/cmy.67103

Liu, L. (2010). New records of Bostrichidae (Insecta: Coleoptera, Bostrichidae, Bostrichinae, Lycinae, Polycyphoninae, Dinoderinae, Apatinae). Mitt. Munch. Entomol. Ges. 100, 103–117.

Liu, L., and Beaver, R. A. (2018). “A synopsis of the woodpesser beetles of the Himalayas with a key to the genera (Insecta: Coleoptera: Bostrichidae)” in Biodiversität und Naturausstattung im Himalaya VI, eds M. Hartmann, M. Barclay, and J. Weipert (Germany: Naturkundemuseum Erfurt), 407–422.

Liu, Y.-J., Whelen, S., and Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol. Biol. Evol. 16, 1799–1808. doi: 10.1093/molbev.12020692

Lynch, S. C., Wang, D. H., Mayorquin, J. S., Ruggmen-Jones, P. F., Stouthamer, R., and Eskalen, A. (2014). First report of Geosmithia pallida causing foamy bark canker, a new disease on coast live oak (Quercus agrifolia), in association with Pseudopothythorax pubipennis in California. Plant Dis. 98:1276. doi: 10.1094/PDIS-03-14-0273-PDN

Machingambi, N. M., Roux, J., Dreyer, L. L., and Roets, F. (2014). Bark and ambrosia beetles (Curculionidae: Scolytinae), their phoretic mites (Acari), and associated Geosmithia species (Ascomycota: Hypocreales) from Virgilia trees in South Africa. Fungal Biol. 118, 472–483. doi: 10.1016/j.fuimbio.2014.03.006

McPherson, B. A., Erbilgin, N., Bonello, P., and Wood, D. L. (2013). Fungal species assemblages associated with Phytophthora ramorum-infected coast live oaks following bark and ambrosia beetle colonization in northern California. For. Ecol. Manage. 291, 30–42. doi: 10.1016/j.foreco.2012.11.010

Miller, M. A., Pfeiffer, W., and Schwartz, T. (2010). “Creating the CIPRES science gateway for inference of large phylogenetic trees,” in Gateway Computing Environments Workshop (GCE) (New Orleans, LA).

O’Donnell, K., and Cigelnik, E. (1997). Two Divergent Intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are Nonorthological. Mol. Phylogenet. Evol. 7, 103–116. doi: 10.1016/0953-8152.1996.0376

Pepori, A. L., Kolarik, M., Bettini, P. P., Vettraino, A. M., and Santini, A. (2013). Are alkalitolerant fungi of the genus Geosmithia of marine origin? Fungal Ecol. 66, 682–700. doi: 10.1016/j.mycres.2007.06.010

Pett, J. J. (1979). Geosmithia gen. nov. for Penicillium lavendulum and related species. Can. J. Bot. 57, 2021–2030. doi: 10.1139/b79-252

Pitt, I. J., and Hocking, A. D. (2009). Fungi and Food Spoilage. Vol. 519. Berlin: Springer.

Rehner, S. A., and Buckley, E. (2005). A Beauveria phylogeny inferred from nuclear ITS and EFL-α sequences: evidence for cryptic diversification and links
to *Cordyceps* teleomorphs. *Mycologia* 97, 84–98. doi: 10.1080/15572356.2006.11382842

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., et al. (2012). MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542. doi: 10.1093/sysbio/sys029

Seifert, K., De Beer, Z. W., and Wingfield, M. (2013). *The Ophiostomatoid Fungi: Expanding Frontiers*. Utrecht: CBS-KNAW Fungal Biodiversity Centre.

Seybold, S. J., Haugen, D., O’Brien, J., and Graves, A. D. (2013). *The Ophiostomatales*. USDA Forest Service, Northeastern Area State and Private Forestry Pest Alert NA-PR-02e10. Milwaukee, WI: USDA Forest Service.

Six, D. L., and Bentz, B. J. (2007). Temperature determines symbiont abundance in a multipartite bark beetle-fungus ectosymbiosis. *Microb. Ecol.* 54, 112–118. doi: 10.1007/s00248-006-9178-x

Skelton, J., Jusino, M. A., Li, Y., Bateman, C., Thai, P. H., Wu, C., et al. (2018). Detecting symbioses in complex communities: the fungal symbionts of bark and ambrosia beetles within Asian Pines. *Microb. Ecol.* 76, 839–850. doi: 10.1007/s00248-018-1154-8

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. doi: 10.1093/bioinformatics/btu333

Stodůlková, E., Kolarík, M., Keesinová, Z., Kuzma, M., Šulc, M., Man, P., et al. (2009). Hydroxylated anthraquinones produced by *Geosmithia* species. *Folia Microbiol.* 54, 179–187. doi: 10.1007/s12223-009-0028-3

Strzalka, B., Kolarík, M., and Jankowiak, R. (2021). *Geosmithia* associated with hardwood-infesting bark and ambrosia beetles, with the description of three new species from Poland. *Antonie Van Leeuwenhoek* 118, 169–194. doi: 10.1007/s10482-020-01510-6

Tisserat, N., Cranshaw, W., Leatherman, D., Utley, C., and Alexander, K. (2009). Black walnut mortality in colorado caused by the walnut twig beetle and thousand cankers disease. *Plant Health Prog.* 10:10. doi: 10.1094/PHP-2009-0811-01-BS

Tisserat, N., Cranshaw, W., Putnam, M. L., Psheidt, J., Leslie, C. A., Murray, M., et al. (2011). Thousand cankers disease is widespread in black walnut in the Western United States. *Plant Health Prog.* 12:35. doi: 10.1094/PHP-2011-0630-01-BS

Utley, C., Nguyen, T., Roubitsova, T., Coggeshall, M., Ford, T. M., Grauke, L. J., et al. (2012). Susceptibility of walnut and hickory species to *Geosmithia morbida*. *Plant Dis.* 97, 601–607. doi: 10.1094/PDIS-07-12-0636-RE

Vannini, A., Contarini, M., Faccoli, M., Dalla Valle, M., Morales-Rodriguez, C., Mazzetto, T., et al. (2017). First report of the ambrosia beetle *Xylosandrus compactus* and associated fungi in the Mediterranean maquis in Italy, and new host-pest associations. *Bull. OEPP* 47, 100–103. doi: 10.1111/eppp.12358

Veselá, T., Skelton, J., Kostovčík, M., Hulcr, J., Balďan, P., Chudíčková, M., et al. (2019). Adaptive traits of bark and ambrosia beetle-associated fungi. *Fungal Ecol.* 41, 165–176. doi: 10.1016/j.funeco.2019.06.005

White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics," in *PCR Protocols: A Guide to Methods and Applications*, Vol. 18, eds M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (New York, NY: Academic Press, Inc), 315–322.

Zheng, S., Johnson, A. J., Li, Y., Chu, C., and Hulcr, J. (2019). *Cryptopus eriobotryae* sp. nov. (Coleoptera: Curculionidae: Scolytinae), a new insect pest of loquat *Eriobotrya japonica* in China. *Insects* 10:180. doi: 10.3390/insects10060180

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