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## PUBIGERA, A NEW GENUS FOR OMBROPHILA SUBVILLOSULA REHM

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ABSTRACT : Based on a study of mostly recent collections and an isotype of *Ombrophila subvillosula*, this species is redescribed and figured with more details. It is mainly restricted to needles of *Picea abies*, and is so far only known from Central Europe. A new monotypic genus *Pubigera* is proposed and its placement in the *Arachnopezizeae* (*Hyaloscyphaceae*) is suggested.

RESUME : (*Pubigera*, un genre nouveau pour *Ombrophila subvillosula* Rehm.) Basée sur l'étude de quelques récoltes, récentes pour la plupart, et d'un isotype d' *Ombrophila subvillosula*, les auteurs redécrivent cette espèce et la figurent en détail. Elle est surtout limitée aux aiguilles de *Picea abies*, et n'est connue, jusqu'à présent, que d'Europe Centrale. Le genre monotypique nouveau, *Pubigera*, est proposé et son classement au sein des *Arachnopezizeae* (*Hyaloscyphaceae*) est suggéré (traduit par la rédaction).

### INTRODUCTION :

During several stays in Legnica (Poland) the second author took the chance to search for inoperculate discomycetes in the same areas where Schroeter collected one hundred years ago. This led not only to several collections of species mentioned by him (Schroeter 1908), but also of some fungi new at least to the silesian area. One of these is an exceptional discomycete growing on spruce needles which was identified as *Ombrophila subvillosula* Rehm. An examination revealed some new details, and since its relationship within the *Leotiales* is ambiguous, a detailed discussion seems to be useful.



geotropic, hymenium slightly concave to flat, greyish-brown to dark grey when fresh {2}, with a distinct purple tint {1}, shining deep ochraceous when rehydrated (dead) {1}, margin scarcely inrolled, strongly protruding by teeth-like tufts of whitish marginal hairs, exterior concolorous or darker, densely covered by a whitish to light grey (brownish) felt of long protruding hairs. **Stipe**  $\approx 3-10/0.2-0.4$  mm {3}, /0.2 mm near base {1},  $\pm$  straight, cylindrical, concolorous, densely felty or downy by  $\pm$  appressed or  $\pm$  protruding hairs, lower part finally  $\pm$  smooth, dark brownish; primordia formed between epidermis and parenchym by tearing off small parts of the epidermis, no blackish stroma. **Ectal excipulum** in cupula of *textura prismatica-porrecta*,  $\approx 15-20$   $\mu$ m thick from base of cupula to margin {1}, cells  $\pm$  thin-walled (0.2-0.4  $\mu$ m), smooth, KOH  $\approx 20-45/4-9.5$   $\mu$ m {1}, cells distinctly warted by red- to fox-brown exsudate, cortical layer of narrower hyphae giving rise to hairs; similar in stipe, cells near base KOH 10-30/6-8(10)  $\mu$ m {1}, exterior covered by reddish-brown exudate. **Medullary excipulum** in cupula two-layered: outer layer of *textura porrecta*, 60  $\mu$ m thick near base, composed of dense or loose parallel hyphae,  $\pm$  well differentiated from ectal excipulum, individual cells KOH (1.5)2.5-5(7)  $\mu$ m broad {2}, thin-walled (0.2-0.3  $\mu$ m), distinctly warted by pale ochraceous-brown exsudate, inner layer of  $\pm$  dense *textura intricata* over 100  $\mu$ m thick in centre; gradually disappearing towards margin, hyphae KOH 2-4(6)  $\mu$ m broad,  $\pm$  rough by deep red-brown exsudate; stipe of parallel *textura porrecta*, cells near base KOH 25-50/5-7  $\mu$ m {1}, with sparse ochraceous exudate. **Subhymenium** not distinct from medulla, of dark red-brown, roughened hyphae. **Hairs**  $\pm$  flexuous, originating superficial,  $\pm$  fasciculate, on margin and flanks KOH  $\approx 100-200/3.5-4.5$   $\mu$ m {4}, /3-3.5(4.3)  $\mu$ m in upper half, terminal cell not or often gradually tapering to /2-3  $\mu$ m, apex obtuse, upper half hyaline to pale ochraceous, wall in KOH 0.3-0.5  $\mu$ m thick, lower part pale to light ochraceous, wall 0.5-1  $\mu$ m {1}, marginal hairs sometimes interwoven, forming protruding teeth-like triangles; hairs on stipe of  $\pm$  equal size ( $\approx 50-25/2.5-3.5$   $\mu$ m {2}), rarely branching {3}. Numerous octaedric or irregular shaped crystals of presumably calcium oxalate present on the stipe at the hair base deposited in groups on the ectal exciple {2}, these rare or absent on margin and flanks, sometimes also absent on the stipe {1}. Distantly septate (cells  $\approx 23-48$   $\mu$ m long {3}, terminal cell  $\approx 40-43$   $\mu$ m {1}, septa thin), only outer layer of wall (0.2  $\mu$ m) strongly refractive, this layer of entire hair in H<sub>2</sub>O, KOH or MLZ distinctly and densely roughened by usually firmly adhering, irregularly shaped, low warts, rarely  $\pm$  smooth in upper part, no color change in KOH, base often covered by pale to deep red-brown exsudate. **Paraphyses** filiform, KOH /1-1.8  $\mu$ m {2} in the long unbranched middle part, near apex rarely {1} or often {1}  $\pm$  repeatedly branching, here  $\pm$  flexuous to curved (similar as in *Polydesmia pruinosa* (Jerdon in Berk. & Br.) Boud.), KOH /0.8-1.5  $\mu$ m {2}, sometimes swollen up to 1.7-2.3(3)  $\mu$ m {2}, branching also at base, sparingly septate, terminal cell  $\approx 40-60$   $\mu$ m long, no septa at apical branches, protruding 0-15  $\mu$ m beyond dead asci (in CR or KOH), apex immersed in a hyaline to pale yellowish-ochraceous-greyish gel (in H<sub>2</sub>O or KOH, gel TB-) limited by a 0.3  $\mu$ m thick refractive line, no refractive cell contents seen in living state, no carotenoids. **Asci** mature in H<sub>2</sub>O 100-150/(10)11-11.5  $\mu$ m {2, vital state}, KOH (88)97-120(135)/(7.5)8-9.8  $\mu$ m {3}, CB 91-113/8.3-9.2(10)  $\mu$ m {1}, pars sporifera MLZ/CB 65-90  $\mu$ m {2}, spores obliquely biseriata in both living and dead state. Apex strongly conical to slightly mammiform,

