HIRSUTELLA GUIGNARDII AND STILBELLA KERVILLEI, TWO TROGLOBIOtic ENTOMOGENOUS HYphomycetes

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Descriptions of two synnematous, entomogenous hyphomycetes occurring on insects in caves are presented, and their taxonomy and nomenclature clarified. New combinations are proposed for Isaria guignardii and Stilbum kervillei. The biological interaction between these two fungi is described.

The fungal flora of caves has been extensively investigated by Lagarde (1913, 1917a and b, 1922), a work that has been largely overlooked by the mycological community. Unfortunately Lagarde's rich collections have apparently been lost, but the excellent illustrations render his papers quite valuable. Among the fungi Lagarde discussed are several entomogenous fungi found on troglobiotic insects.

During some recent excursions in caves in Southern Limburg (Netherlands), numerous insects were observed which were heavily parasitized by various Hyphomycetes. Most of these were parasitized by the ubiquitous Beauveria bassiana and Paecilomyces farinosus, but two other Hyphomycetes with distinct synnemata were also encountered. These species are usually referred in the literature as Isaria guignardii and Stilbum kervillei. Although several authors (Maheu 1906; Lagarde 1917a, 1917b; Petch 1932, 1937; Pacioni 1980) have reported on these cave-dwelling fungi, the taxonomy and nomenclature is still poorly defined. In this paper, the nomenclature of both fungi is discussed and descriptions in vivo and in vitro are given. The interaction between the two fungi is also discussed. Terminology for phialides is from Evans & Samson (1982).

Hirsutella guignardii (Maheu) Samson, Rombach & Seifert, comb. nov. — Figs. 1–2


Conidiomata either stromatous or synnematous, one or both types frequently occurring on the same host. Stromata highly developed on specimens infected with S. kervillei, emerging between segments of the host cadaver, spreading to form a crust-like, adpressed, smooth, golden brown, irregular mass over much of the exoskeleton of the abdomen and parts of the thorax and head, up to 200 μm thick; composed of a surface layer, up to 25 μm thick, of textura epidermoidea in surface view, of golden brown
cells with thin, smooth walls; below this is a white plectenchyma of interweaving hyphae which are hyaline, branched, septate, and 1.5–2 μm wide with smooth, thin walls. Synnemata emerging singly or in groups of up to 6 from the thorax and abdomen or from the stromata, pendulous from the host which is usually adhering to the wall of the cave, filiform, acicular, or terete, curved or sinuous, yellow-brown, pallid brown to grey; simple or composed of several individual fascicles which emerge independently on the host and then twist together to form a compound stalk which ultimately fuses into a single unit, usually unbranched or bifurcately branched one or two times at the base in specimens infected with S. kervillei, but uninfected specimens may have perpendicular branches up to 800 μm long emerging from the main synnema; surface smooth and spirally twisted, hirtellous in fertile regions; up to 6 cm long, 75–250(–800) μm wide at the base; fertile portions of the synnemata irregularly scattered along the synnemata,
or synnemata entirely sterile in specimens infected with S. kervillei, or forming a more or less continuous layer in uninfected specimens, the bases of the synnemata usually sterile. Hyphae of the synnemata arranged in a parallel ascending spiral, somewhat divergent at the growing terminus, hyaline to subhyaline apically, becoming yellow-brown in the basal parts of the synnemata, septate, septa more frequent in basal parts of the synnemata, 2–4 μm wide, walls smooth and thin. Phialides arising singly and laterally from the hyphae of the stroma, or terminally at the apex of the synnema, originating as lateral bumps on the hyphae which elongate, develop a basal septum, then develop a neck, lageniform to subulate, subhyaline, (13–)20–35(–50) μm long in entirety, 4–6 μm wide at base, transition from the basal portion to the neck gradual or relatively abrupt, necks cylindrical, acicular to subulate, 15–25(–45) μm long, 0.5–1 μm wide at the tip. Conidia usually developing in a viscous mucus, 1–2 per phialide, the poles of the conidia often extending slightly beyond the mucous membrane, ellipsoidal-fusiform, hyaline, 7.5–10 × 2.5–3 μm, the walls smooth and thin, the mucus translucent grey, ovoid to globose, 7–13 × 4–6 μm.

