

Two uncommon fungal species from Italy

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Introduction

The Italian peninsula and the two largest islands (Sardinia and Sicily) are characterised by a high level of fungal diversity (Venturella *et al.* 2011). The variety of ecosystems present often leads to collections of species not yet reported or infrequent, some of them very rare even at European level (Karadelev *et al.* 2017b; Venturella 2017). Moreover, the increasingly variable climatic conditions of the Mediterranean environment, with the alternation of long dry periods followed by drastic drops in temperature and intense rainfall, allow the unexpected appearance of species previously reported only in other areas (Karadelev *et al.* 2017a). Recent field excursions in natural forest ecosystems and mixed reforestation with a prevalence of *Cedrus atlantica* have allowed a study of two infrequent species in Italy: *Hebeloma subtortum* P. Karst. and *Boletopsis grisea* (Peck) Bondartsev & Singer.

Material and Methods

Basidiomata were collected in the field and then identified while fresh under a Leica microscope. The microscopic characters were observed in water, spore size measurements were based on 50 observations (100× objective lens) and compared with Jülich (1984), Vesterholt (2005) and Beker *et al.* (2016). Chemical reactions on fresh basidiomata were carried out on *B. grisea* only. Nomenclature of plants follows Euro+Med PlantBase while the nomenclature of fungi follows Index Fungorum. The herbarium samples are kept in the Herbarium SAF of the Department of Agricultural, Food and Forest Sciences (University of Palermo, Italy, SAF 00140, SAF 00141).

Hebeloma subtortum P. Karst.

H. subtortum P. Karst. (Hymenogasteraceae) (Fig. 1) is a member of sect *Hebeloma* (Fr.) P.

Kumm. which is likely to be more widely distributed in Europe than reported in literature and on various mycological websites. Also, descriptions of the morphological characters have been discordant and at times referred to both *H. subtortum* and *H. mesophaeum* (Pers.) Quél. The two species were recognized, for a long time, as synonyms. According to Beker *et al.* (2016) the main morphological characters of *H. subtortum* are:

Pileus, 3-5 cm, viscous, whitish cream, pale brick-red or pallid, disc darker, convex in young basidiomes then plane, obtuse, even, glabrous, irregular at first, silky near edge from veil; **Gills** white then brownish, adnate-subdecurrent, crowded, edge crenate; **Stipe** subconcolorous to pileus, soft and fibrillose, with a clear cortina that can form an annular zone; hollow, usually twisted, whitish. **Basidiospores** 7–12.5 x 4–7 µm, not dextrinoid, cheilocystidia present. Smell mixed of turnip and cocoa.

H. subtortum differs from *H. mesophaeum* by its more robust basidiomes, with stipe up to 4 mm wide, gills more numerous, basidiospores ovoid rather than ellipsoidal, pale yellow. The current distribution in Europe of *H. subtortum* is known from studies carried out by some specialists of the genus *Hebeloma* and by reports by amateur mycologists and university researchers. The Danish Mycological Society's dataset checklist (Læssøe *et al.*, 2017) reports a collection by Clausen & Christensen of *H. subtortum* in a *Pinus* wood, on the island of Anholt (Denmark) during autumn. According to these authors, *H. subtortum* is common in Southern Europe but apparently rare in Northern Europe (presence reported only in Finland, Germany and, Poland) where it can also be found in deciduous forests. Zibarová (2015) reports two localities for *H. subtortum* in the Czech Republic (Dobrin and Deštnice) in *Pinus* woods and mixed forests of *Quercus* and *Populus* respectively. In the Iberian

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Fig. 1. *Hebeloma subtortum* collected in *Pinus pinaster* and *P. pinea* woodland, near Castell'Umberto (Messina), Sicily, 2016. Photograph © G. Venturella.

Peninsula, *H. subtortum* is reported for Spain and Portugal respectively in the provinces of Zaragoza (Asociación Micológica San Martín de Moncayo) and Monsanto Forest Park (Lisbon) in *Quercus* wood mixed to scattered pines. Other collections of *H. subtortum* from Belgium, Cyprus, Denmark, Finland, France, Germany, Hungary, Macedonia, Spain and, Turkey are included in Beker *et al.* (2016) and also from Great Britain.

Due to the lack of specialists of the genus *Hebeloma* in Italy, especially following the premature death of the mycologist Livio Quadraccia (University of Rome) taxonomic studies as well as analysis of the distribution of different species has been rather limited and for identification the monographs of Vesterholt (2005) and Beker *et al.* (2016) have been taken as a reference.

