FRUIT-BODIES OF BOLETUS AMARELLUS IN PURE-CULTURE

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ABSTRACT. A collection of *Boletus amarellus* Quélet, 1882 from Attiki, Greece, is described from fresh and cultured fruit-bodies; cultural characters of the vegetative mycelium and the development of fruit-bodies are outlined. The relationships between this collection, *B. rubinellus* Peck and *B. piperatus* Fries are discussed.

A very striking bolete was found growing in large numbers in a forest of *Pinus halepensis* at lower Parnis, Attiki, Greece on 12 Dec. 1970. Suitable material was selected the same day for culture by the tissue-plug method using pieces of inner tissue. Isolation was carried out on Hagem's medium as modified by Modess, and as outlined in previous studies by Pantidou (1961). The bolete has been identified as *Boletus amarellus* Quélet, and is the subject of this communication.

Boletus amarellus Quélet in II suppl., Ass. Fr. Av. Sci. 398 (1882).
Syn.: *Suillus amarellus* (Quélet) O. Kuntze, in Rev. Gen. Pl. 3, 2: 538 (1898).
Versipellis amarellus (Quélet) Quélet, Enchir. 158 (1886).
Xerocomus amarellus (Quélet) Quélet, Flor. Myc. 418 (1888).
Ixocomus amarellus (Quélet) Gilbert, Les Bolets 136 (1931) [Ex Vassilieva, Utch. Zap. Kaz. Gos. Un. 105: 44, 1939 fide Singer].
Suillus piperatus var. *amarellus* (Quélet) Singer in Farlowia 2: 278 (1945).

Pileus up to 30 mm diam., convex becoming expanded, straw-colour then buff, flushed with salmon-colour, dry, smooth but surface cracking slightly with age. *Stipe* 15–30/5–7 mm, pink to rose at the apex, straw-colour elsewhere either smooth or dotted with minute red punctae. *Pores* large, approximately boletinoid, coral-colour when young, becoming apricot-colour at maturity; *tubes* concolorous with pores. *Flesh* whitish in pileus, rose near tubes and in upper part of stipe, buff below, not bluing; *taste* mild; *smell* not distinctive.

Basidia 2–4 spored. *Basidiospores* 9–14 (–15) × 4–5 (–6·5) μm, mostly 10 × 4·5 μm.

In forest of *Pinus halepensis*, lower Parnis, Attiki, 12 xii 1970, legit *E. Tjamos*; Pantidou MPIH 1142, culture B-84-70.

CULTURAL CHARACTERISTICS

Macroscopic characters. Mycelial mat of fairly slow growth, 30–50 mm diameter in four weeks, yellow-brown, often entirely immersed in the substrate, rarely with aerial hyphae; lemon-chrome pigment diffusing readily and abundantly into medium.

Microscopic characters. Hyphal system with very few cylindrical hyphae; sap hyphae rare; strands few; clamp-connections not seen; paarige branching

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very rare; immersed hyphae short with dendritic branching, often forming rounded cells, and some end-cells cystidioid.

Description of fruit-bodies in culture. Fruit-body 20–40 mm high. *Pileus* 10–20 mm diam., salmon-colour, flushed with peach- to rust-colour, or vinaceous to rusty tawny, often with a paler narrow marginal zone, almost always tomentose. *Stipe* 20–30/2–5 mm, smooth or with vinaceous punctae, pale yellow with pinkish tints to straw-colour or lemon-chrome, except for vinaceous red or rust-coloured apex, often strongly lemon yellow at very base. *Pores* pale-coloured, becoming rust-coloured to fulvous; *tubes* concolorous depressed around the stipe. *Flesh* red in pileus down to hymenophore when young, later fading. *Spore-print* rust-colour 'on agar surface'.

Basidia pale honey-colour in ammoniacal solutions, (1–)2–4 spored, clavate, 35 × 4·5–6·5 *µm*. *Basidiospores* 9–10 × 4–4·5 *µm*, smooth, apical pore absent, elongate ellipsoid in face-view, flattened on one side in side-view, profile obscurely inequilateral, very slightly constricted and bent at half way and flattened in hilar area, dingy honey-colour in water and ammoniacal solutions, slightly red-brown in Melzer's reagent.

Pileus cuticle of smooth, cylindric, thin-walled hyphae forming a collapsed surface with individual end-cells or hyphae 5·5–8 *µm* broad projecting through the collapsed elements, with little or no gelatinised matrix evident; end-cells thin-walled, smooth, or very rarely faintly and irregularly punctate from attached debris, obtuse rounded at the apex not tapered and not constricted from main hyphae.

