

A new unusual species of *Phialina*

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A new species *Phialina anomala* growing on dead stems of *Equisetum hiemale* in Switzerland is described and its taxonomic position discussed. The species is exceptional in having constantly both 4-spored and 8-spored asci in the same apothecium.

Keywords: Ascomycetes, Discomycetes, *Equisetum*, Hyaloscyphaceae, *Phialina*, taxonomy.

The literature on equiseticolous microfungi is sparse (Dennis, 1978; Holm & Holm, 1981; Ellis & Ellis, 1985) and it seems that living or dead parts of *Equisetum* species are only rarely examined for their fungal companions. However, as known for most of the cormophytes, more than one parasitic or saprophytic fungal species may occur on any given plant host. Some of the fungi so far observed on *Equisetum* seem to be host specific, whereas others are found on different taxa of the genus (Holm & Holm, 1981). The new discomycete species described here was observed by one of us (J. S.) for some years on dead stems of *Equisetum hiemale* L. only and could not be identified with any known species. Therefore the material was sent for identification to the senior author who concluded that it should be recognized as a new species. We present here a description of this new taxon, compare it with other *Phialina* Höhn. species and discuss its extraordinary characteristics.

Material and methods

Dead stems of *Equisetum hiemale* bearing apothecia of the unknown discomycete were collected during different times of the season. The humid stems were air-dried and then kept in paper envelopes under room temperature.

Dry herbarium material was rehydrated in 3% aqueous solution of KOH. Melzer's reagent (MLZ), 1% I + 3% IKI Lugol's solution

(IKI), ammoniacal Congo Red (CR) and Cotton Blue (CB) were used for histochemical reactions. Mounts were examined using a Nikon Labophot-2 microscope equipped with a drawing tube. Microscopic structures were measured in 3% KOH using a 100x oil immersion objective lens unless otherwise stated.

Results and discussion

Phialina anomala Raitv. & Schneller sp. nov. – (Figs. 1–16).

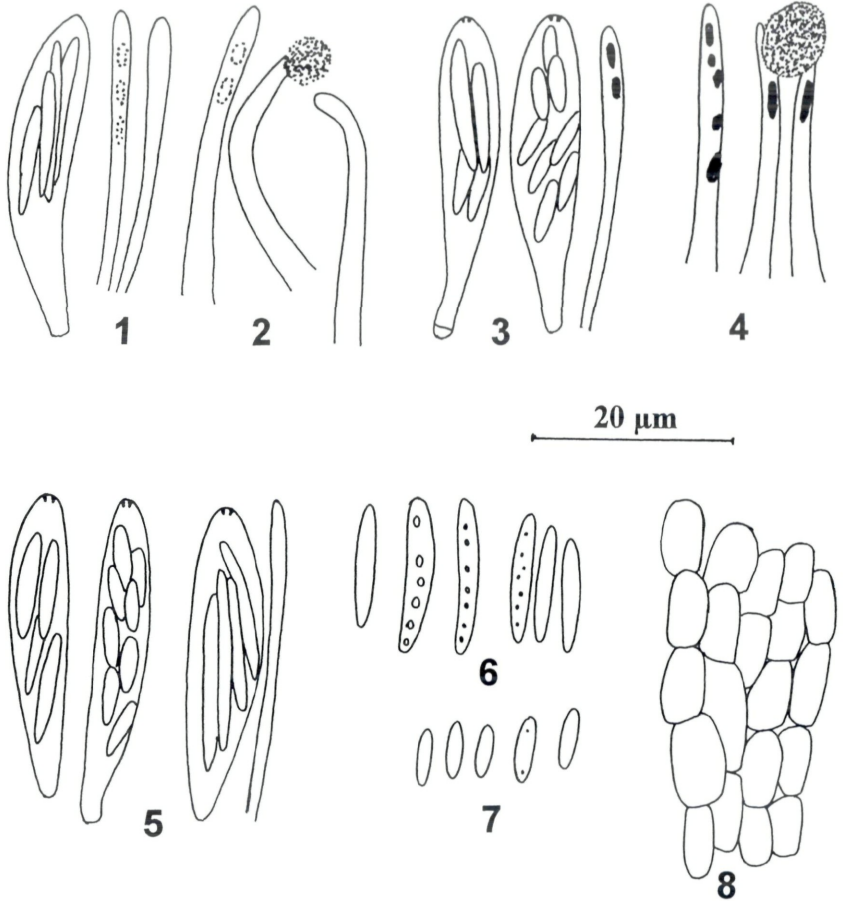
Apothecia superficialia, sessilia, dispersa vel gregaria, 0.1–0.3 mm in diametro, primo subsphaeroidea, dein cupulata usque ad patellata, sicca extus albida, cremea, pallide lutea, pallide ochracea vel pallide luteo-brunnea, disco albido, pallide luteo vel pallide ochraceo. Excipulum ectale ex textura prismatica compositum, cellulis hyalinis, tenuiter vel subcrassiter tunicatis, interdum amyloideis. Pili anguste conici vel cylindraco-conici, curvati vel uncinati, raro recti, apicibus obtusis, raro subacutis, guttulis pallide luteis continentes, substantia resinosa ochracea incrustati, 15–35 × 2–3 µm. Asci uncinati, clavati, 4- et 8-spori, 25–35 × 4.5–7 µm, poro iodo caerulescenti. Sporae ex ascis tetrasporis cylindracoae vel ellipsoideo-cylindracoae, interdum subclavatae, multiguttulatae, aseptatae vel raro uniseptatae, 11–17.5 × 1.5–2.5 µm. Sporae ex ascis octosporis ellipsoideae, aseptatae, 6.5–10 × 1.5–2.5 µm. Paraphyses cylindracoae vel subclavatae, apicibus 2–3 µm latis, guttulis pallide luteis continentes.

