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# *Cortinarius ayanamii* (*Fungi*, *Cortinariaceae*). The first record of a *Cortinarius* species for the Maltese Islands

**Abstract**

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A small cluster of yellowish caramel-brown macromycetes growing under *Cistus monspeliensis* L. in Nadur, Gozo, has been identified as a *Cortinarius* species for its rough morphology, weak cortina on the stipe, arid taste, spore print and shape. DNA sequencing using ITS primers has further resolved the determination to species level where a 100% match correspond to *Cortinarius ayanamii* A.Ortega, Vila, Bidaud & Llimona. *Cortinarius* is the largest genus in terms of species diversity in Europe, however, this finding is the first record of *Cortinarius* for the Maltese Islands. The ecology of the Maltese population matches with that reported in the species description, specifically as a mycorrhizal species with *C. monspeliensis* and *C. albida* from Catalonia, Spain. The lack of other records from mainland Europe may indicate that the species is rare in Europe, and its distribution is poorly known. A detailed account of the finding in Gozo is given in this work, including the habitat and morphology of the individuals accompanied by relevant photographs.

*Key words*: *Myxacium*, *Vibratilis*, *Cistus*, Malta, Gozo.

## Introduction

The study of fungi in the Maltese islands is much less widespread and intense than that of plants and animals, and the systematic work carried out by Saccardo (1912–15) at the beginning of the 20$^{th}$ century remained more or less unaltered until mycological research resumed in the last three decades. Most records of macromycetes were reported from mainland Malta; hence a proper investigation on the mycobiota of the island of Gozo was needed and has been recently commenced as an M.Sc. degree by the present senior author at the Institute of Earth Systems, University of Malta. Only 40 species of macrofungi are recorded from Gozo despite the fact that the island has a sizable area of ca. 67 km$^2$; 32 of these were only recorded between 2015-2021 (e.g. Mifsud 2017a, 2017b, 2019; Fournier & al. 2021).

During forays in a phrygana habitat of an *Erico multiflorae-Antyllidetum melitensis* (Brullo & al. 2020) dominated by *Cistus monspeliensis* L. in Nadur, Gozo, three yellowish to light olive-brown basidiomata were observed under *C. monspeliensis* on 19 January 2021. Their pileus was 5–6 cm wide, moderately viscous, and possessed adnate to subemarginate, light brown lamellae. The delicate and fugacious rust-coloured cortina around the stipe, the light cinnamon-brown spore print, the almond-shaped spores with a thick wall and, the extremely bitter taste strongly indicated that the specimens belong to a species of *Cortinarius* Gray (1821) (Boccardo & al. 2008).

In Malta, most of the recorded *Basidiomycota* are members of the families of *Psathyrellacae* s.l. Vilgalys, Moncalvo & Redhead (2001), *Agaricaceae* Chevall. (1826) and *Bolbitiaceae* Singer (1948), but despite *Cortinariaceae* R.Heim ex Pouzar (1983) is the largest family in Europe, with some 2000 recognized species (Kirk & al. 2008; Gibby & al. 2009), no *Cortinarius* records have ever been noted from the Maltese Islands. Hence this finding from Nadur, Gozo elicited a comprehensive analysis to determine this population down to species level.

## Materials and methods

The colour, size, texture, and other organoleptic and physical characteristics of the specimens were examined *in situ*. In addition, the habitat and plant community around the population was recorded, and the specific plant species they were associated with was ascertained. A sample was collected for further observation, and the tissue from the hymenium, cap cuticle, stipe and, the spores were further examined under the microscope (Zeiss AxioLab) using standard staining and microscopy techniques (Clémençon 2009).

Moreover, genetic sequencing was employed to provide an accurate identification. Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona 1987) included 35 cycles with an annealing temperature of 54 ºC. The primers ITS1F and ITS4 (White & al. 1990, Gardes & Bruns 1993) were employed to amplify the ITS rDNA region. PCR product was checked in 1% agarose gels, and the amplicon was sequenced with primer ITS4.

## Results

The genetic sequence was obtained successfully from ITS primers, but it did not match any data from major gene banks such as Unite and GenBank to determine the species. Since several ITS sequences of *Cortinarius* are yet unpublished (Alvarado 2021, pers comm.), contact was made with Dr Bálint Dima, a European expert on *Cortinarius*, and the ITS sequence was sent to him for comparison. A 100% successful match resulted for the species *Cortinarius ayanamii* A.Ortega, Vila, Bidaud & Llimona. The authors of *C. ayanamii* have not shared the sequence data in public mycological gene banks. The morphology and ecology of the population in Malta match with the species description (Ortega et al. 2000).

