Xylaria xylarioides (*Xylariaceae*), a subtropical species reported for the first time from Europe

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Abstract: *Xylaria xylarioides* is reported for the first time from Europe based on two recent collections from Catalonia and Galicia in Spain, and three previous unpublished records including one from Corfu (Greece) and two from Tenerife (Canary Islands) made by Dr. Walter Jaklitsch. An illustrated description of the material collected in Galicia is provided together with comments on the morphological variations encountered in *X. xylarioides*, its known distribution worldwide, ecology and taxonomic affinities.
Keywords: Ascomycota, Catalonia, Corfou, Galicia, Greece, pyrenomycetes, saproxylic fungi, Spain, subtropical climate, taxonomy, Tenerife, *Xylariales*.

Resumen: La *Xylaria xylarioides* fué encontrada por primera vez en Europa por el Dr. Walter Jaklitsch, tres muestras que no fueron publicadas, una de las muestras proviene de Corfú (Grecia) y las otras dos muestras de Tenerife (Canarias). Recientemente ha sido localizada en Cataluña y Galicia en España. En este estudio hacemos una descripción ilustrada de la muestra localizada en Galicia (España). Se hace un relato de las variaciones morfológicas presentadas por la *X. xylarioides*, su reparto conocido a nivel mundial, su ecología y sus afinidades taxonómicas.
Palabras claves: Ascomycota, Cataluña, clima subtropical, Corfu, España, Galicia, Grecia, pyrenomycetes, setas saprófitas, taxonomía, Tenerife, *Xylariales*.

Résumé : *Xylaria xylarioides* est signalée pour la première fois d'Europe sur la base de deux récoltes récentes en Espagne (Catalogne et Galice) et de trois récoltes antérieures non publiées, une provenant de Corfou (Grèce) et deux de Tenerife (Iles Canaries) faites par le Dr. Walter Jaklitsch. Une description illustrée de la récolte de Galice est proposée. Les variations morphologiques présentées par *X. xylarioides*, sa répartition connue au niveau mondial, son écologie et ses affinités taxinomiques sont commentées.
Mots-clés : Ascomycota, Catalogne, champignons saproxyliques, climat subtropical, Corfou, Espagne, Galice, Grèce, Iles Canaries, pyrénomycètes, taxinomie, Ténérife, *Xylariales*.

Introduction

In February 2016, a puzzling xylariaceous fungus was posted on the forum of Ascofrance (http://www.ascofrance.fr/search_forum/) by one of us (JC), on behalf of the collector (MAD). The microscopic characters suggested a xylariaceous species but the very small size of the stromata did not clearly evoke a known European taxon. It was first assumed that it might be a stunted form of a *Xylaria* Hill ex Schrank species usually featuring stouter stromata, but the ascospore size and shape did not match with those of *Xylaria* spp. commonly encountered in Europe.

As the material was collected in Galicia, a region from northwestern Spain known to enjoy a mild and rainy climate, in which subtropical or undescribed ascomycetes are not uncommon (E. Rubio, pers. comm.), we were prompted to take a closer look at this sample.

Although most stromata were indeed atypical and difficult to link with a species featuring a similar ascospore morphology, a thorough examination of the whole collection revealed that some subglobose to conical stromata, often with a pointed apex bearing a tan outer layer splitting into wide strips were reminiscent of a small penzigioid *Xylaria* species recently redescribed from Argentina by Hladki & Romero (2010). As *X. xylarioides* (Speg.) Hladki & Romero is a distinctive species and as the material from Galicia conformed with it both macro- and microscopically, we submitted our identification to Dr. Yu-Ming Ju who kindly confirmed it.

We provide an illustrated description of the Galician material, stressing the high variability of stromatal shape, and comments on its affinities with the most closely related *Xylaria* species in the *X. arbuscula* aggregate as defined by Hsieh *et al*. (2010). Its known distribution in Europe with regard to geographical and climatic data is likewise discussed.

