Plectania harnischii, a new North American species of the *Plectania melastoma* lineage (*Pezizales*, *Sarcosomataceae*)

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Abstract: *Plectania harnischii* is here proposed as a new species collected in the coniferous forests of the Pacific Northwest in USA. This species is characterised by its distinct morphology and genetic profile. A discussion on its affinities with the other two members of the so-called "*Plectania melastoma* clade" is presented. Colour pictures of fresh samples as well as microscopical features are provided.
Keywords: *Ascomycota*, ITS rDNA, 28S rDNA, Pacific North West, phylogeny, *Plectania melastoma*, *P. zugazae*.

Riassunto: Il nuovo nome *Plectania harnischii* viene qui proposto per una specie rinvenuta nei boschi di conifer del Pacific North West (USA). Questa specie è distinta per la sua morfologia e il suo profilo genetico. Vengono inoltre discusse le affinità con gli altri due membri del cosiddetto "*Plectania melastoma* clade". Vengono aggiunte foto a colori di esemplari freschi e di microscopia.
Parole chiave: *Ascomycota*, ITS rDNA, 28S rDNA, filogenesi, Pacific North West, *Plectania melastoma*, *P. zugazae*.

Introduction

In the phylogenetic study of Carbone *et al.* (2013) on *Sarcosomataceae*, *Plectania melastoma* (Sowerby) Fuckel was considered a single lineage of genus *Plectania* Fuckel. Later, Carbone *et al.* (2015a) confirmed that *Plectania zugazae* Calonge & Alb. García represents an independent lineage sister to *P. melastoma*, characterised by its genetic profile and lower length/width ratio (Q-value) of its ascospores. None of these works addressed the status of a deviant sample, labelled *Plectania* cf. *melastoma*, which displayed an isolated phylogenetic position sister to *P. melastoma* and *P. zugazae*.

In the present work, we further investigate this sample and others coming from the same geographical region to ascertain their most suitable taxonomic status.

Material and methods

Morphological study. — The microscopical studies were based on both fresh and dried specimens. Microscopical characters were studied in living (*) cells and tissues and rehydrated material (†) at magnifications up to 1000×. Three optical microscopes were used: Olympus CX41 trinocular, Olympus CH-2 binocular and Optika B353 trinocular with plan-achromatic objectives 10×, 40×, 60× and 100× magnifications in oil immersion. The following reagents were used: Melzer's reagent to test the amyloid response, cotton blue to check the spores surfaces, Congo red to highlight micro-structures. Water mounts were used for the observation of the pigmentation and measurements. At least 50 ascospores were measured from each apothecium.

DNA extraction, amplification and sequencing. — Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona, 1987) included 35 cycles with an annealing temperature of 54° C. The primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) were employed to amplify the internal transcribed spacer rDNA region (ITS), while LR0R and LR5 (Vilgalys & Hester, 1990; Cubeta *et al.*, 1991) were used for the 28S rDNA region (LSU). PCR products were checked in 1% agarose gels, and amplicons were sequenced with one or both PCR primers. Sequences were corrected to remove reading errors in chromatograms.

Phylogenetic analyses. — BLASTn (Altschul *et al.*, 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration public database (INSDC, Cochrane *et al.*, 2011) and UNITE (Nilsson *et al.*, 2018), which are summarised in Table 1. Sequences first were aligned in MEGA 5.0 (Tamura *et al.*, 2011) with its Clustal W application and then realigned manually as needed to establish positional homology. The final alignment included 89/518 (ITS) and 28/813 (LSU) variable sites. The aligned loci were loaded in MrBayes 3.2.6 (Ronquist *et al.*, 2012), where a Bayesian analysis was performed (partitions: ITS, LSU, two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until the average split frequencies between the simultaneous runs fell below 0.01 after 0.28 M generations. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis, 2014) using the standard search algorithm (same partitions, GTRGAMMAI model, 2000 bootstrap replications). The significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Phylogenetic results

Phylogenetic inference based on ITS and LSU sequences (Fig. 1) suggests that samples identified as *Plectania melastoma* represent at least two significantly distinct clades, one of them exclusively containing samples from North America (USA), and another one containing samples collected worldwide, including North America (Canada, Mexico, USA), temperate or montane habitats of Europe (France, Germany, Italy, Luxembourg, Portugal-Azores, Slovenia, Spain, UK) and Asia (China, Japan). On the other hand, all samples collected in Mediterranean Europe (Cyprus, Greece, Spain) belong to *P. zugazae*. Finally, an undetermined sample obtained from leaf tissue of *Taxus fuana* in an alpine region of Pakistan (KC812764, Fatima *et al.* 2016) seemed to represent a fourth independent lineage, probably an undescribed species.

