Xylaria violaceorosea sp. nov. (*Xylariaceae*), a distinctive species discovered in Spain

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Summary: *Xylaria violaceorosea* is described as a new species based on the combination of distinctive macroscopic and microscopic characters. It is a lignicolous *Xylaria* with stromata roughly recalling those of *X. hypoxylon* but with a purplish pink outer layer that yields olivaceous yellow pigments in 10% KOH and relatively large ascospores provided with gelatinous secondary appendages.
Keywords: Ascomycota, Asturias, Cuevas de Andina, taxonomy, *Xylariales*.

Resumen: Se describe *Xylaria violaceorosea* como nueva especie en base a sus peculiares caracteres macro y microscópicos. Esta *Xylaria* lignícola se caracteriza por formar estromas rugosos que recuerdan vagamente los de *Xylaria hypoxylon*, con un estrato externo con tonalidades sonrosadas o purpúreas que desprende pigmentos de color oliváceo o amarillo en KOH al 10% y por sus ascósporas relativamente grandes provistas de apéndices gelatinosos secundarios.
Palabras clave: Ascomycota, Asturias, Cuevas de Andina, taxonomia, *Xylariales*.

Résumé: *Xylaria violaceorosea* est décrite comme une espèce nouvelle pour ses caractères macroscopiques et microscopiques remarquables. C'est une espèce lignicole dont les stromas rappellent de loin ceux de *X. hypoxylon* mais qui s'en distinguent par un revêtement rose-pourpre qui libère un pigment jaune olivacé dans la potasse à 10 % et des ascospores relativement grandes pourvues d'appendices secondaires gélatineux.
Mots-clés: Ascomycota, Asturias, Cuevas de Andina, taxinomie, *Xylariales*.

Introduction

Xylaria Hill ex Schrank is the largest genus of the family *Xylariaceae* Tul. & C. Tul., with over 700 binomials listed in Index Fungorum. It is widespread and cosmopolitan but particularly well represented in the tropics. The whole genus is taxonomically poorly understood owing to a high number of species based on immature or inadequately described material and the difficulty to find stable and discriminant morphological characters allowing a safe segregation of species. The taxonomic tangle lying under the name *Xylaria* was highlighted by the phylogenetic work of Hsieh *et al.* (2010) who showed that the genus itself is paraphyletic. As a result a world monograph is still wanting, which does not facilitate the creation of new taxa without revising many type specimens of possible earlier synonyms.

Although on a smaller scale, this likewise applies to European species that feature four widespread and well-known species, i.e., *X. carpophila* (Pers.: Fr.) Fr., *X. hypoxylon* (L.: Fr.) Grev., the type species (Peršoh *et al.*, 2009; Stadler *et al.*, 2014), *X. longipes* Nitschke and *X. polymorpha* (Pers.: Fr.) Grev., along with over twenty further species either recently described (Fournier *et al.*, 2011) or rarely recorded or still ill-defined. In this context, the delimitation of new species of *Xylaria* is most often challenging unless very distinctive characters allow a safe characterization.

A *Xylaria* with a striking purplish pink surface was encountered by one of us (A. R.) in Asturias (Spain) and two subsequent collections of immature stromata were made in Navarra that prompted us to study it thoroughly and to recognize it as a hitherto undescribed species.

Material and methods

The observations were carried out on dry material. Measurements of asci and ascospores were made in water and ascospores measurements processed with the free software Piximetre 5.2 (http://ach.log.free.fr/Piximetre/). In the formula given by this software N represents the number of ascospores measured, Me the mean values of length × width, Q the ratio length/width and Qe its mean value.

Asci were mounted in chlorazol black to take photomicrographs and ascal apical apparati were tested for amyloidity in Melzer's reagent. Ascospores were mounted in aqueous nigrosin or dilute India ink to show the secondary appendages.

