

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. putterillii* and four new species which were described as *G. juliashanensis*, *G. jiangxiensis*, *G. formosana*, and *G. pulvere* (*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 (Kolařík 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. microcorthylis*). Colony color could be in shades of white, yellow, brown or red, but newer bluish green or green (Kolařík 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 (Kolařík 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; Kolařík 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; Kolařík and Jankowiak 2013; Kolařík 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 (Kolařík and Jankowiak 2013; Kolařík 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 coinfesting the same host tree have similar *Geosmithia* assemblages (Kolařík et al. 2008; Machingambi et al. 2014).

At present, most of 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 luofuense* (*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 *Acanthotomicus suncei* (*Curculionidae-Scolytinae*), *Scolytus juliashanensis* (*Curculionidae-Scolytinae*), *Crossotarsus 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

province, China. Isolates linked to type specimens of the fungal species were deposited in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. Holotype specimens (dry cultures) were deposited in the Herbarium Mycologicum, Academiae Sinicae (HMAS), Beijing, China.

DNA extraction, amplification, and sequencing.

DNA was extracted by scraping fresh fungal tissue from pure cultures and adding to 50 μ L 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 μ L PCR reaction mixture consisting of 50–100 ng template DNA, 1.25 U Taq polymerase (Vazyme Biotech Co., Ltd, China), 200 μ M dNTP, 0.5 μ M 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, 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 (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) × (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) × (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) × (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 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 erose; 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 styraciflua*, *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. jiangxiensis* 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°5'2.4"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 (SNM882).

CHINA, Shanghai, from gallery of *Acanthotomicus suncei* on *Liquidambar styraciflua*, 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

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 249920 - holotype, SNM279 = CGMCC3.20253 - ex-holotype culture).

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 terverticillate, mostly symmetrical, rami (1st branch) in whorls of X-Y, (4.2-) 5.2–7.8 (-10.6) \times (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) \times (1.3-) 1.7–2.6 (-3.3) μm . Phialides in whorls of X-Y, (3.9-) 4.6–6.2 (-7.7) \times (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) \times (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 only 1 mm and 1–4 mm respectively. The optimal temperature for growth is 25°C. Colonies at 25°C, 8 d, plane, slightly raised centrally, velutinous, with slight overgrowth of aerial mycelium, with floccose and funiculose areas; substrate mycelium white, aerial mycelium hyaline; sporulation moderate to heavy, pale cream; vegetative mycelium hyaline; reverse lighter; soluble pigment and exudate absent. When incubated at 35 °C, colonies rising, slightly sunken in the center, furrowed or irregularly fringed; the substratum hyphae was dense and formed a tough basal felt. The colony is dark and yellowish-brown. MEA, 37°C, 8 d: no growth.

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

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

Distribution

Jiangxi

Notes

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

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 juianshanensis* 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°38'2"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) \times (1.0-) 1.7-3.0 (-3.5) μm ; penicillus (21.2-) 41.0-88.8 (-113.9) μm long, penicillate conidiophores (penicilli) 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) \times (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) \times (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) \times (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) \times (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 floccose 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 quaterverticillate, 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 verruculose 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. 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, L. styraciflua.

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 *Geosmithia* sp. 3 on MEA, but *Geosmithia* sp. 3 was darker and wrinkled (Kolařík et al. 2004). *Geosmithia pulverea* seems to have smaller stipe size, but other features fit to 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 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, Shangsì 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°5'2.4"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 styraciflua*, 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. jiulianshanensis*, *G. jiangxiensis*, *G. formosana* and *G. pulvereae* 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. jiulianshanensis* and *G. pulvereae*, 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 Altingiaceae; 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. pulvereae*.

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 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. jiulianshanensis* 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. styraciflua* and *L. formosana*. The beetle was recorded as an agent of great damage to the imported American sweetgum *L. styraciflua* 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 jiulianshanensis* on *Ulmus* sp, which suggests that *G. jiulianshanensis* might colonize wide range tree hosts.

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

Geosmithia pulvereae, is a species closely related to *Geosmithia* sp. 3 and *Geosmithia* sp. 23 which are known 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. pulvereae* 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. jiulianshanensis*, *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 Xiaoqian 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

