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

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Abstract

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 were reported from Taiwan, China. The aim of this study was to investigate the diversity of *Geosmithia* species in southern China. Field surveys in Guangdong, Guangxi, Hunan, Jiangxi and Shanghai yielded a total of 76 fungal isolates from six beetle species. Isolates were grouped based on morphology. The ITS, β-tubulin and elongation factor 1-α gene regions of representatives of each group were sequenced. Phylogenetic trees were constructed based on those sequences. In total five species were identified, with one previously described species *G. putterilli* and four new species which were described as *G. jiulianshanensis*, *G. jiangxiensis*, *G. formosana*, and *G. pulvereae* (*Geosmithia* sp. 3 and *Geosmithia* sp. 23) sp. nov., in this paper.

Introduction

Members of *Geosmithia* are widely distributed fungal associates of phloem- and xylem-feeding beetles (Kolařík et al. 2007, 2017; Lin et al. 2016; Pitt 1979), 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 (Kolarík 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 2012). 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 curved basal cell, verrucous conidiophores (incl. phialide), cylindrical phialide shape with very short and cylindrical neck (collula) and by ellipsoidal or cylindrical conidia (except of globose conidia in *G. eupagioceri* and *G. microcorthyli*). Colony color could be in shades of white, yellow, brown or red, but newer bluish green or green (Kolarík et al. 2004; Kolařík and Kirkendall 2010).

The spores of *Geosmithia* may be transmitted by attaching to the surface of beetle vector, but the ecological role of most *Geosmithia* species in symbiosis with bark beetles is still unclear. Some species serve as a main food source or supplementary nutrition for the beetles (Kolarík and Kirkendall 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; Huang et al. 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* (Kolařík et al. 2011). Following high density colonization by its beetle vector, the walnut twig beetle (WTB, *Pityophthorus juglandis*), in the phloem of walnut (*Juglans* spp.) or wingnut (*Pterocarya* spp.) trees, *G. morbida* causes numerous small lesions which eventually girdle the vascular tissue (Hishinuma et al. 2015; Kolarík et al. 2011; Tisserat et al. 2009, Seybold et al. 2013; Utley et al. 2013). TCD has affected many walnut trees in North America, especially in the western United States (Tisserat et al. 2009; Tisserat et al. 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* (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 (Huang et al. 2019; Jankowiak et al. 2014; Kolarík and Jankowiak 2013; Kolarík et al. 2004, 2005, 2007, 2008; Kubátová et al. 2004; Machingambi et al. 2014; McPherson et al. 2013; Pepori et al. 2015; 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. 2019). 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 (Kolarík and Jankowiak 2013; Kolarík et al. 2008, 2017; Jankowiak et al. 2014; Veselská et al. 2019). Other factors affecting the fungal community structure include beetle ecology, the surrounding host tree community, and climatic factors (Jankowiak et al. 2014; Six and Bentz 2007). These factors also influence the communities of *Geosmithia*, most notably by the fact that different beetles coexisting the same host tree have similar *Geosmithia* assemblages (Kolarík et al. 2008; Machingambi et al. 2014).

At present, most studies of *Geosmithia* were conducted from North and South America and Europe, but the mycoflora of Asian bark beetles remains understudied. The purpose of this study is to investigate the *Geosmithia* species from southern China using phylogenetic analysis and morphological and physiological features to fill the gap in our understanding of the global *Geosmithia* diversity.

Materials and Methods

**Sampling, isolating, and preserving of fungal isolates.**

The beetle gallery samples were collected in Guangdong, Guangxi, Hunan, Jiangxi and Shanghai Province from plant hosts of *Altingia gracilipes* (*Altingiaceae*), *Gnetum luzhouense* (*Gnetaceae*), Lauraceae sp., *Liquidambar formosana* (*Altingiaceae*), *L. styraciflua* (*Altingiaceae*) and *Ulmus* sp. (*Ulmaceae*) and kept individually in sealable bags. Adult beetles were individually placed in Eppendorf tubes. Both galleries and adult beetles were kept at 4°C for further isolation. The beetle vectors were *Acanthotomus suenel* (*Curculionidae-Scolytinae*), *Scolytus jiulianshanensis* (*Curculionidae-Scolytinae*), *Crossotosaurus emancipatus* (*Curculionidae-Platypodinae*), *Dinoderus* sp. L489 (*Bostrichidae*), *Microperus* sp. L589 (*Curculionidae-Scolytinae*) and *Phloeosinus* sp. (*Curculionidae*) (Table 1). The fungal isolates were obtained by using method of scraping wood tissue from the beetle galleries and inoculated on 2% malt extract agar (MEA: 20 g agar [Solarbio, China], 20 g malt extract [Hopebio, China], 1 L deionized water). The cultures were purified by hyphal-tip subculturing and incubated at 25 °C. All the cultures obtained in this study were deposited in culture collection (SNM) of Shandong Normal University, Jinan, Shandong.
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). Samples were vortexed after incubated at 100 °C for 10 min and then centrifuged at 5000 rpm for 5 min. The supernatant was transferred to a new Eppendorf tube and used as template for polymerase chain reaction (PCR) amplification.

The rDNA region of the 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). Translation elongation factor 1-α gene (TEF1-α) was amplified using 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). PCR amplification was 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, China), 200 µM dNTP, 0.5 µM of each primer, and 5% (v/v) dimethyl sulfoxide (DMSO). The PCR conditions were as follows: 95°C for 3 min, followed by 30 cycles of 95°C for 1 min, 50–55°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). Reference sequences of Geosmithia species were retrieved from GenBank (Table 2). Emericellopsis pallida CBS 490.71 was chosen as the phylogenetic outgroup. Sequences were aligned by using the online version of MAFFT v. 7 (Katoh and Standley 2013) with default setting. The best nucleotide substitution model for each partition was determined in jModelTest v. 2.1.1 (Darriba et al. 2012). 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 recommended partition parameters to assess the tree topology and bootstrap values from 1000 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). MCMC runs of four chains were executed simultaneously from a random starting tree for five million generations, every 100 generations were sampled resulting in 50000 trees, and 12500 trees were discarded during burn-in. Posterior probabilities were estimated from the retained 37500 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 (https://www.treebase.org/, nos.: 28242).

Morphological study

Morphological characters were observed and recorded using the Olympus BX61 microscope (Olympus Corporation, Japan). The images were analyzed using ImageJ (https://imagej.net/). 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). 

