Xylaria melitensis (*Xylariaceae*), a new penzigioid species from the Maltese Islands

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Abstract: Based on detailed morphological observations of both sexual and asexual morphs *in vivo*, cultures *in vitro* and molecular phylogenetic data based on rDNA ITS and LSU sequences, we document herein a penzigioid *Xylaria* repeatedly collected in the Maltese islands of Gozo and Malta. Though it morphologically resembles *X. sibirica* by stromatal habit and ascospores in the same size range with a long sigmoid germ slit, some significant differences could be detected after comparison of this *Xylaria* with an isotype collection of *X. sibirica*. Further differences in cultural characteristics and occurrence of a distinctive asexual morph *in vivo* preceding the development of the sexual morph, along with a molecular phylogenetic analysis clearly showing that they are distantly related, strongly support the recognition of this penzigioid *Xylaria* as a distinct species with phylogenetic affinities with the *X. polymorpha* aggregate. We therefore introduce *X. melitensis* sp. nov. to accommodate it.
Keywords: *Ascomycota*, Gozo, Malta, Mediterranean mycobiota, ribosomal DNA, taxonomy, *Xylariales*.

Résumé : en nous fondant sur les observations morphologiques détaillées de ses stades sexués et asexués *in vivo* et des cultures *in vitro*, ainsi que sur les données phylogénétiques fournies par l'analyse de ses séquences ITS et LSU, nous documentons une xylaire penzigioïde récoltée à plusieurs reprises dans les îles maltaises de Gozo et Malte. Malgré sa ressemblance avec *X. sibirica* due à des stromas d'allure similaire et des ascospores de dimensions comparables présentant également un sillon germinatif sigmoïde, certaines différences significatives furent observées après comparaison avec un isotype de *X. sibirica*. Des différences supplémentaires concernant les caractéristiques en culture et la présence *in vivo* d'un stade asexué remarquable se développant avant l'apparition du stade sexué, ainsi que les résultats de l'analyse phylogénétique moléculaire montrant clairement qu'elles ne sont que faiblement apparentées étayent la reconnaissance de cette xylaire penzigioïde comme une espèce distincte, ayant des affinités phylogénétiques avec les espèces du groupe de *X. polymorpha*. En conséquence, nous proposons de la nommer *X. melitensis* sp. nov.
Mots-clés : ADN ribosomal, *Ascomycota*, Gozo, Malte, mycobiote méditerranéen, taxinomie, *Xylariales*.

Introduction

The term "penzigioid" is applied to members of the genus *Xylaria* Hill ex Schrank whose stromata are wider than high and sessile to substipitate. It is derived from the genus name *Penzigia* Sacc. that is now abandoned after having been synonymized with *Xylaria* by Ju & Rogers (2001). Though lacking taxonomic relevance, it is still used for convenience sake, as commented on by Fournier *et al.* (2018).

Known penzigioid *Xylaria* taxa predominantly have a tropical distribution (Ju & Rogers, 1999; Ju *et al.*, 2012; Ma *et al.*, 2012; Huang *et al.*, 2015; Tapia *et al.*, 2017; Fournier *et al.*, 2018) except for "*Rosellinia*" *callosa* Winter (Petrini, 1992; Fournier, 2014), and *X. crozonensis* Leroy & Mornand (Leroy & Mornand, 2001; 2004), both known from Europe, as well as *X. sibirica* Y.-M. Ju, H.-M. Hsieh, Lar. N. Vassiljeva & Akulov known from Russian Far East (Ju *et al.*, 2009). In this context, a stunted xylariaceous fungus that was repeatedly collected by two of us (SM and CS) in the Maltese islands of Gozo and Malta could not be unambiguously identified. Some photos were posted on the forum of Ascofrance (http://www.ascofrance.fr/) and our colleague Dr. Walter Jaklitsch suggested *X. sibirica* based on its small pulvinate and sessile stromata and ascospores in the same size range provided with a sigmoid germ slit. Despite a striking morphological resemblance, we were somewhat reluctant to equate without further investigations this Maltese *Xylaria*, coming from a Mediterranean subtropical environment (https://en.wikipedia.org/wiki/Climate_of_Malta), with *X. sibirica* coming from a humid continental climate (https://en.wikipedia.org/wiki/Kedrovaya_Pad_Nature_Reserve).

The material collected in Malta was compared to an isotype of *X. sibirica* kindly communicated by A. Akulov and illustrated in Fournier (2014) and to the original description by Ju *et al.* (2009). Two fresh collections of the Maltese *Xylaria* were cultured and the cultures were compared to those described in the above publications; DNA extracted from the mycelium produced in culture was sequenced, allowing the comparison of the two markers ITS and LSU with those of *X. sibirica* deposited in GenBank.

In the following we present and discuss our morphological, cultural and molecular phylogenetic results supporting the recognition of *X. melitensis* sp. nov as a distinct species, unexpectedly distantly related to *X. sibirica* based on phylogenetics.

Materials and methods

The observations were carried out on fresh or dry material rehydrated in water or 1% SDS. Measurements of asci and ascospores were made in water and ascospores measurements were processed with the free software Piximetre 5.2 (http://ach.log.free.fr/Piximetre/). 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 ascospore appendages, asci and paraphyses was carried out after 1 min in 1% SDS and mounting in black Pelikan ink, diluted blue Pelikan ink or diluted India ink. Measurements of stromata, asci and ascus apical apparati are recorded as height × width. Nomenclature follows MycoBank. 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 digitized photographs were processed with Adobe Photoshop Elements 10 and the plates assembled with the same software. The specimens are deposited in LIP fungarium (University of Lille, France). Initials JF, CL, SM and CS refer to the authors and their personal fungaria.

