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Current status of *Asplenium sagittatum* (*Aspleniaceae*) in the Maltese islands

Abstract

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Asplenium sagittatum is a rare, indigenous fern which was assumed extinct in the dated Maltese Red Data book until 2008 when a small number of individuals was rediscovered. New records of this fern are reported, including a large and important population located in the north of mainland Malta. For the first time, the anatomical characters of Maltese material are described and compared with those of material from central Europe. The paper also presents taxonomical clarifications dealing with *A. scolopendrium*, as also the results of a local Red List assessment for this endangered fern.

Key words: *Asplenium scolopendrium*, flora of Malta, Central Mediterranean region, Red List, pteridophytes, ferns.

Introduction

Ten species of ferns are listed in the inventory of Maltese flora, with the latest addition being an endemic subspecies of *Polypodium vulgare* L. (Peroni & al. 2013). The following pteridophytes are known to occur in the Maltese islands: *Selaginella denticulata* (L.) Spring (rare), *Equisetum ramosissimum* Desf. (frequent but of localized distribution), *Adiantum capillus-veneris* L. (frequent), *Anogramma leptophylla* (L.) Link (locally frequent), *Asplenium sagittatum* (DC) Bange (rare), *A. ceterach* L. (rare), *A. trichomanes* L. (rare), *A. marinum* L. (very rare), *Pteridium aquilinum* (L.) Kuhn (very rare) and *Polypodium vulgare* subsp. *melitensis* Peroni A., Peroni G. & Mifsud S. (very rare). *Salvinia natans* (L.) All. was recorded by Gulia (1909) but has not been recorded since and it is hence assumed that this species has either been extirpated within its range in the Maltese Islands or that its presence has, over the course of over a century, been overlooked; an alternative possibility is that the 1909 record was misidentified. Similarly, the authenticity of old records of *Asplenium scolopendrium* L. recorded by Gulia (1909) and Borg (1927) is uncertain, and there is assumed to be misidentification (with *A. sagittatum*), as noted by Sommier & Caruana Gatto (1915) and discussed in further detail below.

In Malta's dated Red List (Lanfranco 1989), *Asplenium marinum* and *A. sagittatum* were listed as possibly extinct, due to lack of substantiated records. However, these species have been rediscovered in small numbers in recent years (Lalov & al. 2008; Mifsud 2010a, 2010b). Recent findings include a large population of *A. sagittatum* at Qammieh, Mellieha, which is described in this contribution. We also present the findings of cytological examination of individuals from this population, to increase knowledge of Maltese material of this species, and to enable comparison with *A. scolopendrium*, which was reported over a century ago (Gulia 1909).

Distribution

Asplenium sagittatum is a species of pan-Mediterranean distribution, found in Spain (including the Balearic islands), France (including Corse), Italy (including Sardinia and Sicily), as well as in Malta, Croatia, Greece, Lebanon, Syria, Israel, Jordan, Libya, Algeria, Tunisia and Morocco (Greuter & al. 1984; GBIF, 2013), Palestine, the Anatolian plateau, and the Aegean islands (GBIF 2013). It has an array of synonyms (= *A. hemionitis* Sw. [non L. 1753]; *Phyllitis hemionitis* Kuntze; *Phyllitis sagittata* (DC.) Guinea & Heywood; *Scolopendrium sagittatum* DC. and *S. hemionitis* Lag.). There has not been full consensus regarding the separation of the genus *Phyllitis* from *Asplenium*; notwithstanding, and without going into the merits of classification and nomenclature, *Asplenium sagittatum* is used in this communication, in concurrence with established classifications such as Euro+Med (2006-), Tropicos (2015), and The Plant List (2013) and in line with recent treatments, for example, by Hassler (2015).

