

***Coprotus granuliformis* (P. Crouan & H. Crouan) Kimbr. – like [AEB 1359 (= PDD 120027)]**

Collected: March 2023

Substrate: wild red deer (*Cervus elaphus*) dung

Collection site: forested bush area several kilometers north of higher-altitude Moonshine Hill Road, Upper Hutt, New Zealand

Collectors: Ian Flux & Merryl Park; **Identifiers:** Ann Bell & Dan Mahoney

Voucher materials: No dried herbarium specimen but 5 Shear's mounting fluid (SMF) semi-permanent microscope slides; in-situ photographic views of fresh fruiting bodies on the dung and microscopic views of apothecium details; Dan's comments.

References consulted: (listed in chronological order)

1) Kimbrough J.W., Luck-Allen E.R. & Cain R.F. 1972. North American species of *Coprotus* (*Thelebolaceae*, *Pezizales*). *Canadian Journal of Botany* 50: 957–971. **Their complete descriptions and illustrations of *Coprotus granuliformis* and *C. breviascus* are reproduced on the following pages of this pdf.**

2) Bell A. & Kimbrough J.W. 1973. *Coprotus trichosurus* sp. nov. from New Zealand. *Transactions of the British Mycological Society* 61(1): 190–193. **Their complete description and illustrations of *Coprotus trichosuri* (as *C. trichosurus*) are reproduced on a following page of this pdf.**

3) Spooner B.M. & Butterfill G.B. 1999. Coprophilous Discomycetes from the Azores. *Kew Bulletin* 54, No. 3: 541–560. **They provide descriptions and illustrations of *Coprotus breviascus* & *C. granuliformis*.**

4) Doveri F. 2004. *Fungi Fimicoli Italici*. Vicenza, A.M.B.-Fondazione Centro Studi Micologici, 1104 p., 158 pl. **Includes an updated key to *Coprotus* (after Kimbrough, Luck-Allen & Cain, 1972) and a thorough description with illustrations of *C. granuliformis*.**

5) *Fungi of Great Britain and Ireland*. 2016. Key partially adapted from an unpublished document by Yi-Jan Yao and description of *C. granuliformis* adapted from Kimbrough et al. (1972) and Doveri (2004) by Paul Cannon.

Continued on the next page:

6) Kušan I., Matočec N., Jadan M., Tkalčec Z. & Mešić A. 2017. An overview of the genus *Coprotus* with notes on the type species and description of *C. epithecioides* sp. nov. MycoKeys 29: 15–47. **This more recent reference is invaluable. It provides a historical summary, a key to accepted species of *Coprotus*, tables comparing their various morphological features and a phylogram with those species sequenced. Below and on the next page I have extracted their morphological information on the 3 species most closely related to AEB 1359: *Coprotus granuliformis*, *C. trichosuri* & *C. breviascus*. None of these have been sequenced to date with *C. granuliformis* recorded most frequently and *C. breviascus* and *C. trichosuri* a distant 2nd and 3rd, resp.**

Comparison of AEB 1359, which I am treating as *Coprotus granuliformis*-like, with 3 closely related morphological species treated by Kušan I. et al. 2017; i.e. *C. granuliformis*, *C. trichosuri* & *C. breviascus*.

1) Apothecium:

AEB 1359 – margins n/a, diam. $187.5 \times 125 \mu\text{m}$ & $130 \times 65 \mu\text{m}$ with outer excipulum textura angularis, shape glob-lent in an overhead view, pigment white then faintly yellowish, substrate red deer dung

C. granuliformis – margins \pm isodiametric cells, diam. 0.2–0.6 mm, shape glob-lent, pigment white to yellowish, substrate ruminant dung

C. trichosuri – margins n/a, diam. 0.1–0.2 mm, shape n/a, pigment always hyaline to white, substrate *Trichosurus vulpecula* dung

C. breviascus – margins elongated cells, diam. 0.2–0.6 mm, shape disc-lent, pigment always yellow to orange or always yellowish orange, substrate ruminants & Equus dung

2) Ascus:

AEB 1359 – $32.5\text{--}37.5\text{--}(40) \times 10\text{--}14\text{--}(16.5) \mu\text{m}$, broad clavate, no bluing in Melzer's

C. granuliformis – $38\text{--}58 \times 14\text{--}20 \mu\text{m}$, broad clavate

C. trichosuri – $50\text{--}60 \times 20\text{--}26 \mu\text{m}$, broad clavate

C. breviascus – $45\text{--}60 \times 20\text{--}28 \mu\text{m}$, short cylindrical & $45\text{--}65 \times 11\text{--}15 \mu\text{m}$; broad clavate

3) Ascospores:

AEB 1359 – ellipsoid, obtuse, $7.5\text{--}10 \times (4.5\text{--})5\text{--}6 \mu\text{m}$

C. granuliformis – ellipsoid, narrowly-ellipsoid, obtuse, $9.5\text{--}14.5 \times 6\text{--}9.5 \mu\text{m}$

C. trichosuri – narrowly-oblong, obtuse, $9\text{--}14 \times 5\text{--}6 \mu\text{m}$

C. breviascus – ellipsoid, narrowly-ellipsoid, tapered, $12.5\text{--}18 \times 7.5\text{--}12 \mu\text{m}$ & $9.8\text{--}11.1 \times 6.5\text{--}7.2 \mu\text{m}$

4) Paraphyses:

AEB 1359 – apices clavate & bent apically (also simple & cylindrical), width n/a, branching n/a, globules none to diffuse, pigment white becoming faintly yellow

C. granuliformis – apices clavate, width $4\text{--}8 \mu\text{m}$, bent, branching below, globules none to diffuse, pigments none to yellow