Cultures of the living specimens were made on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 5 cm diam. incubated at 25°C.

DNA extraction, amplification, and sequencing: Total DNA was extracted from pure culture blending a portion of them using a micropestle in 600 µl CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min. at 65°C. A similar volume of chloroform:isoamylalcohol (24:1) was added and carefully mixed with the samples until

their emulsion. It was then centrifuged for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µl ddH_2O. PCR amplification was performed with the primers ITS1F and ITS4 (White *et al*., 1990; Gardes & Bruns, 1993) for ITS. Chromatograms were checked, searching for putative reading errors, and these were corrected.

Molecular phylogenetic analyses were conducted using MEGA version 6 (Tamura *et al*., 2013).

Taxonomy

Xylaria melitensis J. Fourn., Lechat, Mifsud & Sammut, *sp. nov.* – Figs. 1–2; Plates 1–3; Table 1 – MycoBank MB838608

Diagnosis: Differs from *X. sibirica*, the most resembling species, by a brown and scaly-cracked stromatal surface when young, papillate ostioles occasionally seated on a basal disc or surrounded by a paler halo and inequilateral, blackish brown ascospores.

Typification: Malta: Siggiewi, Buskett, 35° 51.37′ N, 14° 25.93′ E, 195 m asl, under *Quercus ilex* (*Fagaceae*), *Laurus nobilis* (*Lauraceae*) and *Rhamnus alaternus* (*Rhamnaceae*), on a dead corticated branch of *L. nobilis* on the ground, 17 May 2019, *leg.* C. Sammut, CS1223 (LIP *holotypus*); GenBank ITS sequence MW513722, LSU sequence MW513725.

Etymology: The specific epithet is derived from Melita, the Latin name of Malta.

Stromata superficial, scattered or in small or large clusters, discrete to coalescent, glomerate to pulvinate, with slightly to strongly exposed perithecial contours, (0.8–)1–2(–2.7) mm diam., 1–1.7 mm high, sessile or attached to the substrate by a narrow central connective; surface light brown and scaly-cracked on immature stromata, dull brown to dark brown at maturity, smooth on top, often with a paler halo around the ostioles, frequently roughened at sides by low ridges, becoming blackish with age; subsurface a thin black

