

Notes on *Urnula hiemalis* Nannf.

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Summary: The authors report a detailed morphological study of *Urnula hiemalis*, based on recent Finnish collections. Colour pictures of fresh and dried material, microscopy, as well as B/W drawings are provided. The taxon is also compared with the closest species like *U. craterium*, *U. groenlandica* and *U. brachysperma*.

Keywords: Ascomycota, Pezizales, Sarcosomataceae, Plectania, *Urnula craterium*, *Urnula groenlandica*, *Urnula brachysperma*, taxonomy.

Riassunto: Gli autori presentano lo studio morfologico di *Urnula hiemalis* basato su recenti raccolte provenienti dalla Finlandia, corredando l'articolo con foto in habitat, foto in studio, foto di microscopia e disegno al tratto. Il taxon è confrontato con specie vicine quali *U. craterium*, *U. groenlandica* e *U. brachysperma*.

Parole chiave: Ascomycota, Pezizales, Sarcosomataceae, Plectania, *Urnula craterium*, *Urnula groenlandica*, *Urnula brachysperma*, tassonomia.

Due to this special issue focusing the family *Sarcosomataceae*, we thought it would be useful to propose an English version of the article: CARBONE M. & AGNELLO C. 2012. — Appunti di studio su *Urnula hiemalis*. *Ascomycete.org*, 4 (5): 99-108.

According to the papers here proposed and also further (not yet published) studies, some names changed and some different features have been found. For that reason many footnotes not present in the Italian version have been added. The authors thank Chris Yeates for reviewing this translation.

Introduction

From the very beginning of our study of the family *Sarcosomataceae* Kobayasi, we always had a particular interest in this poorly known northern European species. At present, in Italy the genus *Urnula* Fr. is represented only by the type species¹ *Urnula craterium* (Schwein.) Fr., which will be treated in a forthcoming paper. The genus *Urnula* was at first defined by morphological features only, such as habit, flesh consistency and lack of a gelatinised flesh (NANNFELDT, 1949; LE GAL, 1958). It was later characterised by several cytological studies focusing on the spore nuclei (BERTHET, 1964; DONADINI, 1987), or the ultrastructure of the ascus apex (BELLEMÈRE *et al.*, 1990). The phylogenetic studies published so far support the independence of the genus, as do our genetic results (see in this issue). Although in the past many *Urnula* species have been published (ca. 20 validly and one ad interim according to Index Fungorum and MycoBank), nowadays the genus *Urnula* seems to be restricted to a very limited number of species. According to DISSING (1981) only four species remain, of which one is not yet described: *U. craterium*, *U. hiemalis* Nannf., *U. groenlandica* Dissing and *Urnula sp.* [= *U. hiemalis sensu* KEMPTON & WELLS (1974), from Alaska]².

Materials and methods

Microscopic characters are based on fresh and dry specimens. Two optical microscopes were used: Olympus CX41 trinocular and Optika B353 trinocular with plan-achromatic objectives 4×, 10×, 40×, 60×, and 100× in oil immersion. Primary mounting media were Melzer's reagent to test amyloid reaction; cotton blue to test the spore surface; Congo red to stain the hymenial elements and the excipulum. Water mounts were used for all measurements and observations of pigments. At least 30 spores were measured from each apothecium examined. The choice of the spores in dried specimens has been made on those already out of the asci.

Taxonomy

Urnula hiemalis Nannf., *Svensk bot. Tidskr.*, 43: 471 (1949).

Original diagnosis

U. craterio valde affinis sed differt: apotheciis sessilibus – substipitatis, terram adfixis, infundibuliformibus, saepe mutua compressione irregularibus; hymenio tarde et raro maturescente; ascosporis brevioribus (sporis apicalioribus circ. 20 μ longis).

Description

Apothecium cupulate to funnel-shaped, up to 5 cm in diameter, more or less stipitate. **Hymenium** smooth, sometimes gently folded, pure black to blackish. **External surface** rough, warty in places, blackish but often with pale to dark copper shades. **Stipe** very short and mainly subterranean, but sometimes also well developed, up to 2.5 cm high (see collection TUR 196076); covered at the base by a thick subiculum.