O Grove (Province of Pontevedra), where *X. xylarioides* was collected, is a small peninsula on the coast of Galicia, north of Portugal, where the vegetation is largely dominated by plantations of *Eucalyptus* sp. and ferns in open grounds, with scattered *Acacia* sp., *A. dealbata* Link, *Genista scorpius* (L.) DC. and *Quercus* sp. Due to the oceanic influence and the latitude (42° 29' 43'' N), O Grove and the Province of Pontevedra enjoy an exceptionally mild and humid climate with an annual average temperature of 20.5 °C and an annual average rainfall of 1,691 mm, calculated over the period 1971–2000 (https://en.wikipedia.org/wiki/Galicia_%28Spain%29#Climate). The most distinctive climatic character of this region is the relatively high temperature in winter, averaging 9.5°C in January, with only two days with frost, which most likely accounts for the presence of rare xylariaceous ascomycetes like *Annulohypoxylon michelianum* (Ces. & De Not.) Y.M. Ju, J.D. Rogers & H.M. Hsieh (Rubio & De la Peña, 2016) and *X. crozonensis* Leroy & Mornand (Rubio *et al.*, 2016) and several others that are still under investigation.

Material and methods

The observations were carried out on dry material rehydrated in water. Measurements of asci and ascospores were made in water or 1% SDS, with the ascospore measurements processed with the free software Piximetre 5.2 (http://ach.log.free.fr/Piximetre/). In the formula given by this software the values within brackets represent the extreme values (20%) that are not taken into account for the calculation; N represents the number of ascospores measured, Q the quotient length/width, Me the mean values of length × width, and Qe the mean value of quotient length/width. The amyloid reaction of the ascus apical apparatus was tested by adding a drop of Melzer's reagent to a water mount of perithecial contents. Microscopic observation of asci, ascospores and paraphyses was carried out in water or after mounting in blue Pelikan® ink diluted in 1% SDS or in India ink to check the presence of slimy material. Measurements of perithecia, asci and ascal apical apparati are recorded as height × width.

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 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 images were processed with

Fig. 1. Overview of the collecting site, the exact location of the pile of branches indicated by the red arrow. Photo M-A Delgado.

Adobe Photoshop Elements 10, and the figures were assembled with the same software.

The material from Catalonia and Galicia was deposited in LIP herbarium (University of Lille, France) and a duplicate of the specimen from Galicia was sent to Dr. Yu-Ming Ju (HAST, Taiwan). Nomenclature follows MycoBank. Initials JC, JF and MAD refer to the authors of this paper.

Taxonomy

Xylaria xylarioides (Speg.) Hladki & Romero, *Fungal Diversity*, 42: 86 (2010). Fig. 1. Plates 1–2. Table1.

Basionym: *Hypoxylon xylarioides* Speg., *Anales Soc. Ci. Argent*., 9 (4): 179 (1880).

Stromata scattered, separate, upright, 0.75–1.35(–3.4) mm high × 0.75–1.9 mm diam, fertile head subglobose to broadly conical, rarely narrowly fusiform or flattened, few peritheciate, with a conical sterile apex or apically obtuse, with perithecial contours slightly to strongly exposed, subsessile to short-stipitate, rarely with a narrow stipe up to 1.8 mm high; surface blackish, glabrous, obscurely cracked and roughened, apex typically overlain by a tan to brownish black horny outer layer splitting downwards into strips or plaques, black crust 35–50 µm thick, leathery; interior white, brownish grey in the stipe, cheesy, solid. **Perithecia** subglobose, 0.45–0.6 mm diam. **Ostioles** obtusely papillate, black, often inconspicuous.