The pigments released by the outer stromatal layer were observed through the stereomicroscope by adding a small fragment of this tissue to a drop of 10% KOH or aqueous NH_3 on a glass slide lying on a white sheet of paper and recorded within one minute.

Photomacrographs were taken with a Nikon Coolpix 995 digital camera either directly mounted on a stand or, for higher magnifications, through the eyepiece of an Olympus SZ60 stereomicroscope, by the means of a 30 mm diameter adapter. Photomicrographs were taken with the same camera mounted on the trinocular port of a Leitz Orthoplan microscope. The digitalised photographs were processed with Adobe Photoshop Elements 10 and the plates assembled with the same software.

The holotype material was deposited in University of Lille (LIP) and a duplicate is kept in the personal herbaria of JF and ERD. Initials ERD, JBJ and JF with herbarium numbers refer to Enrique Rubio, Javier Balda and Jacques Fournier respectively.

Taxonomy

Xylaria violaceorosea J. Fourn., A. Román, J. Balda & E. Rubio sp. nov. — MB 808092

Plates 1-2

Holotype: Spain: Asturias, La Andina, cuevas de Andina (El Franco), 43º 29.580 N 6º 49.826 W, 29T PJ 7540 1785 (UTM coordinates), alt. 100 m asl, on dead corticated twigs and branchlets of *Castanea sativa* 0,8–1,5 cm diam, 22 Jan. 2010, *leg*. A. Román, communicated to JF by E. Rubio, ERD-5046 (LIP).

Diagnosis: *Xylaria violaceorosea* differs from other known lignicolous species in the genus by the combination of purplish pink stromata and ascospores averaging 16 × 6 µm with persistent mucilaginous secondary appendages.

Etymology: the epithet *violaceorosea* refers to the purplish pink outermost stromatal layer.

Stromata first entirely white at asexual state (A, centre) gradually turning purplish pink from the base upon maturation (C); mature stromata (A, D-K) upright, simple, rarely fasciculate on a long rooting stipe (C), scattered to clustered in small groups, 7–52 mm total height, the fertile head 6–40 mm high × 1,5–3 mm diam, cylindrical to slightly flattened in places, typically with a mucronate sterile apex, rarely apically ramified (C), sometimes with a rounded apex especially on small stunted sessile stromata; stromatal surface conspicuously nodulose with deep wrinkles and perithecial contours slightly to conspicuously exposed, with a pink to purplish pink peeling outer layer somewhat powdery and fragile, splitting into elongated strips (H-J), vanishing with time; aged specimens dark grey to blackish with a faint purplish tone in places (E, G); the coloured granules present in the peeling outer layer are vinaceous when observed in water and turn violet blue when 10% KOH is added to the slide; when added to a drop of 10% KOH or aqueous NH3, a fragment of the peeling outer layer yields olivaceous yellow pigments within one minute (B); crust leathery ca. 40 µm thick, black; interior fibrous to cheesy, solid, white with a yellowish core (F, K); the stipes ill-defined, irregularly cylindrical to strap-like, at times much reduced, occasionally long rooting when originating from buried wood, concolorous or turning blackish, puckered, glabrous, hardly swollen at base. **Perithecia** immersed, their contours hardly to partly exposed, subglobose, 0.6–0.7 mm diam (K). **Ostioles** conic-papillate, black, ca. 80 µm diam (H-J).

