

First European records of *Xylaria apiculata* (Xylariales) from Spain

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Abstract: *Xylaria apiculata*, so far known to have a pantropical distribution, is reported here for the first time from Europe, based on two collections from Asturias (Spain). The author presents a macro- and microscopic description of the fungus and its differences with the most closely related species within the “*Xylaria arbuscula* complex” are discussed. ITS and LSU rDNA sequences were generated during this study and deposited to enable further phylogenetic studies in this taxonomic group.

Keywords: Ascomycota, Asturias, chorology, fungi, taxonomy, Xylariaceae.

Resumen: En base a dos recolecciones procedentes de Asturias (España), se describe *Xylaria apiculata*, un hongo con una bien conocida distribución pantropical. El autor presenta una descripción macro y microscópica del hongo, a la vez que lo compara y distingue de otras especies relacionadas dentro del llamado “complejo de *Xylaria arbuscula*”. Las secuencias obtenidas de las regiones ITS y LSU del rDNA han sido depositadas con el fin de favorecer ulteriores estudios taxonómicos dentro de este grupo.

Palabras clave: Ascomycota, Asturias, corología, hongos, taxonomía, Xylariaceae.

Introduction

The number of records of tropical and subtropical fungal species has increased continuously in Europe in recent years (RUBIO *et al.*, 2016, 2018), likely because of both the significant increase in average temperatures on the continent and the number of researchers working in this region.

The climate of Asturias, a province located in the northwest of Spain, is typically oceanic, with mild summers and winters, generally rainy, at least in the coastal regions of the province. The sheltered, shadowy and humid places located on the banks of rivers are very appropriate for the discovery of pyrenomycetes and especially Xylariaceae.

Material and methods

The samples described here have been examined at fresh state, without the help of rehydrating agents that could change both the morphology and the dimensions of their structures. However, additional examinations in Melzer’s reagent and in an aqueous solution of India ink, have also been necessary to enable the observation of respectively the amyloidity of the apical apparatus of the asci and the possible existence of appendages or gelatinous envelopes to the ascospores.

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,



Plate 1 – *Xylaria apiculata* ERD-7166. Stromata *in situ*. Photo E. Rubio.

1987) included 35 cycles with an annealing temperature of 54 °C. Primer ITS4 (WHITE *et al.*, 1990; GARDES & BRUNS, 1993) was employed to amplify the ITS rDNA region, while LR0R (VILGALYS & HESTER, 1990; CUBETA *et al.*, 1991) was used for the LSU 28S rDNA region. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

The sequences of the ITS and LSU regions of the ERD-7166 sample are deposited in GenBank respectively with the following accession numbers: MN644466 and MN644468. The samples are preserved in the author's personal herbarium.

Taxonomy

Material studied: SPAIN: Asturias, Soto del Barco, Riberas de Pravia, road to Los Veneros, 43° 24' 53" N; 6° 03' 14" W; 115 m, on in-

determinate hardwood partially submerged in river water, 7-IX-2017, *leg.* & *det.* E. Rubio, ERD-7166. *Ibid.*, 15-VII-2019, ERD-8011 Asturias, Villaviciosa, Valbúcar, road to 'Molinos del río Profundo', 43° 27' 10" N; 5° 26' 22" W; 82 m, 28-VIII-2019, on wood of *Acer pseudo-platanus*, *leg.* J. Linde, *det.* E. Rubio, ERD-8013. Pontevedra, Vigo, Coruxo, 16-XI-1993, on wood of *Quercus* sp., *leg.* M. Carballo, *det.* F. Candoussau as *X. apiculata*, deposited in the herbarium of Lourizán (Pontevedra) 6786 (= *X. cinerea* J. Fourn. & M. Stadler). Canary Islands, Tenerife, Parque rural de Teno. Monte de Aguas y Pasos, 2-II-1985, *leg.* & *det.* A. Bañares, TFC-Mic: 2391-1, as *X. apiculata* (= *X. arbuscula* var. *plenofissura*). *Ibid.*, 2-II-1985, TFC-Mic: 2434-1 as *X. apiculata* (= *X. arbuscula* var. *plenofissura*). *Ibid.*, 16-VI-1986, TFC-Mic: 2927-1 as *X. apiculata* (= *X. arbuscula* var. *plenofissura*).

Description of ERD-7166

Stromata superficial, solitary or clustered in small groups, erect, stipitate, 9–30 mm in total height, the fertile head subcylindrical to slightly fusiform or narrowly clavate, constricted in places, simple to



Plate 2 – *Xylaria apiculata* ERD-7166. Stromata. Close up. Photos E. Rubio.