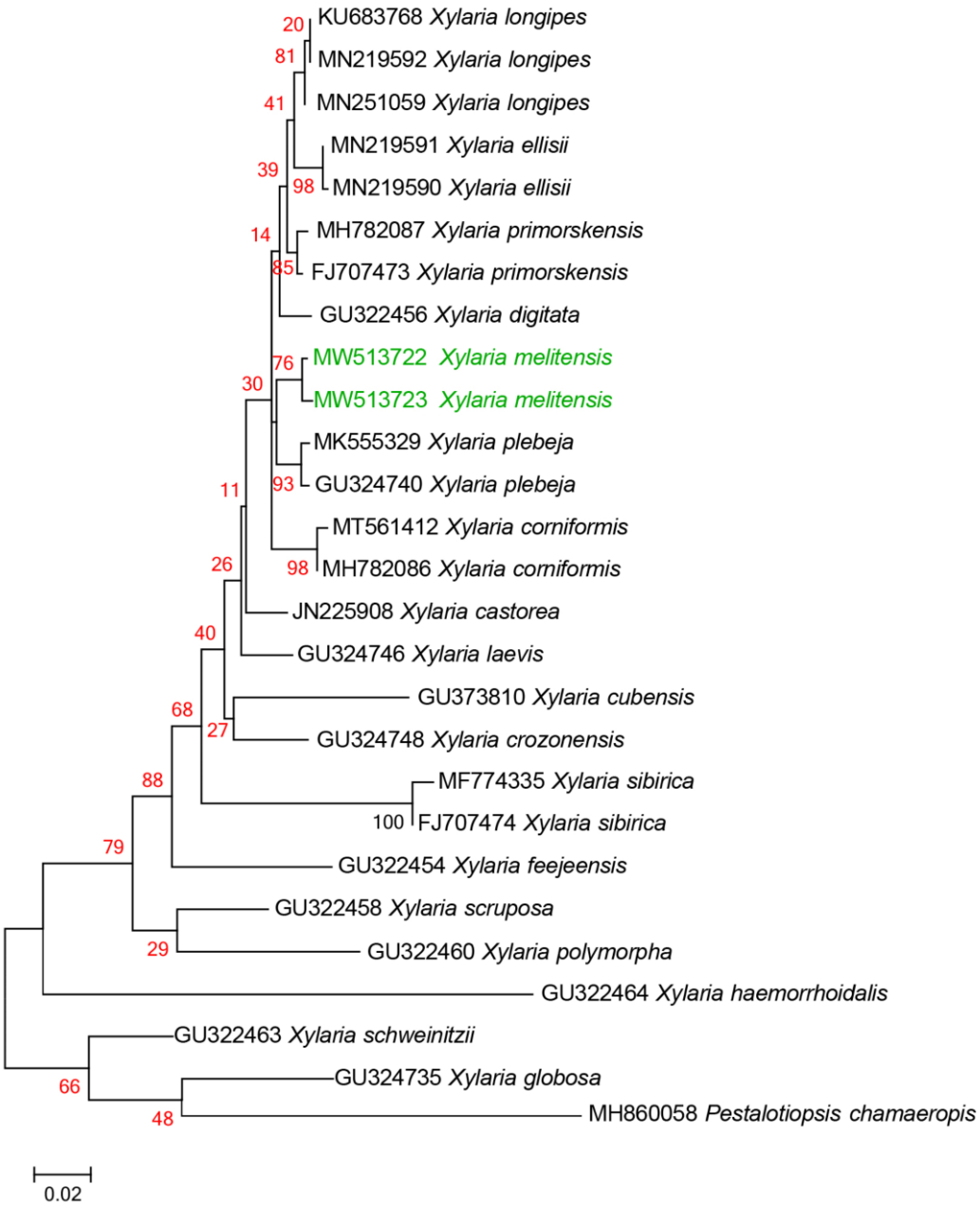

Fig. 1 – Maximum likelihood phylogeny (-lnL = 3633.23) of *Xylaria* spp. inferred by using the method based on the Tamura-Nei model, from a 565 bp matrix of ITS sequences, rooted with *Pestalotiopsis chamaeropsis*.

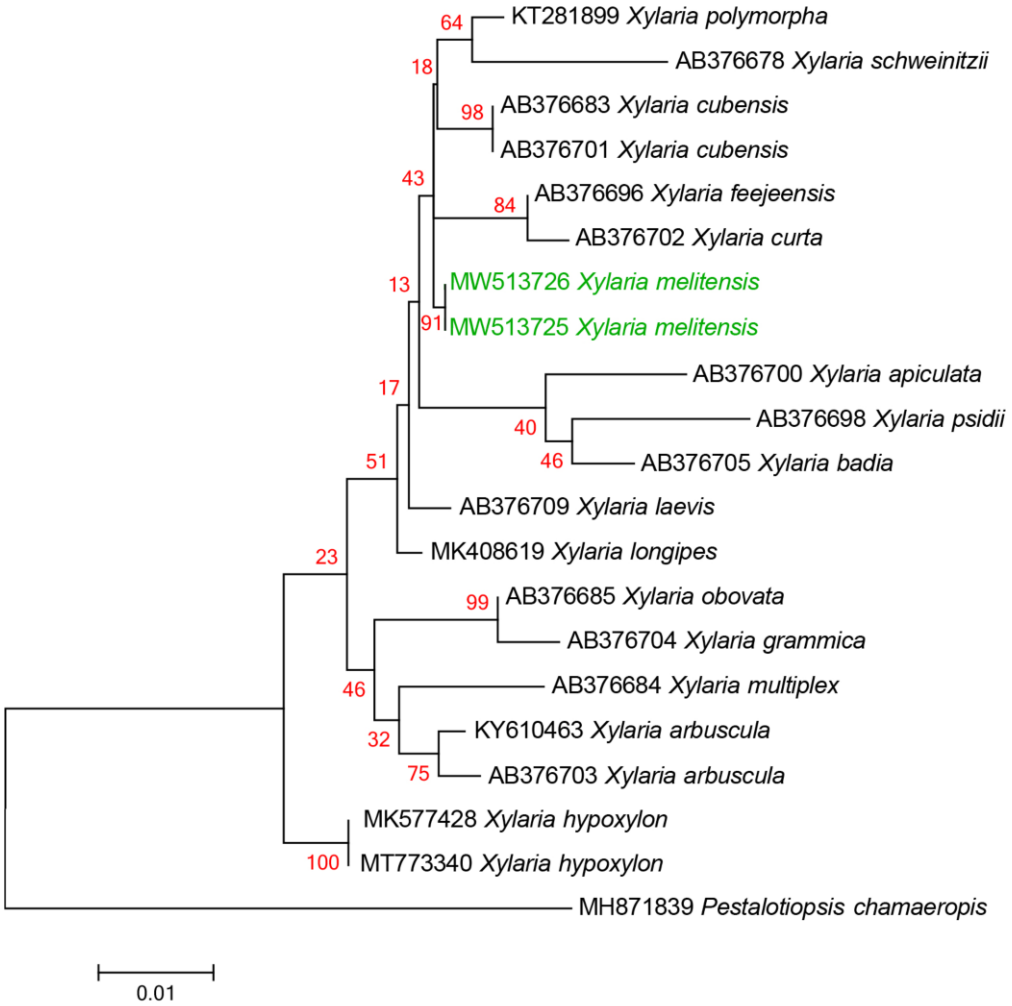

Fig. 2 – Maximum likelihood phylogeny (-lnL = 2425.52) of *Xylaria* spp. inferred by using the method based on the Tamura-Nei model, from a 568 bp matrix of LSU sequences, rooted with *Pestalotiopsis chamaeropsis*.

leathery crust 0.20–0.35 mm thick; internal tissue soft, crumbly, pure white, turning greyish with age, extending into the basal connective where it occasionally appears interspersed with brown host fibres, remaining on host surface as a white scar after removal of the stroma. **Perithecia** subglobose, 0.4–0.6 mm diam., (1–)5–12(–20) per individual stroma. **Ostioles** bluntly to sharply conic-papillate, black, slightly shiny, porate on aged stromata, most often at the centre of a dull black, discoid, occasionally slightly raised or convex area 100–170 µm diam.

