Contribution to the knowledge of *Plectania milleri* (Ascomycota, Pezizales), a Northwestern American species

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Summary: Two collections of *Plectania milleri* from Montana and Washington State (U.S.A.) are studied in depth. Color pictures of fresh specimens, microscopic morphology, and B/W drawing are added.

Keywords: Ascomycetes, *Plectania* sect. *Plectania*, taxonomy, United States of America.

Riassunto: Viene presentato uno studio dettagliato di Plectania milleri, raccolta in Montana e nello stato di Washington (U.S.A.). Il tutto viene corredato da fotocolor di esemplari freschi e del quadro microscopico, nonché da una tavola al tratto.

Parole chiave: Ascomiceti, *Plectania* sect. *Plectania*, tassonomia, Stati Uniti d'America.

Résumé : Deux récoltes de *Plectania milleri*, des états du Montana et de Washington (États-Unis), sont étudiées en profondeur. Des images en couleur de spécimens frais et de la morphologie microscopique, ainsi qu'un dessin en N&B, sont proposés.

Mots-clés : Ascomycètes, *Plectania* sect. *Plectania*, taxinomie, États-Unis d'Amérique.

Introduction

During their studies on the genus *Plectania* Fuckel, the Italian authors found on the "Mushroom Observer" web site, some very good pictures of a collection that seemed to fit the characteristics of *Plectania milleri* perfectly, at least from the macroscopical point of view. The Canadian author immediately agreed to send the specimens to Italy, where once the specimens were microscopically studied they appeared to match typically *Plectania milleri*.
Because of the lack of an in-depth study of this species in literature, aside from the protologue, we agreed that this collection deserved a more detailed description, and also a discussion on its position in the genus *Plectania* after the new described species of the past forty years.
During the final revision of this paper, thanks to the courtesy of Andrew Parker (Washington State, USA), another collection has been studied and confirmed to be a typical *P. milleri*. The results led to an emendation of some microscopical features, useful to a better knowledge of the interspecific variability of this rare species.

Materials and methods

Microscopic characters are based on dry specimens. Two optical microscopes were used: Olympus CX41 trinocular and Optika B353 trinocular with plan-achromatic objectives 4×, 10×, 40×, 100× in oil immersion. Microscopic pictures were made using a Nikon 4500 camera and a Nikon Coolpix. The following main reagents were used: Melzer's reagent, cotton blue, Congo red. Water mounts were used for the observation of the pigmentation and spore size. At least 30 spores were measured from each apothecium.

Description

Plectania milleri Paden & Tylutki, *Mycologia*, 61: 683 (1969).

Original diagnosis

Apothecia gregaria, cupularia, margo stellata, brevistipitata vel sessilis, 5-10 *mm alta*, 10-40 *mm lata, hymenium fuscum; externa atra; asci cylindracei, suboperculati, octospori, in basim sensim angustati,* 380-425 × 12-15 µ; *ascosporae ellipsoideae, hyalinae, laeves, eguttulatae,* 21-25,5 × 9-10,5 µ; *paraphyses tenues,* 1.5-3 µ *latae; excipulum medullare ad usque 1.2 mm crassum, e textura intricata hyalina, in matrice gelatinosa situm, penetratum hyphis ramosis fuscique; excipulum exterius e textura angulari; pili atri laevesque, raro ramosi septatique.*
Hab. in ligno putrido.
Loc. Bonner et Clearwater Counties, Idaho, U.S.A.
Typus: exsiccatum in Herb. Washington State University, WSP #56273.

Typification

The original material (holotype) does still exist in the Washington State University Herbarium (WSP) under the number WSP 56273. We have not personally studied it and so do not know its condition. Jack D. Rogers, Regents Professor,

Director of WSP, kindly told us: "We have the type collection of *Plectania milleri*. We do not have other collections of this species. I looked at the material and it seems in excellent condition".

Brief description: Small, dark black cup shaped fungus with a stellate margin, smooth spores and brown hairs crossing through the medullary excipulum; specimens found growing clustered or scattered, on sandy, rocky soil with conifer debris from *Larix* sp., and also from *Abies grandis*, early to mid-spring, North-Western America.