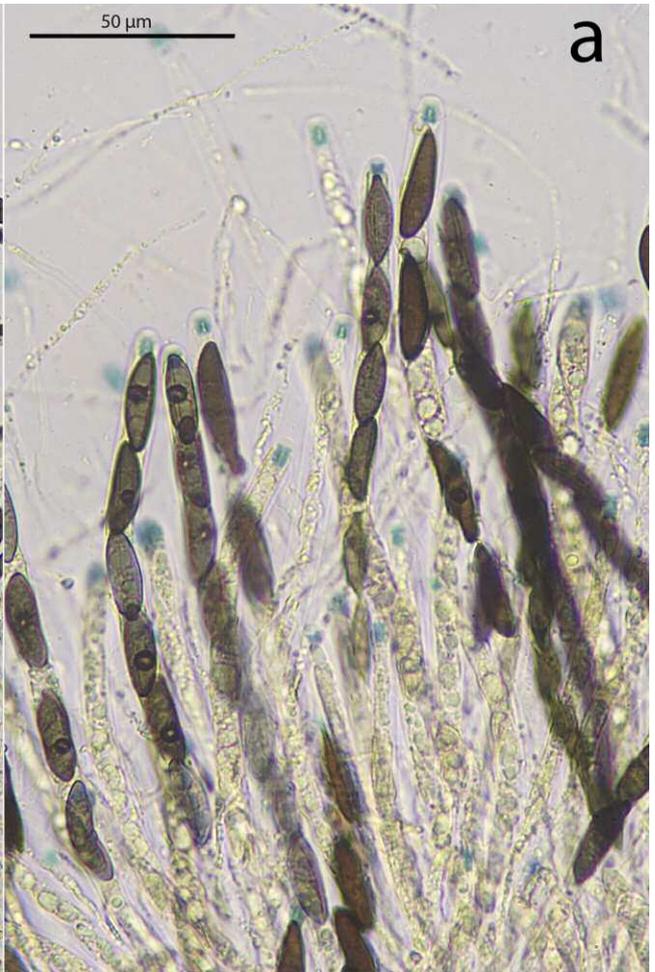
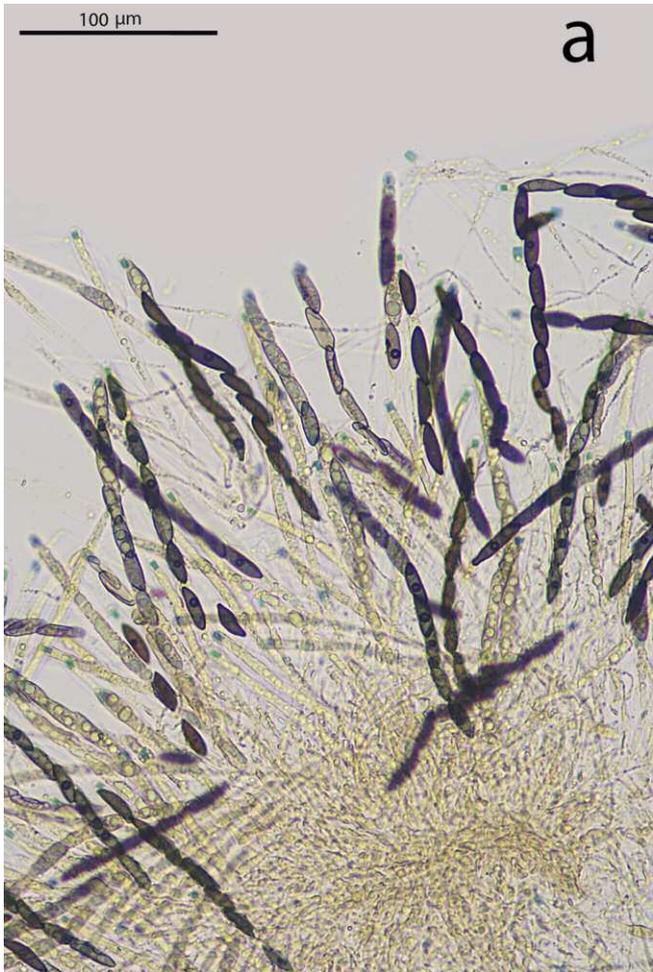


Plate 3 – *Xylaria apiculata* ERD-7166. a: Hymenium in Melzer's reagent. b: Hymenium in Congo red. Photos E. Rubio.

rarely branched, 5–24 mm high × 2.5–3 mm diam, apically ending into a short, narrow, fragile, sterile extension 1–2 mm long; surface blackish, occasionally with a purplish tone, hard-textured, slightly nodulose with perithecial contours not to faintly exposed, strongly roughened by a blackish grey outer layer splitting into elongated, longitudinally oriented scales delimiting narrow cracks; the stipe sharply-defined, 3–12 mm high × 0.8–1.0 mm diam, slightly swollen at base, black, densely covered in short light brown hairs; subsurface black, carbonaceous, 75–100(–120) μm thick, finely longitudinally grooved; interior solid, white, cheesy to fibrous, with a light brown inner core in lower part; stromata lack KOH-extractable pigments.

Perithecia subglobose to slightly depressed-spherical, often in contact, (750–)820–1000(–1005) μm high × (800–)1000–1100(–1200) μm diam. **Ostioles** inconspicuous, faintly conic-papillate, up to 80 μm diam at base.