In the Maltese Islands, *Asplenium sagittatum* has been recorded under different synonyms from coastal rocks and wells in various locations (Fig. 1 and Table 1). These include the vicinity of an area referred to as **Mistra rocks,** on the northern coastal stretch of **Nadur,** better known as **Rdum il-Kbir** (Duthie 1872; Caruana Gatto 1892; Sommier & Caruana Gatto 1915; Borg 1927), **Mellieħa** (Caruana Gatto 1892; Gulia 1909; Sommier & Caruana Gatto 1915; Borg 1927), **Għajnsielem** (Borg 1927), **Dwejra** in Gozo (Gulia 1909; Sommier & Caruana Gatto 1915; Borg 1927), **Nadur valley** (Gulia 1909; Sommier & Caruana Gatto 1915), **Xlendi valley** (Gulia 1909; Sommier & Caruana Gatto 1915; Borg 1927**), Ras il-Kala, Qala** (Gulia 1909; Sommier & Caruana Gatto 1915; Borg 1927), **Wied Babu** (Gulia 1909; Borg 1927), **Wied Għomor** (Gulia 1909; Borg 1927), **Għajn Tuffieħa** (Borg 1927), **Wied il-Għasel** (Borg 1927), and wells in **Birkirkara, Lija**, and **Mosta** (Borg 1927).

The lack of substantiated records for several decades after Borg's (1927) records led to the assumption that *A. sagittatum* had possibly become extirpated (Lanfranco 1989), and as a result of its extended absence, this was subsequently considered to have become extinct locally (Tabone 2007). However, in April 2008, Lalov & al. (2008) carried out numerous field searches which led to its rediscovery at five different sites (Table 1: pop 1-5). Their records consisted of populations with 25 individuals or less. In March 2009, one of the authors [SM] found a sizeable population of 30–40 specimens (including young sporophytes) in a deep fissure within the scree at Mistra rocks in Gozo (Table 1: pop6), and an additional small cluster of four specimens two months later (Table 1: pop7). The extent of its local distribution was further broadened when the same author discovered scattered specimens within the scree of Rdum Majjiesa on October 2010 (Table 1: pop8).

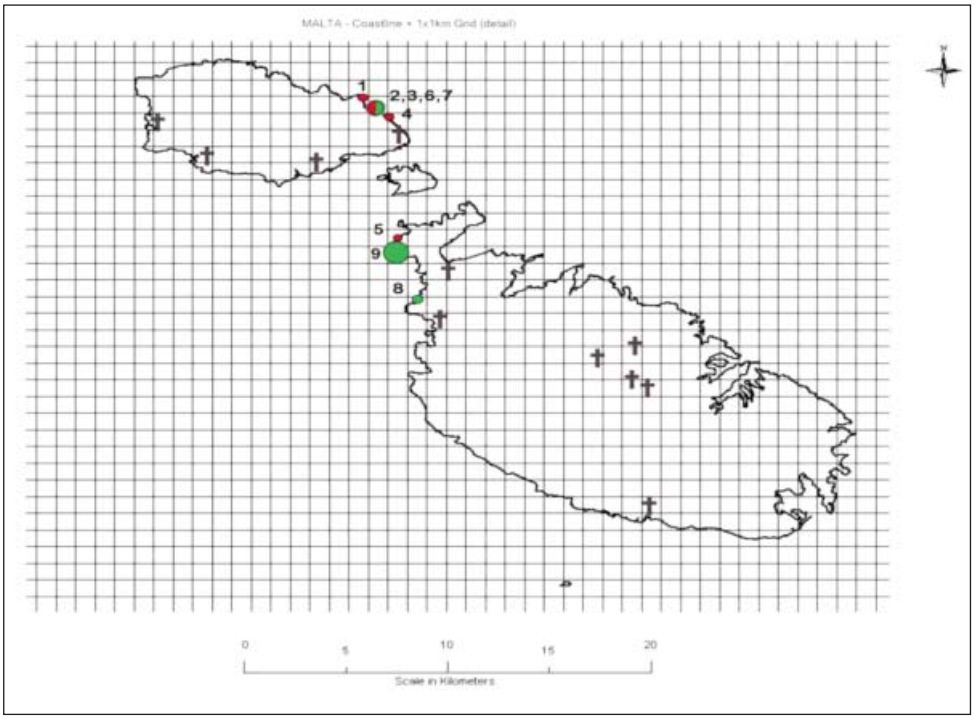

Fig. 1. Distribution of *Asplenium sagittatum* in the Maltese islands. Legend: Circles indicate populations which have been rediscovered during the last eight years - red by Lalov et al. (2008) only; green by present authors during the last six years (of which some have already been reported by Lalov & al (2008)); numbers refer to toponyms in table 1; † indicate historical records which have not been reconfirmed in recent years.