Paraphyses copious, hyphal, thin-walled, much longer than asci, remotely septate, branched at base, 7–9 µm wide at base, tapering to 1.5–2 µm wide above asci, embedded in mucilaginous material. **Asci** cylindrical to subclavate, with (4–)8 slightly overlapping uniseriately arranged ascospores, thin-walled and fragile, the spore-bearing parts 112–132 × 11.5–13 µm, the stipes 45–58 µm long, with apical apparatus short-cylindrical to tubular with a marked upper rim, 4.2–6.4 × 3.3–4.1 µm (Me = 5.2 × 3.7 µm, N = 60), blueing in Melzer's reagent. **Ascospores** (13.9–)14.6–21.6(–23.6) × (6.3–)7.3–10.6(–11.4) µm, Q = (1.7–)1.8–2.4(–2.8), N = 420 (Me = 17.8 × 8.7 µm, Qe = 2), fairly variable in shape and dimensions, ellipsoid-inequilateral, with narrowly to broadly rounded ends, frequently heteropolar with one end broadly rounded, the other narrowly rounded, unicellular, dark brown to blackish brown, with a strongly sigmoid germ slit on the less convex side, slightly less than full length, fairly inconspicuous at maturity; occasionally with a minute unipolar secondary appendage visible in India ink; epispore smooth.

Asexual morph on the natural substrate occurring in autumn, consisting first of filiform, white, upright sterile synnemata 1–3 mm high arising either directly from the wood or from a black bulbous base, gradually covered by a thick olivaceous grey fertile layer and reaching a diameter of 0.25–0.5 mm; conidiophores dull olivaceous grey, 4–5 µm diam., in palisadic arrangement, sparingly ramified; conidiogenous cells terminal, cylindrical, light grey in 3% KOH, 20–34 × 3.5–4 µm, flexuous, frequently with a transverse septum, bearing terminal and scattered lateral secession scars; conidia produced holoblastically in sympodial sequence, hyaline, olivaceous grey in mass, 7–9 × 2.5–3.5 µm (N = 30), ellipsoid to faintly clavate, attenuated at base and slightly truncate. A similar conidiogenous structure occasionally arises through the ostioles of overmature stromata, forming olivaceous hemispherical to pulvinate masses.

Cultural characteristics: Colonies 30–40 mm diam. at four weeks, white to greyish white, cottony, azonate, with irregular outgrowths or furrows, with a peripheral fan-shaped extension spreading toward the edge of the Petri dish, reaching it at six weeks; medium unstained; reverse orange brown. At this time short, upright, white synnemata 0.5–1 mm high × 0.2–0.4 mm wide develop on the fan-shaped region of the colony, developing a superficial layer of olivaceous brown hyphal tissue that remains sterile at four months incubation.

Additional specimens examined (paratypes in bold): Malta, Gozo, Nadur, Wied ta' Binġemma, on bark of a dead fallen branches of *Prunus dulcis* (*Rosaceae*), *Olea europaea* (*Oleaceae*) and decaying canes of *Arundo donax* (*Poaceae*) on humid ground, 29 Mar. 2020, *leg*. S. Mifsud, **SM467** (LIP), GenBank ITS sequence MW513723, LSU sequence MW513726; Gozo, Kerċem, Wied il-Lunzjata (is-Sellum),

Fig. 3 – Overview of the site where the holotype of *X. melitensis* was collected, a shady grove dominated by *Laurus nobilis* lined by a dense undergrowth of *Acanthus mollis* L. (*Acanthaceae*). (Photo CS)

on decaying fallen branches of *Ceratonia siliqua* (*Fabaceae*), 29 Oct. 2020, *leg*. S. Mifsud, **SM510** (SM); Gozo, Xagħra, Wied tal-Egħżien, fallen and decayed twigs of *Eucalyptus gomphocephala* (*Myrtaceae*) in a damp area, 31 Oct. 2019, *leg*. S. Mifsud, SM417 (SM); Siggiewi, Buskett, 35.856131° N, 14.399269° E, 200 m asl, on a rotting branch under *Quercus ilex* (*Fagaceae*) and *Laurus nobilis*, 8 Jan. 2014, *leg*. C. Sammut, **CS484** (LIP) (barely mature); *ibid*., 35.856167° N, 14.398833° E, 193 m asl, on a dead corticated branch under *Quercus ilex*, *Laurus nobilis* and *Rhamnus alaternus*, 25 Feb. 2017, *leg*. C. Sammut, CS1096 (LIP); *ibid*., on a dead corticated branch of *Laurus nobilis* 2 cm diam., 22 Mar. 2017, *leg*. C. Sammut, **CS1115** (LIP); *ibid*., on a dead corticated branch, 7 Apr. 2018, **CS1221** (CS).

Isotype of *Xylaria sibirica*: Russia. Primorsky Territory, natural reserve Kedrovaya Pad, vicinity of reserve office, a slope of the Gakkilevsky Ridge, 500 m upstream the Kedrovaya River, on branches, 19 Aug. 2005, *leg*. E. Popov, CWU (Myc) AS-2065, communicated by A. Akulov to JF.

Known distribution: Maltese Islands.

Habitat: on dead, mostly corticated rotten branches of various trees (*Eucalyptus gomphocephala*, *Ceratonia siliqua*, *Laurus nobilis*, *Prunus dulcis*, *Olea europaea*) or rotten canes of *Arundo donax*, lying on the ground in shady and relatively humid groves.

Comments: *Xylaria melitensis* is characterized by small pulvinate stromata with slightly to strongly exposed perithecial contours and a light brown, scaly-cracked surface before maturity, turning dark brown to blackish at maturity, frequently with low ridges in lower half. In contrast, the stromata of *X. sibirica* have a smooth surface lacking cracks or ridges, with a superficial, long persistent, white powdery to downy layer and less exposed perithecial contours. The ascospores of *X. melitensis* are blackish brown at maturity, slightly inequilateral and fairly variable in shape and dimensions, while those of *X. sibirica*, though in a similar size range (Table 1) are paler brown at maturity, making the germ slit more conspicuous, equilateral and consistently feature broadly rounded ends; the sigmoid germ slit is likewise more slanted and tends to be spiralling (Ju *et al*., 2009; Fournier, 2014).

