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CLITOPILUS ARGENTINUS IN NORTH AMERICA*

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Clitopilus argentinus is reported for the first time from North America. A full description and illustrations of the basidiomata and microscopic structures are provided. An X-ray elemental analysis of the diagnostic 'hyaline' incrustations of the pileus surface shows these extracellular structures to be composed mainly of calcium and magnesium. In addition, a multispore culture of this collection produces basidiomata in vitro. *Clitopilus incrustatus* Singer and *C. fuscogelatinosus* Redhead & Baroni are also discussed.

Clitopilus argentinus Singer was originally described by Spegazzini in 1898 from Argentina as *Pleurotus pusillimus* Speg. and was reported as growing on living bryophytes (Farr, 1973). Singer (1952), after a study of the type collection of *P. pusillimus*, concluded that it belonged in the genus *Clitopilus* and therefore had to provide a new name for this taxon since the epithet *pusillimus* was preoccupied (i.e. *Clitopilus pusillimus* (Speg.) Sing., an *Omphalia* as described by Spegazzini). In Singer's (1952) paper he reported two further collections (*Singer T. 822* and *T. 861*) of *C. argentinus* from the montane and subalpine regions in Argentina from wood and bark substrata of dead trees, i.e. *Podocarpus parlatorei* and *Polylepis australis*. Since those two widely scattered reports, nothing further has appeared in the literature about this species.

The following report provides a description of the North American collection of *C. argentinus*, and adds new information to our understanding of the diagnostic microscopic character of this species, the hyaline encrusting material on the hyphae of the pileipellis. These diagnostic extracellular structures are illustrated with light and SEM photomicrographs and were also analyzed with the aid of an X-ray probe. *Clitopilus incrustatus* Sing. (Singer, 1948) and *C. fuscogelatinosus* Redh. & Baroni (Redhead & Baroni, 1986), taxa of similar macromorphology and ones which also produce encrusting materials on the hyphae of their pileipelles are discussed. In addition, this is the first report of successful pure culture and basidiome production of *C. argentinus* in vitro.

MATERIALS AND METHODS

For the production of the macro- and microscopic descriptions, methods employed were those previously cited in Baroni (1990), with the exception that E (the spore length divided by the spore width) is now designated as Q and E^m (the mean of E) is now designated as Q^m.

* Entolomataceae in North America II.

