THE STATUS OF BOLETUS CALOPUS FR. IN NORTH AMERICA

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Boletus calopus Fr. is a well-known European bolete and a characteristic member of *Boletus* section *Calopodes*. Its occurrence in North America has been discussed by several authors but no evidence to support their conclusions has been presented. Snell (1936) suggested that both *Boletus calopus* and *B. pachypus* Fr. were present in North America; in addition he considered that *B. calopus* and *B. pachypus* were probably synonymous.

However, it was not until Imler (1950) studied *Boletus frustosus* Snell & Dick and *B. calopus* that evidence for a possible relationship between all three taxa was discovered. Imler utilised Melzer's reagent to study the amyloid tissue present in certain boleti and carried out an extensive survey of the reaction found in various species from Europe, North America and elsewhere. In only two species i.e. *B. calopus* and *B. frustosus*, did he observe amyloid septa in otherwise non-amyloid cells of the trama. He noted that the spores were also the same size and shape and concluded that they were probably very closely related.

Darkly amyloid hyphal septa appear therefore only to be common to these last two taxa and have not been observed in any other bolete during this study except perhaps very faintly in some collections from Michigan, U.S.A. of so-called *Boletus subvelutipes* Peck analysed by one of us (R.W.); the colouration produced in this fungus however is very weak and forms more of a halo or haze of pigment about the septa. After the uniqueness of the reaction was fully appreciated and the taxonomic significance understood various modifications of the Iodine reaction were tested primarily to observe whether this staining is localised entirely at the septa, but also to ascertain whether it was general for reactions involving Iodine complexes and whether it was really an amyloid reaction parallel to that which is found in some agaric spores.

Watling (1966) has already pointed out that the colour range one accepts under the term amyloid is an extremely broad spectrum and the reaction may not be as simple as it at first appears; it may involve different substrates with similar stereo-chemical properties, different quantities of the same substrate or a mixture of both these factors. A procedure outlined by Johansen (1940) was utilised to test these questions and involved the placing of fresh sections of the carpophore in a I-KI solution for fifteen minutes and then transferring and mounting them in a fresh solution of the same mixture to which was added a drop of a 65% aqueous solution of sulphuric acid; the acid was allowed to influx under the cover-slip. Gradually a bright cobalt blue developed at the septa indicating the presence at this location of a long chain polysaccharide with similar chemical composition and/or stereo-chemical properties to starch or cellulose.

Applying the zinc-chlor-iodine-cellulose reaction outlined by Rawlins & Takahashi (1952) a positive result was again obtained. Here similar sections

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as above were mounted in several drops of zinc-chlor-iodine.* A blue colour was clearly seen at the septa in hyphae of *Boletus calopus* and a closely related bolete from Idaho to be discussed below. Both the above reactions imparted rather different shades of blue to the septa of hyphae taken from the same area of the same carpophore; in Melzer's solution parallel septa turned blue-black. Throughout the above experiments controls were run on all the reagents used and the solutions utilised in their preparation. Only in an aqueous solution of KI was a slight reaction recorded and this was only after a twenty-four hour incubation period during which time the sections were exposed for over twelve hours to diffuse light. In a fresh solution of chloral hydrate and KI a yellow colour was exhibited but if the same solution was tested after a month an amethyst colour was immediately produced and after prolonged incubation some bluing was observed.

The rather more simple but traditional use of the KI-zinc reaction immediately gave a rich red brown colour but this gradually became slightly lilaceous in places and after 5–10 minutes a blue-black colouration resulted.

One of us (R.W.) during a symposium held jointly by the Society for General Microbiology and the British Mycological Society called the reaction between Melzer's solution and bolete tissues the Imler reaction in honour of L. Imler who as noted above was the first to appreciate the significance of this reaction in the taxonomy of the boleti.