Growth study

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

Results

Collection of samples and isolation of fungi

A total of 76 strains in the genus Geosmithia were isolated from 6 beetle species and their galleries. The 73 strains were from the galleries and three strains (SNM887, SNM886, SNM885) from the beetles. Sixty-three strains were from Jiangxi, nine from Shanghai, two from Guangxi, one from Guangdong and one from Hunan (Table 1).

Phylogenetic analysis

The preliminary classification was carried out by BLAST on NCBI GenBank using the ITS marker. Subsequently, 20 representative strains were selected for multi-gene phylogenetic analysis and 10 strains were screened for morphological studies (Table 2). Aligned sequences including gaps yielded 562 characters for ITS, 907 characters for TEF1-α, and 632 characters for TUB2. The best substitution model for ITS, TEF1-α and TUB2 was GTR + I + G. For all datasets (ITS, TUB2, TEF1-α), ML, MP and Bayesian inference produced nearly identical topologies, with slight variations in the statistical support for each of the individual sequence datasets. Phylograms obtained by ML are presented for all the individual datasets.

Taxonomy

Among the 76 strains obtained in this study, five species were identified. Four of these species are new to science, and are described as follows:

Geosmithia jiulianshanensis R. Chang & X. Zhang, sp. nov. (Fig. 4)

Mycobank MB839256
**Etymology.** *jiulianshanensis*, referring to the predominant beetle vector *Scolytus jiulianshanensis*.

**Diagnosis**

The stipe of *G. jiulianshanensis* is slightly thicker and shorter than that in other species. *Geosmithia jiulianshanensis* can grow at 5 and 35°C, even grow slowly at 37°C.

**Type**

**CHINA.** Jiangxi Province, Ganzhou City, Longnan county, Jiulianshan National Nature Reserve (24°34′1″N, 115°30′E), from gallery of *Scolytus jiulianshanensis* on *Ulmus* sp., 5 May, 2020, S. C. Lai, Y. Xu, S. Liao, Y. Wen & T. Li (HMAS 249919 - holotype, SNM261 = CGMCC3.20252 - ex-holotype culture).

**Description**

Sexual state not observed. Asexual state penicillium-like. Conidiophores borne mostly from aerial fungal hyphae, erect, determinate, solitary, sometimes funiculose, with all parts verrucose; base often consisting of 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 (19.0) 29.6–61.5 (85.0) µm long, biverticillate to quaterverticillate (penicilli of conidiophores on aerial funiculose mycelia are monoverticillate or biverticillate), symmetric or asymmetric, often irregularly branched, rami (1st branch) in whorls of X-Y, (4.1-) 5.2-7.0 (8.7) x (1.2) 1.7–2.5 (-3.2) µm, metulae (last branch) in whorls of X-Y, (4.0-) 4.9–6.5 (7.6) x (1.4) 1.8–2.3 (2.6) µm; phialides in whorls of X-Y, cylindrical, without or with short cylindrical neck and smooth to verruculose walls, (4.2-) 5.1–7.5 (10.2) x (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) x (0.9-) 1.2–1.7(2.2) µm, produced in non-persistent conidial chains. Substrate conidia absent.

MEA, 8 d: Colony diam 59–64 mm at 20°C, 65–78 mm at 25°C, and 66–70 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 d were appressed, velutinous or occose with raised mycelial cords; colony margin smooth, lamentous, diffuse; aerial mycelium sparse; substrate mycelium sparse; conidiogenesis at 5 and 35°C. After 8 days of culture, the colony diam was 1.5-4 mm and 11–14 mm respectively. The optimal temperature for growth was 25°C. Conidia borne mostly from aerial fungal hyphae, erect, determinate, solitary, sometimes fuscous; base often consisting of 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 (19.0) 29.6–61.5 (85.0) µm long, biverticillate to quaterverticillate (penicilli of conidiophores on aerial funiculose mycelia are monoverticillate or biverticillate), symmetric or asymmetric, often irregularly branched, rami (1st branch) in whorls of X-Y, (4.1-) 5.2-7.0 (8.7) x (1.2) 1.7–2.5 (-3.2) µm, metulae (last branch) in whorls of X-Y, (4.0-) 4.9–6.5 (7.6) x (1.4) 1.8–2.3 (2.6) µm; phialides in whorls of X-Y, cylindrical, without or with short cylindrical neck and smooth to verruculose walls, (4.2-) 5.1–7.5 (10.2) x (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) x (0.9-) 1.2–1.7(2.2) µm, produced in non-persistent conidial chains. Substrate conidia absent.

MEA, 8 d: Colony diam 59–64 mm at 20°C, 65–78 mm at 25°C, and 66–70 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 d were appressed, 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; absence of exudate; no soluble pigment. When incubated at 35 °C, colonies raised, slightly depressed at center, rugose or irregularly furrowed; margin undulate somewhat erosive; aerial mycelia sparse to moderate; substratum mycelia dense, forming a tough basal felt; the colony is darker and yellowish brown; soluble pigment is brown. MEA, 37°C, 8 d, germinating only.

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

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

**Distribution**

Currently only known from Jiangxi and Shanghai

**Notes.** *Geosmithia formosana, G. jiulianshanensis* and *G. jiangxiensis* are phylogenetically close to each other on ITS, TUB2 and TEF1-α trees. The colony morphology of *G. formosana, G. jiulianshanensis* and *G. jiangxiensis* are also similar, but there are many differences among those three species. First of all, their sequences are quite different (Table 3). And then, under the microscope, the morphological differences between them are more obvious. The spore of *G. jiangxiensis* is thicker than the other two species. The stipe of *G. formosana* is thinner and longer than other two species, the stipe of *G. jiulianshanensis* is obviously thicker than the other two species, and the stipe of *G. jiulianshanensis* is slightly thicker and shorter than that of *G. formosana*. Moreover, their growths at different temperatures are also different (Table 4). *Geosmithia formosana* cannot grow at 5 and 35°C while *G. jiulianshanensis* can grow at both temperatures, especially at 35°C, even grow slowly at 37°C. *Geosmithia jiangxiensis* only grows a little at 5°C, and grows slowly at 35°C. The growth speed of *G. jiulianshanensis* is faster than other two species (Table 4).