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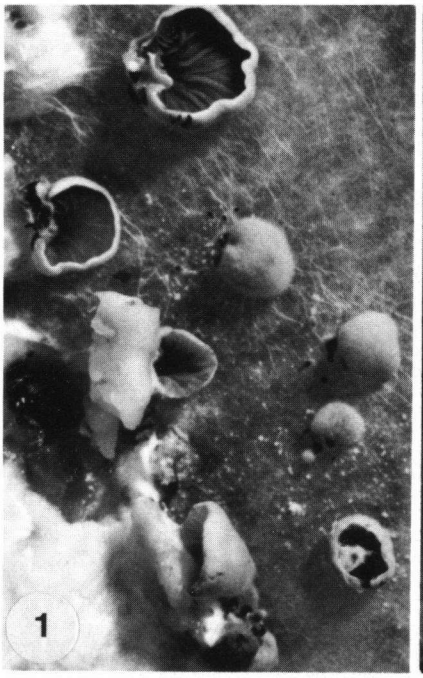
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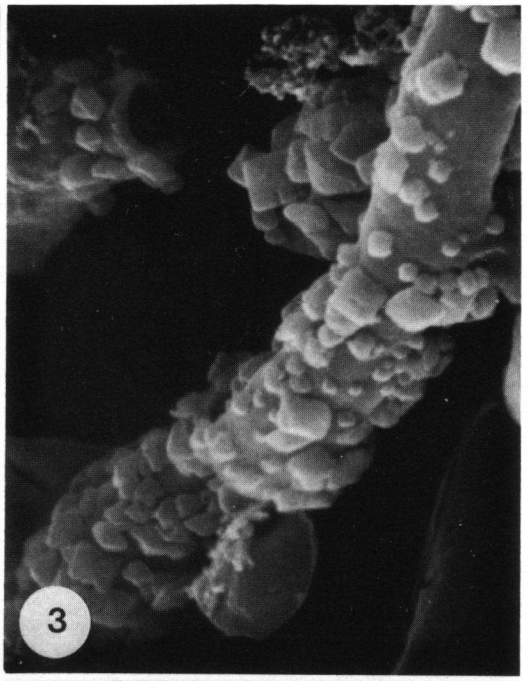
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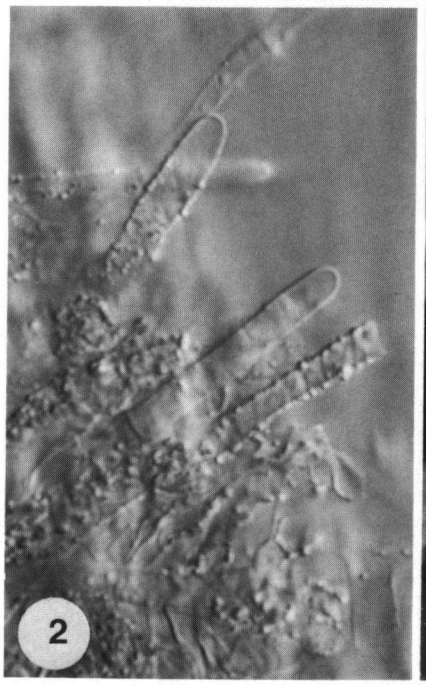
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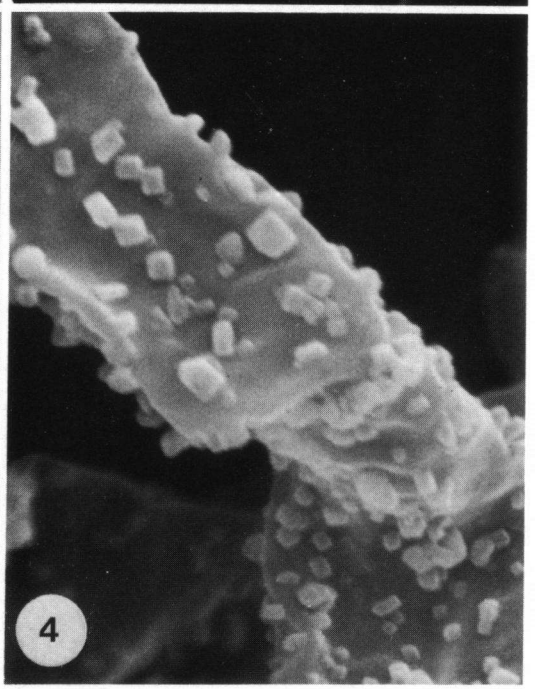
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In the materials studied section the collection designated (CORT), which is our institutional herbarium, is currently not listed in Holmgren & Keuken (1974).

Cultures were grown on PDA (Difco) and MEA (M-35, Stevens, 1974) in plastic petri dishes and kept in an incubator held at 24°C with a light/dark cycle of 11/13 hrs per day. Five plates were inoculated per study and two separate studies were performed. Radial growth measurements and morphological features were recorded every 4 to 6 days until the culture covered two thirds of the agar surface.

The light photomicrographs of the crystal encrusted hyphae were produced using Hoffman Modulation Optics. The SEM photomicrographs and the X-ray elemental analysis profiles were taken on an ETEC SEM operated at 20 keV and interfaced with a KEVEX X-ray analysis system.

RESULTS

A. Taxonomic studies

Clitopilus argentinus Sing.—Figs. 1–9

Clitopilus argentinus Sing. *in* Lilloa 25: 513–514. ('1951') 1952. ≡ *Pleurotus pusillimus* Speg. *in* An. Mus. nac. Hist. nat. B. Aires 6: 108–109. 1898 (fide Farr, 1973).

Pileus pale greyish (5B2) to darker greyish (5C2), densely whitish or pale greyish hispid pubescent overall and this vestiture easily squashed and then showing the darker greyish hues, 1–10 mm broad, convex becoming broadly convex to plano-convex, some +/- conchate, dimidiate or subspathulate, margin strongly inrolled and densely hispid. Lamellae greyish as pileus (mostly 5C2), adnate to adnexed, not decurrent, close (L = 6–20, l = 1–2 tiers), narrow or moderately broad; edges even and concolorous. Stipe (when present!) coloured as pileus, central at first in youngest buttons, always becoming eccentric to nearly lateral, typically disappearing at maturity, if present at maturity very reduced and at most 0.5 wide and 1 mm long, equal, hispid pubescent overall, moderately strigose at base.