Asci narrowly fusiform, the spore-bearing parts (70–)90–100 µm long × 12.5–18 µm wide, the stipes 54–68 µm long, with eight obliquely to transversely uniseriate ascospores, biseriate in places, with apical apparatus tubular with a flat apical rim, 3.9–5.1 × 2.8–3.7 µm (Me = 4.4 × 3.2 µm, N = 22), bluing in Melzer's reagent. **Paraphyses** hyphal, septate, thin-walled, 4.5– 7.5 µm wide at base, tapering to 2–2.5 µm wide above asci, embedded in gelatinous matrix.

Ascospores (16.4–)17.3–20.2(–20.8) × (5.9–)6.4–7.6(–7.7) µm, Q = (2.2–)2.5–3(–3.3); N = 60 (Me = 18.9 × 6.9 µm; Qe = 2.7), fusiform-inequilateral with narrowly rounded to subacute or slightly pinched ends, lower end with a small hemispherical refractive cellular appendage stained in blue ink, wall olivaceous brown, with a conspicuous, straight to slightly undulating germ slit slightly less than spore-length on the ventral side; epispore smooth; no sheath observed in India ink.

Asexual morph not seen on the natural substrate. Sterile asexual stromata 1.8–4.7 × 0.25–0.35 mm, upright, subcylindrical-flattened, blackish to dark grey in lower part, pale grey to whitish above, hairy at base.

Known distribution: Argentina, ?Brazil (as *X. venosula*, Hladki & Romero, 2010), Greece (Corfu, this paper), New Zealand North Island (Rogers & Samuels, 1986, as *X. schreuderiana*), South Africa (as *X. schreuderiana*, Hladki & Romero, 2010), Spain (Canary Islands, Catalonia, Galicia, this paper), and Taiwan (Ju & Rogers, 1999, as *X. papillata*).

Specimens examined: Spain: Catalonia, Barcelona Province, Barcelona, Cerdanyola del Vallès, ca. 150 m elevation, on dead stems of *Rubus fruticosus*, 8 Apr. 2016, *leg*. Pascal Ribollet, JF 16031 (LIP); Galicia, Pontevedra Province, O Grove peninsula, 6 m elevation, on dead corticated twigs 0.3–1 cm diam (possibly *Acacia* sp.) in a pile of dead branches in a clearing, associated with an effete nectriaceous ascomycete, 22 Feb. 2016, *leg*. Miguel-Angel Delgado, communicated by Joseba Castillo, JF 16004 (LIP).

Discussion: In their survey of *Xylaria* in Tucuman Province of Argentina, Hladki & Romero (2010) resurrected a xylariaceous species formerly described in 1880 by Spegazzini as *Hypoxylon xylarioides* and recombined it with *Xylaria* as *X. xylarioides* (Speg.) Hladki & A.I. Romero based on morphological characters. In the same study,