C. trichosuri – apices cylindric-obtuse, width $3\text{--}4 \mu\text{m}$, branched, straight, globules & pigments none

C. breviascus – apices cylindric-clavate, width $3\text{--}4 \mu\text{m}$, simple, straight-bent, globules yellowish, pigments n/a

other *C. breviascus* – apices filiform, width $1.5\text{--}2 \mu\text{m}$, branching n/a, bending uncinata, globules yellowish, pigment granules

Comments: Relatively few patches of the AEB 1359 apothecia were observed on the red deer dung. Ann recognized these as a *Coprotus* species and suggested *C. trichosuri*. I then spent several days preparing slides, measuring and searching for publications with photos and descriptive detail. These were readily available for *C. granuliformis* (with 162 records in the Mycoportal website) but fewer for *C. breviascus* (10 records) and *C. trichosuri* (5 records). Morphological information on the latter two species is scant so my comparison of AEB 1359 with these 3 similar species really requires more information on what variability exists within *C. trichosuri* and *C. breviascus*. Added to this is the lack of any culturing or sequencing for all 3. Comparative information in Kušan I. et al. would seem to eliminate *C. breviascus*, leaving *C. granuliformis* & *C. trichosuri*. AEB 1359 apothecia best match the size and early coloration of *C. trichosuri*, but its asci are smaller and its ascospores slightly smaller than those of both *C. trichosuri* and *C. granuliformis*. The paraphyses of AEB 1359 best tie AEB 1359 to *C. granuliformis* – but is this significant enough to call AEB 1359 *C. granuliformis*? Probably not. In the meantime, until more collections come to light with good illustrations, descriptions and sequencing, I have designated AEB 1359 as *Coprotus granuliformis*-like.

8. *Coprotus granuliformis* (Cr. & Cr.) Kimbr. Figs. 25-29

≡ *Ascobolus granuliformis* Crouan & Crouan, *Ann. Sci. Nat.* IV, 10: 195. 1858.

≡ *Ascophanus granuliformis* (Cr. & Cr.) Boudier, *Ann. Sci. Nat.* V, 10: 245. 1869.

≡ *Coprotus granuliformis* (Cr. & Cr.) Kimbrough, *Am. J. Bot.* 54: 22. 1967.

= *Ascobolus argenteus* Currey, *Trans. Linn. Soc.* 24: 496. 1864.

≡ *Ascophanus argenteus* (Curr.) Boud., *Ann. Sci. Nat.* V, 10: 245. 1869.

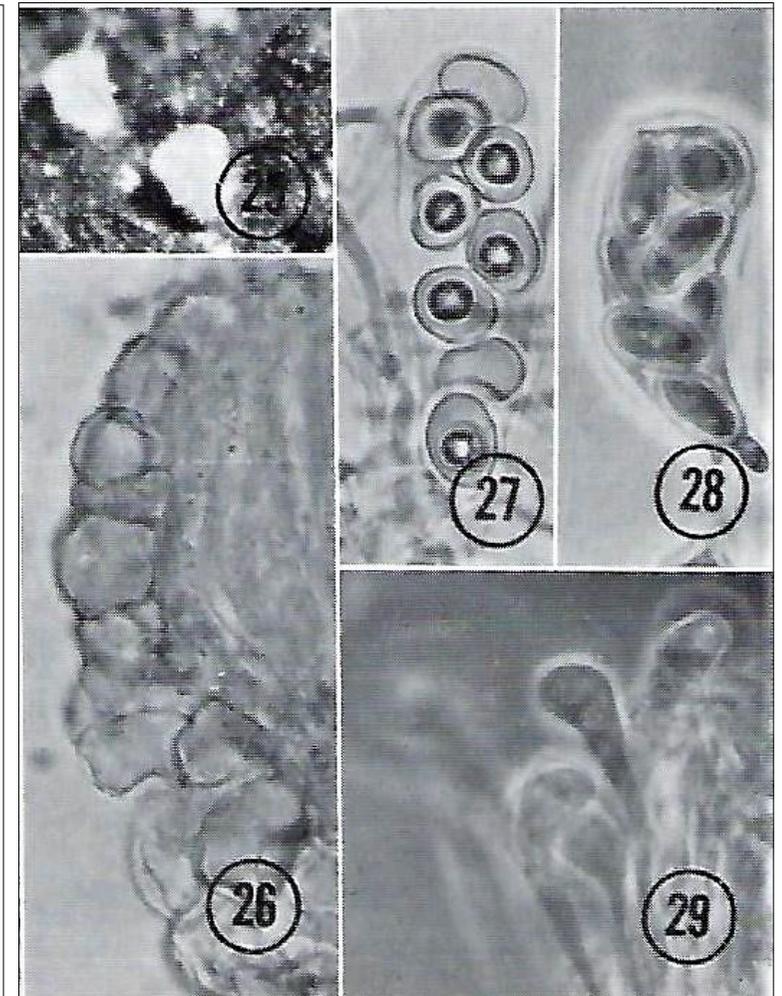
= *Ascophanus rosellus* Starbäck, *Bot. Notis.* 216. 1898.

Apothecia cupulate to discoid, white to pale yellow, margins darker in color than hymenium, 0.2-0.6 mm in diam; excipulum of a *textura angularis* to *globulosa*, marginal cells non-elongated, almost isodiametric, reaching 10–12 µm diam, with cell walls slightly thickened and pigmented with age; **asci** eight-spored, broadly clavate, 40–55 X 15–30 µm, almost truncate above, sharply tapering to a short stalk below; **ascospores** mostly biseriate, broadly ellipsoid, 9.0–15.0 X 6.5–9.5 µm, each with one large de Bary bubble; **paraphyses** filiform, septate below, inflated at apices to 5.0–8.0 µm, hyaline to slightly pigmented, often with minute oil guttules.

HABITAT: Mostly on cow dung, but also on deer and sheep dung.

TYPE: On cow dung, Brest, Finistère, France, *Crouan* (examined and figured by Le Gal (1961)).