The blue colouration discussed above which is localised in the hyphal septa is very easily demonstrable; however it cannot be the only source of a substrate which blues with iodine complexes because the flesh of *B. calopus* turns a deep blue-black, a stronger colour than can be accounted for simply by the presence of amyloid septa. Part of this colour fades, both as the carpophore matures and as the sections are allowed to stand in the mountant; thus the blue colour noted is in addition due either to the fleeting amyloid reaction mentioned by Smith (1965) and so common in boletes and related fungi e.g. *Gyrodon meruloides* (Schw.) Singer or the extreme blue-green colouration found in *Boletus rubinus* W.G.Sm. when the flesh is treated with similar reagents.

It was necessary as a first step in the study of the status of *B. frustosus* to examine the type material and compare it with a bolete we had collected and studied in the Western United States and believed to be closely related to it and to *B. calopus*. *B. frustosus* is characterised by possessing very large frusta in the pileus (Plate 14B & D). In order to study the 'cuticle', sections were made through the flat upper surface of these frusta.

The 'cuticle' of our western collections was compared with sections of the surface of the frusta of the type of Snell and Dick's bolete; the 'cuticle' in both was identical in every way. In addition it was noted that one side of the pileus of the type of *B. frustosus* possessed frusta whilst the other portion of the pileus showed a minutely tomentose to smooth surface as is shown in the plate. This portion of the pileus is also identical in every way with the sections prepared from the frusta and the 'cuticle' of other non-frustose western collections (Plate 14C). It is quite likely that the type of *B. frustosus* was baked in the sun or dried *in situ* prior to collection. A similar situation appears to have arisen in *Boletus stellenbossiensis* v.d. Byl. from South

* 50g $ZnCl_2$; 16g KI; 17 ml distilled water: mix, add excess iodine and stand for several days.

Africa which Pearson (1950) considers to be simply *Boletus edulis* Fr. with the development of frusta on the pileus due to becoming sun-dried.

No field notes on the fresh condition and colour of the type collection of *B. frustosus* accompany the herbarium material and thus it is necessary to rely on the characters as published by Snell (1941) and by Slipp & Snell (1944). In both descriptions the characters listed vary only slightly from those of typical *B. calopus*. We have collected many times in Idaho particularly in the two hundred year old *Tsuga heterophylla* stand along Sands Creek mentioned by Slipp & Snell (1944), a bolete which can only possibly be conspecific with *B. frustosus*. It cannot be distinguished microscopically from *B. calopus* and certainly had many of the macroscopic characters outlined for *B. frustosus* with the exception of the large frusta. One cap in one collection was rimose-areolate i.e. frustose but all our other collections from the Western United States were smooth to subtomentose (Plate 14A and C). There is little doubt therefore that the formation of large frusta is the exception to the rule. Thus some questions exist concerning the status of *B. frustosus* as it occurs in the Western United States.

In Europe *B. calopus* has a distinctly white or pinkish netted, ± swollen stipe which is yellow only at the very apex if at all and carmine, or some similar intense purple-red colour elsewhere. In the Western United States it is only faintly red-coloured if red at all and hardly noticeably netted when young whilst the stipe is broad to nearly bulbose at the base often with a tendency to become subradicate. In Europe the pileus is usually pale grey or light brown ('light drab') although a darker more olivaceous coloured form is infrequently seen; this form probably gave rise to the taxon *B. olivaceus* Fr. now considered synonymous with *B. calopus*. In the Western United States it is much more characteristically darker brown, 'olive-brown' to 'buffy-brown' or 'clove brown'. The differences between the taxa are therefore of degree and warrant recognition only at varietal rank; it is proposed therefore that the taxon in question be recognized as a variety of *B. calopus*. The authors are aware that there is a very slight difference in spore length of up to 2μ in 15μ between the two taxa but we do not believe this is significant when general shape and width are also considered. The epithet *olivaceus* has not been used, although the North American fungus is often quite olive, because the original description under this name does not cover all the features mentioned above. It must be emphasised that the microscopic characters of *B. calopus* var. *calopus* and the new variety are identical. The reticulum although distinct in collections from Western United States forms late in the carpophore's development and then frequently only at the stipe apex. We have not seen European collections of this fungus lacking a reticulum although such forms are known (cf. Pearson, 1946). The problems surrounding the significance of the presence or absence of a stipe reticulum in classification occurs in several boletes; for instance it causes considerable confusion in the *Boletus erythropus—subvelutipes—luridus* complex. Bertaux's (1960) notes on this subject are relevant in placing these non-reticulate forms of *B. calopus* in what appears to be usually a very constantly netted bolete.

