

# Typification of *Hymenoscyphus sulphuratus* (Ascomycota, Helotiales)

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**Summary:** *Hymenoscyphus sulphuratus* is an uncommon species growing on conifer litter, but is typically found on *Picea abies* needles. As with many other historically described species, this name lacks a clearly defined type. The purpose of this note is to provide a type which covers all the features that agree with the protologue and our modern interpretation of this name.

**Keywords:** *Helotiaceae*, conifer needles, neotypification, epitypification.

**Résumé :** *Hymenoscyphus sulphuratus* est une espèce peu commune se développant sur la litière de conifères, typiquement sur aiguilles de *Picea abies*. Comme d'autres espèces décrites par les auteurs anciens, ce nom manque d'un type clairement défini. L'objectif de cette note est de fournir un type qui couvre tous les caractères en accord avec le protologue et avec notre conception moderne de ce nom.

**Mots-clés :** *Helotiaceae*, aiguilles de conifère, néotypification, épitypification.

## Introduction

In a previous article (VAN VOOREN & CHEYPE, 2008), a thorough description was given off a *Hymenoscyphus s.l.* species growing on decaying conifer needles, which was identified as *Helotium sulphuratum*. Since this date, other collections of this taxon were made by these two authors but the question of the typification of this species remains unresolved. As indicated in VAN VOOREN & CHEYPE (*op. cit.*), no type material can be attached to the basionym, *Peziza sulphurata* Fr., so a neotypification is needed. This article provides revisions of collections suitable to provide a type specimen to this taxon and our conclusions.

## Materials and methods

The following revisions were made on dried material; the specimens were rehydrated in water during several hours. Microscopic characters were studied with an optical microscope (Olympus CX-31) under different objectives. Water mounts were used for the observation of microscopic characters and measurements, and iodine (Lugol's solution/IKI) for testing the amyloid reaction of asci, with and without KOH-pretreatment. Ascospore measurements were made on free spores; X is the arithmetic mean of spore dimensions, and Q the ratio "length divided by width", the value in italics represents the mean value of this ratio.

## Revision of different collections

HENGSTMENGEL (1984, 1985) proposed to put *Helotium sulphuratum* in synonymy with *Hymenoscyphus epiphyllum* var. *acarius* because of the close similarity of *H. sulphuratum* with *Phaeohelotium epiphyllum* and its occurrence on decaying needles. We therefore decided to revise the holotype of this variety.

***Phaeohelotium epiphyllum* var. *acarium*** (P. Karst.) Hengstm., *Mycotaxon*, 107: 273 (2009).

Basionym: *Peziza epiphylla* var. *acaria* P. Karst., *Not. Sällsk. Fauna Fl. Fenn. Förhand.*, X: 143 (1869).

≡ *Hymenoscyphus epiphyllus* var. *acarius* (P. Karst.) Hengstm., *Per-soonia*, 12 (4): 490 (1985).

**Holotype:** collection from Mustalia, Tammela, South Häme, Finland, leg. P.A. Karsten, 5 Aug. 1886, on needles of *Pinus sylvestris*, first named *Helotium epiphyllum* f. *pineti* by Karsten on the label. H 6003460. The microscopic characters, after our revision, are as follows:

**Asci** cylindrical, 114–125 × 8–10 μm, 8-spored, apex conical, with an apical ring reacting blue (bb) in IKI without KOH-pretreatment, of the *Hymenoscyphus* type, occupying only the lower part of the apical thickening (which is 2–3 μm thick); base arising from croziers.