The comparison of our cultures with those of *X. sibirica* is hampered by the fact that they were obtained on PDA instead of OMA, which may explain their morphological differences as the fan-shaped lateral extensions and the orange brown reverse of the colonies (Plate 3). Cultures of both species produce synnematous conidiomata but while those of *X. melitensis* are scattered and simple and remain sterile, those of *X. sibirica* are flabellate, assignable to xylocoremium-like and yield a fertile conidiogenous structure.

Conidiomata occurring on the natural substrate prior to the development of the sexual morph were observed in two collections (SM467 and SM510). These conidiomata differ from the flabellate xylocoremium-like asexual morph of *X. sibirica* by small, upright white synnemata remaining simple, occasionally arising from a black bulbous base, coated with a dense olivaceous grey conidiogenous layer yielding conidiophores and conidiogenous cells arranged in a palisadic pattern typical for *Xylaria* (Plate 3). Olivaceous grey hyphae could be observed on some synnemata in culture but they did not develop further to conidiogenesis after four months incubation; an olivaceous grey fertile conidiogenous structure similar to that observed on conidiomata on the natural substrate could likewise be encountered emerging from the ostioles of overmature stromata. This type of asexual morph is clearly different from the xylocoremium-like asexual morph of *X. sibirica* and most unusual within *Xylaria* where a greenish asexual morph, when present, develops on immature stromata of the sexual morph, especially in some species of the *X. polymorpha* aggregate as defined by Hsieh *et al.* (2010).