Asci cylindrical, 200–230 µm total length, spore-bearing parts 115–130 × 8-9 µm, stipes 80–110 µm long, with eight uniseriate ascospores (L), with apical apparatus tubular with a rim at upper end, 2.5–4 × 2.2–2.5 µm, bluing in Melzer's reagent (M). **Paraphyses** filiform, copious, embedded in mucilage. **Ascospores** (14,1–)15,8–16,3 (–17,9) × (5–) 5,9–6,1 (–7) µm ; Q = (2,1–) 2,6–2,8 (–3,3) ; N = 60; Me = 16 × 6 µm ; Qe = 2,7, fusiform slightly inequilateral with narrowly rounded ends (N), the lower end with a fugacious hyaline cellular appendage visible on hyaline immature ascospores (Q, arrowhead), at times still present on pigmented ascospores (M, S, arrowhead), often mucronate when pigmented (O), dark olive brown when fresh, turning dark brown, smooth, with a mucilaginous sheath stained in aqueous nigrosin (Q, R) and visible in India ink (S-U), forming two persistent pads at both ends connected by a thin layer on the less convex side that gradually disappears with maturation, with a conspicuous straight germ slit spore-length on the less convex side, parallel to the sides (P) or slightly oblique and/or undulate (U).

Anamorph unknown, the white immature stromata were sterile.

Other specimens examined: Navarra, Olagüe, 30 T XN 14 56 (UTM coordinates), alt. 550 m asl, in a *Quercus* woods lining a small brook, on unidentified branches in a pile, 17 Jan. 2010, *leg*. Javier Balda, JBJ 01710 (immature); same location, 24 Nov. 2010, on a branch of *Quercus* sp., *leg*. Javier Balda, JBJ 024110 (immature).

Discussion: This *Xylaria* is very distinctive in the purplish pink colour of the peeling outer layer and the secondary appendages of ascospores. Such a colour is unknown in temperate species of *Xylaria* (Ju *et al.*, 2009; Læssøe, 1987, 1992, 1993; Rogers, 1983, 1984; Rogers & Callan, 1986; Rogers & Ju, 1998) but has been reported for a few tropical species listed below and briefly commented on.

Xylaria ianthino-velutina Mont. : Fr. is known to have purplish stromata, at least when young, but this is due to a dense purplish brown tomentum, not an amorphous outer layer and it lacks the rosy colour encountered in *X. violaceorosea*; moreover it is a tropical taxon occurring on woody pods and its ascospores are 9–12 × 3.5–5 µm (Rogers, 1979). Interestingly, ascospores of *X. ianthino-velutina* likewise bear hyaline secondary appendages on each end (Ju & Rogers, 1999).

According to its original description from Paraguay, *X. violaceo-pannosa* Starb. (Starbäck, 1901) features dark violet to blackish stromata that are flattened-clavate and possess a verruculose-roughened surface like species referable to *X. polymorpha* (Pers.: Fr.) Grev. and its tropical relatives. In addition to this difference with *X. violaceorosea* in stromatal morphology, ascospores of *X. violaceo-pannosa* are reported as 10–11 × 5–5.5 µm, thus much smaller. Moreover, Dr. Yu-Ming Ju who studied the type of *X. violaceo-pannosa* stated that it is a later synonym of *X. holmbergii* Speg. (pers. comm.), a taxon belonging to the *X. corniformis* (Fr.) Fr. complex, differing from other members of this complex by the short germ slits of ascospores (Ju *et al.* , 2009).

Xylaria moliwensis Læssøe (Læssøe & Cheek, 2002) is a terrestrial taxon known from Cameroon, with a pinkish squamulose coating on the stromata, but the fertile heads are clavate nodulose and its ascospores are much larger (av. 36.2 × 8.8 µm) than those of *X. violaceorosea* and lack mucilaginous secondary appendages.

Our attention was drawn by Dr. Yu-Ming Ju (pers. comm.) on a species with pinkish stromata called *X. rosea* Beeli, collected in Congo (Beeli, 1926). Based on the original description and drawing it appears that the stromata of *X. rosea* are cylindric-clavate with broadly rounded apices, simple to branched and at conidial state. It is even noted that the conidia were pink in mass, forming a pink dust on the substrate. It can therefore be assumed that the conidia accounted for the pink colour, unlike in *X. violaceorosea*. Dr. Yu-Ming Ju, after examination of the type specimen of *X. rosea*, stated that the stromata were immature, making any further comparison impossible (pers. comm.).

