Urnula padeniana (Pezizales) sp. nov. and the type study of *Bulgaria mexicana*

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**Summary:** *Urnula padeniana* is described as new although it is a common northwestern American species known for years as *Sarcosoma mexicanum* or *Plectania mexicana*. A morphological in-depth study of this species is reported. The type of *Bulgaria mexicana* is also revised, and turns out to be a different species, being a member of the *Urnula campylospora* complex. The new combination *Urnula mexicana* is proposed. Color pictures of fresh and dried specimens, microscopic morphology, and a black and white plate of hymenial elements are included. Related or similar species are also discussed.

**Keywords:** Ascomycota, Sarcosomataceae, Urnula campylospora, Pseudosarcosoma latahensis, Urnula hie-malis, Sarcosoma globosum, taxonomy.

**Riassunto:** *Urnula padeniana* viene descritta come nuova sebbene rappresenti la specie nordamericana conosciuta da anni sotto il nome di *Sarcosoma mexicanum* o *Plectania mexicana*. Viene presentato un suo approfondito studio morfologico. Viene inoltre revisionato il tipo di *Bulgaria mexicana*, che è risultata essere una specie differente, essendo membro dell’*Urnula campylospora* complex. La nuova combinazione *Urnula mexicana* viene proposta. Vengono inclusi fotografie di materiale fresco e secco, quadro microscopico, ed una tavola al tratto degli elementi microscopici. Sono inoltre discusse specie vicine o simili.

**Parole chiave:** Ascomycota, Sarcosomataceae, Urnula campylospora, Pseudosarcosoma latahensis, Urnula hie-malis, Sarcosoma globosum, tassonomia.

**Résumé :** *Urnula padeniana* est décrite comme nouvelle bien qu’il s’agisse d’une espèce commune du nord-ouest américain, connue depuis des années sous le nom *Sarcosoma mexicanum* ou *Plectania mexicana*. Une étude morphologique complète est présentée. Le type de *Bulgaria mexicana* a également été révisé et fait ressortir qu’il s’agit d’un taxon différent, membre du complexe *Urnula campylospora*. La nouvelle combinaison *Urnula mexicana* est proposée. Des photographies en couleur de spécimens frais ou secs, des caractères microscopiques et une planche en noir et blanc des éléments hyméniaux sont incluses. Les espèces similaires sont également évoquées.

**Mots-clés :** Ascomycota, Sarcosomataceae, Urnula campylospora, Pseudosarcosoma latahensis, Urnula hie-malis, Sarcosoma globosum, taxonomie.

**Introduction**

From the beginning of our studies in *Sarcosomataceae* Kobayasi, we noticed many discrepancies between the *Bulgaria mexicana* Ellis & Holw. original diagnosis and the features of the common northwestern American species known as *Sarcosoma mexicanum* (Ellis & Holw.) Paden & Tylutki (=*Plectania mexicana* (Ellis & Holw.) Paden). Therefore, we thought that they could represent two different, independent species. Besides, phylogenetic studies conducted by CARBONE et al. (2013) have demonstrated that the lineage of the latter species places it in the *Urnula* clade. For that reason it became necessary to clarify the position of the epithet “mexicana”, and so *Urnula padeniana* is described as new to replace the incorrect name of the northwestern American species.

*Urnula padeniana* is a common species in the American north-west, known mainly from the studies made first by PADEN (1967), then published by PADEN & TYLUTKI (1969) and finally again by PADEN (1983). This species had been misinterpreted by SEAVER (1942, as *Bulgaria globosa*) who reported, along with a photograph, some collections from Oregon and Idaho. The first one to use the epithet “mexicana” for this species was apparently SMITH (1948, as *Bulgaria hie-malis*) who gave a “technical description” almost identical to the original diagnosis of *Bulgaria mexicana*. It has since been described, also with photographs, as *Sarcosoma mexicanum* again by SMITH et al. (1973), and many other American authors such as ARORA (1986), TYLUTKI (1993), CASTELLANO et al. (1999), PHILLIPS (2005), MILLER & MILLER (2006) and TRUDELL & AMMI RATTI (2009).