In September 2003, during a series of mycological excursions carried out in Sicily in the autumn by our research group together with the late J. Vesterholt, a collection of *Hebeloma* species was carried out, including *H. subtortum*. The first collection of this species in Italy dates back to the spring of 1983 at Castelfusano (Rome) under *Pinus pinea*. After about thirteen years, a second finding was reported at Orgosolo (Sardinia), in autumn and, for the first time, under a broad-leaved tree (*Quercus ilex*). Many other collections are reported by Beker (2016) for

Sicily as a result of the excursions carried out by J. Vesterholt together with one of the authors of this publication (G. Venturella). These collections were all carried out, in autumn, in reforestation with *Pinus pinaster* and *P. pinea* and undergrowth characterized by small shrubs of *Cistus salvifolius* near Castell'Umberto (Messina) (Fig. 1). Additionally, in 2010, *H. subtortum* was reported from Piedmont, in autumn, in a chestnut forest (*Castanea sativa*).

A recent collection of *H. subtortum* carried out in autumn 2017 is here reported for Sicily. The collection area falls again in Castell'Umberto, in the province of Messina (north-eastern Sicily, southern Italy) in the so-called Pinewood of Castell'Umberto (38°4'53.299"N, 14°48'59.138"E). The habitat (Fig. 2) is mixed pine forest on leached brown soils at an altitude of about 700m.

H. subtortum is therefore confirmed as an infrequent species, with a very localized distribution in Italy, and with habitat not necessarily limited to conifers. Furthermore, its fructification seems to be more concentrated in autumn, with some rare appearances in spring. In Sicily, the presence of *H. subtortum* in coniferous reforestation is not constant and basidiomes occur mainly in less rainy years and characterized by periods with higher temperatures followed by drastic decreases.



Fig. 2. *Pinus pinaster* and *P. pinea* woodland: the growth environment of *H. subtortum* in Sicily.

As demonstrated by Venturella *et al.* (2011) other fungal species, rather rare at European level, show similar behaviour in Sicily to *H. subtortum* and a close relationship between anomalous climatic conditions and fruiting capacity.

In the case of *H. subtortum*, the term infrequent has been used, taking into account the fluctuating fruiting over the years due in most cases to seasonal weather conditions, and also the difficulties in macroscopic recognition due to similarity with related species.

Finally, since there are few specialists of the *Hebeloma* genus, the correct taxonomic identification of each taxon and consequently their distribution and ecological characterization remain at the moment a critical point in mycological studies.

***Boletopsis grisea* (Peck) Bondartsev & Singer**

B. grisea (Peck) Bondartsev & Singer (*Bankeraceae*) (Figs 3 & 4) is one of the three European species of the genus *Boletopsis* Fayod [the other two are *B. leucomelaena* (Pers.) Fayod and *B. perplexa* Watling & Jer. Milne] which is recognized in the literature in Europe (Niemelä & Saarenoska 1989). *B. perplexa* is a taxon associated with *Pinus sylvestris*, reported only for

Scotland (Watling & Milne 2008). *B. perplexa* is similar to *B. grisea*, but has bigger pores and smaller spores while *B. leucomelaena* differs by its hyphal structures and association with *Picea*. In 1992 and 2006, *B. perplexa* was erroneously assessed as *B. leucomelaena* (Ing 1992, Evans *et al.* 2006) while, more recently, it has been classified as Endangered (EN D) (Smith *et al.* 2016). The similar macroscopic features of *B. grisea* and *B. leucomelaena* make identification of the two species rather difficult. Some authors (Niemelä & Saarenoska 1989) dwell on colour differences in the pileus: blackish, with strong contrast between colour of the cuticle and poroid hymenium (*B. leucomelaena*); pale greyish; light contrast between the colour of pileus and the hymenium (*B. grisea*). But in the basidiomata we have assigned to *B. grisea*, the colour of the pileus varies from the white, then greyish white, finally smoky grey, turning to grey when rubbed. In some cases, there are also pinkish shades. The size of the pileus is 6–18 cm, initially convex, then plano-convex with smooth surface. The margin is wavy and remains inrolled for a long time. The hymenium is curved, with very short tubules, not separable, decurrent on the stem, of a cream-yellowish colour. The pores are very dense, indistinct, then polygonal, for a long time

white. The stem, 3–8 x 1–3 cm, is short, smooth, thinned upwards, widened in the median part and attenuated at the base, almost all rooted in the ground, white, then darkening starting from the base. The flesh is compact, with a slight fungal odour, not rotten, white, slightly turning pinkish grey when cut, with an unpleasant taste, bitterish after long chewing. The flesh changes to green when in contact with KOH. The basidiospores are angular, nodulose, of a more or less irregularly width, 4–7 μm . The basidia are clavate, bisporic and tetrasporic.