In May of 2015, three of the present authors [SF, SM and LFC] were conducting fieldwork to assess the status of *Hyoseris frutescens* Brullo & Pavone within il-Qammieh area, Mellieha (Fenech 2015), when a large population of ferns with long, unlobed fronds was observed at the base of a relatively dark, inaccessible limestone fissure. (Table 1: pop9). Due to the inaccessibility of the population, a second visit was planned with appropriate abseiling equipment. This latest discovery, on which the present communication is based, comprises a large population within a deep crevice at Qammieh, L-Ahrax tal-Mellieha.

Habitat context

The Maltese islands, comprised almost entirely of sedimentary rocks of Tertiary age, lie on the Siculo-Tunisian sill, (Schembri 1997; Cassar 2010). The islands support no perennial fluvial sources (beyond sparse ephemeral streams, fed by seepage from spring-lines) and few permanent bodies of water (with the exception of few small wetlands dependent on seasonal run-off during the wet season). The highest elevations are 253 m a.s.l. and 190 m a.s.l. on the islands of Malta and Gozo, respectively (Schembri 1997; Cassar 2010) and the indigenous vegetation

is thus within the limits of the Thermo-Mediterranean zone. The climate is typified by dry, hot summers and mild, wet winters, with the landscape largely influenced by this biseasonality (Cassar 2010). The ensuing semi-aridity and the pervasive lack of woodlands do not provide abiotic conditions that favour widespread colonization by ferns.

The Qammieh region, which lies on the northern Ahrax promontory, is characterized by exposed Upper Coralline Limestone, with karstic features predominating. The wind-swept (due to exposure to northerly winds) plateau surface is colonized by a suite of biotopes typical of garrigue and phrygana assemblages. The underlying Blue Clay, a more dynamically malleable stratigraphy and which is thus prone to inducing mass movement, is largely responsible for slippage; such processes lead to the formation of boulder screes and deep limestone crevices on and around the plateau escarpment, within which species like *Asplenium sagittatum* thrive.

Material and Methods

A visit to the site of this newly-discovered population (Table 1: pop9) was conducted in July 2015, when one of the authors (MN) descended into the rocky limestone fissure, using specialized abseiling equipment. Natural belay formations within the karstic Upper Coralline Limestone were used to secure abseiling lines, while another of the authors (LFC) acted as belayer. Dyneema slings of 120 cm and 2 locking carabiners were used for this purpose.

During this sampling exercise, three fronds were collected for identification and taxonomical purposes. It was noted that the fern population colonized the lower-most sloping southern face (north-facing) of the fissure, where conditions were rather humid, and its immediate, lower sides, where substrate had accumulated; this substrate consisted of a combination of fugitive sediments, comprising terrarossa and palaeosol, conveyed into the 15 m deep fissure from the Upper Coralline Limestone karstic surface via freshwater runoff and aeolian dynamics. Owing to the dense population, and to the relatively inaccessible location and conditions in the gorge, it was difficult to accurately count individuals and population size was thus estimated. The conditions at the site were noted to be ideal for growth of *Asplenium sagittatum.* In addition to it at the bottom of the fissure, *Adiantum capellus-veneris* L. was also present in small numbers, as were other rupestral species, namely *Hyoseris frutescens* Brullo & Pavone and *Hypericum aegyptium* L., on the upper, exposed reaches of the limestone fissure (albeit in limited number). Another site visit was carried out by one of the authors [SM] on April 2nd 2016, to survey the north-facing scree of Rdum il-Qawwi.

Anatomical examinations focused on the spores and the epidermis of fronds of specimens collected during this abseil The stomata and cells of the abaxial epidermis are of diagnostic value (Peroni & al. 2008) and were examined using a × 100 and × 400 light microscope. The epidermis was peeled carefully under a dissecting microscope (× 16) using a razor blade and mounted in 10% glycerol aqueous solution. Stomata types referred to are based on Cotthem van (1970) and Peroni & Peroni (2004).

