Article

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Investigation on *Pisolithus* (*Fungi*, *Sclerodermataceae*) occurring in the Maltese Islands

Abstract

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The investigation of 20 populations of *Pisolithus* spp. collected from the Maltese Islands resulted in three different records. *Pisolithus albus* and *P. marmoratus* are new to Malta and were found associated with *Eucalyptus* spp. The third species, *P. arhizus*, was confirmed from two new stations and both were associated with *Quercus ilex*. A brief morphological and ecological comparison of the three species is given and supplemented by images and a dichotomous identification key.

Key words: *Pisolithus albus*, *Pisolithus arhizus*, *Pisolithus marmoratus*, *Eucalyptus*, *Quercus*, Malta, Gozo, gasteromycetes, macrofungi.

Introduction

The genus *Pisolithus* (*Sclerodermataceae*) currently comprises 20 legitimate species (Mycobank, 2022), distributed globally, but many are described from and native to geothermal areas (arid, temperate and subtropical regions), namely, Southeast Asia and Australasia (Lebel & al. 2018; Martin & al. 2002). Yet, the type species - *P. arenarius* Alb. & Schwein was collected from Lusatia, an area situated between Germany and Poland (Albertini & Schweinitz 1805). The genus is not well resolved and consists of some undescribed clades and accessions, especially in the 'tinctorius' and the 'albus' complex clades (e.g. Phosri & al. 2012; Martin & al. 2013; Lebel & al. 2018). Some of these clades have been described during the last decade. The latest work by Lebel & al. (2018) provided two new additions from Australasia: *P. thermaceus* T.Lebel, Pennycook & Beever associated with *Kunzea* spp., and *P. tympanobaculus* T.Lebel & M.D Barrett associated with *Eucalyptus* spp. In addition, *P. calongei* M.P. Martín, Phosri & Watling was described from Spain and is associated with *Cistus* spp. (Martin & al. 2013) and *P. orientalis* Watling, Phosri & M.P. Martín was described from Thailand is associated with *Pinus kesiya* Royle ex. Gordon (Phosri & al. 2012).

Pisolithus species are mycorrhizal and often show host-specificity to one or a few genera of trees such as *Eucalyptus, Acacia, Quercus, Pinus, Leucopogon, Hopea* and some woody shrubs such as *Leptosperum, Kunzea* and *Cistus* (Diez & al. 2001; Phosri & al. 2012; Martin & al. 2013; Lebel & al. 2018).

The first records of *Pisolithus* from the Maltese Islands were reported by Mifsud (2017), two from Malta (SM035, SM085) and one from Gozo (SM036) (see Table 1). Only SM085 was examined carefully and corresponded to *P. arhizus* (Scop.) Rauschert. The other two collections were presumed to be the same species for their close macromorphological resemblance and further influenced by the fact that almost all *Pisolithus* records from south Europe were ascribed to *P. arhizus* (GBIF, 2022). Recent studies in the Mediterranean region revealed the presence of other *Pisolithus* species, such as *P. albus* (Cooke & Massee) Priest from the Italian regions of Calabria and Sicily (Gargano & al. 2018) and from Tunisia (Jaouani & al. 2015), growing under *Eucalyptus* plantations.

Gargano & al. (2018) further advised that the distribution of *P. albus* is wider than assumed, and records of *P. arhizus* in the Mediterranean region should be re-analysed due to possible incorrect determinations. A similar situation stood in Australia, where the commonly reported *P. arhizus* (often reported as *P. tinctorius* (Pers.) Coker & Couch) was a misidentification to other Australasian native species and it was eventually excluded from the mycobiota of this continent and New Zealand (Lebel & al. 2018). *P. arhizus* is now confined as a northern hemisphere species. Based on these revisions and papers, the Maltese material was re-examined in surveys between 2018 and 2021 to assess better the inventory and associations of *Pisolithus* populations occurring in the Maltese Islands.