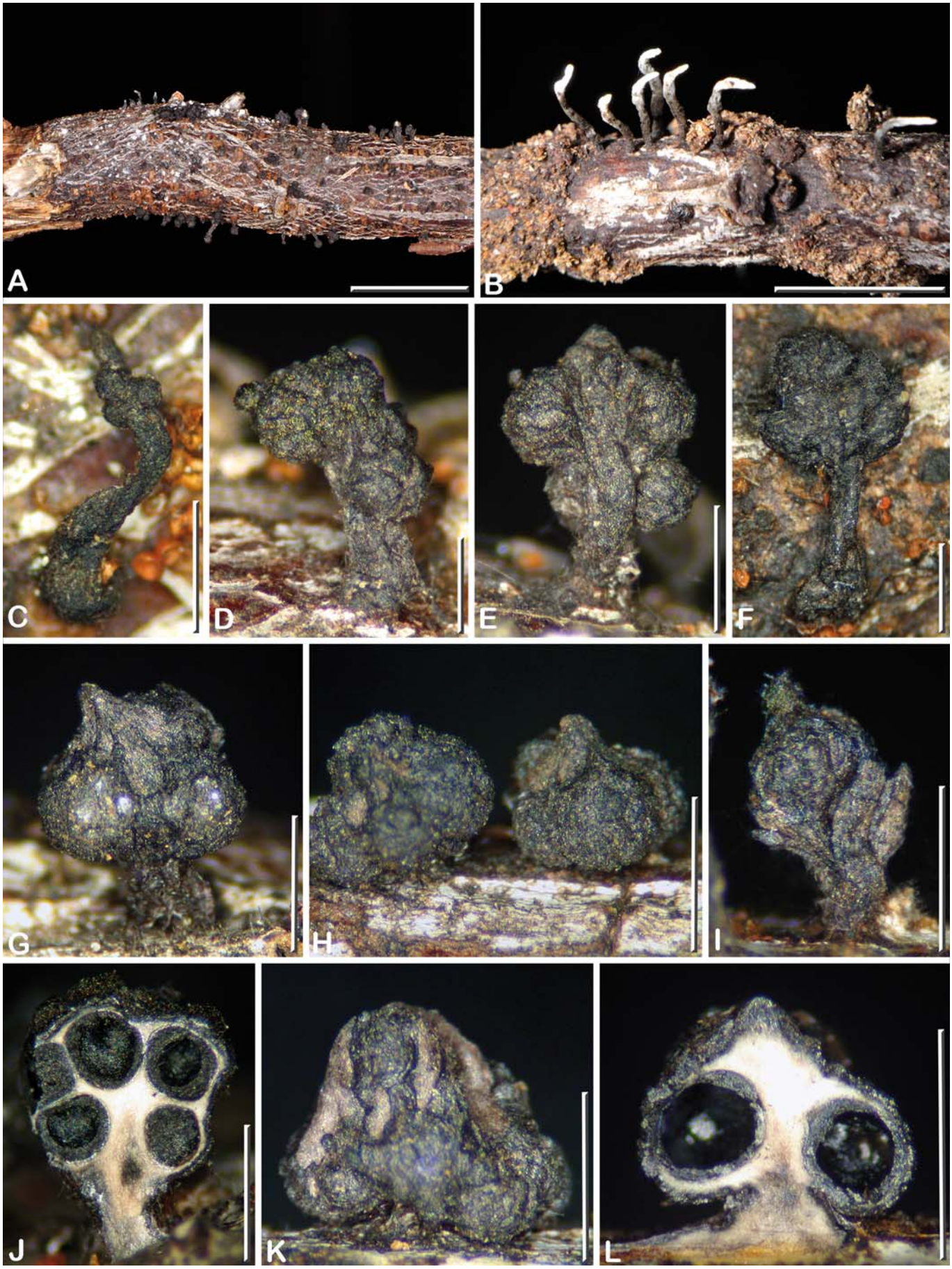

Plate 1 – *Xylaria xylarioides*

JF 16004. A: Stromata on host surface; B: Supposed asexual stromata on host surface; C-F: Variously shaped abnormal stromata; G-I, K: Typical stromata; J, L: Stromata in vertical section showing the perithecia, the white interior and the thin black outer crust. Scale bars: A = 10 mm; B = 5 mm; C-L = 1 mm. A, B: Photos by M.-A. Delgado.