It is pertinent in this discussion to mention *Boletus inedulis* (Murr.) Murr. because this taxon appears to be closely related to *B. calopus*. Singer (1947) has described the fungus in some detail based on collections from Florida

and one collection from South Carolina originally determined as *B. pachypus*. Part of the type and two collections determined by Murrill at the National Fungus Collections (BPI) were studied by one of us (O.K.M.). These were (part of type) Ex Herb. Univ. Fla., Coll. & Det. W. A. Murrill; Missouri Bot. Garden, no. 150910; Gainesville, Florida, 11 iv 1941, Det. W. A. Murrill. *B. inedulis*, however, has neither amyloid septa nor amyloid hyphal walls in the context; it differs from *B. calopus* also in the colours of the stipe and its reticulation which are 'lemon chrome' to 'light cadmium' yellow and 'acajou red' or 'pomegrade purple' respectively. In the publication cited above Singer discussed both *B. frustosus* Snell & Dick and *B. radicans* Fr. in the sense of Kallenbach. He recorded the latter from Maine and the former from Idaho where non-frustose specimens were collected and reported as 'hard to distinguish from good specimens of *B. radicans* in dried condition'. We have collected in Idaho specimens which could possibly agree with *B. albidus* Rocq. (=*B. radicans* sensu Kallenbach) but we are of the opinion that the collections from Selway National Forest, Rossbach, cited by Singer could well represent the taxon we are dealing with here.

A. H. Smith has kindly sent us two collections of Kauffman's, one which was named *Boletus calopus* and the other *B. pachypus*; they were collected at Coy Glen, Ithaca, New York State (9 viii 1904) and Sault St. Marie, Michigan (11 vii 1906) respectively. Neither of the collections give the characteristic amyloid reaction; in fact, anatomically they are quite different to *B. calopus*; both collections possess distinct pileocystidia. Five collections named by Ravenel *B. pachypus* have also been examined; three housed at Edinburgh (E) and two at the Farlow Herbarium, Cambridge, Massachusetts (FH). Two of the former collections are mouldy and are certainly not *B. calopus* and the latter collections have longitudinally striate spores and are possibly *Boletellus chrysenteroides*; a few similar alien spores are also found in the Edinburgh specimens. A further collection of Ravenel's from S Carolina in the herbarium of the National Fungus Collections, Beltsville, Maryland (BPI) also has longitudinally striate spores but the stipe is lacerate and the specimens are probably referable to *Boletellus russellii* Frost. as is the third collection at Edinburgh.

The authors conclude from all the reports of *B. calopus* (or *B. pachypus*) which were uncovered and studied that this taxon has not been found as yet in eastern North America. In addition, closely related species in *Calopodes* reported from eastern North America and elsewhere have been examined but none have the distinctive darkly amyloid septa and correlated characters of *B. calopus* var. *calopus* and *B. calopus* var. *frustosus*.

It is also necessary to mention here the taxon *B. pachypus* Fr. described by Fries (1821) in the same publication as *B. calopus* and indicated by him as very close to it. *B. pachypus* differs mainly in the alutaceous pileus, the free tubes, the round pores and the stipe being 'flavo-rubro', as opposed to 'coccineo' as indicated for *B. calopus*. Pearson (1946) simply states:- Identity doubtful; either *calopus* or *albidus*: Dennis, Orton & Hora (1960) consider it simply a synonym of *B. calopus*, a result also arrived at by other European workers e.g. Pilat (1958). The present authors support this conclusion for it has been seen in the field that the extent of yellow at the stipe apex may be quite small, the tubes frequently become free and the pores angular in some carpophores only at maturity; the colour range exhibited by the pileus includes alutaceous.