Finally an unnamed *Xylaria* still under investigation, known from Ecuador (Læssøe, pers. comm.), French Guiana (Ju, pers. comm.), Panama and West Indies, also features purplish stromata with whitish apices when young. It deviates from *X. violaceorosea* in that its stromata vary from clavate with rounded fertile apices to cylindric with faintly mucronate apices, they are hard-textured with a thick carbonaceous crust and unexposed perithecial contours, and its ascospores average 10 × 4 µm and lack secondary appendages.

The presence of secondary appendages on ascospores encountered in *X. violaceorosea* deserves special attention since it is unknown in temperate lignicolous species and fairly rarely occurs in tropical lignicolous species. In contrast it is not uncommon in tropical foliicolous or fruit-inhabiting species but typically consists of two more or less developed mucilaginous pads that are not united by a lateral "bridge" on the less convex side of ascospores like in *X. violaceorosea*. This configuration recalls what is encountered in species of *Rosellinia* De Not. like *R. britannica* L.E. Petrini, O. Petrini & S.M. Francis and *R. necatrix* Berl. ex Prill. and their relatives, and thus its occurrence in a *Xylaria* makes this character distinctive.

Chemotaxonomy

The chemotaxonomic approach based on stromatal pigments released in 10% KOH proved highly effective when applied to the *Xylariaceae* and helped resolve taxonomic issues in *Hypoxylon* Bull. (Ju & Rogers, 1996) and in *Daldinia* Ces. & De Not. (Ju *et al.*, 1997). Further progress was made by the use of High Performance Liquid Chromatography (HPLC), allowing a characterization of molecules involved in the chemical reactions (Stadler *et al.*, 2004, Stadler & Fournier, 2006, Stadler *et al.*, 2014) but those results were largely restricted to the subfamily *Hypoxyloideae*.

The first report of stromatal pigments released by the action of a chemical occurring in a *Xylaria* species was that of Gunawan *et al.* (1990). These authors observed that fragments of stromata of *X. polymorpha* released a purple pigment in 25% NH_3 and they gave the name xylaral to the compound responsible for this reaction. The presence of xylaral was further investigated in the *Xylarioideae* and appeared restricted to a small number of species including the relatives of *X. telfairii* (Berk.) Fr. along with *X. aenea* Mont., *X. mesenterica* (A. Möller) M. Stadler, Læssøe & J. Fourn., *X. polymorpha* and *X. scruposa* (Fr.) Fr., and to a lesser extent in *Stilbohypoxylon quisquiliarum* (Mont.) J. D. Rogers & Y.-M. Ju and *Nemania diffusa* (Sowerby) S. F. Gray (Stadler *et al.*, 2008). Aside from the purple or rather vinaceous pigment obtained in aqueous NH_3, the xylaral yields olivaceous yellow pigments in 10% KOH. The coloured reactions are easily observable in species related to *X. telfairii* but are very faint or even absent in *X. aenea*, *X. polymorpha* and *X. scruposa* where xylaral can only be detected by HPLC. The stromata of *X. badia* Pat., a tropical bambusicolous species, feature colours in the same range as *X. telfairii* but this species deviates in that its pigments vary from