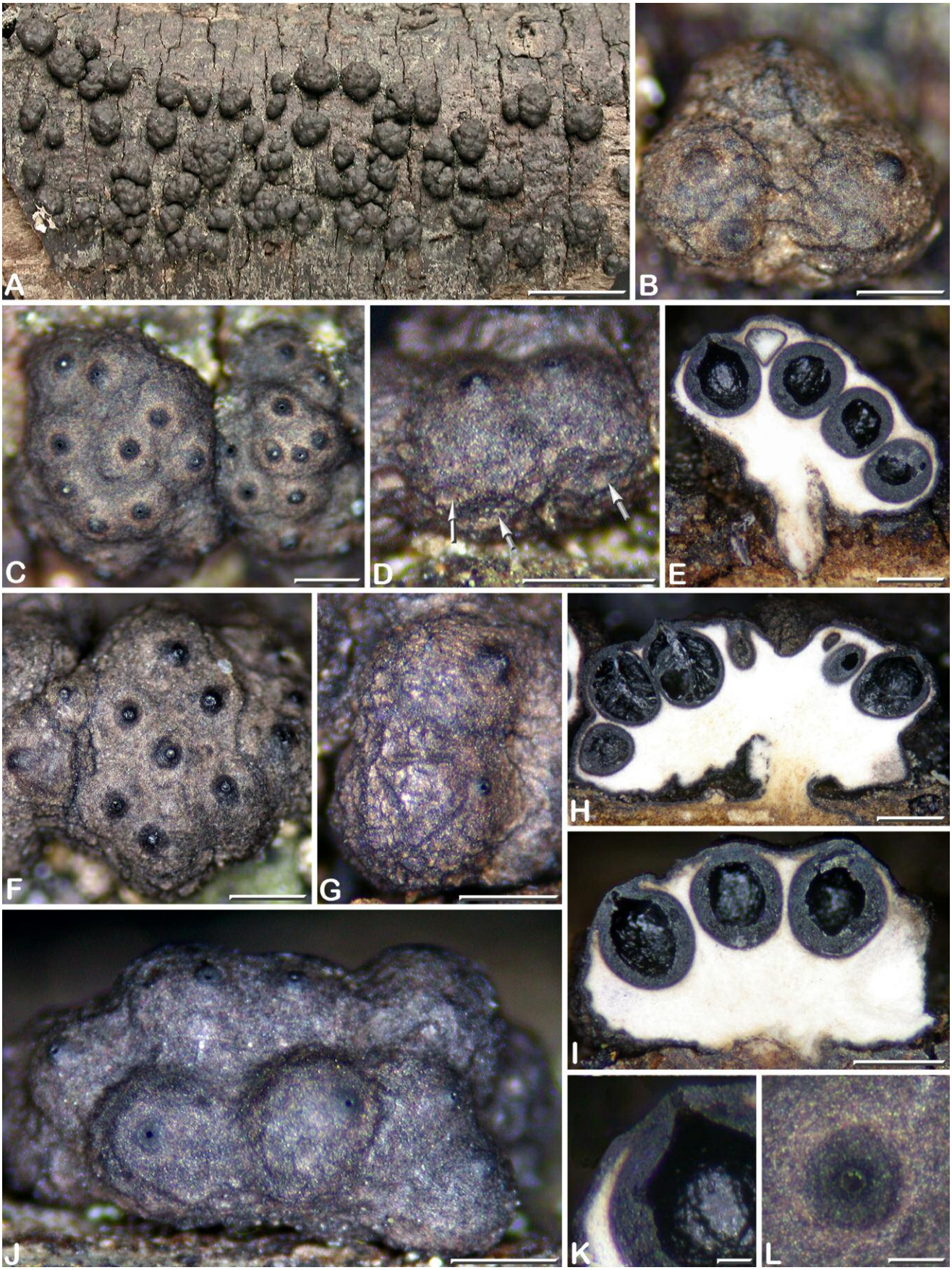

Plate 1 – *Xylaria melitensis* macroscopic characters

A-E, I, K, L: CS1223 (holotype); F-H: SM467 (paratype); J: CS1115 (paratype). A: Habit of mature stromata; B: Immature stroma with a light brown and cracked surface; C: Mature stromata with ostioles surrounded by a paler discoid halo; D: Mature stroma in side view showing a papillate ostiole and a lower half roughened by low ridges (arrows); E, H: Shortly stipitate stromata in vertical section; F: Mature stroma in top view showing shiny black papillate ostioles and inconspicuous perithecial contours with an even surface; G: Mature stroma in side view showing a finely cracked surface; I: Sessile stroma in vertical section; J: Blackish mature stroma with strongly exposed perithecial contours and porate ostioles; K: Vertically cut perithecium in close-up showing a thin stromatal crust; L: Papillate ostiole on a black discoid base. Scale bars: A = 5 mm; B-J = 0.5 mm; K, L = 100 µm.

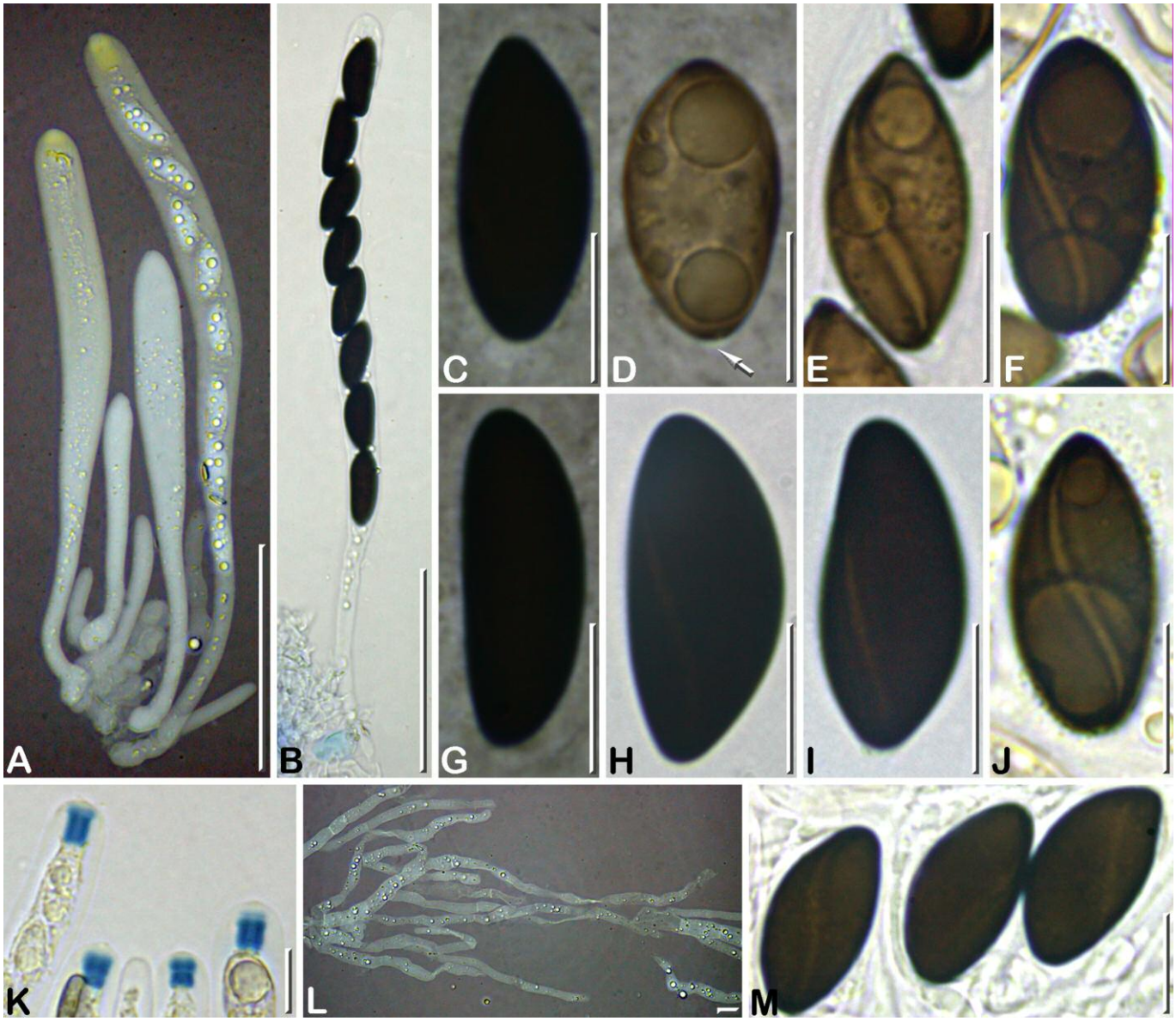

Plate 2 – *Xylaria melitensis* microscopic characters

A, K: CS 484 (paratype); B-J, M: CS1223 (Holotype); L: SM467 (paratype). A: Bundle of immature asci, in black Pelikan ink; B: Mature ascus, in diluted blue Pelikan ink; C: Heteropolar mature ascospore in diluted India ink; D: Immature ascospore showing a minute basal secondary appendage (arrow), in diluted India ink; E, F, J: Immature ascospores showing a sigmoid germ slit, in Melzer's reagent; G: Mature ascospore with broadly rounded ends, in diluted India ink; H, I: Mature ascospores in 1% SDS; K: Ascal apical apparati of immature asci, in Melzer's reagent; L: Basal portion of paraphyses, in black Pelikan ink; M: Mature ascospores, in 1% SDS. Scale bars: A, B = 50 µm; C-M = 10 µm.