Some clades displayed a significant internal structure resulting from the intraspecific genetic variability. The clade endemic to North America had a 99.7% average similarity of ITS rDNA between samples, while samples of *P. zugazae* had a 99.5% average similarity, and those of *P. melastoma* 98.5%. On the contrary, average ITS rDNA similarity between these clades was much lower: 89.2% (*P. melastoma* vs. *P. harnischii*), 93.3% (*P. melastoma* vs. *P. zugazae*) and 91.4% (*P. zugazae* vs. *P. harnischii*). The variability within these clades is similar to the average displayed by most species of fungi, while that observed between the same clades exceeds these values, resembling the distribution displayed by reproductively isolated species. Therefore, on the basis of genetic data, the three clades probably represent distinct species, and so a new name is proposed below for the undetermined one.

Table 1 – Sequences used for this study and GenBank accession numbers, in bold the newly generated. (T) means "Type".

Species	Herbarium Ref.	ITS	LSU
Ascomycota sp.	M52 XS-2012	JX298924	-
Fungal sp.	422	MT820110	-
Fungal sp.	M17 XS-2012	JX298890	-
Pezizomycetes sp.	ARIZ ML0055	KJ508269	KJ508269
Pezizomycetes sp.	ARIZ ML0098	KJ508302	KJ508302
Pezizomycetes sp.	ARIZ ML0133	KJ508330	KJ508330
Pezizomycetes sp.	ARIZ ML0136	KJ508333	KJ508333
Plectania harnischii	CBS 918.72 (as *Plectania melastoma*)	MH860629	MH872322
Plectania harnischii	**TUR-A 209455**	**MZ713185**	**MZ713200**
Plectania harnischii	**WTU-F-17145**	**MZ713188**	**MZ713203**
Plectania harnischii	**WTU-F-17179**	**MZ713187**	**MZ713202**
Plectania harnischii	**WTU-F-68864**	**MZ713186**	**MZ713201**
Plectania harnischii (T)	TUR-A 195785 (as *Plectania* cf. *melastoma*)	JX669804	JX669840
Plectania melastoma	K-M 191454	MZ159505	–
Plectania melastoma	LUX F. Lommer 20-04-2016-2	MF000911	MF000911
Plectania melastoma	K. Yoshino 18052604	LC431569	–
Plectania melastoma	**TMI-26361**	**MZ713193**	**MZ713207**
Plectania melastoma	TUR-A 195783	JX669805	JX669841
Plectania melastoma	TUR-A 195784	JX669814	JX669850
Plectania melastoma	**TUR-A 209456**	**MZ713189**	–
Plectania melastoma	**TUR-A 209457**	**MZ713192**	**MZ713206**
Plectania melastoma	**TUR-A 209458**	**MZ713190**	**MZ713204**
Plectania melastoma	**TUR-A 209459**	**MZ713191**	**MZ713205**
Plectania melastoma	**WTU-F-17169**	**MZ713194**	**MZ713208**
Plectania melastoma	**WTU-F-762**	**MZ713195**	**MZ713209**
Plectania milleri	ASR_H68_Ant	JX421749	–
Plectania milleri	SGLMf02	GQ377485	GQ377485
Plectania sp.	NFL1	KC812764	–
Plectania sp.	PA39	MG543948	–
Plectania zugazae	AVM 2086	JX669818	JX669855
Plectania zugazae	TUR-A 199784	KM610323	KM610325
Plectania zugazae	TUR-A 199785	KM610322	KM610324
Plectania zugazae	**TUR-A 204704**	**MZ713196**	**MZ713210**
Plectania zugazae	**TUR-A 209460**	**MZ713197**	**MZ713211**
Plectania zugazae (T)	AVM 1467	JX669817	JX669854
Plectania zugazae (T)	MA-F 53068	NR120217	NG042729
Sarcosomataceae sp.	45-1-8-1	KP109690	-
Uncultured fungus	AIASOILG03	JN889853	JN889853
Uncultured fungus	AIASOILG08	JN889858	JN889858
Uncultured fungus	KTF784	MG417882	-
Uncultured fungus	S264	FJ820751	-
Uncultured *Pezizomycotina*	LTSP_EUKA_P3A05	FJ553323	-

Taxonomy

Plectania harnischii M. Carbone, Agnello, A.D. Parker & P. Alvarado, *sp. nov.* – MB 841928 – Pl. 1–2.

Diagnosis: Besides its genetic profile, it differs from the very similar *Plectania melastoma* and *P. zugazae* by its thinner ascospores with a higher Q-value.

Holotype here designated: USA, Montana, Cedar Creek Trail, moist areas along the trail, under conifers as well as on decaying wood, 31 Jul. 2011, *leg*. J. Harnisch (TUR-A 195785).

Etymology: dedicated to our Canadian friend Johannes Harnisch, who first brought this species to our attention and who miraculously survived the attack of a Grizzly.