*Bulgaria mexicana* was originally described by ELLIS & HOLWAY (1897) from Mexico, Cuernavaca, with the following diagnosis: “Ob-conic stipitate, 2.5-3.5 cm across, carnose-gelatinous, becoming hard and rigid when dry, with the margin involute and much wrinkled, globaceous, olive-black : hymenium reddish-brown, cracking and showing the white substance of the ascoma: stipe central, short, stout, wrinkled (when dry) : asci cylindrical, stipitate, 8-spored, 250-270 by 15 μ : paraphyses filiform, slightly thickened above and brownish : sporeidia uniseriate allantoid, rounded at the ends, slightly curved, mostly with a large vacuole in the center, sub-hyaline, 25-34 by 8-10 μ. – Cuernavaca. Sept. Has the general appearance of *B. spongiosa* Pk., but with different sporidia”.

Although on many occasions PADEN reported to have revised the holotype of *Bulgaria mexicana*, it is clear that the general features displayed in the diagnosis, above all size, hymenial color and allan-toid spores, are better aligned with two other species: *Urnula campylospora* (Berk.) Cooke and *Plectania chilensis* (Mont.) Gamundi. The term “allantoid” derives from ancient Greek *allantoeidés* that means “sausage shaped” and, according to SNELL & DICK (1957), it is used in mycology for spores that are cylindrical and somewhat curved, with rounded ends, as exactly described by ELLIS & HOLWAY (1897). In *U. padeniana* the spores differ greatly from this definition, at most they could be regarded as slightly inequilateral. ELLIS & HOLWAY (1897) also reported an important consideration, i.e. that *Bulgaria mexi-cana* “has the general appearance of *Bulgaria spongiosa* Peck, but with different sporidia”. According to Peck’s protologue (PECK, 1881) — and to the type revision made by PADEN (1967) —, *Bulgaria spongiosa* Peck seems to be a possible synonym of *Pseudoplectania melaena* (Fr.) Sacc. [= *P. vogesiaca* (Pers.) Seaver], which due to its stipitate habit definitely resembles more the two above mentioned species than *U. padeniana*. Furthermore, PADEN (1983) stated that the original collection of *B. mexicana* was not from Cuernavaca because the northwestern American species is not tropical and thus he suggested that the type collection possibly came from a different, more temperate, part of Mexico. We never agreed with his position because there was no good reason to doubt the provenance reported by ELLIS & HOLWAY (1897). We have always believed that Paden tried to justify the presence of *U. padeniana* in a tropical area, simply because he misinterpreted the original concept of *Bulgaria mexicana*. Lastly, another striking difference that attracted our at-
tention was the phenology because *Bulgaria mexicana* was collected in September while *U. padeniana* usually fruits from spring to early summer.

All these incongruities led us to revise the type specimen of *Bulgaria mexicana* housed in the Herbarium of the New York Botanical Garden (NY). After this revision, all these doubts have been confirmed in that *Sarcosoma mexicanum* sensu Paden and other authors represents a different, still undescribed species.

Materials and methods

Microscopic characters are based on dry specimens. Three optical microscopes were used: Olympus CX41 trinocular, Olympus CH-2 binocular, and Optika B333 trinocular with plan-achromatic objectives 4x, 10x, 40x, 100x in oil immersion. 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. Spores were mainly measured from spore print.

Description

**Urnula padeniana** M. Carbone, Agnello, A.D. Parker & P. Alvarado, sp. nov. – MB 801355


Original diagnosis

Ascom blackish, massive, fleshy, rubbery, up to 9 cm wide and up to 7 cm high, context gelatinous. Spores (23–) 25–30 (–32) × 11–13 μm, elliptical to slightly subfusoid, mainly slightly inequilateral, hyaline, smooth, with 1–3 (–4) guttules; paraphyses closely septate especially toward the tips; hymenial hairs straight, not-septate; flesh divided into a brownish subhymenium of thick texture intricata, a medullary excipulum of loose, hyaline, highly gelified *textura intricata*, a brown ectal excipulum of *textura globulosa* to *subglobulosa*; external hairs mainly hyphoid, heavily encrusted by a brownish crystalloid pigment, but also olivaceous true hairs are present, although in a less percentage. Differs from *Sarcosoma globosum* in black colors and the absence of moniloid external hairs; from *Plectania* species in bigger size, more gelatinous flesh, and a different morphology of the external hairs. Holotype in Washington University Herbarium, accession number WTU-F-33051.

Etymology: We named it in honor of the American mycologist John Wilburn Paden, definitely the major researcher who treated this species.