Macroscopical features

Apothecia nearly round at first, expanding as they mature, up to 3 cm across, and up to 1 cm high; margin stellate, incurvate when young but less so with age. Base with very little if any stalk, attached to substrate with very numerous dark black, threadlike filaments (subiculum). **Hymenium** very dark brown or black, also slightly flushed of purple, smooth but uneven. **External surface** very dark brown or black, rough, with small pimples or bumps at least when young. **Flesh** tough, rubbery or leather like, not brittle or fragile, center filled with a light whitish colored substance, darker near external surfaces; the fertile surface is dark all the way through.

Microscopic features

Asci 400–600 × 13–14 µm, cylindrical, operculate, with a slightly excentric operculum, inamyloid, eight-spored, with walls up to 1.8 µm thick and a tapered, flexuous base. Most of the bases are simple, but some appeared "forked" due to a generative hypha that connected more or less distantly from the basal septum. **Paraphyses** not exceeding the length of the asci, 1.8–2.5 µm wide, cylindrical, septate, sometimes anastomosing, branched below but often also at the top, multiguttulate by very small oil drops. The apex is usually more or less diverticulate, lobed, ramose, in some cases resembling the deer antlers. Extracellular, amber-brown, gluey pigment is present in the upper part of dried specimens observed in water mounts. **Setae** cylindrical, as long as the paraphyses, 3–3.5 µm wide, with a simple apex, and a single septum at the very base. **Spores** ellipsoid, sometimes only very slightly inequilateral, 22–31 × (8–) 9–11 µm, most frequently 27–28 × 9–10 µm, very few spores up to 37 µm in length have been observed, Q = 2.4–3.2, hyaline, without oil drops but filled with a granular content, walls less or equal to 1 µm thick; sometimes a thin gelatinoid sheath has been observed covering the spores. Unripe spores show some oil drops and walls up to 1.5 µm thick. In young asci, the very unripe spores are globose. **Subhymenium** composed by a dense *textura intricata* of cylindrical, frequently septate, hyphae, with thickened, more or less dark brown walls. At low magnifications, it appears uniformly brown. **Medullary excipulum** of *textura intricata* with cylindrical, septate, hyphae, 4–10 µm wide, with walls on average 0.5–0.8 µm thick but also up to 1.5 µm in the wider hyphae, and immersed in a gelatinous matrix. In water mounts at low magnification, the medullary excipulum is uniformly very pale yellowish colored but also with a pale greenish shade. It is crossed by brown, cylindrical, smooth, septate, more or less twisted and sometimes slightly diverticulated hairs, up to 7 µm wide and with walls thickened up to 1 µm. **Ectal excipulum** of *textura angularis* made up of elements up to 15 (–18) µm wide or high, very dark brown due to the colored walls and the presence of an incrusting pigment. **External hairs** cylindrical, septate, up to 8 µm wide, very long[1], straight, smooth, originated in the ectal excipulum where they can be encrusted at the very base. They are brown due to an epimembranaceous pigmentation, with walls thickened up to 1 µm. In non-squashed mounts, they are mainly lying on the external surface.

Studied collections and habitat

United States of America: **Montana**, Trego, GPS Coordinates: 48.6685863, -114.9677634 (= N 48° 40' 06", W 114° 58' 03"); elevation: 3,950 feet (=1200 m). Among Larch trees (Larix spp.); the soil composition below the humus layer of needle debris, to which this epigeous, cup-like ascocarp was attached by very numerous dark, hair like, rhizoid, filaments, is mostly rock and sand. As would be expected, these specimens where found in groups or clusters on conifer debris, in a wilderness environment. 11.VI.2011, *leg*. J. Harnisch, det. M. Carbone, C. Agnello & J. Harnisch (herbarium TUR-A 190823). **Washington State**, Pend Oreille Co., FS-Rd 190 North of Slate Creek; elevation: 2550 feet (=780 m). In mixed, dense, coniferous forest, on Abies grandis debris. 07.VI.2011, *leg*. et det. Andrew Parker, rev. M. Carbone (herbarium Andrew Parker 110607-1, duplicate in TUR-A 190835).

Discussion

Notes and emendations

As said in the introduction, our study points out some interesting notes and emendations (of the protologue) that need to be reported.