Asci cylindrical, 330–400 × 12–16.5 μm, inamyloid, operculate, 8-spored, with walls up to 1 μm thick; the base long, flexuous and aporhynchous³. **Spores** hyaline, globose at first with walls up to 2 μm thick, becoming elliptical with walls up to 1 μm thick, finally definitely elliptical to subcylindrical, 24–31 (–32) × (10.6–) 12–15 μm, Q = (1.6–) 2–2.4 (–2.5); thin walled, with 1–5 small oil drops at the poles. **Paraphyses** filiform, mainly 2.5–3.5 (–4) μm in diameter, although some elements are inflated up to 8 μm (probably due to fortoulism⁴), closely septate and so composed by many elements 10–25 (–40) μm long, and slightly restricted at the septa; branched below and in the middle-upper part, sometimes anastomosing, with some diverticulate to lobed elements; tips simple to forked or laterally diverticulate; in the upper part a dark brown amorphous extracellular pigment is present. **Hymenial hairs** infrequent, sometimes apparently absent, cylindrical, av. 4 μm in diameter, straight, not branched, not septate but at the base (septum of origin), concolor to the

¹ After this special issue also *Urnula mediterranea* must be considered.

² Also in this case our genetic studies reveal more species.

³ The asci match perfectly to those described and figured by BERTHET (1964, Pl. XI, fig. 21) regarding *Urnula craterium*.

⁴ The term “fortoulism” is commonly used in French and Italian languages when some elements of paraphyses are inflated (i.e. appearing moniloid) although the paraphyses are not normally so.

paraphyses. **Subhymenium** of a thick *textura intricata* made up of cylindrical, smooth, closely septate hyphae; uniformly brown at low magnification. **Medullary excipulum** of *textura intricata* composed by cylindrical, smooth, septate hyphae, 4–6 µm in diameter, with slightly thickened hyaline walls; some elements are inflated to globose. **Ectal excipulum** of *textura angularis* made up of elements up to 25 µm long and/or wide, with thick walls, dark brown to blackish due to an epimembranaceous pigment, while an incrusting pigment is present mainly in the outermost layers. **External hairs** of two types, although intermediates forms may be present: 1) hyphoid, thin walled, av. 3 µm in diameter, mainly hyaline heavily encrusted by an extracellular amber to dark brown pigment; 2) true hairs, av. 5–6 µm wide, smooth, with walls up to 1 µm thick, uniformly dark brown for an epimembranaceous pigment. **Basal tomentum** composed of cylindrical hairs, 8–9 µm wide, brown, straight, septate, with walls up to 1 µm thick, smooth, but in places a very light greenish extracellular amorphous pigment is present.

Studied collections: *Urnula hiemalis*. FINLANDIA. Etelä-Häme, Tammele, Syrjänharju, lakeshore under *Picea abies*, *Betula* and *Alnus glutinosa*, on calcareous soil with debris, 09.IV.2000, leg. M.-L. Heinonen & P. Heinonen, det. S. Huhtinen (TUR 136909). Hämeenlinna, Ilamo, on soil on a pond shore, 22.V.2000, leg. et det. H. Paavola (TUR 157025). Orivesi, Lyytikälä, Perkiö, on soil. 17.IV.2009, leg. et det. L. Kosonen (TUR 193255). Hattula, Ilamo, fish pond, 23.IV.2010, leg. et det. M. Lahti (TUR 191432). Orivesi, Lyytikälä, mossy soil in a garden, 27.XI.2011, leg. et det. L. Kosonen (TUR 194147). Tampere, Vestonkatu, 10 m south of Kaukajärventie, 1 m from the street, in a small area between some gardens and the street, on soil near *Picea abies*, *Betula pendula* and also *Alnus glutinosa*, 22.IV.2012, leg. et det. S. Kytöharju, rev. M. Carbone (H). Perä-Pohjanmaa, Rovaniemi, Pullinpuoli, in an old garden with *Picea*, *Sorbus* and *Sorbaria sorbifolia*, 26.V.2011, leg. et det. T. Kekki (TUR 196076). Uusimaa, Porvoo, Saksala, in a grassy forest with *Acer platanoides*, *Betula* and *Prunus padus*, on sandy soil, 10.V.1994, leg. et det. P. Höijer (TUR 111561). Varsinais-Suomi, Naantali, Kukonpää, mixed wood, 24.IV.2011, leg. M. Viljanen, det. S. Huhtinen (TUR 193902).

Urnula aff. *groenlandica*. FINLANDIA. Inarin-Lappi, Utsjoki, Kevo, Kotkapahta, sandy soil on riverside, pH 5.2, among *Polytrichum* and *Vaccinium vitis-idaea* and other sparse vegetation, 16.VI.1982, leg. et det. S. Huhtinen ut *Urnula hiemalis* (TUR 071079).

Discussion

Brief historical-nomenclatural note

Urnula hiemalis was described by NANNFELDT (1949) based on some winter/spring Swedish collections. The author decided to place this new species into the genus *Urnula* due to its “tough and leathery” consistency and the lack of a “gelatinous layer”. All these features match the genus concept perfectly. According to the protologue, the main features can be summarized as follows: sessile to substipitate apothecium, dark-grey to black hymenium, asci 400–450 × 12–14 µm, spores 18–26 × 12 µm, growing on the ground (“*terram adfixis*”), very slow spore maturation, and growth from December to May.