COMMENTS: *Coprotus granuliformis* is easily distinguished from other species of the genus by its short, very broadly clavate asci and by paraphyses which are greatly inflated at their apices.



Coprotus granuliformis. Fig. 25. Apothecia on dung, X30. Fig. 26. Section of apothecium showing globose excipular cells, X800. Fig. 27. Ascus with ascospores, X800. Fig. 28. Young ascus with thick-walled spores, X800. Fig. 29. Strongly inflated paraphyses, X800.

Bell A. & Kimbrough J.W. 1973. *Coprotus trichosurus* sp. nov. from New Zealand. Transactions of the British Mycological Society 61 (1): 190–193.

COPROTUS TRICHOSURUS SP. NOV. FROM NEW ZEALAND

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Coprotus Korf & Kimbrough (*Thelebolaceae*, *Pezizales*) is a segregate of the coprophilous genus *Ascophanus* Boud. characterized by minute, translucent, white to yellow apothecia, operculate, non-amyloid, eight-to multi-spored asci, and smooth, hyaline, thin-walled ascospores that contain a refractive bubble. Kimbrough & Korf (1967) transferred six species previously belonging to *Ascophanus* or *Ryparobius* Boud, to *Coprotus* and selected *C. sexdecimsporus* (Cr. & Cr.) Kimbrough & Korf as the holotype. In a current treatment of the genus, 18 North American species of *Coprotus* are recognized (Kimbrough, Luck-Allen & Cain, 1972). In a study of coprophilous discomycetes of New Zealand, a collection of *Coprotus* was found on faecal pellets of the Brush-tailed opossum, *Trichosurus vulpecula* Kerr., that appeared different from previously described species. For this we propose the following new specific name.

Coprotus trichosurus Bell & Kimbrough sp. nov. – Collected Orongorongo Valley, Wellington, New Zealand. Dec. 1971, by A. Bell, holotype (PDD 30082).

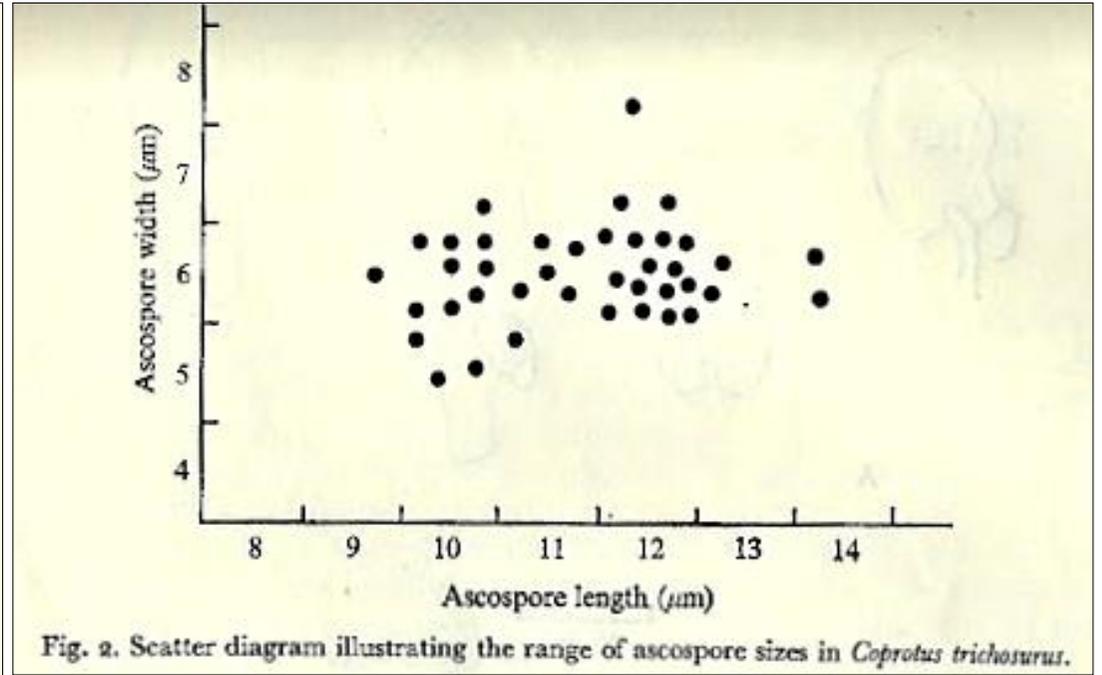
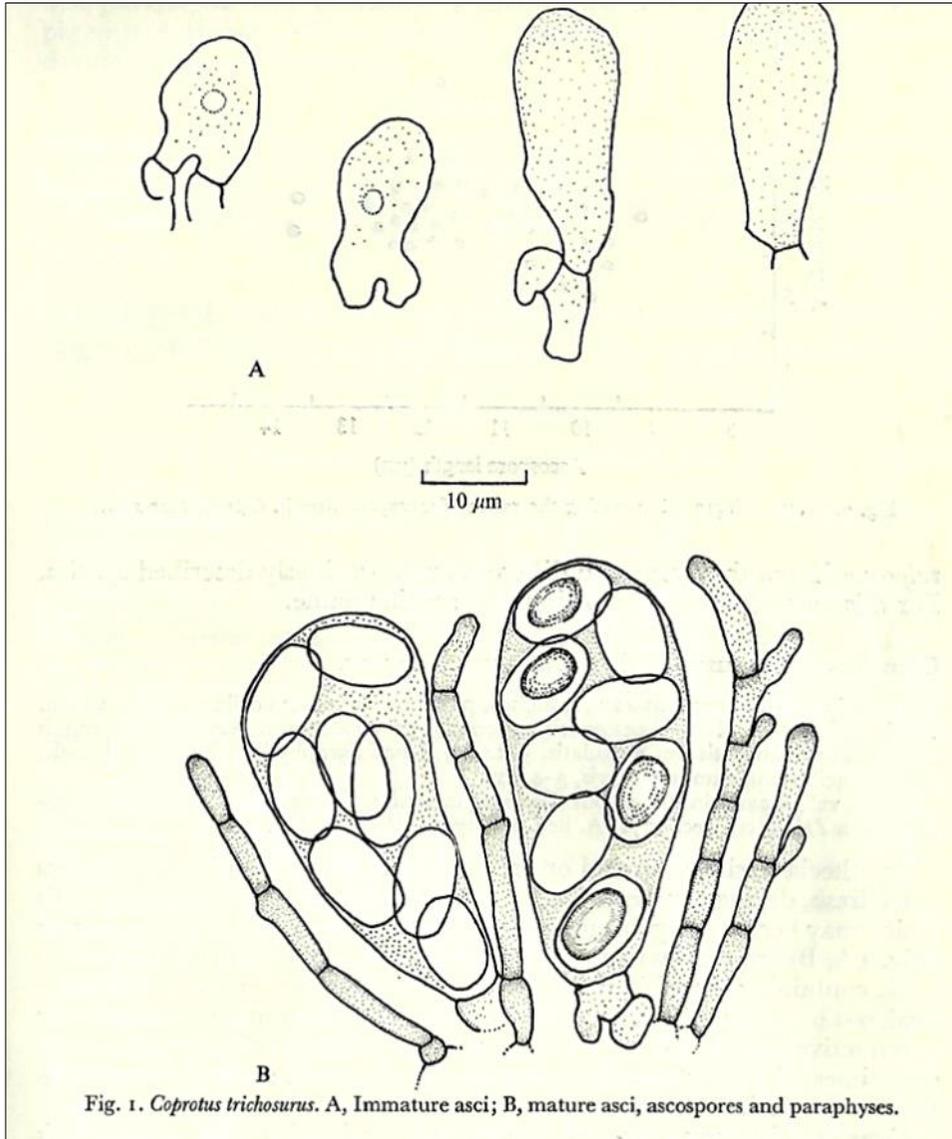
Apothecia sessile, scattered or gregarious, 125–175 µm diam, colourless when fresh, drying to pale yellow. Apothecial wall composed of globose cells which may become angular through mutual pressure. **Asci** broadly clavate (Fig. 1A, B) approximately 50–60 x 20 µm when mature, with little or no stalk, containing eight biserially arranged ascospores. **Ascospores** bluntly oval, 9–14 × 5–6 µm (Fig. 2), smooth-walled, hyaline, sometimes containing a refractive bubble (Fig. 1B). **Paraphyses** hyaline, cylindrical, septate, sometimes with short branches, 3–4 µm in diam and similar in length to the mature asci.