apical ring IKI pale to distinctly blue-grey (euamyloid, BB) in IKI {5}, MLZ very pale or distinctly blue-grey, reaction not enhanced after KOH-treatment, 2-2.5  $\mu$ m wide,  $\approx 0.2-0.3$   $\mu$ m thick (dead state in IKI) {2}, visible as two tiny, inconspicuous dots within the ascus wall in optical cross-section (easily overlooked), apical thickening absent, or inconspicuous (easily overlooked, inamyloid, total wall 0.7-1  $\mu$ m thick (KOH/MLZ) {1}), no periscus, lateral wall in immature asci 0.5-0.7  $\mu$ m thick (MLZ), -1.5  $\mu$ m (KOH), CRB-. Emptied asci with immarginate foramen, no eversion seen. Base with a short to long stalk, arising from simple septa {4}. **Ascospores** in living state (17)18-21.5-23(27)/(4)4.5-5.1-5.5(6)  $\mu$ m {2}, Q (l/b): 3.6-4.2-5.2, dead in H<sub>2</sub>O/CB/MLZ 15-22/(3) 3.5-4.3(4.5)  $\mu$ m {2}, KOH (14.5)16-21.5(23.5)/(3.8)4-4.5(4.8)  $\mu$ m {3},  $\pm$  straight, oblong-ellipsoid(-fusoid) or mostly  $\pm$  clavate: the end toward ascus apex mostly wider and more obtuse, lower end more tapered. Thin-walled, hyaline, non-septate, wall surface (perispore) very slightly roughened, with a very delicate but distinct longitudinal striation {4} (visible in living turgescient spores in CRB, less so in H<sub>2</sub>O; also visible in dead shrunken spores in H<sub>2</sub>O, more visible in KOH, MLZ or CRB), striae unstained in IKI, CRB, CR, CB or TB, invisible in CB (heated or not) {2}; without appendages, wall CRB pale violet (ends CRB-) {1}, IKI-. With 1-3 refractive lipid bodies (0.6-0.9  $\mu$ m {1}) close to the ends and some minute ones, or only with minute LBs (KOH-inert), with one central nucleus (visible in IKI) and 2-4 regions of glycogene  $\approx 1.3-1.7$   $\mu$ m  $\emptyset$  visible by red-brown stain in IKI.