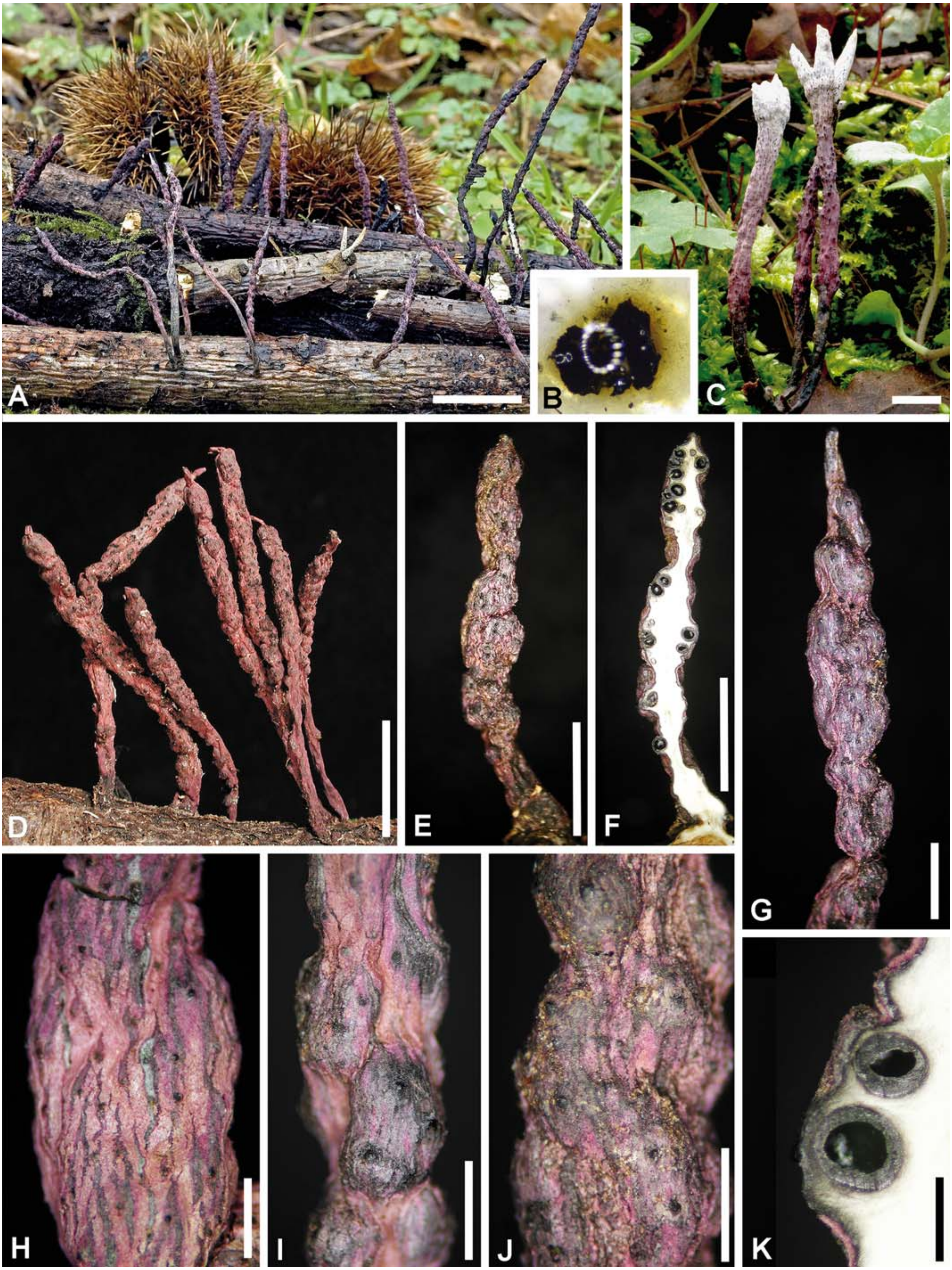

Plate 1 – *Xylaria violaceorosea*

A, B, D-K: holotype ERD-5046; C: JBJ 01710. A: Fresh stromata *in situ* (photo E. Rubio); B: yellowish pigments released in 10% KOH; C: immature stromata (photo J. Balda); D, E,G: mature stromata with mucronate sterile apices; F: mature stroma in vertical section; H-J: stromatal surface in close-up; K: vertical section of a stroma showing perithecia lying under the black leathery crust and the outermost pink layer. Scale bars: A = 2 cm; C, D = 1 cm; E, F = 5 mm; G = 2 mm; H-J = 1 mm; K = 0.5 mm.