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

Table 2 Cultures examined in this study and their GenBank accession numbers

Species	Isolation no	Beetle vectors	Tree host	ITS	GenBank accession no		Referen
					TEF1- α	TUB2	
<i>G. brunnea</i>	CBS 142634	<i>Xylosandrus compactus</i>	<i>Liquidambar styraciflua</i>	KY872741	KY872746	KY872751	Present study
	CBS 142635	<i>X. compactus</i>	<i>L. styraciflua</i>	KY872742	KY872747	KY872752	Present study
	CBS 142633 ^T	<i>Hypothenemus dissimilis</i>	<i>Quercus</i> sp.	KY872743	KY872748	KY872753	Present study
<i>G. cnesini</i>	CCF 3753	<i>Cnesinus lecontei</i>	<i>Croton draco</i>	AM947670			Kolařík al. (201
	MK 1820	<i>C. lecontei</i>	<i>C. draco</i>	AM947671			Kolařík al. (201
<i>G. eupagioceri</i>	MKA1-b	<i>Eupagiocerus dentipes</i>	<i>Paullinia renesii</i>	AM947666			Kolařík al. (201
	CCF 3754				LR535705		Kolařík al. (201
<i>G. fagi</i>	CCF 6235	<i>Taphrorychus bicolor</i>	<i>Fagus sylvatica</i>	LR812775	LR813193	LR813119	Strzalka al. 2021
	21114TBb	<i>T. bicolor</i>	<i>F. sylvatica</i>	LR812776		LR813120	Strzalka al. 2021
	CCF 6234 ^T	<i>T. bicolor</i>	<i>F. sylvatica</i>	LR812785	LR813141	LR813129	Strzalka al. 2021
<i>G. fassatiae</i>	AK 31/98	<i>S. intricatus</i>	<i>Quercus</i> sp.	AM421039	MH580557		Kolařík al. (200
	CCF 4331			HF546239		KF853894	Kolařík al. (201
	CCF 4340			HF546247		KF853895	Kolařík al. (201
	CCF 3334 ^T		<i>Quercus pubescens</i>		MH580530		Kolařík al. (200
<i>G. flava</i>	CCF 3333 ^T	<i>Xiphydria</i> sp.	<i>Castanea sativa</i>	AJ578483	MH580541		Kolařík al. (200
	CCF4337	<i>Cerambycidae</i> sp.	<i>Pseudotsuga menziesii</i>	HF546244	MH580542	KF853897	Kolařík al. (200
<i>G. formosana</i>	SNM256= CGMCC3.20254	<i>A. suncei</i>	<i>L. formosana</i>	MW222401	MW592423	MW592403	
<i>G. jiangxiensis</i>	SNM279= CGMCC3.20253	<i>A. suncei</i>	<i>L. formosana</i>	MW222397	MW592420	MW592402	
	SNM280	<i>S. jiulianshanensis</i>	<i>Ulmus</i> sp.	MW222396	MW592409	MW592401	
	SNM883	<i>A. suncei</i>	<i>L. formosana</i>	MW222407	MW592412	MW592399	
	SNM884	<i>A. suncei</i>	<i>L. formosana</i>	MW222406	MW592411	MW592400	
<i>G. jiulianshanensis</i>	SNM261= CGMCC3.20252	<i>S. jiulianshanensis</i>	<i>Ulmus</i> sp.	MW222399	MW592410	MW592395	
	SNM246	<i>Acanthotomicus suncei</i>	<i>Liquidambar formosana</i>	MW222403	MW592425	MW592393	
	SNM260	<i>A. suncei</i>	<i>L. formosana</i>	MW222400	MW592422	MW592394	
	SNM226	<i>A. suncei</i>	<i>L. styraciflua</i>	MW222404	MW592426	MW592392	
	SNM210	<i>A. suncei</i>	<i>L. styraciflua</i>	MW222405	MW592427	MW592391	
	SNM285	<i>A. suncei</i>	<i>L. styraciflua</i>	MW222395	MW592408	MW592396	
	SNM286	<i>A. suncei</i>	<i>L. styraciflua</i>	MW222394	MW592407	MW592397	
	SNM287	<i>A. suncei</i>	<i>L. styraciflua</i>	MW222393	MW592406	MW592398	
	SNM882	<i>A. suncei</i>	<i>L. formosana</i>	MW222408	MW592413	MW592390	
<i>G. lavendula</i>	CCF 3051		Laboratory	AF033385			Kolařík

			<i>contamination</i>				al. (200
	CCF 3394	<i>Carphoborus vestitus</i>	<i>Pistacia terebinthus</i>	AM421098			olarik e (2007)
	Hulcr_17347				MH580484		
	CCF 4336					KF853902	Hameli al. (201
<i>G. langdonii</i>	CCF 3332 ^T	<i>Scolytus intricatus</i>	<i>Quercus robur</i>	KF808297	HG799876	HG799887	Kolařík al. (200 2017)
	CCF 4338	<i>Cryphalus pubescens</i>	<i>Sequoia serpervirens</i>	HF546245	HG799877	HG799881	Kolařík al. (201
<i>G. longistipitata</i>	RJ278m	<i>Pityophthorus pityographus</i>	<i>Picea abies</i>	HE604124			Strzalka al. 2021
	CCF 4210 ^T	<i>P. pityographus</i>	<i>P. abies</i>	HE604154	LR813194	LR813140	Strzalka al. 2021
<i>G. microcorthyli</i>	CCF 3861 ^T	<i>Microcorthylus</i> sp.	<i>Cassia grandis</i>	FM986798	MH580560	FM986793	Kolařík Kirkend (2010)
<i>G. morbida</i>	CBS 124664		<i>Juglans nigra</i>	FN434081		KF853912	Kolařík al. (201
	CCF 3881	<i>Pityophthorus juglandis</i>	<i>J. nigra</i>	FN434082	MH580543	KF853911	Kolařík al. (201
	CCF 4576	<i>P. juglandis</i>	<i>J. nigra</i>		MH580544		Kolařík al. (200
<i>G. obscura</i>	CCF 3422 ^T	<i>S. intricatus</i>	<i>Q. robur</i>	AJ784999	MH580539		Kolařík al. (200
	CCF 3425	<i>S. carpini</i>	<i>Carpinus betulus</i>	AM181460	MH580540	KF853914	Kolařík al. (200
<i>G. omnicola10</i>	MK 1707	<i>Hylesinus orni</i>	<i>Fraxinus</i> sp.	AM181452	MH580558		Kolařík al. (200
	CNR115		<i>Ulmus minor</i>			KP990607	Pepori, et al. (2015)
	CNR5		<i>Ulmus glabra</i>			KP990546	Pepori, et al. (2015)
	IMI 194089	<i>H. orni</i>	<i>Fraxinus</i> sp.	AM181450			Kolařík al. (200
	CCF 3553	<i>H. orni</i>	<i>Fraxinus</i> sp.	AM181433			Kolařík al. (200
<i>G. pallida</i>	CCF 3053 ^T		<i>Cotton yarn</i>	AJ578486		HG799817	Kolařík al. (200 2017)
	CCF 3324		Soil		HG799846	HG799809	Kolařík al. (200 2017)
<i>G. pazoutovae</i>	22015aSI	<i>S. intricatus</i>	<i>Q. robur</i>	LR812786		LR813130	Strzalka al. 2021
	24Wa14SI	<i>S. intricatus</i>	<i>Q. robur</i>	LR812787		LR813131	Strzalka al. 2021
	CCF 6233 ^T	<i>S. intricatus</i>	<i>Q. robur</i>	LR812796	LR813142	LR813138	Strzalka al. 2021
	CBS 142636 ^T	<i>Phloeotribus frontalis</i>	<i>Acer negundo</i>	KY872744	KY872749	KY872754	Present study
	CBS 142637	<i>P. frontalis</i>	<i>A. negundo</i>	KY872745	KY872750	KY872755	Present study
<i>G. pulvereae</i>	SNM885=	<i>Dinoderus</i> sp.	<i>Gnetum luofuense</i>	MW222410	MW592415	MW592388	