Paraphyses filiform, longer than asci, 1–3 μm wide, sparsely guttulate, embedded in slimy material. **Asci** narrowly cylindrical, (125–)132–137(–150) μm in total length, with a *pars sporifera* (100–)107–112(–125) μm long, containing eight uniseriately arranged, slightly overlapping ascospores; apical apparatus tubular, apically flattened with a narrow rim, 4–6 × 3–4 μm, euamyloid in Melzer's reagent and Lugol's solution. **Ascospores** (20.5–)24.0(–28.0) × (6.5–)7.3(–7.9) μm, $Q = (2.9\text{--})3.3\text{--}(4.4)$, narrowly fusiform-inequilateral, straight to slightly curved, with narrowly rounded to subacute, occasionally beaked or obtuse ends, olivaceous brown turning dark brown at maturity, smooth-walled, multi-guttulate, lacking a sheath or secondary appendages; a tiny hyaline, rounded, cellular appendage up to 1 μm diam can occasionally be detected at one or both ends.

No associated **asexual morph** observed.



Plate 4 – *Xylaria apiculata* ERD-7166. Ascospores 1000× in Melzer's reagent. Photo E. Rubio.

Discussion

Xylaria apiculata was first described from New Zealand by COOKE (1879). This species has been extensively documented by ROGERS & SAMUELS (1986), based on material from New Zealand where these authors report it as one of the most commonly encountered *Xylaria* species. They tentatively accepted *X. zealandica* Cooke as a distinct species, based on slightly larger ascospores, but with reservations. *Xylaria apiculata* was reported from various tropical zones, including South America by DENNIS (1956), ROGERS *et al.* (1988), VANDEGRIFT (2019), from Indonesia by ROGERS *et al.* (1987), from Papua New Guinea by VAN DER GUCHT (1995), from Taiwan by JU & ROGERS (1999) and from Hawaii Islands by ROGERS & JU (2012). These data are suggestive of a pantropical known distribution, however apparently excluding Africa so far.

Our collections conform well with the concept of *X. apiculata* delineated by ROGERS & SAMUELS (1986), with an overall morphology typical of those species accommodated in the "*Xylaria arbuscula* species complex" by the presence of cylindrical mucronate stromata with sharply defined stipes, an outer stromatal layer cracking into elongated scales and mostly unexposed perithecial contours, its ascospores 24 × 7.3 μm on average with a long straight germ slit clearly setting it apart from its relatives. The members of this complex include *X. arbuscula* Sacc., *X. arbuscula* var. *plenofissura* Y.-M. Ju & Tzean, *X. bambusicola* J.D. Rogers & Y.-M. Ju, *X. papillata* Syd., *X. pseudoapiculata* Hamme & Guerrero, *X. schreuderiana* Van der Bijl, *X. smilacicola* Speg., *X. venosula* Speg. and *X. xylarioides* (Speg.) Hladki & Romero. *Xylaria bambusicola* is set apart by its host-specificity for bamboo (JU & ROGERS, 1999) and *X. papillata*, *X. schreuderiana*, *X. smilacicola* and *X. xylarioides* by their penzigoid stromata, the former differing by a sigmoid germ slit (FOURNIER *et al.*, 2018) and the three latter being most likely synonyms (FOURNIER *et al.*, 2016; MARCOTE & COSTA, 2017). *Xylaria arbuscula* is not well circumscribed but its ascospores are significantly shorter than those of *X. apiculata* and their germ slit is rarely over $\frac{2}{3}$ – $\frac{3}{4}$ of the spore length (JU & ROGERS, 1999; FOURNIER, 2014). Its variety *plenofissura* differs by larger ascospores 18–20 × 6–7 μm with a long straight germ slit, approximating those of *X. apiculata* but regarded by most authors as significantly different (JU & ROGERS, 1999; FOURNIER, 2014). Ascospores of *X. pseudoapiculata* are in the same size range but they differ by a long sigmoid to spiralling germ slit (HAMME & GUERRERO, 1997) and *X. venosula* is regarded as a large-spored form of *X. arbuscula* with which it shares almost similar ITS sequences (FOURNIER *et al.*, 2016).

The material from previous citations of *X. apiculata* in Spain, from the Canary Islands (TFC-Mic), that we have been able to examine, contains small-sized mucronate stromata with the typical cracked surface but with spores that rarely reach 20 μm in length and that fits well the current concept of *X. arbuscula* var. *plenofissura*, while that from Galicia shows the peculiar appearance of the stromata of *X. cinerea*, a fungus very common throughout the oceanic coasts of Spain. Finally, the material, from Galicia, that we have not been able to examine personally, is discarded because it is described as having ascospores different from those of typical *X. apiculata*, i.e. 14–16 × 5.5–6.0 μm, with germ slit that does not cover the entire length of the spore (RODRÍGUEZ & CASTRO, 1996).

This taxonomic overview of the species currently accommodated in the "*Xylaria arbuscula* species complex" shows that it is not yet fully resolved but that the status of *X. apiculata* as a distinct species, characterized by ascospores 20–24.5 × 6.7–8.0 μm with a long straight germ slit (ROGERS & SAMUELS, 1986) is well established. As a result, our collections from Asturias can be confidently presented as the first records from the European continent.

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