Coprotus trichosurus appears most closely related to *C. granuliformis* (Cr. & Cr.) Kimbrough and *C. breviascus* (Vel.) Kimbrough, Luck-Allen & Cain. All three species have very short, broad asci (45–60 x 15–30 µm), and ascospores that approach 15 µm in length. *Coprotus trichosurus* may be distinguished from these species, however, in that its apothecia are usually smaller and devoid of pigments, its spores are from 1.0–5.0 µm smaller in diameter, and its paraphyses are neither broadly inflated nor filled with lipid bound pigments. Although certain collections of *C. granuliformis* appear colourless, small lipid droplets may still be found in the paraphyses. The paraphyses in *C. breviascus* are slightly uncinuate and filled with large pigmented droplets, while those of *C. trichosurus* are straight, cylindrical and devoid of pigmented droplets.

References:

KIMBROUGH J.W. & KORF R.P. 1967. A synopsis of the genera and Species of the tribe *Theleboleae* (= *Pseudoascoboleae*). American Journal of Botany 54: 9–23.

KIMBROUGH J.W., LUCK-ALLEN E.R. & CAIN R.F. 1972. North American species of *Coprotus* (*Thelebolaceae*, *Pezizales*). Canadian Journal of Botany 50: 957–971. **Figures on the next page:**



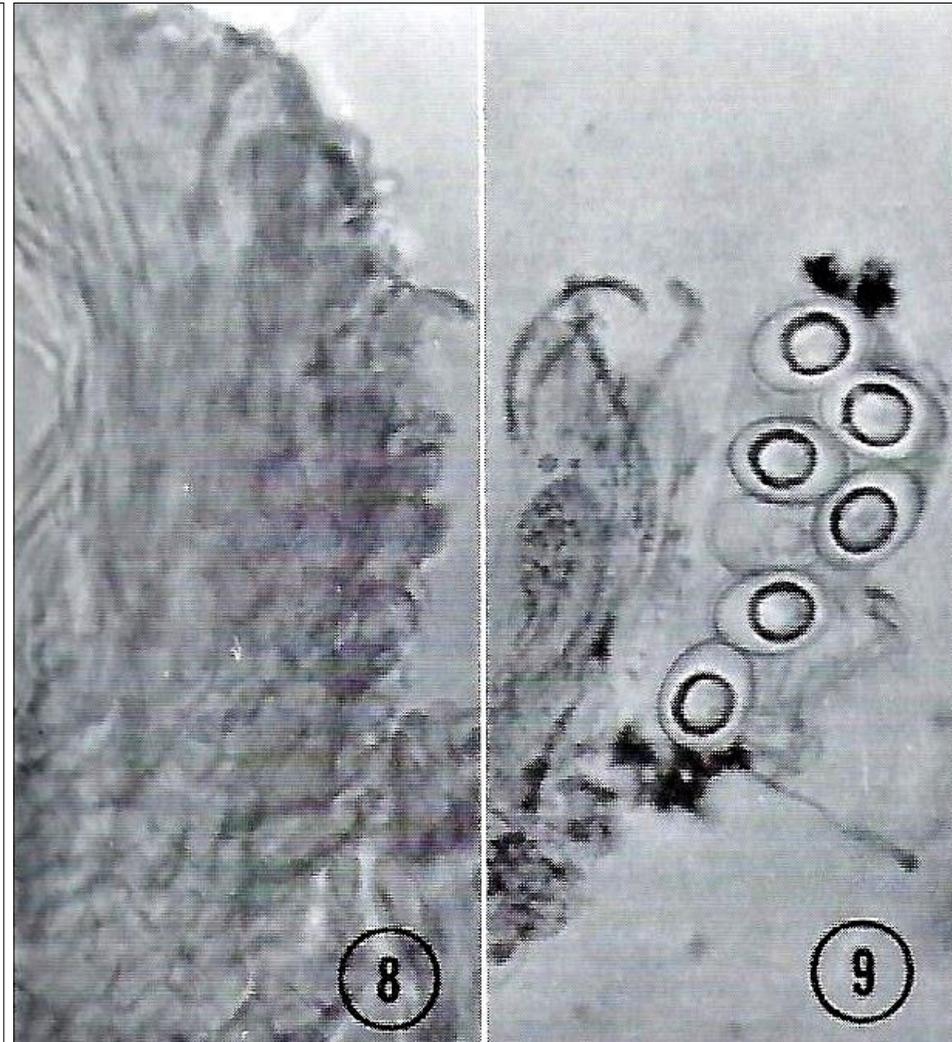
3. *Coprotus breviascus* (Vel.) comb. nov. Figs. 8, 9