Conidia of H. guignardii do not germinate on agar, but the fungus may be isolated by plating out hyphal bodies from inside the host. The fungus grows slowly on 2% malt extract agar, attaining a diameter of 2–5 mm after 2 weeks at 12° to 18°C, much slower than other species of Hirsutella. The colony is originally whitish, but the colour changes to straw yellow to dark brown after three to four weeks. Phialides and conidia identical to those produced on the host are produced from the scanty brown aerial mycelium, but no synnematal formation occurs.

Fig. 2. Hirsutella guignardii. Camera lucida drawings of phialides and conidia. — a. Part of tip of synnema. — b. Phialides occurring along the synnema. — c. Conidia.
Notes.—The material examined is listed after the description of *Stilbella kervillei*.

*Hirsutella guignardii* has been reported only from cavernicolous dipteran and coleopteran hosts in Europe. The long phialides and the ellipsoid-fusiform conidia, 7.5—10 × 2.5—3 μm, distinguish this species from others in the genus.

Maheu (1906) described *Isaria guignardii* from the beetle *Quedius mesomelinus* from catacombs in Paris. Lagarde (1913) identified several specimens on coleopteran hosts as *I. guignardii*. Recently Pacioni (1980) examined and redescribed several Italian specimens of *I. guignardii*. Both Maheu and Pacioni erected new genera for *I. guignardii* because of the branching of the synnemata, but for reasons outlined in the general discussion, we reject this concept. As the holotype of Maheu and the specimens of Lagarde are not known to exist, Pacioni neotypified *I. guignardii* with one of his specimens on a coleopteran host.

*Hirsutella dipterigena* was briefly described by Petch (1937) from cave-inhabiting flies in England. The holotype material of *H. dipterigena* is in very poor condition, but several other specimens in Petch’s herbarium (K) are in better condition. Specimens of *H. guignardii* identified by Pacioni on helomyzid flies from caves in Switzerland differ from specimens of *H. dipterigena* in Petch’s herbarium only by the highly branched synnemata. The characteristic long phialides (30–60 μm long) giving rise to large conidia (8—10 μm long) are present in all collections. *H. dipterigena* Petch is therefore synonymous with *H. guignardii*. Pacioni (1980) did not consider Petch’s species.

*Hirsutella eleutheratorum* (Nees) Petch, commonly found on coleopteran larvae and adults throughout the world, is very similar to *H. guignardii*. Although Lagarde (1917) placed *I. guignardii* in a separate genus *Mahevia*, he later (1922) considered it a synonym of *I. eleutheratorum*, based on personal communication with P. Vuillemin, but called the species *Tilachlidium eleutheratorum*. Petch (1932) considered *I. guignardii* synonymous with *H. eleutheratorum*. Pacioni (1980) after examining the holotype and several of his own collections, considered it distinct from *H. guignardii*. We agree with this conclusion since the species differ in ecology as well as in the dimensions of the phialides and conidia.

No type material of *I. guignardii* forma *major* Martinez & Guinea could be obtained. However, judging from the protologue it is indistinguishable from *H. guignardii*.

*Stilbella kervillei* (Quélet) Samson, Rombach & Seifert, *comb. nov.* — Figs. 3—5