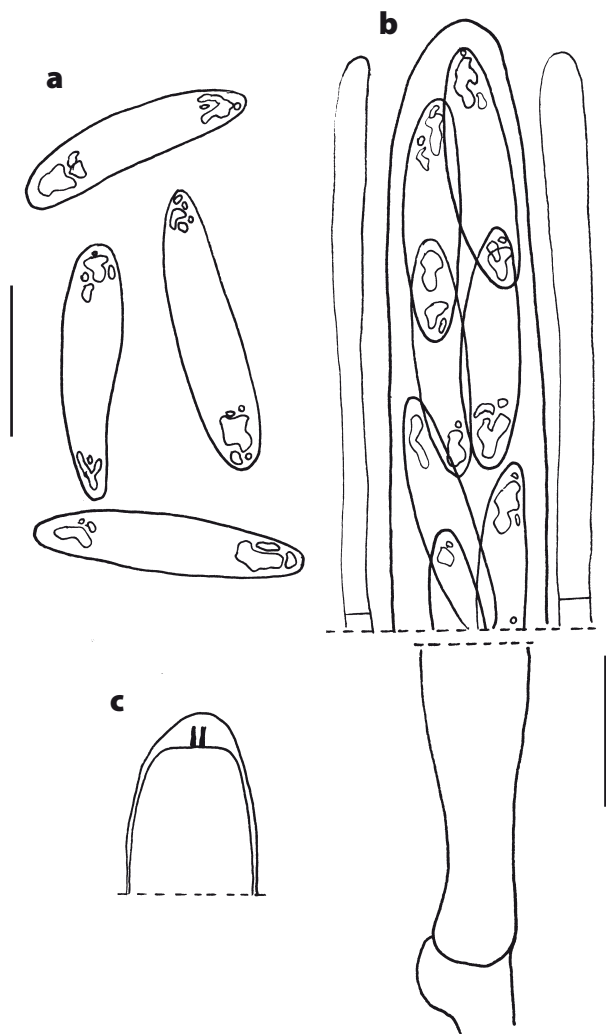
**Paraphyses** numerous, straight, cylindrical, not enlarged at the apex (here 2–3 μm wide), hyaline, without visible contents, as long as the asci. **Ascospores** biseriolate in asci, fusoid, often slightly curved or inequilateral, (15.5–) 16–19 × 3.5–4 (–4.2) μm [X = 17.07 × 3.80 μm], Q = 3.8–4.5–5.1, hyaline, with some lipid bodies, aseptate but sometimes with one septum in overmature spores, forming microconidia on very short germ tubes. **Medullary excipulum** of *textura intricata*. **Ectal excipulum** of *textura angularis* at the lower flanks of the apothecia, with cells 20–36 × 15–18 μm, but near margin of *textura prismatica*, upper margin of *textura porrecta*.

The microscopic features are compatible with the genus *Phaeohelotium* Kanouse emend. DENNIS (1959) and HENGSTMENGEL (2009); the taxon also clearly fits with *Phaeohelotium epiphyllum*, at least *sensu lato*. Indeed, this latter species grows on a multiple variety of hosts (rotten wood of different trees, *Castanea* chestnut husk, decaying leaves, and herbaceous stems), and the variety *acarium* seems to differ only by the host. However, *P. epiphyllum* was also reported on needles and cones of *Abies*, *Picea* and *Pinus* (BARAL, 1985), and on *Larix* needles by SVRČEK (1985).

DENNIS (1956: 102) placed *Helotium sulphuratum* in synonymy with *H. epiphyllum* after the revision of two collections growing on *Pinus* needles, in particular part 189 of Phillips' *Elvellacei Britannici Exsiccati*. However, even if this latter collection is not cited by PHILLIPS (1887: 162), there is no reason to exclude it from the material representing the *sulphuratus* concept of this author. Besides, it seems impossible that the material collected by Phillips could have inamyloid asci, as seen in our collections of *Hymenoscyphus sulphuratus*, because of the revision made by DENNIS (*loc. cit.*).

Another collection under the name *Peziza acaria* is conserved in Karsten's herbarium: collection from Kytö, Tammela, South Häme, leg. P.A. Karsten, 9 Jul. 1869, H 6003461. The microscopic characters, after our revision, are the following:

**Asci** cylindrical, 115–130 × 8–11 μm, 8-spored, *pars sporifera* 65–75 μm, with an apical ring reacting blue (bb) in IKI without KOH-pretreatment, of the *Hymenoscyphus* type; base arising from croziers. **Paraphyses** numerous, straight, cylindrical, slightly rounded at the apex (here 2–2.5 μm wide), hyaline, without visible contents, protruding slightly beyond the asci. **Ascospores** biseriolate in asci, fusoid, 16–21 × 4–4.5 μm [X = 18.53 × 4.17 μm], Q = 3.8–4.4–5.3, hyaline, with many minute lipid bodies near each end (hard to see), aseptate. **Medullary excipulum** of *textura intricata*. **Ectal excipulum** near margin of *textura prismatica* with large cells.



**Fig. 1** – *Phaeohelotium epiphyllum* var. *acarium*. Collection H 6003460 (holotype). a. Ascospores (in water). b. Ascus and paraphyses (in water). c. Ascus apex (in Lugol's solution). Scale bars = 10  $\mu$ m.