≡ *Ascophanus breviascus* Velenovsky, Monogr. Discom. Bohem. 360. 1934.

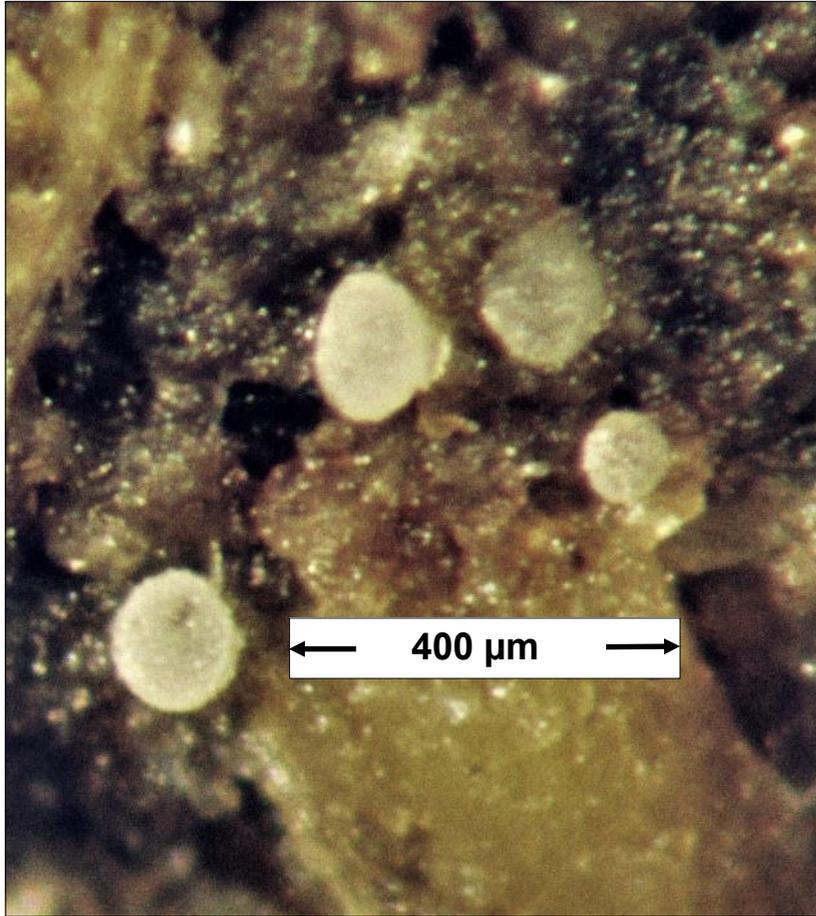
Apothecia scattered to gregarious, yellowish to orange, discoid to lenticular, 0.2–0.6 mm in diam; excipulum slightly pigmented, non-cyanophilous, cells of a *textura angularis* and elongated at the tips; **asci** eight-spored, broadly clavate, 45–60 X 20–28 μm , abruptly attenuated at base; **ascospores** biseriate, 12.0–16.0 X 8.5–12.0 μm , hyaline to pale yellow, smooth, broadly ellipsoid, with perispore layer thin and cyanophilous; **paraphyses** filiform, septate, simple or branched, 1.5–2.0 μm below, slightly inflated, uncinuate at their apices and containing yellowish oil droplets.

TYPE: On cow dung, Radotin, Mnichovice, Czechoslovakia, Velenovsky (PR).