Phialinae ulmariae (Lasch) Dennis similis, pilis obtusis extus incrustatis et ascis dimorphis differt.

In caulibus emortuis Equiseti hiemalis crescit.

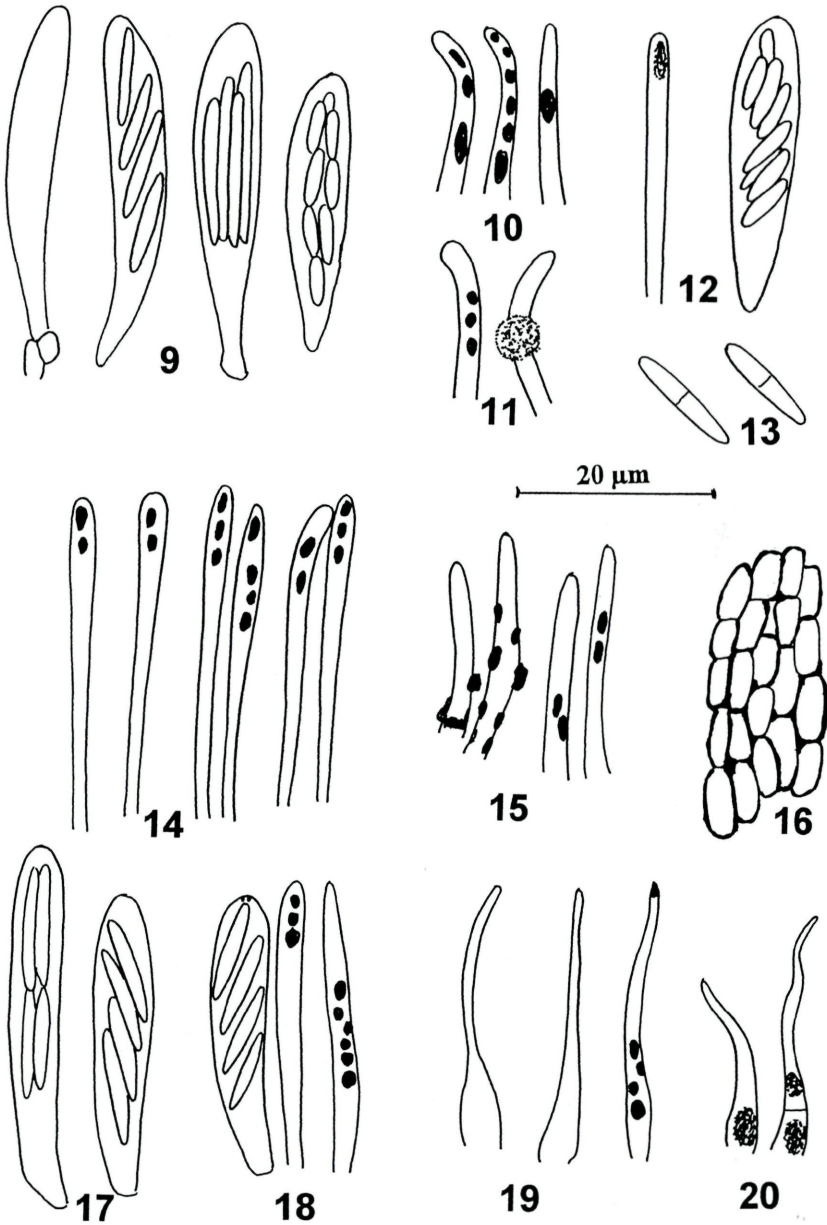
Holotypus.– In caulibus emortuis Equiseti hiemalis, Küssnacht, Tobel, ZH, Helvetia, coord. 68734/241400, alt. 490 m, 08. V. 1986, J. Schneller, sub numero 86–183 legit (Z, isotypus TAA).