Red listing of the species was conducted according to IUCN guidelines (IUCN 2012a) and Categories and Criteria (IUCN 2012b), with a grid of 2 km × 2 km used for determining Area of Occupancy.

Results

Overview

The population at il-Qammieh was noted to be quite dense in comparison with the other Maltese populations, with as many as 10 mature ferns/m^2 colonizing a stretch of about 25 m in length and of a varying width of 1–2 m, totalling an estimated 300–400 individuals. This effectively appears to be the largest population of *Asplenium sagittatum* within the Maltese islands recorded to-date and, as a consequence, is of conservation significance, both in terms of species and habitat. Population 5 (Table 1), also from the same area, suggests that other pockets with this fern may be present in non-accessible sites along the scree and cliffs of this area. During the second site visit of the 2nd April 2016, some 40 specimens of *Asplenium trichomanes* were discovered at Rdum il-Qawwi. This species is also rare and endangered in the Maltese islands; it was listed as 'possibly extinct' in the Red Data Book (Lanfranco 1989), but its presence was subsequently reconfirmed by Tabone (2007) and by one of the present authors (Mifsud 2009).

Habitat preference

The microhabitats of the various populations of *A. sagittatum* in Malta share common characteristics of a damp, shaded, sheltered location in chambers or caverns formed by scree, in caves within shattered rock profiles, or in rock fissures of Upper Coralline Limestone in coastal scree areas. Thus far, only the population at Qammieh is known to receive direct sunlight for a short period of time; this was measured on 10th October 2015 by [SF], with a total of 57 min of direct sunlight (between 11:50 and 12:47) recorded.

Taxonomy and anatomy

The following morphological description of *A. sagittatum* is based on Population 9 (Table 1, Fig. 2), since, as noted above, this is a relatively large population of about 400 individuals, and the examination of three fronds in this case did not cause any harm to the population.

Ferns have 6–10 tufted leaves, with laminae measuring between 8–24 cm long; they are dark green, glossy above, narrowly deltoid or oblong-deltoid with an entire margin, with a sub-acute tip and auricled base with a broad-cordate to typical hastate shape, usually cordate in young fronds and becoming hastate in mature fronds, forming two oppositely divergent, broadly-triangular lobes up to 6 cm from tip to midrib. The petiole is slightly shorter from the lamina, is dark-green to black at the lower half and with slender, brownish, hair-like scales. Sori are cinnamon brown when mature, narrowly elliptical to rectangular, with each receiving two veins at the base and apex, located in a row between the frond's margin and midrib at an angle of 45 °, usually with another series of shorter sori in the lower half of large fronds. Sporangia are 180–220 µm in diameter with about 20 amber to brown annulus cells. The size of the spores is (31.3–) 34.9 (–39.8) × (23.2–) 26.5 (– 28.7) µm [n = 25] including the winged exospore, which is about 2 µm broad. The stomata of the abax-

Table 1. List of substantiated records of *Asplenium sagittatum* and related population sizes from Malta.

Population ref. no. and date	**Site, Locality, Island**	**Size** (no. of individuals)	**Landform**	**Observed by**
1. Apr. 2008	Western part of Mistra rocks, Nadur, Gozo	25	n/a	Lalov & al. (2008)
2. Apr. 2008	Central part of Mistra rocks, Nadur, Gozo	4	n/a	Lalov & al. (2008)
3*. Apr. 2008	Eastern part of Mistra rocks, Nadur, Gozo	20	Rocky cavities between large displaced boulders forming a scree (= mass movement dynamics)	Lalov & al. (2008)
4. Apr. 2008	Rdum San Filep, Nadur, Gozo	5	Rocky cavities between large displaced boulders forming a scree	Lalov & al. (2008)
5. Apr. 2008	Rdum il-Qawwi, Mellieha, Malta	22	Shaded cliff side	Lalov & al. (2008)
6*. Mar. 2009	Eastern part of Mistra rocks, Nadur, Gozo	35**	Deep limestone fissures	SM ^
7. May. 2009	Central part of Mistra rocks, Nadur, Gozo	3	Rocky cavities between large displaced boulders forming a scree	SM ^
8. Oct. 2010	Rdum Majjiesa	5	Rocky cavities between large displaced boulders forming a scree	SM ^
9. May 2015	Rdum il-Qawwi/Qammieh area Mellieha, Malta	300-400**	Deep limestone fissure receiving partial sunlight	SM, LFC, SF ^
*Populations 3 and 6 are likely the same location. ^ Unpublished new records ** Mats of prothallic growth and many young specimens were observed				