CGMCC3.20255

	SNM270	<i>A. suncei</i>	<i>L. formosana</i>	MW222398	MW592421	MW592387	
	SNM248	<i>A. suncei</i>	<i>L. styraciflua</i>	MW222402	MW592424	MW592386	
	SNM886	<i>Crossotarsus emancipatus</i>		MW222411	MW592416	MW592385	
	SNM887	<i>C. emancipatus</i>		MW222412	MW592417	MW592384	
	SNM888	<i>Microperus</i> sp.	<i>Choerospondias axillaris</i>	MW222409	MW592414	MW592389	
<i>G. putterillii</i>	CCF 3052 ^T		<i>Beilschmiedia tawa</i>	AF033384	HG799853	HG799816	Kolařík et al. (2017)
	U 307		<i>B. tawa</i>	HF546306	MH580529		Kolařík et al. (2017)
	SNM402	<i>Phloeosinus</i> sp.		MW584874	MW592419	MW592405	
	SNM436	<i>Phloeosinus</i> sp.		MW584873	MW592418	MW592404	
<i>G. rufescens</i>	MK 1800	<i>C. lecontei</i>	<i>C. draco</i>	AM947667			Kolařík et al. (2017)
	MK 1803	<i>C. lecontei</i>	<i>C. draco</i>	AM947668			Kolařík et al. (2017)
	MK 1821	<i>C. lecontei</i>	<i>C. draco</i>	AM947669		KF853927	Kolařík et al. (2017)
	CCF 3752				LR535709		Kolařík et al. (2017)
<i>G. ulmacea</i> 13	CCF 3559	<i>S. multistriatus</i>	<i>Ulmus</i> sp.	AM181439	MH580535		Kolařík et al. (2017)
	1226	<i>S. schevyrewi</i>	<i>Ulmus</i> sp.	KJ716463			Zerillo et al. (2017)
	CNR23		<i>U. minor</i>			KP990560	Alessia et al. (2015)
	CNR24		<i>U. minor</i>			KP990561	Alessia et al. (2015)
<i>G. sp. 2</i>	U107	<i>Scolytus rugulosus</i>	<i>Prunus</i> sp.	HF546256	HG799855	HG799818	Kolařík et al. (2017)
	MK 642	<i>H. orni</i>	<i>Fraxinus ornus</i>		HG799852		Kolařík et al. (2017)
<i>G. sp. 3</i>	CCF 4298	<i>S. intricatus</i>	<i>Quercus dalechampii</i>	AM181436	HG799851	HG799814	Kolařík et al. (2017)
	CCF 3481	<i>Scolytus carpini</i>	<i>C. betulus</i>	AM181467	HG799842	HG799805	Kolařík et al. (2017)
<i>G. sp. 4</i>	CCF 4278	<i>Pteleobius vittatus</i>	<i>Ulmus laevis</i>	AM181466	HG799850	HG799813	Kolařík et al. (2017)
<i>G. sp. 5</i>	CCF 3341	<i>S. intricatus</i>	<i>Quercus petraea</i>	AJ578487	HG799837	HG799801	Kolařík et al. (2017)
	CCF 4215	<i>P. pityographus</i>	<i>P. abies</i>	HE604117			Kolařík and Jankowiak (2013)
	AK192/98	<i>S. intricatus</i>	<i>Q. robur</i>		HG799835		Kolařík et al. (2017)
<i>G. sp. 8</i>	CCF 3358	<i>S. intricatus</i>	<i>Q. petraea</i>	AM181421	MH580559	FM986788	Kolařík et al. (2017)
<i>G. sp. 9</i>	CCF 3564			AM181428			Kolařík et al. (2017)
	CCF 3702			AM746018			Kolařík and Jankowiak (2013)
	RJ0266	<i>Ips cembrae</i>	<i>Larix decidua</i>		MH580551		Kolařík and Jankowiak (2013)
<i>G. sp. 11</i>	CCF 3555	<i>S. intricatus</i>	<i>Q. pubescens</i>	AM181419	MH580545	KF853931	Kolařík et al. (2017)
	CCF 3556	<i>S. intricatus</i>	<i>Q. pubescens</i>	AM181418			Kolařík et al. (2017)
<i>G. sp. 12</i>	CCF 4320	<i>Hylesinus oregonus</i>	<i>Fraxinus</i> sp.	HF546229	MH580532	KF853932	Kolařík et al. (2017)
	CCF 3557	<i>Leperisinus orni</i>	<i>F. excelsior</i>	AM181431	MH580531		Kolařík et al. (2017)
<i>G. sp. 16</i>	CCF 4201	<i>P. pityographus</i>	<i>P. abies</i>	HE604146	HE604206	HE604181	Kolařík and Jankowiak (2013)
	RJ34m	<i>P. pityographus</i>	<i>P. abies</i>			HE604182	Kolařík and Jankowiak (2013)