**Additional cultures examined**

**CHINA.** Jiangxi Province, Ganzhou City, Longnan county (24°52′4″N, 114°47′2.4″E), from gallery of *Acanthotomicus suncei* on *Liquidambar formosana*, 5 May, 2020, S. C. Lai (SNM260, SNM246).

**CHINA.** Jiangxi Province, Ganzhou City, Xunwu county (24°57′N, 115°38′2″E), from gallery of *Acanthotomicus suncei* on *Liquidambar formosana*, 5 May, 2020 (SNM287).

**CHINA.** Shanghai, from gallery of *Acanthotomicus suncei* on *Liquidambar styraciua*, April 2019, L. Gao (SNM210, SNM226, SNM285, SNM286, SNM287).

*Geosmithia jiangxiensis* R. Chang & X. Zhang, sp. nov. (Fig. 5)

MycoBank MB839257

**Etymology: jiangxiensis, referring to the place where this species was isolated, Jiangxi Province.**

**Diagnosis**

The spore and the stipe of *G. jiangxiensis* is thicker than close related species. *Geosmithia jiangxiensis* only grows a little at 5 and 35°C.

**Type**
**Description**

Sexual state not observed. Asexual state penicillium-like. Conidiophores borne from 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 (22.6-) 35.6–85.7 (-119.3) µm long, with walls thick, septate; penicillus terminal, mostly biverticillate, rarely triverticillate, mostly symmetrical, rami (1st branch) in whorls of X-Y, (4.2-) 5.2–7.8 (-10.6) × (1.3-) 2.1–3.5 (-4.8) µm, metulae (last branch) in whorls of X-Y, (2.6-) 3.9–5.8 (-7.3) × (1.3-) 1.7–2.6 (-3.3) µm. Phialides in whorls of X-Y, (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 verruculose 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 d: Colony diam 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 less than 1 mm and close to 0 mm, respectively. At 35°C, there was little or no growth. The optimal growth temperature is 25°C. Colonies

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

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

**Distribution**

Jiangxi

**Notes**

See comparisons between Geosmithia jiulianshanensis, G. jiangxiensis and G. formosana below the description of G. jiulianshanensis.

**Additional cultures examined**

**CHINA. Jiangxi Province, Ganzhou City, Longnan county, Jiulianshan National Nature Reserve (24°34′1″N, 114°30′E), from gallery of Scolytus jiulianshanensis on Ulmus sp., 5 May, 2020, S. C. Lai, Y. Xu, S. Liao, Y. Wen & T. Li (SNM280).**

**CHINA. Jiangxi Province, Ganzhou City, Xunwu county (24°57′N, 115°38′E), from gallery of Acanthotomicus suncei on Liquidambar formosana, 5 May, 2020 (SNM883, SNM884).**

Geosmithia formosana R. Chang & X. Zhang, sp. nov. (Fig. 6)

MycoBank MB839258

**Etymology.** formosana, referring to the tree host of Liquidambar formosana where this species has been isolated.

**Diagnosis**

The stipe of G. formosana is thinner and longer than close related species. Geosmithia formosana cannot grow at 5 and 35°C.

**Type**

**CHINA. Jiangxi Province, Ganzhou City, Longnan county (24°5′2.4″N, 114°47′2.4″E), from gallery of Acanthotomicus suncei on Liquidambar formosana, 5 May, 2020, S. C. Lai (HMAS 249921 - holotype, SNM256 = CGMCC3.20254 - ex-holotype culture).**

**Description**

Sexual state not observed. Asexual state penicillium-like. Conidiophores borne from substrate or aerial mycelium, erect, determinate, solitary, with all parts verrucose; base often consisting of curved and atypically branched cell, stipe (9.2-) 16.7–62.6 (-108.0) × (1.0-) 1.7–3.0 (-3.5) µm; penicillus (21.2-) 41.0–88.8 (-113.9) µm long, long, penicillus terminal, biverticillate to quartermocillate, terminal, biverticillate to quaterverticillate (penicilli of conidiophores on aerial funiculose mycelia are monoverticillate or biverticillate), symmetric or asymmetric, often irregularly branched, rami (1st branch) in whorls of X-Y, (5.1-) 5.7–7.8 (-9.6) × (1.3-) 1.6–2.5 (-3.9) µm, metulae (last branch) in whorls of X-Y, (4.4-) 5.1–6.5 (-7.3) × (1.1-) 1.6–2.4 (-2.9) µm; phialides in whorls of X-Y, cylindrical, without or with short cylindrical neck and smooth to verruculose walls, (3.0-) 4.7–6.9 (-8.1) × (1.1-) 1.5–2.4 (-3.2) µm. Conidia hyaline to subhyaline, smooth, narrowly cylindrical to ellipsoidal, (2.3-) 2.7–3.7 (-4.4) × (0.8-) 1.2–1.8 (-2.2) µm, produced in non-persistent chains. Substrate conidia absent.

MEA, 8 d: Colony diam 50–54 mm at 20°C, 58–64 mm at 25°C, and 44–52 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 mm and close to 0 mm, respectively. At 35°C, there was little or no growth. The optimal growth temperature is 25°C. Colonies
at 25°C, 8 d, appressed, white velutinous or flocose with raised mycelial cords; colony margin smooth, filamentous, diffuse, pale yellow; aerial mycelium hyaline, sparse; substrate mycelium hyaline, sparse; conidiogenesis moderate; light yellow to brown; absence of exudate; no soluble pigment. MEA, 37°C, 8 d: no growth.

Host: *Liquidambar formosana*.

**Beetle vectors: Acanthotomicus suncei**

**Distribution**
Jiangxi

**Notes**
See comparisons between *G. jiulianshanensis*, *G. jiangxiensis* and *G. formosana* below the description of *G. jiulianshanensis*.

*Geosmithia pulverea* R. Chang & X. Zhang, sp. nov. (Fig. 7)

MycoBank MB839259

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

*Diagnosis:* *Geosmithia pulverea* produces long spore chain while its close related species does not.