Apothecia superficial, scattered to gregarious, sessile. Disc 0.1–0.3 mm, whitish to pale yellowish or pale ochraceous when dry. Receptacle at first sessile on a narrow base, almost spherical, becoming deeply cupulate, later broadly sessile, cupulate to shallow cupulate or saucer-shaped, whitish, cream-coloured, pale yellowish, pale ochraceous or light yellowish brown, externally, particularly at the margin, minutely downy. Ectal excipulum composed of hyaline textura prismatica, cells with thin to moderately, sometimes irregularly thickened walls, 5.5–11 × 3–5 µm in MLZ, 5–7 × 2.5–4 µm in CB. In some populations the ectal excipulum turns partly pale blue in MLZ, in others it stains deep blue in IKI or remains inamyloid. Hairs narrowly conical or cylindric-conical, with thin hyaline walls showing no reactions in IKI and MLZ, mostly curved or apically hooked, more rarely straight, with pointed to blunt apices, generally aseptate but sometimes 1-septate, 15–35 × 2–3 µm, apically 0.8–1.5 µm, containing pale yellowish pigment hardly visible in KOH but turning deep blue in CB, deep reddish brown in IKI and golden brown in



Figs. 1–8. *Phialina anomala*. – 1. An ascus and two paraphyses. – 2. Three hairs, the middle one bearing a ball of amorphous matter. – 3. Two asci showing apical pore and a paraphysis with stained inclusions. – 4. Three hairs with stained inclusions, two of them agglutinated into a ball of resinaceous matter. – 5. Three asci showing apical pore and a paraphysis. – 6. Spores from 4-spored asci. – 7. Spores from 8-spored asci. – 8. Cells of ectal excipulum. – 1, 2: in KOH; 3–8: in MLZ; 1–4: from 87–110; 5–8: from 87–315).