G. sp. 19	CCF 3658	<i>Hypoborus ficus</i>	<i>Ficus carica</i>	AM421085	MH580546		Kolařík et al. (2017)
	CCF 3655	<i>H. ficus</i>	<i>F. carica</i>	AM421075			Kolařík et al. (2017)
G. sp. 20	CCF 4316	<i>Phloeosinus fulgens</i>	<i>Calocedrus decurrens</i>	HF546226	MH580547		Kolařík et al. (2017)
	U193	<i>Scolytus schevyrewi</i>	<i>Ulmus pumila</i>	HF546287	MH580548		Kolařík et al. (2017)
G. sp. 21	CCF 5270	<i>Scolytus oregoni</i>	<i>P. menziesii</i>	HF546289	MH580534		Kolařík et al. (2017)
	CCF 4280	<i>H. ficus</i>	<i>F. carica</i>	AM421049	MH580533		Kolařík et al. (2017)
G. sp. 22	CCF 3645	<i>Phloeotribus scarabeoides</i>	<i>Olea europaea</i>	AM421061	MH580552	KF853941	Kolařík et al. (2017)
	CCF 3652	<i>P. scarabeoides</i>	<i>O. europaea</i>	AM421062	MH580553		Kolařík et al. (2017)
G. sp. 23	CCF 3318	<i>Scolytid beetles</i>	<i>Persea gratissima</i>	AJ578489		HG799808	Kolařík et al. (2017)
	CCF 3639	<i>Scolytus rugulosus</i>	<i>Prunus armeniaca</i>	AM421068	HG799838	HG799802	Kolařík et al. (2017)
	U160	<i>Scolytus multistriatus</i>	<i>U. pumila</i>	HF546284			Kolařík et al. (2017)
G. sp. 24	MB136	<i>Orthotomicus erosus</i>	<i>Pinus halepensis</i>	KP691926		KP691936	Dori-Bachash et al. (2015)
	MB242	<i>Pityogenes calcaratus</i>	<i>Pinus brutia</i>	KP691927		KP691937	Dori-Bachash et al. (2015)
	MB322	<i>O. erosus</i>	<i>P. brutia</i>	KP691928		KP691938	Dori-Bachash et al. (2015)
	CCF 4294	<i>Pityogenes quadridens</i>	<i>P. sylvestris</i>	HE604165	MH580555		Kolařík and Jankowiak (2017)
	MK1772	<i>P. pityographus</i>	<i>P. sylvestris</i>	HE604164	MH580556		Kolařík and Jankowiak (2017)
G. sp. 25	MK1832	<i>Cryphalus abietis</i>	<i>Abies alba</i>	HE604128	HE604218	HE604186	Kolařík and Jankowiak (2017)
	CCF 4205	<i>Cryphalus piceae</i>	<i>A. alba</i>	HE604127	HE604219	HE604187	Kolařík and Jankowiak (2017)
G. sp. 26	CCF 4222	<i>Pinus sylvestris</i>		HE604158	LN907595		Kolařík et al. (2017)
G. sp. 27	CCF 4206	<i>Pityogenes bidentatus</i>	<i>P. sylvestris</i>	HE794978	HG799839		Kolařík et al. (2017)
	CCF 4605	<i>Pityophthorus</i> sp.	<i>Pinus ponderosae</i>	HF546309		HG799827	Kolařík and Jankowiak (2017)
G. sp. 29	CCF 4221	<i>C. piceae</i>	<i>A. alba</i>	HE604125	HE604233	HE604184	Kolařík and Jankowiak (2017)
G. sp. 30	CCF 4288	<i>I. cembrae</i>	<i>L. decidua</i>	HE604132	HE604216	HE604193	Kolařík and Jankowiak (2017)
G. sp. 31	CCF 4196	<i>P. pityographus</i>	<i>P. sylvestris</i>		HE604230	HE604176	Kolařík and Jankowiak (2017)
G. sp. 32	CCF 3554	<i>Phloeosinus thujae</i>	<i>Chamaecyparis pisifera</i>	AM181426	HG799874	HG799885	Kolařík et al. (2017)
	CCF 5242	<i>Phloeosinus sequiae</i>	<i>S. serpervirens</i>	HF546265	HG799873	HG799886	Kolařík et al. (2017)
G. sp. 33	CCF 4598	<i>Scolytus praeceps</i>	<i>Abies concolor</i>	HF546331	HG799869	HG799831	Kolařík et al. (2017)
G. sp. 34	CCF 4604	<i>Ips plastographus</i>	<i>C. decurrens</i>	HF546295	HG799866	HG799826	Kolařík et al. (2017)
	U417	<i>S. praeceps</i>	<i>A. concolor</i>	HF546330	HG799868	HG799830	Kolařík et al. (2017)
G. sp. 35.	U196	<i>Pityophthorus</i> sp.	<i>P. menziesii</i>	HF546231		HG799823	Kolařík et al. (2017)
G. sp. 36	CCF 4328	<i>Pityophthorus</i> sp.	<i>Pinus muricata</i>	HF546236			Kolařík et al. (2017)
	MK1814		<i>Cedrus atlantica</i>		MH580538		present study
G. sp. 37	U197	<i>Pityophthorus</i> sp.	<i>P. menziesii</i>	HF546288	HG799862	HG799824	Kolařík et al. (2017)
G. sp. 38	U79	<i>Pseudopityophthorus pubipennis</i>	<i>Notholithocarpus densiflorus</i>	HF546346	MH580537		Kolařík et al. (2017)

	CCF 5241	<i>P. pubipennis</i>	<i>Quercus acrifolia</i>	HF546251	MH580536		Kolařík et al. (2011)
<i>G. sp. 39</i>	U323	<i>P. juglandis</i>	<i>Juglans hindsii</i>	HF546314		KC222335	Kolařík et al. (2011)
<i>G. sp. 40</i>	CCF 5250	<i>Pityophthorus sp.</i>	<i>Pinus ponderosa</i>	HF546273	MH580550		Kolařík et al. (2011)
	CCF 5245	<i>I. plastographus</i>	<i>Pinus radiata</i>	HF546304	MH580549		Kolařík et al. (2011)
<i>G. sp. 41</i>	U215	<i>C. pubescens</i> <i>Cossoninae sp.</i>	<i>Artemisia arborea</i>	HF546292	HG799865	HG799825	Kolařík et al. (2011)
	CCF 4342	<i>Bostrichidae sp.</i>	<i>Toxicodendron diversilobum</i>	HF546249	HG799871	HG799833	Kolařík et al. (2011)
	U64	<i>Scobicia declivis</i>	<i>Umbellularia californica</i>	HF546342	HG799870	HG799832	Kolařík et al. (2011)
<i>G. sp. 42</i>	U166	<i>Phloesinus canadensis</i>	<i>Chamaecyparis sp.</i>	HF546279	HG799860	HG799821	Kolařík et al. (2011)
	CCF 5251	<i>S. rugulosus</i>	<i>Prunus sp.</i>	HF546285	HG799861	HG799822	Kolařík et al. (2011)
<i>G. sp. 43</i>	CCF 4203	<i>Pityogenes knechteli</i>	<i>P. ponderosae</i>	HF546223	HG799864		Kolařík et al. (2011)
<i>G. sp. 44</i>	CCF 4333	<i>Pityophthorus sp.</i>	<i>Pinus sabiniana</i>		LN907598		Kolařík et al. (2011)
	CCF 4332	<i>Pityophthorus sp.</i>	<i>P. sabiniana</i>		LN907599		Kolařík et al. (2011)
<i>G. sp. 45</i>	Hulcr 17004	<i>Pityophthorus annectens</i>	<i>Pinus taeda</i>		MH580482		present study
	Hulcr 17006	<i>P. annectens</i>	<i>P. taeda</i>		MH580487		present study
	Hulcr 18823	<i>Pityophthorus pulicarius</i>	<i>P. taeda</i>		MH580505		present study
<i>G. sp. 46</i>	Hulcr 11575	<i>Pseudopityophthorus minutissimus</i>	<i>Quercus laurifolia</i>	MH426748	MH580479		present study
	Hulcr 18077	<i>Hypothenemus eruditus</i>	<i>J. nigra</i>	MH426766	MH580490		present study
	Hulcr 18201	<i>H. eruditus</i>	<i>J. nigra</i>	MH426776	MH580501		present study
<i>G. sp. 47</i>	Hulcr 11904	<i>H. dissimilis</i>	<i>Q. laurifolia</i>	MH426749	MH580480		present study
	Hulcr 19182	<i>H. dissimilis</i>	<i>Carya illinoensis</i>	MH426789	MH580510		present study
<i>G. sp. 48</i>	Hulcr 19190	<i>Phloeosinus dentatus</i>	<i>Juniperus virginiana</i>	MH426796	MH580514		present study
	Hulcr 19192	<i>P. dentatus</i>	<i>J. virginiana</i>	MH426797	MH580515		present study
<i>Emericellopsis pallida</i> ^a	CBS 490.71 ^b	<i>Pityophthorus sp.</i>		NR_145052.1	KC998998.1	KC987138.1	Grum-Grzhimay et al. (2013)