Macroscopical features

Apothecia nearly spherical at first, at maturity expanding and becoming cup-shaped, up to 1,5 (–2) cm in diam. and 0,8 (–1) cm high,

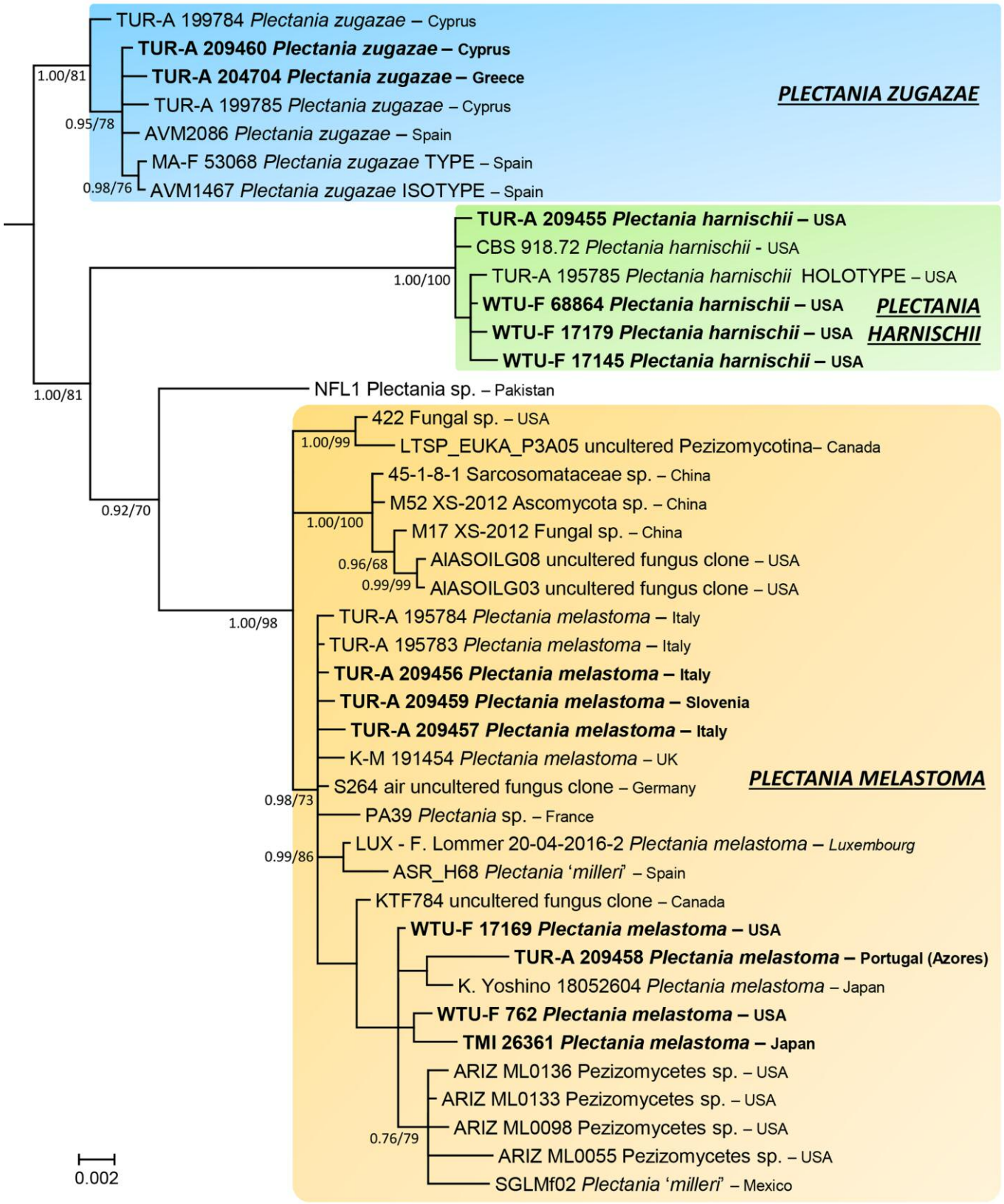

Fig. 1 – A 50% majority rule ITS-LSU consensus phylogram of *Plectania melastoma* and the most closely related species (with *Plectania zugazae* as outgroup) obtained using MrBayes from 2100 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Sequences newly generated in this study are in bold.

Plate 1 – *Plectania harnischii*. A–C: TUR-A 195785 (holotype, photo J. Harnisch); D: TUR-A 209455 (photo A. Parker); E–I: WTU-F-074325, H and I evolution of the same apothecia (photo A. Parker).