ECKBLAD (1957) reported the first Norwegian record, based on a collection made in Oppland county, in April 1950.

KEMPTON & WELLS (1974) named many collections coming from central-southern Alaska (USA) as *Urnula hiemalis*. By contrast, as can be seen below, DISSING (1981) considered that the Alaska collections could not be regarded as conspecific with *U. hiemalis*.

ULVINEN (1976) included it in his key on “*Sarcosomataceae*”, and reported the first records for Finland.

NEUENDORF (1979) traced the history of this species, analysed the phenological and morphological characteristics, and extended its distribution to Sweden.

When DISSING (1981) established his new species *Urnula groenlandica*, he also revised Swedish samples of *Urnula hiemalis* and reported in them the absence of the so-called “hymenial hairs”. According to this author, this feature distinguishes it from *Urnula craterium*, so that the presence or absence of hymenial hairs is at the base of his key to the genus *Urnula*.

DISSING & ECKBLAD (2000) separated it from *Urnula craterium*, and distinguished it on macroscopic features and spore size only. Nothing is said about the presence or absence of hymenial hairs.

More recently, ZETTUR & KULLMAN (2011) recorded it from Estonia and reported a detailed area of distribution based on all the collections housed in many Scandinavian herbaria (although they did not revise them). From a morphological point of view, they did not add any details, nor said anything on the presence of hymenial hairs.

These few references seem to confirm the rarity of this species but, in the meantime, they all report macro-micromorphological characters which make it pretty easy to be recognized. Also the current iconography is very poor. A good picture with many apothecia in different stages of growth can be found in NILSSON & NILSSON (2008), other photos are in NEUENDORF (1979), RYMAN & HOLMÅSEN (1984), NYLÉN (2001) and SALO *et al.* (2006)

Observations

As we have found in other *Sarcosomataceae* species, the maturing time of the spores is very long. According to the protologue (NANNFELDT, 1949) this seems not to be an exception because, as observed by us, many collections of this species seem not to easily reach complete spore maturity. We can confirm this character, 6 of the 9 collections here studied being immature, even when some of them consisted of medium to large apothecia. Due to this slow process and the degree of maturity of the studied samples, much care must be taken because it could lead to errors in measurements of spore size. As reported by KEMPTON & WELLS (1974), observed by us in the present work, and also in CARBONE *et al.* (2011a, regarding *Plectania milleri* Paden & Tylutki), the spores are rounded and thick-walled in the beginning; then they extend to a distinctly elliptical shape, maintaining the thickened walls; finally, upon attaining complete maturity, the walls become thinner. In our collections we have noticed, above all in TUR 193902, that almost all the spores show one (or also 2-3 smaller) oil drop at each pole. This feature was already well underlined by NANNFELDT (1949), but probably it could not be noticed on old dried material.

Another interesting feature that we wish to stress is that, contrary to what was reported by DISSING (1981), in the collections studied here we have noticed the presence of hymenial hairs. As we already had the opportunity to point out in our previous papers, the hymenial hairs (or setae, as they are termed by some authors) in the *Sarcosomataceae* are a secondary type of paraphyses generated by the same hyphae. Hymenial hairs are slightly wider and without septa except the basal one. We must admit that their frequency in the studied collections is very rare, in fact in some mounts we had some difficulty in finding them. By contrast, in S. Kytöharju’s collection, the hymenial hairs were slightly more frequent⁵. In any case, their rarity or infrequency are clearly the reason behind the statement by DISSING (1981) of their absence. We think that this feature cannot be used as a differentiating character, and that it needs to be further investigated. Certainly, our personal experience led us to state that hymenial hairs are definitely frequent in *Urnula craterium* and easily visible in every mount of material taken from any part of the hymenium.

Even if the sum of the microscopic characters, the general morphology, the area of distribution, and the phenology avoid confu-

⁵ This collection has been sequenced, see the genetic work in the present issue.



Urnula hiemalis

Pictures: TUR 136909 - P. Heinonen (left) and TUR-A 195795 - S. Kytöharju (right).

sion with the other species, we think that some of the latter definitely deserve to be mentioned.