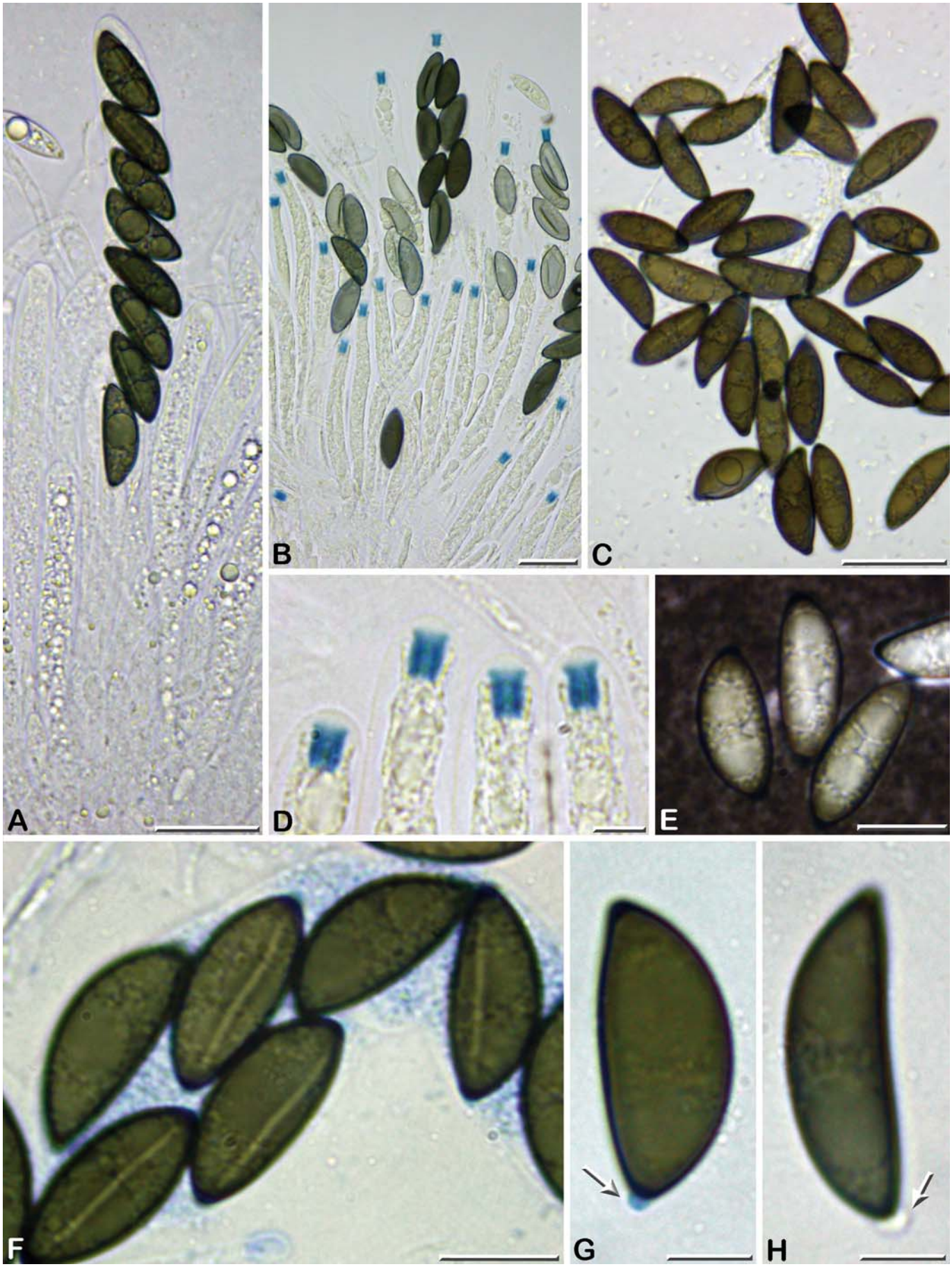

Plate 2 – *Xylaria xylarioides*

JF 16004. A: Mature ascus, in water; B: Mature and immature asci with uniseriate to biseriate ascospores, in Melzer's reagent; C: Variously shaped ascospores, in water; D: Ascal apical apparati of immature asci, in Melzer's reagent; E: Ascospores in India ink, showing the lack of slimy sheath; F: Ascospores in ventral view showing the germ slit, in diluted blue Pelikan® ink; G, H: Ascospores in side view showing the basal cellular appendage (arrows), in diluted blue Pelikan® ink and water, respectively. Scale bars: A-C = 20 µm; D, G, H = 5 mm; E-F = 10 µm.

X. smilacicola Speg., also collected by Spegazzini in 1909 in Tucuman Province, later assessed to be a synonym of *X. xylarioides*.