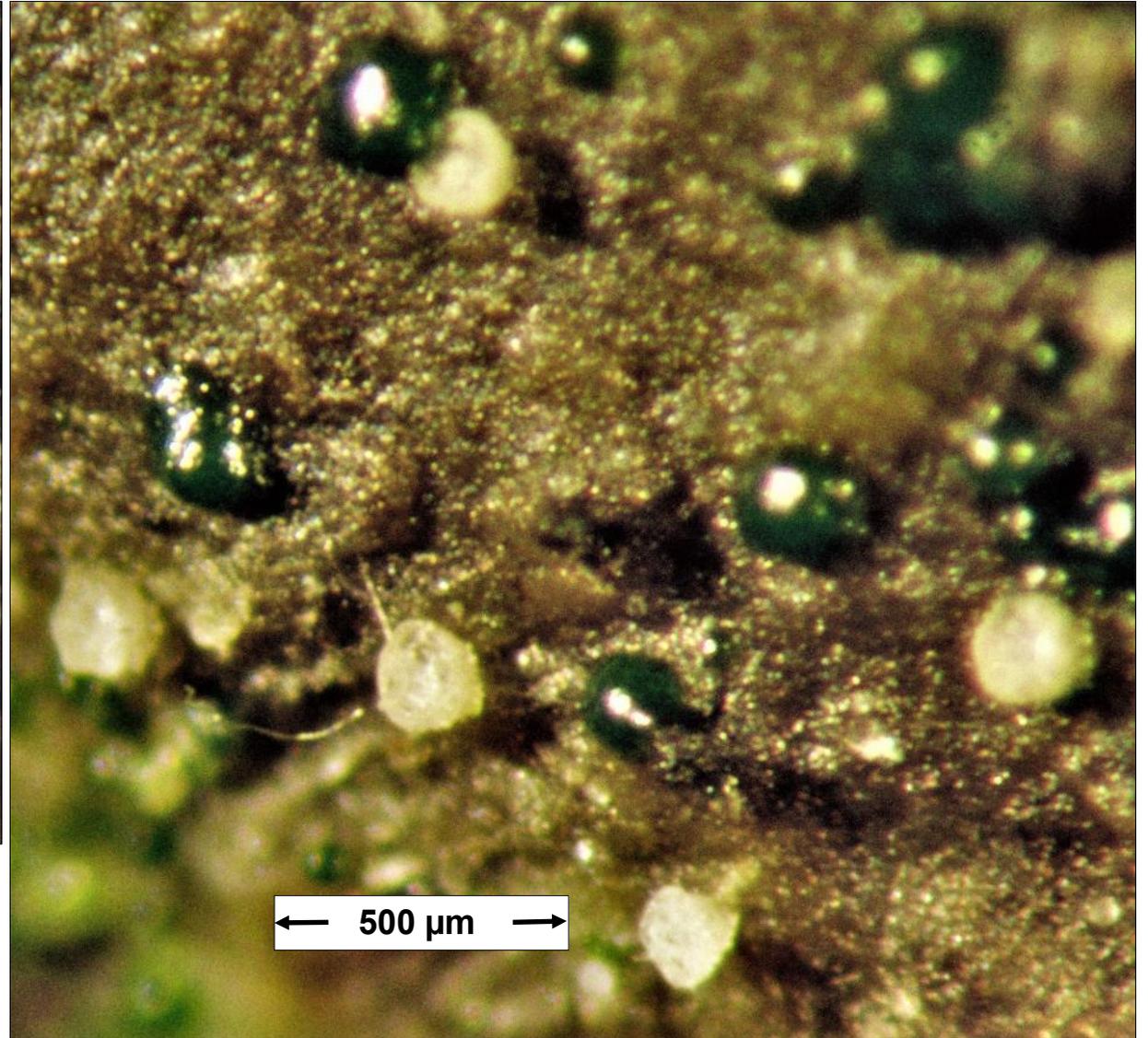
COMMENTS: *Coprotus breviascus* is morphologically similar to *C. granuliformis* and *C. ochraceus*. The broadly clavate asci and broadly ellipsoid ascospores are similar to those of *C. granuliformis*, but the latter species can be distinguished by its strongly inflated, hyaline paraphyses and larger, more globose excipular cells. *Coprotus ochraceus* has both larger asci and ascospores. Velenovsky (1934) cites the same figures for *C. breviascus* and *A. bilobus* but the latter appears to be a synonym of *C. ochraceus*. Contrary to the original description, we find oil droplets in the paraphyses of *C. breviascus*.