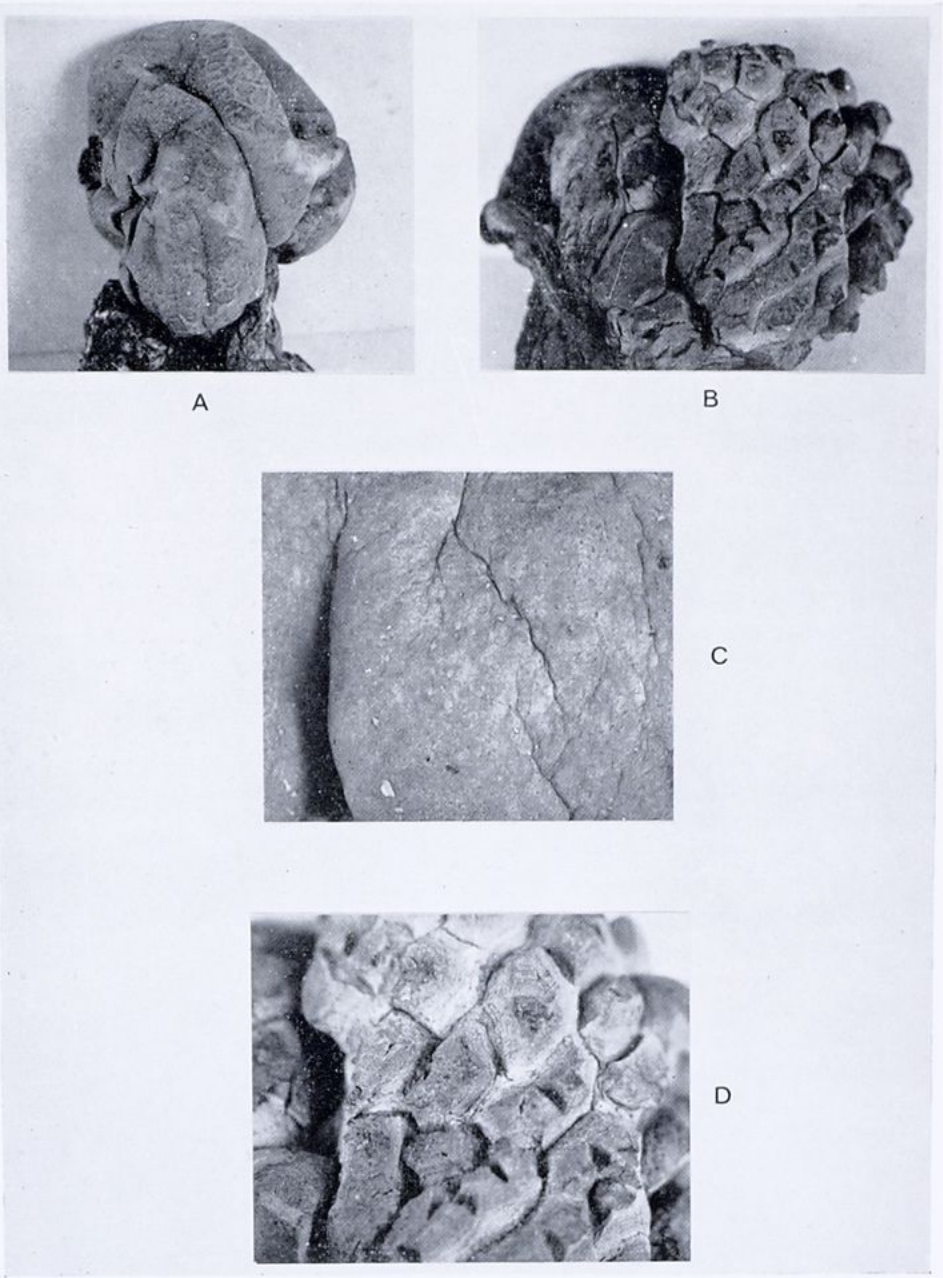

PLATE 14. *Boletus calopus* Fr. var. *frustosus* (Snell & Dick) Miller & Watling.
A & C. *Boletus calopus* from Sands Creek, Deception Creek Experimental Forest, Coeur d'Alene, Idaho, ix 1966; A, general facies; C, close-up of pileus surface in A: B and D, *Boletus frustosus* Snell & Dick (type); B, general facies showing large frusta; D. close-up of B. (A & B approx. × ½).