Morphology: **Pileus** (Fig 1a) 61–72 mm wide, rounded and broadly convex then applanate when ripe, margin raised up and visibly undulate and irregular in outline, fleshy,

viscid then dry with many soil particles and debris firmly attached on the surface, not hygrophanous, ochre or pale caramel-yellow to light brown, drying to light caramel brown when overmature. **Stipe** 40–48 × 15–17 mm, clavate-cylindrical, broadest at the top and narrowing lightly; pale cinnamon-ochre colour, with a silvery or ash-gray hues especially below the pileus, finely fibrillose in texture, veil fugaceous and remnant as tobacco-brown lining. **Lamellae** (Fig 1b) 4–5 mm wide, convex, adnate-emarginate, tobacco-curry brown, edge concolorous and generally smooth, medium crowded. **Context** solid and compact, cream-white. **Odour** insignificant or earthy. **Taste** extremely bitter. **Macrochemical** reactions: KOH (10%) cap darkens gradually to greyish-brown, while insignificant change on the context. **Basidia** (Fig. 1d) 2-spored and less frequently 4-spored, 24–28 × 7–9 µm. **Cystidia** infrequent to rare at the edge, absent on the faces, not conspicuous and almost same size as basidia, cylindrical to subclavate, sometimes lageniform, flexuose, smooth, hyaline. **Spores** (Fig. 1c) (6.8) 7.2 – 10 (11.1) × (4.6) 5.3 – 7.3 (8.1) µm [mean = 8.5 × 6.1 µm], Q = (1.2) 1.3 – 1.6 (1.7), [mean = 1.4] ellipsoid-subamygdaloid, rough verrucose and bumpy, sometimes showing what appears to be shallow, short ridges; apiculum distinct and central; contents with one or two guttulae appearing dull olive-green, spore print tobacco-brown (Fig. 1b). **Pileipellis** composed of two layers, the outer an intricate trichoderm made of elongated, curved thread-like hyphae, 1–5 µm wide with slightly inflated heads. Clamp junctions present. Below, the subpellis is composed of a layer of tubular-cylindrical hyphae, 10–15 µm wide, also with clamp junctions but less frequent than in the suprapellis.

Habitat and distribution: In calcareous phyrganae-garigue mosaic habitat specifically under *Cistus monspeliensis* fruiting in mid-January (rather late in season for Malta). Relative accompanying woody species included *Thymus capitatus* L., *Anthyllis hermaniae* subsp. *melitensis* Brullo & Giusso*., Erica multiflora* L. and, occassionally *Pistacia lentiscus* L. and *Ruta chalepensis* L.

## Discussion and Conclusion

*Cortinarius ayanamii* is classified under the subgenus Myxacium and Section Vibratilis. Members of Myxacium are characterized by viscid pileus and stipe, although specimens dry up when humidity or rain is scarce, whereas Vibratilis is a section with ellipsoid (-sub-amigdayloid) spores seldom more than 9 µm long (Kibby & al. 2009).

The macro- and micro-morphological characters of the Maltese population closely match with the description of the holotype from Catalonia (Spain) (Ortega et al. 2000) with slight variations in colour tonalities of the pileus and the stipe. The spore size, for example, is almost the same: mean of 8.5 × 6.1 µm for the Maltese population and 8.6 × 6.4 µm for the type (Ortega & al. 2000). Likewise, both populations exhibited mycorrhizal associations with *Cistus monspeliensis* (and also *C. albidus* in Spain).

Literature review on the distribution of the species resulted in deficient data. No distribution data was provided by global biodiversity databases such as MNHN (2021) and GBIF (2021) except for the type specimen at Catalonia same as recorded in the protologue. The record from Gozo within the Maltese archipelago is hence the second known station for the species since its description (Oretega & al. 2000), suggesting that the fungus is rare and/or, understudied.

