NOTES ON SOME BRITISH AGARICS: IV

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ABSTRACT. *Boletus edulis* subsp. *trisporus* is described from Britain and *Squamanita paradoxa* is recorded for the first time from the British Isles.

A NEW SUBSPECIES OF THE STEINPILZ

Visiting agaricologists, particularly European, are always dismayed by the British interpretation of the much cherished *Boletus edulis*—the penny bun, le cep, steinpilz, porcino, brisa, etc. It is supposedly a common fungus throughout Europe; so much so it is assumed that everyone knows its characteristics. Difficulties in Britain have been experienced in distinguishing it from the closely related *Boletus reticulatus* Boudier; the complexity of the confusion becomes very apparent when one attends the familiar autumnal gatherings of mycologists. Very probably some of this confusion has arisen through the belief that *Boletus edulis* should not have a strongly reticulate stipe—and uncertainty whether, 'reticulatus' refers to the pileus surface or stipe surface! Many have wrongly assumed it refers to the stipe.

With the introduction of anatomical details to the study of boletes certain field characters can now be based on firm microscopic observation and not simply on the 'feel' or 'appearance' of a given surface. In the *British Fungus Flora* part I, some of these characters have been emphasised and the epithet 'aestivalis' was adopted therein for the fungus generally known in Britain as *B. reticulatus*. In the same work five sub-species of *B. edulis* were tabulated including the type form with white network at the apex of its stipe, buff colouration below and unchanging flesh. Bridging the morphological gap between these subspecies and *B. aestivalis* Fries a three-spored bolete in the *B. edulis* agg. has been noted and was associated provisionally with *B. aestivalis* (under *B. reticulatus* Boud.). Indeed the uncertainty as to the presence in Britain of two boletes, one growing in summer (*B. aestivalis*) and the second in late autumn, has masked the autonomy of this latter 3-spored bolete which in Scotland is widespread and characteristic of windbreaks of the introduced beech, or of conifer plantations particularly of Sitka spruce (*Picea sitchensis*). Mycologists familiar with this taxon, particularly Drs E. G. Duncan and I. Alexander, have not seen it outwith artificial plantings; this is a very significant observation.

The fungus is typified by the greasy reticulate pileus which may be strongly wrinkled, the distinctly reticulate, clavate and robust stipe and the predominance of three-spored basidia in the hymenium, the percentage of which never falls below 90%. Smith & Singer (1948) and Smith & Thiers (1971) indicate the basidia of typical *B. edulis* as 4-spored and I have had an opportunity to confirm this in fresh collections made in spruce woods of Norway, Sweden, Finland and Switzerland in the summer and autumn of 1971. I conclude that the information reported here is constant and diagnostic for this bolete; there is no indication that normal spore production is in any way hindered, indeed the development of individual spores has been followed

in this species more carefully than in any other bolete. It is this species which has been studied both cytologically at St Andrews University and ecologically at Edinburgh University.

Boletus edulis subsp. **trisporus** Duncan & Watling, **subsp. nov.** Fig. 1, f–i.

Pileus 80–300 mm latus, pulvinatus, glaber vel rugosus, demum subviscidus, ochraceo-brunneus, fulvus vel ferrugineo-aurantiacus demum badius. *Stipes* 45–70 (60–80) × 100–175 mm, pallidus vel pallido-alutaceus, albo-reticulatus. *Caro* albido demum in pileo subvinaceo. *Tubuli* pallide lutei immutabiles; *pori* albido, lutei tarde luteo-brunnei. *Sporae* 13·5–15·5 (–16) × 4–5 μm, subfusoideae, laeves. *Basidia* trisporigera.

Typus: Scotland, Fife, St. Andrews, Tentsmuir, 10 x 1965, *Watling* C. 2493 (holo. E).

