

New species of *Geosmithia* and *Graphium* associated with ambrosia beetles in Costa Rica

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Geosmithia cnesini sp. nov. is a dominant symbiont of the ambrosia beetle *Cnesinus lecontei* collected from *Croton draco* in Costa Rica. This fungus is characterised by whitish colonies and penicillate conidiophores with extraordinary large catenate conidia. *Graphium scolytodis* sp. nov. is described here from the galleries of ambrosia beetle *Scolytodes unipunctatus* collected from the trunk of a fallen *Cecropia angustifolia* tree in Costa Rica. This species does not seem to be a nutritional mutualist but rather a stable associate of unknown function. It produces mononematic conidiophores only and is related to *Graphium penicillioides*.

Key words: ambrosia fungi, *Hypocreales*, *Scolytodes*, *Cnesinus*, ophiostomatoid fungi.

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Geosmithia cnesini sp. nov. je dominantním symbiontem ambrosiového brouka *Cnesinus lecontei*, sbíraným v Kostarice na *Croton draco*. Tato houba je charakterizována bělavými koloniemi a penicilátními konidiofory s mimořádně velkými řetízkovitými konidii. *Graphium scolytodis* sp. nov. je popsán z galerií ambrosiového brouka *Scolytodes unipunctatus* v kmeni padlého stromu *Cecropia angustifolia*, též v Kostarice. U tohoto druhu se nezdá, že by jeho výživa byla založena na mutualistickém vztahu; spíše je zde funkčně neznámá, byť stabilní asociace. Druh vytváří pouze mononematické konidiofory a je příbuzný *Graphium penicillioides*.

SHORT TAXONOMIC REPORT

***Geosmithia cnesini* M. Kolařík & Kirkendall, sp. nov.**
(Mycobank MB 811256)

Fig. 1

Typus. Costa Rica, Heredia Province, town of Birrí, 10°03'28.2" N, 84°08'30.0" W, alt. 1350 m; gallery of *Cnesinus lecontei* Blandford, in pith of *Croton draco* Schltld. & Cham.; coll. L.R. Kirkendall and A. Herrera as No. 060722-7a (L.R. Kirkendall herbarium), Aug 2007; isol. M. Kolařík, Sept 2007; PRM 933242 (National Museum, Prague, holotype, cultura sicca), ex-type culture CCF 4292 (= MK1820); sequence accession numbers: AM947671 (ITS and partial LSU rDNA). Additional material examined: CCF 3753 (= MK1802), substrate, collectors and location as previous collection, but collected in Aug 2006.

E t y m o l o g y. Named after the genus name of its vector beetle, *Cnesinus lecontei*.

Description of ex-type culture. Methods follow Kolařík et al. (2011), including culture media recipes for Czapek yeast agar (CYA), Malt extract agar (MEA) and Czapek-Dox agar (CDA). Dimensions of conidia are given as minimum, average \pm standard deviation and maximum. Colony diameter (7 d, 25 °C): MEA – 35 mm, CYA – 30 mm, CDA – 20 mm. Colony diameter (14 d, 25 °C): MEA – 70 mm, CYA – 70 mm, CDA – 55 mm. Colony characteristics on MEA: margin narrow and diffuse; substrate mycelium hyaline, not forming tough basal felt; aerial mycelium hyaline; surface low and plane, velutinous with conidial crust in the central area and overgrowth of aerial mycelium in other parts; sporulation abundant, spore mass white; reverse yellowish; soluble pigment and exudate absent. Colony characteristics on CYA: margin narrow and diffuse; colony plane with radial rows and slightly raised centrally, consisting of dense felt of aerial mycelium, texture floccose to funiculose, with velvety area in the centre; sporulation moderate, spore mass yellowish to ochre; reverse yellowish to slightly avellaneous brown; soluble pigment and exudate absent. Colony characteristics on CDA: margin narrow, diffuse; colony low and plane, velutinous; sporulation abundant, spore mass white; reverse uncoloured; soluble pigment and exudate absent.