From our knowledge, it seems that no one has ever made any investigation of the nature of the medullary excipulum "dark hyphae". Paden & Tylutki (1969) first, and Tylutki (1993) later, did not report any consideration about their origin or structure. Our results led us to consider the dark hyphae not morphologically distinct from the hairs of the external surface, whereas both originate in the ectal excipulum. Nevertheless, at present, it is not easy to explain why some hairs should grow downwards. Obviously more studies on other collections are required to obtain a final opinion with certainty. A primary hypothesis could be that during its growth many hairs are incorporated in the medullary excipulum. This could be a good explanation for their wrinkled, flexuous to twisted appearance and also for those that are present only as pieces. In this latter case, in fact, we can suppose that, during the thickening of the flesh, some hairs are broken and remain as pieces without a defined point of origin or end.

Another good argument of discussion is the spore size of these collections. Paden & Tylutki (*op. cit.*) reported "21–25.5 × 9–10.5 µm" and Tylutki (*op. cit.*) slightly extended it up to "26 × 11 µm"; our measurements are definitely big-

[1] It is difficult to determine the real length because, in non-squashed mounts, they are lying and appressed, and once squashed they easily break.

Fig. 1 & 2 – *Plectania milleri.* Photos: J. Harnisch.

Fig. 3 & 4 – *Plectania milleri.* Photos: A. Parker.