Pileus up to 300 mm broad, humid, strongly reticulate, becoming less so especially in the central areas, at the margin wrinkled with raised lines like a roughened skin, becoming shiny and tacky with age although never strongly viscid, ochraceous brown at the margin, darker towards the centre, dark sienna or fulvous with flush of rust-colour then more rusty tawny towards disc and at very centre dark rusty tawny with flush of bay brown; young pilei paler than that of the mature pilei although basically of the same colouration, strongly wrinkled except at very centre; margin of pileus entirely powdery white at first but easily lost and mature pilei appearing only as if hoary, in very young specimens pileipellis overhanging tubes to form a regular but narrow skirt. *Stipe* 70 (–80 at base) × 175 mm, very pale creamy tan or pale buff with many small raised rectangles at the very apex which are slightly larger towards central zone; network whitish or pale pinkish buff at apex, composed of fine lines with sharp but powdered white edges, angular to lozenge shaped, 1–2 mm broad at apex, more elongate downwards and with smaller lines within the main reticulum, clearly differentiated and almost lacunose to ⅓ way from apex, more elongate and pale buff-colour to half way, white reticulate to base but net-work as shallow depressions, passing into distinct white, mycelial tomentum. *Tubes* very pale pallid ochraceous finally dark olivaceous lemon-yellow, depressed about the stipe apex; *pores* pure white at first, stuffed, small, finally angular-ellipsoid and sulphur yellow but bruising rust-colour at maturity. *Flesh* at least 30 mm deep in pileus, white with distinct pinkish or wine-colour under the pileipellis and staining slightly rust-colour or yellowish in stipe-base, soft in pileus and fibrous in stipe; *taste* pleasant, *smell* very slightly acidulous in base of stipe. *Colour changes* of flesh with aqueous solutions of potassium hydroxide, phenol and formaldehyde—negative; with ammonium hydroxide faintly pinkish but soon fading; with Melzer's reagent yellow brown.

Basidia 3-spored, ochraceous yellow in Melzer's reagent, hyaline in water and aqueous ammoniacal and alkaline solutions, clavate with distinct pedicel and sterigmata up to 5 μm long. *Basidiospores* olive-brown in mass; 13·5–15·5 (–16) × 4–5μm, subfusiform in face view, elongate elliptic in side-view with a distinct broad hilar depression, flattened on upper side (boletoid), smooth, with hardly thickened wall, honey-coloured in ammoniacal and alkaline solutions and ochraceous in Melzer's reagent, lacking apical pore

or thinning. *Pleurocystidia* scattered, prominent, 35–45 × 5–7·5*µm*, narrowly fusoid ventricose, hyaline or pale yellowish in water and aqueous ammoniacal and alkaline solutions, pale yellowish in Melzer's reagent; *cheilocystidia* similar to pleurocystidia up to 40 *µm* long, loosely grouped in small bundles. *Hymenophoral trama* of gelatinised, hyaline or pale yellow hyphae divergent from gelatinised and similarly coloured central strand. *Pileipellis* a trichoderm of tangled ± erect hyphae 4–10 *µm* broad which soon collapses and becomes gelatinised; constituent hyphae yellowish in alkaline solutions and in Melzer's reagent and end-cells tubular. *Pileus trama* of anastomosing, tangled hyphae with numerous laticiferous hyphae, pale yellowish in ammoniacal solutions, yellow in Melzer's reagent. *Clamp-connections* absent. Sandy pathside under mixed conifers and beech in amenity belt; good edible fungus.

SCOTLAND. Fife, St. Andrews, Tentsmuir, 10 x 1965, *Watling* C. 2493 (holo. E).

The description is based entirely on the type collection which agrees in all ways with material collected over the past five years in the same place from late August until late October. It seems to be one of the latest appearing boletes in the flora and is therefore useful as teaching material. The type collection agrees in all ways with material from West- and Mid-lothian Dumfries, Perthshire, Aberdeenshire, etc. The Fife material has been selected as type as it is the subject of several student degree projects and publications.

The flesh of the fruit-body of *B. edulis* subsp. *trisporus* is typically composed of thin-walled hyphae lacking clamp-connections. The hyphae vary in width but usually they may be placed in one of two size ranges, 3–5.5(–7) *µm* and 8–12(–14) *µm*. They are interwoven in the pileus-trama but take on a more parallel and less random distribution in the stipe flesh particularly in the cortical areas (the rind, or stipitipellis). Distributed amongst these long cells are irregularly twisted swollen cells up to 20 *µm* broad which in ammoniacal or alkaline solutions are yellower than the surrounding cells (Fig. 1, h).