Basidiospores 5.6–7.2(–8) × 4–4.8(–5.2) µm (Q = 1.17–1.8, Q^m = 1.45, L^m = 6.57, W^m = 4.45, n = 23), broadly ellipsoid to oblong in lateral and face views with faint longitudinal lines running from apex to apiculus, with distinct to obscure angular appearance in polar view (8–10 facets) because of pronounced to obscure ridges; walls evenly cyanophilic, sordid melleous in NH_4OH, inamyloid; deposit not successful. Basidia 21.9–32 × 7–8.8 µm, 4-sterigmate, a very few 2-sterigmate, less frequently 1-sterigmate, elongate-clavate, with widely scattered and small, dark cyanophilic bodies, thin-walled. Hymenial cystidia lacking. Lamellar trama of strongly interwoven cylindric to inflated hyphae producing a cellular appearance, 2.4–12.1 µm in diameter. Pileus context of radially arranged, hyaline hyphae of the same diam. as lamellar trama. Pileipellis two layered; suprapellis of loosely interwoven and often erect, hyaline, tapered, projecting, filamentous end cells which are occasionally branched, 1.6–4 µm in diameter at widest point; subpellis an interwoven layer of cylindric hyphae, 2.4–4 µm in diameter; walls of hyphae pale brownish and covered with

Figs. 1–4. *Clitopilus argentinus*. — 1. Basidiomata produced in vitro, × 1.5. — 2. Hyphae from pileipellis, × 1500. — 3. SEM of hyphae of pileipellis, × 8000. — 4. SEM of hyphae of pileipellis, × 9000. (Figs. 1, 2 and 4 from *Baroni 6057*; fig. 3 from *Dumont PA 921*.)

abundant, small, hyaline, refractive, crystalline-like encrusting material. Clamp connections absent.

Habitat.—Lignicolous, on decaying periderm and exposed wood of (?) *Ostrya virginiana* (Mill.) K. Koch.

Collections examined.—U.S.A: New York, Tompkins Co., vicinity of Ithaca, McGowan's Woods, 29.VIII.1988, *Baroni 6057* (CORT). — ARGENTINA: prov. Tucuman, Cerro Grande del Taficillo, 6.I.1950, *Singer T822* (MICH). — PANAMA: prov. San Blas, trail from Puerto Obaldia to Darien, approx. 300 ft above sea level, no date on collection label, *Dumont PA 921*, conf. Singer (NY).

Collections examined of *Clitopilus incrustatus* Sing.— U.S.A: Florida, Alachua Co., Gainesville, *Singer F1981* (Holotype, FH); Texas, Grimes Co., Sam Houston National Forest, near Richards, 17.V.1953, *Thiers 1872* (MICH). — ARGENTINA: prov. Tucuman, Yerba Buena, 4.XI.1950, *Singer T1005* (MICH).

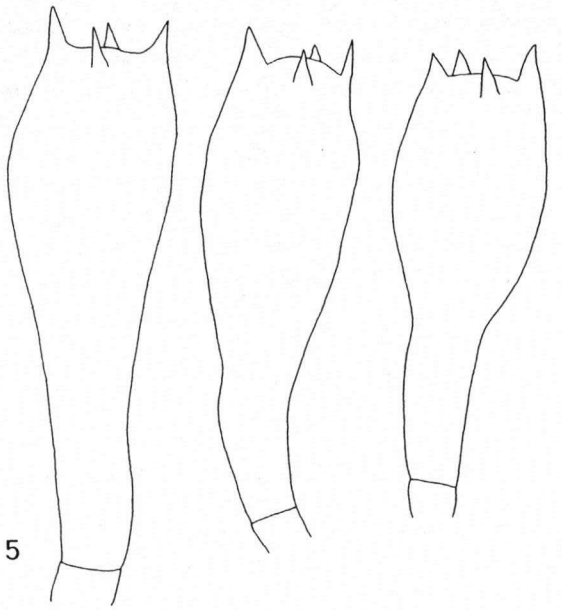
Clitopilus argentinus is recognized in the field by its pleurotoid habit, the pale to moderately greyish, small pilei which are adorned with a dense hispid vestiture, the greyish lamellae and the lack of a stipe at maturity. The hyaline, refractive, crystalline-like encrusting material which covers the hyphae of the subpellis (Fig. 2) is diagnostic for this species.