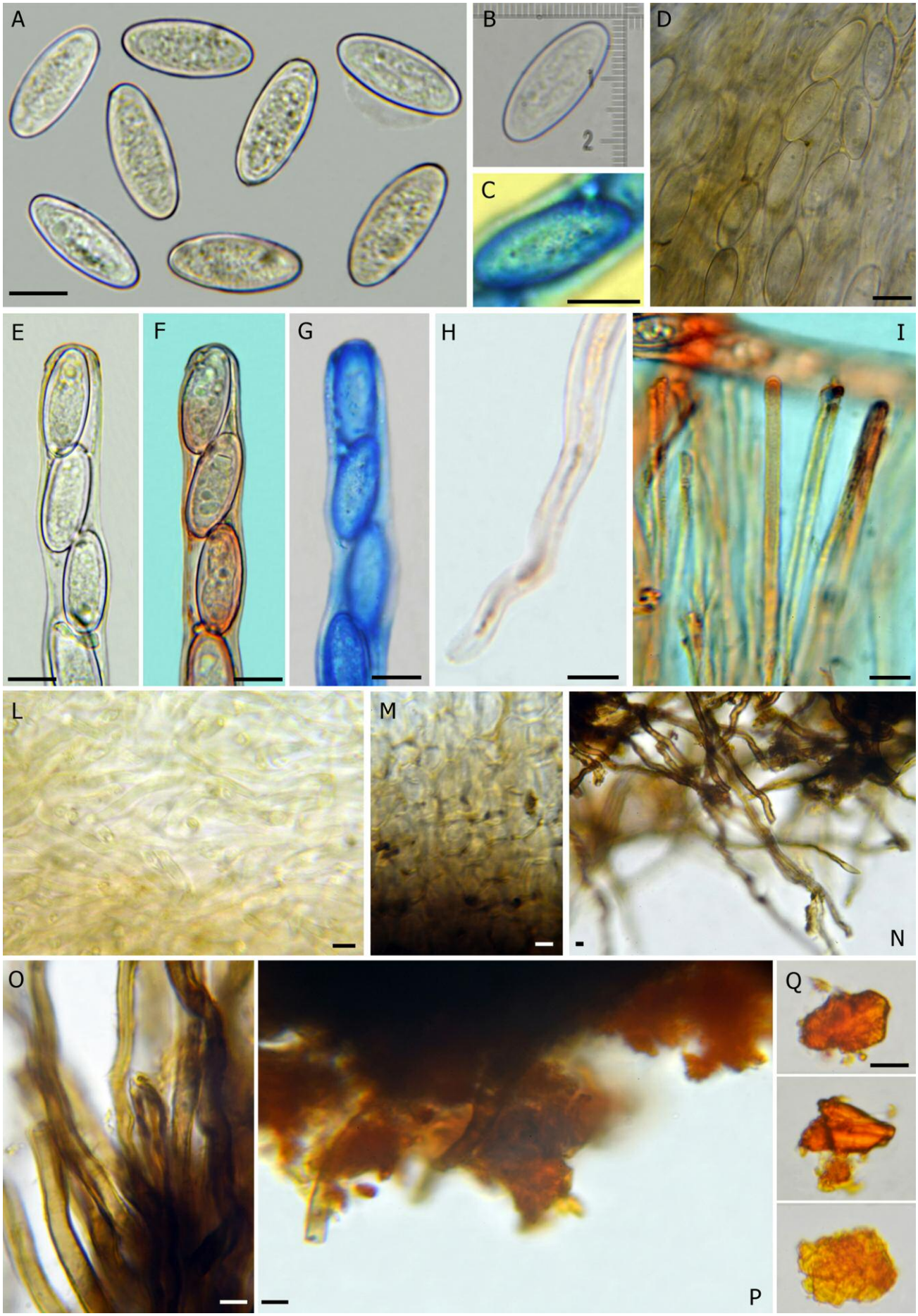

Plate 2 – *Plectania harnischii*. A, B: Ascospores in water; C: Ascospores in cotton blue; D: Ascospores inside the asci in water; E–G: Asci tips in water, Congo red and cotton blue; H: Ascus base in Congo red; I: Hymenial hairs and paraphyses in Congo red; L: Medullary excipulum in water; M: Ectal excipulum in water; N, O: External hairs in water; P, Q: Crystals on the external surface. Bars = 10 µm. Photos by C. Agnello.

attached to the substrate by a black tomentum. **Hymenium** blackish to greyish-brown. **External surface** more or less concolorous to the hymenium, rough, with often indistinct, pale orange to orange-reddish granules, more frequent at the cup margin, but also present in the entire external surface. **Margin** more or less even to occasionally serrate, with a concentration of pale orange to reddish-orange or brownish granules. **Flesh** greyish, rubbery.