If we compare *B. leucomelaena* with *B. grisea*, the identification of the latter is facilitated by the use of chemical reagents and in particular by the use of KOH at 30% and NaOH at 25%, which show an immediate colour change of the flesh towards green (Fig. 4).

B. grisea is distributed in Europe, Canada and the United States (Richter 2004) and it is recognized as a mycorrhizal species with pines (Watling & Milne 2008).

Niemelä & Saarenoska (1989) collected *B. grisea* exclusively in dry pine forests of Finland. In Bohemian Forest (Czech Republic) it was again observed (more than 14 years later) by Holec (2004) among needles in a mixed conifer forest. The appearance of *B. grisea* in Estonia and Sweden is strongly related to the presence of older pine forests (Bresinsky 2006, Eilertsen 2014). Its southernmost distribution limit in Europe includes Italy and the island of Malta. In Italy, it has been reported only in the northern and

central regions, so our new report for Sicily further extends southwards the distribution area of *B. grisea* in Italy. In addition, for the first time in Europe, the presence of a mycorrhizal association with *Cedrus atlantica* is reported (Fig. 3).

Conclusions

Mediterranean forests continue to be an inexhaustible reservoir of fungi of considerable ecological and biogeographical interest. In the current scientific context in which molecular studies prevail, field mycology remains an essential basic activity that allows: a) the characterisation of new habitats for fungal species; b) the possibility of extending the known area of distribution of fungal species hitherto considered to be confined to environments very different from Mediterranean ones. This is the case for the two species reported in this publication. Furthermore, *B. grisea* is a threatened species in Europe while *H. subtortum* is infrequent.

Acknowledgements

We would like to dedicate this study to the memory of two talented mycologists and friends Livio Quadraccia (Italy) and Jan Vesterholt (Denmark).



Fig. 3. *Boletopsis grisea* collected in Sicily. Photograph © G. Venturella.

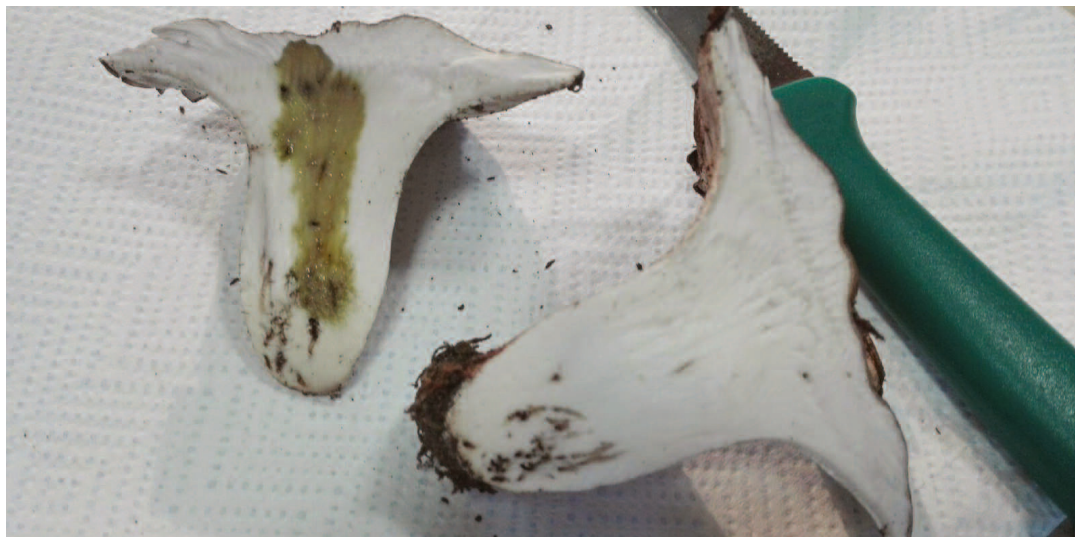


Fig. 4. The characteristic change in the colour of the flesh of *B. grisea* on contact with KOH.

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