Hyphae hyaline, smooth, 3–6 μ m wide. Conidiophores on MEA *Penicillium*-like, all parts verrucose, arising from substrate or aerial mycelium; base often consisting of curved and atypically branched cell, conidiophores 40–250 μ m tall; stipe 15–100 \times 3–6 μ m; penicillus 30–150 μ m tall, symmetric or asymmetric, often irregularly branched, 3–4 \times , rarely more, first branch 15–25 \times 2–3 μ m, last branch (metula) 4–11 \times 1.5–2.5 μ m; phialides cylindrical or ellipsoidal, 6.5–15 \times 1.5–2.5 μ m. Conidia hyaline, smooth, narrowly cylindrical to ellipsoidal, (4.8)6.4 \pm 0.7(9.2) \times (2.7)3.4 \pm 0.3(4.6) μ m. Conidial chains up to 200 μ m long, persistent. Substrate conidia absent. Conidia fallen from conidiophores form a mass of inflated and budding cells at the colony base.

Strain CCF 3753 has the same morphology as the ex-type strain.

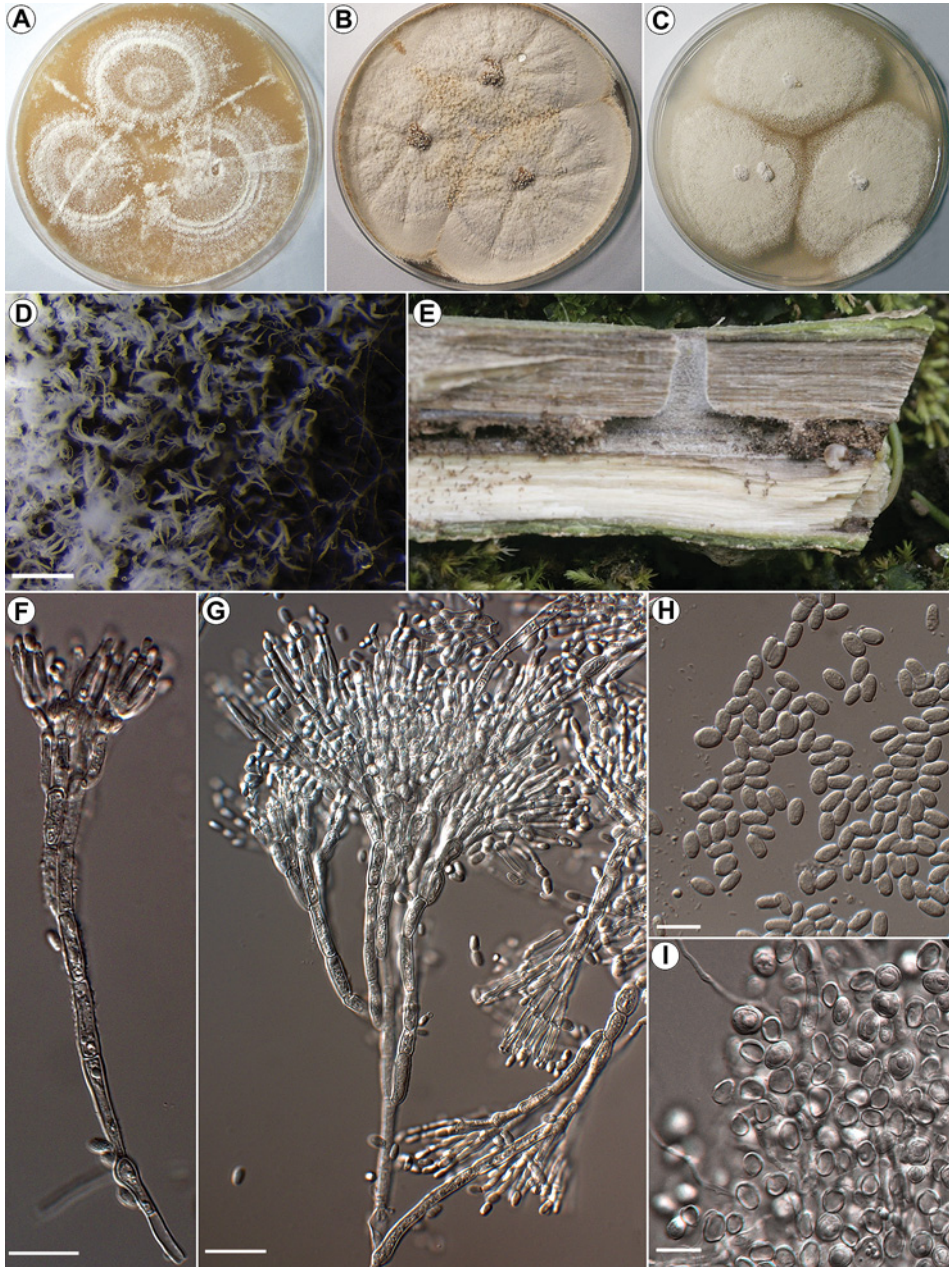


Fig. 1. Morphology of *Geosmithia cnesini*. Colony morphology of CCF 4292 after 14 d, 25 °C: **A** – Malt extract agar; **B** – Czapek yeast agar; **C** – Czapek-Dox agar. **D** – Details of conidial chains on MEA. **E** – Strain CCF 3753 growing in gallery of *Cnesinus lecontei*. Micromorphology of CCF 4292 on MEA, 7 d, 25 °C: **F**, **G** – conidiophores; **H** – conidia; **I** – inflated conidia from colony base. Bar: **D** = 200 µm; **F**, **G** = 20 µm; **H**, **I** = 10 µm. Photo L.R. Kirkendall (F), M. Kolařík (others).