Table 1 – Ascospore dimensions from seven collections of *X. melitensis* showing a fairly wide range of intraspecific variations, compared with those of the holotype of *X. sibirica*.

Collections numbers	Ascospore measurements with extreme values in parentheses (µm)	Q = quotient l/w N = number of measurements	Mean values (µm)
CS484	(16.3–)18.4–21.6(–22.8) × (7.6–)8.7 × 10.6(–11.4)	Q = (1.8–)1.9–2.3(–2.7), N = 60	Me = 19.9 × 9.5, Qe = 2.1
CS1096	(16.4–)17.1–20.6(–23.6) × (7.4–)8.1–9.4(–10.2)	Q = (1.8–)1.9–2.4(–2.8), N = 60	Me = 18.8 × 8.8, Qe = 2.2
CS1115	(14.9–)16.1–18.1(–18.5) × (7.6–)7.9–9.5(–9.7)	Q = (1.7–)1.8–2.1(–2.2), N = 60	Me = 17.1 × 8.7, Qe = 2.0
CS1223 holotype	(15.8–)16.5–19.4(–20.3) × (6.9–)7.5–8.7(–9)	Q = (1.9–)2–2.4(–2.7), N = 60	Me = 17.9 × 8.1, Qe = 2.2
SM417	(15.6–)17.3–17.8(–19.5) × (8.4–)9.0–9.8(–10.4)	Q = (1.7–)1.8–2.0(–2.2), N = 60	Me = 17.5 × 9.4, Qe = 1.9
SM467	(13.9–)14.6–17.8(–20.3) × (6.3–)7.3–8.6(–9.1)	Q = (1.7–)1.8–2.3(–2.7), N = 60	Me = 16.4 × 8, Qe = 2.1
SM510	(14.7–)15.6–18.4(–20.0) × (6.9–)7.6–9.1(–9.4)	Q = (1.8–)1.9–2.2(–2.7), N = 60	Me = 17.1 × 8.4, Qe = 2.0
Cumulated values	(13.9–)14.6–21.6(–23.6) × (6.3–)7.3–10.6(–11.4)	Q = (1.7–)1.8–2.4(–2.8), N = 420	Me = 17.8 × 8.7, Qe = 2.0
X. sibirica Ju *et al.* (2009)	(14–)15–19.5 × (6–)7–9 µm		Me = 17.3 × 8, Qe = 2.2