Macroscopic characters

**Apothecium** epigeous, irregularly radically symmetrical, turbinate to discoid and deflexed in age, up to 9 cm in diameter and up to 7 cm high. **Hymenium** plane to somewhat concave with an upturned margin, brownish-black to blackish, glabrous, folded in age. **External surface** with large radial folds not reaching margin, surface tending to form cracks exposing the gelatinous interior, brownish-black, glabrous at the naked eye but finely rough if magnified, with patches of very fine grey matted tomentum visible within the folds. **Stipe** not present; overmature specimens which have lost all the watery interior could show a small central pseudostipe at their base. **Flesh** solid, highly gelatinous, translucent with a slight olive-brown tint.

**Microscopic characters**

**Spores** (23–) 25–30 (–32) × 11–13 μm, Q = 1.91–2.75, Qm = 2.32, heterogeneous in shape depending on the position of the spores inside the asci, but mainly elliptical to slightly subfuscoid, often slightly inequilateral, hyaline, smooth, with 1–3 (–4) guttules (one bigger than the others), content granular, walls thickened up to 0.8 (–1) μm. **Asci** cylindrical, operculate, inamyloid, eight-spored, 415–590 × 13–15 (–20) μm, tapering toward the base. **Paraphyses** 2.5–3 (–3.5) μm in diameter, cylindrical, anastomosing, some branched, closely septate especially in the upper part; elements can be 7.5–12 μm long but also up to 55, slightly swollen, regular or with nodules and irregular protuberances especially toward the tips; some paraphyses are affected by fourtoulism being the elements inflated up to 10 μm and constricted at the septa; in dried specimens the paraphyses are united in bundles by an amorphous extracellular amber-brown pigment. **Hymenial hairs** cylindrical, 3–4.5 μm in diameter, apex only slightly enlarged, with a sole septum at the very base; they share the same pigment as the paraphyses. **Subhymenium** of thick *textura intricata* composed by cylindrical, septate hyphae; brown at low magnifications. **Medullary excipulum** of loose *textura intricata* made up by, cylindrical, hyaline, septate hyphae, 3–5 μm in diameter, with walls on average 0.3–0.4 μm thick, immersed in a gelatinous matrix; in dried specimen some hyphae are twisted and other, especially in the lower half, are covered by subhyaline to light greenish pyramidal crystals. **Ectal excipulum** very thin, of *textura globulosa* to *subglobulosa* made up of elements spherical to elliptical and constricted in the middle, with walls up to 0.8 (–1) μm thick, brown due to both epimembranaceous and extracellularg pigmentation. **External hairs** mainly hyphoid, 3–5 μm in diameter, thinned, subhyaline but heavily encrusted by a brownish crystalloid pigment; although in a less percentage, true hair type is also present, 6–8 μm in diameter, olivaceous due to an epimembranaceous pigmentation, with smooth walls up to 0.5 μm. **Subiculum** 4–6 μm in diameter, cylindrical, septate, smooth, walls up to 1 μm thick, brown.

**Studied collections and ecology**

USA, Washington, Pend Oreille County, Roosevelt Cedar Grove, Forest Service Rd. 302, 48° 46’ 04” N, 117° 03’ 38” W, on needle duff under old growth *Thuja plicata*, at 1185 m. a.s.l., 11 June 2006, leg. et det. A.D. Parker (WTU-F-33051, holotype). Pend Oreille County, Roosevelt Cedar Grove, Forest Service Rd. 302, 48° 46’ 04” N, 117° 03’ 38” W, on needle duff under old growth *Thuja plicata*, at 1185 m. a.s.l., 30 June 2012, leg. et det. A.D. Parker (WTU-F-33050). Pend Oreille County, Upper Cedar Creek, 48° 49.711 N, 117° 29.860 W, cespitose to scattered in widely spaced groups under old growth *Thuja plicata* and *Tsuga heterophylla*, at 1416 m. a.s.l. on a north facing slope; understory was open and consisted of low shrubs and herbs. In the immediate vicinity were *Oplonapax horridus*, Gymnocarpium dryopteris, *Trillium ovatum* and *Clintonia uniflora*, 24 June 2012, leg. et det. A.D. Parker (WTU-F-33052).

Discussion

**Similar or related species**

As stated in the introduction, Paden & Tylutki (1969) rightly noted that *Seaver* (1928) first recorded *Bulgaria globosa* from Ontario (Canada) that seems to be the real *Sarcosoma globosum* but then *Seaver* (1942) reported and figured (again as *Bulgaria globosa*) some collections from Oregon and Idaho definitely representing *Urnula padeniana*. *Sarcosoma globosum*, or at least a very similar macro-morphological species, is surely present in northeastern America, as can be seen in Besse et al. (1997) and previously reported by

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1 Constricted at regular intervals so as to resemble a string of beads of a necklace.
**Urnula padeniana**

Top: Mature ascomata *in situ* (holotype).
Bottom left: Fresh ascomata (paratype). Bottom right: Section of a fresh apothecium (paratype).