Similar species

The most similar species, at least from a microscopic point of view, is undoubtedly *Urnula groenlandica* Dissing. It was based on material collected in Greenland (DISSING, 1981) and published with the following, detailed, original description: “*Carposoma* 0.6–1.8 cm latum, subglobulare vel cupulatum, margine incurvo, elevato, sessile vel base sulcata subsessile, hymenio laevi, obscure fusco, extra eodem fere colore, ad marginem versus saepe rubro affectum, supra oculo inermi glabrum apparens, infra tomento fulvo-cinnabarino vestitum. Excipulum exterius 130–200 μm crassum, textura angulari, infra e seriebus cellularum 16.5–33 \times 33–60 μm magnis ad superficiem versus sub angulo recto directis, extra e cellulis 6–20 \times 6–26 μm magnis compositum. Pili dimorphi. Alii forma ordinarii, septati, ramificati, achroi vel fuscidi, substantia fusca etiam superficiei haerente, irregulariter dispositi, alii capillitio similes, fusci, laeves, foveolati, septis nullis, non ramificati, paulum undulati, superficiei paene paralleli. Excipulum medullare 380–450 μm crassum. Hymenium 370–400 μm crassum. Ascii 12.5–14 μm crassi, cylindrici, in bases angustissimas acrorhynchis sensim attenuati. Paraphyses pervariae, septatae, supra fuscidae, substantia fusca etiam superficiei haerente paraphyses in fasciculos conglutinante, infra inflatae, saepe mammillosae. Sporae 21.5–25.7–27.1 \times 10.0–11.2–13.2 μm magnae, ellipsoides vel ovoides, hyalinae, glabrae, guttulas nullas sed saepe substantiam refringentem ad polos versus sitam continentes. Holotypus die 11 Julii anni 1972 in Saliceto glaucae perhumili cum Chamaenerio latifolio, Empetro hermaphrodite, Equiseto arvensi, Luzula confusa, Pyrola grandiflora, Vaccinio uliginoso prope oppidum Groenlandiae Occidentali Qeqertarsuaq (Godhavn, lat. bor. 69° 14', long. occ. 53° 31') sub numero 72.14 a P. Milan Petersen lectus, siccus in Museo Botanico Hauniensi (C) depositus”. The main characters can be summarised as follows: subglobose to cupulate ascomata, 0.6–1.8 cm in diameter, sessile to substipitate, with dark brown hymenium, concolorous external surface often with reddish-brown shades especially toward the margin; medullary excipulum of *textura intricata*; ectal excipulum of *textura angularis*; external hairs of two types, one hyphoid, encrusted, and the other “capillitium-like”; lack of hymenial hairs; branched, septate paraphyses, brown in the upper part due to an amorphous pigment uniting them in bundles; spores 21.5–27.1 \times 10–13.2 μm , hyaline, smooth, ellipsoid, with refracting content at the poles, ma-

turing readily; growth from June to August. It is thus evident how the microscopic features are pretty much identical to *U. hiemalis*, in fact DISSING (*op. cit.*), in his observations and key, uses macroscopic and phenological features only as distinguishing characters. As already mentioned, and also observed by us, in the studied material of *U. hiemalis*, it must be stressed that the absence of hymenial hairs needs to be better investigated. ZETTUR & KULLMAN (2011) mentioned a collection of *Urnula hiemalis* from Utsjoki (Finland, Inarin-Lappi, 69° 54' 25" North), which very probably is the one here studied as TUR 071079. This collection, determined by S. Huhtinen as *U. hiemalis*, in contrast to the others has shown: small apothecium, 1.3 cm in diameter (dry), cupulate, blackish; very rare hymenial hairs; all asci were full of spores; the latter were not fully mature but 20–26 \times 11–12 μm ; growing in mid-June at a latitude almost identical to that of the original site of *U. groenlandica* (Greenland, Godhavn-Qeqertarsuaq, 69° 14' 50" North). All these characters lead us to think that this collection could well represent Dissing's species. Unfortunately we have not revised the type specimens of *U. groenlandica* and so, at present, we can only use the detailed protologue for a distinction with *U. hiemalis*. What is certain is that the collection TUR 071079 shows many features definitely similar to *U. groenlandica*, while not so different microscopically from the *U. hiemalis* collections here studied. The rare presence of hymenial hairs found in this collection is not surprising if we consider that DISSING (1981) reported their absence for both the species. Future studies will clarify whether the macroscopic and phenological differences of these two species will be taxonomically relevant.

Although not validly published (*ad interim*), another very similar species is *Urnula brachysperma* (BRUNELLI, 1998). It was described from Switzerland and, according to the protologue it is characterised by: hymenium “brun-noir”, a small stipe 2–3 mm long supporting a 20–25 mm wide cup; eguttulate spores, with a very high Q ratio; closely septate paraphyses with monilioid appearance probably for fortoulism; lack of hymenial hairs. We do not know if this latter feature is reliable, having not yet examined the original material. The general features are definitely reminiscent of *Urnula hiemalis*. A revision of the original material is needed to solve this issue⁶.

Urnula craterium is very different for many macro-micromorphological features, among which we find: a different habitus due to a long stipe and an urceolate cup; a margin heavily crenulate to odontoid and frequently deflexed at maturity; slightly larger spores and very abundant hymenial hairs.