ial surface were (42.3–) 49.9 (–57.2) × (34.8–) 40.7 (–47.3) µm [n = 27], polocytic (3-4(5) cells), or less frequently anomocyctic ((4)5–7 cells). The epidermal cells are deeply sinuous and approximately 80–120 µm in length along their longest axis. In comparison with the abaxial epidermis, the adaxial surface is without stomata, and consists of smaller (c. 80 µm along their longest angle) cells that are less sinusoidal and usually with angular wide lobes (Figure 3a-d).

The macro-morphological features of the Maltese population correspond to those of *A. sagittatum* (e.g. Pignatti (1982); Crabbe & al. (1993); Ferrarini & al. (1986); Ormonde (1998); Peroni & Peroni (2004)); however, cellular anatomy discrepancies were noted when compared with reports of Peroni & Peroni (2004), Ferrarini & al. (1986); and Rossello & al. (1990) in Peroni & Peroni (2004). The stomata and spores sizes measured from *A. sagittatum* (Malta) are reported and compared in Table 2.

It was found that the material of *A. sagittatum* from Qammieh, Malta has remarkably larger stomata, slightly larger spores, and different morphology of the epidermal layers. The spore ornamentation of the Maltese material seems to be more pronounced than that illustrated by Peroni & Peroni (2004), with a broader exospore wing of about 2–3 µm (Figs. 3H and 3I). The upper epidermis consists of cells that are larger (60–80 µm) and much more lobed (Figs. 3A and 3C) from 27–45 µm, as documented by Peroni & Peroni (2004) and illustrated in Figs. 3F and 3G. The abaxial epidermis cells (Figs. 3B and 3D) are more similar to those illustrated for *A. scolopendrium* than to those illustrated for *A. sagittatum* (Peroni & Peroni 2004), being more deeply sinuous and with longer lobe-like