These have been called laticiferous hyphae but it is doubtful whether this is really the correct term; Smith & Thiers (1971) have also used the term repository hyphae. In *B. edulis* ssp. *trisporus* these hyphae are very prominent and although they are not confined in the Boletaceae to this fungus the opportunity is here taken to describe their structure and possible development. In *B. edulis* ssp. *trisporus* the laticiferous hyphae are of two general sizes similar to the ranges in breadth found in the structural hyphae. Under as yet unspecified conditions it would appear that the contents of the structural (background) hyphae become modified in colour and texture. The contents become yellowish and oily, and lose their granular appearance. This visual change in contents is associated with the breakdown of the septa between the cells in any one hypha; finally a continuous tube filled with clear, yellowish contents results. Presumably at the same time as the disorganization of the septa the hyphal wall plasticises, for individual units swell irregularly. In fact the wall is apparently irregular in thickness (or chemistry) along its length, for simultaneously or certainly at some period very close in time the yellow contents balloon out of the hyphae especially in the vicinity of the now disrupted septa. However, although there is an apparent change in the wall

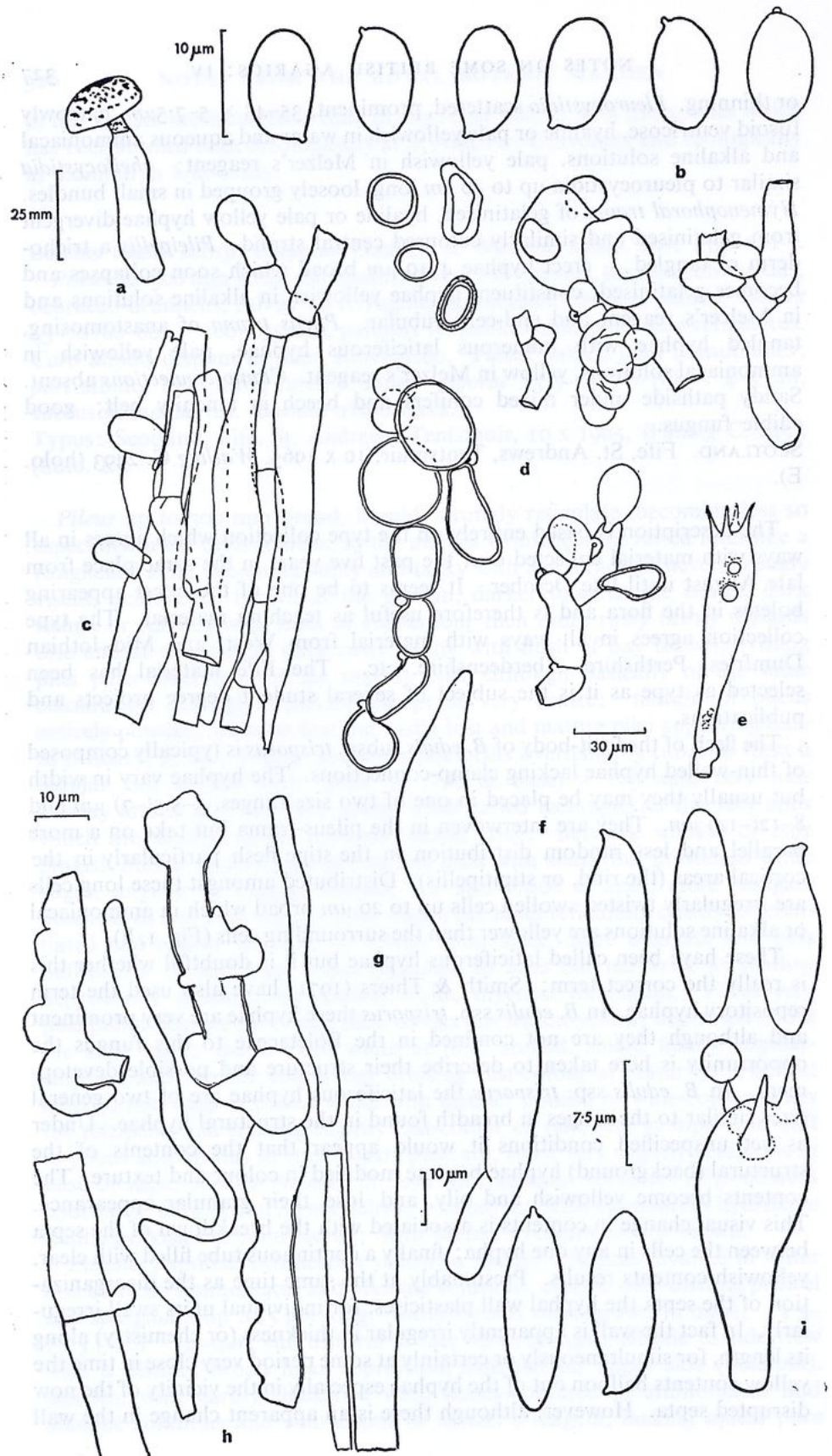

FIG. 1. a-e, *Squamanita paradoxa*: a, field sketch; b, basidiospores; c, cortical zone of stipe; d, chlamydospores from basal rim of stipe; e, basidium. f-i, *Boletus edulis* subsp. *trisporus*: f, basidiospores; g, pleurocystidium; h, laticiferous hyphae from stipe context; i, trisporic basidium. Magnification as indicated.

at these points there is no associated fracturing as the contents are retained and not dispersed irregularly into the surrounding flesh or in microscopic mounts into the mountant. There must be a slightly different innermost layer to the wall or resistant outermost part to the protoplast. The ballooning continues and further areas of swelling appear resulting in a chain of ellipsoid, ovoid or globose units jointed by sections of less modified original cell-wall.

At first these hyphae appear to be indistinguishable from the background hyphae and apparently only differentiate when the primordia have been formed and the fruit-bodies commence to expand. It is suggested that they are simply modified tramal hyphae, a fact which would be supported by Smith's observation (in Smith & Thiers, 1971) that sometimes in boletes such laticiferous hyphae end in hymenial elements.