Pictures: Andrew Parker

**Smith et al.** (1973). It is a very distinct species mainly for its general color, habit and above all the external moniliform hairs.

Another very similar species, present in the same geographical area as *U. padeniana*, is surely *Pseudosarcosoma latahensis* (Paden & Tylutki) M. Carbone, Agnello & P. Alvarado (≡ *Sarcosoma latahensis* Paden & Tylutki; *Plectania latahensis* (Paden & Tylutki) M. Carbone, Agnello & Baglivo), which 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 Lincoff, 1981; Tylutki, 1993). Originally invalidly described as *Plectania latahense* (Paden, 1967), then validly published as *Sarcosoma latahense* Paden & Tylutki (Paden & Tylutki, 1969), it was lastly transferred to *Plectania* by **Carbone et al.** (2009) because they thought it did not completely fit the concept of the genus *Sarcosoma* Casp. as pointed out first by **Paden** (1983), and more recently by **Carbone** (2009). **Carbone et al.** (2013) finally demonstrated that it represents an independent lineage in the family *Chorioactidaceae* Pfister.

**Urnula hiemalis** Nannf. is another blackish species that can reach up to 5 cm in width. According to **Dissing** (1981) it was erroneously recorded from Alaska by **Kempton & Wells** (1974). Anyway, it definitely can be distinguished by many macroscopic and microscopic...
Fig. 1 – Urnula padeniana. Microscopic characters of the holotype.
A: Apothecium in section; B: Spores; C: Ascus tip with spores; D: Paraphyses; E: Hymenial hair; F: External hairs.
Drawing: C. Agnello
Fig. 2 – *Urnula padeniana*. Microscopic characters of the holotype. Upper row: Ascus apical part, paraphyses, hymenial hair. Bottom row: Spores in water mount. Scale bars = 10 μm. Pictures: C. Agnello & M. Carbone.
Fig. 3 – *Urnula padeniana*. Microscopic characters of the holotype (in water mount).
A: Upper half part of the apothecium in section; B: Crystals in the lower medullary excipulum; C: Ectal excipulum; D-F: External hairs, hyphoid type; G: External hair, true hair type; H: Subiculum. Scale bars = 10 μm, except A = 100 μm. Pictures: M. Carbone.
features (see Carbóne & Agnello, 2012, 2013a) and also genetically (Carbóne et al., 2013).

At present we believe that no other “sarcosomataceous” species could be mistaken for our species because too many differences separate them.

**Revision of the holotype of *Bulgaria mexicana***

**Specimen examined:** *Bulgaria mexicana*. Cuernavaca, Mexico, Sept. 1896, legit E.W.D. Holway (NY Herbarium, barcode 01169254).

**Description:** The type specimen is in very good condition. It consists of one apothecium broken into two main parts and other smaller pieces. It is about 2.5 cm in diameter, definitely cup-shaped with a brownish to slightly orangish hymenium, inrolled margin and a dark brown, wrinkled, external surface; the flesh is very thin and whitish. A very small stipe can be seen once the apothecium is reconstructed.

In the herbarium packet there is a note by Holway: “Asci cyl. 250–270 × 15. Paraph. filiform, slightly thickened above straight brownish. Spores 25–34 × 8–10 uniseriate, hyaline” and two spores are depicted. There is also an annotation slip on which Paden had written: “*Plectania* sp. Sect. *curvatispora*; 25th Oct. 1984.”

**Microscopic characters.** *Asci* 400–450 × 17–20 μm, cylindrical, operculate, inamyloid, eight-spored, with walls up to 1.5 μm thick and a tapered, flexuous, apparently aporhynchous base. *Paraphyses* not, or only slightly, exceeding the length of the ascii, 2–3 (–3.5) μm wide, cylindrical, closely septate, sometimes anastomosing, pale brownish; some with a flexuous and lobed apex have been observed; an extracellular, brownish, amorphous pigment is present in the upper part (observed in water mounts), uniting them in bundles. *Hymenial hairs* cylindrical, straight to rarely slightly bent toward the tip, little longer than the paraphyses, 4–5 μm wide, with a simple to slightly subcapitate apex and single septum at the very base, pale brownish. *Spores* smooth, slightly curved with rounded poles to definitely allantoid (bean-shaped), av. 29–33 × 11.5–12 μm, Qm = 2.5–2.6 [some spores reach 34–37 (~42) × 13–16 μm], smooth, slightly curved with rounded poles to definitely allantoid (bean-shaped).