**Type**

**CHINA**, Guangdong Province, Shenzhen City (22°37′54″N, 114°27′16″E), from gallery 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. *Conidiophores* arising from substrate or aerial mycelium with all parts verrucose, 40–250 μm tall; 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 (17.5-) 30.9–84.3 (-120.1) μm long, biverticillate to quaternverticillate, symmetric or asymmetric, often irregularly branched, 2–3×, rarely more, rami (1st branch) in whorls of X-Y, (8.2-) 10.2–14.4 (-18.9) × (2.2-) 2.5–3.3 (-3.9) μm, metulae (last branch) in whorls of X-Y, (6.3-) 7.5–10.9 (-15.8) × (1.8-) 2.1–2.8 (-3.5) μm; phialides X-Y, cylindrical or ellipsoidal, without or with short cylindrical neck and smooth to verrucose walls, (5.3-) 7.0-9.6 (-12.3) × (1.5-) 2.1–2.8 (-3.5) 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. Conidia formed in long, non-persistent conidial chains. Substrate conidia narrow, with free or with short conidiophores arising from substrate or aerial mycelium with all parts verrucose, 40–250 μm tall; 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 (17.5-) 30.9–84.3 (-120.1) μm long, biverticillate to quaternverticillate, symmetric or asymmetric, often irregularly branched, 2–3×, rarely more, rami (1st branch) in whorls of X-Y, (8.2-) 10.2–14.4 (-18.9) × (2.2-) 2.5–3.3 (-3.9) μm, metulae (last branch) in whorls of X-Y, (6.3-) 7.5–10.9 (-15.8) × (1.8-) 2.1–2.8 (-3.5) μm; phialides X-Y, cylindrical or ellipsoidal, without or with short cylindrical neck and smooth to verrucose walls, (5.3-) 7.0-9.6 (-12.3) × (1.5-) 2.1–2.8 (-3.5) 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. Conidia formed in long, non-persistent conidial chains. Substrate conidia absent.

MEA, 8 d: Colony diam 23–29 mm at 20°C, 30–37 mm at 25°C, and 31–36 mm at 30°C. No grow at 5°C. At 35°C, mycelia grew slowly. After 8 days of culture, the colony diameter was 1.5-4 mm, with yellow soluble pigment. The optimal growth temperature is 25–30°C. Colonies at 25°C, 8 d, plane with radial rows and slightly raised centrally, texture velutinous (powdery); sporulation abundant, spore mass 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 above. MEA, 37°C, 8 d: no growth.

Host: *Gnetum luofuense*, *Liquidambar formosana*.

**Beetle vectors:** *Acanthotomicus suncei*, *Crossotarsus emancipatus*, *Dinoderus sp.*, *Microperus sp.*

**Distribution**
Gungdong, Guangxi, Hunan, Jiangxi, Shanghai

*Notes: Geosmithia pulverea* 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 was clustered with *Geosmithia* sp. 3, and SNM886, SNM887 and SNM270 were clustered with *Geosmithia* sp. 23 (Fig. 1). However, in the trees with TUB2 and TEF1-α, these strains did not have clear subclassification (Fig. 2 and Fig. 3). It was consequently recognized, using multigene phylogeny, together with *Geosmithia* sp. 23, as a well-defined phylogenetic species inside the *G. pallida* species complex (Kolařík et al. 2017; Huang et al. 2017). The colony of *G. pulverea* was very similar to *G. jiangxiensis* 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 the Temperate Europe in case of *Geosmithia* sp. 3 (Kolařík et al. 2004, 2008; Strzalka et al. 2021) or seems to have global distribution and many bark beetle hosts across Temperate Europe (Strzalka et al. 2021), Mediterranean basin (Kolařík et al. 2007), Northern America (Kolařík et al. 2017; Huang et al. 2017, 2019) and Seychelles (Kolařík et al. 2017). The further study is needed to assess the taxonomic relationships between *G. pulverea*, *Geosmithia* sp. 3 and *Geosmithia* sp. 23.

**Additional cultures examined:** CHINA, Guangxi Province, Shangsi City (21°54′12″N, 107°54′14″E), from body surface of *Crossotarsus emancipates*, 27 March, 2018, Y. Li (SNM887, SNM886).
China Hunan Province, Changsha City (28°10′56″N, 112°55′41″E), from gallery of Microperus sp. L589, 15 July, 2019, Y. Li (SNM888).

China Jiangxi Province, Ganzhou City, Longnan county (24°52.4″N, 114°47′2.4″E), from gallery of Acanthotomicus suncei on Liquidambar formosana, 5 May, 2020, S. C. Lai (SNM270).

China Shanghai, from gallery of Acanthotomicus suncei on Liquidambar styrraciflua, April 2019, L. Gao (SNM248).

Discussion

A total of 76 strains of Geosmithia were isolated in this study. Analyses of ITS, TUB2 and TEF1-α showed those isolates were separated into five taxa, with one of these strains has been named in previous, G. putterillii, and the other four were novel species, described as G. jiangxiensis, G. formosana and G. pulverea in this study. Those species were isolated from larvae, frass and wood dust in beetle galleries of dying, stressed or weakened broad-leaf tree host, such as Liquidambar spp. and Ulmus sp.

The dominant species obtained in this study were G. jiangxiensis and G. pulverea, with 38 and 18 strains respectively (Table 1). The reason for their abundance in our dataset is the fact that our study focused on sampling from Altinginaceae; it does not mean that the fungus is dominant in other tree taxa. Four species, G. putterillii, G. jiangxiensis and G. formosana have only been isolated in Jiangxi (Table 1). The samples collected from Guangdong, Guangxi and Hunan only yielded G. pulverea.

Geosmithia putterillii was isolated from bark beetles feeding on plants from the family of Rossaceae (Kolařík et al. 2008) and Lauraceae in Europe (Kolařík et al. 2004) and on various families of Angiosperms and Gymnosperms in the Western U.S. (Kolařík et al. 2017). The type strain was isolated from the timber in the New Zealand (Pitt 1979). In this study, G. putterillii was isolated from gallery of Phloeosinus sp. on Lauraceae sp. 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.

Most of G. jiangxiensis 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. styrraciflua and L. formosana. The beetle was recorded as an agent of great damage to the imported American sweetgum L. styrraciflua in Shanghai and neighbouring Jiangsu province (Gao and Cognato 2018). The role of the fungus in this outbreak and in the tree pathology remains uninvestigated, though the authors of this paper noted small lesions around the beetle galleries. The other five isolates were isolated from the galleries of Scolytus jiiulianshanensis on Ulmus sp, which suggests that G. jiangxiensis might colonize wide range tree hosts.

Geosmithia jiangxiensis was only isolated in samples from Jiangxi province, from two plant families: Altinginaceae and Ulmaceae (Table 1). The colony of G. jiangxiensis is similar to G. jiiulianshanensis in morphology, but the difference can be seen in the growth rate and micromorphology.