Materials and methods

Surveys were carried out in the three main islands of the Maltese archipelago (Malta, Gozo, and Comino). The fruiting bodies start to emerge from the soil as early as the end of August, well before the onset of rain precipitation, and they persist till December. Encountered populations and representative specimen(s) were first photographed, and then one sample was collected and cut in half to examine the peridium's thickness; the morphology of peridioles; the size of the stipe and the colour of the rhizomorphs and the base of the stipe. The trees present close by, and the general habitat (usually the soil type) were also noted. The collected samples were then further examined in the laboratory.

The spores were gathered in a mass, and the colour was noted. A pinch of spores was then transferred in a droplet of 4% KOH on a glass slide and added with a small amount of anti-surfactant (soap water) to ease the dispersion of the spores in the aqueous medium due to their strong hydrophobic character. Microscopy was carried out with a compound light microscope (Karl-Zeiss AxioLab RE), where the size of the spores and the morphology of the warts/spines on the exospore were given the most importance and viewed at ×1000 magnification. Identification was based on morphology by consulting the identification keys of Leonard (2011) and Leonard & McMullan-Fisher (2013), and the species descriptions and host-specificity provided by Martin & al. (2002); Phosri & al. (2012) and in particular, Lebel & al. (2018).

Table 1. Examined material from the Maltese Islands denoted by M (mainland Malta); G (island of Gozo) and C (island of Comino). The location of population SM522 was previously mentioned to one of the authors [SM] by Marica Lewis (pers. comm.).

Record Code	Collection Date	Locality and Toponym	Associated Plant Species	Soil type
SM035	11/01/15	Mġarr (Wied Gerżuma) [M]	*Eucalyptus gomphocephala*	Clayey
SM036	08/11/15	Qala (Wied Ħondoq ir-Rummien) [G]	*E. gomphocephala*	Clayey
SM085	31/12/16	Msida (University Campus) [M]	*Quercus ilex*	Loamy, partly clayey + stones
SM280	06/01/18	Għajnsielem (Żewwieqa) [G]	*E. gomphocephala*	Clayey + stones
SM292	19/01/18	Victoria (Triq Marsalforn) [G]	*E. gomphocephala*	Clayey + stones
SM331	23/10/18	Saint Paul's Bay (Ballut tal-Wardija) [M]	*Q. ilex*	Loamy
SM355	24/12/18	Sannat (Ta' Cenc) [G]	*E. gomphocephala*	Clayey + stones
SM391	04/02/19	Comino (Fuq San Niklaw) [C]	*E. gomphocephala*	Clayey
SM454	15/12/19	Xewkija (fuq tal-Gruwa) [G]	*E. gomphocephala*	Clayey
SM477	29/08/20	Xagħra (Wied tal-Egħżien [G]	*E. gomphocephala*	Clayey + stones
SM483	27/09/20	Xagħra (Sinet tal-Egħżien) [G]	*E. gomphocephala*	Clayey + stones
SM484	27/09/20	Xagħra (Daħla tal-Bullara) [G]	*E. gomphocephala*	Clayey + stones
SM485	27/09/20	Żebbuġ (Wied ta' Mforn) [G]	*E. gomphocephala*	Clayey
SM488	01/10/20	Kerċem (Ghar Ilma area) [G]	*E. gomphocephala*	Clayey + stones
SM489	01/10/20	Kerċem (Is-Sidir) [G]	*E. gomphocephala*	Clayey + stones
SM504	23/10/20	Xagħra (Ta' Sruġ) [G]	*E. gomphocephala*	Clayey
SM522	09/11/20	Msida (Għajn tal-Ħasselin near water reservoir) [M]	*E. camendulensis*	Clayey & slightly loamy
SM621	17/02/21	Victoria (Ta' Cianti) [G]	*E. gomphocephala*	Clayey
SM623	20/03/21	Comino (Art Ħażina) [C]	*E. gomphocephala*	Clayey
SM627	16/10/21	Siġġiewi (Wied il-Luq) [M]	*Q. ilex* & *Populus alba*	Loamy

Results

Surveys between 2015 and 2021 resulted in examining and collecting material of *Pisolithus* spp. from 20 different sites. Details of each record, including those reported in Mifsud (2017), are given in Table 1. This Table includes the date when the material was found in situ, the locality, the edaphic conditions and the species of tree(s) that the fruiting bodies were assumed to be associated with. All reported locations reside between 25 – 200 m above sea level.