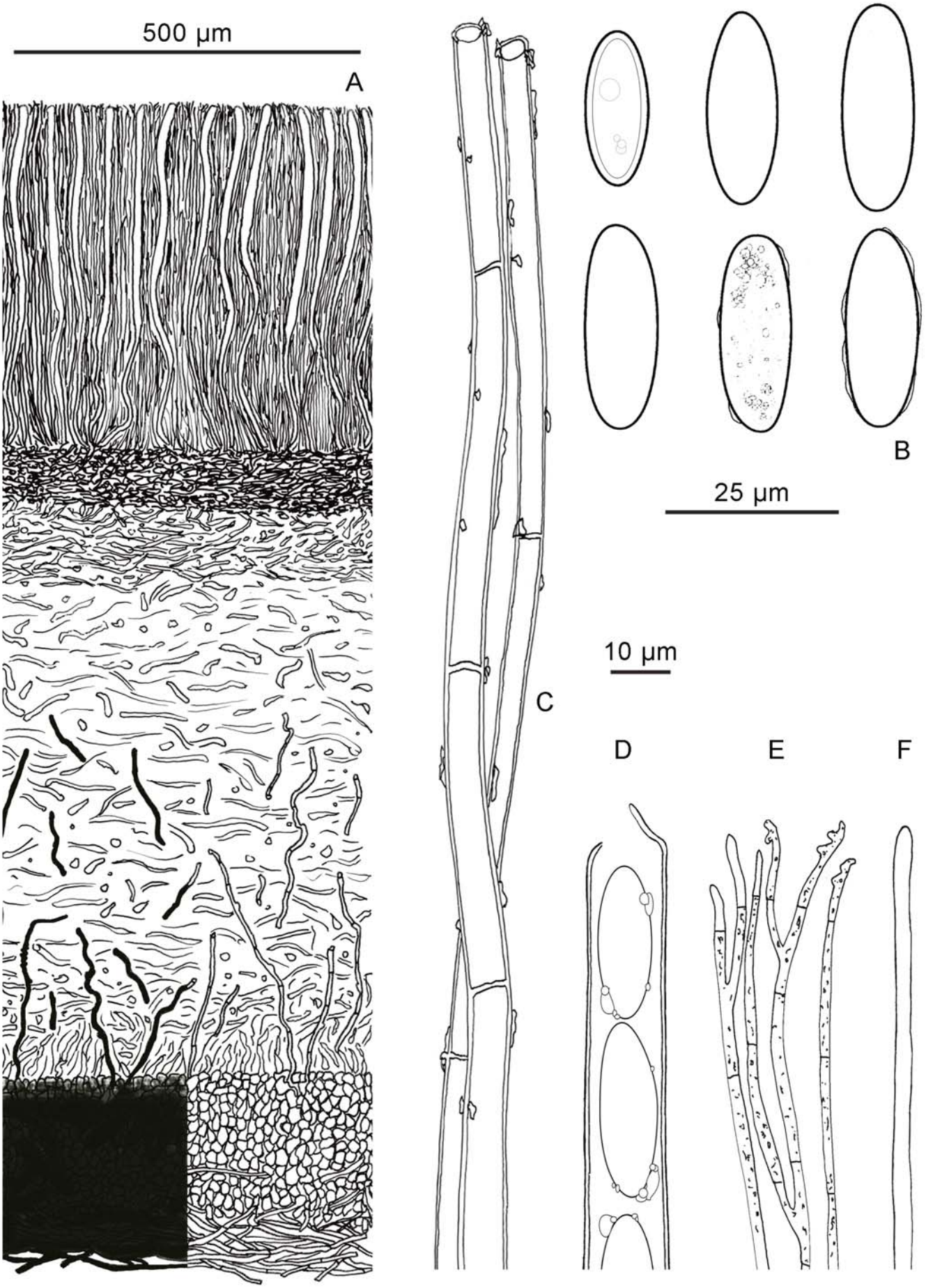

Fig. 5 — *Plectania milleri.* Microscopic features. Drawing: C. Agnello.
A: Section of the apothecium; B: spores; C: external hairs; D: upper part of the ascus; E: paraphyses; F: seta.