Geosmithia pulverea, is a species closely related to Geosmithia sp. 3 and Geosmithia sp. 23 which are know from various bark beetle hosts in Europa, USA and Seychelles (Kolařík et al. 2007, 2008, 2017; Huang et al. 2017, 2019), and further study need to clarify among these three lineages. In this study, we isolated G. pulverea from A. gracilipes, Gne. luofuense, L. formosana 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, Jiangxi, and Shanghai (Table 1) and vectored by several beetle species, such as, S. jiiulianshanensis, A. suncei, C. emancipatus, Dinoderus sp. Microperus sp. and Phloeosinus sp. (Table 1). Moreover, the abundant of Geosmithia species associated with Acanthotomicus suncei in the current study was also consistent with the frequent occurrence in Shanghai and Jiangxi (Gao et al. 2021).

Conclusions

This study does not provide sufficient data to determine the structure of the Geosmithia community in southern China, as was inferred in Europe and USA after a significantly greater sampling effort (Kolařík et al. 2007, 2008, 2013, 2017; Huang et al. 2017, 2019; Jankowiak et al. 2014). Fungal communities are regulated by a number of factors, including geographic location, host tree species and bark beetle vectors, and 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.

Abbreviations

BI: Bayesian inference; ITS: Nuclear ribosomal internal transcribed spacer; TEF1-α: Translation elongation factor 1-α; TUB2: β-tubulin ; ML: Maximum likelihood; PCR: Polymerase chain reaction; CGMCC: China General Microbiological Culture Collection Center; HMAS: Herbarium Mycologicum, Academiae Sinicae; TCD: Thousand cankers disease

Declarations

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Adherence to national and international regulations

Not applicable.

Authors’ contributions

Runlei Chang Meixue Dai and You Li designed the research. You Li, Hongli Si and Gouyan Zhao collected samples. Xiuyu Zhang, Runlei Chang and You Li isolated and purified fungal cultures. Xiuyu Zhang, Runlei Chang and Xiaojian Jiang completed the data acquisition, analyses and interpretation. Xiuyu Zhang and Runlei Chang completed the writing of the paper. Miroslav Kolařík, Jiri Hulcr and You Li revised text, taxonomy and phylogeny. All authors approved the manuscript.

Availability of data and materials

The datasets generated for this study (Table 2) can be accessed via GenBank: https://www.ncbi.nlm.nih.gov/genbank/. Alignments used during the current study are available at TreeBase: https://www.treebase.org/.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Distribution and number of species of Geosmithia among 76 isolated strains

| Geosmithia specie | Location | Tree host | Beetle species | Number(76) |
|------------------|----------|-----------|----------------|------------|
| G. formosana (1) | Jiangxi  | Liquidambar formosana | Acanthotomicus suncei | 1 |
| G. jiangxiensis (14) | Jiangxi | Liquidambar formosana | Acanthotomicus suncei | 7 |
|                  |          | Ulmus sp. | Scolytus jiulianshanensis | 1 |
| G. jiulianshanensis (38) | Jiangxi | Liquidambar formosana | Acanthotomicus suncei | 25 |
|                  |          | Ulmus sp. | Scolytus jiulianshanensis | 5 |
|                  | Shanghai | Liquidambar styraciflua | Acanthotomicus suncei | 8 |
| G. pulverea (18) | Guangdong | Gnetum luofuense | Dinoderus sp. | 1 |
|                  | Guangxi  | unknown   | Crossotarsus emancipatus | 2 |
|                  | Hunan    | unknown   | Microporus sp. | 1 |
|                  | Jiangxi  | Liquidambar formosana | Acanthotomicus suncei | 1 |
|                  | unknown  | unknown   | Phloeosinus sp. | 6 |
|                  | Ulmus sp. | Scolytus jiulianshanensis | 1 |
|                  | Altingia gracilipes | Acanthotomicus suncei | 4 |
| G. putterillii (6) | Jiangxi | Laraceae | Phloeosinus sp. | 6 |
|                  | Shanghai | Liquidambar styraciflua | Acanthotomicus suncei | 1 |