Synnemata solitary, crowded, or caespitose, arising from all parts of the insect corpse or from synnemata of *H. guignardii*, unbranched, or branched, the branches perpendicular to the main axis and curving antorsely, or branched palmately with 3—5 synne-
SAMSON & AL.: Hirsutella guignardii and Stilbella kervillei
mata arising from one stalk, cylindrical and capitate, or subulate and capitate, straight, nodding or sinuous, pubescent to subvillose, white with a yellow to yellow orange terminal spore mass, 800—6000 μm tall, 50—300(—600) μm wide. Hyphae of the synnemata interweaving, individually hyaline, septate, 1.5—4 μm wide with smooth, thin walls, with B-phialides arising laterally along the entire stalk. Phialides of two types, A-type phialides terminal on conidioma, B-type phialides lateral on conidioma, producing A- and B-conidia respectively. A-phialides produced in a divergent, terminal capitulum on the synnemata or its branches, lateral or terminal on the conidiophores, or in whorls
of 3–4, narrowly lageniform, hyaline, with thin smooth walls, 7–24 μm long, 1.5–2 μm wide at base, often with cylindrical phialides inserted laterally below the septa, up to 14.5 μm long and 0.5 μm wide. Spore mass terminal, translucent or opaque, yellow to orange-yellow, mucoid, globose to subglobose, 150–1000 μm diam., containing both A- and B-conidia. A-conidia obovate to broadly obpyriform, hyaline, aseptate, with smooth, thin walls, 2–3 x 1–2 μm. B-phialides arising laterally from hyphae of the synnemata or from branches thereof, or in terminal whorls of 2–4 on short conidiophores, narrowly lageniform or subulate, curved or straight, symmetrical or asymmetrical, hyaline, with thin smooth walls, 2–3.5 μm wide at the base, 6–27 μm long, with a cylindrical to acicular neck, up to 12 μm long, 0.5–1 μm wide, terminating in an inconspicuous collarette. B-conidia aseptate, hyaline, fusiform, catenulate, 3–4.5 x 1.5–2 μm, with smooth, thin walls.

Cultures isolated from either A- or B-conidia grow and produce synnemata readily on 2% malt extract agar or on sterilized rice (Fig. 4). Synnemata formed in culture may be up to 4 cm long, if sufficient space to allow for this growth is provided, are typically filiform, unbranched, more villose than those produced on insects, and have a terminal spore mass, although some lack this mass of A-conidia. Both A- and B-phialides are formed in single conidium isolates. Cultures on sterilized rice have abundant fluffy, white, superficial mycelium. Synnemata are produced in complete darkness. The fungus is capable of growing and producing synnemata as low as 9°C.


Hirsutella guignardii only present: Troglobiomyces guignardii (Maheu) Pacioni, on Heleomyza serrata, Y. Basset, Grotte de Chemin de Fer NE 14, 4.III.1980 (AQUI); Hirsutella dipterigena, holotype, on flies, L. Armstrong, Pinhole Cave, Creswell, England, March 1934 (K).

Living cultures: CBS 426.82, Stilbella kervillei, pure culture isolated from conidia of herb CBS 003324; CBS 611.83, Hirsutella guignardii, pure culture isolated from internal hyphal material from fly, CBS 003328.

Notes.—Stilbella kervillei has been reported only from European limestone caves, from various species of flies in the genera Blepharoptera, Heleomyza and Scoliocentra. It is distinguished from other species of Stilbella by its consistent association with H. guignardii on dipteran hosts, the pubescent white synnemata with yellow spore masses, the B-type phialides which produce catenulate conidia, and the Sesquicillium-like A-type phialides which produce small obovoid conidia.

The nomenclature of the species is somewhat confused. It was originally described by Quélet (in Gadeau de Kerville, 1884) in Stilbum, but Stilbum has been replaced by Stilbella Lindau as the genus for these hyphomycetes (Benjamin, 1968). When Lingelsheim (1921) described Stilbella arndtii, he discussed Stilbum kervillei as if it were a species of Stilbella, but did not in any way suggest a generic transfer. Mason (1931) referred to
the species as *Stilbella kervillei* (Quélet) Lindau, apparently implying that the fungus was a member of *Stilbella* sensu Lindau, but Lindau made no such transfer. Petch (1937) referred to the fungus as *Stilbella kervillei* (Quélet) Lingelsheim, again a transfer that was never made. As *Stilbella* is currently the most suitable genus for *Stilbum kervillei*, we have formally proposed the transfer above.