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

Table 3 Summary of the variability between species of the *Geosmithia juliashanensis* 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)	
	<i>G. jiangxiensis</i>	<i>G. juliashanensis</i>	<i>G. jiangxiensis</i>	<i>G. juliashanensis</i>	<i>G. jiangxiensis</i>	<i>G. juliashanensis</i>
<i>G. formosana</i>	5 (0.94 %)	4 (0.75 %)	5-6 (0.56-0.67 %)	5 (0.56 %)	3 (0.60%)	4 (0.81%)
<i>G. jiangxiensis</i>		5 (0.94%)		8-9 (0.89-1.0 %)		4 (0.81%)

Table 4 After 8 days of culture in MEA medium, the colony diameter (unit: mm) of *Geosmithia juliashanensis* species complex and *Geosmithia pulvereana* at different temperatures.

Species/T	<i>G. formosana</i>	<i>G. jiangxiensis</i>	<i>G. juliانشanensis</i>	<i>G. pulvereae</i>
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

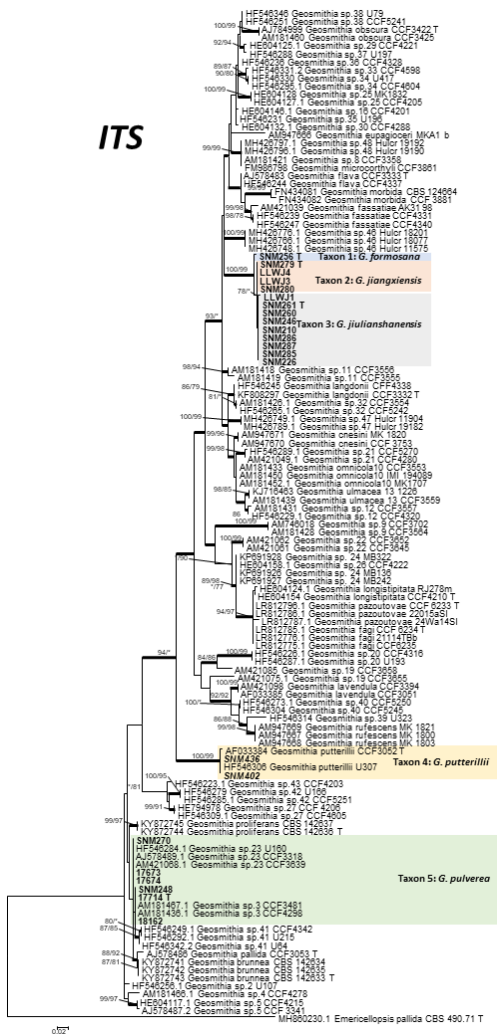


Figure 1

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 ≥ 0.9 . Bootstrap values of ML/MP $\geq 75\%$ are recorded at the nodes. T = ex-type isolates

TUB2

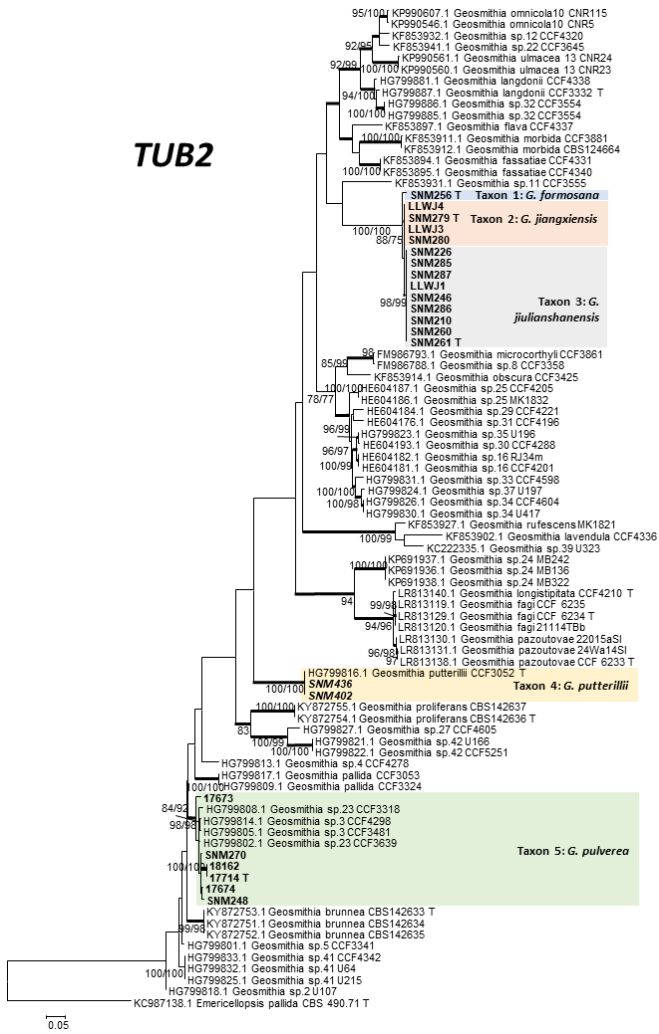


Figure 2

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 $\geq 75\%$ are recorded at the nodes. T = ex-type isolates