Three samples were recorded on rather loamy to peaty soil under *Quercus ilex*. In contrast, the remaining 17 collections were found in clayey soil (sometimes compact and with several stones) under *Eucalyptus* plantations.

Based on the ecology of the examined material, two main groups of *Pisolithus* are present: those associated with *Q. ilex* (native to the Maltese Islands) and those with *Eucalyptus* spp. (alien species). The morphological findings of these two groups were distinct and are summarised in Table 2.

Discussion and Conclusion

Records SM035, SM331, SM627 found associated with *Quercus ilex* are identified as *P. arhizus*, characterised by reddish and sombre colours of the peridium (Fig. 1a), spore mass or mature peridioles with a reddish-brown colour (Fig. 1b, Fig. 4a), a distinctly large, brown stipe without amber hues at the base (Fig. 1b), and spinulose spores measuring 10.2–12.7 µm (mean 11.6 µm) in diameter with isolated spines that are distinctly curved at the upper half and about 1.5–2.0 µm long (Fig. 5a). This species has been found in the old forest of Buskett close to Wied il-Luq (SM627) and il-Ballut tal-Wardija – the oldest oak forest in the Maltese Islands, around 900 years old. The location of *P. arhizus* in these old forests corroborates that the species is native and autochthonous, and Malta lies well within the distributional range of the species, currently recorded from many Mediterranean countries, namely Portugal, Spain, France (including Corse), Italy (including Sardinia and Sicily), Greece (including Crete), Israel and Syria (GBIF 2022).

Of the 17 *Pisolithus* populations, all found specifically under trees of *Eucalyptus* spp., 16 were more or less characterised with basidiomes having a white, ash-grey or cream, tough and thick peridium (Fig. 2a) forming mature peridioles or a spore mass with a distinct curry or olivaceous brown colour (Fig. 2b, Fig. 4a). The spores of these 16 collections had a homogenous spore morphology: globular, 8.9–11.2 µm in diameter (mean 10.2 µm), with a spinulose ornamentation formed by isolated and slightly curved, sharp-tipped teeth about 1.0 µm long (Fig. 5c). The stipe of these collections was short, often submerged in the soil and had a distinct mustard-yellow colour at the base (Fig. 2b). According to literature (Leonard & McMullan-Fisher 2013; Gargano & al. 2018; Lebel & al. 2018), these 16 populations (all group 1 records in Table 1 except SM355) correspond to *Pisolithus albus*.

Record SM485 deviated by having a dull greyish peridium with white or pale grey streaks (Fig. 4b), and hence different from the typical whitish or pale peridium of *P. albus. P. microcarpus* (Cooke & Massee) G. Cunn. was also considered, but this species has spores less than 8 µm across (Lebet & al. 2018), – far smaller than 10 µm in the examined material. The darker peridium of SM485 might have been attained by weathering (excess heat), fire or deposit of

Table 2. Macromorphological findings and comparison between the *Eucalyptus*-associated and the *Quercus*-associated specimens.