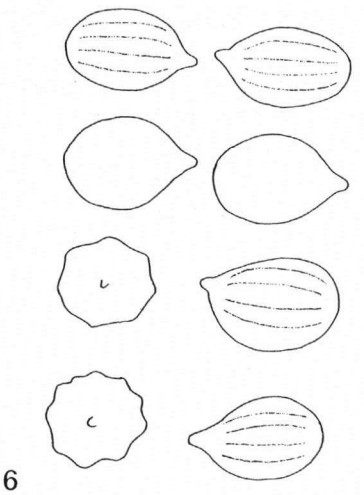
Clitopilus incrustatus was examined for comparison because of the similarity in macroscopic morphology to *C. argentinus*. *Clitopilus incrustatus* differs most notably from *C. argentinus* by its paler coloured lamellae ('Lamellis sordide albidis, dein albo-cremeis vel alutaceo-albidis' Singer, 1948) and by the obvious, dark brown, plate-like incrustations on the hyphae of the pileipellis. Otherwise these two taxa are similar in size and shape, basidiospore size, general construction of the pileipellis (i.e. a two-layered pellis as described for *C. argentinus* above) and their preference for decaying woody substrata. Interestingly, numerous, small, hyaline, refractive crystalline-like bodies were found scattered over the hyphae of the pileipellis of *C. incrustatus* in addition to the obvious, dark brownish, plate-like, extracellular pigments. Also, large (5–10.5 × 2–5.6 µm) hyaline prismatic crystals can be found generally distributed among the tissues of the pileus surface and context and the lamellar faces in the type collection. *Clitopilus argentinus* and *C. incrustatus* are apparently closely related based upon morphological similarities. However, they are readily separated by the colour of their lamellae and by the type of encrusting material on the hyphae of the pileipellis.

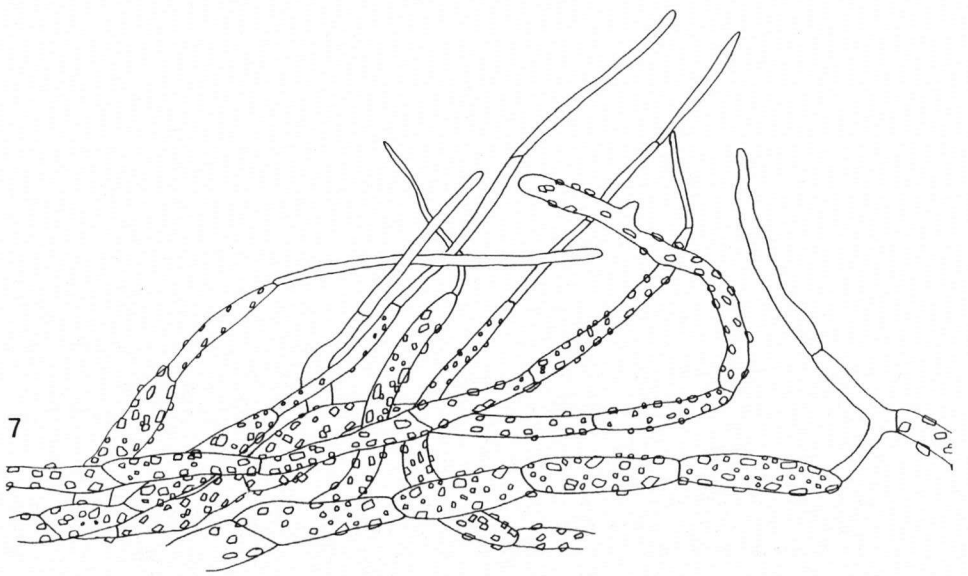
Clitopilus fuscogelatinosus, another small pleurotoid *Clitopilus* found on decaying wood, which possess a greyish pileus, darkly coloured lamellae and crystalline material in the pileipellis, can be readily recognized by its whitish floccose lamellar edges which produce a sterile layer of filamentous cheilocystidia and by the distinctive gelatinized tissues of the pileus and lamellar tramae. Also, the larger basidiospores (7.5–8.3 × 4.9–5.5 µm) and the bacilliform crystals found on the hyphae of the pileipellis of *C. fuscogelatinosus* serve to further distinguish this species from *C. argentinus*.