⁶ We have revised this collection and we can confirm the presence of hymenial hairs, a paper on this species is under writing.



A



B



C



D

Plate 1 – Exsiccata

Urnula hiemalis. A: TUR 193902; B: TUR 196076; C: TUR 194147. *Urnula aff. groenlandica*. D: TUR 071079.
Scale bars = 1 cm. Pictures: M. Carbone.

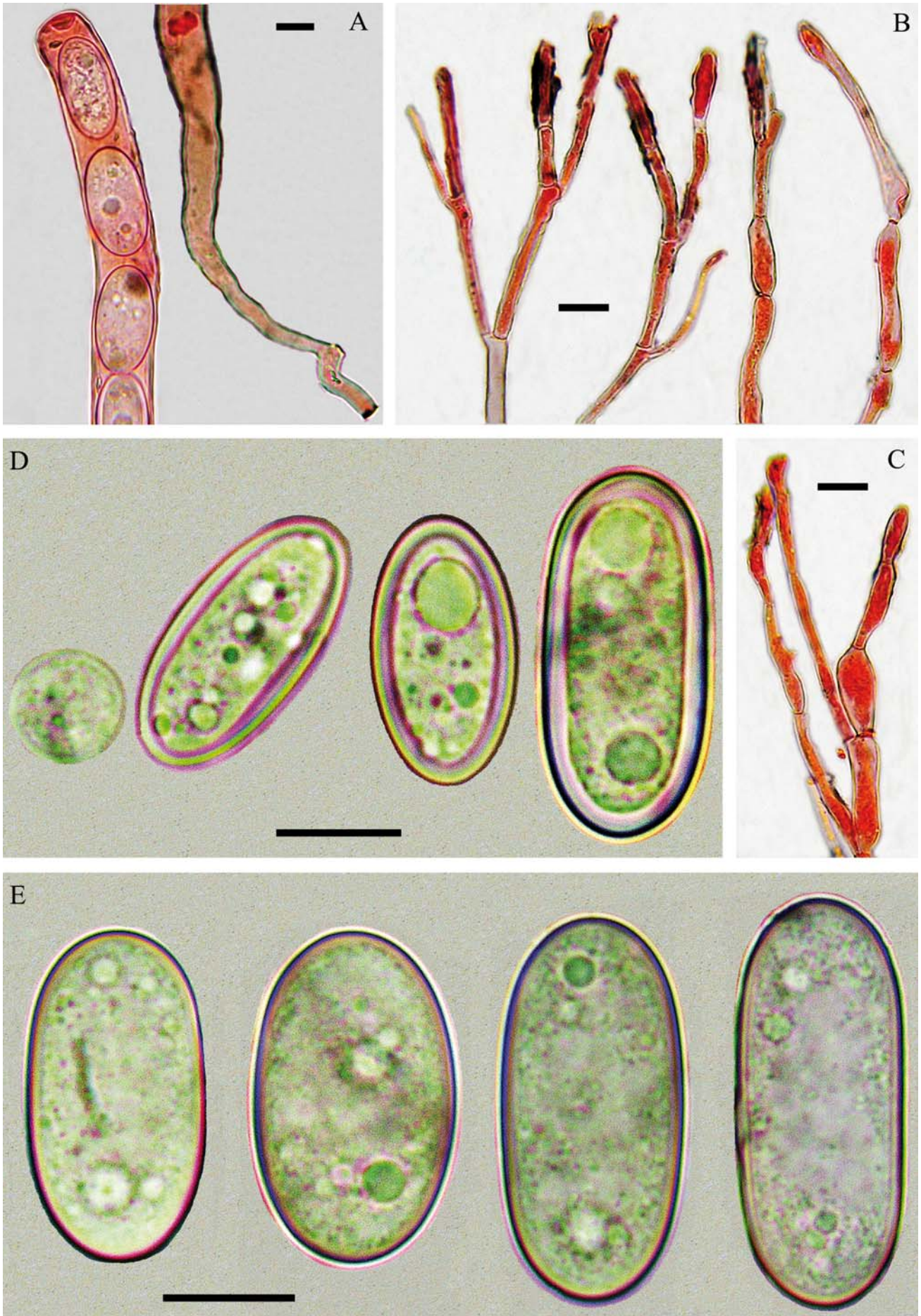


Plate 2 – *Urnula hiemalis*. Microscopic characters.

A: apex and base of an ascus in Congo red; B-C: paraphyses in Congo red; D: immature spores in H₂O; E: mature spores in H₂O. Scale bars = 10 μm. Pictures: M. Carbone & C. Agnello.