Notes. The fungus was isolated during the study by Kolařík & Kirkendall (2010) and its phylogenetic identity has been confirmed repeatedly (Kolařík & Kirkendall 2010, Kolařík et al. 2011, Kolařík & Jankowiak 2013). The ITS rDNA sequence (AM947670, AM947671) has the closest BLASTn match of 99% (560/564 bp) with various sequences of *Geosmithia* sp. 10. The strain belongs to the group of *Geosmithia* species comprising the species 10, 21, 11, 12 and 13 (numbering according to Kolařík et al. 2007, 2008), which have white, yellowish or brown colonies, catenate conidia not exceeding 5.5 µm (mean length). Among them, *G. cnesini* is unique by its longer and wider conidia. The closest formally described species, *G. langdoini* (ex-type strain, KF808297) has a similarity of 98% (555/565 bp). *G. cnesini* is a dominant fungus in the galleries of *Cnesinus lecontei* and is most probably its principal nutritional symbiont (Kolařík & Kirkendall 2010, Veselská & Kolařík 2015).

***Graphium scolytodis* M. Kolařík & Hulcr, sp. nov.**

(Mycobank MB 811227)

Typus. Costa Rica, Heredia Province, Finca Murillo farm, 10°14' N, 84°06' W, mountain cloud forest, alt. 1500 m; on adults of *Scolytodes unipunctatus* Blandford, in sapwood of *Cecropia angustifolia* Trécul, 30 Sep 2005; coll. L.R. Kirkendall & J. Hulcr, isol. M. Kolařík; PRM 858085 (holotype, cultura sicca), CCF 3566 (= S4, culture ex-type); sequence accession numbers: AM267264, AM267265 (ITS rDNA), AM267263 (partial LSU rDNA) and AM267260 (SSU rDNA). Additional material examined: S20 (PRM 858086), isolated from the same collection.

Etymology. Named after its beetle vector, *Scolytodes unipunctatus*.