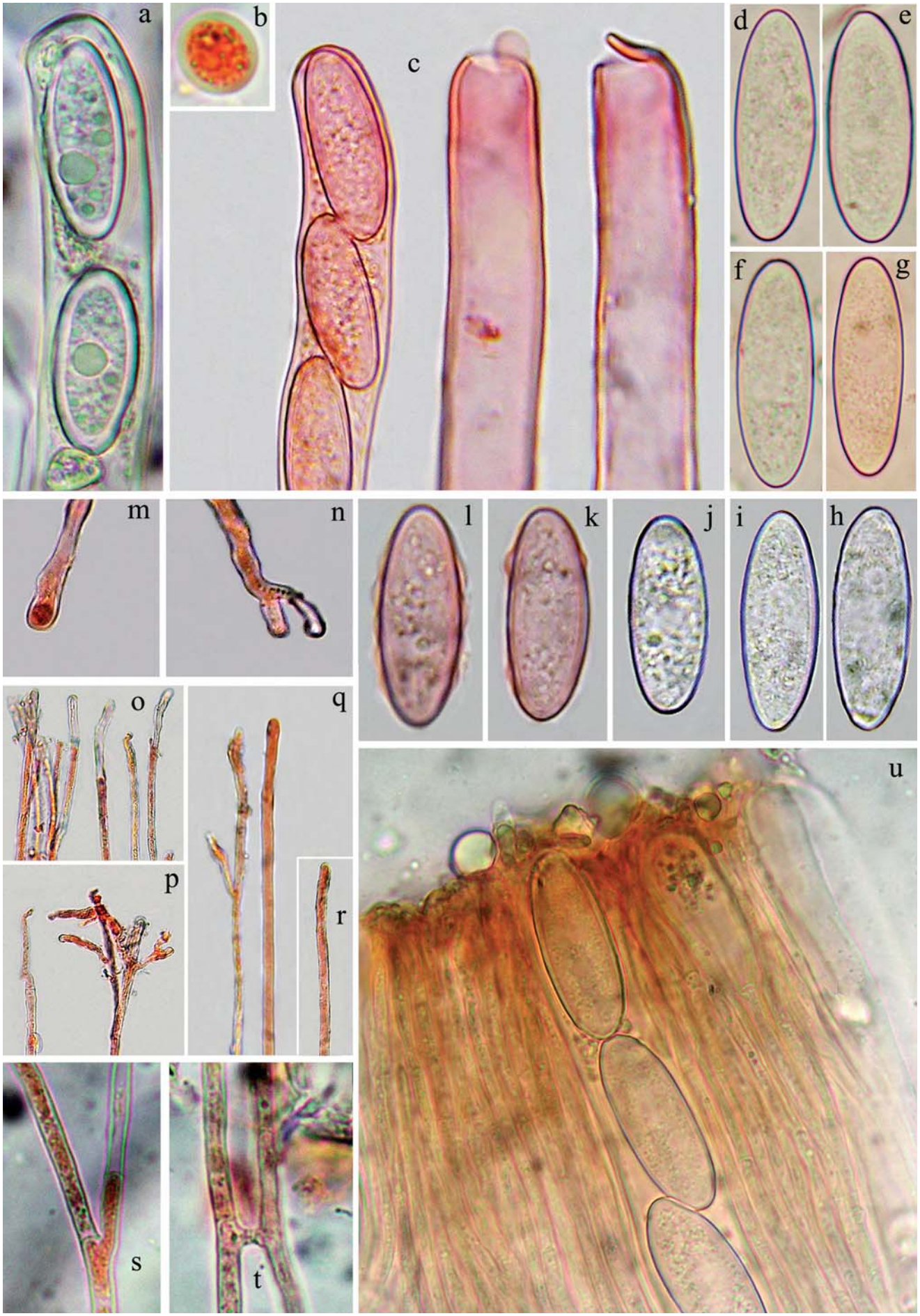

Fig. 6 — *Plectania milleri.* Hymenial elements. Photos: C. Agnello & M. Carbone.
a: unripe spores; b: very unripe spore; c: ascus tips; d-l: spores; m-n: ascus bases; o-p: paraphyses tips; q: paraphyses and setae; r: setae tip; s-t: paraphyses; u: upper part of the hymenium.

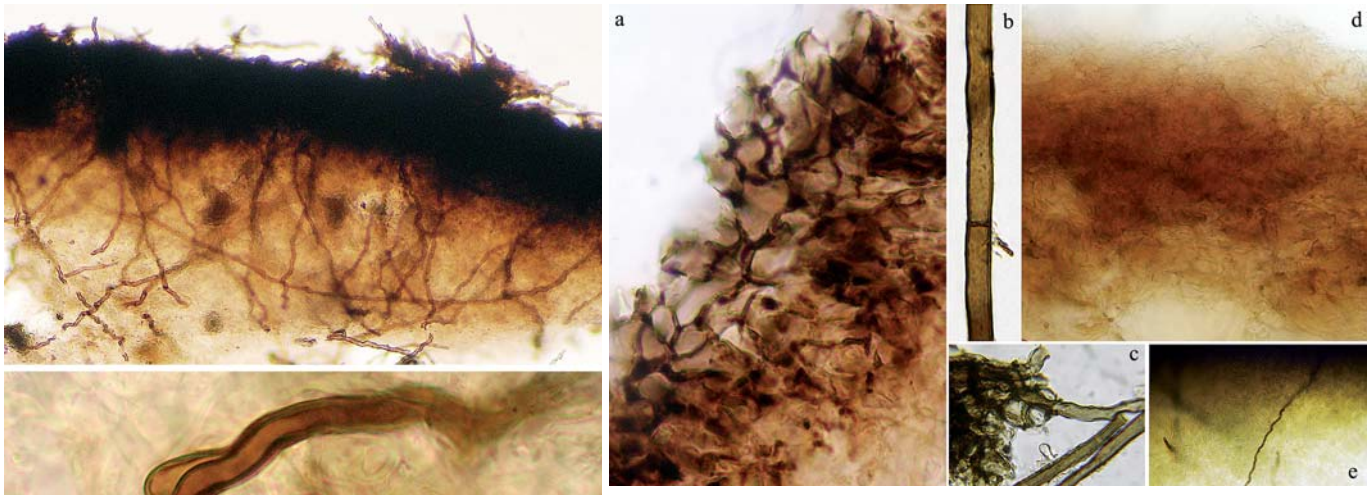

Fig. 7 — *Plectania milleri.* Photos: C. Agnello & M. Carbone.
a: ectal excipulum in water mount; b: external hair; c: base of the external hairs; d: subhymenium in water mount; e: medullar excipulum in water mount at low magnifications.

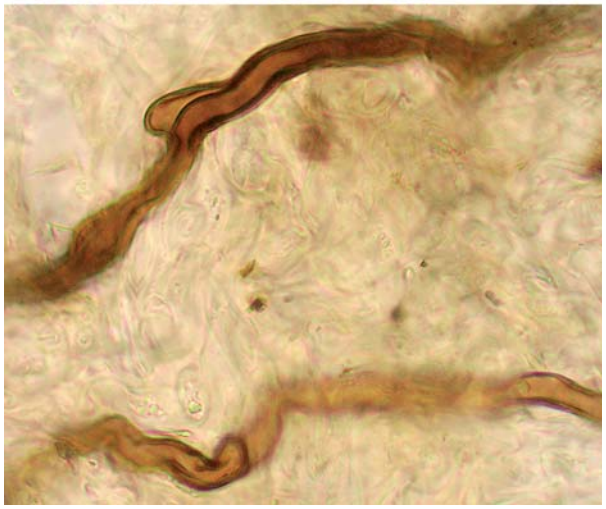

Fig. 8 — *Plectania milleri.* Photo: M. Carbone.
Top: ectal and medullary excipulum in water mounts at low magnifications. Bottom: medullary excipulum at 1000× oil immersion.