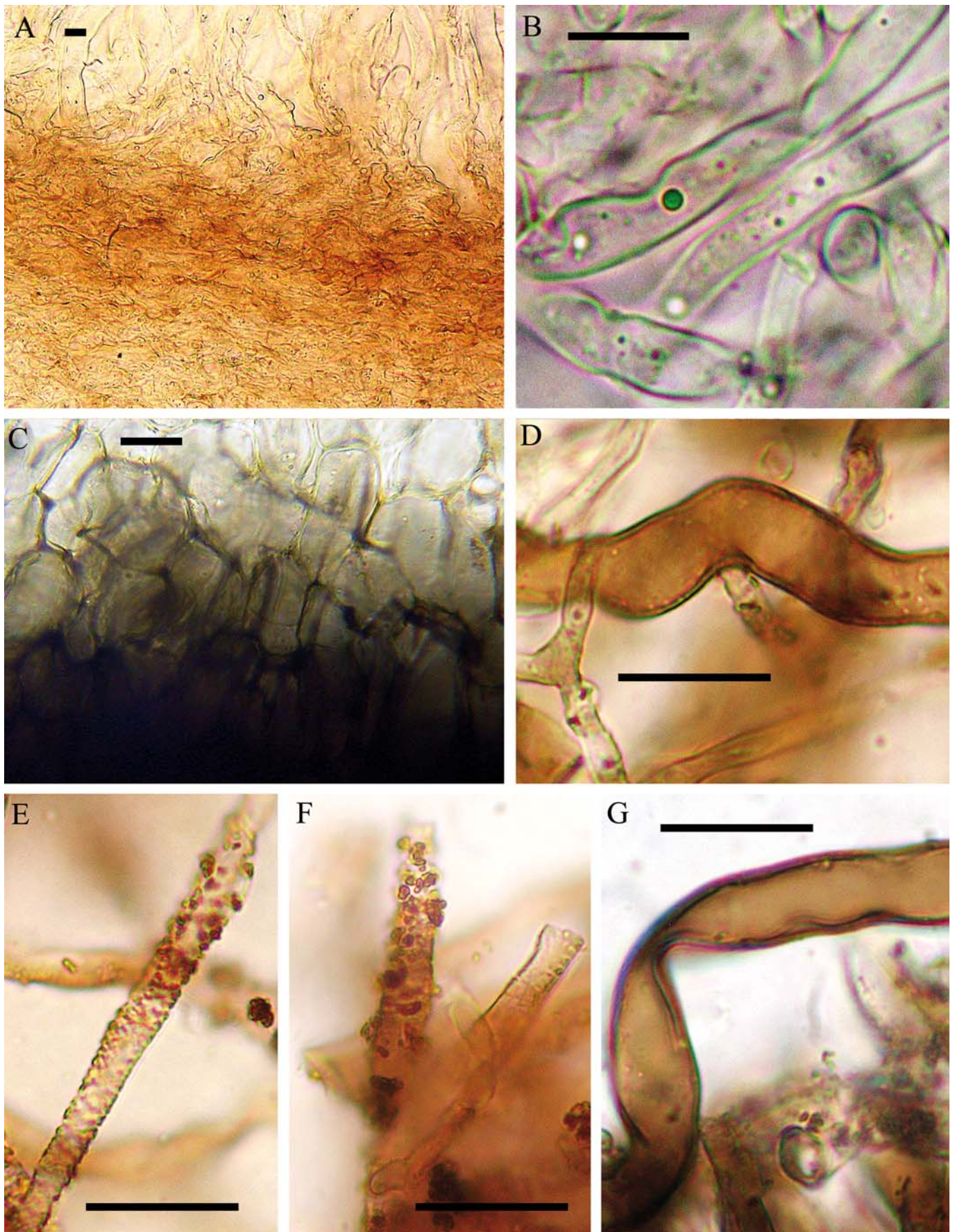


Plate 3 – *Urnula hiemalis*. Microscopic characters (in water).

A: Subhymenium; B: hyphae of the medullary excipulum; C: Ectal excipulum; D-G: hairs of the ectal excipulum.
Scale bars = 10 μ m. Pictures: M. Carbone & C. Agnello.