Colours referred to in quotations (e.g. 'Olive-brown') in the text and in the descriptions which follow are from Ridgway (1912). The national herbaria from which specimens have been borrowed and examined are abbreviated as outlined by Lanjouw & Stafleu (1964). Both authors are grateful to the respective directors of institutes from which specimens have been borrowed.

Boletus calopus Fries in Syst. Mycologicum: 390 (1821).

var. **calopus** Fr. ibid. Fig. 1a–a″.

Syn.: *Boletus pachypus* Fr. in Syst. Mycologicum: 390 (1821).

Boletus olivaceous Fr. in Epicrisis Syst. Mycologicum: 416 (1838).

Pileus 6–21 cm wide, almost globose then convex, dry, subtomentose then smooth, whitish grey or pallid tan, 'drab-grey', 'drab' when young to greyish olive, at first gradually darkening but finally paling and becoming flushed with light brown to ochraceous, sometimes slightly minutely cracked scaly at disc when old, infrequently cracking deeply to form 'lesions' and so exposing the pallid flesh beneath. *Stipe* robust, nearly equal or slightly swollen towards the base, not infrequently distinctly obconic, carmine red to almost scarlet, deeper tints especially towards the base where it may also be brownish, paler about the centre and usually with the very apex distinctly yellow, conspicuously reticulate over upper half or completely to the base with white or pinkish network, infrequently non-reticulate. *Tubes* depressed, adnate, up to 20 mm deep, dirty sulphur-yellow becoming blue when exposed to the air and fading finally to olivaceous, characteristically joining up with the reticulation at the stipe apex; *pores* 2 per mm on average at maturity, round, dirty sulphur yellow becoming flushed greenish and bruising bluish green and in age yellowish with brown tint. *Context* firm, pale sulphur or deeper sulphur to chrome yellow in places in the stipe, pallid or pale lemon in pileus which immediately becomes whitish then rapidly azure blue, becoming stronger blue in stipe particularly the base where it is also often mosaiced with red; yellow brown with alkali, olive green with Fe SO_4 and dark blue black with Melzer's solution. *Taste* bitter, fading slightly when old; *odour* strong, distinct although not unpleasant.

Basidia 21–35 × 6·5–11μ, clavate, thin-walled, 4-spored, hyaline in both KOH and Melzer's solution. *Spores* 10–16 × 5–5·5μ, thin-walled, subfusiform in profile, elliptical in face-view, yellow brown in Melzer's solution, light yellowish in KOH. *Pleurocystidia* 24–38 × 5–8·5μ, fusiform to narrowly fusiform or narrowly clavate to lageniform, thin-walled, widely distributed but not frequent, solitary or in loose fascicles, hyaline in KOH and Melzer's solution; *cheilocystidia* similar in all respects to pleurocystidia or slightly more variable, usually in fascicles protruding 1/4 to 1/5 of total length above the hymenium. '*Cuticle*' *of pileus* a compact decumbent layer of tightly packed, ± ornamented hyphae 2·5–9μ in diam. × 17–60μ long, thin-walled, yellow brown in Melzer's solution although dark blue black particles which have similarities to those described by Harrison (1964) in *Hydnum* spp. may be seen adhering to the cell walls. Directly beneath this outer layer is a reddish brown pigmented zone and an amyloid (blue) layer with scattered amyloid septa; this is a distinct layer which is darker amyloid than the trama beneath. *Trama of pileus* consisting of loosely interwoven hyphae 3–13μ in diam. nearly cylindric to somewhat inflated, yellowish with darkly amyloid

septa becoming amyloid throughout the pores. *Trama of pores* consisting of nearly parallel, slightly diverging hyphae, 3–6μ in diam. with deeply amyloid septa which become so numerous in subhymenium that it appears like an amyloid zone although few cells are amyloid throughout. No clamp-connections seen.