As a result of this revision of Spegazzini's material and the examination of plentiful fresh material collected in Tucuman province, *X. xylarioides* was well defined as having small short-stipitate subglobose to conical stromata less than 2 mm wide, with a conical sterile apex coated by a light brown peeling outer layer splitting into bands and navicular ascospores 17–21 × 6.5–9 µm with a germ slit almost full-length (Hladki & Romero, 2010). Its occurrence on monocot (often *Smilacaceae*) or dicot twigs likewise appears to be a consistent ecological character of this species.

The material collected in Galicia (Spain) described above fits well the concept of *X. xylarioides* as defined by Hladki & Romero (2010), with the exception of numerous deviating stromata, deformed or with unusually high stipes, illustrated in Plate 1 (A, C-F). Since the morphology of ascospores appeared strictly consistent amongst typical and less typical stromata and in close agreement with that assigned to *X. xylarioides* by Hladki & Romero (2010), there is little doubt that the stromatal morphology of this taxon is highly variable probably depending on environmental conditions, a fact which is familiar to the students of this challenging genus. The only minor difference that we observed is the presence of a small cellular appendage on immature and mature ascospores not reported by Hladki & Romero (2010), but this character is known to be variable within a species, frequently disappearing with maturity or in dried material (Fournier *et al*., 2011; Peršoh *et al*., 2009). The collection from Barcelona features the same high proportion of atypically shaped stromata along with some distinctly stipitate stromata with stipes 4–11 mm high. As overall morphology of stromata and ascospore morphology conform well with that of the Galician collection, including the presence of a cellular appendage on ascospores, both are regarded as conspecific.

Hladki & Romero (2010) listed *X. papillata* Syd., known from Congo (Dennis, 1958), *X. schreuderiana* Van der Bijl, known from South Africa and New Zealand (Rogers & Samuels, 1986), and *X. venosula* Speg., known from Argentina and Brazil (Hladki & Romero, 2010) and Hawaii (Hsieh *et al*., 2010; Rogers & Ju, 2012), as possible synonyms of *X. xylarioides*. According to Dr. Yu-Ming Ju (pers. comm.), who revised the type collections of these species, *X. papillata* differs from *X. xylarioides* by "a white outer layer and light brown to brown ascospores with a sigmoid germ slit", and therefore "our 1999 report on *X. papillata* from Taiwan should be corrected to *X. xylarioides*", while *X. venosula* "is by no means a penzigioid fungus and may be viewed as a large-spored *X. arbuscula*, with which it shares a nearly identical ITS sequence". On the other hand, based on the material of *X. schreuderiana* collected in New Zealand and studied and illustrated by Rogers & Samuels (1986), *X. schreuderiana* is indeed very likely a synonym of *X. xylarioides*.

These data suggest a subtropical rather than typically temperate or tropical distribution for *X. xylaroides*. This view is confirmed by information provided by Dr. Yu-Ming Ju (pers. comm.) about the presence of *X. xylarioides* in the Canary Islands, based on two unpublished records from Tenerife, viz.: Spain, Islas Canarias, Tenerife, Macizo de Anaga, Chinobre, El Pijaral, on twigs of *Laurus novocanariensis*, *leg./det*. W. Jaklitsch, 15 Apr. 2010, HAST 139905; *ibid*., Macizo de Anaga, Pico del Ingles, on twigs of *Laurus novocanariensis*, *leg./det*. W. Jaklitsch, 11 Apr. 2010, HAST 139958. Although the Canary Islands politically belong to Spain and thus to Europe, their position off the coast of Morocco at a latitude of about 28° clearly set them in a subtropical zone separate from mainland Europe. To stress their particular ecology, the Canary Islands are usually regarded as part of the ecoregion Macaronesia, also including the Azores, Cape Verde and Madeira. Therefore, we first regarded the collections of *X. xylarioides* from Galicia and Barcelona as the first records from mainland Europe, until Dr. Jaklitsch informed us about a further unpublished collection of this *Xylaria* in the island of Corfu: Greece, Corfu, Skripero, NE Poulades, opposite of the marble quarry, on *Hippocrepis emerus*, 21 Apr. 2012, *leg./det*. W. Jaklitsch & H. Voglmayr (WU 32021).