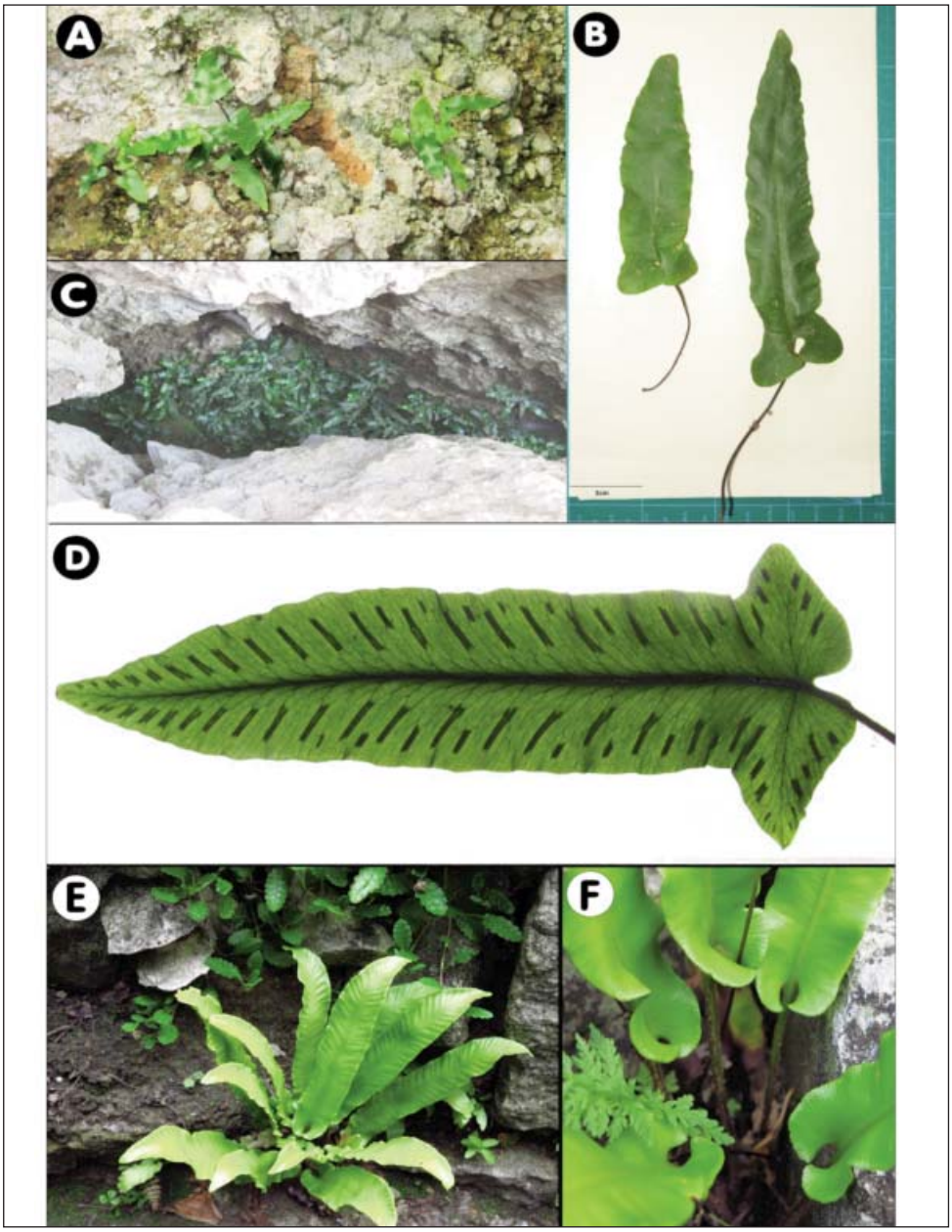

Fig. 2. *Asplenium sagittatum,* Malta. **A**. Eastern part of Mistra Rocks, Nadur, Gozo (March 2009); **B.** Young and mature fronds (Rdum il-Qawwi, Qammieh, Mellieha, July 2015); **C.** Rdum il-Qawwi, Qammieh, Mellieha, largest population ever recorded in Malta represented by over 300 plants (July 2015); **D.** Mature frond showing sori, venation, and basal divergent triangular lobes (Rdum il-Qawwi, Qammieh, Mellieha, July 2015); 2E-2F *Asplenium scolopendrium,* Warsaw, Poland. **E.** Whole plant (Jul 2015); **F.** Leaf bases showing cordate base (July 2015). Photos by Stephen Mifsud.

Table 2. Measurements of stomata and spores from *A. sagittatum* in Qammieh, Mellieha, Malta and comparison against reported measurements.

	Stomata length (µm)	**Stomata width** (µm)	**Spore length** (µm)	**Spore width** (µm)
Studies specimens				
A. sagittatum, Qammieh, Mellieha	(42.3-) **49.9** (- 57.2)	(34.8-) **40.7** (- 47.3)	(31.3-) **34.9** (- 39.8)	(23.2-) **26.5** (- 28.7)
Reported measures				
Peroni & Peroni (2004)	(33-) **40.9** (-48)	(27-) **31.7** (-36)	(27-) **30.8** (-36)	–
Ferrarini & al. (1986)	–	–	(24-) **29.7** (-34)	–
Rossello & al. (1990)	–	–	**27.3**	–
Ormonde (1998)	–	–	–	(24-) **27-30** (-33)

projections. This overall foliar anatomy of the Maltese *A. sagittatum* is hence more similar to *A. scolopendrium* sensu Peroni & Peroni (2004) (Fig. 3G). Since currently these foliar variabilities are not of any taxonomic importance, even at an infraspecific level, the examined Maltese material was treated as *Asplenium sagittatum*, the morphological plasticity of which was already demonstrated by Ferrarini & al. (1986).