Habit, Habitat & Distribution: In frondose woods under beech (*Fagus sylvatica*) and oak (*Quercus robur*), usually on siliceous soils; July to October. Material examined: Germany, x 1927, *Lohwag* (MICH); Downs south of Horsley, Surrey, England, 6 viii 1950, *R. W. G. Dennis* (MICH); Gifford East Lothian, Scotland, 8 ix 1953, *I. M. Cowan in Henderson* 772 (as *Boletus appendiculatus*); Loch an Eilean, Inverness-shire, Scotland, 13 ix 1957, *Watling* 217C; Derwentwater, Cumberland, England, 17 vii 1958, *Watling* 260C; Dinnet Wood, Aberdeenshire, Scotland, 26 viii 1962, *Watling* 2650C; same locality different dates, *Watling* 2651C & 2652C; Crathes Castle, Aberdeenshire, Scotland, 24 viii 1963, *Watling* 2660C; South Devon, England, 6 ix 1966, *Freystag* in *Watling* 4131 (all in E); Lundell & Nannfeldt Exsiccati 2609, Sweden (BPI); Italy, G. Bresadola, Trento (BPI).

var. **frustosus** (Snell & Dick) Miller & Watling **comb. nov.**

Plate 14A & C; fig. 1c–c″: (Type) Plate 14B & D; fig. 1b–b″.

Basionym: *Boletus frustosus* Snell & Dick in Mycologia 33: 194 (1941).

Pileus 5–12(–25) mm wide, dry, convex, light to dark brown, 'wood brown' to 'Saccardo's umber' if moistened or at maturity 'bister' to 'chestnut brown' somewhat areolate in age, turning dull blue when bruised and slowly fading. *Stipe* 7–9 cm long, 3·5–4(–5) cm wide, widest in middle tapering towards apex and base or nearly equal, reticulate in all mature caps (young buttons show no reticulation) in some strongly reticulate to the base, 'barium yellow' to 'straw yellow' becoming salmon to reddish at base with pallid reticulations. *Tubes* depressed; *pores* 2–3 per mm at maturity, nearly round, very fine in buttons, yellow with greenish cast, 'citron yellow' to 'barium yellow' changing to blue when bruised at or near maturity, drying brown.

Basidia 27–77 × 5·0–15μ clavate, thin-walled, 4-spored, hyaline with scattered granular yellow contents in KOH, yellowish to hyaline in Melzer's solution. *Spores* (10–)12–14 × 4·0–5·0μ subfusoid in profile, elliptical in face view, thin-walled, hyaline in KOH and Melzer's solution, scattered or in fascicles. '*Cuticle*' *of pileus* consisting of interwoven faintly to strongly banded hyphae 3·5–15μ in diam. mostly hyaline to yellowish with a few amyloid septa, some with amyloid particles adhering to the cell walls in Melzer's solution, yellowish in KOH, lactifers occasionally terminate in cystidioid end-cells which appear as dermatocystidia. *Trama of pileus* consisting of interwoven hyphae (1·5–)3·5–8·5(–18)μ in diam., thin-walled yellow to yellow brown near cuticle with yellow or amyloid (deep purple) septa or occasionally amyloid (light blue) walls in Melzer's solution. *Tube trama* consisting of nearly parallel hyphae that diverge slightly from a central strand; hyphae 3·5–8·5μ in diam., deep yellowish with amyloid (deep purple) septa which are sometimes numerous near hymenium and appear as an amyloid zone in Melzer's solution, light yellowish to nearly hyaline in KOH. No clamp-connections seen.