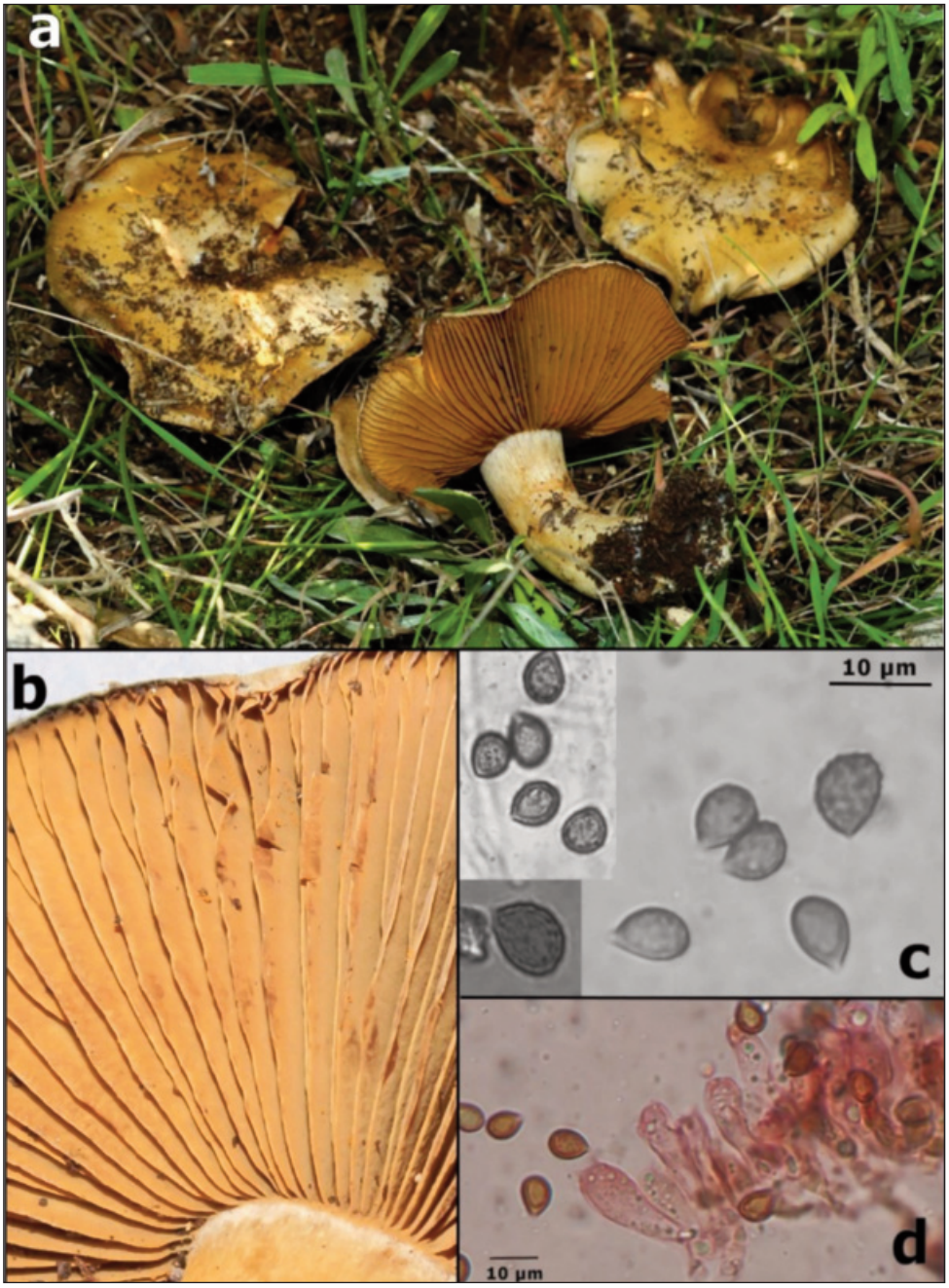


Fig. 1. *Cortinarius ayanamii* A.Ortega, Vila, Bidaud & Llimona from Nadur (Gozo), Malta (19-Jan-2021): 1a. three specimens in habitat under *Cistus monspeliensis* L.; 1b. close up of hymenium showing the cinnamon-brown spore mass deposit on some lamellae; 1c. ellipsoid-subamagdyloid spores with verrucose surface (mean = 8.5 × 6.1 µm); 1d. hymenium showing basidia, several being 2-spored.

Although it is expected that there are unpublished data by European experts of *Cortinarius*, the record from Malta currently represents the southernmost finding for the species in Europe and the first confirmed record of a *Cortinarius* species for the Maltese Islands.

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