For reasons mentioned in the general discussion, *S. kervillei* might ultimately be regarded a species of *Polycephalomyces*, because of the distinctive arrangement of phialides it shares with *P. cylindrosporus* Samson & Evans (Samson & al., 1981). The correct generic placement is further confused by the occurrence of two kinds of phialides, pro-

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**Fig. 5.** *Stilbella kervillei*, camera lucida drawings. — a—c. Southern Limburg specimens. — a. A-type phialides. — b. Conidia from A-type phialides. — c. Portion of synnema with B-type phialides and chains of conidia. — d. Holotype of *Stilbum kervillei*, showing whorls of B-type phialides arising from *Hirsutella* synnema.
Producing conidia either in chains or in slime, on the same conidiomata. This is unknown in other species of *Stilbella*, including the entomogenous taxa, but is known for some species of *Hirsutella* (Evans & Samson, 1982). Catenulate conidia, as produced by the B-type phialides of *S. kervillei* are also unique in *Stilbella* sensu stricto. Petch (1938) observed the B-type phialides of *S. kervillei*, but interpreted them as the phialides of the *Hirsutella* host. However, the conidia produced by the *Hirsutella* are much larger, and are produced in slime rather than chains. The production of both A- and B-type phialides in single conidium isolates of *S. kervillei* proves they are part of the same genome.

*Stilbella arndtii* was described from the fly *Blepharoptera serrata* in caves in Poland (Lingelsheim, 1921). It was separated from *S. kervillei* on the basis of its smooth stalk, white head, and the absence of superficial mycelium. We have not been able to locate the type specimen, but follow Petch (1937) in considering the two fungi conspecific.

Petch (1937) examined several collections of a fungus he called *Stilbella ramosa*, transferring the species from *Stilbum* where it had originally been described by Peck (1874). He concluded that it was an earlier name for *S. kervillei*, but did not examine Peck's type specimen. Ten years later, Mains (1948) examined Peck's specimen and transferred the species to *Polycephalomyces*, making no mention of *S. kervillei*. Although we have not examined the holotype, we consider Petch's conclusion that these two fungi are conspecific dubious for several reasons. *Stilbella ramosa* has synnemata with a brown coloration at the base; it grows on larvae rather than mature insects; and it has never been recorded as occurring in caves.

**OBSERVATIONS AND GENERAL DISCUSSION**

Parasitism of *Hirsutella guignardii* by *Stilbella kervillei*

Petch (1937) was the first to observe that *S. kervillei* was invariably associated with *Hirsutella guignardii* and that synnemata of the *Stilbella* frequently grew directly from the brown clavae of the *Hirsutella*, which were often covered by a scanty white mycelium. In the original description of *S. kervillei*, Quélet (in Gadeau de Kerville, 1884) mentioned a filiform *Isaria* species very similar to *I. eleutheratorum* Nees, and in fact both synnemata and stromata of *H. guignardii* occur on the holotype of *S. kervillei*. Invariably on the specimens we have examined, if the *Stilbella* is present so too is the *Hirsutella*. However, the *Hirsutella* is known to occur independently, as is shown by Pacioni's specimens, and the successful isolation of this species from hyphal bodies in host cadavers convinces us that it is a true entomopathogenic species. The tendency of *Stilbella* B-type phialides to occur in whorls on the *Hirsutella* clavae (Fig. 5d), the scanty white mycelium that covers these clavae, and the frequent growth of *Stilbella* synnemata from those of the *Hirsutella*, suggest that the *Stilbella* is in fact a mycoparasite rather than an insect parasite. In mixed culture, our isolate of *S. kervillei* overgrows *H. guignardii*, but this may reflect a difference in competitive ability on agar rather than actual parasitism, as no haustoria or penetration pegs were observed in these mixed cultures. Although it seems
relatively clear that the *Stilbella* is a mycoparasite, the overall similarity with *P. cylindro- sporus* suggests it may have evolved from an entomogenous ancestor.

Specimens of *H. guignardii* infected with *S. kervillei* display several morphological anomalies when compared with uninfected specimens. Synnemata of infected specimens lack a regularly developed conidiogenous zone, instead having scattered phialides along the stipe, or no phialides at all. The stromata are much more highly developed when the *Stilbella* is present, and are often quite inconspicuous on healthy specimens. Lateral perpendicular branches on the synnemata, as seen on Pacioni's specimens of *H. guignardii*, are not seen on *Hirsutella* infected with *Stilbella*.