Table 2 Cultures examined in this study and their GenBank accession numbers
| Species           | Isolation no | Beetle vectors          | Tree host              | ITS        | TEF1-α     | TUB2      | GenBank accession no                  | Referen     |
|-------------------|--------------|-------------------------|------------------------|------------|------------|-----------|---------------------------------------|-------------|
| *G. brunnea*      | CBS 142634   | *Xylosandrus compactus* | Liquidambar styraciflua | KY872741   | KY872746   | KY872751 | KY872741, KY872746, KY872751          | Present study|
|                   | CBS 142635   | *X. compactus*          | *L. styraciflua*       | KY872742   | KY872747   | KY872752 | KY872742, KY872747, KY872752          | Present study|
|                   | CBS 142633   | Hypothenemus dissimilis| *Quercus sp.*          | KY872743   | KY872748   | KY872753 | KY872743, KY872748, KY872753          | Present study|
| *G. cnesini*      | CCF 3753     | *Cnesinus lecontei*     | *Croton draco*         | AM947670   | LR535705   |           | AM947670                             | Kolařík al. (201)|
|                   | MK 1820      | *C. lecontei*           | *C. draco*             | AM947671   | LR535705   |           | AM947671                             | Kolařík al. (201)|
| *G. eupagioceri*  | MKA1-b       | *Eupagiocerus dentipes* | *Paulinia renesi*      | AM947666   | LR535705   |           | AM947666                             | Kolařík al. (201)|
|                   | CCF 3754     |                         |                        | LR535705   |           |           | LR535705                             | Kolařík al. (201)|
| *G. fagi*         | CCF 6235     | *Taphrotrychus bicolor* | *Fagus sylvatica*      | LR812775   | LR813193   | LR813119 | LR812775, LR813193, LR813119          | Strzałka al. 2021|
|                   | 21114TBb     | *T. bicolor*            | *F. sylvatica*         | LR812776   | LR813120   |           | LR812776, LR813120                    | Strzałka al. 2021|
|                   | CCF 6234     | *T. bicolor*            | *F. sylvatica*         | LR812785   | LR813129   |           | LR812785, LR813129                    | Strzałka al. 2021|
| *G. fassatiae*    | AK 31/98     | *S. intricatus*         | *Quercus sp.*          | AM421039   | MH580557   |           | AM421039                             | Kolařík al. (200)|
|                   | CCF 4331     |                         |                        | HF546239   | KF853894   |           | HF546239                             | Kolařík al. (200)|
|                   | CCF 4340     |                         |                        | HF546247   | KF853895   |           | HF546247                             | Kolařík al. (200)|
|                   | CCF 3334     |                         | *Quercus pubescens*    | MH580530   | LR535705   |           | MH580530                             | Kolařík al. (200)|
| *G. flavia*       | CCF 3333     | *Xiphydria sp.*         | *Castanea sativa*      | AJ578483   | MH580541   |           | AJ578483                             | Kolařík al. (200)|
|                   | CCF4337      | *Cerambycidae sp.*      | *Pseudotsuga menziesii*| HF546244   | MH580542   | KF853897 | HF546244                             | Kolařík al. (200)|
| *G. formosana*    | SNM256=      | A. suncei               | L. formosana           | MW222401   | MW592423   | MW592403 | MW222401, MW592423, MW592403          | Kolařík al. (200)|
|                   | CGMCC3.20254 |                         |                        |            |           |           | SNM256=CGMCC3.20254                   | Kolařík al. (200)|
| *G. jiangxiensis* | SNM279=     | A. suncei               | L. formosana           | MW222397   | MW592420   | MW592402 | MW222397, MW592420, MW592402          | Kolařík al. (200)|
|                   | CGMCC3.20253 |                         |                        |            |           |           | SNM279=CGMCC3.20253                   | Kolařík al. (200)|
| *S. jiulianshanensis* | SNM280 | S. jiulianshanensis | Ulmus sp.               | MW222396   | MW592409   | MW592401 | MW222396, MW592409, MW592401          | Kolařík al. (200)|
| *A. suncei*       | SNM883       | L. formosana           |                        | MW222407   | MW592412   | MW592399 | MW222407, MW592412, MW592399          | Kolařík al. (200)|
| *A. suncei*       | SNM884       | L. formosana           |                        | MW222406   | MW592411   | MW592400 | MW222406, MW592411, MW592400          | Kolařík al. (200)|
| *S. jiulianshanensis* | SNM261= | S. jiulianshanensis | Ulmus sp.               | MW222399   | MW592410   | MW592395 | MW222399, MW592410, MW592395          | Kolařík al. (200)|
|                   | CGMCC3.20252 |                         |                        |            |           |           | SNM261=CGMCC3.20252                   | Kolařík al. (200)|
| *Acanthotomicus suncei* | SNM246 | A. suncei               | Liquidambar formosana  | MW222403   | MW592425   | MW592393 | MW222403, MW592425, MW592393          | Kolařík al. (200)|
| *A. suncei*       | SNM260       | L. formosana           |                        | MW222400   | MW592422   | MW592394 | MW222400, MW592422, MW592394          | Kolařík al. (200)|
| *A. suncei*       | SNM226       | L. styraciflua         |                        | MW222404   | MW592426   | MW592392 | MW222404, MW592426, MW592392          | Kolařík al. (200)|
| *A. suncei*       | SNM210       | L. styraciflua         |                        | MW222405   | MW592427   | MW592391 | MW222405, MW592427, MW592391          | Kolařík al. (200)|
| *A. suncei*       | SNM285       | L. styraciflua         |                        | MW222395   | MW592408   | MW592396 | MW222395, MW592408, MW592396          | Kolařík al. (200)|
| *A. suncei*       | SNM286       | L. styraciflua         |                        | MW222394   | MW592407   | MW592397 | MW222394, MW592407, MW592397          | Kolařík al. (200)|
| *A. suncei*       | SNM287       | L. styraciflua         |                        | MW222393   | MW592406   | MW592398 | MW222393, MW592406, MW592398          | Kolařík al. (200)|
| *A. suncei*       | SNM882       | L. formosana           |                        | MW222408   | MW592413   | MW592390 | MW222408, MW592413, MW592390          | Kolařík al. (200)|
| *G. lavendula*    | CCF 3051     | Laboratory             | AF033385               |           | LR535705   |           | AF033385                             | Kolařík al. (200)|

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| Species                        | Accession Numbers | Authors     |
|-------------------------------|-------------------|-------------|
| Carphoborus vestitus          | CCF 3394         | Hulcr et al. (2007) |
| Pistacia terebinthus          |                   |             |
| AM421098                      |                   |             |
| Pistacia terebinthus          |                   | Hamelin et al. (2011) |
| CCF 4336                      |                   |             |
| Carphoborus vestitus          | CCF 3322T        | Kolařík et al. (2020) |
| Scolytus intricatus          |                   |             |
| Quercus robur                | KF808297         |             |
| HG799876                      | HG799877         |             |
| Picea abies                  | HE604124         |             |
| P. pityographus              |                   | Strzalki et al. 2021 |
| Pityophthorus pityographus   |                   |             |
| RJ278m                       |                   |             |
| P. abies                     | HE604154         |             |
| LR813194                     | LR813140         |             |
| Microcorthylus sp.           | CCF 3861T        | Kolařík et al. (2020) |
| Cassia grandis               |                   |             |
| FM986798                     | FM986793         |             |
| J. nigra                     | FN434081         |             |
| Kolařík et al. (2020)        |                   |             |
| Pityophthorus juglandis      | CCF 3881         | Kolařík et al. (2020) |
| J. nigra                     | FN434082         |             |
| MHS80543                     | KF853911         |             |
| P. juglandis                 | CCF 4576         | Kolařík et al. (2020) |
| J. nigra                     |                   |             |
| MHS80544                     |                   |             |
| Q. robur                     | CCF 3422         | Kolařík et al. (2020) |
| S. intricus                  |                   |             |
| Carpinus betulus             | CCF 3425         | Kolařík et al. (2020) |
| AM181460                     | MHS80540         |             |
| Kolařík et al. (2000)        |                   |             |
| Hylesinus omi                | MK 1707          | Kolařík et al. (2020) |
| Fraxinus sp.                 |                   |             |
| AM181452                     | MHS80558         |             |
| Kolařík et al. (2000)        |                   |             |
| Phloeotribus frontalis       | CCF 3324         | Kolařík et al. (2020) |
| Acer negundo                 |                   |             |
| Aj578486                     |                   |             |
| Kolařík et al. (2000)        |                   |             |
| Dinoderus sp.                | SNM885           | Kolařík et al. (2000) |
| Gnetum luofuense             |                   |             |
| MW222410                     | MW592415         |             |
| Kolařík et al. (2001)        |                   |             |