ger (at least on the average, see above), even more than those reported by Castellano *et al.* (1999) "(24.4–) 26.3–27.6 (–28.9) × 10.5–12.5 µm in fresh material from Oregon", but we do not consider this so taxonomically relevant to justify the creation of a different taxon based on larger spores. In fact, we believe that the lack of further in-depth studies (besides the protologue) and the rarity of the species are the main reasons for a previously ambiguous spore size range. Our collections are undoubtedly naturally matured and, as far as we know from our studies on the genus, *Plectania* spp. spores usually take a long time to reach complete maturity (this goes for all the *Sarcosomataceae* species spores). In this genus we have also found that unmature spores are easily released from the asci when we try to obtain a spore print, therefore it is not always easy at first sight to know if a spore is completely mature. In the Montana collection we find (as well as in other *Plectania* species) that unripe spores show thicker spore walls and bigger oil drops, therefore it must be regarded (at least for *P. milleri*) that mature spores are, obviously, the ones with thinner walls and lacking oil drops. Anyway, we would like to underline that the protologue's spore size is covered in the wide size range between very unmature spores and overmature ones.

Our collections shows the presence of an unreported "microscopic cell", i.e. the setae. In Carbone *et al.* (2009) and Carbone *et al*. (2010), the Italian authors used the words "peli imeniali" that could be translated as "hymenial hairs" but, since we were not sure if this translation is sufficiently correct, we decided to use setae, following Paden (1983). Setae must be considered as a slightly different kind of paraphyses. They are present (in variable number) in all the *Plectania* ssp. that we have studied, and they are mainly distinguished by the presence of a septum only at the very base, and a wider diameter (if compared to the paraphyses). Our findings show that setae are definitely present in *P. milleri*, and we can speculate that they had also been observed by Paden & Tylutki (*op. cit.*) but maybe they considered them falling in the morphological variability of the paraphyses. The American authors in fact, reported a range of 1.5–3.0 µm for the paraphyses, thus covering the range of both paraphyses and setae.

Lastly, Paden & Tylutki (1969) state "It should be noted that in Seaver *P. milleri* will key out to *P. melastoma* (as *Bulgaria melastoma*)". This could be possible but, for the moment, we have no data supporting this statement. A revision of the material of *Bulgaria melastoma* in the sense of Seaver (1928) is required.

Similar species

Although it is a well defined species for reasons mentioned above, we think that it could be useful to make a short survey on the allied species, but only those belonging to *Plectania* sect. *Plectania* Korf (Korf, 1957).

From the continent of North America there are four other species: *Plectania melastoma* (Sowerby) Fuckel, *P. mexicana* (Ellis & Holoway) Paden, *P. latahensis* (Paden & Tylutki) M. Carbone, Agnello & Baglivo and *P. nannfeldtii* Korf.

Plectania melastoma, *typus generis*, is easily distinguished due to its orange-red warts on the external surface, which are more abundant on the margin of the black apothecia (see, *inter alia*, Mauer, 1992).

Plectania mexicana is a common species, apparently recorded from Mexico, India, western U.S.A. and Canada (British Columbia). Originally described under the name *Bulgaria*

mexicana Ellis & Holway (Ellis & Holway, 1897), it was later transferred to *Sarcosoma* (Paden & Tylutki, 1969) and finally to *Plectania* (Paden, 1983) due to the non-watery flesh and the non-monilioid hairs of the external surface. Macroscopically, it produces large ascomata, up to 14 cm in diameter, turbinate to discoid in age, but always with a truncated, inverted, conic shape, black hymenium, rugose and black external surface. Microscopically, it shows smooth, ellipsoid, with 1-3 oil drops, sometimes slightly inequilateral spores, 23–34 × 10–14 µm. No setae seems to be present in this taxon (Paden, 1983; Arora, 1986; Tylutki, 1993; Miller & Miller, 2006) but, as stated above, we think that the presence or absence must be checked with care.

Plectania latahensis is characterized by a dark purple to black hymenium, external surface (usually) dark greyish to black, non-guttulate spores at maturity, large medullary hyphae and usually light coloured (subhyaline) hairs paler at the distal end (see also Tylutki, 1993). Originally described as *Sarcosoma latahense* Paden & Tylutki (Paden & Tylutki, 1969), it was transferred to *Plectania* by Carbone *et al*. (2009) because it (together with the previous species) does not fit completely the concept of the genus *Sarcosoma* Casp., as pointed out first by Paden (*op. cit*.) and more recently by Carbone (2009).