**Ecology**: on fallen needles of *Picea abies* {5}, but (according to the Bohemian collections) sometimes passing over to needles of *Abies alba* or *Pinus sylvestris* when growing in close vicinity, lying in litter on the ground {2} in or at the border of pure or mixed spruce forests (*Piceetum nudum*) {2}, on acid and calcareous soils, optimal phase, needles not blackened, not covered by *Thysanophora*. **Drought tolerance**: All elements were dead at least after c. 8 weeks in the herbarium, except for the spores, some of which survived upto 3 months. **Phenology**: The data available indicates occurrence in early spring: (Jan.) Feb.-April. A search for it at the locality near Jawor end of May was unsuccessful, although five weeks earlier a great number of fruitbodies were found there. Occurrence from lowlands to hill-country, 220-500 m NN.

**Specimens examined**: **CZECH REPUBLIC**: (herbarium specimens in PRM, except 1st. specimen) BÖHMISCHES ERZGEBIRGE, no locality, spring 1912, *Th. Kupka* 1919 No. 5, teste Rehm, M. **CENTRAL BOHEMIA**: Chrustenice near Loděnice, *Picea abies* et *Abies alba*, 300 m NN, 27.III.1949, *M. Svrček* (Svrček 1951). Slapy near Štěchovice, *Picea abies* et *Abies alba*, 400 m NN, 18.IV.1949, *M. Svrček* (Svrček 1951). Horní Jirčany near Praha, *Picea abies* and *Pinus sylvestris* (!), 01.IV.1956, 10.II.1957 and 08.III.1959, 420 m NN, *K. Poner, I. Charvát and M. Svrček* (Poner 1959). Srbsko near Karlštejn, valley of the brook Bubovický potok, *Picea abies*, 220 m NN, calcareous soil, 30.III.1958, *M. Svrček* (Svrček 1959b, 1960). Brdské hřebenys Mts., between Řevnice and Skalka, 500 m NN, *Picea abies*, 500 m NN, 13.04.1958, *M. Svrček* (Svrček 1959b). Brdské hřebenys Mts., Dobřichovice, 470 m NN, *Picea abies*, 24.IV.1986, *M. Svrček*. **SOUTHERN BOHEMIA**: Tréboň, at the margin of pond Stupský rybník, *Picea abies*, 430 m NN, 02.II. and 16.II.1957, *V. Ježek and J. Kubicka* (Svrček 1978). Paseky near Protivín, *Picea abies*,

500 m NN, 01.II.1976, *J. and J. Kubička* (Svrček 1978). -young fruitbodies have been found in good condition in frozen soil on *Picea*-needles (*Piceetum nudum*)- WEST BOHEMIA : Koryta near Plzeň, *Picea abies*, 350 m NN, 24.02.1980, *Z. Hájek Jr.* (Svrček 1986). GERMANY : BADEN-WÜRTTEMBERG : Stuttgart-Feuerbach, Heimberg, MTB 7220/2, 340 m NN, Schilfsandstein, 23.I.1976, *H.O. Baral*, HB 587, Duplum in AG 95/171. BAVARIA : Bau Reichenhall, «Kirchholz», 530 m, needles of *Picea abies*, 29.III.1964, *F. Oberwinkler*, FO 6778. SACHSEN-ANHALT : Dresden, Königstein/ Elbe, 14.III.1885, *W. Krieger*, Fungi saxonici 677 (2 Isotypes), M. POLAND : Jawor, between Paszowica and Lipa, MTB 5062/2, *Picea abies*, 320 m NN, basalt, 17.IV.1995, *A. Gminder*, 95/022 AG.