Red Listing and conservation

In Malta, *A. sagittatum* was historically recorded as *Scolopendrium hemionitis* Sin. (Duthie 1872; Caruana Gatto 1893; Gulia 1909; Caruana Gatto 1915), while *A. scolopendrium* was recorded as *Scolopendrium vulgare* L. (Gulia 1909; Borg 1927). None of the aforementioned pioneering naturalists recorded both species concurrently in their respective works, with the exception of Gulia (1909); (it should nevertheless be noted that Gulia also catalogued previous records in his account of Maltese ferns). While it is difficult to ascertain if these early records truly represent two different species of spleenworts in Malta, it can be safely assumed that *A. scolopendrium* was misidentified and confused with *A. sagittatum*, owing to the fact that recent records all attest to *A. sagittatum*. Such a conclusion has already been suggested by various authors, including Sommier & Caruana-Gatto (1915), Lanfranco (1989), and Lalov & al. (2008). Such misidentification may have also arisen because young fronds of *A. sagittatum* have cordate leaf bases that look superficially like *A. scolopendrium* (Ferrarini & al. 1986; Marchetti 2004). Confusion of *A. scolopendrium* with *A. sagittatum* was also reported in the past in Italy (Marchetti 2004).

Using the current substantiated records of *A. sagittatum*, in Malta the Extent of Occurrence (EOO) is calculated to be 7.5 km^2 while the area of Occupancy (AOO) is 16 km^2. Population trend cannot be estimated since no counts have ever been published. Assuming that several historically recorded populations have become extinct, the number of locations has decreased by more than half over a 100 years; however, the rate of decrease over the last 10 years is uncertain with reference to application of criteria A. The current population size is estimated to be > 300 but < 800 individuals.