Plectania nannfeldtii is a long and slender stipitate species, very different from *P. milleri* in many respects, and it is one of the so called "snowbank mushrooms". It is a well known taxon, and so for a comparison we invite the reader to take a look at the following references: Seaver (1928, 1942), Seaver & Shope (1930), Kanouse (1947), Miller (1965, 1967), Arora (1986), Li & Kimbrough (1995), Evenson (1997), Smith *et al.* (1981), Tylutki (1993) and Cripps (2009). An in-depth study of this species complex is in print (Carbone *et al*.)

Concerning the rest of the world, several species apparently belonging to *Plectania* sect. *Plectania* have been described. In Europe, we find *Plectania mediterranea* M. Carbone, Agnello & Baglivo and *Plectania zugazae* Calonge & A. García (and also *P. melastoma*); in Asia, we find *Plectania yunnanensis* W.Y. Zhuang and *Plectania modesta* Otani.

Plectania mediterranea is a big, fleshy species with a vivid brick red to brown blackish hymenium, and smooth spores, distributed in the Mediterranean area (Italy and Corsica). It is a very different species from any other of the genus *Plectania*, and so it definitely could not be confused with *P. milleri* already from the macroscopical point of view (see the color photos in Carbone *et al*., 2009).

Plectania zugazae is described from Spain and considered a distinct species on the account of the combination of the following features: black, gregarious, sessile apothecia with rhizomorph hyphae, asci 250–300 × 16–18 µm, smooth ellipsoid spores, 18–22 × 12–14 µm, with a granulose content and covered by a gelatinoid sheath, filiform and simple paraphyses (Calonge *et al*., 2003).

Plectania yunnanensis has been described from China (Zhuang & Wang, 1998), according to the protologue, it possesses a "dark black" hymenium, small apothecia (10–16 mm), short asci (330–345 µm) and 2-4-guttulate spores, 35–42 × 13–18 µm, some with a slightly flat side. The original diagnosis compares it to *Plectania campylospora* (Berk.) Nannf. and *Plectania chilensis* (Mont.) Gamundí, due to the tendency of the spores to be slightly inequilateral. In this case, however, it seems to be closer to *Plectania* sect. *Curvatisporae* Korf.

Plectania modesta, described from Japan (Otani, 1973), is distinguished by entirely black, cup-shaped, subsessile or shortly stipitate apothecia, 2–4 × 0.2–0.5 cm (diam. × height), asci 350–400 × 12–15 µm and smooth ellipsoid biguttulate spores, 16–25 × 8–10 µm. In this case as in the two previous ones, different features enable us to clarify the distinction with *P. milleri*.

Final considerations

In conclusion, *Plectania milleri* can definitely be regarded as a rare species, only recorded from the north-western part of the United States. It was first described from Idaho (Paden & Tylutki, 1969) and then reported from Oregon (Tylutki, 1993). We also have currently recorded findings only from the north-western part of the U.S.A. and from Canada, British Columbia (Andrew Parker, pers. comm.), therefore we can consider it an endemic species of that part of the world. According to the cited literature, it was certainly a novelty to see this rare discomycete in Montana (not far south of the Canadian, USA border, west of Glacier National park in the Kootenai Forest District) and also in Washington State. Due to its rarity, the real habitat is still not well known, although it seems to be associated with conifer litter. The studied collections match perfectly the habitat and also the period of growth, being reported "May and June" by Paden & Tylutki (*op. cit.*) and "late spring" by Tylutki (*op. cit.*). Because of its north western situation in the USA, where spring comes later than in other areas, it's not surprising to find our "spring collections'' around June 12.

Plectania milleri is a well defined species in the genus *Plectania* Fuckel, and a perfect member of *Plectania* sect. *Plectania* due to the smooth regular ellipsoid spores (Korf, 1957). As seen above, it is easily distinguished from the allied species by macroscopic and microscopic morphology, such as the "stellate-dentate" margin of fresh black apothecia and, at the same time, smooth spores, non encrusted hairs, and above all a unique type of medullary excipulum. In fact, this latter feature seems to give to *P. milleri* an isolated position in section *Plectania*, and apparently also in the entire genus. This fact must be well investigated to point out if it deserves its own section or maybe the creation of a subsection.