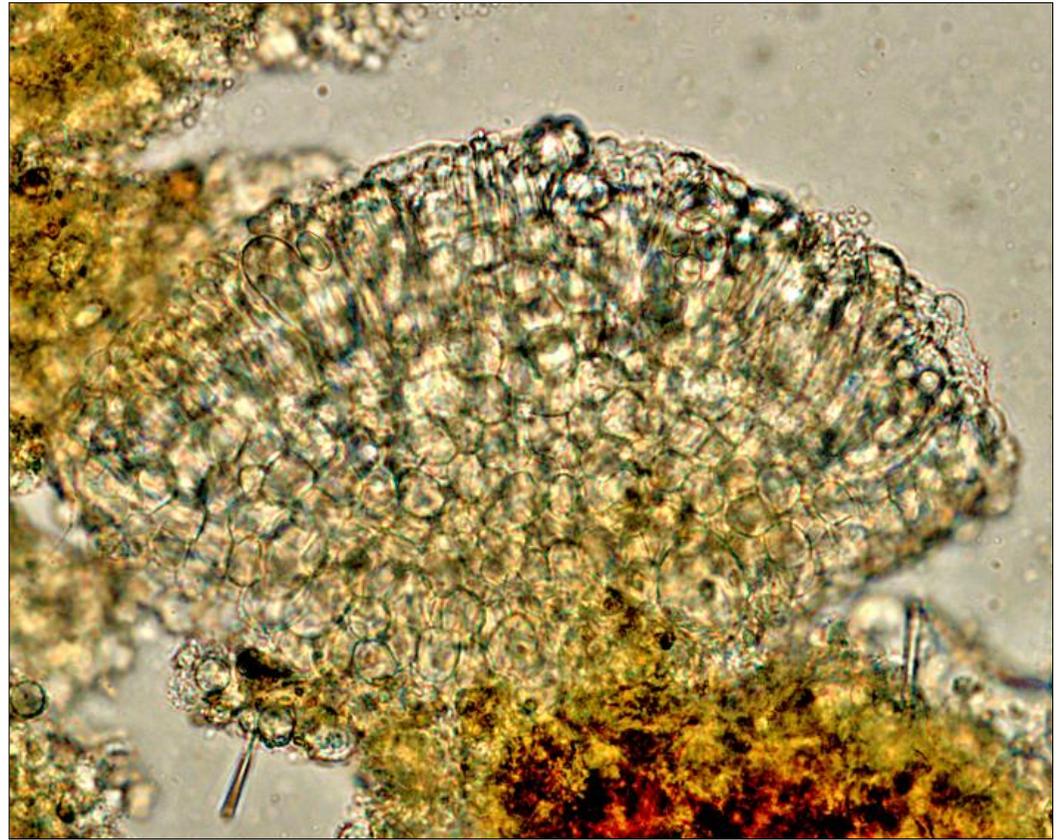
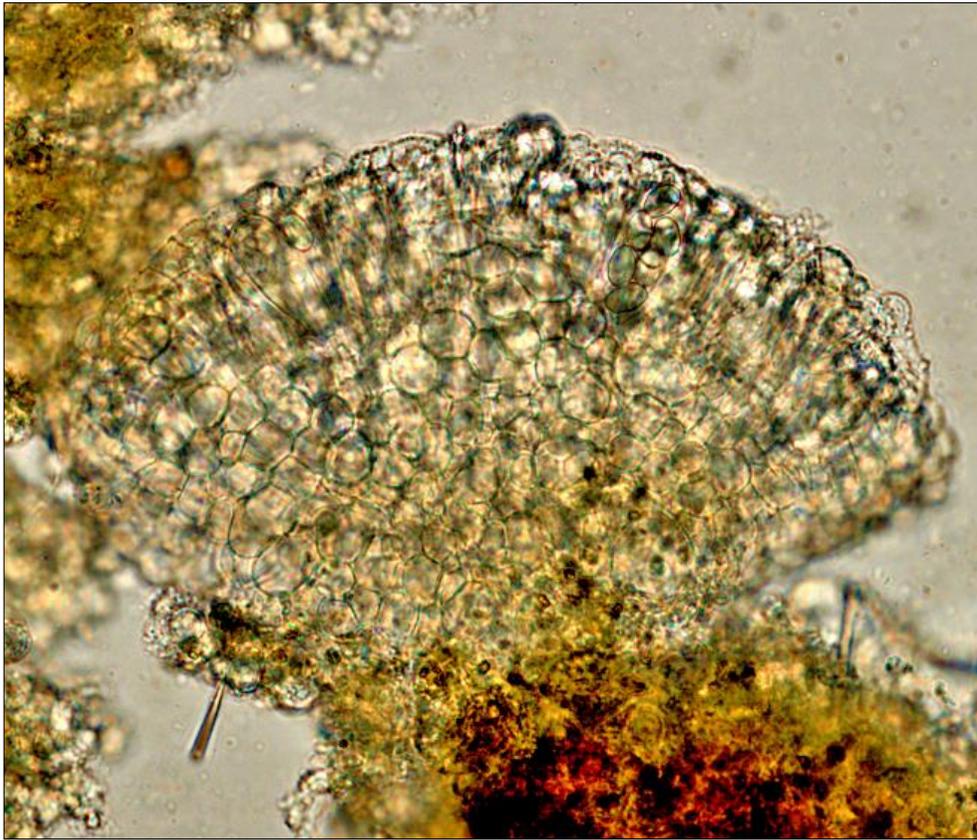


Coprotus breviascus. Fig. 8. Section of apothecium showing excipulum, X800. Fig. 9. Ascus, ascospores and paraphyses, X800.