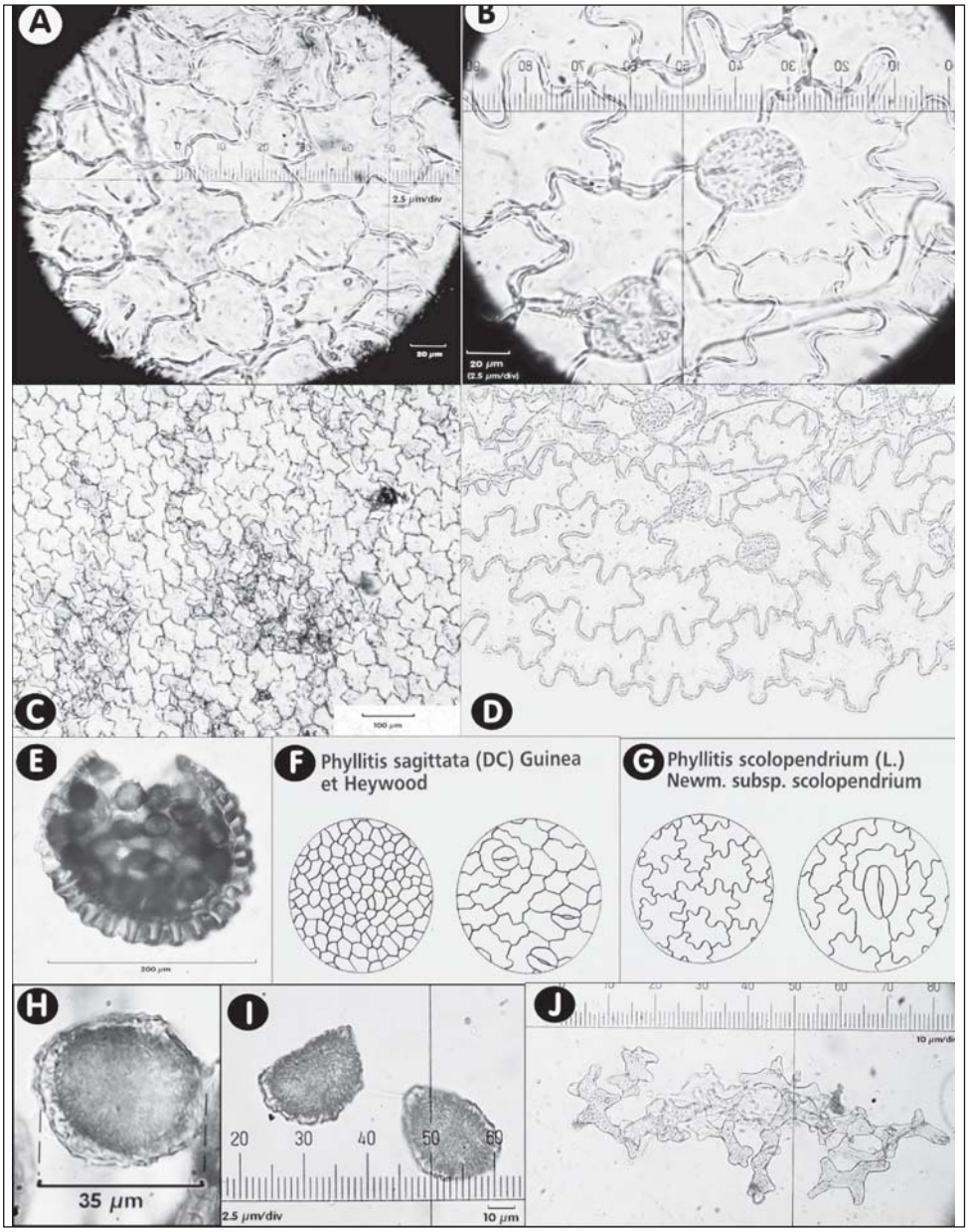

Fig. 3. Anatomical examination of *A. sagittatum*, from material at Rdum il-Qawwi, Qammieh, Mellieha, July 2015. **A.** Epidermis cells of adaxial face frond; **B.** Epidermis cells and stomata of abaxial face; **C.** Adaxial epidermis (low magnification); **D.** Abaxial epidermis (low magnification); **E.** Sporangium; **F.** Illustration of epidermis (left abaxial, right adaxial) of *Asplenium sagittatum*; **G.** Illustration of epidermis (left abaxial, right adaxial) of *Asplenium scolopendrium*; **H-I.** Spores; **J.** Chlorenchyma cells from the mesophyll layer. Photos by Stephen Mifsud. Illustrations **F** and **G** adapted from Peroni & Peroni (2004, pp 95-96).

Since *A. sagittatum* resides in inaccessible cavities and fissures in cliff screes, mostly located in areas that are protected at international level (Natura 2000 sites), the populations are not endangered by direct anthropogenic activities such as picking, land reclamation, grazing, construction works, or invasion by alien species. However, one major threat is habitat loss as a result of stochastic events, such as a tremor resulting in mass movement (landslide or scree collapse). Moreover, the decline of subpopulations during the last few decades, leading to 'presumed extinction' (Lanfranco 1989) must also be considered. It is unclear why so many populations are not extant, but one feasible explanation may relate to the reduction of natural water supply from springs emanating from perched aquifers, mainly due to the latter's continuous deterioration and exploitation by human agency, especially during the late 20th century.

Based on available data for the Maltese Islands, the Red List assessment of *Asplenium sagittatum* is EN B1ab (i,ii,iii); due to the small geographic scale of Malta, the resulting criteria obtained from the current AOO and EOO were downgraded by one level in accordance with IUCN (2012a) (assessor: Stephen Mifsud, September 2015).

Conclusions

Two new records of *Asplenium sagittatum* have been discovered from the boulder scree and rock fissures at Rdum Majjiesa and il-Qammieh, respectively. The latter comprises about 300-400 mature specimens, making it the most significant population in the Maltese Islands when compared to other reported populations that comprise of only a few individuals. Moreover, *Asplenium trichomanes* was also discovered at Rdum il-Qawwi, limits of Qammieh.

The cytology of the epidermis and the morphology of the spores were found to differ somewhat from documented descriptions of this species, but these slight differences are not considered to be of any taxonomic importance. A Red List assessment was carried out on the existing populations reported in the last ten years, resulting in 'endangered' status; this is an upgrade from Lanfranco (1989) assessment ('presumably extinct'). Given that all current records refer to *A. sagittatum*, there is considerable doubt over the listing of *A. scolopendrium* in historical records and the current authors assume that these records should be considered as referring to *A. sagittatum.*

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