Hymenophoral trama divergent from a central, floccose, very slightly gelatinised strand of filamentous hyphae, darker in colour to the lateral strata. *Pileus-trama* of strongly anastomosing hyphae 3–6·5 *µm* broad. *Cheilocystidia* numerous, variable, forming a fringe to the pore, subcylindric, obtuse, slightly curved, slightly inflated below and/or very slightly swollen towards the apex, smooth, thin-walled, very pale honey-colour in water and ammoniacal solutions, slightly darker in Melzer's reagent; *pleurocystidia* numerous, pale yellow, fusiform to subcylindric, with obtuse apex, not encrusted, 36–61 × 5–11 *µm* (apex 2·5–5 *µm*). *Stipe-cortex* of parallel, cylindric hyphae 4·5–8 *µm* broad, frequently with glassy yellow septa; *caulocystidia* sparse, cylindric-fusiform, 4·5–6 *µm* broad, in small discrete bundles. *Stipe-trama* of cylindric hyphae intermixed with a few laticiferous hyphae filled with honey-coloured to yellow contents. *Clamp-connections* not seen.

DEVELOPMENT OF FRUIT-BODIES

Primordia develop all over the mycelial mat in 10–15 days after culturing, and normally mature fruit-bodies form in 2–3 weeks after primordial initiation. Fruiting continued after several transfers and this ability to fruit has been retained undiminished until the present, i.e. two years after the initial isolation. The cultures were maintained at room temperature which fluctuated between 5–10°C at night and 20–25°C during the day, and were placed near a window with a northern exposure. Usually 10–15 primordia developed in each culture; however, as is common in many agarics, only 1–2, rarely up to 5, mature to normal, sporulating fruit-bodies were formed.

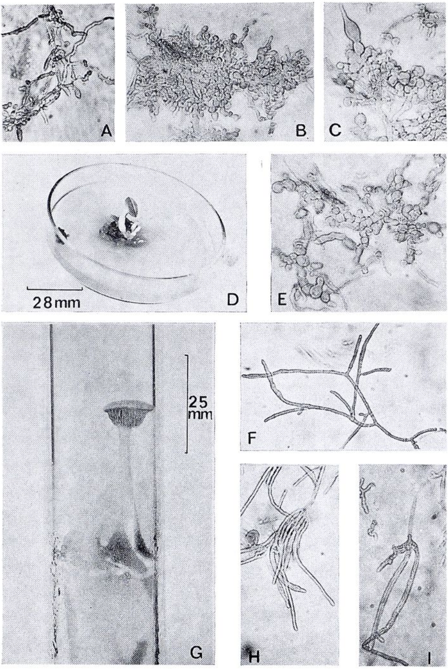

PLATE 11. *Boletus amarellus* Quélet: A, Hyphal system and branching; B, Hyphal tissue at base of well differentiated primordium; C, as B, but earlier stage; E, still earlier stage where hyphae are massing to form primordia (in C note the presence of cystidiform cells); F-I, Hyphal system and branching; D & G, Mature sporulating fruit-body in petri-dish and test-tube culture. Microscope details × 370: D & G as indicated.

The primordia when first observed are knot-like tufts of hyphae mostly consisting of flattened, rounded cells which gradually fuse and lose their identity. The tuft soon elongates to form a pale yellow stipe which, on reaching 10–14 mm in height, becomes slightly constricted at the apex. After a further 24 hours signs of differentiation into a pileus are apparent. In the following 24-48 hours both pileus-expansion and stipe-elongation occur simultaneously. The rate of expansion of the pileus was 1 mm in 24 hours. A limited cleavage was apparent at the point of constriction separating the pileus from the stipe. Twenty-four hours after the beginning of the pileus expansion the pileipellis becomes pale cinnamon-colour and the tubes delimit as a gelatinous layer on the lower surface of the pileus. The primordia are fully mature in 3–6 days after their initiation and their development at all times is gymnocarpic.

DISCUSSION

As is common in many basidiomycete cultures the variability of fruit-body characteristics is greater in culture than in nature. The fruit-bodies from culture in the present study were somewhat smaller than most of those seen in nature. The colours of the pileus and stipe were more variable in culture than in nature although it must be emphasised that, although consisting of many individuals, only one original collection has been examined in the fresh state whereas in culture several crops of fruit-bodies have been obtained. The punctae on the stipes were rarely found in fruit-bodies from culture, whereas they were constant in those from nature. This is surprising as the punctae are apparently 'islands' of hymenium consisting of cystidiform and basidial units intermixed with cells similar to those of the subhymenium and one would have expected this character to be constant. However, it emphasises once again that differences found between fresh collections of fleshy fungi may not be as significant as first thought and that the taxonomic significance of the character in which differences are found should be analysed whenever possible under standardised conditions. The stipe acts as an incipient hymenium and it is possible that the buffered conditions found in the culture-vessels do not favour the minor dissection of the outer layer to form punctae. In fact in culture the hymenium, although thin, completely girdles the stipe.