Adaptations to life in caves

The limestone caves are relatively cool habitats, where the only light is provided by flashlights of human intruders. According to Teenstra-Eeken & Engel (1967), the temperature in caves in southern Netherlands is 5–12°C and the relative humidity 90–100% throughout the year. Cultures of both *S. kervillei* and *H. guignardii* are capable of growing and sporulating at relatively low temperatures. *H. guignardii* grows well at temperatures as low as 12°C. *S. kervillei*, although growing more slowly, produces synnemata as low as 9°C, and as high as 25°C, with optimal growth occurring at 21°C.

The formation of synnemata in the absence of light for both fungi is worthy of note. Although no synnemata of *H. guignardii* were formed in our pure cultures, *S. kervillei* produced fertile synnemata when grown in complete darkness. The ability to produce synnemata in complete darkness is not unusual, but is shared by *Penicillium clavigerum* (Carlile & al. 1962), *Beauveria felina* (= *Isaria cretacea*) (Taber & Vining, 1959), *Trichurus spiralis* (Fahmy & Yusef, 1974) and *Stilbella thermophila* (Al-Hassan & Fergus, 1967). Light is essential for synnematal initiation or production in many hyphomycetes, including most other species of *Stilbella* known in pure culture (Seifert, unpublished data). Production of synnemata in some entomogenous fungi is not a simple matter of presence or absence of light. Samson & Evans (1976) described synnema development in the entomogenous species *Paecilomyces fumosoroseus*. This fungus may produce synnemata in nature on a host which is completely buried and hence hidden from light. Cultures of this species, however, require light for synnema formation.

Branching of synnemata as a generic character

The genus *Polycephalomyces* was established by Kobayasi (1941) for *P. formosus*, growing on coleopterous larvae in Japan. The genus was said to differ from *Stilbella* by possessing branched, many-headed synnemata. *Stilbella kervillei* often possesses polycephalous synnemata on natural substrata, but rarely produces branched synnemata in culture. In fact, specimens of *S. kervillei* have been identified in the past as *Polycephalomyces* (Teenstra-Eeken & Engel, 1967). The reverse situation is true in *Stilbum albobactritiun* Ellis & Everhart, which produces only unbranched synnemata in nature, but may produce highly branched synnemata when grown in culture (Seifert, unpublished data). Clearly, synnematal branching alone is not sufficient to distinguish genera in this group.
Unfortunately, it is not possible to make a conclusive statement on the tenability of *Polycephalomyces* at this time. Neither Kobayasi (1941) nor Mains (1948), who added two species to *Polycephalomyces*, illustrated the conidiophores of their fungi. The conidiogenous cells and conidiophores of *S. kervillei* are remarkably similar to those of the only species of *Polycephalomyces* available to us, *P. cylindrosporus*, and distinctly different from those of known saprobic species of *Stilbella*. It is possible that *Polycephalomyces* may be distinguished from *Stilbella* by these phialides, but as the specimens of Kobayasi and Mains are too fragile to be sent on loan, we are presently unable to make conclusions.

A similar situation occurs with *H. guignardii*, which may produce racemously branched synnemata in healthy specimens, but rarely produces branched synnemata when infected by the *Stilbella*. Lagarde (1922) and later Pacioni (1980) used this branching as a diagnostic character of their genera *Mahevia* and *Troglobiomyces*. As both genera have *Isaria guignardii* as type species, *Troglobiomyces* is an obligate synonym of *Mahevia*. *I. guignardii* as illustrated by Lagarde and by Pacioni is clearly *A Hirsutella*, and we cannot accept *Mahevia* as a distinct genus based on synnematal branching alone.

ACKNOWLEDGEMENTS

We would like to thank Dr. U. Passauer (W), Dr. G. Pacioni (AQUI), and the curators of K, PC and CMI for the loan of specimens. Mr. P. J. Bels and Mr. E. de Grood guided us through the caves of Maastricht, and we are grateful for their hospitality. K. A. Seifert wishes to thank the Natural Sciences and Engineering Research Council of Canada for financial support in the form of a postgraduate scholarship.

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