**Contamination**
| CGMCC3.20255                                      | SNM270 | A. suncei | L. formosana | MW222398 | MW592421 | MW592387 |
|--------------------------------------------------|--------|-----------|--------------|----------|----------|----------|
| SNM248                                           | A. suncei | L. styraciflua | MW222402 | MW592424 | MW592386 |
| SNM886                                           | Crossotarsus emancipatus | MW222411 | MW592416 | MW592385 |
| SNM887                                           | C. emancipatus | MW222412 | MW592417 | MW592384 |
| SNM888                                           | Microperus sp. | Choerospondias axillaris | MW222409 | MW592414 | MW592389 |
| G. putterillii                                    | CCF 3052 | Beilschmiedia tawa | AF033384 | HG799853 | HG799816 | Kolarík et al. (2017) |
| U 307                                            | B. tawa | HF546306 | MH580529 | Kolarík et al. (2017) |
| SNM402                                           | Phloeosinus sp. | MW584874 | MW592419 | MW592405 |
| SNM436                                           | Phloeosinus sp. | MW584873 | MW592418 | MW592404 |
| G. rufescens                                     | MK 1800 | C. lecontei | C. draco | AM947667 | Kolarík et al. (2017) |
| MK 1803                                          | C. lecontei | C. draco | AM947668 | Kolarík et al. (2017) |
| MK 1821                                          | C. lecontei | C. draco | AM947669 | Kolarík et al. (2017) |
| CCF 3752                                         | LRI55709 | Kolarík et al. (2017) |
| G. ulmacea 13                                     | CCF 3559 | S. multistriatus | Ulmus sp. | AM181439 | MH580535 | Kolarík et al. (2017) |
| 1226                                             | S. schevyrwi | Ulmus sp. | KJ716463 | Zerillo et al. (20) |
| CNR23                                            | U. minor | KP990560 | Alessia et al. (2015) |
| CNR24                                            | U. minor | KP990561 | Alessia et al. (2015) |
| G. sp. 2                                          | U107 | Scolytys rugulosus | Prunus sp. | HF546256 | HG799855 | HG799818 | Kolarík et al. (201) |
| MK 642                                           | H. orni | Fraxinus orinus | HG799852 | Kolarík et al. (201) |
| G. sp. 3                                          | CCF 4298 | S. intricatus | Quercus dalechampii | AM181436 | HG799851 | HG799814 | Kolarík et al. (2017) |
| CCF 3481                                         | Scolytus carpini | C. betulus | AM181467 | HG799842 | HG799805 | Kolarík et al. (2017) |
| G. sp. 4                                          | CCF 4278 | Pteleobius vittatus | Ulmus laevis | AM181466 | HG799850 | HG799813 | Kolarík et al. (2017) |
| G. sp. 5                                          | CCF 3341 | S. intricatus | Quercus petraea | AJ578487 | HG799837 | HG799801 | Kolarík et al. (2017) |
| CCF 4215                                          | P. pityographus | P. abies | HE604117 | Kolarík and Jankowiak (201) |
| AK192/98                                         | S. intricatus | Q. robur | HG799835 | Kolarík et al. (201) |
| G. sp. 8                                          | CCF 3358 | S. intricatus | Q. petraea | AM181421 | MH580559 | FM986788 | Kolarík et al. (2017) |
| G. sp. 9                                          | CCF 3564 | AM181428 | Kolarík et al. (201) |
| CCF 3702                                          | AM746018 | Kolarík and Jankowiak (201) |
| RJ0266                                           | Ips cembrae | Larix decidua | MH580551 | Kolarík and Jankowiak (201) |
| G. sp. 11                                         | CCF 3555 | S. intricatus | Q. pubescens | AM181419 | MH580545 | KF853931 | Kolarík et al. (201) |
| CCF 3556                                         | S. intricatus | Q. pubescens | AM181418 | Kolarík et al. (201) |
| G. sp. 12                                         | CCF 4320 | Hylesinus oregonus | Fraxinus sp. | HF546229 | MH580532 | KF853932 | Kolarík et al. (201) |
| CCF 3557                                         | Leperisinus orni | F. excelsior | AM181431 | MH580531 | Kolarík et al. (201) |
| G. sp. 16                                         | CCF 4201 | P. pityographus | P. abies | HE604146 | HE604206 | HE604181 | Kolarík and Jankowiak (2013) |
| RJ34m                                            | P. pityographus | P. abies | HE604182 | Kolarík and Jankowiak (201) |
| G. sp. 19 | CCF 3658 | Hypoborus ficus | Ficus carica | AM421085 | MH580546 | Kolařík et al. (2015) |
| G. sp. 20 | CCF 3655 | H. ficus | F. carica | AM421075 | | Kolařík et al. (2015) |
| G. sp. 21 | CCF 4316 | Phloeosinus fulgens | Calocedrus decurrens | HF546226 | MH580547 | Kolařík et al. (2015) |
| | U193 | Scolytus scheidwieri | Ulmus pumila | HF546287 | MH580548 | Kolařík et al. (2015) |
| G. sp. 22 | CCF 4196 | Scolytus oregoni | P. menziesii | HF546289 | MH580534 | Kolařík et al. (2015) |
| | CCF 4200 | H. ficus | F. carica | AM421049 | MH580533 | Kolařík et al. (2015) |
| G. sp. 23 | CCF 4206 | Phloeotribus scarabeoides | Olea europaea | AM421061 | MH580552 | Kolařík et al. (2015) |
| | CCF 3654 | Scolytus rugulosus | P. menziesii | AM421062 | MH580553 | Kolařík et al. (2015) |
| | CCF 3652 | P. scarabeoides | O. europaea | AM421062 | MH580553 | Kolařík et al. (2015) |
| G. sp. 24 | CCF 4218 | Scolytus multistriatus | P. sylvestris | HE604168 | HG799838 | Kolařík et al. (2015) |
| | U160 | Scolytus multistriatus | U. pumila | HF546284 | | Kolařík et al. (2015) |
| G. sp. 25 | MB136 | Orthotomicus erosus | Pinus halepensis | KP691926 | KP691936 | Dori-Bachash et al. (2015) |
| | MB242 | Pityogenes calcaratus | Pinus brutia | KP691927 | KP691937 | Dori-Bachash et al. (2015) |
| | MB222 | O. erusus | P. brutia | KP691928 | KP691938 | Dori-Bachash et al. (2015) |
| | CCF 4294 | Pityogenes quadridens | P. sylvestris | HE604165 | MH580555 | Kolařík and Jankowiak (2017) |
| | MK1772 | P. pitiographus | P. sylvestris | HE604164 | MH580556 | Kolařík and Jankowiak (2017) |
| G. sp. 26 | CCF 4205 | Cryphalus abietis | Abies alba | HE604128 | HE604218 | HE604186 | Kolařík and Jankowiak (2017) |
| | CCF 4222 | Pinus sylvestris | A. alba | HE604127 | HE604219 | HE604187 | Kolařík and Jankowiak (2017) |
| G. sp. 27 | CCF 4206 | Phloeosinus bidentatus | P. sylvestris | HE794978 | HG799839 | Kolařík et al. (2017) |
| | CCF 4605 | Pityophthorus sp. | Pinus ponderosa | HF546309 | HG799827 | Kolařík et al. (2017) |
| G. sp. 28 | CCF 4221 | C. piceae | A. alba | HE604125 | HE604233 | HE604184 | Kolařík and Jankowiak (2017) |
| G. sp. 29 | CCF 4228 | I. cembrae | L. decidua | HE604132 | HE604216 | HE604193 | Kolařík and Jankowiak (2017) |
| G. sp. 30 | CCF 4196 | Phloeosinus thuje | Chamaeyparis pisifera | AM181426 | HG799874 | HG799885 | Kolařík et al. (2017) |
| | CCF 4605 | Phloeosinus sequiae | S. serpervirens | HF546265 | HG799873 | HG799886 | Kolařík et al. (2017) |
| G. sp. 31 | CCF 4598 | Cryphalus piceae | P. menziesii |HF546231 | HG799869 | HG799831 | Kolařík et al. (2017) |
| G. sp. 32 | CCF 4604 | Phloeotribus pubipennis | P. menziesii | HF546295 | HG799866 | HG799826 | Kolařík et al. (2017) |
| G. sp. 33 | CCF 4598 | Scolytus praeceps | A. concolor | HF546331 | HG799869 | HG799831 | Kolařík et al. (2017) |
| G. sp. 34 | U193 | S. praeceps | A. concolor | HF546330 | HG799868 | HG799830 | Kolařík et al. (2017) |
| G. sp. 35 | CCF 4238 | Pityophthorus sp. | Pinus murticata | HF546236 | | Kolařík et al. (2017) |
| | MK1814 | C. atlantica | | | MH580538 | | present study |
| G. sp. 36 | U79 | Pseudopityophthorus pubipennis | Notolithocarpus densiflorus | HF546346 | MH580537 | Kolařík et al. (2017) |