## DISCUSSION AND CLASSIFICATION :

Re-examination of two isotype specimens deposited in M revealed full agreement with our recent collections. Rehm's placement of the species among those in *Ombrophila* with inamyloid asci proved erroneous : also in the type specimen, the asci have a distinctly blue but tiny apical ring in MLZ or IKI (Fig.21).

The hairs of *P. subvillosula* are very similar to those found in genera e.g. *Trichopeziza* Fuckel, *Lasiobelonium* Ellis & Everh., and in *Arachnopeziza aurelia*. The size of  $\approx 100\text{--}200/3\text{--}4.5\ \mu\text{m}$ , the flexuous shape, the gradually narrowed obtuse apex, and the irregular granulation on the thick (upto  $\approx 1\ \mu\text{m}$ ) hair walls appear to be the same as in the mentioned taxa. However, the granules on the hairs may be lower, more dense, and more firmly attached in *Pubigera*. Our new genus differs from these taxa (characters in brackets [ ]) as follows : (1) the hairs originate from a prosenchymatic ectal excipulum [parenchymatic or at least very short-celled, but mostly prosenchymatic in *A. aurelia*], (2) the amyloid ring is very thin [thick], (3) the spores are ornamented [smooth], (4) the apothecia are long-stalked [sessile], (5) the filiform paraphyses tend to be branched and flexuous at the apex and are embedded in gel [filiform or lanceolate, unbranched at apex, without gel], (6) the hyphae of the medulla have a dark brown incrustation [smooth, hyaline]. Asci arising from simple septa occur rarely in these taxa, e. g. in *Trichopeziza sulphurea* (Pers.) Fuckel (Baral, ined.).

Similar hairs are also known from several species in further genera, e. g. *Perrotia* Boud., *Lachnellula* P. Karst., *Capitotricha* (Raitv.) Baral. In *Solenopeziza* Sacc. (= *Niveostoma* Svrček), the hairs on the flanks are similar while the terminal and inner hairs of the strongly protruding margin largely differ. All of these genera differ from our genus by several features : e.g., the inner medullary excipulum is hyaline in all of them, spore ornaments are unknown, and amyloid rings are either thick or absent.

In his key to the European genera of *Hyaloscyphaceae*, Svrček (1987) did not mention this species although it is easily taken for a member of this family.

The peculiar type of apical apparatus of *P. subvillosula* is characterized by a wide but very thin euamyloid apical ring (dead state). It strongly resembles that of the undoubtedly unrelated *Nimbomollisia melatephroides* (Rehm) Nannf. (However, the very close *N. eriophori* (Kirchn.) Nannf. has thick apical rings of the *Laetinaevia*-type). *Nimbomollisia* Nannf. is clearly a member of the *Dermateaceae* and is considered a synonym of *Niptera*

Fr., with the type species having either an apical ring of the *Laetinaevia*-type or completely lacking a ring (Baral 1994:116). A worker stressing the apical apparatus as a taxonomically relevant feature, would probably consider the asci of *Nimbomollisia melatephroides* and *Pubigera* as one and a single type of apical apparatus, thinking of a phylogenetic relationship. Most probably, the apical apparatus of *Pubigera* evolved independently, and is derived from a type of apical apparatus with a thick amyloid ring which occurs in the obviously closely related genera *Trichopeziza* and *Arachnopeziza* (*Bulgaria*-type).

As a very striking feature in the *Leotiales*, the ascospores in *P. subvillosula* show a delicate ornamentation of the epispore when viewed in a water mount of living mature (recently discharged) spores with the light microscope at 1500x. The outline of the spores therefore appears finely roughened. This ornament consists of thin longitudinal ridges which are unstained in all tested reagents. It is also easily visible in herbarium specimens using KOH or MLZ as mountant. Nevertheless, this ornament was not reported for this species by other workers. A similar ornament is known for the ascospores of *Ascotremella faginea* (Peck) Seaver, but in this species, the ridges stain red in toluidine blue while the rest of the spore remained blue (Matheis 1984).