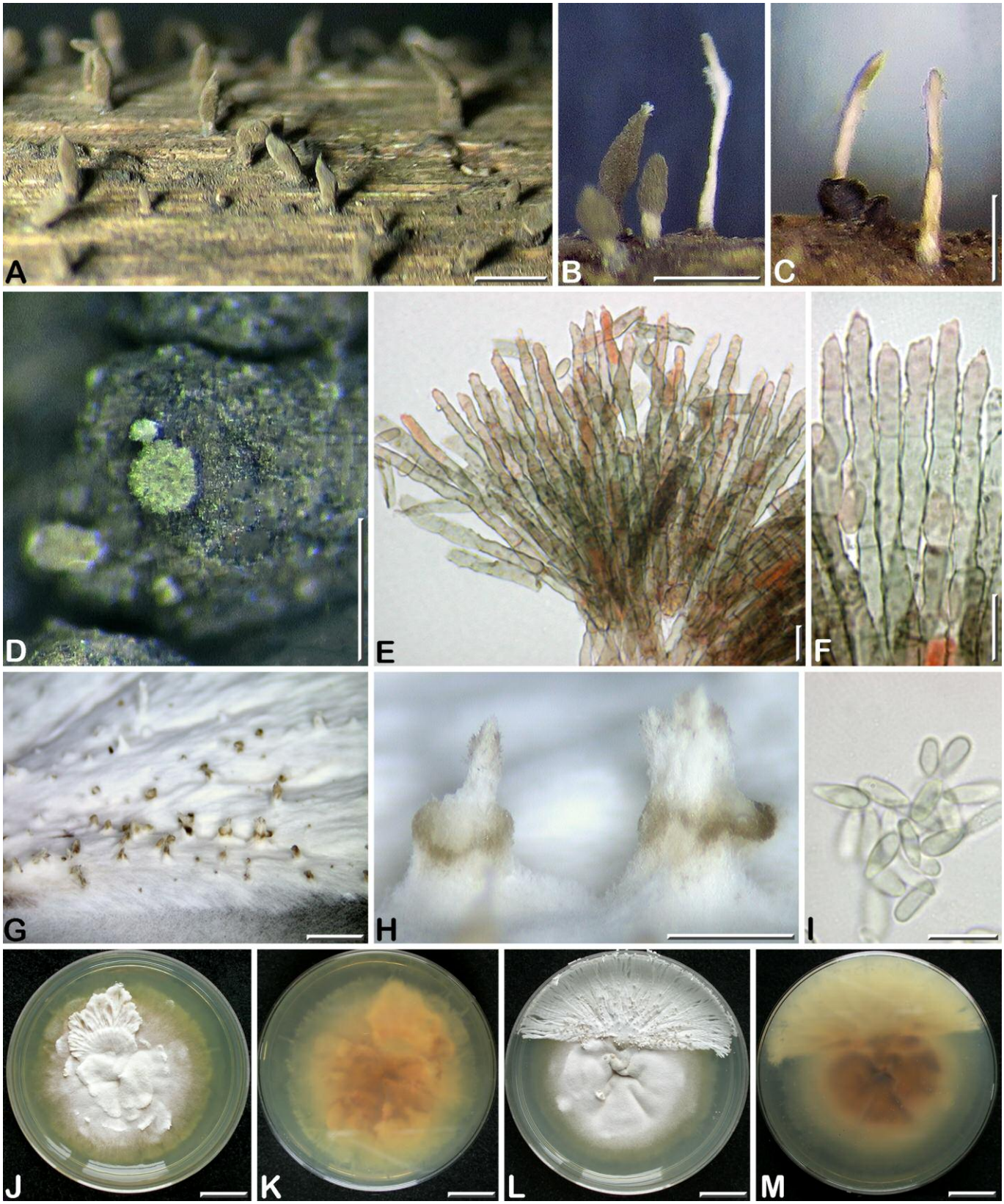

Plate 3 – *Xylaria melitensis* asexual morph and cultures

A-I, L, M: SM 467; J, K: CS1223. A: Fertile conidiomata on host surface; B: Three fertile conidiomata associated with a sterile white synnema; C: Two sterile synnemata, one arising from a black bulbous base; D: Pulvinate mass of fertile conidiophores arising from the ostiole of an overmature stroma; E: Bundle of conidiophores and conidiogenous cells; F: Conidiophores in palisadic arrangement showing secession scars (E and F in Congo red followed by 3% KOH); G, H: Sterile synnemata in culture at four months incubation; I: Conidia in 3% KOH; J, L: Colonies at six weeks; K, M: Reverse of colonies at six weeks. Scale bars: A-C, H = 1 mm; D = 0.5 mm; E, F, I = 10 µm; G = 5 mm; J-M = 10 mm. (Images A-D by SM)

Morphological features of both asexual and sexual morphs appear fairly conclusive to distinguish *X. melitensis* from *X. sibirica* and are supported by the analysis of both ITS-based and LSU-based phylogenies (Figs 1 and 2). They show the same unexpected results with the two strains of *X. melitensis* clustering on the same isolated branch among species of the *X. polymorpha* aggregate while both strains of *X. sibirica* cluster on a distant branch that appears basal to the *X. polymorpha* aggregate. However, these conclusions should be re-evaluated on the basis of a multigene phylogenetic analysis similar to that carried out by Hsieh *et al.* (2010) and U'Ren *et al.* (2016), providing a better phylogenetic resolution than ITS and LSU sequences as to generic affinities (Ju, pers. comm., Jan. 2021).

ITS sequences of collections CS1223 and SM467 have 99.5% similarity, and those of the two strains of *X. sibirica* have 99.1% similarity. Those of the strains of *X. melitensis* and *X. sibirica* have only 89.5–90% similarity, strongly supporting the recognition of *X. melitensis* as a distinct species. In the ITS-based phylogenetic tree (Fig. 1), *X. plebeja* Ces. appears as the closest relative of *X. melitensis*. Though phylogenetically related to the same aggregate of taxa related to *X. polymorpha* (PO clade, Hsieh *et al.*, 2010), *X. plebeja* is a taxon known from South East Asia that clearly differs from *X. melitensis* by upright cylindrical to fusiform or subclavate stromata with a surface roughened by small black scales and a faintly yellow subsurface tissue, along with smaller ascospores 8.8 × 4.2 µm on average with a straight germ slit (JF, unpublished data based on material from Taiwan communicated by Dr. Ju).

As mentioned in the introduction, two penzigioid species known from North temperate regions must be considered for comparison with *X. melitensis*, namely "*Rosellinia*" *callosa* and *X. crozonensis*. The former awaits molecular data to be formally transferred to *Xylaria* but shares some similarity with *X. melitensis* in having small, few-perithecate, pulvinate, sessile, soft-textured stromata with a cracked surface. It can be distinguished by its ecology mostly restricted to damp riparian stations and larger, fusoid-inequilateral ascospores 25.5 × 7.2 µm on average with acute ends and with a germ slit much less than spore length (Fournier, 2014). *Xylaria crozonensis* can be distinguished by peltate stromata with a black, undulate carbonaceous surface and equilateral ascospores 14.6 × 8.2 µm on average with narrowly rounded to subacute ends and an inconspicuous straight germ slit less than spore length. It is so far only known from regions under strong oceanic influence as Brittany (France), Cornwall (U.K.) and Galicia (Spain) (Fournier, 2014; Rubio *et al.*, 2016).

Other members of the *Xylariaceae* so far reported from the Maltese Islands are *X. arbuscula* Sacc. (Saccardo, 1912), *Anthostomella appendiculosa* (Berk. & Broome) Sacc. (Saccardo, 1915), *Dematophora necatrix* R. Hartig (Porta-Puglia & Mifsud, 2006) and *Xylaria sicula* Pass. & Beltrani (Sammut, 2016). The paucity of reports of xylariaceous fungi from the Maltese Islands listed above highlights how their dry climate is likely unfavourable to these fungi, making the numerous collections of *X. melitensis* reported herein all the more remarkable.

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Authors' contributions

JF was responsible for the study conception, morphological studies, plates design, registration in MycoBank and writing a first draft. CL performed *in vitro* cultures, phylogenetic analyses, figures design and registrations in GenBank. SM and CS collected material and provided voucher specimens, photos and ecological information, and made preliminary morphological observations and constructive comments and suggestions on the first draft. All authors read and approved the final manuscript.

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