Figs. 9–20. *Phialina anomala*, *Phialina ulmariae*. – 9–16. *P. anomala*. – 9. Four asci. – 10. Three hairs with stained inclusions. – 11. Two hairs, the left one with stained inclusions, the right one bearing a ball of amorphous matter in its middle part. – 12. A paraphysis with stained inclusion and ascus. – 13. Two septate spores from 4-spored asci. – 14. A group of paraphyses with stained inclusions. – 15. A group of hairs, the left two of them showing external encrustation, right two with stained inclusions. – 16. Cells of ectal excipulum with irregularly thickened walls. – 9: in CR; 10: in MLZ; 11–13: in IKI; 14–16: in CB; all from 86–183. – 17–20. *P. ulmariae*. –



17. Two asci. – 18. An ascus and two paraphyses with stained inclusions. – 19. Two hairs. – 20. Three hairs with stained inclusions. – 17, 19: in KOH; 18, 20: in MLZ; 17, 18: from TAA-61445; 19, 20: from TAA-43765.

MLZ. Hairs bear either spherical lumps of pale yellow to ochraceous resinous matter or are encrusted with irregular pieces of the same matter, which is persistent in all embedding media. Resin exuded from the hairs is often very abundant and forms extensive shields agglutinating the upper parts of marginal hairs. This resin reacts with CB, IKI and MLZ in the same way as pigment inside of the hairs. – Ascii arising from croziers, clavate, always both 8- and 4-spored asci present in the same apothecium, $25\text{--}32 \times 5\text{--}7 \mu\text{m}$ in KOH and MLZ, $26\text{--}35 \times 4.5\text{--}6 \mu\text{m}$ in CR, apical pore MLZ+. – Spores in 8-spored asci biseriate or irregularly biseriate, ellipsoid, aseptate, containing two small polar lipid globules in living material, in dead material mostly without inclusions in all embedding media, $6.5\text{--}10 \times 1.5\text{--}2.5 \mu\text{m}$. Spores in 4-spored asci fasciculate or obliquely uniseriate, cylindrical to cylindric-ellipsoid, often slightly clavate, sometimes slightly curved, containing a variable number of small lipid globules, mostly aseptate, rarely becoming 1-septate, $11\text{--}17.5 \times 1.5\text{--}2.5 \mu\text{m}$. – Paraphyses cylindrical to subclavate, not exceeding the asci, apically $2\text{--}3 \mu\text{m}$ wide, often containing pale yellowish pigment turning deep blue in CB, deep reddish brown in IKI and golden brown in MLZ.

On dead stems of *Equisetum hiemale*.

Specimens examined.– SWITZERLAND: Canton Aargau. Baden, Tüfelscheller, on dead stems of *Equisetum hiemale*, coord. 665200/256950, alt. 430 m, 8.1.1990, coll. J. Schneller. – Canton Fribourg. Marly, on dead stems of *Equisetum hiemale*, coord. 578600/181850, alt. 550 m, 24.9.1987, coll. J. Schneller, no. 87–268. – Canton Zurich. Küsnacht, Ravine: on dead stems of *Equisetum hiemale*, coord. 687340/241400, alt. 490 m, 8.5.1986, coll. J. Schneller, no. 86–183 (Holotype in Z, isotype in TAA); coord. 687400/241420, alt. 500 m, 5.5.1986, coll. J. Schneller, no. 86–171; coord. 685500/242120, alt. 580 m, 16.6.1986, coll. J. Schneller, no. 86–278; coord. 687540/241560, alt. 470 m, 14.10.1986, coll. J. Schneller, no. 86–399; coord. 687200/241420, alt. 470 m, 17.4.1987, coll. J. Schneller, no. 87–110; coord. 687560/241580, alt. 470 m, 31.5.1987, coll. J. Schneller, no. 87–169; coord. 688260/241780, alt. 530 m, 10.10.1987, coll. J. Schneller, no. 87–315; coord. 687240/242420, alt. 470 m, 4.12.1988, coll. J. Schneller, no. 88–276; coord. 687550/241570, alt. 470 m, 1.5.1990, coll. J. Schneller, no. 90–14; coord. 688280/241750, alt. 540 m, 17.10.1993, coll. J. Schneller, no. 93–22. Küsnacht, Haselstuden: on dead stems of *Equisetum hiemale*, coord. 687250/241420, alt. 470 m, 4.4.1987, coll. J. Schneller, no. 87–94. Küsnacht, Hesli-bachtobel: on dead stems of *Equisetum hiemale*, coord. 687550/240690, alt. 480 m, 5.4.1987, coll. J. Schneller, no. 87–98; Küsnacht, near Wulp, on dead stems of *Equisetum hiemale*, coord. 688250/241670, alt. 540 m, 12.4.1987, coll. J. Schneller, no. 87–107; Küsnacht, Itschnach: on dead stems of *Equisetum hiemale*, coord. 687970/241830, alt. 520 m, 17.10.1989, coll. J. Schneller, no. 89–175; Sihlwald: on dead stems of *Equisetum hiemale*, coord. 684920/235620, alt. 490 m, 26.8.1987, coll. J. Schneller, no. 87–238; Thalwil: on dead stems of *Equisetum hiemale*, coord. 684200/237000, alt. 480 m, 9.12.1986, coll. J. Schneller, no. 86–441.