Characteristic	**Group 1**	**Group 2**
Association	*Eucalyptus* spp.	*Quercus ilex*
Record code	SM036, SM085, SM280, SM292, SM391, SM454, SM477, SM483, SM484, SM485, SM488, SM489, SM504, SM522, SM621, SM623	SM035, SM331, SM627
Peridium colour when young	Whitish to ash grey with few dark grey or brown patches or scars; (reddish-brown with black patches in SM355; dull grey with pale streaks in SM485)	Dark reddish-brown
Peridium texture	Leathery, tough (more fragile in SM355)	Thin, skin-like and easy to tear apart
Stipe	Short and stout, ca 10–15 mm long, partly buried, gleba often resting on the soil surface.	Distinct, ca 20–40 mm long, gleba elevated from the ground.
Stipe base and rhizomorphs	Yellowish mustard brown (more vibrant and bright in SM355)	Dark brown
Spore mass colour	Curry/olivaceous brown (tan to light clay brown in SM355)	Chocolate-cinnamon brown, rather dark

soot, amongst other abiotic factors. It may as well correspond to some undescribed species possessing that specific colour, but it is currently retained as *Pisolithus* cf. *albus*.

Finally material SM355 with its fragile to slightly toughened peridium having a typical mosaic-like pattern alternating between dark polished grey, reddish-brown and mottled with golden or amber colours (Fig. 3a), cannot be ascribed to any of the species mentioned above. The colour of the spore mass or the mature peridioles are tan to sand-brown (Fig. 3b). Moreover, the spores are slightly shorter, measuring 7.8–10.4 µm (mean 9.5 µm) in diameter, and covered with short spines (1.0 µm or less) that seem to form small crests due to the fusion of their base (Fig. 5b). These characteristics matched with *P. marmoratus* in the keys by Leonard (2011) and Leonard & McMullan-Fisher (2013); in species descriptions (Leonard & McMullan-Fisher, 2013; Lebel & al., 2018); and when compared with images (e.g. the peridium, cross-section showing the peridioles and spores) as provided by Lebel & al. (2018). Closely related is the newly described *P. tympanobaculus* T. Lebel & M.D. Barrett, which is also associated with *Eucalyptus* spp., but Lebel & al. (2018) emphasised on the distinct drumstick-like (prolate) shape of the basidiomes of this species, which was not observed for SM455 where instead, it was rather spherical. A summary of the morphological findings of *P. albus*, *P. marmoratus* and *P. arhizus* is provided in the Electronic Supplementary File (ESF 1).

Fig.1. Basidiome of *Pisolithus arhizus* Wardija, Malta. A. in situ; B. dissected (grid =1cm).

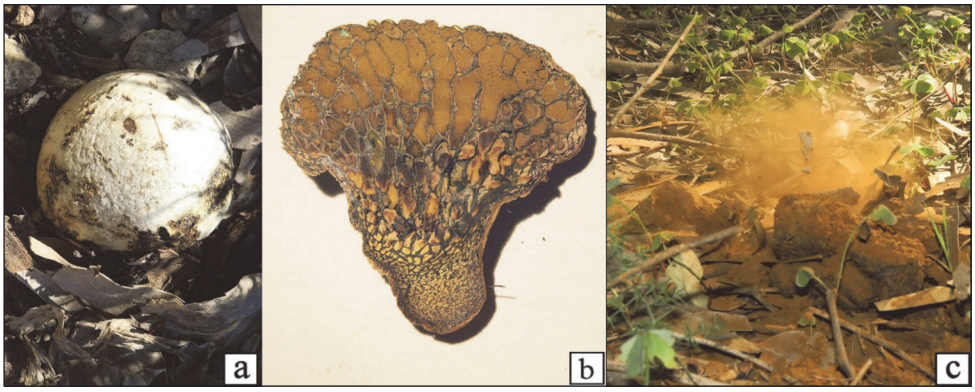

Fig. 2. Basidiome of *Pisolithus albus*, Wied tal-Egħżien, Gozo. A. in situ; B. dissected; C. spore cloud.