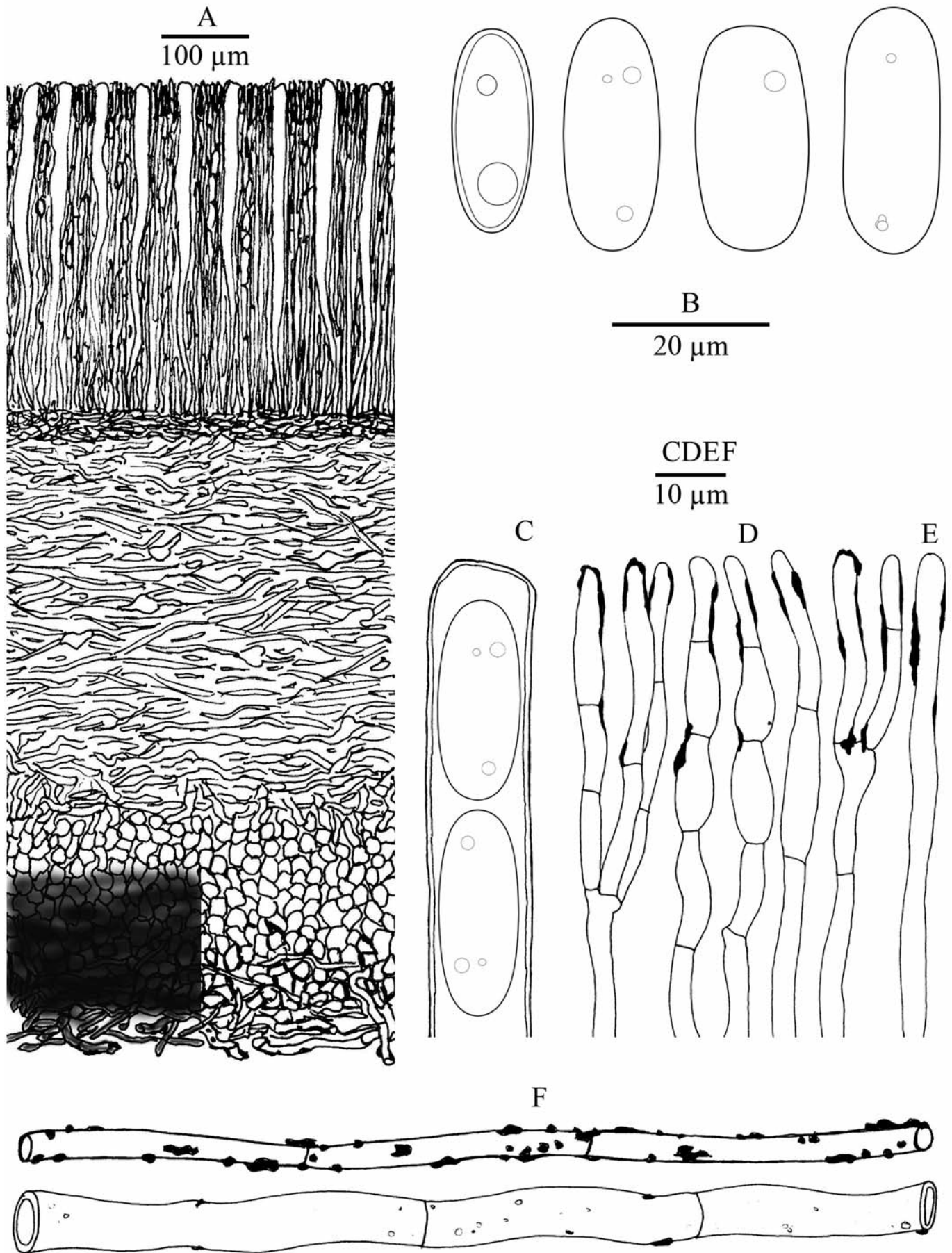


Fig. 1 – *Urnula hiemalis*. Microscopic characters.

A: Apothecium in section; B: Spore; C: Apical part of an ascus; D: Paraphyses; E: Hymenial hairs; F: Hairs of the ectal excipulum.
Drawing: C. Agnello.