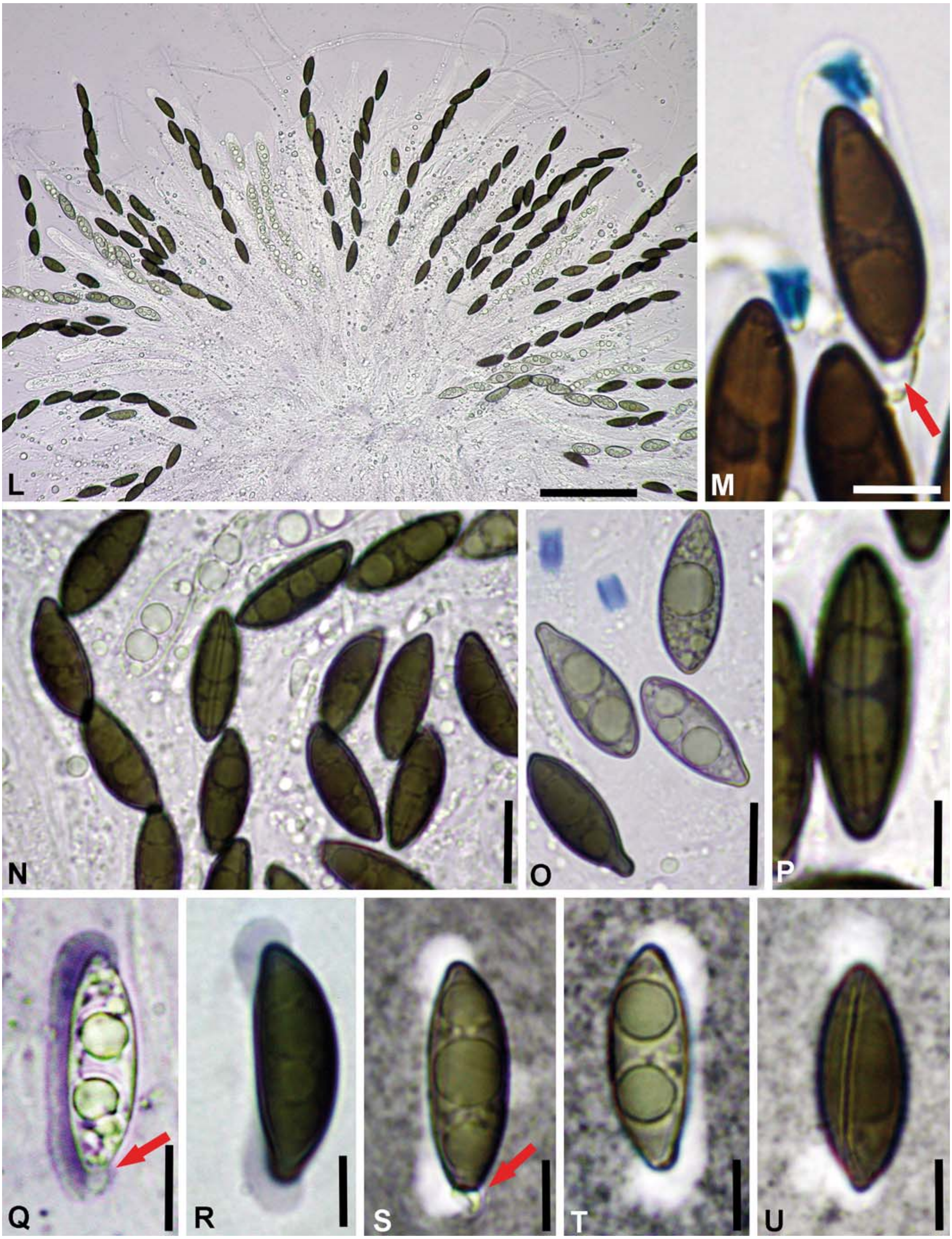

Plate 2 – *Xylaria violaceorosea*. Microscopic characters of holotype ERD-5046

L: Asci and paraphyses in chlorazol black; M: apical apparati in Melzer's reagent; N: ascospores in water; O: immature and mature ascospores with mucronate lower ends (in Melzer's reagent); P: germ slit (in water); Q: hyaline immature ascospore in aqueous nigrosin, showing the partial mucilaginous sheath and the cellular appendage; R: mature ascospore in aqueous nigrosin, showing the mucilaginous appendages on both ends; S-U: mature ascospores in dilute India ink, showing the mucilaginous appendages, with a slightly oblique germ slit in U. Scale bars: L = 50 μm; M = 5 μm; N, O = 10 μm; P-U = 5 μm.

amber in aqueous NH_3 to olivaceous yellow in 10% KOH. HPLC revealed that stromata of *X. badia* do not contain xylaral but different unknown compounds (Stadler *et al.*, 2008).

Xylaria violaceorosea likewise yields olivaceous yellow pigments in 10% KOH and aqueous NH_3 and like *X. badia* its stromata do not contain xylaral but unknown compounds (Stadler, unpublished results). This unique character among temperate species of *Xylaria* is a further discriminating feature that clearly sets *X. violaceorosea* apart from its relatives.

Distribution, ecology and chorology.

The fact that such a distinctive species that can be readily recognized in the field was so far unknown to science suggests its rare occurrence. The causes of rarity may be various but most often rare species have very specific ecological requirements that are only met occasionally.

The site of Cuevas de Andina in Asturias, from which *X. violaceorosea* was collected for the first time, is fairly peculiar in being located in a karstic depression under both oceanic and Mediterranean influence. The limestone soil is shaped into sinkholes, abrupt peaks and caves, associated with karst aquifers and disappearing streams. The forest cover is dense with upper parts of the site colonized by *Laurus nobilis* and *Castanea sativa*, at times by *Arbutus unedo*, while *Fraxinus excelsior*, *Corylus avellana*, *Ilex aquifolium*, *Taxus baccata* and *Quercus* sp. are encountered at lower altitudes. When it is combined with mild temperatures, this type of habitat usually is highly favourable to saproxylic fungi and especially *Xylariaceae* by the occurrence of shadowy micro-habitats characterized by semi-permanent humidity.

In terms of climate the second collection site in Navarra is similar though predominantly oceanic and at higher altitude. One can speculate from these two records that *X. violaceorosea* might be present along the whole Cantabrian range and further records should help define more accurately its ecology.

It is noteworthy that all the material studied was collected in winter and the holotype material was consisting of immature stromata at asexual state mixed with hardly mature and mature stromata. This configuration is reminiscent of what is encountered in *X. hypoxylon*, in contrast to most other temperate species that occur at asexual state in spring and gradually mature until late autumn (Peršoh *et al.*, 2009).

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References

Beeli M. 1926. — Contribution nouvelle à l'étude de la flore mycologique du Congo. *Bulletin de la Société Royale Botanique de Belgique*, 58 (2): 203-215.

Fournier J., Flessa F., Peršoh D. & Stadler M. 2011. — Three new *Xylaria* species from southwestern Europe. *Mycological Progress*, 10: 33-52.

Gunawan S., Steffan B. & Steglich W. 1990. — Xylaral, ein Hydroxyphtalid-Derivat aus Früchtkorpen von *Xylaria polymorpha* (Ascomycetes). *Liebigs Annalen der Chemie*, 1990 (8): 825-827.

Hsieh H.-M., Lin C.-R., Fang M.-J., Rogers J.D., Fournier J., Lechat C. & Ju Y.-M. 2010. — Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily *Xylarioideae* (*Xylariaceae*) and phylogeny of the taxa involved in the subfamily. *Molecular Phylogenetics and Evolution*, 54: 957-969.