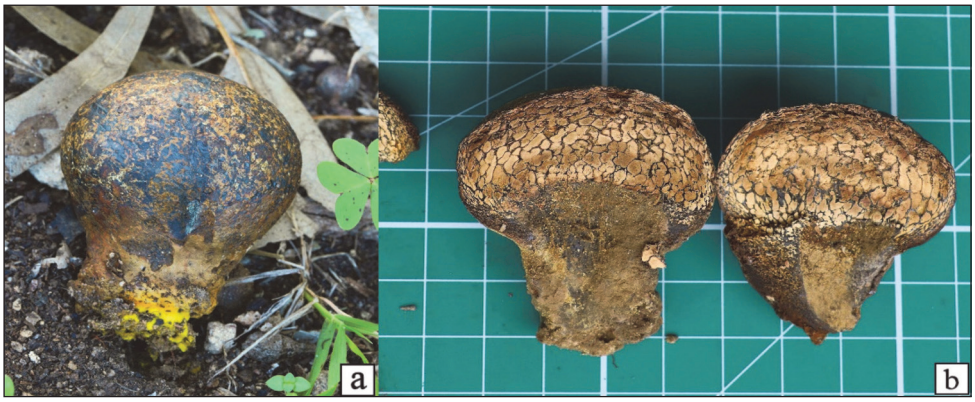

Fig. 3. Basidiome of *Pisolithus marmoratus,* Ta' Ċenċ, Gozo. A. in situ; B. dissected (grid=1cm).

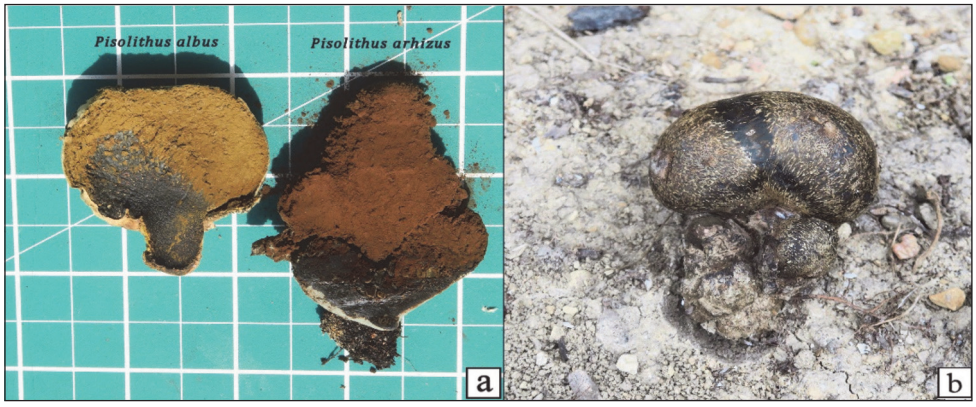

Fig. 4a. Comparison of the curry-brown coloured spore mass of *Pisolithus albus* (left) and the chocolate-cinnamon brown coloured spore mass of *P. arhizus* (right); 4b. an atypical form of *Pisolithus* cf. *albus* with medium-dark grey peridium mottled and with beige streaks.

The three *Pisolithus* species present in the Maltese islands can be identified using the following key (* new records for the Maltese Islands):

1A. Associated with *Quercus* spp., never *Eucalyptus* spp.; spore mass reddish cinnamon brown, stipe distinct ..... ***P. arhizus***

1B. Associated specifically with *Eucalyptus* spp.; spore mass lighter in colour, tan or curry brown, stipe short or hypogeal (hidden below ground) **2**

2A. Peridium white, ash or cream, tough; spore mass curry-olivaceous brown ..... ***P. albus****

2B. Peridium darker, mottled in blackish, brown and amber, fragile; spore mass tan to sand brown (not so olivaceous) ..... ***P. marmoratus****

Concurring with Martin & al. (2002), Jaouani & al. (2015) and Gargano & al. (2018) *P. albus* and *P. marmoratus* were introduced in Europe through contaminated soil of introduced *Eucalyptus* trees. Both species are poorly reported in Europe, with singular reports from Spain and Italy (Gargano & al. 2018; GBIF 2022). Hence, this work should encourage further research on *Pisolithus* spp. in other European territories, where it is likely that records under *P. arhizus* might correspond to other introduced species.