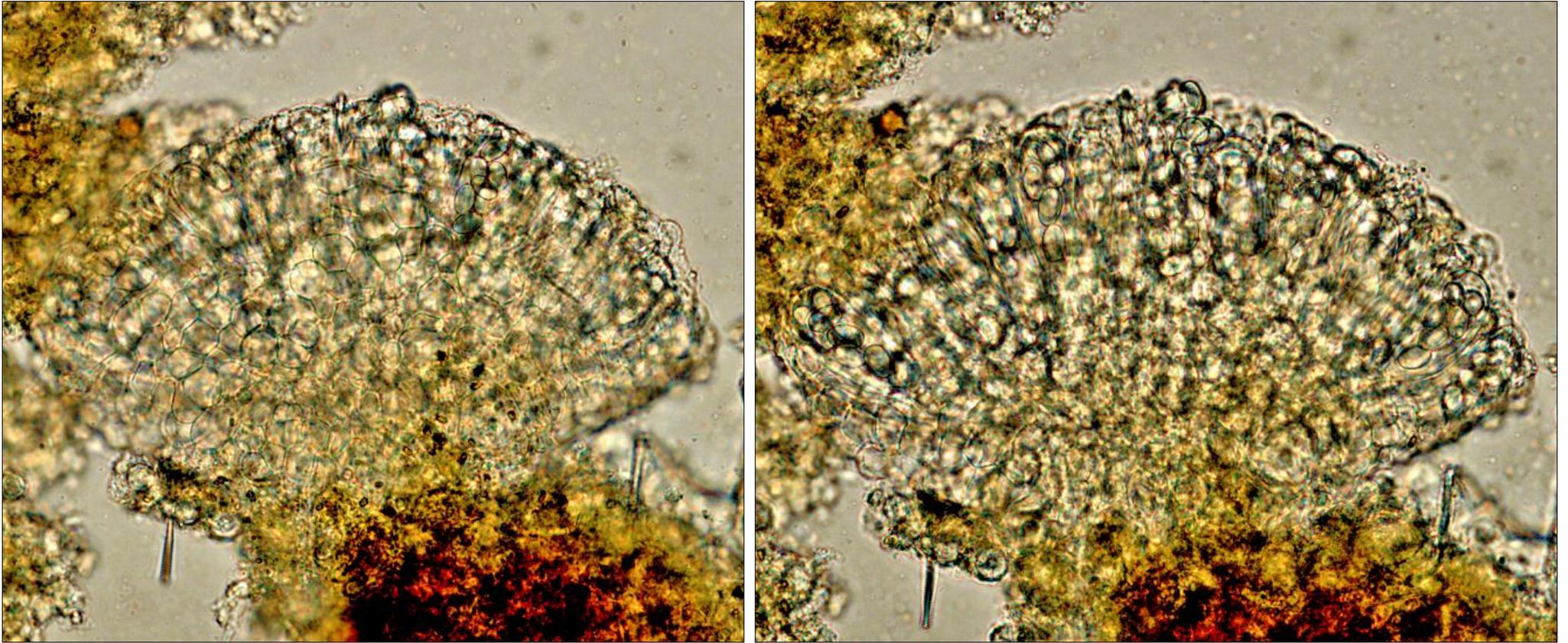


AEB 1359 apothecia on red deer dung incubated 2 weeks in a moist chamber. Their small size and lack of color made them a difficult photographic subject.





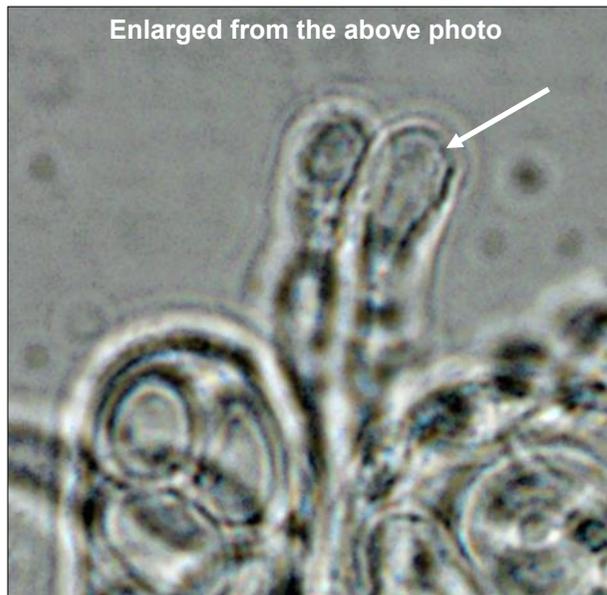
AEB 1359 apothecia. Photographed from fresh water mounts under the X40 objective using brightfield microscopy. These photos left to right and continuing on the next page were taken of the same ascoma (187.5 × 125 μm) at successively deeper foci from an outermost focus (emphasizing the globular to angular-celled outer excipulum tissue) to inner areas of the ascoma (emphasizing the asci and ascospores located there).



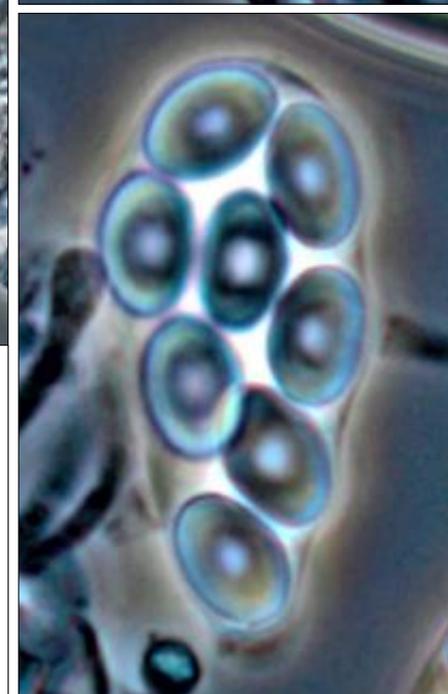
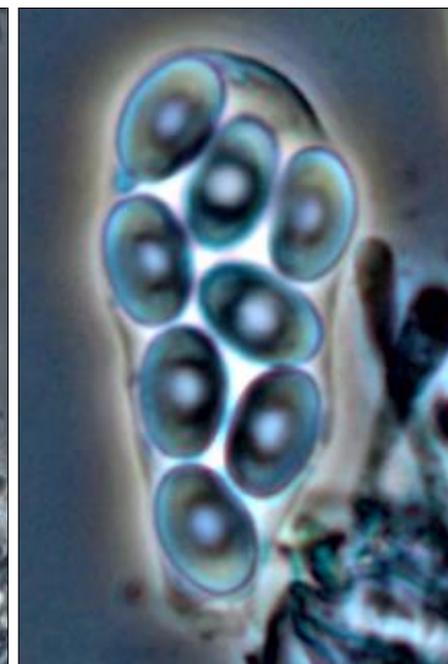
AEB 1359 apothecia. Photographed from fresh water mounts under the X40 objective using brightfield microscopy. These photos beginning left to right on the previous page and continuing here were taken of the same ascoma ($187.5 \times 125 \mu\text{m}$) at successively deeper foci from an outermost focus (emphasizing the globular to angular-celled outer excipulum tissue) to inner areas of the ascoma (emphasizing the asci and ascospores located there).



AEB 1359 apothecium. Photographed from a fresh water mount under the X40 objective using phase microscopy. The apothecium shown measures $130 \times 65 \mu\text{m}$ and emphasizes the *textura angularis* tissue of the outer excipulum.



AEB 1359 emphasis paraphyses. All photos from SMF slide mounts using the X100 objective. Left 3 photos brightfield, upper right photo phase. Note that paraphyses can be \pm straight to curving and somewhat capitate or not. In the brightfield photos, capitate portions are spinulose (arrowed).



AEB 1359. All photos taken using the X40 objective and phase microscopy. Left photo from a water mount, right 2 from SMF. Asci overall: 32.5–37.5 (–40) × 10–14(–16.5) μm. Ascospores overall: 7.5–10 × (4.5–)5–6 μm.



AEB 1359 fertile asci and a paraphysis. Both photos from the same SMF mount under the X100 objective using phase and brightfield microscopy, resp. The ascospores in these photos measured $9 \times 5 \mu\text{m}$.