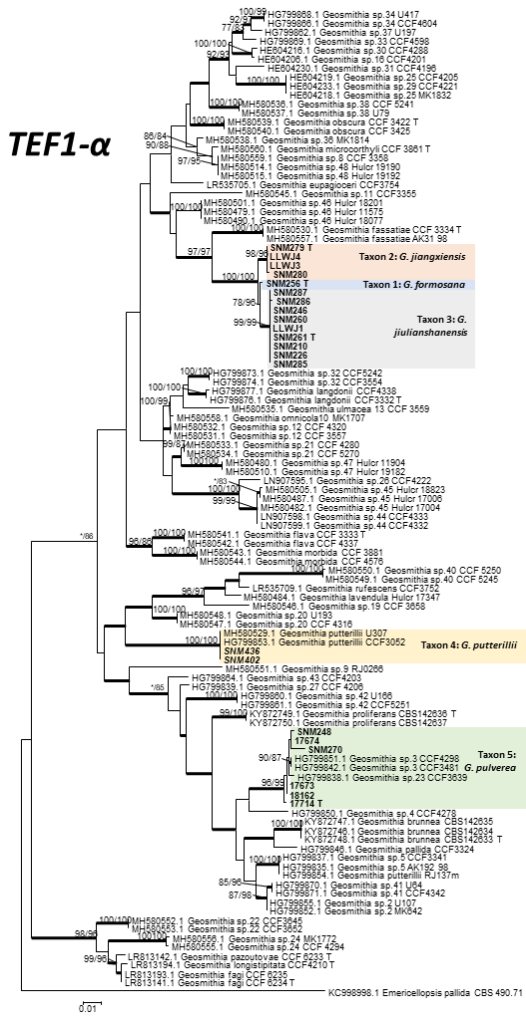


Figure 3

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 $\geq 75\%$ are recorded at the nodes. T = ex-type isolates