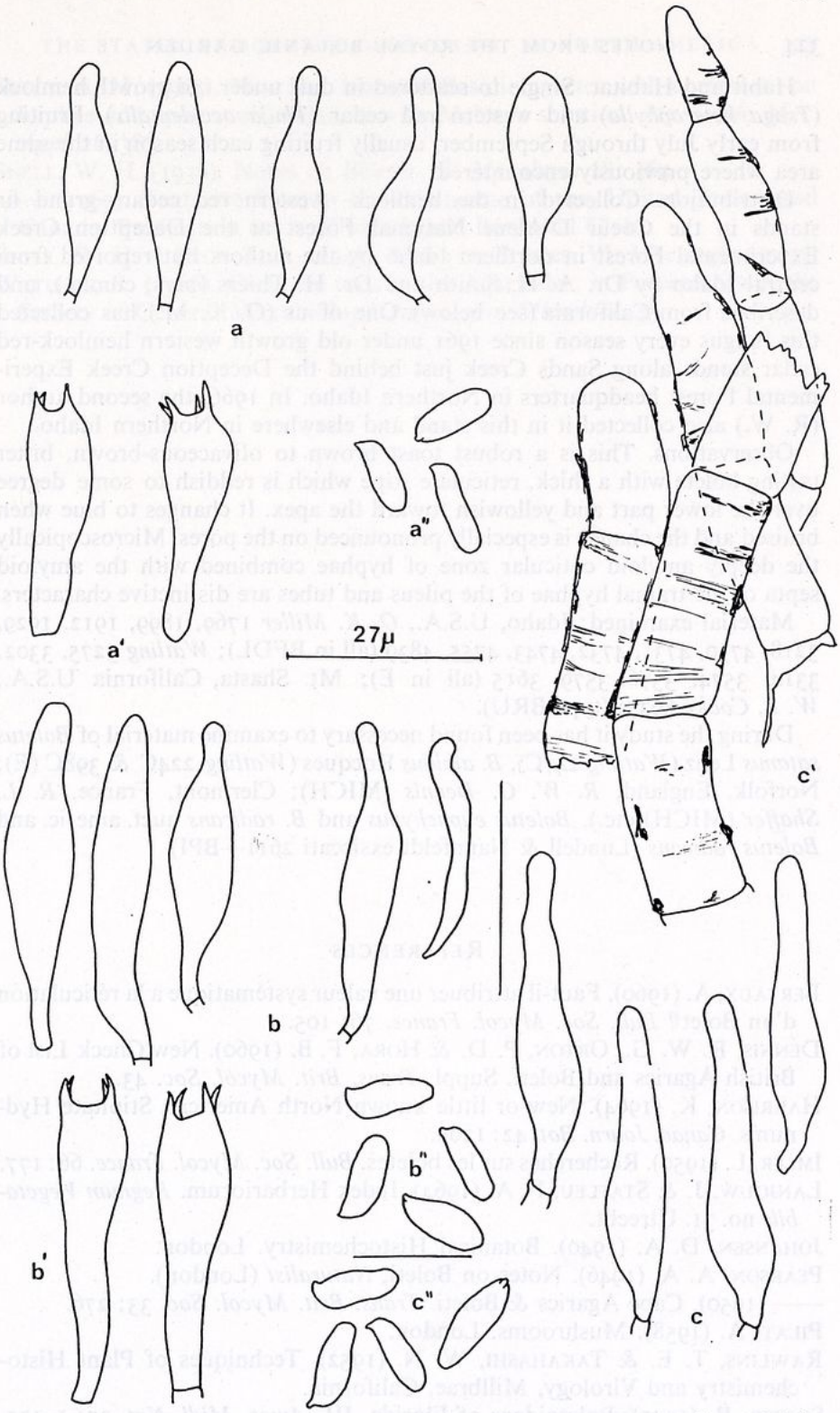

FIG. 1. *Boletus calopus* Fr. var. *calopus* and var. *frustosus* (Snell & Dick) Miller & Watling. a–a″ var. *calopus*, Lundell & Nannfeldt Exsiccati 2609; a, cystidia; a′, basidia; a″, basidiospores. b–b″ *Boletus frustosus* Snell & Dick (type): b, cystidia; b′, basidia, b″, basidiospores. c–c″ var. *frustosus*, Sands Creek, near Deception Creek, Couer d'Alene, Idaho-30 x 1966, *Watling* 3576: c, cystidia; c′, elements of the pileus 'cuticle' showing end-cells; c″, basidiospores.

Habit and Habitat. Single to scattered in duff under old growth hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja occidentalis*). Fruiting from early July through September, usually fruiting each season in the same area where previously encountered.