Lastly, during our studies, we have considered many ancient names and we have looked at many ancient drawings but, at present, we have not found any old species that could be a synonym of *P. milleri* having priority over it.

Aknowledgements

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References

Arora D. 1986. — *Mushrooms demystified*. Berkeley, Ten speed Press, 959 p.

Calonge F.D., García A., Sanz M. & Bastardo J. 2003. — *Plectania zugazae* (Ascomycotina), especie nueva para la ciencia. Boletín *Sociedad Mycológica de Madrid*, 27: 17-20.

Carbone M. 2009. — Contributo alla conoscenza di *Sarcosoma globosum*. *Bolletino A.M.E.R.*, 77-78: 32-43.

Carbone M., Agnello C. & Baglivo A. 2009. — *Plectania mediterranea* una nuova specie dell'Italia mediterranea, con storia e circoscrizione del Genere *Plectania*. *Rivista di Micologia*, 52 (3): 245-266.

Carbone M., Agnello C. & Baglivo A. 2010. — Appunti su *Plectania rhytidia* e studio del typus di *Urnula platensis*. *Rivista di Micologia*, 53 (2): 119-135.

Castellano M.A., Smith J.E., O'Dell T., Cázares E. & Nugent S. 1999. — *Handbook to strategy 1 fungal taxa from the Northwest Forest* Plan. Gen. Tech. Rep. PNW-GTR-476. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 195 p.

Cripps C. 2009. — Snowbank fungi revisited. *Fungi*, 2 (1): 47-53.

Ellis J.B. & Holway E.W.D. 1897. — Mexican fungi. *Botanical Gazette*, 24 (1): 23-38.

Evenson V. 1997. — *Mushrooms of Colorado and the southern rocky mountains*. Denver, Denver Botanic Gardens & Denver Museum of Natural History.

Kanouse B.B. 1947. — A survey of the Discomycete flora of the Olympic National Park and adjacent areas. *Mycologia*, 39: 635-689.

Korf R.P. 1957. — Two bulgarioid genera: *Galiella* and *Plectania*. *Mycologia*, 49: 107-111.

Li L.T. & Kimbrough J.W. 1995. — Spore wall ontogeny in *Pseudoplectania nigrella* and *Plectania nannfeldttii* (Ascomycotina, Pezizales). *Canadian Journal of Botany*, 73(11): 1761-1767.

Mauer B. 1992. — *Plectania melastoma* (Sow.: Fr.) Fuckel - ein in Rheinland-Pfalz lange verschollener Ascomycet in der Westpfalz gefunden. *Rheinland-Pfälzisches Pilzjournal*, 2 (1): 28-30.

Miller O.K. Jr. 1965. — Snowbank Mushrooms in the Three Sisters Wilderness Area. *Mazama*, 47: 38-41.

Miller O.K. Jr. 1967. — Notes on Western Fungi. I. *Mycologia*, 59: 504-512.

Miller O.K & Miller H.H. 2006. — *North American Mushrooms*. A field guide to edible and inedible fungi. Falcon guide, Morris Book publishing.

Otani Y. 1973. — On *Pseudoplectania* and *Plectania* collected in Japan. *Report of Tottori Mycological Institute*, 10: 411-419.

Paden J.W. 1983. — *Sarcosomataceae* (Pezizales, *Sarcoscyphineae*). *Flora Neotropica*, 37: 1-17.

Paden J.W. & Tylutki E.E. 1969. — Idaho Discomycetes II. *Mycologia*, 61: 683-693.

Seaver F.J. 1928. — *North American cup fungi (Operculates)*. New York.

Seaver F.J. 1942. — *North American cup fungi (Operculates)*. Supplemented edition, New York.

Seaver F.J. & Shope P.F. 1930. — A mycological foray through the mountains of Colorado, Wyoming and South Dakota. *Mycologia*, 22: 1-8.

Smith A.H., Smith Vendler H. & Weber N.S. 1981. — *How to Know the Non-Gilled Mushrooms*. 2nd Edition, Pictured Key Nature.

Tylutki E.E. 1993. — *Mushrooms of Idaho and the Pacific Northwest*. Vol. I, Discomycetes. Univerity of Idaho Press.

Zhuang W.y. & Wang Z. 1998. — Sarcosomataceous discomycetes in China. *Mycotaxon*, 67: 355-364.