B. Morphological and elemental analysis studies

The extracellular crystals covering the hyphae of the pileipellis of *C. argentinus* (Figs. 2–4) were subjected to X-ray elemental analysis and found to consist mainly of calcium and magnesium and perhaps some potassium elements (Figs. 8, 9). These crystals are also known







Figs. 5–7. *Clitopilus argentinus*. — 5. Basidia, × 1000. — 6. Basidiospores, × 2500. —7. Pileipellis, × 1000. (All figs. from *Baroni 6057*.)

to dissolve readily in hydrosulfuric acid (Singer, 1952). It is likely that these small (typically less than one μm) cuboidal to tetragonal crystals (Fig. 3, 4) are a form of oxalate crystal since oxalate crystals have been widely reported from various groups of fungi (Horner & al., 1983). These crystals are also produced on the hyphae of the basidiomata that develop from in vitro agar cultures of either PDA or MEA.

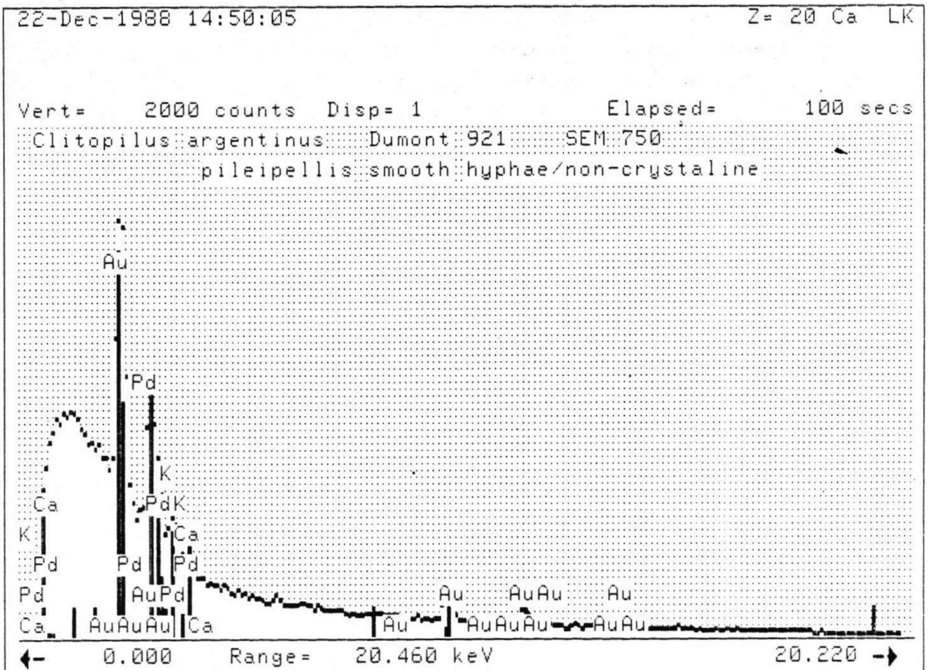
Specific morphological forms of crystals have been described for various groups of fungi and given some emphasis as useful taxonomic characters (e.g. in recent literature Baroni, 1981; Methven, 1989; Redhead & Baroni, 1986). As noted above, *Clitopilus incrustatus* and *C. fuscogelatinosus* produce crystalline material on their hyphae, and these crystals are morphologically distinctive for each of these taxa. Certainly more attention should be given to these structures by agaric taxonomists.

C. Culture mat characteristics and basidiome production in pure culture

Under constant conditions, all colonies grew at a rate of 3–4 mm/day over a 14 day period. Generally the surface of the colonies produced a thin, white, fluffy aerial layer. After day 5, most colonies produced obscure subzonate rings which consisted of flats alternating with thin, fluffy aerial hyphae, while the area around the point of inoculum became more densely cottony and turned pale cream. The reverse side of the colony also appeared pale cream mimicking the colour and extent of pigmentation of the surface hyphae. By day 14, fluffy white primordia had begun to form around the point of inoculation on most of the plates (nearly 80 percent). Mature basidiomata, i.e. those with a hymenium and producing basidiospores, could be found on the plates 2–3 months after inoculation (Fig. 1). From 4 to 25 mature basidiomata were produced per plate.