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Table 3 Summary of the variability between species of the *Geosmithia jiulianshanensis* species complex. Numbers of changes (substitutions and indels) and corresponding relative percentage dissimilarity values are presented.

| Species         | ITS rDNA (531 bp) | TEF1-α (899 bp) | TUB2 (496 bp) |
|-----------------|-------------------|-----------------|--------------|
| *G. formosana*  | 5 (0.94 %)        | 4 (0.75 %)      | 5-6 (0.56-0.67 %) |
| *G. jiulianshanensis* | 5 (0.56 %)    | 3 (0.60 %)      | 4 (0.81 %)   |
| *Emericellopsis pallida* | 8-9 (0.89-1.0 %) | 4 (0.81 %)      |              |

Note. Isolates recovered in present study are in bold. a G. pallida selected as outgroup of phylogenies. T = ex-type isolates.

Table 4 After 8 days of culture in MEA medium, the colony diameter (unit: mm) of *Geosmithia jiulianshanensis* species complex and *Geosmithia pulverea* at different temperatures.
| Species/T | G. formosana | G. jiangxiensis | G. jiulianshanensis | G. pulverea |
|-----------|--------------|-----------------|---------------------|-------------|
| 5°C       | 1            | 1               | 1.5-4               | 0           |
| 20°C      | 50-54        | 50-58           | 59-64               | 23-29       |
| 25°C      | 58-64        | 59-69           | 65-78               | 30-37       |
| 30°C      | 44-52        | 49-60           | 66-70               | 31-36       |
| 35°C      | ≈0           | 1-4             | 11-14               | 1.5-4       |
| 37°C      | 0            | 0               | 1                   | 0           |

**Figures**

**Figure 1**

ML tree of Geosmithia generated from the ITS sequence data. Sequences generated from this study are printed in bold. Bold branches indicate posterior probability values $\geq 0.9$. Bootstrap values of ML/MP $\geq 75\%$ are recorded at the nodes. T = ex-type isolates.
Figure 2

ML tree of Geosmithia generated from the TUB2 sequence data. Sequences generated from this study are printed in bold. Bold branches indicate posterior probability values ≥ 0.9. Bootstrap values of ML/MP ≥ 75% are recorded at the nodes. T = ex-type isolates
Figure 3

ML tree of Geosmithia generated from the TEF1-α sequence data. Sequences generated from this study are printed in bold. Bold branches indicate posterior probability values ≥ 0.9. Bootstrap values of ML/MP ≥ 75% are recorded at the nodes. T = ex-type isolates
Figure 4

Morphological characters of Geosmithia jiulianshanensis sp. nov. (CGMCC3.20252) a. 8 days old culture on 2% MEA; b–e. Conidiophores and conidia. Scale bars: b–e=10μm
Figure 5

Morphological characters of Geosmithia jiangxiensis sp. nov. (CGMCC3.20253) a. 8 days old culture on 2% MEA; b–e. Conidiophores and conidia. Scale bars: b–d=10μm, e=20μm
Figure 6

Morphological characters of Geosmithia formosana sp. nov. (CGMCC3.20254) a. 8 days old culture on 2% MEA; b–e. Conidiophores and conidia. Scale bars: b–e=10μm
Figure 7

Morphological characters of asexual structures of Geosmithia pulvorea sp. nov. (CGMCC3.20255) a. 8 days old culture on 2% MEA; b–e. Conidiophores and conidia. Scale bars: b–e=10μm