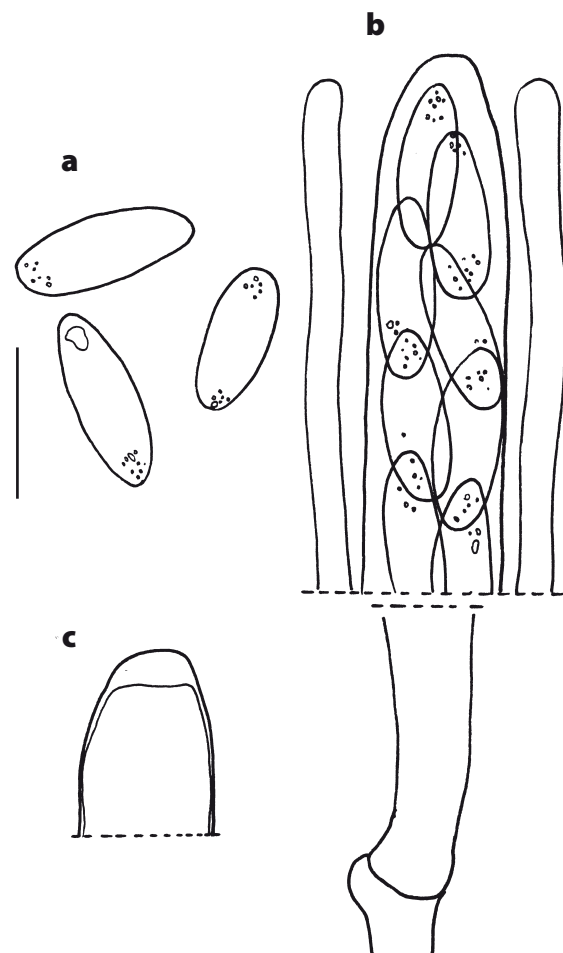
Even if this collection differs from the previous one by the lack of a real *textura angularis* at the base of the apothecia, we think that it could belong in the scope of *Phaeohelotium epiphyllum*.

We also revised the holotype of *Helotium piceae* (Kauffman) Kanouse, a North-American species that was originally treated as a variety of *Helotium sulphuratum* which has also been found on dead needles of different *Picea* species.

***Helotium piceae*** (Kauffman) Kanouse, *Mycologia*, 33: 465 (1941).  
Basionym: *Helotium sulphuratum* var. *piceae* Kauffman, *Pap. Michigan Acad. Sci.*, 1: 107 (1923).

**Holotype:** collection from Tolland, Colorado, USA; leg. C.H. Kauffman, 12 Sept. 1920, on decaying needles of *Picea engelmannii*; conserved in MICH herbarium (14186). The microscopic characters, after our revision, are the following:

**Asci** cylindrical, 100–130  $\times$  7.5–9  $\mu$ m, 8-spored, apex conical, inamyloid; base arising from croziers. **Paraphyses** straight, cylindrical, not enlarged at the apex (about 2  $\mu$ m wide), hyaline, probably with vacuole bodies. **Ascospores** irregularly biseriate in dead asci, elliptical with a more acute end, inequilateral, 8–12  $\times$  3.5–5  $\mu$ m [ $X = 9.80 \times 4.36 \mu$ m],  $Q = 1.8$ –2.3–2.5, hyaline, with one or two large lipid bodies; under the action of the Lugol, some ascospores present two small red spots (due to glycogen). **Medullary excipulum** of *textura intricata*. **Ectal excipulum** of *textura subglobulosa/angularis*, with



**Fig. 2** – *Hymenoscyphus sulphuratus*. Collection PC 0096442. a. Ascospores (in water). b. Ascus and paraphyses (in water). c. Ascus apex (in Lugol's solution). Scale bars = 10  $\mu$ m.

pale yellowish cells up to 20–25  $\mu$ m diam., becoming more elongated near the margin of the apothecia.

The collection is probably a little immature due to the low number of ejected ascospores found in the studied specimens. The inamyloid asci in this collection match *Hymenoscyphus sulphuratus* as redefined by VAN VOOREN & CHEYPE (2008). Ascospore size is smaller than in the other collections known under the name *Helotium sulphuratum*, as noted by KANOUSE (1941: 465) who also cited another collection made in the Michigan state with the same characters (i.e. smaller ascospores). Since this morphological character may be consistent, it is treated provisionally here as a possible variety of *Hymenoscyphus sulphuratus*, characterized by smaller ascospores and a North American provenance.

The fourth collection that we revised was reported by LE GAL (1954: 204) under the name *Helotium sulphuratum*: collection from Sörenberg, Switzerland, alt. 1200 m, leg. G. Malençon, 18 Sept. 1953, on *Picea abies* needles, PC 0096442. The microscopic characters, after our revision, are the following:

**Asci** cylindrical, 130–150  $\times$  7–9 (–11)  $\mu$ m, 8-spored, apex conical, inamyloid; base arising from croziers. **Paraphyses** numerous, straight, cylindrical, not enlarged at the apex (here 2.5–3  $\mu$ m wide), hyaline, without visible contents, septate in the lower part, as long as the asci. **Ascospores** biseriate in asci, elliptic-fusoid or ciborioid, 10–12.2 (–13)  $\times$  4–5  $\mu$ m [ $X = 11.07 \times 4.32 \mu$ m],  $Q = 2.2$ –2.6–3.1, hyaline, without or with a few small lipid bodies, but with indistinct gra-





*Hymenoscyphus sulphuratus*. Collection NV 2011.09.11

nular content, aseptate. **Subhymenium** of *textura intricata*. **Medullary excipulum** of *textura prismatica*, mixed with some subangular cells especially in the pseudostipe. **Ectal excipulum** of *textura prismatica* towards the margin of the apothecia but with a texture more complex at the base, with enlarged cells, and with emerging hyphae.