Microscopic features

Asci on average †330–370 × 10–13 µm, cylindrical, operculate, inamyloid, eight-spored, with walls up to 1 µm thick and tapered, flexuous, arising from a simple septum (no crozier). **Paraphyses** not exceeding the length of asci, †1,5–2,5 µm wide, cylindrical, septate, sometimes anastomosing, branched at the base, with a simple or lobed apex, pale brownish at low magnification, presenting a gluey extracellular brown pigment in the upper part of dried specimens observed in water mounts. **Hymenial hairs** cylindrical, similar in length to paraphyses, 3–4 µm wide, with a simple apex, and a single septum at the very base, generally pale brown but sometimes also with a brownish gluey pigment in the upper part. **Ascospores** ellipsoid, †20,2–24,8 × 8,1–11,2 µm (av. 22,7 × 9,9 µm), Q = 2–2,53 (Qm = 2,35), [in A.P. 210608-1 collection, overmature ascospores were found: *19–28 × 8,5–11 µm (av. 23,5 × 9,8 µm), Q = 2–2,95 (Qm = 2,4)], hyaline, with tiny low and narrow warts (< 0,5 µm) visible only in lactic cotton blue mounts, lacking oil drops but filled with a granular content, presenting thick walls and sometimes covered with a thin gelatinous sheath, especially while still inside the ascus. **Subhymenium** arranged as a dense *textura intricata* made of cylindrical, frequently septate, brownish hyphae. **Medullary excipulum** arranged as a *textura intricata* composed of cylindrical, septate, yellowish hyphae *2,5–5 (–6) µm wide, immersed in a gelatinous matrix, arranged more or less parallel with the hymenium near the ectal excipulum, showing inflated or globose elements. **Ectal excipulum** of *textura angularis* made up of elements up to 20 µm wide, very dark brown due to the thick walls presenting an incrusting pigment. **External hairs** cylindrical, septate, †5–7(–8) µm wide, up to 600 µm long, straight, sometimes notched, smooth, originating in the deeper layers of the ectal excipulum, brown in color due to an epimembranaceous pigmentation, with walls thickened 0,5–0,8 (–1) µm. **Crystals** present among hairs and in the outer surface of the ectal excipulum, amber red or amber orange in color.

Ecology and known distribution

Growing solitary or in small groups from midwinter to spring, among mosses on woody debris of conifers. So far found in Montana, Oregon and Washington (USA), but likely reaching also California, Idaho and British Columbia, Canada (see discussion).

Studied and/or sequenced collections

***Plectania harnischii*.** USA. Montana, Cedar Creek Trail, moist areas along the trail, under conifers as well as on decaying wood, 31.VII.2011, *leg*. J. Harnisch (TUR-A 195785, holotype). Oregon, Clackamas County, west side of Trillium lake, near the shore in a mixed conifer forest, 30.V.2015, *leg*. A.D. Parker (TUR-A 209455, immature!). Washington, Pend Oreille County, State Route 31, ~9 miles north of Metaline Falls, N 48.958°, W 117.32°, 762 m a.s.l., on soil/moss under mixed conifers, *Tsuga heterophylla*, *Thuja plicata*, *Abies grandis*, *Pinus monticola*, 06.VIII.2021, *leg*. A. Parker (WTU-F-074325). Washington, Skamania County, Gifford Pinchot National Forest, Road 42, in gravel on shoulder of road in mixed conifers and *Alnus*, 01.VII.1999, *leg*. J. Lindgren (WTU-F-17179). Washington, Skamania County, Gifford Pinchot National Forest, Cowlitz Valley Ranger District, Spur 022 off FR 77, on mossy soil in mixed conifer forest with *Pseudotsuga menziesii*, *Tsuga heterophylla*, *Abies* sp. and *Thuja plicata*, 07.VI.2016, *leg*. S. Trudell & L. Stingley (WTU-F-068864, not perfectly mature). Washington, Whatcom County, East Creek Trail, Forest Service n. 756, 860 m a.s.l., under *Picea engelmanii*, *Tsuga* and *Abies amabilis*, 6.V.1999, *leg*. M. T. Seidl & J.F. Ammirati (WTU-F-17145, immature!).

Plectania melastoma. Italy. Emilia-Romagna, Parma, Albareto, roadside in a moist spot under *Abies alba* with some *Fagus sylvatica* and *Castanea sativa*, 650 m a.s.l., 24.III.2016, *leg*. M. Bianchi (TUR-A 209457). Liguria, Genova, Davagna, Loc. Piancarnese, among moss under *Castanea sativa*, 20.V.2018, *leg*. M. Carbone (TUR-A 209456). Puglia, Brindisi, Bosco Preti, on dead wood of *Erica arborea*, 54 m a.s.l., 21.IV.2014, *leg*. C. Agnello (AMB 18755). *Ibidem*, 16.III.2017 (AMB 18756). Japan. Kyoto Prefecture, Funai-gun, Kyotanba-cho, on dead *Cryptomeria japonica* sticks and needle litter on the floor of a *C. japonica* artificial forest, 1.V.2017, *leg*. T. Nakanishi, *det*. E. Nagasawa (TMI-26361). Portugal. Azores archipelago, Terceira island, Lagoa do Negro, on dead wood of *Juniperus brevifolia*, 11.VII.2015, *leg*. E. Campo (TUR-A 209458). Slovenia. Središče ob Dravi, Šalovci, in *Picea* and *Abies* forest, 28.V.2020, *leg*. E. Zupan (TUR-A 209459). USA. Washington, King County, Saddle Swamp, on moss covered