Latitudinal and climatic data on the four European regions where the studied collections of *X. xylarioides* originate are summarized in Table 1. They reveal that latitude and average annual rainfall seem much less significant than average winter temperature with respect to the occurrence of *X. xylarioides*. Therefore, it could be proposed that this species is possibly present in those European regions with mild winters with no frost, either under Mediterranean or Atlantic influence, irrespective of the rainfall. This last observation is fairly unexpected since most *Xylaria* spp. are usually considered to thrive in moist environments.

The known European distribution of *X. xylarioides* inferred from these collections demonstrates that the distribution of fungi often extends beyond the boundaries of artificially defined ecoclimatic zones. Two other members of the genus *Xylaria* also provide good examples: while *X. cinerea* J. Fourn. & M. Stadler extends in Europe from the UK to Spain, it is also commonly found in the Canary Islands and even in New Zealand (Fournier *et al*., 2011). Conversely, the subtropical to tropical taxon *X. arbuscula* var. *plenofissura* Y.-M. Ju & Tzean known from Martinique (FWI), Mexico (San Martín *et al*., 2001, as *X. juniperus* Starb. var. *asperula* Starb.) and Taiwan (Ju & Rogers, 1999) was also shown to be present in France (Fournier *et al*., 2011).

Interestingly, the stunted stromata of *X. arbuscula* var. *plenofissura* that were collected in France (Fournier, 2014) might be confused with some of the atypical stromata of *X. xylarioides* like the one illustrated in Plate 1 (fig C), with their ascospores averaging 19 × 6.8 µm and having a germ slit slightly less than spore-length resemble those of *X. xylarioides*. In such borderline cases, the shape of ascospores, more strongly inequilateral and with more acute ends in *X. xylarioides* appears to be the most discriminant morphological character from *X. arbuscula* var. *plenofissura*.

Owing to several morphological similarities, *X. xylarioides* probably belongs to the *X. arbuscula* aggregate as defined by Hsieh *et al*. (2010) based on multigene phylogenetic analyses, which includes *X. arbuscula* Sacc., *X. arbuscula* var. *plenofissura*, *X. bambusicola* Y.-M.

Table 1 – Comparison of some geographical and climatic data on the European regions where *X. xylarioides* was collected

	Latitude	Temperatures in January (night/ average/ day)	Average annual temperature	Average annual rainfall	Climate type (Köppen classification)
Barcelona https://en.wikipedia.org/wiki/Climate_of_Barcelona	41°23′ N	5–9.4–14 °C	16.5 °C	529 mm	Csa subtropical- mediterranean
Corfu https://en.wikipedia.org/wiki/Corfu#Climate	39°35′ N	5.1–9.7–13.9 °C	17.5 °C	1097 mm	Csa hot mediterranean
Galicia (Pontevedra) http://www.pontevedra.climatemps.com/	42° 29′ 43″ N	7.5–9.5–12.3 °C	14.7 °C	1691 mm	Csb warm-summer mediterranean
Tenerife (Santa Cruz) https://en.wikipedia.org/wiki/Tenerife#Climate	28° 17′ N	15.4–18.2–21 °C	21.5 °C	225.7 mm	BSh hot steppe

Ju & J.D. Rogers, *X. striata* Pat. and *X. venosula*. This aggregate is a well-supported subclade of the "HY clade" (Hsieh *et al*., 2010) including the type species *X. hypoxylon* (L. : Fr.) Grev. and related species but also some morphologically unrelated tropical species of *Xylaria* and members of the genus *Kretzschmaria* Fr. It is hoped that further field and molecular investigations will help resolve better the complicated relationships within this aggregate.

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