Specimens examined of *Phialina ulmariae*: Estonia: Harjumaa County, Riisipere, on dead stems of *Filipendula ulmaria*, 05.07.1965, coll. & det. A. Raitviir; TAA-43765; Russia: the Sakhalin island, Homutovo, on dead herbaceous stems, 03.08.1970. coll. & det. A. Raitviir, TAA-61445.

This species gives at the first sight the impression of a member of *Hyaloscypha* subgen. *Eupezizella* (Huhtinen, 1989) due to abundant resinaceous matter on the hairs. On closer examination, however, it is evident that the species is better accommodated in the genus *Phialina* Höhn., because the yellowish pigment reacting with CB, IKI and MLZ, typical for this genus, is present in the hairs and paraphyses. In this genus it occupies, however, a rather isolated position due to its heavily encrusted, blunt, curved hairs. Hairs of this form are also found in species of *Hamatocanthoscypha* Svrček, but in that genus they are typically unencrusted and lack yellow pigment. The present species is perhaps more similar to *P. pseudopuberula* (Graddon) Raitv., which also has blunt and sometimes curved hairs which can bear small amount of resinous encrustation and lacks the long cilium-like appendix present in some other species of *Phialina*. *P. pseudopuberula* has, however, much larger asci (51–88 µm long *vide* Huhtinen, 1989) and ellipsoid spores. The hymenial features of *P. anomala* are very similar to those of *P. ulmariae* (Lasch) Dennis, particularly with regard to the size of the asci and spores from 4-spored asci. Asci of *P. ulmariae*, however, are constantly 4-spored, and they also differ in their more cylindrical shape (Figs 17, 18). Furthermore, the hairs of these two species are quite different. *Phialina ulmariae* has hairs typical of the genus, equipped with a long and narrow whip- or cilium-like appendix (Figs 19, 20), and lacks any resinous encrustation.

The dimorphism of asci and ascospores is a very special feature of *P. anomala*. There are always 10–15% of 8-spored asci present in all apothecia of *P. anomala*. The ascus pore is invariably blued in MLZ and IKI in both types of asci and there is nothing to indicate that ascus dimorphism should be due to the presence of some intrahymenial parasitic fungus. In two other species of *Phialina*, *P. lachnibrachya* (Desm.) Raitv. and *P. pseudopuberula* (Graddon) Raitv., some occasional variability of spore number in ascus has been reported (Huhtinen, 1989), but the presence of two kind of asci in all apothecia is not their regular feature. It is remarkable that the spore shape in 4-spored and 8-spored asci is completely different. In other cases of variable spore number in asci, for example in *Hyphodiscus gemmarum* (Boud.) Raitv. & R. Galán, the spores in 4- and 8-spored asci differ only very slightly in their size (Raitviir & Galán, 1994). Further investigations on the biology of *P. anomala* and the mycelia formed by the two spore types is needed.

This fungus has proved to be quite common in the type locality. More extensive collecting is required throughout the range of its host, *Equisetum hiemale*, to ascertain its distribution and frequency.

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