The function of these hyphae is unknown although it has been suggested that they are conducting vessels or storage organs. They are not the same as the laticifers found in the Russulaceae and although some can be found attached to pleurocystidia in no way can the latter be considered gloeocystidia or chrysocystidia. Singer (1947) has described coscinoids but their actual structure has been re-investigated by Petersen (1968) and they are now thought of as gloeovessels. A further general term i.e. oleiferous hyphae, is used for non-reacting hyphae in *Amanita* and those hyphae reacting with sulpho-aldehydes in *Russula*. However, no published report is available as to their formation or function.

Petersen & Olexia (1967) have indicated that the presence of laticiferous hyphae in the genus *Ramaria* is very important in classifying species and in suggesting evolutionary trends (pers. communication; 1971). Petersen has suggested the use of phase contrast microscopy in order to ease the observation of these structures in the fairy club fungi and although this technique has been used in the present study, because the structures are so conspicuous in *B. edulis* subsp. *trisporus* it was not found really essential.

In *Boletus edulis* the laticiferous hyphae have little or no colouration in reagents; this is in common with most British members of the genus *Boletus*. In *Leccinum*, however, it is the substances contained within these hyphae which darken considerably when the tissue is bruised or exposed to the air. The flesh of *Leccinum percandidum* (Vassilkov) Watling incorporates a very great development of laticiferous hyphae and all elements darken very strongly. Indeed so numerous are they that they must share some of the tensions normally upheld by the structural hyphae in related boletes. In this way there is some parallelism in this fungus with members of the genus *Lactocollybia* (Tricholomataceae).

A third British Squamanita

During the examination of unidentified material from Mull as part of the British Museum botanical survey of the island a collection came to hand which was described on the packet as '*Lepiota amianthinum* with mutant cap'. The dried material was recognised immediately as a species of *Squamanita* and the recent key by Bas (1965) was consulted particularly as two earlier British collections labelled *S. odorata* one at Kew, and one at Edinburgh, had been each described as new species. Unfortunately the Edinburgh material was badly damaged and the name *S. scotica* was only provisionally

adopted; the second species was called *S. pearsonii*. However, close examination of the description of these two taxa indicated that the Mull material did not refer to either species; indeed it keyed with ease to *S. paradoxa* (Smith & Singer) Bas, a fungus originally described in *Cystoderma*. The field notes describing the yellow brown 'scruffiness' and the reference to *Cystoderma amianthinum* supported what is a most unlikely record.