Boletus amarellus approaches very closely the north American *B. rubinellus* Peck and it is fortunate that this latter species also has been described in detail from cultures (McLaughlin, 1964, 1970). Although there are similarities and the two species are undoubtedly closely related, *B. rubinellus*, unlike *B. amarellus*, produces in culture thick, yellow superficial hyphal strands connected to the base of the fruit-body. Also, McLaughlin describes a white pilosity clothing the lower part of the stipe but this has not been found in our specimens neither in nature nor in culture. The minimum temperature of fruit-body initiation in *B. rubinellus* is given by McLaughlin as 17°C but, although no parallel experiments have as yet been carried out with our material, initiation of fructification at room temperatures in the laboratory may indicate that the requirements as to the temperature in *B. amarellus* might be different.

Unlike *Boletus sulphureus* and *B. lignicola* (as *Phlebopus*, Pantidou, 1961, 1962) the present isolate does not have the pileus expansion as a direct continuation from the stipe. A limited cleavage is present but is not as pronounced, however, as that in *Boletus illudens* (Pantidou, 1964). McLaughlin (1964) in his study of *B. rubinellus* describes what must be a similar line of cleavage when he reported a "groove below the expanding pileus".

B. rubinellus differs from the present collection in the distinctly reddish colour to the pileus, the deeper coloured tubes and the slightly different combinations of colours in the stipe. It also differs in the cultural characters mentioned above. There is some hesitation in American literature as to the correct usage of the name *B. rubinellus* and whether it should also include Kauffman's taxon *B. rubritubifer* (Coker, 1943) or not. Smith & Thiers (1971) prefer to retain as distinct not only both these but also *B. amarellus* until further work is carried out. There is little doubt that, with McLaughlin's close association with Walter Snell, the fungus he used in his experiments is the same as that described in Snell & Dick (1970), and it is on this description and Smith & Thiers' valuable information on the microscopic characters of Peck's type material that we have built our discussion.

Although there is some variation between the variously published descriptions of *B. amarellus* in European literature the Attiki collection agrees very closely with most of the available information. *B. amarellus* is apparently a rare fungus and it is because of this that the descriptions are not only dispersed but only recent finds are fully documented (Kühner, 1962). The Greek material, excepting for the much paler and slightly larger pores, agrees in all ways with Kühner's description; in the size of the pores our collection agrees with Quélet's diagnosis (1888). However, Quélet's original description (1882) differs in minor ways from other, more recent, descriptions and our own material.

The red punctae at the apex of the stipe appear to be very significant in identification (Leclair & Essette, 1969; Heinneman, 1961; Blum 1962) but as indicated in the present study in culture these same structures may be present or absent. The pileus-colour, flushed but not entirely some shade of rose, coral etc., i.e. faintly red only, separates this taxon from both *B. rubinus* and *B. piperatus* (red colour absent), and *B. rubinellus* (red colours distinct).

We agree with Kühner (1962) and Moser (1967) that *B. amarellus* is less close to *B. piperatus* than to *B. rubinus* with which one of us (R.W.) is very familiar. The present collection should be assigned to *Boletus* subgenus. *Xerocomus* and not subgenus *Chalciporus*, and in no event be placed in *Suillus* (or *Ixocomus*). In the latter respect we would refer the reader to Quélet's placement of the species in 1882. It is not a variety of *Boletus piperatus* (see Singer, 1945 & 1965) from which it differs in microscopic characters. Quélet's original collections were from pine woods, 'Alpes-Maritimes'; this is very similar to the habitat of the present collection.

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BOOK REVIEW

Malaysian boletes. The format of this book* is modelled on earlier publications for which the author is very well-known although there are a few minor differences which go to improve the text. Although the emphasis is on tropical boletes there is much of value to those working on temperate boletes and to those interested in the development of the hymenomycete fruit-body. The text is supported by excellent figures and reproductions of field paintings executed in the skill which one now associates with Professor Corner. However, what is a new innovation is the inclusion of some photographs (coloured and black and white). The book, like its predecessors, is a major contribution to our knowledge of higher fungi and is particularly helpful to the tropical botanist who has no modern texts when dealing with the bewildering number of unfamiliar taxa he meets: the introduction hints at these problems.

The book commences with a brief introduction to the classification of the boletes and continues by tracing the history and relationship of sixteen genera and a discussion about whether the author accepts them or not. The taxonomic significance of various characters formally used in defining genera and species are dealt with in turn—tramal structure of the tubes, the lacunose-reticulate stem, surface of the pileus and morphology of the spores; included in this section are the identities of boletes referred to only by number by Corner in his earlier papers on spores. Two artificial but useful units conclude this chapter, i.e. those species with dark brown or ferruginous spores and those with a strong smell. After this, Corner deals with the evolution of boletes, illustrated by an interlacing table, and then the growth of the fruit-body in selected Malaysian species. The latter studies are very useful and are supported by graphical information. The next sixteen pages are devoted to

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* Boletus in Malaysia by E. J. H. Corner. Published under the auspices of the Botanic Gardens, Singapore; printed at the Government printing office, Singapore 263 pp. 16 colour plates, 7 photographs (3 col.) 80 text figs., 1972. Price $50 Singapore.