Our revision is in agreement with the data given by Le Gal in her publication and with our own collections on spruce needles that we identified as *Hymenoscyphus sulphuratus*.

### Typification of *Hymenoscyphus sulphuratus*

*Peziza sulphurata* Fr. (FRIES, 1822: 72) is a new name for *Peziza sulphurea* Schumacher. (SCHUMACHER, 1803) which is illegitimate. As a sanctioned name (ICN Art. 15), it is protected against earlier homonyms or competing synonyms. There is no type material preserved of *Peziza sulphurata* in C (Copenhagen, Denmark) nor in UPS (Uppsala, Sweden). Unfortunately, among the references cited by FRIES (*loc. cit.*) there is no plate to serve as a lectotype (ICN Art. 9.10). So we propose to define a neotype for this name. To be sure that the typification of the name is based on material "approved" by Fries, we propose to designate as neotype (ICN Art. 9.7) the figure 2, plate 1915, from the *Flora Danica* (see discussion in VAN VOOREN & CHEYPE, 2008), which was probably supervised by Fries himself.

As outlined before, there are two opposing concepts of the original *Peziza sulphurata*. The first places the taxon as a possible synonym of *P. epiphyllum*, the second places it as an independent species. Following BARAL & KRIEGLSTEINER (1985), VAN VOOREN & CHEYPE (*op. cit.*) developed their arguments for the second concept. After a complete revision of material, we think it is possible to keep the epithet *sulphuratus* for the species growing on decaying *Abies* and *Picea abies* needles, with inamyloid asci, and to identify as *Phaeohelotium epiphyllum* var. *acarium* those collections growing on pine needles,

with amyloid apical ring and longer spores. In this context, the chosen neotype remains partially ambiguous, so we propose to designate Le Gal's collection PC 0096442 as the epitype of *Peziza sulphurata*.

Neotype of *Peziza sulphurata* Fr. designated here: HORNEMANN (1827), plate 1915, figure 2; Mycobank MBT200224. Epitype designated here: Switzerland, Sörenberg, on *Picea abies* needles, 18 Sept. 1953, *leg.* G. Malençon, *det.* M. Le Gal, PC 0096442; Mycobank MBT200225.

**Other known collections:** FRANCE: Jura, Longchaumois, route forestière du bois des Ban, alt. 1120 m, *leg.* N. Van Vooren, 20-IX-2007, on decaying needles of *Abies*, on calcareous soil, pers. herb. NV 2007.09.31. Haute-Savoie, Plaine-Joux, plateau d'Assy, alt. 1300 m, *leg.* J.-L. Cheype, 11-X-2008, on needles of *Picea abies*, pers. herb. CJL 081011-08. Savoie, Beaufort, col du Pré, alt. 1700 m, *leg.* N. Van Vooren, 16-IX-2011, on needles of *Picea abies*, pers. herb. NV 2011.09.11. Isère, Lans-en-Vercors, bois de Chabaud, route forestière de Servagnet, alt. 1258 m, *leg.* N. Van Vooren, 26-IX-2013, on needles of *Picea abies*, pers. herb. NV 2013.09.29.

### Systematics

BARAL *et al.* (2013) presented new phylogenetic data based on ITS1-5.8S-ITS2 region of rDNA concerning *Hymenoscyphus s.l.* and allied genera, especially *Phaeohelotium* and *Cudoniella* species; two groups where *Hymenoscyphus sulphuratus* could be placed due to its morphological characters. In this study, the genus *Phaeohelotium* is restricted to a clade comprising the type-species, *P. monticola* (Berk.) Dennis, *P. geogenum* (Cooke) Svrček & Matheis and some *Eucalyptus*-inhabiting species. *Phaeohelotium epiphyllum* which shares affinities with *H. sulphuratus* is clearly excluded from this clade. The

genus *Cudoniella* appears also paraphyletic. In this context, it seems premature to propose a new generic placement for *H. sulphuratus* without phylogenetic investigations of this species, and a detailed revision of *Hymenoscyphus s.l.* and allied genera; both were beyond the scope of the present study. This species is therefore retained in *Hymenoscyphus s.l.* pending further work.

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