Distribution. Collected in the hemlock—western red cedar—grand fir stands in the Coeur D'Alene National Forest at the Deception Creek Experimental Forest in northern Idaho by the authors but reported from central Idaho by Dr. A. H. Smith and Dr. H. Thiers (pers. comm.), and described from California (see below). One of us (O. K. M.) has collected this fungus every season since 1961 under old growth western hemlock-red cedar stands along Sands Creek just behind the Deception Creek Experimental Forest headquarters in Northern Idaho. In 1966, the second author (R. W.) also collected it in this stand and elsewhere in Northern Idaho.

Observations. This is a robust toast brown to olivaceous-brown, bitter tasting bolete with a thick, reticulate stipe which is reddish to some degree over the lower part and yellowish toward the apex. It changes to blue when bruised and the change is especially pronounced on the pores. Microscopically the deeply amyloid cuticular zone of hyphae combined with the amyloid septa of the tramal hyphae of the pileus and tubes are distinctive characters.

Material examined: Idaho, U.S.A., *O. K. Miller* 1769, 1899, 1912, 1929, 3218, 4730, 4731, 4732, 4743, 4755, 4839 (all in BFDL); *Watling* 3275, 3302, 3314, 3574, 3576, 3579, 3615 (all in E); Mt. Shasta, California U.S.A., *W. B. Cooke* 8637; (type BRU).

During the study it has been found necessary to examine material of *Boletus satanus* Lenz (*Watling* 247C), *B. albidus* Rocques (*Watling* 224C & 398C (E); Norfolk, England, *R. W. G. Dennis* (MICH); Clermont, France, *R. L. Shaffer* (MICH) etc.), *Boletus eupachypus* and *B. radicans* auct. americ. and *Boletus radicans* (Lundell & Nannfeldt exsiccati 2611—BPI).

References

Bertaux, A. (1960). Faut-il attribuer une valeur systématique a la réticulation d'un Bolet? *Bull. Soc. Mycol. France.* 76: 105.

Dennis, R. W. G., Orton, P. D. & Hora, F. B. (1960). New Check List of British Agarics and Boleti. Suppl. *Trans. Brit. Mycol. Soc.* 43.

Harrison, K. (1964). New or little known North American Stipitate Hydnums. *Canad. Journ. Bot.* 42: 1205.

Imler, L. (1950). Recherches sur les boletes. *Bull. Soc. Mycol. France.* 66: 177.

Lanjouw, J. & Stafleu, F. A. (1964). Index Herbariorum. *Regnum Vegetabile* no. 31. Utrecht.

Johansen, D. A. (1940). Botanical Histochemistry. London.

Pearson, A. A. (1946). Notes on Boleti, *Naturalist* (London).

—— (1950). Cape Agarics & Boleti. *Trans. Brit. Mycol. Soc.* 33: 276.

Pilat, A. (1958). Mushrooms. London.

Rawlins, T. E. & Takahashi, W. N. (1952). Techniques of Plant Histochemistry and Virology, Millbrae, California.

Singer, R. (1947). Boletoideae of Florida, III. *Amer. Midl. Nat.* 37: 1–135.

Slipp, A. W. & Snell, W. H. (1944). Taxonomic-ecologic studies of the Boletaceae in Northern Idaho and adjacent Washington. *Lloydia* 7: 1–66.

SMITH, A. H. (1965). New and Unusual Basidiomycetes with comments on hyphae and spore wall reactions with Melzer's solution. *Mycopath. Myc. Appl.* 26: 385.

SNELL, W. H. (1936). Notes on Boletes, V. *Mycologia* 28: 464.

—— (1960). Tentative keys to the Boletaceae of the United States and Canada. Publication no. 1, Rhode Island Botanical Club.

SNELL, W. H. & DICK, E. A. (1941). Notes on Boletes, VI. *Mycologia* 33: 23.

WATLING, R. (1966). Chemical tests in the Agaricales, in Proceedings of the Society of General Microbiology. *Journ. gen. Microbiol.* 45.