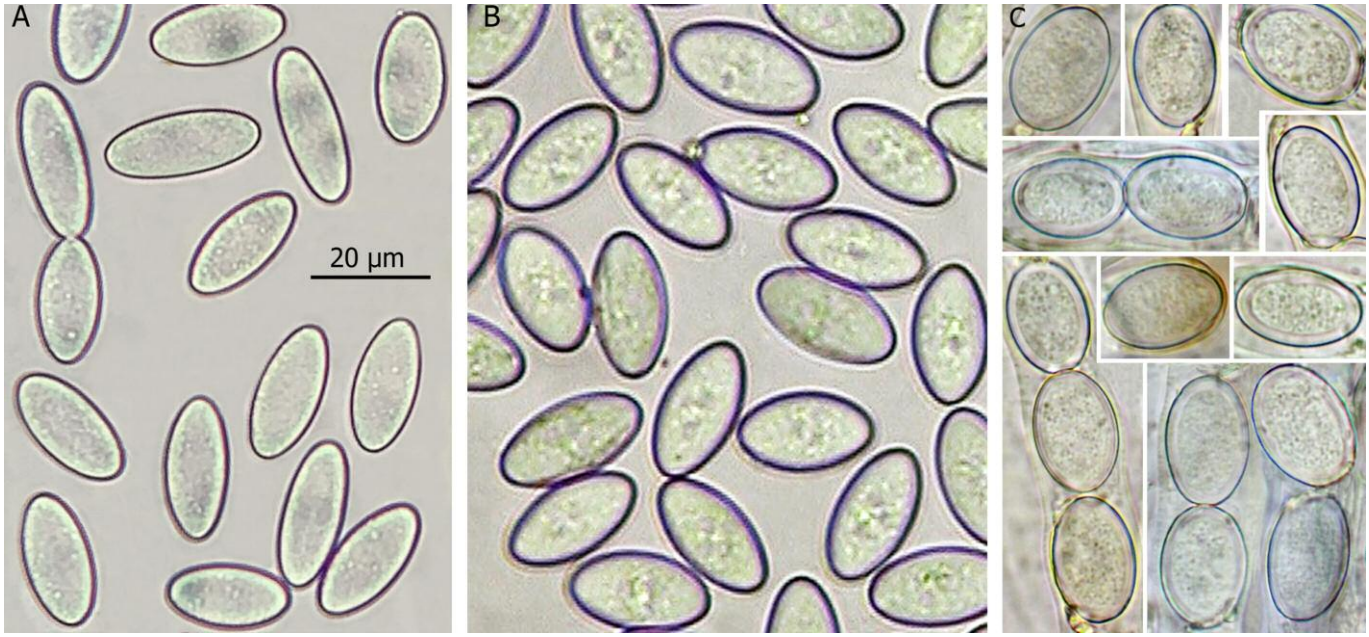

Plate 3 – Comparison of the ascospores. A: *Plectania harnischii* (A. Parker); B: *Plectania melastoma* (C. Agnello); C: *Plectania zugazae* (M. Carbone). Scale bar valid for A, B and C.