**Comment.** It was a big surprise to see that Paden, one year after his publication on the tropical Sarcosomataceae (Paden, 1983), annotated on the type specimen of *Bulgaria mexicana* that it represents a species of *Plectania* sect. *Curvatispora*. In fact, our revision of the *B. mexicana* type specimen confirms that this species belongs to the genus *Urnula* Fr. in the sense of Carbóne et al. (2013) (i.e. *Plectania* sect. *Curvatispora*) and precisely a species in the *Urnula campylospora* complex, as already hinted by us (see introduction). Most of the original features are so confirmed and some other microscopic ones are amended. It is worth noting that the description of *Urnula campylospora* in the sense of Pompa-Gonzáles & Cifuentes (1991, as *Plectania campylospora*) fits perfectly with *Urnula mexicana* and, at the same time, slightly differs in some ways from our data of New Zealand material of *P. campylospora* (see Carbóne & Agnello, 2013b) having the latter two types of external hairs, curved hymenial hairs and a lower Q of spores. In addition, the Mexican collection of Pompa-Gonzáles & Cifuentes (1991) comes from Guerrero State, ca. 150 km south-west of the type locality of *Bulgaria mexicana*. Unfortunately we are not able to confirm the identity of the collections cited in the check-lists of Frutis & Guzmán (1983), Díaz-Barriga et al. (1988) and Heredia (1989) as *Sarcosoma mexicanum*, due to the lack of any morphological details.

With regards to *Plectania chilensis*, although we still had no opportunity to study it in depth, in 2009 the Italian authors had a chance to observe macro- and microphotographs of a collection made by Prof. Goetz Palfner, University of Concepción (Chile). This species, although possessing quite similar colors to *Urnula mexicana*, seems to show spores less curved, mostly asymmetrical, and with a tendency to be narrower (Spegazzini, 1918, as *Ombrophila chilensis*; Le Gal, 1962, as *Sarcosoma chilensis*; Gamundi, 1971). This is a feature common also to *Urnula padeniana* and *Urnula mediterranea* (M. Carbóne, Agnello & Baglivo) M. Carbóne, Agnello & P. Alvarado, but at present we prefer not to transfer it into *Urnula* until a genetic study confirms its true position.

It is obvious that further studies are required in order to establish how many cryptic taxa actually exist in the “*U. campylospora*-complex” species recorded in the neotropics, such as the Jamaican collection studied by Paden (1983). For the reasons above, at present we prefer to keep *U. mexicana* separate from *U. campylospora* and “*Plectania* chilensis”.

Furthermore, the revision of *Urnula mexicana* confirms that the northwestern American species *Urnula padeniana* is extremely different with regard to macro- and micromorphological, ecological and phenological characters.

**Table 1** – Main distinguishing morphological characters between *U. padeniana* and *U. mexicana*

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<th><em>Urnula padeniana</em></th>
<th><em>Urnula mexicana</em></th>
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<tr>
<td>Apothecium</td>
<td>Blackish, massive, fleshy, rubbery, up to 9 cm wide and up to 7 cm high</td>
<td>Brownish, about 2.5 cm in diam., definitely cup-shaped; flesh very thin</td>
</tr>
<tr>
<td>Spores</td>
<td>(23–)25–30 (~32) × 11–13 μm, Qm = 2.32, heterogeneous in shape, but mainly elliptical to slightly subfusoid, often slightly inequilateral</td>
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</tr>
<tr>
<td>External hairs</td>
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<td>Hyphoid</td>
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Fig. 4 – *Urnula mexicana*. Holotype and its annotations. Scale bar = 1 cm. Pictures: M. Carbone.
Fig. 5 – *Urnula mexicana*. Spores from the holotype. Upper row: Spores in asci. Bottom row: free spores in water. Scale bars = 10 μm. Pictures: M. Carbone.
Fig. 6 – *Urnula mexicana*. Parts of the excipulum from the holotype (in water mount).
A: Subhymenium and upper part of medullary excipulum; B: Ectal excipulum; C-D: External hairs.
Scale bars = 10 μm. Pictures: M. Carbone.
Acknowledgements

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References


Fig. 7 — Urnula mexicana. Paraphyses and hymenial hairs from the holotype. Scale bars = 10 μm. Pictures: M. Carbone.

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