S. paradoxa was originally described from several collections from Oregon, USA (Smith & Singer, 1948). Since then it has been recorded by Horak from Switzerland and by Zd. Shaeffer from Czechoslovakia. The present specimen was unfortunately solitary and lacks extensive field data and ecological information but the field sketch and dried material resembles Smith & Singer's illustration very closely.

It is here unnecessary to add to Bas' macroscopic description as the field-data is scanty but microscopic details (fig. 1, b-e) support the determination and confirm the characters first demonstrated by Bas.

James' field notes accompanied by a sketch (fig. 1, a) are :—
"Curious and anomalous: base seems to be *Lepiota amianthina* with a mutant cap. Cap dilute purplish brown, shaggy fibrillose, with purple-brown fibrils. Stem shaggy fibrillose and similar to cap, fibrils with purple-brown. Lower part golden brown, scurfy. Gills whitish grey. Cap c. 1", umbonate".

Basidia 4-spored, 35–40 × 7·5–10 *μm*, with clamp-connections at base, hyaline in water and ammoniacal solutions, yellowish in Melzer's reagent. *Basidiospores* 8–10·5 × 4·5–6 *μm*, ellipsoid, slightly flattened in one plane in side-view, smooth fairly thin-walled, hyaline in water and ammoniacal solutions. Very slightly dextrinoid in Melzer's solution, only slightly pink in congo red (pale), wall red-purple in cresyl blue, contents blue in cotton blue. *Cheilo-pleurocystidia* absent. *Pileipellis* of radially arranged, pale brownish-grey hyphae 8–15 *μm* broad, slightly more pigmented and perhaps broader in scales, obscured by veil in irregularities of pileus. *Veil* of chains of hyaline, pale yellow to distinctly ochraceous cells, end-cells frequently brown in ammoniacal solutions, smooth or with only one or two obtuse papillae, thin to slightly thick-walled, ellipsoid, ovoid or broadly cylindric, found on stipe as narrow concentric ochraceous brown or rust-coloured zones or in ridge at apex of pseudorhizoidal structure, and in depressions on pileus. *Conidia*, solitary aleuriospores, hyaline, with glassy appearance, thick-walled (bitunicate), lumen often considerably reduced, lacking infra structure, borne on simple, smooth, broad finger-like conidiophores, with branches often reduced, 10–15 *μm* × 7·5–12·5 *μm*, cask-shaped, ellipsoid or ovoid. *Clamp-connections* numerous. *Hymenophoral trama* regular.

Amongst *Calluna* in bog at edge of mixed woodland of *Betula*, *Pinus* and *Quercus* in association with luxuriant material of *Cystoderma amianthinum*. SCOTLAND, Argyllshire, Mull, on north side of Loch Ba, slopes above Grulene House, Garbh Choire, 500 ft, 8 x 1969, *P. James*.

The British species of *Squamanita* can be separated as follows:—

1. Cortical layers of stipe base and pileus covered in chains of rounded cells and abundant smooth, thick-walled chlamydospores
   Basidiospores with moderately thickened wall
   S. paradoxa (Smith & Singer) Bas

+ Cortical layers lacking chains of rounded cells but chlamydospores may be present at stipe-base 2
2. Basidiospores globose, amyloid, slightly thick-walled; chlamydospores lacking . . . . . *S. scotica* Bas nomen prov.
+ Basidiospores ellipsoid, pseudo-amyloid, rather thick-walled; chlamydospores thick-walled with embedded warts . *S. pearsonii* Bas

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Addendum

Since this paper was prepared, Singer (*Sydowia Beiheft* 7:1–106, 1973) has erected the new genus *Nissoderma*, formerly a subgenus of *Cystoderma*, to accommodate the species described above. The present author, however, still wishes to follow Bas's treatment.