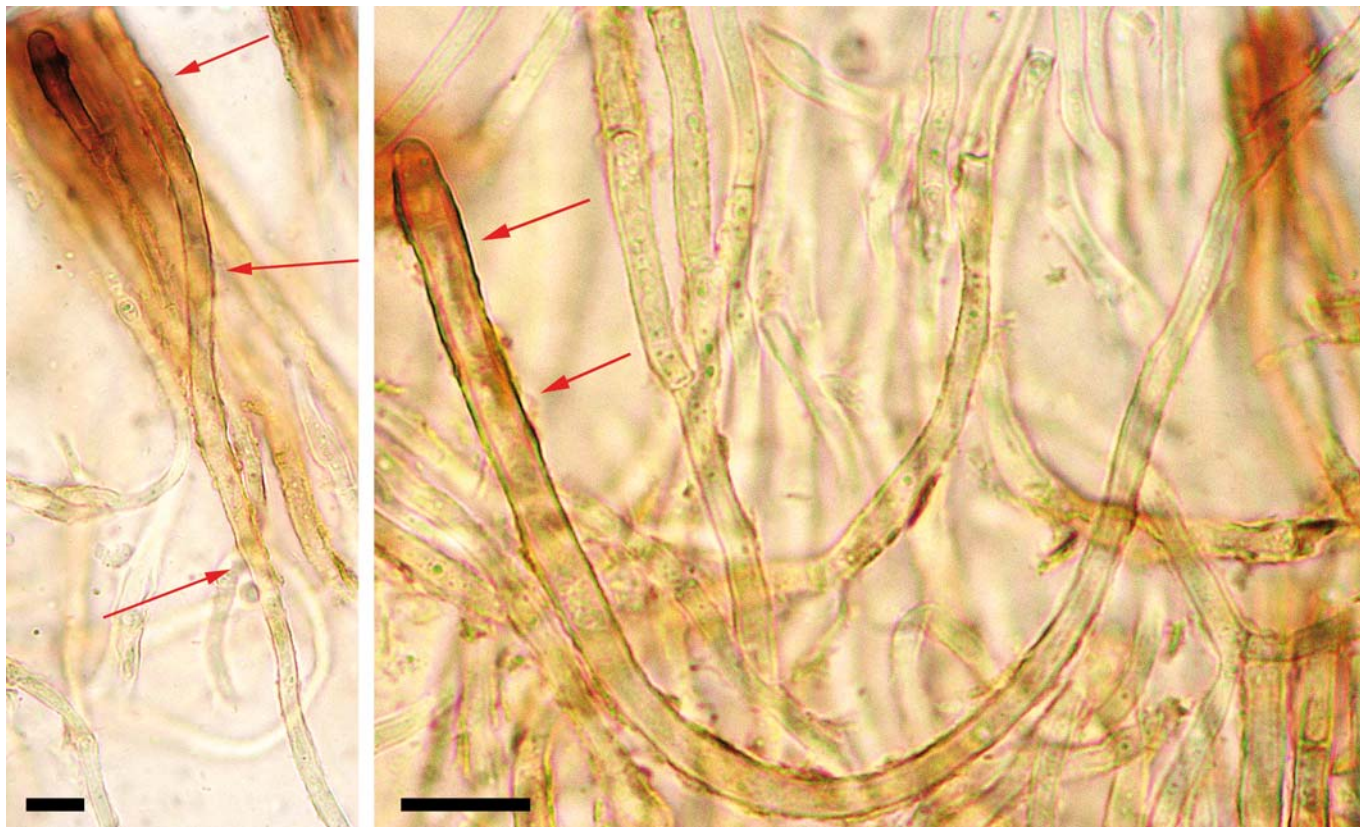


Plate 4 – *Urnula hiemalis*. Hymenial hairs in water (indicated by red arrows). On the right TUR 193902. Scale bars = 10 μ m. Pictures: M. Carbone.

As seen by us after the study of a North American collection, a black sarcosomataceous species, which shows rare hymenial hairs, is the taxon known as *Plectania mexicana*⁷ (Ellis & Holway) Paden [= *Sarcosoma mexicanum* (Ellis & Holway) Paden & Tylutki], which is easily distinguished for many macro-micromorphological characters, such as the larger size of the ascomata and the highly gelatinised flesh (PADEN & TYLUTKI, 1969; SMITH *et al.*, 1973; PADEN, 1983; ARORA, 1986; TYLUTKI, 1993; CASTELLANO *et al.*, 1999; PHILLIPS, 2005; MILLER & MILLER, 2006; TRUDELL & AMMIRATI, 2009).

Urnula hiemalis was also cited in the protologues of two species: *Plectania megalocrater* (Malençon & Le Gal) M. Carbone, Agnello & Konstantinidis (≡ *Urnula megalocrater* Malençon & Le Gal, in LE GAL, 1958) and *Plectania mediterranea*⁸ M. Carbone, Agnello & Baglivo (CARBONE *et al.*, 2009). The one potentially more similar by way of its cupulate habit and black colors is definitely *Plectania megalocrater*, which however is very different even to a non-expert's eye (see LE GAL, *op. cit.*; CARBONE *et al.*, 2011b).

Distribution

Urnula hiemalis is known from many localities in Norway, Sweden, Finland and also from one in Estonia (ZETTUR & KULLMAN, 2011). As already mentioned, the Alaska collections of KEMPTON & WELLS (1974), according to DISSING (1981), should be regarded as erroneous. At present we think that a new study of that material is required to determine their real (and correct) identity.

In Wien University Herbarium (WU) is housed a specimen no. WU-Mykologicum 0016145, named *Urnula hiemalis*, legit et det. W. Klofac, 23.IV.1996, collected in Austria, Groß-Enzersdorf, Herrnau, Steinbühelau. We have not yet studied it, but it seems necessary to examine it before confirming its identity and then its presence in Austria. If

confirmed, it could represent the most southern collection currently known.

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⁷ Now *Urnula padeniana*, see the article in this issue.

⁸ Now *Urnula mediterranea*, see the article in this issue.

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