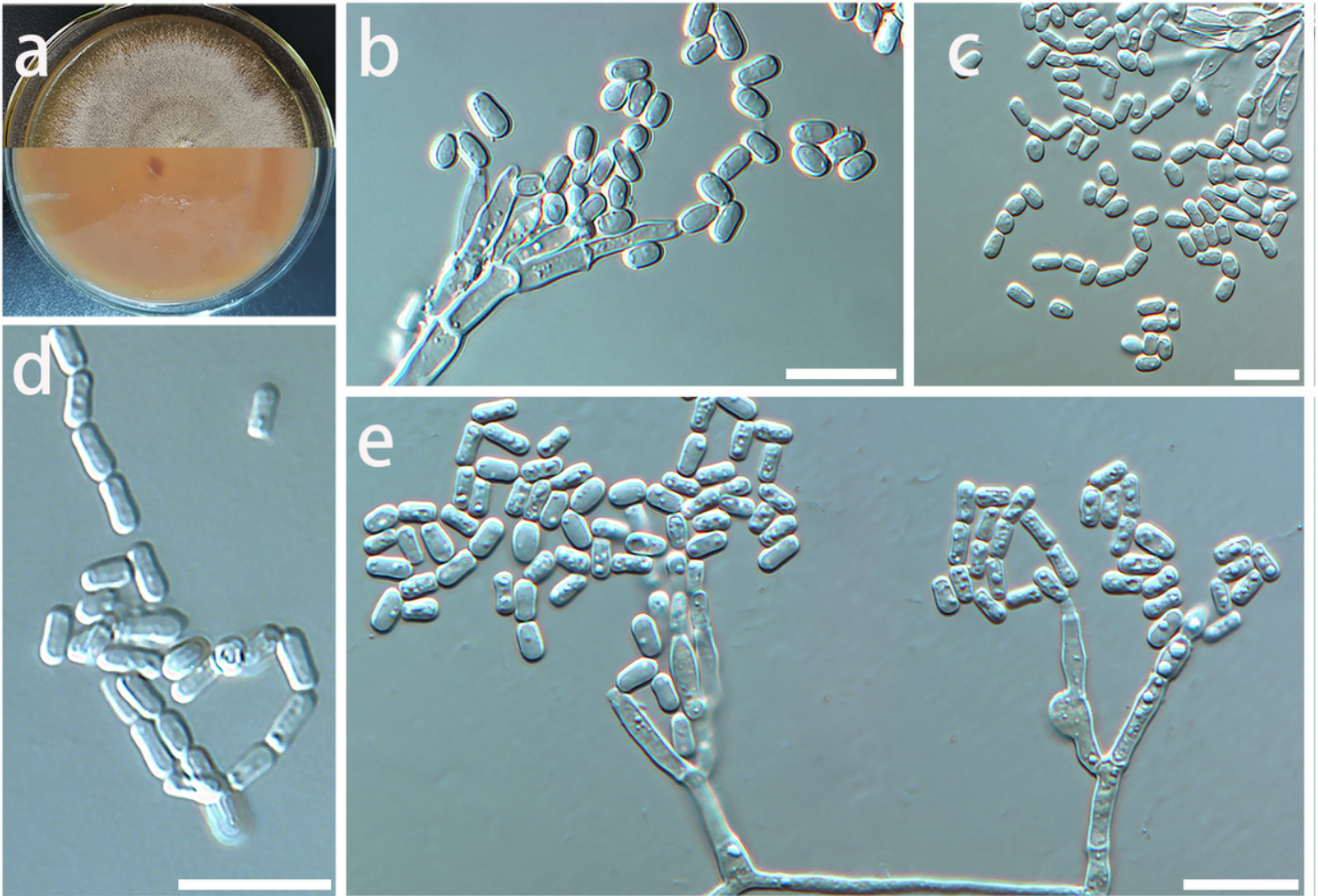


Figure 4

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

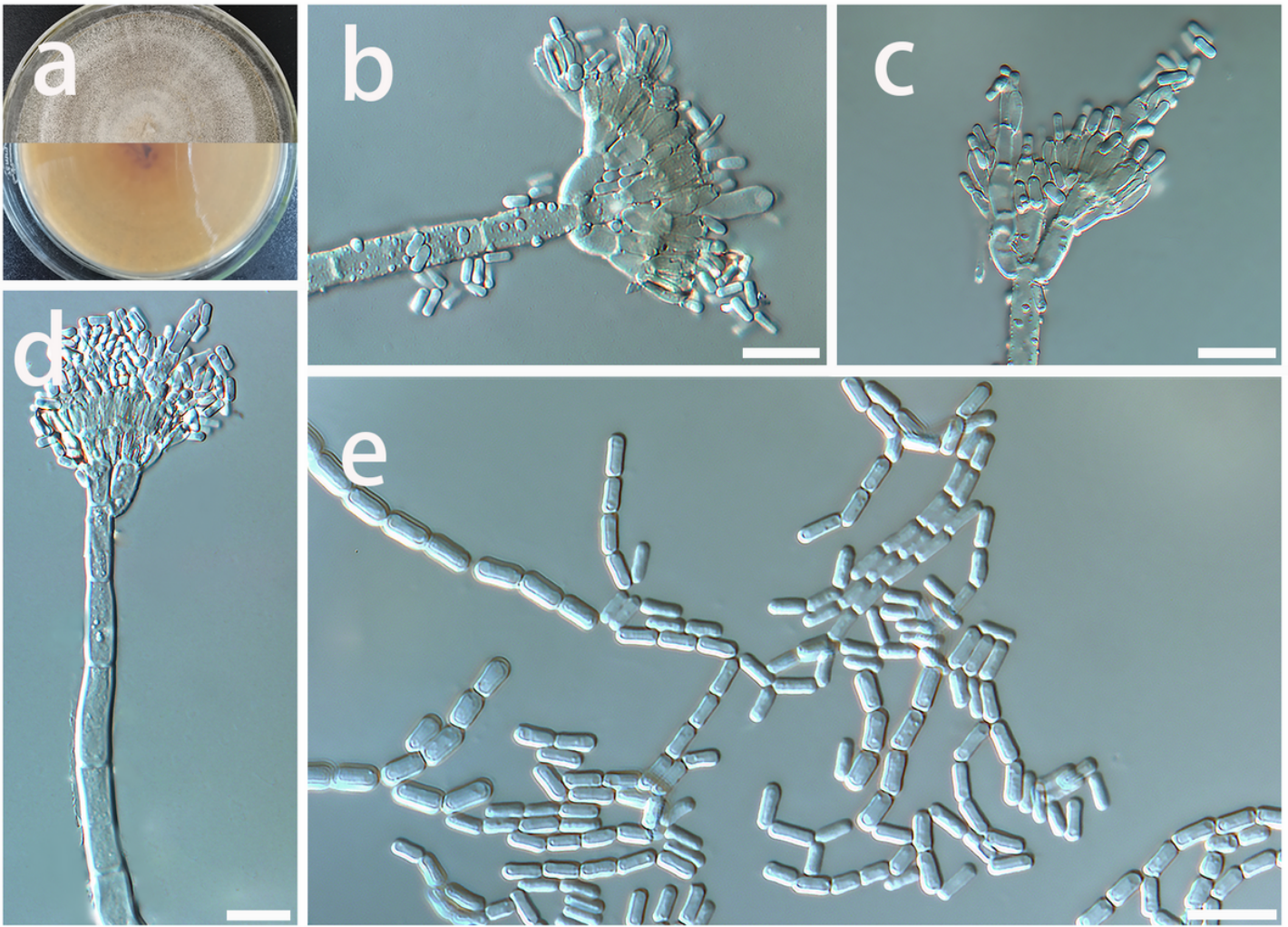


Figure 5
Morphological characters of *Geosmithia jiangxiensis* sp. nov. (CGMCC3.20253) a. 8 days old culture on 2% MEA; b–e. Conidiophores and conidia. Scale bars: b–d=10µm, e=20µm