The macroscopic features of these basidiomata produced in culture matched exactly the features recorded for the specimens found on their natural substrata, with just a few exceptions. In many cases delicate thin white rhizoids could be found radiating from the base of the basidiomata grown in culture. This feature was not observed on specimens of the original collection. As found with these organisms in their natural setting, many basidiomata developed a small distinct central stipe at first which elevated the pileus above the agar surface. The stipe then became eccentric and eventually lateral as the pileus enlarged unequally. However, the majority of basidiomata that developed in culture formed with their strongly inrolled pileus margins tightly appressed to the agar surface, never becoming elevated by a stipe, and therefore appeared as greyish 'sclerotial-like' masses on the surface of the agar. Once these pilei were turned over, the well-developed lamellae and reduced stipes became obvious (Fig. 1).

Figs. 8–9. *Clitopilus argentinus*. — 8. X-ray analysis of smooth, non-crystal encrusted hyphae of the pileipellis. Note the lack of elevated peaks for magnesium (Mg) and calcium (Ca). — 9. X-ray analysis of crystal encrusted hyphae. Note the arrows indicating significantly increased levels of Mg and Ca. Also note a marked increase in potassium (K). In both profiles, the constant levels of gold (Au) and pallidium (Pd) are the result of the standard coating which is applied to biological specimens before they are studied ultrastructurally under the SEM. (All figs. from *Dumont PA 921*.)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pileipellis smooth hyphae/non-crystaline
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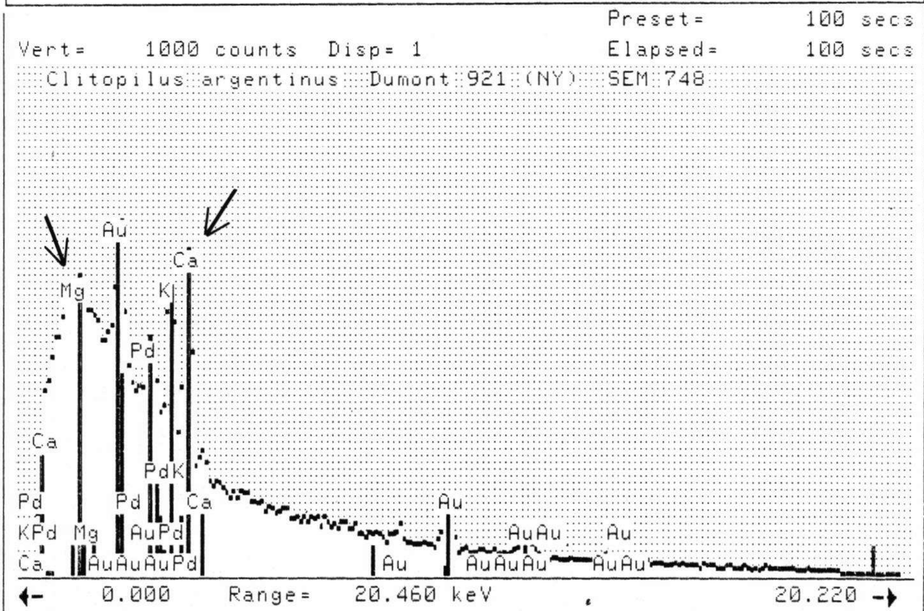
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The microscopic features of the basidiomata produced in culture also matched the form and dimensions of those observed from the original collection, with again just a few exceptions. The basidiospores tended to be slightly larger (6.4–9.7 × 4.8–5.6 µm, Q = 1.33–2.02, Q^m = 1.53, L^m = 7.75, W^m = 5.07) and the crystals on the hyphae of the pileipellis were not as abundant as in the original collection.

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A grant from the Buffalo Museum of Science supporting continuing research on the New York State agaric and bolete mycota is directly responsible for this work and is gratefully acknowledged. The following curators and directors of herbaria are sincerely thanked for their help in securing loans: Drs. R.E. Halling (NY), D.H. Pfister (FH) and R.L. Shaffer (MICH). Ms. Dawn Van Hall, photographic department SUNY – College at Cortland, provided technical assistance in reproducing the X-ray analysis print-outs and with production of black and white negatives of the basidiomata from colour slides. The use of the SEM facilities at the Center for Ultrastructural Studies, Environmental Science and Forestry College – SUNY at Syracuse, is also gratefully acknowledged with special thanks due Mr. Arnold Day for producing the X-ray analysis profiles.

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