*Arachnopeziza aurelia* has, according to recent studies, spores of approximately the same size and shape, differing in having a smooth wall, a median septum, and elongate polar appendages. The hairs form distinct, bright orange, teeth-like fascicles on the exterior, and mainly originate from a textura *intricata-porrecta*. The arachnoid felt on the stipe of *P. subvillosula* is also reminiscent of *Arachnopeziza*. We therefore place our new genus in the family *Hyaloscyphaceae*, and consider a preliminary placement in the *Arachnopezizeae*. The presence of a long apothecial stipe showing a negative geotropism was so far unknown in this group, while being typical for such groups as the *Sclerotiniaceae*. Obviously the occurrence of apothecia of *Pubigera subvillosula* in litter affords a habitate in which an elongate stipe benefits spore dispersal.

A brownish incrustation by warts or small patches on the excipular and medullary hyphae is a characteristic feature in both *Pubigera subvillosula* and members of the *Sclerotiniaceae*. However, the apical apparatus of the latter family differs radically from that in *Pubigera subvillosula* : it shows a high conformity and serves as a very useful tool in recognizing a sclerotiniaceous discomycete (Baral, 1987: figs. 9, 15-16 ; Verkley 1993: figs. 41-56) : the apex is broadly truncate and only slightly conical. The apical thickening is very abundant and the amyloid ring is even taller showing an annular protrusion (dead state). This type of apex is also present in the type species of *Lambertella* Höhn., *L. corni-maris* Höhn. The absence of black stroma in *Pubigera* is not a mandatory reason to exclude this family, but certainly most of its members show such stromatic parts on the substrate.

*Lambertella carpatica* has a macroscopic habit and hairs comparable to *Pubigera*. It is, however, certainly a true member of the *Sclerotiniaceae*, differing from *P. subvillosula* in e.g. (1) amyloid subhymenium, (2) an apical apparatus of the *Sclerotinia*-type, (3) a hyaline medullary excipulum and (4) a brown to purplish-violet pigmentation of the outer ectal excipulum, and (5) a black stroma within the host tissue. In addition, it

differs from *Pubigera* in having 4-spored asci and smaller ascospores. The species was accepted and incorporated in the synoptic key to *Lambertella* by Korf & Zhuang (1985).

#### GEOGRAPHIC DISTRIBUTION :

*Pubigera subvillosula* is hitherto only known from few localities in Czech Republic, Germany and Poland from colline to submontane regions. It is apparently a rare species, nevertheless it might easily be overlooked due to the minute fruitbodies and the unusual ecology of the species.

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Fig. 1-21 : *Pubigera subvillosula* (Rehm)Baral, Gminder & Svrček ; Fig.1. Fresh apothecia, 0,7x ; Fig.2. Fresh apothecia, 10x ; Fig.3. Radial section of receptacle, KOH, 67x ; Fig.4. Radial section of stalk, basal part, and cross section of needle, dead in H<sub>2</sub>O, 33x ; Fig.5. Radial section of ectal excipulum giving rise to hairs, and part of outer medullary excipulum, lower flanks, KOH, 1000x ; Fig.6. Groups of octaedric crystals on exterior of stalk, 1000x ; Fig.7. Upper part of hair with octaedric crystals, dead in H<sub>2</sub>O, 1330 x ; Fig.8-9. Detail of hair (middle part), KOH, 2650x ; Fig.10. Part of medullary hypha, surface view, KOH, 2650x ; Fig.11. Living ascospores, H<sub>2</sub>O, right : surface view showing longitudinal striations, 2000x ; Fig.12. Dead ascospores, KOH, left : surface view, 2000x ; Fig.13. Living ascus and paraphyses, H<sub>2</sub>O, 1000x ; Fig.14. Dead paraphysis, MLZ, 1000x ; Fig.15-17. Ascus bases with simple septa, KOH [fig.15 : 1000x ; Fig.16-17 : 1330x] ; Fig.18-21. Apices of dead asci with amyloid ring, 2650x [fig.18 : prior to spore formation, fig.19, 21 : with mature spores, fig.20 : after discharge, figs.18-20 : IKI, fig.21 : MLZ].

Origin of drawings : HB 587 : Figs. 1, 2, 4, 7, 13, 15 ; AG 95/022 : Figs. 8, 11, 14, 16, 18, 19, 20 ; Krieger 677 (Isotype) : Figs. 3, 5, 6, 9, 10, 12, 17, 21.