Although *Eucalyptus* trees have been introduced abundantly during the last 70 years in Malta, and the large sporocarps of *Pisolithus* are easy to spot, the first records were reported from Malta only recently (Mifsud 2017). This may indicate that *Pisolithus* was recently introduced, and the numerous local populations resulted from a rapid natural propagation from spore dispersal. In this regard, the mycorrhizal activity of *P. arhizus* on native trees, namely *Quercus* and *Pinus* spp. (Diez & al. 2001; Martin & al. 2002; Phosri & al. 2012)

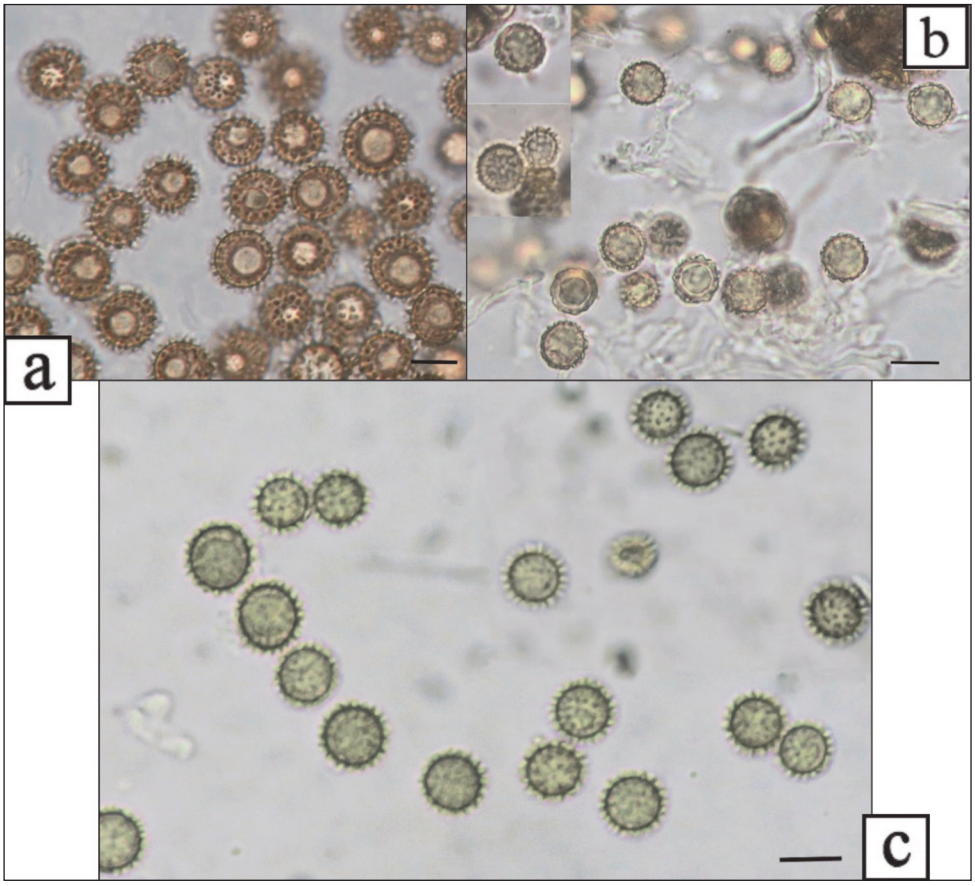

Fig. 5. Spores of *Pisolithus* spp. from Malta: a. *P. arhizus*; b. *P. marmoratus*; c. *P. albus* (Scale bar = 10 µm).

should be more exploited given that *Pisolithus* spp. populate quickly in a relatively short period of time, and their abundant spores are easy to harvest and introduce in afforested areas or nurseries growing oaks, pines and possibly other native or archaeophytic trees.

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