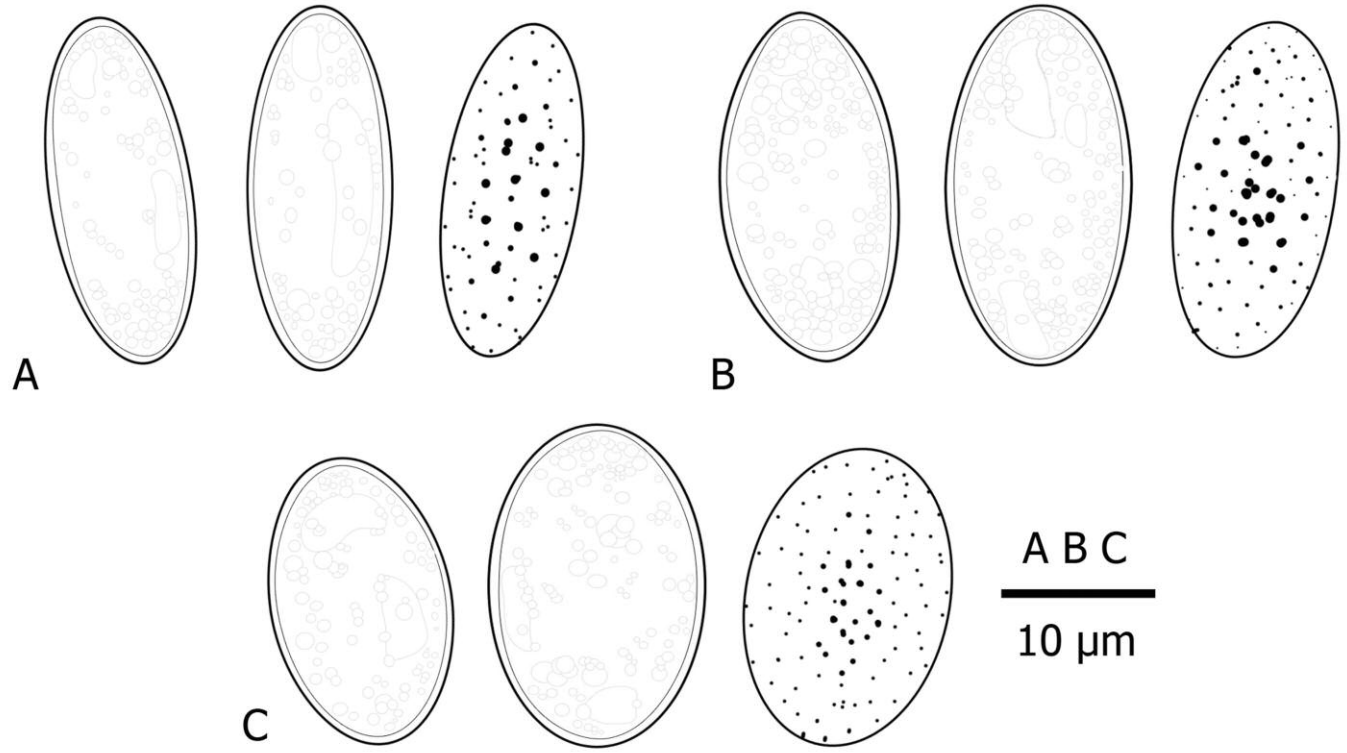

Plate 4 – Comparison among the ascospores. A: *Plectania harnischii*; B: *Plectania melastoma*; C: *Plectania zugazae*. Drawing by C. Agnello.

downed *Alnus*, 16.V.1997, *leg*. P. B. Matheny (WTU-F-000762). Washington, Pacific County, Bone river upland, on needles, 15.IV.2000, unknown collector (WTU-F-17169, not fully mature).

Plectania zugazae. Cyprus, Paphos district, Pera Vasa, ca 550 m a.s.l., in open *Pinus brutia* forest among mosses, 08.II.2015, *leg*. M. Loizides (ML5128PZ, TUR-A 209460). Greece. Attica, Nea Makri, in forest with *Pinus halepensis*, *Cistus monspeliensis* and *Erica arborea*, 06.III.2014, *leg*. V. Kaounas (TUR-A 204704).

Discussion

Synonyms of *Plectania melastoma* have been treated in detail by Agnello & Carbone (2012). No distinct names for the American collections seem to exist but *Plectania rhizomorpha* (Ellis & Everh.) Sacc. [≡ *Peziza rhizomorpha* Ellis & Everh., whose original collections were found in Louisiana, USA] was considered a synonym of *P. melastoma* first by Seaver (1928) and later by Paden (1983). *Peziza rhizomorpha* was described upon samples growing on *Rhizomorpha* with the following description: "Turbinate, with a thick stipe-like base, carnose, deeply sulcate outside and covered with a thin coat of tomentum, slaty-black throughout, 4–6 mm. diam., margin strongly incurved, substrigose-tomentose at base. Asci cylindrical, about 200 × 10 micr., with filiform olivaceous somewhat branched paraphyses, which are scarcely thickened at the tips. Sporidia uniseriate, elliptical about 20 × 10 micr. granular. Allied to *P. melastoma* Sow. & *P. hirtipes* Cke." (Ellis & Everhart, 1888). The original description did not mention orangish or reddish colors and these seem absent also from the type specimen (https://mycoportal.org/portal/collections/individual/index.php?occid=7587368&clid=0). The type collection has the typical and unmistakable habit of *Plectania rhytidia* (Berk.) Nannf. & Korf with the vertical furrows running along the external surface (see for example Carbone *et al*., 2010).

Today we know that Seaver's concept of *Plectania melastoma* was definitely heterogeneous. In fact, he listed both *Peziza hirtipes* Cooke and *Sarcosoma cyttarioides* Rehm (*in* Durand, 1903) as synonyms of *P. melastoma* (Seaver, 1928, 1942). Regarding *P. hirtipes*, Carbone *et al.* (2015b) agreed with Rifai's proposal (Rifai, 1968) to consider it a synonym of *Galiella rufa* (Schwein.) Nannf. & Korf. Cooke's plate and description (Cooke, 1876), as well the English and Latin description (Cooke, 1875a, 1875b), support this view. Regarding *Sarcosoma cyttarioides*, the ITS sequences we obtained from original collections (CUP-A-012278b and CUP-A-012281) confirm it belongs to the clade of *Plectania rhytidia* (results not shown here). Lastly, Carbone *et al.* (2012) also suggested that Seaver's concept of *P. melastoma* hid a different species now known as *Donadinia seaveri* (M. Carbone, Agnello & LaGreca) M. Carbone, Agnello & P. Alvarado (see also Pfister & LoBuglio, 2018).