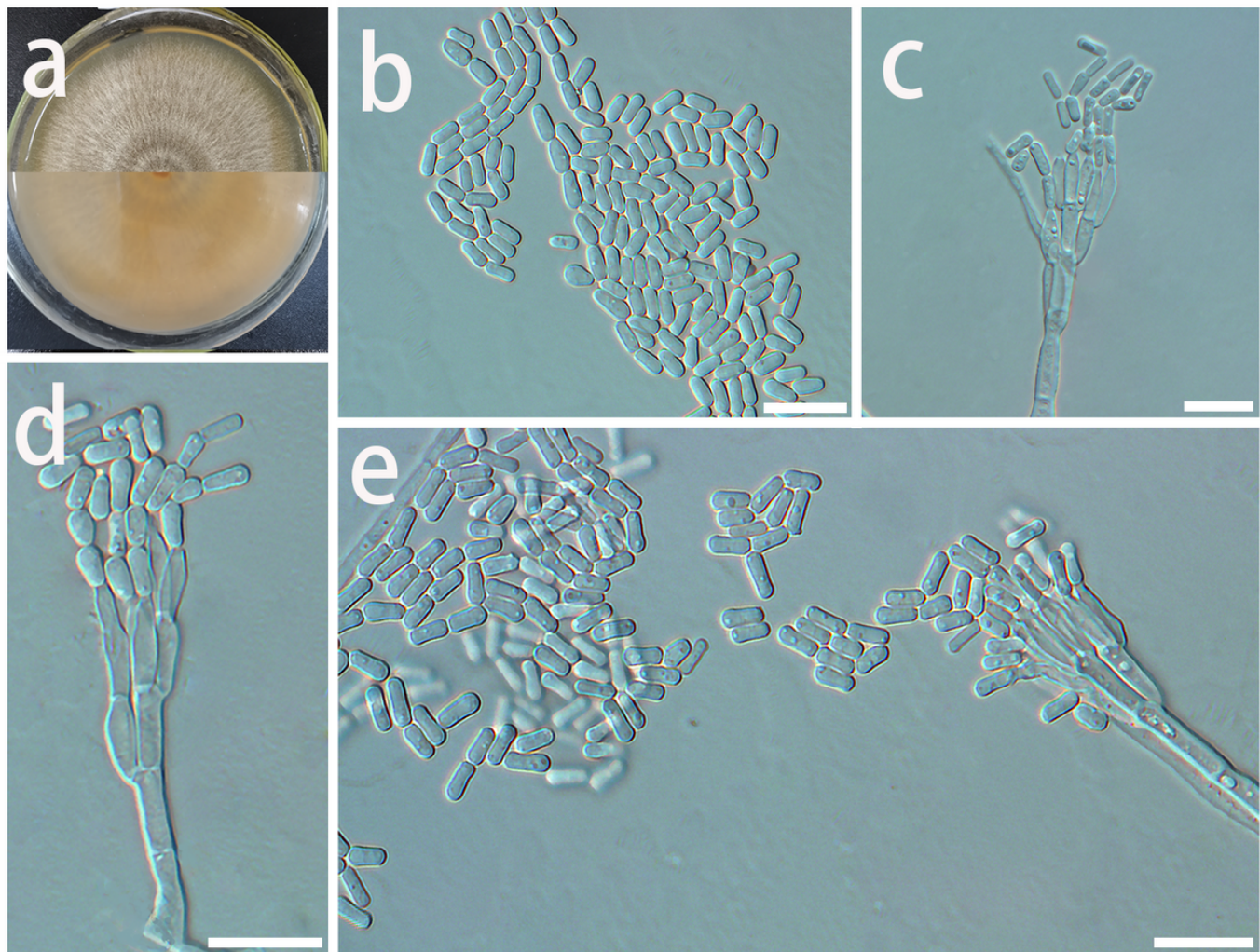


Figure 6
Morphological characters of *Geosmithia formosana* sp. nov. (CGMCC3.20254) a. 8 days old culture on 2% MEA; b–e. Conidiophores and conidia. Scale bars: b–e=10 μ m

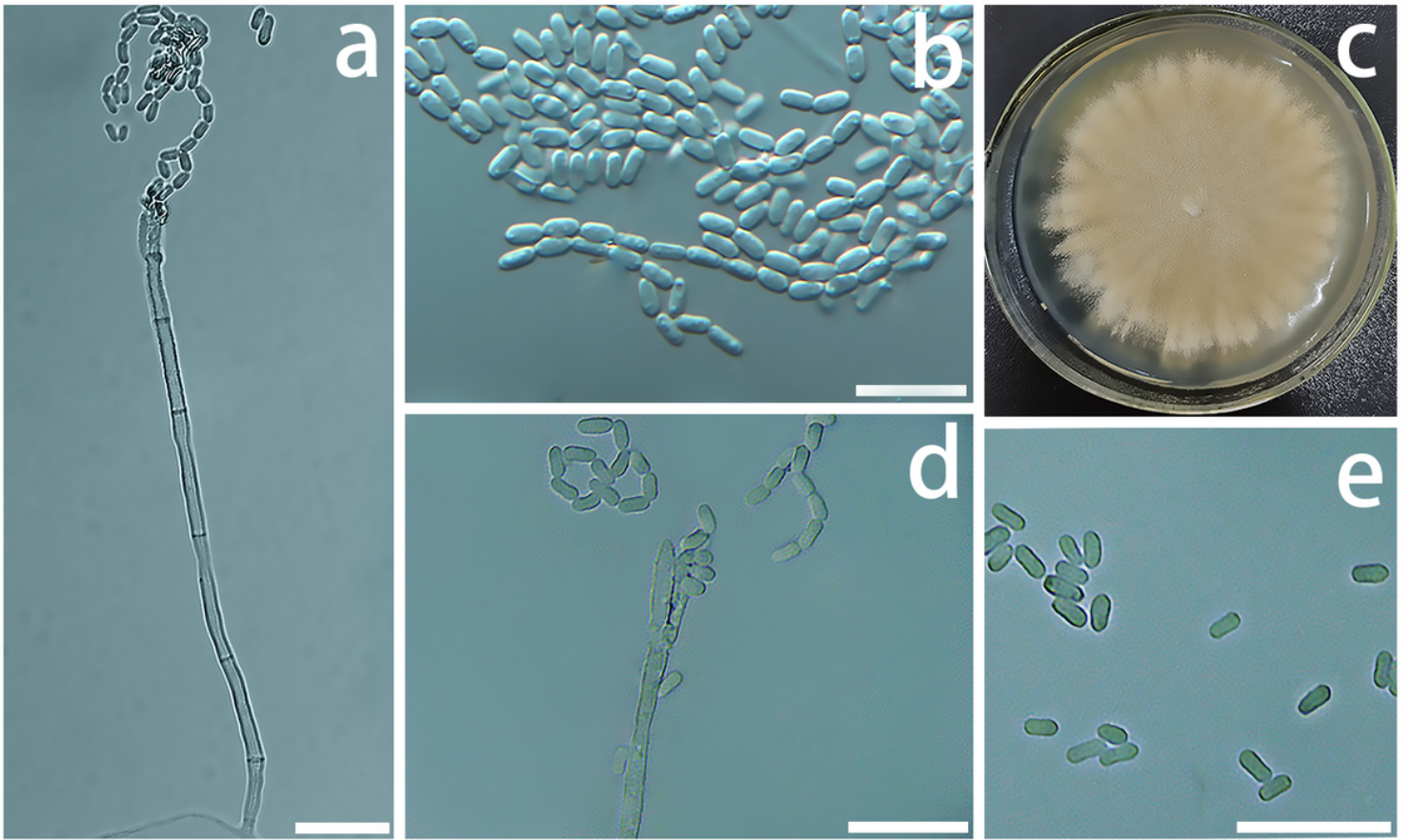


Figure 7
Morphological characters of asexual structures of *Geosmithia pulverea* sp. nov. (CGMCC3.20255) a. 8 days old culture on 2% MEA; b–e. Conidiophores and conidia. Scale bars: b–e=10 μ m