Description of ex-type strain. Its morphology is described on Malt extract agar (MEA, see Kolařík & Hulcr 2009) and its dimensions are given as minimum, average ± standard deviation and maximum. Colony reaching 25–35 mm diam., plane, covered by simply branched conidiophores with slime masses of conidia and swollen cells and with aerial mycelium forming overgrowing hyphae and funicles (indeterminate synnemata). All hyphal segments and conidia smooth-walled, hyaline, becoming dark with age. Sporulation abundant. Funicles up to 1 cm long, consisting of smooth-walled hyphae, bearing conidiophores on the whole surface. Mononematous conidiophores arise from basal mycelium, from masses of sprout cells or from funicles; monoverticillate, biverticillate or more and irregularly branched with different numbers of elements and conidiogenous cells arising at various branching levels. Conidiogenous cells discrete, 2–5 per branch, tapering distally, neck curved, (8.5)9.5 ± 1.3(12) × (1.5)1.6 ± 0.2(2) µm, conidiation annellidic, conidial dehiscence sometimes delayed and thus giving the impression of sympodial proliferation. Conidia abundant, single celled, clavate, variable in size (3.5)4.5 ± 0.7(6) × (1.3)1.5 ± 0.2(2) or larger (6)8 ± 1(11) × (2.5)2.8 ± 0.3(5) µm; larger conidia are typical of young conidiophores, smaller conidia of older conidiophores, but sometimes conidia are mixed in one

penicillus, accumulating in hyaline mucilaginous masses. Swollen ‘sprout’ cells, $(5)13 \pm 3(20) \times (3.5)5 \pm 1(8) \mu\text{m}$, originate from conidia, ellipsoidal, lemon-shaped or irregular, thick-walled with a lumen intensively stained by cotton blue. These cells germinate into hyphae, phialides, or bud as yeast cells. Intolerant to low concentrations of cycloheximide ($100 \text{ mg}\cdot\text{l}^{-1}$). Teleomorph absent.

Notes. The ITS rDNA sequence (AM267265) and partial LSU rDNA (AM267263) have the closest BLASTn match of 89–92% and 95%, respectively, to various species of the *Graphium penicillioides* species complex. SSU rDNA sequence (AM267260) has the highest similarity (1638/1652 bp, 99.2%) with *G. penicillioides* AB038423. A detailed characterisation, including phylogeny and comparison with related species, was presented by Kolařík & Hulcr (2009) and Cruywagen et al. (2010). The latter study provides a comparison of *Graphium* species with known DNA data and places *G. scolytodis* into the genus *Graphium*, close to *G. penicillioides* and *G. basitruncatum*. *G. scolytodis* was frequently isolated from adults, but not from galleries, which suggests that it may not be the primary nutritional ambrosia fungus but rather a regular associate with unknown ecological strategy (Hulcr et al. 2007). Kolařík & Hulcr (2009) did not provide a formal description because of absence of true synnemata, typical of the genus. Since then, numerous attempts to stimulate synnemata production have been conducted using natural substrates (wood), different temperatures and light regimes as well as a synnema-stimulating medium (see Kolařík & Hulcr 2009 for details) but without success. Convergent size reduction or absence of synnemata as a response to a subcortical lifestyle has been observed in other fungi (Batra 1966), so reduced morphology may be a typical feature of this species.

In *Graphium scolytodis* indeterminate synnemata with mononematous conidiophores bearing conidia of the shape and size similar to those known from determinate synnemata of *G. penicillioides* aggregate (Okada et al. 2000, Cruywagen et al. 2010) are present. This is congruent with the situation in *G. penicillioides*, where degenerate synnemata or mononematous conidiophores are also present, with conidiophores and conidia basically identical in shape to those found in synnemata, but much more variable in conidial dimensions (Okada et al. 2000). The morphology of these conidiophores is different from the *Scedosporium* (or *Scedosporium*-like) synanamorph which is present in *Graphium basitruncatum* (Okada et al. 2000) or species outside of the *G. penicillioides* aggregate (see below). Conidiophores of the *Scedosporium* type are in comparison with *G. scolytodis* simply branched, with less abundant and generally larger conidia, which are ovoid and different in size and shape from those present in the *Graphium* synanamorph of the same species. Most species of the *G. penicillioides* aggregate have been isolated from insect galleries or ex-

posed sapwood of trees and the entire group is strictly asexual (Okada et al. 2000, Cruywagen et al. 2010). The genus *Graphium* has not been monographed yet and contains numerous poorly characterised species. Among them, species from insect galleries have been relatively well studied, but *G. scolytodis* with its specific ecology has most probably not yet been described. Phylogenetically related lineages of *Parascedosporium*, *Petriella*, *Pseudallescheria* and *Scedosporium* occur on mammals, in dung, soil and wood, often produce *Graphium* and *Scedosporium*-type conidial morphs and form sexual stages (reviewed in Gilgado et al. 2007, Cruywagen et al. 2010, Seifert et al. 2011). The phylogenetic position (known in most of the species), presence of *Scedosporium* or *Sporothrix* type of conidiophores, and ecology are characters distinguishing species of the above-mentioned genera from *G. scolytodis*.