Ju Y.-M. & Rogers J.D. 1996. — *A revision of the genus Hypoxylon*. Mycologia Memoir No. 20. St Paul MN, APS Press, 365 pp.

Ju Y.-M., Rogers J. D. & San Martín F. 1997. — A revision of the genus *Daldinia*. *Mycotaxon*, 61: 243-293.

Ju Y.-M. & Rogers J.D. 1999. — The *Xylariaceae* of Taiwan (excluding *Anthostomella*). *Mycotaxon*, 73: 343-440.

Ju Y.-M., Hsieh H.-M., Vasiljeva L. & Akulov A. 2009. — Three new *Xylaria* species from Russian Far East. *Mycologia*, 101: 548-553.

Læssøe T. 1987. — *Xylaria corniformis* reconsidered. *Mycotaxon*, 30: 81-85.

Læssøe T. 1992. — *Xylaria digitata* and its allies-delimitation and typification-I. *Persoonia*, 14: 603-613.

Læssøe T. 1993. — *Xylaria digitata* and its allies-delimitation and typification-II. *Persoonia*, 15: 149-153.

Læssøe T. & Cheek M. 2002. — A new *Xylaria* (*Xylariaceae*, Ascomycota) from Cameroon. *Kew Bulletin*, 57: 149-153.

Peršoh D., Melcher M., Graf K., Fournier J., Stadler M. & Rambold G. 2009. — Molecular and morphological evidence for the delimitation of *Xylaria hypoxylon*. *Mycologia*, 101: 256-268.

Rogers J.D. 1979. — *Xylaria magnolia* sp. nov. and comments on several other fruit-inhabiting species. *Canadian Journal of Botany*, 57: 941-945.

Rogers J.D. 1983. — *Xylaria bulbosa*, *Xylaria curta* and *Xylaria longipes* in continental United States. *Mycologia*, 75 (3): 457-467.

Rogers J.D. 1984. — *Xylaria acuta*, *Xylaria cornu-damae* and *Xylaria mali* in continental United States. *Mycologia*, 76 (1): 23-33.

Rogers J.D. & Callan B.E. 1986. — *Xylaria polymorpha* and its allies in continental United States. *Mycologia*, 78 (3): 391-400.

Rogers J.D. & Ju Y.-M. 1998. — Keys to the *Xylariaceae* (excluding *Anthostomella*) of the British Isles. *Botanical Journal of Scotland*, 50: 153-160.

Stadler M., Wollweber H. & Fournier J. 2004. — A host-specific species of *Hypoxylon* from France, and notes on the chemotaxonomy of the "*Hypoxylon rubiginosum* complex". *Mycotaxon*, 90: 187-211.

Stadler M. & Fournier J. 2006. — Pigment chemistry, taxonomy and phylogeny of the *Hypoxyloideae* (*Xylariaceae*). *Revista Iberoamericana de Micología*, 23: 160-170.

Stadler M., Hawksworth D.L. & Fournier J. 2014. — The application of the name *Xylaria hypoxylon*, based on *Clavaria hypoxylon* of Linnaeus. *IMA Fungus*, 5 (1): 57-66.

Stadler M., Fournier J., Læssøe T., Lechat C., Tichy H.-V. & Piepenbring M. 2008. — Recognition of hypoxyloid and xylarioid *Entonaema* species and allied *Xylaria* species from a comparison of holomorphic morphology, HPLC profiles and ribosomal DNA sequences. *Mycological Progress*, 7: 53-73.

Stadler M., Læssøe T., Fournier J., Decock C., Schiemschek B., Tichy H.-V. & Peršoh D. 2014. — A polyphasic taxonomy of *Daldinia* (*Xylariaceae*). *Studies in Mycology*, 77: 1-143.

Starbäck K. 1901. — Ascomyceten der Ersten Regnellschen Expedition II. *Bihang til Kongliga Svenska Vetenskaps-akademiens Handlingar*, 27, Afd. 3 (9): 1-26.

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