Plectania japonica (Berk. & M.A. Curtis) Cooke was considered a synonym of *P. melastoma* by Pfister (1977). This species was described from Japan as *Peziza japonica* Berk. & M.A. Curtis (Berkeley & Curtis, 1860) with a very short diagnosis: "*fusco-atra; cupulis congestis subpedicellatis plicato-rugosis, e floccis brunneis in fasciculum unitis oriundis*. On roots, Japan. Allied to *P. melaena*". In this study, the Japanese collection TMI-26361 (Nagasawa & Nakanishi, 2017) nests inside the "*melastoma*" clade, in agreement with its microscopical characters. This seems to support the synonymy proposed by Pfister (*op. cit*.) and discourages its use for *Plectania harnischii*.

Agnello & Carbone (2012) overlooked *Bulgaria sydowii* Henn. in their list of synonyms of *Plectania melastoma*. *Bulgaria sydowii* was described by Hennings (1898) from specimens growing on *Ericaceae* in Germany, whose phenology and morphology point undoubtedly to *Plectania melastoma*.

According to our results, ascospores of *Plectania harnischii* usually reach 10 µm in width and never exceed 11 µm, so they are the thinnest (with the highest Q ratio) if compared to the much larger, 12,5–15 µm of *Plectania zugazae* (see Calonge *et al*., 2003; Carbone *et al*., 2015a) and to those of *P. melastoma* which are frequently 10–12,5 µm (see for example: Glejdura *et al*., 2011; Agnello & Carbone, 2012; Nagasawa & Nakanishi, 2017). This observation seems consistent with the measures reported by many American mycologists [21–24 × 8,5–10 µm (Paden, 1967); 21–24 × 8–10 µm (Tylutki, 1993); 20–28 × 9–11 µm (Bessette *et al*., 1997); 21–24 × 8–10 µm (Castellano *et al*., 2003); 21–24 × 8–10 (Desjardin *et al*., 2015); 23–28 × 10–11 µm (Siegel & Schwarz, 2016)]. The wide range of spore sizes reported by

Plate 5 – *Plectania melastoma*. A: TUR-A 209456 (M. Carbone); B: TUR-A 209457 (M. Bianchi); C: TUR-A 209458 (E. Campo); D: TUR-A 209459 (E. Zupan); E: AMB 18755 (C. Agnello); F: AMB 18756 (C. Agnello). ***Plectania zugazae*.** G: TUR-A 209460 (M. Loizides); H: TUR-A 204704 (V. Kaounas).

Amendment of the key to *Plectania* in Carbone *et al.* (2015a)

1a. Ascospores inequilateral, transversally striated and furrowed on a side, apothecia black, vertically wrinkled on the external surface ..... ***Plectania rhytidia*** [= *P. platensis*]
1b. Mature ascospores very minutely verrucose (Cotton Blue, 100 × in oil immersion) **2**
1c. Mature ascospores smooth **3**

2a. Apothecia external surface and margin covered by orange granules, mature spores 8–10 (–11) µm wide; North America ..... ***P. harnischii***
2b. Apothecia external surface and margin covered by orange granules, mature spores 10–12 (–12.5) µm wide; Europe, North America, Japan ..... ***P. melastoma***
2c. Apothecia external surface covered or not by orange or brownish granules often restricted at the margin, mature spores > 12 µm wide; Spain, Greece, Cyprus ..... ***P. zugazae***

3a. Ascospores 22–31 × (8–) 9–11 µm, dark hyphae running from the external layer into the medullary excipulum; North Western America ..... ***P. milleri***
3b. Ascospores 20.5–29.5 × 9–13 (15) µm (Le Gal, 1958); 19.4–28 × 7.4–11.8 µm (Carbone *et al.*, 2011b), no dark hyphae running in the flesh; so far known from Morocco, Greece and Spain ..... ***P. megalocrater***

Beug *et al.* (2014) could be due to the presence of both *P. melastoma* and *P. harnischii* among the samples studied by them. Arora (1986) treating *P. melastoma* reports: "Our oak-loving version usually lacks visible granules, but orange granules can be seen under the microscope". However, the picture published recalls *Plectania rhytidia* very much more than *P. melastoma*, and this could justify the wide range of spore widths, being 8–12 µm.

Plectania melastoma was described from material found in the United Kingdom (Northumberland) growing on *Erica vulgaris* (Sowerby, 1799). Although it is a common species in Europe, a formal epitypification is still needed to clarify its concept both microscopically and genetically. According to our phylogenetic results, the British collection K(M) 191454 definitely belongs to the *P. melastoma* s. str. clade and so it is here regarded as a topotype representing this species. British samples housed in K are being studied in order to ascertain if they are mature enough to select an epitype among them.

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Authors' contributions

Matteo Carbone was responsible for the study conception. Morphological analyses were performed by the authors on their collections. Molecular analyses and all the phylogenetic chapters and notes were performed by P. Alvarado (ALVALAB). Macroscopic plates were assembled by M. Carbone and microscopic ones by C. Agnello. The first draft of the manuscript was written by M. Carbone and was subsequently updated by all authors. All authors read and approved the final manuscript.

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