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REFERENCES

- BATRA L.R. (1966): Ambrosia fungi: extent of specificity to ambrosia beetles. – *Science* 153: 193–195.
- CRUYWAGEN E.M., DE BEER Z.W., ROUX J., WINGFIELD M.J. (2010): Three new *Graphium* species from baobab trees in South Africa and Madagascar. – *Persoonia* 25: 61–71.
- GILGADO F., GENE J., CANO J., GUARRO J. (2007): Reclassification of *Graphium tectonae* as *Parascedosporium tectonae* gen. nov., comb. nov., *Pseudallescheria africana* as *Petriellopsis africana* gen. nov., comb. nov. and *Pseudallescheria fimeti* as *Lophotrichus fimeti* comb. nov. – *Int. J. Syst. Evol. Microbiol.* 57: 2171–2178.
- HULCR J., KOLAŘÍK M., KIRKENDALL L.R. (2007): A new record of fungus-beetle symbiosis in *Scolytodes* bark beetles (*Scolytinae*, *Curculionidae*, *Coleoptera*). – *Symbiosis* 43: 151–159.
- KOLAŘÍK M., KOSTOVČÍK M., PAŽOUTOVÁ S. (2007): Host range and diversity of the genus *Geosmithia* (Ascomycota: *Hypocreales*) living in association with bark beetles in the Mediterranean area. – *Mycol. Res.* 111: 1298–1310.
- KOLAŘÍK M., KUBÁTOVÁ A., HULCR J., PAŽOUTOVÁ S. (2008): *Geosmithia* fungi are highly diverse and consistent bark beetle associates: evidence from their community structure in temperate Europe. – *Microb. Ecol.* 55: 65–80.
- KOLAŘÍK M., HULCR J. (2009): Mycobiota associated with the ambrosia beetle *Scolytodes unipunctatus* (*Coleoptera*: *Curculionidae*, *Scolytinae*). – *Mycol. Res.* 113: 44–60.
- KOLAŘÍK M., KIRKENDALL L.R. (2010): Evidence for a new lineage of primary ambrosia fungi in *Geosmithia* Pitt (Ascomycota: *Hypocreales*). – *Fungal Biol.* 114: 676–689.
- KOLAŘÍK M., FREELAND E., UTLEY C., TISSERAT N. (2011): *Geosmithia morbida* sp. nov., a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis*) on *Juglans* in USA. – *Mycologia* 103: 325–332.
- KOLAŘÍK M., JANKOWIAK R. (2013): Vector affinity and diversity of *Geosmithia* fungi living on subcortical insects inhabiting *Pinaceae* species in Central and Northeastern Europe. – *Microb. Ecol.* 66: 682–700.

- OKADA G., JACOBS K., KIRISITS T., LOUIS-SEIZE G.W., SEIFERT K.A., SUGITA T., TAKEMATSU A., WINGFIELD M.J. (2000): Epitypification of *Graphium penicillioides* Corda, with comments on the phylogeny and taxonomy of *Graphium*-like synnematous fungi. – Stud. Mycol. 45: 169–188.
- SEIFERT K.A., MORGAN-JONES G., GAMS W., KENDRICK B. (2011): The genera of Hyphomycetes. – 997 p. CBS-KNAW Fungal Biodiversity Centre, Utrecht.
- VESELSKÁ T., KOLAŘÍK M. (2015): Application of flow cytometry for exploring the evolution of *Geosmithia* fungi living in association with bark beetles: the role of conidial DNA content. – Fungal Ecol. 13: 83–92.